Volume 1 | Number 1 | Jan 2013 | Pages 1–100

ChemComm

Chemical Communications
www.rsc.org/chemcomm

ROYAL SOCIETY OF CHEMISTRY

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard **Terms & Conditions** and the **Ethical guidelines** still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

www.rsc.org/chemcomm

Journal Name

ARTICLE

Near IR Excitation of Heavy Atom Free Bodipy Photosensitizers Through the Intermediacy of Upconverting Nanoparticles

Seda Demirel Topel,[a] Gunseli Turgut Cin[a] and Engin U. Akkaya*[b,c]

Orthogonal dimers of Bodipy were recently shown to be efficient generators of singlet oxygen. However, these dyes require green light for excitation, which would be very quickly attenuated inside the mammalian tissues. We now demonstrate that when these dyes are covalently attached to UCNPs, near IR irradiation results very efficient generation of singlet oxygen.

Recent years witnessed an exponentially growing interest in a class of compounds known as Bodipy dyes (a.k.a., boradiazaindacenes or borondipyrrin dyes).[1] It is remarkable that every single member of the tricyclic boradiazaindacene ring system, including the components of the difluoroboron bridge can be derivatized, substituted or otherwise functionalized in a straightforward manner, yielding new spectral features, including red shifted absorption and emission,[2] or large intersystem crossing rates.[3] The latter is of particular importance if these dyes are to be proposed as potential singlet oxygen generators in a photodynamic therapeutic regime. Photodynamic therapy (PDT) is a non-invasive treatment modality for certain cancers and non-cancerous disease states.[4] The treatment is based on the cytotoxicity of singlet oxygen at the region where it is generated, following sensitization of the dissolved oxygen by a dye with high intersystem crossing efficiency. There is an important caveat however; the dye has to have a strong absorption in the red or near IR region of the spectrum, otherwise the excitation of the dye will be highly ineffective beyond the first few millimeters of the tissues.[5] This is due the fact that, mammalian tissues are highly scattering and absorbing for light with wavelengths shorter than 630 nm.[6]

Historically, porphyrins and derivatives were considered as the photosensitizers of choice for this purpose, but the extinction coefficients in the therapeutic window are not very high. Over the years, a number of classes of compounds were proposed as PDT photosensitizers, and within the last few years Bodipy dyes are being considered as viable alternatives to established examples.[7] Although the parent dye absorbs in the middle of the visible region, it is possible to move the absorption band up to near IR region with straightforward transformations.[8]

Parent (unmodified) Bodipy dyes typically are not effective photosensitizers. As a general rule, heavy atom substitution on the π-system results in enhanced intersystem crossing.[9] However, the incorporation of heavy atoms for photosensitizers geared for photodynamic therapy wouldn't be the optimal choice, because while the sensitization of molecular oxygen is very likely with this approach, increased dark toxicity is typically an undesired outcome. Dark toxicity limits the inherent selectivity of photodynamic therapy agents, which rests on the fact that PDT agent is expected to be non-toxic unless/until it is at the right location for absorbing the light of excitation.

In our earlier work,[10] followed up by computational studies,[11] and later corroborated by Zhang,[12] it was demonstrated that orthogonal-Bodipy dyes, whether connected in a 8,2- or 8,8- fashion, resulted in a very peculiar first excited states with tetraradicalic character and had very high propensity to undergo intersystem crossing. This finding was noteworthy, because in principle, the design is applicable to essentially all other chromophores, offering an alternative to heavy atom substitution as a safe bet for enhanced intersystem crossing (another approach for heavy-atom free photosensitization was developed by Zhao and co-workers[13]). While the detailed photophysical experimentation eliminating other possibilities, such as singlet fission is awaited, one limitation expressed in the previous work,[10] was that the dimeric Bodipy dyes needed excitation around 500 nm, however for true phodynamic therapy potential, the excitation should be done in the therapeutic window, i.e., between 650-1000 nm. With Bodipy dyes, conjugation extension by styryl modification has been shown to yield bathochromic shifts in the absorption band, but unfortunately, computational and experimental work showed that the excited state loses its tetraradicalic character in the longer wavelength absorbing derivatives of the dimeric chromophores, becoming much less efficient singlet oxygen generators.

In consideration of this, we investigated the possibility of using upconverting nanoparticles (UCNPs) as a solution to this problem. UCNPs can be excited with near IR irradiation and depending on the

exact composition, emission at shorter wavelength regions of the spectrum is obtained. UCNPs have this remarkable properties due to the presence of trivalent lanthanide ions as dopant (as absorbers and emitters) in an inorganic matrix.[14] This combination provides a multitude of metastable states with relatively long lifetimes. The main difference between two-photon absorbance (TPA) and upconversion is that, in upconversion, the virtual states of TPA, are replaced by actual states, which are long-lived enough to be excited once more, under intense irradiation. The typical emitters are erbium, thulium and holmium ions due the availability of equally spaced energy levels which would be optimal for absorption and energy transfer processes.[15]

The first goal of the present work was to synthesize appropriately derivatized orthogonal Bodipy dimers (Scheme 1). First of all, a positive control compound **1** was synthesized (ESI†). This compound has two iodo substituents on the Bodipy core, ensuring good singlet oxygen generation on excitation in the presence of dissolved molecular oxygen. Nagano,[16] and others[17] demonstrated that such halogenated Bodipy compounds are indeed efficient photosensitizers.

As for the dimeric compounds, we have previously reported the synthesis of the dimeric parent compounds,[10] but for covalent attachment, additional functionalization is needed. In the orthogonal dimer **2**, carboxylate anchor is placed on the *meso*-phenyl substituent. Formylation and the construction of the second Bodipy core in the usual manner, yields the desired compound. The other dimer **3**, carries the carboxylate on the 2-position of a Bodipy core, which is easily placed by a reaction with acrylic acid under palladium(II) catalysis (ESI†). Absorption spectra of the Bodipy compounds show peaks, in the visible region. Compound **1** shows a major peak at 638 nm, red shifted due to dimethylaminostyryl conjugation. The dimers **2** and **3** show a major band at peaking at 508 and 509 nm, respectively, corresponding to S_0-S_1 transitions and the bands in the visible region are somewhat broadened. The peak shapes and positions are not affected by concentration changes, eliminating the possibility of any aggregation induced changes for spectral differences.

There are many successful techniques for the preparation of lanthanide-doped upconversion luminescence NPs, such as coprecipitation[18] and thermal decomposition.[19] However, these methods involve organic solvent or high temperatures and usually require thermal after treatment or produce toxic byproducts.[20] Therefore, in this work, we preferred to prepare upconverting nanoparticles by solvothermal method[21] which employs milder conditions and water based reaction medium leading to the formation of polyethyleneimine (PEI) covered nanoparticles for further functionalization with BODIPY derivatives. PEI is biocompatible[22] and PEI-nanoparticles are soluble in aqueous systems. It has been reported that free PEI can be toxic to cells, but when it is in in particulate form, the hazardous effects are greatly decreased.[23] When used for DNA delivery PEI was reportedly non-toxic at concentrations used in that study.[24] In principle, the functional groups or water solubility conferring groups can be incorporated during synthesis or can be placed by post-synthetic modifications. We opted for *in situ* preparation which includes the addition of polyethyleneimine (PEI) groups during the nanoparticle synthesis. The synthesis procedure of the upconverting nanoparticles is presented in detail in ESI. The size and morphology of the UCNPs were examined by TEM which shows an average size of 35 nm and this size distribution was also determined by DLS measurements. TEM-EDAX spectrum shows that the rare earth cations were successfully doped into the UCNPs (Fig.1). When excited using near IR laser at 980 nm (fluence rate 50 mW/cm²),
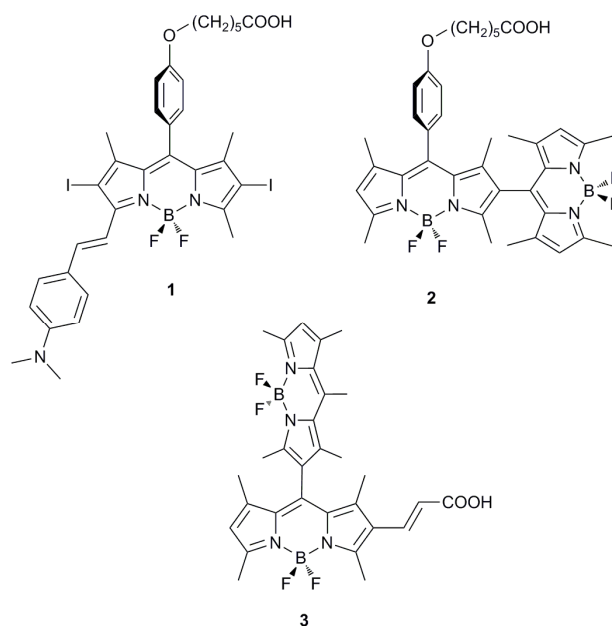
Scheme 1 Strucures of the carboxy-functionalized sensitizers: styryl-Bodipy **1**, orthogonal Bodipy dimers **2**, and **3**.
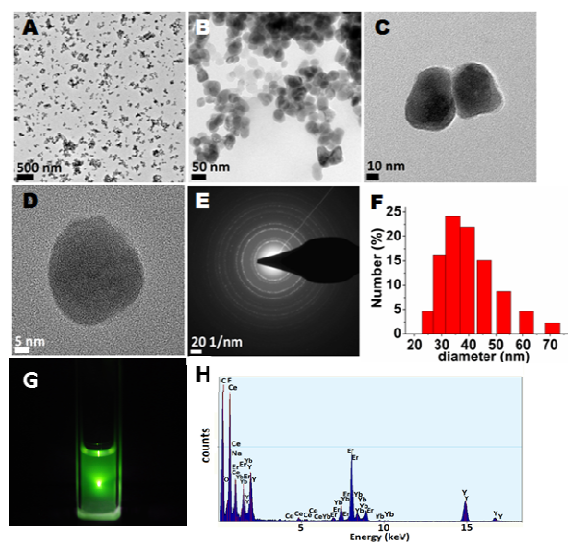

Fig. 1 TEM images of PEI-coated NaYF₄:(Yb⁺³,Er⁺³)/Ce⁺³ nanoparticles (A-D), E: SAED (Selected area electron diffraction) pattern, F: Histogram of the particle size distribution from DLS measurements, G: Emission from UCNP solution in methanol (1.0 mg/1.0 mL) on excitation with 980 nm laser, H: TEM-EDAX spectrum of the PEI-coated UCNP.

green emission from a solution containing these nanoparticles is clearly observed which is composed of two main peaks at 540 and 654 nm (Fig. S28, ESI† and Fig.1G). Further characterization at this stage, was done by acquiring XPS and XRD data (Fig.S26, Fig.S25, ESI,†respectively). XPS shows a particular peak at 402 eV, clearly belonging to N (1s) which corroborates that UCNP was covered by PEI *in situ*. XRD spectrum also shows that UCNPs are highly crystalline form including both hexagonal and cubic phase (Fig. S25, ESI†).

For UCNP functionalization, the photosensitizers have to be prepared in an appropriately derivatized form. Orthogonal Bodipy dimer and styryl-Bodipy functionalization are shown in Scheme S1, ESI. Detailed synthesis procedures are provided, here it will suffice to say that orthogonal Bodipy dimers with such remarkable excited state properties proved to be amenable to modifications leading to derivatives with conjugatable side groups. The next step was to conjugate these dyes on to the PEI coated UCNPs. This step proceeded smoothly, leading to sensitizer dye functionalized UCNPs (ESI, Fig. S37) in the presence of DCC/ DMAP coupling reagents.

BODIPY conjugated nanoparticles obtained this way, were first characterized physically. Following attachment of the BODIPY dyes to the UCNPs, no appreciable changes in the size was observed based on TEM analysis and also TEM image (Fig. 2B) shows that there are fringes on the UCNPs, which prove that crystallinity is preserved, this also corroborated with the SAED image (Fig. 2C). EF-TEM (Energy Filtered-TEM) map shows (Fig. 2, bottom three pictures) a halo of boron nuclei around the nanoparticles following the conjugation procedure.
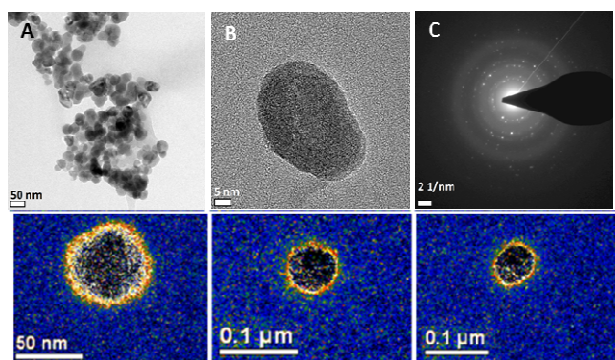

Fig. 2 TEM images (A, B) SAED (Selected Area Electron Diffraction) image (C) and EF-TEM Boron mapping of BOD**1**-UCNP, BOD**2**-UCNP and BOD**3**-UCNP, left to right. Orange halo indicates Boron nuclei (bottom three pictures).

On the other hand, FTIR spectrum also support covalent modification: there are new peaks at 1692-1686 cm^{-1} (1st amide bond, ν(C=O)), 1665-1656 cm^{-1} (2nd amide bond, ν(N-H)) and 1428-1422 cm^{-1} (3rd amide bond, ν(C-N)) attesting to the formation of the amide functionality between BODIPY dyes and UCNP. Before the conjugation reaction (i.e., PEI-covered UCNP) the nanoparticles show bands corresponding to the internal vibration of NH$_2$ bands at 1630,1420 cm^{-1} and CH$_2$ stretching at 2930 and 2850 cm^{-1} (Fig. S27, ESI†).

The emission wavelength of UCNPs matched with the absorption wavelength of BODIPY dyes resulting in reasonable spectral overlap, and since the distance between the core and the dyes is short, efficient energy transfer is to be expected on excitation of the UCNPs using 980 nm laser (ESI, Fig. S38).

Finally, we wanted to demonstrate the efficiency of singlet oxygen generation with specifically designed Bodipy dyes attached covalently to the UCNPs. The generation of singlet oxygen was monitored by using 1,3-diphenylisobenzofuran (DPBF) as a trap.[25] When DPBF reacts with singlet oxygen, it is initially converted to its endoperoxide form, and then further transformed through ring opening into 1,2-dibenzoylbenzene, resulting in a decrease of the absorbance peak of DPBF. Therefore, singlet oxygen production rate can be assessed following the change in the absorption of DPBF at the wavelength of absorption maximum ($\sim$ 410 nm) (Fig. S30-32, ESI†).

The procedure we followed for studying the relative rates of singlet oxygen generation with functionalized UCNPs (Fig. S39) is as follows: All BODIY conjugated UCNPs were exposed to NIR laser (50 mW/cm^2) for 36 min (following 9 min dark incubation to eliminate any source for absorbance change due to a dark reaction). As expected, the adsorption of DPBF in the iodinated BOD**1**-UCNP ($\sim$26%) showed a decrease, but dimeric BOD**2**-UCNP showed a rapid decrease ($\sim$36 %) during irradiation. The smallest change in the absorbance was observed in BOD**3**-UCNP ($\sim$23%). These results show that Bodipy dyes were effectively sensitized by the energy transfer from UCNPs. It is suprising that even a minimal overlap between the UCNP emission and the Bodipy emission in BOD**2**-UCNP is sufficient for effective sensitization. This is probably due to large extinction coefficients of the orthogonal Bodipy dyes and short distance between the dye and the particles.

In order to eliminate other possibilities for singlet oxygen generation, control experiments were carried out as follows: unmodified UCNPs and Bodipy dyes alone were subjected to under near IR irradiation (980 nm) separately, no singlet oxygen generation (Fig. S33, S34 ESI†) was observed under either of these conditions.

In summary, we were able to show conclusively that UCNP-conjugated orthogonal Bodipy dimers which require excitation around 500 nm were successfully excited by much more tissue penetrating 980 nm laser through the intermediacy of upconversion process. Thus, Bodipy dimers with no heavy atoms can be considered as potential photosensitizers with practical utility when used in conjunction with UCNPs. Work towards making biocompatible nanoparticles with sensitizer functionality, aiming this particular goal is in progress in our laboratory.

Notes and references

^{a} Department of Chemistry, Faculty of Science, Akdeniz University, 07058, Antalya, Turkey

^{b} UNAM-National Nanotechnology Research Center, Bilkent University, 06800, Ankara, Turkey.

^{c} Department of Chemistry, Bilkent University, 06800, Ankara, Turkey.

1   (a) A. Kamkaew, S.H. Lim, H.B. Lee, L.V. Kiew, L.Y. Chung and K.Burgess, *Chem. Soc.Rev.*, 2013, **42**, 77; (b) S.G. Awuah and Y.You, *RSC. Adv.* 2012, **2**, 11169; (c) G. Ulrich, R. Ziessel and A. Harriman, *Angew. Chem. Int. Ed.*, 2008, **47**, 1184.

2   (a) T. Mizuno, K. Umezawa, Y. Shondo, D. Citterio, K.Oka and K. Suzuki, *J. of Fluoresence*, 2013, **23**, 1007; (b) A. Poirel, A. De Nicola and R. Ziessel, *J. Org. Chem*, 2011, **76**, 4489; c) S. Kolemen, Y. Cakmak, S. Erten Ela, Y. Altay, J. Brendel, M. Thelakkat and E.U. Akkaya, *Org. Lett.*, 2010, **12**, 3812; (b) Z. Dost, S. Atilgan and E.U. Akkaya, *Tetrahedron*, 2006, **62**, 8484.

3   M.J. Ortiz, I. Garcia Moreno, A.R. Agarrabeita, G. Duran Sampedro, A. Costela, R. Sastre, F. Lopez Arbeloa, J. Banuelos Prietoand and I. Lopez Arbeloa, *Phys. Chem. Chem. Phys.*, 2010, **12**, 7804.

ChemComm Accepted Manuscript

4   (a) D.E.J.G.J. Dolmans, D. Fukumura and R.K. Jain, *Nature Rev. Cancer*, 2003, **3**, 380; (b) Z. Huang, *Technol. Cancer Res. Treat.* 2005, **4**, 283.

5   K. Plaetzer, B. Krammer, J. Berlanda, F. Berr and T. Kiesslich, *Lasers Med. Sci.* 2009, **24**, 259.

6   T. Vo-Dinh, Editor-in-Chief, Biomedical Photonics Handbook, CRC Press, Boca Raton, Florida, 2003.

7   (a) Y. Cakmak, T. Nalbantoglu, T. Durgut and E.U. Akkaya, *Tet. Lett.*, 2014, **5**, 538; (b) S. Erbas Cakmak and E.U. Akkaya, *Angew. Chem. Int. Ed.* 2013, **52**, 11364; (c) S. Erbas, M. Kocakusakogullari and E.U. Akkaya, *Chem. Commun.*, 2009, 4956.

8   S. Ozlem and E.U. Akkaya, *J. Am. Chem. Soc.*, 2009, **131**, 48.

9   N. Adarsh, R.R. Avirah and D. Ramaiah, *Org. Lett.*, 2010, **12**, 5720.

10 Y. Cakmak, S. Kolemen, S. Duman, Y. Dede, Y. Dolen, B. Kilic, Z. Kostereli, L. Tatar Yildirim, A.L. Dogan, D. Guc and E.U. Akkaya, *Angew. Chem. Int. Ed.*, 2011, **50**, 11937.

11 S. Duman, Y. Cakmak, S. Kolemen, E.U. Akkaya and Y. Dede, *J. Org. Chem.*, 2012, **77**, 4516.

12 X.F. Zhang and X. Yang, *J. Phys. Chem. B.*, 2013, **117**, 9050.

13 L. Huang, X. R. Yui W.H. Wu and J. Z. Zhao, *Org. Lett.*, 2012, **14**, 2594.

14 (a) F. Wang, D. Banerjee, Y. Liu, X. Chen and X. Liu, *Analyst*, 2010, **135**, 1839; (b) H. Schäfer and M. Haase, *Angew. Chem. Int. Ed.*, 2011, **50**, 5808.

15 H.X. Mai, Y.W. Zhang, R. Si, Z.G. Yan, L.D. Sun, L.P. You and C.H. Yan, *J. Am. Chem. Soc.*, 2006, **128**, 6426.

16 T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 12162.

17 (a) C.S. Zhang, J.Z. Zhao, S. Wu, Z.L. Wang, W.H. Wu, J. Ma, S. Guo and L. Huang, *J. Am. Chem. Soc.*, 2013, **135**, 10566-10578. (b) S. Atilgan, Z. Ekmekci, A.L. Dogan, D. Guc and E.U. Akkaya, *Chem. Commun.*, 2006, 4398.

18 (a) S. Heer, K. Kömpe and H.U. Gudel, *Adv. Mater.* 2004, **16**, 2102; (b) J.H. Zeng, J. Su, Z. H. Li, X. Ja and Y.D. Li, *Adv. Mater.* 2005, 2119.

19 (a) J.C. Boyer, F. Vetrone, L.A. Cuccia and J.A. Capobianeo, *J. Am Chem Soc.*,2006, **128**, 7444; (b) H.X. Mail, Y.W. Zhang and R. Si, *J. Am. Chem. Soc.* 2006, **128**, 6426.

20 F. Wang and X. Liu, *Chem. Soc. Rew.*, 2009, **38**, 976.

21 (a) X.F. Yu, Z. Sun, M. Li, Y. Xiang, Q.Q. Wang, F. Tang, Y. Wu, Z. Cao and W. Li, *Biomaterials*, 2010, **31**, 8724; (b) X. Yu, M. Li, M. Xie, L. Chen and Q. Wang, *Nano.Res.*, 2010, **3**, 51.

22 F. Wang, D.K. Chatterjee, Z. Li, Y. Zhang, X. Fan and M. Wang, *Nanotech.*, 2006, **17**, 5786.

23 W.T. Godbey, K.K. Wu and A. Mikos, *J. Control Rel.*, 1999, **60**, 149.

24 R.C. Lambert, Y. Maulet, J.L Dupont, S. Mykita, P. Craig, S. Volsen and A. Feltz, *Mol.Cell Neurosci.*, 1996, **7**, 239.

25 S. Erbas Cakmak, O.A. Bozdemir, Y. Cakmak and E.U. Akkaya, *Chem. Sci.*, 2013, **4**, 858.

ChemComm Accepted Manuscript