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# Fabrication of a Novel Cholic Acid Modified OPE-Based Fluorescent Film and Its Sensing Performances to Inorganic Acids in Acetone

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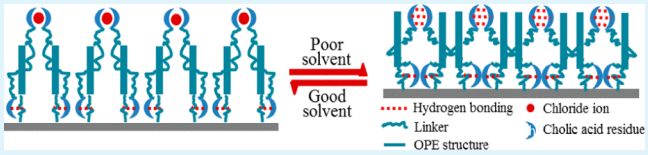
Supporting Information

**ABSTRACT:** A self-assembled monolayer (SAM)-based fluorescent film was designed and prepared by chemical immobilization of a novel oligo(*p*-phenylene- ethynylene) (OPE) with cholic acid moieties at the ends of its side chains (Film 1). As a control, a similar film, Film 2, of which OPE brings no side chains, was also prepared. The structures of the films were characterized by contact angle, XPS, ATR-IR and fluorescence measurements. Fluorescence studies revealed that the emission of Film 1 is sensitive to the presence of trace amount of some inorganic acids in acetone, such as HCl, $H_2SO_4$, $HNO_3$, and $H_3PO_4$, etc., whereas the acids as studied showed little effect on the emission of Film 2. The difference in the sensing performances of the two films have been rationalized by considering presence or absence of a possible cavity, a substructure appearing above the OPE adlayer which is something like a dimer of cholic acid (CholA) formed at specific environment.




**KEYWORDS:** *oligo(p-phenyleneethynylene) (OPE), cholic acid (CholA), organic medium, pH determination, fluorescence films, self-assembled monolayer (SAM)*

## 1. INTRODUCTION

The application of organic solvents in enzymology has attracted increasing attention during the last few decades.[1] This is because enzymes in organic solvents may offer unique properties, such as increasing solubility of substrates, realizing reactions that may not be feasible in water, and enhancing overall conversion due to favorable equilibrium shift, etc.[2−5] In fact, nonaqueous enzymology has become an important field of biotechnology recently.[6] However, as reaction media, organic solvents are very different from water, which is the natural medium for enzymes to play their functions, and thereby it is not difficult to understand why it it is essential to elucidate the influencing factors on the stability of enzymes first before them can be used in the nonaqueous media.[7,8] In fact, for every enzyme, there is an optimum pH value at which the specific enzyme functions most actively. Any change to the pH will significantly affect its activity and the rate of the reaction under its catalysis. Therefore, it is of great importance to realize online and real-time monitoring of pH in organic solvents.

Ion-selective electrodes have been well-recognized as the most effective pH monitoring technique as evidenced by the widely adopted pH-sensitive glass electrode, which is usable both in aqueous and nonaqueous media.[9] However, the method has suffered a few serious drawbacks, for example: (1) response speediness of the electrode in nonaqueous medium is often very slow, and in some cases, it may take more than one hour to reach equilibrium,[10] (2) interferences from other ions have long been a troublesome issue,[11] and (3) the electrode is fragile and easily contaminated in organic medium. It is because of these reasons that various efforts have been made to improve the performances of the ion-selective electrode but without significant progress. Therefore, it still remains a big challenge to develop sensitive, selective, fast responsive and nonaqueous medium usable pH monitoring techniques.

Fluorescence methods are widely used in detections because of their high sensitivity, great selectivity, no need of references, and abundant signals.[12−18] Of organic compounds-based fluorescence methods, two kinds of them, including low-molecular mass fluorophores and conjugated polymers (CPs)/oligomers have been widely used or created as sensing elements.[19] Compared to low-molecular-mass fluorophores, CPs and their relevant oligomers possess an important advantage, the so-called super-quenching effect, which means that the backbones of them can act as molecular wires, enabling rapid propagation of an exciton throughout an individual polymer chain.[20−23] The CPs and their relevant oligomers-based fluorescent sensing films are so far usually prepared via spin coating, dip coating, casting or other physical methods.[24] The films as prepared, however, suffer from several drawbacks, such as self-quenching, slow responding, leaching, and contamination of the samples under study, etc.[25−28] Our group started to develop monolayer chemistry-based fluorescent sensing films with CPs or the oligomers as sensing elements a few years ago.[29] On the basis of the strategy, several fluorescent films with superior sensing properties to nitro-aromatic compounds (NACs) either in vapor or in solution states, and to formaldehyde vapor have been designed and prepared.[30] These efforts have opened a new way to generate

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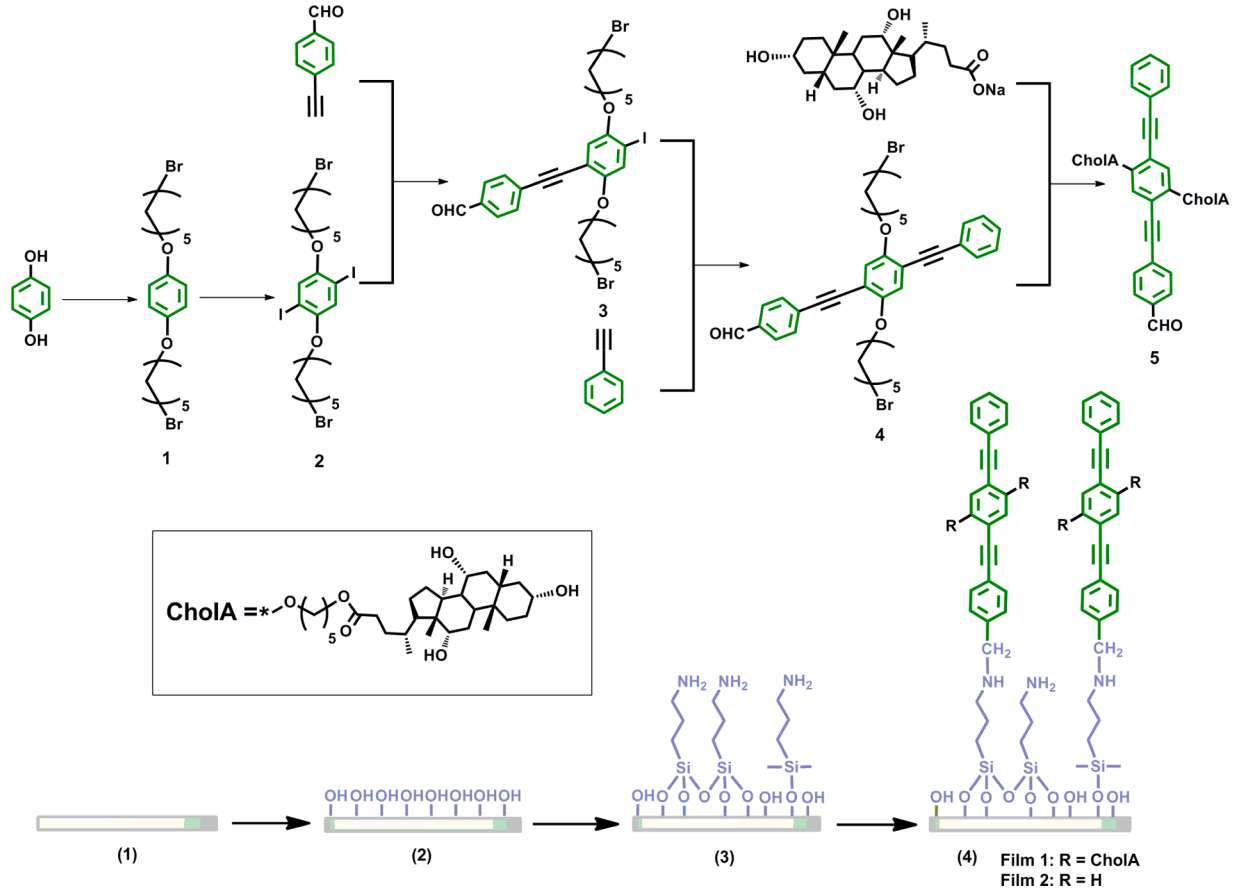
**Scheme 1. Synthesis Procedures of CholA-OPE (5) and Its Chemical Immobilization onto Glass Plate Surface**




fast response, very sensitive and highly stable fluorescent sensing films. However, every coin has two sides. Unlike monomolecular layer chemistry-based fluorescent films with low-molecular-mass fluorophores as sensing elements, creation of CPs or their relevant oligomers-based fluorescent films in the same way suffers from a serious problem that is creation of new fluorescent films by simple variation of the structures of the linkers connecting the sensing elements and substrates may not work due to difficulty in polymerization at the substrate surfaces. Accordingly, a new strategy for creation of the films was proposed recently.[31] In the strategy, suitable structures were specially introduced as side chains into the backbones of the CPs or the relevant oligomers. It is believed that variation of the side chains must result in new fluorescent films without the need of change of the backbones of the sensing fluorophores. In this way, a long flexible alkyl, hexadecyl, was introduced as side chains of poly(*p*-phenyleneethynylene) (PPEs) to obtain novel PPE-based fluorescent films via the monomolecular layer chemistry way.[31]As it is expected, the fluorescent behavior of the film in a "good" solvent is very different from that in a "poor" solvent. In a similar way, cholesterol terminated alkyls were also introduced as side chains to a conjugated oligomer, and the cholesterol-modified oligomer was chemically bonded to a glass plate surface.[32] Fluorescence studies revealed that the side chains exhibit significant effect to the fluorescence behavior of the film. Furthermore, the film is supersensitive to the presence of picric acid (PA) in aqueous phase.

Cholic acid (CholA), which is the most abundant bile acid in humans, has attracted great interest during the past few years mainly because of its superior biocompatibility, unique amphiphilic structure, chirality, and in particular its capability to aggregate in various solvents.[33−35] It is these properties that make CholA an ideal building block for creating novel supramolecular structures. Therefore, it is expectable that introduction of the structure into the side-chains of a suitable conjugated oligomer, and then chemically binding it to a substrate surface would produce a novel fluorescent films with unique structures, and of course unique properties. This article reports the preparation, the characterization, the fluorescence behavior and the sensing applications of the film.

## 2. EXPERIMENTAL SECTION

**2.1. Materials.** 1,4-Dihydroxybenzene (TCI, 99%), 4-bromobenzaldehyde (Alfa aesar, 99%), phenylacetylene (Alfa aesar, 99%), (3-aminopropyl)trimethoxysilane (APTMS, Alfa aesar, 97%), $Pd(PPh_3)_4$ (Alfa aesar, 99%), and CuI (Alfa aesar, 98%) were used as received. All manipulations for the preparation of the samples were performed using standard vacuum line and Schlenk technique under a purified argon atmosphere. Toluene was distilled from sodium benzophenone ketyl under argon prior to use. Acetone was also distilled from potassium under argon before use.

**2.2. Measurements and Characterization.** Fluorescence measurements were performed at room temperature on a time-correlated single photon counting Edinburgh Instruments FLS 920 fluorescence spectrometer with a front-face method. The fabricated film was inserted into a quartz cell with its surface facing the excitation light source. The position of the film was kept constant during each set of measurements. Contact angles of the films were measured on a Dataphysics OCA20 contact-angle system at ambient temperature. $^1$H NMR spectra were measured on Bruker AV 300 NMR spectrometer. The MS were acquired in ESI positive mode and MADI-TOF mode using a Bruker maxis UHR- TOF mass spectrometer. X-ray

photoelectron spectroscopy (XPS) measurements were carried out on an ESCAPHI5400 photoelectron spectrometer. Pressed KBr disks for the powder samples were used for the FTIR spectroscopy measurements. The FTIR spectra were obtained with a Bio-Rad FTIR spectrometer, and the attenuated total reflection infrared (ATR-IR) spectra of the films were recorded with a Bruker VERTEX 70v spectrometer.

**2.3. Synthetic Procedures of Compounds 1−7.** The details of the synthesis are provided in the Supporting Information.

**2.4. Activation and Silanization of the Glass Plate Surface.** A glass plate (0.9 cm ×2.5 cm) was treated in a "piranha solution" (7/3, V/V, 30% $H_2O_2$/98% $H_2SO_4$) (***Warning:*** *Piranha solution should be handled with extreme caution because it can react violently with organic matter*) at 98 °C for 1 h, then rinsed thoroughly with plenty of water, and finally dried at 100 °C in a dust-free oven for 1 h. The activated glass plate was placed in a flask charged with a warm (50 °C) toluene solution (30 mL) of APTMS (0.15 mL) containing trace amount of water (10 μL) for 3 h. After being cooled to room temperature, the plate was removed from the flask, successively washed with copious amounts of toluene and $CH_2Cl_2$, and then dried under a gentle stream of nitrogen.

**2.5. Chemical Coupling of OPE on a Glass Plate Surface.** The APTMS-modified glass plate was further macerated into a $CHCl_3$ solution of compound **5** or **6** ($1 \times 10^{-3}$ mol/L) under reflux for 12 h to generate corresponding Schiff's base. The glass plate was then washed by $CHCl_3$ and directly reduced with $NaBH_4$ in anhydrous methanol for 1 h. To remove unreacted compound **5** or **6**, we rinsed the glass plate with plenty of $CHCl_3$, THF, and water, respectively. The synthesis route of the compounds and the fabrication process were schematically shown in Scheme 1.

## 3. RESULTS AND DISCUSSION

**3.1. Characterization of the Films.** Contact angle (CA) measurement is a simple but efficient way to characterize the SAM surfaces that reflects the polarity and the changes in chemical composition of the examined surfaces.[23] The values of CA ($\theta$) of the surfaces to water at ambient temperature were monitored at different stages of the functionalization process, and the results are shown in Figure 1. It can be seen that the


**Figure 1.** Static contact angles ($\theta$) to water for various glass plate surfaces, where the meanings of (1), (2), (3), and (4) are the same with those explained in Scheme 1.

activation process made the contact angle of the glass wafer surface decrease significantly from 35 ± 1.2° to 10 ± 0.5°, indicating that more hydroxyl groups were generated after treatment with the "piranha solution", which should be favorable for the silanization of the surface. The silanization process reduced the surface wettability as the contact angle rises to 40 ± 1.0°, suggesting the formation of a silane adlayer on the surface. The angle increased further with the introduction of CholA-OPE and reached a value of 70 ± 0.6°. These results are consistent with the expectation from the changes of chemical compositions of the surfaces (c.f. Scheme 1).

XPS is an ideal analysis technique to evaluate the elemental composition of surface-supported organic adlayers.[36] Chemical coupling of CholA-OPE on the glass plate surface was further confirmed by XPS measurements. Figure 2 shows the XPS spectra of each oxidized silicon surface in the process of developing fluorescent SAMs. It can be seen that the signals of C1s and N1s become stronger, compared with the one covered with hydroxyl groups, a direct evidence for the coupling of the silanization agent on the substrate surface. As expected, immobilization of CholA-OPE on the surface made the signals of C1s and O1s even stronger, indicating the covalent attachment of the structure of CholA-OPE. Analysis of the high- resolution XPS spectra of the films could provide useful information on the recognition of the changes in the oxidation state of N1s, which is directly relevant to the compositions of the surface structures of the films. From Figure 2b, it can be seen that for Film 1, about 32.6% of the amino groups reacted with CholA-OPE, whereas only 12.7% of them reacted with OPE for Film 2, indicating that introduction of CholA as side chains to the backbone of the conjugated oligomer improved its immobilization density, which is believed to be favorable for sensing. From another perspective, the result can be also taken as evidence to support the tentative conclusion that the silane adlayer and CholA-OPE derivatives were chemically attached on the substrate surfaces.

As for the immobilization density of CholA-OPE or OPE on the substrate surface, we were quite confused initially because compared to OPE, CholA-OPE carries two bulky side chains, which might cause steric hindrance to the immobilization of the compound onto substrate surface, and would result in low immobilization density. However, experimentally the result is just the opposite. This rather surprising result might be understood by considering that introduction of the side chains may prohibit the aggregation of the OPE backbones of the molecules of CholA-OPE, and favors its dissolution in organic solvents. In fact, OPE, which has no side chains, would stay in monomolecular state due to the insolubility of its aggregates as evidenced by the fact that its emission appears at shorter wavelengths (c.f. Figure 4). But for CholA-OPE, introduction of the side chains promotes the dissolution of its backbone, OPE, due to the hydrophobic nature of the side chains. Good solubility leads to more opportunities for the compound to react with the terminal groups of the silane adlayer on the substrate surface, resulting in a greater density.

The ATR-IR spectra of APTMS-modified glass plate and CholA-OPE-modified glass plate were collected from 4000 to 1250 $cm^{-1}$, as shown in Figure 3. In the figure, it is seen that the existence of a broad peak centering at 3300 $cm^{-1}$ for the APTMS-modified glass plate and the one modified by CholA-OPE may be originated from absorbed water and unreacted OH. But for the later one, there is a high and sharp specific absorption centering at 2850 $cm^{-1}$, which may originate from C−H stretching vibration. In addition, an absorption around 1700 $cm^{-1}$ is observable, which can be assigned to the stretching vibration of C═O in the CholA-OPE structure, a direct evidence to confirm the covalent attachment of CholA-OPE moieties on the glass plate surface.

**3.2. Steady-State Excitation and Emission Spectra of the Films.** The fluorescence excitation and emission spectra of the two films immersed in water or acetone were recorded separately and shown in Figure 4. The maximum excitation of Film 1 in acetone appears at 370 nm, and the maximum emission at 446 nm. Whereas, the maximum emission shifts to 469 nm and the excitation shows no significant change when acetone is replaced by water. In contrast, the position and profile of the excitation and emission spectra of Film 2 recorded are very similar either in acetone or water, indicating that the fluorophore experiences similar microenvironment in the two solvents.

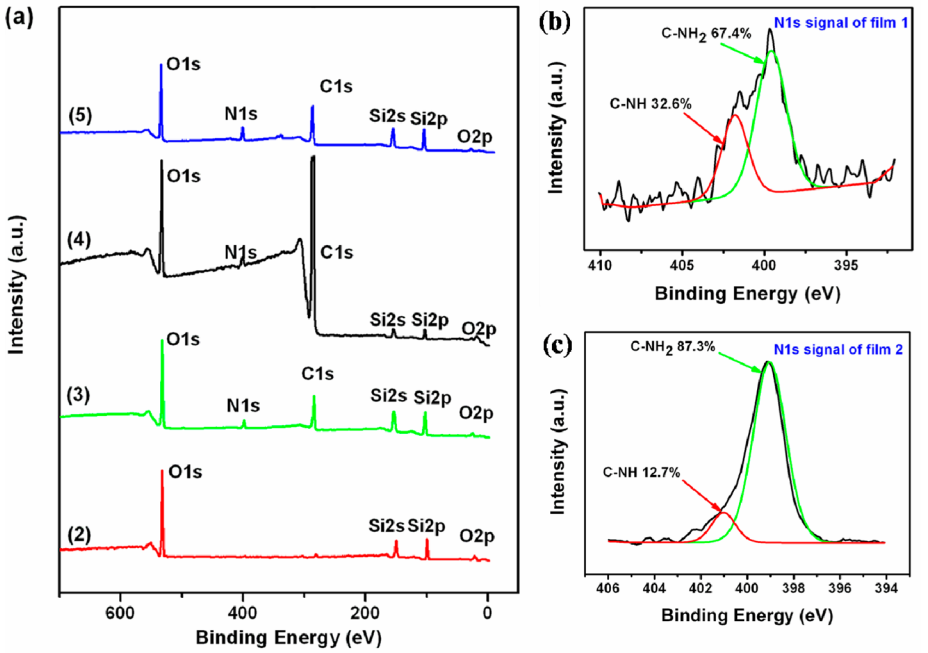



**Figure 2.** XPS traces of Film 1, where the meanings of (2), (3), and (4) are the same with those explained in Scheme 1,a–c are the results from spectroscopy simulation of the signals of N1s from Film 1 and Film 2, respectively.

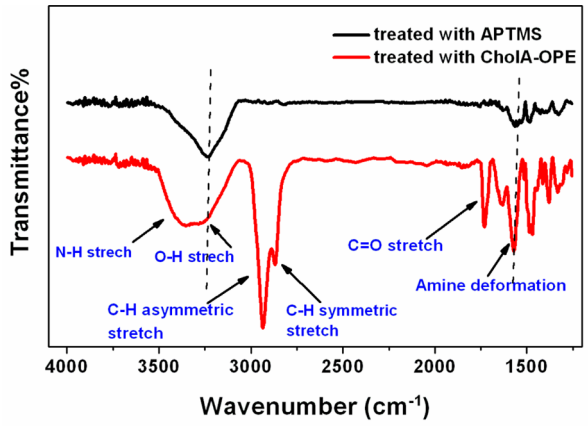



**Figure 3.** ATR-IR spectra of Film 1, where the meanings of (3) and (4) are the same with those explained in Scheme 1.

The leaching issue is an obstacle for the solution sensing applications of fluorescent films. Therefore, it is worthwhile to investigate the fluorescence emission of residual solvent after immersion of the two films in acetone or water for 24 h. No evidence of existence of free CholA-OPE or OPE in the solvents was observed, suggesting that the leaching is negligible for the two films. In other words, CholA-OPE or OPE had been chemically and successfully coupled on the substrate surface, and thereby the modified films can be used for exploring their fluorescent sensing applications.

**3.3. Sensing Performance Studies.** As shown in Figure 5, it is seen that presence of HCl can quench the fluorescence emission of Film 1 in acetone significantly along with increasing HCl concentration from 0 to 40 $\mu$M. The quenching results can be quantitatively treated with the Stern–Volmer equation, $I_0/I = 1 + K_{sv}[HCl]$, where $I_0$ and $I$ stand for the fluorescence intensity of the film in the absence and presence of HCl, respectively, and $K_{sv}$ is the Stern–Volmer constant. It can be seen that the Stern–Volmer plot is down-curved, suggesting heterogeneous of the microenvironment of the fluorophore immobilized, as discussed by Lackwicz and co-workers.[37] As expected, other inorganic acids, including $H_2SO_4$, $HNO_3$, and

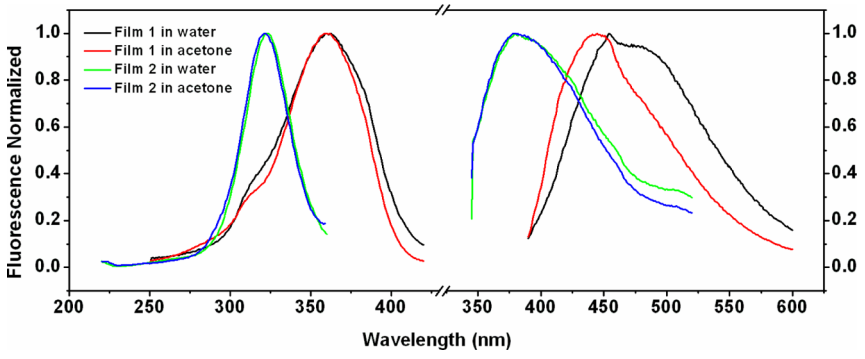



**Figure 4.** Excitation and emission spectra of Film 1 and Film 2 recorded in water and acetone, respectively (Film 1: $\lambda_{ex}$ = 370 nm, $\lambda_{em}$ = 450 nm; Film 2: $\lambda_{ex}$ = 340 nm, $\lambda_{em}$= 390 nm).

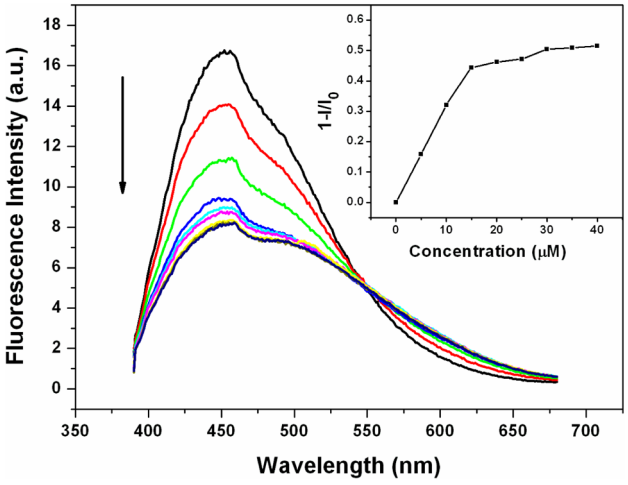
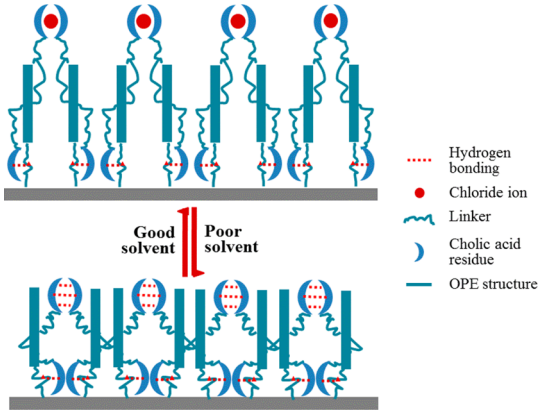



**Figure 5.** Fluorescence emission spectra of Film 1 in the presence of different concentrations of HCl in acetone (from top to bottom, 0, 5, 10, 15, 20, 25, 30, 35, and 40 $\mu$M) ($\lambda_{ex}$ = 370 nm).

**Scheme 2. Cartoon Illustrating the Possible Change of the Conformations of the Surface-Immobilized Molecules of CholA-OPE with its Medium Being Changed from a Good Solvent (acetone as an example) to a Poor One (water, for example)**




$H_3PO_4$ also quench the fluorescence emission of the film but with different efficiencies. The acids like HBr, HI, HCOOH and $CH_3COOH$, however, show little effect to the fluorescence emission of the film in acetone. By contrast, the inorganic acids mentioned above basically show no influence on the fluorescence emission of the film in aqueous medium. For comparison, similar experiments were conducted for Film 2, and it was found that almost all of the acids tested show no effect on the fluorescence emission of this film (cf. Figure 6).

**3.4. Sensing Mechanism Studies.** As will be demonstrated later, the reason for the quenching of the fluorescence emission of the OPE functionalized film is the protonation of the imino group next to the OPE segment (cf. Scheme 1). In this way, only those of the acids that can protonate the OPE layer may behave like fluorescence quenchers. Accordingly, a structural model was proposed in order to understand the sensing performance of the film (cf. Scheme 2).

In the model, it is seen that two CholA residues adjacent to each other could form a hydrophilic pocket at a molecular level with their $\alpha$ faces, the so-called hydrophilic sides of the residues, when the film is inserted in acetone. This pocket should extend to the outer of the OPE adlayer due to the hydrophobic nature of the $\beta$ face of CholA and the linker connecting the CholA residue with the backbone of OPE. Furthermore, it is understandable that the CholA pocket should be pulled into the OPE adlayer when the film is used in water due to collapse of the linkers and the hydrophobic nature of the CholA residues. In this way, the pocket could function as a holder for the anions of some halogens, such as $Cl^-$, particularly when they are dissolved in acetone-like non/less-polar solvents. This binding, of course, must favor the protonation of the imino groups next the OPE segments, which is, as stated already, a prerequirement for the quenching of the fluorescence emission of the film. As for the fact why HBr or HI shows much smaller quenching efficiency to the fluorescence emission of the

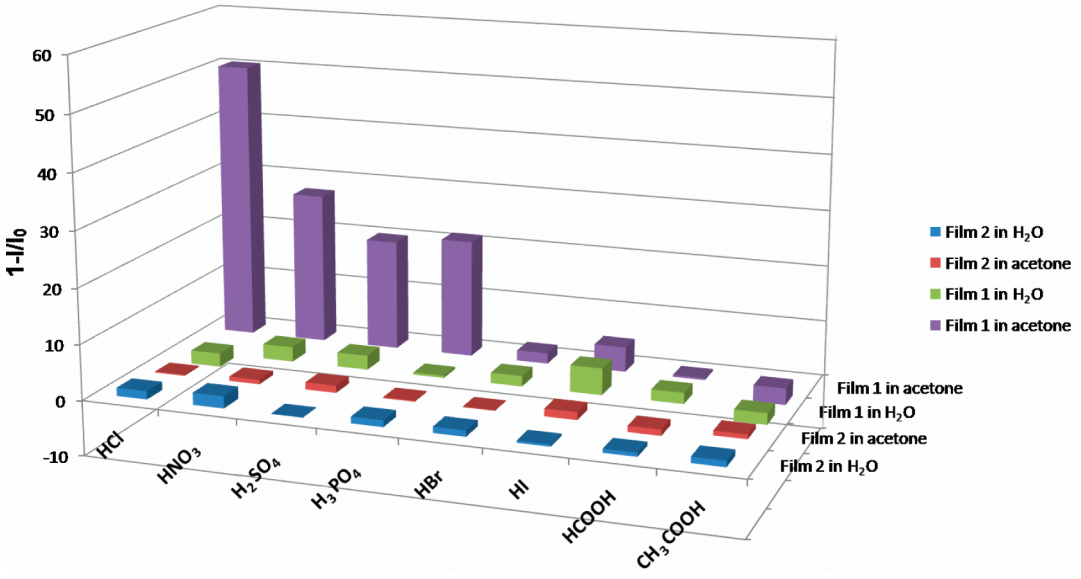



**Figure 6.** Quenching efficiencies of various acids to the fluorescence emission of Film 1 and Film 2 in water and acetone, respectively (concentration of acids are 20 $\mu$M).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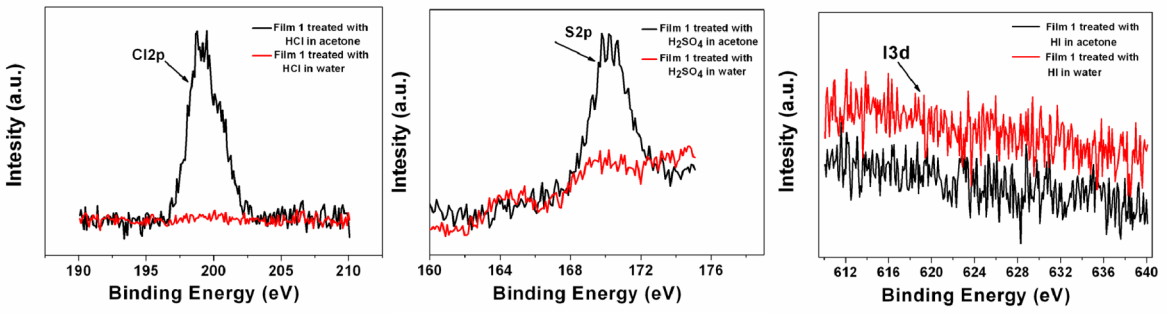


**Figure 7.** XPS spectra of Film 1 treated with the acetone or water solution of HCl, $H_2SO_4$, or HI, respectively.

film, it might be explained by the differences of the sizes of them. It is known that the size of $Cl^-$ is significantly smaller than that of $Br^-$ or $I^-$ and the volume of the pocket may not be big enough to host the bigger ions of $Br^-$ or $I^-$, and thereby the two compounds show lower quenching efficiency to the emission of the film. As for aerobic acid, such as $HNO_3$, $H_2SO_4$, and $H_3PO_4$, however, hydrogen bonds between the anions of the acids and the CholA moieties may exist when they are dissolved in acetone, which must promote the association of the anions to the CholA moieties, and thereby favor protonation of the imino groups next to the OPE segments, explaining why these acids show greater quenching efficiencies to the emission of the film in acetone. It is to be noted that the binding of the quencher, including any of the compounds under discussion, should be a result of synergic process of the protonation and the hosting or association via hydrogen bonding between the aerobic acids and the CholA moieties. Furthermore, not only the side chains of the fluorescent active CholA-OPE show response to the change in the polarity of the medium, but also its backbones. In this way, the backbones would prefer to aggregate in water rather than in acetone because the former is a poor solvent, and correspondingly the emission shifted to longer wavelengths as that shown in Figure 4.

The model and the tentative explanation were confirmed by XPS measurements. Figure 7 depicts the XPS traces of Cl2p, S2p, and $I3d^3/I3d^5$, which are the characteristic signals of HCl, $H_2SO_4$, and HI, respectively, the acids used to treat Film 1 in acetone or water before the measurements. With regards to the the traces, it is seen that the signals of Cl2p, and S2p appear significantly in the traces of the film treated with HCl or $H_2SO_4$ in acetone, but the same signals are absence or much weaker when the film was treated with the same compounds but water was employed as the medium. As for the film treated with HI, no obvious signals of $I3d^3$ or $I3d^5$ either the film was treated with the compound in acetone or in water. These results demonstrate clearly that HI is hard to diffuse into the CholA-modified OPE layer, but in contrast, the other two compounds could be physically adsorbed by the layer when the experiments were conducted in acetone, explaining why HCl and $H_2SO_4$ are efficient quenchers of the fluorescence emission of the film, but they are not efficient in quenching the emission of the film in water. As for HI, it is not an efficient quencher to the fluorescence emission of the film in either acetone or water because of its relatively larger size and weaker hydrogen bond forming ability.

As mentioned already, the protonation of the imino group next to the OPE segment within the CholA-modified OPE adlayer (c.f. Scheme 1, surface structure 4) might be the main reason to lead the fluorescence quenching of the film. To have direct evidence to support the guesswork, we calculated the energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of a compound (cf. the inset of Figure 8), which is a representative

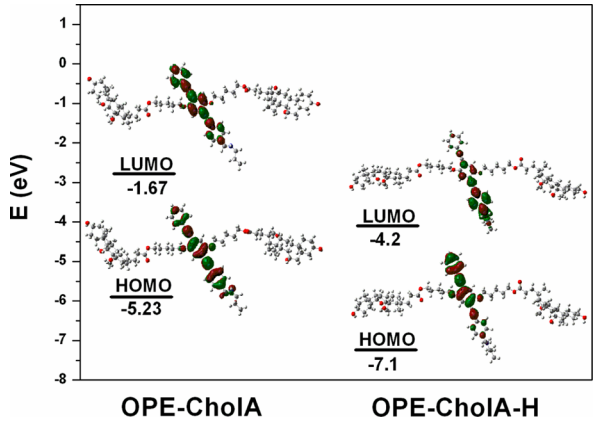


**Figure 8.** Frontier orbital energy correlation diagram and the frontier molecular orbitals of compound 7 in its neutral and protonated states.

of the molecular structures of CholA-modified OPE functionalized film, and those of the protonated state of the compound with the help of quantum-chemical calculations at the level of DFT/B3LYP/6-31G* in a suite of Gaussian 09 programs. The results are shown in Figure 8. The figure reveals that protonation alters the structures of the frontier orbitals greatly, and in particular the gap between the two orbitals decreases from 3.56 eV to 2.90 eV, which explains why protonation quenches the fluorescence emission at the original position.

## 4. CONCLUSIONS

A novel fluorescent sensing film with OPE as sensing element was fabricated in a monolayer manner. Interestingly, introduction of two CholA structures as the side groups of an OPE unit affects its fluorescence behavior significantly when the OPE unit is chemically attached to a glass plate surface. The film as fabricated responses sensitively and selectively to the presence of HCl and some aerobic acids, including $HNO_3$, $H_2SO_4$ and $H_3PO_4$, provided acetone is used as the medium. The selectivity has been rationalized by proposing that presence of CholA favors formation of some hydrophilic pockets above the OPE adlayer when the film is used in acetone. It is the pockets and the imino groups next to the OPE segments that provide the film with special attractions to some proton-containing compounds and lay the foundation for the selective

response of the film to them. The quenching, however, has been attributed to the protonation of the imino groups next to the OPE units. The sensitivity of the emission of the film to the presence of HCl and the aerobic acids in acetone seems to be a combined result of the selective binding of the film to the compounds and proton transfer from the compounds to the imino groups within the OPE adlayer. The work as presented shows again that introduction of side groups with special structures or properties to conjugated polymers/oligomers is a simple but effective way to create novel monomolecular layer chemistry-based fluorescent sensing films.

## ■ ASSOCIATED CONTENT

### Supporting Information

The synthesis procedures of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

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