



Live Webinar Q&A Sheet: Characterizing Protein-Nucleic Acid Interactions by Light Scattering

The recorded webinar may be viewed from the [CG-MALS](#) 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 with any additional questions.

Q: How does the analysis depend on molecular conformation? Can it determine the geometry of a protein-DNA or DNA-DNA complex; for example, can it discriminate compact from elongated geometries?

A: The molar mass and RMS radius measured by MALS is independent of the shape of the molecule or complex. Both SEC-MALS and CG-MALS would report accurate molar masses for a protein, DNA, or complex regardless of the molecular conformation. By combining size and molar mass data, we can characterize the shape of the molecule or particle. For example, conformation plots of RMS radius vs. molar mass can be created from SEC-MALS data for a polymer to determine how compact or elongated its structure is. Similarly, by incorporating dynamic light scattering (DLS) or intrinsic viscosity (IV) with MALS, you can measure hydrodynamic radius simultaneously with molar mass to determine shape information. More information can be found at www.wyatt.com/Conformation.

Q: If I am analyzing a protein-DNA complex by SEC, should I use protein calibration standards or DNA calibration standards?

A: With SEC-MALS, you don't need to use any molar mass calibration standards! The MALS detector directly measures the molar mass of the protein, DNA, or other macromolecule. The measurement is independent of the elution time. Whether your molecule has a different density or conformation, compared to your standard, and whether your molecule interacts with the column (thus influencing its elution time) does not matter for SEC-MALS.

Q: How do the equilibrium constants (K_d) determined by CG-MALS compare with those obtained by surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), equilibrium sedimentation (AUC-SE) or similar techniques?

A: The equilibrium constants determined by CG-MALS compare very well with those obtained by other techniques. In particular, CG-MALS agrees strongly with solution-based methods like ITC and AUC-SE. Because CG-MALS directly measures the molar mass of the complexes in solution, it provides the added benefit of determining stoichiometry directly, which can be difficult or only



indirectly measured by other techniques. Examples of CG-MALS data complementing ITC and AUC can be found in [EMBO J. 2013, 32, 424-436](#) and [J. Gen. Physiol. 2014, 143, 231-252](#), respectively.

We also see good agreement with surface-based measurements, such as SPR and BLI. An example of complementary data can be found in [Nat. Microbiol. 2016, 1, 16128](#). Where steady state and kinetic experiments do not agree, CG-MALS often provides key information, such as complex stoichiometries inducing avidity effects on the surface of the SPR chip or reversible self-association of the analyte.

More information can be found at www.wyatt.com/Interactions.

Q: What are the maximum and minimum molar masses that can be measured with these light scattering techniques? Are there limitations on the affinity?

A: Multi-angle light scattering can measure molar masses down to a few hundred g/mol and up to $\sim 10^6$ g/mol using a 3-angle miniDAWN or microDAWN detector or up to $\sim 10^9$ g/mol using an 18-angle DAWN detector.

For SEC-MALS, the “modifier” (the DNA in this webinar) must comprise at least 5% of the molar mass of the complex to measure the amount of modification and molar mass of each species using protein conjugate analysis. To quantify the affinity and stoichiometry of an interaction by CG-MALS, at least a 10% increase in molar mass must occur upon binding. CG-MALS can be used to measure interactions with K_d as low as ~ 100 pM to as high as several mM.

Q: Can we determine molecular weight (molar mass) without knowing the sample concentration?

A. The light scattering I intensity is related to the molar mass M according to the relationship $I \propto Mc(dn/dc)^2$, where c is the weight concentration and dn/dc is the refractive index increment. Therefore, in order to measure molar mass by light scattering, we must know the concentration. In a SEC-MALS experiment, the concentration is usually measured simultaneously with the light scattering signal using a UV detector, refractive index detector, or both. In a batch CG-MALS experiment, the concentration may be measured online using a concentration detector, or the concentration of the stock solutions may be measured offline and input into the software.

Q: How do you measure extinction coefficient for single components in ASTRA?

A: The extinction coefficient can be measured in ASTRA from any SEC-MALS data file that contains both UV and RI data. When the dn/dc of molecule (0.185 mL/g for most proteins), UV cell path length, and UV analog constant are known, the extinction coefficient can be measured using the method *UV extinction from RI peak*. More information can be found at www.wyatt.com/ASTRA.



Q: Is there a generic dn/dc value for DNA/RNA much like there is for protein? How accurate do these values need to be for conjugate analysis?

How do differences in dn/dc for proteins vs. nucleic acids influence the analysis?

A: Like most macromolecules, the dn/dc for DNA and RNA is constant for a given solvent, wavelength of light, and above a certain threshold molar mass (typically a few thousand g/mol). The dn/dc used for all the DNA data in this presentation was 0.17 mL/g. The dn/dc is required to determine the molar mass via light scattering and also to determine the concentration from the refractive index detector, so an error in dn/dc will affect both calculations.

The fact that protein and nucleic acids have different dn/dc values is one of the reasons that the protein conjugate analysis works so well for complexes containing both species. In order to use the protein conjugate analysis, the “protein” and “modifier” must have different extinction coefficients, different dn/dc , or differences in both values.

Q: Would the Cre:loxP 4:2 stoichiometry be more observable with longer binding times in the CG-MALS experiment?

A: CG-MALS experiments are designed to allow the binding reactions to come to equilibrium within the MALS flow cell. This can be observed directly and in real time during an experiment. At equilibrium the light scattering intensity no longer changes with time, and this “plateau” is consistent with the fact that the net concentration of each complex is no longer changing. Depending on the affinity and kinetics of the interaction, equilibration times can span anywhere from a few seconds to an hour.

In the Cre-*loxP* experiment, equilibrium was reached after ~20 minutes. At this point, the weight-average molar mass of the solution (light scattering intensity) was no longer changing. The formation of product was occurring at the same rate as the dissociation into reactant, and the net concentration of each complex was not changing. The only way to increase the concentration of the complex would be to increase the overall concentrations of Cre and *loxP* and force the equilibrium to favor the bound state even more.

Q: How do you measure the extinction coefficient without dRI? Can it be done with an ordinary spectrophotometer?

A: The webinar featured a useful feature in ASTRA that uses the measured concentration by dRI to calculate the UV extinction coefficient when UV and RI data are collected simultaneously. Extinction coefficients may also be measured by preparing several known concentrations of solution and measuring the UV absorbance in batch mode. Extinction coefficients measured this way may also be used for protein conjugate analysis. Often, the protein extinction coefficient can



be estimated from the sequence, but as was demonstrated in the webinar, this value may not always be accurate and should be verified.

Q: Can we measure the hydrodynamic radius of this DNA-Protein interaction?

A: Yes, the hydrodynamic radius of the DNA-protein complex may be measured simultaneously using online dynamic light scattering (DLS). An internal DLS detector may be added to all Wyatt MALS detectors in order to measure molar mass and size simultaneously in the same measurement volume. For the SEC-MALS example, the hydrodynamic radius may also be measured by viscometry if an inline differential viscometer like the Wyatt ViscoStar is plumbed in line with the MALS and concentration detectors.

Q. What A_2 value do you use for protein-DNA analysis?

A. In these examples, the concentration of protein and DNA were far below where nonspecific interactions described by A_2 become important. For all the analyses, no A_2 was left at 0. Typically, nonspecific interactions can be ignored for protein or DNA concentrations below 1 mg/mL. More information on specific and nonspecific interactions can be found at www.wyatt.com/Interactions or on our Support Center in Technical Note TN9102 "[Assessing Nonspecific Interactions with Light Scattering: \$A_2\$ by SLS and \$k_D\$ by DLS.](#)"

In addition, the CG-MALS experiment in the webinar was designed specifically to measure both self and hetero-associations. The molar mass the Cre recombinase and *loxP* DNA were each measured separately as a function of concentration. Since the molar mass was constant for each species, we can conclude there are no significant self-interactions. The A_2 can be ignored, and no specific monomer-oligomer equilibrium needed to be considered.