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Dual kinetic and mechanistic profiling of rolling circle amplification using real-time optomagnetic studies

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We demonstrate measurements of the hydrodynamic size of magnetic nanoparticles (MNPs) using an optomagnetic (OM) technique and present for the first time, real-time results on the binding of functionalized MNPs to DNA rolling circle amplification products (RCPs) synthesized *in vitro* and loaded onto the measurement chamber. RCPs are long single-stranded DNA coils produced by amplification of a circularized padlock probe, therefore they contain sequence repeats. Measurements of the hydrodynamic size during formation of the RCPs on the MNPs, real-time in the measurement chamber, are shown. These results illustrate the use of the OM technique for studies of both kinetics and mechanistics of rolling circle amplification (RCA).

The OM technique relies on measurements of the 2nd harmonic modulation of the light transmitted through a suspension of MNPs vs. frequency of an applied oscillating magnetic field. In the resulting OM spectra, a peak is centered at a frequency, which is inversely proportional to the hydrodynamic volume of the MNPs.[1] In this work, we studied the OM behavior of RCPs produced from a sequence targeting hemagglutinin gene of Victoria, a type-B influenza virus strain and recorded spectra in real-time. First, we applied the OM technique to detect binding of MNPs labelled with capture probes to RCPs (6 nM, 25 min RCA time). Fig. 1A shows the kinetic information, where the signal changes from 100 Hz to 3 Hz, corresponding to the free MNPs and MNPs bound to RCPs, respectively, within the recorded time of 15 min. Next, we applied the OM technique to obtain mechanistic details in monitoring the size of the RCP during RCA on a target pre-attached to MNPs in real-time at 37°C (Fig. 1B). Fig. 1C shows the corresponding hydrodynamic MNP diameter vs. RCA time. Furthermore, mechanistic studies performed at temperatures between 30° and 75°C will be presented. Such kinetic and mechanistic insights help in better understanding and tailoring of RCA steps towards diagnostics.

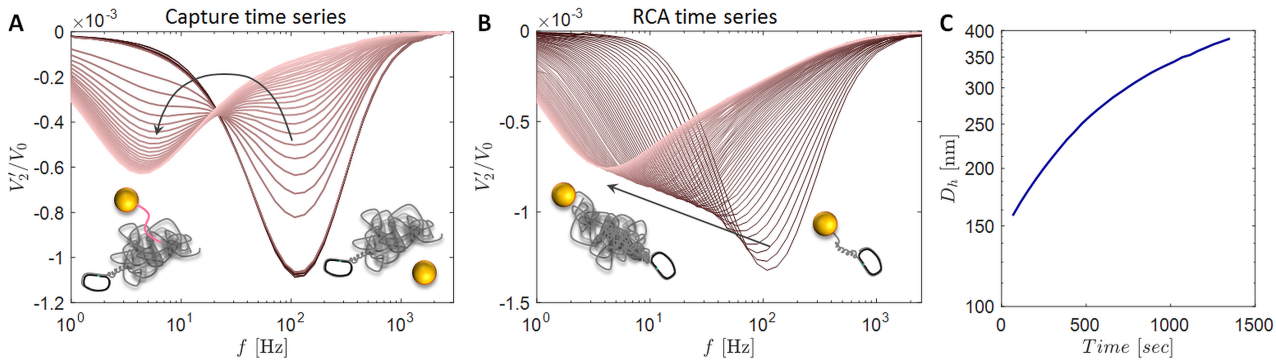

Fig.1. OM detection of hydrodynamic size of MNPs during real-time experiments (peak position inversely proportional to hydrodynamic volume): (A) Binding of capture-probe functionalized MNPs to synthesized RCPs. (B)-(C) Change of MNP hydrodynamic size during RCA on target pre-attached to MNPs.

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1 J. Fock et al., *Phys. Chem. Chem. Phys.*, 2017, **19**, 8802–8814.