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Mitochondrial ATP synthase is the major producer of ATP in respiratory cells and maintains the mitochondrial membrane potential $\Delta \Psi_{m}$ in glycolytic cells. For these purposes, it works in forward (ATP synthesis) or reverse mode (ATP hydrolysis). These two modes are not necessarily antagonistic. We provide evidence that both functional types can be found within the same mitochondria.

We combined inhibitor studies with high-resolution microscopy, which reveals the mobility and localization of mitochondrial ATP synthase in living cells under different conditions. We found that the distribution of ATP synthase and its mobility in the inner membrane, especially the cristae, was altered when either ATP synthase or ATPase function was inhibited. These results were consistent and independent of whether chemical inhibitors (oligomycin, BMS) were used, whether the level of the inhibitory factor IF1 was altered, or whether changes in ATP synthase activity were induced by metabolic modifications. Inhibition of ATPase resulted in increased confinement of ATP synthase. Transient overexpression of constitutively active IF1 (IF1-H49K), which inhibits ATPase function, reduced mobility, while knockdown of IF1 led to increased mobility of ATP synthase. In addition, IF1 strongly influenced mitochondrial pH values. When substrate for respiration was limited, in tendency more IF1 bound to the ATP synthase and the mobility of the enzyme decreased probably due to higher oligomerization. In summary, we found that a change in ATP synthase versus ATP hydrolysis activity was associated with a change in the localization and mobility of ATP synthase, with ATPase being more mobile. This suggests, consistently, the activity of ATP synthase and ATPase is possible in different sub-compartments of the IMM, preventing futile cycles. Furthermore, IF1 directs not only the activity but also the spatio-temporal organization of ATP synthase.

938-Plat

Tracking Motility and Morphology of Individual Mitochondria Reveals Large Heterogeneities in Breast Cancer Subtypes

Austin E.Y.T. Lefebvre1,2, Dennis Ma1, Kai Kessenbrock1, Devon A. Lawson1, Michelle A. Digman1,2,3

1Biomedical Engineering, University of California Irvine, Irvine, CA, USA, 2Laboratory for Fluorescence Dynamics, Irvine, CA, USA, 3Biological Chemistry, University of California Irvine, Irvine, CA, USA.

Mitochondrial dysregulation has long been investigated on the linear microtubules of axons as it makes exploring trafficking relatively simple. In contrast, non-neuronal cells form complex networks of microtubules on which mitochondria can move, and the gap in publications on mitochondrial dynamics between these cell types illustrates these difficulties. Despite this, recent publications suggest that the dysregulation of mitochondrial motion may fuel some types of metastatic cancer. As mitochondria exist in complex shapes and move erratically across the cell, automated segmentation and tracking of individual mitochondria are challenging. Consequently, time-consuming manual morphological measurements and tracking remain the gold standard. These user-dependent methods introduce bias to the data and make them unreliable. Here, we introduce an algorithm for fast, unbiased, and automated segmentation and spatiotemporal tracking of mitochondria in live-cell fluorescence 2D and 3D time-lapse images. The inputs of pixel size and time between frames (and in 3D, the distance between stacks) are the only required inputs from the user and allow identification of changes in mitochondrial morphology, motion, and fusion and fission dynamics. Using a method that our lab has pioneered for assessing metabolic shifts in living cells and tissues, the phasor-approach to the fluorescence lifetime imaging (FLIM) of the reduced form of nicotinamide adenine dinucleotide (NADH), we assess the spatiotemporal metabolic changes of individual mitochondria in living cells. We further compare metabolically heterogeneous populations of mitochondria across breast cell lines of various metastatic potential. Furthermore, using a novel technique that we have developed for generating patient-derived xenograft (PDX) spheroids, we probe, in 2D and 3D, the morphologic, motile, and dynamic differences between mitochondria from normal breast epithelia, receptor-positive, and triple-negative breast cancer patients. This work is supported by grant NIH P41-GM103540, and NSF GRFP DGE-1839285.

939-Plat

Wetting Transitions in the ATP Synthase C-Subunit Ring, a Large Conductance Ion Channel

Rachel J. Dotson1, Nelli Mirtsakanyan1, Elizabeth A. Jonas1, Sally C. Pias1,2

1Department of Chemistry, New Mexico Institute of Mining and Technology (New Mexico Tech), Socorro, NM, USA, 2Department of Internal Medicine (Endocrinology), Yale University, New Haven, CT, USA.

Apart from its known role in proton transport, the mammalian ATP synthase c-subunit ring (F$_c$ domain) has been observed in recent electrophysiology experiments to form a high-conductance voltage-gated ion channel ($\sim$1.5 nS). Yet, the ring’s hydrophobic central lumen has been assumed to be dry, precluding a physical explanation for the observed conductance. We present an atomistic molecular dynamics simulation study of lumen wetting for mammalian and yeast c-subunit rings. Consistent with prior work, we find that the $\sim$12 Å diameter yeast ring remains dry on the several-hundred-nanosecond timescale of the simulations. In contrast, the narrower, $\sim$8 Å mammalian ring undergoes rapid ($\sim$50 ns) liquid-vapor transitions, forming a transient but continuous water column across the lumen. Modifying the charge on the glutamate residues of the proton-transport pathway at the membrane-protein interface affects the duration and extent of lumen wetting, as do in silico mutations at various sites. The wetting variations are qualitatively consistent with electrophysiology experimental findings for wild-type and mutant forms of the mammalian c-subunit ring. We are encouraged to think that the simulations will serve as a valuable “computational microscope” for understanding how charge conductance can occur in the ATP synthase c-subunit ring. To determine the mechanism of wetting and its modulation, we are pursuing analysis of the surface hydrophobicity of the lumen lining and its response to the mutations. The simulation finding of reversible lumen wetting is supported by published work indicating c-subunit ring wetting in the intact ATP synthase. New experiments and published data additionally indicate that the F$_c$ domain may exist at least partly dissociated from the physiology on recombinant VDACs reconstituted into planar lipid membranes in CaCl$_2$ gradient to perform direct measurements of VDAC permeability to Ca$^{2+}$ in the open and blocked states. We found that under these conditions, VDAC high conducting or open state is highly anion selective, and CT/Ca$^{2+}$ permeability ratio strongly depends on CaCl$_2$ concentration and on channel orientation in salt gradient. To investigate the effect of $\alpha$Syn on Ca$^{2+}$ flux through VDAC, we measured ion selectivity of VDAC state blocked by $\alpha$Syn. We established that when the highly negatively charged C-terminal tail of $\alpha$Syn enters VDAC pore, it reverses ion selectivity, making VDAC more permeable for Ca$^{2+}$ and thus increasing its net flux. We found that the three mammalian isoforms of VDAC differ significantly in Ca$^{2+}$ permeability, with VDAC1 being more CT and VDAC3 more Ca$^{2+}$ permeable among the three. We also studied the effect of the knockdown of each VDAC isoform and $\alpha$Syn overexpression on Ca$^{2+}$ influx into the mitochondria using genetically encoded calcium sensor, GCamp6f. These results suggest that $\alpha$Syn interaction with VDAC might have important consequences for Ca$^{2+}$ flux through the pore, with possible implications in both normal mitochondrial function and PD associated dysfunction.

Platform: Molecular Dynamics and Bioinformatics II

941-Plat

Response in Immune Repertoires

Aleksandra M. Walczak1, Vicente M. Aguilella2, Tatiana K. Rostovtseva1, Sergey M. Bezrukov1

1Section on Molecular Transport, National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA, 2Department of Physics, Universitat Jaume I, Castellón, Spain.

Calcium (Ca$^{2+}$) signaling plays a key role regulating mitochondrial function in response to constantly changing cell demands. The pivotal role of the Voltage Dependent Anion Channel (VDAC), the most ubiquitous protein in the outer mitochondrial membrane, in Ca$^{2+}$ homeostasis and mitochondrial metabolism is well established. However, the exact biophysical mechanism of how VDAC mediates Ca$^{2+}$ uptake and thus is involved in mitochondrial Ca$^{2+}$ homeostasis remains poorly understood. Previously, we found that the Parkinson’s disease (PD) related neuronal protein, alpha-synuclein ($\alpha$Syn), reversibly and partially blocks ion current through VDAC. In this study we utilized single-molecule electrophysiology on recombinant VDACs reconstituted into planar lipid membranes in CaCl$_2$ gradient to perform direct measurements of VDAC permeability to Ca$^{2+}$ in the open and blocked states. We found that under these conditions, VDAC high conducting or open state is highly anion selective, and CT/Ca$^{2+}$ permeability ratio strongly depends on CaCl$_2$ concentration and on channel orientation in salt gradient. To investigate the effect of $\alpha$Syn on Ca$^{2+}$ flux through VDAC, we measured ion selectivity of VDAC state blocked by $\alpha$Syn. We established that when the highly negatively charged C-terminal tail of $\alpha$Syn enters VDAC pore, it reverses ion selectivity, making VDAC more permeable for Ca$^{2+}$ and thus increasing its net flux. We found that the three mammalian isoforms of VDAC differ significantly in Ca$^{2+}$ permeability, with VDAC1 being more CT and VDAC3 more Ca$^{2+}$ permeable among the three. We also studied the effect of the knockdown of each VDAC isoform and $\alpha$Syn overexpression on Ca$^{2+}$ influx into the mitochondria using genetically encoded calcium sensor, GCamp6f. These results suggest that $\alpha$Syn interaction with VDAC might have important consequences for Ca$^{2+}$ flux through the pore, with possible implications in both normal mitochondrial function and PD associated dysfunction.

Platform: Molecular Dynamics and Bioinformatics II

940-Plat

$\alpha$Synuclein Regulates Mitochondrial Calcium Transport through the Voltage Dependent Anion Channel

William M. Rosenca1ns, Vicente M. Aguilella2, Tatiana K. Rostovtseva1, Sergey M. Bezrukov1

1Department of Physics, University of California Irvine, Irvine, CA, USA, 2Department of Physics, Universitat Jaume I, Castellón, Spain.

Voltage Dependent Anion Channel (VDAC) is a high-conductance ion channel that mediates the entry of Ca$^{2+}$ into the mitochondrial matrix. This influx of Ca$^{2+}$ could have significant implications for cell biology. The Voltage Dependent Anion Channel (VDAC) is a high-conductance ion channel that mediates the entry of Ca$^{2+}$ into the mitochondrial matrix. This influx of Ca$^{2+}$ could have significant implications for cell biology.
properties, statistical analysis is needed to identify responding clones. Using such methods I will describe the repertoire level response to the SARS-CoV-2, among other perturbations. More generally, I will show how immune repertoires provide a unique fingerprint reflecting the immune history of individuals, with potential applications in precision medicine.

942-Plat
Development of a Hybrid Neural Network/Molecular Mechanics Approach for Metalloprotein Simulations
Bettina Lie1, Peter Poljak1, Julia Wessendorf2, Philipp Marquetand1, Chris Oostenbrink1.
1Institute for Molecular Modeling and Simulation, University of Natural Resources and Life Sciences, Vienna, Austria, 2Department of Chemical Physics, Slovak University of Technology in Bratislava, Bratislava, Slovakia.

Metalloproteins are surprisingly common, the inclusion of metal ion cofactors results in amazingly diverse proteins that are involved in virtually all biological processes. Investigation of metal-ligand interactions is particularly important for drug design of metalloenzyme inhibitors and metallo-drugs. Molecular dynamics (MD) simulations of metalloproteins based on molecular mechanics (MM) force fields are challenging due to the intricate nature of transition metals. Despite improvement of force fields, electronic effects demand a full quantum mechanical (QM) description. QM calculations are limited to a few hundreds of atoms, therefore not suitable for biomolecular systems. Multiscale QM/MM methodologies overcome this bottleneck but remain computationally demanding. Here we introduce a machine learning based method as an efficient alternative to conventional hybrid techniques. Instead of expensive quantum chemistry during MD simulations, atomistic neural networks (NNs) learn the relationship between molecular conformations and corresponding energies based on QM reference data. In particular, the NNs are trained on the interaction energies of the metal, the difference in QM energy with and without the metal present. The resulting energies are representative of the interactions between the ligands and the metal, as well as the influence of the metal on the ligand-ligand interactions. The remaining interactions of the system as well as long-range metal interactions are evaluated by classical force-field terms. This coupling is very similar to mechanical embedding, with the advantage that overpolariization at the boundary is not an issue. We implemented this hybrid NN/MM approach using existing QM/MM features in the GROMOS software. Besides, we started to train NNs on small metal coordination complexes, beginning with hexa-aqua iron up to porphyrins. We have performed NN/MM-MD simulations, where the trained NNs were utilized to describe the metal-ligand interactions. Very promising preliminary results of our approach were obtained.

943-Plat
Enhanced Sampling of RNA Folding/Unfolding Configurations using Machine Learning/Grand Canonical Monte Carlo
Mert Y. Sengul, Abhishek A. Kognole, Alexander D. MacKerell.
Department of Pharmaceutical Sciences, University of Maryland Baltimore, Baltimore, MD, USA.

Ribonucleic acids (RNAs), as one of the most versatile and important molecules in molecular biology, are associated with an extensive range of functions to understand ion-driven folding can help uncover deeper insights in RNA folding pathways.

944-Plat
Amplification-Free Detection of Viruses in Minutes using Single-Particle Imaging and Machine Learning
Nicolas Shiuells1, Leon Peter2, Andrew McMahon3, Chritof Hepp1, Erica Bickerton1, Cyril Favard1, Delphine Muriaux5, Monique Andersson2, Alison Vaughan2, Philippa Matthews4, Nicole Stoesser4, Derrick Crook4, Achillefs N. Kapanidis5, Nicole C. Robb1.
1Physics, University of Oxford, Oxford, United Kingdom, 2Medical Sciences Division / NDM Experimental Medicine, University of Oxford, Oxford, United Kingdom, 3The Pirbright Institute, Surrey, United Kingdom, 4UMR 6133, CNRS - Institute Fresnel, Marseille, France, 5University of Montpellier, Montpellier, France.

The increasing frequency and magnitude of viral outbreaks in recent decades, epitomized by the current COVID-19 pandemic, has resulted in an urgent need for rapid and sensitive viral diagnostic methods. To address this need, we developed a novel method to rapidly detect and identify intact virus particles using wide-field fluorescence imaging, advanced image analysis and deep learning. Our method utilises cation-mediated labelling of enveloped viruses, a labelling method not specific to any virus. We subsequently immobilise double labelled viruses on the surface of a glass slide, collect diffraction-limited images containing thousands of labelled particles, and finally use image analysis and deep learning to identify different viruses in biological and clinical samples. The software initially segments the images and only keeps the signals present in both the green and the red channel. Those signals are then saved as individual images and fed into a custom convolutional neural network for classification. The network exploits the differences between the labelling efficiency for different viruses, as well as their size and shape differences. Our assay achieves labelling, imaging and virus identification in less than five minutes; further, the whole procedure does not require any lysis, purification or amplification steps. The trained neural network was able to differentiate SARS-CoV-2 from negative clinical samples, as well as from other common respiratory pathogens (such as influenza and seasonal human coronaviruses). The sensitivity and specificity for lab-adapted strains exceeded 90% per signal, and the overall validation accuracy for Flu A clinical samples was 86.5% per signal. Single-particle imaging combined with deep learning therefore provides a promising alternative to traditional viral diagnostic methods and has the potential for significant impact.

945-Plat
Computing Absolute Free Energy with Deep Generative Models
Xinqiang Ding, Bin Zhang.
Massachusetts Institute of Technology, Cambridge, MA, USA.

Fast and accurate evaluation of free energy has broad applications from drug design to material engineering. Computing the absolute free energy is of particular interest since it allows the assessment of the relative stability between states without intermediates. In this work, we introduced a general framework for calculating the absolute free energy of a state. A key step of the calculation is the definition of a reference state with tractable deep generative machine learning models using locally sampled configurations. The absolute free energy of this reference state is zero by design. The free energy for the state of interest can then be determined as the difference from the reference. We applied this approach to both simple model systems and biological systems and demonstrated its effectiveness. It was found that the Bennett acceptance ratio method provides more accurate and efficient free energy estimations than approximate expressions based on work. Compared with traditional methods such as umbrella sampling and alchemical free energy calculation, the method presented here provides a new strategy for computing free energy differences between states without sampling from intermediate states and has important applications in drug design.

Platform: Bioengineering, Biosurfaces, and Biomaterials

946-Plat
Membrane Fission: Insights from Reconstituting Organelle Form and Chemistry
Thomas Pucadyil.
Biology, Indian Institute of Science Education and Research, Pune, India.

The lipid bilayer is highly resilient to rupture and explains why it was selected over the course of evolution to serve a barrier function. Yet fission, or the