ROYAL SOCIETY OF CHEMISTRY

ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Open Access Article. Published on 31 March 2023. Downloaded on 12/31/2025 7:57:32 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Check for updates

Cite this: *Chem. Commun.*, 2023, 59, 5543

Received 9th March 2023,
Accepted 23rd March 2023

DOI: 10.1039/d3cc01181j

rsc.li/chemcomm

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

Varun Rawat, ‡[a] Abhishek Baheti,‡[a] Om Shanker Tiwari [b] and
Arkadi Vigalok *[a]

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 hosts[17] 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 (O_2, CO, CO_2) 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 wavelengths would add more steps to an already complex multistep synthesis. In addition, long wavelength-emitting poly(aryleneethynylenes) tended to exhibit diminished quantum yields in aqueous solutions.[21]

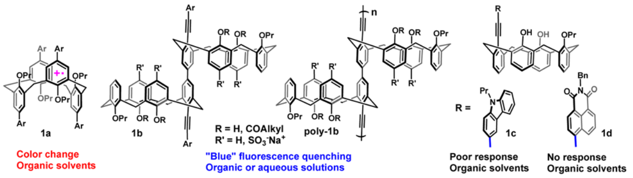


Fig. 1 Examples of calixarene hosts for the encapsulation and sensing of NO gas.

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 monocalixarene 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

---

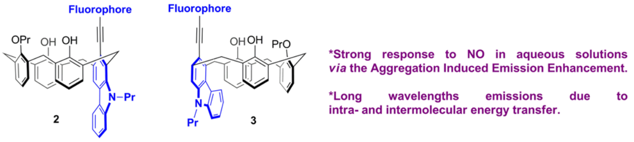
[a] *School of Chemistry, The Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 69978, Israel. E-mail: avigal@tauex.tau.ac.il*

[b] *The Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 6997801, Israel*

† Electronic supplementary information (ESI) available. See DOI: **https://doi.org/10.1039/d3cc01181j**

‡ Equal contribution.

Open Access Article. Published on 31 March 2023. Downloaded on 12/31/2025 7:57:32 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.



*Strong response to NO in aqueous solutions *via* the Aggregation Induced Emission Enhancement.

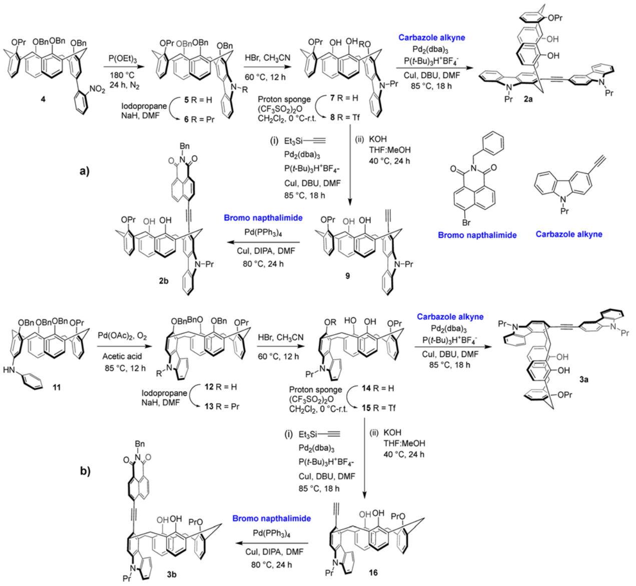
*Long wavelengths emissions due to intra- and intermolecular energy transfer.

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 (λ_{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 ($Et_2N–N(N{=}O)O\text{-}Et_2NH_2^+$, DEA NONOate, **10**), and studied the fluorescence response of 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.



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

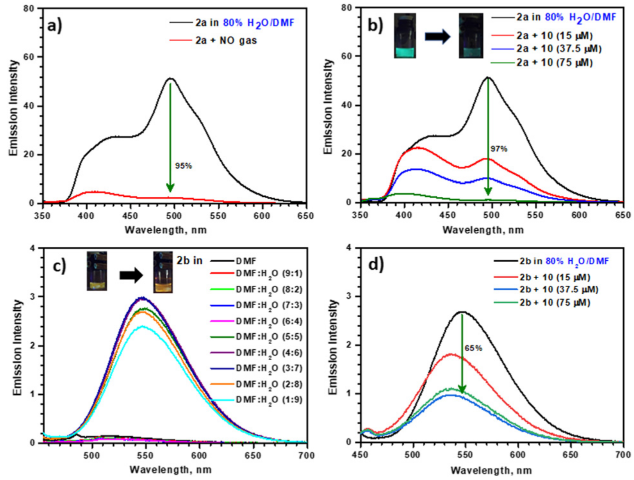


Fig. 3 Emission spectra of **2a** (10 μM): (a) in an 80% H_2O–DMF solution before and after passing NO gas; (b) before and after addition of **10** in an 80% H_2O–DMF solution. Emission spectra of **2b** (10 μM): (c) at different DMF:H_2O ratios; (d) before and after addition of **10** in an 80% H_2O–DMF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

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 ∼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 ∼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.

This journal is © The Royal Society of Chemistry 2023

Open Access Article. Published on 31 March 2023. Downloaded on 12/31/2025 7:57:32 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

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).

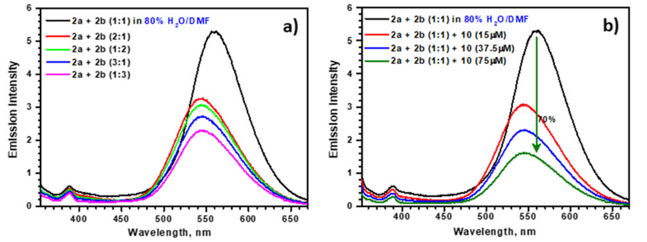


Fig. 4 (a) Emission spectra of **2a + 2b** mixtures at different ratios in 80% H_2O–DMF (λ_{ex} 345 nm); (b) emission spectra of a **2a + 2b** (1:1) mixture in 80% H_2O–DMF before and after addition of different concentrations of **10**.

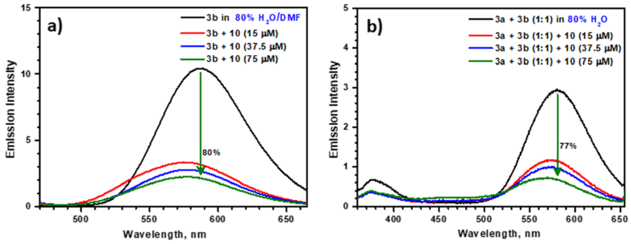


Fig. 5 (a) Emission spectra of **3b** in 80% H_2O–DMF before and after addition of different concentrations of **10**; (b) emission spectra of a 1:1 **3a + 3b** mixture in 80% H_2O–DMF before and after addition of different concentrations of **10**.

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-μM 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).

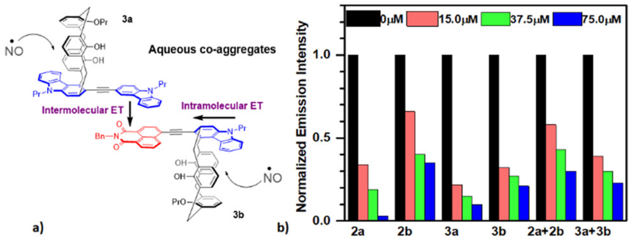


Fig. 6 (a) Proposed effect of NO host–guest complexation on the intra- and intermolecular energy transfer in calixarene co-aggregates. (b) Normalized fluorescence quenching for the aggregates and co-aggregates of **2a** and **b** and **3a** and **b** (10 μM in 80% H_2O–DMF) at different concentrations of **10**.

Open Access Article. Published on 31 March 2023. Downloaded on 12/31/2025 7:57:32 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

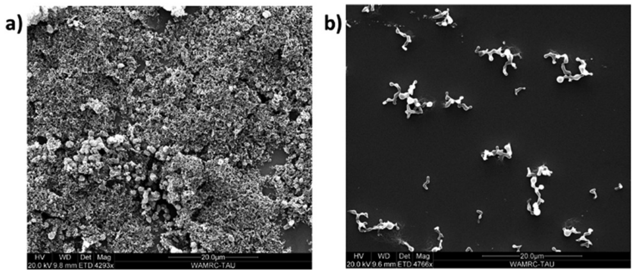


Fig. 7 SEM images of **3b** at 10 μM in: (a) 80% H_2O–DMF solvent mixture; (b) 100% DMF.

To further investigate the aggregation properties of carbazole-fused calixarenes, we undertook scanning electron microscopy (SEM). Accordingly, samples of **3b** were dissolved in two solvents (80% H_2O–DMF and in 100% DMF) at the same concentration (10 μM). The resulting solutions were drop-cast on a silicon wafer, and the aggregation properties of both samples were examined by SEM. Compound **3b** exhibited highly aggregated particles in the sample drop-cast from 80% H_2O–DMF (Fig. 7a), whereas negligible aggregation was observed in the SEM image of the sample obtained from pure DMF solvent (Fig. 7b).

Overall, we showed that the fluorescence of aqueous AIEgen aggregates could be affected by the host–guest complexation occurring at the peripheral conjugated cavity, as demonstrated by the detection of NO gas at micromolar concentrations. A combination of an electron-rich and electron-poor AIEgens led to ordered co-aggregates which allow shifting the analyte detection to longer wavelengths using intra- and intermolecular energy transfer.

This work was supported by the Israel Science Foundation grant (1328/20). We thank Professor Ehud Gazit for help with SEM measurements.

Conflicts of interest

There are no conflicts of interest to declare.

Notes and references

1 H. Prast and A. Philippu, *Prog. Neurobiol.*, 2001, **64**, 51.
2 C. Bogdan, *Nat. Immunol.*, 2001, **2**, 907.
3 J. Garthwaite, *Eur. J. Neurosci.*, 2008, **27**, 2783.
4 M. Yang, J. Fan, J. Du and X. Peng, *Chem. Sci.*, 2020, **11**, 5127.
5 D. Andina, J.-C. Leroux and P. Luciani, *Chem. – Eur. J.*, 2017, **23**, 13549.
6 N. Kumar, V. Bhalla and M. Kumar, *Coord. Chem. Rev.*, 2013, **257**, 2335.
7 J. Krämer, R. Kang, L. M. Grimm, L. De Cola, P. Picchetti and F. Biedermann, *Chem. Rev.*, 2022, **122**, 3459.
8 E. M. Hetrick and M. H. Schoenfisch, *Annu. Rev. Anal. Chem.*, 2009, **2**, 409.
9 M. H. Lim and S. J. Lippard, *Acc. Chem. Res.*, 2007, **40**, 41.
10 J. Alday, A. Mazzeo and S. Suarez, *Inorg. Chim. Acta*, 2020, **510**, 119696.
11 For general issues with the fluorescent detection of NO in biological systems see: A. K. Vidanapathirana, P. J. Psaltis, C. A. Bursill, A. D. Abell and S. J. Nicholls, *Med. Res. Rev.*, 2021, **41**, 435.
12 R. Rathore, S. V. Lindeman, K. S. S. P. Rao, D. Sun and J. K. Kochi, *Angew. Chem., Int. Ed.*, 2000, **39**, 2123.
13 R. Rathore, S. H. Abdelwahed and I. A. Guzei, *J. Am. Chem. Soc.*, 2004, **126**, 13582.
14 G. V. Zyryanov, Y. Kang, S. P. Stampp and D. M. Rudkevich, *Chem. Commun.*, 2002, 2792.
15 E. Wanigasekara, C. Gaeta, P. Neri and D. M. Rudkevich, *Org. Lett.*, 2008, **10**, 1263.
16 The resulting adduct is better viewed as a charge transfer complex between the nitrosonium cation (NO+) and an electron-rich aromatic ring: R. Rathore, S. V. Lindeman and J. K. Kochi, *J. Am. Chem. Soc.*, 1997, **119**, 9393.
17 For the original synthesis of 5,5′-Bicalixarenes see: P. Neri, A. Bottino, F. Cunsolo, M. Piattelli and E. Gavuzzo, *Angew. Chem., Int. Ed.*, 1998, **37**, 166.
18 A. Molad, I. Goldberg and A. Vigalok, *J. Am. Chem. Soc.*, 2012, **134**, 7290.
19 B. B. Ahuja and A. Vigalok, *Angew. Chem., Int. Ed.*, 2019, **58**, 2774.
20 A. Baheti, R. Dobrovetsky and A. Vigalok, *Org. Lett.*, 2020, **22**, 9706.
21 X. Zhao, M. R. Pinto, L. M. Hardison, J. Mwaura, J. Müller, H. Jiang, D. Witker, V. D. Kleiman, J. R. Reynolds and K. S. Schanze, *Macromolecules*, 2006, **39**, 6355.
22 Z. Zhao, H. Zhang, J. W. Y. Lam and B. Z. Tang, *Angew. Chem., Int. Ed.*, 2020, **59**, 9888.
23 L. Xu, Z. Wang, R. Wang, L. Wang, X. He, H. Jiang, H. Tang, D. Cao and B. Z. Tang, *Angew. Chem., Int. Ed.*, 2020, **59**, 9908.
24 P. Wei, Z. Li, J.-X. Zhang, Z. Zhao, H. Xing, Y. Tu, J. Gong, T. S. Cheung, S. Hu, H. H.-Y. Sung, I. D. Williams, R. T. K. Kwok, J. W. Y. Lam and B. Z. Tang, *Chem. Mater.*, 2019, **31**, 1092.
25 Y.-Y. Chen, X.-M. Jiang, G.-F. Gong, H. Yao, Y.-M. Zhang, T.-B. Wei and Q. Lin, *Chem. Commun.*, 2021, **57**, 284.
26 D. Dai, J. Yang and Y.-W. Yang, *Chem. – Eur. J.*, 2022, 28.
27 M. Gao and B. Z. Tang, *ACS Sens.*, 2017, **2**, 1382.
28 K.-W. Lee, H. Chen, Y. Wan, Z. Zhang, Z. Huang, S. Li and C.-S. Lee, *Biomaterials*, 2022, **289**, 121753.
29 J. Qi, L. Feng, X. Zhang, H. Zhang, L. Huang, Y. Zhou, Z. Zhao, X. Duan, F. Xu, R. T. K. Kwok, J. W. Y. Lam, D. Ding, X. Xue and B. Z. Tang, *Nat. Commun.*, 2021, **12**, 960.
30 M. N. Möller and A. Denicola, *Biol. Med.*, 2018, **128**, 137.
31 P. Lhoták, *Org. Biomol. Chem.*, 2022, **20**, 7377.
32 M. Tlusty, H. Dvorakova, J. Cejka, M. Kohout and P. Lhotak, *New J. Chem.*, 2020, **44**, 6490.
33 W. Hüggenberg, A. Seper, I. M. Oppel and G. Dyker, *Eur. J. Org. Chem.*, 2010, 6786.
34 R. Miao, Q.-Y. Zheng, C.-F. Chen and Z.-T. Huang, *J. Org. Chem.*, 2005, **70**, 7662.
35 O. G. Barton, B. Neumann, H.-G. Stammler and J. Mattay, *Org. Biomol. Chem.*, 2008, **6**, 104.
36 F. Elaieb, D. Sémeril, D. Matt, M. Pfeffer, P.-A. Bouit, M. Hissler, C. Gourlaouen and J. Harrowfield, *Dalton Trans.*, 2017, **46**, 9833.
37 S. Chowdhury and P. E. Georghiou, *J. Org. Chem.*, 2002, **67**, 6808.
38 See ESI† for the details.
39 Y. Yoon, J. Song, S. H. Hong and J. Q. Kim, *Clin. Chem.*, 2000, **46**, 1626.
40 E. L. Kanabrocki, M. George, R. C. Hermida, H. L. Messmore, M. D. Ryan, D. E. Ayala, D. A. Hoppensteadt, J. Fareed, F. W. Bremner, J. L. H. C. Third, P. Shiraz and B. A. Nemchausky, *Clin. Appl. Thromb./Hemostasis*, 2001, **7**, 339.
41 C.-W. Wan, A. Burghart, J. Chen, F. Bergström, L. B.-Å. Johansson, M. F. Wolford, T. G. Kim, M. R. Topp, R. M. Hochstrasser and K. Burgess, *Chem. – Eur. J.*, 2003, **9**, 4430.
42 D. Cao, L. Zhu, Z. Liu and W. Lin, *J. Photochem. Photobiol., C*, 2020, **44**, 100371.
43 Naphthalimide and carbazole co-aggregation has very recently been reported in the design of photoredox catalysts: H. Lin, J. Wang, J. Zhao, Y. Zhuang, B. Liu, Y. Zhu, H. Jia, K. Wu, J. Shen, X. Fu and X. Zhang, *Angew. Chem., Int. Ed.*, 2022, **61**, e202117645.

This journal is © The Royal Society of Chemistry 2023