Biopolymers in Vivo

568-Pos Board B348
A Sensor for Quantification of Macromolecular Crowding in Living Cells
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The cell is highly crowded with biomacromolecules, and the excluded volume influences processes such as diffusion, folding, conformation, and aggregation or association of proteins and polynucleic acids. In Escherichia coli, the values reported for the total macromolecular content range from 200 to 400 mg/mL. Knowledge of the macromolecular crowding is needed to understand behavior and especially interactions of biomolecules in vivo, be it for drug development, fundamental knowledge, or to support computational efforts to model the living cell. Direct spatiotemporal readout of the crowding would be a powerful asset to unravel the structure of the cytoplasm and the impact of excluded volume on protein function in living cells. Here, we introduce a Förster resonance energy transfer (FRET) sensor for quantification of the macromolecular crowding and apply the sensor in living cells.

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Desolvation Energy: A Rationale for Changes in Binding Affinity as Measured by ITC
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This study tests a new thermodynamic framework for aqueous binding equilibria that features an explicit consideration for the change in hydration that occurs when two solvated surfaces come into contact. As an outcome of this approach, the standard state free energy of binding is defined by the summation of two terms, the traditional term (-RTln$K$) plus a desolvation free energy term that is weighted by the number of complexes formed at equilibrium. The governing equation suggests that the equilibrium ratio (K) is not a constant; this equation is supported firmly by results from isothermal titration calorimetry using the chelation of calcium(II) by EDTA as a model binding reaction. In addition, we demonstrate that secondary solutes can shift the equilibrium by altering the average free energy of bulk water; molar solutions of urea, sucrose, and trehalose result in significant changes in the equilibrium ratio without altering the standard state free energy, as defined by our working equation. This investigation provides a fresh approach for characterizing concentrated, nonideal solutions, as relevant for understanding the driving forces behind molecular interactions in a cell or tissue. If the desolvation equation is demonstrated to be correct for other binding reactions in general, this project could ignite a renaissance in the application of aqueous solution thermodynamics.

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Rapidly Inducible De Novo Synthesis of Hydrogels in Living Cells
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We report a design, synthesis and characterization of hydrogels inside living cells which will serve as a biomolecular sift. We achieve this by rapid induction of a sol-gel phase transition. A hydrogel is defined as a non-fluid, cross-linked polymer network. Inducing polymerization of soluble multivalent molecules could thus lead to in situ hydrogel formation. To probe a spatiotemporally dynamic cellular processes, the phase transition must take place at a specific subcellular location in a rapidly inducible manner. To achieve this, we utilized a chemically inducible dimerization technique, through which a pair of protein domains bind to each other only in the presence of the corresponding chemical dimerizer. Our choice of protein pairs are FKBP (FK506 binding protein) and FRB (FKBP-rapamycin biding domain) which dimerize in the presence of rapamycin. For the in-cell hydrogel formation, we induced polymerization of the multivalent FKBP (FKBPX) and FRB (FRBX) molecules spaced with a long peptide linker by adding a chemical dimerizer. By targeting the FRBX to a particular cellular location and FKBPX to the cytosol, we were able to rapidly produce a hydrogel in a spatio-temporally controlled manner. Subsequently, we performed biophysical characterization of the synthetic gels both in vitro and in vivo and found that the gels allow passive diffusion of most of the proteins but not larger molecular complexes or cellular organelles. The present study is highly unique in its quality execution of rapidly inducing de novo synthesis of hydrogels in living cells.

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Excluded Volume Effects Inside the Living Cell
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Proteins and nucleic acids fold and behave in a highly occupied matrix of macromolecules, the cellular environment. Although this environment is filled up to a volume of 40% with macromolecules, the effect of crowding on biochemical reactions as well as biophysical properties of proteins has been rarely considered. Therefore, studies on macromolecular crowding are mainly conducted using artificially created polymer based substances as mimics of the in cell environment. Still, a common concept how the excluded volume effect in the cell affects protein folding, function and structure is lacking. Understanding these properties will be important to unravel the underlying mechanism of protein misfolding and aggregation as well as the behavior of intrinsically disordered proteins in a living cell. We introduce a FRET based random coil polymer to characterize crowding both in vitro and inside a living cell. We find different compression of the polymer in artificial crowding substances compared to protein crowders such as BSA or oocyte lysate. Injection of the probe inside cells confirms this result and reveals a heterogeneous environment which, on average, shows comparable polymer conformations as in diluted buffer. The polymer conformation is used to quantify crowding differences in the cytosol and the nucleus as well as to identify crowding in cells influenced by different extrinsic conditions. Severe osmotic stress is used to probe compression of the polymer in the highly concentrated cytosolic environment.
We conclude that the FRET labelled polymer provides a new approach to investigate and characterize the cellular solvation properties with high spatio-temporal resolution in a variety of systems and identify crowding differences due to the architecture of the cellular matrix. Therefore, it will help to understand how the cell, as the native environment in which proteins evolved, might modulate and tune biomolecule properties and functions.

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Macromolecular Crowding in the Cytosol: Underappreciated or Overestimated?
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The cytosol of an *E. coli* cell is extremely crowded, containing 20-30 % (v/v) macromolecules. Such conditions are expected to increase activity coefficients and result in excluded volume effects, driving complexation, multimerization and compact conformations of macromolecules. Experimentally, crowding is often simulated by high concentrations of inert, uncharged hydrophilic polymers such as PEG and Ficoll. However, recent studies strongly indicate that excluded volume effects can be attenuated by associative interactions of macromolecules with the crowding agent. To simulate cytosolic crowding *in vitro*, we use cell-free *E. coli* lysate in physiologically relevant concentrations. We study the effects of crowding with cell lysate and polymeric crowding agents on the FRET efficiency of a CFP/YFP crowding sensor and a FRET-based ATP sensor. If strong excluded volume effects are present in cells, quantification of metabolite concentrations *in vivo* by FRET-based constructs is severely complicated by the additional effects of excluded volume on FRET efficiency. We found that synthetic crowding agents not only decrease association constants of the ATP-sensor for ATP significantly, but also induce a strong increase in FRET efficiency by themselves. Our results indicate a strong excluded volume effect from polymeric crowding agents, while cell-free lysate does not show these effects. We hypothesize that *in vivo*, associative interactions can overcome excluded volume effects in some cases, whereas in other interactions depletion forces could be dominant.

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Thermodynamics and Kinetics of Multi-Protein Binding in Crowded Environments
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Protein-protein interactions play an essential role in many biological processes inside a cell. The cellular medium is crowded with an ensemble of