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Live Webinar Q&A Sheet:

Quantifying Affinity and Stoichiometry of Biomolecular Interactions by Composition-Gradient Multi-Angle Light Scattering (CG-MALS)

The recorded webinar may be viewed here. These questions were submitted by live viewers. Additional information on CG-MALS and Wyatt instrumentation may be found in the Wyatt Library under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding Product and Theory pages of our website.

Q: What is the range of molecular weights that can be measured with CG-MALS?
What is the molecular weight range of proteins that can be measured by this light scattering technique?
Can you measure interactions between small molecules and a protein?
Could this technique have applications to small molecule inhibitor studies that shift the protein multimeric equlibrium state in the presence of inhibitor?

A: Multi-angle light scattering (MALS) can measure molar mass for molecules as low as 300 g/mol up to ~10^6 g/mol (3-angle miniDAWN TREOS) or ~10^9 g/mol (18-angle DAWN HELEOS). In order to measure an interaction by CG-MALS, we require the molar mass to change by at least ~10% upon binding. This means we would not be able to see the interaction between a small molecule and macromolecule, such as a protein. However, we would be able to measure the effect a small molecule had on a protein-protein interaction. For example, we have previously measured the effect of the small molecule AEBSF on the dimerization of chymotrypsin (www.wyatt.com/files/literature/app-notes/cg-mals/inhibition-kinetics.pdf).

Q: Can you use this for characterizing DNA-DNA interaction?
Will this work for starches?
Have you seen any examples where CG-MALS was used to study aptamer-target interactions?

A: CG-MALS is not limited to protein-protein interactions. Protein-DNA interactions, DNA-DNA interactions, and polysaccharide interactions can also be measured.

CG-MALS has been applied to aptamer-target interactions. In one application, we measured the interaction between streptavidin and a DNA aptamer: www.wyatt.com/files/literature/app-notes/cg-mals/aptamer-binding.pdf. In this case, we observed a 1:2 streptavidin:aptamer stoichiometry and were able to measure the affinity of the interaction at each binding site.

Q: Are there any solvent or buffer restrictions in a CG-MALS experiment?
Can the technique be used in non-aqueous or mixed solvent solutions?
Can you measure membrane proteins in detergent solutions?

A: The Calypso and MALS detectors are compatible with a wide variety of solvents and buffers. There are practically no buffer restrictions when performing a CG-MALS experiment. However, the buffer should be clean and free of particulates, which can overwhelm the scattering signal from the molecules of interest. In the case of detergent solutions, this may mean keeping the detergent concentration below the CMC.

Q: What is the range of K_D values that can be measured?

A: The range of measurable K_D values is ~0.1 nM to ~1 mM for 100 kDa molecules. For smaller molecules (~10 kDa), the minimum K_D (tightest interaction) is ~1 nM. Even if the K_D is below the limit of detection, a CG-MALS experiment can still provide the stoichiometry of the interaction and an upper limit on the K_D (i.e., $K_D \leq 1$ nM).

Q: What are the average amounts of protein needed based on the sensitivity of the instrument and the number of required data points at various concentrations?
What is the concentration of protein typically used in the experiment, e.g., the Ab/Ag experiment?
How much material would a typical run require?

A: The amount of protein required depends on the strength of the interaction (K_D). A typical antibody-antigen interaction with K_D ~1-10 nM requires ~100 µg of each species. Weaker interactions require higher protein concentrations and, thus, a larger amount of protein.

Q: Can you measure the effect of temperature on an interaction?

A: The measurement volume in the DAWN HELEOS can be temperature controlled. With this MALS detector, a CG-MALS experiment could be repeated several times at different temperatures to assess the effect of temperature on an interaction.

Q: Is there a way to use this method if the hetero-interaction results in a precipitate/particles?

A: Light scattering measurements require all species to remain soluble and not precipitate or form insoluble particles in solution.

Q: Is there an effect of shape (e.g., compact vs. more extended or linear) on M_W determination by MALS?

Could you detect a conformation change?

Can the technique be used to study reactions of biomolecules to follow both size and stochiometry of the products formed?

A: Molecular shape does not affect molar mass determination by MALS. MALS can measure radius of gyration (r_g) for particles with r_g > 10 nm based on the variation in light scattering intensity as a function of scattering angle. However, most protein and protein complexes are smaller than this limit.

By adding simultaneous dynamic light scattering (DLS) measurements with the WyattQELS module, you can measure hydrodynamic radius (r_h) down to ~0.5 nm in addition to molar mass. Depending on the system, this may enable measurement of a large conformational change.

Q: How are K_D values determined for a CG-MALS experiment?

It's likely that various models can fit the data. Please comment on the ability to differentiate among likely models.

A: In the dilute solution limit, the light scattering intensity is proportional to the sum of the molar mass and concentration of each species in solution. For a system of molecules A and B that associate into complexes A_iB_j, we can write this relationship as:

$$\frac{R}{K^*} = \sum_{i,j} (iM_A + jM_B)^2 [A_iB_j]$$

Here, $[A_iB_j]$ are the molar concentrations of each complex, with $[A_1B_0]$ and $[A_0,B_1]$ representing the concentration of A and B monomer, respectively. M_A and M_B are the monomer molar mass of A and B. The concentrations of each species are related to the concentration of monomer via the equilibrium constant, $K_{ij} = [A_iB_j]/[A]^i[B]^j$, and the total concentration of A and B are known or measured during the experiment for each data point. The measured data are fit to the above equations to yield K_A for each complex.

Additional information can be found in the open-access book chapter "Characterization of Protein-Protein Interactions via Static and Dynamic Light Scattering" and references therein.

In some cases, multiple models can fit the data equally well. The CALYPSO software provides a Simulation tool that allows you to compare multiple models and design a follow-up experiment that would distinguish between these possible best fits.

Q: CG-MALS assumes equilibrium. What if the interactions are non-equilibrium? Is the data still valid?

Has kinetics fitting been tried on the pre-equlibrium phase of the data?

A: CG-MALS is not suited for quantifying irreversible aggregates or reaction products in solution. These are better quantified by a separation technique, such as SEC-MALS.

CG-MALS can be applied to interactions with slow kinetics (reaction times longer than a few seconds). In these cases, we can observe the molar mass (light scattering intensity) change as a function of time after the sample has been injected into the MALS detector. When the solution reaches equilibrium, the molar mass will eventually reach a plateau. These equilibrium data can be used to measure the affinity and stoichiometry of the interactions in solution. The CALYPSO software can also extrapolate to equilibrium if the amount of stop-flow time was inadequate.

Fitting kinetic data requires detailed knowledge of the binding mechanism for a particular interaction in addition to the stoichiometry. The CALYPSO software can provide a characteristic equilibration time for a kinetic process, but it does not currently support a more detailed analysis of k_{on} and k_{off}. These data may be fit by a third-party software. In a recent application note, we exported the kinetic data for the slow, covalent binding of thrombin to antithrombin III and fit the data to determine the second-order reaction rate constant (k) for this interaction:
www.wyatt.com/files/literature/app-notes/cg-mals/covalent-antithrombin.pdf.

Q: What is the upper limit of sample concentration (mg/mL) for measuring self-association?

A: There is no practical limit for the maximum sample concentration as long as the sample is soluble, and we have measured self-association for proteins at concentrations up to ~200 mg/mL. However, the Calypso II instrument is limited to delivering samples with viscosity less than ~5 cP. For samples whose viscosity exceeds 5 cP, we can perform manual CG-MALS experiments using a microcuvette. These manual data can be concatenated with automated experiments at lower concentration and lower viscosity to provide a complete picture of the interactions in a macromolecular solution.

Q: How many different macromolecules can be present in solution at the same time that can be measured by this technique?

Is it possible to study interactions between more than 2 proteins simultaneously?

Are you limited to 2, 4, or 10 compounds in solution?

A: The CALYPSO software only supports interactions between two different species: A and B. However, there is no practical limit to the number of complexes that can be formed by these species. This includes self-association complexes and hetero-association complexes.

In certain cases, inferences can be made about interactions between more than two species. For example, we measured whether an α-thrombin antibody could recognize a thrombin-antithrombin complex. In this case, thrombin and antithrombin III form a covalent association. Recognition of the thrombin-antithrombin complex by the antibody would result in an increase in the weight-average molar mass of the solution. You can read more about this system in our application note: www.wyatt.com/files/literature/app-notes/cg-mals/competive-binding-antithrombin.pdf.

Q: How MALS sensitive to impurities?

A: Multi-angle light scattering (MALS) will detect all species in solution—sample and contaminants. MALS is especially sensitive to large particle contaminants, and these must be removed by filtration and/or centrifugation prior to CG-MALS experiments. For CG-MALS experiments, all sample solutions should be ≥95% pure. A small fraction of inactive species, such as a misfolded protein or dimer aggregate, can be accounted for in the CALYPSO software.