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Multisignal Chemosensor for Cr^{3+} and Its Application in Bioimaging

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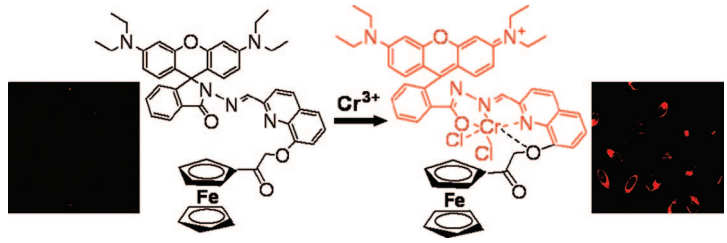
ABSTRACT



A multisignal sensor based on rhodamine B with a ferrocene substituent (FD7) has been developed as a highly sensitive chemosensor for Cr^{3+}. A prominent fluorescence enhancement was found in the presence of Cr^{3+}, which was accompanied by changes in the absorption spectrum and the electrochemical parameters. Furthermore, by means of confocal laser scanning microscopy experiments, it has been demonstrated that FD7 can be used as a fluorescent probe for monitoring Cr^{3+} in living cells.

Trivalent chromium, Cr^{3+}, is involved in some biochemical processes at the cellular level.[1] The mechanism by which Cr^{3+} affects human metabolism is based on modulation of the action of insulin through glucose tolerance factors (GTF), thereby activating certain enzymes and stabilizing proteins and nucleic acids.[2] A deficiency of chromium in the human body would lead to a variety of diseases, including diabetes and cardiovascular disease. Recently, Cr(III) has also been shown to adversely affect cellular structures, although its in vivo toxicity observed is much lower than that of Cr(VI).[2] To date, however, few methods have been developed whereby intracellular Cr(III) levels may be monitored or its distribution in living systems mapped, although organically bound Cr(III) derivatives might be transported across cell membranes by some as yet unknown mechanism.[3] Accordingly, it is of necessity to be able to monitor the intracellular Cr^{3+} with the help of some effective sensors.

In this regard, by virtue of its highly sensitive and high-speed spatial analysis of cells, fluorescence bioimaging with selective chemosensors offers a potentially powerful approach for detecting the transformation and distribution of low molecular-weight analytes (especially metal cations) in living samples.[4] Such reagents have facilitated the bioimaging Ca^{2+},[5] Zn^{2+},[6] Hg^{2+},[7] Cu^{+},[8] and Pb^{2+}[9] ions in living cells, but analogous tools for intracellular Cr^{3+} remain underdeveloped owing to the fluorescence quenching of paramagnetic Cr^{3+} and the lack of a selective ligand system. To the best of our knowledge, only two examples of Cr^{3+}-

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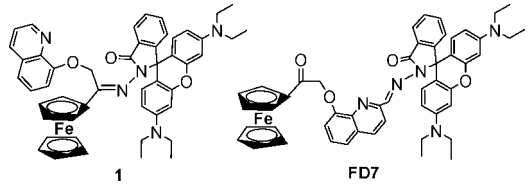
selective fluorescent chemosensors have been reported. One was reported by Samanta et al., who used di(2-ethylsulfanylethyl)amine as a receptor moiety in organic solvents.[10] In the other case, Liu et al. synthesized two fluorescent chemosensors capable of discriminating Fe^{3+} and Cr^{3+}.[11] However, these Cr^{3+}-sensors only functioned in organic solvent and thus needed to be further developed if they are to be applied in bioimaging.

Owing to their excellent spectroscopic properties of visible wavelength excitation and emission profiles, large molar extinction coefficients, and high fluorescence quantum yields, rhodamine derivatives have been utilized as important fluorescent probes. On the basis of the spirolactam (nonfluorescence) to ring-open amide (fluorescence) equilibrium of rhodamine, several rhodamine derivatives have been successfully fabricated as the OFF−ON-type fluorescent chemosensors for Cu^{2+},[12] Pb^{2+},[13] Fe^{3+},[14,15] and Hg^{2+}.[16,17] Recently, we have reported that a rhodamine B derivative (**1**, Figure 1) attached an 8-hydroxyquinoline moiety with



1 **FD7**

Figure 1. Chemical structures of **1** and **FD7**.

δ-bond could be used as a multisignaling chemosensor for Hg^{2+}.[17b] In the present study, an 8-hydroxyquinoline group as a conjugated moiety was further attached to the rhodamine derivative **FD7** (Figure 1). Interestingly, **FD7** could selectively recognize Cr^{3+} over other metal cations (including Hg^{2+}), with suitably large variations in absorption, fluorescence emission, and electrochemical parameters. Further fluorescence bioimaging investigations have indicated that **FD7** may be used as a fluorescent probe for monitoring Cr^{3+} in living cells.

The rhodamine derivative **FD7** (Figure 1) was prepared from rhodamine B hydrazide by a two-step procedure in a yield of 60%. The structures of **FD7** and other intermediate products were confirmed by ^{1}H NMR, ^{13}C NMR, and MS data (Supporting Information).

For practical application, the appropriate pH conditions for successful operation of the sensor were evaluated. Ring opening of the rhodamine occurred under acidic conditions (pH < 5.0) as a result of protonation.[14] At pH > 5.0, no obvious ring opening was observed. However, the addition of Cr^{3+} led to the fluorescence enhancement over a comparatively wide pH range (5.0−10.0), which is attributed to opening of the rhodamine ring (Figure S1, Supporting Information). Consequently, **FD7** may be used to detect Cr^{3+} over a wide pH range since there is a Cr^{3+}-induced OFF−ON fluorescence change throughout this range. A similar result was found by examining the UV/vis spectra rather than fluorescence over the same pH range (Figure S2, Supporting Information).

An optimized solvent system of ethanol/H_2O (1:1, v/v, pH 7.4) solution was selected for the spectroscopic investigations. Upon binding with Cr^{3+}, the absorption peak of **FD7** (20 μM) at 565 nm in ethanol/H_2O (1:1, v/v, pH 7.4) solution increased evidently. The colorless solution gradually turned to pink, clearly indicating the ring-opening process of the rhodamine B unit in **FD7**. The association constant for Cr^{3+} was estimated to be 7.5×10^3 M^{-1} in the ethanol/H_2O (1:1, v/v, pH 7.4) solution on the basis of nonlinear fitting of the titration curve assuming a 1:1 stoichiometry (Figure 2 inset).

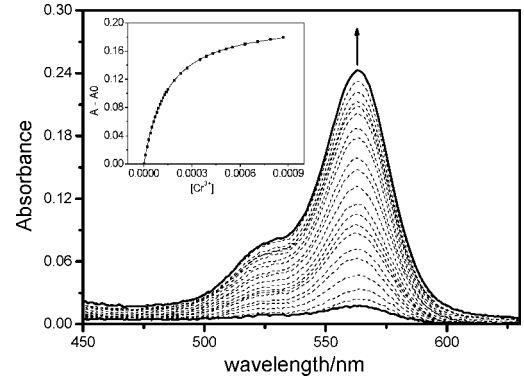


Figure 2. Changes in UV−vis absorption spectra of **FD7** (20 μM) in ethanol/H_2O (1:1, v/v, pH 7.4) solutions with various amounts of Cr^{3+} ions (0−45 equiv). Inset: Absorbance at 565 nm of **FD7** as a function of Cr^{3+} concentration.

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As designed, **FD7** shows an distinct current change of the electric currency in its reversible ferrocene/ ferricinium redox cycles upon complexation. Differential pulse voltammetry (DPV) curves of **FD7** were recorded in ethanol solution containing 0.1 M *n*-tetrabutylammonium hexafluorophosphate (*n*-Bu_4NPF_6) as supporting electrolyte in the absence and presence of Cr^{3+}. As shown in Figure 3, a significant

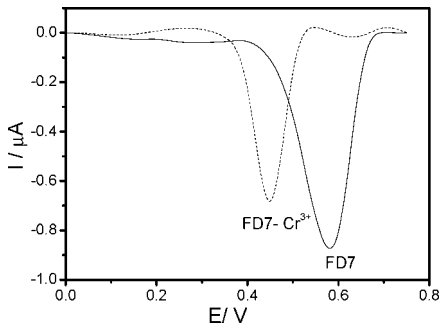

Figure 3. DPV of **FD7** (100 μM) in ethanol solution in the absence and presence of 1.6 equiv of Cr^{3+} with *n*-Bu_4NPF_6 as supporting electrolyte.

displacement was observed upon addition of Cr^{3+}. The peak was cathodically shifted upon complexation ($\Delta E_{1/2}$ = 140 mV) in ethanol. This fact indicated that **FD7**, like other chemosensors with ferrocene units,[18] had the potential to be a redox chemosensor of Cr^{3+}.

In investigating the fluorescence, the spectrum of **FD7** (20 μM) in ethanol/H_2O (1:1, v/v, pH 7.4) showed a weak emission at around 580 nm (λ_{ex} = 530 nm), since the spirocyclic form of rhodamine prevailed.[14] Upon addition of Cr^{3+}, an emission band peaked at 587 nm attributable to delocalization in the xanthene moiety of the rhodamine significantly increased in intensity. The solution showed an intense red fluorescence (Figure 4), with a quantum yield of 0.18 and an approximately 15-fold enhancement in the fluorescence intensity at 587 nm. This fact means that FD7 could be used as an off−on fluorescent chemosensor for Cr^{3+}.

To obtain an excellent chemosensor, high selectivity is essential. To validate the selectivity of **FD7** in practice, some other metal ions including alkali, alkaline earth, and transition-metal ions were added into a solution of **FD7** under the same conditions (ethanol/H_2O, 1:1, v/v, pH 7.4). Only Hg^{2+} elicited a slight fluorescence intensity enhancement, while the other metal ions did not cause any discernible changes (Figure 5). Moreover, the enhancement of the fluorescence intensity resulting from the addition of Cr^{3+} was not influenced by subsequent addition of other metal ions. All of these results indicate the high selectivity of **FD7** for Cr^{3+} over other competing cations in ethanol/H_2O (1:1, v/v, pH 7.4) solution.

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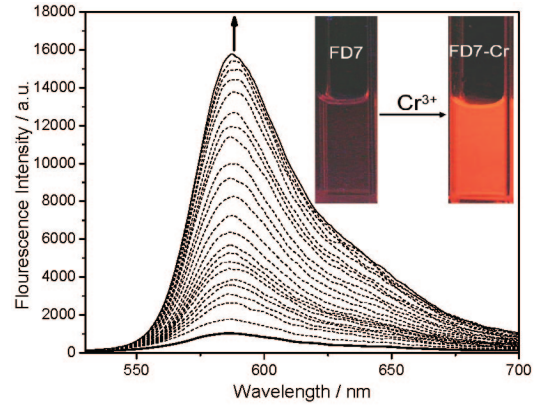

Figure 4. Fluorescence emission changes of FD7 (20 μM) in ethanol/H_2O (1:1, v/v, pH 7.4) solutions upon addition of Cr^{3+} (0−45 equiv). Each spectrum was acquired 1 min after Cr^{3+} addition, λ_{ex} = 530 nm. Fluorescence photograph was obtained under irradiation of UV light (365 nm).

The probable complexation mechanism of **FD7** with Cr^{3+} was validated by XPS and ESI-MS. The XPS of **FD7**-Cr indicated the sole presence of Cr^{3+} during this process, that is, no redox process but only complexation, albeit with the rather electron-with rhodamine B hydrazide moiety generally behaving as an electron donor (Figure S8, Supporting Information).[19] Moreover, this binding mode is supported

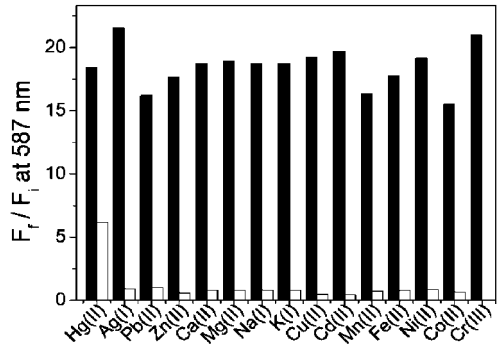

Figure 5. Fluorescence responses of **FD7** (20 μM) to various metal ions (5 mM for Ca^{2+}, Mg^{2+}, Na^+, K^+, and Zn^{2+}; 900 μM for all other cations) in ethanol/H_2O (1:1, v/v, pH 7.4). Bars represent the final integrated fluorescence response (F_f) over the initial integrated emission (F_i). White bars represent the addition of metal ions to a 20 μM solution of **FD7**. Black bars represent the addition of Cr^{3+} (45 equiv) to the solution. (λ_{ex} = 530 nm).

by the presence of a peak at *m*/*z*962.0 (calcd 962.7) corresponding to [**FD7** + $CrCl_2$]$^+$ in the ESI-MS spectrum of a mixture of **FD7** and 200 μM $CrCl_3$ (Figure S9, Supporting Information). Thus, in accordance with the

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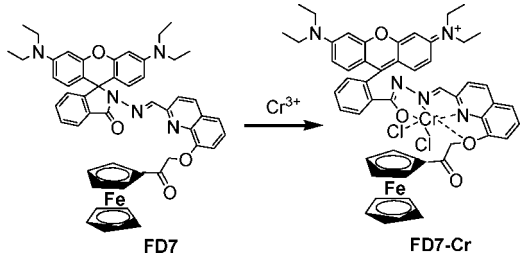
coordination number of 6 of Cr^{3+}, the most likely binding sites for $Cr^{3+}$are the conjugated moiety including carbonyl O, imino N, and quinoline N and O atoms of **FD7** (Scheme 1).

Scheme 1. Probable Complexation Mechanism of **FD7** with Cr^{3+}



To test the sensitivity of the interaction between **FD7** and Cr^{3+}, we also investigated the time course of the response of **FD7** (20 μM) in the presence of 45 equiv of Cr^{3+} in ethanol/H_2O (1:1, v/v, pH 7.4). It was found that the obvious spectral change was observed within 1 min (Figure S5 and movie, Supporting Information) upon addition of 45 equiv Cr^{3+}. Therefore, this system is suitably predisposed for the detection of Cr^{3+}.

In view of its favorable spectroscopic properties and the instantaneous interaction with Cr^{3+}, **FD7** should be well-suited for fluorescence imaging in living cells. As determined by laser scanning confocal microscopy, staining HeLa cells with a 20 μM solution of **FD7** in ethanol/PBS (1:99, v/v) buffer for 30 min at 25 °C led to very faint intracellular fluorescence (Figure 6a1). The cells were supplemented with 0, 5, 20, and 100 μM $CrCl_3$ in the growth medium for 2.5 h at 37 °C and then loaded with **FD7** under the same conditions, whereupon a significant increase in the fluorescence from the intracellular area was observed (Figure 6a1−d1). Moreover, after incubation with 50 μM $CrCl_3$, an obvious increase in the intracellular Cr(III) concentration was observed by ICP-AEC (Supporting Information). Bright-field measurements after treatment with Cr^{3+} and **FD7** confirmed that the cells remained viable throughout the imaging experiments (Figure 6a2−d2). As depicted in Figure 6b3−d3, the overlay of fluorescence and bright-field images revealed that the fluorescence signals were localized in the perinuclear

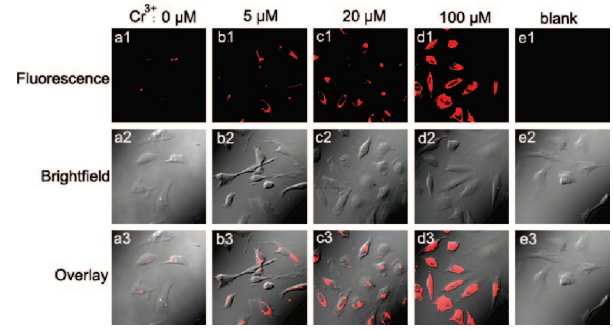


Figure 6. Confocal fluorescence, brightfield, and overlay images of HeLa cells. The cells were supplemented with 0 μM (a1−a3), 5 μM (b1−b3), 20 μM (c1−c3), or 100 μM (d1−d3) $CrCl_3$ in the growth media for 2.5 h at 37 °C and then were stained with 20 μM **FD7** for 30 min at 37 °C (λ_{ex} = 543 nm). e1−e3 were reference cells without Cr^{3+}.

area of the cytosol, indicating a subcellular distribution of Cr^{3+}. Taken together, the results indicate that the **FD7** is cell-permeable and can respond to changes in intracellular Cr^{3+} concentration within living cells.

In summary, we have utilized the ring-opening of a rhodamine B derivative and the electrochemical characterization of a ferrocenyl group to develop a novel multisignal probe for detecting the transition-metal ion Cr^{3+} in ethanol/H_2O (1:1, v/v, pH 7.4) solution. The new fluorescent sensor showed an excellent selectivity for Cr^{3+} over other metal ions examined in similar solutions. Confocal laser scanning microscopy experiments have proven that **FD7** can be used to monitor Cr^{3+} in living cells and to map its subcellular distribution.

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Supporting Information Available: Synthetic details, NMR spectra, and additional spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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