



Live Webinar Q&A Sheet:

Applications of Light Scattering to HIV Integrase Structural Biology and Drug Discovery

The recorded webinar may be viewed from the [SEC-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.

General SEC-MALS questions

Q: Does HPLC standard protein mix give accurate values on MALS?

A: Yes, standard proteins have been evaluated by MALS for decades and are typically accurate to within one or two percent.

Q: Can glycosylation patterns or unstructured parts of proteins be identified or differentiated using MALS?

A: Yes, this is one of the most powerful aspects of MALS analysis. If you do standard gel filtration analysis and the protein appears to be a dimer by retention time but MALS tells you it is actually a monomer, this is a good sign of a partially unfolded protein. In some instances, measurements of diffusion coefficient and hydrodynamic radius by online DLS can help nail down size as well, which can be compared to molar mass to assess overall structure.

Glycosylation patterns per se cannot really be assessed by these techniques, since they do not have the resolution to determine where glycosylation occurs on the protein. However, SEC-MALS can determine overall degree of glycosylation using the Protein Conjugate analysis method in ASTRA, which combines UV, RI and MALS signals to calculate the protein molar mass and glycan molar mass in the conjugate at each elution volume.

Q: *What can we say about the shape or “health” of a monodisperse protein that elutes late in SEC but the MALS analysis shows a higher molecular weight than expected?*

A: A non-globular protein will usually elute *earlier* than expected due to a larger hydrodynamic radius associated with a non-compact conformation, and MALS shows this quite readily. If the protein elutes *later* than expected, and MALS confirms the higher molar mass, the cause is usually column interaction – typically a hydrophobic or electrostatic interaction of the protein with the resin.

We can also learn something about the “health” of the protein from the breadth of the peak and the apparent variation of molar mass across it. If the peak is relatively broad and the molar mass appears to track the concentration – i.e., has a maximum value at the peak and decreases towards either edge – this is a sign of moderate self-association. If this is the case, then by running SEC-MALS with a series of sample concentrations you can parse out the association behavior.

Combining SEC-MALS with other biophysical techniques

Q: *Why do you see aggregates in light scattering but not in UV?*

A: Like SAXS, light scattering intensity varies with the square of the molecular volume – in other words, it is proportional to the product of molar mass and concentration – whereas UV absorption is only proportional to the concentration. Therefore light scattering is very sensitive to large aggregates which occur at concentrations too low to detect by UV, and even small amounts will produce a large signal.

Q: *If the aggregate concentration is very low in UV, why do these affect SAXS measurements?*

A: The overall intensity recorded in SAXS is the summation of intensities of the component parts, so these aggregates – which scatter well in excess of their proportion in solution – contribute significant signal that cannot be deconvolved from the signal due to monomers. The final result will be corrupted correspondingly.

Q: *Can molar mass measured by MALS and protein size measured by DLS be used to constrain SAXS/SANS reconstructions?*

A: We take into account all the data available when choosing the most appropriate structures, including MALS data for oligomeric state and complex stoichiometry, and DLS data for size, and not just in SAXS/SANS reconstructions. For example, when choosing models to fit our sedimentation equilibrium data, I will take into account the range of molecular weights that appeared in the MALS data. For structures I will make use of DLS online size measurements and the SEC elution time, as well as evidence I might have from cryo-EM and other techniques.

In the end all the biophysical characterization is combined in a self-consistent manner to provide a complete, rigorous picture. If there are inconsistencies then I need to investigate and find why that is, reconcile the different pieces of information and perhaps come up with a better understanding of the biomacromolecule.

Q: *How did you determine that the PFV intasome complex stoichiometry was 4:2?*

A: Since this was a protein-DNA complex, it was a little more complicated than for a standard protein complex because the dn/dc value was not just the well-known, universal value for proteins of 0.185 mL/g. We combined the SEC-MALS data with sedimentation equilibrium experiments to confirm that all the data pointed to the same result.

Experimental details

Q: *Do you run SEC-MALS at room temperature?*

A: Yes, for convenience we generally run SEC-MALS at room temperature. Some of the complementary analyses are performed at 4 °C.

Q: *What types of size exclusion columns work best or are most popular to use for SEC MALS analysis?*

A: While Superdex 200 and Superdex 75 may be the first choice for separation of biomacromolecules, these media do have a tendency to shed fines (fine particles) that adversely impact the noise in the light scattering signal and should be washed overnight before the light scattering experiment. The silica columns, if well-maintained, do not shed so many particles and tend to have much lower background noise and so are more appropriate for SEC-MALS.

Q: *What is your philosophy on pre-filtering or centrifuging samples prior to SEC-MALS?*

A: I always spin the sample for ten minutes on a tabletop centrifuge at maximum speed, at 4 °C. I tend not to filter because some of the large complexes I work with stick to the filter. However, some proteins do not behave well under centrifugation and then I do filter the samples.