The Power of Polypeptides from a Molecular Perspective

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Polypeptides (also called proteins) are linear biopolymers composed of amino acid residues that fold into well-defined structures depending on their amino acid sequence. Folding is essential for the function of a protein whereas misfolding can cause severe diseases. Our aim is to understand protein function and diseases on a molecular level. The information on the molecular dynamics of polypeptides accessible experimentally is very limited. Therefore we employ molecular dynamics (MD) simulation techniques to model the process by which polypeptides sample conformational space. Here, the polypeptide and its solvent environment are described in atomic or near-atomic detail. Currently we try to understand how polypeptides may drag cellular organelles along filaments, recognize and kill bacteria as part of the immune defense, or cause neurodegenerative diseases.

Amyloid Peptides – Origin of Neurodegenerative Diseases

Amyloid diseases including Alzheimer's or Creutzfeld-Jakob disease are associated with the conversion of a protein from a soluble (functional) form into higher order fibrillar aggregates rich in β-sheet structure. The toxic species, though, seemingly, are not the mature fibrils but early oligomers. To understand the origin of their toxicity and to develop drugs against amyloid diseases requires to comprehend the structure of these species. We study the folding and aggregation of small amyloid peptides in solution. The systems investigated include the model amyloid peptide B18 as in **Fig. 1**(a,b) **[1]**, as well as the 25-35 **[2]** and the 10-35 fragment of the Amyloid β (Aβ) peptide associated with Alzheimer's disease **[3]**, as in **Fig. 1**(c-e) and (f-k), respectively.

The amyloid peptides form various β-sheets consisting of different sets of residues with comparable statistical weight. Aggregation is largely driven by the hydrophobic effect. Disordered conformations are stabilized entropically whereas fibril-like, β-sheet rich structures exhibit a lower energy. The Aβ (10-35) dimers show a larger conformational diversity than observed in previous simulations using a (less accurate) implicit solvent model, highlighting the need of the (more accurate but also computationally more expensive) explicit solvent model. In a running project, we also study the full length Aβ (1-40) peptide in terms of the effect of an interface on the peptide's conformation and the peptide's ability to induce membrane pores, as a possible origin of its toxicity. In collaboration with Gerald Brezesinski, the structure of larger aggregates, peptide monolayers with β-sheet structure at a water/air interface, has been studied **[4]**.

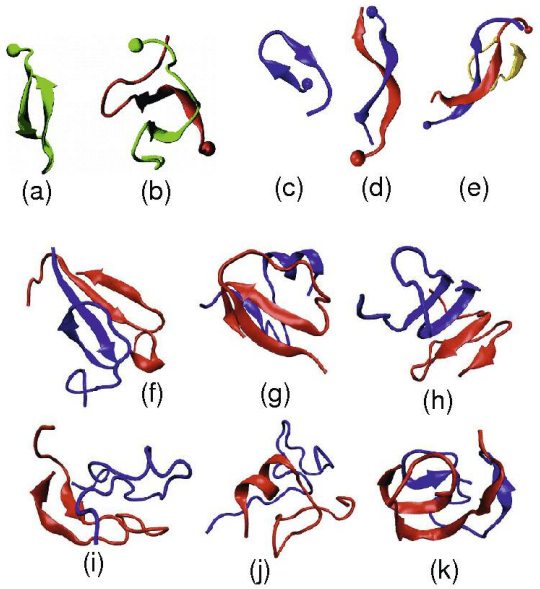

Fig. 1: β-hairpin folding and aggregation of fibrillogenic peptides in explicit water in molecular dynamics simulations. The model peptide B18 (a,b), as well as the Aβ (25-35) and Aβ (10-35) peptides associated with Alzheimer's disease, are depicted. In detail, (a) a B18 monomer **[1]** *and (b) dimer* **[1]**, *(c) an Aβ (25-35) monomer, (d) dimer* **[2]**, *and (e) trimer, as well as (f-k) Aβ (10-35) dimers* **[3]**, *are shown in ribbon representation.*

Antimicrobial Peptides – Smart Weapons of Immune Defense

Antimicrobial peptides (AMPs) are an evolutionary conserved component of the innate immune system found among all classes of life; their main function is the recognition and inactivation of invading pathogens like bacteria, viruses, or fungi. The mode of action of most AMPs is the permeabilization of the cell membrane via the formation of pores. AMPs are toxic against bacteria without affecting cells produced naturally in multicellular organisms, likely due to specific binding to lipids contained in the extracellular leaflet of probut not eukaryotic cell membranes. In vitro experiments of the antimicrobial peptide NK-2 indicate that the discrimination between zwitterionic lipids with phosphatidylethanolamine (PE) head groups exposed by prokaryotes and phosphatidylcholine (PC) head groups exposed by eukaryotes plays an important role. We have conducted molecular dynamics simulations in conjunction with a coarse grained model confirming that NK-2 binds more strongly to PE than to PC and revealing the underlying mechanism **[5]**. As indicated in **Fig. 2**, we find that the transfer of NK-2 from POPE to POPC is favored because of a better shielding of nonpolar groups from the water and increased electrostatic interactions

between the cationic and anionic portions of the lipid headgroups. We also find that the adsorption of a cationic peptide to an anionic lipid is governed by a complex interplay of competing interactions. In a related project we reveal the driving forces of molecular recognition of pathogens in the form of proteins or lipids by antibodies **[6]**.

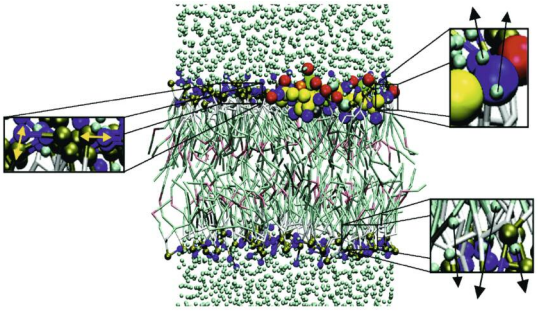

Fig. 2: Configuration of antimicrobial peptide NK-2 (large spheres) at a POPE bilayer from an MD simulation using a coarse grained model **[5]**. *The main contributions to the favorable transfer of NK-2 from POPE to POPC are highlighted; these are a removal of water particles from the hydrophobic core and the nonpolar side chains and an increase in the number of interlipid salt bridges.*

Molecular Motors – Force Generators of the Cell

Kinesin motors use the chemical energy supplied by ATP hydrolysis to transport cargo along microtubules (MTs). Because of the ATP hydrolysis, the motor assumes different nucleotide states during its processive motion. These three states differ in their affinities to the microtuble; strong binding of the motor domain to tubulin is observed when the nucleotide-binding pocket is empty or contains ATP whereas weak binding, leading to detachment, occurs when ADP is bound. The catalytic cycle of the motor domains is out-of-phase which facilitates kinesin's walk along the MT. Phosphate release is believed to trigger conformational changes in the motor head leading to detachment of the head from the MT and the undocking of the neck linker from the motor domain. The neck linker is a 10 amino acid residue peptide at the carboxy terminal of the motor domain and, because of its nucleotide dependent flexibility, is proposed to generate a force that brings the trailing motor head to the leading position.

Our simulations of a kinesin head attached to tubulin provide strong evidence for a specific allosteric coupling mechanism that consists of several subsequent molecular transitions which finally lead to the detachment of the neck linker from the motor domain **[7]**. The initial steps in this cascade of conformational changes induced by phosphate release are indicated in **Fig. 3**. Interestingly, our simulations reveal conformational changes of the proteins that are quite different from rigid-body transformations as previously assumed when high-resolution X-ray structures were fitted into low-resolution cryo electron microscopy images. In running projects we study the free energy differences between the different nucleotide states assuming a local equilibrium for pairs of states. As the motor energetics depend on the equilibrium constant for the hydrolysis reaction which we would like to understand from ab initio calculations, we are currently testing quantum mechanical methods, starting with the calculation of free energies of formation for gas phase reactions of small molecules. Finally, we investigate the mechanical step itself in full atomic detail with explicit solvent.

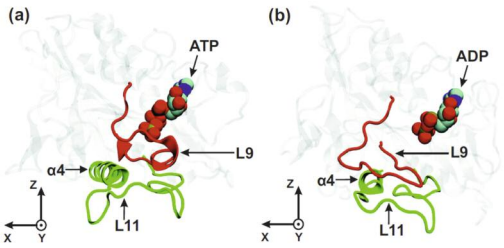

Fig. 3: Dominant conformations of the loops L9 (red) and L11 (green) for kinesin KIF1A attached to tubulin with (a) ATP and (b) ADP in the binding pocket, as obtained from MD simulations **[7]**.

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References:

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[7] Aliaksei Krukau, Volker Knecht, Reinhard Lipowsky: Conformational changes of ATP hydrolyzing enzymes from atomistic simulations: Kinesin's motor domain as a case study. Submitted.