Optomagnetic Sequence-Specific Detection of Dengue Target DNA

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Optomagnetic (OM) measurements, probing changes in the rotation dynamics of magnetic nanoparticles (MNPs) in response to an oscillating magnetic field, were recently used to detect products of loop-mediated isothermal amplification (LAMP) originating from Newcastle disease virus under ideal, clean lab conditions with a limit of detection (LOD) of 10 aM for 30 min assay time[1]. There, a fraction of the forward inner primers was biotinylated, and the end-point OM readout was carried out after mixing the sample with streptavidin MNPs. Here, we report on the first real-time OM measurements during LAMP of Dengue target DNA under non-ideal lab conditions where the reaction and detection took place repeatedly in a single lab. Using an alternative detection scheme, we discriminated between spurious amplification products from previous reactions and specific amplification products from the actual sample. In this reaction, MNPs functionalized with capture probes, targeting the loops of specific LAMP amplicons (Fig 1A), were premixed with the sample. Capture probes bound to spurious LAMP products containing mutations had a 20°C lower melting point (Fig. 1C) allowing for discrimination between the two types of products at 67°C (Fig. 1A-B). In LAMP, strand displacement and self-priming lead to long single stranded tracts containing repeats of the loop sequences[2]. Specific binding of MNPs to these resulted in MNP clustering (signal at f <20 Hz) and a depletion of free MNPs (signal at f ≈300 Hz), see Fig. 1A. Fig. 1D shows the decrease of the single MNP signal vs. Dengue target concentration observed at 67°C. By this, we found an LOD of about 100 fM after 20 min of amplification.

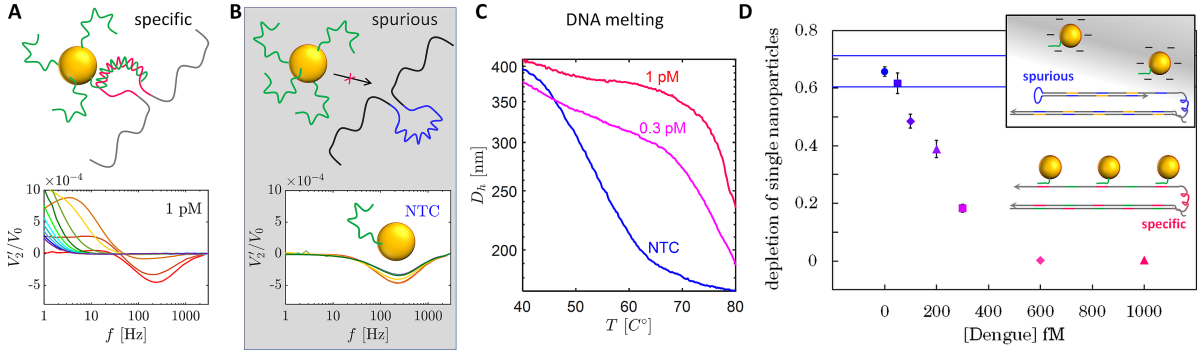

Fig. 1: Sequence-specific validation of Dengue amplicons in LAMP detected in the OM signal. (A) Capture probe (green) binding to the complementary DNA sequence of specific Dengue amplicons in the LAMP elongation phase. (B) Capture probe do not bind to spurious amplicons with mutations (blue). Curves show the optomagnetic signal at different times. (C) Melting of DNA bridges between amplicons and MNPs via probing the median hydrodynamic diameter of the MNPs. (D) Dose-dependent analysis of Dengue target amplification at a LAMP threshold time of 20 min (67°C). Two blue lines represent 3σ rule of thumb for calculation of LOD.

1 B. Tian et al., *ACS Sensors*, 2016, **1**, 1228–1234.
2 T. Notomi, Y. Mori, N. Tomita and H. Kanda, *J. Microbiol.*, 2015, **53**, 1–5.