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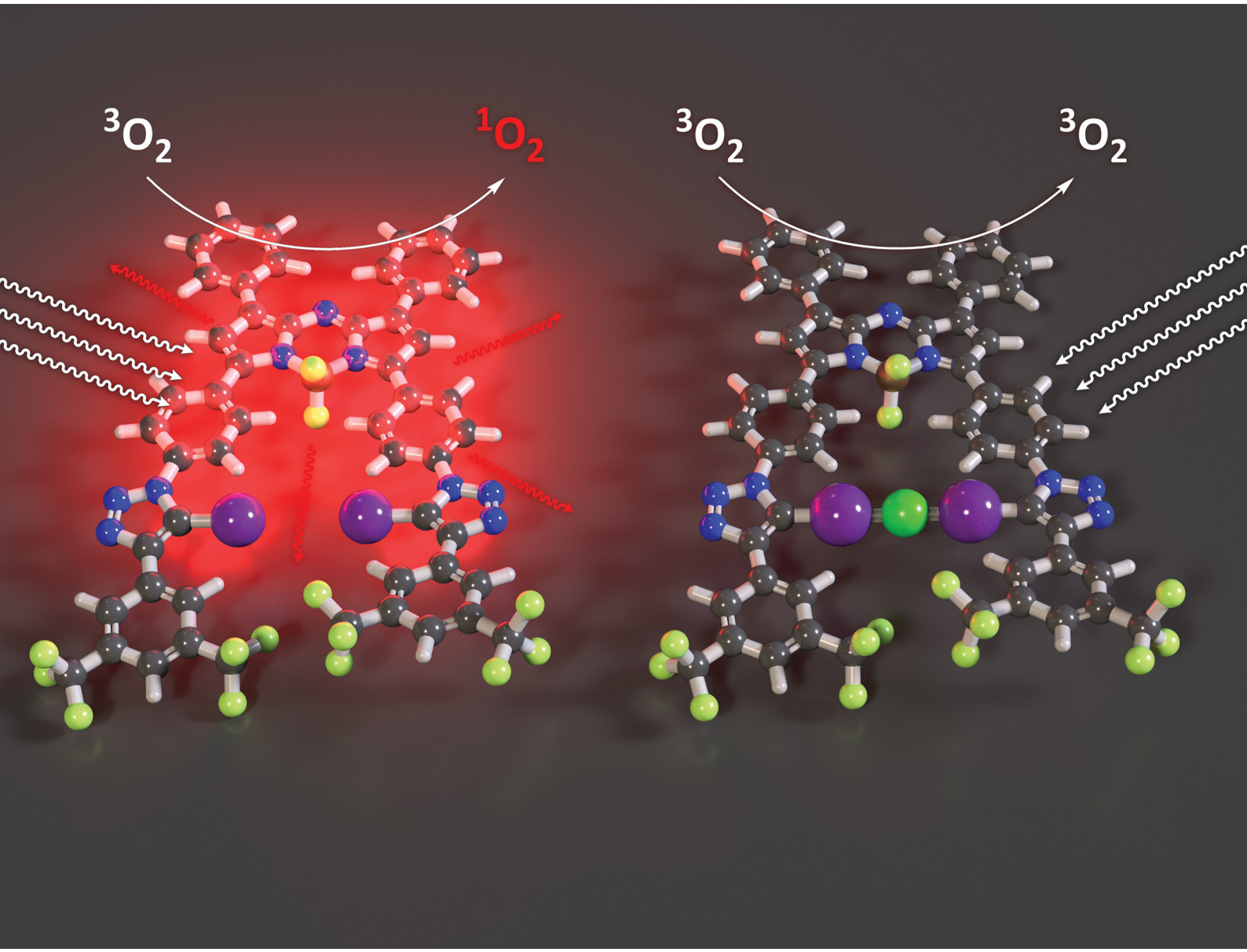

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Halogen bonding aza-BODIPYs for anion sensing and anion binding-modulated singlet oxygen generation†

Andrew J. Taylor and Paul D. Beer *

Two novel aza-BODIPY based anion sensors, decorated with halogen bonding recognition sites, are capable of detecting halide anions at biologically-relevant near-IR wavelengths. With potential application for improving the selectivity of photodynamic therapy agents, unprecedented supramolecular host–guest anion binding-modulated singlet oxygen generation is demonstrated.

Following their first report in 2002,[1] aza-boron-dipyrromethene (aza-BODIPY) compounds have attracted much interest in recent years due to their long-wavelength absorption and emission features and photosensitizing properties,[2,3] which renders them promising photodynamic therapy (PDT) and cellular imaging agents.[4–7]

The combination of favourable optical properties and synthetic flexibility means that aza-BODIPY compounds are propitious candidates for supramolecular sensing materials. Indeed, aza-BODIPY compounds have found application as sensors for alkali metal,[8] mercury,[9] and copper cations,[10] as well as pH sensors.[11–13] Aza-BODIPY based chemodosimeters for anions including NO_2^-,[14] F^-,[15] CN^-,[16] and ClO^- [17] have also been reported, however these compounds do not allow for reversible, supramolecular sensing – which remains exceedingly rare for aza-BODIPY based probes.

Over recent years, halogen bonding (XB) interactions have been exploited for anion recognition due to their advantages in affinity, directionality and selectivity over traditionally employed interactions, such as hydrogen bonding (HB).[18,19] Within the context of optical sensing, XB interactions have additionally shown enhanced signal transduction,[20] leading to the development of an array of optical sensors for anions and other analytes.[21–25] However, optical sensors employing XB interactions for anion recognition which operate at biologically-relevant wavelengths remain scarce, and the use of aza-BODIPY compounds within this context is unprecedented.

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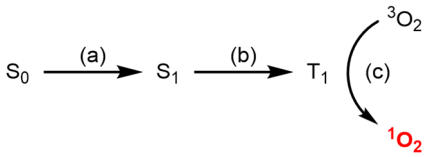


Fig. 1 Photophysical pathway for the generation of $^1O_2$. (a) Excitation, (b) intersystem-crossing, (c) energy transfer.

The use of aza-BODIPY compounds for PDT exploits their ability to generate reactive oxygen species (ROS), in particular singlet oxygen ($^1O_2$). The generation of $^1O_2$ is mediated by the triplet state of the aza-BODIPY fluorophore, which is itself generated by intersystem crossing (ISC) (Fig. 1).[26] The use of heavy halogen atom substituents has been shown to improve the efficiency of ISC and hence $^1O_2$ generation.[4,26] Therefore, the judicious combination of iodine-based XB donor motifs with an aza-BODIPY fluorophore could conceivably facilitate both fluorescent anion sensing and also efficient $^1O_2$ generation. Methods to reversibly modulate the generation of $^1O_2$ are highly sought after, because they allow the selectivity of PDT techniques to be improved.[27] Existing methods have focused on

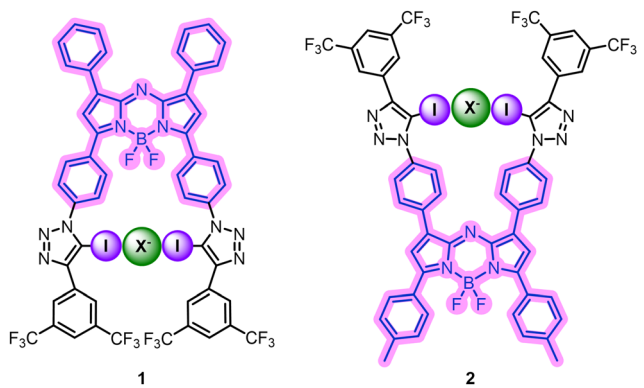
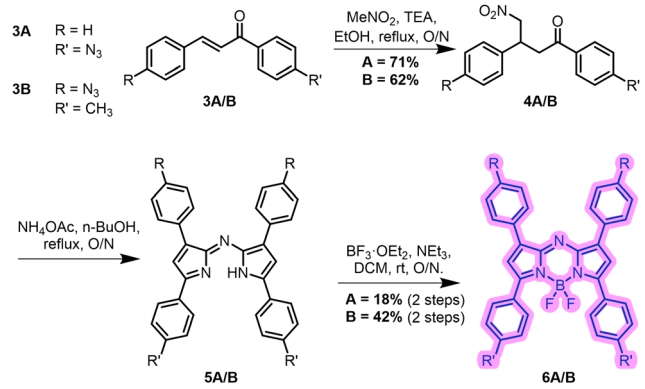


Fig. 2 Structures of the title compounds **1** and **2**, showing the proposed convergent binding mode.

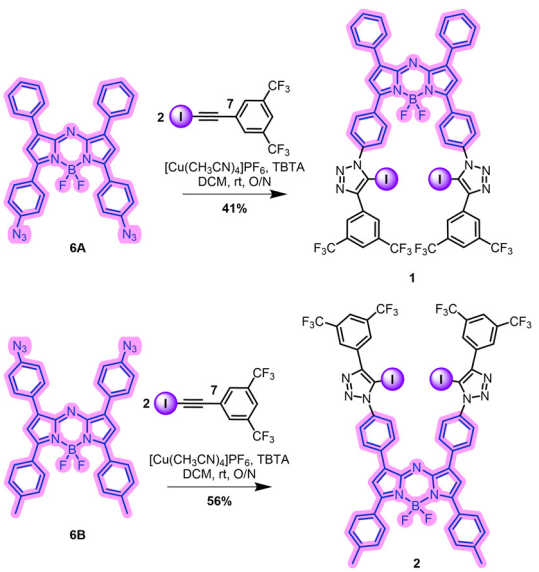


Scheme 1 Synthesis of precursor azides **6A** and **6B**.

activatable photosensitisers which respond, often irreversibly, to stimuli including pH,[28] H_2O_2[29] and thiols.[30] Anion activated photosensitisers are very rare, and previous reports have focused on irreversible activation methods.[31,32]

Herein, we report the synthesis of two novel, XB aza-BODIPY based anion sensors **1** and **2** and investigate the relationship between the structure of the sensor, its anion binding response, and anion affinity. Through optical fluorescence titration studies, we demonstrate that the aza-BODIPY fluorophore displays strong anion binding responses in the near-IR (NIR) wavelength window, which is promising for biological applications. Furthermore, we show that one of the XB aza-BODIPY sensors displays photosensitising ability and demonstrate, in a proof-of-concept, unprecedented reversible anion binding-modulated $^1O_2$ generation, highlighting a potential novel supramolecular host–guest approach for tuning and improving PDT selectivity.

The target anion sensors **1** and **2** feature two iodotriazole XB donor moieties appended to a planar aza-BODIPY core, allowing for anion binding in a convergent configuration (Fig. 2). It was anticipated that anion binding in close proximity to the aza-BODIPY core would induce changes in the fluorophore's emission spectrum, enabling optical sensing. Electron-withdrawing aromatic motifs, bearing $-CF_3$ groups, were used to polarise the iodotriazole XB donors to improve anion binding affinity.[33]



Scheme 2 Synthesis of title compounds **1** and **2**.

Table 1 Photophysical properties of sensors **1** and **2** in acetone

Compound	**1**	**2**
$\lambda_{max,abs}$ (nm)	656	670
ε (M^{-1} cm^{-1})	30 000	33 000
$\lambda_{max,em}$ (nm)	684	701
Stokes shift (nm)	28	31

The synthetic route undertaken to prepare the two XB aza-BODIPY receptors initially targeted the parent aza-BODIPY azides, **6A** and **6B**, beginning from the aldol condensation products **3A** and **3B** (Scheme 1).[34] Michael addition of nitromethane afforded intermediates **4A** and **4B** in 71% and 62% yields respectively.[35] **4A** and **4B** were refluxed in *n*-BuOH with NH_4OAc to afford intermediates **5A** and **5B**. These were used without further purification and the chelation of the BF_2 unit was accomplished *via* treatment of **5A** and **5B** with $BF_3{\cdot}OEt_2$, giving **6A** and **6B** in 18% and 42% yields over two steps respectively.[35] The target XB aza-BODIPY anion receptors **1** and **2** were synthesised in 41% and 56% yield respectively *via* CuAAC methodology, combining **6A** and **6B** with two equivalents of the electron-withdrawing iodo-alkyne **7** (Scheme 2).[36] Novel compounds were characterised by, ^1H, ^{13}C, ^{19}F and ^{11}B NMR, UV-vis spectroscopy and HR-MS (see ESI,† Section S2).‡

Both receptors displayed characteristic aza-BODIPY optical properties,[26] with absorption and emission features in the NIR biologically-relevant wavelength range and typical Stokes shifts (Table 1 and Fig. S28, S29, ESI†).

The anion sensing properties of the receptors were investigated *via* fluorescence studies in acetone, by addition of aliquots of a variety of tetrabutylammonium salts (TBAX, X = Cl, Br, I, HSO_4, OAc, H_2PO_4). Upon addition of halide anions, both receptors exhibited a significant turn-off quenching response with negligible changes in either absorbance or emission wavelength maxima (Fig. 3 and Fig. S30, ESI†), whereas no response was observed upon addition of $TBAHSO_4$ or $TBAH_2PO_4$,§ in line with the frequently observed preference of XB sensors for halide anions.[37]

Receptor **1** displayed a relatively larger turn-off response to all halide anions. Invariably, I^- produced the strongest

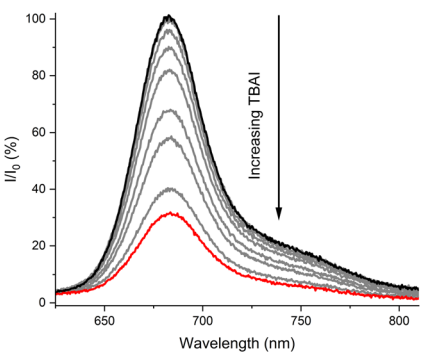


Fig. 3 Normalised fluorescence emission response of 1 μM sensor **1** upon addition of increasing concentrations of I^- (up to 2.8 mM) in acetone at 298 K.

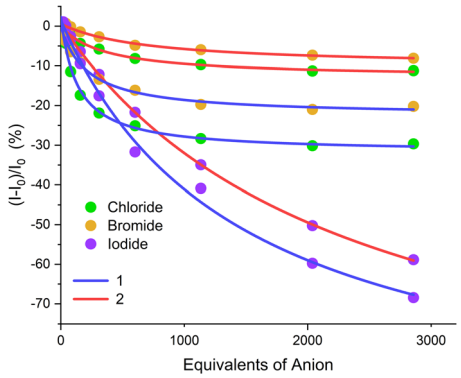


Fig. 4 Representative fluorescence isotherms for the titration of halide anions with receptors **1** (blue) and **2** (red) (1 μM, acetone, 298 K).

Table 2 Halide association constants (M^{-1}) of sensors **1** and **2**

Anion	1	2
Cl^-	7100	2900
Br^-	4100	1200
I^-	730	440

Association constants (M^{-1}) in acetone determined by global fitting to a 1 : 1 binding model. All errors <10%. Acetone, 298 K.

quenching, which was ascribed to a heavy atom effect enhancing ISC, with receptor **1** showing up to a 70% diminishment in fluorescence intensity in the presence of 2.8 mM TBAI (Fig. 4). Cl^- and Br^- produced smaller, but still significant quenching effects.¶ Pleasingly, the receptors remained sensitive to Cl^- in the presence of water in a 9 : 1 acetone/water (*v*/*v*) mixture (the halide anion with the highest hydration enthalpy tested; see Fig. S31 and S32, ESI†).

Global fitting of the fluorescence isotherms to a 1 : 1 stoichiometric binding model determined the association constant values shown in Table 2. It is important to note that the magnitude of the fluorescence turn-off response is not correlated with the strength of anion binding. Indeed, the binding constants indicate that the halide anions are bound with affinities correlating to their charge-density, as expected in organic solvent media.[38] It is noteworthy that receptor **1** exhibited stronger halide binding, which may be due to the convergent orientation of the iodo-triazole motifs of the XB donor binding cavity being relatively closer together and of a more complementary geometry in receptor **1** compared to receptor **2**, determined from a computational study of similar compounds.[39]

Having established the halide anion sensing properties of both receptors, the photosensitising abilities of receptor **1** were investigated, as it exhibits a relatively greater magnitude of fluorescence response. $^1O_2$ generation studies in acetone were conducted with receptor **1**, using diphenylisobenzofuran (DPBF), a dye known to be a trap for $^1O_2$ and other ROS.[40,41] DPBF has a characteristic absorbance at 410 nm that diminishes when it reacts with $^1O_2$, forming non-absorbing products. The rate at which the absorbance of DPBF decreases is therefore correlated to the $^1O_2$ quantum yield of the photosensitising compound under investigation.[42]

In a typical experiment, an acetone solution of receptor **1** (20 μM) and DPBF (30 μM) was irradiated at 625 nm and the absorbance at 410 nm monitored. Receptor **1** proved capable of generating $^1O_2$, shown by the decrease in DPBF absorbance (Fig. 5). No changes in the aza-BODIPY absorbance were observed, indicating the photostability of receptor **1**.

Attention then turned to determining whether anion binding could modulate $^1O_2$ generation efficiency. Cl^- was used, because the halide had shown a large magnitude of fluorescence anion sensing response.‖ In these experiments, an acetone solution of receptor **1** (20 μM), DPBF (30 μM) and TBACl (2 mM) was irradiated and the rate of diminishment in absorbance of the DPBF chromophore was monitored (Fig. 6). Relative to receptor **1** in the absence of any anion, the presence of Cl^- decreased $^1O_2$ quantum yield by 37%. Notably, control experiments showed the non-binding precursor azide **6A** did not demonstrate anion binding-modulated $^1O_2$ generation and the $^1O_2$ generation of receptor **1** was not affected by the presence of the non-coordinating PF_6^- anion, ruling out any non-specific effects (Fig. S33–S35, ESI†).

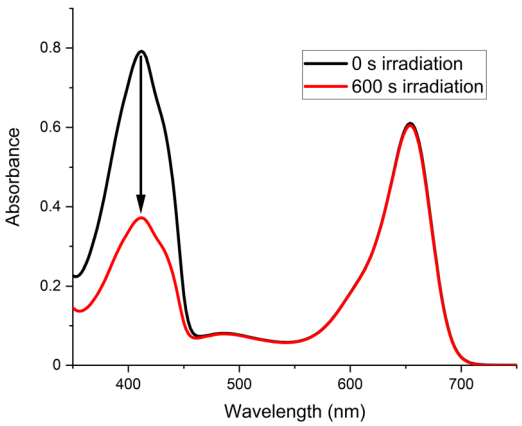


Fig. 5 Absorbance of a solution initially containing DPBF (30 μM) and receptor **1** (20 μM), before and after 600 s irradiation at 625 nm (acetone, 298 K).

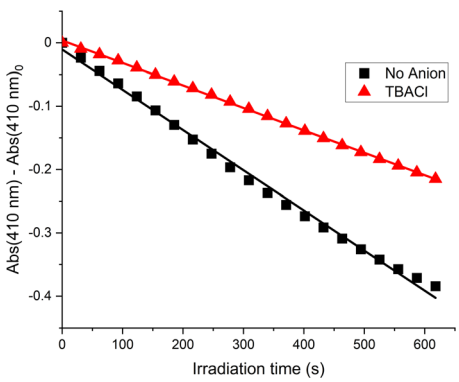


Fig. 6 Plot of DPBF absorbance against irradiation time, in the presence and absence of TBACl (receptor **1**, acetone, 298 K).

Table 3 Fluorescence lifetimes (ns) of sensor **1** with and without 2 mM TBACl

Anion	τ_F
No anion	1.13
Cl^-	1.07

Determined in acetone at 298 K. Errors $<5\%$.

To gain insight into the mechanism of host–guest anion binding-modulated $^1O_2$ generation, measurements of the fluorescence lifetime of receptor **1** in the presence and absence of 2 mM TBACl were conducted (Table 3). The lifetime was not significantly affected by the presence of TBACl, despite the emission intensity decreasing, which indicates the rate of non-radiative decay processes from the excited S_1 state is increased in the presence of TBACl (Section S5, ESI†).[23] Conceivably therefore, these non-radiative decay processes may increasingly compete with ISC upon anion binding, reducing the triplet quantum yield and hence the $^1O_2$ quantum yield, which have been shown to be highly correlated.[26]

In summary, we report the synthesis of two novel XB aza-BODIPY fluorescent anion sensors **1** and **2**, both of which demonstrate significant turn-off quenching responses to halide anion binding, extending the optical range of XB fluorescent sensors to the biologically-relevant NIR window. Notably receptor **1** displayed both larger magnitude fluorescence changes and stronger anion binding, which will inform the design of future aza-BODIPY based anion sensors. Receptor **1** was also shown to act as a photosensitiser to generate $^1O_2$. Importantly, in a proof-of-concept, combining the anion sensing and photosensitising capabilities of receptor **1**, we demonstrate unprecedented supramolecular host–guest anion binding-modulated $^1O_2$ generation, opening a new avenue for improving the selectivity of PDT agents.

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Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ Synthesis of hydrogen bonding analogues of receptors **1** and **2** was attempted, but the products were too insoluble to isolate.
§ Addition of TBAOAc caused decomposition of the receptors.
¶ A dynamic quenching mechanism is most likely, as the absorbance spectrum was unchanged in the presence of anions.
‖ ESI,† Section S5 for discussion of other anions.

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