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A label-free conjugated polymer-based fluorescence assay for the determination of adenosine triphosphate and alkaline phosphatase

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Abstract

In this paper, a simple, sensitive, label-free fluorescence sensor for the detection of adenosine triphosphate and alkaline phosphatase was developed, which was based on the water-soluble fluorescent conjugated polymer. Cu^{2+} could efficiently quench the photoluminescence (PL) intensity of fluorescent conjugated polymer $PPESO_3$ due to the strong electrostatic interaction and electron transfer between $PPESO_3$ and Cu^{2+}. However, the addition of adenosine triphosphate (ATP) could disrupt the polymer-metal complex, leading to the recovery of the fluorescence of $PPESO_3$. The PL intensity ratio I/I_0 (I_0 and I are the fluorescence intensity of $PPESO_3/Cu^{2+}$ system in the absence and presence of ATP, respectively) was proportional to the concentration of ATP. The proposed method was successfully applied to the detection of ATP in human serum sample with satisfactory results. Moreover, considering that ATP could be hydrolyzed by alkaline phosphatase (ALP) and the released Cu^{2+} could quench the fluorescence of $PPESO_3$, the enzyme activity of ALP was also studied.

Keywords: Water-soluble conjugated polymer; Adenosine triphosphate; Alkaline phosphatase; Fluorescence sensor.

1. Introduction

In recent years, conjugated polymers (CPs) have attracted considerable attention as promising fluorescence material for biosensors since they coordinate the action of a large number of repeated absorbing units with efficient intrachain and interchain energy transfer mechanisms.[1, 2] The unique π-π* conjugated electronic structure of CPs allows for rapid migration of excitation between each repeating unit along the polymer backbone and energy/electron receptors which results in remarkable amplification of optical signal through a collective response.[3, 4] Thus, a single quencher molecule can cause a superquenching of the entire polymer chain, which can be depicted as "molecular wire". However, for the non-conjugated small molecule compounds, the fluorescence is quenched only in those small molecules combined with the quencher.[5] The superquenching effect of CPs provides remarkable optical properties in highly sensitive chemical and biological sensors to selectively detect metal ion,[6] organic compound,[7] DNA,[8] protein,[9] amino acid,[10] enzyme[11] and so on. Poly(2,5-bis(3-sulfonatopropoxy)-1,4-phenylethynylenealt-1,4-poly(phenylene ethynylene)) ($PPESO_3$) is one of the typical water-soluble fluorescent conjugated polymers with sulfonic group. Tan et al. investigated the quenching effect of N,N'-dimethyl-4,4'-bipyridinium (MV^{2+}) to $PPESO_3$ in different solvent.[12] Zhang et al. successfully utilized BSA-enhanced fluorescence of $PPESO_3$ to monitor trypsin and pepsin activities.[13] Chung et al. realized the label-free spectroscopic detection of human serum albumin (HSA) with high selectivity and sensitivity by monitoring the deaggregation of $PPESO_3$-metal complex.[14] Li et al. designed a highly sensitive optical enzyme-coupled biosensor based on the $PPESO_3$ for the detection of choline.[15]

Adenosine triphosphate (ATP) is generally acknowledged as the major energy carrier of all

living cells and it plays a critical role in the regulation of cellular metabolism and biochemical pathways in cell physiology.[16, 17] The concentration and dissipative rate of ATP have been found to be closely related to many diseases such as hypoxia, hypoglycemia, ischemia and Parkinson's disease.[18] Consequently, as an indicator for cell viability and cell injury,[19] the accurate detection of ATP is an important goal for both biochemical and clinical applications. So far, several approaches have been developed for the detection of ATP, such as chromatography, fluorometry, bioluminescence, chemiluminescence, and electrochemical.[20-23] However, among these methods, the aptamer-based ATP detection is one of the mostly used strategies which generally need labeled aptamer and very circumspect manipulation.[24-27] Thus, it remains urgent to construct simple, label-free and sensitive assay for monitoring ATP.

In this paper, a turn-on fluorescence sensor probe for the detection of trace amount ATP was established based on the water-soluble fluorescent conjugated polymer $PPESO_3$. Compared to most of the other metal ions, Cu^{2+} could efficiently quench the photoluminescence (PL) intensity of $PPESO_3$ due to the strong electrostatic interaction and electron transfer between $PPESO_3$ and Cu^{2+}. Then the following addition of ATP to a $PPESO_3/Cu^{2+}$ system would disrupt the polymer-metal complex, leading to recovery of the polymer's fluorescence. Thus the label-free detection of ATP could be achieved through monitoring the recovered PL intensity of $PPESO_3$.

Alkaline phosphatase (ALP) is commonly used as the biomarker in enzyme immunoassays, gene assays and routine clinical analysis to diagnose different types of diseases because of its involvement in hepatobiliary and bone disorder.[28, 29] The significantly increasing of ALP in serum results in diseases of the skeletal system, such as Paget's disease, osteomalacia, fractures and rickets, as well as with sarcoma and malignant tumors.[30, 31] Given that ALP could remove the

5'-phosphate groups from ATP to convert each of these species into adenosine, with the addition of ALP into the $PPESO_3/Cu^{2+}$/ATP system, ATP could be hydrolyzed and the released Cu^{2+} could quench the fluorescence of $PPESO_3$. The decrease of the fluorescence intensity is quantitatively related to the concentration of ALP, thus realizing a real-time monitor of enzyme activity.

2. Experimental

2.1 Reagents

All chemicals used were of analytical reagent grade and used without further purification. Tetrakis(triphenylphosphine)palladium ($(PPh_3)_4Pd$) and 1,3-propanesultone were obtained from Hangzhou Kaida Metal Catalyst & Compounds Co. Ltd and J&K Chemica, respectively. 2,5-Diiodohydroquinone and dioxane were purchased from Tianjin Guangfu Institute of elaborate chemical industry. 1,4-Diethynylbenzene was obtained from Aldrich Chemical Co. Calf intestine alkaline phosphatase (ALP) was purchased from Shanghai kayon Biological Technology CO. Ltd. ATP was obtained from Beijing Dingguo Changsheng Biotechnology CO. Ltd. Copper(II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$) was purchased from Beijing Chemical Works. All the other chemicals, including CuI, methanol, acetone, diethylether and dimethyl formamide (DMF) were obtained from Arkema Beijing Chemical Co. Ltd. All work solutions were prepared with 10 mM Tris-HCl buffer solution (pH 7.0). The water used in all experiments had a resistivity higher than 18 MΩ/cm. All the water used in the experiments was deaerated by purging with N_2 for 30 min.

2.2 Instrumentation

Fluorescence measurements were performed on a Shimadzu RF-5301 PC spectra fluorophotometer containing a 1 cm path-length quartz cuvette. The fluorescence spectra were

recorded with the excitation wavelength of 400 nm and the fluorescence intensity referred to the maximum emission of $PPESO_3$ at 528 nm. The slit widths of the excitation and emission were both 5 nm. All pH measurements were made with a Starter-2C pH meter obtained from Ohaus Instruments Co. Ltd., Shanghai, China.

2.3 Experimental method

The water-soluble fluorescent conjugated polymer $PPESO_3$ (MW=520) was synthesized according to a previous report.[13]

For the detection of ATP, $PPESO_3$ was diluted to 1.0 μmol L^{-1} followed by the addition of 10 μmol L^{-1} Cu^{2+} and certain amounts of ATP solution. Then the mixture (2.0 mL) was shaken evenly and kept at room temperature for 5 min before recording the spectral information by spectrofluorophotometer.

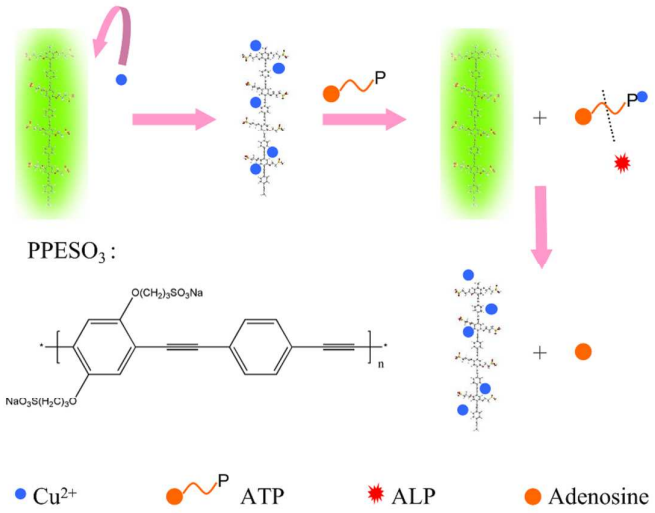
For human serum samples detection, drug-free human blood samples were collected from healthy volunteers at the Hospital of Changchun China Japan Union Hospital. All the blood samples were obtained through venipuncture and centrifuged at 10000 rpm for 15 min. The supernatant were freezed and saved as stock solution of serum samples. When use, the serum samples were added with different concentration of ATP separately to prepare the spiked samples. The serum sample was thawed and deproteinized by adding acetonitrile. After vigorously shaking for 2 min, the mixture was centrifuged at 10000 rpm for 15 min at 4℃. Aliquots of the supernatant were diluted 100 times and adjusted to neutral pH by 10 mmol L^{-1} Tris-HCl buffer. The results from three individual experiments were averaged. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the relevant institutional committees have approved the experiments.

For the detection of ALP, certain amounts of ALP stock solution were mixed with 10 μmol L^{-1} ATP in 100 μL system to thermally equilibrate at 37℃ for 5 min. Then the mixture was added with 1.0 μmol L^{-1} $PPESO_3$ and 10 μmol L^{-1} Cu^{2+} and diluted into 2.0 mL. After shaking and equilibrating for 5 min, the fluorescence spectra were obtained by spectrofluorophotometer.

3. Results and discussion

3.1 Experiment principle

A schematic illustration of the fluorescent sensor for the determination of ATP and ALP is shown in Scheme 1. As depicted in Scheme 1, Cu^{2+} could cause the superquenching effect on the fluorescence of $PPESO_3$ due to the electrostatic interaction and electron transfer between $PPESO_3$ and Cu^{2+}. The addition of ATP to the above mixture would disrupt the $PPESO_3$-Cu^{2+} system and form a more stable complex with Cu^{2+}, resulted in the recovery of the fluorescence of $PPESO_3$. Thus a label-free fluorescence sensor for the determination of ATP could be achieved. Furthermore, the phosphate moiety in ATP would be hydrolyzed when the ALP is introduced into above system, and Cu^{2+} in the ATP-Cu^{2+} complex would be released, which would quench the fluorescence of $PPESO_3$ again. Thus an assay of enzyme activity could be realized by monitoring the relationship between the decreased PL intensity of $PPESO_3$ and the concentration of ALP.



Scheme 1 Schematic illustration of the fluorescent sensor for the determination of ATP and ALP.

3.2 Fluorescence quenching of $PPESO_3$ with Cu^{2+} ions

Recently, many CPs-based fluorescence sensors were prepared due to their high fluorescence quenching response to some metal ions with high selectivity.[32, 33] Fig. 1 exhibited the influence of different metal ions on the PL intensity of $PPESO_3$ solution. We can found that most of metal ions do not have obviously effect on the PL intensity of $PPESO_3$, except for Cu^{2+}, Hg^{2+} and Fe^{3+}. We can utilize triethanolamine as a suitable masking reagent in the real samples containing Fe^{3+}.[34] Then we investigated the relationship between the PL intensity of $PPESO_3$ and Cu^{2+} concentration and the results were shown in Fig. 2. It can be seen that upon the addition of Cu^{2+} ions, the fluorescence of $PPESO_3$ quenched proportionally without obvious maximum PL emission wavelength shift in the range of 0.5 to 15 μmol L^{-1} (inset in Fig. 2) and the linear regression equation is as follows: $I_0/I = 0.9794 + 0.3174\ [Cu^{2+}]$ (μmol L^{-1}), with the correlation coefficient (R) of 0.994. It fitted well with the conventional Stern-Volmer equation:

$$I_0/I = 1 + K_{sv}[Q] = 1 + K_q\tau_0[Q]$$

where I_0 and I are the fluorescence intensity of $PPESO_3$ in the absence and presence of the quencher (Cu^{2+}), respectively; [Q] is the concentration of the quencher (Cu^{2+}); K_{sv} is the Stern-Volmer quenching constant; τ_0 is the fluorescence lifetime of the molecule without a quencher and its value is 10^{-8} s; K_q is the quenching rate constant of molecule and is calculated to be 3.3×10^{13} L mol^{-1} s^{-1}. The detection limit for Cu^{2+} is 0.1 μmol L^{-1} defined by the equation LOD = (3σ/s), where σ is the standard deviation of the blank signals (n=10) and s is the slope of the calibration curve. The strong electrostatic interaction and electron transfer between $PPESO_3$ and Cu^{2+} should be mainly responsible for the remarkable quenching effect of Cu^{2+} ions.

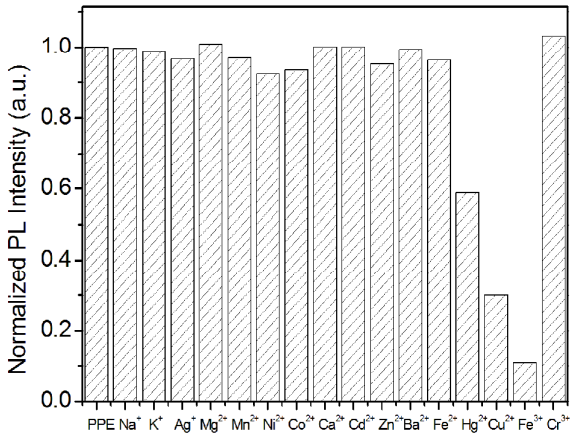


Fig. 1 Effect of metal ions (10 μmol L^{-1}) on the fluorescence of $PPESO_3$ (1.0 μmol L^{-1}) solution at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0.

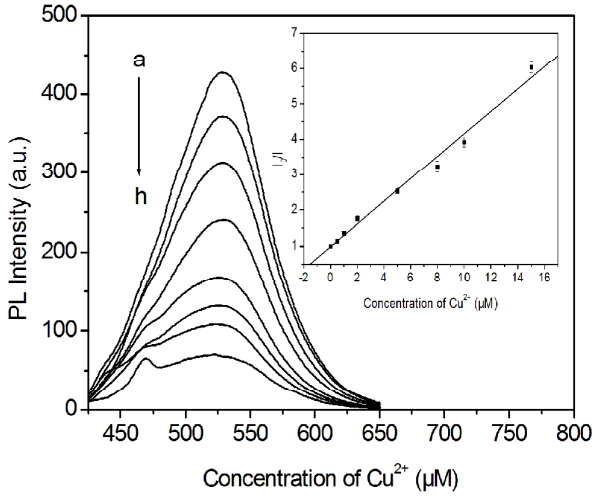


Fig. 2 Effect of Cu^{2+} concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ solution at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-h) represents the concentration of Cu^{2+} ions: 0, 0.5, 1, 2, 5, 8, 10, 15 μmol L^{-1}, respectively. The inset shows the linear relationship between the fluorescence intensity ratio I_0/I and the concentration of Cu^{2+} ions.

3.3 Fluorescence turn-on system for ATP detection

The addition of ATP to a $PPESO_3/Cu^{2+}$ system would disrupt the polymer-metal complex, leading to recovery of the polymer's fluorescence. From Fig. 3, we can see that the addition of 15 μmol L^{-1} ATP to $PPESO_3/Cu^{2+}$ (10 μmol L^{-1}) solution resulted in the fluorescence recovery of $PPESO_3$. The recovery demonstrated that the chelation of the phosphate moiety to Cu^{2+} effectively sequesters the metal ion, disrupting its ability to bind to the sulfonic acid groups of $PPESO_3$, which could quench the fluorescence of $PPESO_3$.[35, 36] Thus a $PPESO_3$ -based fluorescence turn-on label-free sensor for ATP detection could be established.

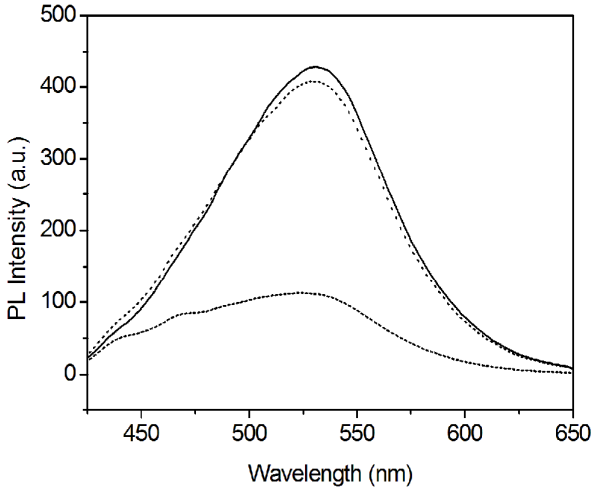


Fig. 3 Fluorescence emission spectra of 1.0 μmol L^{-1} $PPESO_3$ (— Solid), 1.0 μmol L^{-1} $PPESO_3$ and 10 μmol L^{-1} Cu^{2+} (– – Dash), 1.0 μmol L^{-1} $PPESO_3$, 10 μmol L^{-1} Cu^{2+} and 10 μmol L^{-1} ATP (⋯ Dot) at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0.

The reaction time in the detection of ATP by $PPESO_3/Cu^{2+}$ sensor was investigated and the results were shown in Fig. S1 (ESM). It can be seen that the PL intensity of $PPESO_3/Cu^{2+}$ system increased immediately when ATP was added into the $PPESO_3/Cu^{2+}$ solution and then decreased and remained nearly constant after 5 min. Thus 5 min was chosen as the reaction time in the further experiments.

The effect of NaCl concentration in the detection of ATP by $PPESO_3/Cu^{2+}$ sensor was also studied. From Fig. S2 (ESM), we can see that when NaCl concentrations were less than 1×10^{-5} mol L^{-1}, the PL intensity of the system reached the maximum and remained constant. However, it gradually decreased as the concentration of NaCl was more than 1×10^{-5} mol L^{-1}. Thus, we selected 1×10^{-5} mol L^{-1} NaCl as the optimum ionic strength in the further experiments.

Under the optimal conditions, we studied the fluorescence turn-on sensor based on the

conjugated polymer $PPESO_3$. Fig. 4 illustrated the fluorescence spectra of $PPESO_3/Cu^{2+}$ system upon the addition of different concentrations of ATP. The inset in Fig. 4 showed the linear relationship between the fluorescence intensity ratio I/I_0 (I_0 and I are the fluorescence intensity of $PPESO_3/Cu^{2+}$ system in the absence and presence of ATP, respectively) and the concentration of ATP in the range of 0.05-15 μmol L^{-1}. The linear regression equation is as follows:

$$I/I_0 = 0.9590 + 0.1795\ [ATP],\ \mu mol\ L^{-1}$$

The corresponding regression coefficient is 0.998. The detection limit for ATP is 0.03 μmol L^{-1}. A comparison between our $PPESO_3$-based sensor and other reported methods for the determination of ATP in detection limit and linear range was summed up in Table 1. We can found that the sensitivity of our fluorescence sensor was better than most of the other reported methods. Moreover, compared to other ATP sensors, our sensor has the advantages of nontoxic, label-free, facile preparation and low cost.

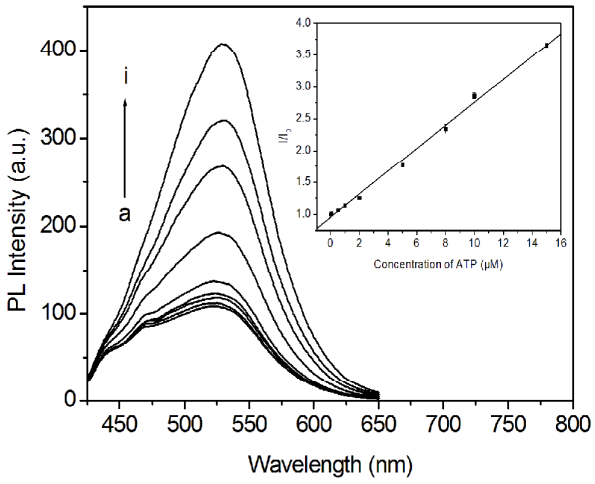


Fig. 4 Effect of ATP concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ -10 μmol L^{-1} Cu^{2+} system at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-i) represents the concentration of ATP: 0, 0.05, 0.5, 1, 2, 5, 8, 10, 15 μmol L^{-1}, respectively. The inset shows the

linear relationship between the fluorescence intensity ratio I/I_0 and the concentration of ATP.

Table 1 Comparison of different methods for the determination of ATP.

Methods	System	Linear range (mol L^{-1})	LOD (mol L^{-1})	References
Potentiometry	Aptasens/ion-selective electrode	5.0×10^{-7} - 3.0×10^{-6}	3.7×10^{-7}	[37]
Chemiluminescence	Aptamer/QDs	5.0×10^{-5} - 2.3×10^{-4}	1.8×10^{-7}	[38]
Fluorometry	QD-cDNA/aptamer-Ab nanoprobes	1.0×10^{-5} - 3.5×10^{-4}	3.7×10^{-6}	[24]
Fluorometry	Aptamer/cDNA duplex	1.0×10^{-7} - 1.0×10^{-2}	2.3×10^{-8}	[39]
Fluorometry	Aptamer/protein	1.0×10^{-6} - 2.5×10^{-5}	5.0×10^{-7}	[40]
Fluorometry	Molecular aptamer beacon/ graphene oxide	5.0×10^{-6} - 2.5×10^{-3}	2.0×10^{-6}	[16]
Fluorometry	Aptamer/molecular beacon	8.0×10^{-7} - 8.0×10^{-5}	5.0×10^{-7}	[41]
Fluorometry	$PPESO_3/Cu^{2+}$	5.0×10^{-8} - 1.5×10^{-5}	3.0×10^{-8}	This work

3.4 Real human serum samples detection

We evaluated the selectivity of the proposed sensor. ATP and other potentially interfering anions at the same concentration were investigated. As shown in Fig. 5, ATP could effectively restore the fluorescence of $PPESO_3$ quenched by Cu^{2+}, while the other anions nearly did not result in obvious fluorescence restoration of the fluorescence of the mixed system. These results indicated that the $PPESO_3/Cu^{2+}$ sensor could be used as a fluorescent probe to detect ATP. Furthermore, since adenosine diphosphate (ADP) always coexists with ATP, The selectivity against ADP was also investigated. From Fig. 6, it could be seen that ADP could also recover the fluorescence of $PPESO_3/Cu^{2+}$ system, but ATP has the stronger recovering ability to the fluorescence of the $PPESO_3/Cu^{2+}$ system. For the system contained both ATP and ADP, we can synchronous determinate ATP and ADP according to the analogous method previously reported in our group.[42]

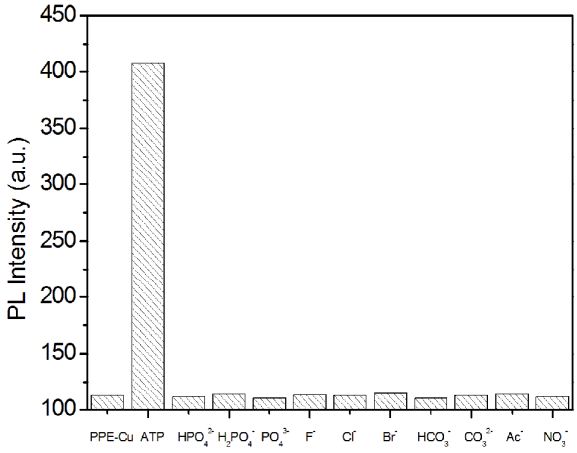


Fig. 5 Effect of ATP (10 μmol L^{-1}) and different anions (1.0 mmol L^{-1}) on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ - 10 μmol L^{-1} Cu^{2+} system (Q: $PPESO_3/Cu^{2+}$) at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0.

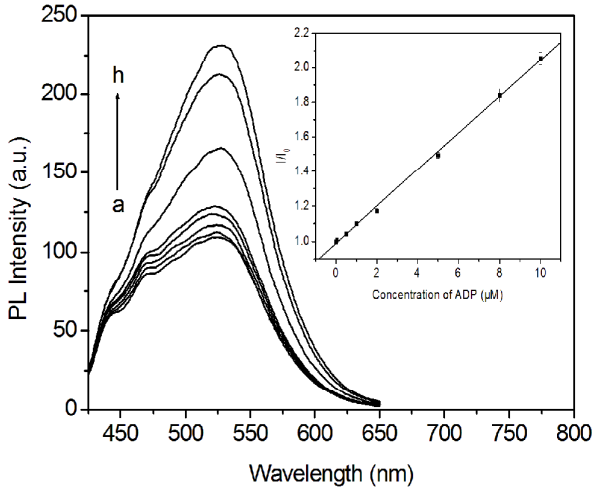


Fig. 6 Effect of ADP concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ in the presence of 10 μmol L^{-1} Cu^{2+} at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-h) represents the concentration of ADP: 0, 0.05, 0.5, 1, 2, 5, 8, 10 μmol L^{-1}, respectively. The inset shows the linear relationship between the fluorescence intensity ratio I/I_0 and the concentration of ADP.

In order to evaluate the feasibility of the proposed method in real samples detection, the developed fluorescence sensor was applied to the determination of ATP in human serum samples and the results were shown in Table 2. The ATP content in the samples was derived from the standard curve and the regression equation. The average recovery test was made by using the standard addition method. From Table 2, we can see that the ATP concentration found were consistent with those obtained by the UV-vis spectrophotometry and the recoveries were found to be in the range 102–106%, the RSD were less than 3.0%. The results indicated that the accuracy and precision of the method were satisfactory. The above results demonstrated the potential applicability of the $PPESO_3$-based fluorescence sensor for the detection of ATP in human serum samples.

Table 2 Determination of ATP in human serum samples by the proposed method and UV-vis spectrophotometry (n = 3).

Sample	Added (μmol L^{-1})	Found (μmol L^{-1})		Recovery (%)	RSD (%)
		Proposed Method	UV-vis spectrophotometry		
ATP	0.100	0.103	0.098	103	0.29
	0.500	0.532	0.542	106	0.48
	1.00	1.04	0.95	104	0.26
	5.00	5.08	5.06	102	1.84
	10.0	10.2	10.6	102	2.21

3.5 Assay for ALP activity

In order to demonstrate the potential of our $PPESO_3$-based ATP sensor in the analytical application, a real-time fluorescence turn-off assay was designed to monitor the activity of ALP. For this purpose, the reaction of 10 μmol L^{-1} ATP and 1 U mL^{-1} ALP proceeded at 37℃ for 0-15 min and then the fluorescence spectra were recorded in the presence of 1.0 μmol L^{-1} $PPESO_3$ and 10 μmol L^{-1} Cu^{2+}. From Fig. S3 (ESM), it can be seen that the reaction of ATP and ALP finished in 5 min. Thus, in our following experiments, 5 min was chosen for ATP-ALP reaction system. Fig. 7 showed that the PL intensity of $PPESO_3$ decreased successively with the increasing concentration of ALP. This phenomenon demonstrated that the chelation of the phosphate moiety to Cu^{2+} was destroyed due to the hydrolysis of ATP by ALP and then the fluorescence of $PPESO_3$

was quenched by the released Cu^{2+} ion again. A good linear relationship was observed between the fluorescence intensity ratio of I/I_0 (I_0 and I are the fluorescence intensity of $PPESO_3/Cu^{2+}$/ATP system in the absence and presence of ALP, respectively) and the concentration of ALP over the range of 0.05 to 1.0 U mL^{-1} (inset in Fig. 6). The linear regression equation is as follows:

$$I/I_0 = 0.9632 - 0.4518\ [ALP],\ U\ mL^{-1}.$$

The corresponding regression coefficient is 0.997. The detection limit for ATP is 0.01 U mL^{-1}, and is comparable to the other reported methods [17, 28]. This initial study clearly demonstrated that the $PPESO_3$-based ATP sensor could provided an effective, real-time fluorescence assay for ALP activity.

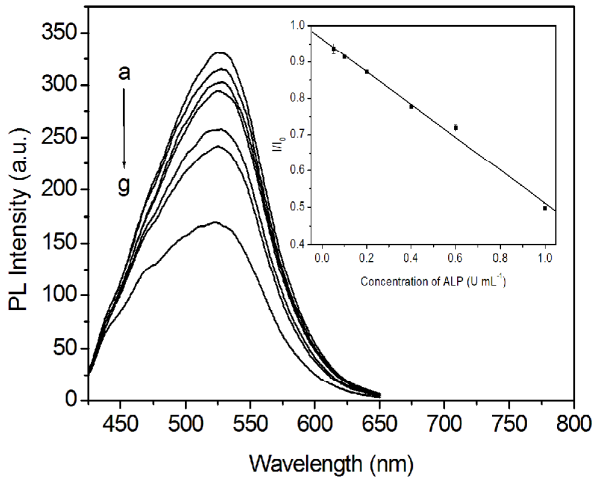


Fig. 7 Effect of ALP concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ -10 μmol L^{-1} Cu^{2+} - 10 μmol L^{-1} ATP system at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-g) represents the concentration of ALP: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 1.0 U mL^{-1}, respectively. The inset shows the linear relationship between the fluorescence intensity ratio I/I_0 and the concentration of ALP.

4. Conclusions

In this paper, a simple, sensitive, label-free fluorescence sensor for the detection of ATP and ALP was developed based on the electrostatic interaction and electron transfer between $PPESO_3$ and Cu^{2+} ions. The recovered PL intensity of $PPESO_3$ had a good linear relationship with the concentration of ATP in the range of 0.05-15 μmol L^{-1}, which demonstrated that the strong chelation of the phosphate moiety in ATP to Cu^{2+} can recover the fluorescence of $PPESO_3$. The detection limit for ATP was down to 0.03 μmol L^{-1}. Furthermore, a fluorescence turn-off assay for the enzyme activity of ALP was achieved in the range of 0.05 to 1.0 U mL^{-1} based on the hydrolysis of ATP by ALP. The detection limit for ALP was down to 0.01 U mL^{-1}.

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Figure captions

Scheme 1 Schematic illustration of the fluorescent sensor for the determination of ATP and ALP.

Fig. 1 Effect of metal ions (10 μmol L^{-1}) on the fluorescence of $PPESO_3$ (1.0 μmol L^{-1}) solution at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0.

Fig. 2 Effect of Cu^{2+} concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ solution at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-h) represents the concentration of Cu^{2+} ions: 0, 0.5, 1, 2, 5, 8, 10, 15 μmol L^{-1}, respectively. The inset shows the linear relationship between the fluorescence intensity ratio I_0/I and the concentration of Cu^{2+} ions.

Fig. 3 Fluorescence emission spectra of 1.0 μmol L^{-1} $PPESO_3$ (— Solid), 1.0 μmol L^{-1} $PPESO_3$ and 10 μmol L^{-1} Cu^{2+} (– – Dash), 1.0 μmol L^{-1} $PPESO_3$, 10 μmol L^{-1} Cu^{2+} and 10 μmol L^{-1} ATP (⋯ Dot) at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0.

Fig. 4 Effect of ATP concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ -10 μmol L^{-1} Cu^{2+} system at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-i) represents the concentration of ATP: 0, 0.05, 0.5, 1, 2, 5, 8, 10, 15 μmol L^{-1}, respectively. The inset shows the linear relationship between the fluorescence intensity ratio I/I_0 and the concentration of ATP.

Fig. 5 Effect of ATP (10 μmol L^{-1}) and different anions (1.0 mmol L^{-1}) on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ - 10 μmol L^{-1} Cu^{2+} system (Q: $PPESO_3/Cu^{2+}$) at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0.

Fig. 6 Effect of ADP concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ in the presence of 10 μmol L^{-1} Cu^{2+} at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-h) represents the concentration of ADP: 0, 0.05, 0.5, 1, 2, 5, 8, 10 μmol L^{-1}, respectively. The inset shows the linear relationship between the fluorescence intensity ratio I/I_0 and the concentration of ADP.

Fig. 7 Effect of ALP concentration on the PL intensity of 1.0 μmol L^{-1} $PPESO_3$ -10 μmol L^{-1} Cu^{2+} - 10 μmol L^{-1} ATP system at room temperature, 10 mmol L^{-1} Tris-HCl buffer, pH=7.0; (a-g) represents the concentration of ALP: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 1.0 U mL^{-1}, respectively. The inset shows the linear relationship between the fluorescence intensity ratio I/I_0 and the concentration of ALP.

Scheme 1:

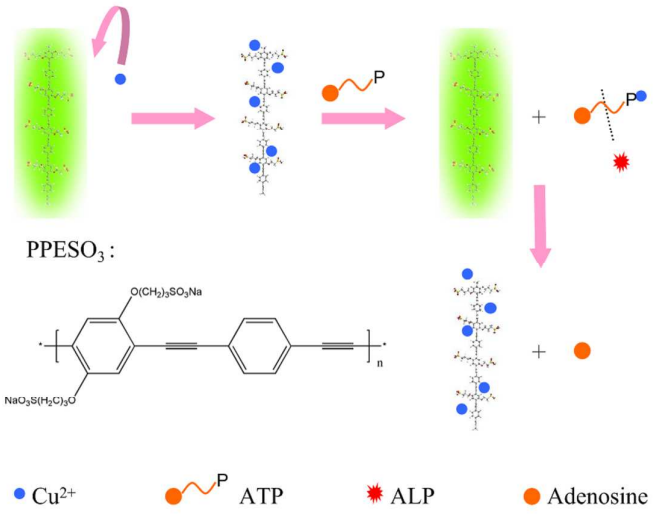
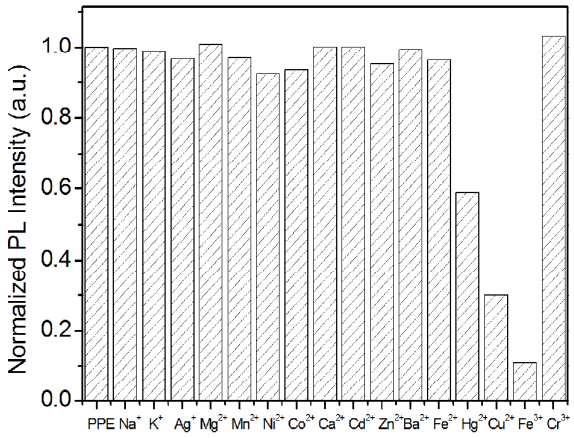


Fig. 1:



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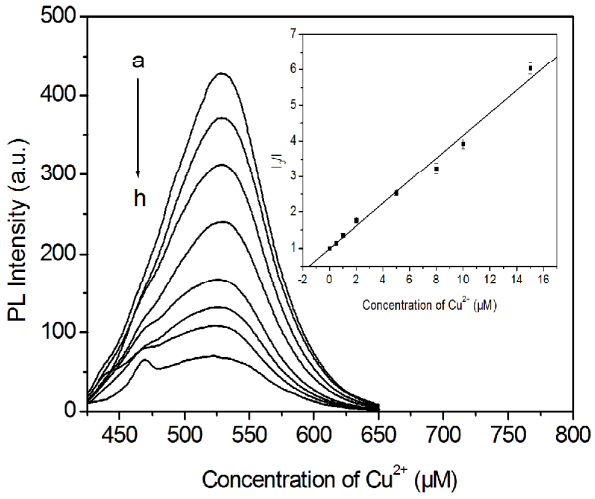

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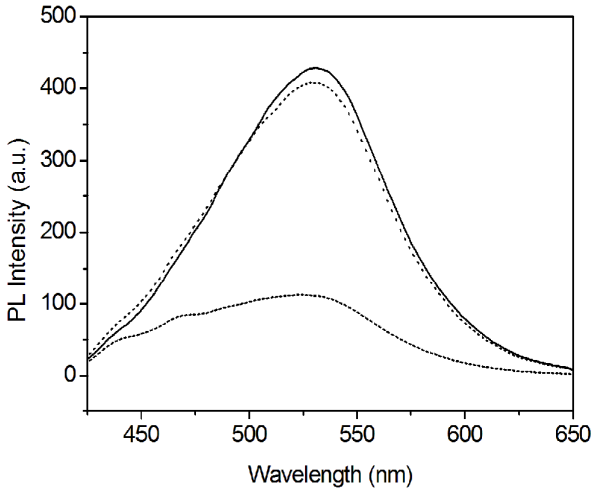

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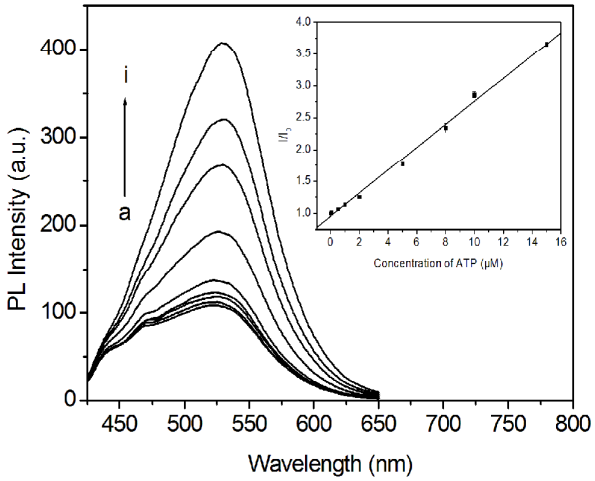

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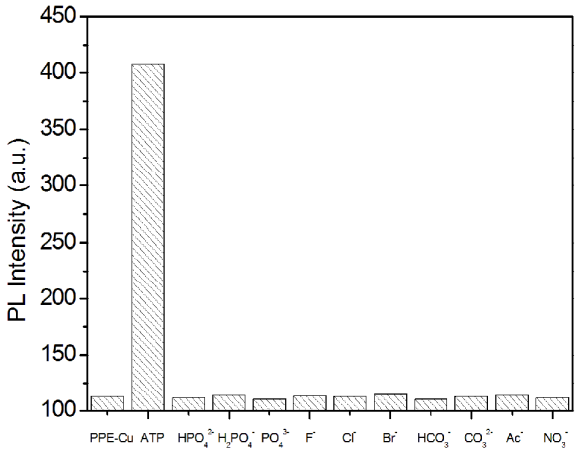

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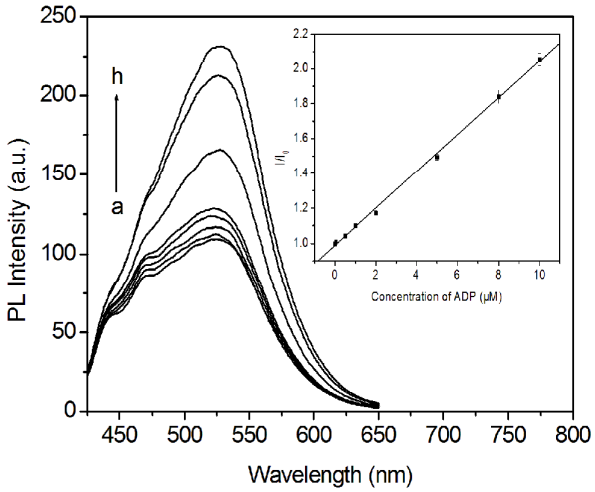

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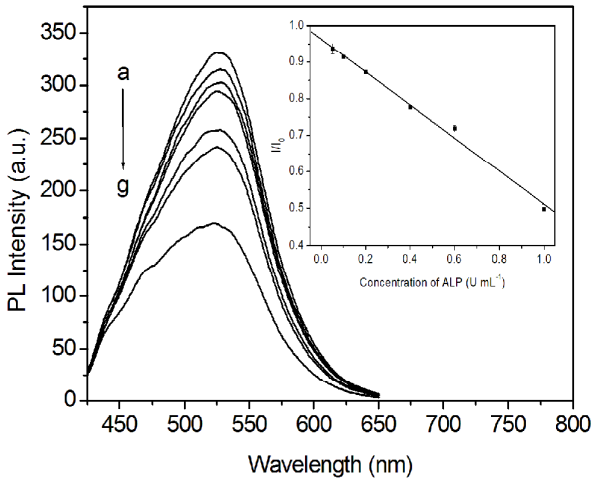

Number of Figures: 8.

The sensor was based on the quenching ability of Cu^{2+} on $PPESO_3$ and the hydrolysis of ATP by ALP.