LSPR nanobiosensor for the detection of DNA hybridization events at room temperature

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Gold nanoparticles (AuNP) exhibit a localized surface plasmon resonance (LSPR) band when excited by an electromagnetic field. These resonances are responsible for the unique optical properties and effects, namely light scattering, absorption and extinction, which depend on the morphology, size and also on the physicochemical characteristics of the immediate surroundings of the nanoparticle. These features can lead to multiple applications in medical diagnosis procedures, developing highly sensitive and specific sensors for biological targets. The preparation, deposition and optical characterization of well-defined AuNPs on suitable optical substrates have shown great potential for future generations of integrated photonic microfluidic circuits and biosensors on chips.

We present a specific type of DNA biosensor, which is based on the binding of fully complementary targets and/or not binding of a mismatch sequence to a nanoprobe. This nanoprobe consists of a AuNP (nanosphere or nanotriangle) functionalized with a thiolated oligonucleotide with specific characteristics, such as, base sequence, length and presence of a spacer; and is immobilized on a borosilicate glass surface previously treated with APTES.
Spectrophotometric measurements of LSPR were made by dark field microscopy, a technique that allows following individual nanoparticles in terms of their surface changes. [1]
Therefore, after several optimization procedures, such as, hybridization temperature, incubation time and salt concentration, the binding of synthetic targets (50 bp) or PCR products (345 bp) can be detected by the nanoprobes. This is promoted by a DNA-DNA interaction, which is directly related to a difference in the refractive index of the material around the AuNP and simultaneously to an associated resonance plasmonic shift to higher wavelengths (Figure 1). This phenomenon is verified after the functionalization of the nanoparticle (from original capping to first layer of DNA molecules) and also when the target is detected, after the hybridization. This mechanism is validated when in the presence of a non-complementary sequence, hybridization does not occur and consequently, there is not a plasmonic shift.
Considering the two nanoparticle morphologies, nanospheres and nanotriangles, coated with different capping agents (citrate and CTAB, respectively), it is possible to conclude that this is a robust mechanism of DNA detection at room temperature. Moreover, due to the different chemical surface and mainly, since gold nanotriangles have strong dipolar fields concentrated at the tips and edges (“hot spots”) [2] they have demonstrated higher LSPR band shifts upon probe functionalization, in comparison with spherical AuNPs. Beside DNA-DNA interaction, the versatility of the principle behind LSPR nanobiosensors can be further extended to immunodiagnostics using specific antibodies for several diseases, such as Alzheimer. [3]

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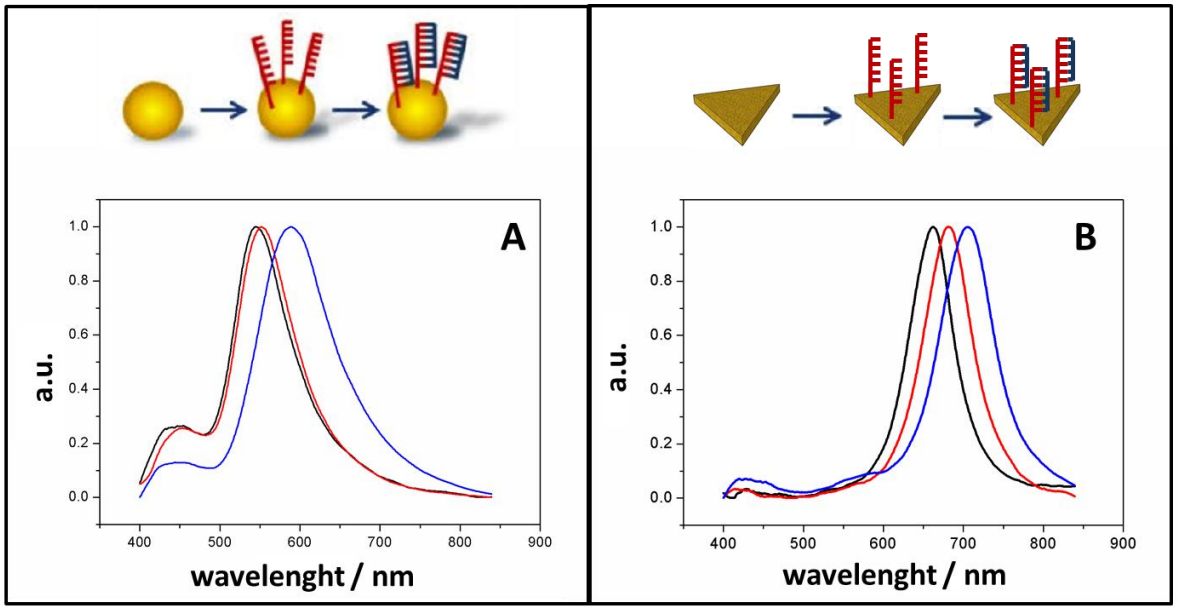

Figure: Schematic representation of the detection assay using 80 nm citrate-gold nanospheres **(A)** and 90 nm CTAB-gold nanotriangles **(B)**, after functionalization with thiolated oligonucleotides (**red**) and after hybridization with a fully complementary target (**blue**). As an example, LSPR spectra of two single nanoparticles show the successive red-shifts after each step.