RAPID AND CONTINUOUS ON-CHIP MULTISTEP CHEMICAL PROCESSING ON MAGNETIC PARTICLES

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ABSTRACT

We present on-chip continuous flow DNA hybridisation and chemical synthesis of peptides. All the necessary washing and reaction steps are achieved by pulling magnetic particles across laminar streams with processing times of < 1 minute.

KEYWORDS: DNA hybridisation, peptide synthesis, magnetic particles, continuous flow

INTRODUCTION

Magnetic particles are commonly used in bioanalysis due to their versatility, range of surface functionalities and high surface to volume ratios. They are easily manipulated by magnetic fields, and as such are becoming frequently used for microfluidic applications. However, many particle-based biochemical procedures involve tedious, repetitive reaction and washing steps, rendering these processes both time consuming and labour intensive. Previously, we have demonstrated that such operations can be performed with significantly reduced procedural time and reagent volume by pulling magnetic particles across several laminar flow streams containing reagents and washing buffer via an external magnet [1] and applied this concept to a two-step assay [2]. Here, we investigate the use of this technique for performing one-step and two-step DNA hybridisation as well as one-step chemical synthesis of peptides.

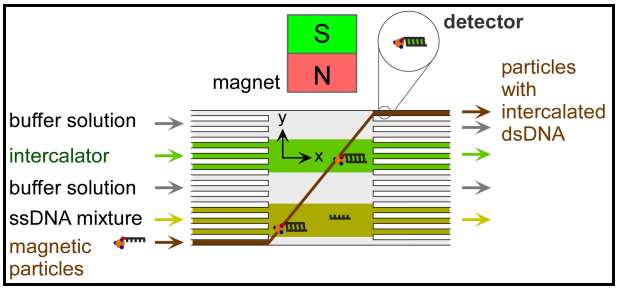

Fig. 1: Principle of our multi-step processing system shown for DNA hybridisation and intercalation. Particles are magnetically deflected through streams containing reagents or washing buffer.

THEORY

The principle of the multi-step processing system is illustrated in Fig. 1. Laminar flow streams containing reagents and washing buffer are generated in a deflection chamber. Particles introduced into the chip move in the x axis direction with the laminar flow. The application of a magnetic field gradient along the y axis results in the exertion of a magnetic force on the particles. The magnitude of the force depends on the properties of the particles and the magnetic field [3]. This results in a diagonal

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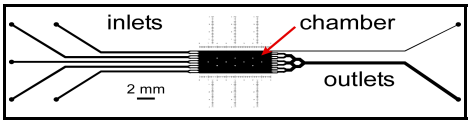
trajectory of the particles across the chamber, causing them to pass through alternating laminar flow streams and to react consecutively with the reagents present.

EXPERIMENTAL

The chip design and setup are shown in Fig. 2. The 22 µm deep chip featuring five inlets, two outlet channels and a 8x3 mm^2 deflection chamber was fabricated in glass. For DNA hybridisation, streptavidin functionalised particles (Dynabeads M270) were incubated off-chip with a biotinylated capture probe. In the one-step hybridisation, particles were deflected through a stream containing complementary ssDNA labelled with AlexaFluor555. The two-step hybridisation comprised of pulling particles through unlabelled cDNA, washing buffer and PicoGreen streams, respectively. For the peptide synthesis reaction, carboxylic acid modified 2.8 µm Dynabeads were reacted with EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride) off-chip, then pumped into the device and deflected across a stream containing fluoresceinyl-glycine-amide.



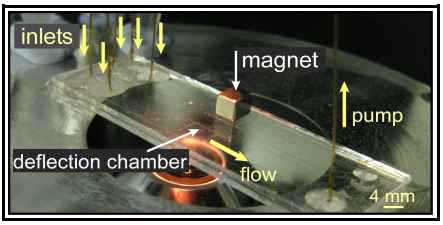


Fig. 2: Chip design (above) and setup (right) showing the NdFeB magnet (4x4x5 mm^3) used for deflection.

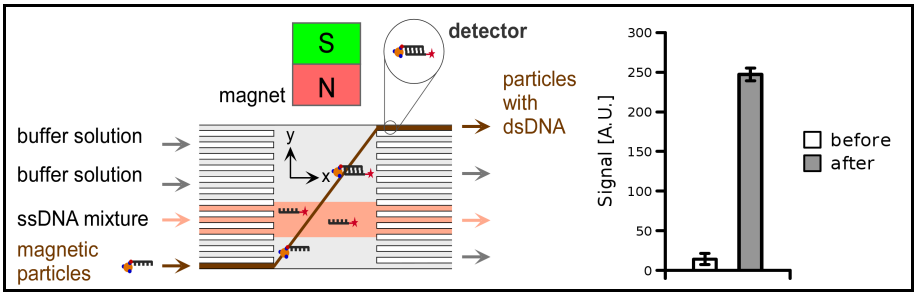


Fig. 3: Schematic (left) and results (right) for one-step DNA hybridisation. Fluorescence intensities of particles were measured before and after passing the reagent stream containing fluorescently labelled target ssDNA.

RESULTS AND DISCUSSION

The results of the one-step DNA hybridisation detection are shown in Fig. 3. From the increase in fluorescence it is clear that even a relatively short residence time of particles in the reagent stream (about 30 s) was sufficient for hybridisation. Fig. 4 shows the results for the two-step reaction, where an increase of fluorescence intensity indicated successful hybridisation and subsequent staining with PicoGreen. Target ssDNA concentration in the reagent stream was 1 nmol.mL^{-1} and the experiment was conducted at room temperature (24°C).

Fig. 5 illustrates an increase in the fluorescence of EDC-reacted magnetic particles

after passing through a reagent stream of labelled glycine, indicating that peptide bond formation had been achieved.

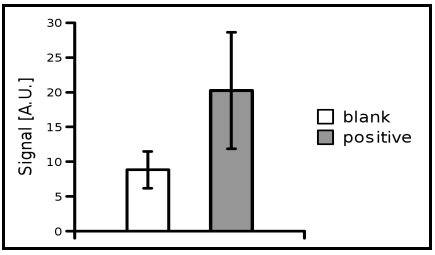


Fig. 4: Results for two-step DNA hybridisation and intercalation. Particles were pulled through streams containing cDNA and PicoGreen intercalator. For the blank, the cDNA stream was replaced with washing buffer.

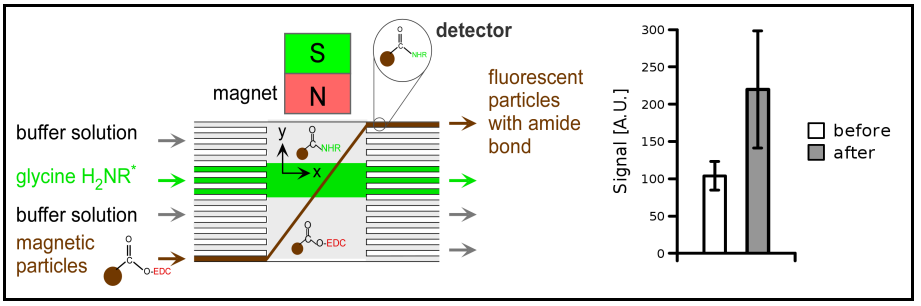


Fig. 5: (left) Principle of peptide synthesis in continuous flow. (right) Measured increase in fluorescence on particles after passing through the glycine stream.

CONCLUSIONS

We have successfully demonstrated DNA hybridisation and peptide bond formation on particle surfaces, using our continuous flow system, whereby particles are deflected through alternating streams of reagents and washing buffers. These reactions display the versatility of our microfluidic platform, which can perform particle-based processes with greatly reduced procedural time and minimal reagent usage.

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