1. PrP aggregations on SLBs at pH 7.0.

The AFM images show that two layers of PrP were observed at pH 7.4 (Figure S1A). A defect in the first layer (the green arrow in Figure S1A) shows the height of ~ 5 nm (Figure S1A’), which is equivalent to the height of lipid bilayers. This result suggests that the PrP molecules were absorbed on the SLBs. To obtain more details of the PrP aggregation, the enlarged AFM image was provided, which shows that PrP molecules form ball-like aggregates (Figure S1B). In addition, we randomly measured 150 PrP aggregates from 20 enlarged images. The most probable height value is 3.8±0.1 nm (Figure S1C), which is very similar to our previous experimental results obtained on mica substrate. Because of the highly soluble structure of the monomeric PrP molecule at neutral pH, it is not able to transform to a β-sheet isoform. Moreover, the PrP has the weak affinity to lipid membranes at pH 7.4. These properties could make the PrP molecule freely aggregates with each other via intermolecular hydrogen bonding in the solution before depositing on the SLBs. This experiment indicates that SLBs have no effect on PrP aggregation at pH 7.4.
Figure S1. The aggregation of PrP on lipid membrane at pH 7.4. (A) The topography of PrP aggregation on lipid membrane. (A’) The cross-section profile of the dark blue line. (B) AFM image of PrP patterns enlarged from the area inside the blue frame in (A). (C) The height distribution of PrP aggregates.

2. Image the lipid membrane before injecting PrP.

Fig. S2. (A) The AFM image of lipid bilayer at pH 5.0. (B) The cross section analysis shows that the height of the lipid bilayer is about 5 nm.
3. **PrP aggregations on lipid membrane at 40nM**

The AFM images show that two layers of PrP were also observed at a PrP concentration of 40nM (Figure S3A). A defect in the first layer (the green arrow in Figure S3A) shows the height of the lipid bilayer is ~ 5 nm (Figure S3B). This result suggests that the PrP molecules were absorbed on the SLBs (Figure S3A I). Flat and compact PrP monlayer was formed as the first layer (Figure S3A II). A second layer of PrP aggregates was also formed (Figure S3A III). The enlarged AFM images show that these aggregates are oligomers (Figure S3C), which is similar to the phenomenon detected at a concentration of 4nM.

![Figure S3](image1.jpg)

**Figure S3.** The aggregation of PrP in concentration of ~ 40nM on lipid membrane at pH 5.0. (A) The topography of PrP aggregation on lipid membrane. (B) The cross section analysis shows that the height of the lipid bilayer is about 5 nm. (C) AFM image of PrP patterns enlarged from the area inside the blue frame in (A).

4. **Simulation details**

The PrP monomer (23-231) was modified from PDB entry 2LSB, the NMR structure of the human prion (90-231). The sequence 23-89 was attached to the 2LSB structure by connecting the residue Trp89 to Gly90 with the amide bond. All of the Histidine residues were modified to be protonated for the simulation of PrP folding under pH 4.5. In Amber 11, the entire PrP (23-231) structure was minimized in vacuum, neutralized with counter ions Cl\(^-\), and put into a water solvent box. This system was heated up to 300 K and applied with the pressure of 1 atm. The equilibrium under 300 K and 1 atm was conducted for 20 ns. The stable folding structure of PrP (23-231) is shown in Figure S4.
Figure S4. The folding structure of PrP (23-231) obtained by Amber molecular dynamic simulations. The α-helices are in red, β-sheets in yellow, and the N-terminal domain Gly23-Trp89 in blue.

In the docking simulation, the residues in the β-sheets were used as the predicted binding domains, which are the same ones as previous publications. The monomer structure shown in Fig. 1 was uploaded to HADDOCK webserver, and the results were downloaded for analysis. The binding interfaces of dimer D are shown in Figure S5, and trimer T in Figure S6. The residues at each interface and their hydrogen bonds are shown in Table S1.
Figure S5. The docking result of D, the monomer M1 in yellow, M2 in blue. The binding residues at the interface are shown in stick representation.

Figure S6. The docking result of T, the monomer M1 in yellow, M2 in blue, and M3 in magentas. The binding residues at the interface are shown in stick representation. (A) M1-M2 interface, (B) M2-M3 interface, (3) M1-M3 interface.
Table S1. The details of binding residues involved in PrP(23-231) dimer and trimer binding.

<table>
<thead>
<tr>
<th>Interface</th>
<th>Interface between two monomers</th>
<th>Predicted binding residues</th>
<th>Residues involved in hydrogen bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (M2-M3)</td>
<td></td>
<td>N/A</td>
<td>Thr107, Asn108, Ala118, Gly119, Ala120, Val121, Val122.</td>
</tr>
</tbody>
</table>
5. Typical Force curves at different loading rate.

Figure S7. Typical force curves and force distributions at different loading rate.

\[ \langle F \rangle = F_{eq} + F_{\beta} \ln \left( 1 + e^{-\gamma R(F_{eq})} \right) \]

\[ R(F_{eq}) = \frac{r}{k_{eff}(F_{eq})F_{\beta}} \]

(EqS1)

where \( F_{\beta} = k_b T / x_{\mu} \) is the thermal force, \( k_b = 1.38 \times 10^{-23} \) is the Boltzmann constant,

\( T = 298 \) is the absolute temperature, \( F_{eq} \) is the equilibrium force for the bond-transducer system,

\( \gamma = 0.577 \) is the Euler’s constant.

\[ F_{eq} = \sqrt{2k_{eff} \Delta G_{bu}} \]

(EqS2)

\[ \Delta G_{bu} = k_b T \times \ln(0.018 K_d) \]

(EqS3)

where \( k_{eff} \) is the effective spring constant of the cantilever and linker molecule (Fig. S8), \( \Delta G_{bu} \) is the binding equilibrium free energy, 0.018 l mol\(^{-1}\) is the partial molar volume of water.

7. Determining the effective spring constant \( k_{eff} \) of the cantilever-PEG linker system.

The effective spring constant \( k_{eff} \) can be calculated from the following equation:

\[ k_{eff} = \frac{k_c k_{PEG}}{k_c + k_{PEG}} \]

(Eq S4)

where \( k_c \) is the spring constant of the cantilever and \( k_{PEG} \) is the stiffness of the linker. The stretching of the PEG linker under force \( F \) is described WLC model:

\[ F = \left( \frac{k_b T}{L_p} \right) \left[ \frac{1}{4(1-x/L_0)^2} - \frac{1}{4} + \frac{x}{L_0} \right] \]

(Eq S5)

were \( F \) is the force applied on glucose, \( k_b \) is the Boltzmann constant, \( T \) is the temperature, \( L_p \) is the persistence length, \( L_0 \) is the contour length of the PEG, \( K_0 \) is the enthalpic correction\(^{10}\). The contour length of PEG2000 (\( L_0 \)) is estimated as 20 nm, the persistence length \( L_p \) is 3.8 Å, and the \( K_0 \) is 1561 pN\(^{11}\). In this study, two PEGs are involved in the calculation.

The PEG elasticity can be described by the following equations\(^{12}\):
\[ L(F) = L_c(F) \times \left( \coth \left( \frac{F L_K}{k_B T} \right) - \frac{k_B T}{F L_K} \right) + \frac{n_m F}{K_S} \]  
(Eq S6)

Where the Kuhn length \( L_k \) is 0.7 nm, the chain stiffness \( K_s \) is \( \sim 150 \) pN/nm, \( n_m \) is the PEG monomers, and \( L_c(F) \) is contour length that is dependent on force, which can described by

\[ L_c(F) = n_m \left( \frac{L_p}{e^{\Delta G/k_B T} + 1} + \frac{L_h}{e^{-\Delta G/k_B T} + 1} \right) \]  
(Eq S7)

Where \( L_p \) and \( L_h \) are the monomer length in planar and helical configuration, respectively, the energy between the planar ad the helical conformation state \( \Delta G \) is \( 3 \) \( k_B \)T. Under applied load this energy difference can be described by

\[ \Delta G(F) = \Delta G - F \times (L_p - L_h) \]  
(Eq S8)

Figure S8 shows the relationship between the force and \( k_{\text{eff}} \). From the models described above, the corresponding \( k_{\text{eff}} \) to the \( F_{eq} \) can be calculated.

![Figure S8. The relationship of force and \( k_{\text{eff}} \)](image)

**Materials and Methods**

**Lipids and PrP**

Both the zwitterionic lipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and the anionic lipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS) were purchased from
sigma. Frozen full-length human recombinant prion protein (sequence: 23-231, theoretical PI/Mw: 9.39/23571.92) was purchased from Calbiochem® in Germany with a concentration of 2mg/mL, which was from E. coli expression and purified as previously described. The purity of >95% was determined by SDS-PAGE. The protein was stored at -20°C in 10 mM sodium acetate buffer at pH 4.0 before usage. Before each experiment, purchased prion protein was centrifuged (20000g) for 30 min at 4°C to remove pre-existing aggregates. Phosphate buffered saline (PBS: 100mM sodium phosphate, 150 mM sodium chloride, pH 7.4, added with 0.05% sodium azide) was purchased from Pierce (Thermo Scientific, Waltham, MA, USA). Triple deionized water was provided by a Barnstead Nanopure Diamond Laboratory Water System. Mica was purchased from TED PELLA, INC (Product No: 56).

**Preparation of SLBs and AFM**

All vesicles were prepared using the ‘dry’ method. Briefly, 10 μl POPC chloroform solution (10.0 mg/mL) and 10 μL POPs chloroform solution (10.0 mg/mL) were measured into a small glass bottle and the chloroform removed using a stream of dry nitrogen. The dry POPC/POPS was then suspended in PBS buffer (containing 2 mM CaCl₂) to a final concentration of 0.5 mg/mL. After stirring 30 min, the solution was incubated at 4 °C for 1 hour, and then incubated at 60 °C for 1 hour. Then, 50 μL lipid solutions are deposited on the freshly cleaved mica for about 5 min. Excess vesicles and salt were removed by exchanging the buffer PBS buffer. The bilayer was then equilibrated with 10 mM sodium acetate, pH 5.0, and imaged to ensure that the bilayer was intact. Then, PrP(23-231) in 10 mM sodium acetate, pH 5.0, was injected into the liquid cell and incubated for 40 min before imaging. All the images were acquired by AAC mode using the AFM 5500 (Agilent Technologies, Chandler, AZ). The whole system was shielded from environmental interference by a PicoPlusIsolation Chamber. Silicon cantilevers tip with spring constant of around 0.1 N/m were used for experiments.

**Modify PrP on the AFM tip and substrate**

Bare AFM tips were first cleaned and coated with Au on the tip side by using an E-beam evaporator. The tip coated with gold was first immersed in a DMSO solution containing 0.5 mM HS-PEG-COOH linker and 0.2 mM 1-dodecanethiol for 6 hours. After rinsing with DMSO and water, the carboxyl groups were activated to form N-hydroxysuccinimidyl ester by reacting with 10 mM fresh EDC/NHS mixture solution for 30 min. Then the tip was further thoroughly washed
with 10 mM sodium acetate, and then dipped into PrP solution (10ng/mL, in sodium acetate buffer) for 2 hours. PrP was linked on AFM tip by the reaction between its amino group and N-hydroxysuccinimidy ester. The PrP were modified on gold coated mica in the same way. Functionalized tips and samples were stored in PBS before use.

References