

PhD bursary position “Design of bio-inspired nanopores”

Place: Molecular Dynamics Group, University of Groningen

Promotor: Prof. S.J. Marrink

Duration: 4 years

Start: july-dec 2009

Applications: email CV to s.j.marrink@rug.nl

Requirements: solid background in biophysics

Abstract: In a collaborative project, pH-sensitive, light-modulated and redox switches will be conjugated into pore forming peptides, peptide mimics and channel proteins to engineer selectivity and to allow controlled opening and closing of the pore. Lipids will be designed and synthesized that form robust membranes which are suitable hosts for the peptides and proteins. The PhD student will apply multi-scale Molecular Dynamics simulations techniques to analyse the pore properties and help in the design of this promising class of bio-inspired nanopores.

Introduction: Antimicrobial peptides (AMPs) are small molecules produced by a wide range of organism ranging from fungi to humans in order to kill microbes [1]. The interest in AMPs originates from their potential application as alternative to conventional antibiotics in fighting bacterial infections and also in cancer treatment [1-3]. Moreover, their structural simplicity is ideal for elucidating mechanisms of pore formation and engineering of channel properties. Currently, there are three general mechanisms of action proposed for AMPs (fig. 1) [1-4]. Two of them assume the formation of a pore (‘toroidal’ and ‘barrel-stave’); the third one, the ‘carpet-like’ mechanism, assumes a detergent-like action. In the toroidal pore mechanism, membrane lipids bend to form the pore together with AMPs, while in the barrel-stave mechanism the pore is lined solely by peptides. Pore forming AMPs can be regarded as ‘the minimal transmembrane proteins’ [5] and might mimic the channels that facilitated the movement of nutrients in the earliest forms of life. Of the three mechanisms, the barrel-stave pores are structurally most similar to channel-forming proteins. However, they do not have a selectivity filter and gating mechanism.

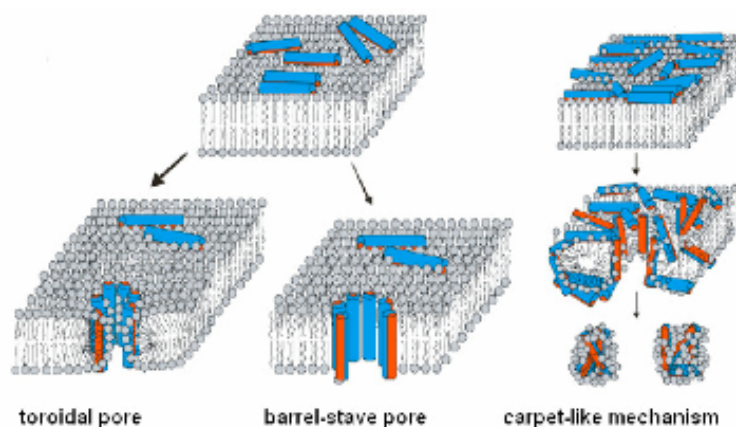


Figure 1. Three models of action mechanisms of antimicrobial peptides. [4]

Mechanosensitive (MS) ion channels on the other hand sense membrane tension in all species ranging from bacteria to man. Due to their relative simplicity, bacterial MS channels have been the object of study for the past decade. The channels sense changes in membrane tension invoked by osmotic stress and as a response they open and create a transient pore in the membrane. The best studied MS channel so far is the “Mechanosensitive channel of large conductance (MscL)” (Figure 2). The open MscL pore has a diameter of about 3 nm and allows the passage of not only ions and small molecules but also small proteins [6]. It has been recognized already years ago that MS membrane channels can in principle be used as “valves” in various applications and devices [7]. When reconstituted into artificial membranes, MS channels are still capable of mechano-sensing and responding to the alteration in membrane tension [8].

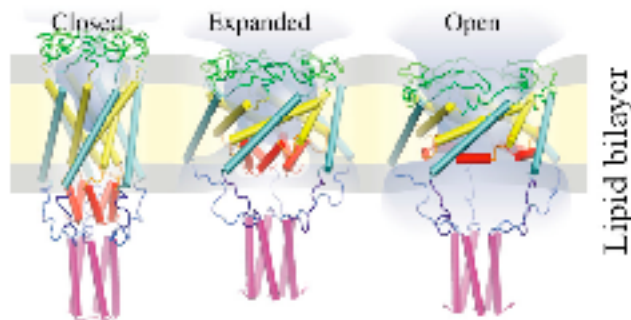


Figure 2. MscL is a homopentamer with two transmembrane helices, M1 (yellow) and M2 (blue), per subunit. During opening, it undergoes dramatic structural changes from a 2 Å closed to a 35 Å open state.

Aim: Inspired by the concept of redesigning life and the tools offered by nanobiotechnology [9], new semi-synthetic channels are proposed, based on AMPs and engineered with chemical switches and selectivity functionalities. The incorporation of synthetic molecular switches into AMPs would allow the modulation of opening and closing of pores by light (photochemical switches) or by a change of pH (pH switches). The strategic engineering of re-entrant loops will be used to add specificity to the system, similar to the selectivity filters in natural proteins. Such hybrid constructs containing AMP as the protein element and a chemical switch would form a synthetic membrane protein that can be opened and closed like real membrane proteins, but in a designed, controlled way. In addition, controlling the behaviour of MS channels and AMPs by modifying the membrane has hardly been studied. This is an excellent opportunity, as membranes composed of artificial lipids can be systematically varied to regulate their flexibility, thickness, charge, and even tension profile. From an application point of view, such a tunable environment is crucial for the incorporation of channels and pores without impeding their function. We propose therefore to develop membranes that on the one hand are considerably more stable and have a much longer life time than natural membranes and at the other hand provide an optimal and controllable environment for the imbedded MS channels and AMPs. The design of the membrane lipids will be strongly bio-inspired by the lipids occurring in *Mycobacteria* and *Acidobacteria* as these organisms have evolved to live under harsh environmental conditions.

Tentative Workplan: Simulations of pore formation by AMPs [5, 10] will be used to guide the engineering of peptides. Nowadays the spontaneous formation of pores by AMPs can be simulated at atomic detail [9] (see figure 3). Recent advances in coarse grained (CG) modeling [11] allow these simulations to be further extended in both time- and length scale. With the CG models small liposomes can be modeled and the collective action of 10s to 100s of peptides can be simulated. Using this combined, multi-scale, approach we will simulate the pores formed by peptides (focusing on alamethicin and ceratotoxin A, since melittin has been studied recently in our group [12]) and their engineered variants. At a later stage, the MD simulations will be used to unravel the structural changes in the modulated protein nanopore upon gating and the influence of methyl-branching on the permeability of the membrane. The proposed MD simulations will provide structural and dynamical detail of the nanopores at the atomic level. Predictions from the simulations will be tested by the proposed experimental work, and used to guide the refining of the modulated protein nanopores.

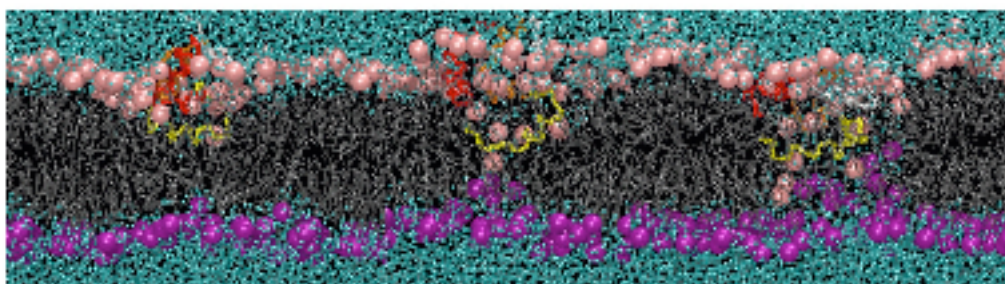


Figure 3: Simulation at atomic resolution of the specific mode of action of a particular member of the magainin family [10]. A time sequence is shown illustrating how four peptides upon binding to the membrane aggregate spontaneously and induce the formation of a transmembrane toroidal pore. The peptide backbones are shown in yellow, red, orange and white, the lipid tails grey, the water cyan. Lipid headgroups are represented by pink/purple spheres depending on the monolayer in which they initially reside. Total simulation time depicted is 200 ns.

Embedding: The research will take place within the Molecular Dynamics group, attached to the Groningen Biomolecular Sciences and Biotechnology Institute (GBB). The MD group, headed by S.J. Marrink, concentrates on dynamical simulation of biopolymers and lipid aggregates. The aim is to understand and predict macroscopic behaviour of complex biomolecular systems on the basis of the effective interactions between atoms. The MD group is home to the new MARTINI coarse grained forcefield for biomolecular simulations. Furthermore, the MD group remains closely linked to the ongoing development of the GROMACS software, the origins of which are found in this group. The group currently consists of 12 students/postdocs from all over the world.

The PhD position is part of a collaborative project funded by the Zernike Institute of Advanced Materials. It involves a close collaboration between the groups of A.J. Minnaard (Bio-organic Chemistry), B.L. Feringa (Synthetic Organic Chemistry), and A. Koçer and B. Poolman (Membrane Enzymology). The focus of the project is on the next stage of the challenging goal to control transport through membrane channels and pores. There is tremendous joined expertise in modifying membrane proteins, membrane structure and functioning, self-assembly, synthesis of switches and photo-and redox active compounds, transport phenomena and molecular modeling. The Poolman/Koçer group will bring the knowledge and facilities on protein isolation, characterization and modification as well as membrane reconstitution and fluorescence measurements. The Feringa group will contribute with synthesis expertise, photochemical and electrochemical studies, protein functionalization and study of isomerization processes. The Minnaard group brings in the expertise on the synthesis of membrane lipids and the knowledge on translating membrane properties to molecular design. The PhD student employed on this project is expected to be in close contact with the other groups involved.

References:

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