



**WYATT**  
TECHNOLOGY

6300 Hollister Avenue  
Santa Barbara, CA 93117  
Tel: +1 (805) 681-9009  
Fax: +1 (805) 681-0123  
Web: [www.wyatt.com](http://www.wyatt.com)

Live Webinar Q&A Sheet:

## **Biotherapeutics Form and Function: Case Studies in Light Scattering**

The recorded webinar may be viewed from the [Biotherapeutics webinars page](#). These questions were submitted by live viewers. Additional information on MALS, DLS, and FFF may be found on the Wyatt web [Library](#) under webinars, application notes, featured publications and bibliography, as well as on the corresponding [Product page](#) and [Theory](#) page of our web site.

Please contact [info@wyatt.com](mailto:info@wyatt.com) with any additional questions.

*Q: What is the typical range of  $dn/dc$  you find experimentally for biotherapeutics in aqueous PBS buffer? What impact on size is there in just using a literature value versus experimentally derived  $dn/dc$  in similar conditions?*

*A: The literature value for the  $dn/dc$  of protein in water is 0.185 mL/g, and this can be applied to the vast majority of proteins. Depending on the sequence, the  $dn/dc$  can vary, and the classic example is  $\alpha$ -crystallin, found in the lens of the eye ( $dn/dc \sim 0.2$  mL/g).*

If the protein is being analyzed in a buffer whose refractive index is much different from water (e.g., due to high concentrations of excipient), you may need a new  $dn/dc$  for the protein in that buffer. It is possible to estimate the effect based on the refractive index of the formulation buffer.

Modifications, such as PEGylation or glycosylation, can change the  $dn/dc$  value. The Protein Conjugate Analysis in ASTRA is perfect for determining the mass fraction of protein, mass fraction of modifier, and corrected conjugate  $dn/dc$ . An example of Protein Conjugate analysis can be found in this [application note](#).

For SEC-MALS-RI, an error in  $dn/dc$  is proportional to an error in molar mass. For example, if the  $dn/dc$  used for analysis is 3% higher than the actual  $dn/dc$  for that molecule, the measured molar mass will be 3% too low. Batch MALS with nominal concentration or SEC-MALS-UV will be more affected by an error in  $dn/dc$ .

*Q: Why would molar mass vary across the peak?*

A: There are several reasons molar mass might vary across the peak. Polydisperse species, like polysaccharides and other polymers, will have a variable molar mass that can be measured with MALS. An example is shown in the application note, [“Polyvalent Pneumococcal Polysaccharide Vaccine.”](#)

For proteins and other molecules that should be monodisperse, there are a few common reasons for non-constant molar mass. There may be a small amount of aggregate or fragment co-eluting at the leading or tailing edge of the peak, which can be observed by MALS. Alternatively, reversible oligomerization may cause a concentration-dependent molar mass across the peak. At the apex of the peak, where the concentration is highest, the molar mass may be higher due to the presence of reversible oligomers. At the edges of the peak, where the concentration is lower, the equilibrium is pushed to the monomer, and the molar mass is slightly less. Examples of both of these behaviors can be found in the application note, [“Identification of Insulin Oligomeric States Using SEC-MALS.”](#)

*Q: What range of flow rates are used for your detectors?*

A: The detectors are compatible with a full range of flow rates used in analytical chromatography and for batch applications. Typical flow rates range from 0.1 mL/min to 1 mL/min depending on the column and mobile phase being used. More information regarding typical back pressure and other instrument specifications can be found at [www.wyatt.com/Products](http://www.wyatt.com/Products).

*Q: The Plate Reader collects scattered light at only one angle. What limitations does this introduce?*

A: The DynaPro Plate Reader measures dynamic light scattering (DLS) at a single scattering angle. DLS analyzes the fluctuations in the scattering intensity as a function of time; from the rate of fluctuations, we can calculate the diffusion coefficient and, thus, the hydrodynamic radius. Additional information as a function of angle is not required for this measurement. The Plate Reader can measure a full range of hydrodynamic radii from 0.5 nm to 1000 nm. For more information about the range of sizes that can be measured by DLS, see our [Products page](#).

*Q: Is there any way to study ternary/quaternary complex formation and stoichiometry using CG-MALS? What if one or two components in the ternary/quaternary complex are small molecules or peptides?*

A: The CALYPSO software can analyze data for multiple interactions among one or two species (e.g., A and B). This includes any combination of self-association (e.g., dimers of B) in addition to hetero-association (e.g., formation of AB, A<sub>2</sub>B, and A<sub>2</sub>B<sub>2</sub>). Analysis of ternary complexes (e.g., ABC) is more complex and cannot be done within the CALYPSO software.

The molar mass of the complex must increase by at least 10% in order to quantify it by CG-MALS. Typically, CG-MALS cannot be used to measure the binding of small molecules to proteins. Peptide-peptide and peptide-protein interactions can be measured as long as the total molar mass increase is at least 10%.

*Q: For the Wyatt  $\mu$ DAWN and HELEOS, how important it is to know the initial dn/dc values, buffer viscosity, etc. parameter? What if you have molecules where the parameters are not very straight forward?*

A: The molar mass determined by static light scattering does require some knowledge of the refractive index of the solvent and the dn/dc of the solute in that solvent. The relationship can be found on our [MALS Theory page](#). With the ASTRA software, a Wyatt MALS detector and (U)T-rEX refractive index detector, these properties can be easily measured in order to refine the analysis. Refined estimates of these parameters can be applied to previously collected data to determine their effect.

Solvent viscosity is only required for calculating the hydrodynamic radius from DLS, and this can also be measured using a DynaPro NanoStar or Plate Reader. More information can be found in our [DLS Theory page](#).

*Q: Can we use/control the Wyatt detectors with Empower?*

A: Empower can be used to control the appropriate HPLC and UHPLC pumps and autosamplers. However, Empower cannot be used to collect and analyze data from the Wyatt detectors. Data collection and analysis can only be done with ASTRA. An analog light scattering signal from one of the photodiodes of the Wyatt MALS detector in the MALS detector and/or from the Wyatt (U)T-rEX may be sent out to the Waters e-SAT/IN module and read into Empower for recordkeeping purposes.

*Q: Do you have an instrument that can be used to distinguish the size of a monomer (7 kDa), dimer (14 kDa) and tetramer (28 kDa)?*

A: Multi-angle light scattering (MALS) can easily be used to distinguish the molar mass of monomers and oligomers both with chromatography ([SEC-MALS](#)) and in batch mode. If the oligomer is reversible, CG-MALS can be used to determine the affinity and stoichiometry of that interaction, as in the application note, "[Self-association of Insulin Quantified by CG-MALS](#)." Dynamic light scattering ([DLS](#)) can also be used to measure the hydrodynamic size of proteins, peptides, and complexes.

*Q: Can light scattering techniques be used to study small peptides (<2kDa) and their potential aggregates?*

A: Multi-angle light scattering can be used to study molecules with molar mass as low as a few hundred Dalton. We have regularly applied MALS to the study of peptides, such as insulin and exenatide. Specifications, including molar mass range, can be found on our [Product page](#).

*Q: What  $dn/dc$  might you use for PEG-20000, and for a PEGylated protein, is it okay to use a weighted average of the overall mass to estimate the overall  $dn/dc$ ?*

A: The best way to measure the molar mass of a PEGylated protein is to use the Protein Conjugate Analysis in ASTRA. This method is for SEC-MALS combined with both UV and dRI detection. The software uses the dRI signal from the T-rEX, the absorbance from a UV detector, the  $dn/dc$  for the protein (typically 0.185 mL/g) and PEG (typically 0.11 mL/g), and the extinction coefficient of the protein and PEG in order to determine the amount of PEG, protein, weight-average molar mass, and corrected  $dn/dc$  and extinction coefficient for the conjugate. An example of Protein Conjugate analysis can be found in this [application note](#).