

Challenges and dreams: physics of weak interactions essential to life

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ABSTRACT Biological systems display stunning capacities to self-organize. Moreover, their subcellular architectures are dynamic and responsive to changing needs and conditions. Key to these properties are manifold weak “quinary” interactions that have evolved to create specific spatial networks of macromolecules. These specific arrangements of molecules enable signals to be propagated over distances much greater than molecular dimensions, create phase separations that define functional regions in cells, and amplify cellular responses to changes in their environments. A major challenge is to develop biochemical tools and physical models to describe the panoply of weak interactions operating in cells. We also need better approaches to measure the biases in the spatial distributions of cellular macromolecules that result from the integrated action of multiple weak interactions. Partnerships between cell biologists, biochemists, and physicists are required to deploy these methods. Together these approaches will help us realize the dream of understanding the biological “glue” that sustains life at a molecular and cellular level.

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INTRODUCTION

The ability to self-organize is a hallmark of living systems. Moreover, living systems reorganize cellular components in response to changing needs. Although the orchestrated rearrangements occurring during cell division provide a vivid example, responses to altered metabolic states are equally impressive. The ultrastructure of cells is a consequence of exquisitely nonrandom arrangements of macromolecules. Some subcellular architectural elements, such as membranes and the cytoskeleton, are maintained as stable elements held together by sets of relatively strong and thus persistent interactions (e.g., the interaction energy of tubulin dimers is ~10–15 kilocalories/mole; Caplow and Fee, 2002), whereas others, such as signaling networks, rely on weak, transient interactions (e.g., fractions to a few kilocalories/mole, in the realm of van der Waals interactions). Because weak interactions are numerous and in many cases cooperative (all surfaces are sticky!), when taken together they have a profound effect on the temporal and spatial

distribution of macromolecules in the cell. All these interactions are under evolutionary pressure because they are essential for the fitness of an organism. McConkey (1982) coined the term “quinary” to describe the set of evolutionarily selected weak interactions that are essential to maintaining the functional organization of biological macromolecules in living cells and eloquently pointed out that even the gentlest separation methods may disrupt them. Thus efforts to describe and quantitate weak, “quinary” interactions of importance to living systems must respect their fragility.

Challenge #1: To study the roles of weak, quinary interactions (biological “glue”), we need to interrogate them without significantly perturbing them, which generally requires that we keep the living system intact (Gierasch and Gershenson, 2009; Wirth and Gruebele, 2013). Quinary interactions must be dynamically regulated, as cells reorganize in response to signals. Therefore we need not only knowledge of the network of weak interactions in which any biomacromolecule participates, but also the time evolution of these interactions, particularly in response to a trigger (e.g., stress or normal conditions). Whereas the physics of weak interactions is a mature science for well-defined molecular systems, the heterogeneity and complexity of cellular assemblies present significant challenges, such as measuring these interactions in vivo without additional perturbations unavoidably generated by introducing fluorescent reporters (Landgraf *et al.* 2012; Wang *et al.* 2014). We need to engage physicists who can grapple with this challenge!

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Abbreviation used: AAA+, ATPases associated with diverse activities.

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Challenge #2: To follow the network of weak interactions over time in response to changing cellular states, we need experimental methods that provide dynamic information about weak interactions. This challenge is driving the development of new optical methods and new biochemical tools to determine interactomes, but these tools are still in their infancy. The experimental data will feed physical analyses that can in turn address the temporal and spatial complexity of simultaneous interactions with energies that are only fractions of a kilocalorie/mole.

Challenge #3: Living systems function over a broad range of length, time, and energy scales. To elucidate the physical underpinnings of biology requires that one comprehend phenomena over these broad ranges. One must decipher the extent to which events at one length, time, or energy scale affect those at others, and, conversely, determine when it is justified to consider only a subset of the length, time, and energetic ranges in analyzing a given process. Of importance, interactions at the short range (angstrom/molecular level) have long-range implications (micrometer/cellular level) when considered in the proper cellular context, as illustrated later by the cellular cell wall biosynthesis machinery.

These challenges are daunting. Fortunately, we are witnessing impressive progress in several labs that are tackling cell biological questions linked to quinary interactions. These studies are yielding both new methods and new experimental data about weak interactions and their biological roles. In the following vignettes, we briefly describe three examples in which the biological consequences of weak interactions are being explored and revealed.

CHURNING THE CYTOPLASM

The concentration of proteins in the bacterial cytosol reaches a staggering 250 mg/ml by some estimates (Li *et al.*, 2014); factoring in other biological molecules (e.g., nucleic acids), the concentration of macromolecules within the cellular environment begins to reach ~400 mg/ml, resulting in substantial loss of free water. At first glance these concentrated conditions should result in catastrophic aggregation or extremely high viscosity, yet enzyme complexes orchestrate complicated chemistry requiring free diffusion and mobility. Of interest, recent work from the Jacobs-Wagner lab has shown that under ATP-limiting conditions, the bacterial cytoplasm indeed forms a glassy state, where motions of particles are severely restricted (Figure 1A; Parry *et al.*, 2014). In ATP-replete conditions, the cytoplasm becomes more fluid. Their work suggests that this fluidization is driven through the action of multiple energy-dependent enzymes and their dynamics, and thus the cell is investing energy to maintain cellular fluidity. This work is in excellent agreement with a prior report from the Theriot lab showing that jiggling motions of chromosomal loci in eukaryotic nuclei and in bacterial nucleoids are fueled by ATP-dependent processes (Weber *et al.*, 2012). For both cases, increases in motion cannot be explained by a simple increase in thermal fluctuations due to heat release by energy consumption. Instead, these experiments point to a model in which energy-dependent increases in other dynamical processes drive intracellular fluidity.

We suggest that some aspect of this increased motion may arise from changes in quinary interactions upon changes in metabolism. Proteins and protein complexes constantly undergo changes in structure that can stem directly or indirectly from ATP consumption. For example, in the bacterium *Caulobacter crescentus*, the ATP-dependent AAA+ protease ClpXP unfolds, remodels, and degrades hundreds of proteins during differentiation and the cell cycle (Bhat *et al.*, 2013). This oligomeric enzyme undergoes dramatic conformational changes upon ATP hydrolysis and also increases protein

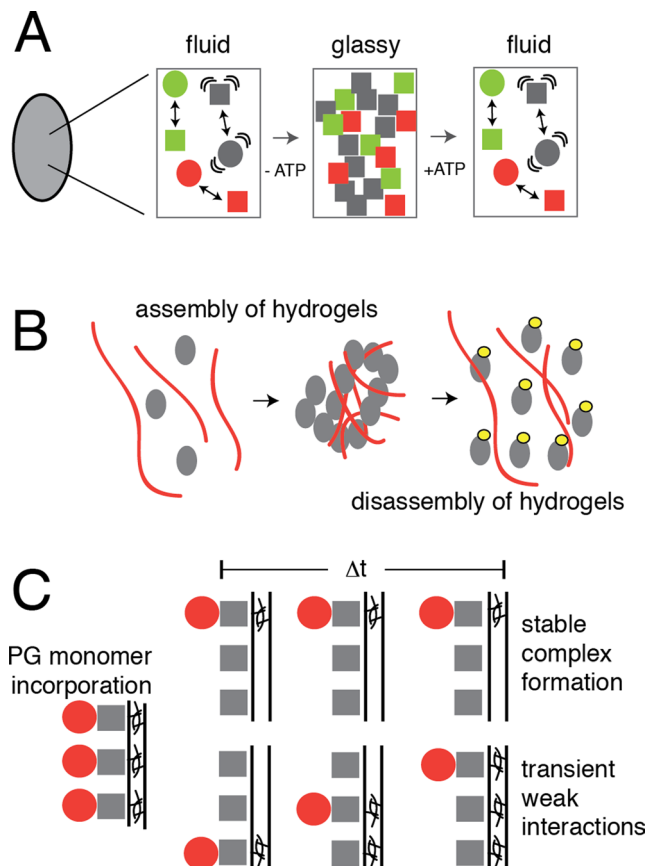


FIGURE 1: (A) Local energy consumption modulates global cellular dynamics. Proteins are constantly undergoing conformational changes (squares/circles) and breathing (wavy lines). In many cases these dynamics are fueled directly by energy consumption (e.g., conformational changes in AAA+ proteins) or indirectly (e.g., client protein remodeling by ATP-dependent chaperones). As illustrated in the work of Parry *et al.* (2014), in the absence of ATP, these interactions may be dampened, freezing proteins into static conformations. At the high solute concentrations in the cell, weak quinary interactions between these proteins can force a phase transition into a more “glass-like” state. On restoration of ATP, motions resume, breaking these weak interactions and fluidizing the entire pool of cellular biomolecules. (B) Formation of large-scale biomolecule assemblies can occur with a collection of weakly interacting proteins. At low concentrations, RNA and weakly interacting proteins circulate freely. At higher concentrations or higher valency, weak interactions cooperate to generate a higher-order assembly (Li *et al.*, 2012). In the case of RNA granules, low-complexity regions within multiple proteins produce a dynamic hydrogel that cages RNA (Han *et al.*, 2012). Hydrogels can be disassembled rapidly upon phosphorylation of the proteins (as shown by yellow circles) or by changes in temperature and concentration (Kato *et al.*, 2012). (C) Transient short-range interactions can tune long-range effects across the cell. Proper incorporation of peptidoglycan monomers into bacterial cell envelopes requires cross-linking enzymes (red) recruited by a moving assembly platform (gray). At high concentrations (left), there are sufficient cross-linking enzymes to saturate the platforms. If the interaction of cross-linking enzymes with the platform is strong/stable, then a decrease in enzyme levels would result in regions that lack the cross-linking enzyme and thus are unable to add monomers. Over a long enough time interval (Δt), this would result in loss of cell wall integrity at those unfulfilled points. By contrast, dynamic weak interactions enable transient association of cross-linking enzymes with platforms and thus buffer changes in enzyme concentration so as to sustain cell-wide synthesis by rapid redistribution of enzymes across many platforms (Lee *et al.*, 2014).

dynamics more globally due to its actions on its client proteins. On depletion of ATP, these various direct and indirect dynamics are suppressed, paving the way for weak interactions among multiple unrelated proteins to gradually accrue and locally cage biomolecules, essentially freezing out movement during energy depletion. Metabolic restart would increase the supply of ATP, fueling protein conformational changes that lead to fluidization of the cytosol. In this light, quinary interactions can be viewed as angstrom-scale frictional forces that hold proteins within micrometer-scale subcellular neighborhoods. Energy-dependent protein dynamics break these weak interactions to promote fluidity.

SUBCELLULAR PHASE TRANSITIONS

Inherently weak interactions can produce long-lived associations especially when present in multivalent complexes and suitably high concentrations. Similar to the phase transitions seen in any chemical solution close to saturation, the crowded environment of a cytoplasm can foster the abrupt condensation of biomolecules via quinary interactions into stable intracellular clusters. Work from the Rosen lab recapitulated these phase transitions and showed how they may be controlled by signals such as phosphorylation (Li *et al.*, 2012). Using tandem repeats of weakly interacting peptide ligands and their binding partners, this lab demonstrated how multivalent display can partition proteins into discrete assemblies converting low-affinity interactions into stable complexes (Figure 1B). The speed and degree of assembly can be tuned by valency and post-translational modifications of the binding partners.

Evidence of these phase transitions has also been seen in heterogeneous biomolecular systems, as shown by the recent characterization of eukaryotic RNA granules by the McKnight lab (Han *et al.*, 2012; Kato *et al.*, 2012). These granules are critical for spatial and temporal control of translation, and thus their assembly can profoundly alter programming of the entire cell. Collections of RNA are caged by dynamic protein hydrogels formed in a concentration-, temperature-, and modification-dependent manner by coalescence of weakly interacting, intrinsically unstructured, low-complexity protein regions found within multiple proteins. Both of these studies illustrate how short-range weak interactions presented at high enough valency can drive collections of biomolecules to generate large-scale assemblies.

BRIEF BUT MEANINGFUL INTERACTIONS

Macromolecular complexes that perform biosynthetic operations are generally considered to be stable assemblies optimized not only for chemical reactivity, but also for overall stability (e.g., the ribosome). In contrast to this notion, quinary interactions can promote the formation of transient complexes assembled for specific functions. For decades it has been known that dynamic clustering of enzymes required for sequential steps of a metabolic process into a “metabolon” promotes substrate reactivity, presumably through a combination of substrate channeling and limiting diffusion of intermediates (Srere, 2000; Clegg *et al.*, 2001). However, these individual enzymes interact only weakly in dilute solution, requiring immobilization or enzyme fusions to capture this enhancement *in vitro*.

Recent work from K. C. Huang’s lab illustrates how similar assemblies built from transient weak interactions orchestrate bacterial cell wall synthesis (Lee *et al.*, 2014). Formation of the peptidoglycan mesh of proteins and glycans that maintains bacterial cell shape requires multiple enzymes to break the existing mesh, insert new monomers, and cross-link those subunits into the growing matrix. A protein platform coordinates cell wall-synthesizing enzymes by moving circumferentially around the inner membrane and guiding

monomer insertion. The enzyme responsible for cross-linking newly inserted monomers is required for cell wall integrity, and inhibition of this enzyme blocks cell wall synthesis.

Of interest, single-particle tracking in living *Escherichia coli* shows that the cross-linking enzyme exhibits rapid diffusive motions, in contrast to the slower, circumferential motions of the assembly platform (Lee *et al.*, 2014). These highly dynamic interactions turn out to be beneficial for the cells, as there are only ~100 copies of cross-linking enzyme per cell. If they were tightly associated with the assembly platform, then small decreases in enzyme concentration would result in empty assembly platforms that lack the ability to properly synthesize peptidoglycan due to the long-lived nature of the existing loaded platforms (Figure 1C). By contrast, weak binding to the platforms enables the cross-linking enzyme to cross-link the inserted monomers upon binding to a platform at one site and immediately hop to the next unoccupied platform to perform its function there. In this case, substantial decreases in enzyme concentration would be buffered by these dynamics, ensuring robust cell growth. Thus the transient quality of the binding interactions of single proteins at the molecular level is responsible for long-range effects across the entire cell wall.

WHERE SHOULD WE GO NEXT? DREAM EXPERIMENTS

As the foregoing examples show, combinations of powerful new microscopy methods, genetic strategies, and proteomics have revealed the spatial and dynamic organization and reorganization of cellular components during different physiological events. These approaches have pointed out the complexity of the highly concentrated and organized cellular contents. Furthermore, these examples have helped the field to realize and appreciate the complexity of biology that arises from collections of weak interactions. These scenarios illustrate the profound concepts that can be more deeply informed by understanding biological glue—the panoply of weak “quinary” interactions.

This leaves us with a yearning for the realization of dream experiments: We can imagine the insights that could be gained by knowing the nearest-neighbor distribution for each macromolecule in a cell as a function of time and physiological condition. Who spends time with whom? For how long? In what orientation, and with which partners? Are the interactions multivalent or one-to-one? Is the picture that emerges one of zones at higher interaction density than other zones? Is energy consumed to generate or disrupt these zones?

How do we achieve this goal? Methods to detect these interactions could include in-cell nuclear magnetic resonance to follow isotopically labeled molecules (Freedberg and Selenko, 2014); particle tracking of fluorescently labeled molecules using light microscopy (Huang *et al.*, 2009); *in situ* measurements of pairwise interactions using fragment complementation at a systems level (Remy and Michnick, 2004; Tchekanda *et al.*, 2014); cross-linking studies that reveal neighboring proteins both in stable complexes and those that are only transiently interacting; proximity-induced chemical modifications to capture accumulated snapshots of transient interactions (Slavoff *et al.*, 2011); and more that we expect are in development. The outcome of these approaches must be incorporated into robust computational models to frame the results in the proper complex cellular context (Chang *et al.*, 2013; Bordbar *et al.*, 2014; Hallock *et al.*, 2014). All of these approaches and more will help shed light on the fabric of weak interactions controlling biology. New and more powerful physical understanding must accompany the development and application of these experimental methods so that we can elucidate how higher-order levels of cell biology can derive from these weak molecular interactions.

REFERENCES

- Bhat NH, Vass RH, Stoddard PR, Shin DK, Chien P (2013). Identification of ClpP substrates in *Caulobacter crescentus* reveals a role for regulated proteolysis in bacterial development. *Mol Microbiol* 88, 1083–1092.
- Bordbar A, Nagarajan H, Lewis NE, Latif H, Ebrahim A, Federowicz S, Schellenberger J, Palsson BO (2014). Minimal metabolic pathway structure is consistent with associated biomolecular interactions. *Mol Syst Biol* 10, 737.
- Caplow M, Fee L (2002). Dissociation of the tubulin dimer is extremely slow, thermodynamically very unfavorable, and reversible in the absence of an energy source. *Mol Biol Cell* 13, 2120–2131.
- Chang RL, Andrews K, Kim D, Li Z, Godzik A, Palsson BO (2013). Structural systems biology evaluation of metabolic thermotolerance in *Escherichia coli*. *Science* 340, 1220–1223.
- Clegg J, Kell D, Knull H, Welch GR, Wilson J (2001). Macromolecular interactions: tracing the roots. *Trends Biochem Sci* 26, 91.
- Freedberg DI, Selenko P (2014). Live cell NMR. *Annu Rev Biophys* 43, 171–192.
- Gierasch LM, Gershenson A (2009). Post-reductionist protein science, or putting Humpty Dumpty back together again. *Nat Chem Biol* 5, 774–777.
- Hallock MJ, Stone JE, Roberts E, Fry C, Luthey-Schulten Z (2014). Simulation of reaction diffusion processes over biologically relevant size and time scales using multi-GPU workstations. *Parallel Computing* 40, 86–99.
- Han TW, Kato M, Xie S, Wu LC, Mirzaei H, Pei J, Chen M, Xie Y, Allen J, Xiao G, *et al.* (2012). Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. *Cell* 149, 768–779.
- Huang B, Bates M, Zhuang X (2009). Super-resolution fluorescence microscopy. *Annu Rev Biochem* 78, 993–1016.
- Kato M, Han TW, Xie S, Shi K, Du X, Wu LC, Mirzaei H, Goldsmith EJ, Longgood J, Pei J, *et al.* (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell* 149, 753–767.
- Landgraf D, Okumus B, Chien P, Baker TA, Paulsson J (2012). Segregation of molecules at cell division reveals native protein localization. *Nat Methods* 9, 480–482.
- Lee TK, Tropini C, Hsin J, Desmarais SM, Ursell TS, Gong E, Gitai Z, Monds RD, Huang KC (2014). A dynamically assembled cell wall synthesis machinery buffers cell growth. *Proc Natl Acad Sci USA* 111, 4554–4559.
- Li GW, Burkhardt D, Gross C, Weissman JS (2014). Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell* 157, 624–635.
- Li P, Banjade S, Cheng HC, Kim S, Chen B, Guo L, Llaguno M, Hollingsworth JV, King DS, Banani SF, *et al.* (2012). Phase transitions in the assembly of multivalent signalling proteins. *Nature* 483, 336–340.
- McConkey EH (1982). Molecular evolution, intracellular organization, and the quinary structure of proteins. *Proc Natl Acad Sci USA* 79, 3236–3240.
- Parry BR, Surovtsev IV, Cabeen MT, O'Hern CS, Dufresne ER, Jacobs-Wagner C (2014). The bacterial cytoplasm has glass-like properties and is fluidized by metabolic activity. *Cell* 156, 183–194.
- Remy I, Michnick SW (2004). Mapping biochemical networks with protein-fragment complementation assays. *Methods Mol Biol* 261, 411–426.
- Slavoff SA, Liu DS, Cohen JD, Ting AY (2011). Imaging protein-protein interactions inside living cells via interaction-dependent fluorophore ligation. *J Am Chem Soc* 133, 19769–19776.
- Srere PA (2000). Macromolecular interactions: tracing the roots. *Trends Biochem Sci* 25, 150–153.
- Tchekanda E, Sivanesan D, Michnick SW (2014). An infrared reporter to detect spatiotemporal dynamics of protein-protein interactions. *Nat Methods* 11, 641–644.
- Wang S, Moffitt JR, Dempsey GT, Xie XS, Zhuang X (2014). Characterization and development of photoactivatable fluorescent proteins for single-molecule-based superresolution imaging. *Proc Natl Acad Sci USA* 111, 8452–8457.
- Weber SC, Spakowitz AJ, Theriot JA (2012). Nonthermal ATP-dependent fluctuations contribute to the in vivo motion of chromosomal loci. *Proc Natl Acad Sci USA* 109, 7338–7343.
- Wirth AJ, Greubele M (2013). Quinary protein structure and the consequences of crowding in living cells: leaving the test-tube behind. *Bioessays* 35, 984–993.