Modular Protein Engineering Approach to the Functionalization of Gold Nanoparticles for Use in Clinical Diagnostics

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Supporting Information

ABSTRACT: Functional protein−gold nanoparticle (AuNP) conjugates have a wide variety of applications including biosensing and drug delivery. Correct protein orientation, which is important to maintain functionality on the nanoparticle surface, can be difficult to achieve in practice, and dedicated protein scaffolds have been used on planar gold surfaces to drive the self-assembly of oriented protein arrays. Here we use the transmembrane domain of Escherichia coli outer membrane protein A (OmpA_TM) to create protein−AuNP conjugates. The addition of a single cysteine residue into a periplasmic loop, to create cysOmpA_TM, drives oriented assembly and increased equilibrium binding. As the protein surface concentration increases, the sulfur−gold bond in cysOmpA_TM creates a more densely populated AuNP surface than the poorly organized wtOmpA_TM layer. The functionalization of AuNP improved both their stability and homogeneity. This was further exploited using multidomain protein chimeras, based on cysOmpA(TM) which were shown to form ordered protein arrays with their functional domains displayed away from the AuNP surface. A fusion with protein G was shown to specifically bind antibodies via their Fc region. Next, an in vitro selected single chain antibody (scFv)-cysOmpA(TM) fusion protein, bound to AuNP, detected influenza A nucleoprotein, a widely used antigen in diagnostic assays. Finally, using the same scFv-cysOmpA(TM)−AuNP conjugates, a prototype lateral flow assay for influenza demonstrated the utility of fully recombinant self-assembling sensor layers. By simultaneously removing the need for both animal antibodies and a separate immobilization procedure, this technology could greatly simplify the development of a range of in vitro diagnostics.

KEYWORDS: gold nanoparticles, self-assembly, outer membrane proteins, single chain variable fragment, LSPR, lateral flow assay, biosensing

INTRODUCTION

The generation of functional AuNP-protein conjugates is of great interest and importance for many bioscience applications, particularly diagnostics and therapeutics.1,2 AuNPs are an attractive vehicle for biomolecules as they can be made in well-defined sizes,3 are biocompatible,4 and exhibit unique optical properties that provide an intrinsic ability to sense changes in the local environment5 and have also been utilized for therapeutic purposes.6−8 Protein, i.e., mostly antibody, conjugation is generally used to bestow biological functionality to the AuNP surface, such as targeting of a particular protein or antigen of interest9,10 or carrying out therapeutic processes, including gene editing.11,12 Numerous protein attachment strategies have been developed, usually via chemically modified linker molecules that are assembled on the AuNP surface through terminal thiol groups.13 These linker chemistries can be quite widely applicable, such as coupling to amines or hydrazide coupling of antibody glycans. Furthermore, it has been shown that more directed coupling, providing orientational control, improves functionality of the protein−nanoparticle conjugates.5,10 Control over antibody orientation has been shown to enhance antigen binding when immobilized on planar surfaces.14 Previously, it has been shown that outer membrane β-barrel proteins from Escherichia coli can be
engineered to self-assemble into oriented monolayers on planar gold surfaces. An upright orientation was driven by both the addition of a single cysteine residue into a periplasmic turn and coassembly with membrane mimicking thioAlkylPEG molecules. The transmembrane domain of E. coli outer membrane protein A (OmpATM) can be used as such a protein engineering scaffold when circularly permuted to enable modification of its extracellular loops. This allows heterologous functional domains, such as antibody binding domains from protein A and protein G, to be fused to OmpATM and displayed away from the surface. The utility of such protein arrays on planar gold surfaces for biological sensing has been previously presented. AuNP−protein conjugates are widely used in diagnostic assays, particularly for lateral flow tests, where they are used to detect many different antigens, e.g., hormones and viral proteins. While protein binding to AuNPs has been extensively researched, most studies have focused on the stoichiometry and thermodynamics of globular model proteins such as serum albumins and ubiquitin.
control over protein orientation, with changes in AuNP surface charge and site-directed insertion of cysteine residues being shown to influence α-synuclein and E. coli PPase orientation on the AuNP surface, respectively. Here we present the self-assembly of engineered OmpATM proteins on the surface of 20 nm AuNPs (Scheme 1). Protein binding could be sensitively monitored by observing changes in the localized surface plasmon resonance (LSPR) peak, and the protein layer structure was investigated by dynamic light scattering (DLS). Furthermore, we introduce chimeric, single chain antibody-OmpATM proteins as a simple method to functionalize AuNP conjugates to detect a clinical antigen, influenza A nucleoprotein (FluA NP). NP is the preferred target for diagnostic assays due to its highly conserved nature and type specificity for type A and B influenza.

■ RESULTS AND DISCUSSION

Cysteine Mediated Binding and Assembly of OmpATM. Initially we investigated the effect of a single cysteine residue, inserted into a periplasmic turn of OmpATM, on the binding of detergent solubilized OmpATM to the surface of AuNPs. We present data exclusively for 20 nm AuNPs (Scheme 1). Protein binding could be sensitively monitored by observing changes in the localized surface plasmon resonance (LSPR) peak, and the protein layer structure was investigated by dynamic light scattering (DLS). Furthermore, we introduce chimeric, single chain antibody-OmpATM proteins as a simple method to functionalize AuNP conjugates to detect a clinical antigen, influenza A nucleoprotein (FluA NP). NP is the preferred target for diagnostic assays due to its highly conserved nature and type specificity for type A and B influenza.

Protein binding was measured by the shift in the barycentric mean wavelength (Δλm) of the LSPR absorption peak. Higher equilibrium binding was observed for the cysOmpATM mutant than for the wild type protein (wtOmpATM), with an increase in the Δλm of 0.93 nm (Figure 1A). The increased binding was also observed by fluorescence spectroscopy experiments which recorded the reduction in concentration of free protein (Figure 1B). To confirm the formation of a sulfur–gold bond, the stabilities of the WT and cysOmpATM protein–AuNP conjugates were probed by challenging the particles with borohydride ions (BH4−), which have a high affinity for the nanoparticle surface. This revealed a significant increase in the stability of the conjugated particles when the cysteine residue was present (Figure 1C).

A stark difference between the two particle types was revealed by agarose gel electrophoresis where the cysOmpATM–AuNP conjugates migrate faster than the wild type–AuNP conjugates, indicating that they had an increased net negative charge (OmpATM has an estimated charge of −3.6 at pH 8) (Figure 2A). Characterization of these protein–AuNP conjugates by DLS showed that the diameters of the wtOmpATM and cysOmpATM particles were remarkably similar; however the latter sample had a greater LSPR λm shift indicating greater protein binding (Figure 2B and Figure 2C). The assembly for this experiment was carried out at a 10 times higher overall concentration of both protein and AuNP compared to Figure 1, and while the LSPR shifts for wtOmpATM increase from 1.4 to 2.0 nm, the cysOmpATM

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Figure 2. Characterization of AuNPs (preconjugated with wild type and cysOmpATM) before and after overnight thioalkyPEG filler incubation. 20 nm AuNPs at OD525nm = 10 were incubated with 8 μM of either protein for 30 min, followed by coassembly with filler by mixing the protein–AuNP conjugates with 8 μM filler and incubating overnight. (A) 0.5% agarose gel of wtOmpATM particles before (1) and after filler addition (2) and cysOmpATM particles before (3) and after (4) filler addition (F). Corresponding LSPR absorbance peak barycentric mean wavelength Δλm (B) and change in hydrodynamic radius (ΔDHyd) measured by DLS (C) data (where ΔDHyd = 0 corresponds to bare particles) for the same protein–AuNP conjugate samples used in (A). Error bars represent the standard error of the mean based upon three measurements.
values are 2.4 and 2.1, respectively, confirming the apparent surface saturation observed at 0.8 μM cysteine-ATM and the less specific binding of the WT protein. Infilling of the nanoparticle surface with an uncharged lipid mimicking molecule, 1-mercaptoundecyl-11 hexa(ethylene glycol) (filler), increased the size of both wild type and cysteine-ATM particles, as seen by DLS, and retarded the electrophoretic migration of the particles (Figure 2A and Figure 2C). The effect of filler addition on both the size of the particles and the increase in the Δλm was much more pronounced for the cysteine-ATM particles. These results suggest that insertion of the cysteine residue plays a role in both protein binding and organized assembly of OmpA-ATM on the nanoparticle surface. Interestingly, the binding kinetics of the wild type and cysteine-ATM proteins were strikingly similar, with pseudo-second-order rate constants of 14.76 ± 1.12 and 14.86 ± 1.09 × 10⁻³ s⁻¹ nm⁻¹ respectively (Supporting Information). This would suggest that electrostatic interactions dominate the early stages of protein binding, i.e., in the seconds to minutes range, followed by reorganization of the protein layer to maximize the number of favorable interactions. Protein binding to AuNPs has been shown to obey equilibrium dynamics, therefore the formation of a thermodynamically stable thiol–gold bond between the protein and AuNP would shift the binding equilibrium toward associated and oriented protein. Outer membrane β-barrels consist of a hydrophobic core region, which normally resides in the lipid membrane, and hydrophilic loops at their periplasmic and extracellular ends. A standing orientation, driven by the location of the cysteine in a periplasmic loop, as shown by neutron scattering experiments on planar surfaces, maximizes favorable hydrophobic interactions with filler molecules and neighboring proteins while minimizing the surface footprint of bound proteins, thus increasing the maximum adsorption capacity of the nanoparticle surface. A more organized cysteine-ATM array on the AuNP surface would explain the increase in the apparent
protein binding and also the increased filler binding observed compared to a wtOmpATM array with poorly oriented proteins in many different conformations. The high wtOmpATM binding is most likely due to exposed lysine residues, which have been shown to mediate binding to AuNP;30 in particular, residues K73 and R102 form a positively charged surface at the extracellular end of the barrel. The N-terminal 6xHis-tag may also play a role in AuNP binding.31 The protein binding behavior observed in this study is consistent with a protein binding model proposed by Wang et al., where initial association is dynamic and reversible, followed by reorganization of the protein layer to maximize the available AuNP surface and, finally, irreversible binding of the protein which is accelerated here by the exposed cysteine residue.25

Surface Assembly of Antibody Capture Domains. To test the utility of cysOmpATM−AuNP conjugates as a diagnostic tool, a multidomain version termed GGzOmpATM was used. This protein combines tandem B-domains from protein G (GG)19 and a Z-domain from protein A (z)18 bound to the N-terminus of a circularly permuted OmpA transmembrane domain (OmpATM)20 and has a total length of approximately 135 Å when standing on a gold surface 20 (Figure 3 A). Both B- and Z-domains bind to the constant regions of immunoglobulin G (IgG) enabling oriented presentation of antibodies on surfaces so that their binding sites are exposed. As expected, gel electrophoresis and DLS measurements showed a greater increase in particle size on addition of GGzOmpATM when compared with cysOmpATM (Figure 3B and Figure 3C). Negative stained transmission electron microscopy images of GGzOmpATM−AuNP conjugates also revealed material bound to the surface that was not visible on untreated particles (Figure 3D). These features are too large to be individual GGzOmpATM proteins and may be groups of closely associated proteins since at around 11 nm in length they are consistent with the height of GGzOmpATM. Further analysis of GGzOmpATM−AuNP conjugates was carried out using sedimentation velocity analytical ultracentrifugation. One-dimensional sedimentation coefficient, c(s), distribution analysis32 showed broad single peaks for each sample (Figure 3E, top panel). The c(s) distributions of the protein coated AuNPs were clearly affected by changes in their size and density when compared with the untreated AuNPs. More in-depth analysis using a two-dimensional “size and shape” distribution, c(s/f0), demonstrated a higher fraction of aggregated, asymmetrical species (with a friction ratio significantly above 1) before addition of the filler molecule on to the AuNP surface (Figure 3E, bottom panel). This suggests that incorporation of filler molecules into the GGzOmpATM layer results in a more ordered protein-filler array. The resulting particles were more spherical in shape and had a lower level of inhomogeneity when compared with AuNPs coated in just GGzOmpATM (more details in the Supporting Information).
Assembly of GGzOmpATM−AuNP and subsequent binding of a monoclonal antibody were followed by DLS (Figure 4A and Figure 4C). This showed a surface layer thickness of 10.1 ± 0.53 and 12.13 ± 0.68 nm after addition of GGzOmpATM and the thioAlkylPEG filler, respectively, confirming that infilling of the surface may encourage a more perpendicular protein orientation. This observation is consistent with previous AFM and neutron reflection studies of engineered OmpATM arrays on planar gold surfaces which showed an increase in orientation and stability after filler assembly. The protein layer thickness and antibody binding function were thus indicative of ordered protein assembly where the antibody binding domains are displayed away from the particle surface.

Incubation with a mAb formed a combined thickness of 19.26 ± 2.04 nm protein on the AuNP surface, confirming that the B-domains were free to bind antibody. The increase in standard error from ±0.68 to ±2.04 nm in the DLS measurements after mAb binding was suggestive of a less homogeneous population of particles, possibly due to differing amounts of mAb bound. Antibody binding was specific to GGzOmpATM coated AuNPs with wild type and cysOmpATM particles showing low levels of nonspecific binding (Figure 4B).

Surface Assembly of Antibody Domains for Influenza Detection. The most common application of conjugated AuNPs is in diagnostic tests where they are used to detect a number of different antigens such as proteins and DNA. Therefore, the ability of the modular OmpATM system, assembled on AuNPs, to detect a clinical antigen was tested. For this we used a different OmpATM fusion protein which contained at its N-terminus an antigen binding domain derived from an antibody. Since the antigen binding or complementarity-determining regions (CDRs) of antibodies consist of two separate proteins, engineered versions called single chain variable fragments (scFv) have been developed which combine the recognition domains of immunoglobulins into a single polypeptide chain. These can be derived from known antibodies, or novel functions can be developed using combinatorial selection procedures to select the scFv of choice from a random library. This protein (scFvOmpATM) consisted of a bespoke single chain variable fragment (scFv domain), selected to bind influenza A nucleoprotein (FluA NP) using a bacterial retained display platform and affinity selection techniques, fused to the N-terminus of circularly permuted cysOmpATM via an α-helical linker domain. This engineered protein eliminates the need for both animal derived antibodies and immobilization chemistries, allowing a highly selective AuNP surface to be manufactured using a single recombinant protein easily manufactured by bacterial fermentation. A second protein (*OmpATM), which consisted of just the cysOmpATM and α-helical linker domains, was used as a negative control (Figure 5A and Figure 5B). Binding of recombinant FluA NP to scFvOmpATM conjugated particles was observed by UV−vis spectroscopy as a red shift in the LSPR peak and DLS as a large increase in the particle size (Figure 5C and Figure 5D). TEM images of scFvOmpATM particles after incubation with FluA NP revealed large objects bound to the surface of the particles that were not present before antigen addition (Figure 5E and Figure 5F). These are larger than expected for NP; however the monomeric species...
exists in equilibrium with trimers, which at 150 kDa approximate to the structures seen here. This flu NP quaternary structure also explains both the cross-linking of AuNP by the flu antigen observed in EM (Figure 5F) and, by forming multi AuNP-FluA NP complexes via multivalent interactions, the strong binding response seen by spectroscopy and DLS. This effect has also been exploited in AuNP LSPR based assays of intact flu virus where binding to the hemagglutinin proteins on the viral surface brings many AuNP into close proximity. Here, samples of the complexes were observed by TEM with FluA NP apparently bridging between different scFvOmpATM–AuNP conjugates (Figure SF). Detection of FluA NP was thus robust and specific. Control particles conjugated with *OmpATM, the negative control protein, did not show any FluA NP binding by either UV−vis spectroscopy or DLS. Finally, FluA NP binding to scFvOmpATM−AuNP conjugates was tested using a lateral flow assay (LFA). LFAs are widely used in diagnostics and comprise a porous membrane, commonly nitrocellulose, to which antibodies are immobilized in defined lines. Samples suspected of containing the antigen are mixed with visible particles, usually AuNPs or latex beads that have been conjugated with a second antibody, which recognizes a secondary site on the antigen. This solution is allowed to flow along the strip, with any bound antigen forming a cross-link between the particle and the immobilized antibody, leading to the formation of a characteristic colored line. A second antibody line on the strip acts as a control, binding to the antibody on the particle surface. A prototype lateral flow assay which used α-FluA NP and α-human IgG antibodies, as the capture and control lines respectively, could detect a minimum of 500 ng/mL FluA NP when incubated with scFvOmpATM coated particles (Figure 5G). This is comparable sensitivity to commercially available anti-NP antibodies used in diagnostic assays. Detection was specific to FluA NP with no capture line observed for scFvOmpATM particles incubated with recombinant respiratory syncytial virus nucleoprotein (RSV NP) (Figure 5G). Detection was also accurate and discriminatory since all samples containing FluA NP were positive and no capture or control binding was observed for AuNPs conjugated with the *OmpATM control protein after incubation with FluA NP (Figure 5G). Comparison of the detection limits with other methods is somewhat difficult as they are not always directly comparable. It is also difficult to find primary data for the detection limits of commercial LFAs. The detection limit presented here is comparable to a lateral flow assay using silica nanoparticles and a double antibody sandwich enzyme-linked immunosorbent ELISA assay. However, more complex detection methods, such as two-photon fluorescence excitation, have been able to detect nucleoprotein concentrations down to 50 ng/mL. The experimental conditions used here, while not using patient samples, are comparable to the clinical situation where the tests use diluted washes of nasal swabs. These samples are free of the complications of blood samples and, compared to tests for other biomarkers, contain relatively large amounts of shed virions and viral proteins.

**CONCLUSION**

The work presented here describes the simple creation of gold nanoparticles decorated with functional protein domains suitable for diagnostic applications. The addition of a single cysteine residue in a periplasmic loop of OmpATM plays an important role in its assembly on the surface of AuNPs. Although significant amounts of WT protein bind to AuNP, the addition of the cyste residue leads to larger amounts of bound protein, greater stability (Figure 1), more efficient assembly of the membrane mimicking thioAlkylPEG layer (Figure 2), and improved protein orientation, consistent with previous studies. We then used two different engineered multidomain OmpATM protein chimeras. GGzOmpATM, which contains antibody binding domains, was shown to form ordered protein arrays on AuNPs with the functional G-domains displayed away from the surface and able to display monoclonal IgG antibodies. Next we fused a single domain antibody, which recognizes the influenza antigen FluA NP, to OmpATM (scFv-OmpATM). FluA NP is an important diagnostic antigen used in the detection of influenza infections. Nanoparticles decorated with anti-FluA NP scFv-OmpATM were used to detect FluA NP using UV−vis spectroscopy, dynamic light scattering, and electron microscopy. Furthermore, it was used in a lateral flow assay (Figure 5) as proof of concept for using self-assembled engineered membrane proteins in a clinically relevant diagnostic test. By fusing recombinant single chain antibodies with a self-assembling scaffold protein, this approach removes the need for complex and inefficient conjugation of animal derived antibodies. It has the scope to detect a wide variety of antigens with antibody-like specificity while being economically produced on a large scale by bacterial fermentation. Additional applications could include gold labeling of cellular or engineered (FLAG) antigens in electron microscopy.

**MATERIALS AND METHODS**

**Materials.** Gold nanoparticles were purchased from BBI Solutions (Cardiff, U.K.). Antibodies were purchased from HyTest (Turku, Finland) and Abcam (Cambridge, U.K.). Molecular biology and protein purification materials were purchased from Invitrogen, Generon, and GE Healthcare (U.K.). All other materials were purchased from Sigma-Aldrich unless otherwise stated.

**Production and Purification.** wtOmpATM and cysOmpATM were expressed, purified, and refolded from inclusion bodies as described previously for OmpAZ. GGzOmpATM was expressed, purified, and refolded as described previously. scFvOmpATM was expressed, purified, and refolded as described for GGzOmpATM. The bespoke scFv domain was purchased from Affinity Bio (Scoresby, Australia). OmpATM was also expressed, purified, and refolded as described for GGzOmpATM. Recombinant influenza A nucleoprotein expression and purification were carried out as described previously. Recombinant respiratory syncytial virus nucleoprotein expression and purification were carried out as for influenza A nucleoprotein.

**Equilibrium Binding Measurements.** wtOmpATM and cysOmpATM were buffer exchanged into DDM buffer (0.5% w/v docetyl β-maltoside, 10 mM Tris-HCl, pH 8) using a PD10 desalting column (GE Healthcare). The resulting protein solution was incubated in 5 mM TCEP (tris(2-carboxyethyl)phosphine) for 30 min before use. 20 nm AuNPs at OD 525 nm = 1 were mixed with protein at concentrations between 0.016 and 0.8 μM before overnight incubation at room temperature. UV−vis spectra were acquired with a Cary 4E spectrophotometer between 400 and 800 nm. The barycenter mean wavelength (λm) was calculated between 500 and 600 nm using the following equation:

$$\lambda_m = \frac{\sum I(\lambda) \lambda}{\sum I(\lambda)}$$

where I(λ) is the absorbance at wavelength λ. The shift (∆λm) was calculated as the difference from the λm of nonfunctionalized AuNPs.
Fluorescence Spectroscopy. Overnight assembly of wtOmpATM and cysOmpATM–AuNP conjugates was carried out as for the equilibrium binding experiments with protein concentrations between 0.08 and 0.8 μM. Before carrying out fluorescence measurements of protein concentration, the protein–AuNP conjugates were removed by centrifugation at 20,000g for 10 min. Protein binding was calculated by measuring the residual protein concentration using the intensity of the tryptophan fluorescence of the supernatant. Fluorescence spectra were acquired using a Cary Eclipse fluorescence spectrophotometer using 5 nm path length quartz cuvettes (Hellma 111.057). Excitation was at 280 nm and emission scanned between 300 and 450 nm. Total fluorescence was calculated by integrating the intensities between 300 and 400 nm for each sample and the protein concentration calculated from a calibration curve made using protein standards between 0.0125 and 1 μM.

Protein–AuNP Conjugate Stability. Overnight preparation of wtOmpATM and cysOmpATM–AuNP conjugates was carried out as for the equilibrium binding experiments with a protein concentration of 0.8 μM. Protein–AuNP conjugates at OD525nm = 1 were mixed with NaBH4 at concentrations between 0.05 and 1.5 mM before incubating at room temperature for 3 h. UV–vis spectra were acquired with the Cary 4E spectrophotometer for the protein–AuNP conjugates before and after NaBH4 addition. Stability was measured as the ratio of the shift in the λmax before and after NaBH4 addition.

Agarose Gel Electrophoresis. Protein–AuNP conjugates were made by mixing 20 nm AuNPs at OD530nm = 10 with 8 μM protein before overnight incubation. Coassembly of the 1-mercaptoundecylamine–AuNP conjugates at OD525nm = 1 were mixed with 7.5 mM boric acid) and run in TB buffer supplemented with 30% w/v glycerol and 0.05% Tween 20 and resuspended in Nanopure water. For antibody binding experiments, protein conjugated particles were incubated at 16 900 × g for 10 min before centrifugation at 16 900 × g. The resulting images were processed using the ImageJ software.

Dynamic Light Scattering. DLS measurements were carried out in a 45 μL cuvette (Hellma) with a Malvern Zetasizer Nano S. Residual protein was removed by centrifugation of the protein–AuNP conjugates at 16 900 g and resuspension in Nanopure water. For antibody binding experiments, protein conjugated particles were mixed with 1 μM monoclonal antivinculin antibody and incubated for 10 min before centrifugation at 16 900 g and resuspension in Nanopure water.

FluA NP Binding Experiments. scFvOmpATM–AuNP conjugates were made with protein and AuNP concentrations of 0.8 μM using the same method as for cysOmpATM–AuNP. Residual protein was removed by centrifugation and resuspension in Nanopure water. The resulting scFvOmpATM–AuNP conjugates at OD530nm = 1, were mixed with 150 nM of FluA NP and incubated for 10 min before residual protein was removed by centrifugation and resuspension in Nanopure water. UV–vis spectroscopy, DLS, and TEM were then carried out on the protein–AuNP conjugates before and after FluA NP incubation.

Analytical Ultracentrifugation (AUC). Sedimentation velocity (SV) experiments were carried out with a Beckman Coulter Optima XL-A analytical ultracentrifuge (Palo Alto, CA, USA) using both absorbance at 530 nm and interference optics at a rotation speed of 5000 rpm and experimental temperature of 20 °C. The AuNPs were diluted in water to a final concentration of OD530nm = 1.5 with a path length of 1 cm. A sample volume of 400 μL was used. Sedimentation velocity profiles were analyzed using both 1D size-distribution c(s) and 2D distribution c(s,θ) models implemented in the program SEDFIT. Each peak on the distribution plot was integrated in order to obtain the weight-averaged values for sedimentation coefficient. In brief, sedimentation of the material is described by the Svedberg equation as follows:

\[ M = \frac{sRT}{D(1 - \frac{\bar{v}_{\text{particle}}\rho_{\text{solvent}}}{\bar{v}_{\text{solvent}}\rho_{\text{solvent}}})} \]

where \( M \) is the particle’s molecular mass, \( s \) is its sedimentation coefficient, \( D \) is the diffusion coefficient, \( \bar{v}_{\text{particle}} \) is the partial specific volume of the sedimenting particle, \( \rho_{\text{solvent}} \) is the density of the solvent, \( T \) is the absolute temperature, \( R \) is the ideal gas constant.

The partial specific volume can be represented in terms of the particle’s density, \( \rho_{\text{particle}} \) as

\[ \bar{v} = 1/\rho_{\text{particle}} \]

The density of the particle can, in turn, be expressed as a function of its hydrodynamic radius, \( R_h \), and sedimentation coefficient, \( s \), by

\[ \rho_{\text{particle}} = \rho_{\text{solvent}} + \frac{18\eta_{\text{solution}}s}{(2R_h^2)} \]

where \( \rho_{\text{solvent}} \) is the density of the solvent and \( \eta_{\text{solution}} \) is its viscosity.

Lateral Flow. Lateral flow strips were made from nitrocellulose membrane (4 cm × 30 cm) with capture and control antibody lines deposited by hand using a pipet tip at 0.5 mg/mL in binding buffer (10 mM NaHCO3, pH 9.5). The strips were blocked overnight with 2% w/v BSA at 4 °C and cut into 4 cm × 0.5 cm segments before use. An amount of 7.5 μL of protein–AuNP conjugate samples at OD530nm = 10 was mixed with 7.5 μL of antigen solution and incubated for 5 min before applying to the opposite end of the nitrocellulose strip to the antibody lines. The solution progressed through the membrane via capillary action and the strips were washed 5 times with 20 μL of washing buffer (50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.8) before imaging with a digital camera.

ASSOCIATED CONTENT

Supporting Information

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Protein binding kinetics and AUC analysis of the AuNPs (PDF)

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Notes

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ABBREVIATIONS

AuNP = gold nanoparticle
LSPR = localized surface plasmon resonance
DLS = dynamic light scattering
AUC = analytical ultracentrifugation
OD = optical density
TEM = transmission electron microscopy
mAb = monoclonal antibody
OmpA_TM = transmembrane domain of E. coli outer membrane protein A
cysOmpA_TM = cysteine mutant of OmpA_TM
GGzOmpA_TM = N terminal tandem pair of B domains from protein G and Z domain from protein A fused to a cysOmpA_TM scaffold
scFvOmpA_TM = N-terminal single chain variable fragment domain fused to an OmpA_TM scaffold
*OmpA_TM = α helical linker domain fused to cysOmpA_TM
FluA NP = influenza A nucleoprotein

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