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OPTOMAGNETIC STUDIES OF PH-SWITCHABLE NANOPARTICLE AGGLUTINATION VIA TRIPLEX DNA FORMATION

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ABSTRACT

Polypurine-polypyrimidine sequences found in eukaryotic genomes can fold into a triple-helical structure if the sequences exhibit mirror symmetry [1]. They have been shown to stop DNA replication *in vitro* and in cell cultures and play a significant role in genetic regulation. New methods are needed to investigate switching behavior of triplex DNA complexes under controlled conditions.

KEYWORDS: DNA triplex, nanoparticle, biosensor, pH

INTRODUCTION

We present measurements of the pH-dependent switching of DNA triplex structures using an optomagnetic technique, which is simple to implement and can be applied on any transparent microfluidic chip. The 2nd harmonic modulation of 405 nm laser light transmitted through the sample container is measured as a function of the frequency f of an applied oscillating magnetic field. This modulation arises from the coupled magnetic and optical anisotropies of 100 nm magnetic nanobeads (MNBs). The frequency spectra reflect the rotation response of the MNBs and show characteristic features at frequencies related to their inverse hydrodynamic size. This method was recently used for the sensitive detection of the DNA-target induced agglutination of two populations of MNBs functionalized with different DNA probes [2]. Here, we use a single population of MNBs functionalized with palindromic polypyrimidine DNA oligonucleotides that may spontaneously fold in the presence of polypurine DNA [3] to form a triplex structure at pH < 6 that links MNBs.

EXPERIMENTAL

Intermolecular triplex folding (Fig. 1a) was investigated in a cuvette setup (Fig. 1b), with 10 nM polypyrimidine probe DNA attached to the 100 nm MNBs (0.1 mg/ml) in the presence of 5 nM polypurine target in binding buffer (8 mM Tris, 4 mM EDTA, 0.1 % Tween-20, and 0.8 M NaCl).

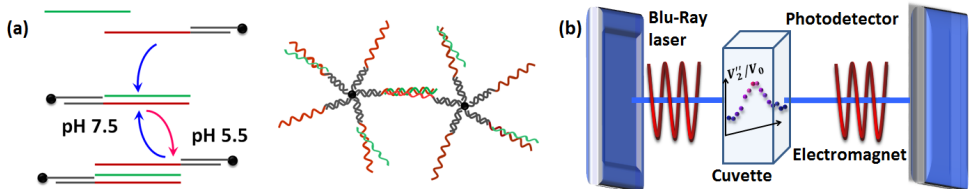

***Figure 1**: Approach used for detection of particle assembly. **(a)** Schematic illustration of pH-dependent triplex folding of biotinylated pyrimidine sequences (red) bound to streptavidin coated MNBs via adaptor strands (grey). At low pH, pyrimidine sequences on separate MNBs form links in the presence of the complementary purine DNA target (green). **(b)** Schematic of setup for optomagnetic detection of nanoswitches.*

RESULTS AND DISCUSSION

We first measured the optomagnetic spectra at pH 5.5 and pH 7.5 on a sample of MNBs and DNA target (polypurine DNA) in a cuvette without magnetic incubation. Data are shown as spectra of the in-phase (Re) and out-of-phase (Im) 2nd harmonic of transmitted light signal V_2 normalized with average intensity of transmitted light, V_0, (Fig. 2a-c). Essentially no signal difference between the two pH values was observed after 5 min (no clustering) and a large effect is observed at low frequencies after 2 hr (strong clustering at pH 5.5). The reaction time was reduced to 1 min using magnetic incubation in 20 mT (Fig. 2c) such that the total time for incubation and measurement was about 3 min. The change in $\mathrm{Re}(V_2)/V_0$ (Fig. 2c) is different from that in (b) due to the different cluster sizes. The largest effect of the cluster formation was observed in $\mathrm{Im}(V_2)/V_0$, at f = 1-10 Hz. For further analysis, we therefore used the average signal in this frequency range. Fig. 2d shows the optomagnetic signal from an experiment commencing at pH 8 (blue), where no triplex DNA was formed.

Subsequently, acid and base were sequentially added (left to right in figure) to vary the pH between stable and unstable conditions for triplex formation. The switches are observed to behave reversibly and to be clearly detectable in the optomagnetic signal. The effect is reduced upon repeated pH switching due to screening from salt formed from the acid-base reaction. This could be avoided by electronically switching pH [3].

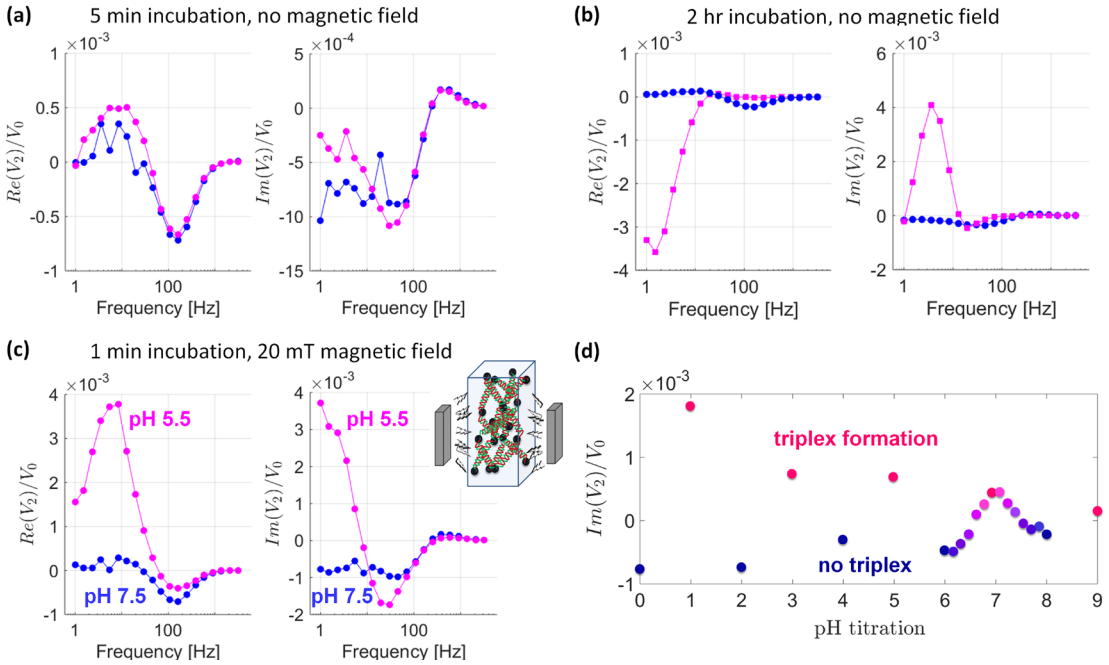

***Figure 2**: pH-dependent optomagnetic detection of triplex nanoswitches. Spectra obtained after **(a)** 5 min and **(b)** 2 hours of incubation with no applied magnetic field for pH 7.5 (blue) and 5.5 (pink). **(c)** Corresponding spectra obtained after only 1 min of magnetic incubation in 20 mT. A large signal difference is observed at low frequencies in $Im(V_2)/V_0$. **(d)** Analysis of DNA mediated assembly upon pH titration from pH ~ 5 (pink) to pH ~ 8 (blue) by sequential addition of 10 mM HCl and 10 mM NaOH, respectively, followed by 1 min magnetic incubation. For gradual pH changes, series of 1.4 mM acid and base were added to the system.*

CONCLUSION

The presented MNB-based readout has the advantages that a sensitive lock-in based detection scheme can be applied and that the magnetic incubation reduces the assay time to a few minutes compared to for example 12 hr, which has been reported for the assembly of gold nanoparticles [4].

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