Introduction:

CG-MALS is a technique used for characterizing interactions of macromolecules in solution by measuring and analysing the light scattering and refractive index signals from a series of solutions comprising different compositions / concentrations of those macromolecules.

CG-MALS employs a Calypso connected to a MALS instrument and optional concentration detector. Calypso performs sample preparation and delivery, combining up to three different solutions in precise mixing ratio and injecting them into the detector.

Features of Calypso II CG-MALS:

Wyatt Calypso II

- Syringe pump for appappropriate flow rate ratios.
- Vacuum degasser and filter (0.1 µm or 0.03 µm) for reliable light scattering measurements.
- Convenient sample reservoir configuration using standard conical tubes of 5, 15 or 50 mL.
- Affinity range from pM to mM.
- Coupling to MALS/QELS and dRI detectors.

Wyatt miniDAWN TREOS MALS/QELS Detector

- Uses light scattering detectors at 3 angles (49˚, 90˚, and 131˚) integrated Quasi-Elastic Light Scattering (QELS) module to calculate the absolute molecular mass and hydrodynamic radius (R_h).
- Determine the molar masses from 200 Da to 10 MDa and radii from 10 to 50 nm.

Wyatt Optilab T-rEX RI Detector

- Measures differential refractive index within a flow cell. Refractive index detection is often used for samples with weak or no UV absorption.
- Temperature range from 4 °C - 65 °C with ±0.005 °C regulation.

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Calypso II GC-MALS

Calypso II Composition Gradient Multi-Angle Light Scattering - Wyatt Technology

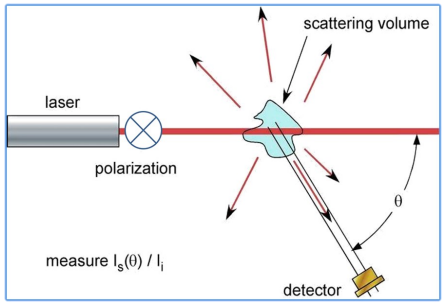
- Determination of label-free and immobilization-free protein-protein and other macromolecular interactions from pM to mM affinities.
- Stoichiometric determination of reversible self- and hetero-associations.
- Measurement of weight-average molar mass and dn/dc for biomolecules, polymers and other molecules.
- Determination of non-specific interaction parameters and colloidal stability (virial coefficients A_2/B_{22}, k_D).

Characterization of Protein-Protein Interactions

Theoretical Background

MALS Detector (miniDAWN TREOS)

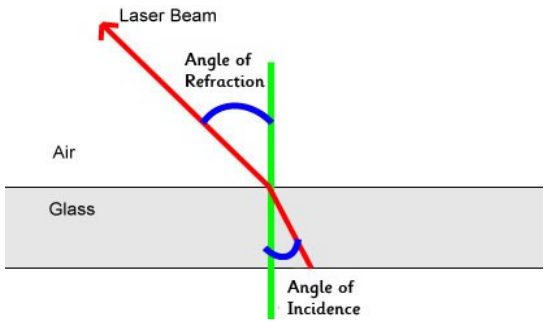
When oscillating polarized light hits a macromolecule, the resulting intensity of light scattered is proportional to concentration and the molar mass which can be determined. Random Brownian motion is corrected by averaging intensity over time while the root mean square (RMS) radius (mass-weighted mean distance from the core to each mass element) can be calculated.



Concentration Detector (Optilab T-rEX)

Differential RI can be reduced to a measure of how much light bends when it enters a medium of a different density. Absolute RI refers to when the difference is between a medium and a vacuum.

As the sample enters the flow cell, the change of the refraction angle is measured against that of the reference cell, containing the sample solvent. Using Snell's Law, this appears as a peak or a trough in the resulting chromatogram.



Reversible Self- and Hetero-association of Proteins

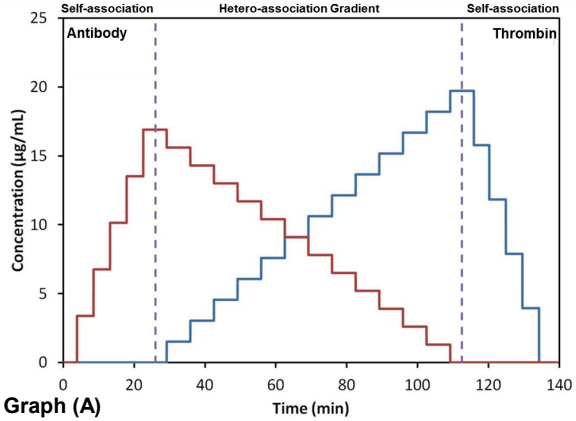
The reversible self-association and hetero-association of two proteins like antibody (Ab) and Thrombin (Thr) were determined by using the following procedure.

Hetero-association Concentration Gradient

Antibody (nM)	123	113.5	104.1	94.6	85.14	75.68	66.22	56.78	47.3	37.84	28.38	18.92	9.46	0
Thrombin (nM)	0	41.3	82.6	123.9	165.2	206.5	247.8	289.1	330.4	371.7	413	454.3	495.6	537
Mole Fraction (Thr)	0	0.267	0.445	0.567	0.66	0.732	0.789	0.84	0.875	0.908	0.936	0.96	0.98	1

Self-association Concentration Gradient

Antibody (nM)	0	24.6	49.2	73.8	98.4	123
Thrombin (nM)	537	429.6	322.2	214.8	107.4	0

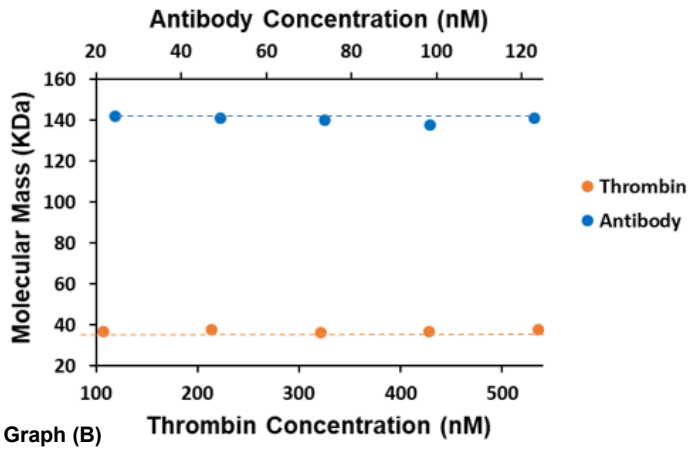


Graph (A)

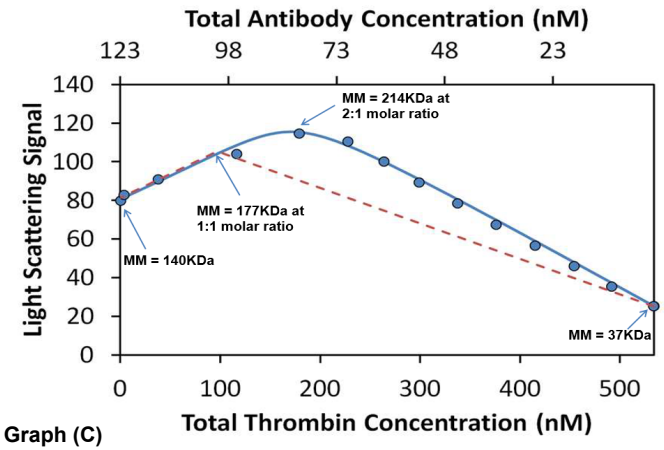
Graph (A): For the experiment, a single ascending component concentration series of Ab, followed by a crossover composition series of Ab and Thr varying from 100% Ab and 0% Thr to 0% Ab and 100% Thr, then a descending concentration series of Thr was performed. The single component stepwise gradients assist to characterize the monomer and any self-association that may be present, while the crossover gradient probes the entire range of possible hetero-association stoichiometry.

Graph (B): Shows that there is no self–association because the molecular mass (MM) of each individual protein (MM of antibody is 140 KDa and thrombin is 37 KDa) remains constant as the concentrations are varied.

Graph (C): The light scattering intensity of the thrombin-antibody complex is observed to be at its maximum value at 214 KDa. This is indicative of a 2:1 interaction. The red dotted line represents the light scattering signal if there was a 1:1 interaction. The calculated Kd value is 8 nM.



Graph (B)



Graph (C)