

# **Pressure Perturbation Calorimetry and the Effects of High Hydrostatic Pressure on the Conformational Equilibrium of Biomacromolecules**

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Temperature and pressure, T and P, are important environmental variables that play an essential role in the life and death of organisms on Earth. From a practical perspective, the sensitivity of microorganisms to P and T has led to significant progress in civilization through the ability to provide food safety via canning, pasteurization, and pascalization processes. From a molecular perspective, the physicochemical mechanisms that control survival are the effects of pressure and temperature on biomacromolecules (proteins, DNA, RNA, lipid membranes). Biomacromolecules adopt a unique three-dimensional structure (native or folded state) that is required for their biological function. The stability of this structure is very important for the function, and thus biomacromolecules need to evolve to remain folded under living conditions. Increasing pressure, much like increasing temperature, perturbs the thermodynamic equilibrium between the native folded state and the denatured unfolded state. In thermodynamic terms, stability is defined by the Gibbs energy,  $\Delta G$ , which is a function of both temperature and pressure and is related to the population of the native, N, and unfolded, U, states as shown in Equation (1).

$$\Delta G(T, P) = -RT \ln(K_{eq}(T, P)) = -RT \ln([U]/[N]) \quad (1.)$$

where  $K_{eq}$  is equilibrium constant. The pressure dependence of  $\Delta G$  is defined by the volume changes between the unfolded and native states,  $\Delta V$ :

$$\Delta V = \left( \frac{\partial \Delta G}{\partial P} \right)_T \quad (2.)$$

Thus, the response of the system to changes in pressure is driven by Le Chatelier's principle. If  $\Delta V$  is positive, the stability will increase with an increase in temperature, and thus the thermodynamic equilibrium will be shifted towards the native state. If  $\Delta V$  is negative, an increase in pressure will shift the equilibrium towards the unfolded state, and thus at some pressure, all molecules are expected to be in the unfolded state. The method of choice to experimentally measure  $\Delta V$  is pressure perturbation calorimetry (PPC). I will discuss the principles of PPC and highlight some important findings using this calorimetric method. Finally, I will also discuss novel computational methods to predict the values of  $\Delta V$  from the knowledge of three-dimensional coordinates of proteins and nucleic acids.

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