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Catalyzed hairpin assembly of magnetic nanoclusters with single nucleotide discrimination

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We demonstrate detection and single nucleotide discrimination of DNA targets based on hairpin assembly (HA) combined with an optomagnetic (OM) readout detecting the clustering of magnetic nanoparticles (MNPs). The HA assay[1] is based on two initially 'locked' hairpin stems *H1* and *H2* that are grafted onto two separate populations of 100 nm MNPs (Fig. 1a). A fully matching DNA target *C1* 'unlocks' the stem of *H1* via toehold strand displacement. The unlocked *H1* probe subsequently unlocks the stem of *H2* and displaces the *C1* target to form a stable *H1-H2* hybrid linking two MNPs. The clustering state of the MNPs is detected using the OM technique, which probes the rotational dynamics of MNPs in response to an applied oscillating magnetic field at frequency *f*.[2] The MNPs have a magnetic moment and a linked optical anisotropy and the magnetic field results in a modulation of the intensity of light transmitted through the MNP suspension (Fig. 1a). Depending on the hydrodynamic volume V_h, the MNP response shows a phase lag φ with respect to the field excitation. Clustering increases V_h and a phase lag is observed at lower frequencies.[2] The response of single MNPs was mainly detected at $f > 40$ Hz, whereas that from MNP clusters was detected at $f < 40$ Hz. As the HA signal, we take the average phase at $f < 40$ Hz after 50 min of HA reaction at 40°C. We studied the dose-response curve and limit of detection (LOD) for MNP hairpin probe densities of 100% and 40% and the specificity of the assay by comparing *C1* and *Mut* targets, where *Mut* had a single base mismatch in the toehold-recognizing part of *H1*. Figs. 1b and c show the average phase lag for $f < 40$ Hz vs. concentration of *C1* and *Mut* targets for the two probe densities. For 100% probe density, we found *C1* and *Mut* LODs to fall within the range of 1-10 nM and 10-100 nM, respectively, and 16-fold discrimination of *C1* from *Mut* at 10 nM. For 40% probe density (Fig. 1c), the *C1* LOD was improved to 0.1 nM, but the specificity was reduced. Less accessible toehold regions of highly 'crowded' *H1* and *H2* give better discrimination of the single base mutation in the DNA target.

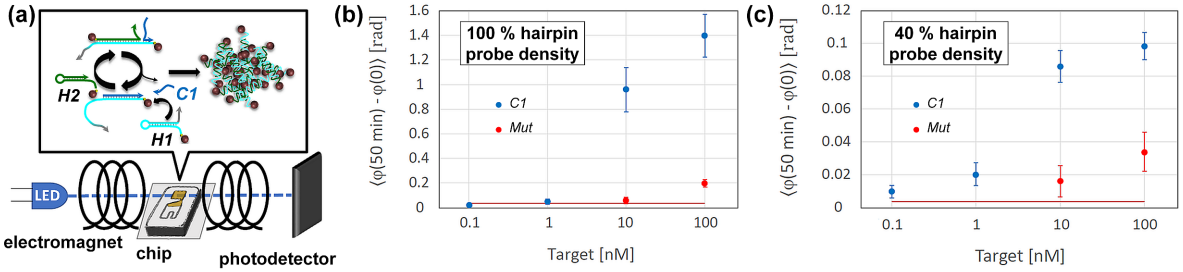

Fig. 1: **OM-HA detection.** (a) Schematic of assay and OM setup. Measurements were performed using plastic chips sandwiched between two heaters at 40°C. (b)-(c) Dose-response analysis of the HA triggered by *C1* as well *Mut* targets at the indicated hairpin densities.

References

[1] B. Li *et al*. (2011) *Nucleic Acids Res*. **39**, e110.
[2] A. Mezger *et al.* (2015) *ACS Nano* **9**, 7374-7382.