

# Controlled Assembly of Magnetic Biomolecular Nanostructures

Hui Wang, Harish Padmanabhan, David Thomson, Krassen Dimitrov

**Abstract**—Two optimized strategies were successfully established to develop biomolecule-based magnetic nanoassemblies. Streptavidin-coated and amine-coated magnetic nanoparticles were chosen as model scaffolds onto which double-stranded DNA and human immunoglobulin G were specifically conjugated in succession, using biotin-streptavidin interaction or covalent cross-linkers. The success of this study opens the prospect of developing selective and sensitive nanoparticle-based structures for diagnostics or drug delivery.

**Keywords**—Antibody, DNA, linker, magnetic nanoparticles, streptavidin

## I. INTRODUCTION

GREAT efforts have been invested to develop high throughput proteomics methods for detection and analysis of specific proteins, which could greatly benefit in the diagnosis and treatments of multiple diseases [1]. Antibodies, due to their large diversity and specific binding properties, have been widely utilized for target protein identification and characterization [2, 3]. Some technologies, especially those for multiplexed protein analysis; rely on DNA conjugation to the antibody, either as universal linkers [4, 5] or as functional barcode [6, 7]. In addition, DNA conjugation can significantly reduce non-specific binding, thus improving the sensitivity of certain bioassays [8, 9].

DNA-antibody conjugation could be achieved either by non covalent coupling or covalent coupling chemistry. For example, the extraordinary affinity of biotin and avidin ( $Ka=10^{15} M^{-1}$ ) has been widely utilized in biomolecule conjugation system. Biotin is a small molecule (244 Da), which could be conjugated to the target antibody without significant alteration of antibody's activity. In addition, DNA synthesized with functional groups can also be covalently conjugated on the surface of target protein using various conjugation chemistries. For example, N-hydroxysuccinimide (NHS) is one of the most frequently used cross linking groups in the conjugation of amine groups in proteins, which are present on most antibodies due to the abundance of lysine side chain  $\epsilon$ -amine and N-terminal  $\alpha$ -amine. Beyond NHS, sulfhydryl or aldehyde groups can also be generated by reducing disulfide bonds in the hinge region or by oxidizing carbohydrate residues of antibody,

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respectively. Free sulfhydryl group or active aldehyde group can then react with maleimide or amine group on target molecule to form stable thioether bond or amide bond, respectively. A number of these cross linking chemistries are commercially available. For example, companies such as SoluLink, Merck, Abgent, etc., utilize hydrazine and aldehyde as cross-linkers in conjugation reactions and they offer more promises in tailoring target-specific bio-conjugates.

As versatile molecular tools, DNA-antibody conjugates have been widely applied in planar-based [10] or beads-based assays [11] for the detection of DNA, proteins or cell sorting. Among these methods, nanoparticles (NPs), typically ranging in size from 1 nm to 1 micron, have drawn considerable attention as an attractive structural scaffold with wide functional diversity [12, 13]. When their surface is modified with biomolecules, these biomolecule-functionalized NPs can offer unique advantages for developing selective and highly sensitive nanoparticle-based diagnostics and therapeutics. Thus, biomolecule modified NPs have been widely used in the fields of biotechnology and biomedicine, as selective and ultra-sensitive probes [14-16].

In this work, two optimized strategies were successfully established to tailor biomolecule-functionalized magnetic NPs for inclusion into complex nanostructures. Both streptavidin (SA)-coated and amine-coated magnetic NPs were used as scaffolds onto which double stranded DNA (dsDNA) and human immunoglobulin G (IgG) were conjugated in sequence via biotin-SA linkages and commercially available covalent cross-linkers.

## II. MATERIALS AND METHODS

### A. Reagents

Amine- and SA-coated magnetic TurboBeads<sup>®</sup> ( $\phi=50$ nm, TurboBeads Ltd., Zurich), human IgG (Sigma-Aldrich, Australia), Sulfo-Succinimidyl 4-formylbenzoate (Sulfo-S-4FB, Solulink, USA), Sulfo-succinimidyl 6-hydrazinonicotinate acetone hydrazone, (Sulfo-S-HyNic, Solulink, USA), Sulfo-NHS-LC-Biotin (Thermo Scientific, Australia), 6x Loading Dye (Promega, USA), 100bp DNA Ladder (Promega, USA), BenchMark Ladder (Invitrogen, USA), SYBR Safe DNA Gel Stain (Invitrogen, USA), NuPage LDS Sample Buffer (Invitrogen, USA), SYBR Green DNA Gel Stain (Invitrogen, USA), SYPRO Ruby Protein Gel Stain (Invitrogen, USA), Tris Acetate SDS Buffer (Invitrogen, USA), MES buffer (Invitrogen, USA).

ssDNA with either biotin (Bio-) or amine functional group was synthesized by IDT, USA. Nucleotide sequences were as following:

**Bio-AAAC:** /5Biosg/AAA CAA ACA AAC AAA CAA  
ACA AAC AAA CAA ACA AAC AAA CAA ACA AAC  
AAA CAA ACA AAC AAA CAA ACA AAC AAA CAA  
ACA AAC AAA CAA ACA AAC AAA CAA ACA AAC  
AAA CAA ACA AAC AAA CAA ACA AAC AAA CAA  
ACA AAC AAA CAA ACA AAC AAA CAA ACA AAC  
AAA CAA ACA AAC AAA CAA ACA AAC AAA CAA AC

**Bio-GTTT:** /5Biosg/GTT TGT TTG TTT GTT TGT TTG  
TTT GTT TGT TTG TTT GTT TGT TTG TTT GTT TGT  
TTG TTT GTT TGT TTG TTT GTT TGT TTG TTT GTT  
TGT TTG TTT GTT TGT TTG TTT GTT TGT TTG TTT  
GTT TGT TTG TTT GTT TGT TTG TTT GTT TGT TTG  
TTT GTT TGT TTG TTT GTT TGT TTG TTT GTT TGT  
TTG TTT GTT TGT TTG TTT GTT TGT TTG TTT GTT  
TGT TTG TTT GTT TGT TT

**Amine-GTTT:** /5AmMC6/GTT TGT TTG TTT GTT TGT  
TTG TTT GTT TGT TTG TTT GTT TGT TTG TTT GTT  
TGT TTG TTT GTT TGT TTG TTT GTT TGT TTG TTT  
GTT TGT TTG TTT GTT TGT TTG TTT GTT TGT TTG  
TTT GTT TGT TTG TTT GTT TGT TTG TTT GTT TGT  
TTG TTT GTT TGT TTG TTT GTT TGT TTG TTT GTT  
TGT TTG TTT GTT TGT TT

**Bio-CL-Rev2:** /5Biosg/GCG GCA CTA CGG CAA TAA  
TCC

#### B. Modification of Amine-coated TurboBeads<sup>®</sup> with Sulfo-S-HyNic

The process of DNA and antibody conjugation on amine-coated NPs was shown in Fig. 1. Briefly, amine-coated TurboBeads<sup>®</sup> solution was vortexed for 1min and 100 μL of the well-suspended colloid was taken for further manipulation. NPs were separated from supernatant using a magnet and re-suspended in 500 μL of 5x SSC (0.75M NaCl, 75mM sodium citrate, Sigma-Aldrich, Australia). 10 μL of 20 mg/mL S-Hynic dissolved in Milli Q water was added into NPs solution and incubated in a sonicating water bath (Unisonics, Australia) for 1 h at room temperature with shaking at regular intervals (to ensure the NPs were always in suspension). After modification, the NPs were washed 3 times with 5x SSC via magnetic separation and eventually re-suspended with 500 μL of 5x SSC.

#### C. Modification of Amine-GTTT with Sulfo-S-4FB

40 mole equivalents of 20 mg/mL Sulfo-S-4FB was added to Amine-GTTT and mixed well in Milli Q water. The mixture was incubated for 2 hours at room temperature.

#### D. 4FB-modified DNA Precipitation

After 4FB modification, 20 μL DNA was purified by sodium acetate-ethanol precipitation procedure. Briefly, 10% (v/v) of saturated sodium acetate (pH 6.0) and 75% (v/v) of 100% ethanol (ice cold) were added into 4FB-modified ssDNA solution following by vortexing and centrifugation at 20,000 rpm for 10min. The supernatant was discarded and 70% Ethanol (ice cold) was added. The mixture was centrifuged at 20,000 rpm for 10 min again. The pellet obtained was air dried and was suspended in 20 μL Conjugation Buffer (100mM sodium phosphate, 50mM Sodium Chloride, pH 6.0). The concentration of DNA was determined using the NanoDrop

1000 Spectrometer (Thermo Scientific, USA).

#### E. DNA Conjugation on Amine-coated TurboBeads<sup>®</sup>

20 μL of 50 μM of 4FB-modified ssDNA were added into 500 μL NPs solution (2x molar excess). The mixture was incubated in a water-bath sonicator for overnight. NPs were then washed with 5x SSC by using magnet and supernatant was discarded. NPs were re-suspended in 500 μL of 5x SSC. 8 μL of 100 μM of Bio-AAAC was then added into 500 μL NPs solution. The mixture was incubated in water-bath sonicator for 1 h with shaking at regular intervals to ensure NPs were in suspension, followed by additional three washes with 5x SSC. The NPs were eventually re-suspended in 500 μL of 5x SSC, and 100 μL of well-suspended NPs mixture was taken out for DNA conjugation analysis.

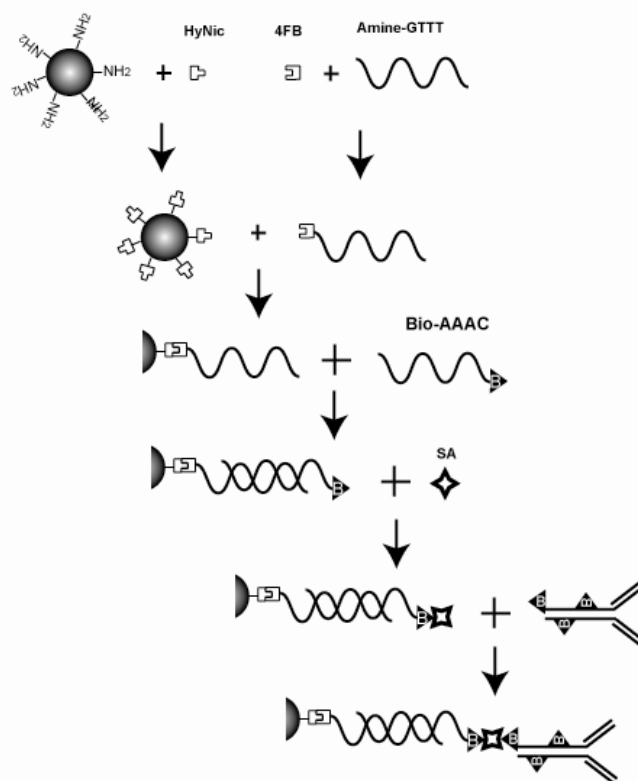


Fig. 1 The schematic diagram of DNA-antibody conjugation on amine-coated magnetic NPs.

#### F. DNA Conjugation on SA-coated TurboBeads<sup>®</sup>

The process of DNA and antibody conjugation on SA-coated NPs was shown in Fig. 2. Briefly, SA-coated TurboBeads<sup>®</sup> solution was vortexed for 1min and 100 μL of the well-suspended colloid was taken for further processing. NPs were separated from supernatant using magnet and re-suspended in 500 μL of 5x SSC, and 8 μL of 100 μM of Bio-AAAC were added into 500 μL NPs suspension (2x molar excess). The mixture was incubated in water-bath sonicator for 1 h at room temperature. After incubation, NPs were washed with 5x SSC by using magnet to recover the NPs, while the supernatant was discarded. NPs were then re-suspended in 500

$\mu\text{L}$  of 5x SSC. 5  $\mu\text{L}$  of 150  $\mu\text{M}$  Bio-CL-Rev2 was added into 500  $\mu\text{L}$  NPs mixture in order to block unoccupied SA binding sites. After 1 h incubation in water bath sonicator and three washes with 5x SSC, the NPs were re-suspended with 500  $\mu\text{L}$  of 5x SSC. 8  $\mu\text{L}$  of 100  $\mu\text{M}$  of Bio-GTTT, as complementary DNA, was then added and the mixture was incubated in water-bath sonicator for 1 h, followed by three washes with 5x SSC. The NPs were eventually re-suspended in 500  $\mu\text{L}$  of 5x SSC and 100  $\mu\text{L}$  of the well-suspended NPs mixture was taken out for DNA conjugation analysis.

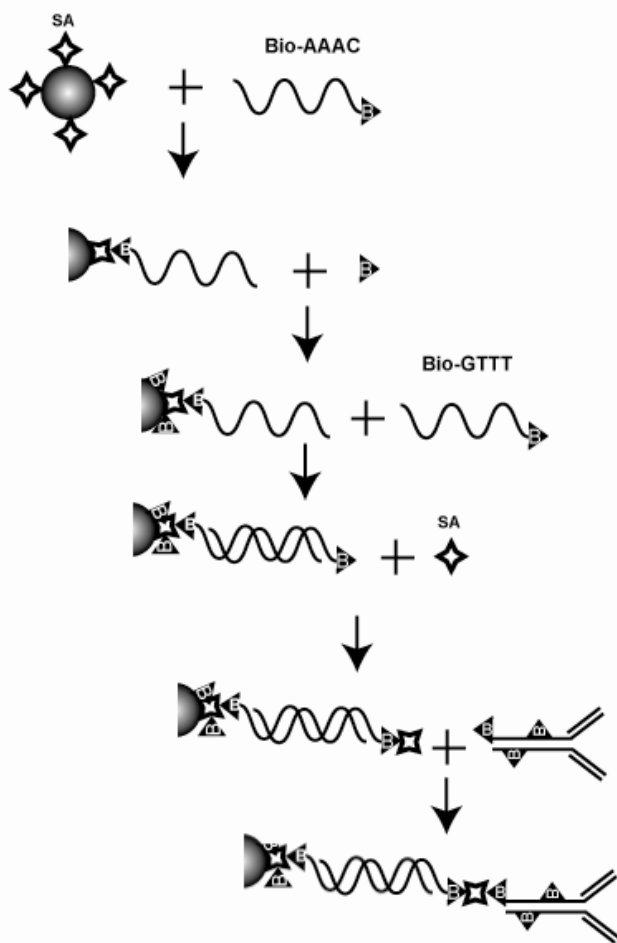


Fig. 2 The schematic diagram of DNA-antibody conjugation on amine-coated magnetic NPs.

#### G. SA Conjugation on dsDNA Modified TurboBeads®

65  $\mu\text{L}$  of 1  $\mu\text{g}/\mu\text{L}$  SA was added into 400  $\mu\text{L}$  NPs solution (molar access of protein/DNA is 2:1 assuming 100% DNA was attached on NPs). The mixture was then incubated in water bath sonicator for 1 h, followed by wash with TE buffer (10 mM Tris-Cl, pH 7.5. 1 mM EDTA). NPs were re-suspended in 400  $\mu\text{L}$  of TE buffer after 3 times wash. 10  $\mu\text{L}$  of supernatant and 10  $\mu\text{L}$  of well-suspended NPs mixture were taken for SA analysis, respectively.

#### H. Biotinylation of IgG

1 mg sulfo-NHS-LC-Biotin was dissolved in 180  $\mu\text{L}$  of deionized water to a final concentration of 10 mM. 28  $\mu\text{L}$  of fresh prepared reagent added into 500  $\mu\text{L}$  of 4.2  $\mu\text{g}/\mu\text{L}$  IgG solution. The mixture was mixed and incubated on ice for two hours. After incubation, biotinylated IgG was purified using Zebra Spin column (MWCO 50 kDa, Thermo Scientific, USA). The degree of biotinylation was then determined using Pierce Biotin Quantitation Kit (Thermo Scientific, USA).

#### I. Conjugation of biotinylated IgG on dsDNA and SA Modified TurboBeads®

28  $\mu\text{L}$  of 4.2  $\mu\text{g}/\mu\text{L}$  biotinylated human IgG was added into 400  $\mu\text{L}$  NPs solution prepared as in E. and F. (molar access of protein/DNA is 2:1). The mixture was then incubated in water bath sonicator for 1 h, followed by 3 washes with TE buffer. The NPs were then re-suspended in 400  $\mu\text{L}$  of TE buffer. 10  $\mu\text{L}$  of supernatant and 10  $\mu\text{L}$  of well-suspended NPs mixture were taken for IgG analysis, respectively.

#### J. DNA sample analysis

DNA modified NPs were separated from 100  $\mu\text{L}$  sample solution and re-suspended in 20  $\mu\text{L}$  5x SSC to yield a 5 times concentrated aliquot. The NPs were magnetically separated and 10  $\mu\text{L}$  of the supernatant was labeled as S1. The other un-separated DNA sample was labeled as S2. 3.3  $\mu\text{L}$  of LDS sample buffer was added (Invitrogen, Australia) into S1 and S2 and heated at 70°C over 10 min. NPs of S2 were separated from supernatant (10  $\mu\text{L}$ ) immediately after heating. 10  $\mu\text{L}$  of S1 and S2 supernatant were loaded on NuPAGE® Novex 3-8% Tris-Acetate gel (Invitrogen, Australia) and stained with SYBR™ Safe DNA Gel Stain (Invitrogen, Australia) according to the manufacture's instructions.

#### K. Protein sample analysis

3.3  $\mu\text{L}$  of LDS sample buffer (Invitrogen, Australia) was added to 10  $\mu\text{L}$  of SA and IgG samples, followed by heating at 70°C for 10 min. 10  $\mu\text{L}$  supernatant was immediately taken after heating and loaded on NuPAGE® Novex 4-12% Bis-Tris Gels (Invitrogen, Australia). The gel was stained with SYPRO® Ruby Protein Stain according to the manufacture's instructions for protein analysis.

### III. RESULTS AND DISCUSSION

#### A. DNA modification on Amine-coated TurboBeads®

In this study, commercial cross-linkers, HydraLink™ (Sulfo-HyNic and Sulfo-4FB), were used as a pair to modify amine-coated TurboBeads® and Amine-GTTT ssDNA, respectively. Both Sulfo-HyNic and Sulfo-4FB contain N-hydroxysuccinimide ester (NHS), which could be attached to NPs and target ssDNA respectively by interacting with amine group and forming stable amide bonds. Modified magnetic NPs and ssDNA were purified via magnetic separation and DNA precipitation, respectively. After purification, modified ssDNA was attached to magnetic TurboBeads® via the formation of bis-aryl hydrazone bond between Sulfo-4FB and Sulfo-HyNic.

The complementary DNA, Bio-ACCC, was then added to form dsDNA on the NP's surface (Fig. 1). The samples were analyzed on NuPAGE® Novex 3-8% Tris-Acetate gel. As a control, Sulfo-4FB modified Amine-GTTT was also hybridized with its complementary DNA, Bio-AAAC, in 5x SSC solution without NPs. As shown in Fig. 3, Lane 1, a band around 200bp was found on the gel, which confirmed the success of hybridization of Sulfo-4FB modified Amine-GTTT and Bio-AAAC without NPs. The same conditions were applied for DNA hybridization on the NPs' surface. As showed in Fig. 3, the supernatant (Lane 2) was clear after intensive washing. On the other hand, a band around 200 bp was observed after heating the NPs with LDS buffer (Lane 3), which was target dsDNA released from NPs after heating with denaturing reagents. This result clearly confirmed the attachment of dsDNA on magnetic NPs via the covalent cross-linkers.

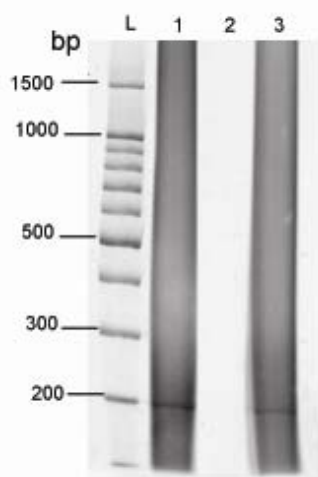


Fig. 3 DNA assembly on amine-coated magnetic NPs. Lane description: lane L: 100 bp DNA Ladder. Lane 1: hybridization of Sulfo-4FB modified Amine-GTTT and Bio-AAAC without NPs. Lane 2: supernatant of dsDNA modified magnetic NPs solution after washing. Lane 3: Mixture of homogeneous dsDNA modified magnetic NPs solution after washing.

#### B. Antibody attachment on dsDNA modified amine-coated TurboBeads®

Before conjugating IgG to dsDNA modified NPs, biotinylation of IgG was performed. Accessible amino-groups of IgG were modified with Sulfo-NHS-biotin. The molar ratio of biotin to antibody was investigated using Pierce® Biotin Quantitation Kit. Based on measurements of UV absorbance, 5 biotin molecules in average were successfully attached on each IgG molecule.

In the next step, biotinylated IgG was conjugated to dsDNA modified magnetic NPs via biotin and SA interactions (Fig. 1). As mentioned above, the success of dsDNA assembly on magnetic NPs provided numerous biotin molecules on the surface of NPs, since single biotin molecule was synthesized on the end of complementary Bio-AAAC. The addition of SA

specifically interacted with those biotin molecules and formed strong non-covalent bonds. SA is known to be a tetramer and each SA has four biotin binding sites. Thus, the attachment of SA on dsDNA provided abundant biotin binding sites on magnetic NPs' surface, which enables attachment of biotinylated IgG in the final step. Homogeneous NPs solution, as well as NP-free supernatant from each step were collected and heated at 70°C for 10 min with LDS sample buffer. Under these conditions, SA was denatured from tetramer (MW= 52.8 kDa) to monomer (MW=13.2 kDa), which also allowed conjugated SA and IgG to strip off from NPs and come into supernatant. As shown in Fig. 4, in the final step, there were two major bands, which respectively stood for IgG and SA, were seen in heated NPs sample solution (Lane 4) but not the supernatant without NPs (Lane 3). The results clearly confirm that both SA and IgG were successfully attached on magnetic NPs.

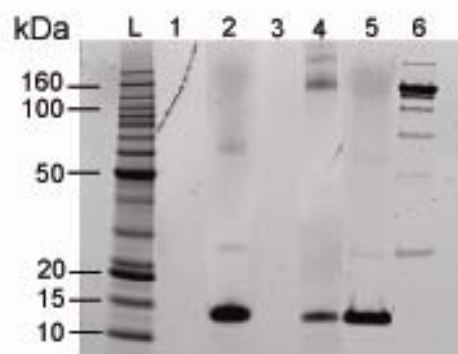


Fig. 4 Antibody attachment on dsDNA modified amine-coated magnetic NPs. Lane description: Lane L, protein ladder (BenchMark™). Lane 1, the supernatant without NPs after SA addition. Lane 2, the supernatant with NPs after SA addition. Lane 3, the supernatant without NPs after SA and IgG addition. Lane 4, the supernatant with NPs after SA and IgG addition. Lane 5, SA. Lane 6, IgG.

#### C. Biomolecular modification on SA-coated TurboBeads®

Another biomolecular modification strategy was completely developed via biotin-SA interaction, which is one of most common affinity interactions widely utilized in purification and detection system. In this study, Bio-AAAC (synthesized ssDNA with a biotin molecule at one of ends) was first assembled on SA-coated magnetic NPs. Since the complementary DNA, Bio-GTTT, also has a biotin molecule on its distal end, additional blocking step was conducted by using free biotin molecules to block unoccupied SA binding sites before the addition of complementary DNA. SA and biotinylated IgG were successively attached onto dsDNA modified NPs after the DNA hybridization step. As shown in Fig 5, no band was detected in lanes A2, B1, and B4, containing the NP-free supernatant after washing. However, after boiling the whole sample including the NPs, bands were appeared with the expected electrophoretic mobilities (Lane A3, B2, and B3).

The results convincingly showed that both dsDNA and target IgG had been successfully assembled on the surface of SA-coated magnetic NPs via biotin and SA specific interactions.

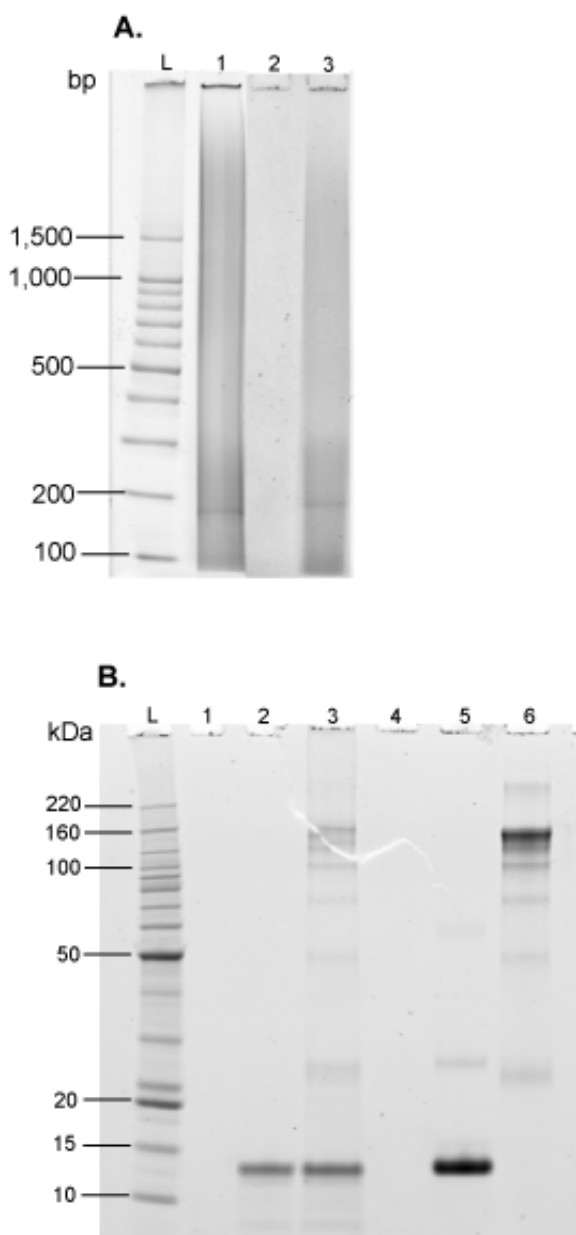


Fig. 5 Biomolecular modification on SA modified magnetic NPs. A). Lane L: 100 bp DNA Ladder. Lane 1: hybridization of Bio-GTTT and Bio-AAAC in 5x SSC without NPs. Lane 2: supernatant of dsDNA modified TurboBeads® after washing. Lane 3: Mixture of homogeneous dsDNA modified TurboBeads® after heating. B). Lane L, protein ladder (BenchMark™). Lane 1, supernatant of mixture after SA addition. Lane 2, whole mixture including NPs after SA addition. Lane 3, whole mixture including NPs after SA and IgG addition. Lane 4, supernatant of mixture after SA and IgG addition. Lane 5, SA. Lane 6, IgG.

#### IV. CONCLUSION

In this work, two strategies were developed to conjugate biomolecules on the surface of magnetic NPs. Both amine- and SA-coated magnetic NPs were successfully modified. In one of methods, commercial cross-linkers, Sulfo-S-HyNic and Sulfo-S-4FB, were utilized to modify amine-coated NPs. Stable bis-aryl hydrazone bonds were formed and enabled controlled assembly of target DNA on NPs' surface. The bonds formed between aromatic hydrazine (HyNic) and aromatic aldehyde is specific and offers stable and long shelf life. This covalent bond is stable for 2 hours at 94°C<sup>[17]</sup>, which could prove highly usable in biomolecular conjugation studies. The other NPs modification method takes advantage of biotin-streptavidin interactions. The affinity of avidin to biotin is the strongest known non-covalent interactions between protein and ligand, which has also been successfully applied in NPs modification in this investigation. The results in this study confirmed that, via those two methods, the process of magnetic NPs modification with biomolecules could be well controlled. The target DNA and antibody were successfully attached on two different magnetic NPs, which offer potential for applications in more sophisticated nanoparticle-based assemblies.

#### ACKNOWLEDGMENT

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