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Combination of a Fluorescent Dye and a Zn−S Cluster and Its Biological Application as a Stain for Bacteria

Jingli Xie,*,†,‡ Siyu Cao,§ David Good,§ Mingqian Wei,§ and Xiaoming Ren⊥

†*School of Chemistry & Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia,* §*School of Medical Science, Griffith University, Gold Coast, Queensland 4222, Australia, and* ⊥*College of Science, Nanjing University of Technology, Nanjing 210009, People's Republic of China.* ‡*Present address: School of Chemistry, Monash University, Clayton, Victoria 3800, Australia.*

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An ionic-pair charge-transfer salt $[C_{15}H_{16}N_3]^+[Zn_8S(SC_6H_5)_{15}\cdot H_2O]^-$ (**1**) featuring a fluorescent dye and a wurtzite-like octanuclear Zn−S cluster shows high stability when staining bacteria *Escherichia coli*, *Salmonella typhimurium*, and *Clostridium novyi NT*.

Semiconductor nanocrystals have certain advantages over conventional fluorescent dyes because of intrinsic properties such as high photostability (long fluorescence lifetime), large Stokes shifts, and a relatively narrow range emission. The past two decades have seen the fast evolution of nanocrystals from mere electronic materials to materials with biological applications.[1−3] Crystalline semiconducting II−VI chalcogenide clusters have received widespread attention because of their unique optical properties.[4] One approach to further promoting the optical activity of crystalline chalcogenide clusters is to combine them with other optically active species such as the metal chelate dyes $[M(1,10\text{-phenanthroline})_3]^{2+}$ (M = Fe^{2+}, Ru^{2+})/$[Fe(2,2'\text{-bipyridine})_3]^{2+}$ or organic cations such as the methyl viologen dication. The anticipated synergistic effects between organic and inorganic components could pave the way for novel applications.[5] It is strongly believed that the combination of fluorescent dyes and chalcogenide clusters will provide great potential for biological applications such as biolabeling, -imaging, and -sensing, with the enhanced optical properties making the overall materials more practical and useful.[2a,6]

In recent work, we were able to assemble neutral crystalline zinc sulfide clusters with wurtzite-like cores in a controlled and purposeful fashion. The photoluminescence properties of the three clusters $Zn_8S(SC_6H_5)_{14}L_2$ [L = 3-aminopyridine, 4-(dimethylamino)pyridine, and 4-methylpyridine] reported originally depended upon the terminal pyridine ligand substituents.[7] Following this research line with fused-ring aromatic ligands such as Acridine Yellow G to assemble target molecules is a very attractive proposition because these compounds themselves are colorful fluorescent dyes. To our best knowledge, there have not yet been any reports on crystalline Zn−S clusters combined with fluorescent dyes, let alone on their biological application.

Herein we report the ionic-pair charge-transfer (IPCT) salt $[C_{15}H_{16}N_3]^+[Zn_8S(SC_6H_5)_{15}H_2O]^-$ (**1**) featuring a wurtzite-like octanuclear Zn−S cluster as the electron donor and its application as a stain for bacteria.

*To whom correspondence should be addressed. E-mail: xij@unimelb.edu.au.

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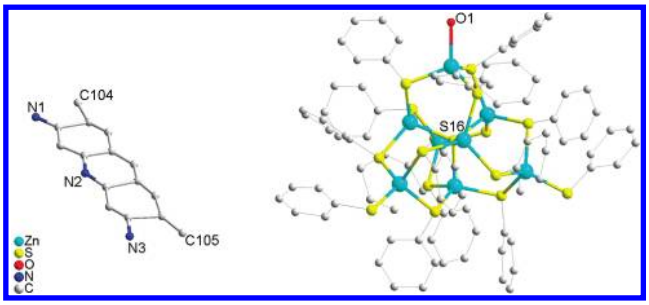

Figure 1. Molecular structure of **1**. H atoms are omitted for clarity.

Compound **1** was obtained by hydrothermal synthesis,[8] and its structure was determined from single-crystal X-ray diffraction data collected at 130 K.[9−11] The asymmetric unit contains one $[C_{15}H_{16}N_3]^+$ cation and an octanuclear zinc cluster $[Zn_8S(SC_6H_5)_{15}H_2O]^-$ anion to achieve a IPCT salt (Figure 1). As with the reported structures,[7] all Zn atoms in **1** adopt a four-coordinated tetrahedral coordination. The central S^{2-} ion (S16) is coordinated to four Zn atoms (Zn2−Zn5) to form a central tetrahedron, and the SZn_4 unit is capped on four faces by three ZnS_4 units and one ZnS_3O unit. The coordinated S atoms surrounding Zn6, Zn7, and Zn8 define regular ZnS_4 tetrahedral units, while the remaining Zn1 atom features a ZnS_3O mode because of the involvement of one coordinated water molecule. The Zn−S bond lengths range from 2.307(8) to 2.408(6) Å, and the Zn−O distance is 2.224(11) Å. The O−Zn−S and S−Zn−S angles are within the ranges 107.7(4)−112.2(3)° and 101.7(2)−119.2(2)°, respectively. It should be noted that while the two pyridine ligands directly coordinated with Zn atoms in the previously reported cluster $Zn_8S(SC_6H_5)_{14}L_2$ neutralize the overall charge,[7] the water molecule in **1** acts as a neutral ligand. Thus, the overall cluster $[Zn_8S(SC_6H_5)_{15}H_2O]^-$ is anionic, and as a consequence, a $[C_{15}H_{16}N_3]^+$ cation is required to maintain neutrality. At room temperature, **1** displays a powder electron spin resonance (ESR) spectrum, as depicted in Figure S1 in the Supporting Information (the **g** tensor is 2.0023), further indicating the essential property of a charge-transfer salt. The broadening of the spectrum shows that the electron is delocalized.[12]

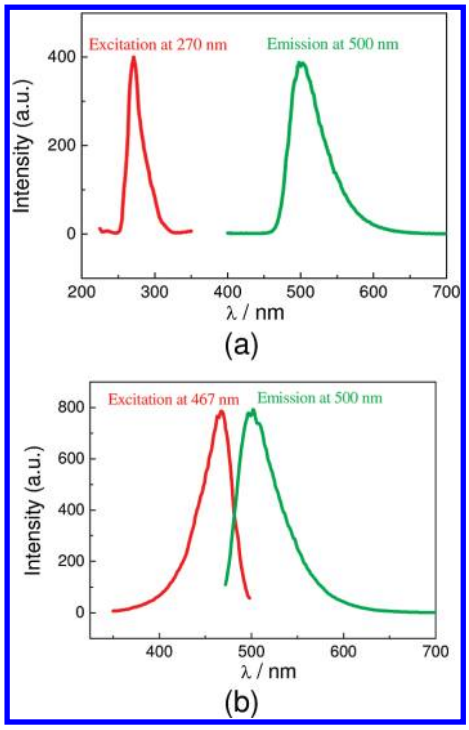

Figure 2. Excitation and emission spectra of **1** in DMSO ($c = 1.0 \times 10^{-5}$ M): excited at (a) 270 nm and (b) 467 nm.

Because of the involvement of the $[C_{15}H_{16}N_3]^+$ cation, the UV−vis spectrum of **1** in dimethyl sulfoxide (DMSO; $c = 1.0 \times 10^{-5}$M) showed maximum absorption at around 267 and 467 nm. Compared to the original dye, the peak at 267 nm suggests that, within the UV range, the $[C_{15}H_{16}N_3]^+$ cation and Zn−S cluster absorb together[7] (Figure S2 in the Supporting Information). It should be particularly noted that the fluorescence emission at 500 nm of **1** occurs as a result of excitation over a relatively wide range down to at least 270 nm (Figure 2), suggesting its potential application for biological imaging and detection.[14]

We have carried out preliminary research to determine the possibility of labeling bacteria with compound **1**. The compound was suspended in DMSO (Figure S4 in the Supporting Information) and tested in three species of bacteria, *Escherichia coli*, *Salmonella typhimurium*, and *Clostridium novyi NT*, using a protocol described below.[15] In brief, 200 μL of **1** was added to 250 μL of bacteria (during their logarithmic growth phase) and 0.8 mL of a Langmuir−Blodgett medium

(8) To prepare crystals of **1**, $Zn(CH_3COO)_2 \cdot 2H_2O$ (0.878 g, 4.0 mmol), thiourea (0.038 g, 0.5 mmol), and Acridine Yellow G (0.274 g, 1.0 mmol) were dissolved in H_2O (15 mL) in a Teflon-lined stainless steel autoclave (23 mL). Thiophenol (0.770 g, 7.0 mmol) was added to the clear solution, and the mixture was stirred efficiently for 15 min, leading to the formation of an orange-red sticky suspension. The sealed vessel was then heated at 165 °C for 7 days. After cooling to room temperature, pink block crystals of **1** (suitable for X-ray analysis) were obtained (weight, 0.815 g; yield, 67% based on the Zn source). Anal. Calcd for $C_{105}H_{93}N_3OS_{16}Zn_8$ (2449.13): C, 51.49; H, 3.83; N, 1.72; O, 0.65. Found: C, 51.91; H, 4.08; N, 2.86; O, 0.72 (Chemical & MicroAnalytical Services Pty. Ltd., Belmont, Victoria, Australia). Notes: There are discrepancies between the experimental elemental analyses and the theoretical values. This is not unusual for this class of Zn−S cluster compounds.[7,13] ε_{267}: 7.73×10^4 M^{-1} cm^{-1}, ε_{467}: 3.60×10^4 M^{-1} cm^{-1}. FTIR data (cm^{-1}): 3461 (m, $\nu(NH_2)$), 3048 (m, $\nu(=CH)$), 1626 (vs, $\nu(NH_2)$), 157(vs, $\delta(C=C)$), 1564 (s, $\nu(NH_2)$), 1476 (vs, $\delta(C=C)$), 1436 (vs, $\delta(C=C)$), 1389 (m, $\nu(CH_3)$), 1085 (s, $\nu(C-S)$), 1069 (m, $\nu(C-S)$), 1023 (vs, $\nu(C-S)$), 734 (vs, $\delta(=CH)$), 685 (vs, $\delta(=CH)$). ^1H NMR (400 MHz, $(CD_3)_2SO$, 25 °C): δ 2.23 (s, $2CH_3$, 6H), 6.79 (s, 1CH, 1H), 6.82 (t, J = 6.8 Hz, 15CH, 15H), 6.95 (m, $15C(CH)_2$, 30H), 7.27 (m, $15C(CH)_2$, 30H), 7.37 (t, J = 8.0 Hz, 2CH, 2H), 7.51 (t, J = 4.0 Hz, 2CH, 2H).

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(11) Crystal data for **1**: $C_{105}H_{93}N_3OS_{16}Zn_8$, M = 2449.13, monoclinic, $C2/c$, a = 27.752(9) Å, b = 25.752(8) Å, c = 33.771(11) Å, β = 103.656(5)°, V = 23453(13) Å^3, Z = 8, T = 130(2) K, D_c = 1.334 g cm^{-3}, $F(000)$ = 9240, μ(Mo Kα) = 1.934 mm^{-1}, GOF = 1.012. A total of 67 119 reflections measured, 26 441 unique (R_{int} = 0.2729), that were used in all calculations. R_1 = 0.1640, wR_2 = 0.3800 [$I > 2\sigma(I)$], CCDC 731860. The surface $-SC_6H_5$ ligands have severe disorder, and this is responsible for the elevated agreement values (R values) found for this structure.

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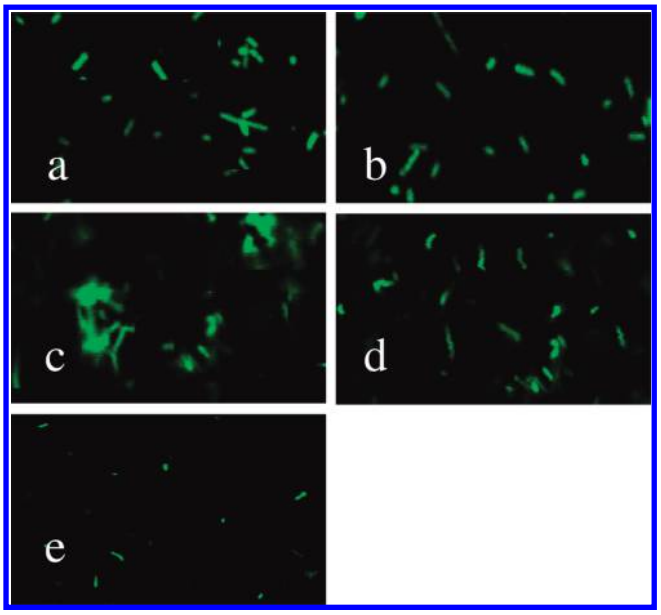

Figure 3. Images of compound **1** achieving stable bacterial staining under confocal microscopy: (a and b) *E. coli*; (c and d) *S. typhimurium*; (e) *C. novyi NT*.

(final concentration: 100 nM for compound **1**). After incubation, bacterial cultures were pelleted at 14000 rpm in a microcentrifuge and washed once in 1% saline to eliminate unbound compound **1**. The resulting suspension was subjected to epifluorescence and confocal microscopy. Intense staining was observed using **1** for all three strains of bacteria used (Figure 3), whereas bleaching of fluorescence was clearly observed when only the original fluorescent dye (Acridine Yellow G) was used for staining (data not shown). Thus, compound **1** stains, combining a dye and a Zn−S cluster, showed minimal bleaching over time, indicating more stable fluorescent imaging. Significantly, intense staining could still be observed after 3 months. It is clear that incorporation of the Zn−S cluster greatly helps stabilization of the overall compound's fluorescence, namely, providing a longer lifetime.

The ability to stain bacteria shows the potential to meet the specific needs of bacterial detection in a variety of environmental settings. It also suggests the potential benefits of staining and identification of microbial communities, such as biofilms on medical devices, infections resulting from various bacteria, and the use of bacteria in gene delivery and gene therapeutical applications.[16] For practical applications, the study about the toxicity of **1** in anoxic/oxic aqueous environments is currently underway.[17]

In conclusion, we have successfully combined a fluorescent dye with a wurtzite-like Zn−S crystalline cluster, and it shows high stability when staining bacteria. It is believed that this alternative strategy, which incorporates the coordination of a water molecule rather than pyridine ligands, should provide a more diverse range of IPCT salts. Further study is underway to explore the mechanism of the Zn−S cluster's ability to help to inhibit photobleaching. Concomitantly, insights into the relationship between the structural characteristics and fluorescent properties will be gained via spectroscopic methods for potential applications. Furthermore, this synthesis as "proof-of-concept" would help to establish viability, as well as provide feedback for choosing different fluorescent dyes to use in the areas of biological application.[18]

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Supporting Information Available: Materials and general procedures, structure solution and refinement, table of crystal data, ESR, UV−vis, and IR spectra of **1**, photos of **1** in DMSO under daylight and UV irradiation, and an X-ray crystallographic file (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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