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SENSITIVE BLU-RAY DETECTION OF CLUSTERED ROLLING CIRCLE PRODUCTS FOR MOLECULAR DIAGNOSTICS

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In this paper we present a method for low cost and rapid diagnostic sensing of nucleic acids (NA) where rolling circle amplification (RCA) products for the first time are analyzed on a novel magneto-optical platform using a blu-ray pickup head.

Molecular diagnostics may provide tailored and cost efficient treatment of infectious disease and thus novel integrated tests for point-of-care analysis are desirable. In our previous work we have proven that molecular assays based on padlock probe ligation and RCA are fast, highly sensitive and specific for NA, protein and bacterial detection in a digital fashion down to single nucleotide resolution [1,2,3]. Detection of RCA product coils has among others been achieved by measuring the changes in magnetic properties of magnetic nanoparticle (MNP) labels, both at a macro scale and in a microfluidic chip [4,5]. These sensors are simple but currently encompass limited sensitivity. **In this work we greatly improved the limit of detection using a novel low-cost magneto-optical readout technology to analyze monomerized RCA coils, based on dynamic light modulation due to formation and disruption of chains of MNPs [6,7].**

A molecular protocol for circle-to-circle amplification (C2CA) was established for detection of the human pathogen Pseudomonas Aeruginosa as a model (Fig1). The procedure involves specific target capture and probing by ligation of padlock probes followed by RCA which generates coils comprising ~1000 copies of the probe in 1 hour. We digested the coils and conducted a second amplification round on re-circularized monomers with subsequent digestion. In our design the C2CA coils or monomers are bound to MNPs conjugated with two different detection probes (Fig1f) and the difference in light modulation due to the rotation of free or bound MNPs is measured. The magneto-optics set-up uses electromagnets to generate an AC field which drives MNPs dynamic rotation which modulate the transmitted light. A commercial blu-ray pickup head is used both as a laser source and as detector (Fig2). The technique is sensitive to the presence of clustered particles in the sample which is related to the DNA target concentration. **We hypothesized that monomers would more efficiently cross-link nano particles and form bead clusters as compared to coils leading to an increased signal shift**.

We hybridized triplicate samples of monomers and coils at three concentrations and negative control samples to MNBs and measured the in-phase second harmonic signal at different frequencies (Fig 3). From the plots it is clear that the coils even at high concentrations coincide with the negative control while monomers result in a detectable shift in signal even for the lowest measured concentration (90 pM). The limit of detection is hence 50 zmol of initial DNA target concentration providing clinically relevant sensitivity.

The molecular assays involve serial additions of reagents and isothermal incubation with no fast temperature cycling needed, which is favorable for integration into microfluidic devices. Our goal is now to integrate the complete C2CA protocol with improved magneto-optic sensing into a centrifugal disc for rapid and automated on-site analysis in infectious disease diagnostics.

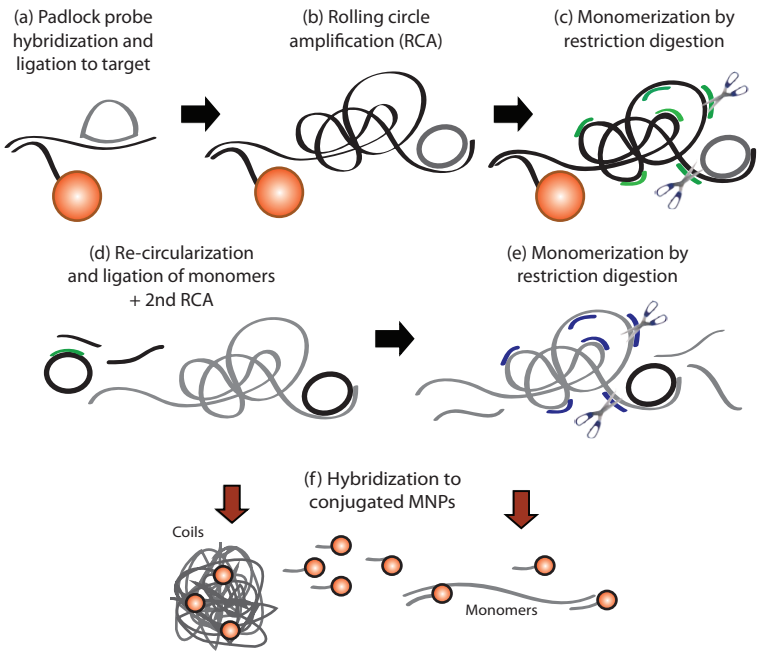

Figure 1: Schematic C2CA protocol (a) A target sequence is captured to a magnetic bead. Upon recognition of the target the padlock probe is circularized and enzymatically ligated. (b)The padlock is amplified by RCA using the target as template. (c) The concatenated RCA product is restriction digested to monomers. (d) The monomers are re-circularized and amplified in a second RCA before (e) second digestion of the C2CA coils. (f) The C2CA coils and monomers are finally hybridized to MNPs, conjugated with capture probes and then detected in the magneto-optical system.

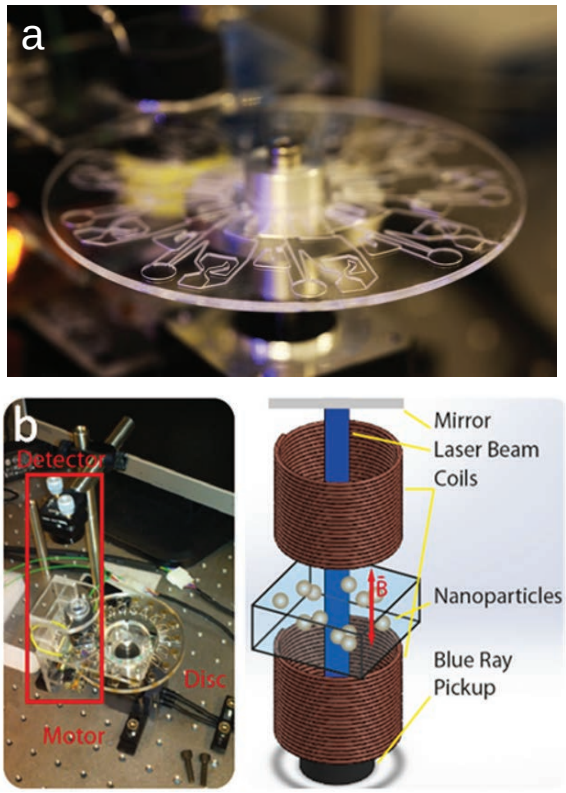

Figure 2: (a) Disk for magneto-optical measurements on RCA products (b) overall platform comprising a motor, a SonyBlu Ray pickup head, coils for magnetic field excitation and a mirror used to reflect the light back to the photodetector. A customized electronic board is used to extract the signal from the Blu-Ray photodetector.

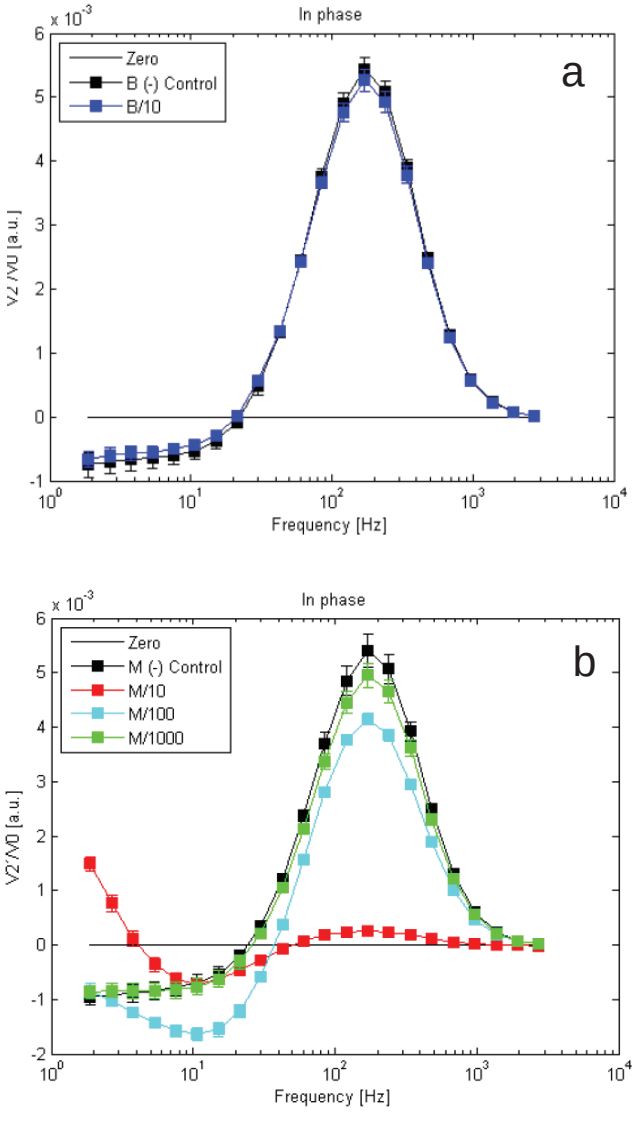

Figure 3: In phase second harmonic photodetector signals plotted vs. frequency for (a) coils B to the left and (b) monomers M to the right. The coils were measured at 33pM concentration (B/10) and the monomers at 9nM (M/10), 900pM (M/100) and 90pM (M/1000) concentration. The (-) negative control is from a template free C2CA reaction.

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