Improved Gold Nanoparticle Probes for Molecular Detection of Metabolic Diseases

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There is an overall increase in life expectancy associated to high costs in health care for an ageing population in the industrialized world. Consequently, there is a demand for fast, unexpensive and easy to use diagnostic tests that are essential in identifying patients, determining prognosis, monitoring treatment and assessing the efficacy of prevention. [1] Here, nanotechnology has a prominent role, with special emphasis on gold nanoparticles (AuNP) for detection of Single Nucleotide Polymorphisms (SNP) associated with metabolic diseases with high prevalence, such as lactose intolerance. [2, 3]
The aim of the current project is (i) to synthesize and characterize spherical-AuNPs with different diameters; (ii) to functionalise AuNPs with specific oligonucleotide in order to obtain stable Au nanoprobes; (iii) development and optimization of a nanotechnology-based colorimetric assay for detection of an SNP related to lactose intolerance (LCT13910C>T) (Figure 1).
Spherical AuNPs with two distinct diameters (15 and 40 nm) were successfully synthesized, based on a citrate-reduction method and further characterized by Ultraviolet-visible spectrophotometry, Dynamic Light Scattering (DLS), Electrophoretic Light Scattering (ELS) and Nanoparticle Tracking Analysis (NTA). Their functionalization was further performed based on either a salt ageing or a pH method, using a thiol-modified oligonucleotides (5'-(SH-C_6)-AGTTCCTTTGAGGCCAGGG-3'). The pH method allowed Au nanoprobes with improved colloidal stability, and using less oligonucleotides for full capping, in comparison with the more widely used Salt ageing functionalization method. In the colorimetric assays, when nanoprobes were incubated with target DNA and upon salt induced aggregation, the colour of nanoprobes visibly changes from red (negative) to blue (positive) depending on the type of DNA present (Complementary = lactose intolerance, Mismatch = lactose tolerance or Noncomplementary). In conclusion, we successfully establish an innovative, faster, and simpler colorimetric molecular assay for SNP detection associated to lactose intolerance. The validated assay using synthetic DNA target and PCR products from sample patients will be considered for prototype at STABVida, and apply for detection of other single nucleotide polymorphisms related to metabolic and genetic diseases.

REFERENCES

[1] F.B. Myers, R.H. Henrikson, J. Bone, L.P. Lee, PLOS one, 8 (2013) 70266.
[2] G. Doria, R. Franco, P. Baptista, IET Nanobiotechnology, 4 (2007) 53.
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FIGURES

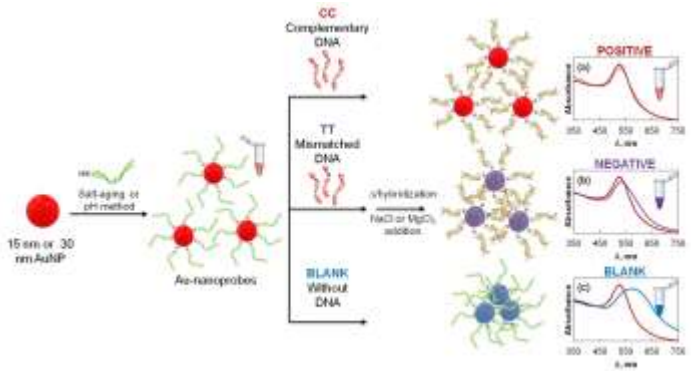

Figure 1: Scheme of the proposed colorimetric assay for SNP detection.