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# Quantitative and ultrasensitive detection of multiplex cardiac biomarkers in lateral flow assay with core-shell SERS nanotags

Di Zhang[a,b,c], Li Huang[a,b,d], Bing Liu[a,b], Haibin Ni[a,b], Liangdong Sun[a,b], Enben Su[d], Hongyuan Chen[e], Zhongze Gu[a,b], Xiangwei Zhao[a,b,c,*]

[a] State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China
[b] National Demonstration Center for Experimental Biomedical Engineering Education, Southeast University, Nanjing 210096, China
[c] Key Laboratory of Environmental Medicine Engineering of Ministry of Education, Southeast University, Nanjing 210009, China
[d] Getein Biotechnology Co., Ltd., Nanjing 210000, China
[e] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

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ABSTRACT

Rapid and sensitive quantification of multiplex proteins in a wide concentration range is challenging in high throughput analysis. Herein, we proposed a lateral flow assay (LFA) based on core-shell surface enhanced Raman scattering (SERS) nanotags for multiplex and quantitative detection of cardiac biomarkers for the early diagnosis of acute myocardial infarction (AMI). In practice, Raman dyes (RDs) were embedded into the interior-gap of silver core and gold shell nanoparticles (NPs) to form SERS nanotags as labels instead of gold colloids and three test lines were employed in the strip for the detection of three cardiac biomarkers, Myo, cTnI, and CK-MB, respectively. Due to the amplified signal of the SERS nanotags and the high surface area to volume ratio (SVR) of porous nitrocellulose (NC) membrane, ultrasensitive quantification of protein markers with wide linear dynamic range (LDR) was realized, which is crucial for the quick detection of multiplex biomarkers in the same sample without pretreatments at bedsides. This method makes it possible for LFA in point of care testing (POCT) to be comparable with chemiluminescence immunoassay (CLIA) used in labs.

## 1. Introduction

Taking into account individual variability in genes, environment, and lifestyle for each person, precision medicine (PM) will revolutionize the disease prevention and treatment by big data collected from over millions of patients. It is evident that PM highly depends on how well the individual health condition can be interrogated and monitored and how much information could be obtained. Therefore, point of care testing (POCT) technologies, aiming to the rapid and facile health information acquirement almost anywhere in a cost-effective way, becomes one focus of current health monitoring. In consequence, new POCT techniques with much higher accuracy and throughput become one direction of future development (Parolo and Merkoci, 2013; Xia et al., 2016; Jung et al., 2015; Yetisen et al., 2013).

Lateral flow assay (LFA) based on immunostrip and gold colloids is the most widely used POCT technology owing to its simple operation, rapid detection and robustness in various applications (Zhu et al., 2011; Song et al., 2014; Lee et al., 2016; Chen et al., 2016; Zarei, 2017). However, intrinsically the sensitivity and quantification ability of LFA are limited due to the colorimetric signal readout. Recently, quantitative LFAs utilizing fluorescence, Raman or magnetic labels instead of gold colloids attracted much more attentions (Taranova et al., 2015; Hu et al., 2016; Xu et al., 2009, 2014; Wang et al., 2017b; Fu et al., 2016). But fluorescence labels are amenable to quenching and bleaching and usually suffer from the background of the strips. In addition, the sensitivity of fluorescence and magnetic labels still need to be improved. On the other hand, more and more analytes are needed to be detected in LFA and the multiplex assays integrated on a single immunostrip will apparently increase the information throughput and reduce the time and cost, which will improve the accuracy of health monitoring (Wang et al., 2017a). For example, multiplex cardiac biomarkers like myoglobin (Myo), cardiac troponin I (cTnI), and creatine kinase-MB isoenzymes (CK-MB) are needed to be detected for the quick diagnosis of acute myocardial infarction (AMI), one of the leading causes of death and the most immediately life-threatening problem in the world (Rezaei and Ranjbar, 2017). Quantitative and multiplex detection of the three biomarkers with rapid LFA will greatly leverage the AMI monitoring level and rescues lives more efficiently (Ryu et al., 2011). However, the

* Corresponding author at: State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China.
*E-mail address:* xwzhao@seu.edu.cn (X. Zhao).

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normal concentration of cTnI is 0.01 ng mL$^{-1}$ and will increase to 30 ng mL$^{-1}$ after AMI (Apple et al., 2009; Jaffe and Apple, 2012). For CK-MB, the concentration is 1 ng mL$^{-1}$ and increase 5 – 20 times when AMI occurs (Lai et al., 2016; Qureshi et al., 2012). For Myo, the concentration is 50 – 100 ng mL$^{-1}$ and increases 10 – 500 times in AMI (Sallach et al., 2004). The concentration of three markers spans 6 orders of magnitude from pg mL$^{-1}$ to sub μg mL$^{-1}$. Therefore, for the quantitative analysis of multiplex analytes with concentrations in a wide range covering orders of magnitude, an ultrasensitive LFA method with wide linear dynamic range (LDR) is in great demand.

In recent years, SERS nanotags were proved to be good candidates as labels, in which the signal of Raman Dyes (RDs) with large Raman scattering cross sections is enhanced by plasmonic nanoparticles (NPs) of gold or silver (Wall et al., 2017; Yang et al., 2017). In comparison with fluorescence, SERS signals coming from the vibration and rotation of dye molecules are much more stable and single nanotag may be detected due to the high enhancement factor of rationally designed plasmonic nanostructures (Lim et al., 2011; Liu et al., 2017). Herein, we construct a core-shell SERS nanotag-based multiplex LFA (SERS LFA) for rapid and quantitative detection of three cardiac biomarkers. As illustrated in Fig. 1, silver-gold core-shell bimetallic nanotags with Nile blue A (NBA) encapsulated in the interior-gap between the two metals ($Ag^{NBA}$@Au) are used instead of gold colloids in the conjugate pad. Detection antibodies for three biomarkers are conjugated with SERS nanotags respectively and three test lines are fabricated on the nitrocellulose (NC) membrane for multiplex detection. After the flow of sample from sample pad to absorption pad, Raman signals of three test lines are measured for the quantification of cardiac biomarkers. Owing to the high surface area to volume ratio (SVR) of porous NC membrane and strong signal of SERS nanotags, an ultrasensitive LFA with a wide LDR is realized. To the best of our knowledge, this is the first report of simultaneous quantitative detection of three different biomarkers using one SERS LFA strip.

## 2. Material and methods

### 2.1. Materials and chemicals

Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), tetrachloroaurate (III) trihydrate ($HAuCl_4{\cdot}3H_2O$), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$), trisodium citrate ($Na_3C_6H_5O_7$), bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Thiolated-carboxylated PEG (HS-PEG-COOH, MW ~5 kDa) was commercially available from Laysan Bio, Inc. (USA). Nile blue A (NBA), sucrose, tween-20, ethyl dimethylaminopropyl carbodiimide (EDC) and sulfo-N-hydroxysuccinimide (NHS) were received from Alfa Aesar. The nitrocellulose (NC) membrane was purchased from Whatman-GE Healthcare Company (UK). Sample pad and gold conjugate pad were purchased from PALL Corporation (USA). Absorption pad and backing pad were purchased from Shanghai Goldbio Tech Co., Ltd (China). Myo, cTnI, CK-MB, a pair of monclonal antibodies against Myo, a pair of monclonal antibodies against cTnI, a pair of monclonal antibodies against CK-MB and anti-mouse IgG antibody produced in goat were provided by GeteinBiotech (China). Phosphate buffer (0.05 M, pH 7.0) was prepared in-house. Clinical serum samples were obtained from Nanjing First Hospital, approved by its Institutional Ethics Committee. All water was distilled and subsequently purified to Millipore Milli-Q quality. Glassware was cleaned in a bath of freshly prepared aqua regia solution ($HCl/HNO_3$, 3:1) then rinsed thoroughly with $H_2O$ before use.

### 2.2. Instruments

The SERS nanotags were characterized by transmission electron microscopy (TEM; JEOL, JEM-2100). The UV–vis adsorption spectra were recorded using a Hitachi 5000 UV/Vis/NIR spectrophotometer.

Raman spectra of SERS nanotag and test lines of the SERS LFA strip as well as Raman mapping images were acquired using an In Via Renishaw Raman microscope system (Renishaw, New Mills, UK). The different lasers used were 532 nm, 633 nm, and 785 nm. Baseline correction of each Raman spectrum was performed using Renishaw Wire 4.2 software, and the baseline was corrected as zero. In this work, a 50× objective lens with the numerical aperture of 0.75 was used. For $Ag^{NBA}$@Au NPs, 10 μL of NPs was transferred to a capillary tube, the Raman spectra were measured by focusing a laser spot on the tube. The acquisition time was 10 s. The characteristic Raman shift of NBA is at 592 cm$^{-1}$. Raman spectra and Raman mapping images for the test lines of SERS LFA strips were measured using 785 nm laser and integration time was 1 s. A holographic notch filter was placed in the collection path to remove the Rayleigh line from the collected Raman data. The spectral resolution can reach 1 cm$^{-1}$. A computer-controlled x-y translational stage was scanned in 10 μm × 10 μm steps over a 200 μm (x axis) and 200 μm (y axis) range (total 441 pixels). The data acquisition time at each pixel is 1 s, and the total image acquisition time is about 7 min. For real sample comparative experiment, Myo detection

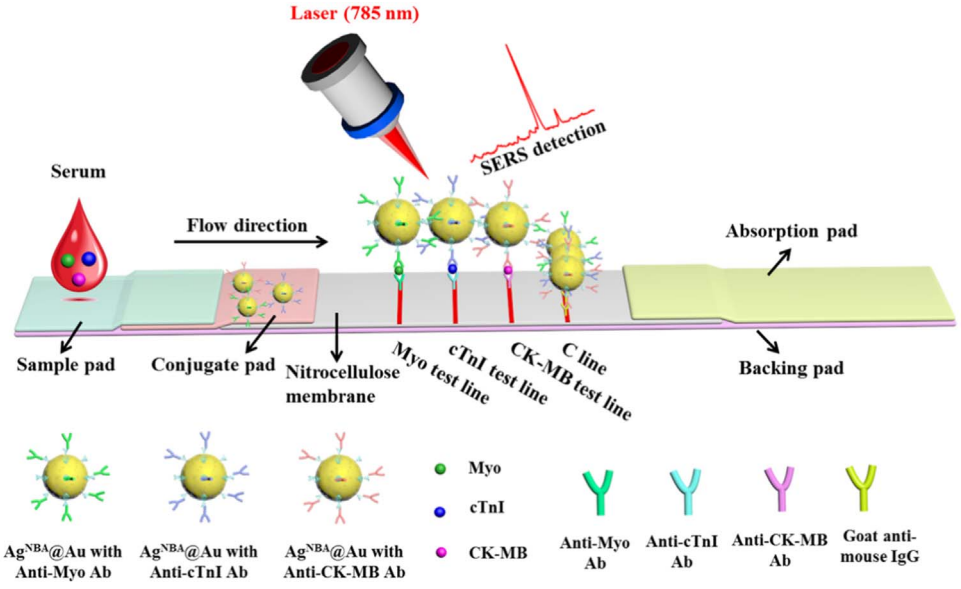


**Fig. 1.** Schematic illustration of the core-shell SERS nanotag-based multiplex LFA.