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Anthracene-based fluorescent nanoprobes for singlet oxygen detection in biological media

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ABSTRACT

We have developed a novel singlet oxygen nanoprobe based on 9,10-anthracenedipropionic acid covalently bound to mesoporous silica nanoparticles. The nanoparticle protects the probe from interactions with proteins, which detract from its ability to detect singlet oxygen. *In vitro* studies show that the nanoprobe is internalized by cells and is distributed throughout the cytoplasm, thus being capable of detecting intracellularly-generated singlet oxygen.

1. Introduction

Singlet molecular oxygen (hereafter $^1O_2$) has gained ample attention due to its pivotal role in a large variety of chemical and biological processes [1–3], e.g., plant signaling [4], organic synthesis [5], food and beverage oxidation [6], or photodynamic therapy [2,7].

This versatility stems from the broad reactivity of $^1O_2$ towards a vast array of electron-rich molecules and biomolecules, particularly lipids, proteins and nucleic acids [8–10]. $^1O_2$ is very often generated in photosensitized processes, in which a light-responsive photosensitising agent (PS) absorbs light and funnels its acquired electronic energy to molecular oxygen, thereby generating $^1O_2$ [11].

On account of its relevance, a number of methods have been developed to detect and quantify the production of $^1O_2$. It can be detected directly through its intrinsic phosphorescence at 1275 nm [12–14]. This direct method is robust, specific and non-invasive. However, it suffers from weak sensitivity due to the low efficiency for $^1O_2$ emission in aqueous media. This drawback is specially critical in biological media, where the $^1O_2$ lifetime (τ_Δ) is very short due to the presence of $^1O_2$ quenchers [1,11,15]. An alternative to circumvent these shortcomings is to trap $^1O_2$ with suitable chemical acceptors where the precursor and/or oxidation products can be conveniently monitored through absorption [16], fluorescence [17,18], or ESR [19].

Polycyclic aromatic hydrocarbons such as anthracenes or rubrene are commonly used as $^1O_2$ traps [20]. Among them, anthracene dipropionic acid (ADPA), which can be monitored by both absorption and/or fluorescence spectroscopies [21–24], is highly reactive against $^1O_2$ (reactive rate constant, $k_r = 8 \times 10^7$ - M^{-1} s^{-1} in heavy water) [25]. ADPA shows structured absorption and fluorescence spectra with maximums around 380 nm and 430 nm, respectively. Upon reaction with $^1O_2$, its characteristic absorption/fluorescence is bleached concomitant with the formation of an endoperoxide adduct (Scheme 1) [25].

A main problem from molecular $^1O_2$ fluorescent traps is complex-formation with proteins [26,27], which affects their response to $^1O_2$ and often prevents their uptake by cells. A novel anthracene derivative have been developed that aim at alleviating these problems [28,29].

An alternative strategy is to embed the $^1O_2$ traps into nanoparticles (NPs) that preserve their reactivity towards $^1O_2$ while the interaction with proteins is minimized. This concept was pioneered by Kopelman et al., who studied several fluorescent probes inside ORMOSIL NPs [30]. One such probes was the $^1O_2$ trap dimethylanthracene, unfortunately no data in biological media was reported [31].

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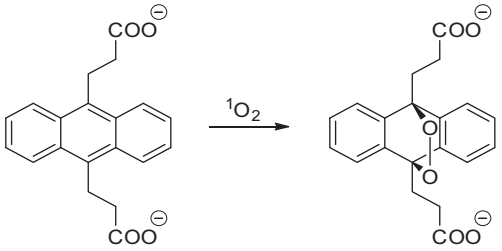


Scheme 1. ADPA's reactivity in the presence of $^1O_2$.

Although several nanomaterials have been explored to design and improve different protective delivery systems [32] silica nanoparticles (SNPs) appear of great interest for biological media due to their biocompatibility, ease of internalization by an ample number of cell lines [33,34], high loading capacity, chemical inertness and versatile surface derivatization [35–37].

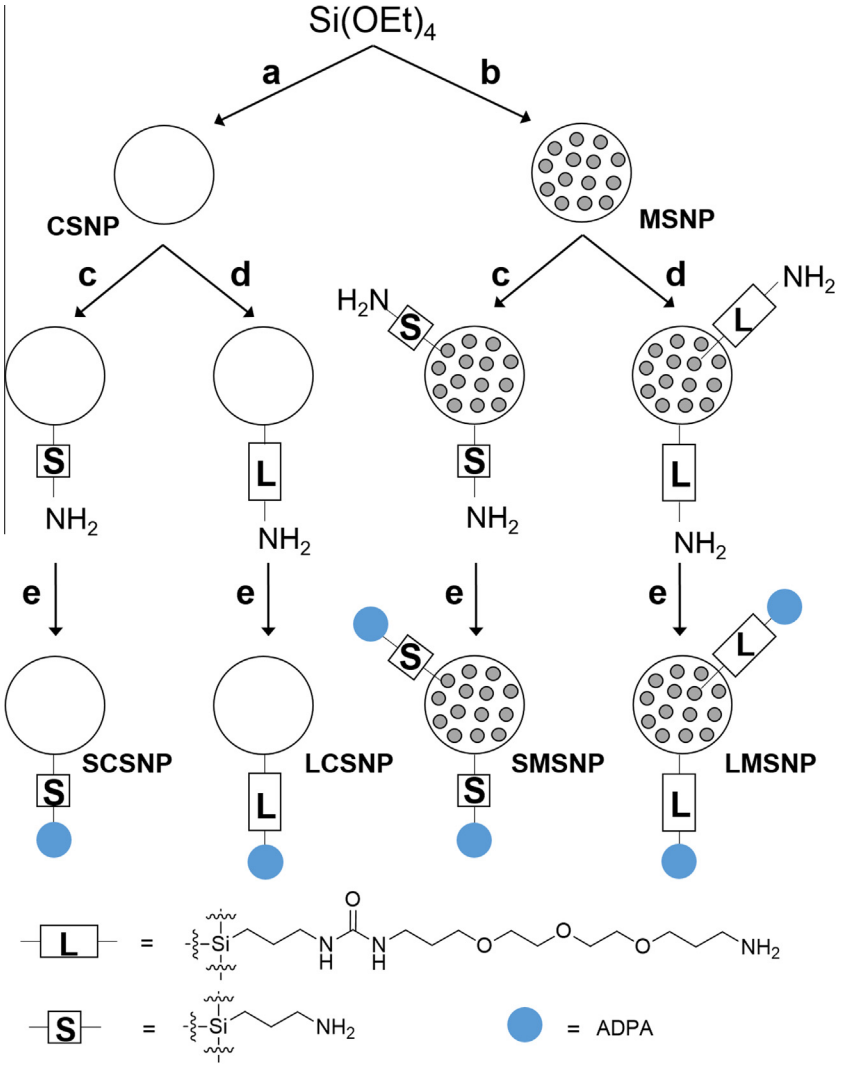
In this manuscript, we have engineered, synthesized and optimized a new nanodevice for $^1O_2$ detection based on SNPs. The nanosensor behavior has been tested in ethanol solutions, phosphate buffered saline and HeLa cells.

2. Results and discussion

2.1. Preparation and characterization of the nanoprobes

In a first step, we developed a simple nanoconjugate in which ADPA is directly attached covalently to the pre-formed SNPs, as depicted in Scheme 2. Two different types of SNP were synthesized: compact (CSNP) [38] and mesoporous (MSNP) [37]. Each type was functionalized with short (S) and long (L) linkers with terminal amino groups and finally ADPA was covalently linked by Steglich amidation [39].

All synthesized SNPs were characterized by their size and ζ-potential (Table 1) as well as by infrared spectroscopy (Fig. S3). Dynamic light scattering shows that the starting CSNP and MSNP rendered a hydrodynamic diameter of 100 and 140 nm, respectively, and a ζ-potential of −30 and −41 mV, respectively. After surface functionalisation and ADPA conjugation, the diameter of the NPs increased up to 30 nm and the ζ-potential became less negative and even turned positive when the external amines were protonated (Table 1). ADPA concentration was quantified for these NPs, ranging from 0.02 to 0.18 μmol per mg of silica (Fig. S4). MSNP showed the highest loading capacity.



Scheme 2. Synthesis, derivatization and ADPA conjugation. Reagents and conditions: a): CTAC, EtOH, H_2O, NH_3, 80 °C, 12 min, then HCl (37%), reflux, 24 h. b): IprOH, EtOH, H_2O, NH_3, room temperature (rt). 72 h. c): APTES, EtOH, rt, 24 h. d): Si-Linker, EtOH, rt, 24 h. e): ADPA, EDC, NHS, Na_2CO_3, CH_3CN:CH_2Cl_2 (1:1), rt, 24 h.