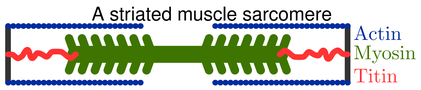
Experimental Estimation of the Free Energy Landscape Roughness of Protein Molecules

W. Trevor King, Guoliang Yang
Department of Physics, Drexel University

Motivation

Understanding a protein's free energy landscape is important to efficiently modeling protein folding behaviors. Due to polymer nature of the polypeptides, the protein folding free energy landscape is rugged. Knowledge of this roughness can enhance our understanding of the protein folding/unfolding mechanisms. We experimentally probed the free energy landscape for the muscle protein Titin domain I27, using single-molecule mechanical unfolding using an atomic force microscope. The unfolding forces of I27 were measured as a function of temperature and unfolding rate. These measurements not only provide information on the roughness of the proteins energy landscape, but also yield possible molecular mechanisms for determining how muscle stiffness changes with temperature.



Theory

We have a well defined reaction coordinate in the protein's end-to-end distance x. For Titin, which is naturally tethered at both ends, this reaction coordinate has the added benefit of direct biological significance. The free energy $F(x)$ is given by $F(x) = F_0(x) + F_1(x)$, where $F_0(x)$ is the smooth free energy funnel, and $F_1(x) \sim \varepsilon$ is the local roughness in free energy. The frustration $F_1(x)$ serves to limit the diffusion of proteins on the free energy landscape, increasing the force or time necessary to unfold the protein.

We measure the most common unfolding force f^* under a range of loading rates at two temperatures T_1 and T_2. We then estimate ε using

$$\varepsilon^2 \approx \frac{k_b^2 T_1 T_2}{\Delta x(T_1) T_1 - \Delta x(T_2) T_2} \cdot \left[\Delta x(T_2) T_1 \log\left(\frac{r_f(T_1)\Delta x(T_1)}{k_0(T_1) k_B T_1}\right) - \Delta x(T_1) T_2 \log\left(\frac{r_f(T_2)\Delta x(T_2)}{k_0(T_2) k_B T_2}\right)\right]$$

where $r_f(T_1)$ and $r_f(T_2)$ are loading rates selected from the data such that

$$f^*(r_f(T_1), T_1) = f^*(r_f(T_2), T_2),$$

$k_0(T)$ is the unfolding rate without applied force, and $\Delta x(T)$ is the distance between the bound state and the transition state[1][2]. Note that the sign of the prefactor is different from Nevo's Eqn. 2 and that there is no explicit $\Delta F^{\ddagger}$ term.

We estimate $k_0(T)$ and $\Delta x(T)$ by fitting measurements at a single temperature to

$$f^* = \frac{k_B T}{\Delta x(T)} \ln\left(\frac{r_f \Delta x(T)}{k_0(T) k_B T}\right),$$

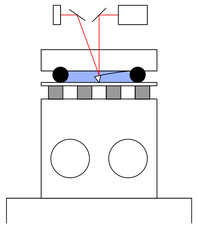
which is a simplified form of Hyeon Eqn. 8[1].

Method

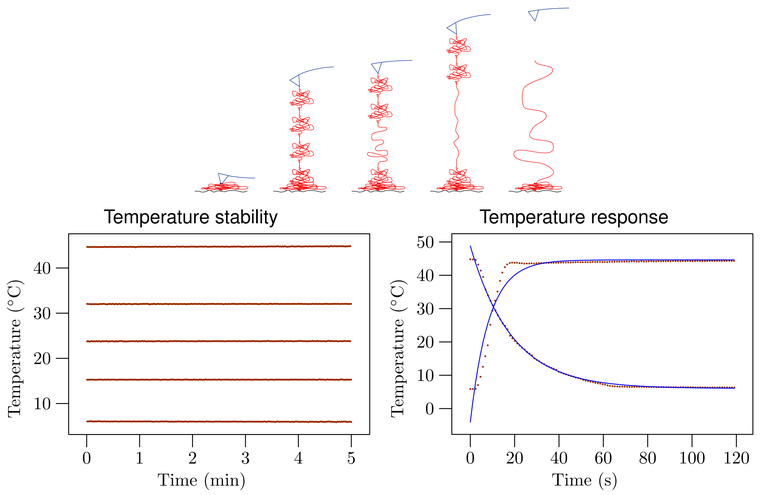
Titin I27 octomers have a Cysteine capped end bound to a gold substrate (AthenaES, Baltimore MD). The proteins are mechanically unfolded by pulling one end with the AFM cantilever (DI, Santa Barbara, CA). Clean unfolding curves were determined by visual inspection and fitted to a worm-like chain model[4]. Unfolding curves were taken at 6 and 45°C, at pulling speeds ranging from 0.37 to 5 μm/s. The cantilever was calibrated using the equipartition method[5] with a measured photodiode sensitivity of $\sigma_p \approx 6.25$ mV/nm.

The software controlling the experiment and analyzing the data was written primarily in Python and C. Analog I/O was via a National Instruments PCI card with Comedi drivers.

Temperature Control



The substrate temperature is controlled using a water-cooled Peltier thermoelectric device[3]. A commercial thermoelectric controller (Melcor, Trenton, NJ) monitors the sample temperature using a thermocouple and powers the Peltier as required to maintain a set temperature. The temperature control system is stable to ~ 0.05°C (max. range 0.25°C over 5 minutes) and responds to setpoint changes on a timescale of 20 seconds.
Note that the temperature response time is the same order of magnitude as the system dead-time, which is ~ 4 s, and is just visible in the temperature response figure. Because of this, the response time cannot be reduced much below the current level without overdriving.



Analysis

Selecting $f^* = 160$ pN, we extract $T_1 \approx 45.7$°C, $T_2 \approx 6.0$°C, $v_1 \approx 2.80$ μm/s, $v_2 \approx 0.499$ μm/s, $k_0(T_1) \approx 112$ 1/s, and $k_0(T_2) \approx 38.7$ 1/s, and $\Delta x(T_1) \approx 1.11$ Å. $\Delta x(T_2) \approx 0.854$ Å, so $r_f(T_1) = k v_1 \approx 252$ nN/s, and $r_f(T_2) = k v_2 \approx 44.9$ nN/s. The k_0 approximations are off by $\mathcal{O}(10^4)$ from traditional estimates of $k_0 = 3.3 \cdot 10^{-3}$ 1/s[6].

With these the strange k_0 and Δxs we estimate

$$\varepsilon \approx 1.16 k_B T_{avg},$$

which seems reasonable.

Using the traditional $T = 25$°C $k_0 = 3.3 \cdot 10^{-3}$ 1/s and $\Delta X = 2.5$ Å[6], we estimate loading rates of $r_f(T_1) = k v_1 \approx 9.24$ nN/s, and $r_f(T_2) = k v_2 \approx 1.65$ nN/s, and estimate $\varepsilon \approx 4.8 k_B T_{avg}$, so the measurement seems insensitive to k and Δx.

Conclusions

Mechanical unfolding experiments can provide an estimate of the energy landscape roughness of protein molecules. For I27, the energy landscape roughness was determined to be on the order of $k_B T$. However, the measurements are still preliminary and more data will be acquired to provide a more reliable measure of r_1 and r_2, as well as allow several different methods of estimating k_0 and Δx.

We would like to compare the results of estimating k_0 and Δx using Monte Carlo simulations of the $f^*(r_f)$[4] and the width of the unfolding force distribution for a given temperature and loading rate[6], as well as extrapolation and fitting[2][7].

All estimates would also be improved by adding data over a longer range of pulling speeds. Reducing our signal noise and drift would allow measurements extending into slower pulling speeds. Other groups have acquired clean data at pulling speeds an order of magnitude smaller than our minimum[6][4], so speeds at least that slow are achievable.

To extend our measurements into higher pulling speeds, a better understanding of the hydrodynamic effects on the AFM cantilever is required. We are carrying out parallel experiments attempting to characterize these effects on our system.

References

[1] C. Hyeon and D. Thirumalai. *Can energy landscape roughness of proteins and RNA be measured by using mechanical unfolding experiments?* PNAS **100**, 18 10249-10253 (2003).

[2] R. Nevo, V. Brumfeld, R. Kapon, P. Hinterdorfer, and Z. Reich. *Direct measurement of protein energy landscape roughness.* EMBO Rep. **6**, 482-486 (2005).

[3] Y. Yang, F. Lin, and G. Yang. *Temperature control device for single molecule measurements using the atomic force microscope.* Rev. Sci. Instrum. **77**, 063701 (2006).

[4] M. Rief, M. Gautel, F. Oesterhelt, J. Fernandez, and H. Gaub. *Reversible Unfolding of Individual Titin Immunoglobulin Domains by AFM.* Science **276**, 1109 (1997).

[5] J. Hutter and J. Bechhoefer. *Calibration of atomic-force microscope tips.* Rev. Sci. Intrum. **64**, 3342 (1993).

[6] M. Carrion-Vazquez, A. Oberhauser, S. Fowler, P. Marszalek, S. Broedel, J. Clarke, and J. Fernandez. *Mechanical and chemical unfolding of a single protein: A comparison.* Proc. Natl. Acad. Sci. USA **94**, 3694-3699 (1999).

[7] Y. Sheng, S. Jiang, and H. Tsao. *Forced Kramers escape in single-molecule pulling experiments.* J. Chem. Phys. **123**, 091102 (2005).

Biophysical Society Annual Meeting, February 2008, Long Beach, California