

# Selective DNA Adsorption on Layered Double Hydroxide Nanoparticles

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We investigated the selective deoxyribonucleic acid (DNA) adsorption on layered double hydroxide (LDH) nanoparticles via studying the interaction between positively charged LDH nanoparticle as adsorbent and negatively charged adsorbates such as methyl orange (MO), fluorescein (FL), and DNA strands. The size controlled LDH ( $\text{Mg}_{0.78}\text{Al}_{0.22}(\text{OH})_2(\text{CO}_3)_{0.11} \cdot m\text{H}_2\text{O}$ ) was prepared by conventional coprecipitation method, followed by the hydrothermal treatment. According to the adsorption isotherms, the adsorbed amounts of MO and FL were similar, however, that of DNA were much larger. The adsorption behaviors were well fitted to Freundlich adsorption model. The concentration dependent adsorption behavior on LDH surface was described in order to verify the selective DNA separation ability. The result showed that the LDH has advantages in selective adsorption of DNA competing with single molecular anions.

**Key Words :** Layered double hydroxide, Deoxyribonucleic acid, Adsorption, Separation

## Introduction

Recently, DNA separations and gatherings are attracting tremendous attentions in biotechnologies and forensic sciences.<sup>1-5</sup> In biotechnologies, DNA is selectively separated from cells or tissues in order to verify the genetic disorder or to modify the genetic information.<sup>1,2,4</sup> More specifically, gathering trace amount of DNA from bloodstain or separating artificially encoded DNA strands plays an important role to solve cases in forensic science.<sup>3,5</sup> Numerous inorganic adsorption systems including silica, metal oxide, and hydroxide are extensively studied and some of them are already commercialized.<sup>6</sup> Among those inorganic adsorbents, layered double hydroxide (LDH) nanoparticles, which are also called anionic nanoclay,<sup>7,8</sup> are considered as potent DNA collection material as they have tunable physicochemical properties like surface area, particle size, and charge density as well as biocompatibility.<sup>9,10</sup>

The general formula of LDH is  $[\text{M(II)}_{1-x}\text{M(III)}_x(\text{OH})_2]^{x+}(\text{A}^{n-})_{x/n} \cdot m\text{H}_2\text{O}$ , where M(II) and M(III) are divalent and trivalent metals and,  $\text{A}^{n-}$  stands for anion species.<sup>11</sup> The edge sharing array of  $\text{M(II)}(\text{OH})_6$  octahedrons make 2-dimensional sheets and the isomorphic substitution of M(II) with M(III) produces permanent positive charge which is compensated by the interlayer anions through electrostatic interaction. Due to their positive zeta potential ( $\sim +40$  mV of  $\xi$  potential) and specific surface charge density ( $1 \times 10^{20} \sim 5 \times 10^{20} \text{ e}^-/\text{g}$ ), LDHs have already been studied as scavengers of hazardous substances with negative charge in aqueous environments.<sup>12</sup> Their controllable particle size (from tens of nanometers to a few micrometers) and flexible metal composition make it possible to utilize them in various appli-

cations such as biological and environmental system.<sup>13</sup>

Although LDHs have a variety of advantages as bio- and eco-friendly anionic adsorption system, as far as we know, there have been few researches on the adsorption behavior of LDHs with various types of anionic molecules. It is expected that the molecular shape and charge density of adsorbate anions affects on both adsorption kinetics and thermodynamics. In this report, we are going to describe the concentration dependent adsorption behavior of various kinds of anionic molecules on LDH surface in order to verify the DNA separation ability of LDH nanoparticles.

## Experimental

**Reagents.** Magnesium nitrate hexahydrate ( $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), aluminum nitrate nonahydrate ( $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ), sodium bicarbonate ( $\text{NaHCO}_3$ ), methyl orange ( $\text{C}_{14}\text{H}_{14}\text{N}_3\text{NaO}_3\text{S}$ ), fluorescein ( $\text{C}_{20}\text{H}_{12}\text{O}_5$ ), deoxyribonucleic acid sodium salt (Type XIV: From Herring Testes), chloroform:isoamyl alcohol (24:1), and phenol solution were purchased from Aldrich. Sodium hydroxide pellets ( $\text{NaOH}$ ) were supplied by Daejung Chemicals & Metals Co., Ltd.

**Preparation LDH Nanoparticle.** The LDH nanoparticle ( $\text{Mg}_{0.78}\text{Al}_{0.22}(\text{OH})_2(\text{CO}_3)_{0.11} \cdot m\text{H}_2\text{O}$ ) was prepared by conventional coprecipitation.<sup>10</sup> Typically, alkaline solution (0.9 M  $\text{NaOH}$ /0.675 M  $\text{NaHCO}_3$ ) was added dropwisely to a mixed metal solution (0.3 M  $\text{Mg}^{2+}$ /0.15 M  $\text{Al}^{3+}$ ) until pH  $\sim 9.5$ . The mixture was hydrothermally treated at 373 K for 48 hrs to obtain size controlled LDH nanoparticles. The white precipitate was centrifuged, repeatedly washed with deionized water and then freeze-dried.

Powder X-ray diffraction pattern was recorded by a (XRD;

Rigaku D/Max-2000/PC) diffractometer with Ni-filtered Cu-K $\alpha$  radiation ( $\lambda = 1.5418$  Å). The fourier transform infrared (FT-IR) spectrum for LDH was obtained by Perkin Elmer spectrum one FT-IR spectrometer using KBr pellet method. The morphology and particle size of the prepared LDH were investigated by scanning (SEM; JEOL JSM-6700F) and transmission electron microscopy (TEM; JEOL JEM 1010). The chemical composition of the LDH was analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; PerkinElmer/Optima-4300 DV).

**Purification of Adsorbates.** MO and FL were used without further purification. Type XIV crude DNA was purified through conventional phenol extraction method.<sup>14</sup> The extraction solution containing phenol solution, chloroform, and isoamyl alcohol with 25:24:1 ratio was well mixed with aqueous DNA solution (2 mg/mL) and then centrifuged at 4,000 rpm for 15 min. The supernatant was collected and the same process was repeated 4 times. The final supernatant

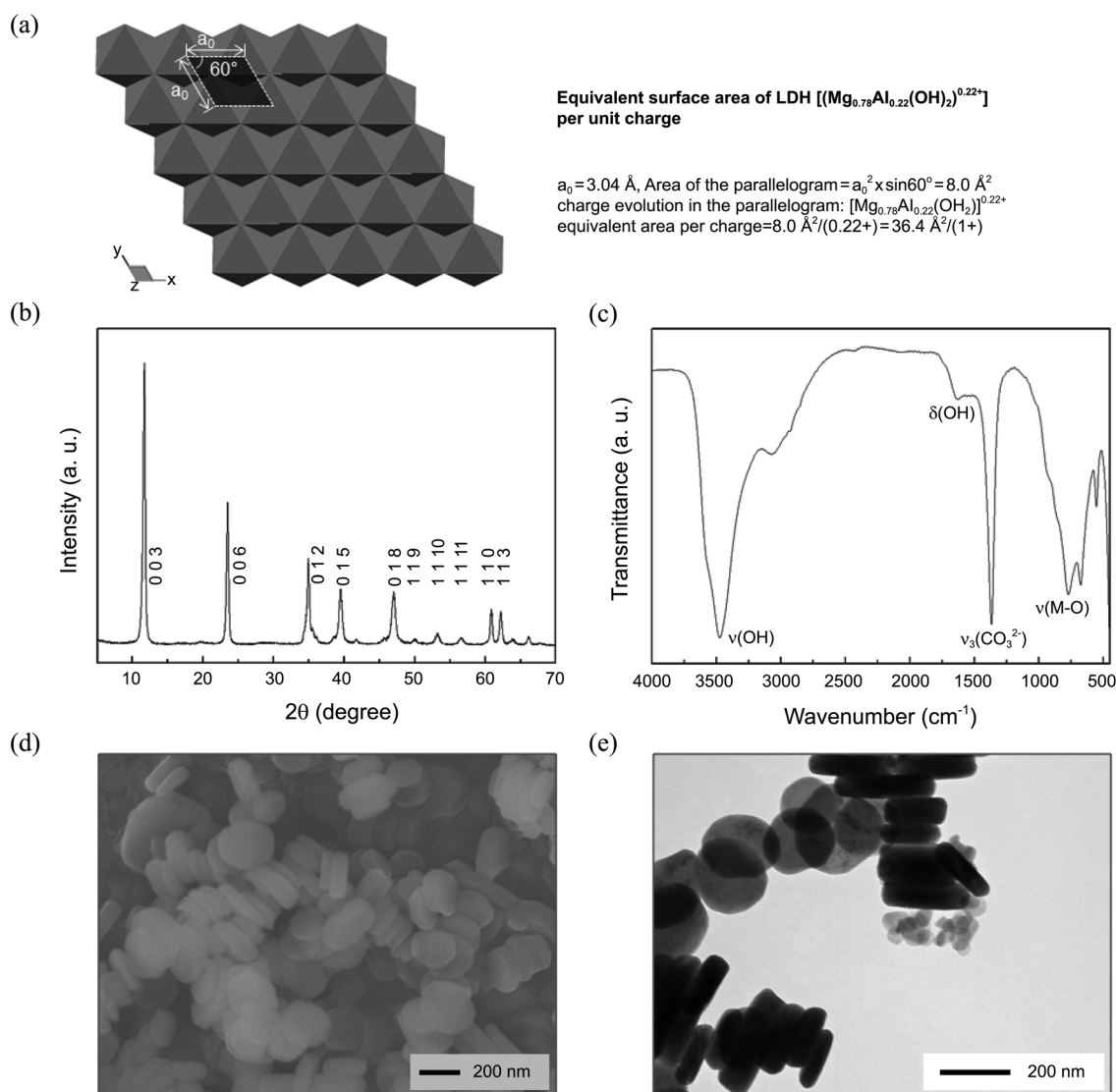
was mixed twice volume of ethanol and a small amount of NaCl for 30 min in 268 K refrigerator. Thus obtained recrystallized DNA precipitate was centrifuged, repeatedly washed with ethanol.

**Adsorption Study.** Powdered LDH was dispersed in deionized water (0.25 mg/mL) and 1 mL of the suspension was added to 10 mL of each adsorbate solution such as MO, FL, and DNA solution with various concentrations from  $1.93 \times 10^{-6}$  to  $1.60 \times 10^{-4}$  M. The mixture was gently shaken at room temperature for 24 h and then centrifuged at 12,000 rpm for 10 min in order to separate supernatant.

The adsorbed amount was determined by UV spectrometer (SHIMADZU/UV-1800) at the wavelength of 464, 233, and 260 nm for MO, FL, and DNA, respectively.

## Results and Discussion

**Characterization of LDH.** According to the X-ray diffr-



**Figure 1.** (a) The schematic structure of LDH (top-view) and the equivalent surface area of LDH per unit charge (Å<sup>2</sup>/(+)), (b) X-ray diffraction pattern, (c) fourier transform infrared spectrum, (d) the scanning electron microscopic image, and (e) the transmission electron microscopic image of size controlled LDH.

**Table 1.** Chemical formula and the surface area of LDH per unit charge

Chemical formula <sup>a</sup>	Cell parameter <sup>b</sup> (Å)	Equivalent surface area of LDH per charge (Å <sup>2</sup> /(+))
Mg <sub>0.78</sub> Al <sub>0.22</sub> (OH) <sub>2</sub> (CO <sub>3</sub> ) <sub>0.22</sub>	a <sub>0</sub> = 3.04 c <sub>0</sub> = 22.74	36.4

<sup>a</sup>Determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). <sup>b</sup>Obtained from X-ray diffraction (XRD).

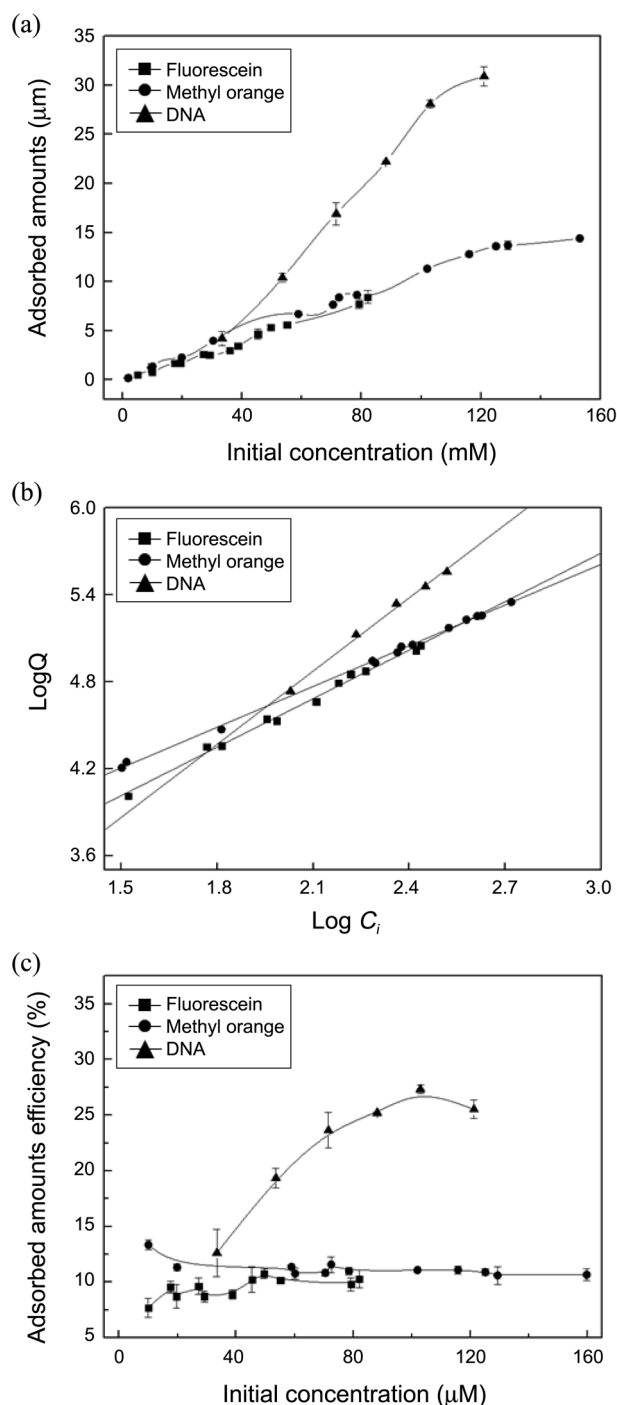
action patterns shown in Figure 1(b), the prepared LDH turned out to be well crystallized with interlayer carbonate ion having hexagonal cell parameters of  $a_0 = 3.04$  and  $c_0 = 22.74$  Å of which results are in good accordance with the previously reported LDH<sup>15,16</sup> (Table 1). As shown in Figure 1(c), the FT-IR spectrum exhibits all the characteristic stretching vibrations of LDHs. Peaks at 3400 and 800 cm<sup>-1</sup> are attributed to the  $\nu(\text{OH})$  and  $\nu(\text{M-O})$  in the metal hydroxide layers whereas those at 1630 and 1370 cm<sup>-1</sup> are explained by the  $\delta(\text{OH})$  and  $\nu_3(\text{CO}_3^{2-})$  modes of interlayer water molecules and carbonate ions, respectively.<sup>11</sup>

The chemical composition of the LDH was determined to be Mg<sub>0.78</sub>Al<sub>0.22</sub>(OH)<sub>2</sub>(CO<sub>3</sub>)<sub>0.11</sub>·*m*H<sub>2</sub>O by the ICP-AES (Table 1). The equivalent surface area of LDH per unit charge can be calculated to 36.4 Å<sup>2</sup>/(1+) as described in Figure 1(a). The SEM and TEM images of LDH shown in Figure 1(d) and (e) revealed that the nanoparticles have primary particle size of  $\sim 200 \pm 20$  nm with circular-lamellar morphology.

**Adsorption Study.** The adsorbed amount ( $Q$ ) of MO, FL, and DNA were plotted versus initial adsorbate concentration ( $C_i$ ). The adsorbed amount per gram LDH ( $Q$ ) was calculated as follows;  $Q$  (mol/g) =  $\{(C_i - C_e) \times V\}/m$ , where  $C_i$ ,  $C_e$ , and  $m$  stand for the initial concentration, equilibrium concentration, and the mass of LDH adsorbent, respectively.<sup>17</sup> As shown in Figure 2(a) and (b), regardless of adsorbate type, the adsorbed amount increases depending on the initial concentration. It is worthy to note here that the DNA adsorption on LDH is approximately twice compared to MO or FL, while the adsorbed amounts of MO and FL are similar. In other words, the adsorption of DNA on the LDH is significantly higher than single molecular adsorbates. In order to explain the difference in adsorption behavior in detail, the isotherms were fitted to well known adsorption models such as Freundlich, Langmuir, and the linear kinetics.

The Freundlich equation is represented as  $Q = k \cdot C_i^{1/n}$  ( $Q$ : adsorbed amount in  $\mu\text{g/g}$  unit,  $k$ : the adsorption coefficient ( $k = k_{\text{adsorption}}/k_{\text{desorption}}$ ), and  $n$ : a correlation coefficient indicating the adsorption behavior).<sup>17</sup> Langmuir equation is  $C_i/Q = 1/q_m \cdot k + C_i/q_m$  where  $q_m$  and  $k$  are adsorption coefficients. Linear model is simply expressed as  $Q = K \cdot C_i$ . For the linear regression, the Freundlich equation was modified to  $\log Q = 1/n \cdot \log C_i + \log k$ . As shown in Table 2, the isotherms are best understood with Freundlich model having  $R^2 > 0.99$  therefore we analyzed the adsorption behavior with Freundlich kinetics.

The correlation coefficients are displayed in Table 3 showing  $k$  values of 627, 217, and 22 and  $n$  values of 1.07, 0.90,

**Figure 2.** (a) The adsorption isotherms, (b) fitting results according to Freundlich model, and (c) the adsorption efficiency of MO, FL, and DNA.

and 0.59 for MO, FL, and DNA, respectively. The significantly different correlation coefficients result from the different shape and charge density of each adsorbate.

Methyl orange and fluorescein exist as single molecule with characteristic shapes and anionic functional groups; MO has cylindrical shape with sulfonate ( $-\text{SO}_3^-$ ) group while FL has spherical shape with carboxylate ( $-\text{CO}_2^-$ ) group (Fig. 3(a) and (b)). In contrast, DNA strands are biopolymers having helical structure and phosphate ( $-\text{PO}_3^-$ )

**Table 2.** The correlation coefficients and regression equation of isotherm models such as Freundlich, linear, and Langmuir

Adsorbate	Isotherm models	Regression equation	$R^2$
Methyl orange	Freundlich	$y = 0.938 \cdot x + 2.797$	0.999
	Linear	$y = 435.45 \cdot x$	0.997
	Langmuir	$y = 7 \times 10^{-7} \cdot x + 0.002$	0.575
Fluorescein	Freundlich	$y = 1.117 \cdot x + 2.337$	0.993
	Linear	$y = 395.47 \cdot x$	0.984
	Langmuir	$y = -2 \times 10^{-6} \cdot x + 0.003$	0.458
DNA	Freundlich	$y = 1.685 \cdot x + 1.334$	0.995
	Linear	$y = 996.88 \cdot x$	0.937
	Langmuir	$y = 9 \times 10^{-6} \cdot x - 0.002$	0.229

in the backbone (Fig. 3(c)). Typically, a base pair contains two negatively charged nucleotides which periodically repeat every 3.4 Å. The required area per charge of each anionic species can be calculated by assuming the simplest molecular shape described in Figure 3. The estimated values for the equivalent area per charge are 23.8, 95.0, and 34.0 Å<sup>2</sup>/e<sup>-</sup> for MO, FL, and DNA, respectively.

As the adsorption between LDH and anionic adsorbates

**Table 3.** Adsorption coefficients obtained from Freundlich model

	$k$	$n$	$R^2$
Methyl orange	627	1.07	0.999
Fluorescein	217	0.90	0.993
DNA	22	0.59	0.995

are based on cation (LDH)-anion (adsorbate) interaction, the  $k$  values are thought to be highly dependent on the equivalent area per unit charge. However, DNA shows much smaller  $k$  value than expected. Smaller molecules like MO and FL move actively to adsorb on LDH and be stabilized. On the other hand, once adsorbed DNA can be also detached due to the physical strain of the dangling part, if only a small portion of strand is attached on the LDH surface.

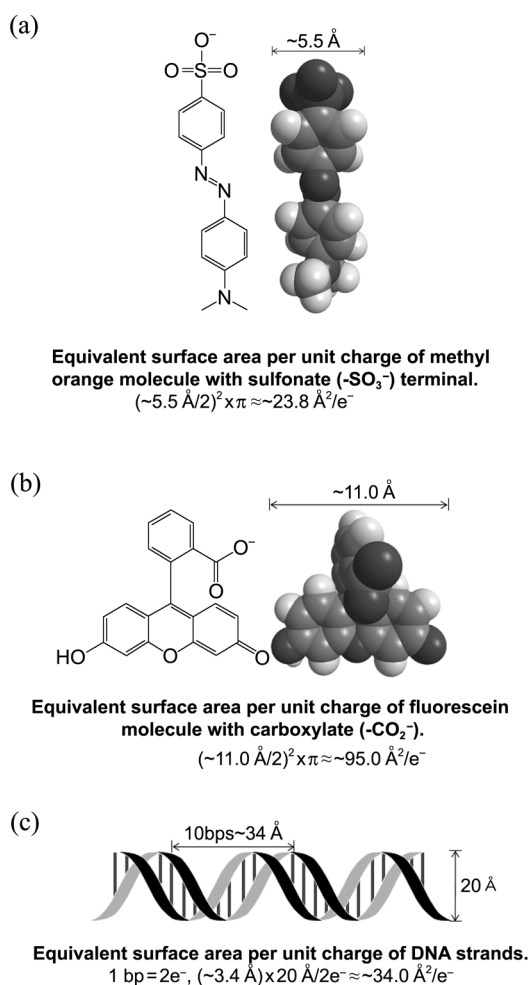
As shown in Table 3, the  $n$  values of MO and FL are 1.07 and 0.90, respectively. Both  $n$  values are approximately 1, meaning that their adsorption behavior in Freundlich model is fairly similar to linear one (see the  $R^2$  values in Table 2). The  $n$  value of DNA is 0.59 with which we could expect the exponentially increasing adsorption with respect to the initial concentration. It means that the adsorbed amount of DNA strands rise rapidly according to the initial concentration increase since the polymeric DNA strands have more chance to interact with LDH surface than single molecules. Furthermore, LDH and DNA have similar equivalent area values per charge (36.4 Å<sup>2</sup>/e<sup>-</sup> and 34.0 Å<sup>2</sup>/e<sup>-</sup>, respectively) exhibiting similar charge periodicity. This regularity in charge is thought to accelerate the adsorption of DNA on LDH surface.

It is also cross-confirmed by the adsorption efficiency estimation<sup>18</sup> (Fig. 2(c)). The adsorption efficiency  $E$  is calculated as  $E = (C_e - C_i)/C_i \times 100$ . As shown in Figure 2(c), the efficiency of DNA adsorption increase dramatically (upto 25%) upon  $C_i$ , while those of MO and FL are just fluctuating around 10%. It is therefore concluded that the LDH has higher advantages in adsorbing DNA compared with single molecules. Even though the  $k$  value for DNA is smaller than the single molecules, the final adsorption after equilibrium would be much higher.

## Conclusion

In this report, we have investigated the adsorption behaviors of three kinds of anionic molecules such as MO, FL, and DNA strands on the size controlled LDH nanoparticles. It was revealed that the difference of adsorption amount among the adsorbates is mainly dependent on the area demand per unit charge (Å<sup>2</sup>/e<sup>-</sup>) for single molecules. In the case of DNA strands, adsorbed amount of DNA strands depended on initial concentration and exceeded single molecules due to the concurrence of equivalent area per charge and charge periodicity. We were successful to demonstrate that the LDH has advantage in selective adsorption of DNA competing with single molecular anions.

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**Figure 3.** Schematic structure and equivalent surface area per unit charge (Å<sup>2</sup>/e<sup>-</sup>) of anionic molecule adsorbates (a) MO, (b) FL, and (c) DNA strands were calculated.

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