1073-Plat
Unraveling Chromatin Structure Using Magnetic Tweezers
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To understand the relation between gene regulation and chromatin structure it is essential to uncover the mechanisms by which chromatin fibers fold and unfold. We used magnetic tweezers to probe the mechanical properties of individual nucleosomes and chromatin fibers consisting of a single, well-defined array of 25 nucleosomes. Neighboring nucleosomes stabilize DNA folding into a nucleosome relative to isolated nucleosomes. When an array of nucleosomes is folded into a 30 nm fiber, representing the first level of chromatin condensation, the fiber stretched like a Hookian spring at forces up to 4 pN. Together with a nucleosome-nucleosome stacking energy of 14 kT this points to a solenoid as the underlying topology of the 30 nm fiber. Surprisingly, linker histones do not affect the length or stiffness of the fibers, but stabilize fiber folding up to forces of 7 pN. Fibers with a nucleosome repeat length of 167 bp instead of 197 bp are significantly stiffer, consistent with a two-start helical arrangement. The extensive thermal breathing of the chromatin fiber that is a consequence of the observed high compliance provides a structural basis for understanding the balance between chromatin condensation and transparency for DNA transactions.

1074-Plat
Spatio-Temporal Plasticity in Chromatin Assembly & Transcription Control within Living Cells
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Recent evidence suggests that the spatio-temporal organization of gene position and its interaction with the transcription apparatus within the crowded 3D architecture of the cell nucleus is vital to orchestrating gene regulation, yet the biophysical basis of this assembly is unclear. Using live-cell imaging combined with fluorescence spectroscopy and biomechanics experiments; we probe the dynamic nature of transcription compartments within living cells and the underlying transitions in chromatin organization during cellular differentiation. Our studies reveal an intimate coupling between chromatin plasticity and transcription compartment dynamics in the regulation of gene function.

Platform Q: Member-Organized Session: Diverse Views of VDAC Structure & Functioning: Quest for the Native Conformations

1075-Plat
Electron Microscopy of VDAC Membrane Crystals Redux. Pore Shape, Size, and Location(s) of the N-Terminal Domain
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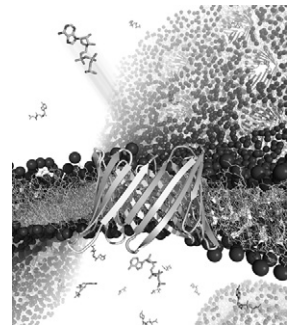
Atomic structures of mammalian VDAC, the mitochondrial outer membrane channel protein, have recently been determined by three groups using NMR and x-ray crystallography. The three structures are similar, showing a 19-strand β-barrel into which the N-terminal domain inserts. That this common protein fold was determined independently in three different labs strongly suggests that it is an easily accessible low-energy state of the protein. Nonetheless, concerns have been raised whether the atomic structures obtained using bacterially expressed proteins refolded in detergent micelles or lipid bicelles correspond to physiologically relevant states of this integral membrane protein. The definitive answer to this question will require detailed information about the topology of VDAC in its native mitochondrial outer membrane environment. Some insights can be provided by past electron microscopic (EM) studies of ordered arrays of fungal VDAC in isolated mitochondrial outer membranes. Projected density maps of the VDAC protein in frozen-hydrated membranes are consistent with a circular β-barrel having a diameter of 3.6+/-0.2 nm at the alpha-carbon backbone, in good agreement with the atomic models. The low resolution of the EM density maps may have precluded detection of the N-terminal domain within the pore lumen. However, antibodies against the N-terminal domain bound well both to isolated mitochondria and outer membranes, and the corresponding Fab fragments mapped to membrane regions adjacent to the pore. These data indicate that the N-terminus of VDAC in the mitochondrial outer membrane occurs outside the β-barrel at least some of the time. That the amphipathic N-terminal domain might move in and out of the lumen is suggested by changes in the pore detected by multivariate statistical analysis and difference imaging of VDAC arrays embedded in gold-glucose. (Supported by NSF grants and NIH/NCRR grant RR01219.)

1076-Plat
The Crystal Structure of the Murine Voltage Dependent Anion Channel 1 (mVDAC1) (Likely) Represents a Native Conformation
Jeff Abramson, Rachna Ujwal.
David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.
The Voltage Dependent Anion Channel (VDAC) constitutes the major pathway for the entry and exit of metabolites across the outer membrane of the mitochondria and can serve as a scaffold for molecules that modulate the organelle. After decades of trials, three groups independently reported the 3D structure of this eukaryotic membrane protein consisting of a 19-stranded β-barrel. Our group resolved the mVDAC1 structure at 2.3Å resolution revealing a high-resolution image of its architecture including the position of the voltage sensing N-terminal α-helix segment- oriented against the interior wall causing a partial narrowing at the center of the pore. Recently, however, the overall relevance of these structures and whether they represent a native conformation has been called into question. This presentation will highlight our assertion that this fold is indeed the native structure and stress: a) the bicelle crystallization method, b) electrostatic experiments corroborating an 'open' conformation, and c) new ongoing experiments to validate gating and conformation observed in the crystal structure.



1077-Plat
VDAC Studied by Solution NMR: Implications for the Native Structure
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The voltage-dependent anion channel (VDAC) is the main pathway for metabolites, small molecules and ions across the eukaryotic outer mitochondrial membrane. VDAC has been extensively studied for over thirty years and recently, high-resolution structures of VDAC were determined by X-ray and NMR methods (1-3). These studies used recombinant, refolded protein in membrane mimicking environments and thus the valid question arises how well the resulting atomic structure might resemble the "native" structure of VDAC in the mitochondrial outer membrane (4).
Here, we describe implications from solution NMR experiments to this question. Recombinant human VDAC-1 is stably folded in LDAO detergent micelles. Well resolved NMR spectra, including four-dimensional NOESYs, yielded a consistent set of more than 1000 spatial spin-spin correlations that unambiguously define the three-dimensional structure of VDAC-1 (1). The protein forms a 19-stranded beta-barrel with 18 antiparallel and 1 parallel strand pairing. The N-terminal 25 residues are not part of the beta-barrel and solution NMR data link the dynamic properties of this segment to the well-known voltage gating process. The inner diameter of the VDAC-1 barrel is about 25 Å, in consistence with published micrographs of native or native-like preparations. The entire outside perimeter of the barrel is hydrophobic and covered by detergent molecules, compatible with a membrane bilayer topology. NMR measurements also revealed interactions of VDAC-1 with beta-NADH and cholesterol, providing a functional connection to experiments on native states of the protein. Furthermore, we can link the micelle-bound state of VDAC structurally and functionally to preparations in phospholipid bilayers by comparing NMR spectra and electron micrographs.
(1) Hiller et al. Science 321, 1206 (2008).
(2) Bayrhuber et al. PNAS 105, 15370 (2008).
(3) Ujwal et al. PNAS 105, 17742 (2008).
(4) Colombini. Trends Biochem. Sci. 34, 382 (2009).

1078-Plat
Using VDAC-Tubulin Interaction to Assess VDAC Orientation in the Mitochondrial Membrane
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Recently we have found that dimeric αβ-tubulin at nanomolar concentrations induces reversible partial blockage of VDAC channel, reconstituted into a planar lipid membrane (Rostovtseva et al., PNAS 2008). Tubulin induces characteristic, well resolved fast blocking events with the highly voltage-dependent binding parameters. Tubulin interaction with VDAC requires the presence of C-terminal tails (CTT) of tubulin. Tubulin with truncated CTT did not induce reversible blockage typical for intact tubulin. We propose a model in which

negatively charged CTT of tubulin penetrates into VDAC pore, reaching through the channel at application from both sides of the membrane and interacting with high affinity with the positively charged channel lumen. We used the VDAC-tubulin specific interaction feature to probe orientation of VDAC in a planar membrane and extrapolate the results to the mitochondria outer membrane. We found that after *in vitro* phosphorylation by PKA or GSK3β cytosolic kinases the tubulin binding to VDAC (from rat liver mitochondria) becomes highly asymmetrical. When tubulin was added to the *cis* side of the membrane (side of VDAC addition) k_{on} was more than 10 times higher than with tubulin added to the *trans* side. Untreated VDAC interacts symmetrically with tubulin. Considering putative PKA and GSK3b phosphorylation sites on the cytosolic loops 3, 5 and 7, we conclude that these loops face *cis* side in the VDAC reconstitution system. Our preliminary data show that some of the specific antibodies raised against different VDAC peptides, compete with tubulin-VDAC binding when added to the membrane bathing solution and therefore could be employed to probe VDAC orientation and positioning of the loops. Recent VDAC three-dimensional structures are compared with the functional data of VDAC-tubulin binding.

1079-Plat

Electrostatic Properties of VDAC Channel: Structure Vs. Selectivity

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The measurement of ionic selectivity of large channels has been demonstrated as a useful tool for exploring their structure and their electrostatic properties. This is true even in channels whose crystal structure has been resolved down to a few angstroms by X-ray or NMR techniques. The comparison of structural data with measurements of channel conductance and reversal potential performed under a variety of experimental conditions adds valuable information about the role and/or position of key ionizable residues in the protein channel. The recently published 3D structure of the mitochondrial channel, VDAC, has challenged a large body of experimental evidence accumulated over the years about the channel functional properties. Here we analyze several experiments performed with VDAC (isolated from mitochondria of N. Crassa and rat liver) where the net charge of the channel has a key role. We discuss in detail whether measurements of selectivity, conductance, gating and tubulin-induced partial blocking of the channel are basically consistent with the same picture of the channel structural and electrostatic properties.

1080-Plat

Role of the N-Terminal Moiety in VDAC Isoforms

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VDAC or mitochondrial porin is the most abundant transmembrane protein in the mitochondrial outer membrane. Similarly to bacterial porins that are formed of a typical transmembrane β-barrel, the mitochondrial VDAC structure has been also found to form a β-barrel [1-3]. Interestingly the VDAC N-terminal 20 amino acids fold as an amphipathic α-helix [1-3]. We have synthesized the human VDAC1 (HVDAC1) N-terminal peptide Ac-AVPPTYADLGK-SARDVFTK-NH2 (Prn2-20) and determined its structure by CD and NMR. Our studies show that the Prn2-20 peptide exists as an unstructured peptide in aqueous solvent but it is structured in a hydrophobic environment [4]. Transfection of eukaryotic cells with recombinant humanVDAC1 devoid of N-terminal amino acids reduced the number of COS cells with depolarised mitochondria. These mutants showed an unaffected mitochondrial targeting [4].
Two more VDAC isoforms exist in mammals. 3D structural prediction showed that they have high probability to assume the same structure than VDAC1. N-terminal deletion experiments were performed in VDAC2 and VDAC3 and the effect of such N-terminal ablation was compared.
The overall picture emerging from our experiments is that the VDAC N-terminal peptide plays a role in the proper function of this protein during cell life events.
[1] Hiller et al (2008) Science 321, 1206; [2] Bayrhuber et al PNAS (2008) 105, 15370; [3] Ujwal et al (2008) 105, 17742; [4] De Pinto et al Chembiochem (2007) 8, 744.
Acknowledgements: The authors acknowledge the financial support of University of Catania and FIRB RBRN07BMCT.

1081-Plat

Investigating VDAC1 Orientation *In Vivo*

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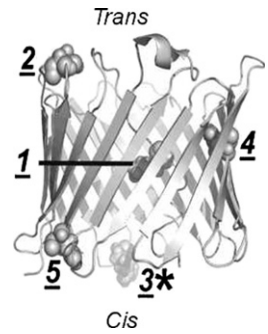
VDAC is an important and abundant ion channel in mitochondrial outer membranes but its structure remained unclear until recent structures of human VDAC1 revealed a 19 stranded β-barrel. Nevertheless there are still unresolved questions concerning the orientation of VDAC in the membrane and its fine structure. Starting from a bacterial OMP perspective we employ topology predictions based upon bacterial porins studies and orientation determination using measurements of antibody epitope exposure. We probe here the topology and orientation of yeast scVDAC1 *in vivo*. Five FLAG epitopes were independently inserted into scVDAC1 and their surface exposure in intact and disrupted mitochondria detected by immunoprecipitation. Functionality of the FLAG inserts was confirmed by measurements of respiration. Two epitopes suggest that scVDAC has its C terminus exposed to the cytoplasm whilst two others are more equivocal and, when combined with published data, suggest a dynamic behaviour.



References.

McDonald, B.M et al., (2009). *Febs Letters* **583**: 739–742.
Bayrhuber, M., et al., (2008) *PNAS* **105**: 15370–15375.
Hiller, S et al., (2008) *Science* **321**: 1206–1210.
Ujwal, R. et al., (2008) *PNAS* **105**: 17742–17747.

1082-Plat

Hard Constraints on the Structure of VDAC from Functional Studies

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Three different laboratories have solved the 3D structure of mammalian VDAC1 and obtained essentially the same result, a 19-stranded beta barrel. However, this structure is in serious conflict with published results of many experiments performed over the last 25 years. The 3D structures are of VDAC expressed in E. coli and refolded from inclusion bodies, and, despite the achievement of a uniform population of refolded protein, upon reconstitution into planar membranes, the conductances observed lack the characteristic electrophysiological properties of VDAC. It seems unlikely that the these properties of VDAC are an artifact and the refolded protein represents the native structure because these properties are highly conserved in at least one VDAC isoform from all species tested, from humans to potatoes to fungi to protists. Moreover, one single point mutation can drastically alter each of VDAC's characteristic properties: selectivity, steepness of voltage gating, and single-channel size. The strict evolutionary conservation compels the conclusion that the characteristic properties of VDAC are essential to optimal cell survival. Thus structural constraints deduced from studies on VDAC channels with these characteristic properties must be used as tests for any proposed structure of VDAC whether determined experimentally or theoretically. On this basis, the published 3D structures do not represent the native structure. (Supported by NSF grant: MCB-0641208)

Workshop 1: Applied Single-Molecule Techniques

1083-Wkshp

Single Molecule DNA Sequencing: from Demonstration to Application

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In recent years, single molecule DNA sequencing by cyclic synthesis has progressed from the demonstration stage [1] to a working system with high throughput DNA [2,3], cDNA [4], and direct RNA [5] unbiased sequencing capabilities. In this system, fluorescence microscopy is used to individually monitor tens of millions of immobilized DNA or RNA molecules for incorporation of labeled nucleotides. This process yields read lengths with sufficient sequence information to allow reliable and unique alignment of most tested fragments to a reference sequence, supporting a sequencing method that is amplification-free, fast and cheap. In this presentation, various aspects of single molecule sequencing by cyclic synthesis will be discussed. Low cost and high throughput DNA and RNA sequencing methods will usher in a new era of personal medicine.
1. Braslavsky I, Hebert B, Kartalov E, Quake SR: Sequence information can be obtained from single DNA molecules. Proceedings Of The National Academy Of Sciences Of The United States Of America 2003, 100:3960–3964.
2. Harris TD, Buzby PR, Babcock H, Beer E, Bowers J, Braslavsky I, Causey M, Colonell J, Dimeo J, Efcavitch JW, et al.: Single-molecule DNA sequencing of a viral genome. Science 2008, 320:106–109.