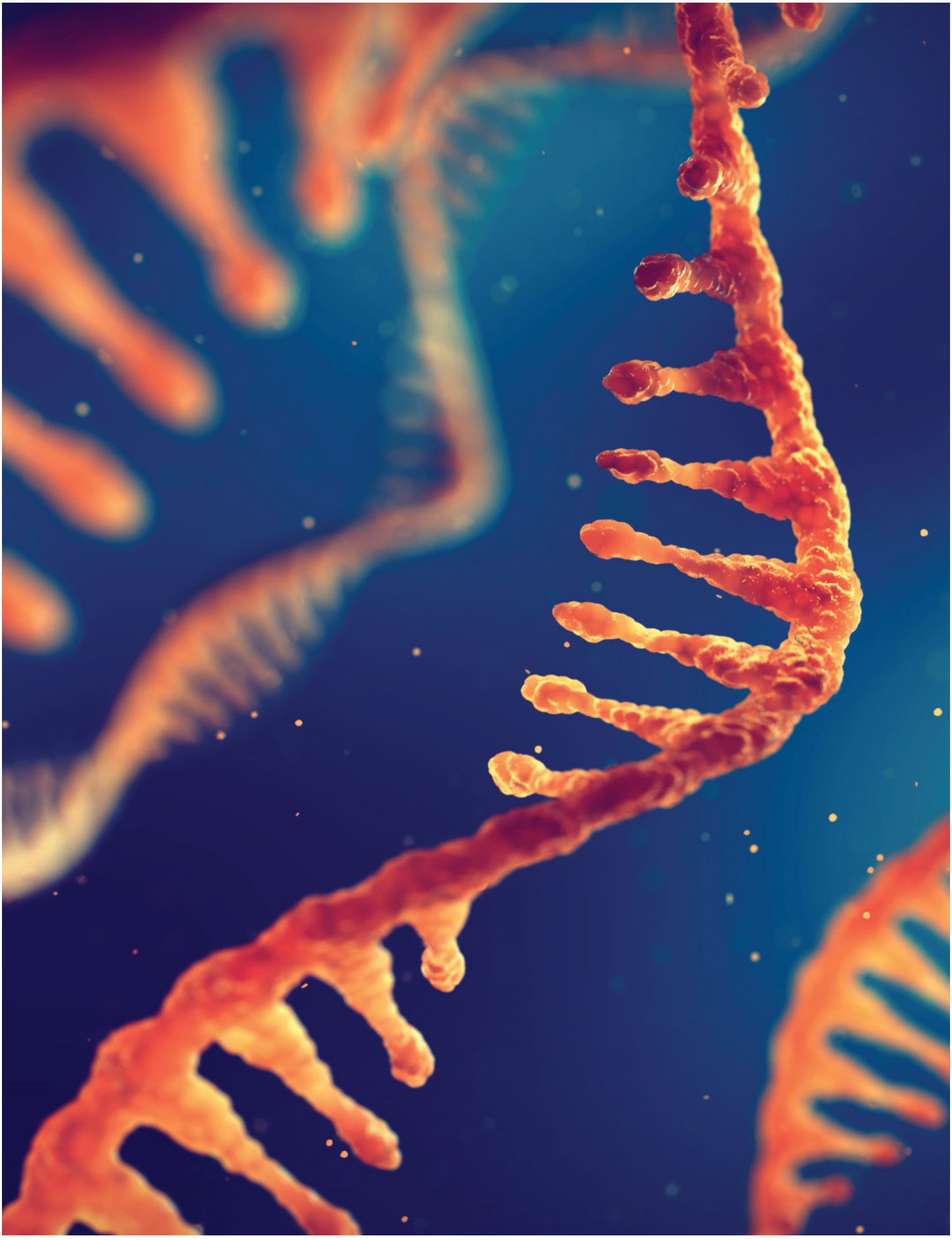
Agilent InfinityLab

Enhance Biopharma Insights with Advanced Size Exclusion Chromatography

Agilent Biopharma SEC Solutions

Agilent

Trusted Answers

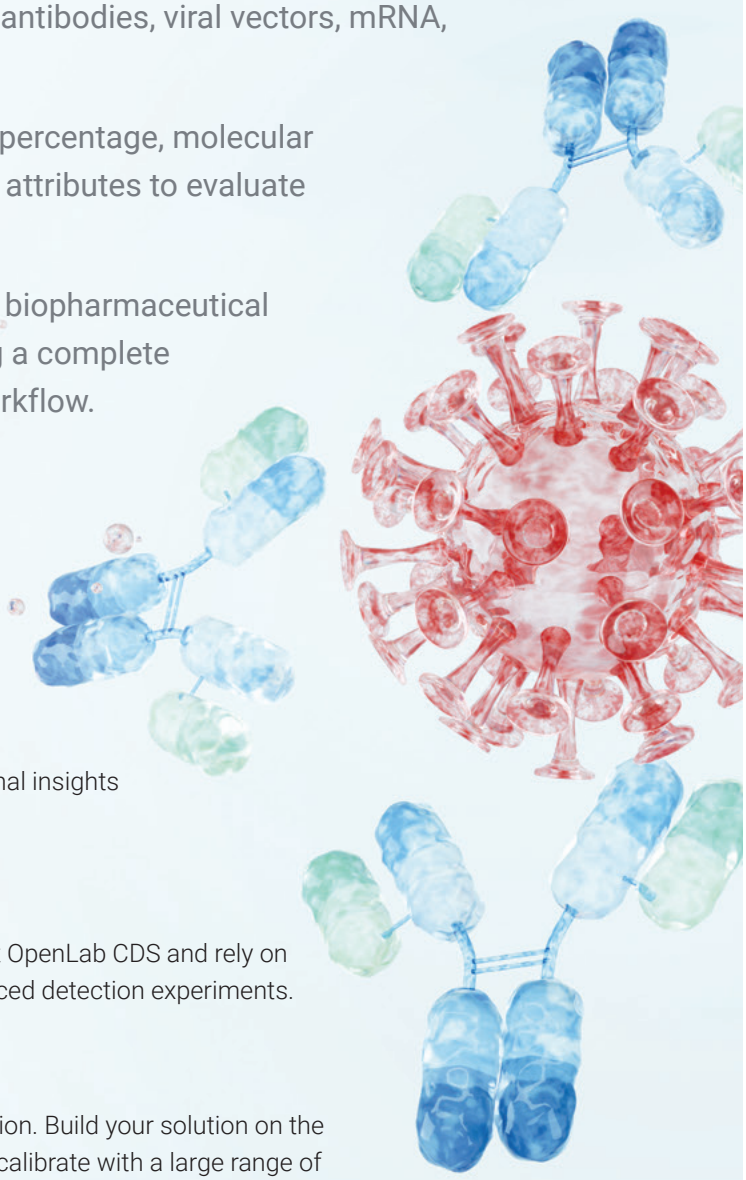


Agilent Biopharma SEC Solutions

Size exclusion chromatography (SEC) plays an essential role in the analysis of biopharmaceutical modalities like monoclonal antibodies, viral vectors, mRNA, and other.

SEC enables the determination of aggregation percentage, molecular weight, and size, as some of the critical quality attributes to evaluate stability and integrity.

The challenges when analyzing these complex biopharmaceutical components can only be addressed by offering a complete solution that covers the entire SEC analysis workflow.



Advanced detection

Get absolute molecular weight information and gain additional insights into size, structure, and aggregation behavior.

Powerful and user-friendly software

Have your SEC workflows seamlessly integrated into Agilent OpenLab CDS and rely on intuitive, user-friendly workflows even for challenging advanced detection experiments.

Comprehensive columns and standards offerings

The SEC column is always key for reliable SEC characterization. Build your solution on the broadest SEC column portfolio on the market and precisely calibrate with a large range of dedicated SEC standards.

Reliable Hardware — The Foundation of Every Successful SEC Analysis

Agilent InfinityLab LC series instrumentation provides the base for all SEC workflows

Leverage the full potential of the Agilent Infinity III portfolio for SEC analysis. Rely on robust pump technology, flexible sampling devices, and high-resolution detection. The Agilent biocompatible and bio-inert SEC modules provide additional robustness and reliability for the analysis of biopharmaceutical modalities.

Bio LC solutions

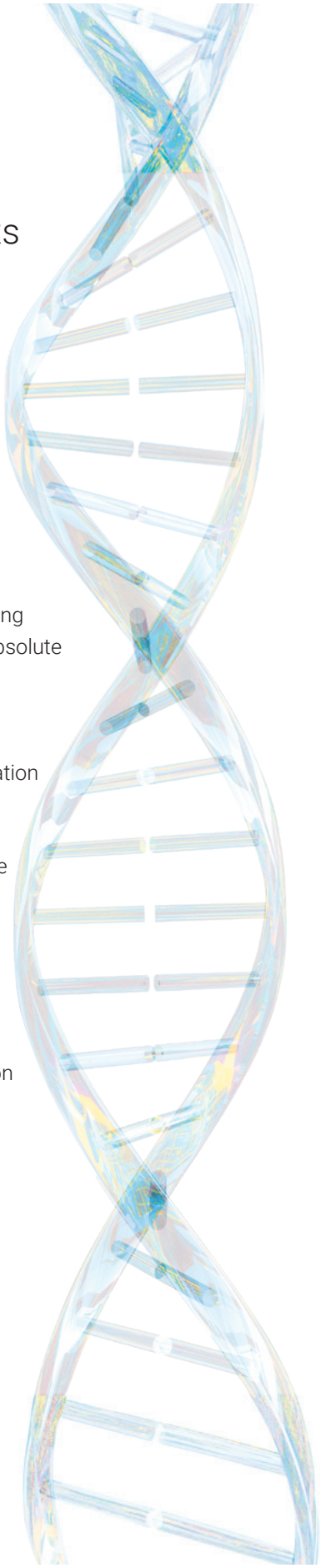
Bio LC solutions comprise a full range of entirely biocompatible and bio-inert LC systems that enable robust, accurate bioanalysis from drug discovery and development to QA/QC, plus matching columns.

BIO BIO INERT

SEC Advanced Detection

Derive absolute molecular weight and structural insights

Powerful additions to classic SEC are static and dynamic light scattering detection, enabling absolute determination of molecular weight and molecular size.



Why light scattering?

Traditional SEC relies on calibration standards, which can introduce uncertainty when analyzing complex biomolecules. Light scattering detection eliminates these limitations by providing absolute molecular weight and size measurements, independent of column calibration.

Static light scattering techniques like multi-angle light scattering give you highest accuracy molecular weight information of large biopharma molecules and provides additional size information by measuring the radius of gyration of the analytes.

The Agilent 1260 Infinity II Multi-Angle Light Scattering Detector enables high-precision determination of absolute molecular weight and radius of gyration via light scattering detection from up to 20 angles.

Dynamic light scattering provides size information via the hydrodynamic radius by measuring the time-dependent fluctuations in scattering intensity. This allows you to precisely determine size and aggregation behavior of biopharma molecules like proteins and antibodies.

The Agilent 1260 Infinity II Bio-SEC Multidetector System's market-leading, low-volume detector flow cell minimizes any peak band broadening to ensure the highest precision for molecular weight and size determination.

Agilent 1260 Infinity II Multi-Angle Light Scattering Detector

Agilent 1260 Infinity II Bio-SEC Multidetector System

Powerful and Seamlessly Integrated Software

Advanced GPC/SEC software for OpenLab CDS

Data processing

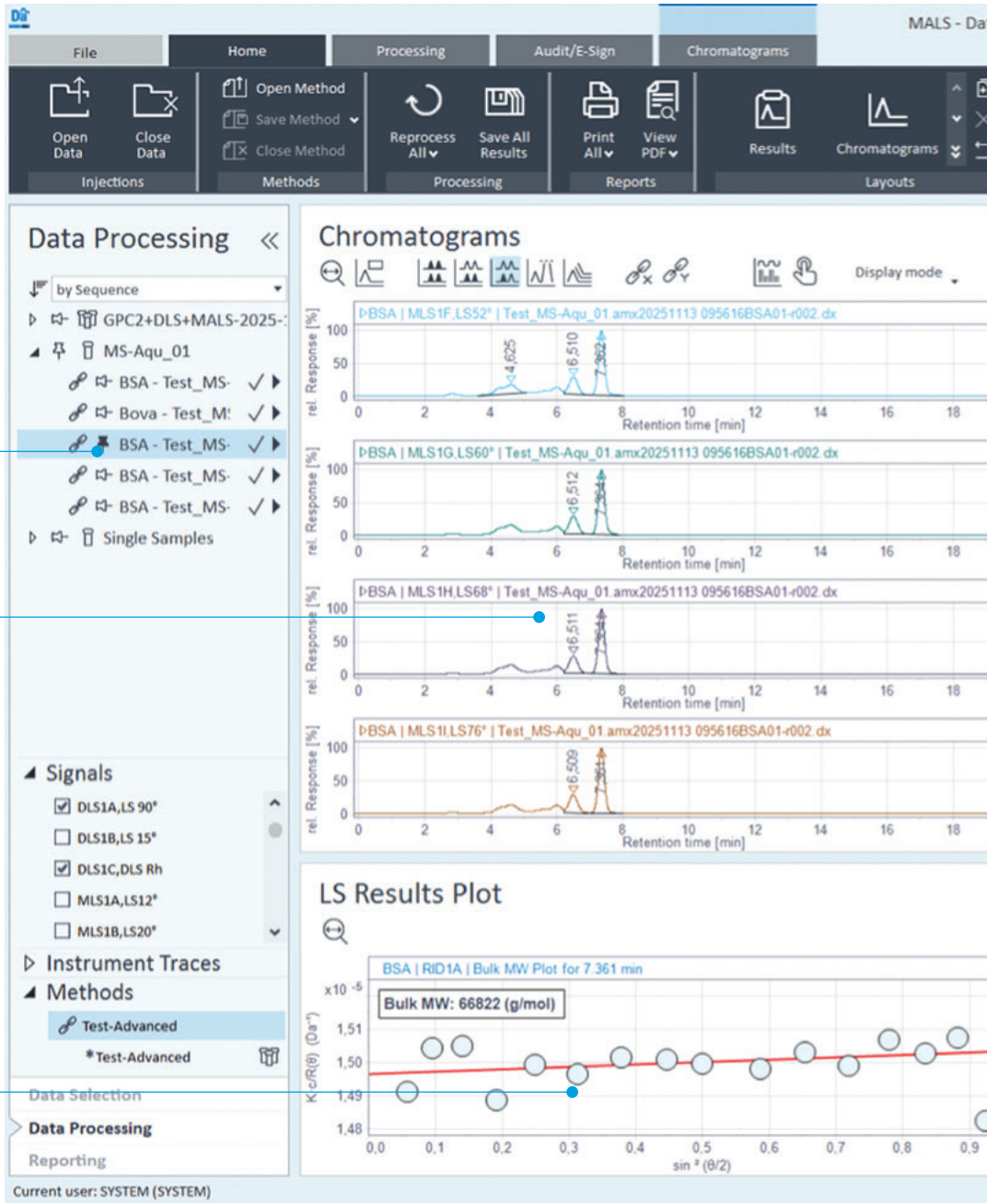
Open and organize your data in the familiar Agilent OpenLab framework.

Chromatograms window

Organize and overlay your data according to signal/detector values.

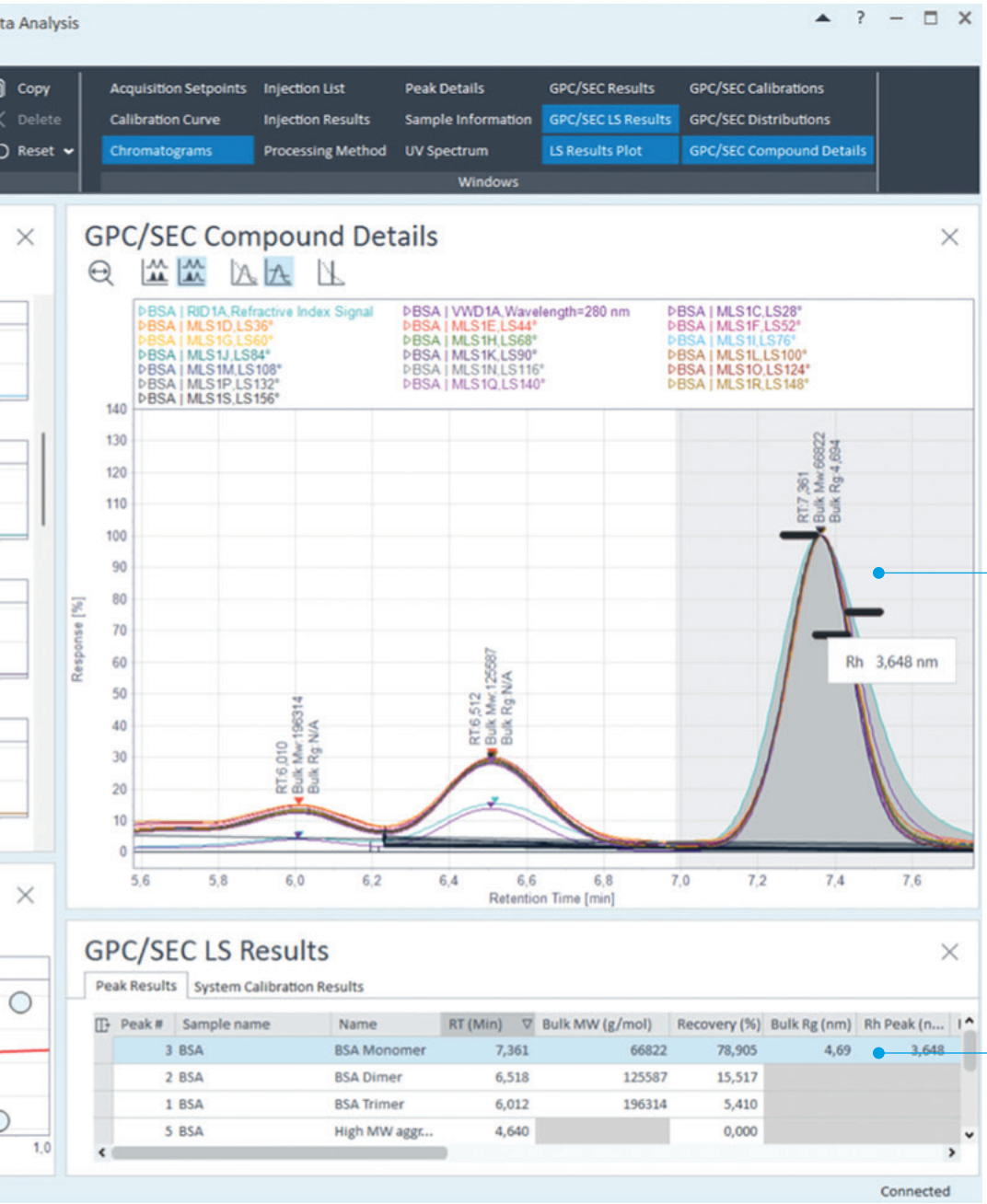
LS results plot

All light scattering angles in a comprehensive plot let you calculate absolute molecular weight.



Screenshot of the Agilent Advanced GPC/SEC Software for OpenLab CDS data analysis software interface.

SEC advanced detection does not need to be complex. Rely on a familiar UI for advanced SEC light scattering workflows. Perform all your analyses on a single software platform. From advanced or conventional SEC to powerful liquid chromatography data analysis, OpenLab CDS provides a one-stop solution for size exclusion chromatography and much more.



GPC/SEC compound details

Overlaid light scattering and concentration detector signals with optional display of molecular weight and size information.

GPC/SEC light scattering results

Overview table lets you review all important GPC/SEC light scattering results and parameters.

Application example 1

SEC Light Scattering Analysis of Monoclonal Antibodies with Advanced GPC/SEC Software for OpenLab CDS

Size exclusion chromatography, in combination with static and dynamic light scattering detection enables accurate and reliable separation with molecular weight and size determination of monomer and aggregate protein species.

The Advanced GPC/SEC Software for OpenLab CDS, in combination with the Agilent 1260 Infinity II Bio-SEC Multidetector System featuring dual-angle and DLS detection enables light scattering capabilities in a compliance-supporting CDS environment and provides a single platform for advanced SEC analysis of biopharmaceuticals.

Figures 1A and 1B show the characterization of the mAb Rituximab in the GPC/SEC compound details window. The light scattering analysis revealed a molecular weight of about 148 kDa for the monomeric peak. The precision of molecular weight determination of seven consecutive runs was excellent, with 0.253% relative standard deviation (RSD).

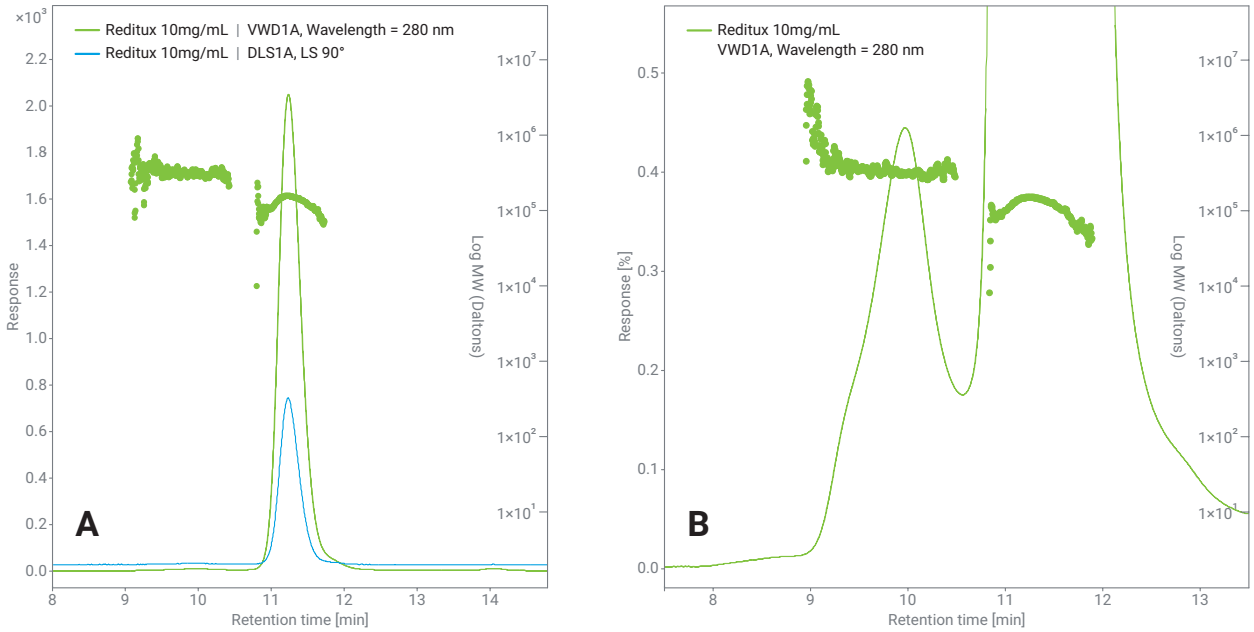
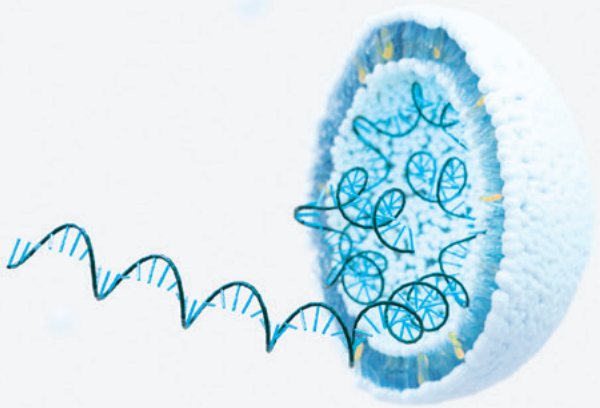


Figure 1. SEC light scattering analysis of Rituximab biosimilar with UV (green) and light scattering signal at 90° (blue). The molecular weight was determined at ~148 kDa for the monomer peak. Figure 1B shows a zoomed in version, with more details.

mRNA Analysis with MALS



The characterization of messenger ribonucleic acid (mRNA) with SEC-MALS enables the analysis of both biophysical and structural attributes of the molecules. Aggregation, molecular weight, and size can be determined to study the impact on these attributes from different conditions during production, formulation, and storage.

The Advanced GPC/SEC Software for OpenLab CDS, in combination with the Agilent 1260 Infinity II Multi-Angle Light Scattering Detector and the Agilent 1290 Infinity II Bio LC System enables light scattering analysis in a compliance-supporting CDS environment and provides a single platform for advanced SEC analysis of biopharmaceuticals.

Figure 2 shows the characterization of an IVT mRNA sample in the GPC/SEC Compound Details Window. Here, the MW distribution can be displayed over the different peaks of interest. The calculated MW, as well as Rg can be annotated directly over the peaks in the window. In addition, all parameters are displayed in the GPC/SEC LS Results table.

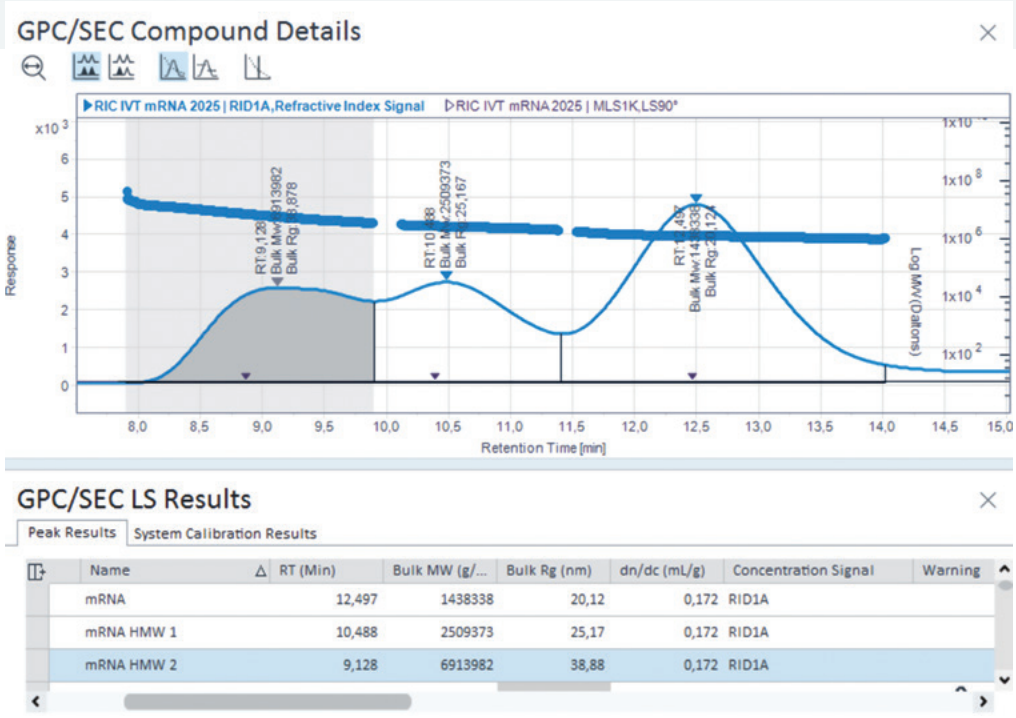
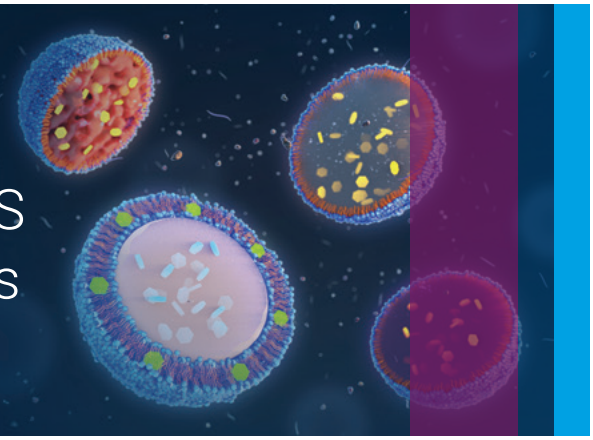


Figure 2. Screenshot of the GPC/SEC Compound Details Window, as well as the GPC/SEC LS Results Table.

Application example 3

Advanced SEC with Online LS/DLS Detection for the Analysis of LNPs



The size of lipid nanoparticles (LNPs) is a fundamental critical quality attribute that directly impacts their biological performance. Precise control and analytical characterization of LNP size is essential for optimizing drug delivery systems and ensuring the safety and effectiveness of biopharmaceutical formulations.

In this application note, we present the size exclusion chromatography (SEC) analysis combined with online dynamic light scattering (DLS) and static light scattering (LS) of various LNP samples, including both loaded and empty vesicles. This approach yields precise and reproducible sizing data, along with information on stability and aggregation. A comparison with batch DLS measurements demonstrated a strong correlation with the online SEC-LS/DLS setup.

In addition to the calculated Rh values, radius of gyration and shape factors were also calculated for the tested LNP samples. The shape factor is defined as Rg/Rh = ρ. For a sphere with a dense core, ρ is expected to be 0.77 or less. All calculated values were evaluated for precision (n = 5) as relative standard deviation (RSD) (see Table 1 and Figure 3).

Table 1. Size in Rhp and Rgp and shape factor plus precision values in % RSD for n = 5.

Sample Name	Size Rhp (nm)	RSD (%)	Size Rgp (nm)	RSD (%)	Shaple ρ
LNP A mRNA	38	5.0	22.9	8.6	0.608
LNP B mRNA	58	2.2	37.4	12.6	0.646
LNP A (Empty)	30	2.5	25.2	15.1	0.851
LNP B (Empty)	47	3.2	35.1	2.1	0.752

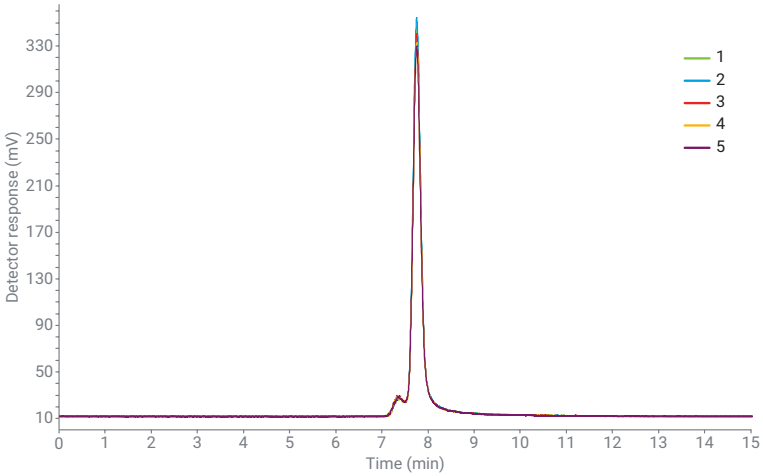
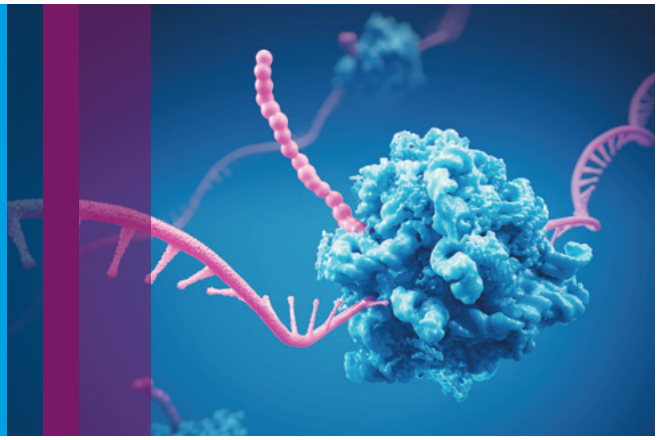
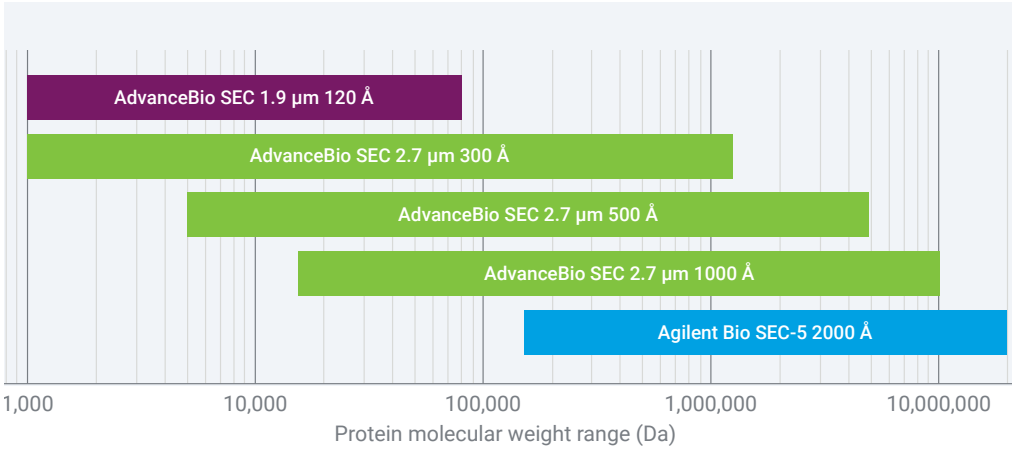


Figure 3. Overlay of five consecutive injections of LNP A empty.

Choosing an SEC Column to Complete Your Workflow



Agilent offers a variety of size exclusion columns suitable for use with light scattering detection. Pore size is arguably the most critical aspect of SEC column selection, as it dictates which analytes can be separated.



Protein standards for SEC

Ensure column performance and system suitability.

Column	Application	Particle Size	Pore Size	Sample Size Range	Maximum Pressure
Agilent AdvanceBio SEC	mAbs and other proteins, oligonucleotides	2.7 µm	300 Å	1 kDa to 1.25 MDa 10-300 bp DS oligo; 25-1000 nt SS oligo	400 bar
		1.9 µm	200 Å	2-700 kDa	620 bar
	AAVs, small VLPs, oligonucleotides	2.7 µm	500 Å	5 kDa to 5 MDa; Rh 3-25 nm 20-1000 bp DS oligo; 60-3000 nt SS oligo	400 bar
	VLPs, large oligonucleotides such as mRNA	2.7 µm	1000 Å	5 kDa to 10 MDa; Rh 5-75 nm 50-1000 bp DS oligo; 150-10000 nt SS oligo	400 bar
Agilent Bio SEC-5	VLPs, very large oligonucleotides	5 µm	2000 Å	150 kDa to 10 Mda; Rh 5-75 nm	240 bar
Agilent PROTEEMA Lux	Natural and synthetic polymers and proteins	5 µm	1000 Å	1 kDa to 7.5 MDa	150 bar

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