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para-Sulfonatocalix[4]arene stabilized gold nanoparticles multilayers interfaced to electrodes through host-guest interaction for sensitive ErbB2 detection

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ABSTRACT

Nanoparticle (NP) structure, compositing and the nature of the NP-functionalized electrode interface have a strong influence upon electrochemical properties that are critical in applications such as sensing, photocatalysis and electrocatalysis. Existing methods to fabricate NP-functionalized electrodes do not allow or precise control over all these variables, especially the NP-electrode interface, making it difficult to understand and predict how structural changes influence electrode activity and consequently limit the application. To conquer this problem, in this study, we fabricated a stepwise construction of a novel supramolecular stabilized gold nanoparticles (AuNPs) multilayer mediated by guest molecules, yielding 3D AuNPs assembly at the electrode interface. *para*-Sulfonatocalix[4]arene (pSC_4), a water soluble macrocyclic synthetic receptor, has been served as a stabilizing ligand for preparation and gaining new insights into pSC_4 stabilized gold nanoparticles (pSC_4-AuNPs) tethered on the electrode interface through host-guest interaction. We investigated the electrochemical properties of multilayer pSC_4-AuNPs modified gold electrode using different core size of AuNPs with varying layer number. The electron transfer ability was characterized by electrochemical impedance spectroscopy (EIS). Electrochemical signals are significantly enhanced through the layer-by-layer assembly of pSC_4-AuNPs due to its high conductivity and high effective area. With this innovative method, by taking the assay of a tumor marker as an example, human epidermal growth factor receptor 2 (ErbB2) was successfully measured with a detection limit of 0.5 ng/mL. Taking the advantage of the pSC_4-AuNPs multilayer's good biocompatibility, high effective area and high electronic transmission, 3D AuNPs multilayer produced on the electrode interface suggests a portable synthetic pathway for the application into sensitive electrochemical biosensor.

1. Introduction

Gold nanoparticles (AuNPs) has important effect in the fields of nanotechnology and nanomaterials on account of their importance in biosensors (Siwy et al., 2005), nanoelectronics (Hassenkam et al., 2004; Zheng et al., 2011; He et al., 2017), catalysis (Chen and Goodman, 2008) and so on. Their good biocompatibility, high chemical stability, surface functionalization, and facile synthesis make them considerable building blocks for novel hybrid nanomaterials (Ofir et al., 2008; Klajn et al., 2010). Macrocycles, such as cyclodextrins, cucurbiturils, and calixarenes still remain major to supramolecular chemistry (Dsouza et al., 2011; Yang, 2011; Sun et al., 2012a). Lately, progress has been created in the synthesis and assembly of metal nanoparticles capped with cyclodextrins (Liu et al., 1999; Heo et al., 2012), cucurbiturils (Lee and Scherman, 2010, 2012), and calixarenes (Wei, 2006). Construction of AuNPs and supramolecular macrocycles expressively combines and enhances the features of the two entities (Crespo-Biel et al., 2005; Li et al., 2013), for instance the thermal, electronic, and catalytic properties of AuNPs and molecular recognition of the macrocyclic hosts, extending potential applications as biosensors (Sun et al., 2012b; Liu et al., 2005; Kim et al., 2010). Among them, several colorimetric and fluorescence sensors which combine the

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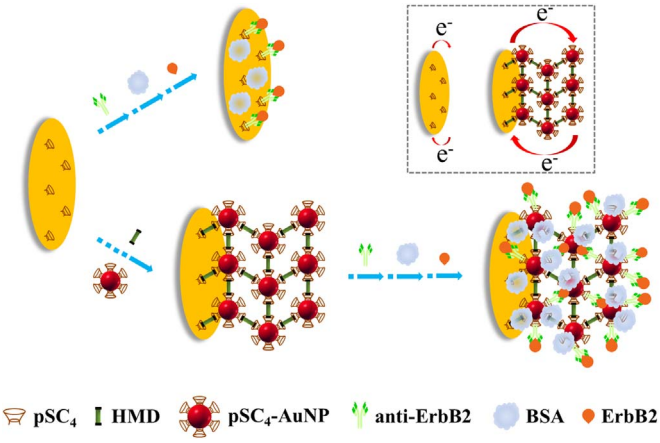
recognition function of supramolecular and SPR of AuNPs were reported recently (Lazar et al., 2016; Guo et al., 2013). However, these methods are suffering the higher detection limit and cannot be applied to the sensitive clinical requirement. Applying supramolecules modified AuNPs as sensors' interface can solve this problem. As an alternative technique to photometry, electrochemical approach potentially provides a more attractive method for biomarker assessment (De Leener et al., 2016). Electrochemical detection of noble metal ions (Wei et al., 2015), small bioactive molecules (Nguyen et al., 2010), DNA (Chakraborty et al., 2015), enzymes (Xianyu et al., 2015) and cancer marker proteins (Lu et al., 2015) have been reported with ultrahigh specificity and sensitivity. Electrochemical method also cost efficient and easier to miniaturize in a compact device, so it has received great interests (McWilliams et al., 2015).

Recently, the principle of nanoparticle-functionalized electrode has been reported to enhance the electrochemical properties that are critical in applications such as amperometric sensing, photocatalysis and electrocatalysis (Young et al., 2016). Unfortunately, disordered arrangement of NP on electrode has restricted enhancement of electrochemical properties. It cannot still get a stabilized functionalized film and make a slight signal enhancement. Young et al. (2016) found the molecular tethered AuNP-electrode interface results in more efficient electron transfer than solution deposited samples. Therefore, in comparison with one layer molecular tethered AuNP electrode interface, self-assembled multi-AuNP layers can be adapted to fabricate more efficient electrochemical activity interface in several aspects. First, conductivity in 3D assemblies of AuNPs can increase comparing with 2D AuNP. Wessels et al. characterize the optical and electrical properties of three-dimensional interlinked AuNPs assemblies (Wessels et al., 2004; Midtvedt et al., 2016). They present linker molecules that enable tuning of the conductivity in 3D assemblies of AuNPs from the semiconducting to the metallic limit by varying the degree of conjugation of the linker molecule and the nature of its metal binding groups. Second, 3D assemblies of AuNPs can be constructed through the self-assembled NP multilayers based on host-guest interaction. Crespo-Biel et al. developed the stepwise construction of a self-assembled organic/inorganic multilayers based on multivalent supramolecular interactions between guest-functionalized dendrimers and host-modified AuNPs, yielding a controlled supramolecualr layer-by-layer assembly (Crespo-Biel et al., 2005; Lu et al., 2016). Third, calixarene derivatives have been confirmed as proteins' linker by binding the amine groups of proteins' through several noncovalent soft bindings (Chen et al., 2010). Additionally, increasing amounts of AuNPs are gathered onto the electrode surface, which significantly amplifies the amount of probe proteins. On the basis of the four aforementioned aspects, a general method is first put forward for convert self-assemble multilayer of supramolecular-AuNP into a more sensitive surface-tethered electrochemical analysis interface.

Here, we represent the stepwise construction of a novel kind of self-assembled supramolecular functionalized AuNPs (pSC_4-AuNPs) multilayers based on supramolecular host-guest interactions between guest molecules and host-modified nanoparticles. Such protocols can potentially be used for obtaining particular structures, whose assembly is determined by multiple supramolecular interactions (Li et al., 2013). The ultimate tridimensional control, when combined with top-down surface patterning strategies such as monomolecular film for surface control, can lead to 3D nanofabrication schemes.

Human epidermal growth factor receptor 2 (ErbB2 or HER2) is a tyrosine kinase receptor vesting in the epidermal growth factor receptor (EGFR) family and is related to cellular signaling pathways, which result in cell proliferation, growth, apoptosis and differentiation (Özcelik et al., 2002). These processes are vital to life, but loss function of control within these pathways is continually tied to several diseases, including cancer (Harari and Yarden, 2000). As the overexpression of ErbB2 is observed in some of breast cancer conditions (thus cataloged as ErbB2 positive) and therapies using a monoclonal antibody to this molecule is present in use, it is only valid in patients with excess receptor levels (Negro et al., 2006). As it is, it is requested to screen the patients before therapy to find those who are qualified for such administrations. The blood ErbB2 content of breast cancer patients is generally > 0.015 μg/mL, which demands assay methods competent to undoubtedly measure such low concentration levels in this sophisticated biological sample (Martin et al., 2006). Current diagnostic tests for ErbB2 comprise the use of immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH), which are optical semi-quantitative techniques (Jiang et al., 2008; Portier et al., 2013). Additionally, both procedures are detailed and time consuming, demand exhaustive sample preparations and desire specially trained personnel to perform the corresponding multi-step procedure (Kallioniemi et al., 1992; Ouyang et al., 1999). In this work, we have fabricated a simple and efficient, label-free and highly sensitive electrochemical method for ErbB2 detection. As presented in Scheme 1, a supramolecule *para*-sulfonatocalix[4]arene (pSC_4) with a pore size of 3.8 Å is employed in this work as the substrate (Shinkai et al., 1988). After being covalently modified onto the gold electrode surface, pSC_4 was used here to play a role of the substrate layer from forming host-guest interaction. The 1,6-hexanediamine (HMD) solution was then added to react with pSC_4 through host-guest recognition for specific binding. The HMD and pSC_4-gold nanoparticles (pSC_4-AuNPs) were added one by one to assemble a layer-by-layer thin film. Electrochemical impedance spectroscopy (EIS) was used investigated the electrochemical properties of multilayer pSC_4-AuNPs modified gold electrode using different core size of AuNPs with varying layer number. Electrochemical signals are significantly improved through the layer-by-layer assembly of pSC_4-AuNPs. Own to the pSC_4-AuNPs multilayer's good biocompatibility and high electronic transmission, ErbB2 was quantified with a concentration range from 0.001 μg/mL to 10 μg/mL. The signal amplification is a novel label-free detection concept for the design of highly sensitive analytical methods for the detection of biomarker.



Scheme 1. Schematic of pSC_4 monolayer and pSC_4-AuNPs layer-by-layer signal amplification on the electrode surface.

2. Experimental section

2.1. Materials and apparatus

Human ErbB2/HER2 protein (10004-HCCH) and its mouse monoclonal antibodies (10004-MM03) were purchased from Sino Biological (Beijing, China). Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Sigma-Aldrich, Inc. (St. Louis, USA). Sodium borohydride was purchased from Aladdin, Inc. (Shanghai, China). Bovine serum albumin, vascular endothelial growth factor and 1,6-hexanediamine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *para*-Sulfonatocalix[4]arene was purchased from TCI (Shanghai) Development Co., Ltd. (Shanghai,

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