PROTEINS UNDER CONFINEMENT: STRUCTURE, DYNAMICS AND THERMODYNAMICS

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ABSTRACT

The structure, dynamics and stability of proteins are affected by the physical environment that surrounds the protein. The influences are both enthalpic and entropic. For example, the cellular environment is a crowded environment with high concentration (up to 400 grams per liter) of macromolecules including proteins, nucleic acids and complex sugars. These macromolecules can take up to 40% of the total cellular volume, and significantly reduce available volume for proteins. Such effect is called the excluded volume effect. Excluded volume effect has been known to influence protein folding rates and stability. Macromolecular crowding can also reduce protein diffusion rate, force folding of marginally stable proteins, change protein shape, and affect protein dynamics. As one type of crowding, confinement has been widely adopted to study crowding effects. The methods to introduce confinement include using sol-gel silica glass and reverse micelles.

Reverse micelles (RM) have been used to study confinement effects because of many of their advantages. RMs are thermodynamically stable nanometer-scale structures that can be formed by many kinds of amphiphilic surfactants such as Sodium bis(2-ethylhexyl) sulfosuccinates (AOTs). RMs have the ability to encapsulate biomolecules into its water pool. Confined waters in RMs are often proposed to mimic confined waters in biological systems. The size of RMs can be well controlled which depends on the water to surfactant ratio w_0, so the confinement size and hydration level can be controlled through the change of w_0. Protein-RM systems have been studied for many proteins, however the detailed interaction and dynamics for protein inside RM are still not clear. We are interested in using AOT RM as a model of confinement to study the effects of encapsulation on peptide/protein structure, hydration, location and dynamics.

We studied the system of an alanine rich alpha-helix peptide encapsulated in a self-assembled RM and found that the peptide prefers to be located at the water/AOT head groups interface. The driving force for this configuration is the gain in entropy by released water molecules that otherwise would solvate the protein and

surfactant head groups. The peptide is dehydrated compared to the peptide in bulk water. Charged Lys side chains interact strongly with the surfactant head groups. We studied the system of ubiquitin protein encapsulated in a self-assembled RM and found that ubiquitin binds to the RM interface under low excess salt concentration in the RM water pool. The same hydrophobic patch that is recognized by ubiquitin binding domains *in vivo* is found to make direct contact with the surfactant head groups, hydrophobic tails, and the isooctane solvent. The fast backbone N-H relaxation dynamics show that the fluctuations of the protein encapsulated in the RM are reduced when compared to the protein in bulk, in agreement with experimental data. This reduction in fluctuations can be explained by the direct interactions of ubiquitin with the surfactant and by the reduced hydration environment within the RM. At high concentrations of excess salt, the protein does not bind to the RM interface and the fast backbone dynamics are similar to that of the protein in bulk. These two studies are the most detailed and comprehensive studies of the protein-RM system as far as we know. They give us an atomic level picture of the interactions of the protein-RM system and improve our understanding of how confinement affects the protein structure and dynamics. The results in these two studies can also can serve to guide future studies on protein-RM system.

Numerous proteins fold spontaneous *in vitro*, while a large portion of newly synthesized proteins in the cell require the assistance of molecular chaperone to reach their folded states efficiently. The Escherichia coli chaperonin GroEL is the best characterized molecular chaperone that assists protein folding *invivo*. GroEL assists protein folding by two mechanisms: cis for small proteins and trans for large proteins. Both of the mechanisms include nucleotide cycling and the cycles of polypeptide binding and release. However, whether the GroEL plays an active role toward the polypeptide folding or a passive role by isolating the polypeptide during this process is still an unanswered question. We studied mini-protein Trp-cage folding under fullerene inert and polar confinements to understand how GroEL confinement assists protein folding. Replica exchange molecular dynamics simulations enable us to reversibly fold and unfold proteins using detailed atomic level model of proteins. We found that that the system under inert confinement stabilizes Trp-cage folding

while the system under polar confinement makes the free energy landscape of Trp-cage folding smooth. Trp-cage inside these confinement volume has a preferred orientation, and it's in contact with the fullerene wall under inert confinement, and is not in contact with the fullerene wall under polar confinement. The entropic effect, enhanced hydrophobic effect, and interaction between the peptide and fullerene wall play together to determine the stabilization (or destabilization) of Trp-cage under confinement.