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Evidence for Diffusing Atomic Oxygen Uncovered by Separating Reactants with a Semi-Permeable Nanocapsule Barrier

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Ground-state atomic oxygen [O(³P)] is an oxidant whose formation in solution was proposed but never proven. Polymer nanocapsules were used to physically separate dibenzothiophene *S*-oxide (DBTO), a source of O(³P), from an O(³P)-accepting molecule. Irradiation of polymer nanocapsules loaded with DBTO resulted in oxidation of the O(³P)-acceptor placed outside nanocapsules. The results rule out a direct oxygen atom transfer mechanism and are consistent with freely diffusing O(³P) as the oxidant.

Ground state atomic oxygen, O(³P), is an attractive reactive oxygen species for exploitation and is the smallest diradical known. The UV irradiation of dibenzothiophene *S*-oxide (**DBTO**) results in unimolecular S—O bond cleavage to form dibenzothiophene (**DBT**) and an oxidant whose reactivity resembles that of O(³P). A bimolecular mechanism of deoxygenation, generating other reactive oxygen species such as $^1O_2$, was inconsistent with the experimental findings.[1,2] However, to date it has not been possible to rule out a viable "oxenoid" alternative involving oxygen atom transfer directly from **DBTO**.[1,2] In the absence of steric burdens within the molecule to be oxidized, the reactivities of the conceivable oxidants are expected to be indistinguishable. Thus, a key mechanistic question is whether the observed oxidations are the result of a freely diffusing oxidant, such as O(³P), or oxygen atom transfer directly from **DBTO**.

The absence of spectroscopic techniques for condensed-phase detection is an obstacle in studying very small and relatively short-lived intermediates such as O(³P). Kautsky and de Brujin faced the same problem in their efforts to uncover singlet oxygen.[3,4] Their solution was a "three-phase test" involving the photosensitizer dye, trypaflavine, and an oxygen acceptor dissolved separately on SiO_2 gel beads, which allowed for a millimeter of air separating the two molecules. By physically separating the site of oxidant generation from the site of oxidation, the experiment elegantly demonstrated that the oxidant produced upon irradiation was capable of diffusing through air.

Another challenge in studying highly reactive oxidants like O(³P) is the need for very short distances between reactants, which cannot be achieved with Kautsky's three-phase test. Porous shells of polymer nanocapsules offer a barrier in solution. These nanocapsules are capable of physically separating relatively large molecules but allow for diffusion of small molecules through very small pores (diameter, <1 nm) in the nanocapsule shell.[5] For example, nanocapsules loaded with pH–sensitive indicator dyes showed unhindered transport of protons while being impermeable to molecules larger than the estimated pore size.[6,7] Small-angle neutron scattering revealed that the thickness of the shells in these vesicle-templated capsules is 1.0 ± 0.1 nm.[8] Long-term stability studies of nanocapsules showed no measurable efflux of molecules larger than the pore size over five years.[9,10]

Using nanocapsules as a barrier, we proposed an experimental design involving the irradiation of **DBTO**-loaded nanocapsules in the presence of an O(³P)-acceptor molecule, referred to herein as an O(³P)-trap. The nanocapsules act as a barrier that selectively allows the passage of small freely diffusing molecules such as the putative O(³P) or similar reactive species, through the holes (or pores) in the nanocapsules' shells. Therefore, if the oxidation mechanism involved only oxygen atom transfer from **DBTO**, the barrier would prevent **DBTO** from oxidizing the O(³P)-trap. In contrast, the formation of oxidized O(³P)-trap following irradiation would confirm a freely diffusing oxidant.

Cargo-loaded nanocapsules are typically prepared by building nanocapsules around the pre-assembled cargo, using surfactant vesicle templating in water.[11,12] Therefore, we prepared a water-soluble DBTO derivative (**1a**, Fig. 1).[13] The functionalization was shown to have no significant ameliorating effect on photodeoxygenation properties (Table S1). The optimal O(³P)-trap, **2a**, was synthesized using a known

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procedure.[14] **2a** offered three sulfides, which are known to have high reaction rate constants with O(^{3}P);[15,16] symmetry, which allowed for a single oxidation product; and several chromophores, which allowed for UV detection. An authentic sample of the anticipated O(^{3}P) oxidation product, **2b**, was also prepared.

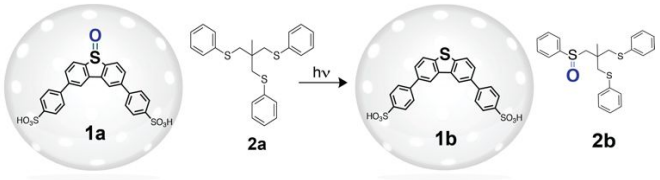

Figure 1. Experimental design. **1a**-loaded nanocapsules irradiated with UV light in the presence of **2a**.

The synthesis of polymer nanocapsules was accomplished using an aqueous suspension of self-assembled surfactant vesicles as templates with bilayers loaded with hydrophobic, methacrylate monomers and cross-linkers.

To prepare nanocapsules, an aqueous solution of 0-10 mM of cargo, (i.e. a molecule to be encapsulated) was used as the solvent (Fig. 2A). Prior to polymerization, the vesicle size was monitored using dynamic light scattering (DLS) (Fig. 2B). A small size distribution centered at 100-200 nm was achieved either by 2 h of equilibration or via extrusion. Following thermal polymerization (65°C for 8-12 h), nanocapsules were precipitated and then separated from the reaction solution. The nanocapsules were washed extensively and resuspended in water or acetonitrile.

SEM analysis of freeze-dried[17,18] nanocapsules confirmed the presence of spherical nanocapsules within the desired size range (Fig. 2C). Nanocapsule pore sizes were estimated by encapsulation of three dyes (Fig. S2) that were used as size probes: Procion Red (1.1 nm), Nile Blue A (1.0 nm), and 4-(phenylazo)benzoic acid (0.6 nm) in a similar fashion to a previously published size probe retention assay.[5,19,] Following washing, Procion Red and Nile Blue A were shown to be retained by the nanocapsules, while 4-(phenylazo)benzoic acid was not suggesting an average nanocapsule pore size >0.6 nm and <1.0 nm. Using M06-2X/6-31G(d,p) geometry optimization and frequency calculations, the calculated diameters of the smallest cross-section of **1a** and **2a** were 1.01 and 1.40 nm, respectively, both of which were larger than the estimated nanocapsule pore size.

We used fluorescent derivatives to demonstrate that **1a** and **2a** were unable to pass through the nanocapsules barrier. Like **DBTO**, **1a** was also found not to be fluorescent; however, the deoxygenation product of **1a**, i.e. the sulfide **1b,** fluorescence upon excitation with 270 nm light.

Nanocapsules loaded with **2a** could not be prepared since **2a** is not readily soluble in water; however, a water-soluble derivative of **2a**, i.e. **3**, was prepared and found to be fluorescent. Using an excitation wavelength (λ_{ex}) of 270 nm, emission peaks of 368 and 344 nm were observed for free **1b** and **3**, respectively. As shown in Fig. 3A-B, similar peaks at 368 and 344 nm were observed in the fluorescence spectra of **1b**- and **3**-loaded nanocapsules, respectively, confirming that **1b** and **3** were successfully encapsulated retained after extensive washing. **3**-loaded nanocapsules were prepared at low concentrations (≤1 mM) due to the low solubility of **3** in water, which resulted in a weak emission peak for **3**-loaded nanocapsules. Nonetheless, the successful encapsulation of **1b** and **3** suggested that **1a** and **2a** are too large to pass through the pores in the nanocapsule barrier, and thus, sufficient separation of **1a** and **2a** can be achieved.

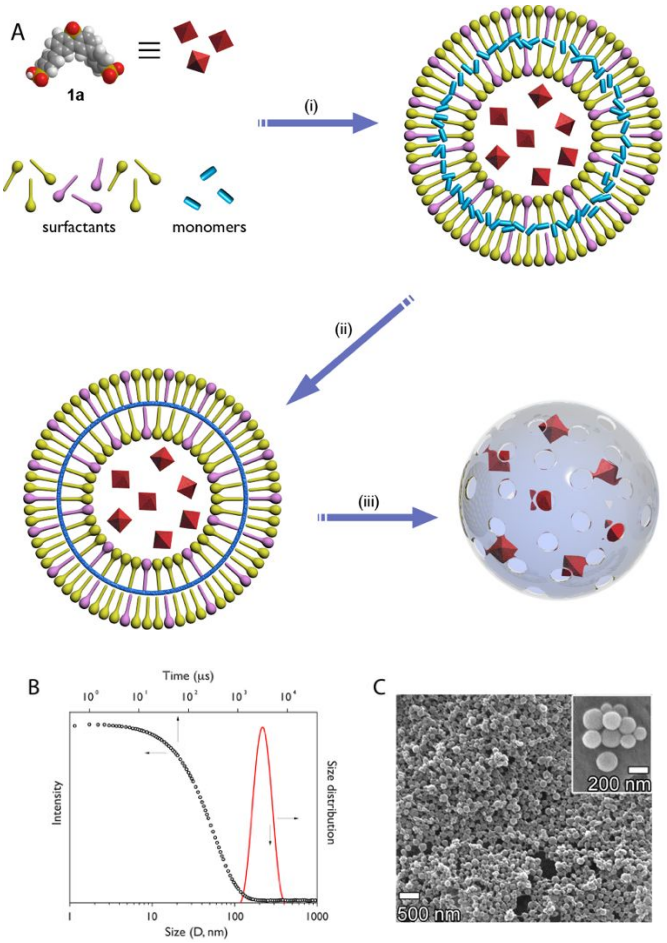

Figure 2. Preparation of **1a**-loaded nanocapsules: (A) Self-assembly of surfactant vesicles with monomers migrating to the interior of the bilayers (i), polymerization (ii), washing step to remove surfactants and free **1a** (iii); (B) typical size distribution (solid line) and autocorrelation function (open circles) of vesicles; and (C) Characterization of nanocapsules (freeze-dried) by SEM. The autocorrelation shown in (B) indicates the correlation of scattering intensity at one time with itself at a different time, which is closely related to the vesicle size.

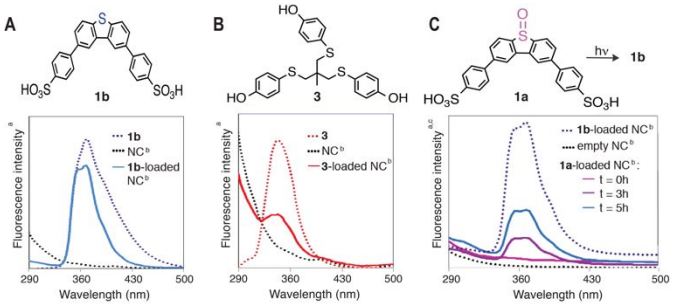

Figure 3. Evidence for separation and encapsulation. Fluorescence spectra (λ_{ex}, 270 nm) showing (A) encapsulation of DBT derivative, **1b**; (B) encapsulation of O(^{3}P)-trap derivative, **3**; and (C) the various stages of irradiation of **1a**-loaded nanocapsules with comparisons to the "empty nanocapsules" and **1b**-loaded nanocapsules. [a] The fluorescence intensity scale was normalized to show all spectra. [b] Nanocapsules represented with the abbreviation, NC. [c] The relative fluorescence intensity for photolysis of **1a**-loaded nanocapsules are depicted. All solutions were prepared in acetonitrile.

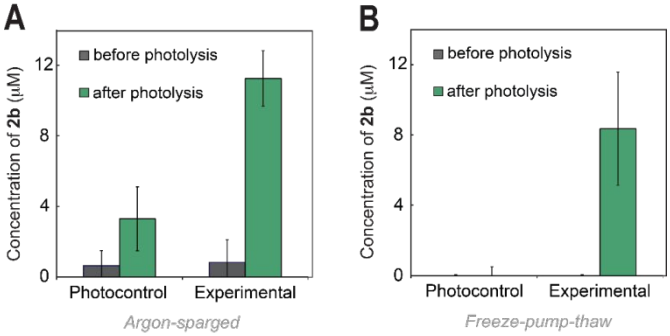

Figure 4. Evidence for a diffusing oxidant. The concentration of 2b is given before and after photolysis of experimental solution, i.e. **1a**-loaded nanocapsules in the presence of 2a, and photocontrol solution, i.e. 1b-loaded nanocapsules of "empty" nanocapsules in the presence of 2a; photolyzed 5 h using broadly emitting fluorescent bulbs (fwhm, 325-375 nm). All error bars are given at a confidence level of 95%. Under argon-sparged conditions (A), each bar represents ≥6 trials. In freeze-pump-thaw trials (B), the concentration of 2b was zero at t = 0 h, and each bar represents three trials.

1a-loaded nanocapsules were irradiated with broadly emitting UV light (fwhm, 325-375 nm), and fluorescence spectroscopy was performed at three time points: 0, 3, and 5 h (Fig. 3C). A fluorescence spectrum consistent with **1b**, increased over time, which demonstrated that encapsulation did not prevent photodeoxygenation of **1a**. After the last fluorescence spectra was taken, the nanocapsules were filtered off, and HPLC analysis of the supernatant revealed no trace of **1a** or **1b**, indicating that leakage did not occurred during the photolysis. Together these results confirmed that **1a** is encapsulated and retained in the nanocapsules.

To determine if the photodeoxygenation of **1a** generates a small diffusing oxidant, **1a**-loaded nanocapsules were added to a solution of **2a** (i.e. **2a** is not encapsulated) and then irradiated as shown in Fig. 1. Nanocapsules loaded with **1a** were used for experimental trials, while **1b**-loaded nanocapsules (type-I) and "empty" nanocapsules (type-II) were used in two different types of photocontrol trials. Both experimental and photocontrol solutions contained nanocapsules and 20 ± 2 mM of **2a** dissolved in acetonitrile. By a procedure described in SI, the maximum concentration of **1a** in experimental solutions, where **1a** is only found inside the shell of the nanocapsules, was estimated to be between 1 and 8.5 mM. Two different degassing methods were examined. Degassing via argon-sparging is known to leave behind residual O_2, while a freeze-pump-thaw (FPT) method results in insignificant concentrations of O_2.[1] Degassed solutions were irradiated using broadly emitting fluorescent bulbs (fwhm 325-375 nm) for 5 h.

A total of twelve experimental and ten photocontrol trials were performed. Overall, the photolysis of **1a**-loaded nanocapsules dissolved in a solution of **2a** resulted in the formation of 8-11 μM of **2b**; and in photocontrol trials (both type-I and type-II), there was very little (0-3 μM) increase in **2b** concentration.

For argon-sparged solutions (Fig. 4A), ≥6 experimental trials were performed and the average change in concentration of **2b** was 10.4 μM, amounting to a 4x increase **2b** formation for experimental trials relative to the photocontrols. On average, the increase in **2b** observed in type-II photocontrol trials ("empty" nanocapsules) was 2.7 μM. For type-I photocontrol solutions (**1b**-loaded nanocapsules), there was no increase in **2b** concentration.

Using the FPT method, three photocontrol trials and three experimental trials were performed (Fig. 4B); and the initial concentration of **2b** was found to be zero for each trial. The average increase in **2b** concentration observed in experimental trials (FPT method) was 8.4 μM. We observed no change in **2b** concentration in any of the three photocontrol trials using the FPT method, where O_2 concentration is very low.

By comparing the trial results for the two degassing methods, we could attribute the formation of **2b** observed in the argon-sparged control experiments (Fig. 2A, photocontrol) to the presence of residual O_2 in solution. In the absence of O_2, the oxidation of **2a** was only observed upon photolysis of **1a** (not encapsulated) or **1a**-loaded nanocapsules, confirming that the oxidant resulted from **1a** photodeoxygenation.

As a control, we examined if **2b** could be the result of a thermal reaction or direct photoproduct of **2a**. Minor photochemical degradation of **2a** was observed; however, **2b** was not observed in the absence of O_2 (i.e. photocontrol trials using FPT). Using GC-MS, products of degradation were identified as thiophenol, diphenyl disulfide, 2,2-bis((phenylthio)methyl)-propane (**4a**), and 2,2-bis((phenylthio)methyl)-cyclopropane. In the dark under ambient air, **2a** was found to oxidize to **2b**, although, very slowly over a period of a month.

In an additional control, a solution containing 80 μM **1a**, "empty" nanocapsules, and **2a** (degassed by FPT) was photolyzed for 5 h, and resulted in complete conversion of **1a** to **1b**. Following photolysis, the observed increase in **2b** concentration was 7.4 μM, or slightly less than the average observed in the experimental trials (i.e. with **1a**-loaded nanocapsules). If **1a** leaked from **1a**-loaded nanocapsules to cause the oxidation of **2a** observed in experimental solutions, then the concentration of leaked **1a** would have to be at least 80 μM. The detection limit of **1a** and **1b** was found to 0.5 μM.

Since neither **1a** or **1b** were observed in the supernatant following irradiation, the increase in **2b** observed in the experimental trials cannot be explained by leakage of **1a**.

Polymer nanocapsules have a nanometer-thick shell,[8] which is also about the size of the smallest cross-section of **1a** and **2a**. In the synthesis of rotaxane-like structures, a short linker threaded through a nanopore was not able to connect two molecules located on opposite sides of the shell.[20] This observation suggested that direct physical contact between **1a** and **2a** was extremely unlikely. Nevertheless, we considered the hypothetical scenario of physical contact between **1a** and **2a** through a collision event involving the partial insertion of **1a** and **2a** into the same small hole (or pore) on either side of the nanocapsule shell. Since **1a** has a rod-like shape and **2a** has a dendritic shape, the most likely collision within the pore would be between the phenyl groups of **1a** and **2a**. In this unlikely event of direct oxygen atom transfer, we would expect to generate arene oxide intermediates, but no phenolic products of **2a**, which would be expected for arene oxide intermediates, were observed in any experiment. Additionally, a collision event between the sulfoxide of the excited **1a** and the sulfide of **2a** would be required for direct oxygen atom transfer resulting in **2b**. Thus, the probability of a productive collision event within a pore of the nanocapsule shell is very small and cannot explain the 8-11 μM increase in **2b**.

The results from the experiments described above demonstrate that photodeoxygenation of **1a** inside of the nanocapsules generates a freely diffusing intermediate that oxidizes **2a** to **2b**. Since the diffusion distance of $O(^3P)$ in this system is predicted to be 65 nm,[16] a freely diffusing $O(^3P)$ would be capable of traversing the nanocapsule intact.

Because **2a** is oxidized to **2b** upon irradiation in the presence of O_2 (Fig. 2A, photocontrol), the possibility of O_2 as the oxidant should be examined. While the current experiments cannot rule this out, the preponderance of evidence from previous studies have led to the conclusion that the direct irradiation of **DBTO** and its derivatives result in photodeoxygenation by a unimolecular mechanism.[1,2,] A biomolecular mechanism of deoxygenation leading to O_2 was inconsistent with several different experiments. Photodeoxygenation was observed when **DBTO** was isolated in a solid matrix to prevent bimolecular collisions.[1,2] Additionally, the selective irradiation of **DBTO** in the presence of diphenyl sulfoxide produced no diphenyl sulfide, which would be expected if a bimolecular exciplex was involved in the photodeoxygenation mechanism.[1] The possibility of two $O(^3P)$ combining to form O_2 is unlikely due to the low steady-state concentration of $O(^3P)$ in these conditions.

As described in the supporting information (Table S1, Fig. S1), a common intermediate and isolation experiment were performed and indicated that **1a** and **DBTO** generate an oxidant with the same chemoselectivity and that **1a** undergoes photodeoxygenation by a unimolecular mechanism.

Applying Occam's razor, the simplest explanation for the oxidation of **2a** by **1a** through an impermeable barrier upon irradiation of **1a** is that the photodeoxygenation of **1a** generates a small freely diffusing oxidant. Since **1a** undergoes deoxygenation by a unimolecular mechanism and has the same chemoselectivity as **DBTO**, these results are consistent with the notion that the freely diffusing oxidant is $O(^3P)$.

The employed experimental scheme answers a key mechanistic question about the nature of the oxidant in aryl sulfoxide photooxygenations. The scheme also offers a viable method for studying other short-lived reactive intermediates.

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Conflicts of interest

There are no conflicts to declare.

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