VISION 3
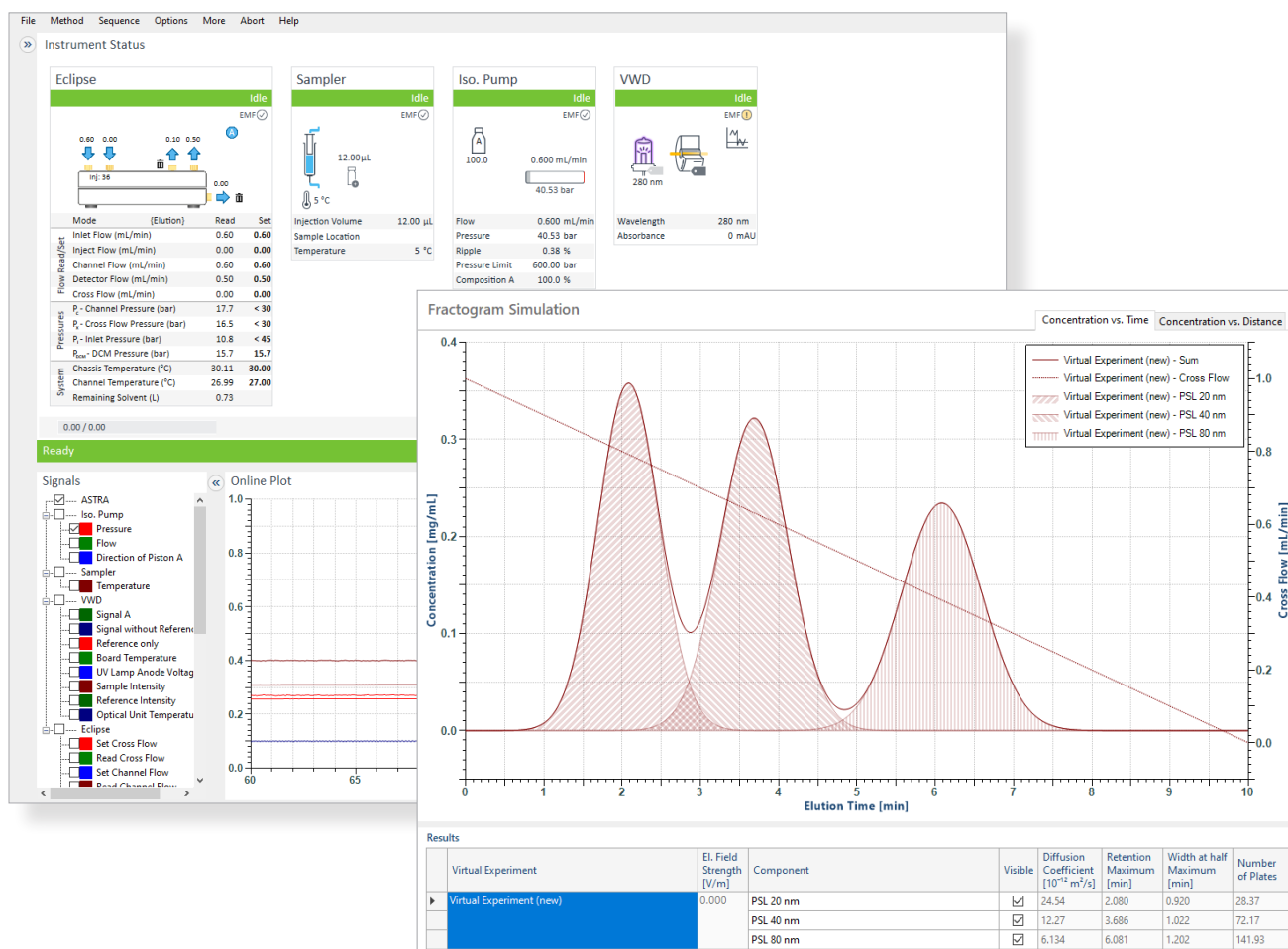
Intelligent design, operation and analysis for field-flow fractionation
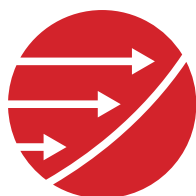
VISION™

Look ahead, see more

VISION, the brains behind FFF

VISION

FFF-MALS—field-flow fractionation with online multi-angle light scattering detection—is a powerful tool for characterizing all types of macromolecules and nanoparticles in solution or suspension. VISION software is the intelligent human interface to an FFF-MALS system built on Wyatt Technology's Eclipse™ FFF controller and DAWN® MALS instrument.

VISION comprises two primary modules:

VISION DESIGN™ carries out physics-based FFF simulations for *in silico* method design and optimization. The physics engine also calculates diffusion coefficients and zeta potential from FFF and EAF4 measurements.

VISION DESIGN

VISION RUN™ is comprehensive software for running FFF methods. It seamlessly coordinates the pump and autosampler front-end with the Eclipse, detectors and ASTRA®, and records FFF, electrical and UV signals for diagnostics and analysis in VISION DESIGN.
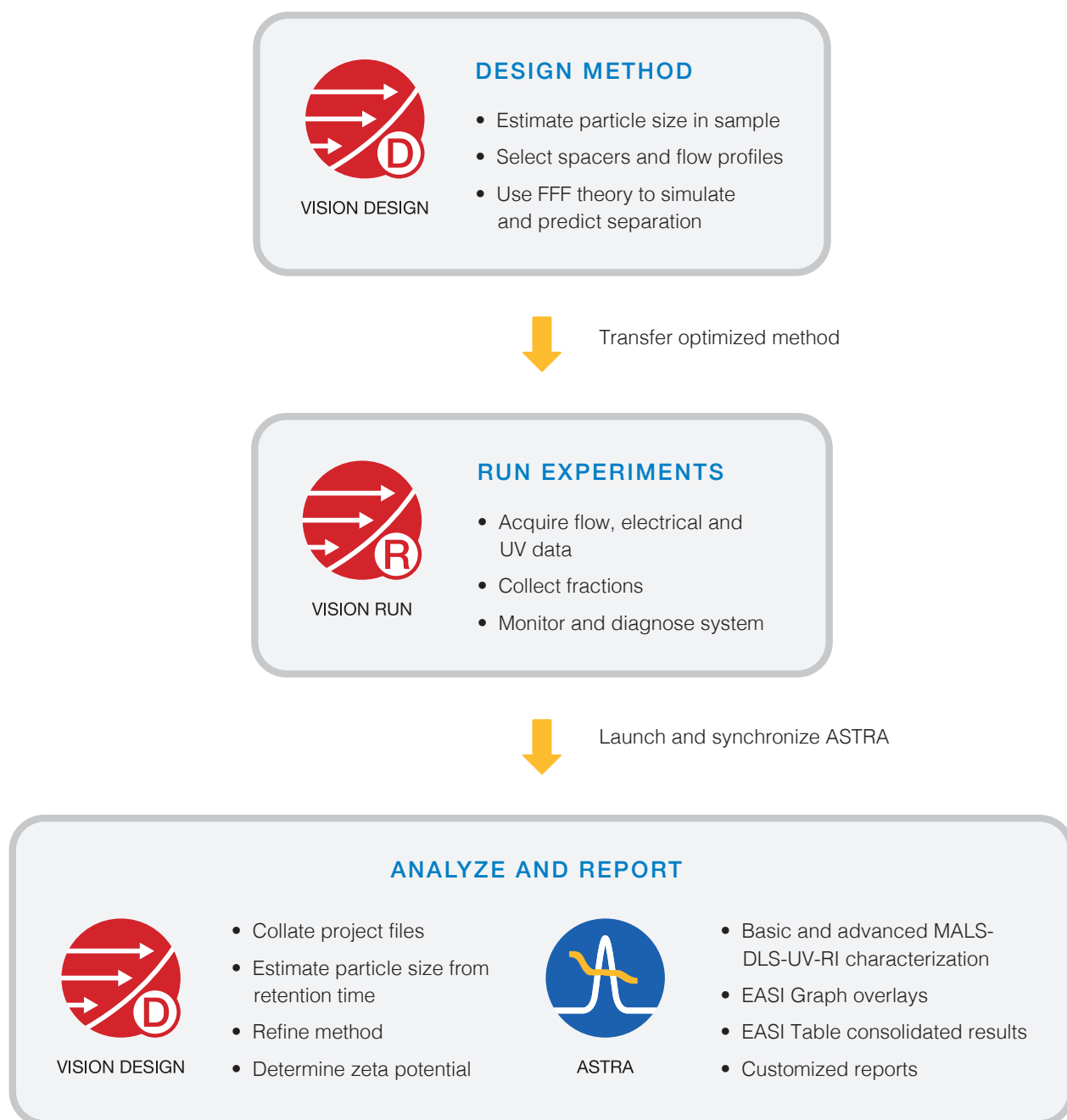

VISION RUN

VISION interfaces with **ASTRA**, the industry-leading light scattering analysis software for determination of molar mass, size, conformation, composition, particle concentration and more.

Integrated Workflow
From method development to final results

VISION offers a seamless workflow in a few simple steps, starting with developing and optimizing the flow program *in silico*, then running a sample sequence, followed by data processing, refining the separation method, reporting and consolidating the data files in projects.
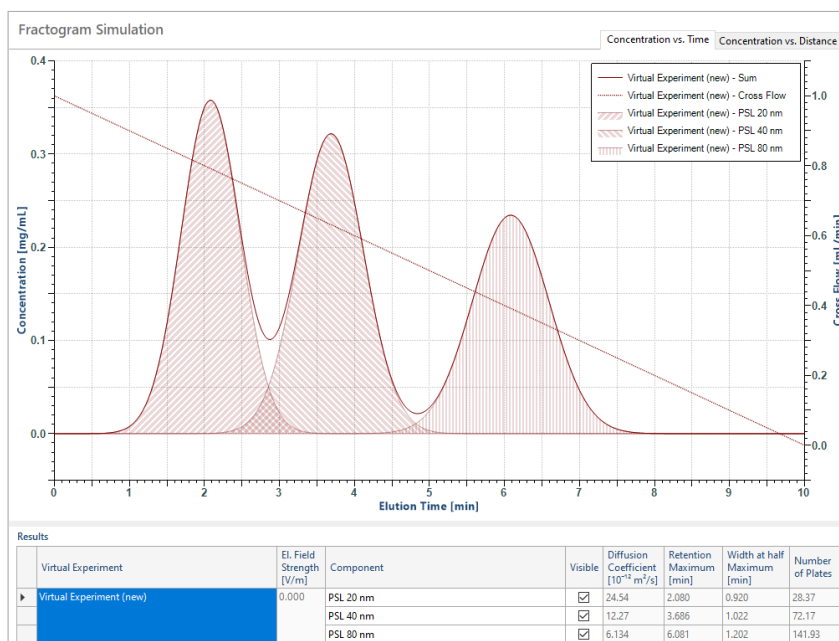
DESIGN METHOD

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

FFF Method Development

Let your PC do the work

Have a new sample to analyze? VISION DESIGN eliminates the need to spend inordinate amounts of time running a matrix of experiments to develop an FFF method. Just list the estimated particle sizes, select your FFF channel, membrane and spacer, and specify a cross-flow gradient.
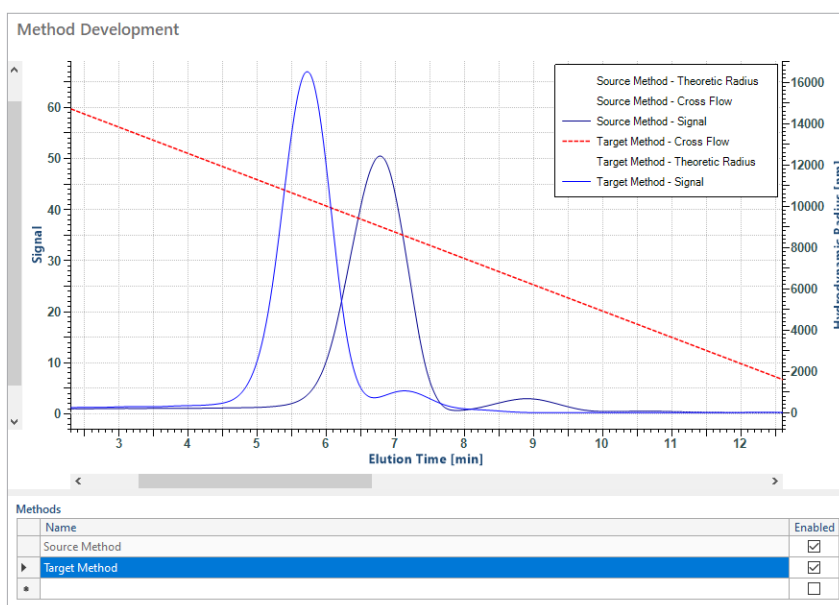
VISION DESIGN immediately displays the predicted fractogram using fundamental FFF theory to calculate retention times. It only takes a few minutes to explore the effect of different cross flow rates and timings to improve resolution and find the best method - all from the comfort of your desk.

Fractogram Simulation — Concentration vs. Time | Concentration vs. Distance

Legend:
- Virtual Experiment (new) - Sum
- Virtual Experiment (new) - Cross Flow
- Virtual Experiment (new) - PSL 20 nm
- Virtual Experiment (new) - PSL 40 nm
- Virtual Experiment (new) - PSL 80 nm

Results

Virtual Experiment	El. Field Strength [V/m]	Component	Visible	Diffusion Coefficient [10^{-12} m²/s]	Retention Maximum [min]	Width at half Maximum [min]	Number of Plates
Virtual Experiment (new)	0.000	PSL 20 nm	☑	24.54	2.080	0.920	28.37
		PSL 40 nm	☑	12.27	3.686	1.022	72.17
		PSL 80 nm	☑	6.134	6.081	1.202	141.93

Refine the method

The final design can be transferred in a click to VISION RUN to test your method.

Your initial guess of sample and channel parameters may have been inaccurate, but have no concern — results of a physical experiment can be fed back into VISION DESIGN to further refine the method. Usually a single test run suffices to give VISION DESIGN the information it needs to help you fully optimize your method.

Method Development

Legend:
- Source Method - Theoretic Radius
- Source Method - Cross Flow
- Source Method - Signal
- Target Method - Cross Flow
- Target Method - Theoretic Radius
- Target Method - Signal

Methods

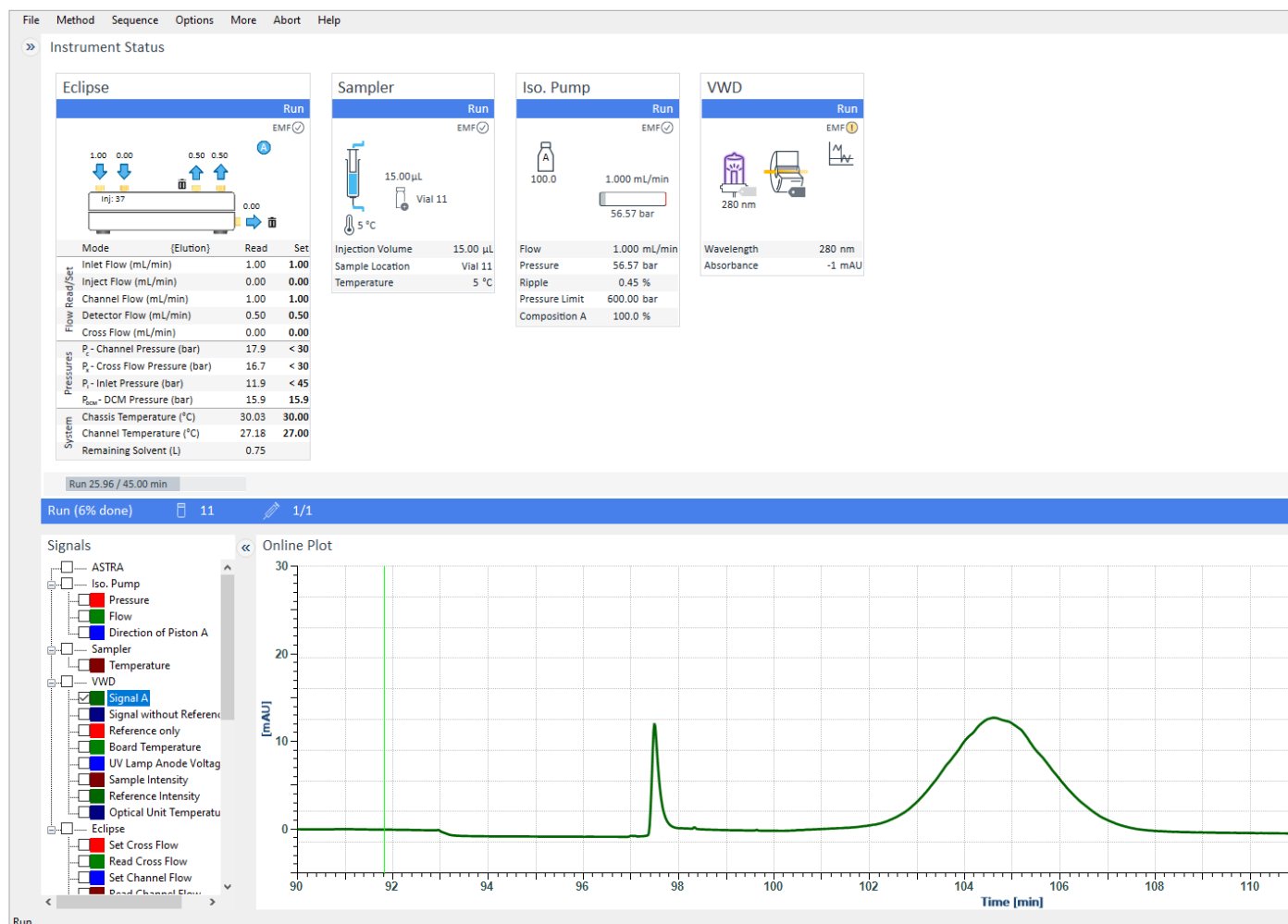|	Name	Enabled
	Source Method	☑
▶	Target Method	☑
*		☐

FFF Instrument Control

Enter the cockpit

VISION RUN is a sophisticated control center for the Eclipse FFF system. The dashboard shows the system status in real time with direct access to instrument configurations and sample sequences. Status information from the Eclipse instrument's Health Indicators are displayed; should any warnings come up, you will receive actionable recommendations.

Expand your horizons

VISION RUN uses the ICF instrument control framework for Agilent® modules from the 1260 series. The complete feature sets of autosamplers, isocratic or quaternary pumps, diode array detectors, fluorescence detectors and fraction collectors are supported to extract the maximum from FFF experiments.

Automated Sample Sequences

VISION RUN

Fly on autopilot

Once your sample sequence table is defined in VISION RUN, including FFF operation and MALS analysis methods, it's time to take flight. Sit back and trust VISION to run on autopilot, orchestrating the entire FFF-MALS measurement.

Should the need arise, system health indicators will alert you to intervene and prevent wasted runs. Decided to add more samples to a running sequence? Simply create another line in the table and VISION RUN will incorporate the additional samples.

Regulatory Compliance

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

- Administrator, researcher, technician and guest access levels
- Full audit trails
- Secure, joint SQL database for VISION and ASTRA data

- Local or remote database connectivity
- Data integrity validation
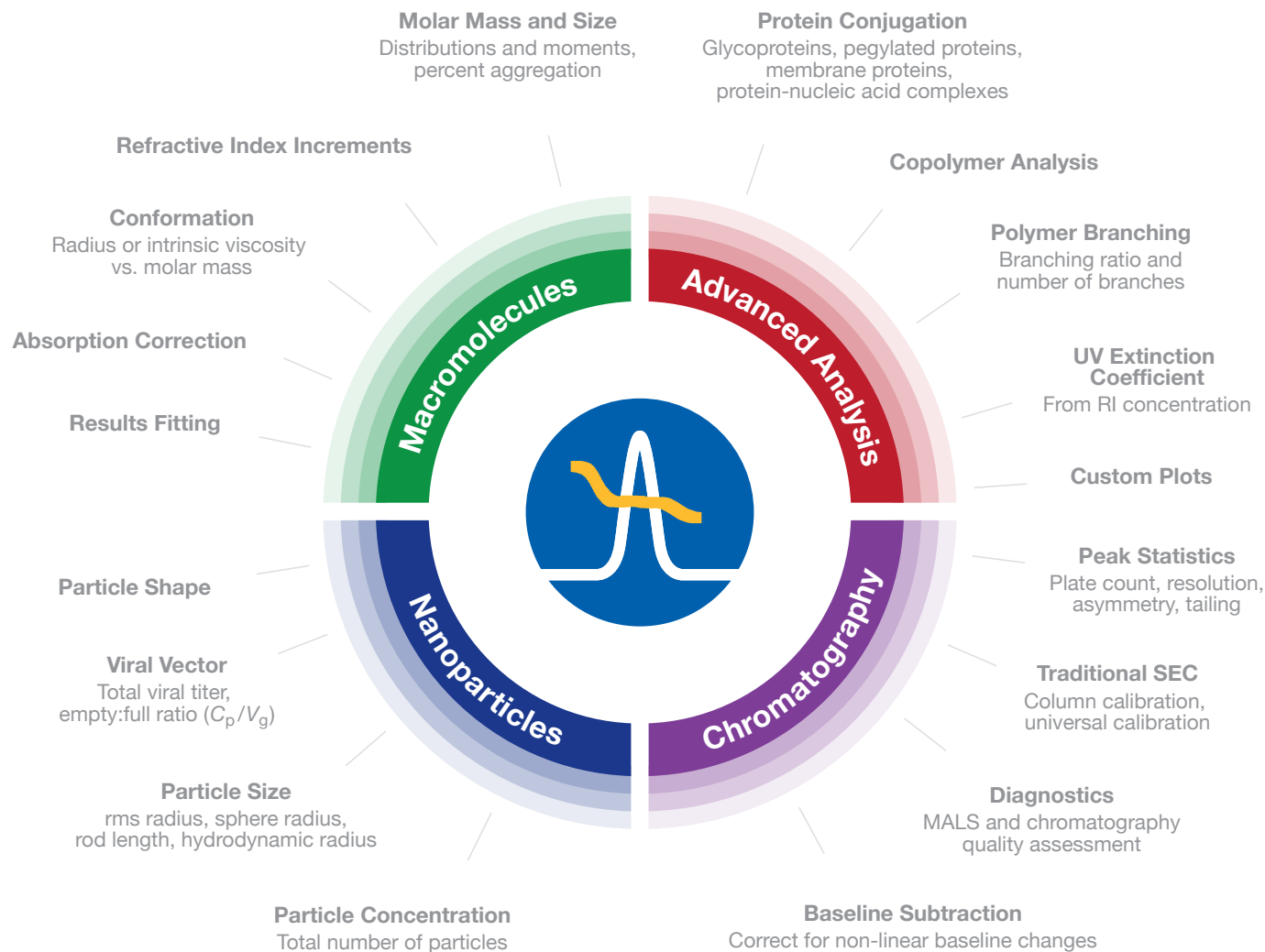- Full IQ/OQ procedures and documentation validation

ASTRA
Comprehensive characterization of FFF-MALS data

ASTRA

Macromolecules

Molar Mass and Size
Distributions and moments, percent aggregation

Refractive Index Increments

Conformation
Radius or intrinsic viscosity vs. molar mass

Absorption Correction

Results Fitting

Advanced Analysis

Protein Conjugation
Glycoproteins, pegylated proteins, membrane proteins, protein-nucleic acid complexes

Copolymer Analysis

Polymer Branching
Branching ratio and number of branches

UV Extinction Coefficient
From RI concentration

Custom Plots

Chromatography

Peak Statistics
Plate count, resolution, asymmetry, tailing

Traditional SEC
Column calibration, universal calibration

Diagnostics
MALS and chromatography quality assessment

Baseline Subtraction
Correct for non-linear baseline changes

Nanoparticles

Particle Shape

Viral Vector
Total viral titer, empty:full ratio (C_p/V_g)

Particle Size
rms radius, sphere radius, rod length, hydrodynamic radius

Particle Concentration
Total number of particles

Building on over 40 years of light scattering research and development, ASTRA includes an unrivaled range of analysis features to characterize macromolecules and nanoparticles.
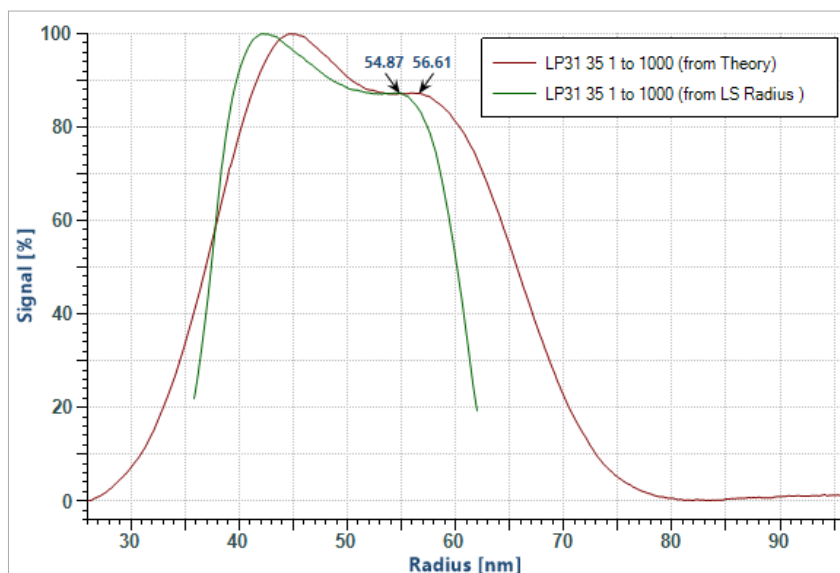Learn more about ASTRA's capabilities at www.wyatt.com/astra-benefits

FFF Data Analysis

Particle size distributions

Even without light scattering, VISION DESIGN can read in FFF data collected by VISION RUN to determine the hydrodynamic radius (R_h) and its distribution in the sample based on FFF retention time.
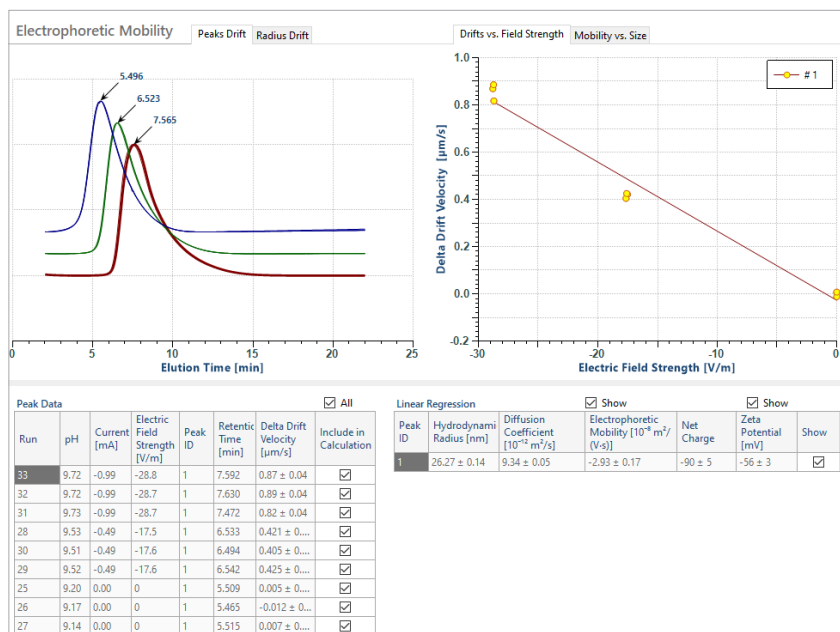
Choose from a calculation based on fundamental FFF theory or calibration with reference standards. The detailed, high-resolution R_h distribution complements FFF-MALS, batch dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA).

VISION compares hydrodynamic radius distributions from FFF theory (red) with geometric radius distributions from MALS (green).

Electrophoretic mobility and zeta potential

VISION calculates zeta potential and electrophoretic mobility from EAF4 measurements made with different applied electric fields. Data treatment is fully automated, including baseline and peak selection plus determination of peak retention time and its shift with the electrical field.

VISION DESIGN

Project and Data Management

System data traces

An invaluable function of VISION DESIGN is the overlay of system data traces for experiments within a sequence or project. Deviations in flow rate, pressure, or any other data trace are readily identified to validate results and facilitate troubleshooting.

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.

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.

VISION is one of many tools in Wyatt's Light Scattering Toolkit used to characterize proteins, polymers and nanoparticles in solution.
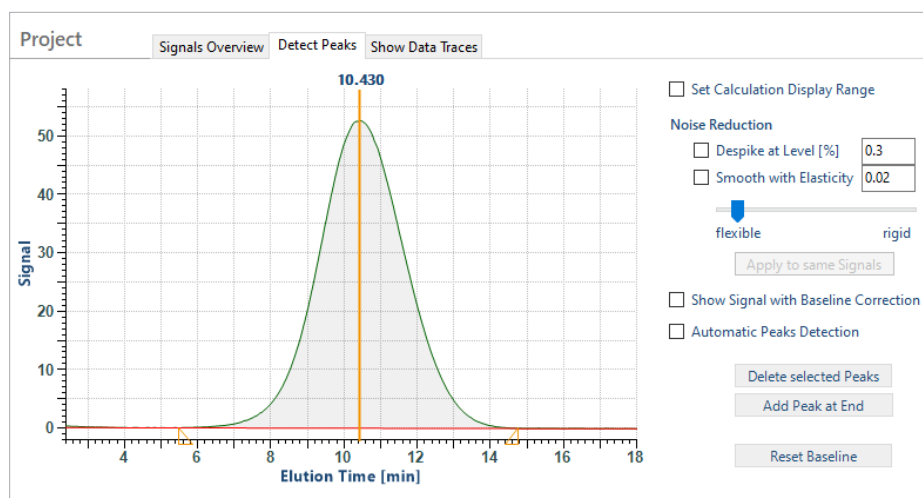
Learn more at www.wyatt.com

Learn more about FFF-MALS: www.wyatt.com/fff-mals

WYATT TECHNOLOGY