thermal isomerization, and Schiff base hydrolysis of WT, S186W, and D190N rhodopsin. Using UV-visible spectroscopy, we observed that the D190N mutant and WT rhodopsin do not decay over 24 hours at 37°C, whereas the S186W mutant decays with a half-life of 36 ± 4 min. We also measured the half-lives at 55°C, which are 70 ± 2 min for WT, 2.4 ± 0.2 for D190N, and 0.43 ± 0.03 min for S186W. Using HPLC and the acid denaturation assay, we measured the rates of thermal isomerization of 11-*cis* retinal and hydrolysis of the Schiff base linkage between retinal and opsin. We found that the mutations also increase these rates by 1-2 orders of magnitude. Because thermal isomerization of rhodopsin generates the same physiological response as photoisomerization, we suggest that the higher thermal isomerization rate in the mutants increases the level of dark noise, which desensitizes rhodopsin and causes the early symptom of night blindness. Because the drastic destabilizing effect of the mutations is likely correlated with the progressive deformation of the outer segment and subsequent loss of rod cells in RP, we propose that a future systematic study of the thermal stability of the RP-causing mutations can potentially provide more accurate predictions of the pace of vision loss in patients and guide strategies for treatment.

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Assembly and Function of the Transmembrane Domain of the Two-Component System PhoQ from E.coli

Matteo Dal Peraro, Thomas Lemmin.

In bacteria, two-component systems (TCS) detect the environmental changes via a sensor kinase at the periplasmic level, which triggers a phosphorelay cascade mediating specific gene transcriptions. PhoP/PhoQ is a TCS, which detects and responds to divalent cations and antimicrobial peptides, initiating a resistant response for the reshaping of the bacterial membrane. The PhoQ sensor kinase assembly is characterized by a homo-dimeric structure, which spans the bacterial inner membrane. The multidomain structure of PhoQ from E.coli is largely unresolved, and consequently the mechanism by which the chemical signal is transferred across the membrane remains unclear. We used all-atom molecular dynamics (MD) techniques to assemble the PhoQ transmembrane (TM) domain, which is still structurally unexplored, but appears to be crucial for signal transmission. We observed that the conserved polar amino acids at the TM domain are important for the stability of the tetramer, and are directly involved in rotation of the helical bundle upon solvation. Thus, our computational results, which are consistent with experimental cross-linking data, support a rotating four-helix TM region and give a rationale for the mechanistic transmission of the signal through the bacterial membrane.

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Dissecting the Oligomeric Behavior of Caveolin-1 using the Analytical Ultracentrifuge

Monica D. Rieth.

The cell membrane is involved in a variety of cellular functions such as signal transduction, membrane trafficking, calcium signaling, and lipid recycling. One unique feature of the cell membrane is small invaginations called caveolae. Caveolae are comprised primarily of an integral membrane protein called caveolin-1, and it is the oligomerization of this protein that is thought to play a role in the formation of the caveolae structure in cell membranes. The caveolin-1 protein has four distinct domains: a soluble N-terminal domain, a short amino acid stretch called the scaffolding domain, a transmembrane domain that is hypothesized to form a putative hairpin-like loop in the bilayer of the plasma membrane, and a C-terminal domain that is believed to closely associate with the surface of the cell membrane. Understanding the oligomerization process of caveolin-1 is critical to understanding the biological role of this protein. We are investigating the self-association of each of the four domains of the caveolin-1 protein to determine the contribution of each domain to the oligomerization process. By performing sedimentation equilibrium experiments in the analytical ultracentrifuge and employing a technique called density matching, it is possible to determine which domains in the caveolin-1 protein are responsible for the oligomerization of the protein in a detergent micelle solution. Using a wide range of concentrations, we can determine which domain contributes most significantly to the oligomerization process by closely monitoring changes in the molecular weight of each domain as a function of increasing peptide concentration. With this comprehensive data we can begin to understand how caveolin-1 behaves in the cell membrane and therefore learn more about its biological role.

2971-Pos Board B76

Synthetic Adhesion and Migration Models of Living Cells

Christian Kreidler, Michael Bärmann, Joachim P. Spatz.

Cells are indivisible units of life. Therefore, mimicking single functions of cells has attracted major interest within the so-called synthetic biology field. In particular, cell adhesion, spreading and migration are fundamental functions for cells, which are worthwhile goals to aim for from a synthetic point of view. In addition, such biomimetic model systems allow for the quantitative description of cellular functions and hence contribute to the physical understanding of living cells.

In our approach, the adhesion protein integrin $\alpha_{IIb}\beta_3$ is reconstituted in giant unilamellar vesicles (GUVs) containing G-actin in buffer solution. For the imitation of the cell's cytoskeleton, actin can be polymerized by the addition of Mg^{2+} ions which can cross the membrane by means of an incorporated ionophore. To mimic the glycocalyx of a cell and to prevent unspecific binding, PEG-functionalized lipids are also included in the membrane.

This bottom-up artificial system could be further developed by adding more proteins successively, for example motor proteins or focal adhesion proteins and is aiming to the synthetic reconstruction of the adhesion and migration apparatus of living cells.

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Effect of FGFR3 Juxtamembrane Domain on FGFR3 Dimerization

Sarvenaz Sarabipour, Edwin Li, Kalina Hristova.

Receptor Tyrosine Kinases have four distinct domains: extracellular (EC), transmembrane (TM), juxtamembrane (JM), and catalytic (CAT). Receptor Tyrosine Kinase (RTK) dimerization is critical for RTK function, and disregulation of ligand-independent dimerization of RTKs is known to be the underlying cause for a number of human pathologies. Yet, the exact mechanism of RTK dimerization, and the roles of the four RTK domains in the dimerization process are unknown.

To investigate the role of the juxtamembrane domain in ligand-independent homodimerization of RTKs, we are comparing the dimerization of two truncated FGFR3 constructs which both lack the catalytic domain, EC+TM+JM and EC+TM. We assess dimerization in single membrane-derived vesicles using the quantitative imaging FRET (QI-FRET) method [Li et al., 2008, Chen et al., 2010]. The results show that the juxtamembrane domain of FGFR3 increases the measured FRET efficiency and hence contributes to the energetics of lateral dimerization of the FGFR3 receptor.

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2973-Pos Board B78

Unraveling Two Distinct Binding Interfaces for E Cadherin Dimerization: A Structural Study Using Single-Molecule Super-Resolved Fluorescence Imaging

Yunxiang Zhang, Alexandros Pertsinidis, W. James Nelson, Steven Chu.

Cadherins are homophilic adhesion molecules mediating Ca++ dependent cell-cell adhesion. It is not very clear how exactly cadherins interact and promote cell adhesion at the molecular level. We have previously characterized cadherin interactions with single molecule FRET and single molecule AFM and found cadherins interact mostly through outermost EC1 domains (Zhang & Sivasankar et al 2009). We then developed super-resolution localization microscopy that achieved sub-nanometer precision and accuracy and applied it to measurements of the end-to-end distances of E-cadherin dimers (Pertsinidis & Zhang et al 2010). The majority of the wild type cadherin dimers exhibits an extended EC5-EC5 distance of 32nm matching a strand-swapped trans-dimer model. A smaller population of the wild type dimers shows shorter distances of around 25nm suggesting an alternative binding configuration. We extended this work further by characterizing mutations in E-cadherin that prevent formation of the the strand-swapping interface. Interestingly, we observed that W2A dimers exist in a more compact conformation, similar to the minor population in the wild type dimers. The compact conformation is consistent with a so-called X-dimer interface discovered in recent crystal structures (Ciatto et al 2010, Harrison et al 2010). These new data refine our previous induced-fit dimerization pathway (Sivasankar & Zhang et al 2009) and are consistent with the X-dimer being the initial encounter complex. Furthermore, these results provide evidence that, at near-physiological Ca++ concentrations, E-cadherins can utilize two distinct binding interfaces to facilitate cell adhesion.