Mechanism Underlying Specificity of Proteins Targeting Inorganic Materials
(Supporting Information)

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Sample preparation

Au(111) substrates were prepared by evaporating gold onto freshly cleaved mica substrates. Prior to use, we baked the gold substrate at 400 °C for 4 hours. SAMs of ODT and MUOH were formed by immersing gold substrates in the ethanol solution containing the corresponding molecules at a concentration of 1 mM for 24 hours. The Si substrates were cleaned by ultrasonication in an acetone bath for 5 min and then in ethanol for 5 min, immediately after which they were rinsed thoroughly with pure water and air-dried under flowing nitrogen. They were then further cleaned by UV-ozone exposure (UV-3000, SAMCO, Tokyo, Japan) for 15 min at room temperature. The Ti substrates were rinsed with ethanol and then cleaned by UV-ozone exposure for 15 min. HOPG substrates were peeled with an adhesive tape prior to each experiment. Static water contact angles were measured with contact angle meter (DSA 10 Mk2, Krüss, Hamburg, Germany).

In the procedure, 1 mg PBLH was solubilized in 0.68 µl chloroform containing dichloroacetic acid (0.54 mg/mL). The desired amount (10 µl) of PBLH was
then spread over the recombinant ferritin, Δ1-LF or minT1-LF solution (1 mg/mL, Tris-HCl buffer at pH 8.0) in a Langmuir trough (20 x 45 x 2 mm) and allowed for condensed ferritin monolayer to adsorb onto the PBLH film for 3 hours at room temperature. The obtained hetero-bilayer of ferritin and PBLH was horizontally transferred onto the AFM tip, and the tip was then immediately stored in deionized water. To check the density of ferritins immobilized on the AFM tip, we performed an AFM observation of the hetero-bilayer of ferritin and PBLH on the V-shaped cantilever surface. A close-packed monolayer of ferritin, Δ1-LF or minT1-LF was always imaged under air. We therefore concluded that the AFM tip is fully covered with the monolayer of ferritin molecules.¹

**Stability of the hetero-bilayer of PBLH and ferritin**

We also checked the stability of the bilayer during the AFM measurements very carefully using the variation of adhesion force between the tip and substrate. In this work, we carefully selected and employed AFM tips showing similar adhesion forces against HOPG (1.5 nN) to avoid the effect of the differences in tip geometry. The selected tip fully covered with Δ1-LF or minT1-LF, showed the adhesion forces against Si substrates about 0.5 and 2.5 nN, respectively, at the same loading rate. After many approach and retract cycles, the adhesion force between the tip and Si substrate sometimes showed a sudden rise to a much higher value (above 4 nN). This is explained by the exfoliation of ferritin molecules from PBLH film, because the tip covered with PBLH showed similar adhesion force and a bare Si₃N₄ tip exhibited almost no adhesion force. In most cases, the hetero-bilayers were stable for more than 100 cycles of approaches and retractions. With these findings taken together, we concluded that the ferritin is rigidly immobilized on the AFM tip.²

**Effect of solution conditions on the adhesion force**

We checked the effect of salt and pH value. Figure 1 and 2 show the adhesion force measured in Tris-HCl buffer (pH 8.0) with NaCl (150mM) and in this buffer containing TWEEN20 (0.5 wt%), respectively. We found there is little effect of the condition of the solution on the adhesion force, indicating that ions in solution do not play an important role in the adhesion event.


References & Comments

(2) In the case of TWEEN20 added to the solution, we checked the immobilization of ferritin on the tip by going back to pure water and clean substrates, and examined the adhesion force. In most of the case, the adhesion force represented same value as before, indicating that the surfactant molecules are easily removed from the surface of ferritin molecules.
Figure Captions

Figure 1s the adhesion forces measured in Tris-HCl buffer (pH 8.0, 50 mM) with NaCl (150 mM).

Figure 2s the adhesion forces measured in Tris-HCl buffer (pH 8.0, 50 mM) with NaCl (150 mM) containing TWEEN20 at a concentration of 0.5 weight percent.
Figures 1s

Hayashi et al.
Figure 2s

Hayashi et al