Viral Membrane Proteins: Flexibility and Assembly

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Membrane bound and pore forming viral proteins like M2 from Influenza A, Vpu from HIV-1 or 3a from SARS-CoV show in their monomeric form high flexibility and adaptability in different lipid bilayer environments. Their conformational space under these conditions has been studied by ample molecular dynamic simulations.

The understanding of the abilities of the monomeric units is used to further explore the energy landscapes of the molecular assembly of multiple monomers. The newly developed protocol screens the full high dimensional search space leading to highly reliable pore models. After minor refinement e.g. with short molecular dynamics simulations they are suitable for the use in drug screening. Furthermore the evaluation of the energy landscapes allows drawing conclusions about the gating mechanism of the pores.

1 Introduction

The genome from different viruses encodes a series of membrane bound or attached proteins. These proteins fulfill a broad range of functions and are often essential in the virus reproductive life cycle (see also Patargias et al. in this proceedings p. 93). Some of these proteins include Vpu from HIV-1 which helps degrading the CD4 receptor and enhances the particle release (virion budding).[1,2] M2 from Influenza A which facilities the viral entry into the host cell via the endocytosis pathway.[3] 3a from SARS-CoV plays an important role during the virion release.[4] All of these proteins have in common that they assemble within a lipid environment to form multimeric homooligomers which function as pore or ion channel. Unfortunately all membrane proteins are barley crystallisable which makes it a challenging task to get atomistic x-ray data for this kind of proteins. Currently the best source for structural data are NMR studies, which are usually limited to monomeric forms and can only provide information about the assembled pore under special conditions.[3]

2 Computational Method

We herby present an approach to derive structural data for the assembly of viral membrane proteins within a two dimensional lipid bilayer environment. Starting point is to screen with experimental and computational methods the viruses protein sequence for putative transmembrane regions, which consequently could form a pore.

2.1 Monomer Molecular Dynamics

The transmembrane spanning parts are modeled as ideal helices and embedded into a phospholipid bilayer (POPC, but also DPPC, DDPC and DTPC). After stepwise minimization

they undergo multiple 10 ns GROMACS MD simulation with full pressure and temperature coupling. By applying a principal component analysis (PCA) the conformational space of the monomer is analyzed and an average structure is generated.[1] As the different proteins can develop significant kinks and bends this step is essential to derive a good starting structure for the following assembly.

2.2 Assembly Protocol

To sample the whole conformational space of a pore assembly the lipid environment is considered as two dimensional space in which the assembly takes place. Furthermore homooligomer pores are considered symmetrical towards their central pore axis. Monomers are placed around the central pore axis, while the following degrees of freedom are sampled in a systematic way. The distances between packed helices in transmembrane proteins usually show values around 10 Å. To cover weak and tight packing interhelical distances in the range from 8 to 12.5 Å are sampled. The expression 'angle' is used to describe the rotation of each monomer around its own helical axis. In the case of homooligomers there is only one value per conformation, as due to symmetry all monomers are oriented in the same way towards the central pore axis. In the case of heterooligomers it is necessary to sample multiple angles, one for each non-symmetrical monomer. In some cases like M2 from Influenza A it is possible to narrow the search space significantly, as it is known that His-37 and Trp-41 play an important role in the proton conductance through the pore. They have to face inwards which narrows the search by at least 2/3 to 120 °. The tilt describes the orientation of the helical axis towards the membrane normal. As membrane proteins can develop significant tilts up to 50 °,[1] it is also required to sample this dimension of the conformational space in a sufficient way. Finally the sidechains are optimized and the energy for each conformer is evaluated. The geometrical and energetic operations have been implemented with SVL in MOE (Chemical Computing Group, Montreal `http://www.chemcomp.com/`).

3 Pore Assembly

The quality of the pore models generated by the here presented protocol have been evaluated for M2 from Influenza A. The C_α-RMSD of the best generated pore compared to the established model 2H95 is 2.086.[3] This excellent agreement speaks for the strength of the approach. To illustrate the abilities of the above described assembly protocol detailed data for Vpu from HIV-1 is shown in Figure 1. In this case more than one minimum appears on the high-dimensional energy landscape. While some of the 5 resulting models may be excluded due to experimental evidence, some may represent alternative conformations, responsible for multiple conductance states.[2,5] To push the method to its limits it was attempted to generate a full pore model for 3a from SARS (data not shown). After assembling the monomeric unit with three membrane spanning parts, it was assembled to form a tetrameric pore.

4 Conclusion

Here we present a sophisticated approach to screen the conformational space of a protein on the basis of a forcefield which enables to scan the whole search space with an accept-

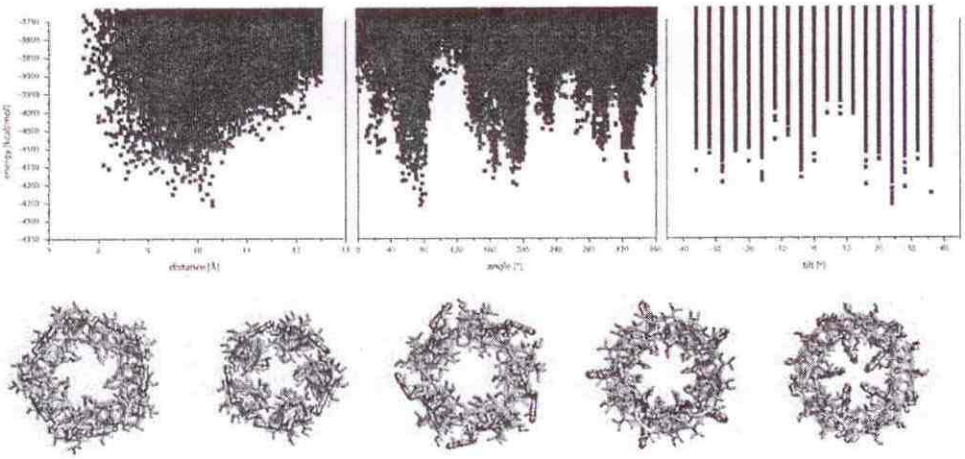

Figure 1. In the top row representative energyplots for the assembly of Vpu from HIV-1 are shown. The pore models which correspond to the minima in the plots are shown in the lower row. Trp-23 (blue) and Ser-24 (red) are highlighted. Both residues play an important role for the stability of the pore and the conductance of ions.

able resolution. By reasonable simplification and consideration of symmetry of the studied proteins a significant confinement of the search space can be made. This enables the resolvability of the search in an acceptable sampling time. The quality of the constructed structural models does not rank behind any experimental technique. In fact the careful optimization leads to more consistent models. When experimental results are taken into account e.g. in the form of a distance restraint, significant further confinements of the search space can be made. This boosts the sampling speed while at the same time an improved quality can be achieved.

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