Avoiding Thiol Compound Interference: A Nanoplatform Based on High-Fidelity Au–Se Bonds for Biological Applications

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Abstract: Gold nanoparticles (Au NPs) assembled through Au–S covalent bonds have been widely used in biomolecule-sensing technologies. However, during the process, detection distortions caused by high levels of thiol compounds can still significantly influence the result and this problem has not really been solved. Based on the higher stability of Au–Se bonds compared to Au–S bonds, we prepared selenol-modified Au NPs as an Au-Se nanoplatform (NPF). Compared with the Au-S NPF, the Au-Se NPF exhibits excellent anti-interference properties in the presence of millimolar levels of glutathione (GSH). Such an Au-Se NPF that can effectively avoid detection distortions caused by high levels of thiols thus offers a new perspective in future nanomaterial design, as well as a novel platform with higher stability and selectivity for the in vivo application of chemical sensing and clinical therapies.

Thiol-functionalized gold nanoparticles (Au NPs) have been extensively used in fluorescence, opto-acoustic, Raman, and electrochemistry biosensors due to their excellent biocompatibility and unique, tunable optical properties. However, under physiological conditions, with a comparably low loading capacity of the thiolated recognition elements on the surface of Au NPs, the ligands can be replaced by abundant biological thiols, thereby causing detection distortions. Avoiding such interference from the high concentrations of biothiols in living cells thus becomes a great challenge. Recently, Wang and co-workers utilized two fluorophore-labeled “flares” to build a ratio approach that indirectly calibrates the distorted false-positive signals from Au-S probes. However, by better directly solve the instability of Au–S bonds, a more stable Au nanoplatofrm (NPF) for high-fidelity imaging biomolecules under physiological conditions urgently needs to be developed. Our previous study established a near-infrared fluorescent nanosensor based on thiol-functionalized Au NPs for selenol detection, and we found that selenol could break the Au–S bonds to form more stable Au-Se bonds on the surfaces of the Au NPs. Since the higher stability of the Au–Se bond compared with the analogous Au–S bond has been demonstrated, a new strategy to reconstruct the conventional Au–S-bonding NPF, using Au–Se bonds instead of Au–S bonds was envisaged. Such an Au–Se-bonding NPF is expected to possess a better performance in avoiding detection distortions caused by biological thiols, thereby allowing high-fidelity cell imaging.

We created an Au-Se NPF by attaching selenol-modified peptides with a fluorescence dye (FITC) but no cleavage site (FITC-Ser-Gly-[Se-Cys]) onto Au NPs through Au–Se bonds. For comparison, the Au-S NPF was also prepared with thiol-modified peptides (FITC-Ser-Gly-Cys) and Au NPs through Au–S bonds (Scheme 1). First, the GSH response of the two NPFs was investigated through 5 mM GSH treatment. No obvious change in fluorescence intensity was observed before and after addition of GSH into the solution of the Au-Se NPF, but the Au-S NPF did show an evident fluorescence enhancement, which confirms that the Au-Se NPF displays a higher stability than the Au-S NPF. In addition, the temperature dependence of the two NPFs was also tested, and the Au-Se NPF exhibited better thermal stability. Hence, the new Au-Se NPF can effectively avoid interference from GSH in the bioimaging process.

Caspases are a family of proteases that are only activated during cell apoptosis. Since the upstream caspase-9 plays an essential role in the induction of apoptosis, it is critical for cytochrome c dependent apoptosis. To investigate the sensing ability of the Au-Se NPF, a nanoprobe (Au-Se probe) for detecting and imaging caspase-9 in living cells...
was designed and synthesized by assembling selenol-modified peptides (FITC-Leu-Glu-His-Asp-Ser-Gly-[Se-Cys]) onto Au NPs. For comparison, an Au-S probe for caspase-9 detection was also prepared in the same way. The fluorescence of the fluorophore in the two nanoprobes is quenched through surface-energy transfer to the Au NPs, and is recovered upon cleavage of the caspase-9 site (LEHD). Parallel experiments demonstrated that the Au-Se probe has a high-fidelity fluorescent signal, whereas an obvious detection distortion occurred for the Au-S probe caused by GSH in physiological environments. In addition, the Au-Se probe was successfully applied in monitoring the changes of caspase-9 levels in MCF-7 cells (breast cancer cell line) treated with staurosporine (STS). We thus believe that nanosensors based on the Au-Se NPF will certainly have wide application in the near future.

To synthesize the two NPFs, the Au NPs were first prepared,[21–22] and then the selenol-modified and thiol-modified peptides with FITC (Figures S1, S2 in the Supporting Information) were assembled onto the surface of the Au NPs through Au–Se and Au–S bonds to form the Au-Se NPF and Au-S NPF, respectively. High-resolution transmission electron microscopy (HRTEM) images of the Au NPs and the two NPFs showed no morphology change after the modifications (Figure S3a). The sizes of the Au NPs determined by dynamic light scattering (DLS) was changed from 14.6 ± 2.1 nm to 20.3 ± 2.3 nm (Au-Se NPF) and 19.8 ± 3.1 nm (Au-S NPF) after peptide conjugation (Figure S3b). The Zeta potential value of the Au NPs changed from −13.8 ± 2.6 mV to −6.3 ± 1.8 mV (Au-Se NPF) and −7.9 ± 1.6 mV (Au-S NPF) due to the surface modification. Afterwards, the optical properties of the two NPFs were characterized by UV/Vis spectroscopy, and a red shift of the maximum absorption from 519 to 524 nm was obtained, thus indicating a successfully functionalized with peptides attached to the Au NPs (Figure S4). The loading capacity of the peptides were calculated by using a previously established method (Figure S5).[22] As shown in Table S1, with the same concentration, there are more selenol-modified peptides attached than thiol-modified peptides, which reflects easier formation of the Au–Se bond. To further verify the accuracy of the following comparative experiments, similar loading amounts of selenol-modified and thiol-modified peptides on each Au NP were selected for the Au-Se NPF and the Au-S NPF (107 ± 2 peptides).

Compared to the conventional Au-S NPF, the novel Au-Se NPF was expected to have a superior performance in terms of resisting interference from high concentrations of biological thiols in simulated physiological conditions. The Au-Se NPF and Au-S NPF were incubated with 5 mM GSH for increasing times (0–12 h) or with different concentrations of GSH (0–5 mM) for 12 h. The fluorescence intensities barely changed when 5 mM GSH was mixed with the Au-Se NPF (Figure 1a); in contrast, a distinctly enhanced fluorescent signal was observed for the Au-S NPF (7.9-fold at 12 h, Figure 1b) due to breaking of the Au–S bond by GSH. The same tendency was observed with increasing GSH concentrations for 12 h (Figure S6a), which demonstrates that the Au-Se NPF is more stable in the presence of high levels of GSH than the Au-S NPF. Meanwhile, it was also established that the Au-Se NPF shows a high level of resistance to physiological levels of selenol (Figure S6b), thus making it applicable for in vivo applications. The changes in the background signals caused by thermodynamic fluctuations in the two NPFs were also evaluated (Figure 1c,d). As the time (0–12 h) and temperature (10–80 °C) increase, the stable fluorescent signals of the Au-Se NPF were in stark contrast to the gradually increasing fluorescent signals of the Au-S NPF. This unusual behavior suggests that the Au-Se NPF has better thermal stability. The nuclease stability of the Au-Se NPF was also investigated (Figure S7), and the results show that the Au-Se exhibits high resistance to nucleases.

To further investigate the effect of biothiols on the Au-Se NPF and Au-S NPF in living cells, MCF-7 cells were pre-treated with N-ethylmaleimide (NEM; an irreversible thiol scavenger)[23–26] or without NEM, followed by incubation with the two NPFs. After treatment with NEM, no fluorescence change of the Au-Se NPF was observed (Figure S2a). Statistical data of the cell fluorescence intensities were consistent with the above results (Figure 2b), which confirms the stability of the Au-Se NPF in biological systems. In contrast, the fluorescence signal from the NEM-treated cells incubated with the Au-S NPF was weaker than that from the cells only treated with the Au-S NPF (Figure 2c,d), which indicated lower stability. The results indicate that the Au-Se NPF possesses superb stability and strong resistance against biothiol interference. Furthermore, the cytotoxicity of the two NPFs was also tested with MCF-7 cells by using the MTT assay (Figure S8), and the results revealed that both NPFs show low toxicity and can be used in living cells.

In order to confirm the practicality of the Au-Se NPF for use in sensors, we designed two nanoprobes (Au-Se probe and Au-S probe) for imaging caspase-9 in living cells, based on caspase-9 specifically cleaving the LEHD site. The two probes were synthesized by attaching the FITC-labeled peptides (FITC-Leu-Glu-His-Asp-Ser-Gly-[Se-Cys]) and FITC-Leu-Glu-His-Asp-Ser-Gly-Cys) onto the Au NPs through Au–Se or Au–S bonds (Figure S9–12). Similar amounts of selenol-
The influence of GSH on the detection of caspase-9 with the acquired m due to 520 nm interference from intracellular biothiol for the Au-Se NPF (a) (Figure 3d and Figure S17). Furthermore, the performance of the Au-Se probe in serum was studied (Figure S18), and the fluorescence responses were similar to those in PBS solution, which demonstrated that the Au-Se probe could be applied in a complex biological environment to monitor caspase-9.

Finally, specific detection of caspase-9 in MCF-7 cells using the Au-Se probe was explored. The cellular cytotoxicity of the Au-Se probe was determined using a cell viability assay in MCF-7 cells (Figure S19), with the results suggesting that the Au-Se probe has low toxicity towards living cells. Afterwards, MCF-7 cells were treated with STS or caspase-9 inhibitors (Z-LEHD-FMK) followed by STS. Then the treated cells were divided into two groups to detect caspase-9. One group was incubated with the Au-Se probe (Figure 4a, b), and the other was lysed and then incubated with a commercial caspase-9 activity assay kit (colorimetric; Figure 4c). STS is known to be a strong apoptosis inducer in MCF-7 cells via activation of upstream components in the intracellular caspase-9 cascade.57–59 Figure 4a shows that clear fluorescence signals in the STS-treated MCF-7 cells were observed, thus indicating that FITC-labeled peptides were released from the Au NPs due to the expression and activation of caspase-9. Moreover, a weak fluorescence signal was observed when the cells were exposed to STS treatment in the presence of the caspase-9 inhibitors. Statistical data for the cell fluorescence intensity is given in Figure 4b. The enzymatic activity of caspase-9 was also confirmed using a commercial caspase-9 activity assay kit (Figure 4c), and the results are consistent with data from the Au-Se probe. This experiment further validates the practicality of the Au-Se probe for specific sensing of caspase-9 levels in living cells.

In conclusion, we have developed a novel Au-Se NPF by utilizing Au-Se bonds to reconstruct the conventional Au-S NPF. The Au-Se NPF exhibits excellent thermal stability and resistance to biothiol interference compared to the Au-S NPF. The Au-Se probe also displays a high-fidelity fluorescent signal under physiological conditions. Moreover, the Au-Se probe was stable in the presence of 5 mM GSH, and the changes in the fluorescent intensity of the Au-Se probe solution triggered by caspase-9 was obvious (1.4-fold). However, under the same conditions, the background signal of the Au-Se probe was clearly enhanced in the presence of GSH, thus resulting in an inconspicuous increase in fluorescent intensity triggered by caspase-9 (0.4-fold) (Figure 3b). The results suggest that physiological levels of GSH can competitively displace the thiol-modified peptides on the surface of the Au NPs to decrease the detection sensitivity, whereas the Au-Se probe exhibits a high-fidelity fluorescent signal.

The response of the Au-Se probes to caspase-9 protein was then studied. After incubating a mixture of the nanoprobe and caspase-9 in PBS at 37 °C for 1.5 h, a significantly enhanced fluorescence signal at 520 nm was acquired due to the enzymatic reaction release of the FITC dyes from the Au NPs (Figure 3c). A specific inhibitor of caspase-9 (Z-LEHD-FMK) was also added to the study for comparison. The responses of the Au-Se probe to various concentrations of caspase-9 were measured, and a decent linear relationship was observed between the fluorescence intensity and caspase-9 concentration from 0–20 unit mL⁻¹ (Figure 3d and Figure S17). Furthermore, the performance of the Au-Se probe in serum was studied (Figure S18), and the fluorescence responses were similar to those in PBS solution, which demonstrated that the Au-Se probe could be applied in a complex biological environment to monitor caspase-9.

modified and thiol-modified peptides on each Au NPs were selected (110 ± 1 peptides, Figure S13). Interference experiments and the thermodynamic stabilities of two probes were applied to demonstrate better resistance to GSH interference and higher thermal stability for the Au-Se probe (Figure S14–S16).

The influence of GSH on the detection signals from the Au-Se and Au-S probes to caspase-9 was examined as well. Under simulated physiological conditions, the recombinant caspase-9 protein (20 Unit mL⁻¹) was added into the two probe solutions. In Figure 3a, the background signal of the Au-Se probe has low toxicity towards living cells. Afterwards, MCF-7 cells were treated with STS or caspase-9 inhibitors (Z-LEHD-FMK) followed by STS. Then the treated cells were divided into two groups to detect caspase-9. One group was incubated with the Au-Se probe (Figure 4a, b), and the other was lysed and then incubated with a commercial caspase-9 activity assay kit (colorimetric; Figure 4c). STS is known to be a strong apoptosis inducer in MCF-7 cells via activation of upstream components in the intracellular caspase-9 cascade.57–59 Figure 4a shows that clear fluorescence signals in the STS-treated MCF-7 cells were observed, thus indicating that FITC-labeled peptides were released from the Au NPs due to the expression and activation of caspase-9. Moreover, a weak fluorescence signal was observed when the cells were exposed to STS treatment in the presence of the caspase-9 inhibitors. Statistical data for the cell fluorescence intensity is given in Figure 4b. The enzymatic activity of caspase-9 was also confirmed using a commercial caspase-9 activity assay kit (Figure 4c), and the results are consistent with data from the Au-Se probe. This experiment further validates the practicality of the Au-Se probe for specific sensing of caspase-9 levels in living cells.

In conclusion, we have developed a novel Au-Se NPF by utilizing Au-Se bonds to reconstruct the conventional Au-S NPF. The Au-Se NPF exhibits excellent thermal stability and resistance to biothiol interference compared to the Au-S NPF. The Au-Se probe also displays a higher-fidelity fluorescent signal under physiological conditions. Moreover, the Au-Se probe was stable in the presence of 5 mM GSH, and the changes in the fluorescent intensity of the Au-Se probe solution triggered by caspase-9 was obvious (1.4-fold). However, under the same conditions, the background signal of the Au-Se probe was clearly enhanced in the presence of GSH, thus resulting in an inconspicuous increase in fluorescent intensity triggered by caspase-9 (0.4-fold) (Figure 3b). The results suggest that physiological levels of GSH can competitively displace the thiol-modified peptides on the surface of the Au NPs to decrease the detection sensitivity, whereas the Au-Se probe exhibits a high-fidelity fluorescent signal.
probe was successfully used to monitor the changes in caspase-9 in MCF-7 cells after STS treatment. Overall, such a novel Au-Se NPF that effectively avoids detection distortions to realize high-fidelity imaging in living cells with higher stability and selectivity offers new perspectives in future nanomaterial designs for biological applications such as drug delivery and chemical sensing.

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Conflict of interest

The authors declare no conflict of interest.

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