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Shape anisotropy enhanced optomagnetic measurement for prostate-specific antigen detection via magnetic chain formation

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ABSTRACT

We demonstrate a homogeneous biosensor for the detection of multivalent targets by combination of magnetic nanoparticle (MNP) chains and a low-cost 405 nm laser-based optomagnetic system. The MNP chains are assembled in a rotating magnetic field and stabilized by multivalent target molecules. The number of chains remaining in zero field is proportional to the target concentration, and can be quantified by optomagnetic measurements. The shape anisotropy of the MNP chains enhances the biosensor system in terms of providing efficient mixing, reduction of depletion effects (via magnetic shape anisotropy), and directly increasing the optomagnetic signal (via optical shape anisotropy). We achieve a limit of detection (LOD) of 5.5 pM (0.82 ng/mL) for the detection of a model multivalent molecule, biotinylated anti-streptavidin, in PBS. For the measurements of prostate-specific antigen (PSA) in 50% serum using the proposed method, we achieve an LOD of 21.6 pM (0.65 ng/mL) and a dynamic detection range up to 66.7 nM (2 μg/mL) with a sample-to-result time of approximately 20 min. The performance for PSA detection therefore well meets the clinical requirements in terms of LOD (the threshold PSA level in blood is 4 ng/mL) and detection range (PSA levels span from $< 0.1–10^4$ ng/mL in blood), thus showing great promise for routine PSA diagnostics and for other *in-situ* applications.

1. Introduction

Due to their large surface-to-volume ratio, simplicity of bio-functionalization, low background signal in biological samples, and cost-efficiency, magnetic particles have been widely used in biosensors ranging from *in-situ* decentralized diagnostics to centralized laboratory based high-throughput assays (Lee et al., 2015; Tekin and Gijs, 2013). Manipulated by external magnetic fields (static or dynamic actuation), magnetic particles have long been utilized for extraction, enrichment and stirring (mixing) to accelerate the reaction kinetics and improve the sensitivity of biosensors (van Reenen et al., 2014). In particular, the application of a rotating magnetic field (RMF) can induce formation of one-dimensional nanostructured assemblies of magnetic particles (Vuppu et al., 2003). The rotation of the formed magnetic particle chains results in effective mixing and accelerates reactions that are otherwise limited by diffusion (Fermigier and Gast, 1992; Martin et al., 2009). Moreover, the angular velocity of the magnetic particle chains reduces the depletion layers of target molecules around the particles, thereby increasing the association constant of the reaction (van Reenen et al., 2017).

Magnetic field-induced particle chains can be stabilized by the binding of multivalent molecules (Furst et al., 1998; Goubault et al., 2005). The relationship between the concentration of multivalent molecules and the shape anisotropy of the chains has been utilized to design RMF-based biosensors (Park et al., 2010a, 2010b; Ranzoni et al., 2011; Vuppu et al., 2004). These RMF and chain formation based biosensors analyze the transmitted (or scattered) laser light to quantify the target molecules in the sample. Park et al. (2010a) reported an RMF-based optomagnetic biosensor to monitor the changes of the transmitted laser light intensity at different angles between the direction of the magnetic chains and that of the light, thereby achieving a limit of detection (LOD) of 100 pM avidin. Ranzoni et al. measured the scattered light from particle dimers and achieved an LOD of 5 pM biotinylated BSA in serum (Ranzoni et al., 2011). However, some other important properties of the suspension, such as the hydrodynamic size of the chains and the concentration of unbound magnetic particles,

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were not monitored due to the design of the sensors and the utilization of superparamagnetic particles. Therefore, these optomagnetic biosensors have limited linear detection ranges (no more than 3 orders of magnitude) and lack the potential for multiplexing. In addition, both of these works only demonstrated the capability of their biosensors with biotin-avidin/streptavidin model reaction systems, which are much stronger and faster than common biological reactions.

The 405 nm laser-based optomagnetic sensor, first reported by Donolato et al. (2015a), is a rapid and low-cost volumetric magnetic nanoparticle (MNP) detection system that provides information about the hydrodynamic size of suspended MNPs. By measuring the alternating current (AC) magnetic field-induced modulation of the optical transmission signal, this optomagnetic sensor has been utilized to detect a variety of analytes including proteins (Antunes et al., 2015; Fock et al., 2017b; Uddin et al., 2016), bacteria (Tian et al., 2016a), microRNA (Tian et al., 2017), DNA (Minero et al., 2017), and amplicons of different DNA amplification methods (Donolato et al., 2015b; Mezger et al., 2015; Tian et al., 2016b). In previous work, magnetic incubation was employed to accelerate the formation of MNP clusters having a random morphology and to minimize nonspecific binding between MNPs (Uddin et al., 2016). The magnetic incubation was performed by incubating and shaking the samples between two magnets (Baudry et al., 2006; Daynes et al., 2015; Ranzoni et al., 2012). The linear detection range for these magnetic cluster-based optomagnetic biosensors is around 2 orders of magnitude, which is relatively good but not enough for clinical quantitative analysis.

Herein, for the first time, we demonstrate a shape anisotropy enhanced protein bioassay using the 405 nm laser-based optomagnetic setup. Recognition molecule coated MNPs are mixed with multivalent target proteins and incubated in an external RMF. Induced by the RMF and stabilized by the binding of target proteins, MNP chains can be quantified after incubation. The shape anisotropy of magnetic chains enhances the AC magnetic field-induced modulation of the optical transmission, and leads to an increase of the signal amplitude (and accuracy) compared to previous work, which does not utilize this shape anisotropy (Antunes et al., 2015). We demonstrate the detection principle by analysis of a model protein, biotinylated anti-streptavidin, with streptavidin coated 250 nm MNPs. The signal of the MNP chains presents in the low-frequency region of the optomagnetic spectrum and is used to detect low target concentrations. We also determine the concentration of unbound MNPs by monitoring the high-frequency region of the optomagnetic spectrum. Since the reduction of the concentration of unbound MNPs upon chain formation is not influenced by the size or shape of the aggregates that are formed, the signal from unbound MNPs can be used to detect high target concentrations, thereby extending the linear detection range of the assay.

Prostate-specific antigen (PSA), a serine protease indicator that relates to many prostate diseases including prostate cancer of all grades and stages, is widely detected as a cancer biomarker both for initial diagnosis and for monitoring the response to treatment (Lilja et al., 2008). PSA levels span from $< 0.1–10^4$ ng/mL in blood and the median PSA level for healthy adult males aged ≤ 50 years is approximately 0.6 ng/mL (Savblom et al., 2005). The traditional threshold PSA level (in blood) for detecting prostate cancer is 4 ng/mL, and levels above 10^2 ng/mL have been found almost exclusively related to advanced prostate cancer (Lilja et al., 2008). The low threshold level and large concentration span of PSA require detection methods with both high sensitivities and wide detection ranges, which is challenging especially for *in-situ* diagnostics. Furthermore, analysis of a single biomarker may give misleading diagnostic results for prostate cancer detection (Harris and Lohr, 2002), meaning that the capability for multiplex detection of PSA and other biomarkers is important for biosensors. To demonstrate the applicability of the shape anisotropy enhanced optomagnetic sensor for *in-situ* clinical applications, we evaluate our biosensor for the detection of serum samples spiked with PSA.

2. Materials and methods

2.1. Reagents

Ultrapure grade phosphate buffered saline (PBS, 20 ×) and bovine serum albumin (BSA) were purchased from AMRESCO (Solon, USA). Biotinylated goat anti-streptavidin antibody was purchased from Vector Laboratories Inc. (Burlingame, USA). Native human PSA (30 kDa), polyclonal sheep anti human PSA, and biotin conjugation kit (type 2) were purchased from Bio-Rad Laboratories (Kidlington, UK). Streptavidin coated 250 nm MNPs (multicore magnetic beads containing clusters of small single domain particles, product code 09-19-252, 10 mg/mL, 4.9×10^{11} particles/mL) were purchased from Micromod (Rostock, Germany). Fetal bovine serum was purchased from Sigma-Aldrich (St. Louis, USA). UV-transparent cuvettes (REF 67.758.001) for optomagnetic measurements were purchased from SARSTEDT (Nümbrecht, Germany). Particles and biotinylated anti-streptavidin were suspended or diluted in 1 × PBS containing 0.1% (1 mg/mL) BSA.

2.2. Antibody conjugation of MNPs

The polyclonal sheep anti-PSA antibody was biotinylated using the biotin conjugation kit according to the instructions provided by the manufacturer. Antibody-conjugated 250 nm MNPs were prepared by adding 100 μg of biotinylated sheep anti-PSA antibody into 0.1 mL of 250 nm streptavidin coated MNPs (10 mg/mL, 1.5–2 μg streptavidin/mg MNP) followed by incubation at room temperature for 1 h. After washing three times with PBS using a magnetic stand, the Ab-MNPs were resuspended at a concentration of 1 mg/mL in PBS (containing 0.1% BSA) and stored at 4 °C.

2.3. RMF incubation platform and optomagnetic setup

The RMF platform contains a pair of computer-controlled, perpendicular iron-core magnetic circuits that can generate a homogeneous magnetic field in the central part of the platform. The chain formation process in an RMF was observed using an Olympus BX60 microscope equipped with a 10× objective and a digital camera, and images were analyzed using the public domain Java image processing software ImageJ (Bejhed et al., 2015; Schneider et al., 2012). For the optomagnetic setup, a detailed description as well as the underlying theory can be found in our previous publications (Fock et al., 2017a; Tian et al., 2017). Briefly, an AC magnetic excitation field, $H(t) = H_0 \sin(2\pi ft)$, with H_0 = 2.1 kA/m was applied parallel to the laser beam (λ = 405 nm, a light beam diameter of 2 mm), and the optical path through the cuvette was 10 mm. The real ($\sin(4\pi ft)$ part of the second harmonic component of the transmitted light intensity, V'_2, was recorded and normalized with respect to the simultaneously measured total intensity of transmitted laser light, V_0, to compensate for the variations in laser light intensity, particle concentration and cuvette reflection/absorption.

2.4. Reaction and optomagnetic measurement

Streptavidin coated MNPs and anti-PSA antibody-conjugated MNPs were utilized for the detection of biotinylated anti-streptavidin and PSA, respectively. The sample (95 μL) was mixed with 5 μL of biofunctionalized 250 nm MNPs to a final MNP concentration of 50 μg/mL (4.07 fM), followed by incubation at room temperature in the RMF platform. A rotational frequency of 1 Hz was applied. For plotting dose-response curves of biotinylated anti-streptavidin and PSA, the incubation time was 15 min and the RMF strength, H_{RMF}, was 10 kA/m. Thereafter the suspension was measured by the optomagnetic setup. Twenty-five logarithmically equidistant frequency points were recorded in the frequency range of 0.3–100 Hz, and the data acquisition time was 270 s. The cutoff value was calculated as the average value

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