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Redesigning Photosynthetic Membranes: Development of Bio-Inspired Photonic Nanomaterials
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Biological cell membranes rely upon hierarchical organization to elicit functional responses. In natural photosynthetic membranes, light harvesting (LH) membrane protein complexes act as a framework for coordination of chromophores which absorb solar energy and channel it to downstream bioenergetic processes. Supported lipid bilayers (SLBs) are established as simple model membranes, into which membrane proteins can be incorporated. As an alternative to lipids, certain diblock copolymers can from membrane-mimetic systems with potential advantages (increased robustness, functionality, responsivity). Here, we present research into two different bio-inspired LH systems.
Firstly, a modular, artificial LH system is described, where amphiphilic diblock polymers, poly(ethylene oxide)-block-poly(butadiene), act a matrix for noncovalent arrangement of BODIPY energy donor and bacteriochlorin energy acceptor chromophores. The polymer/ chromophore composites form nanoscale micelles in aqueous solution and defect-free monolayer and bilayer films on solid substrates. Donor-acceptor Forster resonance energy transfer is shown by steady state and time-resolved fluorescence spectroscopy and the system is modelled by theoretical calculations. Supported polymer bilayers demonstrated energy transfer efficiency up to 90%. Secondly, we present ongoing research into the redesign of protein/ lipid LH systems. Purified plant proteins and lipids are used as building blocks to form novel reconstructed protein/SLBs with defined compositions and 3-D organization using a combination of surface patterning and photolithography techniques. Atomic force and fluorescence microscopy and spectroscopy show protein arrangement and light harvesting functionality can be controlled. These new protein/chromophore and polymer/chromophore bio-inspired systems could act as a platforms to investigate membrane self-assembly and organization and could lead to applications in chip-based nanodevices.

106-Plat
Engineered AAA^+ Proteases Reveal Mechanisms of Degradation at the Mitochondrial Inner Membrane
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The Yme1 protease is a hexameric membrane-anchored AAA^+ machine that derives energy from ATP to control proteostasis in the inner membrane and intermembrane space of mitochondria. Substrates of Yme1 include soluble, peripheral membrane, and integral membrane proteins but the mechanisms of substrate recognition and degradation remain elusive. Detailed solution studies of this class of membrane-bound AAA^+ enzyme have been hampered by the requirement for an insoluble transmembrane span to drive oligomerization and thus form the ATPase sites. By replacing the transmembrane span with a soluble coiled-coil analogue, we have developed a method for driving assembly of soluble active hexameric Yme1 proteases that are fully competent for ATP-dependent protein degradation in vitro.
Using these engineered enzymes with model substrates we show that Yme1 requires substrates to present an accessible peptide signal to initiate degradation and displays clear preferences in the amino acid sequence of the degradation signal. Furthermore, the protease is capable of delivering a significant unfolding force to processively unravel and translocate stable protein substrates. These principles are extended to physiological substrates of Yme1 by examining the degradation of the intermembrane-space protein transport complex Tim9/10. We demonstrate the existence of a mitochondrial degradation signal that is necessary and sufficient to target proteins for proteolysis by Yme1.

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ER Calcium Release is Tuned by Mitochondrial Redox Nanodomains
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Spatial and temporal increases of H_2O_2 have emerged as an intracellular signal. We hypothesized that ROS and Ca^{2+} interact locally, in the confined volume of the ER-mitochondrial interface. These physically tethered structures host enrichments of ion transport proteins such as the IP_3 receptor (IP_3R), which support elevated nanodomains of Ca^{2+} during signalling events and are sensitive to H_2O_2. We used the genetically encoded H_2O_2 sensor HyPer incorporated into an inducible linker system to probe the redox environment at the ER-mitochondrial interface in HepG2 cells. We found a moderately elevated H_2O_2 nanodomain which developes into a H_2O_2 transient following IP_3R-mediated ER Ca^{2+} release. Pharmacological inhibition showed that the transient was dependent upon ER Ca^{2+} release via the IP_3R, mitochondrial membrane potential and a functional electron transport chain. HyPer measurements of the mitochondrial intermembrane space (IMS) revealed significantly elevated H_2O_2 within this volume. Using transmission electron microscopy we found that HepG2 mitochondria possess a cohort of dilated cristae, which disappeared following IP_3-linked Ca^{2+} release. Paxilline which inhibits BKCa channels, blocked the cristae reshaping and abolished the H_2O_2 transient at the interface. Furthermore, paxilline supressed IP_3-linked Ca^{2+} oscillations. Conversely, interface targeted Killer Red, a localized H_2O_2 source, induced sensitization to the IP_3-linked agonist. We conclude that the IMS/cristae volume of mitochondria represents an oxidized pool fed by the electron transport chain. Ca^{2+}-uptake stimulates expansion of the mitochondrial matrix via K^+ and concomitant water uptake, squeezing the oxidized volume of the cristae to the interface. Here, a transient H_2O_2 nanodomain sensitizes IP_3Rs to further stimulation. Thus, Ca^{2+} release may autoregulate using mitochondrial ROS released from the cristae during physiological calcium signaling.

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Unexpected Modifications of Cysteines in VDAC3: Indication that VDAC3 may Signal the Mitochondrial Intermembrane Redox State
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The accumulation in mitochondria of oxidative agents must be signaled to the cell. Neverthless the organelle is the source of redox species. The accumulation in mitochondria of oxidative agents must be signaled to the cell and an organelle heavily loaded with ROS evidenced. We addressed specific structural questions related to the function of VoltageDependentAnion-selectiveChannel isoform 3 (VDAC3) cysteines. We show that VDAC3 (1–3) may be relevant for signaling the redox potential existing in the mitochondrial intermembrane space. We found that VDAC3 can be progressively modified by an accumulation of ROS, resulting in the oxidation at different extents of the exposed cysteine residues. We discovered that each single VDAC3 molecule in the membrane can contain a differently oxidated set of cysteine residues, thus giving rise to what we call "redox isomers" (4). A disulfide bridge was evidenced by mass spectrometry (5). A recent paper indicated a putative disulfide bridge that we did not find by MassSpec (6), neither the authirs detected intermediate oxidation states. Since this complex oxidation pattern is a consequence of the ROS level in the IMS, VDAC3 monitores the redox homeostasis. In our opinion this work represents a pathbreaking finding in the field of mitochondrial redox sensing.
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109-Plat
Membrane Lipid Composition Regulates Alpha-Synuclein Blockage of and Translocation through the Mitochondrial Voltage-Dependent Anion Channel
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Alpha-synuclein (α-syn), an intrinsically disordered protein, is a major component of Lewy bodies, a hallmark of Parkinson's disease (PD). α-Syn has been found to interact with both inner and outer mitochondrial membranes (MOM),