# Eclipse

The premier solution for versatile separations of proteins, polymers and nanoparticles


WYATT TECHNOLOGY

# Eclipse™

## The foundation for absolute characterization by FFF-MALS

Field-flow fractionation (FFF) is a unique method for separating macromolecules and nanoparticles by size. Covering a range of 1 to 1000 nm and beyond, the power of FFF resides in its essential tunability. Simply by changing flow rates, a single separation channel can be used to explore complex samples, comprising molecules, particles and emulsions across the entire size range, with superb resolution.

## A complete solution: FFF-MALS

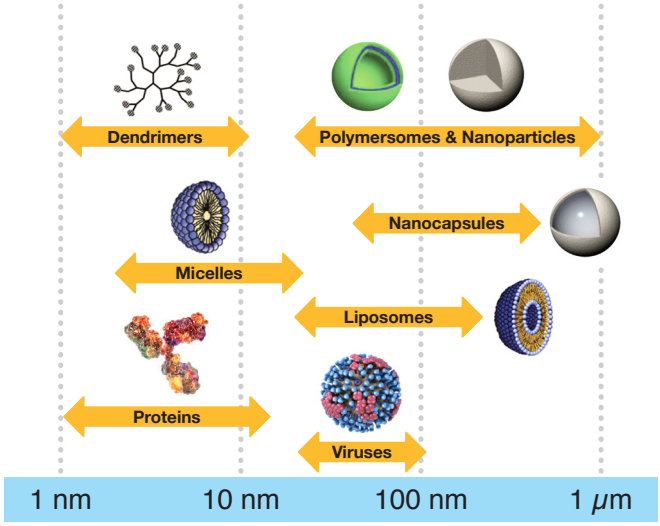
Separation is only half the battle. For comprehensive characterization, an FFF–MALS system combines an Eclipse FFF instrument with a DAWN® online multi-angle light scattering (MALS) instrument, refractive index and UV/Vis detectors to analyze molar mass, size, concentration, conformation and composition.

**FFF-MALS fractionates and characterizes:**

- Gene vectors, viruses and nanodrug carriers
- Nanoplastics and metallic tracer nanoparticles
- Proteins, aggregates and assemblies
- High-molecular-weight as well as standard polymers

Beyond online analysis, size fractions can be collected for additional offline characterization.

**Left stack: Agilent 1260 Infinity II quaternary pump, Autosampler, UV detector Center: Separation channel Right stack: DAWN MALS, Optilab® dRI detector, Eclipse**




**Working range of FFF-MALS: Sizes and applications**

## Advanced Technology

Following major advances in sensitivity, reproducibility, functionality and usability, Eclipse represents the state of the art for FFF. Exciting new features:

- Dilution Control Module™ (DCM) enhances sample concentration up to 10x for higher sensitivity and fraction titer
- Mobility™ implements electrical/asymmetric-flow FFF for determining size-resolved zeta potential
- Improved channel design with temperature control for higher recovery, reproducibility and pressure stability
- Information-rich touch screen with System Health Indicators

The Eclipse interfaces with industry-leading Agilent 1260 Infinity II modules (sold separately from the Eclipse) serving as the FFF front end. This creates a robust FFF-MALS system that serves the needs of scientists and engineers across research, development and quality control.

# Eclipse Advantages

## Engineered for reliability, versatility and simplicity of use

Industry leading Agilent 1260 Infinity II front-end with one pump for maximum accuracy and reliability

System health indicators and pop-up troubleshooting guidance help ensure smooth runs with no guesswork

Compatible with most aqueous buffers and many organic solvents including THF and toluene


SEC switching option enables automated switching between FFF-MALS and SEC-MALS

Intelligent solvent management system with configurable recycling minimizes solvent consumption

Real-time measurement of all flow rates for unprecedented precision and reproducibility

# Experience Intelligence

## Eclipse's interactive front panel is the gateway to Smart Services™

### Channel Status

Need to know what's happening? An intuitive graphical representation of the separation channel indicates the current status of each flow path, while the strip chart graphs show how the system parameters have evolved. Deviations to the set values are graphically indicated.

### System Ready Monitor

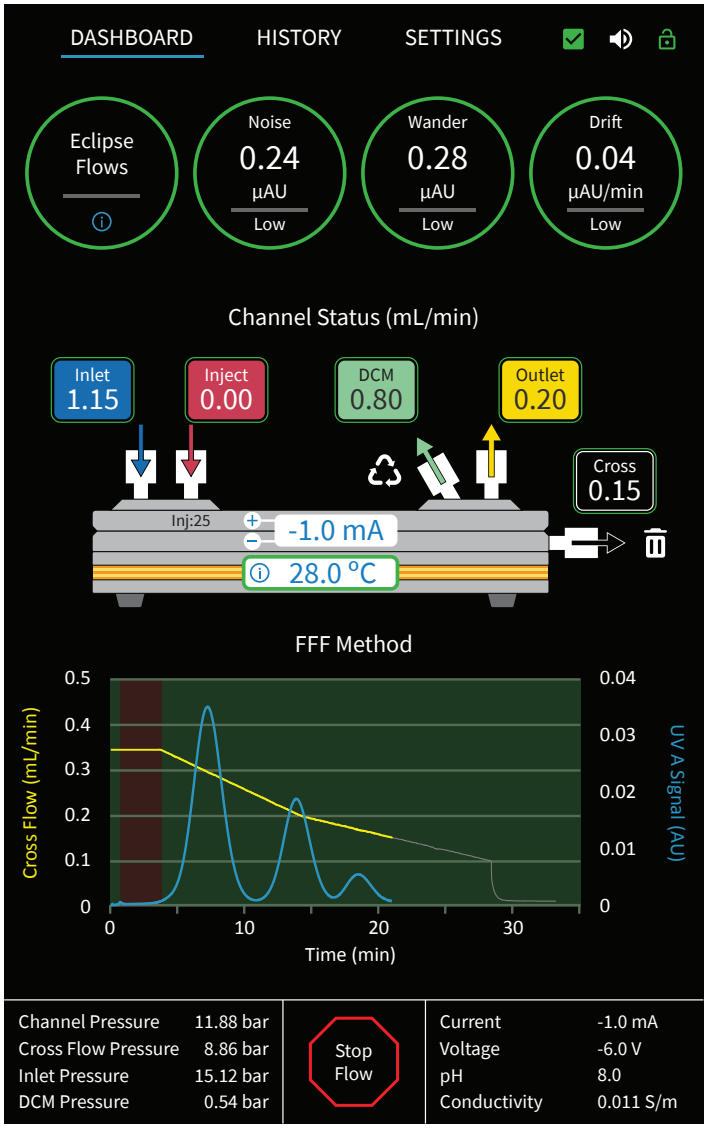
Never waste a run due to incomplete equilibration or past-due system maintenance! The System Ready Monitor continuously checks if all parameters are optimal. If problems do arise, it alerts you and provides actionable guidance on what needs to be done to bring the system to peak performance.

### Real-Time Health Indicators

System Ready Monitor indicating that not all is well? For more detailed information on what's holding you back, review the Real-Time Health indicators. They clearly describe not only what the problem is, but also how to fix it. A great help to reduce down-time and maintain a high level of productivity.

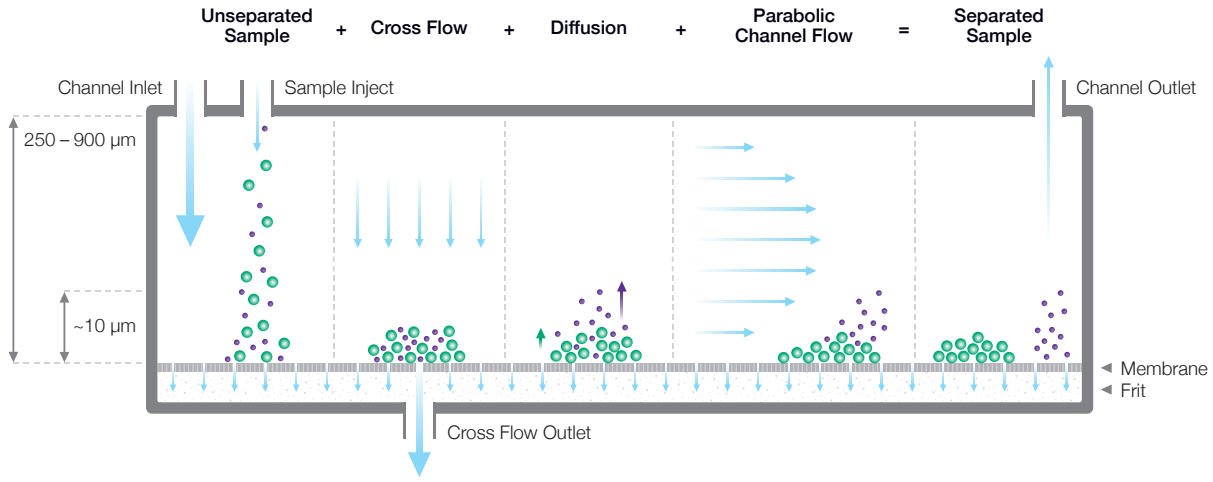
### Collection Mode

When a method is running, the display provides comprehensive, easy-to-read information on the run status including a progress bar indicating the status of the current experiment, and the time remaining to completion of the sample sequence.



# How FFF works

## Gentle and fast one-phase separation




Asymmetric-flow field-flow fractionation (herein FFF) is a one-phase separation technique, implemented in the Eclipse FFF system. High-resolution separation is achieved in a thin, ribbon-like channel by controlling two flow streams:

- Channel flow, in the longitudinal direction parallel to the membrane; and
- Cross-flow, in the perpendicular direction, which permeates through the nano-porous membrane.

The cross-flow acts as a force field, concentrating the sample against the bottom wall. Diffusion presents a counter force, dispersing the sample back up towards the middle of the channel. The balance of these forces produces an exponential concentration profile above the membrane that depends only on the diffusion coefficient of the particles, and therefore their hydrodynamic size.

Channel flow is laminar, leading to a parabolic differential velocity profile. Hence smaller particles, which are on average higher above the membrane, elute first, followed by the larger components which experience a lower longitudinal flow rate.

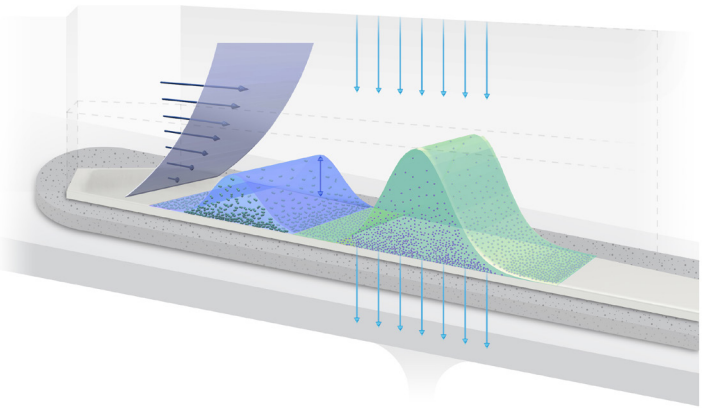
Eclipse implements both focus-zone and tip injection modes. Focus-zone injection minimizes potential loss of sample and resolution for standard AF4 channels; tip injection is necessary for SEC mode.

## Method optimization

The physics behind FFF show that retention time $t_R$ is related only to sample diffusion coefficient $D_t$, height of the channel $w$, and the ratio of cross flow to channel flow $F_x / F_{ch}$.

$$t_R = \frac{w^2}{6D_t} ln\left(1 + \frac{F_x}{F_{ch}}\right)$$

This enables separations to be optimized simply by modulating the cross flow, even on-the-fly during the method. Built-in cross-flow programs include linear and exponential gradients.



**With no stationary phase and very little surface area, separation by FFF creates no shear on the sample and relatively little surface interaction compared to size-exclusion chromatography.**

# Enhanced Functionality

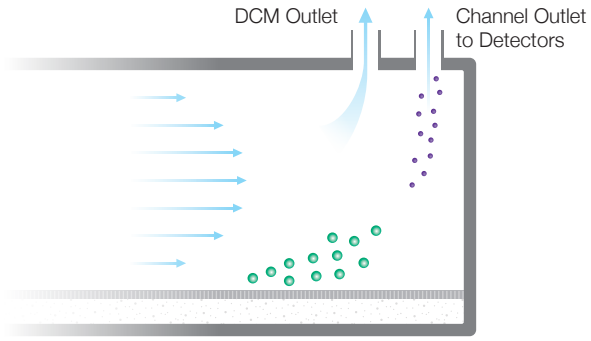
## Optional modules add new levels of performance

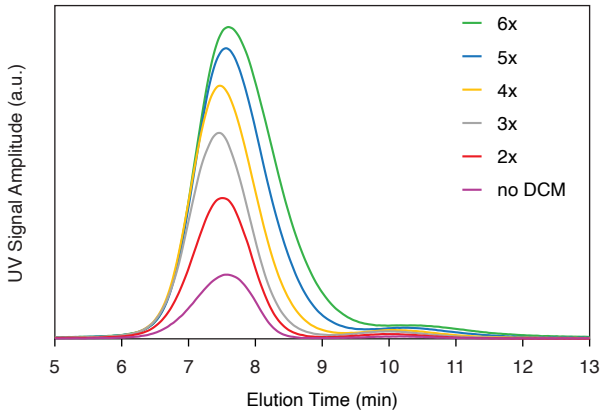
## Dilution Control Module

The Dilution Control Module (DCM) increases the concentration of sample eluting from the channel by a factor of up to 10 over standard FFF, with little to no loss in resolution up to 5x. This is achieved by splitting away a fraction of the channel flow close to the upper wall, which does not contain sample, in a tightly regulated fashion.

**Benefits of the DCM include:**

- Higher detection sensitivity for low-concentration samples
- Higher concentration of sample in collected fractions
- Improved dynamic light scattering measurements resulting from a lower detector flow rate
- Increase reproducibility of retention time, calculated recovery and collected fractions resulting from active control of the detector flow rate



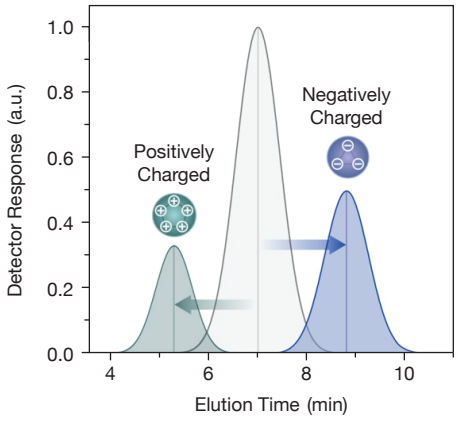

**The DCM increases sample concentration at the detector by removing extraneous solvent with zero loss of sample or resolution. The graph shows overlaid UV signals for BSA, exhibiting a gain in amplitude proportional to increasing split ratio.**

## Mobility

Electrical/asymmetric-flow field-flow fractionation (EAF4) separates by both size and particle charge to determine zeta potential distributions, even of multimodal and polydisperse populations. Mobility is an add-on feature that combines an innovative EAF4 channel design with outstanding software control and analysis.

**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




**In EAF4, the zero-field retention time (grey peak) is determined by the particle's hydrodynamic radius. Application of an electric field to the EAF4 channel shifts the retention time according to the sign and magnitude of the particle's charge.**

# Versatile FFF Channels

## Engineered for improved performance and ease of use

### A channel for every application

Eclipse FFF channels are available in a range of footprints for different sample loads and separation requirements. The Short Channel is the workhorse for FFF, suitable for most applications. With the Long Channel, separation of large particles or polymers can be achieved at low cross flow to minimize membrane interaction. The Semi-Prep Channel separates sample loads up to the milligram range. Standard inject-and-focus FFF may not be appropriate for some challenging samples. The Dispersion Inlet Channel eliminates focusing and is the right choice for aggregation-prone samples.


**Exploded view of the fixed-height Long Channel**

### Superior, lasting performance

Constructed from 316 stainless steel, Eclipse channels are designed for rigidity with no flex or creep, during or between runs. This translates to unprecedented reliability and detector signal quality, while maintaining compatibility with all types of solvents. All Eclipse channels, including the Mobility Channel, are DCM-capable.

### Tailored to your needs

Channels with interchangeable spacers of variable heights serve the most versatile applications for research and method development. The spacer design ensures optimal sealing with no microscopic leaks and provides maximum sample recovery. For routine and QC applications, fixed-height top plates eliminate the need for a spacer, resulting in the highest reproducibility while offering easy assembly and maintenance.

### Unmatched reproducibility

Stable temperature is critical in ensuring highly reproducible retention times. That's why all Eclipse channels come with built-in temperature regulation, from ambient up to 50 °C. This feature is especially important in long sample sequences carried out overnight or over the weekend, when lab temperatures can fluctuate by several degrees.

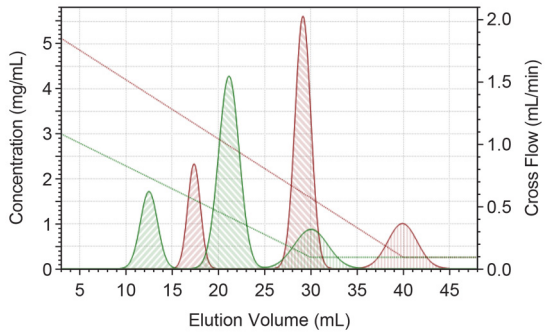
**Left to right: Short, Long, Dispersion Inlet, Semi-Prep and Mobility Channels**

# See the light with VISION and ASTRA

## Powerful tools to design, run and analyze FFF-MALS experiments


## VISION

### The gateway to successful FFF-MALS

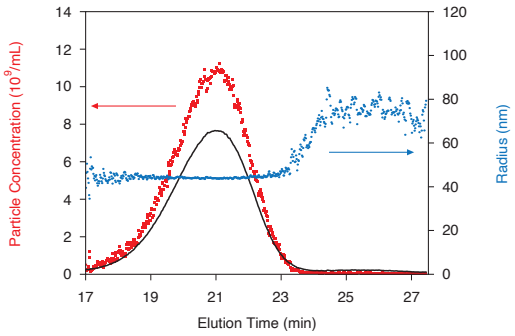



VISION™ is comprehensive software for designing, optimizing, performing and recording FFF-MALS methods.

- VISION DESIGN provides intelligent, in-silico FFF method development, plus visualization and analysis of FFF and EAF4 signals
- VISION RUN implements FFF methods, controls the entire FFF system, and acquires fluidic and UV data for post-run diagnostics
- VISION RUN interacts with ASTRA software for FFF-MALS measurements. It seamlessly launches ASTRA®, synchronizes sequences and displays ASTRA data during the run.

## ASTRA

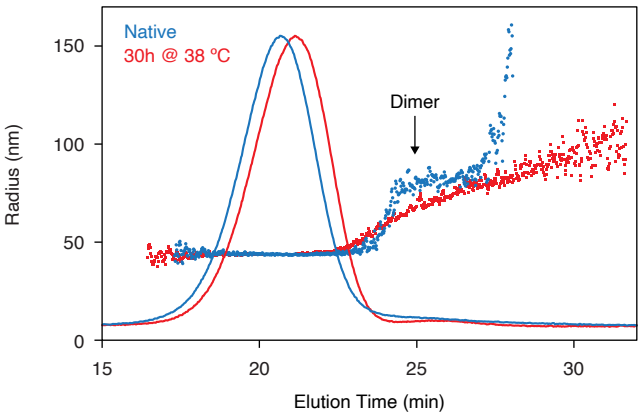
### Gain unique insights from FFF-MALS




ASTRA collects and analyzes signals from online MALS, DLS, RI, UV and/or viscometry detectors. In addition to determining absolute molar mass and size distributions and moments, with ASTRA you can:

- Calculate particle concentration and evaluate particle shape or structure
- Determine the intrinsic viscosity, conformation and branching ratio of polymers
- Assess the nucleic acid content of viral vectors or the PEG content in PEGylated proteins
- Pull in graphical or tabulated results from multiple sample runs, calculate averages and standard deviations, and create custom reports

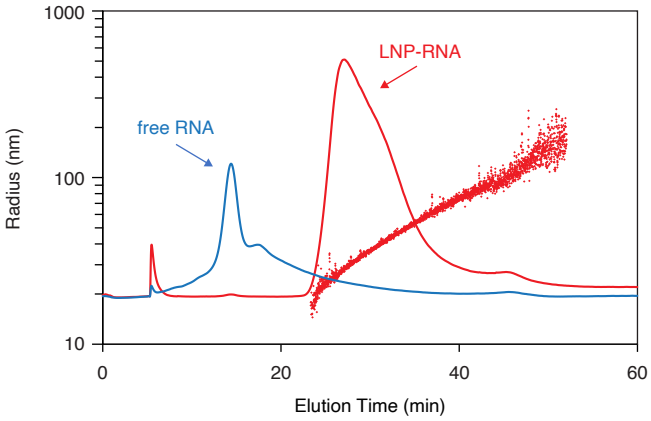
## Viral vector and aggregates




FFF-MALS provides high-resolution size distributions and particle concentration measurements of viruses and viral gene vectors such as AAV, adenovirus and lentivirus. Shown in this graph are fractograms from fresh and aged adenovirus samples. MALS characterizes and quantifies the aggregates to ensure the safety of these gene therapy products.

Small viruses such as AAV may be characterized by SEC-MALS, but still benefit from FFF-MALS analysis since large aggregates that are removed by the SEC column elute well from the FFF channel.

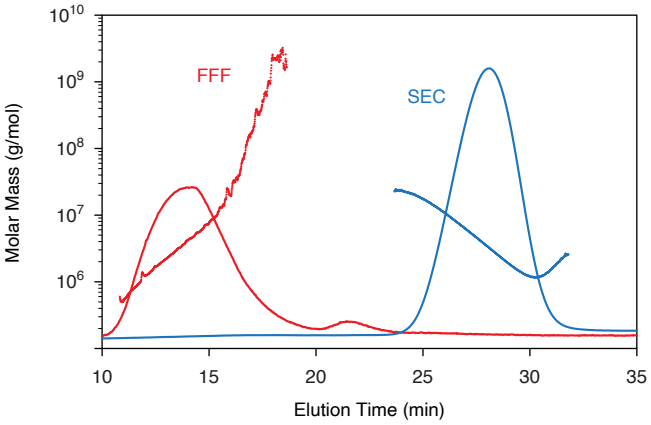
## DNA/RNA lipoplex




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 inside 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.

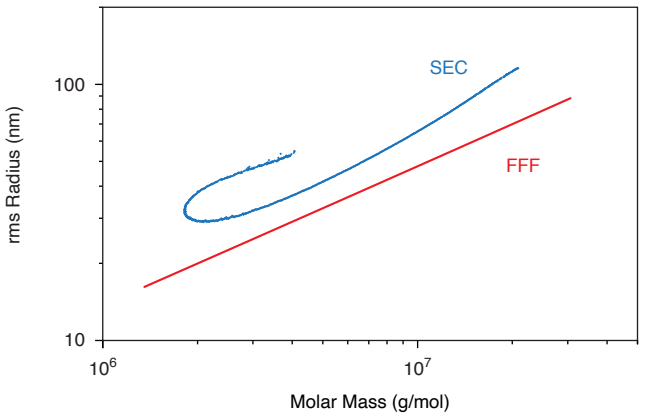
MALS-UV-RI analysis of the lipoplex peak provides additional information such as distributions of size and nucleic acid payload.

## HMW complexes and conjugates



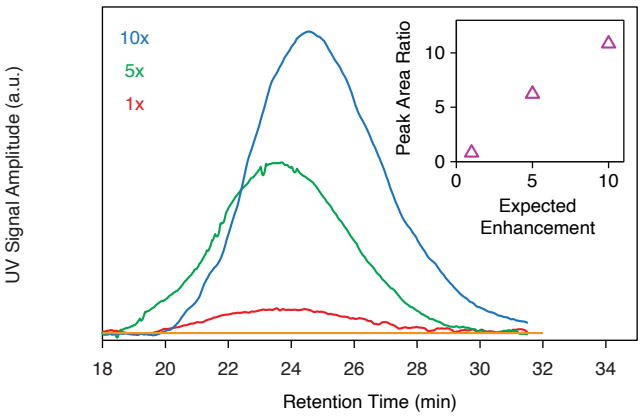

Large biomacromolecules are challenging for SEC. A high molecular-weight protein-polysaccharide conjugate (PPC), spanning four orders of magnitude in MW, was characterized by both FFF-MALS (red) and SEC-MALS (blue). It is evident that the fraction above ~$10^7$ g/mol was removed by the SEC columns. SEC separation was further compromised by the non-ideal SEC effect (MW curves up at the tail of the blue peak). However, FFF provides near-ideal fractionation of this large conjugate and is conducive to accurate MALS analysis.

## Architecture of large polymers



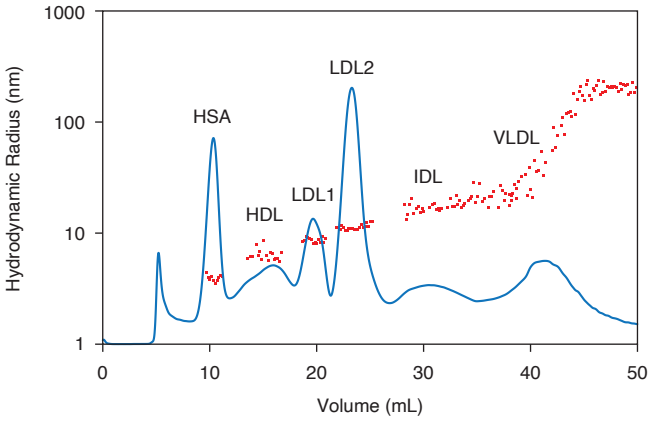

Correct separation of macromolecules is essential for proper characterization of molecular architecture. Here we see conformation plots of the same PPC sample shown to the left, separated by FFF (red) and SEC (blue). The plot from SEC is non-monotonic, making it impossible to get a proper interpretation of this PPC's conformation. On the other hand, the FFF plot shows excellent linearity; the slope suggests a slightly branched structure. FFF-MALS is the tool of choice for separating and characterizing such large macromolecules.

## Enrichment of EVs using DCM



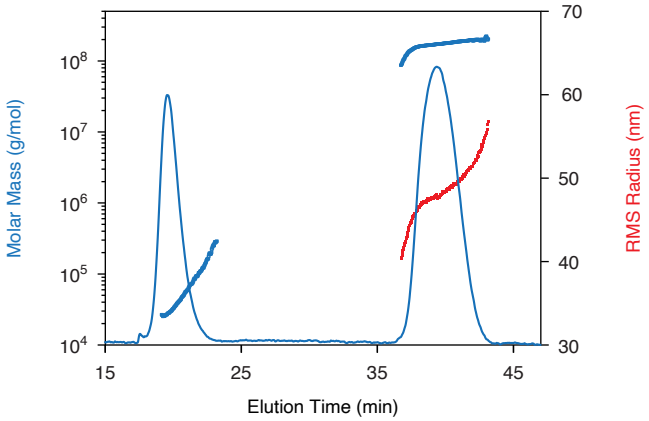

When working with extracellular vesicles (EV), limitations on sample quantity are often challenging. The DCM dilution control module increases both detector signals and the concentration of collected fractions. Here split ratios of 5x (green) and 10x (blue) were applied, increasing the effective peak area of the RI signal accordingly, relative to 1x separation (red). The increased concentrations translate to fractions that are isolated downstream by a fraction collector.

## Analysis of complex samples



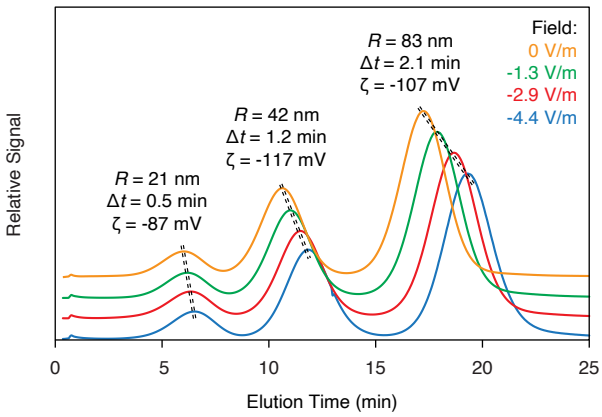

AF4-MALS-DLS separation of whole serum, with distinct peaks for serum albumin, IgG, and various types of lipoproteins. Hydrodynamic radii ($R_h$) were determined by online dynamic light scattering embedded in the DAWN MALS detector. Not shown, MALS determines molar masses of each peak; it also determines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

## Polymer/nanogels solutions




RI fractogram of methyl methacrylate-butyl acrylate-ethylene glycol dimethacrylate separated in THF. The solution is turbid due to the scattering of the 40 to 55 nm radius gel nanoparticles that elute at 38 minutes. A column could be damaged by blocking of pores by the nanogels, but FFF separates the nanogels from the dissolved macromolecules and allows quantification of both fractions.

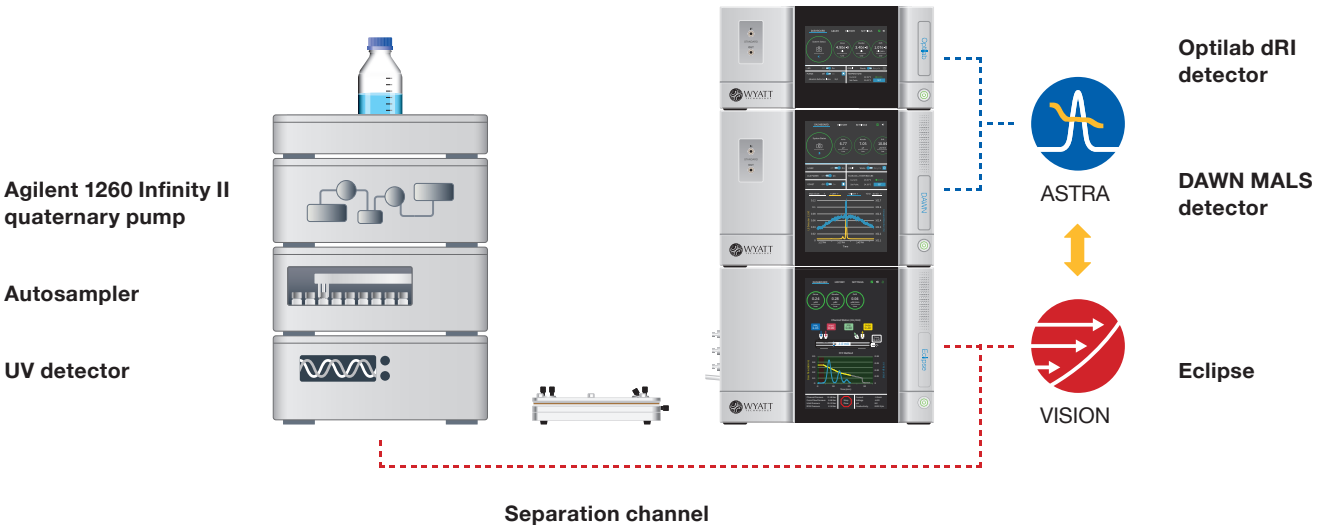
## Zeta potential of particle mixtures




Mobility applies both electrical and cross-flow fields during separation (EAF4). The shift in retention time due to the electrical field is analyzed to determine zeta potential and electrophoretic mobility of each eluting peak. Shown here is EAF4 analysis of a mix of polystyrene nanoparticles with 21, 42 and 83 nm hydrodynamic radius. Zeta potentials are determined for each size as they elute in the mix.

# FFF-MALS Systems

## Components and options providing powerful capabilities




## Separation system

The upstream, separation part of FFF-MALS consists of an Eclipse FFF instrument and separation channel, plus a single pump and an autosampler (pump and autosampler sold separately from the Eclipse).

### Pump and autosampler

An Agilent 1260 quaternary pump and autosampler comprise the liquid-handling front end. Other Agilent pumps and autosamplers are also supported. The sample loop can be underfilled, enabling a broad range of injection volumes: 0.1 to 900 µL.

Options

- Standard 100 µL loop or 900 µL loop
- Agilent multi-draw kit for higher injection volumes, i.e., 3.6 mL

### FFF controller

The Eclipse regulates channel, focus, cross, and injection flows by splitting, configuring and controlling flow paths from a single HPLC pump. The single-pump configuration results in excellent signal-to-noise ratios because 1) pump pulses co-occur in the various flow paths and therefore are self-compensated, and 2) the Agilent pump itself is state-of-the-art, with internal compressibility compensation for minimal pump pulsation.

Options

- Dilution Control Module: Factory-installed option. Increases sample concentration at the detector by up to a factor of 10 (up to 5 with negligible loss of resolution), controlled by software.
- SEC Switching Option: Factory-installed option. Allows for automated software-controlled switching between SEC mode and FFF mode.
- Mobility: Add-on option. Includes Mobility Module for performing electrical/asymmetric-flow field-flow fractionation (EAF4), pH sensor and conductivity sensor with software supported calibration and Mobility channel. See Mobility brochure for more information.

### FFF channels

The most commonly used analytical FFF channel is the Short Channel, with the fixed-height version preferred for most applications. It may be exchanged with other channels and versions directly by the user. All channels include a DCM port and are temperature-controlled to ensure unsurpassed repeatability of elution profiles.

Options

- Channel types: Short Channel (analytical), Long Channel (analytical), Semi-prep Channel (semi-preparative), Dispersion Inlet Channel (analytical, eliminates the focusing step for aggregation-prone analytes), Mobility Channel (for use with EAF4 measurements).
- Channel models: Short, Long, and Semi-prep channels are offered as fixed-height, for maximum ease of use and reproducibility, and variable-height, using spacers for maximum flexibility in separation method. The Dispersion Inlet and Mobility Channels are only offered as variable-height option.

### Software

The VISION RUN module (sold separately from the Eclipse) provides complete control over the entire FFF-MALS system, and controls data acquisition by ASTRA analysis software from a single sequence table.

The VISION DESIGN module is used for in silico method design and method refinement. VISION DESIGN also analyzes Eclipse and HPLC data, including flows, pressures, UV, FLD, and mobility; it can display 3D spectral plots of UV and FLD data, perform advanced effective height and zeta potential calculations, and more.

## Detection system

The downstream, detection part of a basic FFF-MALS system includes a DAWN MALS instrument and either UV or dRI detector (detectors are sold separately from the Eclipse).

### MALS instrument

DAWN quantifies molar masses of 200 to 1 x $10^9$ g/mol and radii of 10 to 500 nm. DAWN independently determines nanoparticle size (radius in nm) and particle concentration (particles/mL). For analysis of molar mass, a concentration detector must be added. See DAWN page for more information.

Options

- WyattQELS™ embedded dynamic light scattering module for measuring particle size from 0.5 to 50 nm (standard MALS flow cell) or 0.5 to 300 nm (wide-bore MALS flow cell). Alternatively, a DynaPro® NanoStar® or Mobius™ may be connected via optical fiber to the MALS flow cell to serve as an inline DLS detector
- Standard or wide-bore flow cell (standard is preferable unless wide-bore is required for online DLS measurement of larger particles)
- Fused silica or F2 flow cell (F2 is only recommended for with high-refractive-index solvents like DCB or TCB)

### Concentration detector

Add an Agilent 1260 VWD, MWD or DAD for proteins, nucleic acids and other molecules with known UV extinction coefficients.

Add an Optilab differential refractive index detector for polymers and other analytes (including indeterminate proteins) that do not contain reliably known chromophores. Learn more about Optilab.

For analysis of conjugation ratios of glycoconjugates, protein-DNA complexes, co-polymers and other binary macromolecules, or payload analysis of small viruses such as adeno-associated virus or RNA-bearing lipid nanoparticles, MALS must be combined with both UV and RI detectors.

Options

- Agilent variable wavelength UV/Vis detector, multi-wavelength UV/Vis detector or diode array detector. Multi-wavelength UV data may only be acquired from the MWD and DAD units.
- Agilent fluorescence detector
- ICP-MS detection of metallic nanoparticles to determine composition and size

### Software

ASTRA software acquires and analyzes MALS and concentration data as well as DLS data (sold separately from the Eclipse). See ASTRA page to learn more.

### Additional options

An Agilent fraction collector, controlled by VISION RUN, may be included for automated fraction collection following size-based FFF separation.

# Specifications

| | |
|---|---|
| **Eclipse** | Compatible with aqueous and organic solvents. Corrosion-resistant from pH 2 to 12. Wetted materials: 316 stainless steel, FFKM perfluoroelastomer, FR3 stainless steel, Vespel. |
| Separation Range by Radius | 1 nm to 1000 nm |
| Typical Injection Volume | 1 to 100 µL (0.1 to 900 µL, depending on Agilent autosampler configuration |
| Typical Injected Mass | 5 to 100 µg (1 µg to 15 mg, depending on channel configuration and analytical detectors) |
| **FFF-MALS Solution** | A basic FFF-MALS system includes an Eclipse instrument, one separation channel (matched to customer application), Agilent 1260 Infinity II quaternary pump and autosampler, DAWN MALS instrument, VISION software suite and ASTRA light scattering analysis software. Each component and software must be ordered individually and are not included in the cost of Eclipse. See FFF-MALS Systems on page 11 for descriptions and optional modules. |
| **Channels** | |
| Selection | Short, Long and Semi-Prep channel types are offered as fixed-height and variable-height options. The Dispersion Inlet Channel is offered as variable-height option (aqueous solvents only). Maximum pressure 30 bar. |
| Temperature Regulation | Ambient to 50 °C |
| Wetted Materials | 316 stainless steel, FFKM perfluoroelastomer, alumina ceramic. Polycarbonate or Mylar according to the selected spacer for variable-height channels. |
| Spacers | Selection ranges from 250 µm to 1000 µm, depending on channel. FFKM perfluoroelastomer-coated polycarbonate for aqueous solvents, Mylar for organic solvents for variable-height channels. |
| Membranes | Pre-cut polyethersulfone or regenerated cellulose with molecular-weight cutoff from 2 to 30 kDa. Please contact Wyatt Technology if alternative material membranes are required. |
| **Dimensions** | 58 cm (L) x 36 cm (W) x 26 cm (H) |

Wyatt Technology is committed to continual improvement. Specifications subject to change without notice.

Warranty: All Wyatt instruments are guaranteed against manufacturing defects for 1 year.





**Left to Right**
Geofrey K. Wyatt, Chief Executive Officer
Dr. Philip J. Wyatt, Chairman of the Board
Clifford D. Wyatt, President

Wyatt Technology provides absolute macromolecular and nanoparticle characterization solutions by developing the finest instrumentation and services to chemical, petrochemical, pharmaceutical, biotechnological and academic laboratories worldwide. We delight our customers with unparalleled levels of service and support, facilitating their cutting-edge research and development efforts.

Eclipse is one of many tools in Wyatt's Light Scattering Toolkit used to characterize proteins, polymers and nanoparticles in solution.

**Learn more at www.wyatt.com**

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