Supporting Information for

Quantifying interactions between singlet oxygen and aquatic fulvic acids

Rose M. Cory,[a,b,c] James B. Cotner[a]*, and Kristopher McNeill [b]*

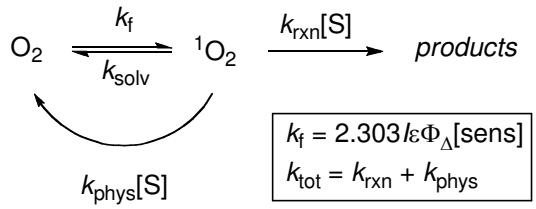
[a] Dept. of Ecology, Evolution & Behavior, 1987 Upper Buford Circle, St. Paul, MN, USA.

Fax: 612-624-6777; Tel: 612-625-1706

[b]Dept. of Chemistry, 207 Pleasant St SE, Minneapolis, MN, USA.

Fax: 612-626-7541; Tel: 612-625-0781

Section S1. Kinetic model for the formation and loss of $^1O_2$



O_2 is molecular oxygen. S is substrate, k_f is the zero-order formation rate constant, I is light intensity, ε is the absorption coefficient for the sensitizer, is the quantum yield for $^1O_2(^1\Delta_g)$ formation, and sens is sensitizer. The zero-order $^1O_2$ formation rate constant, k_f , is proportional to I ε Φ_Δ [sens]. The rate constants k_{solv}, k_{phys}, and k_{rxn} are for deactivation of $^1O_2$ by solvent, physical quenching by S, and chemical reaction with S, respectively.

Section S2. Chemical characteristics of end-member fulvic acids

Table S1. Fulvic acid chemical characteristics

	Suwannee River Fulvic Acid	Pony Lake Fulvic Acid	Reference
SUVA (m^2 g-C^{-1})	3.2	1.7	This work
Fluorescence index	1.24	1.51	This work
%N	0.72	6.0	IHSS
%O	43	31	IHSS
% aromatic carbon	24	12	IHSS
% aliphatic carbon	33	61	IHSS
Φ_Δ $^1O_2$ (%)	0.47	0.69	This work

[a] SUVA = specific absorbance at 254 nm; %N and %O are percent of the fulvic acid by mass; Fluorescence Index = Em 520/470 for Ex=370 nm (McKnight et al. 2001). IHSS = http://www.ihss.gatech.edu/

Section S3. Experimental solution preparation & analysis

Stock solutions of the fulvic acid isolates were prepared in the range of 500 mg-C L^{-1} (42 M-C) by dissolving the lyophilized material in nanopure water or deuterium oxide (D_2O) in amber glass bottles, stirring for 24 hours, and adjusting to pH 6-7 using 0.1 N HCl or NaOH (nanopure) or DCl or NaOD (D_2O). Aliquots of the stock solutions were diluted in Nanopure (Barnstead) water or D_2O to reach the desired concentration of organic matter (2-8 mM-C). Concentrations of dissolved organic carbon (DOC) in the stock and experiment solutions were measured by a Shimadzu TOC 5000 analyzer as non-purgeable organic carbon (NPOC) after acidification to pH 2.0 with concentrated hydrochloric acid. Potassium-hydrogen phthalate solutions were used as standards for the DOC analyzer. Standard deviation in DOC concentrations for samples and standards analyzed in triplicate ranged from ± 0.7 M-C (stock solutions) to ± 0.04 M-C (experiment solutions).

To investigate the effect of β-carotene, a hydrophobic quencher of $^1O_2$ insoluble in water, fulvic acid solutions were spiked with β-carotene prepared from β-carotene stock solutions in THF (Latch & McNeill, citation 4 in manuscript text).

Fulvic acid solutions were analyzed by UV-Vis absorbance and fluorescence using 1-cm pathlength quartz cuvettes on a Cary 50 Bio Spectrophotometer (Varian) and a Fluoromax-3 fluorometer (Jobin-Yvon Horiba), respectively. Excitation-emission matrices (EEMs) for the fulvic acid solutions were collected with excitation range of 240-400 nm, emission 320-550 nm in reference beam mode, which corrects for first-order variation in the xenon lamp output. Excitation was incremented by 5 nm and emission by 2 nm. EEMs of MilliQ water were subtracted to remove Raman scattering and each EEM was then corrected for the wavelength-dependent contribution that instrumental components have on the measured signal using the emission and excitation correction files provided by the manufacturer (Cory & McKnight, 2005; citation 12 in manuscript text). Concentrated fulvic acid solutions were diluted in Nanopure water and mathematically corrected for the inner-filter effect (McKnight et al. 2001; citation 10 in manuscript text). Intensities were converted to Raman units (Cory & McKnight, 2005; citation 12 in manuscript text). Based on fulvic acid solutions analyzed in triplicate differences in emission intensity less than 10% were determined to be within instrumental error.

Singlet oxygen quantum yield values (Φ_Δ $^1O_2$; Table S1) were measured for each fulvic acid solution by FFA degradation. Briefly, fulvic acid solutions spiked with 100 μM FFA were irradiated in a Rayonet photochemical chamber (Southern New England Ultraviolet Company) containing UV light bulbs with a maximum intensity centered at 350 nm. Sub-samples for FFA degradation were collected from each fulvic acid solutions during light exposure as a function of time. Quantum yields were determined through comparison to perinaphthenone as a quantum yield standard (Φ = 0.98; citation 14 in manuscript text).

Section S4. Photochemical uptake of $^1O_2$ as quantified by membrane inlet mass spectrometry (MIMS): controls

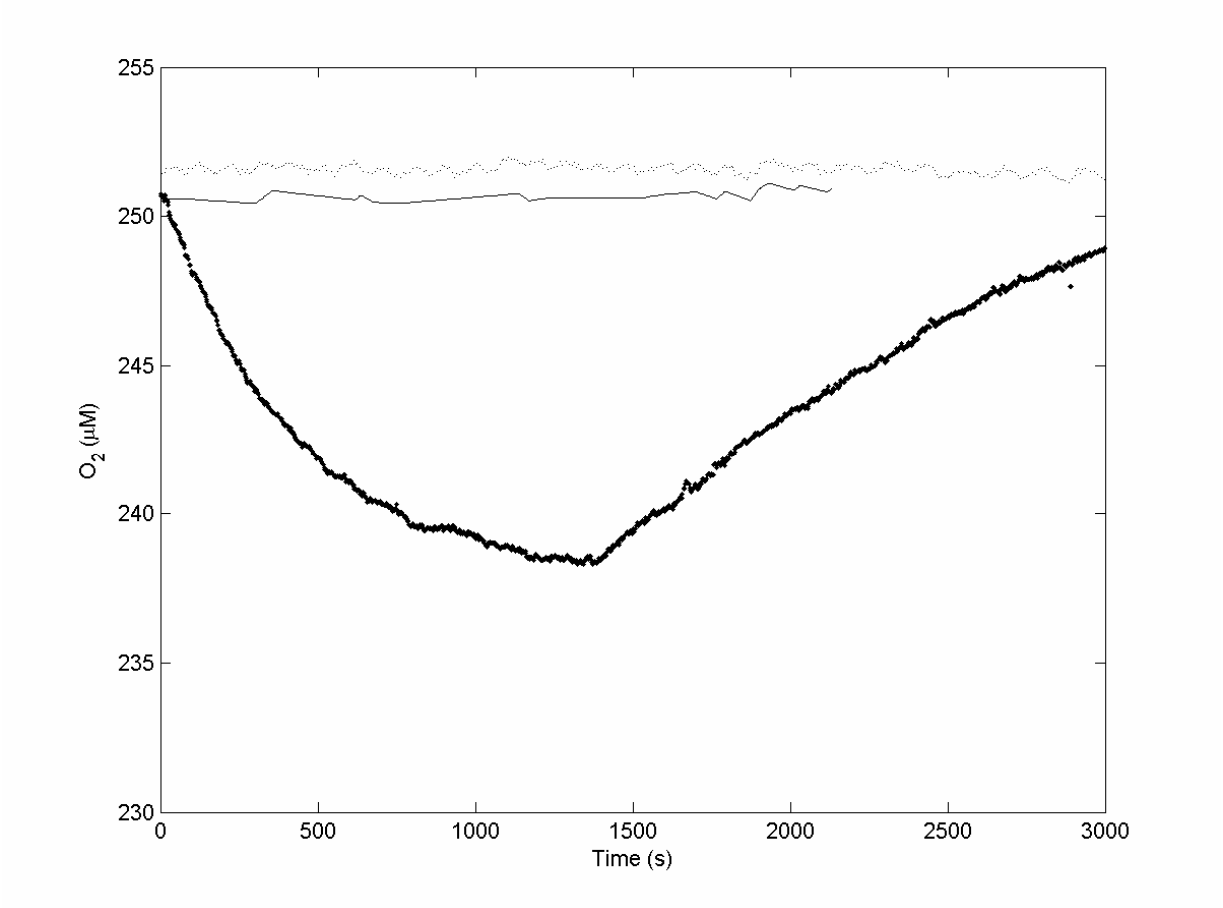


Fig.S1 O_2 vs. time. SRFA (3.3 mM-C), H_2O + Rose Bengal + light(▬), Rose Bengal, dark (▬), SRFA only (– – –). Note that the light was switched off at 1400 s.

This figure shows that only in the presence of the $^1O_2$ sensitizer (Rose Bengal) and the substrate (SRFA) was detectable loss of dissolved O_2 observed. The benefit to using the photochemical oxygen demand to quantify the reaction between $^1O_2$ and fulvic acid is that only net consumptive reactions will result in oxygen uptake. Other interactions, such as quenching of $^1O_2$ by the fulvic acid, should not result in oxygen consumption. The dependence of the replenishment rate of O_2 on the oxygen concentration gradient can be seen in Figure S1, after the light was switched off at 1400 s.

Section S5. Calculation of enhancement and quenching factors on rates of $^1O_2$ uptake

The theoretical, or expected, enhancement or quenching factors on the observed rate of oxygen uptake were calculated as follows using the expected enhancement of $^1O_2$ process in solution in D_2O relative to H_2O (kinetic isotope solvent effect, KSIE) as an example. The ratio of Eqn. 1 (in manuscript text) is calculated for H_2O relative to D_2O, with k_{phys} = $2.5x10^5$ s^{-1} and $1.6x10^4$ s^{-1} for H_2O and D_2O, respectively. In the case of substrate (S) = FFA (Figure 1 in manuscript text), a 10 mM FFA in H_2O stock solution was used for both the D_2O and H_2O experiments. Thus, 50 μM FFA in D_2O (20 mL) contained 0.5% H_2O by volume (the FFA experimental solution in H2O was 100% H_2O). Therefore, the expected enhancement factor is 12:

$$\frac{k_{obs,D_2O}}{k_{obs,H_2O}} = \frac{0.5\% \times k_{phys,H_2O} + 99.5\% \times k_{phys,D_2O} + k_{rxn}[S]}{100\% \times k_{phys,H_2O} + 0\% \times k_{phys,D_2O} + k_{rxn}[S]} \qquad \text{(S1)}$$

In this case, we included the $k_{rxn}[S]$ term for 50 μM FFA in Eqn. S1 because it was not << k_{solv}.

Stock solutions of each fulvic acid were prepared in either H_2O or D_2O, thus any H_2O contamination in the D_2O experimental solutions likely originated from trace amounts of H_2O in the D_2O or the fulvic acid itself.

The effects of $^1O_2$ quenchers, sodium azide and β-carotene, were also calculated in a similar manner, using quenching constants cited in the manuscript text.

Section S6. Effect of $^1O_2$ DOC concentration, absorbance and emission spectra of SRFA

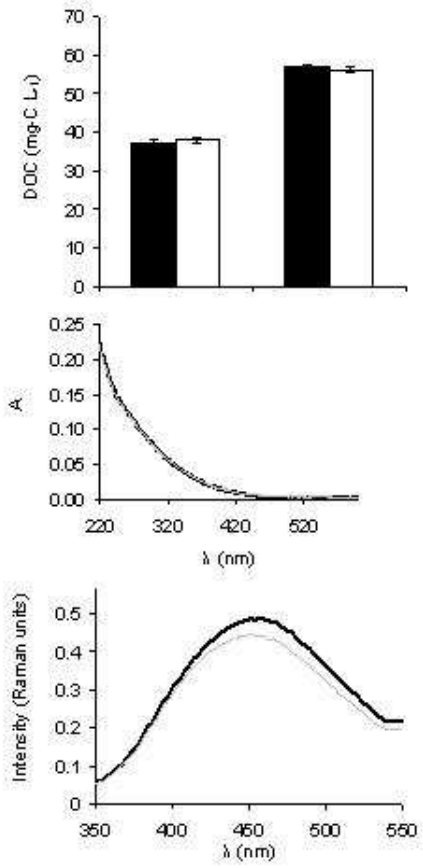


Fig. S2 Effect of $^1O_2$ on SRFA in D_2O on DOC (top), absorbance (A; middle) and fluorescence (bottom). DOC: control = shaded, reacted = white for two different SRFA concentrations. For absorbance and fluorescence, control = ▬ reacted = ▬

To assess how this reaction alters the chemical quality of the fulvic acid, the absorbance and fluorescence spectra of each fulvic acid were measured before and after reacting with $^1O_2$ for 30 minutes. A lower concentration of Rose Bengal (1 μM Rose Bengal, k_f = 1.5 μM s^{-1}) was used for these experiments due to significant interference by 40 μM Rose Bengal in the absorbance and fluorescence spectra.