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FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Chemistry/Life Science Department

Gold NanoBeacon For Universal Spectral Codification - Application as DNA Sensor

Photochemistry Group, REQUIMTE

NanoTheragnostics Group, CIGMH

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2010 - 2012: Master in Biotechnology at FCT/UNL.

2006 - 2010: Graduate in Molecular and Cellular Biology at FCT/UNL.

Objectives

The main objective of this work is to develop a Universal Codifying Gold-nanobeacon coupled to a wavelength shift mediated by Förster Resonance Energy Transfer (FRET), suitable for application as molecular (nano)diagnostics. For the success of this work, several intermediate goals need to be achieved:

Evaluate the hairpin opening ability and determine the occurrence of quenching by the nanoparticle, optimize the open sensor status identification through the specific hybridization to acceptor oligonucleotide complementary, ascertain FRET efficiency to the acceptor molecule and verify wavelength shift. This system will be validated in biological samples.

Methodology

The gold nanoparticles (AuNP) will be synthetized by the optimized Turkevich method (citrate reduction). The AuNP will be characterized by Transmission Electron Microscopy (TEM), Uv-Vis spectrophotometry and Dynamic Light Scattering (DLS). The functionalization of AuNP with the double labeled hairpin structure oligonucleotide will be performed by the salt aging method, leading to the formation of a Au-nanobeacon. The quantification of the number of oligonucleotide per AuNP will be performed by steady-state spectroscopy.

The photophysical characterization and the identification of the specific fluorescence signature of each donor/acceptor pair will be performed by steady-state and time resolve fluorescence spectroscopy, fluorescence/confocal microscopy. PCR amplification and other molecular biology techniques will allow production of targets with precise size.

Expected Results

In the absence of the complementary target, the hairpin structure will be in its close conformation, thus the donor fluorophore will be in the vicinity of the AuNP surface, leading to a quenching of its fluorescence (minimization of background signal). Upon hybridization to the target sequence, the hairpin opens and the donor breaks away from the AuNP surface. This allows for partial recover of fluorescence emission from the donor, thus allowing FRET to occur once the acceptor labeled oligonucleotide hybridizes to the exposed palindrome sequence.

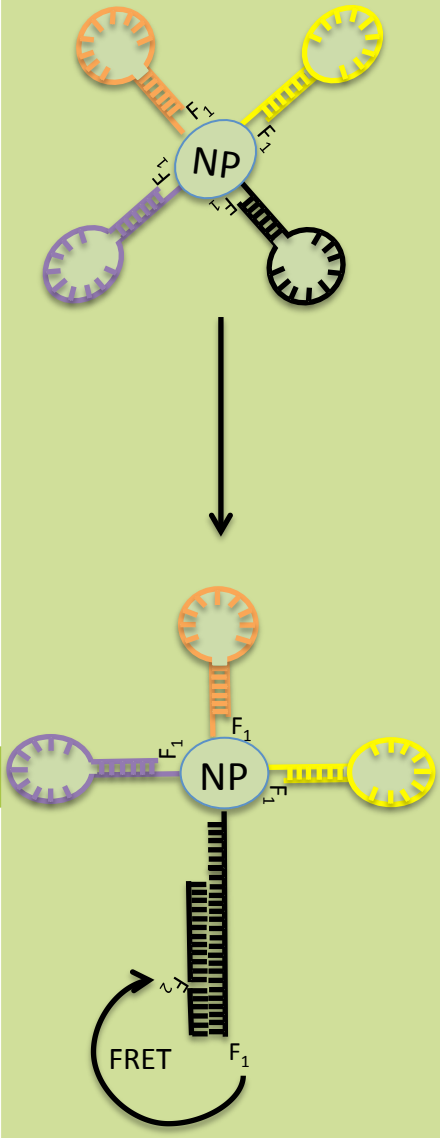

Figure 1. Schematic representation of the opening of the Au-nanobeacon in the presence of target sequence and hybridisation of acceptor labeled oligonucleotide.

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