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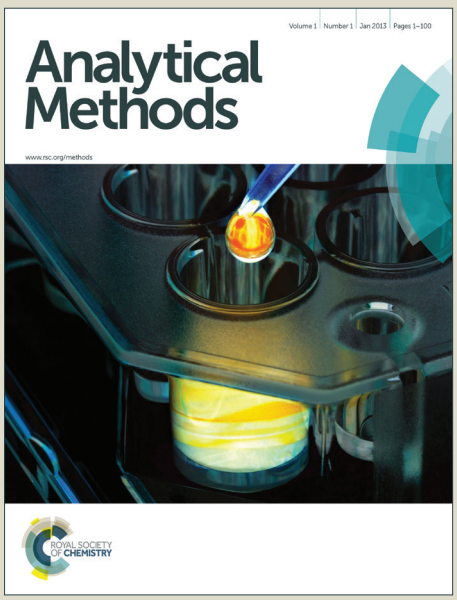

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ARTICLE TYPE

Rapid Screening of Antibody-Antigen Binding using Dynamic Light Scattering (DLS) and Gold Nanoparticles

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A rapid one-step screening method to evaluate the specificity of antibody-antigen binding was developed using antibody-conjugated gold nanoparticles (Ab-AuNPs) and dynamic light scattering (DLS). Influenza A virus was used as a model antigen to develop this platform and antigen-specific antibodies were attached to the surface of AuNPs. Introduction of virus to the nanoparticle solution resulted in aggregation of the AuNP probes provided that the antibody had an affinity for the virus. A fundamental concern of the work was to ensure aggregates only formed in the presence of the antigen. Therefore, optimal conditions for synthesizing and maintaining the stability of the Ab-AuNP conjugates were investigated by varying pH and antibody concentration, and a protocol for preparing stable Ab-AuNP conjugates is presented. Thereafter the AuNP probes were exploited in a DLS assay to screen the binding specificity of four antibodies to two different isolates of influenza virus (subtype H1N1). The DLS data for antibody binding were in concordance with the results obtained with a conventional ELISA, thereby validating the DLS platform. Importantly, the DLS assay was completed in 30 minutes relative to 24 hours via ELISA.

Introduction

Nanoparticle–based immunoassays have emerged as a great tool for rapid, sensitive, and low–cost point of care diagnostic tests due to the selective molecular recognition based on antibody–antigen specificity and unique properties of nanoparticles (NP). Antibodies are immobilized onto the surface of nanoparticles that vary in material, size, and shape which can be tailored to improve the detection of pathogens and biomarkers. Despite the advantages provided by NPs and readout technology, detection improvement is also inherently governed by the antibody. There is a direct correlation between assay performance and antibody affinity, regardless of the readout technology.

Advances in genetic engineering of antibodies have led to the development of many recombinant monoclonal antibodies (mAb) highly specific to many targets. In antibody engineering and production, several antibody selection platforms such as phage/ribosome and mRNA/microbial cell displays,[1, 2] flow cytometry,[3] and protein arrays,[4] etc. have been employed to isolate potential antibodies for maturation and to build an antibody library for target binding. As an outcome of the selection process, a mixture of antibody clones needs to be individually analyzed to obtain certain target binding properties. Additionally, many mAbs developed using traditional means (i.e. hybridomas) are commercially available yet vendors provide little information on antibody affinity. Thus, the burden to evaluate mAb affinity and specificity falls on the user to identify the most appropriate mAb candidate for a particular study, and the ability to rank the affinity of mAbs in addition to specificity towards the molecule of interest becomes essential.

Enzyme–linked immunosorbent assay (ELISA) is a primary method to screen the specificity and affinity of mAbs.[2] ELISA has many attributes such as sensitivity due to enzyme driven amplification, and low cost of analysis;[5, 6] however, it possesses certain limitations. For example, ELISAs require multiple steps of incubation and washing cycles that are labor–intensive and time–consuming. Moreover, results are often irreproducible and matrix dependent because plasma constituents often impact enzyme activity. A single ELISA assay usually takes up to 24 h for analysis; a major portion of assay time is consumed by incubation due to small diffusion coefficients of biomacromolecules.

Additional challenges may be encountered when an ELISA is used to screen antibodies to be incorporated into emerging AuNP-based immunoassays. Conjugation of the antibody to AuNPs may affect the bioactivity of the antibody which will not be detected by ELISA. Thus, ELISA may not accurately select for the most suitable antibody in AuNP-based immunoassays. An alternative method to screen and characterize mAbs that overcomes the limitations of ELISA is needed.

Herein a rapid screening method for determining antibody-antigen binding specificity and affinity was developed using AuNPs and dynamic light scattering (DLS). Briefly, AuNP probes are produced by the immobilization of antibodies onto AuNP. The probes are then mixed with the specimen, and the presence of the target antigen induces aggregation of the AuNP probe. The formation of aggregates is detected as an increase in hydrodynamic diameter by the DLS instrument.[7-12] DLS detection

of agglutination assays offers many advantages over the more conventional colorimetric readout; most notably, DLS provides much better detection limits and higher sensitivity.[8, 12] To develop the DLS-based mAb screening method and establish proof-of-principle, four anti-influenza virus monoclonal antibodies were directly adsorbed onto the surface of AuNP. Each of the antibodies was developed against influenza virus A/New Caledonia/20/99 and directed towards the H1 hemagglutinin, a surface protein on the virus. Next, a series of dilutions of the corresponding antigen (influenza virus A/New Caledonia/20/99 (H1N1)) was mixed with the AuNP probes. Calibration curves for each of the antibodies were constructed to rank the specificity and affinity of their binding. Owing to single–step and wash–free procedure, the screening time using DLS was significantly reduced to 30 min in comparison to 24 h by ELISA.

Experimental

Reagents

Gold nanoparticles (60 nm; 2.6 × 10^{10} particles/mL) were purchased from Ted Pella, Inc (Redding, CA). Phosphate buffered saline (PBS) was purchased from Thermo Scientific (Logan, UT). Borate buffers were prepared from sodium borate obtained from Fisher Scientific (Fair Lawn, NJ). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO).

Antibodies

Mouse monoclonal anti-influenza A antibodies (InA4, InA16, InA88, and InA97) specific to native HA from influenza virus A/New Caledonia/20/99 (H1N1) were purchased from Novus Biological. The antibodies were purified by protein A affinity chromatography and supplied in PBS, pH 7.4.

Viruses

Human influenza virus isolates (both H1N1 subtypes) A/New Caledonia/20/99 and A/Puerto Rico/8/34 were grown in 9 to 11-day old embryonated chicken eggs for 48 to 72 hours at 37 °C. Fertile eggs were obtained from a flock of specific pathogen-free leghorn chickens (Merial Select, Gainesville, GA and Sunrise Farms, Catskill, NY). Allantoic fluid from infected eggs was then collected and pooled for each strain, divided into aliquots, and stored at -80 °C. The 50% tissue culture infectious dose ($TCID_{50}$) of the stock viruses was determined by the Reed and Meunch method on MDCK cells.[13] Two independently prepared virus samples were used, one was propagated in-house (UIUC) and a second stock was graciously provided by collaborators at the University of Georgia (UGA). A/New Caledonia/20/99 (UGA) titer was 1.75 × 10^7 $TCID_{50}$/mL and A/New Caledonia/20/99 (UIUC) was 3.00 × 10^5 $TCID_{50}$/mL. A/ Puerto Rico/8/34 titer was 3.70 × 10^7 $TCID_{50}$/mL.

Optimization of pH for AuNP-mAb conjugation

100 µL of 60 nm gold nanoparticle suspension was added to separate 0.50 mL microcentrifuge tubes (5 tubes in total). The pH of the colloidal gold sol was adjusted to 5.5, 6.5, 7.5, 8.5, and 9.5 by adding 4 µL of 50 mM phosphate buffer pH 5.5, 6.5, and 7.5, and borate buffer 8.5 and 9.5 into each tube, respectively. The antibody (30 µg/mL) was mixed with the pH adjusted suspension 15 min. DLS was used to monitor the hydrodynamic diameter of the particles. 10 µL of 10% NaCl was added to each tube to verify the stability of AuNP conjugates in saline environment. DLS measurement was conducted again to determine the appropriate pH for stabilization.

Optimization of Ab concentration for AuNP-mAb conjugation

100 µL of 60 nm gold nanoparticle suspension was added to separate 0.50 mL microcentrifuge tubes (11 tubes in total). The pH of the colloidal gold was adjusted to the optimal pH by adding 4 µL of 50 mM borate buffer at the appropriate pH into each tube. Different amounts of the antibody were added into each tube to obtain a wide range of concentrations (namely, 0, 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0 µg/mL). The solutions were mixed well. After 15 min, the hydrodynamic diameter of antibody-conjugated AuNPs was measured via DLS. Next, 10 µL of 10% NaCl was added to each tube to verify the stability of AuNP conjugates in saline environment.[14] The changes in size of the particles were measured again by DLS. The amount of antibody added at the stabilization point plus 10% should be used to produce the final antibody-AuNP conjugate.[15]

Preparation and characterization of Ab-AuNP

4 µL of 50 mM borate buffer (at the optimal pH for the antibody adsorption) was added to 100 µL AuNP to adjust the pH. The stabilization amount of mAb plus an additional 10% was added to the AuNP for 15 min. 33.3 µL of 1% BSA in borate buffer or phosphate buffer (at the optimal pH for the antibody absorption, 2 mM) was added to bring the concentration of BSA to 0.25% in the Ab-AuNP suspension. BSA helps to further stabilize the sol against aggregation and also blocks nonspecific binding sites. After 5 min, excess antibody was removed via centrifugation at 5000 rpm for 5 min. The conjugate was resuspended in 100 µL PBS (pH 7.4, 10 mM) with 0.25% BSA to mimic physiological conditions and promote mAb-virus binding.

Immunoassay protocol

A previously described procedure for the assay was used with slight modifications.[8] Four-fold serial dilutions of virus stocks were prepared in 10 mM PBS (pH 7.4). A total of 90 µL of virus dilutions were added per well of a 96-well round-bottom microliter plate (Corning, Corning, NY). PBS served as negative control. 10 µL of antibody-modified gold nanoparticles made by the above procedure was added to each well and allowed to incubate for 30 min at room temperature. The AuNP reagent/sample mixture was then transferred to a 70 µL small volume disposable cuvette (Eppendorf, Germany) for DLS measurement.

DLS measurement

A BI-90Plus (Brookhaven Instruments Corporation, NY) equipped with a 658 nm laser and avalanche photodiode detector (Perkin) was used to measure hydrodynamic diameters of AuNP for all DLS measurements. The backscattered light collection angle was set at 90°. Each sample was analyzed in triplicate and each measurement was an average of three 30-s runs. Data were collected and analyzed using MAS OPTION particle sizing

software. Hydrodynamic diameters were referred to as the effective diameter by cumulants analysis.

ELISA

Virus (diluted 1:100) or PBS (negative control) was added 100 µL per well in a 96 well Immulon 2HB microtiter plates and incubated 18 hours at 4 °C. Wells were washed and then blocked with BSA/non-fat milk. Serial dilutions (1:4) of anti-influenza mAbs were applied to pre-adsorbed plates, starting with a dilution of 1:100. After washing, HRP labelled goat anti-mouse IgG (Thermo-Fisher) was applied as the secondary antibody 1:1000 to all wells. After the final washing step, 1-step Turbo TMB ELISA (Thermo-Fisher) was added for the substrate and the reaction was stopped with 1 M H_2SO_4. Absorbance was measured at 450 nm on a 96-well format plate reader (SpectraMax Gemini reader and SpectraMax software). Absorbance readings for mAbs against PBS were subtracted from absorbance readings for mAbs against virus and plotted.

Results and Discussion

Adsorption of proteins onto gold nanoparticles

One major concern relating to any AuNP–based immunoassay is the stability of antibody-AuNP conjugates in biological environments of high ionic strength. Antibodies can be immobilized onto a gold surface via a cross-linker or directly adsorbed to the surface. Regardless of the immobilization strategy, the conjugate needs to be protected from salt-induced aggregation.[16, 17] In this study, direct adsorption was applied. Direct adsorption to form antibody-AuNP conjugates has historical precedence and is a straightforward method requiring minimal expertise in synthesis; thus, this method is easily adopted by a broad audience to screen antibodies. The behaviors of different subclasses of antibody in the direct conjugation are distinct.[14] The direct adsorption of proteins onto the gold surface is a complex process dependent on several parameters such as the concentration, isoelectric point of protein, bound fraction of segments, ionic strength, and pH.[9, 15, 18] However, it is not practical to simultaneously study all the factors for the optimization of this process. Herein DLS was employed to investigate the immobilization of mouse monoclonal IgG1 (InA4 and InA97) and IgG2 antibodies (InA88 and InA16) on gold nanoparticles primarily in relation to pH and concentration of the protein; the other parameters will be correspondingly discussed.

The pH has great influence on the hydrogen bonds and overall charge of the biomacromolecule. Extremely high or low pH can cause a dramatic change in molecular configuration and perhaps its bioactivity. Therefore, the pH range selected for this study was 5.5 to 9.5, which minimizes the likelihood of damage to the molecular activity. It is well established that the pH slightly above or equal to isoelectric point of the biomolecule is the optimal pH for protein adsorption.[18] However, we suggest that pH dependent study is more broadly applicable since the surface charge distribution is necessary to be taken into account. Zhang S. et al. 2014 indicated that proteins and AuNP can be alike in charge at a certain pH and still interact with each other, e.g. negatively charged BSA, can still interact with citrate–capped gold particles via its positive patches.[19]

Previous studies by our group and others suggest 30 µg/mL

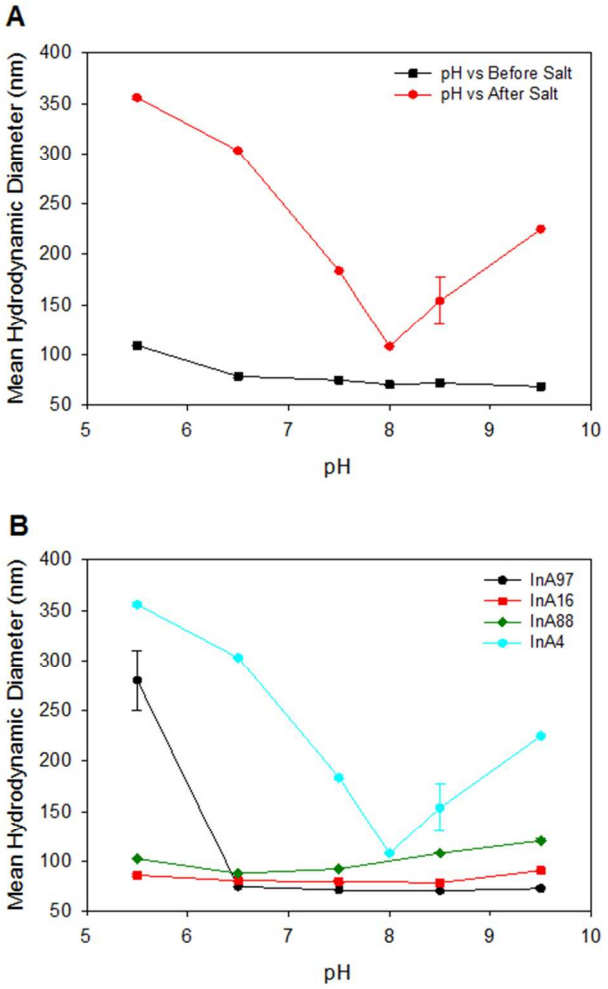

Fig. 1. DLS aggregation curves to assess pH-dependent adsorption of mAb onto AuNP, the mAb concentration is fixed at 30 µg/mL. (A) Mean hydrodynamic diameter of InA4-AuNP conjugate as a function of pH before and after addition of 150 mM NaCl. (B) Mean hydrodynamic diameter of four mAb-AuNP conjugate as a function of pH after addition of 150 mM NaCl.

IgG will fully coat AuNP.[9, 20] Therefore, as a starting point to investigate pH dependent adsorption, 60 nm AuNP were mixed well with each antibody (30 µg/mL) at the adjusted pH. DLS was used to measure the mean hydrodynamic diameter (D_H) and monitor antibody adsorption. The adsorption curve for InA4, plotted as D_H versus pH is displayed in Fig. 1A. The D_H of unconjugated AuNP measured 60 nm and the adsorption of the antibody caused the D_H to increase by 10 nm to 40 nm, depending on the pH, relative to the unconjugated AuNP. We have previously demonstrated that D_H increases by ~20 nm when the AuNP is fully saturated with a layer of antibody.[9] Thus, we speculate that at pH 5.5, in which the D_H increased by 40 nm, the charge of the antibody was sufficiently positive that the antibody itself destabilized the AuNP to induce aggregation. Conversely, at pH 9.5, in which the D_H only increased by 10 nm, the antibody had sufficient negative charge that a full monolayer of antibody was not adsorbed on the negatively charged citrate-capped AuNP. NaCl was then added to a final concentration of 1% to confirm antibody adsorption and establish stability of the conjugate in a solution of high ionic strength. Fig. 1A shows the D_H as a

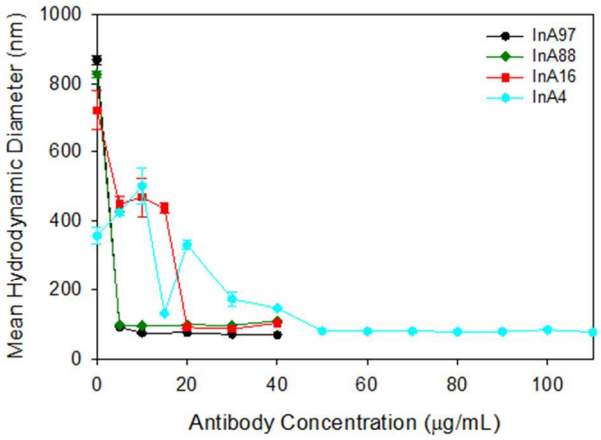

Fig. 2. Mean hydrodynamic diameter of mAb-AuNP conjugates as a function of mAb concentration after the addition of 150 mM NaCl.

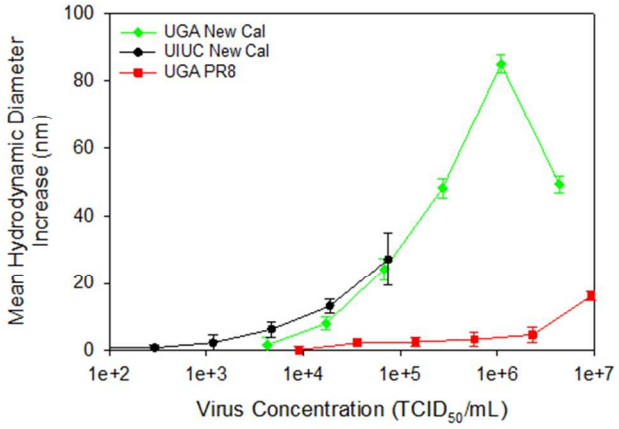

Fig. 3. DLS response curves to evaluate the specific binding of influenza A viruses with InA97-AuNP probes. Two assays were performed on independent preparations of New Caledonia virus and one assay was performed on the PR8 strain of influenza virus.

function of pH for the InA4-AuNP conjugate after the addition of NaCl. It is evident that at the extreme pHs, e.g., 5.5 and 9.5, not enough antibody adsorbed onto the AuNP to render the particles stable. However, at pH 8.0 the InA4-AuNP conjugates were least affected by the electrolytes and the data confirm complete antibody adsorption for stability. Additional antibody-AuNP conjugates were prepared with three other antibodies and the coagulation curves for the conjugates in a saline environment are provided in Fig. 1B. The adsorption of IgG1 antibodies (InA4 and InA97) were highly unstable at pH lower than 6.5. InA97 was quite stable at pH higher than 6.5 whilst InA4 reached the smallest diameter of 107 nm at only pH 8.0 (the expected diameter of AuNP-InA4 is ~80 to 90 nm). It is worth mentioning that the stability of InA4-AuNP conjugate was not obtained at the optimal pH since the adsorption also relies on a sufficient amount of antibody required for full protection. This will be further discussed in the following section. As for IgG2 (InA16 and InA88), the stability of nanoparticles after adsorption did not undergo such a dramatic change for the tested pH range (Fig. 1B). The adsorption of IgG2 was optimized at pH 6.5-7.5. These results showed good agreement with the optimal pH for IgG subtypes adsorbed on gold nanoparticle concluded by William D. G. et al. 1980.[14] Moreover, as previously noted, protein adsorption is maximized when the pH is adjust slightly above or equal to the protein pI.[18] The pI of IgG1 antibodies ranged from 7.5-8.0 while the pI of IgG2 antibodies ranged from 6.6-6.9 as determined by isoelectric focusing. Thus, the pI data is consistent with the optimal pH for conjugation determined by the pH-dependent coagulation curves in Fig. 1.

In addition to pH, the adsorption and subsequent stabilization of gold nanoparticles are also affected by antibody concentration. To determine the minimum amount of antibody required for adsorption and stabilization of AuNP, various amounts of antibody (0-110 µg/mL) were incubated for 15 min with AuNP adjusted to the optimal mAb-dependent pH, followed by the addition of NaCl. DLS was then used to measure the D_H as a means of evaluating AuNP-antibody stability (Fig. 2). At optimal pH, InA97 and InA88 only required 5.0 µg of antibody per ml of AuNP to protect against salt-induced aggregation and the stability was also maintained at higher concentrations of the antibody. A slightly greater concentration of InA16 was required for stabilization (20 µg/ml) while InA4 required 50 µg/mL to stabilize the gold suspension. It is likely that InA97, InA16, and InA88 have confirmations that form more contacts with the gold surface so that the surface rapidly reaches saturation by these antibodies at lower coverage. On the other hand, InA4 has fewer contacts to the gold surface per molecule.

It was reported that the addition of 110% of the minimum amount of antibody is sufficient for conjugate stabilization.[15] In general, it is more favorable to use secondary stabilizer such as BSA to obtain the desired stability of the colloidal gold conjugation in saline environment for longer storage time due to steric stabilization created by the adsorbed layer.[18, 19] Therefore, to prepare AuNP probes for use in DLS assays the AuNP suspension was adjusted to the optimal pH, the requisite amount of antibody was mixed with colloidal gold, and 0.25% BSA was added to the suspension without the removal of excess antibody. Centrifugation before adding BSA may bring the antibody-AuNP proximal to each other and cross-link therefore is not recommended. The unbound antibodies were then removed by centrifugation three times and the colloidal gold nanoparticles were resuspended in PBS (10 mM) buffer containing 0.25% BSA. No significant increase in size indicated that the conjugate was stable in the saline solution. Based on a geometric analysis of the surface area of a 60 nm AuNP and the footprint of an antibody (7-10 nm in diameter), we estimate a maximum surface coverage of 150-290 antibodies per AuNP for the fully coated AuNP. Bradley et al. experimentally measured and reported the adsorption of 190 antibodies per 60 nm AuNP when relying on direct adsorption to the nanoparticle surface.[21] The stable, fully coated conjugates were stored at 4 °C for 5 days without aggregation or a loss in activity.

Validating DLS assay to monitor antibody–antigen binding

To establish this platform as a means of monitoring antibody-antigen interaction, AuNPs were modified with the mAb InA97 using the optimized conjugation procedure detailed in the previous section. The InA97-AuNP probes suspended in PBS measured 82.9 nm in diameter via DLS, consistent with the

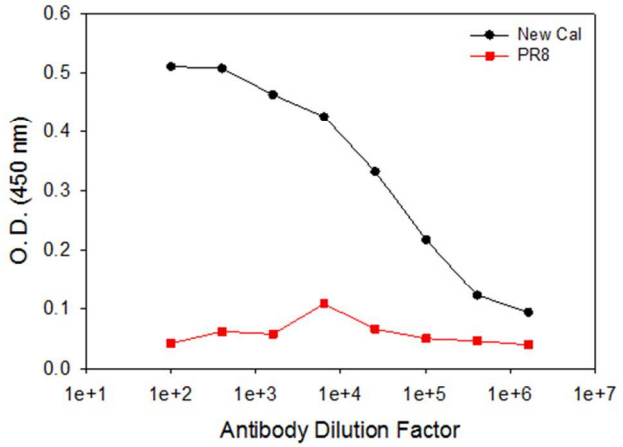

Fig. 4. ELISA results to evaluate InA97 binding specificity towards New Caledonia and PR8 strains of influenza A viruses.

expected size of a 60 nm AuNP coated with a protective IgG layer. The InA97–AuNP probes were mixed with dilutions of influenza A/New Caledonia virus or PBS, e.g. negative control, for 30 min and the mean hydrodynamic diameter (D_H) was measured via DLS. The calibration curve, constructed as a plot of D_H increase versus virus concentration, is displayed in Fig. 3. Fig. 3 shows a detectable increase in D_H relative to the D_H of the InA97-AuNP probe at a New Caledonia virus concentration of 10^3 $TCID_{50}$/mL. Moreover, the increase in D_H correlates with an increase in New Caledonia virus concentration reaching a maximum value at a virus concentration of 10^6 $TCID_{50}$/mL. The "hook effect" was observed at the highest concentration of New Caledonia virus as a decrease in diameter. This phenomenon was previously reported and discussed in detail for DLS assays.[8, 12]

The robustness of the assay was evaluated by analyzing a second, independently prepared New Caledonia virus stock. This virus was propagated using the same procedure and yielded a viral titer of 3.00×10^5 TCID50/mL. InA97-AuNP probes were mixed with 4-fold dilutions of this New Caledonia stock and DLS was used to measure the formation of aggregates resulting from virus-antibody binding. The results are displayed in Fig. 3 and the measured DH provided a similar concentration-dependent response to that obtained for the original virus stock.

Upon mixing the InA97-AuNP probes with influenza A/Puerto Rico/8/34 virus, no significant aggregates were detected via DLS (Fig. 3). It is worth noting that a slight increase in D_H was measured for InA97 probes when mixed with the highest concentration of influenza A/Puerto Rico/8/34 virus. It is possible that this increase is due to a specific interaction between this strain of influenza virus and InA97, albeit very weak (low K_a). However, it is more likely that the increase in D_H at this virus concentration is caused by the matrix. While AuNP is highly efficient at light scattering and is the primary source of light scattering in the DLS measurement,[22, 23] the high concentrations of large particulate in undiluted allantoic fluid is likely the cause of the measured increase in D_H for this concentration. Thus, we conclude that the InA97 mAb selectively binds influenza A/New Caledonia virus and does not have an affinity for influenza A/Puerto Rico/8/34 virus.

ELISA is the gold standard platform for testing antibody-

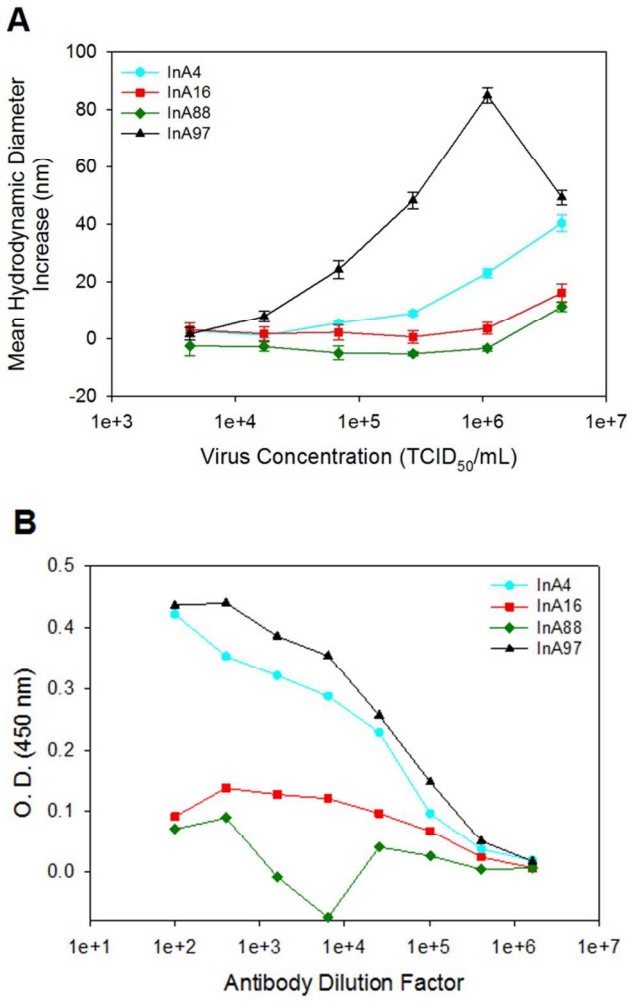

Fig. 5. Evaluation of antibody binding to influenza A New Caledonia virus. (A) DLS response curves to evaluate the specific binding of influenza A New Caledonia virus with five Ab-AuNP probes. (B) ELISA.

antigen binding. Therefore, an ELISA was performed to evaluate the binding of InA97 to New Caledonia and PR8 viruses, and the results were compared to the DLS-derived binding specificity results. The ELISA results are presented in Fig. 4. ELISA confirmed that InA97 specifically binds to New Caledonia; however, InA97 does not have a specific interaction with PR8 as minimal binding of the antibody is detected even for high antibody concentrations. These results are in agreement with the DLS results for InA97 binding specificity, and serve as validation of the DLS platform.

Screening and evaluating antibody–virus binding specificity

The selection of the antibody is critical to any antibody-based detection method. The assay performance is governed by the antibody-antigen binding; thus, it is essential to understand the specificity and affinity of this interaction. To this end, the DLS assay was explored as a potential candidate for rapidly screening antibody-antigen specificity and affinity. The antibody-antigen binding assay is reduced to 30 min using the DLS assay compared to 24 h using ELISA. Moreover, the DLS assay may be better suited for rapidly screening antibodies intended for use in AuNP-based immunoassays. During conjugation to AuNP

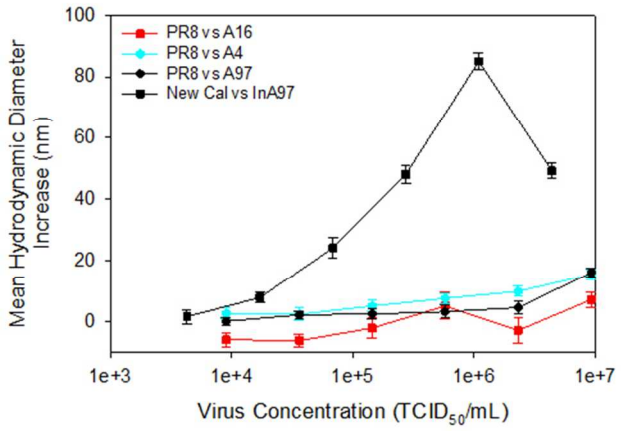

Fig. 6. DLS response curves to evaluate the specific binding of influenza A PR/8 virus with four Ab-AuNP probes. The DLS curve for the binding of InA97 to New Caledonia is included as a reference to illustrate specific binding.

conformational changes may affect the antibody bioactivity relative to the free antibody that is evaluated for binding in the ELISA format.[24] Thus, this novel DLS-based screening method may be a better alternative to ELISA with respect to time and effectiveness.

Three additional monoclonal anti-influenza antibodies InA4, InA16, and InA88 were investigated to evaluate specific binding interactions with intact influenza A/New Caledonia/20/99 (H1N1). All four antibodies were developed using influenza A/New Caledonia/20/99 as the immunogen and the vendor advertises the antibodies as broadly cross-reactive with H1 subtype influenza viruses. Fig. 5A shows the DLS response curves of the four antibody-AuNP probes incubated with 4-fold dilutions of the New Caledonia strain. The extent of aggregation was used to evaluate the interaction between the antibody and the virus. Only InA97 and InA4 antibodies were found to specifically bind to New Caledonia virus. The assay suggests that InA97 had a greater affinity toward the New Caledonia virus strain compared to InA4, given that aggregation of the InA97-AuNP probes was detected at a lower virus concentration than for the InA4-AuNP conjugates. Interestingly, no increase in D_H was measured for the antibody-AuNP conjugates for the other two antibodies, InA16 and InA88. The small increase in D_H a the highest concentration of virus was similar for each antibody probe and was likely due to particulate in the undiluted matrix as discussed above. These data suggest that these two antibodies do not specifically bind New Caledonia virus and is in contrast to the expected results as both of these antibodies were also developed with New Caledonia as the immunogen.

One explanation for the unexpected results is that the InA16 and InA88 antibodies lost bioactivity upon adsorption to the AuNP, but in the unconjugated state do specifically bind to New Caledonia virus. To test this possibility each antibody was evaluated using an ELISA and the results were directly compared to the DLS assay. The ELISA results are presented in Fig. 5B. The direct correlation between the mAb dilution and absorbance demonstrate that InA4 and InA97 are the only antibodies that specifically bind New Caledonia virus. These results are consistent with the DLS results, provide evidence that the lack of InA16 and InA88 antibody affinity to the immunogen is not a result of conjugation to the AuNP, and ultimately validate DLS as a rapid and effective platform for screening antibody-antigen binding.

The DLS assay was also conducted using the three antibody probes and influenza A/PR/8 virus to determine the antibody specificity towards a different H1N1influenza virus. The DLS response curves are plotted in Fig. 6, along with one New Caledonia calibration curve as a point of reference. As is evident in the data, no significant binding of these antibodies to PR8 was detected. While PR8 is the same subtype as New Caledonia, it is not surprising that the antibodies do not bind PR8 as it was not the immunogen used to develop the antibodies. It is probable that the epitopes to which InA97 and InA4 bind are specific to the New Caledonia strain and not conserved across all H1 subtype influenza viruses.

Conclusions

In this investigation, we demonstrate a simple, rapid, and cost–effective method to screen specificity of antibodies in a single–step homogeneous assay using mAb–AuNP probes and DLS. This novel method offers a significant improvement in terms of screening time compared to ELISA assays, while providing the same accurate results as the conventional method. This platform could be easily implemented in most laboratories to select antibodies for a wide variety of targets. This screening method has the potential to expedite the development and optimization of antibody-based diagnostics and antibody therapeutics. In addition, a straightforward protocol to synthesize antibody-AuNP conjugates was presented.

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Notes and references

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Rapid Screening of Antibody-Antigen Binding using Dynamic Light Scattering (DLS) and Gold Nanoparticles

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TOC ABSTRACT

Antigen-mediated aggregation of antibody-gold nanoparticle conjugates is detected with dynamic light scattering to evaluate antibody-antigen binding.