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Expanding Octet® Applications in Downstream Biologics Characterization: Stability, Formulations and Aggregation Studies

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Abstract

Structure is an important characteristic for protein activity and function. Structural alterations due to protein mis-folding, denaturation or unfavorable conditions can lead to the formation of aggregate species that can affect the efficacy and safety of biologic drug candidates. While many biophysical analytical tools are well suited for detecting these aggregates, few offer a combination of structural and functions assessment in the same system. Sartorius' Octet® platform is an easy-to-use analytical tool that can detect the presence of aggregated species while monitoring biologics functional activity at the formulation stage of biologics drug development process.

Introduction

The prediction of how a drug substance performs *in vitro* and *in vivo* is a critical process in the development of the drug product's final formulation. Pre-formulation studies aimed at identifying the physicochemical characteristics of a drug candidate may include the evaluation of solvent solubility, pH stability, size distribution and structural and functional stability in different excipients. Structural stability may be assessed through biophysical characteristics such as un-folding or mis-folding properties or through the analysis of presence of aggregation. Protein or antibody aggregation is common and is often associated with physiological conditions. Aggregation in cells indicates cellular inability to maintain proteostasis. Aggregation often occurs as a result of association of the protein into larger assembly resulting from loss of secondary, tertiary or quaternary structures which in turn leads to compromised biological activity [1,2]. There is, therefore, a clear relationship between the aggregation and molecular structure.

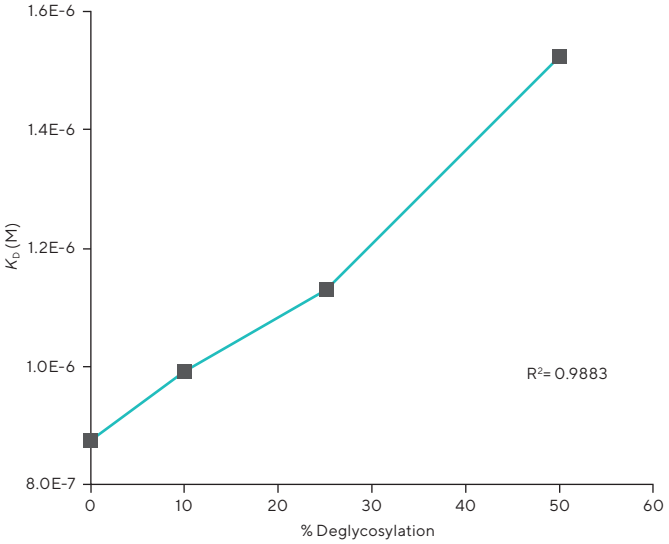

Figure 1: Affinity analysis of the binding of an IgG1 to an Fc-receptor as a function of deglycosylation.

Traditional technologies for downstream assessment of biological drug candidate stability include dynamic light scattering (DLS), multiple-angle light scattering (MALS) and circular dichroism (CD). These techniques predict stability either through the detection of presence and on-set of aggregation, molecular secondary structure changes due to instability or melting temperatures, a predictor of thermal stability, amongst other biophysical parameters. However, they cannot provide a functional assessment. The Octet® platform gives you the capability for multiple applications in one system. It enables critical functional stability studies, where the presence of aggregation can be monitored in tandem with more traditional binding analysis such as target binding, potentially saving some users the need for multiple technologies

Octet® as a Stability Indicating Analytical Tool

Octet® platforms can be used as stability-indicating tools and are suitable for measuring and distinguishing between fully functional drug products and those whose binding activities have been affected by degradation. In one study, the Octet® system was used to distinguish between native and deactivated antigen and showed reduced binding activity of the deactivated antigen, proving the assay was stability indicating. In another study, the affinity of an IgG1 to an Fc gamma receptor IIIa molecule was shown to decrease with increasing deglycosylation percentages (Figure 1), further indicating Octet® system suitability for use in developing stability-indicating methods.

Furthermore, Okbazghi et al. has shown that the Octet® platform can be used to develop analytical methods well suited for the assessment of a variety of biologics for their biosimilarities. They used the Octet® platform to evaluate potential biological activity of four different IgG1 Fc glycoforms (HM-Fc, Man5-Fc, GlcNAc-Fc and N297Q-Fc) using two different binding formats; one utilizing Protein G Biosensors to immobilize IgG1 Fc, and the other utilizing C-terminally biotinylated FcγRIIIa produced by a novel method to immobilize FcγRIIIa on Streptavidin Biosensors. The use of a series of well-defined glycoproteins in these studies allowed for the identification of important structural and biological features for comparability and biosimilarity analyses. These two assay formats were used to assess the affinity of the IgG1 Fc glycoforms for FcγRIIIa. The study demonstrated complementary information about binding and concluded that the Octet® method could be applied to future studies of full-length antibodies, antibody-drug conjugates and antibodies with more complex glycosylation patterns than the types they studied. From the perspective of developing a model system for biosimilarity analysis, the binding studies also identified members of the model system that exhibited highly similar biological activity and those with distinct differences.

Octet® Potency Assays with Aggregated Samples

The Fc region of human IgG molecules contribute to a number of beneficial biological and pharmacological characteristics of therapeutic antibodies. One of the most important characteristics is the ability to prolong plasma half-life, due to its unique, pH-dependent interaction with the neonatal Fc-receptor (FcRn). Because altered FcRn binding can impact drug efficacy by increasing or decreasing the serum half-life of Fc-containing therapeutics, FcRn binding interactions are assessed at multiple stages of biologic drug development. FcRn-Fc activity and binding assays are performed as part of characterization studies that enhance overall product understanding and demonstrate comparability in the development of biosimilars. Commonly used *in vitro* methods for analysis of FcRn binding include ELISA, SPR, and bead-based proximity assays. However, some of these techniques may not deliver the desired accuracy. In a recent potency assay study, Barjardi-Taccioli[3] et al. demonstrated that the presence of aggregates in samples significantly increased binding potency values in AlphaScreen-based FcRn binding assays, sometimes masking the loss of potency, suggesting that the AlphaScreen method overestimated potency values. They observed even more enhanced aggregates in an AlphaScreen®-based FcγRIIa binding assay for a monoclonal antibody with strong effector function. To resolve this issue, a novel Octet-based FcγRIIa binding assay was developed and qualified. The Octet® assay measured association binding responses and calculated the binding potency of the samples relative to the standard using Parallel Line Analysis. This method overcomes interference of aggregates present in the samples, distinguishes different Fc glycosylation patterns and is stability-indicating. It can be used for sample characterization, drug product release and stability testing. In this study, Barjardi-Taccioli group used thermally stressed mAb samples with % aggregates ranging from 2.7 to 6.9%, a monomeric mAb with only 0.1% aggregates and a reference sample containing 1.8% aggregates. The AlphaScreen and BLI methods were both used to evaluate antibody potency against the reference sample. An increase in potency was observed with increasing aggregates population using the alpha-LISA assay, while the mAb potency stayed the same despite the presence of up to 5% aggregates using the Octet® instrument. This finding reveals that the Octet® platform data is not affected by interference from aggregates and is sensitive to changes in activity due to post-translational modifications.

Probing Structurally Altered and Aggregated States in Therapeutic Proteins

In a study designed to probe the potential use of label free technologies in the detection of structurally altered and or early aggregate species of therapeutic protein candidates, Volkin et al. demonstrated the analytical development of a novel chaperonin-based Octet® assay (GroEL-BLI) that can be used to probe for transiently exposed hydrophobic surfaces[4]. They used several pharmaceutically relevant proteins as model systems to demonstrate the ability of the GroEL-BLI biosensor methodology to rapidly detect pre-aggregate/aggregate formation in protein solutions during slightly elevated (40–45°C) to moderate (55°C) thermal stress (Figure 2).

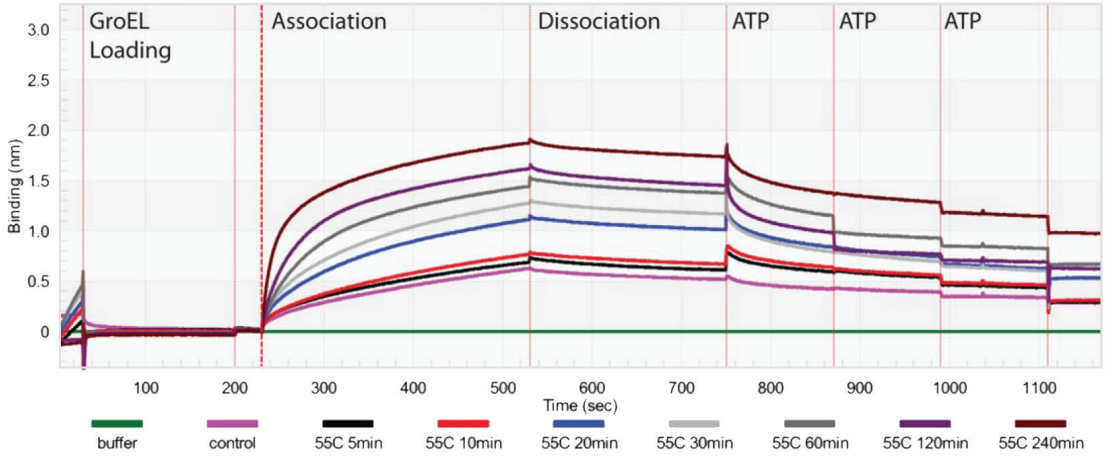

Figure 2: Octet-GroEL assay monitoring changing polyclonal IgG binding amplitude with increasing temperature[4].

The group proposed two potential methods for monitoring the interaction of unfolded protein substrates; one where the test protein substrate is immobilized onto a biosensor surface and then allowed to interact with a chaperonin protein (GroEL in their study) in solution and another where the chaperonin (GroEL) is immobilized onto the biosensor followed by interactions with the test protein substrate in solution. The former method is suitable when the test protein is prone to aggregation that can lead to loss in solution. In a fairly detailed study, the Volkin group compared aggregation data obtained using the Octet® method with data obtained using size exclusion chromatography (SEC) for pre-aggregated samples and samples stressed at high temperatures. The SEC data was used to confirm the presence of a known % of aggregates for the polyclonal IgG sample used in the study, where the presence of soluble aggregates in the heated sample is associated with the disappearance of specific peaks in the chromatogram. The Octet® system data was monitored through the increase in binding amplitude upon aggregate formation and was used to show changes in the polyclonal IgG binding profile to the GroEL loaded biosensor concurrently with changes in the levels of soluble dimeric and multimeric aggregate species as measured by SEC.

Measuring Nanoparticle Scaffold Stability[5]

Nanoparticles (NP) exhibit properties desirable for drug delivery tools. They provide better drug pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity and biorecognition and improve drug efficacy when used as carriers[6]. In a study that measured NP scaffold stability and established the Octet® platform's versatility for stability assessment method development for a diverse range of molecules, Marcandalli et al. utilized an Octet® assay to assess the physical stability of a self-assembling NP presenting a pre-fusion stabilized variant of an F-glycoprotein trimer (DS-Cav1). The NP assembly was 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 resulted in the assembly of an icosahedral NP. An Octet® assay was used to determine the stability of DS-Cav1, a key component of antigenic activity of the NP assembly. Protein A (ProA) Biosensors were used to immobilize AM14, a DS-Cav1 reactive IgG, and binding response was measured 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. Activity was assessed based on binding response levels in the association binding phase at 1500 sec. Binding response comparisons were then used to compare binding activity as they relate to the overall affinity of the interaction[7]. The data indicated that the NP assemblies were stable at these conditions and this conclusion was further supported with SEC, Guanidinium-HCl denaturation and HDX-MS studies.

Formulations Studies on Octet® Systems

The *in vivo* function of a protein can be influenced by the presence of hundreds of different other molecules in the crowded and complex environment of the human body. An understanding of the non-specific interactions between proteins under crowded environments is critical to gaining a more complete insight into protein function in a biological context. Popular techniques for monitoring protein function in crowded environments include analytical ultracentrifugation (AUC), which can take to generate results, and NMR, which is subject to interference by the ionic strength of the sample. Kim et al demonstrated that the Octet® system is a viable alternative for examining the impact of non-specific interactions on specific biologically relevant interactions, providing a direct method to assess binding events in crowded conditions[5] without the same limitations as AUC and NMR. The group performed a series of experiments including the data shown in Figure 3 to examine how crowding reagents such as Human Serum Albumin (HAS) and Ficoll70 affect the binding of mAbs to their specific antigens at low (10 mM NaCl) or high (137 mM NaCl) ionic strengths. Their findings highlight the potential use of the Octet® platform as a convenient and high-throughput method to assess binding interactions with the inherent flexibility to test many different conditions including crowding agents, and that this approach is an easy and efficient way to eliminate mAbs or other molecules from consideration during early discovery work.

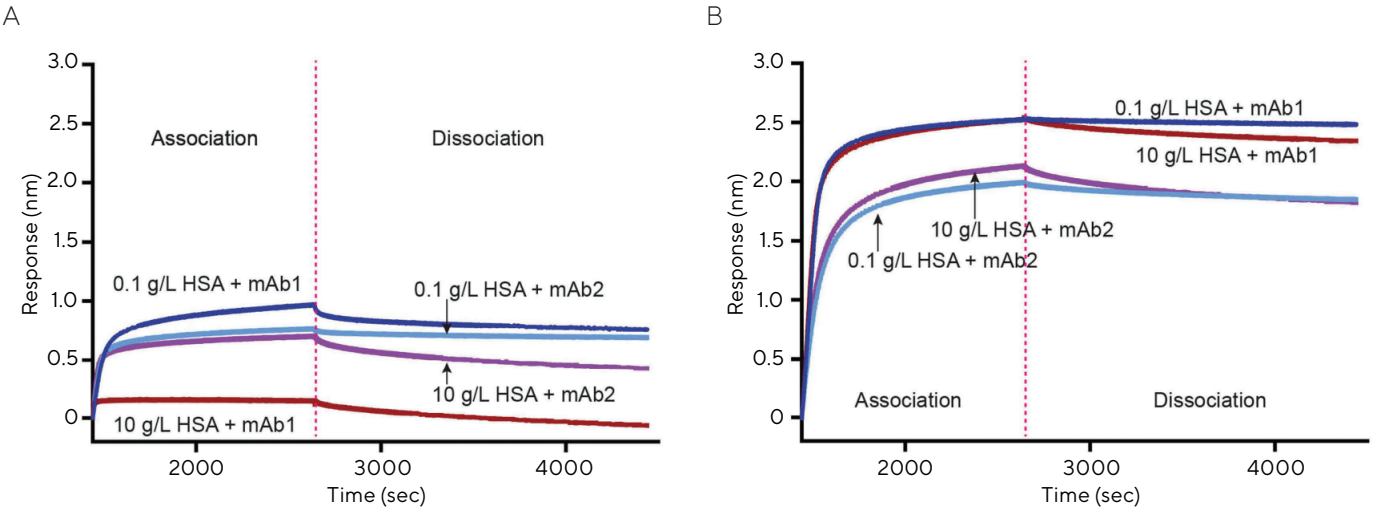

Figure 3: Effect of mAb binding in the presence of low and high HAS at low ionic strength (a) and high ionic strength (b) monitored on the Octet[8].

Summary

The Octet® system's unique Bio-Layer Interferometry detection system can be used in applications that analyze biophysical characteristics of biologics beyond typical bio-molecular interactions. A hydrophobic based probe can be used to screen for and to evaluate molecular structure including unfolded proteins as well as to differentiate between pre-aggregates and aggregate formation. In addition, the plate design and the use of multiple biosensors in parallel can be taken advantage of to screen for buffers or formulations at that may accord the developed biologics their most optimal functional environment.

Kim[8] et al, in their article on measuring the effects of macromolecular crowding on antibody function, further conclude that the application of the Octet® platform to a variety of biological and drug discovery problems is expanding; and that as an early discovery research screening tool, the Octet® system can be used to more quickly eliminate candidates from the pipeline and can be beneficial in diversifying the types of assays used in discovery research. This applies for downstream applications as well.

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