

Cycloadditions of Singlet Oxygen for Responsive Fluorescent Polymers

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Abstract: This article describes progress in the author's laboratory in the area of new fluorescent polymers that respond to the reactive oxygen species $^1\text{O}_2$. Key to the development of these materials are the [4+2] cycloaddition reactions between $^1\text{O}_2$ and dienes such as acenes and furans. When covalently bound to conjugated polymer backbones, cycloadditions of these dienes with $^1\text{O}_2$ can yield dramatic changes in the wavelength and intensity of luminescence: three such examples are given here. The article also summarizes our work to understand how changing the chemical structures of acenes affect reactivity with $^1\text{O}_2$ as well as the cycloreversion of the resulting endoperoxides.

Key words: cycloaddition, singlet oxygen, fluorescence, polymers, acenes

Introduction

This paper describes work in the author's lab regarding the development of fluorescent materials that respond to the important reactive oxygen species (ROS) singlet oxygen ($^1\text{O}_2$). Fluorescent polymers, especially conjugated polymers (CPs), have received much attention as high performance, chemically sensitive fluorophores.¹ Much of this attention stems from the properties of amplification that these materials offer, resulting from light harvesting and exciton mobility characteristics.² In comparison to a more traditional, small molecule fluorophore-receptor pair, in which each binding event or reaction of analyte yields a change in a single chromophore, the harvesting of photons together with transport of excited states through, for example, energy transfer mechanisms, allows a single excited state to sample many potential reaction sites. The result is a funneling of excited states generated far away from the site of reaction or binding to a low-energy trap. These processes of light harvesting and exciton mobility of CPs have proven useful in not only chemical and biological sensing, for which the sensing of nitroaromatic explosives through amplified photoinduced electron transfer quenching has been particularly successful,^{3, 4} but are also the initial steps in the operation of bulk heterojunction organic photovoltaics.⁵

One of the rewarding aspects of working with singlet oxygen is the direct connection to lessons taught in introduction chemistry on molecular orbital theory and electronic structure. There are a number of sources with excellent details on the physical and

chemical characteristics of $^1\text{O}_2$,⁶⁻¹¹ only brief descriptions are given here. Two low energy electronic configurations with singlet multiplicity exist above the ground state triplet of O_2 , as shown in Figure 1. The higher energy of the two, in which electrons with opposite spin occupy different π^* antibonding orbitals with term symbol $^1\Sigma_g$, has a very short lifetime, and quickly undergoes internal conversion to the lowest energy electronic excited state with paired electrons with term symbol $^1\Delta_g$. In the remainder of this paper, $^1\text{O}_2$ refers to this lowest energy excited state of O_2 . The excited state energy of $^1\text{O}_2$ is relatively low (~ 1 eV), giving weak phosphorescence at 1270 nm that is an important observable for monitoring excited-state processes of $^1\text{O}_2$. The lifetime of $^1\text{O}_2$ is also strongly dependent on solvent, ranging from 3 μs in H_2O to 87 ms in CCl_4 . A related unique aspect of $^1\text{O}_2$ quenching dynamics is also the strong dependence of lifetime on solvent deuteration due to decreased coupling to bond vibrations of deuterated solvents.¹² as an example, $^1\text{O}_2$ decays one order of magnitude more slowly in D_2O than in H_2O .

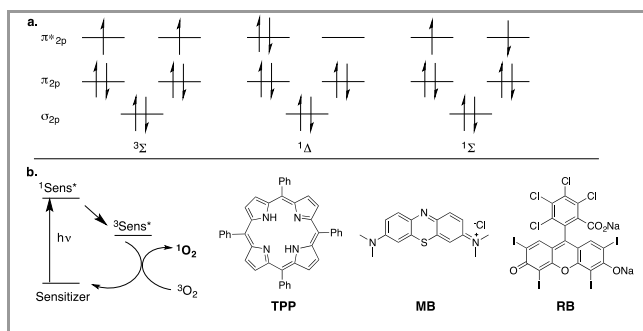


Figure 1. a) Electronic configurations of the electronic ground state ($^3\Sigma$) and the two lowest energy electronic excited states ($^1\Delta$ and $^1\Sigma$) of O_2 ; b) General scheme for preparation of $^1\text{O}_2$ by photosensitization, and the structures of some generally useful and commercially available photosensitizers.

As a spin forbidden transition, the direct absorbance of photons to promote $^3\text{O}_2$ to $^1\text{O}_2$ is not efficient. Given the low excited-state energy of $^1\text{O}_2$, however, its generation via energy transfer from other molecules can, in contrast, be highly efficient.¹³ Although it is possible to transfer energy from singlet excited states to $^3\text{O}_2$ to produce $^1\text{O}_2$, most highly efficient sensitizers proceed through excited triplet states in a triplet annihilation process, as shown in

Figure 1—excitation of a sensitizing chromophore and intersystem crossing yields the triplet state of the sensitizer, which upon energy transfer to O_2 regenerates the ground electronic state of the sensitizer and 1O_2 . Efficiencies of this process have been tabulated for a wide variety of sensitizers.¹⁴ An important consequence of this efficient sensitization process and the ubiquity of O_2 is that in those samples that are not deoxygenated, 1O_2 is a commonly generated reactive oxygen species: 1O_2 is commonly blamed culprit for the photo-oxidative decomposition of materials.^{15, 16} The stoichiometrically consumed reagents are photons, and O_2 if the 1O_2 goes on to react by some other pathway other than unimolecular or bimolecular physical quenching. This process does not consume the sensitizer, although sensitizer decomposition by other excited state pathways can of course occur. Therefore, substoichiometric quantities of the sensitizers can be used to generate 1O_2 and downstream products.

In addition to its interesting unimolecular behavior and method of production, 1O_2 has rich palette of organic reaction chemistry, which is usually broken into three classes: ene reactions to form hydroperoxides,¹⁷ as well as [2+2] cycloaddition reactions with electron-rich alkenes^{18, 19} and [4+2] cycloaddition reactions with dienes,²⁰ both of which yield peroxides. These reactions, especially [4+2] cycloadditions between 1O_2 and furan moieties, have found great utility in the preparation of a significant number of natural products.²¹ For applications in organic electronics, however, this reaction is generally considered a nuisance. Pentacene, one of the most thoroughly studied organic semiconductors, is an excellent exemplar of this concept. Organic thin film transistors fabricated using pentacene represent a benchmark in terms of performance, with hole mobility values among the highest reported.²² What is often considered an unfavorable side-effect of pentacene and many of its derivatives is its rapid rate of [4+2] cycloaddition with 1O_2 to form the 6,13-endoperoxide (Figure 2), the bimolecular rate constant of which is approximately $4 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$.²³ Shorter acenes, such as tetracene or anthracene, are also highly reactive ($k \sim 10^5\text{--}10^7 \text{ M}^{-1} \text{ s}^{-1}$),¹² giving the corresponding endoperoxides with high yields. Given the ease with which light, O_2 and many chromophores (including acenes themselves) generate 1O_2 , this reactivity and the resulting destruction of the highly conjugated structures of acenes presents a challenge for their use in electronic devices. As a result there exist a number of strategies relying on substituent effects to reduce the observed reactivity of acenes with 1O_2 by up to several orders of magnitude.²⁴

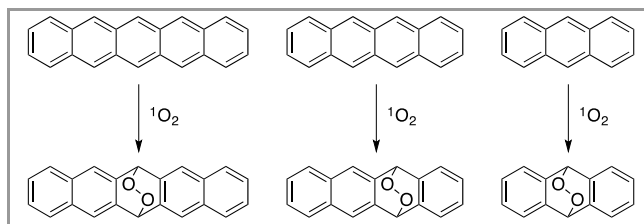


Figure 2. Endoperoxide formation through [4+2] cycloadditions of 1O_2 and linear acenes.

For our group, however, these characteristics—i) large changes in optical and electronic properties, ii) the use of reactants that are ubiquitous in ambient conditions, iii) rapid and clean addition chemistry, and iv) the chemical amplification derived from very small amounts of a sensitizer to produce large quantities of the reactive intermediate—make acene- 1O_2 cycloadditions attractive for stimuli-responsive materials. In fact, these types of [4+2] cycloaddition reactions, typically with anthracenes or isobenzofurans, are responsible for the most well known molecules that show colorimetric or fluorescent responses to 1O_2 .²⁵ Fluorescent sensors or dosimeters for 1O_2 are important due to its central nature in both a variety of biological processes, and as a key cytotoxic ROS in photodynamic therapy.^{26, 27} In addition, due to the fact that the production of 1O_2 is chemically amplified relative to the amount of sensitizer, reactions of 1O_2 that produce a change in fluorescence can be used as replacement for enzymatic catalysis of formation of a luminescent product in bioassays, as had been demonstrated in the bead-based luminescent oxygen channeling immunoassay,^{28, 29} commercialized as the AlphaLISA platform.

At the time that we started this research in 2010, the molecule DMAX from the group of Nagano was a benchmark fluorescent dosimeter for 1O_2 , comprising a 9,10-dialkylanthracene moiety bound to a xanthene dye.³⁰ Before cycloaddition with 1O_2 , the anthracene moiety efficiently quenches the luminescence of fluorescein through photoinduced electron transfer (Figure 3) from the anthracene HOMO to the lower energy singly occupied orbitals of the excited xanthene. Anthracene- 1O_2 cycloaddition, however, lowers the HOMO energy to such an extent as to render this photoinduced electron transfer endergonic, resulting in a “turn-on” of fluorescence from the fluorescein dye in response to 1O_2 . DMAX was an improvement over an earlier analog (DPAX), with similar mechanism, that used 9,10-diphenylanthracene as the reactive quencher;³¹ this anthracene derivative reacts with 1O_2 more slowly than the dialkyl analog used in DMAX. This technology has been commercially successful as well, with an analog of DMAX currently available as “Singlet Oxygen Sensor Green” (SOSG).³²

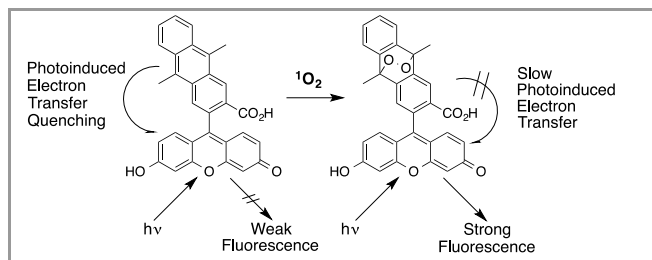


Figure 3. Oxidation of DMAX with $^1\text{O}_2$ to its endoperoxide with increases the energy of the LUMO of the anthracene. This eliminates the driving force for fluorescence quenching by photoinduced electron transfer from the anthracene to the xanthene fluorophores endergonic, and increases the observed fluorescence quantum yield from the xanthene.

Concurrent with our work described below, several important improvements on the DMAX/SOSG design have been reported, including i) “Aarhus Sensor Green” from the group of Ogilby, a DPAX analog that solves the problem of $^1\text{O}_2$ sensitization by the endoperoxide product through fluorination of the fluorescein,³³ ii) “Si-DMA” from the group of Majima, which combines both minimal self-oxidation and far-red emission,³⁴ and iii) a series of ratiometric probes for $^1\text{O}_2$ based on interruption of the conjugation of extended diarylisobenzofurans from the group of Nam.³⁵

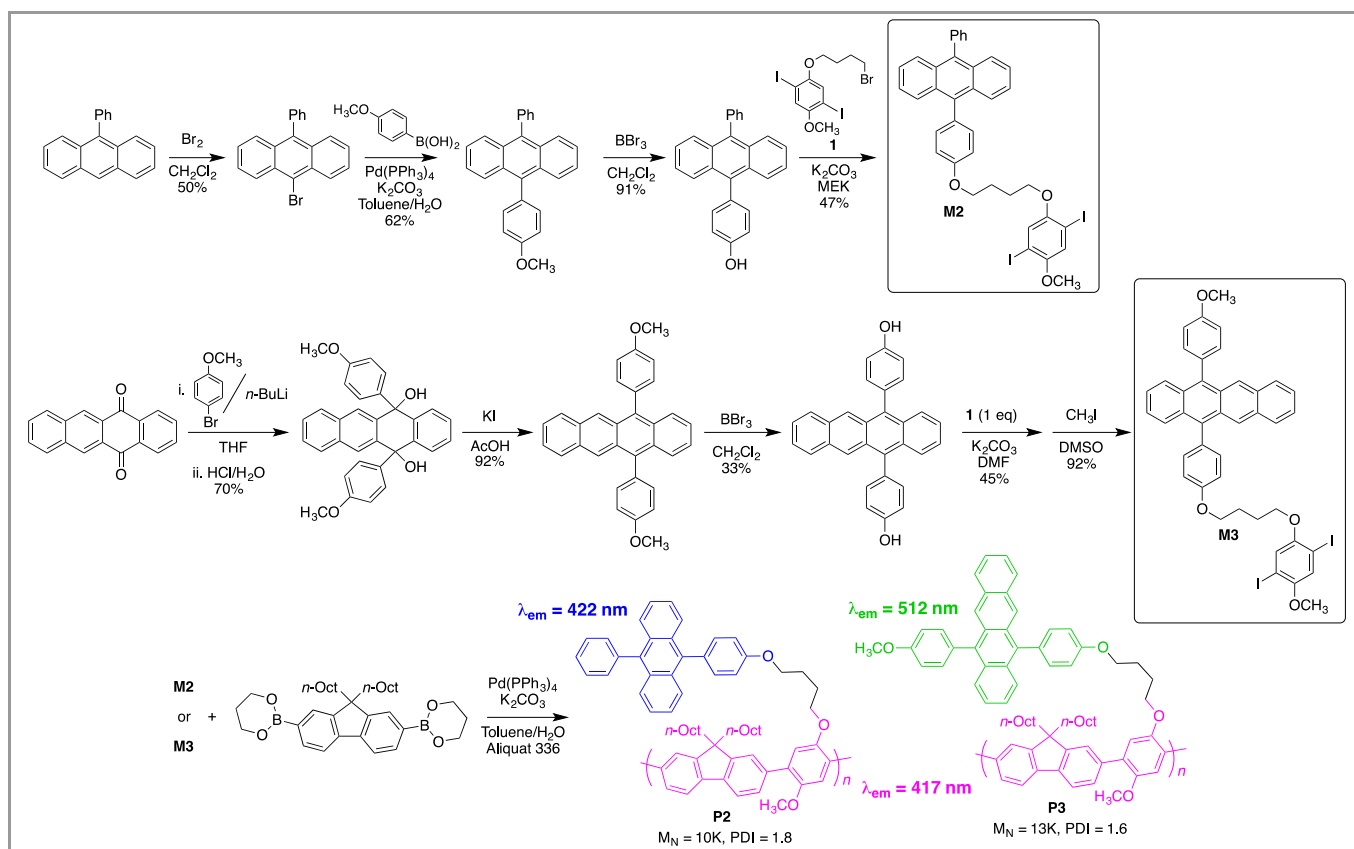
Motivation

Keeping in mind the advantages of CPs as amplifying fluorescent sensing materials, it seemed to us that two potential routes for innovation in $^1\text{O}_2$ -sensitive fluorophores would be i) to use a CP backbone instead of a simple fluorescent small molecule dye to enable light-harvesting and exciton mobility to improve performance in response to $^1\text{O}_2$, and ii) expand the structural space of $^1\text{O}_2$ -reactive acenes used in $^1\text{O}_2$ -responsive materials beyond anthracenes, as longer acenes generally tend to be more reactive with $^1\text{O}_2$ than shorter acenes. In addition to CPs providing a pathway for improving $^1\text{O}_2$ -responsive materials, we found tantalizing the general challenge of making a CP that responds to a reactive oxygen species through increased fluorescence intensity, without degradation, as fluorescent conjugated polymers—especially the highly fluorescent 9,9-dialkylfluorene-based CPs—do have a reputation for being photo-oxidatively unstable.³⁶ Finally, as a side note it is important to mention in this context that initial inspiration for this work derived from Thomas’s unexpected observation as a graduate student in the laboratory of Prof. Tim Swager, in which thin films of an anthracene-substituted poly(phenylene-ethynylene) showed an *increase* in fluorescence intensity and a hypsochromic shift upon acquisition of emission spectra. Whether that exact observation was due to endoperoxide formation or some other photoreaction such as [4+4] anthracene dimerization remains unknown.

Organic Soluble Diene-Linked CPs

There are a number of strongly fluorescent polymer backbones from which to choose in designing a CP-based sensing material. Based on some previous experience with sensors for nitroalkanes, we chose a highly fluorescent poly(fluorene-*alt*-phenylene) conjugated polymer backbone.³⁷ The reasons for this choice were: i) they are highly fluorescent both in solution and as solids, ii) 2,7-diboronates of 9,9-dialkylfluorene are commercially available for use in Suzuki polymerization reactions, iii) the HOMO-LUMO gaps of these CPs are large; the maximum wavelength of emission is ~ 420 nm, which maximized the probability that modification of the fluorescence of the polymer by electron transfer or energy transfer would be energetically favorable, iv) dialkoxyphenylene backbone units are readily functionalized through alkylation of the hydroquinones. We therefore first prepared polymer **P2** as shown in Scheme 1.³⁸ In this design, the conjugated polymer backbone takes the place of the fluorescein dye in DPAX. The synthesis of this polymer was quite straightforward, mainly because of the ease of preparing anthracene derivatives that are unsymmetrically substituted about the long axis. Simple bromination of commercial 9-phenylanthracene and Suzuki coupling with 4-methoxyphenylboronic acid were known procedures at the time, as was the synthesis of the alkylating agent **1** from MEHQ. The assembly of **M2** and its copolymerization with a commercially available fluorene diboronate monomer went quickly.

Although it was quite easy to synthesize **P2**, the responsive characteristics were, predictably, poor. Before exposure to $^1\text{O}_2$, the fluorescence spectrum of **P2** matched almost perfectly with that of 9,10-dianisylanthracene, which indicated that energy transfer from the CP backbone to the anthracene was occurring. Upon exposure to $^1\text{O}_2$ in organic solvent, the change in the fluorescence spectrum was *so small* that with just a quick glance one could miss it. A slight blue shift in λ_{max} of emission from 422 nm to 417 nm occurred, with almost no change in intensity. Another disadvantage was that readily perceptible responses required ≥ 20 minutes of irradiation of methylene blue. These spectra observed did indeed match those of the anthracene (before exposure) and CP backbone (after exposure). We therefore developed a model in which energy transfer from the CP backbone to the pendant acene occurred before exposure to $^1\text{O}_2$. After endoperoxidation of the acene pendant, however, the much larger HOMO-LUMO gap of the endoperoxide makes this ET unfavorable, resulting instead in luminescence from the polymer. Therefore, it became clear that in order for an acene-linked CP to show rapid, clear response to $^1\text{O}_2$, we needed acene pendants that were both red-shifted from diarylanthracenes and more reactive.



Scheme 1: Synthesis of acene-linked diiodophenylene monomers for cross-coupling polymerizations (**M2** and **M3**) and resulting anthracene-linked (**P2**) and tetracene-linked (**P3**) poly(fluorene-*alt*-phenylene)s

The use of longer linear acenes was the obvious solution to this problem, but posed a greater challenge for synthesis. Unlike anthracenes, for which there is a plethora of literature precedent and commercially available starting materials for easy synthesis of elaborated, unsymmetrically substituted derivatives, there are far fewer easy options for unsymmetrically substituted tetracene derivatives. The lower solubility of tetracene derivatives and their more reactive nature with respect to photooxidation presented additional challenges. The obvious starting material to us was 5,12-tetracenequinone, but we struggled for a while to prepare a tetracene analog of **M2** from it. Initial efforts focused on early introduction of an unsymmetrical substitution pattern by adding only one equivalent of 4-methoxyphenyllithium to the quinone, but all such attempts gave only very poor yields of the γ -hydroxyketone. The same was true at that time for attempting to deprotect only one of the methyl ethers with BBr_3 , so we settled on isolating a singly alkylated product of the tetracene diol with **1**, followed by methylation of the remaining phenol. Upon isolating **M3** by column purification the first time, we did not have much material, perhaps only 20-

30 mg, and the compound was contaminated with 10% of some unidentified impurity. The temptation to carry this into the polymerization to see if our idea would work was enormous, given the several months of effort it took to sort out the conditions of synthesis. The demanding nature of stoichiometry control of step-growth polymerization, however, required that we further purify this first batch of tetracene monomer through three painstakingly careful recrystallizations. In the end, we were able to polymerize about 15 mg of **M3** with the fluorene comonomer, which yielded **P3** with a number average molecular weight of 13 KDa g/mol.

Data of the type in Figure 4, collected using the newly characterized **P3**, are among the most gratifying ever recorded in our lab, particularly because it was the very early days of the lab's existence. It was clear that the diaryltetracene allowed for a fast, clear ratiometric fluorescence response to $^1\text{O}_2$ due to acene- $^1\text{O}_2$ cycloaddition interrupting energy transfer from the CP backbone to the tetracene pendants. We submitted the paper after a flurry of confirmation, control, and characterization experiments that lasted about a month or two.

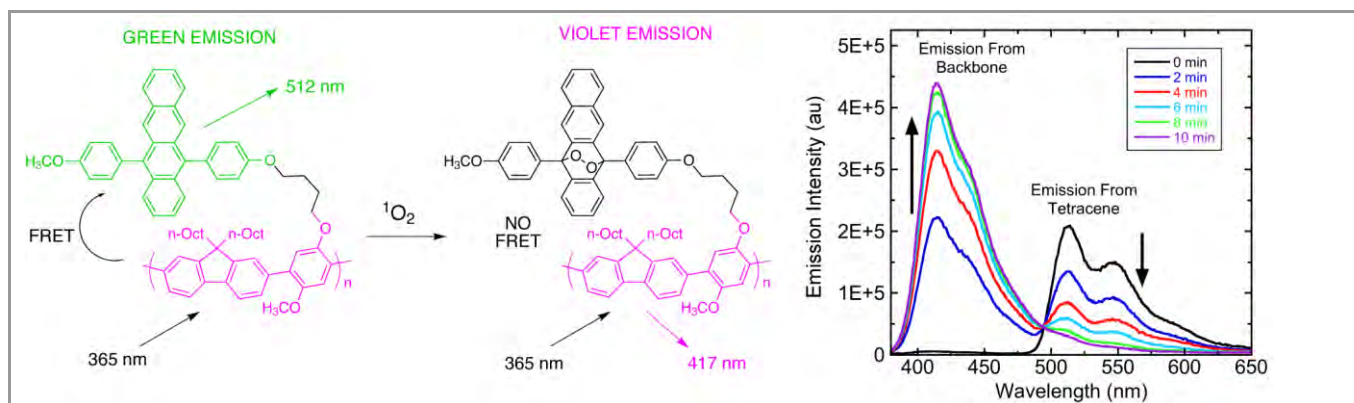
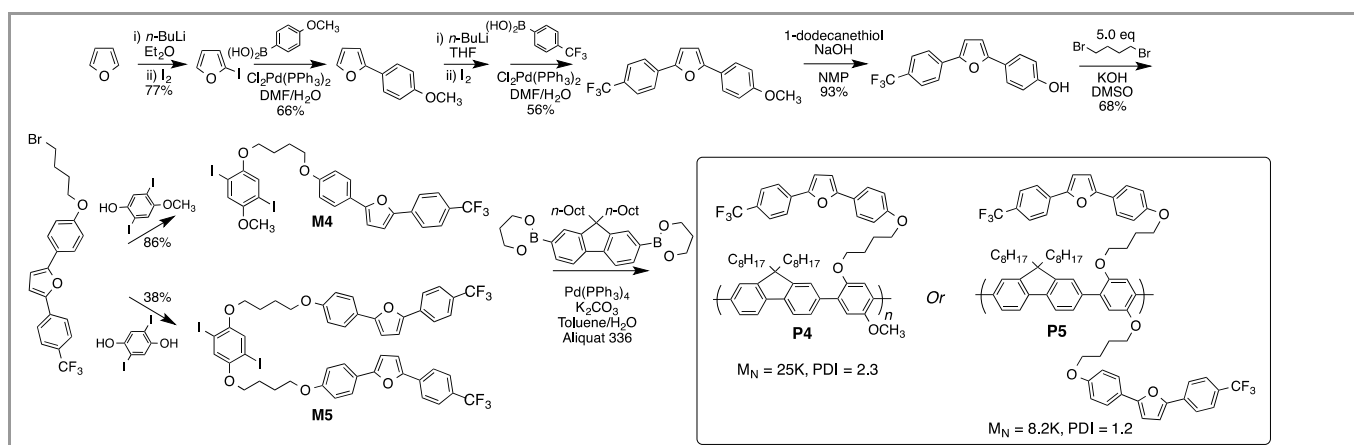


Figure 4. Ratiometric fluorescence response of tetracene-linked conjugated polymer **P3** to photochemically generated $^1\text{O}_2$ in CH_2Cl_2 . Reproduced in part from Ref. 28 with permission from the Royal Society of Chemistry.



Scheme 2. Example synthesis of trifluoromethyl-substituted diarylfuran-linked poly(fluorene-*alt*-phenylene)s with one furan per repeating unit (**P4**) or two (**P5**).

Not long after, we investigated a related series of diene-linked CPs for $^1\text{O}_2$ responsiveness, this time using 2,5-diarylfurans,³⁹ endoperoxides derived from which are not stable at room temperature and fragment into several electron-poor products.⁴⁰ Inspired by the mechanism through which nitroaromatics quench CPs,⁴ we predicted that the electron-poor products would be good fluorescence quenchers by accepting excited electrons from the CP backbone (Figure 5). We used the same type of poly(fluorene-*alt*-phenylene) backbone, but replaced the acene with a diarylfuran as shown in Scheme 2. Introducing unsymmetric substitution on the diarylfuran is easier than on tetracenes, but the iodofurans are highly unstable and decompose completely within a couple of days. This instability required us to perform cross-coupling reactions with them immediately upon isolation. It is also worth mentioning the alkylation of the phenol-substituted diarylfurans with 1,4-dibromobutane, which is necessary to link the furan pendant to the polymerizable diiodoarene. Our initial efforts to link these two units with a butyl chain were exactly analogous to the acene pendants, but reactions of

phenol-substituted furans with **1** gave yields so low that it was difficult to prepare the actual CP, which was extremely frustrating especially as it was so late in the synthesis. For reasons we do not understand, the approach shown in Scheme 2, which just swaps which unit we alkylate first with dibromobutane, gave good yields (58-93%) for each of the two alkylation steps.

These polymers behaved as predicted upon exposure to photochemically generated $^1\text{O}_2$, showing strong fluorescence quenching due to oxidation of furan pendants (Figure 5). There was a tradeoff in terms of substituent effects, with increasingly electron-poor furans showing more efficient quenching upon oxidation, due to the products being better electron acceptors, but slower rates of reaction with $^1\text{O}_2$, as one expects from typical Diels-Alder reactions. Polymers with two furans per repeat unit showed more fluorescence quenching upon complete furan oxidation (**P5** showed 93% quenching). Key to these materials was that the rates of reaction between $^1\text{O}_2$ and the furans were so fast as to preclude decomposition of the CP backbone from contributing to the observed quenching.

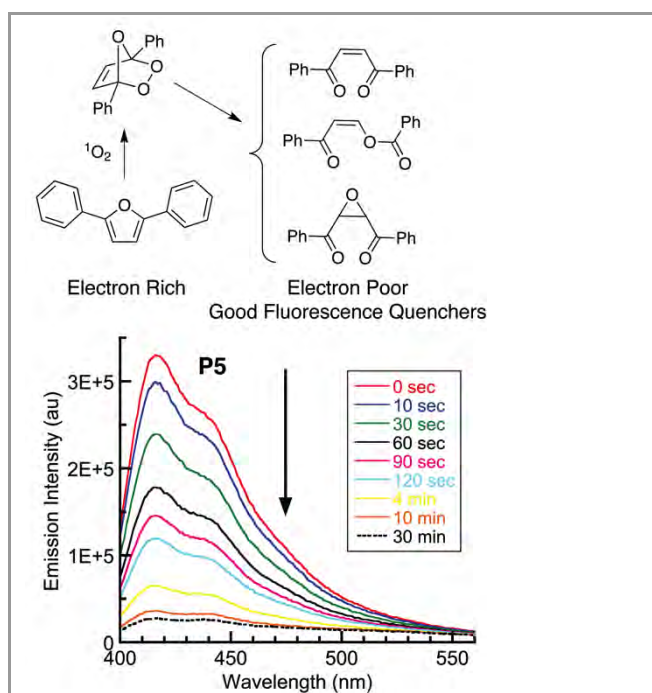


Figure 5. *Top:* Endoperoxides of diarylfurans yield various electron-poor quenchers. *Bottom:* Fluorescence quenching of **P5** due to oxidation of furan pendants with $^1\text{O}_2$. Adapted with permission from Ref. 29. Copyright 2013 American Chemical Society

Red-Shifting Woes Lead to Reversibility

Having investigated two of the most popular classes of dienes for cycloaddition with $^1\text{O}_2$, we decided to focus on acenes because of the ratiometric responses we were able to observe and the large degree to which substituents can tune their luminescence and reactivities. Our efforts then turned to the preparation of CPs that incorporate acenes that were i) more red-shifted and ii) more reactive than the diaryltetracene used in **P3**. Although the obvious candidates were rubrene (5,6,11,12-tetraphenyltetracene) and 6,13-diarylpentacene derivatives, we have been unable to prepare CPs that contain these moieties as $^1\text{O}_2$ -reactive pendants. Contributing to this has been the relative insolubility and reactivity of the 6,13-diarylpentacene derivatives. Beyond these types of structures, however, not much had been reported quantitatively on the reactivity of highly red-shifted acenes, which we attribute in part to the requirement for electronics applications to *prevent* this reaction. Therefore, an important phase of our research at this time turned to understanding what other sorts of novel acene structures could fulfill our goals of red-shifted emission, faster reaction with $^1\text{O}_2$, or both.

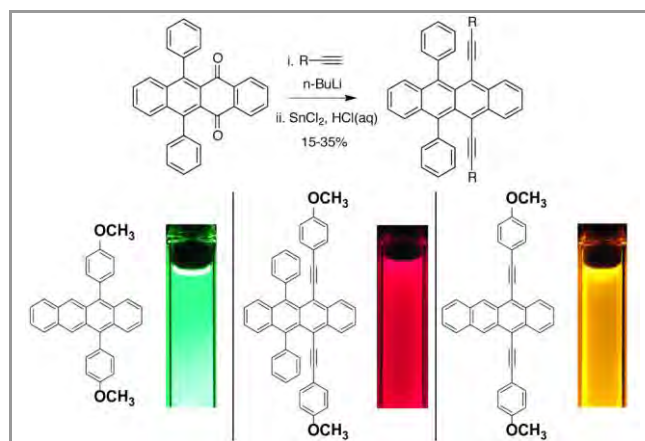
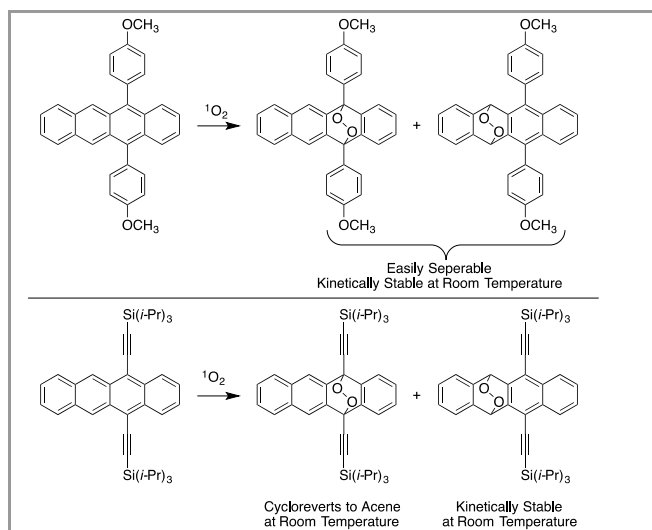


Figure 6. *Top:* Synthesis of tetrasubstituted tetracenes with both phenyl and ethynyl substituents for increased conjugation. *Bottom:* Photographs of fluorescence from solutions of three substituted tetracene derivatives in solution under illumination with UV light, showing increased red-shifted emission from the tetrasubstituted derivative. Adapted with permission from Ref. 31 with permission from the Royal Society of Chemistry.

Our initial targets were related to rubrene, but instead substituted two alkynes for two of the phenyl substituents for increased conjugation and easy synthetic manipulation (Figure 6).⁴¹ Access to these novel acene structures was possible through double addition of alkynyllithium reagents to diphenyltetracenequinone, followed by reduction of the diol intermediate with SnCl_2 in aqueous HCl . In line with our design principles, these tetra-substituted tetracene derivatives had HOMO-LUMO gaps that were significantly lower than those of the diaryltetracene from **P3**; in fact, their onset of absorbance and fluorescence spectra matched almost exactly those of 6,13-dianisylpentacene. Figure 6 shows visual comparisons of the fluorescence of disubstituted and tetrasubstituted tetracenes. From the point of view of their reactivity with $^1\text{O}_2$, however, they were actually less reactive than 5,12-dianisyltetracene by 2.5x-5x. It is indeed well known that the reactivity of alkyne substituted acenes is suppressed relative to unsubstituted analogs; Fudikar and Linker have elucidated many of the reasons for this, which turn out to depend on the length of the acene.²³ Another approach to accessing increasingly red-shifted tetracenes would be substitution with methoxy groups as reported by Anthony and coworkers.⁴²



Scheme 3: The regioisomeric mixture of endoperoxides from 5,12-diaryltetracenes is easily separable, while the endoperoxide formed on the ethynylated positions of diethynyltetracenes cycloreverts to the acene at room temperature.

As a part of this work we had a goal of separating and characterizing the two endoperoxides that could be formed upon oxidation with $^1\text{O}_2$. In previous experiments on dianisyltetracene, we were able to do so easily by flash chromatography (see Scheme 3). In these cases, however, we were unable to do so: although, based on NMR and TLC analysis of the crude oxidation mixture, there was clearly a mixture of endoperoxides formed, we were only able to isolate one by chromatography, along with significant amounts of the starting acene which formed on the silica gel with a distinctive red color. We then noticed that upon standing neat, in the dark, the mixture of endoperoxides from the tetrasubstituted tetracenes turned red over the span of several days and showed resonances in the NMR spectrum attributed to the starting acene. By studying the evolution of the mixture of endoperoxides from bis(trimethylsilyl)ethynyltetracene, we found that the regioisomer oxidized at ethynylated ring cycloreverts to the starting acene at room temperature, while the regioisomer at the unsubstituted ring did not, suggesting that ethynyl substitution lowers the barrier for cycloreversion of endoperoxides. Around the same time, Fudikar and Linker reported that diethynylanthracene derivatives cyclorevert rapidly at room temperature (much like the endoperoxide of 1,4-dimethylnaphthalene), attributing the behavior to increased stability of propargyl radical intermediates.⁴³ Clearly, then, ethynylated acene-endoperoxides show promise as substrates for reversible photoreactions. Although we have yet to capitalize on this discovery for $^1\text{O}_2$ -responsive polymers and other materials, it opens possibilities to move beyond dosimeters to dynamically-responsive, reversible sensors based on $^1\text{O}_2$ -acene cycloaddition chemistry.

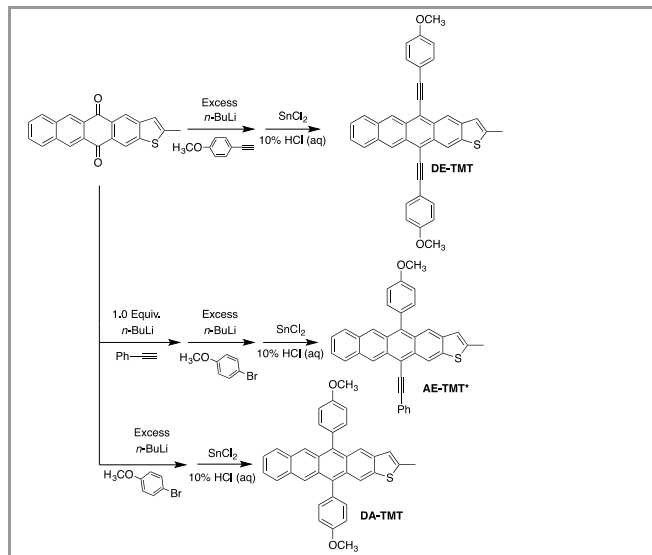
Building a Database

Our group's efforts in rational tuning of properties of a tetracene derivative by extending conjugation through ethynyl substitution opened our eyes to the challenges inherent in trying to improve the properties of a molecule along multiple axes simultaneously. In the case of the 5,12-diethynyl-6,11-diphenyltetracenes, the desired characteristic of red-shifted emission also yielded a decrease in reactivity, which is unfavorable for maximum sensitivity to $^1\text{O}_2$. Therefore, our strategy shifted from the design of individual acene cores to a systematic study of how important variations in key structural elements affect the properties important to our applications: absorbance and fluorescence properties, frontier molecular orbital energies, and reactivity with $^1\text{O}_2$. Much of this information has been reported for a large number of anthracene derivatives,^{12, 44} a variety of symmetrically substituted pentacene derivatives,^{45, 46} and diethynyl anthradithiophenes.⁴⁵

We decided to examine how these properties depended on two common structural elements of elaborated acenes.⁴⁷ One was whether substitution directly on the acene core was diaryl, diethynyl, or a mixture of the two; in general, we expected that unsymmetric substitution of acenes with one aryl and one ethynyl group, examples of which are rare for acenes longer than three rings, would yield spectral properties and reactivities intermediate between corresponding diaryl and diethynyl derivatives. The other was whether a conjugated acene core was extended by either a fused thiophene or benzene ring; in this case, we expected that the smaller resonance energy of thiophene compared to benzene would result in thienoacenes having properties intermediate between an unextended acene and the corresponding acenes extended with a benzene ring.

A thorough study of such a line of inquiry therefore required the synthesis of a significant number of compounds, several of which constituted the first examples of their subclass of substituted acenes and thienoacenes. The basic strategy for synthesis was highly conserved (see Scheme 4 for an example with "TMT" derivatives—Tetracene-MonoThiophene). All compounds were prepared using nucleophilic addition of the appropriate organolithium reagents to quinones, followed by reduction. In these syntheses, we faced two significant challenges. The first was a general approach to preparing unsymmetrically substituted aryl-ethynylacenes; our previous attempts (*vide supra*) to prepare unsymmetric diaryl acenes from quinones failed. The work of Tykwinski and coworkers, in which monoadducts of 6,13-pentacenequinone and alkynyllithium reagents were prepared in high yield as intermediates in the synthesis of unsymmetric diethynylpentacenes, gave us an easily implemented and practical method for preparing such compounds.^{48, 49} We found that this approach was both i) generally useful for preparing monoethynyl

adducts for all the quinones we tested (anthracene, tetracene, pentacene, thienoanthracene, thienotetracene, and anthradithiophene), and ii) useful for preparing arylethynyl derivatives by exposing the intermediate γ -hydroxyketones to excess aryllithium salt.



Scheme 4. Example syntheses of diethynyl, aryl-ethynyl, and diaryl derivatives of the tetraceno[thiophene] backbone. Reproduced with permission from Ref. 35. Copyright 2014 American Chemical Society.

Our second challenge involved the somewhat labile C-H bond on the 2-position of terminal fused thiophene rings. Most previous reports of substituted derivatives of the heteroacene cores under investigation here utilized ethynyl substituents on a central ring, installation of which required alkynyllithium salts that are not sufficiently basic to deprotonate the thiophene moieties. Analogous efforts in our lab to prepare aryl-substituted derivatives of, for example, anthradithiophene, yielded only poor yields of butylated products, presumably through deprotonation terminal thiophene rings and subsequent alkylation with the 1-bromobutyl side-product of halogen-metal exchange. We solved this problem through methylation of the 2-position of all terminal thiophene rings early in the synthesis of the quinones,⁵⁰ resulting in clean thienoacenes in acceptable yields for our purposes (30–50%). Although substitution with other groups, such as fluorine atoms or nitriles has also been reported to yield highly fluorescent thienoacene derivatives,⁵¹ we chose alkylation to prevent the possibility of electron withdrawing substituents slowing the rate of oxidation by $^1\text{O}_2$. This approach of installing alkyl groups on terminal thiophene rings of these heteroacenes also brings the additional possibility of increasing solubility of these derivatives and their synthetic intermediates, which can be a limiting factor in the practical synthesis of large, elaborated acenes.

Trends in the key properties of these acenes for $^1\text{O}_2$ -responsive fluorophores (optical properties and reaction rates) generally adhered to what we predicted. Lengthening of acenes through addition of a thiophene ring yielded red shifted spectra, mostly through an increase in HOMO energies. This shift was this than that due to addition of a benzene ring, which both increased HOMO energies and decreased LUMO energies. In addition, increasing conjugation of these acenes by substituting ethynyl groups for aryl groups also yielded red-shifted spectra, primarily through a lowering of the LUMO energies. In terms of absorbance and fluorescence, these compounds together cover nearly the entire visible spectrum from violet to red emission (see Figure 7). The rates of reactivity with $^1\text{O}_2$ also corresponded to our expectations, with longer acenes generally reacting faster than shorter acenes, and addition of fused thiophenes contributing less to the observed increase in rate than addition of fused benzenes. Double ethynyl substitution also lowers the rate of reaction of an acene core with $^1\text{O}_2$ by approximately 3–10x, with single ethynyl substitution yielding a smaller decrease in rate. The most obvious exception to this is diethynylpentacenes, which show a $\sim 10^3$ decrease in reactivity, which Linker has discovered is due to a competitive quenching of $^1\text{O}_2$.²³ Our calculation of the triplet energy (0.74 eV) of this pentacene suggests that this may be due to energy transfer from $^1\text{O}_2$ to the pentacene, as is known to occur for β -carotene. Finally, this work uncovered that the diaryl and aryl-ethynyl derivatives of tetraceno[thiophene] react with $^1\text{O}_2$ with observed rate constants and spectral positions close to that of 6,13-diarylpentacene, but with much higher fluorescence quantum yields (0.4–0.5 vs. 0.1), highlighting these and related structures for potential use in $^1\text{O}_2$ -responsive fluorescent materials.

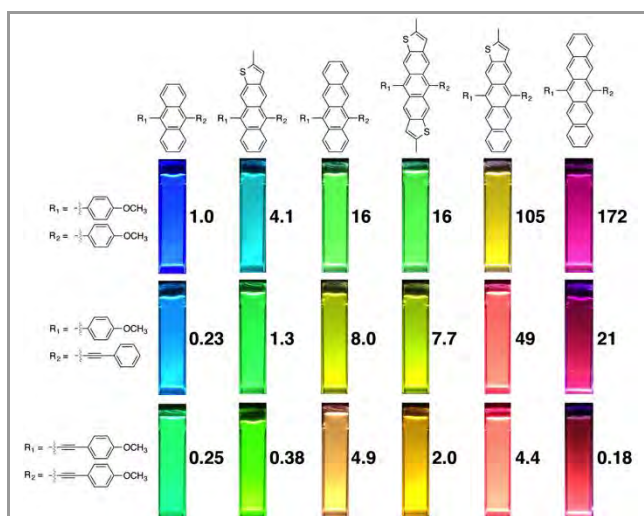
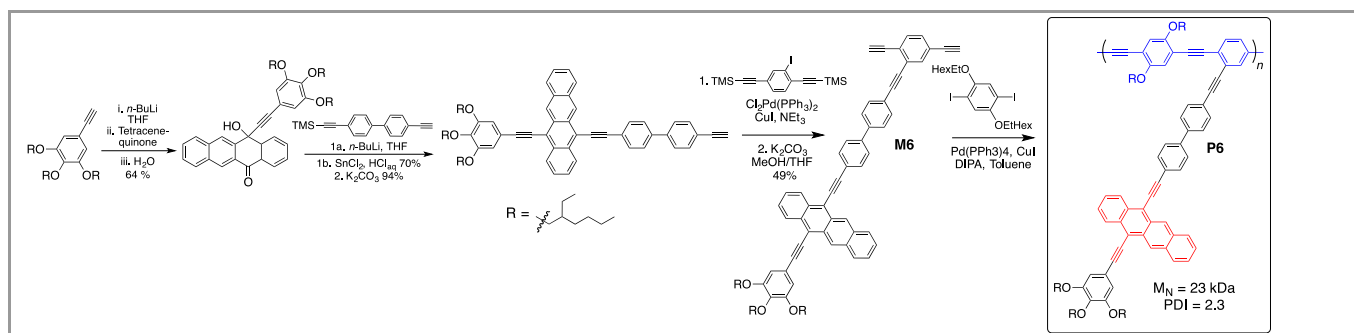


Figure 7. Trends of emission colors, visualized using photographs of acene solutions under UV illumination, together with relative observed rates of oxidation for each acene. . Adapted with permission from Ref. 35. Copyright 2014 American Chemical Society.



Scheme 5. Synthesis of two-dimensional, ¹O₂-responsive conjugated polymer **P6**.

Integrating Other Acenes

Having quantitative reactivity data in hand for a broad range of substituted acenes, our goal has been to integrate ¹O₂-reactive acenes into polymers in new ways. One recently reported effort centered on linking an acene to a CP backbone through a conjugated linker, as such linkers have been reported to increase energy transfer rates in a number of examples of “through bond” energy transfer cassettes.⁵² We therefore targeted the poly(phenylene ethynylene) shown in Scheme 5, which uses a biphenyl-bridged oligo(phenylene-ethynylene) as a conjugated linker between the CP backbone and the pendant diethynyltetracenes.⁵³ We chose tetracenes in this case as they react the fastest with ¹O₂ than any other of the diethynylacenes we examined above. The first key intermediate in this synthesis is the 3,4,5-tri(2-ethylhexyl)ethynylbenzene; the long, branched alkyl chains are required not only for solubility of the final CP, but also for solubility of the monomer and other extended, rigid intermediates. Analogous routes that did not include these solubilizing chains resulted in low yields and difficult purifications because of the insolubility of many of the reaction products. Again critical to our success was the selective mono-addition of alkynyllithium salts to tetracenequinone, which was followed by excess lithiated 4-ethynyl-4'-trimethylsilyl ethynylbiphenyl, SnCl₂/HCl reduction, and deprotection of the TMS group to yield a monomer precursor. Finally, Sonogashira cross-coupling with 2,5-bis(trimethylsilyl)ethynyl iodobenzene and TMS deprotection yielded the dialkyne monomer **M6** suitable for Sonogashira polymerizations. When conducted at ambient temperature with the comonomer 1,4-di(2-ethylhexyl)-2,5-diiodobenzene comonomer, these reactions worked well, yielding polymers with *M_N* values of 20–70 kDa. At 65 °C, however, these reactions yielded only insoluble highly colored gelatinous precipitates that were insoluble in every organic solvent investigated.

The 50-50 alternating copolymer (**P6**) showed what was, for us at the time, peculiar behavior upon

exposure to ¹O₂. The UV/vis spectrum behaved as one would expect, with decreasing absorbance of bands attributed to the extended tetracene moiety. The fluorescence spectrum, on the other hand, showed not a decrease in fluorescence intensity from the tetracene at 580 nm, but an *increase*, even though the concentration of tetracene was decreasing with increasing exposure time to ¹O₂ (see Figure 8). This increase continued until approximately 75% conversion of tetracene pendants to endoperoxides, at which time it decreased and fluorescence from the polymer backbone started to emerge from the baseline at 470 nm. An observed increase in average fluorescence lifetime of **P6** with increasing endoperoxide formation indicated that dynamic self-quenching of the tetracenes occurs with a large local concentration with tetracenes on every repeating unit, while decreasing the local tetracene concentration with endoperoxide formation inhibits this self-quenching. This interpretation explains the unexpected fluorescence response of **P6** to ¹O₂. Finally, we conducted a random copolymerization with **M6** diluted four-fold with a ¹O₂-inert dialkyne monomer so that the overall loading of the tetracene pendant was decreased by 4x relative to the 1:1 copolymer **P6**. In an illustration of the amplifying nature of CPs, this polymer with an average of one tetracene for every eight conjugated aromatic rings along the polymer backbone showed nearly complete energy transfer before exposure to ¹O₂, and a faster ratiometric fluorescent response to ¹O₂ than **P6**, as there was not a large excess of tetracenes that had to be oxidized before fluorescence from the CP backbone could compete with energy transfer.

Conclusion and Looking Forward

Our group has created a general construct comprising conjugated polymers with ¹O₂-reactive pendants. We have demonstrated that such materials can demonstrate ratiometric fluorescent responses to ¹O₂, and that such responses can be amplified relative to analogous small molecules. Through fundamental studies of the structure-property relationships of acenes and heteroacenes, we have uncovered structural elements that enable reversibility of ¹O₂-mediated oxidation, created a library of substituted

acene derivatives that span a range of luminescence colors and reactivities, and developed guidelines for predicting the effects of additional fused rings and different substituents on frontier molecular orbital energies. We are currently combining these lines of inquiry into next-generation fluorescent materials with the goals of increased sensitivity to photosensitized $^1\text{O}_2$ through incorporation of maximally reactive (hetero)acenes and improvement of energy transfer efficiencies. We are also developing $^1\text{O}_2$ -responsive materials that work in aqueous environments, building upon previous success in CP/acene thin film composites that respond ratiometrically to $^1\text{O}_2$ generated using sensitizers bound to proteins.

As have our achievements to this point, our future success will rely upon the ever-expanding toolbox of synthesis to control molecular structure. Key design elements such as controlling the electronic coupling between donors and acceptors, developing $^1\text{O}_2$ -reactive units that can also be integrated into polymer structures in new ways, and enhancing performance in water will require the efficient syntheses of new molecules and materials that combine these individual design elements. As practitioners of synthetic chemistry in pursuit of novel functional materials, the ever-increasing scope of structures accessible with modern synthetic methods is both tantalizing and overwhelming, which results in feeling like the proverbial “kid in a candy store”, with our imaginations the largest hurdle to new discoveries.

Acknowledgment

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References

1. Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339-1386.
2. Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201-207.
3. Yang, J. S.; Swager, T. M. *J. Am. Chem. Soc.* **1998**, *120*, 5321-5322.
4. Yang, J. S.; Swager, T. M. *J. Am. Chem. Soc.* **1998**, *120*, 11864-11873.
5. Dennler, G.; Scharber, M. C.; Brabec, C. J. *Adv. Mater.* **2009**, *21*, 1323-1338.
6. Turro, N. J.; Ramamurthy, V.; Scaiano, J. C., *Modern molecular photochemistry of organic molecules*. University Science Books: Sausalito, CA, 2010.
7. Clennan, E. L. *Tetrahedron* **2000**, *56*, 9151-9179.
8. Kearns, D. R. *Chem. Rev.* **1971**, *71*, 395-&.
9. Clennan, E. L.; Pace, A. *Tetrahedron* **2005**, *61*, 6665-6691.
10. Ogilby, P. R. *Chem. Soc. Rev.* **2010**, *39*, 3181-3209.
11. Ogilby, P. R. *Photochem. Photobiol. Sci.* **2010**, *9*, 1543-1560.
12. Wilkinson, F.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1995**, *24*, 663-1021.
13. Schweitzer, C.; Schmidt, R. *Chem. Rev.* **2003**, *103*, 1685-1757.
14. Wilkinson, F.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1993**, *22*, 113-262.
15. Abdou, M. S. A.; Holdcroft, S. *Macromolecules* **1993**, *26*, 2954-2962.
16. Koch, M.; Nicolaescu, R.; Kamat, P. V. *J. Phys. Chem. C* **2009**, *113*, 11507-11513.
17. Prein, M.; Adam, W. *Angew. Chem. Int. Ed.* **1996**, *35*, 477-494.
18. Zaklika, K. A.; Kaskar, B.; Schaap, A. P. *J. Am. Chem. Soc.* **1980**, *102*, 386-389.
19. Maranzana, A.; Ghigo, G.; Tonachini, G. *J. Am. Chem. Soc.* **2000**, *122*, 1414-1423.
20. Aubry, J. M.; Mandardcazin, B.; Rougee, M.; Bensasson, R. V. *J. Am. Chem. Soc.* **1995**, *117*, 9159-9164.
21. Montagnon, T.; Kalaitzakis, D.; Triantafyllakis, M.; Stratakis, M.; Vassilikogiannakis, G. *Chem. Commun.* **2014**, *50*, 15480-15498.
22. Anthony, J. E. *Angew. Chem. Int. Ed.* **2008**, *47*, 452-483.
23. Fudickar, W.; Linker, T. *J. Am. Chem. Soc.* **2012**, *134*, 15071-15082.
24. Thorley, K. J.; Anthony, J. E. *Isr. J. Chem.* **2014**, *54*, 642-649.
25. Wu, H. Y.; Song, Q. J.; Ran, G. X.; Lu, X. M.; Xu, B. G. *Trends Anal. Chem.* **2011**, *30*, 133-141.
26. Lucky, S. S.; Soo, K. C.; Zhang, Y. *Chem. Rev.* **2015**, *115*, 1990-2042.
27. Dolmans, D. E. J. G. J.; Fukumura, D.; Jain, R. K. *Nat. Rev. Cancer* **2003**, *3*, 380-387.
28. Poulsen, F.; Jensen, K. B. *J. Biomolec. Screen.* **2007**, *12*, 240-247.

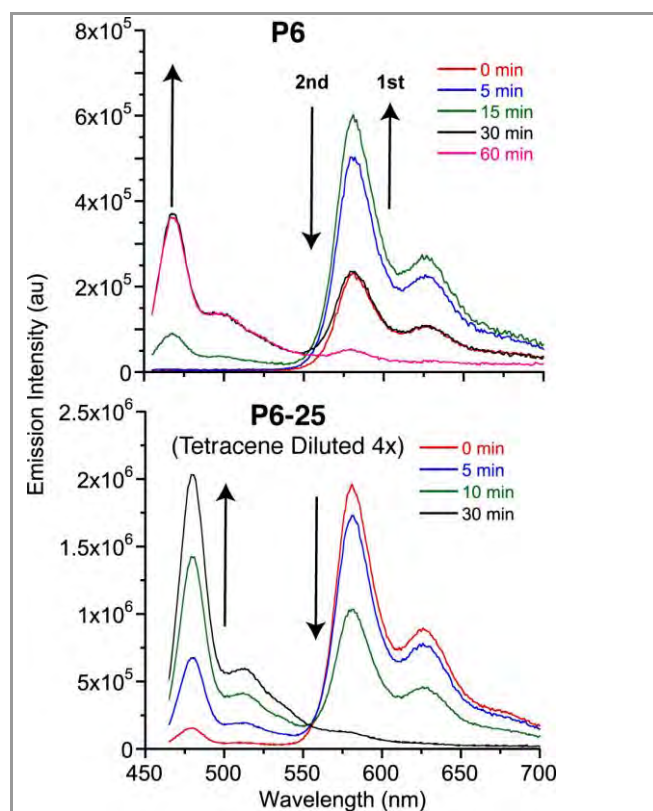
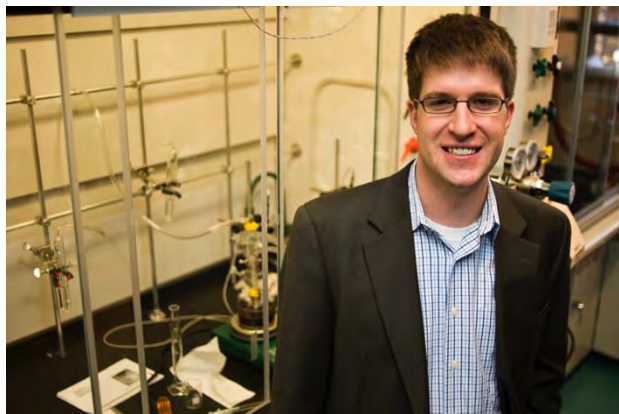


Figure 8. Due to the amplifying properties of CPs, the ratiometric response to $^1\text{O}_2$ of **P6** accelerates when the tetracene content is reduced four-fold. Copyright 2015 American Chemical Society.

29. Ullman, E. F.; Kirakossian, H.; Singh, S.; Wu, Z. P.; Irvin, B. R.; Pease, J. S.; Switchenko, A. C.; Irvine, J. D.; Dafforn, A.; Skold, C. N.; Wagner, D. B. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5426-5430.
30. Tanaka, K.; Miura, T.; Umezawa, N.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T. *J. Am. Chem. Soc.* **2001**, *123*, 2530-2536.
31. Umezawa, N.; Tanaka, K.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T. *Angew. Chem. Int. Ed.* **1999**, *38*, 2899-2901.
32. Kim, S.; Fujitsuka, M.; Majima, T. *J. Phys. Chem. B* **2013**, *117*, 13985-13992.
33. Pedersen, S. K.; Holmehave, J.; Blaikie, F. H.; Gollmer, A.; Breitenbach, T.; Jensen, H. H.; Ogilby, P. R. *J. Org. Chem.* **2014**, *79*, 3079-3087.
34. Kim, S.; Tachikawa, T.; Fujitsuka, M.; Majima, T. *J. Am. Chem. Soc.* **2014**, *136*, 11707-11715.
35. Song, D.; Cho, S.; Han, Y.; You, Y.; Nam, W. *Org. Lett.* **2013**, *15*, 3582-3585.
36. Chan, K. L.; Sims, M.; Pascu, S. I.; Ariu, M.; Holmes, A. B.; Bradley, D. D. C. *Adv. Funct. Mater.* **2009**, *19*, 2147-2154.
37. Thomas, S. W.; Amara, J. P.; Bjork, R. E.; Swager, T. M. *Chem. Commun.* **2005**, 4572-4574.
38. Zhang, J. J.; Sarrafpour, S.; Pawle, R. H.; Thomas, S. W. *Chem. Commun.* **2011**, *47*, 3445-3447.
39. Altinok, E.; Friedle, S.; Thomas, S. W. *Macromolecules* **2013**, *46*, 756-762.
40. Graziano, M. L.; Iesce, M. R.; Scarpati, R. *J. Chem. Soc. Perkin Trans. 1* **1982**, 2007-2012.
41. Zhang, J. J.; Sarrafpour, S.; Haas, T. E.; Muller, P.; Thomas, S. W. *J. Mater. Chem.* **2012**, *22*, 6182-6189.
42. Odom, S. A.; Parkin, S. R.; Anthony, J. E. *Org. Lett.* **2003**, *5*, 4245-4248.
43. Fudickar, W.; Linker, T. *Chem. Eur. J.* **2011**, *17*, 13661-13664.
44. Fudickar, W.; Linker, T. *Chem. Commun.* **2008**, 1771-1773.
45. Kim, C.; Huang, P. Y.; Jhuang, J. W.; Chen, M. C.; Ho, J. C.; Hu, T. S.; Yan, J. Y.; Chen, L. H.; Lee, G. H.; Facchetti, A.; Marks, T. J. *Org. Electron.* **2010**, *11*, 1363-1375.
46. Kaur, I.; Jia, W. L.; Kopreski, R. P.; Selvarasah, S.; Dokmeci, M. R.; Pramanik, C.; McGruer, N. E.; Miller, G. P. *J. Am. Chem. Soc.* **2008**, *130*, 16274-16286.
47. Zhang, J. J.; Smith, Z. C.; Thomas, S. W. *J. Org. Chem.* **2014**, *79*, 10081-10093.
48. Lehnher, D.; Gao, J.; Hegmann, F. A.; Tykwinski, R. R. *Org. Lett.* **2008**, *10*, 4779-4782.
49. Lehnher, D.; McDonald, R.; Tykwinski, R. R. *Org. Lett.* **2008**, *10*, 4163-4166.
50. Anthony, J. E.; Subramanian, S.; Parkin, S. R.; Park, S. K.; Jackson, T. N. *J. Mater. Chem.* **2009**, *19*, 7984-7989.
51. Platt, A. D.; Day, J.; Subramanian, S.; Anthony, J. E.; Ostroverkhova, O. *J. Phys. Chem. C* **2009**, *113*, 14006-14014.
52. Gong, Y. J.; Zhang, X. B.; Zhang, C. C.; Luo, A. L.; Fu, T.; Tan, W. H.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2012**, *84*, 10777-10784.
53. Altinok, E.; Smith, Z. C.; Thomas, S. W. *Macromolecules* **2015**, DOI: 10.1021/acs.macromol.5b01076.

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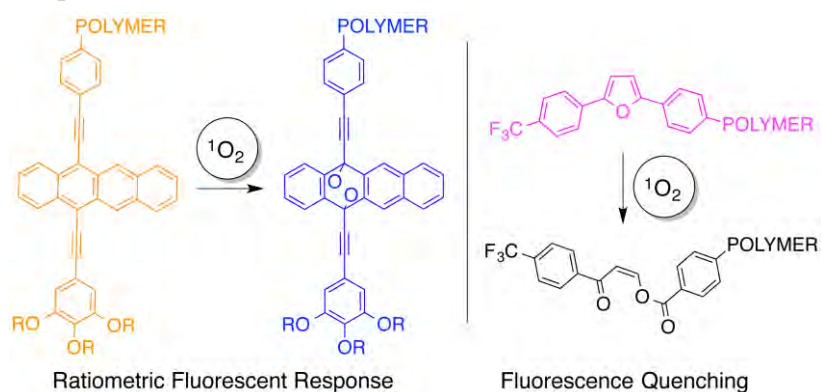
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Graphical Abstract:



Short Title: $^1\text{O}_2$ -responsive fluorescent polymers