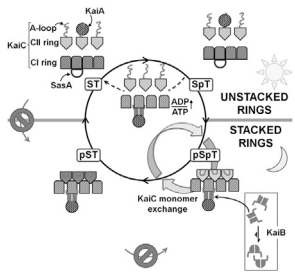
synchronizing them to a ~24-h biochemical rhythm. Here, we will show in a model system that structural gymnastics by proteins make the clock mechanism go round and round. The ''gears'' of the cyanobacterial clock are composed of three proteins, KaiA, KaiB, and KaiC. Remarkably, when they are mixed together in a test tube with ATP, they generate a circadian rhythm of (auto) phosphorylation and (auto)dephosphorylation of the KaiC protein for many days. KaiA stimulates KaiC phosphorylation, whereas KaiB antagonizes KaiA to promote KaiC dephosphorylation. The field has been attempting to crack the mechanism using published crystal structures to model complexes of these proteins. However, we will show that surprisingly large conformational changes (not captured in the crystal structures) determine when and how the KaiABC complexes assemble and disassemble, setting up time steps in the clock. We can also explain how the oscillator is entrained by environmental light/dark cycles, and how the clock transmits timing signals.



Platform: Bioenergetic Processes in Bacteria, Chloroplasts, and Mitochondria

152-Plat

Single-Molecule Live-Cell Imaging of Bacterial Respiratory Complexes Indicates OXPHOS Delocalization

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Chemiosmotic energy coupling through oxidative phosphorylation (OXPHOS) is crucial to life, requiring coordinated action of membrane-integral protein complexes whose architecture and dynamics in functional membranes are poorly understood. We explore the nanoarchitecture, molecular stoichiometry and real-time dynamics in functional *Escherichia coli* cells using genomic fluorescent protein fusions to 5 key OXPHOS complexes with *in vivo* single-molecule superresolution imaging and nanoscale localization microscopy. 10s to 100s of complexes cluster in mobile cytoplasmic membrane domains 100-200 nm in diameter. Domains of different complexes do not co-localize, indicating that electron transport and proton circuitry are delocalized over the whole membrane surface. We measured long-range diffusion of ubiquinone in the membrane, consistent with a role as a carrier shuttling electrons between islands of different complexes. Our results give a insight into the functional organization of a cell membrane, and indicate an OXPHOS strategy very different to that in mitochondria.

153-Plat

Cardiolipins at the Interface of Supercomplexes in the Respiratory Chain

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Mitochondria produce most of the ATP consumed by the cells through the so-called respiratory chain.

It is now well established that the protein complexes forming the respiratory chain assemble into larger structures, the supercomplexes. Cardiolipin (CL), signature phospholipid of mitochondria, composes ~20% of the mitochondrial inner membrane and is crucial for the formation and the functionality of these supercomplexes. Using coarse-grained molecular dynamics simulations we have previously shown that CL forms specific binding sites on the membrane exposed surface of the complex III (CIII) and complex IV (CIV) suggesting an involvement in proton delivery and supercomplex formation (Arnarez, J.Am.Chem.Soc. 2013; Arnarez, Sci.Rep. 2013). The fast exchange (~μs) of these CLs with the bulk membrane explains their absence in the crystal structures.

Here we investigate the role of CLs and these bindings sites in the formation and stabilization of supercomplexes by comparing self-assembly simulations of CIIIs and CIVs with and without CLs. We find a significantly larger protein burial formed in the absence of CLs. This difference results from a larger number of CIV/CIV interfaces formed in the absence of CLs. The number of CIII/CIV interfaces is not affected. In contrast the topology of CIII/CIV interfaces is affected by the presence of CLs but CIV/CIV interfaces are not. Notably CLs strongly populate CIII/CIV interfaces with a significant involvement of CL binding sites in the contacts formed, suggesting a role in forming and strengthening the interfaces, and potentially guiding the relative orientation of the complexes in supercomplexes. Our data suggest that CL acts as lubricant during the formation of supercomplexes, and seem to stabilize them by bridging the complexes together.

154-Plat

Elucidation of the Photodynamics of Single Photosynthetic LH2 Complexes in Solution

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Photosynthetic organisms function under low light by converting photoenergy to chemical energy with near-unity quantum efficiency and under high light by dissipating unused photoenergy to prevent formation of deleterious photoproducts. There is widespread interest in how this dual functionality is achieved, because this balance enables efficient light harvesting under the fluctuating intensities of sunlight at the earth's surface. One obstacle to characterizing the molecular mechanisms responsible for this balance is that the excited-state properties of photosynthetic proteins vary drastically between individual proteins (due to static heterogeneity) and even within a single protein over time (due to dynamic heterogeneity). In ensemble measurements, these excited-state properties appear as a static, average value. To overcome this averaging, we investigate light-harvesting complex 2 (LH2), the primary antenna in purple bacteria, at the single-molecule level. Using a novel technique, the Anti-Brownian ELectrokinetic (ABEL) trap, we study individual LH2 complexes in a solution-phase environment to eliminate perturbations due to immobilization schemes, which can alter the protein structure and function. Furthermore, we perform the first simultaneous measurements of fluorescence intensity, lifetime, and emission spectra from individual proteins. We identify three distinct functional conformations of LH2, two of which correspond to a quenched and an unquenched form, and observe transitions occurring between these forms on a timescale of seconds. Our results reveal that individual LH2 complexes undergo photoactivated switching to the quenched state, and thermally revert to the ground state. This is a previously unknown, reversible mechanism for dissipation of excitation energy activated by high light conditions, and may be one component by which photosynthetic organisms flourish under varying light intensities.

155-Plat

Mechanism of Water Splitting by Photosystem II

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The light driven water splitting achieved in the oxygen evolving complex (OEC) of Photosystem II is a critical process that sustains our biosphere. Photosynthetic water splitting is fascinating in its complexity, inspiring due to its practical applications in designing artificial photosynthesis, and not yet understood. At the heart of the water splitting process is the Mn_4Ca cluster embedded in a fine tuned protein environment.

The electronic structure and geometry of this cluster were probed by X-ray spectroscopy at the functional room temperature state.[1, 2] Detailed kinetic analysis of the X-ray induced damage was performed and allowed selection of undamaging conditions for experimentation. High-quality extended X-ray absorption fine structure (EXAFS) spectra of the OEC at room temperature will be presented and compared with XRD and DFT derived molecular models of the dark stable S_1 state. The determined Debye-Waller factors, sensitive to dynamic processes, support the rigid structure of the Mn_4Ca cluster at room temperature.

Laser-pump X-ray probe time-resolved X-ray emission measurements (XES) allowed monitoring of changes in the electronic structure of the OEC in real time during the catalysis. Using time-resolved XES we monitored the evolution of the electronic structure of the OEC of Photosystem II during the most critical S_3 to S_0 transition which results in O2 evolution. Our data show no oxidation but only a gradual reduction of the Mn centers after excitation of the complex past the S_3 state. These observations allow us to propose a unique O-O bond formation and water splitting mechanism. Combined with DFT modeling, our analysis reveals the mechanism of catalytic water splitting.

1. Davis et al. *J. Phys. Chem. B* 117, 9161-9169 (2013).
2. Davis et al. *J. Phys. Chem. Lett.* 3, 1858-1864 (2012).