Fluorophore-Induced Plasmonic Current: Generation-Based Detection of Singlet Oxygen

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ABSTRACT: In this work, we report the surface-based electrical detection of singlet oxygen using the emerging fluorophore-induced plasmonic current (PC) technique. By this method, we utilize the fluorescent “turn on” response of the well-known singlet oxygen sensor green (SOSG) singlet oxygen (1O2) fluorescent probe for the generation of fluorophore-induced PC in a silver nanoparticle film. To demonstrate the potential utility of this new technique, a photosensitizing molecule is used to generate 1O2 in a solution containing the SOSG probe. The resulting change in SOSG fluorescence quantum yield and extinction coefficient permits stronger energy transfer from the SOSG probe to a proximal silver nanoparticle island film located in the near-electric field of the probe. This yields an increase in the induced electric current flow, allowing for the detection of the 1O2 analyte. To the author’s knowledge, this represents the first detection of the reactive oxygen species 1O2 utilizing fluorophore-induced PC methodology and even broader electrical detection of 1O2. This is significant as it opens the possibility for 1O2 detection methods which do not require a traditional “photodetector” and associated optics, simplifying the instrumentation over existing fluorescence detection methods and potentially even lowering the cost.

KEYWORDS: plasmonic current, plasmonic electricity, plasmonics, reactive oxygen species, singlet oxygen, singlet oxygen sensor green, fluorescence detection, sensing

Rеactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, hydrogen peroxide, and notably singlet oxygen (1O2) have implications and impact in a broad array of fields, with perhaps the strongest interest lying in health and medicine.1−3 These species are derived from molecular oxygen and are well known to have important roles in cell signaling and maintaining regular biological function, yet oxidative stress can be severely damaging to organic and biological materials.4,5 As such, the sensing of ROS to monitor the health of systems is needed. Conversely, the deleterious properties of ROS have generated research interest in the controlled production of such species; technologies such as photodynamic therapy, for example, employ the targeted generation of ROS to inflict oxidative damage on cancer cells, thereby providing an additional treatment option in localized cancers.1,2 Therefore, detection of ROS has been vital in research settings for some time.

The generation of singlet oxygen is a key aspect of photodynamic therapies, and the mechanism of photosensitization includes the need for light-activated photosensitizing (PS) molecules to generate this species.6 In brief, photosensitizers absorb light and undergo intersystem crossing to the triplet excited state; from this point, the molecule may relax via typical pathways such as radiative decay or interact with ground state molecular oxygen to alter its electronic spin state.6 A number of detection methods for singlet oxygen have been employed. Direct detection of 1O2 is possible as the molecule phosphoresces at 1275 nm.7 This signal is typically weak and also is only observable in real-time, requiring sensitive fluorescence detection equipment and the limitation of constant monitoring of the system for detection. As an alternative, researchers have developed fluorescent probes such as singlet oxygen sensor green (SOSG) for the irreversible detection of singlet oxygen in the visible spectral region.8−10 This probe consists of a fluorescent fluorescein-based moiety, covalently modified to include an anthracene substituent, as shown in Scheme 1A. This molecule has a low quantum yield because of excited state quenching via a photo-induced electron transfer (PET) mechanism.11 In the presence of 1O2, SOSG forms an endoperoxide in its anthracene moiety, preventing PET quenching, resulting in a large increase in fluorescence quantum yield and extinction coefficient.6 To demonstrate the potential utility of this new technique, a photosensitizing molecule is used to generate 1O2 in a solution containing the SOSG probe. The resulting change in SOSG fluorescence quantum yield and extinction coefficient permits stronger energy transfer from the SOSG probe to a proximal silver nanoparticle island film located in the near-electric field of the probe. This yields an increase in the induced electric current flow, allowing for the detection of the 1O2 analyte. To the author’s knowledge, this represents the first detection of the reactive oxygen species 1O2 utilizing fluorophore-induced PC methodology and even broader electrical detection of 1O2. This is significant as it opens the possibility for 1O2 detection methods which do not require a traditional “photodetector” and associated optics, simplifying the instrumentation over existing fluorescence detection methods and potentially even lowering the cost.
Scheme 1. Schematic for Detection Methods of Singlet Oxygen (^1O_2)\textsuperscript{a}

\textsuperscript{(A)} Schematic demonstrating the turn-on detection of ^1O_2 by SOSG) including both the weakly fluorescent and reacted, highly fluorescent endoperoxide (SOSG-EP) structures. (B) Schematic of fluorophore induced plasmonic current set up, depicting excitation and subsequent nonradiative energy transfer from a fluorophore to metal nanoparticle islands. Electron flow is indicated, along with concurrently detected metal-enhanced fluorescence. The blue coloring represents a liquid solvent. Electrodes are in simultaneous contact with the metal film and solvent. (Redrawn from ref 12). (C) Modification of the PC set up from (B) for detection of singlet oxygen. The PS molecules are included.

fluorescence emission, and permitting the subsequent detection of ^1O_2.\textsuperscript{11}

Both direct and fluorescence probe detection methods necessitate expensive and sensitive optical equipment, which is limiting in the case of wide-spread detection techniques, point-of-care testing, and in low resource settings. To address this limitation, herein, we investigate for the first time the use of an emerging phenomenon called plasmonic current (PC) or plasmonic electricity (PE) for the surface-based detection of ^1O_2. Fluorophore-induced PC is generated when an excited fluorescent molecule nonradiatively transfers energy to a nearby metallic nanoparticle film, generating a measurable electrical current through the film (Scheme 1B).\textsuperscript{12} The generated electrical current is due to electron transport between discreet metal nanoparticles in the film, also known in the literature as electron “hopping” or “tunneling” for closely spaced particles.\textsuperscript{13–17} The ability of nanoparticles in the film to support electron transport is dependent on the charging energy required for a nanoparticle to gain an electron, which is related to the nanoparticle capacitance, that is,

\[ E_C = \frac{\varepsilon^2}{2C} \]  

(1)

where \( E_C \) is charging energy, \( \varepsilon \) is the elementary electric charge, and \( C \) is capacitance of the particle.\textsuperscript{13–16}

We have previously modeled the metal nanoparticle capacitance in our films utilizing the concentric sphere model for nanoparticle capacitance, which is a commonly used approach throughout the literature,\textsuperscript{16,18–21}

\[ C = 4\pi\varepsilon_0\varepsilon r_0(r_0 + s)/s \]  

(2)

where \( \varepsilon_0 \) is the vacuum permittivity, \( \varepsilon \) is the relative permittivity of the medium surrounding the particle, \( r_0 \) is the particle radius, and \( s \) is the distance between two neighboring particles. Upon excitation of a proximal fluorophore, energy transfer to the metal is expected to help overcome the columbic gap to electron transport, increasing electron transport in the film. Previous work from our laboratory has demonstrated the effect of particle size and spacing in the fluorophore-induced current. Here, it was found that larger and more closely spaced particles lead to an increase in particle capacitance and a decrease in the columbic gap required for electron transport, providing for increased current generation with fluorophore excitation.\textsuperscript{22} In addition, changing the particle size will cause a shift in the plasmon resonance frequency of the particle; however, this effect on the current is currently thought to be minor compared to the change in particle capacitance with changing particle size. The strength of direct coupling between metal nanoparticles and far-field excitation light decreases as nanoparticles grow in size toward the wavelength of incoming radiation.\textsuperscript{23} Representative scanning electron microscopy (SEM) images of metal nanoparticles in our films have been collected in previous work;\textsuperscript{12} the particles are relatively large (~50 nm diameter) and only weakly couple with direct far-field excitation light. These nanoparticles may still couple strongly with local near field molecular emitters,\textsuperscript{20} generating an increase in electrical current through the film.

As described in recent work by our laboratory, this electrical current is dependent on a number of factors including not only nanoparticle size and spacing in the film but also solvent permittivity, applied voltage, temperature, and the fluorophore extinction coefficient.\textsuperscript{12,22} In our studies of extinction coefficient effects, an increase in the current was observed with increasing fluorophore extinction coefficient.\textsuperscript{12} This is thought to be due to the high extinction fluorophore absorbing more radiative energy from the far-field light (laser or LED) and bringing more energy into the near-electric field of the nanoparticles. Assuming no change in the fluorophore quantum yield, this leads to more energy transfer from the molecule to the metal and a subsequent increase in electron transport through the film. In a similar manner to fluorescence resonance energy transfer, we predict that a lower quantum yield will diminish energy transfer from the fluorophore to the metal, decreasing the observed induced PC. Essentially, the induced current is dependent on the fluorophore brightness, that is,

\[ \text{Brightness} = \Phi_{F} \varepsilon_0 \]  

(3)
where $\Phi_\text{fl}$ is the fluorophore quantum yield and $\varepsilon_\text{fl}$ is the extinction coefficient. In other words, the induced current in the nanoparticle film contains photophysical information pertaining to the fluorophore. This provides for a molecular fingerprint, opening the possibility for detection assays which distinguish species based on a change in extinction coefficient and quantum yield, as compared to actual radiative emission from a traditional fluorophore. This is significant as it represents a low-cost analytical method that does not require a photodetector and associated optics, thereby simplifying instrumentation over existing $\text{O}_2$ spectrophotometric detection methods.

In this manuscript, we describe the utilization of the SOSG singlet oxygen probe as a molecular energy donor for PC generation on a silver nanoparticle substrate, expanding beyond its intended capacity as a fluorescence-on probe. It is clear that long-term use of silver films for biological sensing applications would not be preferred to substrates such as gold, which are superior in stability. In this instance, silver films were chosen due to their simplicity of fabrication and the significant overlap between the silver nanoparticle films’ extinction spectra and the emission profile of the commercially available SOSG fluorescent probe. In addition, the relatively large size of the particles (>50 nm from SEM) confer additional stability that is not inherent to smaller sized silver particles. Upon excitation of the SOSG molecule with far-field light (laser or LED), energy is readily transferred to the metal nanoparticle film, allowing for the generation of PC (Scheme 1C). In the presence of $\text{O}_2$, generated by the light activation of the photosensitizer (PS), SOSG displays a change in fluorescence brightness because of an alteration of both extinction coefficient and fluorescent quantum yield, which is expected to result in an increased PC as compared to SOSG without $\text{O}_2$ exposure. We subsequently investigated the detection of $\text{O}_2$ via the SOSG induced PC, an unreported sensing modality until now.

**MATERIALS AND METHODS**

**Silver Nanoparticle Island Film Preparation: Thermal Vapor Deposition.** Silver nanoparticle island films were prepared using thermal vapor deposition and silver pellets (99.999%, Research and PVD Materials Corporation), as described in previous work from our laboratory. Brieﬂy, silane-prep glass microscope slides (Sigma-Aldrich) were first cleaned with methanol and dried under N$_2$; these were subsequently used as a substrate for vapor deposition using an Edwards BOC Auto 306 Vapor Deposition Unit, at a pressure of $9 \times 10^{-6}$ Torr. The deposition rate was held constant at 0.1 Å/s with a deposition time of 28 min. Prepared films were cooled to room temperature and stored in a dessicator under vacuum until use.

**Silver Nanoparticle Film Characterization.** Silver nanoparticle island films utilized for SOSG induced current measurements were characterized, as described in a previous manuscript from our laboratory. Brieﬂy, absorption spectra of prepared silver films were collected using a single beam Varian Cary 50-Bio UV–VIS spectrophotometer. SEM of vapor deposited films was performed using a Nova NanoSEM 450 with secondary electron imaging, with representative film surface images displayed in previous work. All dry silver nanoparticle island films were characterized as electrically nonconductive, displaying zero measurable current under zero applied bias voltage.

**Instrumentation.** Fluorescence and absorption measurements of rose bengal and methylene blue (Sigma-Aldrich) solutions were performed in a quartz cuvette. Fluorescence was measured using a Fluoromax-4P spectrophotometer; absorption spectra were collected using the Varian Cary 50-Bio UV–VIS spectrophotometer, as mentioned previously. Electrical current through the system was measured with a Keithley 6487 picoameter, in either an open circuit configuration or under an applied potential, with digital output to an external computer, as described previously. Electrode materials were selected to match the metal nanoparticle film (e.g., silver-on-silver). The electrodes were positioned to make simultaneous contact with the metal nanoparticle film and the liquid solvent. Conductivity measurements were carried out with a Fisher Scientific Accumet Conductivity Meter.

**Photostimulation of SOSG and Induced PC Measurements.** In order to determine the detectability of $\text{O}_2$, using SOSG induced PC measurements, solutions of SOSG were first prepared by performing various dilutions of stock SOSG (5 mM) with methylene blue, previously prepared in de-ionized water to known concentrations. To perform the photostimulation step, the solutions were divided into two aliquots (Scheme S1); one aliquot was transferred to a black centrifuge tube and received no light exposure, thereby avoiding photostimulation. The other aliquot was added to a 500 µL quartz cuvette, capped, and exposed to white light using a ThorLabs OSL1 fiber illuminator. Following exposure, the aliquot was transferred to a black centrifuge tube for PC detection.

Following photostimulation, aliquots were pipetted onto an electrically noncontinuous silver nanoparticle island film and allowed to diffuse into the gaps between separate nanoparticle islands. Upon addition of solution to the metal, electrical current due to photosensitized convection was allowed to stabilize for approximately 5 min. Both photostimulated and control solutions were then excited with a 473 nm LED directed at the film surface. Change in electrical current through the fluorophore-metal system was monitored in an open circuit configuration at room temperature (20 °C) and reported as the absolute value of current change, $\Delta I$, with application of the excitation source. This method of reporting accounts for any background current that may already be inherent to the system, for example, due to ions in solution. Similarly, background current changes are monitored with application of the excitation in the absence of the fluorophore; this background may be subtracted from the fluorophore-induced signal. Statistical analysis of all results reported herein are the result of a minimum of $n = 3$ measurements and were performed using Student’s $t$-test, assuming equal variances. Model fits and corresponding equations and values were determined using the LINEST function in Microsoft Excel.

**Role of Photosensitizer in Fluorophore-Induced PC.** In order to generate the highly fluorescent endoperoxide form of SOSG, a photosensitizer molecule is required to first produce $\text{O}_2$ (Scheme 1). Rose bengal and methylene blue are two well-known $\text{O}_2$ photosensitizers and were both therefore considered for this proof-of-concept study. Both photosensitizers absorb in the visible region (Figure 1a) and therefore can generate $\text{O}_2$ via white light exposure. It is also desirable for the photosensitizer to not be excited by the SOSG excitation source (473 nm LED), as this could lead to energy transfer from the photosensitizer itself to the nanoparticles, potentially causing background interference in the SOSG-induced PC. Figure 1a shows the normalized absorption spectra of both photosensitizers; methylene blue shows less overlap with the LED source emission spectrum as compared to rose bengal; at 473 nm, the extinction coefficient of the latter ($\varepsilon_{\text{MB},473} = 6200 \pm 100$ M$^{-1}$ cm$^{-1}$, $n = 3$) is considerably higher than that of the former ($\varepsilon_{\text{RB},473} = 1600 \pm 600$ M$^{-1}$ cm$^{-1}$, $n = 4$. Figure S1). This opens the possibility for excitation of rose bengal during PC detection and subsequent background rose bengal current interference in the desired SOSG-induced electrical signal. This is observable also in the fluorescence emission spectra of each photosensitizer when excited at 473 nm; while emission from rose bengal is detectable in the combined solution of SOSG and rose bengal...
Detection of Singlet Oxygen via SOSG-Induced PC.

SOSG is known to detect \(^{1}\text{O}_2\) because of a significant quantum yield increase following reaction with \(^{1}\text{O}_2\), with reported values of \(0.009\) and \(0.45\) for unreacted SOSG and the endoperoxide, respectively.\(^{11}\) In the low quantum yield state, intramolecular PET in the SOSG molecule from the anthracene to fluorescein moieties prevents relaxation via fluorescence emission.\(^{11}\) After \(^{1}\text{O}_2\) photosensitization and endoperoxide formation, the relative energies of the highest occupied molecular orbital and lowest unoccupied molecular orbital of each moiety are altered and PET is no longer favorable; radiative decay therefore becomes a competitive relaxation pathway. In a modality similar to its fluorescent function, this increase in brightness ultimately leads to the increase in the induced PC. Quantum yield change in the probe alone is not the only potential mechanism for \(^{1}\text{O}_2\) detection via SOSG-induced PC. Given that the mechanism of \(^{1}\text{O}_2\) detection involves chemical modification of the probe itself, a change in ground state properties is possible. Similar to another report on SOSG,\(^{27}\) we have observed an increase in extinction of SOSG after photosensitization, when the endoperoxide is formed in solution (Figure 2, Table S1).

Before photosensitization, the extinction coefficient of SOSG at 473 nm is \(18,000 \pm 1000 \text{ M}^{-1} \text{ cm}^{-1}\), a value >10-fold greater than methylene blue. Previous reports from our lab have shown PC detection of fluorophores to be much more favorable for molecules with higher extinction values,\(^{12}\) indicating that SOSG induced detection has the potential to...
be discernible from the methylene blue background. Because the extinction coefficient of photosensitized SOSG increases by 11% relative to the “dark” solution (Δε473 = 2000 ± 1000 M⁻¹ cm⁻¹), by extinction values alone, we could predict ¹O₂ detection via SOSG-induced PC should be possible.

To determine optimal experimental parameters for SOSG-induced plasmonic detection of ¹O₂, we first prepared a series of SOSG solution concentrations in 50 μM methylene blue. Each solution was exposed to white light for 10 min in a closed system, as described in previous sections, and the fluorescence emission detected, as shown in Figure 3. As expected, quantum yield increases are observed in the exposed versus dark solutions. This effect appears concentration dependent for 10 versus 20 μM solutions but plateaus at higher concentrations of SOSG. This is likely due to the closed nature of the experimental system; in this case, molecular oxygen rather than SOSG is the limiting reagent. These SOSG concentrations were subsequently tested for PC generation to minimize background current generated from unreacted SOSG.

Having established the parameters, the photosensitized solutions were then analyzed using the surface-based PC detection method on silver nanoparticle film substrates. Figure 4 demonstrates the fluorophore-induced current increase between the low-fluorescence (“dark” conditions with no ¹O₂ generation) and high-fluorescence (“exposure” conditions leading to ¹O₂ generation and endoperoxide formation) forms of SOSG at varying concentrations. While a photosensitizer-containing control solution without SOSG displayed no significant difference in the induced current when exposed to the ¹O₂ generation source, a 20 μM SOSG solution was found to display a very large (228%) current increase in the presence of ¹O₂. This 228% increase in the induced electrical signal is correlated with an increase in fluorescence brightness (Figures 4 and S4).

These results are most significant, as they demonstrate the utility of the fluorophore-induced PC platform in the detection of singlet oxygen via the large “turn on” fluorescence response of the SOSG molecular probe. We were subsequently curious if the exposure time dependence of photosensitization could be monitored using SOSG-induced PC detection. Figure 5 shows the induced plasmonic current with varying exposure time, using a concentration of 20 μM SOSG and 50 μM methylene blue as the photosensitizer. Here, as the white light exposure time is increased for the photosensitizer, more ¹O₂ is generated. This increase ultimately leads to a greater number of SOSG molecules in the highly fluorescent, endoperoxide form. Figure 5a shows the corresponding increase in fluorescence intensity, which progresses in a linear fashion (Figure S5). Figure 5b shows the correlation in the induced current, which displays a positive and also linear trend with increasing exposure time with an approximate 3-fold increase in signal over 600 s of exposure (statistical analysis in Figure S6, Table S2). The successful detection of ¹O₂ using SOSG-induced plasmonic current described herein reinforces the vast
potential that this surface detection method may exhibit in future applications.

CONCLUSIONS

Herein, we have reported the successful detection of singlet oxygen using the novel phenomenon of fluorophore-induced plasmonic current (PC). Singlet oxygen was detected using the well-known fluorescence-on probe SOSG, which in turn was able to induce plasmonic current in silver island films, which was then detected by an ammeter. Both an increase in extinction coefficient and quantum yield associated with the mechanism of singlet oxygen detection by SOSG are implicated as favorable properties for the use of fluorescent probes in plasmonic current-based detection applications. The results reported herein provide a promising foundation for future development of probes specific to plasmonic current in a new chapter of detection and sensing research. Future sensing fluorophores developed specifically for induced plasmonic current will potentially require metal films beyond silver, depending on their extinction/fluorophore emission spectral properties. This aspect opens the door for optimization of substrate characteristics—including stability for long-term or repeated use—as the field develops. In terms of ROS detection, it is interesting to note that generation of singlet oxygen has no considerable impact on the absorption properties of silver island films used in this study (Figure S7, Table S3). The fact that these substrates are stable in the presence of such highly reactive species provides further support for their use in future sensing applications such as plasmonic current. Described herein is only one example of how fluorescence-induced plasmonic current may be used in sensing applications. Plasmonic current detection bypasses the need for traditional optics, reducing the cost and footprint associated with traditional fluorescence detection and microscopy methods. The simplicity of plasmonic current could serve to reduce the cost of detection devices, simplify design for innovative point-of-care testing, and broaden the scope of sensing capabilities beyond what is currently possible using fluorescence detection methods. Implications of plasmonic current extend far beyond ROS into fields from quality control to diagnostics.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.0c00377.

Diagram of the experimental procedure, absorption intensities of methylene blue and rose bengal photosensitizers of varying concentrations, normalized fluorescence spectra of SOSG (20 μM), conductivity values of methylene blue solutions in deionized (DI) water, extinction Coefficients (ε) of SOSG solutions at different wavelengths, statistical analysis (T-test, equal variance) of detection by plasmonic current of singlet oxygen (1O2) photosensitized with methylene blue (50 μM) and white light, fluorescence detection of singlet oxygen (1O2) by SOSG (20 μM) photosensitized with methylene blue (50 μM) and white light, statistical analysis for plasmonic current (PC) detection of singlet oxygen (1O2) by SOSG (20 μM) photosensitized with methylene blue (MB, 50 μM) and white light, confidence intervals for statistical analysis of singlet oxygen detection by plasmonic current, absorption spectra of Silver Island Films on glass substrates, and changes in spectral properties of silver island films following exposure to variation solution conditions (PDF).

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Author Contributions

All experiments were designed and executed by J.M. and R.K. under the mentorship of C.D.G. E.H. aided in the execution of experiments and data analysis.
photosensitization procedures. The manuscript was written collaboratively by J.M. and R.K., edited by C.D.G., J.M. and R.K. contributed equally to this work.

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**Notes**

The authors declare no competing financial interest.

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**ADDITIONAL NOTE**

“The reported extinction coefficients are not meant to be analytical values for pure SOSG and pure SOSG-EP but are rather intended to demonstrate the photophysical effects following photosensitization of and reaction with singlet oxygen.

**REFERENCES**
