Special Research Seminar

日時 ： 2022年9月7日（水） 午後 2 時 - 3 時

場所 ： 理学館506講義室

Probing the energetics and hidden dynamics of bacteriorhodopsin by single-molecule force spectroscopy

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The forces and energetics that stabilize membrane proteins remain elusive to precise quantification. Single-molecule force spectroscopy can yield kinetic rate constants, energetics, intermediate states, unfolding pathways, and even a projection of the underlying free-energy landscape. Using recently developed focused-ion-beam (FIB) modified cantilevers[1], we reexamined the unfolding of individual molecules of bacteriorhodopsin (bR) embedded in its native lipid bilayer with a 100-fold improvement in time resolution and a 10-fold improvement in force precision [2]. 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. We next integrated these technical advances with site-specific covalent coupling of bR to an ing of bR [3]. The resulting records revealed rapid near-equilibrium dynamics between three states spanning a mere 8 amino acids. The third of these states corresponded to Lys216, where bR's retinal is covalently bound. Dynamic force spectroscopy revealed this previously unobserved state was retinal-stabilized and, indeed, the most mechanically robust state in bR's extensively characterized unfolding pathway. These rapid and reversible dynamics allowed us to measure the equilibrium energetics of a membrane protein in its native lipid bilayer [4,5], an advance over traditional results obtained by chemical denaturation in nonphysiological mixed micelles. Finally, the metrological enhancements enabled by FIB-modified cantilevers are not limited to force spectroscopy but are broadly applicable across diverse AFM studies.

References

[1] D. T. Edwards & T. T. Perkins. Optimizing force spectroscopy by modifying commercial cantilevers: improved stability, precision, and temporal resolution. J. Struct. Biol.. 197, 13 (2017).

[2] H. Yu, et al., Hidden dynamics in the unfolding of individual bacteriorhodopsin proteins. Science 355, 945 (2017).

[3] H. Yu, et al., Quantifying the initial unfolding of bacteriorhodopsin reveals retinal stabilization. Angew. Chem. Int. Ed. 58, 1710 (2019).

[4] H. Yu, et al., Quantifying the native energetics stabilizing bacteriorhodopsin by singlemolecule force spectroscopy. Phys. Rev. Lett. 124, 068102 (2020).

[5] D. R. Jacobson & T. T. Perkins, Free-energy changes of bacteriorhodopsin point mutants measured by single-molecule force spectroscopy. PNAS 118, e2020083118 (2021).

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