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Short communication

Fluorometric sensing of intracellular thiols in living cells using a AuNPs/**1**-PR_3^+ adsorbate

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ABSTRACT

A straightforward, sensitive, and selective fluorescent turn-on probe based on the AuNPs/**1**-PR_3^+ electrostatic adsorbate was applied to the detection of intracellular thiols such as cysteine, homocysteine, and glutathione in both aqueous buffer solutions (pH 7.4) and living cells.

1. Introduction

Intracellular thiols such as cysteine (Cys), homocysteine (HCys), and glutathione (GSH) play crucial roles in numerous biological processes, including redox homeostasis, detoxification, and cellular metabolism [1]. Abnormal levels of certain thiols such as HCys are linked to a number of diseases, including cancer, Alzheimer's disease, and cardiovascular disease [2,3]. GSH, the most abundant thiol species present in cells, is involved in antioxidant cellular defense through the balancing of the GSH/oxidized glutathione (GSSG) equilibrium [4]. Consequently, a number of interesting fluorometric approaches have been described for the *in vitro* and *in vivo* detection of thiols, as recently reviewed by Yoon and co-workers [5].

Gold nanoparticles (AuNPs) are becoming increasingly attractive materials for the design of sensory systems and drug carriers because of their large surface area, good biocompatibility, and unique size- and shape-dependent optical and electronic properties [6–8]. One of the most important properties of AuNPs is their strong surface plasmon resonance (SPR) absorption, with extremely high molar extinction coefficients (10^8–10^{10} M^{-1} cm^{-1}) and a broad energy bandwidth [9]. These advantageous optical characteristics allow for AuNPs to act as excellent fluorescence quenchers *via* electron- and/or energy-transfer processes [10]. This strong fluorescence quenching ability has been employed to develop many AuNP-based fluorescent assays for the detection of metal ions and biological molecules [11–17]. Recently, we made use of this superquenching property of AuNPs for the detection of polyamines by modulating the photoluminescence quenching efficiency of the cationic boron-dipyrromethene (BODIPY) chromophore, which adsorbs onto anionic citrate-capped AuNPs through electrostatic interactions [18]. The interesting results of that study prompted us to investigate a new homogenous sensing scheme based on modulation of the efficiency of energy transfer between fluorescent dyes and AuNPs. In this paper, we report the extension of this strategy to the highly sensitive, selective, and convenient fluorescence turn-on sensing of thiols in aqueous buffer solution and living systems.

2. Experimental

2.1. General methods, instrumentation, and measurements

Absorption spectra were obtained on a Shimadzu UV-2501 spectrophotometer. Fluorescence measurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer using 10 mm quartz cuvettes with a path length of 1 cm. Fluorometric assays with various analytes were measured by monitoring changes in fluorescence intensity using a Synergy Mx Microplate Reader (BioTek, USA). Both excitation and emission slit widths were 9 nm.

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According to a previously reported method [18], citrate-capped AuNPs, and cationic BODIPY derivatives (**1**-PR_3^+ and **2**-NR_3^+) were prepared.

2.2. Fluorometric assay studies

For assays, a solution of AuNPs/**1**-PR_3^+ adsorbate was prepared by mixing AuNPs (180 μL, 3.33 nM) and BODIPY dye **1**-PR_3^+ (20 μL, final concentration 1 μM) in HEPES buffer (pH 7.4) containing 5% ethanol. To a solution of AuNPs/**1**-PR_3^+ adsorbate (200 μL) was added 20 μL solution containing each analyte and the absorption and emission spectra of the resulting solution were then measured.

3. Cell imaging studies

HeLa cell line (human cervical carcinoma) was obtained from the American Type Culture Collection (Rockville, MD), and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% antibiotic–antimycotic in a humidified incubator containing 5% CO_2 at 37 °C. For fluorescence imaging study, HeLa cells were seeded at a density of 5×10^4 cells/well into 12-well plate and incubated for 24 h for cell attachment. Then, the cells were washed and treated with AuNPs/**1**-PR_3^+ adsorbate in HEPES buffer (10 mM, pH 7.4). The fluorescence images (Ex. 470/40 nm, Em. 525/50 nm) were obtained at every 30 s for 20 min using cell observer system (Axio observer Z1, 10×, NA 0.55, Carl Zeiss, Germany).

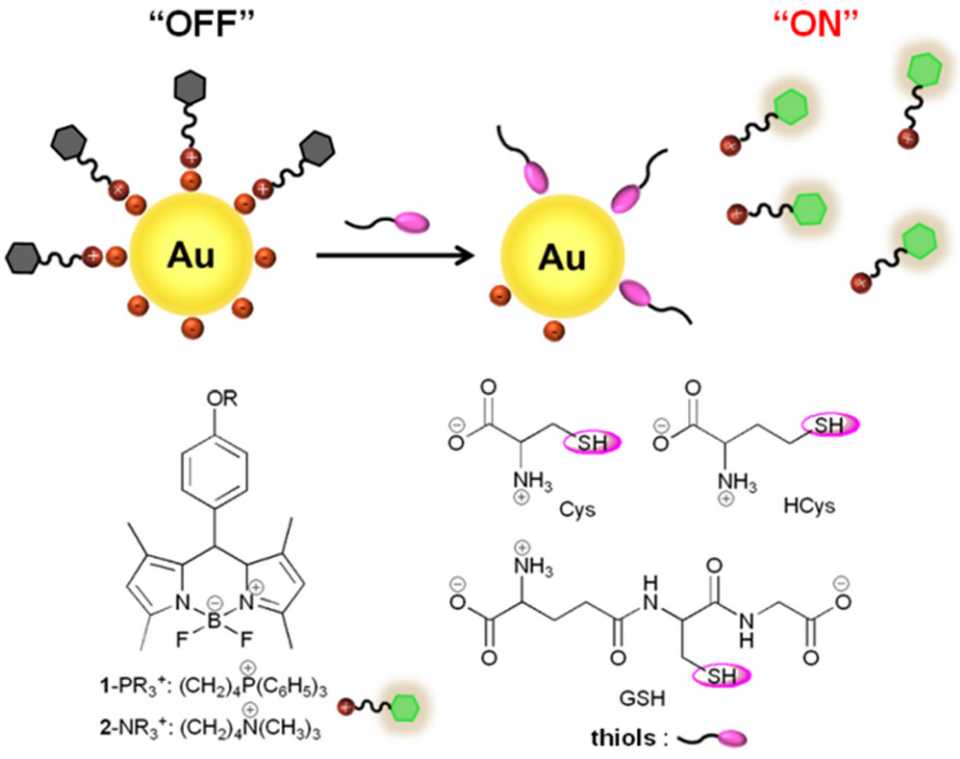
4. Results and discussion

Our sensing protocol is based on the strong affinity of Au for the thiol group. As proposed in Scheme 1, a positively charged BODIPY dye, **1**-PR_3^+, is adsorbed onto the negatively charged citrate-capped AuNPs through electrostatic interactions, and the fluorescence of **1**-PR_3^+ in the AuNPs/**1**-PR_3^+ adsorbate is quenched efficiently as a result of electron and/or energy transfer. Addition of thiols to the AuNPs/**1**-PR_3^+ adsorbate causes displacement of the citrate-**1**-PR_3^+ ion association complex from the AuNP surface, and the liberated fluorophore **1**-PR_3^+ rapidly recovers its original fluorescence intensity, thereby enabling efficient determination and quantification of the thiols.

The strong binding affinity of AuNPs for thiols has previously been utilized for the preparation of AuNP-based colorimetric assays of thiols [19,20]. Recently, Chang and co-workers reported the fluorometric determination of thiols using AuNPs adsorbed on Nile red through weak Au–N interactions [21]. Thiol-containing compounds displaced the fluorophore from the AuNP surface due to stronger Au–S interactions, leading to an enhancement of the fluorescence signals. Another related example of homogeneous assay for thiols is based on modulation of the quenching efficiency of acridine orange by AuNPs [22]. The enhanced fluorescence of these AuNP/dye assemblies is strongly affected by the pH of the assay solution; that is, they work under conditions of low pH (pH 4–4.5), which limits their widespread biological applications.

To obtain a AuNP/dye electrostatic assembly that is stable over a wide pH range, a lipophilic, fully cationic salt-based fluorophore may be an excellent choice. We have explored the properties of both an alkyltriarylphosphonium (**1**-PR_3^+) and a tetraalkylammonium (**2**-NR_3^+; see ESI) BODIPY dyes, and found that the former exhibited the desired characteristics. Consequently, we selected the AuNPs/**1**-PR_3^+ fluorometric system, which is quite stable in various intracellular pH environments, shows fast response to biological thiol-containing amino acids (HCys and Cys), and gives high turn-on signals.

The AuNPs/**1**-PR_3^+ adsorbate, which consists of 13-nm citrate-capped AuNPs and **1**-PR_3^+, was prepared under optimized conditions to maximize the fluorescence quenching efficiency and sensitivity toward thiols (see ESI). The as-prepared AuNPs/**1**-PR_3^+ adsorbate in the presence of small amounts of **1**-PR_3^+ (1 μM), was dispersible in aqueous solvents, as was evident from the negligible



Scheme 1. The proposed sensing approach and the chemical structures of fluorophores, cysteine (Cys), homocysteine (HCys), and glutathione (GSH).