Graph theoretical analysis of the network defined by inter-water H-bonds shows that residues in Hx3 and Hx7 (L3.43, N7.49 and Y7.53) interrupt the network targeting two isolated clusters - one large encompassing the ligand binding pocket and a small one in the G protein site - preventing inter-site communication. Inclusion of protein sites that H-bond waters establishes a continuous but very weak connectivity between the isolated clusters in the complex with an antagonist. In contrast, the presence of an agonist creates multiple pathways and increases network connectivity by ca. 30-fold, establishing a allosteric link between the ligand and G protein. We are currently investigating the network connectivity in the ternary complex agonist-receptor-Gprotein.

1826-Plat
Insertion of β-Barrel Proteins in Gram-Negative Bacteria
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Gram-negative bacteria possess two membranes, the inner and outer of which contain primarily α-helix and β-barrel proteins respectively. In recent years, significant progress has been made in understanding insertion and assembly of proteins into the inner membrane, while the same process in the outer membrane has remained elusive. In 2013, the crystal structure of BamA, the central and essential component of the β-barrel assembly machinery (BAM), was released, paving the way for rapid progress in understanding the insertion and assembly process. All-atom molecular dynamics simulations have been performed, revealing many novel features including lateral gate opening between the first and last barrel strands, and a significantly thinner, destabilized membrane region near the putative insertion site. However, many questions remain, including the role of the periplasmic domains, the mode of substrate recognition, and the energetic factors driving function in the absence of both ATP and an electrochemical gradient. We have performed novel equilibrium simulations of the protein in its native lipopolysaccharide environment including its essential periplasmic domain. Here, we present a comparison of free energy associated with lateral-gate opening for native systems, as well as systems with strand modifications and augmentations which yield insight into driving energetics and substrate recognition.

1827-Plat
Super-Resolution Mapping of the Dynamics of Periodic Structural Defects in Collagen Fibrils
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Single-molecule tracking of matrix metalloproteinases (MMPs) moving on fibrillar collagen reveals a regular binding pattern with a 1.1 nm periodicity. The binding sites exhibit collective motion that preserves the distribution but merge. The dynamic nature of the binding sites suggests that they correspond to transient local defects in the collagen fibril structure. However, the long-range order of their pattern, exceeding any known structural scale of the fibril, indicates a collective defect formation process. We propose a model in which internal strain energy in fibrillar collagen is relieved by the formation of defects that are distributed along its length. This model falls into the general class of mechanical instabilities that generate long-range spatial patterning in physical systems ranging from mud cracking to skin wrinkling. However, unlike cracks and wrinkles that are stable structures, the microscopic fibril features thermally excited structural dynamics and self-healing of defect states. One physiological consequence of the proposed model is that external tension opposing the internal strain in the fibril can suppress defect formation and exposure of the MMP binding sites. This experimental finding that external loading attenuates the enzymatic degradation of fibrillar collagen are consistent with this prediction of the model. More generally, many aspects of collagen degradation, including cleavage initiation, processivity, and kinetics, may largely be a consequence of a previously unrecognized structural heterogeneity in the underlying fibrillar substrate. Thus, mapping the periodic array of defects in the molecular architecture of collagen eludes a key feature regulating enzymatic activity and remodeling of the extracellular matrix.