From this asymmetric location, the cell wall starts to constrict and a septum forms. Our results show that the cellular structure of bacteria has a high degree of plasticity in coping with lateral stress and confinement.

[1] Bacterial growth and motility in sub-micron constrictions, J. Männik, R. Driessen, P. Galajda, J.E. Keymer and C. Dekker, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14861.

2151-Plat

Morphology, Growth and Size Limit of Bacteria

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Bacterial cell wall is the main structure to maintain a specific cell shape and to resist the osmotic pressure of several atmospheres. Despite many research, some basic questions remain unsolved: Out of many possibilities, why do bacteria only have several defined shapes? What is the relation between growth and morphology of a bacteria? How do rod-like bacteria select and maintain a specific radius, but grow in the axial direction? Is there any size limit for bacteria? What factors determine the size limit if it exists? In this paper, we set up a general growth model for bacterial cell wall and try to answer the previous questions. We found the growth modes and the size limits for coccus, bacillus, vibrio and spirillum, which are consistent with the experiments well.

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Structure and Assembly of CFA/I pili from Enterotoxigenic Escherichia Coli That Cause Traveler's Diarrhea

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Enterotoxigenic Escherichia coli (ETEC) bacteria that cause traveler's diarrhea utilize pili to initiate infection via pilus binding to epithelial cells in the small intestine. According to the World Health Organization, ETEC cause the largest number of recorded community-acquired cases of childhood diarrhea in the developing world, and are the most common cause of Traveler's diarrhea. Through a multi-disciplinary approach that includes x-ray crystallography, electron microscopy, site-directed mutagenesis, and genetic sequence analysis we elucidate the structure and assembly of CFA/I pili expressed on ETEC. We show that the distinction between Class I pili from the chaperone/usher pathway (e.g., P-pili from uropathogenic bacteria) and Class 5 pili from the alternate chaperone pathway (e.g., CFA/I pili), which was based on the lack of genetic sequence homology, does not correlate with any major structural or functional differences between these classes of pili. Pilin subunits transit the outer membrane through an usher that can accommodate single subunits, but not the assembled helical filament. We identify a proline residue in the major pilin, CfaB, that appears to isomerize from the trans to the cis conformation, producing the conformational change required for assembly of the mature pilus filament comprising about 1,000 subunits. Lastly, analysis of genetic variability among clinical strains representative of the eight discrete Class 5 fimbrial subtypes, in combination with structural data, show that each bacterial strain presents a distinct outer surface of CfaB, while the interior and protein-protein interface residues are more highly conserved. These data suggest that protein surface variability facilitates evasion of the immune system by ETEC.

2153-Plat

Force Generation by Type IV pili of *Neisseria Gonorrhoeae*

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Type IV pili are major bacterial virulence factors supporting adhesion, surface motility, and gene transfer. During infection they mediate attachment to mammalian host cells and elicit downstream signals. The polymeric pilus fiber is a highly dynamic molecular machine that switches between elongation and retraction. We used laser tweezers to investigate the dynamics of individual pili of the human pathogen *Neisseria gonorrheae*. We found that the retraction velocity of bacteria adhered to an abiotic surface is bimodal and that the bimodality depends on force and on the concentration of the putative motor protein PilT [1]. When adhered to host cells the bimodality persisted at higher forces compared to an abiotic environment. This increase in average velocity is consistent with an up-regulation of PilT due to interaction with host cells. Bacteria generated considerable force during infection but the maximum force was reduced from (120 ± 40)pN on abiotic surfaces to (70 ± 20)pN on host cells, most likely due to elastic effects. Velocity and maximum force of pilus retraction were independent of the infection period within 1h and 24h post infection [2]. Thus the force generated by type IV pili during infection is high enough to induce cytoskeletal rearrangements in the host cell.

[1] M. Clausen, M. Koomey and B. Maier, Biophys. J. 2009, 96, 1169-1177
[2] D. Opitz, M. Clausen and B. Maier, ChemPhysChem 2009, 10, 1614-1618

Platform AG: Membrane Protein Structure I

2154-Plat

Molecular Modeling and Simulations of the Transmembrane Domain of Human Growth Hormone Receptor

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How transmembrane (TM) domains of membrane proteins transmit the signal across the cell membrane has long been a subject of keen interest in biology. There is a recent paradigm shift in the mechanism of activation for the cytokine receptor superfamily. The role of cytokine hormone binding to the extracellular domain is now recognized as an ''inducer'' of the conformational change of pre-dimerized TM domains that triggers subsequent intracellular responses. This is drastically different from its traditional role as an ''organizer'' whose sole function was to initiate the receptor TM dimer formation. Toward quantitative understanding of the mechanisms and accompanying energetics of TM-induced signaling of various single-pass TM receptors, we have generated TM homodimer models of human growth hormone receptor (hGHR) from primary sequence information using the GBSW implicit membrane model and replica-exchange molecular dynamics (REX-MD) simulations. The conformational clustering shows that hGHR forms right-handed TM dimers with two different interfacial motifs, i.e., LFFQ and GxxG. To test such prediction, we first carried out TOXCAT experiments of two hGHR TM mutants: Gly256Ile and Gly259Ile. Mutation of either position to isoleucine disrupts dimer formation. These results suggest the involvement of the glycine residues in the TM helix interaction through the GxxG motif, although we need more extensive experiments to examine the involvement of other residues in the TM dimer interface, or the existence of an alternate dimerization point. In addition, we have performed MD simulations of various hGHR dimer models extracted from GBSW REX-MD in explicit POPC membranes. The stability and orientational changes of hGHR TM dimers as well as various helix-lipid interactions will be also presented and discussed.

2155-Plat

Molecular Dynamics Simulations of the Dimerization of Transmembrane α-Helices

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The lateral association of transmembrane (TM) α-helices within a lipid bilayer environment is a key stage in the folding of membrane proteins. It may also play a role in signalling across cell membranes. Dimerization of TM helices provides a simple example of such lateral association. Direct atomistic (AT) resolution MD simulation of self-assembly of a TM helix bundle remains challenging. AT-MD may be complemented by coarse-grained (CG) simulations. We demonstrate how CG-MD may be used to simulate formation of dimers of TM helices. We also show how a serial combination of CG and AT simulation provides a *multi-scale* approach for generating and refining models of TM helix dimers. This approach has been applied to a number of examples, including the glycophorin TM helix dimer (a paradigm for helix/helix packing) [1], and the TM domain of the syndecan-2 receptor protein, which contains a GxxxG motif comparable to that of glycophorin. The multi-scale approach has also been applied to a more complex system, the heterodimeric αIIb/β3 integrin TM helix dimer.

[1] Psachoulia, E., P. J. Bond, P. W. Fowler, and M. S. P. Sansom. 2008. Helix-helix interactions in membrane proteins: coarse grained simulations of glycophorin helix dimerization. Biochem. 47:10503-105012.

2156-Plat

Aromatic Interfaces between Transmembrane Helices M1/M4 and M3/M4 Play a Key Role in Cys-loop Receptor Assembly

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Cys-loop receptors, also designated pentameric ligand-gated ion channels (pLGICs) include nicotinic acetylcholine receptors (nAChRs), serotonin type 3 receptors ($5HT_3$Rs), γ-amino butyric acid type-A receptors ($GABA_A$Rs) and glycine receptors (GlyRs). pLGICs function as obligate pentamers linked by non-covalent interactions between the N-terminal extracellular domains of identical or homologous subunits. Here we show that expression of GlyR α1 or $5HT_3$A subunits in two separate fragments (one containing the ectodomain

and transmembrane domains M1-M3, and the other the fourth transmembrane domain M4) results in the assembly of functional pLGICs indistinguishable in their electrophysiological properties from wt pLGICs assembled from contiguous subunits. Alanine scanning of M1, M3 and M4 of the GlyR α1 subunit identified a total of 12 aromatic residues as important or crucial for pentameric assembly. The assembly-relevant aromatic residues cluster in one face of each helix. Homology modelling based on crystal structures (Hilf & Dutzler 2008; Bocquet et al 2008) predicted π- π interactions between the aromatic face of the M4 helix and three or two aromatic residues located in the M1 helix (Tyr228, Trp239, and Phe242) and the M3 helix (Trp286, Phe293), respectively . The loss of homopentamer formation and function seen upon alanine replacement of any of these contact residues strongly supports the existence of a membrane-embedded network of pairwisely interacting aromatic side chains that compacts and stabilizes the membrane core region of the GlyR. We infer from these results that a precise geometric arrangement of transmembrane helices defined by the tri-helical aromatic network is a prerequisite to allow the circular arrangement of the subunits stabilized essentially by earlier occurring random subunit interactions between the ectodomains.

2157-Plat

Conformational Changes in GPCR Surface and Core Probed by [^{13}C]-Methyl NMR Spectroscopy

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Recent crystal structures reveal the inactive states of non-rhodopsin G-protein coupled receptors (GPCRs) in beautiful detail. Solution NMR spectroscopy is ideally suited to contribute dynamic information regarding GPCR activation. However, these eukaryotically-expressed membrane proteins remain challenging NMR targets. We apply selective labeling with [^{13}C]methyl probes and two-dimensional NMR to analyze ligand-induced conformational changes in beta2-adrenergic receptor (b2AR).

Lysine side chains were labeled with [^{13}C]dimethyl probes to explore conformational changes in the b2AR extracellular surface. Lys305 forms a salt bridge connecting the extracellular end of transmembrane (TM) helix 7 with extracellular loop 2. The Lys305 NMR resonances are sensitive to conformational changes in the receptor extracellular surface. Using NMR, we observe disruption of the Lys305 salt bridge upon receptor activation by agonist. Computational modeling suggests that a lateral displacement of TM7 occurs in concert with an inward motion at the extracellular end of TM6 (thus extending the "global toggle switch" model of Schwartz (2006) *Annu. Rev. Pharmacol. Toxicol.*) Different conformational changes occur upon inverse agonist binding. Molecular dynamics simulations suggest that a conserved phenylalanine (Phe193) in the orthosteric ligand binding site is key for inverse agonism. Taken as a whole, these results demonstrate conformational coupling between the GPCR extracellular surface and orthosteric ligand binding site within the transmembrane domains (Ahuja (2009) *Nat. Struct. Mol. Biol.*) This provides rationale for developing allosteric pharmaceuticals targeting the GPCR extracellular surface.

Conformational changes within the b2AR transmembrane core are also observed by NMR using selective epsilon-[$^{13}CH_3$] labeling of methionines. While assignments are pending, clear conformational changes are seen with activation or inverse agonist binding. [^{13}C]methyl NMR spectroscopy, in combination with crystal structures and molecular dynamics simulation, provides a dynamic view of the conformational changes intrinsic to GPCR function.

2158-Plat

Observation of Structural Changes on Activation of the NTS1 G-Protein-Coupled Receptor on DNA-Templated Protein Arrays by cryo-EM

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We report the use of self-assembled DNA templates to create dense, orientationally disordered protein arrays that are optimized for single-particle cryo-EM. The nanostructure templates dramatically simplify data collection and have allowed us to obtain the first structures of a wild-type, neuropeptide-binding GPCR, the rat neurotensin receptor type 1 (NTS1), in both its ligand-free and liganded forms. Resolution better than 7Å allows clear identification of the 7 trans-membrane (TM) alpha-helices. Comparison of the structures provides the first direct observations and measurements of helix excursions during the conformational changes associated with activation of a ligand-binding GPCR. Conformational changes in the TM helices are observed upon ligand binding, namely shifts in TM1 and TM2 at the extracellular side of the membrane and pronounced shifts in TM5 and TM6 on the intracellular, G-protein interacting side that are the hallmark of the GPCR-activated state. Our results suggest a mechanism for ligand activation of a class A GPCR.

This is the highest resolution achieved to date by single-particle cryo-EM of a membrane protein, and NTS1 (43kDa) is an order of magnitude smaller than the nearest similar existing reconstruction of an asymmetric protein at comparable resolution. Our results suggest that the use of DNA-templated protein arrays has the potential to make high-resolution structure determination for small, asymmetric and hard-to-crystallize proteins routinely achievable.

2159-Plat

The Structure and Transport Mechanism of AdiC - an Arginine/agmatine Antiporter

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AdiC transports arginine and agmatine (the decarboxylation product of arginine) across the membrane of certain enteric bacteria including *E. coli*, as well as pathognic organisms such as *Salmonella*. It belongs to the APC (amino acids, polyamines and organic cations) superfamily. Its major role is to maintain the internal pH of the cell in the acidic environment (such as stomach) by functioning as a virtual proton pump - transporting arginine (+1 charge) into and agmatine (decarboxylated arginine, +2 charge) out of the cell, resulting in export of 1 proton out during each turnover.

We recently solved the crystal structure of AdiC with a Fab fragment at 3.2 Å. The protein is captured in an outward-open, substrate-free conformation. Both structure and functional data have shown that some aromatic residues (Y93, W293 and Y365) are important for the substrate binding and transport, yet we don't have the direct picture of the structure in the presence of either arginine or agmatine. Therefore, our current work is focused on obtaining the conformation with substrate-bound. One approach is to design pair wise cysteines that can crosslink and mimic the conformation with substrate. Comparing the structure of AdiC and several other proteins with similar fold, we are targeting TM2 and TM8 for the cysteine crosslinking now. A second approach, based on our experience with Fab production, is to find another Fab that can stabilize the substrate-bound conformation. Currently we have twelve more monoclonal antibodies that bind AdiC and need to be tested.

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Crystal Structure of the Membrane Fusion Protein CusB from Escherichia Coli

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Gram-negative bacteria, such as Escherichia coli, frequently utilize tripartite efflux complexes belonging to the resistance-nodulation-division family to expel diverse toxic compounds from the cell. These systems contain a periplasmic membrane fusion protein that is critical for substrate transport. We here present the x-ray structures of the CusB membrane fusion protein from the copper/silver efflux system of E. coli. This is the first structure of any membrane fusion proteins associated with heavy-metal efflux transporters. CusB bridges the inner membrane efflux pump CusA and outer membrane channel CusC to mediate resistance to Cu+ and Ag+ ions. Two distinct structures of the elongated molecules of CusB were found in the asymmetric unit of a single crystal, which suggests the flexible nature of this protein. Each protomer of CusB can be divided into four different domains, whereby the first three domains are mostly β-strands and the last domain adopts an entirely helical architecture. Unlike other known structures of membrane fusion proteins, the α-helical domain of CusB is folded into a three-helix bundle. This three-helix bundle presumably interacts with the periplasmic domain of CusC. The N and C-termini of CusB form the first β-strand domain, which is found to interact with the periplasmic domain of the CusA efflux pump. Atomic details of how this efflux protein binds Cu+ and Ag+ were revealed by the crystals of the CusB-Cu(I) and CusB-Ag(I) complexes. The structures indicate that CusB consists of multiple binding sites for these metal ions. These findings reveal novel structural features of a membrane fusion protein in the resistance-nodulation-division efflux system, and provide direct evidence that this protein specifically interacts with transported substrates.