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# Highly selective and sensitive surface enhanced Raman scattering nanosensors for detection of hydrogen peroxide in living cells

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ABSTRACT

Determination of hydrogen peroxide ($H_2O_2$) with high sensitivity and selectivity in living cells is a challenge for evaluating the diverse roles of $H_2O_2$ in the physiological and pathological processes. In this work, we present novel surface enhanced Raman scattering (SERS) nanosensors, 4-carboxyphenylboronic acid (4-CA) modified gold nanoparticles (Au NPs/4-CA), for sensing $H_2O_2$ in living cells. The nanosensors are based on that the $H_2O_2$-triggered oxidation reaction with the arylboronate on Au NPs would liberate the phenol, thus causing changes of the SERS spectra of the nanosensors. The results show the nanosensors feature higher selectivity for $H_2O_2$ over other reactive oxygen species, abundant competing cellular thiols and biologically relevant species, as well as excellent sensitivity with a low detection limit of 80 nM, which fulfills the requirements for detection of $H_2O_2$ in a biological system. In addition, the SERS nanosensors exhibit long term stability against time and pH, and high biocompatibility. More importantly, the presented nanosensors can be successfully used for monitoring changes of $H_2O_2$ levels within living biological samples upon oxidative stress, which opens up new opportunities to study its cellular biochemistry.

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## 1. Introduction

Hydrogen peroxide ($H_2O_2$), one of the major reactive oxygen species (ROS) in living organisms, has diverse physiological and pathological consequences (Marco et al., 2007). Emerging evidence supports a physiological role for $H_2O_2$ as a second messenger in cellular signal transduction (Reth, 2002). However, over accumulation of $H_2O_2$ causes oxidative stress which can induce damage to proteins, DNA, and lipids, and is associated with aging and severe human diseases including cardiovascular disorders, cancer, and neurodegenerative diseases (Toren and Nikki, 2000; Imlay et al., 1988). Therefore, a selective and accurate method to measure $H_2O_2$ is useful for underlying molecular mechanisms and elucidating the biological roles of $H_2O_2$.

Over the past decades, several elegant methods have been developed for detection of $H_2O_2$, such as electron spin resonance (ESR) spectroscopy (Tetsuya et al., 2003), chemiluminescence (Hu et al., 2008), electrochemical sensing (Kafi et al., 2008) and chromatography (Toshimasa et al., 2003). Nevertheless, these methods often cause destruction of tissues or cells, which is generally conflicting with the in-situ monitoring of $H_2O_2$ in live biological samples (Lin et al., 2013; Xuan et al., 2012). By contrast, molecular fluorescence imaging through staining with a $H_2O_2$-responsive fluorescent indicator offers an attractive approach for the in vivo detection of $H_2O_2$ owing to its high sensitivity and aptness for living cells (Lippert et al., 2011; Van de Bittner et al., 2010; Lee et al., 2007; Karton-Lifshin et al., 2011). However, the live-cell fluorescence imaging is usually limited severely by the photobleaching and phototoxicity induced by the excitation light (Magidson and Khodjakov, 2013).

Surface enhanced Raman scattering (SERS), which can provide molecular vibrational information of the analytes, has been proved to be a powerful detection technique and extensively applied in various fields such as diagnosis, biosensing (Lee et al., 2014; Ye et al., 2014; Dong et al., 2015). The typical application of SERS is the direct probing of organic molecular systems which attached to metallic SERS substrates and had high Raman cross-sections (Li et al., 2010, 2011). In this scenario, the use of SERS for the direct sensing of inorganic species hit a snag due to their small Raman scattering cross-sections. To overcome this limitation, novel SERS nanosensors that combine metallic nanoparticles and specific organic Raman reporter molecules with both high Raman cross-section and recognizable ability to inorganic species have been designed (Wang et al., 2013; Yin et al., 2011; Zamarion et al., 2008). Especially, SERS signals of the Raman reporter molecule vary depending on the concentrations of the target inorganic species, which is the ideal criterion to determine the target inorganic species. For example, mobile and biocompatible pH nanosensors

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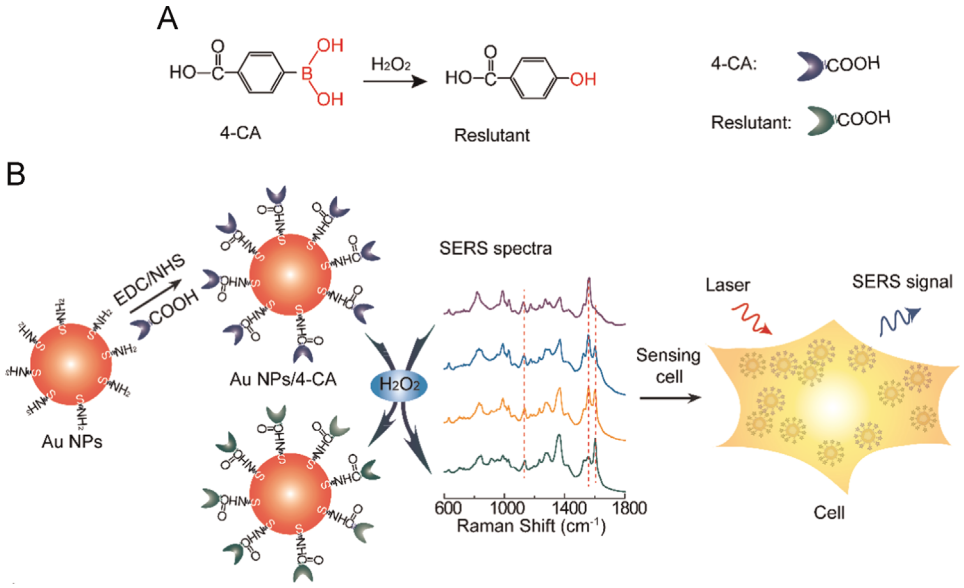


**Scheme 1.** Illustration of the Au NPs/4-CA nanosensors for sensing $H_2O_2$ in living cells.

have been designed by modifying 4-mercaptobenzoic acid on gold nanoaggregates (Kneipp et al., 2007; Kennedy et al., 2009). Redox potential SERS nanosensors have been developed by decorating a noble-metal nanoshell with redox-responsive small molecules (Auchinvole et al., 2012; Thomson et al., 2015). Also, we have fabricated a novel SERS nanosensor by functionalizing gold nanoparticles with oxidized cytochrome c for the selective and sensitive monitoring of intracellular superoxide anion radical (Qu et al., 2013). However, to the best of our knowledge, SERS nanosensors for selective and sensitive detection of the $H_2O_2$ have not yet been reported.

With the idea of developing SERS as a new approach for rapid determination of $H_2O_2$ concentration under physiological conditions in mind, we embarked on a search to find a selective chemosensing agent for $H_2O_2$. Recent researches show that arylboronates could be oxidized by $H_2O_2$ selectively to provide phenols under mild conditions (Weinstain et al., 2014; Sun et al., 2013; Miller et al., 2005). Based on this reaction, we fabricated SERS nanosensors by modifying Au NPs with 4-CA (Au NPs/4-CA) for the real-time determination of $H_2O_2$ in living cells (Scheme 1). The Au NPs/4-CA nanosensors can rapidly and selectively respond to $H_2O_2$ in physiological solutions with tens of nanomolar sensitivity, and can be used for real-time SERS detection of $H_2O_2$ in living cells, demonstrating their practical functionality in complex biological systems.

## 2. Experimental

### 2.1. Reagents and materials

All reagents were of analytical grade and used without further purification. Hydrogen peroxide ($H_2O_2$, 30 wt%), sodium hypochlorite ((NaClO), ferrous ammonium sulfate ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$) and sodium chloride (NaCl) were bought from Aladdin Chemical Company (Shanghai, China). 4-Carboxyphenylboronic acid (4-CA, 97%), $HAuCl_4 \cdot 3H_2O$, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), tert-butyl hydroperoxide (t-BuOOH), pyrogallic acid, phorbol myristate acetate (PMA), glutathione (GSH), tris(hydroxymethyl)aminomethane (Tris), L-cysteine (L-cys), S-nitroso-N-acetyl-dl-penicillamine (SNAP, the source of NO), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS) were obtained from Sigma-Aldrich (St. Louis, MO). All reactions using air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry $N_2$. Deionized water (18 MΩ $cm^{-1}$) used throughout the work was obtained from a Water Pro water purification system (Billerica, MA, USA). HeLa and normal human liver cells were purchased from the Chinese Academy of Sciences in Shanghai originally from American Type Culture Collection (Manassas, VA, USA).

### 2.2. Instruments

Dynamic light scattering (DLS) measurements were performed using a Zetasizer Nano ZS (Malvern Instruments, Southborough, UK). Transmission electron microscopy (TEM) images of Au NPs were acquired from a JEM-1011 electron microscope (JEOL, Japan) with an accelerating voltage of 100 kV. HPLC–MS was implemented on a Hewlett Packard Series 1100 HPLC (column: Agilent Prep-C18, 5 μm, 4.6 × 250 mm). All pH measurements were performed using a pH-3c digital pH meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. An inverted microscope (Ti U, Nikon, Japan) with an external triple channel optical system was equipped with a 40 × plan fluor objective (N.A. 0.75, Nikon, Japan). The use of dark-field condenser (N.A. 0.80–0.95, Nikon, Japan) with 100 W halogen tungsten lamp enables excitation light scattering from NPs. In the front of a filter turret (TI-FLC Epi-FL, Nikon, Japan), a 785 nm laser was used for Raman excitation. The spectrograph with a resolution of 2 $cm^{-1}$ was equipped with a back-illuminated deep depletion CCD (PIXIS 400BR, Princeton Instruments, USA) which measures Raman spectra from 400 to 1800 $cm^{-1}$ with a high sensitivity and fast acquisition rate. An automation controller system (MAC 6000, Ludl Electronic Products, Ltd., USA) was coupled to the fine adjustment knob of the microscope to precisely control the stage position in the *z*-direction with a resolution of 0.1 μm.

### 2.3. Fabrication of Au NPs/4-CA

Au NPs were prepared by a modified citrate reduction method (Lee and Meisel, 1982). All the glass wares were immersed into aqua regia (HCl: $HNO_3$ in a ratio of 3:1) over night and washed with plenty of deionized water for at least 5 min. 4.8 mL of

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