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Intracellular Glutathione Detection Using MnO_2-Nanosheet-Modified Upconversion Nanoparticles

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Supporting Information

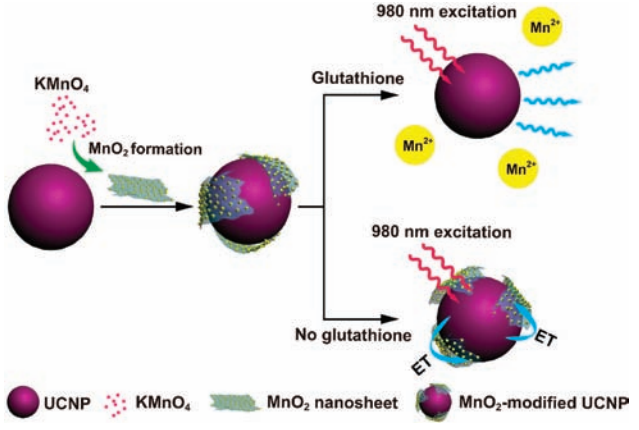
ABSTRACT: We report a novel design, based on a combination of lanthanide-doped upconversion nanoparticles and manganese dioxide nanosheets, for rapid, selective detection of glutathione in aqueous solutions and living cells. In this approach, manganese dioxide (MnO_2) nanosheets formed on the surface of nanoparticles serve as an efficient quencher for upconverted luminescence. The luminescence can be turned on by introducing glutathione that reduces MnO_2 into Mn^{2+}. The ability to monitor the glutathione concentration intracellularly may enable rational design of a convenient platform for targeted drug and gene delivery.

Glutathione (GSH, L-γ-glutamyl-L-cysteinyl-glycine) is the most abundant thiolated tripeptide in mammalian and eukaryotic cells. GSH is an essential endogenous antioxidant that plays a central role in cellular defense against toxins and free radicals.[1] GSH levels are implicated in many diseases typically associated with cancer, aging, or heart problems.[2] Thus, it is important to be able to monitor the change of GSH concentration in real time. Conventional methods for monitoring cellular GSH levels are generally based on thiol-sensitive organic fluorophores.[3,4] Despite their usefulness, these organic fluorophores, however, are unsuitable for long-term assays due to photobleaching and for sample labeling at a substantial depth because of low-depth tissue penetration by UV/visible excitation light sources.

The use of lanthanide-doped upconversion nanoparticles (UCNPs), which convert near-infrared (NIR) radiation to visible light,[5] provides an alternative method for GSH monitoring. In contrast to organic fluorophores, UCNPs have several outstanding features: (i) they offer high photostability and thermal stability; (ii) the NIR excitation source (typically 980 nm) offers a substantially higher tissue penetration depth and causes less damage to biological samples than UV excitation source; and (iii) the NIR-excitation technique features nonblinking[6] and non-autofluorescence assays, resulting in significantly improved signal-to-noise ratios. These advantages make the UCNPs particularly attractive for biolabeling and biosensing.[7]

During the course of our investigation on energy migration upconversion,[8] we discovered that the upconverted luminescence can be effectively quenched by MnO_2 nanosheets formed in a solution of UCNPs. The quenching is highly efficient, as 20 mol % of MnO_2 nanosheets almost completely quench the upconverted emissions. Interestingly, this MnO_2-induced quenching effect can be reversed by adding a small amount of GSH to the particle solution. On the basis of these findings, we propose a hybrid system, based on MnO_2-modified UCNPs, for rapid screening and quantification of GSH levels occurring in cancer cells (Scheme 1).

Scheme 1. Experimental Design for GSH Detection Using MnO_2-Nanosheet-Modified Upconversion Nanoparticles



As a proof-of-concept experiment, Yb/Tm codoped hexagonal-phase core−shell $NaYF_4$ UCNPs (~30 nm) were first prepared according to the literature.[8,9c] The core−shell design provides the benefit of minimizing surface quenching-induced emission loss.[9] The oleic acid ligand capped on the surface of core−shell nanoparticles was further oxidized to afford azelaic acid by using a technique developed by Li and co-workers.[10] This modification provides hydrophilic nanoparticles without notable effects on the resulting particle shape, size, and optical property (Supporting Information, Figures S1−S3).

The as-prepared core−shell $NaYF_4$:Yb/Tm@$NaYF_4$ nanoparticles were then used to direct the growth of MnO_2 nanosheets by addition of an aqueous $KMnO_4$ solution in the presence of 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer at pH 6. $KMnO_4$ was reduced by MES to form amorphous MnO_2 nanosheets. The formation of upconversion nanoparticle/MnO_2 nanosheet assemblies, dominated by electrostatic interactions, was confirmed by transmission electron microscopy (TEM) (Supporting Information,

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Figures S4–S5). Compositional analyses of the hybrid nanomaterials by X-ray photoelectron spectroscopy (XPS) and energy-dispersive X-ray spectroscopy (EDS) revealed the presence of MnO_2 (Supporting Information, Figures S6–S7).

We next assessed the optical properties of MnO_2-modified UCNPs. UV–vis absorption spectroscopy showed an intense broad band centered at 380 nm, characteristic of MnO_2 nanomaterials (Supporting Information, Figure S8).[11] This absorption can be attributed to d–d band transitions, caused by the ligand field of MnO_6 octahedra in MnO_2 crystal lattices, between the lower energy (3d t_{2g}) and higher energy (3d e_g) levels of manganese ions.[11] Notably, the absorption band of MnO_2 nanosheets overlaps with the emissions of the core–shell nanoparticles in the UV and blue spectral regions, thereby enabling Förster resonance energy transfer. The energy transfer is confirmed by the observation of emission quenching of the particle solution upon NIR excitation. For example, when the molar ratio of MnO_2 nanosheets and UCNPs reaches 0.25, the solution exhibited a weak red color emission (Figure 1a). In contrast, the core–shell

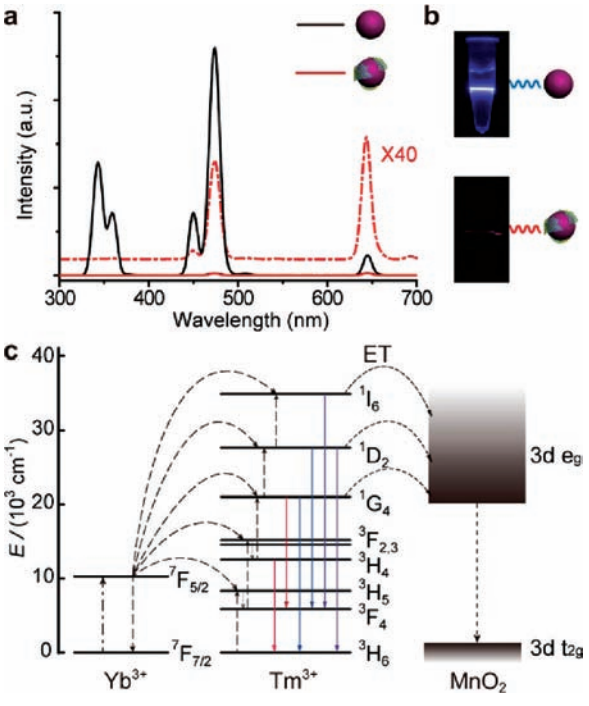

Figure 1. (a) Upconversion emission spectra of core–shell $NaYF_4$:Yb/Tm@$NaYF_4$ nanoparticles (black line) and MnO_2-modified nanoparticles with a UCNP/MnO_2 molar ratio of 1:0.25 (red lines). The dotted line, enhanced 40 times, reveals the higher emission intensity at 645 nm relative to that at 476 nm. (b) Corresponding photographs of UCNP (top) and MnO_2-modified UCNP (bottom) solutions under the 980 nm excitation. (c) Proposed excitation and energy-transfer mechanisms in MnO_2-modified UCNPs. Note that the samples in (a) and (b) were dispersed in deionized water, and the concentrations of lanthanide ions were adjusted to 0.01 M. The emission spectra and photographs were recorded with a diode 980-nm laser at a power density of 10 W cm^{-2}.

nanoparticles without the MnO_2 modification exhibited a blue color emission (Figure 1b), which can be attributed to the dominant blue emission ($^1G_4 \rightarrow {}^3H_6$) at around 476 nm.

We next tested GSH detection in aqueous solutions using these hybrid nanoparticles. We added GSH at different concentrations to the nanoparticle solution. As anticipated, upconversion luminescence was gradually recovered with increasing amounts of GSH (Figure 2). Remarkably, we observed a 100-fold enhancement of

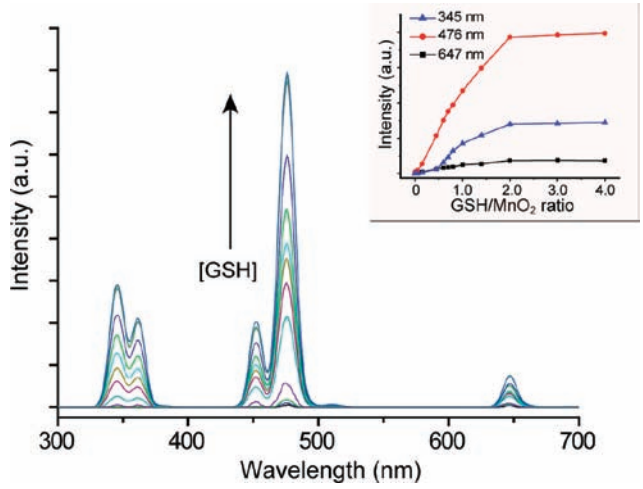

Figure 2. Photoluminescence response of MnO_2-modified $NaYF_4$:Yb/Tm@$NaYF_4$ nanoparticles as a function of GSH concentration (0–10 mM) in an aqueous solution. (Inset) Plot of luminescence intensity at 345, 476, and 647 nm, respectively, against the GSH/MnO_2 molar ratio. The spectra were recorded under excitation of a 980-nm laser at a power density of 10 W cm^{-2}.

upconversion emission in the blue spectral region after 2 equiv of GSH were added. Because of the low background signal by NIR excitation, this method has a low detection limit of 0.9 μM. We attributed the recovery of upconversion emission to the GSH-mediated reduction of MnO_2 to Mn^{2+}, leading to the decomposition of the MnO_2 nanosheets. During this redox reaction, GSH was oxidized to generate glutathione disulfide (GSSG) through thiol-disulfide exchange as shown in eq 1.

$$MnO_2 + 2GSH + 2H^+ \rightarrow Mn^{2+} + GSSG + 2H_2O \qquad (1)$$

Importantly, this chemical response of MnO_2-modified UCNPs is highly selective toward GSH relative to other biomolecules or different electrolytes. This investigation revealed that a wide range of electrolytes and weakly reducing bioagents such as glucose and fructose did not lead to notable optical responses (Figure 3).

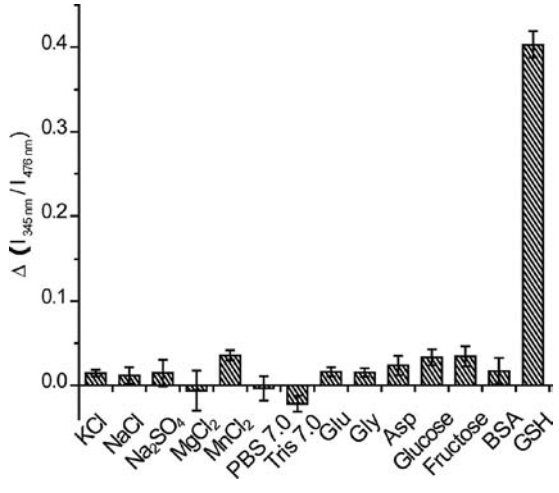

Figure 3. Photoluminescence response of MnO_2-modified $NaYF_4$:Yb/Tm@$NaYF_4$ nanoparticle solutions in the presence of different electrolytes and biomolecules (0.1 M each; for bovine serum albumin: 1 mg/mL). The photoluminescence response was evaluated by calculating the change in the relative luminescence intensity ratio (I_{345nm}/I_{476nm}) of MnO_2-modified nanoparticles.

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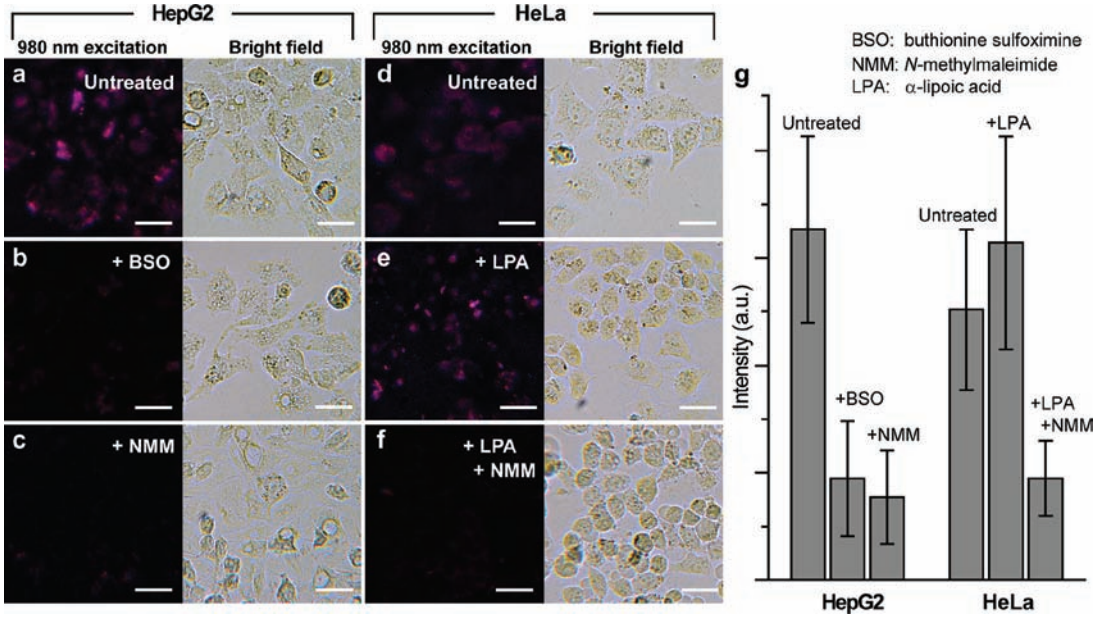

Figure 4. Intracellular GSH detection in HepG2 and HeLa cell lines using MnO_2-modified $NaYF_4$:Yb/Tm@$NaYF_4$ nanoparticles. (a–c) Upconversion luminescence and bright-field microscope images showing (a) nanoparticle-labeled HepG2 cells, (b) HepG2 cells pretreated with BSO (100 μM) for 1 h followed by incubation with the nanoparticles, and (c) HepG2 cells pretreated with NMM (500 μM) for 20 min followed by incubation with the nanoparticles. (d–f) Upconversion luminescence and bright-field microscope images showing (d) nanoparticle-labeled HeLa cells, (e) HeLa cells pretreated with LPA (500 μM) for 24 h followed by incubation with the nanoparticles, and (f) HeLa cells pretreated with LPA for 24 h and then NMM (500 μM) for 20 min, followed by incubation with the nanoparticles. Both cell lines were washed with cell culture media and incubated with MnO_2-modified nanoparticles (100 μg/mL) for 3 h at 37 °C. The scale bars are 10 μm. (g) Photoluminescence intensity of HepG2 and HeLa cells measured after treatment with different chemicals and upconversion nanoparticles.

To investigate the capability of MnO_2-modified UCNPs for monitoring intracellular GSH levels in cancer cells, we incubated two types of cancer cell lines (HepG2 human hepatoblastoma and HeLa cervical carcinoma cells) with the nanoparticles and subsequently analyzed the results under an optical microscope equipped with a 980-nm laser. As shown in Figure 4, HepG2 cells incubated with the nanoparticles (100 μg/mL) for 3 h exhibited a strong luminescence emission, indicating a high level of GSH in hepatoblastoma cells.[12] However, addition of buthionine sulfoximine (BSO, a glutathione synthesis inhibitor:[13] 100 μM) or *N*-methylmaleimide (NMM, a GSH scavenger:[4a] 500 μM) resulted in a significant decrease of the luminescence intensity. In parallel experiments, we observed a clear emission enhancement for HeLa cancer cells treated with α-lipoic acid (LPA, a GSH synthesis enhancer:[14] 500 μM). When NMM (500 μM) was further added to LPA-treated HeLa cells, a distinct decrease of luminescence intensity was observed. Taken together, these results strongly support the feasibility of intracellular GSH monitoring by MnO_2-modified UCNPs.

In conclusion, we have developed a novel method through use of MnO_2-nanosheet-modified upconversion nanoparticles for rapid screening of glutathione molecules in aqueous solutions. By taking advantage of the highly sensitive GSH/MnO_2 redox reaction and the nonautofluorescent assays offered by the upconversion nanoparticles, we have also demonstrated the monitoring of GSH levels in living cells. These findings are important not only for enabling promising applications in target drug and gene delivery but also for providing a sensor platform useful for photocatalytic and photoelectric studies when one considers the rich electrical and catalytic properties of MnO_2 nanomaterials.[15]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, Figures S1–S11. This material is available free of charge via the Internet at http://pubs.acs.org.

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