shape of self-assembled fibers is determined by competition between the elastic costs of inter-filament frustration, bending deformation of constituent filaments and surface energy of fibers. We find that for sufficiently large twist, isotropic (cylindrical) bundles are generically unstable to developing anisotropic cross-sections (helical tapes). Critically, the anisotropy of fiber cross-sections is found to give a direct measure of the anisotropy of inter-filament vs. intra-filament elasticity. We corroborate the universal predictions of our theory with numerical simulations of self-twisting fibers and compare the morphology diagram structural observations of anisotropy of micron-scale amyloid fibers assembled from hydrolyzed protein fragments.

**2661-Pos Board B91**

**Novel Single-Molecule Resolution Method for Spatio-Temporal Simulations of Protein Binding and Recruitment on the Membrane**

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In the early stages of clathrin-mediated endocytosis (CME), a variety of distinct proteins can bind to the membrane and engage in further interactions with proteins on the membrane and in solution. Understanding the dynamics of this process requires correctly accounting for the behavior of protein interactions while restricted to the 2D membrane surface, as it is fundamentally distinct from binding in solution due to changes in the dynamics of the proteins. Here we introduce the 2D Free-Propagator Reweighting (2D-FPR) method that accurately models the spatial and temporal dynamics of proteins as they are recruited to the membrane surface and as they interact with one another while bound to the membrane. In this method the position of each diffusing protein is tracked, and reactions between binding partners can occur upon collisions. Reaction probabilities are determined by the solution to the 2D Smoluchowski diffusion equation with reactive boundary conditions, allowing us to take large time steps. Molecule positions are propagated by free diffusion, but by using a trajectory reweighting approach we can recover the exact association rates for all reactive pairs. This approach is uniquely able to capture the changes in protein binding dynamics that can occur upon membrane binding because it accounts for both the diffusional motion of proteins and their binding reactions. These important details are absent from models that lack spatial resolution. We present our simulation results on modeling adaptor protein interaction dynamics, and discuss the effects of varying local protein concentration on both recruitment to the membrane and complex formation in the confined 2D geometry.

**2662-Pos Board B92**

**Stoichiometry as a Key Modulator of PIP Plant Aquaporins Biological Activity**

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The specific self-association of membrane channels to form oligomeric assemblies is a biological relevant event, which usually confers functional advantages to biological systems. Evidences strongly support that plant plasma membrane aquaporin (PIP) can physically interact to form oligomers by combining PIP2 and PIP1 monomers; however, the kind of oligomer and/or its stoichiometry has not been experimentally elucidated yet.

Along this research, we aim at defining whether aquaporins are able to form functional hetero-tetramers (different PIP monomers organized in a single tetramer) with a given stoichiometry that determines their activity and regulation.

To achieve this goal, we examine the functional properties of heterotetramers comprising different PIP2-PIP1 ratios. Our experimental approaches include: i) designing mutants to alter PIP2-PIP1 interaction; ii) performing homo and heterodimeric constructs made of either PIP1 or PIP2, as well as both subunits, iii) analyzing PIP location by confocal fluorescence microscopy; iv) measuring water transport in control and inhibited conditions (citosolic acidification); and v) studying PIPs in silico.

Results show that PIP heterotetramers with different stoichiometries can be functional in a heterologous cellular system since they are able to assemble by expression of PIP2-PIP1 tandem dimers, and by co-expression of those dimers plus PIP2 or PIP1 monomers. Interestingly, the composition of these heterotetramers can modify water transport activity and pH sensitivity by shifting the EC50 of the inhibitory response. Moreover, the first extracellular loop of PIP2 acts as a crucial structural element to achieve PIP heterotetramerization.

Thus, our findings throw light not only on PIP heterotetramerization as a novel regulatory mechanism to adjust water transport across the plasma membrane but also on the stoichiometry of PIP heterooligomers, issues that had been unclear for many years in the biophysical field of plant aquaporins.

**2663-Pos Board B93**

**Landscapes of Membrane Protein Interactions from High-Throughput MD Simulations using the Daft Approach**

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Interactions between membrane proteins are key in many biological and pathological processes and offer potential targets for pharmacological intervention. Unfortunately, the complex environment makes it difficult to explore these in high detail. In addition, the time scales of binding and unbinding pose difficulties for molecular simulations to probe such interactions. Over the past few years, we have developed an approach using large numbers of simulations, which avoids the problem of unbinding, allowing rapid building of a detailed map of the interaction landscape. The method, called Docking Assay For Transmembrane components (DAFT), has to date been used to investigate a range of 23 Glycophorin A mutants, a set of 40 receptor tyrosine kinase (RTK) pairs, SNARE protein TM helices, the DesK minimal thermosensor and GPCRs, accounting for >15M CPU hours and representing a total simulation time of more than 30 milliseconds. The results show that several hundreds of simulations are necessary for a converged view and that the time scales required range from 300 ns per simulation for simple helices to microseconds for larger and more complex systems. Yet the results also provide unique views on the convergence properties of ensembles of simulations, yield detailed maps of interaction landscapes, and allow 2D-PMFs to be derived. Furthermore, the comparison of different members of the RTK family of or wild type proteins and mutants gives insight in the mechanisms underlying the relative propensities to dimerize.

**2664-Pos Board B94**

**Increased Signaling Efficiency of Conventional PKC through Self-Assembled Clustering on the Plasma Membrane**

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The Ca sensitive conventional Protein Kinases C (cPKCs) play critical roles in signaling processes. cPKCs are recruited to the plasma membrane by Ca binding to its C2 domain. Diacylglycerol (DAG) at the membrane enforces cPKC-membrane binding via its C1 domain and activates the kinase. Phosphorylation rates of cPKCs are around 6 per second. During the average Ca-dependent membrane residence time of some tens of milliseconds at most one phosphorylation event may take place. How cells overcome this apparent limitation is still unclear. We used quantitative video- and confocal FRET measurements of PKCα-CFP and -eYFP and mathematical modeling of putative PKC-PKC interactions at the plasma membrane to address this. Following ATP stimulation, membrane recruitment of PKCα was accompanied by substantial increases in FRET. The FRET signal decayed significantly slower than the underlying Ca transient. Sole increases in the intracellular Ca concentration were also sufficient to induce long-lasting FRET signals resulting from membrane-bound PKCα. Since the FRET signal spontaneously decayed at high Ca concentrations, we could exclude the possibility of molecular crowding as the underlying FRET mechanism. We defined a computational model of Ca dependent PKCα attachment to the membrane, which included formation of PKCα clusters. Assuming cooperative detachment from the membrane of PKCα in a cluster we could reproduce all experimental observations. Ca dependent formation of clusters on the membrane is not limited to PKCα but appears as a universal property of C2-domain containing proteins, including the sole C2-domain, PKCßII and Synaptotagmin-1. This mechanism leads to an increased membrane residence time of signalling proteins and could thus represent a universal feature of cellular signaling. Supported by the DFG (SFB1027).