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Signal Ratio Amplification via Modulation of Resonance Energy Transfer: Proof of Principle in an Emission Ratiometric Hg(II) Sensor

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Fluorescent chemosensor design is an active field of supramolecular chemistry, not only because of potential practical benefits in cell physiology, analytical, and environmental chemistry,[1] but also as a proving ground for manipulation and/or engineering of various photophysical processes toward an ultimate goal of selective and sensitive signaling of targeted molecular or ionic species.[2] Recently, boradiazaindacenes have become the fluorophore of choice in many chemosensor designs,[3] not only because of their exceptional properties as fluorophores, but also as a result of their remarkably rich chemistry.[4] Previously, we reported[3d] a dimeric boradiazaindacene, which can be converted into an energy transfer cassette and furthermore into a ratiometric ICT (internal charge transfer) based cation sensor, selective for silver ions, all through simple structural modifications. In that design the two fluorophores were kept very close to each other, so that the through-space EET was nearly 100% efficient, thus creating a large pseudo-Stokes' shift chemosensor. ICT based chemosensors typically have an advantage of two distinct emissive states (analyte-free and analyte-bound), which makes these chemosensors potentially wavelength-ratiometric, that is, internal referencing of the signal is possible, eliminating potential artifacts.

In designing ratiometric chemosensors, one of most important parameters is the magnitude of the range of signal ratios of emission intensities at two different wavelengths for the analyte-free and analyte-bound chemosensor, preferably excited at the isosbestic point. This parameter determines the dynamic range and the sensitivity of the chemosensor to the analyte concentration.[5] A large range of ratios could be obtained, only when the emission peaks for the bound and free chemosensor are well-resolved and if both forms have reasonable emission intensities. However, this ratio is an inherent property of the particular chemosensor-analyte interactions, and, until now, a systematic methodology for improving the signal ratio was unavailable. We now introduce a new strategy in ratiometric chemosensor design which would make this possible: the range of ratios can be significantly improved, if the chemosensor is designed as an energy transfer dyad, and once the interchromophoric distance is carefully adjusted, binding of the analyte increases the spectral overlap between the donor emission and the acceptor absorption peaks. Thus, more efficient Förster type energy transfer in the bound state results in higher emission intensity for the analyte-bound chemosensor, effectively increasing the signal ratio for the two states.

To demonstrate the feasibility of this approach, we set out to synthesize a series of boradiazaindacene dyads, with increasing interchromophoric distance. In chemosensors in which the metal receptor is an electron donor and also part of the π-system of the chromophore, metal binding always causes a blue shift in the absorption spectrum, and in our systems increase the EET efficiency. In the compound **2b**, the energy transfer efficiency was almost 100%, and increasing the interchromophoric distance should decrease the EET efficiency to levels appropriate for cation modulation. Thus, we synthesized new dyes **3**−**4** shown in Figure 1. Boron to boron distance in dyads **2b**, **3b**, and **4b** is estimated as 11.9, 25.5, and 39.1 Å (Hyperchem version 7.5, PM3 semiempirical module). For compounds **3b**, **3c**, **4b**, and **4c**, the absorbance spectra very clearly show two prominent peaks in the visible region, as expected (Supporting Information). The emission spectra of these compounds are very informative. The changes in the emission characteristics reflect the changes in the EET efficiency. In compound **2b**, there is very weak emission from the shorter wavelength emitting dye, but in compounds **3b** and **4b**, a progressively larger fraction of excitation energy appears as emission at 540 nm (Figure 2). This is also in accordance with the decreased emission intensity at the longer wavelengths (670 nm). Energy transfer efficiencies were calculated using quantum yields of emission (Supporting Information). In order to obtain a cation responsive chemosensor, we took advantage of our modular design and switching to a dithiaazacrown-substituted benzaldehyde instead of 4-dimethylaminobenzaldehyde, we obtained compounds **3c** and **4c**. The specific dithiazacrown we selected, is reportedly selective for Hg(II) ions in a range of solvents.[6] This is apparent in our experiments (Figure 3) as well, the emission ratio changes 35-fold on binding to Hg(II). This is a remarkable value for a ratiometric fluorescent chemosensor. The emission response of **4c** to Hg(II) ions is also shown in Figure 4. Clearly, Hg(II) binding (K_d in THF was determined to be 4.5×10^{-7} M, see Supporting Information) causes a spectral shift in the absorbance of the longer wavelength dye, increases the spectral overlap between the energy donor and the acceptor, and an enhanced emission peak is observed. More efficient energy transfer is corroborated with the changes at the 540 nm.

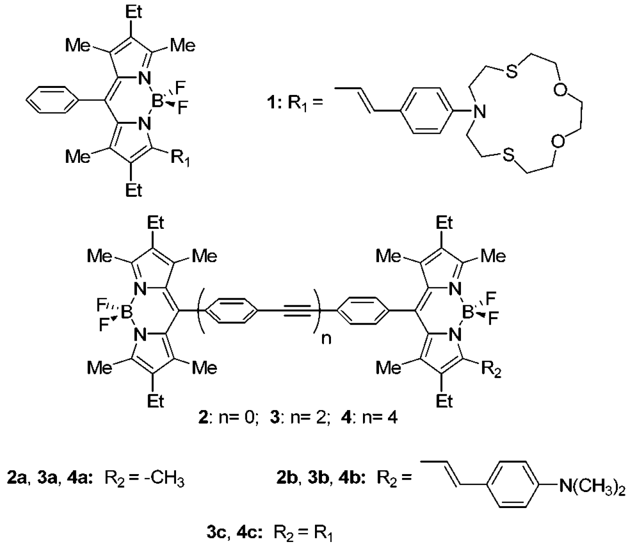

Figure 1. Structure of the boradiazaindacene dyads and the reference compound **1**.

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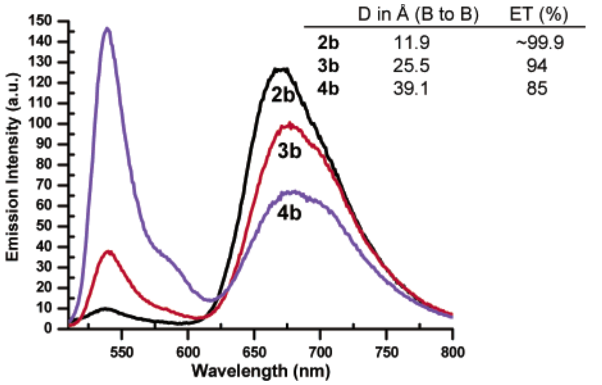

Figure 2. EET efficiency as a function of interchromophoric distance: as the distance between the two boradiazaindacene chromophores increases, the emission peak at 540 nm become more prominent. Concentrations of the dyes (**2b**, **3b**, and **4b**) were set at 1.0 μM, and the excitation was at 500 nm with 5 nm slit widths. The inset shows the boron to boron distance in Å and the corresponding EET efficiency.

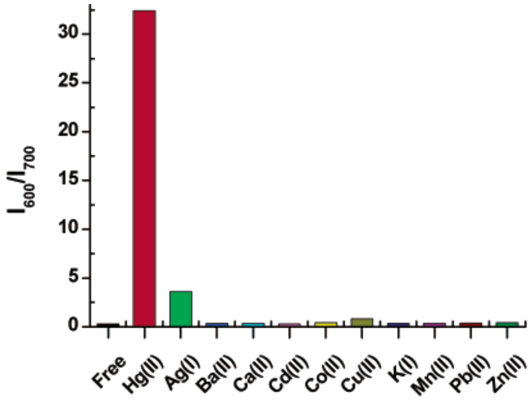

Figure 3. Emission ratios (I_{600}/I_{700}) for the boradiazaindacene dyad **4c** (1.0 μM) on excitation at 500 nm. Hg(II) causes the largest spectral shift, and hence results in the most efficient energy transfer for the bound state. All metal ions were at 50 μM concentration. The data were collected in THF and metals were introduced as perchlorates, except for silver (triflate).

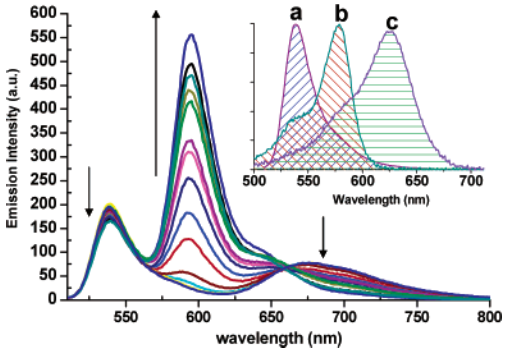

Figure 4. The emission response of the dyad **4c** in THF (1.0 μM) to an increasing concentration (0−25 μM) of Hg(II) ions. A small decrease in the intensity at 540 nm is indicative of larger EET. The inset shows the increasing spectral overlap on metal binding: The emission peak (a) of the compound **4a** and absorption peak of metal-bound compound (b) and free (c) **1** were normalized while keeping the peak ratios between **1**-Hg and free **1** unchanged. The absorption peaks were moved 7 nm to account for the spectral difference between **1** and **4c**.

The spectrum between 450 and 575 nm is the sum of the emission peak at 540 nm and the shorter wavelength extension of the major emission peak at 600 nm. Even without accounting for this contribution from the major metal-bound emission peak, increasing concentrations of metal ion result in a small, but reproducible apparent reduction in the emission intensity at 540.

Normalized emission spectrum of the energy donor dye (estimated using the emission spectrum of compound **4a**) and the absorption spectrum of the energy acceptor dye obtained from the absorption spectra of metal-bound **4c** and free **4c** are shown in the inset of Figure 4; the increased spectral overlap on Hg(II) binding is obvious. Now, if we were to calculate the peak emission ratios using the emission intensity values at 600 and 700 nm, for the reference compound **1**, a ratio of 2.8 is obtained. Modulated EET yields higher values for this ratio, the values are 5.9 for compound **3c** and 7.2 for compound **4c**. This means that cation modulated excitation energy transfer, through increased spectral overlap, generates a large enhancement of signal ratios, which translates to drastically improved sensitivity to metal ion concentrations. Thus, we satisfactorily demonstrate that in judiciously designed energy transfer dyads, chemosensors of larger intensity ratio changes can be obtained, if the binding of the analyte modulates the energy transfer efficiency. We are confident that in due course this general and modular approach in ratiometric chemosensor design would lead to novel and useful chemosensors for many biologically and environmentally relevant analytes.

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Supporting Information Available: Syntheses, experimental details, ^{1}H, ^{13}C NMR spectra, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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