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Development of Fluorescent Film Sensors for the Detection of Divalent Copper

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Abstract: Monolayers of several peptide lipids at air−water and air−solid interfaces were prepared using Langmuir and Langmuir−Blodgett (LB) film techniques, and tested as fluorescent sensors for copper ions in aqueous phase. In one method, both the ionophore and the fluorophore were in the same molecule (lipid **A)**, so intramolecular interaction was responsible for the fluorescence quenching of monolayers of this lipid. In the other method, ionophore and fluorophore were located on two different molecules (lipids **B** and **C**) so the intramolecular coupling does not exist; instead the fluorescence quenching was realized by a through-space interaction mechanism. Several experimental techniques, including π−A isotherm, epifluorescence microscopy, and absorption and emission spectroscopies were used to study the different characteristics of copper ion effect on the properties of the lipid monolayers. Additionally, the fluorescence quenching properties of the Langmuir monolayers were found to be transferred to the one-layer LB films. On LB films, the fluorescence response presented a clear selectivity for copper ions in comparison with several other transition metal ions. Further, an excellent reversibility was observed: the fluorescence was switched OFF by immersing the solid substrate in copper ion solution and ON by washing with HCl solution. The intermolecular approach used here seems to be a very flexible and general method to design surface-oriented fluorescent sensors to meet different analytic purposes.

Introduction

The past few years have witnessed a large number of reports on the design of fluorescent sensors for the detection of divalent copper ions.[1−10] This intense research interest is strongly related to the analytical significance of trace transitional metal ions found in environmental and biological processes. The molecular sensors that have been developed so far are commonly composed of two structural subunits: a fluorophore (for signal transduction) and an ionophore (for selective recognition of metal ion).[11−16] The two components are intramolecularly correlated together such that the binding of the target metal ion causes significant changes to the photophysical properties of the fluorophore such as emission intensity, wavelength, or lifetime of the excited state.

Most of these studies on copper ion sensors are stressed on the design of selective fluorescent probes used in aqueous or polar organic solvents. In contrast, the investigation of sensors on a surface has been rarely studied.[17,18] In terms of practicability, a surface sensor possesses more favorable properties than a fluorescent probe used only in solution phase. For example, the surface sensor can be used conveniently to achieve real-time and real-space measurements. Reversible switches of the sensor between ON and OFF states are technically easier to realize if the working units are immobilized on a solid surface. Moreover,

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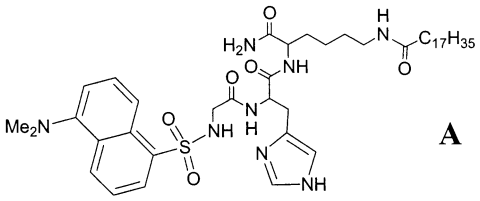
molecular interaction on a surface often exhibits conspicuous divergence from that in solution, thus should be given adequate attention. For instance, we once showed that peptide lipids which have no binding affinity for copper ions in solution can interact with the metal ion significantly at the air−water interface.[19] With this idea in mind, we reason that not only could the surface sensor be designed using the common intramolecular ionophore-fluorophore coupling mechanism, but also the special characteristics of surface chemistry may endow alternative strategies for making sensors with new or improved functionalities.

In the current study, we have developed Langmuir and Langmuir−Blodgett (LB) films of several peptide lipids to detect copper ions in aqueous subphase. We have recently reported on two peptide structures, i.e., glycine-histidine[20] and dansyl-glycine-histidine[21] which showed selective binding toward copper ion. In our studies, dansyl group (5-(dimethylamino)-naphthalene-1-sulfonamide, Dns) was used for fluorescent transduction due to its amiable reactivity in amino modification in solid-phase peptide synthesis. Dansyl fluorophore has a large Stokes shift, thus its fluorescence is not prone to self-quenching in Langmuir and Langmuir−Blodgett films.[22] This is strikingly advantageous when compared to many other fluorophore lipids such as fluorescein or anthrancene lipids, which show serious self-quenching upon compression and have to be used in a very low fraction at air−water interface.[23,24] Herein, the dansyl-glycine-histidine unit is present in lipid **A** (Dns-Gly-His-Lys-(C_{18})). Therefore, fluorescence quenching on the surface can be achieved through intramolecular ionophore−fluorophore interaction. In the monolayers composed of lipids **B** (Gly-His-Lys(C_{18})) and **C** (Dns-Lys(C_{18})), the ionophore and fluorophore are located on two different molecules, so the intramolecular ionophore-fluorophore coupling does not exist any more. However, taking into account the fact that lipid molecules are pressed closely adjacent to one another at air−water interface, we speculate that the ionophore-fluorophore coupling could be worked out by a through-space interaction mechanism.



Experimental Section

Materials. The Rink amide resin and amino acids used for peptide synthesis were purchased from Advanced Chem Tech (Louisville, KY) or Novabiochem (San Diego, CA). Other chemicals and solvents were purchased from Aldrich (St. Louis, MO) or Acros (Pittsburgh, PA) and used without further purification. Inorganic salts used for fluorescence measurements were analytical grade pure. The water used (pH = 5.8) was purified by a Modulab 2020 water purification system (Continental Water Systems Corp., San Antonio, TX). It has a resistance of 18 MQ.cm and a surface tension of 72.6 mN/m at 20 °C.

Synthesis of Peptide Lipids. All the peptide lipids were synthesized via standard solid phase 9-fluorenylmethoxycarbonyl (Fmoc) chemistry.[25,26] Rink amide resin was used as the solid phase; therefore the C-terminal end of the products is carboxamide. Originally, Wang resin was used and the obtained peptide lipids have a free carboxyl end group. This carboxyl group (pK_a around 3.0) is negatively charged in solution at neutral pH, thus the peptide lipids would have electrostatic interactions with metal cations and their binding selectivity toward copper ions may be compromised. On the other hand, the carboxamide group remains uncharged under the experimental conditions used in this study and so avoids the problem of electrostatic interactions. Three amino acids (Fmoc-Gly-OH, Fmoc-L-His(Boc)-OH and Fmoc-L-Lys-(Dde)-OH) were used to construct the peptide backbone. Diisopropylcarbodiimide (DIC) and 1-hydroxylbenzotriazole (HOBt) in situ activation method was used for the coupling reactions. Dansylation was done with 1.5 equiv of dansyl chloride in the presence of 2 equiv of diisopropylethylamine (DIEA) for 30 min. The Dde group was removed with 2% hydrazine in DMF for 4 min.[27] The hydrophobic long chain (C_{18}) was introduced by reacting stearic acid activated with DIC with the peptide. Cleavage of peptides from the resin was completed with CF_3COOH/H_2O (95/5, v/v) for 2.5 h. After the synthesis, TFA in the residue was removed with a N_2 stream. The crude products did not precipitate in ether, instead water was used for the precipitation (sometimes pH needed to be adjusted). The crude product was lyophilized under vacuum. Semipreparative RP-HPLC was performed on a Waters 2690 separations module. A reversed-phase diphenyl column was used (219TP1010, Vydac Inc). Two solutions were prepared: A: 0.1% TFA in water, B: 0.1% TFA in 2-propanol/acetonitrile (1:1, v/v). Typically a linear gradient (30∼70% B within 40 min, flow rate at 2 mL/min) was used.

Analysis of Peptide Lipids. The purities of synthesized lipids were characterized with analytical HPLC, ^{1}H NMR, and mass spectrometry. Analytical HPLC was conducted on a small-scale column (219TP5415, Vydac Inc). The same gradient as the semipreparative method was used (flow rate at 1 mL/min). ^{1}H NMR data were taken on Bruker 300 or 500 MHz spectrometers. Low resolution FAB was recorded on a VG-Trio 2000 mass spectrometer. High-resolution FAB was conducted on a 70−4F instrument in the Mass Spectrometry Lab, University of Illinois at Urbana-Champaign. **Lipid A**. ^{1}H NMR (500 MHz, MeOD): δ = 8.79 (1H, s), 8.59 (1H, d), 8.32 (1H, d), 8.19 (1H, d), 7.62 (2H, m), 7.38 (1H, s), 7.31 (1H, d), 4.71 (1H, m), 4.31 (1H, m), 3.47 (2H,

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s), 3.35 (2H, m), 3.27 (2H, m), 3.17 (2H, m), 2.90 (6H, s), 2.16 (2H, t), 1.85 (1H, m), 1.75 (1H, m), 1.62−1.35 (6H, m), 1.35−1.21 (28H, m), 0.89 (3H, t). FAB-MS: 839.5310 (MH^+, calcd 839.5217). **Lipid B**. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.53 (1H, d), 8.17 (1H, d), 7.76 (2H, m), 7.68 (1H, s), 7.04 (1H, s), 6.90 (1H, s), 4.60 (1H, m), 4.09 (1H, m), 3.58 (2H, m), 2.97 (4H, m), 2.02 (2H, t), 1.70 (1H, m), 1.52 (1H, m), 1.46 (2H, m), 1.38−1.12 (32H, m), 0.86 (3H, t). FAB-MS: 606.4705 (MH^+, calcd 606.4707). **Lipid C**. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.40 (1H, d), 8.29 (1H, d), 8.09 (1H, d), 8.02 (1H, d), 7.54 (3H, m), 7.21 (1H, d), 7.11 (1H, s), 6.83 (1H, s), 3.50 (1H, m), 2.78 (6H, s), 2.63 (2H, m), 1.97 (2H, t), 1.42 (4H, m), 1.23 (28H, m), 1.11−0.92 (4H, m), 0.86 (3H, t). FAB-MS: 645.4413 (MH^+, calcd 645.4414).

Surface Chemistry and Photophysical Measurements. Fluorescence measurements were performed on a Spex Fluorolog 1680 0.22m double beam spectrometer (Spex Industries, Inc., Edison, NJ). Langmuir monolayers were prepared on a Kibron mini-trough (Kibron Inc., Helsinki, Finland). The peptide lipids of appropriate compositions were dissolved in chloroform/methanol (9:1, v/v). The injected volumes for spreading at air−water interface were usually 20 to 40 μl depending on the concentration of the samples. After the sample had been spread, the solvent was allowed to evaporate for 10 min. Then the barriers were compressed to obtain Langmuir monolayers. Epifluorescence images of Langmuir monolayers under different subphases were recorded with an Olympus IX-FLA epifluorescence microscope (Olympus America Inc, Melville, NY). UV light was selected for the excitation and an Optronic Magnafire TM CCD camera (Meyer Instruments, Houston, TX) was used to detect the fluorescence emission. Absorption spectra of monolayers at air−water interface were recorded with a custom-made Hewlett-Packard 8452A diode array spectrophotometer through a quartz window in the center of a KSV mini-trough. The fluorescence spectra of Langmuir monolayers at air−water interface were measured via an optical fiber detector on the top of the KSV mini-trough, which was connected to the Spex Fluorog fluorospectrometer. The optical fiber had an area of 0.25 cm^2 and was placed 1 mm above the surface of the subphase. The excitation light was transmitted through the optical fiber from the light source to the monolayer, and the emission light from the monolayer was also sent back to the detector through the optical fiber. LB films were made by transferring the Langmuir monolayers onto a hydrophobic quartz slide by up-to-down vertical deposition. The deposition ratios for the LB films were about 1.0. For the fluorescence measurements of LB films, the loaded slide was put in a 1-cm fluorescence cuvette with an angle of 45° facing both the incidence and emission light beams to ensure maximum collection of fluorescent signals.

Results and Discussion

Isotherms of Langmuir Monolayers. Surface pressure−molecular area (π−A) isotherms provide useful information on the thermodynamic properties of Langmuir monolayers of peptide lipids, and can be used to evaluate the interaction between the monolayers and copper ions in aqueous subphase. When copper ions are bound to lipid molecules, the limiting molecular area of the monolayer will most likely become larger due to the metal ion insertion within the monolayer composition. The isotherm of lipid **B** on aqueous subphase (phosphate buffer, pH = 7.0) showed a stable solid-condensed phase with a limiting molecular area of 35 Å^2/molecule and a collapse surface pressure of 41 mN/m (Figure 1, curve a). When copper ions were present in the subphase, the monolayer showed a characteristic of liquid-expanded phase (Figure 1, curve b). The collapse surface pressure decreased to 37 mN/m, and meanwhile, the limiting molecular area increased to 61 Å^2/molecule, a change of 26 Å^2/molecule compared with that on aqueous subphase. These

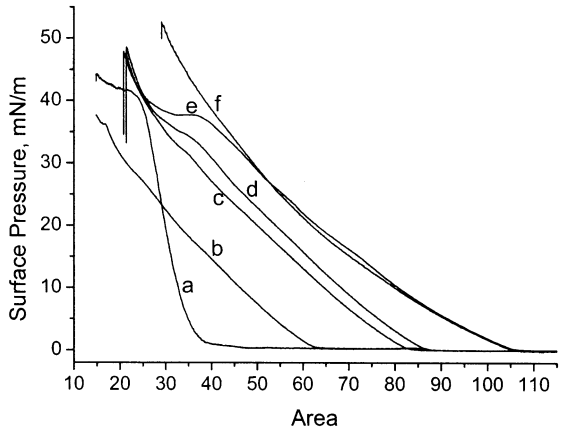

Figure 1. Surface pressure−area (π−A) isotherms of peptide lipid monolayers at air−water interface in the absence and presence of copper ions (10^{-5} M) in the aqueous subphase. (a) Lipid **B** on aqueous buffer, (b) lipid **B** on copper ion solution, (c) lipid **C** on aqueous buffer, (d) lipid **C** on copper ion solution, (e) lipid **A** on aqueous buffer, (f) lipid **A** on copper ion solution. All subphases were phosphate buffered to pH 7.

observations show that copper ions were strongly bound to the monolayer and affected its thermodynamic properties.

The Langmuir monolayer of lipid **C** presented a liquid-expanded phase characteristic and had a limiting molecular area of 82 Å^2/molecule (Figure 1, curve c). Copper ions only had a slight effect on the isotherm (Figure 1, curve d). The limiting molecular area became a bit larger (86 Å^2/molecule) and the collapse surface pressure increased by 3 mN/m (from 32 to 35 mN/m). This small change is reasonable because lipid **C** does not possess any binding site for copper ions.

The isotherm of lipid **A** exhibited a characteristic of liquid-expanded phase (Figure 1, curve e), which is similar to lipid **C**. The monolayer had a limiting molecular area of 103 Å^2/molecule and a collapse surface pressure of 38 mN/m. The large value of limiting molecular area is not surprising because this lipid possesses a tripeptide moiety and a bulky dansyl group. When copper ions were present in the subphase, the collapse surface pressure went up to 53 mN/m, indicative of enhanced film stability. Unexpectedly, there was no marked change to the limiting molecular area (105 Å^2/molecule) of the lipid. In fact, as shown in the next sections, the photophysical properties of lipid **A** monolayers were hugely affected by copper ions. To explain why the limiting molecular area of lipid **A** monolayer was not affected by copper ions, we considered two factors involved in the lipid-metal interaction. The first factor is the metal-binding interaction. Because lipid **A** possesses a peptidyl motif, which can strongly bind with copper ions, the bound metal ion will increase the limiting molecular area of the monolayer. The increasing value induced by copper binding can be estimated to be 26 Å^2/molecule as seen from the monolayer of lipid **B**. The other determining factor is the conformation of dansyl group. It is reported that monolayers of dansyl containing lipids at air−water interface can exist in two kinds of conformations: horizontal and vertical.[28,29] The conformational change from horizontal to vertical orientation reduces the limiting molecular area (25−30 Å^2/molecule). Most likely, the two counteracting factors work together during the compression, and

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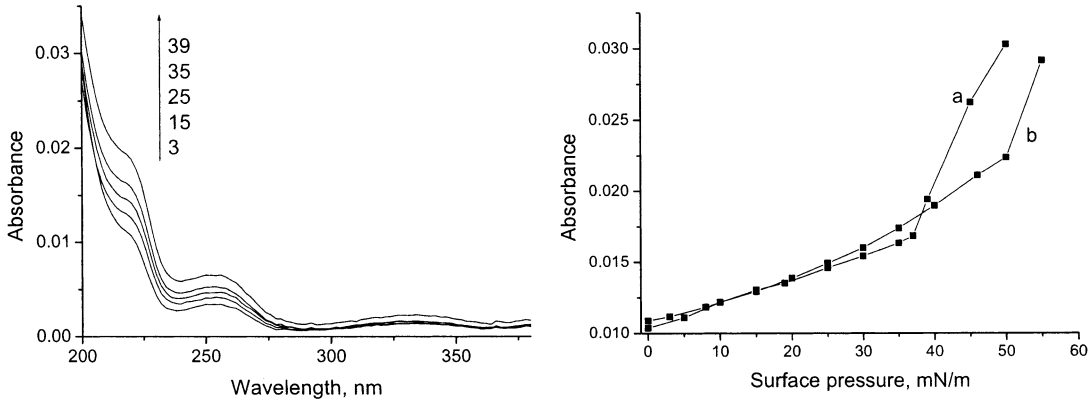


Figure 2. Relationship of UV−vis absorption of lipid **A** monolayer at air−water interface with surface pressure. Left: absorption spectra of the monolayer on water subphase at surface pressures of 3, 15, 25, 35, and 39 mN/m. Right: The absorbance at 218 nm of the monolayer with surface pressure in the absence and presence of copper ions (10^{-5} M). (a) Lipid **A** on water, (b) lipid **A** on copper ion solution (pH = 7.0).

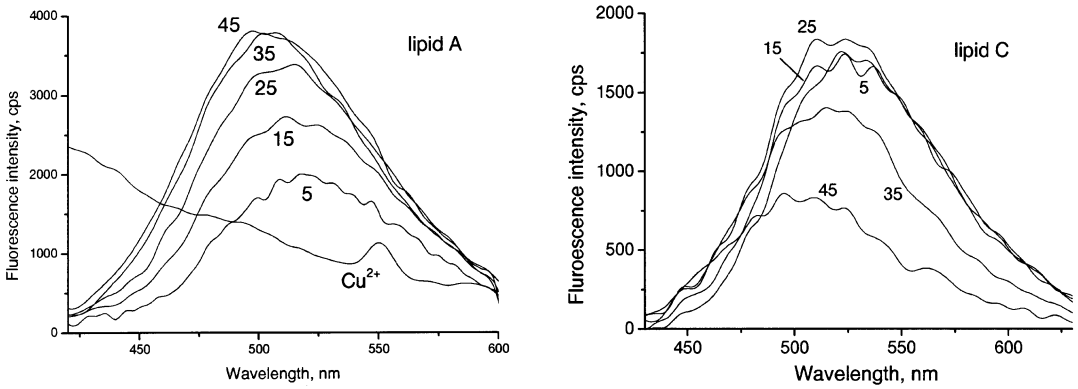


Figure 3. Fluorescence of Langmuir monolayers of lipids **A** and **C** at different surface pressures. Left: fluorescence of lipid **A** monolayer on water subphase at 5, 15, 25, 35, and 45 mN/m, respectively, and on copper ion subphase (surface pressure at 15 mN/m; copper concentration at 10^{-5} M; pH = 7.0). Right: fluorescence of lipid **C** monolayer on water subphase at 5, 15, 25, 35, and 45 mN/m, respectively.

as a consequence no manifest measurable change in the limiting molecular area was observed.

Absorption Spectra of Langmuir Monolayers. Absorption data offer additional information on the properties of the Langmuir monolayers of various lipids. As a typical example, we show here the absorption spectra of the lipid **A** monolayer at air−water interface (Figure 2). The spectra have three maximum absorption bands at 218, 254, and 334 nm. Upon compression of the monolayer, the absorbance increased steadily and a linear relationship between the absorbance and surface pressure was observed. This is understandable because the number of molecules per unit area increased during compression. An inflection point at 37 mN/m was seen on the absorbance−surface pressure curve, after which absorbance increased with a much sharper slope. In the presence of copper ions, the linear increase in absorbance lasted up to a surface pressure value of 50 mN/m. As noted above, π−A isotherms of lipid **A** showed a collapse surface pressure at 38 mN/m in the absence (53 mN/m in the presence) of copper ions. Therefore, it is concluded that these inflection points are related to the collapse of the lipid **A** monolayers.

Fluorescence Spectra of Langmuir Monolayers. Fluorescence is one of the most important properties of dansyl-labeled lipids. We recorded the fluorescence spectra of lipid monolayers at air−water interface under different surface pressures. For the monolayer of lipid **A**, with the increase of surface pressures, the fluorescence intensity increased and the maximum emission wavelength was blue-shifted (Figure 3, left graph). The increase in fluorescence intensity is mainly due to the increase in the number of lipid molecules per unit area, which is similar to its absorption spectra. Grieser et al.[28] proposed that the blue shifts of dansyl in a liquid-expanded phase are caused by changes in the solvation of the dansyl groups as the monolayer becomes progressively denser. If copper ions were present in the aqueous subphase, the fluorescence of the monolayer was largely quenched due to the binding between Cu^{2+} and the lipid molecules (indicated in Figure 3, left graph). When the surface pressure changed, the quenched spectra did not show any difference and were overlapped together (see the Supporting Information).

The fluorescence intensity of lipid **C** monolayer increased with surface pressure until 25 mN/m (Figure 3, right graph). After that, the intensity began to decrease upon further compression. This self-quenching phenomenon under high surface pressure is attributed to the formation of aggregates of dansyl groups. Compared with lipid **A**, lipid **C** does not have the Gly-His-Lys motif, so dansyl groups in lipid **C** monolayer can be compressed more closely, leading to aggregation and fluorescence self-quenching. In the presence of copper ions, the fluorescence of lipid **C** monolayer was almost the same as that in aqueous solution and exhibited a similar dependence on the surface pressure (see the Supporting Information). This gives further proof that copper ions have little effect on the lipid **C** monolayer.

Epifluorescence of Langmuir Monolayers. The effect of copper ions on the fluorescence of Langmuir monolayers of

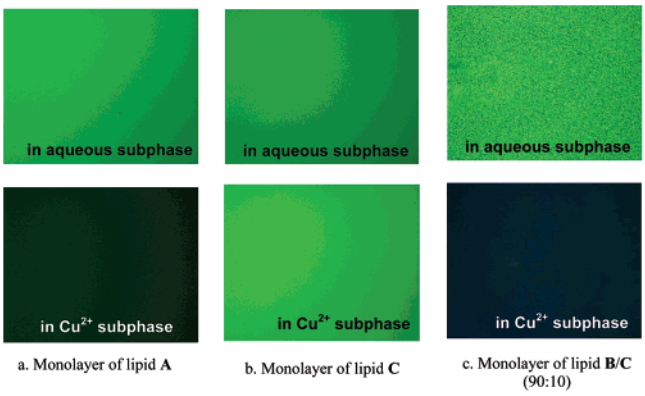
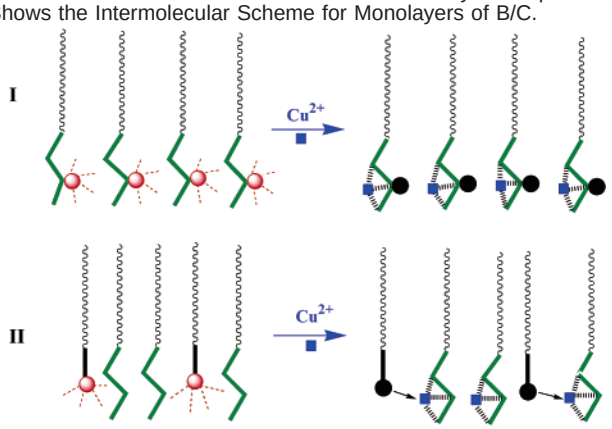


Figure 4. Epifluorescence images of Langmuir monolayers of peptide lipids **A**, **C**, and **B**/**C** (90:10, molar ratio) in the absence and presence of copper ions (10^{-5} M) in the subphase (image size 895 × 713 μm). Experimental conditions: surface pressure = 15 mN/m; pH = 7.0 (phosphate buffer); UV light was used for excitation.

Scheme 1. Proposed Mechanisms on the Fluorescence Quenching of Langmuir Monolayers Caused by Copper Ions. I Shows the Intramolecular Scheme for the Monolayer of Lipid A. II Shows the Intermolecular Scheme for Monolayers of B/C.



various peptide lipids was also demonstrated by epifluorescence microscopy. Figure 4a shows the epifluorescence images of lipid **A** with and without Cu^{2+} in the aqueous subphase. The drastic change from a bright green color to a dim one illustrates the strong fluorescence quenching of the Langmuir monolayer caused by copper ions.

Epifluorescence of lipid **C** is different from **A**. It is seen that copper ions did not cause any quenching to the epifluorescence image of the Langmuir monolayer of lipid **c** (Figure 4b). This is actually what we expected because it proves that dansyl group itself in the lipids was not affected by the presence of copper ions and therefore dansyl lipid such as **C** can be used as a general probe in combination with other recognition motifs to prepare sensors for this target ion. This idea is clearly illustrated in the mixed monolayer of lipid **B** and **C** (90:10 in molar ratio). The epifluorescence images of this **B**/**C** monolayer are shown in Figure 4c. They were strongly fluorescent in aqueous buffer subphase but quenched when copper ions were present. Obviously, it is the binding of lipid **B** that caused the fluorescence quenching of **C**. This intermolecular working scheme is strikingly different from that of lipid **A** which is based on intramolecular ionophore−fluorophore coupling mechanism. We present the two different mechanisms in Scheme 1.

Fluorescence of LB Films. Langmuir monolayers of peptide lipids were transferred onto hydrophobic quartz slides to obtain fluorescent Langmuir−Blodgett (LB) films. These LB films are used as solid-supported sensors for the detection of copper ions in aqueous subphase. The fluorescence spectra of the LB films of lipids **A** and **C** are shown in Figure 5. The fluorescence of lipid **A** has a maximum emission at 524 nm (excited at 350 nm). When the slide was put in an aqueous solution containing several miscellaneous ions (Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}, each 10^{-5} M), the fluorescence intensity only slightly decreased indicating that the LB film has a very weak interaction with these competitive ions. Then the slide was put in a copper ion solution (10^{-5} M), and a strong quenching was observed (the intensity at 524 nm was reduced to 30%). So the LB film of lipid **A** showed a clear selectivity toward copper ions. After that, the quartz slide was put in HCl solution (pH = 2) and then rinsed with aqueous buffer (pH = 7). It is seen that the fluorescence of the LB film was efficiently restored, indicating that the bound copper ions were washed out of the film by the acid treatment.

Unlike lipid **A**, the fluorescence of the LB film of lipid **C** was affected very little by copper or other transition metal ions (Figure 5, right graph). This is in accordance with the aforementioned copper ion effect on the Langmuir monolayers of lipid **C** and gives further proof that the fluorescence of the dansyl lipid itself was not affected by metal ions. Therefore, all the fluorescent changes caused by copper or other metal ions should be attributed to the specific interaction between the peptidyl binding motifs and the metal ions.

The fluorescence of LB films of **B**/**C** was also efficiently quenched by copper ions in aqueous subphase (Figure 6). In the LB film of **B**/**C** (90:10, molar ratio), the intensity at 500 nm decreased to 35% in the presence of copper ions (10^{-5} M). Comparatively, the other metal ions have a less degree of quenching (64% of intensity remained at 500 nm). Therefore, a clear selectivity was seen. Further, the slide was washed with acid, and then buffer solution; the fluorescence again was restored substantially demonstrating the reversibility of the LB film. We also prepared LB film of **B**/**C** in different composition (100:1, molar ratio) and measured its fluorescence (Figure 6, right graph). The experimental results such as the effects of copper and other transition metal ions, and the acid treatment are all the same as the former **B**/**C** (90:10) film. The only difference is the lower fluorescence intensity because a quite small fraction of lipid **C** was used in the LB film.

Like Langmuir monolayers composed of **B**/**C**, the fluorescence quenching observed in LB films of **B**/**C** was also attributed to through-space ionophore-fluorophore interaction. It seems to us that this intermolecular method could be used generally to design LB film-based sensors with improved efficiency and simplicity. Most of currently studied fluorescent sensors are composed of intramolecularly linked receptor unit and signal transduction unit. Therefore, organic syntheses of these sensor molecules are often lengthy and complex. In our proposed method, the receptor and transduction molecules are designed and prepared independently, so the synthesis process is greatly simplified. Only at the surface chemistry stage are the two parts with appropriate compositions dissolved in organic solvent and compressed to form Langmuir and LB films. The other advantage of this method is that it provides great convenience and flexibility for sensor design. The same fluorophore lipid can be combined with different receptor molecules

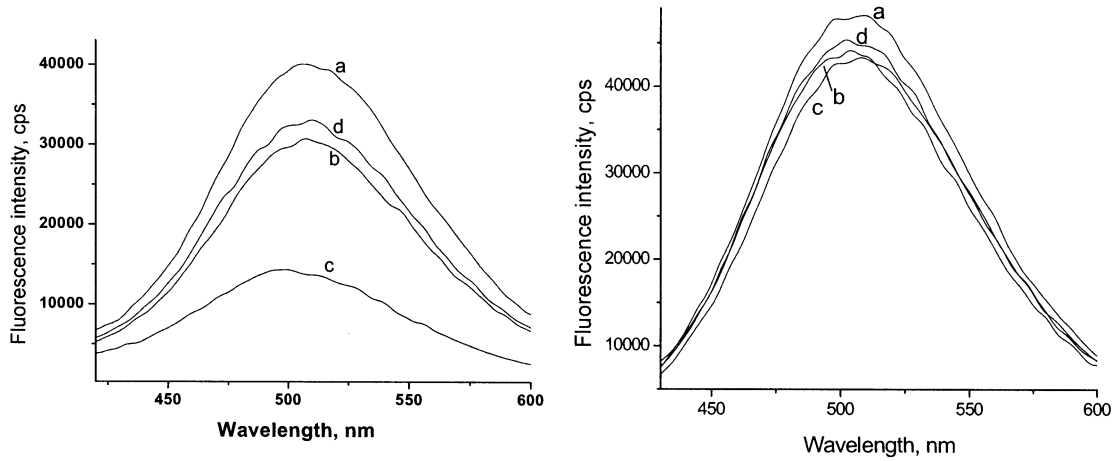


Figure 5. Fluorescence of LB films of **A** (left) and **C** (right) in different subphases. (a) in aqueous buffer; (b) in a solution containing Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}, and each ion has a concentration of 10^{-5} M; (c) in copper ion solution (10^{-5} M); (d) washed with HCl (pH = 2) and then in buffer. All of the solutions (except HCl solution) are phosphate buffered to pH 7.0. Excitation wavelength is 350 nm.

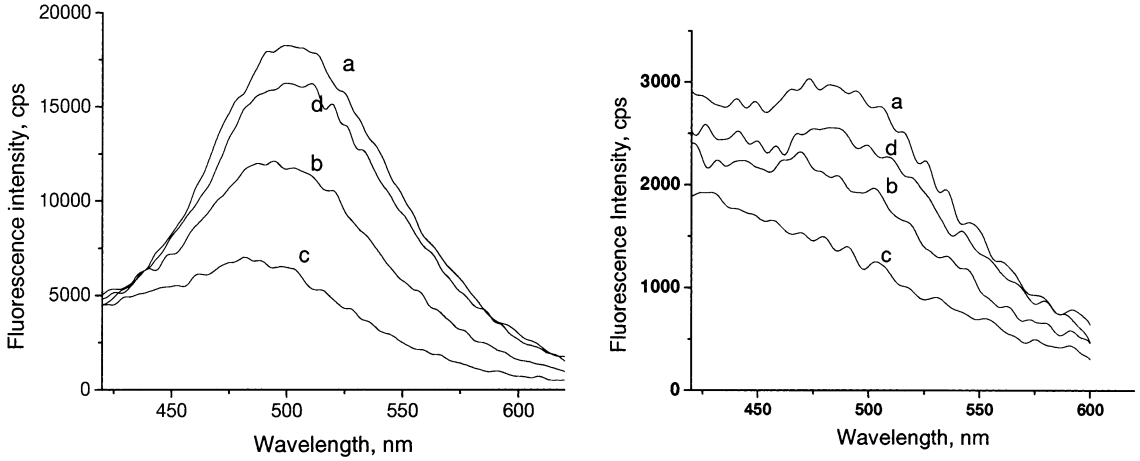


Figure 6. Fluorescence spectra of LB films composed of lipids **B** and **C**. Left: **B**/**C** (90:10, molar ratio) and Right: **B**/**C** (100:1, molar ratio)). (a) in aqueous buffer; (b) in a solution containing Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}, and each ion has a concentration of 10^{-5} M; (c) in copper ion solution (10^{-5} M); (d) washed with HCl (pH = 2) and then in buffer. All the solutions (except HCl solution) are phosphate buffered at pH 7.0. Excitation wavelength is 350 nm.

for various purposes of analysis; and vice versa, the same receptor can work with different fluorophore lipids to obtain fluorescent signals with different parameters (like different excitation or emission wavelengths). To illustrate this idea, we prepared a LB film composed of lipid **B** and a fluorescein lipid, i.e., 5-octadecanoylaminofluorescein (100:1, molar ratio). The fluorescence spectra of the LB film were shown in Figure 7 (excited at 467 nm). Obviously, this fluorescein lipid is more fluorescent than lipid **C**. The maximum emission of the LB film was observed at 524 nm. When copper ions were present in the subphase, as expected, the fluorescence intensity dramatically decreased (8% of intensity remained at 524 nm). The other transition metal ions also caused a quenching to the fluorescence but not as much as Cu^{2+}. The low selectivity could be ascribed to the nonspecific interaction between the fluorescein motif and the metal ions. And again, the fluorescence was found to be efficiently restored by washing out the bound copper ions with HCl. These results together with those of **B**/**C** lipids, demonstrate the validity of the intermolecular receptor-fluorophore coupling mechanism in the fluorescent sensor design.

It is of interest that Tonellato and co-workers have exploited a self-assembly strategy for the design of selective copper ion sensors.[30,31] In their method, the ligand (receptor) and fluorophore units self-assembled intimately within surfactant ag-

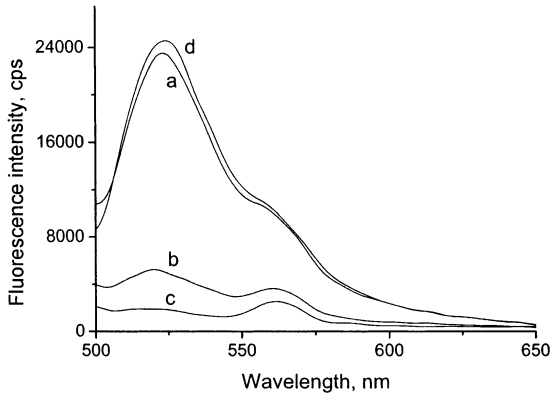


Figure 7. Fluorescence spectra of LB film of lipid **B** and the fluorescein lipid (100:1, molar ratio). (a) in aqueous buffer; (b) in a solution containing Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}, and each ion has a concentration of 10^{-5} M; (c) in copper ion solution (10^{-5} M); (d) washed with HCl (pH = 2) and then in buffer. All the solutions (except HCl solution) are phosphate buffered at pH 7.0. Excitation wavelength is 467 nm.

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gregates in water, and communication between the binding site and the signaling unit occurred effectively. Obviously, the signal transduction in their study was also worked out via intermolecular receptor−fluorophore interaction. We envision that the two techniques, i.e., self-assembly and surface chemistry technique (Langmuir and Langmuir−Blodgett films) could be used complementarily to design fluorescent sensors for chemical species of interest such as transition metal ions.

Conclusion

We have used two different strategies to make Langmuir and Langmuir−Blodgett films whose fluorescence was effectively quenched by copper ions in aqueous subphase. In lipid **A**, the ionophore and fluorophore are intramolecularly linked together so intramolecular ionophore-fluorophore coupling is responsible for the fluorescence quenching of the monolayers. In the other method, the ionophore (lipid **B**) and fluorophore (lipid **C** or fluorescein lipid) are two different molecules, so the intramolecular interaction does not exist; instead the copper-sensing functionality was realized by a through-space interaction mechanism. For the LB films, the fluorescence quenching exhibited a clear selectivity toward Cu^{2+} in comparison with several other transition metal ions. Moreover, the fluorescence can be effectively restored by washing out bound metal ions with HCl, therefore the LB films presented an ideal reversibility. The intermolecular signaling method used in this study provides great advantages for the sensor design like simplicity and flexibility.

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Supporting Information Available: Additional absorption and fluorescence spectra of lipid monolayers at the air−water interface. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0293610

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