A PCR-Au-nanoprobes combined approach for detection of mutations associated with antibiotic resistance in *Mycobacterium tuberculosis*

Bruno Veigas[1]; Diana Machado[2,3]; João Perdigão[4]; Isabel Portugal[4]; Isabel Couto[2,5]; Miguel Viveiros[2,6]; Pedro Baptista[1]

[1] CIGMH/DCV,Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal;
[2] Unidade de Micobactérias, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL);
[3] UPMM (IHMT/UNL);
[4] Centro de Patogénese Molecular/URIA, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal;
[5] Centro de Recursos Microbiológicos (CREM), Universidade Nova de Lisboa
[6] COST ACTION BM0701 (ATENS)

Contact: bmrveigas@gmail.com

Tuberculosis (TB) is one of the leading causes of infection in humans, causing high mobility and mortality all over the world. The rate of new cases of multi and extensively drug resistant tuberculosis (MDR/XDR-TB) continues to increase [1], representing a serious health problem with serious implications in the containment of the disease [2]. In most cases, anti-TB drug resistance has been related to mutations in several loci within the pathogen's genome. The development of fast, cheap and simple screening methodologies would be of paramount relevance for the early detection of these mutations, and essential for the timely and effective diagnosis and management of MDR/XDR-TB patients.

The use of gold nanoparticles derivatized with thiol-modified oligonucleotides (Au-nanoprobes) has lead to new approaches in molecular diagnostics - nanodiagnostics. Based on the differential non-cross-linking aggregation of Au-nanoprobes we were able to develop a colorimetric method for the detection of specific sequences with a single base resolution at room temperature [3,4] – Figure 1.

Here, we present a simplified approach for the rapid detection of *M. tuberculosis* complex (MTBC) strains and for the simultaneous detection of of mutations associated with rifampicin resistance. This low-complexity assay enabled for detection of mutations D516V and S531L from MTBC clinical specimens with remarkable sensitivity in a few hours. This approach is being extended to further relevant mutations at other loci. This type of assay may prove to be useful in the initial management of suspected TB cases, especially from areas known to harbor high rates of MDRTB.

References:

[1] World Health Organization. Anti tuberculosis drug resistance in the world: report No. 4. Geneva, Switzerland: WHO; (2008).
[2] Martins M, Viveiros M, Couto I and Amaral L. Int J Tuberc. Lung. Dis. (2009) 13:569 73.
[3] Baptista PV, Montewka MK, Oles JP, Doria G and Franco R. Clin. Chem. 52, No. 7, (2006).
[4] Doria G, Franco R and Baptista PV. IET Nanobiotechnology, 1(4) (2007) 53-57.

Acknowledgments:

We acknowledge Fundação para a Ciência e a Tecnologia, Portugal for funding (CIGMH).This work was partially supported by Project PTDC/BIA-MIC/71280/2006 and PTDC/SAU-BEB/66511/2006; and PTDC/FIS/74274/2006 (BV). D. Machado was supported by grant SFRH/BD/65060/2009.

Figures:

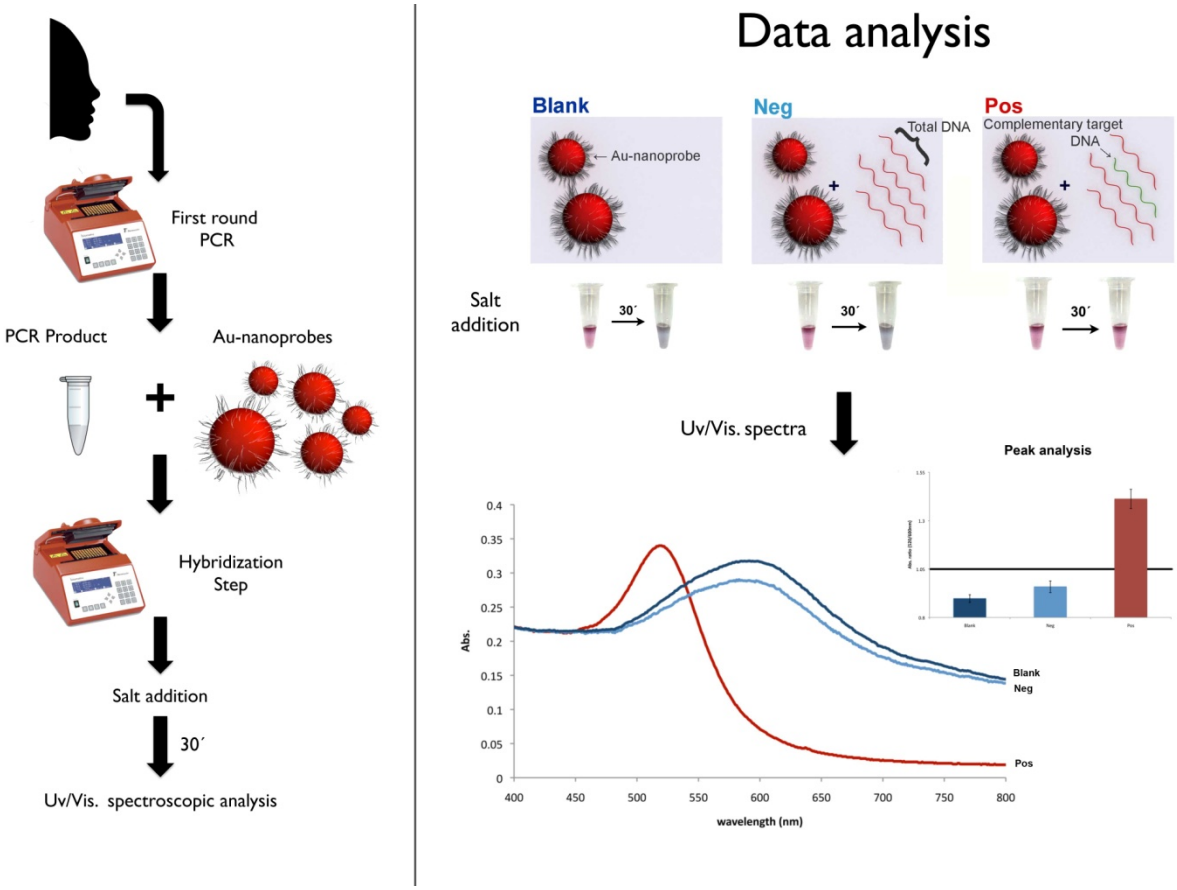

Figure 1- Au-nanoprobe strategy for detection of MTBC members and mutations associated with rifampicin resistance. Schematic representation of the detection with gold nanoprobes. The assay consists on visual comparison of test solutions before and after salt induced Au-nanoprobe aggregation: Au-nanoprobe alone – Blank; in the presence of a complementary DNA sequence – “POS”; and in the presence of a non-complementary DNA sequence – “NEG”.