large ensemble of emitters near a metallic nanoparticle. The preferential emission into a single plasmonic mode also makes possible the concept of a spaser, an amplifier and coherent generator of plasmons (*7*). To understand plasmons in complex nanoparticle architectures for spasers or other applications such as single-molecule detection, it is important to incorporate a quantum-mechanical model of the electron density in the analysis. Effects such as tunneling of electrons between coupled nanostructures can considerably affect the nature of plasmons (*8*). Such a microscopic approach will be critical to develop a complete understanding of quantum plasmonics.

Efforts in quantum optics have been directed toward overcoming decoherence effects and achieving scalability of quantum bits (qubits) for practical applications. One approach is to achieve parallelism and communication between quantum bits of different nature (e.g., spin qubits and photonic qubits; akin to our current use of optoelectronics for computation and communication). The nitrogen-vacancy (NV) center in diamond is a promising choice for the robust solid-state quantum bit because it can show single-photon emission as well as long spin coherence times (*9*). The ability to make these two degrees of freedom interact rests on efficient single-photon emission beyond that available in bulk diamond NV centers. Resonant cavity approaches to enhancing the optical emission are incompatible with these sources, which have a broad emission spectrum. The broadband enhancement of spontaneous emission enabled by nanoplasmonic approaches allows the possibility of coupling to such emitters, which was otherwise difficult to achieve by conventional quantum optical techniques (*10*).

Another unorthodox approach of enhancing the nanoscale light-matter interaction in a broad bandwidth is to provide the quantum emitter with a plethora of electromagnetic states (*11*). Current nanofabrication technologies allow the engineering of the dielectric constant with metamaterials, transforming the space perceived by light to be metallic in one direction and dielectric in another. This lifts the restriction on the well-known closed spherical dispersion relation of an isotropic medium into a hyperboloid, leading to electromagnetic states unique to the metamaterial (*12*, *13*). An infinite number of metamaterial states can lie on this hyperboloid (in the low-loss, effective-medium limit), increasing the interaction with the quantum emitter while simultaneously channeling the light into a subdiffraction single-photon resonance cone (*12*) (see the figure, panel B). Currently, losses present a formidable challenge to practical applications, but the new class of alternate plasmonic materials can lead to quantum-vacuum engineered devices with these "hyperbolic" metamaterials (*14*).

The future of nanophotonics is bright, with many possibilities of interfacing with quantum optics to address challenges of qubit scalability and communication. One topic to be addressed in the near future is single-photon switching and routing. Single photons do not talk to each other, but efforts are under way to use plasmon-mediated interactions for this purpose (*15*). It is quite likely that the hybrid excitation that combines photons and electrons will be the carrier of choice in future quantum information systems.

References and Notes

1. M. L. Brongersma, V. M. Shalaev, *Science* **328**, 440 (2010).
2. A. V. Akimov *et al*., *Nature* **450**, 402 (2007).
3. R. Kolesov *et al*., *Nat. Phys.* **5**, 470 (2009).
4. S. Fasel *et al*., *Phys. Rev. Lett.* **94**, 110501 (2005).
5. A. Huck *et al*., *Phys. Rev. Lett.* **102**, 246802 (2009).
6. E. Altewischer *et al*., *Nature* **418**, 304 (2002).
7. D. J. Bergman, M. I. Stockman, *Phys. Rev. Lett.* **90**, 027402 (2003).
8. J. Zuloaga *et al*., *Nano Lett.* **9**, 887 (2009).
9. I. Aharonovich *et al*., *Nat. Photonics* **5**, 397 (2011).
10. A. Huck *et al*., *Phys. Rev. Lett.* **106**, 096801 (2011).
11. Z. Jacob *et al*., http://arxiv.org/abs/0910.3981 (2009).
12. Z. Jacob *et al*., *Appl. Phys. B Lasers Opt.* **100**, 215 (2010).
13. M. A. Noginov *et al*., *Opt. Lett.* **35**, 1863 (2010).
14. A. Boltasseva, H. A. Atwater, *Science* **331**, 290 (2011).
15. D. E. Chang *et al*., *Nat. Phys.* **3**, 807 (2007).

16. This work was supported in part by Army Research Office–Multidisciplinary University Research Initiative (ARO-MURI) grant W911NF-0910539, ONR-MURI grant N00014-010942, and Natural Sciences and Engineering Research Council of Canada Discovery grant 402792. Z.J. acknowledges input from W. Newman.

10.1126/science.1211736

BIOCHEMISTRY

How Proteins Fold

Computational and experimental results provide support for defined protein folding pathways.

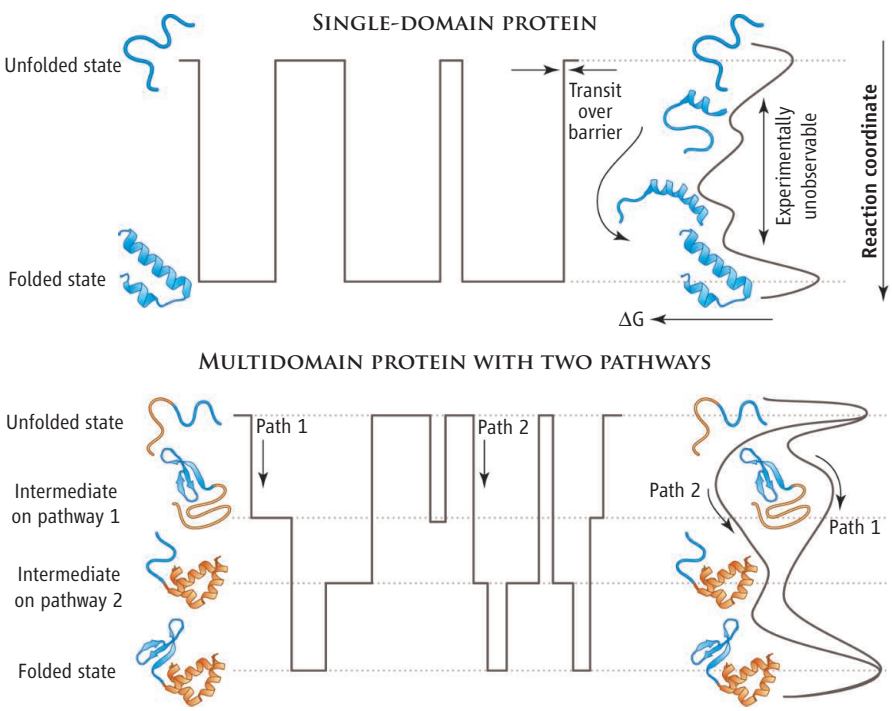
Tobin R. Sosnick[1] and James R. Hinshaw[2]

Two reports in this issue probe single protein chains as they spontaneously unfold and refold. On page 517 of this issue, Lindorff-Larsen *et al*. (*1*) use state-of-the-art molecular dynamics (MD) simulations to elucidate the folding mechanisms of 12 different proteins. On page 512 of this issue, Stigler *et al*. (*2*) study the folding and unfolding of single calmodulin domains with single-molecule force spectroscopy. The results provide remarkable views of the folding process and address basic questions, such as whether proteins fold along pathways.

[1]Department of Biochemistry and Molecular Biology, Institute for Biophysical Dynamics, Computation Institute, Chicago, IL 60637, USA. [2]Department of Chemistry, University of Chicago, Chicago, IL 60637, USA.

The Shaw group previously succeeded in modeling the folding of a 35-residue protein in the presence of water molecules (*3*). Lindorff-Larsen *et al*. now show that these methods are suitable for probing the folding of larger, more complex proteins. In addition to matching experimental folding rates, the authors obtain native-like models for 12 proteins, which contain helices and sheets and are up to 80 residues long. For most of the proteins, the trajectories contain discrete transitions between the native and unfolded states. This behavior is consistent with barrier-limited cooperative folding, the hallmark of the experimental folding reaction.

Whether folding occurs along a diverse set of routes elicits diverse opinions, with many researchers favoring extensive pathway heterogeneity due to the complexity of the system (*4*). Yet, Lindorff-Larsen *et al*. find that for nine of their proteins, heterogeneity is minimal, with the routes typically sharing over 60% of their native contacts. They conclude that these routes are best viewed as variations of a single folding pathway. This lack of pathway diversity is consistent with experimental studies where transition state heterogeneity was not observed (*5*). However, in the simulations of two β sheet–containing proteins, the order of strand formation can vary. For a protein G variant, the observation of two pathways is consistent with experimental work, which found that the folding order of the two hairpins can be manipulated (*6*). Generally, symmetric (*7*, *8*) and multidomain proteins are strong candidates for multiple pathways, because different portions can form first.



Folding of one- and two-domain proteins. (**A**) Lindorff-Larsen *et al*. (*1*) show that the spontaneous folding and unfolding of a single-domain protein is best described by a single pathway for most proteins studied. (**B**) For a two-domain protein, there are two pathways, reflecting the presence of two intermediates with one or the other domain folded. This is the behavior found by Stigler *et al*. (*2*) for calmodulin, although its two domains are of similar size and the steps are thus of similar length. In addition, the authors observe two non-native intermediates.

Stigler *et al*. observe this behavior in their study of calmodulin, a protein composed of two nearly identical domains. They use ultrastable high-resolution optical tweezers to pull the protein's ends to a particular tension level at which the protein repeatedly folds and unfolds. With this powerful protocol, they simultaneously track changes in the extension and force to provide two reaction coordinates in a single measurement.

Stigler *et al*. detect a limited set of pathways and four intermediates. Either domain can fold independently, accounting for two intermediates; each domain folds in a single kinetic step, as found by Lindorff-Larsen *et al*. for single-domain proteins. The third, off-pathway intermediate combines half of each subdomain. The fourth intermediate contains one domain plus part of the other in an unproductive conformation. The identification of defined intermediates along with their interconversion rates and stabilities highlights the power of single-molecule techniques.

Both studies (*1*, *2*) observe sharp folding transitions indicative of a free energy barrier between well-defined thermodynamic states. Although a folding time constant may be milliseconds, the transit time over the barrier is much faster, generally within less than a microsecond (*1*, *3*). This time range is out of reach for current experimental single-molecule methods (*9*), but MD simulations provide detail at the nanosecond level.

Lindorff-Larsen *et al*. find that during the barrier crossing, the long-range contacts that establish the protein's overall fold form early along with a considerable amount of secondary structure and surface burial. This amount of residual structure is higher than suggested by experimental studies for small proteins where the denatured state is largely unstructured (*10*), although this issue is not fully resolved (*5*). This difference could be a result of inadequate energy functions, which generally are calibrated on folded proteins. The presence of residual structure could affect the folding mechanism observed in a simulation.

Nevertheless, notable agreement exists between the simulations and experimental studies beyond those already noted. Native-state hydrogen-exchange studies have shown that folding is not completely cooperative but is punctuated by small-scale events reflecting the stepwise addition of secondary structural elements termed "foldons" (*11*). Because these elements often are intrinsically unstable, low-energy pathways are likely to involve foldons building on top of existing structures in a process of sequential stabilization (*12*). Thermodynamic biases and topological considerations, including chain connectivity, imply that some structures are more populated and can serve as better templates for additional foldons, resulting in preferred folding routes. Ample evidence exists for this process late in the pathway, and it may also apply to the early portion (*13*).

The simulations of Lindorff-Larsen *et al*. appear consistent with this mechanism of sequential stabilization. For most proteins, the sequence of structure formation is the same in all pathways and correlates with unfolded state structural propensities. This behavior is observed despite the high level of residual structure, suggesting that the templating process with only a few low-energy routes is a robust phenomenon.

Non-native structure has minimal influence on the pathway. If non-native contacts are also insignificant, the widespread use of Go-models [which assume that only native-like contacts are kinetically relevant (*14*)], would be justified. Such models would, however, need to be modified (*15*) to apply to calmodulin because of the two non-native intermediates found by Stigler *et al*.

Currently, it is extremely difficult to compare MD simulations and experimental data for the species on the high-energy portion of the reaction surface. Obtaining experimental information about these transiently populated intermediates requires the development of new experimental methods. Computationally, larger proteins may become foldable as computing power increases, but issues may arise due to minor inaccuracies in the energy functions, and the presence of multiple and possibly non-native minima, as observed by Stigler *et al*. and in de novo structure prediction (*16*). Hopefully, progress on these fronts will be forthcoming.

References

1. K. Lindorff-Larsen *et al.*, *Science* **334**, 517 (2011).
2. J. Stigler, F. Ziegler, A. Gieseke, J. C. M. Gebhardt, M. Rief, *Science* **334**, 512 (2011).
3. D. E. Shaw *et al.*, *Science* **330**, 341 (2010).
4. M. O. Lindberg, M. Oliveberg, *Curr. Opin. Struct. Biol.* **17**, 21 (2007).
5. T. R. Sosnick, D. Barrick, *Curr. Opin. Struct. Biol.* **21**, 12 (2011).
6. S. Nauli *et al.*, *Nat. Struct. Biol.* **8**, 602 (2001).
7. D. K. Klimov, D. Thirumalai, *J. Mol. Biol.* **353**, 1171 (2005).
8. T. Shen *et al.*, *Biochemistry* **44**, 6433 (2005).
9. H. S. Chung, J. M. Louis, W. A. Eaton, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 11837 (2009).
10. M. C. Baxa *et al.*, *J. Mol. Biol.* **381**, 1362 (2008).
11. Y. Bai, T. R. Sosnick, L. Mayne, S. W. Englander, *Science* **269**, 192 (1995).
12. H. Maity, M. Maity, M. M. Krishna, L. Mayne, S. W. Englander, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 4741 (2005).
13. B. A. Krantz, R. S. Dothager, T. R. Sosnick, *J. Mol. Biol.* **337**, 463 (2004).
14. S. Takada, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 11698 (1999).
15. S. Yang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 13786 (2004).
16. J. Moult, K. Fidelis, A. Kryshtafovych, B. Rost, A. Tramontano, *Proteins* **77** (suppl. 9), 1 (2009).

10.1126/science.1214018