Plasmonic nanocomposites: Synthesis, characterization and applications in biosensing

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Plasmonic nanomaterials have attracted much attention worldwide. The Localized Surface Plasmon Resonance (LSPR) effect occurs when the frequency of incident photon is resonant with the collective oscillation of conduction electrons in metallic nanoparticles. LSPR can be tuned by varying nanoparticles' size, shape and dielectric environment and is quite sensible to variations on these properties. These features have attracted much attention worldwide due to their feasibility for applications in chemical and biological sensing. Present work deals with the colloidal synthesis of plasmonic nanocomposites that results from the modification of anisotropic metallic nanoparticles covered by polyelectrolyte and conjugated to specific anti-bodies. The characterization of the resulting composite will be presented and discussed by means of electronic microscopy and absorption spectroscopy measurements. Application of the resulting nanocomposite as a sensing platform for the cardiac biomarker Troponin I (cTnI) will also be presented and discussed.

Keywords: composites; nanoplasmonics; biosensing

1. Introdutory remarks

The obtaining of biocompatible nanostructured materials has increasingly attracted the attention of research groups worldwide [1-3]. *In vitro* diagnostics is one of the very promising applications for these materials. Many times biocompatibility is achieved by modifying nanoparticles' surface, *via* the controlled addition of layers of organic polyelectrolites, peptides and/or other biomolecules, such as mono or polyclonal antibodies [4-5]. Metallic nanocomposites may act as chemical transducers in which the signal may be collected by distinct analytical methods [6]. This class of nanomaterials has shown to be very useful as biosensing platforms, mostly due to the Localized Surface Plasmon Resonance effect (LSPR). Isotropic and anisotropic plasmonic nanomaterials demonstrate very unique optical and structural behavior that highly depends on their size and shape. Some examples may be cited, such as: anisotropic silver and gold nanoprisms, they tend to present hot spots, while anistotropic silver and gold nanorods usually present high polarizability [4-5]. These distinct responses of the nanoparticles within a dielectric medium to the interaction with a polarized electromagnetic source give rise to significant differences in their respective absorption spectra, which are very distinct for instance, of the absorption spectra obtained for spherical (isotropic) nanoparticles having the same composition and size range. When an electromagnetic field interacts with the surface of metallic nanoparticles, a collective oscilation of the particles' surface electrons occurs. This is the so called LSPR effect [6-7]. Figure 1 shows a typical absorption spectrum for metallic nanorods. $\lambda1$ corresponds to the longitudinal oscillations and $\lambda2$ corresponds to the transversal oscillations of the surface plasmons. Thus, $\lambda1$ and $\lambda2$ are the plasmon bands.
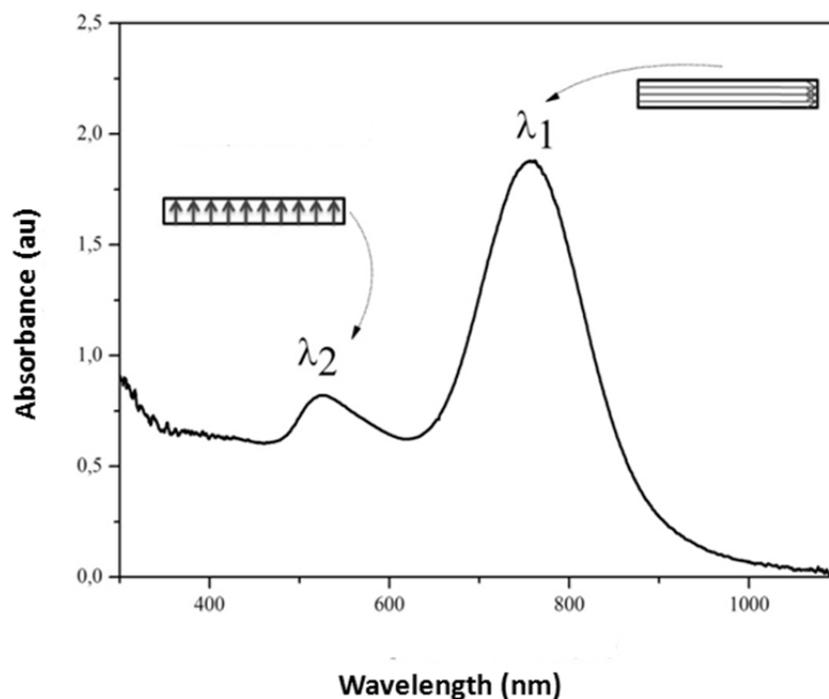
Fig. 1   Representation of a typical absorption spectrum for metallic nanorods. λ1 corresponds to the longitudinal oscillations and λ2 corresponds to the transversal oscillations of the surface plasmons.

Under the influence of LSPR effect slight changes in the chemical surroundings of the particles will correspond to measurable changes in their corresponding absorption spectra. This feature is quite suitable to the development of nanosensing platforms, which can be able to have very low limit of detection and very high sensibility [8]. Thus, proteins that in some situations are circulating in human blood may be detected by LSPR based nanosensing platforms, even if their concentrations are very low. LSPR sensing based techniques takes into account the optical properties of the samples (analytes and media), such as the variations in the refraction index. These changes may occur due to interactions of the metallic nanocomposites to nitrile (-CN), thiol (-SH) and amine ($-NH_2$) groups, that are present in many biomolecules [9].

2. Cardiac biomarkers sensing

Taking the cardiovascular system as a case of study: when the myocardial muscle is injured, even in small amplitude, natural cardiac biomarkers (proteins) are released into the blood stream. Myoglobin, Creatino-kinase and Troponins (cardiac Troponin I (cTnI) and cardiac Troponin T (cTnT), simultaneously) start to circulate in very low concentration ranges (0,1-10 ng mL^{-1}) [10-14], indicating that an acute myocardial infarction (AMI) is taking place. Despite of cTnI (cardiac Troponin I) and cardiac Troponin T (cTnT) beeing released in blood stream as a consequence of of the same event (the breakdown of the Troponin Complex) [15] cTnI is the golden standard biomarker for AMI [16-19]. Nowadays there are c.a. 18 commercially available tests for its quantification in whole blood or plasma [18-20]. These tests make use of anti-bodies that interact with cTnI. The larger the amount of antibodies the higher is the test sensibility. However, the search for high sensibility may lead to a lack on specificity, as almost all the available tests for cTnI quantification make use of organic molecules (fluorescent and non-fluorescent markers) that may interact with distinct parts of the anti-body, making these parts unavailable to correctly interact with cTnI [21-25]. When using a nanosensing platform controlling the amount of anti-body linked to each particle is not so simple, but is completely possible. Stoichiometric funcionalization and conjugation of the plasmonic nanoparticles give rise to plasmonic nancomposites that make possible to quantify biomolecules such as cTnI, without losing sensibility and specificity.

3. Synthesis, functionalization and conjugation of the sensing platform

Otherwise mentioned, all the chemicals used were purchased from Sigma-Aldrich and used without further purification. Gold nanorods with aspect ratio (a.r.) = 4.5 nm were prepared in aqueous medium by colloidal seed mediated method as described previously [5,8-9]. Figure 2 shows absorption spectra obtained for gold nanorods of distinct aspect ratio (B1=4.5 nm; B2=2.5 nm; B3=3.5 nm; B4=.0 nm). After synthesis, the functionalization of gold nanorods was preceded by the removal of the excess of cetyl-trimethyl-ammonium bromide (CTAB) via centrifugation of the samples (1 mL

each) at 12000 rpm for 20 minutes, the samples were rewashed until pH=6, in order to avoid unwanted effects such as precipitation, which can be induced by CTAB at higher pH values. The supernatant was discarded and the sample was diluted in milli-Q ultra-pure water (1mL). Further, under gentle stirring they were added 400 µL of Poly-styrene sulphonate (PSS) at 2 mg mL^{-1}. Once again the samples were rinsed and centrifuged, in order to remove the excess of PSS. Then, each sample was diluted in milli-Q ultra-pure water (15 mL). An excess of anti-Troponin I was added to each sample under gentle stirring. Further, the samples were kept undisturbed for 30 minutes. The samples now contain gold nanorods functionalized by PSS and conjugated to Anti-Troponin I. The samples were then centrifuged again at 12000 rpm for 20 minutes, in order to remove the non-bound Anti-Troponin I molecules. All the samples were resuspended in ultra-pure milli-Q water (pH=5.8) and PBS buffer (pH= 7). Then, the samples were stored at 4°C.
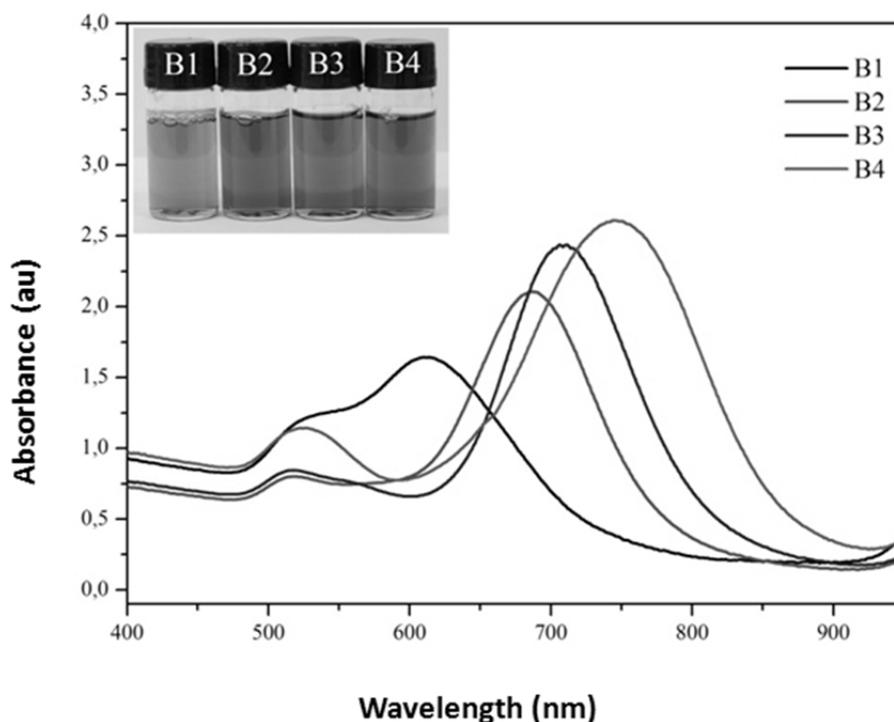

Fig. 2   Absorption spectra obtained for gold nanorods of distinct aspect ratio (B1=4.5 nm; B2=2.5 nm; B3=3.5 nm; B4=.0 nm). Aspect ratio (a.r.) corresponds to the result of the ratio: larger edge (nm) divided by smaller edge (nm).

The morphology of the resulting nanocomposites was investigated by Transmission Electronic Microscopy at a FEI Morgagni 268D microscopy, under voltage acceleration of 100 kV. In order to obtain the average aspect ratio and other image parameters, the software Image-Pro Plus 6 (Media Cybernetics) was used. Figure 3 shows a Transmission Eletronic Micrography of nanocomposite obtained by functionalization and conjugation of sample B1 (a.r.=4.5 nm).
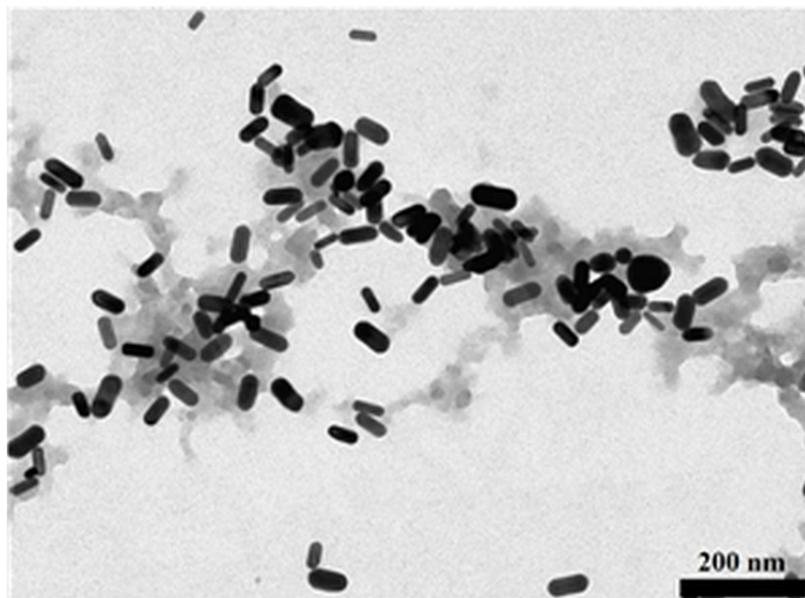

Fig. 3   Transmission Eletronic Micrography obtained for gold nancomposites (AuNRs-PSS-Anti Trponin I). The average aspect ratio of the initial nanoparticles was 4.5 nm.

4. The biosensing platform

Early stage detection methods that can quickly measure very low concentrations of cTnl with high sensitivity are needed for the early diagnosis of Acute Myocardial Infarction (AMI) and progress management of patients. Current cTnl detection methods are based on traditional enzyme linked immunosorbent assay (ELISA) methods which capture anti-cTnl antibodies against cTnl that are firstly immobilized onto the surface of a plastic

well, followed by adding a patient sample containing cTnI for binding to the anti-cTnI antibody. Then an enzyme labeled detector anti-cTnI antibody is allowed to bind with the immobilized cTnI. In each step mentioned above, numerous wash cycles are necessary in order to remove the unbound antibody or antigen from the well. Eventually, enzymatic substrate is added and cTnI is detected by an enzyme-dependent color change reaction. The entire ELISA process can take several hours to days to accomplish, which is labor-intensive and time consuming. Early detection and treatment of AMI substantially improves a patient's prognosis. A need therefore exists for a reliable, quick test to determine the presence of cTnI and thus the occurrence of an AMI [26]. One promising means of biological and chemical sensing is plasmonics. Plasmonics is the study of light-matter interactions in which materials that possess a negative real and small positive imaginary dielectric constant are capable of supporting surface plasmon resonance (SPR). This resonance is a coherent oscillation of the surface conduction electrons excited by electromagnetic (EM) radiation. Propagated surface plasmon resonance (PSPR) relates to a phenomenon when an incidence light emitted from a light source reaches the surface of a metal film at a fixed incident angle. In such an instance the light intensity reflected from a surface of the metal film picked up by a photo detector is approaching zero, i.e., the reflectance of the metal film is approaching zero while the light beam not reflected propagates at a given speed in a direction along the interface and excites the plasmon on the surface of the metal film to resonate. However, light in a sample medium cannot naturally excite PSPR and a high refractive index prism or grating is required for coupling. By comparison, Localized Surface Plasmon Resonance (LSPR) is defined as collective charge density oscillations restricted in the neighborhood of nanoparticles excited by an electromagnetic field with a specific frequency. LSPR may be set without utilizing the prism or grating for light coupling. LSPR is a possible excited state of the metallic nanoparticle electron system, which can be excited by photons or, equivalent, by an electromagnetic field of light incident on the particle. LSPR excitation is a consequence of the inter-electronic (collective) interactions of the electrons combined with spatial confinement of the conduction band electron system within a conductive nanoparticle volume [27]. An electron density wave is formed with a frequency/wavelength/energy that depends on the electronic structure of the nanoparticle, its geometry, size and dielectric environment. The spectral sensitivity of the LSPR, that is the amount of spectral shift of the LSPR along the wavelength/frequency/energy axis (alternatively, a spectral sensitivity of a LSPR) is measurable to events taking place on the surface of and close to the nanoparticles). This characteristic creates the possibility to use "plasmonic" nanoparticles as transducers in sensors. Most of these reported applications of LSPR in plasmonic biosensors rely on the sensitivity of the nanoparticle LSPR to the dielectric constant of the surrounding medium, opening up a route to "refractive index sensing" where adsorbate-induced changes in the local dielectric environment are utilized for detection of e.g. molecular binding events on the nanoparticle surface and in the particle nano-environment [26].

In order to proceed the calibration of the sensing platform, five vials (5 mL each) were filled with 3 mL of the nanocomposite aqueous suspension (gold nanorods functionalized with PSS and conjugated to monoclonal anti-body Anti-Troponin I (AuNRs/Anti-TnI)). One vial was filled with a stock solution without Troponin I. To each one of the remaining five vials, 1 mL of Troponin I stock solution of different concentrations was added (0.1; 0.5; 0.8; 1 and 2 ng mL^{-1}). Before performing the absorption spectra measurements, the samples were kept undisturbed by twenty minutes. This procedure was adopted according to the standard protocols [26] to calibrate the platform, once the concentrations of the stock solutions are known. As it can be seen at Figure 4, the sensing platform was able to distinguish different concentrations of Troponin I.
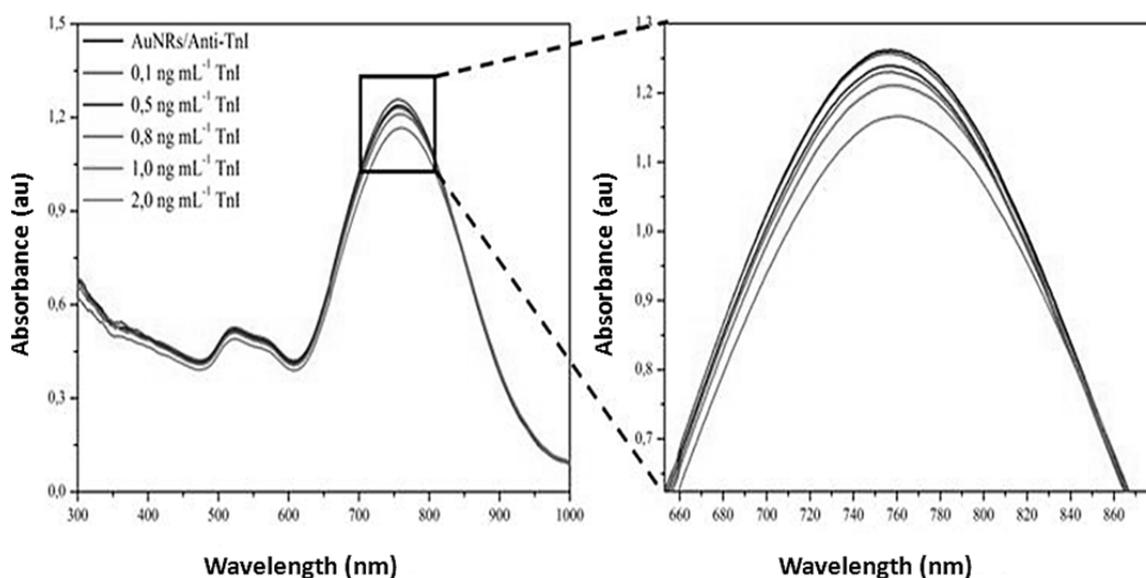

Fig. 4 Transmission Eletronic Micrography obtained for gold nancomposites (AuNRs-PSS-Anti Trponin I). The average aspect ratio of the initial nanoparticles was 4.5 nm.

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