New and Notable

SAXS/SANS Probe of Intermolecular Interactions in Concentrated Protein Solutions

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Most biochemical and biophysical studies are conducted under relatively dilute concentrations, typically micromolar for proteins and nanomolar for nucleic acids. However, proteins and other macromolecules are present at high (up to 400 mg/mL) total concentrations in all cellular compartments. High protein concentrations are also present in therapeutic formulations (>100 mg/mL), such as for monoclonal antibodies, necessitated by the volume limit (<1.5 mL) of subcutaneous injections and high dosage (~100 mg) of antibody therapies. These concentrations provide many opportunities for chance intermolecular encounters. Such weak, nonspecific interactions of a protein with the macromolecular milieu may facilitate its functions (1,2). Undoubtedly, the concentrated bystander macromolecules (i.e., crowders) would have significant effects on the stability, conformational ensembles, and dynamics of the protein. With high concentrations of a single species (such as hemoglobin in red blood cells and antibodies in therapeutics), self-interactions could increase the propensities of polymerization and aggregation. Quantifying these intermolecular interactions will bring us closer to elucidating biochemical processes in their native environments and improve protein therapeutics.

However, many biophysical techniques useful for dilute-solution studies are confounded by complexities of concentrated macromolecular solutions. For example, multiple scattering in light-scattering techniques and the nonlinearity of optical signals in methods employing fluorescence, absorption, or refractive index changes (e.g., in analytical ultracentrifugation) can complicate data interpretation at high sample concentrations. Fortunately, as illustrated by two articles of Goldenberg and Argyle (3,4) in this issue of *Biophysical Journal*, with small-angle x-ray and neutron scattering (SAXS and SANS), one can overcome these limitations and obtain valuable information on intermolecular interactions in concentrated protein solutions. Goldenberg and Argyle used SAXS to probe solutions of myoglobin and bovine pancreatic trypsin inhibitor (BPTI) (3), and used SANS to probe an intrinsically disordered protein (N protein of bacteriophage λ, or λ N) in myoglobin and BPTI solutions ((4), and see Fig. 1). The functions of intrinsically disordered proteins are of intensive contemporary interest, but characterizing their conformational ensembles remains a challenge even in dilute solutions. Because of their conformational malleability, these proteins are expected to be particularly susceptible to the influences of macromolecular crowding (5).

SAXS and SANS signals of a protein solution are due to interference of waves scattered from atoms in the same protein molecule as well as those from different protein molecules. They contain information about the distribution of interatomic distances over a broad range of length scales, typically from ten to hundreds of Ångstroms. Under dilute conditions, as SAXS and SANS are usually applied, the scattering profile is dominated by intramolecular interference, because individual protein molecules are far from each other. The scattering profile can be used to obtain a low-resolution structural envelope of the protein. At higher concentrations, destructive interference from intermolecular scattering centers leads to reduced scattering intensity that can be used to obtain information about deviations from solution ideality and quantitatively probe intermolecular interactions.

In their first article, Goldenberg and Argyle (3) carried out SAXS measurements at a series of concentrations up to 400 mg/mL for myoglobin and 150 mg/mL for BPTI. The departure from ideality at high protein concentrations was used to provide a measure of the second (and higher-order) virial coefficients and assess the extent of intermolecular interactions.

In the second article, the authors used SANS to probe the conformational ensembles of λ N under high concentrations of myoglobin and BPTI. One of the challenges of probing a tracer protein in the presence of crowding agents is in avoiding signals from the latter. In their SANS study, the scattering of the crowder (myoglobin or BPTI) was masked by uniformly labeling λ N with deuterium and adjusting the solution D_2O concentration to contrast match the crowding protein. This approach avoids the need for labeling the tracer protein (e.g., with a fluorophore as in Förster resonance energy transfer) and allows the use of biological crowders (i.e., proteins) instead of synthetic polymers. The SANS profile of λ N results from averages over conformations of the disordered protein, which in turn are influenced by interactions with the crowder proteins.

Meaningful interpretations of the SAXS and SANS data require theoretical models for calculating

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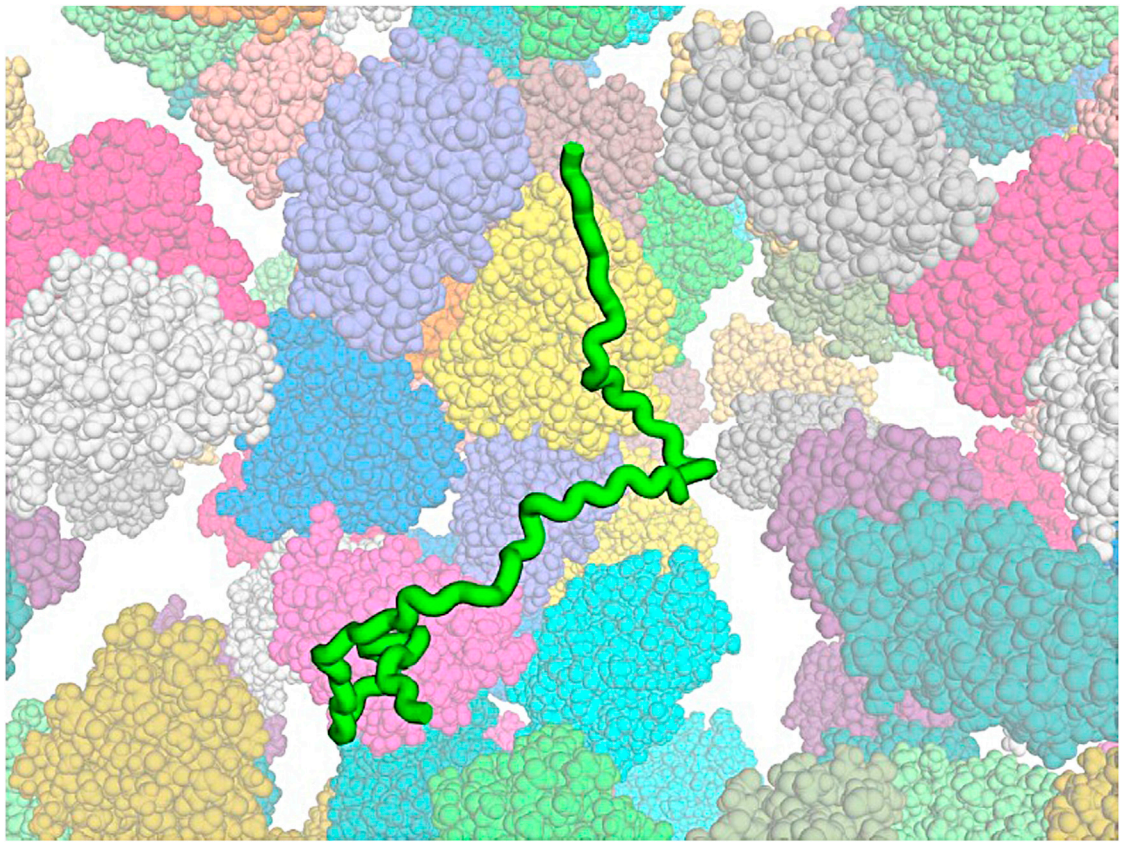

FIGURE 1 A disordered protein (*green tube*) in a concentrated myoglobin solution. To see this figure in color, go online.

intermolecular interactions. The modeling done by Goldenberg and Argyle provides a first approximation that is based on treating globular proteins as hard spheres. It will be possible to use more sophisticated modeling to fully exploit the data, thanks to two recent developments. The first is the simulation of molecular configurations in concentrated protein solutions (6,7). The second is the rapid evaluation of interactions between a tracer protein molecule and crowder molecules, based on fast Fourier transform (8).

Some specific findings of the two articles are worth noting:

1. The authors found evidence that phosphate ions may mediate electrostatic interactions between BPTI molecules. If more sophisticated modeling and further experiment confirm this mediating role, then it is indication that the weak interactions between protein molecules may be nonspecific but not necessarily random. The nonrandom nature of weak interactions between macromolecules has already been suggested in other recent studies (1,2).
2. Crowding proteins did not seem to affect the overall size of the disordered protein λ N. Other studies of disordered or unfolded proteins have uncovered evidence that crowding may induce conformational compaction (9–12). Compaction can result from repulsive interactions with the crowders, as shown by Goldenberg and Argyle (4) as well as another recent computational study (5), but such an effect could be countered by attractive interactions.

It will take additional integrated experimental and computational studies to untangle the various effects produced in concentrated macromolecular solutions and overcome challenges toward gaining insights into in vivo biochemical processes.

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