Electronic Supporting Information For:

Site-Specific Conjugation of Antibody on Gold Nanoparticle Surface for One-Step Diagnosis of Prostate Specific Antigen with Dynamic Light Scattering

Nur Mustafaoglu[a], Tanyel Kiziltepe[a,c] and Basar Bilgicer[a,b,c,d,e,f]*

[a] Department of Chemical and Biomolecular Engineering,

[b] Department of Chemistry and Biochemistry,

[c] Advanced Diagnostics and Therapeutics,

[d] Mike and Josie Harper Cancer Research Institute,

[e] Center for Rare & Neglected Diseases,

[f] ND Nano Center for Nano Science and Technology, University of Notre Dame, University of Notre Dame, Notre Dame, IN 46556, USA

Corresponding Author

* Basar Bilgicer
Associate Professor
Department of Chemical and Biomolecular Engineering
Department of Chemistry and Biochemistry
Advanced Diagnostics & Therapeutics Initiative
Mike and Josie Harper Cancer Research Institute
Center for Rare & Neglected Diseases
Center for Nano Science and Technology
University of Notre Dame
205 McCourtney Hall
Notre Dame, IN 46556-5637
Voice: 574-631-1429
Fax: 574-631-8366
bbilgicer@nd.edu

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Fig. S10: Absorption spectra of 20 nm AuNPs and antibody functionalized AuNPs

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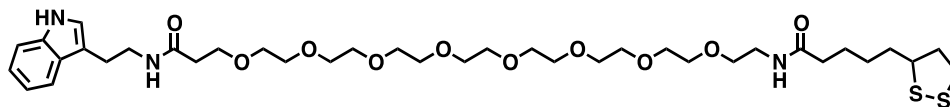
Fig. S12: Testing for antigen binding ability and structure stability of antibody functionalized AuNPs (20 nm) via an ELISA assay.

**SUPPORTING INFORMATION TABLES:**

Table S1: Maximum absorbance wavelengths of antibody functionalized AuNPs (5 nm).

**Table S2:** Maximum absorbance wavelengths of antibody functionalized AuNPs (20 nm).

Electronic Supporting Information

Fig. S1 Molecule structure and characterization of Tryptamine-EG8-TA molecule. Tryptamine-EG$_8$-TA is synthesized by conjugating tryptamine to the lipoamido-dPEG8-acid. The product is purified via RP-HPLC on the Zorbax C18 column and characterized with MICRO-TOF MS (calculated mass is 771.38; found 772.42). The purity of the Tryptamine-EG$_8$-TA was confirmed using RP-HPLC on an analytical Zorbax C18 column to be >95%.
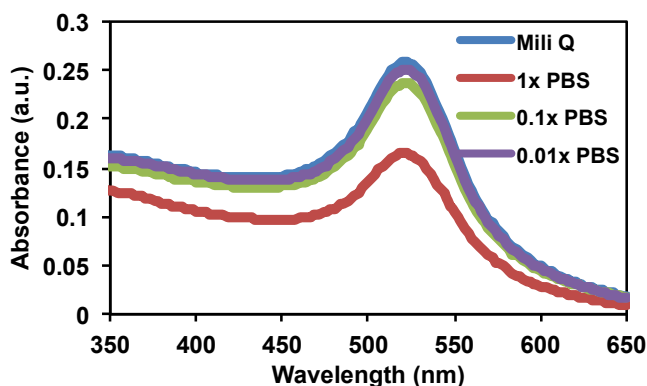

Fig. S2 Stability test of citrate stabilized AuNPs in various buffer conditions: Absorbance spectra of citrate stabilized gold nanoparticles (AuNPs) in Mili Q water, 1x PBS, 0.1x PBS, 0.01x PBS. 0.01x PBS is found to be the most appropriate buffer condition for functionalization of AuNPs with antibodies while preserving stability and providing ionic strength for antibodies.
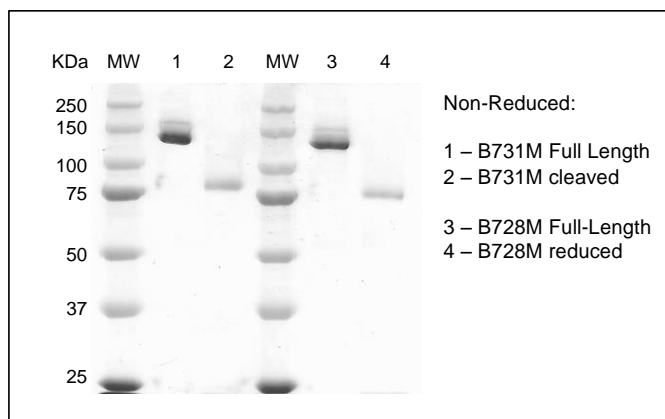
Fig. S3 Characterization of antibody disulfide bond reduction reaction. Both intact and reduced B731M and B728M antibodies were run on 10% SDS-PAGE under non-reducing conditions. The bands for the intact antibodies were observed at ~150 KDa. The bands at ~75 kDa were attributed to the reduced half antibodies.
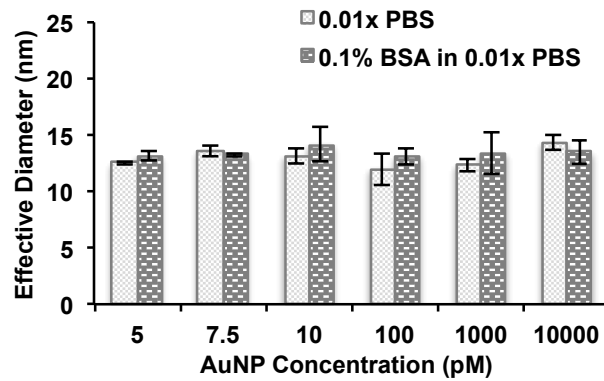
Fig. S4 Testing the lowest AuNP concentration that can be measurable on DLS instrument. We tested various concentration of citrate stabilized AuNPs from 5 pM to 10 nM in two different buffer: 0.01x PBS and 0.1% BSA in 0.01x PBS. The rest of the DLS experiments were carried out using 50 μl of 5 pM antibody functionalized AuNPs in 0.1% BSA in 0.01x PBS.
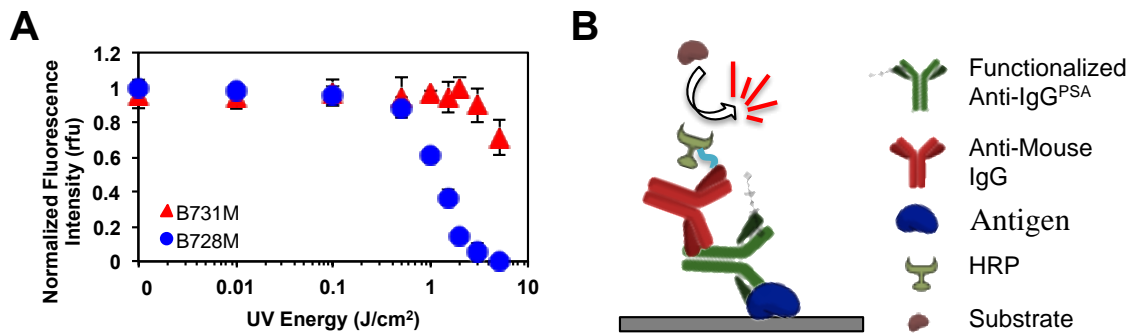

Fig. S5 Characterization of site-specific photocrosslinking reaction with Trypramine-EG$_8$-TA linker via the UV-NBS method. A) Antigen binding site activity and antibody structure stability of both B731M and B728M antibodies were investigated with an ELISA assay. Tryptamine-EG$_8$-TA ligand was incubated with the antibody and then desired amount of UV-energy $(0 - 5 \text{ J/cm}^2)$ was exposed on the ligand-antibody complex to form a covalent bond. B) TA functionalized antibodies were incubated on PSA immobilized 96-well ELISA surface, and then a Fc specific anti-Mouse IgG antibody was used to determine the structure stability of the antibody Fc fragment. Amplex Red, an HRP substrate, was used to form fluorescent product, then fluorescent intensity was measured using a Molecular Devices SpectraMax M5 plate reader (ex. 570 nm, em. 592 nm). All data represent means (±SD) of triplicate experiments.

**Table S1:** Maximum absorbance wavelengths of antibody functionalized AuNPs (5 nm).

	Max. λ (nm)	
AuNP (5 nm)	522	
	B731M	**B728M**
Ionic Interaction	523	523
Thiol Reduction	525	526
EDC-NHS	524	522
UV-NBS	524	524

A

$y = 0.616x + 7.7565$
$R^2 = 0.99847$

B
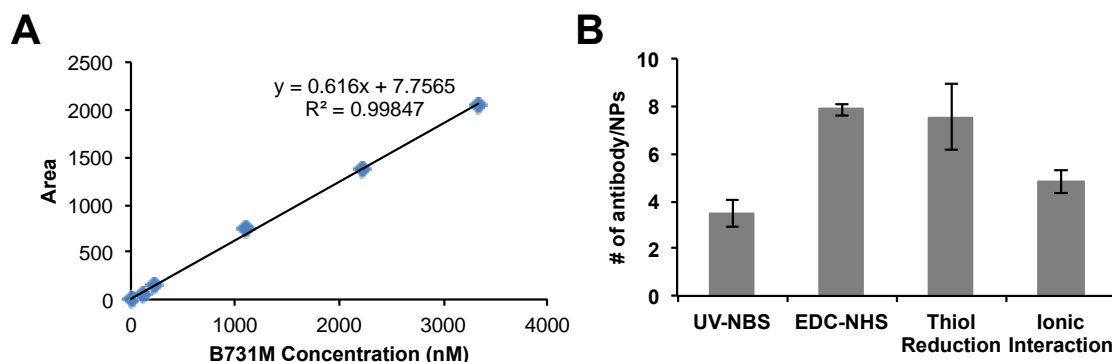
Fig. S6 Number of antibodies per AuNPs was determined by running antibody functionalized AuNPs on size exclusion column (SEC). A) First, known amounts of antibody were injected to the column to obtain a standard curve. Each SEC run was achieved using a 25 min isocratic gradient of 50 mM PBS at pH 6.8 with 370 mM NaCl and 0.1% Tween 20. All samples were analyzed at 220 and 274 nm to detect antibody content and at 522 nm to detect gold nanoparticles. Each absorbance spectrum was integrated on Chemstation LC software and used to calculate total antibody content and total gold nanoparticle content, and then compared to the standard curve to determine the average number of antibody per gold nanoparticle. B) Average number of antibody per AuNP for each method: the UV-NBS, EDC-NHS, thiol reduction, and ionic interaction.
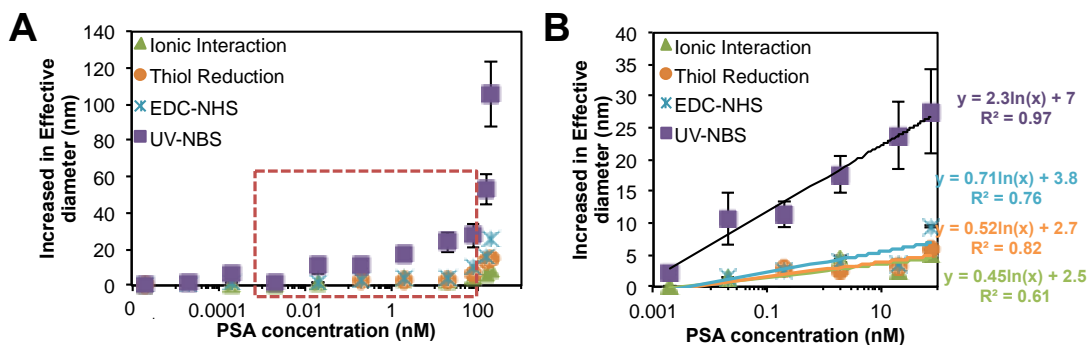
Fig. S7 A) DLS measurements to compare the cluster formations of all tested methods: UV-NBS, EDC-NHS, Thiol Reduction and Ionic Interaction B) The equation of the linear part of the regression line was used to determine the limit of detection value. The coefficient of the natural logarithm from the linear part of the regression line was used to calculate the antigen binding sensitivity for each method.
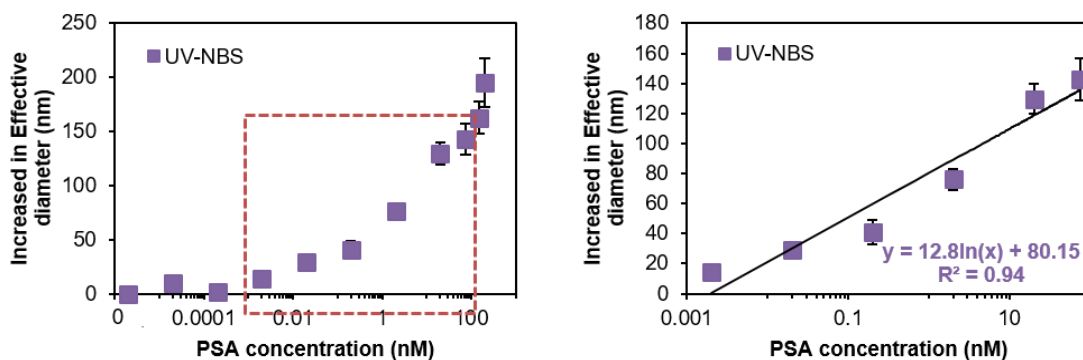

Fig. S8 A) DLS measurements of antibody functionalized AuNPs via the UV-NBS method to detect PSA in human plasma. Size of the antibody functionalized AuNPs without the addition of any PSA was subtracted from to obtain size differences of aggregations with the addition of increased amounts of PSA. B) The equation of the linear part of the regression line was used to determine the limit of detection (LOD) value. The coefficient of the natural logarithm from the linear part of the regression line was used to calculate the antigen binding sensitivity.
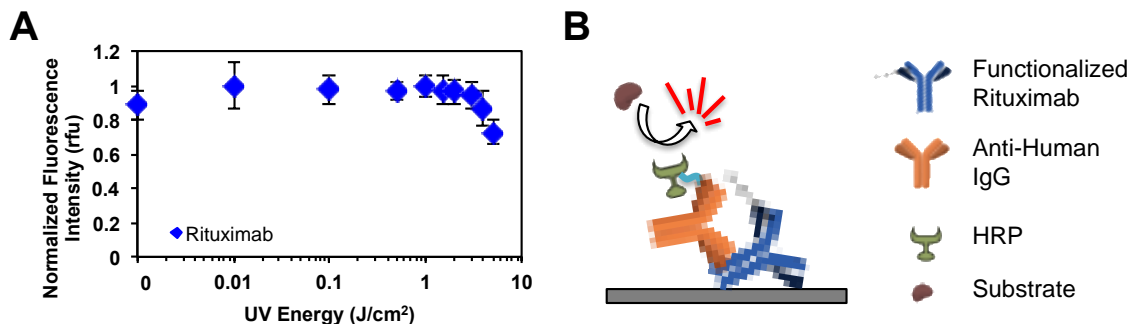
Fig. S9 Characterization of Rituximab photocrosslinking reaction with Trypramine-EG$_8$-TA linker via the UV-NBS method. A) Antibody structure stability of Rituximab was investigated with an ELISA assay. Tryptamine-EG$_8$-TA ligand was incubated with the antibody and then desired amount of UV-energy (0 – 5 J/cm^2) was exposed on the ligand-antibody complex to form a covalent bond. B) TA functionalized Rituximab were incubated on a high-binding 96-well ELISA surface, and then a Fc specific anti-Human antibody was used to determine the structure stability of the antibody Fc fragment. Amplex Red, an HRP substrate, was used to form fluorescent product, then fluorescent intensity was measured using a Molecular

Devices SpectraMax M5 plate reader (ex. 570 nm, em. 592 nm). All data represent means (±SD) of triplicate experiments.
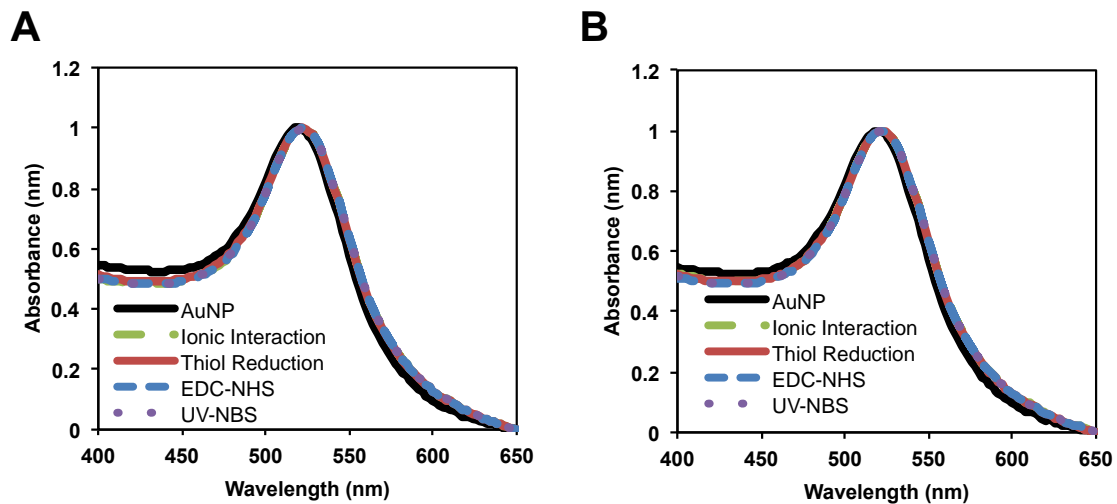
Fig. S10 Absorption spectra of 20 nm AuNPs and A) B731M functionalized AuNPs and B) B728M functionalized AuNPs via the Ionic interaction, Thiol reduction, EDC-NHS and UV-NBS methods.

Table S2: Maximum absorbance wavelengths of antibody functionalized AuNPs (20 nm).

	Max. λ (nm)	
AuNP (20 nm)	520	
	B731M	**B728M**
Ionic Interaction	522	522
Thiol Reduction	522	522
EDC-NHS	522	522
UV-NBS	522	522
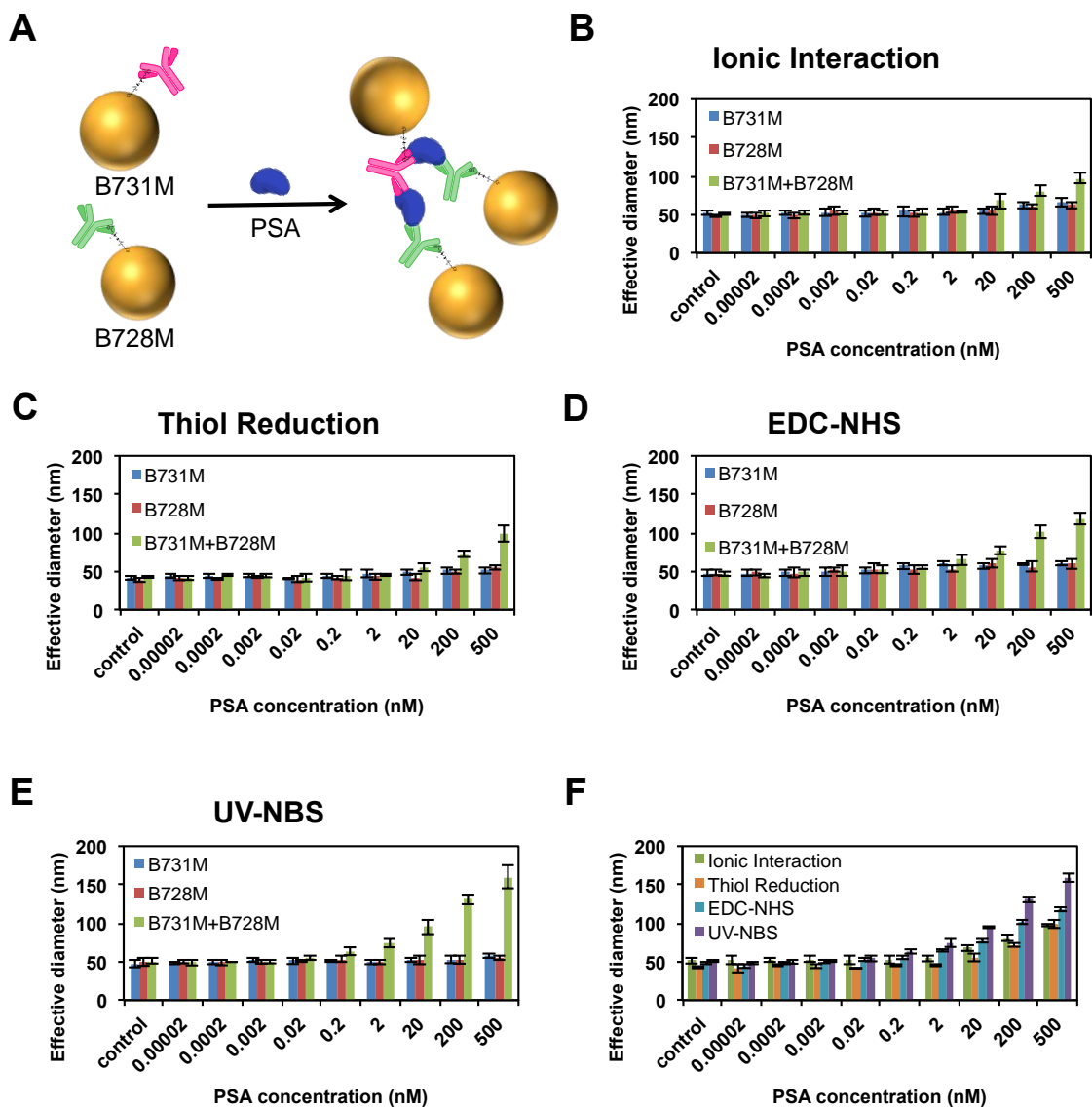
Fig. S11 DLS measurements for detecting cluster formation of antibody functionalized AuNPs (20 nm) upon binding on PSA. A) Schematic representation of antibody functionalized AuNP cluster formation upon binding of antigen. DLS measurements of antibody, B731M and B728M, immobilization on gold nanoparticles and their cluster formations utilizing B) the ionic interaction, C) Thiol Reduction, D) EDC-NHS, and E) UV-NBS methods. F) Comparison of cluster formations utilized with all methods. All data represent means (±SD) of triplicate experiments.
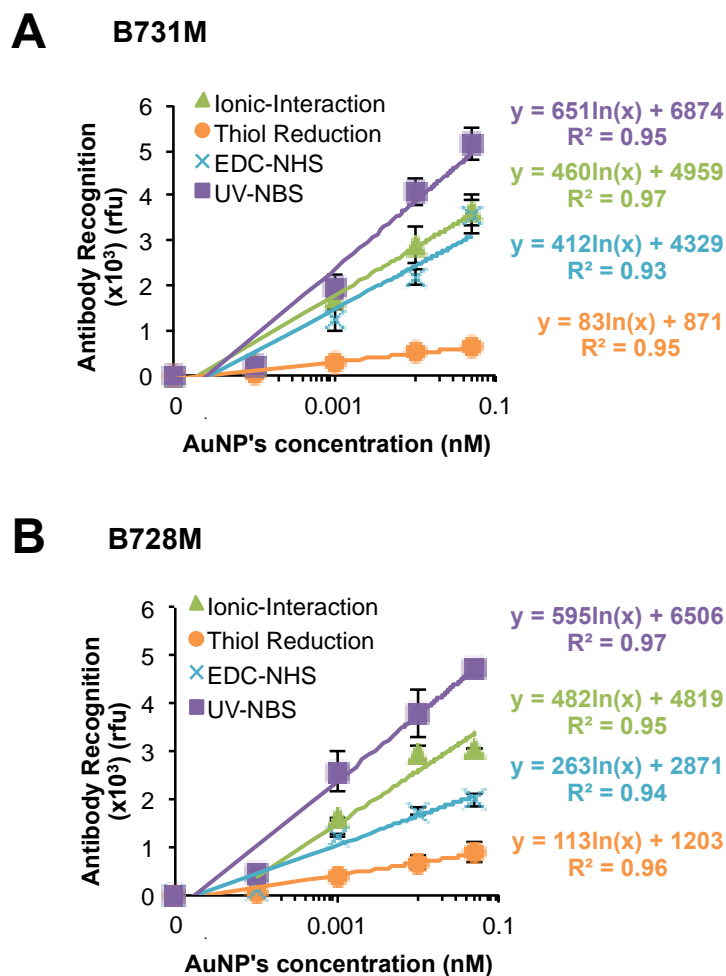
A B731M

B B728M

Fig. S12 Testing for antigen binding ability and structure stability of A) B731M and B) B728M antibody functionalized AuNPs (20 nm) utilizing the UV-NBS, EDC-NHS, Thiol Reduction and Ionic interaction immobilization methods via ELISA assay. For this experiments, antibody functionalized 20 nm AuNPs were incubated on PSA coated 96-well ELISA plate for 1 h. A HRP conjugated Fc specific anti-Mouse antibody was used to determine the stability of the antibodies on AuNPs surface. Amplex Red was used to quantify antigen bound structurally stable antibodies on AuNP surface by forming fluorescent product. Then, fluorescent intensity was measured using a Molecular Devices SpectraMax M5 plate reader (ex. 570 nm, em. 592 nm). The UV-NBS method provided the most active and stable antibodies immobilized on AuNP surface. All data represent means (±SD) of triplicate experiments.