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Garbarino, Francesca; Minero, Gabriel Khose Antonio; Fock, Jeppe; Nilsson, M.; Hansen, Mikkel Fougt

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On-chip optomagnetic detection and discrimination of single base mutation in *Mycobacterium tuberculosis*

F. Garbarino[a], **G.A.S. Minero**[a], **J. Fock**[a], **M. Nilsson**[b], **M.F. Hansen**[a]

[a]Department of Micro- and Nanotechnology, Technical University of Denmark, Building 345B, DK-2800 Kgs. Lyngby, Denmark

[b]Science for Life Laboratory, Department of Biochemistry and Biophysics,Stockholm University, Box 1031, Se-171 21 Solna, Sweden

We demonstrate the ability of an on-chip optomagnetic (OM) detection scheme based on the rotation response of magnetic nanoparticles (MNPs) to detect synthetic *Mycobacterium tuberculosis* DNA targets and to discriminate between the wild-type (WT) variant and the single point mutation mutant-type (MT) variant responsible for resistance against the first-line antibiotic, rifampicin.

The assay is based on rolling circle amplification (RCA) with MT- and WT-specific padlock probes (PLPs). Upon addition of ligase, PLPs on matching targets are enzymatically joined to form circles, whereas those on mismatching targets remain open. In a subsequent amplification using phi29 polymerase, targets on circular PLPs are extended to form a long concatemer of the sequence complementary to the PLP, whereas non-circular PLPs are not extended (Fig. 1a).[1] The resulting rolling circle products (RCPs) form ≈0.5 μm coils that are detected using 100 nm MNPs functionalized with a detection probe with a sequence overlapping part of the PLP backbone. Upon mixing of an MNP suspension with the RCPs, MNPs are bound to the RCPs and thus experience a significant increase of their hydrodynamic size; the fraction of these MNPs is quantified using OM measurements. In these, a magnetic field alternating at a frequency f is applied and the modulation of the light transmitted through the suspension is measured as function of f. The MNPs have a linked magnetic moment and an optical anisotropy. In the alternating magnetic field they periodically try to reorient themselves to align along the magnetic field causing a change in the transmitted light intensity. The timescale of particle reorientation depends on their hydrodynamic size.[2] We detect the depletion of free MNPs as the reduction of the OM signal at high frequency as function of the time after mixing of the MNPs and RCPs.

The assay was based on previously published PLP sequence.[3] The PLP-target hybridization, ligation and RCA were performed sequentially in test tubes after which the RCP-containing solutions were mixed with the MNP suspension in plastic chips, which were quickly mounted for readout in the previously described OM setup capable of performing four parallel measurements.[4] All four combinations of WT and MT targets and WT and MT PLPs were studied simultaneously for a target concentration of 100 pM. Fig. 1b shows the signal from free MNPs vs. time after mixing. A depletion of free MNPs of more than 80% was observed for the matching target-PLP combinations, whereas the mismatching combinations showed a signal decrease of about 4%, which is comparable to the observation for the no target control sample. This demonstrates the ability of the assay with on-chip OM readout to detect M. tuberculosis and the mutation responsible for rifampicin antibiotic resistance with high specificity.

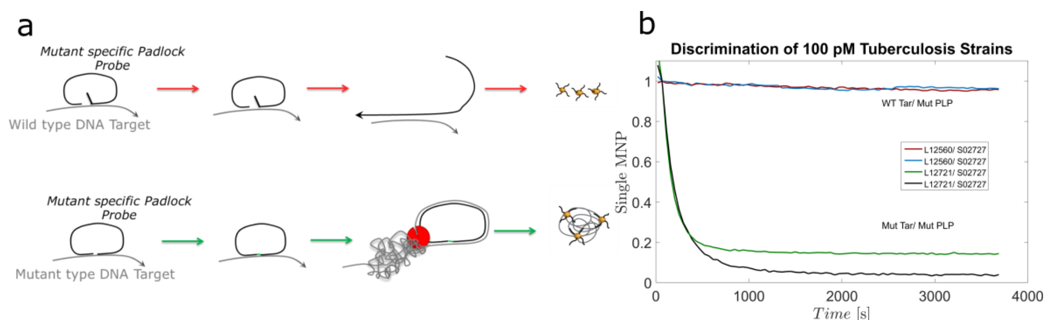

Figure 1 (a) Multistep assay (PLP hybridization, ligation and RCA) for detection of target with single nucleotide specificity. A matching PLP-target pair produces an RCA product, whereas a mismatching pair does not. The RCPs are detected via the binding (depletion) of MNPs functionalized with probes targeting a sequence complementary to a part of the backbone of the PLPs. (b) Signal from free MNPs vs. time after mixing with the post-RCA sample.

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