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SHORT COMMUNICATION

Metal-enhanced Singlet Oxygen Generation: A Consequence of Plasmon Enhanced Triplet Yields

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Abstract In this Rapid Communication, we report the first observation of Metal-Enhanced singlet oxygen generation (ME^1O_2). Rose Bengal in close proximity to Silver Island Films (SiFs) can generate more singlet oxygen, a three-fold increase observed, as compared to an identical glass control sample but containing no silver. The enhanced absorption of the photo-sensitizer, due to coupling to silver surface plasmons, facilitates enhanced singlet oxygen generation. The singlet oxygen yield can potentially be adjusted by modifying the choice of MEF (Metal-Enhanced Fluorescence) & MEP (Metal Enhance Phosphorescence) parameters, such as distance dependence for plasmon coupling and wavelength emission of the coupling fluorophore. This is a most helpful observation in understanding the interactions between plasmons and lumophores, and this approach may well be of significance for singlet oxygen based clinical therapy.

Keywords Metal-Enhanced Fluorescence (MEF) · Metal-Enhanced Phosphorescence (MEP) · Metal-Enhanced $^1O_2$ generation (ME^1O_2) · Photodynamic Therapy (PDT) · Surface enhanced fluorescence · Plasmons · Plasmonics · Radiative Decay Engineering (RDE) · Plasmon enhanced luminescence · Plasmon enhanced fluorescence · Fluorescence · Singlet oxygen

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Abbreviations

GR	Green sensor
MEF	Metal Enhanced Fluorescence
MEP	Metal Enhanced Phosphorescence
ME^1O_2	Metal Enhanced Singlet Oxygen Generation
PDT	Photodynamic Therapy
RB	Rose Bengal
SiFs	Silver Island Films
UV	Ultraviolet

Introduction

Molecular oxygen has a unique electronic configuration characterized by a partially filled set of antibonding π* orbitals. As predicted by Hund's rule, the lowest energy state of the molecular oxygen has maximum multiplicity, i.e. is a triplet ground state [1, 2]. Molecular oxygen has two excited singlet states, $^1\sum_g^+$ and $^1\Delta_g$, whose electronic energies are 158 and 95 kJ/mol higher than that of the triplet ground state $^3\sum_g^-$, respectively. The electronic configuration of $^1\sum_g^+$ is very similar to that of the ground state, except the fact that the last two electrons in $^1\sum_g^+$ have antiparallel spins while these electrons in the ground state have parallel spins. The other excited singlet state, $^1\Delta_g$, is commonly called singlet oxygen ($^1O_2$) and is more important than $^1\sum_g^+$ because of its longer lifetime (lifetime of $^1\Delta_g$ is 45 min and of $^1\sum_g^+$ is 7–12 s) [3]. The

physical, chemical and biological properties of singlet oxygen attracted serious attention from researchers during the 1960's despite its discovery in 1924 [4]. Since singlet oxygen can readily react with many biological targets and destroy a wide variety of cells, the photo-sensitized production of singlet oxygen has significance in a range of areas, especially in photodynamic therapy (PDT) [5].

PDT encompasses the combined use of light, a light-sensitive drug / compound (photosensitizer) and singlet oxygen, and has been widely used in the treatment of several diseases such as diabetes, peripheral vascular diseases, cerebrovascular, cardiovascular [5] and cancer [6–8]. PDT is applied in multiple steps for the treatment of patients with cancer. In the first step, a photosensitizing agent is deposited on to surface tumors after its injection into the bloodstream. Then, the photosensitizer-deposited cancer tumor is exposed to light. Here, the excited photosensitizer transfers its energy to molecular oxygen while returning to the ground state, which results in the production of singlet oxygen. Subsequently, singlet oxygen destroys nearby cancer cells [6–8].

It is widely accepted that singlet oxygen is the primary cytotoxic agent responsible for photobiological activity [9]. Since singlet oxygen plays a very important role in cell damage, an abundant supply of oxygen is required. In addition, photodynamic therapy is currently limited by the insufficient generation of singlet oxygen while reacting with biological targets. A method to possibly resolve these problems is to increase the triplet yield of sensitizers, by coupling to surface plasmons, which invariably results in more singlet oxygen generation.

In this regard, we have recently reported the first observation of metal-enhanced phosphorescence (MEP) [10, 11] at low temperature, where non-radiative energy transfer is thought to occur from excited distal triplet-state luminophores to surface plasmons in non-continuous silver films, which in turn, radiate fluorophore/lumophore phosphorescence emission efficiently. This observation suggests that photon-induced electronic excited states can both induce and couple to surface plasmons (mirror dipole effect) facilitating both enhanced S_1 fluorescence [12], and phosphorescence, T_1, emission. Thus, MEP (i.e. enhanced triplet yields) provides us with an opportunity to test whether singlet oxygen is also enhanced when a photosensitizer is in close proximity to the metallic nanoparticles.

Subsequently, in this Rapid Communication, we report the first observation of Metal-Enhanced singlet oxygen generation (ME^1O_2). A photosensitizer sandwiched between silver island films (SiFs) enables more singlet oxygen to be produced due to the enhanced triplet excited state yield of the sensitizer [11]. Figure 1 shows a modified Jablonski diagram showing the possible mechanisms for the fate of an excited fluorophore/ lumophore when in close proximity to a plasmon resonance particle. Firstly, a photosensitizer is excited from the ground state S_0 to an excited state, S_n, where the metal readily allows for enhanced absorption [11] i.e. an enhanced excitation rate. S_n then relaxes to the lowest excited singlet state S_1, facilitating intersystem crossing. More singlet oxygen is subsequently generated due to the enhanced intersystem crossing and enhanced triplet yield. This observation of Metal-Enhanced $^1O_2$ generation is not only helpful to further our understanding of plasmon-lumophore interactions, but suggests that this approach may be of significance for singlet oxygen based clinical therapy, where an abundant supply of $^1O_2$ is required [6–8].

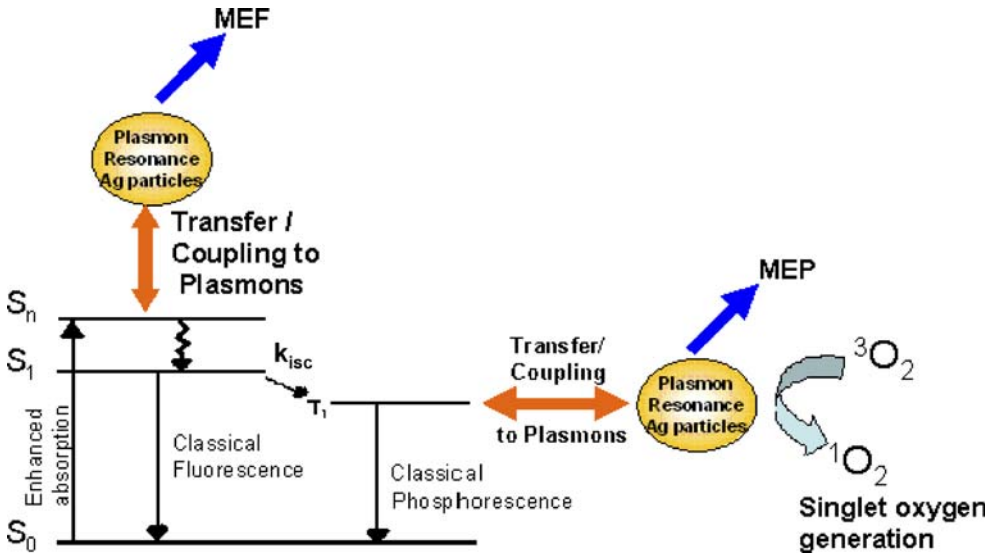

Fig. 1 Schematic Jablonski diagram for the Photosentisitizer and surface plasmon enhanced Singlet Oxygen generation. *MEF*—Metal Enhanced Fluorescence. *MEP*—Metal Enhanced Phosphorescence. $^3O_2$ triplet ground state oxygen. $^1O_2$ singlet oxygen. *isc*—intersystem crossing

Experiment

The singlet Oxygen Sensor Green reagent (GR) obtained from Molecular Probes (Invitrogen) is highly selective for $^1O_2$. In the presence of singlet oxygen, it emits a green fluorescence (excitation/emission 504/525 nm). Rose Bengal is a well known $^1O_2$ sensitizer, with high singlet oxygen yield (0.8) [13]. All chemicals were used as received.

Materials and methods

Silver Island Films (SiFs) were prepared according to the previously published procedures [14]. A solution of 500 µl of Rose Bengal (0.1 mM) in ethanol and a solution of 500 µl GR (4.5 mM) also in ethanol was dropped in a sandwich format between the glass slides and the silver island films (SiFs), respectively. Figure 2-Top shows the experimental sandwich sample geometry. The glass/SiFs surfaces were exposed to UV-light (365 nm (100 W)) for 2 min for singlet oxygen generation, with the green sensor dye fluorescence collected at 45° to the excitation through a long pass filter, using a Fiber Optic Spectrometer (HD2000) from Ocean Optics, Inc.

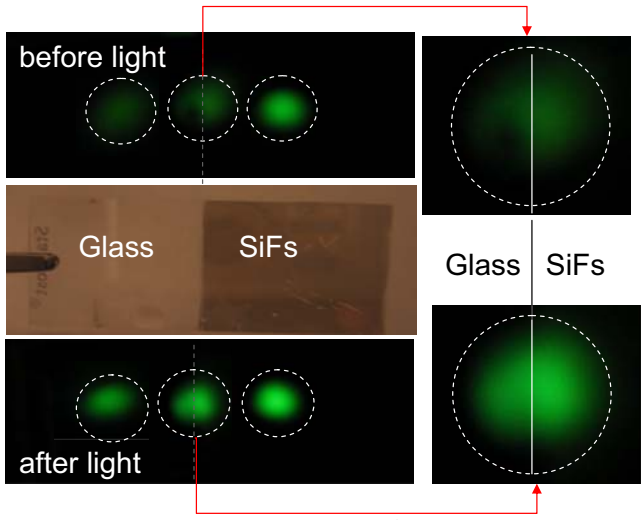

Fig. 3 Real color photographs of Green $^1O_2$ sensor (*GR*) and Rose Bengal (*RB*) emission from glass and SiFs, before and after 2 min light exposure. Light exposure source was a 100 W mercury lamp. λ_{ex}=473 nm. *SiFs*—Silver Island Films. Note! As the excitation (*circles*) spots are moved from the glass to the SiFs, more emission is observed. The right hand side photographs show the sample half glass and half SiFs

Results and discussion

Figure 2-Bottom shows the fluorescence emission spectra of a mixture of GR and Rose Bengal solutions on glass and SiFs, before and after UV light exposure. While the peak at 588 nm is the Rose Bengal fluorescence emission and not related with singlet oxygen [11], the peak at 525 nm originates from the GR sensor dye emission and directly correlates with singlet oxygen yield. It can be seen that the fluorescence emission intensity at 525 nm is increased after UV light exposure, which is indicative of singlet oxygen generation [15]. On SiFs, the fluorescence intensity at 525 nm was increased ≈3.3 fold, as compared to that on glass. The increased intensity signifies that more singlet oxygen was generated from the Silver-Rose Bengal system after light exposure. The spectra are corrected for the

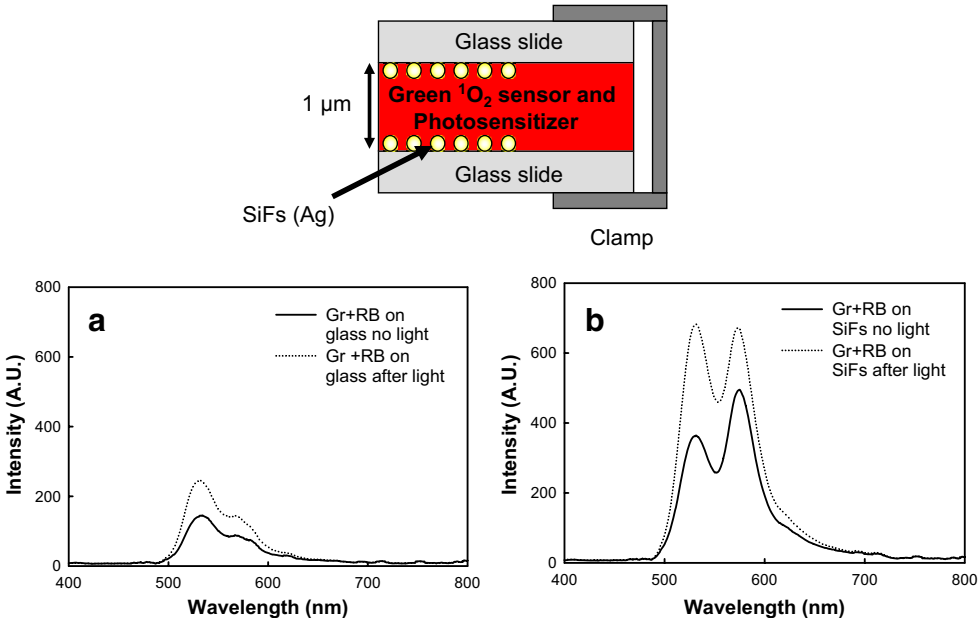

Fig. 2 Schematic representation of the sample geometry (*Top*), and fluorescence emission spectra (*Bottom*) of a mixture of the green sensor (*GR*) and Rose Bengal (*RB*) on glass **a**, mixture on SiFs **b**, before and after light exposure (2 min) at room temperature. Light source is 100 W mercury lamp. λ_{ex}=473 nm. The spectra have been corrected for the MEF effect on the GR emission

increased intensity of the GR sensor by the MEF effect, so that the ME $^1O_2$ can be solely observed.

At this time we believe that the mechanism of singlet oxygen generation from the photo-sensitizer is an energy transfer process during a collision of the excited lumophore with $^3O_2$. Since the lifetime of the triplet excited T_1 state (micro or milli-second range) is much longer than that of the singlet excited S_1 state (nanosecond), it is long enough for the triplet excited state to react with (ground-state triplet oxygen) $^3O_2$ to form $^1O_2$, in essence competing with the more favorable phosphorescence emission. At first consideration one may be surprised by the presence of both enhanced MEP [11] and ME$^1O_2$ in the same system, as both processes are competitive and provide a route for the deactivation of electronic excited triplet states. However, it seems reasonable that an enhanced absorption of Rose Bengal near to silver [10, 11] (i.e. an enhanced excitation rate) would facilitate both MEP and ME$^1O_2$ simultaneously in the same system.

Previous studies of both the MEF & MEP phenomenon have reported that the enhancement factor values are dependent on distance between the lumophore and nanoparticle [16], nanoparticle size [17], surface roughness [17] and temperature [10]. With regard to singlet oxygen generation, it should also be noted that the true metal-enhanced singlet oxygen enhancement factor is ≈25 times larger than observed by us. This is because the ME $^1O_2$ phenomenon, like MEF and MEP, is through-space, with an interaction distance of less than 20 nm. With a sample thickness of ≈1 μ, only 4% of the sample is within the plasmon enhancement region (See Fig. 2-Top). In addition to close proximity nanostructures, we believe other factors such as temperature, surface roughness and indeed shape of SiFs or nanostructures can also influence the extent of ME$^1O_2$, effectively providing us with tools to manipulate the ME$^1O_2$ yield, and to some degree, therefore PDT therapy.

It is interesting to visually observe the enhanced ME$^1O_2$ yield in the presence of the green sensor, Fig. 3. It can be seen that the GR fluorescence emission intensity on glass before light exposure is dim; However on SiFs before light exposure it looks brighter than on glass due to MEF. However, after light exposure, the GR fluorescence emission on glass was easily observed. This indicates the generation of singlet oxygen after the exposure of RB to UV radiation. The GR fluorescence emission was more intense on SiFs after light exposure, indicating that more singlet oxygen is generated on SiFs than on the glass slide, Fig. 3.

Conclusion

In this Rapid Communication, we report the first observation of plasmon assisted ME$^1O_2$ generation. Rose Bengal in close proximity to SiFs can generate more singlet oxygen, a three-fold increase observed as compared to an identical control sample. Singlet Oxygen Yield can be adjusted by adjusting the MEF & MEP parameters, such as distance dependence, shape, size of nanoparticles and wavelength emission of the coupling fluorophore.

In addition, our finding suggests that ME$^1O_2$ probes and $^1O_2$ generation have additional advantages:

1. The ME$^1O_2$ probes tested to date remain photostable, due to a shorter lifetime (less oxidization) even in the presence of an increased singlet oxygen yield.
2. Silver is readily used for numerous device applications [18] and is even ingested. Therefore silver is not likely to be deemed toxic for ME$^1O_2$ biological applications, such as photodynamic therapy.
3. ME$^1O_2$ probes can potentially be developed for cellular applications. Current MEF/MEP probes are small enough for cellular entry, vascular diffusion, and are therefore potentially valuable for photodynamic therapy. In this regard, we recently developed fluorophore-doped core-shell nanoparticles that can potentially be doped with a suitable sensitizer and used for cancer cell therapy [19].
4. This approach and technology looks likely to be compatible with the other $^1O_2$ technologies, such as $^1O_2$ for water purification [20].
5. The Ag surfaces are not perturbed by $^1O_2$. In this regard, the plasmon absorption spectrum was identical both before and after ME $^1O_2$ generation (data not shown).
6. Our group has recently shown Metal-Enhanced Fluorescence from large gold colloids [21]. Given the significant amounts of gold surface chemistries available, coupled with the long-term stability of gold, it is therefore likely that ME $^1O_2$ generation would be highly suited for use with gold nanoparticles. Further studies are underway in this regard, and will be reported in due course.

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