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Journal Name

ARTICLE

Selective sensing of citrate by supramolecular ensemble formed by phenazine copper(I) complex and perylene diimide derivative

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A fluorescent sensing ensemble for citrate has been developed, which is composed of an meta-phenylboronic acid substituted dipyrrodo[3,2-a:2',3'-c]-7-aza-phenazine copper(I) complex ($[\text{Cu}(\text{L}^1)_2]$) and aspartic acid modified perylene diimide (PASP). The complex $[\text{Cu}(\text{L}^1)_2]$ can bind to PASP with a molar ratio of 2:1 ($[\text{Cu}(\text{L}^1)_2]/\text{PASP}$), and the fluorescence of PASP is efficiently quenched due to photoinduced electron transfer (PET) from PASP to $[\text{Cu}(\text{L}^1)_2]$ ($\Phi_{\text{ET}} = 0.53$). Highly selective "turn-on" type fluorescence changes are observed upon addition of citrate, with emission enhancement (F/F_0) of about 1500-fold when the concentration of citrate is 40 mM. The fluorescence enhancement shows an excellent linear relationship ($R^2 = 0.9972$) with the concentration of citrate in the range of 25 μM to 1 mM, indicating that the $\text{PASP}/[\text{Cu}(\text{L}^1)_2]$ ensemble is sensitive to citrate. In contrast, there is little change in the emission intensity of the $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ ensemble upon addition of the other α -hydroxycarboxylates, dicarboxylates or monosaccharides ($\leq 1\text{mM}$). The phenylboronic acid substituent and copper(I) ion are essential for the sensing selectivity.

Introduction

Interest in the molecular sensing of anions is growing due to their fundamental role in chemical as well as in biological processes.¹ Citrate is an organic tricarboxylate playing a central role in biochemical processes like Krebs cycle.² Citrate can also be used as a biomarker for the diagnosis of prostate cancer and urological diseases, since its concentration can be related to some pathological state.³ For example, citrate concentrations in prostatic fluid samples decrease from the normal range of 50–200 mM in healthy males to cancer levels of 2–20 mM.⁴ Thus, the development of selective chemosensors to detect citrate in biological and other fluid samples is of considerable significance.

The conventional analytical methods for the determination of citrate are gas chromatography, high performance liquid chromatography, spectrometry and capillary electrophoresis.⁵ In contrast to other analytical methods, fluorescence based citrate sensor systems have attracted particular attention because of their high selectivity, simplicity and short response time. Fluorophores, such as anthracene,⁶ pyrene,⁷ coumarin,⁸

europium complex,⁹ and rhodamine,¹⁰ have been used in the fluorescent chemosensor for citrate. The photoinduced electron transfer (PET) mechanism between receptor and fluorophore is frequently employed in citrate sensor.¹¹ Accordingly, the recognition event induces a change of the electronic properties of the receptor, which leads to either enhanced or reduced fluorescence by blocking or promoting PET process.

Recently, chemosensors noncovalently formed by receptor and chromophores have been used in citrate detection. However, many of the receptors display low affinities for citrate in aqueous solution due to the strong solvation effects of water, and the tests are carried out in organic solvents, thus limiting their applications.¹² It is still necessary to develop citrate receptors that can work in aqueous solutions with high affinity. Perylene diimide derivatives (PDIs), with the advantage of chemical and photochemical stability and fluorescence quantum yield close to unity, have been used as prominent chromophores in biological and chemical fields.¹³ Their rigid, large π -cores are particularly interesting for their ability to efficiently construct supramolecular ensemble in aqueous media.¹⁴ The supramolecular assemblies, constructed by water soluble perylene diimides, can be disaggregated upon binding to the substrate, and have been used as "off-on" fluorescence sensor.¹⁵ Our previous studies suggest that the cationic phenazine derivatives may bind to aspartic acid modified perylene diimide (PASP) and form a stable charge-transfer ensemble in buffer solution.¹⁶ In this paper, the meta- and para-phenylboronic acid substituted dipyrrodo[3,2-a:2',3'-c]-7-aza-phenazines

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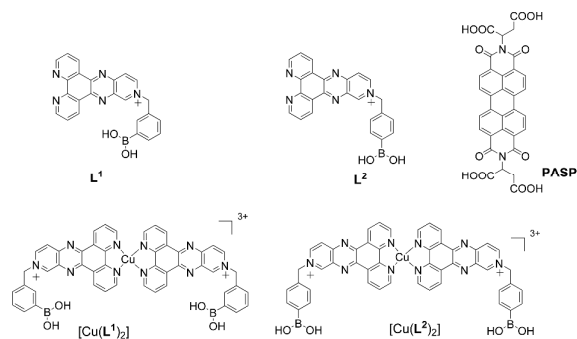


Fig. 1 Chemical structure of PASP, phenylboronic acid modified phenazines (**L¹**-**L²**) and their copper(I) complexes $[\text{Cu}(\text{L}^1)_2]$ and $[\text{Cu}(\text{L}^2)_2]$.

(**L¹**-**L²**) and their copper(I) complexes ($[\text{Cu}(\text{L}^1)_2]$ and $[\text{Cu}(\text{L}^2)_2]$) (Fig.1) are synthesized. The phenylboronic acid in these ligands and complexes can act as receptor for citrate,¹⁷ and PASP is employed as a fluorescent reporter for detection of citrate. The photophysical properties and the citrate sensing selectivity of supramolecular ensembles, constructed by PASP and phenylboronic acid modified phenazines or their copper(I) complexes, are studied.

Experimental

Materials

Compound **1** (1,10-phenanthroline-5,6-dione),¹⁸ **2** (dipyrido[3,2-a:2',3'-c]-7-aza-phenazine, dpapz),¹⁹ and N,N'-Di(2-succinic acid)-perylene-3,4,9,10-tetracarboxylic diimide (PASP)²⁰ were prepared according to the literature procedures. All the other reagents were commercially available and used without further purification. All experiments involving buffer solution were performed in 10 mM Tris-HCl (pH 7.4), unless otherwise noted.

Synthesis of ligand **L¹**

To a solution of compound **2** (0.066 g, 0.23 mmol) in 5 mL of chloroform, 3-(bromomethyl)phenylboronic acid (0.05 g, 0.233 mmol) was added, and the mixture was stirred at 50 °C for 24 h. The precipitate was separated and washed with THF, to yield **L¹** as dark green solid (42.7 mg, yield 37 %). ¹H NMR (MeOD, 400 MHz) δ : 10.52 (s, 1H); 9.58 (m, 2H); 9.17(m, 2H); 9.07 (m, 1H); 8.80 (m, 1H); 7.92 (m, 3H); 7.81(s, 1H); 7.67(m, 1H); 7.45 (s, 1H); 6.14 (s, 2H).

Synthesis of ligand **L²**

The procedure was similar to that for **L¹**, except 4-(bromomethyl)phenylboronic acid was used instead of 3-(bromomethyl)phenylboronic acid (47.0 mg, yield: 41.1%). ¹H NMR (MeOD, 400 MHz) δ : 10.64 (s, 1H); 9.59 (m, 2H); 9.21 (m, 3H); 8.88 (s, 1H); 7.99 (m, 3H); 7.75 (m, 3H); 6.27 (s, 2H).

General procedure for synthesis of copper(I) complexes

The solution of **L¹** or **L²** (12.1 mg, 0.024 mmol) in 10 mL of MeOH was stirred and degassed with argon for 20 min, followed by the addition of $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ (4.47 mg, 0.012

mmol) in 2 mL of MeOH. The resulting mixture was degassed for another 5 min. After the Teflon screw of Schlenk tube was closed, the contents of the tube were stirred at 60 °C overnight. After cooling to room temperature, the reaction mixture was evaporated to dryness. The crude product was dissolved in a small amount of DMSO and a solution of sodium hexafluorophosphate (0.20 g, 1.20 mmol) in water was added. The product was isolated and repeatedly washed with water, methanol and diethyl ether.

$[\text{Cu}(\text{L}^1)_2]$ (10.8 mg, yield 67.4 %), ¹H NMR (MeOD, 400 MHz) δ : 10.42 (m, 2H); 9.11 (m, 2H); 8.70 (m, 2H); 7.90 (m, 4H); 7.77-7.59 (m, 8H); 7.45 (m, 4H); 7.36 (m, 4H); 6.16 (s, 4H). MS (MALDI-TOF): $[\text{M-PF}_6]^+$ 1189.6, calcd for $\text{C}_{48}\text{H}_{34}\text{B}_2\text{CuF}_{12}\text{N}_{10}\text{O}_4\text{P}_2$, 1189.0; elemental analysis calcd (%), $\text{C}_{48}\text{H}_{34}\text{B}_2\text{CuF}_{18}\text{N}_{10}\text{O}_4\text{P}_3$: C 43.19, H 2.57, N 10.49, found: C 43.12, H 2.70, N 10.55.

$[\text{Cu}(\text{L}^2)_2]$ (10.2 mg, yield 63.7 %), ¹H NMR (MeOD, 400 MHz) δ : 10.43 (m, 1H); 9.12 (m, 2H); 8.697 (s, 1H); 7.72 (m, 3H); 7.59 (m, 4H); 7.33 (m, 2H); 6.16 (s, 2H). MS (MALDI-TOF): $[\text{M-PF}_6]^+$ 1189.1, calcd for $\text{C}_{48}\text{H}_{34}\text{B}_2\text{CuF}_{12}\text{N}_{10}\text{O}_4\text{P}_2$, 1189.0; elemental analysis calcd (%), $\text{C}_{48}\text{H}_{34}\text{B}_2\text{CuF}_{18}\text{N}_{10}\text{O}_4\text{P}_3$: C 43.19, H 2.57, N 10.49, found: C 43.28, H 2.80, N 10.68.

General information

¹H NMR spectra were measured on a Bruker Avance 400 spectrometer. Chemical shifts δ were given in parts per million (TMS as internal standard). Steady state absorption and fluorescence spectra were recorded with a Hitachi UV-3010 UV-Vis spectrophotometer and Hitachi F-4600 spectrofluorimeter, respectively.

Cyclic voltammetry (CV) experiments were performed on a CHI660D electrochemical workstation by a cyclic voltammetry (CV) technique, using two platinum wires as the working and counter electrodes, respectively, and a saturated calomel electrode (SCE) as the reference electrode in the presence of 1 mM n-tetrabutylammonium perchlorate as the supporting electrolyte.

Fluorescence lifetimes were measured by time-correlated single-photon counting technique with Edinburgh FL-900 spectrophotometer upon 400 nm laser pulse irradiation.

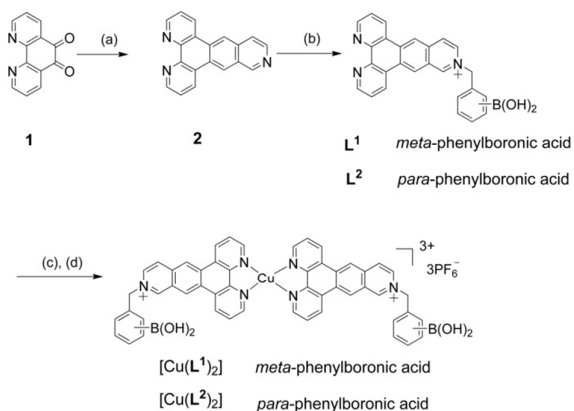
UV-Vis Job's Plot of copper(I) complexes with PASP

Stock solutions of copper(I) complex (200 μM) and PASP (200 μM) were prepared, and solutions with a molar fraction of PASP from 0.1 to 0.9 were prepared in 5 mL volumetric flasks by diluting the required amount of the stock solutions. The total concentration of copper(I) complex and PASP was fixed at 20 μM . The vertical coordination (ΔA) in differential absorption spectra represented the difference between the absorption of the mixed solution and that of the neat copper(I) complex and PASP solutions at corresponding concentrations.²¹

Fluorescence measurements for quenching studies

The titrations were performed by adding the required volumes of a solution of ligand or copper(I) complex (1 mM) into the buffer solution of PASP (4 μM). Under experimental

conditions, the fluorescence of PASP decreased with increasing concentration of ligand or copper(I) complex.



Scheme 1 The synthetic route of ligands L^1 - L^2 and their copper(I) complexes $[Cu(L^1)_2]$ and $[Cu(L^2)_2]$. Reagents and conditions: (a) 3,4-pyridinediamine, ethanol, reflux for 6 h; (b) (bromomethyl)phenylboronic acid, chloroform, 50 °C, 24 h; (c) $[Cu(CH_3CN)_4]PF_6$, methanol, 60 °C, overnight; (d) DMSO, NaPF₆.

Fluorescence measurement for citrate sensing studies

Solutions of PASP/quencher ensemble were prepared by adding L^1 , L^2 , $[Cu(L^1)_2]$, or $[Cu(L^2)_2]$ to 2 mL buffered solution of PASP (4 μ M). The changes in the emission are monitored by addition of different concentrations of citrate and various other analytes.

Results and discussion

Synthesis and characterization

Boronic acids have been incorporated into various receptors for molecular sensing and recognition.²² In combination with salt bridges and hydrogen bonding, the ensembles containing boronic acid moiety have been developed for the selective sensing of citrate,²³ tartrate,²⁴ malate,²⁵ triphosphate,²⁶ and heparin.²⁷ The two-component fluorescence probe, comprising a fluorescent dye that serves as the reporter unit and a diboronic acid functionalized benzyl viologen that acts dually as a fluorescence quencher and a receptor, has been applied for detection of monosaccharides and α -hydroxycarboxylates.²⁸ The reduction potential of viologen and the number of anionic groups on the dye is important for fluorescence quenching efficiency and sensing selectivity. Dipyrido[3,2-a:2',3'-c]-7-aza-phenazine (compound **1**, Scheme 1) and its pyridinium conjugated derivatives are novel electron acceptors.^{16,19} It can be anticipated that the synthesis of boronic acid modified dipyrido[3,2-a:2',3'-c]-7-aza-phenazine may provide opportunities for further design and construction of new supramolecular ensemble with molecular sensing properties. The ligands L^1 and L^2 can be synthesized by the alkylation of **2** with meta- or para-(bromomethyl) phenylboronic acid, respectively. It has been reported that the alkylation of nitrogen

atoms in position N-7 of **2** instead of nitrogen atoms in position N-1 (or N-1') can be accomplished under low reaction temperature (50 °C) in chloroform.¹⁶ The resulting quaternary pyridinium L^1 - L^2 can react with $[Cu(CH_3CN)_4]PF_6$ under inert atmosphere. This affords $[Cu(L^1)_2]$ and $[Cu(L^2)_2]$ in moderate yield, which are isolated as their PF₆⁻ salts. The chemical structures of L^1 - L^2 , $[Cu(L^1)_2]$ and $[Cu(L^2)_2]$ are characterized by ¹HNMR, MALDI-TOF and elemental analysis.

Absorption studies

The UV-Vis absorption spectra of ligands L^1 and L^2 in buffer solution present two intense bands at about 285 and 445 nm, respectively (Table 1 and Fig. S1). The absorption band at about 285 nm is assigned to the $\pi \rightarrow \pi^*$ transition of the ligands,

Table 1 Photophysical data of phenylboronic acid modified phenazines and their copper(I) complexes in Tris-HCl buffer (10 mM, pH 7.4) (λ_{ex} =365 nm)

Compound	$\lambda_{abs}(nm)$	$\epsilon (\times 10^4 L \cdot mol^{-1} \cdot cm^{-1})$	$\lambda_{em(max)}(nm)$
L^1	283, 446	1.94, 1.22	420
L^2	285, 449	1.72, 1.05	422
$[Cu(L^1)_2]$	286, 426	1.99, 0.94	420
$[Cu(L^2)_2]$	288, 433	2.91, 1.29	419

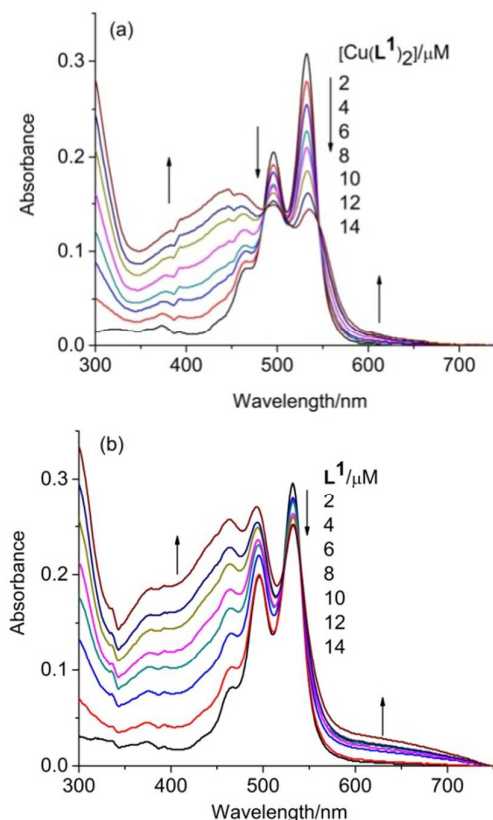


Fig. 2 UV-Vis absorption spectra of PASP (8 μM) in the presence of (a) $[\text{Cu}(\text{L}^1)_2]$ and (b) L^1 in Tris-HCl (10 mM, pH 7.4) buffer solution.

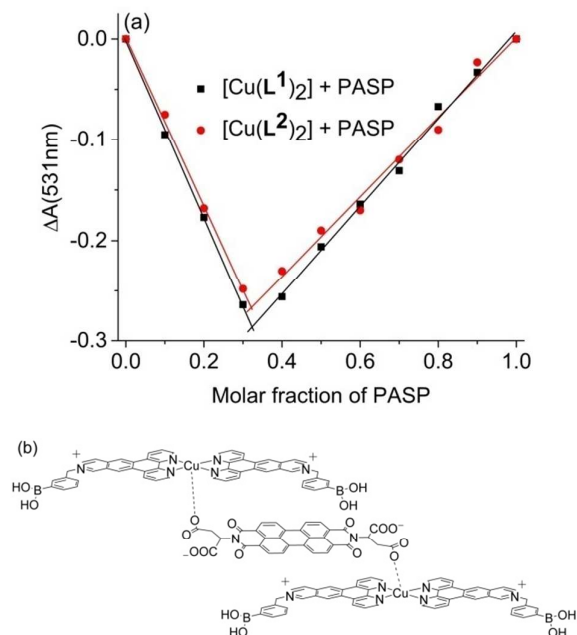


Fig. 3 (a) Job's plots for the interaction between copper(I) complexes and PASP in Tris-HCl buffer (10 mM, pH 7.4) obtained by the absorbance difference (ΔA) at 531 nm. (b) Proposed pentacoordinate structure for the interaction of $[\text{Cu}(\text{L}^1)_2]$ with PASP.

and the band at 400–500 nm is attributed to intramolecular charge transfer (ICT) transition.²⁹ The absorption band at 400–500 nm of copper(I) complexes $[\text{Cu}(\text{L}^1)_2]$ and $[\text{Cu}(\text{L}^2)_2]$ is blue-shifted relative to their corresponding ligands, indicating some interference at the ICT process of the ligands after coordination.³⁰ The spectral data of the ligands and their copper(I) complexes are summarized in Table 1. The copper(I) complexes $[\text{Cu}(\text{L}^1)_2]$ and $[\text{Cu}(\text{L}^2)_2]$ are stable in organic solvents, such as DMF, DMSO and methanol, for several weeks. The absorption spectra of the aqueous solution of copper(I) complexes show almost no changes in several hours, indicating that they are relatively stable in aqueous solution (Fig. S2). It has been reported that the copper(I) complex of 1,10-phenanthroline can be oxidized to copper(II) complex upon irradiation.³¹ To avoid oxidation, the samples are protected from light during incubation.

Interaction of PASP with the ligands L^1 – L^2 , and their copper(I) complexes has been studied with absorption spectra. PASP has two absorption peaks at 496 nm and 532 nm (Fig. 2), respectively. Addition of $[\text{Cu}(\text{L}^1)_2]$ (14 μM) into the solution of PASP leads to a hypochromic change of about 54% and a red shift of the absorption maximum from 532 to 536 nm (Fig. 2a). In addition, the absorption spectra of PASP in the presence of $[\text{Cu}(\text{L}^1)_2]$ show two isobestic points at 486 nm and 545 nm. Similar results have been obtained when $[\text{Cu}(\text{L}^2)_2]$ is added into the solution of PASP, with a hypochromic change of about 52% and an isobestic point at 483 nm (data not shown).

Addition of the ligands, also leads to the decrease of the peak at 532 nm, but no red-shift in the absorption spectra of PASP is observed (see Fig. 2b for L^1). These results indicate

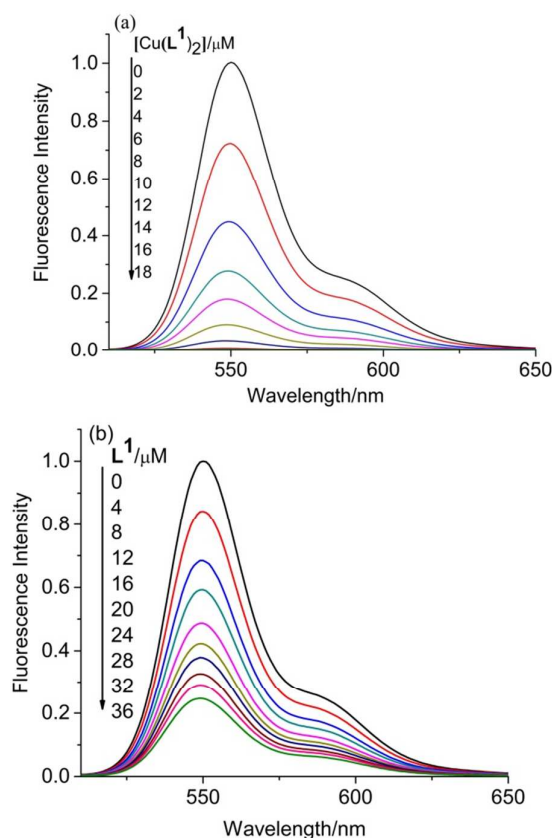


Fig. 4 Fluorescence emission spectra of PASP (4 μM) in the presence of (a) $[\text{Cu}(\text{L}^1)_2]$ and (b) L^1 in Tris-HCl buffer (pH 7.4, 10 mM). ($\lambda_{\text{ex}} = 496 \text{ nm}$)

that the ligands L^1 – L^2 associate with PASP mainly through electrostatic interaction.³² In all spectra, the broad absorption bands around 440 and 650 nm may originate from the addition of the ligands or their copper(I) complexes.

Job's plots are constructed to determine the stoichiometry between copper(I) complexes and PASP by monitoring the absorbance at 531 nm. As shown in Fig. 3a, the minimum ΔA occurs at a molar fraction of around 0.32 in the Job's plot, suggesting a 2:1 molar ratio of copper(I) complexes to PASP in supramolecular assemblies. The $\text{pK}_{\text{a}1}$ and $\text{pK}_{\text{a}2}$ of succinic acid is 4.21 and 5.64, respectively, and in Tris-HCl buffer (pH 7.4) the carboxyl groups in PASP can be ionized. The carboxylate of the PASP can coordinate with the copper(I) complex, resulting in a pentacoordinate structure (Fig. 3b).³³ These results indicate that both $[\text{Cu}(\text{L}^1)_2]$ and $[\text{Cu}(\text{L}^2)_2]$ can form stable ground-state complex with PASP through π - π stacking and coordination interaction.³⁴ The stoichiometry between the ligands L^1 – L^2 and PASP could not be obtained by Job's plot, indicating that the copper(I) ion plays critical role in the interaction between the copper(I) complexes and PASP.

Fluorescence quenching studies

Along with the absorption spectral changes, titration of PASP with the ligands **L**¹–**L**² and their copper(I) complexes leads to a fluorescence quenching. The emission spectrum of PASP shows a band with a maximum at 551 nm in buffer solution. The fluorescence intensity of PASP decreases upon addition of [Cu(**L**¹)₂] or **L**¹ (Fig. 4). The two copper(I) complexes have higher fluorescence quenching efficiency towards PASP than their corresponding ligands, maybe due to the coordination of the PASP to the copper(I) complex.²¹

In principle, both energy transfer and electron transfer could be responsible for the fluorescence quenching of PASP. However, the absorption maximum of the copper complexes and their ligands appear at a shorter wavelength relative to the emission maximum of PASP (Table 1), thus ruling out the possibility of energy transfer.

Table 2 The redox potentials of compounds and the calculated results of the free energy changes (ΔG) of photoinduced electron transfer reactions involving PASP and electron acceptors

Compound	E_{red} (V vs SCE) ^a	ΔG (eV)
L ¹	-0.417	-0.233
L ²	-0.430	-0.220
[Cu(L ¹) ₂]	-0.529	-0.121
[Cu(L ²) ₂]	-0.567	-0.083

Cyclic voltammetric studies are performed to visualize the redox properties. The intermolecular electron transfer from the excited state of PASP to the ligands or their copper(I) complexes is thermodynamically favourable process ($\Delta G < 0$) (Table 2),¹⁶ estimated by the Rehm–Weller equation (eqn (1)).³⁵

$$\Delta G = E_{\text{ox}}(\text{donor}) - E_{\text{red}}(\text{acceptor}) - E_{0-0}(\text{Excited state energy}) - C \quad (1)$$

where $E_{\text{ox}}(\text{donor})$ is the oxidation potential of perylene diimide core of the donor PASP (1.66 V vs. SCE, oxidation to cation of the aromatic ring),³⁶ $E_{\text{red}}(\text{acceptor})$ is the reduction potential of the acceptors (Table 2). E_{0-0} is the singlet excited energy of PASP (2.25 eV), calculated from the formula $E_{0-0} = 1240/\lambda_{\text{em}}$ ($\lambda_{\text{em}} = 551$ nm) (Fig. 4). C is the static Coulomb energy of $D^{+}A^{-}$ (can usually be regarded as 0.06 eV in polar solvents). No obvious changes in the lifetime of PASP can be observed when addition of **L**¹ or **L**² (Fig. 5), and the ligands can form stable ground-state complexes with PASP (Fig. 2b), indicating that **L**¹ and **L**² quench the fluorescence of PASP mainly through static quenching mechanism.³⁷ The fluorescence time

Table 3 Fluorescence life time (τ_f), electron transfer rate constants k_{ET} ($\times 10^8$ s⁻¹) and electron transfer quantum yields Φ_{ET} for PASP in the absence and presence of electron acceptors

Compound	τ_f/ns (fra %)	k_{ET}	Φ_{ET}
PASP	4.65	ND ^a	ND ^a
PASP/ L ¹	4.55	ND ^a	ND ^a
PASP/ L ²	4.62	ND ^a	ND ^a
PASP/[Cu(L ¹) ₂]	4.65 (23.8%) 1.44 (76.2%)	2.38	0.53
PASP/[Cu(L ²) ₂]	4.65 (17.1%) 1.46 (82.9%)	2.84	0.57

^aND means not determined.

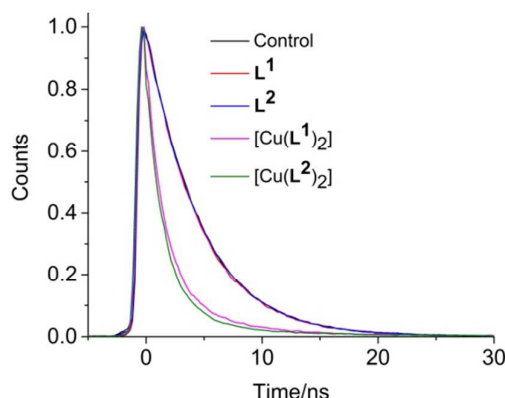


Fig. 5 Fluorescence decay profiles of PASP (4 μM) in Tris-HCl buffer solution (10 mM, pH 7.4) in the presence of **L**¹, **L**², [Cu(**L**¹)₂] and [Cu(**L**²)₂]. ($\lambda_{\text{ex}} = 496$ nm).

profiles of PASP in the presence of [Cu(**L**¹)₂] and [Cu(**L**²)₂] could be fitted satisfactorily to biexponential decay (Fig. 5). The longer lifetime component can be assigned to the singlet state of pristine PASP, and the shorter one may be due to photoinduced electron transfer (PET) from the singlet excited PASP to [Cu(**L**¹)₂] or [Cu(**L**²)₂]. The rate constant (k_{ET}) and the efficiency (Φ_{ET}) for the electron transfer can be calculated from the lifetime of PASP (τ_f)_{PASP} and the average lifetime of its complexes (τ_f)_{complex} according to eqn (2) and (3), respectively, which are all summarized in Table 3. The data in Table 3 show that [Cu(**L**¹)₂] and [Cu(**L**²)₂] display similar k_{ET} and Φ_{ET} , in agreement with their reduction potentials (Table 2).

$$k_{\text{ET}} = 1/(\tau_f)_{\text{complex}} - 1/(\tau_f)_{\text{PASP}} \quad (2)$$

$$\Phi_{\text{ET}} = [(\tau_f)_{\text{PASP}} - (\tau_f)_{\text{complex}}]/(\tau_f)_{\text{PASP}} \quad (3)$$

Anion-sensing studies

The two receptors [Cu(**L**¹)₂] and [Cu(**L**²)₂] present similar reduction potential (Table 2), but differ in their fluorescence quenching strengths. To balance out the quenching difference, a higher concentration of [Cu(**L**¹)₂] (18 μM) is used to quench the fluorescence emission of PASP (Fig. 6a), while a lower concentration (8 μM) of [Cu(**L**²)₂] is used to achieve similar degree of quenching (Fig. 6b). When each of these PASP/quencher solutions is titrated with citrate, a significant

fluorescence turn-on response at 551 nm is observed (Fig. 6a and 6b).

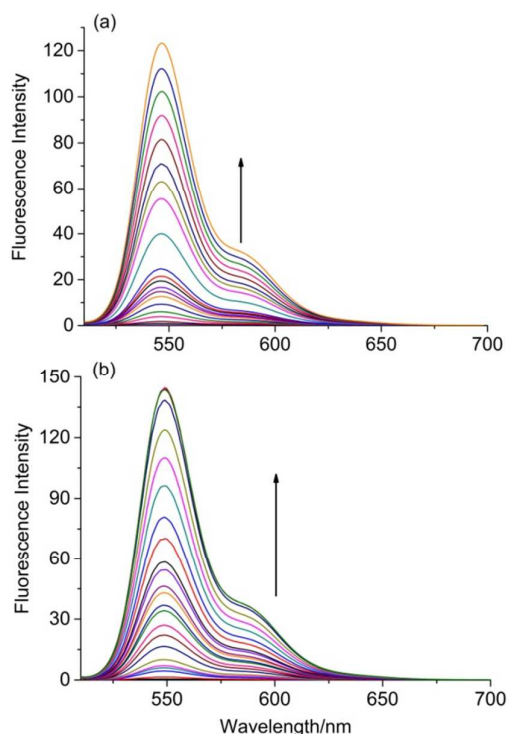


Fig. 6 Changes in fluorescence emission spectra of PASP (4 μM) in Tris-HCl buffer in the presence of (a) $[\text{Cu}(\text{L}^1)_2]$ (18 μM) and (b) $[\text{Cu}(\text{L}^2)_2]$ (8 μM) upon addition of citrate. [citrate] = 0, 0.0025, 0.005, 0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.5, 5.0, 10, 15, 20, 25, 30, 35, 40 mM.

A more than 1500-fold and 250-fold increase in emission intensity (F/F_0) is observed upon addition of citrate to the PASP/ $[\text{Cu}(\text{L}^1)_2]$ and PASP/ $[\text{Cu}(\text{L}^2)_2]$ solution, respectively (Fig. 7a), where F_0 is the fluorescence intensity of the quenched PASP, F is the fluorescence intensity after addition of citrate. However, when PASP/ L^1 and PASP/ L^2 are titrated with citrate, the emission intensity increase is only within 5-fold (Fig. 7a and Fig. S3). These results suggest that the copper(I) cation plays a critical role in citrate sensing. Furthermore, for the PASP/ $[\text{Cu}(\text{L}^1)_2]$ system, the fluorescence enhancement (F/F_0) at 551 nm shows an excellent linear relationship ($R^2 = 0.9972$)

with the concentration of citrate in the range of 25 μM to 1 mM (Fig. 7b), indicating that the PASP/ $[\text{Cu}(\text{L}^1)_2]$ ensemble is highly sensitive to citrate. However, as for the PASP/ $[\text{Cu}(\text{L}^2)_2]$ system, non-linear response is observed when citrate (0–1 mM) is added (Fig. 7b).

The selectivity of the PASP/ $[\text{Cu}(\text{L}^1)_2]$ for citrate determination has been evaluated by measuring the increase in fluorescence intensity (F/F_0) with the other carboxylates. As shown in Fig. 8, citrate binding results in the most significant emission enhancement. No significant fluorescence increase takes place with tartrate, malate, lactate, acetate, oxalate, malonate, succinate, gluconate and adipate. Given the fact that boronic acid can bind monosaccharides, such as glucose and

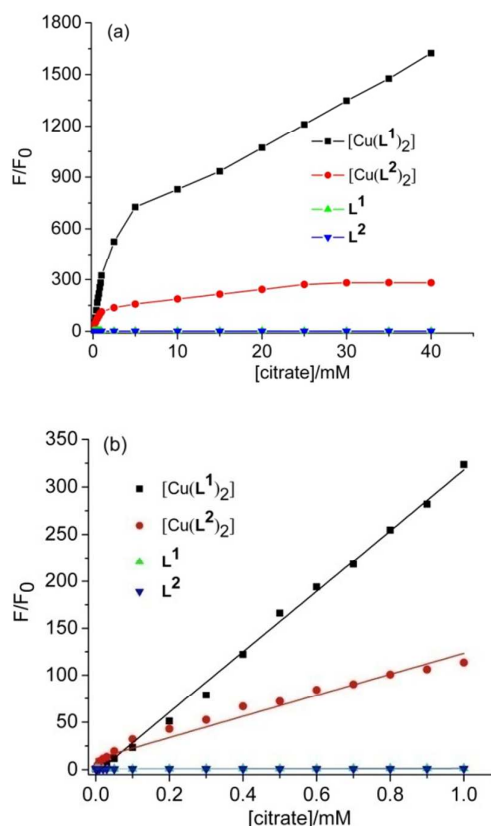


Fig. 7 Relative fluorescence intensity (F/F_0) of PASP (4 μM) in the presence of $[\text{Cu}(\text{L}^1)_2]$, $[\text{Cu}(\text{L}^2)_2]$, L^1 and L^2 plotted as a function of citrate concentration ranges in (a) 0–40 mM and (b) 0–1 mM. The concentrations of $[\text{Cu}(\text{L}^1)_2]$, $[\text{Cu}(\text{L}^2)_2]$, L^1 and L^2 are 18, 8, 36 and 16 μM , respectively.

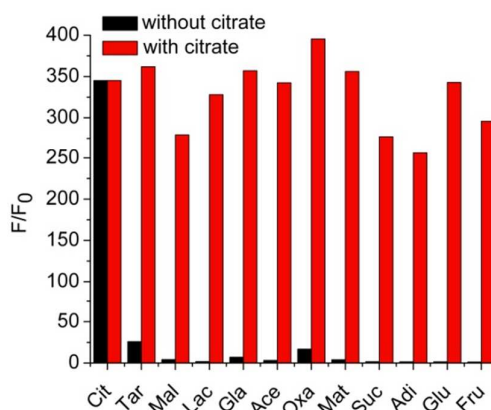


Fig. 8 Fluorescence response of $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ towards various analytes (1 mM) in the absence (black bars) or presence of citrate (red bar, 1 mM). Cit: citrate, Tar: tartrate, Mal: malate, Lac: lactate, Gla: gluconate, Ace: acetate, Oxa: oxalate, Mal: malonate, Suc: succinate, Adi: adipate, Glu: glucose, Fru: fructose. The concentrations of $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ are 18 and 4 μM , respectively.

fructose,^{28,38} the sensing ability of $\text{PASP}/[\text{Cu}(\text{L}^1)_2]$ towards glucose and fructose has also been examined. As shown in Fig. 8, fructose and glucose induce no obvious fluorescence increase of $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$. In order to examine the practical applicability of $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ as citrate selective fluorescence chemosensor, competition experiments are carried out. $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ is titrated with 1 mM citrate in the presence of the other analytes (1 mM). As shown in Fig. 8 (red bar), the other analytes have small or no obvious interference with the detection of citrate. These results suggest that $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ could be used as potential citrate-selective fluorescent chemosensor.

A proposed mechanism accounting for citrate-sensing is depicted in Fig. 9. Supramolecular ensemble formation between the anionic PASP and cationic complex $[\text{Cu}(\text{L}^1)_2]$ facilitates photoinduced electron transfer (PET) from the singlet excited state of PASP to $[\text{Cu}(\text{L}^1)_2]$, resulting in a dramatic decrease in the fluorescence intensity of PASP. When citrate is added to the $\text{PASP}/[\text{Cu}(\text{L}^1)_2]$ ensemble, citrate can chelate with copper(I) in $[\text{Cu}(\text{L}^1)_2]$, thus release free PASP and drastically increase fluorescence intensity¹⁵. On the other hand, the boronic acid moieties in $[\text{Cu}(\text{L}^1)_2]$ are converted into anionic boronate esters,^{23,39} partially neutralizing the net charge of the cationic $[\text{Cu}(\text{L}^1)_2]$, which may further reduce the interaction between $[\text{Cu}(\text{L}^1)_2]$ and PASP.

The $[\text{Cu}(\text{L}^1)_2]$ and $[\text{Cu}(\text{L}^2)_2]$ based sensors have been shown to display α -hydroxycarboxylate selectivity in the order of citrate>tartrate>malate≈lactate (Fig. 8 and Fig. S4). At lower concentration (≤ 1 mM), the difference is even larger, with a much stronger fluorescence enhancement for citrate than that for tartrate, malate or lactate (Fig. S4b). The coordination ability of other organic compound used in this study is much weaker than citrate, which cannot replace PASP, thus making it possible to detect citrate with relative high selectivity.

The strong and optically visible fluorescence of PASP allows a direct naked eye detection of citrate using a standard UV-lamp. As can be seen in Fig. 10, the orange fluorescence of PASP is completely quenched in the presence of $[\text{Cu}(\text{L}^1)_2]$. The addition of citrate can restore the fluorescence of PASP, and the fluorescence can be nicely seen by the recurrence of the orange emission of PASP under UV light. However, the same amount of the other carboxylates or monosaccharides cannot recover the orange fluorescence despite the large excess of substrate.

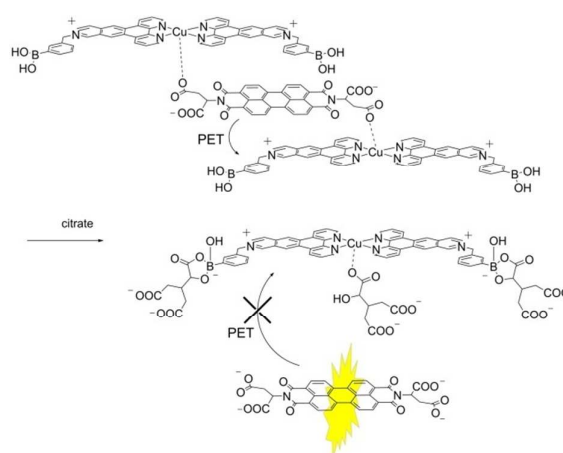


Fig. 9 Proposed citrate-sensing mechanism based on the receptor $[\text{Cu}(\text{L}^1)_2]$ and the anionic fluorescent dye PASP.



Fig. 10 Fluorescence photograph of $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ ensemble upon addition of various analytes (5 mM) under a portable UV lamp at 365 nm. Vials from left: (a) PASP only, (b) $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ ensemble only, (c) citrate, (d) tartrate, (e) malate, (f) lactate, (g) acetate, (h) oxalate, (i) malonate, (j) succinate, (k) adipate, (l) glucose, (m) gluconate, (n) fructose. The concentrations of $[\text{Cu}(\text{L}^1)_2]/\text{PASP}$ are 18 and 4 μM , respectively.

Conclusions

In conclusion, we have synthesized phenylboronic acid modified phenazine derivatives and their copper(I) complexes. The copper(I) complexes can form ensembles with aspartic acid modified perylene diimide (PASP). The fluorescence of PASP can be dramatically quenched due to photoinduced electron transfer (PET) from PASP to the copper(I) complexes. The supramolecular ensembles, comprised of the copper(I) complexes and PASP, can act as PET based “off-on” fluorescent sensor for citrate in buffer solution. The copper(I) cation and phenylboronic acid substituted position plays important role in selectivity of citrate sensing.

Acknowledgements

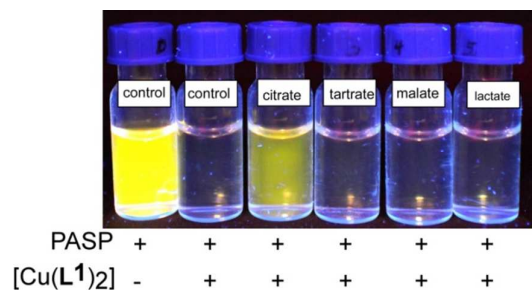
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Selective sensing of citrate by supramolecular ensemble formed by phenazine copper(I) complex and perylene diimide derivative

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A PET based “off-on” fluorescent sensor for citrate has been developed, displaying low interference by other α -hydroxycarboxylates, dicarboxylates and monosaccharides.