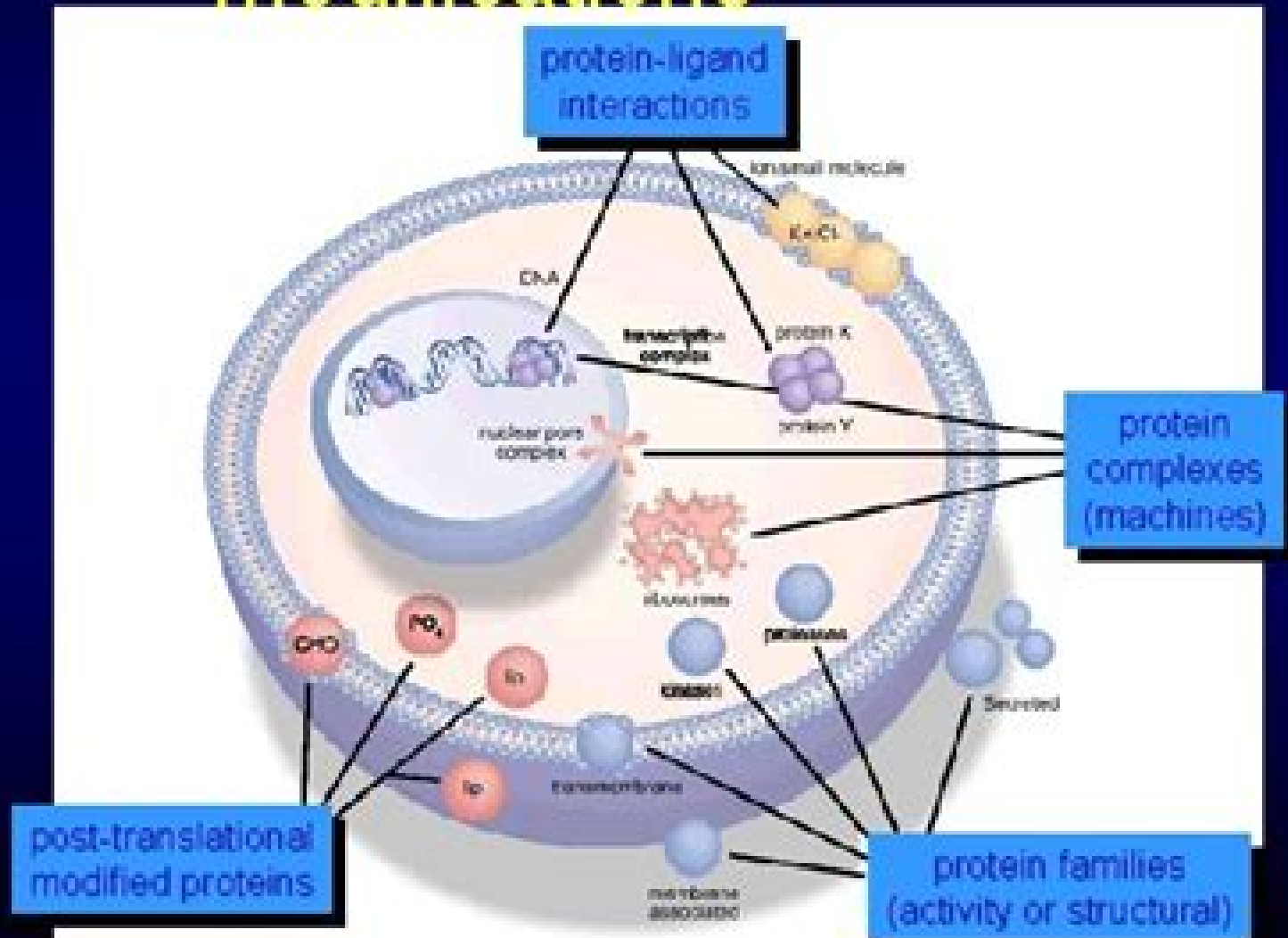


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Patterson and Aebersold, Nature Genetics (supp.), 33, 311 (2003)

Proteinligand Interactions

**Babur Z. Chowdhry, Stephen E.
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computational design was used to engineer a zinc binding site in the enzyme ATPase. As a result, zinc acts as an allosteric regulator of the enzymatic activity. Computational design was further applied to the redesign of the binding specificity of glucose and ribose binding proteins to bind pinacolmethylphosphonic acid (PMPA), a degradation product of the nerve agent soman. The computationally redesigned binding proteins were labeled with a thiol-reactive fluorophore at a unique cysteine position, and as a result, a change in fluorescence is exhibited by the protein fluorophore conjugate in response to ligand binding. The results demonstrate that the engineered proteins act as reagentless fluorescent biosensors for PMPA and exhibit a range of affinities between 0.045 and 10 μ M. Protein engineering techniques were used to extend the ability of a single biosensor element to distinguish between several similar target ligands by incorporating many sensor elements in a multisensor system. The protein PhnD, a periplasmic binding protein that binds many phosphonates, was characterized, and variants were constructed by introducing point mutations in its binding pocket. The PhnD variants exhibit differential binding affinities to several similar molecules and were used as sensor elements in a fluorescent multisensor system. The multisensor can be used to determine the concentrations of many analytes in a solution and can detect the presence of an interferent for which it has not been characterized by taking advantage of the non-linear nature of the fluorescent response to ligand binding.

Computational Analyses of Protein-ligand Interactions, 2010 **Computational Insights Into Protein-ligand Interactions** L. M. Birch, 2006 An Investigation Into the Role of Protein-ligand Interactions on Obligate and Transient Protein-protein Interactions [microform] Quinlan, Robert Jason, 2005. Protein-ligand and protein-protein interactions are critical to cellular function. Most cellular metabolic and signal transduction pathways are influenced by these interactions; consequently, molecular-level understanding of these associations is an important area of biochemical research. We have examined the thermodynamics of several protein-protein associations and the protein-ligand interactions that mediate them. Using Fluorescence Correlation Spectroscopy, we have examined the putative interaction between pig heart malate dehydrogenase (MDH) and citrate synthase (CTS). We demonstrate a specific, low-affinity interaction between these enzymes. The association is highly polyethylene glycol (PEG)-dependent, and at high concentrations of NaCl or PEG, non-specific aggregates are formed. We demonstrate that oxaloacetate, the intermediate common to both CTS and MDH, induces the association at concentrations below the K_m of CTS, suggesting that the open conformation of CTS is involved in the association. Using several biophysical techniques, we have examined the subunit associations of *B. stearothermophilus* phosphofructokinase (PFK). We demonstrate that the inhibitor-bound conformation of the enzyme has reduced subunit affinity. The kinetics and thermodynamics of the phosphoenolpyruvate (PEP)-induced dissociation of PFK have been quantified. Binding substrate fructose 6-phosphate (F6P) stabilizes the enzyme to inhibitor-induced dissociation by 132-fold. These data suggest that subunit associations may play a role in the allosteric inhibition of PFK by PEP. The thermodynamics of the protein-ligand associations and allosteric inhibition of *E. coli* phosphofructokinase have been examined using intrinsic fluorescence and hydrostatic

pressure Both ligand binding affinity and PEP inhibition are diminished by pressure whereas substrate binding affinity for inhibitor bound enzyme is pressure insensitive Larger entropic than enthalpic changes with pressure lead to the overall reduction in free energies Using a fluorescence based assay we have developed a series of baroresistant buffer mixtures By combining a buffer with acid dissociation of negative volume with a buffer of positive volume a pressure resistant mixture is produced Alteration of the molar ratio of the two component buffers yields mixtures that are pressure insensitive at pH values around neutrality

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