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Octet® Bio-Layer Interferometry as a Tool for Determining Nanoparticle Vaccine Construct Design, Stability and Antigenic Efficiency

Nilshad Salim, PhD, Sartorius, Fremont, CA

Correspondence
Email: octet@sartorius.com

Abstract

Nanoparticle (NP) vaccine and antigen delivery platforms have emerged as a promising approach due to their ability to interact with immune components and induce humoral and cellular immune responses. NP arrangements have also been shown to increase antigen stability and to provide greater flexibility allowing the incorporation of diverse antigen conformations and immunostimulants compared to traditional vaccines. However, NP formulations' optimal performance depends on antigen presentations and nanoparticle physicochemical properties such as core size, charge, surface properties, and chemical compositions. In this article, we review a few selected examples to showcase how Octet® Bio-Layer Interferometry (BLI) platforms can be used to optimize NP attributes such as polymer lengths, size antigen spacing, presentation, and stability to select ideal NP vaccine candidates based on binding activity and target recognition. Additionally, we discuss how quantitation capabilities on the Octet® system are used to determine downstream vaccine efficacy by measuring NP vaccine-derived humoral responses.

Vaccination is currently the most cost-effective and efficient strategy to control infectious diseases and is a major topic of discussion during the current COVID-19 pandemic. Lack of an effective vaccine towards COVID-19 has already cost worldwide economies trillions of dollars and counting. While numerous concerted efforts by private industries and governments are underway to develop vaccines and biotherapeutics to control the current pandemic, this has also highlighted the need to shorten traditional timelines to produce a vaccine successfully. Therefore, it is essential to focus on resolving current challenges in vaccine development to respond better to a future pandemic.

While numerous vaccines have allowed controlling infectious diseases such as rubella, yellow fever, polio and measles, many diseases still lack an effective vaccine. Some of the main challenges in developing vaccines against certain pathogens are antigenic variability (HIV), availability of multiple serotypes or genotypes (Dengue), or the scarcity of the adjuvants and delivery systems suitable for human applications. Nanoparticles (NPs) have emerged as a promising approach for vaccine delivery and as an antigen delivery platform due to its ability to interact with immune components and induce humoral and cellular immune responses. Additionally, its amenability to being successfully administered through different routes and the ability to easily control physicochemical properties such as size, shape, charge, surface chemistry and composition provides additional flexibility in vaccine development for generating immune responses. NPs are also known to increase antigen stability (heat, proteolysis) and provide substantial flexibility to vaccine formulation, allowing the incorporation of diverse antigens and immunostimulants, compared to conventional subunit/adjuvant vaccines. To date, about 30 nanoparticle medications have been approved by the Food and Drug Administration (FDA) or the European Medicines Agency (EMA) and over 75 new nanoparticles have entered clinical trials in just the past three years[1]. In addition, Moderna's mRNA based COVID-19 vaccine that is currently on clinical trials utilizes a nanoparticle delivery system while Novavax is developing a nanoparticle-based vaccine for COVID-19[2].

Development of NP vaccine or immunostimulant constructs that often display ligands in multimeric arrangements may require identifying optimal NP physiochemical properties and ligand display conformations (polymer length, spacing, orientation, etc.) for best target recognition. Several studies have documented both NP core size and ligand conformations to be important determinants in NP construct design[3-6]. Binding affinity and kinetics can be a reliable indicator for optimal ligand presentation, spacing and accessibility and NP ligand stability.

As described in this short review using a few selected examples, Octet® BLI platforms can serve as an invaluable tool in NP development workflows providing several measurable NP quality attributes such as binding affinity and kinetics, stability and quantitation of humoral responses exerted by NP vaccines. Unlike traditional surface plasmon resonance (SPR) biosensor systems, Octet® systems do not use fluidics systems to transfer samples to the biosensors. This provides users greater flexibility in the types of samples that can be used in experiments, such as cell lysates or aggregation-prone formulations, without fluidic clogging concerns or the requirement of extensive sample preparation procedures. Additionally, Octet® platforms enable simultaneous analysis of data from up to 96-biosensors that allow for rapid screening, characterization and assay development capabilities. Octet® instruments operate using Dip and Read Biosensors where the biosensor travels to samples housed in microwell plates. Due to the lack of sample movement out of the microplate, samples can often be reused for other experiments or analysis in low sample availability situations. Dip-and-read biosensor architectures also enable Octet® systems to be readily utilized as a robust and convenient immunoassay quantitation platform for the determination of antibody and virus titers in vaccine development workflows. This document highlights the versatile use of Octet® label-free binding assays in NP vaccine development workflows using a few published examples. Octet® binding assays were adapted for optimizing nanoparticle core size and surface properties to maximize antigen accessibility[7], to determine efficient antigenic constructs displayed on NPs[8,9], antigen stability in NP displays[10] and as a tool to measure humoral responses trigged by NP vaccine candidates[11]. These are in addition to the vast applications space where Octet® platforms can be utilized for research involving analytes from small-molecules, proteins, peptides, nucleic acids, liposomes, VLP to cells, adding value to any research or bioprocess lab as a multipurpose tool.

Learn More About Octet® Technology and Applications

Determination of Antigenic Activity from HIV Spike Conformations Displayed in Nanoparticles

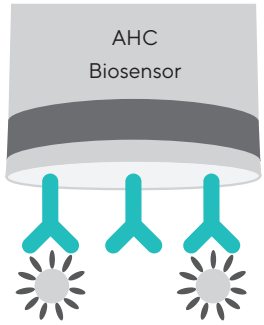
A vaccine for HIV-1 that produces broadly neutralizing antibodies (bNAbs) in naïve individuals is highly desirable. Several studies have isolated a broad range of bNAbs from HIV-1 infected individuals exposing multiple vulnerabilities on the HIV-1 envelope (Env) glycoproteins. In HIV-1, the Env trimeric structure is composed of heterodimers, each containing a receptor-binding protein (gp120) and a transmembrane fusion protein (gp41) that constitutes the

viral spike conformation. The trimeric conformation is deemed critical for robust humoral responses as vaccines designed based on soluble trimers alone have shown poor humoral responses. He *et al.,* reported the multivalent presentation of HIV-1 spikes in 24-meric ferritin (FR) and two 60-meric lumazine synthase (LS) and dihydrolipoyl acetyltransferase (E2p) nanoparticles that can mimic native-like conformations and trigger B-cell activation as a vaccine strategy[8]. This study indicated for the first time that V1V2 and gp120 can form native-like trimeric spike conformations on FR and E2p NPs as potential HIV-1 vaccine antigens.

The antigenic efficiency of these NP presentations was measured *in vitro* using an Octet® binding assay. To test if the NP constructs based on V1V2 and gp120 fused to N-terminus of the FR subunit can mimic native trimeric recognition, a nanoparticle-IgG binding assay was utilized using antibodies PG9 and PGDM1400 that were reactive towards specific trimeric conformations. PG9 recognizes V1V2 in both monomeric and native trimeric states whereas PGDM1400 is sensitive to only native-like trimeric antigen presentations. Therefore, the binding activity of NPs with a variety of antigen presentations to PG9 and PGDM1400 can indicate if these presentations are closely related to monomeric or native-like trimeric conformations. NP-IgG binding assays were constructed using anti-human Fc (AHC) Biosensors where IgGs were immobilized and NP formulations were titrated for binding assessment (Figure 1A). Octet® assays validated that V1V2 monomers bound to PG9 with low affinity and had no detectable binding to PGDM1400, as expected. However, when presented V1V2 in NPs (multivalent presentations), sub-nanomolar binding affinities were measured to both IgGs albeit with notably different kinetics. The authors attributed faster on-rates observed for V1V2-NPs binding to PGDM1400 compared to PG9 to well-formed trimeric conformations efficiently recognized by the PGDM1400 antibody. Additionally, reduced antibody binding affinities were observed with V1V2sht NPs compared to V1V2Ext NPs. Here the V1V2sht NP construct represents the use of truncated V1V2 antigens. Octet® kinetic assays were able to quantitate the consequential reduction in binding due to the shortened presentation of the V1V2.

One of the key aspects of assay development involving NPs as analytes is biosensor loading optimization due to the larger analyte size. Higher density ligand loading can lead to steric hindrance diminishing the NP analyte's ability to access the immobilized ligand. An example ligand loading scouting experiment is shown in Figure 1B and 1C. Figure 1B indicates a simple ligand loading assay setup where the IgG immobilization concentrations are varied, starting from 5 µg/mL. A single NP concentration is then used to assess loading conditions. Analyte concentration for loading

A



B

Biosensor	Assay Buffer	IgG (Ligand)	Nanoparticle
A5	Buffer	5 µg/mL	25 nM NP
B5	Buffer	2.5 µg/mL	25 nM NP
C5	Buffer	1.25 µg/mL	25 nM NP
D5	Buffer	0.625 µg/mL	25 nM NP
E5	Buffer	0.312 µg/mL	25 nM NP
F5	Buffer	0.156 µg/mL	25 nM NP
G5	Buffer	0.078 µg/mL	25 nM NP
H5	Buffer	0.039 µg/mL	25 nM NP

C

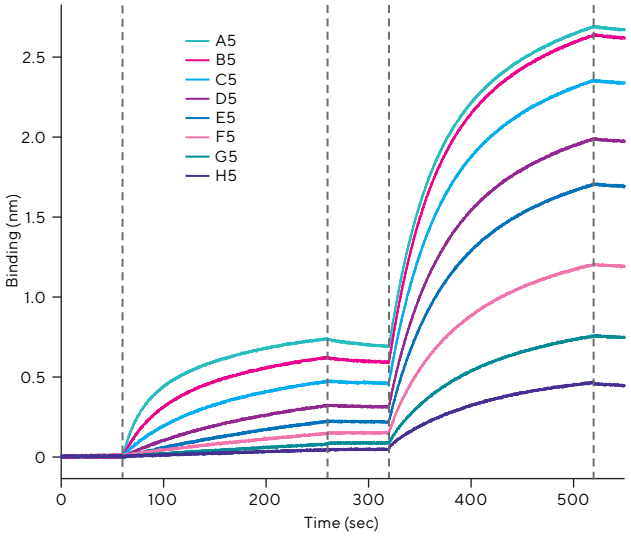


Figure 1: IgG - Nanoparticle binding assay development. A critical step in NP binding assay development is optimizing ligand loading levels. Due to the larger size of NPs, higher densities can cause steric crowding on the biosensor inhibiting NP access to ligand binding sites. (A) Assay workflow is depicted where a human IgG is immobilized on Anti-human Fc (AHC) Biosensors followed by NP binding. (B) Assay sample plate map for ligand concentration scouting experiment where 25 nM of NP binding is tested against varying loading concentrations of IgG starting at 5 µg/mL. (C) Representative data from the scouting experiment. The top two ligand concentrations indicated signal saturation and should not be considered. Ligand concentrations of 0.312 µg/mL or 0.156 µg/mL can be selected as an optimal IgG loading concentrations.

scouting is typically selected based on the approximate K_D, which can be anywhere between 1 to 5 x K_D. As depicted in Figure 1C, the top two loading concentrations reach response saturation at 25 nM NP concentration and are not ideal despite providing a higher signal. An acceptable loading concentration from this assay would be either the 0.312 µg/mL or 0.156 µg/mL. The optimized loading concentration can now be used to titrate a NP concentration series for affinity and kinetic determination.

Octet® Screen to Assess Optimum Nanoparticle Core Size and Polymer Linker Lengths for Antigen Recognition

To understand the recognition of influenza hemagglutinins (HA) to multivalent sialic acid isomers in host receptors, Richards *et al.*, developed a sialic acid nanoparticle system. While all HAs are known to bind sialic acids, human influenza targets terminal α2,6-sialic acids and avian influenza targets α2,3- sialic acids in host receptors. For interspecies transmissions (zoonosis), HAs must be able to adapt to these or any other variations in host glycans. As such, a platform that can mimic host antigen presentations and test variations can help elucidate virus receptor recognitions that contribute to zoonosis. The binding affinity of hemagglutinin towards an individual sialoside is reported to be ~2 mM[12]. However, the biologically relevant interaction takes place at a vastly different landscape with hemagglutinins displayed at ~200–1000 copies per virus, binding to a dense layer of sialic acids in host cell surfaces significantly increasing the overall affinity of the interaction by the *cluster glycoside effect*[13]. Therefore, a proper *in vitro* representation of the interaction would have to be established to mimic the cellular complexity and understand receptor recognition by Influenza hemagglutinins. Therefore, in this work, sialic acids were assembled onto NPs to imitate a multivalent environment and create a platform to understand and predict sialic acid interactions and zoonosis in a range of influenza viruses.

While NPs allows for presenting ligands in multivalent configurations that increase the density and avidity binding effects, this can also sterically hinder access to ligands and increase aggregation propensities. Therefore, to identify suitable NP formulations for measuring sialic acid interactions with HA, a library of NP configurations was screened that differed in core particle size and linker lengths. The linker lengths were controlled by varying degrees of polymerization (DP) and NP size was controlled by kinetic seed growth[7]. Three different NP sizes together with nine different polymer lengths where generated that constituted a library of 27 multivalent glyconanoparticles. First, the colloidal stabilities of NPs in buffers were measured using a colorimetric aggregation assay. Aggregation can often lead to false-positive binding data by increasing non-specific binding accumulation on biosensors. NP aggregation was measured in NaCl and HEPES buffer gradients (0–1 M). NP formulations that did not show aggregation propensities were moved forward to test for optimal NP size and polymer linker lengths using an Octet® binding assay (Figure 2).

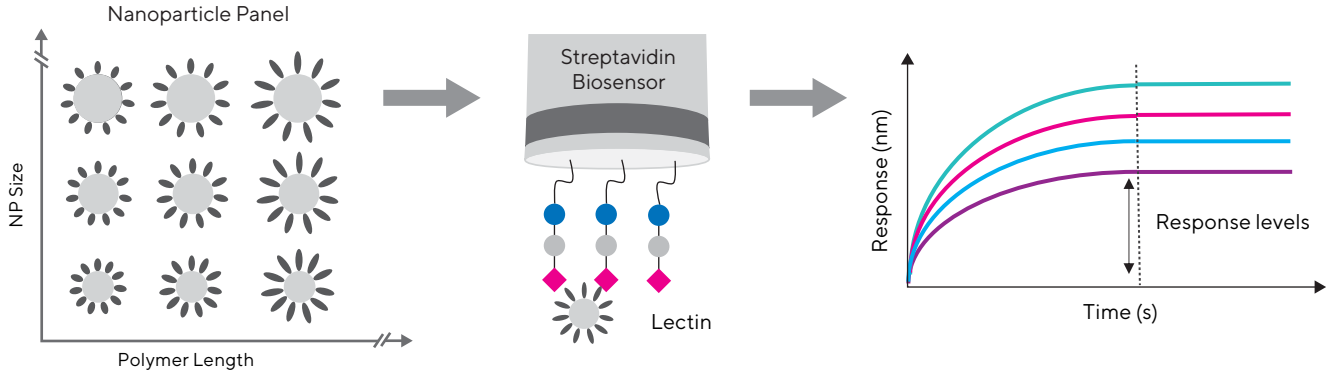


Figure 2: Nanoparticle (NP) binding assessment to lectin for particle size and polymer length optimization. A library of NPs varying in particle size and ligand polymer lengths were assayed for binding with a lectin using the Octet® instrument. The binding responses were used as an indication for binding affinity levels to measure the NP platforms' suitability to mimic high-affinity binding interactions in multivalent ligand presentations. The lectin soybean agglutinin (SBA) was immobilized onto Streptavidin Biosensors and assayed for binding with glyconanoparticles. NP construct that produced higher binding affinities were used to measure 2,3-sialic acid and 2,6-sialic acid interactions with Influenza hemagglutinins to study zoonosis.

As depicted in Figure 2, biotinylated glycans were immobilized on to a Streptavidin Biosensor and binding levels were measured with NPs that differed in particle size and polymer length. Typically, an Octet® binding assay can be used to measure binding affinities (K_D) and kinetics (k_{on}, k_{off}). However, in this screen, a single NP analyte concentration was used and binding levels were compared using signal response levels. One should note that response levels measured in the Octet® system experiments are proportional to the thickness of the bio-layer generated by the binding complex. This is dependent on the size of the analyte, in this case the NP. However, binding responses are also proportional to the affinity of the interaction at equilibrium (R_{eq}). Therefore, when affinity levels are estimated based on binding response levels obtained from equimolar analyte concentrations, both analyte and ligand sizes should be similar in size to avoid misrepresentation of affinities.

As noted above binding signal responses were probed to gauge NP-lectin affinity and favorable binding recognition of glyco-NP conformations. Binding levels (changes in binding affinities) or lack of binding can indicate steric hindrances or less than favorable conformations of sialic acids attained during NP assembly. A range of NPs with linker lengths with DP (25–50) and NP sizes (30, 50 and 70 nm) were analyzed using the Octet® screen. Interestingly, it was observed that the shorter polymers of all NP sizes gave the largest signal outputs tested, suggesting that the lower density of longer polymers were reducing overall affinity and multivalent enhancement. These optimization screens led the authors to select telechelic poly (N-hydroxyethyl acrylamide) (PHEA) with a DP of 25 conjugated to 70 nm gold NPs for investigating multivalent sialic acid-binding to influenza hemagglutinins.

The selected NP formulation was then subjected to another Octet® binding assay against a panel of Influenza hemagglutinins from a variety of strains that were immobilized onto Streptavidin Biosensors. Varying levels of binding responses and binding rates were observed upon binding to 2,3- and 2,6-sialyllactosamine functionalized NPs. Interestingly, many avian hemagglutinins deemed selective towards α2,3 based on monomeric evaluations showed potent recognition in multivalent NP presentations. This shows the importance of sialic acid presentations in NP platforms, as it indicates changes in glycan specificity when presented in multivalent formats, due to enhancement of weak interactions, as well as confirming evidence for avian influenza origins being able to infect human hosts. Overall 2,3- and 2,6-sialyllactosamine conjugated NPs systems investigated in this study were able to successfully evaluate glycan-binding to haemagglutinins and unravel cross-species affinities from multivalent presentations, correlating with biological cross-infectivity observations and serving as a platform to recognize zoonosis.

Measuring Nanoparticle Scaffold Stability

Marcandalli *et al.* utilized an Octet® assay to assess physical stability of a self-assembling NP presenting a pre-fusion stabilized variant of an F-glycoprotein trimer (DS-Cav1)[10]. The NP assembly is tested as an approach to generate an amplified neutralization antibody response against the respiratory syncytial virus (RSV) compared to trimeric subunit vaccines. The DS-Cav1 protein was fused to the I53–50 trimeric component to maximize antigen density and would result in the assembly of an icosahedral NP. Here the I53–50 is a computationally designed co-assembling, two-component protein complex that would form 120-subunit icosahedral nanostructures[14]. To determine the stability of DS-Cav1, a key component of antigenic activity of the NP assembly, an Octet® BLI assay was used. As also noted in the above examples, antigen activity displayed on NPs can be measured by an antibody binding assay. Here, Protein A (ProA) Biosensors were used to immobilize AM14, a DS-Cav1 reactive IgG and measure binding responses with DS-Cav1-I53-50A NPs. A single 200 nM NP concentration was assayed with samples that were either incubated at 37°C or stored at -80°C for 1 and 2 weeks. The activity was assessed based on binding response levels in the association binding phase. As described above, binding response comparisons can be used to compare binding activity as it relates to the overall affinity of the interaction. The data indicated that the NP assemblies were stable at these conditions, which was also supported by SEC, Guanidinium-HCl denaturation and HDX-MS experiments.

Octet® assays have been used similarly to measure vaccine potency and stability. Read the Application Note on developing a vaccine potency and stability indicating assay.

Assessment of Immune Responses Generated by a Nanoparticle Vaccine

The 60 sub-unit self-assembling NP eOD-GT8 is a B cell receptor germline targeting immunogen. It has been shown to activate naïve precursor B cells in murine models, with epitope specificities similar to HIV VRC01-class broadly neutralizing antibodies. However, challenges remain in human germline targeted vaccines. This is partly because germline centers (GCs) are not located in the blood but lymph nodes (LNs), making quantifying the success of the vaccine difficult. As such clinical trials have only been able to indirectly infer GC or germline center B cell activity. Following immunization with a NP vaccine, Havenar-Daughton *et al.*, used LN fine needle aspirates (LN FNAs) to follow the germinal center response in the LNs of rhesus monkeys[11]. The group used an Octet® assay to evaluate serum antibody titers before and after immunizations. Octet® platforms provide a convenient assay solution to rapidly measure vaccine or disease-induced humoral responses in serum and plasma samples[14,15]. Octet® systems and biosensors are readily applied in crude sample analysis, unlike other fluidics-based biosensor detection platforms. The rapid dip-and-read measurement can provide titer measurements within minutes (Sartorius AAV app note). In this work, Streptavidin Biosensors were loaded with biotinylated eOD-GT8 to evaluate serum antibody levels.

Summary

In this short communication, we have highlighted how Octet® platforms can be used in nanoparticle-based vaccine research and development workflows. Nanoparticles are emerging as an effective vaccine strategy due to its ability to present antigens in multimeric conformations that often mimic native-like conformations. However, studies have shown that optimal antigenic presentation is dependent on several NP physiochemical properties such as size, charge, surface chemistry and composition. Methods and assays are needed that can optimize these variables for constructing ideal immunogenic antigen presentations. Octet® assays were used to evaluate a wide range of native-like HIV-1 spike protein constructs displayed on NPs and their binding profiles to bNAbs and non-NAbs to elucidate the structure-based design of HIV-1 vaccine candidates with information on specific epitope presentations that generates B-cell responses *in vivo*. In another study, an Octet® binding screen was used to determine optimal NP core size and formulation that can best mimic host sialic acid environments for influenza hemagglutinin recognition to understand zoonosis. Similarly, Octet® instrument binding assays were also used to establish antigen stability of NP presentations and measure humoral responses in serum samples in response to a NP vaccine strategy. These few examples highlight the versatile use of the Octet® system from screening and identification of optimal NP physical properties, evaluation of antigenic profiles, antigen stability and measurement of humoral responses in NP vaccine development workflows.

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Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 888 OCTET 75
Or +1 650 322 1360

For further contacts, visit
www.sartorius.com/octet-support

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