Communication

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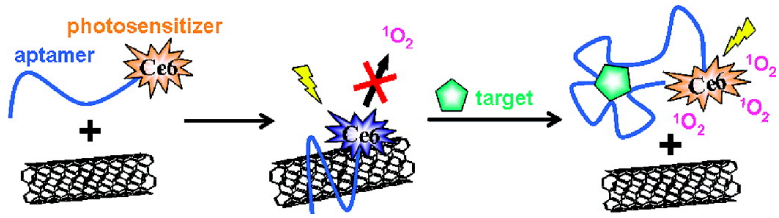

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Regulation of Singlet Oxygen Generation Using Single-Walled Carbon Nanotubes

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We have engineered a novel molecular complex of a photosensitizer, an ssDNA aptamer, and single-walled carbon nanotubes (SWNTs) for controllable singlet oxygen ($^1O_2$) generation. $^1O_2$ is one of the most important cytotoxic agents generated during photodynamic therapy (PDT), which is gaining wide acceptance as an alternative noninvasive treatment of cancers.[1] Briefly, PDT involves a two-step process whereby a nontoxic photosensitizer is delivered to an organism and then activated by an appropriate harmless light source. The photosensitizer, generally a chemical, transfers the light energy to tissue oxygen to generate highly reactive $^1O_2$, an aggressive chemical species, which can react rapidly with cellular molecules and mediate cellular toxicity to cause cell damage, ultimately leading to cell death.[2]

Because the lifetime and diffusion distance of $^1O_2$ is very limited, a controllable singlet oxygen generation (SOG) with high selectivity and localization would lead to more efficient and reliable PDT, as well as fewer side effects. This is where careful molecular engineering can play a major role in designing PDT. Several research groups have now taken this approach to develop selective PDT agents that can be triggered by protease digestion,[3] pH change,[4] or DNA hybridization.[5] For instance, Zhang and coworkers[3b] have reported a photodynamic molecular beacon, in which a photosensitizer and a $^1O_2$ quencher were kept in close proximity by a disease-specific peptide sequence. Upon enzyme cleavage, the photosensitizer was freed from the quencher, leading to an increase in the amount of SOG. We believe that aptamers can be effectively used to control $^1O_2$ generation upon target binding. Aptamers are synthetic DNA/RNA probes that can recognize and bind their targets with high affinity and specificity.[6] These targets range from small molecules to proteins and even to disease cells.[7] Aptamers rival other molecular probes by their intrinsic advantages, such as reproducible synthesis, easy manipulation, excellent stability, and nontoxicity.[8−10] In addition, DNA aptamers have a large variety of adaptability for molecular engineering, making the design of controllable PDT feasible.

We propose a new molecular design for regulating SOG by SWNTs. SWNTs have already proved to be efficient quenchers of fluorescence probe design.[11,12] Based on these findings and that both the fluorescence process and SOG share a similar photophysical mechanism, we anticipated that SWNTs could quench SOG, replacing organic molecular quenchers. Meanwhile, interactions of SWNTs with biomolecules, such as proteins and DNA,[11,12] have been intensively studied and applied to biosensing[11a,b,12b] and as intracellular transporters.[11c,12c] This indicates SWNTs may also protect DNA probes from digestion by nuclease. This notion is given further support by the recent demonstration that single-stranded DNA interacts noncovalently with SWNTs[12a] such that ssDNA will wrap onto the SWNT surface by means of π-stacking interactions.

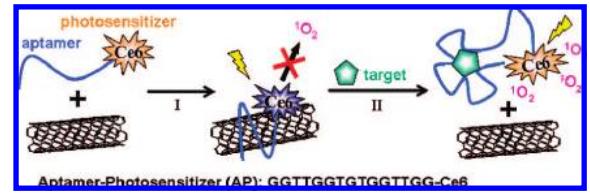

Figure 1. Schematic of aptamer−photosensitizer−SWNT complex and the regulation of SOG upon target binding: (I) AP and SWNTs were mixed together to form AP−SWNT complex. The ssDNA aptamer is wrapped on the surface of SWNTs, which brings the photosensitizer close to the SWNTs to quench SOG. (II) Target binding with aptamers can disturb the interaction between AP and SWNTs, resulting in the restoration of SOG.

Figure 1 shows how we combined the significant features of SWNT, aptamer, and photosensitizer to form a simple, but efficient and elegant PDT agent, named AP-SWNT. First, the aptamer was developed by an in vitro process known as SELEX (systematic evolution of ligands by exponential enrichment).[6] Second, a photosensitizer is covalently attached to one end of the DNA aptamer that wraps onto the SWNT surface. In the absence of a target, the design becomes operational by close proximity of the photosensitizer to the SWNT surface, which causes efficient quenching of SOG. Importantly, the conformation of the probe can be altered upon target binding. Thus, in the presence of its target, the binding between the aptamer and target molecule will disturb the DNA interaction with SWNTs and cause the DNA to fall off the SWNT surface, resulting in a restoration of SOG for PDT applications. $^1O_2$ can thus be regulated by target binding.

Thrombin aptamers have been widely investigated for the past decade. Specifically, α-thrombin is a trypsin-like serine protease that has many effects in the coagulation cascade and relates to a multitude of diseases. For these reasons, human α-thrombin (Tmb) aptamer was chosen as the model probe construct by which to investigate the photodynamic process that takes place under the conditions suggested above. Chlorin e6 (Ce6), a second generation and easily modifiable photosensitizer, was selected because of its high photosensitizing efficacy and low dark toxicity. Since it is highly selective for $^1O_2$, singlet oxygen sensor green (SOSG) was employed to quantify SOG by fluorescence enhancement.

We covalently linked Ce6 to DNA aptamer; therefore, the stronger binding affinity of DNA with SWNTs should enable high quenching efficiency. In our experiment, up to 98% quenching was observed. Meanwhile, the AP−SWNT complex had significant fluorescence enhancement upon target binding. This was illustrated by the Ce6 fluorescence which increased up to 20-fold after the addition of 2.0 μM thrombin (Figure S1). SOG was tested by mixing all the samples with 2.0 μM SOSG and irradiating at the maximum

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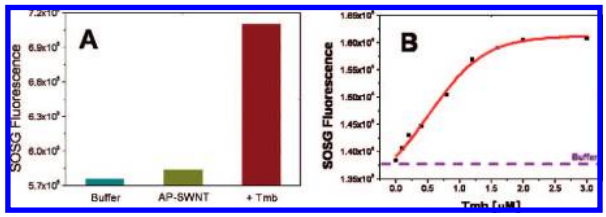

Figure 2. SOG regulation by aptamer-binding protein. (A) The SOSG signal readout after 10.5 min of irradiation with excitation at 404 nm. SWNTs show great quenching for SOG. After introduction of 2.0 μM thrombin, SOG is increased significantly. (B) The SOSG signal plotted as the function of thrombin concentration. The purple line indicates the buffer's SOSG signal.

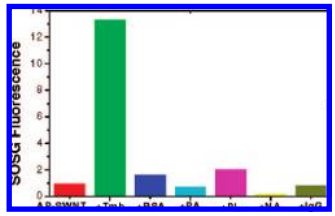

Figure 3. SOG specificity. The SOG selectivity of AP−SWNT toward different proteins: thrombin, bovine serum albumin (BSA), protein A (PA), protein L (PL), NeutrAvidin (NA), and IgG. The SOSG fluorescence signals were normalized to AP−SWNT.

absorption of Ce6, which was 404 nm, for 10.5 min. SOG was then evaluated in terms of signal-to-background ratio by comparing the SOSG fluorescence enhancement after subtracting the buffer background. In Figure 2A, the SOSG fluorescence of AP−SWNT did not change much compared to buffer solution. However, the SOSG fluorescence of AP−SWNT exhibited a 13-fold enhancement upon introduction of 2.0 μM thrombin. This demonstrates that SWNTs can efficiently turn off SOG and, more importantly, that it can be reversibly mediated by its target binding event, that is, the binding between the aptamer and thrombin, which also has been confirmed by gel electrophoresis (Figure S3).

The SOG of AP−SWNT with a series concentration of thrombin has also been investigated. In Figure 2B, a linear increase of SOSG fluorescence intensity was observed for [thrombin] range 0.1−1.6 μM. It then reached a plateau. Even with [thrombin] as high as 6.0 μM, 30 times higher than that of AP−SWNT, no more fluorescence increase could be observed. The SOG of the AP−SWNT complex could thus be quantitatively mediated.

As noted above, aptamers have high binding affinity and specificity, and our results showed that AP−SWNT maintained this advantage and presented excellent specific response toward thrombin. In Figure 3, when tested with bovine serum albumin (BSA), protein A, protein L, NeutrAvidin, and IgG at 2.0 μM, the AP−SWNT only gave little SOSG fluorescence response to these proteins compared with that of AP−SWNT only. However, significant SOSG enhancement occurred after the introduction of 2.0 μM thrombin. This clearly demonstrated that the production of $^1O_2$ by our AP−SWNT could only be triggered by the specific target protein, without the interference of other proteins.

In conclusion, we have successfully constructed a novel PDT that can be selectively triggered by target proteins based on SWNTs. To the best of our knowledge, this is the first study to report on the quenching of SOG by SWNTs and the restoration by specific target proteins. The AP−SWNT design is based on the attachment of DNA aptamer and photosensitizer on SWNTs, with the aptamer interacting noncovalently with SWNTs by π-stacking between nucleotide bases and SWNT side walls. This design has several significant advantages. First, it can be a general approach for SOG regulation by a variety of targets. There is no strict requirement for this design: no need for hairpin structure[5] or peptide self-folding.[3] Different aptamer sequences can be used for a variety of targets. For example, we have also designed other ssDNA with the same scheme and all worked for the regulation of SOG by their corresponding targets (Figures S4 and S5). These features establish the universality and simplicity of AP−SWNT as a PDT agent. Second, the SOG of AP−SWNT can be specifically triggered by a target of interest. By simply changing the sequences of the ssDNA to a specific aptamer, a selective PDT agent can be created to trigger SOG with that specific target. This is important because many biomolecules, such as thrombin in our model, play significant roles in many life processes and various diseases. In the absence of a target, even with light irradiation, only minimal $^1O_2$ would be generated. This can reduce side effects. Third, SWNTs deployed as cargo carriers for probe delivery can protect recognition ligands, such as ssDNA aptamers, from enzymatic digestion or degradation in the biological environment. It can also be used for multiple ssDNA probes for high capacity in delivery of PDT agents or in multivalent binding and delivery. Overall, this could have the potential to generate a wide range of PDT agents, making them useful for selective and controllable treatments. Our preliminary cellular experiments with the PDT confirm these predictions (Figure S6).

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Supporting Information Available: Synthesis, experimental details, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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