Hydrophobic enhancement of Dopa-mediated adhesion in a mussel foot protein

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Mfp3 *slow* content estimation in mussel plaque

Amino acid composition of Mfp3 *fast*, Mfp3 *slow*, mussel plaque, and also lysozyme were measured by amino acid analyzer after hydrolyzing proteins using methanesulfonic acid (MES) and hydrochloric acid (HCl) at 110□ for 22 hrs respectively. For each protein sample, MES and HCl hydrolysis give similar results for most of the amino acids except Tryptophan (Trp). MES is capable of protecting Trp from being destroyed in some extent while there is almost no recovery of Trp during HCl hydrolysis. The normalized mole concentration of Trp in Mfp3 *fast*, Mfp3 *slow*, mussel plaque, and lysozyme are 4.5% and 4.7%, 1.1%, and 3.1% respectively. cDNA-deduced sequence gives about 8% Trp content in both Mfp3 *fast* and *slow*,[1] while Trp content of lysozyme calculated from its sequence is 4.7%.[2] By comparing the measured and calculated Trp content of Mfp3 *fast*, *slow* and lysozyme, it seems MES hydrolysis give consistent Trp recovery percentage. Mfp3 is the only known protein that contains Trp in mussel plaque. Assuming Mfp3 is the only source of Trp for plaque, the amount of Mfp3 content in plaque may be estimated from the measured Trp value to be 24%. By comparing the area of assigned peaks in shodex spectra, the amount ratio of Mfp3 *fast* and *slow* in plaque is less than 1:2. A figure of at least 16% may be calculated for Mfp3 *fast* content in plaque.

The influence of $NaIO_4$ on Mfp3 *slow* adhesion to mica surface

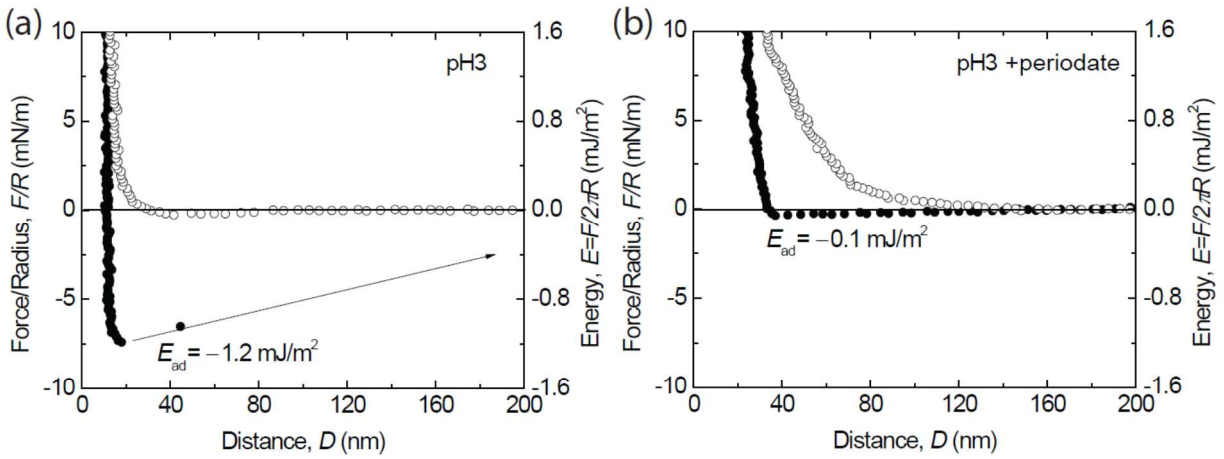

Figure S1. Interaction between mica and Mfp3 *slow* adsorbed on mica at pH 3 (a) before and (b) after adding excess amount $NaIO_4$. Approach *(unfilled symbols)*; separation *(filled symbols)*.

The influence of $NaIO_4$ on Mfp3 *slow* cohesion

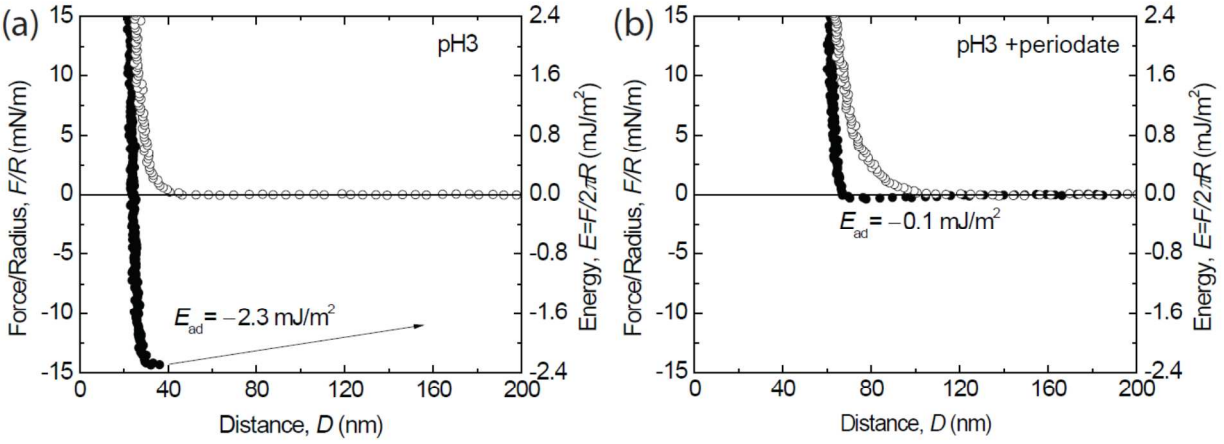

Figure S2. Interaction measured at pH 3 between two mica surfaces with Mfp3 *slow* absorbed symmetrically (a) before and (b) after adding excess amount $NaIO_4$. Approach *(unfilled symbols)*; separation *(filled symbols)*.

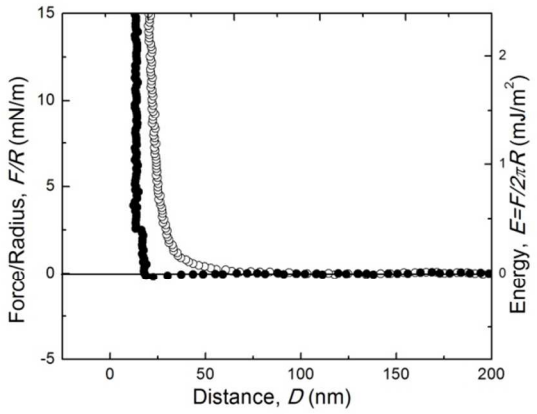

Figure S3. Interaction measured at pH 5.5 between two mica surfaces bridged with Mfp3 *slow* after being exposed to excess amount $NaIO_4$ for 1 hr to allow $NaIO_4$ diffusion. $NaIO_4$ was injected between two mica surfaces after they are in contact position. Approach *(unfilled symbols)*; separation *(filled symbols)*.

Interactions between Mfp3 *slow* with other Mfps

Besides with itself, Mfp3 *slow* also interacts with Mfp3 *fast* and Mfp2 which are localized at the plaque-substratum interface and overlying plaque layers, respectively. The interactions between Mfp3 *slow* /Mfp3 *fast* (Figure S4) and Mfp3 *slow* /Mfp2 (Figure S5) were studied by depositing Mfp3 *slow* on one mica surface and a film of Mfp3 *fast* or Mfp2 deposited on the other mica surface.

At pH 5.5, Mfp3 *slow* showed strong binding to Mfp3 *fast* with an adhesion energy of ~ -1.6 mJ/m^2 (Figure S4b) after a short 3 min contact. The interaction between Mfp3 *slow* and Mfp3 *fast* showed very strong pH dependence: increasing the buffer pH to 7.5 greatly diminished the binding strength in the SFA measurement with the adhesion energy decreasing from -1.6 mJ/m^2 to -0.3 mJ/m^2 (Figure S4b).

For the interactions between Mfp3 *slow* and Mfp2, a weak adhesion energy between the two protein layers (-0.4 mJ/m^2) was detected after 3 min contact under pH 5.5 buffer condition (Figure S5b). A longer contact time (13 min) did not lead to significantly stronger adhesion (Figure S5c). Previous SFA studies had showed that Mfp2 cannot bind to Mfp3 *fast*, leaving open the question of how a load is transferred this interfacial adhesive protein to other proteins in the plaque. The answer may be via Mfp3 *slow* as seen by the strong interaction between Mfp3 *slow* and Mfp3 *fast* as well as the interaction between Mfp3 *slow* and Mfp2.

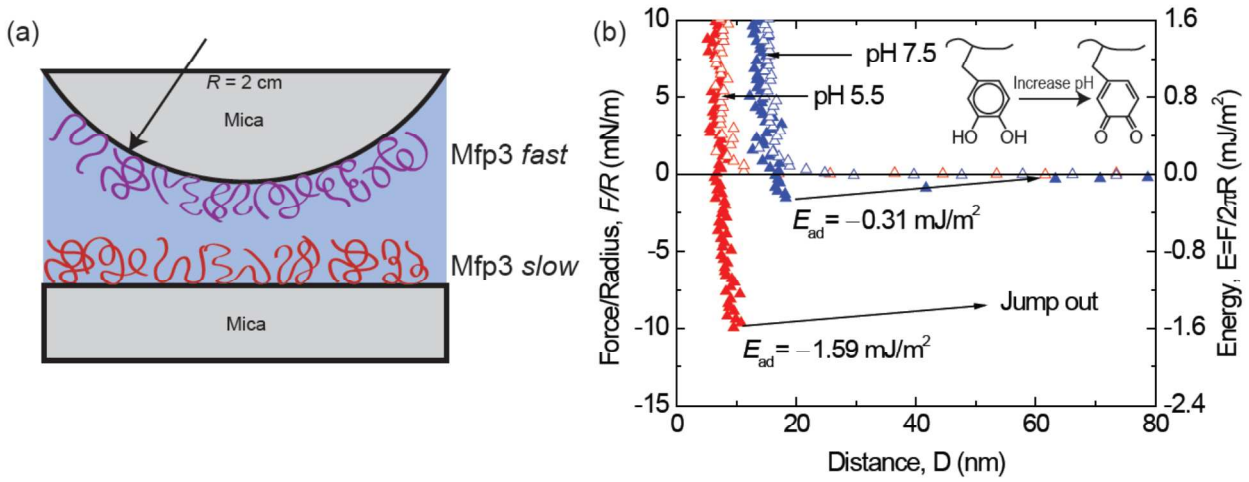

Figure S4. Interaction between Mfp3 *fast* and Mfp3 *slow* adsorbed on two facing mica surfaces at (b) pH 5.5; (c) pH 7.5. Approach *(unfilled symbols)*; separation *(filled symbols)*. Separation was after a brief (~3 min) contact.

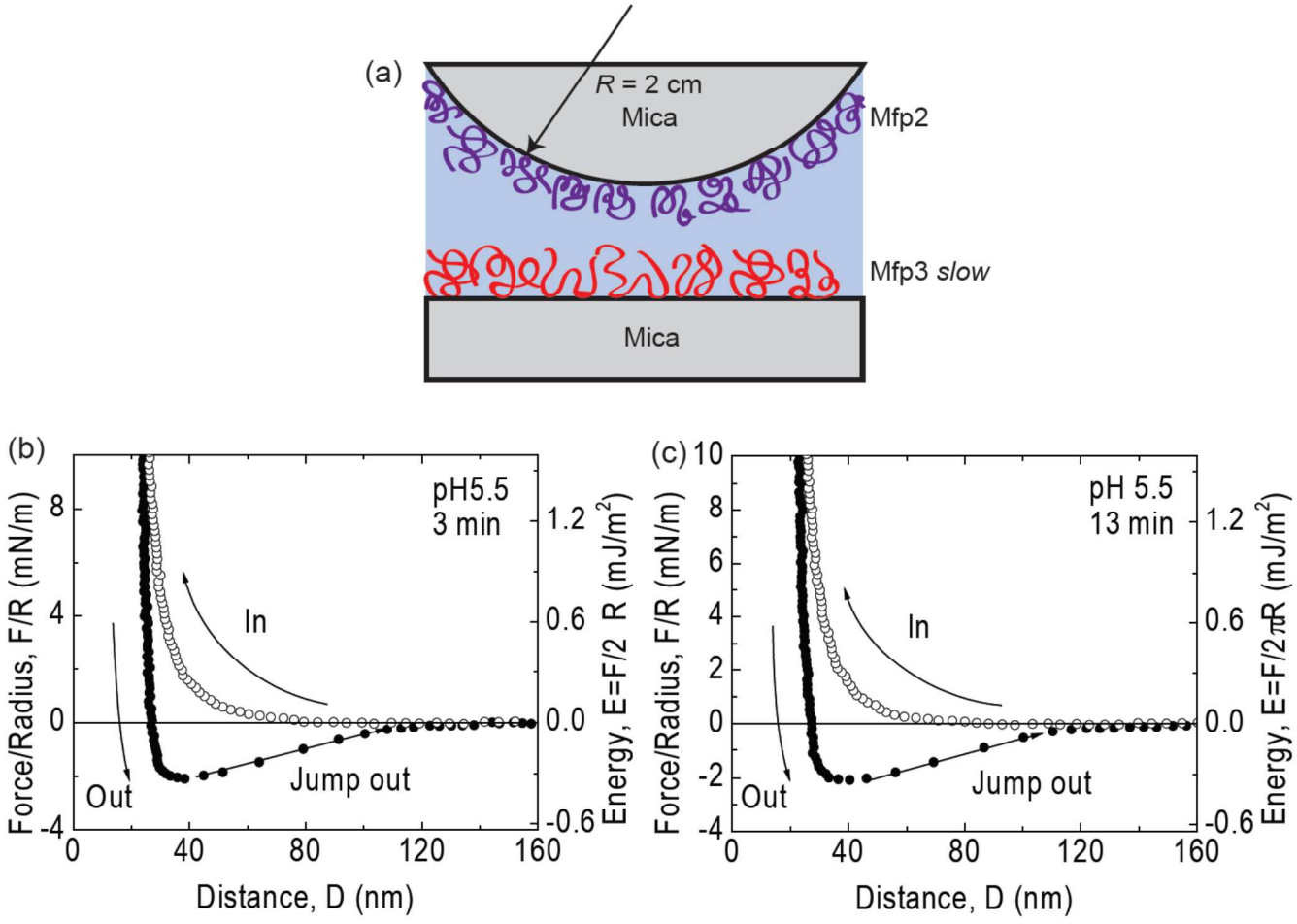

Figure S5. Interaction between Mfp3 *slow* and Mfp2 adsorbed on two facing mica surfaces. Contact times were as indicated. Approach *(unfilled symbols)*; separation *(filled symbols)*.

References

(1) Yu, J.; Wei, W.; Danner, E.; Israelachvili, J. N.; Waite, J. H. *Adv Mater* **2011**, *23*, 2362.
(2) Canfield, R. E. The amino acid sequence of egg white lyzosyme. *J Biol Chem* **1963**, *238*, 2698.