15TH HORIZONS IN MOLECULAR BIOLOGY

Max Planck Institute for Biophysical Chemistry
Göttingen, Germany
10-13 September 2018

Presented by the students of the IMPRS for Molecular Biology at the Georg-August Universität Göttingen

15TH HORIZONS IN MOLECULAR BIOLOGY

International PhD Student Symposium
and
Career Fair for Life Sciences

10th-13th September 2018
Göttingen, Germany

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We would like to warmly thank all our old and new sponsors and donors without whom this conference would not have been possible. Luckily, our sponsor list is expanding and this is only giving us more and more support to make Horizons a joyous ride! We wholeheartedly thank Peter Lösel ,Christian Hartig and Frederik Köpper for helping us all throughout. A warm gratitude to Uwe Krüger and his team for making sure we have food and coffee during the entire conference. We thank the IT department of the MPI-BPC for the technical support.

Many thanks to Prof Mary Osborn for kindly agreeing once again to be the moderator of the panel discussion. Talking with her is always quite an insightful experience. Special thanks to our honorary guests – Dr Katharina Seitz for kindly providing and showing us the Indesign templates for the booklet and Claudia Schmidt for helpful consultations during the entire year. Special thanks to Irene Böttcher-Gajewski for being there to capture the Horizons moments through her camera and an extended gratitude to our official student photographers Taras Velychko and Kristina Stakyte. Furthermore, we would like to thank Claus-Peter Adam for compiling this booklet and being there all the time when we needed him. In the end, to all our participants, a warm Thank You,

From Horizons Organizing Committee

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An International Scientific Conference from PhD students for PhD students

Welcome to the 15th Horizons in Molecular Biology!

Fifteen years ago, Horizons in Molecular Biology symposium was conceived by the graduate students of International Max Plank Research School in Molecular Biology to widen their own horizons beyond the rut of classes and lab work. Ever since, Horizons has grown from a humble idea to one of the most sought-after events in Göttingen and Germany. Horizons aims to bridge the gap between young scientists and experienced researchers by promoting them to engage in a productive dialog and exchange information. This year, we have planned four exhilarating days of presentations, poster sessions, panel discussions, and workshops aimed to inform, inspire and pique the interest of the young, scientific mind.

One could describe Horizons as a platform built to facilitate interactions between participants and speakers! We therefore invite you to engage with our roster of distinguished speakers from diverse fields of work during their presentation or even over a cup of coffee. Do not miss this opportunity to network with your favourite speakers and participants from around the world. Should you require an introduction, we are glad to help you! From academia to far and beyond! Our Career Fair is specially tailored to meet the needs and expectations of a budding researcher. In addition to our informative talks and educational workshops, we also have a 'Speed Dating' event where you can gain valuable insights from the diverse speakers in a personalized and informal setting. Make sure to sign up for this unique experience!

The life of a PhD student can be full of many rewarding moments driven by curiosity and hardships. However, in the journey towards a PhD, the highs are often fraught with disheartening lows too. Being all too familiar with the experience, we thought that advice from those who persevered and succeeded could shed some light on the 'tricks of the trade'. To this aim, the Panel Discussion titled 'Troubles of a young scientist: Fantastic ideas and where to Find them' will take place on the final day of the conference.

Learning from the experiences of accomplished scientists is of vital importance, however, here at Horizons, we believe it is equally imperative to support and network with our peers. The Poster Sessions and Awarded Student Talks provide just such an opportunity. We encourage everyone to actively participate in the sessions and vote for which poster appealed to you the most!

We look forward to having you here with us and hope to make this not only an educational experience, but also a memorable one.

Horizons in Molecular Biology 2018 Organizing Team

Organizing Committee

The 15th PhD Student Symposium Horizons in Molecular Biology is organized by a group of PhD students of the International Max Planck Research School for Molecular Biology in Göttingen.

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Partners

The 15th International PhD Student Symposium "Horizons in Molecular Biology" is financially supported by the following institutions through the International Max Planck Research School for Molecular Biology.

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

MAX-PLANCK-GESELLSCHAFT

Donors

The PhD Student Organizing Committee would like to thank the following companies and organizations for their support:

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Program overview

Monday, September 10th

09:15 *Registration*
09:45 **Opening Ceremony Career fair**
10:00 - 10:30 **Daisy Robinton** – *WandW*
10:30 - 11:00 **Ben Glick** – *SnapGene*
Coffee break
11:20 - 11:50 **Stephanie Boue** – *sbv IMPROVER*
11:50 - 12:20 **Adrian Schomburg** – *Proteros Biostructures*
12:20 - 13:30 **Speed Dating / *Lunch break***
13:30 - 14:00 **Maria Loidolt-Krüger** – *PicoQuant*
14:00 - 14:30 **Wolfgang Grosse** – *CureVac*
14:30 - 15:30 **Marie-Laure Yaspo** – *Alacris Theranostics*
15:30 - 15:45 **Horizons Opening Ceremony**
15:45 - 16:30 **Osamu Nureki** – Molecular mechanism of CRISPR and structure based development of genome editing tool towards medical applications
16:30 - 17:15 **Juliane Liepe** – Peptide splicing at the hedge between self and non-self
17:15 - 18:00 **Leo James** – Trim-Away: Targeted degradation of pathogens and proteins by the cytosolic antibody receptor TRIM21

19:00 *Join us for a beer!* (Z.A.K., Am Wochenmarkt 22, 37073 Göttingen)

Tuesday, September 11th

09:00 - 09:30 *Registration*
09:30 - 10:15 **Roeland Nusse** – Gene signatures and stemness in the liver controlled by local Wnt signals
10:15 - 11:00 **Neville Sanjana** – New frontiers for pooled screens: Finding regulatory elements in the noncoding genome and capturing multi-cell interactions
Coffee break
11:30 - 12:15 **Peter Lenart** – The same, only different: How does the cell division machinery adapt to divide the large oocyte?
12:15 - 13:00 **Daniel Gerlich** – Chromosome mechanics during nuclear assembly
Lunch
13:50 - 14:35 **Michael Sheehan** – Genomic insights into the evolution of social intelligence and phenotypic diversity
14:35 - 15:20 **Ulrike Kutay** – Taking apart the nuclear envelope during open mitosis
Coffee break
15:35 - 15:55 **Awarded Student Talk**
15:55 - 16:40 **Floyd Romesberg** – A semi-synthetic organism that stores and retrieves increased genetic information
16:40 - 17:25 **Elizabeth Villa** – Opening windows into the cell: Revealing molecular architecture of the nuclear periphery
17:25 **Poster Session 1** ***(wine and cheese)***

Wednesday, September 12th

09:00 - 09:30 *Registration*
09:30 - 10:15 **Katherine Pappas** – From tequila shots to chassis genomics: works and days of an ethanol producer
10:15 - 11:00 **Florian Jug** – Content-Aware Image Restoration and Quantitative Downstream Analysis
Coffee break
11:30 - 12:15 **Yamuna Krishnan** – DNA nanodevices as probes for cell signaling
12:15 - 13:00 **Bianxiao Cui** – The role of membrane curvature at the nano-bio interface
13:00 - 14:30 **Poster Session 2 / *Lunch break***
14:30 - 15:15 **Andy Heron** – Senior Director, Advanced Research, Oxford, UK
15:15 - 16:00 **Danielle Laurencin** – Looking into calcium and oxygen local environments using solid state NMR spectroscopy
Coffee break
16:20 - 17:05 **Sjors Scheres** – The atomic structures of Tau filaments from Alzheimer's and Pick's disease brains
17:05 - 17:20 **Awarded Student Talk**
17:20 - 18:05 **Anne-Claude Gavin** – A network of lipid-transfer proteins at the crossroads between metabolism and signalling

19:00 City tour

20:00 Horizons Dinner & Party

Thursday, September 13th

09:00 - 09:30 *Registration*
09:30 - 10:15 **Food for Thought: *Horizons Breakfast***
10:15 - 11:00 **Polly Matzinger** – The Danger model of immunity
Coffee break
11:30 - 13:00 **Panel Discussion**
Troubles of a young scientist: Fantastic ideas and where to find them
Lunch
14:15 - 14:35 **Awarded Student Talk**
14:35 - 15:20 **Mara Dierssen** – Remodelling brain plasticity in intellectual disability
Coffee break
15:40 - 16:25 **Anna Marie Pyle** – Harnessing the RIG-I innate immune sensor to control antiviral and antitumor responses

16:25 **Closing Ceremony**
16:45 **Champagne and Goodbye**

GENERAL INFORMATION

Admission to Plenary Lectures

Admission to plenary lectures is restricted to registered participants.

Name badges and Conference Package vouchers

You will receive your name badge and vouchers for the conference package (if you chose it) during registration. The conference package includes lunches, city tour and conference dinner and party. Please wear your name badge at all times during the symposium. It will be required for admission to both the scientific and social programs.

Poster Sessions

If you are presenting a poster you will be given your poster number during registration. Make sure that you put up your poster on the right poster stand. The number will serve as an identification mark for our poster committee. Pins for putting up the poster will be available in the room of the poster session.

Poster Session I: Tuesday, 17:25-18:55, odd numbers *(Wine and Cheese)*
Poster Session II: Wednesday, 13:00-14:30, even numbers *(Lunch Buffet)*.

Poster Prizes

A poster prize will be awarded to the top three participants selected by our jury. All registered participants presenting a poster are eligible for the prize. The participants must be available and present their poster during one of our poster sessions to the jury. The jury will comprise a speaker of the Horizons symposium, a faculty member of the IMPRS Molecular Biology program and an organizer of the symposium. Our prizes for this year include:

- a Nikon Coolpix camera
- a set of pipettes signed by our Göttingen Nobel laureate Erwin Neher
- an Amazon voucher.

Travel Grants

One of the main objectives of Horizons is sharing the research of young scientists at various stages of their career. The travel grants give selected PhD students the opportunity to come from different parts of the world and present their work in the plenary lecture or the poster sessions of the conference. This year we received more than 70 applications from all over the world representing 22 countries of 5 continents. After a rigorous selection process, we would like to congratulate the following awardees.

Awarded Student Talks

- **Mireia Sola (11th Sept, 15:35)**
  Dissecting nuclear pore assembly with intermediate-arresting nanobodies

- **Ivan Sorokin (12th Sept, 17:05)**
  Ribosomes and Polyribosomes imaging via super-resolution microscopy

- **Harvijay Singh (13th Sept, 14:15)**
  Structure of chikungunya virus nsP2 cysteine protease and its inhibition by structure-based peptidomimetics inhibitors

Poster Presentation

- **Yelyzaveta Snihirova**
- **Ekaterina Zhigalova**
- **Mariacristina Ciccioli**
- **Surabhi Swarnkar**
- **Roza Szatkowska**
- **Polina Pikus**
- **Kateryna Uspenska**

Diversity of applications received for Horizons 2018

Social Events

Horizons is not only about scientific exchange but also building personal contacts with people in your field. A long standing tradition of the Symposium is to organize social events so that the participants and speakers get a chance to interact in an informal setting. This year we will be hosting the following activities:

Social Mixer: Join us for a Beer!

After an engaging day of Career Fair and scientific discussions, we invite the participants and speakers to join us in exploring the colorful nightlife of Göttingen. We will visit one of the local's favorite bars to experience the lively atmosphere. It is a great opportunity to meet new people over a glass of traditional German beer and food from 19:30 on Monday, the 10th of September. This event is not included in the conference package.

Location: *Z.A.K.* (Am Wochenmarkt 22, 37073 Göttingen)

Wine and Cheese

During one of the poster sessions, a wine and cheese tasting is organized, where a great variety of cheese from different places will be served. With a glass of wine in hand, you can have forthright discussions about the research of young scientists from all parts of the world.

City Tour

Göttingen is known as the city of Science, it dates back to middle Ages and boasts its well preserved half-timbered houses. Join us in a short city tour combining the fantastic architecture and the scientific historical overview. The guided tour will take place on Wednesday, September 12th, starting at 19:00 h. The tour is in English. We will meet at the Gänseliesel fountain in the city center and end the tour at *Paulaner* where we will have the conference **dinner and party**. The tour is for participants who opted for the conference package.

Starting point: Gänseliesel fountain in the city center

Dinner and Party

As every year, we invite you to a night of informal interaction with dining and merriment. It is a good occasion to get to know the other participants and speakers and to celebrate new friendships over a couple of drinks, food, games and music and dancing starting at 20:00 on the 13th of September

Location: *Paulaner - Zur alten Brauerei Göttingen* (Düstere Straße 20A, 37073 Göttingen)

"Food for Thought" Breakfast

On Thursday morning we invite you to our science breakfast, to enjoy breads, pastries and castagnes with a cup of coffee or tea on our last conference day! We thank our sponsors *Ruch* and *Küsters* for sponsoring the breakfast.

History of Horizons

December 4th, 2003. The seminar room in the Göttingen Center for Molecular Biosciences was bustling with activity. Organizers darted in and out of the room, making sure everything was in order and as it should be. Speakers attempted to look through their talking points, whilst entertaining the many curiosities of the next generation gathered around them. The graduate students themselves, whispered to each other and waited apprehensively, with thinly veiled excitement, for the program to begin. There were just under a hundred eager minds crowded into that one room. Students and researchers; all brought together by a shared passion: a passion for science. This was the inaugural **Horizons in Molecular Biology Conference**: An International PhD Student Symposium!

The conference has come a long way since then. Over the years, the number of participants grew steadily and increased in variety. Horizons now regularly attracts around *three hundred participants from over thirty countries*. The symposium has featured numerous renowned researchers, representing a wide range of fields in the life sciences. This exhaustive list also included several Nobel Laureates, namely: Professors **Martin Chalfie**, **Sir John Walker**, **Ada Yonath**, **Thomas Südhof**, **Kurt Wüthrich**, **Carol Greider**, **Venki Ramakrishnan, Sir John Gurdon**, and **Tim Hunt**.

Horizons has also evolved continuously as each new batch of student organizers brings a fresh perspective and new ideas, proving consistently that you *can* make a good thing better! In 2006, the organizers introduced a **Poster Session** accompanied by **Student Talks**. PhD students presented *over a hundred posters* and a select few were given the chance to present their work to an audience of peers and professors. Building on this, in 2007, Horizons launched its first **Career Fair** in conjunction with the conference. The fair offered budding scientists with an opportunity to connect with industry and network with life science professionals with a different backgrounds and perspectives. Representatives from over *twenty companies* were present at the inaugural Career Fair, conducting live interviews and CV checks. Today, the Career Fair has adopted a major role in the conference, offering **workshops** held by professionals, and interactive discussions with key figures in the modern life science industry. All catered to the needs of a young scientist.

At the heart of Horizons, is an idea: **An International Scientific Conference *from* PhD students *for* PhD students**. Organized by students from Göttingen's Intetrnational Max Plank Research School for Molecular Biology, Horizons in Molecular Biology provides an interactive experience in a relaxed environment. It aims to bridge the gap between young scientists and experienced researchers and bring together experts and novices from various fields of the life sciences to engage in a productive dialog and exchange information. Modern discovery is persistently progressing from exploring rainforests in faraway lands to observing the nature of life under a microscope. We are the modern explorers, and are committed to keep progressing forward, in the pursuit of science, towards the Horizon.

IMPRS for Molecular Biology

For the 15th time, the Horizons Symposium is organized by students of the International Max Planck Research School (IMPRS) for Molecular Biology at the University of Göttingen. The Molecular Biology Program is jointly conducted by many different departments of the University of Göttingen, the Max Planck Institutes for Biophysical Chemistry and Experimental Medicine, the European Neuroscience Institute as well as the German Primate Center. The common aim is to offer an intensive, research-oriented education in order to prepare the students for a professional career in the life sciences. This broad spectrum of topics is mirrored in the fields covered in Horizons. In addition to the small classes and specialized hands-on laboratory courses, the intercultural experience is extraordinary. Excursions, culture nights and workshops with students from all over the world naturally become a "seminar" on intercultural communication. Students can join the program after completing their Bachelor studies. During the first year, students receive a broad education in molecular biology through lectures, lab rotations and methods courses delivered by the participating institutions. From the second year, the students conduct their own research during their MSc or PhD thesis projects and receive further training in specialized methods and skills courses within the Göttingen Graduate School for Neurosciences and Molecular Biosciences (GGNB), which is funded by the German Excellence Initiative.

In September 2000 the first brave students from as far away as Ghana, China, Mexico and Malaysia came to Göttingen to join the new International MSc/PhD Molecular Biology Program. Today, with the fifteenth batch of twenty students joining the IMPRS, the program has become very successful as demonstrated by excellent evaluations, awarded prizes, and an ever increasing number of applicants. By now, more than 100 students have successfully defended their PhD theses within the program and kissed the Gänseliesel. Kissing this statue that stands on a fountain on the market place in the middle of the city and giving her flowers is an old tradition for Göttingen PhD students (read more about this in the text about Göttingen, page 21) For more detailed information about the International MSc/PhD Molecular Biology Program in Göttingen, please take a look at our website:

www.gpmolbio.uni-goettingen.de

About Göttingen

The renowned university town of Göttingen lies in the center of Germany in southern Niedersachsen (Lower Saxony) between the Harz mountains and the Weser river. Its establishment dates back to 953, it was chartered in 1210 and flourished as a member of the Hanseatic League.

A glimpse of this hanseatic opulence is still present today in the medieval Town Hall, the splendid churches and quaint half-timbered houses in Gothic, Renaissance and Baroque styles. Göttingen's landmark is the "Gänseliesel", an art nouveau statue of a girl herding geese on top of the fountain on the market square, outside the Old Town Hall. Traditionally, all new doctoral graduates of the University kiss the cheeks of the statue after passing their examinations, making her "the most kissed girl in the world".

The Georg-August University was founded in 1734 and today has 14 faculties with 24,000 students. The university sends forth many famous scientists – amongst its alumni are 42 Nobel laureates. Especially many famous mathematicians come from Göttingen: Carl Friedrich Gauss, Bernhard Riemann and David Hilbert, to name only a few.

Apart from the university, the city is also home to many research institutes, such as Max Planck Institutes, and international companies.

Nearly untouched by bombings in World War II, the city center is now an attractive place to live in with many shops, small cafes and chic bars. Consequently many students live here, giving Göttingen a young face: in 2003, almost half of the population of the inner city was between 18 and 30 years of age. Today Göttingen has approximately 130,000 inhabitants including 24,000 students, giving it the charm of a small and lively university city.

You can find further information on Göttingen in the yellow booklet in your bag, during the city tour and of course online (*www.goettingen-tourismus.de*).

Practical Information

Certificate of Attendance

Certificates of attendance will be handed out during registration upon request.

Insurance

The Organizing Committee accepts no responsibility for accidents or damage to participants' private property. Please make your own arrangements for all the necessary insurance.

Internet

Free Wi-Fi internet (eduroam) is available throughout the symposium in the lecture facilities. If you do not have access to eduroam, please contact the registration desk.

Parking

Participants can use the parking lots available near the MPI for Biophysical Chemistry.

Buses

The buses number 21, 22 and 23 to the city center leaves just outside the symposium venue at the bus stop „Faßberg". Bus schedules are available at the registration desk.

Taxi

Taxis to the city center can be arranged by calling one of the many Göttingen taxi companies:

- *Göttinger-Funk-Taxi-Zentrale:* 0049-551-69300
- puk minicar: 0049-551-484848
- *City-Taxi Göttingen GmbH:* 0049-551-380840
- *Night & Day Taxi Göttingen GmbH:* 0049-551-65000

Please note that taxis are considerably more expensive than public transport. Also, keep in mind that taxis only accept cash.

Services

Registered participants are entitled to the following:
Admission to plenary lectures, conference material (certificate of participation, name badge, conference bag) and refreshment during coffee breaks.
Participants who opted for the conference package, in addition to the above get a voucher for lunch, city tour, conference dinner and party and a hardcopy conference booklet.

CAREER FAIR

Career Fair - Schedule

CAREER FAIR

9:15 Registration

9:45 Opening Ceremony
Career Fair

10:00 Daisy Robinton
WandW

10:30 Ben Glick
SnapGene

11:00 Coffee Break

11:20 Stephanie Boue
sbv IMPROVER

11:50 Adrian Schomburg
Proteros Biostructures

12:20 Speed Dating
Lunch

13:30 Maria Loidolt-Krüger
PicoQuant

14:00 Wolfgang Große
CureVac

14:30 Marie-Laure Yaspo
Alacris Theranostics

15:30 Opening Ceremony
Horizons

19:30 Join us for a beer!*
*not included in conference package

WORKSHOPS

Parallel Workshops

10:00 David Giltner
TurningScience

"How to be more employable in the private sector"

"Telling better stories with the same facts"

12:30

13:00 Monica Feliu-Mojer
iBiology

"Storytelling: Crafting narratives to share your science"

15:30

Welcome to the 12th Career Fair for Life Sciences

One of the major aims of our conference is to prepare young researchers for their future in the scientific community, be it in academia or in the private sector. With this in mind, the Career Fair was launched in conjunction with our conference in 2007 and has been a resounding success ever since! The Horizons organizing team would like to welcome you to the 12th Horizons in Molecular Biology Career Fair.

Our roster of speakers is specifically tailored to inform and inspire the next generation of scientific professionals. From biotech, to successful start-ups, to science communication; do not miss this opportunity to engage, and network with, a variety of experts that encompass the growing spectrum of scientific industry.

The presentations are sure to provide valuable insights into life in the private sector, however it is at the Career Fair workshops where you will learn, and develop the skills, to succeed in such an environment. *How does one transition from lab to industry? What should be considered when writing a scientific article?* These are some of the questions we hope to address during the course of the workshops this year!

We hope to see you at the Career Fair and invite you to make the most of this opportunity!

Career Fair Organizers

Monday
10:00 am

Can we engineer the end of ageing

"Interdisciplinary" for us, as scientists, typically refers to cross-pollinating amongst other hard science fields. However, there is value to be had in building skills in disciplines outside of science itself. Stepping outside of the ivory tower of academia allows bigger, freer, and often more creative thinking that can energize and inform your work in lab. Along my academic career path I have explored and pursued many other interests, including an active modeling career that has spanned over a decade. I have spent a considerable amount of time traveling and speaking to non-science audiences about exciting research and progress in biomedical science, and even had the opportunity to work with entertainers like Craig Ferguson and Steve Coogan to bring science to the masses. I love bridging seemingly disparate areas and working across multiple disciplines to synthesize ideas and broaden my own perspective. In this talk I'll share my journey weaving together my careers in science, modeling, and public speaking, and discuss how cultivating interests and success outside of the lab can elevate your science and enrich your life.

Daisy Robinton
WandW

Dr. Daisy Robinton is a Hearst Fellow and Postdoctoral Research Scientist in Beth Stevens' lab at Boston Children's Hospital. Her current research focuses on neurodevelopment and the cell-to-cell interactions that contribute to neurodegenerative diseases. Daisy completed her PhD in Human Biology and Translational Medicine in the laboratory of Dr. George Q. Daley at Harvard University. Her doctoral work focused on mechanisms of stem cell identity at the intersection of cancer and developmental biology. Daisy's passion for the effective translation of science has fueled her years of teaching, speaking, and consulting on numerous projects in the US and abroad. In addition, she is a co-founder of Weird and Wonderful, a production company aimed at bridging the knowledge gap in science by connecting with creativity and entertainment to engage, educate and inspire people from all walks of life.

Benjamin Glick
Snap Gene

Ben Glick majored in Neuroscience and Mathematics at Amherst College. His graduate work with Jim Rothman at Stanford University focused on intracellular membrane transport in mammalian cells, and his postdoctoral work with Jeff Schatz at the Biozentrum in Basel, Switzerland focused on protein import and sorting in yeast mitochondria. He is now a Professor at the University of Chicago, where his lab studies the biogenesis and dynamics of membrane compartments using molecular genetics and 4D imaging. A major part of his research program is an ongoing analysis of cisternal maturation in the Golgi apparatus.

In 2004, he co-founded GSL Biotech LLC. Their core product is the molecular biology software called SnapGene. As Chief Scientist of this company, his major role is to serve as lead designer of the software. By building on his own experience as well as feedback from colleagues and customers, he guides the developers to create an intuitive, versatile, and responsive software interface.

Creating SnapGene

As a cell biologist, I became frustrated by inefficiencies in how my group performed DNA cloning. We used those procedures routinely, but mistakes were common and sequence records were poor or nonexistent. Those issues cost time and resources, and could even jeopardize the reliability of experiments. Many other researchers expressed similar frustrations.

I realized that good software could alleviate many of the problems with DNA cloning. Several colleagues joined with me in forming a company to create the software that became known as SnapGene. The goal was to make SnapGene easier to use than paper notebooks so that researchers would switch to electronic methods to plan, visualize, and document their DNA manipulations. Our software design process adheres to the principles of human-computer interaction, which focuses on how to meet users' needs as effectively and painlessly as possible. We have consistently maintained high standards by imagining how an ideal software interface would look and feel, and then doing everything possible to achieve that vision.

Our business strategy is rather unconventional. We have not sought external investment, and as a result, the founders retain full creative and financial control of the company. Initial development of SnapGene was funded by Small Business Innovation Research (SBIR) grants from the NIH. SnapGene 1.0 was released when the SBIR grants ended, and the company became profitable within a few months. Active ongoing development of the software has led to robust growth in market share. We have gradually expanded the development and sales teams in accord with our increasing needs and financial resources.

Two strategic decisions have been key. First, we provide a free version of the software called SnapGene Viewer. As a result, the SnapGene file format has become a standard for sharing annotated sequence data. Second, instead of paying to advertise, we provide an online collection of map/sequence files for commonly used plasmids. This collection brings us customers who discover SnapGene through web searches. The net result is that by investing sustained effort but very little money, we have been able to build a successful software company.

Monday
11:20 am

The systems biology verification endeavour – Harness the power of the crowd to address computational and biological challenges

Systems biology relies on large numbers of data points and sophisticated methods to extract biologically meaningful signal and mechanistic understanding. For example, analyses of transcriptomics and proteomics data enable to gain insights into the molecular differences in tissues exposed to diverse stimuli or test items. The sbv IMPROVER crowdsourcing project (*https://sbvimprover.com*), developed by Philip Morris International as a mean to verify methods and data in systems biology, has already proven its usefulness in benchmarking computational methods used in diagnostic signature discovery or the assessment of species translatability for example. Five challenges have already been successfully conducted, allowing to gain insights into key topics in systems biology and confirmed that the aggregation of predictions often leads to better results than individual predictions and that methods perform best in specific contexts.

Stéphanie Boue
sbv IMPROVER

Stéphanie has a background in biotechnology (Engineering degree in Biotechnology from the Ecole Supérieure de Biotechnologie de Strasbourg (ESBS)), molecular biology, and bioinformatics and obtained her PhD in bioinformatics from the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. After a postdoc on pluripotent cells at the Center for Regenerative Medicine in Barcelona (CMRB), she joined Philip Morris International R&D as a computational biologist in 2010. She now manages scientific transparency and verification aspects in the biomedical research department and is responsible for the development of INTERVALS, a platform to share data, methods, and results, and for the Systems Biology Verification project.

Stephanie Boue currently works at the Biological Systems Research, Philip Morris International (PMI). She is representing sbv IMPROVER, which stands for Systems Biology Verification combined with Industrial Methodology for Process Verification in Research. It constitutes crowdsourcing approaches to provide a measure of quality control of industrial research and development. The sbv IMPROVER approach was initially developed by PMI and IBM Research in 2011-2013 and is now a collaborative effort led and funded by PMI Research and Development.

Adrian Schomburg
Proteros Pictures

Dr. Adrian Schomburg is the CTO of Proteros, a biotechnology company that discovers and develops novel epigenetic medicines based on disease relevant screening methods. Adrian oversees the target identification, validation and early drug discovery areas. As a project manager of collaborations with international pharma partners, Adrian aligns the project teams for joint and productive drug discovery. As a trained biologist, Adrian completed the IMPRS for molecular biology in Göttingen, followed by a master Thesis with Carl Wu at the NIH, a PhD with Wolfgang Fischle at the Max Planck Institute for biophysical chemistry and a research scientist role with Pfizer.

New ways of drug discovery: Between the big and the small

As an early stage researcher, ample opportunities for pursuing your personal goals exist both in the academic as well as the industry sector. However, this is not a black and white picture: The novel way of doing drug discovery research is highly decentralized. Big Pharma collaborates with biotech and institutions. Start-up companies pursue challenging and novel concepts to then acquire venture capital funding, partner with the big once or become acquired themselves. This talk will illustrate this novel "matrix" approach of drug discovery and highlights opportunities to position yourself – as an early stage career researcher – in this matrix.

Monday 1:30 pm

PicoQuant – Advanced photon counting technologies in service of life sciences

PicoQuant develops and produces components as well as instrumentation for time-resolved applications. Our philosophy is to provide robust, state-of-the-art technology that is easy to use and tailored to the users needs in both academia and industry. We are a world-leading company in the fields of pulsed diode lasers, time-resolved data acquisition, single photon counting, and fluorescence instrumentation.

Our products are tested by and often co-developed with renowned scientists. Thanks to the ease-of-use of our instruments, researchers can focus their attention on the questions under study in biology, medicine, chemistry, environmental or materials science instead of wrangling the machines.

We at PicoQuant are always on the look out for talented individuals interested in:

- developing novel optical concepts, fluorescence techniques and applications
- consulting customers regarding technical questions and new research applications
- supporting and training researchers which use our microscopes and spectrometers.

My role as microscopy application specialist involves primarily communicating with customers. Starting with advising them on basic questions regarding the principles and applications of our time-resolved techniques, I then assist them in tailoring the desired microscope system to meet their research needs. I also participate in designing custom solutions for novel techniques together with our development team.

Maria Loidolt-Krüger
PicoQuant

Maria studied biophysics at the Technical University Kaiserslautern (Germany). During her diploma and PhD studies, she worked in the group of Prof. Stefan W. Hell at the Max Planck Institute for Biophysical Chemistry in Göttingen. The subject of her Diploma thesis was developing a multicolor STED microscopy technique with corresponding live cell staining protocol. During her PhD work, she investigated the feasibility of performing STED microscopy on FRET pairs.

In January 2018, Maria joined PicoQuant as an application specialist for confocal fluorescence microscopy. Her background in method development enables her to advise customers not only regarding technical questions but also with new research applications or custom developments. She is also involved in the product management of the SymPhoTime 64, PicoQuant's data acquisition and analysis software for time-resolved microscopy.

Wolfgang Grosse
CureVac

Dr. Wolfgang Grosse is a Scientist at CureVac AG, the company of mRNA people. We are fighting for human health by using natural mRNA as a data carrier to instruct the human body how to produce its own proteins to fight a wide range of diseases. At CureVac Wolfgang uses his passion for protein structure by working in the field of protein design assuring the desired function is achieved by the mRNA encoded protein in the rather early stages of projects. Wolfgang completed his diploma in Chemistry at the Philipps-Universität Marburg where he discovered his interest in biochemistry. He completed his thesis in the field of Ion-Channel Engineering in the group of Prof. Dr. Lars-Oliver Essen. Before joining CureVac in 2014 he was performing work in the area of expression and purification of G-protein coupled receptors in the lab of Prof. Dr. Horst Vogel at the EPF Lausanne.

CureVac – mRNA delivered as a message to the body to cure and protect itself from diseases

Despite the tremendous achievements in medicine in the last century there is still a lot of room for improvement towards current medical treatments, especially in cancer and treatments that are applicable in the third world. CureVac explores the diverse therapeutic possibilities (= Cure) based on revolutionary vaccination (= Vac) and therapeutic treatment based on mRNA. We use unmodified mRNA to give the body the exact information it needs to produce its own medicine, naturally and safely.

On the path to bring the first mRNA-based drug to the market many exciting tasks have to be tackled. Starting from the first proof of principle, over the development of robust and scalable methods towards the actual permission to produce drugs that might be administered to human beings and performing the clinical trials to prove efficacy. Together we manage that task and many different company units cover all aspects of this process on this incredibly potent technology platform.

We believe in the revolution of mRNA! We fight for human health, because it's worth it!

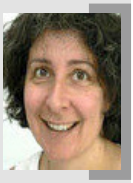
Monday
2:30 pm

Gene regulation and systems biology of cancer

The Yaspo research group focuses on cancer genomics and system biology of cancer, with a translational perspective in personalized medicine. Based on NGS technologies, our interests are centered on dissecting molecular landscapes of tumors for identifying pathway components and biomarkers associated with malignancy,, and on exploring gene regulation networks operating in specific cancer entities. Onging cooperative projects address various aspects of cancer genomics in metastatic melanoma, early-onset prostate cancer, medulloblastoma, pediatric leukemia, and colorectal cancer. We have developed powerful integrative NGS analysis pipelines exploiting simultaneously genome and transcriptome information. In particular we promote the use of RNAseq in tumor profiling, detecting the consequences of somatic events at the gene expression levels, such as gene fusions, alternatively spliced isoforms, long non-coding RNAs, and epigenetic dysregulations. Beyond primary tumor chracterisation, we are interested in identifying the nature of the stromal niche and immune infiltrates in various tumors, and in evaluating to which extend patient-derived model systems cell or xenografts, used in drug sensitivity assays, recapitulate the features of their donors.

Besides, we are developing NGS-based methods allowing a deep characterisation of human immune cell repertoires and therefore the immune status in health and disease.

Marie-Laure Yaspo
Alacris Theranostics



Marie-Laure Yaspo heads an independent research group at the Max Planck Institute for Molecular Genetics in Berlin. She is also one of the founders and Chief Scientific Officer of Alacris Theranostics, a Berlin based company founded in 2008. Alacris Theranostics works towards developing novel approaches in precision medicine with long-standing expertise in interdisciplinary approaches, combining sequencing technologies, large-scale operations, advanced computing, high dimensional data analytics and bioinformatics systems.

David Giltner
TurningScience

David Giltner is the author of the book Turning Science into Things People Need, and is an internationally recognized speaker and mentor for early career scientists and engineers seeking careers in industry. He has spent the last 20 years commercializing photonics technologies in a variety of roles for companies, including JDS Uniphase and Ball Aerospace.

David began lecturing on technical career building in 2010. In 2017 he started TurningScience to provide tools and advice for making the transition from academia into the private sector.

David has developed the unique ability to function well in both highly technical and business circles, and has often functioned as an interpreter to help these two words communicate more productively. He now uses this skill to help scientists and engineers understand the world of product development so they can design and build rewarding careers in industry.

David has a BS and PhD in physics and holds six patents in the fields of laser spectroscopy and optical communications.

David is an internationally recognized author, speaker, and career coach He works with students and early career professionals to help them design exciting careers making things people need!

How to be more employable in the private sector

Working in industry is very different than working in academia! An advanced degree in science or engineering gives you many technical skills that are valuable in the private sector, but this is only 90% of what you need to be successful. That missing 10% is very important, and it is not taught in university.

Being successful in industry, either as an employee or an entrepreneur, requires understanding what behavior is rewarded, and developing new habits accordingly.

In this seminar I discuss some important ways that industry is different than academia, and I use stories from my own career to illustrate exactly why these differences are so important.

I will leave you with three important takeaways:

1. 4 important ways working in industry is different than academia
2. 5 essential habits that successful engineers learn quickly
3. 1 very useful technique for making difficult decisions and accelerating your career

Make these habits part of your daily working life and you will be much more employable and successful in the 'real world.'

Monday
1:00 pm

Storytelling: Crafting narratives to share your Science

Storytelling is a powerful tool for scientists to convey the importance of their work and create meaningful connections. It helps them make their skills and expertise valuable; humanize science; and inspire others to support or pursue careers in science.

The main goal of this workshop is to teach participants how they can share their science effectively and bolster their career development via storytelling. After being introduced to storytelling, participants will use a worksheet to learn how to craft narratives that they can use for multiple professional purposes, from communicating science to non-experts to writing compelling statements for job or fellowship applications. The facilitator will share her own sample narratives and experiences and offer resources to help participants continue the development of their storytelling skills.

Monica Feliu-Mojer
iBiology

Mónica grew up in rural Puerto Rico, catching lizards and with a cow in her backyard, which sparked her interest in all things biology. A PhD scientist-turned-communicator, she uses online technologies, storytelling, community-building, and cultural relevance to make science more accessible and inclusive. Her work focuses on empowering individuals, both scientists and non-experts, through bilingual science outreach, communication, education, and mentoring. Mónica is the Director of Communications & Science Outreach for Ciencia Puerto Rico, a non-profit organization democratizing science, supporting the career development of young scientists, and transforming science education and training in Puerto Rico. She is also Associate Director of Diversity & Communication Training for iBiology, a non-profit organization that produces and distributes free online videos about research, the process of science, and professional development featuring the world's leading biologists. Mónica earned her B.S. in Human Biology at the University of Puerto Rico in Bayamón, and her Ph.D. in Neurobiology at Harvard University. You can find her on Twitter: *@moefeliu*.

Speed Dating

Along with talks and exciting workshops, we have a 'Speed Dating' event in the lineup. Here, the participants get a chance to talk to the speakers of choice face to face. During the lunch break, this event gives you an opportunity to interact with the speakers in a personal and informal environment. In small groups, you will get to meet two speakers and discuss for half an hour all the questions that pop in your heads.

PLENARY LECTURES

Osamu Nureki

The University of Tokyo
Tokyo, Japan

Monday
3:45 pm

Professor Nureki is a structural biologist who has published numerous articles on prestigious scientific journals in the CRISPR field (e.g. Cell, 2014, 2015 & 2016). Dr. Nureki has a long-lasting collaboration relationship with Dr. Feng Zhang at Broad Institute, one of the key discoverers of CRISPR technology. Dr. Nureki and Dr. Zhang co-publish many scientific papers and co-file key critical patents on CRISPR and gene editing technologies. Dr. Nureki receives his PhD from University of Tokyo. He is currently a Professor at Department of Biological Sciences, Graduate School of Science in the University of Tokyo.

He was awarded the JSPS PRIZE in 2008, the Mochida Memorial Research Prize in 2009 and the Inoue Prize in 2011 for his research into "the genetic code translation and the definition of protein synthesis mechanism", and was awarded the Uehara Prize and Takeda Medical Prize in 2014 for his research into "molecular mechanism of membrane transport."

http://www.nurekilab.net/index.php/en?Member%2FStaff%2FNureki

Molecular mechanism of CRISPR and structure-based development of genome editing tool towards medical applications

The CRISPR-associated endonuclease Cas9 can be targeted to specific genomic loci by single guide RNAs (sgRNAs). We solved the crystal structure of Cas9, from 4 sources (984 a.a. to 1,629 a.a.), complexed with sgRNA and its target DNA at atomic resolutions. These high-resolution structures combined with functional analyses revealed the generality and diversity of molecular mechanism of RNA-guided DNA targeting by Cas9, and uncovered the distinct mechanisms of PAM recognition. On the basis of the structures, we succeeded in changing the specificity of PAM recognition, which paves the way for rational design of new, versatile genome-editing technologies. Recently, our high-speed atomic force microscopy (HS-AFM) analysis of SpCas9 visualized real-space and real-time dynamics of Cas9. We further solved the crystal structure of type-V CRISPR, Cpf1 in complex with crRNA and target dsDNA. The structure explains striking similarity and major differences between Cas9 and Cpf1.

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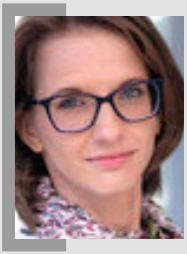


Juliane Liepe

MPI for Biophysical Chemistry
Göttingen, Germany

Wednesday 3:30 pm

I studied Biochemistry (2004-2008) at the University of Potsdam (Germany). During this time, I also studied two years of Mathematics. I completed my studies in Biochemistry with the diploma thesis on "Identification and characterisation of proteasome-catalyzed spliced peptides" at the University Hospital Charité Berlin. In 2009, I joint the 1+3 years Welcome Trust PhD program at Imperial College London under supervision of Professor Michael PH Stumpf on the topic "Novel descriptive and model based statistical approaches in immunology and signal transduction". After completing my PhD I was awarded the NC3Rs David Sainsbury research fellowship, continuing my work on understanding signalling processes involved in the innate immune response. During my time at Imperial college I worked on the analysis of life-fluorescent microscopy images describing innate immune cell migration during acute inflammation by developing and applying automated image processing tools. Random walk models and statistical inference approaches were used to elucidate the signalling cascades during wound healing. I furthermore developed Bayesian approaches for model selection and parameter inference as well as Bayesian experimental design with applications in signal transduction and in immunology. After studying the signalling processes of the innate immune response for several years, I became interested again in the adaptive immune response

Peptide splicing at the hedge between self and non-self

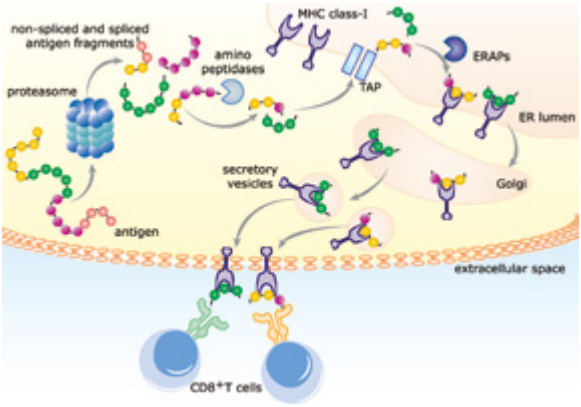
The Research Group *Quantitative and Systems Biology* employs in silico approaches using *in vitro* and *ex vivo* as well as *in vivo* experimental data to study the pathways of the proteasome that regulate the human immune response.

The proteasome is a multicomplex enzyme that catalyzes protein degradation. Apart from its function in protein metabolism, the proteasome regulates the immune system through antigen presentation, where the proteasome produces most of the epitopes presented in the MHC-class I pathway. These epitopes can be generated by simple cut, or cut-and-paste events. Latter so-called proteasome-generated spliced peptides represent more than one third of all epitopes bound to MHC-class I molecules.

We have developed a set of mathematical and bioinformatics tools to study the details of proteasome-catalyzed hydrolysis and peptide splicing and its importance in the MHC-class I pathway. This includes algorithms to identify spliced peptides from mass spectrometry data and methods to classify and characterize spliced peptides. In the future, this will result in the development of algorithms to predict spliced peptide sequences and their relevance during an immune response.

Our group therefore focuses on the development and application of diverse approaches, ranging from efficient mass spectrometry search algorithms and machine learning algorithms to dynamical modelling and model calibration approaches.

The proteasome already is a target for therapeutic trails against cancer and infectious diseases, but its full potential still needs to be explored. This research and the in silico tools developed here will aid such translational aspects and advance the on-going research in systems immunology.



and more specifically, the role of proteasome-catalyzed peptide splicing during antigen presentation. In 2017, I became the head of the research group "Quantitative and Systems Biology" at the Max Planck institute for Biophysical Chemistry in Göttingen (Germany) where, together with my new research team, we continue the research on the proteasome.

Leo James

MRC Laboratory of Molecular Biology
Cambridge, UK

Wednesday 3:30 pm

Leo received his Ph.D. from Cambridge University in 2000, where he worked on antibody structure and design including the first humanised antibody CAMPATH. He post-doc'd in the labs of Prof. Dan Tawfik and Sir Greg Winter, investigating molecular mechanisms of antibody pathogenicity. In 2007, Leo established an independent group at the Laboratory of Molecular Biology in Cambridge (http://www2.mrc-lmb.cam.ac.uk/group-leaders/h-to-m/l-james). His lab studies intracellular host-pathogen interactions using a broad range of in vitro and in vivo techniques. In 2010, Leo discovered TRIM21, the most conserved and highest affinity antibody receptor in mammals and unique for its cytosolic expression. Since then, his lab has shown that TRIM21 prevents infection by intercepting viruses, bacteria and pathogenic proteins inside the cell and targeting them for rapid degradation. This work has also led to the development of 'TrimAway', a technique which exploits TRIM21 for the rapid and specific degradation of cellular proteins. Leo's lab is now working to define the molecular mechanisms that underpin TRIM21 activity to further develop this technology and understand how intracellular antibody immunity is regulated. Leo also investigates HIV post-fusion biology; recent work includes identifying the HIV capsid interface used to recruit cofactors for nuclear import and discovering dynamic pores in the capsid that are essential for HIV infection.

Trim-Away: Targeted degradation of pathogens and proteins by the cytosolic antibody receptor TRIM21

TRIM21 is a recently discovered mammalian Fc receptor and E3 ubiquitin ligase expressed in the cytosol of all cells. The highest affinity IgG receptor in man, it mediates an intracellular humoral response against antibody-opsonized pathogens that invade the cytosol during infection. Upon detection, TRIM21 activates key immune transcription pathways to induce a potent antiviral state. Simultaneous with, and independent of, immune activation, TRIM21 recruits cellular degradation machinery, which catalyse the disassembly and destruction of cytosolic virions to prevent their replication. This rapid virion destruction also exposes the genomes of RNA and DNA viruses, allowing TRIM21 to potentiate the activity of RIG-I and cGAS to promote a rapid antiviral response. TRIM21 is effective against diverse viruses but also bacteria and proteopathic agents like tau. In my talk I will summarize key aspects of TRIM21 biology and focus on recent work showing how its inflammatory signaling is regulated and how we have repurposed its activity to perform targeted protein depletion. This latter technology, called 'Trim-Away', uses off-the-shelf antibodies to rapidly and acutely degrade cellular proteins in diverse cells.

This work was funded by the Medical Research Council (UK U105181010), European Research Council (IAI 281627) and the Wellcome Trust (Investigator Award).

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Roeland Nusse

Stanford University
Stanford, U S A

Tuesday 9:30 am

Roel Nusse was born in Amsterdam, The Netherlands. He received his PhD from the Netherlands Cancer Institute and the University of Amsterdam in 1980. He completed postdoctoral studies at the University of California, San Francisco in 1982 working with Dr. Harold Varmus. After several years as head of the molecular biology department at the Netherlands Cancer Institute, he returned to the Bay Area and joined the Stanford faculty in 1990 as a Professor of Developmental Biology. He has been a Howard Hughes Medical Institute Investigator since 1990. In 1999 he was appointed as chair of the department of Developmental Biology at Stanford, a position he still holds. Currently, he is the Virginia and Daniel K. Ludwig Professor of Cancer Research. In 2010, he was elected as a member of the National Academy of Sciences. Roel Nusse is also a fellow of the American Academy of Arts and Sciences and a member of the Royal Dutch Academy of Sciences. In 2016, he received the Breakthrough Prize in Life Sciences. He has made major discoveries in developmental biology and adult stem cell research. His pioneering research has elucidated the mechanism and role of Wnt signaling, one of the paradigms for the fundamental connections between normal development and cancer.

Gene signatures and stemness in the liver controlled by local Wnt signals

Wnt signaling is widely implicated in stem cell control, as a mechanism to regulate the number of stem cells in tissues. Using various cell labeling and lineage tracing methods, we have described novel populations of stem cells in various tissues, including in the liver. In that tissue, we found that hepatocytes that reside in the pericentral domain of the liver demonstrate stem cell behavior. Although these cells are functional hepatocytes, they are diploid and thus differ from the mostly polyploid mature hepatocyte population. They are active in homeostatic cell replacement and therefore distinct from oval cells, which require injury for their induction. Through transcriptional gene profiling, we have identified a molecular mechanism that maintains the stem cells in a diploid state, involving a transcriptional repressor, Tbx3, a Wnt target gene. Based on additional gene expression data, we propose that Tbx3 defines a state of stemness in the liver. It is noteworthy that liver cancer is often characterized by loss of function mutations in negative components of the Wnt pathway, including Axin and APC. Human liver cancer cells (HCC) often express Tbx3 as well. We suggest that peri-central hepatocyte stem cells, normally controlled by a paracrine Wnt signal, are precursors to liver cancer. Ongoing research includes developing methods to expand mouse and human hepatocytes in culture, based on hepatocyte growth promoting factors we have identified in vivo.

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Neville Sanjana

New York University
New York, U S A

Tuesday 10:15 am

Neville Sanjana, PhD, is a Core Faculty Member at the New York Genome Center and Assistant Professor in the Departments of Biology and of Neuroscience and Physiology at New York University. As a bioengineer, Dr. Sanjana creates new tools to understand the impact of genetic changes on the nervous system and cancer evolution. His lab has harnessed high-throughput gene editing to pinpoint which regions of the genome, including both protein-coding genes and noncoding regulatory elements, are involved in diverse diseases.

Dr. Sanjana is a recipient of the NIH's New Innovator Award and Pathway to Independence Award, the AAAS Wachtel Prize for Cancer Research, the DARPA Young Faculty Award, the Sidney Kimmel Scholar Award, the Melanoma Research Alliance Young Investigator Award, and the Allen Institute for Brain Science Next Generation Leader Award. Previously, he was a Simons Postdoctoral Fellow at the Broad Institute of Harvard and MIT. Dr. Sanjana holds a PhD in Brain and Cognitive Sciences from MIT, a BS in Symbolic Systems and a BA in English from Stanford University.

New frontiers for pooled screens: Finding regulatory elements in the noncoding genome and capturing multi-cell interactions

Forward genetic screens using CRISPR (clustered regularly interspaced short palindromic repeats)–associated nucleases are a powerful tool to pinpoint genes involved in disease. Initial screens capitalized on genome-scale libraries to perturb nearly all protein-coding genes in the human genome to examine therapeutic resistance and gene essentiality in cancer cell lines. Recently, our lab has further developed the CRISPR screening toolbox in several new directions, such as in vivo screens to understand drivers of lung metastasis and *in vitro* saturation mutagenesis of noncoding regions to identify functional elements that drive chemotherapeutic resistance in melanoma. But certain disease-relevant phenotypes are difficult to probe *in vitro* in cell lines or *in vivo* in the complex multicellular environment.

To bridge the gap between the reductionism of *in vitro* screens and the full *in vivo* environment, we have recently developed two-cell type (2CT) whole-genome CRISPR screens to dissect the complex interactions between tumor cells and primary immune cells in cancer immunotherapy. Using primary human cytotoxic T cells, we identify loss-of-function mutations genome-wide that drive resistance to immunotherapy with T cells engineered with a transgenic, antigen-matched T cell receptor. We validate several novel immunotherapy resistance mechanisms across different melanomas, different cancers, and different antigens in human and mouse models. We also find that the enriched genes in our CRISPR screen correspond with mutated genes in a meta-analysis of cancer

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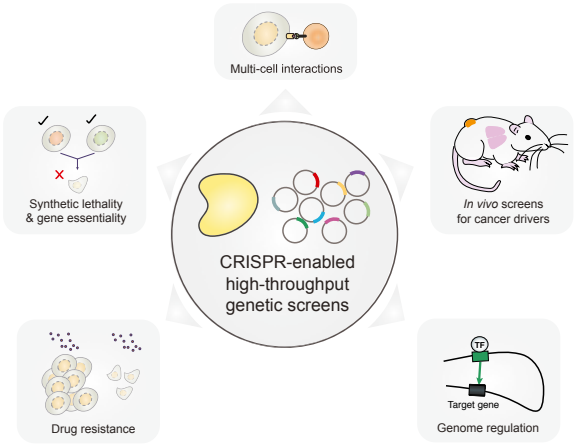
immunotherapy non-responders, suggesting that forward genetic screens could help predict which tumors would respond to therapy in advance of treatment.

In addition to genome-scale screens for protein-coding genes, we have recently adapted pooled CRISPR screens into noncoding regions of the genome, where it can be challenging to identify functional elements. We find that mutations at specific noncoding elements lead to changes in transcription factor occupancy and in the local epigenetic landscape and that these changes are coincident with modulation of nearby gene expression. Taken together, these new frontiers expand the potential of CRISPR screens for fundamental genomic discovery, gene regulation, and therapeutic development to overcome cancer resistance.



Peter Lenart

MPI for Biophysical Chemistry
Göttingen, Germany

Tuesday 11:30 am

I have just moved to the Max-Planck Institute for Biophysical Chemistry in Göttingen, Germany where I will set up a research group and coordinate the Live-cell Imaging Facility. Before, I was staff scientist (2008) and then group leader (2011) at the European Molecular Biology Laboratory (EMBL), Heidelberg Germany. Previously I spent three years as postdoctoral fellow in the laboratory of Jan-Michael Peters at the Research Institute of Molecular Pathology (IMP), Vienna, Austria, preceded by doctoral work with Jan Ellenberg at EMBL, Heidelberg. I am originally from Budapest, Hungary where I studied biology at the Eötvös Loránd University.

I have always been fascinated by the internal dynamics of cells, cell division in particular, and by having the possibility to visualize this dynamics directly in the live cells by light microscopy. I first started by looking at fertilization in plants, and spent significant time looking at mitosis in cultured mammalian cells, but then finally settled on studying meiosis in starfish oocytes. This somewhat exotic model system is exceptionally suited for live imaging, and thereby it allowed us over the past years to reveal key mechanisms underlying these specialized divisions producing the fertilizable egg.

The same, only different: How does the cell division machinery adapt to divide the large oocyte?

We study the specialized oocyte divisions that produce the fertilizable egg. Our general aim is to understand how the cell division machinery adapted to this specialized function, dividing the very large oocyte in a very asymmetric manner to retain all nutrients stored for the embryo in a single large egg. We will demonstrate the underlying principles through a couple examples for such adaptations to meiosis-specific functions.

Recently we revealed a novel, actin-driven mechanism required to break the exceptionally large oocyte nucleus. This involves the assembly of a massive but very transient actin 'shell' under the nuclear envelope mediated by the Arp2/3 nucleator complex. We could show that the actin shell forces apart the nuclear membranes and the underlying lamina, and thereby leading to destabilization of the nuclear envelope.

As another example, we showed how the capture of chromosomes scattered in the very large oocyte nucleus is coordinated in order to incorporate each and every one of them into the forming meiotic spindle. Surprisingly, this is again mediated by the actin cytoskeleton, which transports chromosomes near to microtubules and at the same time coordinates the timing of their capture.

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Daniel Gerlich

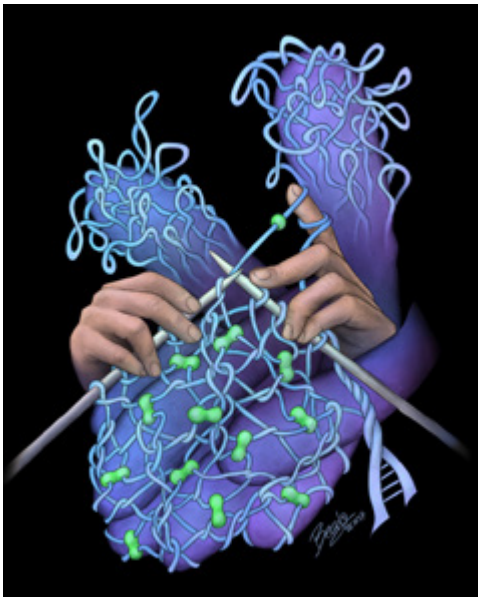
IMBA, Vienna BioCenter
Vienna, Austria

Tuesday 12:15 pm

Daniel Gerlich studied Biology at the University of Freiburg and completed his PhD thesis at the German Cancer Research Center and the University of Heidelberg in 2002. Following postdoctoral research in Jan Ellenberg's laboratory at EMBL Heidelberg, Dr. Gerlich established his independent laboratory as Assistant Professor at ETH Zurich in 2005. He moved to the Institute of Molecular Biotechnology (IMBA) at the Vienna BioCenter to take a position as senior research group leader in 2012. Dr. Gerlich's research combines cell biology, biophysics, biochemistry, and computer science approaches to address fundamental questions about cellular self-organization and biomechanics during cell division. Throughout his career, Dr. Gerlich has developed technology for high-throughput microscopy, computer vision, and machine learning. With this, his team has revealed new principles underlying mitotic chromosome mechanics, including a surfactant-like protein that establishes a repulsive surface on mitotic chromosomes and a chromatin network that specifies the geometry of a single nucleus during mitotic exit. He further discovered a new filament type that splits cells apart during the final stages of cell division. Dr. Gerlich's research has been honored by various awards, including a European Young Investigator Award of the European Research Council (2005), an EMBO Young Investigator Program membership (2009), an ERC Starting grant (2012), and an EMBO membership (2017).

Chromosome mechanics during nuclear assembly

A hallmark of eukaryotic cells is that they store all their chromosomes in a single nucleus. This is important for the maintenance of genomic integrity, as individual chromosomes packaged into separate micronuclei are prone to massive DNA damage. Animal cells undergo an open mitosis, in which the cell disassembles its nucleus to release a set of individualized chromosomes. At the end of mitosis, cells reassemble a single nucleus around a complete set of chromosomes, utilizing endoplasmic reticulum-derived membranes. How cells restrict the nuclear envelope to the surface around the set of anaphase chromosomes has remained unclear. By a live-cell microscopy RNAi



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screen, we identified the protein barrier-to-autointegration factor (BAF) to be a major factor in shaping a single nucleus. Using mutagenesis and CRISPR-mediated genome engineering, we found that BAF provides this function by cross-bridging distant DNA segments into a dense chromatin network. *In vitro* characterization of purified chromatin and recombinant BAF proteins with atomic force microscopy showed that BAF forms a rigid shell at the chromatin surface. In cells, this restricts membranes to the surface formed by the set of anaphase chromosomes, thereby preventing the formation of micronuclei. Thus, BAF regulates chromosome mechanics to shape a single nucleus during mitotic exit.

Reference:

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S. Cuylen, C. Blaukopf, A. Z. Politi, T. Müller-Reichert, B. Neumann, I. Poser, J. Ellenberg, A. A. Hyman, D. W. Gerlich. Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. Nature. (2016). 535(7611).

Michael Sheehan

Cornell University
New York, USA

Tuesday 1:50 pm

Michael Sheehan is an assistant professor and Nancy and Peter Meinig Investigator in the Life Science in Neurobiology and Behavior at Cornell University. His lab examines the reciprocal relationships between social behavior and diversity. Much of his work has focused on the ecology and evolution of individual recognition. Previous studies in paper wasps, mice and humans have demonstrated that individual recognition can drive the evolution of extreme phenotypic variability, allowing for efficient recognition. Additionally, Sheehan has demonstrated that paper wasps use face-specific processing mechanisms to recognize individuals, analogous to facial processing in humans and primates. Ongoing work in the lab integrates approaches from behavioral ecology, population genomics and increasingly neuroscience to address a range of mechanistic and evolutionary questions regarding recognition systems in both paper wasps and house mice.

Sheehan received his PhD in 2012 in Ecology and Evolutionary Biology from the University of Michigan and received and NIH NRSA postdoctoral fellowship to purse training in population genomics at the University of California at Berkely. Sheehan's work has been recognized with the Allee Award from the Animal Behavior Society (2011) and the Young Investigator Award from the American Society of Naturalists (2014). Recently Sheehan was awarded an NIH Director's New Innovator Award (2017) for examine the genetic basis of facial processing in paper wasps.

Genomic insights into the evolution of social intelligence and phenotypic diversity

Individual recognition is the most specific and precise form of recognition. It requires that individuals have distinctive phenotypes to allow for recognition, which itself requires flexible learning and memory. Here I report insights into how individual recognition is driving the evolution of phenotypic diversity and social intelligence in a unique species of paper wasp. The Northern paper wasp, *Polistes fuscatus*, displays a wide diversity of color facial color pattering, which they use to recognize individuals within their nests. By combining comparative, population and functional genomic approaches we are describing the genetic mechanisms and selective pressures maintaining extreme phenotypic diversity in this species. Individual recognition and extreme diversity is in *P. fuscatus* is especially notable since its close relatives lack color pattern variation and do not recognize individuals.

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We have taken advantage of the recent evolution of recognition in *P. fuscatus* to examine patterns of selection on genes associated with learning and memory. Population genomic analyses reveal that the strongest selective events in the recent history of *P. fuscatus* have been associated with selection on learning, memory and vision. We do not find these same patterns in closely related species, suggesting that social behavior and recognition in particular can be extreme potent forces driving the rapid evolution of diversity and cognition.

Ulrike Kutay

ETH Zurich,
Zurich, Switzerland

Tuesday 2:35 pm

Ulrike Kutay is a Full Professor at the Institute of Biochemistry (IBC) of the ETH Zurich. Her research is centered on the structure, function, biogenesis and dynamics of the cell nucleus. Ulrike studied Biochemistry in Berlin. During her dissertation work (Berlin, Boston), she characterized integration of tail-anchored proteins into the ER membrane. As a postdoc in Heidelberg, Ulrike investigated the mechanism of nuclear transport and identified RanGTP-controlled exportins. As an independent researcher, her lab initially deciphered various nuclear transport pathways, such as nuclear import of histones, export of miRNAs and ribosomal subunits. Current research topics include the dynamics of the nuclear envelope during mitosis, the structure and function of linker of nucleo- and cytoskeleton (LINC) complexes, the sorting of membrane proteins to the inner nuclear membrane and ribosome synthesis in mammalian cells. The Kutay group is tightly embedded into the Swiss National Centre of Competence in Research 'RNA biology and disease'. The work of the Kutay lab relies on a wide repertoire of approaches, ranging from biochemical reconstitution of cellular processes, imaging-based (screening) technologies to mathematical modeling.

Ulrike is an elected member of EMBO, the Leopoldina, the Academia Europaea and she has been awarded an ERC Advanced grant for her research on the nuclear envelope. Ulrike Kutay is a co-founder of the Zurich Molecular Life

Taking apart the nuclear envelope during open mitosis

NE breakdown (NEBD) is a major event during the drastic intracellular reorganization in preparation of mammalian cells for division. Disassembly of the nucleus exploits the activity of protein kinases involved in mitotic entry and is supported by microtubule-dependent restructuring of the NE. In my talk, I will cover two different aspects of NEBD, namely disassembly of nuclear pore complexes (NPCs) and the removal of membranes from chromatin. To study NPC disintegration, we had previously established a visual in vitro assay relying on semi-permeabilized cells. Exploiting this system, we have reconstituted the initial steps of mitotic NPC disassembly using purified soluble factors. We could demonstrate that the combined action of multiple mitotic kinases is sufficient to drive mitotic NE permeabilization. We further showed that hyperphosphorylation of both the gate-keeper nucleoporin Nup98 and the central scaffold component Nup53 is required for timely NPC disassembly. Secondly, we have assessed the importance of membrane dissociation from chromatin for chromosome segregation and cell division. When cells were allowed to enter mitosis with NE membranes tethered to chromatin, we observed strong chromatin segregation defects leading to a complete failure in cytokinesis. Our findings bear important further implications, as impaired cell division is known to promote genomic instability and tumorigenesis. Further, our observations indicate that cells must take great care to dissociate interactions between inner nuclear membrane proteins and chromatin. Notably, the dissociation of NE–chromatin contacts in not limited to organisms undergoing open

mitosis but rather a general feature accompanying spindle formation in all eukaryotes.

Science PhD program, and she serves in the advisory boards of other graduate schools in Europe. Ulrike Kutay actively participates in the mentoring of junior researchers and is a member of the ETH Women Professors Forum. At ETH, she takes major administrative responsibilities, for instance as a vice chair of IBC, as a presidential delegate for faculty recruitment, and as a member of the ETH Zurich Strategy Committee.

Floyd Romesberg

Scripps Research Institute
La Jolla, CA, U S A

Tuesday 3:55 pm

Floyd E. Romesberg received a B.S. and M.S in chemistry from The Ohio State University, and a Ph.D. in physical organic chemistry from Cornell University. He was an NIH postdoctoral fellow at UC Berkeley with Peter Schultz, and in 1998 joined the faculty at The Scripps Research Institute, where he is currently Professor of Chemistry.

Romesberg's research laboratory applies organic chemistry, microbiology, non-linear optical spectroscopy, and genetics, to study different aspects of evolution. Of particular significance is his work to expand the genetic alphabet and code through the development of a third DNA base pair that relies on hydrophobicity as opposed to hydrogen bonding for selective pairing.

Romesberg has been recognized by several awards including the Camille Dreyfus Teacher Scholar Award, the NSF CAREER Award, the Discover Magazine Technology Innovation Award, and the ACS Nobel Laureate Signature Award. His research has been funded by NIH, NSF, Office of Naval Research, and Defense Advanced Research Projects Agency. Romesberg is also a scientific founder of several biotech companies, including Achaogen, Inc., RQx Inc., and Synthorx, Inc.

A semi-synthetic organism that stores and retrieves increased genetic information

Since the last common ancestor of all life on earth, the biological diversity has been encoded in a four letter, two base pair genetic alphabet. Expansion of the genetic alphabet to include a fifth and sixth letter than for a third, unnatural base pair not only has immediate utility for a number of applications, such as site-specific oligonucleotide labeling, but also serves as the foundation for an organism with an expanded genetic code. Toward this goal, we have examined a large number of different unnatural nucleotides bearing mainly hydrophobic nucleobase analogs that pair based on packing and hydrophobic interactions rather than H-bonding. Optimization based on extensive structure-activity relationship studies and two screens resulted in the identification of a class of unnatural base pairs that are well recognized by DNA and RNA polymerases. More recently, we have engineered E. coli to import the requisite unnatural triphosphates and shown that DNA containing the unnatural base pair is efficiently replicated, transcribed, and translated within the cell, resulting in the first semi-synthetic organism that stores and retreives increased information.

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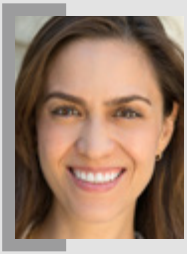

Elizabeth Villa

University of California
San Diego, USA

Tuesday
4:40 pm

Elizabeth Villa, Ph.D. is an Assistant Professor in the Division of Biological Sciences at the University of California San Diego. She completed her PhD in Biophysics at the University of Illinois at Urbana-Champaign as a Fulbright Fellow. She was a Marie Curie Postdoctoral Fellow in the Max Planck Institute of Biochemistry in Munich. In 2016, she was granted an NIH Director's New Innovator Award, which allows her to pursue high-risk high-reward research developing tags for cryo-electron tomography (cryo-ET), and developing new technological and computational techniques to advance structural cell biology. In 2017, she was named a Pew Scholar.

Dr. Villa's laboratory has a strong focus on building tools for quantitative cell biology, using cryo-electron microscopy and tomography, cell biophysics, computational analysis, and integrative modeling. This potent combination enables the observation of macromolecular complexes in their native environment and derive their structure, context, and interaction partners. Her current research is focused on studying the nuclear periphery, as nuclear biology remains one of the most exciting challenges in the cell, and it is uncharted territory structurally. Her lab also pursues many collaborations aimed at understanding the bacterial cytoskeleton, studying Parkinson's disease.

Opening windows into the cell: Revealing molecular architecture of the nuclear periphery

To perform their function, biological systems need to operate across multiple scales. Current techniques in structural and cellular biology lack either the resolution or the context to observe the structure of individual biomolecules in their natural environment, and are often hindered by artifacts. Our goal is to build tools that can reveal molecular structures in their native cellular environment. Using the power of cryo-electron tomography (CET) to image biomolecules at molecular resolution in situ, we are building tools to make compatible with, and directly comparable to, biophysical and cell biology experiments, capturing the structural behavior of macromolecules in action under controlled conditions. I will show how we use these techniques to study the molecular of the nuclear periphery, to understand Parkinson's disease at the molecular level, and to peek on the inner life of bacteria.

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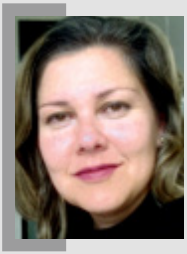


Katherine M. Pappas

Faculty of Biology, NKUA
Athen, Greece

Wednesday 9:30 am

Katherine M. Pappas obtained her BSc degree in Biology and PhD in Genetics at the National & Kapodistrian University of Athens (NKUA). She did her postdoctoral studies at the Department of Microbiology, College of Agriculture & Life Sciences, Cornell University. She is currently Assistant Professor of Molecular Microbial Genetics in the Department of Genetics & Biotechnology, Faculty of Biology, NKUA. Her research interests lie in the fields of genomics, transcriptional regulation, cell-cell signaling, plasmid biology, and strain engineering. She has mostly worked with model biotechnological organisms such as *Agrobacterium tumefaciens*, a plant pathogen and a transgenic technology vector, and *Zymomonas mobilis*, a bioethanol producer. Her work with *Agrobacterium tumefaciens* contributed to the understanding of the molecular mechanisms underlying quorum-sensing dependent activation of Ti plasmid replication, a phenomenon with ecological repercussions now thought to characterize other proteobacterial plasmids as well (Nature 417: 971-974; Nature Reviews Microbiology 10: 755-765). Work with *Zymomonas mobilis* has been multi-faceted and addresses important aspects of the organism's biology and applicability, not least of which, 'omics' aspects (Nature Biotechnology 27: 893-894). Apart from conducting own research, K. M. Pappas reviews for many journals and has also served as Senate member in NKUA, consultant for the Greek Ministries of

From tequila shots to chassis genomics: works and days of an ethanol producer

Zymomonas mobilis is a bacterium long known to be involved in the production of alcoholic beverages in the tropics. However, when tested in the laboratory, it is also found to outcompete yeasts in ethanol fermentations. Given the global mandate for cleaner, safer and renewable energy, *Z. mobilis* has been studied in academic and industrial establishments as a platform catalyst for first and second generation bioethanol. Of interest to my laboratory has been the understanding of the *Z. mobilis* genome to a comparative, structural and functional level. This helps in discriminating essential from accessory genes, observing genomic division between the chromosome and extrachromosomal elements, gaining evidence for gene flows and horizontal transfer events, and ultimately choosing the most

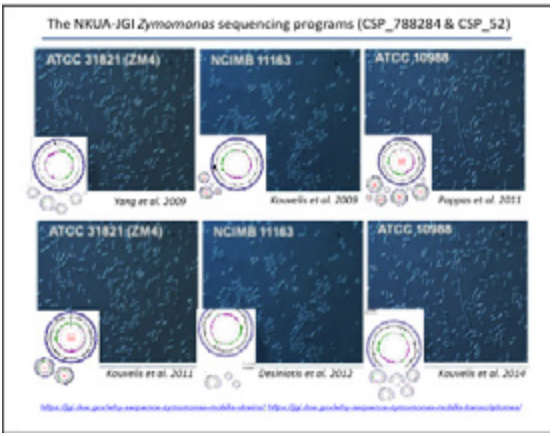
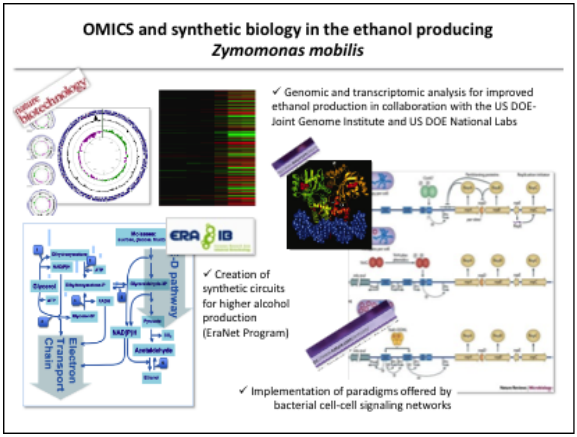


Illustration of DNAseq and RNAseq programs carried out between the NKUA and the US DOE-Joint Genome Institute.

suitable modules – genes or cis-acting elements – in order to enhance the bacterium's performance. Gene networks that act in cell-cell signaling and mutagenic stress are also of interest, since they reveal important facets of the organism's physiology. Particularly in terms of elucidating responses that follow mutational challenge, transcriptional profiling has been carried out and has revealed the vast numbers of genes being implicated in SOS induction, in DNA repair and cell-cycle regulation, and in multitudes of other activities expected or unforeseen. Transcriptomics also reveal growth-dependent gene expression variability, which reflects the ways and needs of the organism at different fermentation regimes. Overall, we believe that addressing the organism's fundamental biology is the first step towards doing insightful engineering and producing novel strains.



Overall activities.

Health and Education, proposal and institutional evaluator for Greek, EU and British authorities, as well as international mentor and country ambassador for the American Society of Microbiology.

Florian Jug

MPI of Molecular Cell Biology and Genetics
Dresden, Germany

Wednesday 10:15 am

Florian Jug is a research group leader at the Center for System Biology Dresden and the Max-Planck Institute of Molecular Cell Biology and Genetics. The overarching goal of his research is to push the boundaries of what image analyses and machine learning can do for quantifying biological data. Research in the Jug Lab is aiming at finding ways to efficiently analyze large amounts of microscopy data, while avoiding impossible amounts of manual analyses and curation tasks – often a major bottleneck in biomedical research projects. Besides finding novel algorithms and machine learning methods, the Jug Lab is also critically involved in the development of Fiji, a popular and widely applicable open software tool for biomedical image analysis and the ideal way to disseminate newly developed methods.

Content-aware image restoration and quantitative downstream analysis

In recent years, fluorescent microscopy saw tremendous advances. Today we routinely image beyond the resolution limit, acquire large volumes at high temporal resolution, and capture many hours of video material showing processes of interest inside cells, in tissues, and in developing organisms. Despite these possibilities, the analysis of raw datasets is usually non-trivial and cumbersome.

In my talk, I will show how machine learning can help to tap the full potential of fluorescent microscopy by first enhancing raw data, followed by joint segmentation and tracking of objects in time-lapse data. Although being powerful in their own right, the combination of such approaches unrolls the full potential of (semi-)automated image quantification pipelines.

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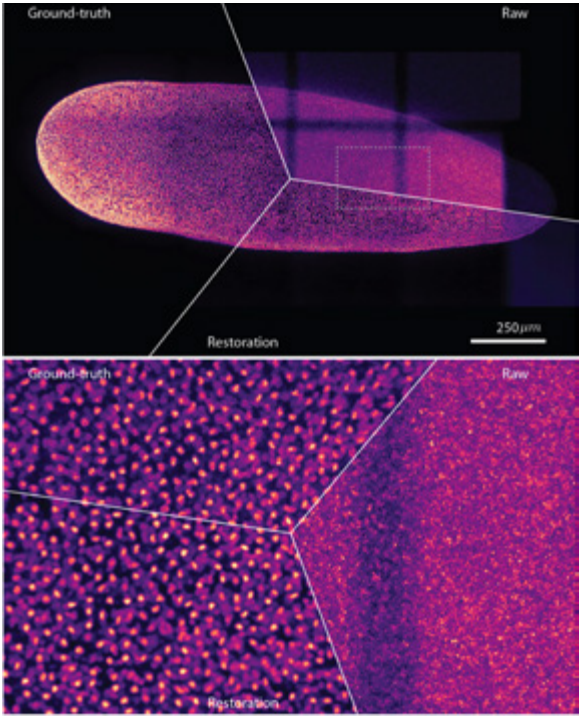
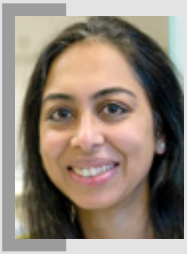


Restoration of low-SNR multi-tiled acquisitions of a flatworm (*Schmidtea mediterranea*). The top image shows a comparison of low SNR input data (RAW) with restoration results as we obtain them with CARE (Restoration) and a high-SNR acquisitions (Ground- truth). The bottom image shows a zoomed comparison, as indicated by dashed box in the top image. My talk will argue that CARE can improve/enable the analysis of microscopy data in various ways.



Yamuna Krishnan

University of Chicago
Chicago, U S A

Wednesday 11:30 am

Yamuna Krishnan, is a Professor and Brain Research Foundation Fellow of Chemistry and the Grossman Institute of Neuroscience at the University of Chicago.

Research in her laboratory spans organic synthesis, nucleic acid biophysics, biochemistry, molecular biology, and cell biology. Her lab has pioneered the deployment of DNA nanodevices as quantitative fluorescent reporters of second messengers for in vivo imaging.

The youngest woman recipient of India's highest scientific prize, the Shanti Swarup Bhatnagar Award, featured in *Cell's* 40 under 40 - a list of young scientists who are shaping current and future trends in biology. She has received the Infosys Prize for Physical Sciences, the Wellcome Trust Senior Fellowship, the AVRA Young Scientist Award, the Innovative Young Biotechnologist Award, the INSA Young Scientist Medal, the Chemical Sciences Lectureship and the AWIS Chicago Innovator of the Year 2016.

DNA nanodevices as probes for cell signaling.

DNA has taken on a new aspect as a construction element to create synthetic architectures on the nanoscale of exquisite structural complexity and *in vitro* functionality.[1] However, till 2009, the functionality of such synthetic DNA-based devices in living organisms remained elusive.[2] Work from my group has bridged this gap by showing the functionality of architecturally simple DNA-based nanodevices in living systems.[3,4] I will describe our recent work that uses a cell targetable icosahedral DNA nanocapsule4 as a versatile probe for live cell imaging to study membrane initiated steroid signaling.[5,6]

Notes

References:

[1] Chakraborty, K., et al. Nucleic acid based nanodevices in biological imaging. *Ann. Rev. Biochem.* **2016**, 85, 349

[2] Surana, S., et al. Designing DNA nanodevices for compatibility with the immune system of higher organisms. *Nat. Nanotechnol.* **2015**, 10, 741

[3] Bhatia, D. et al, A synthetic icosahedral DNA-based host–cargo complex for functional in vivo imaging *Nature Communications*, **2011**, 2, 339

[4] Bhatia, D. et al Quantum-dot loaded, monofunctionalized DNA icosahedra for single particle tracking of endocytic pathways. *Nat. Nanotechnol.* **2016**, 11,1112

[5] Veetil, A. T. et al. Cell targetable nanocapsules for spatiotemporal release of caged bioactive small molecules. *Nat. Nanotechnol.* **2017**, 12, 1183

[6] Veetil, A. T. et al. Chemical Control over membrane-initiated steroid signaling with a DNA nanocapsule *Proc. Natl. Acad Sci. USA* **2018**, in press.

Bianxiao Cui

Stanford Neuroscience Institute
Stanford, U S A

Wednesday 12:15 am

Bianxiao Cui is an associate professor in the Department of Chemistry at Stanford University and Stanford Neuroscience Institute. She holds a Ph.D. degree from the University of Chicago and a B.S. degree from University of Science and Technology of China. She worked as a postdoctoral scholar with Prof. Steven Chu before joining the faculty of Stanford University, Department of Chemistry in 2008. Her main area of interest is to develop nanoscale tools to study electrophysiology and signal transduction in neurons at normal conditions and in neurodegenerative diseases. In particular, she focuses on developing (1) nano-electrodes to detect action potential propagation in neurons and cardiomyocytes and (2) nanoparticle sensors to measure cargo transport in axons. Her recent awards and distinctions include Barany Award from the Biophysical Society, NIH New Innovator Award, NSF CAREER award, NSF Inspire award, Packard Fellowships in Science and Engineering, Hellman Scholar, Searle Scholar Award and Dreyfus New Faculty award.

The role of membrane curvature at the nano-bio interface

The interaction between the cell membrane and the contacting substrate is crucial for many biological applications such as medical implants. We are interested in exploring nanotechnology and novel materials to improve the membrane-surface interactions. Recently, we and other groups show that vertical nanopillars protruding from a flat surface support cell survival and can be used as subcellular sensors to probe biological processes in live cells. Vertical nanopillars deform the plasma membrane inwards and induce membrane curvature when the cell engulfs them, leading to a reduction of the membrane-substrate gap distance. We found that the high membrane curvature induced by vertical nanopillars significantly affects the distribution of curvature-sensitive proteins and stimulates several cellular processes in live cells. Our studies show a strong interplay between biological cells and nano-featured surfaces, which is an essential consideration for future development of interfacing devices.

Notes

References:

1. Zhao W, Hanson L, Lou HY, Akamatsu M, Chowdary P, Santoro F, Marks JR, Grassart A, Drubin DG, Cui Y, Cui B, Nanoscale manipulation of membrane curvature for probing endocytosis in live cells, ***Nature Nanotechnology***, 12, 750-756 (2017).
2. Hanson L, Zhao W, Lou HY, Lin ZL, Lee SW, Chowdary P, Cui Y, Cui B, Vertical nanopillars for in situ probing of nuclear mechanics in adherent cells, ***Nature Nanotechnology***, 10, 554-562, (2015).
3. Lin ZL, Xie C, Osakada Y, Cui Y, Cui B, Iridium Oxide Nanotube Electrodes for Intracellular Measurement of Action Potentials, ***Nature Communications***, 5, 3206 (2014).
4. Xie C, Lin ZL, Hanson L, Cui Y, Cui B, Intracellular recording of action potentials by nanopillar electroporation, ***Nature Nanotechnology***, 7, 185-190 (2012).
5. Hanson L, Lin ZL, Xie C, Cui Y, Cui B, Characterization of the Cell-Nanopillar Interface by Transmission Electron Microscopy, ***Nano Letters***, 12, 5815-5820 (2012).

Andy Heron

Advanced Research at
Oxford Nanopore Technologies Ltd,
United Kingdom

Wednesday 2:30 pm

Andy Heron is the Senior Director of Advanced Research at Oxford Nanopore Technologies, with responsibility for development of various aspects of Oxford Nanopore's DNA and RNA sequencing chemistry, and also development of new sensing strategies for nanopore-detection of proteins and small-molecules.

Andy's interests lie in the field of single-molecule sensing and the development of new detection technologies to address unmet needs in fields such as healthcare, environment and science. Andy has a degree and PhD in Chemistry from Imperial College London, which was followed by 5 years of post-doctoral research at Oxford University in the laboratories of Prof. Hagan Bayley and Prof. Mark Wallace, where he worked on single-molecule optical and electrical detection methods with nanopores. During this time Andy explored the use of nanopores for DNA sequencing, working as part of the NHGRI's "$1000 Genome" program and as a consultant and collaborator with Oxford Nanopore Technologies. After significant advances in the lab and wider community Andy joined Oxford Nanopore in 2010 to continue development of nanopore sequencing. At Oxford Nanopore Andy has been involved in developing many aspects of Oxford Nanopore's technology, including nanopores, enzyme motors, chip and informatics. Andy continues to lead efforts to further develop Oxford Nanopore's technology, with a focus

Oxford Nanopore Sequencing

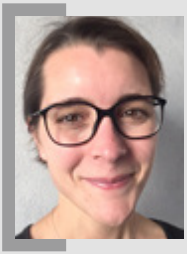
Oxford Nanopore Technologies Ltd. is a private company headquartered in Oxford that was founded in 2005 to develop a disruptive, electronic, single-molecule sensing system based on nanopore science. Oxford Nanopore has developed the world's first and only nanopore DNA and RNA sequencers, and the key features of our technology, such as portability, low capital cost, ultra-long read lengths, and real-time data are enabling a revolution in the sequencing market that cover diverse areas such as science, healthcare and the environment. Our goal is to disrupt the paradigm of biological analysis by making high performance DNA/RNA sequencing technology that is accessible and easy to use, ultimately to enable anyone to sequence anything, anywhere.

Oxford Nanopore's first sequencer, the MinION, has been available since 2014, and is already being used in more than 70 countries for a range of applications, including large scale human genomics, cancer research, microbiology, plant science and environmental research. The MinION is a portable pocket-sized device with powerful and versatile sequencing performance that can deliver high volumes of long read sequence data for everything from in-field sequencing to large genomic projects. Oxford Nanopore's sequencing technology is also fully scalable, and we have continued to develop a range of platforms to address diverse sequencing requirements. For example, smaller and cheaper formats such as Flongle address the need for on-demand rapid tests for labs or in the field, and benchtop devices such as the GridION and

PromethION provide scalable and very high throughput sequencing to address service centre requirements and large genomic projects. Oxford Nanopore have also developed a wide range of sequencing kits and products to complement the strengths of our technology. For more information visit *https://nanoporetech.com/*

Andy Heron will provide an overview of Oxford Nanopore's technology and how it is currently being used, and a view towards the future of the technology.

on extending the capabilities of this disruptive technology to enable sensing and sequencing for many more applications.



Danielle Laurencin

Charles Gerhardt Institut
Montpellier , France

Wednesday 3:15 pm

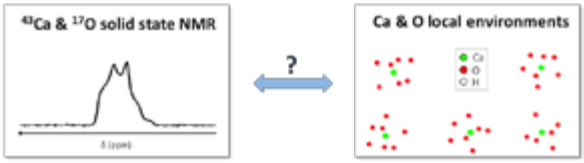
Danielle Laurencin is a CNRS researcher at the Institut Charles Gerhardt in Montpellier (France). Her research activities are at the interface between materials chemistry and solid state Nuclear Magnetic Resonance (NMR), with a particular focus on (i) calcium phosphate materials (including hydroxyapatite and bone), (ii) organic-inorganic hybrid materials (involving in particular organoboron molecules like boronic acids and benzoxaboroles), and (iii) solid state NMR of alkaline earth metals (especially calcium-43). More recently, she has also started to look into the use of mechanochemistry as a new means of labeling organic and inorganic compounds of synthetic interest in oxygen-17, in view of 17O solid state NMR analyses.

Danielle received her PhD in 2006 from the University Pierre et Marie Curie (Paris, France) in the field of inorganic molecular chemistry (functionalization of polyoxometalates for catalytic applications). Then, she was awarded a Marie-Curie fellowship to perform a 2-year post-doc at the University of Warwick (UK), during which she worked on apatite-related biomaterials and more specifically on their characterization by solid state NMR. She was then recruited at the CNRS in 2009, and passed her habilitation in 2015.

Danielle is co-author of over 70 peer-reviewed international publications and 1 patent. She has given more than 15 invited presentations in international conferences and workshops, as well as over 50 other talks on her

Looking into calcium and oxygen local environments using solid state NMR spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy has emerged over the years as an invaluable technique for studying the atomic-level structure of a variety of biological systems, ranging from membrane proteins and viruses to tissues like bone. While high resolution ^{1}H, ^{13}C, ^{15}N and ^{31}P NMR experiments have been mainly used for this purpose, other essential nuclei like oxygen and calcium have been left out of most investigations. This is mainly due to the unfavorable properties of the corresponding NMR-active isotopes, oxygen-17 and calcium-43, which are both poorly-receptive quadrupolar nuclei. Indeed, ^{43}Ca is a spin-7/2 isotope of low natural abundance (0.14%) and very low resonance frequency (ν_0 ~ 40 MHz on a 600 MHz NMR spectrometer), while ^{17}O is a spin-5/2 isotope of very low natural abundance (0.04%) and low resonance frequency (ν_0 ~ 81 MHz on a 600 MHz NMR spectrometer).



Although calcium-43 and oxygen-17 are still generally considered as "exotic" nuclei for NMR spectroscopy, several recent advances are making their analysis by solid state NMR become not only more accessible but also more informative. Here, our latest contributions along these lines will be presented, which include *(i)* new labeling schemes for the preparation of ^{17}O-en-

riched compounds; *(ii)* high-resolution experiments for ^{43}Ca (including ^{43}Ca…^{1}H and ^{43}Ca…^{13}C correlation experiments), and *(iii)* natural abundance ^{43}Ca NMR studies performed at ultra-high magnetic fields (35 T) or using dynamic nuclear polarization (DNP). To illustrate these points, spectra of both natural and synthetic materials will be presented, with the idea of showing the level of structural insight that can currently be reached. The compounds discussed will include substituted hydroxyapatites (which are related to bone and teeth), calcium pyrophosphates (which are related to the pathological calcifications involved in a disease called "pseudo-gout"), and calcium oxalates (which are related to kidney stones).

work. Since 2007, she has been successful in obtaining funding at the national (ANR, Labex ChemiSYST), European (Marie Curie IEF; Marie Curie ERG; ERC Consolidator grant) and international (PUF) levels to develop her research projects. In 2013, she was awarded the CNRS Bronze medal, which is the highest distinction given by the CNRS to the most promising young researchers in France.

Sjors Scheres

Laboratory of Molecular Biology, Cambridge, United Kingdom

Wednesday 4:20 pm

Sjors studied chemistry at Utrecht University, The Netherlands, where he also obtained his PhD in protein crystallography. He was a post-doc in the group of Jose-Maria Carazo in Madrid, before he started his group at the LMB in 2010. His main interest is currently in the development of new methods for high-resolution cryo-EM structure determination and in the cryo-EM structure determination of amyloid fibrils.

The atomic structures of Tau filaments from Alzheimer's and Pick's disease brains

The assembly of microtubule-associated protein tau into abundant filamentous inclusions underlies many neurodegenerative diseases called tauopathies. Tau inclusions display distinct neuroanatomical and cellular distributions between different tauopathies. Morphological and biochemical differences suggest that tau filaments adopt disease-specific molecular conformations. There are six tau isoforms - three isoforms with four microtubule-binding repeats each (4R tau) and three isoforms lacking the second repeat (3R tau). This gives rise to tauopathies with filaments composed of only 3R tau, only 4R tau or both 3R and 4R tau. Molecular conformers of filamentous tau may give rise to different neuropathological phenotypes, similar to prion strains, but the underlying structures are not known. Using electron cryo-microscopy (cryo-EM), we previously determined the structures of tau filaments from Alzheimer's disease, which contain both 3R and 4R tau. More recently, we also determined the structures of tau filaments from Pick's disease and show that they define a second tau protein fold consisting of residues K254-F378 of 3R tau, thereby proving the existence of molecular tau conformers. I will present both folds and discuss how they explain differences in isoform incorporation and phosphorylation observed between Alzheimer's disease and Pick's disease.

Notes

Anne-Claude Gavin

EMBL
Heidelberg, Germany

Wednesday 5:20 pm

Dr. Gavin is group leader and senior scientist in the Structural and Computational Biology Unit at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. Before moving to the EMBL in 2005, she was Director of the Molecular and Cell Biology division of the biotech company Cellzome Inc. Previously she spent four years as a postdoctoral fellow at the Department for Physiology, University of Basel, Switzerland and at the EMBL Heidelberg, Germany. She received her baccalaureate from the college Voltaire in Geneva, Switzerland, her M.S. and PhD in Biology/Biochemistry from the University of Geneva, Switzerland.

Dr. Gavin is an established scientist working on proteomics, biological networks and systems biology. Gavin's research programs integrate biochemical, mass spectrometry, structural and computational methods to characterize cellular networks and circuitry at molecular levels, both spatially and temporally. Her research aims at understanding how cellular components work collectively and achieve biological function.

A network of lipid-transfer proteins at the crossroads between metabolism and signalling

Lipid metabolism attracts much attention owing to the pleiotropic roles of lipids and their implication in human pathologies, and this interest motivates growing efforts dedicated to cataloguing the lipidome. However, all aspects of lipid function rely on their heterogeneous distribution in living systems and the formation of molecular signatures that define organelle membranes or microdomains. Lipid metabolism – and its associated disorders – needs to be understood in the context of this functional, three-dimensional organization. An emerging player in these processes is a group of disease-associated proteins known as lipid-transfer proteins (LTPs). They spatially organize lipids and connect lipid metabolic pathways that are distributed across distinct organelles, but our knowledge of these transport mechanisms remains fragmented. I will present a number of new large-scale biochemical methods designed to systematically characterize the pathways of LTP-mediated lipid movements.

Notes

Polly Matzinger

National Institute of Allergy and Infectious Diseases
Bethesda (Maryland), U S A

Thursday 10:15 am

Polly Matzinger PhD, Honorary D Phil, is a world renowned immunologist, who began a new paradigm in Immunology with the publication of her "Danger Model" of immunity. and has won several awards, as well as been featured in several films because of her work, including a one-hour BBC documentary on the Danger model titled "Turned on by Danger". In her pre-scientific life she worked as a bartender, carpenter, jazz musician, playboy bunny, and dog trainer.

She is currently the chief of the Ghost lab, and the section on T cell Tolerance and Memory at the National Institutes of Health. She worried for years that the dominant model of immunity does not explain a wealth of accumulated data and proposed an alternative, the Danger model, which suggests that the immune system is far less concerned with things that are foreign than with those that do damage.

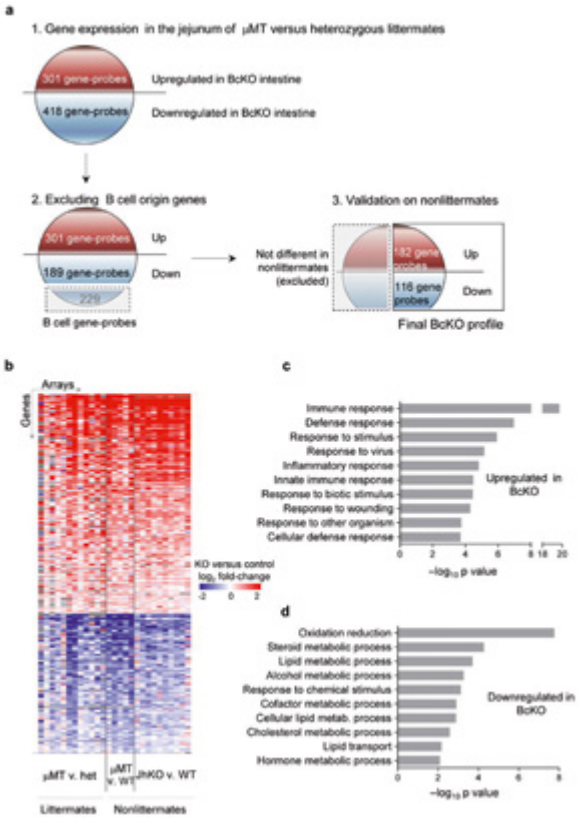
This model, whose two major tenets were conceived in a bath and on a field while herding sheep, has very few assumptions and yet explains most of what the immune system seems to do right, as well as most of what it appears to do wrong, covering such areas as transplantation, autoimmunity, and the immunobiology of tumors. The model has been the subject of a BBC "horizon" film and has featured in three other films about immunity, and countless articles in both the scientific and the lay press.

The Danger model of immunity

Most textbooks teach that the immune system functions by discriminating self (defined early in life) from nonself (what comes later). Although this elegantly simple idea once seemed sensible, it has failed over the years to explain a great number of findings. For example, how do organisms go through puberty, metamorphosis, pregnancy, and aging without attacking newly changed tissues? Why do mammalian mothers not reject their fetuses or attack their newly lactating breasts, which produce milk proteins that were not part of earlier "self"? Why do vaccines need adjuvants? Why do tumors grow, even when many express new or mutated proteins? Why do most of us harbor autoreactive lymphocytes without any sign of autoimmune disease, while a few individuals succumb?

The Danger model suggests that immune responses are initiated by alarm signals from injured tissues, rather than by the recognition of nonself. Although this basic assumption is very simple, it allows us to explain most of what the immune system does right……and also most of what it seems to get wrong. Although many immunology classes now include parts of the Danger model, it is regrettably still not being applied to a wide range of immunological phenomena.

In this talk I will cover some of those, comparing the predictions of the self-non-self models of the '50s, the "extended non self" model of Janeway, and the Danger model.



In her spare time, Polly trains border collies for competitive shepherding trials, composes songs that are not really worth listening to, and is working on the next major question in the immune system, namely "once it decides to respond, how does the immune system know what kind of response to make?"

A first answer to this question seems to be “local tissues send instructions to immune cells, guiding them to make the right kind of response”. This has major implications for autoimmunity, cancer immunotherapy, and vaccine design.

Finally, she is also a sheep breeder, and is part of a small group bringing Gotland sheep into the USA, using frozen embryos from New Zealand and semen from Sweden.

She is currently president of the Gotland Sheep Breeders Association of North America, and vice president of the Frederick Sheep Breeders Association.

Mara Dierssen

Center for Genomic Regulation
Barcelona, Spain

Thursday 2:35 pm

Dr Dierssen leads a Systems Neuroscience Lab at the Center for Genomic Regulation. She is interested in understanding how genetic perturbation in mental disorders modifies the way the brain integrates information. The main question in the field remains how the disturbances of dendritic tree architecture observed in most mental disorders constrain the network activity and influences cognition (Dierssen, 2012, Nat Rev. Neurosci). We use a systems neuroscience approach that combines behavioral and molecular neurobiology analyses in genetic mouse models and cellular models. This systematic and wide-angled approach with different levels of description, has led us to build tools for gathering an integrated view of how the phenomic profiles correlate with cellular and molecular alterations in the neurons of these mouse models. This is a realistic stepping-stone towards unravelling the biological codes behind mental disorders.

Remodelling brain plasticity in intellectual disability

Intellectual disabilities (ID) are chronic diseases that place a disproportionate burden on medical and social care, and educational systems, and rates of ID are on the increase. People with ID are vulnerable and many need lifelong assistance in most aspects of daily life. Cognitive deficits are a prominent feature and severely compromise their quality of life. The reduction in health inequalities and the improvement of health for people with intellectual disability has become a priority worldwide. In spite of the broad spectrum of genetic and environmental aetiologies, the disruption of neural plasticity can be viewed as a sign of cognitive impairment across ID. Our observations that brain plasticity is effective in the recovery from some of the cognitive deficits associated with ID open a window of opportunity for a novel therapeutic concept: therapies that improves and stabilizes physiological (experience-dependent) plasticity in genetic ID syndromes. Thus, targeting core molecular substrates of neuroplasticity with specific drugs, in combination with non-pharmacological interventions. We have identified an extremely powerful target for drug development, DYRK1A (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A), a serine/threonine kinase that plays key roles in cell proliferation, survival and neural plasticity.

Notes

Anna Marie Pyle

Yale University
New Haven, U S A

Thursday 3:40 pm

Anna Marie Pyle is Professor of Molecular, Cellular and Developmental Biology and Professor of Chemistry at Yale University and she is a Howard Hughes Medical Institute Investigator. Dr. Pyle obtained her undergraduate degree in Chemistry from Princeton University and received her Ph.D. in Chemistry from Columbia University in 1990, where she worked with Professor Jacqueline K. Barton. Dr. Pyle was a postdoctoral fellow at the University of Colorado in the laboratory of Thomas Cech. Dr. Pyle formed her own research group in 1992 in the Department of Biochemistry and Molecular Biophysics at Columbia University Medical Center.

In 2002, she moved to Yale University, where she leads a research group that specializes in determining the structure and function of large RNA molecules and protein enzymes that operate on RNA. Dr. Pyle teaches the undergraduate Molecular Biology course at Yale, she serves on the Yale University Budget Committee and University Science Strategy Committee.

Dr. Pyle has been a chair and active member of numerous NIH study sections. She serves on the Science and Technology Steering Committee and the NSLS-II Biology Beamline Advisory Team at Brookhaven National labs. Dr. Pyle is President-Elect of the RNA Society and she is on the scientific advisory board of Arrakis Pharmaceuticals.

Harnessing the RIG-I innate immune sensor to control antiviral and antitumor responses

The Pyle lab studies a large family of mechanical enzymes that hydrolyze ATP upon binding RNA (known as helicase Superfamily 2, or SF2 proteins). Some of these enzymes act as nucleic acid unwinding motors, while others undergo ATP-dependent conformational changes upon binding viral RNA, thereby leading to a signaling cascade that induces an immune response. We use a combination of experimental biochemistry, cell biology and crystallography to study the functional properties of these proteins. Recent studies have focused on structures and mechanism of RIG-I and related antiviral sensors. We solved the first crystal structures RIG-I, thereby defining the unusual molecular architecture and RNA recognition properties of the RLR subfamily (which includes RIG-I, the related MDA-5 and LGP-2 innate immune sensors, and the small RNA processing enzyme Dicer) (see papers by Luo et al, 2011-2013). We conducted complementary thermodynamic analyses to dissect the energetic basis for binding and detection of viral RNA by RIG-I (Vela et al, 2012) and we have elucidated the role of ATP in RIG-I mediated signaling and (Kohlway, 2013, Rawling 2015 and Fitzgerald 2017). Our structural and biochemical studies have led to the development of specific RIG-I agonists that specifically control the function of this receptor *in-vivo* (Linehan, 2018). We are using these findings to develop new therapeutic strategies for the treatment of viral infection, cancer and and autoimmunity.

Dr. Pyle is the Co-Editor of Methods in Enzymology and serves on the editorial boards of eLife and other journals. Dr. Pyle is the author of over 170 publications and has mentored more than 40 graduate students and postdocs.

PANEL DISCUSSION

Panel Discussion

The panel discussion has been organized every year in Horizons since its conception. It provides a channel for lively interactions in a less formal setting. Typically, we have six guest speakers from diverse disciplines on the panel taking questions from a moderator and from the audience. The idea is to illuminate different aspects of the topic from various perspectives. Horizons strives to have a diverse range of speakers to guide us with their unique experiences. For our 15th anniversary Horizons in Molecular Biology symposium, the following speakers will join the panel:

1. Dr. David Giltner
2. Dr. Elizabeth Villa
3. Dr. Sjors Scheres
4. Dr. Mara Dierssen
5. Dr. Polly Matzinger
6. Dr. Yamuna Krishnan

This year, Emeritus Professor Mary Osborn has once again kindly agreed to moderate the discussion. The panel discussion is titled

"Troubles of a young scientist: Fantastic ideas and where to find them".

Peter Medawer preached that "science at all levels of endeavour is a passionate enterprise and the pursuit of natural knowledge a sortie into the unknown". Most of us dive into the world of scientific research with a positive outlook either looking to satisfy our curiosities, revolutionize the world or any other variation in between of the two extremes. It is only with time that we can appreciate that the trajectory of this path is far from a straight line. Even in its most rewarding times, a career in science can be riddled with moments of despair and confusion, leaving even the brightest minds to question their merit. Am I asking the right question? Am I answering it the right way? What does *right* mean?

In this panel discussion, we will explore how to develop ideas, maximize efforts and grab opportunities. What are the skillset that a 21st century scientist needs to achieve this? Is it more than just dumb luck? This would entail a discussion about how to make the right step at the right time, and how this has significantly changed over the past decades.

Mary Osborn

Mary Osborn received a BA in Physics from the University of Cambridge and a PhD in biophysics from Pennsylvania State University. She was a post doc in Jim Watson's lab at Harvard University and a scientific staff member in Sydney Brenner and Francis Crick`s division at the MRC Laboratory of Molecular Biology in Cambridge, U.K. She then joined the staff at Cold Spring Harbor Laboratory. In 1975, she moved to the Max Planck Institute for Biophysical Chemistry in Göttingen and in 1989 was appointed an honorary professor at the University of Göttingen.

Her research has focused on different areas in the life sciences. Her 1964 paper with Klaus Weber, on the use of SDS gels to determine molecular weight is a Citation Classic. Her work on the use of antibodies in immunofluorescence microscopy allowed the definition of the arrangements of microfilaments, microtubules and intermediate filaments in cells and tissues .The 1978 demonstration of a 1:1 correspondence between microtubules viewed in immunofluorescence and electron microscopy in the same cell was a major breakthrough as was the work showing that antibodies specific for the different types of intermediate filament proteins are powerful reagents in human tumor diagnosis.

She has been an EMBO member since 1978 and she holds an honorary doctorate from the Pomerian Medical Academy in Szczeczin, Poland. She was a trustee of the Swedish Foundation on the Environment, MISTRA, and has chaired the Scientific Advisory Boards of the European Molecular Biology Laboratory (EMBL) in Heidelberg and the Pette Institute in Hamburg. She has also chaired the Cell Biology Section of Academia Europaea. She has been a member of the European Strategy Forum on Research Infrastructures (ESFRI) Group. She has participated in several juries concerned with awarding substantial grants to young scientists including the BioFuture program and the Helmholtz Young Investigator Program.

She has been awarded the Meyenburg Prize and the L'Oreal/UNESCO Prize for her work on the cytoskeleton and the use of antibodies in tumor diagnosis. Her research and her efforts for Women in Science, were honored by the award of the Dorothea Schlözer Medal from the University of Göttingen in 2007 and by the award of the Bundesverdienstkreuz (1st class) in 2014.

With her classic profile, we are thrilled to have her as a moderator in the panel discussion. With this, we would like to invite you all to a brainstorming question and answer session where you can feel free to seek fantastic ideas to sort your scientific enigma.

STUDENT POSTER ABSTRACTS

1

Muhammad Ali Khan

Isolation and Functional Characterization of an Ethylene Response Factor (RhERF092) Transcription Factor From Rose (Rosa Hybrid)

Muhammad Ali Khan1*, Gao Junping[2], Muhammad Imtiaz[1], Adil Hussain[1], Ma Nan[2], Sayed Hussain[1], Fazal Jalal, Fazal Said, Xiao Xu[2]

[1)] Department of Agriculture, Abdul Wali Khan University Mardan Pakistan;
[2)] College of Horticulture, China Agriculture University Beijing, P.R. China;
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Rose is one of the most important ornamental plants around the world with a huge aesthetic and market value. However, improper postharvest handling and transportation often results in great loss to the quality of cut roses. The phytohormone ethylene plays a key role in opening of rose flowers as well as in the vegetative and reproductive growth and development. Ethylene biosynthesis and homeostasis is regulated by plethora of biomolecular cues within the plant as well as affected by different biotic and abiotic factors. In this study we isolated and characterized Ethylene Response Factor (RhERF092) from the rose (Rosa hybrida) cv. "Samantha" and investigated its role in flower opening and senescence. ERF is a group of transcriptional factors that generally encode transcriptional regulators involved in several physiological processes. The RhERF092 was originally detected in a microarray experiment with a significant increase in its expression after 1hr of ethylene treatment in rose petals. Sequence analysis showed the presence of the canonical AP2/EREBP domain and a C-terminus trans-activation domain. Phylogenetic analysis showed that RhERF092 is an ortholog of the Arabidopsis ERF1 (AT3G23240)belonging to subgroup IX of the ERF gene family. Confocal laser scanning microscopy showed that the gene is localized in the nucleus. Analysis of basal expression patterns through qRT-PCR showed the highest expression at stage 0 of flower opening with a gradual decrease till senescence. However, rapid increase in transcript accumulation was observedup to 1hr of ethylene treatment. Ectopic expression of RhERF092 in Arabidopsis caused various ethylene-related aberrations in plant development including stunted growth, abortion of apical dominance, production of lateral tillers from rosette nodes, branches from the aerial nodes, and sterile inflorescence. Arabidopsis RhERF092-OX plants were found to be sensitive to the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) with significantly reduced hypocotyl and root system as compared to WT plants with significant reduction in the expression of genes involved in cell proliferation, cell expansion and cell cycle such as ARGOS, ARGOS-LIKE, SIM, JAGGED, AN3, CYCD3-1, and CYCD3-2. Taken together these results indicate that RhERF092 regulates ethylene-specific responses in rose.

KeyWords: Rose; Flower opening; Ethylene; RhERF092; Transcriptional factor

2

Ata Efes

Effects of Nano-Hydroxyapatite paste on Remineralisation of Initial Enamel Carious Lesion

Ata Efes[1], Sevdiye Burke[2], Begum Guray Efes[2]

[1)] Department of Molecular Biology and Genetics, College of Sciences, Koç University;
[2)] Department of Restorative Dentistry, Faculty of Dentistry, Istanbul University

AIM: The aim of this *in vitro* study was to compare the effectiveness of nano-hydroxyapatite (n-HAp) paste, fluoride gel and Sodium fluoride dentifrice in remineralizing, artificially induced initial enamel carious lesion by using caries detection device

MATERIALS AND METHODS: 21 healthy orthodontically extracted premolar teeth were used. The surfaces of specimens were coated with one layer of acid-resistant varnish (Max Factor, France) except for a 3x3 mm window on the buccal enamel surface; which continued intact. Artificial enamel carious lesions were created by inserting the specimens in demineralization solution (2H2O x CaCl2= 2.2 mM, 2H2O x NaH2PO = 2.2 Mm, Lactic acid= 0.05 M) for 3 consecutive days. Then teeth were kept in sterilised saliva for a day.

Specimens were divided into three groups (n=7): (1) Sodium fluoride dentifrice (Opalescence Whitening Toothpaste, Ultradent), (2) fluoride gel (2% Neutral Sodium Fluoride, Imicryl), and (3) home-made n-HAp paste (Geistlich, Bio-Oss powder + olive oil).

Artificial demineralization was followed by remineralization using dentifrice slurry (group 1) contacted for 2 min. to the buccal enamel surface. The slurry of toothpaste was prepared by a 3:1 weight ratio of deionized water to toothpaste in accordance with EN ISO 11609-2017 (Dentistry - Dentifrices - Requirements, test methods and marking).

Neutral sodium fluoride applications (group 2) contacted for 4 min.to the tooth surface according to manufacturer instructions and n-HAp paste (group 3) 1:1 weight ratio of olive oil to n-HAp powder (Geistlich, Bio-Oss) without any fluorides were applied 2 min on specimens.

Baseline, after lesion formation, immediately after remineralization, caries detection and remineralization were performed for all the enamel specimens using caries detection device the DIAGNOdent (KaVo, Germany) Data were statistically analysed with Tukey HSD Test, using SPSS version 11 software.

RESULTS: The degree of demineralization (12,57; 12,28; 12,14) was significantly elevated compared to baseline (3; 2,5; 2,28) however, no significant difference was found between Sodium fluoride dentifrice (6) fluoride gel group (7) and n-HAp paste group (5.5)($p<0.05$)

CONCLUSIONS: Within the limitations of this in vitro study, the homemade n-HAp paste has a beneficial effect on remineralization of initial enamel carious lesion.

Keywords: Demineralization, dentifrice, remineralization, artificial caries, n-HAp paste

3

Vladyslav Holiar

Knockout of the ribosomal protein S6 kinase 1 isoform, p85, in the MCF-7 cell line leads to decreased cell growth, survival and migration

V. V. Holiar, I. V. Zaiets

Institute of Molecular Biology and Genetics, NAS of Ukraine

S6 ribosomal protein kinase 1 (S6K1) is an extensively studied serine/threonine protein kinase which functions as a component of the mTOR/S6K-dependent signaling pathway. It is involved in regulation of numerous cellular processes including protein synthesis, cell growth, motility and survival. Moreover, S6K1 is responsible for the development of a number of disease states such as obesity, diabetes and cancer. The S6K1 gene encodes two major isoforms, p70 and p85, which differ only by the N-terminal 23 amino acid extension unique to p85. Despite plenty of data that characterize their shared role in control of cell physiology, the exact difference in functioning of these isoforms remains unclear. Although, recent studies suggest that p85 is a secreted protein which facilitates cell growth and migration, and can also drive tumorigenesis through acting on surrounding normal cells within the tumor microenvironment.

The aim of the study was to generate p85 knockout MCF-7 cells using the CRISPR/Cas9 genome editing technology and evaluate an ability of the cells to survive, grow and migrate compared to wild-type MCF-7 cells. At the first step, we applied the CRISPR/Cas9 system to selectively disrupt expression of the p85 isoform but not p70, thereby targeting the p85-specific N-terminal extension. Knockout of the p85 isoform in MCF-7 was confirmed by immunoblotting with antibodies specific to both the S6K1 C-terminus and p85-specific amino acid sequence. After selection of several clones with a complete loss of p85 we tested their viability in response to hydrogen peroxide treatment, as well as cell proliferation and migration rate. Cell survival and proliferation were examined by using the MTT assay. The obtained results indicate that the MCF-7 cells with a loss of p85 display an impaired ability to survive since all the generated clones showed a decrease in resistance to oxidative stress. In addition, we observed a reduced proliferation rate in comparison to the control MCF-7 cells. To estimate cell motility, we performed the scratch assay which revealed the inhibition of migration rate in the generated clones.

In summary, our findings suggest that p85 isoform can promote malignant cell behavior regulating cell survival, growth and migration. Furthermore, the generated MCF-7 cell line could be used to get deeper understanding of separate impact of different S6K1 isoforms on control of cell function.

4

Ka Lok Kam

The Study of GCAL Model for Visual system

Ka Lok Kam

University of Göttingen

The GCAL model is a stimulus-driven model which mimics the development of visual system from retina to V1 cortex (primary visual cortex). The maps of V1 created by the model shows some import properties given by earlier studies , such as the aperiodic structure of the orientation maps in primary visual cortex and the pinwheel density. Although the mechanics and learning rules used by the model are familiar, an explicit study of it is still challenging due to the sheer amount of control-parameters. During the study both theoretical approach and simulation are used to identify some import parameters and their corresponding roles in the model.

5

Tomasz Wenta

Physiological characterization of the proapoptotic HtrA4 protease

Tomasz Wenta*, Monika Borysiak, Anna Latała, Karol Jasiński, Małgorzata Sakowicz, Barbara Lipińska

Department of General and Medical Biochemistry, Faculty of Biology, University of Gdańsk, 80-308 Gdańsk, Poland; **tomasz.wenta@biol.ug.edu.pl*

The HtrA (high temperature requirement A) family proteins are serine proteases very well conserved in evolution. Their dysfunction may cause severe diseases, e.g. the neurodegenerative diseases and cancer. There are four human HtrA proteins, HtrA1-4, and changed levels of these proteins have been found in many cancer tissues. The knowledge concerning the function of the HtrA4 protein is limited. It is known that it participates in embryo implantation and formation of placenta. So far, only three HtrA4 cellular partners have been identified: the HtrA1/3 and syncytin-1 proteins. The aim of this study was to identify cellular partners of the HtrA4.

Using pull down and LC/MS techniques we identified a panel cellular proteins interacting with HtrA4 which belong to the four main groups: structural cytoskeleton proteins and proteins involved in its formation, proteins participating in maintaining cellular homeostasis and cell death, proteins involved in cellular transport and proteins connected with DNA replication. Using western blotting and immunoprecipitation we found that HtrA4 forms complexes with actin, β-tubulin, TCP1α, S100A6, XIAP both *in vitro* and *in vivo*. We also showed that HtrA4 protease promote etoposide cytotoxicity and cleaved recombinant XIAP. Collectivelly, our results suggest that HtrA4 is involved in modulation of cytoskeleton stability and regulation of cell death. We believe that these results provide new insight into the function of HtrA4 in the cell.

Acknowledgements

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6

Artem Mansurkhodzhaev

The mechanisms of peptide bond cleavage and Proteasome-Catalysed Peptide Splicing in Humans

Artem Mansurkhodzhaev

Max Planck Institute for Biophysical Chemistry

Introduction: The activation of cells of the adaptive immune system is achieved by the presentation of antigen associated peptides (epitopes) on the surface of antigen presenting cells. The presentation of epitopes on the cell surface for T cell receptors (TCRs) is performed by Major Histocompatibility Complex (MHC) molecules. MHC class I (MHCI) presents epitopes to CD8+ T cells. MHCI presentation involves degradation of proteins by the ubiquitin-proteasome system (UPS). The key element of the UPS is 26S proteasome. Its core, the 20S particle performs peptide bond cleavage by catalytically active β1/β2/β5 subunits carrying amino terminal threonine. In addition to a peptide bond cleavage, 20S core can catalyse a ligation of two non-continuous peptides in a transpeptidation reaction. This novel activity is called Proteasome-Catalysed Peptide Splicing (PCPS). Spliced peptides are hypothesised to significantly enlarge the antigenic repertoire of a cell and putatively could be used in a variety of translational aspects.

There are still many unanswered questions regarding the mechanisms and relevance of PCPS. It is known that PCPS is most likely a carefully regulated process with its own cleavage and sequence preferences. Our goal is to elucidate the cleavage and splicing sites preferences and determine whether there are quantitative differences in PCPS displayed by β1/β2/β5 and β1i/β2i/β5i subunits of a standard- and immunoproteasomes, respectively.

Methods and results: Three synthetic peptides were digested with purified 20S standard- and immunoproteasome from T2 and LcL cancer cell lines respectively in presence/absence of specific β1/β2/β5 subunit inhibitors at several time points. The digestion mixtures were then measured on Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (MS) coupled to high-performance liquid chromatography (HPLC) (ThermoFisher). The identification of the peptides in the digestion mixtures and label-free quantification based on MS signals intensities was performed using Mascot search engine (version 2.6) and Mascot Distiller add-on, respectively (Matrix Science). To obtain absolute peptide quantities we further developed and will apply a quantitative method called quantification with minimal effort (QME), which is based on the conservation of mass in the in vitro digestions.

We are currently performing data acquisition and analysis. So far, we determined a complete generation dynamics for spliced and nonspliced products produced by 20S proteasomes from T2 (standard proteasome) and LcL (immunoproteasome) cell lines currently for one substrate. Following label-free quantification, we determined the quantitative dynamics of the generation of all products (spliced and nonspliced) by the standard T2 20S proteasome with the differentially inhibited subunits. In this way, we are hoping to get an idea of the kinetics of cleavage and splicing catalysed by each active β subunit, as well as their overall preference for PCPS. Next, we intend to find the probabilities of cleavage and splicing for every residue of the substrates by calculation of site-specific cleavage strength (SCS). With this, we are planning to elucidate residues preferentially utilized for cleavage and splicing to understand why the frequently used cleavage sites do not correspond to the sites used for PCPS.

7

Timur Tuganbaev

Weizmann Institute of Science

The intestinal microbiota undergoes diurnal compositional and functional oscillations that affect metabolic homeostasis. Disruption of microbial circadian rhythms leads to the development of metabolic syndrome. But the mechanisms by which the rhythmic microbiota influences host circadian activity remain elusive. Using integrated multi-omics and imaging approaches, we have demonstrated that the gut microbiota features oscillating biogeographical localization and metabolome patterns that determine the rhythmic exposure of the intestinal epithelium to different bacterial species and their metabolites over the course of a day. This diurnal microbial behavior drives, in turn, the global programming of the host circadian transcriptional, epigenetic, and metabolite oscillations. Surprisingly, disruption of homeostatic microbiome rhythmicity not only abrogates normal chromatin and transcriptional oscillations of the host, but also incites genome-wide de novo oscillations in both intestine and liver, thereby impacting diurnal fluctuations of host physiology and disease susceptibility. As such, the rhythmic biogeography and metabolome of the intestinal microbiota regulates the temporal organization and functional outcome of host transcriptional and epigenetic programs.

8

Hagar Meltzer

A CRISPR *in vivo* screen aims to uncover the mechanisms underlying neuronal remodeling

Hagar Meltzer[1], Efrat Marom[1], Idan Alyagor[1], Netta Segal-Gilboa[2], Tamar Unger[2], Oren Schuldiner[1]

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[2)] Center for Structural Proteomics, Weizmann Institute of Science, Israel

Neuronal remodeling is a late developmental process that serves as a mechanism to refine neural circuits, and is crucial for sculpting the mature nervous systems of both vertebrates and invertebrates. Understanding the molecular basis of neuronal remodeling may offer insight into neural degeneration and regeneration during development and disease.

Drosophila mushroom body (MB) γ neurons undergo highly stereotypical remodeling during metamorphosis, including elimination of exuberant neural connections, followed by regrowth of adult-specific ones. Despite recent progress, the molecular basis for MB remodeling remains largely unknown. In search of new genes involved in MB remodeling, I am conducting a large-scale CRISPR/Cas9-mediated in vivo screen. Our novel screening method enables simple and efficient generation of tissue-specific biallelic gene disruption, and is ideal for high-throughput application. For the screen, we are constructing a library of gRNA-expressing transgenic Drosophila lines, which will cover several hundred genes, and would pose an extremely valuable resource to the worldwide fly community.

So far our screen identified several new genes as potentially required for remodeling of MB γ neurons. Among these is the F-BOX protein CG9003, that functions as part of the Skp, Cullin, F-BOX (SCF) E3 ubiquitin ligase complex. I am now delineating the precise role of CG9003 in MB γ neurons and attempting to uncover the substrates it targets for degradation. While further pursuing this direction I am also continuing the large-scale screening process, in the overall goal of promoting our understanding of the molecular events that govern neuronal remodeling.

9

Elinor Breiner Goldstein

Structural Studies on Biosynthetic Antibiotics that Target The Protein Exit Tunnel in The Ribosome.

Elinor Breiner-Goldstein, Zohar Baram, Donna Matzov, Yehuda Halfon, Ella Zimmerman, Haim Rozenberg, Anat Bashan and Ada Yonath

Department of Structural Biology, the Weizmann Institute of Science, Rehovot, Israel

The ribosome, a multi component universal assembly of RNA and proteins, translates the genetic code into proteins in all living cells. Active ribosomes are essential for cell life therefore inhibiting their function will damage the cell. Currently, about 40% of the clinically used antibiotic compounds target functional centers in the ribosome.

The macrolides have been known as antibacterial drugs from the beginning of the 1950s and are used to treat gram positive bacterial infections as well as a limited number of gram negative bacteria. They are well established as clinically used antibiotics and known for their broad antimicrobial spectrum as well as relatively high margin of safety. Their structure is composed of a macrolactone ring with variety of ring sizes, to which one or more sugars as well as various short chemical segments are attached.

Macrolides were shown to inhibit protein biosynthesis by blocking the nascent peptide exit tunnel (NPET) close to the peptidyl transferase center (PTC), thus interfering with nascent chain progression.

We have determined the crystal structure of the D. radiodurans large ribosomal subunit in complex with three biosynthetic macrolides, shedding light on their binding, inhibition, selectivity and resistance modes of action.

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Olesia Taburets

Molecular markers during dynamics of wound healing and with the treatment of melanin

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The adverse physiological and psychological effects of scars formation after healing of wounds are broad and a major medical problem for patients.We have previously shown that melanin, which is produced by Antarctic black yeast-like fungi Pseudonadsoniella brunnea, strain X1-M, from Galindez island's vertical cliffs (Ukrainian Antarctic Station "Akademik Vernadsky") has expressed cytoprotective effect, promotes rapid healing of wounds of various etiology and can be offered as a new dermotropic drug. One of the main focuses of recent research has been the role played by the Tlr2, Tjp1, Ptgs2 ,Tgfb1 and Slc9a3 in the process of wound healing and scar formation.

Methods: Study was conducted on rat model of purulent necrotic wound. In each model one group was a control, while in others wound healing occurred without drugs or with administration of 0,5 % carbopol or with both 0,5 % carbopol and 0,1% melanin. Level of genes mRNA was determined with quantitative RT-PCR.

Results: TGF-β1, Tlr2, COX2, ZO-1, Slc9a3 are factors identified as reduced in scarless healing. An increase in Tlr2, Ptgs2, Tgfb1, Slc9a3 gene expression and Tjp1 reduction is shown during healing of the f purulent necrotic wounds. Under the influence of the pharmaceutical composition, the expression level of these genes changed in the direction of the control group of rats, whose wounds were not treated with a pharmacological composition.

As for the possible mechanisms of melanin influence as polyphenolic compounds on analyzed gene expression during the healing of skin lesions of various etiologies, first of all, its frank cytoprotective effect should be noted. Namely, it reduces the activity of lipid peroxidation, increases the activity of enzymes of antioxidant system, prevents DNA damage; influences the cytokines production: TNF-α, IL-6, VEGF, etc. by, for example, the impact on the expression of nuclear receptors PPAR, increases eNOS expression and outflow of anti-inflammatory cytokines to reduce the intensity of inflammation in wound healing

Thus, our results may indicate the advisability of applying pharmaceutical compositions based on melanin for the treatment of inflammatory processes in the skin wounds.

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Janith Manahara Bandara Wanigasekara Wanigasekara Mudiyanselage

Cloning and Over Expression of Bromelain Gene from Annanas Comosus into an *E. Coli* Expression System

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A gene encoding bromelain enzyme has been cloned from Annanas comosus Red Ceylon (Mauritius) into an E.coli expression system. There are several different types of bromelain enzymes such as stem bromelain, fruit bromelain, ananain and comosain. However fruit bromelain is not commercially available. Bromelain has wide application in medicine as a therapeutic supplement, an anti-inflammatory agent and proteolytic agent as well as in food and leather industry. Development of a novel method to mass-produce bromelain is therefore important.

Total RNA was extracted from pineapple fruits and first strand synthesis was carried out using oligo dT primer. The second strand synthesis and subsequent PCR amplifications were carried out using designed primers, BromFP1, BromFP2, BromRP1. The complete bromelain gene initially cloned into pGEMT-easy vector and transformed into E.coli JM109 followed by sequence confirmation and protein blast analysis by NCBI. Thereafter, the bromelain gene was re-cloned into PET28a+ vector and transformed into E. coli BL21 (DE3) pLysS expression host. Recombinant E.coli clones were confirmed by colony PCR. Sequence analysis confirmed the presence of the complete gene (1200bp) including start and stop codons. The complete protein sequence consists of 399 amino acids.

Protein blast (NCBI) was obtained following schematic line diagram of query amino acid sequence indicating the position of active sites in peptidase C1 super family peptidase, composed of cysteine peptidases (CPs). A search of the protein data base at Swissprot revealed a high degree of similarities (99%) to fruit bromelain precursor.

The over expressed bromelain protein was purified from culture by using MagneHisTM protein purification system while the crude bromelain protein from Annanas comosus Red Ceylon (Mauritius) was purified by using ion exchange chromatography. The activity of over expressed bromelain protein was measured by activity assay. The amount of bromelain protein produced in the recombinant increased by 87 % relative to the original strain. SDS-PAGE analysis indicated a molecular weight of 31kDa.

The recombinant bromelain protein was stable over a broad pH range (2.0 to 13.0) and an optimum pH value was pH 9.0. bromelain protein activity was partially inhibited by EDTA and was completely inhibited by PMSF. The bromelain protein activity was increased with 2.5mM of Ca2+ incubation and activity was reduced with other metal ions (Cu2+, Mg2+and Zn2+). The highest bromelain protein activity was observed in phosphate buffer and it was also stable with organic solvents, surfactants & bleaching agents (except 0.5% w/v SDS) tested.

In conclusion, bromelain genes from Annanas comosus Red Ceylon (Mauritius) was successfully cloned, expressed, protein purified and characterized in E.coli.

Keywords: Bromelain, Bacillus licheniformis, recombinant expression.

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Kalpani Yashodara Perera Devaligoda Gamage

Troubleshooting in RNA extraction from human tissue

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Extracting high-quality RNA from tissue samples especially adipose tissue is challenging because of high fat content and low cell number. Most protocols for RNA extraction from adipose tissue (AT) results in degraded or poor yield RNA. The objective was to extract RNA from tissue samples (AT and placenta) for gene expression analysis.

AT and placenta were collected from pregnant women undergoing caesarean section at De Soysa Maternity hospital. RNA was extracted in AT by optimizing TRIzol® Reagent protocol. 1st method: mechanical homogenizer was used. 2nd method: conducted in cold conditions. 3rd method: homogenized in liquid nitrogen. 4th method: RNA extraction by; powdering in liquid nitrogen and vortexing for 1/2hr. 5th method: Chloroform was added twice and samples left overnight for precipitation. 6th method: RNA extracted using isolated cells. 7th method: AT was placed on TRIzol® Reagent for 1 week before extraction, minced into pieces and homogenized using needle. Fresh and snap frozen placental tissue was used to extract RNA by method 1. RNA was quantified and DNase treated.

Degraded RNA was resulted from all methods however a higher concentrated band was observed when using method 7 (A260/A280 1.92). The fresh placental tissue yield more concentrated RNA than t snap frozen tissue.

RNA extraction from tissue samples is tedious process, while homogenizing is the most crucial step. RNA extraction from placental tissue was less demanding due to the less fat content when compared to AT. The real time PCR can be performed although degraded RNA was resulted.

Key words: RNA, trouble shooting, adipose tissue, placenta

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Yajie Zhu

Caspase signaling promotes SOP cell development by cleaving Arm

Yajie Zhu, Yu Pan, Lei Xue

IMPRS Molecular Biology

Cell fate determination is crucial for building a functional organism. Drosophila SOP cell is a powerful model for studying neural cell fate determination. Wnt pathway plays a role in SOP cell fate determination. Caspase signaling is found to regulate cell fates in some cases, but its function and mechanism remain largely elusive. Here, we reveal that caspase signaling promotes SOP cell emergence by cleaving Arm. Downregulation of caspase signaling alleviates the Wnt-induced ectopic bristle phenotype in wings while upregulation of caspase signaling alleviates loss-of-wg induced margin bristle loss. Besides, deviation of caspase signaling leads to abnormality in bristles and veins. These findings demonstrate that caspases pathway has a role in regulating SOP cell fate. Then we found that caspase signaling has an effect on influencing Achaete level. Finally, we found that the function of caspases in cell fate is achieved by cleaving Arm. Our study not only reveals a novel function of caspase signaling, but also contributes to the understanding for neural fate determination, which may serve for providing the theoretical basis for clinical treatment of neurodegeneration in the future.

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David Wiener

N6-methyladenosine shapes poly(A) tail length in yeast meiosis

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RNA is modified by >100 chemical modifications. These modifications are catalyzed post-transcriptionally across different classes of RNA and thus have the potential of imposing a unique and poorly characterized regulatory code. N6-methyladenosine(m6A) is the most prevalent mRNA post-transcriptional modification. Although methylation sites were mapped in different species and across heterogeneous classes of genes, the function of the modification remains poorly understood. Interestingly in mammals, there exists a negative correlation between methylation status and RNA stability. Mechanistically, reporter assays suggested that m6A causes recruitment of the CCR4-NOT deadenylase complex resulting in shortening of the tail and subsequent RNA decay. However, it remains unclear how general this effect is, in particular given that high throughput studies in various systems generally find only very poor correlation between tail length and RNA stability. Whereas m6A is ubiquitous across all mammalian tissues, in yeast m6A is highly dynamic, with mRNAs only undergoing methylation during meiosis providing a unique biological context to study the impact of the modification on poly(A) tail length.

The aim of this work is to systematically evaluate the cross-talk between m6A and regulation of poly(A) tails in yeast meiosis. To measure poly(A) tail length we developed a new high-throughput sequencing based method and generated detailed maps of poly(A) lengths across yeast meiosis in both WT and methylation deficient strains. We observed an inverse correlation between methylation levels and length of the poly(A) tail. This correlation was specific to meiosis and absent under vegetative growth conditions, and was eliminated in deadenylation machinery mutants, suggesting an interaction between both levels of post transcriptional processing. Consistently, we found that poly(A) tail correlates with mRNA half-life exclusively in meiosis, where the mRNA is methylated, but not under vegetative growth conditions, when it is not. These results, based on systematic and genome-wide analyses, thus highlight coupling between two key post-transcriptional layers regulating a primordial biological process.

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Joshua Philippe Olorocisimo

Psychobiotic effects of *Lactobacillus* species on anxiety and memory of *Danio rerio*

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Psychobiotics are probiotics which may confer mental health benefits. As a relatively new field, only few bacteria have been characterized as psychobiotics, and their exact mechanisms of actions are still unknown. Here, we fed Danio rerio with three Lactobacillus species isolated from the Philippines. Behavioral analysis was done to determine if these species can improve memory or reduce anxiety in zebrafish. Treatment with Lactobacillus sp. strain 1 increased the left eye preference of the zebrafish in the visual lateralization novel object recognition (VL-NOR) test, indicating better memory performance. Lactobacillus sp. strains 2 and 3 increased fish exploration rate, indicating anxiolytic effects. In addition, Lactobacillus sp. strain 3 increased time spent in the upper half of the novel tank diving test and induced an upregulation in gad brain gene expression, suggesting anxiolytic effects in zebrafish through the GABAergic pathway. Thus, we discovered three different Lactobacillus species as possible psychobiotics which can improve memory and reduce anxiety in zebrafish.

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Harold Carag

Psychobiotic effects of *Lactobacillus* species on anxiety and memory of Danio rerio

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Lyly Madiluabu Mutombo

Effects of the Essential Oil of Rosemary (Rosmarinus Officinalis) on the Conservation of Tomato Fruits (Solanum Lycopersicum L.)

Lyly Madiluabu Mutombo

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The fruits and the vegetables provide a source of abundant and cheap energy, nutritive substances of growth, vitamins (A, B6, C, E) and minerals (Ca, Fer, K, Mg, Na6...); their food value is higher when they are fresh, but it is not always possible to make an immediate consumption of it. Although the fruit and vegetables are significant for human health; however, they EP do not uvent being preserved for a long time because of their perishable nature.

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Scott Harroun

DNA Probes for Monitoring Enzyme Activity

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To measure enzymatic activity, one may need a different assay for each reaction catalysed by the enzyme. Therefore, a universal approach to monitor catalysis of any substrate of a given enzyme is desirable, especially for applications such as high-throughput screening. In this study, we employ fluorescent DNA-based molecular probes to measure enzymatic activity via their attachment to an enzyme. We demonstrate that our DNA probe only reports signal change due to a local effect during catalysis, possibly from structural destabilization. Our approach correlates well with traditional measurements of enzymatic activity (i.e. KM), thereby confirming its validity to measure kinetics. We optimized various conditions, including the attachment strategy, DNA composition and length, the effect of steric hindrance, and the ratio of DNA and enzyme added. Our system performs well for at least 100 days, making it convenient once prepared. Furthermore, by employing several fluorophores possessing differing structures and hydrophobic properties, it may be possible to probe various regions of the enzyme surface during catalysis.

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Shaymaa Abdulmalek

Boosting brain insulin signaling to combat neurotoxicity arising in type 2 diabetes

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Insulin signaling reveals to be a very promising pathway for the prevention and treatment of Alzheimer's Disease (AD). Available data have indicated that brain insulin resistance may contribute to neurodegenerative diseases. Aim: The present work aimed to study the role of combined metformin with natural oil to enhance brain insulin signaling in type 2 diabetic (T2D) rats as well as study its role on the expression profile of AD-related miRNA that is possibly related to AD pathology and its impact in the early diagnosis of AD in T2D. Methods: After intraperitoneal injection of AG538, an insulin receptor substrate inhibitor, the induced rats were orally and daily administrated metformin and oil for 21 days. Results: We identified significant disturbances of insulin signaling in the brain of induced rats, including the inhibition of physiological, p-IRS1, p-AKT and p-GSK3β, as well as the enhancement of tau protein phosphorylation; these effects were clearly attenuated by treatment. Remarkably, AD-associated pathological proteins, such as oxidative stress, inflammation, BACE-1, APP and Aβ42 were noticeably increased, and these effects were significantly revoked after treatment. Interestingly, the expression profile of AD-related miRNAs in sera and brain tissues displayed its neuro-protection role. Conclusion: These findings shed the light on the specific roles of insulin signaling in T2D-mediated AD-like neurotoxicity. Thus, an understanding of the regulation of brain insulin signaling may provide novel insights into potential therapeutic targets for T2D-mediated AD-like neurotoxicity.

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Maria Loidolt-Krüger

Quantitative Ultra-fast FLIM

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Increasing the speed of Fluorescence Lifetime Imaging (FLIM) is essential to cementing its importance as a tool in the Life Sciences. This technique is already well established, but imaging dynamic processes requires shorter acquisition times. Our novel rapidFLIM approach dramatically reduces the acquisition time through a combination of fast scanning, hybrid photomultiplier detectors which are capable of handling very high count rates, and TCSPC modules with ultra short dead times. With the new FLIMbee fast scanning add-on for the MicroTime 200, this technique can be used with our microscopy platform as well as being offered as an upgrade kit for conventional Laser Scanning Microscopes (LSMs).

With this hardware combination, excellent photon statistics can be achieved in significantly shorter time spans, allowing fast processes to be measured with the high resolution achieveable in confocal microscopy. Depending on the image size, rapidFLIM allows imaging at a rate of several frames per second, enabling dynamic processes, such as protein interactions, FRET dynamics, or chemical reactions to be imaged in a time-resolved manner. With these high frame rates, FLIM can also be used on highly mobile species such as cell organelles and for other live cell imaging applications.

Recently, we have further pushed the limits of this method by systematically reducing the effects of decay distortions at very high count rates, allowing quantitative data analysis to be performed even at count rates >> 10 Mcps. This technique has been applied to quantitatively analyze FRET measurements using fluorescent proteins.

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Zhengzhong QU

Validation of NGS Clinical Assays Based on the Guidelines of College of American Pathologists

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Genome Institute of Singapore

Targeted next-generation sequencing (NGS) is becoming increasingly common in the daily clinical usage. NGS assays can be used in the early screening, diagnosis, prognosis, and selecting of targeted therapies for cancer patients as well as other diseases. POLARIS (Personalized OMIC Lattice for Advanced Research and Improving Stratification) is a strategic program to pilot the application of clinical genomics in the treatment and diagnosis of medical diseases in Singapore. Here, we describe two custom-developed, next-generation sequencing tests. One for detecting single-nucleotide variants (SNVs) and short insertions and deletions (indels) in 740 genes related to various types of cancers from routine shock-frozen clinical specimens. Another for detecting germ line inhereted SNVs and short indels in the whole exome range to obtain a diagnosis in patients with undiagnosed genetic disorders.

Both two NGS clinical assays were validated based on the guidelines of College of American Pathologists. We implemented a strategy by using real clinical samples as well as synthetic reference DNA derived from cell lines with known genetic variants which model a broad range of allele frequencies. For our oncology panel, test sensitivity achieved 99.4% for SNVs, 96.5% for indels, with high specificity (98.9 % for SNVs and 88.7 % for indels) for allele frequencies >10% in synthetic reference DNA. We further confirmed test accuracies using real shock-frozen clinical specimens characterized by alternative and conventional clinical diagnostic technologies. Robust performance was observed: sensitivity was 100% and specificity was 100%. For our inhereted disease panel, test sensitivity achieved 95% for SNVs, 89% for Indels, with high specificity (100% for SNVs and 100% for Indels) for allele frequencies >10% in synthetic reference DNA. Test accuraries were further confirmed using real clinical blood samples with Sanger sequencing, and 100% sensitivity and specificity were observed. We also observed high intra-run and inter-run reproducibility, as well as low cross-contamination rates in both two assays. By using synthetic reference DNA, we also showed that our assays have consistent analytical sensitivity down to 10% minor allelle frequencies for the detection of somatic mutations.

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Dominik Botermann

A new role of Hedgehog signaling pathway in folliculostellate cells of the adult murine pituitary gland

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The essential role of the Hedgehog (Hh) signaling pathway during the embryonal development of the pituitary gland is well established but the knowledge about its function in the adult pituitary and the respective tumors is still sparse. Recently our group has shown that excessive Hh signaling in murine pituitary explants (e.g. by inducible homozygous Ptch depletion) leads to enhanced growth hormone (Gh), prolactin (Prl), and adrenocorticotropic hormone (Acth) secretion and an enhanced proliferation of Sox2+/Sox9+ adult stem cells, which contribute to the regeneration/maintenance of endocrine pituitary cells. Moreover Gli1+ cells of the anterior pituitary lobe express simultaneously Sox2. These data prompted us to the assumption that Hh signaling plays also a fundamental role in the adult pituitary. Thus we narrowed down the cellular population which is directly regulated by Hh signaling. Lineage tracing experiments by using an inducible Gli1-CreERT2/Rosa26-tdTomato mouse model revealed that the progeny of Gli1-expressing cells contribute to folliculostellate (FS) cells of the adult pituitary gland that regulate the activity of hormone secreting cells and supposedly harbors stem cell-like features. These conclusions were based on the morphological appearance, the close proximity to hormone secreting cells, the absence of hormone expression and the typical expression profile (e.g. S100, Sox2) of the progeny of Gli1-expressing cells. Taken together these data presume that Hh signaling indeed impacts hormone secretion but rather in an indirect than in a direct fashion via regulating FS cells. Moreover, since it has been assumed that FS cells harbors stem cell-like features it seems to be obvious that Hh signaling additionally regulates the fate of FS cells. Finally, the fact that Hh signaling is involved in the formation/maintenance of a variety of tumor entities these data suggest that it might be also implicated in tumorigenesis of pituitary adenoma or spindle cell oncocytoma which show highest FS cell infiltration or develop from FS cells, respectively.

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Nadeem Khan

Genome-Wide Identification, Classification, and Expression Divergence of Glutathione-Transferase Family in Brassica rapa under Multiple Hormone Treatments

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The GSTs is one of the most important multifunctional protein families which has been playing a crucial role in the different aspects of plant growth. This extensive study about GSTs may establish a solid foundation for the brief functional analysis of BraGSTs in future. In this study, a total of 75 genes were identified in B. rapa. Phylogenetic analysis characterized them into eight different sub-classes, while Tau and Phi sub-class being the most numerous. The exon-intron structure and the motif composition of BraGSTs were exhibited accordingly to its sub-classes. Notably, we also investigated 15 tandem paralogous pairs of genes, which highlighted that all the pairs were purifying in nature as their synonymous values were lower than 1.00. Duplication analysis indicated that about 45.33 % of genes were mainly occurred through tandem duplication in B. rapa. Predominately, the tandem cluster of genes in sub-class Tau was greater than other sub-classes. Furthermore, among eight multiple hormonal treatments (ABA, GA, BR, ETH, IAA, IBA, NPA and JA), most number of BraGSTs were activated by NPA, BR and ABA treatments. This analysis has provided comprehensive information about GSTs family which may assist in elucidating their exact functions in B. rapa.

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Raghupathi Kummari

Deciphering the Mode of Regulation and Functional Redundancy of HtrA4 – a Serine Protease Implicated in the Cell Death Pathway.

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HtrA4 (High-temperature requirement factor A4) is a nuclear-encoded serine protease that shares significant sequence and structural similarity with its other human homologs (HtrA1-3). HtrA4 that is associated with various diseases including cancer, and a biomarker for pre-eclampsia, is a promising therapeutic target. Unlike other HtrAs, little information is available on HtrA4. Therefore in this study, using functional enzymology, computational, biochemical and spectroscopic probes, we aimed at elucidation of its mechanism of action, substrate recognition and identification of novel binding partners. Here, we have characterized different domains and mutants of HtrA4 to delineate their structural plasticity as well as intra/intermolecular cross-talks that are essential for maintaining its functional integrity. Our findings distinctly demonstrate the importance of N-terminal region and critical residues involved in its trimerization, which in turn is a prerequisite for its activity. Molecular modelling in combination with Molecular Dynamics Simulation (MDS) demonstrated overall structural conservation within the human HtrA family members. However, subtle structural variations in the dynamic loop regions and around the active-site might be responsible for its slower protease activity (compared to HtrA2), and hence functional diversity within the protease family. For the first time, comprehensive interaction and enzymatic studies identified X-linked inhibitor of apoptosis protein (XIAP) to be an HtrA4 substrate thus implicating its role in apoptosis. Extensive enzymology studies have provided insights into the substrate specificity of HtrA4 as well. Further studies are underway to determine its structure-function correlates that would lead toward modulating the protease with

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Amina Habbeche

Production and characterization of keratinase from a newly thermophilic actinvomycete Actinomadura keratinilytica strain Cpt29 isolated from Algeria

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An extracellular thermostable keratinase (called KERAK-29) was purified and biochemically characterized from a thermophilic actinomycetes Actinomadura keratinilytica strain Cpt29 newly isolated from Algerian poultry compost. The isolate exhibited high keratinase production when grown in chicken-feather meal media (24,000 U/ml). Based on matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) analysis, the purified enzyme is a monomer with a molecular mass of 29233.10-Da. The data revealed that the 25 b , N-terminal residue sequence displayed by KERAK-29 was TQADPPSWGLNNIDRQTAFTKATSI, which showed high homology with those of Streptomyces proteases. This keratinase was completely inhibited by phenylmethanesulfonyl fluoride (PMSF) and diiodopropyl fluorophosphates (DIFP), which suggests that it belongs to the serine protease family. Using keratin azure as a substrate, the optimum pH and temperature values for keratinase activity were pH 10 and 70 °C, respectively. KERAK-29 was stable between 20 and 60 °C and pH 3 and 10 for 5 and 120 h, respectively, and its thermoactivity and thermostability were enhanced in the presence of 5 mM Mn2+.

KERAK-29 was also noted to show high keratinolytic activity and significant stability in the presence of detergents, which made it able to accomplish the entire feather-biodegradation process on its own. The ability of the Actinomadura keratinilytica strain Cpt29 to grow and produce substantial levels of keratinase using feather as a substrate could open new promising opportunities for the valorization of keratin-containing wastes and reduction of its impacts on the environment.

Key words: Keratinase; Protease; KERAK-29; KERAB; Actinomadura; Streptomyces; Keratin hydrolysis.

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Eman Abbas

Chromatin remodeling BAF complexes control oligodendrogenesis in the developing forebrain

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In the developing mammalian brain, the ventral telencephalic neural stem cells (NSCs) produce several kinds of cells such as GABA-secreting interneurons, astrocytes, and oligodendrocytes (OLs). OLs could uniquely myelinate axons in the CNS that lead to accelerating their signal transduction. Epigenetic approaches, which govern OLs formation, are based on many extrinsic and intrinsic cues. However, little is known about the epigenetic regulation mechanisms that control the oligodendrogenesis. In this study, we focused on the role of the chromatin remodeling BAF complex in the OLs production. Our findings revealed that BAF complex is highly expressed in the oligodendrocyte lineage including oligodendrocyte precursor cells (OPCs), immature oligodendrocytes (iOLs) as well as, mature oligodendrocytes (mOLs). To delete BAF 155/BAF170 subunits in the ventral NSCs in the forebrain, we generated the conditional BAF155/BAF170 double knockout mice. Remarkably, this conditional BAF complex loss resulted in a depleted pool of iOLs and mOLs in both ventral and dorsal telencephalon. Furthermore, our data revealed that the BAF complex is required for the proper proliferation of OPCs. Thus, our findings implicated that the BAF complex has a crucial function to regulate the OLs development during the embryogenesis.

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Sophia Hernandez

The Immune Response of Mice to Immunization with Pf-MSP1 Block 2 RO33 is affected by sex and strain

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Malaria continues to threaten half of the world's population. The disease manifests itself in either mild or severe forms, however the molecular mechanisms behind the development of severe forms of the disease are still unknown. Elucidation of these mechanisms and the factors that affect disease outcome can provide important insights on how to manage and minimize the impact of the disease. Pf-MSP1 is a large protein believed to be involved in host erythrocyte invasion. It can be divided into 17 blocks based on sequence conservation, with block 2 being the most polymorphic, having 3 allelic variants, namely MAD20, K1 and RO33. Previous studies have observed that natural antibodies against this region are protective against clinical malaria. However, the RO33 variant has been correlated with increased levels of TNF-α in some populations, which in turn is correlated with severe disease. Previous studies in our lab using BALB/c mice have shown a sex-specific antibody response to Pf-MSP1 Block 2 RO33 with female mice having a lower antibody response against the antigen than males. In this study, the humoral and cytokine response of a different mouse strain to Pf-MSP1 Block 2 was measured by immunizing male and female C57Bl/6 mice with recombinant Pf-MSP1 Block 2 RO33. ELISA results from blood collected during week 0 and week 3 showed no significant sex-specific responses to the antigen. Levels of cytokines IFN-γ, IL-4, IL-6, IL-1ß, IL-17, TNF-α, TGF-ß, and IL-10 produced by splenocytes were also measured using quantitative PCR (qPCR). Again, no sex-specific differences were observed in this mouse strain. Taken together, these results show that (1) C57Bl/6 mice mount a significant antibody response against the antigen and (2) no sex-specific response is observed unlike in Balb/c mice indicating that a combination of MHC haplotype and sex play a role in the immune response against PfMSP-1 RO33.

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Salma Sohrabi-Jahromi

Transcriptome maps of the general RNA degradation machinery

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RNA needs to get degraded with fine regulation to maintain a functional cellular concentration and to remove functionally defective transcripts. Moreover, some transcripts need trimming by the degradation machinery to gain their physiological activity. In eukaryotes, removal of the cap and poly-(A) tail is known to play key roles in initiating degradation of stable RNAs. In cytosol, translationally deficient transcripts due to premature stop codon are targeted by the nonsense mediated decay (NMD) pathway and passed on to exosome or Xrn1 for degradation from 3' and 5' end, respectively. While individual pathways for RNA degradation have been characterized, we lack an insight in the interplay between different factors and the differences in their specificities.

By transcriptome-wide mapping of protein-RNA interactions, we identified binding profiles for 30 RNA degradation factors involved in deadenylation, decapping, exosome, TRAMP complex, Ski complex, and NMD in S. cerevisiae. We show that different degradation complexes, and sometimes different subunits in these complexes regulate the degradation of different classes of RNA. We observed that Pop2, Not1, TRAMP4, and the exosome degrade antisense transcripts originating from bidirectional transcription together with Nrd1/Nab3. Moreover, degradation of other non-coding RNAs, as well as nuclear mRNA surveillance is mainly controlled by Ccr4/Not complex, Upf1, TRAMP5, Rrp6, and Csl4. For cytosolic transcripts, we showed that decapping and subsequent 5' degradation is the prominent degradation pathway terminating translationally inefficient mRNAs. Alternatively, mRNA can undergo deadenylation and exosome mediated 3' 5' degradation. In contrast to previous studies, we observed significant differences in transcripts processed by distinct deadenylation complexes.

Our results propose novel functions and interactions between RNA degradation factors. By mapping degradation factors on the yeast transcriptome, we provide a dataset of binding profiles that can serve as a resource for the advancement of our understanding of eukaryotic RNA degradation.

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To form a synaptic terminal, various components have to be targeted to specific sites in the axon. Presynaptic proteins are synthesized in the cell soma, packaged in vesicular cargos and transported by the motor complexes traveling along microtubules. During synapse development and maturation, the intracellular transport of proteins is highly regulated by mechanisms governing the interactions and movement of motor proteins. Additional specificity and regulation is provided by interaction of motor proteins with their cargos through adaptor and scaffolding proteins.

Previous studies have shown that FEZ1 acts as an adaptor for kinesin-1 and its binding to the motor is regulated by serine-58 phosphorylation. FEZ1/kinesin-1 complex partakes in presynapse formation by transporting syntaxin and Munc18 in the axon. Identification of numerous synaptic vesicle and active zone proteins in the FEZ1/kinesin-1 immunoisolated vesicles suggested the association and transport of these components with this complex. Although functional studies in Caenorhabditis elegans showed synaptic disorganization, the relevance of mammalian FEZ1 in neurons have not yet been investigated.

In this study, the mobility of FEZ1 and its phosphomutants were characterized in mammalian neurons. Furthermore, the role of FEZ1-mediated transport in formation of presynaptic and postsynaptic specializations was investigated by ablating the expression of FEZ1 using CRISPR/cas9 knockdown system. The data in this study show that mobility of neuronal FEZ1 is a result of precise modulation of its binding to kinesin-1 by serine-58 phosphorylation. This study also provides evidence for FEZ1 role in transport of active zone components and synaptic vesicle precursors, albeit in distinct mechanisms. Additionally, FEZ1 impacts neuronal development by regulating dendritic branching in young neurons, and dendritic spine formation during later stages.

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Alexander Rotsch

Effects of fusion tags on the ATP sensor ATeam

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It is known that ATP can be stored in secretory vesicles, as synaptic vesicles and chromaffin granules. A putative vesicular ATP transporter (VNUT) and a preliminary characterization of it have been presented. However, many questions about the loading of ATP into vesicles are still open, especially due to the limitation of the classical techniques of ATP concentration determiantion. Therefore, we attempted to stablish a new method to detect specifically intravesicular ATP in order to better characterize ATP transport into vesicles. For this, we took advantage of a FRET based ATP sensor (ATeam 1.03), consisting of a mse CFP with mVenus, each at either the N- or C terminus of a FoF1-ATP synthase subunit. In order to increase ATeam concentration inside vesicle, ATeam was fused to Tags, which can bind modified lipids or artificial membrane proteins. Furthermore, the effect of pH on the FRET signal was characterized to differentiate between signals based on ATP loading and signals based on acidification prior ATP loading.

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Wei-hsuan YU

Proteolytic EGFR fragments translocating into mitochondria involved in the EGFR axes modulate mitochondria proliferation

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EGFR pathway, involved in cancer cell migration, proliferation and survival drives a lots attentions of cancer biologist in searching the therapeutic targets. Tyrosine kinase inhibitor (TKI)-resistant small lung carcinoma cells and recurrent cancer stem cell sub population with EGFR mutations have been quite frustrated approaches by anti-EGFR based therapy. In the synthetic EGFR mutant axils enlightening Mitochondria-desRed small lung carcinoma CL1-0 cell line revealing an interesting findings well correlating the active EGFR or spontaneous active EGFR mutant T790M/L858R with high energy demanding status. In our EGFR axes-mitochondria synthetic cell based model, we first observe a phenomenon that EGF treatment enhances the amount of mitochondria per cell which is correlated to up regulation of MMP-7 expression. The MMP-7 mediated proteolytic processing substrates could potentially activate mitochondria proliferation. Not only EGFR can be cleaved by MMP-7 and release proteolytic fragments of EGFR translocated to mitochondriaand further can be transported into mitochondria and initiate mitochondria proliferation.

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Ekaterina Zhigalova

Anti-CTLA4 therapy polarizes B16 melanoma-infiltrating B lymphocytes towards the antibody-producing phenotype

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Therapeutic antibodies targeting CTLA-4 promote robust immune response against malignant melanoma in the fraction of patients. However, the mechanisms underlying the antitumor effect of CTLA-4 blockade are under debate. The main effects of aCTLA-4 therapy are intratumoral Treg depletion and cytotoxic and helper T cell activation. Other players of melanoma immune microenvironment expressing CTLA-4 are B lymphocytes. The function of CTLA-4 on B cells remains unclear, as well as the potential influence of aCTLA-4 therapy on intratumoral B-cell mediated immune response, which investigation was the aim of the study.

We used several approaches on the syngeneic C57Bl/6 mouse model of B16 melanoma. Firstly, we applied RNA-Seq of FACS-sorted CD19+ B cells to analyze the changes in the expression profile of intratumoral B lymphocytes in the response to aCTLA-4 therapy. Secondly, we performed RNA-Seq of the bulk tumors to determine how the treatment affects lymphoid structures formation. Finally, we sequenced immunoglobulin repertoires from bulk tumors and TCR repertoires from FACS-sorted melanoma-infiltrating FoxP3- CD4+ T cells.

CTLA-4 blockade enriched melanoma microenvironment with plasma cells producing large amounts of antibodies, particularly of IgA and IgG2 isotypes. On the contrast, the signatures of activated and germinal center B cell subsets were depleted in the treatment conditions. Interestingly, BCR signaling pathway, follicular helper T cell signatures, and local lymphoid structures formation processes were downregulated. The last findings are in concordance with the previous studies suggesting the negative loop between plasma cell enrichment and the suppression of germinal centers activity. Additionally, aCTLA4 treatment promotes the clonal expansion of intratumoral helper T lymphocytes that may indicate enhanced T cell activation via cross-presentation by plasma B cells. Thus, we have described the previously unknown effect of aCTLA-4 therapy on the B cell-mediated intratumoral immunity that lay a foundation for further studies.

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Ayman Ahmed

First detection and isolation of dengue serotype 2 in Kassala state, Sudanese-Eritrean borders

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Background: Dengue is one of the most rapidly spreading diseases around the world with a remarkably growing incidence and expanding distribution. Dengue is caused by one of four closely-related dengue virus serotypes (DENV1-4) belonging to the genus flavivirus and family Flaviviridae. The disease is characterized by most of its infections being asymptomatic and its symptoms ranging from a flu-like to a very severe form of dengue haemorrhagic fever with neurological involvement. In Sudan, dengue is a major public health problem in the Red Sea coastal region of Sudan, Port Sudan state. Recently, dengue emerged in Kassala, a border state with Eritrea.

Methods: A cross-sectional, hospital-based study was conducted in Kassala state, from October 2016-January 2017. One hundred and six blood samples were collected from patients admitted to Kassala Teaching Hospital. RT-PCR with specific primers for DENV1-4 were used for testing the samples for DENV and identifying the virus serotype. Following the Applied Biosystems standard protocol, the RT-PCR positive samples were prepared for sequencing using ABI 3730 genetic analyzer (ThermoFisher). DNA sequences were BLASTed, cleaned, and aligned with 61 DENV serotypes retrieved from the GenBank database. The MEGA 7 software was utilized for establishing the phylogenetic tree and molecular evolutionary analyses applying the maximum likelihood model.

Results: Out of 106 blood samples from febrile patients, DENV was detected in 4 (3.7%). DNA sequences of E/NS1 gene were successfully obtained from the four samples. The assigned GenBank accession numbers of the four sequences are MF574722, MF574723, MF574724, and MF574725. The BLAST search showed that all four DNA sequences belonged to DENV serotype 2; this was confirmed by phylogenetic analysis. The Phylogenetic tree for genotype analysis showed clearly that the studied sequences of serotype 2 belong to Cosmopolitan genotype. They correspond to the same genotype with strains from India, Pakistan, China and Jeddah-Saudi Arabia.

Conclusion: This is the first study to identify and report the circulation of the DENV-2 in the Kassala state in the Sudanese- Eritrean borders. This finding urges the need for genetic epidemiological study of dengue virus in the region.

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Aarthy Murali

Comparative analysis of the E7 oncoprotein in high and low risk type Human Papillomavirus: Structural Dynamics approach

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vIntrinsic disorder proteins (IDP) are common in numerous cancer associated proteins and involves in molecular signalling, regulation and recognition. It is found that the intrinsic disorder regions present in the viral protein are greater when compared with the human proteins. The intrinsic disorder is comparatively abundant in several diseases and plays a noteworthy role in oncogenesis. IDs are also identified in the human papillomaviruses which is a double stranded DNA viruses that causes diverse cancers. More than 160 types of HPV has been identified that are responsible for carcinomas, dysplasias, severe lesions and warts. HPVs have been progressed as the common sexually transmitted pathogens and can promulgate in several animal species including humans. Cervical cancer is the leading cause of cancer in women worldwide caused by HPV. These viruses has been classified into the high and low risk based on the efficiency to develop transformation or malignancy. Apparent results on the analysis of intrinsic disorder states that E6 and E7 proteins of high-risk HPVs contain increased amount of disordered region. The involvement of E7 protein in the cell cycle control depicts it to be the drug target. It is primarily located in the nucleus and associates with the gene product namely retinoblastoma and expedites the progression of S phase of the cell cycle. Expression of E7 oncoprotein stimulates cell proliferation and are responsible for the genomic instability of the infected cells. The MoRFs predicted for the HPV type 16 E7 states that ID regions are potential binding sites for other cellular proteins. Our study focuses on the intrinsic disorder analysis along with the MoRF prediction of the low risk types (HPV 6a, HPV 11, HPV 40, HPV 42, HPV 44, HPV 61) and high risk types (HPV 16, HPV 18, HPV 31, HPV 35, HPV 39, HPV 45) followed by the homology modelling and molecular dynamics simulation studies. The dynamic behaviour of the disordered region shows the variation throughout the simulation. These studies helps to understand the conformational stability and changes of the protein E7 of various types. The prediction of MoRFs could pave way for the disordered based drug targeting approaches. The analysis of the disordered regions and the prediction of the three dimensional structure and conformational stability of the structure contributes to the new avenues targeting the oncoprotein E7 of the low risk and high risk HPV for the treatment of HPV associated lesions and carcinomas.

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Fouzia Qamar

Isolation, Characterization and Identification of Beneficial Microbes from Different Sources and their Antimicrobial Potential against Pathogens

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Probiotics are reported to have antimutagenic, anticarcinogenic, hypocholestrolemic, antihypersensitive, antiosteoporosis and immunomodulatory effects. Most commonly used probiotics are Lactic Acid Bacteria (LAB). During the present study 10 strains of LAB were isolated from diverse sources. Strains were identified morphologically, physiologically and biochemically. To characterize the isolates at molecular level, 16SrRNA sequencing was carried out that helped to determine genetic variability among strain. The isolates were identified to be Lactobacillus fermentatum(3) , Weissella confusa (1),Lactobacillus pentosus (1),Lactobacillus lactis (1), Lactobacillus plantarum (1), Lactobacillus rhamnosus(1) and Leuconostoc mesenteroides (1) and Weissella cibaria. Strains were also tested for their resistance against antibiotics, thereby showing their ability to survive in the harsh environment of gastrointestinal tract. Antimicrobial potential of these strains were investigated against five target pathogens i.e B.subtilis, L.monocytogenes, S.aureus, E.coli and Klebsiella which will help explore bioprotective nature of the compounds exhibited by Lactic acid bacteria.

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Malgorzata Majewska

Characterisation of HMGR4 promoter from *Salvia miltiorrhiza*

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Salvia miltiorrhiza is one of the most commonly used traditional medicines in China. In vitro studies, animal models and clinical trials indicated that it may be a promising treatment for coronary heart disease, cerebrovascular disease, Alzheimer's disease, Parkinson's disease, neuropathic pain, diabetes mellitus, cancer, hepatocirrhosis, acute lung injury, renal injury, fibrosis, alcohol dependence. Secondary metabolites are considered to be responsible for the activity of S. miltiorrhiza.

The aim of the study is to investigate promoter region of 3-hydroxy-3-methylglutaryl coenzyme A reductase 4 gene (HMGR4) from S. miltiorrhiza. HMGR4 reductase plays an important role in the biosynthesis of mevalonic acid, the precursor of diterpenoid quinones.

At first, HMGR4 promoter was isolated using genome walking technique and for the first time sequenced. The sequence was then in silico analysed for the presence of TATA box, tandem repeats, CpG islands, target sites for miRNAs, cis-active elements and interacting transcription factors. Obtained results were verified by co-expression studies based on Arabidopsis thaliana microarray data. Moreover, the activity of HMGR4 promoter in response to abiotic factors is being evaluated using in vitro cultured transformed S. miltiorrhiza plants.

Obtained data related to the structure and activity of HMGR4 promoter are of importance for production of modified or synthetic promoters. As a result, greater concentrations of medically important secondary metabolites can be achieved.

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Damilola Dawodu

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In chronic kidney disease (CKD), hyperlipidemia is a common occurrence with total cholesterol and LDL cholesterol been the two independent risk factors for cardiovascular morbidity and mortality. There is evidence that LDL can be oxidized invivo to oxLDL leading to a signaling cascade that induces foam cell formation, inflammation and plaque formation in the vessel walls. Cardiovascular complications are also associated with an impaired mineral bone metabolism a term known as the CKD-mineral bone disorder (CKD-MBD) which could be due to a dysfunctional bone remodeling process. Studies have shown that oxLDL can disturb the delicate balance between bone forming cells and bone resorbing cells, however the mechanisms by which oxLDL mediates these effects are poorly explored. This present study aims at investigating the mechanism by which oxLDL interferes with the process of bone remodeling (osteoclastogenesis and osteoblastogenesis) in the advent of CKD.

Methods: The effects of oxLDL on bone remodeling and its downstream signaling were assessed by gene expression analysis, enzyme activity assay, immunoblotting, gelatin zymography, immunohistochemistry and confocal microscopy.

Results: We show that oxLDL inhibits bone remodeling. We demonstrate that oxLDL inhibits the process of osteoclastogenesis and the functional activities of osteoclasts by disabling the expression and more importantly, the secretion of CatK through autophagy regulation.

Conclusion & Significance: A great percentage of CKD patients are known to have a variety of bone disorders ranging from osteoporosis to osteopetrosis. Our research work shows for the first time, a possible mechanistic insight on the effect of oxLDL on both osteoclastogenesis and osteoblastogenesis suggesting that this compound by itself could play important roles in the progression of bone-mineral disorder in CKD patients.

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Shraddha Digole

Anticancer potential of Mycophenolic acid: *in vitro* and *in silico* approach.

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Mycophenolic acid (MPA) is a secondary metabolite produced by various Penicillium sp. It has various biological activities including immunosuppressive, anti-inflammatory, antiviral, antineoplastic, antipsoriasis, antiangiogenic and antitumor activity etc. Cancer is one of the most dreaded diseases of the 20th century and spreading further continuously and increasing incidence in 21st century. Breast cancer is the commonest form of malignancies in females and metastasis of breast cancer is common. In present work anticancer activities of MPA were evaluated. Effect of MPA on breast cancer cell line (MCF7) and colon cancer line (COLO-205) was studied. The cell viability was measured using SRB assay. Results showed that MPA has very mild cytotoxic effect on colon cancer cell line COLO-205. Whereas MPA showed cytotoxic effect on breast cancer cell line (MCF7) suggesting that it can be used as a potential anticancer molecule for breast cancer. In order to evaluate and predict probable mechanism of anticancer activity of MPA against human breast cancer cells, Ligand-protein molecular docking simulation was used to preliminarily investigate and to confirm the potential molecular targets for Mycophenolic acid. The study concluded that MPA has affinity for receptors like estrogen receptor α, estrogen receptor β and topoisomerase II; this can be a significant lead for further investigation of its potential. The results of molecular docking studies indicate efficient computational tools are capable of identifying potential targets for ligand such as Mycophenolic acid. In vitro and in silico studies revealed promising anticancer potential of Mycophenolic acid.

Keywords: Mycophenolic acid, anticancer activity, SRB assay, Molecular docking

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Jose Gabriel Garcia Hilario

Investigating the role of ING1b in regulating p16INK4a- and p14ARF-mediated pathways of neuronal senescence and apoptosis

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X-linked dystonia-parkinsonism (XDP) is a progressive neurodegenerative disease endemic to the Philippine island of Panay. Characterized by early-onset dystonia followed by parkinsonian symptoms, the disease has been stigmatized in the region for its overt physical manifestation. However, an influx in XDP research in recent years has revealed much more about the disease beyond its traditional reputation. The prevailing hypothesis for XDP pathogenesis is that an insertion of a SINE-VNTR-Alu (SVA) element within the TATA-Box Binding Protein Associated Factor I (TAF1) gene causes its dysregulation, leading to the loss of medium spiny neurons. However, the biphasic nature of XDP suggests that other mechanisms may play a role in its pathogenesis. In this study, XDP-associated dysregulation of the noncoding Inhibitor of Growth Family, X-linked (INGX) is hypothesized to regulate Inhibitor of Growth Family Member 1 isoform b (ING1b), leading to ING1b-mediated programs of cellular senescence and apoptosis. To test if INGX can regulate ING1b in a neuronal background, qRT-PCR of INGX and ING1b was performed in endogenous conditions as well as upon INGX overexpression in SH-SY5Y neuroblastoma cells. Results showed that INGX was differentially expressed across a serum concentration gradient, and that INGX overexpression led to changes in ING1b transcript levels. Moreover, to determine the consequences of ING1b derepression, ING1b was overexpressed in SH-SY5Y followed by qRT-PCR of p16INK4a and p14ARF, two downstream targets implicated in senescence and apoptosis, respectively. Altered levels of p16INK4a and p14ARF expression upon ING1b overexpression suggest that ING1b may regulate these targets. However, fine-tuning of ING1b overexpression must be performed to more definitively see its effects on p16INK4a and p14ARF transcript levels in a neuronal context. In addition, the effect of ING1b on p16INK4a expression was also analyzed through the generation of a p16INK4a promoter reporter luciferase construct. Overexpression of ING1b alongside the reporter showed consistent upregulation of p16INK4a, indicating that ING1b may positively regulate p16INK4a in a neuronal context. Taken together, these results contribute to the elucidation of a previously unexplored mechanism for XDP pathogenesis.

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Anjana Ghelani

Marine Metagenomics Based Strategy for the Cultivation of Rare Actinobacteria and their Exploration for the Multiple Valuable Metabolites

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Microorganisms play a crucial role in mediating global biogeochemical cycles in the marine environment. Since marine environments differ significantly from terrestrial habitats, the biological characteristics of marine actinomycetes and their distribution are expected to be different from those of soil actinomycetes and therefore, might produce different types of bioactive compounds. Unique features are usually exhibited by the marine actinobacteria to survive in harsh condition. However, the cultivation and detection of the rare marine actinomycetes are challenging task using traditional microbiological methods. Presently, the metagenomics based high-throughput environmental DNA sequencing has revealed the extensive microbial diversity and clarified the role of microbial communities. Shotgun metagenomic DNA sequencing is a relatively new and powerful environmental sequencing approach that provides insight into community biodiversity and function. In the present study, the coastal soil of western India was collect and whole isolated DNA was subjected to the shotgun sequencing using MiSeq Sequencer. Approximately 1 gb datasets was uploaded for the taxonomic and functional analysis using EBI metagenomics, MG-RAST, and One Codex online server. Comparative analysis using the reference metagenomes of the same habitats but different location has been performed using Megan and STAMP. The metagenome revealed the dominance of bacteria (98.50%) and Archaea (0.8%). Total 28 different bacterial phyla were reported among them Proteobacteria (48%) Firmicutes (16%), Bacteriodetes (14%), Fusobacteria (13%) and Actinobacteria (2%) were dominated. Total 1462 bacterial species were found and out of them, 172 actinomycetes species recorded in taxonomic profiling at the species level with the Nocardioides JS614 as a prominent species. Furthermore, 29 different species of Streptomycetes were also reported. Functional analysis using subsystem level 1 indicated 2% stress response genes. In subsystem level 3 analyses revealed the various valuable traits i.e. presence of the resistance to antibiotics and toxic compounds, extracellular polysaccharides, iron acquisition and metabolisms, metabolisms of aromatic compounds, secondary metabolisms. Due to reported ample diversity of actinobacteria, cultivable diversity approaches was focused for isolation of Actinomycetes in the present study. More than 50 different bacterial strains were isolated and 6 actinomycetes i.e. Streptomyces violascens, Nocardiopsis dassonvillei subsp. Albirubida, Streptomyces albus, Streptomyces somaliensis, Streptomyces longissimus, and Nocardiopsis lucentensis were identified based on the 16S-rRNA gene sequencing. All the strains were explored for the multiple biotechnological vital traits including 8 plant growth promoting features, 2 pesticide degradation, 6 industrial enzymes, 2 compatible solutes, CO2 sequestration, and AntiMRSA Activity. So, the exploration of marine microbial diversity with a combination of cultivable and uncultivable approach from Dandi coastal region of India suggest the inputs of potent actinomycetes of marine habitats for maintaining ecological balance in the marine environment and bright application prospectus in agriculture biotechnology, environmental and industrial biotechnology.

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Pravin Dudhagara

Reconstruction of Pseudomonas mendocina Genomes from Metagenomic Shotgun Nanopore Reads of Alkaline Hot Spring of India using Binning-Assembly Approach

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Multiple challenges in the cultivation of microorganisms have restricted the phylogenetic diversity of microbial genomes. However, It is being resolved by next generation sequencing technologies and computational techniques that allow for the cultivation-independent sequencing and recovery of genomes from metagenomes. Here, we provided a synthesis of available methods to bin metagenomic contigs into species-level groups and reconstructed the of metagenome-assembled Pseudomonas mendocina genomes of two strains. This specie was found dominated in the metagenome with 9.44% classified reads using RefSeq complete genome database. The water sample was subjected to the metageomic DNA isolation and sequencing using MinION Nanopore platform. The nearly 1gb dataset was analyzed using MG-RAST, One Codex and EBI Metagenomics to decode the taxonomic and functional diversity of the alkaline hot spring of Unapdev, India. The metagenomic reads were binned using Markov chain approach (MBMC) and annotation was done using RAST server. Two strains genome- P. mendocina 1 and P. mendocina 2 were constructed with the size of 6.08 and 4.97 million bp respectively. Subsystem feature analysis suggests the presence of the plenty of stress response genes and genes encoded the metabolisms of aromatic compounds in the genome of both strains. More than 70% complete draft genome sequences could be reconstructed for both the strains. Closest neighbor of both strains was P. mendocina ymp strain. The comparative metabolic reconstruction with closest P. mendocina ymp strains reveals various new metabolic genes applicable for the biotechnological industries. The protein and metabolite profiling was done using FIGfams and KEGG to decode the pathways and the proteins responsible for the primary and secondary metabolites synthesis. To the best of our knowledge, this is the first study to extract the P. mendocina genomes from the alkaline hot spring using metagenomics-assemblies. The study provides useful information on binning and assembly of the Unapdev hot spring metagenome for P. mendocina and possibilities to extract the more draft genomes from the metagenomes.

Keywords: Metagenomics, Binning-Assembly Approach, Pseudomonas mendocina

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Malaria is major killer of human worldwide and strongest known force for evolutionary selection in the recent history of human genome. One of the most important human virulent malaria parasite, Plasmodium falciparum that infect human red blood cells. Malaria is transmitted by inoculation of sporozoites during the bite of an infected female Anopheles mosquito. P. falciparum the overall goal is reproduction, and thus biomass production on the metabolic level. At the same time the parasite has an interest to keep its host cell alive for the period of replication which sets an upper limit on the replication rate. The use of antimalarial drugs is an important component of malaria control programme. These drugs class broadly into quinolones, artemisinins, antifolates and antibiotics. Drug resistant malaria has become one of the most important problem in malarial control in recent years. The development of new antimalarials and improvement of existing ones is therefore crucial to reduction of the increasing disease burden and economic loss due to malaria. Our data uses in silico molecular modeling methods, such as docking, SAR/QSAR model building, statistical analysis that can aid the drug discovery process by ascertaining the binding affinities of existing and hypothetical compounds towards naphthoquinone and also elucidate the origin behind the observed inhibition. A combined experimental and computational effort may counterbalance the antimalarials and provide an enhanced avenue for inhibitor development. The mathematical model developed can be further used to develop and synthesize novel naphthoquinone analogs and validation of the same can be done by testing the molecule on microbial assay.

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Mariia Usenko

Production of IL7-fusion recombinant proteins and their application for affinity chromatography resin creation

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Interleukin-7 (IL-7) is a type I glycoprotein produced primarily by stromal cells and exerts its effects through a receptor complex consisting of IL-7 R alpha and common gamma-chain/ IL-2 R gamma. Investigations IL-7 and IL-7R have shown that their levels vary in different diseases such as viral infections (HIV, CMV, HCV), multiple sclerosis, Type 1 diabetes and others. Monitoring of IL-7R concentrations appears to play an important prognostic value. IL-7-His can be a perspective component of test-systems for IL-7R detection. Moreover, IL-7 genetically fused with cellulose-binding domain (CBD – a component of the cellulase complex of Clostridium cellulovorans) gives an opportunity to bind IL-7-CBD to cellulose sorbent, thus developing rather cheap and convenient test-system. Also IL-7-His and IL-7-CBD can be used as ligands for purification of polyclonal antibodies specific to IL-7. The aim of the study was to obtain IL-7-His and IL-7-CBD fusion proteins in Escherichia coli, to prove functionality of both molecules and to receive stable immunosorbents with them.

The DNA sequences encoding human IL-7 was subcloned into pET24a(+) expression vector under control of T7 promoter and upstream of the vector-derived 6xHis-tag. For obtaining IL-7-CBD fusion corresponding DNA sequences were genetically fused and also subcloned into pET24a(+). pET24-IL7-His and pET24-IL7-CBD were transformed into E. coli, protein synthesis was induced with IPTG and with auto-induction protocol. Use of the auto-induction protocol provided significantly higher protein yield as compared to IPTG induction. Proteins of interest were obtained in the form of inclusion bodies. Immobilized-metal affinity chromatography was used for purification of solubilized proteins with subsequent renaturation of IL-7-His. In the case of IL-7-CBD, refolding of purified solubilized protein was developed using modifications of dialysis strategies. Functions of both proteins were tested in ELISA. Additionally, IL-7-CBD was immobilized on the microcrystalline cellulose CC31 using high affinity of CBD to the sorbent, thus proving the bifunctional activity of fusion protein. Purification and immobilization of IL-7-CBD were essentially one step, thus significantly reducing the cost of production. Resulted affinity mediums – IL-7-His on the Ni2+-sepharose and IL-7-CBD on the microcrystalline cellulose – was successfully used for purification of polyclonal antibodies.

Fully functional IL-7-His and IL-7-CBD fusion proteins were obtained and applied for purification of high specific polyclonal antibodies. IL-7-His, IL-7-CBD fusion protein and purified polyclonal antibodies can be used in capture sandwich immunoassays. In addition, our results may potentially contribute to the development of diagnostic tools for IL-7R detection.

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Chaimaa Moundir

Association between IL-17 and MIF polymorphisms and the risk of Gastrointestinal cancer in a Moroccan population

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Macrophage migration inhibitory factor (MIF) is a versatile cytokine that mediates both innate and adaptive immunity and plays a role in Chronic inflammation that is the hallmark of the pathogenesis of Helicobacter pylori-induced gastric cancer. Interleukin IL-17 are inflammatory cytokines expressed by a novel subset of CD4+ Th cells and play critical function in inflammation and probably in cancer.

We conducted a study to investigate the role of common SNPs in the IL-17A and the macrophage migration inhibitory MIF (rs2275913G>A and rs755622 G>C) in the development of Gastrointestinal cancer in Moroccan populations, and their interaction with H.pylori infection.

A total of 120 patients with Gastrointestinal and 120 control subjects were consecutively recruited between March 2017 and April 2018. Genotyping of IL-17A and MIF was performed by real-time polymerase chain reaction (RT-PCR) using specific TaqMan predesigned probes. Logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals (CI).

We found that an increased Gastrointestinal risk was independently associated to IL-17A (rs2275913G>A), the adjusted Ors (95% CI) were 1.47 (1.04-2.07) for GA genotype and 2.58 (1.52-4.44) for AA genotype. We observed that the GA+AA genotype of rs2275913 was associated with an increased risk of Gastrointestinal among H.pylori infection subjects (OR=2.22, 95% CI=1.30-3.80).

In conclusion, we found that there was a significant association between L-17A rs-2275913G>A polymorphism and increased Gastrointestinal risk, especially in H.pylori infection subjects.

Keywords: IL-17, polymorphism, MIF, H.pylori infection, Gastrointestinal

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Kaoutar Idouz

ACE I/D polymorphism in Moroccan children with hypertrophic and dilated cardiomyopathies.

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Cardiovascular diseases are the principal threat to global health worldwide, including North Africa. However, the pediatric cardiomyopathies are an uncommon and heterogeneous group. The renin-angiotensin-aldosterone system (RAAS) plays a critical role in regulation of normal cardiovascular function, yet the influence of RAAS in the pediatric population is unknown. Angiotensin-converting enzyme (ACE) is an exopeptidase that catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. ACE is also involved in the inactivation of bradykinin, a potent vasodilator. These mechanisms highlight the importance of angiotensin-converting enzyme ACE in cardiovascular pathophysiology and motivate the exploration of the genetic variants associated with cardiovascular disease including cardiomyopathies. A common variant in ACE gene (The I/D polymorphism: insertion (I) or deletion (D) of a 287 bp fragment (Alu repeat) in intron 16 was shown in previous studies to be associated with a modifying effect on cardiomyopathy phenotypes in adults. Therefore, we investigated whether the ACE I/D polymorphism influences cardiomyopathies risk among Moroccan patients. To the best of our knowledge, this is the first pediatric population based study in North Africa.

The present study included 40 clinically diagnosed patients (30 dilated and 10 dilated) and 30 healthy controls. DNA samples were genotyped for the presence of ACE gene polymorphism by the PCR method. Genotypes were defined as DD, II and ID according to the presence of the D (deletion) and I (insertion) alleles. ACE genotype frequency in cases (ID -61,11 %, DD- 30,56 %, II- 8,33 %) has a total frequency in cases D < I. Statistical analyzes were performed using SPSS software.

Key words: Dilated cardiomyopathy, Hypertrophic cardiomyopathy, ACE, RAAS, Pediatric cardiomyopathies.

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Dounia Chraa

Anti-tumor immune response status analyzed through differential gene expression in patients with breast cancer.

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Background: Women with breast cancer appear to exhibit distinct expression levels of immune genes. In particular, cytokines from TH1, TH2 and TH17 subpopulations within tumor microenvironment seem to be closely related to disease prognosis. Gene expression profiling of cancerous tissues revealed that many of these immune signatures are related to propitious outcomes. This indicates that some of the patients might benefit from immunotherapy through targeting of pro-tumorigenic candidates, allowing efficient anti-tumor immune response to take place.

Methods: Tumor tissues and uninvaded control regions were collected from 96 women with breast cancer. At this point, total RNA was extracted from 32 tumor and healthy tissues using TRizol protocol and cDNA were synthetized using reverse transcriptase. The expression of IL17A gene was evaluated using real time polymerase chain reaction (qRT-PCR) using Syber Green PCR Master Mix. We further evaluated the effect of recombinant IL17A on proliferation of breast cancer cell lines, MCF7, MDA-MB-231 and T47D, in vitro using Alamar blue Assay.

Concomitantly, we asked whether IL-17A receptor blockade with anti-IL-17A receptor neutralizing antibody would suppress the IL-17A-triggered proliferation of different breast cancer cell lines. Further tests of the blockade of IL-17A with a generated anti-IL17A are being carried out, in order to detect the effect of the monoclonal anti-body on the proliferation of breast cancer cell lines.

Results: Our preliminary data suggest that IL17A transcripts exhibit significantly higher expression in tumor tissues compared to healthy controls. Clinical parameters such as histological type of the tumor, the stage and the age of the patients seem to be tightly linked to the expression of IL17A. Interestingly, a more thorough analysis showed that the IL17A expression is inversely associated with estrogen and progesterone receptor expression in patients. Similarly, we attempted to unravel the oncogenic properties of IL17A in breast cancer. We therefore tested whether IL17A could promote proliferation of breast cancer cell lines. As expected, our results suggest that IL17 indeed significantly promotes the proliferation of these cell lines, in a dose dependent manner, and its blockade lead to the diminution of breast cancer cell proliferation compared to the control.

Conclusion: Taken together, our data suggest that IL17A could be considered as a potential therapeutic target at least for some breast cancer patients.

Key words: Breast cancer, TH17, gene expression, proliferation, IL17A, anti-IL17A.

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Olha Krynina

Role of the sHB-EGF heparing-binding domain in EGFR intracellular trafficking.

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Heparin-binding EGF-like growth factor (HB-EGF) is a member of EGF-like growth factors family. It is synthesized in as a transmembrane precursor (proHB-EGF) that can be proteolytically cleaved by metalloproteinases with forming of a soluble HB-EGF (sHB-EGF). Both forms of HB-EGF can activate and promote clathrin-dependent endocytosis of ErbB receptor class 1 (Epidermal growth factor receptor, EGFR) and class 4. Downstream EGFR trafficking after HB-EGF stimulation can be ended with lysosomal degradation of a receptor or its nuclear localization.

The main goal of this research is to reveal a role of the heparin-binding domain (HBD) of HB-EGF in a process of its binding to receptors and trafficking to the cytoplasm.

To determine how the absence of HBD in the structure of HB-EGF affects its receptor binding and intracellular transport dynamics we use fluorescently labeled recombinant derivatives: EGFP-sHB-EGF and EGFP-sHB-EGFΔ84-106 with the HBD deletion. Firstly, we perform the saturation ligand binding assay with A431 cell line. According to the flow cytometry data, the fluorescence intensity of cells stained with full-size sHB-EGF is twice as high as the fluorescence intensity of cells stained with the HBD-deficient form of sHB-EGF. This difference is caused by binding both EGFR receptors via an EGF-like domain, as well as to cell membrane HSPGs. Next, we investigate internalization dynamics of two forms of growth factor using confocal microscopy. In more detail, the process of endocytosis at each interval of time was characterized by quantitative parameters that were obtained after confocal images processing with Fiji. It was shown, that endocytic trafficking can be observed following 15 min of internalization for both forms of sHB-EGF and increased with incubation time passing. However, the process of endocytosis is more intense for the HBD-deficient form of sHB-EGF comparing to the full-size form. The average endosomal size formed after EGFP-sHB-EGFΔ84-106 or EGFP-sHB-EGF EGFR stimulation almost was not differ. Also, we examine the trafficking route of full-size and truncated forms of sHB-EGF by comparing their colocalization to a lysosome (LAMP1) and endosomal pathway (Rab5, Rab7a, Rab11a) markers.

The key finding is that HB-EGF interaction with HPSGs on a cell surface can facilitate the more prolonged formation of ligand-receptor complex and leads to later its internalization. Thus, differences in the trafficking dynamics of full-size or truncated forms of sHB-EGF in the cytoplasm cells, as well as the endosome colocalization pattern, may reflect the mechanisms of biological activity of sHB-EGF, as well as its dependence on the specificity of an extracellular matrix organization.

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Tetiana Borikun

miRNA profiling: from in silico target prediction to practical usage for breast cancer prognosis

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Exploration of the possibilities of circulating miRNA usage in the clinic is one of the newest trends in modern science. Our research started in silico searching for the most suitable miRNA to predict the drug resistance development in breast cancer (BC) cells. As the result, we made a list of miRNAs, that are proven to be involved in regulation of proliferation, migration, drug resistance etc. that counted 24 miRNAs. Next step was their validation on in vitro and in vivo systems to find out their relevance with breast cancer features, including changes during treatment with cytostatics and tumor growth. After in vitro experiments we shortened the list to the 10 miRNAs, which expression changed extremely and explored their expression in serum and tumor tissue from Walker-256 carcinosarcoma bearing rats. The most significant changes were found for miRNA-205, -21, -182 and -155 between sensitive and doxorubicin and cisplatin-resistant tumor strains, as well as during tumor growth. The third part of our research was to define the significance of miRNA-205, -21, -182 and -155 expression both in tumor tissue and serum obtained from BC patients from Ukrainian population. Our results proved the involvement of miRNA-205 and miRNA-21 in TNBC subtype formation, as well as the development of resistance to anthracycline- and platinum-based neo- and adjuvant therapy. Also, we established the prognostic role of miRNA—205 and -182 in breast cancer course and validated their prognostic significance to monitor the therapy effectiveness.

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Viktoriia Yavorovska

Tetrahydroxythiacalix[4]arene-tetraphosphonate C-800 remove inhibitory effects of Pb and Cd cations on myosin ATPase activity and reduce proliferation of the cultured rat uterine smooth muscle cells induced by this heavy metals

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Nowadays, the problem of the environmental pollution with heavy metals is very considerable. Therefore, searching for ways to reduce their impact on living objects is relevant. Pb2+ and Cd2+ are the most common pollutants of the environment among heavy metal cations abundantly used in industry. These metals are very deleterious with considerable ability to accumulate in the tissues of living organisms including the uterus. Pb and Cd cations are associated with many disorders of the female reproductive system. These heavy metals can cause the impairment of the contractile function of uterine smooth muscle that can lead to pathologies during pregnancy and childbirth. The uterine contraction is associated with the activity of the actomyosin complex containing myosin and actin. The catalytic domain of myosin subfragment-1 hydrolyzes ATP transforming chemical energy of macroergic bonds into mechanical movement.

Therefore, it is important to develop chemical matters that can eliminate harmful effects of these heavy metals. One of such promising compounds is tetrahydroxythiacalix[4]arene-tetraphosphonate C-800. This thiacalix[4]arene has hydroxyl groups and divalent sulfur atoms on the lower rim of macrocycle that can chelate transition and heavy metals to form metal complexes. The phosphonate groups of C-800 whereas improve its solubility.

It was shown the inhibitory effect of heavy metal cations Pb2++and Cd2+ on ATPase activity of uterine smooth muscle myosin subfragment-1 at the concentration ranging from 30 to 300 μM for Pb2+and Cd2+. Inhibitory impact for Pb2+ and Cd2+ at concentration 30 μM was approximately 25% and 20% compared to a control, respectively. In particular, the inhibitory effect of Pb2+ and Cd2+was the most notable at the 300 μM concentration (on average 88% and 56%, respectively) in comparison with control ATPase activity. The values of an inhibition coefficient I0.5 for these cations were 0.08+0.01 mM for Pb2+and 0.30 +0.03 mM for Cd2+

It was shown that 100 μM C-800 restored myosin subfragment-1 ATPase activity to the control level in the presence of 0.1 mM Pb2+ and Cd2+. Also, we studied using MTT assay the effect of Pb2+ and Cd2+ on the viability of the rat uterine smooth muscle cell in the culture at concentrations ranging from 2,5 mM to 160 μM. It was found that Pb and Cd cations facilitated smooth muscle cells viability and increased their proliferation rate twice as much compared with control ones. The most prominent effect of Pb2+ and Cd2+ on the uterine myocytes viability was established at 2,5 mM and 1,25 mM concentrations, correspondingly. While 100 μM C-800 removed the activating effect of heavy metals on the growth of cultured myometrium cells to control level.

The demonstrated results can be used for further research aimed to use C-800 as chelation agent that can prevent impairment of myometrium contraction caused by Pb2+ and Cd2+ and the accumulation of these heavy metals in the organism.

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Yelyzaveta Snihirova

Functionalized benzothiazole styryl cyanine dyes for fluorescent detection of nucleic acids

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Fluorescent dyes for the nucleic acids (NA) detection are widely used in molecular biology, i.e. for gel electrophoresis, real-time PCR, sequencing, chromatography, chromosome staining, cell organelles visualization [1]. For one of the most widespread routine techniques, gel electrophoresis, DNA is commonly visualized by well-known low-cost dye ethidium bromide (EtBr). Its main disadvantage is high toxicity as a mutagen. The fluorescent dyes belonging to styryl cyanines have ability of effective NA sensing, earlier their low toxicity was shown [2].

Here, we characterized a series of novel benzothiazole styryl cyanines with different N-alkyl-aryl tail groups as probes for NA detection by fluorescent and absorption spectroscopy. The efficiency of the dyes for post-electrophoretic visualization was investigated in comparison with EtBr. The photostability of the styryl cyanines was studied upon continuous irradiation with LED lamp (470 nm).

In-solution the studied styryl cyanines are weakly fluorescent (6-35 a.u.) and they noticeably increase emission intensity upon the binding to dsDNA/RNA (in 14-78 times). The dyes in complex with dsDNA have moderate quantum yields (up to 25 %). The VIS excitation and (524-544 nm) and emission maxima (591-601 nm) of the dyes are close to that of EtBr (em. 590 nm).

The dyes applicability for post gel electrophoresis DNA staining, i.e. visualizing of DNA fragments in the range of 50-1031 bp under UV-transilluminator (312 nm) was shown. The staining efficiency was determined by the nature of N-alkyl tail group, the higher DNA discernibility by the dye with N-alkyl-dipuridyl group at low DNA concentration (4 ng per lane) in comparison with EtBr was observed. The dyes do not demonstrate bleaching after repeating UV-irradiation during ~30 min, which points on their sufficient photostability.

The photostability of the dyes upon continuous irradiation with LED lamp (470 nm) was evaluated by control of VIS-absorbance at (0 min and 30, 90, 150 min). The dyes significantly enhance the photostability (up to 31.8 %) upon the binding to DNA. The better photostability of dyes in the DNA presence could be explained by the intercalation of this dye in the DNA macromolecule, which causes the "isolation" of the dye molecule against external influences and the decreasing of contacts between the dye molecules and surrounding molecules of oxygen [3]. Besides, the nature of N-alkyl substituent could determine efficiency of sensing of dye to NA type: the higher preference for RNA (53 times emission increase) over dsDNA (14 times increase) was possessed by the dye with N-alkyl-phenantroline group.

The efficiency of the functionalized styryl cyanines for post gel electrophoresis DNA visualization and their sufficient photostability were shown. This cause an interest in further characterization of styryl cyanines as low-cost dyes for routine techniques of DNA detection.

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Andrei Smid

Investigating the pre-TCR complex and its intracellular trafficking – clues for lineage commitment in T-cell development

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Cell signalling is a fundamental mechanism in biology, underpinning complex processes such as development, immunity or tissue repair. At the same time, defects in signal transduction or processing often lead to diseases such as cancer or autoimmunity. T-cells, a member of the lymphocyte family, are crucial cells of the adaptive immune system which rely on signalling through the αβ-T-cell receptor (TCR) to identify and kill virus-infected or cancer cells. Aside from cytotoxic functions, signalling through the TCR also drives the development of T-cells. During development, precursor T-cells, called thymocytes, express a different form of the TCR composed of a newly V(D)J-recombined β-chain and the invariant pre-Tα chain. Despite being different to the mature αβ-TCR, the precursor TCR (pre-TCR) uses the same signalling relays as the mature receptor, generating the same input signal. Surprisingly, there is evidence to suggest that thymocytes can differentiate between signals coming from the pre-TCR or from the mature TCR. Moreover, making this distinction is crucial, as it determines which T-cell lineage the thymocytes progress towards, while aberrations in signal processing can lead to the development of T-cell lymphoblastic leukaemia. However, the molecular mechanisms which enable thymocytes to differentiate between these signals are poorly understood, mainly due to the difficulty of studying thymocytes in vitro. In this study, we overcome this challenge by expressing the pre-TCR in immortalised cell lines to more easily investigate its expression and intracellular trafficking. Using tools from molecular biology, cell biological assays and microscopy, we show that the pre-TCR is mainly retained inside the cell, while a fraction travels to the cell surface. Upon reaching the cell surface, the receptor is actively internalised through an unknown mechanism. We also show that the intracellular localisation of the pre-TCR is determined by the structure of its extracellular domain. These findings point towards the existence of a yet uncharacterised cell surface protein quality control machinery specific for proteins without a cytoplasmic domain. Our observations also give us clues as to how thymocytes may regulate pre-TCR signalling to generate a distinct signal.

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Ivan Sorokin

Ribosomes and Polyribosomes imaging via super-resolution microscopy

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Polyribosomes (or polysomes) are formed in the process of protein synthesis. Their existence is essential for eukaryotic cells. Several specific modes of spatial organization of polyribosomes have been found already in early electron microscopy studies. Latest cryo electron tomography (cryo-ET) analyses corroborate the existence of circular, zigzag and helical conformations of polyribosomes. However, EM data give us information only about structural aspects of polyribosomes being. In its turn, functional information may be obtained only from experiments without structural data. In order to obtain structural and functional data at once, we have implemented imaging of ribosomes and polyribosomes with super-resolution microscopy.

Our experiments revealed the ribosomes and polyribosomes at the nanometer scale. The achieved spatial resolution has allowed us determination of each ribosome position in single polysome. Ribosomes and polysomes were visualized by an organic cyanine dyes and also by a photoactivatable fluorescent proteins (PAtagRFP). PALM/STORM method were used.

Our investigation demonstrates possibility to study translational apparatus (emerging structural and functional data) via super-resolution fluorescence microscopy. Our works are the first step to approach of translational apparatus studying with the highest spatiotemporal resolution and determination of the functional origination of each ribosome, ribosome`s subunit, or even translational factor.

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Xavier Eugenio Asuncion

ConoTarget: a tool for predicting conopeptide targets using molecular descriptors and machine learning

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Venomics-based bioprospecting, which integrates genomic, transcriptomic and proteomic data, has emerged as a promising tool for drug discovery and serves as a powerful complement to bioassay-guided fractionation strategy. Advances in sequencing technologies have allowed venomics research to hasten the drug discovery process through large-scale identification of novel venom components. However, to date, identifying the molecular target of venom peptides is still a major challenge due to the demanding nature of laboratory experiments and limited bioinformatics tools that can provide direct information on the possible function of venom peptides. With the development of publicly available databases that combine sequence and experimental data, primary sequence can be a rich source of valuable information that can be utilized to predict the potential target of an uncharacterized venom peptide. In this work, we used a straightforward approach to develop a tool, called ConoTarget, for predicting five biomedically important targets of Conus venom peptides (conopeptides) using machine learning and sequence-derived features, generally called molecular descriptors, that capture both structural and physicochemical properties of a sequence. Our model is trained on a high-quality benchmark dataset using a combined Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) approach. Results based on rigorous cross-validation show that our approach reaches an overall accuracy of 92.5%, which is highly comparable to previously reported methods. The model will be useful as a preliminary analysis tool to perform high-throughput functional annotation of newly sequenced conopeptides. Currently, we are developing a web server for the predictor that can be easily and freely accessed by other researchers in the future.

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Zahra Shojaei Jeshvaghani

In silico and experimental evaluation of hsv1-mir-H2 effect on MAPK1 and SMAD2

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During its latency in neural ganglia, HSV-1 produces a primary miRNA precursor, (LAT), encoding six distinct miRNAs. these miRNAs not only regulate viral gene expression but also have the potential to regulate the expression of host cell genes. In silico and empirical investigations have shown that LAT has a regulatory effect on TGF-β pathway which is a popular drug target in disease states.

Using TargetScan which is an established miRNA prediction software, we decided to find a cellular target involved in TGF-β pathway for one of these six miRNAs. We chose hsv1-mir-H2 due to its high efficiency in loading into RISC, then we examined whether the miRNA could target the candidate proteins in practical.

Considering the miRNA-targets interactions information produced by TargetScan, MAPK1 and SMAD2 were selected as targets. After cloning of mir-H2 into pCDH-CMV-MCS-EF1-cGFP-puro, the miRNA-expressing construct, and the backbone plasmid, as negative control, were transfected individually into LX-2 cells (a highly transfectable human hepatic stellate cell line). After 48 hours, the total RNA from the H2-transfected cells and control groups was extracted . the cDNAs encoding mir-H2, snord47 (as internal control), and total cDNA (using Random Hexamer primer) were synthesized. The cDNAs were used as templates for qRT-PCR amplification. Using qRT-PCR, mir-H2 overexpression in treated cells was confirmed, then relative expression of MAPK1 and SMAD2 at mRNA level, were measured in both treated and control cells. In addition, luciferase assay was performed to confirm the results gained from qRT-PCR.

The experimental results show that contrary to in silico predictions, mir-H2 can't make any significant difference in the expression level of SMAD2 and MAPK1 as TGF-β related genes. The results presented herein indicate genes cannot be always predicted correctly by online software tools, and experimental verification tests are essential to confirm the outcomes.

Keywords: Herpes simplex virus 1 (HSV-1), Mitogen-activated protein kinase 1(MAPK1), Mothers against decapentaplegic homolog 2 (SMAD2), Latency associated transcript (LAT), Transforming growth factor beta (TGF-β), RNA-induced silencing complex(RISC)

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Mariano Martín

Uncovering the Mechanisms Underlying Na+/I- Symporter (NIS) Transport to the Plasma Membrane

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The Na+/I- symporter (NIS), a 643 amino acid-long glycoprotein expressed at the basolateral plasma membrane of thyroid follicular cells, mediates iodide accumulation for thyroid hormonogenesis. Underscoring the significance of NIS for thyroid physiology, loss-of-function mutations in NIS-coding SLC5A5 gene cause iodide transport defect (ITD) resulting in dyshormonogenic congenital hypothyroidism. Moreover, NIS-mediated iodide accumulation constitutes the cornerstone for radioiodide ablation therapy for differentiated thyroid carcinomas. Thyroid tumors often exhibit reduced iodide transport compared to normal thyroid tissue. Surprisingly, NIS expression is frequently increased although mainly intracellularly retained, suggesting the presence of post-translational abnormalities in the transport of the protein to the plasma membrane.

Regarding structural determinants that control NIS transport to the plasma membrane, new avenues were opened after the functional characterization of the ITD-causing NIS mutant S509RfsX6 lacking the last transmembrane segment and the carboxy-terminus of the protein. S509RfsX6 NIS is fully intracellularly retained, thus suggesting that the cytosol-facing carboxy-terminus may contain crucial information for proper NIS transport to the plasma membrane. Moreover, in silico computational analysis of NIS carboxy-terminus revealed several putative short linear motifs involved in plasma membrane proteins trafficking. Therefore, we generated partial deletion mutants of the carboxy-terminus and site-directed mutants of putative sorting motifs. Functional evaluation revealed that the NIS mutants Δ546-578 and W565A/D566A are intracellularly retained in non-polarized MDCK cells. The NIS mutants Δ578-583 and I582A/L583A, disrupting a diacidic-monoleucine motif, are transported to the apical membrane in polarized MDCK cells. Moreover, the NIS mutant missing a putative PDZ binding-motif (TNL643)—dispensable for NIS sorting to the plasma membrane in non-polarized MDCK cells—is intracellularly retained in polarized MDCK cells.

Significantly, here, we report the identification of the ITD-causing NIS pathogenic variants G561E and L562M—located in the carboxy-terminus of the protein—in two pediatric patients with dyshormonogenic congenital hypothyroidism. Functional studies in non-polarized MDCK cells revealed that G561E and L562M NIS are partially and fully retained in the endoplasmic reticulum, respectively. Moreover, bioinformatics and biochemical analysis indicates that these mutants disrupt the recognition of the adjacent tryptophan-acidic motif W565D566 by the kinesin light chain 2, thus impairing mutant NIS exit from the endoplasmic reticulum and subsequent transport to the plasma membrane. Of note, comparison of SLC5A5 gene sequence across different species indicates a full conservation of the kinesin light chain 2-recognized tryptophan-acid domain.

Although the molecular mechanisms that determine NIS intracellular retention in thyroid cancer cells remain elusive, here, we provide evidence regarding the importance of short linear motifs for NIS functional cell surface expression under physiological conditions. A thorough comprehension of the mechanisms that regulate NIS transport to the plasma membrane would have multiple implications for radioiodide therapy, opening the possibility to identify new molecular targets to treat radioiodide-refractory thyroid tumors.

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Aysha Kamran

Carbonate biomineralization process for the preservation of organic solids

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Biomineralization has a wide application in the removal and recovery of various metals under the shadow of environmental biotechnology. Biologically induced mineralization also triggered the preservation of organic compounds in specific strata. Microbial communities and activities nurtured under this specific conditions, however, is not well understood. One enigmatic biogenic process is the formation of carbonate concretions in association with fatty acids (FA, cf. Thiel and Hoppert 2018). FA may act as "scavengers" for calcium/iron ions in an initial stage of concretion formation and then slowly release calcium during long time periods. This may be activated, in shallow marine sediments, by microbial degradation of FA. In order to contribute to the further understanding the role of biominerals such as siderite (FeCO3) in this process, we prepared mesocosms with marine sediments, artificial seawater and various FA, lipids or lipid-like substrates. Mineral precipitates were observed and analyzed by (scanning) transmission electron microscopy (STEM). Fluorescence in situ hybridization (FISH) analysis was performed with specific bacterial probes. Mesocosms with Span 80 and 20 (sorbitan monolaurate) as substrates exhibited more than 50% of retrieved ribosomal RNA sequences related to the sulfate reducer Desulfofrigus. Electron microscopy revealed nanoparticles decorating the cell periphery of the bacterial morphotype. Elemental analysis of these nanoparticles by STEM shown the presence of Fe, S, C and Ca in the sample. FISH confirmed the presence of specific bacterial species (SRB). These findings point to the relevance of lipid substrate for initial biominerals formation by sulfate reducers. SRB may have influence on the nucleation and growth of iron sulfide or iron carbonate such as siderite (FeCO3) under specific environmental conditions. In future, we will further investigate the mineralogical characteristics and interaction of microorganisms within the sphere of siderite.

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Viktor Dmytryk

Plasma levels of heat shock proteins in bladder cancer patients depends on the tumor stages

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Urinary bladder cancer (UBC) is a common disease worldwide with high mortality rate. Heat shock proteins (Hsps) are members of a number of families of stress-induced proteins, whose main intracellular functions are as molecular chaperones. Hsps are potent inducers of an antigen-specific immunological response. Hsps are capable of promoting antigen presentation of chaperoned peptides through interaction with the receptors on APCs (i.e., antigen cross-presentation). Hsps have essential roles in a number of pathophysiologic conditions including carcinogenesis.

The aim of this study was to determine the correlation between the level of Hsp60 and Hsp70 in the blood plasma and different stages of urinary bladder cancer.

The study enrolled 29 patients with UBC. Clinical stages of bladder cancer as per TNM 7th (2009) were spread the following way: Stage I – 6 patients; Stage II - 5; Stage III - 10; Stage IV – 8 patients. All patients had transitional cell carcinoma of the bladder, G3-4. The control group consisted of 15 healthy individuals. Plasma levels of Hsp60 and Hsp70 were determined by ELISA before the curative procedure and correlated afterwards with clinical parameters of the patient. Data analysis was conducted using Microsoft Excel 2010.

The level of Hsp60 was higher by 2 times compared to control in patients with I Stages of UBC. Hps70 level was was up by 1.7 times in patients with Stage I of UBC. It was shown a slight increase plasma levels of Hsp60 and 70 in patients with Stage II and III of UBC. The levels of Hsp60 and Hsp70 were increased by 1.7 and 1.4 compared to controls value, recently. Many studies have demonstrated that increase levels of HSPs and their expression has been associated with cell proliferation and disease prognosis in cancer patients. The significance of the researches of HSPs in clinical management of tumoral patients is now expanding. These molecules play a major role in protecting cells against damage in stress conditions, but they also have essential roles under a number of physiological conditions. Experimental data shown that Hps may suppress apoptosis and thus promote tumorigenesis. Nevertheless, some HSPs may have a pro-apoptotic action as well as play important roles in the immune response against cancer. The literature data have demonstrated that high expression of HSPs has been associated with cancer metastases, poor prognoses and resistance to chemotherapy and radiation therapy.

Hps 60 and 70 protect tissues from spontaneous apoptosis, which is accompanied by oncogenesis. Therefore, increase the level of Hsp 60 and 70 in the blood plasma of patients is one of the factors of tumor growth and metastasis.

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Mireia Sola

Dissecting nuclear pore assembly with intermediate-arresting nanobodies

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Nuclear pore complexes (NPCs) are large multi-protein assemblies that control the transport of macromolecules between the nucleus and the cytoplasm. Their formation from ~ 1000 individual polypeptides is an impressive and still enigmatic example of self-assembly. NPCs are inserted during interphase into an intact nuclear envelope (NE), which requires a pore-forming fusion event between inner and outer nuclear membrane. In higher eukaryotes, NPCs also assemble upon mitotic exit concomitantly with the reformation of the NE.

Either pathway is still poorly understood, foremost because it has been very difficult to identify assembly intermediates, put them into a temporal order, and characterize them structurally. To tackle this problem, we generated nanobody-based inhibitors of discrete NPC assembly steps and used them to trap intermediates of this process.

We selected nanobodies (Nbs) targeting specific nucleoporins (Nups) by phage display. Then, we screened for Nbs that interfere with in vitro NPC assembly in the Xenopus nuclei reconstitution system. This strategy allowed us to identify Nbs targeting three different Nups that hinder the formation of functional nuclear pores. Next, we unveiled the Nup composition of each potential trapped intermediate by immunofluorescence, and we assessed the morphology of the assembled nuclear pores by scanning- electron microscopy. In the next steps, we would like to solve the crystal structures of the inhibitory Nbs-Nup complexes to reveal the epitopes that the Nbs target, and define the essential Nup-Nup interaction that they prevent.

Overall, we present Nup-specific Nbs as novel tools to block NPC assembly at specific steps. We believe that these tools will significantly contribute to the understanding of one of the most crucial processes in biology: how nuclear pores are formed to provide order and organization in cells.

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Ben Vezina

Development of a live bacterial vector for vaccine antigen delivery to the gastrointestinal tract of chickens

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The gastrointestinal tract of chickens can be colonised and adversely affected by a variety of pathogenic bacteria. Traditional injectable vaccines have been largely ineffective against such gastrointestinal pathogens and the diseases they cause in poultry. Failure has probably been due to an inability to induce an appropriate immune response that is effective at mucosal surfaces. A promising modern twist on vaccination lies within applied microbiology: utilising bacteria to express pathogen antigens and then deliver these recombinantly-expressed antigens directly to the mucosal surface of the gastrointestinal tract. This concept is known as a live vaccine, or paratransgenesis. Lactobacillus agilis La3, a commensal chicken isolate has been identified as a suitable candidate for use as a live vaccine vector due to the combination of several rare characteristics: strong colonisation and lifetime persistence within the chicken, being capable of genetic manipulation, and being a member of the L. agilis species, which have demonstrated probiotic effects in chickens.

Through this work, a combination of bioinformatics, microbiology and molecular biology was used to characterise L. agilis La3 and construct several live vaccine strains capable of recombinant-antigen expression. Initially, the genome of L. agilis La3 was completed and RNA sequencing was used to characterise the transcriptome of L. agilis La3 and identify genes that were constitutively expressed at high levels. Generally, the host (chicken) immune response is proportional to the antigen dose regarding vaccination. Given this, the predicted promoter regions from the most abundant, constitutively expressed genes were cloned into expression cassettes containing a fluorescent reporter gene. This was done to confirm the predicted strength of these promoters, which was based on the corresponding relative transcript abundance. A chromosomal integration plasmid was then constructed, allowing perfectly stable integration of these expression cassettes into the L. agilis La3 chromosome via double crossover homologous recombination. The predicted strengths of the promoters were found to have a moderate, positive relationship with protein expression levels, indicating that strong promoters had been identified. Finally, stable, persistent expression of a recombinant antigen from the chicken pathogen Clostridium perfringens was successfully demonstrated using several of these promoters.

The major outcomes of this project include the development of knowledge and a set of molecular tools and methods to advance L. agilis La3's potential as a live vaccine vector. Choosing an indigenous and persistent bacterium from the host gut microbiota for use as a vaccine vector, using native, homologous promoters to express an L. agilis La3 codon-optimised pathogen antigen, and stably expressing it from within the chromosome should provide the live vaccines developed here a significant edge over other vaccination strategies. We believe this holistic and isolate-driven approach should inform future live vaccine or paratransgenic vector development.

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Ruta Paulauskaite

Impact of BaSO4 Particles on the Viability of *Escherichia Coli*

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With the constant advances in nanotechnologies and emerging nanoproducts market, the need to investigate not only chemically active but also stable particles such as BaSO4 effects on the environment become evident. Such breakthroughs may result in undesirable environment developments and negatively affect bacteria, plant life, humans. The inevitable process of car stopping is related with the BaSO4 particles emission because of break pad waning. Approximately 220000 tons of breaking pads per year worldwide are grinded and their small particles spread around the environment. Such enormous quantities may result in biological effect on the environment and human well-being. Risk assessment of barium sulphate nanoparticles require careful evaluation of its mobility, reactivity, environmental toxicity, and stability. Thus far, few studies have been conducted on the toxicity to the environment caused by direct and indirect exposure to barium sulphate nanoparticles. Until now, no clear studies have been carried out to evaluate the effects of barium sulphate nanoparticles on bacteria. This work contains information on the stability of the barium sulfate nanoparticle suspension, the minimum inhibitory concentration for gram-negative E. coli bacteria and zeta potential differences.

Keywords: Barium sulphate nanoparticles, Zeta potential, minimal inhibitory concentration.

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Mariacristina Ciccioli

WIP regulates myeloid cells migration and invasion through changes in lipid membrane composition in response to hyperthermia

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Local mild hyperthermia and fever stimulate the activity of immune cells including prompting leukocyte migration and trafficking but the molecular mechanisms involved in this process are poorly understood. The WASP interacting protein (WIP) plays an important role in assembly and disassembly of podosomes (migratory adhesions assembled by myeloid cells) by binding both F-actin and other actin-related proteins. In addition, WIP can also regulate actin dynamics by modulating the lipid composition of the plasma membrane, as recently shown in neurons. In various organisms, the lipid composition and fluidity of the cell membrane work as a thermosensor that regulates the cellular response to changes in temperature. Our study aims to investigate whether WIP plays a role in mediating changes in actin dynamics by regulating membrane lipid composition in response to a febrile event.

Our results showed that hyperthermia (40°C) promoted random cell migration (distance travelled and velocity) in parental THP1 cells, while this effect was not observed in WIP KD THP1 cells. When incubated at 40°C, parental THP1 cells assembled more robust and numerous podosomes with increased turn over dynamics that correlated with increased matrix degradation and production of active MMP-9. Hyperthermia also induced highly significant changes in the total levels of certain lipids in a WIP-dependent manner. Parental cells increased the amount of saturated fatty acids and reduced the non-saturated ones, a common response seen in various microorganisms to compensate the initial heat-induced increase in membrane fluidity. However, WIP KD cells did not respond in the same way. For instance, lipidomics analysis showed that, in response to exposure to 40°C, two Phosphatidyl Inositol (PI) non-saturated derivatives, PI_24:6 and PI_24:5, whose levels decreased in parental THP-1 cells, and one PI long chained monounsaturated derivative (PI_34:1), whose levels increased in parental THP-1 cells, were highly de-regulated in the WIP KD cells. Furthermore, the change in membrane fluidity can trigger cell response by modulating the expression of Heat Shock Genes. Interestingly, we found that the Heat Shock protein 90 (HSP90), previously associated with podosomes formation and amplification of N-WASP-induced actin polymerization, is upregulated in THP-1 cells exposed to hyperthermia.

Taken together, our findings suggest that following hyperthermia WIP delivers changes in monocytic cells' membrane fluidity leading to increased HSP90-WASP/WIP dependent actin polymerization that may be required for the enhancement of the immune response in febrile conditions.

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Mona Tamaddon

MiR-24 and miR-374 involve in splicing regulation of oncogenes in breast cancer by targeting of CFIm25

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Breast cancer is the most prevalent occurring cancer in women and ranks second among causes for cancer related death in women worldwide. Today, there are many studies have devoted significant efforts to characterize mechanism associated with breast cancer initiation and development. Alternative polyadenylation (APA) represents an exciting case in gene expression regulation which needs to be studied further. In this regard, APA adds a new regulatory level to gene expression mechanism by the function of the genes Cleavage Factor I 25kDa (CFIm25), as mRNAs with shorter 3' UTRs due to APA will no longer be regulated by microRNA. The aim of the present study is to Investigation of the miRNAs interaction on the master regulator of CFIm25 and to clarify which microRNAs modulate CFIm25 gene level in breast cancer.

Material and Method: Using a various bioinformatic algorithm, 10 potential miRNAs that target CFIm25 were selected and profiled in 50 breast cancer and 50 normal samples. Next, human breast cancer cell line of MDA-MB-231 was infected with lentiviruses containing predicted miRNAs precursor sequence. Then, RNA and protein level of CFIm25 genes were analyzed by QRT-PCR and western blotting. luciferase assays were performed for confirmation of miRNAs binding to CFIm25. Finally, the effect of miR-24 and miR-374 down-regulation was monitored in MDA-MB-231.

Results: We identified that ectopic expression of miR-23, miR-24, miR-27, miR-135, miR-182, miR-221, miR-374 have inverse relationship with CFIM25 level in clinical samples. Real-time PCR showed that miR-24 and miR-374 knockdown enhanced CFIm25 expression level in MDA-MB-231 treated cells. Western blot and luciferase assay results also confirmed that downregulation of miR-24 and miR-374 in MDA-MB-231 cell lines dramatically enhanced CFIm25 expression.

Conclusions: These experiments indicated that in addition to CFIm25 could play important role in regulation of genes through 3' UTR length and action of miRNAs; miRNA also can directly control the CFIm25 expression level and indirectly affect the expression of the oncogenes. So, regulation of CFIm25 expression level through modulation of miRNAs may lead to improve treatment response in breast cancer.

Key words: Breast cancer, Alternative polyadenylation, microRNA, Tumor suppressor, CFIm25

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Gelareh Shokri

Non-viral Generation of Functional Patient-Specific Induced Pluripotent Stem Cells as an *In Vitro* Model of p47-phox deficient CGD

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Background: Chronic granulomatous disease (CGD) is an inherited monogenetic immunodeficiencies characterized by recurrent and life-threatening infections in early childhood due to defects in the production of microbicidal reactive oxygen species (ROS) by phagocytes.

Methods: In this study, we optimized reprogramming protocols to differentiate induced pluripotent stem cells (iPSCs) from wild type and patient's fibroblasts (p47phox with a GT-dinucleotide deletion (ΔGT) mutation in p47phox encoding NCF1 gene) to pluripotency by nucleofection of four transcription factors, OCT4, SOX2, KLF4 and c-MYC using pMASTER non-viral vector.

Results: All generated iPSC lines showed morphological features, profile of human embryonic stem cells and express the key pluripotency factors. Furthermore, all iPSC lines formed embryoid bodies in vitro containing originating from all three primary germ layers and were capable of teratoma formation in vivo. The iPSC line retained p47phox mutation found in the corresponding patient's neutrophils.

Discussion: Our results suggest that CGD-patient specific iPSC lines represent an important tool for modeling disease phenotypes. This new cellular model for the p47phox -CGD has not been described before. iPSC lines generated from CGD patients represent a unique resource to investigate the pathophysiology of primary immunodeficiencies and may potentially use to development of correction-based therapies.

Key words: Chronic granulomatous disease, induced pluripotent stem cells, Reprogramming

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Fatemeh Kouhkan Kahangi

Investigation on the EGFR-dependant expression of miRNA-34a and shRNA-CDK6 in GBM cells using highly efficient knock-in by a double cut donor HDR vector

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Glioblastoma multiforme (GBM) is one of the most malignant forms of the brain tumors. The most familiar genetic aberration associated with malignant glioma is the amplification of the epidermal growth factor receptor, EGFR, with a frequency of about 50%. Currently, there is a great attention on using gene therapy methods like herpes simplex virus type 1 thymidine kinase (HSV1-tk) to treat GBM. However, because of the immunostimulatory problems of viruses and random integration of foreign genes in other neural cells, this therapeutic approach is not satisfying.

In the present study, we developed a system which not only can specifically target cancer cells but also can increase the efficiency of homologous recombination of a transgene or a regulatory RNA molecule at our target site without disrupting the functionality of any gene in the target cells. In order to achieve these goals, we used CRISPR-based gene editing to introduce miR-34a and shRNA-CDK6 into one of the introns of the EGFR gene in GBM cells. By using this strategy our construct was integrated into the 7th intron of the EGFR gene. Insertion of regulatory RNAs in one of the introns of EGFR gene can create a co-expression system, needless of any excess gene regulatory elements, for simultaneous expression of the target regulatory RNAs and EGFR gene. This strategy helped us to achieve a high expression of the regulatory RNAs in the GBM cells compared to the healthy cells, which don't express EGFR gene in such a high level. This high expression of regulatory RNAs in GBM cells can act as an indirect approach to specifically target tumor cells.

We designed a PX-459-based vector with a guide RNA which targets the 7th intron of EGFR gene. We also designed a double-cut donor HDR vector, the transgene is flanked by two gRNA cut site on both sides, to deliver a miR-34a and shRNA CDK6 construct flanked by sequences homologous to those surrounding the intron. Treatment with both vectors resulted in EGFR-dependent expression of miR-34a and shRNA CDK6.

The data from quantitative polymerase chain reaction (qPCR) showed that the expression of EGFR gene, after adding our construct in the intronic site, wasn't changed significantly, which proved our idea of the non-disrupting effect of our construct on EGFR gene. The apoptosis and cell cycle assays showed a higher rate of apoptosis and a lower rate of growth in the treatment group.

Thus, Cas9-mediated intronic insertion may be a viable approach in cancer therapy.

Keywords: GBM cancer treatment, Mirtron, Crispr/Cas-9, gene therapy, miRNAs.

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Vishnu K. Sharma

Identification of novel, selective Inhibitors of LdDHFR using Computer Aided Drug Design Techniques

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Among the various known targets for the treatment of Leishmaniasis, Dihydrofolate reductase (DHFR) is an essential, validated drug target which plays an important role in the folate metabolic pathway. However, limited leads or inhibitors of the Leishmania donovani DHFR (LdDHFR) are reported in the literature, thus there is an urgent need to identify new inhibitors. In this research work, the binding mode analysis was performed to understand the mode of interaction of substrate and lead inhibitor in the active site of the LdDHFR using the molecular docking and MD simulation approaches. Later on, two different virtual screening approaches (Shape-based virtual screening: SBVS, Docking-based virtual screening: DBVS) were utilized to identify potential LdDHFR inhibitors using ZINC, ChEMBL and DrugBank publicly available databases. A reported, potent LdDHFR inhibitor, 5-(3-(octyloxy)benzyl) pyrimidine-2,4-diamine (lead) was utilized as the query for SBVS while MD simulated homology model of LdDHFR, was utilized for DBVS. In silico ADMET factors were also considered during virtual screening. These two screening processes produced 25 suitable hits, which were further confirmed for their selectivity towards LdDHFR using molecular docking and Prime-MM/GBSA analysis (as against human DHFR). Best 5 hits, which found selective and energetically favored for the LdDHFR, were chosen for MD simulation analysis. The MD analysis suggested that the selected hits exhibit very good binding affinity and H-bonds as well as hydrophobic interactions were also comparable to that of the lead molecule. The current study, thus identifies hits, which can be further optimized as potent LdDHFR inhibitors.

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Harvijay Singh

Structure of chikungunya virus nsP2 cysteine protease and its inhibition by structure-based peptidomimetics inhibitors

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Chikungunya virus (CHIKV), a mosquito born pathogenic virus, is responsible for causing rashes, arthritis, encephalitis, and death in humans. In recent years, CHIKV has re-emerged as a global public health threat and has caused several outbreaks around the world. Presently, there are no vaccines or specific drugs/antiviral are available against CHIKV infection. The replication machinery of CHIKV consists of four nonstructural proteins (nsP1 to nsP4), produced as a single nonstructural polypeptide (nsP1234) through direct translation of positive sense single stranded RNA. Processing of nsP1234 is carried out by the virus specific nsP2pro cysteine protease through autoproteolysis, by which long nonstructural polyprotein is spliced into individual nsPs. Furthermore, these nsPs assist the virus replication by forming a viral replication complex. Therefore nsP-2pro constitutes a promising drug target for antiviral drug development to check CHIKV infection in humans.

In the present study, we have cloned, purified and crystallized the nsP2pro of chikungunya virus and have elucidated the crystal structure of this enzyme. Furthermore, structure-based peptidomimetic inhibitors of CHIKV nsP2pro were identified and tested for enzyme inhibition. CHIKV nsP2pro structure revealed that the protein is comprises of two subdomains; N-terminal protease and a C-terminal MTase-like subdomain. Active site of the protein, which is consist of a catalytic dyad made up of cysteine and histidine is located at the interface of both the subdomains. Further structural analysis has shown that the active site of the protein is gated by a flexible loop which posses an Asn residue interacting with incoming substrate and somehow regulating its entry into the active site. Substitution of this Asn residue with Ala has marked a significant reduction in the activity of enzyme, indicating the functional specificity of this residue in substrate recognition by the enzyme. Based on the crystal structure of CHIKV nsP2pro (PDB ID: 4ZTB), a series of peptidomimetic compounds were identified and these compounds were further assessed by molecular docking and molecular dynamics simulation for structural stability and conformational flexibility. Additionally, a FRET based nsP2 protease activity assay is developed by using flurogenic substrate oligopeptides. Two of the identified peptidomimetic compounds were screened for the inhibition of nsP2pro enzymatic activity by using this proteolytic activity assay. Inhibition of CHIKV nsP-2pro by the tested compounds PepI & PepII was observed with IC50 values of 34μM and 42μM, respectively. The inhibition kinetic studies showed that the inhibition constant (Ki) is 33.34±2.53 μM for PepI and 45.89±4.38 μM for PepII. Additionally, these two compounds were further validated by cell culture based plaque reduction assay and found to be significantly inhibiting CHIKV replication in BHK-21 cells at concentrations much lower than their cytotoxic concentrations. This approach of structure-based identification of peptidomimetics compounds and evaluating their in vitro & in vivo efficacies for inhibiting CHIKV nsP2pro may further lead to novel antiviral drugs against chikungunya infection.

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Siranjeevi Nagaraj

Circulatory miRNAs in AD as indicators: as potential diagnostic marker; in shedding pathogenic insights; pioneering personalized medicine

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Alzheimer Disease (AD) is the most common age-related dementia worldwide. Among its major challenges is identifying molecular signatures characteristic for the early AD stage which could serve for deciphering the AD pathomechanism and also as non-invasive, easy-to-access biomarkers. Recently several studies propose miRNA as epigenetic regulator implicated in AD pathogenesis. Also it is now evident that blood brain barrier(BBB) is impaired that suggest AD as a systemic disease. This avenue opens up the field of circulatory miRNA as potential marker in non-invasive samples such as blood. Moreover, unravelling functional role of these miRNA is key to appreciate the pathways involved in AD pathogenesis to improve causative or symptomatic treatments.

To this end, using panel of 179 miRNAs in plasma of AD and CTR subjects (1st group of cohorts), we screened for miRNAs differentially expressed in AD patients by quantitative real-time polymerase chain reaction (qRT-PCR). From this group, we selected the 15 most differentially expressed miRNAs in AD and validated them in 2nd independent group of cohorts. Further we selected consistently observed 6miR panel based on its diagnostic predictability and utilized softwares such as miRTarBase, Target scan and KEGG database to obtain putative targets. Later, we selected one particular miRNA hsa-miR-483-5p and analyzed its ability to bind/regulate targets such as Mitogen-activated protein kinase 3 (MAPK3) and microtubule-associated protein tau (MAPT), proven to be the culprits in AD. For this approach we used dual luciferase and immunoblots techniques to test our hypothesis. To add, not only limiting our understanding pertaining to a single study (our previous study), we widened our horizon in understanding circulatory miRNAs published in AD blood based studies till date to arrive at miRNA-mRNA network. In conclusion, minimally invasive blood based miRNA biomarkers could be a potential alternative to existing sophisticated (brain imaging) and invasive (CSF withdrawal) procedures for AD diagnosis and moreover these miRNAs could be used as an indicator for understanding pathology and also to pioneer personalized medicine.

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Ifeoluwa Adefila

Prevalence of Cryptosporidium Infection among HIV Sero-positive Patients Attending the HAART Clinic of University of Ilorin teaching Hospital, Ilorin, Nigeria.

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Cryptosporidium, an obligate opportunistic intestinal, the progression and severity of this parasitic infection are closely linked to adversely suppressed immune conditions of the infected persons. A hospital based cross-sectional study was conducted from May-July, 2016 at the Highly Active Anti-Retroviral Therapy Clinic (HAART) of University of Ilorin Teaching Hospital to determine the prevalence of Cryptosporidium infection among HIV sero-positive patients. Stool specimens from 196 consenting HIV sero-positive and 196 sero-negative (control group) patients were collected, stained and examined. The conclusive diagnosis of Cryptosporidium infection was based on the presence of characteristic oocysts under both light compound microscope and florescent microscope (magnification X1000). The overall prevalence of Cryptosporidium infection among the 196 HIV sero-positive patients was 3.1%. None of the 196 HIV sero-negative patients examined were positive for Cryptosporidium infection. HIV sero-positive females had higher prevalence (3.6%) of Cryptosporidium infection than males (2.3%), but the difference was not significant. Of the 6 positive cases for Cryptosporidium infection, 15.4% had diarrhoea, 12.5% had CD4+ T-Lymphocyte Count less than 200 cells/ µl while 3.4% had been on Anti-Retroviral Therapy for more than 6 months. The prevalence of Cryptosporidium infection (3.1%) recorded in this study is seemingly low but because Cryptosporidium is a leading cause of chronic life threatening diarrhoea, especially among immunosuppressed individuals globally and because of accelerated morbidity and mortality among HIV/AIDS patients when complicated by Cryptosporidium and other opportunistic infections, this result should stimulate greater concern whereby concerted studies should be carried out, using uniformly standardized procedures to ascertain the prevalence and implications of Cryptosporidium infection nationwide.

Key Word: Cryptosporidium, Oocyst, HIV/AIDS

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Jason Charamis

Construction and study of a new species-specific shuttle vector for the biofuel-producing *Zymomonas mobilis*

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Zymomonas mobilis is an alphaproteobacterium that is implemented in the production of first and second generation bioethanol, as well as high added value compounds deriving from its powerful Entner-Doudoroff glycolytic pathway. The application of Z. mobilis in various bioprocessing procedures often requires its metabolic broadening, which is achieved via genetic engineering and foreign gene introduction. Shuttle vectors that have been hitherto used for the engineering of Z. mobilis are, in their vast majority, based on a scarce number of suitable broad-host-range plasmids or on native cryptic plasmids, small in size (1.6 to 2.7 kb) and contributing mostly with their replication functions.

In this work, we created an alternative shuttle vector for Z. mobilis that makes use of a 4.5-kb native plasmid of Z. mobilis strain NCIMB 11163, pZA1003 (GeneBank acc. no. CP001725). pZA1003 harbors an α-proteobacterial type replicator initiator protein (RepB), a plasmid addiction stabilization system (DinJ-YafQ toxin-antitoxin system), and a four-gene-member mobilization region (mobA-mobD), homologous to those of colicinogenic plasmids. The shuttle vector that was created, namely pJC1 (8,002 bp), is a fusion product between pZA1003 and a 2.7-kb derivative of pBluescript II KS (+) that, for reasons of compactness, lacks the f1 ori. It additionally carries a chloramphenicol resistance gene (catE), which is suitable for plasmid selection in Z. mobilis. pJC1 proved to be over 90% stable in the two most applied and patented Z. mobilis strains, industrial strains ZM4 and CP4, for 100 generations without selection pressure. It was also highly transferrable to E. coli and Z. mobilis recipients via TraP-mediated conjugation, at transconjugant per recipient frequencies of 10-1 and 10-5, respectively. Importantly, pJC1 was found to relatively stably co-exist with plasmids of the broad-host-range pBBR1MCS lineage in Z. mobilis, thus enabling their successive use in the organism for gene stacking purposes. The pBluescript-originating multiple cloning site (MCS) of pJC1 retains a considerable number of unique restrictions sites, while effort is being made nowadays to restore its blue/white screening capacity by translocating catE in the vector backbone, as well as improve the transcriptional strength of genes cloned into the MCS by introducing the robust Z. mobilis pyruvate decarboxylase (pdc) promoter. Other current optimization efforts entail the substitution of catE with other suitable for Z. mobilis selection markers and, finally, the creation of a vector derivative depleted for the plasmid addiction region, in hopes of reducing vector size without compromising its stability.

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Mohd Yaqub Mir

Gal-1 Expression Profile in CNS

Mohd Yaqub Mir

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The galectin-1 protein contains a single carbohydrate recognition domain through which it can bind to glycans both as a monomer and a homodimer. Microglia organizes the immune functions in the CNS. They have pro and anti-inflammatory phenotypes depending on the expressed cytokine profile.

Aims: The main aim is to examine the expression of Gal-1 in microglial cells in different in-vitro condition.

Methods: We use different methods such as double immunofluorescence labeling to examine the galectin-1 expression in primary mixed neuronal cultures and western blot to quantify the Gal-1 expression in the cultures.

Results:

Gal-1 is expressed in some cells in the white and grey matter of the brain, No significant Gal-1 expression in astrocytes and oligodendrocytes after 7 day culturing, The Gal-1 expression is increasing with the cultring time, Galectin-1 is expression is low in astrocytes and very high in microglia cells derived from 14-day old primary neuronal cell culture, and Neither immunomodulators nor the neuronal modulators modify the Gal-1 expression in primary neuronal cell culture.

Conclusion: Galectin-1 expression is mostly connected to the old strongly attached amoeboid form fragmented microglial population which derived from the mixed cultures. The form of these cells can be quite similar to the gitter microglias. Gitter microglia cells have an anti-inflammatory phenotype, and according to our results, one of the factors responsible for this inflammatory inhibiting phenotype is possibly galectin-1.

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Dushyantkumar Dudhagara

Bioremediation of Poly Aromatic Hydrocarbons (PAHs) from contaminated soil by Marine microorganisms

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Marine environment is one of those areas which show the dynamic fluxes encompassing half of the world's population. Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants resulting from incomplete combustion of organic materials. Microbial degradation is the major route for its removal from the environment. The present study represents the assessment of PAHs from contaminated sites near Bhavnagar coast, Gujarat, India. The results showed that 2-3 rings containing LMW PAHs (Nap, Ace and Phe) predominated over 4 ring containing HMW PAHs (Pyr, Flt and Chr). Sources identified by using various diagnostic ratios, indicated pyrogenic and petrogenic source of contamination, due to ship breaking activities. In addition, the ecological risk assessment was carried out using RQNCs and RQMPCs. The results indicated that PAHs posed adverse ecological risk, which can due to ship breaking and petroleum transportation at the sites, providing to be harmful for marine biota and human health. Thus, elevated levels of ∑PAHs need to be treated by devising effective bioremediation strategies. Furthermore, selective isolation approach was employed to isolate multiple PAHs degrading bacteria. multiple PAHs degrading bacteria was isolate and identified as Mycobacterium litorale. Furthermore, optimization of BH medium components viz. MgSO4, CaCl2, KH2PO4, K2HPO4, NH4NO3 and FeCl3 were primarily screened using Plackett-Burman (PB) design, followed by RSM and ANN approaches. The optimization resulted in, 51.53% fluoranthene degradation on 3rd day. The ANN model had been developed as it is an efficient method for empirical modeling and optimization, especially for non-linear systems. The predictive capabilities of RSM and ANN model were compared on the basis of R2, RMSE, and MAPE values. The R2 values for predicted models RSM and ANN were 0.988 and 0.997, respectively; RMSE value for RSM and ANN were 0.940 and 0.3234; and MAPE value for RSM and ANN were 1.432 and 0.5715. Further, the enzyme Catechol 1,2-dioxygenase involved in fluoranthene degradation were purified and categorized. The purified product had showed single band on SDS PAGE corresponding to the molecular mass of 32 KDa. Furthermore, amplification of catabolic genes responsible for hydrocarbon degradation was detected using PCR. The designed primers were applied to amplify catabolic genes viz. alkB, nahAc, PAH-RHDα, C12O and C23O. The results showed three catabolic genes i.e. alkB, PAH-RHDα, and C12O that were successfully amplified. PAHs degradation by M. litorale was examined in different biostimulated soil conditions using a microcosm experiment. Biostimulating agents used were BH medium, Triton X-100, agriculture compost and mixture of all. The addition of BH medium and agriculture compost enhanced biodegradation of both LMW and HMW PAHs, whereas, addition of Triton X-100 as surfactant had a negative effect on biodegradation. The biodegradation rate (K) was higher in soil treated with BH and compost with apparent lower t1/2. The microcosm approach thus provides an effective strategy to designing bioremediation techniques. The study would surely lay the foundation for bioremediation and ecological restoration of our coastal sites.

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Natalia Pavlova

Ion Channels in the Nuclear Envelope of Cardiomyocytes

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The perinuclear space is an intracellular calcium storage and Ca2+ ions are known to regulate gene activity. Nuclear membrane is permeated with a large number of ion channels, but their properties and role in the cell are still insufficiently investigated. The goal of this work was to estimate the conductance, selectivity and pharmacological sensitivity of ion channels in the nuclear envelope of cardiomyocytes.

In the experiments, two-three-week-old Wistar and Fisher rats were used. The nuclei of cardiomyocytes were isolated by myocardium homogenization and centrifugation of the resulting suspension. Single ion channels were recorded from nucleus-attached and excised patches of the nuclear membrane in the voltage-clamp mode of the patch-clamp technique. The bath and glass pipettes were filled with solution containing (mM): KCl–150; HEPES–8; HEPES-K–12; EGTA–1; pH 7,2. The results were processed using Clampfit and Origin software. All experiments were performed according to the relevant national and international ethical requirements.

We confirmed the presence of inositol-1,4,5-triphosphate receptors in the nuclear membrane of cardiomyocytes (384±5 pS). Their activity depends on the IP3, Ca2+ and ATP concentrations.

In the nuclear membrane of cardiomyocytes we recorded ion channels with conductance of 210±12 pS and properties similar to previously described LCC-channels (Marchenko et al., 2005) in the nuclear membrane of neurons. The kinetics of these channels was slow, at positive potentials they were almost always active, but at negative potentials their activity decreased. We found out that LCC-channels are impermeable to Ca2+ and Cl-, but permeable to K+ and Na+. We have found that blockers of nACh-receptors, in particular tubocurarine-Ki=0.2 mM, hexamethionium-Ki=22.6 mM, atracurium-Ki=1.27 mM and dytilin-Ki=1.64 mM, have inhibitory effect on LCC-channels.

There are also less common spontaneously active ion channels in the nuclear membrane. We recorded a cationic channel which resembles a typical LCC-channel, with similar kinetic characteristics, but lower conductance – 169±6 pS. We also recorded a chlorine ion channel with conductance 319±10 pS.

In addition to the above channels, we have recorded ion channels with low conductance (10-90 pS), which differ in conductance and kinetic parameters. We also have recorded very rare channels, including ion channel with the conductance of 429±14 pS.

Consequently, in the nuclear membrane of cardiomyocytes we recorded inositol-1,4,5-triphosphate receptors (384±5 pS), LCC channels (210±12 pS), cationic channels (169±6 pS), chlorine channels (319±10 pS), channels with high conductance (429±14 pS) and group of channels with low conductance (10-90 pS).

We hypothesize that nuclear ion channels are an integral part of Ca2+ signaling machinery and, as such, play an important role in the functioning of the heart muscle. Ion channels involved in intracellular Ca2+ signaling may be a target of completely new types of drugs for treatment of heart diseases.

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Ahmed Soliman

Chemotherapy Induced Peripheral Neuropathy (CIPN) is a common problem resulting from some widely used chemotherapeutic agents

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Chemotherapy Induced Peripheral Neuropathy (CIPN) is a common problem resulting from some widely used chemotherapeutic agents as: Vinca alkaloids (e.g. Vincristine), Taxanes (e.g. Paclitaxel), and Platinum compounds (e.g. Oxaliplatin). The exact mechanism behind CIPN is not known.

The research constitutes two drugs; the first one is Vincristine (VCR) that is widely used against many types of Cancer (e.g. Colon cancer), one of the most known side effects to Vincristine is CIPN. Second, Melatonin which is a neurohormone that acts as an antioxidant and has an anti-inflammatory effect; therefore, Melatonin is believed to enhance the CIPN condition. Another interesting property of Melatonin is its ability to potentiate the effect of chemotherapeutic agents (e.g. VCR) relying on its antioxidant property.

For the experimental design, first, Melatonin administration effect on VCR-Induced-CIPN; developing a CIPN mouse model using Wistar Albino mice (20-25gm) with 0.1 mg/kg IP Vincristine sulfate injections once daily over 7 days to induce CIPN. Melatonin will be administered after the murine model is developed. Second, Melatonin effect when added to VCR; to examine the in-vitro efficacy of Melatonin when added to VCR on the apoptosis level.

Finally, the oxidative stress markers will be measured; SOD, MDA, CAT and GSH using the homogenate of the sciatic nerve of the sacrificed mice. Also, MTT and TUNEL assays will be done for the HCT-8 cell line along with DNA extraction to check the amount of P53 expression of the cells using QPCR.

The main aim is to provide a recommendation of Melatonin administration along with Vincristine according to two reasons: The anti-CIPN effect of Melatonin and the potentiation of Vincristine work that may lead to a decrease in its dose and its side effects accordingly.

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Yassine Kasmi

Methylome Restriction-Modification Sites Prediction in Bacteria using Markov Models

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The restriction-modification (RM) is ubiquitous enzymatic system, it is found in bacteria where it destroys foreign DNA entering into bacteria's cell and presented as immunological system in prokaryote. Its main function is to protect cells from foreign genetic material such as bacteriophages and plasmids. The system's components are characterized by two types of activities - methyltransferase (MTase) and endonuclease (REase) which are specific to certain nucleotide sequences in DNA called restriction sites. Biologically, RM system is affected by the methylase, which is considered as the main enzyme of this system. Some MTases exist alone, without an apparent cognate REase partner. These so-called "orphan" MTases include DNA adenine methylase (Dam), which modifies the adenine N-6 in the GATC motif. Methylome sites "Dam" plays several key roles in bacterial processes, including mismatch repair, the timing of DNA replication, and gene expression. The localization of the Dam sites in bacterial genome is still a challenge due to the complexity of experimental tests - which explains the limited number of studies dealing with this aspect-, and their limitations. Hence, the importance of developing a computational model that can predict Dam's methylome regions from genomics sequences.

Here, we propose a new stochastic model, that predicts the methylome sites using Hidden Markov Chain (HMM). HMM model was generated by different emission and transition matrices. We also adopt a Bayesian approach and sample the parameters of the model from the posterior distribution with Markov Chain Monte Carlo (MCMC), using a Metropolis-Hastings and Gibbs-within-Gibbs scheme.

The prediction model was generated and then applied on the genome sequence of E. coli. We do a comparison of the results generated by the current computational method descripted in this work and the experimental results of the whole genome sequence obtained by the single-molecule real-time sequencing (SMRT) method for E. coli (access number CP027599).

The model demonstrates that we can reveal 90% of sensitivity by using known methylome fragments, which are included in non-methylatable DNA regions. To validate these models, they were applied on the methylome regions of the E. coli genome. The simulation results show a high specificity and similarity between results generated by both techniques: Computational model developed in this study and the results of SMRT DNA's technique described in previous experimental studies. The model has the ability to distinct the high methylation of the GACT sites from other types of methylation in E coli genome. The stochastic model presented in this study permits the prediction of Dam methylome as RM motifs present in bacterial genome sequences.

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Heorhi Selikhanau

Crystallization and X-RAY studies of mutant I(L177)Y photosynthetic reaction center of *Rhodobacter sphaeroides*

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In purple bacteria, the primary process of photosynthesis occurs in a pigment-protein complex termed the reaction center (RC). This membrane structure converts solar energy into chemical bond energy through a series of electron and proton transfer reactions. It is remarkable that the quantum efficiency of energy conversion is close to 100%. An amino acid environment of pigments involved in electron transfer plays an important role in the functioning of these complexes and therefore investigations of protein-pigment interactions are obligatory for an understanding of the mechanisms of RCs functioning. A mutant RC from Rhodobacter sphaeroides with the replacement of isoleucine to tyrosine in the 177 position of the L subunit was obtained. This amino acid residue is located near the special pair of bacteriochlorophylls and the bacteriochlorophyll monomer of the inactive electron transfer branch. This mutation effected spectral and functional characteristics of the RC. To determine the reasons for the changes, the high-resolution spatial protein structure is necessary. We isolated, purified and crystallized RC I(L177)Y using various crystallization methods: a vapor diffusion technique with the addition of a detergent and using a lipid sponge phase. The diffraction data sets were collected from the obtained crystals with the use of the ESRF synchrotron. The best resolution for crystals obtained through the use of vapor diffusion technique with the addition of detergent was 2.6 Å and for the sponge phase crystals, it was 2.1 Å. Collected datasets were successfully used to solve and refine protein structures.

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Preethi Basavaraju

Sorting out the Link Between AD and Diabetes Using *Drosophila* as a Novel Animal Model

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Background: Alzheimer's Disease (AD) is a chronic neuro-degenerative disorder in which the individual suspects have loads of plaques and tangles deposited in the brain. Tangles are twisted fibres of a protein called tau that builds up inside cells and β-Amyloid plaques or senile plaques are clumps of insoluble peptides that result from the proteolytic cleavage of β-amyloid amyloid precursor protein (APP) by BACE1 enzyme and are found in extracellular deposits throughout the central nervous system (CNS). Apart from the fact that AD is neurodegenerative disorder there are many controversial talks on a crucial point that Type 2 Diabetes mellitus might play a key role in serving as a contributing factor for the disease pathology and progression. Though much work is in progress on this particular area, there are no confirmatory results stating that this fact is true or not till date.

On the other hand metal homeostasis can be modulated by APP and its cleavage product Aβ, the elevated levels of the Aβ production and the expression levels of metals such as copper, zinc and iron are found in AD plasma and brain tissues. Relatedness of these parameters with amyloid plaque formation in AD brain is more interestingly sorted out with the fact that metal homeostasis also plays a key role in diabetes mellitus. The associations of zinc with various metabolic pathways include functions such as insulin storage and secretion. The reluctant deposition of zinc in case of diabetic patients caused due to low absorption might reciprocate itself with increased deposition of it gradually with increase in the production of Aβ through hippocampal circulations that occur centripetally or centrifugally in the brain. Hence in the current study we focus on Drosophila melanogaster models to intervene these associations.

Methods: In AD individuals, the variant of γ-secretase cleaves APP at the wrong position yielding a 39-42 amino acid peptide called Aß42 or Aß, which is insoluble and aggregates as identical clumps of peptides termed the β-Amyloid plaques. In the present study we have taken adult fly models of human wild-type and mutant Tau strain to reveal the interconnections between AD – Aß/ Aß42 – Zinc deposition.

Results: Depositions of Aß/ Aß42 and Zinc metal ions were seen in all the strains except for the adult wild type flies.

Conclusion: Drosophila is a promising *in vivo* model for studying the depositions of Aβ production and taupathies but still the aggregates formed are not much nearly associated with the human Aβ production. Since, the depositions of zinc metal ions were seen in the mutated strains we can come to a brief conclusion that the Glut- isomers play a key role in AD progression.

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LLokesh Thangamani

A Theranostic Approach to Intervene the Link between Parkinson's Disease (PD) and LRRK – 2 Mutation

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Background: Parkinson's disease is a neurodegenerative disorder in which several regions of the brain are affected mainly, the cerebral cortex, peripheral nervous system, pigmented nuclei seen in the midbrain and brainstem and also in parts of the olfactory tubercle. The major characteristic features of the disease are the prominent degeneration of dopaminergic neurons seen in the midbrain specially the Substantia Nigra pars compacta (SNc). Patients represented with mutated gene are seen for excess alpha synculein presence and this might be due to an altered protein product formed. These protein molecules accumulate in the cell and get deposited as junks which further leads to the damage of the neuron. Sometimes these altered proteins may be because of alterations in several genes which are responsible for many neuronal functions, out of which mutations in the DNA of Parkinson's disease (PD) patients located in the leucine rich repeat kinase 2 regions (LRRK-2) represent the most common genetic cause of Parkinson's disease.

LRRK2 is a kind of protein that belongs to the family of kinase enzymes, its main function in the cells is to tag molecules with chemicals called phosphate groups. This process is termed as phosphorylation and it regulates basic nerve cell function and health. However, when mutated LRRK-2 tags proteins, it results in over-manufacturing certain products in a cellular process. Mutated LRRK-2 performs its tagging function by attaching phosphate groups and leads to increases in proteins through its action on a part of the cell called ribosomal s15. The removal of phosphate groups that tag ribosomal s15 actually prevents degeneration of cells in the brain.

Methods: The study includes 21 subjects of PD patients belonging to late onset PD. All of these subjects were screened for the presence of G2019S mutation occuring in LRRK2 gene. The positive subjects are then studied for the phosphate tagging group of ribosomal s15 presence using biochemical analysis.

Results: In the present study the mutational analysis show positive results for majority of cohorts belonging to that particular region and have been also studied for the phosphate tagging group of ribosomal s15 presence.

Conclusion: Previous studies suggest that the disease-causing mutations, like the G2019S mutation, increase the rate at which LRRK2 tags molecules. Identifying the molecules that LRRK2 tags provides clues as to how nerve cells may die during Parkinson's disease. Though these findings are exciting, it has not been still implemented on humans. Since, there are several genes and susceptibility factors that have been reported to be the causative for the progression of Parkinson's disease (PD) in the past decades.

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Rifaldy Fajar

Nonlinear Mathematical Modeling and Simulation of Blood Cell Production (Hematopoiesis) with Time Delay

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Mathematical modeling is a way to explain the reality to the mathematic equations. One thing which can be construct to the mathematica models ia about blood cells production located in bone marrow. Blood cells production model has formulated in a system of differential equation consist of two nonlinear ordinary differential equation with time delay. This research purpose is to study deeply about the formation of mathematical model of blood cells production, find the steady state, analyze the stability and graphic of the model. This research is a literature study and using comprtment model to construct the model for blood cells production. The result of this research is description about the model of total hematopoietic stem cells and non proliferating stem cells population. There are a necessary condition to obtain a globally asymptotic stable and locally asymptotic stable for blood cells production model. Graphic of the numerical solution of the model show the system of hematopoietic model will oscillate at $3 \leq \varphi < 5{,}5$ but it will be stable again at $\varphi \geq 5{,}5$. Oscillation in this case mean that blood cells production in bone marrow is unstable so the circulating blood cells count in human body is unstable.

Keywords : Hematopoiesis, Mathematical Modeling, Time Delay

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Thaddeus Gugu

Combretum dolichopetalum leaf extracts potentials in the management of some tropical diseases

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Introduction: The emergency of drug resistance and proliferation of incessant infection and diseases has considered worrisome in most tropical rain forest zone and among rural dwellers as a results of drug abuse and poor health care delivery. This necessitated consideration of use of some herbal and medicinal preparation of plant origin as emergence alternative in controlling and treatment of these current trends to ensure quality of life and economic dealing in the tropics. Medicinal plant in this case can be termed as the plant of natural origin that contain an effective and active principles useful in the management and treatment of diseases with low or minimal toxicity. Materials: Combretum dolichopotelum, drugs (Ciprofloxacin, Ketoconazole and loperamide, Diminazene), extracting and fractionating solvents, media and reagents, glassware, instruments and other equipments. Methods: The active principle of the plant was extracted and fractioned using cold maceration and volume-liquid chromatography after pulverization, the phytochemical screening was done to estimate the presence of secondary metabolites using standard procedure and high performance liquid chromatography (HPLC) was carried out on the fractions to authenticate the presence of compounds. Acute toxicity, in vitro- in vivo anti-trypanosomiasis, anti-leishmaniasis, anti-Malarial, and cytotoxicity studies were carried out on both crude and fractions following standard methods. Results: The HPLC results showed there is presence of unknown compounds as regards to the peaks of the chromatograms of the fractions..The LD50 >5000 mg/kg. The in vitro and in vivo anti-trypanosoma, anti-malarial and anti-leishmania study revealed that, there was significant reduction of parasite (P>0.05) within 8 days of post infection treatment with both crude and fractions. The cytotoxicity studies showed that both crude extract and some fractions are safe. Conclusion: the study showed effective activity of Combretum dolichopotelum against tested organisms which are responsible for some of tropical diseases as it can be advice to be used in the management of such ailments in various standard dosage form.

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Idowu Aimola

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Gamma globin induction remains a promising pharmacological therapeutic treatment mode for sickle cell anemia and beta thalassemia, however Hydroxyurea remains the only FDA approved drug which works via this mechanism.In this regard,we assayed the γ-globin inducing capacity of Cis-vaccenic acid (CVA). CVA induced differentiation of K562, JK1 and transgenic mice primary bone marrow hematopoietic progenitor stem cells.CVA also significantly up-regulated γ-globin gene expression in JK-1 and transgenic mice bone marrow erythroid progenitor stem cells (TMbmEPSCs) but not K562 cells without altering cell viability. Increased γ-globin expression was accompanied by KLF1 suppression in CVA induced JK-1cells.Erythropoietin induced differentiation of JK-1 cells 24h before CVA induction did not significantly alter CVA induced differentiation and γ-globin expression in JK-1cells. Inhibition of JK-1 and Transgenic mice bone marrow erythroid progenitor stem cells Fatty acid elongase5 (Elovl5) and Δ9 desaturase suppressed the γ-globin inductive effects of CVA.CVA treatment failed to rescue γ-globin expression in Elovl5 and Δ9-desaturase inhibited cells 48h post inhibition in JK-1cells. The data suggests that CVA directly modulates differentiation of JK-1 and TMbmEPSCs, and indirectly modulates γ-globin gene expression in these cells.Our findings provide important clues for further evaluations of CVA as a potential fetal hemoglobin therapeutic inducer

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Praveen Kumar

Anti Microbial Activity of Peptide Folding Propensities for Cancer Drug Target Improvisation and Library Construction

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Peptide plays characteristic role in Drug Discovery, Development and Drug Improvisation purpose. Experimentally, the peptide has different monomeric domains to occupy the structure which is inevitably making a peptide functional. Molecular cause of a peptide secondary structure profile has more than one function when it interacts with target protein or act as a linker or anchored one. Solvent property influence the parameter of a peptide structure eg: Temperature, pH, ratios of solvent volume, multimeric composition in the solvent. Before to design druggable molecule and to study drug likeliness disease, the properties of peptide and functions should be studied. But there is no proper computational annotations for peptide nature and behavior in different solvent. So this study targets to annotate functional monomeric peptides that could target "dis-ease" and "dis-order" profile. To probable short sequence of residues, Microbial Surface proteins were retrieved from Meta-Protein sequence Database. Library of Protein Sequence were subjected to variate differentially in length and functional features. There are 1809 peptide sequence were retrieved and analyzed features of is same. Totally six features were adopted from obtained peptide sequence such as length, residual classification, secondary structure properties, conformational features (co-ordinates), volumes of peptide structure and functional features. Output of this study concentrates on making a peptides confined to make use for Cancer Drug Discovery purpose and Library Construction.

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Margarita Chudenkova

Changes in mitochondrial respiratory chain supercomplexes in human tumor tissues.

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Mitochondria are multifunctional organelles responsible for energy metabolism via oxidative phosphorylation. This process is carried out by protein complexes present in the inner membrane of mitochondria. Recently, it has been shown that three of these complexes (NADH-dehydrogenase (CI), coenzyme Q-Cytochrome C reductase (CIII) and Cytochrome-C-oxidase (CIV)) can be associated together to form supercomplexes (SCs); SCs that comprise all three complexes are called respirasomes. While the exact function of these structures remains unknown, there is evidence that SC abnormalities may affect tumorigenesis. However, little data is available concerning the exact molecular changes in structure and formation of SCs in cancer due to lack of studies with patients' biopsy samples.

The present work was carried out using biopsy materials from patients suffering from gastric and colorectal cancers acquired in close collaboration with surgical oncologists. The goal of this research was to study changes in supercomplex components and activities of individual complexes in mitochondria isolated from human tumor tissues.

We used two-dimensional Blue-Native polyacrylamide gel electrophoresis to separate and identify the supercomplexes present in healthy and tumor human tissues, as well as applied in-gel and spectrophotometric CI and CIV activity assays to measure the activities of these complexes in the analyzed tissue samples.

Our results demonstrate the absence of high molecular weight respirasomes and the decrease in the content of low molecular weight respirasomes in tumor tissues as compared to healthy gastric tissue. This finding correlates with the results acquired in previous studies on human cell lines (Baracca et al., 2010). Moreover, our study showed an overall decrease of CI activity and, in contrast, increase in CIV activity in tumor tissues compared to healthy gastric tissue. These changes in activities of two respiratory chain complexes are in line with the data of Feichtinger et al., 2017, which were performed on biopsy materials from patients with gastric cancer. In addition, we found a redistribution of both protein and CI activity staining in-gel from respirasomes in healthy tissues into supercomplexes comprising CI and a dimer of CIII in both tumor tissues.

Our study addresses the significance of biopsy materials in research. In spite of the heterogeneity of tumor cells even within a single population, we were able to show and explain some alterations in the analyzed tissues. However, further studies of supercomplex formation and factors controlling this process are needed to gain a profound understanding of molecular properties of tumor cells.

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Hemant Arya

Natural product inspired drug discovery from *Clerodendrum colebrookianum Walp.*: Fragment-based drug design for anticancer drug lead

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Rho-associated coiled-coil protein kinase (ROCK) is a serine/threonine protein kinase which plays an important role in actin organization, proliferation, apoptosis, cell invasion and metastasis. Altered ROCK pathway is implicated in the development and progression of cancer. Our previous in silico studies have revealed that constituents from the plant Clerodendrum colebrookianum Walp., such as acteoside, martinoside and osmanthuside β6 could interact with ROCK I/II. In this study, three analogs of acteoside, martinoside and osmanthuside β6 were designed using fragment-based drug design approach and their interactions with ROCK I/II were investigated using docking and molecular dynamics simulation studies. The designed analogs made stable hydrogen bond interactions with the hinge region residues Met156/ Met157 of ROCK I/II. Based on the encouraging insights from in silico studies, the three analogs were synthesized in two steps and tested for in vitro anticancer activity along with their intermediates on different cancer cell lines (SW480, MDA-MB-231 and A-549) using MTT assay. The in vitro results indicated that the acteoside analog 3a (4-[3-(3,4-di-hydroxy-phenyl)-acryloyl]-[1,4] diazepane-1-carboxylic acid tert-butyl ester) has highest anticancer activity in all the three tested cell line, virtually equal to fasudil (standard ROCK inhibitor). These results suggest that the natural product inspired drug design could be a useful strategy for identifying lead molecules to cure cancer. Further studies are required to delineate the full potential of the lead 3a as an anticancer molecule.

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Olayinka Aina

Synthesis, Drug Likeness and Antioxidant Activity of Analogues of L-Arginine

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Free radicals damage is responsible for the development many chronic health problems. 3 analogues of L-arginine were synthesized and their structures were confirmed with FTIR, 1H and 13C NMR. Molecular properties and bioactivity prediction of the synthesized compounds was carried out with molinspirationsoftware. The antioxidant activity of the synthesized compounds was evaluated in vitro using DPPH radical scavenging assay. The compounds obeyed Lipinsky rule of five and showed good bioactivity scores. The analogues A1-A3 exhibited low antiradical activity against with IC50 value of 631.06, 501.19 and 537.02μg/ml respectively.

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Surabhi Swarnkar

Cardiac progenitor stem cells and growth factor cocktail as therapy in Myocardial Infarction

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Recent strides in regenerative medicine have established the potential scope of stem cells as a therapeutic modality for myocardial infarction (MI). Stem cell therapy has the potential to limit the extent of post infarction cardiac damage by accelerating the normal process of healing, improving vascularization, inhibiting apoptosis, and potentially regenerating cardiac muscle. However, the efficacy of this approach is limited due to the hostile microenvironment of the acutely injured myocardium. Some putative secreted paracrine factors are involved that confer cardioprotective benefits like migration, homing, cell proliferation, cytoprotection, contractility, angiogenesis, and stabilization of both native and non- native progenitor cell types. We therefore hypothesized that a combination of these growth factors along with the stem cells should be a more superior therapeutic modality as it would provide a conducive milieu for the stem cells and may be able to circumvent the limitations of cell based therapeutics.

Rat models of myocardial infarction were created to assess the in vivo efficacy of this combinational therapy and its superiority as compared to stem cell or growth factor therapy alone. Subsequently after MI, interventions were given intra-myocardially and the animal models were then followed for a period of twenty eight days which is considered equivalent to two years in human context, to assess cardiac function by echocardioraphy. At the end of follow-up, the animal models were sacrificed and histopathology was performed on the infarcted tissue.

Serial echocardiography revealed that the combinational therapy group had improved cardiac function twenty eight days after MI as compared to stem cell or growth factor group, with heart function parameters reaching towards their baseline values. Histopathological analysis of infarcted tissue revealed lesser extent of typical MI characteristics like replacement fibrosis, presence of granulomatous tissue and granulocytic infilterates in the combinational therapy group. Altogether, the findings substantiate the in vivo efficacy of the combinational therapeutic modality and as evidenced by animal model experiments, it holds great translational value in a clinical MI setting.

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Sababil Ali

Sensitive and less invasive confirmatory diagnosis of visceral leishmaniasis in Sudan using loop-mediated isothermal amplification (LAMP)

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Background: Confirmatory diagnosis of visceral leishmaniasis (VL), as well as diagnosis of relapses and test of cure, usually requires examination by microscopy of samples collected by invasive means, such as splenic, bone marrow or lymph node aspirates. This causes discomfort to patients, with risks of bleeding and iatrogenic infections, and requires technical expertise. Molecular tests have great potential for diagnosis of VL using peripheral blood, but are expensive, require well-equipped facilities and trained personnel. More user-friendly, and field-amenable options are therefore needed. One method that could meet these requirements is loop-mediated isothermal amplification (LAMP) using the LoopampTM Leishmania Detection Kit, which comes as dried down reagents that can be stored at room temperature, and allows simple visualization of results.

Methodology/Principal Findings: The LoopampTM Leishmania Detection Kit (Eiken Chemical Co., Japan), was evaluated in the diagnosis of VL in Sudan. A total of 198 VL suspects were tested by microscopy of lymph node aspirates (the reference test), direct agglutination test-DAT (in house production) and rK28 antigen-based rapid diagnostic test (OnSite Leishmania rK39-Plus, CTK Biotech, USA). LAMP was performed on peripheral blood (whole blood and buffy coat) previously processed by: i) a direct boil and spin method, and ii) the QIAamp DNA Mini Kit (QIAgen). Ninety seven of the VL suspects were confirmed as cases by microscopy of lymph node aspirates. The sensitivity and specificity for each of the tests were: rK28 RDT 98.81% and 100%; DAT 88.10% and 78.22%; LAMP-boil and spin 97.65% and 99.01%; LAMP-QIAgen 100% and 99.01%.

Conclusions/Significance: The excellent performance of LAMP using peripheral blood indicates that it can be included in the algorithm for diagnosis of VL, avoiding the need for invasive lymph node aspiration. The simplicity of the test makes it a promising candidate for confirmatory diagnosis in settings that are lower than the reference laboratory

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Kehinde Aina

Malaria and West –Nile Virus Co-infection amongst Febrile HIV Infected Patients attending a Tertiary Hospital in Abuja, Nigeria.

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Background: Malaria and West Nile fever has ubiquitous distribution in Africa, mostly in sub-Saharan Africa where the prevalence of HIV infection is increasing. Many febrile patients are most times underdiagnosed or misdiagnosed with malaria due to striking similarities, such as fever shared by malaria and certain arboviral infections. Clinical symptoms of WNV fever often overlap with other agents of febrile illnesses. Over the years, the geographical range of WNV activity has increased and the virus has become established even in non-endemic areas where it has not been previously detected.

Methods: This serological-survey investigated the prevalence of anti-WNV IgM and Malaria among HIV infected patients with febrile illnesses at Gwagwalada metropolis, Abuja. Between the period of May and August 2016, a total of 171 participants attending the University of Abuja Teaching Hospital were recruited for the study. Serum samples were immediately harvested, stored and analyzed using the indirect ELISA for anti-WNV IgM antibodies using kits endorsed by the World Health Organization and also Microscopy and RDTs for Malaria. Socio-demographvariables and clinical data was gotten using a self-administered interviewer-based questionnaires.

Results: Out of the 171 febrile participants, the overall prevalence of WNV IgM antibodies was 66.1%. whereas 29.2% were positive for Plasmodium falciparum. About 31.4% were positive for both WNV virus and P. falciparum. Significant association was observed in prevalence of WNV IgM and Malaria/WNV co-infection ($p< 0.5$). Sixty two (54.9%) of WNV seropositive females and 51/113 (45.1%) seropositive males was recorded. With regards to participants' knowledge, attitude and practice towards preventive measures against WNV, significant association was observed between the WNV IgM seropositivity and the use of mosquito repellents ($p =0.016$).

Conclusions: Findings from this study necessitate the need for routine diagnosis and surveillance of WNV as possible agents of febrile illness in Nigeria. More so, infected patients should be closely monitored in order to detect possible associated sequelae.

Keywords: (West-nile Virus, Prevalence, Endemicity,)

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Abdelrahim Hamad

The transmission rate of the zica virus from mother to child

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Al Neelain University

The transmission rate of the zica virus from mother to child .The research describes the rate of transmission of ZIKA from infected mothers to their children during and after childbirth and how it is transmitted and inherited if found

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Mohd Shamoon Asmat

Elucidating binding interactions of high performance lipase with novel fabricated nanocellulose fused polypyrrole/graphene oxide nanocomposite as promising nanobiocatalyst: Multi-spectroscopic and molecular level insights

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Enzymes perform an essential role in catalysing extensive reactions and enzymatic biocatalysis has a vast reputation on an industrial scale. The major hindrance faced by enzymes is their denaturation under various conditions. This work was performed to describe the facile procedure of a novel nanobiocatalyst, nano cellulose fused polypyrrole/graphene oxide nanocomposite for the efficacious immobilization of lipase, a versatile hydrolytic enzyme having potential applications in industries. The fabricated nanocomposite was characterized using Fourier transform infrared spectroscopy, differential thermal analysis, thermogravimetric analysis, X-ray diffraction, scanning electron microscopy, atomic force microscopy, transmission electron microscopy, and Candida rugosa lipase was immobilized onto nanocomposite through physical adsorption. The catalytic efficiency and operational stabilities of immobilized lipase were improved significantly compared to the free lipase. The reusability profile outcomes showed that the immobilized lipase formulation was an outstanding nanobiocatalyst as it retained 85% of its original catalytic activity after 10 cycles of application. The nanobiocatalyst was employed for the synthesis of the fruit flavour compound, ethyl acetoacetate. The immobilized lipase successfully synthesised flavour compound in solvent free media and n-hexane having 27.5% and 75.5% ester yields respectively. Moreover, these outcomes demonstrating graphene oxide modified carrier induced stabilization, amended solvent tolerance and operational stability of immobilized enzyme, will have quintessential influence on practical scale up of biotechnological industries. Molecular docking outcomes confirmed the successful binding of the enzyme-nanocomposite macromolecular complex.

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Frank Richter

ROMO1 Links Protein Import to Quality Control and Morphology in Mitochondria

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Mitochondria are vital organelles that perform fundamental functions such as ATP synthesis, iron-sulfur cluster formation and apoptosis regulation. Their importance is highlighted by the fact that mitochondrial dysfunction can lead to a plethora of diseases, especially affecting the brain, muscle or heart.

While mitochondria have maintained their own genome, more than 99% of its proteome is imported by dedicated translocation machineries. Different translocation complexes ensure that every protein reaches its right compartment within mitochondria. Regarding proteins destined for the mitochondrial matrix or the inner membrane, their import is facilitated by the presequence translocase of the inner membrane (TIM23 complex). Most of its functional characterization has been carried out in S.cerevisiae.

We purified the human TIM23 complex using its FLAG-tagged components TIM23, TIM21 and TIM50 upon differential stable isotope labeling with amino acids in cell culture (SILAC). In the overlapping population of all three analyses we observed a high enrichment for a protein named ROMO1 (Reactive Oxygen Species Modulator 1).

The upregulation of the small inner membrane protein ROMO1 has been correlated with high levels of reactive oxygen species (ROS) especially in cancer cells and tissue. Furthermore, it plays a role in the regulation of mitochondrial morphology. However, the molecular function of ROMO1 is unknown.

Here, we elucidated the molecular function of ROMO1 in mitochondrial protein import using a CRISPR/Cas9 mediated knockout as well as siRNA mediated knockdown approach. We show that ROMO1 interacts with the TIM23 complex and couples TIM21 and TIM23 to one another. While ROMO1 does not play a role in general protein import, we established an interesting link to protein quality control and inner membrane morphology.

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Daryna Demenko

Molecular Mechanism of Factor Xa-Inhibitors Interaction

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Cardiovascular related diseases, being the major cause of mortality in developed countries, demand development of new anticoagulant drugs. Factor Xa (FXa), interconnecting internal and external blood coagulation pathways, plays the important regulatory role in blood coagulation functionality. Therefore, FXa is considered as one of the main targets for therapeutic blood investigations.

However, it is important not only to conduct biological tests, but also to discover and understand the key interactions between protein and ligand.

In the present study we provide detail description of mechanism of molecular interaction between factor Xa of blood coagulation cascade and its direct inhibitors.

For our research several software packages and databases were used. Structural compounds of Xa inhibitors taken from ChEMBL and filtered according to ADME parameters that resulted in decreasing Enamine database from 2.5m to 1.75m compounds. Also, stereoisomers were generated for them and the structures were translated into a format suitable for docking.

A Schrodinger program was used to prepare binding site of the FXa from PDB crystal structures (entry 1KSN and 1F0S). After analysis subpockets' structure and interactions with native ligands, 4 main constrains within S1 and S4 were posed and docking was performed.

Molecular docking was based on 2 pharmacophore models: either presence of an amine component as imitation of arginine or a polarized aromatic fragment or a halide occupying the volume of S1.

This resulted in virtually generated target library of 1397 inhibitors, based on all aforementioned criteria. Those compounds belong to different chemical classes.

Selected series for each model were tested using a modified RVV test (RVVT) and inhibitor activity.

Biological screening on purified FXa within compounds showed activity ranging 40-60%. The most effective among them inhibitors were used in the RVVT on human blood plasma. The result of test emerged efficiency of 17 substances within 70-100% and 2 inhibitors reached the highest capability to reduce FXa activity.

Further, related substances with the highest and the lowest efficiency were identified and dynamic analysis was performed based on them. Such test leaded to discovering mechanism of interaction within subpockets and influence of different functional groups on a compound's ability to decrease enzyme's activity.

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Maryia Fando

Crystatallization of a LSM Protein from *Halobacterium Salinarium*

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Proteins of Lsm (Sm-like) family are found in all the three domains of life. They provide biogenesis and functioning of various RNA molecules in the cells. Bacterial Lsm protein called Hfq exhibit chaperone activity promoting interaction between regulatory sRNA and mRNA during regulation of translation. Eukaryotic Sm proteins are core proteins of the spliceosome while eukaryotic Lsm proteins are involved in the mRNA degradation. Functions of archaeal Lsm proteins (SmAP) in the cells have been studied pitiable, although there is some data on their participation in processing of some RNA (such as tRNA).

Our work concerns with structural and functional studies of SmAP protein from Halobacterium salinarum. This protein has remarkable difference of the sequence compared with homologues and, in fact, represents a minimal Lsm core. Our current task is to determine the structure of this protein and its complexes with ribonucleotides and short RNA to define the specificity and structural aspects of the SmAP-RNA interaction. We have obtained a genetic construct carrying the gene of the SmAP from H. salinarum (HsaSmAP). The protein has been isolated and purified in preparative scale, then it has been crystallized and a high resolution diffraction dataset has been collected at ERSF in Grenoble. At this moment, the HsaSmAP structure is under determination and refinement.

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Abu Imran Baba

Comparative analysis and subcellular localization of CDPK Related Kinase (CRK) family in *Arabidopsis thaliana*

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The CDPK-Related Kinase (CRK) family is closely related to CDPKs and consists of eight members in Arabidopsis plant. Unlike CDPKs limited information is available about the functional role of plant CRKs in vivo in Arabidopsis thaliana, only their membrane localization have been predicted so far. The substrate specificities of Arabidopsis CRKs are also not well known, except for AtCRK1 which regulates heat and salt tolerance and AtCRK3 involved in leaf senescence. Additionally, AtCRK5 was shown to have a direct role in the regulation of root gravitropic response involving polar auxin transport. In this study we report the comparative analysis of all Arabidopsis CRK genes involving their biological function and subcellular localization. Morever the investigation of gravitropic features of the all the CRK family members reported delayed gravitropic responses upon gravistimulation and the cloned versions of all 35S::cCRK-GFP N-Terminal tagged Green Fluorescent protein (GFP) localization pattern indicated that all Arabidopsis CRK protein kinases have altogether plasmamembrane localization based on transient protoplast and inplanta microscopic analysis. While Atcrk mutants were indistinguishable from wild type plants in short day conditions, but Atcrk1-1 mutant had serious growth defects under continuous illumination. Semi-dwarf phenotype of Atcrk1-1 was accompanied with disturbed photosynthetic fitness, accumulation of singlet oxygen, and enhanced cell death in photosynthetic tissues. AtCRK1 is therefore important to maintain cellular homeostasis during continuous illumination.

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Kateryna Uspenska

Role of Mitochondrial Nicotinic Acetylcholine Receptors in Knock-Out Mice and after Partial Hepatectomy in Rats

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Nicotinic acetylcholine receptors (nAChRs) are classically regarded as ligand-gated ion-channels located in plasma membrane, regulating neurotransmitter and cytokine release and supporting the viability of many cell types. We have found that α7β2, α3β4, α4β4 nAChR subtypes are present in intracellular compartment – in the outer membrane of mitochondria. Mitochondrial nAChRs regulate the early stage of mitochondrial apoptosis, namely, cytochrome c (Cyt c) release under the effect of apoptogenic agents like Ca2+ or H2O2. Cyt c release from mitochondria can be attenuated by either nAChRs agonist or antagonist indicating that the nAChR signaling in these intracellular organelles is ion channel-independent and is mediated through conformational changes of the nAChR molecule upon specific ligand binding. The aim of the present study was to evaluate the role of nAChRs in mitochondria under different physiological conditions.

Experiments have been performed in C57Bl/6, a7-/-, a3+/-, a7b2-/-, b4-/- mice or Wistar rats. Mitochondrial and non-mitochondrial fractions were isolated from either the liver or brain by differential centrifugation according to standard procedures. The level of nAChR subunits was studied by Sandwich-ELISA using subunit-specific antibodies. The apoptotic resistance of mitochondria was evaluated based on the level of Cyt c released under the effect of Ca2+ or H2O2.

It was found that the lack of α7, α3, β2 or β4 nAChR subunits didn't change dramatically the sustainability of mitochondria to apoptogenic doses of Ca2+. We found that the absence of nAChR subunits in mitochondria of knockout mice was compensated by significant increase of α9 and/or β4 subunits. α-conotoxin PeIA, specific ligand of α9 nAChRs, attenuated Cyt c release more effectively from α7-/- mitochondria than from wild-type, especially affecting Ca-stimulated Cyt c release. In contrast, it was found that α7-, α3-, α4- and, especially, α9-containing nAChRs increased in the rat liver mitochondria 3-6h after partial hepatectomy (when 70% of liver is removed) resulting in increased mitochondria resistance to 0.1-0.9μM Ca2+ and 0.1-0.5mM H2O2. 12 hours after partial hepatectomy the level of nAChRs in mitochondria returned to per-operational level.

It is concluded that the presence of several nAChR subtypes in mitochondria provides an effective protection of mitochondria from different apoptogenic factors. Mitochondrial nAChRs play an important role in cell survival after partial hepatectomy, but not in cell proliferation.

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Polina Pikus

Comparison of the efficacy of Free and encapsulated human umbilical cord MSCs in the correcting rat liver cirrhosis induced by CCl4.

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Mesenchymal stem cells (MSCs) represent an attractive tool for stem-cell-based therapy of liver diseases. We aimed to evaluate the therapeutic potential of MSCs from human umbilical cord, a readily available source of MSCs, in the CCl4-induced liver injury model.

MSCs are the most promising candidates for regenerative medicine due to their unique properties. In particular it is the possibility to use MSCs from different sources to cure liver damage. The aim of this study was to compare the efficiency of human umbilical cord MSCs transplantation in the conditions of intravenous injection of MSCs as cell suspension and intraperitoneal injection of MSCs encapsulated in alginate microcapsules for the treatment of rat liver cirrhosis. MSCs were isolated from human umbilical cord using explants method. After culturing in vitro MSCs meet minimum criteria accepted by The International Society for Cellular Therapy.

Wistar rats liver cirrhosis was caused by intraperitoneal injections of CCL4 solution in olive oil (1: 1) according to a particular scheme. After 13 weeks, the histological study showed the presence of changes in the structure of liver parenchyma typical for cirrhosis. At the same time, the level of EGF (epidermal growth factor) and αSMA (α-Smooth muscle actin) genes expression has sharply increased. At this stage, one group of animals (n = 6) was injected human umbilical cord MSCs (6-7x106 cells/kg of rat weight) of the second passage into the tail vein, another group was intraperitoneally injected with the same amount of MSCs encapsulated in alginate microcapsules. Control animals received either intravenously normal saline or intraperitoneally "empty" alginate microcapsules. Histological studies showed that in 2 weeks after MSCs transplantation, in both groups the amount fibrosis tissue in the liver begins to decrease and, by the 13-th week, amount collagen and others morphological parameters of liver almost returns to normal level.

A study of the EGF and αSMA expression in the liver of experimental animals showed that it begins to fall in both transplantation cases. Reduction of both gene expression levels occurs faster in the case of encapsulated MSCs administration. In particular, in 6 weeks after the encapsulated MSCs transplantation, EGF is practically not expressed in the rats' liver. Transplantation of the cell suspension into the tail vein results to the disappearance in the expression of EGF after 12-13 weeks. In control animals the liver recovery process is very slow and after 13 weeks there are clear signs of cirrhosis in the liver of those animals.

Studies have shown that human umbilical cord MSCs demonstrate therapeutic activity in the treatment of rats' liver cirrhosis induced by CCl4, both in cell suspension transplantation and in transplantation in the form of cells encapsulated in alginate microcapsules.

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Andrii Zinchenko

Chromosomal abnormalities of *Allium cepa (L.)* meristematical cells, caused by Manganese and Copper nanoparticles

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Nowadays, in crop production usage of colloidal solutions of nanometals are widely apply. However, data on the phytotoxicity of nanoparticles is still insufficient. That is why the potential impact of nanoparticles on biota requires careful study. Toxicity research of nanoparticles Mn, Cu, and their citrate forms can justify the expediency of using colloidal solutions of nanoparticles in agriculture considering of their safety for organisms and the natural environment.

We conducted ana-telophase method of chromosome aberration analysis and it shows that there is a low mutagenic effect under treatment of free citric acid, but Mn and citrate forms of both forms of nanoparticles showed absence of any level of mutagenic effects. Withal intensity of mutagenic effect of Cu were three times higher comparatively to the control. Which made it very high level of mutagenic effect.

For the action of nanoparticles of copper, a significant increase in the number of mitosis anomalies were observed. The most frequent of them were the loss of fragments of chromosomes in the metaphase, violations of the spiralization, chromosome retardation in metaphase and the formation of double or triple anaphase and telophase bridges.

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Oleksandr Savytskyi

MolDynGrid Virtual Laboratory: web-oriented grid-service dedicated to computational structural biology

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MolDynGrid (http://moldyngrid.org) is web-oriented virtual laboratories (VL) in Ukrainian National Grid (UNG, http://ung.in.ua/) and European Grid Infrastructure (https://www.egi.eu/) dedicated to computational structural biology particularly to molecular dynamics (MD) simulations of biological macromolecules and their complexes. In addition to standard MD analysis techniques provided by the GROMACS package, MolDynGrid VL features advanced analysis tools such as Pteros molecular modeling library (http://pteros.sourceforge.net/) and Distributed Analyzer Script (DAS) [Savytskyi et al., 2011].

Currently, MolDynGrid VL is used for MD simulations of various proteins with an emphasis on tyrosyl-tRNA synthetases (TyrRS) and its mutant forms associated with neurodegenerative disease (Charcot-Marie-Tooth disorder, DI-CMTC) in complex with substrates. TyrRS one of the key enzyme of protein biosynthesis, which catalyzes the aminoacylation of tRNA(Tyr). In this work we constructed the model of the full-length HsTyrRS structure, DI-CMTC mutant forms of HsTyrRS using structure templates and studied its putative compactization by all-atom MD simulations. Twenty-six independent 100 ns MD trajectories of HsTyrRS and its mutant forms were computed using GROMACS software. The Contacts Analyzer Script (CAS) and the tRMSF tool of the Pteros molecular modeling library were used for analysis.

The strongest binding energy of ~1000 kJ/mol is observed for the second C-module of HsTyrRS. Antiparallel β-sheet formation in Ala 355 – Val 363 region was revealed for 3-100 ns time interval (~ 85 % of time). Extensive MD simulations, which were possible in the ARC-based UNG infrastructure, allowed us observing the hydrogen bonding between the residue R93 of the ELR motif and the residues A340 and E479 in C-module and finding local conformational changes (antiparallel β-sheet formation) in Ala 355 – Val 363 region. These findings support the idea that the full- length TyrRS lacks its cytokine activity because of the interactions between N-terminal and the C-terminal modules, which protect the ELR cytokine motif [Savytskyi et al, 2013, J Mol Recognit, 113-120]. We found the new role of connective peptide 1 (CP1) in mammalian tyrosyl-tRNA synthetase. Formation of strong interaction between Lys154 (located in CP1) and γ-phosphate suggests the additional role of CP1 insertion as an important factor for ATP binding. [Kravchuk, Savytskyi et al., 2017, J. Biomol. Struct. Dyn., 2772–2788]. Novel β-sheet formation was observed in the K147-E157 region in G41R and 153-156delVKQV mutant forms of HsTyrRS in the CP1 region of the Rossmann fold. The results support the idea, that defects of the intermolecular interfaces of complexes of mutational forms of HsTyrRS with cognate tRNA(Tyr) and/or translation elongation factor eEF1A may be a common molecular mechanism of Charcot-Marie-Tooth disorder.

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Leila-Anastasiia Karpets

The role of diacylglycerol kinase in the process of formation of phosphatidic acid induced by brassinosteroids in *Arabidopsis thaliana L*

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Brassinosteroids (BRs) have a profound impact in the formation of protective reactions in plants to the action of abiotic and biotic factors. The current direction of modern research is to find out the mechanisms of transduction of the signal of the BRs and its subsequent metabolic changes in the cell. An important part of this mechanism might be the phospholipid signaling network, which is one of the most important components of the transduction of signals.

In this work we investigated an effect of one of the most active BRs - 24-epibassinolide (EBL) on the formation of lipid second messengers, - diacylglycerol (DAG) and phosphatidic acid (PA). By using fluorescently labeled phosphatidylcholine (PC-BODIPY) we analyzed products of phosphatidylcholine hydrolysing PLC (EC 3.1.4.3) that hydrolyzes PC-BODIPY into DAG-BODIPY and PA-BODIPY. Hydrolysis of labeled lipids leads to the formation of DAG, which is subsequently, can be phosphorylated by diacylglycerol kinases (DAG-kinase) to PA. Phosphatidic acid, in turn, take its part signaling and regulation in plant cells. The transgenic plants dgk3,7, dgk1,2 and dgk5,6 of A. thaliana L. with double mutations in the diacylglycerol kinase genes were used in the experiment.

The results of our studies showed the involvement of DAG-kinases in the process of PA formation in response to EBL treatment. In wild type plants brassinosteroids induced formation of second messengers, while transgenic plants showed a decrease or no changes in the level of phosphatidic acid in response to EBL treatment. With the usage of the specific inhibitor of diacylglycerol kinase - R59022, a decrease in the phosphatidic acid level in response to EBL treatment was also observed comparing to untreated with inhibitor plants. dgk3,7, dgk1,2 and dgk5,6 lines accumulated less PA in response to treatment with R59022 and EBL comparing to wild type plants.

We also checked the importance of different isoforms of DAG-kinases and the effect of the loss of functional genes of DAG kinases on the growth and germination of plants under optimal and stress conditions. Under optimal conditions, the effect of the specific inhibitor of BRs biosynthesis – brassinazole, did not reveal a significant difference between wild type plants and transgenic plants with mutations in DAG-kinases genes. In the same time, under mild salinity (75 mM of NaCl), the germination of mutant plants was significantly reduced, which could be partially restored with the application of exogenous EBL. The obtained results indicate that the number of isoforms of DAG-kinases are involved in the formation of the PA in the response to the action of the EBL and loss of some isoforms of DAG-kinases are critical for induction of germination program stimulated by BRs.

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Jyoti Aggarwal

Low-Technology water purification techniques

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A goal of ENVS 600, Concepts in Earth and Environmental Science is to learn to utilize the scientific method in teaching elementary and secondary students the techniques of doing science. Key to this goal is demonstrating that teachers have the capacity to develop appropriate and relevant scientific studies with easily available resources and techniques. Inexpensive and convenient water purification is a necessity of life in many developing countries, and among such groups as backcountry hikers and others living off the grid in the U.S. This project tests effectiveness of low-technology water purification techniques. Several simple chemical mechanical technique are compared in their capacity to remove harmful bacteria, and produce good tasting clean water. The environmental science results of this project are also considered with the economics and logistics to draw conclusions about the feasibility and value of these water purification techniques. The results also are related to the goal of teaching the scientific method by doing relevant, logistically easy classroom scientific studies. This research focuses on *E.coli* bacteria present in water.

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Rispah Nyambura Ng'ang'a

Characterization of Wild Baboon's' Plant Diet through DNA Barcoding

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DNA barcoding involves the standardized use of one or a few DNA regions to distinguish species. DNA barcodes can be applied as tools for addressing fundamental questions in diet analysis, which is a prerequisite for fully understanding the biology of a species and the functioning of ecosystems. The most efficient and accurate method to identify the taxa consumed by a particular animal is to compare DNA sequences extracted from gut contents and scats directly to a reference DNA barcode library of the food items. The quality of the identification will depend on the completeness and accuracy of the sequences included in the reference library. When DNA sequences for potential food items are missing in the database, the contents of the gut and scats can be misidentified. The aim of this research is to develop a reference set of DNA sequences to characterize the plants that comprise the diets of wild baboons (*Papio cycnocephalus*) living in the Amboseli ecosystem in Kenya.

The Amboseli baboons, have been observed to consume a diverse diet, including the fruits, leaves, stems and seeds of several trees, bushes, herbs, and grasses. To create the reference library, we will analyze two commonly used plant DNA barcodes, namely the intergenic spacer trnL-p6 and the internal transcribed spacer 1 (ITS1) regions. DNA will be extracted from forty-six plant samples, collected from the ABRP study site. Next, trnL-p6 and ITS1 loci will be amplified and sequenced. The data generated will be used to establish an open access library, for reference by other studies involving diet analysis of East African animals.

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Farahnoosh Doustdar

Analysis of Quorum sensing related genes in Pseudomonas aeuroginosa isolates from Cystic fibrosis patients

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Introduction: Chronic Pseudomonas aeruginosa infections cause significant morbidity in patients with cystic fibrosis (CF). The pathogenesis of these infections is multifactorial and the production of many virulence factors is regulated by quorum sensing (QS), a cell-to-cell communication mechanism. The two well defined QS systems in P. aeruginosa, the las and rhl systems, rely on N-acyl homoserine lactone signal molecules, also termed auto inducers. QS regulates the production of pathogenic virulence factors and biofilm formation in P. aeruginosa. The four genes lasR, lasI, rhlR and rhlI were found to regulate this QS system. On the hand it is shown that despite the pivotal role of QS in the pathogenesis of P. aeruginosa respiratory infections, QS-deficient strains are still capable of causing infections and tend to be less susceptible to antimicrobials and also non-functional LasR isolates show a selective advantage in the late stationary phase of disease. To elucidate the dynamics of P. aeruginosa QS systems during chronic infection of the CF lung, we have investigated QS Genes in isolates obtained from CF patients.

Methods: Thirty six P. aeruginosa clinical isolates were collected from 6 months to twelve years old CF patients. Susceptibility to different antibiotics was tested using disk diffusion method. QS Genes including lasR, lasI, rhlR and rhlI were amplified by polymerase chain reaction (PCR), and PCR results subsequently underwent sequencing.

Results and Conclusions: The most resistance was against Amikacin (72.22%) and Ceftazidime (55.55%) and the most susceptibility was observed to Ciprofloxacin (75.00%) and Gentamicin (61.11%) respectively. All of 36 clinical isolates harbored all of quorum sensing related genes including lasR, lasI, rhlR and rhlI genes. Sequence analyses of these isolates showed that 41.66 and 33.33 percent of isolates had point mutations in lasR and lasI genes respectively. These results emphasize the importance of QS genes in establishment and virulence of P. aeruginosa isolates in cystic fibrosis, however QS deficient clinical isolates occur and are still capable of causing clinical infections in these patients. It could be concluded that during the chronic lung infection of patients with cystic fibrosis (CF), P. aeruginosa can survive for long periods due to adaptive evolution mediated by genetic variation. Hyper mutability and loss of quorum sensing is considered to play an important role in this adaptive evolution.

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Olga Zaitseva

The features of protein modifications formation in rats with vitamin D insufficiency

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Today vitamin D3 insufficiency is a worldwide problem. The lack of D3 causes mineral metabolism disturbance, immune system weakening and leads to different disease development in particular osteoporosis. By turn D3 deficiency reduces endothelial nitric oxide (NO) synthase expression and NO production.

In this case the aim of the research was to analyze the effect of D3 deficiency and additional enhanced nitric oxide production on the oxidative processes activity (by EPR spectroscopy using) and to investigate possible formation of posttranslational modifications (PTMs) in proteins (by UPLC method using).

D3 deficient experimental model (duration for 45 days) was performed on female Wistar rats by using of a synthetic diet without D3 and combined with balanced calcium (1.2%) and phosphorus (0.7 %) content. For NO synthesis activation D3 deficient rats were intraperitoneal injected with 0.05 mg of Bacillus Calmette-Guerin (BCG) vaccine at 30 day of experiment.

In liver of D3 deficient rats was elevated dinitrosyl iron complexes (DNIC) level (2 times vs. control) that may indicate about endogenous NO synthesis activation as compensatory reaction. It was also observed the decrease cytochrome P450 level in liver and increase methemoglobin level in blood vs. control. Activation of NO synthesis by BCG causes the elevation of free mitochondrial radical compounds (1.7 times) and DNIC level (2.5 times) vs. D3 deficient rats. Analysis of the amino acids UPLC chromatograms of acidic hydrolyzed blood proteins has shown that lack of D3 causes the enhancing of proteins PTMs. We have found the elevation (up to 7 times) of modified amino acid vs. control. Under NO hyperproduction significant effect hasn't been shown.

Thus, obtained results indicate that vitamin D3 deficit leads to formation of new PTMs in proteins. In our opinion PTMs can be the target for novel approaches development to normalization of disturbances that occur at vitamin D3 insufficiency.

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Brijesh Yadav

Molecular Network and Structural Insights into Interleukin-18 (IL-18) gene in Mus masculus

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Interleukin-18 (IL-18) protein is a pleiotropic pro-inflammatory cytokine belonging to the IL-18 superfamily. IL-18 plays an important role in host innate and adaptive immune defense but its aberrant activities are also associated with inflammatory diseases such as rheumatoid arthritis and Crohn's disease. In this study Interleukin-18 gene from mice was isolated, cloned and sequenced. Consequently the network analysis was done to explore the functional role of IL-18 protein in animals. The protein three dimensional structure of IL-18 protein wasgenerated. Further three dimensional structure of IL-8 was docked with CPS ligand ((3-[(3- cholamidopropyl) dimethylammonio] -1-propanesulfonate)) and later the compex was subjected to molecular dynamics simulation for 50 ns.The network analysis explore the correlation of IL-18 protein with others proteins and their involvement in different significant pathway to defense the cell from various diseases. Until now, no structural details were available for smaller animal IL-18 proteins, hence this study will broaden the horizon towards understanding the structural and functional aspects of this proteins in pet animals. The docked IL-18 protein and CPS ligand structure was more reliable and stable and it was confirmed by molecular dynamics simulation method.

IL-18 protein interacted with other proteins to protect animal from different immune related diseases. The three dimensional structure of IL-18 was generated and its interactions with CPS ligand demonstrates the role of key binding residues. CPS ligand has ability to future drug molecule to counteract IL-18 activity.

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Volodymyr Kavetskyi

Polymorphism of 5S rDNA intergenic spacer in some *Gentiana* species

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Gentiana L. plants are able to accumulate biologically active substances such as glycosides, alkaloids, xanthones, and flavonoids. Today, many species of *Gentiana* are under threat of extinction. They are subject of protection worldwide. The number of species within the *Gentiana* genus, the systematic value and degree of variability of morphological characters, and the taxonomic status of some polymorphic species remain unresolved. These questions can be solved by using molecular markers such as 5S rDNA sequences. Thus, our goals were to determine the nucleotide sequence of non-transcribed spacer (NTS) of 5S rRNA genes in some *Gentiana* species of the Ukrainian flora and to study the organization of this rDNA region.

DNA was isolated using CTAB method followed by PCR-analysis with primers for 5S rDNA, and agarose gel electrophoresis. Isolated PCR-fragments were cloned and sequenced. DNA sequences were aligned in Unipro UGENE software.

Our study showed that the length of non-transcribed spacer of *G. acaulis* was 503 bp. Four clones of *G.asclepiadea* non-transcribed spacers had length of 411 bp. The size of *G.lutea* NTS was 402 bp.

On the basis of alignment and comparative analysis of 5S rDNA NTS of these and additional 11 gentians, found in GenBank, two groups of species were identified, which differed significantly in the organization of this region. The first group included three East-Asian species of Pneumonanthe section, and the second comprised 7 species of *Calathianae* section, mainly from Europe, and three species under study. The feature of the first group is a CT-rich site of about 80 bp in length located behind the termination site, which is followed by a GC-rich region of approximately equal size. All representatives of the second group had a relatively conservative site with high homology to the coding portion of the 5S rRNA gene in this part of the NTS.

Non-transcribed spacer regions of *G.asclepiadea* and *G.acaulis* had sequence identity of 94%. Meanwhile percent of NTS sequence identity of *G.asclepiadea* and *G.lutea* was 97 %. Identity of the NTS regions of three species of the Pneumonanthe section *G.triflora*, *G.scabra* and *G.manshurica* was 98%. Species from the *Calathianae* section also showed a high level of similarity. Their sequence identities were at least 98%.

Nucleotide sequence of 5S rDNA NTS region was determined for three *Gentiana* species of Ukrainian flora. The features of the organization of the 5S rDNA NTS region are identified in European and Asian species of gentians.

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Faramarz Mehrnejad

Antimicrobial Peptide Pleurocidin-Membrane Interactions: A Molecular Dynamics Simulation Study

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Antimicrobial peptides (AMPs) have a clinical value that involve in immune response to the bacterial aggression and act as the first-line defense against invading microorganisms. Pleurocidin, a 25-mer cationic peptide, isolated from the winter flounder, has broad antimicrobial activity and has very low hemolytic activity against the human Red Blood Cells (RBC). In this research, we investigated the interactions of pleurocidin with the different membrane models using all-atom molecular dynamics (MD) simulation to clarify the selectivity of pleurocidin with different membranes. The results indicated that pleurocidin interacted weakly with a neutral phospholipid but strongly with an acidic phospholipid. MD results revealed that for early insertion peptide into membrane the tryptophan and phenylalanine residues in the N-terminal domain have a major role. The cationic residues have the most favorable energy for interaction in anionic membranes, but in the DOPC membrane, these residues induce repulsion between the peptide and the membrane. This study provides useful information on the

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Ziad Khattari

Interfacial behavior of a Myristic acid in mixtures with DMPC and Cholesterol

Ziad Khattari

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Binary mixture monolayers of Myristic acid (MA) with the same length of saturated acyl chain lipid viz 1,2-myristoyl-sn-glycero-3-phosphocholine (DMPC) and Cholesterol (Chol), were investigated under different experimental conditions using Langmuir monolayers (LMs). The interfacial pressure-area (π-A) isotherms, excess molecular area, excess free energy and fluorescence microscopy (FM) images were recorded at the air/water interface. Monolayers of both systems (e.g. MA/DMPC, MA/Chol) reach the closest acyl hydrophobic chain packing in the range 0.20 < xMA< 0.70. Thermodynamic analysis indicates miscibility of the binary mixtures when spread at the air/water interface with negative deviation from the ideal behavior. Morphological features of MA/DMPC systems were found to depend strongly on MA mole fraction and pressures by showing two extreme minima in Gibbs free energy of mixing, while MA/Chol systems showed only an effective condensing effect at xMA =0.90. In the whole range of compositions studied here, the liquid-expanded (LE) to liquid-condensed (LC) phase transition occurs at increasing xAM as it accomplished by a huge increase in the inverse compressibility modulus. FM observations confirmed the phase-transition and condensing effects of both mixture monolayers as evidenced by Gibbs free energy of mixing in a limited range of compositions.

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Andrii Siromolot

HB-EGF neutralizing antibodies could block mitogenic effects of HB-EGF and its binding with receptors

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Heparin-binding EGF-like growth factor (HB-EGF) belongs to the epidermal growth factor receptor family and is synthesized as a transmembrane precursor proHB-EGF. HB-EGF is involved in the regulation of many vital processes such as implantation of the blastocyst, placentation and protection of trophoblast from hypoxia, wound healing and post trauma recovery, angiogenesis, hematopoiesis, plasticity of nerve synapses, etc. However, HB-EGF in many cases plays a key role in acquiring cells of the malignant phenotype, in particular the ability to oncogenicity, invasiveness, metastasis, and resistance to chemotherapy.

The promising direction of modern oncotherapy is the search for specific blockers of growth factors and signaling pathways that they activate. For this purpose, we have obtained polyclonal and single chain Fragment variable (scFv) HB-EGF neutralizing antibodies to investigate their anti-mitogenic effect and test the ability to block the signaling pathways that are activated by HB-EGF and are responsible for cells survival and proliferation.

Mouse and rabbit immune sera containing polyclonal antibodies against HB-EGF have been obtained, among which was chosen the most specific to recombinant antigen sHB-EGF-TRX. HB-EGF neutralizing antibodies have anti-mitotic and cytotoxic effects in relation to A431cell line which origin from human adenocarcinoma and express proHB-EGF, as well as HER1 and HER4 receptors. It has been shown that immune serum containing polyclonal antibodies against HB-EGF is able to inhibit HB-EGF binding to surface cellular receptors and block the mitogenic activation with HB-EGF of cells by deactivating Ras-MAPK/ERK1/2. These antibodies can be used to create new approaches for cancer treatment therapy.

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Sarah Henze

Kinetic analysis of signaling cascades involved in the adaptive immune response

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The adaptive immune system provides a specific and directed response against pathogens. T lymphocytes are activated upon recognition of nonself antigens presented on a cell's surface by a Major Histocompatibility Complex I (MHC I) molecule. The process resulting in this presentation is called MHC-I-restricted antigen presentation pathway. It follows five steps: proteasomal processing of the antigen into fragments (epitopes), TAP-mediated transport of these peptides into the ER, ERAP-mediated processing, loading of the peptides onto the MHC complex and presentation on the cell surface. To understand the relevance of each step, a quantitative modelling approach is mandatory. The model shall be able to estimate the intensity of CD8+ T cell clone responses towards an epitope by providing the relative amount of each component measured through proteomics in the target cell. It will be calibrated against the experimental data of six selected target epitopes using likelihood-based Bayesian inference methods for deterministic descriptions, and using approximate Bayesian computation (ABC) for stochastic descriptions. The model will allow us to understand why certain epitopes are able to elicit an efficient immune response, while others are not. This is of particular interest in the design of peptide based vaccines against pathogens and the development of targeted immunotherapies.

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Utkarsh Kapoor

Global Interplay of A-to-I RNA Editing and pre-mRNA Splicing

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Adenosine deamination type editing and pre-mRNA splicing are tightly interlinked processes. Adenosine deaminases acting on RNA (ADARs) recognize double stranded structures, typically formed between adjacent complementary sequences within one RNA. Frequently, editing events that lead to the recoding of mRNAs are defined by base pairing between the exonic editing site with an adjacent intronic editing complementary sequence (ECS). Consequently, editing needs to occur before the ECS is removed by splicing, implying that the speed of splicing affects the extent of editing. Conversely, it has been show that inhibition of editing can interfere with splicing. Lastly, nuclear and cytoplasmic editing levels can vary, indicating selective processing and / or export of mRNAs, depending on their editing status. Using inhibitors of splicing and genetic mouse models in which either one of the two editing enzymes ADAR1 or ADAR2 are deleted and carrying out RNA-Seq, we determine the interplay of pre-mRNA splicing and RNA editing. Our studies show an unexpected high level of intronic RNA editing events, exceeding the previously known number of editing events in the mouse. We show further, that inhibition of pre-mRNA splicing increases the rate of RNA editing, primarily in introns and UTRs. Lastly, we can show that inhibition of editing by ADAR1 can strongly affect alternative splicing while ADAR2 only has a minor impact on splicing. Currently, we are determining whether tissue specific splice rates can affect editing rates in vivo and explain the observed tissue specific variation in editing patterns despite relatively constant levels of the editing enzymes.

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Azat Kuluev

The Research of the T. Sinskajae on the Content of Immunogenic Peptides

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It is widely known that such species as wheat, rice and barley contain in the grains proteins of gluten, which belong to α-gliadins. These proteins are responsible for such pathologies as celiac disease (CD), food enteropathy with a prevalence of about 0.7-2% in the human population in genetically predisposed individuals and sensitivity to gluten. Proteins of α-gliadins contain three main immunogenic peptides: p31-43, which induces a congenital immune response; 33-mer peptide, which causes the most powerful immune response; as well as an additional epitope of DQ2.5-glia-a3, which overlaps partially with the 33-mer peptide. We conducted a study on the content of gluten in plants T. sinskajae. To do this, we selected the primer sequences for the α-gliadins of wheat. After amplification, we sequenced the sequences in CJSC "Evrogen" (Moscow). According to the data obtained, it was found that T. sinskajae contains the epitope DQ2.5-glia-a3, which is the least immunogenic. Also, a 33-mer peptide region was found, but it turned out to be reduced, not canonical. Thus, we assume that it is possible that T. sinskajae can be used as a hypoallergenic culture (for celiac disease and sensitivity to gluten). In studies of other scientists, it is indicated that T. aestivum is the most allergenic. A study of other diploid wheat shows that other species, such as T. monococcum, T. boeoticum, have altered areas of the 33-mer peptide. But, first, more careful studies of diploid species on the content of gluten are needed. Secondly, only T. sinskajae is the only easily threshed species among diploid wheat, so this species can be recommended as an agricultural crop with reduced allergenic properties.

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Omid Mashinchian

3D-Derivation of Uncommitted Human Muscle Stem Cells from iPSCs

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One of the most fundamental problems associated with stem cell therapy of skeletal muscle is the limited availability of cells that can robustly engraft into the stem cell compartment. It has extensively been attempted to isolate adult muscle stem cells (MuSCs) and expand them in culture to obtain sufficient cell numbers for such treatments.

The challenge associated with this approach is that, once isolated from their niche and maintained in culture, MuSCs become terminally committed to myogenic differentiation and show a dramatically reduced engraftment potential. However, the recent discovery of induced pluripotent stem cells (iPSCs) has opened new avenues for the in-vitro derivation of cell types that are more suitable for transplantation.

Here, we report a highly efficient approach for the scalable derivation of uncommitted MuSCs from human iPSCs in a biologically faithful 3D environment. We employed human iPSCs and a spectrum of immortalized cell lines to generate 3D aggregation conditions promoting mesoderm formation and subsequent specification to the myogenic lineage without the parallel upregulation of myogenic commitment markers.

Taken together, our project has revealed a novel highly efficient derivation protocol for uncommitted MuSCs from human IPSCs that can easily be scaled up to bioreactor level. Our novel protocol has fundamental implications for cell therapy of muscular dystrophy and will inspire future preclinical studies that will pave the way towards trails in human patients.

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Yana Raetska

Proteasomes activity in the rat's esophagus under acid burns of II stage.

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Acid esophagus burn causes different shifts in organs functions accompanied by pathology states such as inflammation or toxicity. Damages, which appear due burn, are stimulating proteasome-dependent proteins degradation.

The activity of proteasome in the esophagus was determined by the method of cleavage of fluorogenic peptides. Caspase-like (CP) and chymotrypsin-like (CT) activity were determined using Z-LLE-AMC and Suc-LLVY-AMC peptide substrates, respectively. The 7-amino-4-methylcumarin (AMC) product formed during the cleavage was measured at λex = 375 nm, λem = 440 nm on Synergy HT (BioTek, USA) microplateletspectrofluorometer.

CT activity by proteasome (26S) for 1 day after AEB decreased by 2.9 times relative to control. At 7 and 15 days after burn, this indicator was higher than control at 1.8 and 2.3 times respectively. At the 21st day of the CT activity begins to decrease, but remains higher than control, 1.4 times. A similar trend was observed in the study of 20S CT activity.

CP activity of proteasome (26S) and 20S tended to increase throughout the study period. Thus, for 1 day after AEB, an increase in CP activity was observed 2.1 times for 26S proteasome and 2.2 times for 20S relative to control. At 7, 15 and 21 days there was a further increase in CP activity. The maximum value of this indicator was at 21st day, 7 times higher than control.

According to the literature, statistically significant increases in activity of proteasome in plasma were observed in all groups of children 12-16 hours after the thermal burn.

The increase in proteasome activity promotes the formation of fibrosis in post-defective wounds, which occurs as a result of hyper proliferative growth of fibroblasts, and is associated with activation of the signaling pathway of TGF-β. As a rule, the level of intracellular signaling protein (Smad) that activates transcription of target genes in TGF-β stimulation is regulated by proteasome activity. Treatment for burn patients is often complicated by fibrosis, and the development of means using proteasome inhibitors is becoming increasingly popular as a way of preventing or reducing scarring.

Therefore, the obtained dates show that activity of proteasome in rat's esophagus increase after acid burn of II stage in compare with intact tissues. Such an increase in proteasome activity is due to an increase in the processes of intracellular proteolysis in burn wounds. In order to cell survive the activity of the system that deals with the degradation of damaged protein and peptide material increases. It should also be noted that the most significant changes were detected for CP activity by proteasome.

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Roza Szatkowska

Carbon flow through tricarboxylic acid cycle (TCA) is changed in yeast strains with altered RNA Polymerase III activity

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Citric acid cycle, also known as Krebs or tricarboxylic acid cycle (TCA), plays a key role in aerobic metabolism. The TCA cycle is responsible for the total oxidation of acetyl-CoA that is derived mainly from the pyruvate produced by glycolysis. Beyond that, Krebs cycle provides metabolic intermediates - precursors for the biosynthesis of ten amino acids.

Mutations causing perturbations in TCA cycle are nonlethal and display phenotypic effects only during growth on non-fermentable carbon source. According to Przybyla-Zawiślak et al. 1999 [1] null mutants of ADH1, SDH1, SDH2-4, FUM1 genes do not grow on glycerol as a carbon source (YPGly) and deletion of IDH1-2, KGD1-2 or LPD1 displayed slow growth phenotypes on YPGly. Similar phenotype display yeast MAF1 knockout mutant.

Maf1 protein is the only negative regulator of RNA Polymerase III (RNAP III) in yeast Saccharomyces cerevisiae [2]. Deletion of MAF1 causes up-regulation of RNAP III activity and tRNA accumulation on glycerol-based medium. One of the suppressors, that restores the ability of Maf1 deficient cells to grow in medium with glycerol as a sole carbon source at non-permissive temperature, is rpc128-1007 mutant [3]. A single nucleotide change, localized close to the nucleotide binding motif in the C terminus of the RNAP III catalytic subunit C128, causes a decrease of total tRNA amount in rpc128-1007 [3].

To investigate the intracellular distribution of metabolites in maf1-Δ and rpc128-1007, 13C NMR-based isotopomer analysis was performed. For this purpose, different isotopic tracers, [U-13C]-glycerol and [1,2-13C]-labelled glucose, were used for experiments performed in triplicate batch cultures for each case. The differences in labelling distribution in amino acid pool depend on 13C profile of TCA cycle intermediates. Alanine was used as a reporter molecule on isotopic labelling in pyruvate – the end point of glycolysis.

Our results suggest that both, up- and down-regulation of RNAP III activity, have a critical impact on carbon flux distribution in yeast cells. Changes in RNAP III activity result in significant decreased carbon flux through the TCA cycle, not observed in WT, while grown on glucose-based medium. Asparagine isotopomers 13C splitting suggest an anaplerotic pathway activation in both mutant strains.

Remarkably, maf1-Δ and rpc128-1007 yeast cells showed different patterns of TCA activity, while grown on non-fermentable carbon source. Maf1 deficiency resulted in Krebs cycle dysfunction. While, down-regulation of RNAP III activity was correlated with increased citric acid cycle activity.

Moreover, in maf1-Δ cells grown on 2% [U-13C]-glycerol supplemented medium at 30°C and shifted to 37°C for 1 h, the presence of [1,2-13C]-alanine isotopomer was observed. Therefore, it may indicate that the growth defect, of MAF1 lacking cells, result from changes in lower glycolysis.

[1] Przybyla-Zwislak B et al. 1999.
[2] Boguta M et al.1997
[3] Cieśla M et al. 2007

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Anna Bakhmachuk

Method for determination of Ig concentration using recombinant protein A as bioselective component of SPR biosensor

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Quantitative serum immunoglobulin (Ig) tests are used to detect levels of the three major classes (IgG, IgA and IgM) for the evaluation of possible antibody immunodeficiencies. Also, serum Ig tests may be used in the assessment of conditions associated with chronic inflammation (e.g. rheumatoid arthritis, systemic lupus erythematosus, autoimmune liver disease) or chronic infections (e.g. hepatitis C, HIV). Together with other tests and clinical history, serum Ig tests are used to help diagnose such diseases as myeloma, lymphoma or chronic lymphoid leukemia. IgG, IgA, and IgM levels are usually evaluated together.

Comparing to conventional analytical approaches, biosensor measurements are easy, fast, accurate, sensitive, specific, and cheap. Surface plasmon resonance (SPR) spectrometry is a real-time method that does not require the use of any molecular labels, so it can overcome such drawbacks as time and cost consuming preparation of labeled components, possible influence of labels on interacting biomolecules, multi-step detection protocols, etc. Staphylococcal immunoglobulin-binding protein A (SPA) is one of the best candidates for creation of the bioselective element of SPR biosensor, which can measure the Ig concentration in real biological samples. To ensure the efficient immobilization of SPA via a strong interaction of thiol group with gold sensor surface and improve its Ig-binding activity, one cysteine residue was introduced into a non-essential part of the recombinant protein A (SPA-Cys).

According to the literature data, SPA mostly binds to IgG molecules and has variable affinity to IgM and IgA. For this reason, solutions of human IgG have been used to construct calibration curve and to calculate Ig concentration in blood samples.

The study of immobilization processes and intermolecular interactions has been performed by using the measuring flow cell of SPR spectrometer "Plasmon-4m" developed at V.E. Lashkaryov Institute of Semiconductor Physics of NAS of Ukraine. A successful immobilization of SPA-Cys on the gold surface of SPR biosensor while preserving its high Ig-binding activity has been performed. Interactions of immobilized SPA-Cys with human IgG have shown the linear dependency of SPR biosensor response on IgG concentration in range from 0.5 to 5 μg/ml. This calibration curve has been used for determination of Ig concentration in blood serum samples. The obtained values of Ig in serum samples were ~2.4 times higher than the concentration determined by another method (RID). This can be partially explained by greater molecular weight of IgM and IgA than IgG. Anyway, we believe that using this factor an actual concentration of Ig in serum samples will be possible to determine.

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Nataliia Chornenka

Molecular markers of effective healing process under chemical burn of the esophagus in use of melanin

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According to modern concepts about burn disease, one of the main causes that burden the burns course - is the development of inflammation. Pathological change inflammatory pathways can also be linked to hypertrophic scarring and delay wound healing. The correct approach for the treatment of burns may effectively influence the clinical outcome. To the promising substances belongs melanin, which is produced of yeast fungi Nadsoniella nigra strain X-1.

In experiments were used immature rats in compliance with general ethical principles of experiments of animals. The animals were experimentally simulated the alkali burn of esophagus (ABE) with 20% NaOH. Scheme of the experiment was as follows: Group 1 – healthy rats (intact control); group 2 - rats, which modeled ABE, which was administered saline in the appropriate dose and timing (burn control); Group 3 - ABE rats, which was injected melanin at a dose of 1 mg / kg during 14 days. The material for the research selected at 7th, 15th and 21st days. We had determined total proteolytic activity in the blood. Serum cytokines level (IL-1β, IL-12, IF-γ, IL-6, TNF-α) and matrix metalloproteinases level (MMPs 1, 2, 8, 9) were analyzed by ELISA using sets of reagents Biotrack ELISA System. To test the genes expression (Ptgs2, Tgfb1) were used Real-time PCR using sets of reagents Thermo Scientific Verso SYBR Green 1-Step qRT-PCR ROX Mix. To determine the reliability of the differences between the two samples we used the two-way ANOVA and Student test (t), differences $p < 0.05$ were deemed reliable.

The level of all pro-inflamatory cytokines under modeling ABE were higher on the all researching days after burn, in comparison with the control. The used of melanin contributed to the normalization of these indicators. The level of expression of the Ptgs2 gene in the group of animals with esophageal burns, was higher at 3.1 and 2.0 times on the 7th and 15th day of healing, respectively, in comparison with the control. In the melanin rats, the level of expression of the Ptgs2 gene was already at the control level by 15th day. Under the conditions ABE was increased total proteolytic activity and concentration the MMPs in comparison with the control. With the introduction of melanin these indicators decreased in animals with burn. The expression level of the Tgfb1 gene in the esophageal burn group was higher at 3 and 2.9 times on the 7th and 15th day of healing, respectively, compared to control. In rats receiving melanin, this figure was 1.4 times lower than 7 days than in animals with burn.

The data indicate at the inflammatory process and hight level of proteolytic activity after burn. The use of melanin a contributed to the normalization of these indicators and reduced of the inflammation. The given researches indicate that melanin could be a promising drug for the prevention of post-burn complications.

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Vahid Tavakolpour

RNA sequences in the 3.-untranslated region (3. UTR) of their mRNA targets

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In multiple sclerosis (MS) disease, one of the earliest events observed in brain is the trafficking of peripheral activated leukocytes via disruptions in the blood-brain barrier (BBB) which leads to formation of multifocal lesions in the brain. Matrix metalloproteinases (MMPs), particularly MMP-9, effectively mediate BBB disruption and cell migration through the BBB secreted by activated T cells and macrophages in response to inflammatory cytokines. Several studies have been shown increased levels of MMP9 in peripheral blood mononuclear cells (PBMCs) and serum of MS patients even prior to or around clinical or magnetic resonance imaging (MRI) relapse. However, the activation process of MMPs regulated at the transcriptional and posttranslational level by tissue inhibitors of MMPs (TIMPs). Importantly, among the TIMP family TIMP3 inhibits MMP9 activity which is the main inducible MMP upregulated during BBB disruption. Recently, however, it has been shown that miR-206 by inhibiting TIMP3 promotes the MMP 9 expression levels. MiRNAs are non-coding highly conserved regulatory small RNAs which negatively regulating gene expression at the post-transcriptional level by binding to complementary RNA sequences in the 3'-untranslated region (3' UTR) of their mRNA targets.

In the current study using quantitative real time PCR method we evaluated the expression of miR-206, TIMP3 and MMP9 in PBMC of 38 relapsing-remitting MS (RRMS) patients including two groups of patients in relapse (n=18) and remitting (n=20) phases in comparison to 20 age and sex-matched healthy volunteers. We also studied the MRI and disease activity in MS patients. The results showed that the expressions of miR-206 and MMP9 in relapse phase of MS patients were significantly increased relative to healthy controls and remitting phase patients ($P < 0.05$). In contrast, TIMP3 expression analysis showed a significant down-regulation in relapse phase of RRMS patients ($P < 0.05$). Furthermore, the TIMP-3 expression was statistically associated with high expression levels of miR-206 and MMP9 in relapse phase group and disease activity. Using TargetScan prediction analysis we found TIMP3 as a potential target of miR-206. Subsequently, luciferase assay results demonstrated that TIMP3 is an actual target of miR-206 in human genome.

Taken together, in addition to underlining the significance of TIMP3, our findings suggest that miR-206 may function as an inflammatory regulator and by targeting TIMP3 promotes the expression of MMP9 in PBMCs of RRMS patients with clinical relapse.

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Ronja Rehm

Investigating the fate of synaptotagmin-IV harbouring dense core vesicles after fusion

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Dense core vesicles (DCVs) are neurosecretory vesicles, which transport di erent types of neurotrophins and neuropeptides and consequently play an important role in regulation of synaptic transmission. Their tra cking, fusion and cargo release properties are still an area of open research. DCVs are synthesised within the cell soma followed by long range tra cking in processes along sequential release sites until they reach the distal end, from where they return to the soma (Sushi model; Wong et al., 2012). In this study, we investigated the fate of DCVs after fusion. We performed single and double antibody uptake assays on GFP-syt IV transfected rat hippocampal neurons in combination with both, xed sample and time lapse imaging, where syt IV serves as a speci c DCV marker protein (Dean et al., 2009). Results show that DCVs travel long distances with multiple pauses after fusion and accumulate inside the cell soma over time. Furthermore, we found single DCVs to be able to fuse multiple times in a row within their journey along the axon. Multiple methods like STED microscopy, nanobody uptake and surface/internal assays were used to avoid possible misinterpretations. To summarize, DCVs are highly mobile neurosecretory vesicles that hold the potential of multiple fusion.

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Amr Al Shebel

A new method for expressing the ribosome silencing factor of *Staphylococcus Aureus*

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Staphylococcus aureus is one of the most common pathogens. It is well-known as a nosocomial infection with multi-drug resistance strains, and for its ability to hibernate under unfavorable conditions. Ribosome Silencing Factor (RsfS) associates with the protein L14 of the ribosomal large subunit L50 and prevents formation of the functional ribosome, so RsfS depletes ribosomes from translation pool cutting down energy spent. Since L14 protein of bacterial species is very conservative heterologous expression of staphylococcal RsfS in *E.coli* also results in slowing down the translation and gives little yield of this protein. Moreover RsfS is poorly soluble in majority of buffer systems. To produce RsfS, two variants expression cassettes containing rsfS and l14 genes of *S.aureus* were constructed using pACYCDuet-1 vector and expressed in *E.coli*. The first construct was expressing RsfS tagged with 6 histidine residues on its N-terminus (RsfS- 6xhis) and intact L14, second construct expressed intact rsfS and L14 tagged with 6 histidine residue on its N-terminus. The constructs did not influenced growth of producing *E.coli* cells in contrast expression of single rsfS nearly ceased the growth of *E.coli* culture. Both constructs demonstrated expressed RsfS-L14 complex effectively and in contrast to the construct, producing single RsfS, the complex was soluble. Stability of the complexes was confirmed using size exclusion and ion exchange chromatography methods. We also separated RsfS and L14 using denaturation in urea, in complex RsfS-6xhis-L14 effective dissociation was observed when 4M urea or higher concentration was used. After that pure RsfS-6xhis was recovered from affinity column. In this report we demonstrate that ribosome hibernation protein RsfS of *S.aureus* can influence protein synthesis machinery even in heterologous expression in *E.coli*. Nevertheless RsfS can be produced effectively when co-expressed with its target protein L14 without remarkable repression of producing *E.coli* culture growth. Since these two proteins form stable complex it is possible to purify them using affinity chromatography and further separate them using mild denaturation conditions. Work supported by program of Competitive Growth of KFU.

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Sindhuja Gowrisankaran

Regulation of vesicle acidification at the neuronal synapse

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The regulation and timing of vesicle acidification is essential for numerous cellular processes, from macromolecule degradation in lysosomes to refilling of synaptic vesicles (SVs) at the neuronal synapse. Acidification of vesicles is achieved by vacuolar ATPases (vATPases), a family of proton pumps that controls the pH gradient across organelle membranes. Despite their critical importance at the synapse and in many intracellular trafficking routes, the regulation of vATPase activity is poorly understood. Recently, it was shown that clathrin coat controls SV acidification by blocking vATPase activity (Farsi et al., eLife 2018). In a further search for the vATPase regulators, we cloned human Dmxl2 gene encoding Rabconnectin-3a (Rbcn-3a).

Rbcn-3a, a 340kDa synapse-enriched protein with unknown function, was reported initially as an interaction partner of Rab3 GTP/GDP exchange factors (Rab3 GEF) and Rab3 GTPase activating protein (Rab3 GAP) (Nagano et al 2002, Kawabe et al 2003). Rbcn-3a was also found to regulate the function of v-ATPase in trafficking organelles and suggested to regulate organelle acidification (Einhorn et al 2012, Tuttle et al 2014). Given that animals without Rbcn-3a are lethal, and that the mutations in Rbcn-3a are reported as a cause of polyendocrine-polyneuropathy syndrome (Tata et al., 2014), the protein seems to be relevant for the proper brain function and we characterized its function at the neuronal synapses. We found it to be present on every organelle that acidifies, including SVs. When Rbcn-3a is eliminated from neuronal cells in culture, neurons developed normally, yet their activity was perturbed. Neurons without Rbcn-3a failed to fully acidify SVs, and showed altered SV recycling. Curiously, the synapses of neurons without Rbcn-3a also accumulated lysosome-like structures and lysosomal markers, suggesting an unanticipated connection between the machineries for endocytosis, acidification and proteostasis.

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Claudia Schmidt

Reconstitution of Doa10-mediated ER-associated protein degradation with purified components

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Proteins of the secretory pathway fold and assemble into multiprotein complexes in the endoplasmicreticulum (ER). Unassembled subunits and terminally misfolded proteins are side-products of this process. They are removed from the ER and degraded by the cytosolic ubiquitin proteasome system. This quality control pathway is called ER-associated protein degradation (ERAD). It is conserved in eukaryotes. During ERAD, substrates are recognized, ubiquitinated by a set of enzymes called E1, E2, and E3 enzymes and retrotranslocated into the cytosol for proteasomal degradation.

In S. cerevisiae, ERAD substrates are degraded by two membrane embedded E3-ligase complexes, Hrd1 and Doa10. They differ in their substrate specificity. Doa10 targets ER membrane proteins with misfolded cytoplasmic or transmembrane domains for degradation. It has a broad substrate range: beside its role in ERAD, Doa10 also degrades cytosolic and nuclear substrates. During ubiquitination, Doa10 acts with two E2 enzymes. One of those E2 enzymes, the tail-anchored protein Ubc6 is itself unstable and degraded in a Doa10-dependent manner.

To gain mechanistic insight into Doa10-mediated degradation, we have developed a reconstituted system that recapitulates ubiquitination and extraction of Ubc6 using artificial phospholipid vesicles and purified proteins. When Ubc6 and Doa10 are co-reconstituted, Ubc6 is ubiquitinated in the presence of the ubiquitination machinery. The AAA ATPase Cdc48 together with its cofactors Ufd1 and Npl4 unfolds and extracts ubiquitinated substrates. This reaction is facilitated by the transmembrane domain of Doa10. Our results define the minimal machinery for Doa10-mediated ERAD and provide mechanistic insight into the retrotranslocation process.

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Sebastian Grosse

Gbp2 and Hrb1, two yeast nuclear mRNA quality control factors continue their surveillance function in the cytoplasm

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In eukaryotic cells, mRNAs undergo various processing steps before they are translated. Furthermore, these mRNAs are quality controlled to prevent translation of aberrant transcripts and thereby the production of potentially harmful polypeptides. Correct mRNA-capping, splicing, and polyadenylation is quality controlled by guard proteins. However, mRNA quality control is not limited to the nucleus, but rather continues in the cytoplasm. Here we present two guard proteins of the nuclear surveillance system that continue their quality control function in the cytoplasm. Gbp2 and Hrb1 interact with the late spliceosome and help to retain unspliced pre-mRNA in the nucleus. After correct splicing, they shuttle on the mRNA into the cytoplasm, where they are a part of the mRNP until translation. While, so far, no function during translation was described for the two proteins, we gathered evidence that Gbp2 and Hrb1 are involved in Nonsense Mediated mRNA Decay (NMD). NMD is a cytoplasmic surveillance pathway that targets premature termination as a quality control system as well as a means of regulation in some cases. Our data indicates that Gbp2 and Hrb1 promote both mRNA decay and translation inhibition on spliced reporter-mRNAs with a premature termination codon. Furthermore, they physically interact with the main NMD factors Upf1 Upf2 and Upf3 as well as the decapping and degradation machinery. A major group of NMD targets are un- or mis-spliced mRNAs; hence we propose that these guard proteins continue their surveillance function on spliced mRNAs in the cytoplasm.

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Yajie Zhu

Caspase signaling promotes SOP cell development by cleaving Arm

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Cell fate determination is crucial for building a functional organism. *Drosophila* SOP cell is a powerful model for studying neural cell fate determination. Wnt pathway plays a role in SOP cell fate determination. Caspase signaling is found to regulate cell fates in some cases, but its function and mechanism remain largely elusive. Here, we reveal that caspase signaling promotes SOP cell emergence by cleaving Arm. Downregulation of caspase signaling alleviates the Wnt-induced ectopic bristle phenotype in wings while upregulation of caspase signaling alleviates loss-of-wg induced margin bristle loss. Besides, deviation of caspase signaling leads to abnormality in bristles and veins. These findings demonstrate that caspases pathway has a role in regulating SOP cell fate. Then we found that caspase signaling has an effect on influencing Achaete level. Finally, we found that the function of caspases in cell fate is achieved by cleaving Arm. Our study not only reveals a novel function of caspase signaling, but also contributes to the understanding for neural fate determination, which may serve for providing the theoretical basis for clinical treatment of neurodegeneration in the future.

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Zhengzhong QU

Validation of NGS clinical assays based on the Guidelines of College of American Pathologists

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Targeted next-generation sequencing (NGS) is becoming increasingly common in the daily clinical usage. NGS assays can be used in the early screening, diagnosis, prognosis, and selecting of targeted therapies for cancer patients as well as other diseases. POLARIS (Personalized OMIC Lattice for Advanced Research and Improving Stratification) is a strategic program to pilot the application of clinical genomics in the treatment and diagnosis of medical diseases in Singapore. Here, we describe two custom-developed, next-generation sequencing tests. One for detecting single-nucleotide variants (SNVs) and short insertions and deletions (indels) in 740 genes related to various types of cancers from routine shock-frozen clinical specimens. Another for detecting germ line inhereted SNVs and short indels in the whole exome range to obtain a diagnosis in patients with undiagnosed genetic disorders.

Both two NGS clinical assays were validated based on the guidelines of College of American Pathologists. We implemented a strategy by using real clinical samples as well as synthetic reference DNA derived from cell lines with known genetic variants which model a broad range of allele frequencies. For our oncology panel, test sensitivity achieved 99.4% for SNVs, 96.5% for indels, with high specificity (98.9 % for SNVs and 88.7 % for indels) for allele frequencies >10% in synthetic reference DNA. We further confirmed test accuracies using real shock-frozen clinical specimens characterized by alternative and conventional clinical diagnostic technologies. Robust performance was observed: sensitivity was 100% and specificity was 100%. For our inhereted disease panel, test sensitivity achieved 95% for SNVs, 89% for Indels, with high specificity (100% for SNVs and 100% for Indels) for allele frequencies >10% in synthetic reference DNA. Test accuraries were further confirmed using real clinical blood samples with Sanger sequencing, and 100% sensitivity and specificity were observed. We also observed high intra-run and inter-run reproducibility, as well as low cross-contamination rates in both two assays. By using synthetic reference DNA, we also showed that our assays have consistent analytical sensitivity down to 10% minor allelle frequencies for the detection of somatic mutations.

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Alexander Rotsch

Effects of fusion tags on the ATP sensor ATeam

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It is known that ATP can be stored in secretory vesicles, as synaptic vesicles and chromaffin granules. A putative vesicular ATP transporter (VNUT) and a preliminary characterization of it have been presented. However, many questions about the loading of ATP into vesicles are still open, especially due to the limitation of the classical techniques of ATP concentration determiantion. Therefore, we attempted to stablish a new method to detect specifically intravesicular ATP in order to better characterize ATP transport into vesicles. For this, we took advantage of a FRET based ATP sensor (ATeam 1.03), consisting of a mse CFP with mVenus, each at either the N- or C terminus of a FoF1-ATP synthase subunit. In order to increase ATeam concentration inside vesicle, ATeam was fused to Tags, which can bind modified lipids or artificial membrane proteins. Furthermore, the effect of pH on the FRET signal was characterized to differentiate between signals based on ATP loading and signals based on acidification prior ATP loading.

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Margarita Chudenkova

Changes in mitochondrial respiratory chain supercomplexes in human tumor tissues.

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Mitochondria are multifunctional organelles responsible for energy metabolism via oxidative phosphorylation. This process is carried out by protein complexes present in the inner membrane of mitochondria. Recently, it has been shown that three of these complexes (NADH-dehydrogenase (CI), coenzyme Q-Cytochrome C reductase (CIII) and Cytochrome-C-oxidase (CIV)) can be associated together to form supercomplexes (SCs); SCs that comprise all three complexes are called respirasomes. While the exact function of these structures remains unknown, there is evidence that SC abnormalities may affect tumorigenesis. However, little data is available concerning the exact molecular changes in structure and formation of SCs in cancer due to lack of studies with patients' biopsy samples.

The present work was carried out using biopsy materials from patients suffering from gastric and colorectal cancers acquired in close collaboration with surgical oncologists. The goal of this research was to study changes in supercomplex components and activities of individual complexes in mitochondria isolated from human tumor tissues.

We used two-dimensional Blue-Native polyacrylamide gel electrophoresis to separate and identify the supercomplexes present in healthy and tumor human tissues, as well as applied in-gel and spectrophotometric CI and CIV activity assays to measure the activities of these complexes in the analyzed tissue samples.

Our results demonstrate the absence of high molecular weight respirasomes and the decrease in the content of low molecular weight respirasomes in tumor tissues as compared to healthy gastric tissue. This finding correlates with the results acquired in previous studies on human cell lines (Baracca et al., 2010). Moreover, our study showed an overall decrease of CI activity and, in contrast, increase in CIV activity in tumor tissues compared to healthy gastric tissue. These changes in activities of two respiratory chain complexes are in line with the data of Feichtinger et al., 2017, which were performed on biopsy materials from patients with gastric cancer. In addition, we found a redistribution of both protein and CI activity staining in-gel from respirasomes in healthy tissues into supercomplexes comprising CI and a dimer of CIII in both tumor tissues.

Our study addresses the significance of biopsy materials in research. In spite of the heterogeneity of tumor cells even within a single population, we were able to show and explain some alterations in the analyzed tissues. However, further studies of supercomplex formation and factors controlling this process are needed to gain a profound understanding of molecular properties of tumor cells.

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Surabhi Swarnkar

Cardiac progenitor stem cells and growth factor cocktail as therapy in Myocardial Infarction

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Recent strides in regenerative medicine have established the potential scope of stem cells as a therapeutic modality for myocardial infarction (MI). Stem cell therapy has the potential to limit the extent of post infarction cardiac damage by accelerating the normal process of healing, improving vascularization, inhibiting apoptosis, and potentially regenerating cardiac muscle. However, the efficacy of this approach is limited due to the hostile microenvironment of the acutely injured myocardium. Some putative secreted paracrine factors are involved that confer cardioprotective benefits like migration, homing, cell proliferation, cytoprotection, contractility, angiogenesis, and stabilization of both native and non- native progenitor cell types. We therefore hypothesized that a combination of these growth factors along with the stem cells should be a more superior therapeutic modality as it would provide a conducive milieu for the stem cells and may be able to circumvent the limitations of cell based therapeutics.

Rat models of myocardial infarction were created to assess the in vivo efficacy of this combinational therapy and its superiority as compared to stem cell or growth factor therapy alone. Subsequently after MI, interventions were given intra-myocardially and the animal models were then followed for a period of twenty eight days which is considered equivalent to two years in human context, to assess cardiac function by echocardioraphy. At the end of follow-up, the animal models were sacrificed and histopathology was performed on the infarcted tissue.

Serial echocardiography revealed that the combinational therapy group had improved cardiac function twenty eight days after MI as compared to stem cell or growth factor group, with heart function parameters reaching towards their baseline values. Histopathological analysis of infarcted tissue revealed lesser extent of typical MI characteristics like replacement fibrosis, presence of granulomatous tissue and granulocytic infilterates in the combinational therapy group. Altogether, the findings substantiate the in vivo efficacy of the combinational therapeutic modality and as evidenced by animal model experiments, it holds great translational value in a clinical MI setting.

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Kateryna Uspenska

Role of Mitochondrial Nicotinic Acetylcholine Receptors in Knockout Mice and after Partial Hepatectomy in Rats

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Nicotinic acetylcholine receptors (nAChRs) are classically regarded as ligand-gated ion-channels located in plasma membrane, regulating neurotransmitter and cytokine release and supporting the viability of many cell types. We have found that α7β2, α3β4, α4β4 nAChR subtypes are present in intracellular compartment – in the outer membrane of mitochondria. Mitochondrial nAChRs regulate the early stage of mitochondrial apoptosis, namely, cytochrome c (Cyt c) release under the effect of apoptogenic agents like Ca^{2+} or H_2O_2. Cyt c release from mitochondria can be attenuated by either nAChRs agonist or antagonist indicating that the nAChR signaling in these intracellular organelles is ion channel-independent and is mediated through conformational changes of the nAChR molecule upon specific ligand binding. The aim of the present study was to evaluate the role of nAChRs in mitochondria under different physiological conditions.

Experiments have been performed in C57Bl/6, a7-/-, a3+/-, a7b2-/-, b4-/- mice or Wistar rats. Mitochondrial and non-mitochondrial fractions were isolated from either the liver or brain by differential centrifugation according to standard procedures. The level of nAChR subunits was studied by Sandwich-ELISA using subunit-specific antibodies. The apoptotic resistance of mitochondria was evaluated based on the level of Cyt c released under the effect of Ca^{2+} or H_2O_2.

It was found that the lack of α7, α3, β2 or β4 nAChR subunits didn't change dramatically the sustainability of mitochondria to apoptogenic doses of Ca^{2+}. We found that the absence of nAChR subunits in mitochondria of knockout mice was compensated by significant increase of α9 and/or β4 subunits. α-conotoxin PeIA, specific ligand of α9 nAChRs, attenuated Cyt c release more effectively from α7-/- mitochondria than from wild-type, especially affecting Ca-stimulated Cyt c release. In contrast, it was found that α7-, α3-, α4- and, especially, α9-containing nAChRs increased in the rat liver mitochondria 3-6h after partial hepatectomy (when 70% of liver is removed) resulting in increased mitochondria resistance to 0.1-0.9μM Ca^{2+} and 0.1-0.5mM H_2O_2. 12 hours after partial hepatectomy the level of nAChRs in mitochondria returned to per-operational level.

It is concluded that the presence of several nAChR subtypes in mitochondria provides an effective protection of mitochondria from different apoptogenic factors. Mitochondrial nAChRs play an important role in cell survival after partial hepatectomy, but not in cell proliferation.

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Svetlana Ohiienko

Low-molecular weight cow colostrum components regulate bone marrow functions, modeling the redox-system of organism

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