Solutions for Macromolecular and Nanoparticle Characterization

Waters™ | WYATT TECHNOLOGY

A Word from Dr. Philip Wyatt, Founder

It is my great pleasure to welcome you to the pages of this booklet which describe Wyatt Technology™ and its products.

For most of my adult life, I've led companies developing and producing light scattering instruments—as well as a few other analytical devices. From commercializing the very first scientific instruments incorporating lasers and microprocessors to overseeing the introduction of the very first multi-angle light scattering (MALS) detectors, I've been at the nexus of some remarkable organizations. Wyatt Technology is a private family business—not beholden to outside shareholders, private equity ownership or short-term profitability. Our first commitment is to our customers, and our mission to delight them. But in order to do this, our second pledge is to our employees who enable us to indulge in this old-fashioned approach to customer service. Without the team of extraordinarily talented, diverse and passionate people we have, we could not have thrived for the past 40 years.

More than two decades ago, I established what has become one of the crown jewels of Wyatt Technology—a course we call Light Scattering University (LSU). This class, which typically runs three days, is taught monthly by our distinguished technical staff and designed to ensure that our customers get the most out of their Wyatt instruments.

I take enormous pleasure in personally interacting with our participants during lunches and dinners, not to mention leading them through our Light Scattering Instrument Museum with a highly-personalized tour. LSU really is the starting point for our successful, life-long relationships with our customers.

I would love to have you visit us here in Santa Barbara by enrolling in an LSU class, or by planning a visit to see our company and our manufacturing facilities, as well as meeting our incredible people. In the meantime, I hope that the following pages will help you learn more about our products, which have been referenced in more than 19,000 peer-reviewed scientific papers, used by Nobel laureates and installed in most major academic and corporate macromolecular characterization laboratories in the world.

Wyatt Technology

SEC-MALS Products for HPLC & UHPLC

RT-MALS Products for Process Analytics

Dynamic & Electrophoretic Light Scattering Products

Field-Flow Fractionation

CG-MALS Products

Training, Service & Support

This Time, It's Personal

For over forty years we have brought family commitment to the analytical industry business, and that's not going away.

From the beginning, Wyatt Technology has been a family – both literally, through its founder Dr. Philip Wyatt and his sons Geofrey and Clifford, and metaphorically as well. All of our customers and staff are considered part of the extended family, and we take the work of our customers personally; when they succeed, we couldn't be prouder.

Through four decades, Wyatt Technology has grown organically and has recently joined Waters™ Corporation, another organization that primarily focuses on the customers and their science. We remain committed to our mission of delighting our customers. Developing and manufacturing our own hardware and software is our passion, which we personalize through peer-level customer contact, Light Scattering University lunches and dinners and unprecedented relationship-building.

We invite you to join us and experience our refreshingly different corporate philosophy of emphasizing *you!*

Clifford D. Wyatt, former President, Wyatt Technology
Dr. Philip J. Wyatt, Founder and former Chairman of the Board, Wyatt Technology
Dr. Udit Batra, President & CEO, Waters Corporation
Geofrey K. Wyatt, former CEO, Wyatt Technology

OUR PURPOSE

We delight our customers
by providing outstanding analytical tools,
as well as unparalleled levels of personal service.
We support life-enhancing macromolecular
and nanoparticle science.

Growth & Cutting-Edge Technological Innovation

2020

2023 DynaPro™ ZetaStar™ is the first DLS/SLS/ELS instrument offering both walk-up and automated measurement modes
2022 Next-generation DynaPro NanoStar™ II introduces walk-up measurements with DYNAMICS™ Touch™
2020 Next-generation Eclipse™ adds dilution control, EAF4 and advanced ease of use
2019 ultraDAWN™ for process monitoring and control is introduced
2019 Next-generation DAWN™, miniDAWN™, microDAWN™, Optilab™ and ViscoStar™ instruments are introduced
2018 Integration of Field-Flow Fractionation technology with acquisition of Superon GmbH
2017 DynaPro Plate Reader III, with true molar mass capability, launched

2010

2017 miniDAWN TREOS™ II, with field-serviceability and upgradeability to µDAWN™, introduced
2017 DAWN HELEOS™ II wins Scientist's Choice Award® from SelectScience for Instrument of the Year
2017 Wyatt Technology™ expands headquarters by 50% to 45,000+ square feet
2016 Completely re-engineered ViscoStar III revealed
2014 First MALS detector for UHPLC, the µDAWN, featured
2011 Tibbetts Award for exemplifying notable lifetime achievements in innovation
2010 Mobius™ zeta potential instrument, first with flow through and pressurized capabilities, introduced
2008 DynaPro NanoStar DLS detector introduced with front panel computer

2000

2008 Scientist Magazine Award: Best Places to Work in Industry—also awarded in 2009, 2010 and 2012
2007 miniDAWN TREOS introduced with front panel computer
2007 Calypso™ (Composition-Gradient) system introduced for reversible and irreversible interactions
2005 R&D 100 Award for Optilab rEX™ RI detector
2005 DAWN HELEOS (18-angle) instrument introduced with front panel computer
2005 First DynaPro Plate Reader for automated DLS measurements introduced
2004 Optilab rEX (Extended Range) array diode RI detector arrives
2004 Wyatt Technology China office formed
2004 ViscoStar viscometer enters the market
2004 ASTRA GPC software with 21 CFR Part 11 compliance released

1990

1999 DAWN EOS (18-angle Enhanced Optical System) introduced with solid state laser
1995 Optilab DSP (Digital Signal Processing) RI detector comes to market
1994 Major sensitivity improvements arrive with the DAWN DSP (Digital Signal Processing)
1993 Wyatt Technology Europe formed in Germany
1992 miniDAWN (3-angle) GPC detector introduced with solid state laser

1980

1989 ASTRA™ 1.0 GPC software released
1988 Optilab differential refractive index detector line acquired from Perstorp Analytical, Sweden
1986 First high temperature (150 °C) DAWN F instrument placed
1985 DAWN B (Batch-mode) instrument introduced
1984 AMOCO Production Company orders 1st DAWN 16-angle GPC detector
1983 SC Johnson & Son orders 1st DAWN F with 7-angle flow-through detector
1982 Wyatt Technology formed with $50,000 contract to detect toxicants in drinking water

Wyatt Technology's Rich History

In 1970, Wyatt Technology's founder, Philip Wyatt, and some of his colleagues, formed a company that developed the world's very first multi-angle light scattering instruments using a laser as the light source. In addition, they developed instrumentation that was the first to incorporate microprocessors.

Since those days, Dr. Wyatt spearheaded the definition and redefinition of state-of-the-art analytical instrumentation at Wyatt Technology. The company's light scattering lore runs deep, and with a team of now more than 200 people, including many of which are Ph.D.'s, we ensure that Dr. Wyatt's expertise is multiplied and perpetuated.

What can I measure?

Particle Concentration

Physical titer and quantitative particle size distributions

Molar Mass

Absolute molecular weight from 200 to 1,000,000,000 g/mol

Conformation

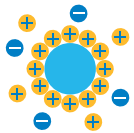
Shape, structure and branching parameters

Size

RMS radius from 10 to 500 nm and hydrodynamic radius from 0.2 to 5,000 nm

Interactions

Binding affinity from pM to mM and absolute stoichiometry of complex interactions



Charge

Zeta potential and net molecular charge for particles from 2 nm to 100 µm

Conjugation

Molecular weight and fraction of each constituent in a binary conjugate

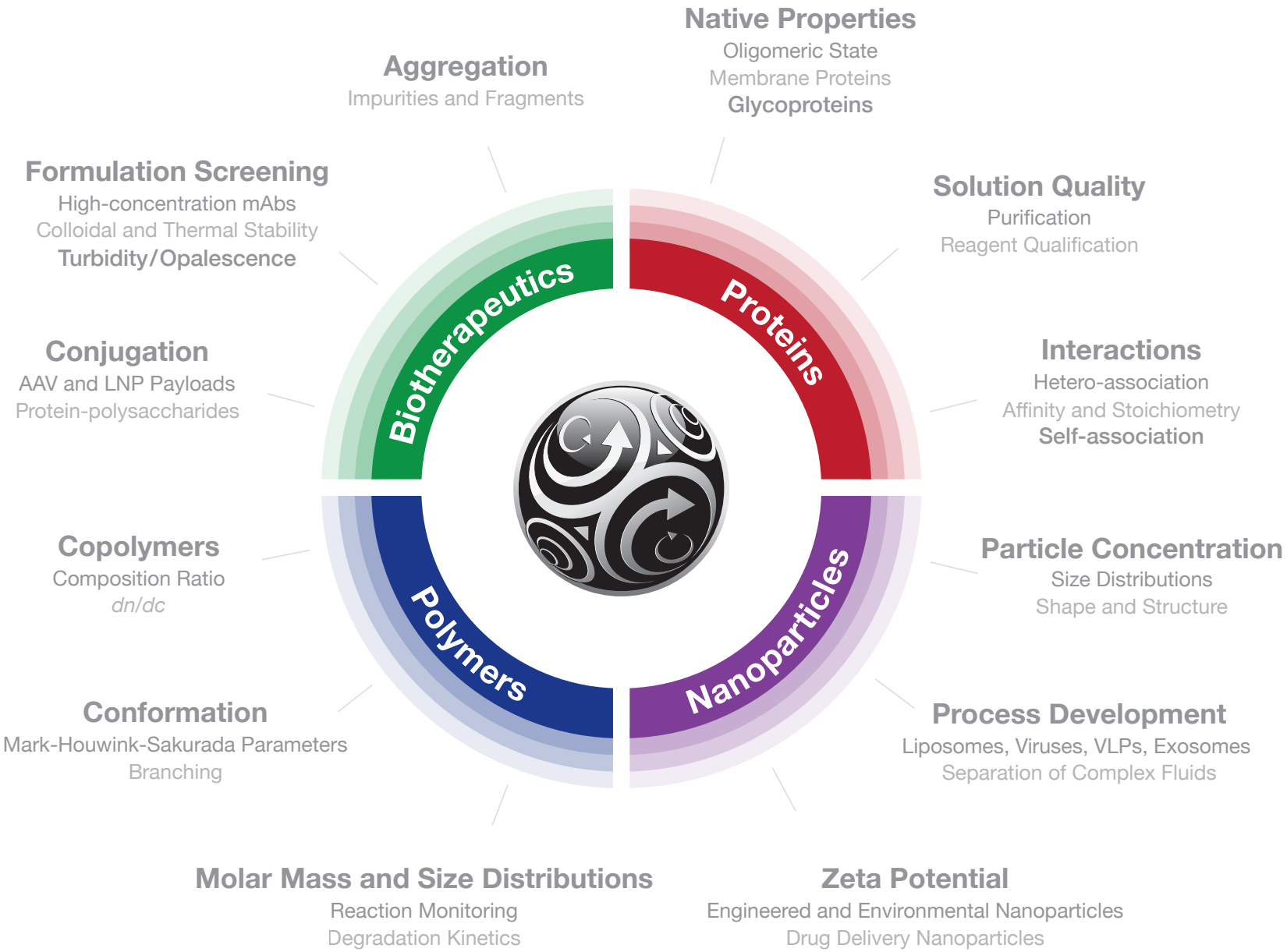
Turbidity

Turbidity/opalescence from 1 to 80 NTU

Payload

Content of gene vectors or nanoparticles

What can I analyze?




DASHBOARD
HISTORY
SETTINGS
System Status
Noise
6.77
µV
Low
Wander
7.05
µV
Low
Drift
10.84
µV/min
Low
LASER
Off
On
DLS POWER
Off
On
COMET
Off
On
ORBIT
Waste
Recycle
FLOW CELL TEMPERATURE
GOOD
Current:
23.60 °C
Set Point:
24.39 °C
SET
Time:
30 min
Main Chart
Y LEFT
Y RIGHT
LS Detector 11 (V)
Forward Monitor (%)
0.12
0.1
0.08
0.06
0.04
0.02
0
102.7
102.6
102.5
102.4
102.3
102.2
102.1
3:25 PM
3:35 PM
3:45 PM
Time
IN
STANDARD
OUT
DAWN
WYATT
TECHNOLOGY

SEC-MALS Products

For HPLC & UHPLC

Characterize molar mass, size and conformation

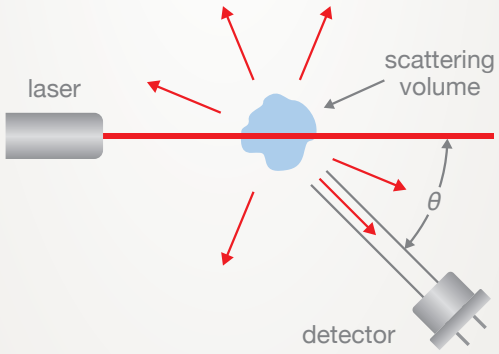
MALS

multi-angle light scattering

Based on first principles, MALS determines the molar mass and size of macromolecules and nanoparticles in solution.

Characterize:

- Peptides and proteins
- Conjugated proteins
- Polymers and copolymers
- Nanoparticles
- Viral vectors and VLPs
- Liposomes, LNPs and exosomes



Multi-angle light scattering determines molar mass from the scattered intensity and the molecular radius from the angular scattering pattern.

DAWN

Premier family of MALS detectors

Choose between DAWN for the highest sensitivity and widest measurement range or miniDAWN for fundamental analysis of proteins and small polymers. Also available is microDAWN, uniquely suited for UHPLC.

SCIENTISTS' CHOICE AWARDS WINNER

Wyatt's MALS detectors interface to most industry-standard HPLC, GPC and FPLC systems.

	DAWN	miniDAWN	microDAWN
Description	The premier SEC-MALS detector for absolute molar mass and size, offering the highest sensitivity	The best in fundamental multi-angle light scattering	The only MALS detector uniquely designed for UHPLC with superb sensitivity
Applications	Peptides, proteins and polymers; plus viruses, vesicles and nanoparticles up to 500 nm in radius	Peptides, proteins small polymers, small viruses, VLPs and nanoparticles	Peptides, proteins and small polymers compatible with UHPLC
Molar Mass Range	200 Da to 1 GDa	200 Da to 10 MDa (proteins) or 1 MDa (polymers)	200 Da to 10 MDa (proteins) or 1 MDa (polymers)
Molecular Size Range (MALS — R_g)	10 to 500 nm	10 to 50 nm	10 to 50 nm
Molecular Size Range (DLS —R_h)*	Flow: 0.5 to 300 nm Batch: 0.5 nm to 1 µm	Flow: 0.5 to 50 nm Batch: 0.5 nm to 1 µm	Flow: 0.5 to 30 nm 0.5 nm to 1 µm
Compatibility	HPLC	HPLC	UHPLC/APC
Flow Cell	Standard and high-temperature flow cells, COMET cell cleaning module included	Standard flow cell, COMET cell cleaning module included	Micro flow cell, COMET cell cleaning module included
Detectors	18 angles	3 angles	3 angles
MALS Sensitivity: BSA in Aqueous Buffer	0.2 µg typical, 30 cm SEC column	0.5 µg typical, 30 cm SEC column	70 ng typical, 15 cm UHPLC-SEC column
MALS Sensitivity: 100 kDa Polystyrene in THF	10 ng typical, 30 cm GPC column	25 ng typical, 30 cm GPC column	3.5 ng typical, 15 cm UHPLC-SEC column
Temperature Control	Ambient; Heated/cooled from -15 °C to 150 °C; Ultra-high: 20 °C to 210 °C	Ambient only	Ambient only
Options	Temperature control, Fluorescent polymer configuration, WyattQELS embedded DLS	WyattQELS embedded DLS	WyattQELS embedded DLS

* Size range will depend on flow rate, application and instrument configuration.

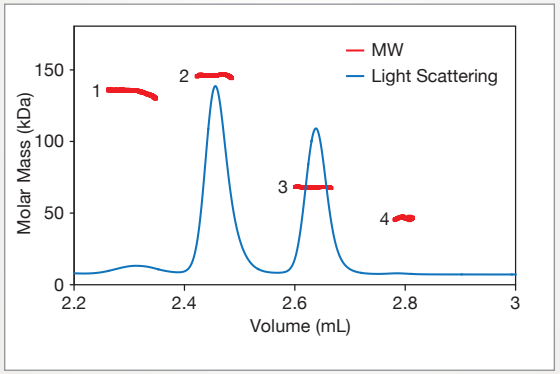
SEC-MALS

size exclusion chromatography combined with multi-angle light scattering

SEC-MALS is an absolute method that does not rely on column calibration for analyzing:

- Molar mass
- Size distributions
- Oligomeric state
- Conformation
- Conjugation ratio
- Polymer branching

SEC-MALS combines MALS, intrinsic viscosity (IV) and differential refractive index (dRI) instruments with SEC separation.



Even though Peak 1 elutes earliest, MALS shows that it does not have the largest molar mass for this example of protein aggregates and fragments.

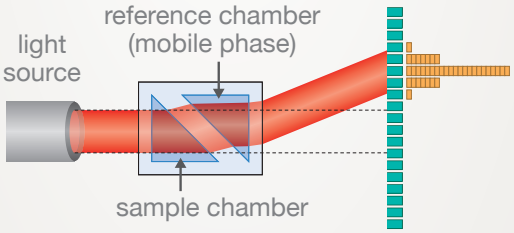
dRI

differential refractive index

dRI is a universal concentration measurement technique that does not depend on chromophores or fluorophores.

Optilab online dRI instruments are used in:

- MALS analysis of molar mass
- Intrinsic viscosity determination for polymer conformation and branching
- Triple-detection characterization of copolymers and protein conjugates
- Basic quantitation of chromatographic peaks
- Measurement of *dn/dc* in different mobile phases
- Determination of solvent absolute refractive index



Optilab's 512-detector array means it can reliably quantify a tiny peak at the nanogram level superimposed on a milligram-level peak!

Optilab

Extended dRI measurement range

The only RI detector designed to operate at the same wavelength as the MALS detector for *dn/dc* measurements, Optilab is available in a variety of configurations depending on your application. It can also measure the absolute refractive index (aRI) of the solvent.

	Optilab	Optilab HC	microOptilab
Description	dRI detector for standard HPLC, offering the highest sensitivity and dynamic range	dRI detector for CG-MALS, protein purification and other high-concentration analyses	dRI detector for UHPLC, offering the highest sensitivity and dynamic range
Application	Quantify a few ng/mL up to 25 mg/mL	Measure proteins up to 180 mg/mL	UHPLC/APC
dRI Range	-4.7×10^{-3} RIU to $+4.7\times10^{-3}$ RIU (refractive index unit)	-2.6×10^{-3} RIU to $+3.4\times10^{-2}$ RIU	-4.7×10^{-3} RIU to $+4.7\times10^{-3}$ RIU
Dynamic Range	12,000,000:1	23,000,000:1	6,000,000:1
dRI Sensitivity	0.75×10^{-9} RIU	1.5×10^{-9} RIU	1.5×10^{-9} RIU
aRI Range	1.2 to 1.8	1.2 to 1.8	1.2 to 1.8
aRI Sensitivity	± 0.002	± 0.002	± 0.002
Temperature Control	4 °C to 65 °C	4 °C to 65 °C	4 °C to 65 °C

ViscoStar

Unsurpassed differential viscometer

Incorporating patented thermal bridge balancing, as well as proprietary technology to suppress pressure pulse noise and temperature gradients, ViscoStar offers the best performance in differential viscosity measurements.

	ViscoStar	microViscoStar
Description	The ultimate differential viscometer for GPC	Differential viscometer for UHPLC/APC
Applications	Polymers below ~ 1 MDa for conformational analysis; all polymers for Mark-Houwink-Sakurada parameters	Polymers suitable for UHPLC-SEC separation
Sensitivity	0.1 µg of 100 kDa polystyrene in THF	5 ng of 100 kDa polystyrene in THF
Dynamic Range	135,000:1	135,000:1
Drift	2.5 Pa/hr	1.25 Pa/hr
Temperature Control	4 °C to 70 °C	4 °C to 70 °C
Capillary Bridge Tuning	Automated thermal tuning	Automated thermal tuning
Pump Pulse Suppression	Full impedance matching of the capillary bridge and proprietary software algorithms	Full impedance matching of the capillary bridge and proprietary software algorithms
Delay Column Options	8.1, 5.4 or 2.7 mL standard; 16.2 mL optional	5.4 mL

ΔP

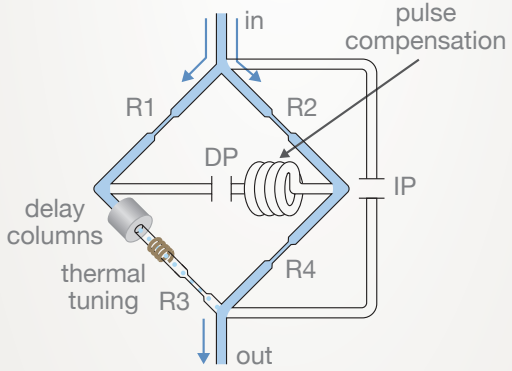
IV

intrinsic viscosity

Differential viscometers are used in conjunction with SEC to measure the specific and intrinsic viscosities of polymer solutions.

Combined with a MALS instrument, SEC-MALS-IV determines:

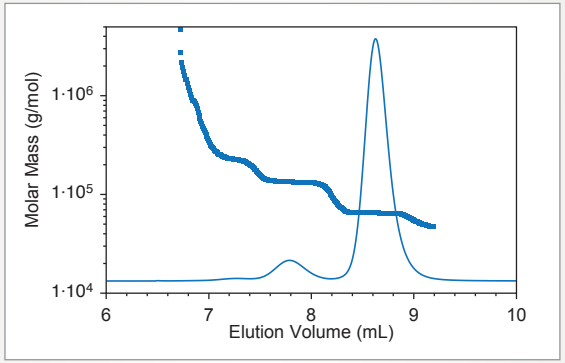
- Intrinsic viscosity
- Conformation
- Branching analysis
- Hydrodynamic radius
- Mark-Houwink-Sakurada parameters



Without delay columns, the impedance of the capillary bridge would be fully balanced. The pulse compensation element matches the additional impedance of the delay columns, eliminating the effect of pump pulses on the DP transducer.

ASTRA

advanced software
for macromolecular and
nanoparticle characterization



Absolute molar mass analysis

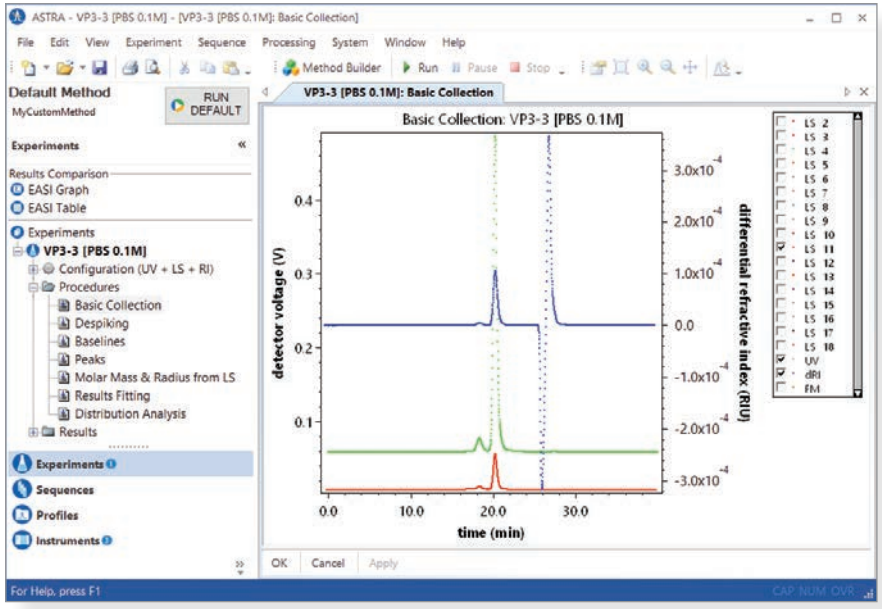
ASTRA's Band Broadening Correction accounts for interdetector dispersion to match signals from each detector in the chromatographic elution series.

This algorithm is responsible for proving uniform molecular weights across the BSA monomer, dimer and trimer peaks.

ASTRA

The premier software for analyzing macromolecules and nanoparticles by multi-angle light scattering

ASTRA integrates MALS, UV, refractive index, dynamic light scattering and intrinsic viscosity data for comprehensive characterization of the physical properties of materials in solution/suspension.



ASTRA provides absolute determination of:

- Molar mass and size
- Conformation, shape and conjugation ratio
- Differential and cumulative distributions; moments of the distribution and polydispersity
- Intrinsic viscosity and Mark-Houwink-Sakurada parameters
- Nanoparticle concentration, total viral titer; viral and drug nanocarrier payload

Compile key results:

ASTRA gives you a quick and easy overview of your most important results in one compact table.

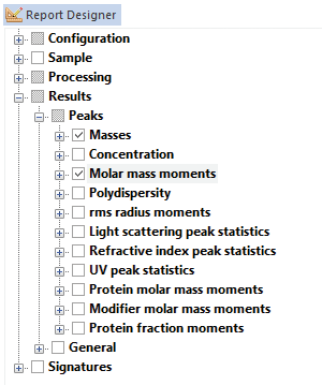
EASI Table

Experiments: All Peaks: All Abscissa: min

	Peak 1		
	Mn (kDa)	Mw (kDa)	Polydispersity (Mw/Mn)
sample01	66.8 (±0.2%)	66.8 (±0.2%)	1.00 (±0.23%)
sample02	65.9 (±0.0%)	65.9 (±0.0%)	1.00 (±0.05%)
sample03	65.7 (±0.4%)	65.7 (±0.4%)	1.00 (±0.54%)
sample04	66.6 (±0.0%)	66.6 (±0.0%)	1.00 (±0.07%)
sample05	66.8 (±0.2%)	66.8 (±0.2%)	1.00 (±0.26%)
sample06	65.8 (±0.2%)	65.8 (±0.2%)	1.00 (±0.29%)
Average	66.3	66.3	1.00
Standard deviation	0.5	0.5	0.00
% Standard deviation	0.8	0.8	0.00
Minimum	65.7	65.7	1.00
Maximum	66.8	66.8	1.00

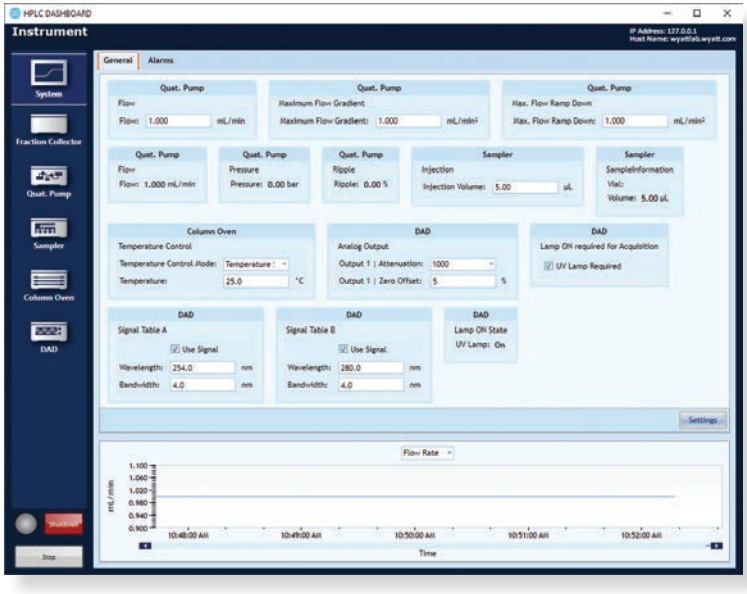
Customized reports:

ASTRA provides customized reporting options so you can export exactly the information you need. It even allows you to customize the report with your company's logo and descriptive text.



Molar mass in a single click

1. Select experiment type
2. Input parameters
3. Click 'Run'



HPLC CONNECT

- ASTRA performs all SEC-MALS control, data acquisition and analysis
- One ASTRA configuration for HPLC modules and Wyatt detectors
- Full digital synchronization of the HPLC pump, autosampler and detectors
- Direct digital acquisition of multiple wavelengths from the HPLC UV/Vis

Regulatory Compliance

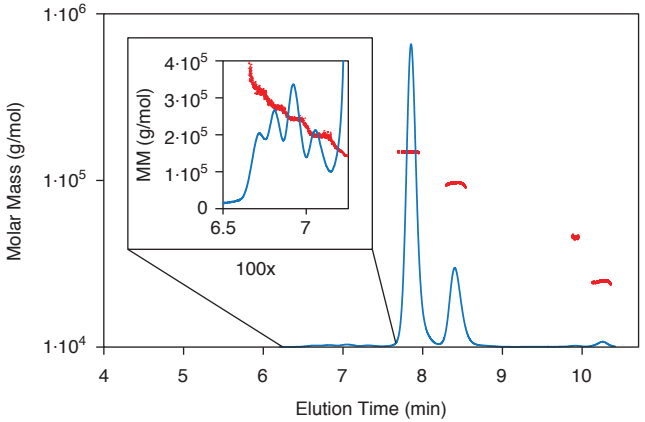
Following industry standards, ASTRA offers an optional 21 CFR Part 11 compliance package, including IQ/OQ documents and procedures.

ASTRA's Security Pack includes:

- Administrator, researcher, technician and guest access levels
- Full audit trails
- Electronic signatures
- Sign-in/sign-out during a run
- Secure SQL server database
- Local or remote database connectivity
- Data integrity validation
- Full IQ/OQ procedures and documentation validation

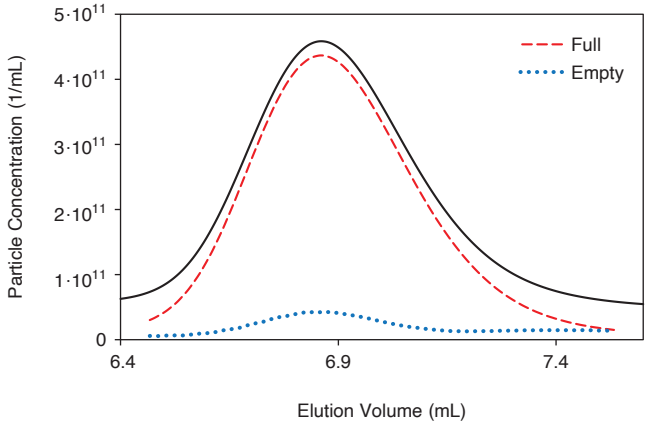
SEC-MALS Applications

Aggregates and Fragments



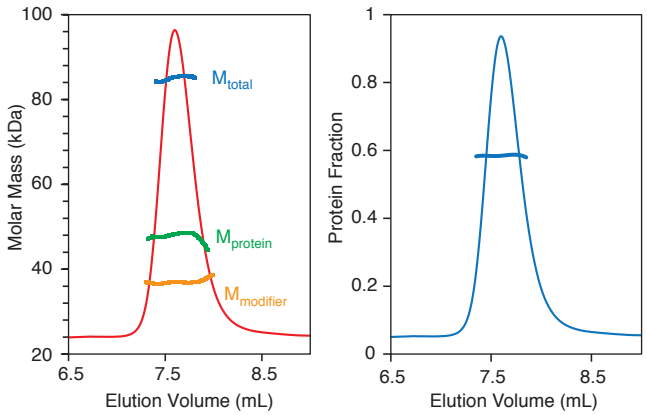
The power of UHPLC for separating aggregates and fragments combines with MALS to unequivocally identify small quantities of impurities in an IgG sample. Each of the aggregate peaks shown in the 100x inset represent a fraction of one percent of the monomer total mass yet is well-quantified by MALS.

Viral Vector Particle Concentrations



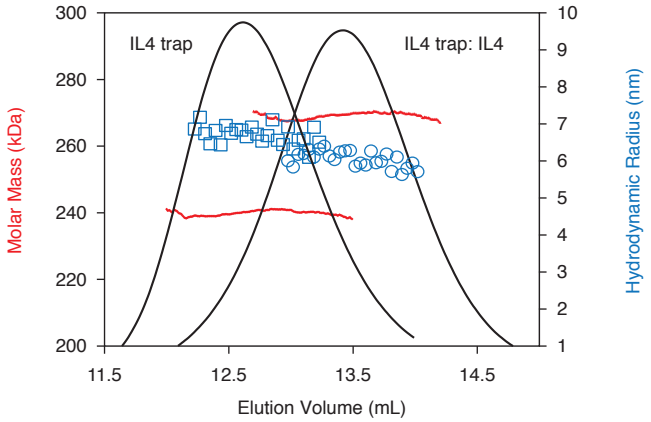
The *Viral Vector Analysis* method determines multiple critical quality attributes. This graph shows an overlay of the size-exclusion chromatogram of an adeno-associated virus (black solid line) with particle concentrations determined at each data slice for sub-populations of full capsids (red, long dash) and empty capsids (blue, dotted).

Protein Conjugate and Copolymer Analysis



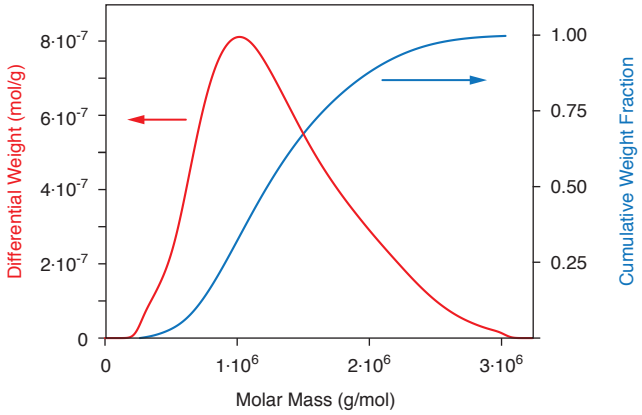
ASTRA's Protein Conjugate algorithm makes use of data from MALS, UV and RI detectors to characterize conjugated proteins and copolymers. This analysis determines the molecular weights of the protein, modifier and complete conjugate as well as average extinction coefficient and *dn/dc*.

Protein Complexes and Conformations



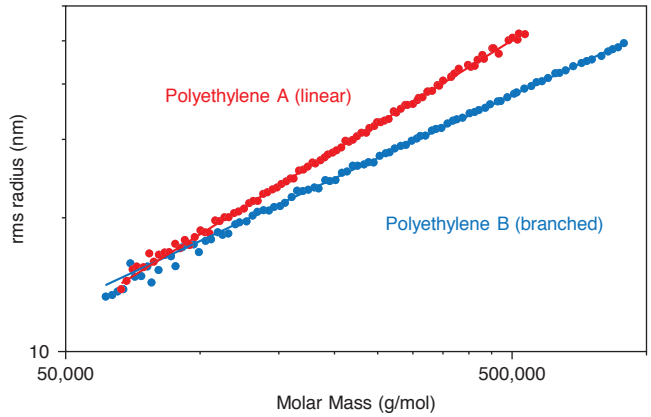
Pure interleukin 4 trap (IL4-trap) elutes earlier than the IL4 : IL4-trap complex, despite its lower molecular weight. MALS MW analysis (small red symbols) indicates the expected MW values. Online DLS R_h data (open blue symbols) show the reason for the late elution: IL4 stabilizes the trap to form a compact IL4 : IL4-trap complex.

Molar Mass and Size Distributions



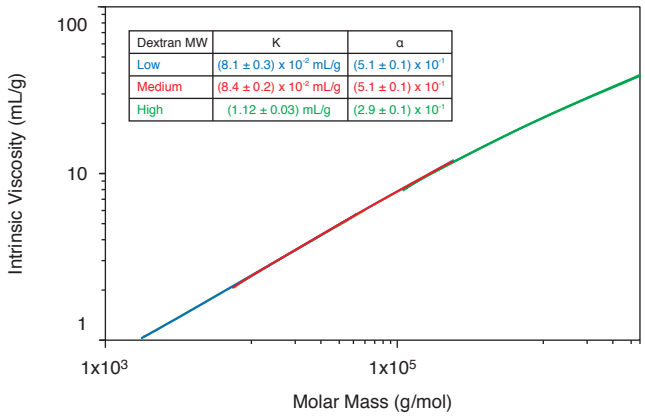
In addition to plotting the molar mass and size determined by multi-angle light scattering over a chromatogram or fractogram, ASTRA can convert the data into distributions. These graphs show differential and cumulative distributions of molar mass as measured for hyaluronic acid.

Polymer Branching



A MALS instrument measures rms radius vs. molar mass to reveal a polymer's branching properties. Here, the branching of Polyethylene B is apparent by its significantly lower slope in relation to Polyethylene A, which is known to be linear.

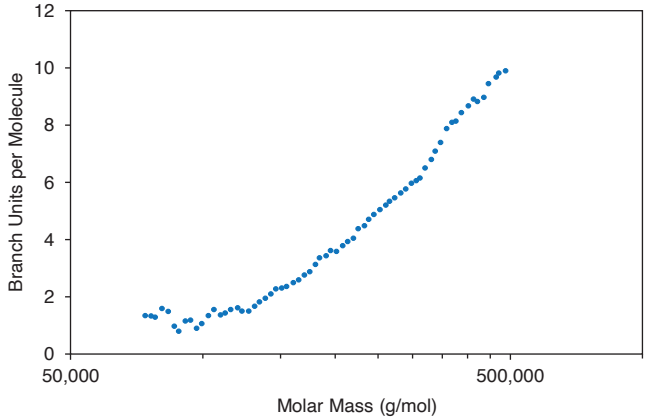
Conformational Change with MW



Dextran MW	K	α
Low	(8.1 ± 0.3) x 10^{-2} mL/g	(5.1 ± 0.1) x 10^{-1}
Medium	(8.4 ± 0.2) x 10^{-2} mL/g	(5.1 ± 0.1) x 10^{-1}
High	(1.12 ± 0.03) mL/g	(2.9 ± 0.1) x 10^{-1}

A Mark-Houwink-Sakurada (MHS) plot shows intrinsic viscosity as a function of molar mass—revealing the polymer conformation. The MHS plots of low, medium and high MW dextrans, shown here, indicate conformational change with increasing molar mass of the molecules.

Branching Calculations



ASTRA compares linear and branched polymers in order to determine branching ratio. The data in the top chart (Polymer Branching) were analyzed to yield the average number of branching units per molecule and its dependence on molar mass. Branching begins above a molar mass of ~100,000 g/mol.


DASHBOARD
GRAPH
HISTORY
SETTINGS
Y LEFT
Y RIGHT
Molecular Weight, MW (kDa)
Radius Rg (nm)
Time (minutes)
Current Mw:
686.1 kDa
Current Rg:
10.2 nm
Run Status
Running
IN
STANDARD
OUT
ultraDAWN
WYATT
TECHNOLOGY

RT-MALS Products

For Process Analytics

Monitor molar mass, size and particle concentration

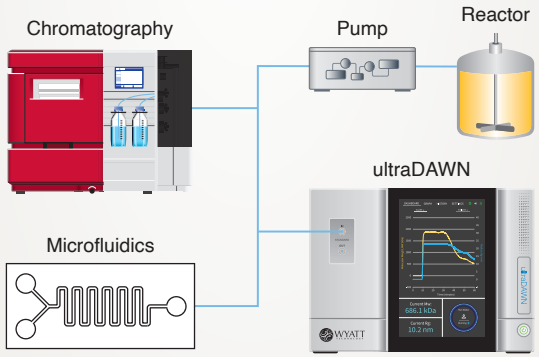
RT-MALS

real-time multi-angle light scattering

RT-MALS monitors molar mass, size and particle concentration for quality assurance and control of production processes.

Optimize and control:

- Viral vector downstream processing
- Nano-pharmaceutical formulation
- Protein or nucleic acid purification
- Non-viral vector production
- De-polymerization and conjugation



ultraDAWN supports in-line measurements with flow rates up to 150 mL/min. On-line operation utilizes a pump to transfer fluid from a vessel or to sample a high-flow-rate process to the ultraDAWN.

ultraDAWN

Measure the product, not the process

ultraDAWN is a breakthrough in process analytical technology for nanomedicines, gene vectors, vaccines and biotherapeutics. It measures key product attributes in real time for process development, scale-up and production.

OBSERVER Software

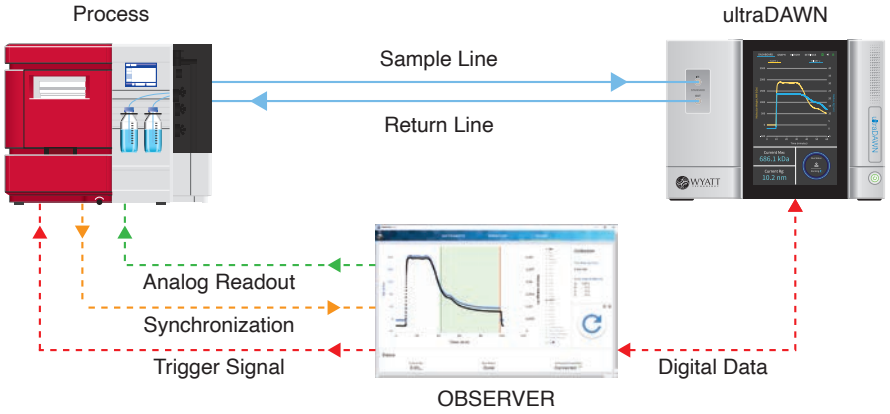
Built-in workflows for flexible process integration

OBSERVER monitors MALS data from the ultraDAWN, calculating MW, radius and/or particle concentration up to 300 times per minute. PAT workflows let users specify product attribute criteria for trigger activation.

OBSERVER communicates digitally via OPC-UA with process-control software such as SIPAT or DeltaV, or via analog signals with lab-scale equipment such as FPLC, TFF or microfluidic nanoparticle production.

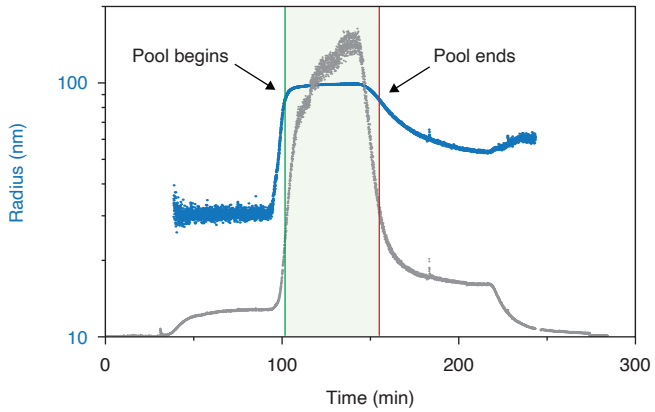
Measurement capabilities for ultraDAWN:

- **Macromolecules:** Weight-average molar mass from 10^3 to 10^9 g/mol and rms radius from 10 to 250 nm
- **Nanoparticles:** z-average radius from 10 to 250 nm, and corresponding particle concentration
- **Viral Vectors:** Vg/Cp (full:total ratio); full, empty and total viral concentrations; capsid, genome and total molar masses. For viruses and VLPs smaller than 20 nm in radius



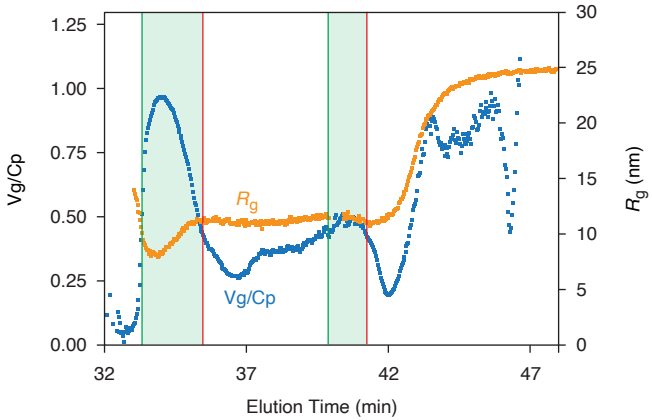
RT-MALS Applications

Chromatographic purification of viruses



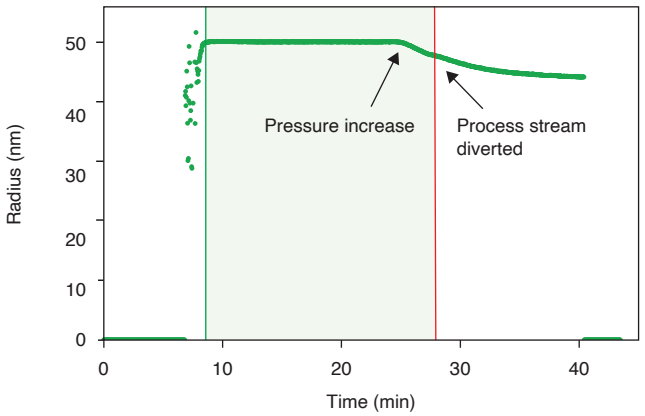
In an ion-exchange purification process, the viruses are distinguished by their size, which is much larger than other species. OBSERVER triggers pooling of fractions for which the radius is between 85 and 105 nm. Final particle concentration and total particle count are calculated as well.

AAV capsid content and aggregation



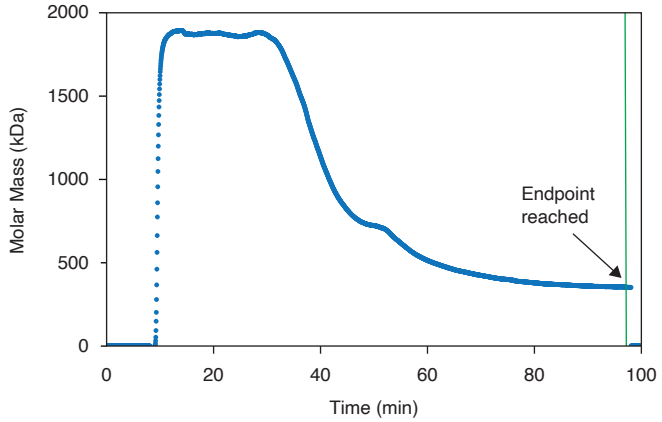
Enrichment of full AAV vectors is often done by ion-exchange chromatography, where RT-MALS determines the full:total ratio Vg/Cp, empty and full titers and more. Increasing particle radius is useful in identifying aggregates. Here the trigger was set when Vg/Cp was between 0.4 and 1.2.

In-line monitoring of liposome size



Heterogeneous liposomes with sizes from 150 nm to 800 nm were passed through a microfluidizer in order to reduce the diameter to 100 nm, then to the ultraDAWN. OBSERVER was programmed to trigger a diversion of the exit stream if particle size deviated by 2 nm from the nominal CQA value.

End-point determination of a polysaccharide depolymerization process



A critical depolymerization step must reduce the polysaccharide's initial molar mass M_w from over 1800 kDa down to less than 350 kDa. RT-MALS tracked reduction of M_w and triggered reaction shutdown once it fell below 350 kDa.

The case studies are for illustrative purposes only and based on a theorized use case for ultraDAWN. They do not represent specific customer experiments, results or conclusions.


DYNAMICS Touch
Measure Size
Molar Mass & Size
Particle Conc. & Size
Results
Presets
Settings
ELS & Size
Particle Conc. & ELS
ZetaStar
WYATT
TECHNOLOGY
DynaPro

DLS & ELS Products

Measure in Cuvettes and Well Plates

Characterize size, zeta potential, turbidity and stability

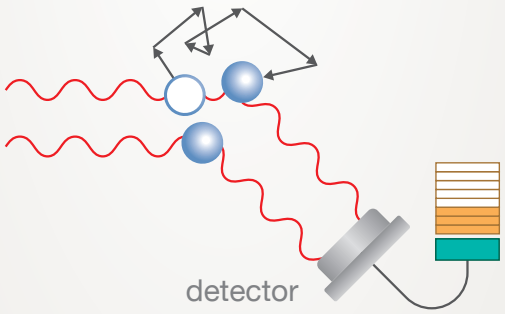
DLS

dynamic light scattering

DLS determines the diffusion coefficients, size and size distributions of particles in a fluid by measuring the light intensity fluctuations arising from their Brownian motion.

In addition to basic sizing applications for sub-micrometer macromolecules and nanoparticles, DLS measures:

- Quality
- Aggregation
- Stability
- Propensity for aggregation
- Particle concentration
- Turbidity



Brownian motion of sub-micrometer particles gives rise to intensity fluctuations in the scattered light. The rate of fluctuation is analyzed to determine the diffusion coefficient.

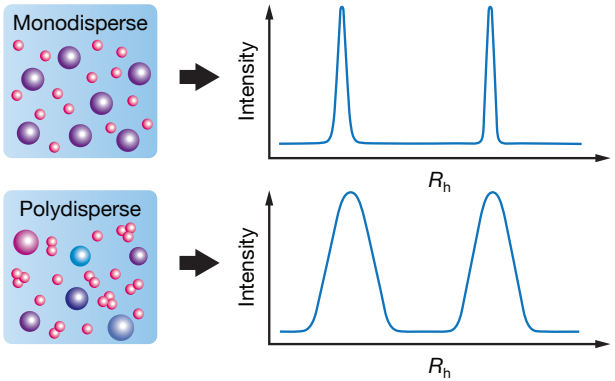
DynaPro

Unrivaled DLS/SLS detection

Perform fully automated DLS and SLS with the breakthrough DynaPro Plate Reader III in standard 96, 384 or 1536 well plates or use the NanoStar cuvette-based instrument for minimum sample volume and maximum results.

	DynaPro Plate Reader III	DynaPro NanoStar II	WyattQELS
Description	Automated DLS measured directly in standard microwell plates	Traditional cuvette-based DLS, walk-up operation	Embedded DLS module for any Wyatt MALS detector
Application	High-throughput screening and other automated measurements of multiple samples	Low-volume, high-quality measurements of size, MW and nanoparticle concentration. Also supports online measurements.	Online DLS for high-resolution size distributions, simultaneous with MALS MW analysis
Plate Scan Time	As little as 1.5 hours for a 384 well plate	n/a	n/a
Hydrodynamic Radius Range (R_h)	0.5 nm to 1 µm	0.2 nm to 1 µm	Flow: see page 11 Batch: 0.5 nm to 1 µm
Sensitivity (R_h)	0.125 mg/mL lysozyme	0.1 mg/mL lysozyme	0.1 mg/mL lysozyme
Molar Mass Range	1000 g/mol to 10^6 g/mol*	300 g/mol to 10^7 g/mol†	n/a
Turbidity/Opalescence	n/a	1 to 80 NTU	n/a
Minimum Sample Volume	4 µL (1536 well plate), 10 µL (384 well plate), 60 µL (96 well plate)	2 µL (quartz cuvette), 4 µL (disposable cuvette)	Flow: n/a DAWN microCuvette: 10 µL Flow cell: 300 µL
Temperature Control	4 °C to 85 °C	-10 °C to +120 °C	Depends on MALS detector

*Upper limit depends on conformation: It is limited to a maximum R_h of 12 nm. †Upper limit depends on conformation: It is limited to a maximum R_h of 50 nm.



DLS determines size distributions without fractionation, providing polydispersity estimates as well as hydrodynamic radii.

Automation	Automated analysis of dozens of samples with an autosampler
Additional Options	• Disposable Flow Cell • Dip electrode cell • Disposable cuvettes for DLS • MALS connectivity kit
Fluorescence Suppression	• 785 nm laser rarely excites fluorescence

DynaPro ZetaStar

Walk-up or automated measurements of size, particle concentration and zeta potential

Designed for the casual user and expert alike, the ZetaStar is the only DLS/SLS/ELS detector offering simultaneous DLS and ELS, and a pressurizable flow cell for zeta potential in high-salt buffers. The intuitive touch-screen app guides users through each type of analysis.

	DynaPro ZetaStar
Description	DLS/SLS/ELS instrument for batch, flow mode and online measurements
Application	Particle size & concentration, polydispersity, zeta potential, molar mass and turbidity
Hydrodynamic Radius Range (R_h)	0.2 nm to 400 µm (flow cell), 0.2 nm to 1000 nm (cuvette)
DLS Sensitivity	90°: 0.1 mg/mL lysozyme Backscatter: 1 mg/mL lysozyme
ELS Sensitivity	Dip or disposable cell: 2 mg/mL BSA Flow cell: 1 mg/mL lysozyme
ELS Conductivity Range	0 to 7 mS/cm; 0 to 100 mS/cm with flow cell pressurization
Molar Mass Range	300 to $1x10^7$ g/mol†
Turbidity Range	0 to 100 NTU
Minimum Sample Volume	4 µL (DLS, disposable cuv.), 2 µL, 45 µL (DLS, quartz cuv.), 65 µL (ELS, quartz cuv.), 350 µL (flow cell)
Temperature Control	-10 °C to 120 °C*

*4 °C to 70 °C for ELS measurements. †Upper limit depends on conformation: It is limited to a maximum R_h of 50 nm.

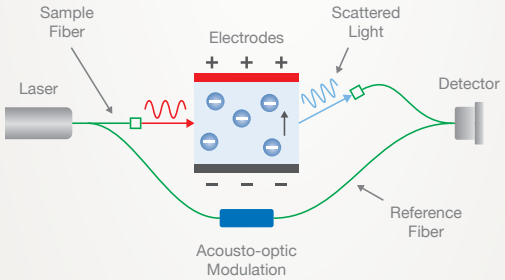
ELS

electrophoretic light scattering

ELS determines the electrophoretic mobility of particles in a fluid by measuring their velocity under an applied electric field. With additional measurement of R_h by DLS, zeta potential and the net charge are calculated.

ELS is used to study:

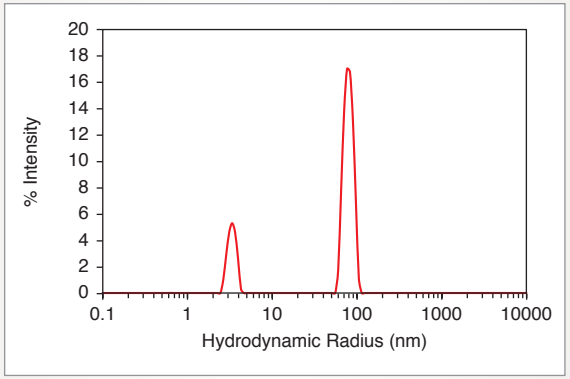
- Stability against flocculation of colloids and emulsions
- The isoelectric point of protein formulations in native formulation buffer or under physiological conditions
- Surface charges that impact gene and drug delivery



Wyatt's advanced FIDELIS (fiber-Interferometric Doppler electrophoretic light scattering) technology employs fiber-coupled elements, including high-frequency acousto-optic phase modulators, for a robust ELS optical system providing excellent sensitivity and speed.

DYNAMICS

comprehensive software for dynamic and electrophoretic light scattering



Size distributions from sub-nanometers to micrometers

Dynamic light scattering determines size distributions without any separation. This regularization graph shows the presence of an 80 nm nanoparticle in a protein solution. The particle concentration of each peak may be determined and displayed as well.

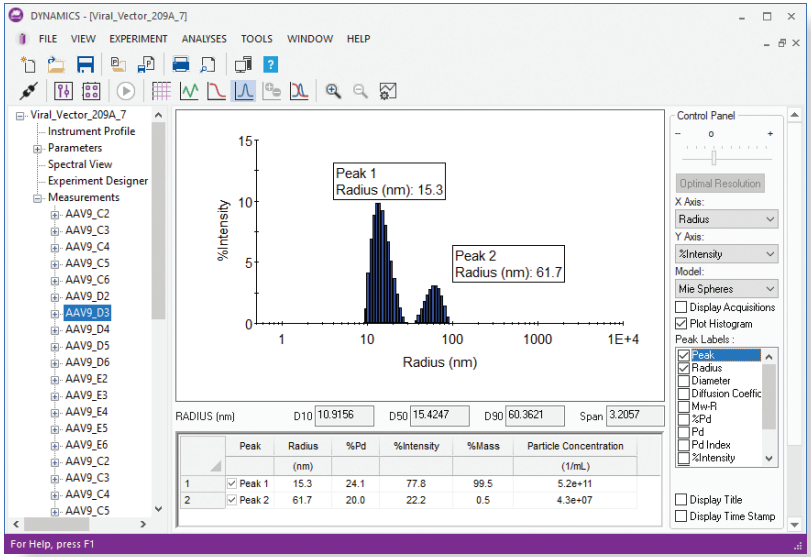
Essential Size and Zeta Potential

Intuitive yet powerful, DYNAMICS gives you access to all the information needed to ensure correct and thorough analysis of dynamic light scattering (DLS) and electrophoretic light scattering (ELS) data:

- Autocorrelation function from raw DLS data
- Size distributions
- Datalog table of all parameters, results and goodness-of-fit indicators
- Raw electrophoresis data for zeta potential analysis
- Data quality assessment and assistance

Regulatory Compliance

DYNAMICS offers an optional 21 CFR Part 11 compliance package, including IQ/OQ documents and procedures.



DYNAMICS Regularization View offers many ways to analyze and display multimodal size distributions.

From Mobility to Stability

Collect, display and analyze batch DLS, ELS and static light scattering (SLS) measurements.

Size and Size Distributions

Average size from cumulants, distributions from regularization, polydispersity index or cumulative size distributions D10/D50/D90. Analyze by %Intensity, %Mass or %Number.

Zeta Potential or Net Charge

Electrophoretic mobility for nanoparticles or proteins vs. pH or salt concentration.

Particle Concentration

Particles/mL for each peak in the distribution.

Molar Mass

Average solution molecular weight from SLS or estimated from DLS.

Turbidity/Opalescence

Nephelometric measurements from SLS

Thermal and Parametric Analysis

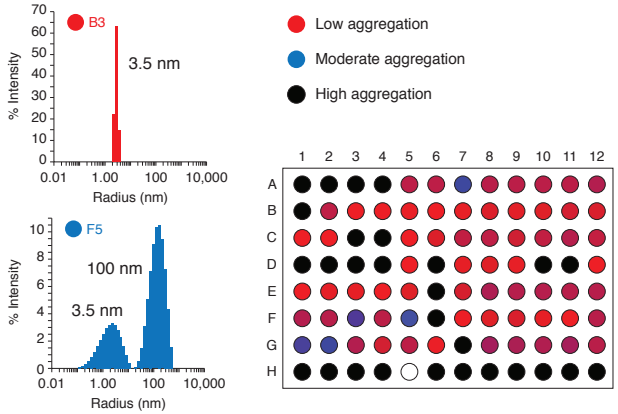
Determine dependence on temperature, concentration or time for stability analysis.

Full Automation

- Program automated temperature profiles
- Select wells and measurement cycles in the DynaPro Plate Reader
- Program vial selection in a ZetaStar autosampler sequence

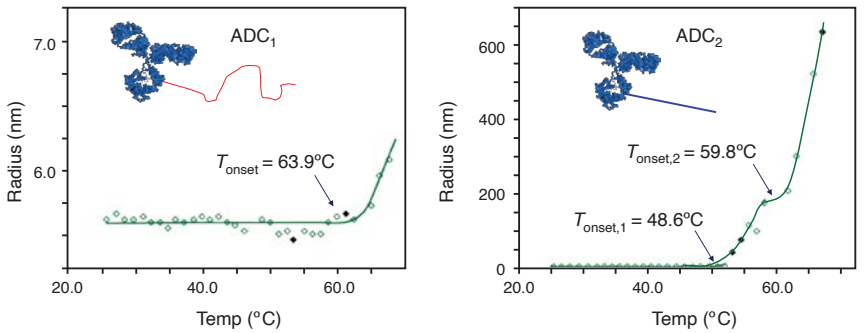
DLS Applications

Aggregation in a 96 Well Plate



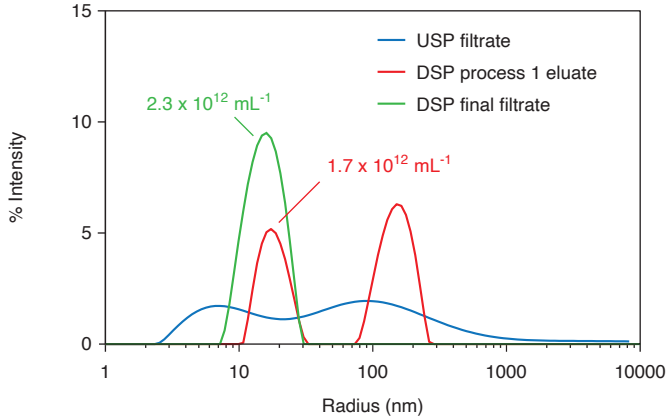
The SpectralView feature in DYNAMICS supports color-coded visualization of the results of a plate scan, which might include hundreds of samples. Here the visualization represents the degree of aggregation for a rapid, intuitive assessment of the optimal formulation.

Conformational Stability



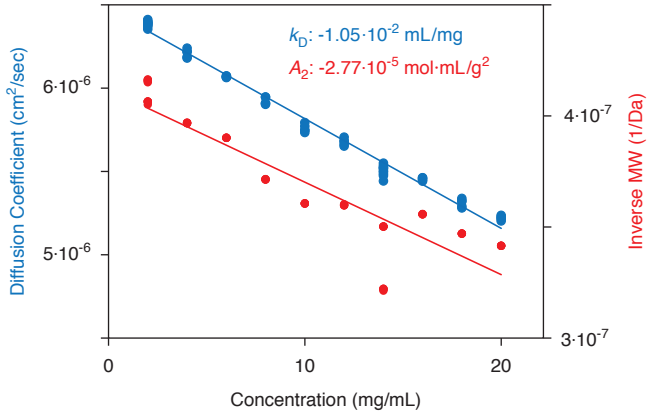
Conjugating the same monoclonal antibody and drug via different linkers can have significant impact on stability. Here, ADC_2 exhibits two thermal transitions, one at 60 °C, similar to ADC_1, while the other is near 50 °C. DLS highlights the degree of thermally-induced aggregation, negligible in ADC_1 yet rapid and extensive in ADC_2.

In-process AAV Quantitation



Direct measurement of AAV attributes during upstream and downstream processing (USP and DSP) can be accomplished at-line with a NanoStar. Sample purity progresses from crude USP product (blue) to the first DSP purification (red) and the final DSP filtrate (green), where a single size population is present with concentration of 2×10^{12} particles/mL.

Aggregation Propensity



Non-specific protein-protein interactions, important for selecting and optimizing biotherapeutic candidates and formulations such as IgG, are characterized by means of a concentration series. Both static light scattering (A_2) and dynamic light scattering (k_D) may be used.


DASHBOARD
HISTORY
SETTINGS
Eclipse Flows
Noise 0.24 µAU Low
Wander 0.28 µAU Low
Drift 0.04 µAU/min Low
Channel Status (mL/min)
Inlet 1.15
Inject 0.00
DCM 0.80
Outlet 0.20
Cross 0.15
-1.0 mA
28.0 °C
FFF Method
Cross Flow (mL/min)
UV A Signal (AU)
Time (min)
Channel Pressure 11.88 bar
Cross Flow Pressure 8.86 bar
Inlet Pressure 15.12 bar
DCM Pressure 0.54 bar
Stop Flow
Current -1.0 mA
Voltage -6.0 V
pH 8.0
Conductivity 0.011 S/m
Eclipse
WYATT TECHNOLOGY

FFF Products

Advanced Separation Technology

Separate complex samples for extended characterization

FFF

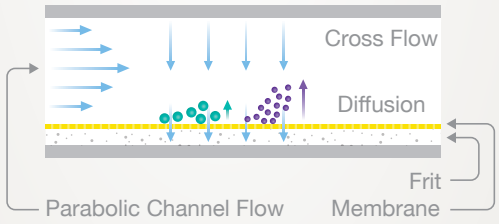
field-flow fractionation

FFF is a powerful separation technique covering a size range of 1 to 1000 nm and beyond. Having very low surface area and no stationary phase, FFF generates very little shear and is an excellent choice when non-ideal sample-surface interactions are a concern. MALS, DLS and dRI detectors are placed downstream of the separation channel for complete characterization.

FFF fractionates and characterizes:

- Colloids and nanoparticles
- Macromolecules and assemblies
- Complex samples

Field-Flow Fractionation Channel



FFF separation power can be tuned by changing the ratio of cross flow to channel flow.

	Feature	Benefit
Single-pump technology	Standard	High system reliability
System Ready Monitor and Health Indicators	Standard	High productivity: eliminate bad runs and shorten troubleshooting
Injection method	Tip or Focus-zone	Supports different FFF separation techniques and SEC
FFF-SEC switching	Optional	Share FFF system with SEC
Dilution Control Module	Optional	Higher sensitivity, fraction concentration and repeatability
Mobility	Optional	Measure zeta potential of each fraction

Eclipse

Advanced FFF technology

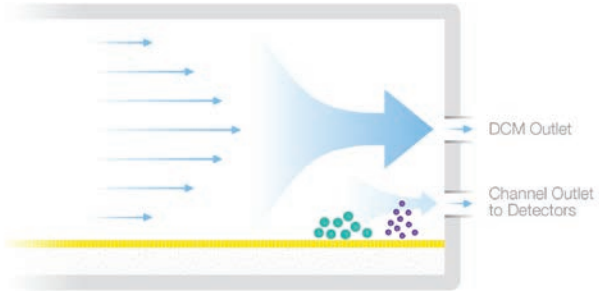
Eclipse is a sophisticated system for performing analytical and semi-preparative separations over a wide range of analytes.

The Eclipse system combines an industry-standard autosampler and pump for maximum reliability, convenience, repeatability and intelligence. With multiple online detectors, FFF-MALS provides extended characterization.

Dilution Control Module

The Dilution Control Module (DCM) increases the concentration of sample eluting from the channel by a factor up to 5x or more. Benefits of the DCM:

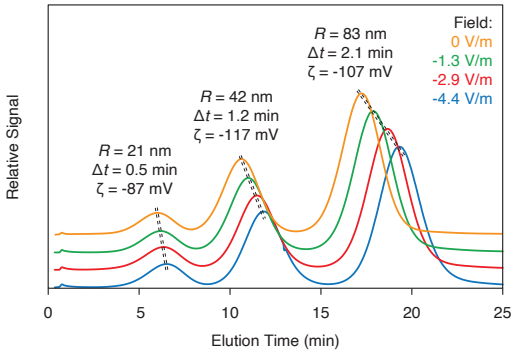
- Higher sensitivity at the detector
- Higher concentration in collected fractions
- Extended dynamic light scattering size range
- Highly reproducible retention time



Mobility

Mobility combines an innovative EAF4 channel design with precise current control, and the pH and conductivity measurements essential for zeta potential interpretation.

The Mobility channel is rigorously engineered for long life and high reliability. It incorporates a DCM port to increase retention accuracy, sensitivity and DLS measurement range.



Channel Type	Benefits	Applications
Analytical Short	Rapid nano/microgram separations	Versatile all-purpose
Analytical Long	Nano/microgram separations	Polymers, less prone to overloading
Semi-Prep	Milligram separations	Extracellular vesicle, virus and LNP isolation
Dispersion Inlet	For aggregation-prone samples	Monoclonal antibodies, liposomes
Mobility EAF4	Separate by size and charge	Zeta potential distributions

EAF4

electrical/asymmetric-flow FFF

EAF4 separates by both size and charge to determine zeta potential distributions, even for multimodal and polydisperse populations.

Use Mobility to:

- Understand if all components in the sample have similar surface chemistry
- Evaluate N/P ratio of drug and gene delivery nanoparticles
- Assess biopharmaceuticals for chemical and physical degradation

Electrical/asymmetric-flow field-flow fractionation (EAF4) channel

EAF4 retention time is set by the balance between cross flow, diffusion and electrophoretic mobility. Sample does not come in contact with the electrodes.

VISION

intelligent design, operation and analysis for field-flow fractionation

In silico method development

VISION DESIGN eliminates long cycles of trial-and-error method development. Its FFF simulation engine predicts the fractogram based on the specified separation conditions, leading to method optimization with a single sample run.

Regulatory Compliance

VISION offers an optional 21 CFR Part 11 compliance package, including IQ/OQ documents and procedures.

VISION

The brains behind FFF

VISION software is the intelligent human interface to FFF-MALS. It streamlines complex procedures and provides critical diagnostics to ensure simplicity and productivity. VISION turns FFF-MALS into a routine analytical tool for scientists and technicians alike.

VISION DESIGN helps users design optimal FFF separation methods from their desks. It also calculates diffusion coefficients and zeta potential from FFF and EAF4 measurements.

VISION RUN is comprehensive software for running FFF methods. It seamlessly coordinates the pump, autosampler, Eclipse FFF controller, detectors and ASTRA, and records FFF and UV signals for diagnostics and analysis in VISION DESIGN.

DESIGN METHOD

VISION DESIGN

- Estimate particle size in sample
- Select spacers and flow profiles
- Use FFF theory to simulate and predict separation

Transfer optimized method

RUN EXPERIMENTS

VISION RUN

- Acquire flow, electrical and UV data
- Collect fractions
- Monitor and diagnose system

Launch and synchronize ASTRA

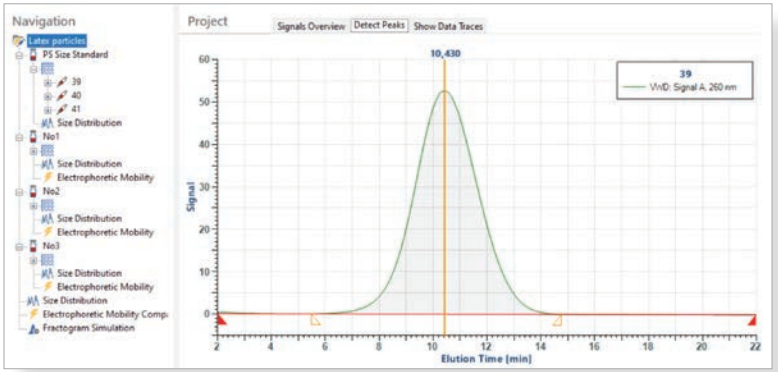
ANALYZE AND REPORT

VISION DESIGN

- Collate project files
- Estimate particle size from retention time
- Refine method
- Determine zeta potential

ASTRA

- Basic and advanced MALS-DLS-UV-RI characterization
- EASI Graph overlays
- EASI Table consolidated results
- Customized reports

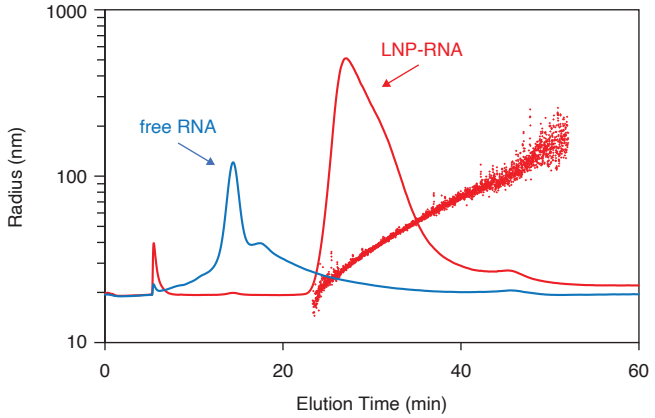


Smart project administration

VISION organizes all FFF and MALS data as projects, for convenient review and replication, as well as comparison and reporting. Projects can be merged and experiments added or deleted at will, making this a powerful and flexible way to handle large sets of FFF experiments.

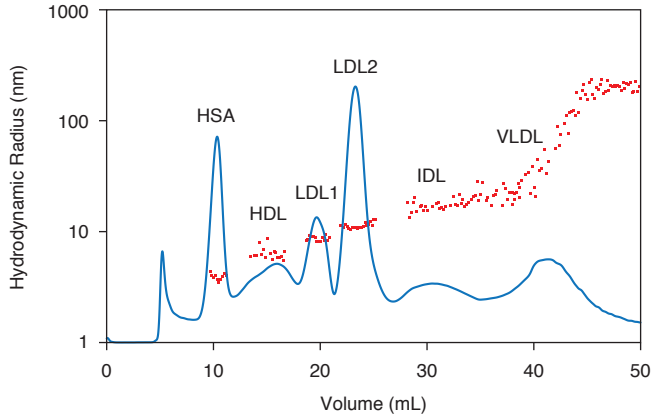
FFF Applications

DNA/RNA Lipoplex



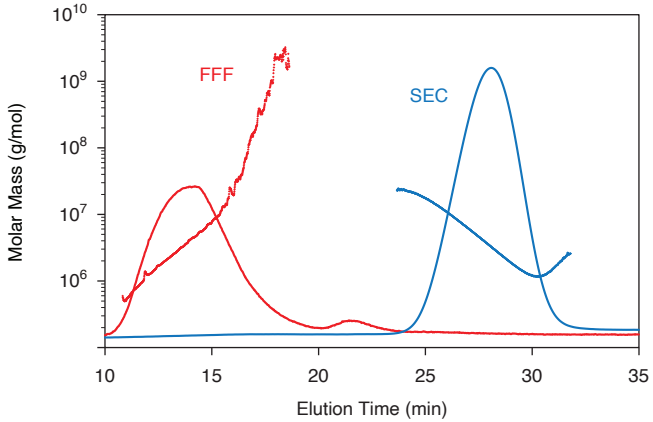
mRNA, siRNA, and plasmid DNA are often formulated into and delivered by non-viral vectors such as lipid nanoparticles (LNP). For such lipoplexes it is important to know the amount of DNA/RNA encapsulated by the particle. In this example, free RNA is well resolved from the mRNA-LNP complex by FFF separation and therefore the amount of free RNA is readily quantified.

Blood Serum Components



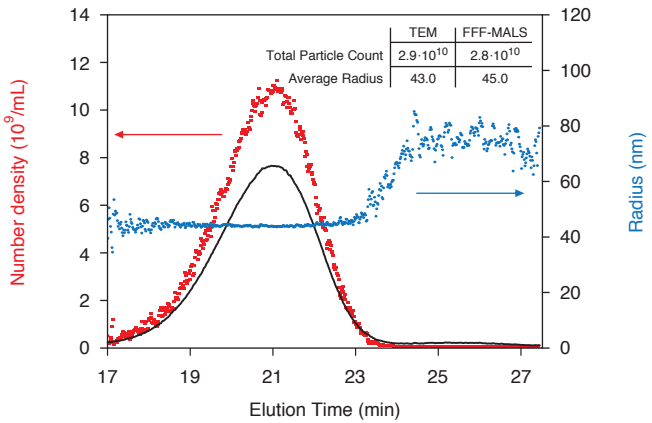
AF4-MALS-DLS separation of whole serum, with distinct peaks for serum albumin, IgG, and various types of lipoproteins. Hydrodynamic radii (R_h) were determined by online dynamic light scattering embedded in the DAWN MALS detector. Not shown, MALS determines molar masses of each peak; it also determines rms radius R_g for species larger than ~10 nm.

HMW complexes and conjugates



A high-molecular-weight protein-polysaccharide conjugate (PPC), spanning four orders of magnitude in MW, was characterized by both FFF-MALS (red) and SEC-MALS (blue). The SEC elution was non-ideal and HMW fractions were removed by the column, while FFF provides near-ideal fractionation of this large conjugate and is conducive to accurate MALS analysis.

Viruses and Viral Vectors



FFF-MALS provides quantitative, high-resolution size distributions based on large particle ensembles. This adenovirus analysis indicates the concentration (number density) in billion/mL at each elution time along with the radius. The LS fractogram is overlaid in black. The results compare well with TEM analysis.


Calypso-II
WYATT
TECHNOLOGY
PUMP 1
PUMP 2
PUMP 3
DASHBOARD
HISTORY
SETTINGS
WYATT

CG-MALS Products

Analyze biomolecular interactions

Label-free, in solution, from pM to mM

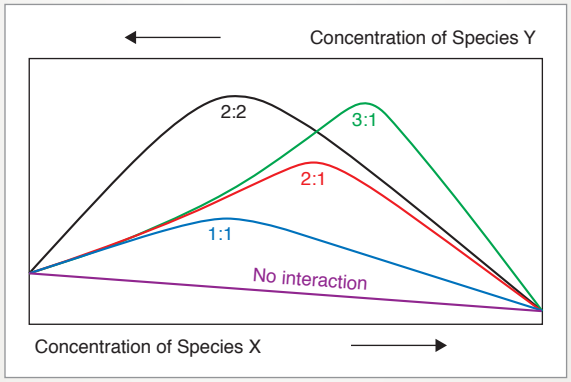
CG-MALS

composition-gradient multi-angle light scattering

CG-MALS is a label-free, immobilization-free technique for characterizing:

- Protein-protein interactions
- Protein-DNA complexes
- Other macromolecular interactions

CG-MALS characterizes biomolecular interactions from first principles by measuring the change in the weight-average molar mass (M_w) of a solution as a function of concentration and composition.



CG-MALS analyzes the light scattering signals from composition gradients to calculate K_d and absolute stoichiometry. It can differentiate between complexes with the same stoichiometric ratio but different overall number of bound monomers.

Calypso

Composition-gradient stop-flow system for biomolecular interactions and reaction kinetics

- K_d from pM to mM
- Reaction times from seconds to hours
- Self- and hetero-associations
- Interfaces with DAWN, miniDAWN and Optilab instruments for automated MALS and concentration measurements.

CALYPSO Software

Comprehensive set of association models covering simple to complex interactions

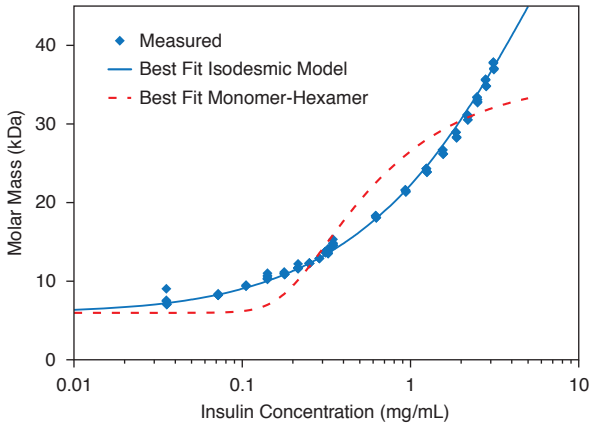
- Versatile, easy-to-use method programming for multiple gradient types, system preparation and post-experiment cleanup
- Simulation capabilities for experiment design and interpretation

Versatile association model design for:

- Standard homodimer, heterodimer and progessive self-association
- Multivalent interactions and multiple oligomers in equilibrium
- Simultaneous self- and hetero-association
- High-concentration proteins
- Non-specific interactions of cosolutes

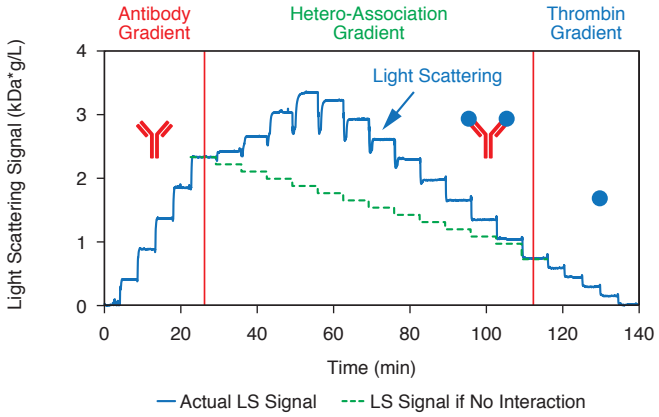
CG-MALS Applications

Insulin Self-Association



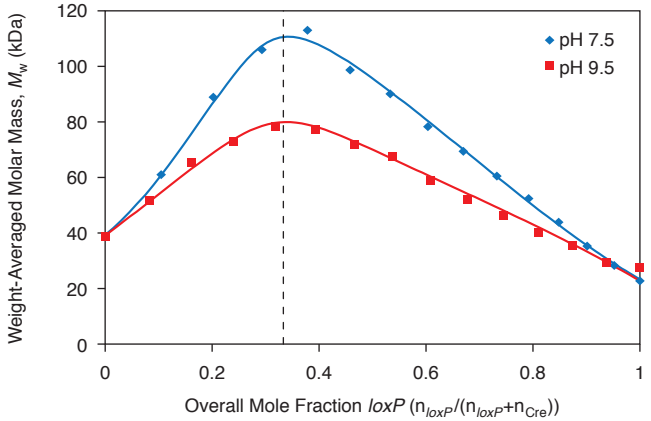
CG-MALS analyzes self-association by measuring the weight-average molar mass over a concentration series. In the absence of zinc, insulin is found to self-associate isodesmically (progressively) with a K_d of 52 µM. A monomer-hexamer model fits poorly and can be ruled out.

Antibody-Antigen Binding



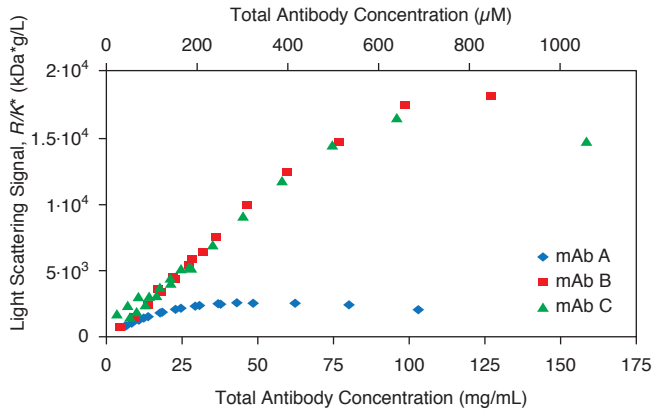
A Calypso stop-flow measurement of antibody-antigen interactions. Here the CALYPSO software found that thrombin binds to an anti-thrombin monoclonal antibody with K_d=9 nm at two equivalent, non-cooperative binding sites on the mAb and no self-association.

Cooperative Binding vs. pH



Cre recombinase binds to the *loxP* DNA segment in a pH-dependent manner. CG-MALS determines that at pH 7.5, each *loxP* binds two Cre molecules with positive cooperativity, and the 2:1 complex dimerizes to form a synapse tetramer; while at pH 9.5, cooperativity and synapsis are lost.

High-Concentration IgG



mAbs A, B and C exhibit widely varying viscosities at high protein concentration, a consequence of differing degrees of self-attraction. CG-MALS is one of very few techniques capable of analyzing protein self-interaction at high concentrations. For these mAbs, self-interaction correlates well with viscosity.

Service & Support Plans

continued instrument service and software support

Silver Service Plan

Full instrument calibration and quality control testing. Priority service including parts, labor, shipping, and loaners based on availability. Comprehensive first-priority technical and application support by phone, email and screen sharing sessions.

Gold Service Plan

All that a Silver Plan offers, plus comprehensive on-site annual preventive maintenance and repair services along with software updates for increased productivity through continual enhancements. Guaranteed loaner units should an instrument require factory repair.

Platinum Service Plan

For our customers who need semi-annual preventative maintenance in addition to all our Gold Plan features.

Service & Support

Customer Service

Our team of support specialists and application scientists will help you get the most out of your Wyatt instruments and software. All new Wyatt instruments come with a full year of unlimited telephone and e-mail support.

SCIENTISTS' CHOICE AWARDS WINNER

Wyatt Technology is committed to your continued success by offering three levels of comprehensive service plans: Platinum, Gold and Silver. We offer installation, preventative maintenance and qualification (IQ/OQ), as well as training and consulting.

In our online support center, you'll find a wealth of technical notes, application guides, software and instrument firmware downloads, manuals, tutorials, training videos and more.

We look forward to meeting you at Light Scattering University!

Application Support

Our dedicated and helpful application scientists, with diverse scientific and cultural backgrounds, are not only enthusiastic about Wyatt's analytical technologies, but also curious about your applications. Whether you're working with synthetic polymers, polysaccharides, therapeutic proteins or nanoparticles, we're committed to helping you solve real world problems.

We're also the liaison between you and our product development team, ensuring continuous improvements of our instruments and software to meet your application needs.

Our newly expanded application lab in Santa Barbara showcases our state-of-the-art static and dynamic light scattering instruments, either stand-alone or connected to HPLC, UHPLC and field-flow fractionation systems.

We welcome customers and collaborators from around the world to visit our lab!

Michelle Radeke, Ph.D.
Director of Customer Service
Joined Wyatt Technology 2015

Michelle Chen, Ph.D.
Vice President of Analytical Services
Joined Wyatt Technology 1996

Light Scattering University

Demystify light scattering and get the most out of your Wyatt instruments

"I wanted to thank you for the tremendous training experience with the Wyatt staff. It has been the most remarkable and useful training session that I've ever completed. Truly first class."

Dr. InKwan Han,
Merck & Co. Inc.

Highlights of LSU

Many trainees come away from LSU inspired with new ideas for how light scattering can solve some of their analytical challenges. One of the most popular aspects of LSU is the opportunity to meet and work with the scientists and engineers behind the products, as well as get acquainted with support staff that they usually only contact over the phone.

Another not-to-be missed session (available only in Santa Barbara) is the Light Scattering Museum tour, led by Dr. Philip Wyatt, the inventor and pioneer of MALS detectors.

LSU

light scattering university

Often described by participants as the best instrument user training they have ever attended, Light Scattering University (LSU) is an intensive experience that combines hard work, good food and a friendly atmosphere.

You'll learn about:

- Light scattering theory and applications
- How to interpret your data
- Instrument best practices
- History of light scattering

World Wide Support

Global Offices

North and South America
(Corporate Office)
Wyatt Technology LLC
Santa Barbara, CA, USA
Tel: +1 805 681 9009
info@wyatt.com
www.wyatt.com

Northeast U.S. Applications Lab
Woburn, MA, USA
Tel: +1 805 681 9009 x212
info@wyatt.com
www.wyatt.com

Germany, Austria, BeNeLux, Scandinavia, Switzerland, Russia, Czech Republic and all Eastern European and Middle East countries
Wyatt Technology Europe GmbH
Dernbach, Germany
Tel: +49 2689 925 0
info@wyatt.eu
www.wyatt.com

China
Wyatt Technology China
Beijing, China
Tel: +86 10 8229 2806
info@wyatt.com.cn
www.wyatt.com.cn

France, Portugal and Spain
Wyatt Technology France
Toulouse, France
Tel: +33 (0)5 34 55 99 28
wtf@wyatt.com
www.wyatt.com

United Kingdom and Ireland
Wyatt Technology UK Ltd.
Surrey, United Kingdom
Tel: +44 1440 705229
wtuk@wyatt.com
www.wyatt.com

World Wide Support

Click here to find a distributor near you.

W1000W | November 2023

Waters™ | WYATT TECHNOLOGY