Supporting Information for "SpyTag/SpyCatcher Tether as a Fingerprint and Force Marker for Single-Molecule Force Spectroscopy Experiments"

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Materials and Methods

Proteins Expression

Bacteria cells of E. coil BL21 strain were transformed with pQE80L plasmid with insert of His6-AviTag-GB1$_8$-SpyTag and BirA plasmid. The cells grew at 37 °C in LB medium for 6-8 h. After the optical density (OD) of bacterial suspension reaches 0.8, protein began expressing and lasted for 10 h at 20 °C with 0.5 mM IPTG in medium. The protein was harvested by centrifugation at a speed of 5000 rpm and then resuspended in lysis buffer. The cells were sonicated on ice for 7 minutes, then centrifuged at 12000 g for 40 min at 4 °C. The proteins were purified by Ni-NTA resin then stored in lysis buffer with 5 mM β-ME at -80 °C.

Single Molecule Measurement

Home-made magnetic tweezers setup was built on an inverted optical microscope (IX73,Olympus). Detailed setup can be found in our previous publication[1]. We made flow channel by sandwiching a piece of APTES-functionalized coverslip and another piece of coverslip with parafilm in between and then flowed polybead Amino Microspheres (cat. 17145, Poly-sciences) with diameter of 3.0 μm into chamber to get

them stuck on the coverslip. Rinsing the chamber by 200 μL PBS buffer was followed by flowing 1% Sulfo-SMCC (SE 247420, Thermo Science) in DI water into the chamber and incubated for 20 min. After that, N-SpyCatcher protein in PBS was flowed into chamber and incubated for 1 h. Then 1% BSA solution in PBS was flowed into chamber to block the nonspecific interaction overnight. Before magnetic tweezers experiment, around 1 nM protein $GB1_8$ in PBS buffer was flowed into the chamber and incubated for 10 min, then streptavidin-coated paramagnetic beads Dynabead M280 (cat. 65305, Invitrogen) were flowed into the chamber to form protein tethers. To prevent the protein from being oxidized during long time measurement, the phosphate buffer was added with 5 mM sodium ascorbate.

Steered Molecular Dynamics Simulation

The steered molecular dynamics (SMD) simulation of SpyTag/SpyCatcher complex (pdb:4mli) unzipping from N-N* termini was performed by the software package GROMACS with the GROMOS96 53A6 force field. The spring constant is 100 kJ/mol/nm². The moving speed of the restraint point is 0.01 nm/ps. The whole process of simulation lasted for 850 ps.

Theoretical Modeling

As a two state process of the unzipping dynamics, Bell's model was used to fit force-dependent folding and unfolding rates of $k_f(f)$ and $k_u(f)$:

$$k_u(f) = k_u^0 exp(f x_u / k_B T) \,, \qquad (S1)$$

$$k_f(f) = k_f^0 \exp(f x_f / k_B T) \,, \qquad (S2)$$

where f denotes the stretching force, k_u^0 the unfolding rate at zero force, k_f^0 the folding rate at zero force, x_u the unfolding distance, x_f the folding distance, k_B the Boltzmann constant, and T the absolute temperature.

Native state of SpyTag/SpyCatcher complex is modeled as a solid body. Therefore, its extension along force direction, x_N, changes due to orientation fluctuation:

$$x_N(f) = l_0 \coth\left(\frac{fl_0}{k_BT}\right) - \frac{k_BT}{f}, \qquad \text{(S3)}$$

where l_0 (1.66 nm) is the distance between N-termini of SpyTag and SpyCatcher, which can be measured from resolved crystal structure of SpyTag/SpyCatcher complex (PDB: 4mli) (Fig. S1). The unzipped state of N-SpyTag/N-SpyCatcher complex comprises a rigid body of SpyCatcher and an unzipped peptide, which can be modeled as a Worm-like chain (WLC) and its force-extension curve is given by:

$$\frac{fA}{k_BT} = \frac{x_p}{L} + \frac{1}{4(1 - x_p/L)^2} - \frac{1}{4}, \qquad \text{(S4)}$$

where x_p is the extension of unzipped peptide along force direction, A the persistence length, and L the contour length of peptide. Persistence length of A = 0.82 nm is used based on former works[2, 3]. Contour length of peptide is 2.80 nm as measured from MD simulation structure at 700 pN. The extension of remaining SpyCatcher, x_c, follows Eq. (S3) with contour length of 3.11 nm (Fig. S1). The unzipping step size Δx can be calculated from the extension difference between native SpyTag/SpyCatcher complex and the unzipped conformation:

$$\Delta x(f) = x_p(f) + x_c(f) - x_N(f). \qquad \text{(S5)}$$

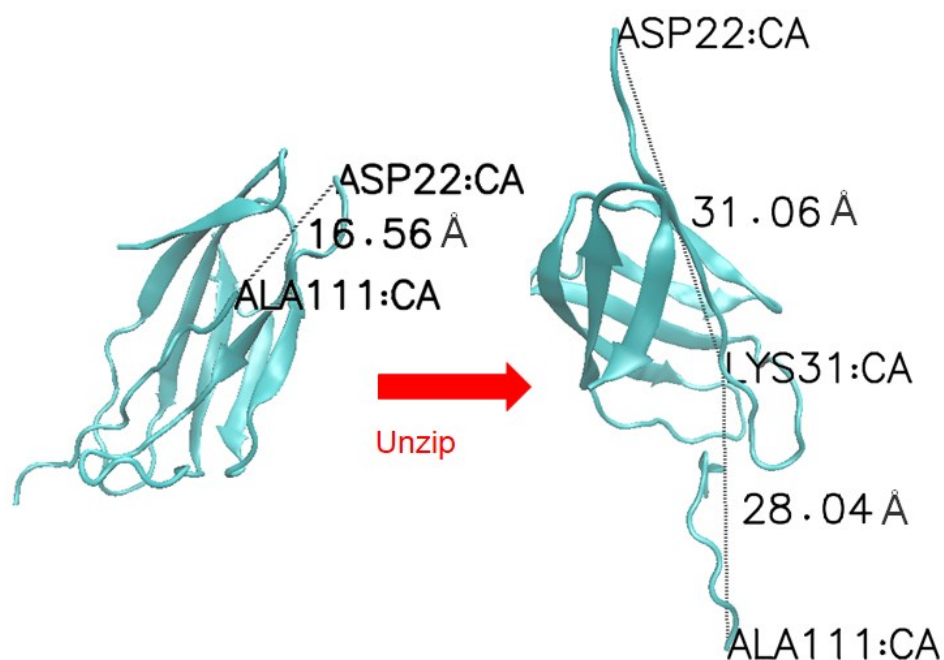At 30 pN, Δx is calculated to be 3.4 nm.

Supporting Figures

Figure S1: Native structure (PDB: 4mli) and structure of unzipped state at 700 pN from SMD simulation. We measured length between corresponding C_α atoms by software PyMOL.
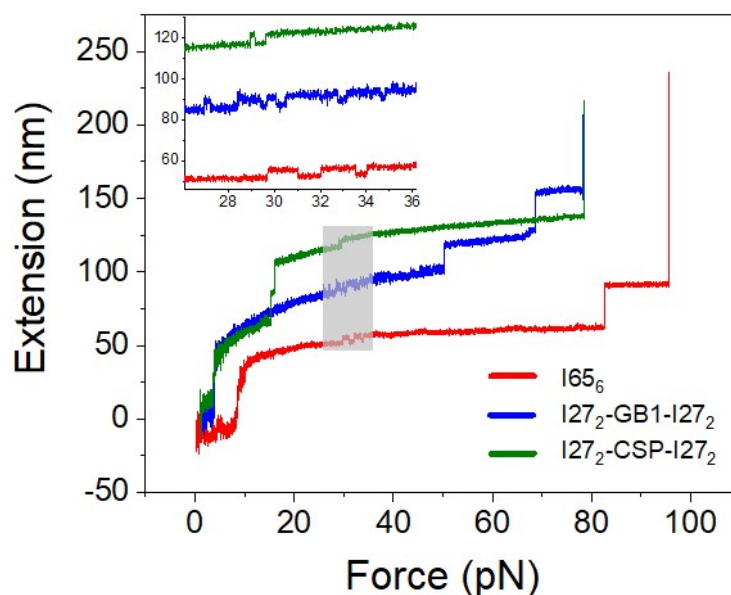

Figure S2: Force-Extension curve of three protein tethers containing N-SpyTag and N-SpyCatcher. Three different kind of protein constructs containing SpyTag at C-terminus were stretched by constant loading rate of ~1 pN/s up to forces around 80 pN. The insert figure shows the zoomed-in signals of SpyTag/SpyCatcher complex
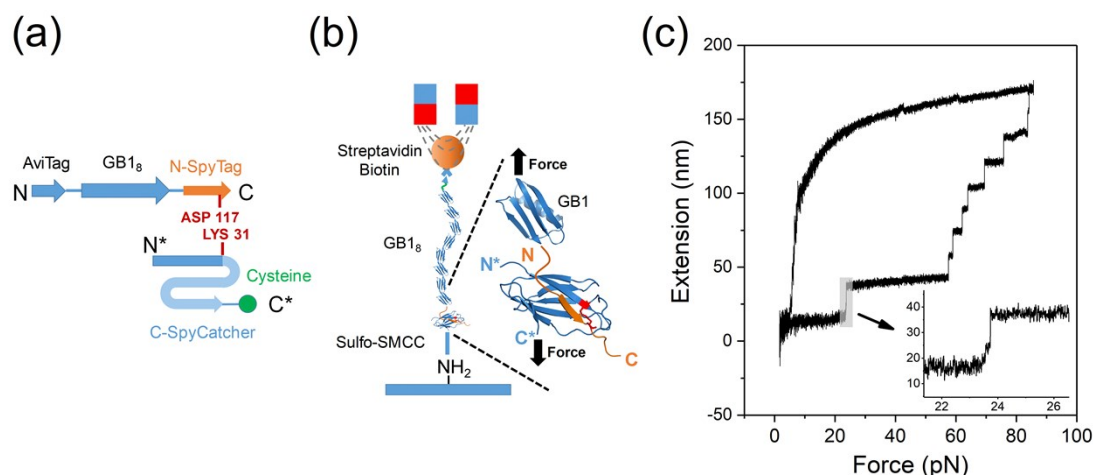
unzipping transition at stretching force of around 30 pN.

Figure S3: Unfolding transitions of the SpyTag/SpyCatcher complex by magnetic tweezers. (a) Sketch of the protein construct of $GB1_8$ with N-SpyTag and SpyCatcher with C-terminal cysteine (C-SpyCatcher). (b) Sketch of stretching N-SpyTag/C-SpyCatcher. SpyCatcher with C-terminal cysteine is covalently attached to glass surface by Sulfo-SMCC, and forms covalent bond with SpyTag at C-terminus of $GB1_8$ tandem repeats. (c) Force-extension curve shows single unfolding step of SpyCatcher at force ~ 24 pN with step size of ~20 nm together with regular unfolding steps of GB1.
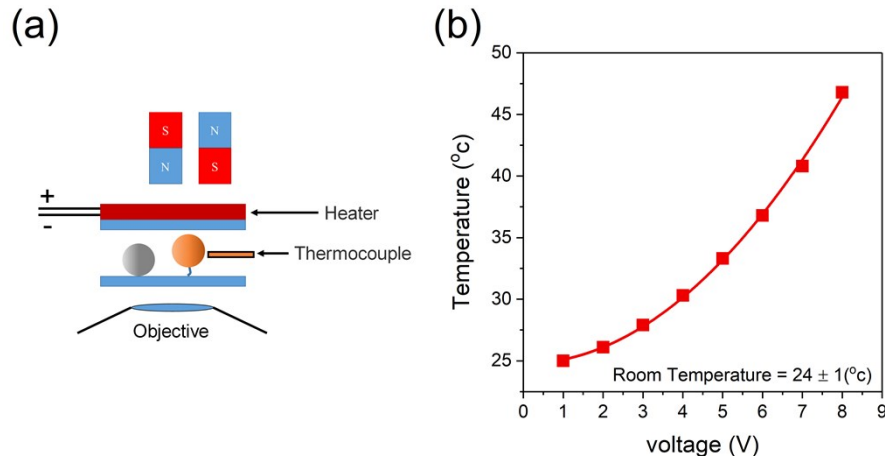

Figure S4: (a) Temperatures in the channel were measured as a function of voltage applied on the heater, as show in Fig. 4(c). (b) Quadratic function fits the data points well.
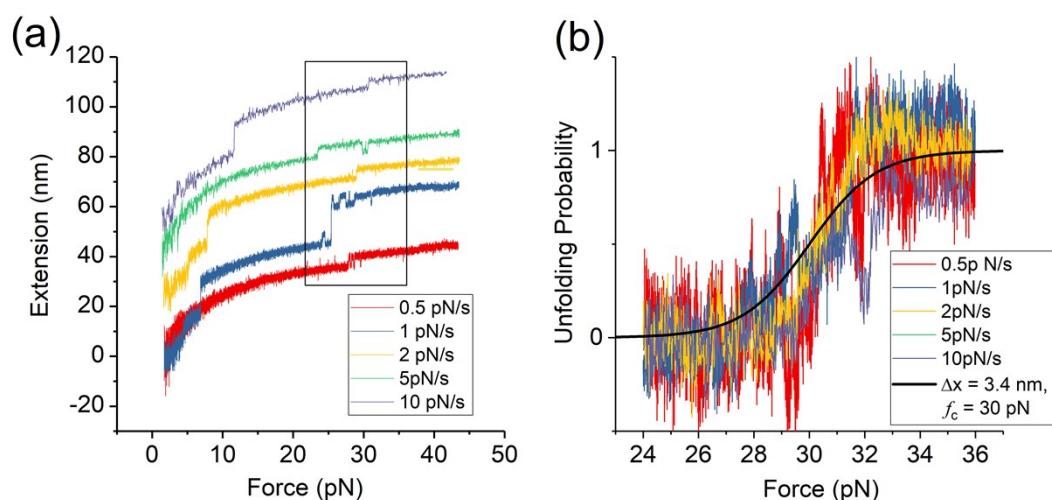
Figure S5: (a) The force-extension curves are measured from constant loading rate experiments with loading rates ranging from 0.5 pN/s to 10 pN/s on a tether containing SpyTag/SpyCatcher complex. (b) At least ten force-extension curves are averaged to obtain the force-dependent unfolding probability at each loading rate. The black line indicates the unfolding probability predicated by Eq.1 with parameters $\Delta x = 3.4$ nm and $f_c = 30$ pN.

References

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