Supporting Information

Orientation-Controlled Bioconjugation of Antibodies to Silver Nanoparticles

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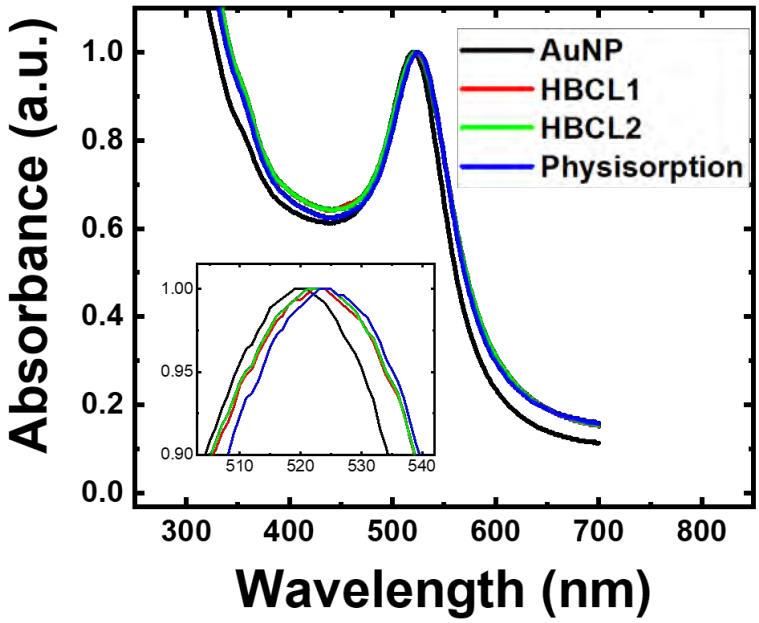

Figure S1. Normalized UV-Vis spectra of 20 nm-diameter citrate-capped AuNPs before (black) and after bioconjugation with HBCL1-modified Abs (red), HBCL2-modified Abs (green), and unmodified Abs (physisorption, blue). The AuNP-HCBL-Ab conjugates were prepared using the procedure described in the Experimental Section of the main text. The inset is an expanded view of the maxima of the plasmon peaks. λ_{max} = 520, 523, 523 and 525 nm for citrate-capped AuNPs, AuNP-HBCL1-Ab, AuNP-HBCL2-Ab and physisorbed conjugates, respectively. The experiment was carried out by adding 100 μL of each solution (containing the NPs and SBB) to the wells of a microtiter plate. For clarity, only one spectrum of triplicate measurements is depicted here. λ_{max} did not shift within the precision of the measurement (accuracy within 1 nm). The shift in the plasmon peak corresponds to a change in the refractive index of the environment of the NP[2,3] and is in agreement with literature results.[1]

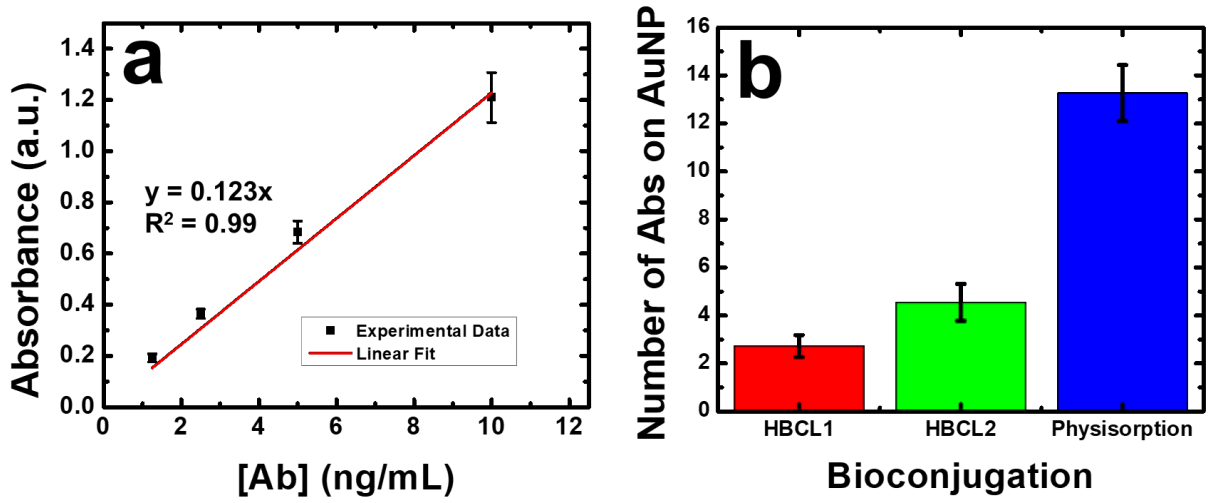

Figure S2. UV-Vis spectrophotometric data collected at 450 nm in triplicate using an indirect enzyme-linked immunosorbent assay (ELISA) to determine the number of Abs per AuNP. (a) Calibration curve obtained *via* indirect ELISA of unmodified Abs. (b) Histogram showing the number of Abs bound per AuNP, which was determined by performing an indirect ELISA with the supernatant of the first washing step (first centrifugation) following bioconjugation of Abs to AgNPs. The complete procedure for this experiment is described in the Experimental Section of the main text.

Sample	Change in hydrodynamic diameter (Δ_H)
Physisorbed conjugate in SBB	24.6 ± 1.2 nm
AgNP-HBCL1-Ab in SBB	9.4 ± 0.7 nm
AgNP-HBCL2-Ab in SBB	7.1 ± 1.3 nm

Table S1. Hydrodynamic diameter measurements obtained for various AgNP conjugates using dynamic light scattering spectroscopy (DLS). The experiment was performed as follow: 1 mL of 0.25 nM AgNP solution was added to a quartz cuvette through a PTFE 0.2 μm membrane. The DLS measurement was performed in triplicate. A change in the hydrodynamic diameter was reported in comparison to the hydrodynamic diameter of AgNP in SBB. All the quartz cuvettes were washed with aqua regia (3 HCl: 1 HNO_3) prior to being used to get rid of any residual NPs. The HBCL itself was used as the "backfill" ligand in these experiments in place of the mPEG-SH. The mPEG-SH used in the conjugate protocol has a molecular weight of 5 kDa, which would generate false increased diameters with this technique.

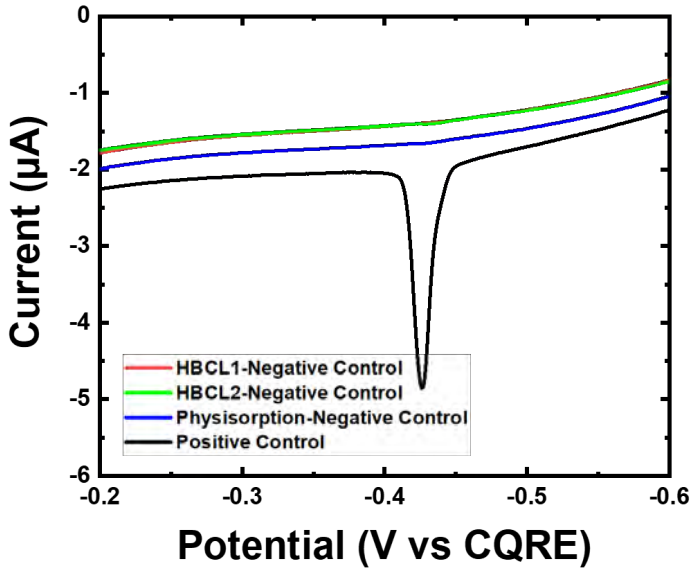

Figure S3. ASV traces obtained by forming a half-metalloimmunoassay for the detection of NT-proBNP with NT-proBNP off-target conjugates. The Abs used here are α-anti- human chorionic gonadotropin (hCG). The AgNP-Ab conjugates were formed following the same procedure as described in the experimental section, *via* bioconjugation with HBCL1, HBCL2, or physisorption technique. After the half-metalloimmunoassay was formed, the electrochemical protocol, also described in the experimental section, was performed. As expected, the 3 off-target conjugates (denoted as negative control) traces (red, green, blue) showed no Ag peak, confirming that the α-anti-hCG Abs do not recognize the NT-proBNP on the plate. Additionally, this proves that the HBCLs do not lead to Ab binding to the NT-proBNP on the plate. The positive control in this experiment consisted of hCG being bound to the plate to ensure the conjugate components were functioning properly. For clarity purpose, only 1 of the 3 individual replicates is depicted here.

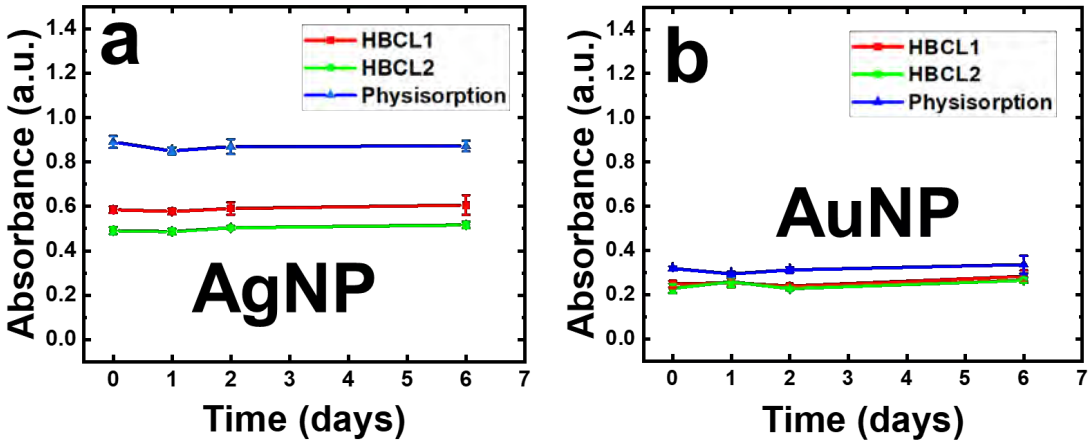

Figure S4. Spectrophotometric data related to the stability of AgNPs and AuNPs. The absorbance spectra were measured at (a) 395 nm for AgNPs and (b) 520 nm for AuNPs. This experiment was performed by freshly preparing the conjugates on day 0 and then analyzing the solutions on day 0, 1, 2, and 6. The NPs were suspended in SBB for analysis. 70 μL of each sample were added to the wells of a microtiter plate on each testing day and absorbance determined at the indicated wavelengths. The overall trend is that there is no change for either NP solution throughout the duration of the experiments. A decrease in intensity would be indicative of NP aggregation and because this is not observed, we conclude there is no detectable aggregation.[1–3]

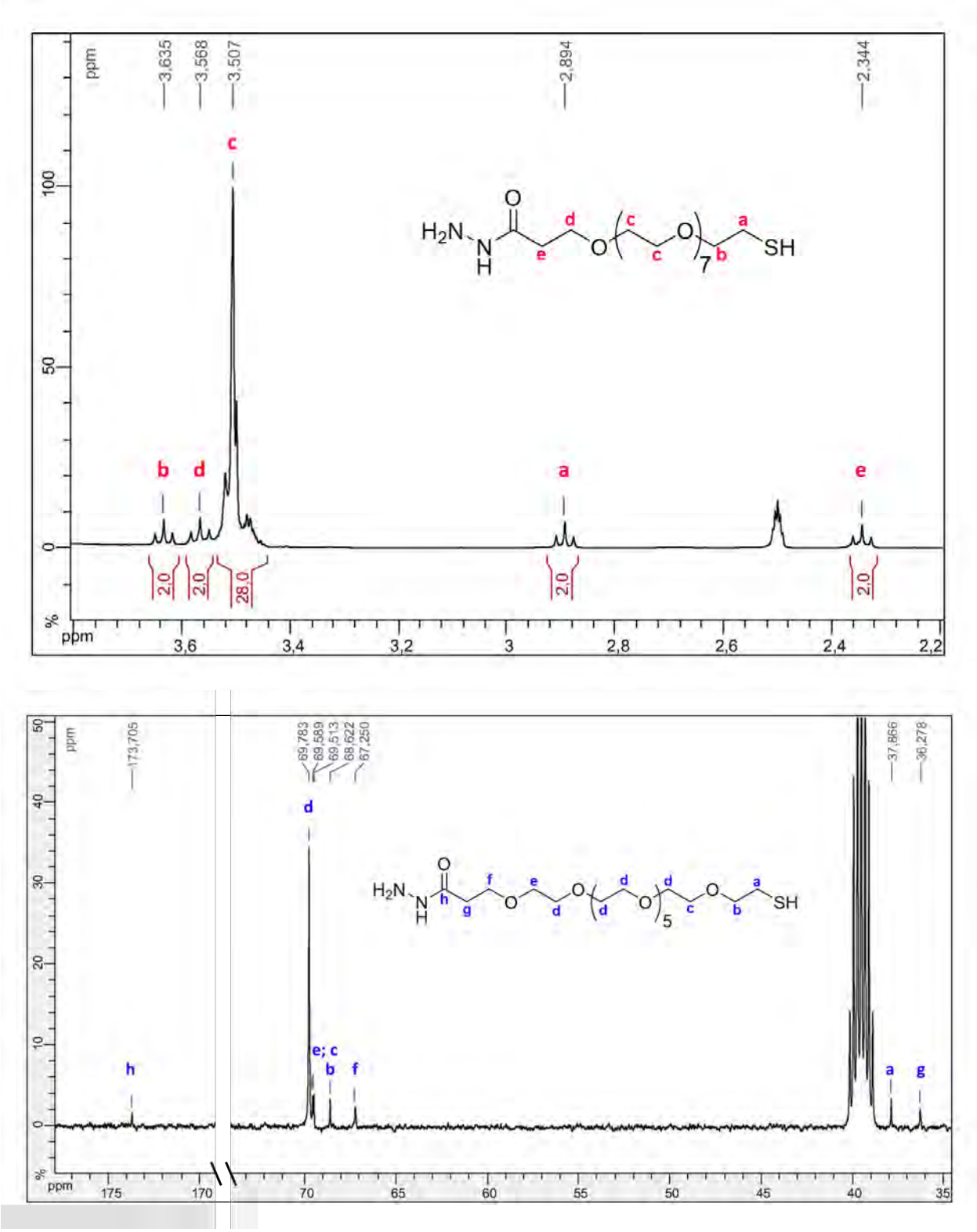

Figure S5. (top) ^{1}H NMR (400 MHz, $DMSO_{d6}$) and (bottom) ^{13}C NMR (400 MHz, $DMSO_{d6}$) spectra of heterobifunctional cross-linker 1 (HBCL1).

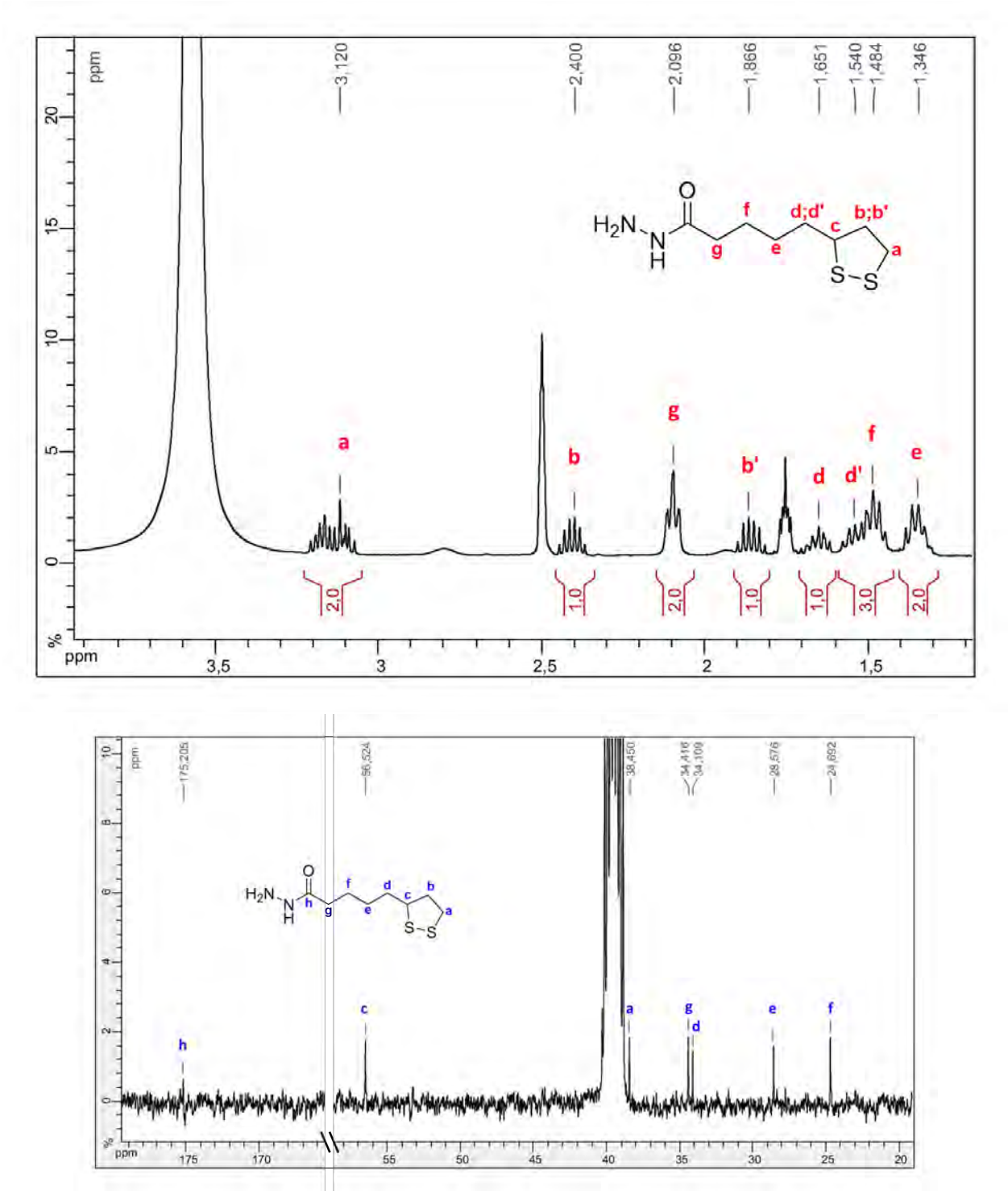

Figure S6. (top) ^{1}H NMR (400 MHz, $DMSO_{d6}$) and (bottom) ^{13}C NMR (400 MHz, $DMSO_{d6}$) spectra of HBCL2. Note, the spectra of HBCL2 for day 90 are identical and therefore not shown.

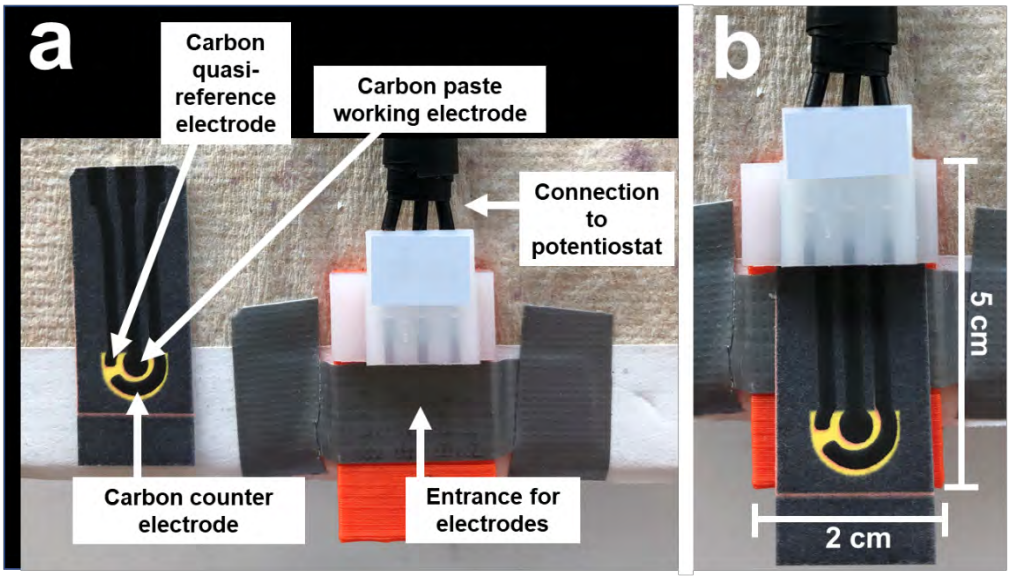

Figure S7. Description of the electrochemical cell used for determining the activity of the Abs on the conjugates. Ag electrodeposition and anodic stripping voltammetry (ASV) were performed with this device. (a) Photograph of the electrodes and the electrochemical cell. (b) Photograph of the assembled cell. The white portion of the electrochemical cell incorporates metal prongs within the enclosure to provide ohmic contact between the carbon paste electrodes and the potentiostat. Duct tape is placed across the orange platform of the cell to keep it stable during analysis. The electrodes were stencil printed with conductive carbon paste, as mentioned in the Experimental Section of the main text. The regions printed in yellow wax are slightly more hydrophilic compared to the black wax regions, allowing for the sample solution to span all three carbon electrodes. The yellow region can hold an aqueous volume of 50-80 μL. In the present article, 76 μL of sample solution were added to the electrode region.

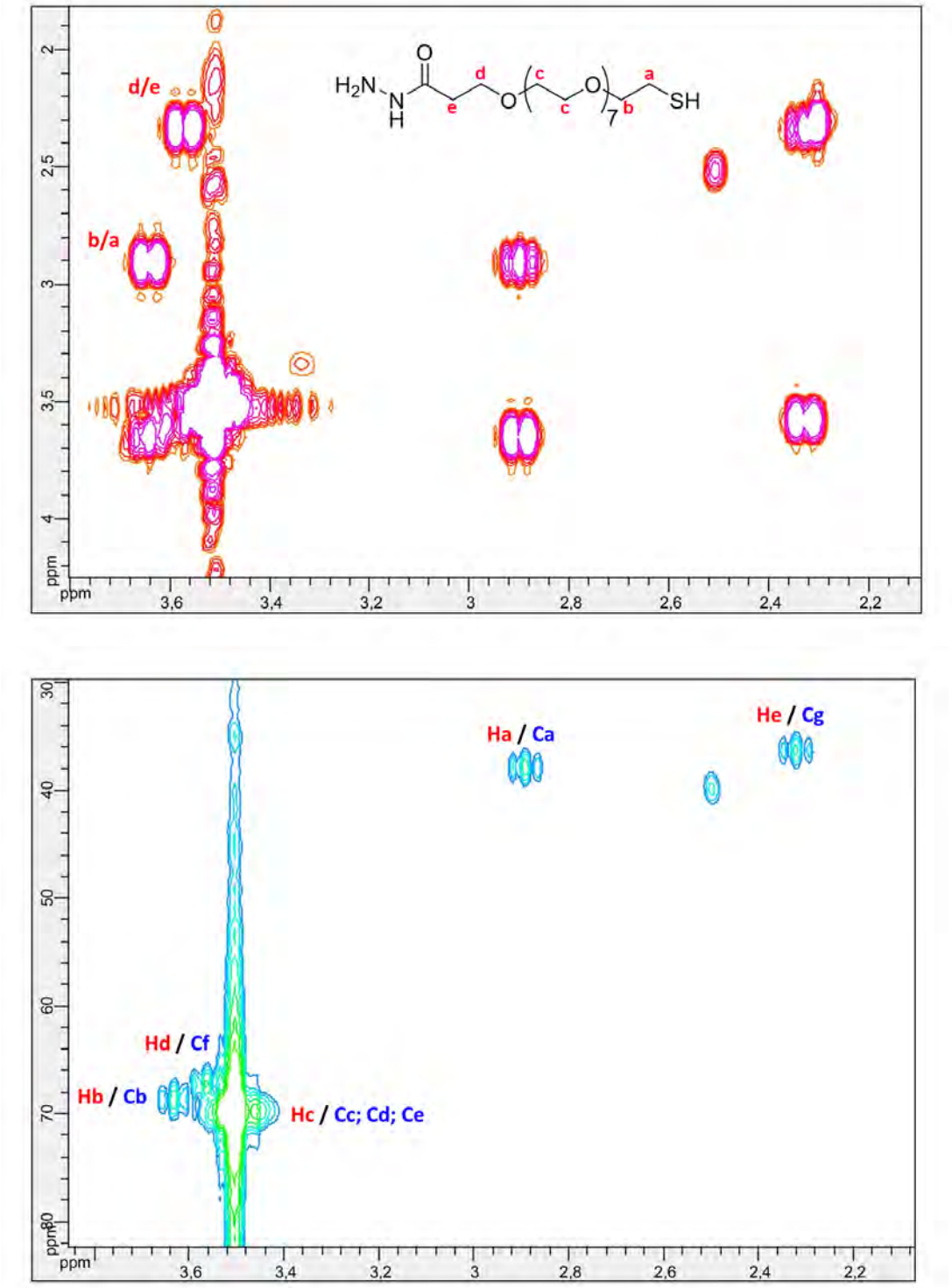

Figure S8. (top) COSY ($^{1}H/^{1}H$) 2D NMR (400 MHz, $DMSO_{d6}$) and (bottom) HSQC ($^{1}H/^{13}C$) 2D NMR (400 MHz, $DMSO_{d6}$) correlations for HBCL1.

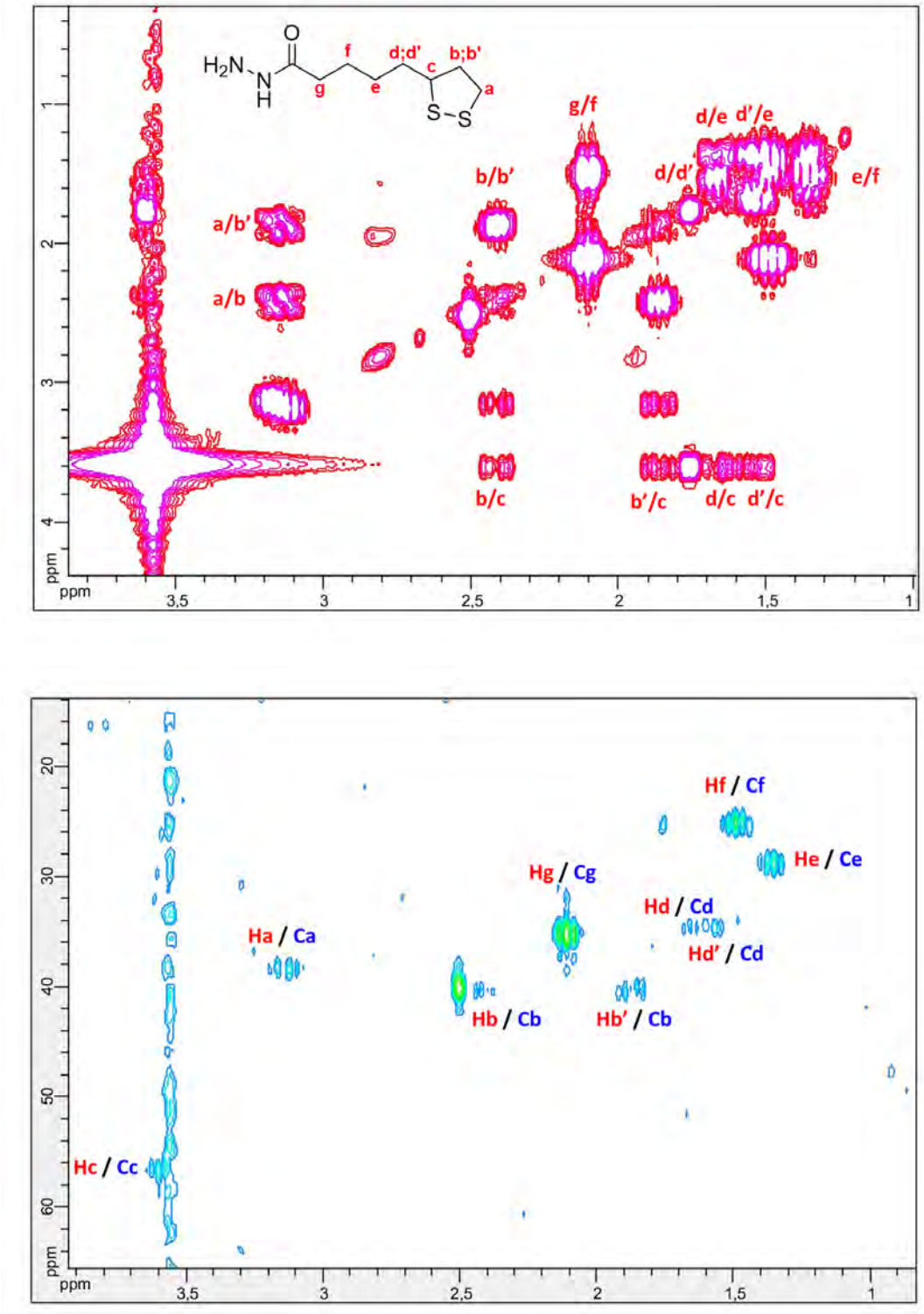

Figure S9. (top) COSY ($^{1}H/^{1}H$) 2D NMR (400 MHz, $DMSO_{d6}$) and (bottom) HSQC ($^{1}H/^{13}C$) 2D NMR (400 MHz, $DMSO_{d6}$) correlations of HBCL2.

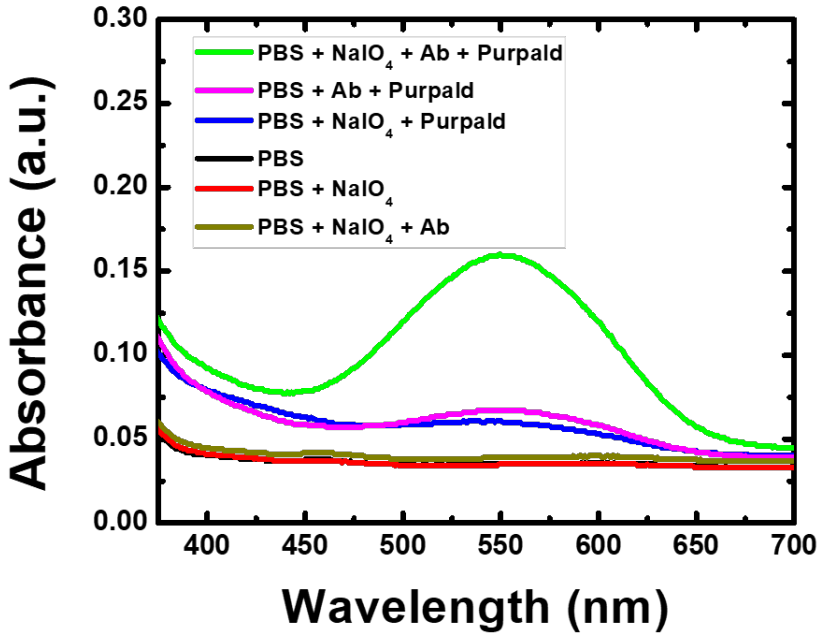

Figure S10. Colorimetric determination of polysaccharide chain content of 13G12 anti-N-terminal prohormone brain natriuretic peptide antibody (Ab) by UV-Vis spectroscopy using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald). This experiment was carried out by adding 4 μL of 5.4 μM Ab to 4 μL of 50 mM $NaIO_4$, incubating the mixture in the dark for 30 min at 22 ± 3 °C, and agitating at 600 rpm to oxidize the polysaccharide chains of the Ab. Oxidation was quenched by adding 100 μL of 1x concentrated phosphate buffered saline (PBS). 75 μL of a freshly prepared 68.4 mM solution of Purpald in 1 M NaOH were then added to the wells of a microtiter plate immediately prior to spectrophotometric analysis. The solution was monitored at 550 nm, which is characteristic of the Purpald hydrazone product resulting from the reaction between the oxidized polysaccharide chains of the Ab and Purpald. The spectrum corresponding to this experiment was recorded, and an absorbance intensity of ~0.15 a.u. was obtained (green spectrum), confirming the presence of the polysaccharide chains on the Ab. Several controls were also performed to ensure that the absorbance intensity at 550 nm was only due to the hydrazone formation. The black, red, and brown spectra were recorded without Purpald, and as expected there is no elevation in the baseline. The pink spectrum corresponds to Purpald in the presence of non-oxidized Ab, and it shows a slight increase in the baseline at 550 nm due to the background of Purpald degradation.[1] The blue spectrum corresponds to Purpald in presence of $NaIO_4$ and here again only the background signal of the degradation of Purpald is observed.

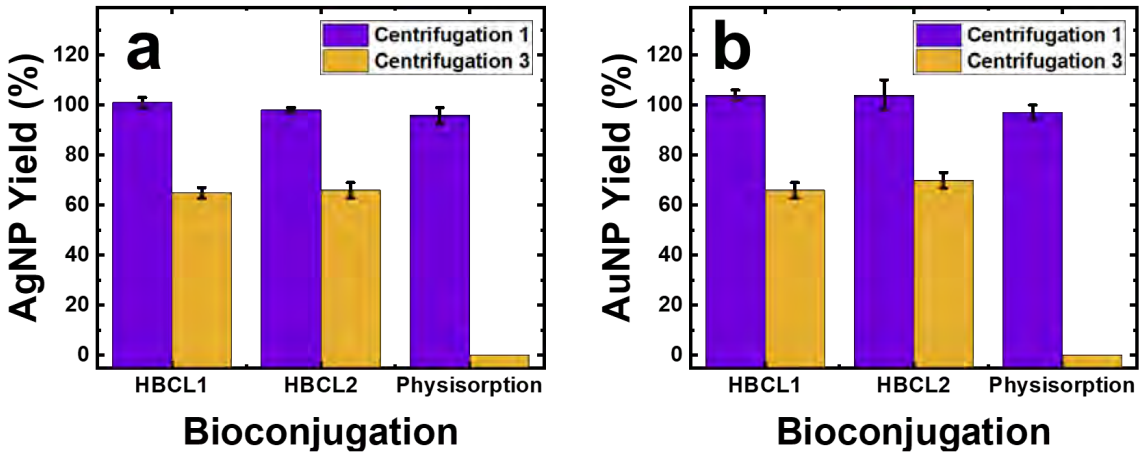

Figure S11. Histograms showing the percentage yield of (a) the AgNPs and (b) the AuNPs, after functionalization with Ab-HBCL1, Ab-HBCL2 and unmodified Abs. This experiment was carried out as follows. First, the absorbance intensity at λ_{max} was measured for each conjugate (*i.e.* NP-HBCL1-Ab, NP-HBCL2-Ab and physisorbed conjugates). Second, the absorbance value was converted to a NP concentration (NPs/mL) using the calibration curve shown in Figure S9. Third, this concentration was divided by the concentration of NPs before bioconjugation and was multiplied by 100. For all conjugates, the yields reflect those after the first (blue) and second (orange) centrifugation (conditions: 25 min, 4 °C, 16,600 g). The absorbance at λ_{max} is directly correlated to the number of NPs in solution, and a decrease is usually representative of NP aggregation.[2] Therefore, this analysis provides a means for determining the extent of NP aggregation that results after Ab conjugation. The data indicates that almost no aggregation of the conjugates occurs after the first centrifugation. In contrast, the NP-Ab conjugates aggregate significantly after the third centrifugation. Note that, the physisorbed conjugates aggregate completely under the same conditions.

Protocol for physisorption of Abs to NPs

Using a microcentrifuge tube blocked with Superblock blocking buffer (SBB), 500 μL of nanoparticle (NP) stock solution (4.9 x 10^{11} AgNPs/mL or 7.4 x 10^{11} AuNPs/mL) were added to 5 μL of 6.7 μM Ab. The mixture was incubated for 30 min at RT and 1400 rpm. Unbound Abs were removed with one centrifugation step (25 min, 4 $^{\circ}$C, 16,600 g,), after which the supernatant was removed to leave behind only 5.0 μL of the initial solution (so as not to disturb the pellet). The NP pellet was then resuspended into a total volume of 500 μL of SBB. This solution was stored in the dark at 4 $^{\circ}$C. Note, the physisorbed AgNP-Ab conjugates promptly aggregate after a second centrifugation step. This shows that the physisorbed NP-Ab conjugates exhibit weak electrostatic interactions between the Abs and the metallic surface and are not very stable.

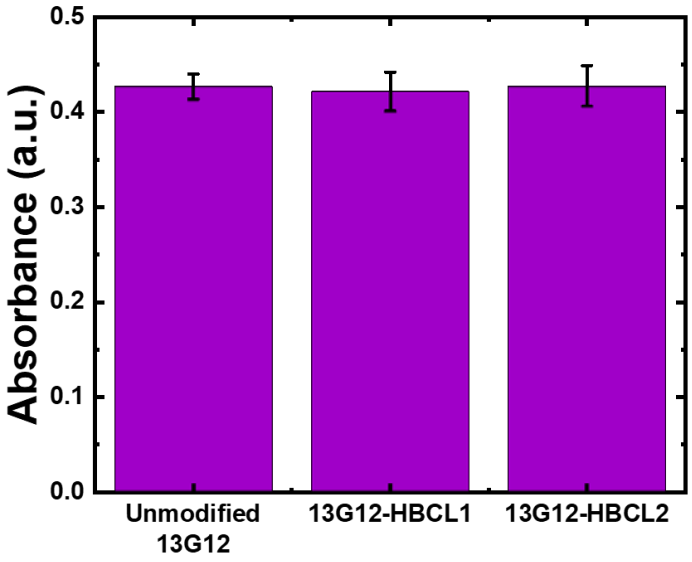

Figure S12. ELISA results for the comparison of Ab activity before and after using hydrazide chemistry to conjugate Ab with HBCL1 and HBCL2. This experiment was performed by adding 100 µL of 58.2 nM NT-proBNP to a plate for 14 h at 4 °C to immobilize target, followed by washing with PBS and blocking with 2% (w/v) casein solution in PBS. Next, each well received 100 µL 33.3 pM of Ab, including unmodified, HBCL1- and HBCL2- modified Abs. Each well was washed with PBS before 100 μL of 6.7 μM of secondary Ab label with HRP were added, incubated and washed again. Finally, 100 μL of the 1-Step Ultra TMB solution were added and 50.0 μL of 2 M H_2SO_4 were used to quench the reaction after it reached the desired color intensity (~3 min). These results show that the activity of the Abs was unaffected by the method used to attach the HBCLs, nor did the attachment of the HBCLs have a negative effect on the Ab activity, as the absorbances observed are the same within error. The error bars represent the standard deviation from the mean for three independent experiments.

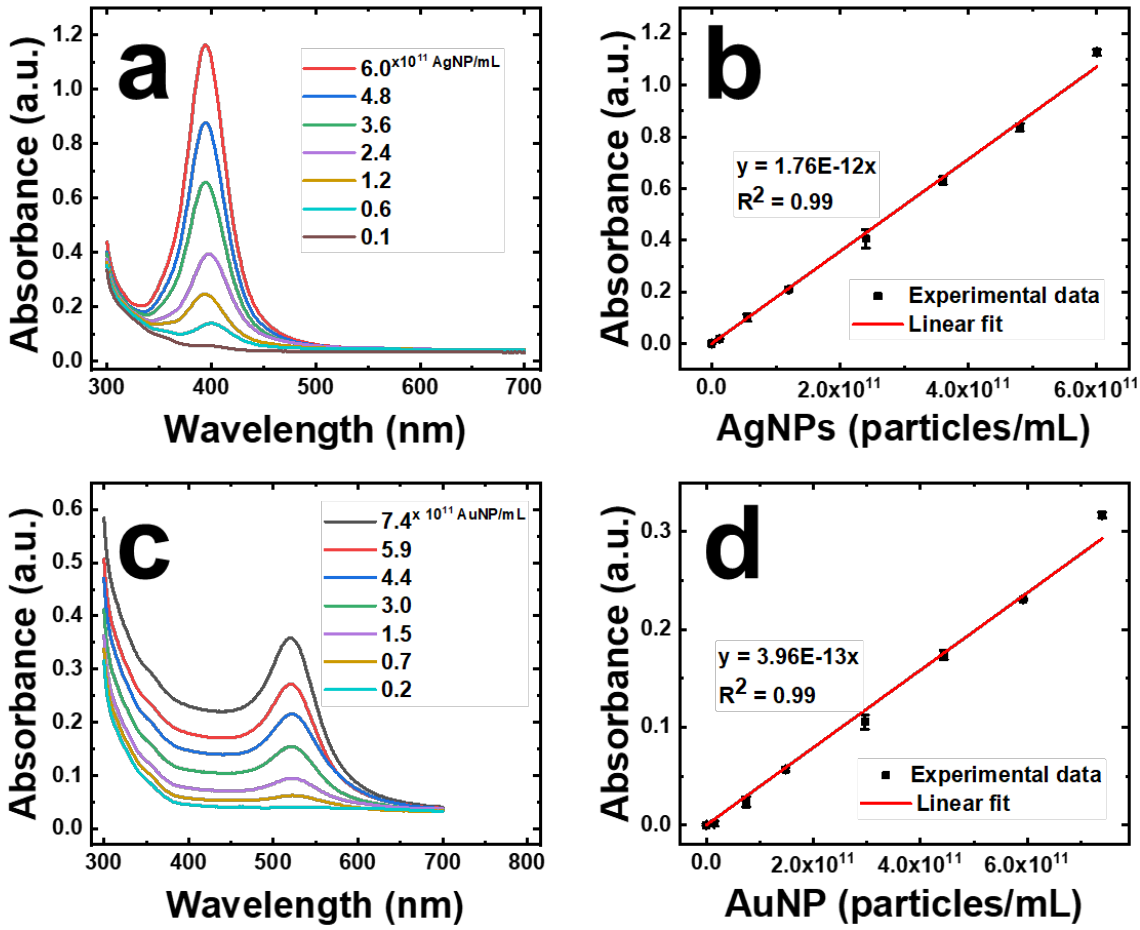

Figure S13. UV-Vis calibration data for quantification of Ag and Au NPs. (a) UV-Vis spectra of decreasing concentrations of AgNP. (b) AgNP calibration curve generated from the absorbance values at 395 nm. (c) UV-Vis spectra of decreasing concentrations of AuNP. (d) AuNP calibration curve generated from the absorbance values at 520 nm. These experiments were performed by diluting the stock solution of 20 nm-diameter, citrate-capped AgNPs and AuNPs with deionized water. 100 μL of each solution were added to the wells of a microtiter plate and the absorbance spectra were recorded. The error bars represent the standard deviation from the mean for three independent experiments.

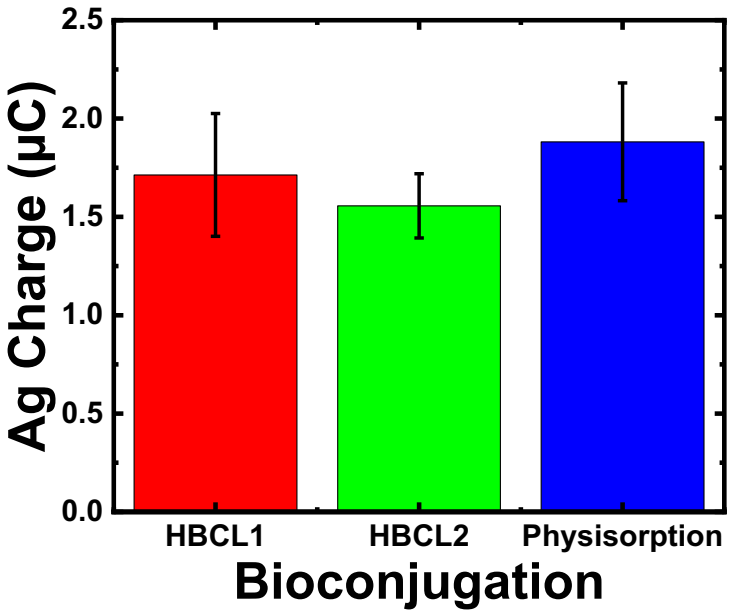

Figure S14. Ag charge obtained for the conjugates in solution following electrochemical analysis. This experiment was performed by freshly preparing the conjugates, diluting them all to the same 0.23 μM AgNP concentration, incubating with 30 μL of 0.20 mM $KMnO_4$ for 15 min, and then carrying out the electrochemical protocol described in the Experimental Section of the main text. The Ag charges obtained for each conjugate for the same concentration of AgNP are, within error, equivalent. This shows that the three AgNP functionalization protocols do not affect the electrochemical analysis. The error bars represent the standard deviation from the mean for three independent experiments.

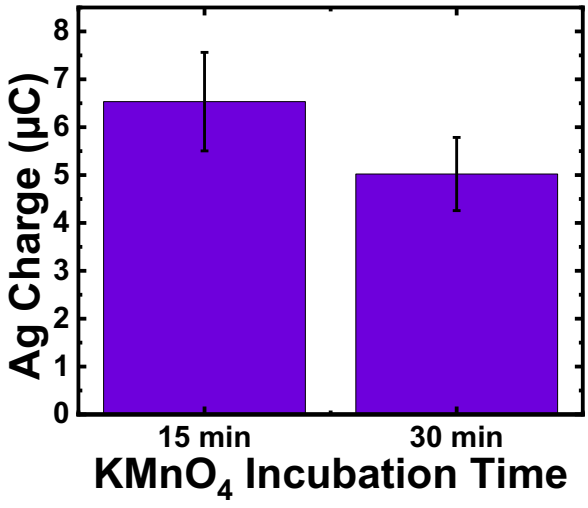

Figure S15. Electrochemical detection of unmodified, 20 nm-diameter, citrate-capped AgNPs incubated with $KMnO_4$ for the indicated times. The electrochemical procedure described in the Experimental Section of the main text was used. Briefly, however, 46 μL of 0.60 μM AgNPs in 1xPBS were incubated with 30 μL of 0.2 mM $KMnO_4$ for 15 and 30 min at 1000 rpm in the wells of a microtiter plate. Note that, here no target was immobilized on the wells of the microtiter plate. The entire content of each well was then placed on an electrode and the electrochemical protocol described in the main text was performed. The resulting Ag charges are not statistically different with a *p-value* greater than 0.05 (0.2380). This experiment proves that all of the AgNPs are oxidized within a 15 min incubation period. Thus, the experimental procedure used to obtain the ASV results discussed in the main text results in complete oxidation of the AgNPs. Therefore, the Ag charge detected corresponds to the binding activity of each conjugate for the immobilized target (N-terminal prohormone brain natriuretic peptide) on the plate. The maximum amount of target immobilized on the plate during the Ab activity experiments corresponded to 0.60 μM, and therefore a molar equivalent of AgNPs were used for this control experiment. Note that, the $KMnO_4$ solution was in excess, which was confirmed by the presence of the strong pink color observed throughout the experiment.

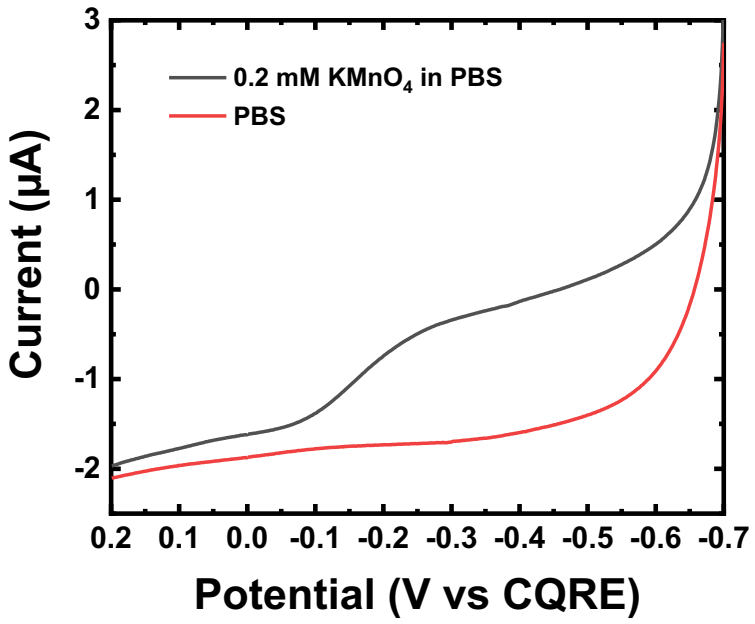

Figure S16. ASV results obtained using a solution containing only $KMnO_4$ and PBS. The electrochemical protocol described in the Experimental Section in the main text was used for these experiments. The electrochemical analysis was carried out in solutions containing: (black trace) 30 μL of freshly prepared 0.20 mM $KMnO_4$ and 46 μL of PBS; (red trace) 76 μL PBS. The purpose of this experiment was to evaluate the background current arising from the presence of $KMnO_4$. The black background trace was subtracted from all ASV data presented in the main text.

References

(1) Kumar, S., Aaron, J., and Sokolov, K. (2008) Directional Conjugation of Antibodies to Nanoparticles for Synthesis of Multiplexed Optical Contrast Agents with Both Delivery and Targeting Moieties. *Nat. Protoc. 3*, 314—320.

(2) Haiss, W., Thanh, N. T. K., Aveyard, J., and Fernig, D. G. (2008) Determination of Size and Concentration of Gold Nanoparticles from Extinction Spectra. *Anal. Chem. 80*, 6620—6625.

(3) Rahme, K., Chen, L., Hobbs, R. G., Morris, M. A., O'Driscoll, C., and Holmes, J. D. (2013) PEGylated Gold Nanoparticles: Polymer Quantification as a Function of PEG Lengths and Nanoparticle Dimensions. *RSC Adv. 3*, 6085—6094.