61-Symp The Molecular Mechanism of the Activation of Myosin Filaments

Raul Padron, Lorenzo Alamo, BS[1], Willy Wriggers, PhD[2], Antonio Pinto, E.E.[1], Fulvia Bártoli, MSc[1], Leiria Salazar, PhD[1], Raúl Padrón, PhD[2]

Venezuelan Institute for Scientific Research (IVIC) Caracas, Venezuela.
[1] *IVIC, Venezuela*
[2] *U. Texas, U.S.A.*

Muscle contraction involves interaction of myosin heads of thick filaments with actin thin filaments. Myosin-linked regulation can occur by phosphorylation of the myosin regulatory light chains (RLC). EM of 2D-crystal vertebrate smooth muscle myosin provided insight into the relaxed (switched-OFF) myosin structure, achieved by asymmetric intra-molecular interactions between both heads, switching them OFF. Cryo-EM revealed that this interacting-heads structure is present in thick filament of tarantula striated muscle. We calculated an IHRSR reconstruction of frozen-hydrated tarantula thick filaments including the filament tilt, which increased the yield of images used, reaching 2nm resolution. The reconstruction reveals new densities: one between the free-head and its S2, and two between the free-head and the neighbor blocked-head. To interpret these intermolecular interactions, we sequenced the tarantula RLC. Using SITUS we fitted an interacting-heads atomic model that included the tarantula RLC homology model, its 52aa N-terminal fragment predicted model and the human cardiac S2 crystal structure. The fitting revealed an intramolecular interaction between the cardiomyopathy loop (Arg-411) of the free-head and its S2 and two intermolecular interactions between the cardio-loop (Arg-371) and the 297–326 loop of the free-head with the N-terminal fragments of the blocked-head essential and regulatory light chains. These intermolecular interactions help to establish the thick filament switched-OFF relaxed state. Phoshorylation of Ser-45 of the RLC N-terminal fragment could weaken this intermolecular interaction, as suggested by secondary structure predictions; helping to release both heads, activating the thick filament.

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62-Symp Navigating the Cytoskeleton with Myosin X

Ronald S. Rock

University of Chicago, Chicago, IL, USA.

Eukaryotic cells organize their contents through trafficking along cytoskeletal filaments. The leading edge of a typical metazoan cytoskeleton consists of a dense and complex arrangement of cortical actin. A dendritic mesh is found across the broad lamellopodium, with long parallel bundles at microspikes and filopodia. It is currently unclear if and how myosin motors identify the few actin filaments that lead to the correct destination, when presented with many similar alternatives within the cortex. Here we show that myosin X, an actin-based motor that concentrates at the distal tips of filopodia, selects the fascin-actin bundle at the filopodial core for motility. Myosin X moves individual actin filaments poorly in vitro, often supercoiling actin into plectonemes. However, single myosin X motors move robustly and processively along fascin-actin bundles. This selection requires only parallel, closely spaced filaments, as myosin X is also processive on artificial actin bundles formed by molecular crowding. Myosin X filopodial localization is perturbed in fascin-depleted HeLa cells, demonstrating that fascin bundles also direct motility in vivo. Our results demonstrate that myosin X recognizes the local structural arrangement of filaments in long bundles, providing a mechanism for sorting cargo to distant target sites.

Minisymposium 1: Structure-Function of Oxidative Pathway Proteins

63-Minisymp Building a Function into Simplified Structures: Membrane Spanning Maquettes with Chains of Redox Cofactors

Maxim Nikiforov, J. L. Ross Anderson, Elizabeth Sweeny, Viraj P. Parekh, Vidya Karunakaran, Paul A. O'Brien, Dawn A. Bonnell, P. Leslie Dutton, Bohdana M. Discher

University of Pennsylvania, Philadelphia, PA, USA.

Heme proteins play a pivotal role not only in energy generation but also in controlling cell life and death. Many of the key functions are accomplished through complicated system of enzymes and redox carrier molecules that control electron and proton transport. Even though significant number of these enzymes has been structurally characterized, the actual mechanism of redox catalysis is not always understood. Therefore we have adopted a different approach to address the structure-function of redox proteins: we aim to uncover the assembly instructions of function in proteins using smaller, simpler, more robust model proteins, maquettes. Our questions ask how many engineering elements are required to achieve a particular biological function, what are the individual biochemical and structural tolerances of these elements and how much of a protein infrastructure is consumed in accommodating the function. To start answering these questions, we have synthesized a set of amphiphilic maquettes (AP6 series). The 44 amino acid long, tetrameric AP-6 maquettes assemble with up to six ferric hemes b per tetramer in three different positions (two hemes per position). We will report how these maquettes transfer electrons across membranes, bind O2 and CO. These AP6 maquettes also co-solubilize with diblock copolymers or lipids and on an air-water interface, they compress to specific surface pressure, and can be transferred by Langmuir - Blodgett technique on HOPG (highly ordered pyrolytic graphite) surface for single molecule measurements of electrical properties by scanning probe microscopy.

64-Minisymp Structure, Function and Evolution of Reaction Center Bound Cytochrome c

Oliver Hucke[1], Gernot Kieseritzky[2], Ernst-Walter Knapp[2], Stefan Herter[3], Gerhart Drews[3], Andreas Labahn[1]

[1] *Institut für Physikalische Chemie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany*
[2] *Institut für Chemie und Biochemie, Freie Universität Berlin, Berlin, Germany*
[3] *Institut für Biologie II, Mikrobiologie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany.*

Photosynthetic bacterial reaction centers (bRC) from several species are equipped with a cytochrome C (CytoC) firmly attached at the protein complex. To these belong for instance bRC from the species *Blastochloris viridis* (*B. viridis*), *Thermochromatium tepidum* and *Roseobacter denitrificans* (*R. denitrificans*). In tetra-heme CytoC from *B. viridis* there are two high and two low potential hemes forming an electron transfer (ET) chain with alternating redox potentials recently called "ET roller coaster" . Under physiological conditions, CytoC functions as a two electron storage device, needed to perform a complete photocycle where two electrons move from the primary electron donor via a bacteriopheophytin and the primary electron acceptor (Q_A) to the secondary quinone (Q_B). But, the exact role of the tetra-heme ET chain and the energetic arrangement as a roller coaster is unclear. Recently, the energetics of ET in CytoC from *B. viridis* was computed successfully explaining how the protein environment is tuning the heme redox potentials to generate the ET roller coaster [1]. In the present contribution, we investigated the energetics of ET for the tetra-heme CytoC from *R. denitrificans*. Since no crystal structure is available the structure of the bRC was determined by homology modeling. FTIR difference spectroscopy revealed that the binding pockets of the reaction center cofactors are highly similar to those of *Rb. sphaeroides.* The energetic assignment of the hemes exhibited the same type of ET roller coaster, which can therefore be considered to play a functional role. We show that the ET roller coaster is likely a device to prevent photo-damage. Furthermore, we provide evidence that the tetra-heme CytoC emerged from a precursor two-heme CytoC by gene-duplication.

References

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65-Minisymp Electron Transfer from Cytochromes b_L to b_H Facilitates Head Domain Movement of Iron Sulfur Protein in Cytochrome bc_1 Complex

Xiawei Cen, Linda Yu, Chang-An Yu

Oklahoma State University, Stillwater, OK, USA.

Intensive biochemical, biophysical and structural studies of the cytochrome bc_1 complex in the past have led to the formulation of the "protonmotive Q-cycle" mechanism for the electron and proton transfer in this vital important complex. The key step of this mechanism is the bifurcated oxidation of ubiquinol at the Qp site with both electrons from the substrate molecule being transferred to ISP and cytochrome b_L simultaneously when the head domain of ISP is located at b-position. Pre-steady kinetics of reduction of cytochromes b_H and c_1 suggests that the reduced head domain of ISP moves to the c-position to reduce c_1 after the reduced cytochrome b_L is oxidized by cytochrome b_H. X-ray crystallography analyses of Pm and Pf inhibitors loaded cytochrome bc_1 complex crystals reveal that P_f inhibitor causes the fixation of head domain of ISP at the b-position whereas P_m inhibitor promotes the movement of the head domain of ISP. Analysis of inhibitory efficacy of Pm and Pf on the cytochrome bc_1 complex under different redox states reveals that stigmatellin (P_f) has a higher inhibitory efficacy when ISP in cytochrome bc_1 complex is in the reduced state, whereas MOAS (P_m) binds better when cytochrome b_H in the complex is in the reduced state. These results confirm that the electron transfer form b_L to b_H is the driving force for releasing the reduced ISP head domain from the b-position to the c-position to reduce cytochrome c_1.

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66-Minisymp Trapping oxygen-intermediates of bovine Cytochrome *c* Oxidase by rapid freeze-quench

Michelle A. Yu[1], Tsuyoshi Egawa[1], Gary J. Gerfen[1], Robert B. Gennis[2], Syun-Ru R. Yeh[1], Denis L. Rousseau[1]

[1] *Albert Einstein College of Medicine, Bronx, NY, USA*
[2] *University of Illinois - Urbana-Champaign, Champaign, IL, USA.*

The structure and function of intermediates formed in the catalytic reaction of molecular oxygen with fully reduced bovine Cytochrome *c* Oxidase (bC*c*O) was studied using a custom rapid freeze-quench technique, designed to trap fast biochemical reactions on the 50 μsec to several msec time scale. Fully reduced bC*c*O was mixed with O[[Unsupported Character - Codename]]$_2$-saturated buffer at room temperature. Reaction intermediates were freeze-quenched at 77 K at several time points and examined by optical absorption, EPR, and resonance Raman spectroscopy. A carbon-based radical with a narrow, symmetrical signal was detected and assigned to known intermediates based on characteristic optical absorption and resonance Raman spectra of P and F species. The results from bCcO are compared to equivalent measurements done on Cytochrome *c* Oxidase from *Rhodobacter sphaeroides*. The mechanism of the catalytic reaction was re-assessed based on these results.

67-Minisymp Net Proton Uptake is preceded by Multiple Proton Transfer Steps upon Electron Injection into Cytochrome c Oxidase

Kristina Kirchberg[1], Elena Olkhova[2], Hartmut Michel[2], Ulrike Alexiev[1]

[1] *Freie Universitaet Berlin, Berlin, Germany*
[2] *Max-Planck-Institute of Biophysics, Frankfurt, Germany.*

Cytochrome *c* oxidase (COX), the last enzyme of the respiratory chain of aerobic organisms, catalyzes the reduction of molecular oxygen to water. It is a redox linked proton pump, whose mechanism of proton pumping is controversially discussed and the coupling of proton and electron transfer is still not understood. Here, we investigated the kinetics of proton transfer reactions following the injection of a single electron into the fully oxidized enzyme and its

transfer to the hemes by using time-resolved absorption spectroscopy and pH indicator dyes. By comparison of proton uptake and release kinetics observed for solubilized COX and COX-containing liposomes, we conclude that the 1-μs-electron-injection into Cu_A, close to the positive membrane side (P-side) of the enzyme, already results in H^+-uptake from *both* the P- and N (negative)-side (1.5 H^+ and 1 H^+, respectively). The following 10-μs-transfer of the electron to heme *a* is accompanied by the release of one proton from the P-side to the aqueous bulk phase, leaving about 0.5 H^+ at this side to electrostatically compensate the charge of the electron. With about 200 μs, all but 0.3 H^+ at the N-side are released to the bulk, and the remaining proton is transferred towards the hemes to a so called "pump site". Thus, this proton may already be taken up by the enzyme as early as during the first electron transfer to Cu_A. The results of continuum electrostatic calculations support our conclusions by identifying residues which undergo protonation changes and may act as primary proton acceptor sites.

68-Minisymp A Serine to Aspartate Mutation Close to Heme a Results in a Slow, Proton Dependent Electron Transfer from CuA to Heme a and an Altered EPR Spectrum

Ling Qin[1], Denise A. Mills[1], Shujuan Xu[2], Lois Geren[3], Carrie Hiser[1], Martyn A. Sharpe[1], John McCracken[2], Francis Millett[3], Shelagh Ferguson-Miller[1]

[1] *Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA*
[2] *Department of Chemistry, Michigan State University, East Lansing, MI, USA*
[3] *Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.*

The difference observed in the heme *a* EPR spectra between cytochrome *c* oxidases (C*c*O) from mammalian mitochondria and various bacteria can be explained in part as due to a different amino acid, $G30_I$ in bovine C*c*O vs. $S44_I$ in *Rhodobacter sphaeroides* (*Rs*) C*c*O, hydrogen bonded to one of the heme *a* ligands, $H102_I$. Analysis of two site-directed mutants of *Rs*C*c*O, S44G and S44D, shows that S44G has native characteristics, but displays a shifted heme *a* EPR signal, closer to that of the bovine C*c*O. The S44D mutant has lower activity than wild-type and a shifted, split heme *a* EPR signal moved to a lower value, further from bovine. The splitting of the EPR signal is sensitive to pH, as is the intrinsic rate of electron transfer from Cu_A to heme *a.* At pH 8, the heme *a* EPR signal is most intense at g_z=2.72, and the electron transfer from Cu_A to heme *a* is less than 90 sec^{-1}, compared to 90,000 sec^{-1} for wild-type; whereas at pH 5.5, the g_z=2.77 peak is more intense, and close to 50% of the Cu_A to heme *a* rate is wild-type, while the rest remains slow. It is proposed that the protonated/deprotonated states of D44 result in drastically different redox potentials of heme *a,* by as much as 300 mV. This accounts for the markedly different electron transfer rates from Cu_A to heme *a*, where the slow rate is dependent on proton uptake to the internal S44D residue. The source of the proton is under investigation.

The different protonation states of residue D44, and an otherwise unaltered structure, are supported by preliminary X-ray crystallographic analysis.

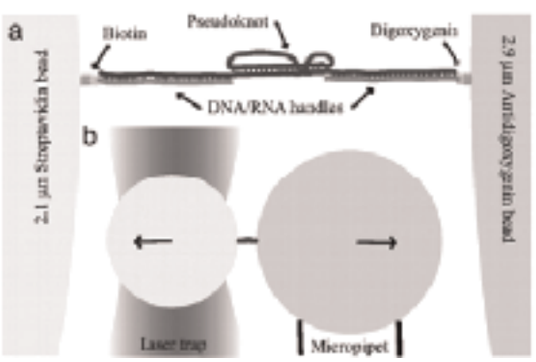
Platform F: DNA, RNA, Structure & Conformation

69-Plat Correlation Between Mechanical Strength Of mRNA Pseudoknots And Ribosomal Frameshifting

Thomas M. Hansen[1], Nader S. Reihani[2], Lene Oddershede[1], Michael A. Sørensen[1]

[1] *University of Copenhagen, Copenhagen, Denmark*
[2] *IASBS, Zanjan, Iran (Islamic Republic of).*

Programmed ribosomal frameshifting is often used by viral pathogens including HIV. Slippery sequences present in some mRNAs cause the ribosome to shift reading frame. Although the mechanism is not well understood, frameshifing is known to be stimulated by an mRNA structure such as a pseudoknot. Here, we show that the efficiency of frameshifting relates to the mechanical strength of the pseudoknot. Two pseudoknots derived from the Infectious bronchitis Virus were used, differing by one base pair in the first stem. These two pseudoknots caused framsshifting frequencies that differed by a factor of two. We used optical tweezers to unfold the pseudoknots. The pseudoknot giving rise to the highest degree of frameshifting required a nearly 2 fold larger unfolding energy than the other. We propose that the degree of ribosomal frameshifting is related to the mechanical strength of RNA pseudoknots (PNAS vol. 104 p. 5830, 2007). Our observations support the 9 Å model that predicts some physical barrier is needed to force the ribosome into the 1−1 frame. The result has implicatiomns for the understanding of genetic regulation and unwinding of mRNA secondary structures by ribosomes.



70-Plat Translation by Single Ribosomes One Codon at a Time

Jin-Der Wen[1], Laura Lancaster[2], Courtney Hodges[1], Harry Noller[2], Carlos Bustamante[1], Ignacio Tinoco[1]

[1] *University of California, Berkeley, Berkeley, CA, USA*
[2] *University of California, Santa Cruz, Santa Cruz, CA, USA.*

The ribosome and the mechanism of translation have been extensively studied by biochemical, genetic, and structural approaches. To explore the dynamic nature of translation, we have used optical