macromolecules, e.g., proteins, nucleic acids, sugars, lipids, etc. These macromolecules occupy as much as 30% of the cell volume, thereby affecting the stability and rate of multi-protein binding. We investigate the effect of macromolecular crowding on the protein complex formation, using coarse-grained simulation models for proteins and macromolecular crowders. Proteins are represented by a residue-level coarse-grained model that has been shown to yield binding affinities and native structures of various weakly binding protein complexes, in good agreement with experimental data. Macromolecular crowders are modeled as spherical particles or polymeric chains that interact with proteins via repulsive as well as attractive interactions. Repulsive crowders, interacting via excluded volume interactions, of various types stabilize the formation of the protein complex, but the attractive protein-crowder interactions are shown to destabilize the protein complex above moderate attraction strengths. We find that the translational and rotational diffusion for both proteins are slowed down with both the repulsive and attractive crowders, although the anisotropy of the rotational diffusion coefficient increases for both cases. Consequently, the dissociation rate decreases with increasing crowder volume fraction. But the protein association rate is found to increase as a function of the crowder volume fraction in the presence of repulsive crowders, while attractive protein-crowder interactions decrease the association rate. Interestingly, we find that the polymeric crowders can change the protein binding behavior in a complex manner depending on the degree of crowder polymerization and conformational flexibility. We develop a theory, with physically meaningful parameters, that can describe the simulation data very well and provide further insights into the observed results.

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Real-Time Transcription Initiation by E. coli RNA Polymerase in vitro and in vivo

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Transcription initiation is the most important step in gene regulation and is orchestrated by RNA polymerase (RNAP). However, its molecular mechanisms have remained unclear due to the presence of transient intermediates and heterogeneity.

To characterize such mechanisms, we used an *in vitro* real-time FRET assay on immobilized transcription complexes for minute-long observations of DNA scrunching and unscrunching. We characterized the kinetics of abortive initiation (by following single cycles of abortive synthesis) and promoter escape, and identified functionally important heterogeneity. We observed, for the first time, extensive RNAP pausing (lasting for ~15 s) and backtracking during initiation; such behaviors may play regulatory roles.

We also studied initial transcription *in vivo* using electroporation to internalize doubly-labeled promoter DNA fragments into live *E. coli* and track them using TIRF microscopy. We observed low-FRET species of 0.18 ± 0.05 (corresponding to duplex DNA) and fluctuations to higher FRET-states, which we attribute to RNAP promoter binding, open complex formation and initial transcription. Specifically, we see FRET of 0.35 ± 0.10 (linked to initiation pausing), and 0.85 ± 0.06 (linked to promoter escape); both levels are absent in non-promoter DNA.

Our work reveals the detailed kinetics of initial transcription *in vitro* and offers the first such observations in living cells, which opens exciting avenues to study gene regulation *in vivo*.

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A Disorder-Based Strategy for the Introduction of Allosteric, "Hill-Type" Cooperativity into Artificial Receptors

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Control over of the sensitivity with which biomolecular receptors respond to small changes in target molecule concentration is crucial to many biological processes. The ability to introduce cooperativity into artificial bioreceptors would likely prove useful for applications such as biosensors, genetic logic gates, and "smart" materials, in which highly responsive behavior is of value. In Nature, this control is commonly achieved via allosteric, "Hill-type," cooperativity, in which binding events on a multivalent receptor are coupled so that the first event enhances the affinity of subsequent events, producing an "all-or-nothing" binding response and, in turn, a higher-order, steeper, dependence on target molecule concentration. Here we use an intrinsic-disorder-mediated approach to rationally and quantitatively introduce this useful property into several normally non-cooperative biomolecular receptors. Specifically, we fabricate a tandem repeat of the receptor that is destabilized by the inclusion of a disordered loop. The first target binding event pays an entropic cost to close this loop, thus forming a structured site for a second target molecule to bind with comparatively higher affinity. By changing the length of the loop, we can quantitatively change the energetic cost of closing the loop and in turn the extent of cooperativity, and thus the order of the binding curve and sensitivity to small changes in concentration. Using this approach we have rationally introduced cooperativity into three unrelated aptamers, achieving in the best of these a Hill coefficient experimentally indistinguishable from the theoretically expected maximum. Furthermore, the extent of cooperativity, and thus the steepness of the binding transition, are, moreover, well modeled as simple functions of the energetic cost of binding-induced folding, speaking to the quantitative nature of this design strategy.

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Structure-Function Relations and Rigidity Percolation in Biopolymer Networks in Live Tissue under Shear: Bovine Articular Cartilage as a Model System

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Studies of hierarchical structure in biological tissues are fundamental to identifying useful design principles that can be transferred to both biological and non-biological engineered systems. Among mammalian soft tissues, articular cartilage is particularly interesting, as it can endure 60+ years of daily mechanical loading despite having minimal regenerative capacity. This remarkable resilience may be due to the depth-dependent mechanical properties, which are known to localize strain and energy dissipation to the tissue's surface. Based on qualitative observations, it has been proposed that these properties arise from the depth-dependent collagen fiber orientation. Nevertheless, this structure-function relationship has not yet been quantified. Here, we use a combination of a computational model made of a collagen network and a hydrated aggrecan matrix, and confocal elastography experiments on live tissue to look for constitutive relations between mechanical and structural quantities. Surprisingly, we find weak correlations between the shear modulus and the collagen fiber orientation. Instead, we find a much stronger correlation with the concentration of collagen fibers, which shows a 2-fold variation in collagen volume fraction correlates with a 100-fold variation in the modulus, and follows a scaling law relation. Such dependencies are observed in the rheology of in-vitro cytoskeletal networks that exhibit a rigidity percolation phase transition. Along these lines, we propose that the collagen network is near a percolation threshold that gives rise to these large mechanical variations and strain-localization at the tissue's surface. The interplay between this criticality, and the co-operative interaction between the collagen network and the aggrecan background underlie the observed mechanical response.

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Nuclear Damage in Highly Constrained Migration: From Lamina Defects to DNA Breaks

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Cells in vivo are sometimes required to migrate through tight spaces that are much smaller than the largest organelle, their nucleus. Micro-pore migration of lung cancer cells causes nuclear blebs with segregated lamins as well as DNA tethering and breaks. Nuclear blebs seen in the majority of cells are enriched in lamin-A and deficient in both lamin-B and DNA, but the cells are viable with a normal rate of post-migration proliferation. Phosphorylation of lamin-A, which relates to turnover under low stress, decreases with migration, while phosphomimetic and progeria mutants of lamin-A exhibit distinct differences. Knockdown of lamin-A induced the frequent formation of DNA tethers that extrude from the main nuclear body through a gap in lamin-B and to the

pore that the cell migrated through. Double strand breaks also increased and are consistent with subsequent cell death. The findings reveal a crucial role for the lamins in cell migration and survival, likely through DNA protection.

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The Kinetics of Nascent Protein Folding upon Release from the Ribosome

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Very little is known about the way proteins attain their native structure within the context of the living cell. In addition to the ribosome's well-established role in peptide bond formation, recent studies suggest that ribosomes play an important role in the early stages of protein folding in the cell and may be crucial for the production of folded bioactive proteins. Importantly, little is known about the impact of the mechanism of protein release from the ribosome on the attainment of a correctly folded conformation. Here, we present a kinetic study on the release time-course of fully synthesized ribosome-bound nascent proteins upon addition of the antibiotic puromycin. We focus these studies on the E. coli globin ApoHmpH. By time-resolved gel electrophoresis, we are able to follow puromycin's hydrolysis of the ester bond linking nascent polypeptides to the 3' end of tRNA. Steady-state fluorescence anisotropy allows us to follow the escape and folding of ApoHmpH from the ribosome. Finally, time decay fluorescence anisotropy analysis in the frequency domain complements the above techniques by providing insights into the local motions experienced by the nascent protein before and after release from the ribosome. Under experimental conditions where puromycin reacts at rates comparable to the naturally occurring release factors, we show that protein release from the ribosome is rate-limited by the C-terminal ester bond cleavage, and that escape from the ribosome and completion of folding occur quickly following this step. This result shows that the ribosomal context promotes a particularly ''temporally efficient'' folding upon nascent protein release. An important consequence of this phenomenon is the prevention of undesirable diffusion- and concentration-dependent phenomena such as aggregation.

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Diffusion Coefficient as a Function of Mass for Globular Biomolecules

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How well can the diffusion coefficient D of a globular biomolecule be predicted from its molecular mass MW? In ''Wanted: Scalable Tracers for Diffusion Measurements'' [J Phys Chem B, submitted], I propose that diffusion measurements in heterogeneous systems can be improved by the use of scalable tracers, in which the size is varied alone at constant shape, surface properties, diffusion mechanism, deformability, and other properties affecting diffusion. Before trying to design a de novo series of scalable globular proteins, it is appropriate to examine how scalable the commonly used de antiquo globular proteins are [ibid., supporting information]. The widely-used compilation of experimental diffusion coefficients by Tyn and Gusek [Biotech Bioeng 35 (1990) 327] was examined. This set – ranging from ribonuclease, 12640 Da, to tobacco mosaic virus, 50 MDa – was plotted as D versus log MW. The obviously linear species were removed, and values of D and MW for the outliers were examined. The plot yields a cloud of values of D versus log MW. In this plot, rigorously scalable tracers are expected to give a single smooth curve of D versus log MW, and the extent of the cloud represents scatter due to nonscalablity in the other properties, and to experimental error. Values of D from hydrodynamic calculations from various laboratories are remarkably consistent with the cloud. The cloud prediction is certainly good enough for semi-quantitative estimates or for designing single-particle tracking experiments. For a diffusion-controlled reaction in dilute solution, the prediction is close enough that the standard analysis of propagation of errors can be used. But arbitrary cloud proteins are not adequate for, say, measuring the percolation threshold of cytoplasm. The incomplete examination of the question here indicates what would be required for a complete examination.

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Developing a Nanocarrier for Targeted Delivery of Cardio-Protective Agents

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Background: Ischemic heart disease (IHD) is a leading cause of death worldwide. Pre-clinical drugs for IHD have largely failed in human clinical trials, highlighting the importance of developing vehicles to selectively deliver optimal therapeutic agents to the ischemic myocardium. Hydrophobins are fungal proteins that self-assemble into robust amphipathic monolayers that can encapsulate and solubilise hydrophobic drugs.
Aim: Develop the non-immunogenic hydrophobin RodA as a targeted-drug delivery vehicle.
Methods: Three recombinant RodA variants were prepared: RodA-MY, engineered to contain the peptide CSTSMLKAC that selectively binds to ischemic myocardium and RodA with or without a FITC-conjugate. RodA monomers and polymers were produced with and without the targeting sequence. All variants were tested for the ability of self-assemble into fibrillar (nanocarrier) structures. Monomeric and polymeric RodA variants were tested for cardiotoxicity using cardiomyocyte-like H9c2 cells. Targeting ability was tested using ischemia-challenged H9c2 cells and ischemic myocardium from a rat model of myocardial ischemia reperfusion.
Results and conclusions: Hydrophobins were well-tolerated by cultured H9c2 cells. Exposure of cells to both monomeric RodA or RodA polymers (50-100μg/mL) had no effect on cell viability or cell cycle profile. Immunocytochemistry demonstrated unaltered cell surface receptor distribution and activity as judged by binding and uptake of FITC-labelled transferrin. Consistent with previous data, the levels of the pro-inflammatory gene nFkB were unchanged suggesting that hydrophobins did not elicit inflammation in cardiac cells. Although, RodA-MY-nanocarrier has advantages over the native RodA-nanocarrier in targeting both normoxic and injured H9C2 cells, pilot work has revealed that RodA-MY-nanocarrier preferentially binds ex vivo to ischemic rat myocardium. Together the data indicate that hydrophobin-based polymers may act as drug-delivery vehicles for myocardial ischemic pathologies. **Future work:** studying the vehicles targeted ability of passing the vasculature barriers.

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The Nonrandom Nature of Weak Interactions between Proteins and Bystander Macromolecules in Cellular Environments

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Biochemical studies have mainly focused on specific interactions between ''reactant'' molecules, even though these interactions occur in cellular milieus crowded with ''bystander'' macromolecules. There is now growing experimental evidence that weak interactions of reactant proteins with macromolecular crowders may regulate biochemical processes [Miklos and Zhou, PLoS ONE 8, e74969 (2013)]. Computationally, our recent development of the FFT-based method for Modeling Atomistic Protein-crowder interactions (FMAP) has made it feasible to investigate the nature of such weak interactions [Qin and Zhou, JCTC 10, 2824 (2014)]. In FMAP, protein-crowder interactions, both hard-core and soft, are represented as correlations functions and evaluated via FFT, leading to the chemical potential of the protein in a crowded solution. Here we applied FMAP to three proteins whose interactions with crowders were subject to recent experimental studies. While the chemical potential comes from averaging all possible protein-crowder arrangements, we found that in each of the three cases a few ''hot'' regions on the protein surface make dominant contributions. Specifically, (1) a mutation of Ser16 to Glu on the Pin1 WW domain significantly reduces the magnitude of its chemical potential in concentrated ovalbumin solutions; (2) the chemical potential of a concentrated human growth hormone solution is dominated by two ''hot'' regions; (3) the domain cleft of the bi-lobed maltose binding protein is a ''hot'' region for interacting with bovine serum albumin as a crowder. These results are generally in line with experimental observations, though quantitative agreement will require further parameterization of protein-crowder interactions. The picture emerging from the computational and experimental studies is that weak protein-crowder interactions are nonrandom such that crowders and ligands may compete for the same sites for interacting with protein receptors, thus blurring the divide between specific and nonspecific interactions.

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A Computational Model for E. coli Cytoplasm: Diffusion and Hydrodynamics

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The dynamics of proteins is essential for the quantification of various cellular processes like rates of enzymatic reactions, signal transduction and protein