

**UNDERSTANDING THE EFFECTS OF PRESSURE ON
BIOLOGICAL SELF-ASSEMBLY: ROLE OF
HYDRATION AND WATER-MEDIATED
INTERACTIONS**

By

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ABSTRACT

Pressure has recently emerged as a new and promising dimension to perturb and study the structure, thermodynamics, dynamics, and kinetics of various biological systems. The advances in high pressure experimental techniques and their integration to powerful imaging methods have resulted in an explosion of experimental studies of biology under pressure. A fundamental framework for understanding and predicting effects of pressure on these systems is missing, however. The availability of wealth of experimental data presents a unique opportunity for obtaining new fundamental, molecular level insights through statistical mechanical and computer simulation studies.

Effects of high pressure on globular proteins in solution are particularly intriguing. Instead of being mechanically compressed at high pressures, experiments reveal that proteins unfold into water swollen denatured states! Also, the kinetics of protein folding/unfolding is found to slow down significantly at high pressures. At the multi-protein level, high pressures dissociate complexes (especially those with hydrophobic interfaces). How do we explain these puzzling observations from a fundamental perspective?

We address this problem using a two-pronged approach. At one level, we focus on the pressure-dependent behavior of the fundamental interactions that govern protein stability. We have characterized hydrophobic hydration and interactions, ion hydration, and ion-ion interactions in a broad space of molecular (such as solute lengthscale, strength of solute-water interactions) and thermodynamic parameters (e.g. temperature and pressure). Water-mediated interactions between hydrophobic solutes are weakened with increasing pressure. The pressure sensitivity of these interactions is expected to be dependent on the solute lengthscale. Our results show that water is more compressible around large hydrophobic solutes. This indicates that clusters of larger solutes will dissociate more easily than those of smaller solutes at high pressures. Folded as well as unfolded states of proteins and surfaces of protein complexes contain large hydrophobic patches with different curvatures. Our results

have implications on understanding their pressure sensitivity. Pressure response of water-mediated interactions between ions is dependent on the charge density. Although, in general ion-ion interactions are insensitive to pressure, the behavior is different for strongly hydrated ions. Association of strongly charged ions is weakened with increasing pressure. These results suggest that pressure can disrupt protein structure through destabilization of both hydrophobic interactions and salt-bridges.

The second level of our approach comprises detailed molecular simulation studies of realistic protein-water systems. We have developed a method to access pressure unfolded conformations of proteins in simulations, otherwise inaccessible to straightforward molecular simulations. We used this method to generate pressure unfolded conformations of a globular protein, Staphylococcal nuclease (Snase). Using the unfolded conformations of Snase, we estimated the thermodynamic properties associated with pressure denaturation – the change in volume up on unfolding and the Gibbs free energy of unfolding. These calculations clearly show that the volume of unfolding is negative making free energy of unfolding negative, and hence unfolding favorable, at higher pressures. Our two-scale approach comprehensively provides a molecular level framework to understand and predict the behavior of biological systems under pressure.