Controlled charged amino acids of Ti-binding peptide for surfactant-free selective adsorption

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Abstract

The adsorption mechanism of titanium-binding peptide (TBP) on metal oxide substrates was investigated by evaluating the adsorption behavior of ferritins with various alanine-substituted TBPs. Results revealed that (a) a positively charged amino acid, lysine (K) or arginine (R), in TBP can anchor ferritin to negative zeta-potential substrates, (b) the adsorption force of K is stronger than R, and (c) local electrostatic interactions and flexibility of TBP directly affect adsorption. Based on these findings, selective ferritin adsorption on SiO_2 with TiO_x patterned surfaces in a surfactant-free condition was demonstrated. Alanine-substituted TBP with one positively charged amino acid (K) and one negatively charged amino acid (D), achieved ferritin-selective adsorption without a surfactant. The importance of controlled electrostatic forces between TBP and a substrate for selective adsorption without a surfactant was clearly demonstrated.

Graphical abstract

Keywords

Titanium-binding peptide; ferritin; adsorption mechanism; selective adsorption

1. Introduction

Nanoparticles (NPs) are fundamental elements in nanodevices [1-4] and nanotechnology [5-8]. Unlike bulk materials, NPs exhibit extraordinary optical [9-12], electronic [10,11], magnetic [11,13] and catalytic [14,15] properties and are thus now acknowledged as powerful tools in the fabrication of novel nanodevices such as solar cells, floating gate memory devices, and single electron transistors. A wide variety of NP-fabrication methods have been studied, including laser ablation [16,17], liquid-phase synthesis [18,19], chemical vapor-phase synthesis [20,21] and ferritin biomineralization [22-26]. Due its advantage of mass production of homogeneous NPs in one-batch, production of NPs by ferritin biomineralization has been actively researched. Ferritin is a cage-shaped protein that stores iron, and its native protein shell is composed of 24 light (L) and heavy (H) subunits that respectively have molecular weights of around 18 and 21 kDa, and has an outer and inner diameter of 12 and 7 nm, respectively. The mechanism of biomineralization in an approximately 7-nm cavity of ferritin *in vivo* and *in vitro* has been intensively studied [22,23]. Based on the resulting knowledge, many types of inorganic NPs have been synthesized in ferritin cavities by artificial biomineralization [24-26].

From the NP application point of view, it is crucial that NPs are placed and arrayed properly for effective use of their characteristic functions in nanodevices [27-30]. For NP arrangement, material-binding peptides have been attracting attention [31-35], particularly an adsorption mechanism of titanium-binding hexapeptide (TBP; RKLPGA) [36], identified by Sano et al.[37-44]. Our research group genetically modified the outer surface of a ferritin composed of only 24 L-subunits of horse spleen ferritin, called Fer0, with TBPs and synthesized various types of NPs in ferritin cavities because we expected the recognition ability of TBP to achieve a target NP arrangement for device fabrication [39]. The Fer0s with various NPs were successfully delivered to titanium oxide (TiO_x) patterns on a SiO₂ substrate with the help of a Tween20 surfactant [37,45].

As described above, Tween20 plays an important role in selective adsorption of material-binding peptides [30,37,40,45-47]. Although surfactants reportedly suppress non-specific adsorption of biomolecules [48-50], their use might be a drawback in nanodevice fabrication due to possible organic contamination that usually severely hinders device performance. Even in the arrangement of biomolecules, surfactants can affect biomolecule conformation, denature the proteins, and finally lead to unpredictable effects [51,52]. Therefore, surfactant-free selective material-binding peptide adsorption is needed.

The general adsorption mechanism of TBP, in which the amino acid arginine (R) and aspartic acid (D) interact with local charges of a TiO₂ substrate, was suggested by Sano et al. [36]. Hayashi et al. investigated the binding forces of Fer0 and Fer0s with alanine-substituted TBPs to TiO₂ substrates by using force curve measurements via atomic force microscopy (AFM) [41]. Their results revealed

the effect of each charged amino acid (R, lysine (K) and D) on the adhesion force and indicated that two electrostatic interactions are crucial. One interaction is between the positively charged R residue and an acidic site on a Ti substrate, and the other is between the negatively charged D residue and a basic site. The K residue has only a minor contribution to adsorption. Molecular dynamics (MD) simulations indicate that R is the main contributor to adsorption [42,43], and that K and D contribute to equilibrium adsorption on a Ti substrate.

In this study, we investigated surfactant-free TBP selective adsorption based on the above described TBP binding mechanism. First, we quantitatively evaluated the TBP binding mechanism by using AFM observation to determine the adsorption amounts of Fer0s and five types of Fer0 mutants having TBP or alanine-substituted TBPs (Table 1). The focus was the role of each single charged amino and charged amino acid combination in TBP. Then, we analyzed the elution curves of the mutant Fer0s by ion exchange chromatography to evaluate the electrostatic interaction of these Fer0s on charged surfaces. Finally, based on the revealed mechanism, we demonstrated surfactant-free selective adsorption of mutant Fer0s onto TiO_x patterns on a SiO_2 substrate.

2. Materials and methods

2.1. Substrate preparation

A thermally oxidized silicon substrate [p-type Si(100) with a 3-nm-thick thermal oxide layer] was dipped in acetone for a few minutes, rinsed with ultrapure water, immersed in piranha solution [H_2SO_4 : 30% H_2O_2 = 3 : 1 (V : V)] at 110°C for 15 min, and carefully rinsed again with ultrapure water for a few minutes. After washing, the substrate was blow-dried using nitrogen gas. A Ti layer (5 nm) was deposited on the SiO_2 substrate by using a sputtering apparatus (E-200S, Canon ANELVA), and then exposed to air to form a native oxide layer (TiO_x).

Ti disk patterns were then fabricated on a SiO_2 surface using electron beam (EB) lithography and a lift-off process. A positive EB resist (ZEP-520A, ZEON) was spin-coated onto the thermally oxidized Si substrate and baked at 150°C for 3 min. Nano-holes (100-nm diameter with 250-nm pitch) were patterned using EB lithography (ELS-7500, ELIONIX. INC.). Exposed samples were developed in a solution of n-Amyl acetate (ZED-N50, ZEON) for 1 min and blow-dried with nitrogen gas. A 1.5-nm-thick Ti layer was deposited on the EB resist pattern using EB deposition (VPC-1100, ULVAC KIKO, Inc.). The samples were dipped in Dimethylacetamide (ZDMAC, ZEON) at 40°C for 5 min to remove the EB resist and then rinsed with methanol and ultrapure water. After the lift-off process, the surfaces of the samples were cleaned by UV/ozone treatment at 70°C for 20 min, and thus the top Ti surface was oxidized TiO_x .

2.2. Fer0 with alanine-substituted TBPs

A group of alanine substitution mutants of RKD(TBP)-Fer0 in TBP, including D-, K-, R- and

KD-Fer0, were constructed genetically by replacing target amino acid(s) with alanine residue(s), as summarized in Table 1. Briefly, synthetic 5'-phosphorylated oligonucleotide duplexes were ligated to the EcoRI- and SacI-digested RKD-Fer0s expression vector (pKIS1), which codes RKD-Fer0 protein, as previously reported [39]. The oligonucleotides used for each mutant were as follows: (a) for D-Fer0, 5'-AA TTC ATG GCC GCA CTT CCG GAT GCG AGC T-3' & 5'- CGC ATC CGG AAG TGC GGC CAT G-3'; (b) for K-Fer0: 5'- AA TTC ATG GCC AAA CTT CCG GCT GCG AGC T-3' & 5'- CGC AGC CGG AAG TTT GGC CAT G-3'; (c) for R-Fer0: 5'- AA TTC ATG CGC GCA CTT CCG GCT GCG AGC T-3' & 5'- CGC AGC CGG AAG TGC GCG CAT G-3'; and (d) for KD-Fer0: 5'-AA TTC ATG GCC AAA CTT CCG GAT GCG AGC T-3' & 5'-CGC ATC CGG AAG TTT GGC CAT G-3'. All these alanine substitution mutants were expressed in E. coli and purified, as previously reported [39].

2.3. Adsorption method

The Fer0s with alanine-substituted peptides, RKD-Fer0s and Fer0s, were diluted with a 50 mM Tris-HCl buffer (pH 8) to a final concentration of 20 µg/ml with and without 0.5% Tween20. The substrate was dipped into the respective ferritin solution for 3 min, and then rinsed with the buffer solution (pH 8) three times and ultrapure water two times. After rinsing, the substrate was blow-dried using nitrogen gas.

2.4. AFM observation

The absorption densities of the ferritin on both SiO₂ and TiO_x substrates were evaluated using AFM with SPM400 (SII NanoTechnology Inc.) and SPI3800 (SII NanoTechnology Inc.). Selective adsorption of KD-Fer0s was observed using AFM with SPM9600 (SHIMADZU). The AFM observation was conducted in dynamic force mode in air at room temperature. The adsorption densities were evaluated at least five times on each sample.

2.5. Chromatography

The elution curves of all the ferritin samples were obtained using the AKTA Explorer 10S chromatography system (GE healthcare) with a Q Sepharose High Performance and a SP Sepharose High Performance (GE Healthcare), which were pre-equilibrated with buffer A [50 mM Tris-HCl (pH 8.0)]. Ferritin peptide was adjusted with 50 mM Tris-HCl buffer (pH 8.0) to a final concentration of 5 mg/ml. A 500 µl ferritin peptide solution was loaded into the column. The flow rates in Q Sepharose and SP Sepharose were controlled at 2 ml/min and 1 ml/min, respectively. Gradient buffer B was 1 M NaCl in the equilibration buffer at pH 8.0 in Q Sepharose. The ionic gradient ran from 0 to 300 mM sodium sulfate. For SP sepharose, buffer B did not contain NaCl, i.e., buffer A was used. Absorbance of the protein was monitored at 280 nm. We confirmed that the

elution curves by cation- and anion-exchange chromatography have the same trend by repeating the measurements twice.

3. Results and Discussion

3.1. Adsorption of Fer0 with alanine-substituted TBPs on SiO₂ and TiO_x

Fer0s were genetically modified with TBP and four types of alanine-substituted TBPs (Table 1), and the peptides were added to each N-terminus of a Fer0 subunit. Fig. 1 is the space-fill model of unmodified L-ferritin, showing the N-terminus in yellow where peptides are attached, and showing the evenly distributed acidic amino acids in red and basic amino acids in blue. Fer0 has a net negative charge at pH 8, and thus Fer0s have repulsive intermolecular forces at pH 8.

Our focus here was the role of each single charged amino and charged amino acid combination in TBP. The D-Fer0 has one negatively charged amino acid (D) in the peptide, and the K- and R-Fer0s each have one positively charged amino acid (R or K) in the peptide. The KD-Fer0 has one positively charged amino acid (K) and one negatively charged amino acid (D) at the 2nd and 5th positions, respectively. The six types of Fer0s were adsorbed on acidic oxide (SiO₂) and amphoteric oxide (TiO_x) substrates at pH 8 and the adsorption densities were measured by AFM. At pH 8, the SiO₂ and TiO_x substrates had negative zeta-potential (approximately -45 mV and -20 mV, respectively) [44], as did the Fer0s and TBP-Fer0s [41].

Fig. 2 shows the adsorption densities of the Fer0, D-, K-, R-, KD- and RKD-Fer0s on SiO₂ and TiO_x substrates. (Typical AFM images are given as supplementary data.) The Fer0s did not adsorb on the SiO₂ substrate, and scarcely adsorbed on the TiO_x substrate. A SiO₂ substrate has only negative charges, and although a TiO_x substrate also has negative zeta potential it has local negative and positive charges. The strong repulsive force between the Fer0s and both substrates inhibited Fer0 adsorption. The D-Fer0s did not adsorb on either substrate. Because D-peptide did not have positive charges, it is reasonable to assume that D-Fer0s could not adsorb on a SiO₂ substrate. However, although the D-peptide can theoretically interact with a local positive charge on a TiO_x substrate by a local negative charge of D, our results showed that the repulsive force between the Fer0 surface and this substrate governed adsorption and that the adsorption force between D and a local positive charge on the TiO_x substrate was weak.

The K- and R-Fer0s were adsorbed on both the SiO₂ and TiO_x substrates (Fig. 2). Because Fer0s and RKD-Fer0s have negative zeta potential at pH 8, the zeta potentials of K- and R-Fer0s are theoretically negative, which means that there were repulsive forces between the mutant Fer0s and substrates. Contrary to expectation, K- and R-Fer0s were adsorbed on the substrates. This indicates that each single positively charged amino acid (K or R) of the peptides anchored mutant Fer0 on a substrate by overcoming the repulsive force. Although it has been reported that R is mainly responsible for adsorption onto a surface and that K assists the adsorption of TBP, we clarified that K

alone has sufficient ability to adsorb Fer0.

The adsorption densities of KD-Fer0s on SiO₂ and TiO_x substrates were drastically different. The KD-Fer0s adsorbed well on the TiO_x substrate, but only slightly on the SiO₂ substrate. This difference was caused by the difference in substrate surface charges. In a pH 8 solution, SiO₂ substrates have only negatively charged sites, whereas TiO_x substrates have both negatively and positively charged sites. With the TiO_x substrate, both K and D anchored the ferritin molecule to the substrate. However, with the SiO₂ substrate, only K anchored the ferritin to the substrate. Moreover, D acted as a repulsive force on the SiO₂ substrate. As a result, the KD-Fer0 could be adsorbed only on the TiO_x substrate.

The adsorption density of RKD-Fer0s on the SiO₂ substrate was lower than that of K- and R-Fer0s, although the RKD-peptide had two positive charges. This suggests that the repulsive force acted between the D of the RKD-peptide and the substrate, which in turn decreased the adsorption amount. On the TiO_x substrate, the adsorption amount of RKD-Fer0s was similar to that of K- and R-Fer0s. This was unexpected when considering the local charges of the TiO_x substrate. The reason for this similarity in adsorption amount despite the local charges remains unclear.

The above results can be summarized as follows. On SiO₂ substrates, Fer0s with peptides having a negatively charged amino acid did not adsorb. The KD-Fer0s scarcely adsorbed because the D of the peptide caused a repulsive force. When a peptide had only a positively charged amino acid or had positively charged amino acids outnumbering negatively charged amino acid(s), Fer0s with peptides were adsorbed. In conclusion, positively charged amino acid(s) of a peptide are the main contributor to adsorption of Fer0s with peptides on SiO₂ substrates. On TiO_x substrates, Fer0s with peptides having positively charged amino acid were adsorbed. The R or K showed a strong interaction with the local negative charge on the TiO_x substrate. The D-Fer0s were not adsorbed on the TiO_x substrate, indicating that interaction force(s) between aspartic acid(s) of the peptide(s) and local positive charge(s) of a TiO_x substrate is(are) weaker than the overall electric repulsive force between Fer0 and a TiO_x substrate.

3.2. Adsorption force of Fer0 with alanine-substituted TBPs

To better understand the adsorption force of Fer0s with/without peptides, we carried out anion- and cation-exchange chromatography experiments at pH 8.0. Fig. 3 shows the measured elution curves of the six types of Fer0s, and Fig. 4 shows schematics of the electrostatic force direction of these Fer0s on negatively and positively charged substrates based on the results in Fig. 3. In the anion-exchange chromatography (performed with an ionic strength gradient), the elution peak of Fer0s was 79.6 ml (Fig. 3a), and that of D-Fer0 was slightly higher (80.8 ml), indicating enhanced attractive force between D-peptide(s) and anion exchange resin beads, as shown in Fig. 4(a-2). Among the six Fer0s, K-Fer0s were eluted at the lowest ionic strength, and R-Fer0s were at the

second lowest. This suggests that the effect on the adsorption force from R and K was slightly different and that the K-peptide had a higher interaction ability compared with the R-peptide, as shown in Figs. 4(a-3, a-4). The KD-Fer0s were eluted at an intermediate ion strength between D-Fer0 and K-fer0. This is reasonable because the K and D of the peptide act in opposite directions on the anion exchange resin, i.e., repulsive and attractive interactions (Fig. 4(a-5)). The RKD-Fer0s were more easily eluted than KD-Fer0s. The two positively charged amino acids of the RKD-peptide showed stronger repulsive interaction than did KD-Fer0 on the anion exchange resin, and thus led to elution with low ionic strength. However, the elution curve of RKD-Fer0s showed that the repulsive force was weaker than that of K- and R-Fer0s. This also indicates that the negatively charged amino acid (D) of the RKD-peptide acted as an adsorption force (Fig. 4(a-6)).

The results of cation-exchange chromatography (carried out without an ionic strength gradient) for the six types Fer0s are shown in Fig. 3b. The buffer volume required for elution roughly corresponds to the adsorption forces on the negatively charged resin. Ferritins have negative zeta-potential at pH 8, and therefore for all six types of Fer0s, strong repulsive forces existed between the Fer0 and the cation exchange resin beads. However, because the positively charged amino acid(s) in the peptides were expected to interact with negatively charged resins, the buffer volumes for elution would differ. As seen in Fig. 3b, D-Fer0s were eluted with a lower buffer volume than Fer0s, indicating that D enhanced the repulsive force on the negatively charged resin, as shown in Fig. 4(b-2). The D of the peptide added a repulsive force to Fer0s. The elution peaks of K- and R-Fer0s differed, namely, K generated more attractive force than R to the negatively charged resin, as shown in Figs. 4(b-3,b-4). This difference in attractive force also explains the higher adsorption for K-Fer0s than for R-Fer0s on SiO₂ substrates (Fig. 2). These results are consistent with the anion-exchange chromatography results. On the contrary, elution peaks of KD-Fer0s and Fer0s in the cation-exchange chromatography were the same (Fig. 3b), results that differ from those in the anion-exchange chromatography (Fig. 3a). This inconsistency might be explained as follows: in the case of anion-exchange chromatography, the gross adsorption force between Fer0 and a positively charged resin affects the adsorption force of KD-Fer0, and K acts as a repulsive force (Fig. 4(a-5)). In the case of cation-exchange chromatography, the adsorption force of K is cancelled out by the repulsive force of D, as shown in Fig. 4(b-5). Another inconsistency is that RKD-Fer0s exhibited the strongest adsorption force on negatively charged resins, although based on the results of anion-exchange chromatography it was anticipated that K-Fer0s would have the strongest adsorption force. The adsorption force of positively charged amino acids (R and K) exceeded the repulsive force of the negatively charged amino acid (D) on a negatively charged resin (Fig. 4(b-6)). However, on SiO₂ substrates, the adsorption amounts of RKD-Fer0s were less than those with K- and R-Fer0 (Fig. 2). The reason for this difference in adsorption amount is unclear. The adsorption mechanism revealed by the ion exchange chromatography can be summarized as follows: (a) the adsorption force of K is

1 stronger than R on a negatively charged substrate, (b) D of a peptide enhances the action of the
2 negative potential of ferritins, and (c) the adsorption forces of Fer0s with peptides on SiO₂ substrates
3 are very weak compared with TiO_x substrates.

4 The geometrical configuration of peptides is also important for binding on a flat inorganic material.
5 Proline (P) reportedly determines the conformation of peptide and affects adsorption [36,38,41]. The
6 Fer0s surface has fixed charges, as shown in Fig. 1. In a previous study, we reported that Fer0s do
7 not adsorb on negative zeta-potential substrates, even if such substrates have local positive charges
8 [44]. On the other hand, in this study, Fer0s with peptides have positively charged amino acid(s) that
9 adsorb on negative zeta-potential substrates. These results show that the flexibility of peptides is
10 important for ferritin adsorption. Along with high flexibility, the position of charged amino acid(s)
11 might also affect adsorption of a peptide because the P of a peptide affects adsorption. Proline seems
12 to block interactions among the charged amino acids in the TBP. If charged amino acids interact in
13 the TBP, KD-Fer0 will not be adsorbed on TiO_x, because K interacts with D by electrostatic
14 interaction and the charges of K and D do not affect the adsorption to the substrate. However,
15 KD-Fer0s adsorbed on TiO_x substrate in this study. Thus, it is suggest that charged amino acids did
16 not interact in the TBP.

18 3.3. Effect of Tween20 for selective adsorption

19 We now shift focus to the effect of Tween20 for selective adsorption. It has been generally accepted
20 that Tween20 is necessary for selective binding of TBP [30,37,40,45-47]. To investigate the effect of
21 Tween20, we compared adsorption densities of all six types of Fer0s with and without Tween20 on
22 both SiO₂ and TiO_x substrates (Fig. 5). The Fer0 and D-Fer0 did not adsorb on either substrate,
23 regardless of the presence of Tween20. These results indicate that there was a strong repulsive force
24 between these ferritins and substrates, which hindered their adsorption, and that Tween20 had no
25 effect on their adsorption.

26 On SiO₂ substrates, the adsorption density of K-, R- and RKD-Fer0s drastically decreased to zero
27 with Tween20. The cation-exchange chromatography results (Fig. 3b) suggest that the anchoring
28 forces of K-, R- and RKD-Fer0s to SiO₂ substrates are weak. The overall electrostatic repulsive
29 force between ferritins and substrates weakens the anchoring force of positively charged K and R to
30 negative charges on SiO₂ substrates. Moreover, because Tween20 adsorbs on SiO₂ substrates [40], K
31 and R could not anchor to SiO₂ and thus cannot be adsorbed on SiO₂ substrates.

32 On TiO_x substrates, K-, R-, KD- and RKD-Fer0s adsorbed on the substrates with or without
33 Tween20. It is known that the gross repulsive force between ferritins and a TiO_x substrate is weaker
34 than with a SiO₂ substrate due to the difference in their zeta potential. Furthermore, SiO₂ and TiO_x
35 substrates have different surfactant adsorption amounts [40]. Tween20 reportedly adsorbs less on
36 TiO_x substrates than on SiO₂ substrates. Therefore, K-, R-, KD- and RKD-Fer0s can be adsorbed on

TiO_x substrates in the absence of Tween20.

3.4. Selective adsorption without Tween20 on TiO_x patterned on SiO₂ substrates

Even in the absence of Tween20, KD-Fer0s showed clear difference in adsorption between the SiO₂ and TiO_x substrates (Fig. 2). The KD-Fer0s adsorbed well on the TiO_x substrate, but only slightly on the SiO₂ substrate. Based on the results of anion- and cation-exchange chromatography (Fig. 3), the adsorption mechanism of KD-Fer0 can be schematically illustrated as in Fig. 6. As described above in sections 3.1 and 3.2, the gross repulsive force between ferritins and SiO₂ substrates is strong (Fig. 6a). The K attempts to anchor ferritin to the SiO₂ substrate, while the D sufficiently increases the repulsive force to detach ferritins (Fig. 6a). On TiO_x substrates, the gross repulsive force is weaker than that on SiO₂ substrates (Fig. 6). Both K and D adsorb on local charges of TiO_x substrates (Fig. 6b). The adsorption force of D is weak because ion-exchange chromatography results suggest D affects the interaction force although D-Fer0 could not be adsorbed on TiO_x substrate. In conclusion, the electrostatic forces critically govern the adsorption mechanism.

These results indicate the possibility of selective adsorption of KD-Fer0s on the TiO_x pattern on a SiO₂ substrate without Tween20, which is generally accepted as an indispensable reagent for selective adsorption. To demonstrate such surfactant-free selective adsorption, we fabricated TiO_x patterns on a SiO₂ substrate, and adsorbed KD-Fer0s. Fig. 7 shows an AFM image of KD-Fer0s selectively adsorbed on a TiO_x pattern without Tween20. In conclusion, ferritins having peptides of appropriately controlled amino acid charges can be selectively adsorbed on an amphoteric oxide pattern on an acidic oxide substrate without Tween20.

4. Conclusions

The adsorption mechanism of charged amino acids of the RKD-peptide (TBP) was clarified as follows.

- (a) The adsorption force of K is stronger than that of R, although R reportedly is the main contributor to adsorption.
- (b) Fer0s with peptides that have one positively charged amino acid as the charged amino acid, can be adsorbed onto oxide substrates.
- (c) On a SiO₂ substrate, adsorption of Fer0s requires more positively charged amino acid(s) (R, K) of a peptide than negatively charged amino acids (D).
- (d) On a TiO_x substrate, Fer0s with peptides that have both positively- and negatively-charged amino acids, can also be adsorbed.

Based on these findings, selective adsorption of ferritins was achieved with alanine-substituted TBP (KD-Fer0) in the absence of a surfactant, revealing that highly selective adsorption without a surfactant requires the control of the interaction forces. The achievement of surfactant-free selective

adsorption is a breakthrough for device fabrication because organic contamination by a surfactant is thus eliminated.

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FIGURE CAPTIONS

Graphical abstract. AFM image of selective adsorption and adsorption mechanism schematics of KD-Fer0s on TiO_x and SiO_2 . In the AFM image, small whitish particles denote KD-Fer0, and the larger light brown area (about 100 nm in diameter) denotes a TiO_x pattern. In the adsorption mechanism schematics, red and blue circles respectively denote negatively and positively charged amino acids, dotted and black arrows respectively denote weak and strong interaction forces, and up and down arrows respectively denote repulsive and attractive forces.

Fig. 1. Illustration of residue at N-terminus of L-subunits (yellow), acidic amino acids (red) and basic amino acids (blue) on Fer0, and the gray shows amino acids except the residues and the acids. This figure was created using RasMol.

Fig. 2. Adsorption densities of Fer0, D-, K-, R-, KD- and RKD-Fer0s on SiO_2 and TiO_x substrates. Asterisks (*) represent no ferritin adsorption. Error bars represent mean $\pm$ standard deviation from five independent experiments.

Fig. 3. Elution curves of Fer0, D-, K-, R-, KD- and RKD-Fer0s by (a) anion- and (b) cation-exchange chromatography performed with and without ionic strength gradient, respectively. Numbers denote elution peak volume.

Fig. 4. Schematics of electrostatic force direction of 1) Fer0, 2) D-, 3) K-, 4) R-, 5) KD- and 6) RKD-Fer0s on (a) positively and (b) negatively charged substrates based on results of elution curves of each sample by anion- and cation- exchange chromatography in Fig. 3. Large red circles denote Fer0 molecules. Small blue and red circles respectively denote positively and negatively charged amino acids. White arrows denote total electrostatic force of Fer0. Black arrows denote local electrostatic force between charged amino acids and substrates. Arrow width denotes force strength. Dotted lines denote peptides, but peptide conformation was not certain.

Fig. 5. Effect of surfactant Tween20 on adsorption densities of Fer0, D-, K-, R-, KD- and RKD-Fer0s on (a) SiO_2 and (b) TiO_x substrates. Asterisks (*) represent no ferritin adsorption. Error bars represent mean $\pm$ standard deviation from five independent experiments.

Fig. 6. Schematic of KD-Fer0 adsorption mechanism on (a) SiO_2 and (b) TiO_x substrates. Components defined same as in Fig. 4, except small blue and red circles respectively show K (positively) and D (negatively) charged amino acids.

Fig. 7. AFM image of KD-Fer0s adsorption on $\text{TiO}_x/\text{SiO}_2$ pattern without Tween20. Small whitish particles denote KD-Fer0, and larger light brown area (about 100 nm in diameter) show the TiO_x pattern.

1 FIGURES

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3 Fig. 1.

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Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

1 TABLE

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3

4 **Table1.** Name and amino acid sequence of TBP and alanine-substituted TBPs

5

Name	Amino acid sequence
RKD (=TBP)	R K L P <u>D</u> A
D	AAL P <u>D</u> A
K	A K L P A A
R	R AL P A A
KD	A K L P <u>D</u> A

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7 Bold and underlined letters indicate positively and negatively charged amino acids, respectively.

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