Bringing a Greater *in vivo* Relevance to *in vitro* Measurements

Large amounts of thermodynamic and kinetic data related to biochemical reactions are available in the literature. The future goal is to be able to use these data to predict physiological processes or homeostasis in real systems. However, currently, there is a significant barrier in to predict their use this context. Addressing these needs is well matched to NIST's existing expertise in both biochemical measurements and solution physics.

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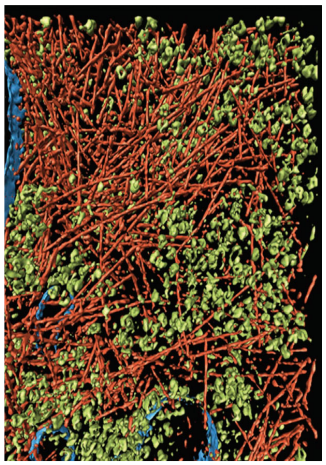
NIST aims to facilitate the use of the large amounts of available thermodynamic and kinetics data related to biochemical reactions by providing the link to *in vitro* measurement *in vivo* systems. The wealth of data currently in the literature were obtained in very dilute solutions, known as ideal conditions, whereas in the real situation physiological fluids are very concentrated with a large total concentration of biological molecules that are not involved in the process of interest. These "crowded" system conditions can affect greatly the properties of the reaction of interest. The scattered investigations of this effect that have been conducted indicate that equilibria can change by orders of magnitude upon going from the idealized dilute solution to the concentrated conditions. The changes in equilibria are entropic, caused by the system rather than by changes in the average energy of the individual molecules of the reaction. Before embarking on a significant program of research, we examined the state of knowledge in this field and found a real need and a rich opportunity for a NIST contribution.



The goal of the NIST research is to adapt available thermodynamic data to complex physiological fluids in which the reaction of interest is "crowded" in a sea of biological material that is not relevant to the process under study.

In order to make use of the dilute solution measurements one needs predictive methods for calculating the changes in the properties of the reaction upon going from the ideal state to a real physiological fluid. Although some preliminary attempts at judging the size of the effect have been made using homopolymer theories, predictive methods for biopolymers do not yet exist. Also not existing is a systematic body of quantitative data against which theoretical predictions can be tested.

The work described here is timely, as a consensus is coalescing that system non-ideality effects are not only important but provide the missing explanations for previous questions of mismatch of *in vitro* data with *in vivo* activity. "Crowding" now appears as a key controlling factor in several biochemical and pathological events. We also have begun exploratory measurements of the stability of protein molecules in "crowded" conditions using calorimetry. Our first measurements of the stability of the protein Ribonuclease A disagreed with theoretical predictions of the magnitude of destabilization of the unfolded state. However, we surmise that the disagreement found does not invalidate the theory, *per se*, but it is more likely that an improper radius of gyration of the native state had been used in the theoretical predictions.

Future Plans:
Calorimetry has been chosen as the principal measurement method for the systematic studies that are required to test theory, and we have begun the process of obtaining the necessary equipment. Calorimetry allows determination of both the enthalpy and equilibrium for a reaction, and from those, the entropy change that accompanies the reaction. Calorimetry allows for direct determination of non-specificity of chosen crowding agents. We will begin systematic studies as tests of theory and for use in the development of improved theory that includes both the entropic crowding effects and the electrostatic effects that arise from charge distributed on the surface of a protein. We also plan to investigate the development of a new crowding agent that will be a better mimic of physiological proteins than are the polymers currently used for that purpose.

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