BOOK OF ABSTRACTS

BOOK OF ABSTRACTS

The 3rd COST-sponsored ARBRE-MOBIEU plenary meeting

Molecular Biophysics: ABC of the puzzle of Life
March 18-20, 2019
Hotel Panorama, Zagreb, Croatia

Published by: Ruđer Bošković Institute and Croatian Biophysical Society
Editors: Nadica Ivošević DeNardis, Ramon Campos-Olivas, Adriana E. Miele, Patrick England, Tomislav Vuletić

ISBN 978-953-7941-28-4

Circulation 200 copies

Scientific Organizing Committee:

Isabel D. Alves, *University of Bordeaux, Pessac, France*

Margarida Bastos, *Faculty of Sciences, University of Porto, Porto, Portugal*

Ramon Campos-Olivas, *Spanish National Cancer Research Center, Madrid, Spain*

Tina Daviter, *Birkbeck, University of London, United Kingdom*

Mauro Dalla Serra, *CNR - Institute of Biophysics, Trento, Italy*

Patrick England, *Institut Pasteur, Paris, France*

Nadica Ivošević DeNardis, *Ruđer Bošković Institute, Zagreb, Croatia*

Thomas Jowitt, *University of Manchester, Manchester, United Kingdom*

Stefan Knauer, *University of Bayreuth, Bayreuth, Germany*

Marlene Martinho, *Aix-Marseille University and CNRS, Marseille, France*

Adriana E. Miele, *Sapienza University of Rome, Rome, Italy*

Bertrand Raynal, *Institut Pasteur, Paris, France*

Wouter H. Roos, *Zernike Institute, Rijksuniversiteit Groningen, Groningen, The Netherland*

Krzysztof Skowronek, *International Institute of Molecular and Cell Biology, Warsaw, Poland*

Ondrej Vanek, *Faculty of Science, Charles University, Prague, Czech Republic*

Tomislav Vuletić, *Institute of Physics, Zagreb, Croatia*

Local Organizing Team:

Tomislav Vuletić, *Institute of Physics, Zagreb, Croatia*

Nadica Ivošević DeNardis, *Ruđer Bošković Institute, Zagreb, Croatia*

Organized by:

Croatian Biophysical Society and ARBRE-MOBIEU COST Action CA15126

CONTENTS

Welcome..... 1
Timetable..... 3
Programme..... 5

Keynote lectures..... 13
Selected talks, Session A..... 19
Selected talks, Session B..... 31
Selected talks, Session C..... 41
ARBRE-MOBIEU meets other COST Actions..... 49

Posters..... 55
Poster sessions..... 145

Sponsor contributions..... 153

Welcome!

Welcome to Zagreb, Croatia, to the 2019 Plenary meeting of the Association of Resources for Biophysical Research in Europe-Molecular Biophysics in Europe (ARBRE-MOBIEU).

This is the 7th meeting of our community and the 3rd annual meeting in the context of the COST Action CA 15126.

In addition to working sessions dedicated to the ongoing work of ARBRE-MOBIEU we will have 4 scientific sessions, 3 poster sessions and plenty of time to network and to solve the puzzle of life:

- **A**ssemblies and Interactions of Bio(macro)molecules
- **B**io-Interfaces, Biomaterials and Membranes
- **C**ontroling Sample Quality, Instruments and Standards
- ARBRE-MOBIEU meets other COST-Actions

The knowledge on the molecular, physical basis of biological mechanisms grows within the rapidly expanding field of bioscience. The principles and methods of biophysics provide the underpinning for all basic bioscience and a rational language for discussion among scientists of different disciplines. Molecular biophysics is a study of biological macromolecules and assemblies as a whole, at an intermediate level between atomic-resolution structural descriptions and cellular-scale observations. We have designed the Program of our meeting in order to reflect this molecular, (bio)physical underpinnings of life with sessions A and B that address the two major (supra)molecular groups that form life. We emphasize also the session C that reflects the need of our community towards the standardization of procedures and experimental approaches and the manner to apply diverse technologies to our problems, which should facilitate our mutual understanding. Indeed, the COST-Action session is here to emphasize this need for always more and more interactions within the community, but also the need to open and link with other communities, in order to discover common interests and possible joint routes for the future.

We hope that you will make the most of your time with us in Zagreb and we thank you for your contribution to the growth and development of ARBRE-MOBIEU. Indeed, this is insofar the largest meeting of our community, with almost 200 participants and over a dozen sponsors and other partners from the industry.

Indeed, ARBRE-MOBIEU community and Croatian biophysical society wish to acknowledge the participation of industrial partners, as well as the assistance provided by the Ruđer Bošković Institute and the Institute of physics, Zagreb.

The organizing committee

SCIENTIFIC PROGRAMME

4 Scientific sessions

- Session A - **A**ssemblies and Interactions of Bio(macro)molecules
- Session B - **B**io-Interfaces, Biomaterials and Membranes
- Session C - **C**ontroling Sample Quality, Instruments and Standards
- ARBRE-MOBIEU meets other COST-Actions

3 Poster sessions

TIMETABLE

Time	Monday, 18.03.2019
10.00-12.30	Core group meeting
12.00-13.00	Registration desk open
12.30-13.30	Lunch
13.30-14.00	Wellcome & Introduction
14.00-15.45	Session A
15.45-16.10	Coffee break
16.10-18.00	Session A
18.00-20.00	Poster session 1

Time	Tuesday, 19.03.2019
9.00-10.40	Session B
10.40-11.10	Coffee break
11.10-12.20	Session B
12.20-13.40	Lunch&poster session 2
13.40-15.15	Session C
15.15-15.45	Coffee break
15.45-17.20	Session C
17.20-19.30	Poster session 3
18.00-19.30	Full MC meeting
20.00	Social Dinner

Time	Wednesday, 20.03.2019
9.00-10.35	COST Actions Session
10.35-11.00	Coffee break
11.00-12.30	ARBRE-MOBIEU General Assembly
12.30	Announcement: best poster, best talk prize
13.00	Departure

PROGRAMME

Molecular Biophysics: ABC of the puzzle of Life
ARBRE-MOBIEU Plenary Meeting. Zagreb, March 18-20, 2019.

Venue: Hotel Panorama, Trg Krešimira Ćosića 9, Zagreb, Croatia

Monday, March 18, 2019

10:00-12:30 Core group meeting (for WG leaders/co-leaders only) & arrival (all)

12:00-13:00 Registration

12:30-13:30 Lunch

13:30-14:00 Welcome & Introduction

Session A - A*ssemblies and Interactions of Bio(macro)molecules*

Chair: Tomislav Vuletić

14:00-14:35 **Keynote Lecture** *Protein synthesis: from ribosome assembly to targeting of membrane proteins*. Nenad Ban, Switzerland.

Selected Abstracts:
14:35-14:55 *Unexpected magnitudes of SPR signals explained by a new model*. Jérôme Dejeu, France.
14:55-15:15 *Biophysics of Viral Assembly: Studies at the Single-Virus Level*. Wouter H. Roos, The Netherland.
15:15-15:35 *Unravelling the Oligomerization State of the T Cell Antigen Receptor*. Mario Brameshuber, Austria.

Sponsor presentation:
15:35-15:45 *EPR: Structural Insight and Sample Quality*. Patrick Carl (**Bruker Biospin**)

15:45-16:10 Coffee Break

Chair: Krzysztof Skowronek

16:10-16:20 *Characterizing conformational changes by second-harmonic generation (SHG) A novel technology for monitoring biomolecule on-target binding and differentiating mechanism of action*. Bruce Mortensen (**Biodesy**).

Selected Abstracts:
16:20-16:40 *Interactions of the disordered oncoprotein Myc - biophysics paves way for understanding and interruption of tumor formation*. Maria Sunnerhagen, Sweden.

16:40-17:00 *Structure-functional study of lectins: key players in a fight between host and pathogen.* Josef Houser, Czech Republic.
17:00-17:20 *Label-free characterization of cell-surface glycans-lectins interactions as test for melanoma development*. Tomasz Kobiela, Poland.
17:20-17:40 *Fluorescence anisotropy based assay for monitoring of interactions between ligand and protein*. Ago Rinken, Estonia.

17:40-18:00 Flash Poster Presentations Session 1

Chair: Isabel D. Alves

1. *Exploring the dynamic structure of bionanocages*. João Jacinto, Portugal.
2. *Long, highly-stable and flexible: what analytical ultracentrifugation can tell us about "random walk" oligomer*. Alexandra Solovyova, United Kingdom.
3. *ITC: theory, practice and pitfalls*. Philippe Dumas, France.
4. *Amyloid β-Protein Inhibition and Interaction with Membranes*. Rita Carrotta, Italy.
5. *Study of The Human Dipeptidyl Peptidase III Interactom*. Sanja Tomic, Croatia.
6. *Heparanase binds substrate via a dynamic binding mechanism*. Aleksandra Marsavelski, Croatia.

18:00-20:00 **Poster Session 1**

Tuesday, March 19, 2019

***Session B - B**io-Interfaces, Biomaterials, and membranes*

Chair: Margarida Bastos

Selected Abstracts:
9:00-9:20 *From ARBRE-MOBIEU networking to regional research cooperation: Multimethod study of algal cell response to laboratory-induced cadmium stress.* Nadica Ivošević DeNardis, Croatia.
9:20-9:40 *Nanoscale Mechanosensing of Natural Killer Cells is Revealed by Antigen-Functionalized Nanowires*. Mark Schvartzmann, Israel.
9:40-10:00 *Deciphering putative lipid membrane interaction sites of the TRAP1 channel by Microfluidic Diffusional Sizing*. Isabel D.Alves, France.
10:00-10:20 *Efficient antimicrobial peptides against multiresistant bacteria presenting also anticancer activity* Nuno Santos, Portugal.

Sponsor presentations:
10:20-10:30 *What happens in solution should stay in solution: Recent needs and how centrifugation can help with preparation and characterisation of proteins and "biological structures"*. Lutz Ehrhardt (**Beckman Coulter**).
10:30-10:40 *The switchSENSE technology as a versatile tool for elucidating the mechanism of action of small molecule drug candidates*. Lena Kilian (**Dynamic Biosensors**).

10:40-11:10 Coffee Break

Chair: Tina Daviter

Sponsor presentations:
11:10-11:20 *Measurement of stoichiometry of protein and membrane protein complexes by light scattering coupled to SEC*. Christoph Albermann (**Wyatt).**
11:20-11:30 *Application of Temperature Related Intensity Change (TRIC) in biophysical drug discovery projects. Matthias Molnar* (**NanoTemper**).

Selected Abstract:
11:30-11:50 *Interaction Between Tethered Bilayer Membranes and s100A9 aggregates*. Rima Budvytyte, Lithuania.

11:50-12:20 Flash Poster Presentations Session 2:

1. *Bead Injection Multiplex Immunoanalysis*. Toonika Rinken, Estonia.
2. *EIS and SERS Analysis of Anchor Molecules for Tethered Bilayer Lipid Membrane Formation*. Indre Aleknaviciene, Lithuania.

3. *Algal cells under heavy metal stress: physiological and morphological response*. Tea Mišić Radić, Croatia.
4. *Quaternary Structure of Human NK Cell Receptor:Ligand complexes by the Looking Glass of Super-resolution Microscopy*. Barbora Kalousková, Czech Republic.
5. *Differential Scanning Fluorimetry: revisiting microtubules assembly*. François Devred, France.
6. *Structural and functional evidence for plasticity of Potato virus Y coat protein*. Marjetka Podobnik, Slovenia.
7. *Ferritin: The most interesting bionano component?* Soumyananda Chakraborti, Poland.
8. *Probing the mechanism of iron release from Dps, a miniferritn*. Ana V Almeida, Portugal.
9. *Plant seryl-tRNA synthetase as a link between translation and metabolism of brassinosteroid hormones*. Jasmina Rokov-Plavec, Croatia.

12:20-13:40 Lunch & poster session 2

Session C - C*ontrolling sample quality, instruments, and standards*

Chair: Bertrand Raynal

13:40-14:15 **Keynote Lecture** *Optimized sample preparation for cryo-electron tomography of cellular lamellas*. Jiří Nováček, Czech Republic.

Selected Abstracts:
14:15-14:35 *An improved facility for rapid-mixing multi-angle light scattering (MALS) coupled to small-angle X-ray scattering (SAXS) at the Synchrotron SOLEIL SWING beamline.* Mattia Rocco, Italy.
14:35-14:55 *ITC benchmark study: test reactions and data analysis - a case study within ARBRE-MOBIEU COST action.* Margarida Bastos, Portugal.

Sponsor presentations:

14:55-15:05 *Microfluidic Diffusional Sizing (MDS) for protein characterization – latest results and next step*. Jonathan Faherty (**Fluidic Analytics Ltd**)
15:05-15:15 *Beyond α-helix and β-sheet: Expanding the Role of Circular Dichroism*. Martin Textor (**Applied Photophysics Inc**).

15:15-15:45 Coffee Break

Chair: Adriana E. Miele

Sponsor presentation:
15:45-15:55 *Flow Induced Dispersion Analysis (FIDA) quantifies proteins, protein-ligand interactions and protein stability under native conditions*. Henrik Jensen (**FIDA-Tech Aps**).

Selected Abstracts:
15:55-16:15 *Kinetic stability of proteins - How stable is stable?* Didier Clénet, France.
16:15-16:35 *Monitoring of a DNA double helix formation: a multi-approach benchmark study.* Carmelo Di Primo, France.

Sponsor presentations:
16:35-16:45 *Integrated Solutions for Early Stage Biologics Discovery*. Hendrik Wünsche (**Pall ForteBio**).

16:45-17:20 Flash Presentations Session 3.

Chair: Ondřej Vaněk

1. *Development of a SANS strategy for the study of membrane proteins: application to a prokaryotic NADPH oxidase homolog*. Christine Ebel, France.
2. *Alzheimer's disease from a single molecule/single cell perspective*. Martino Calamai, Italy.
3. *Cell Adhesion and Mechanics in Fibroblasts and Myofibroblasts*. Manfred Radmacher, Germany.
4. *Controlling enzymatic activity by immobilization*. Marek Wiśniewski, Poland.
5. *Tethered Bilayer Lipid Membranes – a Comprehensive Tool for electrochemical Studies of Pore-Forming Toxins*. Tadas Penkauskas, Lithuania.
6. *Protein-protein interaction standards: The latest news in attempts to make ideal nanobodies*. Tom Jowitt, United Kingdom.
7. *Development of an Infrared Spectroscopy Data Repository*. Tina Daviter, United Kingdom.
8. *Macromolecular interactions in vitro, comparing classical and new approaches*. Paloma Fernandez-Varela, France.
9. *Precision of ITC measurements for target-based drug design*. Daumantas Matulis, Lithuania.

17:20-19:30 Poster Session 3

18:00-19:30 Full Management Committee Meeting (members only)

20:00 Social Dinner

Wednesday, March 20, 2019

COST Actions Session.

Chair: Patrick England

9:00-9:35 **Keynote Lecture** *Structural investigations of striated muscle Z-disks: fuzzy α-Actinin interactome* & *Presentation of COST Action "NGPnet" (Non-Globular Proteins).* Kristina Djinovic-Carugo, Austria.

9:35-9:55 *Presentation of COST Action "BioBrillouin*". Kareem Esayad, Austria.

9:55-10:15 *Presentation of COST Action "Native Mass Spectrometry".* Frank Sobott, United Kingdom.

10:15-10:35 *Presentation of COST Action "Molecular Machines".* Fraser MacMillan, United Kingdom.

10:35-11:00 Coffee break

11:00-12:30 ARBRE/MOBIEU General Assembly: Summary of Achievements during the Third Year of the Action and future perspectives.

12:30 Announcement of best poster & best talk prizes & Concluding Remarks

13:00 Departure

KEYNOTE LECTURES

Protein synthesis: from ribosome assembly to targeting of membrane proteins

Nenad Ban

ETH Zurich, Switzerland

bannenad@gmail.com

We are investigating bacterial and eukaryotic ribosomes and their functional complexes to obtain insights into the process of protein synthesis. Building on our studies of bacterial ribosomes we have increasingly shifted our attention to studying eukaryotic cytosolic and mitochondrial translation and were successful in obtaining first insights into the atomic structures of eukaryotic and mammalian mitochondrial ribosomes (1-3), which pose a significant challenge for structural studies as they are more complex and heterogeneous than their bacterial counterparts. The focus of our research has been to understand eukaryotic translation initiation, targeting of proteins to membranes, regulation of protein synthesis, and the assembly of eukaryotic ribosomes (4-6). The complete molecular structure of the unusual mammalian mitochondrial ribosome specialized for synthesis of membrane proteins was one of the first examples of electron microscopic structure determinations that allowed de-novo building, refinement and validation of the structure. These results revealed the interactions between tRNA and mRNA in the decoding centre, the peptidyl transferase center, and the path of the nascent polypeptide through the idiosyncratic tunnel of the mammalian mitochondrial ribosome. Furthermore, the structure suggests a mechanism of how mitochondrial ribosomes, specialized for the synthesis of membrane proteins, are attached to membranes (3). Recent results on the unique aspects of mitochondrial translation and the divergent structure of mitochondrial ribosomes will also be presented (7-8).

References

[1] Rabl J, Leibundgut M, Sandro F. Ataide SF, Haag A and Ban N. (2010) Nature 331(6018):730-6.

[2] Klinge S, Voigts-Hoffmann F, Leibundgut M, Arpagaus S, Ban N. (2011) Science, 334, 941-8.

[3] Greber BJ, Bieri P, et al. (2015) Science. 348(6232):303-8.

[4] Erzberger JP et al. (2014) Cell 158(5):1123-35.

[5] Greber BJ, et al. (2016) Cell 164(1-2):91-102.

[6] Kobayashi K, Jomaa A, et al. (2018) Science. 360(6386):323-327.

[7] Ramrath DJF, Niemann M, et al. (2018) Science. 362(6413).

[8] Kummer E, Leibundgut M, Rackham O, Lee RG, Boehringer D, Filipovska A, Ban N. (2018) Nature. 560(7717):263-267.

Optimized sample preparation for cryo-electron tomography of cellular lamellas

Radka Dopitová, Jana Moravcová, Jiří Nováček

CEITEC, Masaryk University, Brno, Czech Republic

jiri.novacek@ceitec.muni.cz

Cryo-electron microscopy is a dynamically developing method with increasing utilization in structural biology research. Recent advances in the detector technology enabled near-atomic structural studies of protein complexes including the molecular machines such as RNA polymerases or ribosomes. One advantage of the cryo-electron microscopy with respect to the other high-resolution structural biology methods lies in its capability to structurally characterize pleiomorphic objects and molecular assemblies which are difficult to characterize under *in vitro* conditions by cryo-electron tomography (cryo-ET). Therefore, cryo-ET can provide detailed structural information about macromolecular complexes in their native cellular environement. Since most of the eukaryotic cells are not transpararent for transmission electron microscopy and thus not directly accessible for cryo-ET data collection, additional pre-processing of the cellular samples is necessary prior. Focused ion beam micromachining (FIBM) under cryo-conditions has been developed to generate 100-300 nm thick cellular cross-sections suitable for cryo-ET. The complete sample preparation protocol comprises adhesion of the single layer of the cells to the transmission electron microscopy (TEM) grids, fixation of the cells in frozen hydrated state by vitrification, and FIBM of cellular lamella. Since the sample undergoes multitude transfers and processing in several instruments, the throughput of the whole process in not in general very high and rarely reaches 50%. We systematically study individual steps of the process primarily focussing on the cell adhesion, sample vitrification, and FIBM in order to determine the crucial aspects and parameters which are seminal for increasing the throughput of the lamella preparation workflow.

Structural investigations of striated muscle Z-disks: fuzzy α-Actinin interactome

Kristina Djinovic-Carugo

Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Austria

admin.vbc5@univie.ac.at

The ultra-structure of sarcomere, the basic contractile unit in striated muscle cells, is well organized and delimited by Z-disks, which play a central role in the mechanical stabilization and force transmission. α-Actinin isoform 2 (208 kDa) is a key protein in Z-disk assembly as it crosslinks antiparallel actin filaments from adjacent sarcomeres. It is also a binding platform for a number of other Z-disk proteins such as titin, FATZ-1and ZASP, which participate in Z-disk formation and regulation. FATZ-1/myozenin-1/calsarcin-2 and ZASP/cypher are predominantly intrinsically disordered ad believed to act as adaptors linking α-actinin-2 to other Z-disk proteins, while titin, a giant multidomain protein (~3,800 kDa; including folded and disordered parts) is the molecular ruler that spans half the sarcomere and combines elastic, architectural and signaling functions. Both FATZ-1, ZASP and titin (via its Zq domain) are reported to interact with the rod domain of α-actinin-2, while titin Z-repeats, PDZ domain of ZASP interacts with the calmodulin-like domain of α-actinin-2.

We are addressing the macromolecular assembly of α-actinin-2 with FATZ-1, titin and ZASP in an integrative approach by combining complementary techniques such as crosslinking/MS, NMR, SAXS, optical tweezers and crystallography. Using optical tweezers we studied the mechanics pf titin Z-repeat: α-actinin interaction, and showed that multiple α-actinin/Z-repeat interactions cooperate to ensure long-term stable titin anchoring of the Z-disk. Crystal structure analysis of a FATZ-1 construct in complex with α-actinin-2 shows that FATZ-1 interacts with α-actinin-2 rod, and modulates the EF hand pairs 1-2 the position found in the previously reported structure of full length α-actinin-2. To address the question whether interactions of FATZ-1 and titin with the α-actinin-2 rod are competitive, synergic or independent we determined structures of the complex between titin Zq region and α-actinin-2 rod and characterized the binding affinities, stoichiometry and SAXS derived structures for binary (α-actinin-2/titin and α-actinin-2/FATZ-1) and ternary (α-actinin-2/titin/FATZ-1) complexes. SAXS derived solution structure of binary and ternary assemblies (α-actinin-2/titin/FATZ-1) of display features of fuzzy complexes, with large segments of α-actinin-2 binding partners being intrinsically disordered. These findings will be discussed in terms of Z-disk structure and assembly and Z-disk ultrastructure.

Finally, I will present the COST action Non-globular proteins: from sequence to structure, function and application in molecular physiopathology, its objectives,

activities taken during the funder period (ending in spring 2019), outlook and future plans.

SELECTED TALKS

Session A

Assemblies and Interactions of Bio(macro)molecules

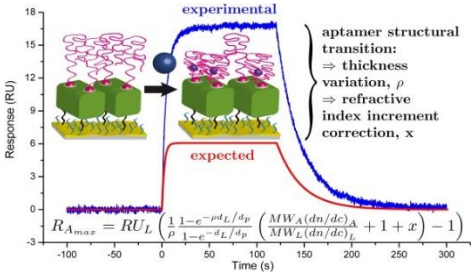
Unexpected magnitudes of SPR signals explained by a new model

H. Bonnet, A. Van der Heyden, N. Spinelli, E. Defrancq, L. Coche-Guérente, J. Dejeu

Université Grenoble Alpes, DCM UMR 5250, F-38000 Grenoble, France

jerome.dejeu@univ-grenoble-alpes.fr

Understanding and quantifying biomolecular interactions represent a major challenge in the field of life sciences, both from the fundamental point of view for the comprehension of biological process, and from the application point of view for the development of new diagnostic and therapeutic tools. Several optical techniques (surface plasmon resonance (SPR), interferometry, ellipsometry, reflectometry) allow monitoring and quantifying in real time and without labeling the interaction between an immobilized ligand on a surface and analytes in solution. These optical methods, based on changes in refractive index and / or thickness of the sensing layer close to the transducer surface have been successfully applied to many types of biomolecules. However, unconventional signals: too high or too low magnitude compared to the theoretically expected ones, or even, negative signals are observed.[1,2] These atypical signals have been explained by the peculiarity of the studied molecules to undergo a conformational change upon their recognition with the analyte. However, this phenomenon has never been quantified and modeled. Recently in the laboratory, we developed a new theoretical model for predicting SPR signals. In addition to contribution of the target binding to the signal, this model takes also into account the contribution of the ligand conformational change that could induce sensing layer thickness variation and/or the non-additivity of refractive index increments (RII) during the target/ligand complex formation.[3,4] This developed model has spin-offs for all optical techniques.



We exemplified this model to the recognition of small molecules by aptamers. Depending of the aptamer sequence, the magnitude of the SPR signal is higher or in opposite sign than one predicted theoretically, even though the aptamer folding was similar.

References

[1] Gestwicki, JE, Hsieh, HV, Pitner, JB, Using receptor conformational change to detect low molecular weight analytes by surface plasmon resonance. Anal Chem, 2001; 73:5732–5737. doi: 10.1021/ac050227i.

[2] Boussaad, S., Pean, J., Tao, NJ, High-Resolution Multiwavelength Surface Plasmon Resonance Spectroscopy for Probing Conformational and Electronic Changes in Redox Proteins. Anal Chem, 2000; 72:222-226. doi: 10.1021/ac990947n.

[3] D.J. Bornhop, DJ Kammer, NM Kussrow, A, Flowers II, RA, Meiler, J, Origin and prediction of free-solution interaction studies performed label-free. PNAS, 2016; E1595-E1604. doi: 10.1073/pnas.1515706113.

[4] Dejeu, J., Bonnet, H., Spinelli, N, Defrancq, E, Coche-Guerente, L, Van der Heyden, A, Labbé, P, Impact of Conformational Transitions on SPR Signals—Theoretical Treatment and Application in Small Analytes/Aptamer Recognition. J. Phys Chem C, 2018; 122:21521-21530. doi: 10.1021/acs.jpcc.8b07298.

Biophysics of Viral Assembly: Studies at the Single-Virus Level

Pedro Buzon[1], Gijs J. L. Wuite[2], Adam Zlotnick[3] and Wouter H. Roos[1]

[1]Molecular Biophysics, Zernike Institute, Rijksuniversiteit Groningen, Groningen, The Netherland, [2]Physics of living systems, Vrije Universiteit Amsterdam, Amsterdam, The Netherland, [3]Physical Virology, Molecular and Cellular Biochemistry Department, Indiana University, Bloomington, USA

w.h.roos@rug.nl

Virus self-assembly is a fascinating process in the viral life cycle, whereby the enclosure of the viral genome occurs concomitantly with capsid formation without any additional source of energy. Next to a fundamental scientific interest, this process is also of importance in the context of the application of viruses in biomedicine and biotechnology. Still the self-assembly process remains a poorly characterized phenomenon, especially at the molecular level. In this context, we present a state-of-the-art biophysical approach to study virus self-assembly at the single-particle level, combining optical tweezers, fluorescence microscopy and atomic force microscopy. These techniques allow us to manipulate one nucleic acid molecule at a time, and track the assembly process by following the changes in the mechanical properties and fluorescence signal of the formed complexes. Herewith, we demonstrate that the self-assembly process of Hepatitis B Virus (HBV) on single-stranded DNA can be followed in real time. Single-molecule traces showed multiple DNA packaging and release events over time, yielding insight into the number of encapsidated nucleotides per packaging event. Also, partial HBV nucleocapsid structures were detected, opening new possibilities to identify intermediate states during assembly. Our approach introduces a new methodology to investigate virus self-

assembly, expanding the field towards the study of the molecular basis of this process.

Unravelling the Oligomerization State of the T Cell Antigen Receptor

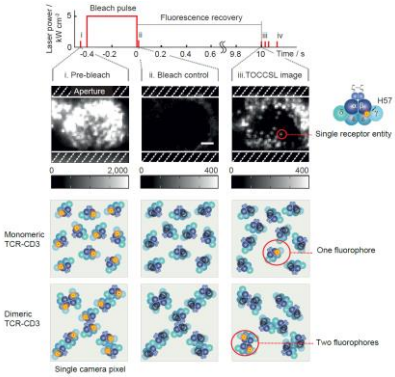
Mario Brameshuber

Institute of Applied Physics – Biophysics, TU Wien, Vienna, Austria

brameshuber@iap.tuwien.ac.at

T cell antigen recognition requires T cell antigen receptors (TCRs) engaging MHC-embedded antigenic peptides (pMHCs) within the contact region of a T cell with its conjugated antigen-presenting cell. Despite micromolar TCR:pMHC affinities, T cells respond to even a single antigenic pMHC, and higher order TCRs have been postulated to maintain high antigen sensitivity and trigger signaling. In my talk I will present how we interrogated the stoichiometry of TCRs and their associated CD3 subunits on the surface of living T cells through single molecule brightness and single molecule coincidence analysis, photon-antibunching based fluorescence correlation spectroscopy and Förster resonance energy transfer measurements. I will focus on an in-house developed imaging modalitiy termed TOCCSL (thinning out clusters while conserving the stoichiometry of labelling [1, 2]), which can reveal the stoichiometry of quantitatively labelled, mobile receptor complexes diffusing on the surface of living cells.

Applied on T cells we found exclusively monomeric TCR-CD3 complexes driving the recognition of antigenic pMHCs, which underscores the exceptional capacity of single TCR-CD3 complexes to elicit robust intracellular signalling [3].



Principle of the TOCCSL approach. T cells were quantitatively labelled via scFvs targeted against the TCR-CD3 beta chain (H57) and placed on supported lipid bilayers featuring the adhesion molecule ICAM-1. The high surface density of TCRs does not allow for observing single receptor entities by using a single molecule fluorescence microscope in total internal reflection (TIR) configuration (i). After full fluorescence ablation of a clearly defined region using a high-power laser pulse (ii), the onset of the recovery process of single receptor signals from the shielded region into the central field is recorded (iii). The brightness of recovered signals finally reveals the TCR oligomerization state.

Scale bar: 2 μm

References

[1] Brameshuber, M. et al. Imaging of mobile long-lived nanoplatforms in the live cell plasma membrane. J Biol Chem 285, 41765-41771 (2010).

[2] Ruprecht, V., Brameshuber, M. & Schutz, G.J. Two-color single molecule tracking combined with photobleaching for the detection of rare molecular interactions in fluid biomembranes. Soft Matter 6, 568-581 (2010).

[3] Brameshuber, M. et al. Monomeric TCRs drive T cell antigen recognition. Nat Immunol 19, 487-496 (2018).

Interactions of the disordered oncoprotein Myc - biophysics paves way for understanding and interruption of tumor formation

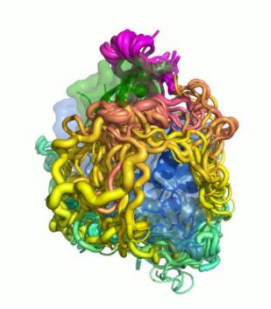
Alexandra Ahlner[1], Sara Helander[1], Diana Resetca[2], Yong Wei[1,2], Vivian Morad[1], Björn Wallner[1], Linda Penn[2], Maria Sunnerhagen[1]

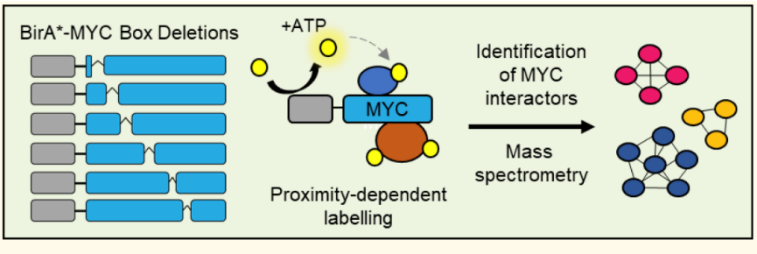
[1]Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden; [2]Department of Medical Biophysics, University of Toronto, and Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada.

maria.sunnerhagen@liu.se

The proto-oncogenic transcription factor c-Myc is a universal regulator of cell growth, apoptosis, and proliferation in both normal and tumor cells. However, the molecular basis for how Myc recognition is linked to its intrinsically disordered properties is unclear. In my group, we showed for the first time how c-Myc interacts with both the WW and PPIase domains of Pin1, and how this interaction is affected by phosphorylation. By SPR, NMR, SAXS, SPR and cellular assays, we show that the transiently ordered, unphosphorylated c-Myc region N-terminal to the phosphorylation site in MBI serves as a dynamic, or 'fuzzy, anchoring site for Pin1 on c-Myc$_{1\text{-}88}$, both in the absence and presence

of phosphorylation. By NMR analysis of CSP binding patterns on Myc binding, we show how its phosphorylation in MBI triggers an alternate allosteric interdomain network in Pin1, which engages its active site loops. Using Rosetta-based ligand docking of the intrinsically disordered Myc together with NMR constraints derived from HNCO intensity ratios and CSPs, we derived a structural ensemble of bound states in the fuzzy Myc-Pin1 pre-ancoring complex. Most recently, we have used proteomic profiling by mass spectrometry of Myc proteins with deleted MBs to investigate the interactomes of each MYC box and their correlations. We have confirmed several of the interactions and further investigated the dynamic interrelation between their activities structurally and biologically. We will describe this together with our ongoing work on characterizing the structural properties of Myc complexes using biophysical techniques.





References

[1] Pre-Anchoring of Pin1 to Unphosphorylated c-Myc in a Fuzzy Complex Regulates c-Myc Activity. Helander S, Montecchio M, Pilstål R, Su Y, Kuruvilla J, Elvén M, Ziauddin JME, Anandapadamanaban M, Cristobal S, Lundström P, Sears RC, Wallner B, Sunnerhagen M. Structure. 2015 Dec 1;23(12):2267-2279

[2] MYC Protein Interactome Profiling Reveals Functionally Distinct Regions that Cooperate to Drive Tumorigenesis. Kalkat M, Resetca D, Lourenco C, Chan PK, Wei Y, Shiah YJ, Vitkin N, Tong Y, SunnerhagenM, Done SJ, Boutros PC, Raught B, Penn LZ. Mol Cell. 2018 Dec 6;72(5):836-848.e7.

Structure-functional study of lectins: key players in a fight between host and pathogen

Josef Houser[1,2], Lenka Malinovska[1,2], Eva Fujdiarova[1,2], Gita Jancarikova[1,2], Jan Komarek[1,2], Jitka Holkova[1], Michaela Wimmerova[1,2,3]

[1]Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic; [3]Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic.

houser@mail.muni.cz

Lectins are proteins that reversibly interact with sugar moieties without chemicaly modifying them. They have been discovered in most of the species including viruses, bacteria, plants, fungi or animals. They functions may range from roles in glycoprotein folding through cell-cell communication upon cell maturation up to recognition of invading pathogens. Our main interest lays in lectins of pathogenic origin – those, that are produced by bacteria [1] of fungi [2] in order to help them invade the host, colonize its tissues and shield them against the immune system. As there are thousands of microbes and the same number of hosts, there is also a huge variety in lectins used in their battles.

To describe a lectin, we apply a broad range of biophysical techniques. In addition to basic protein characterization (temperature and chemical stability, homogeneity, folding), we combine several approaches to assess the binding preferences. While ITC or MST can give us binding affinity in solution, SPR can show how things happen on the surface, where most of lectins are primarily localized. Here additional effects, such as avidity, make generally weak sugar binders much more powerful. All these findings we combine with X-ray crystallography and *in silico* modelling [3] in order to complement knowledge about activity with structural background.

The detailed understanding of lectins and their binding partners does not only allow us to describe the important phenomenon in nature, but also to help with design of potential inhibitors for those pathogens that attack people or important animals or plants [4]. Many lectins can be also applied in biotechnologies, such as basic glycoprotein research or drug targetting.

References

[1] Jančaříková G, Houser J, Dobeš P, Demo G, Hyršl P, Wimmerová M. Characterization of novel bangle lectin from Photorhabdus asymbiotica with dual sugar-binding specificity and its effect on host immunity. PLoS Pathog. 2017; 13(8):e1006564. doi: 10.1371/journal.ppat.1006564.

[2] Houser J, Komarek J, Kostlanova N, Cioci G, Varrot A, Kerr SC, Lahmann M, Balloy V, Fahy JV, Chignard M, Imberty A, Wimmerova M. A soluble fucose-specific lectin from Aspergillus fumigatus conidia--structure, specificity and possible role in fungal pathogenicity. PLoS One. 2013; 8(12):e83077. doi: 10.1371/journal.pone.0083077.

[3] Houser J, Kozmon S, Mishra D, Mishra SK, Romano PR, Wimmerová M, Koča J.

Influence of Trp flipping on carbohydrate binding in lectins. An example on Aleuria aurantia lectin AAL. PLoS One. 2017; 12(12):e0189375. doi: 10.1371/journal.pone.0189375.
[4] Kašáková M, Malinovská L, Klejch T, Hlaváčková M, Dvořáková H, Fujdiarová E, Rottnerová Z, Maťátková O, Lhoták P, Wimmerová M, Moravcová J. Selectivity of original C-hexopyranosyl calix[4]arene conjugates towards lectins of different origin. Carbohydr Res. 2018; 469:60-72. doi: 10.1016/j.carres.2018.08.012.

Label-free characterization of cell-surface glycans-lectins interactions as test for melanoma development

A. Sobiepanek[1], M. Milner-Krawczyk[1], M. Lekka[2], T. Kobiela[1]

[1]Warsaw University of Technology, Laboratory of Biomolecular Interactions Studies, Warsaw, Poland; [2]Institute of Nuclear Physics, Department of Biophysical Microstructures, Cracow, Poland

kobiela@ch.pw.edu.pl

The analysis of the kinetic and thermodynamic parameters of biomolecular interactions has become essential for the development of innovative, more efficient techniques for the diagnosis and prognosis of the cancer progression. Many studies revealed that cancer development and progression, including epithelial-mesenchymal transition and invasion, is accompanied by changes in glycosylation patterns of cell surface and secreted glycoproteins. Other important issue concerns observations that vital cell functions, including proliferation, survival and differentiation correlate with cell shape and orientation and that disturbance of tensional homeostasis plays an important role in initiation of cellular transformation. The aim of presented studies was evaluation of the effects of adhesion and migration of cancer cells inhibitors on their mechanical properties. Investigated were also interactions between specific glycans, present on the surface of primary tumor and metastases of melanoma at various stages of cancer progression, with appropriate lectins, using quartz crystal microbalance with the dissipation monitoring (QCM-D) and atomic force microscopy (AFM). Both methods allow the analysis of the cell membrane surface glycosylation profile with the use of label-free lectins in real time. Previously we have shown that combined AFM/QCM-D measurements of the dissociation process, delivers full quantitative characterization of lectin – carbohydrate complexes [1]. In the other study, on the way to more personalized treatment regimens, we used patient-derived, instead of generic, cell lines and compared the recognition of mannose and glucose type glycans of melanocytes versus patient's melanoma cells originating from the radial growth phase and from lung metastasis [2]. Thus, the effort was undertaken for evaluation of described methodological approach for diagnosis and prognosis of melanoma

patients on the basis of primary melanoma cultures established from dissected tumor tissue. The same concerns the evaluation of adhesion and migration inhibitors (anandamide and FAAH inhibitors) effect on mechanical properties of investigated melanoma samples.

References

[1] E. Senkara-Barwijuk, T. Kobiela, K. Lebed, M. Lekka Reaction pathway and free energy profile determined for specific recognition of oligosaccharide moiety of carboxypeptidase, Biosensors and Bioelectronics, 2012; 36:103-109

[2] A. Sobiepanek, M. Milner-Krawczyk, M. Lekka, T. Kobiela, AFM and QCM-D as tools for the distinction of melanoma cells with a different metastatic potential, Biosensors and Bioelectronics, 2017; 93:274-281

Fluorescence anisotropy based assay for monitoring of interactions between ligand and protein

Sergei Kopanchuk, Ago Rinken

Institute of Chemistry, University of Tartu, Estonia

ago.rinken@ut.ee

Binding of fluorescent ligands (probes) to their targets can be directly monitored in time, as binding of a low-molecular-weight probes to a larger particle limits its rotational freedom and causes increase of fluorescence anisotropy (FA) [1]. The bright fluorophores of probes with low non-specific binding would lead to reasonable signal-to-noise ratio in FA assays even in case of moderate binding affinity. The ratiometric nature of the assay requires the protein concentration to be comparable with the probe's one. In case of membrane proteins, the required high concentration of the protein can be achieved with implementation of budded baculovirus particles, which display the protein of interest on their surfaces [2]. The obtained data enables quantitative assessment of equilibrium binding and kinetic parameters for both probe and competing compounds, as well as estimation of concentration of active protein. We have implemented the fluorescence anisotropy kinetic assay systems for characterization of ligand binding to different G protein coupled receptors like melanocortin (MC_4R) [2], serotonin ($5\text{-}HT_{1A}R$) [3] and dopamine (D_1DAR) [4] receptors, but also for peptide binding to tumor-associated macrophages [5]. The FA assays have clear potential for implementation in drug screening systems, but also in studies of ligand binding mechanisms for particular proteins.

References

[1] Rinken A, Lavogina D, Kopanchuk S. Assays with Detection of Fluorescence

Anisotropy: Challenges and Possibilities to Characterize Ligand Binding to GPCRs. Trends Pharmacol. Sci. 2018; 39: 187-199. doi:10.1016/j.tips.2017.10.004.
[2] Veiksina S, Kopanchuk S, Rinken A. Budded baculoviruses as a tool for a homogeneous fluorescence anisotropy-based assay of ligand binding to G protein-coupled receptors: the case of melanocortin 4 receptors. Biochim. Biophys. Acta - Biomembranes. 2014; 1838: 372–381. doi: 10.1016/j.bbamem.2013.09.015
[3] Tõntson L, Kopanchuk S, Rinken A. Characterization of 5-HT1A receptors and their complexes with G-proteins in budded baculovirus particles using fluorescence anisotropy of Bodipy-FL-NAN-190. Neurochem. Int. 2014; 67: 32-38. doi: 10.1016/j.neuint.2014.01.012.
[4] Allikalt A, Kopanchuk S, Rinken A. Implementation of fluorescence anisotropy-based assay for the characterization of ligand binding to dopamine D1 receptors Eur.J. Pharmacol. 2018; 839: 40-46. doi: 10.1016/j.ejphar.2018.09.008
[5] Scodeller P, Lorena Simon-Gracia L, Kopanchuk S, Tobi A, Kilk K, Säälik P, Kurm K, Squadrito M, Kotamraju VR, Rinken A, de Palmae M, Ruoslahti E, Teesalu T. Precision targeting of tumor macrophages with a CD206 binding peptide. Sci. Reports 2017; 7: 14655 doi:10.1038/s41598-017-14709-x.

SELECTED TALKS

Session B

Bio-Interfaces, Biomaterials and Membranes

From ARBRE-MOBIEU networking to regional research cooperation: Multimethod study of algal cell response to laboratory-induced cadmium stress

Nadica Ivošević DeNardis[1], Jadranka Pečar Ilić[1], Ivica Ružić[1], Nives Novosel[2], Tea Mišić Radić[1], Andreas Weber[3], Damir Kasum[1], Zuzana Pavlinska[4], Ria Katalin Balogh[5], Bálint Hajdu[5], Alžbeta Marček Chorvátová[4], Béla Gyurcsik[4]

[1]Ruđer Bošković Institute, Zagreb, Croatia, [2] Faculty of Science, University of Zagreb, Zagreb, Croatia, [3] Institute for Biophysics, University of Natural Resources and Life Sciences, Vienna, Austria, [4] Faculty of Natural Sciences, University of Ss. Cyril and Methodius, Trnava, Slovakia, [5]Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary

ivosevic@irb.hr

ARBRE-MOBIEU served as the platform to initiate a new research collaboration between partners from Croatia, Czech, Hungary and Slovakia which led to a joint project funded by International Visegrad Fund. In the frame of the corresponding project, we examined algal cells' responses to laboratory-induced cadmium stress, focusing on the biointerface and intracellular changes by employing a multimethod approach. The biointerface is characterized in terms of surface properties (cell stiffness) and adhesion dynamics on a single cell level. The intracellular response was examined in terms of the cells' autonomous feature (autofluorescence) and protein expression obtained from the cell culture. The results show that in spite of very a high cadmium concentration and prolonged cell exposure, the cells grew without significant suppression of growth dynamics. Cell adaptation to cadmium stress is manifested through cell shape deterioration, slower motility and an increase of physiological activity. AFM measurements revealed that cells are significantly stiffer in the presence of cadmium, which influenced the dynamics of the initial contact of the cell at the interface. The difference in adhesion behaviour was determined to be two times slower for initial attachment and deformation, while rate-limiting steps referring to the spreading of released intracellular content are not significantly different in comparison with the control. There was no change in the endogenous fluorescence in the red region (associated with chlorophyll) but there was a change in the green region (possibly associated with cadmium vesicular transport and beta carotene production), which provides insight into the cells' adaptation strategy to maintain photosynthesis. Specific responses of natural fluorescence of cells under the influence of cadmium are most likely associated with the identified chlorophyll a-b binding protein. It seems that another identified protein, carbonic anhydrase, plays role in the photosynthetic pathway. Since production of these proteins can be related to the maintenance of the photosynthesis representing one of the cell defence mechanisms, it may also indicate the presence of toxic metal in seawater. This multimethod study enabled

us to better understand cell responses under laboratory-induced cadmium stress to predict the fate of algae in a marine environment. Our results will substantially contribute to the biophysics of algal cells on a fundamental level.

Reference

[1] N. Ivošević DeNardis, J. Pečar Ilić, I. Ružić, N. Novosel, T. Mišić Radić, A. Weber, D. Kasum, Z. Pavlinska, R. Katalin Balogh, B. Hajdu, A. Marček Chorvatova, B. Gyurcsik, Algal cell response to laboratory-induced cadmium stress: a multimethod approach, Eur. Biophys. J. 2019., doi.org/10.1007/s00249-019-01347-6

Nanoscale Mechanosensing of Natural Killer Cells is Revealed by Antigen-Functionalized Nanowires

G. Le Saux[1,2], N. Bar Hanin[1,2], A. Edri[3], U. Hadad[2], A. Porgador[3], M. Schvartzman[1,2]

[1]Department of Materials Engineering, [2]Ilse Katz Institute for Nanoscale Science & Technology, [3]The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

Cells sense their environment by transducing mechanical stimuli into biochemical signals. Commonly used tools to study cell mechanosensing provide limited spatial and force resolution. Here, we report a novel nanowire-based platform for the detection and monitoring of cell forces, which integrates both mechanical and chemical cues. We used this platform to explore mechano-sensitivity of Natural Killer (NK) cells, whose mechano-regulation has been mostly unexplored up to date. To enable antigen- specific interaction between the nanowires and NK cells, we functionalized the nanowires with Major Histocompatibility Complex I (MICA) – ligands that are recognized by NKG2D activating receptors of NK cells (Fig. 1a). We stimulated NK cells on MICA-functionalized nanowires, and found that nanowires permit enhanced cell contraction, whereas such contraction is impossible on flat surfaces functionalized with MICA. We used high resolution fluorescence microscopy and SEM to discover that NK cells anchor and bend nearby nanowires during their stimulation (Fig. 1b). The nanometric radius and ultra-high aspect- ratio of nanowires allowed us to monitor cell forces with ultra-fine mechanical and spatial resolutions Based on the magnitude of the nanowire bending, we assessed that the mechanical load applied by NK cells on a single nanowire is of the order of 10pN, which is the smallest mechanical force recoded for a spreading cell. Finally, we studied the effect of nanowire topography and MICA functionalization on the immune function NK cells. We found that, whilst each of these two factors alone was insufficient to stimulate significant cell immune

response, their combination substantially boosted NK cell degranulation (Fig. 1c). This finding indicates that NK cells use mechanical forces to sense their environment, and that this sensing is based on an independent mechanotransduction pathway which is costimulatory to the chemical signaling. In this sense, NK cells can be analogous to a Boolean AND gate, whose independent mechanical and chemical signaling provides two logic inputs. Our findings provide an important insight into the underlying mechanism of NK cell immune function, as well as demonstrate a novel toolbox for detecting cellular forces with an unprecedented spatial and mechanical resolution.

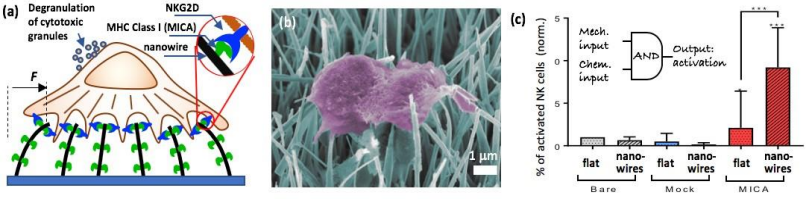


Fig.1. (a) Scheme of nanowire functionalization. (b) Scanning electron microscope of NK cells bending the nanowires. (c) Enhanced immune stimulation of NK cells by antigen-functionalized nanowires

Reference

[1] G. Le Saux, N. Bar Hanin, A. Edri, U. Hadad, A. Porgador, M.Schvartzman. Adv. Mater. 2018, 1805954, https://doi.org/10.1002/adma.201805954

Deciphering putative lipid membrane interaction sites of the TRAP1 channel by Microfluidic Diffusional Sizing

Lucie Macikova[1], Alexandre Ciaccafava[2], Viktorie Vlachova[1], Jonathan Faherty[3], Isabel D. Alves[2]

[1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic; [2]CBMN, U. of Bordeaux, Pessac, France; [3]Fluidic Analytics, Cambridge, United Kingdom

i.alves@cbmn.u-bordeaux.fr

The TRAP1 channel is a chemosensitive ion channel involved in nociception and inflammatory pain. Current structural information, although incomplete, reveals a putative PIP_2 interaction site strategically located in a key region where it may contribute to conformational changes implicated in channel gating [1]. The C-term region presents a high propensity for lipid membrane interaction and in a previous study we have shown part of this region to exhibit high affinity to

lipid membranes containing PIP_2 [2]. With that in mind, in the present investigation we have enlarged the C-term region screened to include two additional sequences, L992-N1008 and T1003-P1034 (Fig. 1A). As the approaches employed previously (Plasmon Resonance, IR spectroscopy and CD for studies of affinity and peptide secondary structure in the lipid-bound state) failed to provide a solid result regarding the interaction of these two new peptides, in part due to their superficial and non-lipid perturbing interaction, a new method to characterize the interaction was needed. We have chosen to use a novel technology named Microfluidic Diffusional Sizing (MDS) developed by Fluidic Analytics to follow peptide interaction with polymer based nanodiscs (SMALPs) of small size. MDS allows one to determine the concentration and size of proteins or peptides or any primary amine containing molecule [3]. The binding of the two peptides to membranes composed of POPC and POPC/PIP2 was followed and K_D values for the interaction obtained (Fig1B and C). Overall the results indicate that although the interaction is strong, it is rather superficial, most probably involving electrostatic interactions and minor lipid rearrangement [manuscript under revision].

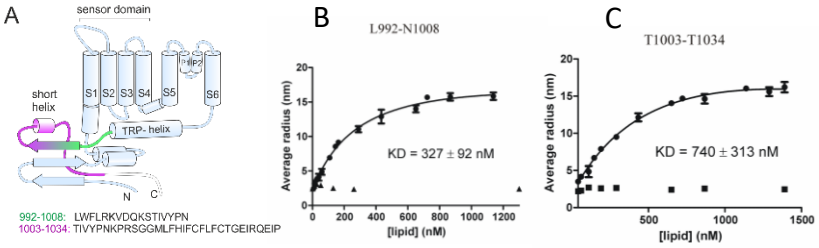


Figure 1. A) Schematic representation of the TRPA1 receptor showing the regions predicted to interact with lipids and those investigated herein. B and C) Interaction of the peptides with POPC (♦) and POPC/PIP2 (●) by MDS.

References

[1] Paulsen CE, Armache JP, Gao Y, Cheng Y, Julius D. Structure of the TRPA1 ion channel suggests regulatory mechanisms. Nature 2015; 520:511-7.

[2] Witschas K, Jobin ML, Korkut DN, Vladan MM, Salgado G, Lecomte S, Vlachova V, Alves ID: Interaction of a peptide derived from C-terminus of human TRPA1 channel with model membranes mimicking the inner leaflet of the plasma membrane. Biochim Biophys Acta 2015; 1848:1147-56.

[3] Arosio P, Muller T, Rajah L, Yates EV, Aprile FA, Zhang Y, Cohen SI, White DA, Herling TW, De Genst EJ, Linse S, Vendruscolo M, Dobson CM, Knowles TP: Microfluidic Diffusion Analysis of the Sizes and Interactions of Proteins under Native Solution Conditions. ACS nano 2016; 10:333-41.

Efficient antimicrobial peptides against multiresistant bacteria presenting also anticancer activity

Mário R. Felício[1], Sónia Gonçalves[1], Octávio L. Franco[2,3], Nuno C. Santos[1]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal. [2]S-INOVA, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, Brazil. [3]Centro de Análises Proteômicas e Bioquímicas, Universidade Católica de Brasília, Brasília, Brazil.

nsantos@fm.ul.pt

The current healthcare scenario is bringing new challenges to the medical field, due to the new threats that are becoming responsible for high mortality ratios. Multiresistant pathogens are one of them, with reports of increased numbers of severe infections on patients that current available drugs cannot fight. The World Health Organization has already pointed out the urgency in finding new molecules against different pathogens, named the *ESKAPE* group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterobacter* species). Furthermore, the number of drug resistance cases in cancer patients has also increased in recent years, associated with the occurrence of hospital infections that debilitate patients' health.

Antimicrobial peptides (AMPs) are pointed out as a rising alternative to conventional drugs in these contexts. AMPs are usually small, highly hydrophobic and with a global positive charge, which promotes the interaction with the pathogens or cancer cells membranes (with negative surface charges). Their mechanism of action is independent of intermediators, transporters or protein channels, being active mostly by peptide-membrane interaction, against bacteria, viruses or cancer cells (anticancer peptides, ACPs). Even so, these mechanisms are not yet fully understood.

In our work, we focused in two AMPs (*Pa*MAP 1.9 and 2), synthetically designed using a natural AMP as template. After initial promising results against bacteria, complemented by computational studies, peptide-membrane interactions were extensively studied, using lipid vesicles and bacterial cells (including clinical multi-resistant strains). Different biophysical techniques, including fluorescence spectroscopy and microscopy, flow cytometry, dynamic light scattering, zeta-potential, circular dichroism and atomic force microscopy allowed to infer about the mechanism and efficiency in promoting bacteria cell death. Data obtained were confirmed and further extend by *in vivo* studies, using animal models of infection. More recently, we hypothesized if any of these AMPs could be good candidates for cancer therapies. Using the same approach, we demonstrated that *Pa*MAP 1.9 has promising anticancer activity.

Interaction Between Tethered Bilayer Membranes and s100A9 aggregates

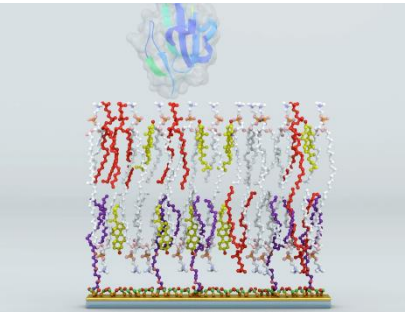
Rima Budvytyte[1], Vytautas Smirnovas[1], Mathias Lösche,[2] and Gintaras Valincius[1]

[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania; [2]Department of Physics, Carnegie Mellon University, Pittsburgh, USA;

rima.budvytyte.@bchi.vu.lt

A central event in pathogenesis of Alzhaimer's diseases are thought to be intracellular and extracellular accumulation, aggregation and misfolding of low molecular mass peptides such as β-amyloid ($A\beta_{1-42}$), tau protein (Tau) and s100A9 [1,2, 6]. Small size aggregates-oligomers were found to be extremely neurotoxic *in vitro* and *in vivo* with the ability to disrupt the major neuron membranes [3,4] and lead to synaptic dysfunction, mithochondrial dysfunction, neuronal apoptosis and brain damage [5].

In this work different sizes of soluble recombinant s100A9 aggregates were used to investigate their interaction with tethered phospholipid membranes (tBLM). The morphology and size of misfolded protein aggregates were monitored by dynamic light scattering (DLS) and atomic force microscopy (AFM). These protein aggregates exhibited the different membrane damaging properties as probed by the electrochemical impedance spectroscopy (EIS). The function and morphology of s100A9 aggregates were depending on different oligomerisation conditions: temperature and time. The interaction between s100A9 and tBLM was monitored by EIS time series measurements. The observed lag phase of this interaction were significantly decreased at s100A9 aggregates concentration level. Membrane composition was found to be one of the important factors affecting the interaction of the s100A9 oligomers to phospholipid membranes.



1pav. Representative image of tethered lipid bilayer membranes (tBLM) interaction with s100A9.

References

[1] Taylor J.P. Toxic proteins in neurodegenerative disease. Science, 2002;296:1991-1995.

[2] Wang C, Klechikov AG, Gharibyan AL, et al. The role of pro-inflammatory S100A9 in Alzheimer's disease amyloid-neuroinflammatory cascade. Acta Neuropathologica. 2014;127(4):507-522.

[3]. Andreasen M, Lorenzen N, Otzen D. Interactions between misfolded protein oligomers and membranes: A central topic in neurodegenerative diseases? Biochimica et Biophysica Acta, 2015;1848:1897–1907.

[4]. Marin R. The neuronal membrane as a key factor in neurodegeneration. Frontiers in Physiology, 2013;188(4):1- 3.

[5] Lippens S, Timmerman V, Janssens S. Mitochondria-associated membranes as hubs for neurodegeneration. Acta Neuropathol., 2016;131:505–523.

[6] Wang C, Klechikov AG, Gharibyan AL, et al. The role of pro-inflammatory S100A9 in Alzheimer's disease amyloid-neuroinflammatory cascade. Acta Neuropathologica. 2014;127(4):507-522.

SELECTED TALKS

Session C

Controling Sample Quality, Instruments and Standards

An improved facility for rapid-mixing multi-angle light scattering (MALS) coupled to small-angle X-ray scattering (SAXS) at the Synchrotron SOLEIL SWING beamline

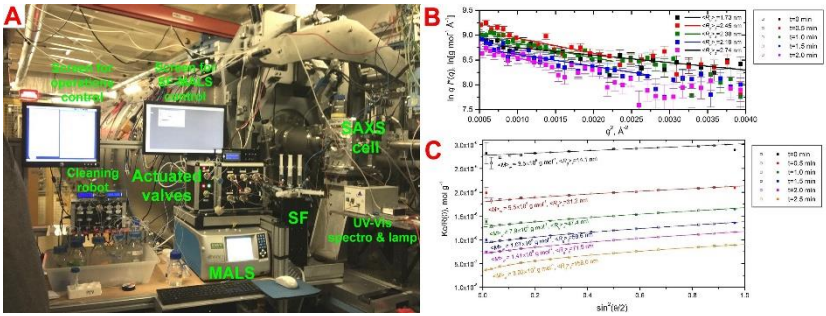
Mattia Rocco[1], Florian Celli[2], Sophie Zinn-Justin[2], Youssef Latimi[3], Pierre Roblin[4], Javier Pérez[3]

[1]Biopolimeri e Proteomica, IRCCS Ospedale Policlinico San Martino, Genova, Italy; [2]I2BC, CEA, CNRS, Université Paris Sud, Université Paris-Saclay, Gif-sur-Yvette, France; [3]SWING beamline, Synchrotron SOLEIL, Gif-sur-Yvette, France; [4]LGC-UMR 5503, CNRS, Toulouse, France

mattia.rocco@hsanmartino.it

Filamentous networks are pervasive elements in biology, either as physiological constituents (*e.g.* actin, tubulin, fibrin) or as pathological by-products (*e.g.* amyloids). Understanding their assembly mechanism and structure is of great biomedical/biotechnological relevance. This can be done by monitoring physical parameters such as fibre length and thickness (which can differ by orders of magnitude), and understanding how and when branching occurs. As demonstrated by Rocco et al. with a prototype experimental set-up developed at the SOLEIL Synchrotron SWING beamline [1], coupling MALS and SAXS with rapid mixing allows the time-resolved determination of the average radius of gyration $\langle R_g^2 \rangle_{z_1}$, molecular weight $\langle M \rangle_w$, and cross-section $\langle R_z^2 \rangle_z$ of polymers during the assembly of filamentous structures in near-physiological conditions.

The recently improved set-up consists in a stopped-flow (SF) mixing device (BioLogic SFM-4) equipped with four 10 mL syringes, providing sample/solutions handling, coupled in-line with a MALS device (WTC HELEOS II) and then with the SWING beamline capillary SAXS cell with integrated UV absorbance detection. All liquid-handling operations are performed using a series of actuated valves mounted on a dedicated table/rack also holding the MALS device. All devices are under control of dedicated software allowing an easy selection of the required steps, interlaced with the software controlling the SF. MALS and SAXS sample cells washing is also provided, either through vacuum suction or with additional syringes, allowing to remotely control all operations (except the SF refilling) without the need of entering the beamline hutch. The SWING beamline (https://www.synchrotron-soleil.fr/en/beamlines/swing) will be shortly accepting beamtime requests involving the use of the SF-MALS-SAXS facility.



A-The SF-MALS-SAXS set up in the SWING beamline hutch; B,C, a selection of SAXS (B) and MALS (C) processed data at 0.5 min intervals derived from a fibrin monomer (0.16 mg/mL) re-polymerization experiment.

Reference

[1] Rocco M, Molteni M, Ponassi M, Giachi G, Frediani M, Koutsioubas A, Profumo A, Trevarin D, Cardinali B, Vachette P, Ferri F, Pérez J. A comprehensive mechanism of fibrin network formation involving early branching and delayed single- to double-strand transition from coupled time-resolved X-ray/light-scattering detection. J. Am. Chem. Soc. 2014; 136:5376-5384. doi: 10.1021/ja5002955.

ITC benchmark study: test reactions and data analysis - a case study within ARBRE-MOBIEU COST action

Adrián Velazquez-Campoy[1], Stefan Knauer[2], Margarida Bastos[3]

[1]Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Spain; ARAID Foundation, Government of Aragon, Zaragoza, Spain; [2]Department of Biopolymers, University of Bayreuth, Bayreuth, Germany; [3]CIQ-UP, Department of Chemistry & Biochemistry, FCUP, University of Porto, Portugal

mbastos@fc.up.pt

The projects being developed at Work Group 4 (Optimization of Data Quality), within COST action *CA15126* Between Atom and Cell: Integrating Molecular Biophysics Approaches for Biology and Healthcare (MOBIEU), are aimed at improving the accuracy, comparability and reproducibility in biophysical research. With this goal, we are setting up SOPs (Standard Operation Procedures) and performing benchmark studies for various biophysical techniques, with results to be available for the scientific community.

As part of this effort, we have been working on a multi-lab ITC study involving

the binding of Ca^{2+} and Mg^{2+} to EDTA in two different buffers. The sample solutions were centrally prepared, sent to 7 participating labs, and were tested on a variety of ITC instruments, following a provided protocol. Additionally, a smaller number of labs prepared their own samples using the same protocol, and performed similar experiments. All raw data was analyzed by 4 different persons, using 4 different software programs.

With the results from this study it will be possible to:
1. compare data handling programs;
2. elaborate on error analysis and reporting;
3. get correct figures for the precision of the thermodynamic data derived from the ITC experiments for the test reactions;
4. suggest test reactions to be used for standardization of ITC data.

This will be very useful for comparing data from different labs, for testing instruments' precision and accuracy, and for assessing the performance of new users/students. The results obtained and the meta data analysis and comparisons performed will be presented and discussed.

Acknowledgments. Thanks are due to Fundação para a Ciência e Tecnologia (FCT), Portugal, for the financial support to Project UID/QUI/0081/2013, Ministero de Ciencia, Innovación y Universidades for the financial support to Project BFU2016-78232-P, and to COST action CA15126.

Kinetic stability of proteins - How stable is stable?

Didier Clenet

Sanofi Pasteur, Marcy l'étoile, France

didier.clenet@sanofi.com

Proteins are fragile macromolecules that required relevant analytical tools and orthogonal approaches to build a broader understanding of their thermodynamic and kinetic stabilities.

First, the Tm value determined by thermal analysis is considered as an indicator of the thermal stability of the protein but it doesn't take into account of the kinetic process. As proteins kinetically stable exhibit high free energy barriers [1], we advantageously used this parameter as an indicator of protein formulations stability. Free energy barriers separating the native state from the non-functional forms were obtained using isoconversional method and heating rate dependence of DSC (differential scanning calorimetry), DSF (differential scanning fluorescence) and nanoDSF profiles.

Second, combining advanced kinetics and statistical analyses, a general

procedure was developed to predict shelf-life of biologics from forced degradation data. This stability modeling approach was successfully applied for vaccine stability predictions [2], providing a time-temperature mapping of product stability able to predict the impact of temperature excursions (cold chain breaks).

References

[1] Sanchez-Ruiz JM, Protein kinetic stability. Biophys. Chem. 2010; 148:1-15. doi:10.1016/j.bpc.2010.02.004.

[2] Clénet D, Accurate prediction of vaccine stability under real storage conditions and during temperature excursions. Eur. J. Pharm. Biopharm. 2018; 125: 76-84. doi: 10.1016/j.ejpb.2018.01.005.

Monitoring of a DNA double helix formation: a multi-approach benchmark study

Carmelo Di Primo[1], Patrick England[2], Thomas Schäfer[3], Iliane Rafaniello[3], Beñat Olave[3], Yves Nominé[4], Radek Bombera[5], Martin Albers[5], Johana Kuncova-Kallio[5], Fabio M. Spiga[6], Karen Mercier[7], Nathalie Vollme [7], Chiraz Frydman[7], Mary Murphy[8], Phillip Page[8], Kristin Fisher[8], Damien Porter[8], Eric Ennifar[9].

[1]INSERM/CNRS/University of Bordeaux, Pessac, France; [2]Institut Pasteur, Paris, France; [3]Polymat, Universidad del Pais Vasco, San Sebastian, Spain; [4]University of Strasbourg, Strasbourg, France; [5]BioNavis Ltd, Tampere, Finland; [6]Creoptix AG, Wadenswil, Switzerland; [7]HORIBA France SAS, Palaiseau, France;[8] Reichert Technologies-AMETEK, Depew NY, USA; [9]CNRS/University of Strasbourg, Strasbourg, France

carmelo.diprimo@inserm.fr

In basic or applied research the characterization of binding parameters of complexes involving biological macromolecules remains a crucial step to understand function, to relate it to the structure, to evaluate whether a specific ligand is of interest for therapeutic purposes, or to validate a molecular probe for biosensing or for more fundamental studies. The determination of the dissociation equilibrium constant, K_D, and if possible the kinetics and the thermodynamic parameters of the binding reaction, is often seen as the Holy Grail. The last decade has seen the emergence of new manufacturers offering biosensing instruments that suit the needs of researchers. There is now a wide range of instruments that are all capable of measuring affinity constants of complexes involving biological macromolecules, with different setups and based on different physical principles. The goals of the present benchmark study was

to analyze how different technical setups performed for analyzing a very simple model, the formation of a 10-mer DNA double helix, the stability of which can be easily tuned by the temperature, the ionic strength or single point mutations. The results show that the measured dissociation constant, K_D, depends to a significant extent on how the DNA formation helix is monitored.

ARBRE-MOBIEU meets other COST-Actions

BioBrillouin: A COST Action to promote applications of Brillouin Light Scattering Microspectroscopy for Life Science and Biomedical Research and Applications

Kareem Elsayad

VBCF, Wien, Austria

kareem.elsayad@vbcf.ac.at

Over the last decade it has become apparent that Brillouin Light Scattering Spectroscopy can have numerous potentially very useful applications in the life sciences. This has motivated the initiation of the "BioBrillouin" COST Action network, with the goal to bring together the community of physicists, engineers, biologists as well as clinicians working or interesting in applying the technique, and thereby promote its implementation for studying biological systems as well as for medical applications. Here I will give a brief overview of the field and its applications in life sciences, focusing on activities, achievements and goals of the BioBrillouin community.

Building networks, capacity and expertise: The case of COST BM1403

Frank Sobott

University of Leeds, Leeds, United Kingdom

f.sobott@leeds.ac.uk

This contribution will take you on a quick journey through the various stages of creating and running COST Action BM1403 (Native Mass Spectrometry and Related Methods for Structural Biology), from its inception to the final report which was just submitted. It will tell the story of how one scientist in the UK encouraged another in Belgium to have a go at this - with one now succeeding the other in their post... The assembled group of Mass Spec's Eleven initial applicants succeeded the first time they tried, blissfully unaware of the overall low success rate of such applications. Maybe the rather unusual gender balance helped - with a slight female majority? Besides training 400+ scientists in ca. 10 training schools and facilitating a. 25 STSM placements, we established a tight network of instrument manufacturers, bio/pharma companies and academics. Working groups in native MS, ion mobility, HDX, crosslinking, and top-down MS/MS established a road map for best practice in experiment and data analysis, as well as setting standards for data deposition and structure modelling. 5 open access papers including in Nature Methods are now the lasting legacy of these efforts.

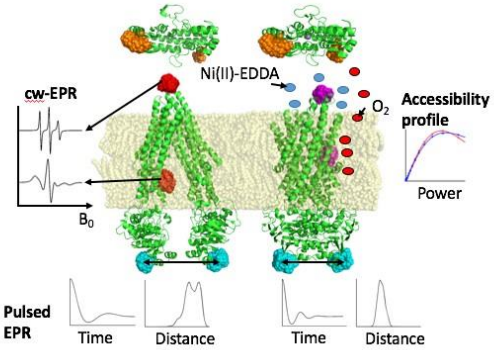
The Henry Wellcome Unit for Biological EPR at UEA: Determining structural features and elucidation of mechanisms in membrane-associated transport proteins

Fraser MacMillan

Henry Wellcome Unit for Biological EPR, School of Chemistry, University of East Anglia, Norwich, NR4 7TJ, UK.

fraser.macmillan@uea.ac.uk

Research in the Henry Wellcome Unit for Biological EPR at UEA focuses on the architecture and functional dynamics of membrane proteins, many medically relevant with a special interest on membrane transport systems and their interaction with intra-cellular signalling pathways. There is increasing evidence that membrane proteins do not act alone, but that they are organised as nano-machineries which function through the concerted action of its individual components with high precision and specificity observed in both time and space. We seek to unravel the principles underlying the architecture and dynamics of these protein nano-machineries as well as their function and regulation. Our experimental approach focuses on the use of Electron Paramagnetic Resonance (EPR) techniques in combination with molecular biological, biochemical and other biophysical methods including theoretical MD approaches. Our expertise lies in the development and application of novel EPR techniques to address these key questions.



EPR spectroscopy is a highly sensitive technique used to study materials containing unpaired electrons; biological examples typically include haem proteins, iron-sulfur centres and transient organic radicals generated by bioenergetic enzymatic processes. In the context of naturally diamagnetic proteins this technique is still viable through the use of site-directed spin labelling

(SDSL). This is the attachment of small, stable spin labels, at specific sites within the protein. This then opens the system up to a range of different magnetic resonance experiments: from room temperature studies for the determination of secondary structural elements and membrane topology to low temperature and pulsed methods that allow determination of structural information (e.g. distances up to 8-10 nm). These experiments in conjunction with computational analyses make EPR a powerful technique for both qualitative and quantitative investigations into the dynamic processes membrane transport systems can undergo.

Here we will use examples from our recent collaborative work on membrane and metallo-proteins, multidrug efflux pumps, membrane transporters and bacterial pathogens to demonstrate the power of this technique to deliver key mechanistic insight into e.g. how to resolve multiple distances in complex macromolecular complexes, how to observe conformational change within membrane proteins at a molecular level and finally to identify the molecular determinants of substrate binding and the potential implications for e.g. host-pathogen interactions.

This research is funded by UEA, The Royal Society and the Wellcome Trust as well as being embedded within the EU COST Action CM1306 “Understanding Movement and Mechanism in Molecular Machines”.

References

[1] J Hall, A Sohail, EJ Cabrita, C Mcdonald, HH Sitte, T Stockner, J Angulo, & F MacMillan; Non-cooperative ligand binding in GltPh: Determination of substrate binding affinity using Saturation Transfer Difference NMR Spectroscopy Angewandte Chemie Submitted (2019)

[2] A Mullen, J Hall, J Diegel, I Hassan, A Fey, F MacMillan: Membrane transporters studied by EPR spectroscopy: structure determination and elucidation of functional dynamics Biochemical Society Transactions 44 (3), 905-915 (2016)

[3] J van Wonderen, DN Kostrz, C Dennison & F MacMillan: REFINE’d Distances Between Paramagnetic Centres of a Multi-Copper Nitrite Reductase Determined by Pulsed EPR (iDEER) Spectroscopy Angewandte Chemie, 52, 1990-1993 (2013)

[4] F MacMillan, S Kacprzak, H Angerer, M Kaupp & H Michel: Using EPR up close and from afar: elucidating mechanisms in haem copper oxidases Faraday Discussions, 148, 315-345 (2011)

[5] Thomas Prisner, Martin Rohrer and Fraser MacMillan: Pulsed EPR spectroscopy: Biological applications Annual Reviews in Physical Chemistry 52, 279-313 (2001)

POSTERS

(listed alphabetically by corresponding author)

P1

Novel molecular interaction controlling yeast cell decision at the transition from fermentative and oxidative metabolism

Malgorzata Adamczyk, Roza Szatkowska

Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland

madamczyk@ch.pw.edu.pl

In *Saccharomyces cerevisiae* cells the switch from respiration to fermentation has been claimed to respond to the external glucose levels. However, several lines of evidence seem to point at cellular metabolism to play the major role in this process. Lack of Maf1, the only negative regulator of yeast RNA polymerase III (RNAP III), as well as point mutation in C128 subunit of RNAP III, have been found to adversely affect metabolic reprogramming, that is correlated with the activity of RNAP III driven transcription of non-coding RNAs, such as tRNAs [1, 2, 3]. The molecular mechanism of the perturbation in switching metabolism in these mutant cells is not well understood.

Cellular localization of Maf1 regulator is glucose dependent [4]. When glucose is abundant, Maf1 is localised in the cytoplasm [4]. Decreased RNAP III activity or the absence of Maf1 elicit broad changes in fundamental metabolism in *S. cerevisiae*. The biological function of Maf1 in the cytosol is still not well defined known.

Here, we report Maf1 new interactions with protein partners in the cytoplasm using Bimolecular Fluorescence Complementation (BiFC) *in vivo*. This novel role of Maf1 as a partner of proteins with intrinsically disordered domains (IDD), recently reported as required for glucose-repressed genes transcription is consistent with the perturbed metabolic reprogramming observed in *maf1-Δ* cells [1, 2, 3].

Intrinsically disordered proteins (IDPs) are crucial for membrane-less organelles formation, such as P-bodies or stress granules (SG). Immunofluorescence of Maf1 deficient strain using the P-bodies marker – Pab1 does not confirm the presence of these structures in the mutant regardless carbon source. However on glucose, we show formation of Dbp2-mediated SGs in *rpc128-1007* the suppressor of *maf1-Δ* growth defect on non-fermentable carbon source [1]. Dbp2 is involved in transcriptional control and glucose metabolism [5]. Recently, the RNA Helicase Dbp2 has been proposed as an intracellular link integrating environmental nutrient conditions with regulation of glucose-repressed, Cys8-targeted genes across the genome [6].

This research received funding from the National Science Centre Poland, grant no 2012/05/E/NZ2/00583 for MA

References
[1] Cieśla M et. al. Mol. Cell. Biol. 2007; 27(12):7693-7702. doi: 10.1128/MCB.01051-07.
[2] Adamczyk M, Szatkowska R. PLoS One. 2017; 12(9):e0185516. doi: 10.1371/journal.pone.0185516.
[3] Szatkowska R, et.al, . Biochem J 2019, under review.
[4] Towpik J et.al. J Biol Chem 2008; 283, 17168-17174. doi: 10.1074/jbc.M709157200.
[5] Xing Z et.al. RNA 2017;1125-1138. doi: 10.1261/rna.060335.116.
[6] Wang S et al.. G3 (Bethesda) 2017; 7(7):2235-2247. doi: 10.1534/g3.117.041814.

P2

EIS and SERS Analysis of Anchor Molecules for Tethered Bilayer Lipid Membrane Formation

Indre Aleknaviciene, Gintaras Valincius

Vilnius university Life Sciences Center, Institute of Biochemistry, Sauletekio av. 7, Vilnius, Lithuania

indre.aleknaviciene@gmc.vu.lt

The tethered bilayer lipid membranes (tBLMs) are considered as perspective experimental platforms for membrane biosensors and may be suitable for a broad spectrum of biophysical experiments such as peptide/membrane interactions, protein/membrane interactions, lipid phase transitions and others. tBLMs attachment to the silver surface allows the monitoring of biologically relevant events with electrochemical impedance (EIS) and surface enhanced Raman (SERS) spectroscopical techniques.

It is known that structure of the self-assembled monolayers (SAMs) used to anchor phospholipid bilayers to surfaces affects the functional properties of the tethered bilayer lipid membranes [1].

To evaluate the differences in tBLM formation on flat silver surface two types of anchor molecules were chosen to form SAMs – long strand thiolipid Wilma's compound (WC14) and three different short strand backfillers 3-mercapto-1-propanol (3-M-1-P), 4-mercapto-1-butanol (4-M-1-P) and 6- mercapto-1-hexanol (6-M-1-H). EIS results showed the ability to successfully form functional SAMs and tBLMs on the plain silver surface, which leads to further experiments with this system using the SERS technique on nanostructured silver surface.

For the SERS analysis the same backfillers (3-M-1-P, 4-M-1P and 6-M-1-H) were used in a pair with WC14 compound to form SAMs with different structural properties. Analysis of SERS spectra form anchor molecules before and after tBLM formation showed, that WC14 and all of the tested short strand

backfiller molecules are suitable for tBLM immobilization on nanostructured silver surface. Furthermore, by using different length backfiller molecules it is possible to control long strand anchor molecules conformation on the surface.

Reference

[1] Rakovska B, Ragaliauskas T, Mickevicius M, Jankunec M, Niaura G, Vanderah DJ, Valincius G. Structure and Function of the Membrane Anchoring Self-Assembled Monolayers. Langmuir. 2015, 31 (2), pp 846–857 doi: 10.1021/la503715b.

P3

Probing the mechanism of iron release from Dps, a miniferritn

Almeida, NR, Almeida, AV., Tavares, P, Pereira, AS

UCIBIO/Requimte Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

acv.almeida@campus.fct.unl.pt

Due to its function as the cofactor of many enzymes related with the metabolism of all living organisms, iron is classified as an essential metal. Despite its biological relevance, iron is highly toxic due to its low solubility under physiological conditions and its role in Fenton reaction, producing reactive oxygen species (ROS) that compromise the integrity of the cell. A large family of proteins called Ferritins have the ability to store this metal within a hollow cavity, controlling its uptake and release into solution, hindering its toxicity. Ferritin family members share a hollow spherical structure formed by identical subunits. Dps (DNA-binding protein from starved cells), a prokaryotic miniferritin, is composed by 12 subunits with the capacity to store up to 500 irons atoms.

The mechanisms through which these proteins store iron in their inner hollow cavity is already well characterized. The initial step of iron intake and oxidation at specific oxidation sites is followed by iron storage in a mineral form known as mineral core[1]. However, the process by which the mineral iron returns to solution is much less understood. Upon low iron availability in the cell, an additional step must occur where iron is released from the Dps core. This process requires the reduction of the iron core ions and subsequent removal of this metal through channels that connect the internal cavity to the outer surface of the protein.

To ensure the reduction step a redox partner is required to allow electron-transfer from an electron donor (such as NADH) to the ferritin mineral core. In this study the redox partner selected to probe this process was a flavoprotein

called WrbA.

To investigate the reduction of ferric core to ferrous ions within the Dps (from *M. hydrocarbonoclasticus*) cavity through the effect of the WrbA flavoprotein (from the same organism) ¯Mössbauer spectroscopy was applied and the kinetic properties of the reduction were observed in anaerobic conditions. The subsequent process of iron release from the inner cavity into solution was monitored by Visible spectroscopy using the *o*-phenanthroline free-iron quantification assay. The kinetic analysis of both processes indicates that iron release is a controlled mechanism ensuring a low and constant iron concentration is released to the cytosol.

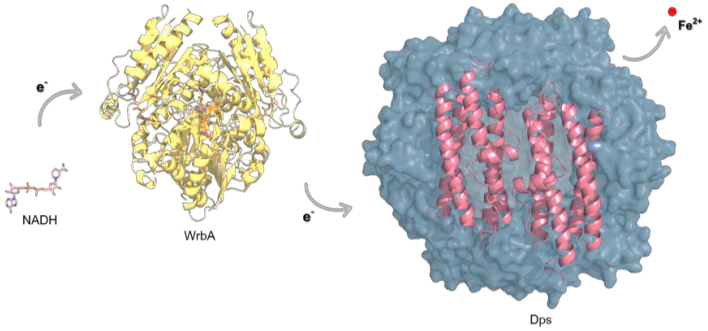


Figure: Iron release mechanism of Dps. For soluble iron to be released, Dps (blue protein) needs to reduce Fe^{3+} to Fe^{2+} requiring an electron transferred from WrbA (yellow), a partner protein. The latter is then regenerated by NADH.

Acknowledgment: This work was supported by the Applied Molecular Biosciences Unit-UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019). This research was supported by Fundação para a Ciência e Tecnologia, Ministério da Educação e Ciência (FCT/MEC), grant PTDC/BIA-PRO/111485/2009 (to P.T.), PTDC/QUI/64248/2006 (to A.S.P). AVA and ANR are supported by the Radiation Biology and Biophysics Doctoral Training Programme (RaBBiT PD/00193/2012) and an FCT/MEC Ph.D. Fellowship PD/BD/135477/2017 (to AVA) and PD/BD/106034/2015 (to NA).

P4

Modification of Magnetic Nanoparticles with DNA Particulates and Their Applications in Magnetic Hyperthermia

Cem L. Altan[1], Seyda Bucak[1], Mehmet H. Ucisik[2,3], S. Sibel Erdem[3,4], Bradley T. Messmer[5,6] Kemal Duzkar[1], Gizem Keceloglu[7]

[1]Department of Chemical Engineering, Yeditepe University, Istanbul 34755, Turkey, [2]School of Engineering and Natural Sciences, Istanbul Medipol University, Istanbul, 34810, Turkey, [3]Medipol Regenerative and Restorative Medicine Research Center (REMER), Istanbul, 34810, Turkey, [4]International School of Medicine, Department of Medical Biochemistry, Istanbul Medipol University, Istanbul, 34810, Turkey. [5]UCSD Moores Cancer Center, University of California San Diego, La Jolla, CA 92093, USA, [6]Abreos Biosciences, San Diego, USA, [7]Graduate School of Health Sciences, Biochemistry, Istanbul Medipol University, Istanbul, 34810, Turkey

cem.altan@yeditepe.edu.tr

For magnetic hyperthermia applications, magnetic nanoparticles are either injected directly into the tumor site or targeted by active molecules having high affinity to the cancer cells. Since, high concentration of these magnetic particles lead to systemic toxicity, the direct injection method is not favored. On the other hand, the active targeting may lead to homogeneous and uniform distribution within the tumor site due to the presence of specific ligands thus requires relatively lower concentrations. As a consequence, it is cruical to develop novel methods for the uniform distribution of magnetic nanoparticles for hyperthermia applications in order to enhance the efficiency of the method and also prevent over use of nanoparticles. DeNAno nanoparticles are synthetic affinity reagents which are composed of highly condensed DNA particulates presenting concatemeric sequence that repeats and acts as ligands to specific molecules [1]. On the contrary to its alternatives, DeNAno binds to its target by the interaction of several active molecules which have surface and morphology recognition properties rather than one to one molecular interactions. Additionally, DeNAno is synthetically formed by using random DNA libraries similar to aptamer technology consequently leading to the formation of multivalent binding nature such that it can act as a ligand to specific targets and be uniformly distributed at the target site. In this study, surface modified magnetite nanoparticles will be synthesized by partial oxidation method and then these magnetic particles will be conjugated with a specific DeNAno DNA sequence that specifically bind to pancreatic cancer cell line Panc-02. The specific absorption rate (SAR) of both surface modified magnetite nanoparticles and the conjugated form will be measured and optimized. Finally, the binding characteristics and affinities of the DeNAno conjugated magnetite nanoparticles to specific cancer cell lines will be investigated in vitro along with their cytotoxic and cell destruction effects caused by magnetic hyperthermia.

The work is supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK), Project Nr.216Z004

Reference

[1] Steiner JM, Sartor M, Sanchez AB, Messmer D, Freed A, Esener S ve Messmer BT. 2010. "DeNAno: selectable deoxyribonucleic acid nanoparticle libraries". Journal of biotechnology, 145(4), 330-333.

P5

Are we observing magnesium (Mg^{2+}) or monovalent (K^+, Na^+) ions in ribosomes?

Pascal Auffinger

Architecture et Réactivité de l'ARN, Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, 67084, France

p.auffinger@ibmc-cnrs.unistra.fr

To comprehend nucleic acid structure and function, it is essential to understand and to precisely model the structure of their solvent shell that usually embed a great diversity of neutral and charged species. Ribosomes are responsible for protein synthesis but are also major antibiotic targets and are key molecular assemblies for understanding the evolutionary process. For a long time, and despite a pioneering study that discussed the importance of mono- and divalent ions in ribosomes (1), most subsequent crystal structures were modeled with a poor ionic environment embedding often an excessive and non-reasonable amount of Mg^{2+} ions. This led to an exaggeration of the role of Mg^{2+} in nucleic acids. Recent reports stress that most of the assigned Mg^{2+} ions in these ribosomal structures are not respecting essential stereochemical constraints and, therefore, correspond to misassigned solvent molecules (2-4). Here, we present data, based on simple stereochemical rules and a precise examination of the electron density patterns of ribosomal systems suggesting that the importance of K^+ ions has been significantly underestimated. To illustrate this observation, I will discuss several examples that stress the importance of K^+ ions. In short, it is proposed: that the monovalent ion (K^+) count in ribosomal structures is largely underestimated at the "profit" of Mg^{2+} ions; *(ii)* that Mg^{2+} ions modeled at the decoding center (Fig.) led to unrealistic models since Mg^{2+}, that is a stronger binder than K^+, would eventually freeze the dynamics of the decoding center while models with K^+ would favor fluidifying essential parts of these molecular machines. Thus, the presence of one or the other ions in structural models will significantly affect our perception of the dynamics of these ribosomal and other RNA systems.

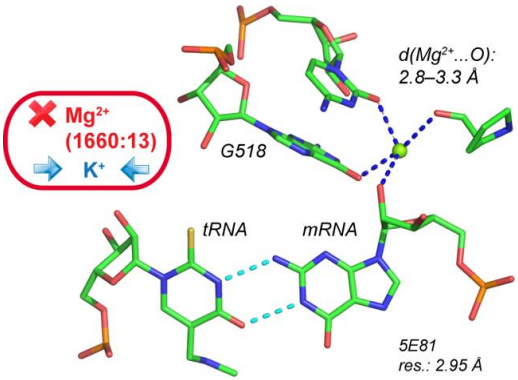


Figure legend – A recurrentlly assigned Mg^{2+} with K^{+} characteristics in the ribosomal decoding center of several T. thermophilus structures with tRNA and mRNA fragments.

References

[1] Klein, D.J., Moore, P.B. and Steitz, T.A. (2004) The contribution of metal ions to the structural stability of the large ribosomal subunit. RNA, 10, 1366-1379.

[2] Auffinger, P., D'Ascenzo, L. and Ennifar, E. (2016) Sodium and potassium interactions with nucleic acids. Met. Ions Life Sci., 16, 167-201.

[3] Leonarski, F., D'Ascenzo, L. and Auffinger, P. (2017) Mg2+ ions: do they bind to nucleobase nitrogens? Nucleic Acids Res., 45, 987-1004.

[4] Leonarski, F., D'Ascenzo, L. and Auffinger, P. (2018) Nucleobase carbonyl groups are poor Mg2+ inner-sphere binders but excellent monovalent ion binders - A critical PDB survey. RNA. In press.

P6

Detection of toxic metal ions by the CueR metalloregulator

Ria Katalin Balogh[1], Edit Mesterházy[1], Kohsuke Kato[2], Kyosuke Nagata[2], Attila Jancsó[1], Béla Gyurcsik[1]

[1]Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, 6720 Szeged, Hungary., [2]Faculty of Medicine, University of Tsukuba, Tsukuba 305-8575, Japan

The understanding of the details of bacterial metal ion regulatory mechanisms may forward the design of molecules for selective metal ion binding/accumulation or the development of sensitive metal ion-detection technics. The $Cu^{I}/Ag^{I}/Au^{I}$ sensing copper efflux regulator CueR is a good example of proteins that regulate the intracellular level of a group of metal ions

at a transcriptional level in several strains of bacteria [1]. The binding of the effector metal ion to CueR induces a conformation change in the protein-bound DNA which ultimately activates the transcription of the downstream metal-resistance genes.

As such, CueR provides a promising platform for the construction of metal-ion selective sensing systems. In the present project, we constructed a fluorescent reporter system by fusing the gene of the enhanced green fluorescent protein (EGFP) downstream to the CueR-responsive promoter region (*PcopA::egfp*). This promoter-reporter sequence was cloned into two different plasmids. One of these encompasses the gene of the CueR protein (pUC119-CueR-copA-EGFP) while the other lacks this gene (pMW219-copA-EGFP). CueR-knocked out ΔCueR BW25 and BL21(DE3) *E. coli* strains were transformed with the reporter plasmids. As a result, the expression of the fluorescent protein EGFP is induced in the presence of effector metal ions in these modified bacteria (Figure 1.).

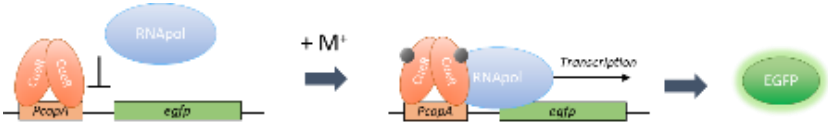


Figure 1: The scheme of CueR based fluorescent reporter system

The characteristics of the metal ion sensing (response time, selectivity, sensitivity) were examined through measuring the fluorescence under different conditions with flow cytometry and fluorescent microscopy. Furthermore, these constructs may be a convenient tool for monitoring the biological functioning of CueR or its mutants that could further our understanding on the metal recognition mechanism of this protein.

Reference

[1] A. Changela, K. Chen, Y. Xue, J. Holschen, C. E. Outten, T. V. O'Halloran, A. Mondrago; Science; 2003 (301) 1383-1387.

P7

Peptide screening using surface sensitive techniques: a way for the development of new antibody mimics

Laure Bar[1], Jérôme Dejeu[1], Rémy Lartia[1], Fouzia Bano[2], Ralf Richter[2], Liliane Guérente[1], Didier Boturyn[1]

[1]Univ. Grenoble Alpes, DCM, CNRS, UMR 5250, F-38000 Grenoble (France), [2]School of Biomedical Sciences, Faculty of Biological Sciences – Leeds (UK)

laure.bar@univ-grenble-alpes.fr

Monoclonal antibody-based treatments have been established as the most successful therapeutic strategies for cancers. They possess excellent specificity and affinity for tumour cell targets (epitope). Despite these advantages, they suffer from limitations that relate mainly to their high molecular weight, their high cost, and polymorphism of their Fc region [1]. The design of entirely synthetic mAb mimics is then an attractive way to bypass these problems [2]. In this context, we decided to design mAb mimics of "Rituximab" which is exploited to treat non-hodgkin lymphoma through the recognition of the CD20 epitope.

The mAb mimics consist of macromolecular constructions of short hexapeptide sequences included in the rituximab CDRs (complementarity determining regions). The selection of peptides presenting the best affinity constants was carried out by using surface plasmon resonance (SPR – Biacore).

For this purpose, we first set up a CD20 grafting protocol to obtain a specific surface recognition for Rituximab. During this study and by coupling quartz crystal microbalance (QCM-D) with spectroscopic ellipsometry (SE), we have shown that the surface CD20 density is a key parameter for the recognition of Rituximab through bivalent interaction. These experiments permitted to extract some parameters of the designed antigenic sensor, such as the inter-ligand spacing or the percentage of available antigens for a binding with Rituximab.

Peptide screening on surface allowed us to highlight the most important and accurate areas of the binding site necessary for the CD20 recognition.

References

[1] Chames, P., Van Regenmortel, M., Weiss, E. & Baty, D. Therapeutic antibodies: successes, limitations and hopes for the future. Br J Pharmacol 2009. 157, 220–233

[2] Park, B.-W. et al. Rationally designed anti-HER2/neu peptide mimetic disables P185HER2/neu tyrosine kinases in vitro and in vivo. Nature Biotechnology 2000; 18, 194–198

P8

Functional dynamics of a non-heme iron(II) enzyme revealed by site-directed spin labeling EPR spectroscopy

E. Fournier[1,2], S. Tachon[2], N. Fowler[3], S.P. de Visser[3], G. Gerbaud[1], E. Etienne[1], J. Simaan[2], V. Belle[1] and M. Martinho[1]

[1]Aix Marseille Univ, CNRS, BIP, Marseille, France, [2] Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, Marseille, France, [3] Manchester Univ, Manchester Institute of Biotechnology, Manchester, United Kingdom

belle@imm.cnrs.fr

ACC Oxidase (ACCO) is a non-heme iron(II) containing enzyme that catalyzes the last step of production of ethylene, a plant phytohormone. Only one crystallographic structure of ACCO has been reported, showing the C-terminal part (C-ter) away from the active site [1]. This might not be the active conformation as it has been shown that the C-ter is essential for activity [2]. A structural model of ACCO has been later proposed in which the C-ter is folded towards the protein core [3]. Two different conformations can thus be hypothesized: either open or closed with respect to the active site, suggesting a dynamic role of the C-ter.

Our study is focused on exploring the functional dynamics of ACC Oxidase C-ter part, using site-directed spin labeling coupled to EPR spectroscopy. This technique relies on the covalent insertion of nitroxide derivative spin labels (EPR active) on selected residues and is well-suited to monitor protein dynamics [4]. Pulsed EPR spectroscopy (DEER experiments) was performed to measure inter-label distances. The comparison between the set of measured distances and the predicted ones using MMM (Multiscale Modeling of Macromolecules [5]) demonstrates that the C-ter part of ACCO in solution is neither in the closed nor in the open conformation but remains highly flexible even in the presence of the different co-factors involved in the catalysis. These findings are reinforced by molecular dynamic calculations giving inter-label distances that are in very good agreement with the experimental results and thus demonstrating that the C-ter keeps high dynamics.

References

[1] Zhang, Z.; Ren, J-S.; Clifton, I. J.; Schofield, C. J. Chem Biol 2004, 11, 1383-1394
[2] Yoo, A.; Seo, Y. S.; Jung, J-W.; Sung, S-K.; Kim, W. T.; Lee, W.; Yang, D. R. J Struct Biol 2006, 156, 407-420
[3] Brisson, L.; El Bakkali-Taheri, N.; Giorgi, M.; Fadel, A.; Kaizer, J.; Réglier, M.; Tron, T.; Ajandouz, E. H.; Simaan, A. J. J Biol Inorg Chem 2012, 17, 939-949
[4] Martinho, M.; Fournier, E.; Le Breton, N.; Mileo, E.; Belle, V. Royal Society of Chemistry book chapter, Electron Paramagn Reson, 2019, 26, 66-88
[5] Polyhach, Y.; Bordignon, E.; Jeschke, G. Phys Chem Chem Phys 2011, 13, 2356-2366

P9

Structure, function and interactions of proteins involved in nickel-dependent carcinogenesis and infections

Ylenia Beniamino, Barbara Zambelli

University of Bologna, Laboratory of Bioinorganic Chemistry

ylenia.beniamino2@unibo.it

The aim of my study is to determine structure, function and interaction of proteins involved in nickel-dependent carcinogenesis and infections.

Nickel is a metallic compound that plays fundamental biological roles for plants, bacteria, archaea and unicellular eukaryotes, catalyzing biochemical reactions in the active site of different metallo-enzymes. Despite this important role, nickel has also a poisoning potential: exposure to nickel is responsible for a variety of adverse effects on humans, such as immune reactions, respiratory and cardiovascular diseases, bacterial infections and carcinogenesis. All hazardous health effects caused by nickel exposure to human health are mediated by the interaction of nickel with macromolecules and by the formation of reactive compounds that mediate cellular damage, through changes in metabolism, inflammation, oxidative stress, cell proliferation and cell death [1].

My work focused on NDRG1 (N-myc downregulated gene 1) is a human protein, whose expression is induced by nickel through the hypoxia pathway. This protein has multiple biological roles (Figure 1) and numerous studies have revealed that the expression levels of this protein are different between tumor and normal tissues, suggesting its function as tumor suppressor in a variety of cancer. Proteins belonging to the NDRG family differ in the C-terminal region and, in particular, NDRG1 features a C- terminal tail, with the three-fold repeat GTRSRSHTSE, a possible nickel binding motif [2][3].

In my work, the protein has been heterologously expressed and purified from *E. coli*. Light scattering, circular dichroism and isothermal titration calorimetry have been applied to determine the biochemical and biophysical properties of the protein in solution.

Figure 1 – Biological function of NDRG1

References
[1] B. Zambelli, V. N. Uversky, S. Ciurli; Nickel impact on human health: an intrinsic disorder perspective; Biochimica and Biophysica Acta (2016) 1714-1731;
[2] Agnieska Kitowska and Tadeusz Pawelczyk. N-myc downstream regulated 1 gene and its place in the cellular machinery. Acta Biochimica Polonica Vol. 57, No 1/2010 15-21.

P10

Characterisation and MDS of SOD mutants

Rosalin Bonetta[1*], Tomasz Borowski,[2] Rebeca Garcia Fandiño,[3] Gary J. Hunter,[1] Thomas Stockner,[4] Chi Trinh,[5] Thérèse Hunter[1]

[1] University of Malta, Msida, Malta, [2] Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland, [3] University of Santiago de Compostela, Santiago de Compostela, Spain, [4] Medical University of Vienna, Vienna, Austria, [5] University of Leeds, Leeds, UK.

rosalin.bonetta@um.edu.mt

The superoxide dismutase (SOD) enzyme acts as the first line of defence against the superoxide free radical, dismutating it into hydrogen peroxide and molecular oxygen. SODs are implicated in longevity pathways and carcinogenesis. Therefore, understanding the functional role of specific amino acids in the protein can help in the future development and application of SODs, which can treat oxidative stress injuries in diseases such as inflammation, diabetes, ischaemia reperfusion injury and cancer [1].

We have generated two site-directed mutants of *C. elegans* MnSOD-3, which modify the metal selectivity of the enzyme. The three-dimensional structure of these mutants was solved via X-ray crystallography at the Diamond Synchrotron Light Source, Oxfordshire, UK. The mutant proteins were characterised by their metal content, circular dichroism spectroscopy and enzyme activity. Finally, molecular dynamics simulations of the second-sphere mutants were performed to elucidate further details on the structure and motions of these proteins.

Native MnSOD-3 functions with manganese in the active site and has no activity when iron is incorporated. However, when histidine replaced glutamine 142 in the active site, the enzyme retained 50% of its activity and became cambialistic for its metal cofactor exhibiting very similar specific activity with either manganese or iron. When asparagine replaced histidine 30 in the active site, metal analysis results of the mutant protein also revealed the spontaneous uptake of Fe in addition to Mn. However, enzyme activity was drastically reduced to 22%. Analysis of the MDS trajectories revealed inner-sphere binding modes of the water molecules that could not be observed via the crystallographic

structures of the two mutant proteins.

Thus, we describe the change in metal selectivity through two single mutations of an MnSOD. However, there seems to be no simple explanation for the metal selectivity during de novo protein folding or specificity (enzyme activity with a selected metal) of this important class of enzymes.

Reference

[1] Bonetta, R., Potential Therapeutic Applications of MnSODs and SOD-Mimetics. Chemistry 2018, 24 (20), 5032-5041. doi: 10.1002/chem.201704561

P11

From gene to biomolecular interaction studies

Deborah Byrne

Protein Expression Facility IMM CNRS, Marseille, France

Byrne@imm.cnrs.fr

In order to perform interaction studies, we need large amounts of stable soluble proteins. However, the challenge we often have is to go from gene to soluble protein. Structural genomics projects were based on low hanging fruit proteins. The strategies we had for these projects are no longer applicable for recalcitrant proteins. We are now confronted with this bottleneck, and some labs are not trained to tackle these obstacles. Our institute set up a core facility to push through this tight jam. We provide a service which not alone improves gene expression and protein production, but we also provide biomolecular interaction studies using DLS (1-3), Blitz (4) and ITC. We have the training and knowledge to improve proteins for interaction studies. This poster will give an overall presentation of the facility and tips to improve protein quality.

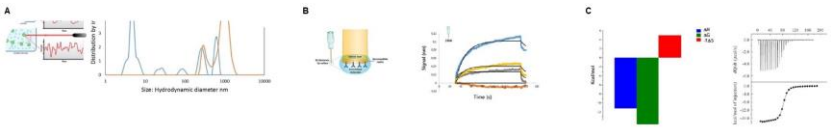


*Figure –**A** DLS for QC & Interaction studies. Interaction studies **B**. BLi, **C**, ITC*

References

[1]Roman, A. Y., Devred, F., Byrne, D., La Rocca, R., Ninkina, N. N., Peyrot, V., & Tsvetkov, P. O. (2018). Zinc Induces Temperature-Dependent Reversible Self-Assembly of Tau. Journal of molecular biology.

[2]Pompeo, F., Byrne, D., Mengin-Lecreulx, D., & Galinier, A. (2018). Dual regulation of activity and intracellular localization of the PASTA kinase PrkC during Bacillus subtilis growth. Scientific reports, 8(1), 1660.
[3]Monsalve, Karen, et al. "Hydrogen bioelectrooxidation on gold nanoparticle-based electrodes modified by Aquifex aeolicus hydrogenase: Application to hydrogen/oxygen enzymatic biofuel cells." Bioelectrochemistry (2015).
[4]Bouillet, Sophie, et al. "The General Stress Response σS is Regulated by a Partner-Switch in the Gram-Negative Bacterium Shewanella oneidensis." Journal of Biological Chemistry (2016): jbc-M116.

P12

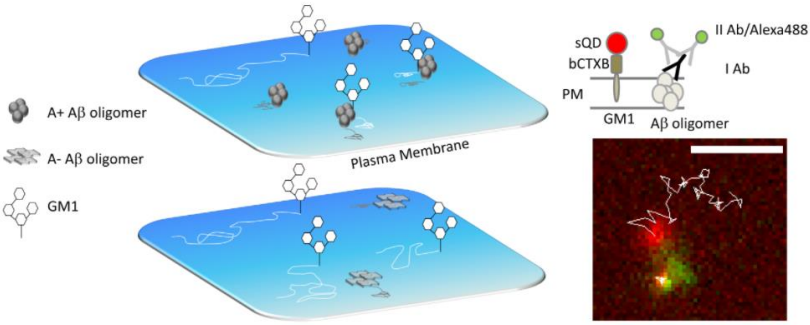
Alzheimer's disease from a single molecule/single cell perspective

Martino Calamai[1,2], Francesco S. Pavone[1,3]

[1]National Institute of Optics, National Research Council of Italy (CNR), Florence, Italy; [2]European Laboratory for Non-linear Spectroscopy (LENS), University of Florence, Florence, Italy; [3]Dipartimento di Fisica e Astronomia, Università degli Studi di Firenze, Florence, Italy.

calamai@lens.unifi.it

We are applying advanced fluorescence microscopy and single molecule tracking techniques to investigate the molecular basis of neurodegenerative diseases, in particular Alzheimer's disease. This kind of approach has allowed discovering new features normally not accessible with standard methods based on data averaging. By studying the dynamic behaviour of toxic Abeta oligomers on the plasma membrane of living cells, and their interaction with specific membrane components, a new potential mechanism of toxicity based on loss of function has been postulated [1-3]. Results similar to those obtained in the case of Abeta oligomers were found for aggregates formed by other proteins or peptides, such as amylin (involved in the development of type II diabetes) and beta2-microglobulin (associated with a familial form of systemic amyloidosis), supporting the hypothesis that amyloid diseases share similar mechanisms of toxicity. Single molecule tracking has also been used to evaluate the impact of cholesterol on the mobility of the transmembrane proteins involved in the production of Abeta peptide. In parallel, we have developed a fluorescence based bioassay to directly measure the proteolytic process preceding the release of toxic Abeta in single living cells, and to evaluate the factors influencing it [4].



Single molecule tracking experiments highlight that Aβ oligomers with different conformations interact differently with GM1, a component of the plasma membrane involved in neuroprotection.

References

[1] Calamai M, Pavone FS. Single molecule tracking analysis reveals that the surface mobility of amyloid oligomers is driven by their conformational structure. J Am Chem Soc. 2011 Aug 10;133(31):12001-8. doi: 10.1021/ja200951f.

[2] Calamai M, Pavone FS. Partitioning and confinement of GM1 ganglioside induced by amyloid aggregates. FEBS letters. 2013 May 2;587(9):1385-91. doi: 10.1016/j.febslet.2013.03.014.

[3] Calamai M, Evangelisti E, Cascella R, Parenti N, Cecchi C, Stefani M, Pavone FS. Single molecule experiments emphasize GM1 as a key player of the different cytotoxicity of structurally distinct Aβ1-42 oligomers. Biochim Biophys Acta. 2016 Feb;1858(2):386-92. doi: 10.1016/j.bbamem.2015.12.009.

[4] Parenti N, Del Grosso A, Antoni C, Cecchini M, Corradetti R, Pavone FS , Calamai M. Direct imaging of APP proteolysis in living cells. Peer J, 2017 Apr 12;5: e3086. doi: 10.7717/peerj.3086.

P13

Amyloid β-Protein Inhibition and Interaction with Membranes

Maria Rosalia Mangione[1], Fabio Librizzi[1], Oscar Moran[2], Vincenzo Martorana[1], Caterina Ricci[3], Maria Grazia Ortore[3], Marco Maccarini[4], Silvia Vilasi[1], Rita Carrotta[1]

[1]Istituto di Biofisica CNR, Palermo, Italy; [2]Istituto di Biofisica CNR, Genova, Italy; [3]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy; [4]Université Grenoble Alpes, CNRS, Grenoble INP, TIMC-IMAG, Grenoble, France

rita.carrotta@cnr.it

The aberrant aggregation of the amyloid β-peptide (Aβ) is an evident hallmark of Alzheimers' disease (AD). Aβ is a 39-42 a.a. peptide, resulting from a complex proteolytic process of the membrane amyloid precursor protein (APP). In particular, transient oligomeric aggregates, on-pathway in the amyloidogenesis, are believed the most reactive and toxic species, able to onset the amyloid cascade and interact with the membrane milieu [1]. We studied the amyloidogenesis inhibition ability of a chaperone-like molecule, α-casein [2], and Hsp 60, a human chaperonin [3]. Results brought to the conclusion that the mechanism of inhibition is based on the ability of recognition and recruitment of the transient reactive oligomers, intermediates in the amyloid pathway [2-4.]. Moreover, studies in the presence of membrane models, such as bilayers and unilamellar vesicles, point out the role of these species in the toxicity, causing in particular a rigidification of the membrane, as seen by atomic force spectroscopy and neutron spin echo [5-7]. The presence of the chaperonin Hsp60 cancels this effect, thus suggesting that Hsp60 assists the stochastic "misfolding events", which in its absence prompt both the amyloid cascade and the insult on the membrane. The ability of Hsp60 to selectively detect and recruit the reactive transient species highlights the importance of further investigation based indeed on chaperonins, due to their potential therapeutic role [7].

References

[1] Walsh D M, Klyubin I, Fadeeva J V, Cullen W K, Anwyl R, Wolfe M S, Rowan M J, Selkoe D J. Naturally Secreted Oligomers of Amyloid □Protein Potently Inhibit Hippocampal Long-Term Potentiation in Vivo. EBioMedicine 2016; 6. 42-49. doi: 10.1038/416535a

[2] Carrotta R, Canale C, Diaspro A, Trapani A, Biagio P L S, Bulone D. Inhibiting Effect of □s1-Casein on Aβ1-40 Fibrillogenesis. Biochim. Biophys. Acta - Gen. Subj. 2012; 1820, 124–132. doi: 10.1016/j.bbagen.2011.11.010.

[3] Mangione M R, Vilasi S, Marino C, Librizzi F, Canale C, Spigolon D, Bucchieri F, Fucarino A, Passantino R, Cappello F et al. Hsp60, Amateur Chaperone in Amyloid-Beta Fibrillogenesis. Biochim. Biophys. Acta - Gen. Subj. 2016; 1860, 2474–2483. doi: 10.1016/j.bbagen.2016.07.019.

[4] Librizzi F, Carrotta R, Spigolon D, Bulone D, San Biagio P L. α-Casein Inhibits Insulin Amyloid Formation by Preventing the Onset of Secondary Nucleation Processes. J. Phys. Chem. Lett. 2014, 5, 3043–3048. doi: 10.1021/jz501570m.

[5] Canale C, Seghezza S, Vilasi S, Carrotta R, Bulone D, Diaspro A, San Biagio P L, Dante S. Different Effects of Alzheimer's Peptide Aβ(1-40) Oligomers and Fibrils on Supported Lipid Membranes. Biophys. Chem. 2013, 182, 23–29. doi: 10.1016/j.bpc.2013.07.010.

[6] Nicastro M C, Spigolon D, Librizzi F, Moran O, Ortore M G, Bulone D, San Biagio P L, Carrotta R. Amyloid β-Peptide Insertion in Liposomes Containing GM1-Cholesterol Domains. Biophys. Chem. 2016, 208, 9–16. doi: 10.1016/j.bpc.2015.07.010.

[7] Ricci C, Maccarini M, Falus P, Librizzi F, Mangione MR, Moran O, Ortore MG, Schweins R, Vilasi S, Carrotta R. Amyloid β-Peptides Interaction with Membranes: Can Chaperones Change the Fate? J Phys. Chem. B 2018, Dec 20. doi: 10.1021/acs.jpcb.8b11719.

P14

Ferritin: The most interesting bionano component?

Soumyananda Chakraborti, Jonathan J Heddle

Malopolska Centre of Biotechnology, Jagiellonian University, 7A Gronostajowa Street, Krakow, Poland

soumyabiochem@gmail.com

Nature uses self-assembly property of proteins to produce wide variety of large, complex, and highly symmetric protein architectures.[1] Understanding the details of self-assembly is not only important for basic science, the knowledge is also essential to build much ambitious and challenging technology, such as programmable nano-machines. Protein cages are known to be one of the most sophisticated and robust protein self-assembled system, and ferritin is a typical example of protein cage. Ferritin is a ubiquitous multi-subunit iron storage protein formed by 24 polypeptide chains that self-assemble into a hollow, roughly spherical protein cage with external and internal diameters of approximately 12 nm and 8 nm, respectively. The interior cavity of ferritin provides a unique reaction vessel to carry out reactions separated from the exterior environment. In nature, the cavity is utilized for sequestration and bio-mineralization as a mechanism to render iron inert and safe by shielding from the external environment. Materials scientists have been inspired by this system and exploited a range of ferritin superfamily proteins as supramolecular templates to encapsulate cargoes ranging from cancer drugs to therapeutic proteins, in addition to using them as well-defined building blocks for fabrication. Besides the interior cavity, the exterior surface and sub-unit

interfaces of ferritin can be modified without affecting ferritin assembly.

Recently a new class of ferritin was discovered in archaea, which shows salt-mediated assembly properties, assembling at high salt and disassembling at low salt.[1] Archeal ferritins are unique in many ways, for example the ferritin isolated from the hyper-thermophilic archaeon *Archaeoglobus fulgidus* (AfFtn) has a novel quaternary structure; its 24 subunits assemble into a shell having tetrahedral symmetry compared to common octahedral symmetry found in all other ferritins, despite having the same type of subunits. The difference in assembly opens four large (~45 Å) pores in the *A. fulgidus* ferritin shell. We recently explored a new archeal ferritin which also shows the interesting property of reversible assembly in solution, forming the canonical cage structure at high ionic strength and get disassembled in low ionic strength solution. This switchable behavior is of note given the proven utility of ferritin as a bionano-building tool. Recently we have successfully shown that our derived ferritin can act as an excellent protein container for capturing protein cargo, and because of its reversibility, encapsulation is switchable, with cargo being released at low salt concentration. Furthermore, we found that the TmFtn system has advantages over the other known salt-ferritin as it forms a complete shell around the cargo and is considerably more heat stable, as demonstrated in experiments where encapsulated enzyme retains close to full activity even at 80°C. In addition, we were also successful in making an enzyme active ferritin superlattice, and we used nano-gold as an anchor molecule for this purpose. In my presentation I will talk about our recent finding related to archaea ferritin and their application as therapeutics.

Reference

[1] Aumiller Jr WM, Uchida M, Douglas T. Protein cage assembly across multiple length scales. Chem. Soc. Rev. 2018; 47: 3433-3469. doi: 10.1039/c7cs00818j.

P15

Inhibitions of carbon monoxide dehydrogenase: spectroscopic investigations.

Alexandre Ciaccafava[1], Daria Tombolelli[1], Lilith Domnik[2], Jochen Fesseler[2], Holger Dobbek[2], Maria-Andrea Mroginski[1], Ingo Zebger[1], and Peter Hildebrandt[1]

[1]Institut für Chemie, Technische Universität Berlin, Sekretariat PC 14, D−10623 Berlin, Germany; [2]Institut für Biologie, Strukturbiologie/Biochemie Humboldt-Universität zu Berlin Unter den Linden 6, 10099 Berlin, Germany

a.ciaccafava@cbmn.u-bordeaux.fr

The O_2-sensitive [Ni-Fe] carbon monoxide dehydrogenase (CODH), is a homodimeric enzyme responsible for the biological interconversion between CO and CO_2 according to the reaction: $CO + H_2O \leftrightarrow CO_2 + 2H^+ + 2e^-$. As a pivotal enzyme of the Wood-Ljungdahl metabolic pathway, it allows microorganisms such as *Carboxydothermus hydrogenoformans* (*Ch*) and *Moorella thermoacetica* to grow on H_2 and CO_2 as electron and carbon source, or to oxidise CO in order to use it as an electron source. Furthermore, the reverse process, *i.e.* the enzymatic reduction of CO_2, is of particularly interest since it may serve as a template for the bioinspired development of catalysts for CO_2 degradation. Despite a large number of biochemical, spectroscopic, and crystallographic investigations, the detailed mechanism of the catalytic process of CODH is not yet fully understood. To gain deeper insight is aggravated by the difficulty to trap substrate-bound intermediate or pure redox states. Thus, as an alternative approach to overcome this limitation, inhibitors such as cyanate (NCO^-) and cyanide (CN^-) mimicking the substrates CO_2 and CO respectively have been employed. In an initial crystallographic study, CN^-, a structural analogue of CO and inhibitor of CODH, was found to possess a bent conformation. Recently, however, an independent re-evaluation of the electron density map did not provide clear evidence for the orientation of the CN^- ligand. In addition, another crystal structure presenting a different binding mode of CN^- has been reported for CODHII$_{Ch}$. While CN^- has been widely studied as an analogue for CO, much less work has been reported on the binding of NCO^-, considered to be an analogue for CO_2. Recent high-resolution structure (d_{min}<1.1Å) suggested that binding of NCO^- to CODHII$_{Ch}$ crystals leads to the formation of Ni-bound a carbamoyl (H_2NCO^+). In analogy to the CO_2 reduction to CO, NCO^- reduction to CN^- by CODH was recently postulated, supported by the NCO^- reduction to CN^- observed in nitrogenases and the observation of a slow oxidative turnover of *n*-butylisocyanide into *n*-butylisocyanate in CODHII$_{Ch}$, as the corresponding reverse reaction.To investigate those inhibitions and elucidate their potential reactivity, we have set up an integral approach including IR spectroscopic, crystallographic, site directed mutagenesis and theoretical analysis. Spectroscopic measurements were carried out with the wild-type and engineered CODHII$_{Ch}$ variant to assess the parameters that control the C≡N and N≡C−O stretching frequencies. This

analysis served as a reference for the quantum-mechanical / molecular mechanics (QM/MM) calculations which were performed on the basis of new high-resolution crystal structures to elucidate details of the active site structure of the CODHII$_{Ch}$-CN and CODHII$_{Ch}$-NCO complexes [1, 2].

References

[1] Ciaccafava A, Tombolelli D, Domnik L, Jeoung J-H, Dobbek H, Mroginski M-A, et al. Carbon Monoxide Dehydrogenase Reduces Cyanate to Cyanide. Angewandte Chemie International Edition. 2017;56:7398-401.10.1002/anie.201703225

[2] Ciaccafava A, Tombolelli D, Domnik L, Fesseler J, Jeoung J-H, Dobbek H, et al. When the inhibitor tells more than the substrate: the cyanide-bound state of a carbon monoxide dehydrogenase. Chem Sci. 2016;7:3162-71.10.1039/c5sc04554a

---

P16

In vitro characterization of the Sulfide Oxidation Unit (SOU) complex using fluorescence methods

Anne-Lise CLAUDEL, Jean-Christophe LEC, Séverine BOUTSERIN, François TALFOURNIER & Sandrine BOSCHI-MULLER

Laboratoire IMoPA Equipe Enzymologie Moléculaire et Structurale, UMR 7365 CNRS-Université de Lorraine, Faculté de médecine, Bâtiment Biopôle, 9 Avenue de la Forêt de Haye, BP 20199 54505 Vandœuvre-lès-Nancy, FRANCE

The Sulfide Oxidation Unit (SOU) complex is a human mitochondrial complex involved in the catabolism of the Janus faced hydrogen sulfide. The SOU comprises the sulfide quinone oxidoreductase (SQOR), a sulfide dioxygenase named ETHE1 and a rhodanese-type sulfurtransferase. SQOR is an inner membrane-anchored flavin disulfide reductase, which catalyzes the initial two-electron oxidation of H_2S into a persulfide entity using sulfite or glutathione as S^0 acceptor and coenzyme Q as final electron acceptor. ETHE1 catalyzes the oxygen-dependent oxidation of glutathione persulfide to sulfite and glutathione, and rhodanese- type sulfurtransferases catalyze the sulfur transfer from a donor substrate to an acceptor one. Although it is accepted that the SOU comprises SOQR and ETHE1, the identity of the rhodanese- type sulfurtransferase and the sulfide oxidation pathway remain to be clarified.

To address these questions, we aim to reconstitute *in vitro* the SOU complex from purified recombinant proteins produced in *Escherichia coli*. Different strategies were thus developed to obtain sufficient amounts of all purified potential partners: SQOR, ETHE1 and the three human mitochondrial sulfurtransferases (i.e. Rhodanese, TSTD1 and 3-MST). A combination of

biophysical methods (fluorescence quenching, fluorescence anisotropy, FRET) was used to study the formation of various binary complexes. Preliminary results will be presented and discussed in relation with the proposed physiological function of the different sulfurtransferases.

P17

Nanoparticle Tracking Analysis (NTA) to sharpen the definition of virus particle stability

Didier CLENET[1], Hadrien KRONENBERGER[1], Aure SAULNIER[2]

[1]Sanofi Pasteur, Bioprocess R&D, Marcy l'Etoile, France. [2] Sanofi Pasteur, Analytical Science Department, Marcy l'Etoile, France.

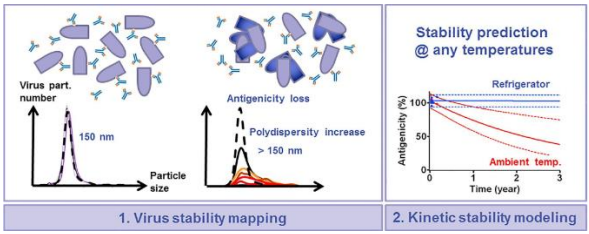
didier.clenet@sanofi.com

Nanoparticle Tracking Analysis (NTA) is a light scattering-based technic used to count nanoparticles while simultaneously determining their size distribution. Using a monoclonal antibody (mAb) which targets the surface G protein of rabies virus (RABV), we demonstrated that antigenic viral particles could be simultaneously counted and sized by NTA [1].

In the aim to integrate this technique in a lab-automation platform, an autosampler (NanoSight Sample Assistant, Malvern Panalytical) that was implemented ensuring automated loading of 96 samples into a NS300 instrument and automated analysis (flow, focus, washes steps…). Furthermore, an automation workflow was developed, including connexion of NTA data to a database through a dataloader (Vault Data Loader, Unchained Labs) and visualization of results in a dedicated software (TIBCO Spotfire®).

RABV degradation was assessed by NTA during thermal stress performed at temperature exposure between 5°C and 45°C, indicating a loss in the number of virus particles at the expected mean size and an increased particle size distribution. A progressive concomitant loss of antigenicity was confirmed by a G-specific enzyme-linked immunosorbent assay (ELISA). These results demonstrated that, in our conditions, RABV particle polydispersity was inversely correlated with antigenicity [1].

Finally, a kinetic-based modeling approach [2], used to explore forced degradation antigenicity data (NTA, ELISA), was able to identify a two-step model accurately describing antigenicity loss. This model predicted a RABV shelf-life of more than 3 years at 5 °C while more than 60% of antigenicity was lost after 2 years at ambient temperature in Guinea [1]. This thorough characterization of RABV forced degradation study originally provided a time-temperature mapping of RABV stability newly obtained with throughput.



From [1]

References

[1] Didier Clénet, Tatiana Vinit, Damien Soulet, Claire Maillet, Françoise Guinet-Morlot, Aure Saulnier, Biophysical virus particle specific characterization to sharpen the definition of virus stability, Eur. J. Pharm. Biopharm. 2018: 132: 62-69, doi: 10.1016/j.ejpb.2018.08.006

[2] Didier Clénet, Accurate prediction of vaccine stability under real storage conditions and during temperature excursions, Eur. J. Pharm. Biopharm. 2018: 125:76–84, doi: 10.1016/j.ejpb.2018.01.005

P18

Biotic Materials for Metal Nanoparticles Synthesis

Msc. Coelho, Nuno, Prof. Tavares, Pedro, Prof. Pereira, Alice

UCIBIO/Requimte, Departamento de Química, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

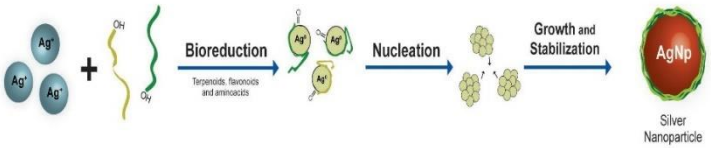
nm.coelho@campus.fct.unl.pt

The Moringa tree is a plant typically grown in semiarid tropical or subtropical regions that recently seized much of the public attention because of the high nutritional value and attributed medicinal and water purifying properties. With such fast growth and widespread interest is not difficult to access the importance of M. oleifera products, from which the protein extracts hold great potential.

Protein extracts can be obtained from different plant parts such as roots, leaves, branches/stems/stalks, flowers, pods and seeds. These extracts already proved to be a hazard-free, safe and economic method to nanoparticle synthesis, mainly due to its enhanced metal ion hyperaccumulating and reductive capacity, fundamental aspects in the nucleation of nanoparticles. All Moringa extracts were obtained through our project partner, Naturinga. Naturinga is a Portuguese leading company in the production, processing and marketing of Moringa oleifera-based products, with a plantation of more than 120,000 trees in

Mozambique.

The “green synthesis” of metallic nanoparticles through M. oleifera extracts is conditioned by several external factors, such as temperature, concentration, pH, reaction time or the velocity of extract addition. These nanoparticles are stabilized due to their inherent biological extract coating, and so, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) were performed in order to access the coating’s morphological properties. This information allows us to fully understand the nanoparticle properties and possibly modified them. The crystallinity of the nanoparticles and their cytotoxicity were also studied.



References

[1] Brilhante, Raimunda; Sales, Jamille; Pereira, Vandbergue et all. Research advances on the multiple uses of Moringa oleifera: A sustainable alternative for socially neglected population. Asian Pacific journal of tropical medicine, ISSN: 2352-4146, Vol: 10, Issue: 7, Page: 621-630

[2] Palaniselvam Kuppusamy, Mashitah M. Yusoff, Gaanty Pragas Maniam, Natanamurugaraj Govindan. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. Saudi Pharmaceutical Journal, Volume 24, Issue 4,2016, Pages 473-484.

[3] Valdez Salas, Benjamin & Arrieta Perez, Cristina & Carrillo, Monica & Curiel, Mario & Mateos, David & Maria Bastidas, Jose & Rosas, Navor. (2017). Silver nanoparticles biosynthesized by secondary metabolites from Moringa oleifera stem and their antimicrobial properties. AFRICAN JOURNAL OF BIOTECHNOLOGY. 16. 400. 10.5897/AJB2016.15840.

P19

Selective levo-thyroxine detection by electrochemical-surface plasmon resonance

Melinda David, Adrian Șerban, Monica Florescu

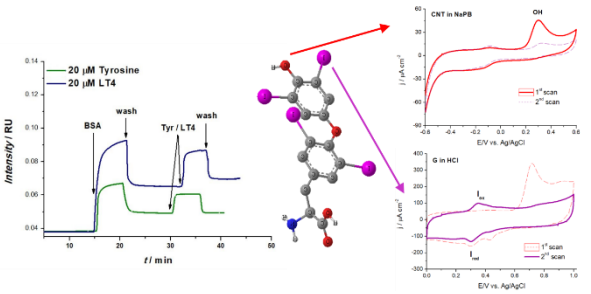
Faculty of Medicine, Transilvania University of Brașov, Brașov, Romania

melinda.dav@gmail.com

Several medical conditions, such as hypothyroidism, require hormone replacement therapy. In this particular case, administration of levo-thyroxine (LT4) plays an important role. Therefore, monitoring of LT4 levels in the human body lacks a simple, selective detection tool. In this work, a simple, rapid and reliable detection tool for LT4 was optimized using two different methods.

Surface plasmon resonance (SPR) is based on refractive index changes, which are sensitive to the adsorption of molecules at a gold coated interface enabling the study of specificity, affinity and kinetics of biomolecular interactions between albumin, LT4 and possible interferents from human blood.

Electrochemistry has also been used as optimizing step. The interfacial changes, which occur due to electrochemical properties of LT4, at different surfaces were studied by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The presence of adsorbed albumin, enables the study of kinetics of LT4 – albumin biomolecular interaction through variation of electrical parameters. The analytical detection was carried out by differential pulse voltammetry (DPV) where a high sensitivity (S = 0.99 μA cm^{-2} $μM^{-1}$) and detection limit of 0.61 μM for LT4 was achieved.



From left to right:

Affinity study using SPR for LT4, LT4 molecule, CV on different surfaces of LT4

Acknowledgements: *This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, Project number PN-III-P1-1.2-PCCDI-2017-0062, contract no. 58, within PNCDI III. We hereby acknowledge the structural funds project PRO-DD (POS-CCE, O.2.2.1., ID 123, SMIS 2637, No 11/2009) for providing the infrastructure used in this work.*

P20

Development of an Infrared Spectroscopy Data Repository

Erik Goormaghtigh[1], Mark Williams[2], Sarah Griffiths[2], Amandine Maréchal[2,3], Peter Rich[3] and Tina Daviter[4]

[1]Faculté des Sciences, Université Libre de Bruxelles, Brussels, Belgium, [2]Department of *Biological Sciences, Birkbeck, University of London, London, UK, [3] Department of Structural and Molecular Biology, University College London, London, UK, [4]ISMB Biophysics Centre, Department of Biological Sciences, Birkbeck, University of London, London, UK*

t.daviter@bbk.ac.uk

We are reporting progress on developing a repository for biological infrared (IR) spectroscopy data. The databank will provide access to the spectral libraries of proteins, nucleic acids, amino acids, peptides, lipids and buffers collected over decades in our laboratories; enabling researchers to much more readily interpret their spectra. We want to make all data accessible under the FAIR sharing principles of Open Science (findable, accessible, interoperable and reusable). This will directly facilitate applications of IR in biology and medicine, including:

- difference spectroscopy analysis of chemical and structural changes such as occur as a result of biomolecular interactions and catalysis,
- protein secondary structure analysis,
- medical diagnostics via detection of changes to spectral features in disease states.

We suggest data standards and metadata to inform best practice in interpretation and introduce transparency in quality control of IR spectra. We have developed the rudimentary web framework to enter and hold the data as well as access it. We are currently evolving the data model, enabling upload of JCAMP files and making the interface more user friendly. We invite input at any stage of these developments and plan to engage ARBRE-MOBIEU members in a first round of testing.

P21

Centromere of the nematode Meloidogyne; characterization of centromere specific H3 protein (CENH3) and underlying DNA sequences

Evelin Despot Slade, Martina Pavlek, Brankica Mravinac, Phillipe Castagnone-Sereno, Miroslav Plohl, Nevenka Meštrović

Ruđer Bošković Institute, Zagreb, Croatia; 2 Institut Sophia Agrobiotech, Sophia Antipolis, France.

evelin.despot.slade@irb.hr

Centromeres are chromosomal domains essential for proper segregation of genetic material in cell divisions. They are defined epigenetically by the presence of centromere-specific histone H3 protein (CENH3). CENH3 histone is a species-specific protein, particularly variable in its N-terminal domain which is associated with centromeric DNA. In contrast to conserved function of the centromere, CENH3 and centromeric DNA evolve rapidly in diverse organisms. Why the centromere is composed of unstable units is still unsolved question. Nematodes Meloidogyne have some exceptional characteristics, such as presumptive holocentric organization of the centromere and specific mode of reproduction by mitotic parthenogenesis, which make them an ideal system to study centromere structure and function. Interestingly, our genome analysis of centromere protein CENH3 in *Meloidogyne incognita* reveal three divergent genes for CENH3 (α,β,γ), which are completely conserved in its related species. Transcriptome data show developmental stage-specific expression of CENH3 variants. Immunostaining of chromosomes with antibodies specific for αCENH3 reveals so far unseen distribution of CENH3 in forms of monocentric, dicentric, polycentric and holocentric centromeres. ChIP experiments suggested that different repetitive sequences with conserved 21-nt motif may be associated with αCENH3 domains.

P22

Differential Scanning Fluorimetry: revisiting microtubules assembly

La Rocca R, Tsvetkov P.O, Devred F.

Aix-Marseille University, CNRS, INP, PINT platform, Marseille, France

francois.devred@univ-amu.fr

Constant progress in biophysics methods is accompanied by an acceleration in data acquisition leading to finer understanding of many biological processes. Recently, a new generation of Differential Scanning Fluorimetry (*nanoDSF*), represented by the Prometheus series, has become available. We have adapted the use of this instrument, originally designed for protein thermal stability characterization, to study the microtubule cytoskeleton. Microtubules are highly dynamic structures participating in such vital processes as cell division and axonal transport. Their dynamics is tightly regulated by Microtubule Associated Proteins (MAPs) and altered by the anti-cancer drugs called microtubule targeting agents (MTAs). Association of several classical biophysical methods has been used to study the interactions between microtubules, MAPs and MTAs. Among them, turbidimetry is commonly used to characterize the pro- or anti-polymerization effect of tubulin interacting partners.

In this work, we use the Prometheus *nanoDSF* instrument, that combines advantages of turbidimetry and fluorescence measurement to monitor both microtubule formation and its interaction with cofactors. While in classical turbidimetry large quantities (250-500 µL) of tubulin solution are necessary to test one condition, the Prometheus enables us to use the same amount of tubulin to test up to 48 different conditions simultaneously. Moreover, it is now possible to study tubulin system in the temperature range from 15 to 115C in the same experiment. In addition, since the intrinsic fluorescence is followed at the same time, this method also provides key information to understand the nature of tubulin polymerization.

In conclusion, *nanoDSF* instrument enabled us to revisit and complement a large amount of data on tubulin polymerization (buffer conditions, impact of MTAs, MAPs, and ions...). We believe that this approach can be extended to study many other complex biological processes with the same success.

P23

Control of reactive oxygen species in mitochondria by cytochrome *c* phosphorylation and respirasome factors

A. Guerra-Castellano, A. Díaz-Quintana, B. Moreno-Beltrán, G. Pérez-Mejías, C.A. Elena-Real, K. González-Arzola, S.M. García-Mauriño, M.A. De la Rosa, I. Díaz-Moreno

Instituto de Investigaciones Químicas, Universidad de Sevilla–CSIC, 41092 Sevilla, Spain

qzaida@us.es

The response of cells to changes in physiological conditions involves a tight modulation of mitochondrial activity to damp reactive oxygen species (ROS) generation. Failure of such a control is associated to hypoxia-reliant disorders like ischemia and cancer. A key factor is keeping electron flow across complex III to avoid the accumulation of semi-Quinone species, which demands an efficient shuttling of electrons to complex IV — a.k.a. Cytochrome *c* oxidase.

Here, we show how the interplay between phosphorylation of cytochrome *c* (C*c*) at tyrosine residues and the expression of a super-complex adaptor such as the hypoxia-inducible domain family member 1A (HIGD1A) modulate ROS generation and the efficiency of the flux of electrons between complexes III and IV. For this purpose, we mimicked such a labile phosphorylation by performing stable substitutions of tyrosine residues by *p*-carboxy-methyl-L-phenylalanine (*p*CMF). The residues targeted in this work are indeed modified during hypoxia response (Tyr 48) and insulin-elicited survival of neurons upon stroke (Tyr 97).

Notably, the mutations hardly affect the structure of C*c*, and affect the dynamics of the protein in different degrees. Moreover, they exert diverse effects on its performance as electron carrier, its affinity towards physiological targets, ROS scavenger activity and role as signalling factor.

Additionally, we detected the interaction between C*c* and HIGD1A, the species mimicking phosphorylation at Tyr97 displaying drop in their affinity towards the assembly factor. In fact, we show the latter facilitates electron flow between complexes III and IV, depending on the presence of modifications in C*c*.

Altogether, these findings yield a better understanding of the molecular basis for mitochondrial metabolism in acute diseases, such as brain ischemia, and thus could allow the use of phosphomimic C*c* as a neuroprotector with therapeutic applications.

References

[1] Guerra-Castellano, A. et al. Chem. Eur. J. 21, 15004-15012 (2015).

[2] Moreno-Beltran, B. et al. Proc Natl Acad Sci U S A 114, E3041-E3050 (2017)

[3] Guerra-Castellano, A. et al. Proc Natl Acad Sci U S A 115, 7955-7960 (2018).

***Acknowledgements**: Work supported by Ministry of Economy and Competitiveness and European Regional Development Fund (MINECO/FEDER) Grant BFU2015-71017/BMC, E. U.; the Ramón Areces Foundation, European Social Fund, Andalusian Government (BIO-198); Waters-TA Instruments, The INSTRUCT platform of Baculovirus expression(expression screening@OPPF) was used for cloning the HIGD1A into the pOPINM vector.*

P24

When spectroscopy meet small-angle scattering
Enhanced information and diagnostics in life sciences and soft matter

Cedric Dicko[1], Tomas Plivelic[2], Ann Terry[2], Javier Perez[3], Adrian Rennie[4], Adam Engberg[4], Andrew Jackson[5], Judith Houston[5], Anders Petersson[5], Robert Dalgliesh[6], Freddie Akeroyd[6], Sarah Rogers[6], Anne Martel[7], Lionel Porcar[7]

[1]Lund University, Lund, Sweden; [2]MAXIV Laboratory, Lund, Sweden; [3]SOLEIL synchrotron, Paris, France; [4] Uppsala University, Uppsala, Sweden; [5]European Spallation Source ESS, Lund Sweden; [6]ISIS – STFC Rutherford Appleton lab, Didcot, U.K.; [7]Institut Laue Langevin ILL, Grenoble, France

cedric.dicko@tbiokem.lth.se

Modern applications and basic research in medicine, biotechnology and materials are often concerned with hybrid (e.g. organic-inorganic) and synergetic systems. In other words, systems that bring enhanced properties and performances. The challenge is now in the understanding of the complex interactions leading to their assembly and operation. Due to their inherent chemical and structural complexities a combination of several techniques is necessary to determine unambiguously the molecular mechanisms of assembly and operation. To address this need, we have designed, built and validated a (quasi) simultaneous measurement platform called SURF that consists of SAXS, UV-Vis, Raman and fluorescence techniques [1, 2].
The SURF platform provides simultaneous measurements on the same sample volume and a multivariate framework to correlate the spectroscopic and X-ray scattering information. The multivariate and correlation analysis enables complex behaviour (structure and chemistry) to be resolved. Additional benefits of SURF are sample quality control and "on line" diagnostics. Recently, an equivalent setup has been developed for small-angle neutron scattering, called NURF, with the addition of in-line density measurements.
In this contribution, we illustrate the concept and benefits of the SURF and NURF on selected examples and provide and update on the current design and status.

References

[1] Haas, S., T. Plivelic and C. Dicko (2014). "Combined SAXS/UV-VIS/Raman as Diagnostic and Structure Resolving Tool in Materials and Life Sciences Applications."

The Journal of Physical Chemistry B 118(8): 2264-2273
[2] Del Giudice, A., C. Dicko, L. Galantini and N. V. Pavel (2016). "Structural response of human serum albumin to oxidation: biological buffer to local formation of hypochlorite." Journal of Physical Chemistry B 120(48): 12261-12271.

P25

ITC: theory, practice and pitfalls

Philippe Dumas

IGBMC-Integrated Structural Biology, F67404 Illkirch, France

dumasp@igbmc.fr

New mathematical methods have been developed for processing titration curves (TC) obtained from Isothermal Titration Calorimetry (ITC). Exact TC equations for the usual multi-injection method (MIM), or for the single-injection method (SIM) with continuous injection, were derived by taking into account rigorously the effect of dilution resulting from the titration process [1]. This analysis identified a systematic error pervading three usual programs out of four, NanoAnalyze from TA being much less in error. An exact TC equation was obtained, which is most easily programmed in common tools like Microsoft Excel and allows fitting an experimental TC by using its Solver functionality (an Excel file is available on https://doi.org/10.1101/512780). This exact analysis was shown to be also applicable for processing data obtained from the single-injection method (SIM). The improvement on existing methods for SIM was assessed with data from [2]; this should incite us to use more widely this method.

It was also examined how seemingly complex mechanisms are in fact thermodynamically equivalent to the simple association/dissociation mechanism and cannot explain atypical titration curves. This will be illustrated with one biologically relevant example encountered in the field of RNA-mediated regulation [3].

Another aspect will be considered about common pitfalls resulting from sloppy requirements on the accuracy of fitting of the experimental TC. As a matter of fact, it is too frequent that the simple association/dissociation mechanism is invoked even though it does not explain well the experimental TC. A striking example of such a problem will be presented, as well as how mass spectrometry gave a decisive clue to the resulting error [4]. Likely, this kind of serious error is not rare in published data.

References

[1] Dumas P. Rigorous equations for isothermal titration calorimetry: theoretical and practical consequences. bioRxiv 512780; doi: https://doi.org/10.1101/512780

[2] Markova N., Hallen D. The development of a continuous isothermal titration calorimetric method for equilibrium studies. Analytical Biochemistry 2004;331, 77-88. doi: 10.1016/j.ab.2004.03.022
[3] Guedich S., Puffer-Enders B., Baltzinger M., Hoffmann G., Da Veiga C., Jossinet F., Thore S., Bec G., Ennifar E., Burnouf D., Dumas P. Quantitative and predictive model of kinetic regulation by E. coli TPP riboswitches. RNA biology 2016; 13, 373-390. doi: 10.1080/15476286.2016.1142040
[4] Wolff P., Da Veiga C., Ennifar E., Bec G, Guichard G., Burnouf D., Dumas P. Native ESI Mass Spectrometry Can Help to Avoid Wrong Interpretations from Isothermal Titration Calorimetry in Difficult Situations. J. Am. Soc. Mass Spectrom. 2016; 28, 347-357. doi: 10.1007/s13361-016-1534-6

P26

Development of a SANS strategy for the study of membrane proteins: application to a prokaryotic NADPH oxidase homolog.

Annelise Vermot[1], Isabelle Petit-Hartlein[1], Cecile Breyton[1], Aline Le Roy[1], Corinne Deniaud[1], Michel Thépaut[1], Michaël Hartlein[2], Susan Smith[3], Christine Ebel[1], Anne Martel[2], Franck Fieschi[1].

[1]IBS, Grenoble, France; [2]ILL, Grenoble, France; [3]Kennesaw State University, Kennesaw, USA.

Christine.ebel@ibs.fr

Because of their hydrophobic surfaces, flexibility and lack of stability, deciphering the structures of membrane proteins remains a real challenge. Small angle neutron scattering (SANS) provides a method to characterize transmembrane systems without necessity for a crystallization step; although of low resolution, SANS furnishes valuable data regarding organization of macromolecular membrane complexes, conformational state modifications from one state to another, etc. Membrane protein solubilization implies the use of amphipathic detergents that form protective belts around hydrophobic patches to maintain the protein's structural integrity outside of the lipid environment. Consequently, the resulting SANS signal includes both protein and detergent belt signatures leading to a global outer shell larger than the envelope expected for the protein alone; masking the detergent signal represents an important issue. Moreover, specific detergent characteristics can disrupt the proper folding of the membrane protein, greatly impacting the structural parameters of the SANS envelope and thus necessitating significant effort in the preparation of an optimized sample for SANS. Thus, a strategy to overcome these specific issues was tested using the LMNG detergent, recently characterized as a strongly stabilizing detergent. As a model protein we used SpNox, a prokaryotic homolog of NADPH oxidase originally identified in phagocytic cells where it produces

ROS. Detergent screening confirmed the improved thermostability of SpNox and limited aggregation when solubilized in LMNG. Through contrast variation experiments, we were able cancel the LMNG contribution to the SANS signal, and we determined the percentage of D_2O corresponding to the buffer matching point. Then, to improve protein contrast, we produced deuterated SpNOX and subsequently solubilized and purified it in LMNG. Finally, since SANS studies are very sensitive to the presence of aggregates or oligomers in the sample, the development of a new in-situ size exclusion chromatography (SEC) system on a SANS instrument enabled data collection on line from an aggregation-free homogeneous sample. This strategy led to the determination of a low resolution envelope of SpNox confirming the monomeric state of the protein in solution. Parallel to the structural study, a homology model of SpNox was developed; the model was successfully docked in the SANS envelope. The general strategy illustrated with the SpNox protein may be applied to many other membrane proteins in the future.

P27

Macromolecular interactions *in vitro*, comparing classical and new approaches

Paloma Fernández Varela, Christophe Velours, Magali Aumont-Niçaise, Michel Desmadril, Philippe Minard, Jean-Baptiste Charbonnier

Institute for Integrative Biology of the Cell, CNRS, Gif sur Yvette, France.

paloma.fernandez-varela@i2bc.paris-saclay.fr

To understand the molecular basis of macromolecules interactions we need to be aware of the advantages and limitations of the available methods. Here we will analyze the principles, strengths and limitations of different methods, classical as analytical ultracentrifugation with or without fluorescence (AUC-FDS), isothermal titration calorimetry (ITC) or size exclusion chromatography coupled to Multi-Angle Light Diffusion (SEC-MALS); and new approaches as bio-layer interferometry (BLI), Microscale Thermophoresis (MST) or switchSENSE (DRX2), for measuring *in vitro* interactions. We will use to different models to study protein-protein interaction (artificial alphaRep proteins [1]) and protein-DNA interactions (DNA repair complexes [2]) that are at the heart of our research projects.

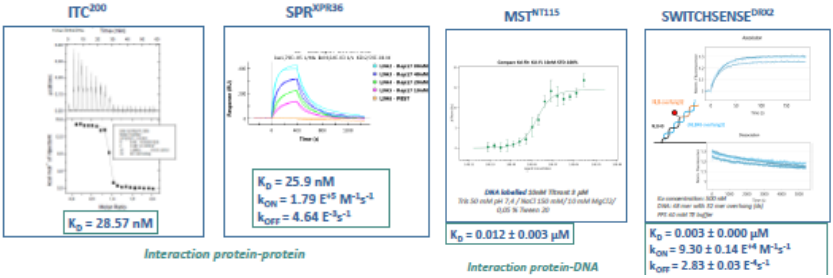


Figure – Samples of protein-protein (alphaRep) to the left and protein-DNA interaction (DNA repair complex) to the right. Classical (ITC, SPR) and new approaches (MST, switchSENSE) have been used, respectively

References

[1] Guellouz A1, Valerio-Lepiniec M, Urvoas A, Chevrel A, Graille M, Fourati-Kammoun Z, Desmadril M, van Tilbeurgh H, Minard P. Selection of specific protein binders for pre-defined targets from an optimized library of artificialhelicoidal repeat proteins (alphaRep).PLoS One. 2013 Aug 27;8(8):e71512. doi: 10.1371/journal.pone.0071512. eCollection 2013.

[2] Nemoz C1, Ropars V1, Frit P2,3, Gontier A1, Drevet P1, Yu J1, Guerois R1, Pitois A1, Comte A1, Delteil C2,3, Barboule N2,3, Legrand P4, Baconnais S5, Yin Y6,Tadi S7, Barbet-Massin E8, Berger I9, Le Cam E5, Modesti M7, Rothenberg E6, Calsou P10,11, Charbonnier JB12. XLF and APLF bind Ku80 at two remote sites to ensure DNA repair by non-homologous end joining. Nat Struct Mol Biol. 2018 Oct;25(10):971-980. doi: 10.1038/s41594-018-0133-6. Epub 2018 Oct 5.

P28

Targeting *de novo* thymidylate synthesis nuclear complex

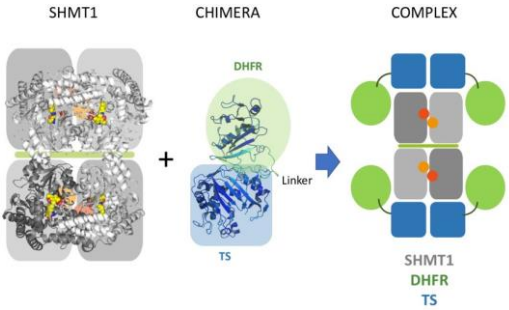
Giorgio Giardina[1], Sharon Spizzichino[1], Giovanna Boumis[1], Serena Rinaldo[1], Alessio Paone[1], Alessandro Paiardini[1], Angela Tramonti[2], Roberto Contestabile[1], Francesca Cutruzzolà[1]

[1]Dep. of Biochemical Sciences "A.R. Fanelli" – Sapienza University of Rome, Italy. [2] Istituto di Biologia e Patologia Molecolari, Italian National Research Council, Rome, Italy

giorgio.giardina@uniroma1.it

Many cancer cells reprogramme one-carbon metabolism (OCM) in order to sustain proliferation. Depending on cell demands, serine hydroxymethyltransferase (SHMT) dynamically changes the fluxes of OCM by

reversibly converting serine and tetrahydrofolate (THF) into 5,10-methylene-THF and glycine. SHMT is a tetrameric enzyme that exists in two isoforms; a cytosolic (SHMT1) and a mitochondrial one (SHMT2). The SHMT1 also translocates to the nucleus where it forms a ternary complex with dihydrofolate reductase (DHFR) and thymidylate synthase (TS) to sustain *de novo* thymidylate synthesis and support cell proliferation [1]. We have recently demonstrated that this nuclear function is crucial for cell survival in lung cancer cell lines (A549; H1299) [2], and that *de novo* thymidylate synthesis requires SHMT1 to be active, regardless of its oligomeric state [3]. We have therefore started the structural and functional characterization of the nuclear tertiary complex SHMT1:DHFR:TS, which represents a promising target for protein-protein interaction (PPI) inhibitors. In order to facilitate complex formation, we downgraded the assembly from a ternary to a binary complex by linking together DHFR and TS in a single chimeric construct. The DHFR-TS fusion protein (CHIMERA) has been successfully expressed and purified. Both the fused enzymes are correctly folded and catalytically active. The progress in the biochemical characterization and structural determination of SHMT1:CHIMERA complex will be discussed.



Crystal structure of SHMT1 (PDBid 1bj4) and homology model of CHIMERAà scheme of a possible complex stoichiometry

References

[1] Anderson DD, Woeller CF, Chiang E-P, Shane B & Stover PJ. Serine hydroxymethyltransferase anchors de novo thymidylate synthesis pathway to nuclear lamina for DNA synthesis. 2012 J. Biol. Chem. 287, 7051–62. doi:10.1074/jbc.M111.333120

[2] Paone A, Marani M, Fiascarelli A, Rinaldo S, Giardina G, Paiardini A & Cutruzzolà F. SHMT1 knockdown induces apoptosis in lung cancer cells by causing uracil misincorporation. 2014 Cell Death Dis. 5- e1525 doi:10.1038/cddis.2014.482

[3] Giardina G, Paone A, Tramonti A, Lucchi R, Marani M, Magnifico MC, Bouzidi A, Pontecorvi V, Guiducci G, Zamparelli C, Rinaldo S, Paiardini A, Contestabile R & Cutruzzolà F. The catalytic activity of serine hydroxymethyltransferase is essential for de novo nuclear dTMP synthesis in lung cancer cells. 2018 FEBS J. 285, 3238–3253. doi:10.1111/febs.14610

P29

CENH3 proteins as epigenetic determinants of *Tribolium* centromeres

Tena Gržan, Nevenka Meštrović, Miroslav Plohl, Brankica Mravinac

Ruđer Bošković Institute, Zagreb, Croatia

tena.grzan@irb.hr

The flour beetles *Tribolium castaneum* and *Tribolium freemani*, the important world-wide pests of stored agricultural products, are able to mate, but produce sterile hybrid progeny in reciprocal crosses. In the context of genetic diversity between closely related species, we focused our research on DNA and protein aspects of their centromere regions as crucial chromosomal structures. By using the histone H3 sequence as query to search genome databases, in both species we first identified the centromeric histone H3 variants (CENH3), epigenetic hallmarks of active centromeres. *T. castaneum* cCENH3 and *T. freemani* fCENH3 H3-like variants share 80% identity in amino acid sequences. Their species-specific N-terminal tails were used to raise antibodies that localized cCENH3 and fCENH3 proteins exclusively to centromeres of *T. castaneum* and *T. freemani*, respectively. We further performed ChIP-Seq experiments, which predominantly associated cCENH3 and fCENH3 nucleosomes with a major satellite DNA in each species. As supported by IF-FISH analyses, highly abundant satellite DNAs, TCAST in *T. castaneum* and TFREE in *T. freemani*, spread both at pericentromeric and functional centromeric regions of all chromosomes. TCAST and TFREE satellite DNAs account for large fractions (>20%) of their indigenous genomes, and they differ dramatically in monomer unit length (360 bp *versus* 166 bp) as well as in nucleotide sequence, thus being fundamentally species-specific. In terms of the reproductive isolation between *T. castaneum* and *T. freemani*, these findings argue in favor of adaptive evolution of cCENH3 and fCENH3 proteins that might be driven by a response to changes in satellite DNA sequences at centromere loci.

***Acknowledments**: This work was supported by Croatian Science Foundation grant IP-2014-09-3183.*

P30

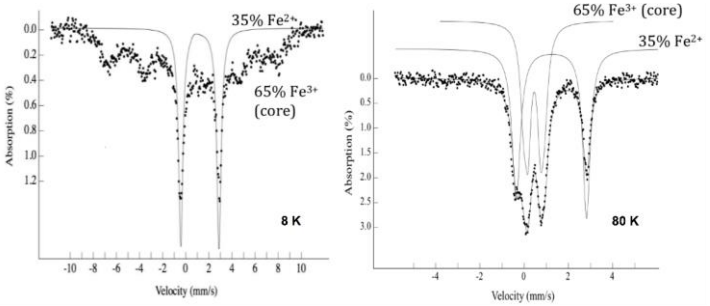
Influence of the cupric ion on the ferroxidation mechanism of Dps, a miniferritin from Marinobacter hydrocarbonoclasticus

Guerra, JP; Pereira, AS; Tavares, P

Applied Molecular Biosciences Unit – UCIBIO, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Caparica, Portugal

jp.guerra@campus.fct.unl.pt

Iron management in bacteria is both a challenge and a necessity: while free iron in both ferrous and ferric forms is insoluble at physiological pH and toxic through harmful Fenton reactions, this element is essential to bacterial metabolism. The DNA-binding-protein from starved cells (Dps) is a prokaryotic miniferritin that presents the unique ability of direct protection of DNA, besides general catalytic abilities of other members of the Ferritin family[1], such as free iron oxidation, storage and controlled release from their hollow-cage assembly, hindering oxidative stress and serving as an iron pool. The Dps[2,3] of *Marinobacter hydrocarbonoclasticus*, a facultative anaerobe of biotechnological interest, oxidizes ferrous ions through the reduction of a co-substrate, either O_2 or H_2O_2, in a relatively slower or faster manner, respectively. Recent results obtained using EPR, Mössbauer, Fluorescence and UV/Vis spectroscopies show that the binding of cupric ions to the Dps dodecamer influences its mechanism: acting as a catalyst, the binding of Cu^{2+} increases the rate of iron-core formation 10-fold when O_2 is used a co-substrate. Also, in the absence of other oxidants and in anaerobic conditions, cupric ions oxidize ferrous ions in a 1:1 stoichiometry, acting as a novel co-substrate. Further work is needed to understand the physiological role of the influence of copper in Dps' mechanism and bacterial iron homeostasis.



Mössbauer spectra and fit of Dps samples incubated with 12 Cu^{2+} and 12 Fe^{2+} per protein in anaerobic conditions for 20 minutes at room temperature. Left panel: Obtained at 8 K; Right panel: Obtained at 80 K.

References

[1] He D, Marles-Wright J. Ferritin family proteins and their use in bionanotechnology. N Biotechnol. 2015;32(6):651- 657. doi:10.1016/j.nbt.2014.12.006.

[2] Chiancone E, Ceci P. The multifaceted capacity of Dps proteins to combat bacterial stress conditions: Detoxification of iron and hydrogen peroxide and DNA binding. Biochim Biophys Acta - Gen Subj. 2010;1800(8):798-805. doi:10.1016/j.bbagen.2010.01.013.

[3] Penas D, Pereira AS, Tavares P. Direct Evidence for Ferrous Ion Oxidation and Incorporation in the Absence of Oxidants by Dps from Marinobacter hydrocarbonoclasticus. 2018:1-7. doi:10.1002/anie.201809584.

Acknowledgements: This work was supported by the Applied Molecular Biosciences Unit- UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019). This research was supported by Fundação para a Ciência e Tecnologia, Ministério da Educação e Ciência (FCT/MEC), grant PTDC/BIA-PRO/111485/2009 (to P.T.), PTDC/QUI/64248/2006 (to A.S.P). JPG is supported by the Radiation Biology and Biophysics Doctoral Training Programme (RaBBiT PD/00193/2012) and an FCT/MEC Ph.D. Fellowship (PD/BD/135476/2017).

P31

Intramolecular allosteric control of NColE7 metallonuclease based on the specific protease action of nickel(II) ions

Béla Gyurcsik, Bálint Hajdu, Enikő Herman[1], Ria K. Balogh, Heba A.H. Abd Elhameed

University of Szeged, Department of Inorganic and Analytical Chemistry, H-6720 Szeged, Dóm tér 7, Hungary

gyurcsik@chem.u-szeged.hu

Artificial nucleases (ANs) can induce DNA repair within cells by hydrolysing the phosphodiester group. This phenomenon can be applied for gene correction. Nevertheless, the medicinal use is still hindered by the cytotoxic effect, related to off-target cleavages. Such side-effects could be obviated by the regulation of the nuclease domain similarly to the enzymes in natural systems. We found that the nuclease domain of colicin E7 would be suitable for the development of such regulated enzymes, as it needs the concerted action of its C and N-termini to cleave the DNA, which property can be exploited for its allosteric control.

By applying various methods of protein purification, we also observed that flanking N or C- terminal sequences due to random mutations or affinity tags may interfere with the catalytic activity of NColE7 nuclease [1,2]. The frequently applied oligohistidine tag used in immobilized metal ion affinity

chromatography may inhibit the catalytic activity of NColE7 or its mutants by various mechanisms. Removal of this inhibitory segment prior to the DNA editing by specific hydrolytic action of Ni(II) ions can be exhausted for additional allosteric control of the NColE7-based artificial enzymes (Figure).

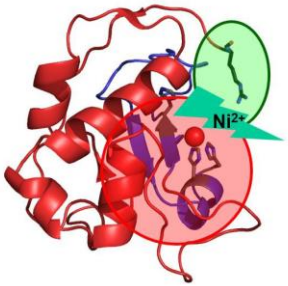


Figure: Cooperation of the C-terminal catalytic centre (in red circle) and the activator N-terminal region (in green circle) can be regulated by flanking terminal sequences, such as protein tags used for affinity chromatography of proteins. Specific cleavage of such segments by nickel(II) ions recovers the catalytic activity.

References

[1] Abd Elhameed HAH, Hajdu B, Balogh RK, Hermann H, Hunyadi-Gulyás É, Gyurcsik B, Purification of proteins with precise sequence using a Ni(II)-cleavable C-terminal hexahistidine affinity tag. Protein Expr. Purif. 2018, submitted.

[2] Németh E, Schilli GK, Nagy G, Hasenhindl C, Gyurcsik B, Oostenbrink C, Design of a colicin E7 based chimeric zinc-finger nuclease. J. Comp-Aid. Mol. Des., 2014, 28:841-850. DOI 10.1007/s10822-014-9765-8

[3] Belczyk-Ciesielska A, Csipak B, Hajdu B, Sparavier A, Asaka MN, Nagata K, Gyurcsik B, Bal B, Nickel(II)- promoted specific hydrolysis of zinc finger proteins. Metallomics, 2018, 10:1089-1098. DOI: 10.1039/c8mt00098k

Acknowledgement: This work was financially supported by the research grants NKFIH K_16/120130 and GINOP 2.3.2-15-2016-00038.

P32

Study of Biocatalytic Systems in Crowded Environments: The Case of Lactate Dehydrogenase

Marin Matić, Josef Hamacek, Francesco Piazza

Centre de Biophysique Moléculaire, CNRS UPR4301 45071, Orléans, France.

josef.hamacek@cnrs.fr

The cell is an extremely complex environment, notably crowded and confining. One third of the cell interior is occupied by biomolecules of all sizes, from a few Ang. to tens of nanometers in size, membrane-bound organelles and a dense meshwork of cytoskeletal structures that form the mechanical backbone of the cell [1]. This macromolecular crowding affects the conformational dynamics and thermodynamics of proteins, and consequently the related reaction equilibria. It might significantly decrease the activity of diffusion-limited enzyme kinetics by slowing diffusion and consequently influence the formation of substrate-enzyme complexes, for instance.

In this contribution, we investigate the effect of macromolecular crowding on the biocatalytic activity of lactate dehydrogenase (LDH). For this purpose, we use different crowders such as Ficoll PM70, PEG 6000 and PEG 35000 to reduce the volume available for enzymatic transformations. The enzyme lactate dehydrogenase carries the interconversion of pyruvate to lactate and NADH to NAD^+, which allows measuring fluorescence changes along the reaction. The crowder concentration and the interactions between different components of the system thus modulate the observed reaction rates, showing an identical decrease of the Michaelis-Menten parameters with crowder concentration, reminiscent of uncompetitive inhibition. The extracted kinetic parameters are discussed in relation with the crowder size and its solution properties.

References

[1] G. Foffi, A. Pastore, F. Piazza and P. A. Temussi, Macromolecular crowding: chemistry and physics meet biology (Ascona, Switzerland, 10-14 June 2012), Physical Biology - 10, 040301 (2013).

P32

Exploring the dynamic structure of bionanocages

João P. Jacinto[1], João P. Guerra[1], Ana V. Almeida[1], Nykola C. Jones[2], Søren V. Hoffmann[2], Alice S. Pereira[1], Pedro Tavares[1]

Laboratório de Biofísica Molecular, UCIBIO/Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal ;[2]ISA, Department of Physics and Astronomy, Aarhus University, 8000 Aarhus C, Denmark

jp.jacinto@campus.fct.unl.pt

Given the vast array of naturally synthesized proteins, the degree of folding, mechanism, location of assembly and size of each protein varies greatly. Amid this multitude of proteins lies the ferritin family of proteins, considered bionanocages that can be classified by size. The DNA-binding proteins from starved cells (DPS) are under 10 nm in diameter and part of this family. These bionanocages are characterized by their globular shape with a hollow interior cavity. DPSs are involved in several metabolic pathways such as detoxification, iron sequestration and oxidative stress and radiation damage prevention. A specially interesting ability of the cavity is the possibility to, without bioengineering, synthesize different types of nanoparticles, forming specific metal cores. Another interesting possibility is the use of the hollow cavity as a drug delivery system. Given the wide array of biotechnology uses that DPS bionanocages can achieve, it is important to have an understanding of the hollow cavity dynamic interactions during synthesis of different compounds inside it. In order to gain a deeper insight into the dynamics of the denaturation of DPS, several techniques were employed such as Synchrotron Radiation Circular Dichroism (SRCD), Circular Dichroism (CD), Differential Scanning Calorimetry (DSC), Dynamic Light Scattering (DLS) and Size Exclusion Chromatography (SEC). This allowed the assessment of melting temperatures, hydrodynamic radii, and secondary structure features providing insights on the cage assembly/disassembly. This information clarifies potential ways on how one can control the opening and closing of the bionanocage by supplying thermal energy to the system and therefore paves the way for novel biotechnological applications.

Acknowledgements: This research was supported by Fundação para a Ciência e Tecnologia, Ministério da Educação e Ciência (FCT/MEC), grant PTDC/BIA-PRO/111485/2009 (to P.T.), PTDC/QUI/64248/2006 (to A.S.P). This work was supported by the Applied Molecular Biosciences Unit- UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019). SRCD measurements were supported by CALIPSOplus EU Framework Programme for Research and Innovation. JPJ, AA, JPG, DP are supported by Radiation Biology and Biophysics Doctoral Training Programme (RaBBiT PD/00193/2012) Ph.D. Fellowships (SFRH/BD/135056/2017), (SFRH/BD/135477/2017), (SFRH/BD/135056/2017) and (SFRH/BD/52535/2014) from FCT/MEC. We would also like to thank to Elisabete Ferreira and Cecilia Bonifácio for the help with CD and DSC measurements.

P34

EPR detection of radical(s) in cytochrome c oxidase

Daniel Jancura[1,2], Marian Fabian[2]

[1]Department of Biophysics, Faculty of Science, Safarik University, Kosice, Slovakia, [2]Center for Interdisciplinary Biosciences, Technology and Innovation Park, Safarik University, Kosice, Slovakia

daniel.jancura@upjs.sk

Catalytic mechanism of cytochrome c oxidase (CcO) involves formation of ferryl intermediates **P** and **F**. The production of these intermediates is accompanied by a formation of protein-based radical(s). The reaction of oxidized CcO with hydrogen peroxide also leads to the formation of **P** and **F** and corresponding radical species. However, the application of electron paramagnetic spectroscopy (EPR) to detect such radical has resulted only in the observation of low amounts relative to the concentration of the **P** intermediate. A possible reason for this fact is a coupling of the unpaired electron of radical with the paramagnetic metal center(s) within the catalytic site of CcO. We have developed a new approach, a moderate destabilization of the enzyme structure by protein denaturant, guanidinium chloride (Gnd.Cl), to detect stoichiometric amount of the radical in CcO. In this situation, a coupling between protein-based radical and a metal center(s) is broken. As our results show, the yield of the EPR observed radical in **P** is significantly increased in the presence of Gnd.Cl relative to that in the absence of denaturant. In a sample with 2 M Gnd.Cl, the yield of the detected radical reached ~50% of the **P** population. The origin of EPR detected radical(s) and their possible roles in the catalytic cycle of CcO is discussed.

Acknowledgement: This work was supported by Slovak Grant Agency (VEGA-1/0464/18).

P35

Protein-protein interaction standards: The latest news in attempts to make ideal nanobodies

Thomas Jowitt and Hilda Ruiz Nivia

Biomolecular Analysis Core Facility, Faculty of Biology, Medicine and Health, University of Manchester

t.jowitt@manchester.ac.uk

As most of you know one of the key directives of Working Group-4 (WG4) within the ARBRE-MOBIEU community has been to develop standard systems for the biophysics community. A survey on this topic was circulated last year and I will present the results of that survey in this presentation. One of the key directives was to design and produce protein-protein interaction standards for use as benchmarking tools, laboratory standards and training tools. In our lab, we have developed several nanobodies to lysozyme as a protein interaction system that fits the criteria of the survey. Nanobodies are small antibody fragments based on the VHH domains of Camelid antibodies. These ~14 kDa protein fragments inherently have high stability and favourable binding properties and more importantly they are easy to produce and are royalty/patent free. The two variable domains which form the interaction sites and the specificity for the antigen can be derived either through phage display or immunisation of a Lama. Our nanobodies have been developed by both rational design and phage display to produce a selection of molecules that have different binding characteristics which makes them particularly interesting when tailoring molecules for certain techniques. However, the goal has been to try to make molecules that are amenable to all techniques, and it is this aspect that has proven to be the most difficult. We have also shown that many nanobodies have very undesirable characteristics and it is only when the binding is investigated with several orthogonal techniques that these undesirable effects can be observed. I will discuss how we have derived our current nanobodies, the results we have gained on different instruments and where we go from here.

P36

Quaternary Structure of Human NK Cell Receptor: Ligand complexes by the Looking Glass of Super-resolution Microscopy

Barbora Kalousková[1], Jan Bláha[1], Ondřej Skořepa[1], Tereza Skálová[2], Jan Dohnálek[2], Ondřej Vaněk[1]

[1]*Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech* Republic; [2]Institute of Biotechnology, The Czech Academy of Sciences, v.v.i., Vestec, Czech *Republic*

barbora.kalouskova@natur.cuni.cz

Natural killer cells (NK cells) play one of the key roles in our immune system. They have ability to eliminate infected, stressed or malignantly transformed cells without prior antigen sensitization. Recognition of the target cell is promoted by surface NK cell receptors and equilibrium between activating and inhibitory signals leads to apoptosis or tolerance. We are focusing on structural description of one such NK cell receptor: ligand complex – human natural killer receptor protein 1 (NKR-P1; gene *klrb1*) and its ligand lectin-like transcript 1 (LLT1; gene *clec2d*).

First, crystal structure of this protein complex was solved and this data were further supported by other biophysical methods (SAXS, AUC, and MST). There are two binding modes between NKR-P1 and LLT1 molecules, which enable not only direct interaction, but also interaction with the neighbouring receptor. These two modes observed within crystal structure led to a hypothesis about NKR-P1 cluster formation upon ligand binding.

Super resolution microscopy was then used to evaluate biological relevance of structural data and reveal nanoscale organisation of NKR-P1:LLT1 complex directly on the cell surface. Using SMLM and cluster analysis of localisation tables, we study reorganisation of NKR-P1 in cell membrane caused by the binding of its biological ligand, LLT1.

Acknowledments: This study was supported by BIOCEV (ERDF CZ.1.05/1.1.00/02.0109), Czech Science Foundation (18-10687S), MEYS of the Czech Republic (LTC17065 in frame of the COST Action CA15126), and Charles University (GAUK 161216). The authors also acknowledge the support and the use of resources of Instruct, a Landmark ESFRI project through the R&D pilot scheme APPID 56 and 286.

P37

Stem Cells Adhesion by Functionalized Nanodiamonds

Nerijus Karalius[1], Vytautas Lapeika[1], Jelena Tamulienė[2], Loreta Rastenienė[1], Rimantas Vaisnoras

[1]Vytautas Magnus University Education Academy, Liquid crystals laboratory, Vilnius, Lithuania [2]VilniusUniversity, Institute of Theoretical Physics and Astronomy, Vilnius, Lithuania

nnerrkka@gmail.com

Cells are surrounded by extracellular matrix in their natural environment [1]; nanoscale topography is observed on the extra–cellular matrix surface. The understanding of these nano–cell interactions is essential if advances in knowledge about cell motility, morphology, proliferation and differentiation are to occur [2]. In this report the interaction between nanodiamond nanoparticles, size 5 nm and human stem cells has been investigated. It has been shown that nanodiamonds with oxygen containing groups on the surface allow for a significantly better stem cells attachment over hydrogen functionalization. Nanodiamonds surface modification by oxygen demonstrate strong resonant as negatively charged structures and strong conjugate with the positively charged extracellular matrix. Contact angle measurements and protein adsorption experiments have enabled the development of a probable explanation as to why this difference is observed. As extracellular matrix used both mixture of the cyanobiphenyl organic compound where banana-shaped molecules (2x0,5 nm) and ball shaped the PPI dendrimer molecules where well oriented around nanodiamond to onion or ball structures, as illustrated in Figure1.

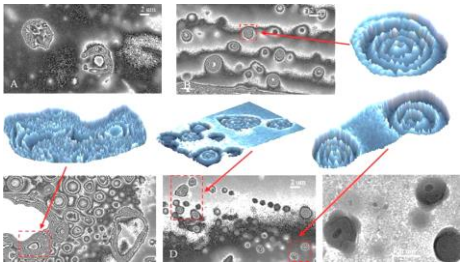


Figure 1. Dynamic formation of the vesicles- nanocarriers in the organic matrix with strong aligning of the molecules in the concentric layers of membranes, much like an onion during time 1-3 min (A, B, C, D) or balls (E). Grey and black images visualized by microscopy.

Vesicles in the red denoted segments were turned into colorized 3-D models to better visualization.

References

[1] Kalra H, Drummen GP, Mathivanan S. Focus on Extracellular Vesicles: Introducing the Next Small Big Thing. Int J Mol Sci. 2016; 17, 170; doi: 10.3390/ijms17020170.

[2] Taylor AC, González CH, Miller BS, Edgington RJ, Ferretti P, Jackman RB. Surface functionalisation of nanodiamonds for human neural stem cell adhesion and proliferation. Scientific Reports 2017; 8, 7307; doi: https://doi.org/10.1038/s41598-017-07361-y.

P38

Changes in the membrane phospholipid composition in erythrocytes in subjects with metabolic syndrome after short term consuming of a pomegranate juice

Kojadinovic Milica[1], Arsic Aleksandra[1], Kardum Nevena[1], Ana Jelenkovic[1], Popovic Milica[2], Martacic Debeljak Jasmina[1], Glibetic Marija[1]

1Center of Research Excellence in Nutrition and Metabolism, Institute for Medical *Research, Univeristy of Belgrade, Belgrade, Serbia; [2]Faculty of Chemistry, Department of Bichemistry, University of Belgrade, Belgrade, Serbia*

milica.kojadinovic.imr@gmail.com

Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors related to insulin resistance, disturbed glucose metabolism, visceral obesity, arterial hypertension and atherogenic dyslipidemia. In these disorders fluidity and structure of cell membrane play a crucial role and it can be damage. [1] Regular consumption of fruits rich in antioxidants, such as pomegranate (*Punica granatum*, L.) may be associated with lower incidences of cardiovascular and other chronic diseases. [2]

In this study were included twenty one subjects (12 woman, 9 man) with MS aged from 40 to 60 years. They were randomly assigned into two groups. The interventional group received 200 mL of pomegranate juice daily for 2 weeks, while control group did not consume the juice.

Phospholipid fatty acid methyl esters in erythrocytes were analyzed by gas-liquid chromatography on Shimadzu chromatograph GC 2014 (Kyoto, Japan).

Phospholipid (FA) composition in erythrocytes was not significantly different between intervention and control group, at baseline. There was a significant increase ($p<0.05$) of relative amounts of dihomo-α-linoleic acid and increase of relative amounts of docosahexaenoic fatty acid in erythrocytes phospholipids in subjects with metabolic syndrome. Also, activity of Δ6 and Δ5 desaturase in erythrocytes were changed in interventional group. Δ6 desaturase were significantly increased and Δ5 desaturase were significantly decreased. Fatty acid profiles in erythrocytes in control group were not significantly changed during the study.

These results suggested that short consumption of pomegranate juice may change phospholipid fatty acid profile and activities of desaturases in erythrocytes in subjects with MS. Considering the limited data on pomegranate impact on FA status, further research is need in order to clarify potential mechanism of action and confirm findings of this study.

References

[1] Eckel RH, Grundy SM and Zimmet PZ, The metabolic syndrome. Lancet. 2005.; 365:1415–1428.

[2] Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA et al. Flavonoids,

flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. Am J Clin Nutr. 2008; 88:38–50.

P39

A biophysical view on the interaction of SSB proteins with single-stranded DNA

Anja Kostelac[1,2], Vlatka Godinić Mikulčić[1], Dušica Vujaklija[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia; [2]Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia.

akostelac@hotmail.com

Streptomyces species are the largest genus of Actinobacteria. This genus is represented by the model organism, *Streptomyces coelicolor*. The replisome of *S. coelicolor* is composed of the following enzymes: DNA polymerase, primase, helicase, ligase, topoisomerase II, SSB and initiation proteins. SSB proteins are essential to all life forms. These proteins bind transiently single-stranded DNA (ssDNA) formed during various cellular events. SSB proteins interact with many different proteins and orchestrate DNA replication, repair and recombination processes [1]. *S. coelicolor* has two paralogous SSB proteins - SsbA and SsbB. SSB proteins are homotetramers that bind ssDNA in multiple binding modes; $(SSB)_{35}$ and $(SSB)_{65}$ [2]. In this project, *SsbA* gene from *S. coelicolor* was cloned in expression vector, overexpressed in *Escherichia coli* and sequentially purified on two chromatographic columns. Binding affinities for GC-rich and poly(dT) oligonucleotides were determined by electrophoretic mobility shift assay (EMSA) for SsbA and SsbB in the presence of different NaCl concentration. The length of the shortest oligonucleotide bound by SsbA was determined by EMSA. Transition between SsbA binding modes was explored as a function of the NaCl concentration. Kinetics of photophysical intramolecular deactivation processes (Stern-Volmer relationship) of SsbA and SsbB were investigated by fluorescence spectroscopy and quenching constants were calculated. In the end, intermolecular competition between SsbA and SsbB for the same ssDNA was analysed in the presence of Mg^{2+} ions and different NaCl concentrations by EMSA and Western blotting. This study provides novel evidence of differential binding affinities of paralogous SSBs to ssDNA in *S. coelicolor* [3, 4, 5]. These results shed the new light upon assembly of SSB-DNA complexes.

References

[1] Shereda RD, Kozlov AG, Lohman TM, Cox MM, Keck JL. SSB as an organizer/mobilizer of genome maintenance complexes. Crit Rev Biochem Mol Biol. 2008; 43:289-318. doi: 10.1080/10409230802341296.

[2] Overman LB, Lohman TM. Linkage of pH, anion and cation effects in protein-nucleic acid equilibria. Escherichia coli SSB protein-single stranded nucleic acid interactions. J Mol Biol. 1994; 236:165-178. doi: 10.1006/jmbi.1994.1126.
[3] Mijaković I, Petranović D, Maček B, Čepo T, Mann M, Davies J, Jensen PR, Vujaklija D. Bacterial single-stranded DNA-binding proteins are phosphorylated on tyrosine. Nucleic Acids Res. 2006; 34:1588-1596. doi: 10.1093/nar/gkj514.
[4] Štefanić Z, Vujaklija D, Luić M. Structure of the single-stranded DNA-binding protein from Streptomyces coelicolor. Acta Crystallogr D Biol Crystallogr. 2009; 65:974-979. doi: 10.1107/S0907444909023634.
[5] Paradžik T, Ivić N, Filić Ž, Manjasetty BA, Herron P, Luić M, Vujaklija D. Structure-function relationships of two paralogous single-stranded DNA-binding proteins from Streptomyces coelicolor: implication of SsbB in chromosome segregation during sporulation. Nucleic Acids Res. 2013; 41:3659-3672. doi: 10.1093/nar/gkt050.

P40

EPR study of ion substituted hydroxy apatites

Timor Grego[1], Božana Čolović[2], Ina Erceg[3], Maja Dutour Sikirić[3], Nadica Maltar-Strmečki[3]

[1]University Hospital Centre Zagreb, Kišpatićeva 12, Zagreb, Croatia; [2]Vinča Institute, Belgrade, Serbia; [3]Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička c. 54, Zagreb, Croatia

nstrm@irb.hr

Since calcium phosphates (CP) constitute the main mineral component of the hard tissues of vertebrates, their synthetic analogues are the most commonly used materials in orthopedics and stomatology [1-3]. Namely, the mineral phase of the bones and teeth is a basic calcium phosphate, which is being equalized with synthetic calcium hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) [2]. However, biological apatites differ from stoichiometric HA in several aspects, such as non-stoichiometry, small crystal sizes and poor crystallinity which are important factors associated with the relatively high solubility of biological apatites compared to stoichiometric HA [2]. Biological apatites are actually carbonated apatites containing also foreign ions, which can be incorporated in or adsorbed on the apatite crystal surface. Synthesis of differently ion substituted HA is attracting attention in developing novel biomaterials with increased bioactivity, as well as possibility to deliver ions for treatment of the diseased bone [2, 4]. Among key questions for optimizing the synthetic procedures and producing efficient biomaterials is how different ions modify crystal and surface structure of HA.

The aim of this study was to determine the influence of Mg and Si on short range ion arrangement in synthetic HA by EPR (electron paramagnetic resonance)

spectroscopy. HA modified with Mg and Si were synthetized by hydrothermal procedure. Their structure and morphology were determined by powder X-ray diffraction and scanning electron microscopy. For EPR investigation, the gamma radiation induced radical were used as probes to follow and control the changes in relation with substituted ions.

The obtained results provide better insight in the influence of ionic substitution on HAP structure and therefore can be used in designing of HAP with specific properties.

References

[1] Dorozhkin S.V. Calcium Orthophosphate-Based Bioceramics and Biocomposites: Wiley-VCH, Weinheim, Germany; 2016.

[2] Boanini E, Gazzano M, Bigi A. Ionic substitutions in calcium phosphates synthesized at low temperature. Acta biomaterialia. 2010; 6(6):1882-94. Epub 2009/12/31. doi: 10.1016/j.actbio.2009.12.041. PubMed PMID: 20040384.

[3] Laskus A, Kolmas J. Ionic Substitutions in Non-Apatitic Calcium Phosphates. Int. J. Mol. Sci. 2017; 18(12). Epub 2017/12/01. doi: 10.3390/ijms18122542. PubMed PMID: 29186932; PubMed Central PMCID: PMC5751145.

[4] Szurkowska K, Kolmas J. Hydroxyapatites enriched in silicon – Bioceramic materials for biomedical and pharmaceutical applications. Progress in Natural Science: Materials International. 2017;27(4):401-9. doi: https://doi.org/10.1016/j.pnsc.2017.08.009.

P41

Heparanase binds substrate *via* a dynamic binding mechanism

F. Hafna Ahmed[1], Aleksandra Maršavelski[1,2], A. Elaaf Moahmed[1], Emily Jamieson[1], Galen Correy[1], and Colin J. Jackson[1]

[1]Research School of Chemistry, Australian National University, Canberra, Australian Capital Territory, Australia; [2]Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia

aleksandra.marsavelski@chem.pmf.hr

Human heparanase (HPSE) cleaves heparan sulfate (HS) localized in the extracellular matrix allowing immune response to occur as well as cell proliferation and angiogenesis. On the other hand, increased HPSE activity promotes a variety of pathological conditions such as arterial thrombosis and tumor metastasis[1], making HPSE an important drug target. Although crystal structures of HSPE are available[2], the nature of interactions with its very flexible and highly negatively charged polysaccharide substrate HS is not fully understood. To better understand processes that govern substrate recognition, we have explored the ligand binding mechanism of HPSE by analysing its

conformational landscape with molecular dynamics (MD) simulations and multi-conformer models generated by crystallographic ensemble refinement. Our results imply that HS binding to HPSE is a complex process. Heparan sulfate is stabilized and immobilized at the active site, whereas the outer regions of the enzyme binding site in complex with HS are characterized with dynamic ionic interactions and exhibit substantial conformational flexibility (Fig. 1). This dynamic substrate binding mechanism allows HSPE to retain high affinity towards its substrate while minimizing the entropic costs associated with binding highly flexible molecules.[3]

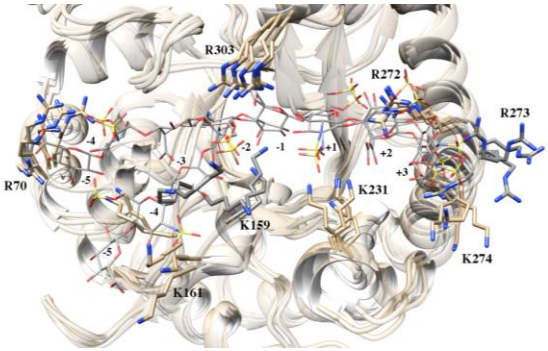


Figure 1. Binding site in the MD model of the HPSE-HS8 complex, represented by an overlay of representative structures of the most populated clusters obtained from cluster analysis. Residues K159 and R273 are given in grey for clarity. HS8 is shown in grey thin sticks.

References

[1] Ilan N, Elkin M, Vlodavsky I. Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. Int. J. Biochem. Cell Biol. 2006; 38:2018–2039. doi:10.1016/j.biocel.2006.06.004

[2] Wu L, Viola CM, Brzozowski AM, Davies GJ. Structural characterization of human heparanase reveals insights into substrate recognition. Nat. Struct. Mol. Biol. 2015; 22:1016–1022. doi:10.1038/nsmb.3136

[3] Ahmed HF, Marsavelski A, Moahmed EA, Jamieson E, Correy G, Jackson CJ. Heparanase binds substrate via a dynamic binding mechanism, manuscript in preparation.

P42

Precision of ITC measurements for target-based drug design

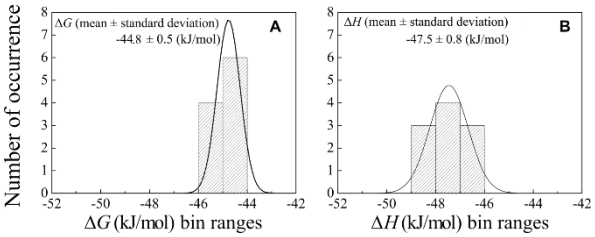
Vaida Paketurytė, Daumantas Matulis

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

matulis@ibt.lt

Target-based rational drug design needs detailed understanding of the correlations between compound chemical structure and thermodynamics of binding. However, pharmaceutical industry still primarily depends on high-throughput screening of random compounds because the correlations are poorly understood. Furthermore, thermodynamic analysis is usually limited to affinity (Gibbs energy change upon binding) while the enthalpy, entropy, heat capacity, and volume of binding together with the analysis of protonation and salvation reactions can provide important details about the protein-ligand recognition.

To study the recognition, we have synthesized a library of 700 compounds bearing a pharmacophoric sulfonamide group that binds directly to the active site Zn(II) of a family of 12 human carbonic anhydrase proteins. Alteration of the compound structure led to micromolar-picomolar variation in affinity [1] and significant enthalpy-entropy compensation. Since it was critical to know the accuracy and precision of our ITC measurements, we repeated the binding reaction of acetazolamide to carbonic anhydrase II numerous times using four isothermal titration calorimetry instruments [2] with the goal to determine the lowest possible and optimal concentrations and standard deviations of our ITC experiments. The ΔG and ΔH of this reaction were determined by repeating the reaction under identical conditions (CA II at 10 μM and 25 ºC, VP-ITC instrument) and were equal to ΔH= –47.5 ± 0.8 kJ/mol and ΔG= –44.8 ± 0.5 kJ/mol. The enthalpies of binding spanned the range between -80 and +10 kJ/mol, much greater than the uncertainty of the measurements thus validating the compensation measurements.



Gaussian distributions of enthalpy and Gibbs energy ITC measurements, obtained by repeating the AZM – CAII reaction 10 times at identical experimental conditions with VP-ITC instrument [2].

References

[1] Linkuvienė, V. et al. Thermodynamic, kinetic, and structural parameterization of human carbonic anhydrase interactions toward enhanced inhibitor design. Q. Rev. Biophys. 51, 2018.

[2] Paketurytė, V., Linkuvienė, V., Krainer, G., Chen, W.-Y. & Matulis, D. Repeatability, precision, and accuracy of the enthalpies and Gibbs energies of a protein–ligand binding reaction measured by isothermal titration calorimetry. Eur. Biophys. J. 2018.

P43

Spectroscopic Studies of Dual Fluorescence in 2-((4-Fluorophenyl)amino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole and other compounds with 1,3,4-thiadiazole

Arkadiusz Matwijczuk[1], Daniel Kamiński[2], Andrzej Górecki[3], Agnieszka Ludwiczuk[4], Andrzej Niewiadomy [5], Sebastian Maćkowski [6]

[1]Department of Biophysics, University of Life Sciences in Lublin, 20-950 Lublin, Poland, [2]Poland Department of Chemistry, University of Life Sciences in Lublin, 20-950 Lublin, Poland, [3]Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University, 31-007 Kraków, Poland, [4]Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki Str., 20-093 Lublin, Poland, [5]Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, 87-100 Toruń, Poland

In this work we presents results of fluorescence analysis of ionic and non-ionic *2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole (FABT)* and another selected compounds with 1,3,4-thiadiazole group in monocrystals and solutions. We obserwd single fluorescence band in the case of FABT crystals grown in methanol (and another crystal which we got in other polar solvents), while for FABT crystals grown in aqueous environment (water in low pH = 1) dual fluorescence emerges. The effect of dual fluorescence is also observed for FABT (and another selected compounds with this group) dissolved in aqueous solutions with pH ranging from ~7,5 to 1. In contrast, FABT dissolved in methanol (apparent pH = 1) exhibited single fluorescence band. The dual fluorescence effect is associated with conformational changes in the FABT molecule, which can be induced by specific aggregation effects. Aggregation for these compounds (especially FABT) can induce specific charge transfer effects (CT) in a molecule that can cause the observed dual fluorescence effects. Based on crystallography data, two types of FABT crystal molecule conformations were distinguished. In methanol FABT molecules: the –OH group from the resorcyl ring oriented towards the sulphur atom from the 1,3,4-thiadiazole ring (are in conformation “S”) , which we have a single fluorescence band in this

case. In water, we have this same –OH group from the resorcyl ring oriented towards the nitrogen atom from the1,3,4-thiadiazole ring due to 180º rotation (conformation "N") – we have two fluorescence bands. This significant finding implies the possibility of performing a rapid analysis of conformational changes in molecules with this groups: 1,3,4-thiadiazoles using fluorescence spectroscopy both in solutions and in biological samples.

P44

A pipeline for testing interaction partners of small GTPases in *Dictyostelium*

Lucija Mijanović, Vedrana Filić, Maja Marinović, Marko Šoštar, Igor Weber

Ruđer Bošković Institute, Division of Molecular Biology, Bijenička 54, 10000 Zagreb, Croatia

Lucija.Mijanovic@irb.hr

Small GTPases from the Ras superfamily are monomeric GTP-hydrolyzing proteins which act as molecular switches, regulating a variety of cellular processes such as cell motility, proliferation, endocytosis, cytokinesis, membrane trafficking etc., by alternating between active (GTP-bound) and inactive (GDP-bound) forms [1]. Since many of them have mutually related domain architecture, regulation and function, it is important to identify specific interactions between a given GTPase and its regulating and effector proteins reliably. In recent years, our group investigated the roles of Rac and Ras proteins and their regulators and effectors in amoeba *Dictyostelium discoideum*. When analyzing interactions and functions of a GTPase, it is crucial to distinguish between its active and inactive forms, since usually only the active form is conveying biological activity of the protein. We therefore introduced a pipeline of assays to determine specific interactions between candidate proteins or protein domains and a panel of small GTPases. Initially, we test potential interactions using yeast two-hybrid (Y2H) screen using constitutively active and inactive GTPase mutants as preys. Next, positive interactions are further tested biochemically using GST pull-down assay, where potential interactions are verified in relation to the activation status of a GTPase. As a final step, the apparent interactions are examined *in vivo*, using fluorescence-based proximity assays which enable discrimination between direct and indirect interactions, such as Fluorescence Resonance Energy Transfer (FRET) measurements realized by sensitized emission of the acceptor [2] or Fluorescence Lifetime Imaging Microscopy (FLIM) [3], and Bimolecular Fluorescence Complementation (BiFC) [4]. We will present the results provided by

application of this pipeline on two examples: development of probes for tracking the intracellular dynamics of active Rac1 proteins [2, 3], and discovery of a specific interaction of IQGAP-related protein IqgC and active RasG [4]. We will provide a comparison of results obtained by equivalent methods in these studies and especially point out certain subtle disparities between the results of different assays. Taken together, results obtained by using this set of methods provide robust data for evaluating the interactions, and their nature, between small GTPases and their potential interaction partners.

References

[1] Wennerberg K, Rossman KL, Der CJ. The Ras superfamily at a glance. J Cell Sci. 2005; 118:843–846. doi: 10.1242/jcs.01660.

[2] Filić V, Marinović M, Faix J, Weber I. A dual role for Rac1 GTPases in the regulation of cell motility. J Cell Sci. 2012; 125:387-398. doi: 10.1242/jcs.089680.

[3] Marinović M, Šoštar M, Filić V, Antolović V, Weber I. Quantitative imaging of Rac1 activity in Dictyostelium cells with a fluorescently labelled GTPase-binding domain from DPAKa kinase. Histochem Cell Biol. 2016; 146:267-279. doi: 10.1007/s00418-016-1440-9.

[4] Marinović M, Mijanović L, Šoštar M, Vizovišek M, Junemann A, Fonović M, Turk B, Weber I, Faix J, Filić V. IQGAP-related protein IqgC suppresses Ras signaling during large-scale endocytosis. PNAS USA. 2019 (in press). doi: 10.1073/pnas.1810268116.

P45

Algal cells under heavy metal stress: physiological and morphological response

Tea Mišić Radić, Andrea Čačković

Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia

tmisic@irb.hr

Heavy metals can cause diverse negative impacts on marine ecosystems including impact on the major primary producers and the base of the food web - algae. The impact of cadmium on lightly silicified marine diatom *Cylindrotheca closterium* growth dynamics and morphological characteristics was studied. *C. closterium* cultures were exposed to 0–1000 $\mu g L^{-1}$ Cd and growth dynamics was followed with optical microscope using haemocytometer. The increased cadmium concentrations from 1 to 1000 μg Cd L^{-1} in *C. closterium* cultures induced decrease of specific growth rates in exponential growth phase. Morphological changes on the cell surface of *C. closterium* exposed to cadmium

were characterized at the nanoscale using atomic force microscope (AFM). The morphological changes were observed as irregular patterns of silica spheres on more silicified parts of the cell, girdle band and around the raphe of the cells that grew at 1000 μg Cd L^{-1}. Cadmium uptake clearly affected the biosilica formation mechanism in *C. closterium*, most probably by interfering in biological and physicochemical processes involved in diatom biosilification.

P46

Biophysical studies on Tau aggregation and metal ion binding

Guilherme G. Moreira[1*], Joana S. Cristóvão[1], Vukosava M. Torres[2], Ana P. Carapeto[1], Mário S. Rodrigues[1], Carlos Cordeiro[2], Isabelle Landrieu[3], Cláudio M. Gomes[1]

[1]Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade Lisboa, Lisbon, Portugal; [2]FTICR e Structural MS laboratory, Faculdade de Ciências, Universidade Lisboa, Lisbon, Portugal; [3]Université Lille, CNRS, UMR 8576 - UGSF - F-59000 Lille, France.

ggmoreira@alunos.fc.ul.pt

Alzheimer's disease (AD) is the most common cause of dementia, affecting millions worldwide. The intracellular aggregation of hyperphosphorylated Tau is a well-known AD hallmark and is directly related to disease progression. Tau hyperphosphorylation leads to intraneuronal microtubule disintegration and destruction of the cytoskeleton resulting in neuronal death [1].

Tau is an intrinsically disordered protein with a high aggregation propensity which in solution is known to form oligomers that evolve into amyloid fibrils. The exact sequence of molecular events starting from monomeric Tau remains to be fully understood, being critical to develop anti-aggregation therapies. It is also critical to understand how metal ion binding to Tau influences its structural and aggregation properties. Indeed, metals are known modulators of AD pathogenesis [2,3].

Towards this goal we here report our investigations using biochemical and biophysical techniques that allowed us to gain insights into Tau structure and aggregation. We report an improvement of an established protocol for the expression and purification of recombinant full-length human Tau (hTau441) [4] obtaining a fraction enriched in monomeric Tau. We also report studies on heparin-induced aggregation experiments of hTau441 monitored by thioflavin-T fluorescence, in the presence and absence of metal ions. We also used ATR-FTIR and native Mass Spectrometry to characterize the structural properties of Tau during aggregation and Atomic Force Microscopy to characterise the morphology of Tau aggregates and fibrils.

References
[1] Burns A, and Iliffe S. Alzheimer's disease. Bmj. 2009; 338, b158.
[2] Cristovao JS, Santos R, and Gomes CM. Metals and Neuronal Metal Binding Proteins Implicated in Alzheimer's Disease. Oxid Med Cell Longev. 2016; 9812178.
[3] Leal, SS, Botelho HM and Gomes CM. Metal ions as modulators of protein conformation and misfolding in neurodegeneration. Coordination Chemistry Reviews. 2012; 256, 2253-2270.
[4] Danis C, Despres C, Bessa, LM, Malki I, Merzougui H, Huvent I, Qi H, Lippens G, Cantrelle FX, Schneider R, Hanoulle X, Smet-Nocca C, and Landrieu I. Nuclear Magnetic Resonance Spectroscopy for the Identification of Multiple Phosphorylations of Intrinsically Disordered Proteins. JoVE. 2016.

P47

Spectroscopic and Theoretical Studies of Dual Fluorescence in 2-Hydroxy-N-(2-phenylethyl)benzamide Induced by ESIPT Process. Solvents Effects

Agnieszka Niemczynowicz[1], Arkadiusz Matwijczuk[2]

[1]Department of Analysis and Differential Equations, Faculty of Mathematics and Computer Science, University of Warmia and Mazury, Słoneczna 54, PL-10-710 Olsztyn, Poland, [2]Department of Biophysics, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

niemaga@matman.uwm.edu.pl

In this work we discusses the theoretical ([TD]DFT) and molecular spectroscopic investigation of *2-Hydroxy-N-(2-phenylethyl)benzamide* (SAL3). We also discuss its physiochemical behaviour in the selected organic solvents (polar and non-polar). The compound demonstrated a very interesting dual fluorescence effect in alcoholic solutions (e.g.: methanol, ethanol or isopropanol), while in acetonitrile (ACN), dimethylsulfoxide (DMSO) and non-polar solvents (like: n-Hexane, n-Heptane, chloroform) only a single emission maximum was observed. The noticeable shortening of average fluorescence lifetime (TCSPC), the employed detailed analysis of solvatochromic shifts of absorption spectra in the function of polarizability related to a change in the induction polarization of the environment and, above all, the performed quantum-mechanical calculations [TD]DFT with a detailed analysis of excited states, clearly indicate a connection between the observed fluorescence effects and processes related to changes in the system's structure in the excited state. We also noticed that the observed fluorescence effects (dual fluorescence) are quenched by aggregation effects of this compounds.
Based on these experimental and theoretical studies, it has been proposed that the environment (solvent) polarity-induced dual fluorescence effect in SAL3 is related to

the Excited-State Intramolecular Proton Transfer (ESIPT) process. Also, the quantum-mechanic studies [TD] DFT point to the specific conformation of SAL3 molecule characteristic of dual fluorescence emission.

P48

Intrinsic affinity of *N*-substituted benzenesulfonamides to CA proteins

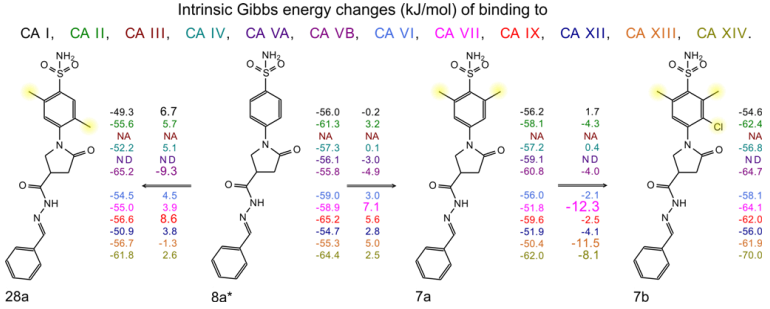
Vaida Paketurytė, Daumantas Matulis

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

vaidapaketuryte@gmail.com

A series of benzenesulfonamides bearing N-substituents at para position and methyl groups or/and halogen atoms at ortho or meta positions were designed, synthesized, and tested as inhibitors of the twelve catalytically active human carbonic anhydrase (CA) isoforms[1-3]. The development of high affinity and selective inhibitors of CA proteins is a need in target based drug design.

We determined the observed binding affinities by fluorescent thermal shift assay, ITC, and enzymatic activity inhibition assay. However, these observed affinities cannot be used in structure-thermodynamics correlations. Therefore, the intrinsic (pH-independent and buffer-independent) binding affinities were calculated representing the interaction of sulfonamide anion (RSO2NH-) to the Zn(II)-bound water form of CA. Some compounds in this series exhibited selectivity for CA VII and CA XIII (up to 500 fold) while other compounds showed low nanomolar dissociation constants and over 10-fold selectivity for mitochondrial isoform CA VB. Determination of intrinsic affinities allowed the development of compounds as inhibitors of CA with higher affinity and selectivity to particular CA isoforms.



Chemical structures of investigated compounds aligned in an order of minimal structural difference. Numbers on the right from structures list the intrinsic Gibbs energy changes upon compound binding to all 12 human CAs (listed in various colors above). Numbers above and below arrows show the differences in binding Gibbs energies between adjacent compounds. Larger font means greater difference leading to chemical groups that contribute to the gain/loss of binding energy.

References

[1] Rutkauskas, K. et al. Benzenesulfonamides bearing pyrrolidinone moiety as inhibitors of carbonic anhydrase IX: synthesis and binding studies. Med. Chem. Res. 2017

[2] Vaškevičienė, I. et al. N-Sulfamoylphenyl- and N-sulfamoylphenyl-N-thiazolyl-β-alanines and their derivatives as inhibitors of human carbonic anhydrases. Bioorg Chem 2017

[3] Vaškevičienė, I. et al. Pyrrolidinone-bearing methylated and halogenated benzenesulfonamides as inhibitors of carbonic anhydrases. Bioorg. Med. Chem. 2018

P49

Application of machine learning approaches for design of more selective herbicides

Vesna Pehar[1], Davor Oršolić[2], Milena Jadrijević-Mladar Takač[3], Višnja Stepanić[2,4]

[1]Croatian Defense Academy "Dr. Franjo Tuđman", Zagreb, Croatia; [2]Centre of Research Excellence for Marine Bioprospecting – BioProCro, Ruđer Bošković Institute, Zagreb, Croatia; [3]Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia; [4]Division of molecular medicine, Ruđer Bošković Institute, Zagreb, Croatia

stepanic@irb.hr

Herbicides are chemical molecules used for destruction of weeds. Massive usage of herbicides has resulted in two global problems: increase in herbicide resistance and harmful impact of human health [1, 2]. In order to facilitate development of novel, more specific herbicides and development of strategies for impeding the weed resistance development, we have carried out extensive *in silico* analysis of the set of herbicides. Herein, we present results revealing links between structural, physicochemical, ADME (Absorption, Distribution, Metabolism, Excretion) and toxic features for herbicides. The analysis has been done by using proper machine learning approaches.

References
[1] Forouzesh A, Zand E, Soufizadeh S, Foroushani SS. W Classification of herbicides according to chemical family for weed resistance management strategies–an update, Weed Res, 2015; 55: 334-358. doi: 10.1111/wre.12153.
[2] Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB. Pesticide toxicity: a mechanistic approach. EXCLI J. 2018; 17:1101-1136. doi: 10.17179/excli2018-1710.

P50

Tethered Bilayer Lipid Membranes – a Comprehensive Tool for Electrochemical Studies of Pore-Forming Toxins

Tadas Penkauskas, Marija Jankunec, Gintaras Valincius

Vilnius university Life Sciences Center, Institute of Biochemistry, Vilnius, Lithuania

tadas.penkauskas@bchi.vu.lt

Formation of plasma membrane is considered as a crucial event during evolution and life, as known today, would not be possible without them. To investigate this complex membrane environment of lipids and membrane proteins significant progress has been made to model native membranes. The most common artificial systems include lipid monolayers, lipid vesicles, and supported lipid bilayers (SLBs) [1]. Distinct group of solid supported bilayers, tethered bilayers (tBLMs), are considered as perspective experimental platforms for membrane biosensors [2]. In particular, the modulation of the ionic conductance of tBLMs may be utilized to develop biosensors of the membrane damaging agents such as pore-forming toxins (PFTs) [3].

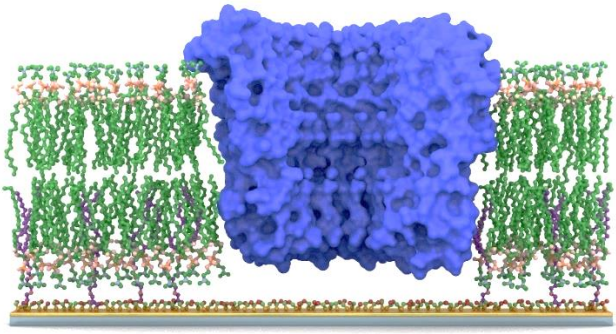


Figure 1. Schematic representation of the tBLM system with an oligomer of pore-forming toxin reconstituted into the lipid bilayer.

We have used the tBLM platform for detection, visualization and investigation of the mechanism of action of cholesterol dependent cytolysins (CDCs) – a class of PFT produced by a large number of Gram-positive pathogens and considered as virulence factors promoting bacterial invasion and infection. Most of the experimental data was obtained by particularly applying electrochemical impedance spectroscopy (EIS) and atomic force microscopy (AFM) techniques. tBLM modification in aqueous environment has been performed, using Methyl-β-cyclodextrin (MβCD) and cholesterol complex, to achieve picomolar sensitivity of CDC detection. Moreover, the additional receptor – human CD59, essential for some of the CDCs to reconstitute, was successfully attached to tBLM, enabling a better insight into different mechanisms of how the same toxin can operate.

References

[1] I. Köper, "Insulating tethered bilayer lipid membranes to study membrane proteins.," *Mol. Biosyst.*, vol. 3, no. 10, pp. 651–7, Oct. 2007.

[2] D. J. McGillivray *et al.*, "Molecular-scale structural and functional characterization of sparsely tethered bilayer lipid membranes.," *Biointerphases*, vol. 2, no. 1, pp. 21–33, 2007.

[3] R. Budvytyte, M. Pleckaityte, A. Zvirbliene, D. J. Vanderah, and G. Valincius, "Reconstitution of cholesterol-dependent vaginolysin into tethered phospholipid bilayers: implications for bioanalysis.," *PLoS One*, vol. 8, no. 12, p. e82536, Jan. 2013.

P51

Structural and functional evidence for plasticity of Potato virus Y coat protein

Andreja Kežar[1], Luka Kavčič[1], Martin Polák[2], Jiří Nováček[2], Ion Gutiérrez-Aguirre[3], Magda Tušek Žnidarič[3], Anna Coll[3], Katja Stare[3], Kristina Gruden[3], Maja Ravnikar[3,4], David Pahovnik[1], Ema Žagar[1], Franci Merzel[1], Gregor Anderluh[1], Marjetka Podobnik[1]

[1]National Institute of Chemistry, Ljubljana, Slovenia; [2]Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [3]National Institute of Biology, Ljubljana, Slovenia. [4]University of Nova Gorica, Nova Gorica, Slovenia

marjetka.podobnik@ki.si

Potato virus Y (PVY) is among top five economically important plant pathogens. It belongs to the genus *Potyvirus* (family Potyviridae), with its 9.7 kb positive-sense single stranded RNA (ssRNA) genome coding for 11 viral proteins. PVY

is encapsidated by roughly 2000 copies of coat protein (CP), thereby forming a flexuous filamentous virion. To address the urgent need to better understand mechanisms of viral infectivity of this important pathogen, we determined the near-atomic structure of PVY's flexuous virions using cryo-electron microscopy (cryo-EM). The structure revealed a novel lumenal interplay between extended C-terminal regions of CP units and viral RNA. These RNA-protein interactions are crucial for the helical configuration and stability of the virion, as further confirmed by the unique structure of RNA-free virus-like particles (VLPs), also determined at near-atomic resolution by cryo-EM. These two structures now offer the first evidence for plasticity of the coat protein's N- and C-terminal regions. In addition, structure-based mutational analysis and *in planta* experiments were performed, which further showed the crucial roles of both terminal extensions in PVY infectivity, and explain the ability of CP to perform multiple biological tasks. These results are important contribution to understating of PVY biology, and its pathogenesis in particular. Moreover, modularity of PVY virus-like particles shown by this work suggests a new molecular scaffold for nanobiotechnological applications.

P52

Cellular responses to proteome-wide isoleucine mistranslation

Marija Pranjić[1], Marko Močibob[1], Maja Šemanjski[2], Boris Maček[2], Ita Gruić-Sovulj[1]

[1]Department of Chemistry, Faculty of Science, University of Zagreb, Croatia;
[2]Proteome Center Tübingen, Eberhard Karls Universität, Tübingen, Germany

marija.pranjic@chem.pmf.hr

Aminoacyl-tRNA synthetases (aaRS) attach amino acids onto cognate tRNAs. Aminoacylated tRNA is delivered to the ribosome where insertion of genetically encoded amino acid at the corresponding place in the proteome takes place. Some aaRSs, like isoleucyl-tRNA synthetase (IleRS), developed hydrolytic editing mechanisms to prevent mispairing of amino acid and tRNA, hence ensuring translational fidelity. Naturally occurring amino acids valine (Val) and norvaline (Nva) are equally good non-cognate substrates for IleRS and therefore represent a threat to faithful protein synthesis in *Escherichia coli*. Inactivation of the IleRS editing mechanisms makes the enzyme error-prone and leads to more frequent incorporation of Nva or Val instead of isoleucine (Ile) in the proteome (mistranslation). Intriguingly, substitution of Ile with Nva appears to be more toxic than Ile-to-Val replacement. [1] Mistranslation affects protein folding resulting in growth defects and neurogenerative diseases in mammals.

We investigated how *E. coli* tunes the apparatus for maintaining proteostasis to minimize the damage related to mistranslation. Two different types of substitutions were explored: canonical (Ile-to-Val) and noncanonical (Ile-to-Nva). Cellular responses to mistranslation were investigated by quantitative proteomics to reveal changes both in the proteome and DnaK clients isolated in pull-down assay. DnaK is the central chaperone in *E. coli* and therefore, chosen to be a model chaperone in the performed experiments. [2] To induce mistranslation in *E. coli* we constructed a strain with editing deficient IleRS and mediated its growth in the media supplemented with Nva or Val (c = 1 mM). Under such conditions, *E. coli* exhibited growth defect and displayed 10 % (Ile-to-Nva) or 16 % (Ile-to-Val) mistranslation frequencies in its proteins. Further proteome analysis revealed significant upregulation of chaperones, among them DnaK and GroES/EL – both part of two main chaperone systems in *E. coli*. However, a protease response seemed to remain unaltered. Intriguingly, mistranslation did not change the identity of DnaK clients and no noticeable difference between cellular responses to canonical and noncanonical mistranslation was observed.

Our data support the notion that proteome-wide substitution of Ile with either Nva or Val caused growth defect and provoked chaperone response that helps *E. coli* to survive at 37 °C. In search for conditions where mistranslation could be more damaging, we tested the growth of the mistranslating strain under error-prone conditions at 42 °C. The lack of growth under these conditions demonstrated that combination of heat-shock and mistranslation highly effects *E coli*. This may originate from the overload of the cell apparatus for maintaining proteostasis and is yet to be investigated.

References

[1] Bilus M, Semanjski M, Mocibob M, Cvetesic N, Zivkovic I, Tawfik DS, Toth-Petroczy A, Macek M, Gruic-Sovulj I. On the mechanism and origin of isoleucyl-tRNA synthetase editing against norvaline. J. Mol. Biol. accepted

[2] Calloni G, Chen T, Schermann SM, Chang H, Genevaux P, Agostini F, Tartaglia GG, Hayer-Hartl M, Hartl FU. DnaK Functions as a Central Hub in the E. coli Chaperone Network. Cell Rep. 2012; 1(3):251-264. doi: 10.1016/j.celrep.2011.12.007

P53

Cell Adhesion and Mechanics in Fibroblasts and Myofibroblasts

Prem Kumar, Manfred Radmacher

Institute of Biophysics, University of Bremen, Bremen, Germany,

radmacher@uni-bremen.de

Fibroblasts play a large role in wound healing, during which they differentiate temporarily in myo-fibroblasts to facilitate wound closure by generation of contractile forces. If myo-fibroblasts due to not differentiate back to the normal state, this may result in stiff scar tissue, or when the behaviour becomes pathological in the manifestation of Dupuytren's disease. We have characterized cells (normal fibroblasts, scar fibroblasts and Dupuytren's fibroblasts obtained from the same patient) in terms of mechanical properties, cell-cell adhesion and migratory properties and could show distinct differences in their properties. Elastic moduli, as determined by Atomic Force Microscopy ranged from 1-2kPa for normal and scar fibroblasts, whereas Dupuytren fibroblasts were much stiffer (5..7kPa). Migration speeds, as determined by a wound healing (scratch assay) were low for Duputren, higher for normal fibroblasts and highest for scar fibroblast.

References

[1] Babu, P. K. V., C. Rianna, G. Belge, U. Mirastschijski and M. Radmacher (2018). "Mechanical and migratory properties of normal, scar, and Dupuytren's fibroblasts." Journal of Molecular Recognition 31(9): 11.

[2] Rianna, C. and M. Radmacher (2016). Cell Mechanics as a Marker for Diseases: Biomedical Applications of AFM. Physics of Cancer: Interdisciplinary Problems and Clinical Applications. E. Y. Gutmanas, O. B. Naimark and Y. P. Sharkeev. Melville, Amer Inst Physics. 1760.

P54

An Enzyme Active Site Loop Revealed as Gatekeeper for Co-factor Flip by Targeted Molecular Dynamics Simulations and FRET-based Kinetics

Sophie Rahuel-Clermont[1], Raphaël Bchini[1], Sophie Barbe[2], Séverine Boutserin[1], Isabelle André[2], François Talfournier[1]*

[1]Université de Lorraine, CNRS, IMoPA, Nancy, France, [2]Laboratoire d'Ingénierie des Systèmes Biologiques et Procédés, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France.

Sophie.rahuel@univ-lorraine.fr

Structural motions are key events in enzyme catalysis, as exemplified by the conformational dynamics associated with the cofactor in the catalytic mechanism of hydrolytic NAD(P)-dependent aldehyde dehydrogenases. We previously showed that after the oxidoreduction step, the reduced cofactor must adopt a flipped conformation, which positions the nicotinamide in a conserved cavity that might constitute the exit door for NAD(P)H. However, the molecular basis that make this movement possible is unknown. Based on the pre- and post-flip X-ray structures, targeted molecular dynamic simulations enabled us to identify the $E^{268}LGG^{271}$ conserved loop that must shift to allow reduced nicotinamide conformational switch. To monitor cofactor movements within the active site, we used an intrinsic fluorescence resonance energy transfer signal between Trp177 and the reduced nicotinamide moiety to kinetically track the flip during the catalytic cycle of retinal dehydrogenase 2 (ALDH1A2). Decreasing loop flexibility by substituting Ala for Gly271 drastically reduced the rate constant associated with this movement that became rate-limiting. We thus propose that the $E^{268}LGG^{271}$ loop acts as a gatekeeper for cofactor flipping. Similar approaches applied to a CoA-dependent aldehyde dehydrogenase showed that cofactor flipping likely extends to the whole ALDH family, thus bridging the gap between the well-studied chemical steps and a conformational transition essential for catalysis.

Reference

[1] Rahuel-Clermont, S, Bchini, R, Barbe, S, Boutserin, S, André, I, Talfournier, F. An Enzyme Active Site Loop Revealed as Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-Based Kinetics. ACS Catal. 2019. https://doi.org/10.1021/acscatal.8b03951.

P55

Structural and Functional Impact of Missense Mutations on Glutaryl-CoA Dehydrogenase

Joana V. Ribeiro[1], Tânia G. Lucas[1], Peter Bross[2], Cláudio M. Gomes[2] and Bárbara J. Henriques[1]

[1]Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; 2Research Unit for Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

majorib18@gmail.com

Glutaric Aciduria Type I (GA-I), is an autosomal recessive neurometabolic disorder caused by mutations in the *GCDH* gene that encodes for Glutaryl-CoA Dehydrogenase [1]. This flavoprotein is involved in amino acid metabolism, more precisely in tryptophan, lysine and hydroxylysine catabolism [1]. Although the clinical features of GA-I are broadly described, studies regarding the impact of the various mutations at the molecular level are rare, and a clear relation between genotype and phenotype constitutes a gap in knowledge. Our project aims to establish a possible correlation between phenotype and genotype, as well as to clarify the mechanism behind the disorder. Here we report our studies on two disease variants, GCDH-p.Val400Met and GCDH- p.Arg227Pro [2, 3] combining biochemical and biophysical methods to discriminate the mutations effects on the GCDH protein folding, stability and function. We showed that the variants retain the overall protein fold, however the thermal stability is slightly altered when compared to wild-type GCDH. Moreover, both variants show compromised enzymatic activity, although more severe for Arg227Pro variant. The data gathered indicates that without extensive disruption of the protein fold, point mutations lead to impaired function. We expect that ongoing studies will clarify on the molecular factors underlying functional deficiency.

References

[1] Hedlund, GL, Longo, N, Pasquali, M. Glutaric Acidemia Type 1. Am J Med Genet C Semin Med Genet. 2006; 142C:86-94 doi: 10.1002/ajmg.c.30088.

[2] Busquets C, Merinero B, Christensen E, Gelpí JL, Campistol J, Pineda M, Fernández-Alvarez E, Prats JM, Sans A, Arteaga R, Martí M, Campos J, Martínez-Pardo M, Martínez-Bermejo A, Ruiz-Falcó ML, Vaquerizo J, Orozco M, Ugarte M, Coll MJ, Ribes A. Glutaryl-CoA dehydrogenase deficiency in Spain: evidence of two groups of patients, genetically, and biochemically distinct. Pediatr Res. 2000; 48(3):315-322. doi: 10.1203/00006450-200009000-00009.

[3] Treacy EP, Lee-Chong A, Roche G, Lynch B, Ryan S, Goodman S. Profound neurological presentation resulting from homozygosity for a mild glutaryl-CoA dehydrogenase mutation with a minimal biochemical phenotype. J Imherit Metab Dis. 2003; 26(1):72-74. doi: 10.1023/A:1024087832406.

P56

Sensing L-arginine in Pseudomonas aeruginosa: a novel link between c-di-GMP and central metabolism?

Giorgio Giardina[1], Alessandro Paiardini[1], Federico Mantoni[1], Paolo Brunotti[1], Laura Cervoni[1], Livia Leoni[2], Gordano Rampioni[2], Alessandro Arcovito[3], Francesca Cutruzzolà[1], Serena Rinaldo[1]

[1]Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Department of Biochemical Sciences Sapienza University of Rome (Italy); [2]Dept. of Science, University Roma Tre (Italy); 3 Istituto di Biochimica e Biochimica Clinica Università Cattolica del Sacro Cuore, Rome (Italy).

serena.rinaldo@uniroma1.it

Cyclic-di-GMP (c-di-GMP) is one of the master regulators in bacteria controlling biofilm formation and persistence in response to environmental and metabolic stimuli. Intracellular levels of c-di-GMP are controlled by the opposite activity of specific diguanylate cyclases and phosphodiesterases (encoded by the GGDEF and the EAL domains, respectively), whose catalytic activities are often allosterically tuned by environmental cues, protein partners and/or metabolic status (1). The variety of phenotypes associated to c-di-GMP modulation likely involves a re-programming of Nitrogen/Carbon metabolism, by mechanisms far to be fully elucidated (2).

C-di-GMP is able to interact with a large variety of macromolecules *via* deeply different binding modes, which lead to multiple allosteric control mechanisms, most of which yet to be characterized in detail biochemically (1).

Here we present mechanistic data on a multidomain protein, i.e. RmcA/PA0575 from *Pseudomonas aeruginosa*. RmcA is a complex protein harbouring a tandem GGDEF-EAL domain downstream to multiple PAS domains, transmembrane helices and a periplasmic sensory domain. We demonstrate that this protein is a one-component antenna able to connect the environmental sensing to the intracellular control of c-di-GMP levels. More in detail, the periplasmic domain perceives L-arginine (a possible carbon/nitrogen and energy source) and the metabolic status (via nucleotide sensing) to finally control the catalytic moiety able to degrade c-di-GMP into the linear pGpG counterpart (leading to biofilm dispersion) (3, 4). Given that in *P. aeruginosa* L-arginine is at the crossroad of many metabolic routes, including alternative energy supply under anaerobic conditions, our data contribute to link re-programming of central metabolism to c-di-GMP signalling and biochemistry.

References

[1] Krasteva, P.V. and Sondermann H. Versatile modes of cellular regulation via cyclic dinucleotides. Nature chemical biology. 2017; 13: 350-359. doi: 10.1038/nchembio.2337.

[2] Rinaldo S., Giardina G., Mantoni F., Paone A,. Cutruzzolà F. Beyond nitrogen metabolism: nitric oxide, cyclic-di-GMP and bacterial biofilms. FEMS Microbiol Lett.

2018; 365: fny029. doi: 10.1093/femsle/fny029.

[3] Mantoni F, Paiardini A, Brunotti P, D'Angelo C, Cervoni L, Paone A, Cappellacci L, Petrelli R, Ricciutelli M, Leoni L, Rampioni G, Arcovito A, Rinaldo S, Cutruzzolà F, Giardina G. Insights into the GTP-dependent allostericcontrol of c-di-GMP hydrolysis from the crystal structure of PA0575 protein from Pseudomonas aeruginosa. FEBS J. 2018; 285:3815-3834. doi:10.1111/febs.14634.

[4] Paiardini A, Mantoni F, Giardina G, Paone A, Janson G, Leoni L, Rampioni G, Cutruzzolà F, Rinaldo S. A novel bacterial l-arginine sensor controlling c-di-GMP levels in Pseudomonas aeruginosa. Proteins. 2018; 86:1088-1096. doi:10.1002/prot.25587.

P57

Bead Injection Multiplex Immunoanalysis

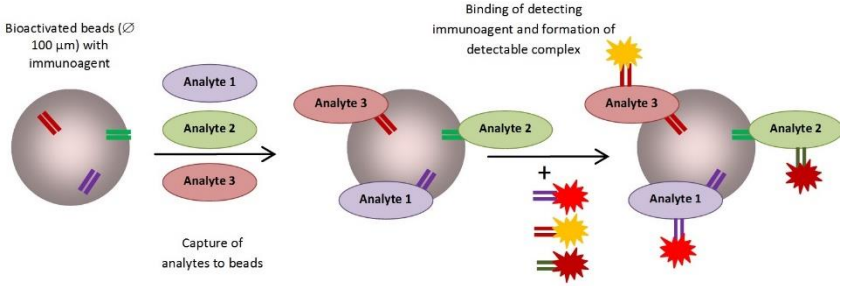
Toonika Rinken

University of Tartu, Tartu, Estonia

toonika.rinken@ut.ee

The key problems of analytical activities include the issues of selectivity and sensitivity. Considering selectivity, one of the top analytical methods allowing specific multiplexed detection of analytes in their natural environment without preliminary treatment of samples is immunoanalysis. However, the performance of immunoassays in natural samples and matrixes is often limited with the sensitivity, as the concentrations of compounds of interest are usually extremely low and it is complicated to collect the signal of antigen/immunoagent interaction. To solve this problem, we have developed a combined method of immunoanalysis and bead injection technique. The principle of the resulting multiplex bead injection immunoanalysis is based on the use of bioactivated beads, which carry one or more immobilized immuno-recognition compounds and form a single-used microcolumn in a flow of a carrier solution. Analytes from the sample flow are selectively bound to the column and the bound analyte is detected with specific immunoreagents, each labelled with a different fluorescence marker. Both antibodies and aptamers can be used as immunoreagents.

We have applied this system for the detection of different analytes in diverse sample matrixes, e.g. pathogens in natural waters, raw milk [1] and urine of patients suffering from cystitis. The analyses can be automatically carried out in on-line mode. The sample volume is determined by analyte concentration and can be varied according to the required limit of detection (LOD) - the bigger the sample, the lower LOD value can be achieved. The analysis time, depending on sample volume and incubation time required for antigen/immunoagent interaction is typically within 5 - 20 min. A common sample volume is <100 µl.



The principle of multiplex bead injection immunoanalysis.

Reference

[1] Juronen D, Kuusk A, Kivirand K, Rinken A, Rinken T Immunosensing system for rapid multiplex detection of mastitis-causing pathogens in milk. Talanta 2018; 178:949-954. doi.org/10.1016/j.talanta.2017.10.043

P58

Plant seryl-tRNA synthetase as a link between translation and metabolism of brassinosteroid hormones

Mario Kekez[1], Vladimir Zanki[1], Ivana Kekez[2], Dubravka Matković-Čalogović[2], Jasmina Rokov-Plavec[1]

[1]Division of Biochemistry, Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia; [2]Division of General and Inorganic Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia.

rokov@chem.pmf.hr

Aminoacyl-tRNA synthetases (aaRSs) are essential cellular enzymes that covalently link specific amino acid to the cognate tRNA, thereby acting as translators of the genetic code. Many aaRSs are involved in diverse cellular processes beyond translation, acting alone or in complex with other proteins. However, studies of aaRS assemblies and noncanonical functions in plants are scarce, as are structural studies of plant aaRSs. We have solved the crystal structure of *Arabidopsis thaliana* cytosolic seryl-tRNA synthetase (SerRS),

which is the first crystallographic structure of a plant aaRS [1]. *Arabidopsis* SerRS displays structural features typical of canonical SerRSs, except for a unique intrasubunit disulfide bridge. Interestingly, cysteines involved in disulfide link are conserved in all SerRSs from green plants, indicating their plant-specific functional importance. In order to identify protein interactors of *Arabidopsis* SerRS, we performed yeast two hybrid screen and identified BEN1, protein involved in metabolism of steroidal plant hormones brassinosteroids that regulate a variety of physiological processes crucial for normal plant growth and development. The SerRS:BEN1 interaction was confirmed using surface plasmon resonance and microscale thermophoresis (MST). To pinpoint regions responsible for interaction, truncated variants of SerRS and BEN1 were created and analyzed using MST. Detailed analysis showed that interaction interface involves SerRS globular catalytic domain and the acidic N-terminal extension of BEN1 protein. BEN1 does not have a strong impact on SerRS aminoacylation activity, indicating that the primary function of the complex is not the modification of SerRS canonical activity. Perhaps SerRS performs as yet unknown noncanonical functions mediated by BEN1. The SerRS:BEN1 complex is a rare example of an aaRSs interacting with an enzyme involved in primary or secondary metabolism. The partnership between SerRS and BEN1 indicates a link between protein translation and steroid metabolic pathways of the plant cell [1].

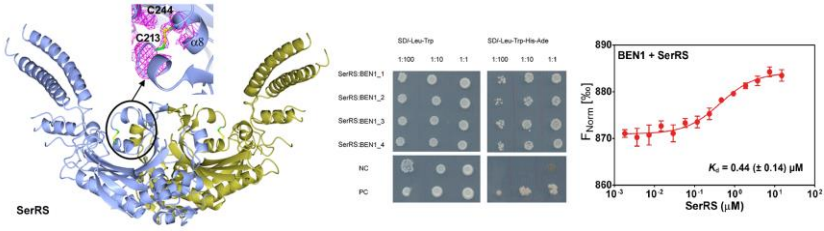


Figure – Left panel: crystal structure of Arabidopsis thaliana SerRS, inset: a detailed view of disulfide link; Middle panel: BEN1 interacts with SerRS in the Y2H assay; Right panel: MST analysis of SerRS:BEN1interaction.

Reference

[1] Kekez M, Zanki V, Kekez I, Baranasic J, Hodnik V, Duchêne A-M, Anderluh G, Gruic-Sovulj I, Matković-Čalogović D, Weygand-Durasevic I, Rokov-Plavec J. Arabidopsis seryl-tRNA synthetase: the first crystal structure and novel protein interactor of plant aminoacyl-tRNA synthetase. FEBS J. doi: 10.1111/febs.14735.

P59

Brain S100A9 forms string-like polymeric assemblies with potential relevance in Alzheimer's Disease

Mariana A. Romão[1,2], Joana S. Cristóvão[1], Gonçalo R. Nogueira[1,2], Isabel Cardoso[3], Maria C. Moraes[4], Bruno Costa-Silva[4], Ana P. Carapeto4, Carlos Marcuello[1], Mário S. Rodrigues[1], Cláudio M. Gomes[1]

[1]Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, [2]BioSYS PhD Program in Biological Systems, Functional & Integrative Genomics; [3]Modulation in Neurodegenerative Disorders (MiND group), Instituto de Biologia Molecular e Celular, Porto, Portugal; [4]Champalimaud Research, Champalimaud Centre for the Unknown, Lisbon.

maromao@fc.ul.pt

Protein aggregation and activation of inflammatory pathways are common features of age-related neurodegenerative disorders. Conversion of misfolded normally soluble proteins into toxic amyloid aggregates is accompanied by upregulation of pro- inflammatory cytokines such as S100 proteins. S100s are small (10-12kDa) dimeric calcium-binding proteins with a dynamic cellular range of intra and extra cellular concentrations (nM to μM) and oligomeric states (2-8mer and higher) which act via regulatory protein : protein interactions, intracellular Ca^{2+} sensing and signalling processes relevant in inflammation, proliferation and differentiation, cancer and neurodegeneration.

S100 proteins are consistently deregulated in pathophysiological conditions, including Alzheimer's Disease (AD). In particular, S100A9 is highly abundant in AD brains[1] and has been shown to be a relevant AD biomarker[2], being upregulated in Tg2576 AD in connection with memory impairment[3]. S100A9 interacts with amyloid-beta playing role in its aggregation process[4].

We gathered evidence that S100A9 forms very stable string-like polymeric structures *in vitro* under physiological conditions. We hypothesize that these polymers, composed by assembly units which can reversibly dissociate, may be a form of protein stabilisation to maximize its extracellular lifetime in the synaptic environment. Our data shows that formation of the S100A9 polymers is a dynamic process which nevertheless does not involve major conformational changes in the S100A9 α-helical fold. The relevance of these S100A9 assemblies in the context of AD will be discussed.

References

[1] Wang, C., et al., Acta Neuropathol, 2014. 127(4): p. 507-22.
[2] Horvath, I., et al., ACS Chem Neurosci, 2016. 7(1): p. 34-9.
[3] Chang, K.A., H.J. Kim, and Y.H. Suh, Neurodegener Dis, 2012. 10(1-4): p. 27-9. [4] Zhang, C., et al. PLoS One, 2012. 7(3): p. e32953

P60

Comparison of graphene oxide and carbon quantum dots as biocompatible supports for adenylate kinase immobilization

Katarzyna Roszek[1], Anna Hetmann[1], Joanna Czarnecka[1], Magdalena Wujak[1], Paulina Bolibok[2], Marek Wiśniewski[2]

[1]Department of Biochemistry, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Torun, Poland; [2]Physicochemistry of Carbon Materials Group, Faculty of Chemistry, Nicolaus Copernicus University, Torun, Poland.

kroszek@umk.pl

Adenylate kinase (AK, EC 2.7.4.3) is a phosphotransferase that, *via* the reversible phosphoryl transfer among adenine nucleotides (AMP, ADP and ATP), can directly control their concentration and participate in energetic metabolism and nucleotide homeostasis. Soluble AK within the cell functions as a regulator of the metabolic sensors activity, whereas in the extracellular space it is a part of purinergic signaling system [1].

The main difficulty in extensive *in vitro* application of enzymes is their sensitivity to environmental conditions and instability. To overcome the limitations, enzymes have usually been immobilized on a solid support [2, 3]. Nanomaterials in general can serve as excellent supporting materials for enzyme immobilization. We have selected two different carbonaceous nanomaterials that fulfil the criterion of non-toxicity for living cells: graphene oxide (GO) and carbon quantum dots (CQD). We have immobilized the recombinant adenylate kinase through adsorption, that turned out to be an effective method for improving the kinetic properties of AK. We determined the kinetic constants (Km, Vmax) of immobilized AK and tested the obtained nanobiocatalytic systems activity in mesenchymal stem cells (MSCs) and neuroblastoma cells (SH-SY5Y) cultured *in vitro*.

The improved kinetic properties of immobilized AK together with substantial increase in MSCs and SH-SY5Y cells viability, especially when exposed to milimolar, potentially cytotoxic ATP concentrations, confirmed the *in vitro* activity of obtained biocatalytic systems. We suppose that these systems efficiently normalize nucleotide concentration disturbances in the extracellular environment The removal of excess ATP, but maintaining low ATP concentrations and restoring the nucleotide balance by studied nanobiocatalysts is particularly promising for their prospective applications in biological systems.

Acknowledgments: this work was supported by the Polish National Science Centre (NCN) grant PRELUDIUM 14 no. 2017/27/N/ST5/02696.

References

[1] Dzeja P, Terzic A. Adenylate kinase and AMP signaling networks: metabolic monitoring, signal communication and body energy sensing. Int J Mol Sci. 2009; 10:1729–1772.

[2] Datta S, Christena LR, Rajaram YRS. Enzyme immobilization: an overview on techniques and support materials. Biotech 2013; 3:1–9.
[3] Pavlidis IV, Patila M, Bornscheuer UT, Gournis D, Stamatis H. Graphene-based nanobiocatalytic systems: recent advances and future prospects. Trends Biotechnol. 2014; 32:312–320.

P61

Oligomerization of NKp30, an activation receptor of human lymphocytes, is dependent on its N-glycosylation

Ondřej Skořepa[1], Jan Bláha[1], Barbora Kalousková[1], Tereza Skálová[2], Samuel Pažický[1], Jan Dohnálek[2], Michal Rosůlek[2], Petr Pompach[2], Alexander Fish[3], Ondřej Vaněk[1]

[1]Department of Biochemistry, Faculty of Science, Charles University, Hlavova 8, 12840 Prague, Czech Republic, [2]Institute of Biotechnology, The Czech Academy of Sciences, v.v.i., Průmyslová 595, 25250 Vestec, Czech Republic, [3]Division of Biochemistry and Center for Biomedical Genetics, The Netherland Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherland.

skorepao@natur.cuni.com

Immune system is able to recognize tumour cells and subsequently eliminate them. Natural killer (NK) cells, a special sort of lymphocytes, provide this function by inducing apoptosis in tumour cells using Fas ligand binding or granzymes. These processes are activated when signals from their inhibitory receptors are decreased and on the other hand, signals from activating receptors are increased. NKp30 is an activating NK cell receptor with one Ig-like extracellular domain. B7-H6 is a membrane protein with two Ig like domains which is present on the surface of some tumour cells. Moreover, BAG6 and galectin 3 were also reported to be NKp30 ligands [1].

Although the NKp30/B7-H6 complex structure has been solved, structural basis of some important features of their binding is not explained yet. Soluble form of NKp30 receptor binding domain creates oligomers, presence of which is dependent on C-terminal stalk domain length and its N-glycosylation; however, structural insight into formation of the oligomers and their significance is not known. Furthermore, binding affinity of NKp30 to its ligands is dependent on presence of its glycosylation and glycosylation type [2,3]. In our laboratory, we found out that the formation of NKp30 oligomers depends on its N glycosylation. NKp30 produced in HEK293S cell line with knocked-out N acetylglucosaminyltransferase I formed oligomers but appeared only as monomer after enzymatic deglycosylation. However, the structural basis of NKp30 oligomerization and basic questions of how stalk domain and

glycosylation impact NKp30 affinity as well as physiological importance of these effects on NK cells remain to be elucidated.

For our studies the extracellular domains of NKp30 and B7-H6 have been cloned into a mammalian expression vector with C-terminal histidine tag. To study the effect of C-terminal region of extracellular domain of NKp30, shorter and longer constructs have been cloned. Both proteins have been produced in human HEK293S GnTI- cell line possessing homogeneous N glycosylation profile, purified by affinity and size exclusion chromatography. Glycosylation of NKp30 was confirmed by mass spectrometry and formation of its oligomers was observed by analytical ultracentrifugation and size exclusion chromatography with multi angle light scattering detection. Impact of glycosylation and C-terminal length of NKp30 construct on B7-H6 binding was measured using analytical ultracentrifugation, surface plasmon resonance and isothermal titration calorimetry. Crystallization screens of the complex with glycosylated NKp30 constructs have been set up, too.

References

1] Vivier E. et al: Immunol Cell Biol, 92 (3), 221-9, 2014
[2] Hartmann J. et al: J Biol Chem. 287(37): 527–39, 2012
[3] Herrmann J. et al: J Biol Chem, 289 (2), 765-77, 2014

Acknowledgment: This study was supported by BIOCEV (ERDF CZ.1.05/1.1.00/02.0109), Czech Science Foundation (18-10687S), MEYS of the Czech Republic (LTC17065 within the COST Action CA15126), and Charles University (GAUK 927916). The authors also acknowledge the support and the use of resources of Instruct, a Landmark ESFRI project through the R&D pilot scheme APPID 56 and 286.

P62

Long, highly-stable and flexible: what analytical ultracentrifugation can tell us about "random walk" oligomer.

Alexandra S Solovyova[1,2], Daniel T Peters[1], Gema Dura[3], Helen Waller[1], Jeremy H Lakey[1] and David A Fulton[3]

[1]Institute for Cell and Molecular BioSciences, Newcastle University Newcastle–upon-Tyne, UK, [2]Newcastle University Protein and Proteome Analysis, Newcastle–upon-Tyne, UK [3]Chemical Nanoscience Laboratory, Department of Chemistry, University of Newcastle, Newcastle–upon-Tyne, UK

alexandra.solovyova@ncl.ac.uk

Analytical ultracentrifugation (AUC) is a fundamental method employed for almost a century to characterise oligomeric biomacromolecules [1]. During the past two decades, the method was also successfully used for the characterisation of extremely flexible biological molecules such as disordered proteins [2, 3]. In this present work, we use analytical centrifugation to characterise the hydrodynamic behaviour of long polymeric chains formed by capsular antigen factor 1 (Caf1), a virulence factor from the plague bacterium, *Yersinia pestis*.Caf1 is a 15 kDa protein subunit which is assembled by the chaperone-usher pathway into flexible, non-covalent, extracellular polymers that can reach up to 1.5 μm in length, and MDa in size [4]. The resulting polymer is a super-stable structure, resistant to high temperatures, detergents, etc [5]. Wild type Caf1 polymers are biologically inert. However, introducing cell-adhesion motifs converts Caf1 into a cell scaffold that has great potential for use in 3D tissue culture techniques [6]. AUC would be an obvious choice of method to characterise oligomeric forms of Caf1 in solution, however the high flexibility and heterogeneous lengths of oligomeric chains of the wild type Caf1 appeared to be a potential pitfalls for hydrodynamic study. An attempt was made to combine the wild-type Caf1 size-and-shape distributions [7] obtained experimentally and calculated hydrodynamic parameters derived from macromolecular trajectory generated by Brownian dynamics technique [8, 9] in order to characterise solution behaviour of Caf1. Recently, we have observed that thermally denatured Caf1 polymers are able to refold and form oligomeric chains of a smaller size than the wild type protein. Developing a hydrodynamic model of oligomer formation through the refolding of thermally denatured Caf1 subunits allows the characterisation of this system, and aids in the engineering of this protein for multiple basic research and biomedical applications.

References

1. Svedberg, T., Nichols, J.B., The application of the oil turbine type of ultracentrifuge to the study of the stability region of carbon monoxide-hemoglobin. Journal of the American Chemical Society, 1927. 49: p. 14.
2. Scott, D.J. and D.J. Winzor, Characterization of Intrinsically Disordered Proteins by Analytical Ultracentrifugation. Methods Enzymol, 2015. 562: p. 225-39.

3. Salvay, A.G., G. Communie, and C. Ebel, Sedimentation velocity analytical ultracentrifugation for intrinsically disordered proteins. Methods Mol Biol, 2012. 896: p. 91-105.
4. Soliakov, A., et al., The structure of Yersinia pestis Caf1 polymer in free and adjuvant bound states. Vaccine, 2010. 28(35): p. 5746-54.
5. Ulusu, Y., et al., Thermal stability and rheological properties of the 'non-stick' Caf1 biomaterial. Biomed Mater, 2017. 12(5): p. 051001.
6. Roque, A.I., et al., Reversible non-stick behaviour of a bacterial protein polymer provides a tuneable molecular mimic for cell and tissue engineering. Adv Mater, 2014. 26(17): p. 2704-9, 2616.
7. Brown, P.H. and P. Schuck, Macromolecular size-and-shape distributions by sedimentation velocity analytical ultracentrifugation. Biophys.J., 2006. 90 (12): p. 4651-4661.
8. de la Torre, J.G., et al., SIMUFLEX: Algorithms and Tools for Simulation of the Conformation and Dynamics of Flexible Molecules and Nanoparticles in Dilute Solution. J Chem Theory Comput, 2009. 5(10): p. 2606-18.
9. Garcia de la Torre, J., et al., Methods and tools for the prediction of hydrodynamic coefficients and other solution properties of flexible macromolecules in solution. A tutorial minireview. Macromol Biosci, 2010. 10(7): p. 721-30.

P63

How oxidized lipids control raft-like domain size in biomimetic membranes

Vesela Yordanova[1], Rusina Hazarosova[1], Victoria Vitkova[2] and Galya Staneva[1]

[1]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, Sofia 1113, Bulgaria; [2]Institute of Solid State Physics, Bulgarian Academy of Sciences, 72 Tsarigradsko Chaussee, 1784 Sofia, Bulgaria

gstaneva@obzor.bio21.bas.bg

Living cells are always exposed to the attack of radicals and therefore they have evolutionarily developed complex systems to cope with them. Brain and nervous tissue are very susceptible to oxidation due to their high lipid content and intense consumption of oxygen. Multiple sclerosis (MS) is a disabling neurodegenerative disease characterized by the presence of demyelinated plaques and axonal degeneration. Since lipids comprise >70% of the myelin sheath, they have been considered capable of inducing autoimmune reactions in MS. Oxidized lipid, palmitoyl-oxo-valeroyl phosphatididylcholine (POVPC) was found to be present in high amounts in brain tissue of MS patients but almost absent in control samples. The *sn-2* acyl chains of oxPLs are shorter in length than the parent non-oxidized phospholipids and bear polar functional group(s) at the end. It is believed that the conformational change in these lipids leads to an alter in several key physicochemical properties of the lipid bilayers: lipid-

lipid interactions, polarity profile, chain order, as well as thermal phase behavior and lateral organization. By using different biophysical methods giving information in different scales, from molecular to micron-scales, we tested the capacity of POVPC to change the membrane properties in biomimetic systems. This molecule, characterized by its truncated molecular shape, alters lipid packing as well as the partition of other lipids into the coexisting liquid-ordered (Lo)/liquid-disordered (Ld) phases mimicking the formation of plasma membrane specialized membrane domains of type "rafts". Lipid domain pattern, the domain size and dynamics are also influenced in the presence of POVPC. The determination of the miscibility temperature of Lo/Ld phases allowed us to estimate how POVPC modulates the line tension of the lipid liquid domains as a function of the surrounding unsaturated PC matrix. This is an essential property of membrane domains because it determines their size and capacity to grow by fusion, as well as their ability to accommodate proteins and other macromolecules. The effect of POVPC on the raft formation and dynamics could be important in early signaling disorders in MS.

Acknowledgment: Financial support from the National Science Fund - Bulgaria (Grants DN 18/15 and DHTC/France 01/4), is gratefully acknowledged.

P64

Analysis of Protein-DNA Interactions by SPR - Simple and cheap quantitation using the ReDCaT Chip

Clare E. M. Stevenson and David M. Lawson

Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

clare.stevenson@jic.ac.uk

The recognition of specific DNA sequences by proteins is crucial to fundamental biological processes such as DNA replication, transcription and gene regulation. The technique of Surface Plasmon Resonance (SPR) is ideally suited for the measurement of these interactions because it is quantitative, simple to implement, reproducible, can be automated and requires very little sample. This typically involves the direct capture of biotinylated DNA to a streptavidin (SA) chip before flowing over the protein of interest and monitoring the interaction. However, once the DNA has been immobilised on the chip it cannot be removed without damaging the chip surface. Moreover, if the protein-DNA interaction is strong, then it may not be possible to remove the protein from the DNA without damaging the chip surface. Given that the chips are costly, this will limit the number of samples that can be tested. Therefore, we have developed a Reusable DNA Capture Technology, or ReDCaT Chip, that enables a single SA chip to be

used multiple times. Results will be presented for two projects where DNA binding sites were identified, footprinted and the binding affinities were quantified using SPR and the ReDCaT chip. This technique, in principle, can be used to study any protein-nucleic acid interaction.

P65

The Protein Facility of Elettra: a platform to support research and development

Barbara Giabbai, Marta S. Semrau, and Paola Storici

Protein Facility, Structural Biology Laboratory, Elettra - Sincrotrone Trieste S.C.p.A., Trieste, Italy.

paola.storici@elettra.eu

A Protein Facility has been recently implemented at the Elettra synchrotron of Trieste taking advantage of the infrastructures and of the expertise existing in the structural biology laboratory of Elettra. The protein production platform is organized for expression in *E.coli*, insect and mammalian cell systems, and is well equipped to go from small-scale constructs screening to mid/large-scale preparations, with strong capacity and competence in protein purification techniques. On average we support about 30 different protein projects a year coming from internal and external users of academic or industrial R&D groups. In particular the lab intent is to facilitate the process to obtain recombinant proteins suitable for analysis with synchrotron radiation techniques (x-ray diffraction, SAXS, FTIR). The lab is also opened to less experienced researchers such as young postdocs and PhD students offering tutoring and knowledge transfer. The active projects are distinguished between collaborative research, industrial services and tutoring activities.

We have a more profound expertise on studying protein targets for drug discovery with specific interests for two big proteins families of druggable targets: the human kinases and the deubiquitinases that are relevant in cancer and neurodegenerative diseases. In addition, our pipeline encompasses between different types of proteins such as membrane proteins, proteases as well as nanobodies. The Facility is an active member of the P4EU Network (Protein Production and Purification Partnership in Europe; www.p4eu.org) contributing also in benchmarking studies[1] that allow technology advancements and maintenance of the state-of-the-art in the protein production field. Moreover the Facility works in close collaboration with Elettra synchrotron beamlines providing samples for structural and biophysical characterization and is working to organize a more robust protein characterization and QC path.

The present poster is intended to introduce the protein production facility of Elettra Sincrotrone Trieste to the ARBRE MOBIEU scientific community, showing the major achievements and future developments of the facility in the field, drawing from a panel of the most relevant results.

References

[1] Stolt-Bergner P, Benda C, Bergbrede T, Besir H, Celie PHN, Chang C, Drechsel D, Fischer A, Geerlof A, Giabbai B, van den Heuvel J, Huber G, Knecht W, Lehner A, Lemaitre R, Nordén K, Pardee G, Racke I, Remans K, Sander A, Scholz J, Stadnik M, Storici P, Weinbruch D, Zaror I, Lua LHL, Suppmann S. Baculovirus-driven protein expression in insect cells: A benchmarking study. J Struct Biol. 2018 Aug;203(2):71-80. doi: 10.1016/j.jsb.2018.03.004. Epub 2018 Mar 12.

P66

Mass spectrometry approach for analysing protein phosphorylation in antibiotic producing bacteria

Ela Šarić[1], Gerre Quinn[1], Maja Šemanjski[2], Tina Paradžik[1], Mladenka Jurin[1], Iain Hunter[3], Paul Herron[3], Boris Maček[2], Dušica Vujaklija[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia, [2]Proteome Center Tuebingen, University of Tuebingen, Tuebingen, Germany, [3]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK

Streptomyces is the largest genus of Actinobacteria, bacteria that exibit complex and tightly regulated life cycle. These bacteria are best known for their ability to produce many bioactive metabolites of medical and biotechnological importance. Approximately 75% of commercially and medically useful antibiotics are produced by different *Streptomyces* species. Antibiotic production generally coincides with the onset of morphological differentiation. *Streptomyces* often exibit growth arrest in the early exponential phase which is important for antibiotic production. During the course of this study, growth arrest was observed in different *S. rimosus* strains. This species is among the best-characterised industrial *Streptomyces* known to produce oxytetracycline (OTC).

Phosphorylation plays critical roles in the regulation of many cellular processes. The aim of this work was to uncover possible regulatory role of protein phosphorylation in OTC production. Analysis of the *S. rimosus* genome has predicted 33 eukaryotic-like protein kinases and 27 eukaryotic type protein phosphatases. Western blot confirmed activity of Ser/Thr/Tyr kinases in cell free extracts collected throughout the growth of six *S. rimosus* strains. Selected strains produced significantly different levels of OTC. For this study we selected

the best OTC producer, strain 23383, and the wild type, strain G7. Taking into account the importance of protein phosphorylation in the regulation of antibiotic production in other bacteria, we had two main objectives. First was to examine the differences in phosphoproteome of OTC producing bacteria at different stages of growth. The second was to find the differences between phosphoproteome of two strains that produce significantly different amounts of antibiotic. The results of comparative proteome/phosphoproteome analysis pointed out several proteins which may be important for the OTC production and responsible for the bacterial resistance to elevated concentration of the antibiotic in the producing media.

P67

Human DPP III - Keap1 interactions

Marko Tomin, Sanja Tomić

Ruđer Bošković Institute, Zagreb, Croatia

marko.tomin@irb.hr

Kelch-like ECH associated protein 1 (Keap1) is a cellular sensor for oxidative stress and a negative regulator of the nuclear erythroid 2–related factor 2 (Nrf2). A cytosollic metallopeptidase dipeptidyl peptidase III (DPP III) has been shown to interact with the Kelch domain of Keap1 [1] via the ETGE motif located in a flexible loop belonging to the upper domain [2]. Using the previously developed models of the DPP III - Keap1 complex [3], we are trying to identify the conformation of the ETGE-containing loop in the complex, as well as the work required to achieve the active conformation for Keap1 binding.

Although the DPP III - Keap1 interaction through the conserved ETGE motif has been experimentally confirmed [4], the extensive MD simulations of the human DPP [2,5] suggest that the loop is attached to the upper domain of DPP III at all times. In order to quantify the thermodynamic barrier and the work required for the loop translocation , as well as the subsequent complex formation we have used steered MD simulations, adaptive steered MD simulations and conventional MD simulations in conjunction with the MM-PBSA energy calculations.

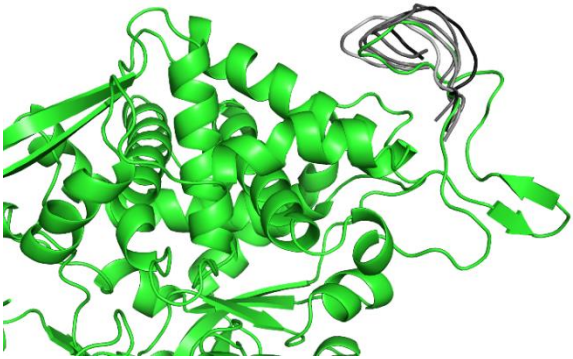


Figure 1: Translocation of the ETGE motif containing loop.

References

[1] Liu Y, Kern JT, Walker JR, Johnson JA, Schultz PG, Luesch H. A genomic screen for activators of the antioxidant response element. PNAS. 2006; 104:5205-5210. doi: 10.1073/pnas.0700898104

[2] Tomić A, González M, Tomić S. The large scale conformational change of the human DPP III-substrate prefers the "closed" form. J Chem Inf Model. 2012; 52:1583-1594. doi: 10.1021/ci300141k

[3] Gundić M, Tomić A, Wade RC, Matovina M, Karačić Z, Kazazić S, Tomić S. Human DPP III – Keap1 Interactions: a Combined Experimental and Computational Study. Croat. Chem. Acta. 2016; 89:217-228. doi: 10.5562/cca2916

[4] Lu K, Alcivar AL, Ma J, Foo TK, Zywea S, Mahdi A. NRF2 induction supporting breast cancer cell survival is enabled by oxidative stress-induced DPP3-KEAP1 interaction. Cancer Res. 2017;77(11):2881-92.

[5] Tomić A, Berynskyy M, Wade RC, Tomić S. Molecular simulations reveal that the long range fluctuations of human DPP III change upon ligand binding. Mol. biosys. 2015; 11:3068-3080. doi: 10.1039/c5mb00465a

---

P68

Denaturation signatures of plasma and cancer diagnostics.

Tsvetkov PO[1], Tabouret E[1], Malezinski S[1], Figarella-Branger D[1], Chinot O[1], Pereira C[2], Peel T[2], Eyraud R[3], Devred F[1].

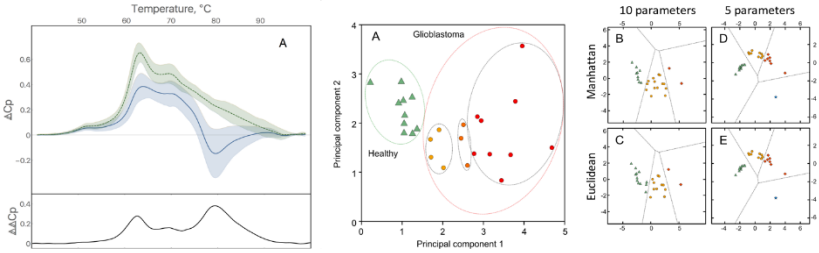
[1]Aix-Marseille Université, CNRS, INP, Marseille, France; [2] EuraNova, Marseille, France; 3 Aix-Marseille Université, LIS, Marseille, France

philipp.tsvetkov@univ-amu.fr

For more than 10 years, several pilot studies have hypothesized that Differential Scanning Calorimetry (DSC) could be used as a potential diagnostic tool for a number of diseases by providing disease-specific calorimetric denaturation

profiles of blood plasma (1). Recently we have shown that this approach can be also used in Glioblastoma, the most frequent and aggressive primary brain tumor in adults (**2**). Comparing the DSC denaturation profiles of plasma samples from patients with glioblastoma with the ones from healthy individuals revealed the existence of a glioblastoma signature, pointing to a potential easy-to-use non-invasive monitoring tool for glioblastoma patients.

Unfortunately, there are several obstacles inherent to the DSC itself that make this approach non-transferable to the clinics. To circumvent these limitations, we proposed to use differential scanning fluorimetry (DSF) rather than DSC to obtain denaturation profiles of plasma samples. Using nanoDSF Prometheus NT instrument, we were able to obtain denaturation profiles of human plasma similar to the ones registered by DSC. The ability of this instrument to run up to 48 disposable capillaries with only 10 µL of undiluted plasma samples makes it not only more suitable for the large-scale experiments necessary for statistically robust results but will also facilitate its transfer to clinic. In order to develop a real diagnostic tool based on nanoDSF, it is necessary to perform the complex analysis of large amount of multiparametric data extracted from these profiles. In collaboration with academic and private scientists (LIS, EuraNova) specialized in Artificial Intelligence solutions we are developing a classification method of the plasma denaturation profiles based on deep learning algorithms to make this profile analysis automatic and applicable to a wider range of pathologies.



References

[1] Garbett NC, Mekmaysy CS, DeLeeuw L, Chaires JB. Clinical application of plasma thermograms. Utility, practical approaches and considerations. Methods. 2015; 76:41–50. doi: 10.1016/j.ymeth.2014.10.030

[2] Tsvetkov PO, Tabouret E, Roman AY, Romain S, Bequet C, Ishimbaeva O, Honoré S, Figarella-Branger D, Chinot O, Devred F. Differential scanning calorimetry of plasma in glioblastoma: toward a new prognostic / monitoring tool. Oncotarget. 2018; 9(10):9391-9399. doi: 10.18632/oncotarget.24317

P69

Controlling enzymatic activity by immobilization

Paulina Bolibok[1], Katarzyna Roszek[2], Marek Wiśniewski[1]

[1]Faculty of Chemistry, Physicochemistry of Carbon Materials Research Group, Nicolaus Copernicus University in Toruń, Gagarin St. 7, 87-100 Toruń, Poland; [2]Department of Biochemistry, Faculty of Biology and Environment Protection, Nicolaus Copernicus University in Toruń, Lwowska 1, 87 – 100 Toruń, Poland

Marek.Wisniewski@umk.pl

During the last decades biocatalysis has become an important technological process making the synthesis of drugs, food, vitamins, and many other chemical compounds more environmentally friendly, economically profitable, and more sustainable than conventional methods. However, independently of many advantages of enzymes, their use on a large industrial scale is restricted mainly due to: (i) enzyme instability in the working conditions or during storage; and/or (ii) high costs of enzymatic processes, that are connected with the inability to efficiently separate catalyst from reaction product. The above disadvantages can be overcome by enzyme immobilization.

Referring to such properties as: the easy-to-control shape and size, low price when compared to majority of other materials, as well as relatively high biocompatibility, the materials most often used in biocatalysis are carbon nanomaterials, among which, the most promising material seems to be graphene oxide (GO). The material proved to be a perfect support for immobilized proteins and enzymes due to its solubility in water and large surface area with oxygen functionalities. Therefore, the immobilization of proteins does not demand prior modifications of the surface.

Obtained results provide important, new insights into the changes of kinetic parameters effected from the structural alterations of enzymes (catalase, β-galactosidase, AK) immobilized on GO.

Our findings reveal e.g. that due to catalase adsorption on GO, the percentage of α-helical and β-sheet structures decreases considerably. The relaxation of secondary structure is followed by the increase in the β-turn regions content. The latter leads to the increase in the catalytic activity. The enzymatic reaction kinetic constants are related to enzyme:matrix ratio. For low ratios, one can obtain a very active enzyme with a lower affinity. Oppositely, for high ratios, the biocatalytic system with activity similar to the native enzyme but with much higher substrate affinity is achieved. It allows for the controlled changing the enzymatic reaction kinetics and for fitting the enzyme parameters to its application. We also confirmed the biological activity of obtained biocatalytic systems.

Acknowledgments: this work was supported by the Polish National Science Centre (NCN) grant PRELUDIUM 14 no. 2017/27/N/ST5/02696.

P70

Targeting protein interactions in *Helicobacter pylori* urease maturation: an in-cell antibacterial screening

Tarsia Cinzia, Danielli Alberto, Florini Francesca, Cinelli Paolo, Ciurli Stefano, Zambelli Barbara.

Dept. of Pharmacy and Biotechnology, University of Bologna, Italy

barbara.zambelli@unibo.it

Helicobacter pylori is a Gram-negative bacterium that colonizes the stomach of half of the human population and is strongly associated with peptic ulcer and gastric cancer. Accordingly, in 1994 *H. pylori* was the first, and so far, unique bacterium classified as a class I carcinogen by IARC. In 2013 its eradication was recommended by WHO as a strategy to diminish the incidence and effects of gastric-associated tumours [1]. Living in the strongly acidic environment of the gastric niche, *H. pylori* developed unique adaptive mechanisms to neutralize extremely low pH for surviving in this challenging setting. For this, the most important player is the nickel-dependent urease, whose catalytic activity increases the pH of the micro-environment around the bacterium [2,3].

The present work aims to provide an innovative strategy for the identification of new antibacterial molecules to the eradication of *H. pylori* by targeting the urease activity. As urease is not present in the human proteome, it represents a promising target for drug development. An in-cell high-throughput urease inhibition assay was developed in the model organism *E. coli* and was validated with known urease inhibitors that selectively bind the enzyme. Subsequently, a two plasmids system was produced for in-cell screening of peptide-based inhibitors that interrupt the protein-protein interaction (PPI) network delivering nickel into the active site, thus preventing enzyme maturation.

A linear segment was identified in the C-terminal region of UreF, a urease accessory protein, undergoing disorder-to-order transition upon interaction with UreD (Figure 1). Its ability to inhibit urease maturation was tested by co-expressing this sequence with the wild-type urease operon. Upon peptide expression, the in-cell urease activity was sensibly reduced indicating that this linear segment is a “hot spot” for the interaction. The developed system is expected to provide, by sequence optimization, the identification of candidates for developing new drugs, to be used, after further experimental evaluation, in *H. pylori* eradication programs.

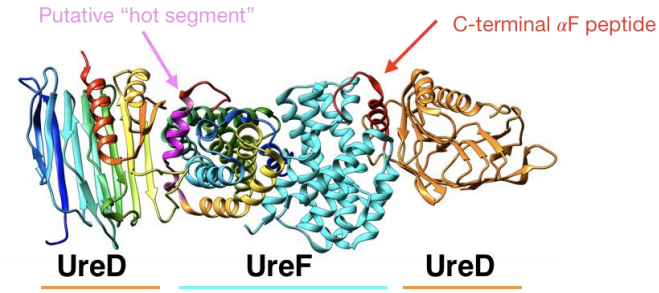


Figure 1. *Crystal structure of the $UreD_2$-$UreF_2$ complex. For each protein, a chain is represented coloring successive residues from blue (N-terminus) to red (C-terminus). The second chain is colored in cyan (UreF) or in orange (UreD). The C-terminal segment of UreF (□F, in red) is sensitive to proteolysis and is preserved only upon the PPI.*

References

[1] IARC W. G. Reports, Helicobacter pylori eradication as a strategy for Preventing Gastric Cancer. 2013. Volume 8.

[2] Zambelli, B.; Musiani F.; Benini; S. Ciurli, S. Acc. Chem. Res. 2011, 44(7): 520-530.

[3] Zambelli, B.; Ciurli S. (2013). Met. Ions Life Sci. 2013, 13: 321-357.

P71

Two IleRSs in *Bacillus megaterium*: is there a difference?

Vladimir Zanki, Ita Gruić Sovulj

Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10 000 Zagreb, Croatia.

vladimir.zanki@chem.pmf.hr

Isoleucyl-tRNA synthetase (IleRS) catalyses covalent coupling of $tRNA^{Ile}$ with cognate isoleucine, thus providing aminoacylated tRNA for protein biosynthesis. During aminoacylation, isoleucine is firstly activated to form isoleucyl-adenylate intermediate. The second step then comprises the transfer of Ile to the cognate $tRNA^{Ile}$. Based on phylogenetic analysis, bacterial IleRSs group in two distinct clades: bIleRS1 (bacteria-like) and bIleRS2 (so-called eukaryote-like) [1]. The two IleRS types differ in mupirocin resistance. Mupirocin is an antibiotic that binds with a high affinity to the synthetic site of

bIleRS1, thus inhibiting formation of Ile-tRNA^{Ile} and blocking protein biosynthesis. At the same time, bIleRS2 is less susceptible to mupirocin inhibition. Generally, either bIleRS1 or bIleRS2 is present in the cell. However, several *Bacilli* species, like *Bacillus megaterium*, are known to have both bacterial IleRS types in their genome.

To gather insight into a difference between two IleRSs in *B. megaterium*, BmIleRS1 and BmIleRS2, both *ileS* genes were cloned, overexpressed in *E. coli*, and purified using Ni-NTA affinity chromatography. Native total tRNA was isolated from *B. megaterium* and specific tRNA$^{Ile}_{GAT}$ isoacceptor was purified using complementary biotinylated oligonucleotide. In parallel, the gene for tRNA$^{Ile}_{GAT}$ was cloned and overexpressed in *E. coli*. Kinetic analysis revealed that BmIleRS2 shows an unusually high Km for isoleucine in the activation step. The Km (Ile) was 30-fold higher compared to the Km (Ile) for BmIleRS1. When aminoacylation was followed, a substantially smaller difference in the Km values for isoleucine between BmIleRS1 and BmIleRS2 was observed. Thus, our results indicate that BmIleRS1 and BmIleRS2 exhibit some differences in the aminoacylation mechanisms. Based on our preliminary data, BmIleRS2 displays lower affinity than BmIleRS1 towards mupirocin. However, detailed kinetic analysis is undergoing to address that issue.

In silico analysis revealed that the gene for BmIleRS1 is under a constitutive promoter, while transcription of BmIleRS2 seems to be induced under stress conditions. This is in agreement with expected lower mupirocin sensitivity of BmIleRS2. We hypothesised that stress conditions could also be related to a temperature stress, so we measured protein's thermal stability. Interestingly, BmIleRS2 possessed a significantly higher Tm (65 °C) compared to BmIleRS1 (55 °C). This is surprising as usually antibiotic resistance evolves at the expense of protein stability. In conclusion, BmIleRS1 and BmIleRS2 differ in some aspects of the aminoacylation mechanism, transcriptional regulation and likely mupirocin susceptibility. Why BmIleRS2 didn't prevail, and both IleRS types exist in *B. megaterium*, remains still unanswered. Further *in vivo* and *in vitro* analyses will show whether the requirement for antibiotic resistance in *B. megaterium* compromised BmIleRS2 catalytic efficiency/accuracy, to a point where presence of BmIleRS1 is essential. However, a possibility that it is an evolution's dead-end could not be excluded at this point.

Reference

[1] Cvetesic, N. et al. Naturally Occurring Isoleucyl-tRNA Synthetase without tRNA-dependent Pre-transfer Editing. JBC. 2016; 291: 8618-8631. doi: 10.1074/jbc.M115.698225

P72

Styrene Maleic Acid Copolymers versus Dodecyl-β-D-Maltoside in Solubilizing Integral Membrane Proteins

Alajos Bérczi[1], Mária Péter[2], Ágota Deá [3], Gábor E. Balogh[2], László Janovák[3], András Kincses[1], László Zimányi[1]

[1]Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; [3]Department of Physical Chemistry and Materials Science, Interdisciplinary Excellence Centre, University of Szeged, Szeged, Hungary

zimanyi.laszlo@brc.mta.hu

Solubilization/dismantling of biological membranes is a prerequisite for separation and purification of any integral membrane protein. Besides the well-known and routinely used ionic, nonionic and zwitter-ionic detergents, new amphiphilic agents have appeared with new properties as compared to those mostly used until now. Styrene maleic acid (SMA) copolymers are among those which have very attractive properties from the point of view of separation of integral transmembrane proteins together with their solvation lipid layers [1]. SMA(2:1) and SMA(3:1) copolymers with molecular mass between 3,000 and 10,000 Da are the two most frequently used SMA copolymers. However, no systematic study has appeared so far to compare the efficiency of these two (and other) copolymers in membrane-protein nanodisc preparation. Here we compare the solubilization efficacy of 3 different SMA copolymers and dodecyl-β-D-maltoside (DDM). We show a lipidomic comparison obtained after solubilization by these agents of the microsomal membrane fraction prepared from *Saccharomyces cerevisiae* cells. We also point out the differences between the solubilizing agents that might be important when planning membrane dismantling experiments with SMA copolymers. As a particular test, we demonstrate and discuss the solubilization of a cytochrome *b*561 protein [2] heterologously expressed in *Saccharomyces cerevisiae* cells. Due to the rather high negative charge density on SMA copolymers, these agents are not recommended when the goal is to study an integral membrane protein in nanoparticles that functions with negatively charged substrate(s).

References

[1] Dörr JM, Scheidelaar S, Koorengevel MC, Dominguez JJ, Schäfer M, van Walree CA, Killian JA. The styrene–maleic acid copolymer: a versatile tool in membrane research. Eur. Biophys. J. 2016; 45(1):3–21. doi: 10.1007/s00249-015-1093-y.

[2] Bérczi A, Zimányi L. (2014) The trans-membrane cytochrome b561 proteins: structural information and biological function. Curr. Prot. Peptide Sci. 2014; 15(8):745-760.

P73

Discrimination of α-aminobutyrate and its fluorinated analogues in the synthetic and editing reactions of isoleucyl-tRNA synthetase

Igor Živković, Ita Gruić-Sovulj

Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia

izivkovic@chem.pmf.hr

Isoleucyl-tRNA synthetase (IleRS) is an enzyme that catalyzes activation of isoleucine (Ile) (Fig, (1)) and its transfer to $tRNA^{Ile}$ (Fig, (2)). Synthetized Ile-$tRNA^{Ile}$ is a substrate for protein synthesis. It has been shown that IleRS can also misactivate and transfer to $tRNA^{Ile}$ non-cognate amino acids valine (Val) and norvaline (Nva), both being one methylene group smaller than Ile. Nva is a non-proteinogenic amino acid that sporadically accumulates in cells. To prevent accumulation of misacylated tRNAs, and thus the error in protein synthesis, IleRS employs pre- and post- transfer hydrolytic editing (Fig, orange/red). In order to explore how substrate hydrophobicity affects amino acid discrimination in the synthetic and editing reactions of IleRS, we now tested non-cognate α-aminobutyrate (α-ABA), which is two methylene groups smaller than Ile. α-ABA is also a metabolic amino acid that accumulates in cells.

IleRS activated and transferred α-ABA to $tRNA^{Ile}$ with the similar rate constants as obtained with Ile. However, IleRS exercised discrimination against α-ABA predominantly at the level of K_m, which is 3500-times greater than the K_m for Ile. This suggests that binding of α-ABA to the active site of IleRS is impaired. Despite α-ABA being activated and transferred to $tRNA^{Ile}$, there was no accumulation of α-ABA-$tRNA^{Ile}$ due to active IleRS pre- and post- transfer editing against α-ABA. Kinetic analysis showed that post-transfer editing is rapid and dominant editing reaction, as previously found for Val and Nva. Our data revealed that decrease in hydrophobicity mainly affects the amino acid binding to IleRS (K_m effect) while the effects on the rate constants of the synthetic and editing reactions were modest.

To explore whether the increase in hydrophobicity by introducing fluorine will affect α-ABA discrimination by IleRS, we investigated aminoacylation and editing of di- and tri- γ-fluorinated analogues of α-ABA. Introduction of fluorine reduced the activation rate up to 5-fold, while surprisingly it had little to no effect on the amino acid binding (K_m) and editing reactions. Thus, in the case of IleRS, fluorination of α-ABA does not compensate the loss of hydrophobic interactions caused by decrease in the size of the amino acid substrate. Even more, our data suggest that "polar hydrophobicity", a peculiar feature of fluorine, produced adverse effect on the binding of fluorinated α-ABA to IleRS. Hence, the unpredictability of fluorine-enzyme interactions could make reengineering of aminoacyl-tRNA synthetases, for the purpose of more efficient incorporation of

fluorinated amino acids into proteins, more challenging than anticipated.

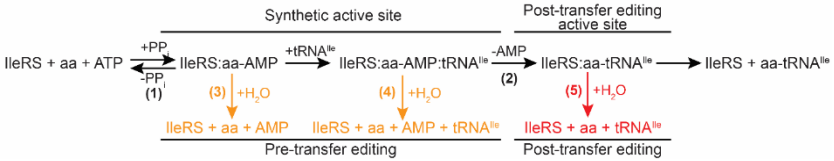


Figure legend – Isoleucyl-tRNA synthetase pathways of aminoacylation and editing.

POSTER SESSIONS

P	1	Novel molecular interaction controlling yeast cell decision at the transition from fermentative and oxidative metabolism	Małgorzata Adamczyk	
P	2	EIS and SERS Analysis of Anchor Molecules for Tethered Bilayer Lipid Membrane Formation	Indre Aleknaviciene	flash
P	3	Probing the mechanism of iron release from Dps, a miniferritn	Ana Almeida	flash
P	4	Modification of Magnetic Nanoparticles with DNA Particulates and Their Applications in Magnetic Hyperthermia	Cem Levent Altan	
P	5	Are we observing magnesium (Mg2+) or monovalent (K+, Na+) ions in ribosomes?	Pascal Auffinger	
P	6	Detection of toxic metal ions by the CueR metalloregulator	Ria Katalin Balogh	
P	7	Peptide screening using surface sensitive techniques: a way for the development of new antibody mimics	Laure Bar	
P	8	Functional dynamics of a non-heme iron(II) enzyme revealed by site-directed spin labeling EPR spectroscopy	Valérie Belle	
P	9	Structure, function and interactions of proteins involved in nickel-dependent carcinogenesis and infections	Ylenia Beniamino	
P	10	Characterisation and MDS of SOD mutants	Rosalin Bonetta	
P	11	From gene to protein interaction	Deborah Byrne	
P	12	Alzheimer’s disease from a single molecule/single cell perspective	Martino Calamai	flash
P	13	Amyloid β-Protein Inhibition and Interaction with Membranes	Rita Carrotta	flash
P	14	Ferritin: The most interesting bionano component?	Soumyananda Chakraborti	flash
P	15	Inhibitions of carbon monoxide dehydrogenase: spectroscopic investigations.	Alexandre Ciaccafava	

P	16	In vitro characterization of the Sulfide Oxidation Unit (SOU) complex using fluorescence methods	Anne-Lise Claudel	
P	17	Nanoparticle Tracking Analysis (NTA) to sharpen the definition of virus particle stability	Didier Clenet	
P	18	Biotic Materials for Metal Nanoparticles Synthesis	Nuno Coelho	
P	19	Selective levo-thyroxine detection by electrochemical-surface plasmon resonance	Melinda David	
P	20	Development of an Infrared Spectroscopy Data Repository	Tina Daviter	flash
P	21	Centromere of the nematode Meloidogyne; characterization of centromere specific H3 protein (CENH3) and underlying DNA sequences	Evelin Despot Slade	
P	22	Differential Scanning Fluorimetry: revisiting microtubules assembly.	François Devred	flash
P	23	Control of reactive oxygen species in mitochondria by cytochrome c phosphorylation and respirasome factors	Antonio Díaz-Quintana	
P	24	When spectroscopy meet small-angle scattering Enhanced information and diagnostics in life sciences and soft matter	Cedric Dicko	
P	25	ITC: theory, practice and pitfalls	Philippe Dumas	flash
P	26	Development of a SANS strategy for the study of membrane proteins: application to a prokaryotic NADPH oxidase homolog.	Christine Ebel	flash
P	27	Macromolecular interactions in vitro, comparing classical and new approaches	Paloma Fernandez Varela	flash
P	28	Targeting de novo thymidylate synthesis nuclear complex	Giorgio Giardina	
P	29	CENH3 proteins as epigenetic determinants of Tribolium centromeres	Tena Gržan	

P	30	Influence of the cupric ion on the ferroxidation mechanism of Dps, a miniferritin from Marinobacter hydrocarbonoclasticus	João Guerra	
P	31	Intramolecular allosteric control of NColE7 metallonuclease based on the specific protease action of nickel(II) ions	Béla Gyurcsik	
P	32	Study of Biocatalytic Systems in Crowded Environment: The Case of Lactate Dehydrogenase	Josef Hamacek	
P	33	Exploring the dynamic structure of bionanocages	João Jacinto	flash
P	34	EPR detection of radical(s) in cytochrome c oxidase	Daniel Jancura	
P	35	Protein-protein interaction standards: The latest news in attempts to make ideal nanobodies	Thomas Jowitt	flash
P	36	Quaternary Structure of Human NK Cell Receptor:Ligand complexes by the Looking Glass of Super-resolution Microscopy	Barbora Kalousková	flash
P	37	Stem Cells Adhesion by Functionalized Nanodiamonds	Nerijus Karalius	
P	38	Changes in the membrane phospholipid composition in erythrocytes in subjects with metabolic syndrome after short term consuming of a pomegranate juice	Milica Kojadinovic	
P	39	A biophysical view on the interaction of SSB proteins with single-stranded DNA	Anja Kostelac	
P	40	EPR study of ion substituted hydroxy apatites	Nadica Maltar-Strmečki	
P	41	Heparanase binds substrate via a dynamic binding mechanism	Aleksandra Maršavelski	flash
P	42	Precision of ITC measurements for target-based drug design	Daumantas Matulis	flash
P	43	Spectroscopic Studies of Dual Fluorescence in 2-((4-Fluorophenyl)amino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole and other compounds with 1,3,4-thiadiazole	Arkadiusz Matwijczuk	
P	44	A pipeline for testing interaction partners of small GTPases in Dictyostelium	Lucija Mijanović	

P	45	Algal cells under heavy metal stress: physiological and morphological response	Tea Mišić Radić	flash
P	46	Biophysical studies on Tau aggregation and metal ion binding	Guilherme G. Moreira	
P	47	Spectroscopic and Theoretical Studies of Dual Fluorescence in 2-Hydroxy-N-(2-phenylethyl)benzamide Induced by ESIPT Process. Solvents Effects	Agnieszka Niemczynowicz	
P	48	Intrinsic affinity of N-substituted benzenesulfonamides to CA proteins	Vaida Paketuryte	
P	49	Application of machine learning approaches for design of more selective herbicides	Vesna Pehar	
P	50	Tethered Bilayer Lipid Membranes – a Comprehensive Tool for Electrochemical Studies of Pore-Forming Toxins	Tadas Penkauskas	flash
P	51	Structural and functional evidence for plasticity of Potato virus Y coat protein	Marjetka Podobnik	flash
P	52	Cellular responses to proteome-wide isoleucine mistranslation	Marija Pranjić	
P	53	Cell Adhesion and Mechanics in Fibroblasts and Myofibroblasts	Manfred Radmacher	flash
P	54	An Enzyme Active Site Loop Revealed as Gatekeeper for Co-factor Flip by Targeted Molecular Dynamics Simulations and FRET-based Kinetics	Sophie Rahuel-Clermont	
P	55	Structural and Functional Impact of Missense Mutations on Glutaryl-CoA Dehydrogenase	Joana Ribeiro	
P	56	Sensing L-arginine in pseudomonas aeruginosa: a novel link between C-DI-GMP and central metabolism?	Serena Rinaldo	
P	57	Bead Injection Multiplex Immunoanalysis	Toonika Rinken	flash

P	58	Plant seryl-tRNA synthetase as a link between translation and metabolism of brassinosteroid hormones	Jasmina Rokov-Plavec	flash
P	59	Brain S100A9 forms string-like polymeric assemblies with potential relevance in Alzheimer's Disease	Mariana Romão	
P	60	Comparison of graphene oxide and carbon quantum dots as biocompatible supports for adenylate kinase immobilization	Katarzyna Roszek	
P	61	Oligomerization of NKp30, an activation receptor of human lymphocytes, is dependent on its N-glycosylation	Ondrej Skorepa	
P	62	Long, highly-stable and flexible: what analytical ultracentrifugation can tell us about "random walk" oligomer	Alexandra Solovyova	flash
P	63	How oxidized lipids control raft-like domain size in biomimetic membranes	Galya Staneva	
P	64	Analysis of Protein-DNA Interactions by SPR - Simple and cheap quantitation using the ReDCaT Chip	Clare Stevenson	
P	65	The Protein Facility of Elettra: a platform to support research and development	Paola Storici	
P	66	Mass spectrometry approach for analysing protein phosphorylation in antibiotic producing bacteria	Ela Šarić	
P	67	Human DPP III - Keap1 interactions	Sanja Tomić	flash
P	68	Denaturation signatures of plasma and cancer diagnostics	Philipp Tsvetkov	
P	69	Controlling enzymatic activity by immobilization	Marek Wisniewski	flash
P	70	Targeting protein interactions in Helicobacter pylori urease maturation: an in-cell antibacterial screening	Barbara Zambelli	
P	71	Two IleRSs in Bacillus megaterium: is there a difference?	Vladimir Zanki	

P	72	Styrene Maleic Acid Copolymers versus Dodecyl-β-D-Maltoside in Solubilizing Integral Membrane Proteins	László Zimányi
P	73	Discrimination of α-aminobutyrate and its fluorinated analogues in the synthetic and editing reactions of isoleucyl-tRNA synthetase	Igor Živković

Session 1 (25 posters)

posters no. 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61, 64, 67, 70, 73

Session 2 (22 posters)

posters no. 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65

Session 3 (26 posters)

posters no. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 68, 69, 71, 72

SPONSOR CONTRIBUTIONS

APPLIED PHOTOPHYSICS Inc

Beyond α-helix and β-sheet: Expanding the Role of Circular Dichroism.

Martin Textor, Application Scientist

Applied Photophysics, Leatherhead, UK

Matrin.Textor@photophysics.com

Circular Dichroism (CD) spectroscopy has long been regarded as a low-resolution technique as its application focused on estimating the α helix and β sheet content of proteins by secondary structure analysis of far UV CD data. This view of CD, though widely held, is no longer accurate as technological advances enable CD spectroscopy to provide more comprehensive structural information including near UV CD to assess minor changes of tertiary structure. The high sensitivity of state of the art CD instrumentation makes such data amenable to statistical analysis, allowing for a more rigorous assessment of higher order structure. This approach allows evaluating if such changes are significant or not and thus provides more confidence than old-fashioned deconvolution analysis limited to far UV data. Using case studies, we illustrate our improvements to CD technology, including enhanced resolution in the near UV, improved detection in the presence of high absorbing buffers/excipients, and the use of Weighted Spectral Difference to demonstrate spectral similarity, with the ultimate purpose of allowing scientists to make informed decisions based on statistically verifiable CD data.

Objective, quantifiable HOS comparisons: a biosimilar case study utilizing circular dichroism

Martin Textor, Application Scientist

Applied Photophysics, Leatherhead, UK

Matrin.Textor@photophysics.com

During biotherapeutic development a wide range of biophysical characterization techniques are required to support informed decision-making and contribute to the totality of evidence in regulatory submissions. Regulatory authorities are increasing their demand for 'state-of-the-art' techniques that can provide statistically-validatable data. To date, obtaining such results for higher order structure (HOS) comparisons has presented challenges in terms of data acquisition and suitability of statistical methods. This poster presents a case study in which an integrated approach to HOS analysis generates an objective,

quantifiable comparison of a commercially-available biotherapeutic (Fab fragment) with a biosimilar currently under development.

More than a-helix and -sheet: expanding the role of circular dichroism

Tony Lester, Sales Manager, Martin Textor, Applications Scientist

Applied Photophysics, Leatherhead, UK

Tony.Lester@photophysics.com

This poster demonstrates how state-of-the-art CD spectrometers go beyond the traditional use of CD. High-quality data now provides unique insights into the higher order structure (HOS) of complex biomolecules. Two applications are presented. 1. A HOS comparison of NIST mAb variants revealed minor differences in secondary and tertiary structure. The statistical significance of these differences was determined using the Tier 2 quality range approach as recommended by FDA guidelines. 2. A multiwavelength, thermal denaturation (temperature ramp) experiment revealed changes to both secondary structure and protein stability in a globular protein after transient exposure to nanoparticles.

BIODESY, INC.

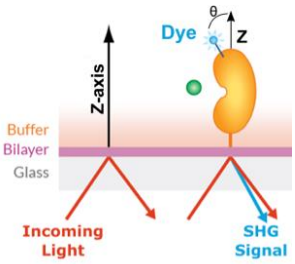
Characterizing Conformational Changes By Second-Harmonic Generation (SHG) A Novel Technology For Monitoring Biomolecule On-Target Binding And Differentiating Mechanism Of Action

David Shaya, Margaret Butko, Gabriel Mercado, Ben Moree, Tracy Young

Biodesy, Inc., South San Francisco, CA-94080, USA
Gabriel.Mercado@Biodesy.com

Second-harmonic generation (SHG) is an optical technique that enables conformational sensing of a target biomolecule upon binding to ligands such as small molecules, fragments, proteins, peptides and oligonucleotides. The technique relies on rendering a target biomolecule SHG-active by labeling with an SHG probe. SHG is sensitive to the angular orientation of the SHG-active probe with respect to the surface to which the labeled biomolecules are tethered. Ligand-induced conformational changes of the labeled biomolecule that result in probe movement are observed as a change in the SHG signal intensity.

Here we describe the SHG characterization of targets such as soluble proteins, oligonucleotides, peripheral and integral membrane proteins upon binding of ligands over a wide range of affinities. In this work we show selected case studies for these types of assays. In addition, we demonstrate how SHG can distinguish different mechanisms of action (e.g., agonist vs. antagonist).



SHG signal intensity depends on physical orientation of the dye. Small discriminatory changes in dye orientation are captured by SHG signal change

References

[1] Moree, B.; Connell, K.; Mortensen, R.B.; Liu, C.T.; Benkovic, S.J. and Salafsky. Protein Conformational Changes Are Detected and Resolved Site Specifically by Second-Harmonic Generation. J. Biophysical Journal. 109, 806 – 815 (2015)

[2] Moree, B.; Yin G.; Lázaro, D.F.; Munari, F.; Strohäker, T.; Giller, K.; Becker, S.;Outeiro, T.F.; Zweckstetter, M. and Salafsky, J. Small Molecules Detected by Second-Harmonic Generation Modulate the Conformation of Monomeric Alpha-Synuclein and Reduce its Aggregation in Cells. The Journal of Biological Chemistry. 290, 27582 – 27593 (2015)

[3] Butko, M.T; Moree, B.; Mortensen, R.B. and Salafsky, J. Detection of ligand-induced conformational changes in oligonucleotides by second-harmonic generation at a supported lipid bilayer interface. Analytical Chemistry. 88 (21), pp 10482– 10489 (2016)

[4] Spagnuolo, L.A.; Eltschkner, S.; Yu, W.; Daryaee, F.; Davoodi, S.; Knudson, S.E.; Allen, E.K.H.; Merino, J.; Pschibul, A.; Moree, B.; Thivalapill, N.; Truglio, J.J.; Salafsky, J.; Slayden, R.A.; Kisker, C. and Tonge. Evaluating the contribution of transition state destabilization to changes in the residence time of triazole-based InhA inhibitors. P.J.; Journal of the American Chemical Society. (2017)

[5] Wong, J.J.W.; Young, T.A.; Zhang, J.; Liu, S.; Leser, G.P.; Kornives, E.A; Lamb, R.A; Hong Zhou, Z.; Salafsky, J.; Jardetzky, T.S. Monomeric ephrinB2 binding induces allosteric changes in Nipah virus G that precede its full activation. Nature Communications. (2017)

BIONAVIS Ltd

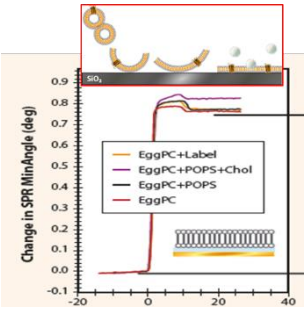
Deciphering biophysics of lipid layers, functional polymers and biomolecules by Multi-Parametric Surface Plasmon Resonance (MP-SPR)

Radek Bombera, Roosa Ståhlberg, Annika Järvinen, Martin Albers, Johana Kuncova-Kallio

BioNavis Ltd., Tampere, Finland

radek.bombera@bionavis.com

Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a surface analysis technique which application goes far beyond the traditional SPR, typically used for biomolecular binding studies. Indeed, this label-free and real-time technique give insight into biophysics of functional layers and interactions between a wide variety of molecules. MP-SPR optical set-up combines wide angular scanning and detection at multiple wavelengths thus providing a unique possibility to assess layers from Ångströms up to tens of microns. It enables to characterize a large variety of materials ranging from thin films (polymers, nanocellulose), biomolecular assemblies, lipid bilayers and up to nanoparticles and live cells. Measurements provide information on kinetic parameters from biointerface interactions as well as calculation of layer refractive index (RI) and its thickness (d) without prior knowledge of either parameter. The performances of MP-SPR technique are demonstrated in such studies as: conformation changes in lipid layers or functional polymers [1-3], cell binding kinetics in biomimetic environments [4], antifouling efficacy in serum [5] or protein corona formation on liposomes [6]. With the ability to characterize both kinetics and nanoscale layer properties, MP-SPR is an effective tool for nanomaterial, biomaterial and biochemical interactions research.



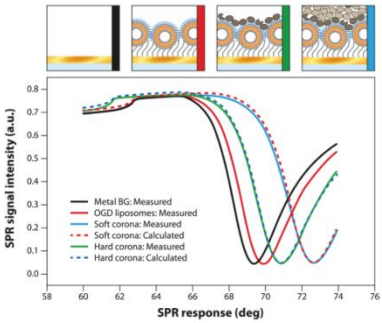


Figure 1 Monitoring of conformational change of liposomes into supported lipid bilayer.

Figure 2 Assessment of protein corona formation when loading serum onto liposomes

References

[1] Granqvist N., Yliperttula M. et al. Control of the Morphology of Lipid Layers by Substrate Surface Chemistry. Langmuir 2014,30(10), 2799–2809.

[2] Parkkila P., Elderfi M., Bunker A., Viitala T. Biophysical Characterization of Supported Lipid Bilayers Using Parallel Dual-Wavelength SPR and QCM Measurements. Langmuir, 2018, Vol. 34 (27), p. 8081-8091.

[3] Ferrand-Drake del Castillo G., Emilsson G., Dahlin A. Quantitative Analysis of Thickness and pH Actuation of Weak Polyelectrolyte Brushes. The Journal of Physical Chemistry C, 2018, 122 (48), p. 27516–27527.

[4] Soler M., Li X. et al. Two-dimensional label-free affinity analysis of tumor-specific CD8 T cells with a biomimetic plasmonic sensor. ACS Sensors, 2018, 3 (11), pp 2286–2295.

[5] Emilsson G., Schoch R.L., et al., Strongly Stretched Protein Resistant Poly(ethylene glycol) Brushes Prepared by Grafting-To. ACS Applied Materials & Interfaces, 2015, 7 (14), pp 7505–7515.

[6] Kari O.K. et al., Multi-parametric surface plasmon resonance platform for studying liposome-serum interactions and protein corona formation. Drug Delivery and Translational Research, 2017, 7(2):228-240.

BRUKER BIOSPIN

Bruker EPR: Structural Insight and Sample Quality

Patrick Carl

Bruker Biospin, Rheinstetten, Germany

Patrick.Carl@bruker.com

Bruker provides a wide range of instrumentation for gaining insight into biological structures. EPR provides techniques for measuring long range distances, interactions as well as dynamics of biomolecules. Key to these techniques is the direct quantification of species present and Bruker EPR systems deliver this without the need of user calibration.

DYNAMIC BIOSENSORS GmbH

The switchSENSE technology as a versatile tool for elucidating the mechanism of action of small molecule drug candidates

Lena Kilian, Daniel Schwarz

Merck KGaA, Discovery & Development Technologies, Discovery Pharmacology; Frankfurter Str. 250, 64293 Darmstadt, Germany

lena.kilian@merckgroup.com

The efficiency of the drug discovery process in the pharmaceutical industry is often reduced by the lack of information on the mechanism of action of drug candidates to their targets. Elucidating the mechanism of inhibition of small molecules towards their target in early stages of the drug discovery process will contribute to reduce attrition rates in later stages.

The switchSENSE technology (Dynamic Biosensors) was used to distinguish different binding modes of identified small molecule inhibitors of a DNA polymerase, an increasingly interesting target class in the field of DNA damage and repair in tumor biology. We characterized the screening hit matter in respect of specificity, activity and mode of inhibition in a single experimental set-up using a fluorescence based read-out.

As a conclusion we see the switchSENSE technology is a versatile and highly valuable tool to impact preclinical drug discovery projects by gaining the understanding of the interaction between a drug candidate and its target protein.

FIDA – TECH APS

Flow Induced Dispersion Analysis (FIDA) quantifies proteins, protein-ligand interactions and protein stability under native conditions

Morten E. Pedersen[1], Sarah I. Gad[1], Nicklas N. Poulsen[2], Brian Sørensen[1], Jesper Østergaard[1,2], and Henrik Jensen[1,2]

[1]FIDA-Tech Aps, Universitetsparken 2 (C/O University of Copenhagen), 2100 Copenhagen, Denmark, [2] Department of Pharmacy, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark;

hj@fida-tech.com

FIDA is a novel capillary-based technology for assessing protein concentration in complex solutions (e.g. plasma samples) and for measuring in-solution binding under native conditions. FIDA is based on quantifying Taylor dispersion in a pressure driven flow of a ligand (indicator molecule) interacting with the protein of interest (e.g. an antibody-based drug). The indicator appears small (i.e. it has a low hydrodynamic radius) when it is not bound to the antibody, but upon binding it will appear larger (i.e. it has a higher apparent hydrodynamic radius). As shown in figure 1, the change in apparent diffusivity/size forms the basis for an accurate measure of protein concentration and interactions [1-3]. In this presentation, FIDA is demonstrated for assessing in-solution protein concentration as shown by the quantification of an antibody-based drug compound (sub-nanomolar sensitivity), and for detection of auto-antibodies [3]. Further, it will be shown that FIDA effectively can probe protein stability by monitoring unfolding processes, loss of binding and changes to intrinsic protein fluorescence (figure 2).

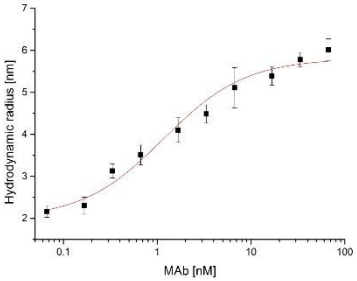


Figure 1. Changes in apparent hydrodynamic radius of the A-beta 1-40 Alzheimer peptide as it binds a MAb drug compound.
Kd = 1,2 nM. Sample matrix: Plasma (10%).

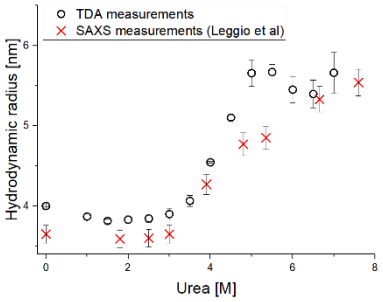


Figure 2. Changes in hydrodynamic radius of Human Serum Albumin as it unfolds in Urea. Data obtained using FIDA and SAXS [4].

Conclusions: FIDA may be used as an alternative to immobilization based methodologies as it provides true native in-solution information on protein size, interactions and concentrations. Key advantages of FIDA include ability to measure in-solution under ***true native*** conditions (plasma, serum, cell lysates, etc) using nano- to microliter sample amounts. FIDA is performed on a fully automated platform as standard (accepting vials and 96 well plates).

References

[1] N. N. Poulsen, et al, Analyst, 2015, 140, 4365-4369.

[2] Henrik Jensen and Jesper Østergaard; Analyte Quantification using Flow Induced Dispersion Analysis, EP2542885B1, US9310359, US20130059313, WO2011107406A1. National Phase.

[3] N. N. Poulsen, et al, Anal. Chem., 2016, 88, 9056-9061.

[4] C. Leggio, et al, J. Phys. Chem. B, 2009, 113, 12590-12602.

FLUENCE ANALYTICS

Peptide stability characterization as influenced by thermal and stir stress using ARGEN

Michael Drenski, Stephanie McCormick

Fluence Analytics, New Orleans, Louisiana, USA

Stephanie.mccormick@fluenceanalytics.com

The ARGEN, by Fluence Analytics, provides researchers the tools to quickly detect changes in the normalized molecular weight of peptide samples in solution, enabling them to identify the onset of aggregation, degradation or phase changes while these phenomena take place. The ARGEN does this by continuously monitoring multiple independent static light scattering experiments, each one with its own set of controlled stressor conditions. These ARGEN controlled stressor conditions include both temperature from 18 ºC – 100 ºC as well as stirring from 0 – 2000 RPM but researchers can additionally screen many common formulation conditions for each sample by adjusting the solvent, pH, salt type and concentration and excipient type and concentration.

The data presented, shows how ARGEN was used to study the stability of peptides as influenced by pH, sample concentration, temperature and stirring stress in real time. This information can be used to preemptively understand how and when aggregation or degradation may occur so that additional formulation development can be focused to minimize these unwanted results.

FLUIDIC ANALYTICS Ltd

Microfluidic Diffusional Sizing (MDS) for protein characterization – latest results and next steps

Sean Devenish, Maya Wright, Luca Gross, Maren Butz, Jonathan Faherty

Fluidic Analytics Ltd, Cambridge, UK

jonathan.faherty@fluidic.com

MDS is an in-solution protein characterisation technique that requires little to no sample preparation, and accurately sizes proteins, oligomers and antibodies over a wide dynamic range[1][2]. Here we compare MDS to established techniques such as size exclusion chromatography and circular dichroism and find comparable results can be achieved in faster time with less sample consumption. In addition, we demonstrate that the protein-specific detection employed enables measurement of protein-lipid interactions by monitoring changes in protein size.

Finally, we share ongoing work to expand the capabilities of the technology to examine protein-protein interactions in solution and at low concentrations.

References

[1] Arosio P, Muller T, Rajah L et al. Microfluidic Diffusion Analysis of the Size and Interactions of Proteins under Native Solution Conditions. ACS Nano. 2016; 10(1), p 333-341. doi: 10.1021/acsnano.5b04713

[2] Yates E, Muller T, Rajah L, De Genst E, Arosio P, Linse S, Vendruscolo M, Dobson C, Knowles T P J. Latent analysis of unmodified biomolecules and their complexes in solution with attomole detection sensitivity. Nature Chemistry. 2015; 7:802-809. doi: 1038/nchem.2344

LUMICKS

z-Movi: A high throughput cell-cell avidity screening and sorting acoustic force based technology

Wouter Scheper[1], James Wilkinson[2], Elena Merino[2], Rens Braster[2], Gerrit Sitters[2], Felix Oswald[2], Rosalie Driessen[2], Ton Schumacher[1], Andrea Candelli[2]

1 Department of Molecular Oncology and Immunology, The Netherland Cancer Institute, Amsterdam, The Netherland;2 LUMICKS b.v., Amsterdam, The Netherland

j.wilkinson@lumicks.com

Adoptive cell therapy can be an effective treatment option in a proportion of cancer patients. Nevertheless, the clinical efficacy of such therapies is likely limited by a lack of tools to quantitatively select and sort the most potently tumor-reactive immune cells at high throughput. Here, we developed a novel platform that uses acoustic force manipulation to query the interaction strengths of cells with their cognate binding partners. This technology, **based on acoustic forces**, is a lab-on-a-chip assay that allows the assessment of thousands of cell-cell interactions in parallel. Moreover, this technology provides an accurate, label-free method to isolate cells based on their avidity to specific targets, such as (tumor) cells as well as proteins, peptides and viruses. As a proof of concept, we validated our technology by analyzing the functional avidity of T-cells towards tumor cells and found that it permits the separation of tumor-specific T-cells from non-specific bystander T-cells. These data demonstrate the potential of this platform in profiling T cell-tumor cell interaction and pave the way to quantitative cell-cell avidity studies as well as selection of patient specific immune effector cells or immune receptors for therapeutic use.

NANION TECHNOLOGIES GmbH

Comprehensive biophysical assays: From single channel electrophysiology to overall cell behavior

Elena Dragicevic[1], Conrad Weichbrodt[1], Ilka Rinke-Weiss[1], Nadine Becker[1], Krisztina Juhasz[1], Ekaterina Zaitseva[2], Gerhard Baaken[2], Alison Obergrussberger[1], Matthias Beckler[1], Michael George[1], Sonja Stoelze-Feix[1], Andrea Brüggemann[1], Niels Fertig[1]

1 Nanion Technologies GmbH, Ganghoferstr. 70a, 80339 Munich, Germany; 2 Ionera Technologies GmbH, Hermann-Herder-Str. 7, 79104 Freiburg, Germany

Integral membrane proteins, predominantly ion channels and transporters, have been the focus of basic biophysical research, as well as drug discovery and safety projects for decades. Electrophysiological experiments on fully functional artificial lipid bilayers enable the investigation of basically any membrane-affecting agent. In combination with automated patch clamp and impedance/electrical field potential (EFP)-like recordings of relevant targets expressed in heterologous systems, as well as of human iPSC-derived cardiomyocytes and neurons, we demonstrate broad biophysical application assays, connecting single channel electrophysiology with overall single cell and cell population behavior.

Here, we present the temperature dependent activation or deactivation of different Transient Receptor Potential (TRP) channels by means of planar patch clamping on our medium and high throughput screening (HTS) platforms Patchliner and SyncroPatch 384PE, as well as with highest resolution on a single channel level on our recently introduced Orbit mini setup. Additionally, the effect of drugs on action potentials as recorded in iPSC-cardiomyocytes is important for assessing the interaction of the cardiac ion channel ensemble. We present our advances in development of iPSC-cardiomyocytes "ready-to-use" assays for automated patch clamp. We also show, short and long-term impedance/EFP-like recordings of diverse cell-types, such as drug safety experiments on iPS cardiomyocytes and cancer tox-assays. In summary, medium and high throughput screening (HTS) assays such as automated electrophysiological patch clamp and impedance-based assays allow for the determination of drug effects on a whole cell level whereas artificial bilayers provide a robust environment for the assessment of single ion channel molecules.

NANION TECHNOLOGIES GmbH

Port-a-Patch mini: The next piece in the APC puzzle

Elena Dragicevic, Patrick Mumm, Conrad Weichbrodt, Matthias Beckler, András Horvath, Michael George, Niels Fertig

Nanion Technologies GmbH, Ganghoferstr. 70a, 80339 Munich, Germany

elena@nanion.de

Patch clamp electrophysiology remains the gold-standard for ion channel research because of the information rich content of the data produced. However, the required skills and complexity of equipment can make this technique intimidating and time consuming for inexperienced scientists. The advances in automated planar patch clamp (APC) electrophysiology have made the technique accessible to a wider audience. Since the introduction of the Port-a-Patch (a single channel semi-automated patch clamp system) over a decade ago, the device has been well accepted in the pharma industry, CROs and academia alike and data produced on the device have been published in various high-ranking journals. The Port-a-Patch mini, it´s younger and smaller brother, is an affordable, plug-and-play patch clamp instrument with an extremely small footprint which, enables every lab to perform high-quality, giga-seal whole-cell voltage clamp recordings with minimal training in a very short amount of time. Operating the Port-a-Patch mini is straightforward and simple – cells and solutions are added onto the disposable NPC-1 chip by the user, where capture and seal formation are automated by a computer-controlled pump. Example recordings of heterologously expressed ion channels, such as Nav1.5 and according pharmacological drug effects, will be shown.

NANOTEMPER TECHNOLOGIES GmbH

Application of Temperature Related Intensity Change (TRIC) in biophysical drug discovery projects

Amit Gupta, Matthias Molnar, Stefan Duhr, Philipp Baaske, Johannes Rieger

NanoTemper Technologies GmbH, Munich, Germany

matthias.molnar@nanotempertech.com

In recent years biophysical screening approaches have received more focus in drug discovery as targets become more diverse, fragment-based projects increase in numbers and the need to identify ligands with different mechanisms of action increases. This is especially true for such targets that were previously considered undruggable or do not exert enzymatic functions. Another class of targets that often requires biophysical instead of biochemical characterization as well as biophysical screening technology are membrane proteins. Biophysical techniques can overcome obstacles that are faced with such challenging targets and result in successful lead identification. Furthermore, biophysical technologies remain a crucial tool in lead validation and optimization. Despite many advantages biophysical tools also have drawbacks and pose obstacles in terms of assay development, throughput and automation. In addition to the existing platforms, MicroScale Thermophoresis has become a complement technology within the last years. MST can overcome some of the obstacles posed by other techniques in that it can harness two distinct physical effects, thermophoresis and temperature related intensity change, making the technique highly sensitive to binding events. TRIC as a part of the MST signal is an intrinsic property of fluorophores to change fluorescence intensity as a function of temperature and is known to be strongly affected by the chemical microenvironment. Recent developments showed that TRIC can be fruitfully applied to make MST based assays more robust, sensitive and higher throughput by reduced measurement times and optimized measurement technology such as improved fluorophores.

Here we present recent developments in TRIC based detection and biophysical characterization of binding events and the direct application of TRIC to screening of diverse protein targets.

Reference

[1] Gupta AJ, Duhr S, Baaske P. MicroScale Thermophoresis. *Enc. Biophys*. **2018** doi:10.1007/978-3-642-35943-9_10063-1

PALL FORTEBIO

Integrated Solutions for Early Stage Biologics Discovery

Hendrik Wünsche

FortéBio provides best-in-class workflow solutions for biologics development, enabling one-point interaction for sales, service, and support. The mission is to fast track timelines by removing hurdles in early stage biologics development through innovative technologies and workflow solutions.

Workflow examples include:

Hybridoma generation and screening or Phage display by screening large library of antibodies to find lead molecules.

Cell line development to find the cell line that produces a large amount of monoclonal antibody or recombinant proteins with desirable quality attributes for a stable period of time.

Above that, the presentation will cover the Advanced Workflow Engineering Solutions (AWES) of automated, customized, and integrated systems to fit customer high-throughput needs.

WYATT TECHNOLOGY

Measurement of stoichiometry of protein and membrane protein complexes by light scattering coupled to SEC

Dr. Christoph Albermann, Dr. Dierk Roessner

Wyatt Technology, Dernbach, Germany

Christoph.albermann@wyatt.eu

What is the oligomeric state of the protein? How many of each molecule are in the complex? How stable is the protein in the buffer? Size Exclusion Chromatography (SEC, also know as GPC or FPLC) combined with Multi Angle Light Scattering (MALS) is a reliable and easy to use tool to answer these questions. In this presentation we will discuss the oligomeric state determination of the heat shock protein GrpE and the oligomeric state determination of the membrane protein AcrB.

Conflicting information about the oligomeric state of proteins are not the exception. Using the heat shock protein GrpE as an example, we will discuss why SEC with conventional column calibration (calibration with protein standards) can provide incorrect information and what the causes are. We will present how MALS coupled to SEC improves the analytics and measures the right stoichiometry.

The Escherichia coli Acriflavine resistance (AcrB) forms a multi-drug efflux system that is believed to protect the bacterium against hydrophobic inhibitors. The AcrB forms a homotrimer of 110kDa per subunit. The three AcrB form a large, 30A-wide central cavity. It is a transporter that shows the widest substrate specificity among all known multidrug pumps, ranging from most of the currently used antibiotics, disinfectants, dyes, and detergents to simple solvents. We will present how SEC with MALS was used to identify the homotrimer in solution.

Reference

[1] Schönfeld H, Behlke J, Molecular chaperones and their interactions investigated by analytical ultracentrifugation and other methodologies, Methods Enzymol. 1998; 290; 269-296

XTAL CONCEPTS GmbH

Sample qualification for membrane protein crystallisation based on protein-detergent-complex identification

A. Eckhardt, A. Meyer, K. Dierks

XtalConcepts GmbH, Schnackenburgallee 13, 22525 Hamburg, Germany

Dynamic light scattering (DLS) is an appropriate method to come up one of the most commonly encountered problems in membrane protein crystallization, the identification of detergents to form protein detergent complexes (PDI). Applying DLS *in situ* compensates some former obstacles of this method e.g. the required sample volume, cuvette cleaning and the limit to one sample at the time. Application of DLS *in situ* makes the use of multi- well plates possible. In combination with the use of very low quantities of protein and an automated way to perform measurements, DLS becomes high throughput capable. These are the prerequisites for a detergent screen in which a PDI can be identified by size distribution and size comparison with the "empty" micelle. Since PDIs are a uniform population of structurally intact macromolecules it provides a clearly distinguishable distribution signature that is easy to identify among many other possible size distributions [1].

The strategy to identify the right detergent to form a PDI based on in situ DLS (SpectroLight 600, XtalConcepts) and the knowledge of pure micelle sizes for comparison starts with a "blind walk". Micelles are highly uniform objects in terms of size and stoichiometry. This fact can be exploited to distinguish "empty" micelles from PDIs. The absolute size of a micelle is highly related to the structure of the detergent molecule forming it. An example for the structure-size relationship of detergents and their micelles are the n-Alkyl-β-D-maltopyranosides a class of detergents that have been used extensively to stabilize membrane proteins for biophysical and structural studies [2].

Assuming that the membrane protein is already dissolved in some kind of Dodecyl-maltoside (DDM) containing buffer this is the initial point. First the membrane protein in its buffer has to get in contact with the new detergent to enable a detergent exchange. The most convenient way to achieve this is by adding an excessing amount of the new detergent buffer to the sample. Since the DLS detection volume is 500 nl and the ratio of new buffer and protein sample is 4:1 only 100 nl sample are required for a single test. A huge variety of detergents of all classes are available and in the majority of our experiments, only a few of them transformed the protein into a potential protein detergent complex identified by its radius distribution signature.

References

[1] J. Birch, D. Axford, J. Foadi, A. Meyer, A. Eckhardt, Y. Thielmann, I. Moraes, in press (2018)

[2] P. Rosevear, T. VanAken, J. Baxter, S. Ferguson-Miller, Biochemistry, 19(17), 4108 (1980)

XTAL CONCEPTS GmbH

A time-efficient identification of the best conditions for cryo-em applying *in situ* dls

A. Meyer, K. Dierks, A. Eckhardt

XtalConcepts GmbH, Schnackenburgallee 13, 22525 Hamburg, Germany

It is been only a few years since single particle 3D cryo-EM has emerged as a method for high resolution structure determination. Some spectacular results on large protein complexes attract broad attention. [1] Astonishingly, a standardized analytic procedure to ensure preservation of native biological state of specimens has not been established yet. Although standard methods like SEC-MALS for sample analysis are applied in early stages of the sample preparation pipeline, the quality of a specimen in terms of molecular complex integrity or absence of aggregates is only examined at the last stage via transmission electron microscopy.

In general, high sample quality in terms of absolute size and homogeneity is required to perform subsequent single particle EM. The optimal single-particle specimen is thus as homogenous as possible. [2] If TEM investigations indicate that a sample does not fulfil the quality criteria, it remains uncertain at which stage the sample might have lost its quality. It might happen after grid loading due to drying, blotting or freezing or even much earlier, depending on the complex stability.

Some already existing methods for sample quality examination, established for a long time in other scientific fields, begin now to adapt to the specific requirements of cryo-electron microscopy in order to address this gap in analytical methods. Among them is dynamic light scattering (DLS), a widely used biophysical method for particle size determination [3]. This method is used for homogeneity, stability analysis during heat treatment and for buffer formulations. In comparison with other biophysical methods, DLS is exceptional in some of its characteristics: namely non-invasiveness and the capability for absolute size determination. Just recently this method appeared in the experimental pipeline of cryo-EM sample integrity determination since a few key improvements were introduced. These key improvements led to a significant reduction of the required sample volume down to 10 nl as well as a high degree of automation enabling high throughput approaches, complementing TEM pre-investigations, which can be performed in small aliquots or even on grids.

References

[1] A. Hahn et al. Structure of a Complete ATP Synthase Dimer Reveals the Molecular Basis of Inner Mitochondrial Membrane MorphologyMolecular Cell 63, 445-456 (2016)
[2] R. F. Thompson et al. Methods Vol.100, p. 3-15 (2016)
[3] B. J. Berne, R. Pecora Dynamic Light Scattering John Wiley and Sons Ltd. Baffins Lane (1977)