Effects of Cationic Proteins on Gold Nanoparticle/Aptamer Assays

Thomas A. Pires,† Conor M. Narovec,‡ and Rebecca J. Whelan*†

Department of Chemistry and Biochemistry, Oberlin College, 119 Woodland Street, Oberlin, Ohio 44074, United States

Supporting Information

ABSTRACT: Gold nanoparticles (AuNPs) and aptamers are compelling building blocks for analytical assays with desired attributes of selectivity and sensitivity and may theoretically form the basis of instrument-free color-changing assays for any target against which a DNA aptamer has been selected. However, assays for proteins based on these components may be subject to significant interferences from the interaction of proteins with nanoparticles. We found that for three representative protein/aptamer systems—thrombin, apolipoprotein E, and platelet-derived growth factor—pH-dependent aggregation occurred, even in the absence of the aptamer, to differing extents. This effect is most pronounced when proteins display net surface charge (i.e., when pH < pI) but can even be observed at pH = pI when the protein retains regions of positive charge. These interactions of AuNPs and cationic regions on proteins may present an important limitation on the development of AuNP-based analytical assays.

INTRODUCTION

As analytical scientists continue to seek methods that are rapid, sensitive, selective, and adaptable to diverse detection challenges in a variety of resource-limited contexts, considerable effort has been invested in the development of novel colorimetric assays.1−5 In particular, gold nanoparticles (AuNPs) are exploited in numerous colorimetric assays developed in recent years.6−8 Owing to their extremely high molar absorptivity (∼2 × 10⁸ M⁻¹ cm⁻¹),9 AuNPs impart a color to solutions that is visible to the naked eye at nanomolar concentrations. Furthermore, the aggregation of AuNPs induces a shift in the surface plasmon resonance of the assembled particles, leading to a dramatic, easily visible color change from red to blue.10 AuNPs prepared via citrate reduction are colloidal, dispersed by charge−charge repulsion of citrate ions on their surfaces. Aggregation of AuNPs can be induced by elevated salt concentrations that screen these surface charges. However, AuNPs may remain dispersed at elevated salt concentrations if they are coated with ssDNA.11,12 The mechanism and kinetics of the interaction between ssDNA and AuNP have been extensively characterized.13 These phenomena have been exploited to develop colorimetric biosensing assays for targets as diverse as dopamine, mercury, and thrombin, wherein specific aptamer/target recognition mediates the extent of salt-induced aggregation.14−16 The assay format capitalizes on the fact that many well-characterized aptamers are ssDNA and therefore stabilize AuNPs against salt-mediated aggregation. Figure 1 illustrates the AuNP/aptamer assay format schematically.

As a step toward developing an instrument-free AuNP aggregation-based assay for clinically relevant biomarker proteins using DNA aptamers as molecular recognition elements, we attempted to reproduce the assay protocol described in a highly cited foundational paper;16 namely, the detection of thrombin using an aggregation mechanism mediated by a thrombin-specific aptamer. Wei et al. described a colorimetric assay for thrombin using unmodified AuNPs and a high-affinity 29-mer thrombin aptamer selected by Tasset and co-workers.17 As described in the Results and Discussion

Figure 1. Scheme of a simple aggregation assay. (Top) AuNPs aggregate in the presence of salts such as sodium chloride, resulting in a color change from red to blue. (Middle) In the presence of a DNA aptamer (blue lines), salt-induced aggregation is mitigated. (Bottom) Addition of the aptamer’s target (green triangles) competes aptamers off the AuNPs and restores salt-induced aggregation. The extent of aggregation therefore indicates the concentration of the aptamer’s target.
section, we find that under the conditions described by Wei et al., thrombin causes AuNP aggregation independent of the involvement of aptamers. We also find that this mechanism operates to different extents for two other protein/aptamer systems. This effect interferes with the straightforward implementation and interpretation of this assay format unless experimental parameters such as pH and ionic strength are well-controlled.

**RESULTS AND DISCUSSION**

A detailed experimental protocol is found in the Experimental Section. Briefly, we synthesized ∼13 nm AuNPs via the reduction of chlorauric acid by citrate. The average size, size distribution, shape, and monodispersity of the resulting AuNPs were confirmed by transmission electron microscopy (Supporting Information Figure 1), and the concentration was determined using measured absorbance at 520 nm and a previously reported value of extinction coefficient. In separate tubes, 200 μL of AuNPs was mixed with 30 μL of thrombin-binding aptamer (5 μM). Thrombin (30 μL) at various concentrations was then added, and the mixture was incubated at room temperature (RT) for 5 min, after which 100 μL of 0.3 M NaCl solution was added to induce aggregation. It is worth noting that this salt concentration is lower than that reported by Wei et al., who used a 0.5 M NaCl solution. In preliminary assays, we observed that the addition of 0.5 M NaCl resulted in an extensive nanoparticle aggregation at all thrombin concentrations; a lower salt concentration was necessary to see any protein-concentration-dependent response. Aggregation was assessed spectrophotometrically by the absorbance ratio $A_{520}/A_{620}$, the ratio most reflective of the visible region changes in absorbance (Supporting Information Figure 3). Aggregation can also be quantified by examining the $A_{720}/A_{520}$ ratio if a spectrophotometer is available; this ratio is more sensitive but lies outside the wavelength range detectable by the eye. As shown in Figure 2 (black circles), the extent of salt-induced aggregation increased with increasing thrombin concentration, ostensibly consistent with the mechanism shown in Figure 1.

We performed a control experiment in which a 29-base ssDNA oligomer with a scrambled sequence was substituted for the thrombin-binding aptamer. By contrast, with the expected negative result, we observed aggregation in the presence of the scrambled sequence, which should not have been driven off the nanoparticles by the presence of thrombin (Figure 2). This observation, along with the positive result for the negative control, suggested an alternative mechanism to that shown in Figure 1. Aggregation was not induced by buffer salts; the results of these control experiments are shown in Figure 3. A proof-of-concept experiment was conducted without thrombin, in which a stoichiometric amount of the aptamer was removed from the solution (replaced by pH-adjusted water) corresponding to the concentrations of thrombin used in the assays reported in Figure 2. This experiment showed that removal of aptamer from the solution (such as would occur in the presence of thrombin through the formation of the thrombin/aptamer complex) has no effect on the state of aggregation of the AuNPs, again suggesting that the aggregation previously reported in the presence of thrombin does not result from the mechanism illustrated in Figure 1. By adjusting the concentrations of aptamer and NaCl to diverge from the conditions reported by Wei et al., we were able to produce the expected trend, confirming that ssDNA confers protection against AuNP aggregation (data not shown).

To test whether thrombin alone has an aggregating effect on AuNPs, an experiment was conducted in which thrombin was added to AuNPs in the absence of oligonucleotides and the addition of NaCl was omitted. The result of this experiment, shown in Figure 4, demonstrates that under the assay conditions reported by Wei et al. (with respect to the buffer composition and AuNP/thrombin concentrations), thrombin itself acts as a coagulating agent, inducing aggregation of citrate-stabilized AuNPs. In light of this result and the observation that pH = 6 in the aforementioned experiments, we hypothesized that proteins bearing a net positive charge (at pH < pI) might be acting as cationic coagulating agents, independent of the aptamer/protein interaction that ostensibly mediates aggregation. This hypothesis motivated us to test three predictions: for

---

**Figure 2.** Salt-induced, thrombin-concentration-dependent aggregation of AuNPs occurs both in the presence of a thrombin-binding aptamer (black circles) and of a scrambled sequence oligonucleotide of the same length (open squares). Aggregation in the presence of a scrambled aptamer is not predicted by the mechanism shown in Figure 1.

**Figure 3.** AuNP aggregation follows the same trend in the presence of a thrombin-binding aptamer (solid circles) and a scrambled control oligonucleotide (open circles) with increasing concentration of thrombin; addition of protein buffer alone (squares) does not induce aggregation. Error bars are standard deviation ($n = 3$).
a given protein, the coagulating (aggregating) effect will be strongest at pH < pI, reduced at pH = pI, and abrogated at pH > pI. We selected two other proteins, apolipoprotein E (ApoE) (pI = 5.6) and platelet-derived growth factor (PDGF) (pI = 9.4) because their pI values (as determined by isoelectric focusing19) bracket that of thrombin (pI = 7.0−7.5). PDGF, which carries the greatest positive surface charge, was predicted to cause the greatest amount of aggregation, whereas ApoE, which carries little if any positive charge, was predicted to cause the least. Each protein was investigated for its ability to aggregate AuNPs at a pH below, near, and above its pI. Buffer cation concentrations were identical across all solutions. Results of these experiments are shown in Figure 5.

ApoE and thrombin produced results that supported our hypothesis, with increasing protein concentrations corresponding to increasing aggregation. Additionally, as predicted, aggregation decreased with decreasing pH. Thrombin causes more aggregation than an equal concentration of ApoE at an identical pH. On the other hand, PDGF, which was predicted to cause the greatest amount of AuNP aggregation, causes the least amount of aggregation of the three proteins tested, though aggregation is greatest at the lowest pH. It is difficult to rationalize why PDGF behaves in this manner. It may be that protein denaturation and interaction with the gold surface counteract the screening effect of cationic proteins. Cysteine residues accessible on the protein surface can immobilize proteins on gold surfaces through gold/thiol interactions. A UniProt Blast search (accessed July 3, 2017) shows that ApoE contains two cysteines; thrombin (peptidase S1) contains 6 cysteines; and one B subunit of PDGF contains 11 cysteines (so that our protein, PDGF-BB, contains 22 cysteines). Crystal structures from the Protein Data Bank (PDB) (1K21 and 1PDG; accessed July 30, 2017) suggest that thrombin contains three disulfide bonds, leaving no cysteine residues available for direct interaction with the gold surface. At the same time, the PDB structure suggests that PDGF contains eight intramolecular disulfide bonds, so that six cysteine residues may be reduced and available for interaction. The larger number of cysteine residues in PDGF and the larger number of these residues not involved in disulfide bonds are consistent with the hypothesis that gold/thiol interactions keep the AuNPs stable as colloids but are not proof that this is the operative mechanism. At the same time, the gold surface is able to interact with many amino acid side chains (anything containing heteroatoms that can act as electron-pair donors). We have not conclusively determined the origin of the unexpected trend observed for PDGF in these assays; more assays with proteins containing different numbers of cysteine residues under both oxidizing and reducing conditions will need to be performed to further substantiate or disprove this possible mechanism.

Other investigators have reported that some biological molecules aggregate AuNPs. Katayama and co-workers20 studied coagulation (their preferred term for aggregation) induced by a variety of biological molecules. They report that cationic biomolecules, including a +3 cationic peptide, spermidine (an oligoamine bearing a +3 charge), and lysozyme (a basic protein), induced aggregation, whereas bovine serum albumin (BSA) (an acidic protein) did not. Li and co-workers21 also observed that lysozyme causes aggregation of citrate-coated AuNPs in a concentration-dependent manner at nanomolar concentrations of protein. The authors attribute the behavior to the high pI of lysozyme, making it cationic under the physiological pH used in the assay. They exploit this phenomenon to develop a surface plasmon resonance light-scattering assay for lysozyme in urine.21 Later, when developing an assay based on the aptamer-mediated mechanism depicted in Figure 1, they needed to completely reoptimize the ionic strengths of the assay.22 This pair of published reports exemplifies the careful consideration of control experiments that must be

**Figure 4.** Thrombin induces aggregation of AuNPs in a concentration-dependent fashion (black triangles). Addition of an equivalent amount of a thrombin buffer (without thrombin) does not induce aggregation of AuNPs, indicating that the aggregation induced by thrombin is protein-mediated and not caused by the addition of buffer components. No oligonucleotides were present in these samples, and no NaCl was added to induce aggregation. Error bars are standard deviation (n = 3).

**Figure 5.** AuNP aggregation (indicated by ΔA620/ΔA520) as a function of protein concentration for ApoE (left), thrombin (middle), and PDGF-BB (right). Each protein was studied at three pH values: below pI (solid squares), near pI (open circles), and above pI (solid diamonds). Protein samples are shown with solid lines; buffer blanks are shown with dashed lines. Error bars are standard deviation (n = 3).
employed when developing assays relying on AuNP aggregation mediated by the aptamer/protein interaction.

We observe that aggregation-mediated color change—ostensibly an indicator of the concentration of a protein analyte—is extremely sensitive to other assay parameters such as concentrations of aptamer, the time delay between mixing and measuring, wavelengths used for detection, and ionic strength. Other investigators have noted similar dependence; sensitivity to ionic strength has been exploited as the basis of a modified version of the aggregation-based assay format. Understanding the nature of the protein/AuNP interaction is the focus of many current lines of research, particularly in the complex biological environment.

■ CONCLUSIONS
We have demonstrated that thrombin coagulates AuNPs, inducing an aggregation-mediated color change, in a concentration-dependent manner. This phenomenon has been observed in other proteins. The ability of some proteins to coagulate AuNPs confounds the straightforward interpretation of assays designed around the protective function of ssDNA (aptamers) on the surface of AuNPs. We argue that rigorous controls to characterize the color change mediated by the protein analyte alone must be part of the process of developing sensor-based assays intended to detect proteins via this aptamer-mediated AuNP aggregation mechanism. Studies that neglect to perform these control experiments may be mischaracterizing the sensing mechanism, inappropriately attributing selectivity to the action of the aptamer. The sensitivity and generality of the aptamer/AuNP aggregation approach is compelling, but the proper controls must be done to ensure reproducible and robust assays.

■ EXPERIMENTAL SECTION
Materials. All oligonucleotides were obtained from IDT (Coralville, IA) as a lyophilized solid and reconstituted to 100 μM in nuclease-free water to make a long-term stock solution. Oligonucleotides used in the study include the 29-mer thrombin-binding aptamer (5′-AGT CGG TGG TGA TCA CT-3′); a random 29-mer oligo control for thrombin experiments (5′-AGT CGG TGG TGA TCA CT-3′); a PDGF-binding aptamer (5′-CAC GGC TAC AAG TTG GGT GTA ACG ATA GGC AAT GAG CTC TCA TTG GGT TAC CTT TAA GGT-3′); and an ApoE-binding aptamer (5′-ACT AGC TAC GGG GGT GGT GGG CGG TGT CAG TTT GTT TAT TGG TGC TAT ACA TCT TCT ATA-3′). All proteins were purchased from Sigma-Aldrich (St. Louis, MO). Thrombin (lyophilized from human plasma) was reconstituted into Milli-Q water to a concentration of 100 U/mL. ApoE3, human, was reconstituted in 0.5 mM DTT and 5 mM NaH2PO4 at pH 7.8 to a concentration of 100 mM. PDGF-BB (human, recombinant) was reconstituted in sterile 4 mM HCl with 0.1% BSA to a concentration of 20 μg/mL. Stainless steel pipette tips and LoBind pipette tips were used in all sample preparation and assay performance to minimize loss of thrombin to surfaces.

Synthesis of AuNPs. AuNPs (13 nm) were synthesized via citrate reduction according to the established literature. All solutions were filtered with a 0.4 μm filter before use, and the glassware was cleaned with aqua regia before use. (Potential hazard: aqua regia solutions are extremely corrosive and may result in an explosion, skin burns, or eye and respiratory tract irritation. Safety goggles, gloves, and other personal protective equipment must be worn and splash hazard protection must be in place when working with aqua regia. Aqua regia solutions should be neutralized by slow addition of sodium bicarbonate before disposal.) Five milliliters of 38.8 mM trisodium citrate was rapidly added to 50 mL of boiling 1 mM HAuCl4 under reflux and under rapid stirring. Upon citrate addition, the solution quickly turned dark blue, then gradually red. The solution was removed from heat 15 min after the initial color change and allowed to continue stirring until it had cooled to RT, when it was subsequently filtered through a single 0.2 μm nylon membrane filter. The AuNP concentration was calculated using the absorbance at 519 nm and an extinction coefficient of 2.0 × 10^4 M⁻¹ cm⁻¹.

AuNP Aggregation Assays. For cuvette-based measurements, 200 μL of AuNPs was mixed with 30 μL of 5 μM thrombin-binding aptamer in 20 mM Tris-HCl, 140 mM NaCl, and 5 mM KCl, pH 7.5; then, 30 μL of thrombin solutions at varying concentrations was added, and the solutions were incubated for 5 min. Then, 100 μL of 0.3 M NaCl was added, and samples were incubated at RT for 30 min before being transferred to a 1 cm path length restricted volume quartz cuvette for measurement of an absorbance spectrum. Plate-based experiments were performed in clear 96-well plates and read in a SpectraMax M5 multimode plate reader ( Molecular Devices, Sunnyvale, CA). To correct the pH of the solution, 440 μL of the appropriate “oligo buffer” was mixed with 2935 μL of stock 13 nm AuNP. The resulting solution (127.9 μL) was then dispensed to each well to be used in a plate. A multichannel pipette was then used to deliver 17 μL of the protein samples (and corresponding blanks) to the appropriate wells simultaneously. The plate was immediately placed in the plate reader and mixed for 30 s. Five minutes after the addition of the first samples, the multichannel pipette was used to deliver 56 μL of water to each well. The plate was immediately transferred to the plate reader, where it was mixed for 30 s before the first spectrum was collected. Subsequent readings were taken at 10, 20, and 30 min after the first addition of water, with 30 s of mixing after each spectrum was collected. These studies were designed so that the concentration of protein exceeded that of AuNPs in all assays, with [protein]/[AuNP] ranging from a minimum of 5 to a maximum of 14 across the assay conditions. This excess of protein is likely sufficient for the formation of a protein monolayer on the AuNP surface in all assays. The proteins are of comparable size. Thrombin was found to have a diameter of 5 nm by electron microscopy. ApoE was found to exist in two major particle groups by dynamic light scattering, the majority of which averaged 4 nm. PDGF-BB was found to be 7.5 nm × 3.5 nm × 2.5 nm by X-ray crystallography.

■ ASSOCIATED CONTENT
* Supporting Information* The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01336.

Transmission electron micrographs of AuNPs; gel images from mobility shift assays; absorbance spectra of AuNPs at different extents of aggregation; and amino acid sequences for the three proteins studied (PDF)
Author Information

Corresponding Author

*E-mail: rwhelan@oberlin.edu (R.J.W.)

ORCID

Rebecca J. Whelan: 0000-0002-9293-1528

Present Addresses

1 Seattle Genetics, 21823 30th Dr SE, Bothell, WA 98021, USA (T.A.P.)

2 Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA (C.M.N.)

Notes

The authors declare no competing financial interest.

Acknowledgments

The project described was supported by a grant number R15CA161970 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. Financial support from a Henry Dreyfus Teacher Scholar Award (to R.J.W.) is gratefully acknowledged. The authors thank Lloyd Smith and Kepler Mears for stimulating discussions.

References

(1) Chen, Z.; Guo, J.; Ma, H.; Zhou, T.; Li, X. A Simple Colorimetric Sensor for Potassium Ion Based on DNA G-Quadruplex Conformation and Salt-Induced Gold Nanoparticles Aggregation. Anal. Methods 2014, 6, 8018–8021.

(2) Chen, Z.; Tan, Y.; Zhang, C.; Yin, L.; Ma, H.; Ye, N.; Qiang, H.; Lin, Y. A Colorimetric Aptamer Biosensor Based on Cationic Polymer and Gold Nanoparticles for the Ultrasensitive Detection of Thrombin. Biosens. Bioelectron. 2014, 56, 46–50.

(3) Liu, Q.; Yang, Y.; Li, H.; Zhu, R.; Shao, Q.; Yang, S.; Xu, J. NiO Nanoparticles Modified with 5,10,15,20-tetrakis(4-carboxyl phenyl)-Porphyrin: Promising Peroxidase Mimetics for H2O2 and Glucose Detection. Biosens. Bioelectron. 2015, 64, 147–153.

(4) McCracken, K. E.; Yoon, J.-Y. Recent Approaches for Optical Detection of Urine Using Membrane Micro-concentrators and Mobile Phone Camera. Sens. Actuators, B 2016, 2, 6591–6601.

(5) Lai, T.-S.; Chang, T.-C.; Wang, S.-C. Gold Nanoparticle-based Colorimetric Methods to Determine Protein Contents in Artificial Urine Using Membrane Micro-concentrators and Mobile Phone Camera. Sens. Actuators, B 2017, 239, 9–16.

(6) Zhao, W.; Brook, M. A.; Li, Y. Design of Gold Nanoparticle-Based Colorimetric Biosensing Assays. ChemBioChem 2008, 9, 2363–2371.

(7) Vilela, D.; González, M. C.; Escarpa, A. Sensing Colorimetric Approaches Based on Gold and Silver Nanoparticles Aggregation: Chemical Creativity Behind the Assay. A Review. Anal. Chim. Acta 2012, 751, 24–43.

(8) Lai, T.-S.; Chang, T.-C.; Wang, S.-C. Gold Nanoparticle-Based Colorimetric Methods to Determine Protein Contents in Artificial Urine Using Membrane Micro-concentrators and Mobile Phone Camera. Sens. Actuators, B 2017, 239, 9–16.

(9) Maye, M. M.; Han, L.; Kariuki, N. N.; Ly, N. K.; Chan, W.-B.; Luo, J.; Zhong, C.-J. Gold and Alloy Nanoparticles in Solution and Thin Film Assembly: Spectrophotometric Determination of Molar Absorptivity. Anal. Chim. Acta 2003, 496, 17–27.

(10) Kreibig, U.; Genzel, L. Optical Absorption of Small Metal Particles. Surf. Sci. 1985, 156, 678–700.

(11) Li, H.; Rothberg, L. J. Label-Free Colorimetric Detection of Specific Sequences in Genomic DNA Amplified by the Polymerase Chain Reaction. J. Am. Chem. Soc. 2004, 126, 10958–10961.

(12) Li, H.; Rothberg, L. J. Label-Free Colorimetric Detection of Specific Sequences in Genomic DNA Amplified by the Polymerase Chain Reaction. J. Am. Chem. Soc. 2004, 126, 10958–10961.

(13) Nelson, E. M.; Rothberg, L. J. Kinetics and Mechanism of Single-Stranded DNA Adsorption onto Citrate-Stabilized Gold Nanoparticles in Colloidal Solution. Langmuir 2011, 27, 1770–1777.

(14) Zheng, Y.; Wang, Y.; Yang, X. Aptamer-Based Colorimetric Biosensing of Dopamine Using Unmodified Gold Nanoparticles. Sens. Actuators, B 2011, 156, 95–99.

(15) Xu, H.; Mao, X.; Zeng, Q.; Wang, S.; Kawe, A.-N.; Liu, G. Aptamer-Functionalized Gold Nanoparticles as Probes in a Dry-Reagent Strip Biosensor for Protein Analysis. Anal. Chem. 2009, 81, 669–675.

(16) Wei, H.; Li, B.; Li, J.; Wang, E.; Dong, S. Simple and Sensitive Aptamer-Based Colorimetric Sensing of Protein Using Unmodified Gold Nanoparticle Probes. Chem. Commun. 2007, 3735–3737.

(17) Tasset, D. M.; Kubik, M. F.; Steiner, W. Oligonucleotide Inhibitors of Human Thrombin that Bind Distinct Epitopes. J. Mol. Biol. 1997, 272, 688–698.

(18) Grabar, K. C.; Freeman, R. G.; Hommer, M. B.; Natan, M. J. Preparation and Characterization of Au Colloid Monolayers. Anal. Chem. 1995, 67, 735–743.

(19) Ahmad, K. M.; Oh, S. S.; Kim, S.; McClellan, F. M.; Xiao, Y.; Soh, H. T. Probing the Limits of Aptamer Affinity with a Microrlfuidic SELEX Platform. PLoS One 2011, 6, e27051.

(20) Oishi, J.; Asami, Y.; Mori, T.; Kang, J.-H.; Niidome, T.; Katayama, Y. Colorimetric Enzymatic Activity Assay Based on Noncrosslinking Aggregation of Gold Nanoparticles Induced by Adsorption of Substrate Peptides. Biomacromolecules 2008, 9, 2301–2308.

(21) Wang, X.; Xu, Y.; Xu, X.; Hu, K.; Xiang, M.; Li, L.; Liu, F.; Li, N. Direct Determination of Urinary Lysozyme Using Surface Plasmon Resonance Light-Scattering of Gold Nanoparticles. Talanta 2010, 82, 693–697.

(22) Wang, X.; Xu, Y.; Chen, Y.; Li, L.; Liu, F.; Li, N. The Gold-Nanoparticle-Based Surface Plasmon Resonance Light Scattering and Visual DNA Aptasensor for Lysozyme. Anal. Bioanal. Chem. 2011, 400, 2085–2091.

(23) Li, N.; Yu, L.; Zou, J. Critical Coagulation Concentration-Based Salt Titration for Visual Quantification in Gold Nanoparticle-Based Colorimetric Biosensors. J. Lab. Autom. 2014, 19, 82–90.

(24) Patra, A.; Ding, T.; Engdah, G.; Wang, Y.; Dykas, M. M.; Liedberg, B.; Kah, J. C. Y.; Venkatesan, T.; Drum, C. L. Component-Specific Analysis of Plasma Protein Corona Formation on Gold Nanoparticle Using Multiplexed Surface Plasmon Resonance. Small 2016, 12, 1174–1182.

(25) Fleischer, C. C.; Payne, C. K. Nanoparticle—Cell Interactions: Molecular Structure of the Protein Corona and Cellular Outcomes. Acc. Chem. Res. 2014, 47, 2651–2659.

(26) Weisel, J. W.; Nagaswami, C.; Young, T. A.; Light, D. R. The Shape of Thermobimodulin and Interactions with Thrombin as Determined by Electron Microscopy. J. Biol. Chem. 1996, 271, 31485–31490.

(27) Chou, C.-Y.; Lin, Y.-L.; Huang, Y.-C.; Sheu, S.-Y.; Lin, T.-H.; Tsay, H.-J.; Chang, G.-G.; Shiao, M.-S. Structural Variation in Human Apolipoprotein E3 and E4: Secondary Structure, Tertiary Structure, and Size Distribution. Biophys. J. 2005, 88, 455–466.

(28) Oelner, C.; D’Arcy, A.; Winkler, F. K.; Eggimann, B.; Hosang, M. Crystal Structure of Human Platelet-Derived Growth Factor BB. EMBO J. 1992, 11, 3921–3926.