Cell Imaging

**MnO$_2$-Modified Persistent Luminescence Nanoparticles for Detection and Imaging of Glutathione in Living Cells and In Vivo**

Na Li, Wei Diao, Yaoyao Han, Wei Pan, Tingting Zhang, and Bo Tang$^{[a]}$

**Abstract:** Persistent luminescence nanoparticles (PLNPs) hold great promise for the detection and imaging of biomolecules. Herein, we have demonstrated a novel nanoprobe, based on the manganese dioxide (MnO$_2$)-modified PLNPs, that can detect and image glutathione in living cells and in vivo. The persistent luminescence of the PLNPs can be efficiently quenched by the MnO$_2$ nanosheets. In the presence of glutathione (GSH), MnO$_2$ was reduced to Mn$^{2+}$ and the luminescence of PLNPs can be restored. The persistent luminescence property can allow detection and imaging without external excitation and avoid the background noise originating from the in situ excitation. This strategy can offer a promising platform for detection and imaging of reactive species in living cells or in vivo.

Glutathione (GSH) is the most prevalent small molecule thiol in living cells$^{[1]}$. The role of GSH is to maintain a cellular reducing environment, cellular signal transduction, and gene regulation$^{[2]}$. Moreover, GSH is an important antioxidant capable of trapping free radicals, thereby effectively protecting the cells from oxidative stress$^{[3]}$. Changes in the level of GSH have been associated with a variety of human diseases, including liver damage, lung disease, Parkinson’s disease, AIDS and cancer$^{[4]}$. Therefore, it is of great importance to monitor the GSH levels in cells and in vivo. In recent years, a number of methods have been exploited to monitor the GSH levels in biological systems$^{[5]}$. Among these techniques, fluorescence measurements have been widely used to detect and image intracellular reactive species. However, several limitations need to be considered, including the low selectivity, long analysis time, the auto-fluorescence from the cells and tissues under external illumination, and weak tissue penetration of low wavelength excitation light. To eliminate the background noise originating from the in situ excitation, we introduced persistent luminescence nanoparticles (PLNPs) as the luminescence unit. As a promising candidate, PLNPs have attracted much attention due to the unique optical properties$^{[6]}$. Yan et al. reported a sensitive and specific nanoprobe based on PLNPs for detection and imaging of α-fetoprotein in serum samples and cancer cells$^{[6a]}$. Richard and co-workers presented a PLNP-based nanoprobe for optical imaging of vascularization, tumours and grafted cells$^{[6f]}$. The PLNPs can be optically excited with a UV lamp before analytical applications. In addition, the persistent luminescence can last several hours, which allows the elimination of the background noise from the in situ excitation. Consequently, the signal-to-noise ratio can be enormously improved when used for the determination and real-time screening of active species in organisms.

Herein, we developed a novel nanoprobe using MnO$_2$-modified PLNPs based on the specific reaction of MnO$_2$ and GSH$^{[7]}$, which was successfully used for detection and imaging of GSH in living cells and in vivo without in situ excitation. The PLNPs were employed as the photoluminescence unit. The MnO$_2$ nanosheets formed on the surface of PLNPs can efficiently quench the persistent luminescence of the PLNPs via the Förster resonance energy transfer (FRET). In the presence of a small amount of GSH, the MnO$_2$ nanosheets can be reduced to Mn$^{2+}$ and the MnO$_2$-induced quenching effect can be reversed, enabling the restoration of the persistent luminescence of PLNPs. The details of this approach are shown in Scheme 1.

![Scheme 1. Schematic illustration of the design for GSH detection using MnO$_2$-modified PLNPs.](http://dx.doi.org/10.1002/chem.201404625)
According to the previous literature, the Eu and Dy-doped Sr$_2$MgSi$_2$O$_7$ PLNPs were synthesized using a sol–gel method with minor modification.$^{[8]}$ Nanometer-sized PLNPs were obtained by basic wet grinding with NaOH solution. Then the PLNPs were characterized by TEM (Figure 1A). The XRD result indicated typical patterns of PLNPs matched well with the standard samples. Figure 1C showed the photoluminescence (PL) of the PLNPs could reach more than 200 min and it was stable in a period range of 55–220 min after excitation (Figure 1D). Next, MnO$_2$ grew on the surface of the as-prepared hydroxylated PLNPs by the addition of KMnO$_4$ solution into 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6). KMnO$_4$ was reduced by MES to form amorphous MnO$_2$ nanosheets on the surface of the PLNPs. The assembly of the MnO$_2$/PLNPs nanoprobe was confirmed by HRTEM (Figure 1B). Composition-analyses of X-ray photoelectron spectroscopy (XPS) further indicated the formation of MnO$_2$ in the hybrid nanoprobe (Figure S2, Supporting Information).

UV/Vis absorption spectroscopy was employed to access the optical properties of the independent materials and the nanoprobe. As can be seen from Figure S3, Supporting Information, the KMnO$_4$ solution showed two broad bands at 310 and 325 nm, when MnO$_2$ nanosheets were formed, the absorption peak was centered at 380 nm, which was consistent with the previous report.$^{[21]}$ Figure S4, Supporting Information, showed that the absorption band of MnO$_2$ overlaps well with the emissions of the PLNPs, which is the key factor for the generation of FRET. As shown in Figure S5, Supporting Information, the nanoprobe showed an intense absorption band centered at 380 nm by comparison to free PLNPs, which was consistent with the characteristic absorption of MnO$_2$, which suggests the successful assembly of the nanoparticles. To further verify the results, a physical mixture of preformed MnO$_2$ nanosheets and the PLNPs was prepared as a comparison. Compared with free PLNPs, the PL intensity of the nanoprobe was significantly reduced because of the FRET effect, while the physical mixture does not lead to an obvious change (Figure S6, Supporting Information).

Then the FRET efficiency of the nanoprobe was investigated by observing the PL quenching of the PLNPs modified with different ratios of MnO$_2$. Figure S7, Supporting Information, demonstrated that the PL intensity of the nanoprobe decreased with the increase of MnO$_2$ concentrations. The maximum quenching efficiency is as high as 96%. Then the FRET nanoprobe was employed to detect GSH. Figure 2A indicated the PL intensity of the nanoprobe increased with the increase of GSH concentrations. The recovery of the PL for the nanoprobe is due to the separation of MnO$_2$ from the PLNPs as a result of the reaction of GSH and MnO$_2$. A good linear correlation (Figure 2B, $R = 0.9990$) was obtained for the enhanced PL intensity and the GSH concentrations. According to the previous protocol,$^{[26]}$ 13 determinations were made according to the experimental method and the standard deviation (SD) was calculated to be 0.16. The regression equation was $F = -0.6515 + 0.5759\times[GSH]$ ($\mu$m). The detection limit was calculated to be as low as 0.83 $\mu$m, because of the low background signal without excitation. Figure S8, Supporting Information, showed that the nanoprobe responded rapidly to GSH within 5 min.

Before applied in living cells, the selectivity of the nanoprobe toward GSH was investigated because the activity of GSH can be influenced by other reactive species in living cells. The selectivity of the nanoprobe towards GSH has been evaluated by monitoring the changes of PL intensity for the nanoprobe upon exposure to various interfering agents and GSH. The results showed that the nanoprobe was highly selective toward GSH compared with other interfering agents, including a wide range of electrolytes and reducing biomolecules (Figure 3). Next, the nanoprobe was used for the determination of GSH in cell extracts. To eliminate the autofluorescence and scattering light from biological matrices produced by in situ excitation, the cell extract solution with the nanoprobe was irradiated for 10 min before analysis. The results showed the signal-to-noise ratio was greatly improved for the sample without in situ excitation compared with the same sample under a 365 nm excitation. (Figure 4A and B) It demonstrated that

![Figure 1](image1.png)

**Figure 1.** A) TEM image of Sr$_2$MgSi$_2$O$_7$: Eu, Dy PLNPs. B) TEM image of the MnO$_2$-modified PLNPs. Scale bars are 100 nm. C) Time-dependent PL intensity of PLNPs (1 mg mL$^{-1}$) without further excitation after direct exposure under a UV lamp for 10 min. D) Time-dependent PL intensity from 55 to 220 min of the PLNPs in (C).

![Figure 2](image2.png)

**Figure 2.** A) PL emission spectra of MnO$_2$-modified PLNPs as a function of GSH concentration (0, 10, 20, 40, 60, 80, 100 $\mu$m) in aqueous solution. B) Plot of enhanced PL intensity (AF) against GSH concentration over the linear range 0–100 $\mu$m.
the nanoprobe using long-lasting PL detection could effectively avoid the fluorescent background noise under in situ excitation. Figure 4C showed that the cell extract solution with PLNPs displayed a bright blue colour long-lasting PL (a), but the cell extract solution with the nanoprobe exhibited a dark view on account of the FRET effect (b and c). The cell extract solution (sample c) also showed a bright blue colour long-lasting PL after the addition of GSH (Figure 4D), which indicated that the nanoprobe could be successfully applied for detecting GSH in cell extracts using the long-lasting afterglow nature.

An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in RAW 264.7 macrophages was carried out to evaluate the cytotoxicity of the PLNPs and nanoprobe.[10] RAW 264.7 macrophages were incubated with the PLNPs and nanoprobe with different concentrations (0, 0.2, 0.5, 1.0, 1.5 and 2.0 mg mL⁻¹). The absorbance of MTT at 490 nm relies on the degree of activation of the cells. The cell viability was then represented by the proportion of absorbance of the cells incubated with the nanoprobe to the cells incubated with culture medium only. Figure S9, Supporting Information, indicated that the cell viability was more than 95% when the concentration of the nanoprobe was up to 2.0 mg mL⁻¹, which suggests that the PLNPs and nanoprobe showed almost no cytotoxicity or side effects in living cells. It indicated that the nanoprobe was an approving candidate to be applied in living cells and in vivo.

Then the nanoprobe was used to detect and image GSH in living cells. RAW 264.7 macrophages were incubated with the nanoprobe (0.2 mg mL⁻¹) at 37°C for 1 h. Before the imaging experiment, the sample was excited by using a UV lamp. To eliminate the autofluorescence and scattering light from the living cells, the sample was no longer excited during the whole imaging process. As shown in Figure 5A, when RAW 264.7 macrophages incubated with the nanoprobe, only negligibly weak PL was observed. Nevertheless, obvious PL was observed when the GSH was added in the same sample (Figure 5B), which indicated that the nanoprobe could detect and image GSH in living cells without excitation. The bright-field images showed that the cells were live throughout the imaging experiments.

We next sought to assess the ability of the nanoprobe to image GSH in a mouse model. Two Kunming mice were chosen in the following experiments. One mouse (the left one) was injected with 0.1 mL normal saline, and another (the right one) was injected with 0.1 mL GSH solution. Next, 0.1 mL MnO₂-modified PLNPs (1 mg mL⁻¹, excited with a 365 nm UV lamp for 10 min before injection) was injected at the same site for the two mice. The PL of the two samples with and without

---

**Figure 3.** PL response of MnO₂-modified PLNPs solutions in the presence of different electrolytes and biomolecules: GSH (100 μM), Fe³⁺ (0.36 mM), Al³⁺, Mg²⁺, Na⁺, Zn²⁺ (0.18 mM), Cu²⁺, K⁺, Ca²⁺ (0.12 mM), Mn²⁺ (0.36 mM), NaClO (1.2 mM), tBuOOH (36 μM), Lys, His, Trp, Hcy and Cys (100 μM).

**Figure 4.** PL spectra of cell extract solutions with detectable GSH (100 μM) in the absence (––) or presence (—–) of the nanoprobe. A) With 365 nm excitation; B) upon removal of the excitation source after excitation using a UV lamp for 10 min. C) Photographs of the cell extract solution with PLNPs (a) and the nanoprobe (b, c) in the dark. D) Photographs of the cell extract solution with PLNPs (a), the nanoprobe (b), the nanoprobe and GSH (c) in the dark. The samples were excited with a UV lamp for 10 min before the photographs were taken in the dark.

**Figure 5.** PL imaging of GSH in living cells. A) PL image of the nanoprobe in RAW 264.7 macrophages; B) PL image of the same sample after the addition of GSH (100 μM); C) Bright-field image of the samples in (A) and (B). Scale bars are 100 μm.
The MnO$_2$-modified persistent luminescence nanoparticles were used for the detection and imaging of GSH in living cells and in vivo using Chem. Eur. J. The PL signals of the nanoparticles can be efficiently quenched by the MnO$_2$. In the control samples under in situ excitation, the nanoprobe was able to monitor GSH in vivo and could effectively eliminate the interference from autofluorescence and scattering light from biological samples under in situ excitation.

In conclusion, we have developed a novel nanoprobe for detection and imaging of GSH in living cells and in vivo using the MnO$_2$-modified persistent luminescence nanoparticles (PLNPs). The PLNPs were served as the luminescence units and the MnO$_2$ nanosheets were used as the quencher. When MnO$_2$ was assembled on the surface of the PLNPs, the luminescence of the PLNPs can be efficiently quenched by the MnO$_2$. In the existence of GSH, MnO$_2$ was reduced to Mn$^{2+}$ and the luminescence of PLNPs can be recovered. Moreover, detection and imaging of GSH in living cells and in vivo without external excitation could be achieved because of the long-lasting afterglow nature, which allows the removal of the autofluorescence and scattering light from biological samples produced by in situ excitation. We envision that the current technique offers new opportunities to detect and image reactive species in living cells or in vivo.

Acknowledgements

This work was supported by 973 Program (2013CB933800) and National Natural Science Foundation of China (21227005, 21390411, 91313302, 21035003, 21422505, 21375081).

Keywords: cell imaging · glutathione · manganese dioxide nanoparticles · nanotechnology