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.
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1081-Plat

Investigating VDAC1 Orientation *In Vivo*

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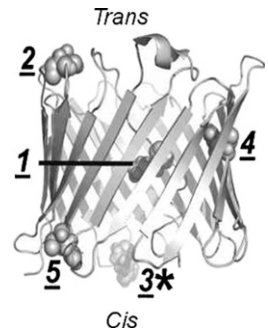
VDAC is an important and abundant ion channel in mitochondrial outer membranes but its structure remained unclear until recent structures of human VDAC1 revealed a 19 stranded β-barrel. Nevertheless there are still unresolved questions concerning the orientation of VDAC in the membrane and its fine structure. 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.



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Bayrhuber, M., et al., (2008) *PNAS* **105**: 15370–15375.
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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.
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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.