Electronic Supporting Information for

The Molecular Basis of Interaction Domains of full-length PrP with Lipid Membrane

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S1. Experimental details on the simulation of PrP stable structure, the AFM tip modification, supported lipid bilayers, and the WLC model of PrP stretching

The full-length PrP monomer (23-231) was modified from PDB entry 2LSB, the NMR structure of the human prion (90-231), as in the previous publication [1]. First, the fragment from Gly23 to Trp89 was manually attached to the 2LSB structure by connecting the residue Trp89 to Gly90 with the amide bond. Next, all of the Histidine residues were set to be protonated when simulating the PrP folding process under pH 5.0. During the molecular dynamics by Amber 11, the PrP (23-231) molecule was first minimized in vacuum, neutralized with counter ions Cl^-, then placed into a water solvent box. The entire system was heated up to 300 K, and applied with the pressure of 1 atm. The equilibrium step under 300 K and 1 atm was conducted for 60 ns, to make sure the folding structure of PrP (23-231) is stabilized. The enlarged PrP structure from the simulation result, in cartoon and surface renderings, is shown in Figure S1, right side. Here, the unstructured N-terminal segment (23-124) is in magenta, including the segment PrP(23-51) with stick rendering to highlight its relatively strong interaction measured in AFM experiments.

For AFM tip modification, the PEG linker ((thiol-(polyethylene1glycol)-acid, HS-PEG-COOH, MW 2000) was purchased from Creative PEGWorks. N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from

Sigma-Aldrich. 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. All chemicals were directly used without further purification. Phosphate buffered saline (PBS: 100mM sodium phosphate, 150mM sodium chloride, pH 7.2, added with 0.05% sodium acetate) was purchased from Pierce (Themo 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). Bare AFM tips were first cleaned and coated with gold on the tip side by ion beam sputtering. The PrP was modified on the AFM tip coated with gold in two ways. (i)The tip 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 (PBS, pH 4.5) and dipped into PrP solution (10 ng/mL, in sodium acetate buffer) for 2 hours. PrP was linked on AFM tip by the reaction between its amino group and N-hydroxysuccinimidyl ester. (ii) The gold coated tip immersed directly into PrP solution (10 ng/mL, in sodium acetate buffer) for 2 hours. Functionalized tips were stored in PBS before use.

The supported lipid bilayers 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 0.5mg/mL. After stirring 30 min, the solution was incubated at 4 ℃ for 1 hour, and then incubated at 60 ℃ for 1 hour. Then, 50 μL lipid solutions are deposited on the freshly cleaved mica for about 15 min. Excess vesicles and salt were removed by exchanging the PBS buffer. The bilayer was then equilibrated with 10 mM sodium acetate, pH = 4.5, and imaged to ensure that the bilayer was intact.

The stretching of this structure is calculated using the WLC model, which treats the molecule as a polypeptide chain, of which the end-to-end distance x and external force F follow the equation shown below [2]:

$F(x) = \left(\frac{kT}{b}\right)[0.25\left(1 - \frac{x}{L}\right)^{-2} - 0.25 + \frac{x}{L}]$ (Eq. S1)where k is Boltzmann's constant, T is room temperature, p is the persistence length of 0.38 nm and L is the contour length between different regions and Cys179 determined by:

$$L = n \times l \quad \text{(Eq. S2)}$$

where l =0.36 nm is the contour length per amino acid, and n is the number of amino acids between different regions and Cys179.

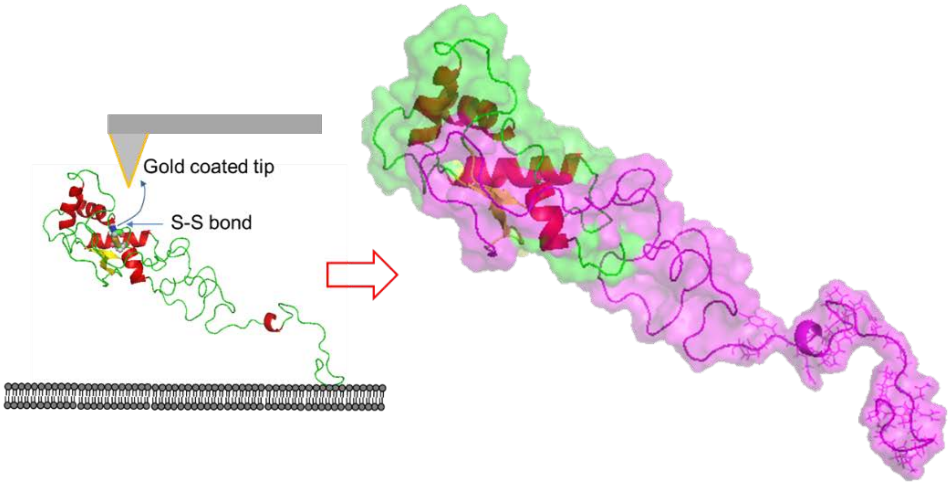

Figure S1. The simulated PrP(23-231) showing the unstructured N-terminal (23-124) in magenta. On the C-terminal side, the α-helices are shown in red, β-sheets in yellow, and other parts of the unstructured fragments in green.

S2: Measuring the distance among PrP fragments

The distance values were calculated for the two stable β sheets in the simulated PrP structure. As shown in Figure S2(A), from Cys179 to Tyr163, n=16, L=0.36 nm, dT=0.5 nm, so ΔL=n×L-dT=5.3 nm. Similarly, in Figure S2(B), from Cys179 to Met134, n=45, L=0.36 nm, dT=1.5 nm, so ΔL=n×L-dT=14.7 nm.

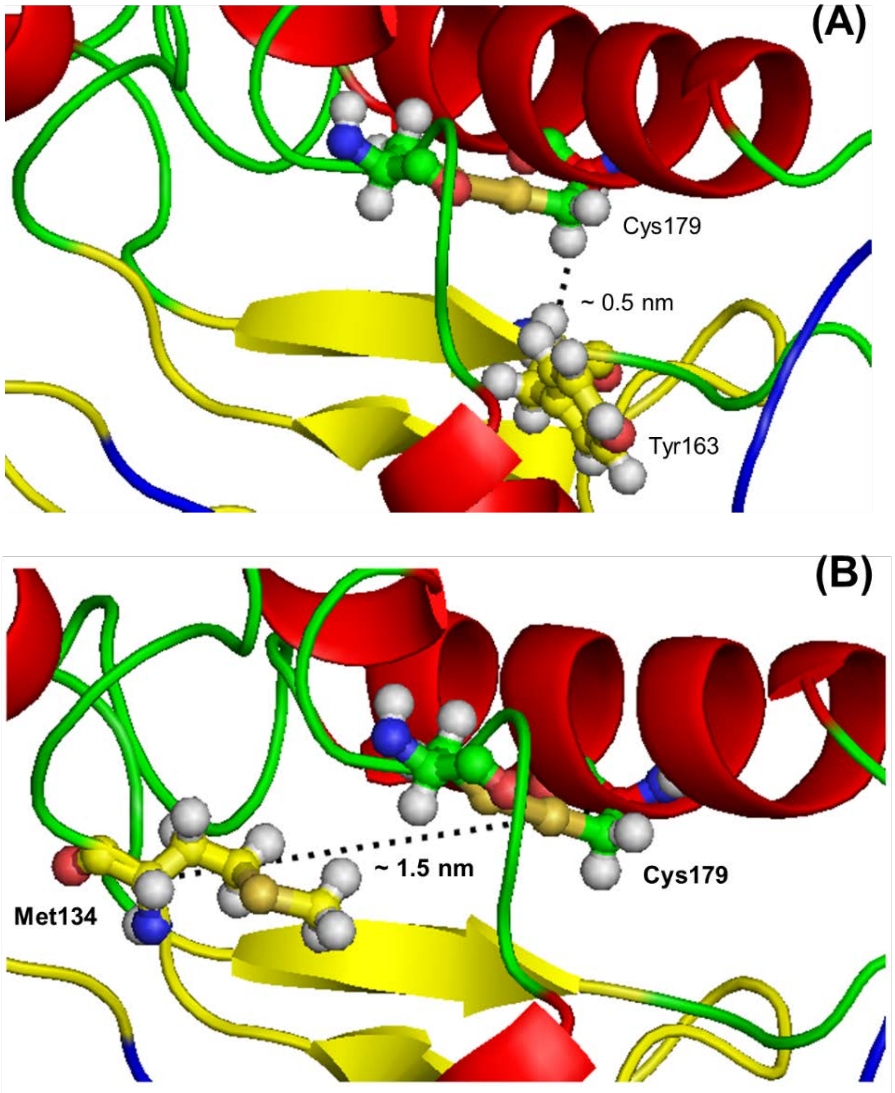

Figure S2. (A) The distance between Cys179 (attached to the AFM tip by S-Au bond) and Tyr164 from one β sheet. (B) The distance between Cys179 and Met134 from the other β sheet. Both of them were measured inside the simulated PrP(23-231) structure.

S3. The structure-function relationship of N-terminal interactions

Currently, the analysis of the interactions among protein and lipid membrane still relies on educated guess. The unstructured N-terminal of PrP further increase the difficulty to understand its interactions to the lipid membrane. Here we simply show the hydrophobicity and charge distribution of PrP segments PrP(23-51) (from Gly23 to Pro51), OR (from Pro51 to Gly90), and CC2 (from Thr95 to Lys110). A surface modeling approach is used here to show the hydrophobicity and charge properties of PrP surface simultaneously.[2] The YRB script was run in PyMOL and generated the surface rendering of PrP molecule according to the "YRB" scheme, where "the carbon atoms not bound to nitrogen and oxygen atoms are highlighted in yellow, nitrogen atoms in the side chains of lysine and arginine are blue, oxygen atoms in the side chains of glutamate and aspartate are red and all remaining atoms white" [3]. However, this approach can't distinguish different protonation states of histidine, which remain neutral under all environment. In our study of PrP molecule under pH 5.0, the histidine residues should be protonated and positively charged. Therefore, all histidine residues were manually set to the color of green after the entire PrP molecule was treated with the YRB script, as shown in Figure S3. One observation is that all negatively charged areas locate on the C-terminal side, and several positively charged areas and hydrophobic areas spread through the relatively more flexible N-terminal side, including PrP(23-51), OR, and CC2. This distribution may facilitate the N-terminal binding to the negatively charged lipid membrane comprised of POPS and POPC. However, this analysis is merely based on fundamental principles of biophysics. The better understanding of the interactions between PrP and lipid membrane requires more powerful tools of molecular modeling, which is beyond the scope of this paper.

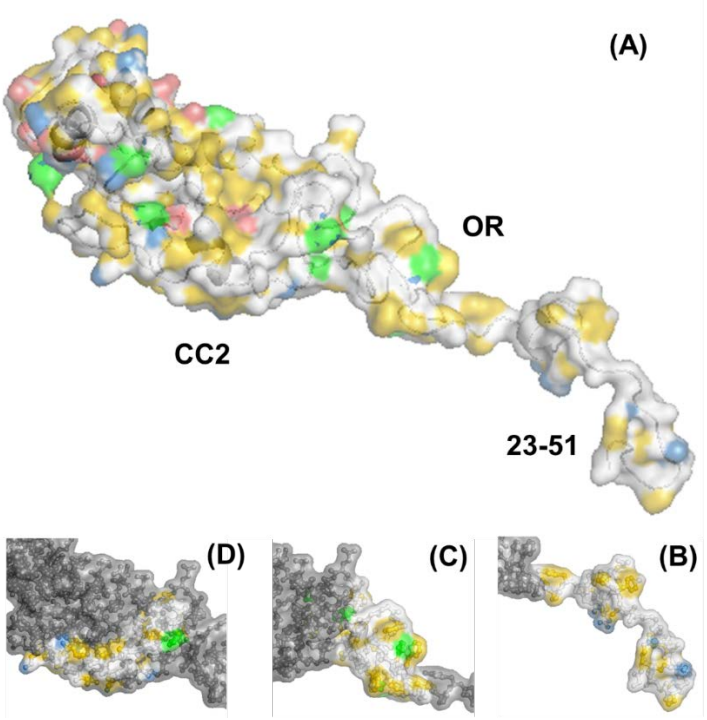

Figure S3. (A) The surface rendering of PrP molecule with YRB scheme, with hydrophobic areas in yellow, negatively charged areas in red, positively charged areas in blue, and the unrecognized positively charged areas covering histidine residues in green. The folding structure is shown in cartoon rendering. (B) The enlarged segment of PrP(23-51) segment, showing its YRB surface scheme and the sticks rendering of each amino acid residue (in gray). (C) The enlarged segment of OR segment, showing its YRB surface scheme and the sticks rendering of each amino acid residue (in gray). (D) The enlarged segment of CC2 segment, showing its YRB surface scheme and the sticks rendering of each amino acid residue (in gray).

Reference:

[1] Nanoscale insights into full-length prion protein aggregation on model lipid membranes. Yangang Pan, Bin Wang, Tong Zhang, Yanan Zhang, Hongda Wang, and Bingqian Xu. *Chem. Commun.*, 2016, 52, 8533-8536.

[2] Reversible unfolding of individual titin immunoglobulin domains by AFM. Matthias Rief, Mathias Gautel, Filipp Oesterhelt, Julio M. Fernandez and Hermann E. Gaub. *Science*, 1997, 276, 1109-1112.

[3] A script to highlight hydrophobicity and charge on protein surfaces. Dominique Hagemans, Ianthe A. E. M. van Belzen, Tania Morán Luengo, and Stefan G. D. Rüdiger. *Front Mol Biosci.,* 2015, 2, 56.