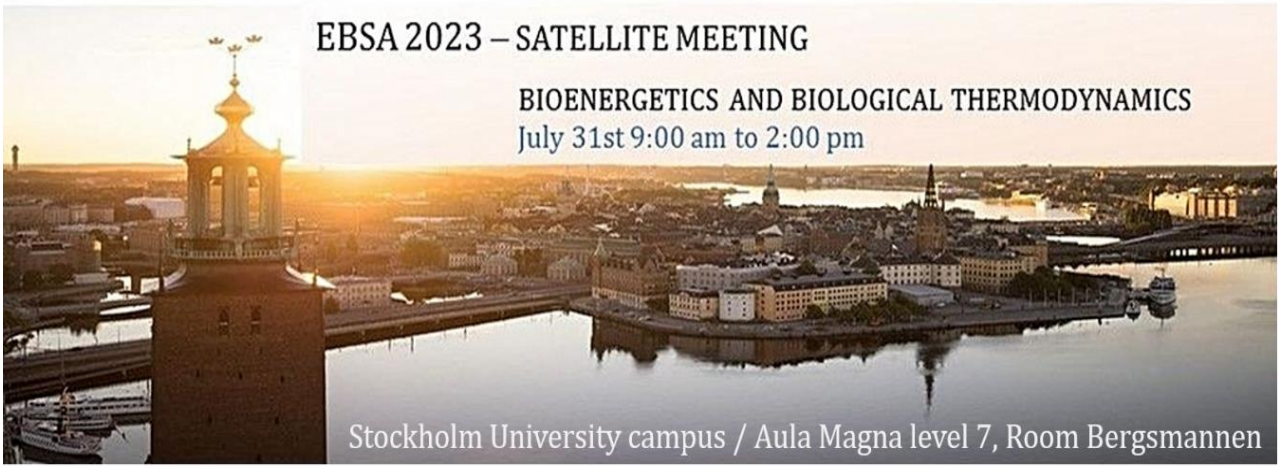

Organizers

- *Karim Fahmy (Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Germany)*
- *Jana Oertel (Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Germany)*
- *Daumantas Matulis (Life Sciences Center, Vilnius University, Lithuania)*

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Invited Speakers

- *Dorothee Kern (Brandeis University, USA)*
- *Johan Åqvist (Uppsala University, Sweden)*
- *Daniel Harries (The Hebrew University of Jerusalem, Israel)*
- *Franz-Josef Meyer-Almes (Darmstadt University of Applied Sciences, Germany)*

Selected Talks

- *Mithun Biswas (Rourkela, India)*
- *Marius Gedgaudas (Vilnius, Lithuania)*
- *Lisa Nucke (Dresden, Germany)*
- *Mona Sarter (Chilton, UK)*
- *Oleksii Zdorevskyi (Helsinki, Finland)*
- *Kristina Zuna (Vienna, Austria)*

Science Coffee Table Presenters

- *Alexey S. Ladokhin (Kyiv, Ukraine)*
- *Eric Pilet (Marseille, France)*
- *Sanja Škulj (Vienna, Austria)*
- *Anaïs Biquet Bisquert (Montpellier, France)*

Scientific Program

09:00-09:05	**Karim Fahmy** Helmholtz-Zentrum Dresden-Rossendorf, Germany	Opening Remarks
09:05-09:30	**Johan Åqvist** Uppsala University, Sweden	Structural Origin of Enzyme Cold-Adaptation
09:30-09:55	**Daniel Harries** The Hebrew University of Jerusalem, Israel	Thermodynamics of Proteins Vitrified in a Glassy Matrix
09:55-10:10	**Mona Sarter** STFC Rutherford Appleton Laboratory, UK	Cooperative change in internal dynamics during streptavidin-biotin binding
10:10-10:25	**Lisa Nucke** Helmholtz-Zentrum Dresden-Rossendorf, Germany	Influence of different membrane mimetic systems on intra-membrane protein hydration and function
10:25-10:40	**Mithun Biswas** National Institute of Technology Rourkela, India	Optimal Resolution to Model Protein Crowding Effects: Lessons from GB1 Dimerization

10:40-11:25 Science Coffee (Rooms Polstjärnan and Mimer)

Discussion Topics:

- **Alexey S. Ladokhin (Palladin Institute of Biochemistry, Kyiv, Ukraine):**
  Successes and challenges in sequence-based thermodynamic predictions of protein-membrane interactions
- **Eric Pilet (Laboratoire de Bioénergétique et Ingénierie des Protéines, Aix-Marseille Université, France):**
  First steps in the characterization of cytochrome bd oxidase from Solidesulfovibrio fructosovorans
- **Sanja Škulj (University of Veterinary Medicine, Austria):**
  Novel Mechanism for the Transport of Fatty Acid Anions Assisted by ANT1
- **Anaïs Biquet Bisquert (University Montpellier, France):**
  Unveiling the dynamic nature of the proton motive force in single Escherichia coli cells temporal and spatial characterization

11:25-11:50	**Dorothee Kern** Brandeis University, USA	Evolution of Allosteric Activation and its Exploitation for Drug Design
11:50-12:15	**Franz-Josef Meyer-Almes** Darmstadt University of Applied Sciences, Germany	Chaperones and protein folding
12:15-12:30	**Kristina Zuna** University of Veterinary Medicine Vienna, Austria	Uncovering the Hidden Role of 2-Oxoglutarate/Malate Carrier in Uncoupling: Implications for Metabolic Disorders and Cancer
12:30-12:45	**Oleksii Zdorevskyi** University of Helsinki, Finland	QM/MM MD simulations of horizontal proton transfer pathways in the antiporter-like subunits of mitochondrial respiratory complex I
12:45-13:00	**Marius Gedgaudas** Vilnius University, Lithuania	Thermott – Tool for Protein-ligand Interactions and Protein–Ligand Binding Database (PLBD) of Thermodynamic and Kinetic Intrinsic Parameters

13:00-14:30 Lunch 15:00 Opening of EBSA-2023 main conference

Structural Origin of Enzyme Cold-Adaptation

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The speed of chemical reactions in water and in enzymes varies with temperature, depending on how the free energy of activation is partitioned into enthalpy and entropy. In enzymes, this partitioning is also optimized as a consequence of the organism's adaptation to the environment. Cold-adapted enzymes from psychrophilic species show the general characteristics of being more heat labile and having a different balance between enthalpic and entropic contributions to free energy barrier of the catalyzed reaction, compared to mesophilic orthologs. It is precisely this altered enthalpy-entropy balance that results in a higher catalytic activity at low temperatures. We will show how the temperature dependence of enzymatic reaction rates can be obtained from brute force computer simulations. Such calculations shed new light on how enzyme structures have evolved in differently adapted species.

Unveiling the Dynamic Nature of the Proton Motive Force in Single Escherichia coli cells: Temporal and Spatial Characterization

A. Biquet-Bisquert[1], B. Carrio[1], N. Meyer[1], T. Fernandes[1], M. Abkarian[1], A.Magalon[2], A. L. Nord[1], and F. Pedaci[1]

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The proton motive force (PMF) is an essential electro-chemical potential established across the membranes of bacteria, mitochondria, and chloroplasts. It serves as a bioenergetic currency that powers an impressive range of physiological processes, including ATP synthesis and bacterial motility. While traditionally considered homeostatic, recent experiments have shown rapid membrane depolarizations on *Escherichia coli* cells [1], indicating a temporally dynamical PMF. Furthermore, polar clustering of respiratory complexes observed in *E. coli* suggests the possibility of a spatial PMF heterogeneity [3]. Such heterogeneity has been recently shown in mitochondria [4]. However, its spatio-temporal behavior in single bacteria remains poorly understood.

We characterized the PMF dynamics on *E. coli* both temporally and spatially. We employed their flagellar motors as local sensors on the membrane, taking advantage from the linearity between their rotational speed and the PMF. We also use spatio-temporally structured laser excitation on single bacteria expressing the light-driven proton pump proteorhodopsin [5] to generate an excess of PMF. We resolved temporal dynamics on the millisecond time scale and observed an asymmetrical capacitive response of the cell. Using localized perturbations on long filamentous cells, we found that the PMF is rapidly homogenized along the entire cell, faster than proton diffusion can allow. Instead, the electrical response can be explained in terms of electrotonic potential spread, similar to that observed in passive neurons and described by cable theory. This implies a global coupling between PMF sources and consumers in the bacterial membrane, excluding a sustained spatial heterogeneity while enabling fast temporal dynamics.

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Optimal Resolution to Model Protein Crowding Effects: Lessons from GB1 Dimerization

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Biochemical reactions occur in a heterogeneous and crowded environment. The crowder molecules exclude the reactant molecules from occupying certain regions of the cell and form soft nonspecific interactions (like hydrogen bonding and hydrophobic interactions), resulting in changes in the reaction thermodynamics and kinetics. Computer simulations are useful tools to obtain mechanistic insights into crowder induced changes to the reaction. However, from a simulation perspective, it is important to decipher the level of structural resolution in a protein-crowder model that can faithfully capture the influences of crowding on protein association with limited computational resources. Here, we investigate the dimerization of model system GB1 in the presence of lysozyme crowders at two structural resolutions. The lower resolution model assumes both protein and crowder species as spherical beads, similar to the analytical scaled particle theory model, whereas the higher resolution model retains residue specific structural details for protein and crowder species. From the higher resolution model, it is found that GB1 dimer formation is destabilized in the presence of lysozyme crowders, and the destabilization is more for the side-by-side dimer compared to the domain-swapped dimer, in qualitative agreement with experimental findings. However, the low resolution CG model predicts stabilization of the dimers in the presence of the lysozyme crowder, similar to the SPT model. Our results indicate a nontrivial role of the choice of model resolution in computer simulation studies investigating crowder induced effects.

Thermott – Tool for Protein-ligand Interactions and Protein–Ligand Binding Database (PLBD) of Thermodynamic and Kinetic Intrinsic Parameters

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Thermal shift assay (TSA, ThermoFluor, differential scanning fluorimetry, DSF) is a technique to determine protein melting temperature for optimal conditions and protein-ligand binding affinities. The technique is easy to perform, but difficult to obtain the dissociation constant. There is a lack of user-friendly tools for regression analysis of complex thermodynamic equations. We developed Thermott — a free and open-source tool for analyzing TSA protein–ligand binding data. The tool is accessible at https://thermott.com.

We have developed and introduce here a Protein-Ligand Binding Database (PLBD) that contains thermodynamic and kinetic data of reversible interactions between proteins and small molecule compounds. The manually curated binding data are linked to protein–ligand crystal structures, enabling the structure-thermodynamics correlations to be determined. The database contains over 5500 binding datasets of 556 sulfonamide compound interactions with the 12 catalytically active human carbonic anhydrase isozymes defined by TSA, ITC, inhibition of enzymatic activity, and SPR. In the PLBD, the intrinsic thermodynamic parameters of interactions are provided which account for the binding-linked protonation reactions. The database provides calorimetrically measured binding enthalpies for additional mechanistic understanding. The PLBD is available at https://plbd.org can be applied for small molecule drug design.

Thermodynamics of Proteins Vitrified in a Glassy Matrix

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Institute of Chemistry and The Fritz Haber Research Center
The Hebrew University, Jerusalem 9190401, Israel

The highly crowded cellular milieu includes countless different solutes that protect proteins and other macromolecules. Of special interest are sugars that can form cellular glassy matrices, corelating with the ability of some yeast, nematodes, and tardigrades to survive desiccation and extreme temperature changes.[1, 2] Yet, although considerable efforts have been directed to discern how the glassy state protects dry proteins, the molecular-level stabilization mechanism has remained elusive. I will describe our combination of synchrotron radiation CD spectroscopy and molecular dynamics (MD) simulations which have allowed us to shed light on molecular origins of protein stability and new emerging nanostructures of proteins when they are embedded in sugar glass.[3] Further resolving the stabilization mechanism may help pave the way to new and improve preservation technologies.

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Evolution of Allosteric Activation and its Exploitation for Drug Design

D. Kern

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Allosteric regulation, the process by which a protein's activity can be modulated by binding of an effector molecule distal to the active site, is vital for cellular signaling. However, its evolution is largely unexplored territory. I will describe our experimental exploration of the evolution over 1.5 billion years of two allosteric regulation mechanisms widely found in the modern protein kinase superfamily, phosphorylation of the activation loop and binding of a regulatory partner protein. Using Ancestral Sequence Reconstruction (ASR) we unravel the origins of allosteric activation including surprising mechanistic features. Moreover, ASR enabled identification of the underlying allosteric network that spans the kinase from the N-terminal to the C-terminal lobes. In the second part of the talk I describe how we exploit this new knowledge for the development of allosteric inhibitors and activators. This latter approach delivered novel kinase inhibitors and activators with extreme specificity and high affinity thereby opening the road to new cancer treatment. Finally, I illustrate our latest vision and results on abolishing the emergence of resistant mutants in cancer treatment by "double drugging" targets simultaneously with allosteric and orthosteric inhibitors. I illustrate how to rationally chose drug combinations with synergy due to positive cooperativity.

Successes and challenges in sequence-based thermodynamic predictions of protein-membrane interactions

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Accurate quantitative estimates of protein-membrane interactions are critical to studies of membrane proteins. Hydropathy plots are a crucial tool to guide experimental design, as they generate predictions of protein-membrane interactions and their topology in the bilayer. The predictions are based on experimentally determined hydrophobicity scales, which provide an estimate for the propensity and stability of these interactions. A significant improvement to the accuracy of hydropathy analyses was provided by the development of the popular Wimley-White interfacial and octanol hydrophobicity scales. These scales have been previously incorporated into the freely available MPEx (Membrane Protein Explorer) online application. Recently, we introduced a substantial update to MPEx that allows for the consideration of electrostatic contributions to the bilayer partitioning free energy [1]. This component originates from the Coulombic attraction or repulsion of charges between proteins and membranes. Its inclusion in hydropathy calculations increases the accuracy of hydropathy plot predictions and extends their use to more complex systems (*i.e.*, anionic membranes).

Another set of recent results demonstrates that thermodynamic analyses based on current hydropathy scales fail to account for the significant and experimentally determined effects that Ca^{2+} or Mg^{2+} have on protein-membrane interactions. We examined distinct modes of interaction (interfacial partitioning and folding and transmembrane insertion) by studying three highly divergent peptides: Bid-BH3 (derived from apoptotic regulator Bid), peripherin-2-derived prph2-CTER, and the cancer-targeting pH-Low-Insertion-Peptide. Fluorescence experiments demonstrate that adding 1-2 mM of divalent cations led to a substantially more favorable bilayer partitioning and insertion, with free energy gains of 5-15 kcal/mol [2].

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Chaperones and protein folding

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The biological functions of proteins are determined by their three-dimensional structure. Protein folding, maintenance of proteome integrity, and proteostasis depend critically on a complex network of protein chaperones. Impairment of proteostasis is associated with aging and numerous degenerative diseases. The observation that even small molecules can support protein folding and rescue protein function, mostly nonspecifically and at higher concentrations, has stimulated the development of more specific bioinspired pharmacological chaperones. Some of them received clinical approval for the treatment of diseases related to protein misfolding, such as Lysosomal Storage Diseases and Cystic Fibrosis. We have studied the effects of inhibitors on human histone deacetylases (HDACs) and found that structurally distinct but rapidly reversible inhibitors are able to stabilize these proteins and also increase their enzyme activity. Importantly, stabilization of HDACs by the inhibitors, as found in thermal shift experiments, does not correlate with increases in enzyme activity. The kinetic data of enzyme activity in the absence and presence of inhibitors are explained by a simplified model. Based on these experiments, we and others propose the use of rapidly reversible HDAC inhibitors at low concentrations in screening campaigns that require reproducible and comparable enzyme activities over a longer period.

Influence of different membrane mimetic systems on intra-membrane protein hydration and function

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Active transport across biological membranes is crucial for the homeostasis of the cell. An often neglected but important molecular constituent in the transport of ions is water. However, it is challenging to monitor, quantitate and site-specifically resolve the functional role of water within membrane proteins.

We have adressed these problems and studied specifically the influence of the lipid environment on ion transport and intra-membrane protein hydration in the copper-transporting ATPase CopA, a system amenable for spectroscopic observations of intra-membrane protein hydration by fluorescence solvatochromism. The recombinant protein was reconstituted into different membrane mimetic systems. Functional assay revealed differences in the activity of CopA in mixed lipid-detergent micelles as compared to protein-based (MSP1E3D1), whereas no activity was detected in polymer-based (DIBMA) nanodisc.

These differences correlated with intra-membrane protein hydration as revealed by spectroscopic response to osmotic pressure changes induced by PEG. The polarity-sensitive fluorophore BADAN was used as a probe for the dielectric environment of one of two conserved cysteine residues at the protein's active (Cu-binding) site. The results show that lipid lateral pressure in MSP1E3D1 nanodiscs reduces the degree of hydration relative to detergent micelles by five to ten internal water molecules. Interestingly, the unexpected lack of functionality of CopA in DIBMA nanodiscs correlated with high lateral pressure on the transmembrane region which may constrain functionally important hydration changes.

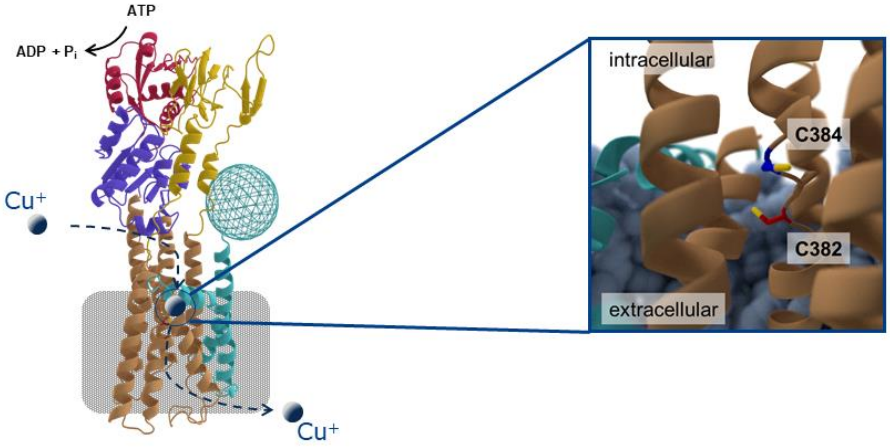

Figure 1: The copper-transporting P-type ATPase CopA from *Legionella pneumophila* with its high affinity binding site, based on pdb file 3RFU.

First steps in the characterization of cytochrome bd oxidase from Solidesulfovibrio fructosovorans

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The cytochrome *bd* oxidase (cyt*bd*) is an O_2 reductase in procaryote respiratory chains. The structures solved so far have given puzzling results about the relation between heme arrangement in the structure, their redox potential, the O_2 and H^+ channel location [(1)]. In such a context, cyt*bd* from *Solidesulfovibrio fructosovorans* (cytbd_{Sdf}) is an interesting enzyme to study: the latest phylogeny study shows that cytbd_{Sdf} is in a different branch that the already characterized enzymes with common features with both mycobacterial enzymes (small Q-loop, absence of additional subunit), and with *E. coli* enzyme (nature of the heme ligand residues).

Here we present our first characterization of cytbd_{Sdf}. When homologously produced in fructose-sulphate medium, with the operon under the control of its native promoter, the enzyme is, indeed composed of two subunits only, as predicted from genome analysis. The purified enzyme displays typical heme signatures, ligands binding and O_2-reductase activities with menaquinol rather than ubiquinol.

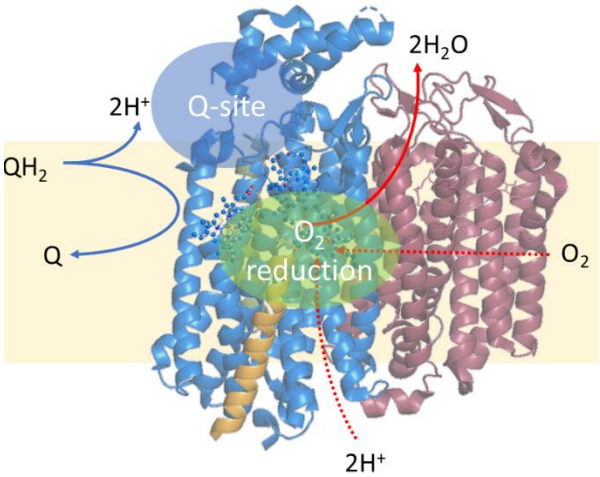

Figure 1: schematic view of *bd* oxidase with quinol oxidation site, in blue, and O_2-reduction site in green.

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Cooperative change in internal dynamics during streptavidin-biotin binding

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Molecular dynamics are vital for the function of proteins. Upon protein ligand binding, changes of conformational entropy occur in the protein and its hydration layer, as well as its internal dynamics. The binding of biotin to streptavidin was investigated using QENS, as well as Thermal Diffusion Forced Rayleigh Scattering (TDFRS) and ITC. This interaction is enthalpy driven, with an opposing entropic component. An experimental investigation of the components of the entropy change, specifically the change in conformational entropy, indicates it is strongly opposed to the binding. This adverse change in entropy has to be compensated, the strongest candidate is a supportive entropy change of the surrounding hydration layer. It is also interesting that the change in conformational entropy upon saturation with biotin is on the same order of magnitude as that of protein folding, without an associated significant structural change.

On the ps timescale the surface residues of free streptavidin show a jump diffusive dynamic, while the fully saturated less flexible streptavidin biotin complex does not exhibit jump diffusive motions. This is indicative of the dynamic of streptavidin being changed for the whole protein upon biotin saturation, instead of only in the binding pocket.

Experiments focusing on the internal dynamics at different degrees of biotin saturation were performed. It was found that the internal dynamics of streptavidin are already drastically altered for only one biotin binding, which corresponds to 25% saturation. Such a suppression of dynamics over the whole protein upon partial saturation is best explained by a cooperative process.

Novel Mechanism for the Transport of Fatty Acid Anions Assisted by ANT1

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Adenine nucleotide translocase (ANT1) is an inner mitochondrial membrane (IMM) protein with the main function of exchanging ADP and ATP for cellular energy supply. An important but less acknowledged function of ANT1 is the transport of protons in the presence of long chain fatty acids (FA). The molecular mechanism of this transport is not understood.[1] FAs act as weak uncouplers, independently of protein. The transport of FA anionic form (FA^-) across the membrane is a rate-limiting step. We hypothesized that ANT1 will accelerate this transport of FA^- across the IMM. Our recent MD simulations, together with experimental results, suggest that anions of arachidonic acid (AA^-) are attracted by the positively charged amino acids cloud at the matrix side and slide between transmembrane helices 2 and 3 to the cytosolic side. The suggested mechanism is energetically favourable because the AA^- "head" interactions with the positively charged amino acids complemented by hydrophobic tail stabilization both within the hydrophobic surface of ANT1 and in the surrounding lipids. The translocation path terminates at R79, where AA^- is protonated with the help of D134. The protonated AA spontaneously exits the protein, flips across the IMM and releases the proton at the matrix side, completing the catalytic cycle. Knowledge of the precise proton transport mechanism will enable the development of effective drugs for the treatment of diseases involving ANT1, such as progressive external ophthalmoplegia, myopathy, and hypertrophic cardiomyopathy.

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QM/MM MD simulations of horizontal proton transfer pathways in the antiporter-like subunits of mitochondrial respiratory complex I

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The molecular mechanism of respiratory complex I (NADH:ubiquinone oxidoreductase) has remained one of the biggest mysteries in bioenergetics. Being the largest enzyme in the respiratory chain, it couples the electron transfer process to proton pumping across the inner mitochondrial membrane, spatially separated by ~200 Å from one another. This phenomenon raises an enigmatic question: how does the energy released from the redox reaction can be transmitted for such long distances? In our work, we advocate the hypothesis that it can be accomplished by the translocation of protons along the central hydrophilic axis of the enzyme - a highly hydrated region revealed by the structural data [1-4]. We utilise state-of-the-art quantum mechanical/molecular mechanical (QM/MM) simulations to study the dynamics of horizontal proton pathways in the high-resolution structure of respiratory complex I from *Yarrowia lipolytica* [4]. We apply QM/MM free energy umbrella sampling simulations to various proton routes bridging highly-conserved protein residues of the ND2 subunit of the enzyme. Our results show low free energy barriers and favourable thermodynamics for the proton pathway laterally spanning the entire ND2 subunit. For the first time, we emphasize the critical role of tyrosine residues in coordinating the transmission, and show reasonable energetics for the proton transfer in interfacial regions of the ND2 subunit with the adjacent ND4L and ND4 subunits. Our results elucidate the long-range energy translocation processes in the membrane part of the enzyme, casting doubts on the canonically admitted proton pumping models in respiratory complex I [5].

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Uncovering the Hidden Role of 2-Oxoglutarate/Malate Carrier in Uncoupling: Implications for Metabolic Disorders and Cancer

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The mitochondrial 2-oxoglutarate/malate carrier (α-ketoglutarate/malate carrier, OGC) transports 2- oxoglutarate for malate across the inner mitochondrial membrane and plays a crucial role in the malate-aspartate shuttle. OGC is ubiquitously expressed in healthy and cancerous tissues, and its gene knockout is embryonically lethal. Despite sharing a high homology with other proteins from the solute carrier 25 family (adenine nucleotide translocase 1 (1) and uncoupling proteins 1-3 (2) that facilitate proton transport mediated by free fatty acids (FAs), the role of OGC in uncoupling has never been studied in a well-defined system. We hypothesized that OGC can directly enhance proton transport in the presence of FAs. To test this, we produced OGC in E. coli and reconstituted it in liposomes. Our experiments show that reconstituted OGC increases the specific membrane conductance of lipid bilayer membranes only in the presence of FAs, proving its involvement in proton transport. OGC's substrates and substrate analogs inhibited this effect, suggesting competition for the same binding site in the protein's cavity. Site-directed mutagenesis revealed that R90 is involved in the FA-mediated proton leak, suggesting a common binding site for substrates and FAs. Understanding the molecular mechanism and role of OGC in uncoupling could have important implications for the development of targeted drug delivery systems for the treatment of metabolic disorders, obesity, and cancer.

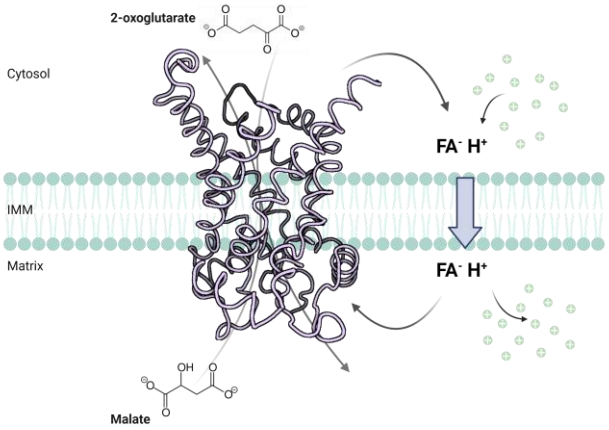

Figure 1: A) Schematic view of OGC's two functions – substrate and FA-mediated proton transport.

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