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Subnanoscale hydrophobic modulation of salt bridges in aqueous media

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1. Introduction

Q. How "Limited variety of 13 polar residues of amino acids" achieves " great diversity of biological molecular recognition on proteins"?

Hypothesis: Modulation of polar interaction by proximal hydrophobic surface

Schellman (1953): A hydrophobic surface near a pair of ions enhances ion pairing by construction of hydrogen bond network.

× No experimental supports;

- Difficulty in preparing a pair of ions at defined distances from a hydrophobic surface.
- And how to evaluate?

This work: Study of the bond strength of a salt bridge depending on spacing from a hydrophobic surface using FRET from mixed SAM (self-assembled monolayer) of anionic head and space-filling alkyl chains to cationic guest (Fig. 1).

- Salt bridge between $FITC^{2-}$ ionic head and cationic guest Gdn-Rho
  => Detection of guest capture by FRET.
- Response to pH titration and electric field.
  => Static & dynamic evaluation of the bond strength of the salt bridge.
- Changing chain length of space-filling hydrophobic SAM (Fig. 2).
  => Distance control between fixed $FITC^{2-}$ ionic head and hydrophobic surface.

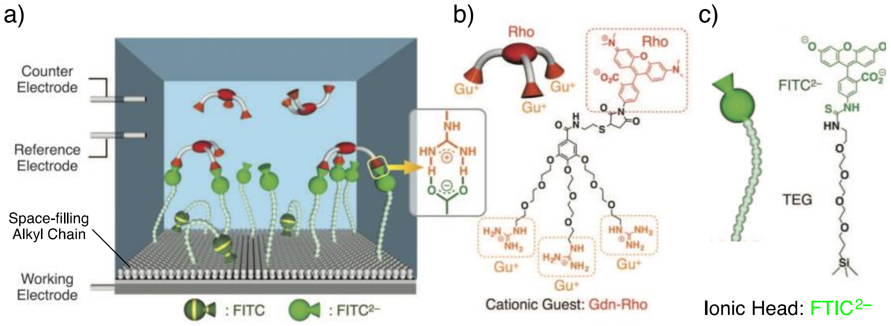

Figure 1. Experimental system. (a) Mixed SAM of space-filling alkyl chain and ionic head was deposited on doped Si electrode. Aqueous solution of the cationic guest was loaded on the SAM to make the salt bridge. (b,c) Chemical structures of the cationic guest and ionic head.

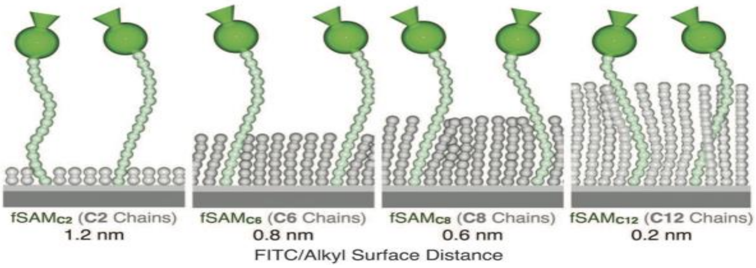

Figure 2✾ The structures of mixed fluorescent SAMs that carry fluorescein isothiocyanate (FITC) head groups via a hydrophilic TEG linker in C2, C6, C8, and C12 space-filling alkyl chains on a silicon substrate, respectively.

2. Results and Discussion

2.1. Characterization of mixed SAMs of FITC ionic head and space-filling alkyl chains

- Fluorescent mixed SAMs are composed of FITC-TEG chain at area-per-molecule density of 2.8-2.9 nm^2 and densely packed C2-12 space-filling alkyl chains.
- Negative fSAM$_{Cn}^{2-}$s, which have anionic FITC^{2-} have larger FL intensity than protonated neutral fSAM$_{Cn}$s.
- Response of fSAM$_{C2}$ and fSAM$_{C12}$, SAM with the shortest and the longest alkyl chain for each, to H$^+$ was investigated using two kinds of modulation method.
  1. Acid-base titration: H$^+$ modulation by bulk pH titration (Fig. 3 ab)
  2. Application of electric field on Si substrate: dynamic H$^+$ modulation near the SAM (Fig. 3 cdef)
     - Positive voltage => low local H$^+$ concentration
     - Negative voltage => high local H$^+$ concentration
- At low H$^+$ concentration, FITC^{2-} is protonated and negative fSAM$_{Cn}^{2-}$s, which have large FL intensity, became dominant.

  => Similar 1.0-1.4 nm thickness of SAM and acidity of FITC moiety independent from length of space-filling alkyl.
- At high H$^+$ concentration, FITC is protonated and neutral fSAM$_{Cn}$s, which have low FL intensity, is generated.

  => FL intensity E$_m^{513}$ and thickness (0.4 nm) of fSAM$_{C2}$ is smaller than that of fSAM$_{C12}$.
- Conformational freedom of hydrophobic non-ionic FITC head in fSAM$_{C2}$ caused them interacts with the hydrophobic alkyl chains and facilitated *nonradiative energy transfer* (Fig. gh).
- Small difference in inflection point between fSAM$_{Cn}$s => *No change in nature of FITC itself.*

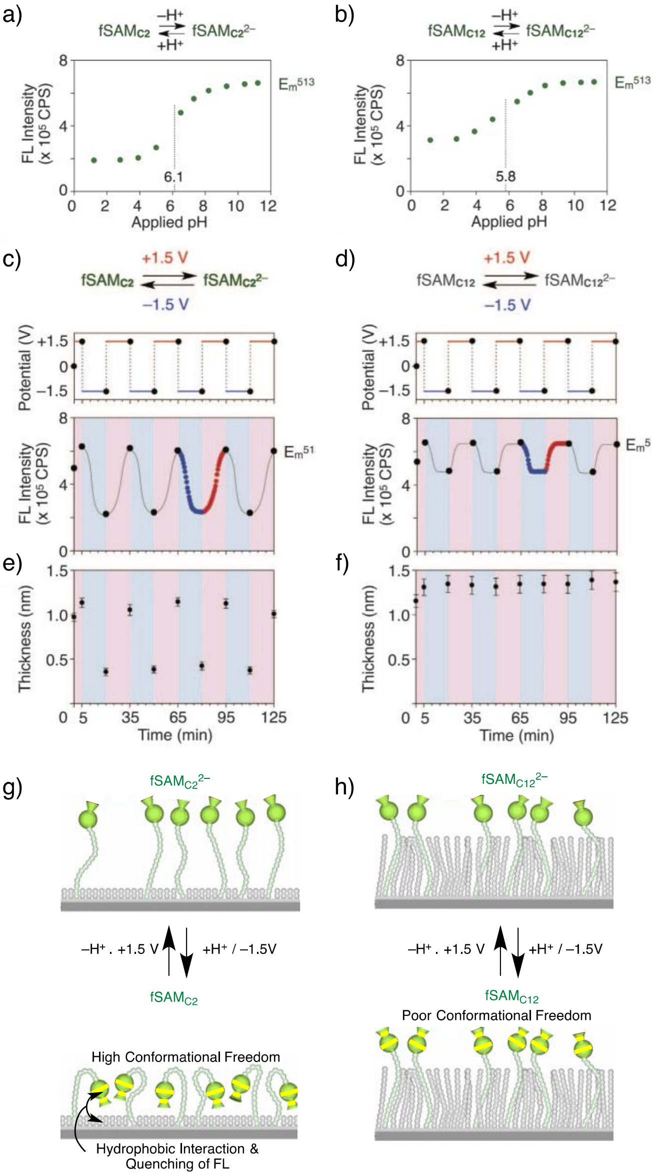

Figure 2. Acid-base, voltage response of mixed SAM of FITC ionic head and space-filling alkyl chains with the lengths of C2 and C12. (a,b) Acid-base titration curve. FL (c and d) and thickness (e and f) responses to the switching voltage between –1.5 and +1.5 V (vs Ag/AgCl). The thickness of SAMs are measured by spectroscopic ellipsometry. (g,h) Models of reasonable explanations of the experimental results a-h.

2.2. FRET study of the strength of salt bridge between fSAM$_{Cn}$s and Gdn-Rho

Response to H$^+$ concentration of fSAMs by capturing and releasesing Gdn-Rho cation was investigated.

- On the fSAM$_{Cn}$s, Gdn-Rho, which is a rhodamine FL dye tethered three Gu$^+$ moieties (10 nM) as a reporter was added (Fig. 1 b).
- A Gu$^+$ moiety and a carboxylate in FITC make a salt bridge (Fig. 4).
- Proximity of FITC and Gdn-Rho by Gu$^+$ bounding causes FRET from FITC to Gdn-Rho. => Evidence for the binding.
- At low H$^+$ concentration, FRET was observed in both C2 and C12 SAMs; Gdn-Rho is bounded to FITC (Fig. 5 ab).
- Switching voltage liberated and recaptured Gdn-Rho on fSAM$_{C2}$. On the other hand, fSAM$_{C12}$ do not liberate Gdn-Rho. Inflection point of fSAM$_{C12}$ is smaller than that of fSAM$_{C2}$ (Fig. 5 cd).

=> *Robustness of salt bridge is reinforced by proximal hydrophobic surface.*

- Difference of binding free energy calculated form that of inflection points: *3.9 kcal/mol*

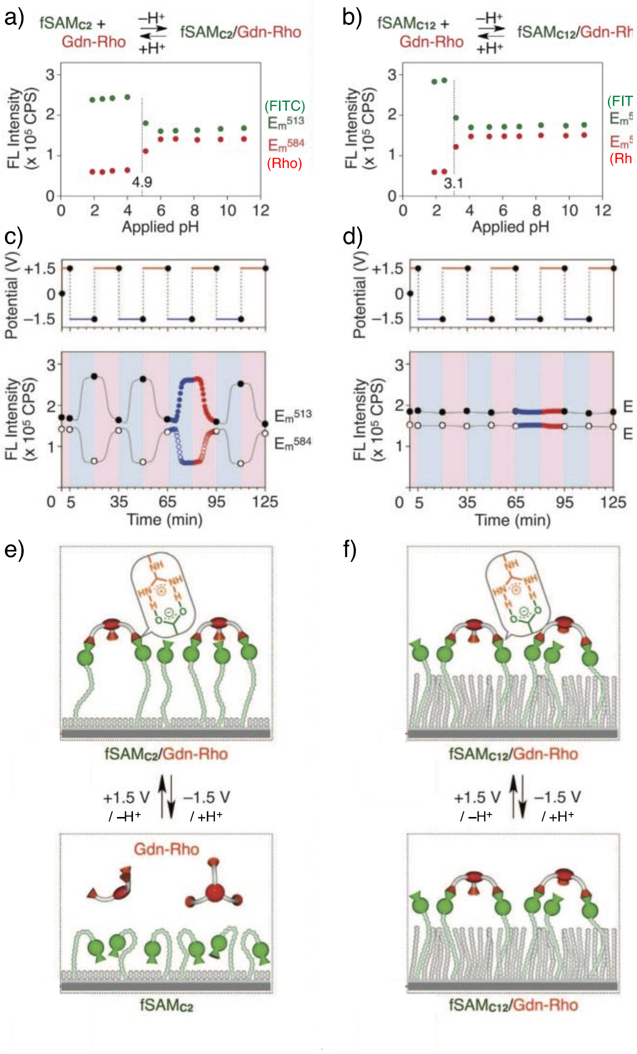

Figure 5. Acid-base, voltage response of mixed SAMs (C2 and C12) and Gdn-Rho. (a,b) Acid-base titration curve. FL (c and d) responses to the switching voltage between –1.5 and +1.5 V (vs Ag/AgCl). (e,f) Models of reasonable explanations of the experimental results a-f.

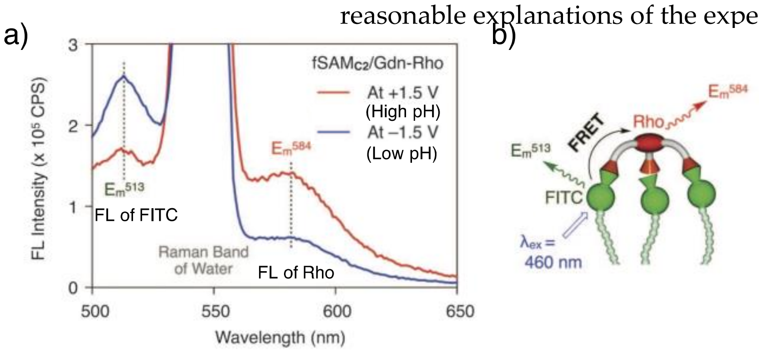

Figure 4✾✾◎a◎◎In situ front-face fluorescence spectra (exited at 460 nm) of fSAM$_{C2}$/Gdn-Rho at –1.5 V (blue curve) and +1.5 V (red curve) (versus Ag/AgCl). FRET takes place from FITC to Rho when fSAMC2 conjugates Gdn-Rho. (b) Schematic image of electron transfer paths in this system

2.3. Hydrophobic modulation of salt bridge

In addition to fSAM$_{C2}$ and fSAMC$_{12}$, intermediate fSAM$_{C6}$ and fSAM$_{C8}$ were also investigated.

- Binding free energies were calculated from the experiments and plotted on Fig 6. The dependency on alkyl length and pH were assumed for the calculation.

=> Nonlinear upward convex

- Binding free energies were calculated from simplified MD calculation. Although absolute value is different, similar convex to the experimental was obtained (Fig. 7).
- Also the local permittivity of water progressively decrease the nearer the water molecule are to a hydrophobic surface.

=> Water molecules are tightly hydrogen bonded near the surface;

A salt bridge breakage against strong hydrogen bond network near a hydrophobic surface is highly energetically demanding.

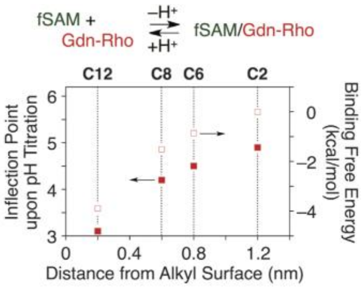

Figure 6. Inflection points upon pH titration of fSAM$_{C2}$, fSAM$_{C6}$, fSAM$_{C8}$, and fSAMC$_{12}$ in the presence of Gdn-Rho (■) and their binding free energies derived from pH titration profiles (□).

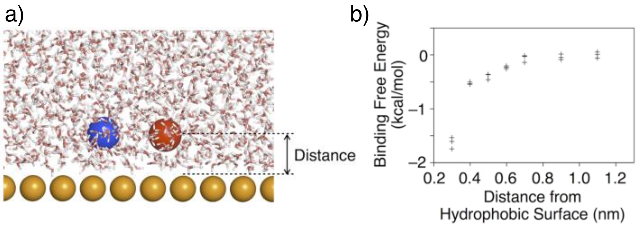

Figure 7. MD simulations of simple model system. (a◎◎A pair of opposite ions located at a certain distance from a hydrophobic surface in water. (B) Relationship between the calculated binding free energy of the ion pair and its distance from the hydrophobic surface.

3. Conclusion

- Ion pairing is reinforced by construction of tight hydrogen bond network near a hydrophobic surface.
- Proteins might employ the mechanism to modulate polar interaction for biological molecular recognition.
- Releasing and reforming of cationic guest Gdn-Rho from fSAM$_{C12}$ was controlled by applied voltage. In stark contrast, fSAM$_{C2}$ never released.