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Metal enhanced fluorescence improved protein and DNA detection by zigzag Ag nanorod arrays

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ABSTRACT

As metal nano-arrays show great potential on metal enhanced fluorescence (MEF) than random nanostructures, MEF of Ag zigzag nanorod (ZNR) arrays made by oblique angle deposition has been studied for biomolecule-protein interaction and DNA hybridization. By changing the folding number and the deposition substrate temperature, a 14-fold enhancement factor (EF) is obtained for biotin-neutravidin detection. The optimal folding number is decided as $Z=7$, owing to the high scattering intensity of Ag ZNRs. The substrate temperature $T=25$ °C and 0 °C slightly alters the morphology of Ag ZNRs but has no big difference in EF. Further, Ag ZNRs deposited on a layer of Ag film have been introduced to the DNA hybridization and a significant signal enhancement has been observed through the fluorescence microscope. Through a detailed quantitative EF analysis, which excludes the enhancing effect from the increased surface area of ZNRs and only considers the contribution of MEF, an EF of 28 is achieved for the hybridization of two single-stranded oligonucleotides with 33 bases. Furthermore, a limit of detection is determined as 0.01 pM. We believe that the Ag ZNR arrays can serve as a universal and sensitive biodetection platform.

1. Introduction

Protein and DNA detection is an important step in biological and diagnostic assays. As one effective method, fluorophore or enzyme labelling are often used for the detection, such as the enzyme-linked immunosorbent assay (ELISA) (Nishi et al., 2015) and DNA hybridization (Xiang et al., 2012). In short, the fluorescent or enzymatic molecules are covalently linked to the antibody or one end of the complimentary single-stranded DNA first. Then when the immune reaction or the hybridization occurs, fluorescence signal or the enzymatic color change can be observed. However, very weak signal is often encountered due to the small amount of samples. To obtain a detectable signal, microgels or liposomes are introduced to the target molecules, with a large amount of signaling molecules encapsulated inside or attached on the particles, (Aliberti et al., 2016; Zimmerman et al., 2010) while this process is often time-consuming and labor-intensive. Modern technologies also provide other label-free solutions to detect protein or DNA, via the electrochemical reaction, (Kerman et al., 2004) electronic signaling, (Adam and Hashim, 2016; Xiao et al., 2006) and waveguide sensor (Toccafondo et al., 2010), though complicated and delicate setup are often needed.

Metal enhanced fluorescence (MEF) will directly enhance the fluorescence (FL) intensity of the fluorophores labelled on the target molecules and can be easily observed by a conventional FL microscope. Based on these merits, MEF has been applied for the detection of virus, protein and DNA (Ahmed et al., 2014; Cao et al., 2015; Fu et al., 2015; Gu et al., 2014; Guo et al., 2014; Chang et al., 2012). MEF refers to the phenomenon where the FL signal can be greatly enhanced if the fluorophores are in the vicinity of metal nanostructures (Geddes et al., 2003; Chen et al., 2012). The mechanism is mostly attributed to localized surface plasmon resonance (LSPR), or, absorption and scattering properties of the nanostructures. (Lakowicz, 2005) In recent years, most MEF applications are performed at a solution basis, where the Au or Ag nanoparticles (NPs) are added into the analyte-contained solution and the fluorescence intensity is measured, with a typical enhancement factors (EFs) ranging from 1.53 to 14 (Gu et al., 2014; Chen et al., 2013; Abadeer et al., 2014; H.X. Zhang et al., 2015; Pang et al., 2015; Nepal et al., 2013; Chen et al., 2015; Sui et al., 2014; Ren et al., 2015; Zhao et al., 2015). In other applications, the NPs are attached to a substrate and FL intensities of analytes immobilized on NPs and a flat substrate are measured. This method can reduce the tedious washing steps but it might be difficult to do the quantitative EF analysis when dealing with the irregular NPs.

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(Yasukuni et al., 2013) Thus, the EFs are normally compared without taking the surface coverage of the analytes into account and ranged from 5.6 to 23 (Yasukuni et al., 2013; Yang et al., 2015; Zhou et al., 2015; Zhou et al., 2014; Y. Zhang et al., 2015; Blake-Hedges et al., 2015). In addition, a few works report single molecule detection with very high EFs such as 30 and 470 (Saito et al., 2011; T.S. Zhang et al., 2015). Though, this method is highly position-dependent and usually results a low average EF (Fu et al., 2015).

While most of the MEF applications are based on the random NPs distribution, a few researchers start to explore the MEF effect of ordered nanostructure arrays, since they own the merits such as better plasmonic coupling between adjacent nanostructures and more hot-spots generation in nanogaps. (Zhou et al., 2015, Zhou et al., 2012). For example, Au nanorods (NRs) array show an EF of 10 for CdSe quantum dots (Zhou et al., 2015) and Pt bowtie array has 30 fold enhancement for a single dye molecule detection. (Saito et al., 2011) Furthermore, Cu nano-arrays show 3.2 EF for TCPP fluorescence probe when compared with the glass substrate while has 89.2 EF when compared with the Cu thin film, which has the quenching effect for the fluorophores. (Sugawa et al., 2013) An even higher EF, 7400 fold, has been reported from a complex structure composed of Au film and Au NP arrays, where human immunoglobulin G has been detected through the protein A attached on the Au surface and the glass reference. (Zhou et al., 2012) Though, the authors have the concern about the different affinity of protein A on Au and glass. However, Ag nano-arrays are seldom studied in the field of MEF, though Ag in general shows higher sensitivity than other materials in plasmonic applications. To our best knowledge, only Akhlesh Lakhtakia's group and our group make Ag NR arrays by oblique angle deposition (OAD) and apply them for MEF applications. The former finds an EF of 15 when coating a layer of dyes on Ag NRs and Ag film reference, without considering the surface coverage of analytes (Abdulhalim et al., 2009) and the latter shows a qualitative enhancement from Ag/Si hetero-NRs (He et al., 2014) and an EF of 6.5 for the amino acids separation in a microfluidic capillary electrophoresis device coated with Ag NRs (Xiao et al., 2014). There is much space left for other biological analysis, such as protein and DNA detection, based on Ag nano-arrays with more plasmonic-active morphology made by OAD.

Here a further study on Ag nano-arrays' MEF property is conducted by introducing a zigzag NRs (ZNRs) structure to biomolecule-protein interaction and DNA hybridization. OAD is physical vapor deposition, where the incident material vapor and the substrate surface normal form a large deposition angle α and tilting NRs arrays grow as a consequence of shadowing effect and surface diffusing. (Robbie et al., 1998; Robbie and Brett 1997) By rotating the substrate 180° at set time, zigzag or folding NRs can be obtained, which are believed to produce more “hot-spots” in nanogaps. These Ag ZNRs are coated with biotin and then capture neutravidin decorated nanospheres. To optimize the structure, the folding number and the substrate temperature are monitored and the corresponding optical properties are measured. Then the optimized Ag ZNRs are applied for two single-stranded oligonucleotides hybridization and a quantitative EF analysis is performed. In detail, the EF is defined as $EF_{MEF}=(I_{MEF}/N_{MEF})/(I_R/N_R)$, where N_R and I_R are the molecule number and FL intensity of analytes without the presence of Ag ZNRs; and N_{MEF} and I_{MEF} are the molecule number and FL intensity of analytes attached onto Ag ZNRs. Finally, a limit of hybridization detection is determined.

2. Material and methods

2.1. Ag zigzag NRs depositions

Ag ZNRs were deposited on Si substrate with a dimension of 0.75 cm by 1.5 cm. Prior to the depositions, the substrates were sonicated in acetone, ethanol and deionized (DI) water for 15 minutes and then dried by nitrogen. A thin layer of Ti film with a thickness of 10 nm was evaporated onto Si surface at $\alpha=0°$ to enhance the adhesion between Ag NRs and Si substrates. By rotating the substrate 180° at each set growth length, Ag ZNRs with folding number $Z=1$, 3, 5 and 7 were deposited at $\alpha=85°$. The nominal thickness was the same and equal to 2 μm. The substrate temperature was monitored at $T=25$ °C and 0 °C by using a built-in cooling system. As a reference, Ag film with a thickness of 500 nm was deposited on the Si substrate at $\alpha=0°$. A quartz crystal microbalance was used to monitor the nominal thickness. The background pressure was about 8×10^{-7} Torr. Ag and Ti were both at 99.99% and were purchased from Kurt J Lesker.

2.2. MEF and optical measurements

The FL spectrum measurement was performed by Luminescence Spectrometer LS 50 B (Perkin Elmer) and the configuration has been described somewhere else (Xiao et al., 2014). Briefly, the sample is mounted onto a home-made holder and forms an incident angle of 60° with the excitation beam. The fluorescence signal is collected at a right angle with respect to the incident beam. The reflected beam is deviated and will not enter the detector. Polarized extinction spectra were measured by PerkinElmer LAMBDA 750 spectrophotometer. Scattering spectra were measured by a reflection stage (STAGE-RTL-T, Ocean Optics). They were taken by a 60° incidence and collected along a direction which forms a right angle with the incidence, same as the FL measurement configuration. The light source was a Xenon lamp and the spectrometer was Ocean Optics USB 4000. The FL images were taken by a Nikon Ni-U upright microscope and the FL intensity was analyzed by ImageJ.

2.3. Biotin-neutravidin interaction

FluoSpheres NeutrAvidin (ex. 488 nm, em. 605 nm) labelled nanospheres with a diameter of 40 nm were attached to Ag ZNRs via biotin-neutravidin interaction. First, a droplet of 100 μl HPDP-biotin of a concentration of 10^{-4} M was dropped onto Ag ZNRs and incubated overnight under room temperature. Then 100 μl bovine serum albumin (BSA) of a concentration of 5% (w/v) was used for non-specific sites and incubated overnight under 4 °C. Finally, 100 μl neutravidin coated FL nanosphere suspension of a concentration of 2.8×10^{12} particles/ml was applied to the sample surface and incubated overnight under 4 °C. After each step, the sample was rinsed by DI water and dried by nitrogen. The samples are transferred to FL spectrum measurements. HPDP-biotin and NeutrAvidin biotin-binding protein were from Thermo Fisher Scientific Inc. BSA was from Sigma.

2.4. Oligonucleotide hybridization

Two single-stranded oligonucleotides hybridize on Ag ZNRs' surface. First, a piece of Ag ZNRs sample chip was immersed into 1 ml thiol modified single-stranded oligonucleotide (molecular probe) solution at a concentration of $C_0=1.0\times10^{-7}$ M. Then the chip was incubated on the shaking bed for 30 min at RT and transferred to 4 °C overnight. After that, the chip was rinsed by DI water, dried by N_2 and immersed into 1 ml Alexa 488 labelled target oligonucleotides solution at the concentration of C_0. Again,