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A BODIPY-based sensor for Hg^{2+} in living cells

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ABSTRACT

A BODIPY-based probe has been investigated for fast response to Hg^{2+} with high sensitivity and selectivity in living cells. This response is attributed to intramolecular charge transfer (ICT) mechanisms. The detection limit is lower than the upper limit (10 nM) that the United States Environmental Protection Agency (EPA) had mandated for Hg^{2+} in drinking water.

1. Introduction

Mercury ion (Hg^{2+}) is one of the most dangerous heavy metal ions.[1–5] Even at a low concentration, mercury ions can induce several human diseases, including acrodynia (pink disease), Hunter-Russell syndrome, and Minamata diseases, which result from the accumulation of mercury through biological chain.[6] Physiologically, mercury ions can also easily pass through biological membranes and cause serious damages to the central nerves and endocrine systems.[7,8] The upper limit of Hg^{2+} in drinking water is 10 nM as mandated by United States Environmental Protection Agency (EPA).[9] Thus, it is highly desirable to develop sensors for Hg^{2+}.

Fluorescence sensors have been widely developed to detect metal ions, organic molecules, and biological analytes. Such fluorescence sensors are superior because of their sensitivity, simplicity, and real-time analysis.[10,11] Especially, the fluorescence sensors are able to modulate the emission wavelength. In far red and near IR-emission region lights can transmit deeply into human body tissues without disturbing the normal physiological activities.[12] It is potential to be well adapted into many applications, such as in vivo biological imaging.[13,14]

BODIPY-based (Boradiazaindacenes, 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene) dyes are expected to be matrix of far red and near-IR dyes, because they possess desired characteristics, such as sharp absorption and fluorescence bands, high extinction coefficients, high fluorescence quantum yields, high stability against light, and easy modification[15–18] to exhibit fluorescence at wavelength beyond 650 nm by extending delocalization of the conjugated system. It is available to synthesize pyrrole derivatives bearing phenyl, vinyl, or thiophene groups at the 3-position to manipulate the characters and properties of this kind of BODIPY dyes.[19]

Knut Rurack et al. had developed BODIPY-based Hg^{2+} sensor by introducing the 1,4,7,10-tetrathia-13-az acyclopentadecane to the *meso* position of BODIPY,[20] however, the sensor emission was far from the far red or Near-IR region.[21] Yuliang Li et al. designed dithia-dioxa-aza macrocycles sensor of Hg^{2+} on the BODIPY chromophore,[1] but its detection limit did not meet the upper limit that the EPA had mandated for Hg^{2+} in drinking water. Our work aimed at the construction of Hg^{2+} sensor based on ICT (intramolecular charge transfer) mechanism The chromophore was designed to shift emission wavelengths to the far red region, i.e., the range beyond 650 nm. Furthermore, it was used for imaging in living cells with excellent performance.

To achieve goals mentioned above, Z1 (Scheme 1) was synthesized featuring a BODIPY fluorophore and a thia aza crown ether receptor (Scheme 1). (The thia aza crown ether receptor was reported to be selectively responsible for Hg^{2+}.[22])

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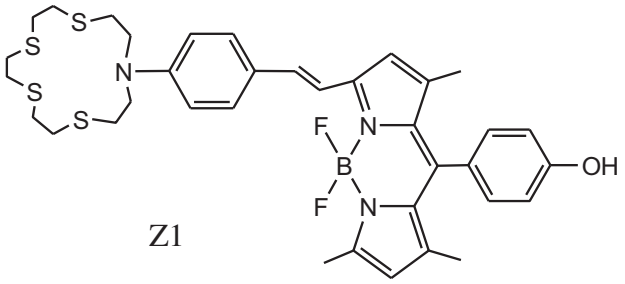

Scheme 1. The molecular structure of Sensor Z1.

2. Result and discussion

2.1. Physical characteristic of Z1

The photophysical properties of Z1 in different solvents were investigated, as shown in Table 1. From cyclohexane to acetonitrile, a significant bathochromic shift in the excitation (586–598 nm) and emission (609–656 nm) were observed along with the increased polarities of the solvents. The absorption maximum is located at 594 nm and has a hypsochromic shift of 12 nm from cyclohexane to acetonitrile–HEPES solution (Table 1), which is weak solvent dependence. In contrast, the fluorescence emission spectra are strongly dependent on the dipole moment of the solvents. The emission maximum shifts from 609 nm in cyclohexane to 656 nm in acetonitrile–HEPES solution.[23] The bathochromic shifts with a concomitant decrease of fluorescence quantum yield are observed (from 0.22 to 0.01). The reducing of the quantum yield is attributed to the acceleration of internal conversion; the bathochromic shift of the emission wavelength is due to the decreasing of the energy gap between the ground state and the excited state.[18] The data above confirmed that Z1 was going through ICT transformation.

Table 1
Absorption and emission properties of Z1 in different solvents

Solvent	λ_{abs} (max/nm)	λ_{em} (max/nm)	Φ_F
Cyclohexane	586	609	0.22
Toluene	596	618	0.15
Chloroform	598	621	0.10
Acetonitrile	591	648	0.02
Acetonitrile/HEPES=4/1	594	656	0.01

2.2. Computational results of Z1

According to the frontier orbital distribution in Fig. 1, the electron cloud density of the LUMO is considerably larger than that of the HOMO, which leads to an expected stabilization of the LUMO through irradiation.[17] The electron cloud density would be disturbed by external interferences. Hence, the energy gap between the HOMO and LUMO is expected to increase owing to the introduction of Hg^{2+} and the absorption has hypochromatic shift.[17] The band gap between LUMO and HOMO is 1.92 eV, which indicates the fluorescence emission of Z1 would be about 650 nm (far red region).

2.3. Spectral response of Z1 to Hg^{2+} and Ag^{+}

Fig. 2a shows the absorption spectral changes of Z1 as a function of the Hg^{2+} concentration in a CH_3CN–HEPES solution (4:1, *v*/*v*, pH=6.86) at room temperature. The UV–Vis spectrum of Z1 is characterized with an intense band centered at 594 nm, which is

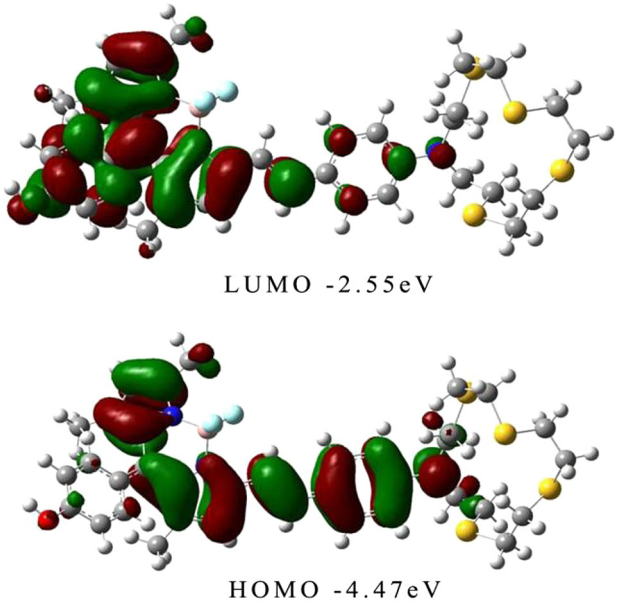

Fig. 1. HOMO (below) and LUMO (above) distributions of Z1.

responsible for the red color of the solution. The absorption maximum of Z1 has a bathochromic shift of about 90 nm in comparison with the standard BODIPY dye.[24] This red shift is assigned to an efficient ICT process from the donor nitrogen atom on the thia aza crown ether receptor that was conjugated to the BODIPY acceptor group.[23,25,26] Upon adding Hg^{2+}, the intensity of the absorption maximum of Z1 at 594 nm gradually decreased along with the formation of a new band centered at 564 nm, indicating that Hg^{2+} binded to thia aza crown ether. The coordination of Hg^{2+} to the ligand reduced the electron-donating ability of the nitrogen atom at the thia aza crown ether, thus the ICT effect decreased. Therefore, the blue-shift in absorption spectra was observed upon Hg^{2+} binding.

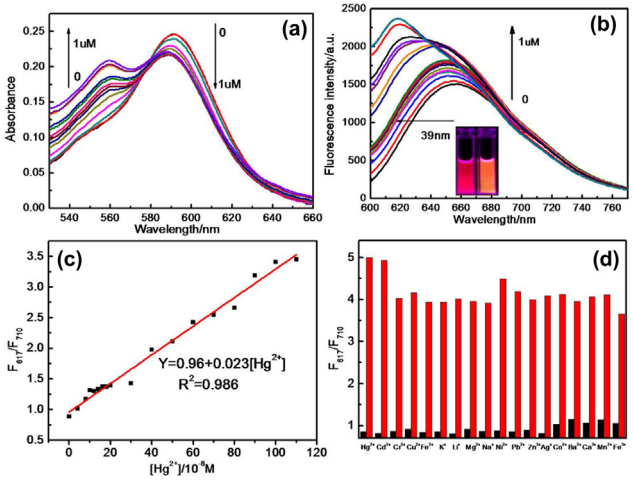

Fig. 2. (a) Absorption spectra of Z1 probe in CH_3CN and HEPES at pH 6.86 upon gradual addition of Hg^{2+} at concentrations of 0–1 μM. (b) Emission spectra of Z1 in the presence of increasing Hg^{2+} concentration (0–1 μM) in a CH_3CN–HEPES solution (*v*/*v*=4/1, pH=6.86). Excitation wavelength was 560 nm. The concentration of Z1 was 1 μM. Inset: the fluorescence color change of Z1 (left) and Z1-Hg^{2+} (right). (c): In emission spectra F_{617}/F_{710nm} as a function of Hg^{2+} concentration (0–1 μM). (d): black bars: In emission spectra F_{617}/F_{710nm} as a function of 50 equiv of Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, K^{+}, Li^{+}, Mg^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Co^{2+}, Ba^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, and Zn^{2+}, 5 equiv of Hg^{2+} and 10 equiv of Ag^{+} in CH_3CN–HEPES solution (*v*/*v*=4/1, pH=6.86). Red bar: to mix 5 equiv of Hg^{2+}.