Supplementary Information For:

Title: Modular Fluorescent Nanoparticle DNA Probes for Detection of Peptides and Proteins

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Supplementary Information

Supplemental Methods

PEG density determination for 40-nm particles

PEGylation was carried out as described in the main text Materials and Methods section "Nanoparticle activation and PEGylation". Particles were resuspended in a 100 mg/mL of a 95:5 mPEG-amine:azide-PEG-amine (MW of PEGs was 2000 g/mol) in PBS at PEG:nanoparticle molar ratios of $1.4x10^5$, $2.1x10^5$, and $2.8x10^5$. Additional sets of 40-nm particles were resuspended in 100 mg/mL of a 99.5:0.5, 99.95:0.05, or 99.995:0.005 mPEG-amine:azide-PEG-amine ratios. The reactions were incubated at 24 °C with shaking at 800 RPM on a ThermoMixer dry block. After 1 hour, 250 µl PBS was added, samples were washed twice, and resuspended in 500 µl PBS. Zeta potential measurements were obtained as described in the main text Materials and Methods section "Fluorescent nanoparticle probe characterization".

Alternative probe conjugation strategies

Oligonucleotides were purchased from Integrated DNA Technologies (IDT) for DNA attachment through a conjugation-annealing handle or from Eurofins Scientific for the direct conjugation of the DBCO-B1 probe (**Table 1**). The attachment of DNA was achieved by pre- or post-annealing the probe to the particle, as described in the main text Material and Methods section "DNA aptamer probe attachment" (**Figure 1A**). Direct conjugation of the probe to the PEG layer was performed as a comparison to more standard approaches. For direct conjugation, 40-nm nanoparticles were PEGylated as described in the main text. The DBCO-B1 probe was reacted with azide-PEGylated particles at a 300:1 ratio in PBS at 24 °C overnight with shaking at 800 RPM. Following incubation, 180 µl PBS was added, and samples were washed twice and resuspended in 500 µl PBS. The final sample was stored at 4 °C.

In-house peptide synthesis

Peptides were synthesized on an Intavis Multipep RSi synthesizer. All fluorenylmethoxycarbonyl protected amino acids (Fmoc-amino acids) and 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) were purchased from AAPPTec. All other solvents and reagents were purchased from Sigma or ACROS. For 2-µmole-scale synthesis on Rink Amide AM resin (200-400 mesh), 0.5 M Fmoc-amino acid solutions in anhydrous dimethylformamide (DMF) were activated by 0.5 M HATU. Activated Fmoc-amino acids were coupled to amine group on N-terminus of the previously added amino acid (or amine group of Rink amide resin in case of first amino acid coupling) using 4.0 N *N*-methylmorpholine. The Fmoc group was removed by incubation in 20% piperidine in DMF prior to addition of next incoming amino acid. The first four cycles of peptide synthesis were double coupled for 15 and 25 minutes followed by double coupling for 20 and 30 minutes for remaining cycles. Deprotection and cleavage of peptides from resin were achieved in 94% trifluoroacetic acid (TFA), 2.5% deionized water, 2.5% 1,2-ethane-di-thiol, 1% triisopropylsilane. Peptides were precipitated by adding tert-butyl methyl ether to concentrated peptidyl-TFA solution. The precipitates were dried in a CentriVap, suspended in 500 µL water, and purified using Waters Sep-Pak C-18 plates[1]. Peptides were identified by their m/z value on AB Sciex MALDI MS at the Canary Center, Stanford University.

Fluorescent stability studies of alternative labels

Biotinylated aptamer probes conjugated to alternative off-the-shelf labels prepared as described in main text Materials and Methods section “Preparation of fluorescent probes with alternative off-the-shelf labels” were stored at concentrations of 1 µM in 10 mM HEPES, 1.2 mM NaCl, 5

mM $MgCl_2$, 5 mM KCl, pH 7.4 at 4 °C. The stability of the conjugated fluorescent entity was determined by performing a 3-fold serial dilution from 300 nM to 137 pM in 100 µL 1X NV buffer in a 96-well black microplate (Corning). The fluorescence intensity of each well was used to generate a linear curve of the fluorescent probe immediately and at 1- and 2-weeks post-conjugation.

Supplemental Results and Discussion

Determination of PEG density for 40-nm particles

To evaluate how PEG density impacted passivation of 40-nm particles, we varied the PEG:nanoparticle ratio and measured the resulting surface charge neutralization using zeta potential measurements. Based on theoretical models, we reduced the PEG:nanoparticle ratio to approximately 10^5 PEG molecules per nanoparticle and tested several concentrations within that order of magnitude. These studies indicated that there was similar surface charge of -10 mV for each experimental group (**Figure S1**). From this, we concluded that $1.4x10^5$ PEG:nanoparticle was sufficient for passivating 40-nm nanoparticles.

Binding assessment of varied functional PEG ratios

To evaluate the ratio of functionalized to non-functionalized PEG required for probe attachment and successful detection, we varied the ratio of amine-PEG-azide to mPEG-amine during nanoparticle fabrication. Fluorescent nanoparticle probes with ratios of mPEG-amine:amine-PEG-azide of 95:5, 99.5:0.5, 99.95:0.05, and 99.995:0.005 were fabricated, conjugated to B1 probes, and evaluated in plate-based binding studies. The ratio of 95:5 showed about 1.5x higher signal than the ratio of 99.5:0.05 (**Figure S2**). As the amine-PEG-azide contains the reactive group for probe attachment, it is expected that if the DNA aptamer probe-complex is in excess of

the amine-PEG-azide, the number of probes per particle will decrease with decreased amine-PEG-azide and thus reduce on-target binding. As this is what we observed, we hypothesize that it may be possible to increase the mPEG-amine:amine-PEG-azide ratio in order to achieve higher binding affinity. For these proof-of-principle studies, we utilized the 95:05 mPEG-amine:amine-PEG-azide ratio for PEGylation of the fluorescent nanoparticle probes.

Pre-anneal versus post-anneal probe attachment

We evaluated two different approaches for probe attachment to the particle: the "pre-anneal" and "post-anneal" approaches. In pre-annealing, the probe is attached to the fluorescent nanoparticle B1 probe by pre-annealing of conjugation-annealing handle to DNA aptamer probe prior to attachment to the particle. In post-annealing, the conjugation-annealing handle is conjugated to the particle and then the DNA aptamer probe is annealed. The pre-annealing and post-annealing methods were compared through assessment of B1 binding to his-tagged Her2 protein (on-target) and myoglobin (off-target). Pre-annealed probes showed higher binding than the post-annealing approach to on-target proteins (5.5-fold and 2.5-fold binding over background, respectively (**Figure S4**). Little non-specific binding was observed irrespective of the attachment strategy. It may be possible to optimize the post-annealing approach in studies beyond the scope of this proof-of-principle analysis. First, the concentration of probe could be increased to ensure attachment to all available conjugation-annealing handles. Second, the annealing handle could include a spacer region to reduce steric hinderance due to the PEG layer. Finally, the annealing region could be extended to increase the likelihood of achieving annealing. For the proof-of-principle studies described in this work, the pre-annealing method was used.

Binding assessment of alternative conjugation strategies

As our probe annealing attachment technique is unique, we compared on-target binding by probe prepared using our pre-annealing attachment approach to a more standard direct probe conjugation attachment. In the direct conjugation strategy, the 5'-DBCO functional group on the DNA probe was conjugated to the PEG-azide. The original design, using the annealing approach for probe attachment, showed higher binding to its target than the direct DBCO conjugation strategy (4.3-fold versus 2.4-fold binding over background, respectively; **Figure S5**). This suggest that our annealing approach works as well or better than more standard approaches.

Fluorescent stability of commercially available probes

Our fluorescent nanoparticle probes bound with higher EC_{50} values than commercially available labels (**Figure 6**). To investigate if this was due to changes in binding affinity or reduction in fluorescent signal, the brightness of probes was assessed immediately after preparation and at 1 and 2 weeks. No change in fluorescent intensities was observed for the commercially available labels over the course of two weeks (**Figure S6**). This study suggests that the reduction in binding signal observed in the commercially available labels is due to loss of binding affinity, not loss in fluorescent intensity. This provides additional motivation to use the fluorescent nanoparticle probes, as they are more stable.

Experimental data replicates

Supplementary **Figures S7-13** show additional replicates of the main text figures. In all cases, at least three replicates were run for all main text figures, even if these replicates were gathered across more than one experiment.

Fluorescent Nanoparticle Probe Protocol

<u>Buffers:</u>
Reaction buffer: 20 mM MES, 500 mM NaCl, pH 6
Wash buffer: PBS; 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4

Nanoparticle Wash:

Nanoparticle washes are carried out throughout this protocol and consist of the following steps:

1. Centrifuge nanoparticles at 31,000 x*g* for 30 minutes prior to PEGylation or 60 minutes post PEGylation in a 1.5-mL tube to form a pellet.
2. Remove supernatant, taking care not to disturb the pellet.
3. Add appropriate buffer and volume to the pellet, as noted in procedure.
4. Redisperse the pellet by pipetting up and down while the nanoparticle tube is partially immersed in a standard laboratory sonication bath (Branson Bransonic Ultrasonic Cleaner 8510R-DTH) until no large aggregates of nanoparticles are visible (usually 10-30 seconds).

Procedure:

This procedure is written for any scale of nanoparticle preparation, but suggested masses, volumes, and concentrations are listed for 40-nm and 200-nm particle preparations used throughout the protocol.

1. Redisperse the stock tube of carboxylate-modified microspheres (see **Table 1** for more information) for 10 seconds following step 4 of "Nanoparticle Wash" to ensure nanoparticles are well distributed in solution.
2. Pipette 5.3×10^{13} 40-nm nanoparticles or 2.13×10^{10} 200-nm nanoparticles into 1.5-mL Eppendorf tubes. (Note: Pellets do not form well in 2 mL tubes.) Suggested volumes:
    a. 40-nm particles: 60 µL (5.3×10^{13} nanoparticles) from a stock of 8.8×10^{14} particles/mL.
    b. 200-nm particles: 6.25 µL (2.13×10^{10} nanoparticles) from a stock of 3.4×10^{12} particles/mL.
3. Activate nanoparticles with EDC/NHS at 5.3×10^{13} 40-nm nanoparticles/mL or 1.7×10^{11} 200-nm nanoparticles/mL in a solution of 50 mM EDC and 100 mM NHS in reaction buffer at 24 °C for 1 hour with shaking at 800 revolutions per minute (RPM) on a ThermoMixer dry block. Dissolve EDC and NHS directly into reaction buffer immediately before use and carry out steps 4 and 5 quickly to reduce NHS hydrolysis. Suggested volumes:
    a. 40-nm particles: Add 500 µL of 100 mM EDC and 200 mM NHS (both dissolved in reaction buffer) to 60 µL of nanoparticle stock.
    b. 200-nm particles: Add 62.5 µL of 100 mM EDC and 200 mM NHS (both dissolved in reaction buffer) to 60 µL of nanoparticle stock.
4. Wash particle pellet and resuspend in 1 mL wash buffer.
5. Wash particles and redisperse in PEG solution:
    a. Dissolve mPEG-amine and azide-PEG-amine (see **Table 1** for details) at 100 mg/mL concentration in wash buffer.
    b. Resuspend particles in 95:5 volume ratio of mPEG-amine:azide-PEG-amine such that PEG:nanoparticle molecular ratio is 3.5×10^{7} and 1.4×10^{5} for 200- and 40-nm nanoparticles, respectively. Suggested volumes:
        i. 40-nm particles: 235 µL 100 mg/mL mPEG-amine and 12.7 µL 100 mg/mL azide-PEG-amine.
        ii. 200-nm particles: 23.5 µL 100 mg/mL mPEG-amine and 1.3 µL 100 mg/mL azide-PEG-amine.
6. Incubate at 24 °C for 1 hour with shaking at 800 RPM on a ThermoMixer dry block. (Note: This incubation step may be allowed to proceed overnight.)

7. Add 1 µL wash buffer/µL PEG solution for 40-nm particles and 7 µL wash buffer/µL PEG solution for 200-nm particles. Suggested volumes:
    a. 40-nm particles: 250 µL wash buffer.
    b. 200-nm particles: 180 µL wash buffer.
8. Wash and redisperse particles in 2 µL wash buffer/µL PEG solution PBS for 40-nm particles and 5 µL wash buffer/µL PEG solution for 200-nm particles. (Note: The pellet will be less defined, and some nanoparticles may remain in solution after this step.) Suggested volumes:
    a. 40-nm particles: 500 µL wash buffer.
    b. 200-nm particles: 125 µL wash buffer.
9. Combine particles with the conjugation-annealing handle or probe for the pre-anneal or post-anneal probe attachment approach. React 125 µM of the DNA aptamer probe-complex or the conjugation annealing handle with particles at a 30,000:1 or 125:1 DNA:nanoparticle molar ratio for 200- and 40-nm particles, respectively:
    a. Pre-anneal:
        i. Pre-anneal the conjugation-annealing handle and probe (see **Table 3** for details) by combining 125 µM conjugation annealing handle and 125 µM DNA aptamer probe in equal volumes and incubate at 95 °C for 5 minutes. Centrifuge briefly to remove condensation from the tube lid. Incubate on benchtop for 10-15 minutes to allow the two complementary regions of DNA to anneal. Suggested volumes:
            1. 40-nm particles: Combine 88 µl each of 125 µM stocks of conjugation annealing handle and probe.
            2. 200-nm particles: Combine 8.5 µl each of 125 µM stocks of conjugation annealing handle and probe.
        ii. Add pre-annealed probe complex to nanoparticle pellet and add appropriate volumes of 1x and 10x wash buffer to achieve a final concentration of 1x wash buffer and a final volume 3.3x the initial nanoparticle volume. Incubate at 24 °C for at least 16 hours with shaking at 800 RPM on a ThermoMixer dry block. Suggested volumes:
            1. 40-nm particles: For a final volume of 200 µl, combine 176 µl probe complex, 17.6 µl 10x wash buffer, and 4 µl wash buffer.
            2. 200-nm particles: For a final volume of 20 µl, combine 17 µl probe complex, 1.7 µl 10x wash buffer, and 1.3 µl wash buffer.
    b. Post-anneal (optimized for 200-nm particles):
        i. Post-anneal the conjugation-annealing handle (see **Table 3** for details) to the particle by adding conjugation annealing handle to nanoparticle pellet and add appropriate volumes of 1x and 10x wash buffer to achieve a final concentration of 1x wash buffer and a final volume 3.3x the initial nanoparticle volume. Incubate at 24 °C for at least 16 hours with shaking at 800 RPM on a ThermoMixer dry block. Suggested volume:
            1. 200-nm particles: For a final volume of 20 µl, combine 8.5 µl conjugation-annealing handle, 0.85 µl 10x wash buffer, and 10.7 µl wash buffer.
        ii. Wash particles and redisperse in wash buffer equal to 28x the initial particle volume twice. Suggested volume:
            1. 200-nm particles: 180 µL.

iii. Wash particles. Redisperse in a solution of 125 µM DNA aptamer probe at equal molar ratio to the conjugation-annealing handle. Bring to 3.3x the initial particle volume using water and incubate at 95 °C for 5 minutes. Centrifuge briefly to remove condensation from the tube lid. Incubate on benchtop for 10-15 minutes to allow the two complementary regions of DNA to anneal. Suggested volumes:
    1. 200-nm particles: Combine 8.5 µl of 125 µM probe and 11.5 µl DNase/RNase free water.

10. Add 1 µL wash buffer/µL PEG solution for 40-nm particles and 7 µL wash buffer/µL PEG solution for 200-nm particles. Suggested volumes:
    a. 40-nm particles: 250 µL wash buffer.
    b. 200-nm particles: 180 µL wash buffer.
11. Wash and redisperse particles in 2 µL wash buffer/µL PEG solution PBS for 40-nm particles and 5 µL wash buffer/µL PEG solution for 200-nm particles. (Note: The pellet will be less defined, and some nanoparticles may remain in solution after this step.) Suggested volumes:
    a. 40-nm particles: 500 µL wash buffer.
    b. 200-nm particles: 125 µL wash buffer.
12. Repeat Step 11. Store nanoparticles in wash buffer at 4 °C.

PEG Density Calculations

The density of PEG-36 (MW of 1.6 kDa) required to achieve brush layer conformation was determined utilizing theoretical and experimental models[2–4]. The PEG conformation depends upon two parameters: 1) the Flory radius (R_F), which is the radius of the PEG coil and is dependent upon molecular weight and 2) the distance between PEG molecule graft sites (D). The relationship between R_F and D dictates the PEG conformation: If $D > R_F$, the PEG layer will be a mushroom conformation; if $D < R_F$, it will be a brush layer; and if $D < 0.36\ R_F$ it will be a dense brush layer[2–4] . R_F can be calculated using the following equations[2–4]:

$$R_F = \alpha N^{3/5}$$

where α = the length of one monomer and N = the number of monomers/polymer chain.

Assumptions:

- D = distance between PEG molecules
- $\alpha = 0.35$ nm

- N = 36
- Nanoparticle diameter = 200 nm
- Each PEG molecule occupies one circular area (A_{PEG}) on the particle surface
- PEG molecules are equally spaced across the surface
- A dense PEG layer requires $D < 0.36R_F$

Calculations:

- Flory Radius:

  $$R_F = (0.35\ nm)\left(36^{\frac{3}{5}}\right) = 3.0\ nm$$

- Surface area of the nanoparticle:

  $$Surface\ area_{NP} = 4\pi r^2 = 4\pi(100nm)^2 = 125{,}663\ nm^2$$

- PEG distance to achieve a dense brush layer:

  $$D < (0.36)R_F, D < 1.08\ nm$$

- PEG distance as a function of PEG spacing:

  Distance between two PEG molecules:

  $$D = Radius\ of\ PEG1 + Radius\ of\ PEG2 = Diameter\ of\ A_{PEG}$$

  $$A_{PEG} = \pi(\frac{D}{2})^2$$

  $$D = 2\left(\frac{A_{PEG}}{\pi}\right)^{1/2}$$

  $$D < 1.08\ nm$$

  $$1.08\ nm > 2\left(\frac{A_{PEG}}{\pi}\right)^{\frac{1}{2}}$$

  $$A_{PEG} < 0.92\ nm^2$$

- Determination of minimum # PEG molecules/particle:

$$A_{PEG} = \frac{Surface\ area_{NP}}{\#\ PEG\ molecules}$$

$$0.92\ nm^2 = \frac{125{,}663\ nm^2}{\#\ PEG\ molecules}$$

$$\#\ PEG\ molecule > 137{,}000, or\ at\ least\ 10^5 PEG\ molecules/particle$$

Supplemental References

1. Pipkorn, R., Boenke, C., Gehrke, M. & Hoffmann, R. High-throughput peptide synthesis and peptide purification strategy at the low micromol-scale using the 96-well format. *J. Pept. Res.* **59**, 105–114 (2002).

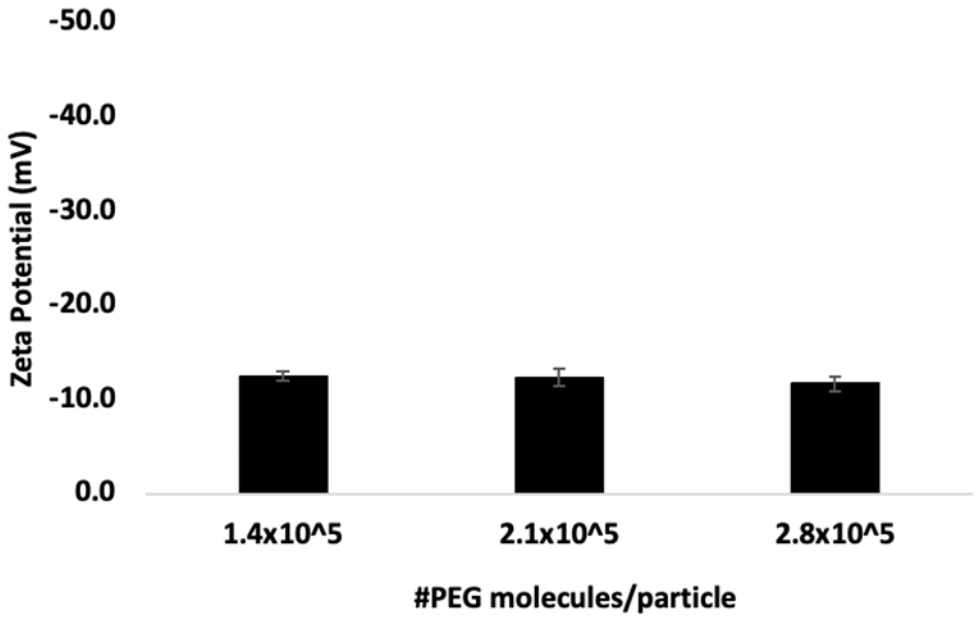
2. Yang, Q. *et al.* Evading immune cell uptake and clearance requires PEG grafting at densities substantially exceeding the minimum for brush conformation. *Mol. Pharm.* **11**, 1250–1258 (2014).

3. Damodaran, V. B., Fee, C. J., Ruckh, T. & Popat, K. C. Conformational Studies of Covalently Grafted Poly (ethylene glycol) on Modified Solid Matrices Using X-ray Photoelectron Spectroscopy. **26**, 7299–7306 (2010).

4. Perry, J. L. *et al.* PEGylated PRINT Nanoparticles: The Impact of PEG Density on Protein Binding, Macrophage Association, Biodistribution, and Pharmacokinetics. (2012).

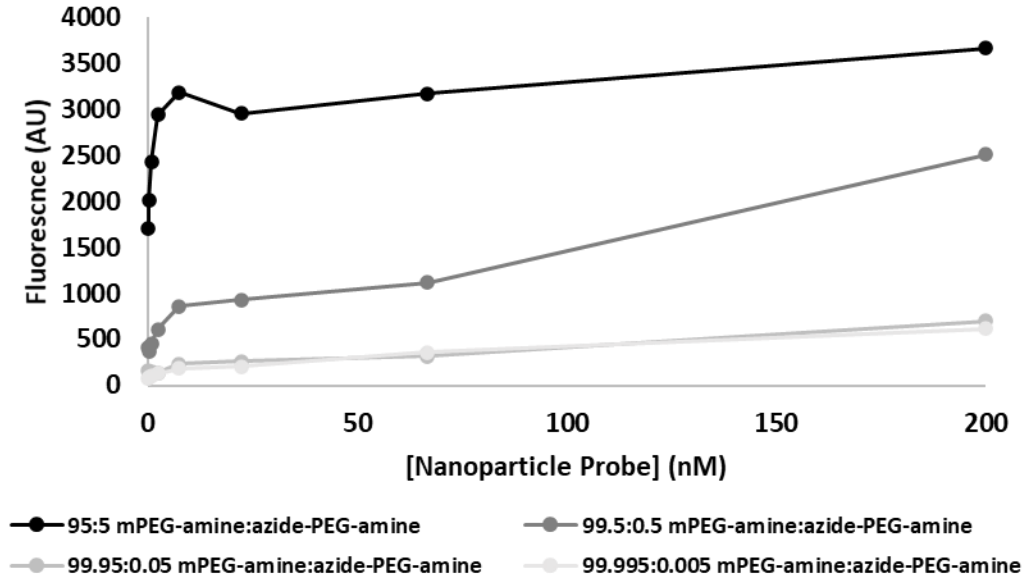
Supplemental Figures and Figure Legends

Figure S1



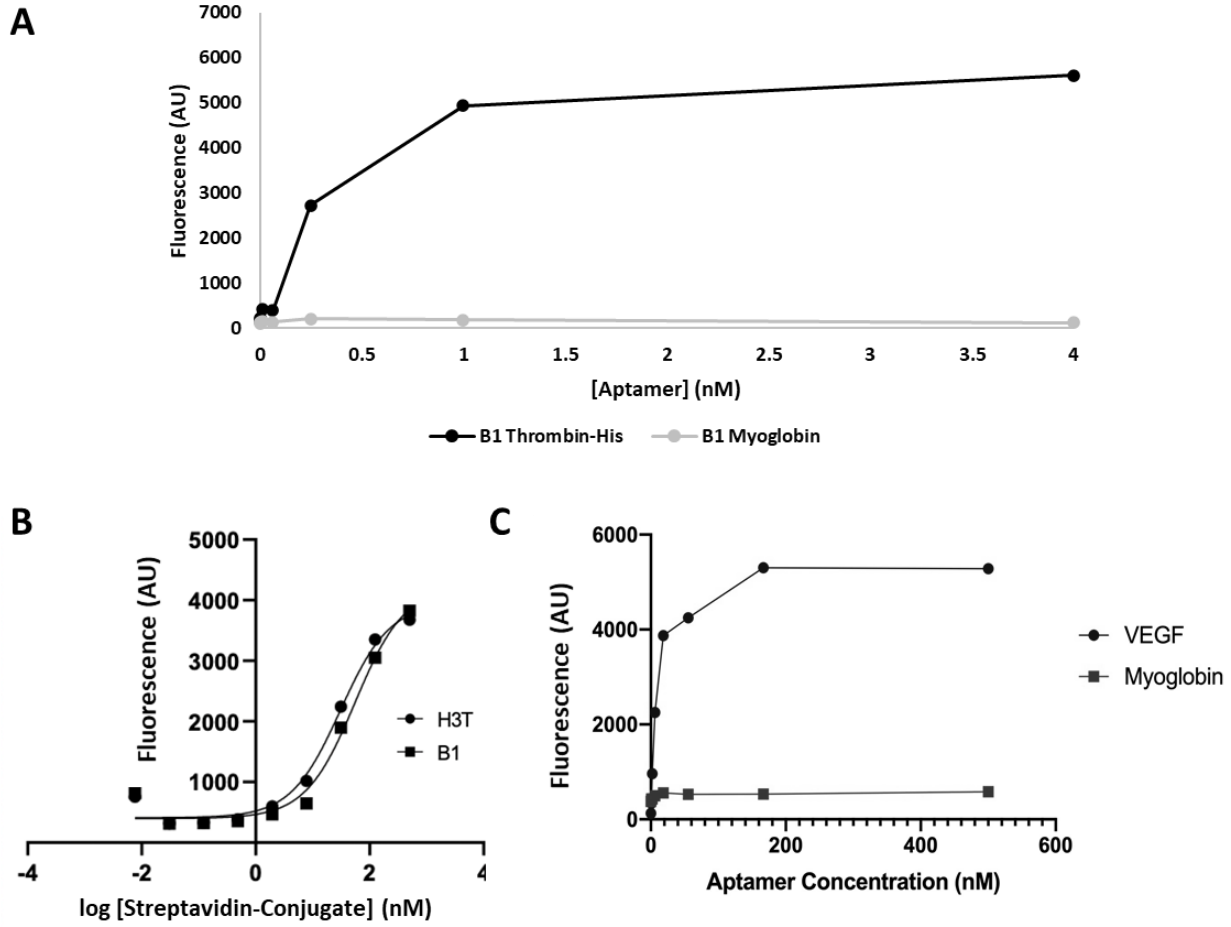
Supplementary Figure S1. PEG density for 40-nm nanoparticles. Zeta potential measurements for 40-nm carboxylated FluoSpheres™ activated with NHS/EDC and reacted with increasing concentrations of mPEG-amine. Data is depicted as means (± standard deviation) of the six measurements taken (three per replicate) from one of two experiments performed in duplicate.

Figure S2



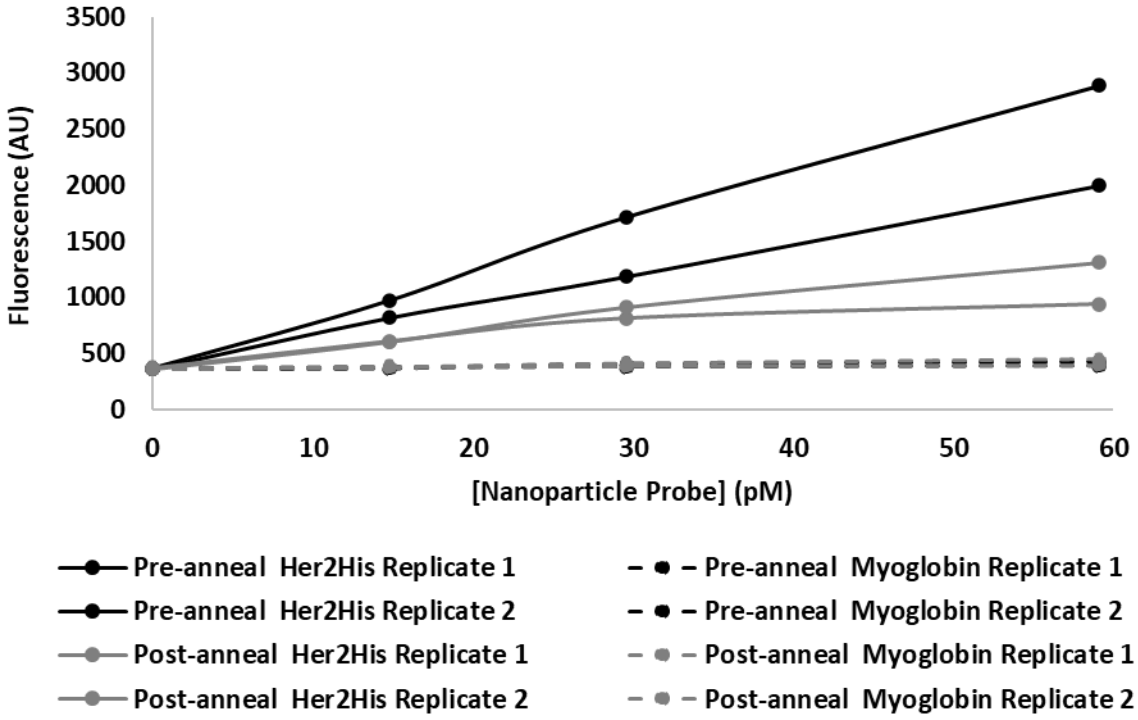
Supplementary Figure S2. On-target binding of nanoparticle B1 probes fabricated with various ratios of mPEG-amine to azide-PEG-amine. Binding of fluorescent nanoparticle B1 probes with varying ratios of mPEG-amine to azide-PEG-amine to his-tagged Her2 protein. Data are from a single experiment.

Figure S3



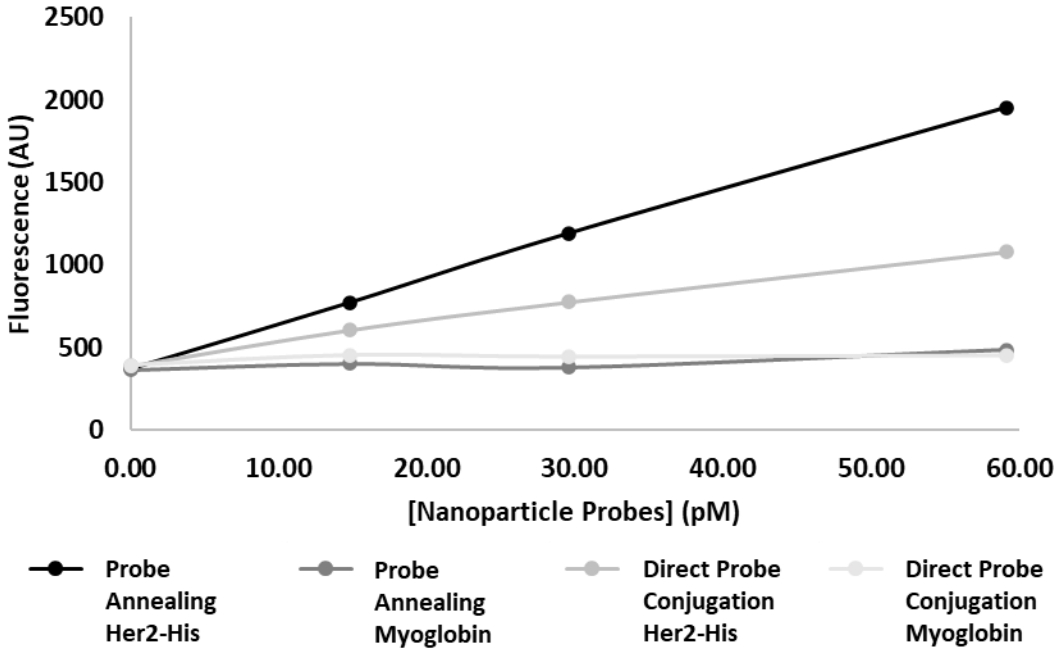
Supplementary Figure S3. Binding of aptamers detected with streptavidin-HRP or streptavidin-conjugates. (A) Binding of a biotinylated B1 aptamer to his-tagged thrombin detected by streptavidin-HRP. (B) Binding of B1 and H3T streptavidin conjugates to HHH peptide, with calculated EC50s of 55 and 30 nM, respectively. (C) Binding of a biotinylated VEGF aptamer to VEGF protein detected by streptavidin-HRP. Data shown are representative of at least three experiments.

Figure S4



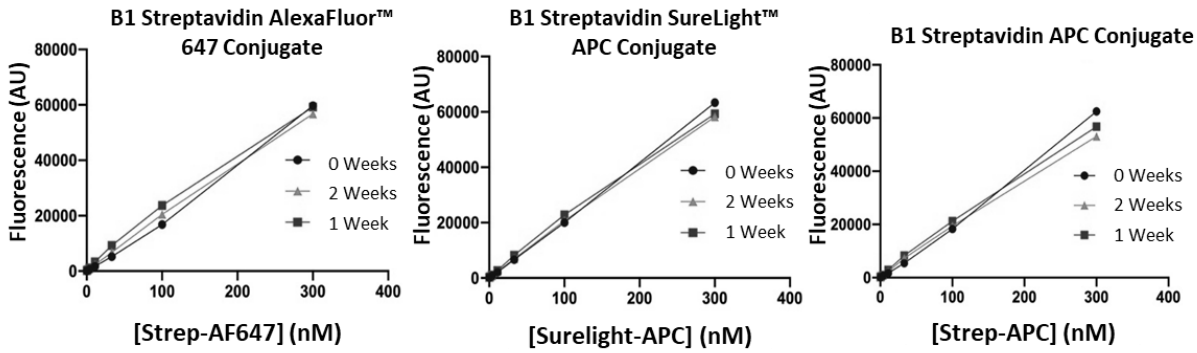
Supplementary Figure S4. Affinities of probes prepared by pre- and post-annealing of DNA aptamers to nanoparticles. Binding of fluorescent nanoparticle B1 probes fabricated with pre- and post-annealing protocols to his-tagged Her2 (on-target) and myoglobin (off-target). Data are representative of a single experiment performed in duplicate.

Figure S5



Supplementary Figure S5. Affinity of probe prepared by direct conjugation of DNA aptamer to nanoparticles. Binding of fluorescent nanoparticle B1 probes fabricated with direct conjugation or with pre-annealing protocol to his-tagged Her2 (on target) and myoglobin (off-target). Data are from a single experiment with the direct conjugation protocol and are representative of at least five experiments for the probe prepared by pre-annealing.

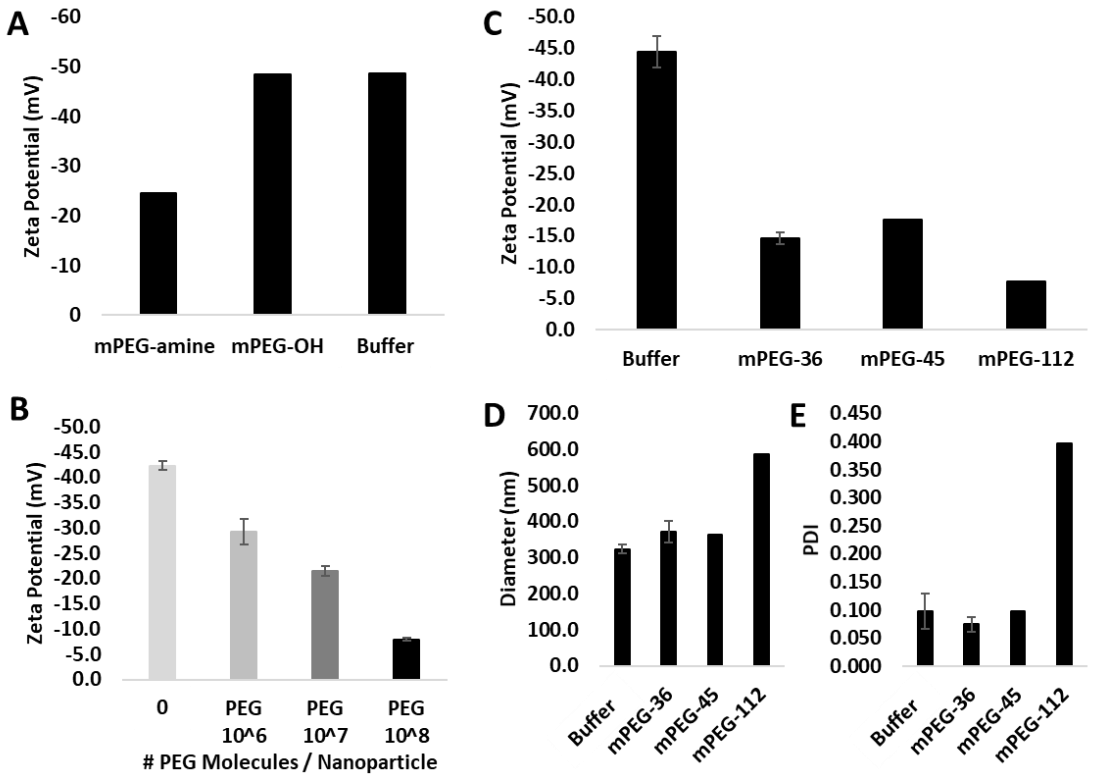
Figure S6



Supplementary Figure S6. Fluorescent intensities of labels are stable over 2 weeks.

Fluorescent intensity readings of commercially available labels conjugated to the B1 aptamer measured over the course of two weeks. Data are representative of two experiments.

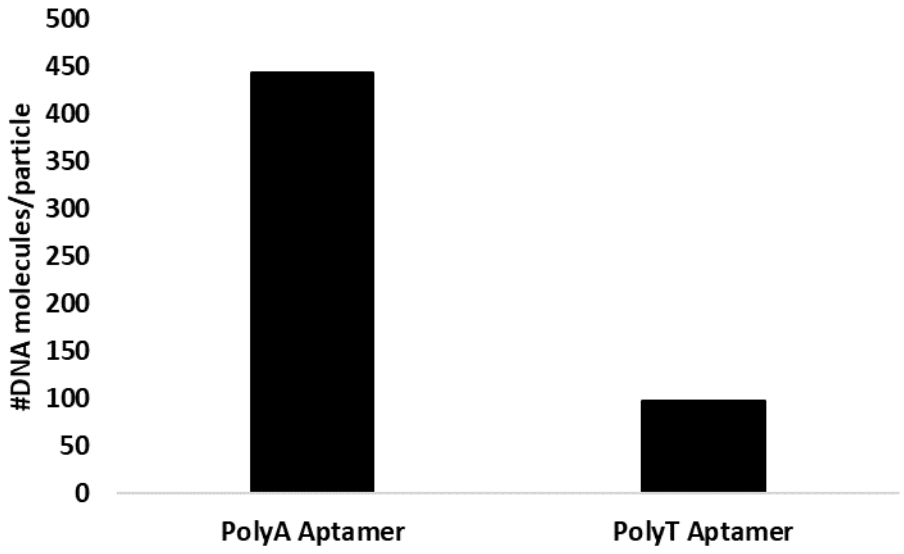
Figure S7



Supplementary Figure S7. Replicates for Figure 2 PEGylation of carboxylate-modified FluoSpheres™. (A) Zeta potential measurements for carboxylated FluoSpheres activated with NHS/EDC and reacted with mPEG-amine, mPEG-methoxy, or buffer only. Data are means of duplicate experiments, analyzed in duplicate. (B) Zeta potential measurements for carboxylated FluoSpheres activated with NHS/EDC and reacted with increasing concentrations of mPEG-amine. Data are means (± standard deviation) of duplicate experiments, analyzed in triplicate measurements. (C-E) FluoSpheres were conjugated with PEGs of indicated molecular weights, and C) zeta potentials, (D) hydrodynamic diameters, and (E) PDI were determined. Data are means (± standard deviations for experiments with three or more replicates) of duplicate

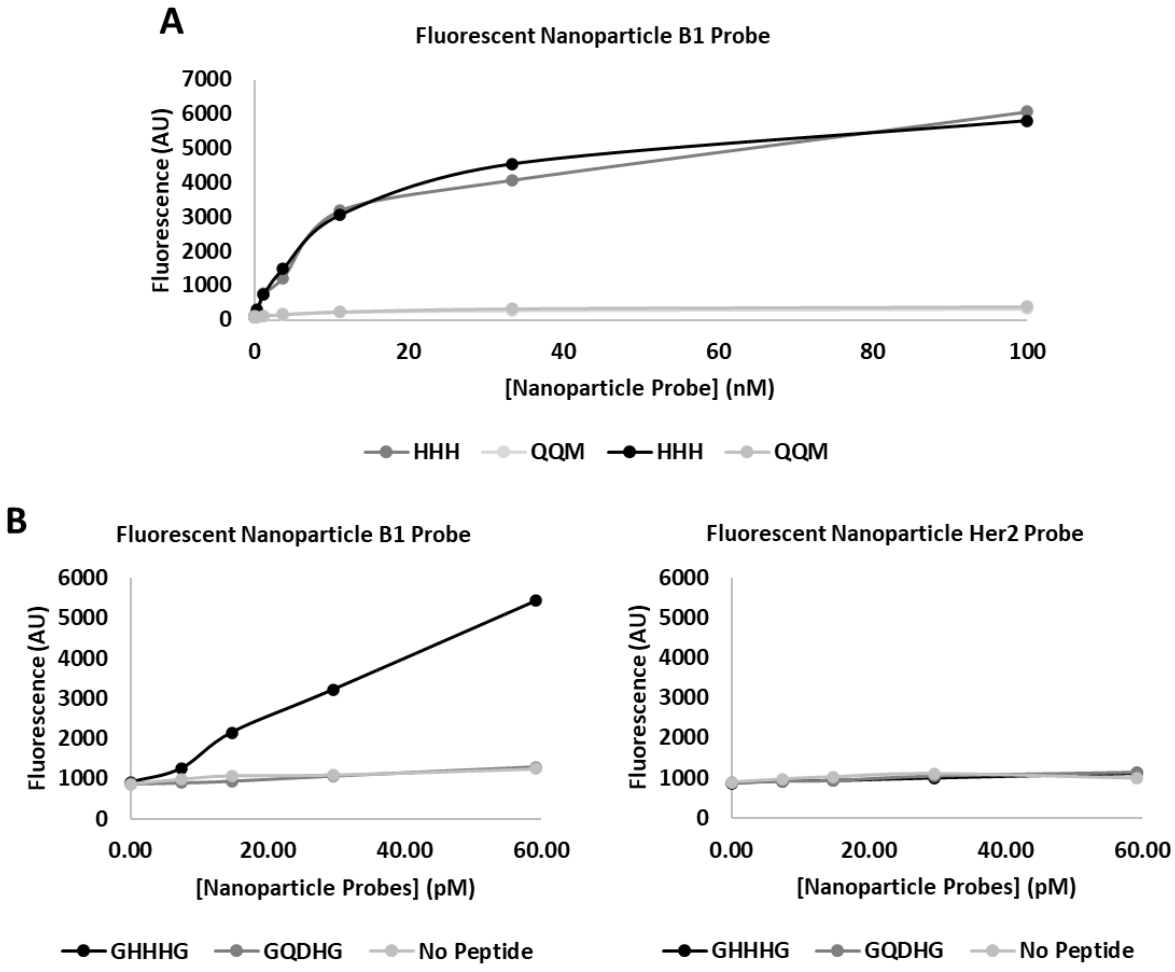
experiment. Replicates per group: 5 for Buffer, 2 for mPEG-12, 2 for mPEG-24, 5 for mPEG-36, 3 for mPEG-45, and 3 for mPEG-112.

Figure S8



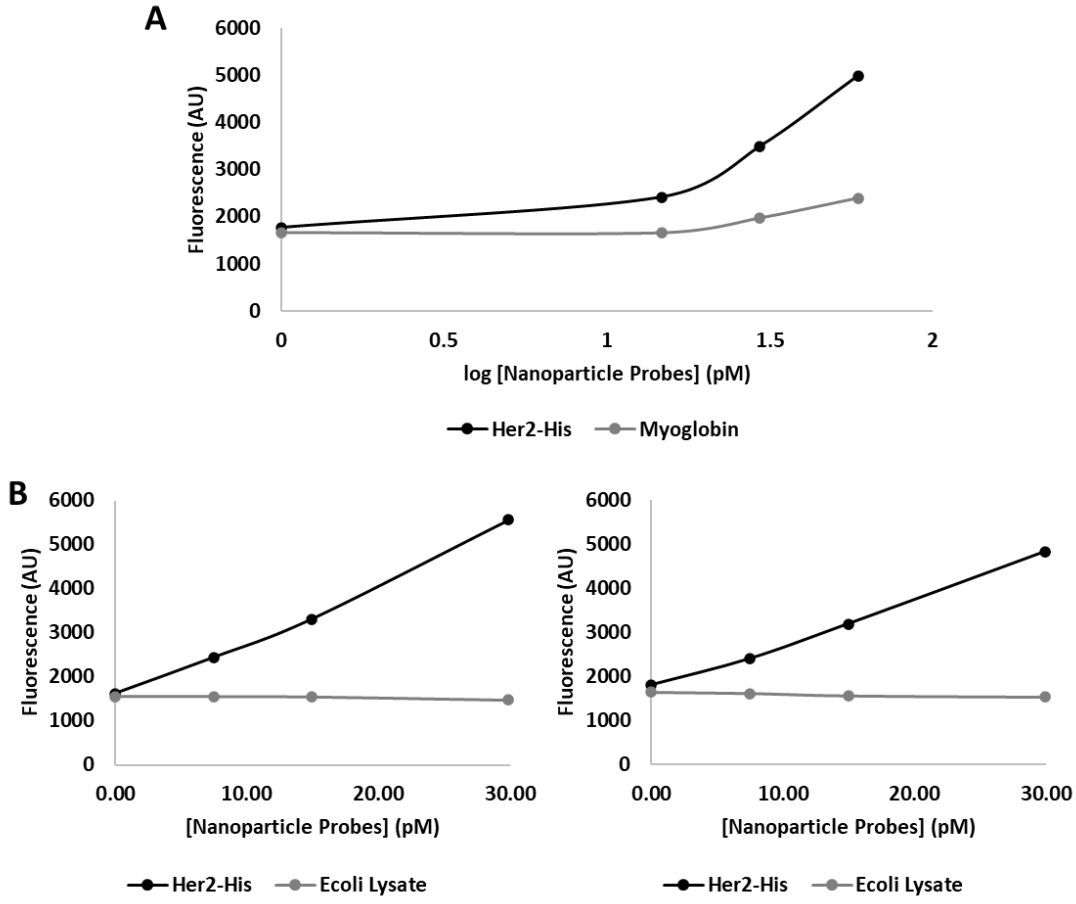
Supplementary Figure S8. Replicate for Figure 3B Optimization of DNA aptamer probe attachment to PEGylated nanoparticle. qPCR quantification of aptamer numbers determined on particle and off particle for particles treated with complimentary polyA aptamer or non-complimentary (negative control) polyT aptamer. Data are means of one of two experiments performed in duplicate.

Figure S9



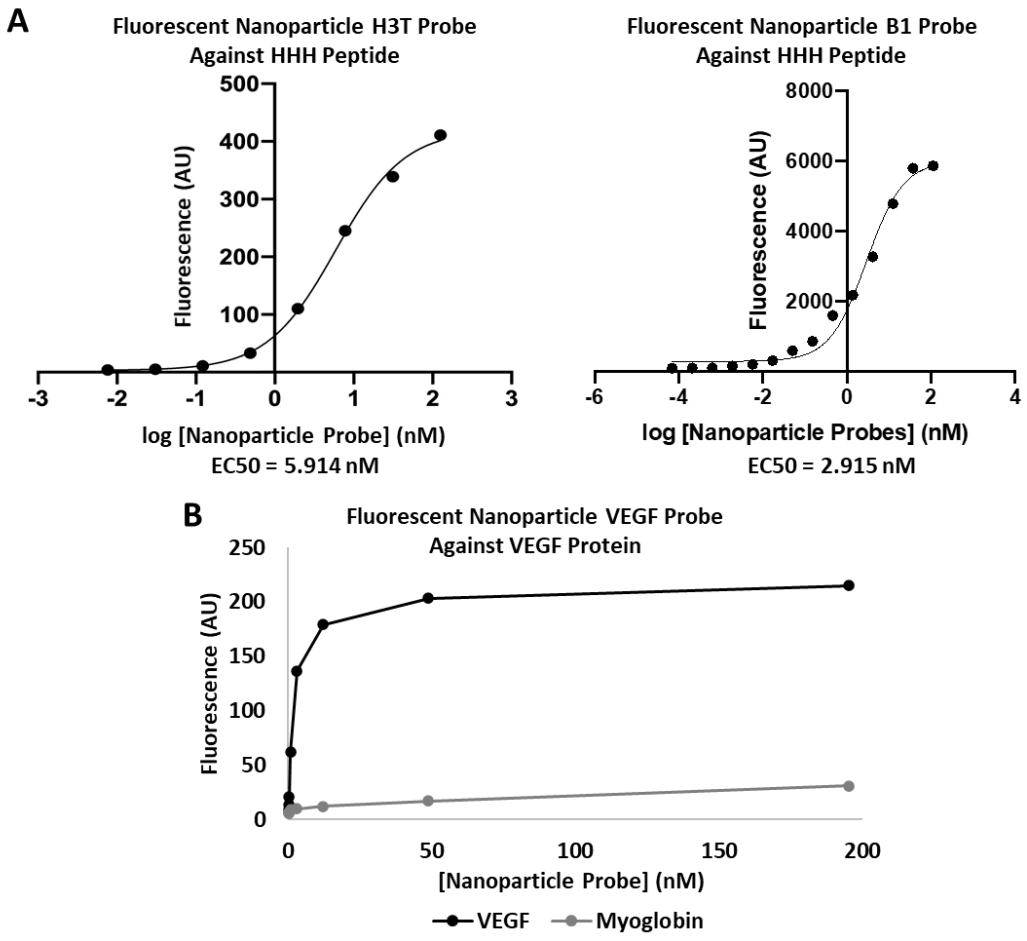
Supplementary Figure S9. Replicates for Figure 4 Fluorescent nanoparticle B1 probe binds specifically to HHH peptide. (A) Fluorescence of B1 probe as a function of concentration againts HHH peptide (on-target) and QQM peptide (off-target). Data are representative of five experiments with HHH and two with QQM. (B) Fluorescence of Her2 probe against HHH and QDH peptides. Data are representative of three experiments for HHH and one for QDH.

Figure S10



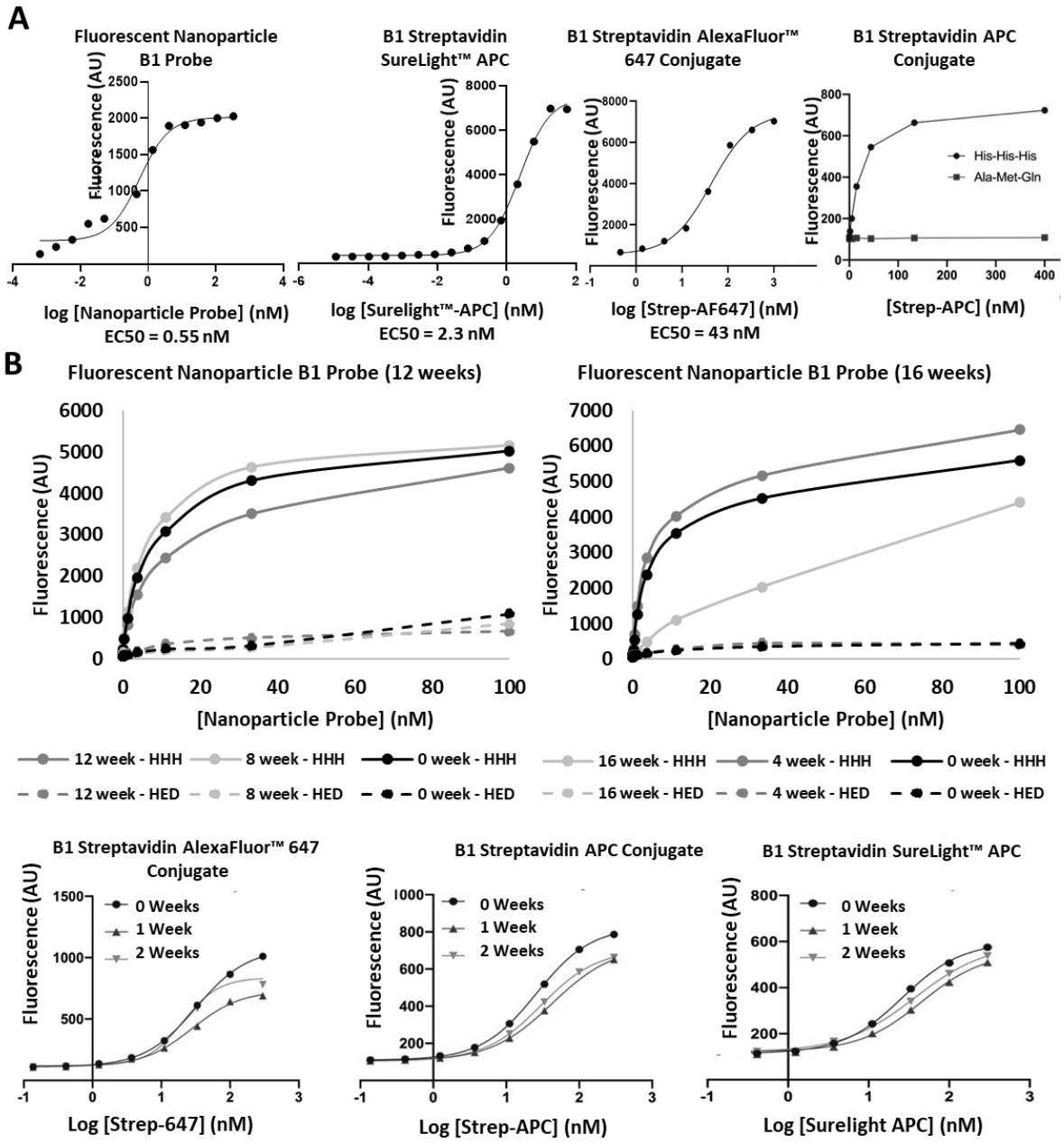
Supplementary Figure S10. Replicates for Figure 4 Fluorescent nanoparticle B1 probe binds specifically to his-tagged proteins. (A) Fluorescence of nanoparticle B1 probe against his-tagged Her2 protein (on-target) and myoglobin (off-target). Data are representative of at least five experiments. (B) Binding of the fluorescent nanoparticle B1 probe to his-tagged Her-2 (on-target) and E. coli lysate (off-target). Data from two representative experiments are shown. The experiment with his-tagged Her2 was repeated five times and that with E. coli lysate was repeated three times.

Figure S11



Supplementary Figure S11. Replicates for Figure 5 Fluorescent nanoparticles functionalized with various aptamers bind specifically. (A) Binding curve for fluorescent nanoparticle H3T and B1 probes against HHH peptide. Data are from one of two experiments performed in duplicate. (B) Binding curve for fluorescent nanoparticle VEGF probe against VEGF (on-target) and myoglobin (off-target). Data are shown as means from one of two experiments performed in duplicate.

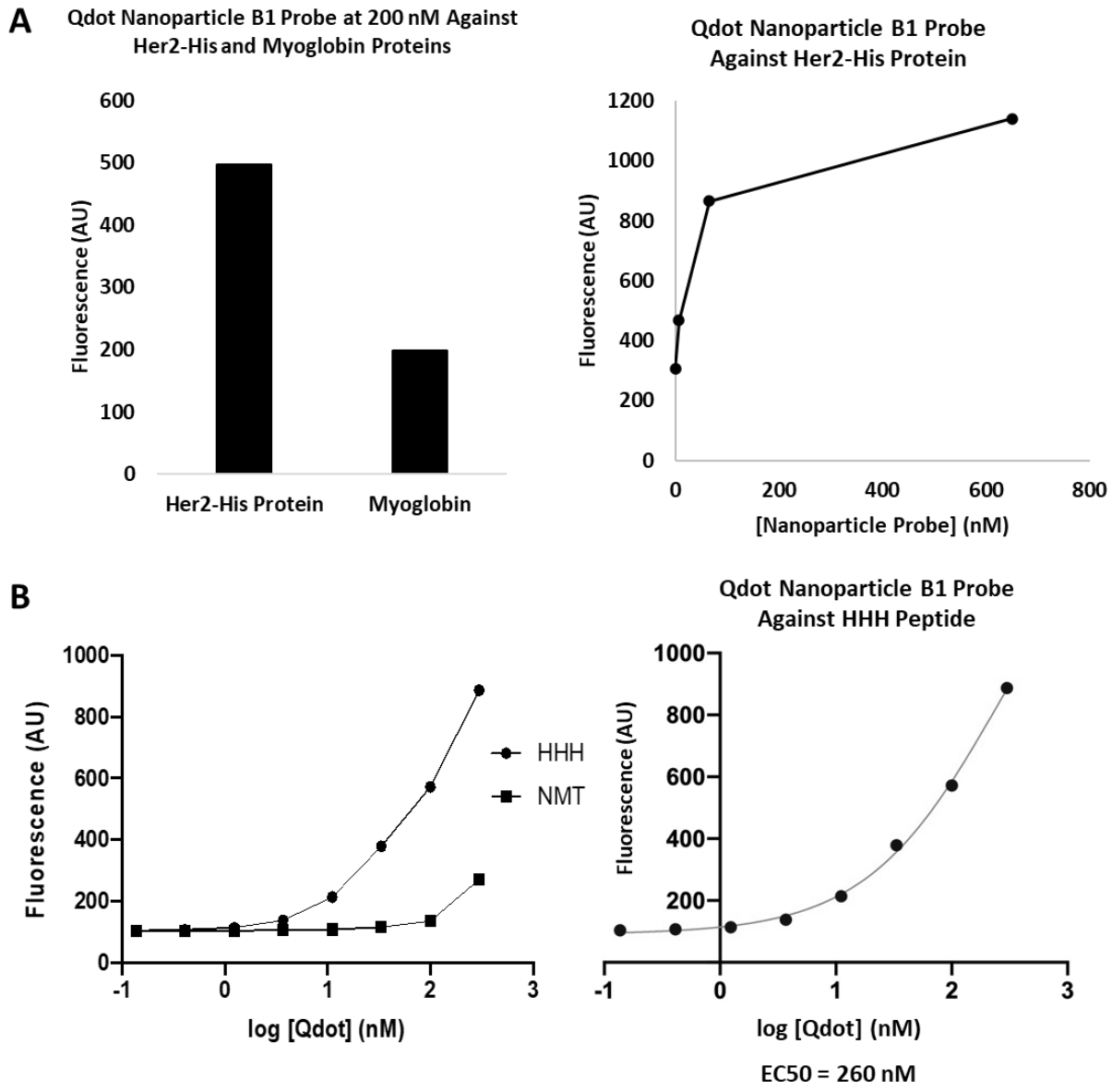
Figure S12



Supplementary Figure S12. Replicates for Figure 6 Comparison of Fluorescent nanoparticle probes with commercially available labels. (A) Binding curves and EC50 values for fluorescent nanoparticle B1 probe, B1 Streptavidin SureLight™ APC, B1 Streptavidin AlexaFluor™ 647 Conjugate, and B1 Streptavidin APC Conjugate against HHH targets. Data

shown are means from one of at least three experiments performed with one to three replicates per experiment. (B) Fluorescence intensities from binding curves of fluorescent nanoparticle B1 probe, B1 Streptavidin AlexaFluor™ 647 Conjugate, B1 Streptavidin APC Conjugate, and the B1 Streptavidin SureLight APC Conjugate against HHH targets at noted timepoints post fabrication. Data shown are from one of two experiments.

Figure S13



Supplementary Figure S13. Replicates for Figure 7 Fluorescent nanoparticle probes with a Quantum Dot core. Qdot™ 655 ITK™ Carboxyl Quantum Dots from Thermo Fisher Scientific were used as an alternative to FluoSpheres™ as the nanoparticle core. (A) Binding data and curves of the Qdot nanoparticle B1 probe to Her2-his (on-target) and myoglobin (off-target) proteins (Experimental repeats: 2. Replicates per experiment: 1). (B) Binding curves of the Qdot

fluorescent nanoparticle B1 probe to HHH peptide (Experimental repeats: 2. Replicates per experiment: 1).