and unfavorable enthalpy, respectively. Furthermore, we characterize dock and lock states of the peptide based on the solvent accessible surface area. We observe that the Lennard-Jones energy of the system increases continuously in lock and dock states as the peptide dissociates. The electrostatic energy in the lock state increases as the peptide dissociates and inter-peptide hydrogen bonds are ruptured while it decreases in the dock state as new peptide-water hydrogen bonds are formed. We also observe that before unbinding from the fibril, the peptide has to overcome an enthalpic barrier of the order of 10 $kJmol^{-1}$. This barrier is associated to interactions between exposed phenylalanine residues of the fibril and the peptide. Implication of these results to fibril growth will be discussed.

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A Computational Study of Amyloid β-Protein Assembly in Crowded Environments

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Alzheimer's disease is strongly associated with aberrant amyloid β-protein (Aβ) assembly into heterogeneous, metastable oligomeric assemblies with structures that have not been experimentally characterized yet. The 40 and 42 amino acids long Aβ40 and Aβ42 are the two predominant Aβ alloforms in the brain. Whereas Aβ40 and Aβ42 oligomer formation from monomeric state is still inaccessible to fully atomistic explicit-solvent molecular dynamics, Aβ40 and Aβ42 oligomers were structurally characterized using discrete molecular dynamics (DMD) and an intermediate-resolution protein model within the DMD4B-HYDRA implicit solvent force field, and the corresponding oligomer size distributions well matched the available in vitro data. In vivo, however, Aβ coexists with other biomolecules in a rather crowded environment. To understand the effect of crowding on Aβ oligomer formation, we used the DMD4B-HYDRA force field and added to an ensemble of 32 monomeric Aβ40 or Aβ42 peptides inert spherical "crowders" with a diameter of 0.5 nm at various concentrations to examine their effect on Aβ40 and Aβ42 oligomerization pathways. Our results show that crowding shifts oligomer size distributions towards smaller oligomer sizes and increases solubility of both peptides in a concentration-dependent way. The effect is stronger for Aβ42, where crowding abolishes the multimodal character of the oligomer size distribution. Our structural analysis revealed that the stability of larger oligomers is compromised by effective osmotic pressure exerted by the crowders, resulting in an increased rate of assembly breakage. While in vivo crowding agents are not inert as the crowders in our study, we here reveal that crowding-induced osmotic pressure strongly affects protein assembly dynamics, which is of significance to the disease.

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Transition of Amyloid Oligomers to Mature Fibrils: Internal Conversion Vs. Competing Assembly Pathways?

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Deposition of protein plaques, rich in long rigid fibrils with a characteristic cross-beta sheet structure, is the pathological marker for human disorders ranging from Alzheimer's disease to type II diabetes and rheumatoid arthritis. Significant evidence has implicated the formation of globular oligomeric amyloids as the main pathogenic agent in amyloid diseases. At the same time, in vitro experiments indicate that amyloid oligomers and rigid fibrils are formed along distinct assembly pathways with characteristic growth kinetics. This raises the questions how these early-stage oligomeric intermediates are converted to the rigid fibrils dominating during the late-stages of most amyloid diseases?

We have investigated the transition from the formation of amyloid oligomers and their curvilinear polymers to the rigid late-stage fibrils using the model amyloid hen egg white lysozyme (hewL). We have shown that hewL oligomers form a distinct aggregate phase with a well-defined transition boundary. However these oligomers and their curvilinear fibrils are metastable against the formation of thermodynamically stable rigid fibrils. We therefore performed experiments to discern whether amyloid oligomer species were directly converted into the stable rigid fibril conformation or whether rigid fibril nucleation proceeded in parallel, i.e. in competition with, oligomer formation. To do so, we monitored the rates of rigid fibril nucleation right outside and inside the transition boundary for oligomer formation. Our data suggest that oligomer formation is in kinetic competition with rigid fibril nucleation for their monomeric growth substrate. Futhermore, we observed no signs that prior formation of oligomeric species accelerated the nucleation of rigid fibrils. The latter would be expected for conformational conversion of oligomers into rigid fibrils. If anything, prior formation of oligomers retards the nucleation of rigid fibrils.

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Structural Variations of Amyloid β-Protein Fibrils Seeded with Extracted Fibrils from Brain Tissue of Alzheimer's Disease Model Mice

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In our previous studies by electron microscopy (EM) and two dimensional infrared spectroscopy of amyloid fibrils prepared *in vitro* from synthetic 40-residue β-amyloid (Aβ40) peptides, it was shown that the Aβ40 fibril structure and its molecular structure are not uniquely determined by amino acid sequence. Instead, the fibril structure is dependent upon the growth conditions. The molecular structures of β-amyloid fibrils that develop in Alzheimer's disease (AD) are therefore uncertain. In this study, fibrils extracted from brain tissue of AD model mice (three strains of aged transgenic mice with increased levels of human amyloid proteins, 3xTg-AD, J20Tg-AD and 5xFAD) were used to seed the growth of synthetic Aβ40 fibrils. Because amyloid fibril structures propagate themselves *via* seeded growth, the structures of seeded Aβ40 fibrils likely reflect structures in AD brain. Negatively stained EM images indicate that seeded fibrils tend to appear twisted like a ribbon, with periodic narrowing or nodes. The distances between nodes (the "internodal" distances) were relatively homogeneous distributions (~145 nm) in the 3xTg-AD and J20Tg-AD mice. In 5xFAD, on the other hand, fibrils having short internodal distances (~30 nm) were observed in addition to the distributions (~145 nm) which were measured in two other strains of mice. The mass-per-length (MPL) evaluated from dark-field EM images indicates that the most prevalent numbers of filaments in fibrils are 3 and 4 but the dominant number of the filaments is dependent on the source of the fibril seeds. Overall, these results demonstrate that AD model mouse brain-derived fibrils have a distinct fibril structures, and the model most relevant to human AD has yet to be determined.

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Determination of Nucleation Mass for Amyloid-β Aggregation

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Protein misfolding and concomitant self-assembly towards ordered aggregates (amyloids) has emerged as an important event governing both functional and pathological events in cells. Both structurally and biophyiscally, amyloid formation is highly conserved involving the conversion of proteins (intrinsically disordered or globular) from their native monomeric states to well-organized, fibrillar aggregates in a nucleation-dependent manner. Although a plethora of literature exists on modeling such aggregations, the molecular mechanisms are poorly understood, especially those leading up to nucleation. In our study we use Aβ as the model system to test our theoretical framework for amyloid aggregation. Specifically, we focus on nucleation, which we believe to be a critical gate-keeping event which controls the dynamics of the entire pathway and determines the physiochemical and biochemical fate of the aggregates formed. In this study we clarify the mechanics of aggregation leading to nucleation, and how fibril morphology depends on size and conformation of the nucleus. The pre-nucleation dynamics are modeled by ODE simulations based upon mass action kinetics and also supported by experimental data. An alternative, novel approach, based upon stability of the equilibria is utilized to identify the optimal nucleation mass range and properties associated with the nucleus.

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Morphology Selection through Geometric Frustration in Twisted Filament Bundles and Fibers

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Rope-like assemblies of twisted protein filaments constitute a common materials archetype appearing in a range of biological contexts from extracellular filament bundles to amyloid fibrils. Owing to the numerous distinctions in molecular structure and interactions underlying these diverse assemblies, a common framework to predict and classify the basic mechanisms of structure formation in twisted filament assemblies is still lacking. In this study, we exploit a recent and surprising connection between the assembly of self-twisting filaments and assembly on spherically-curved 2D surfaces to develop a universal theory of morphology selection in twisted fibers and bundles. This theory shows that the size and cross-sectional

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.

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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.

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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.

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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.

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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).