Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

Adsorption of nucleotides and nucleic acids on goethite nanoparticle: mode, sites and relationship with phosphate and non-phosphate structure

There are 9 texts, 3 tables, and 20 figures.

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Text-S1. Sources of chemical P-OH pK_a values. The pK_a values of P-OH groups for phosphoric acid (PA) are from reference 1. The pK_a values of P-OH for AMP, CMP, GMP, and dTMP are from reference 2. For dUMP, ATP, ADP, CTP and CDP, pK_a values of P-OH groups >4 were determined by acid-base titration (see Fig. S1), while pK_a values <4 were estimated by Marvin Sketch software due to the low accuracy of acid-base titration when pH is low (pH<3). The acid-base titration was conducted in 5.00 mM KCl by a modified batch method.[3] A solution containing 1.00 g/L compound with pH12.00 (adjusted by NaOH) was prepared. Then, 5.00 mL compound solutions were mixed with different amounts of 20 mM HCl (0-5.00mL) and 5.00 mM KCl solution to a final volume of 10.0 mL. After that, the sample vials were shaken and mixed for 1 hour at 150 r/min at 25±1 °C in dark, and the pH of the solution was measured at 25±1 °C. The 5.00 mM KCl solution without the addition of the compound was conducted as a control. The difference of HCl consumed for a final pH was derived to obtain the amount of H^+ consumed by phosphate compounds (Q_H, μmol). The pK_a value of the compound was derived by regression analysis of the measured final pH and Q_H using the proton consumption formula for chemicals (or solids) containing Brønsted acid/base groups [2] as follows:

$$Q_{\mathrm{H}} = \sum_{i=1}^{n} \{C_{\mathrm{T},i} \times [\frac{1}{1+10^{(\mathrm{pH}-\mathrm{p}K_{\mathrm{a},i})}} - \frac{1}{1+10^{(\mathrm{pH}_0-\mathrm{p}K_{\mathrm{a},i})}}]\}$$

where n was the number of acidic functional groups in the compounds, $C_{T,i}$ was the amount of acidic functional groups (μmol), p$K_{a,i}$ was the pK_a value of acidic group i, and pH was the pH value of the solution at acid-base equilibrium, and pH_0 was the pH value when no H^+ was consumed by phosphate compound. n was dependent on the structure of the phosphate compound, the possible pK_a value, and the pH range of acid-base titration. $C_{T,i}$, pH and Q_H were obtained from experiments (Q_H was obtained when the amount of HCl consumed in nucleotide solution subtracted from that consumed in 5.00mM KCl solution at the same final pH), p$K_{a,i}$ and pH_0 were constants estimated by regression analysis. Fig. S1a depicted the determined titration curve of phosphate compound solutions and 5.00 mM KCl solution. The calculated Q_H ~ pH and regression analysis derived results were shown in Fig. S1b.

Text-S2. Preparation of goethite. Goethite was synthesized by a method modified from Cornell and Schwertmann[4]. 90 mL 5 M NaOH (4°C) was mixed with 50 mL 1M $FeCl_3.6H_2O$ solution (4°C) by magnetic stirring, and then diluted to 1 L using deionized water. The magnetic stirring lasted for 10 min, and the derived solution was aged at 70 °C for 60 h. Thereafter, 850 mL supernatant was discarded, and the residual suspension was placed in a dialysis bag of 8000-14000 and dialyzed in deionized water. The deionized water was replaced every 12 hours until the conductivity of external water was ≤ 10 μS/cm.

Text-S3. Adsorption kinetics experiments. The batch adsorption method was employed. Compound solution (1.00 mL, 100±5 mg/L, pH 7.00) was mixed with goethite suspension (pH 7.00), shaken horizontally at 150 r/min in dark at 25 °C for 5, 10, 20, 30, 60, 120, 180, 240 and 300 min. At each time, the suspension was filtered through a 0.22 μm filter, and the phosphorus (or phosphate compound) concentration was measured. Vials containing only compound solutions were also conducted as control groups. No significant change in compound concentration with shaking time was observed in the control groups, suggesting a negligible degradation of compounds.

Text-S4. pH change experiment and the influence of coexisting ions. OH^- release-caused pH change, influence of coexisting ions as a competing solute in NNA adsorption, and effect of PA pre-adsorption on NNA adsorption, were measured to discriminate the adsorption mode and sites of NNAs on goethite. For the pH change experiment, a goethite suspension and a chemical solution with an initial pH of 7.00-7.05 were mixed, and shaken horizontally at 150 r/min in dark at 25±1 °C. The pH of the solution and phosphate concentration were determined after adsorptive interactions. To assess the influence of coexisting ions on adsorption, 2 mmmol/L KCl or 2 mmol/L Na_2SO_4, corresponding to 2-fold of the mole concentration of P-O(H) in the selected chemicals, was added. The solution pH after adsorption was controlled to pH 7.00-7.05 by adding diluted HCl solution. The percentage of NNA adsorbed was derived to examine whether the coexisting Cl^- and SO_4^{2-} could compete against phosphate via electrostatic interactions on goethite. The detailed amounts of goethite suspensions and chemicals added in the pH change and the influence of coexisting ions experiments were listed in Fig. S2. To assess the effect of PA pre-adsorption on organic phosphate adsorption, 100 mmol/L PA (1000 μL) was directly added into goethite suspensions (600 μL), shaken horizontally in dark at 150 r/min at 25±1°C for 120 min (final pH was about pH 7.00-7.05), and then organic phosphate (1000 μL, 1.00 mmol-P/L, pH7.00) was added, shaken horizontally at 150 r/min in dark at 25±1°C for 120 min. The final pH was controlled to pH 7.00-7.05 by adding HCl immediately after addition of organic phosphate solution to consume the released OH^-.

Text-S5. OH^- release and its quantification. Quantification of the amount of OH^- released during adsorption (referred to as Q_1 in Fig. S5a) was necessary for analyzing the stroichiometric relationship between Fe-O-P bonding and OH^- release. An experiment was conducted, which involved buffering OH^- release by HCl titration (Fig. S5). As depicted in Fig. S5a, the OH^- released from goethite (Q_1) was partly consumed by H^+ from the P-OH group of nucleotides (or nucleic acids) (Q_2) involved in the formation of Fe-O-P bonds. The remaining released OH^- (referred to as apparent amount of OH^-

released, Q_a) caused an increase in system pH. Due to the pH buffering effects of goethite, nucleotides (or nucleic acids), and carbonate in the system, a portion of the apparent OH^- release was consumed by these buffering substances ($BiH + OH^- \rightarrow Bi^- + H_2O$, where BiH represents the acid group of the buffering substances). However, it was difficult to accurately quantify the amount of OH^- consumed by BiH due to the lack of precise information regarding the amount and pK_a of the buffering substances involved in pH buffering. Consequently, HCl titration was employed to neutralize the OH^- consumed by BiH (i.e. $Bi^- + H^+ \rightarrow BiH$) in order to determine Q_a (Fig. S5b). In summary, different volumes of 1.86 mmol/L (or 1.82 mmol/L) HCl (given that the HCl concentration was insufficient to dissolve goethite) were mixed with a suspension of goethite (initial pH 7.00-7.02) and a chemical solution (initial pH 7.00-7.02), resulting in dynamic equilibrium pH values ranging from above pH 7.0 to below pH 7.0. The amount of Q_a was calculated based on the volume of HCl consumed at the equilibrium pH (7.00) (please refer to Table S2, Fig. S10, S14 and S16 for more details). It is worth noting that, to verify the accuracy of the Q_a determination method, HCl titration was performed in a system containing goethite, nucleotides (or nucleic acid), and carbonate (Fig. S5b). The results showed that the deviation between the OH^- measured by HCl titration and the added OH^- from NaOH was <5%, confirming the accuracy of HCl titration in measuring Q_a. The amount of OH^- consumed by H^+ from the P-OH group of nucleotides (or nucleic acids) (Q_2) was calculated based on the pH, pK_a and adsorption amount of P-O(H). The procedure for determining Q_2 is described in detail in Text-S7 (please refer to the following section).

Text-S6. Method for quantifying phosphorus concentration in nucleic acid solution and PA solution. Since the examined nucleic acids are a mixture of different sizes and their molecular weight are not definite, the phosphorus concentration in the nucleic acid solution cannot be directly calculated based on the molecular weight and the measured mass concentration of nucleic acids. Thus, we opted to measure the phosphorus concentration directly using an ammonium molybdate spectrophotometric method. This method is in accordance with the National standards of the People's Republic of China (GB/T 11893-1989) and is commonly used for quantifying the total phosphorus content in water. The procedure for measuring the phosphorus concentration involves two steps:

1) Potassium persulfate digestion treatment of the nucleic acid solution: In a 10 mL glass scale tube, 5.00 mL of the nucleic acid solution (with a phosphorus concentration below 2 mg/L) and 800 μL of potassium persulfate (50 g/L) were added. The tube is tightly sealed with a small piece of cloth and wire, placed in a large beaker, and heated in an autoclave at 120 °C for 30 minutes. Afterwards, the solution is diluted to 10 mL with deionized water.

2) Spectrophotometric determination: To the digested solution, 200 μL ascorbic acid solution

(100 g/L) is added and mixed for 30 seconds. Then, 400 μL of molybdate solution (prepared by adding 300 mL (1+1)H_2SO_4 to a mixture of 100 mL of 130 g/L ammonium molybdate and 100 mL of 3.5 g/L potassium antimony tartrate) is added and mixed at room temperature for 15 minutes. The absorbance of the solution at 700 nm is measured using water as a reference. The phosphorus concentration is calculated using the obtained absorbance and a standard curve measured using Potassium phosphate monobasic (KH_2PO_4).

To assess the efficiency of this two-step method, we measured the phosphorus concentration in the solution containing CMP (with a mass concentration of 370 mg/L) and CDP (with a mass concentration of 45 mg/L). Subsequently, we calculated the ratio of the measured phosphate concentration to the phosphate concentration derived from the mass concentration and molecular formula. The obtained ratios were 1.06±0.10 for CMP and 1.04±0.01 for CDP, indicating the effectiveness of this two-step method in accurately measuring the phosphorus content in organic chemical having phosphate ester structures.

The procedure for analyzing the phosphorus concentration in the PA solution is the same as that for nucleic acid solution, with the exception that the potassium persulfate digestion step, which converts nucleic acid phosphate esters into phosphoric acid and its anions, is omited.

Text-S7. Experiment for examining the potential influence of tube adsorption, filtration and degradation in adsorption.

i) Experiment for evaluating the influence of tube adsorption and filtration. A solution of nucleotides, nucleic acids and nucleosides was prepared in 5.00 mM KCl with a pH of 7.0. The concentration of nucleotides and nucleic acids in the solutions was equivalent to 1.00 mmol/L phosphorus, while the concentration of nucleosides was equivalent to 0.500 mmol/L. These concentrations fall within the range of solute concentrations used in the batch adsorption experiment (Table S2). Solutions of 2.00 mL were added to 10 mL polypropylene tubes, shaken at 150 r/min at 25 °C in dark for 2 hours, sampled, and filtered through a 0.22 μm filter. The concentrations of nucleosides, nucleotides, and nucleic acids in the solutions before and after tube adsorption, shaking, and filtration were measured by an ultraviolet-visible spectrophotometer with the wavelengths specified in Table S1. The change in concentrations of nucleotides, nucleic acids and nucleosides (φ) was calculated using the following equation:

$$\varphi = \frac{C_{after}}{C_{before}} \times 100\%$$

where C_{after} and C_{before} are the concentrations of nucleotides, nucleic acids and nucleosides before tube adsorption and filtration, and after tube adsorption and filtration, respctively. The results are presented in Fig. S6.

ii) Experiment for evaluating the contribution of adsorption and degradation of nucleotides and nucleic acids caused by goethite. The decrease in the amount of nucleotides, RNA and DNA3 in solution caused by interactions with goethite, as well as the amount of NNAs extracted from goethite, were analyzed to examine the contribution of adsorption and degradation. In a 10.0 mL polypropylene tube, 1000 μL goethite suspensions (16.65 mg/mLgoethite solid content, 5 mmol/L KCl, pH 7.0) was mixed with 1000 μL solutions of nucleotides, RNA and DNA3 (1.00 mmol-P-O(H)/L, 5 mmol/L KCl, pH 7.0). The tubes were shaken at 150 r/min at 25 °C in the dark for 2 hours and then centrifigued at 5000g for 5 minutes. A volume of 1500 μL supernatant was sampled, and the concentration of NNAs (C_e) were detected by an ultramicro spectrophotometer (Nanodrop 2000, Thermo Fisher). Considering that tube adsorption was found to be negligible based on the results in Fig. S6, the decrease in NNA concentration (C_d) in solution was attributed to the dillution of the NNA solution by goethite suspension and interactions with goethite. The equation used is as follows:

$$C_d = \frac{1.00\ mmol - P - O(H)/L \times 1000\ \mu L}{1000\ \mu L\ +\ 1000\ \mu L} - C_e$$

When 1500 μL of the supernatent was sampled, 1500 μL of a solution composed of 100 mmol/L PA (inorganic phosphate) and 100 mmol/L NaCl with a pH of 7.0 was immediately added to extract the NNAs from goethite. This specific solution was chosen for extraction considering the different roles played by PA and NaCl (as described in reference 32 in the main text). After adding the exatraction solution, the tubes was covered, shaken at 150 r/min at 25 °C in the dark for 24 hours, and then centrifuged at 5000g for 5 minutes. The concentration of NNAs in the supernatant of the extraction solution was measured ($C_{\text{extraction}}$). The ratio (R) of the amount of extracted NNAs to the amount of NNAs decreased after interactions with goethite was calculated using the following equation:

$$R = \frac{2000\ \mu L \times C_{extraction} - 500\ \mu L \times C_e}{2000\ \mu L \times C_d}$$

Text-S8. Quantification of $S_{\text{P-OH~OH}^-}$.

i) $S_{\text{P-OH~OH}^-}$ for single-solute adsorption of PA, nucleotide or nucleic acid. $S_{\text{P-OH~OH}^-}$ is the relation between the amount of P-O(H) engaged in ligand exchange (i.e., forming Fe-O-P) and the amount of OH^- exchanged from goethite (Q_1). PA mainly forms bidentate on goethite at pH7 (two P-OH of PA form Fe-O-P linkages).[5] Therefore, the mole amount of P-O(H) engaged in the complexation of PA [$m(P-O(H)_{PA\ complexation})$] was twice that of PA adsorption capacity [$m((PA)_{adsorbed})$] (Fig. S10a):

$$m(P-O(H)_{PA\ complexation}) = 2m((PA)_{adsorbed}) \quad (1)$$

OH^- exchanged by PA from goethite [$m(OH^-_{PA-motivated\ release\ from\ goethite})$, i.e., the Q_1 in Fig. 3a and Fig. S5a] was composed of apparent OH^- release after adsorption [i.e., Q_a, which was

$m(H^+_{added\ for\ PA})$ determined by HCl titration as shown in Fig. S10b] and OH^- consumed by P-OH engaged in Fe-O-P bonding [$m(OH^-_{consumed\ by\ P-OH\ of\ PA})$, i.e., the Q_2 in Fig. 3a and Fig. S5a]:

$$m(OH^-_{PA-motivated\ release\ from\ goethite}) = m(H^+_{added\ for\ PA}) + m(OH^-_{consumed\ by\ P-OH\ of\ PA}) \quad (2)$$

The OH^- consumed by P-OH engaged in Fe-O-P bonding can be calculated from the mole adsorption amount of PA, the pH value and the pK_a value of P-OH. For PA, the pK_a values for the bidentate at pH7.0 should be 2.21 (denoted as pK_a(i)) and 7.21 (denoted as pK_a(ii)) rather than 12.6 (denoted as pK_a(iii)) because P-OH of pK_a(iii) would be more unfavorable than P-O(H) of pK_a(i) and pK_a(ii) in losing H^+ to form Fe-O-P in thermodynamics. Thus, OH^- consumed by P-OH engaged in Fe-O-P bonding was:

$$m(OH^-_{consumed\ by\ P-OH\ of\ PA}) = m(P-OH(i)_{adsorbed}) + m(P-OH(ii)_{adsorbed}) \quad (3)$$

where, $m(P-OH(i)_{adsorbed})$ and $m(P-OH(ii)_{adsorbed})$ are the mole of protonated form of P-O(H) (i.e., P-OH) engaged in Fe-O-P bonding:

$$m(P-OH(i)_{adsorbed}) = m((PA)_{adsorbed}) \times \frac{K_a(i)}{K_a(i) + 10^{-pH}} \quad (4)$$

$$m(P-OH(ii)_{adsorbed}) = m((PA)_{adsorbed}) \times \frac{K_a(ii)}{K_a(ii) + 10^{-pH}} \quad (5)$$

Fig. S10 summarized the results of $m((PA)_{adsorbed})$ and $m(H^+_{added\ for\ PA})$. Fig. S10 depicted the $S_{P\text{-}OH\sim OH^-}$ of PA [plotted as $m(OH^-_{consumed\ by\ P-OH\ of\ PA})$ versus $m(P-O(H)_{PA\ complexation})$] derived from three replicate experiments. The slope 0.727-0.850 (consideration the standard deviation in linear regression analysis) suggested that iron hydroxyls engaged in LE were composed of 72.7-85.0% Fe-OH (which released OH^- in Fe-O-P bonding, see Fig. 3a) and 15.0-22.3% Fe-OH_2^+ (which released H_2O in Fe-O-P bonding, see Fig. 3a).

The method for calculating $S_{P\text{-}OH\sim OH^-}$ for nucleotide or nucleic acid was the same as PA, except that the pK_a value and/or number of P-O(H) groups engaged in Fe-O-P bonding were different. Fig. S12-S14 depicted the amount adsorbed for phosphorus of nucleotide (or nucleic acid) and OH^- released to solution after adsorption, which were equivalent to $m((PA)_{adsorbed})$ and $m(H^+_{added\ for\ PA})$ of PA in respective, in calculation of $S_{P\text{-}OH\sim OH^-}$. Fig. S15 depicted the relation of apparent OH^- release with an adsorbed mass of nucleotide (or nucleic acid).

ii) $S_{\text{P-OH}\sim\text{OH}^-}$ for PA in bi-solute adsorption (coexistence of PA and nucleotide, or coexistence of PA and nucleic acid). In calculation of $S_{\text{P-OH}\sim\text{OH}^-}$ of PA in bi-solute adsorption, apparent OH^- release from adsorption of NNA was excluded based on the amount of apparent OH^- release in adsorption (estimated by volume of HCl added at pH7.00, Fig. S16), amount of PA adsorbed (Fig. S17), amount of nucleotide (or nucleic acid) adsorbed (Fig. S18), and the relationship between nucleotide (or nucleic acid) adsorbed and apparent OH^- release (Fig. S15). When NNA coexisted, the final release amount of OH^- (determined by acid titration, Fig. S16) was contributed by both nucleotide (or nucleic acid) adsorption (Fig. S18) and PA adsorption (Fig. S17). The mole amount of P-O(H) involved in the complexation of PA [$m'(P-O(H)_{PA\ complexation})$] was twice that of PA adsorption capacity [$m'((PA)_{adsorbed})$]:

$$m'(P-O(H)_{PA\ complexation}) = 2m'((PA)_{adsorbed}) \quad (6)$$

The amount of apparent OH^- release from nucleotide (or nucleic acid) adsorption [$m'(OH^-{}_{organic\ P\ adsorption})$] could be calculated by the amount of nucleotide (or nucleic acid) adsorbed in the bi-solute adsorption [$m(organic\ P\ adsorbed)$], and the relationship between OH^- release and nucleotide (or nucleic acid) adsorption [$f(m(organic\ P\ adsorbed))$] derived in single-solute adsorption of nucleotide (or nucleic acid) (Fig. S15):

$$m'(OH^-{}_{organic\ P\ adsorption}) = f(m(organic\ P\ adsorbed)) \quad (7)$$

The amount of apparent OH^- release from PA adsorption in bi-solute adsorption was calculated by subtracting the apparent OH^- release from nucleotide (or nucleic acid) adsorption from the amount of OH^- release determined by HCl titration in the bi-solute adsorption [$m'(H^+{}_{added})$]:

$$m'(OH^-{}_{PA\ adsorption}) = m'(H^+{}_{added}) - m'(OH^-{}_{organic\ P\ adsorption}) \quad (8)$$

OH^- exchanged by PA from goethite [$m'(OH^-{}_{PA-motivated\ release\ from\ goethite})$] was composed of OH^- released to solution after adsorption [$m'(OH^-{}_{PA\ adsorption})$] and OH^- consumed by P-OH engaged in Fe-O-P bonding [$m'(OH^-{}_{consumed\ by\ P-OH\ of\ PA})$]:

$$m'(OH^-{}_{PA-motivated\ release\ from\ goethite}) = m'(OH^-{}_{PA\ adsorption}) + m'(OH^-{}_{consumed\ by\ P-OH\ of\ PA}) \quad (9)$$

The $m'(OH^-{}_{consumed\ by\ P-OH\ of\ PA})$ can be calculated according to the mole adsorption of PA, the pH value, the pK_a value of P-OH, and the bidentate complexation from P-OH having pK_a(i) and pK_a(ii):

$$m'(OH^-{}_{consumed\ by\ P-OH\ of\ PA}) = m'(P-OH(i)_{adsorbed}) + m'(P-OH(ii)_{adsorbed}) \quad (10)$$

where, $m'(P-OH(i)_{adsorbed})$ and $m'(P-OH(ii)_{adsorbed})$ are the mole P-OH engaged in ligand exchange with pK_a(i) and pK_a(ii), respectively:

$$m'(P-OH(i)_{adsorbed}) = m'((PA)_{adsorbed}) \times \frac{10^{-pH}}{K_a(i) + 10^{-pH}} \quad (11)$$

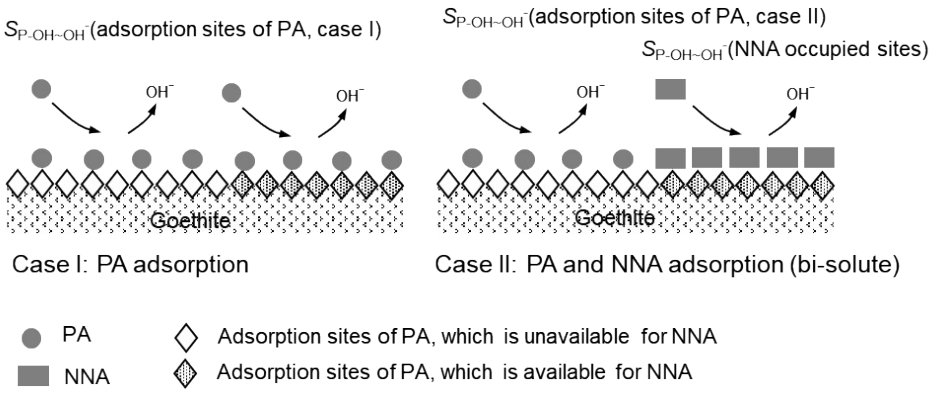
$$m'(P-OH(ii)_{adsorbed}) = m'((PA)_{adsorbed}) \times \frac{10^{-pH}}{K_a(ii) + 10^{-pH}} \quad (12)$$

The $S_{\text{P-OH~OH}^-}$ of PA was derived from the calculated $m'(OH^-_{\ PA-motivated\ release\ from\ goethite})$ and $m'(P-O(H)_{PA\ complexation})$. The results were shown in Fig. 3b.

Text-S9. The logic behind using $S_{\text{P-OH~OH}^-}$ to compare the pK_a values of adsorption sites of NNAs and PA

$S_{\text{P-OH~OH}^-}$ represents the stoichiometric relationship between the OH^- released from goethite (Q_1) and the P-O(H) groups (including P-OH and P-O^-) engaged in Fe-O-P bonding. The value of $S_{\text{P-OH~OH}^-}$ depends on the proportion of Fe-OH (relative to Fe-OH_2^+) involved in Fe-O-P bonding (because the mole ratio of OH^- release to Fe-O-P bond formation is 1 for Fe-OH and 0 for Fe-OH_2^+, while the $S_{\text{P-OH~OH}^-}$ is a sum of them). This proportion is determined by the pH and the pK_a value of Fe-OH_2^+. Therefore, at a given pH, the value of $S_{\text{P-OH~OH}^-}$ depends on the pK_a value of Fe-OH_2^+, suggesting that if two types of sites (Fe-OH_2^+) have the same $S_{\text{P-OH~OH}^-}$ value, their pK_a values would be the same.

The observation that NNA adsorption is blocked by PA (Fig 1d) indicates that the adsorption sites for NNAs are also the adsorption sites for PA. This suggests that the adsorption sites of PA consist of two parts: one part is available for both NNAs and PA adsorption, while the other part is not available for NNAs but only for PA adsorption. Consequently, two types of $S_{\text{P-OH~OH}^-}$ values can be quantified for PA: one from PA adsorption without the coexistence of NNAs (where all adsorption sites are available for PA, case I), and the other from PA adsorption in the presence of NNAs (where some sites are occupied by NNAs and not available for PA adsorption, case II) (see the following scheme).



The $S_{P\text{-}OH\sim OH^-}$ value from case I represents the stoichiometric relationship between the OH^- released from goethite (Q_1) and the P-O(H) engaged in Fe-O-P bonding for all adsorption sites on goethite. This is contributed by the $S_{P\text{-}OH\sim OH^-}$ value from PA adsorption sites and $S_{P\text{-}OH\sim OH^-}$ value from the occupied adsorption sites by NNAs in case II:

$S_{P\text{-}OH\sim OH^-}$(adsorption sites of PA, case I)

$= f \cdot S_{P\text{-}OH\sim OH^-}$(adsorption sites of PA, case II) + $(1\text{-}f) \cdot S_{P\text{-}OH\sim OH^-}$(NNA occupied sites) (13)

where f is the fraction contributed by adsorption sites of PA not occupied by NNA.

If the pK_a values of the adsorption sites for NNAs are the same as (or comparable to) that of PA, the value of $S_{P\text{-}OH\sim OH^-}$ for the NNA occupied sites (case II) would be the same as (or approximate to) the value of $S_{P\text{-}OH\sim OH^-}$ for the adsorption sites of PA (case I). Thus, according to equation (13), the value of $S_{P\text{-}OH\sim OH^-}$ for the adsorption sites of PA (case I) would also be the same as (or approximate to) the value of $S_{P\text{-}OH\sim OH^-}$ for the adsorption sites of PA in the presence of NNAs (case II). This implies that partial occupation of adsorption sites of PA by NNAs would not change the value of $S_{P\text{-}OH\sim OH^-}$ for PA, or it may be altered. In conclusion, the $S_{P\text{-}OH\sim OH^-}$ value can be used to examine whether the pK_a values of adsorption sites are the same (or comparable) for NNAs and PA at the same pH. If the pK_a values of adsorption sites for NNAs are the same as (or comparable to) that of PA, partial occupation of the adsorption sites of PA by NNA would not change the value of $S_{P\text{-}OH\sim OH^-}$ for PA; otherwise, it may be altered.

Table-S1. Molecular weight, sources, purity and Nanodrop detecting wavelength of chemicals

Chemicals listed in Fig. 1	Corresponding chemicals purchased					
	Name	Molecular weight	Source	Purity (%)	Nanodrop detecting wavelength[a] (nm)	Limit of detection[b] (mg/L)
ATP	Adenosine 5′-triphosphate disodium salt hydrate	551.14	Aladdin	98	260	2.40
ADP	Adenosine 5'-diphosphate disodium salt hydrate	471.16	Aladdin	>98	260	2.05
AMP	Adenosine 5′-monophosphate sodium salt	369.2	Aladdin	99	260	5.22
CTP	Cytidine 5′-triphosphate disodium salt	527.12	Aladdin	95	272	4.14
CDP	Cytidine 5'-diphosphate trisodium salt hydrate	469.12	Aladdin	95	272	3.37

CMP	Cytidine 5'-monophosphate	323.2	Macklin	99	271	2.23
GMP	Guanosine 5′-monophosphate disodium salt hydrate	407.18	Aladdin	98	272	3.28
dTMP	Thymidine 5'-monophosphate disodium salt hydrate	366.17	Aladdin	99	265	2.78
dUMP	2′-Deoxyuridine 5′-monophosphate disodium salt	352.15	Yuanye	98	260	2.61
PA	Potassium phosphate monobasic	136.09	Macklin	99.5	-	0.01
DNA1	Deoxyribonucleic acid, low molecular weight from salmon sperm	-	Sigma-Aldrich	≤5% protein	260	3.70
DNA3	Deoxyribonucleic acid sodium salt from herring testes Type XIV	-	Sigma-Aldrich	-	260	2.65
RNA	Ribonucleic acid	-	Yuanye	90	260	2.43
A	Adenosine	267.24	Aladdin	99	260	1.09
C	Cytidine	243.22	Aladdin	99	272	1.62
G	Guanosine	283.24	Aladdin	98	250	1.46
dT	Deoxythymidine	242.23	Aladdin	99	267	2.22
dU	Doxyuridine	228.2	Aladdin	99	260	1.88

[a]The detecting wavelength of DNA2 is 260 nm. [b]The detection of limit is estimated using 5 mM KCl (pH is 7.00 ± 0.10) as control (the average absorbance is about 0.002), the concentration of PA is based on phosphorus atom (i.e., mg-P/L).

Table-S2. Content and volume of goethite suspension and chemical solution used in the adsorption experiment

Category	Experiment for PA	Experiment for nucleotide	Experiment for nucleic acid	Experiment for other chemicals	Experiment for PA and nucleotide (or nucleic acid) coexisted	Corresponding figure
Monitoring pH change in adsorption	1000 μL goethite suspension (solid content 27.3 mg/mL, pH 7.0) + 1000 μL PA solution (1.00 mmol/L P-O(H), pH7.0)	1000 μL goethite suspension (solid content 27.3 mg/mL, pH 7.0) + 1000 μL chemical solution (1.00 mmol/L P-O(H), pH 7.0)	1000 μL goethite suspension (solid content 27.3 mg/mL, pH 7.0) + 1000 μL 800 mg/L nucleic acid solution (pH 7.0)	1000 μL goethite suspension (solid content 27.3 mg/mL, pH 7.0) + 1000 μL chemical (1.00 mmol/L for A, C, G, dT or dU)		Figure 3b

Monitoring effect of coexistence of anions and pre-adsorption of PA on adsorption of nucleotide and nucleic acid at pH7.0	600 μL goethite suspension (solid content 24.9 mg/mL, pH 7.0) + 1000 μL PA solution (1.00 mmol/L PA P-O(H), and 1.00 mmol/L Na_2SO_4 or 2.00 mmol/L KCl, pH 7.0) + 1000 μL HCl (for keeping final pH of 7.0)	600 μL goethite suspension (solid content 24.9 mg/mL, pH 7.0) + 1000 μL chemical solution (1.00 mmol/L nucleotide P-O(H), and 1.00 mmol/L Na_2SO_4 or 2.00 mmol/L KCl, pH 7.0) + 1000 μL HCl (for keeping final pH of 7.0)	600 μL goethite suspension (solid content 24.9 mg/mL, pH 7.0) + 1000μL chemical solution (1.00 mmol/L nucleic acid P-O(H), and 2.00 mmol/L Na_2SO_4 or 2.00 mmol/L KCl, pH 7.0) + 1000 μL HCl (for keeping final pH of 7.0)	600 μL goethite suspension (solid content 24.9 mg/mL, pH 7.0) + 1000μL chemical solution (0.50 mmol/L A, C, G, dT or dU, pH 7.0) + 1000 μL 5mM KCl (pH of 7.0)		Figure 1c, d and e
Monitoring ligand exchange at pH7.0	600 μL goethite suspension (solid content 27.2 mg/mL, 24.9 mg/mL, 21.4 mg/mL ,pH 7.0) + 1000 μL PA solution (0~2.00 mmol-P/L, pH7.0) + 0~1000 μL (1.86mmol/L HCl) + 5 mmol/L KCl (pH7.00) to reach a final volume of 2.60 mL	600 μL goethite suspension (solid content 27.2 mg/mL, pH 7.0) + 1000 μL nucleotide solution (0~2.20 mmol-P/L, pH 7.0) + 0~1000 μL (1.86mmol/L HCl) + 5 mmol/L KCl (pH7.00) to reach a final volume of 2.60 mL	600 μL goethite suspension (solid content 27.2 mg/mL, pH 7.0) + 1000 μL nucleic acid solution (0~1.00 mmol-P/L, pH 7.0) + 0~1000 μL (1.86mmol/L HCl) + 5 mmol/L KCl (pH7.00) to reach a final volume of 2.60 mL		600 μL goethite suspension (solid content 21.4 mg/mL, pH7.0) + 1000 μL PA and nucleotide (or nucleic acid) coexisted solution (nucleotide (or nucleic acid) P-O(H):PA P-O(H)=1:1;1:2;1:3, pH7.0) + 0~1000 μL (1.86mmol/L HCl) + 5 mmol/L KCl (pH7.00) to reach a final volume of 2.60 mL	Figure 3c,d,e,f, Figure 4 and Fig. S10-19

Table-S2 (Continue)

Adsorption isotherm at pH7.0	900 μL goethite suspension (solid content 27.2 mg/mL, pH 7.0) + 3000 μL PA solution (0~200 mg/L, containing HCl for pH control)	900 μL goethite suspension (solid content 27.2mg/mL, pH7.0) + 3000 μL nucleotide solution (0~200 mg/L, containing HCl for pH control)	600 μL goethite suspension (solid content 27.2 mg/mL, pH7.0) + 2000 μL nucleic acid solution (0~200 mg/L, containing HCl for pH control)			Figure 1b and Fig. S9

[a]The content and volume of A, C, G, dT and dU are the same as that of nucleotide.

Table-S3. Relation of P-O(H) engaged in Fe-O-P bonding with molecular structure and phosphorus adsorption capacity*

Name	Molecular weight (g/mol)	Molecular P-OH number	PO(H) number engaged in Fe-O-P bonding in NNA molecule	Percent of P-O(H) engaged in Fe-O-P bonding (%)	NNA mass adsorbed / PO(H) number engaged in Fe-O-P bonding (g/mol)	Q_e at final phosphorus concentration of 50 µmol-P/L (µmol/g)
dUMP	308.15	2	2	100	154	0.20
dTMP	322.17	2	2	100	161	0.33
CMP	323.2	2	2	100	162	0.47
AMP	347.2	2	2	100	174	0.76
GMP	363.18	2	2	100	182	0.73
CDP	403.12	3	3	100	134	1.14
ADP	427.16	3	3	100	142	0.90
CTP	483.12	4	3 or 4	75-100	121-161	1.04

ATP	507.14	4	3 or 4	75-100	127-169	1.15
RNA	8458±4236	61±23	37±14	60.57	633	1.50
DNA1	14966±11633	103±73	70±50	68.01	469	1.29
DNA2	131962±67524	950±370	493±192	51.90	846	1.37
DNA3	590716±497392	40200±31800	11420±9034	28.41	1703	1.23

*Q_e is the amount adsorbed.

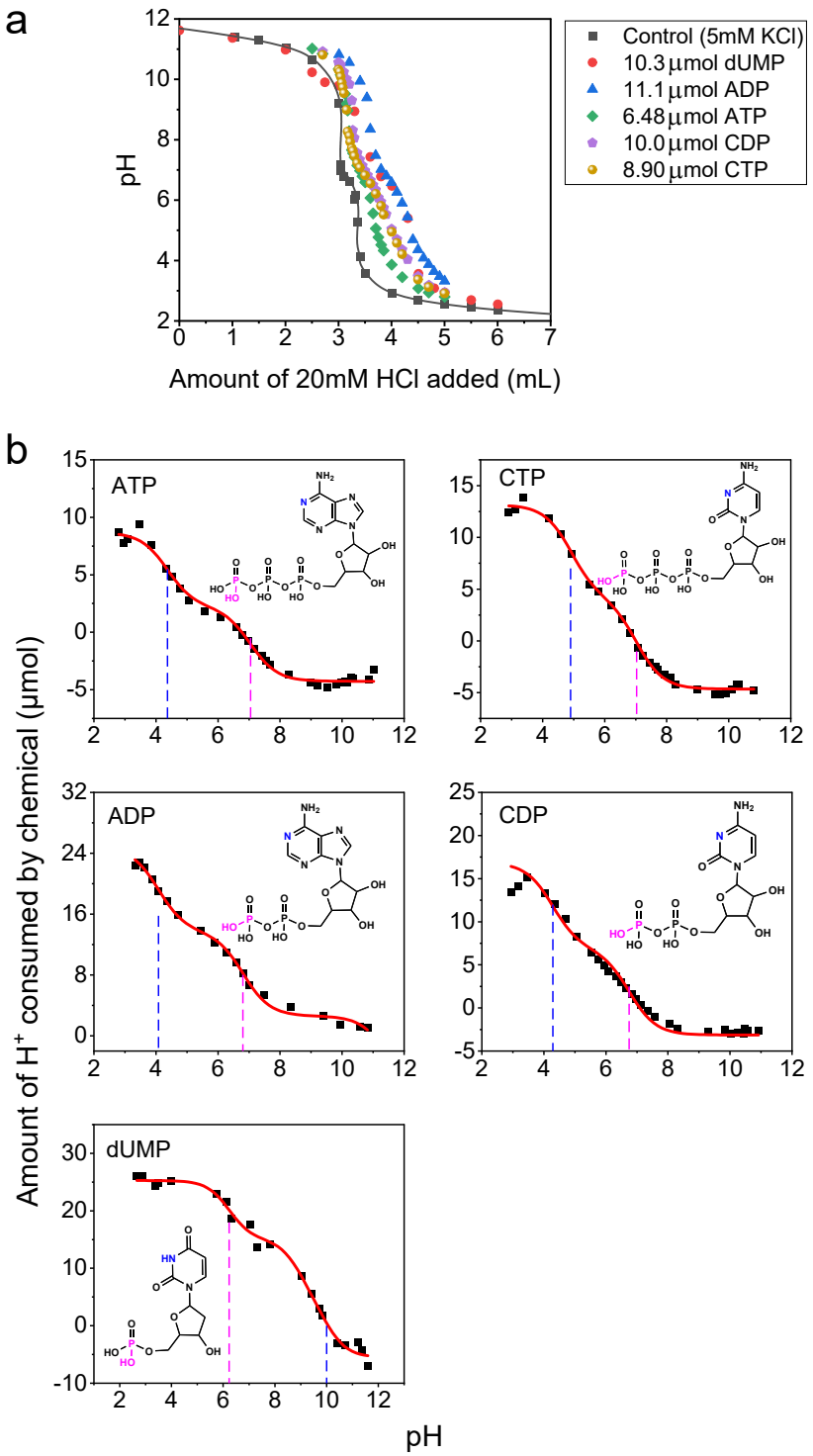


Fig. S1. Acid-base titration results (a) and pH-dependent proton consumption of chemicals (b). The proton consumption curves are derived from regression analysis using the equation listed in Text-S1. The dashed line colorred in pink indicates the pH equaling to the pK_a of nucleotide P-OH. The dashed line colorred in blue indicates the pH equal to the pK_a of nucleotide -NH^+-(OR -NH_2^+-). Since the P-OH groups in the chemicals are used partially or exist as salts (see Table S1), the pH corresponding to the net consumption of H^+ varied for different chemicals.

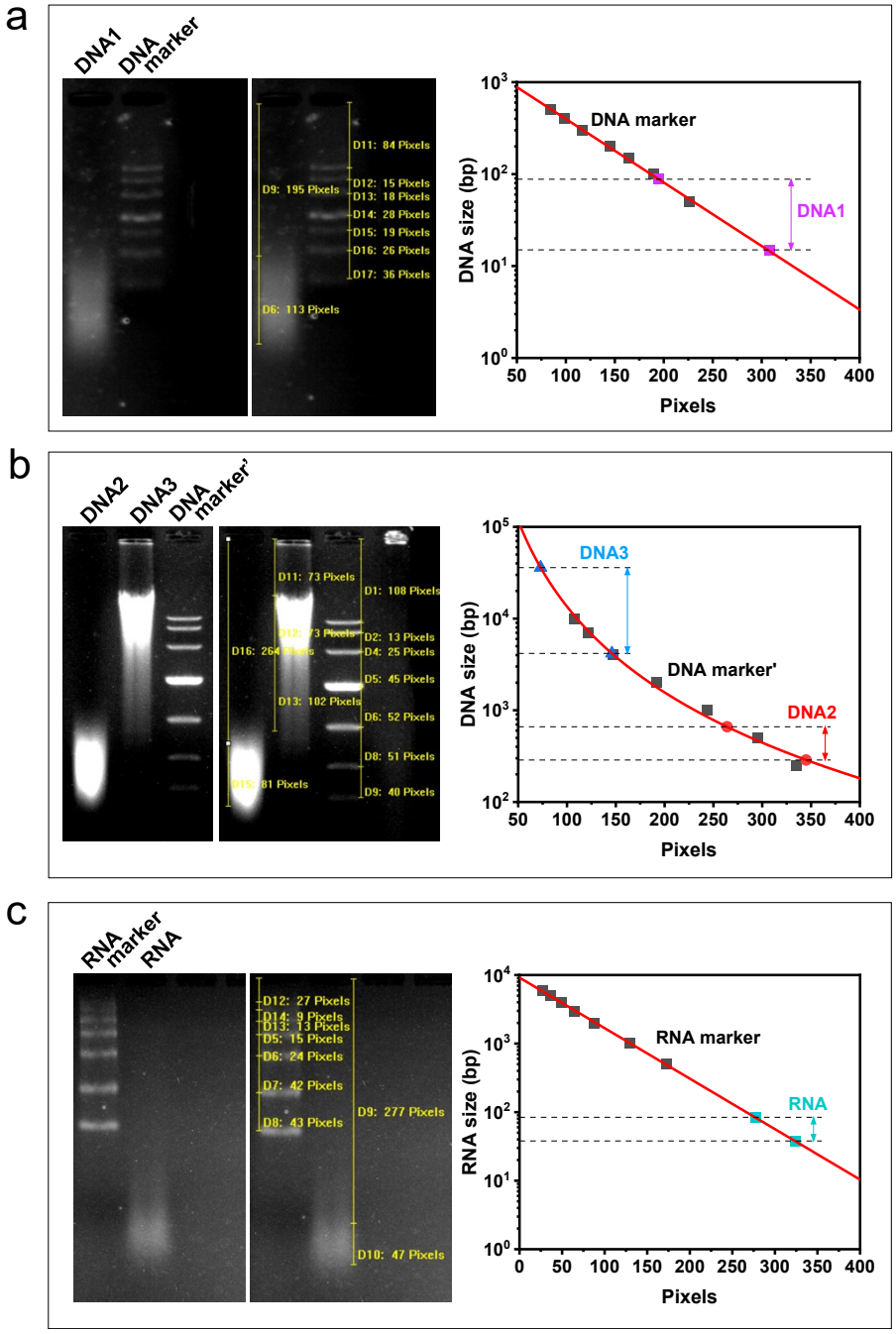


Fig. S2. Gel electrophoresis and estimated sizes of nucleic acids. The running distance of nucleic acids (reflected by software Image-Pro Plus 6.0) and size of nucleic acid (reflected by base pairs, i.e., bp) match exponential relation (i.e., the red curves, regression analysis R^2=0.991-0.999). Based on the exponential relation, the sizes of nucleic acids were estimated.

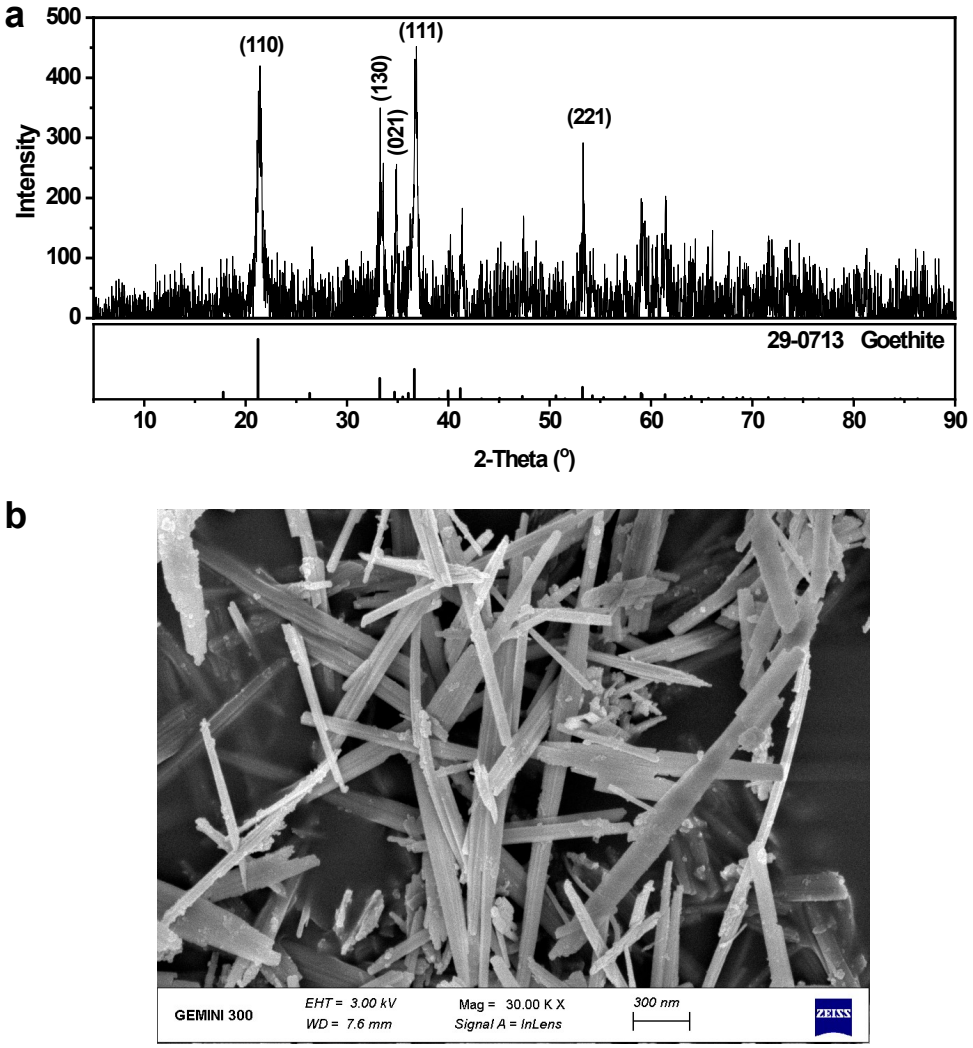


Fig. S3. Characterization of goethite. (a) XRD and (b) SEM

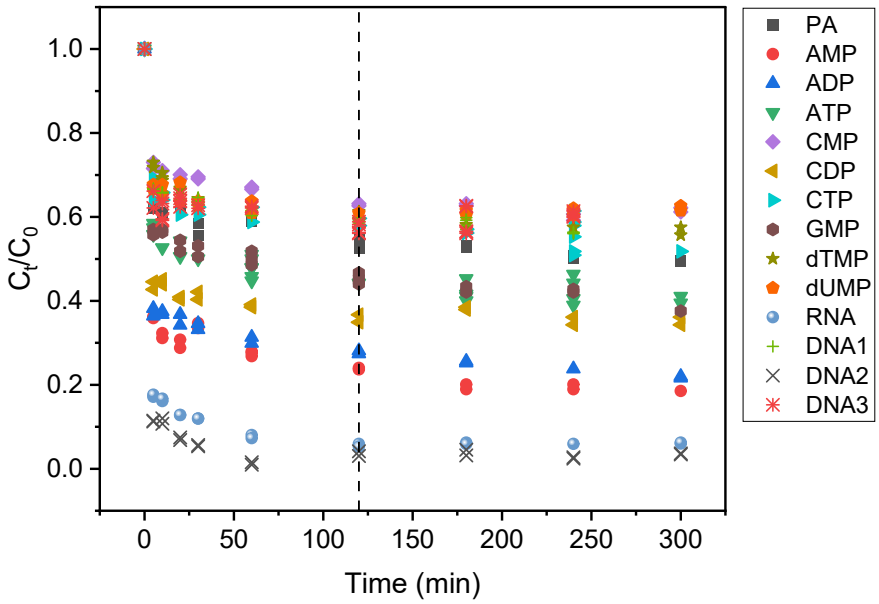


Fig. S4. Adsorption kinetics on goethite. The initial reaction pH is pH7.0, and the initial concentration of PA, nucleotide, and nucleic acid is 100±5 mg/L. The amount of goethite added was 6.23mg/mL for AMP, CMP, GMP, dTMP, ADP, CDP, ATP and CTP, 8.32mg/mL for dUMP, 6.01mg/mL for RNA and DNA2, and 4.50mg/mL for DNA1 and DNA3. C_0: Concentration in solution without adsorption. C_t: Concentration in solution at reaction time *t*.

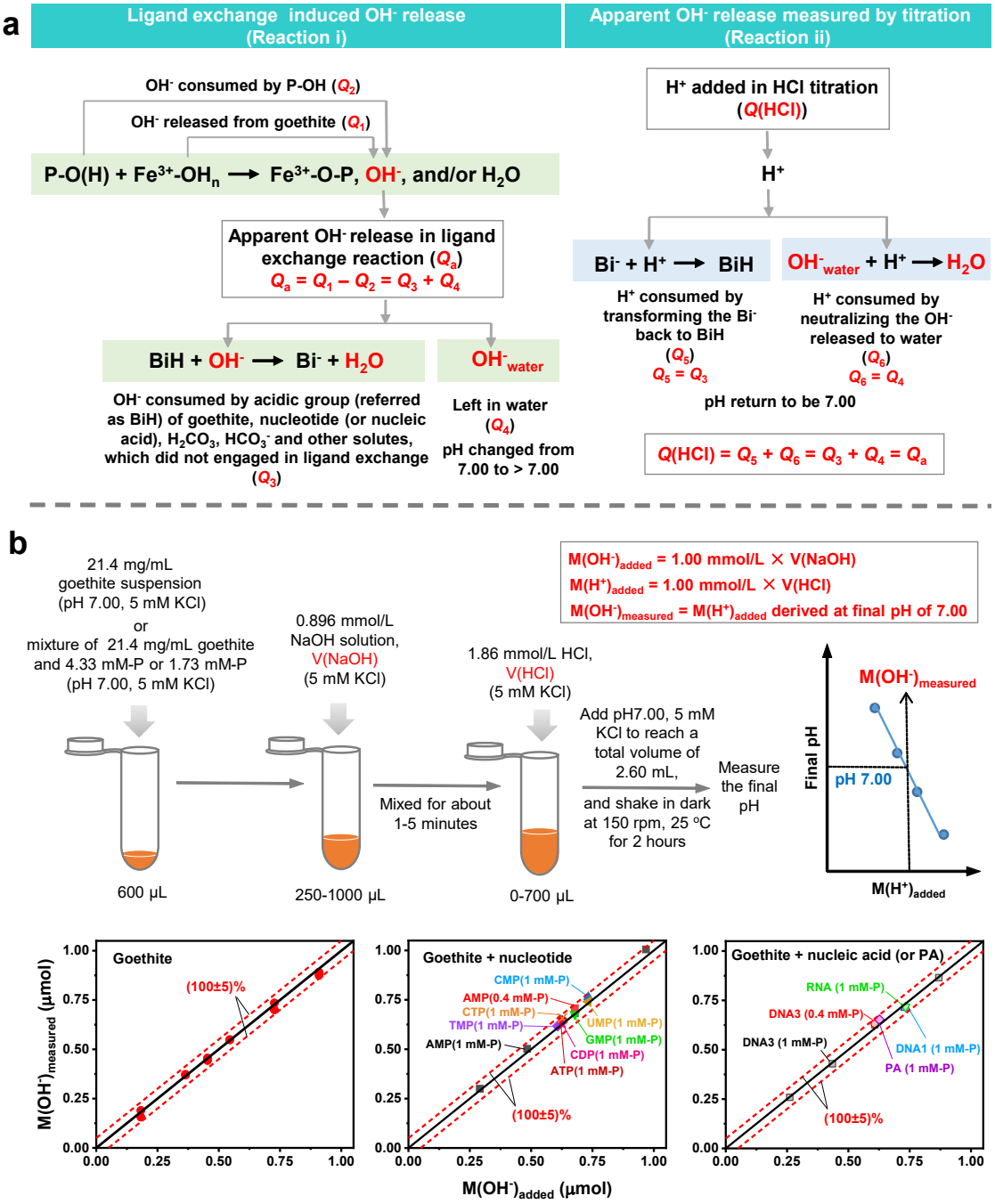


Fig. S5. Apparent OH^- release in the forming of Fe-O-P bonds and theoretical aspects explaining why HCl titration could measure the apparent OH^- release (a). Procedure and results in examination of the accuracy of the HCl titration method by comparing the added amount of NaOH and measured amount of OH^- (b). The amount of goethite added is the same as that in monitoring Fe-O-P bonding at pH7.0 (see Table-S2). The concentration of nucleotide (or nucleic acid, PA, shown as the content of phosphorus) in the reaction systems after considering the dilution effect from addition of NaOH, HCl and KCl solution (i.e., 1 mM-P) approaches the maximum amount of nucleotide (or nucleic acid) added in experiments monitoring pH change or OH^- release listed in Table-S2 (excepting adsorption isotherm experiment). The mole amount of NaOH added for checking HCl titration (0.182-0.968) was overlapped or close to the measured mole amount of apparent OH^- release for nucleotide or nucleic acid (0.051-0.921 µmol).

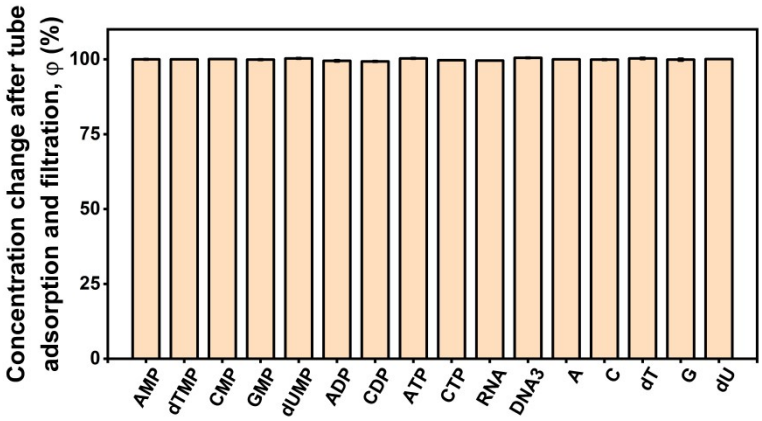


Fig. S6. Concentration change of nucleotide, nucleic acids and nucleoside in solution after tube adsorption and fitration. The procedure of experiment is detailed in Text-S7.

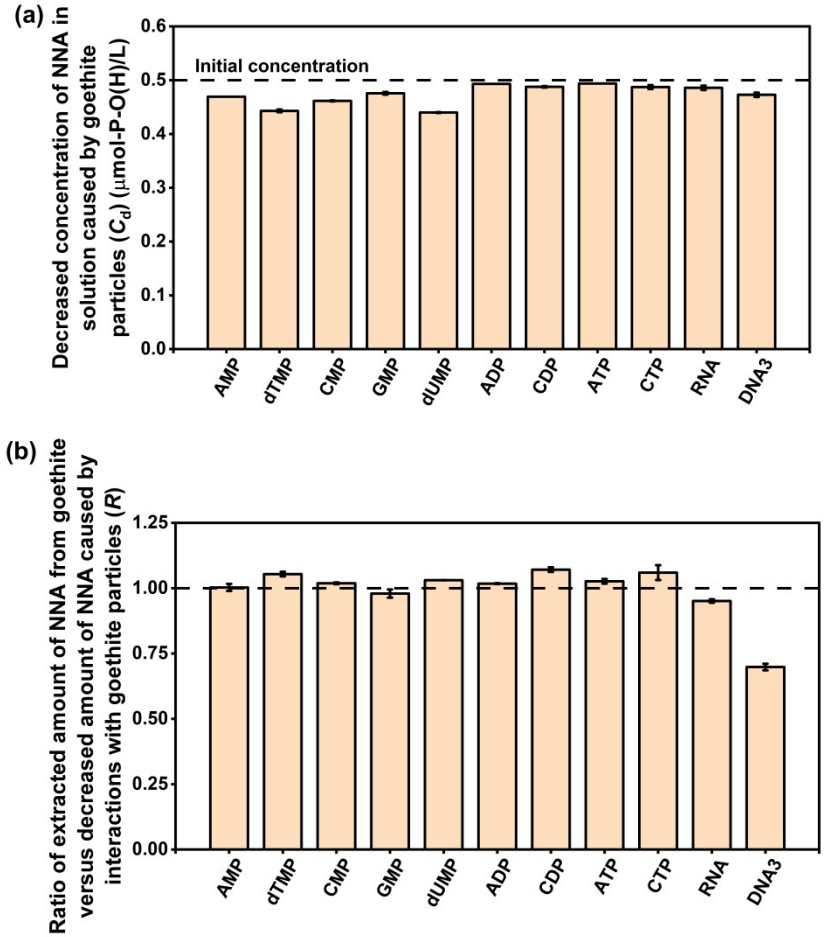


Fig. S7. Decreased concentration of NNA in solution caused by interactions with goethite particles (a) and the ratio of extracted amount of NNA from goehtie versus the decreased amount of NNA caused by interactions with goethite (b). The procedure of experiment is detailed in Text-S7.

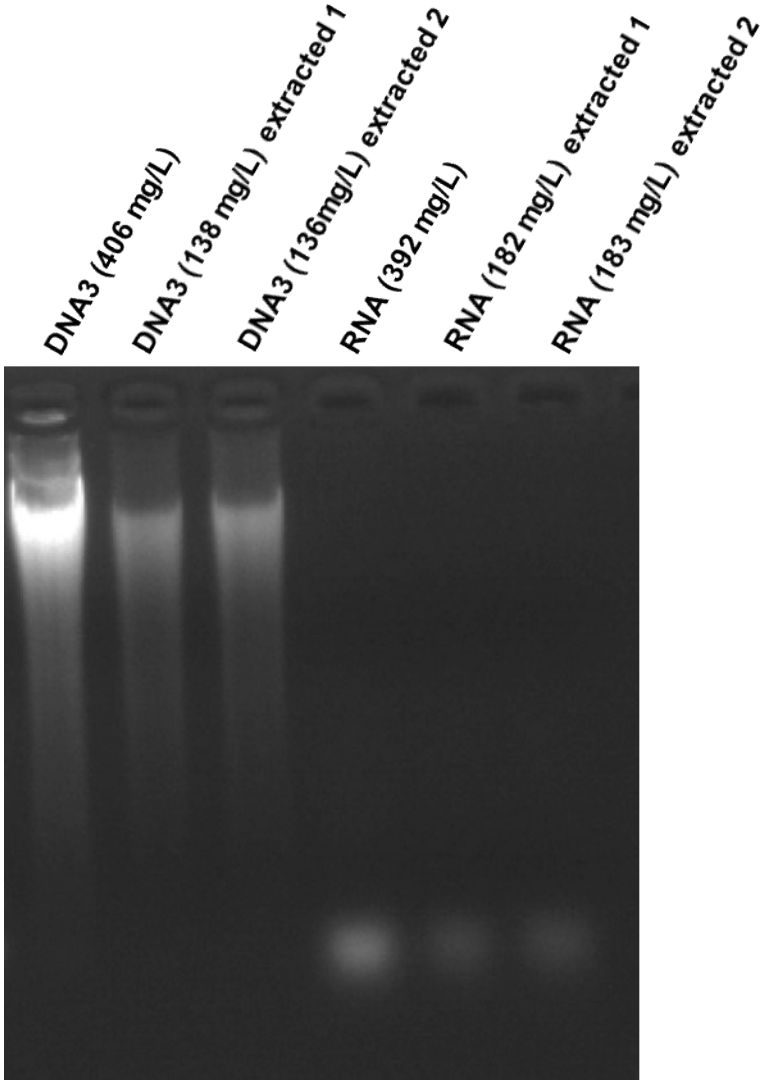


Fig. S8. Gel electrophoresis of RNA and DNA3 extracted from goethite. The extracted 1 and extracted 2 are the results of two repeats. The procedure of experiment is detailed in Text-S7.

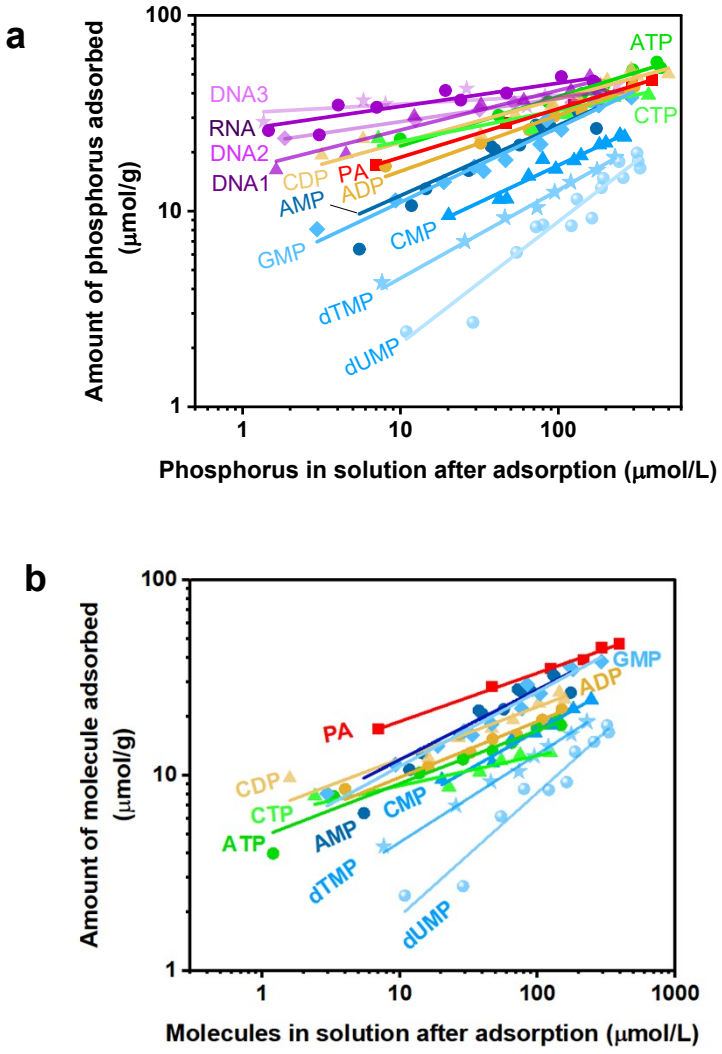


Fig. S9. Adsorption isotherm of PA and NNAs on goethite at pH7.0. (a) Adsorption isotherm derived based on the phosphorus. (b) Adsorption isotherm derived based on phosphate molecule (Since the nucleic acids used are a mixture of molecules of different sizes, the adsorption isotherm of mucleic acids are not derived)

Fig. S10. Solution pH, amount of phosphorus adsorbed and amount of HCl added for PA adsorption on goethite at pH7.0. (a) Amount of phosphorus adsorbed. (b) Amount of HCl (1.86 mmol/L) added. The red solid line is pH 7.0, while the dashed lines are from linear regression analysis of data. PA1, PA2, and PA3 represent the results of three replicates of PA adsorption on goethite. "μM" is μmol/L.

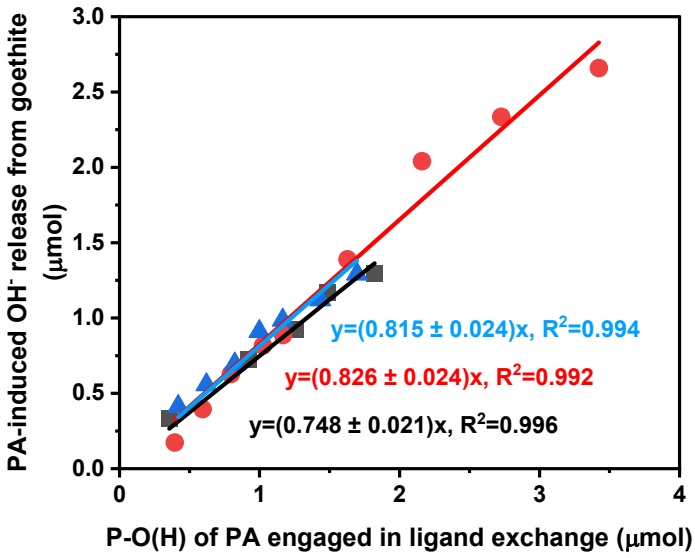


Fig. S11. The linear relationship between the amount of OH^- released from goethite and the amount of PA P-O(H) engaged in Fe-O-P bonding for PA at pH of 7.0. Data points of rectangle, circle, and triangle are the results of three replicates.

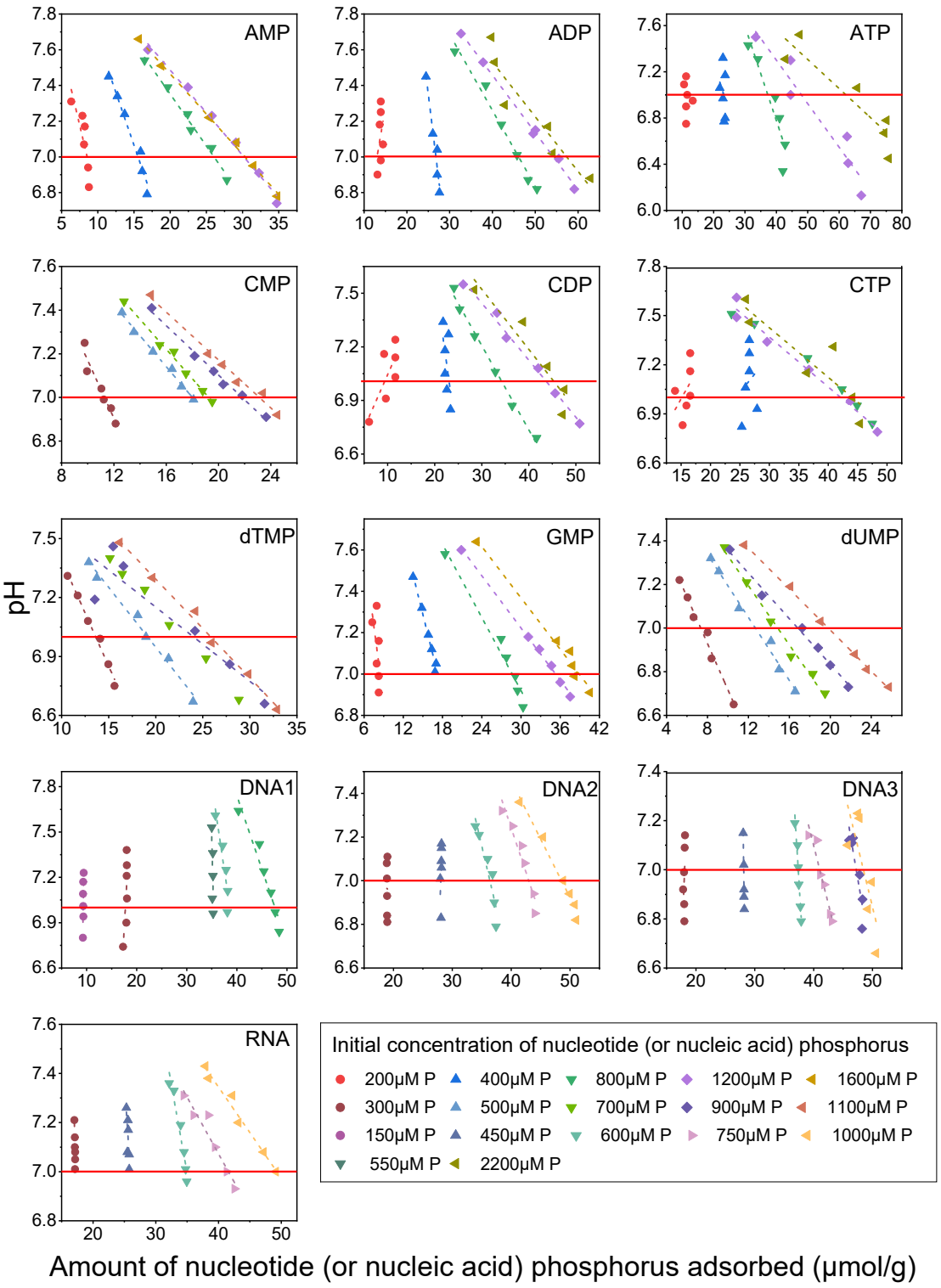


Fig. S12. Solution pH and amount of nucleotide (or nucleic acid) phosphorus adsorbed in the adsorption experiment when only nucleotide (nucleic acid) existed. The red solid line is pH 7.0, while the dashed lines are from linear regression analysis of data.

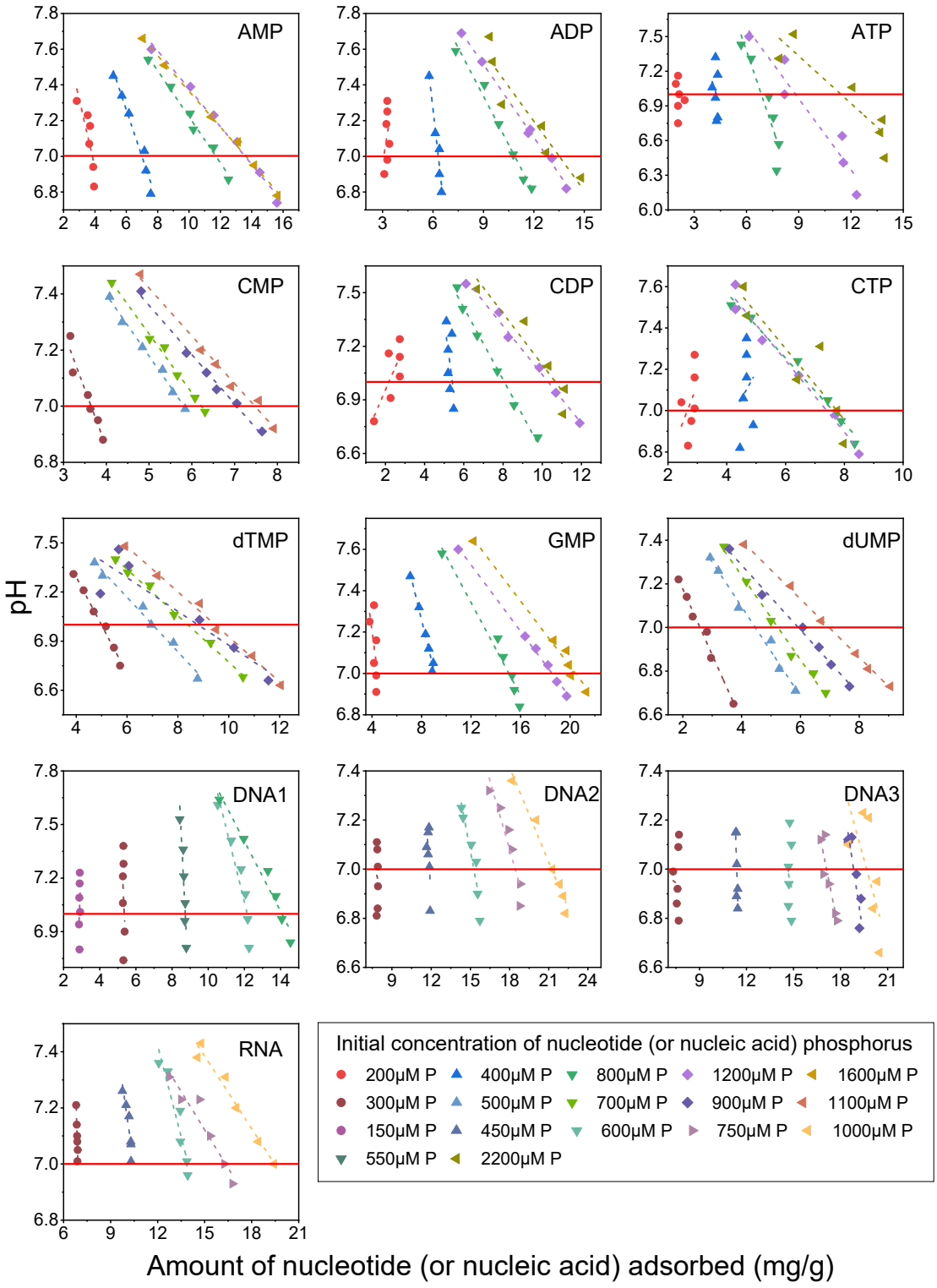


Fig. S13. Solution pH and amount of nucleotide (nucleic acid) adsorbed in the adsorption experiment when only nucleotide (nucleic acid) existed. The red solid line is pH 7.0, while the dashed lines are from linear regression analysis of data.

Fig. S14. Solution pH and amount of HCl added in the adsorption experiments when only nucleotide (or nucleic acid) existed. The red solid line is pH 7.0, while the dashed lines are from linear regression analysis of data.

Fig. S15. The linear relationship between the amount of nucleotide (or nucleic acid) adsorbed and the amount of apparent OH^- release (determined by HCl titration) in the adsorption experiment when only nucleotide (nucleic acid) existed at pH of 7.0.

Fig. S16. Solution pH and amount of 2mM HCl added in the adsorption experiment when PA and nucleotide (or nucleic acid) coexisted. The red solid line is pH 7.0, while the dashed lines are from linear regression analysis of data.

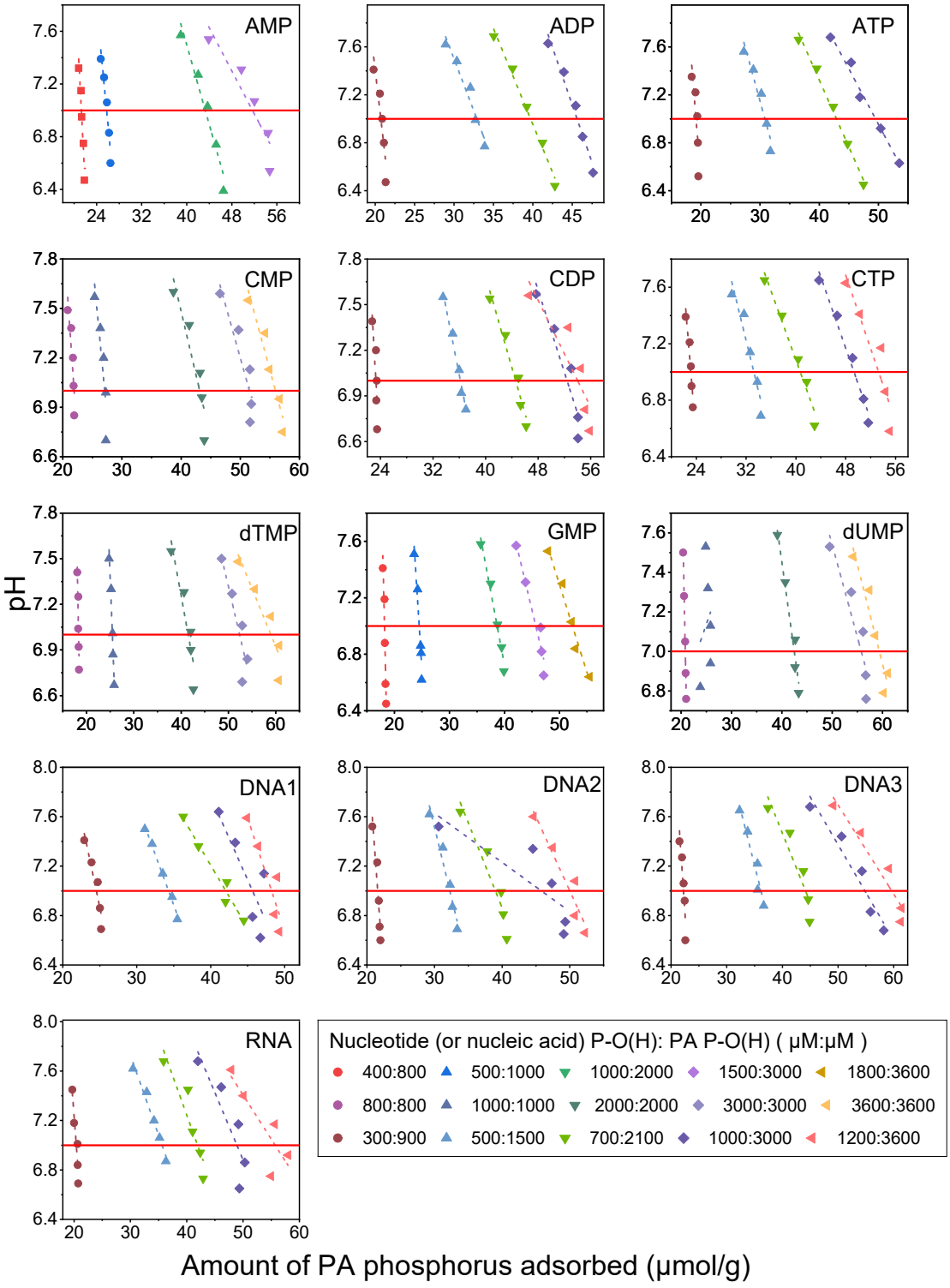


Fig. S17. Solution pH and amount of PA phosphorus adsorbed in adsorption experiment when PA and nucleotide (or nucleic acid) coexisted. The red solid line is pH 7.0, while the dashed lines are from linear regression analysis of data.

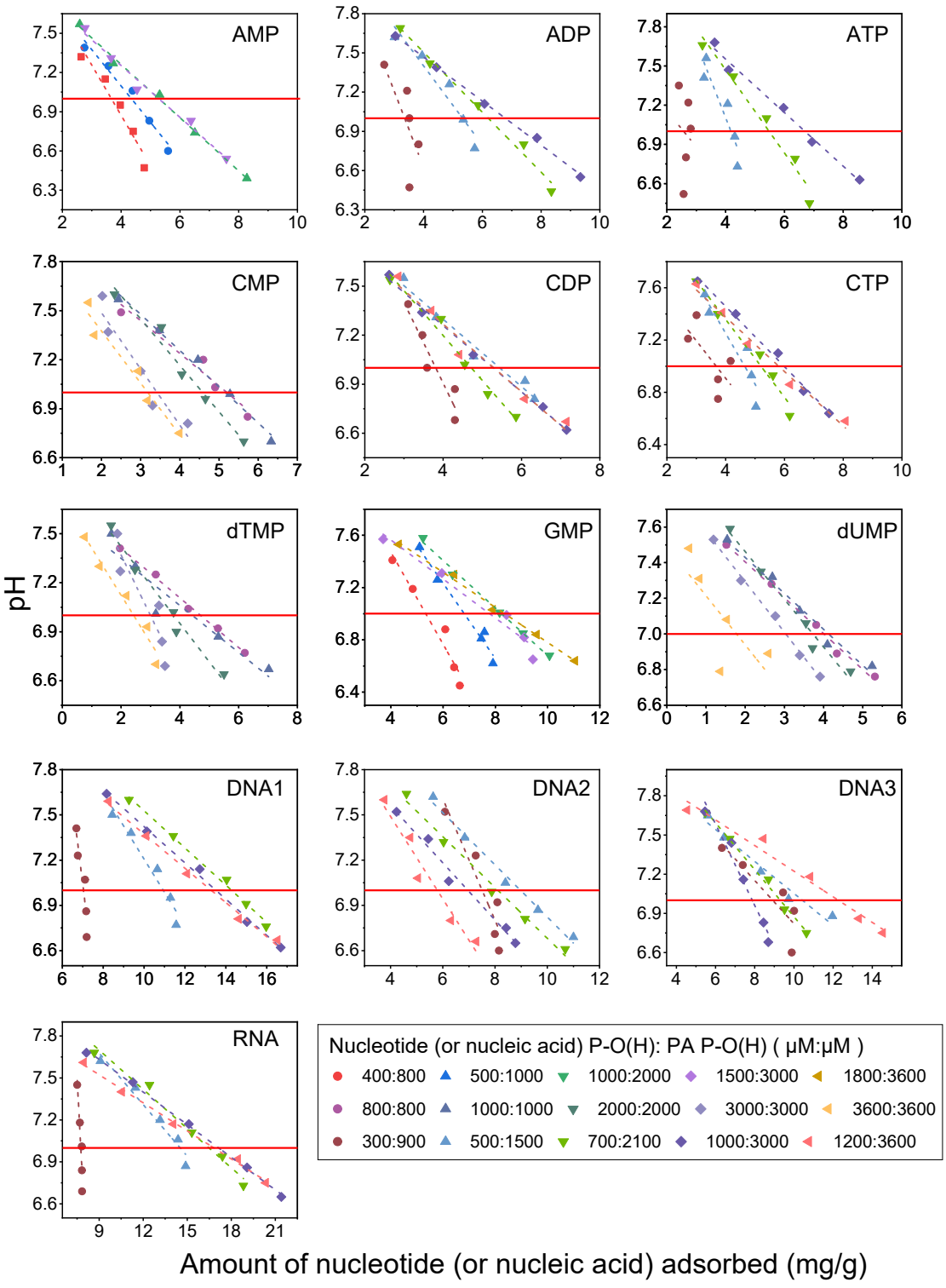


Fig. S18. Solution pH and amount of nucleotide (or nucleic acid) adsorbed in the adsorption experiment when PA and nucleotide (or nucleic acid) coexisted. The red solid line is pH 7.0, while the dashed lines are from linear regression analysis of data.

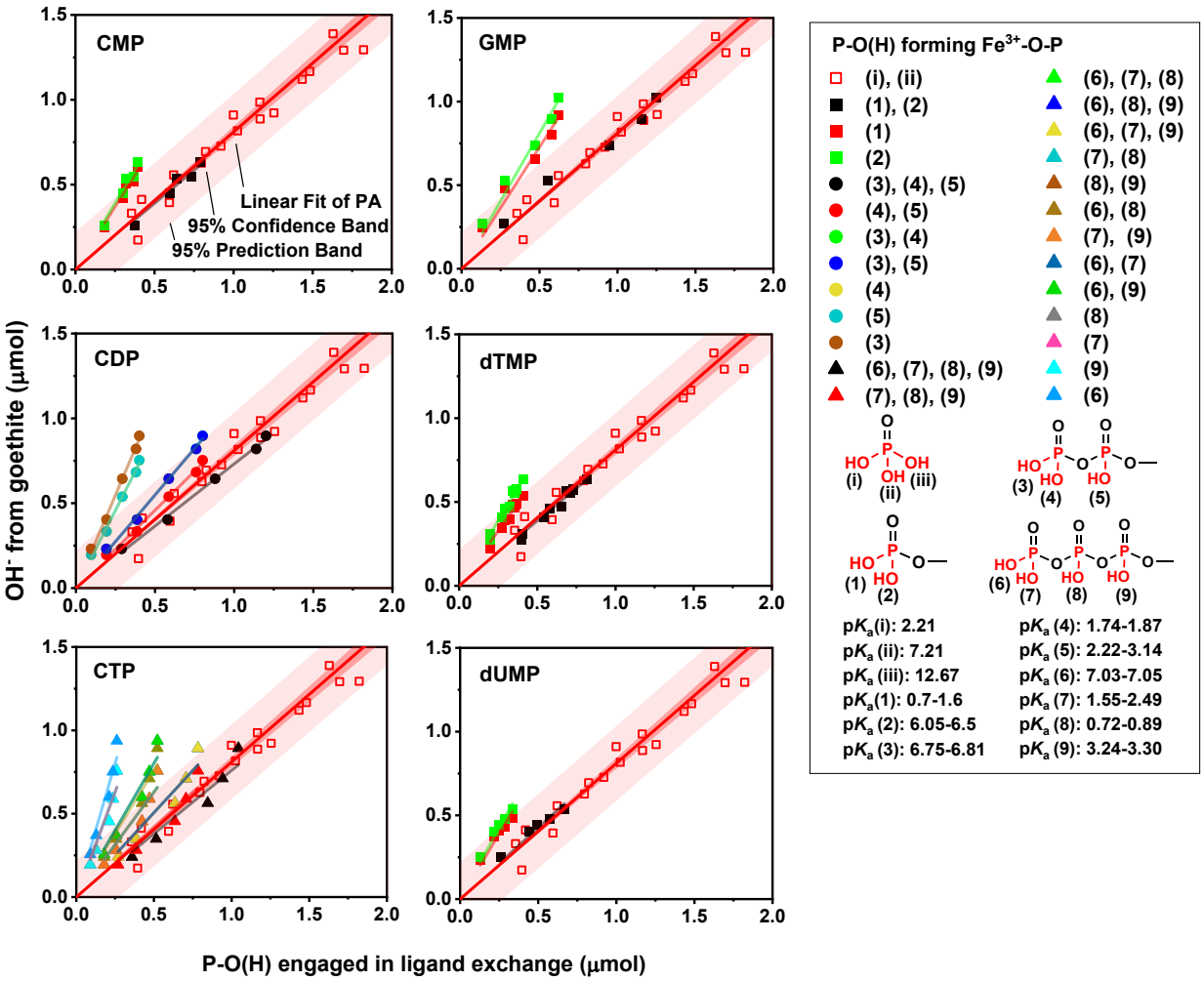


Fig. S19. $S_{\text{P-OH~OH}^-}$ derived with assumed engagement of P-O(H) groups in Fe-O-P bonding for CMP, CDP, CTP, GMP, dTMP, dUMP and PA.

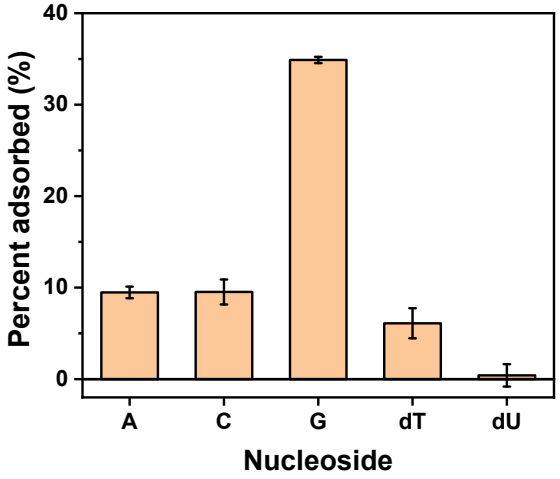


Fig. S20. Adsorption of nucleoside on goethite at pH7.0. The adsorption experiment is conducted by mixing 200 μL goethite suspension (containing 112 mg goethite) with 1000 μL nucleoside solution (concentration is 100 mg/L).

Among nucleotide monophosphate AMP, CMP, GMP, dTMP, and dUMP, the amount of P-O(H) groups engaged in Fe-O-P bonding relative to adsorbed phosphate molecule and relative to adsorbed phosphorus atom were unchanged. The affinity of nucleoside to goethite and the influence of nucleoside structure on the pK_a value of P-OH, instead of molecular P-O(H) groups content and molecular size, played an important role in causing the variation of phosphorus adsorption among nucleotide monophosphate. The adsorption capacity of nucleoside on goethite surface decreased as dU < dT < C ≈ A < G (Fig. S20), matching the decreased adsorption capacity of nucleotide, dUMP < dTMP < CMP < AMP ≈ GMP. The influence of nucleoside A on the pK_a value of P-OH in AMP may further enhance phosphorus adsorption to make AMP comparable to GMP. The smaller pK_a value (6.05) of P-OH in AMP than the pK_a value (6.3) of P-OH in CMP and GMP, made the proportion of P-OH in P-O(H) of AMP (including P-OH and P-O^-) less than that in CMP and GMP (16.6%); In adsorption at pH7.0, less proportion of P-OH favored adsorption because P-OH experienced a free energy-unfavored deprotonation process (to be transformed to P-O^-) in Fe-O-P bonding.

The different adsorption capacity of nucleosides on goethite may be related with the functional groups of these nucleosides. In adsorption on carbon nanostructure adsorbent (e.g., carbon nanotubes), the nucleobases are physisorbed in the order G > T ≈ A > C > U, exhibiting π-π-stacking type of interactions from the aromatic structures.[6] The sequence of adsorption capacity on goethite (e.g., dU < dT < C ≈ A < G) is different from the nuclobases adsorption on carbon nanostructure. Since there is no aromatic structure on goethite, interactions from function groups like -N=, -NH_2, C=O and C-OH with goethite may play an important role.

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