University of Wisconsin-Milwaukee

Dept. of Physics
COLLOQUIUM

Watching Individual Proteins Unfold and Refold Using 1-μs Resolution Force Spectroscopy

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3:30 PM (coffee/cookies at 3:15 PM)

Lapham Hall – Room 160

Protein folding occurs as a set of transitions between structural states within an energy landscape. An oversimplified view of the folding process emerges when transiently populated states are undetected because of limited instrumental resolution. To achieve state-of-the-art performance, we integrated several recent technical advances that improve the precision, stability, and accuracy of AFM-based single molecule force spectroscopy. Using modified cantilevers optimized for 1-μs resolution, we reexamined the unfolding of individual bacteriorhodopsin (bR) molecules in native lipid bilayers. The experimental data revealed the unfolding pathway in unprecedented detail. Numerous newly detected intermediates—many separated by as few as 2–3 amino acids—exhibited complex dynamics, including frequent refolding and state occupancies of <10 μs. Equilibrium measurements between such states enabled the folding free-energy landscape to be deduced. These results sharpen the picture of the mechanical unfolding of bR. Finally, recent efforts to improve the quantity and quality of AFM studies of diverse biomolecules, including nucleic-acid structures and globular proteins, will be discussed.

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