Developing the Statistical Mechanics Theory of Liquids in Chemistry and Biophysics

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"Molecular recognition" is an essential elementary process for protein to function. The process is a thermodynamic process which is characterized with the free energy difference between two states of a host-guest system, namely, associated and dissociated states. It is readily understood that the structural fluctuation of protein gives a big effect on the free energy barrier. In that respect, the "molecular recognition" is a thermodynamic process which is conjugated with the structural fluctuation of protein.

We have been developing a new theory concerning the molecular recognition, based on the 3D-RISM/RISM theory which is a statistical mechanics of liquids. The theory has successfully "probed" small ligands such as water molecules and ions bound in a small cavity of protein.[1–3)]

1. Ligand Mapping on Protein Surfaces by the 3D-RISM Theory: Toward Computational Fragment-Based Drug Design[4)]

In line with the recent development of fragment-based drug design, a novel computational method for mapping of small ligand molecules onto protein surface is proposed in this paper. The method uses the three-dimensional (3D) spatial distribution functions of the atomic sites of ligand calculated by a molecular theory of solvation, known as the 3D reference interaction site model (3D-RISM) theory, to identify the most probable binding modes of the ligand molecule. In this study, the 3D-RISM-based method is applied to the binding of several small organic solvents to thermolysin, in order to evaluate its efficiency. The results demonstrate that our method can reproduce the major binding modes found by X-ray crystallography with sufficient accuracy. Moreover, it is found that the method can successfully indentify some binding modes associated with a known inhibitor, which could not be detected by the experiment. The dependence of ligand-binding modes on the ligand concentration, which can hardly be treated with other existing methods, is also investigated. The results indicate that some binding modes are readily affected by a shift in the ligand concentration, while the others are not substantially altered. An analysis of the water distribution implies that the ligand-binding modes are determined by a subtle balance in the binding affinity between ligand and water.

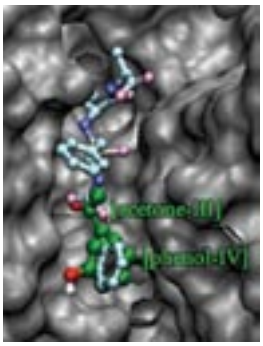

Figure 1. Comparison of two binding modes identified using 3D-RISM with the crystallographic strucre in active site.

2. Molecular Selectivity in Aquaporin Channels Studied by the 3D-RISM Theory[5)]

The three deimensional distribution function (3D-DF) and potential of means force (PMF) of small neutral molecules inside the two aquaporin channels, AQP1 and GlpF, are calculated based on the 3D-RISM theory, the statistical mechanics theory of molecular liquids, in order to investigate the permeability of those ligands through the channels. The ligands investigated are Neon (Ne), carbondioxide (CO_2), Nitrogen oxide (NO), ammonia (NH_3), urea, and glycerol.

Neon showed continuous distribution through out the channel pore in AQP1 as is the case of water, although the PMF of Ne at the selective filter (SF) region is higher than that of water, indicating that the stability of molecules in the channel is determined not only by their size, but also by the charge distribution. The ligand molecules, CO_2, NO, urea, and glycerol have large barrier in PMF at the SF region in AQP1, indicating that those ligands are not permeable through the

channel. On the other hand, NH_3 has only small activation barrier, ~2.5 kJ/mol, to be overcome. Therefore, our theory predicts that a NH_3 molecule can be permeated through the AQP1 channel. In GlpF, all the ligands have negative PMF throughout the channel pore except for glycerol which has a small barrier at the SF area, ~2.1 kJ/mol. The barrier can be readily overcome by the thermal motion. So, our results are quite consistent with the experiments for urea and glycerol, for which the corresponding data are available.

The potential of mean forces of the ligand molecules in GlpF obtained from the MD simulations show distinctly different patterns from our results: PMFs of NH_3 and urea from MD have large positive values throughout the channel, while those of CO_2 and glycerol has some negative regions. In any case, the barriers in PMF at the SF region are so high and large for all the four ligands examined. It seems impossible for them to overcome the barrier in order to be permeated through the channel. This raises a serious question to the results from MD simulations, because the experimental observation indicates that GlpF can conduct glycerol and urea pretty well. (Actually, the name "glyceroporin," came from that function of the channel.)

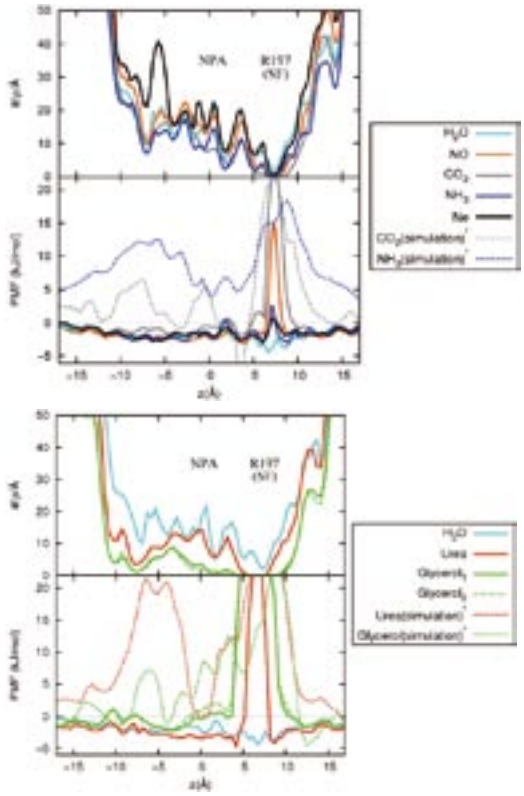

Figure 2. PMFs of water, Ne, CO_2, NH_3, urea and glycerol in AQP1.

3. Proton Transport through the Influenza A M2 Channel: 3D-RISM Study[6)]

The three dimensional distribution and the potential of mean force of water and hydronium ions in five protonated states of the Influenza A M2 channel are calculated by means of the 3D-RISM theory in order to clarify the proton conduction mechanism of the channel. Each protonated state denoted as *i*H, where *i* runs from 0 to 4, has a different number of protonated histidines from 0 to 4. The distribution of water in each state exhibits closed structure of 0H, 1H and 2H, and opened structure in 3H and 4H. In the closed form, the distribution function and potential of mean forces calculated by the 3D-RISM theory indicate that hydronium ions are excluded from the channel. In contrast, the ion can distribute throughout the opened channel. The barrier in potential of mean force of 3H, ~3–5 kJ/mol, is lower than that of 4H, 5–7 kJ/mol, indicating that 3H has higher permeability to proton. Based on the radial distribution functions of water and hydronium ions around the imidazole rings of His37, we propose a new mechanism of proton transfer through the gating region of the channel. In this process, a hydronium ion hands a proton to a non-protonated histidine through a hydrogen-bond between them, and then the other protonated histidine releases a proton to a water molecule via a hydrogen-bond. The process transfers a proton effectively from a water molecule to the other.

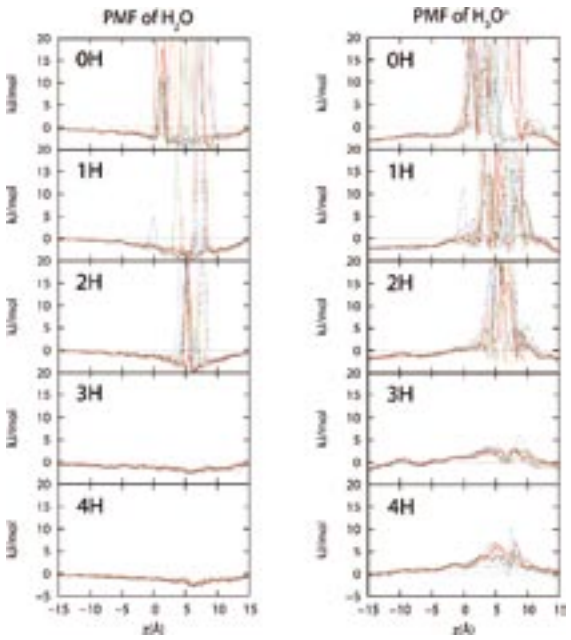

Figure 3. Potential of Mean force of water and hydronium ion in each state, a line represents the PMF for a conformation in the state.

References

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4) T. Imai, K. Oda, A. Kovalenko, F. Hirata and A. Kidera, *J. Am. Chem. Soc.* **131**, 12430 (2009).
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