Regulation of Singlet Oxygen Generation Using Single-Walled Carbon Nanotubes

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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.

We have engineered a novel molecular complex of a photosensitizer, an ssDNA aptamer, and single-walled carbon nanotubes (SWNTs) for controllable singlet oxygen (\( ^1\text{O}_2 \)) generation. \(^1\text{O}_2 \) 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 \(^1\text{O}_2 \), 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 \(^1\text{O}_2 \) 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 co-workers\(^6\) have reported a photodynamic molecular beacon, in which a photosensitizer and a \(^1\text{O}_2 \) 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 \(^1\text{O}_2 \) 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 even to disease cells.\(^7\) Aptamers rival other molecular probes by their intrinsic advantages, such as reproducible synthesis, easy manipulation, excellent stability, and nontoxicity.\(^8\) 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 designs.\(^11,12\) 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,\(^1,12\) have been intensively studied and applied to biosensing\(^11,12,12\) and as intracellular transporters.\(^11,12\) This indicates SWNTs may also protect DNA probes from digestion by nucleases. This notion is given further support by the recent demonstration that single-stranded DNA interacts noncovalently with...
were normalized to AP 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.

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 (PA), protein L (PL), NeutrAvidin (NA), and IgG. The SOSG fluorescence signals were normalized to AP–SWNT.

The SOG 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. 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 could 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).

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