Matching Protein Experiments to Simulations

By Stu Borman

FAST UNFOLDING

This molecular dynamics simulation depicts a titin module as it is stretched out.

Credit: Klaus Schulten/UIUC

The detailed workings of biomolecules as they generate and respond to mechanical forces can be analyzed effectively with computer simulations and experimental techniques. When examining things such as the way proteins unfold when muscles stretch, the two quite different approaches could complement one another. But the techniques up to now have worked on different timescales—experiments are slower—making it hard to combine their results.

A technique called high-speed force spectroscopy now boosts experimental speed, allowing protein-unfolding information from simulations and experiments to be combined directly for the first time.

The method was developed by Simon Scheuring of the French National Institute of Health & Medical Research (INSERM) at Aix-Marseille University and coworkers (*Science* 2013, DOI: 10.1126/science.1239764). The researchers demonstrated high-speed force spectroscopy on titin, a multidomain, springlike protein that unfolds to let muscles extend from a flexed position.

[+]Enlarge

Light from a superluminescent light-emitting diode (SLED) is focused on a cantilever and detected by a photodiode to measure forces on a titin octamer as it is pulled between the cantilever and a stationary support.

Credit: Adapted from *Science*

“High-speed force spectroscopy will enhance experiment-simulation complementarity,” says computational biophysicist Klaus Schulten of the University of Illinois, Urbana-Champaign (UIUC). Development of the new technique “is an exciting milestone,” adds Julio M. Fernandez, a force spectroscopy pioneer at Columbia University.

Molecular dynamics calculations on all the atoms in a protein such as titin are so computationally demanding that even today’s most powerful computers can simulate unfolding only for vanishingly brief moments. Such simulations generate movies of the molecular details of protein unfolding by pulling proteins apart at speeds of a few millimeters per second. Experiments that use atomic force microscopy (AFM) or optical tweezers to measure energies and distances when proteins are pulled apart physically can operate only at speeds of about 100 nm/second at best—three to four orders of magnitude more slowly.

The new technique boosts experimental speed to 4 mm/second, moving experimentation into the same time regime as simulations. Key to the faster speed is a shortened AFM cantilever, a tiny probe that pulls proteins attached to it when it is deflected. The shortened cantilever moves faster and pulls tethered proteins more quickly than conventional ones.

“There has been a lingering doubt about how much molecular dynamics simulations can really tell us because of the huge mismatch in timescale relative to experiments,” comments UIUC’s Taekjip Ha, an expert in single-molecule

imaging and manipulation. Thanks to the new work, Ha says, agreement between simulation and experiment is now “impressive and deeply satisfying.”

The technique can analyze how proteins respond to forces over a range of speeds, yielding what is called an “unfolding energy landscape.” It should also be useful for studying other force-related biological processes such as lipid-membrane dynamics and receptor-ligand unbinding, the researchers note.

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