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# An ultra-sensitive fluorescent "Turn On" biosensor for glutathione and its application in living cells

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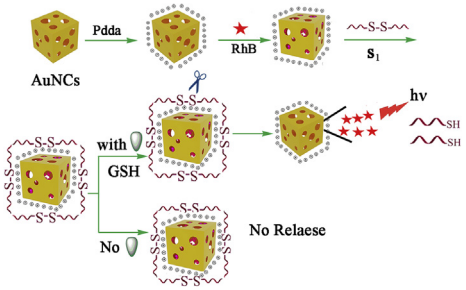
## HIGHLIGHTS

- An effective controlled-release biosensor based on Au nanocages was developed for ultra-sensitive detection of GSH.
- The system can successfully achieve the controlled release of the loaded molecules in response to intracellular target molecules without any external stimuli.
- A detection limit of as low as $4.8 \times 10^{-13}$ M with an excellent selectivity toward GSH could be achieved.
- The fabricated system was successfully employed for fluorescence microscopy imaging of GSH in living cells.

## GRAPHICAL ABSTRACT

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## ABSTRACT

In this work, an effective controlled-release biosensor based on Au nanocages (AuNCs) capped with disulfide-containing DNA molecular gates was developed for ultra-sensitive and highly selective detection of glutathione (GSH). Oligonucleotides containing the S-S bonds were assembled on the surface of the AuNCs by means of electrostatic interactions in order to inhibit the release of fluorescent molecules such as Rhodamine B (RhB) loaded by AuNCs. In the presence of GSH, due to the specific cleavage of S-S bonds in disulfide-containing single-stranded DNAs (ssDNAs) as well as their subsequent departure from the surface of AuNCs, the pores could be opened, and then the dye molecules would be released from AuNCs. The concentration of GSH ranged from $1.0 \times 10^{-12}$ to $6.0 \times 10^{-10}$ M could be detected. The developed amplification strategy based on the controlled-release of fluorescent molecules reached an extraordinary sensitivity of GSH. A detection limit of as low as $4.8 \times 10^{-13}$ M with an excellent selectivity toward GSH could be achieved. The results of fluorescence microscopy imaging of GSH in living cells indicate that the fabricated system is an efficient controlled-release biosensor in response to intracellular target molecules and predict its potential use for *in situ* molecular imaging in living systems.

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## 1. Introduction

Gold nanostructures of different sizes, shapes and composition provide a multifaceted, functional platform can be widely used in bio-medical and imaging field [1]. Compared with other potential nanomaterials used in sensing protocols, Au nanocages (AuNCs) are getting more and more attention due to their significantly physical and unique chemical properties, excellent bio-compatibility and bio-inert nature in bio-medical applications [2,3]. Thanks to their hollow interiors, porous walls, and highly tunable localized surface plasmon resonance (LSPR) into the near infrared (NIR) region ranging from 700 to 900 nm, AuNCs as an attractive platform for optical imaging applications can be widely used in the field of cancer diagnostics [4] and treatment [5]. In addition to the above properties, AuNCs still have many superiorities, such as a compact size, extraordinarily large scattering and absorption cross-sections (almost five orders of magnitude greater than those of conventional organic dyes), low cytotoxicity, excellent bio-compatibility, as well as a robust and straightforward procedure for surface modification using the well-established thiolate-Au chemistry [6–8]. For *in vivo* applications, these significant features of AuNCs can make them as a noticeable material for biomedical applications including imaging [9], diagnosis [10] and treatment [11]. In order to meet both early diagnostic and therapeutic requirements of cancer, it is necessary to develop a composite system that combines all the features of AuNCs.

Recently, AuNCs have been received intensive research as a novel class of controlled-release delivery vehicles by taking advantage of their hollow interiors, porous walls, as well as significant bio-compatibility such as non toxicity and non immunogenicity [12]. It can be functionalized with bio-chemical or supramolecular ensembles than acted as “molecular gates” for the development of stimuli-responsive systems able to release guest molecules due to the presence of certain and well defined stimuli. For example, phase-change material was used as the medium to help load the drug, which can also serve as a “gate-keeper” to control the release of drug in response to temperature up [13,14]. Different external stimuli such as pH or temperature can be used to trigger and regulate the release of filler. In addition, it is possible to vary the power or the duration of exposure to high-intensity focused ultrasound (HIFU) to control the release [15].

Relative to the controlled-release systems triggered by external stimuli, those not requiring any external stimuli can provide more benefits and advantages, including simplicity and practicality, especially *in vivo* biomedical applications. In recent years, our group has proposed a number of controlled-release systems based on AuNCs [16,17], which not requiring any external stimuli and showing promising performance in the field of controlled-release systems. In our reported systems, the controlled release of the guest molecules from the gated AuNCs could be achieved successfully by the challenge with the target biological molecules rather than external stimuli such as pH, temperature, NIR laser irradiation or high-intensity focused ultrasound, and so on. Despite these advances, it is still a challenge to develop more simple and efficient controlled-release AuNCs systems. In present study, we focus on the design and assembly of molecular gates by selecting glutathione (GSH) as a target molecule.

GSH and its oxidized glutathione disulfide (GSSG) as the molecules containing sulfur are known to be closely related with various physiological activities. In particular, GSH as the most abundant cellar thiol is critical for combating oxidative stress and maintaining redox homeostasis, this is because GSH exists in redox equilibrium between sulfhydryl (reduced form GSH) and disulfide (oxidized form GSSG) forms [18]. Recent studies have also shown that the changes in physiological concentrations of GSH are related to a variety of diseases. For example, Alzheimer's disease [19], Parkinson's disease [20–22], arthritis [23], diabetes mellitus [24,25], atherosclerosis [26], epilepsy [27], aging [28] as well as numerous types of cancers. In view of the close relationship between the abnormal concentration of GSH and these diseases development in body fluids, many methods for quantitative detection of GSH have been greatly developed in recent years [29,30], such as electrochemical methods [31], colorimetric methods [32,33], quantum dots-based fluorescence methods [34], and mass spectroscopy [35]. Since GSH can be oxidized, the detection of GSH based on redox reaction has been proposed. In most cases, the processing method requires sophisticated instruments, complicated operation, but with low detection accuracy. To solve these problems, a novel controlled-release biosensor based on AuNCs was designed for accurate and convenient detection of GSH by coupling the controlled-release of cargo molecules from gated AuNCs and the cleavage of S-S bonds in disulfide-containing DNAs by GSH.

Herein, a functional nucleic acid, that is a designed disulfide-bond-modified single-stranded DNA (ssDNA) recognition probe specific for GSH was used as a pore blocker to make AuNC a controllable release system. Based on the design, a novel fluorescent biosensor for GSH was constructed by filling the hollow interiors of AuNCs with fluorescent molecules such as Rhodamine-B (RhB). To the best of our knowledge, a controlled-release biosensor based on signal amplification by the RhB-loaded AuNCs caped with GSH-specific recognition probes and its application in living cells have not yet been reported. Owing to the distinct advantages such as simplicity in molecular gate design and biosensor fabrication, cost-effective and high sensitivity, it thus holds a huge potential for the development of ultra-sensitive bio-sensing platform both *in vitro* and *in vivo* biomedical applications and has a promising future in the fields of cancer theranostics including imaging, diagnosis, drug delivery, therapy and so on.

## 2. Experimental

### 2.1. Materials

Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), polyvinylpyrrolidone (PVP, average Mr ≈ 55,000), silver nitrate ($AgNO_3$), ethylene glycol, sodium chloride (NaCl), poly diallyldimethylammonium chloride (Pdda), N-ethylmaleimide (NEM), hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) were purchased from Sigma-Aldrich (Shanghai, China) and used without further purification. The oligonucleotides used for the detection of GSH were synthesized and purified by Sangon Biotech Co. Ltd. (Shanghai, China), their sequences are as follows: 5′-ACG AGT CA S-S TTT TGT TTG TTC CCC CCT TCT TTC TTA-3' (S1, recognized by GSH), the random DNA, 5′-ACA AGC GCC AAC CCA GAG ACC GAG GAG G-3' (S2, not recognized by GSH). Rhodamine B (RhB) was obtained from Shanghai Aladdin Chemistry Corp (China). Glutathione (GSH), sodium hydrosulfide (NaSH), mercaptohexanol (MCH), thioglycolic acid (TA), cysteine (CYS) and dithiothreitol (DTT) were obtained from Sigma-Aldrich. Silver nanocubes were prepared according to literature procedures [36]. Hela cells were obtained from Chinese Academy of Medical Sciences and Ocean University of China. The 0.01 M PBS buffer (pH 7.4) was prepared by standard methods. Deionized and doubly distilled water was used throughout the experiments.

### 2.2. Apparatus

Magnetic nanoparticles (MNPs) modified with sulfhydryl groups (3.0–4.0 μm, 10 mg $mL^{-1}$) (SH-MNPs) and a magnetic rack were obtained from BaseLine Chrom Tech Research Centre (Tianjin,

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