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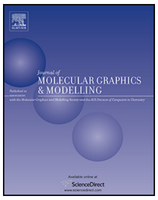

Continuum electrostatic approach for evaluating positions and interactions of proteins in a bilayer membrane

Chirayut Supunyabut, Sunit Fuklang, Pornthep Sompornpisut*

Computational Chemistry Unit Cell, Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Rd. Prathumwan, Bangkok 10330, Thailand

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ABSTRACT

Orientations of proteins in the membranes are crucial to their function and stability. Unfortunately the exact positions of these proteins in the lipid bilayer are mostly undetermined. Here, the spatial orientation of membrane proteins within the lipid membrane was evaluated using a Poisson–Boltzmann solvent continuum approach to calculate the electrostatic free energy of the protein solvation at various orientations in an implicit bilayer. The solvation energy was obtained by computing the difference in electrostatic energies of the protein in water and in lipid/water environments, treating each as an implicit solvent model. The optimal position of transmembrane proteins (TMP) in a lipid bilayer is identified by the minimum in the “downhill” pathway of the solvation energy landscape. The energy landscape pattern was considerably conserved in various TMP classes. Evaluation of the position of 1060 membrane proteins from the orientations of proteins in membranes (OPM) database revealed that most of the polytopic and β-barrel proteins were in good agreement with those of the OPM database. The study provides a useful scheme for estimating the membrane solvation energy made by lipid-exposed amino acids in membrane proteins. In addition, our results tested with the bacterial potassium channel model demonstrated the potential usefulness of the approach in assessing the quality of membrane protein models. The present approach should be applicable for constructing transmembrane proteins–lipid configuration suitable for membrane protein simulations and will have utility for the structural modeling of membrane proteins.

1. Introduction

Membrane proteins play important roles in many biological processes, such as cell communication, signal transduction and regulation, cell adhesion and catalysis. They constitute about one-third of the proteins encoded in prokaryote to eukaryote genomes [1]. Based on the nature of their association with the lipid bilayer, membrane proteins can be grouped into the three major categories of (i) integral, (ii) peripheral and (iii) lipid-anchored membrane proteins. Integral or transmembrane proteins (TMP) are the major class of membrane proteins and exhibit key structural features, such as secondary and tertiary structures as well as orientation and packing of transmembrane segments, to satisfy the interactions with the phospholipid bilayer. Therefore, their spatial position with respect to biological membranes are vital to their function, folding and structure stability [2–6].

A variety of experimental techniques, including, X-ray diffraction XRD, solid-state and solution NMR, spin-labeling electron paramagnetic resonance (EPR), electron microscopy and fluorescence spectroscopy amongst others, have been used to solve the three-dimensional (3D) structures of proteins at various levels of resolution [7–11]. As can be seen by the growth of released entries in Protein Data Bank (PDB), the number of membrane protein structures increases every year. However, because the conventional methods of structure determination of membrane proteins (solution NMR and X-ray crystallography) are typically carried out in detergent micelles, the PDB structure coordinates rarely contain explicit information regarding the orientation of the protein structure with respect to the lipid bilayer. In molecular dynamics (MD) simulations, the generation of reliable trajectory is critically affected by the quality of starting configuration [12]. It would be useful to have a systematic tool for setting up a proper orientation of protein in membrane, whereby the starting configuration is examined energetically without expensive computation. Although

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Abbreviations: TMP, transmembrane protein; NMP, non-transmembrane protein; OPM, orientations of proteins in membranes; PB, Poisson–Boltzmann; RMSD, root-mean-square deviation.

* Corresponding author. Tel.: +66 2 2187604; fax: +66 2 2187598.
E-mail addresses: Pornthep.S@chula.ac.th, spornthe@hotmail.com (P. Sompornpisut).

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the spectroscopic techniques, such as NMR [13], site-directed spin labeling with EPR [14,15], cysteine accessibility mutagenesis [16,17] and tryptophan scanning mutagenesis with fluorescence spectroscopy [18,19], are powerful methods to address the orientation as well as the position of TMP in the bilayer, they require considerable time, effort and expense to study each protein.

Computational methods play an increasing role in studying membrane proteins. A number of computational approaches, such as the use of all-atoms or coarse-grained molecular dynamics (MD) simulation with explicit water–lipid solvents [20–22], solvation free energy calculation in implicit continuum multidielectric treatment [23–26], or the PPM/OPM empirical functions [27,28], have been used to optimize the spatial arrangement of proteins in the membrane. While MD is time consuming and computationally expensive, the latter two approaches allow a rapid assessment for positioning TMP. In particularly, the results of the PPM/OPM method provide a useful guide about positioning proteins in membrane. Interestingly, Choe et al. employed continuum electrostatics in combination with the treatment of membrane deformation and non-polar energy terms to calculate the total insertion energy of transmembrane helical peptide in a membrane [29]. These promising results have stimulated the feasibility of using the continuum electrostatic approach for studying interactions and orientations of proteins in implicit descriptions of the membrane environment which is, however, still not well-established.

The solvent continuum method is a powerful tool for estimating the free energy of binding/solvation in biological systems. Among these methods, the Poisson–Boltzmann (PB) equation is widely used for electrostatic calculations of protein molecules in a continuous dielectric solvent [30–32]. The PB equation allows one to obtain accurate descriptions of the solvent effect on the protein. There have been a number of software packages that implement this technique. Among these popular software is the Adaptive Poisson–Boltzmann Solver (APBS) program. It can be used together with the graphical interface APBSmem, which provides a simple and efficient protocol for calculating the electrostatic solvation energy of membrane protein systems [33,34]. In this protocol, the aqueous and lipid bilayer models are implicitly treated as high- and low-dielectric continuum regions, respectively. The protein orientation in the implicit solvent environments can be graphically monitored and managed by the users. The program offers the “Protein Solvation” module to compute the electrostatic free energy of membrane solvation for transferring the protein from the surrounding aqueous environment to the mixed water-membrane slabs. The available features of the software have made it possible to perform a routine test for searching the optimal positions of membrane proteins within the membrane.

In this study, the PB solvent continuum method was employed to evaluate membrane protein positions in the lipid bilayer. Unless otherwise specified, the term “energy” or any related term used here involve with the electrostatic contribution. For a test case, we initially examined the free energy landscape of membrane solvation for five membrane protein ion channels, whose spatial orientation is fairly well known. The method was then validated with water soluble and peripheral membrane proteins. A total of 1060 membrane proteins from orientations of proteins in membranes (OPM) database (http://opm.phar.umich.edu) were examined for their optimal position by means of the PB approach. As described above, the strategy was to calculate the solvation free energy differences for a given protein immerged in the two solvent environments (water and water–lipid) presented by high (for water) and low (for membrane) continuum dielectric slabs (Fig. 1). The electrostatic free energies of membrane solvation were used to evaluate a variety of positions of proteins within the membrane. Furthermore, the per-residue energy decomposition analysis gave a general picture of the range of the electrostatic solvation cost of

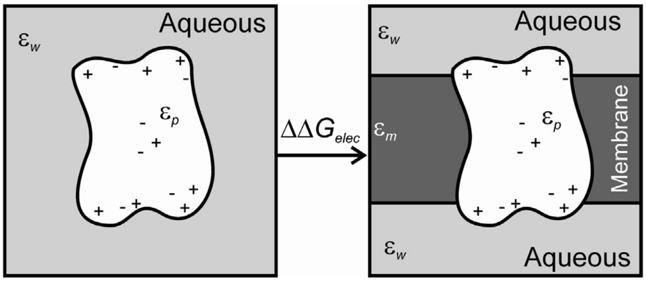

Fig. 1. Schematic representation of the electrostatic free energy ($\Delta\Delta G_{\text{elec}}$) of solvation for a protein transferring from an aqueous environment to an aqueous–membrane environment when treated as continuum solvents. The influence of the solvent is represented by the dielectric continuum, $\varepsilon_{\text{m}} = 2$ for the membrane region and $\varepsilon_{\text{w}} = 80$ for water.

20 common amino acids in membrane. We also showed that the present approach can be used to discriminate 3D protein structure of native fold from incorrect folds.

2. Background

The strategy was to compute the electrostatic free energy of proteins inserted into a lipid bilayer at various orientations and depths to identify the spatial position of the most favorable energy. In an implicit-solvent description of the solvation, we employed the PB method to estimate electrostatic contributions, which is the energy arising from the dielectric response to the electric field produced by the charged molecules. The PB equation representing the relation between the electrostatic potential and the charge distribution in the presence of an electrolyte solution is presented in the following equation:

$$\nabla \times \left[\varepsilon\left(\vec{r}\right) \nabla\varphi\left(\vec{r}\right)\right] - \bar{\kappa}^2\left(\vec{r}\right) \sinh\left[\varphi\left(\vec{r}\right)\right] = -e\beta 4\pi\rho\left(\vec{r}\right), \tag{1}$$

where ε is a dielectric permittivity coefficient, ϕ is the reduced electrostatic potential, κ^2 is the Debye–Hückel screening parameter, e is the unit charge, $\beta = 1/k_BT$ is the inverse temperature and ρ is the charge distribution of the protein. For a given protein configuration, the ionic strength of the salt solution and the surrounding solvent of a known dielectric coefficient, the electrostatic potential can be obtained from solution of the PB equation. In the present study, calculations of the electrostatic free energy of solvation for the protein are carried out with the linearized form of the PB equation according to

$$\Delta G_{\text{elec}} = \frac{1}{2}\sum \Phi\left(\vec{r}\right)\rho\left(\vec{r}\right) \tag{2}$$

3. Methods

3.1. Testing ion channel proteins

Initially, the calculations of the electrostatic solvation energy of proteins inserted into the membrane were performed on five membrane protein channels of KcsA (PDB ID: 1K4C) [35], Kv1.2–2.1 chimera (PDB ID: 2R2R) [36], large-conductance mechanosensitive channel (PDB ID: 2OAR) [37], the voltage-gated sodium channel NaVAb (PDB ID: 3RVY) [38] and the CorA Mg channel (PDB ID: 2IUB) [39]. Starting structure configurations (Fig. S1) was generated by orienting the channels pore with the z-axis and by placing the center of transmembrane segments at the origin of the Cartesian coordinate system (Fig. 2).

The electrostatic free energy of solvation was calculated for various configurations at various positions and angles with respect to the membrane region. The solvent boundaries were generated using a ±20 Å translation about the z-axis at a 1 Å step size and