Molecular diagnostics using magnetic nanobeads

Teresa Zardán Gómez de la Torre¹, Mattias Strömberg¹, Jenny Göransson¹,², Klas Gunnarsson¹, Mats Nilsson², Peter Svedlindh³, and Maria Strømme¹

¹ Department of Engineering Sciences, Division of Nanotechnology and Functional Materials, Uppsala University, Box 534, SE-751 21 Uppsala, Sweden
² Department of Genetics and Pathology, Uppsala University, Rudbeck Laboratory, SE-751 85 Uppsala, Sweden
³ Department of Engineering Sciences, Division of Solid State Physics, Uppsala University, Box 534, SE-751 21 Uppsala, Sweden

teresa.zardan@angstrom.uu.se

Abstract. In this paper, we investigate the volume-amplified magnetic nanobead detection assay with respect to bead size, bead concentration and bead oligonucleotide surface coverage in order to improve the understanding of the underlying microscopic mechanisms. It has been shown that: (i) the immobilization efficiency of the beads depends on the surface coverage of oligonucleotides, (ii) by using lower amounts of probe-tagged beads, detection sensitivity can be improved and (iii) using small enough beads enables both turn-off and turn-on detection. Finally, biplex detection was demonstrated.

1. Introduction

There is an increasing need for simple and cost-efficient bioassay formats for sensitive DNA sequence analysis appropriate for the diagnostic setting. Biofunctionalized magnetic nanobeads are suitable for these applications due to their magnetic properties and biocompatibility [1]. Magnetic bead based bioassays involve labelling of the beads with probe biomolecules, e.g. antibodies or ssDNA (single-stranded DNA). Upon interaction with the target molecule, a change in either static or dynamic magnetic bead properties are detected.

Magnetic bioassays schemes categorize as substrate-based (chip based) or substrate-free. In the former category, if the target molecule is present, the biofunctionalized magnetic beads bind to the sensor surface. Some examples of substrate-based sensors are GMR biosensors [2] and Micro-Hall devices [3]. An example of a substrate-free sensor is the Brownian relaxation biosensor scheme, which detects the decrease of the bead Brownian relaxation frequency caused by the hydrodynamic size increase upon probe-target coupling [4].

We have recently demonstrated a novel nanodiagnostic principle, based on DNA sequence detection; using ssDNA probe-tagged magnetic nanobeads, called the volume-amplified magnetic nanobead detection assay (VAM-NDA) [5]. The bioassay begins with padlock probe target
recognition followed by ligation where the ends of the padlock probe molecule are joined together [6]. The rolling circle amplification (RCA) [7] is then initiated by addition of DNA polymerase where the padlock probes are amplified during a certain time, to form random coiled ssDNA macromolecules (RCA coils). The RCA coils are detected by adding ssDNA tagged magnetic nanobeads. During an incubation step, the beads bind to the RCA coils by base-pair hybridization. The hydrodynamic size of the immobilized magnetic beads is now increased, corresponding essentially to the size of an RCA coil. This causes a strong decrease of the bead Brownian relaxation frequency \( f_B \). Upon immobilization, the magnitude of the imaginary part \( (m''^*) \) of the complex magnetization \( m = m' - im'' \) at \( f_B \) for non-immobilized beads, the high frequency peak level (HFP level), decreases. RCA coils with immobilized beads exhibit a relaxation peak, the low frequency peak (LFP).

Different experimental parameters influencing the outcome of the assay such as bead size, bead concentration and bead surface coverage of oligonucleotides have been investigated in order to improve the understanding of the underlying microscopic mechanisms and for assay optimization. We have also given a proof-of-principle of quantitative biplex detection.

2. Materials and methods

In this study, three aqueous suspensions of amino functionalized cluster-type magnetic beads (nanomag ™-D NH₂; Micromod Partikeltechnologie GmbH) were used. The nominal diameters of the beads were 40, 130 and 250 nm. The oligonucleotide surface functionalization is described elsewhere [5, 8] and the surface coverage was determined by a fluorescence based method [5, 8]. The procedures for performing target recognition, RCA, sample preparation and characterization are described in previous publications [5, 8].

3. Results and Discussion

\( m'' \) vs. frequency spectra at 310 K for samples with 40 nm beads and various RCA coil concentrations are presented in Figure 1. Two batches with different oligonucleotide surface coverages were used. The 0 pM curves correspond to samples with no RCA coils (negative control). Comparing panels a and b, it can be concluded that the bead immobilization efficiency tends to increase with increasing oligonucleotide surface coverage. This is most likely due to that a higher oligonucleotide surface coverage yields more possibilities for a bead to hybridize to an RCA coil. Another observation is that discrimination between a positive and a negative control sample can be made using the HFP and LFP levels, also called “turn-off” and “turn-on” detection, respectively. The “turn-off” effect is the decrease of HFP level due to immobilization of free beads in the RCA coils. The “turn-on” effect is the increase of LFP level when the beads are immobilized in the coils. In ref. [5], where 130 nm beads were used, discrimination between a negative and a positive sample was only possible through the “turn-off” scheme. Thus, using small enough beads may open up the possibility to perform both “turn-off” and “turn-on” detection, whereas for large beads only “turn-off” detection may be possible.
Figure 1. $m''$ vs. frequency spectra at 310 K for samples with 40 nm beads and different RCA coil concentrations (0-300 pM). Batches of beads with 3 and 14 oligonucleotides were used in panel a and b, respectively.

The effect of decreasing the concentration of 250 nm beads is presented in Figure 2, where the $m''$ vs. frequency spectra at 310 K for samples with different RCA coil concentrations are shown. A lower bead concentration is used in panel b than in panel a. The $m''$ curves for the 300 pM sample are small in magnitude, indicating that almost 100% of the beads are immobilized in the coils, which is expected due to the very low bead concentration. Furthermore, no distinct LFPs can be observed even for the highest RCA-coil concentrations despite the fact that the HFP levels are very low. This is probably due to that the 250 nm beads immobilize in a different manner compared to 40 nm beads. Small beads may possibly immobilize in the interior of the coils giving separate coils with beads, all having a relaxation frequency around 1 Hz (see Figure 1). The 250 nm beads probably show preference to bind to the exterior of the coils, thereby linking several coils together and tend to give species with relaxation frequencies lower than 0.5 Hz. Comparing panel a with panel b, it can be observed that the LOD is improved from 11 pM to 3.7 pM. Increased detection sensitivity upon decreasing the bead concentration is expected since the number of beads relative to the number of RCA coils is smaller.

Figure 2. $m''$ vs. frequency spectra at 310 K for samples with 250 nm beads and different RCA coil concentrations (0-300 pM). A lower concentration of beads was used in panel b compared to panel a.

Figure 3 shows composite complex magnetization spectra at 310 K visualizing the results of a quantitative biplex experiment of *Vibrio cholerae* (T130) and *V. vulnificus* (T250) DNA sequences. A batch consisting of a mixture of probe-tagged 130 nm (used for detection of T130 coils) and 250 nm (used for detection of T250 coils) beads was used for simultaneous detection of the two kinds of RCA coils in the same compartment [9]. It can be seen from panel a that the magnitude of $m'$ tends to decrease when the sum of the target concentrations increases. This is reasonable since this quantity should correlate with the total amount of immobilized beads [5]. Furthermore, since each spectrum in Figure 3b is to a good approximation a superposition of two HFPs, a composite $m''$ spectrum can be deconvoluted through a non-linear curve fitting procedure in order to obtain the T130 and T250 HFP levels. This gives simultaneous target quantification [9].
Figure 3. Composite complex magnetization spectra at 310 K visualizing the outcome of biplex detection experiment of Vibrio vulnificus (T250) and V. cholerae (T130) DNA sequences. Panel a and b displays the real and imaginary part of the complex magnetization, respectively.

4. Summary and Conclusion
The effects of varying the bead size, bead surface coverage of probe oligonucleotides and bead concentration on the performance of the volume-amplified magnetic nanobead detection assay were evaluated. It was found that using small enough beads, both turn-off and turn-on detection is possible whereas only turn-off detection is possible using larger beads. This is most likely an effect of different bead immobilization characteristics. It was also found that decreasing the bead concentration and increasing the oligonucleotide surface coverage improves the detection sensitivity. Finally, a proof-of-principle of biplex detection was given.

Acknowledgements
The Knut and Alice Wallenberg Foundation (KAW), the Swedish Foundation for Strategic Research (SSF), the Swedish Defence Nanotechnology programme, and the Swedish Research Council (VR), are gratefully acknowledged for their financial support.

References
[1] Sassolas A, Leca-Bouvier BD and Blum LJ 2008 Chem. Rev. 108 109-39
[2] Schotter J, Kamp PB, Becker A, Puhler A, Reiss G and Bruckl H 2004 Biosens. Bioelectron. 19 1149-56
[3] Lee W, Joo S, Kim SU, Rhie K, Hong J, Shin KH and Kim KH 2009 Appl. Phys. Lett. 94 153903-1 - 03-3
[4] Connolly J and St Pierre TG 2001 J. Magn. Magn. Mater. 225 156-60
[5] Strömberg M, Göransson J, Gunnarsson K, Nilsson M, Svedlindh P and Strømme M 2008 Nano Lett. 8 816-21
[6] Nilsson M, Malmgren H, Samiotaki M, Kwiatkowski M, Chowdhary BP and Landegren U 1994 Science 265 2085-88
[7] Fire A and Xu SQ 1995 Proc. Natl. Acad. Sci. U. S. A. 92 4641-45
[8] Strömberg M, Zardán Gómez de la Torre T, Göransson J, Gunnarsson K, Nilsson M, Strømme M and Svedlindh P 2008 Biosens. Bioelectron. 24 696-703
[9] Strömberg M, Zardán Gómez de la Torre T, Göransson J, Gunnarsson K, Nilsson M, Svedlindh P and Strømme M 2009 Anal. Chem. 81 3398-406