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Localized surface plasmon-enhanced fluorescence spectroscopy for highly-sensitive real-time detection of DNA hybridization

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ABSTRACT

Versatile and highly-sensitive detection of DNA hybridization is described using metal nanostructures-enhanced fluorescence (MEF) emission intensity when fluorescently-labeled DNA oligomers are covalently immobilized on a nanometer-thin amorphous silicon–carbon layer capping the metal nanostructures. The MEF structures are formed by thermal deposition of silver, gold or silver/gold thin films on glass surfaces and post-annealing at 500 °C. The choice of the metal film allows for tuning the optical properties of the interface. The metallic nanostructures are subsequently coated with an amorphous thin silicon–carbon alloy (a-$Si_{0.80}C_{0.20}$: H) layer deposited by PECVD. Carboxydecyl groups are attached on these surfaces through hydrosilylation then reacted with amine-terminated single-stranded DNA oligomers, forming a covalent link. The immobilized DNA is hybridized with its complementary strand carrying a fluorescent label. Through optimization of the thickness of the a-$Si_{0.80}C_{0.20}$: H alloy overlayer and by working close to resonance conditions for plasmon and fluorophore excitation, the hybridization of very dilute oligomers (5 fM) is easily detected, and the hybridization kinetics can be monitored in situ and in real-time.

1. Introduction

The last decade has witnessed a rapid growth in the development of sensors using the properties of noble metal nanoparticles (Anker et al., 2008; Haes et al., 2006; Jain et al., 2007). Especially, gold and silver nanoparticles have been the focus of extensive studies. When metal nanoparticles are excited by an electromagnetic radiation, they exhibit collective oscillation of their conducting electrons, known as localized surface plasmon resonance (LSPR). Sensors have been designed to take advantage of the high sensitivity of the LSPR frequency (usually probed through standard optical absorption measurements) to the structure and environment of the nanoparticles (Hutter and Fendler, 2004).

It is well established that metallic nanoparticles are also able to drastically change molecular fluorescence characteristics by decreasing lifetimes and increasing intensities (Fort and Grésillon, 2008; Lakowicz, 2006), as a result of the enhanced local electric field surrounding the metallic nanoparticles induced by the coupling of the incident light with localized plasmons (Kelly et al., 2003; Pack et al., 2003). The level of enhancement is a strong function of the size, shape and resonance wavelength λ_{max} of the nanoparticles, the distance between the fluorophores and the metallic nanoparticles, as well as the absorption/emission wavelength of the fluorescent molecules (Chen et al., 2007; Lakowicz et al., 2002; Pompa et al., 2006; Ray et al., 2006). Quenching is the dominant effect when the fluorophore is located closer than ~5 nm from the nanostructured metal surface (Anger et al., 2006; Aslan et al., 2005; Thomas et al., 2004). At larger distances, the enhancement starts to override the quenching and the fluorescence reaches its maximum at about 10 nm from the metallic nanoparticles, a distance above which the enhancement effect progressively decreases (Malicka et al., 2003).

A practical strategy for achieving the requested compromise in terms of fluorophore/metal distances is to use a dielectric spacer above the metal nanoparticle layer. The advantages of such an approach are multiple. First, a common drawback of systems based on nanoparticle films is the instability of the metal nanostructures' morphology and optical properties upon immersion in solvents and drying (Luo et al., 2005; Malinsky et al., 2001). This problem can

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be overcome by preconditioning the transducer in the respective solvent or by coating the metallic nanostructures with thin transparent or semi-transparent films (Bendikov et al., 2008; Galopin et al., 2009; Ruach-Nir et al., 2007; Okamoto et al., 2000; Szunerits et al., 2008). Second, insufficient probe attachment yield is another known limitation of LSPR sensors (Blow, 2009). It has recently been shown that coating a random metal nanoparticles assembly with an amorphous silicon–carbon alloy of about 5 nm in thickness leads to the formation of stable LSPR-active structures, on which biomolecules such as DNA can easily be covalently bound (Galopin et al., 2010). On such coatings, a significant increase in DNA attachment yields has also been demonstrated (Touahir et al., 2009). The purpose of this paper is to study whether using a similar spacer made of amorphous silicon–carbon alloy allows versatile and efficient metal enhanced fluorescence structures to be obtained, exhibiting the requested sensitivity for the detection of DNA hybridization in real-time in a conventional epi-fluorescence geometry.

2. Experimental

2.1. Materials

Hydrofluoric acid (HF) and acetic acid were purchased from Carlo Erba and were of VLSI grade. Undecylenic acid (99%) was supplied by Acros organics. *N*-Hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC), phosphate buffer saline (10× PBS), sodium dodecyl sulphate (SDS), salmon sperm DNA, formamide, saline-sodium citrate buffer (2× SCC) were obtained from Aldrich and were used without further purification. Ultrapure Water (MilliQ, 18 MΩ cm) was used for the preparation of the solutions and for all rinses.

The 25 mer oligonucleotides were obtained from Invitrogen. We used labeled probes for fluorescence studies:

5′ Cy5-[AGG-CGT-CGA-TTT-TAA-GAT-GGG-CGT-T]-$(CH_2)_6$-NH_2 3′ (abbreviated as Cy5-ON-L to highlight the presence of a Cy5 label at the 5′ end of the oligonucleotide sequence ON, and that of an amino-linker L at the 3′ end).

5′ Cy3-[AAC-GCC-CAT-CTT-AAA-ATC-GAC-GCC-T]-$(CH_2)_6$-NH_2 3′ (abbreviated as Cy3-$\overline{ON}$-L, the $\overline{ON}$ sequence being complementary to the ON one).

Unlabeled probes were also used: 5′ NH_2-$(CH_2)_6$-[AAC-GCC-CAT-CTT-AAA-ATC-GAC-GCC-T] (abbreviated as L-$\overline{ON}$).

Stock solutions of 5 μM or 10 μM were prepared in PBS buffer (150 mM, pH 8.5) containing 0.01% sodium dodecyl sulphate (SDS).

A complementary strand of Cy3-$\overline{ON}$-L and L-$\overline{ON}$: 5′ Cy5-AGG-CGT-CGA-TTT-TAA-GAT-GGG-CGT-T 3′ (abbreviated as Cy5-ON) and one strand containing 4 mismatches 5′ Cy5-AGG-CGT-GCA-TTT-TAA-GTA-GGG-CGT-T 3′ (abbreviated as Cy5-ON′), were used to study the hybridization reaction.

2.2. Formation of metal nanoparticles on glass

Metal nanostructures deposition was carried out by thermal evaporation of a few nm thick metal films on cleaned glass slides and subsequent annealing. The procedure is given in Supporting Information.

2.3. Deposition of amorphous silicon–carbon alloy overlayers

Amorphous silicon–carbon alloy layers were deposited onto glass/metal nanostructures using plasma-enhanced chemical vapor deposition (PECVD) in a "low-power" regime (Solomon et al., 1988). The procedure is detailed in Supporting Information.

2.4. Monolayer formation on amorphous silicon–carbon alloys

2.4.1. Acid-terminated surface

Procedures for grafting acid groups and activating them into succinimidyl-esters (Voicu et al., 2004; Faucheux et al., 2006; Sam et al., 2010) are given in Supporting Information.

2.4.2. DNA probe immobilization

The succinimidyl-ester-terminated surface was reacted with 10 μM solutions of amine-terminated oligonucleotides Cy5-ON-L, Cy3-$\overline{ON}$-L or L-$\overline{ON}$ in PBS buffer (150 mM, pH 8.5) with 0.01% SDS. This solution was deposited on the activated surface using a pin spotter (Biorobotics MicroGrid II). A commercial slide functionalized with succinimidyl-ester groups served as reference. After spotting, the non amidated ester groups were blocked with ethanolamine (50 mM, 15 min). The resulting surface was copiously rinsed with deionized water and dried under a stream of nitrogen.

2.4.3. DNA hybridization

For end-point measurements, the surface was exposed to the complementary targets at 42 °C during 4 h in a hybridization chamber. The hybridization solutions were made of 2× SSC, 0.1% SDS, 0.1% salmon sperm DNA, 35% formamide and the target oligonucleotides at a concentration chosen in the range from 5 fM to 5 nM. After hybridization, the sample was submitted to three successive wash steps of 2 min each in the following solutions: 2× SSC, 1% SDS, then 1× SSC, 0.1% SDS, and finally 0.1× SSC, all of pH ∼7. The sample was finally dried under a stream of N_2 before fluorescence measurement. For real-time experiments, hybridization was also performed at 42 °C, in the same solution as above.

2.5. Instrumentation

2.5.1. UV/Vis spectrometer

Absorption spectra were recorded with a Cary 50 Scan UV/vis spectrophotometer. The wavelength range was 400–800 nm.

2.5.2. Fluorescence measurements

For end-point measurements, the fluorescence was measured with a scanner (Axon instrumentation Personal 4100A). For real-time, in-situ hybridization monitoring, fluorescence was measured using a Hyblive machine (Genewave, France) (Marcy et al., 2008). In this case, the photoluminescence is continuously monitored, before hybridization (when the measurement cell is filled with a target-free buffer) and during hybridization (for recording kinetic curves). During measurements, the liquid is not circulated but stirred using surface acoustic wave devices.

3. Results and discussion

3.1. Influence of metal nanoparticle composition on the LSPR signal

Fig. 1 shows a schematic presentation of the sensor architecture studied in this work. It consists of a random assembly of metallic nanoparticles (gold, silver or gold/silver) coated with a 5 nm thick amorphous silicon–carbon alloy a-$Si_{0.80}C_{0.20}$: H film, on which 25-mer oligonucleotide probes were covalently immobilized. From SEM and AFM images, the morphology of the nanostructures can be estimated (Supporting Information). In the case of Au nanostructures obtained from a 4 nm gold film, the average diameter of the nanoparticles is $d = 25 \pm 8$ nm with an average interparticle distance $a = 16 \pm 8$ nm and a height $h = 13.6 \pm 3$ nm, as reported previously (Galopin et al., 2009). The final mean aspect ratio is $d/h = 1.8 \pm 0.20$. In the case of Ag nanostructures formed from a 2 nm silver film, the diameter and the height are reduced to $d = 12 \pm 6$ nm