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 an allosteric link between the ligand and G protein. We are currently investigating network connectivity in the ternary complex agonist-receptor-Gprotein.

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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 domain, 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.

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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 μm periodicity. The binding sites exhibit collective motion that preserves the distribution but not the phase of the binding pattern. Over short timescales (~1 s), the motion of individual binding sites is consistent with diffusion in a harmonic potential. At longer timescales (~20 s), the potential wells slowly migrate, bifurcate, and 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, showing 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 elucidates a key feature regulating enzymatic activity and remolding of the extracellular matrix.

1828-Plat

Symmetry and Scale Orient Min Oscillation Patterns in Bacterial Shape Sculptures

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What are the effects of cellular confinement and cell shape on the intracellular organization, and vice versa? Here we present a novel nanostructuring technique to systematically ‘sculpt’ living bacterial cells into defined shapes, e.g. squares and rectangles, to explore the spatial adaptation of Min proteins that oscillate pole-to-pole to assist division-site selection at the mid-plane in rod-shape Escherichia coli.

In a wide geometric parameter space, spanning cell volumes from 2x1x1 to 11x6x1 μm3, Min proteins are found to exhibit versatile oscillation patterns, sustaining rotational, longitudinal, diagonal, stripe, and even transversal modes. Their quantitative distributions reveal two essential properties that orient the Min patterns, viz., aligning to symmetry axes and a characteristic length range of 3-6 μm. Within this range, Min pattern are found to scale in adaptation to the cell length. The finding that Min patterns directly capture the symmetry and scale of the cell boundary to spatially regulate cell division refutes all previous geometry-sensing models that were based on the longest distance, membrane area or curvature.

Using numerical simulations, we find that global symmetry selection and gradient scaling both derive from the local microscopic self-activation and inhibition kinetics, which are key components of the Turing reaction-diffusion mechanism underlying Min oscillatory dynamics. Both geometry-sensing properties only emerge within fully three-dimensionally confined volumes, contrasting in vitro Min waves on planes and theories with a fixed wavelength. Our results show that simple molecular interactions are capable of bridging the characteristics of cell shape to the spatiotemporal regulation of essential cellular processes.

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Quantitative Analysis of RNA Interference by mRNA Counting at Single-Cell Level

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RNA interference (RNAi) is a gene regulation pathway induced by small RNA molecules, microRNA (miRNA) and small interfering RNA (siRNA). It is a multi-step process consisting of the small RNA genesis by Dicer, guide strand selection and the guide strand-mediated gene silencing either through mrna cleavage or translation inhibition by RNA-induced silencing complex (RISC). Even though RNAi is one of the major techniques to regulate gene, the mechanistic details of each step are yet to be determined. Especially, un-veiling which step is the rate-determining step would deepen the understanding about the molecular mechanism of RNAi and could contribute designing more efficient small RNAs for gene silencing. To address the question, the quantitative analysis of RNAi is required, which benefit from the detection of single mRNA at single-cell level. Here, we incorporated single-molecule fluorescence in situ hybridization (smFISH) to count mRNA at single-cell level and screened multiple sets of small RNAs which mimic miRNA, siRNA, pre-miRNA and pre-siRNA. Together with single-molecule fluorescence resonance energy transfer (smFRET) which enables us to quantify the RNA cleavage by Dicer, we discovered that the cleavage step by Dicer controls the overall silencing kinetic. Interestingly, we also found that Dicer is sensitive to 3’ overhang not RISC. The quantitative analysis of RNAi using smFISH together with smFRET would be a powerful platform to study RNAi and other gene-regulating system.

1830-Plat

Environmental Statistics and Optimal Regulation

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Any organism is embedded in an environment that changes over time. The timescale for and statistics of environmental change, the precision with which the organism can detect environment, and the costs and benefits of particular protein expression levels all will affect the suitability of different strategies - such as constitutive expression or graded response - for regulating protein levels in response to environmental inputs. We propose a general framework - here specifically applied to the enzymatic regulation of metabolism in response to changing concentrations of a basic nutrient - to predict the optimal regulatory strategy given the statistics of fluctuations in the environment and measurement apparatus, respectively, and the costs associated with enzyme production. We