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# Synergistic Coupling of Fluorescent "Turn-Off" with Spectral Overlap Modulated FRET for Ratiometric $Ag^+$ Sensor

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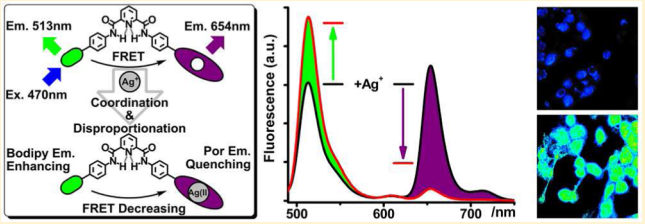
Supporting Information

**ABSTRACT:** A useful strategy for ratiometric fluorescent detecting of $Ag^+$ is demonstrated. Upon selective binding of $Ag^+$ to a BODIPY-porphyrin dyad (**1**), the synergistic coupling of two functions, namely the suppressing of FRET from BODIPY donor to porphyrin acceptor and the fluorescence quenching of porphyrin acceptor, leads to exceptionally large changes in the intensity ratio of two distinct emissions ($F_{513}$/$F_{654}$) which allow for the ratiometric detecting of $Ag^+$ with excellent sensitivity in solution and living cells.

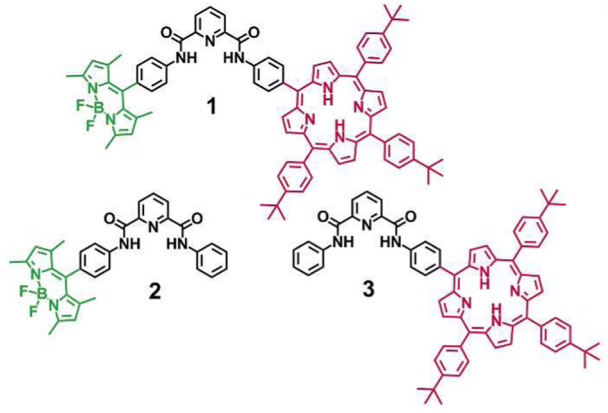



## INTRODUCTION

Fluorescent sensors for detecting transition- and heavy-metal ions in environmental and biological systems have received continuously considerable attention.[1,2] In comparison with single emission intensity-based "turn-off" or "turn-on" sensors, ratiometric sensors can afford self-calibration by dual-channel detection and thus perform precise analyses against various environmental and instrumental factors.[3] One of the most frequently applied mechanisms for ratiometric sensing is the binding-induced modulating of intramolecular fluorescence resonance energy transfer (FRET),[4−6] which is highly dependent on the distance between the donor and acceptor,[7] the overlap integral between the donor emission and acceptor absorption,[8] and the relative orientation of the transition dipoles.[9] Hence the binding-induced changes of the spatial and electronic structure can effectively modulate the FRET efficiency within a donor−acceptor system, consequently leading to change in the fluorescence intensity of the donor and acceptor. However, despite significant efforts,[5−8,10] the design of FRET-based dual-emission sensors with high selectivity and sensitivity still remains a challenging field.

Recently, we reported a FRET-based ratiometric sensor of 8-hydroxyquinoline benzoate (8-HQ-B) linked BODIPY-porphyrin dyad,[11] in which the BODIPY fluorophore[12] serves as a FRET donor and the porphyrin fluorophore[13−15] as an acceptor. In order to develop new ratiometric sensors with improved performance, herein we propose a new strategy for ratiometric fluorescent detecting of metal ions that is based on the rational integration of two sensing mechanisms of a binding-induced modulating of FRET efficiency coupled with a metal-chelating quenching of fluorescence. Synergistic coupling of these two functions may lead to exceptionally large change in the emission ratio, thus allowing for ratiometric detecting of metal ions with improved sensitivity. As a proof-of-concept, a BODIPY-porphyrin dyad (**1**) was designed for the ratiometric sensing of $Ag^+$ ion,[16−18] Scheme 1. As is known, a free base porphyrin fluorophore readily chelates two $Ag^+$ ions to form

**Scheme 1. BODIPY-Porphyrin Dyad (1) and the Reference Compounds 2 and 3**

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dinuclear $[Ag(I)]_2(Por)$, which then undergoes spontaneous disproportionation to produce mononuclear Ag(II)(Por) and Ag(0).[19] Since the fluorescence quantum yield ($\Phi_f$) of Ag(II)(Por) species is remarkably lower than that of free base porphyrin, typically by 1 order of magnitude,[20] this process can be exported as a fluorescence "turn-off" sensing with high selectivity.[21] Furthermore, the absorption shift of the porphyrin unit involved in this process[20] can be programmed into a FRET donor−acceptor system as a modulator for the spectral overlap and in turn for the FRET efficiency.[5,6,10] A BODIPY fluorophore is chosen as the FRET donor because its intense fluorescent emission matches well with the Q-absorption of porphyrin,[13] and the large pseudo-Stokes-shift as well as the emission shift between BODIPY and porphyrin fluorophores are in favor of ratiometric detection.[11] The linker between the donor and acceptor is also crucial in the molecular design. The nonconjugated pyridine-2,6-dicarboxamide spacer, with suitable length[22] and rigidity caused by strong intramolecular hydrogen bonding,[23] not only facilitates the modulation of FRET efficiency but also provides essential solubility in aprotic/aqueous media, and thus is identified as a proper linker.

## RESULTS AND DISCUSSION

The BODIPY-porphyrin dyad (**1**) and the reference compounds **2** and **3** were synthesized by stepwise coupling of pyridine-2,6-dicarbonyl chloride with corresponding amino species (Scheme S1, Figure S1−S4, Supporting Information). The electronic absorption spectrum of dyad **1** is a linear superimposition of those of **2** and **3**, indicating the absence of strong ground-state electronic interaction between the two components in dyad **1** (Figure S5 and Table S1, Supporting Information). Upon excitation at 470 nm, where the BODIPY moiety absorbs most of the light, dyad **1** gives not only the BODIPY emission at 513 nm but also the porphyrin emission at 654 nm with comparable intensities ($F_{513}/F_{654}$ = 0.962). However, the emission intensity of BODIPY moiety in **1** becomes significantly decreased in comparison with that of the reference **2**, while the emission of porphyrin moiety exhibits an obvious enhancement relative to that of the reference **3**, under the same experimental conditions. Furthermore, under 470 nm excitation, a 1:1 mixture of **2** and **3** produces the BODIPY emission with similar intensity to that of **2**, indicating that there is no intermolecular energy transfer between **2** and **3**. The observation suggests the successful construction of intramolecular FRET system in dyad **1** due to the large overlap integral of the emission of BODIPY (513 nm) with the Q-absorption of porphyrin (516 nm) (*vide infra*) as well as the suitable donor−acceptor distance[22] and molecular rigidity provided by the pyridine-2,6-dicarboxamide spacer (Figure S6, Supporting Information). As a result, the efficiency of energy transfer ($\eta_{EET}$) was calculated to be up to 99%.[24] Because of the high fluorescence quantum yield of BODIPY donor, substantial green fluorescence is still observed at 513 nm for dyad **1**. This feature, however, is desirable for the construction of dual-channel fluorescent sensors.

As expected, dyad **1** shows highly sensitive response toward $Ag^+$, Figure 1. Upon addition of $Ag^+$ (0−10 equiv) in a gradual manner, the Soret absorption of porphyrin moiety undergoes bathochromic shift from 420 to 427 nm with a clear isosbestic point observed at 424 nm, indicating the formation of a single component. Fine change in the porphyrin Q absorptions can also be observed, including the raising at 542 nm and

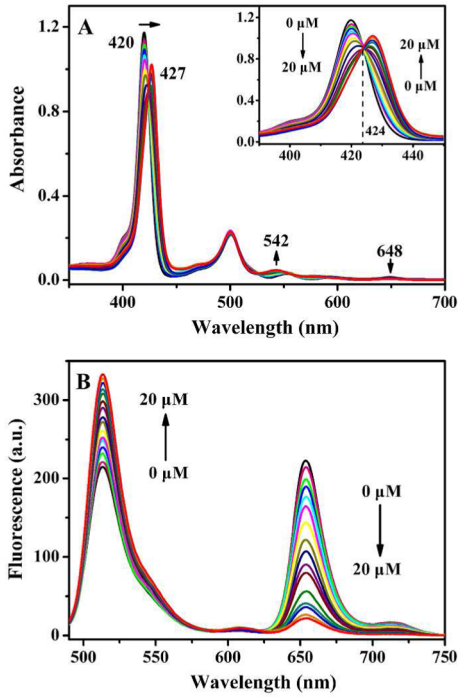


**Figure 1.** Electron absorption spectra (A) and emission spectra (B) of dyad **1** at 2 $\mu$M with excitation of 470 nm upon addition of $Ag^+$ (0−20 $\mu$M) in DMF.

diminishing at 516 and 648 nm. Changes in the fluorescence spectra are more significant. Enhancing of BODIPY emission at 513 nm (by 47.5%) and quenching of porphyrin emission at 654 nm (by 89.7%) result in particularly large emission ratio change, Figure S7, Supporting Information. The $F_{513}/F_{654}$ value experiences remarkable increase from 0.962 to 15.3 along with the addition of $Ag^+$ (0−20 $\mu$M), Figure 2; and the $F_{513}/F_{654}$

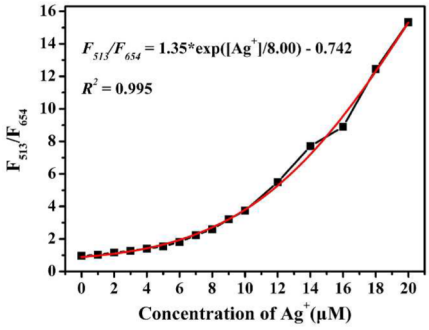


**Figure 2.** Fluorescence intensity ratio ($F_{513}/F_{654}$) of dyad **1** (2 $\mu$M, in DMF) as a function of $Ag^+$ concentration (0−20 $\mu$M).

value is a curvilinear function to $Ag^+$ concentration over the range $1.0 \times 10^{-6}$ to $2.0 \times 10^{-5}$ M, inducing the ratiometric detecting of $Ag^+$ with a detection limit (LOD) of $2.0 \times 10^{-7}$ M ($3\sigma$/slope)[25] in the present conditions (Figure S8, Supporting Information).

The above spectral changes originate from the coordination and disproportionation of silver ions in the presence of free base porphyrin moiety in dyad **1**,[19,20] Scheme 2, which finally leads to the formation of a Ag(II) complex; thus, dyad **1** can be classified as a chemodosimeter.[2] This process induces not only the remarkable quenching of porphyrin emission by Ag(II) but also the substantial shift of porphyrin absorptions, including the


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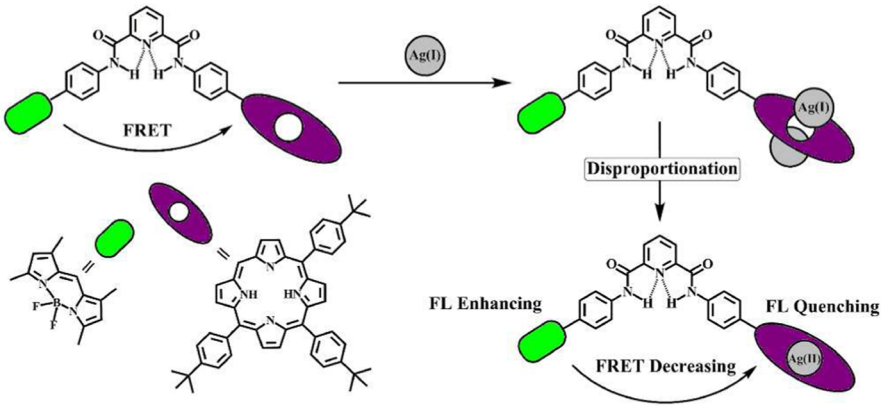
**Scheme 2. Sensing Mechanism of Dyad 1 towards $Ag^+$**




significant bathochromic shift of the most intense Q-absorption band from 516 to 542 nm, which is one of the characteristics for the transformation from a free base porphyrin to a Ag(II) porphyrin complex.[19] This in turn diminishes the spectral overlap between the emission of BODIPY donor and the absorption of porphyrin acceptor, Figure 3, resulting in the slight decrease of FRET efficiency,[8,24] as is revealed by the increase of BODIPY emission at 513 nm (by 47.5%), Figure 1 and Supporting Information Figure S7.

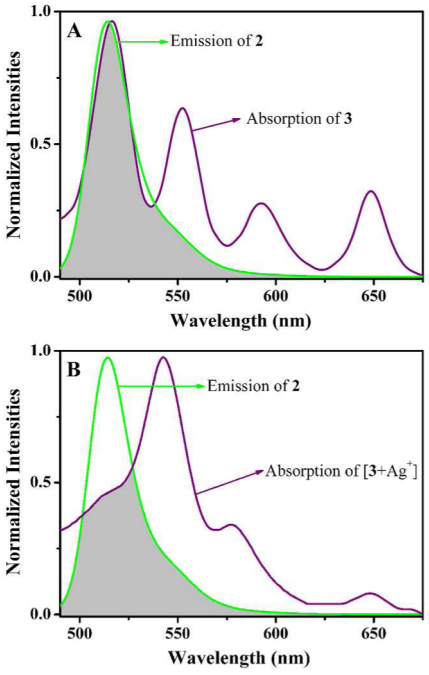



**Figure 3.** Normalized spectral overlap between donor emission and acceptor absorption: (A) emission of **2** and absorption of **3**; (B) emission of **2** and absorption of [**3** + $Ag^+$].

This proposed sensing mechanism was further supported by a range of experimental evidence. Upon adding 10 equiv of $Ag^+$ to dyad **1**, the NMR signal of pyrrol N−H proton ($\delta$ = −2.74 ppm) disappears, and other proton signals become broadened (Figure S9, Supporting Information), suggesting the coordination of silver ions to the inner core of the porphyrin moiety. In addition, the MALDI-TOF mass spectrum of this sample features a clear cluster of isotopic peaks centered at $m/z$ 1376.968 (Figure S10, Supporting Information), which can be exactly identified to the **1**-Ag(II) complex according to the comprehensive previous studies.[19,20] These observations confirm that the binding site for $Ag^+$ is not the pyridine-2,6-dicarboxamide unit but the porphyrin moiety. The high selectivity of porphyrin moiety toward $Ag^+$ is also entirely inherited by dyad **1** (Figure 4 and Supporting Information

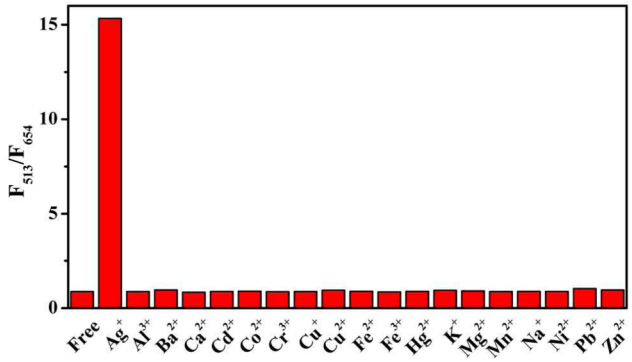



**Figure 4.** Ratiometric fluorescence responses of dyad **1** (2 $\mu$M) to various metal ions (20 $\mu$M). $F_{513}/F_{654}$ data were acquired at 25 °C in DMF, with excitation at 470 nm.

Figure S11). When 10 equiv of metal ions, including $Ag^+$, $Al^{3+}$, $Ba^{2+}$, $Ca^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cr^{3+}$, $Cu^+$, $Cu^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Hg^{2+}$, $K^+$, $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$, $Na^+$, $Pb^{2+}$, and $Zn^{2+}$, were added, respectively, to dyad **1**, only $Ag^+$ induced an obvious change in the absorption and emission spectra. Particularly, the ratiometric emission response ($F_{513}/F_{654}$) of dyad **1** is highly selective for $Ag^+$ over other metal ions. The absorption and fluorescence spectral changes upon addition of a variety of anions to dyad **1** were also evaluated to excluded the suspectable interference by anion coordination[26] to the pyridine-2,6-dicarboxamide unit (Figure S12, Supporting Information). A pH titration revealed that dyad **1** possessed stable fluorescence properties over a wide concentration range of acid/base (from −log[HCl] = 4.0 to −log[NaOH] = 1.3, Figure S13, Supporting Information).

The application of dyad **1** as an intracellular $Ag^+$ sensor was performed with HaCaT cells by confocal fluorescence imaging. After incubation with **1** (10 $\mu$M) for 15 min, HaCaT cells showed not only a green intracellular fluorescence but also a red one of similar intensity, Figure 5A, indicating the cell permeability of **1**. After being incubated with **1** (10 $\mu$M) and $AgNO_3$ (100 $\mu$M) successively, a partial enhancement of the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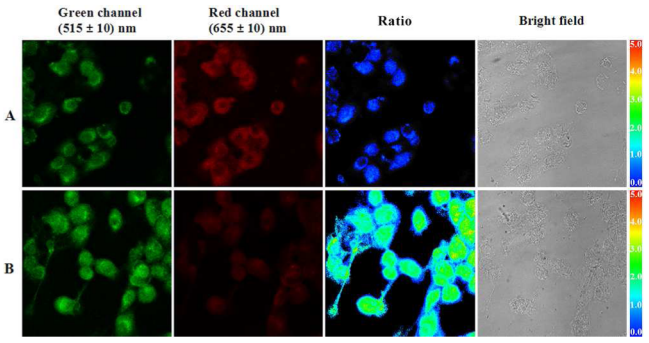


**Figure 5.** Confocal fluorescence and bright field images of HaCaT cells ($\lambda_{ex}$ = 488 nm): (A) incubated with **1** (10 $\mu$M, in DMF:$H_2O$, 1:1 in v/v); (B) incubated with **1** (10 $\mu$M) and then with $AgNO_3$ (100 $\mu$M, in aqueous solution).

green channel and a remarkable quenching of the red one was observed, Figure 5B. The average ratio[27] value of the emission at 515 ± 10 and 655 ± 10 nm also increased from 0.6 to 2.7. The $Ag^+$ triggered obvious color change can be clearly distinguished by comparing the ratio and merged images (Figures 5 and Supporting Information Figure S14). Cell cytotoxicity assay showed that dyad **1** has low cytotoxicity. The cellular viability was estimated to be greater than 85% under the concentration of 40 $\mu$M (Figure S15, Supporting Information). These results demonstrate the potential application of **1** as a ratiometric sensor of $Ag^+$ within living cells.

## EXPERIMENTAL SECTION

**Synthesis of Dyad (1).** Compound **6** (0.056 g, 0.10 mmol) and compound **5** (0.120 g, 0.15 mmol) were dissolved in dry $CH_2Cl_2$ (50 mL) under nitrogen. 4-(Dimethylamino)pyridine (0.012 g, 0.10 mmol) was added. Then the mixture was cooled to 0 °C, and *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (0.016 g, 0.10 mmol) was added. The mixture was stirred at 0 °C under $N_2$ for 10 min before being warmed to rt and stirred for a further 48 h. The solvent was removed on a rotary evaporator, and the residue was purified by column chromatography on silica gel with $CH_2Cl_2$/$CH_3OH$ (100:1, in v/v) as the eluent. The second fraction was collected and evaporated to dryness. The target compound **1** was obtained as orange-red powder (35 mg, 20%). $^1H$ NMR ($CDCl_3$, 400 MHz, 293 K): $\delta$ 9.85 (s, 1H, Amide-H), 9.78 (s, 1H, Amide-H), 8.90 (br, 8H, Pyrrole-$\beta$-H), 8.67 (d, $J$ = 7.2 Hz, 1H, Py-H), 8.62 (d, $J$ = 7.2 Hz, 1H, Py-H), 8.32 (d, $J$ = 8.4 Hz, 2H, POR-Ph-H), 8.28 (t, $J$ = 7.6 Hz, 1H, Py-H), 8.20 (d, $J$ = 8.4 Hz, 2H, POR-Ph-H), 8.15 (d, $J$ = 8.0 Hz, 6H, POR-Ph-H), 8.05 (d, $J$ = 8.4 Hz, 2H, BDP-Ph-H), 7.77 (d, $J$ = 8.0 Hz, 6H, POR-Ph-H), 7.40 (d, $J$ = 8.4 Hz, 2H, BDP-Ph-H), 5.98 (s, 2H, BDP-$\alpha$-H), 2.55 (s, 6H, BDP-$CH_3$), 1.61 (s, 27H, tBu-H), 1.48 (s, 6H, BDP-$CH_3$), −2.74 (s, 2H, N−H). MALDI-TOF-MS: $m/z$ calcd for $C_{82}H_{77}BF_2N_9O_2$ $[M + H]^+$ 1268.6; found 1268.6. Anal. Calcd for $C_{82}H_{76}BF_2N_9O_2$: C, 77.65; H, 6.04; N, 9.94. Found: C, 77.73; H, 6.08; N, 9.82.

**Cell Incubation and Imaging.** HaCaT cells were cultured in DMEM supplemented with 10% FBS. HaCaT cells were seeded on 14 mm glass coverslips. After culturing in an incubator for 12 h, the HaCaT cells were incubated with 10 $\mu$M dyad **1** (DMF:$H_2O$, 1:1 in v/v) for 15 min at room temperature, then washed with distilled water three times. After incubating with 100 $\mu$M $AgNO_3$ aqueous solution for another 15 min at room temperature, the HaCaT cells were rinsed with distilled water three times again. Confocal fluorescence imaging was conducted by a Zeiss LSM-710 confocal microscope with ×20 objectives. The images were recorded at 515 ± 10 (green) and 655 ± 10 nm (red) under the laser excitation of 488 nm. Zen Light Edition and Image Pro-Plus (Universal Imaging Corp.) was used as the analysis software.

## CONCLUSIONS

In summary, we have demonstrated a useful strategy for ratiometric fluorescent sensing of $Ag^+$ by using a BODIPY-porphyrin dyad. The dyad was designed to selectively bind $Ag^+$ in the porphyrin core, which induces a series of synergistically coupled spectral changes, including the following: (1) the quenching of porphyrin acceptor emission; (2) the shift of porphyrin absorption, leading to the decrease of spectral overlap between the emission of BODIPY donor and the absorption of porphyrin acceptor, in turn diminishing the FRET efficiency; and (3) the enhancing of BODIPY donor emission. As a result, remarkable ratiometric fluorescent responses were acquired at two distinct wavelengths ($F_{513}$/$F_{654}$), which allows for the detection of $Ag^+$ in solution and living cells with excellent sensitivity.

## ASSOCIATED CONTENT

### Supporting Information

Details for chemicals and instrumentation, synthesis and characterization for the precursors **6** and **7** and the reference compounds **2** and **3**, DFT calculation of dyad **1**, cytotoxicity assay, optical properties of compounds **1**−**3**, fluorescence intensity ratio changes $F_{513}/F_{654}$ as a rectilinear function of the concentration of $Ag^+$ ions, NMR and mass spectra of **1** and **1** + Ag(II), absorption and fluorescence spectra of **1** with various metal ions and anions, fluorescence intensity ratio $F_{513}/F_{654}$ of **1** with various pH, merged image of HaCaT cells. 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.

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