ChemComm

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Journal:	*ChemComm*
Manuscript ID	CC-COM-05-2019-004123.R1
Article Type:	Communication

ChemComm

COMMUNICATION

Amplifying Fluorescent Conjugated Polymer Sensor for Singlet Oxygen Detection

Chun-Han Wang[b] and Evgueni E. Nesterov*[a,b]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x
www.rsc.org/

A "higher energy gap" concept was applied towards designing an efficient *turn-on* amplifying sensor for singlet oxygen – an important biomedical and environmental monitoring analytical target. The concept is based on modulation of intramolecular energy transfer in fluorescent conjugated polymers through the formation of a higher energy gap "roadblock" upon reaction with a target analyte. The polymer sensor incorporates 1,4-disubstituted tetracene units which act as reactive sites for singlet oxygen. The resulting polymer sensor demonstrates significant fluorescent signal amplification and a broader analyte detection range relative to a corresponding small-molecule sensor.

Conjugated polymers (CPs) make a popular choice for designing fluorescent sensors because of the intrinsic signal amplification associated with intra- and interchain photoexcitation energy (exciton) migration.[1,2] In particular, CP-based sensors deliver better detection sensitivity relative to the corresponding small-molecule sensors. A typical CP amplifying fluorescent sensor utilizes exciton migration to a lower energy gap site created by analyte binding. Such a scheme is common for the detection of fluorescence-quenching electron-deficient analytes (e.g. nitroaromatic explosives) and is used in the design of *turn-off* sensors (i.e. where interaction with analyte results in amplified fluorescence quenching through a photoinduced electron transfer mechanism).[3] However, from a practical standpoint, *turn-on* sensors which increase fluorescent intensity upon analyte binding could be more convenient than *turn-off* sensors, but it still remains a challenge to develop and validate a general design scheme towards *turn-on* sensing which could utilize signal amplification ability of CPs. One previous scheme was based on incorporating an analyte-specific reactive group as part of the CP's π-electron conjugated backbone. Upon reaction with a target analyte, this group would be converted to a lower-energy fluorophore where photoexcitation energy could be funnelled to via intramolecular exciton migration, generating an amplified *turn-on* response.[4] Although promising, this scheme is severely limited by the need to use a reactive group which forms a lower-energy fluorescent chromophore upon reaction with analyte. Indeed, such analytical reactions are not very common, and they are simply not available for many practically important analytical targets.

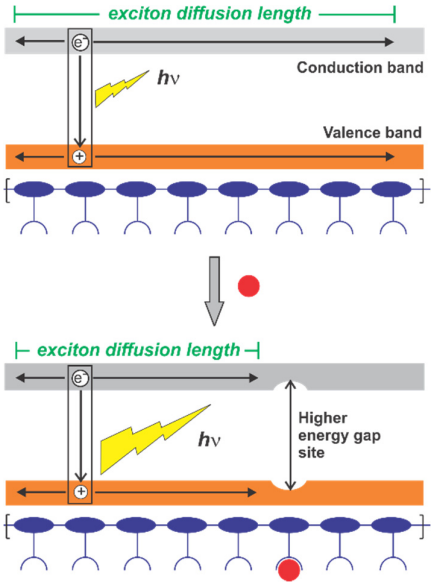


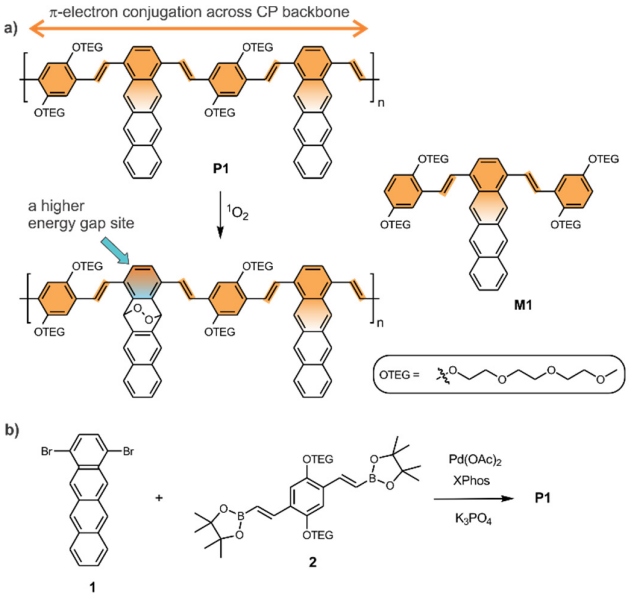
Fig. 1. General mechanism of the "higher energy gap" control of CP fluorescence: reaction of an analyte (red circle) with the CP receptor generates a higher energy gap site in the π-conjugated backbone which decreases the exciton diffusion length in the CP, therefore causing an increase of the intensity of fluorescent emission.

As an alternative to this approach, we recently proposed a "higher energy gap" design concept towards fluorescent *turn-on* chemosensors which is free of the limitations of the previous scheme (Fig. 1).[5] Like in the previous scheme, an analyte-reactive group has to be incorporated as part of the CP π-

[a].Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb, Illinois 60115, USA
[b] Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana, 70803, USA
E-mail: een@niu.edu
Electronic Supplementary Information (ESI) available: Detailed synthetic and experimental procedures, compounds characterization, and additional data and figures. See DOI: 10.1039/x0xx00000x

electron backbone. The critical (and essentially only) requirement to such a group is that, upon reaction with a target analyte, it should generate a species with a higher energy (i.e. HOMO-LUMO) gap. The generated higher energy gap site in the CP backbone would act as a "roadblock" diminishing the efficiency of exciton migration (photoexcitation energy transfer) occurring via a through-bond (Dexter-type) mechanism,[6] thus decreasing the overall exciton migration length. The shorter exciton migration length would result in a fluorescent emission enhancement in a *turn-on* sensor. Since this phenomenon is mechanistically related to intramolecular exciton migration in the CP π-electron system, it will benefit from the same amplification phenomenon as in the case of fluorescent-quenching *turn-off* chemosensing. Importantly, in contrast to the previous approach, there is no need to generate a fluorescent chromophore unit upon the analyte reaction, which opens up a breadth of efficient potentially applicable chemical reactions available for almost any analyte of interest. Earlier, we applied the "higher energy gap" concept towards developing *turn-on* fluorescent sensors for organophosphate agents as well as for hydrogen sulfide.[5] In the present work, we demonstrated how this concept can be used in the design of an amplifying fluorescent sensor for singlet oxygen ($^1O_2$).

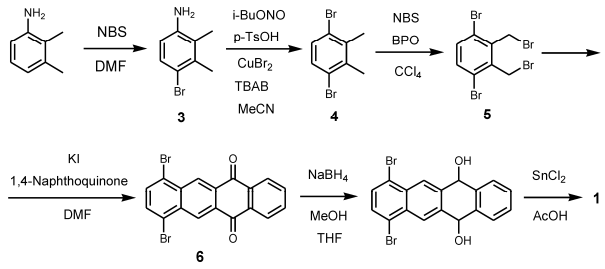
Scheme 1. a) Structures of polymer sensor **P1** and corresponding small-molecule sensor **M1**, as well as the reaction with singlet oxygen $^1O_2$ to generate a higher energy gap site in the CP backbone. b) Synthesis of polymer **P1**.



Singlet oxygen is a highly reactive excited state of molecular oxygen which is responsible for photochemical oxidative damage of biological molecules and organic optoelectronic materials, and its high cytotoxicity makes $^1O_2$ an important species in photodynamic therapy treatment.[7] A number of small molecules and conjugated polymers that sense $^1O_2$ specifically through changes in intensity of fluorescence have been reported.[8,9] These systems contain either a small-molecule or CP chromophore connected to a $^1O_2$-responsive unit such as anthracene which can react with dienophile $^1O_2$ through a [4+2]-cycloaddition (Diels–Alder reaction), yet these sensors normally operate through a *turn-off* pathway, and none of them demonstrate signal amplification.

In this report, we describe an amplifying *turn-on* fluorescent sensor **P1** which was designed using the "higher energy gap" concept (Scheme 1a). The sensor is a poly(arylene vinylene) conjugated polymer which incorporates 1,4-tetracene unit as part of the π-electron conjugated backbone. Reaction of $^1O_2$ with the tetracene unit yields an endo-peroxide moiety which features a higher HOMO-LUMO gap compared to the initial tetracene. A key design approach was to use the 1,4-bisubsituted tetracene unit for the sensor as this unit would preferentially react with $^1O_2$ at a central benzene ring and not at the ring which is part of the CP π-conjugated backbone. In the former case, the reaction with $^1O_2$ would result in increasing HOMO-LUMO gap at the reaction site without disrupting the π-conjugation across the polymer backbone – a main condition for the "higher energy gap" principle to be operative. This preferential reactivity is driven mostly by increasing aromatic stabilization of the corresponding endo-peroxide cycloaddition products. Indeed, DFT computations (at B3LYP/6-31G* level of theory) on a truncated 1,4-bisubstituted tetracene unit indicated significantly higher (12.4-13.0 kcal/mol) stabilization of the endo-peroxides formed at the one of the central benzene rings relative to the endo-peroxide formed at the 1,4-positions (Fig. S1 in the Supporting Information).

Scheme 2. Synthesis of 1,4-dibromotetracene **1**.



The CP **P1** with number-average molecular weight M_n 11.8 kDa (as determined by GPC vs. polystyrene standards, Fig. S2 in the SI) was prepared using Suzuki polymerization of 1,4-dibromotetracene **1** and bis-vinylboronate monomer **2** (Scheme 1b). Preparation of 1,4-dibromotetracene has not been previously described in literature due to the difficulty of selective functionalization at the 1 and 4 positions in tetracene (since direct functionalization of tetracene through electrophilic aromatic substitution occurs at its central benzene rings). Therefore, we developed a convenient indirect method towards 1,4-dibromotetracene (Scheme 2). Bromination of commercially available 2,3-dimethylaniline with NBS occurred selectively and yielded 4-bromo-2,3-dimethylaniline **3**. Diazotization followed by Sandmeyer reaction led to 1,4-dibromo-2,3-dimethylbenzene **4** which was selectively brominated at both benzylic positions to yield 1,4-dibromo-2,3-bis(dibromomethyl)benzene **5**. An *in situ* formation of a reactive 1,4-dibromo-5,6-bis(methylene)-1,3-cyclohexadiene intermediate and its subsequent Diels-Alder reaction with 1,4-naphthoquinone gave 1,4-dibromo-6,11-tetracenequinone **6**.[10]

Reduction of **6** and subsequent dehydroxylation produced **1** in a total 11% yield.

In addition to CP sensor **P1**, we also prepared a corresponding small-molecule sensor **M1** to be used as a benchmark for measuring fluorescent detection amplification by **P1**.

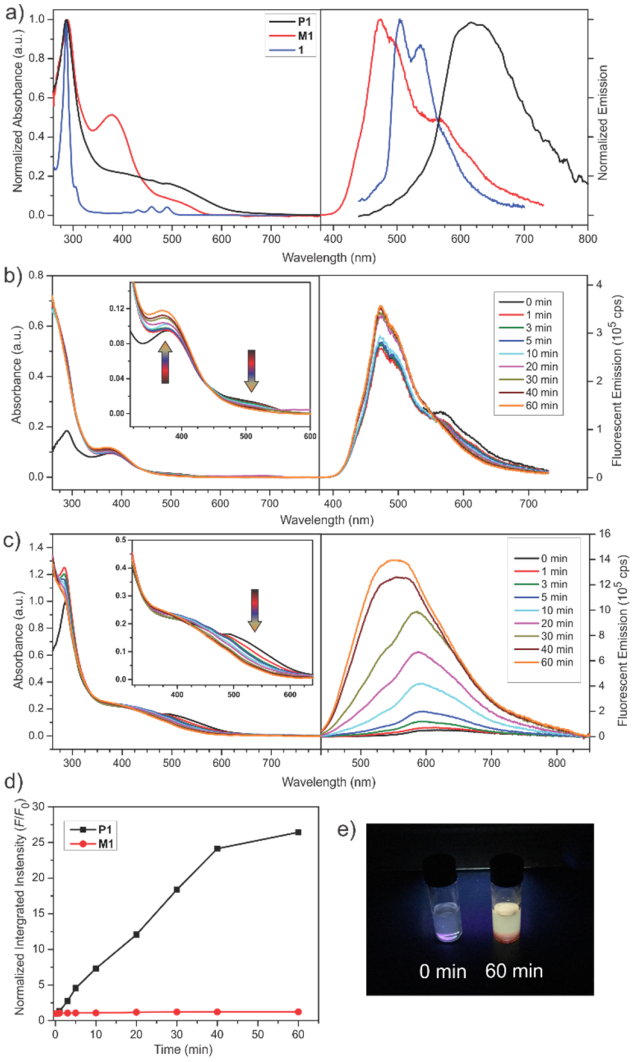


Fig. 2. a) Normalized UV/vis absorption and fluorescence spectra of polymer **P1**, small-molecule sensor **M1** and 1,4-dibromotetracene **1** in DMSO (conc. 0.02 mg/ml). b) Change in absorbance and fluorescence spectra of small-molecule sensor **M1** (10 μM solution in DMSO) upon exposure to $^1O_2$. c) Change in absorbance and fluorescence spectra of polymer **P1** (17 μM solution in DMSO) upon exposure to $^1O_2$. d) Change of fluorescence intensity of polymer **P1** and small-molecule sensor **M1** upon exposure to $^1O_2$. The intensity is expressed as a ratio of integrated area of a fluorescent band at each point divided by the area of the fluorescent band before exposure to $^1O_2$. e) Photograph of a **P1** solution before and after exposure to $^1O_2$ upon irradiation with a hand-held UV lamp.

Despite possessing tri(ethylene glycol) (TEG) solubilizing groups, both **P1** and **M1** showed insufficient solubility in water. Alternatively, we decided to use DMSO solvent in these studies. DMSO is a popular solubility and penetration enhancer in biomedical research,[11] and it has been occasionally used as a medium for $^1O_2$ related previous studies.[12]

The UV-vis absorption spectrum of polymer **P1** showed an intense peak at 290 nm, and a broad lower-intensity shoulder spanning until 630 nm (Fig. 2a). The intense peak at 290 nm is characteristic of tetracene structure,[9] and was also prominent in the absorption spectra of small-molecule sensor **M1** as well as 1,4-dibromotetracene **1**. A characteristic vibronically structured tetracene absorption band between 400 and 520 nm in **1** became a featureless shoulder at around 500 nm in **M1**, and was not easily identifiable in **P1**. The small-molecule compound **M1** also displayed a strong absorption band with a maximum at 385 nm which could be attributed to the π-electron conjugation across the (arylene vinylene) system (Fig. 2a). The longer π-conjugation length in CP **P1** resulted in a bathochromic shift of this band in the polymer's spectrum, where it also overlapped with the tetracene absorption band, producing an overall broad featureless absorption in the spectrum of **P1**.

The fluorescence spectrum of **M1** showed a broad band with a maximum at 475 nm and a shoulder at 575 nm. The polymer **P1** spectrum displayed a significantly bathochromically shifted emission band with an overall very low intensity making **P1** essentially non-fluorescent. The low fluorescent intensity of **P1** was not surprising considering that other, previously described structurally related CPs also showed low fluorescence.[5] However, without additional photophysical studies, we cannot offer a reliable general explanation for this observation.

To test chemosensing response to $^1O_2$, we used photoirradiation of the sensor solution in the presence of Rose Bengal sensitizer. In order to avoid interference of the sensitizer with UV/vis absorption and fluorescence spectroscopy experiments, we prepared Rose Bengal functionalized polystyrene beads.[13] The photoirradiation was carried out in a standard 1 cm rectangular cuvette upon stirring. When the stirring was stopped, the Rose Bengal functionalized beads would rapidly precipitate to the bottom of the cuvette, allowing performing spectroscopic measurements *in situ*, without interference from the sensitizer. Thus, solutions of **P1** or **M1** in DMSO were stirred with Rose Bengal functionalized beads under oxygen and irradiated using a 500 W tungsten-halogen lamp for specified time periods. In excellent agreement with the "higher energy gap" concept, the CP sensor **P1** exhibited a strong and near-linear increase in fluorescent intensity, with the total increase (as an integrated intensity ratio F/F_0) more than 20 times (Fig. 2c, d). The significant enhancement in fluorescent intensity was especially remarkable since it occurred over the essentially dark initial fluorescent background of **P1** (Fig. 2e), thus resulting in a high signal-to-background detection ratio. The control experiments (irradiation with no Rose Bengal sensitizer or exposure to oxygen with no photoirradiation) did not produce any significant increase in fluorescent intensity (Fig. S3 and S4 in the SI). In comparison to the polymer sensor **P1**, fluorescence intensity enhancement of the small-molecule sensor **M1** upon $^1O_2$ exposure in the same conditions was practically negligible (Fig. 2b, d). This illustrated the significant analyte detection amplification in the *turn-on* fluorescent sensor designed using the "higher energy gap" concept.

Further analysis of the spectroscopic results and corresponding ^{1}H NMR data allowed to gain deeper insight into the $^1O_2$ sensing response. Reaction of the small-molecule

sensor **M1** with $^1O_2$ resulted in the gradual decrease of the tetracene absorbance at 500 nm, whereas the absorption band at 385 nm (corresponding to the π-electron conjugation across the (arylene vinylene) system) persisted (and actually increased its absorbance) (Fig. 2b). This indicated that the reaction with $^1O_2$ occurred at one of the central benzene rings of 1,4-disubstituted tetracene system, and the extended π-conjugated (arylene vinylene) system remained mostly unaffected. To determine what specific benzene ring in the 1,4-disubstituted tetracene unit reacted with $^1O_2$, we carried out ^{1}H NMR monitoring of the reaction of **M1** with $^1O_2$. This reaction produced a main product showing a distinct singlet peak at 6.61 ppm (corresponding to the bridgehead protons at the dioxygen bridge in the endo-peroxide). To assign this and other ^{1}H NMR signals to a specific endo-peroxide, we carried out GIAO computational studies on a truncated analogue of **M1** and the corresponding endo-peroxides (Fig. S5 in the SI). The computations confirmed that the dioxygen bridge was more likely to form at the 5,12 positions of the tetracene unit, as the calculated NMR shift for the corresponding bridgehead proton was 6.72 ppm which was close to the experimentally observed value. Other experimental chemical shifts also matched the values calculated for this specific structure. This reactivity pattern with $^1O_2$ also agreed well with the UV/vis absorption spectroscopy observations.

With this information, we could analyse the spectroscopic data obtained for the polymer sensor **P1**. The UV/vis absorption spectrum showed gradual decrease between 480 and 630 nm due to the consumption of tetracene unit through its reaction with $^1O_2$ (Fig. 2c). At the same time, at the earlier stages of photoirradiation, there was no decrease in the absorbance (or even a slight increase) within the range of 360 – 480 nm where the π-electron conjugation in the CP backbone was the main contributor. These changes in the absorption spectra were accompanied by a dramatic intensity increase in the fluorescent emission band, but with essentially no emission wavelength shift. Thus, formation of the endo-peroxide at 5,12 positions of the tetracene unit was responsible for a HOMO-LUMO gap increase which created "roadblocks" for the excitons migrating in the CP π-conjugated backbone, and produced significant *turn-on* fluorescent response. We should also notice that a longer exposure of the **P1** sensor to $^1O_2$ (at the later stages of photoirradiation) led to a noticeable (approx. 35 nm) hypsochromic shift in the maximum of the emission band and a smaller intensity increase as well as absorbance decrease of the 360 – 480 nm absorption band (traces corresponding to 40 and 60 min in Fig. 2c, d). Apparently, this was due to a subsequent reaction of $^1O_2$ at the 1,4 positions of tetracene ring resulting in breakdown of the π-electron conjugation in the CP backbone.

In conclusion, we demonstrated how the simple "higher energy gap" concept can be used in the design of an efficient amplifying *turn-on* CP fluorescent sensor. This concept is based on hindering intramolecular exciton migration occurring via a through-bond (Dexter) mechanism when a higher energy (HOMO-LUMO) gap site is created in the polymer π-electron conjugated backbone upon reacting with a target analyte. From a fundamental standpoint, the success of this design illustrates the importance of the through-bond (Dexter-type) mechanism of intramolecular energy migration in fluorescent CPs. On practical side, this concept can be used for designing amplifying *turn-on* sensors for many other important analytes of interest.

We gratefully acknowledge support of this research by the National Science Foundation (grant number CHE-1362686).

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 T. M. Swager, *Acc. Chem. Res.*, 1998, **31**, 201; X. Feng, L. Liu, S. Wang, D. Zhu, *Chem. Soc. Rev.*, 2010, **39**, 2411; Y. Liu, K. Ogawa, K. S. Schanze, *J. Photochem. Photobiol., C*, 2009, **10**, 173.

2 S. W. Thomas III, D. J. Guy, T. M. Swager, *Chem. Rev.*, 2007, **107**, 1339; S. Rochat, T. M. Swager, *ACS Appl. Mater. Interfaces*, 2013, **5**, 4488.

3 J.-S. Yang, T. M. Swager, *J. Am. Chem. Soc.*, 1998, **120**, 11864; L. Chen, D. W. McBranch, H.-L. Wang, R. Helgeson, F. Wudl, D. G. Whitten, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 12287; X. Sun, Y. Wang, Y. Lei, *Chem. Soc. Rev.*, 2015, **44**, 8019; H. Jiang, P. Taranekar, J. R. Reynolds, K. S. Schanze, *Angew. Chem. Int. Ed.*, 2009, **48**, 4300.

4 T.-H. Kim, T. M. Swager, *Angew. Chem. Int. Ed.*, 2003, **42**, 4803.

5 D. Pangeni, E. E. Nesterov, *Macromolecules*, 2013, **46**, 7266.; C.-H. Chiang, D. Pangeni, E. E. Nesterov, *Macromolecules*, 2017, **50**, 6961.

6 D. Beljonne, C. Curutchet, G. D. Scholes, R. J. Silbey, *J. Phys. Chem. B*, 2009, **113**, 6583; C. F. Wang, J. D. White, T. L. Lim, J. H. Hsu, S. C. Yang, W. S. Fann, K. Y. Peng, S. A. Chen, *Phys. Rev. B*, 2003, **67**, 035202; B. Van Averbeke, D. Beljonne, E. Hennebicq, *Adv. Funct. Mater.*, 2008, **18**, 492; E. E. Nesterov, Z. Zhu, T. M. Swager, *J. Am. Chem. Soc.*, 2005, **127**, 10083.

7 M. C. DeRosa, R. J. Crutchley, *Coord. Chem. Rev.* 2002, **233-234**, 351; P. Agostinis, K. Berg, K. A. Cengel, T. H. Foster, A. W. Girotti, S. A. Gollnick, S. M. Hahn, M. R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B. C. Wilson, J. Golab, *CA Cancer J. Clin.*, 2011, **61**, 250; D. E. J. G. J. Dolmans, D. Fukumura, R. K. Jain, *Nat. Rev. Cancer*, 2003, **3**, 380; R. D. Scurlock, B. Wang, P. R. Ogilby, J. R. Sheats, R. L. Clough, *J. Am. Chem. Soc.*, 1995, **117**, 10194.

8 Y. You, *Org. Biomol. Chem.*, 2018, **16**, 4044.

9 J. Zhang, S. Sarrafpour, R. H. Pawle, S. W. Thomas III, *Chem. Commun.*, 2011, **47**, 3445; E. Altinok, Z. C. Smith, S. W. Thomas III, *Macromolecules*, 2015, **48**, 6825; F. Frausto, S. W. Thomas III, *ChemPhotoChem*, 2018, **2**, 632.

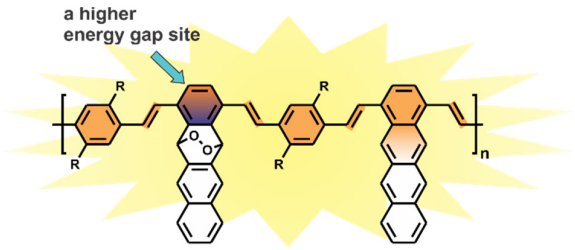
10 M. P. Cava, A. A. Deana, K. Muth, *J. Am. Chem. Soc.*, 1959, **81**, 6458; K. N. Plunkett, K. Godula, C. Nuckolls, N. Tremblay, A. C. Whalley, S. Xiao, *Org. Lett.,* 2009, **11**, 2225.

11 A. C. Williams, B. W. Barry, *Adv. Drug Delivery Rev.*, 2004, **56**, 603.

12 J. Schmitt, V. Heitz, A. Sour, F. Bolze, H. Ftouni, J.-F. Nicoud, L. Flamigni, B. Ventura, *Angew. Chem. Int. Ed.*, 2015, **64**, 169; N. Yesilgul, T. B. Uyar, O. Seven, E. U. Akkaya, *ACS Omega*, 2017, **2**, 1367.

13 R. P. Taylor, J. B. Vatz, *J. Am. Chem. Soc.*, 1973, **95**, 5820.

TOC graphic



A “higher energy gap” concept was used to design an efficient conjugated polymer *turn-on* amplifying fluorescent sensor for singlet oxygen.