FRET based gold nanobeacon for sequence discrimintation

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Abstract

We report development of a gold nanoparticle (AuNP) molecular beacon based biosensor coupled to a wavelength shift mediated by FRET, for the detection of fusion transcripts associated with the development of Chronic Myeloid Leukemia. Citrate capped 14 nm gold nanoparticles were functionalized with thiolade polyethylene glycol and further functionalized with a donor fluorophore labeled ssDNA with a hairpin structure. In the absence of a complementary target, the donor is in close proximity to the surface of the gold nanoparticle, leading to its quenching - gold nanoparticle are know to be fluorescence modulators [1-2]. Upon hybridization to the target sequence the donor breaks away from the surface of the gold nanoparticles due to the disruption of the hairpin structure, leading to a partial restoration of the donor fluorescence. The disruption of the hairpin leads to the exposure of the palindromic sequence, allowing the hybridization of an acceptor labeled oligonucleotide – See figure 1. With the donor and acceptor in such proximity, FRET occurs leading to a wavelength shift of the hybridization fluorescence signal to wavelengths that are not affect by the high absorption of the AuNP.

References

[1] Lakowicz J. R., Radiative decay engineering 5: metal-enhanced fluorescence and plasmon emission, Anal. Biochem., 337: 171–194

[2]- Rosa J., Lima J. C., Baptista P. V.,Experimental photophysical characterization% of fluorophores in the vicinity of gold nanoparticles, Nanotechnology,22:415202(7pp)(2011)

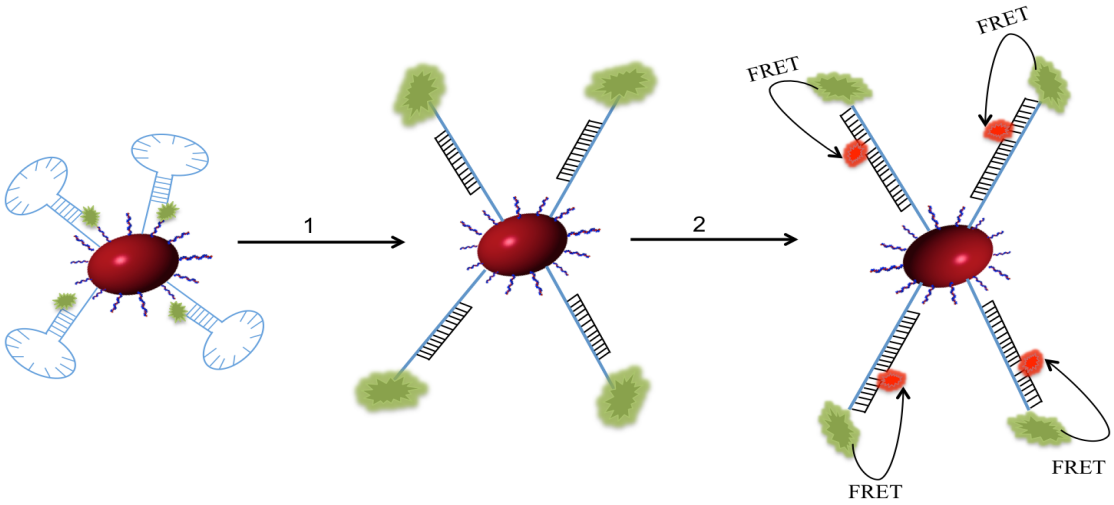

Figure 1. Schematic representation of the FRET based Au-nanoprobe. In the absence of a complementary target, the hairpin is in its closed conformation. Upon addition of complementary target (1), the closed conformation of the hairpin is disrupted and the donor breaks away from the surface of the AuNP, exposing the palindromic sequence that can hybridize to the acceptor labeled oligonucleotide (2).