Electronic Supplementary Information for

Acene-Doped Polymer Films: Singlet Oxygen Dosimetry and Protein Sensing

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Endoperoxide Preparation

The acene was dissolved in $CDCl_3$ and bubbled with O_2. The solution was irradiated with the Hg/Xe lamp using a high-pass filter and plano-convex lens. When all starting material had reacted, the products were analyzed by NMR. The ^1H NMR of the endoperoxide of rubrene matched that reported in the literature,[1] and showed a characteristic signal at 84 ppm in the ^{13}C NMR. The two regioisomeric endoperoxides from the photooxidation of **TET** were separated by flash chromatography on silica gel (230-400 mesh) using CH_2Cl_2/hexanes. Selective irradiation of the sensitizer methylene blue in the presence of **TET** gave the same two regioisomeric endoperoxides.

Film Preparation

The potential film components were **PF** (Sigma Aldrich), rubrene (**RUB**, Sigma-Aldrich) or **TET** (prepared as previously reported).[2] For film preparation, a solution of the polymer and acene was prepared in chloroform as described in the tables below. Spin-casting (Laurell WS-400-6NPP) onto glass microscope cover slips yielded the films. Film thicknesses were measured with profilometry initially, and correlated to optical absorbance for determination of film thickness by UV/vis spectrophotometry.

Recipes for preparing spun-cast films:

100 nm-thick films, 2.5% **TET**/**PF**:

PF	100 mg
TET	2.5 mg
$CHCl_3$	10 mL
Spin speed	800 rpm (3 sec) 1500 rpm (20 sec)

10 nm-thick films, 2.5% **TET**/**PF**:

PF	100 mg
TET	2.5 mg
$CHCl_3$	100 mL
Spin speed	3000 rpm (60 sec)

Optical Measurements

Electronic absorbance spectra were acquired with Varian Cary-100 instrument in double beam mode using a solvent-containing cuvette or clean glass slide for background subtraction spectra. Fluorescence emission spectra were obtained by using a PTI Quantum Master 4 equipped with a 75W Xe lamp. All fluorescence spectra were corrected for the output of the lamp and the dependence of detector response to the wavelength of emitted light. Irradiation for production of singlet oxygen was performed with a 200W Hg/Xe lamp (Newport-Oriel) equipped with a water filter, manual shutter, and either a 546 nm interference filter (for Rose Bengal) or 530 nm high-pass filter (for Eosin) in the light path.

Protein Labeling and Binding to Microspheres

Neutravidin Biotin Binding Protein (10 mg, Thermo Scientific) was dissolved in 1.0 mL of 0.10 M carbonate buffer at pH 9.0. Eosin-5-isothiocyanate (5 mg) was dissolved in 0.5 mL of DMF immediately before it was added to the Neutravidin solution. 100 μL of the eosin solution was added to the protein solution, with continuous stirring for an hour at room temperature. In order to separate the Neutravidin-eosin conjugate from any excess eosin, a Sephadex column was prepared. Approximately 5 g of Sephadex G-50 was allowed to swell overnight in excess distilled water. The Sephadex gel filtration column was prepared using 0.10 M pH 9 carbonate buffer as the eluent. An absorbance spectrum of the band collected containing this conjugate was acquired. The concentration of the labeled protein was 1.5 μM as determined by UV-vis spectrophotometry at 280 nm while accounting for additional absorbance at 280 nm from the bound eosin.

Biotinylated polystyrene microspheres (Bangs Labs), 1% solids in 1 mL with a diameter of 10 μm, were then washed three times with 10 mL of 0.10 M phosphate-buffered saline (PBS), pH 7.4. Centrifugation at 4000 rpm for 5 min yielded the washed microspheres. 10 mL of the solution of Neutravidin-eosin complex (1.5 μM) was added to the suspension of biotinylated microspheres. This mixture was incubated for 30 min at room temperature with

gentle stirring. Washing and centrifuging was done three times using 10 mL 0.10 M PBS at pH 7.4. The Neutravidin-eosin-bound microspheres were stored at 4 ºC in 10 mL PBS.

Supplemental Figures

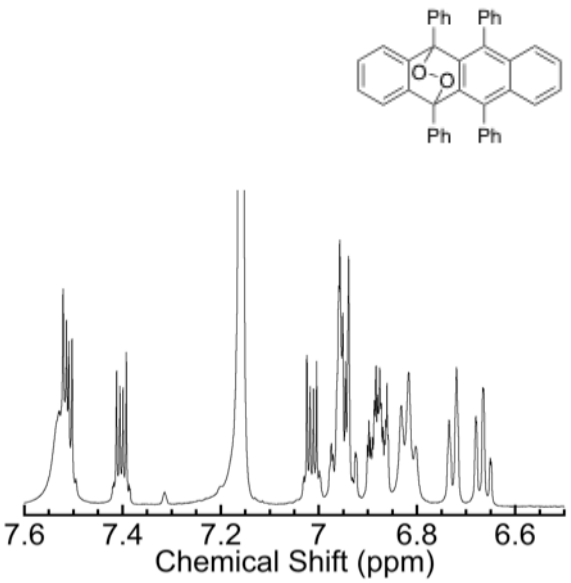

Figure S1. ^{1}H NMR spectra (in C_6D_6) of rubrene endoperoxide generated by direct photo-oxidation.

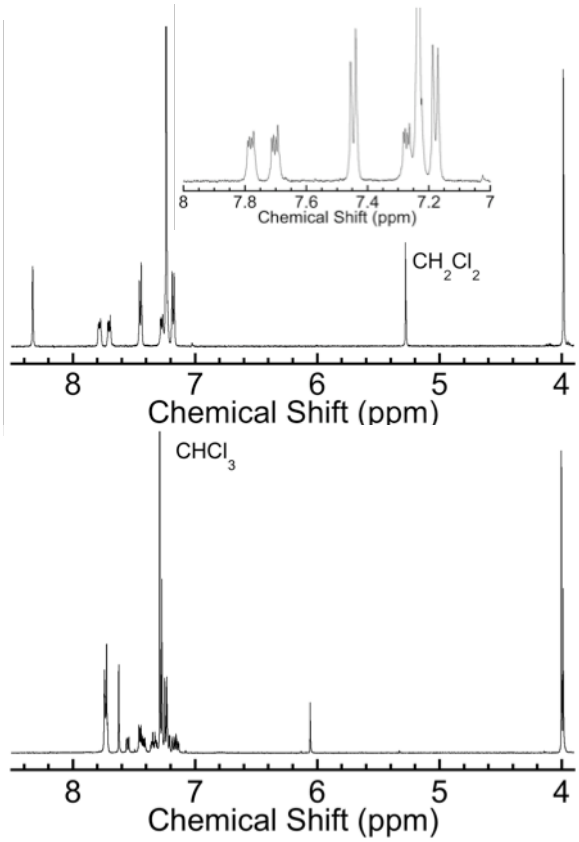

Figure S2. ^{1}H NMR spectra (in $CDCl_3$) of dianisyl tetracene **TET** (*top*), and the mixture of two endoperoxides generated by direct photolysis (*bottom*).

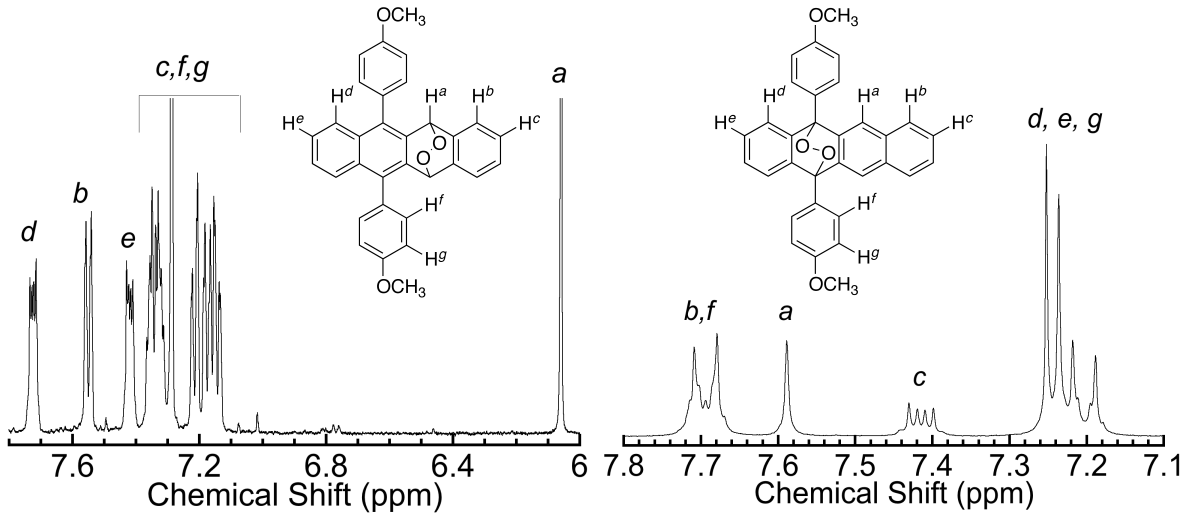

Figure S3. ^{1}H NMR spectra (in $CDCl_3$) of two separated endoperoxides of dianisyl tetracene (**TET**) made by photo-oxidation. These spectra account for all signals in the crude photolysis mixture (Fig. S2).

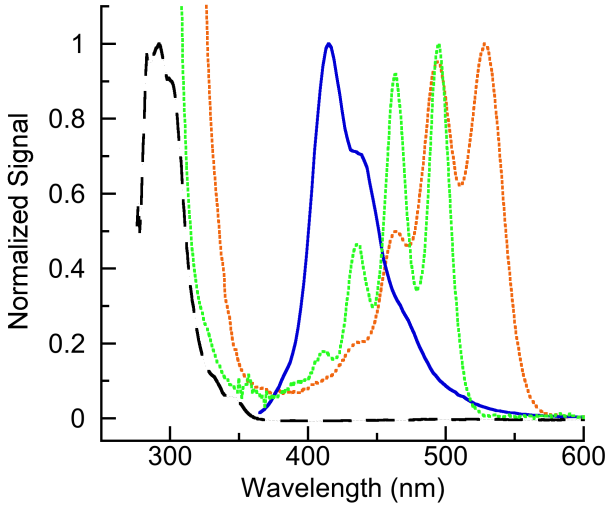

Figure S4. UV/vis spectrum of rubrene endoperoxide (black dashed line), emission spectrum of **PF** (blue solid line), UV/vis spectra of **RUB** (red dotted line) and **TET** (green dotted line). All spectra in CH_2Cl_2.

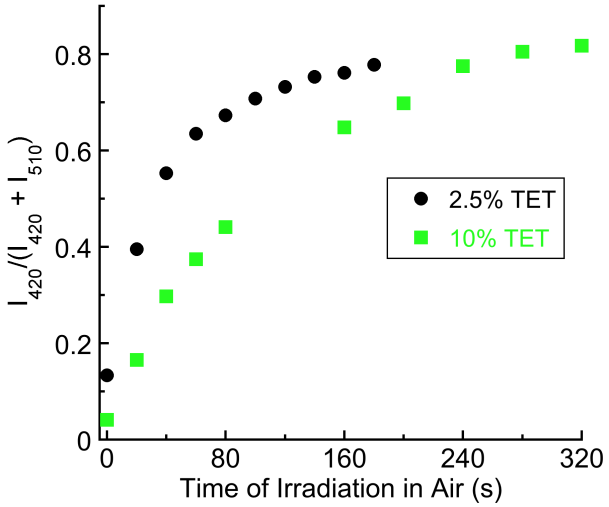

Figure S5. Ratiometric fluorescent response of **TET**-doped 100 nm-thick **PF** films to UV irradiation (λ_{irr} = 350 nm).

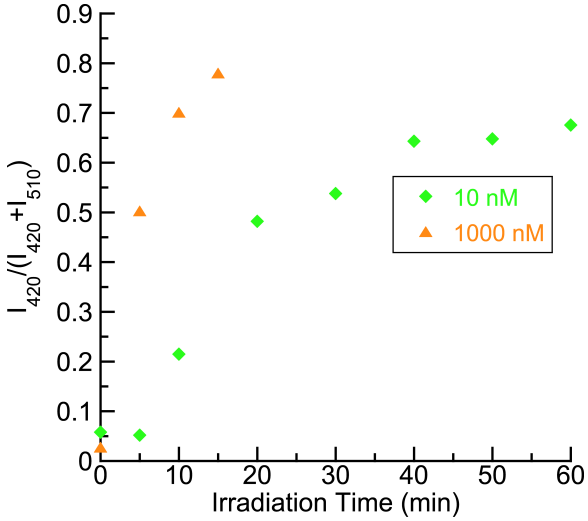

Figure S6. Ratiometric fluorescent response of a 2.5% **TET**/**PF** film to 546 nm irradiation of 10 nM and 1000 nM **RB** in D_2O.

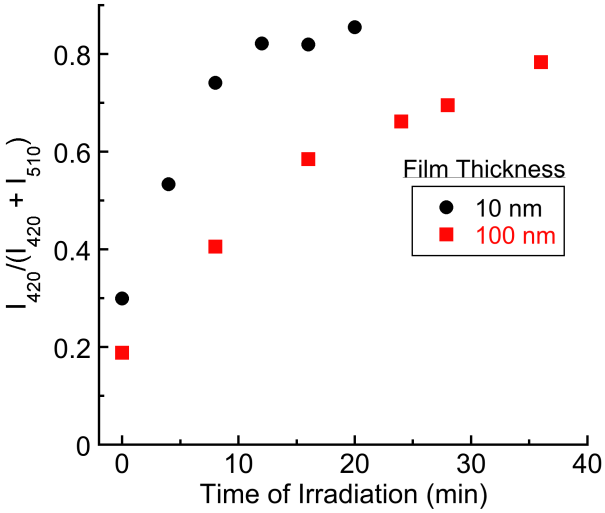

Figure S7. Dependence of ratiometric fluorescent response of 2.5% **TET**/**PF** films to 546 nm irradiation of 100 nM **RB** on film thickness.

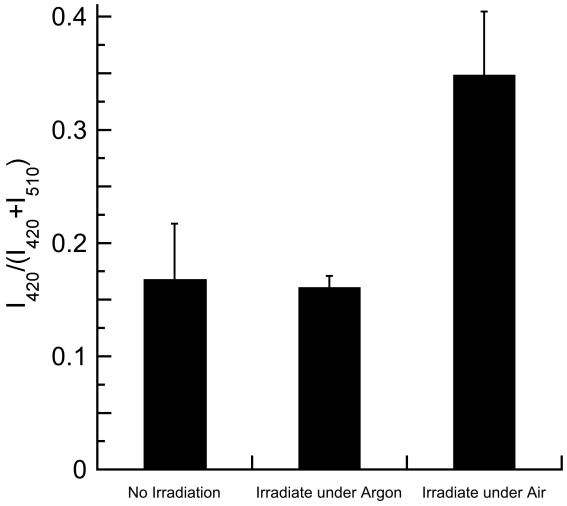

Figure S8. TET/PF films (2.5% w/w) do not respond to irradiation (35 min) of 100 nM RB at 546 nm when the solution of RB is deoxygenated by bubbling with argon for 30 minutes. Heights of the bars represent the mean ratiometric signal from three films; error bars represent one standard deviation.

1. T. A. Singh-Rachford, F. N. Castellano, *J. Phys. Chem. A*, 2008, **112**, 3550–3556.
2. J. Zhang, S. Sarrafpour, R. H. Pawle and S. W. Thomas III, *Chem. Commun.*, 2011, **47**, 3445-3447.