Descriptors for the dihydrofolate reductase-thymidylate synthase (DHFR-TS) systems, whereby methylene tetrahydrofolate is converted to tetrahydrofolate, were found to be a general model for disulfide oxidoreductase function across all domains of life. Despite the similar mechanisms and in vivo roles of the two enzymes, we found vastly different rates of spontaneous release. DHSA releases at a rate of about 0.01 s⁻¹, which is roughly tenfold slower than PDI. This exceedingly slow rate of release suggests that the repertoire of prokaryotic proteins that catalytically oxidized may take longer to fold, on average, than their eukaryotic counterparts.

Kinetics of Sequential Enzyme Reactions and Electrostatic Channelling
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In cells, many enzyme-catalyzed reactions are coupled as a series of biochemical steps. Thus, the reaction rate is dependent on the kinetics of intermediate reaction steps. In this work, we study the case of two coupled enzyme reactions, where the product of the first reaction serves as the substrate of the second reaction. Using a diffusion-limited reaction model, we demonstrate that the over- all kinetics is strongly dependent on the separation distance between two enzymes, although interestingly, we find that in some cases, the maximal rate occurs at a finite, non-smallest separation between enzymes. This is because the second enzyme in proximity can block the access of substrate for the first enzyme reaction, which leads to the decrease of product of the first reaction (substrate for the second reaction). We further demonstrate how this reaction rate is additionally dependent on the nature of electrostatic interactions between reactants and the two enzymes. We demonstrate the interplay of these concepts for the dihydrofolate reductase-thymidylate synthase (DHFR-TS) systems, whereby methylene tetrahydrofolate is converted to tetrahydrofolate. Our study suggests the role of species-specific electrostatic and geometric factors in optimizing reaction rates of substrate-channelling systems.

Synaptotagmin Linker: Tuning of Cooperativity in Calcium Ion Binding
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Synaptotagmin I (Syt I) contains two Ca²⁺ and phospholipid binding domains (C2A and C2B) that function in the regulated exocytosis of neurotransmitters. Syt I participates in the sensing of the Ca²⁺ influx in nerve cells during signal propagation. C2A and C2B are linked to each other with C2A, the domain closest to the vesicle, having a linker region connecting the protein to a vesicle. A combination of differential scanning calorimetry and fluorescence lifetime spectroscopy were used to study free energy of stability of C2A constructs with varying lengths of the linker region. These studies showed a decrease in stability with the inclusion of the linker region. From these findings, we propose that the linker region has an impact on the binding affinity of endogenous ligands (phospholipids and Ca²⁺) by the binding domain. We used isothermal titration calorimetry to investigate and compare the binding of endogenous ligand by the domain with and without the linker. The inclusion of the linker led to a decrease in binding affinity, but the linker promoted cooperativity between binding sites allowing for an increased responsiveness to local ligand concentrations. Although the linker is not in close proximity to the Ca²⁺ binding loops, there is still an effect on binding. This can be linked to the relatively low stability, which allows for small structural changes to make a difference in the ligand binding. The cooperativity between binding sites allows for the saturation of C2A to be more dramatic over a narrower range of ligand concentration. The wider range of responsiveness suggests that Syt I is able to respond better to the influx of Ca²⁺ affecting its ability to participate in exocytotic events.

Molecular Dynamics-Based Predictions of the Structural and Functional Effects of Disease-Causing Cardiac Troponin Mutations
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Cardiac troponin C (cTnC), the regulatory calcium-binding component of the troponin complex, is responsible for the regulation of cardiac muscle contraction in response to varying cytosolic calcium levels. When calcium is bound to cTnC, a conformational change is initiated, which leads to muscle contraction through changes in the interaction with the remainder of the troponin complex and the interaction of the thin filament with myosin. Mutations that alter the ability of cTnC to bind calcium are hypothesized to induce hypertrophic or dilated cardiomyopathies depending on whether calcium affinity is increased or decreased respectively. Several mutations in cTnC have been selected that are associated with these cardiomyopathies. These mutations include A8V, L29Q, L48Q, C84Y, Q122A, E134D, D145E, which are associated with hypertrophic cardiomyopathy. The structural effects of these mutations have been modeled through equilibrium molecular dynamics and their functional impact assessed using free energy calculations. In each mutant that was analyzed, the equilibrated structures were very similar to those of the wild-type according to the following criteria: backbone superposition, site II calcium coordination residues, the size of calcium-Ctnc interface, volume and surface area of the cTnC molecule, and the overall energy score of the structure. The most salient difference between the mutants was the relative orientation of the N & C terminal domains of the proteins. Though the variation across mutants was comparable to the variation between repeated simulations of a given mutation. Despite this very limited structural variation, a measurable change in the free energy of calcium binding was calculated between the mutant and wild-type structures.