Exploring the Scope of Photo-Induced Electron Transfer—Chelation-Enhanced Fluorescence—Fluorescence Resonance Energy Transfer Processes for Recognition and Discrimination of Zn²⁺, Cd²⁺, Hg²⁺, and Al³⁺ in a Ratiometric Manner: Application to Sea Fish Analysis

Milan Ghosh, Sabyasachi Ta, Mahuya Banerjee, Md Mahiuddin, and Debasis Das*®

Department of Chemistry, The University of Burdwan, Burdwan, 713104 West Bengal, India

Supporting Information

ABSTRACT: A rhodamine-based smart probe (RHES) has been developed for trace-level detection and discrimination of multiple cations, viz. Al³⁺, Zn²⁺, Cd²⁺, and Hg²⁺ in a ratiometric manner involving photo-induced electron transfer—chelation-enhanced fluorescence—fluorescence resonance energy transfer processes. The method being very fast and highly selective allows their bare eye visualization at a physiological pH. The optimized geometry and spectral properties of RHES and its cation adducts have been analyzed by time-dependent density functional theory calculations. RHES detects as low as 1.5 × 10⁻⁹ M Al³⁺, 1.2 × 10⁻⁹ M Zn²⁺, 6.7 × 10⁻⁹ M Cd²⁺, and 1.7 × 10⁻¹⁰ M Hg²⁺, whereas the respective association constants are 1.33 × 10⁷ M⁻¹, 2.11 × 10⁵ M⁻¹, 1.35 × 10⁷ M⁻¹, and 4.09 × 10⁴ M⁻¹. The other common ions do not interfere. The probe is useful for intracellular imaging of Zn²⁺, Cd²⁺, and Hg²⁺ in squamous epithelial cells. RHES is useful for the determination of the ions in sea fish and real samples.

INTRODUCTION

A smart probe that converts a highly selective molecular recognition of multiple ions into easily detectable signals is very attractive in the present scenario. The techniques to visualize bioactive and environment-relevant cations have immense importance in biomedical analysis and environmental monitoring. The techniques like inductively coupled plasma mass/atomic emission spectroscopy, atomic absorption spectroscopy, colorimetry, spectrofluorimetry, and voltammetry generally require sophisticated expensive equipment, tedious time-consuming sample preparation procedure, and trained skilled operator. In contrast, fluorescence spectroscopy is very useful to provide instantaneous detection, visual perception, and inexpensive methodology that excludes sample pretreatment. A single probe capable to sense multiple ions is cost-effective and highly desirable for practical applications. Particularly, a probe that selectively and specifically detects and discriminates elements belonging to the same group in the periodic table through the photo-induced electron transfer—chelation-enhanced fluorescence—fluorescence resonance energy transfer (PET—CHEF—FRET) processes in a ratiometric manner is highly demanding as well as difficult to achieve because it rules out the adverse environmental effects like pH, polarity, probe concentration, and excitation power on the emission intensity via built-in correction to the signal ratio of emission intensities at two different wavelengths.

Although Al is extensively used in food packaging, cookware, antiperspirants, drinking water supplies, bleached flour, deodorants, antacids, manufacturing of cars, and computers, it may cause neurotoxicity, disorders of homeostasis, Parkinson’s disease, Alzheimer’s disease (AD), amyotrophic lateral sclerosis (ALS), microcytic hypochromic anemia, myopathy, and anemia. It also inhibits plant growth by increasing the acidity of the soil. Zinc, the most biocompatible metal, is present in all forms of life and plays a vital role in numerous biological processes including brain activity, gene transcription, immune function, etc. Zinc-based compounds are used as radio-protective agents, tumor photosensitizers, anti-diabetic insulin-mimetic, antibacterial, antimicrobial, and anticancer agents. It also reduces cardio- and hepatotoxicity induced by some anticancer drugs. Its abnormal metabolism causes health problems like prostate cancer, delayed sexual maturation and impotence, type 2 diabetes mellitus, ALS, Wilson’s diseases, age-related macular degeneration, AD, cerebral ischemia, epilepsy, and acrodermatitis enteropathica.

On the other hand, Cd occupies the seventh position among the top 20 hazardous substances in the priority list prepared by the Agency for Toxic Substances and Disease Registry and the US Environmental Protection Agency. The World Health Organization allows a maximum of 3 ppb Cd in drinking water. Cd released from Ni–Cd batteries, phosphate fertilizers, pigments, and semiconducting quantum dots/rods causes renal dysfunction, calcium metabolism disorders, reduced lung capacity, risk of cancer, and health disorders. Thus, the
determination of trace-level Cd^{2+} in environmental and biological samples has great significance.

Finally, it is well-known that Hg damages kidney, skin, respiratory system, central nervous system, and other organs.46,47 It is observed in the literature that most of the reported probes relevant to these cations either detect one or dual cations.48-50

Moreover, very few fluorescence probes detect and discriminate Zn^{2+} and Cd^{2+}. In most cases, Cd^{2+} interferes with Zn^{2+} sensing.51 A simple turn-on probe52 for the detection of both Zn^{2+} and Cd^{2+} suffers limitations such as narrow difference in emission wavelengths (λ_{Em} for Zn^{2+}, 572 nm and λ_{Em} for Cd^{2+}, 565 nm) and low detection limit. An amino-terpyridine-based probe53 detects both Zn^{2+} and Cd^{2+} with an incremental enhancement of emission intensity at 535 nm but fails to discriminate Zn^{2+} and Cd^{2+}. Chen et al.54,55 have reported a turn-on fluorescence probe for Zn^{2+}, Cd^{2+}, and Hg^{2+} that also fails to discriminate them.

It is to be noted that the molecular arithmetic that converts a chemically encoded analysis (input) into an optical signal52,53 is potentially an interesting research area in modern unconventional computing system54 that insisted us to construct a scientific logic gate using our present probe.

Rhodamine derivatives possess excellent photophysical properties, viz. high quantum yield, photostability, bioavailability, excitation, and emission in the visible region,56 and undergo an equilibrium between the nonfluorescent "spirolactam ring" and fluorescent "ring-open" forms to allow analyte sensing through "off-on" switching.56 Herein, a smart probe is being reported that selectively detects and discriminates Al^{3+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}, utilizing their significantly different emission wavelengths and eye-catching colors under ultraviolet (UV) and visible light. The ratiometric sensing mechanism involves PET−CHEF−FRET processes. The ability of RHES for imaging intracellular Zn^{2+}, Cd^{2+}, and Hg^{2+} have been demonstrated in live cells.

Results and Discussion

Colorless RHES displays a very weak emission at 397 nm (λ_{Em} = 306 nm, Scheme 1, Figure S1, Supporting Information). In the presence of Al^{3+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}, RHES turns pink (Al^{3+}), green (Zn^{2+}), sky blue (Cd^{2+}), and intense blooded (Hg^{2+}) (λ_{Em} = 306 nm) (Figure 1). Additionally, for Al^{3+} and Hg^{2+}, the solution looks pink and red in bare eye (Figure 1). Interestingly, out of all the common ions tested, the emission intensity enhances for Al^{3+} (51-fold along with 176 nm red shift from 397 to 573 nm), Zn^{2+} (29-fold along with 85 nm red shift from 397 to 482 nm), Cd^{2+} (36-fold along with 2 nm blue shift from 397 to 395 nm), and Hg^{2+} (89-fold along with 180 nm red shift from 397 to 577 nm) (Figure 2).

The spectroscopic properties of RHES depend on the pH of the media as it contains pH-susceptible donor sites. Moreover, the biological application of RHES demands its efficiency at a physiological pH. Hence, RHES is mixed with Al^{3+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} in different sets at different pH values (pH 3.0−12.0). The difference in emission intensities between the free RHES and its metal ion adducts is highest near the physiological pH 7.4, and hence chosen for the entire study (Figure S2, Supporting Information).

Figures 2 and 3a demonstrate the selectivity of RHES for Al^{3+} (λ_{Em} = 573 nm). Figure S3 (Supporting Information) indicates no interference from the common cations. Upon a gradual addition of Al^{3+} (from 0.005 to 1600 μM), the colorless RHES slowly turns sky blue, whereby the emission intensity increases at 362 nm. After 25 min, the emission intensity gradually increases at 573 nm. Further, with increasing time, the emission intensity at 362 nm remains unaltered, whereas it increases at 573 nm. It is proposed that the initial coordination of phenol-O and imine N to Al^{3+} is responsible for the weak emission at 362 nm. Subsequent coordination by the −O donors from the carbonyl functionality of the spirolactam moiety to Al^{3+} leads to fluorescence enhancement at 573 nm along with the opening of the spirolactam ring resulting in pink coloration57 (Figure S4, Supporting Information). The time-dependent color changes of the RHES−Al^{3+} system under a handheld UV lamp are presented in Figure S4 (Supporting Information). Figure 3b shows the fluorescence titration of RHES by Al^{3+}. RHES detects as low as 1.5 × 10^{-9} M Al^{3+} (Figure S5, Supporting Information). Figure 3c shows the [Al^{3+}] versus emission intensity plot at 573 nm (red balls) and 362 nm (inset, sky blue balls). The red shift of the emission band is accompanied by a 51-fold fluorescence enhancement (λ_{Em} = 573 nm; ΔΦ, 6.6-fold enhancement). Moreover, in the presence of Al^{3+}, 1.5-fold increase of F_{573}/F_{362} is observed (Figure S6, Supporting Information). The plot of fluorescence enhancement versus [Al^{3+}] at different wavelengths is shown in Figure S6 (Supporting Information). The UV−vis titration of RHES with Al^{3+} shows the appearance of two new bands at 406 and 555 nm (Figure 3d), indicating the interaction of RHES with Al^{3+}. Job’s plot supports a 1:1 stoichiometry (mole...
The mass spectrum of the $[\text{RHES}^-\text{Al}^{3+}]$ adduct also confirms this composition. The binding constant of RHES for Al$^{3+}$ is $1.33 \times 10^5$ (Figure S8, Supporting Information).

In the presence of Zn$^{2+}$, RHES experiences a red shift of the emission band to bright green along with a 29-fold fluorescence enhancement ($\lambda_{\text{Em}} = 482$ nm; $\lambda_{\text{Ex}} = 306$ nm; $\Phi$, 5.02-fold enhancement, Figure 4a) without any interference from the other common cations (Figure S9, Supporting Information).

With an increasing $[\text{Zn}^{2+}]$, the emission intensity at 342 nm decreases in a ratiometric manner with an isoemissive point at 419 nm (Figure S10, Supporting Information). The plot of emission intensities versus $[\text{Zn}^{2+}]$ at two different wavelengths is shown in Figure S11 (Supporting Information). The fluorescence titration and emission intensities versus $[\text{Zn}^{2+}]$ plots are shown in Figure 4b,c. The absence of bands at 573 nm/577 nm, responsible for bare eye pink/red color, indicates the non-involvement of the rhodamine moiety to coordinate to Zn$^{2+}$. RHES efficiently detects Zn$^{2+}$ at the physiological pH of 7.4 (Figure S2, Supporting Information) and forms a 1:1 (mole ratio) complex at a low $[\text{Zn}^{2+}]$, whereas a 1:2 (mole ratio) complex is formed at a higher $[\text{Zn}^{2+}]$, as revealed from Job’s plot (Figure S12, Supporting Information) and the mass spectrum. The binding constant of $2.11 \times 10^4$ M$^{-1}$ is indicative of a fairly strong interaction between RHES and Zn$^{2+}$ (Figure S13, Supporting Information). The UV−vis titration of RHES versus

---

Figure 2. Emission (left) and absorption spectra (right) of RHES (the same media and pH mentioned above) in the presence of the common metal ions ($\lambda_{\text{Ex}} = 306$ nm).

Figure 3. (a) Emission intensity of RHES in the presence of Al$^{3+}$ and the other common metal ions (i) $\lambda_{\text{Ex}} = 306$ nm, $\lambda_{\text{Em}} = 573$ nm, red bar and (ii) inset $\lambda_{\text{Ex}} = 306$ nm, $\lambda_{\text{Em}} = 362$ nm, sky blue bar; (b) changes in the fluorescence spectra of RHES (20 μM) upon the gradual addition of Al$^{3+}$ (0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10, 50, 100, 200, 500, 800, 1200, and 1600 μM) ($\lambda_{\text{Ex}} = 306$ nm); (c) plot of emission intensities of RHES vs [Al$^{3+}$] at 573 nm (red balls) and at 362 nm (sky blue balls, inset); $\lambda_{\text{Ex}} = 306$ nm; (d) changes in the absorption spectra of RHES in the said media upon the gradual addition of Al$^{3+}$ (same as (b)).
Zn$^{2+}$ is presented in Figure 4d. RHES can detect Zn$^{2+}$ as low as 1.2 × 10$^{-9}$ M (Figure 4c, inset). In the presence of Cd$^{2+}$, RHES shows a sky blue fluorescence ($\lambda_{\text{em}} = 395$ nm, $\lambda_{\text{ex}} = 306$ nm, Figure 5a) without any interference from the common cations (Figure S14, Supporting Information). A gradual addition of Cd$^{2+}$ enhances the emission intensity at 395 nm, while it decreases at 347 nm in a ratiometric manner with an isoemissive point at 368 nm (Figure S15, Supporting Information). The plots of emission intensities versus [Cd$^{2+}$] at two different wavelengths are shown in Figure S16 (Supporting Information). The fluorescence titration with Cd$^{2+}$ (0.05−1600 μM) and concentration versus fluorescence intensity are shown in Figure S20 (Supporting Information). The UV−vis titration of RHES versus [Hg$^{2+}$] is presented in Figure 6d. The optimum pH for the entire study has been determined from Figure S2 (Supporting Information) as 7.4. Figure S21 (Supporting Information) reveals that the common cations do not interfere with the determination of Hg$^{2+}$. Similar to Al$^{3+}$, Hg$^{2+}$ also forms a 1:1 (mole ratio) adduct with RHES, realized from Job’s plot (Figure S22, Supporting Information) and supported by the mass spectrum. The strong interaction between RHES and Hg$^{2+}$ is indicated from the binding constant, 4.09 × 10$^5$ M$^{-1}$ (Figure S23, Supporting Information). RHES detects as low as 1.7 × 10$^{-10}$ M Hg$^{2+}$ (Figure 6c, inset).

As the literature suggests, probably the present study is the first report of a single sensor that detects and discriminates Al$^{3+}$, Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$ through the generation of different colors upon
irradiation of UV light and also observable in bare eye (Figure S24, Supporting Information).

Al$^{3+}$ shifts the emission maxima of RHES from 397 nm (characteristic of PET, from the imine N-to the conjugated 3-ethoxysalicylaldehyde moiety) to 573 nm (characteristic of rhodamine B, FRET) via an intermediate CHEF process having a characteristic emission at 362 nm. Al$^{3+}$ being a hard acid prefers an “O” donor from 3-ethoxysalicylaldehyde. The absorption at 406 nm also indicates the interaction between Al$^{3+}$ and the “O” donor from 3-ethoxysalicylaldehyde (Figure 3d). Thus, upon excitation at 306 nm, the fluorescence at 362 nm enhances because of the CHEF process which subsequently transfers energy to excite the rhodamine moiety.

However, Hg$^{2+}$ being a soft acid does not prefer the hard “O” donor, and hence the CHEF process is absent. Rather, a significant overlap between 3-ethoxysalicylaldehyde emission (donor) and rhodamine absorption (acceptor) has been observed (Figure S25, Supporting Information). Upon the addition of Hg$^{2+}$, the emission maximum of free RHES is redshifted from 397 to 577 nm ($\lambda_{ex}$ = 306 nm), indicating an energy transfer from the donor to the acceptor.

The slow interaction between RHES and Al$^{3+}$ allows the intermediate CHEF process visible. With time, the intensity at 573 nm gradually increases, and finally it moves over to 362 nm. Hence, an intense pink fluorescence and bare eye pink coloration are observed (Figure S26, Supporting Information). The pseudo-first-order rate constant for the interaction has been estimated as 0.0413 min$^{-1}$ by monitoring the changes in the emission intensity at 573 nm (Figure S27, Supporting Information). The proposed binding mechanism is further substantiated by a control experiment using a model compound $R_1$ that lacks −OH functionality. In the presence of Al$^{3+}$/Hg$^{2+}$, the emission spectra of $R_1$ are significantly different (Figures S28, S29, Supporting Information) while their QTOF–MS spectra support the formation of the adduct (Figures S30, S31, Supporting Information). On the basis of these facts, a probable interaction mechanism of RHES with Al$^{3+}$, Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$ is shown in Scheme 2.

As far as the emission profile is concerned (Figure S32, Supporting Information), Zn$^{2+}$/Cd$^{2+}$ do not show any significant interaction with $R_1$, suggesting the necessity and involvement of the phenol −OH for their sensing. For a deeper understanding of the binding mode of RHES and to strengthen the proposed sensing mechanism, $^1$H NMR titration has been conducted in dimethyl sulfoxide (DMSO)-d$_6$. After the addition of 1 equiv Al$^{3+}$/Hg$^{2+}$ to RHES, the alkane protons downfield-shifted from 3.45 to 3.50 ppm, indicating the interaction of the adjacent O donors with the cations. Upon the addition of more Al$^{3+}$/Hg$^{2+}$ (2.0 and 3.0 equiv), these protons further downfield-shifted to 3.54 ppm. Moreover, the ring protons upfield-shifted from 7.0 to 6.95 ppm, which indicates the opening of the spirolactam ring. Interestingly, after the addition of 1 equiv Al$^{3+}$/Hg$^{2+}$ to RHES, the “p” proton downfield-shifted from 8.6 to 10.2 ppm (Al$^{3+}$) and 10.3 ppm (Hg$^{2+}$) because of the close proximity of the rhodamine B unit through the Al$^{3+}$/Hg$^{2+}$-induced folding of RHES. A further addition of Al$^{3+}$/Hg$^{2+}$ (2.0 and 3.0 equiv) shifts...
"p" proton more downfield. The position of the "v" proton of RHES remains unchanged upon the addition of Hg^{2+}, indicating the noninteraction of the −OH group with Hg^{2+}, whereas it shifts downfield from 12.01 to 12.44 for Al^{3+}, confirming the interaction of the −OH group with Al^{3+} (Figures S33, S34, Supporting Information).

Similarly, the addition of 1 equiv of Zn^{2+}/Cd^{2+} to RHES shifts the alkane protons downfield from 3.5 to 3.6 ppm, indicating the interaction of the adjacent O donors to the metal ions. These protons are further downfield-shifted upon the addition of more Zn^{2+}/Cd^{2+} (2.0 and 3.0 equiv). Moreover, the CH=NH proton ("p" proton) is downfield-shifted from 8.75 to 10.25 ppm, whereas the "v" proton shifts downfield from 12.0 to 12.37, indicating the involvement of the −OH group to Zn^{2+}/Cd^{2+} binding (Figures S35, S36, Supporting Information).

To further support the sensing mechanism, fluorescence lifetime measurement is carried out. The average lifetime (τ) of RHES is 0.3852 ns, whereas the corresponding values for RHES−Al^{3+} (λ_{Em} = 573 nm) and RHES−Hg^{2+} (λ_{Em} = 577 nm) systems are 1.7497 and 1.7257 ns, respectively. The τ values for the RHES−Zn^{2+} (λ_{Em} = 482 nm) and RHES−Cd^{2+} (λ_{Em} = 395 nm) systems are 1.2791 and 1.5333 ns, respectively (Table S1, Supporting Information). The τ value of [RHES−Al^{3+}] at 362 nm is 0.8399 ns (Table S1, Figure S37, Supporting Information). The fluorescence lifetime decay curves along with data fitting are shown in Figure 7.

Interestingly, the higher affinity and association constant of RHES for Cd^{2+} allows easy replacement of Zn^{2+} from the [RHES−Zn^{2+}] adduct to form a more stable [RHES−Cd^{2+}] adduct (Scheme 3). Thus, the [RHES−Zn^{2+}] adduct turns out to be a better Cd^{2+} sensor that functions via the displacement interaction.
approach. The gradual addition of Cd\(^{2+}\) to the [RHES\(\text{–Zn}^{2+}\)] system results in a blue shift of the 482 nm emission band to 395 nm along with a fluorescence enhancement (Figure 8).

It is worth mentioning that the simultaneous presence of Al\(^{3+}\) and Hg\(^{2+}\) can be visualized (Scheme 4) using KI to mask Hg\(^{2+}\), while KI does not interfere with the emission profile of RHES (Figure S38, Supporting Information). This fact is also supported from the mass spectrum of the resulting mixture (Figure S39, Supporting Information). Figure S40 (Supporting Information) pictorially represents the reversibility of the probe toward Hg\(^{2+}\) detection. After the addition of the mixture of Al\(^{3+}\) and Hg\(^{2+}\) to RHES, the characteristic emission of the [RHES\(\text{–Hg}^{2+}\)] adduct at 577 nm is observed, attributed to the higher affinity of Hg\(^{2+}\) for RHES. The higher binding constant of [RHES\(\text{–Hg}^{2+}\)] over [RHES\(\text{–Al}^{3+}\)] also supports the observation (Figures S8, S23, Supporting Information). Figure S41 (Supporting Information) also indicates the formation of the [RHES\(\text{–Hg}^{2+}\)] adduct (m/z, 1007.86) when the mixture of Al\(^{3+}\) and Hg\(^{2+}\) is added to RHES. Moreover, after the addition of \(\Gamma\) to this system, the emission shifts to 573 nm, which is characteristic of the [RHES\(\text{–Al}^{3+}\)] adduct. After the addition of \(\Gamma\), it captures Hg\(^{2+}\) while only Al\(^{3+}\) binds to RHES, showing an emission at 573 nm. The \(\Gamma\)-assisted reversible interaction between the probe RHES and Hg\(^{2+}\) is also established from the \(^1\)H NMR titration (Figure S42, Supporting Information). Upon the addition of 1 equiv Hg\(^{2+}\) to the [RHES\(\text{–Al}^{3+}\)] adduct, the "V" proton of RHES upfield-shifted from 12.34 to 12.15 ppm, indicating the noninteraction of the –OH group with Hg\(^{2+}\) and the replacement of Al\(^{3+}\) by Hg\(^{2+}\). Upon further addition of \(\Gamma\) (1 equiv) to the resulting solution, the "V" proton is downfield-shifted from 12.15 to 12.34 ppm, indicating the interaction of the –OH group of RHES. This is due to the
interaction of Al$^{3+}$ present in the system with the free RHES. The formation of this [RHES$-$Al$^{3+}$] adduct is also demonstrated from its Fourier transform infrared (FTIR) spectrum (Figure S43, Supporting Information).

Table S2 (Supporting Information) compares the present probe with the available probes in the literature, although it is not truly a comparison because none of the available single probes can detect all the four cations simultaneously as demonstrated in the present report.

To obtain the energy-optimized geometries of RHES and its Al$^{3+}$, Hg$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$ adducts (Figure S44, Supporting Information), density functional theoretical (DFT) calculations$^{57}$ have been performed. The Gaussian-09 revision C.01 program package is used for all calculations. The geometries in gas phase are fully optimized without any restrictions of symmetry in singlet ground states for RHES and its adducts, viz. [Al(RHES)(NO$_3$)$_3$], [Zn(RHES)$\cdot$(CH$_3$CH$_2$OH)$_2$(CH$_3$OH)(CH$_3$COO)], and [Hg(RHES)(NO$_3$)]$^+$, along with the gradient-corrected DFT level with the three-parameter fit of exchange and correlation functional of Becke (B3LYP), which includes the correlation functional of Lee, Yang and Parr (LYP)$^{57b}$ The basis set LanL2DZ along with an effective core potential is employed for Al, Zn, Cd, and Hg atoms following the associated valence double-$\zeta$ basis set of Hay and Wadt$^{57c}$ along with the 6-31++G** basis set, chosen for hydrogen, carbon, nitrogen and oxygen. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of RHES and its adducts are shown in Figure S45 (Supporting Information). It is clear that the Al$^{3+}$, Hg$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$ adducts are stabilized by 0.06227, 0.03886, 0.0721, and 0.07823 eV, respectively. In [RHES$-$Al$^{3+}$] and [RHES$-$Hg$^{2+}$], the HOMOs are found to be majorly localized on the rhodamine moiety, whereas these are localized on the aldehyde moiety in the

Figure 8. (a) Changes in the fluorescence spectra of the [RHES$-$Zn$^{2+}$] adduct upon the addition of Cd$^{2+}$; (b) changes in the absorbance spectra of the [RHES$-$Zn$^{2+}$] adduct upon the addition of Cd$^{2+}$.

Scheme 4. Role of the Masking Agent (KI) for Simultaneous Detection of Al$^{3+}$ and Hg$^{2+}$
case of the [RHES–Zn²⁺] and [RHES–Cd²⁺] adducts. The corresponding LUMOs are found to be minorly localized on the rhodamine moiety. Time-dependent DFT (TDDFT) calculations have been performed on the excited state based on the optimized ground-state geometry in the Conductor-like Polarizable Continuum Model (CPCM). The electronic transition energies are calculated by the TDDFT method in methanol and gas phase (Tables S3–S7, Supporting Information). The outcomes are in good agreement with our experimental data. The results reveal that the energy gap between HOMO and LUMO of its adducts is reasonably lower than that of the free RHES, leading to a more stable species.

Considering the emission wavelengths of Zn²⁺ and Cd²⁺ and the displacement of Zn²⁺ by Cd²⁺, a binary logic gate has been constructed. There are two input signals, viz. input X (Zn²⁺) and input Y (Cd²⁺), whereas the output signals correspond to the OR and NOT gates along with a switch that describes the situation clearly. When the input is applied through an individual, both ions follow the OR gate, and when both Zn²⁺/Cd²⁺ are the inputs, the pathway is changed (Figure 9).

Fluorescence microscopic images of squamous epithelial cells after incubation for 2 h (100× objective lens): (a) RHES (20 μM); (b) RHES (20 μM) + Zn²⁺ (30 μM); (c) RHES (20 μM) + Cd²⁺ (30 μM); (d) RHES (20 μM) + Hg²⁺ (30 μM).

Figure 10. Fluorescence microscopic images of squamous epithelial cells after incubation for 2 h (100× objective lens): (a) RHES (20 μM); (b) RHES (20 μM) + Zn²⁺ (30 μM); (c) RHES (20 μM) + Cd²⁺ (30 μM); (d) RHES (20 μM) + Hg²⁺ (30 μM).

The cells are washed with phosphate-buffered saline (PBS) (×2), and after washing with PBS (×3), the remaining compounds are removed, and the cells are incubated with the said ions and observed under a fluorescence microscope equipped with a UV filter at ambient temperature. No fluorescence is observed in the cells (Figure 10, RHES) that are not previously exposed to the metal ions, whereas after the addition of the said ions, the nonfluorescence gradually turns green with time for Zn²⁺, sky blue for Cd²⁺, and red for Hg²⁺. Moreover, RHES can easily permeate through the tested living cells without causing any harm (as the cells remain alive even after 2 h of exposure to RHES). The cells treated only with the said ions have no fluorescence. This intracellular imaging clearly shows that RHES has good cell permeability and is efficient for imaging of the said cations.

Application. Real Sample Analysis. To evaluate the practical feasibility of RHES for the determination of Al³⁺, Zn²⁺, Cd²⁺, and Hg²⁺, analyses of the real samples have been performed. The results are summarized in Tables 1–4, respectively. Table 1 indicates the excellent recovery of metal ions from both water and the antacid samples, opening a new avenue for the

Table 1. Determination of Al³⁺ in Real Samples

| sample | Al³⁺ added (μM) | emission intensity (au) | Al³⁺ found (μM) | RSD (%) | recovery (%) |
|--------|----------------|------------------------|----------------|---------|--------------|
| drinking water | 10 | 423 | 9.89 | 1.7 | 98 |
| industrial water | 451 | 10.7 | 1.8 |
| antacid suspension | 12 | 561 | 11.71 | 1.7 | 97 |

Table 2. Determination of Hg²⁺ in Sea Fish Samples

| sample no. | fish name | dry weight of the samples | Hg²⁺ (μg/g) present | emission intensity (au) | RDS (%) |
|------------|-----------|--------------------------|---------------------|------------------------|---------|
| S1 | Subgenus Thunnus | 1.285 | 0.381 | 67 | 1.6 |
| S2 | Eleutheronema tetradactylum | 0.326 | 0.11 | 35 | 1.7 |
| S3 | Loligo duvauceli | 1.321 | 0.11 | 35 | 1.7 |
| S4 | Siganus sp. | 0.3536 | 0.291 | 51 | 1.8 |
| S5 | Johniiceps vogleri | 1.21 | 0.11 | 35 | 1.7 |
| S6 | Stolephorus indicus | 0.571 | 0.239 | 56 | 1.7 |
| S7 | Pampus argenteus | 0.826 | 0.381 | 67 | 1.6 |
| S8 | Parupeneus indicus | 0.467 | 0.167 | 41 | 1.9 |
Table 3. Determination of Zn$^{2+}$ in Real Samples

| sample no. | fish name       | dry weight of the samples (g) | Cd$^{2+}$ found (μg/g) | RDS (%) | recovery (%) |
|------------|-----------------|-------------------------------|-----------------------|---------|--------------|
| drinking water |                |                               |                       |         |              |
| industrial water |                |                               |                       |         |              |
| antacid suspension |            |                               |                       |         |              |

Table 4. Determination of Cd$^{2+}$ in Sea Fish Samples

| sample no. | fish name       | dry weight of the samples (g) | Cd$^{2+}$ found (μg/g) | RDS (%) |
|------------|-----------------|-------------------------------|-----------------------|---------|
| S1         | Stethus           |                               | 1.375                 | 0.381   | 51           |
| S2         | Eleutheronema     |                               | 0.237                 |         |              |
| S3         | Loligo           |                               | 1.323                 | 0.176   | 44           |
| S4         | Setipinnia       |                               | 0.273                 | 0.094   | 21           |
| S5         | Johnieops         |                               | 1.216                 | 0.264   | 32           |
| S6         | Stolephorus       |                               | 0.579                 | 0.291   | 40           |
| S7         | Pampus            |                               | 0.827                 | 0.182   | 28           |
| S8         | Parupeneus        |                               | 0.557                 | 0.161   | 31           |

The synthesis, characterization, and application of a very simple fluorescence and colorimetric probe, RHERS, is described. RHERS is exploited for the detection of nanomolar Al$^{3+}$, Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$ ions in a ratiometric manner involving the PET–CHEF–FRET processes. The method allows their bare eye visualization at a physiological pH. It detects as low as 1.5 × 10$^{-10}$ M Al$^{3+}$, 1.2 × 10$^{-7}$ M Zn$^{2+}$, 6.7 × 10$^{-9}$ M Cd$^{2+}$, and 1.7 × 10$^{-16}$ M Hg$^{2+}$. Furthermore, RHERS successfully images the cations that belong to the same group of the modern periodic table, viz. Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$, in living cells. The developed method is useful for the determination of the said ions in real and sea fish samples. RHERS, a multi-ion sensor, has been used to construct a binary logic gate. The DFT studies support the experimental findings.

**Materials and Methods.** High-purity HEPES buffer, rhodamine B, 3-ethoxysalicylaldehyde, 4-formylbenzonitrile, and 2-[2-(2-amino-ethoxy)-ethoxy]-ethanol have been purchased from Sigma-Aldrich (India). Hg(NO$_3$)$_2$, H$_2$O, Al(NO$_3$)$_3$, H$_2$O, Zn(NO$_3$)$_2$, H$_2$O, and Cd(AcO)$_2$·4H$_2$O are purchased from Merck (India). Spectroscopic grade solvents are filtered, washed with water, and dried under vacuum, and assigned as RHE, 2-[2-(2-aminoethoxy)ethoxy]ethyl]-3′,6′-bis(diethylaminospiro-[isodindole-1′,9′-xanthen]-3-one (yield: 95.8% yield). Analysis. Calcul (C): 71.31; H: 7.74; and N: 9.78. Found: C: 72.05; H: 7.92; and N: 9.25. QTOF–MS ES$^+$ (Figure S48, Supporting Information). The amounts of unknown Al$^{3+}$ and Zn$^{2+}$ have been determined.

**Synthesis of RHERS.** 2-[2-(2-aminoethoxy)-ethoxy]-ethanol (7.0 mL, 12.42 mmol, $\rho = 1.015$ g mL$^{-1}$) is added dropwise to 1.1 g rhodamine B (2.29 mmol) in methanol (30 mL) under stirring condition at room temperature. The mixture is refluxed for 4 days at 60 °C (Scheme 1). The solvent is removed under reduced pressure using a rotary evaporator. Then, HCl (1 mol L$^{-1}$) is added until the solution becomes clear. The pH of the solution is adjusted to 9–10 using NaOH (1 mol L$^{-1}$). A red precipitate that appears is filtered, washed with water, dried under vacuum, and assigned as RHE, 2-[2-(2-aminoethoxy)ethoxy]ethyl]-3′,6′-bis(diethylaminospiro-[isodindole-1′,9′-xanthen]-3-one (yield: 95.8%). Anal. Calcd (C): 71.31; H: 7.74; and N: 9.78. Found: C: 72.05; H: 7.92; and N: 9.25. QTOF–MS ES$^+$ (Figure S48, Supporting Information).
Information): \[ [M + H]^{+} = 573.36 \ (\sim 100\%). \] FTIR (cm\(^{-1}\)) (Figure S49, Supporting Information): \( \nu (N-H, 1^{\text{st}} \text{ amine}) 3289.23; \nu (C-H, aromatic) 2967.21, 2869.56; \nu (C=O, carbonyl, rhodamine spirolactum ring) 1673.81; \nu (C=C, aromatic) 1515.22; \nu (C=C) 1456.23; \nu (C-N, carbonyl group) 1389.69; \nu (C-O, xanthan ring) 1232.10, 1098.78.

**Synthesis of RHES.** The probe, RHES, has been synthesized by refluxing the equimolar mixture of RHE (1.03 g, 1.80 mmol) and 3-ethoxysalicylaldehyde (0.30 g, 1.80 mmol) in methanol for 6 h at 60 °C (Scheme 1). The red gel obtained after the evaporation of the solvent is assigned as RHES, (E)-3′,6′-bis(diethylamino)-2-(2-(2-((2-hydroxy-3-(1H-xanthene)-1,9′-xanthen)-3-one (0.60 g, yield: 96%). Anal. Calcld (%): C, 71.64; H, 7.27; and N, 7.77. Found: C, 71.95; H, 7.37; and N, 7.02. QTOF-MS ES\(^{+}\) (Figure S50, Supporting Information): \([M + H]^{+} = 721.93 \ (\sim 40\%) \text{ and } [M + Na]^{+} = 743.91 \ (\sim 100\%).\]

**Synthesis of the \([\text{RHES} - \text{Zn}^{2+}]\) Adduct.** The methanol solution of Zn(NO\(_3\))\(_2\)-6H\(_2\)O (0.3 g, 1.01 mmol) is added dropwise to a magnetically stirred solution of RHES (0.73 g, 1.01 mmol) in methanol at room temperature. Upon the removal of the solvent by slow evaporation, a light yellow solid appears, which is assigned as the \([\text{RHES} - \text{Zn}^{2+}]\) adduct (yield, 85%). Anal. Calcld (%): C, 59.53; H, 6.95; and N, 7.21. Found: C, 59.12; H, 6.75; and N, 7.21. QTOF-MS ES\(^{+}\) (Figure S60, Supporting Information): \(m/z\) 992.03 is assigned to \([\text{RHES} + \text{Zn}^{2+} + 2\text{CH}_3\text{CH}_2\text{OH} + \text{CH}_3\text{OH} + \text{H}^+\] \(\sim\) (100%), indicating a 1:1 (mole ratio) stoichiometry between RHES and Zn\(^{2+}\), whereas \(m/z\) 1123.04 is assigned to \([\text{RHES} + 2\text{Zn}^{2+} + 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}]^+\) \(\sim\) (40%), indicating a 1:2 (mole ratio) stoichiometry between RHES and Zn\(^{2+}\). FTIR (cm\(^{-1}\)) (Figure S61, Supporting Information): \(\nu (C=H) = 2970.38; \nu (C=O, carbonyl) 1739.19; \nu (C=O, imine bond) 1614.42; \nu (C=C, aromatic) 1377.17; \nu (C-N, attached with the carbonyl group) 137.39; and (C-O, stretch) 1217.08. UV-vis (Figure S62, Supporting Information): \(\lambda (nm)\) in MeOH/H\(_2\)O, 4/1, v/v (\( \epsilon\), M\(^{-1}\) cm\(^{-1}\)): 314 nm (5.6 \times 10^5), 273 nm (7.5 \times 10^5). The excitation spectrum (\(\lambda_{\text{exc}}\) 482 nm) is presented in Figure S62 (Supporting Information).

**Synthesis of the \([\text{RHES} - \text{Cd}^{2+}]\) Adduct.** The methanol solution of Cd(NO\(_3\))\(_2\)-4H\(_2\)O (0.3 g, 0.7 mmol) is added dropwise to a magnetically stirred solution of RHES (0.38 g, 0.7 mmol) in methanol at room temperature. A slow evaporation of the solvent yielded a gray solid, ascribed as the \([\text{RHES} - \text{Cd}^{2+}]\) adduct (yield, 82%). Anal. Calcld (%): C, 52.51; H, 5.33; and N, 7.12. Found: C, 52.93; H, 5.12; and N, 7.02. QTOF-MS ES\(^{+}\) (Figure S63, Supporting Information): \(m/z\) 1020.15 is assigned to \([\text{RHES} + \text{Cd}^{2+} + 2\text{CH}_3\text{COO}^- + \text{CH}_3\text{CH}_2\text{OH} + \text{H}^+\] \(\sim\) (100%), indicating a 1:1 (mole ratio) stoichiometry between RHES and Cd\(^{2+}\), whereas \(m/z\) 1170.21 is assigned to \([\text{RHES} + 2\text{Cd}^{2+} + 2\text{CH}_3\text{COO}^- + \text{CH}_3\text{O}^- + 3\text{H}_2\text{O} + \text{H}^+\] \(\sim\) (50%), confirming a 1:2 (mole ratio) stoichiometry between RHES and Cd\(^{2+}\). FTIR (cm\(^{-1}\)) (Figure S64, Supporting Information): \(\nu (C=H) = 2970.38; \nu (C=O, carbonyl) 1737.86; and \nu (C-O) 1217.08. UV-vis (Figure S18, Supporting Information): \(\lambda (nm)\) in MeOH/H\(_2\)O, 4/1, v/v (\( \epsilon\), M\(^{-1}\) cm\(^{-1}\)): 273 nm (5.6 \times 10^5), 311 nm (7.5 \times 10^5). The excitation spectrum (\(\lambda_{\text{exc}}\) 395 nm) is presented in Figure S65 (Supporting Information).

**Synthesis of the \([\text{RHES} - \text{Hg}^{2+}]\) Adduct.** The methanol solution of Hg(NO\(_3\))\(_2\)-6H\(_2\)O (0.3 g, 0.7 mmol) is added dropwise to a magnetically stirred solution of RHES (0.49 g, 0.7 mmol) in methanol at room temperature. A slow evaporation of the solvent produced bloodred solid (yield, 91%). Anal. Calcld (%): C, 52.51; H, 5.33; and N, 7.12. Found: C, 52.93; H, 5.12; and N, 7.02. QTOF-MS ES\(^{+}\) (Figure S66, Supporting Information): \(m/z\) 1007.77 is assigned to \([\text{RHES} + \text{Hg}^{2+} + \text{NO}_3^- + \text{Na}^+]\) \(\sim\) (100%), indicating a 1:1 (mole ratio) stoichiometry between RHES and Hg\(^{2+}\). FTIR (cm\(^{-1}\)) (Figure S67, Supporting Information): \(\nu (C=H) = 2980.02; \nu (C=O, carbonyl) 1672.28; \nu (C=N) 1377.17; and \nu (C-O, attached with the carbonyl group) 1303.88, 1072.42. UV-vis (Figure S68, Supporting Information): \(\lambda (nm)\) in MeOH/H\(_2\)O, 4/1, v/v (\( \epsilon\), M\(^{-1}\) cm\(^{-1}\)): 555 nm (5.6 \times 10^6). The excitation spectrum (\(\lambda_{\text{exc}}\) 577 nm) is presented in Figure S68 (Supporting Information).
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00266.

1H NMR, MS spectra, and fluorescence lifetime data of the probe and its adducts with the analytes; experimental details of different binding constants; LOD plots; interference studies; optimized structures of the probe and its adducts with the corresponding analytes; and calculated bond lengths and angles (PDF)

Notes
The authors declare no competing financial interest.

Acknowledgments
M.G. and M.M. gratefully acknowledge CSIR and UGC (New Delhi) for the financial support, whereas S.T. acknowledges UGC-DAE-Kolkata for funding. M.B. sincerely acknowledges B.U. for the fellowship. The authors also thank Dr. A. Ghosh, Department of Chemistry, University of Calcutta, Kolkata, for NMR and cell imaging studies.

References
(1) Chatterjee, A.; Santra, M.; Won, N.; Kim, S.; Kim, J. K.; Kim, S. B.; Ahn, K. H. Selective fluorogenic and chromogenic probe for detection of silver ions and silver nanoparticles in aqueous media. J. Am. Chem. Soc. 2009, 131, 2040–2041.

(2) Ni, Y.; Wu, J. Far-red and near infrared BODIPY dyes: synthesis and applications for fluorescent pH probes and bio-imaging. Org. Biomol. Chem. 2014, 12, 3774–3791.

(3) Ohashi, A.; Ito, H.; Kanai, C.; Imura, H.; Ohashi, K. Cloud point extraction of iron(III) and vanadium(V) using 8-quinolinol derivatives and Triton X-100 and determination of 10(7) mol/dm(-3) level iron(III) in riverine water reference by a graphite furnace atomic absorption spectroscopy. Talanta 2005, 65, 525–530.

(4) Liang, Z.-Q.; Wang, C.-X.; Yang, J.-X.; Gao, H.-W.; Tian, Y.-P.; Tao, X.-T.; Jiang, M.-H. A highly selective colorimetric chemosensor for detecting the respective amounts of iron (II) and iron (III) ions in water. New J. Chem. 2007, 31, 906–910.

(5) Lunvongsa, S.; Oshima, M.; Motomizu, S. Determination of total and dissolved amount of iron in water samples using catalytic spectrophotometric flow injection analysis. Talanta 2006, 68, 969–973.

(6) Tesfalet, Z. O.; van Staden, J. F.; Stefan, R. I. Sequential injection spectrophotometric determination of iron as Fe(II) in multi-vitamin preparations using 1,10-phenanthroline as complexing agent. Talanta 2004, 64, 1189–1195.

(7) Gomes, D. M. C.; Segundo, M. A.; Lima, J. L. F. C.; Rangel, A. O. S. S. Spectrophotometric determination of iron and boron in soil extracts using a multi-syringe flow injection system. Talanta 2005, 66, 703–711.

(8) Bobrowski, A.; Nowak, K.; Zarebski, J. Application of a bismuth film electrode to the voltammetric determination of trace iron using a Fe(III)–TEA–BrO(3) catalytic system. Anal. Bioanal. Chem. 2005, 382, 1691–1697.

(9) (a) Nandi, S.; Das, D. Smart Probe for Multianalyte Signaling: Solvent Dependent Selective Recognition of I-. ACS Sens. 2016, 1, 81–87. (b) Zhang, L.; Huang, X.; Cao, Y.; Xin, Y.; Ding, L. Fluorescent Binary Ensemble based on Pyrene Derivative and SDS Assemblies as a Chemical Tongue for Discriminating Metal Ions and Brand Water. ACS Sens. 2017, 2, 1821–1830. (c) Li, S.; Zhang, D.; Xie, X.; Ma, S.; Liu, Y.; Xu, Z.; Gao, Y.; Ye, Y. A novel solvent-dependently bifunctional NIR absorptive and fluorescent ratiometric probe for detecting Fe(3+)/Cu(2+) and its application in bioimaging. Sens. Actuators B 2016, 224, 661–667.

(10) (a) Roat-Malone, R. M.; John, W.; Jersey, J. N. Bioinorganic Chemistry: A Short Course; John Wiley & Sons, Inc., 2002; pp 1–3. (b) Kabat, G. C.; Rohan, T. E. Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis. Cancer Causes Control 2007, 18, 1047–1053.

(11) Mason, W. T. Fluorescent and Luminescent Probes for Biological Activity, 2nd ed.; Mason, W. T., Ed.; Academic Press: London, 1999; p 175.

(12) (a) Kawanishi, Y.; Kikuchi, K.; Takakusa, H.; Mizukami, S.; Urano, Y.; Higuchi, T.; Nagano, T. Design and Synthesis of Intramolecular Resonance-Energy Transfer Probes for Use in Ratiometric Measurements in Aqueous Solution. Angew. Chem., Int. Ed. 2000, 39, 3438–3440. (b) Tang, J.; Ma, S.; Zhang, D.; Liu, Y.; Zhao, Y.; Ye, Y. Highly sensitive and fast responsive ratiometric fluorescent probe for Cu2+ based on a napthalimide-thiodamine dye and its application in living cell imaging. Sens. Actuators B 2016, 236, 109–115.

(13) Fan, J.; Hu, M.; Zhan, P.; Peng, X. Energy transfer cassettes based on organic fluorophores: construction and applications in ratiometric sensing. Chem. Soc. Rev. 2013, 42, 29–43.

(14) Robinson, G. H. Aluminium. Chem. Eng. News 2003, 81, 54.

(15) Scerri, E. R. The Periodic Table: Its Story and Its Significance; Oxford University, 2007; pp 1–329.

(16) (a) Exley, C. Aluminium: Lithosphere to Biosphere (and Back) Sixth Keele Meeting on Aluminium. 26th February to 2nd March 2005, Sixth Keele Meeting on Aluminium. J. Inorg. Biochem. 2005, 99, 1747–1928. (b) Ding, P.; Wang, J.; Cheng, J.; Zhao, Y.; Ye, Y. Three N-stabilization rhodamine-based fluorescent probes for Al3+ via Al3+-promoted hydrolysis of Schiff base. New J. Chem. 2015, 39, 342–348.

(17) Altschuler, E. Aluminium-containing antacids as a cause of idiopathic Parkinson’s disease. Med. Hypotheses 1999, 53, 22–23.

(18) Wang, B.; Xing, W.; Zhao, Y.; Deng, X. Effects of chronic aluminum exposure on memory through multiple signal transduction pathways. Environ. Toxicol. Pharmacol. 2010, 29, 308–313.

(19) Walton, J. R. Aluminium in hippocampal neurons from humans with Alzheimer’s disease. NeuroToxicology 2006, 27, 385–394.

(20) Polizzi, S. Neurotoxic Effects of Aluminium Amongst Foundry Workers and Alzheimer’s Disease. NeuroToxicology 2002, 23, 761–774.

(21) Dhara, A.; Jana, A.; Ghosh, N.; Ghosh, P.; Kar, S. K. Rhodamine-based molecular dips for highly selective recognition of Al3+ ions: synthesis, crystal structure and spectroscopic properties. New J. Chem. 2014, 38, 1627–1634.

(22) Sahana, A.; Banerjee, A.; Das, S.; Lohar, S.; karak, D.; Sarkar, B.; Mukhopadhyay, S. K.; Mukherjee, A. K.; Das, D. A naphthalene-based Al(iii) selective fluorescent sensor for living cell imaging. Org. Biomol. Chem. 2011, 9, 5523–5529.

(23) Delhaize, E.; Ryan, P. R. Aluminum Toxicity and Tolerance in Plants. Plant Physiol. 1995, 107, 315–321.

(24) Vallee, B. L.; Galdes, A. The metallobiology of zinc enzymes. Adv. Enzymol. Relat. Areas Mol. Biol. 1984, 56, 283–430.

(25) (a) Vallee, B. L. Introduction to metallothionein. Methods Enzymol. 1991, 205, 3–7. (b) Matrix Metalloproteinases and Inhibitors; Birkedal-Hansen, H., Werb, Z., Welsg, H., Van Wart, H., Eds; Gustav Fischer: Stuttgart, Germany, 1992; pp 5–19.

(26) Vallee, B. L.; Auld, D. S. Zinc: biological functions and coordination motifs. Acc. Chem. Res. 1993, 26, 543–551.

(27) (a) Hambidge, M.; Krebs, N. Zinc, diarrhea, and pneumonia. J. Pediatr. 1999, 135, 661–664. (b) Ghosh, M.; Ghosh, A.; Ta, S.; Matalobos, J. S.; Das, D. ESP-TBP-Based Nanomolar Zn2+ Sensor for Human Breast Cancer Cell (MCF7) Imaging. ChemistrySelect 2017, 2, 7426–7431. (c) Kumar, B.; Lohar, S.; Ghosh, M.; Ta, S.; Sengupta, A.; Banerjee, P. P.; Chattopadhyay, A.; Das, D. Structurally Characterized Zn2+ Selective Ratiometric Fluorescence Probe in 100% Water for HeLa Cell Imaging: Experimental and Computational Studies. J. Fluoresc. 2016, 26, 87–103. (d) Ghosh, A.; Ta, S.; Ghosh, M.; Karmakar, S.; Banik, A.; Dangar, T. K.; Mukhopadhyay, S. K.; Das, D. Dual mode...
(28) (a) Vallely, B. L.; Auld, D. S. Active-site zinc ligands and activated \( \text{H}_2\text{O} \) of zinc enzymes. *Proc. Natl. Acad. Sci. U.S.A.* 1990, 87, 220–224. (b) Vallely, B. L.; Auld, D. S. Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* 1990, 29, 5647–5659. (c) Vallely, B. L.; Auld, D. S. Cootactic zinc motifs in enzyme catalysis. *Proc. Natl. Acad. Sci. U.S.A.* 1993, 90, 2715–2718. (d) Vallely, B. L.; Auld, D. S. New perspective on zinc biochemistry: Cootactic sites in multinuclear zinc enzymes. *Biochemistry* 1993, 32, 6493–6500. (e) Vallely, B. L.; Falchuk, K. H. The biochemical basis of zinc physiology. *Physiol. Rev.* 1993, 73, 79–118.

(29) (a) Fredericksen, C. J.; Koh, J.-Y.; Bush, A. I. The neurobiology of zinc in health and disease. *Nat. Rev. Neurosci.* 2005, 6, 449–462. (b) Que, E. L.; Domaille, D. W.; Chang, C. J. Metals in neurobiology: probing their chemistry and biology with molecular imaging. *Chem. Rev.* 2008, 108, 1517–1549.

(30) Emmari, S.; Hosseinimehr, S. J.; Taghdisi, S. M.; Akhlaghpoor, S. Kojic acid and its manganese and zinc complexes as potential radioprotective agents. *Bioorg. Med. Chem. Lett.* 2017, 27, 45–48.

(31) Huang, Q.; Pan, Z.; Wang, P.; Chen, Z.; Zhang, X.; Xu, H. Zinc(II) complexes with \( \beta \)-substituted hydroxylporphyrins as tumor photosensitizers. *Bioorg. Med. Chem. Lett.* 2006, 16, 3030–3033.

(32) (a) Nakayama, A.; Hiromura, M.; Adachi, Y.; Sakurai, H. Molecular mechanism of anti diabetic zinc–allixin complexes: regulations of glucose utilization and lipid metabolism. *J. Biol. Inorg. Chem.* 2008, 13, 675–684. (b) Sakurai, H.; Yoshikawa, Y.; Yasui, H. Current state for the development of metallopharmaceutics and anti-diabetic metal complexes. *Chem. Soc. Rev.* 2008, 37, 2383.

(33) (a) Chohan, Z. H.; Arif, M.; Sarfraz, M. Metal-based antibacterial and antifungal amino acid derived Schiff bases: their synthesis, characterization and in vitro biological activity. *Appl. Organomet. Chem.* 2007, 21, 294–302. (b) Katiyar, A.; Singh, V. P. Synthesis, structural studies and bio-activity of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes with \( \beta \)-aminocetophenone salicyloyl hydrazine. *J. Coord. Chem.* 2008, 61, 3200–3212. (c) Kaczmarek, M. T.; Jastrzębski, R.; Holdkarna-Kędzia, E.; Radecka-Paryzek, W. Self-assembled synthesis, characterization and antimicrobial activity of zinc(II) salicyaldehyde dimine complexes. *Inorg. Chem. Acta* 2009, 362, 3127–3133. (d) Anbu, S.; Kamalraj, S.; Varghese, B.; Muthumary, J.; Kandaveloo, M. A Series of Oxime-based Macrocyclic Dinuclear Zinc(II) Complexes Enhances Phosphate Ester Hydrolysis, DNA Binding, DNA Hydrolysis, and Lactate Dehydrogenase Inhibition and Induces Apoptosis. *Inorg. Chem.* 2012, 51, 5580–5592.

(34) Ali, M. M.; Frei, E.; Straub, J.; Breuer, A.; Wiessler, M. Induction of metallothionein by zinc protects from daunorubicin toxicity in rats. *Toxicology* 2002, 179, 85–93.

(35) Dau, Z.; Canary, J. W. Tailoring tripod ligands for zinc sensing. *New J. Chem.* 2007, 31, 1708–1718.

(36) Jobe, K.; Brennan, C. H.; Morevall, M.; Goldup, S. M.; Workentin, M. Modular “click” sensors for zinc and their application in vivo. *Chem. Commun.* 2011, 47, 6036–6038.

(37) Androssov, D. A.; Kishbaugh, T. L. S.; Griible, G. W. Mn (III)-based radical addition reactions of 2-nitroindole with activated CH compounds. *Tetrahedron Lett.* 2008, 49, 6621–6623.

(38) Cuajungco, M. P.; Lees, G. J. Zinc metabolism in the brain: relevance to human neurodegenerative disorders. *Neurobiol. Dis.* 1997, 4, 137–169.

(39) Koh, J.-Y.; Suh, S. W.; Gwag, B. J.; He, Y. Y.; Hsu, C. Y.; Choi, D. W. The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science* 1996, 272, 1013–1016.

(40) Fredericksen, C. J.; Hernandez, M. D.; McGinty, J. F. Translocation of zinc may contribute to seizure-induced death of neurons. *Brain Res.* 1989, 480, 317–321.

(41) Küry, S.; Dréno, B.; Bézieau, S.; Giraudet, S.; Kharfi, M.; Kamoun, R.; Moisan, J.-P. Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. *Nat. Genet.* 2002, 31, 239–240.
16761. (b) Yang, H.; Zhou, Z.; Huang, K.; Yu, M.; Li, F.; Yi, T.; Huang, C. Multi-signaling optical-electrochemical sensor for Hg$^{2+}$ based on a rhodamine derivative with a ferrocene unit. Org. Lett. 2007, 9, 4729–4732. (c) Lee, M. H.; Wu, J.-S.; Lee, J. W.; Jung, J. H.; Kim, J. S. Highly Sensitive and Selective Chemosensor for Hg$^{2+}$ Based on the Rhodamine Fluorophore. Org. Lett. 2007, 9, 2501–2504. (d) Shi, W.; Ma, H. Rhodamine B thiolactone: a simple chemosensor for Hg$^{2+}$ in aqueous media. Chem. Commun. 2008, 1856–1858. (e) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. Switching the Recognition Preference of Rhodamine B Spirolactam by Replacing One Atom: Design of Rhodamine B Thiohydrazide for Recognition of Hg(II) in Aqueous Solution. Org. Lett. 2006, 8, 859–861. (f) Saha, S.; Mahato, P.; Baidya, M.; Ghosh, S. K.; Das, A. An interrupted PET coupled TBET process for the design of a specific receptor for Hg$^{2+}$ and its intracellular detection in MCF7 cells. Chem. Commun. 2012, 48, 9293–9295. (g) Lu, H.; Qi, S.; Mack, J.; Li, Z.; Lei, J.; Kobayashi, N.; Shen, Z. Facile Hg$^{2+}$ detection in water using fluorescent self-assembled monolayers of a rhodamine-based turn-on chemodosimeter formed via a “click” reaction. J. Mater. Chem. 2011, 21, 10878–10882. (h) Kwon, J. Y.; Jang, Y. J.; Lee, Y. J.; Kim, K. M.; Seo, M. S.; Nam, W.; Yoon, J. A highly selective fluorescent chemosensor for Pb$^{2+}$. J. Am. Chem. Soc. 2005, 127, 10107–10111. (i) Xu, L.; Xu, Y.; Zhu, W.; Sun, X.; Xu, Z.; Qian, X. Modulating the selectivity by switching sensing media: a bifunctional chemosensor selectivity for Cd$^{2+}$ and Pb$^{2+}$ in different aqueous solutions. RSC Adv. 2012, 2, 6323–6328. (j) Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. New fluorescent rhodamine hydrazone chemosensor for Cu(II) with high selectivity and sensitivity. Org. Lett. 2006, 8, 2863–2866. (k) Zhang, X.; Shihabuddin, Y.; Hirai, T. Cu(II)-Selective Green Fluorescence of a Rhodamine−Diacetic Acid Conjugate. Org. Lett. 2007, 9, 5039–5042. (l) Dujols, V.; Ford, F.; Czarnik, A. W. A Long-Wavelength Fluorescent Chemodosimeter Selective for Cu(II) Ion in Water. J. Am. Chem. Soc. 1997, 119, 7386–7387. (m) Adhikari, S.; Ghosh, A.; Ghosh, M.; Guria, S.; Das, D. Ratiometric sensing of Fe$^{3+}$ through PET-CHEF-FRET processes: Live cell imaging, speciation and DFT studies. Sens. Actuators, B 2017, 251, 942–950. (n) Huang, J.; Xu, Y.; Qian, X. Rhodamine-based fluorescent off−on sensor for Fe$^{3+}$ – in aqueous solution and in living cells: 8-aminoquinoline receptor and 2 : 1 binding. Dalton Trans. 2014, 43, 5983–5989. (o) Adhikari, S.; Mandal, S.; Ghosh, A.; Das, P.; Das, D. Strategically Modified Rhodamine−Quinoline Conjugate as a CHEF-Assisted FRET Probe for Au$^{3+}$: DFT and Living Cell Imaging Studies. J. Org. Chem. 2015, 80, 8530–8538. (b) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B: Condens. Matter Mater. Phys. 1988, 37, 785–789. (c) Hay, P. J.; Wadt, W. R. Ab initio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals. J. Chem. Phys. 1985, 82, 299. (d) Hay, P. J.; Wadt, W. R. Ab initio effective core potentials for molecular calculations. Potentials for the transition metal atoms Sc to Hg. J. Chem. Phys. 1985, 82, 270–283. (e) Ghosh, M.; Mandal, S.; Ta, S.; Das, D. Detection and discrimination of Al$^{3+}$ and Hg$^{2+}$ using a single probe: nano-level determination, human breast cancer cell (MCF7) imaging, binary logic gate development and sea fish sample analysis. Sens. Actuators, B 2017, 249, 339–347.