the molecular-scale mechanisms of flow mechanotransduction are complex and details remain obscure [1]. It has been observed that large GPI-anchored proteins are reorganized following application of shear flow to living cells [2], but whether this is the result of advection or of active intracellular transport has not yet been determined. Here, we investigate whether physiological levels of fluid flow applied to living cells can redistribute surface proteins. We subject a confluent monolayer of human cells to controlled shear flow. These cells are modified to express a construct comprising extracellular GFP attached to the outer leaflet of the cell plasma membrane through a GPI anchor. We track protein movement across the cells with the goal of estimating the hydrodynamic forces felt by the extracellular domain and the frictional force experienced by the membrane anchor. We relate our results to experiments on supported bilayers. We also investigate the contributions of the cytoskeleton and plasma membrane lipid composition to protein mobility.

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101-Plat

Remodeling of Gamete Membrane during Mammalian Fertilization

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Fertilization is a central event in the life of sexual organisms. It guarantees the survival of species. Close to the oocyte, the spermatozoon still has to interact with the oolemma. For the mammals, the fertilization process is almost doomed to fail without the GPI-AP Juno on the oocyte and the Immunoglobulin-Super Family protein Izumo on the spermatozoon. Nevertheless, the central question about how membranes merge is none answered. Our study aims a better understanding of the membrane dynamics triggered by the adhesion of a sperm on the oocyte membrane leading to gamete fusion.

To do so, we developed two novel approaches. The first one consists in generating a biomimetic pure lipids sperm membrane to measure the fusogenic capacity of the sperm's fatty acids. We found that Nature's wisdom minimizes the spermatozoon's Energy requirement to fuse with standard plasma membrane, compared to other kind of cell lipids composition. Then, incorporating Izumo, we noticed an accommodating oligomerization of this essential protein correlated with the type of lipids. For the second approach, we developed a microfluidic tool to induce a physiological gamete interaction, controlling the time and area of the gamete encounter. This original device offers, in live and full resolution, unique point of view to study live membrane remodeling. We could demonstrate the simultaneity of membrane fusion and spermatozoon internalization as well as a particular way of swimming that seems to be common to sperm which manage to fertilize. These new biophysical tools provide an insight of the dynamics of gamete interplay. They set in motion the usually static biological description of this interaction.

Platform: Bioenergetics and Mitochondrial Signaling

102-Plat

Ultrafast Limits of Photo-Induced Electron Transfer Rates in PPCA, a Multi-Heme C-Type Cytochrome

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Conversion of light energy to electrochemical equivalents is at the core of any photosynthetic system. In our attempts to build enzymes for artificial photosynthesis, we need to have complete control of all charge transfer rates, including the initial charge separation step. Here we demonstrate an unexpectedly large range ($5x10^7$ to $5x10^{11}$ s^{-1}) of the observed photo-induced electron transfer rates between PpcA, a 3-heme member of cytochrome c_7 family from *Geobacter sulfurreducens*, and $Ru(bpy)_2$(4-MeBr-4′ - Mebpy), an artificial photosensitizer covalently-linked to genetically engineered cysteine residues in close proximity to PpcA hemes. HPLC-MS characterization confirmed purity of isolated proteins and their correct assembly, as well as stoichiometric binding of photosensitizers. Combined small- and wide-angle X-ray scattering studies performed at the Advanced Photon Source of Argonne National Laboratory demonstrated the absence of any significant structural changes from the initial compact globular shape in mutated and covalently-labeled protein forms. Ongoing NMR spectroscopy and all-atom molecular dynamics simulations provide further insight into protein structure and dynamics responsible for the large range of electron transfer rates. These results demonstrate the limits of tuning charge transfer rates and offer opportunities to use inexpensive photosensitizers based on abundant elements for the initial charge separation step in artificial photosynthesis.

103-Plat

Infrared Spectroscopic and Electrochemical Approaches for the Study of the Reaction Mechanism of Immobilized Membrane Proteins from the Respiratory Chain

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Although the architectures of several membrane proteins in respiration as well as the basic chemical reactions have been described, the interactions on molecular level, the high diversity and the high efficiency of mechanisms, are under discussion. Experiments have been developed which reveal how protons and other ions are drawn through proteins and how they are coupled to electron transfer.

An electrochemical approach on proteins immobilized on gold nano particles was used to get insight into the redox properties in the presence and absence of substrate and inhibitors to the proton channels (Zn2+).1 Two approaches are presented to functionally probe the enzymes against substrates and inhibitors: i) The redox reaction was IR spectroscopically studied in solution and ii) the proteins are immobilized via their His-Tag on a gold layer, deposited on an ATR-crystal and studied by surface enhanced IR spectroscopy (SEIRAS). 2 The combination of the electrochemical and IR spectroscopic approach allowed the observation of protein action at the level of single functional groups within the large protein studied and thus provides essential knowledge's for the understanding of the mechanism of the studied enzymes.

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104-Plat

Theoretical Investigation into the Color-Tuning Mechanism of Proteorhodopsin

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Retinal proteins are transmembrane proteins with potential applications in optical memory and optogenetics for example. Proteorhodopsin (PR) is a recently discovered microbial retinal protein that acts as a proton pump. Activation of PR occurs when the covalently bound chromophore, retinal, absorbs a photon and undergoes all-trans → 13-cis isomerization. Two variants of PR are tuned to absorb blue (490 nm) and green (520 nm) light. This difference is modulated by a single residue at position 105 that acts as a light-tuning switch—in blue PR it is glutamine whereas in green PR it is leucine. Despite this basic knowledge of the color-tuning mechanism, the electronic environment in the retinal binding pocket that determines the green and blue variants of PR remains poorly understood. Using quantum mechanical/molecular mechanical calculations, we have characterized the color-tuning mechanism of PR at the B3LYP level of theory. We discovered that the difference between blue and green PR depends on a complex interplay between the water-mediated hydrogen-bonded network within the binding-pocket and the twist of the retinal polyene chain. Geometry optimization of the native form of blue PR produced two retinal structures, one with a planar and another with a slightly twisted polyene chain. In both structures a weak hydrogen bond exists between the color-tuning switch, Q105, and the polyene chain. However, the twisted structure is higher in energy, with an elongated N-H bond in the protonated Schiff base (PSB) and shorter hydrogen bond with a coordinated water. Mutation of the color-tuning switch (Q105L) abolishes the hydrogen bond with retinal, leading to a shortened N-H bond in the PSB and a lengthened hydrogen bond with water. Our results provide a detailed understanding of the color-tuning mechanism in PR and can be applied to other retinal proteins as well.