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Novel oxygen-depleted calix[4]arenes containing fused carbazole moieties demonstrate AIEgen behavior in aqueous solutions. This phenomenon leads to highly sensitive detection of nitric-oxide guest molecules because it affects intra- and intermolecular energy transfer within aggregates.

Nitric oxide (NO) is an important gaseous signaling molecule.^{1–3} Other diamagnetic members of this family can be conveniently detected by various fluorescent probes,^{4–7} but direct fluorescent detection of the radical NO represents a significant challenge due to its rapid oxidation to form higher nitrogen oxides. In fact, this oxidation is commonly used to indirectly measure the concentration of NO gas in biological systems.⁸ Direct detection by transition metal-based fluorescent NO probes has also been reported, but selectivity issues and compatibility with biological media remain.^{9–11} Rathore and colleagues, and later Rudkevich and co-workers, demonstrated that oxidized calix[4]arene (calixarene) scaffolds can trap the NO molecule within a hydrophobic cavity (**1a**, Fig. 1), accompanied by a color change,^{12–15} thus becoming supramolecular colorimetric NO probes. Such trapping is accompanied by charge transfer from NO to the oxidized host to give a stable diamagnetic donor–acceptor complex.^{15,16} We showed that conjugated 5,5'-bicalixarene hosts¹⁷ behave as fluorescent NO probes as they undergo blue fluorescence quenching by NO gas in organic solvents or in water, while insensitive to common gases (O₂, CO, CO₂) or ions (**1b**, Fig. 1).^{18,19} Incorporating these scaffolds into conjugated polymers led to a molecular wire-type signal amplification.¹⁹ Attachment of fluorophores at the termini of the conjugated fragment (Ar groups in **1b**) gave access to new fluorescent scaffolds operating at longer wavelengths.²⁰ However, using this approach to make water-soluble polymers emitting at longer

Carbazole-fused calixarene cavities: single and mixed AIEgen systems for NO detection†

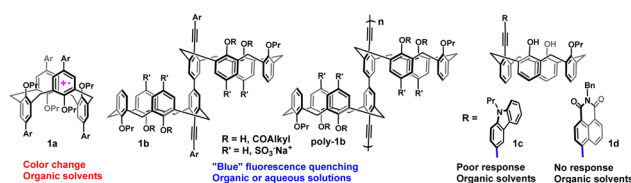
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Fig. 1 Examples of calixarene hosts for the encapsulation and sensing of NO gas.

wavelengths would add more steps to an already complex multi-step synthesis. In addition, long wavelength-emitting poly(aryleneethynylenes) tended to exhibit diminished quantum yields in aqueous solutions.²¹

Thus, we decided to explore a different signal-amplification strategy for fluorescent supramolecular NO detection at longer wavelengths in aqueous media which was based on aggregation-induced emission (AIE).²² Recent studies have demonstrated the ability of guest intercalation to affect the luminescence properties of hosts exhibiting AIE.^{23–26} However, despite a great deal of attention in AIE-based sensing, such molecular probes utilizing a three-dimensional host–guest sensing mechanism are undeveloped in general,²⁷ and for NO sensing, in particular.^{28,29} Here, we present the first examples of such supramolecular AIE-based probes for NO detection in aqueous solutions (Fig. 2).

Considering the weak emission response of small mono-calixarene compounds in organic solvents (**1c,d**, Fig. 1), we hypothesized that their aggregation in aqueous solutions could effectively increase the overall conjugation and, as a result, increase sensitivity. After the initial screening, we found that a carbazole-containing calixarene host **1c** behaves as an AIE luminogen (AIEgen), showing strong AIE changes from blue emission in pure DMF solution to cyan (λ_{em} 450 nm) in a 60% water–DMF mixture. To our chagrin, the new aggregates did not show significant fluorescence changes after NO gas was passed through the solution for 5 min, implying weak interactions of the guest with a single aromatic ring of a calixarene cavity (ESI,† Fig. S52). To facilitate a stronger response, we chose to imbed

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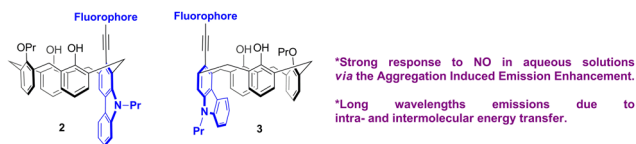


Fig. 2 New design of supramolecular AIE-based calixarene probes.

an electron-rich polycyclic aromatic fragment into the calixarene cavity. It has been reported that polycyclic aromatic compounds interact with NO gas,³⁰ but polycyclic conjugated calix[4]arenes are scarce and have not been employed in host-guest sensing.^{31–37}

The synthetic routes toward compounds **2a** and **b** incorporating a fused carbazole-calixarene unit are shown in Scheme 1a. Importantly, the calixarene intermediate **7** showed fluorescence in organic solvents, but an AIE effect in aqueous solutions was not observed until the phenolic oxygen was replaced by another fluorophore (compounds **2**).

Unlike **1c**, which showed a moderate fluorescence response upon bubbling of NO gas in a dichloroethane (DCE) solution, a solution of **2a** demonstrated nearly complete quenching in the same solvent.³⁸ More interestingly, when aggregated in an 80% water-DMF mixture, compound **2a** exhibited strong fluorescence at longer wavelengths ($\lambda_{\text{em}} = 495$ nm). Contrary to **1c**, upon the exposure to NO gas, aggregates of **2a** demonstrated nearly complete fluorescence quenching (Fig. 3a). This observation provided evidence that the remote host-guest NO complexation had a pronounced effect on the fluorescent properties of the aggregates. In light of this encouraging fluorescence response, we decided to establish whether aggregates of **2a** could be used in quantitative measurements of NO in aqueous solutions. To this end, we prepared stock solutions of a commercial NO source, diethylammonium (*Z*)-1-(*N,N*-diethylamino)diazen-1-ium-1,2-diolate ($\text{Et}_2\text{N}-\text{N}(\text{N}=\text{O})\text{O}-\text{Et}_2\text{NH}_2^+$, DEA NONOate, **10**), and studied the fluorescence response of

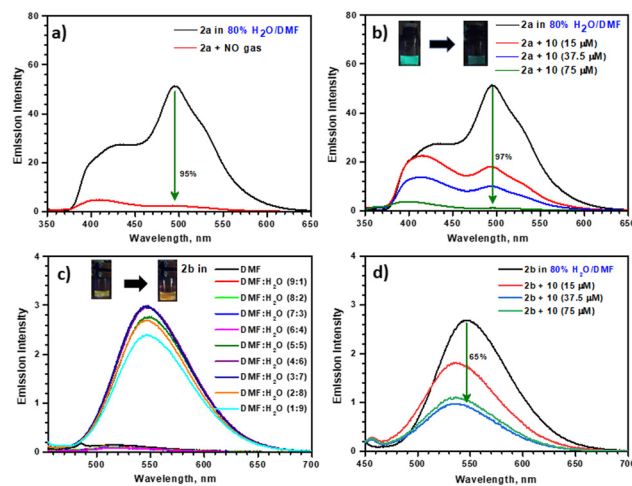
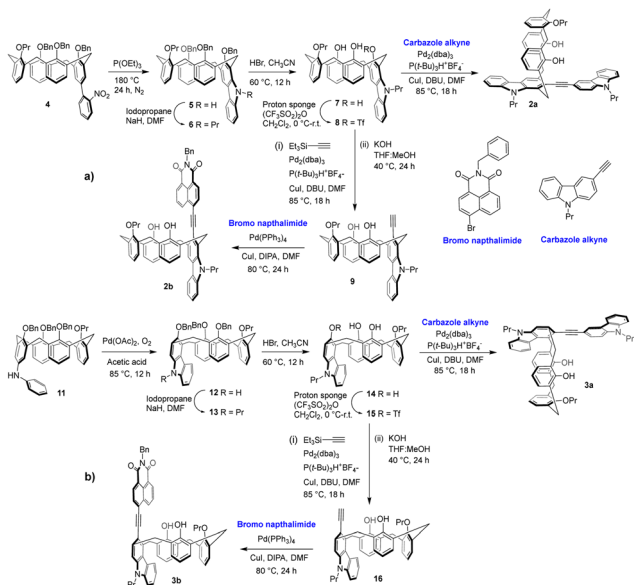


Fig. 3 Emission spectra of **2a** (10 μM): (a) in an 80% H_2O -DMF solution before and after passing NO gas; (b) before and after addition of **10** in an 80% H_2O -DMF solution. Emission spectra of **2b** (10 μM): (c) at different DMF: H_2O ratios; (d) before and after addition of **10** in an 80% H_2O -DMF solution.

aggregates of **2a** to NO generated *in situ*. Gratifyingly, these aggregates showed very strong fluorescence quenching even at concentrations of **10** as low as 15 μM , 1.5 equiv. relative to **2a** (Fig. 3b), which is within the concentration range found in human blood.^{39,40} A comparable sensitivity was observed previously for a conjugated polymer incorporating a water-soluble 5,5'-bicalixarene scaffold (poly-**1b**, Fig. 1).¹⁹ Thus, aggregation of **2a** provided the fluorescence response to NO host-guest complexation on a par with the molecular wire-amplification mechanism, but at significantly longer wavelengths.

A combination of an electron-rich cavity with an electron-poor conjugated fluorophore can lead to probes exhibiting intramolecular effects through transfer of bond energy.^{41,42} 5,5'-bicalixarene scaffolds containing a combination of donor-acceptor fluorophores have demonstrated a ratiometric response to NO gas in organic solvents.²⁰ Interestingly, while compounds **1d** or **1b** (Aryl = naphthalimide, Fig. 1)²⁰ did not show fluorescence quenching upon passing NO through their DCE solutions, the fluorescence intensity of a carbazole-fused calixarene **2b** containing an electron-poor naphthalimide fluorophore decreased by $\sim 37\%$ from the original value under identical conditions.³⁸ Similar to **2a**, compound **2b** gave aggregates in water-DMF mixtures (Fig. 3c). Upon exposure to NO, generated *in situ* from 1.5 equiv. of **10**, these aggregates showed $\sim 33\%$ of fluorescence quenching (Fig. 3d). This decrease in intensity was accompanied by a noticeable blue-shift of emission, indicating loss of donor-acceptor interaction between the electron-rich cavity and naphthalimide fluorophore. A carbazole or naphthalimide fluorescent moiety at the lower rim was essential to observe AIE in the carbazole-fused calixarenes **2a** and **b**, so these flat fragments likely had a key role in aggregate formation.

However, the presence of a calixarene fragment was also crucial because an AIE effect was not observed for simple arylacetylene-substituted carbazole or naphthalimide compounds.



Scheme 1 Synthesis of the calixarenes (a) **2a** and **2b** and (b) **3a** and **3b**.



Thus, we envisioned the formation of co-aggregates incorporating both, donor and acceptor, groups upon mixing of **2a** and **2b** in an 80% water-DMF. Such co-aggregates could exhibit strong donor-acceptor π -stacking and longer wavelength emission due to intermolecular energy transfer.⁴³ In the presence of NO, host-guest interactions between the guest and the cavity of a donor (**2a**) would lead to attenuation of such intermolecular energy transfer and emission shift to shorter wavelengths. Indeed, a 1 : 1 mixture of **2a** and **2b** in 80% water-DMF demonstrated the properties expected from strongly interacting donor-acceptor aggregates. In particular, excitation in the carbazole absorption region (345 nm) led to a weak emission signal from the carbazole fluorophore, whereas the naphthalimide signal became stronger at a slightly longer wavelength (560 nm vs. 550 nm in pure **2b** aggregates, Fig. 4a and 3c). For comparison, the same excitation of a 1 : 1 mixture of **2a** and **2b** in pure DMF solution showed the expected two separate signals for the carbazole fluorophore and naphthalimide fluorophore.³⁸ These findings indicated intermolecular energy transfer between the donor (carbazole) and acceptor (naphthalimide) taking place upon aggregation. Importantly, the largest red-shift and highest fluorescence intensity was obtained when **2a** and **2b** were mixed in a 1 : 1 ratio (Fig. 4a) suggesting that ordered co-aggregate structures involving electron-donating and electron-accepting fluorophores were formed.⁴³ When these co-aggregates were mixed with **10**, substantial fluorescence quenching (70% with 7.5 equiv. of **10**) and blue-shift of the remaining fluorescence was observed (Fig. 4b). The residual fluorescence intensity was similar to that obtained from the partly quenched fluorescence of **2b** aggregates in an 80% water-DMF mixture (*cf.* Fig. 3d). These data indicated that NO complexation fully quenched the fluorescence of the electron-rich **2a**, thereby making it incapable of transferring energy to **2b** in a co-aggregate, and also partially quenched the fluorescence from the electron-poor **2b** (*vide supra*). To obtain further information on the energy-transfer process in calixarene AIE co-aggregates, we prepared calixarenes **3a** and **3b**, isomeric to **2a** and **2b**, respectively (Scheme 1b). The carbazole-fused calixarene scaffold in compounds **3** contained the heterocyclic nitrogen atom in the *para*-position to the lower-rim fluorophore, allowing more efficient conjugation with this donor group than in compounds **2**. Because of that, the emission of **3b** was red-shifted noticeably compared with that of **2b** in organic solvents and a water-DMF mixture (*e.g.*, 585 nm for **3b** vs. 550 nm for **2b** in 80% water-DMF).

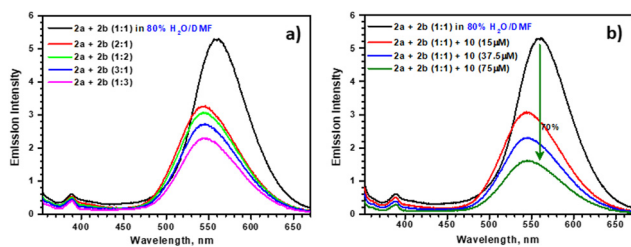


Fig. 4 (a) Emission spectra of **2a** + **2b** mixtures at different ratios in 80% H₂O-DMF (λ_{ex} 345 nm); (b) emission spectra of a **2a** + **2b** (1 : 1) mixture in 80% H₂O-DMF before and after addition of different concentrations of **10**.

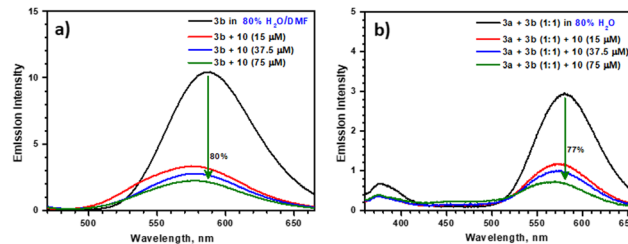


Fig. 5 (a) Emission spectra of **3b** in 80% H₂O-DMF before and after addition of different concentrations of **10**; (b) emission spectra of a 1 : 1 **3a** + **3b** mixture in 80% H₂O-DMF before and after addition of different concentrations of **10**.

Stronger donor-acceptor interactions in **3b** also make it more responsive to the presence of low quantities of NO. For example, addition of 1.5 equiv. of **10** in an 80% water-DMF mixture led to ~70% quenching of fluorescence of the aggregate (Fig. 5a), whereas only ~33% was quenched in the aggregates of **2b** (Fig. 3c). Similar to the isomeric **2**, an 80% water-DMF solution containing a 1 : 1 mixture of **3a** and **3b** gave co-aggregates with the strongest emission and red-shift than solutions prepared with the other ratios, indicating ordered structures. This co-aggregate showed emission at 585 nm, ~25 nm red-shifted compared with the 1 : 1 co-aggregate of **2a** and **2b**. When the former was treated with a 15- μ M solution of **10** (1.5 equiv.), nearly 60% fluorescence quenching was observed, with the remaining fluorescence signal undergoing a blue shift of 10 nm (Fig. 5b). In comparison, only 43% fluorescence quenching was observed for the co-aggregate of **2a** and **2b** under identical conditions. These results showed that, by tuning the donor-acceptor properties of the peripheral three-dimensional host, it was possible to further This tuning influenced the fluorescence properties of supramolecular aggregates. leading to more efficient utilization of intra- and intermolecular energy transfer. Incorporation of the NO guest within the cavity of **3a** affected the intermolecular energy transfer between **3a** and **3b** in the co-aggregate, whereas additional NO complexation within the cavity of **3b** further diminished the intramolecular energy transfer between the carbazole-calixarene cavity and naphthalimide fluorophore (Fig. 6a). Normalized emission spectra of the aggregates of **2a** and **b** and **3a** and **b** and their corresponding 1 : 1 mixtures demonstrated higher sensitivity of compounds **3** to NO, especially at low concentrations (Fig. 6b).

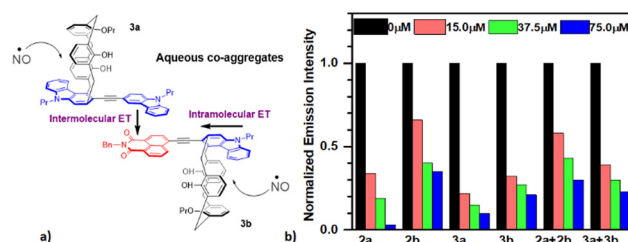


Fig. 6 (a) Proposed effect of NO host-guest complexation on the intra- and intermolecular energy transfer in calixarene co-aggregates. (b) Normalized fluorescence quenching for the aggregates and co-aggregates of **2a** and **b** and **3a** and **b** (10 μ M in 80% H₂O-DMF) at different concentrations of **10**.



