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Fluorescence-On Response via CB7 Binding to Viologen–Dye Pseudorotaxanes

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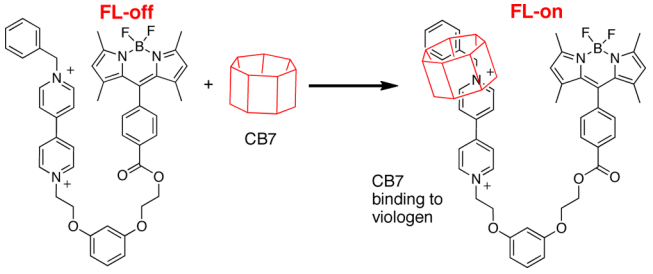
ABSTRACT



Fluorescence-on sensors typically rely on disrupting photoinduced electron transfer quenching of the excited state through binding the electron donor. To provide a more general fluorescence-on signaling unit, a quencher–fluorophore dyad has been developed in which quenching by electron transfer to a tethered viologen acceptor can be disrupted through complexation of the viologen by cucurbit[7]uril (CB7). Dyads of benzyl viologen–rhodamine B or a BODIPY fluorophore gave upon CB7 complexation 14- and 30-fold fluorescence enhancement, respectively.

Molecular fluorescence plays a central role in biological imaging and sensing methods.[1] Cellular imaging often relies on tagging a target with a fluorophore such as green fluorescent protein or small synthetic dyes to enable visualization of the target using optical microscopy.[2] A drawback of this approach is that the background fluorescence of nonspecifically attached fluorophores limits applications to those cases in which the localized emission is sufficiently stronger than the background. This disadvantage can be overcome by using fluorescence-on binding sensors in which emission is substantially quenched until a sensing event takes place to block the quenching. Such fluorescence-on methods have been most frequently applied in metal ion sensors in which quenching by photoinduced electron transfer (PET) from nonbonded electron pairs to the excited state of the fluorophore is shut off by coordinating the donor electron pairs with a metal ion (Figure 1a).[1,3] This approach has been most widely followed to develop selective binding groups for various metal ions. While fluorescence-on ion sensing through disrupting PET from nonbonded electron pairs has been the focus of extensive development, this approach may not lend itself to signaling binding events of a more general nature.

An approach that relies on increasing fluorescence through disruption of contact between a fluorophore and a quencher could provide an entry into a more general signaling method. This fluorescence-on approach has been much less intensively studied, but notable efforts have involved detecting conformational changes in proteins that move a quenching group away from a fluorophore, or disrupt aggregation by binding the fluorophore.[4] A novel and more general fluorescence-on approach would entail disrupting the quenched aggregate through host binding of a guest quencher. When a host is displaced from an initial

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guest complex in response to external stimuli as shown in Figure 1b, a fluorosecence-on signal would result from the host binding the new guest to disrupt the flourescence quenching. Thus, we would have an unprecedented sensor that has one site for interacting with an analyte and a second site that produces the fluorescence signal. Since the fluorescence on the signaling unit is structurally independent of the binding event, variation in the binding unit can be explored without needing to reconfigure the signaling unit in each case.

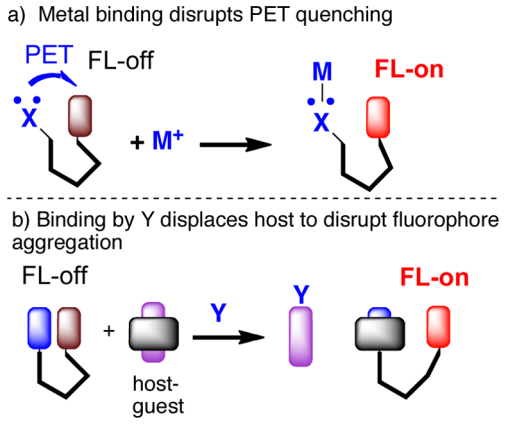

Figure 1. Mechanisms to disrupt fluorescence quenching.

It is well-documented that fluorescence quenched through homoaggregation of fluorophores can be rescued by disrupting cationic fluorophore aggregation through binding with cucurbit[7]uril (CB7).[5] We have reported a 2-fold enhancement of fluorescence of a tethered rhodamine B dyad upon CB7 binding to disrupt a less fluorescent H-dimer aggregate.[6] In order to extend this method toward a more useful, larger change in fluorescence intensity, we have now investigated the disruption of electron transfer quenching upon CB7 binding to a benzyl viologen pseudorotaxane and report our preliminary findings herein. These results show that binding a quencher as shown in Figure 1b can provide a strong fluorescence-on response and provide impetus to extend the method to the shuttle mechanism depicted.

Fluorescence from organic fluorophores such as rhodamine B is known to be quenched by electron transfer from the excited state of the dye to 1,4-dipyridinium viologen dications when the two components are coentrained in a polymer matrix.[7] Given the established binding of CB7 with viologens,[8] we sought to establish an ability to generate a fluorescence-on signal upon addition of CB7 to tethered viologen–rhodamine and viologen–BODIPY[9] dyads.

The preparation of benzyl viologen–rhodamine B (RhB) dyad **3b** is shown in Scheme 1. Alkylation of resorcinol with 2-chloroethanol followed by alkylation with 1,2-dibromoethane provided the tethering anchor **1** that has the desired orthogonal hydroxy and alkyl halide reactive sites. Carbodiimide-promoted esterification of the hydroxy-terminated arm with rhodamine B gave RhB ester **2**.

Alkylation on the bromo-terminated arm by excess 4,4′-dipyridine under mild conditions gave a monosubstituted dipyridyl complex that upon a second pyridine alkylation by methyl iodide or benzyl bromide gave the benzyl viologen–rhodamine B dyads **3a** and **3b**. An analogous set of reactions was used to form benzyl viologen–BODIPY dyad **4**. More forcing conditions in these transformations led to undesired amounts of ester and/or benzylpyridinium cleavage. Isolation of the polycationic complexes was achieved using silica gel chromatography using a methanol–nitromethane–aqueous ammonium chloride eluent followed by dissolving the desired complex away from the excess inorganic salts using dichloromethane.[10]

The aggregation of the viologen–dye dyads and their interaction with CB7 could be modeled by gas phase semiempirical calculations (Spartan 08, PM3) as shown in Figure 2 for the BODIPY fluorophore dyad **4**. The contact needed between the viologen and BODIPY moieties for effective electron transfer was observed in the absence of CB7 and disrupted in the presence of CB7.

The fluorescence brightness of dyads **3a** and **3b** was noticeably lower than that of the corresponding free dye (using RhB ester **2** without the tethered dipyridinium for comparison). As illustrated in Figure 3, CB7 increased the fluorescence intensity of RhB ester **2** about 2-fold as expected due to disruption of RhB aggregation.[6a] Tethering the RhB moiety to either the methyl or benzyl viologen quencher in **3a** or **3b** did result in nearly a 10-fold decrease in the fluorescence intensity of the fluorophore. Addition of CB7 to the methyl viologen–RhB dyad **3a** did not result in much recovery of fluorescence. In contrast, addition of CB7 to benzyl viologen–RhB dyad **3b** gave a much larger, 14-fold increase in fluorescence intensity over **3b** alone. While CB7 is known to bind both methyl viologen and benzyl viologen, the larger hydrophobic group on the latter provides for a stronger association. Thus, known binding

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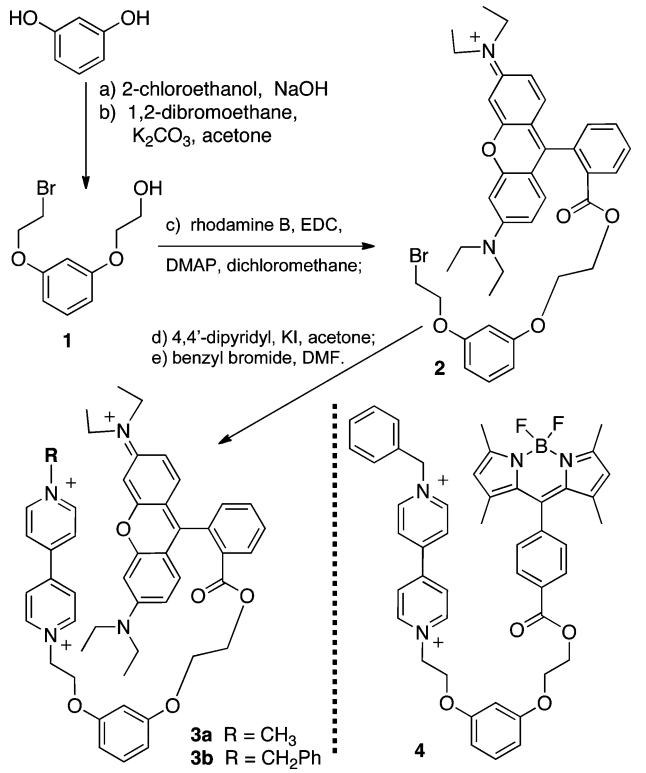
Scheme 1. Preparation of Viologen–Fluorophore Dyads



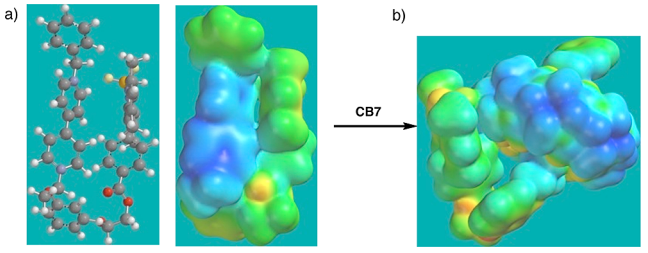


Figure 2. (a) PM3-optimized structure/electrostatic surface potential of **4** before and (b) after binding with CB7.

of the CB7 to cationic RhB would better compete with the methyl viologen than with the benzyl viologen. Binding of CB7 over one diethylamino group of the RhB moiety in the presence of the methyl viologen could still allow the nonincluded portion of the rhodamine chromophore to contact the methyl viologen quencher. Fluorescence titration curves of **2**, **3a**–**b**, and **4** with CB7 and ^{1}H NMR titration spectra for **3a**–**b** are provided in the Supporting Information. Curve fitting analysis gave a good one-site binding model fit with the benzyl viologen and supported one preferential binding site that was effective at disrupting quenching. The methyl viologen data were more consistent with two competing sites.

In order to minimize competitive binding of the CB7 between the viologen and the cationic RhB fluorophore,

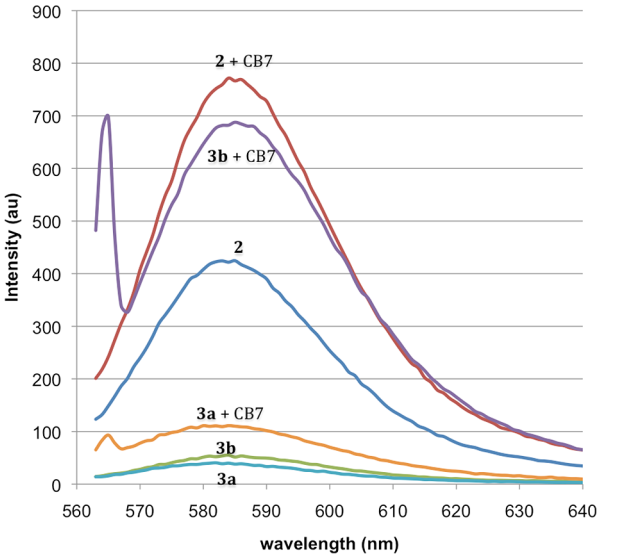


Figure 3. Fluorescence spectra of RhB ester **2**, methyl viologen-RhB **3a**, and benzyl viologen-RhB **3b** (each 5 μM) without and with 8 mM CB7 (excitation 560 nm).

we examined the dyad **4** containing the neutral BODIPY fluorophore. Upon titration of benzyl viologen–BODIPY dyad **4** (5 μM) with CB7 (0 –2 mM) in water, the fluorescence intensity increased a remarkable 30-fold to give a very sizable fluorescence-on response. The titration data fit well to a one-site binding model, which gives $K_a = 1.2 \times 10^4$ M^{-1} for binding CB7 to dyad **4**. Although no association constant between CB7 and BODIPY is available from the literature, we presume that CB7 will bind more effectively to the cationic viologen moiety in preference to the neutral BODIPY group. Our association value with **4** is about 10^2 weaker than typical complexation of CB7 to isolated viologen;[8a,c,11] this difference in binding can be attributed to the penalty required to disrupt the energetically favorable aggregation of the BODIPY with the viologen. If CB7 was indeed bound to the viologen moiety as expected, this would imply that the association constant between BODIPY and viologen should be on the order of 10^2. While solubility limitations hindered titrations with BODIPY dyad **4** in the required aqueous medium, chemical shift changes in ^{1}H NMR spectra of **3b**•CB7 supported inclusion of the benzyl viologen moiety by CB7.

An important aspect of sensor design is illustrated by these results. The complexation of CB7 onto various dyads to disrupt the contact of a quenching unit with the fluorophore is dependent on the different association constants of the various quenching moieties with CB7. Given the similar association constant of CB7 with the benzyl viologen in either the BODIPY **4** or rhodamine B **3b** dyads, we can utilize a common benzyl viologen binding element while still producing a different emission frequency from

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the various fluorophores. The crucial design element would be that the fluorophore be initially quenched through contact with the viologen and that binding of the CB7 to the viologen is roughly independent of the tethered fluorophore

The results presented here demonstrate a novel approach to a fluorescence-on system that relies on shuttling a CB7 onto and disrupting contact in a viologen–fluorophore dyad. While the demonstration of a sensor involved diminishing the binding affinity of CB7 for its initial guest through a deprotonation, one could in principle lower the binding affinity of the CB7 for the initial site through other stimuli while maintaining a common fluorescence-on signaling unit.

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Supporting Information Available. Experimental procedures and characterization of all synthesized compounds. Absorbance and fluorescence titrations of **2**, **3a**, **3b**, **4**; ^{1}H NMR spectral titrations of **3a**, **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.