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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Life Sciences Deparment / Chemistry Department

Nanobiophotonics for biomolecular diagnostics

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Objectives

• To understand the mechanisms involved in localised nanosurface interactions between gold nanoparticles mediated by biomolecules .

• To study and characterise the mechanisms of interaction between gold nanoparticles and fluorophores mediated by nucleic acids – Nanobiophotonics.

• To comprehend the effect of distance and conformation associated with nucleic acids on the spectral cross-talk between noble-metal nanoparticles and fluorophores.

• To develop Nanobiophotonics methodologies suitable to be used for molecular diagnostics in vitro and in situ, and ultimately in vivo.

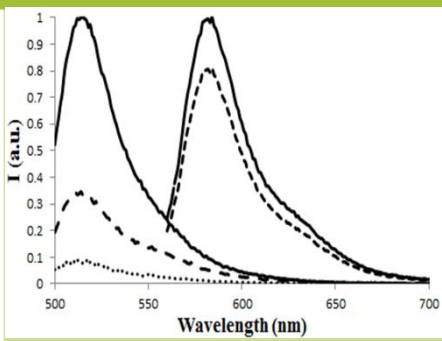

Figure 1. Optical interference of AuNPs on fluorescence.

Methodology

Gold nanoparticles were synthesized following the citrate reduction method and were later functionalized with fluorophores or thiolated ssDNA. UV-Vis spectrophotometry, transmission electron microscopy and dynamic light scattering were used for the characterisation of the AuNP-conjugates. Steady-state fluorescence and time-correlated single photon-counting techniques were performed to assess the fluorescence quantum yield and fluorescence lifetime on the surface of the AuNP and its variation with distance. The characterization was carefully accomplished having in mind the optical interference caused by the presence of AuNPs.
The AuNP-fluorophore system was used in a biosensor using a hairpin oligonucleotide as probe (Au-nanobeacon). The detection potential of such systemof was tested *in vitro* and *in situ* on several nucleic acid targets (DNA, mRNA, siRNA, miRNA

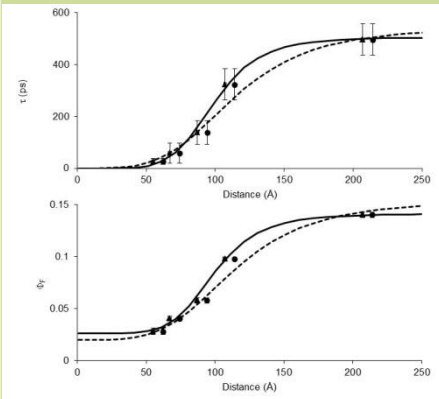

Figure 2. Dependence of fluorescence with distance to AuNPs' surface.

Expected Results

• The optical interference caused by the presence of AuNPs in solution was demonstrated using a two-chambered cuvette – Figure 1.

• A method to properly assess the photophysics parameters underneath the mechanisms of interaction between gold nanoparticles and fluorophores was successfully achieved and the effect of AuNPs on the rate constants was determined.

• The effect of distance and conformation associated with nucleic acids on the AuNP-induced fluorescence modulation was characterised – Figure 2.

• Nanobiophotonics methodologies suitable to be used for molecular diagnostics *in vitro* and *in situ* were sueccessfully created– Figure 3.

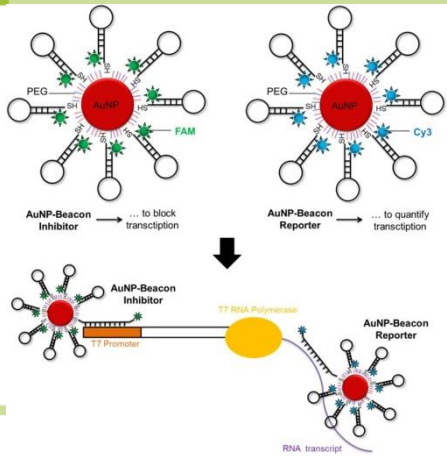

Figure 3. In vitro RNA transcription detection and inhibition using AuNPs.

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