



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, $20\times$) 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 $1\times$ 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 μ g of biotinylated sheep anti-PSA antibody into 0.1 mL of 250 nm streptavidin coated MNPs (10 mg/mL, 1.5–2 μ g 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 $10\times$ 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 ($\lambda = 405$ 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 μ L) was mixed with 5 μ L of bio-functionalized 250 nm MNPs to a final MNP concentration of 50 μ g/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

(measured at 1.3 Hz or 18.4 Hz) of the blank control samples minus three standard deviations, and the LOD was obtained based on the 3 σ criterion.

2.5. Measurement of static magnetization curves

A hysteresis loop starting with a virgin curve was measured at 300 K in the field range –60–60 kA/m for a 10 mg/mL suspension of the non-conjugated MNPs adsorbed and dried on cotton wool. For this, an MPMS-XL SQUID magnetometer (Quantum Design, USA) was used.

2.6. Scanning electron microscopy characterization

The morphology and nanostructure of dispersed 250 nm MNPs (MNPs are of multicore type, *i.e.*, built up of a clusters of small single domain particles) and MNP chains were characterized by scanning electron microscopy (SEM, Zeiss-1530) using an in-lens detector for secondary electrons and the microscope was operated at 10 kV electron beam accelerating voltage.

3. Results and discussion

3.1. Principle of the shape anisotropy enhanced optomagnetic biosensor

The principle of the shape anisotropy enhanced optomagnetic sensor is illustrated in Fig. 1. A video, provided as [Supplementary material](#), illustrates the setup and detection principle. The functionalized 250 nm MNPs form dipolar chains in the presence of the RMF and are subsequently stabilized in presence of the multivalent target.

The RMF is turned off after a short incubation period to allow non-stabilized chains to disassemble. In the absence of multivalent target molecules, the magnetic chains quickly dissociate during the optomagnetic measurement and correspondingly the optomagnetic spectrum contains only the signal from unbound MNPs. In the presence of multivalent target molecules, the optomagnetic spectrum contains contributions from both unbound MNPs and target-stabilized MNP chains. The characteristic valley/peak positions in the optomagnetic V'_2/V_0 spectra are related (but not equal) to the Brownian relaxation frequencies of the particles (Fock et al., 2017a). For particles in liquid, the characteristic frequency for Brownian relaxation is given by

$$f_B = k_B T / (6\pi\eta V_h),$$

where $k_B T$ is the thermal energy, η is the viscosity and V_h is the mean hydrodynamic volume of the particle. Target molecules captured by the chains can be quantified by measuring the V'_2/V_0 spectra of the suspension. A typical V'_2/V_0 spectrum in this study is characterized by a valley in the low-frequency region that represents the response of target-stabilized chains (if present), and a peak in the high-frequency region that represents the unbound MNPs. The sign changes of the characteristic peaks (valleys) are caused by the dependence of the optical extinction coefficient on the sizes of MNPs and chains (Fock et al., 2017b). Target quantification can be accomplished by monitoring either the valley or peak amplitudes.

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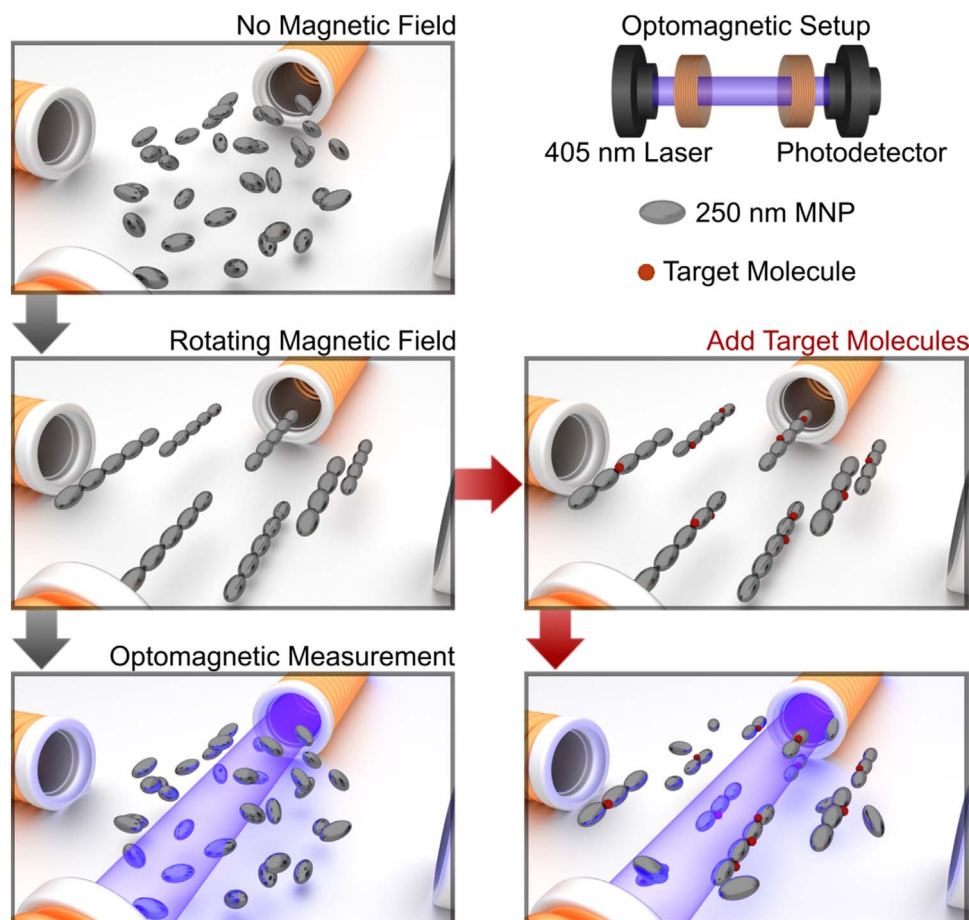


Fig. 1. Schematic illustration of the shape anisotropy enhanced optomagnetic biosensor. The assay includes two independent steps: incubation in an RMF and optomagnetic measurement. Gray and red arrows indicate the assay steps of a blank control sample (the left panels) and of a positive sample (the right panels), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

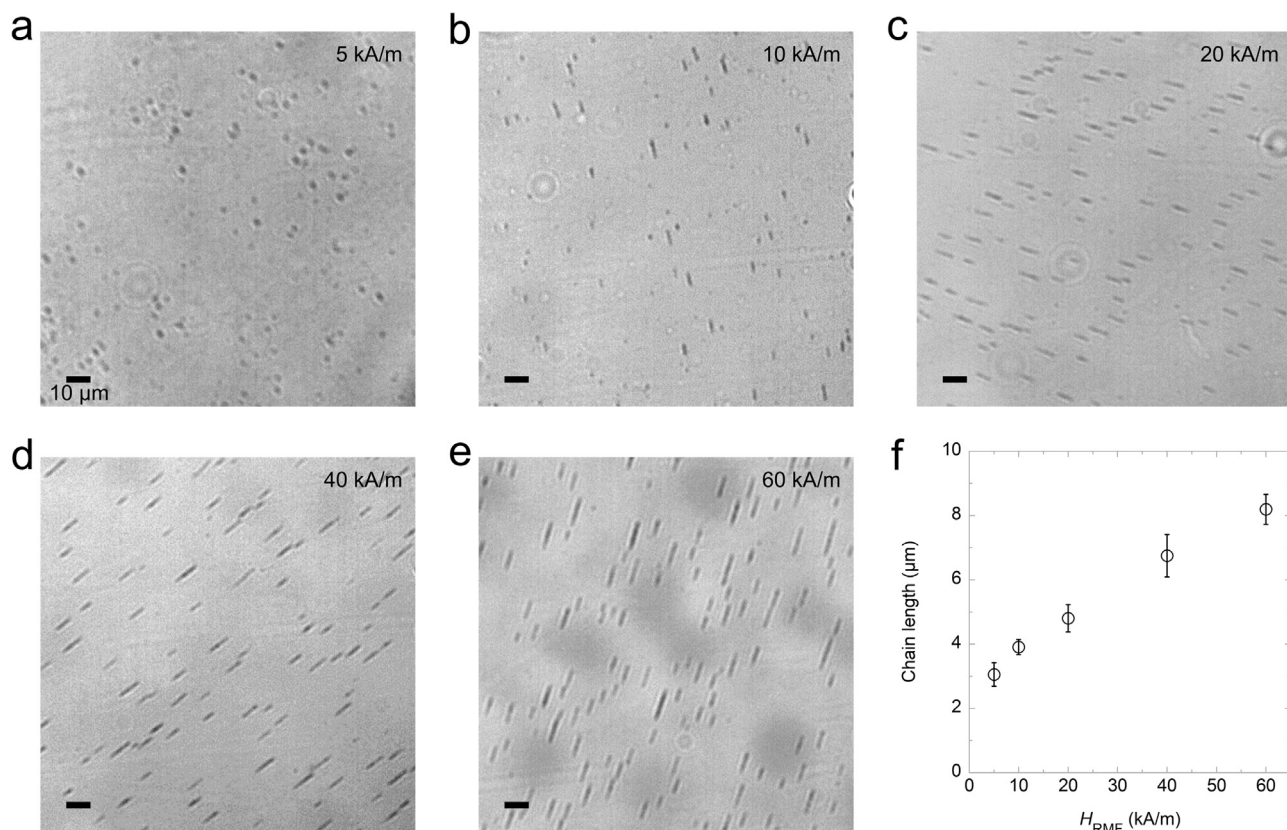


Fig. 2. Observation of magnetic chains in an optical microscope. (a)–(e) Representative top-view images of magnetic chains formed in different H_{RMF} . (f) Effect of H_{RMF} on the average chain length. Error bars indicate the standard deviation of the average chain lengths for four different images.

3.2. Optimization of the assay

During the incubation step in the RMF platform, magnetic chains rotate in-phase with the RMF. This gives rise to a stirring of the fluid that accelerates the reaction speed of the homogeneous assay. The formation of chains of superparamagnetic MNPs can be described by the dimensionless Mason number (Mn) which considers both the rotational shear force and the magnetic interaction force (Melle and Martin, 2003; Petousis et al., 2007):

$$\text{Mn} \equiv \frac{F_{\text{H}}}{F_{\text{m}}} \approx \frac{16\eta\omega}{\mu_0\chi^2 H_{\text{RMF}}^2},$$

where ω is the angular rotation frequency of the RMF, μ_0 is the permeability of free space, and χ is the effective magnetic susceptibility of the MNPs. As shown by the magnetization curve (Fig. S1 in Supplementary material) measured at 300 K for non-conjugated 250 nm MNPs (concentration 10 mg/mL, particles adsorbed and dried in cotton wool), the particles have an approximately superparamagnetic linear behavior (with a small remanence) for fields up to 60 kA/m, and therefore the above expression for the Mason number is qualitatively valid for our study. There is a positive correlation between the average length and H_{RMF} , since the average number of MNPs in a stable chain can be described by (Petousis et al., 2007)

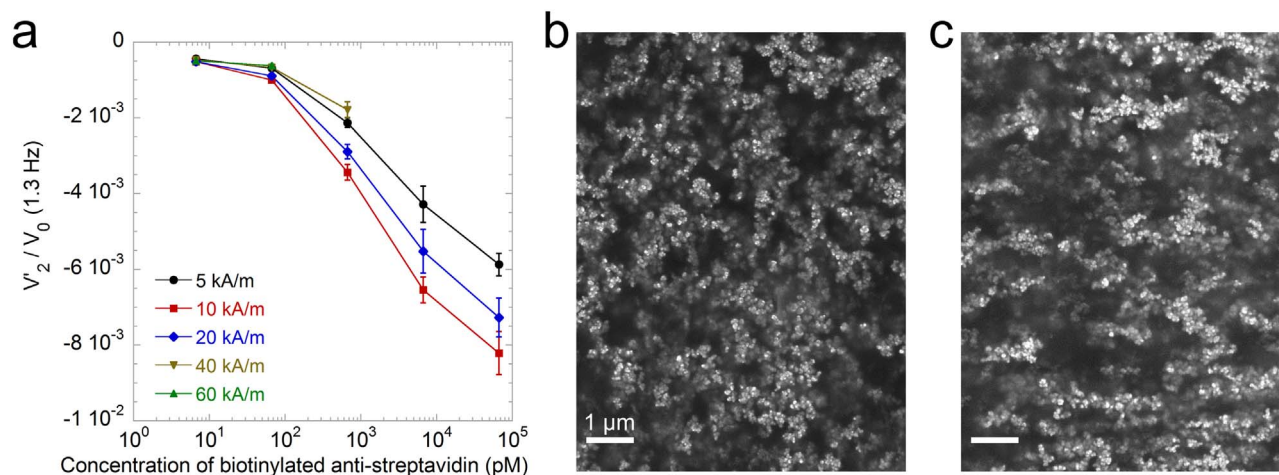


Fig. 3. (a) Relationship between V_2/V_0 valley amplitude (measured at 1.3 Hz) and logarithm of model target (biotinylated anti-streptavidin) concentration at different H_{RMF} (5, 10, 20, 40 and 60 kA/m). Error bars indicate the standard deviation of three independent replicates. (b) Representative SEM micrograph of dispersed MNPs. (c) Representative SEM micrograph of magnetic chains formed at $H_{\text{RMF}} = 10$ kA/m (the concentration of biotinylated anti-streptavidin is 6.7 nM).

$$N \approx Mn^{-0.5}.$$

The binding force induced by the multivalent target molecules, F_{binding} , can decrease the Mason number (Park et al., 2010a), then $Mn \equiv F_H / (F_m + F_{\text{binding}})$. Therefore the average chain length is increased due to the binding force.

The average chain length versus field amplitude was investigated in the RMF platform (Fig. 2). Following optimization routines reported elsewhere (Ranzoni et al., 2011), a rotation frequency of 1 Hz was chosen for the RMF; while H_{RMF} was set to 5, 10, 20, 40 and 60 kA/m (Fig. 2a to Fig. 2e). For each of the five different H_{RMF} , four images (corresponding to four randomly selected places) were taken. The average chain length was calculated for each image. The mean value and the standard deviation of the four average chain lengths for the four different images were calculated for each H_{RMF} (Fig. 2f).

We argue that the target-mediated stabilization of chains occurs mainly in two ways: (1) a multivalent molecule reaches the junction area between two MNPs and forms a sandwich structure; and (2) a multivalent molecule captured on the surface of one MNP encounters another MNP and forms a sandwich structure. The first type of stabilization effect can be enhanced by forming longer chains (providing more junction areas, which need high H_{RMF}), increasing the particle velocities (particle actuation can resolve the limitations of depletion effects), and stirring of the fluid (convection). The second stabilization type is favored for shorter chains. Provided that MNPs in the same chain have fixed positions relative to each other, the second type of sandwich formation mainly occurs at the terminal MNPs of the chain during the repeatedly breaking and reforming of magnetic chains. For a certain MNP concentration, a higher H_{RMF} induces longer chains, leading to fewer terminal MNPs and less stabilization of the second type. From this we argue that there exists an optimum H_{RMF} for chain stabilization and for assay performance. Increasing the concentration of MNPs is not considered in this study since it will decrease the sensitivity of the method (Tian et al., 2016c). Additionally, a too high H_{RMF} leads to the formation of large MNP clusters, which are undetectable in the current optomagnetic setup, since their response fall below the detection frequency window.

In order to optimize the RMF parameters, biotinylated anti-streptavidin was used as a model multivalent target molecule to stabilize magnetic chains formed by streptavidin coated MNPs. Biotinylated anti-streptavidin of different concentrations was mixed with streptavidin coated MNPs and incubated in an RMF for 10 min at room temperature followed by optomagnetic measurement. The V'_2/V_0 valley amplitude at 1.3 Hz, which represents the response from magnetic chains, was recorded at different H_{RMF} and plotted against the target concentration to obtain an optimum H_{RMF} (Fig. 3a). Due to the formation and sedimentation of large MNP clusters formed at high

H_{RMF} , V'_2/V_0 amplitudes of high target concentration samples incubated at 40 and 60 kA/m do not represent magnetic chains and have not been plotted. From Fig. 3a it can be concluded that the optimum H_{RMF} is around 10 kA/m, therefore a H_{RMF} of 10 kA/m was applied in subsequent experiments. The optimum H_{RMF} is caused by a balance between the two stabilization effects discussed above.

The morphology of dispersed 250 nm MNPs (built up of clusters of small single domain particles) and MNP chains was characterized by SEM. Representative micrographs are shown in Fig. 3b and c, respectively. Due to the multicore nanostructure as well as due to drying effects, it is difficult to get any information about the uniformity of the 250 nm MNPs. However, aggregated by 6.7 nM of biotinylated anti-streptavidin in the RMF ($H_{\text{RMF}} = 10$ kA/m), magnetic chains have a clear shape anisotropy compared with dispersed 250 nm MNPs. Since we were unable to perform SEM characterization in the presence of an applied RMF, chains observed in SEM micrographs are much shorter than those in Fig. 2b due to the breakup of magnetic dipolar bindings.

3.3. Quantitative detection of the model target in PBS

Under optimized experimental conditions (RMF of 1 Hz, 10 kA/m), quantitative detection of the model target molecule was investigated. Serial dilutions of biotinylated anti-streptavidin, ranging from 66.7 nM (10 $\mu\text{g/mL}$) to 1 pM (150 pg/mL), were reacted with streptavidin coated MNPs followed by optomagnetic measurements. From the V'_2/V_0 spectra shown in Fig. 4a it can be seen that the valley (measured at 1.3 Hz) and peak (measured at 18.4 Hz) amplitude increases and decreases, respectively, with increasing target concentration. A typical V'_2/V_0 spectrum is characterized by a valley located at around 1.3–1.6 Hz, which represents the stabilized magnetic chains and a peak located at around 14–18 Hz which represents the unbound MNPs. The characteristic valley and peak positions were slightly shifted due to the increase of chain length and the small overlap between the spectra of unbound MNPs and magnetic chains. Interestingly, the hydrodynamic size of the magnetic chains only slightly increased with increasing target concentration, meaning that the increase of the V'_2/V_0 amplitude at 1.3 Hz was mainly caused by the increasing amount of magnetic chains but not by the increase of the chain length. The dose-response curve for biotinylated anti-streptavidin is shown in Fig. 4b. A linear correlation between the V'_2/V_0 valley amplitude and the target concentration can be observed between 1 and 260 pM (see inset in Fig. 4b) with an average coefficient of variation (CV) of 7.4% and an LOD of 5.5 pM obtained according to the 3σ criterion.

As a control experiment, detection of biotinylated anti-streptavidin was performed without RMF, and the results are presented in Fig. 4b (blue squares). Without RMF, the V'_2/V_0 signal at 1.3 Hz is low for all

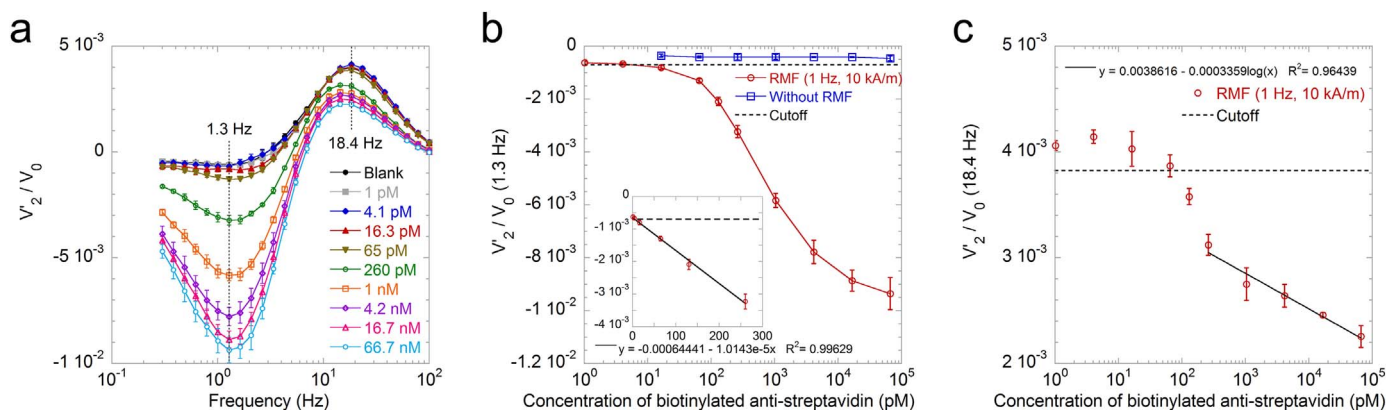


Fig. 4. V'_2/V_0 spectra (a) and dose-response curves (b)–(c) for shape anisotropy enhanced optomagnetic detection of biotinylated anti-streptavidin in PBS. Valley (measured at 1.3 Hz) and peak (measured at 18.4 Hz) amplitudes of the V'_2/V_0 spectra are plotted against the target concentration in panels b and c, respectively. Black lines in panel c and inset of panel b show the linear detection range. Error bars indicate the standard deviation of three independent replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

target concentrations (from 16.3 pM to 66.7 nM). This is caused by depletion effects as well as the slow diffusion of MNPs in conventional (without RMF) particle-based capture experiments (van Reenen et al., 2017). The transport of targets across the local concentration gradient is dominated by diffusion when the particles are at low velocities. The result is in good agreement with a previous study based on magnetic incubation (Antunes et al., 2015).

The V_2/V_0 value at 18.4 Hz, which represents the concentration of unbound MNPs in the suspension, could also be utilized for indirect monitoring of the chain formation reaction (see Fig. 4c). Although the sensitivity of this turn-off detection format is comparatively low, a linear correlation between the V_2/V_0 peak amplitude (measured at 18.4 Hz) and logarithm of target concentration is observed between 260 pM and 66.7 nM (the black line in Fig. 4c) with an average CV of 3.7%. Considering that the reduction of the V_2/V_0 value at 18.4 Hz is not affected by the size or shape of the MNP aggregates, the saturation concentration for V_2/V_0 at 18.4 Hz is much higher than the corresponding value at 1.3 Hz. Therefore, monitoring the concentration of unbound MNPs can be used to detect high target concentrations when the signal at 1.3 Hz is approaching saturation. By combining the turn-on and turn-off read-outs presented in Fig. 4, we have demonstrated a two-section linear detection range of approximately four orders of magnitude (from 1 pM to 66.7 nM). Note that for the low-concentration section, the signal is linear with target concentration, whereas, for the high-concentration section, the signal is linear with the logarithm of target concentration. In addition, since MNPs of different sizes have different characteristic valley/peak frequencies, MNPs of different sizes can be coated with different antibodies for detecting different biomacromolecules simultaneously. This means that the proposed biosensor has the potential for multiplex biosensing which cannot be achieved by other optomagnetic biosensors that only monitor the chains.

3.4. Quantitative detection of PSA in serum

Blood analysis of PSA is performed for initial diagnosis and for monitoring response to treatment. However, it is challenging to perform assays in complex biological matrices due to matrix effects. Here we analyze PSA spiked in fetal bovine serum to demonstrate the applicability of our biosensor for detection in biological matrixes. Fetal bovine serum has three main effects that may influence the assay: (1) inducing aggregation of MNPs, (2) inhibiting the immuno-reaction and (3) increasing the viscosity of the suspension. In previous work, the aggregation effect was evaluated (Tian et al., 2017), and here we chose 50% serum to avoid significant aggregation. The higher viscosity of the serum influences both the Mason number and the Brownian relaxation frequency of MNPs and chains, which could reduce the sensitivity of the system.

Fig. 5a shows V_2/V_0 at 1.3 Hz as a function of RMF incubation time. For the detection of 1 nM of PSA spiked in 50% serum (the concentration of PSA is lower than the surface concentration of antibodies on MNPs), the signal increased dramatically during the first 9 min. After that, non-specific bindings were gradually replaced by antibody-antigen bindings, resulting in a slight increase of the signal. For the detection of 16.7 nM of PSA spiked in 50% serum (the concentration of PSA is much higher than the surface concentration of antibodies on MNPs), the signal kept increasing during the measurement, suggesting that a longer incubation time is needed to reach the signal saturation level for higher target concentrations. Considering that an incubation time of 15 min is enough to reach a saturation signal level for samples of low target concentrations, prolonging the incubation time can hardly improve the sensitivity of the system. Therefore, we chose 15 min as the incubation time to provide a universal biosensing system.

The V_2/V_0 spectra and dose-response curve of PSA detection in 50% serum are shown in Fig. 5b–c, respectively. The result shows that detection of PSA in 50% serum samples can be achieved with an LOD of 21.6 pM (0.65 ng/mL) according to the 3σ criterion and with a dynamic range up to 66.7 nM (2 μ g/mL). The average CV is 5.9%. The sample-to-answer time of PSA detection in serum is approximately 20 min including 15 min for incubation in the RMF and 4.5 min for measurement. The matrix effects do not have an obvious influence on the valley/peak positions or on the shape of the spectra. Considering that serum contains a high concentration of background bio-macromolecules, we did not perform further investigations on the specificity of the proposed biosensor.

The proposed biosensor system has several advantages compared with magnetic cluster-based 405 nm-laser optomagnetic sensors. Firstly, the RMF and the magnetic chains induce efficient mixing and thereby overcome the limitations imposed by target diffusion. According to the theory of actuated particle capture and target depletion zone (van Reenen et al., 2017), applying an RMF can achieve a higher capture efficiency than applying a static field or a field gradient only. However the accelerating effect is difficult to quantify due to the end-point detection format employed in the current work. Secondly, the shape anisotropy enhances the V_2/V_0 signal amplitude, thus increasing the accuracy of measurement. Lastly, by combining the turn-on magnetic chain measurement (monitoring V_2/V_0 at low frequency) with the turn-off unbound MNP measurement (monitoring V_2/V_0 at high frequency), a two-section linear detection range of approximately four orders of magnitude has been achieved, which is much wider than for previous immuno-optomagnetic biosensors. Another PSA detecting optomagnetic biosensor, reported by Ranzoni et al. (2012), measured the scattered light from oscillating magnetic field-induced particle dimers and achieved an LOD of 0.5 pM with a dynamic detection range of 0.5–200 pM (0.015–6 ng/mL). Although our biosensor in comparison with Ranzoni et al. (2012) is less sensitive

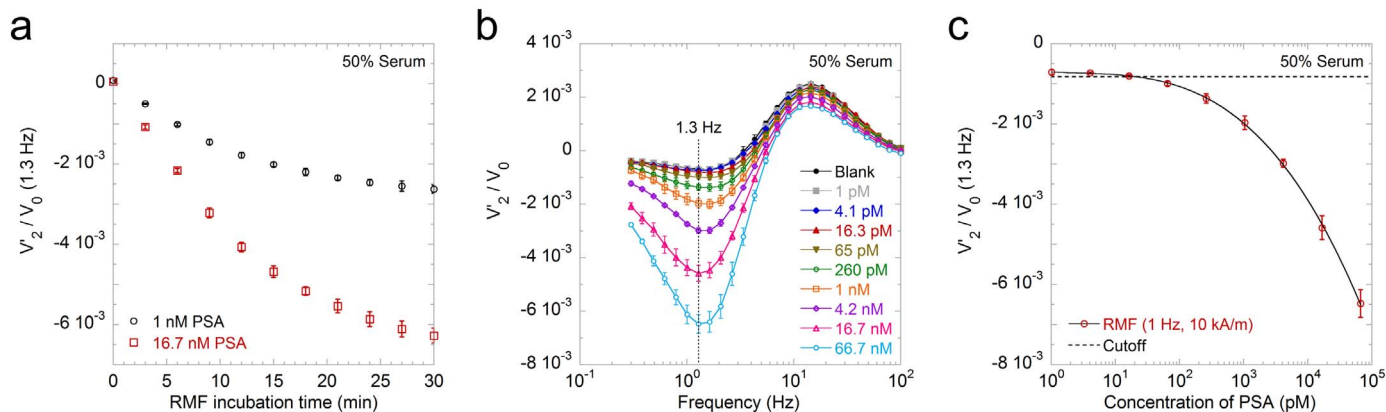


Fig. 5. Effect of RMF incubation time (a), V_2/V_0 spectra (b) and dose-response curve (c) for shape anisotropy enhanced optomagnetic detection of PSA in 50% serum. Error bars indicate the standard deviation of three independent replicates.

due to the lack of dimer detection capability, our LOD for PSA (0.65 ng/mL) is still well below the clinical threshold PSA level in blood (4 ng/mL). In addition, our biosensor provides a wider detection range (up to 2 µg/mL) that is comparable to the clinical concentration range of PSA in blood (up to 10 µg/mL).

4. Conclusion

In summary, we have demonstrated a rapid biosensor principle for bio-macromolecule detection utilizing field-assembly of dipolar magnetic chains and shape anisotropy enhanced optomagnetic technology. The formation of particle chains enhances the biosensor system by mixing (convection) and increasing the optomagnetic signal. Our method allows for the simultaneous detection of MNP chains and unbound MNPs. We have optimized and tested the proposed biosensor by quantifying a multivalent model protein, biotinylated anti-streptavidin, in PBS. Thereafter the biosensor was further evaluated for detection of PSA spiked in 50% serum, for which an LOD of 21.6 pM (0.65 ng/mL) was achieved with a dynamic detection range up to 66.7 nM (2 µg/mL). The performance of our biosensor 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). The detection volume is 100 µL and the sample-to-answer time is approximately 20 min. On the basis of the two protein detection studies, we have shown that the shape anisotropy enhanced optomagnetic biosensor is rapid and sensitive enough for *in-situ* PSA diagnostics and other clinical detections of multivalent molecules. Simultaneous detection of different biomarkers is one of our future topics, which can be achieved by monitoring both chains and unbound MNPs of different sizes. In addition, by performing the measurement on a microfluidic disc (Donolato et al., 2015b), the detection volume can be reduced to a few microliters and the biosensor can enable a detection format close to real-time, which will be another topic for future work.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bios.2017.06.062](https://doi.org/10.1016/j.bios.2017.06.062).

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