

and is known to exchange two chloride ions for one proton with typical turnover of 2000 ions/s for the wildtype protein. The genome databases yield hundreds of putative chloride transporting proteins with a significant bias towards proteins with transporter like sequences. We have functionally reconstituted and determined the structure to 2.5 Å resolution of a second microbial chloride-proton antiporter from *Synechocystis* sp. (ORF sl0855). CLC_{ss1} bears 39% sequence similarity to CLC_{ec1} with the key functional residues being almost identical. Despite this high degree of conservation the observed transport rates of CLC_{ss1} are several fold slower (~100 ions/s). The fold of the dimeric CLC_{ss1} is almost like CLC_{ec1} with an overall backbone rmsd of 1.7 Å. The residues analogous to E148, Y445 and S107 from CLC_{ec1} are situated in analogous positions, with channel lining helices displaying slight movements. No conduit through the protein is observed resulting in a more "inward-open" conformation. When diffraction data from crystals grown in 200 mM Br⁻ were analyzed no evidence for ion binding was detected which correlates with the slow chloride transport displayed by CLC_{ss1}.

1968-Plat

Electrophysiological Investigation of the Lactose Permease from *Escherichia coli* on a Solid-Supported Membrane

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¹Dept. Biophysical Chemistry, Max Planck Institute of Biophysics, Frankfurt (Main), Germany, ²Dept. of Physiology and Microbiology, Molecular Biology Institute, University of California, Los Angeles, CA, USA. Electrogenic events associated with the activity of the wild-type lactose permease (LacY) of *Escherichia coli* were investigated by using proteoliposomes containing purified LacY adsorbed onto a solid-supported membrane. Activation of the proteoliposomes with concentration jumps of different substrates generated transient currents. Analysis of the transient currents at different lipid to protein ratios and different pH values show that the currents represent stationary turnover of LacY. Furthermore, selective inactivation of the substrate binding by alkylation of C148 with *N*-ethyl maleimide (NEM) suppressed the transient currents, indicating that the transients correspond to the electrogenic activity of LacY. Mutant E325A LacY was used to investigate possible electrogenic steps in the transport cycle unrelated with proton translocation. In addition, electrogenic steps taking place before proton translocation were investigated with C154G LacY, which binds sugar as well as the wild-type but catalyzes very little transport activity. Both mutants show electrogenic activity after activation with different substrates. Therefore, either substrate binding or a conformational change following substrate binding is responsible for these electrical transients. As exchange (but not efflux) is almost voltage independent in wild-type LacY, it is concluded that there are at least two electrogenic steps in the transport cycle.

Symposium 15: Awards Symposium

1969-Symp

TBD

Robert Stroud.

University of California, San Francisco, San Francisco, CA, USA.

1970-Symp

Lollipops and Membranes

Stephen H. White.

University of California at Irvine, Irvine, CA, USA.

1971-Symp

Combining Patch-clamp and Fluorescence to Study Structural Dynamics of Ion Channels

Teresa Giraldez.

University of La Laguna, La Laguna-Tenerife, Spain.

1972-Symp

The Biophysics of Neural Computation

Adrienne Fairhall.

University of Washington, Seattle, WA, USA.

1973-Symp

Biophysical Tools Meet Biochemistry in Living Systems: A Tale of Protein Kinases and Second Messengers

Jin Zhang.

University of Maryland School of Medicine, Baltimore, MD, USA.

1974-Symp

Structures and Functions of Large Molecular Assemblies

Keiichi Namba.

Osaka City Univ, Osaka, Japan.

1975-Symp

Connecting Speckles – Relations between Cortical Actin Dynamics and Endocytosis

Gaudenz Danuser.

The Scripps Research Institute, La Jolla, CA, USA.

1976-Symp

Chemistry under Force

Julio Fernandez.

Columbia Univ, New York, NY, USA.

Minisymposium 3: Protein-Ligand Interactions in Cellular Signaling

1977-MiniSymp

Resolving Cadherin Interactions at the Single Molecule Level

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The cadherin family of Calcium-dependent cell adhesion proteins are critical for the morphogenesis and functional organization of tissues in multicellular organisms, but the molecular interactions that are at the core of cadherin binding are poorly understood. The generally accepted model is that cadherins adhere in three stages. First, the functional unit of cadherin adhesion is a cis-dimer formed by the binding of the extracellular regions of two cadherins on the same cell surface. Second, formation of low affinity trans interactions between cadherin cis-dimers on opposing cell surfaces initiates cell-cell adhesion, and third lateral clustering of cadherins cooperatively strengthens intercellular adhesion. Direct molecular proof of these cadherin binding states during adhesion is, however, contradictory, and evidence for cooperativity is lacking. We used single molecule structural (Fluorescence Resonance Energy Transfer) and functional (Atomic Force Microscopy) assays to demonstrate directly that cadherin monomers interact via their outermost domain to form trans adhesive complexes. We could not detect the formation of cadherin cis-dimers, but found that increasing the density of cadherin monomers cooperatively increased the probability of trans-adhesive binding. We also resolved the role of Tryptophan-2 a key amino acid in cadherin trans-binding. These results resolve conflicting data on trans- and cis-cadherin binding states, and provide quantitative evidence for cooperativity in trans-cadherin adhesion.

1978-MiniSymp

A Fluorescence Spectroscopic Approach To Quantify The Binding Characteristics Of The Intracellular Domain Of Crumbs With Pdz Domain-containing Proteins In *Drosophila Melanogaster*

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Formation of multiprotein complexes is a common strategy to pattern a cell, thereby generating spatially and functionally distinct entities at specialized regions. Central components of these complexes are scaffold proteins, which contain several protein-protein interaction domains and provide a platform to recruit a variety of additional components. There is increasing evidence that protein complexes are dynamic structures and that their components can undergo various interactions depending on the cellular context and/or the developmental status. The large transmembrane protein Crumbs is required for the establishment and maintenance of apico-basal polarity in *Drosophila* embryonic epithelia. The short intracellular domain of Crumbs localizes an evolutionary conserved protein scaffold via its interaction with the single PDZ-domain of Stardust. The Crumbs/Stardust/DPATJ complex coexists in *Drosophila* epithelial cells with another apical protein complex consisting of Bazooka, DmPar-6 and DaPKC. The degree of spatial overlap between components of these two complexes found in the subapical regions of many epithelia is striking and several in silico, qualitative in vitro and in vivo interaction experiments performed with constituents of these complexes have all pointed to a direct interaction between Crumbs and DmPar-6. To investigate and compare the interaction of the intracellular domain of Crumbs with the PDZ domains of Stardust and DmPar-6 we labeled the (putative) interaction partners with fluorescent dyes. This enables us to quantify the respective binding characteristics and complex properties with single molecule and ensemble FRET- and anisotropy- as well as with stopped flow-measurements.