ACCELERATED TARGET CAPTURE BY DYNAMIC MAGNETIC PARTICLE ACTUATION

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ABSTRACT

Particle-based capture is a widely used process for target enrichment. The upcoming generation of integrated biosensing systems need target capture technologies that are as efficient and as rapid as possible. Here, we study the effect of magnetic actuation of streptavidin-coated magnetic capture particles on the capture reaction of 200 nm sized biotinylated fluorescent target particles. To overcome limitations due to volume transport and near-surface alignment of reactive particles, and thus to accelerate target capture, various magnetic actuation techniques were developed. Stationary rotation of capture particles was found to increase the rate to obtain near-surface. Furthermore, dynamic actuation of particle chains was found to effectively accelerate both volume transport and near-surface alignment in the capture reaction. We conclude that rotational magnetic particle actuation can be effectively applied to achieve rapid microfluidic particle-based affinity capture.

KEYWORDS: Magnetic particles, Biosensing, Reaction binding kinetics, Magnetic actuation

INTRODUCTION

Ultra-high-sensitivity affinity assays count target molecules with single-molecule resolution.[1-2] Such assays are particularly challenging when a low concentration of target molecules is suspended in a small volume of a complex biological fluid. For example, when the sample volume is 1 µL and the concentration of target molecules is 1 femtomol/L, at maximum 600 molecules are available for the measurement. In such cases biosensing becomes limited by counting statistics and every single target molecule needs to be captured from solution, so the capture efficiency needs to be close to 100%.

Target capture is often performed using affinity molecules that are coated onto nano- or micro-particles, because such particles have a high surface-to-volume ratio.[3] In this paper we report novel methods to achieve *rapid capture of low target numbers from a small sample volume with very effective use of magnetic particles.* We will demonstrate that time-dependent magnetic fields can be used to increase the target capture rate per particle. As capture model system we use streptavidin–biotin molecules, coated onto respectively magnetic capture particles (3 µm; named MC hereafter) and fluorescent target particles (200 nm; FT) to allow for single target resolution quantification of the reaction kinetics. For quantification, we identify the different stages during the reaction, analoguous to a bimolecular binding process [4] (see Figure 1). First (*i*) volume transport of particles is required to obtain encounters between the different particles. As the particles contain discrete binding sites, subsequently (*ii*) near-surface alignment is required of the opposite binding sites. When aligned, a non-diffusional rearrangement of the interacting binding molecules takes place, which is (*iii*) the biochemical reaction process.

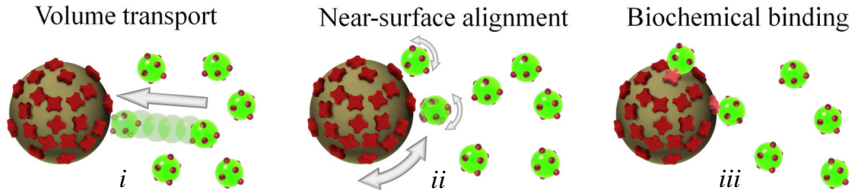

Figure 1: Schematic representation of the different stages in the capture process of targets (in green) by capture particles (in grey). Targets and capture particles are sketched with multiple binding sites (in red).

EXPERIMENTAL

In capture experiments, both capture particles (0.5 µL of ~3000 MC/µL; M270, Dynal) and target particles (8.5 µL of 5×10^5 FT/µL; Fluospheres, Invitrogen) are dispersed in an 9 µL fluid chamber filled with PBS (phosphate buffered saline) containing 1 mg/mL BSA (bovine serum albumin) to reduce non-specific binding. Due to their size and density, the capture particles sediment to the bottom substrate.

First the effect was studied of stationary rotation of single sedimented magnetic capture particles using a horizontal rotating magnetic field with negligible gradients, generated in the center of a quadrupole electromagnet. Images were recorded at random positions at the bottom substrate using an Andor Luca S EMCCD camera, mounted on a microscope (Leica DM6000). To study the effect of rotating single particles, low magnetic particle concentrations were used, and only capture by single magnetic particles was considered.

In further experiments, target capture was studied under magnetic particle chain rotation (at ~4000 MC/µL). To generate the magnetic fields, we use a specifically designed orthogonal arrangement of two quadrupolar electromagnets of which each

pole can be steered separately (see Figure 2a). For quantification of target capture, the particles were concentrated at the bottom substrate by means of a small magnet (see Figure 2b).

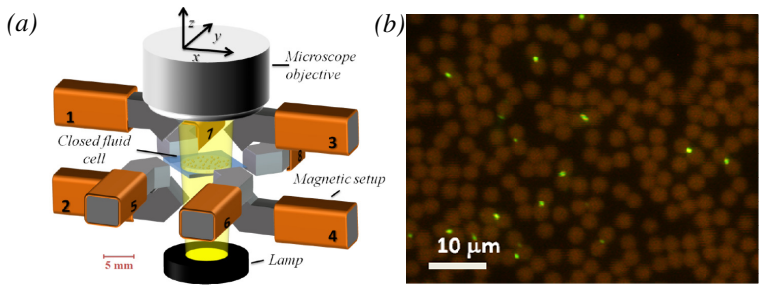

Figure 2: (a) Sketch of the experimental set-up with two quadrupolar electromagnets. In the center a closed fluid cell is located. The set-up is positioned under a microscope to enable imaging during actuation. (b) Typical microscope image of magnetic capture particles (in brown) and captured fluorescent target particles (in green) on the surface of the fluid cell.

RESULTS AND DISCUSSION

The effect of stationary rotation of sedimented magnetic capture particles was studied by applying a rotating magnetic field. First, the response was characterized of sedimented magnetic capture particles in a rotating field [5], as shown in Figure 3a. Up to a field frequency of 1.3 Hz, the average particle rotation frequency is found to be equal to the field frequency, but for higher field frequencies it decreases as the maximum magnetic torque is reached [5].

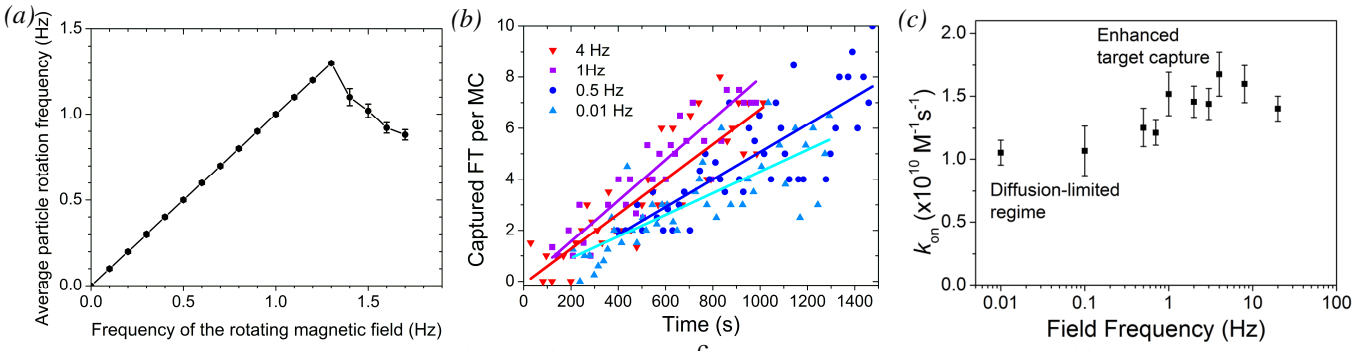

Figure 3: (a) Rotational response of sedimented superparamagnetic particles in a magnetic field rotating at different frequencies at B = 5 mT. Above a critical field frequency of ~1.35 Hz, the particle is unable to completely follow the magnetic field.[5] *(b) Quantified capture for different rotating frequencies. From the slope, the reaction rate constant may be determined. (c) The overall reaction rate constant k_{on} is measured as a function of the rotation frequency of the applied horizontal rotating magnetic field. The amplitude of the applied field was 5 mT.*

Next, biotinylated target particles were added to the solution with sedimented streptavidin-coated capture particles, and the target capture was monitored during the capture reaction under the application of a rotating field (Figure 3b). The surface was randomly scanned for particles with a microscope and every found particle was imaged and the corresponding time was recorded. After the experiment, the images were processed and for each image the average amount of captured targets per capture particle was determined. The large variation is due the small average number of capture particles per image (~1-2 particles). Typically we find that the captured amount of target particles per capture particle increases linearly in time, which correspond to estimations that the loss in targets and capture particle binding sites is negligible. From the slope, the overall reaction rate constant may be determined (Figure 3c) and it is found to increase with increasing field frequency to maximum factor of 1.5 with respect to no rotation (or very slow rotation). Above the critical rotation frequency, the reaction rate constant is found not to increase any further. This caused by the concomitant decrease of the average rotation frequency.

If the reaction rate would be limited only by radial diffusion to the particles (i.e. volume transport), a reaction rate constant would be expected of $k_{dif,surf}$ = 2.1 x10^{10} $M^{-1}s^{-1}$ as was estimated using Comsol Multiphysics simulations of an absorbing sphere near a reflecting surface. In our experiments, for low rotation frequencies, it is found that the reaction rate constant is k_{on} = (1.0±0.2) x10^{10} $M^{-1}s^{-1}$, which is smaller than the rate constant in case only diffusive transport limiting the reaction rate constant. This indicates that the reaction is also limited by another process, which can apparently be accelerated by physically rotating the capture particles (indicating that this process is not the chemical reaction).

The rotational motion of a capture particle is too slow (Re < 10^{-4} at 1 Hz rotation) to cause any significant radial (i.e. volume) transport in the surrounding fluid which is thus dominated by diffusion. The particle rotation can however affect the orientation and the surface area that is probed for binding sites during an encounter with a target particle. In that way, particle rotation enhances the rate at which the particles and thus the reactive molecules align.

An interesting question is why the increase occurs at a frequency near 1 Hz. We can compare the tangential displacement of the capture particles to the diffusional root-mean-squared displacement of a target particle, in the typical time period in which the target and capture particle are near each other (i.e. at approximately one pixel = ~200 nm). From our recordings, we find that this time period is typically on the order of a few hundreds of milliseconds, i.e. 0.44 ± 0.23 s. In this time period, the tangential displacement of the capture particles is 3.9 ± 2.0 μm at a rotation frequency of 1 Hz, while the root-mean-squared displacement of the target particles is 1.3 ± 0.4 μm. Based on these estimations, rotation of the capture particles enhances the area that is probed by the targets during the encounter time. This may explain why particle rotation at these frequencies would increase the rate at which near-surface alignment is obtained.

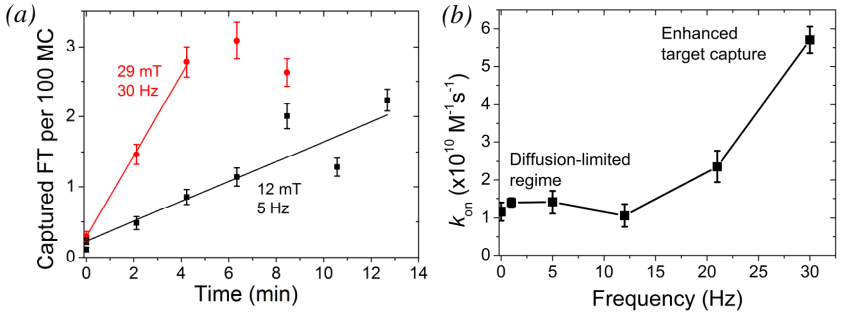

Figure 4: (a) Chain actuated target capture. Each data point corresponds to (10 ± 3) ×10^3 capture particles. Saturation at 30 Hz is due to field-induced drift which brings particles to the fluid chamber wall. (b) The overall reaction rate constant for different frequencies of the rotational field component in the actuation protocol.

To also accelerate radial transport, we have studied the effect of dynamic, i.e. chaotic [6], chain rotation behavior. Experiments were done at different frequencies while keeping the Mason number constant (which is a measure of the magnetic torque with respect to the hydrodynamic drag).[6] In Figure 4a the measured target capture is shown for rotation at 5 Hz and at 30 Hz. From the initial slopes, the reaction rate constant was determined (Figure 4b). At low frequencies the diffusion-limited regime is found, while at higher frequencies it is observed that the overall reaction rate constant increases up to a factor of 4.5±0.5 at 30 Hz horizontal actuation. Apparently, here the diffusion limitations are overcome both for volume and near-surface alignment.

CONCLUSION

We have studied the effect of rotational magnetic actuation of streptavidin-coated magnetic capture particles on the capture reaction of 200 nm sized biotinylated fluorescent target particles. We have shown that the capture reaction is mainly limited by diffusive transport both to generate encounters and to reach near-surface alignment of non-actuated particles. Rotation of individual capture particles enhances near-surface alignment, but not volume transport to create encounters between the particles. Rotational actuation of chains of magnetic particles not only enhances near-surface alignment, but also induces advective particle transport so that the diffusional volume transport limitation is overcome. We conclude that dynamic rotational magnetic particle actuation can significantly accelerate microfluidic particle-based affinity capture.

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