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Evaluation of different strategies for magnetic particle functionalization with DNA aptamers

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Highlights

- Commercial magnetic particles were tested for functionalization with aptamers
- The highest surface coverage with aptamers was found for streptavidin particles
- Streptavidin particles also showed the greatest target capturing efficiency
- The performance of streptavidin particles was tested in aptamer sandwich assay
- The obtained LOD was comparable to that using antibodies as receptors

Abstract

The optimal bio-functionalization of magnetic particles is essential for developing magnetic particle-based bioassays. Whereas functionalization with antibodies is generally well established, immobilization of DNA probes, such as aptamers, is not yet fully explored. In this work, four different types of commercially available magnetic particles, coated with streptavidin, maleimide or carboxyl groups, were evaluated for their surface coverage with aptamer bioreceptors, efficiency in capturing target protein and non-specific protein adsorption on their surface. A recently developed aptamer against the peanut allergen, Ara h 1 protein, was used as a model system. Conjugation of biotinylated Ara h 1 aptamer to the streptavidin particles led to the highest surface coverage, whereas the coverage of maleimide particles was 25% lower. Carboxylated particles appeared to be inadequate for DNA functionalization. Streptavidin particles also showed the greatest target capturing efficiency, comparable to the one of particles functionalized with anti-Ara h 1 antibody. The performance of streptavidin particles was additionally tested in a sandwich assay with the aptamer as a capture receptor on the particle surface. While the limit of detection obtained was comparable to the same assay system with antibody as capture receptor, it was superior to previously reported values using the same aptamer in similar assay schemes with different detection platforms. These results point to the promising application of the Ara h 1 aptamer-functionalized particles in bioassay development.

Key words: Magnetic particles, biofunctionalization, DNA, aptamer

1. Introduction

The field of molecular sensing has been boosted over the years by the advances in bio-functionalized materials[1–3]. In particular, progress in synthesis chemistry has allowed the creation of monodisperse particles of controlled sizes, shapes and compositions, thereby providing a way to precisely manipulate material properties[4]. Specifically, nano- and micrometer-sized magnetic particles have proved to be valuable due to their unique properties, such as magnetization under the influence of an external field and their large surface-to volume ratio. As a result, magnetic particles offer exciting opportunities for technologies at the interfaces between chemistry, physics and biology, and have found application in a broad range of fields, such as electronics[5], therapeutics[6,7] and diagnostics[8–10]. Moreover, they have been shown to be suitable as solid supports in bio-assay development[11] for capture[12,13], preconcentration[14,15], separation[16,17], transportation[18,19] and detection[20,21] of analytes.

Magnetic particles typically consist of a magnetic core and a non-magnetic shell. For bioassay development, this outer shell must be chemically activated to allow binding or adsorption of biomolecules. Functionalization of magnetic particles commonly occurs via covalent binding of a biomolecule to the active surface of the particle using bio-conjugation chemistries, which most commonly relies on bifunctional cross-linkers. A wide range of (bio)chemical reactions are available for coating almost any surface and with most types of molecule[22]. Although it was initially thought that the conjugation method would not influence the assay, it was later found that particular conjugation methods resulted in a higher degree of nonspecific binding than others[23]. Therefore, for an optimal performance of a biosensor, control over the functionality of the magnetic particles (e.g. degree of surface coverage by a biomolecule) is crucial.

Most biosensors nowadays still rely on antibodies as biorecognition elements. In this case, among all conjugation methods, carbodiimide crosslinking is by far the most popular, independent of the application[24–26]. However, in recent years, aptamers have been promoted as ideal aspiring bioreceptors, since they show various advantages when compared to antibodies[27], with the most important being: (i) *in vitro* selection that can be performed in conditions analogous to the real sample matrix, (ii) easy synthesis with high reproducibility and minimal batch to batch variation, (iii) higher stability and consequently longer shelf life, and (iv) easy modification towards custom tailored properties[28,29]. These benefits make aptamers an interesting alternative that is attracting growing interest in the biosensing field. However, although different approaches for conjugation of

DNA probes to gold[30,31] or silica[32] nanoparticles have been published, to the best of our knowledge there are no published studies evaluating different strategies for the conjugation of DNA aptamers to magnetic particles. This information is crucial for the optimal application of aptamer-functionalized magnetic particles in bioassays.

In this context, different surface chemistry protocols for the immobilization of aptamer receptors on magnetic particles have been evaluated here, alongside the target capture efficiency of such aptamer coated magnetic particles. Four different types of magnetic particle were selected according to the most popular and versatile surface chemistry methods employed for biofunctionalization. In order to establish the most suitable approach, three different parameters were evaluated: (1) particle surface coverage with bioreceptor molecules, (2) efficiency in capturing target protein by the functionalized particles and (3) non-specific protein adsorption on the functionalized particles.

A recently developed aptamer against the peanut allergen, Ara h 1 protein, was selected as a model system in this study[33]. Peanut allergy is one of the most serious immediate hypersensitivity reactions to foods, with an increasing prevalence over the past decades[34,35]. Because there is no prophylactic treatment so far, the allergic consumer must avoid peanuts at all time. Hence, labeling legislation is becoming stricter, requiring accurate and reliable product information[36,37]. To date, several immunoassay techniques have been described in literature for detecting Ara h 1 [38–41]. Although their performance is in accordance with new legislations, levels of sensitivity to peanut allergens may vary among individuals, thereby posing new challenges for developing even more sensitive biosensors that can detect ultralow quantities of peanut allergens. Therefore, research in this field is focused on looking for new alternatives, including the introduction of novel bioreceptors, such as aptamers.

2. Materials and methods

2.1 Reagents and biomolecules

All buffer reagents were supplied by Sigma-Aldrich (Norway). All solutions were prepared using deionized water purified with a Milli-RO® 90 from Millipore (Germany). Dithiothreitol (DTT), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were

purchased from Sigma-Aldrich (Norway). LYNX rapid alkaline phosphatase antibody conjugation kit was purchased from AbD Serotec (UK). Natural Ara h 1 protein and monoclonal anti-Ara h 1 antibody were purchased from Indoor Biotechnologies Limited (UK). Human myeloma IgE (hIgE) kappa was supplied by Athens Research and Technology (USA). Superparamagnetic particles of 2.8 μm diameter were obtained from Thermo Fisher Scientific (Norway) with different functional groups: Streptavidin coated (Dynabeads™ M-270 and M-280 Streptavidin) (Table 1), carboxylated (Dynabeads™ M-270 Carboxylic Acid) and maleimide activated (Dynabeads™ M-270 Maleimide, prototype). The prototype Dynabeads™ M-270 Maleimide were provided as a kind gift. Biotinylated, amino and thiolated DNA aptamers against Ara h 1, as well as the corresponding complementary and random DNA sequences (Table 2), were obtained from Integrated DNA technologies, Inc. (Belgium). The aptamers containing thiol modifiers were supplied in their oxidized (disulfide) form.

2.2 Magnetic particle functionalization with Ara h 1 aptamer

2.2.1 Carboxylic acid coated particles

NH₂ terminated aptamer was coupled to carboxylated particles by a single-step carbodiimide reaction (Figure 1A)[42]. 3 mg of the particles were washed twice with 300 μL of 100 mM MES (2-(N-Morpholino)ethanesulfonic acid, pH 4.8) buffer and subsequently resuspended in 100 μL of the same buffer. In a separate tube, a 100 μL of solution containing 15 nmol of EDC and 6 nmol of the aptamer in 100 mM MES buffer was prepared. This EDC/oligo mix was added to the particles solution and incubated on a roller mixer at room temperature (RT) overnight. After incubation, the particles were washed three times with 100 μL of 1x TE (10 mM Tris, 1 mM EDTA, pH 8) buffer and finally resuspended in 300 μL of the same solution, to a final concentration of 10 mg/mL. The functionalized particles were stored at 4°C until use.

2.2.2 Streptavidin coated particles

To couple the biotinylated aptamer to streptavidin coated particles, 3 mg of the particles were first washed twice with 300 μL of 1x TE buffer containing 300 mM NaCl. The particles were then incubated with 1 mL of 1 μM solution of the biotinylated aptamer at RT for 30 min. After functionalization, the particles were washed twice with 300 μL of 150 mM phosphate buffered

saline (PBS), pH 7.4, containing 0.5% BSA and 0.01% Tween 20, and subsequently stored in 300 μ L of the same buffer, at a final concentration of 10 mg/mL, at 4°C until use.

2.2.3 Maleimide-activated particles

Before magnetic particle functionalization (Figure 1B), the protected thiol group in the aptamer sequence was reduced by addition of 30 μ L of 1 M DTT to 27.5 nmol of the aptamer, previously dissolved in 150 μ L water. This mixture was incubated for 5 min at RT and the precipitate removed by centrifugation. The supernatant was transferred to a NAP-5 Column (GE Healthcare Life Sciences, Norway), purified and equilibrated with coupling buffer (1 M NaCl, 10 mM Sodium phosphate, 0.1 M EDTA, pH 8). The eluate from the column was transferred to a clean tube and 1 ml of coupling buffer was added. The maleimide-activated prototype magnetic particles were stored dry at -20°C under N₂ atmosphere. 50 mg of the particles were resuspended in coupling buffer to a final concentration of 50 mg/mL. Subsequently, the previously prepared aptamer solution was added to the magnetic particle solution and mixed by vortexing. This mixture was incubated overnight on a rotator at RT. The particles were then washed several times by incubation for 15 min at RT, once with coupling buffer, three times with 1x TT buffer (250 mM Tris, 0.1% Tween 20, pH 8) and once with 1x TE buffer. Finally, the particles were resuspended in 1x TE buffer to a final concentration of 10 mg/mL and stored at 4°C until use.

2.3 Magnetic particle functionalization with anti-Ara h 1 antibody

Anti-Ara h 1 antibodies were coupled to carboxyl-coated magnetic particles by a carbodiimide reaction[22]. 3 mg of the particles were washed twice with 300 μ L of 100 mM MES buffer. The carboxyl groups were activated for 20 min with 0.4 M EDC and 0.5 M NHS in a 25 mM MES buffer. After washing the particles with MES buffer, they were resuspended in a solution of the same buffer containing 3 mg/mL of the anti-Ara h 1 antibody. Subsequently the functionalized particles were incubated for 30 min with 50 mM cold ethanolamine, followed by washing and storage in 300 μ L of 150 mM PBS containing 0.5% BSA and 0.01% Tween 20.

2.4 Quantification of magnetic particle surface coverage with Ara h 1 aptamer

The amount of aptamer coupled to the magnetic particles was quantified indirectly through a hybridization assay. 10 μ L of the aptamer-functionalized particles were washed twice with 6x SSPE

buffer (0.9 M Sodium Chloride, 60 mM Sodium Hydrogen Phosphate, 6 mM EDTA, pH 7.4), followed by 15 min incubation at RT with 3 nmol of the DNA sequence complementary to the aptamer prepared in the same buffer, a standard hybridization buffer. After incubation, the magnetic particles were washed twice with water and finally resuspended in 100 μ L of water. This solution was incubated for 5 min at 80°C and the amount of DNA melted from the particles was measured in the supernatant.. For that, 2 μ L of the supernatant was incubated for 2 min at RT in 200 μ L of the working solution previously prepared by diluting 1:200 the Qubit® ssDNA reagent (Qubit® ssDNA Assay Kit, Thermo Fisher Scientific, Norway), which consists of a fluorescent ssDNA-binding dye in Qubit® ssDNA buffer. Standard solutions were prepared by diluting 10 μ L of Qubit® ssDNA Standard #1 and #2 in 190 μ L of the working solution and used for calibration of a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Norway). The amount of DNA present in each sample in ng/mL was multiplied by $(200/\chi)$, where χ is the amount of sample used (i.e. 2 μ L), to determine the DNA concentration in the starting sample. Each sample was measured in triplicate.

2.5 Quantification of capture efficiency of target protein

A radioactive assay was performed to quantify the amount of protein captured by the aptamer-functionalized particles. Both the target and the control proteins (Ara h 1 and hIgE, respectively) were labelled with iodine 125 (I-125). Subsequently, the assay solutions were prepared in TGK buffer (25 mM tris, 192 mM glycine and 5 mM K₂HPO₄, pH 8.3) with unlabeled and radioactively labelled protein in a ratio 10000:1. The total amount of 2.5 μ g protein was incubated with 0.25 μ g of aptamer-coupled particles, resulting in a concentration of 10 μ g protein/mg bead. After incubation for 1 h at RT, the radioactivity of the magnetic particle solution was measured (cpm) in a gamma counter (Wallac Wizard® 1470 Automatic Gamma Counter, Perkin Elmer, Norway). Thereafter, the particles were washed three times in PBS buffer containing 0.5 M NaCl and 0.01% Tween 20 to remove unreacted protein and finally resuspended in 250 μ L PBS. The radioactivity of the solution after these washing steps was again measured. The amount of protein captured by the functionalized particles was expressed as a percentage of captured protein over total initial amount of protein calculated by comparing the cpm of the particle solution before and after the washing steps. The amount of protein captured by antibody-functionalized particles (Section 2.3) was also quantified following the same protocol.

2.6 Magnetic particle-based sandwich assay

M-280 Streptavidin microparticles functionalized with Ara h 1 aptamer (Section 2.2.2) were used to develop a sandwich-type assay, in which the aptamer on the particles acted as a capture receptor and an anti-Ara h 1 antibody was used for detection. The particles were diluted to a final concentration of 0.2 mg/mL in TGK buffer and 100 μ L of this particle suspension was mixed with 50 μ L of Ara h 1 with concentrations ranging from 100 to 2500 ng/mL (nM range) in TGK buffer. The assay was performed in individual wells of a 96-well plate for 30 min on a shaker at 37 °C and each sample was assayed in quadruplicate. After incubation, particles were washed once with 200 μ L TGK buffer and twice with 200 μ L TBST buffer (50 mM Tris, 150 mM NaCl and 0.05% Tween20, pH 7.6). Subsequently, they were resuspended in a solution prepared by dissolving 50 μ L of alkaline phosphatase (ALP)-labelled detection antibody (previously diluted 1:1000 from stock, prepared using a commercially available labelling kit, Section 3.3.1) in 100 μ L TBST buffer. Samples were again incubated for 15 min with shaking at 37°C. After this final incubation, the particles were washed twice with 200 μ L TBST buffer and finally resuspended in 20 μ L of the same buffer. After addition of the substrate (DynaLight substrate with Rapid Glow enhancer, Thermo Fisher Scientific, Norway), luminescence of the samples was measured by means of a Synergy 4 spectrophotometer (Biotek, USA). Similarly, a sandwich assay was performed with anti-Ara h 1 antibody as both capture and detection receptor. M270 Carboxylic Acid particles, functionalized with anti-Ara h 1 antibody as in Section 2.3 were used, further diluted to a final concentration of 0.2 mg/mL in TBST. 100 μ L of this bead solution was mixed with 50 μ L of Ara h 1 solution in the same concentration range as for the aptamer assay (100 to 2500 ng/mL), but prepared in TBST buffer. Subsequent assay steps and detection were performed following the same protocol explained above.

3. Results and Discussion

3.1 Quantification of magnetic particle surface coverage

In order to select the optimal surface chemistry for coupling Ara h 1 aptamer to magnetic particles, different parameters were evaluated, namely (1) particle surface coverage with bioreceptor molecules, (2) efficiency in capturing target protein by the functionalized particles and (3) non-specific protein adsorption on the functionalized particles. Four different types of magnetic particles were selected for this study, namely M-270 Carboxylic Acid, M-270 Maleimide prototype, M-270

Streptavidin and M-280 Streptavidin. This selection was based on the most popular and versatile surface chemistry methods, being carbodiimide crosslinking, sulfhydryl crosslinking and noncovalent conjugation through streptavidin-biotin interaction, respectively. Correspondingly, amino-, thiol- and biotin-modified Ara h 1 aptamer probes (Table 2) were bound to the magnetic particles following the protocols in Section 2.2. Because streptavidin-conjugated particles exist on the market as two different types, M-270 and M-280, which are prepared starting from carboxylated and tosyl-activated particles, respectively, they were both included in the study (Table 1).

The amount of Ara h 1 aptamer immobilized per surface area of the different particle types was quantified in an indirect method by measuring the amount of complementary DNA sequence hybridizing to the immobilized aptamer, as explained in Section 2.4. As can be seen in Figure 2, the biotin-streptavidin functionalization strategy using M-270 Streptavidin particles resulted in the highest amount of complementary DNA captured on the particles' surface. The amount of DNA captured when M-280 Streptavidin and M-270 Maleimide prototype particles were employed was similar, whereas a rather low amount of DNA was captured on the surface of M-270 Carboxylic Acid particles. This can be explained by the highly negatively charged surface of these particles repelling negatively charged DNA molecules. Additionally, when the aptamer is attached to the particles, other amino groups in the oligonucleotide might to some extent react with the carboxylic acid groups on the particles, resulting in aptamer coupling via internal bases. Aptamer strands coupled through this suboptimal interaction occupy a larger effective volume on the surface of the magnetic particles, which not only jeopardizes the hybridization with the complementary sequence, but also decreases the total amount of aptamer on the particle surface resulting in fewer DNA strands that can be coupled onto a given space[10]. This suboptimal efficiency of covalent carbodiimide crosslinking for DNA immobilization on a carboxylated surface was previously observed in other applications such as immuno-PCR[43] and DNA microarrays[44], in which parameters such as pH, ionic strength and reaction time seemed to play an important role in immobilization efficiency.

Two negative controls were performed in this experiment: (1) incubation of non-functionalized particles with the complementary DNA sequence in order to quantify non-specific DNA-magnetic particle interactions and (2) incubation of the Ara h 1 aptamer-functionalized particles with a random DNA sequence in order to quantify non-specific DNA-DNA interactions. The fluorescence measured for the first negative control was below the limit of detection (LOD) of

the fluorimeter (i.e. 50 ng/mL) for all selected particles. Thus, unspecific interaction between the DNA and the magnetic particle surfaces was considered negligible in these experiments. The amount of random DNA interacting with the aptamer (i.e. non-specific DNA-DNA interactions) is detailed in Table 3. As can be observed, the amount of random and complementary DNA interacting with the aptamer on M-270 Carboxylic Acid particles was equivalent, excluding the need for these particles to be tested in further experiments. For the other particles, the DNA that specifically interacted with the surface aptamer was estimated by subtracting the amount of random DNA nonspecifically adsorbed onto the functionalized particles from the amount of complementary DNA captured on the particles. This is equivalent to the amount of aptamer on the particles and was used to estimate the approximate surface coverage (see Table 3). As can be seen, coupling biotinylated Ara h 1 aptamer to streptavidin-coated particles (either M-270 or M-280) is the functionalization strategy that led to the highest amount of aptamer immobilized on the particle surface.

3.2 Quantification of target protein capture efficiency

Once the surface coverage of the different coated particles was determined, it was important to evaluate whether the immobilized aptamer was still functional and capable of capturing the target protein in an efficient way. The amount of target protein captured on differently coated particles (i.e. M-270 Streptavidin, M-280 Streptavidin and M-270 Maleimide prototype) was quantified in a radioactive assay, as explained in Section 2.5. I125-labelled IgE, which has a similar molecular weight to Ara h 1 (180 and 195 KDa, respectively), was used as a negative control for evaluating the specificity of the aptamer-coated particles. An additional negative control was performed by incubating non-functionalized particles with the Ara h 1 in order to evaluate non-specific interactions between the protein and the particle surface. Furthermore, the capture efficiency of particles functionalized with anti-Ara h 1 antibody was also tested in a similar experiment, in order to compare the capture efficiency of magnetic particles coated with the two different bioreceptors. The results of all these experiments are summarized in Table 4.

As it can be seen in the table, the amount of Ara h 1 captured on the surface of M-270 Maleimide prototype particles was surprisingly not very different from that of hIgE captured on the same particles, or from the quantity of Ara h 1 binding non-specifically to the surface of the unconjugated particles. These results indicate that the Ara h 1 aptamer immobilized on the surface of M-270 Maleimide prototype particles did not capture the target protein efficiently. This could be

due to the fact that the C6 spacer between the thiol functional group and the aptamer sequence (Table 2) is not long enough to prevent steric hindrance between the DNA probe and the particle, which could interfere with binding of the target protein. It has already been shown that extending the aptamer with a short chain of thymine bases at the thiolated binding site helps to give the aptamer more flexibility for binding with Ara h 1 [31]. With regard to the streptavidin coated particles, both M-270 and M-280 showed a higher capture efficiency, 10.6 ($\pm$ 0.2) % and 8.6 ($\pm$ 0.7) %, respectively. The biotin label at the aptamer 5'-end includes a triethylene glycol (TEG) moiety as a spacer (Table 2), which has proven to be effective in preventing steric hindrance[10]. Also, both types of particle led to a comparable amount of non-specific interaction with the hIgE control, 3.3 ($\pm$ 0.2) % and 2.6 ($\pm$ 0.5) %, respectively. However, non-specific interaction between Ara h 1 and the particle surface was three times higher for M-270 Streptavidin particles compared to the M-280. This was expected since the M-270 Streptavidin particles are prepared from carboxylated particles and consequently present a hydrophilic, highly negatively charged surface (see Table 1). In contrast, M-280 Streptavidin particles are based on tosyl-activated particles and present a hydrophobic, low-charged surface. Moreover, M-280 particles are additionally blocked with BSA. Thus, taking into account all these results, M-280 Streptavidin seem to be the optimal particles for bioassay development, in which complex matrices can lead to unspecific interactions.

As can be also observed in Table 4, the capture efficiency of particles functionalized with anti-Ara h 1 antibody was 6% higher than M-280 Streptavidin particles functionalized with aptamer, whereas the non-specific interaction with the hIgE control was 2.5 times lower. The non-specific particle–protein interactions were comparable for both type of particles (~2%). The calculated capture efficiency values were used to estimate the amount of Ara h 1 captured on the particle surfaces, which was 1.5 and 1 μ g protein/mg of particles functionalized with the anti-Ara h 1 antibody and aptamer, respectively. Based on these results it can be concluded that the binding capacity is similar in both cases. The observed small differences could be expected since the anti-Ara h 1 antibody has a higher affinity than the aptamer towards Ara h 1 with K_D values of 50 nM[45] and 400 nM[33], respectively.

3.3 Magnetic particle-based sandwich assay

To investigate whether the aptamer-functionalized particles are suitable for further implementation in assays for Ara h 1 protein detection, a sandwich assay was developed using Ara h 1 aptamer as capture receptor and ALP-labelled anti-Ara h 1 antibody for detection. Based on the results in

Table 4, M-280 Streptavidin particles were selected as the optimal solid support for aptamer immobilization. A dose-response curve was generated for the detection of Ara h 1 in buffer (Figure 3A). A linear detection range was observed for Ara h 1 concentrations between 100 and 1000 ng/mL ($R^2 = 0.998$) (Figure 3B).

The obtained LOD, calculated by interpolating in the calibration curve a signal equal to 3 times the standard deviation above the mean background value, is 398 (± 14) ng/mL, which is in line with that obtained when anti-Ara h 1 antibody was used as a capture receptor, LOD = 244 (± 66) ng/mL (Figure 4). This is in agreement with previous results indicating that binding capacity of both bioreceptor-functionalized particles is similar (Section 3.2). Additionally, the same Ara h 1 aptamer was previously implemented in a bioassay on an FO-SPR sensing platform[33], acting as a capture receptor on a gold-coated optical fiber, while detection was performed with a gold nanoparticle-labelled anti-Ara h 1 antibody. Most recently, the same aptamer was used for label-free detection of Ara h 1 in heat-transfer resistance measurements[46]. The reported LODs for those two platforms, 15 μ g/mL and 600 ng/mL, respectively, were approximately 40 and 1.5 times higher than the value reported here. The lower LOD shown in this paper suggests that the application of the Ara h 1 aptamer-functionalized magnetic particles is indeed promising for bioassay development.

4. Conclusions

Superparamagnetic particles combine unique magnetic properties with dimensions that are optimal for handling biomolecules in different ways. As a result, they have been consolidated as a distinctive tool in the biosensors field. Whereas magnetic particle functionalization with antibodies is generally well established, immobilization of DNA probes, such as aptamers, is not yet fully explored. Therefore, in this paper, different available strategies were evaluated for the immobilization of a recently developed ssDNA aptamer against Ara h 1 protein on the surface of magnetic particles. Conjugation of biotinylated Ara h 1 aptamer to the streptavidin particles led to the highest surface coverage with receptors, as well as to the highest capture efficiency of Ara h 1 on the particles. The suitability of these functionalized particles for further application in bioassays was supported by the development of a sandwich assay featuring an LOD in line with the one observed when an antibody is used as a capture receptor instead. Moreover, the LOD was better than previous one reported using the same aptamer as a capture receptor in a similar assay scheme

but in other biosensing platforms. Collectively, these results show that the application of the Ara h 1 aptamer-functionalized particles holds great potential for bioassay development.

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7. Legends to Figures

Figure 1: Magnetic particle functionalization with Ara h 1 aptamer. (A) EDC crosslinking reaction scheme. (B) Sulfhydryl/maleimide reaction scheme.

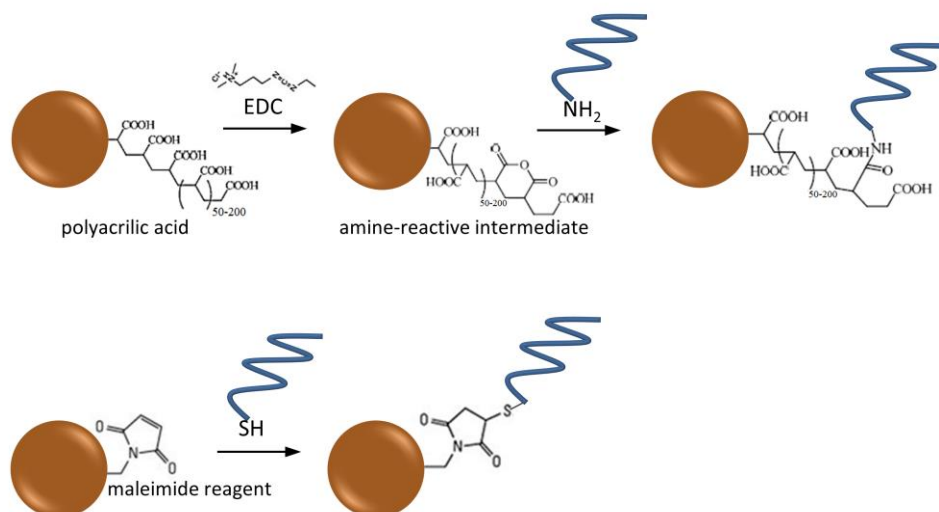


Figure 2: Amount of complementary (blue) and random (red) DNA captured on the surface of aptamer-functionalized magnetic particles, quantified through a hybridization assay. The amount of DNA is expressed in ng DNA/mL particle solution. The error bars represent the standard deviation over four repetitions.

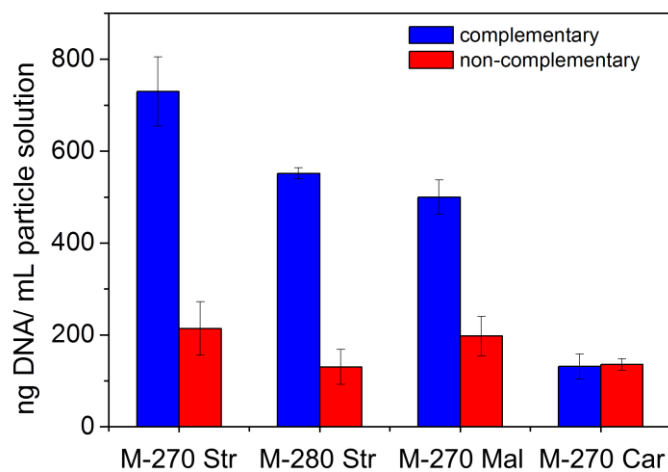


Figure 3: (A) Dose-response curve for the detection of Ara h 1 protein in buffer using a sandwich-type assay with Ara h 1 aptamer as capture receptor on magnetic particles and anti-Ara h 1 antibody as detection receptor. The curve represents measured luminescence intensity as a function of the concentration of target protein. The dashed line represents the curve fitting by a dose-response model. (B) Linear detection range (100-1000 ng/mL) of the dose-response curve represented in panel A ($R^2=0.998$). Error bars represent the standard error over four repetitions.

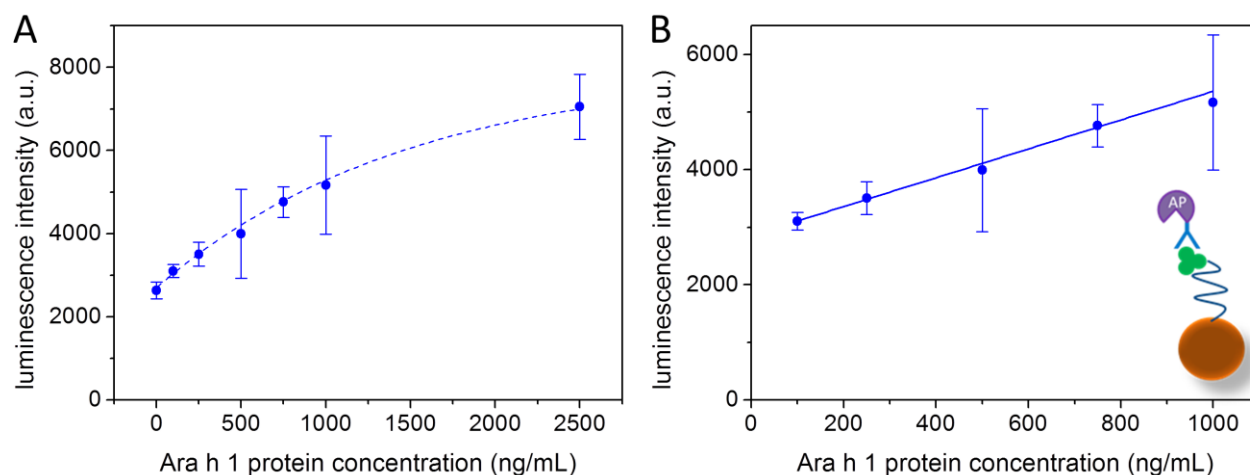


Figure 4: (A) Dose-response curve for the detection of Ara h 1 protein in buffer using a sandwich assay with anti-Ara h 1 antibody as both capture and detection receptors; the capture receptor is immobilized on magnetic particles. The curve represents measured luminescence intensity as a function of the concentration of target protein. The dashed line represents the curve fitting by a dose-response model. (B) Linear detection range (100-1000 ng/mL) of the dose-response curve represented in panel A ($R^2=0.999$). Error bars represent the standard error over four repetitions.

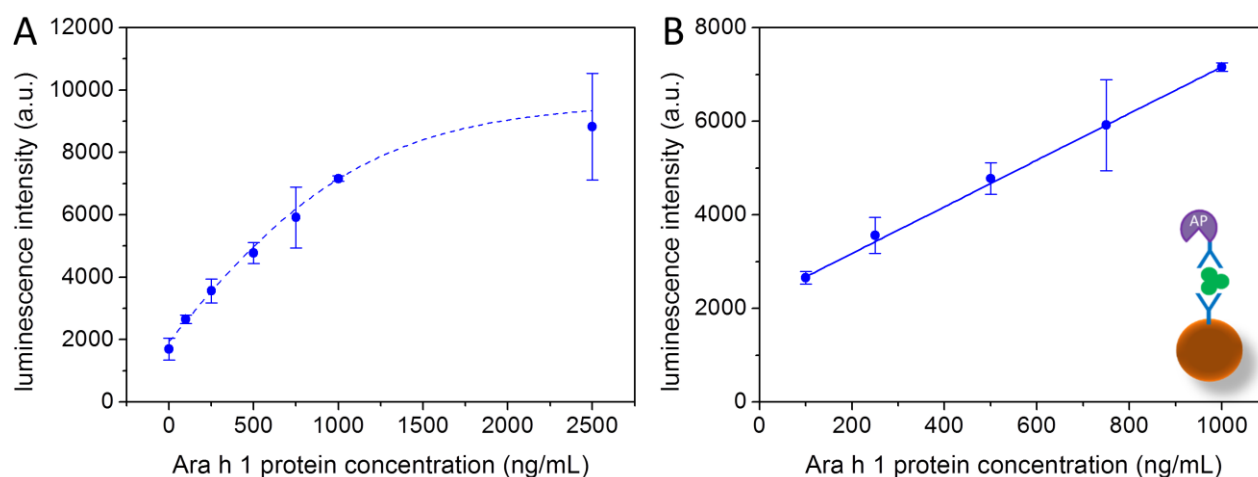


Table 1: Summary of the characteristics of Dynabeads® M-270 and M-280 Streptavidin. Adapted from the Dynabeads® Streptavidin selection guide (<https://www.thermofisher.com>).

Characteristics and properties	
Dynabeads® M-270 Streptavidin	Dynabeads® M-280 Streptavidin
Based on carboxylic acid particles	Based on tosyl-activated particles
Hydrophilic particle surfaces	Hydrophobic particle surface
2.8 µm diameter (CV=3%)	2.8 µm diameter (CV=3%)
No blocking protein	BSA as blocking protein
High negative charge: -50mV at pH=7	Low charge: -10mV at pH=7
Iron content (ferrites): 14% (20%)	Iron content (ferrites): 12% (17%)
Isoelectric point: pH 4.5	Isoelectric point: pH 5.5
Low aggregation of particles in high salt solutions	Not applicable

Table 2: ssDNA sequences used in this paper.

Name	Sequence (5' → 3')	Length (bp)	5' Mod.
Biotin- Ara h 1 aptamer	TCGCACATTCCGCTTCTACCGGG GGGGTCGAGCTGAGTGGATGCGA ATCTGTGGGTGGGCCGTAAGTCC GTGTGTGCGAA	80	Biotin-TEG
Amino- Ara h 1 aptamer	TCGCACATTCCGCTTCTACCGGG GGGGTCGAGCTGAGTGGATGCGA ATCTGTGGGTGGGCCGTAAGTCC GTGTGTGCGAA	80	NH ₂ -C ₆
Thiol- Ara h 1 aptamer	TCGCACATTCCGCTTCTACCGGG GGGGTCGAGCTGAGTGGATGCGA ATCTGTGGGTGGGCCGTAAGTCC GTGTGTGCGAA	80	S-S-C ₆
Complementary DNA sequence	TTCGCACACACGGACTTACGGCC CA	25	-
Random DNA sequence	TGTAACCTATTTGGATCACAACT TA	25	-

Table 3: Measured complementary and random DNA sequences captured on the differently functionalized particles together with estimated amount of bound aptamer and particle surface coverage. The surface coverage (pmol DNA/ cm²) is expressed as a range taking into account the standard deviation of the measurements, which is calculated over four repetitions.

Magnetic particles	Complementary DNA captured on particles (ng/mL)	Random DNA captured on particles (ng/mL)	Estimated bound aptamer (ng/mL)	Surface coverage (pmol/cm²)
M-270 Streptavidin	730 ± 75	214 ± 58	516 ± 95	~0.85-1.25
M-280 Streptavidin	551 ± 12	130 ± 38	421 ± 40	~0.77-0.93
M-270 Maleimide prototype	500 ± 37	197 ± 43	302 ± 57	~0.50-0.72
M-270 Carboxylic Acid	132 ± 27	135 ± 13	-	-

Table 4: Measured capture efficiency for the different coated particles. M-270 streptavidin, M-280 Streptavidin and M-270 Maleimide prototype are coated with Ara h 1 aptamer and M-270 Carboxylic Acid are coated with anti-Ara h 1 antibody. Two negative controls were performed in order to test specificity of the aptamer-functionalized particles and unspecific interactions between target protein and particle surface. Standard deviations are calculated over three repetitions.

Capture efficiency (%)				
	M-270 Streptavidin + aptamer	M-280 Streptavidin + aptamer	M-270 Maleimide prototype + aptamer	M-270 Carboxylic Acid + antibody
Functionalized particles + Ara h 1	10.6 ± 0.2	8.6 ± 0.7	4.3 ± 0.3	14.9 ± 0.6
Functionalized particles + hIgE	3.3 ± 0.2	2.6 ± 0.5	3.3 ± 0.9	1.0 ± 0.1
Bare particles + Ara h 1	6.2 ± 0.3	1.8 ± 0.6	5.6 ± 0.6	2.7 ± 0.2