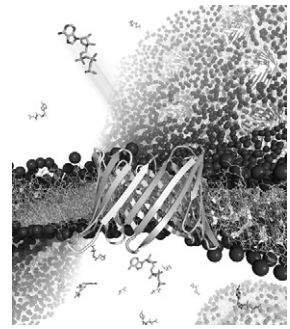
1073-Plat
Unraveling Chromatin Structure Using Magnetic Tweezers
John van Noort.
BF/LION, Leiden University, Leiden, Netherlands.
To understand the relation between gene regulation and chromatin structure it is essential to uncover the mechanisms by which chromatin fibers fold and unfold. We used magnetic 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
G.V. Shivashankar.
National center for Biological Sciences, TIFR, Bangalore, India.
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
Carmen A. Mannella, David Mankus.
Wadsworth Center, Albany, NY, USA.
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
Sebastian Hiller[1,2], Tsyr-Yan Yu[1], Thomas Raschle[1], Amanda J. Rice[1], Thomas Walz[1], Gerhard Wagner[1].
[1]Harvard Medical School, Boston, MA, USA, [2]ETH Zürich, Zürich, Switzerland.
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
Tatiana K. Rostovtseva, Kely Sheldon, Sergey M. Bezrukov.
NICHD, NIH, Bethesda, MD, USA.
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