

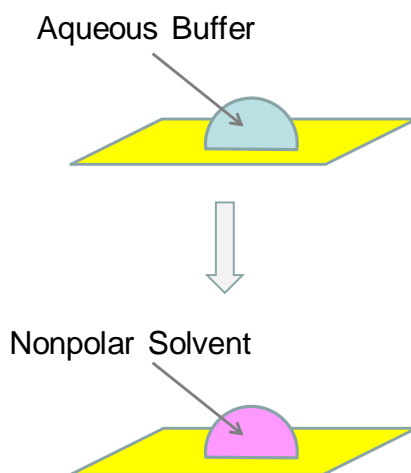
Supporting Information to
**Protein Denaturation at the Single-molecule Level: The Effects
of Nonpolar Environment and Its Implications to the
Unfolding Mechanism by Proteases**

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Scheme S1. Environment change for the SMFS experiments.

The details of QM-WLC model and the process of the generation of a fitting curve

Recently, the inherent single-chain elasticity of a peptide, *i.e.*, the relationship between the force (F) and the contour length of a subunit with F , has been investigated by *ab initio* quantum mechanics (QM) calculations.¹ Since the changes of bond angle and bond length are already considered in the calculations on one subunit, the result can be simply rewritten to describe the whole polymer chain. The chain elasticity is nonlinear, which can be expressed in a polynomial expansion to provide the basis for a numerical fit of the measured force curves:

$$F = \sum_{n=1}^2 \gamma_n \left(\frac{L[F]}{L_0} - 1 \right)^n \quad \gamma_1 = 27.4 \text{ nN}, \gamma_2 = 109.8 \text{ nN} \quad (\text{S1})$$

where γ_1 is the linear elastic modulus, and γ_2 is a non-linear correction, which will be important in the high force region, $L[F]$ is the contour length of the macromolecule upon stretching with F , L_0 is the contour length at zero force.

The worm-like chain (WLC) model² is often used to describe the single-chain elasticity of proteins:

$$F \frac{l_p}{k_B T} = \frac{R}{L[F]} + \frac{1}{4(1-R/(L[F]))^2} - \frac{1}{4} \quad (\text{S2})$$

where R is the end-to-end distance of a polymer chain, l_p is the persistence length, k_B is the Boltzmann constant, and T is the temperature in the Kelvin scale.

In order to integrate the peptide elasticity from QM calculations into the WLC model, L_0 is introduced into the model as below:

$$F \frac{l_p}{k_B T} = \frac{R/L_0}{L[F]/L_0} + \frac{1}{4(1-(R/L_0)/(L[F]/L_0))^2} - \frac{1}{4} \quad (\text{S3})$$

where R/L_0 is the normalized extension of a polymer chain.

During the elastic stretching of a single polymer chain, the value of $L[F]/L_0$, starting from 1, increases with the increasing of F . Therefore, the $L[F]/L_0$ is a monotonic increasing function of F and vice versa. During the elongation of the polymer chain, $L[F]/L_0$ is an ergodic value ranging from 1 to a number corresponding to the rupture of the polymer bridge. Here, we utilize the strength of a typical covalent bond as the upper limit for the stretching force, *e.g.* 2000 pN.³ Thus, the upper limit for $L[F]/L_0$ is about 1.06, according to Eq S1. In the range from 1 to 1.06, any value of $L[F]/L_0$ is reasonable and corresponds to a mapping value of F in the fitting curve, which can be calculated with Eq S1. Since $L[F]/L_0$ is an ergodic value in the proper range (1–1.06 in this study), the model now has only one free parameter left (l_p).⁴

For a given value of l_p , the fitting curve can be generated as described below. In the range of 1 – 1.06, any value of $L[F]/L_0$ will correspond to a point in the force curve. For this point, F can be calculated with Eq S1.

Let $x = \frac{R/L_0}{L[F]/L_0}$, then we have a new form of the QM-WLC model³ as shown below:

$$F \frac{l_p}{k_B T} = x + \frac{1}{4(1-x)^2} - \frac{1}{4} \quad (\text{S4})$$

The value of $F \frac{l_p}{k_B T}$ can be calculated readily. By solving the cubic equation, x can be obtained, and then R/L_0 can be obtained, since $L[F]/L_0$ is a known value. The pair values of F and R/L_0 correspond to one point in the fitting curve. In this way, the whole fitting curve can be generated when the value of $L[F]/L_0$ varies from 1 to 1.06 (see the dotted line in Fig. 4 in the manuscript). The modified WLC model (Eq S3), which are integrated with the QM results, are called QM-WLC model.

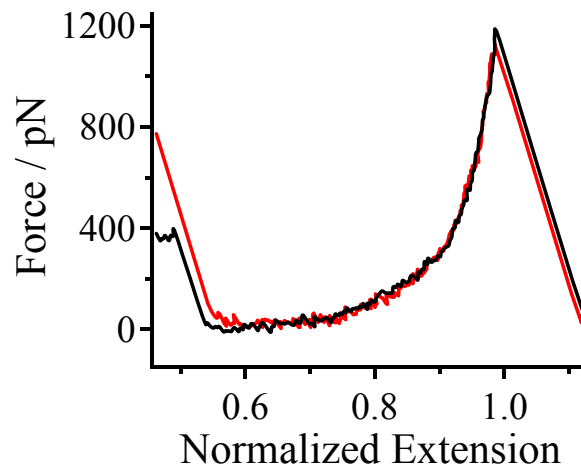


Fig. S1. Normalized F-E curves of polylysine obtained in PBS solution (red line) and I27_s obtained in 6M GdnHCl solution (black line).

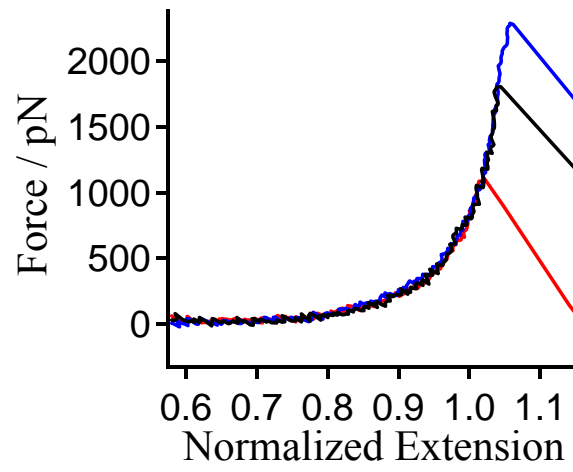


Fig. S2. Normalized F-E curves of polylysine obtained in PBS solution.

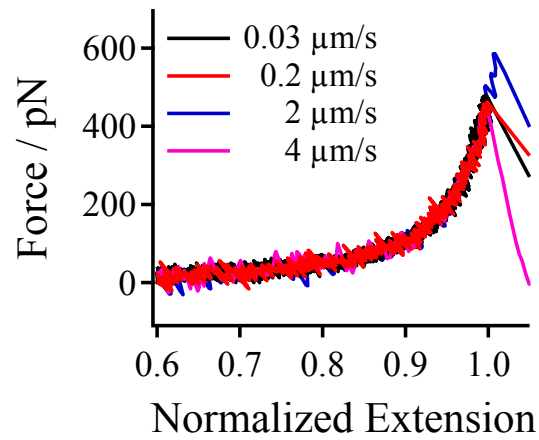


Fig. S3. Normalized F-E curves of I27₈ obtained in octylbenzene at different stretching velocity.

Partial conformation change of one titin domain in octane observed by MD simulation by placing the protein directly into octane and equilibrate the system

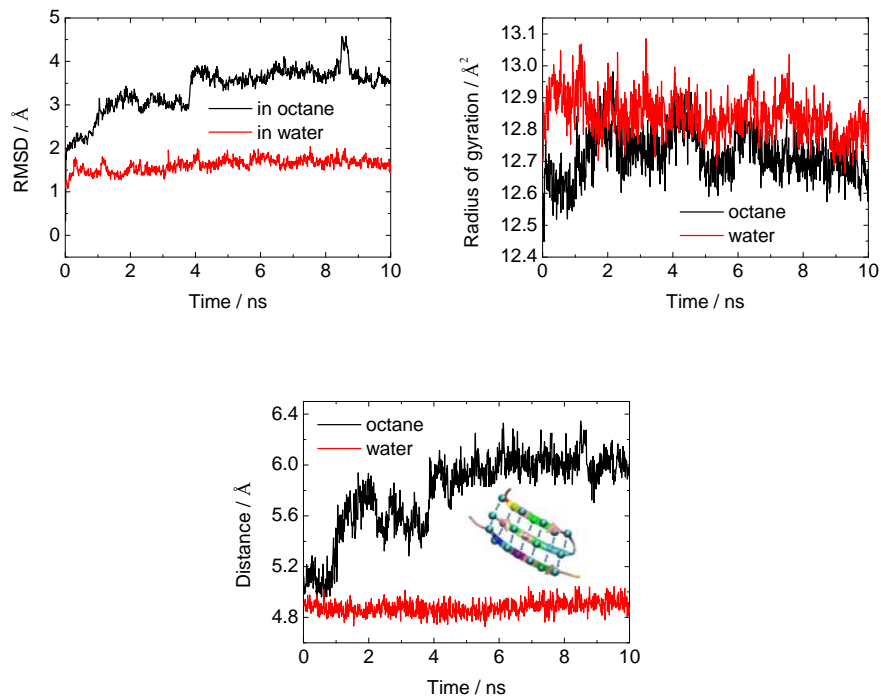


Fig. S4. Root-mean-square-deviation (RMSD) and Radius of gyration (R_{gy}) determined using all the C_{α} atoms in the protein from the pulling trajectory. The starting crystal structure was used as the reference for calculating the RMSD. The average distance between β -pleated sheets (residues 46~52, 55~61, 18~24) is measured to monitor local structure change.

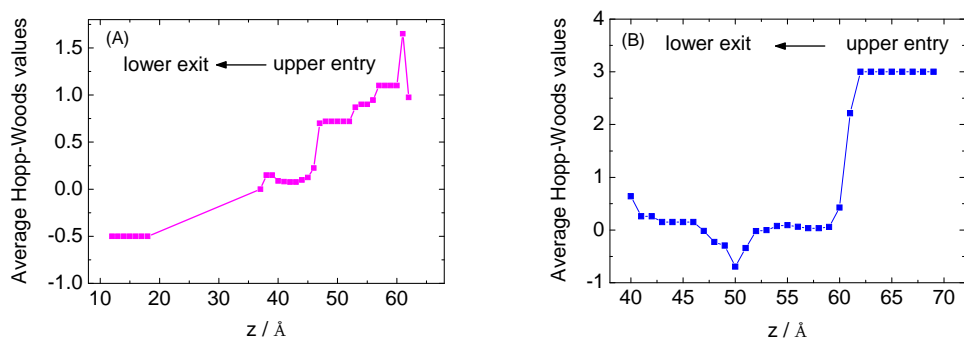


Fig. S5. Average hydrophilicity values (Hopp-Woods index) along the central axis inside A) PAN/20S (PDB ID: 3IPM) and B) HsIU (PDB ID: 1G3I). The calculation was done for amino acids within 0.8 nm of the central axis of the ring. The protein entry is located on the right side. A low average Hopp-Woods value is indicative of a high hydrophobicity.

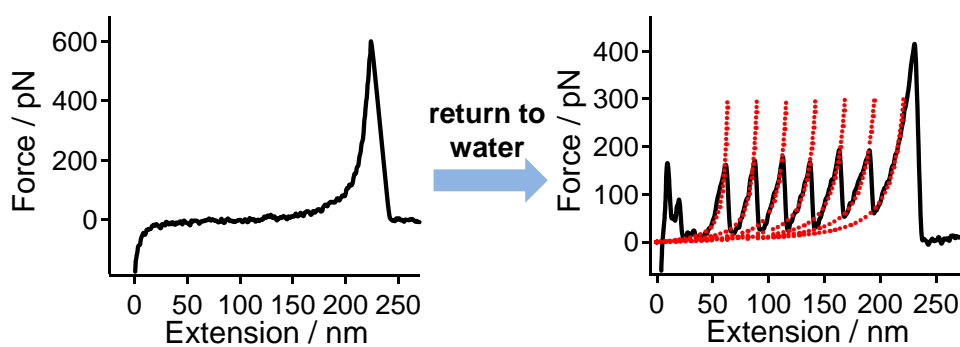


Fig. S6. The obtained F-E curves of I27_s by changing the environment from octylbenzene (left) to PBS buffer (right).

References

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