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Plasmon-mediated fluorescence with distance independence: From model to a biosensing application

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ABSTRACT

In this article, plasmon-mediated fluorescence biosensing is reported to be distance independent through a full-coupling strategy that effectively activates the entire plasmon coupling region. This concept is demonstrated through collecting the directional surface plasmon-coupled emission (SPCE) signal from fluorescent silica nanoparticles with a size that matches the entire coupling region. Based on this design, the spatial distribution of the fluorophores is confined by the dimension of the nanoparticle. Therefore, these encapsulated fluorophores occupy the maximum coupling dominant region and optimally utilize the coupling effect. Being different from the conventional plasmon-mediated fluorescence, the enhanced fluorescence response becomes nearly independent of distance changes on a wide dynamic range from 0 nm to 30 nm between the fluorescent nanoparticles and metal structure. Full-coupling SPCE appropriately enlarges the distribution of fluorophores, ensuring that the coupling dominant region is filled with enough fluorophores at varying distances to create a stable and detectable signal. This scale of distances is well suited for many biorecognition events. Full-coupling SPCE solves signal deviation challenges originating from the susceptible and unpredictable orientation and conformation of biomolecules on the nanoscale. Immunoassays and DNA detection are shown with high and reliable signals, demonstrating the advantages of distance-independent full coupling. Without the need of a complicated and rigorous architecture for precise distance control, full-coupling SPCE offers great promise for a general platform of chip-based biosensing and bioanalysis.

1. Introduction

Fluorescence technology has played an important and indispensable role in bioanalysis, biomedical research, and clinical testing. Efforts that increase the sensitivity and feasibility of fluorescence-based assays are therefore of great significance. The near-field interaction of fluorophores with metallic nanostructures can strongly enhance fluorescence through either localized or propagating surface plasmon resonance (Lakowicz, 2005). The former is usually related to metallic nanoparticles that are much smaller than the optical wavelengths (Mayer and Hafner, 2011; Golberg et al., 2011; Deng and Goldys, 2012). The latter can be realized on a metallic nanofilm with appropriate optical arrangements to fulfill the momentum-matching conditions between the light and the surface plasmons (Neumann et al., 2002; Gryczynski et al., 2004a; Hung et al., 2006). One efficient way to realize this interaction is surface plasmon-coupled emission (SPCE) (Lakowicz, 2004; Aslan and Geddes, 2009; Cao et al., 2012), which is usually carried out using a high refractive index prism attached to the thin metal film. The fluorescence signal coupling Surface plasmons radiates through the prism as p-polarization at a defined emission angle (Gryczynski et al., 2004b; Smith et al., 2007; Jankowski et al., 2010). SPCE has been used as a novel biosensing technique in biomolecular detection (Matveeva et al., 2004; Borejdo et al., 2006; Xie et al., 2009; Yuk et al., 2011; Yuk et al., 2012; Rice et al., 2012; Jin et al., 2012).

From a conventional viewpoint, both localized and propagating surface plasmon effects on coupling fluorophores are distance-dependent (Ray et al., 2007a; Chu et al., 2007; Wilson and Nicolau, 2011). When a fluorophore is located in very close proximity to a metallic nanostructure, quenching is dominant, whereas at large separation distances, spontaneous fluorescence is observed. Effective coupling enhancement is thus squeezed into the middle area between these two distance regions (Lakowicz, 2004). These characteristics are important to the two main pathways that have been developed recently in the plasmophore-based sensing field. One is based on a conformation-switched "molecular beacon" that causes the signal to change from quenching to coupling (Xie et al., 2009; Cheng et al.,

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2011; Peng et al., 2009). This is a promising approach for bioanalysis, though needs additional developments in molecular design. The second area is signal enhancement from binding of fluorophore-labeled biomolecules captured on the surface including classical immunoassay and DNA detection (Touahira et al., 2010; Zhou et al., 2012). Here, effective use of the coupling effect is critical and has attracted considerable attention. This article will focus on the latter.

To establish optimal conditions for strong coupling, numerous efforts have been made to study the behaviors of distance-dependent coupling through various spacing layer controls, including Langmuir–Blodgett films, polymer, SiO_2, and biomolecules (Ray et al., 2007b; Chi et al., 2008; Chan et al., 2009; Guerrero and Aroca, 2011; Yuk et al., 2010), but some intractable problems remain. First, in most of the previous work, the fluorophores were restricted at one optimized distance that is actually dependent on each specific sensing system. Such strict dye-to-metal distance requirements were an awkward compromise among the quenching, coupling, and spontaneous emission conditions (Larmour and Graham, 2011). Consequently, this approach seems to be unsuitable for generalized platforms; this delicate interfacial design must be redesigned for a new system. Second, the coupling efficiency is not constant, but varies considerably with distance at nanometer scale from the metal surface (Lakowicz et al., 2004; Cao et al., 2011). The fluorescence intensity is related not only to the quantity of fluorophores, but also to their spatial positioning, which affects the quantitative relationship between the fluorescence intensity and the number of bound molecules. In the conventional design using small fluorescent molecules as labels, the dye-to-metal distance including the dimension of the connected biomolecule, is readily varied because of the slightly altered orientations and conformations of the biomolecules. This is nearly unavoidable in practical sensing (Touahira et al., 2010; Cao et al., 2011; Yu et al., 2004a, 2004b; Murakami et al., 2012). Furthermore, the inhomogeneity and roughness of the surface also can cause distance changes. Therefore these inconsistent label-to-metal distances lead to signal deviation. Third, the coupling at a specific distance results in limited coupling effects because other distances in the coupling dominant region remain unused.

To better utilize coupling at different distances, some scientists proposed that three-dimensional materials such as dextran chains could be applied as a surface matrix (Yu et al., 2004a, 2004b). Dextran chains are spatially extended from a metal surface into the bulk medium, and their functional groups are ready for the covalent attachment of biomolecules. After bio-reaction, fluorophore-labeled biomolecules are captured along the chains at different distances from the sensor surface, thus increasing the coupling range for fluorescence detection. However, the precise distribution of bound biomolecules along the chains remains unclear, resulting in non-uniform dye-to-metal distances for each binding event. And these chains display steric hindrance that prevents the binding of large molecules (Yu et al., 2004a, b; Yang et al., 2007).

The object of this study is to meet proposed challenges and develop an ingenious approach for plasmon-mediated fluorescence biosensing, which not only can improve the utilization of the coupling effect but also can avoid distance fluctuation-induced signal deviation. Previous reports use small molecules as labels and plasmon-mediated fluorescence is distance-dependent. However, we found that the SPCE signal from a fluorophores-doped layer with a nanoscale thickness matching the coupling region on gold surface displayed a distance-independent behavior when varying the distance between the fluorescent layer and gold surface. The spatial distribution of fluorophores is appropriately enlarged to fill the coupling dominant region with fluorophores at varying distances. The coupling effect was in full use. Then we demonstrated that this distance-independent model could be translated to a biosensing application through silica nanoparticles encapsulating fluorophores as labels. Those nanoparticles were designed to have the dimension similar to thickness of model layer. It is a smart way to naturally restrict the spatial distribution of fluorophores to match the coupling region by the size of the nanoparticle. Through binding event to capture fluorescent nanoparticles on the metal surface, the fluorophores occupy the coupling region. As a result, enhanced fluorescence was always realized independently of the orientations and conformations of biomolecules in the binding interactions. We call this strategy full-coupling because the coupling effect is fully used. Without the need of a complicated and rigorous architecture for precise distance control on metal surface, the full-coupling strategy achieves the spatial control of fluorophores in labeling item in a simple and effective way. Immunoassays and DNA detection through full-coupling SPCE demonstrates the new opportunity to develop chip-based biosensing with enhanced and reliable reporting.

2. Experimental

2.1. Chemicals and materials

Human IgG, anti-human IgG, tetramethylrhodamine isothiocyanate-labeled anti-human IgG, and bovine serum albumin (BSA) were purchased from Xiamen Tagene Biotechnology Co., Ltd. Avidin was purchased from Sangon Biotech (Shanghai) Co., Ltd. DNA oligonucleotides were purchased from Takara Biotechnology (Dalian) Co., Ltd. DNA sequences include: capture DNA: 5'-CGCCT-CACAACCAAAAAA-amido-3'; 5'-CGCCTCACAACCAAAAAA-thiol-3'. Complementary target DNA: 5'-GGTTGTGAGGCGCTGCCCAAGCGA-3'; Non-complementary control: 5'-ACGCTGACTATGAGTTAAAGCTTG-3'; Detection DNA: 5'-Texas Red-AAAAAATCGCTTGGGCAG-3'; 5'-Biotin-AAAAAATCGCTTGGGCAG-3'. All the bioreagents were dissolved in 10 mM phosphate buffered saline (PBS, pH = 7.4) purchased from Sigma-Aldrich.

Tetraethyl orthosilicate and glutaric anhydride were purchased from J&K Chemical Ltd. Lissamine Rhodamine B and Triton X-100 were purchased from Aladdin Chemistry Co., Ltd. The 3-aminopropyltriethoxysiane and glutaraldehyde were purchased from Acros Organics. 2-Morpholinoethanesulfonic hydrate (MES), glycine, cysteamine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), Tris (2,2'-bipyridyl) dichlororuthenium (II), poly (sodium 4-styrenesulfonate) (PSS) and poly (allylamine hydrochloride) (PAH), Rhodamine B were purchased from Sigma-Aldrich. Polyvinyl alcohol 124 (PVA), n-hexanol, cyclohexane, ammonium hydroxide, and sodium metasilicate nonahydrate were purchased from Sinopharm Chemical Reagent Co., Ltd. All other reagents were of analytical grade and used without further purification. Ultrapure water was used throughout the study. The dyes used in the experiments have a near fluorescence emission wavelength (580–600 nm), thus ensuring the similar SPCE property.

2.2. Fabrication of fluorophores-doped nanofilm

1 mM Rhodamine B was dissolved in 0.75% (w/w), 1.5%, and 2.2% PVA solution respectively, and the spin-coating was performed at 3000 rpm for 40 s obtaining PVA films with different thicknesses to simulate the spatial distributions of fluorophores (Gryczynski et al., 2004c; Yan et al., 2011).

2.3. Preparing self-assembled multilayers to simulate distance changes

The quartz substrate was incubated in ethanol with 1% 3 aminopropyltriethoxysiane for 1 h, followed by rinsing and drying at 110 °C. The gold-coated substrate was incubated