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Protein Dynamics in Simulation and Experiment

Nature has solved the problem of chemical complexity in at least two different ways: Small molecules are synthesized not unlike the way the organic chemist thinks about them, by a sequence of enzymatic chiral bond formation processes using all the tricks from modular synthesis to assembly of long polyenes into complex cyclic structures. Large molecules, such as proteins or RNAs, are instead assembled linearly from a very small number of chiral building blocks (4−20), and fold in a self-directed fashion into complex and highly organized structures (assisted by other molecular machinery in the cell to increase efficiency). Instead of highly directed covalent bonds, interactions such as π-stacking, hydrogen bonding, or entropically driven solvent exclusion guide assembly. And supramolecular chemistry has been inspired by these structures, as was covalent synthesis by small natural products before.

The very nature of many weak "bonds" makes proteins highly flexible, whereas small molecules are rigid. Flexibility is critical for the ability of proteins to fold and function: it allows an intervening chain of amino acids with a length scale resolution of 2 Å (about a methyl group) to position charges or hydrogen bond donors/acceptors with sub-angstrom accuracy at an active site, or it allows substrates to flow through fluctuating crevices and cavities in the protein.

Protein dynamics covers a wide range of time scales, from femtoseconds to hours. It involves processes from fast rearrangements following photoexcitation of a chromophore, to prolyl bond isomerization hindered by the high friction of a collapsed protein. These problems are tackled by sophisticated experimental physical chemistry techniques such as X-ray diffraction, NMR, or single-molecule fluorescence, and by sophisticated simulations, sometimes classical such as molecular dynamics, and sometimes quantum mechanical when electron or proton transfer is involved in the dynamics. Often the result is more than the sum of its parts when experiment and computation converge, with an eye kept on simple theoretical models.

This *JACS Select* collection highlights recent work published in the Journal on experiments and simulation of protein dynamics, including papers where both are fruitfully combined. The range of topics is as wide as the field of protein dynamics, and indeed, several virtual issues could have been filled with *JACS* articles on the subject from just the last few months. The publications highlighted here will serve as an illustration of this rich and active field.

The article by **Meech**, **Tonge**, and co-workers nicely illustrates the vast range of time scales in protein dynamics.[1] They study the "proteinquake" that ensues when a flavoprotein is photoactivated by a femtosecond laser pulse that replaces sunlight in the laboratory. The authors demonstrate how a mutation can interrupt the "proteinquake" as it propagates on ever longer time scales from the initial absorption site through the structure, and this interruption short-circuits protein function. Allosteric motions and transient fluctuations are very important when other molecules must get into a protein for its function, and the work by **Persson** and **Halle** analyzes a very long trajectory available from Shaw Research in the literature to simulate magnetic relaxation dispersion NMR data.[2] Their analysis shows what the simulations do well, and where they still need improvement (such as correctly flipping around water molecules that get into the protein interior). **Griesinger** and co-workers take a somewhat different approach with paramagnetic NMR, where calmodulin, a protoype two-domain calcium-sensitive switch, is tagged with paramagnetic lanthanide ions to look at interdomain dynamics.[3] The larger the protein, the more opportunity for rich dynamics, and the papers by **Lewandowski** and **Polenova** and their colleagues look at protein dynamics in two large systems.[4,5] One identifies membrane stiffness as the source for much faster protein conformational dynamics of a rhodopsin (a light-sensitive transmembrane protein), and the other tackles the dynamics of a whole viral capsid, showing that the constituent proteins in an immature capsid must actually disassemble when a signaling peptide is clipped from their tails, leading to reassembly of the capsid into its final structure.

Folding is an important aspect of protein dynamics, and in many ways it is coupled to function. Folding can be frustrated by the presence of necessary functional amino acid side chains that are not optimal for folding, but other factors also control the rate at which proteins fold. **Papoian** and co-workers discuss one of these factors, internal friction.[6] As a polypeptide chain moves about, it encounters friction from the solvent, but also from itself: proteins are large enough molecules to rub against themselves. The authors show in detail how dihedral rotation around bonds in the amino acids contribute to this internal friction. Many small motions can add up to a large effect, and this is also illustrated in the article by **Kasinath**, **Sharp**, and **Wand**, where NMR relaxation measurements are calibrated with molecular dynamics simulations so that the rotation of methyl groups in the amino acids can be used as a quantitative stand-in for protein conformational entropy.[7]

New experimental techniques are constantly coming on-line to study protein folding and protein dynamics. For example, isotope (^{13}C) editing, if cleverly employed, can provide relatively inexpensive yet detailed information on site-specific secondary structure and stability of proteins (see the work by **Kubelka** and **Kubelka**),[8] providing a probe that is useful not just in conventional infrared spectroscopy, but also for the new generation of multidimensional vibrational spectroscopy now on-line in biophysical chemistry laboratories around the world. **Gai** and co-workers, on the other hand, use conventional probes, but they initiate protein folding systematically at different starting temperatures, and then jump to the same final temperature.[9] Such tuning allows them to modulate the initial free energy landscape of the protein, thus unraveling folding mechanisms. **Muñoz**, **Kay**, and collaborators and **Ainavarapu** and co-workers illustrate two more experimental techniques that are proving very fruitful in the study of protein dynamics.[10,11] Relaxation-dispersion NMR can see transiently

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populated, partly unfolded states of proteins. Proteins are constantly in a folding−unfolding equilibrium because the price they pay for high flexibility is low stability. Partly unfolded states that a protein visits again and again during its working life could be functionally important. Single-molecule force spectroscopy, or pulling on a protein, can also reveal heterogeneity in protein unfolding, such as two- and three-state reaction paths in the unfolding of leucine binding protein. Finally, proteins themselves can become tools to study protein folding. **Davis** and **Dyer** use the WW domain from a formin binding protein (FBP) as a tool to study how secondary structure motifs behave when grafted into an "alien" protein.[12] They take the nanosecond-folding β-hairpin CLN025, and show that when it is grafted into the FBP WW domain, it can fold just as fast as when it is free.

Glassy dynamics is one of the underlying principles of modern folding theories, and such ideas can be tested computationally as well as experimentally. **Pande** and co-workers again use the WW domain.[13] They analyze molecular dynamics trajectories with Markov models, which coarse-grain protein dynamics from the atomistic molecular dynamics level to (ideally) the smallest possible number of sub-states that interconvert without having any memory of the interconversions. They identify "active" and "inactive" regions of dynamics, the latter corresponding to glassy behavior, although they do not see a clear-cut "glass transition" in a molecule as small as the WW domain. Some proteins, such as the calmodulin discussed above, visit various metastable conformations so often that we consider them intrinsically disordered proteins. Island amyloid polypeptide (IAPP) is a case in point. Misfolding of this protein results in amyloid aggregation and death in insulin-producing cells. **Huang** and colleagues use Markov models to show that IAPP visits many metastable conformations,[14] some of which are much more prone to aggregation than a more completely unfolded state. Better to be completely folded or completely unfolded, than in the no man's land in-between.

The article by **Kubarych** and co-workers combines sophisticated two-dimensional infrared spectroscopy with molecular dynamics simulations to look not at the protein, but around the protein.[15] They find that when proteins crowd one another at high density (they study a lysozyme), their aqueous solvation shells can be perturbed over distances of 3−4 nm from the protein surface. Water molecules move more collectively near crowded proteins, slowing down hydration dynamics from the usual hundreds of femtoseconds to several picoseconds. Crowding also plays a role in the article by **Gruebele** and colleagues, where protein folding is studied inside live cells as a function of the cell cycle.[16] The probe enzyme phosphoglycerate kinase is more stable in mitotic cells undergoing division than in quiescent interphase cells—and exposure to chromosomal material does not seem to be the reason.

Many of the complex processes that involve proteins in the cell are now amenable to study. The work by **Cooperman**, **Goldman**, and co-workers looks at rhythmic variations during ribosomal synthesis of proteins by single-molecule Förster resonant energy transfer (FRET).[17] Such slow-downs and speed-ups depend on which codon and hence which transfer-RNA is used for an amino acid, and could help regulate folding of proteins as they emerge from the ribosome. Another example, also using FRET, is provided by the collaboration led by **Hyeon**, **Shin**, and **Yoon**.[18] In cells, vesicles must fuse to one another or pinch off from one another during processes such as exocytosis. In some of these cases, the appropriately named SNARE proteins lock vesicles together and make sure the fusion process goes about in an orderly fashion; the report shows that SNAREs can act very cooperatively. **Schulten**, **Roux**, and co-workers use molecular dynamics to study how helices insert into membranes via the translocon.[19] Ribosomes feed the growing amino acid chain into the translocon, which then partitions the chain either into the membrane or into the cytoplasm. They say that kinetics is at the bottom of the process after all: although a quasi-thermodynamic model can fit the data, only kinetic control explains why the apparent free energy scale for membrane insertion of amino acids is compressed relative to the real equilibrium thermodynamic scale. Membranes are not the only surfaces with which proteins can interact: as an example of protein dynamics on non-biological surfaces, **Zanni** and colleagues use sum-frequency generation (SFG) to see how peptides assemble at gold surfaces, and how they orient themselves dynamically.[20] SFG of two different-colored laser beams is a classic technique in surface science, but here it illuminates peptide dynamics.

Protein dynamics is now a major subdiscipline of chemistry and biophysics, drawing on tools from chemical biology, synthesis, and instrumentation and measurement, as well as computational modeling from the highly coarse-grained to atomsitic simulations. Remarkable progress has been made in the past few years with a confluence of new molecular biology or synthetic tools (e.g., click chemistry for FRET labeling), measurement tools (e.g., 2-D infrared spectroscopy or single-molecule FRET), and modeling into the millisecond time scale and beyond. Where computation and experiment in the past covered different length and time scales most accurately, there is now a strong overlap, with simulations providing unprecedented structural insight, and experiments able to validate simulations more directly than ever before.

Martin Gruebele, Associate Editor

AUTHOR INFORMATION

Notes

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