FP7 ITN - SBMPs
Structural Biology of Membrane Proteins

Project for ER (Experienced Researcher):

Investigations the interactions in complexes of membrane proteins by theoretical methods.

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The project is highly complementary to ESR project "Structural and functional characterization of γ-secretase, a core protein complex responsible for Alzheimer's Disease. Theoretical modelling and NMR approach", which is performed in cooperation with Volker Dötsch and Frank Bernhard from Centre for Biomolecular Magnetic Resonance, Institute of Biophysical Chemistry, University of Frankfurt, Germany. The γ-secretase is a complex of four membrane proteins: presenilin, APH-1, PEN-2 and nicastrin. The crystallization of such complex is very complicated, therefore, one possible solution is obtaining the structures of protein fragments using NMR methods and subsequent simulations of them using molecular dynamics methods in lipid bilayers and micelles. Due to a long time scale for such simulations the atomistic approaches are too slow and they can be replaced by much faster coarse-grain methods.

One of such simplified coarse-grain molecular dynamics methods is MARTINI [1, 2] which is part of the GROMACS program. The coarse-graining in this method employs so called 4:1 scaling. It means that 4 heavy atoms (being non-hydrogen atoms) are replaced by one grain. The grains are linked together and can interact with other grains to reproduce real behaviour of molecules especially in membranous environment. The specific force field that govern their motion need to be parametrized to achieve an agreement with experimental data. However, in this method the secondary structure of proteins must be restrained during simulations. This method was designed to investigate lipid membranes and only recently extended toward studying proteins. Therefore, extensive parametrization is needed to achieve reliability and agreement with experimental structural data. Our aim is to make such method useful especially for protein-protein interactions to investigate membrane protein complexes.

The test case for such elucidated method will be the membrane complex of γ-secretase, in which the partial structures will be known from NMR experiments. However, the parametrization process will be limited to known structures of protein-protein complexes in the membrane like bacteriorhodopsin or other membrane protein oligomers. We will also investigate such complexes in continuous environments where the membrane and water are

mimic by suitable potentials [3]. It is because the faint interactions between helices in coarse-grain simulations in MARTINI would be distorted by thermal motion of lipids.

The choice of the target of γ-secretase is because of importance of this enzyme. This complex is a protease performing the last cut in the cascade of proteolytic cleavages of amyloid precursor protein (APP) and produces β-amyloid (Aβ) what can lead to accumulation of the Aβ senile plaques in the brain of Alzheimer's disease patients. The failure of Aβ clearance, mutations in APP or in γ-secretase lead to the pathological aggregation of this peptide [4-5]. Presenilins (PS-1 and PS-2) provide two aspartic acid residues located within the membrane which form a catalytic core for the intramembranous proteolysis of substrates. Genetic studies show that more than 170 mutations in the PS-1 protein were associated with familial forms of Alzheimer's disease and new mutations are still being identified [6]. These pathogenic mutations lead to an overproduction of highly aggregative forms of Aβ within the brain. A vast majority of PS-1 mutations occur within the transmembrane regions indicating that even minute changes in the structure of these regions may radically change properties of the protein including its catalytic characteristics. Residues associated with these pathological mutations appear to form vertical patterns along the helices when mapped on regular α-helices [7-8]. Linear patterns formed by the mutation clusters along transmembrane helices seem to be most likely involved in the inter-helical packing.

Alzheimer's Disease mutations shift this balance toward overproduction of $A\beta_{42}$ form which is highly aggregative. The structure of the membranous part of the complex will be investigated using theoretical methods for wild type and mutated forms. Determined structural fragments of the complex will be simulated using Molecular Dynamics in water-membrane systems to elucidate helix-helix contacts and checking stability of the investigated system.

References:

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