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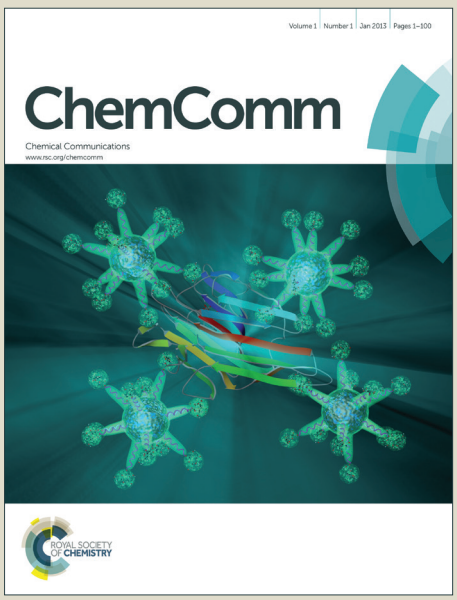

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ARTICLE TYPE

Selective Photosensitization through AND Logic Response: Optimization of pH and Glutathione Response of Activatable Photosensitizers

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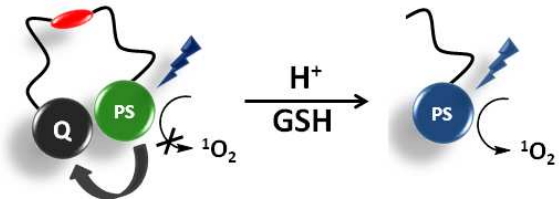
A series of pH and GSH responsive photosensitizers were designed and synthesized. pK_a values were optimized by adjusting the inductive contribution of substituents to reach a pH range (6.0-7.4) relevant to tumour microenvironment. pH-activatable behaviour and redox mediated release of the quencher from the PS by GSH allows the construction of an AND logic operator for selective photodynamic action in aqueous solutions.

The research in molecular logic gates, which was initiated by the seminal work by A. P. de Silva,[1] blossomed in the two decades that followed.[2] In addition, the limitations and the potential of this approach has become more clear. A particularly promising application of molecular logic gates may be in the field of information processing therapeutic agents. Incorporation of Boolean logic ideas in the function of therapeutic agents would be very valuable, if the same results cannot be achieved by random optimization studies. Previously, our group and others provided the early examples of the work in that direction.[3] Our first proof of principle work which linked photodynamic sensitization of a Bodipy based photosensitizer (PS) to the concentrations of sodium ions and the acidity was essentially an AND logic gate, but the system required organic solvents and organic acid to function in the desired manner. While it was considered to be noteworthy, for that approach to have practical potential, AND logic gate based enhanced selectivity should be related to cancer related biological parameters, which can generate significant changes in the photophysical character of the sensitizer in aqueous solutions.

In this work, we took advantage of two characteristics of the tumour microenvironment, lower pH, and higher glutathione concentrations.[4] Difference in pH of cancer tissue and healthy tissue is easily accessible parameter to use in therapeutic activation. A number of pH-responsive polymeric materials, photosensitizers, nanocarriers were studied to control drug release or activation.[5] However, extracellular pH of tumor cells drops to a value not below 6.0.[6] Thus, it is challenging to find a smart therapeutic system responsive to pH within the narrow near neutral range and essentially become active at around pH 6.0-6.5 and stay inactive above pH 7.0. Apart from some,[7] most related works in literature depend on activation at pH below 5.5 which actually requires nonselective lysosomal activation.[8] In this work, properties of the PS is optimized for pH activatability by making rational chemical modification on the pH responsive moiety with electron donating or withdrawing groups to adjust the pK_a to the desired near-neutral value and to get enough spectral shift in acidic aqueous solutions such that protonated PSs are exclusively excited species under the conditions of interest. Thus, the overall design (Scheme 1) involves a pH responsive unit, linked to a quencher, which could be cleaved at elevated GSH concentrations.



Scheme 1 Schematic representation of PS activation by acid and GSH. Protonation causes a spectral shift at near neutral pH to enhance PS excitation by light of specific wavelength, whereas GSH liberates PS from quencher module by reductive cleavage of the disulfide linker.

Previously, GSH has been used as a PS activator mostly through the cleavage of disulfide bond[9] or through reactions with dinitrophenyloxy-tethered moiety.[10] We used redox mediated cleavage of disulfide bond with GSH as an additional mode of activation of the photosensitizer, and attached an electronic energy acceptor module to PS, *via* disulfide bridge to quench the $^1O_2$ production, thus construct an AND molecular logic gate on the PS activation with the other input being acid (Scheme 1).

For both PS and quencher modules, Bodipy derivatives are chosen, since fine tuning the spectral properties and analyte responsiveness of these Bodipy dyes are straightforward as a result of their versatile chemistry.[11]

A spectral shift at the wavelength of excitation upon protonation would be ideal for the photosensitizer to be reversibly activated only in the acidic conditions, as we have previously demonstrated.[12] In order to optimize the pK_a values, a series of water soluble PSs have been synthesized. The structures of the compounds are given in Scheme 2, the complete chemical structures can be found in the ESI.

In order to impart GSH responsiveness, a near-IR absorbing energy acceptor Bodipy dye with an appropriate spectral character for EET is attached to the PS through bioreducible

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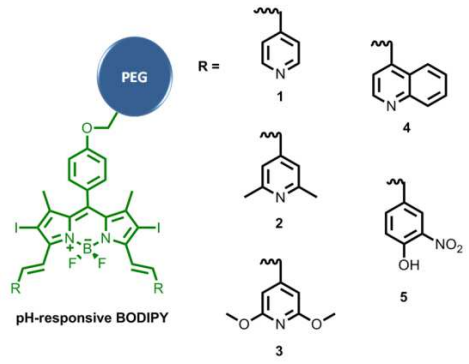
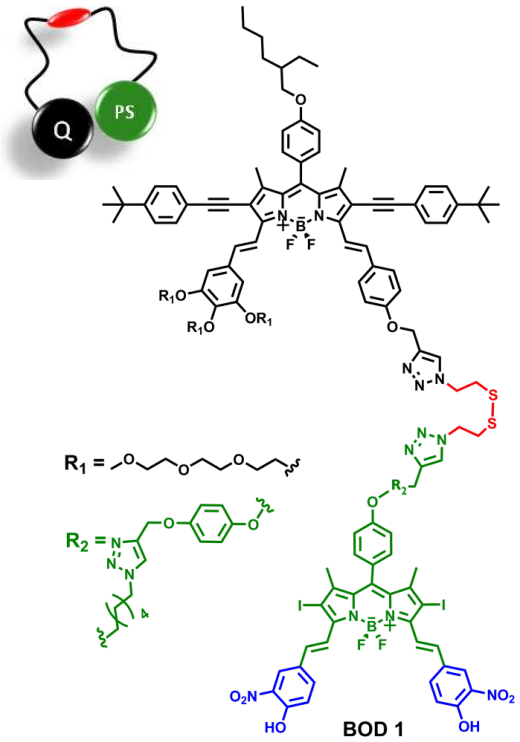
disulfide linker (Scheme 3, black module). To provide relatively milder reaction conditions, the quenching module and the PS are attached to one another through disulfide bridge using copper



Scheme 2 Structures of distyryl-BODIPYs bearing different pH-sensitive groups with polyethylene glycol (PEG) solubilising module depicted in blue.

catalysed Huisgen 1,3-dipolar cycloaddition. The Bodipy dye which was employed as an energy sink, was prepared by the Sonogashira coupling at 2,6-positions followed by Knoevenagel condensation.



Scheme 3 Chemical structure of AND logic construct of photosensitizer BOD 1 with GSH (red) and pH (blue) responsive moieties.

Water soluble distyryl-BODIPY was synthesized through condensation with appropriate aldehydes (e.g., with 4-pyridinecarboxaldehyde for compound **1**). The pK_a value for **1** was determined to be 3.42 with a protonation-induced bathochromic shift from 594 nm to 615 nm (Table 1, Figure S1). In addition to an insufficient spectral shift, compound **1** is not basic enough to be protonated in target biological media.

A list of spectral shifts (on protonation) for all compounds and summary of the data obtained with calculated pK_a values are given in Figure S1 and Table 1.

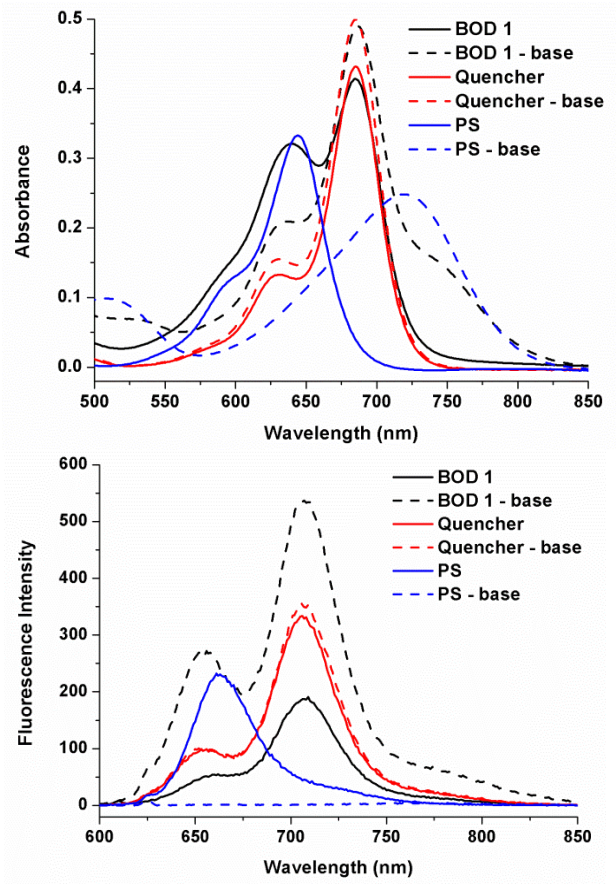


Fig. 1 Electronic absorption (top) and emission (bottom) spectra of 7.50 μM BOD 1 (black), Quencher module (red) and PS module (blue) in THF. Dashed spectra are recorded after addition of base (piperidine) and fluorescence spectra are recorded by excitation at 625 nm.

Since the desired pH-responsive behaviour cannot be reached with pyridine or quinolone derivatives we turned our attention to phenolic groups. In literature, monostyryl-Bodipy with a 3-chloro-4-hydroxyphenyl substituent was reported to have a pK_a of 7.6.[13] As a final attempt, with the same strategy to adjust pK_a through changing inductive/resonance effects, another variation of this phenolic substituent with a stronger electron withdrawing group was targeted with an expectation of decreased the pK_a. Compound **5, BOD 1** and **PS** with a nitro group in place of chloro is synthesized with these considerations (Scheme 1, Scheme S1).

PS is the non-water soluble module of the photosensitizer part of **BOD 1**, exact chemical structure of which is given in supporting information (Scheme S1, green-blue module in Scheme 2). The pH response of **PS** is investigated within a micelle in aqueous solutions, since this compound and the final AND logic gate construct is not soluble in water. Fortunately, in accordance with our expectations, the compound was determined to have a pK_a of 6.92 in Cremophor EL micelles, with a very large spectral change (+81 nm) in absorbance from 649 nm to 730 nm as a result of deprotonation (Figure 1, S2). The spectral data clearly shows that, at the wavelength of light used for PDT measurements (625 nm, indicated with blue dashed line in Figure S2), deprotonated

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compounds have substantially decreased absorbance at the selected wavelength of excitation (625 nm), which ensures selective activation of PDT agent only in acidic solutions.

In order to investigate if the pH response is preserved in non-micellar system, a water soluble version (compound **5**) is made and titrated in 40% THF in water. The pK_a was determined to be 6.62 (Table S1). 0.30 unit difference may result from the fact that, relatively more hydrophobic microenvironment within the micelle may alter the deprotonation due to the fact that the charged species cannot solvated easily in micelle microenvironment. The absorption spectrum of deprotonated compound is essentially the same as it is in micelles, except the minor broadening of the peaks. Absorption spectra of these two compounds are given in Figure 1, S2, S4 and Table S2. With the promising pK_a value obtained, pH dependent component of the molecular AND logic gate is built with distyryl-BODIPYs generated through Knoevenagel condensation reaction with 4-hydroxy-3-nitrobenzaldehyde (Scheme 3).

The electronic absorption spectrum of micellar **BOD 1** in water is given in Figure S5a. Two peaks corresponding to two chromophore modules converge to give an essentially single peak at higher wavelength upon deprotonation, since **PS** shows a bathochromic shift under the conditions applied, whereas quencher module remains the same. For equal concentrations of compounds **PS** and **BOD 1**, emission spectra shows a decrease in the emission of photosensitizer part of **BOD 1** compared to free photosensitizer, **PS** which is an indication of energy transfer (Figure S5b). Since the deprotonated form of free PS is non-emissive, the same spectral analysis cannot be performed for this form. The cleavage of the disulfide bond is analyzed by incubating the micellar **BOD 1** for 12 hour at room temperature with 2.5 equivalents of GSH and comparing it with the GSH-free **BOD 1** both *via* spectroscopic analysis and High Resolution Mass Spectra (HRMS). The thiol form, GSH-conjugate of free photosensitizer and both reduced and disulfide forms of quencher are detected by HRMS after 12 h incubation (Figures S6).

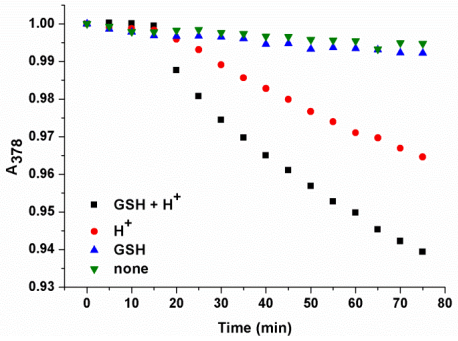

Fig. 2 Comparison of $^1O_2$ generation of micellar forms of molecular AND logic construct (7.50 μM) in the presence of different combinations of inputs as followed by the decrease in $^1O_2$ trap absorbance at 378 nm in water. For the first 15 min, all the samples were kept in dark, followed by irradiation with a 625 nm LED. Acidic solutions are adjusted to pH 6.00.

After resolving the reduction of the disulfide linker by GSH through HRMS analysis, spectral examination was also performed to demonstrate excitation energy transfer (EET). Since the EET efficiency is expected to decrease upon release of the energy donor part, the emission of this part is predicted to increase. An increase in emission of the PS part is clearly observed in fluorescence spectra after GSH treatment (Figure S7) which indicates that the EET is less effective in the free form.

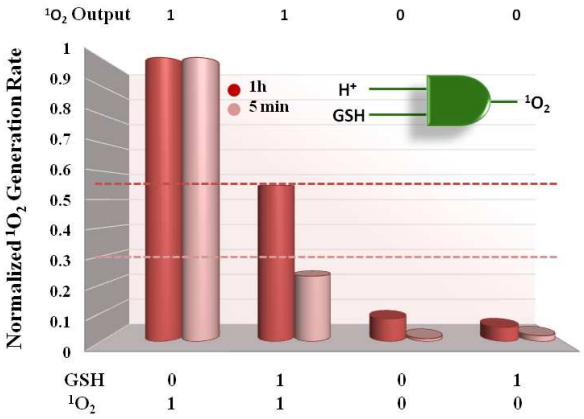

Fig. 3 Comparison of initial $^1O_2$ generation rate of **BOD 1** as measured by the percent decrease in absorbance of trap molecule within 5 min (pink) or 1h (red) of 625 nm light irradiation. 5 minute data is more relevant as the reaction with trap depletes available dissolved oxygen.

$^1O_2$ generation experiments were performed with water soluble $^1O_2$ trap (ESI) and decrease in the absorption at 378 nm was followed as a measure of $^1O_2$ production rate. First, to show that the trap molecule does not decompose in the absence of photosensitizer, control experiments under dark and 625 nm irradiation, were performed in similar conditions using **PS**-free solutions. The trap is stable under experimental conditions (Figure S9). On the other hand, the photosensitizer free from quencher shows a greater extent of $^1O_2$ generation in the presence of slightly acidic media, Figure 2. Although **BOD 1** produces $^1O_2$ to some extent in the absence of GSH, still this is less efficient compared to free PS. The results are depicted as relative initial $^1O_2$ generation rate in Figure 3 as determined by percent decrease of trap absorption at 378 nm for each experimental condition. The threshold value of $^1O_2$ generation efficiency for AND logic gate was set as 0.30 and 0.55 for initial 5 min irradiation and 1h irradiation respectively. Thus, the PS produces $^1O_2$, only in the presence of both inputs acid and GSH.

In this work, a viable alternative for enhanced selectivity for photodynamic action was provided. The designed PS is responsive to acidity comparable found in the tumor regions and higher GSH. Acid induced change in the absorption of the PS allows an increase of extinction coefficient at the wavelength of excitation and thus prepares it for activation. However, an energy acceptor conjugated to the PS via a reducible disulfide bond still quenches the excited state through energy transfer. Singlet oxygen generation activity of the PS was thus, shown to be significantly enhanced only when both cancer related inputs are available at above threshold values. Such AND logic constructs based on cancer related parameters as inputs, should be expected to yield more selective therapeutic agents.

Notes and references

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† Electronic Supplementary Information (ESI) available: Additional analytic and spectral data, and synthesis procedures.

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1 A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, Nature, 1993, 364, 42.

2 A. P. De Silva and S. Uchiyama, *Nature Nanotech*. 2007, **2**, 399; J. Andréasson and U. Pischel, *Chem. Soc. Rev*., 2010, **39**, 174; K. Szaciłowski, *Chem. Rev*., 2008, **108**, 3481; U. Pischel, J. Andréasson, D. Gust and V. F. Pais, *ChemPhysChem*, 2013, **14**, 28; J. Andreasson and U. Pischel, *Chem. Soc. Rev*. 2015, **44**, 1053; B. Rout, P. Milko, M. A. Iron, L. Motiei, D. Margulies, *J. Am. Chem. Soc*. 2013, **135**, 15330.

3 S. Ozlem and E. U. Akkaya, *J. Am. Chem. Soc.* 2009, **131**, 48; S. Erbas-Cakmak, O. A. Bozdemir, Y. Cakmak and E. U. Akkaya, *Chem. Sci.* 2013, **4**, 858; S. Erbas-Cakmak and E. U. Akkaya, *Angew Chem. Int. Ed.* 2013, **52**, 11364; R. J. Amir, M. Popkov, R. A. Lerner, C. F. III. Barbas and D. Shabat, *Angew. Chem. Int. Ed.* 2005, **44**, 4378; S. Angelos, Y. –W. Yang, N. M. Khashab, J. F. Stoddart and J. I. Zink, *J. Am. Chem. Soc*. 2009, **131**, 11344; K. –W., Y. E. Kim, V. Bocharova, J. Halamek, C. –W. Lee, E. Katz, M. –K. Oh, *Chem. Commun*. 2012, **48**, 6918; J. Wang, E. Katz, *Isr. J. Chem*. 2011, **51**, 141; V. Bocharova, O. Zavalov, K. MacVittie, M. A. Arugula, N. V. Guz, M. E. Dokukin, J. Halamek, I. Sokolov, V. Privman, E. Katz, *J. Mater. Chem*. 2012, *22*, 19709; M. Ikeda, T. Tanida, T. Yoshii, K. Kurotani, S. Onogi, K. Urayama, I. Hamachi, *Nat. Chem*. 2014, **6**, 511; I. Takashima, R. Kawagoe, I. Hamachi, A. Ojida, *Chem. Eur. J*. 2015, **21**, 2038; T. Konry, D. R. Walt, *J. Am. Chem. Soc*. 2009, **131**, 13232; M. N. Stojanovic,T. E. Mitchell, D. Stefanovic, *J. Am. Chem. Soc*. 2002, **124**, 3555.

4 R. A. Cairns, I. S. Harris, T. W. Mak, *Nat. Rev. Cancer*, 2011, **11**, 85.

5 J. F. Lovell, T. W. B. Liu, J. Chen and G. Zheng, *Chem. Rev.* 2010, **110**, 2839.

6 R. A. Gatenby and R. J. Gillies, *Nat. Rev. Cancer,* 2004, **4**, 891.

7 X. –J. Jiang, P. –C. Lo, Y. –M. Tsang, S. –L. Sin-Lui Yeung, S. –L.; W. –P. Fong and D. K. P. Ng, *Chem. Eur. J.* 2010, **16**, 4777; M. –R. Ke, D. K. P. Ng and P. –C. Lo, *Chem. Commun*. 2012, **48**, 9065.

8 J. A. Mindell, *Ann. Rev. Phys*. 2012, **74**, 69.

9 J. T. F. Lau, X. –J. Jiang, D. K. P. Ng, and P. –C. Lo, *Chem. Commun*. 2013, **49**, 4274; L. Li, M. Nurunnabi, M. Nafrujjaman, Y. Y. Jeong, Y.-K. Lee, K. M. Huh, *J. Mater. Chem B*, 2014, **2**, 2929; Y. Cho, Y. Choi, *Chem. Commun*. 2012, **48**, 9912; H. Kim, S. Mun, Y. Choi, *J. Mater. Chem. B*, 2013, **1**, 429.

10 J. Bhaumik, R. Weissleder, J. R. McCarthy, *J. Org. Chem*. 2009, **74**, 5894; I. Simsek Turan, F. Pir Cakmak, D. C. Yildirim, R. Cetin-Atalay, E. U. Akkaya, *Chem. Eur. J*. 2014, **20**, 16088.

11 A. Loudet, K. Burgess, *Chem. Rev*. 2007, **107**, 4891; G. Ulrich, R. Ziessel, A. Harriman, *Angew. Chem. Int. Ed*. 2008, **47**, 1184; O. Buyukcakir, O. A. Bozdemir, S. Kolemen, S. Erbas, E. U. Akkaya, *Org. Lett.* 2009, **11**, 4644; O. A. Bozdemir, S. Erbas-Cakmak, O. O. Ekiz, A. Dana, E.U. Akkaya, *Angew. Chem. Int. Ed*. 2011, **50**, 10907; S. Erbas-Cakmak, E. U. Akkaya, *Org. Lett*. 2014, **16**, 2946; Y. Cakmak, S. Kolemen, S. Duman, Y. Dede, Y. Dolen, B. Kilic, Z. Kostereli, L. Tatar Yildirim, L. Dogan, E.U. Akkaya, Angew. Chem. Int. Ed. 2011, **50**, 11937; R. Guliyev, S. Ozturk, Z. Kostereli, E. U. Akkaya, *Angew. Chem. Int. Ed*. 2011, **50**, 9826; Y. Cakmak, T. Nalbantoglu, T. Durgut, E. U. Akkaya, *Tett. Lett*. 2014, **55**, 538; O. A. Bozdemir, R. Guliyev, O. Buyukcakir, S. Selcuk, S. Kolemen, G. Gulseren, T. Nalbantoglu, H. Boyaci, E.U. Akkaya, *J. Am. Chem. Soc*. 2010, **132**, 8029; A. Atilgan, E. Tanriverdi Ecik, R. Guliyev, T. B. Uyar, S. Erbas-Cakmak, E. U. Akkaya, Angew. Chem. Int. Ed. 2014, 53, 10678.

12 E. Deniz, G. C. Isbasar, O. A. Bozdemir and E. U. Akkaya, *Org. Lett.* 2008, **10**, 3401.

13 W. Qin, M. Baruah, W. M. De Borggraeve and N. Boens, *J. Photochem. Photobiol. A* 2006, **183**, 190.

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