Chapter highlights of Volume 2 of the Protein Primer

This list is being updated on 11-17-06 because a number of important concepts in protein and water chemistry have undergone major changes in the last three years. I will list the more important changes here. These have been covered in some detail in new and revised chapters of volume 2 of the protein primer.

A: the two chemical species of water have been fully examined and described although the lower-density form is still subject to some structural uncertainty.

B: The voluminous work of Koga et al have finally led to the explanation for the "magic mole fractions" for aqueous mixtures. With structure-makers of all kinds, pure ions as well as hydrophobes and amphiphiles water formed strong, cooperative clathrates below the magic mole fraction. Near 273 K half of the total water is held in clathrates and half if the higher-temperature water. Relaxation rates are a few picosec which is must slower than the interchanges rat for the pure forms of water.

C. The new facts require a new explanation for the term "hydrophobic hydration". It is the result of competition among solutes for clathrate formation. Small tetrahedral ions of which sulfate is the champion are the most effective clathrate formers.

D/ Clathrate formation is stable below 354K but unstable above because the enthalpy of their formation changes from negative to positive at that temperature.

E 354K has been thought to be the characteristic temperature for stable protein folding but that connection is misleading. It is instead a property of water detected in proteins only because protein hydration depends on the clathrate formation from environmental water. That hydration is responsible for roughly thirty percent of protein stability at 298K and none at 354K usually sufficient to cause denotation to the bubble state usually formed in thermal denaturation. Evolution has found several tricks to raise the melting temperature as much as 20 degrees higher for high ambient temperatures but most proteins are unstable above 354K.

F: The genetic and thermodynamic stability of native proteins is due to the strong knot substructures almost always formed from only 12% of the atoms. The strength of the knots is a consequence of cooperativity in electron redistribution depending

on assemblies of imide groups and interimide hydrogen bonds. Normally only antiparallel association of peptide chains are found in enzymes. The much stronger structures found in amyloid filaments and spider silks appear to be due to parallel association. Kevlar can be explained in the same way but the basic chemistry of such cooperative structures has not been reported.

G: Much larger substructures in enzymes expand and contract in first-order phase transitions that provide pulses of potential energy to reaction sites. Mechanical activation of rates in this way is the basis of all enzymes rates and specificaities thus a major achievement in evolution. The analogy with a nutcracker is close but the enzyme provides the work of closure. In order not to be quickly destroyed nature has evolved the protein process to be almost perfectly reversible.

H. All enzymes apparently use this nutcracker mechanism/ It is not possible with sequence conservation in evolution. Instead nature has depended on the management of free volume. Residues are the building blocks for such experiments. The distribution of free-volume is described by the so-called B or temperatire factors from diffraction studies and tabulated in protein databanks. Virtually all the important information can be extracted from these factors and very little from the pictorial descriptions based on angles and inter-atomic distances.

I: Enzymes as nutcrackers require matched pairs of domains with equal mass and C-2 rotational symmetry so the distributions of free volume in the smaller substructures approach a palindromic pattern. The larger substructure vary depending on substrate, transition-state energy and potential energy required by the reaction catalyzed. In contrast to the current view of function the transient potential energy is used to raise the energy zero of the reaction assembly. Transition-state stabilization has not proved an effective path for enzyme evolution.

J: Very complicated reactions can be carried out with the nutcracker construction. Even with single enzymes multi-stroke mechanisms are common. The central feature in such mechanisms extending up to large multi-enzyme systems appears to be the phase-like behavior of the potential energy reservoir. The near-perfect reversibility of that machine has been found in evolution by adjustments of surface

hydration and other surface interactions by trial and error to achieve that thermodynamic goal.

K. Free energy flows back and forth between mechanical and chemical forms as need by chemical-conformational transducer systems the most familiar of which are the ADP-ATP phosphorylase. A common use is ATP hydrolysis to expand matrices which on subsequent contraction provide a power for muscle function, etc. Enzyme catalysis has been evolved to the point that no power stroke with the accompanying heat productions is required. The heat production and free energy loss takes place only in the substrate-product change. Except for coupling to environment through surfaces each enzyme molecules is an isolated thermodynamic system operating reversibly without heat production.

Now return to the earlier items for comparisons with the new.

1 Persistent errors in protein research. Errors have multiplied and expanded becoming increasingly unfavorable for research. Chief among these are the assumptions that genes conserve sequence information when in fact they conserve patterns of atom free volume arrangement. Second omission is to note the C-2 symmetry of enzymes and many other kinds of proteins. Diffraction studies have too low accuracy and precision to provide essential structural information in the coordinates but do in the temperature factors in the Protein Database. Substructures are ignored as one consequence making the division of labor between folded stability provided by the knots and physiological function primarily dependent on contraction and expansion of matrices. Thermal denaturation in dilute buffers produces small expansion with much increased motility but nothing resembling random-coil conformations.

2 Basic theory. Benzinger's correction for errors in use of thermodynamics, two chemical species of water, basis and applications of enthalpy-entropy compensation behavior. Mean-field potentials and linear-response theory.

3. Free-volume information from temperatures factors. Use of mean and standard deviations in interpretation.

4. Aqueous mixtures. Structure-making and structure breaking solutes, clathrate formation rapidly reduces availability of water so there is very little normal water in most mixtures with

water, structure breakers reduce availability by destroying the cluster species of water, interactions of proteins with water are as important for folded stability and the details of polypeptide conformation but the fine details of these interactions as laid out by Timasheff in particular require reconsiderations to understand how they tune physiological processes.
5. The enzyme mechanism. There does not appear to be more than one mechanism for enzymic catalysis and it depends on mechanical activation of pretransition states and not . on the familiar thermal activation found in small-molecule rate processes. In all cases that mechanism is made possible by extremely fine tuning of structural characteristics in evolution in which enthalpy and entropy changes are exactly balanced and irreversible heat production is minimized in the biosphere. Except for deviations from first and second law requirements the absorbed solar energy not fixed in organisms and their products is conveted to lower and lower temperature finally to be radiated into space.. Most protein machines are multistroke using the expansion-contraction coordinate of matrices more than once to build enzymes and multi-enzymes of ever increasing complexity.
6. Extremophiles. Most extremophiles enzymes can be explained as consequences of dynamic tuning using polypeptide conformation and interactions with water, proteins are linked to their environments in this way allowing increasing complexity in structures and thus in function.
7. Unfamiliar hydrogen-bonded structures. Spider dragline silk is the strongest material in tension known and amyloid filaments have similar properties none of which have been explained, the knots holding proteins in native states with only 12% of the residues probably depend on the same construction principles, the central feature appears to be the cooperative interaction of parallel or perpendicular polypeptide fibers contracted by electrostatic factors into graphite like solids resembling carbon nanotubules
8.Converegent evolution. The single structural and functional feature of the wide range of enzymes indicates that they are evolutionarily product along a common path or a few paths, size and complexity variations may not be entirely rationalized in this way but it is likely that this convergent evolution is the way we have come whether from one initial discovery or several.
9. Fitness of the environment. About the turn of the last century L.J. Henderson published a book with the title "The fitness of the environment" in which he argued that life was possible only because of precise quantitative properties of the earth's surface, an idea and a

book still attracting much attention, but now there has emerged many new numbers describing the foundation from which life has evolved and these considerably supplement the small number of critical observations available to Henderson, new questions some with answers emerge to illuminate arguments about evolution, religion and some other objects of vital argument that may have no rigorous solutions. A few years after this contribution by Henderson J.B.S.Haldane published a very influential book called “the enzymes” also requiring an up-to-date replacement. The Protein Primer comes as close to being that replacement as is now available.