This meeting is supported by the project funded from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860592

EBSA Satellite MEETING

13TH EUROPEAN BIOPHYSICS CONFERENCE

Proton and proton coupled transport

Vienna, 22-24 July 2021

Table of contents:

Program	**p.1**
Abstracts of Talks	**p.4**
Abstracts of Posters	**p.21**

Venue: Arcotel Kaiserwasser, Wagramerstr. 8
Nearest subway station: Kaisermüllen VIC (Line U1)

Program

Zoom link for Online version:
https://jku.zoom.us/j/94898316508?pwd=dE45NIdhcWppUTFzcUZlakt4ZGdCdz09

Meeting-ID: 948 9831 6508
Password: 769779

Thursday, July 22, 2021

13.00 Arrival and Lunch

14.00 Welcome

14.15 Nanoscopy of 2D materials in liquid

Aleksandra Radenovic, Lausanne, Switzerland

14.45 Protein hydration and dynamics probed by time dependent fluorescence shifts: dehalogenases, ATPase and urea channel Hp-UreI

Martin Hof, Prague, Czech Republic

15.15 **Coffee break**

15.45 Facilitation of H^+ conductance by ANT1

Elena E. Pohl, Vienna, Austria

16.15 Measurement of mitochondrial proton gradients in living cells using fluorescent sensors
Karin Busch, Münster, Germany

16.45 The role of a patch of acidic residues in DPP-4 in enzyme activity and specificity and ligand binding

Herbert Nar, Biberach, Germany

19.00 Dinner

Friday, July 23, 2021

08.45 Interfacial proton diffusion

Peter Pohl, Linz, Austria

09.15 Graph-based algorithms for protonation-coupled membrane transporters and receptors

Ana-Nicoleta Bondar, Berlin, Germany

09.45 I am not interested in your results - I am only interested in your controls

Stefan Hannus, Munich, Germany

10.15 **Coffee break**

10.45 Artificial water channels

Mihai Barboui, Montpellier, France

11.15 How zinc affects structure and function of voltage-gated proton channels

Boris Musset, Nurnberg, Germany

11.45 A toolbox to study cellular dynamics – a case study on the architecture of nuclear bodies

Klaus Weisshart, Zeiss Jena, Germany

13.00 Lunch

14.00 **Poster Flashs**

15.00 **Coffee break**

15.30 **POSTER** session

17.00 → Board Meeting

19.00 Dinner

Saturday, July 24, 2021

08.45 Probing the activity of heme copper oxidases at the single enzyme level

Stephan Block, Berlin, Germany

09.15 The 3-0-6 hydration of the $(GPP)_N$ collagen peptide

Noam Agmon, Jerusalem, Israel

09.45 Helicobacter pylori drug discovery using cryo EM:
Targeting the evil duo of a pH-gated urea channel and a cytoplasmic urease

Hartmut Lücke, Oslo, Norway

10.15 **Coffee break**

10.45 Molecular level probing and quantification of lipid droplet, liposome and liquid condensate interfaces in aqueous solution, using nonlinear light scattering

Sylvie Roke, Lausanne, Switzerland

11.15 Interplay of hydration, water mobility, and proton transfer in cytochrome C oxidase

Petra Imhof, Erlangen, Germany

11.45 Delivering drugs with carbon nanotubes, not through them

Gerhard Hummer, Göttingen, Germany

12.15 Closing Remarks

12.30 Lunch

Nanoscopy of 2D materials in liquid

Aleksandra Radenovic

Laboratory of Nanoscale Biology, Institute of Bioengineering, School of Engineering, EPFL, 1015 Lausanne, Switzerland

The simplicity and versatility of optical microscopy make it from the start the workhorse technique in the characterization of 2D materials (*1*). Optical microscopy is used to locate and determine the thickness of the 2D material by measuring its optical contrast with respect to the Si/SiO_2 substrate (*2*). Although in terms of technology, the large-area growth of 2D materials is about to be mastered soon, as-grown 2D materials still host abundant and different types of defects such as vacancies, adatoms, grain boundaries (GBs), edges, and impurities, which strongly influence their properties (*3*). In most cases, the presence of defects is disadvantageous. However, not all defects in 2D materials are detrimental. Some 2D materials have been shown to host defects that can serve as single-photon emitters (SPEs) at cryogenic temperatures for TMDs (*4-7*) and room temperature for h-BN (*8*). This discovery has motivated the search for single-photon sources in other 2D materials and efforts that aim to engineer the defects in well-controlled locations either using strain-induced potential traps (*9*) or via quantum dot confinement (*7*). We have explored the single-molecule localization microscopy to characterize defects in hexagonal boron nitride (*10*). In addition to precise location of the optically active defects, we record their spectral properties using spectral SMLM (11) and use them as a platform that allowed us to track excess protons in interfacial water (12) . Focusing on sulfur-vacancies in TMDs, we demonstrate large-scale mapping of non-emissive defects by coupling single-molecule localization microscopy with fluorescent labelling using thiol chemistry, reminiscent of Point Accumulation for Imaging in Nanoscale Topography (PAINT). We found non-homogenous resonance energy transfer efficiency on MoS2 dependent on the defect structure (grain boundaries and line defects). Moreover, our method allows us to probe the defects that are chemically active in aqueous conditions and reveal insights of the interaction between the thiol group and the sulfur-vacancy. Importantly, our methodology paves the way for in-situ and spatially-resolved monitoring of the interaction between the chemical agent and the defects in 2D materials that has general implications for defect engineering in aqueous conditions. With the versatile chemical labelling strategy, our method is generally applicable to a variety of defects in 2D materials (13).

1. K. S. Novoselov *et al.*, Two-dimensional atomic crystals. *Proc Natl Acad Sci U S A* **102**, 10451-10453 (2005).
2. M. Benameur *et al.*, Visibility of dichalcogenide nanolayers. *Nanotechnology* **22**, 125706 (2011).
3. F. Banhart, J. Kotakoski, A. V. Krasheninnikov, Structural defects in graphene. *ACS Nano* **5**, 26-41 (2011).
4. D. Dumcenco *et al.*, Large-Area Epitaxial Mono layer MoS2. *Acs Nano* **9**, 4611-4620 (2015).
5. C. Chakraborty, L. Kinnischtzke, K. M. Goodfellow, R. Beams, A. N. Vamivakas, Voltage-controlled quantum light from an atomically thin semiconductor. *Nat Nanotechnol* **10**, 507-511 (2015).
6. M. Koperski *et al.*, Single photon emitters in exfoliated WSe2 structures. *Nat Nanotechnol* **10**, 503-506 (2015).
7. A. Srivastava *et al.*, Optically active quantum dots in monolayer WSe2. *Nat Nanotechnol* **10**, 491-496 (2015).
8. T. T. Tran, K. Bray, M. J. Ford, M. Toth, I. Aharonovich, Quantum emission from hexagonal boron nitride monolayers. *Nat. Nanotechnol.* **11**, 37-41 (2016).
9. G. Grosso *et al.*, Tunable and high-purity room temperature single-photon emission from atomic defects in hexagonal boron nitride. *Nat Commun* **8**, 705 (2017).
10. J. Feng *et al.*, Imaging of optically active defects with nanometer resolution. *Nano Lett*, (2018).
11. J. Comtet *et al.*, Wide-field spectral super-resolution mapping of optically active defects in hBN. *arXiv preprint arXiv:1901.06989*, (2019).
12. Comtet J., *et al.*, Direct Observation of Water Mediated Single Proton Transport be-tween hBN Surface Defects. Nature Nanotechnology 2020. DOI 10.1038/s41565-020-0695-4
13. Zhang M., *et al.*, Super-resolved optical mapping of reactive sulfur-vacancy in 2D transition metal dichalcogenides (2020) arXiv:2006.12181

Oral – On-site

PROTEIN HYDRATION AND DYNAMICS PROBED BY TIME DEPENDENT FLUORESCENCE SHIFTS: DEHALOGENASES, ATPASE AND UREA CHANNEL HP-UREI

Abbinhav, Jan Sýkora, Martin Hof

J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences, Dolejškova 2155/3, 18223, Prague 8, Czech Republic

The hydration and mobility of proteins are believed to profoundly affect their function[1]. However, only a few approaches for monitoring these characteristics within the relevant protein regions are available. In our lab we developed „time dependent fluorescence shift“ (TDFS) technique for probing site-specific hydration and mobility[2]. In this contribution the principles of the TDFS technique are outlined and its application presented for 3 different protein classes and their specific regions: The tunnel mouth of dehalogenase enzymes[3], the active site of copper-transporting membrane protein ATPase LpCopA[4], and in ongoing experiments the periplasmic loop of urea channel HpUreI.

(1) Levy, Y.; Onuchic, J. N. Annu. Rev. Biophys. Biomolec. Struct. 2006, 35, 389.

(2) Jesenská, A., J. Sýkora, A. Olžyńska, J. Brezovský, Z. Zdráhal, J. Damborský, M. Hof. J. Am. Chem. Soc., 2009. 131(2): p. 494-501

(3) Sykora, J.; Brezovsky, J.; Koudelakova, T.; Lahoda, M.; Fortova, A.; Chernovets, T.; Chaloupkova, R.; Stepankova, V.; Prokop, Z.; Kuta Smatanova, I.; Hof, M.; Damborsky, J. Nat. Chem. Biol. 2014, 10, 428.

(4) Fischermeier, E.: Pospisil, P.; Sayed, A.; Hof, M.; Solioz, M.; Fahmy, K. Angew. Chemie Int. Ed. 56, 1269–1272 (2017).

Facilitation of H^+ conductance by ANT1

Jürgen Kreiter[1], Zlatko Brkljača[2], Sanja Škulj[2], Mario Vazdar[2,3], Elena E. Pohl[1]

[1]Institute of Physiology, Pathophysiology and Biophysics, Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria
[2]Division of Organic Chemistry and Biochemistry, Rudjer Bošković Institute, Zagreb, Croatia
[3]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

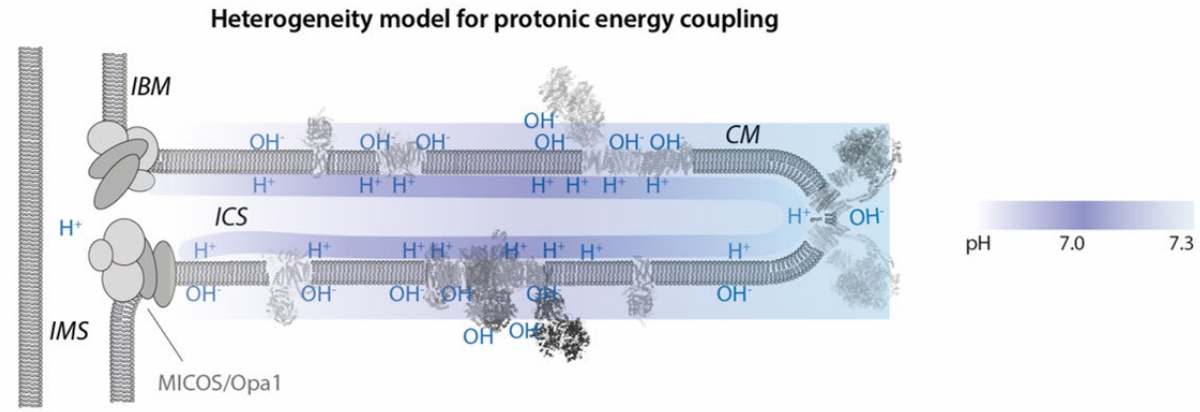
Adenine nucleotide translocase (ANT) is a well-known mitochondrial carrier, which exchanges ATP against ADP. Its proton-transporting function is much less known. Earlier, we have shown that it is well described by the fatty acid (FA) cycling hypothesis. According to this hypothesis, ANT facilitates FA anion (FA^-) translocation across the inner mitochondrial membrane. However, thus far, the detailed molecular mechanism has remained elusive. Here, we employ recombinant murine ANT1, and its mutants reconstituted into planar lipid bilayers to investigate the pathway for the translocation of FA^- alongside ANT1. We show that the FA^- is initially caught by R59 at the matrix-lipid interface, moves on the protein-lipid interface, and binds to R79, where it is protonated in the hydrated cavity with the assistance of D134. Further, we used molecular dynamics simulations to visualize the whole pathway for the first time. We concluded that R79 is a crucial binding site for several ANT1 substrates, including CATR, BA, DNP, purine nucleotides, and fatty acids. Since the identified binding sites are well conserved, we hypothesize a similar FA^- transport mechanism for further proton-transporting SLC25 members, including uncoupling proteins.

MEASUREMENT OF MITOCHONDRIAL PROTON GRADIENTS IN LIVING CELLS USING LOCAL FLUORESCENT SENSORS

B. Rieger[1], J. Villalta[1] and K. B. Busch[1]

Faculty of Biology, Institute of Molecular Cell Biology, Schloßplatz 5, 48941 Münster, Germany.

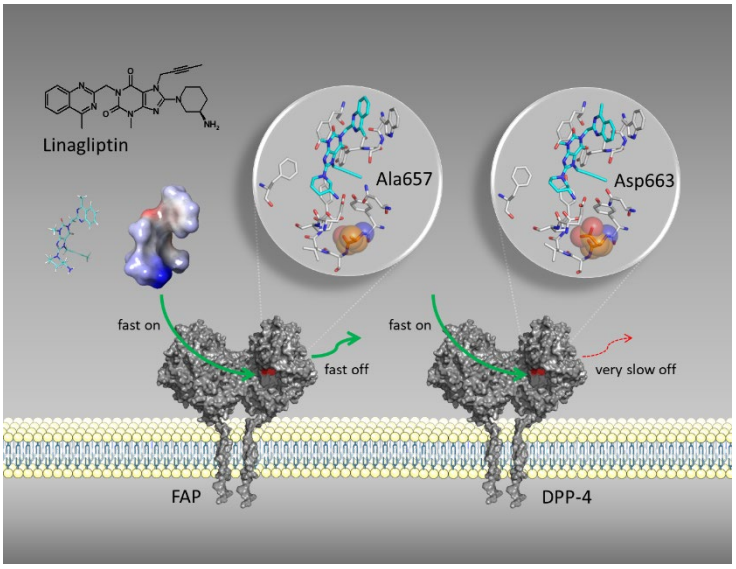
The inner mitochondrial membrane is multiply folded into cristae. Complexes I, III and IV pump protons into the intracristae space thereby generating the proton motor force (PMF). ATP synthase uses PMF to generate ATP. In addition to the transmembrane pH gradient, a lateral pH gradient between the proton pumps and ATP synthase was discovered (Rieger et al., 2014; Sjoholm et al., 2017; Toth et al., 2020). This lateral ΔpH exists because F1FO-ATP synthase is physically separated from the primary proton pumps (Davies et al., 2011, Vogel et al., 2006) and diffusion is apparently insufficient for equilibration. Here, we present pH maps of mitochondria under OXPHOS and glycolytic conditions. Using a ratio-metric pH sensor genetically fused to different subunits of OXPHOS complexes on both sides of the membrane, we obtained the following results: Under hyperglycolytic conditions, the lateral pH gradient intervened (i), the pH at SU e of ATP synthase in the intracristae space was lower than under OXPHOS conditions (ii), and the pH at subunit γ was not in equilibrium with the pH in the matrix. Overall, our data support the model of a localized and heterogeneous proton motive force. Moreover, ATP synthase/hydrolysis activity is a major determinant of PMF.



THE ROLE OF A PATCH OF ACIDIC RESIDUES IN DPP-4 IN ENZYME ACTIVITY AND SPECIFICITY AND LIGAND BINDING

Herbert Nar, Remko A. Bakker, Yvette Hoevels, Thomas Klein, Gisela Schnapp

Department of Medicinal Chemistry and Department of Cardiometabolic Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorferstr.65, 88397 Biberach (Germany)



Human type 4 dipeptidyl peptidase (DPP-4) and fibroblast activation protein alpha (FAP-α) are representatives of the S9B prolyl oligopeptidase subfamily of the SC clan proteases, which typically comprise serine proteases that cleave peptide substrates after a proline residue. Type4 dipeptidyl peptidase is expressed ubiquitously and is an important therapeutic target for Type-2 diabetes mellitus (T2DM) because it cleaves and inactivates insulinotropic peptides such as glucagon-like peptide1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). Inhibition of DPP-4 limits GLP-1 and GIP metabolic breakdown, leading to improved glycemic control in patients with T2DM. Currently there are eight commercially available DPP-4 inhibitors for the treatment of T2DM (gliptins). The DPP-4 binding kinetics of a representative set of gliptins was investigated using surface plasmon resonance. Binding of gliptins to DPP-4 is a rapid process dominated by the electrostatic attraction of the positively charged amino functionality of the gliptins to a glutamate dyad in the active site of DPP-4. Association rates correlate with the pKa of the ligands. Dissociation rates were generally slow partly because of reversible covalent bond formation by some gliptins, and partly because of strong and extensive interactions.

DPP-4 and FAP-α exhibit distinct substrate specificities and pH dependencies of their protease activities despite almost identical active site structures. Likewise, linagliptin binding to DPP-4 and FAP-α is drastically different in terms of affinities and binding kinetics.

We show here that an aspartate residue, located in the second shell around the DPP-4 active site and mutated to alanine in FAP-α, is causal to the observed distinct behavior.

INTERFACIAL PROTON DIFFUSION

Peter Pohl

[1]*Insitute of Biophysics, Johannes Kepler University, Linz, Austria.*

Proton transport displays many peculiarities: (i) Spontaneous translocation across membranes takes place much faster than predicted by Overton's rule[1]; (ii) Facilitated translocation across membrane channels specialized in H^+ transport may occur at a rate beyond the diffusion limit; and (iii) A tenfold reduction in the aqueous proton concentration may produce a less than tenfold drop of the channeled proton flux. Lateral proton movement along the membrane surface towards the channel mouth explains the apparent increase in diffusibility (peculiarity II). It requires an energy barrier for the proton surface to bulk release[2]. This barrier increases the actual proton concentration at the channel mouth above the values predicted from bulk pH. Conducting pH jump experiments at different temperatures allowed us to determine its height[3] for membranes without a net charge, negatively charged membranes, and positively charged membranes. Its enthalpic component is comparable to the energy of one hydrogen bond– the energy of the electrostatic contributions from membrane surface charges not included. Importantly, its entropic component is larger. The less than proportional effect of aqueous H^+ concentration on single-channel conductance (peculiarity III) points toward a rate-limiting transport step inside the channel. Performing water flux measurements confirmed the hypothesis. The voltage-gated proton channel H_v1 is occluded for water in its H^+ - conducting conformation. That is, H_v1's water permeability is immeasurably tiny and, thus, much smaller than predicted from the number of hydrogen bonds between intraluminal water molecules and the channel wall[4]. The identities of (i) the pore blocking amino acid and (ii) the amino acids connecting the intraluminal and membrane surface H^+ wires remain to be identified.

1. C. Hannesschlaeger, A. Horner and P. Pohl, *Chem. Rev.*, 2019, **119**, 5922-5953.
2. A. Springer, V. Hagen, D. A. Cherepanov, Y. N. Antonenko and P. Pohl, *Proc. Natl. Acad. Sci. U.S.A*, 2011, **108**, 14461-14466.
3. E. Weichselbaum, M. Österbauer, D. G. Knyazev, O. V. Batishchev, S. A. Akimov, T. H. Nguyen, C. Zhang, G. Knör, N. Agmon, P. Carloni and P. Pohl, *Scientific Reports*, 2017, **7**, 4553.
4. A. Horner, F. Zocher, J. Preiner, N. Ollinger, C. Siligan, S. A. Akimov and P. Pohl, *Science Advances*, 2015, **1**, e1400083.

Oral – On-site

Graph-based algorithms for protonation-coupled membrane transporters and receptors

Ana-Nicoleta Bondar

[1]University of Bucharest, Faculty of Physics, Str. Atomiştilor nr. 405, Măgurele 077125, Romania
[2]Forschungszentrum Jülich, Institute for Advanced Simulation, Computational Biomedicine, IAS-5/INM-9

Dynamic protein-water hydrogen bond networks participate in allosteric conformational couplings and proton transfers of membrane receptors and transporters whose functioning couples to protonation change. The identity of the proton-binding site, and of paths that connect this site to remote regions of the protein, are of central interest for formulating hypotheses about reaction mechanisms. Addressing these aspects is, however, often challenging given the large number of coordinate snapshots and hydrogen bonds that would need to be analyzed.
Graph-based algorithms we developed enable efficient analyses of dynamic protein-water hydrogen-bond networks, and automated identification of hydrogen-bond motifs commonly present at proton-binding sites, or at other functionally important sites. Computations of conserved protein H-bond graphs allow us to identify core hydrogen-bond networks that inter-connect key functional motifs of G Protein Coupled Receptors, and underline the role of dynamic fluctuations in establishing transient paths for long-distance allosteric coupling.

Research was supported in part by the German Research Foundation (Collaborative Research Center SFB 1078 Project C4, by the European Union's Horizon 2020 Research and Innovation Program, Marie-Sklodowska-Curie grant agreement 860592, Innovative Training Network Proton and Proton-Coupled Transport, and by computing time from the HLRN, The North-German Supercomputing Cluster.

References

Bertalan E, Lesnik S, Bren U, Bondar A-N. Protein-water hydrogen-bond networks of G Protein-Coupled Receptors: Graph-based analyses of static structures and molecular dynamics. *Journal of Structural Biology* 212, 107634 (2020)

Bondar A-N. Proton-binding motifs of membrane-bound proteins: From bacteriorhodopsin to spike protein S. *Frontiers in Chemistry*, doi: 10.3389/fchem.2021.685761 (2021)

Lazaratos M, Karathanou K, Bondar A-N. Graphs of dynamic H-bond networks: from model proteins to protein complexes in cell signaling. *Current Opinion in Structural Biology* 64, 79-87, 2020

Siemers M, Bondar A-N. Interactive interface for graph-based analyses of dynamic H-bond networks: Application to spike protein S. *Journal of Chemical Information and Modeling*, doi: 10.1021/acs.jcim.1c00306 (2021)

"I am not interested in your results - i am only interested in your controls": Lessons learned from bee-keeping

Stefan Hannus

Intana Bioscience GmbH, Martinsried, Germany

Bees are of central importance for food production as pollinators of fruit and vegetable plants. The parasitic mite Varroa destructor is the main cause for the dramatic losses of bee colonies worldwide. An RNAi based therapeutic approach to knockout essential genes in Varroa by horizontal gene transfer yields surprising results and emphasizes the importance of negative controls.

Oral – On-site

ARTIFICIAL WATER CHANNELS

Mihail Barboiu

Institut Europeen desMembranes, ENSCM-UMII-UMR CNRS 5635, Place Eugene Bataillon CC047, 34095 Montpellier, France. Email: mihail-dumitru.barboiu@umontpellier.fr

This lecture discusses the incipient development of the first artificial water channels systems. We include only systems that integrate synthetic elements in their water selective translocation unit. Therefore, we exclude peptide channels because their sequences derive from the proteins in natural channels. We review many of the natural systems involved in water and related proton transport processes. We describe how these systems can fit within our primary goal of maintaining natural function within bio-assisted artificial systems. In the last part, we present several inspiring breakthroughs from the last decade in the field of biomimetic artificial water channels. All these examples demonstrate how the novel interactive water-channels can parallel biomolecular systems.

[1] M. Barboiu and A. Gilles, *Acc. Chem. Res.* 2013, 46, 2814–2823.
[2] M. Barboiu, *Angew. Chem. Int. Ed*. 2012, 51, 11674-11676.
[3] Y. Le Duc, et al., *Angew. Chem. Int. Ed.* 2011, 50(48), 11366-11372.
[4] E. Licsandru, et al., *J. Am. Chem. So*c., 2016, 138, 5403-5409.
[5] M. Barboiu, *Chem. Commun*., 2016, 52, 5657- 5665.
[6] I. Kocsis, et al. *Science Adv.* 2018, 4, eaao5603.
[7] M. Di Vincenzo, et al. *Nat. Nanotechnol.* 2020, *16*, 190-196.

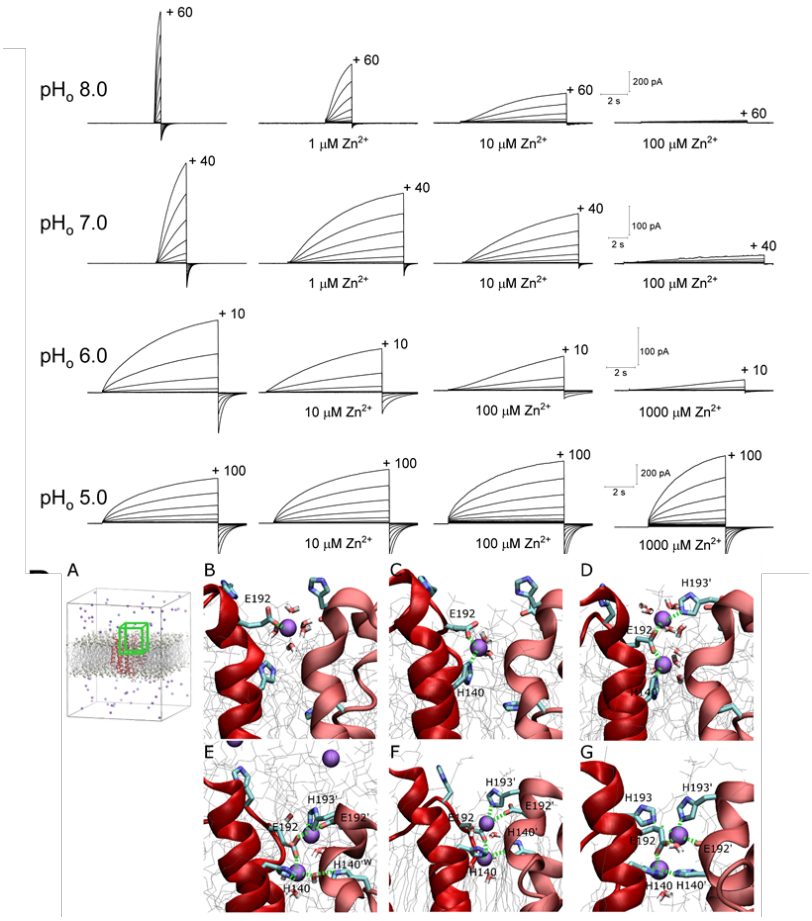
Oral – On-site

How zinc affects structure and function of voltage-gated proton channels

B. Musset

Insitute of Physiology, Pathophysiology and Biophysics, PMU Nuremberg , Nuremberg, Germany.

Voltage-Gated proton channels (H_V1s) are unique in the huge family of voltage-gated ion channels. They consist out of four transmembrane domains homologous to the voltages sensing domains in classical voltage-gated ion channels but they are lacking the pore domain. Structurally, H_V1s are dimers composed out of two homo monomers. They are solely conducting protons and have many implications in physiology. The metal zinc is the most researched inhibitor of voltage-gated proton channels. However, in many species the effect of inhibition of H_V1s by zinc varies drastically. Here, we analyse the effects of zinc on the voltage-gated proton channel of human and insect via different approaches e.g. pH titration of zinc binding, amino acid substitution, HH model, and Molecular Dynamics simulation. Our data disagrees with recent publications using comparable approaches. We see a minor effect on H_V1 function by zinc binding to negative charged amino acids compared to histidines. We measure that zinc binding does not affect the voltage dependence of gating. Our MD simulations suggest a zinc binding sites in between the dimer instead of zinc binding in each monomer. Taken together, zinc inhibition is a tool to decipher structure function relationship of voltage-gated ion channels.



A toolbox to study cellular dynamics – a case study on the architecture of nuclear bodies

Klaus Weisshart, Carl Zeiss Jena GmbH, Carl-Zeiss-Promenade 10, 07745 Jena, Grmany

Microscopic (scanning the laser beam over the sample) as well as spectroscopic (parking the beam at one site) methods contain a plethora of information on the dynamic of a process in the living cell. Either can intensity changes be exploited like in Fluorescence Redistribution after Photobleaching (FRAP), or fluctuation analyses can be performed on a time stack of images like done in Raster Image Correlation Scanning (RICS) or Number and Brightness (N & B) correlative techniques. Likewise, Fluorescence Correlation Spectroscopy (FCS) can yield valuable information on diffusion coefficients, concentrations, and interactions of molecules. We demonstrate the possibilities of these technologies using the nuclear Promyelocytic Leukaemia (PML) bodies and some of their constituent proteins.

Probing the activity of heme copper oxidases at the single enzyme level

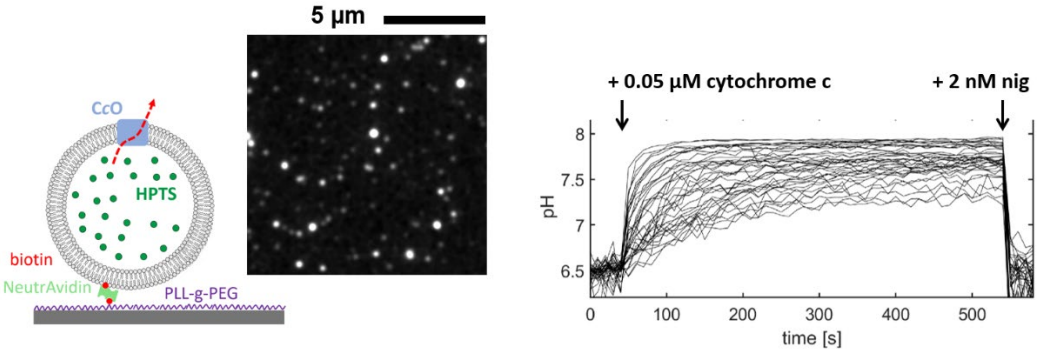
Stephan Block (stephan.block@fu-berlin.de)

Institute of Chemistry and Biochemistry, Freie Universität Berlin, D-14195 Berlin, Germany

Abstract

The A-type heme copper oxidases (HCO) cytochrome *c* oxidase (C*c*O) and cytochrome bo_3 ubiquinol oxidase are terminal oxidases in the respiratory chain and contribute to the generation of an electrochemical gradient across the membrane, which is consumed by ATP synthases to generate ATP from ADP. Due to their high relevance for the bioenergetics of cells, these proteins have been extensively studied, yielding important insights in the intricate interplay of electron uptake, oxygen reduction, and proton translocation, all of which participate in a redox reaction that finally leads to reduction of oxygen to water. Previous research, however, was mainly focused on the turnover of oxygens and electrons by HCOs, while studies reporting the proton turnover rate, that is the rate of proton uptake by the enzyme, are scarce.

In this talk, I will introduce a proteoliposome-based assay, in which HCOs are reconstituted into liposomes containing a pH sensitive dye [1,2]. Immobilizing the proteoliposomes at an interface enables to probe the luminal pH value on the single-proteoliposome level using optical microscopy and thus to quantify changes of the luminal pH value, which are caused by proton uptake by single HCOs. It will be shown that this assay enables to quantify the dynamics of proton uptake by single oxidases and for 100s of individual oxidases in parallel (see figure). Furthermore, this assay enables to systematically vary key parameters of the catalytic reaction, *e.g.*, the type of HCO studied, its lipid environment, as well as the electron donating substrate, and hence to probe their impact on the proton uptake dynamics of single HCOs.



References: [1] Berg J, Block S, Höök F, Brzezinski P*. *Israel Journal of Chemistry* **57**, 437-445 (2017); [2] Hugentobler KG, Heinrich D, Berg J, Heberle J, Brzezinski P, Schlesinger R, Block S. *International Journal of Molecular Sciences* **21**, 6981 (2020).

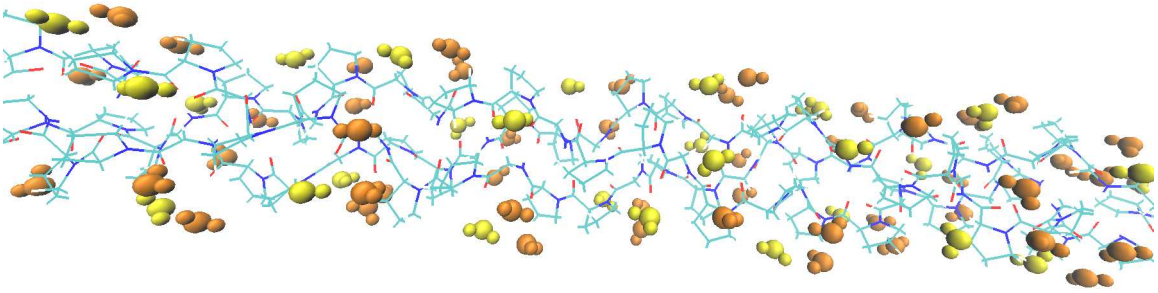
Oral – On-site

THE 3-0-6 HYDRATION OF THE $(GPP)_N$ COLLAGEN PEPTIDE

Satyaranjan Biswal and Noam Agmon

The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

Collagen is the most abundant protein in the body, contributing profoundly to the extra-cellular matrix structure, mechanical properties, and function. Its smallest structural unit is the tropocollagen triple helix, constituting of $(GXY)_n$ polypeptides, where G is glycine, while X and Y have a high probability of being proline or hydroxyproline. As proteins do not function without their hydration layers, collagen hydration has been the subject of extensive research. Several studies suggested that water molecules form bridges connecting different triple-helix chains. Here we focus on the hydration and hydrogen bonding (HBing) properties of the bb-carbonyl oxygen atoms. We have conducted classical molecular dynamics (MD) simulations of the $(GPP)_{10}$ triple helix and calculated the radial distribution function (RDF) of water molecules around each CO moiety and its integral, which gives the number of nearest neighboring water molecules. Gly-CO always has a single water ligand. The situation for the prolines is less trivial. The one in position X forms a strong HB to Gly-NH, and hence is unavailable for binding water. Interestingly, its role is taken over by proline Y, which binds *two* water molecules to its bb-CO moiety. Thus, in the triple helix, the GPP unit attaches 3 (G), 0 (P), and 6 (P) water molecules through strong HBs, and this seems to be the basic hydration pattern of imino-acid rich collagens.



HARTMUT LÜCKE

Oral – On-site

Molecular level probing and quantification of lipid droplet, liposome and liquid condensate interfaces in aqueous solution, using nonlinear light scattering.

S. Roke*

*Laboratory for fundamental BioPhotonics (LBP), Institute of Bio-engineering (IBI), and Institute of Materials Science (IMX), School of Engineering (STI), and Lausanne Centre for Ultrafast Science (LACUS), École Polytechnique Fédérale de Lausanne (EPFL), CH-1015, Lausanne, Switzerland, *sylvie.roke@epfl.ch*

Biomolecular condensates and liquid-liquid phase separated systems are very important players in a plethora of cellular processes. Together with compartmentalized membrane bound systems, such as liposomes and lipid droplets they participate in a wide variety of biological processes. In order to understand the role of these organelles in biology it is necessary to understand the physical and interfacial chemistry of these structures as well as their dynamical interactions. This requires understanding the role of water and the molecular interfacial chemistry and biophysics of these (sub)micron-sized objects. This requires probes of molecular (interfacial) structure that can be applied in-situ.

Vibrational sum frequency scattering and angle resolved second harmonic scattering are two unique nonlinear light scattering techniques that can quantify interfacial structure on the molecular level (see [1] for a perspective). Properties such as the molecular conformation, orientational distribution, relevant chemical interactions and the electrostatic surface potential can be determined in-situ. In this presentation I will introduce these methods and show applications to understand and control the properties of model lipid droplets as well as unilammelar lipid vesicles in aqueous solution.

References

[1] H. I. Okur, O. B. Tarun, S. Roke, J. Am. Chem. Soc (2019), 141, 31, 12168-12181

Oral - Online

INTERPLAY OF HYDRATION, WATER MOBILITY, AND PROTON TRANSFER IN CYTOCHROME C OXIDASE

Rene F. Gorriz[2], Vincent Stegmaier[2], Marco Reidelbach[2], Senta Volkenandt[1,2], Tahereh Ghane[2], and Petra Imhof [1,2]

[1]Friedrich-Alexander (FAU) Universität Erlangen-Nürnberg, Erlangen, Germany

[2]Freie Universität Berlin, Berlin, Germany

Cytochrome c Odixase (CcO) is a protein in the respiratory chain that uses the energy from oxygen reduction to water to pump protons through the membrane. Proton uptake from the inner side of the membrane to the chemical redox centre takes place through two channels, named D or K, after an important Asp or Lys residue, respectively.

Our simulations show that the protonation state of the two channels has an impact on the hydration level within the two channels [1] and of the communication within and between the two channels [2]. For the D-channel, the hydration level is lower when the proton has already reached E286 at the end of the channel as can be explained by the hydrogen-bonded network pointing from E286 to the asparagine gate (formed by N139 and N121), favouring a “closed” conformation [1]. The thus prevented water passage also blocks the most favourable pathway [3] for proton transfer in the D-channel.

In the K-channel, the hydration level depends critically on the position of the excess proton, suggesting that the proton drags its own hydration sphere with it. The probabilities for proton transfer depend on the hydration level and the directionality of the hydrogen-bonded networks which both are coupled to the conformation of K362 [4]. When protonated, K362 is pre-dominantly in an “up” conformation [2] that allows proton transfer only in the upper part of the channel [4], serving as a barrier for passage of an extra proton or back leakage.

The interplay of hydration, water mobilita, and proton transfer in the two channels can thus be regarded as an auto-regulation, allowing proton passage only when required and preventing it, once the proton has reached the upper part of the channel and is therefore close to the redox centre.

[1] T. Ghane, R.F. Gorriz, S. Wrzalek, S. Volkenandt, F. Delatieh, M. Reidelbach, and P. Imhof, J. Membr. Biol. 251 299 (2018).

[2] M. Bagherpoor Helabad, T. Ghane, M. Reidelbach, A.L. Woelke, E.W. Knapp, and P. Imhof, Biophys. J. 113 817 (2017).

[3] M. Reidelbach and P. Imhof, Biochim. Biophys. Acta General Subjects 1864 129614 (2020).

[4] V. Stegmaier, R.F. Gorriz, and P. Imhof, Processes 9 265 (2021).

DELIVERING DRUGS WITH CARBON NANOTUBES, NOT THROUGH THEM

G. Hummer[1,2]

[1]*Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Frankfurt am Main, Germany*

[2]*Institute of Biophysics, Goethe University, Frankfurt am Main, Germany*

Abstract: Carbon nanotubes are nanofluidics channels with unique properties. Embedded into membranes, they can mediate the fast transfer of ions and protons. However, inserted into lipid membranes they act not just as passive pores but as active mediators of membrane remodelling processes. Our earlier molecular dynamics simulations (Bhaskara, Linker et al., ACS Nano 2017; https://doi.org/10.1021/acsnano.6b05434) had suggested that nanotubes could induce membrane fusion. This finding has now been verified by experiments (Ho, Siggel et al., PNAS 2021; https://doi.org/10.1073/pnas.2016974118). By combining experiments and simulations, nanotube dimers were found to be powerful fusogens. Nanotube-mediated fusion of drug-carrying lipid vesicles with cell membranes provides a new platform for the encapsulation and efficient delivery of therapeutics.

Poster - On-site

Hydration and dynamics of function-relevant region of HpUreI

Abhinav, Jan Sykora and Martin Hof

Department of Biophysical Chemistry, J. Heyrovsky Institute of Physical Chemistry, Dolejškova 2155/3, 182 23 Prague 8, Czech Republic

ABSTRACT: Around two-thirds of the world's population have been infected with Helicobacter pylori. This gram-negative bacteria thrives in the digestive tract, causing extreme gastritis, ulcers, gastric adenocarcinoma, and even cancer. To survive in the extremely acidic environment of the stomach, H.pylori produces a proton-gated urea channel HpUreI. This periplasmic inner membrane protein selectively enables only urea molecules to enter cytosol. The urea is then hydrolyzed by urease enzymes producing carbon dioxide and ammonia which are transferred back to periplasm to neutralize the pH (~5). The opening of HpUreI is triggered by the depressed pH in periplasm and majorly 6 important amino acid residues (His-123, His-131, Asp-129, Asp-140, Glu-138 and Lys-132) in periplasmic loop 2 (PL 2) play a vital role in the functioning of this urea channel protein. Our research attempts to link the proton transfer and solvent dynamics to predict the mechanism of involvement of this periplasmic loop 2 in opening and closing of HpUreI by examining the hydration and dynamics in the immediate vicinity of these important amino acid residues. Here we try to label HpUreI protein with highly microenvironment sensitive dye and with the use of time-dependent fluorescence shift data we analyzed the hydration and mobility at these functionally relevant regions. Our studies will elucidate the role of these regions in the proton gating mechanism.

https://cesnet.zoom.us/j/93065118348?pwd=TDZ4ZUJtc240M0pmNmI0cUJNMjFVdz09

Poster - On-site

Self-assembled artificial water and proton channels based on combinatorial self-assembly of tubular Pillar[5]arenes

I.M. Andrei[1], D. Strilets[1], T. Ogoshi[2, 3], M. Barboiu[1]

[1]Institut Europeen des Membranes, Adaptative Supramolecular Nanosystems Group, University of Montpellier, ENSCM-CNRS, Montpellier, France

[2]Department of Synthetic Chemistry and Biological Chemistry Graduate School of Engineering, Kyoto University Katsura, Kyoto, Japan

[3]WPI Nano Life Science Institute, Kanazawa University Kakuma-machi, Kanazawa, Japan

Artificial water channels that can transport water molecules through cell membranes have potential to act as analogues of protein aquaporins. The osmotic permeability of artificial water channels is within the range of aquaporins and so suitable for transporting water at low energy. Compared to aquaporins, artificial water channels can have a similar osmotic permeability, better stability, are simpler to manufacture on a larger-scale, and have a higher functional density when inserted into the cell membrane. However, few artificial water channels are known, most likely due to the difficulty of detecting selective water transport in artificial systems. Here, we report Pillar[5]arenes that form artificial channels with a tubular structure (**PA**) are efficient water transporters in bilayer membranes. Combinatorial self-assembly of functional Pillar[5]arenes A-D lead to synergic increase of water permeabilities, while preventing the translocation of hydrated cations. In our experiments, these artificial water channels presented good selectivity towards water translocation and no transport for Na^+ and K^+ cations. In case of proton transport, the membrane polarization via valinomycin and FCCP generates a strong protonation of PA followed by a low proton antiport rates through the assembled channel.

https://us05web.zoom.us/j/84664550337?pwd=VHdXcEY4cGg2ZnU0akk4YUpQdWorZz09

Meeting ID: 846 6455 0337

Passcode: kH9v7A

Binding mode characterization of PfFNT' inhibitors through Docking and MD simulations.

Martínez-León A. [1], Hub J. S.[1]

[1]Universität des Saarlandes, Saarbrücken, 66123, Germany.
E-mail: alejandro.martinezleon@uni-saarland.de

Malaria is a key threat to public health worldwide. Recently, Plasmodium-faciparum formate-nitrite-transporter (PfFNT) has been identified as the malaria parasite's lactate transporter and as a novel drug target. A few putative inhibitors for PfFNT have been identified. However, their mechanism of binding and inhibition is not well understood. Here, we used molecular dynamics simulation to study the function and inhibition of PfFNT at an atomic level. The ligands MMV007839 and BH267.meta have been identified as potential inhibitors. For these ligands, we derived new parameters based on GAFF2. To do this, we used the HTMD Parameterize tool complemented with Stochastic Conformational Analysis at the semi-empirical level with ab initio refinement. The new parameters reproduce the dihedral potentials of these ligands at the DF-MP2-aug-cc-pVTZ level of theory. This is a remarkable improvement relative to initial GAFF2 parameters. In silico, we docked the ligands into the putative binding site in the PfFNT structure. Our initial simulations agree with the reported experimental results.

https://meet.google.com/cyb-megw-vgi

Poster - Online

GPR68 – AN ORPHAN GPCR

Bhav Kapur[1], Ana-Nicoleta Bondar[2], Alejandro Giorgetti[3], Christopher Tautermann[1], Gisela Schnapp[1], Herbert Nar[1]

[1]Boehringer Ingelheim Pharma GmbH & Co. KG, Germany, [2]Freie Universität Berlin, Germany, [3]Forschungszentrum Jülich GmbH, Germany.

Abstract:

GPR68 belongs to the Class A family of Orphan G-Protein Coupled Receptors (GPCRs). It is a member of sub-family of proton sensing GPCRs. GPR68 is stimulated by the protons in the extracellular environment. Acidification is a classic feature of tumor microenvironment. GPR68 has been associated with multiple types of cancers, brain disorders, Crohn's disease, Amelogenesis imperfecta, bone resorption disorders as well as idiopathic pulmonary fibrosis. The structure of GPR68 is not known and very little is understood about its mechanism of function. Extracellular histidines and buried acidic residues are suggested to play a role in the proton-sensing activity of GPR68. Apart from proton-sensing, GPR68 has also been linked to mechano-sensing. Although, this is not a thoroughly explored territory. Divalent metal ions have shown to modulate the activation and inactivation of GPR68 in a pH and concentration dependent manner. Our aim is to solve the structure of human GPR68 to establish the initial structure-function relationship. Cryo-EM and Lipidic Cubic Phase (LCP) crystallization will be the tools for structure solution of GPR68. Computational studies with homology models based on Proteinase Activated Receptor 1 (PAR1) are being undertaken. Hydrogen bond network analysis using BRIDGE is being carried out for a deeper understanding of the receptor. The 3D structure information and a better understanding of the receptor's function will enable us to design selective antagonists for GPR68, which can help to mitigate the effects of inflammation and fibrosis. Currently, no information is available about antagonists linked to GPR68 and only non-selective positive allosteric modulators like Ogerin and Lorazepam are described for GPR68.

https://teams.microsoft.com/l/meetup-join/19%3ameeting_MjY1NmExMmUtNTE3MS00Mjc5LWEwM2YtMWZhMWZlNzM5Mjly%40thread.v2/0?context=%7b%22Tid%22%3a%22e1f8af86-ee95-4718-bd0d-375b37366c83%22%2c%22Oid%22%3a%223497f415-68e2-48bf-9579-ce7e2cb27a30%22%7d

SURFACE CHARACTERIZATIONS OF SILICA NANOPARTICLES IN AQUEOUS SOLUTIONS UNDER THE EFFECT OF SIZE AND PH

B.Chu[1], A. Marchioro[1], S. Roke[1]

[1]Laboratory for fundamental BioPhotonics (LBP), Institute of Bioengineering (IBI), École polytechnique fedérale de Lausanne (EPFL), Lausanne, Switzerland;

Colloidal silica nanoparticles are important nanomaterials with wide application in the food and healthcare industries and many other emerging fields. When the nanoparticles are in aqueous solutions, the silica-water interface crucially affects its stability and physical chemistry properties. However, the molecular structure of water molecules around the nanoparticles and the role of other chemicals in solution is not sufficiently understood. Here we study the influence of pH and particle size on the silica-water interfacial reaction. We measure the polarimetric angle-resolved second harmonic scattering (SHS) on silica nanoparticle samples of different sizes (100 nm, 177 nm, and 300 nm) and at different pHs. These measurements extract two surface-related parameters of the particles - the surface potential and the second-order surface susceptibility. We find a trend in the extracted surface potential and the second-order surface susceptibility which does not depend on particle size. The SHS intensities are similar for all sizes at pH 7 but vary with size at higher pHs. This experiment improves our understanding of the interfacial properties of silica colloids of different sizes and at different pH environments.

https://us04web.zoom.us/j/6992918324?pwd=OWVDZFowTnQ0dk93OGVNbjRpNmZoQT09

Meeting ID: 699 291 8324
Passcode: 3EZsG7

Poster - Online

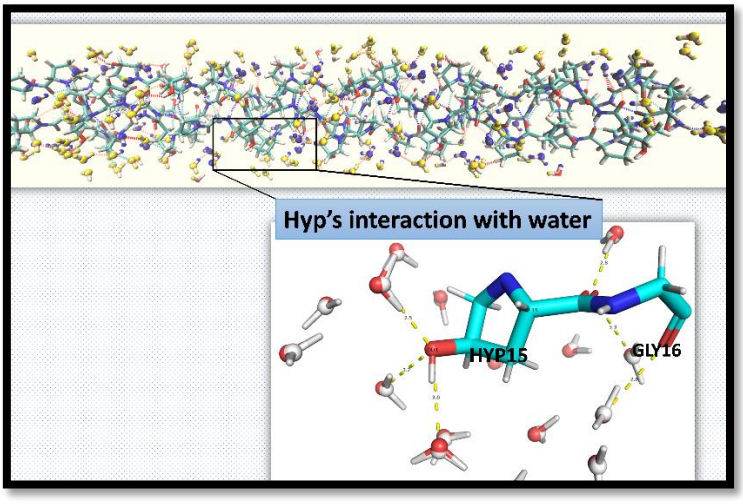
Understanding the hydroxyproline-containing collagen peptide solvation via molecular dynamics simulations

Satyaranjan Biswal and Noam Agmon

The Institute of Chemistry, The Hebrew University of Jerusalem

Jerusalem, Israel

Most collagen fibers are constructed from Gly-Xaa-Yaa triads repeatedly without interruption in invertebrates. The Xaa and Yaa represent predominantly proline (Pro) or a posttranslational modification produced from proline, namely 4-hydroxyproline (Hyp). The 4-hydroxyproline residues are performing a critical role in the correct folding of polypeptide collagen chains. Despite their importance, the hydration property of hydroxyproline and the interaction of its hydroxyl group with water remains unknown. We addressed these issues using molecular dynamics simulations and calculated the radial distribution function (RDF) of water molecules around each -CO group and the -OH group of Hyp, which gives the nearest neighbouring interaction with water molecules. Our analysis showed that the Hyp's carbonyl oxygen forms two hydrogen bonds with water molecules, and Gly's forms only one. On the other hand, the carbonyl oxygen in Pro is directed to the triple helix's center and forms a strong hydrogen bond with the Gly-NH group in the adjacent chain, leaving no room for water molecules. These observations are consistent with the features present in the experimentally derived structure. However, only the -OH group of hydroxyproline creates hydrogen bonds with two or more water molecules during molecular dynamics, while it only has two hydrogen bonds in its crystal structure. It suggests that the hydroxyl group of hydroxyproline has a high tendency for hydration.



https://meet.google.com/mti-vbbz-dpw

Hv1 in cancer cell membranes

Honey Jain and Ana-Nicoleta Bondar
Freie Universität Berlin, Department of Physics, Germany

Cancer cell membranes show a characteristic property of loss of lipid asymmetry due exposure of an anionic lipid phosphatidylserine (PS) on the outer leaflet. This property of cancer cells has become a new potential target for membrane lipid based drug design. Certain types of cancer cells have also shown higher expression of a transmembrane human voltage gated proton channel, Hv1. Hv1 helps highly metabolically active cancer cells in getting rid of excess amounts of acid from its intracellular environment to the negatively charged extracellular environment. Previous studies have shown the proton transfer pathway across the voltage sensing domain of Hv1 in pure lipid bilayers. However, the combined role of lipids and Hv1 in facilitating proton transfer pathways in cancer cell membranes have not been explored. In this study, we use molecular dynamics simulations to investigate the dynamics of the internal H-bond network of Hv1 in leukemia cancer cell membranes. We show that transmembrane helices of Hv1 shift and rearrange along with the formation of a new H-bond network at the extracellular side of Hv1. We suggest that this movement of helices facilitates the change of interaction partners of one of the Arginine present in the voltage sensing domain. This changing of interaction partners might be facilitating the proton transfer through Hv1 in cancer cell membranes. We further investigate the dynamics of H-bond networks and provide a comparison of H-bond networks in MD simulations to NMR ensemble and crystal structure of Hv1.

https://fu-berlin.webex.com/fu-berlin-en/j.php?MTID=mb0e6590a1ce6fe698dacf38fab728376

Poster - On-site

Fluorescence Cross-Correlation Spectroscopy Yields True Affinity and Binding Kinetics of *Plasmodium* Lactate Transport Inhibitors

I. Jakobowska[1], F. Becker[1], S. Minguzzi[1], K. Hansen[1], B. Henke[2], E. Beitz[2]*, and S. Hannus[1]*

[1] *Intana Bioscience GmbH, Lochhamer Str. 29a, 82152 Planegg, Germany, info@intana.de*

[2] *Pharmaceutical Institute, Christian-Albrechts-University of Kiel; ebeitz@pharmazie.uni-kiel.de*

Blocking lactate export in the parasitic protozoan *Plasmodium falciparum* is a novel strategy to combat malaria. We discovered small drug-like molecules that inhibit the sole plasmodial lactate transporter, PfFNT, and kill parasites in culture. The pentafluoro-3-hydroxy-pent-2-en-1-one BH296 blocks PfFNT with nanomolar efficiency but an in vitro selected PfFNT G107S mutation confers resistance against the drug. We circumvented the mutation by introducing a nitrogen atom as a hydrogen bond acceptor site into the aromatic ring of the inhibitor yielding BH267.meta. The current PfFNT inhibitor efficiency values were derived from yeast-based lactate transport assays, yet direct affinity and binding kinetics data are missing. Here, we expressed PfFNT fused with a green fluorescent protein in human embryonic kidney cells and generated fluorescent derivatives of the inhibitors, BH296 and BH267.meta. Using confocal imaging we confirmed the location of the proposed binding site at the cytosolic transporter entry site. We then carried out fluorescence cross correlation spectroscopy measurements to assign true K_i-values, as well as k_{on} and k_{off} rate constants for inhibitor binding to PfFNT wildtype and the G107S mutant. BH296 and BH267.meta gave similar rate constants for binding to PfFNT wildtype. BH296 was inactive on PfFNT G107S whereas BH267.meta bound the mutant protein albeit with weaker affinity than to PfFNT wildtype. Eventually, using a set of PfFNT inhibitor compounds we found robust correlation of the results from the biophysical FCCS binding assay to inhibition data of the functional transport assay.

https://zoom.us/j/7139373709?pwd=S2t1VllWQVEvT0NkbnlJWDV0dExPdz09

Basigin drives intracellular accumulation of *L*-lactate by harvesting protons and substrate anions.

Anna-Lena Köpnick, Annika Jansen, Katharina Geistlinger, Nathan Hugo Epalle, Eric Beitz

Pharmazeutisches Institut, Christian-Albrechts-Universität, Kiel, Germany

Transmembrane transport of L-lactate by members of the monocarboxylate transporter family, MCT, is vital in human physiology and a malignancy factor in cancer. Interaction with an accessory protein, basigin, is required to deliver the MCT to the plasma membrane.
Here, we show that the presence of basigin leads to an intracellular accumulation of L-lactate 4.5-fold above the substrate/proton concentrations provided by the external buffer. We localized the effect to arise from the extracellular Ig-I domain.
Identification of surface patches of condensed opposite electrostatic potential, and experimental analysis of charge-affecting Ig-I mutants indicated a bivalent harvesting antenna functionality for both, protons and substrate anions.
From these data, we conclude that the basigin Ig-I domain drives lactate uptake by locally increasing the proton and substrate concentration at the extracellular MCT entry site. The biophysical properties are physiologically relevant as cell growth on lactate media was strongly promoted in the presence of the Ig-I domain.

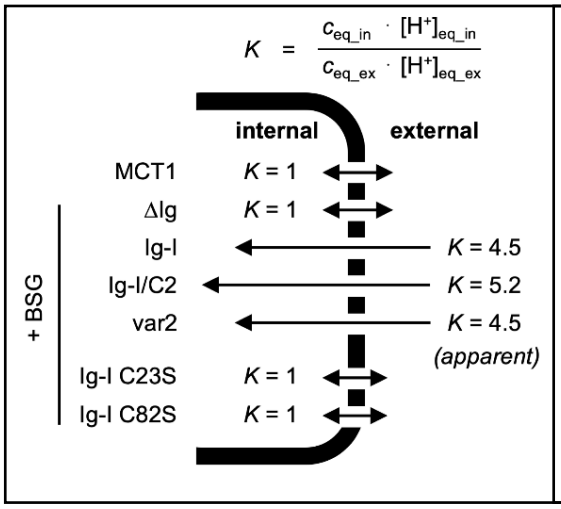


Figure 4A:
Intracellular *L*-lactate accumulation in yeast generated by expression of MCT1 (±BSG variants)

c = internal/external *L*-lactate concentration at transport equilibrium

Ratios (K) of 1 denotes an equilibrium between internal and external lactate concentrations. Greater ratios suggests internal accumulation of the substrate.

https://uni-kiel.zoom.us/j/5280245746?pwd=aGZlc1c0SGFHSHZFajJncVU3NmE1UT09

Meeting-ID: 528 024 5746

Kenncode: 629295

Poster - Online

Dynamic Imaging of Lipid Membrane Hydration Induced by Protons

Seonwoo Lee[a,1] and Sylvie Roke[a,b,c,2]

[a]Laboratory for Fundamental BioPhotonics (LBP), Institute of Bioengineering (IBI), School of Engineering (STI), École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland; [b]Institute of Materials Science and Engineering (IMX), School of Engineering (STI), École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland; and [c]Lausanne Centre for Ultrafast Science, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

The translocation of protons through the lipid membranes plays an important role in physiological processes such as ATP synthesis. These interactions would not be possible without membrane hydration. However, the role of water has not received a lot of attention in membrane studies. Here we investigate the molecular level structural of the hydrated interfacial layers (< 1 nm) of lipid membranes when proton permeation occurs. We use our newly developed high throughput second harmonic imaging technique. This technique allows to probe the interactions of interfacial lipids, water and protons. We observe transient domains of high SH intensity. In these areas, protons bind to the charged head groups leading to charge neutralization on one side of the membrane. Using nonlinear optical theory, we convert the spatiotemporal SH intensity per domain to the membrane potential, surface charge density, and binding free energy per domain. We quantify the proton permeation through the lipid membrane using the capacitance and current measurement,

https://epfl.zoom.us/j/3276823185?pwd=d01uTDJvQnc1UzRHazFNZk93dXovZz09#success

Poster - Online

STRUCTURAL CHARACTERIZATION OF UREA CHANNELS AND THEIR ROLE IN INFECTIOUS DISEASES

F. Kersten[1], J. B. Heim[1], E. Cunha[1], H. Luecke[1]

[1]NCMM, University of Oslo, Oslo, Norway

Abstract: Urea transport across cell membranes is a crucial pathway, particularly for the survival of species in acidic environments such as *Helicobacter pylori*, *Helicobacter hepaticus* and *Streptococcus salivarius*. These organisms are potential pathogens with deadly consequences. For instance, infection of the gastric mucosa by *H. pylori* remains a global health problem and contributes to peptic ulcer disease and gastric cancer. The survival of these organisms depends on the expression of a bacterial urea channel (UreI, >50% sequence identity). It transports urea across the membrane from the periplasm to the cytoplasm, where urease breaks down urea to release ammonia and carbon dioxide. This alkali-generating mechanism maintains a neutral pH in the periplasm allowing the bacteria to survive. Recently published crystal and cryo-electron microscopy (cryoEM) structures of *H. pylori* UreI give some insight into the pH-dependent gating mechanism. However, the full pH-gating mechanism remains elusive. Here, we present a preliminary characterization of UreI from all three microorganisms using new constructs with cleavable affinity tags, closer to their native structure. We hope to be able to structurally characterize the urea channel of these organisms in order to provide a better understanding of the mechanism and therefore set a light on new drug targets.

https://uio.zoom.us/j/3448288722?pwd=eWt1REFDRy81Tmg0NFhscFl6b0pkUT09

Poster – On-site

The effect of membrane composition on proton migration along the membrane-water interface

Anna Maznichenko [1], Ewald Weichselbaum [1], Denis G. Knyazev [1], Timur R. Galimzyanov [2], Oleg V. Batishchev [2], Sergey A. Akimov [2], Peter Pohl [1]

[1] *Johannes Kepler University Linz, Austria*
[2] *A.N.Frumkin Institute of Physical Chemistry and Electrochemistry of the Russian Academy of Sciences, Russian Federation*

Protons are essential for many transport events, e.g., for the uptake of cations, sugars, amino acids, vitamins, as well as for bioenergetics, e.g., ATP synthesis. Their ability to migrate along the membrane surface, i.e., between a proton source and a proton sink, is often crucial for the efficiency of these processes. Interfacial diffusion seldom involves hopping between titratable residues at the membrane surface since H^+release may be too slow[1]. We now monitored diffusion along lipid bilayers of different compositions to test the prevalence of entropy for H^+membrane affinity[2]. We used a caged compound for H^+release from a several micrometer wide spot of a free-standing planar bilayer and recorded H^+arrival in a distant membrane patch. Changing enthalpic H^+-lipid interactions by introducing negative or positive charges to the membrane surface merely delayed or accelerated H^+surface to bulk release, respectively, but did not abolish interfacial H^+ diffusion. In addition, we monitored the effect changes in membrane stiffness exert on H^+membrane interactions. Our results agree with an interfacial entropic H^+trap that opposes irreversible H^+surface to bulk release.

1. *Springer, et al. Proc. Natl. Acad. Sci. U.S.A, 2011, 108, 14461-14466.*
2. *Weichselbaum, et al. Scientific Reports, 2017, 7, 4553.*

https://jku.zoom.us/j/7967811548?pwd=V1BOQm00L3VCdGN3WDNpcXFCWnJQQT09
Meeting-ID: 796 781 1548
Password: 514083

The proton channel H_v1 is water-impermeable.

Danila Boytsov[1], Stefania Brescia[1], Christine Siligan[1], Boris Musset[2], Peter Pohl[1]

[1]*Institute of Biophysics, Johannes Kepler University Linz, Linz, Austria*

[2]*Institute of Physiology, Clinic of Nurnberg Medical School at the Paracelsus Medical Private University, Nurnberg, Germany*

Most ion channels conduct water. Even extremely narrow channels may accommodate an uninterrupted chain of water molecules that connects the two aqueous solutions on both sides of the membrane. The water molecules in this chain may be highly mobile, i.e., their mobility may be close to bulk water molecules. Consequently, such channels, e.g., aquaporins and gramicidin channels, exhibit substantial unitary water permeability. Since interruptions of the water chain diminish the water flux, we used water permeability measurements as a diagnostic tool to distinguish between two proposed mechanisms of proton conduction through the proton channel H_v1: One hypothesis envisioned proton hopping along an uninterrupted water wire. The alternative hypothesis stipulated the existence of titratable amino acids in the proton pathway, interrupting the water wire halfway across the membrane. We reconstituted the purified proton channel into lipid vesicles and monitored their deflation in an osmotic gradient by registering the intensity of light scattered by them. The reconstituted channels were functional, as indicated by fast proton transport kinetics across the reconstituted vesicular membrane, and did not conduct water. Thus, our experiments support the hypothesis that channel occluding amino acids are part of the proton pathway.

https://jku.zoom.us/j/2838686729

Meeting-ID: 283 868 6729