WHITE PAPER

WP9003: VLP Characterization with the Light Scattering Toolbox

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Introduction

Virus-Like Particles (VLPs) are biomolecular nanoparticles formed by controlled self-assembly of viral structural proteins. Lacking viral DNA for replication, VLPs are capable of triggering an immune response without the risk of infection and have been proven to be safe yet highly immunogenic. VLPs are of growing interest in the pharmaceutical and biotechnology industries following the success of blockbuster VLP-based vaccines against hepatitis B and human papillomavirus. These bioparticles are increasingly targeted not only for safe and efficacious immune stimulants but also therapeutic gene delivery agents, taking advantage of their DNA encapsulation and transport capabilities.

With an increasing number of VLPs entering biotherapeutic development pipelines, accelerated development presents several key analytical challenges. Common to all phases of development is the basic biophysical characterization of mass, size, composition and conformation. These and additional, phase-specific properties are required in early stage stability assessment of vaccine candidates; process development; and multiparametric formulation development. In the final product, quality assurance requires identifying and quantifying degradant products such as free capsomeres, partially formed capsids and aggregates.

These challenges are met by a comprehensive suite of analytical tools based on light scattering technologies. Light scattering constitutes a non-invasive probe for characterizing macromolecules and nanoparticles in solution, offering multiple, complementary techniques to assist in the development of VLP-based biotherapeutics. The powerful capabilities of light scattering provide an increasingly popular solution for rapid and effective VLP characterization.

The Characterization Challenge

In some ways, VLP development is similar to that of any biotherapeutic. Functional activity is assessed via the binding of target antibodies to the VLP, though unlike most protein-ligand interactions, the stoichiometry of the interaction may be unknown. Formulation development screens hundreds of combinations of buffer conditions and excipients in order to identify those most promising for maintaining long-term stability. The top formulation candidates undergo accelerated and long-term stability tests. Comparability and lot-release tests of structure and efficacy must verify batch-to-batch consistency via high-resolution characterization and quantification of degradants; incomplete characterization could present a barrier to licensure in the face of tight regulatory control.

A unique challenge is the need to distinguish between unincorporated protomers (capsid proteins), capsomeres (sub-units of the VLP), well-formed capsids (viral envelopes), and intermediate or malformed fragments. Capsids must exhibit specific size, shape and mass values in order to present to the immune system an exterior similar to that of real viruses; partial or misformed VLPs may impact immune stimulus. Additionally, the use of VLPs for delivery of gene therapeutics requires analysis of the oligonucleotide payload inside a VLP scaffold.

A Light Scattering Toolbox

Light scattering for characterization of VLPs comes in three primary varieties. Multi-angle static light scattering (MALS) measures absolute molar mass and size

(root mean square radius, R_g) from first principles. Dynamic light scattering (DLS) measures translational diffusion and hence hydrodynamic radius, R_h. Electrophoretic light scattering (ELS) measures electrophoretic mobility. The measurement ranges of MALS, DLS and ELS are ideally suited to VLPs, which typically fall between 20-50 nm in radius. The combination of MALS, DLS and ELS with different types of sample preparation and delivery creates a comprehensive toolbox of techniques for biophysical characterization.

Wyatt Technology's suite of light scattering and separation technologies addresses to entire range of VLP biophysical characterization. MALS instruments such as the DAWN® couple to size-exclusion chromatography (SEC–MALS) or Eclipse® field-flow fractionation systems (FFF-MALS, described below) in order to characterize the distribution of populations in a VLP sample for structure, aggregation, and the presence of unassembled protomers or capsomeres[1]. Adding a WyattQELS™ embedded DLS module in a DAWN or miniDAWN provides on-line DLS detection to SEC-MALS or FFF-MALS, further refining structural analysis: the relationships between MW, R_h and R_g indicate conformation (shape and compactness).

Coupling a MALS detector to a Calypso® composition-gradient delivery system (CG-MALS) creates a unique means for evaluating process assembly and degradation kinetics, and quantifying biomolecular interactions such as self-association or binding of antibodies to VLP's[2], without labelling or immobilization Since MALS determines absolute molar mass of the solute, it is particularly good at extracting binding stoichiometry (e.g. number of antibodies per VLP, or complex assemblies) as well as affinity.

High-throughput dynamic light scattering (HT-DLS) can very rapidly assess hundreds of formulation conditions for gross aggregation as well as VLP dissociation during thermal or chemical stress tests[3]. A microwell-plate-based, high-throughput in situ DLS plate reader such as the DynaPro® Plate Reader is an ideal tool for time-sensitive development of stable formulations[4].

Rounding out the light scattering toolbox, massively parallel phase analysis light scattering (MP-PALS, a high-sensitivity, high-throughput form of ELS implemented in the Mobius®) in conjunction with sample delivery by an autosampler, facilitates measurement and optimization of surface charge in formulation buffers for enhanced stability.

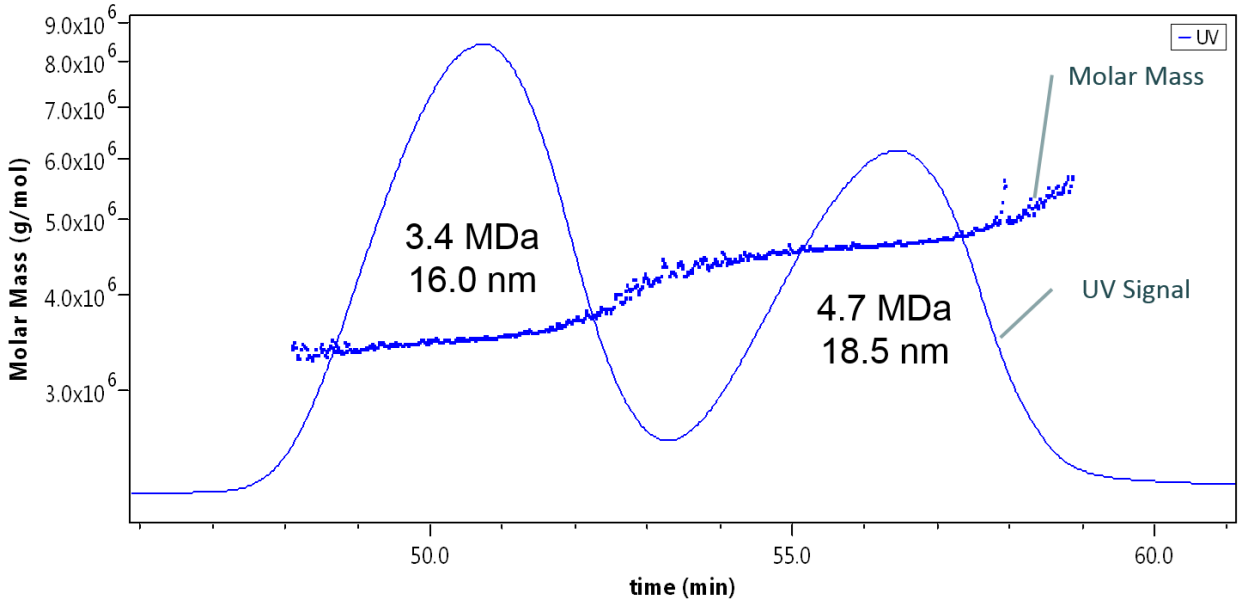

Figure 1. FFF-MALS high-resolution separation and characterization over a small range, using less than 3 µg of VLP. Conversely, FFF may also be tuned to separate a very wide range of sizes.

Very little material is needed in order to characterize VLPs by light scattering – typically sub-microgram quantities for DLS, SEC-MALS and FFF-MALS. CG-MALS requires several µg of VLP and about 100 µg of antibody, though more VLP material may be required to characterize weak self-association.

Separation by FFF-MALS

Unlike typical biotherapeutics, VLPs do not always fall within the range of separation by SEC. In many cases they will not elute from a column, or elute at a time not representative of their true size, due to a variety of non-ideal column interactions.

Field-Flow Fractionation (FFF) overcomes many of the limitations of SEC, providing particle separation from 1 nm to > 1000 nm with no stationary phase and the ability to zoom in on specific ranges, or zoom out over the entire range, simply by modifying flow parameters. The commercial availability of FFF (Wyatt Eclipse) combined with sophisticated MALS and DLS detectors has proven great value in biotechnology, filling in the gaps left by industry standards such as unfractionated DLS and transmission electron microscopy (TEM).

Via FFF-MALS and FFF-DLS, unassembled protomeres, capsomeres, malformed VLPs and aggregates, may be separated and distinguished from well-formed, monomeric VLPs[5]. FFF-MALS systems such as those developed by Wyatt Technology excel at quantifying the variability across different preparations of VLPs before and after stresses such as freeze-thaw or elevated temperature.

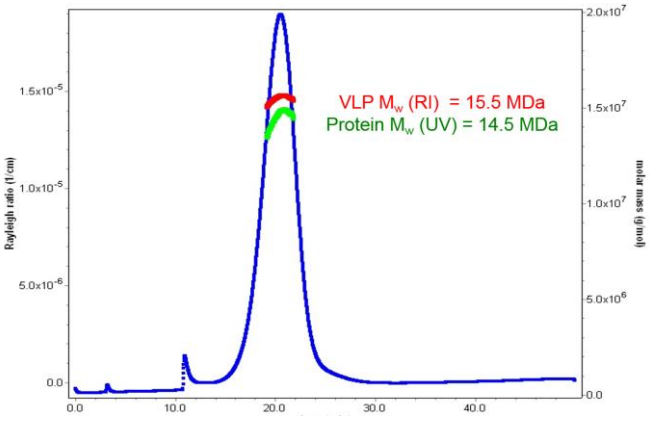

Figure 2. By combining UV, MALS and refractive index (R)I) detection with FFF, a 1 MDa DNA payload is distinguished from the 14.5 MDA protein envelope.

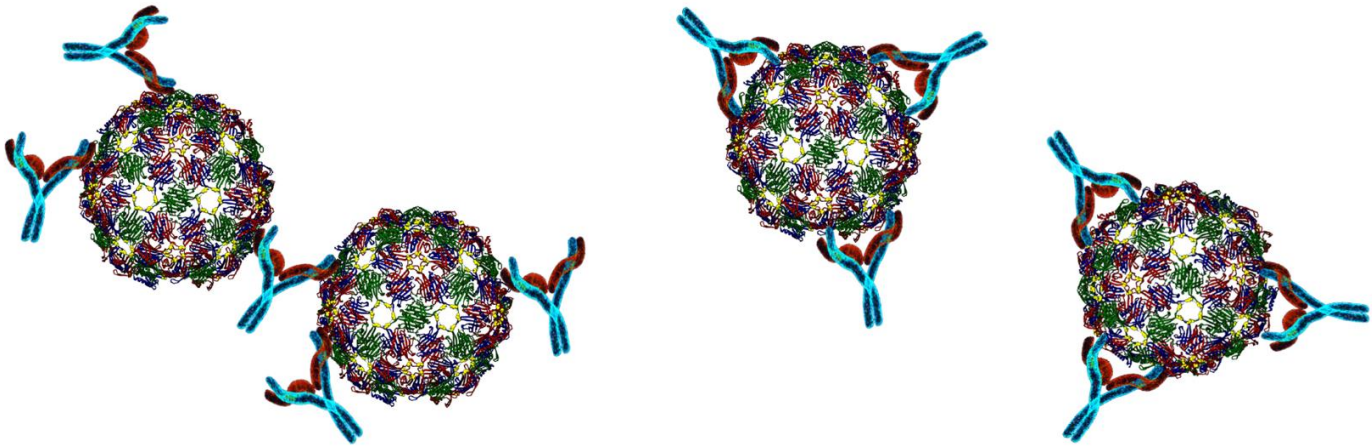

Figure 3. CG-MALS determines binding affinity of antibodies to VLPs, as well as absolute molecular stoichiometry. Hence it distinguishes between complexes where both arms of the antibody bind to a single VLP vs single-arm attachment that can lead to multi-VLP complexes.

Recently, VLP analysis by FFF-MALS has been extended with a powerful new technique: electrical/asymmetric-flow field-flow fractionation (EAF4). The Eclipse Mobility® system separates nanoparticles and macromolecules by size *and* charge. This two-dimensional fractionation method serves two purposes: first it overcomes difficulties in FFF fractionation when the sample tends to adhere to the FFF membrane; and second, as an analytical technique, it can provide detailed size/zeta potential distributions for deeper biophysical characterization.

Conclusions

As has been established both in scientific literature and in biopharmaceutical practice, a comprehensive array of tools exists to support the development of VLP-based vaccines through the characterization of essential biophysical properties: molar mass, size, charge and interactions, as well as derived properties such as conformation and composition. This suite of instrumentation draws on common building blocks based on the basic forms of light scattering (MALS, DLS, ELS), combined with automated techniques for achieving fractionation, composition gradients or high-throughput screening. Researchers in vaccine discovery, process development, formulation development and product characterization can quickly come up to speed on one technique in the light scattering toolbox, then leverage that knowledge to become familiar with the rest of the set and so accelerate the VLP pipeline.

The same light scattering toolbox readily expands to assist in the development of gene delivery technologies, where other methods often fall short due to extensive manual labor, lack of automation, or poor repeatability and reliability. The promise of genetic therapy for some of the most intractable maladies may one day be fulfilled by shining some light on the characterization problems.

A webinar on the topic of VLP characterization by light scattering is available on demand at http://www.wyatt.com/library/webinars/vlp-characterization-light-scattering-biophysical-toolbox.html.

For more information on the range of light scattering products available from Wyatt Technology, please visit our website at www.wyatt.com

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