Size Exclusion Chromatography of Biomolecules: Column Selection and Method Optimization

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Outline

SEC for Biomolecules

Key attributes and testing methods for mAb analysis and characterization

- Aggregates and other impurities

Size exclusion chromatography (SEC)

- Overview, nomenclatures, and why use it?

How to approach SEC for aggregate analysis?

- SEC column characteristics, method development and method optimization

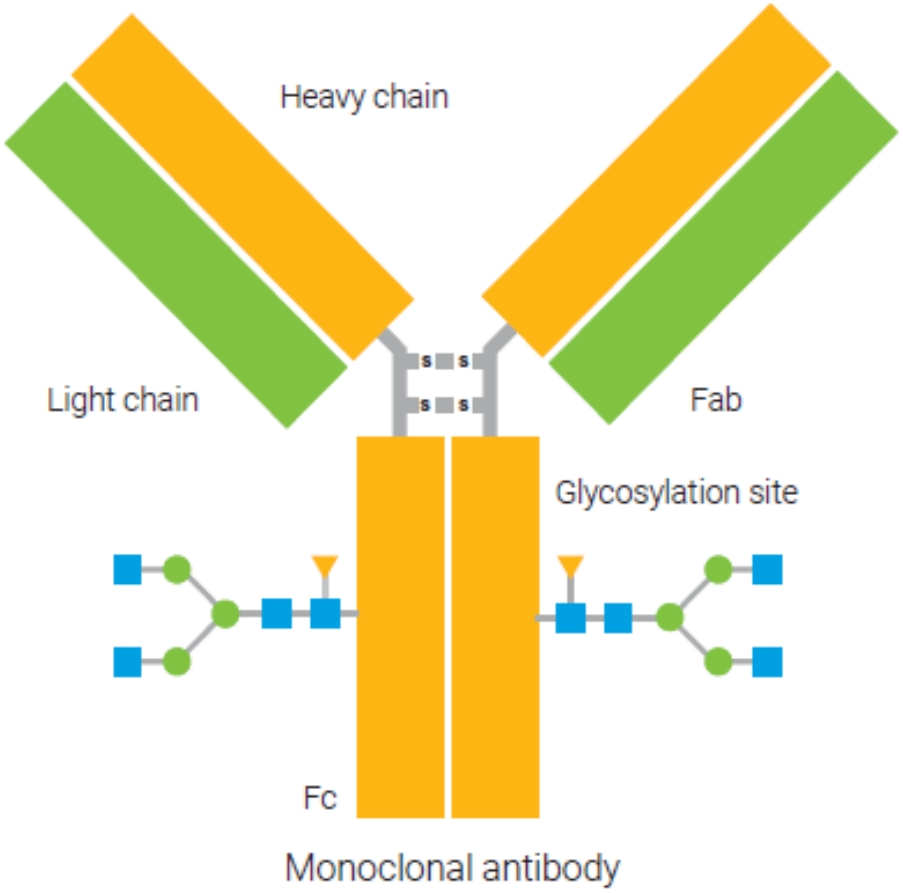
Target workflows

Choice of detector

What Is a Biomolecule?

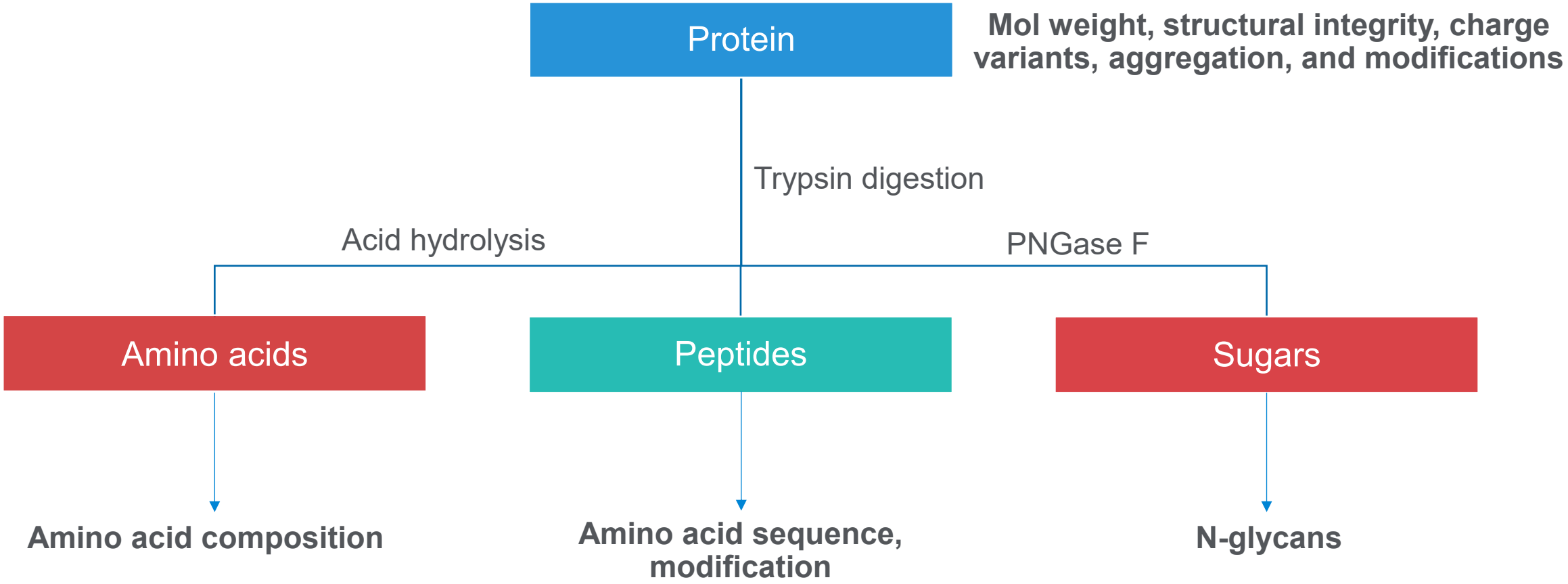
Biomolecules range in size from amino acids and small lipids to large polynucleotides, such as DNA or RNA.

- Proteins
- Peptides
- DNA/RNA oligonucleotides
- Amino acids



Protein Characterization

Eifferent levels

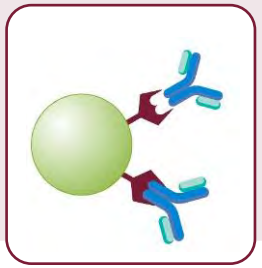


Biochromatography Separation Modes – LC Biocolumn Offerings

Titer Determination

Affinity

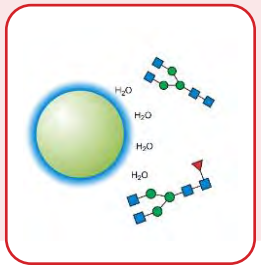
Isolation and quantitation of IgG



Glycan Analysis

HILIC

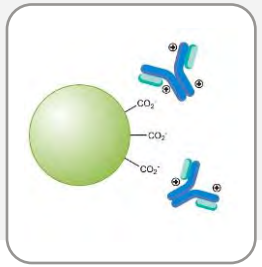
Fast, high-resolution, reproducible glycan separation



Charge Variant Analysis

Ion exchange

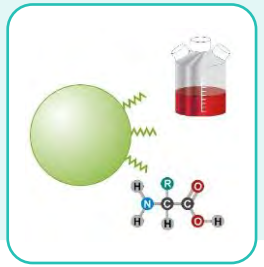
Enhances the accuracy and speed of separation



Amino Acid and Cell Culture

Small molecule (RP)

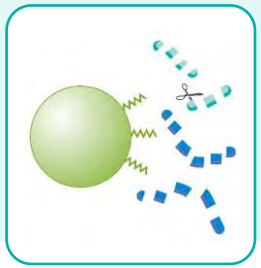
Delivers robust, high-resolution separations



Peptide Mapping

Reversed phase (RP)

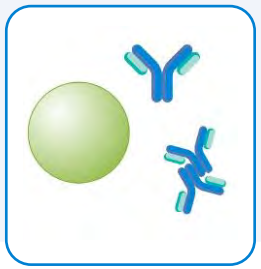
Reliably characterizes primary sequence and detects PTMs



Aggregate/Fragment

SEC

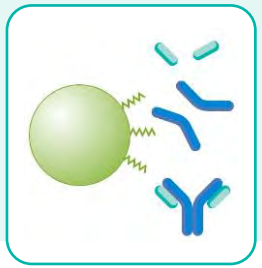
Solution for separating and analyzing intact proteins



Intact and Subunit Purity

Large molecule (RP)

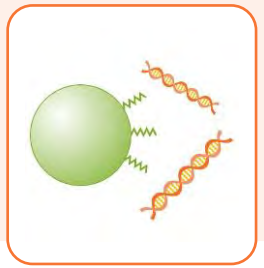
Selective options for every separation need



Oligonucleotide Analysis

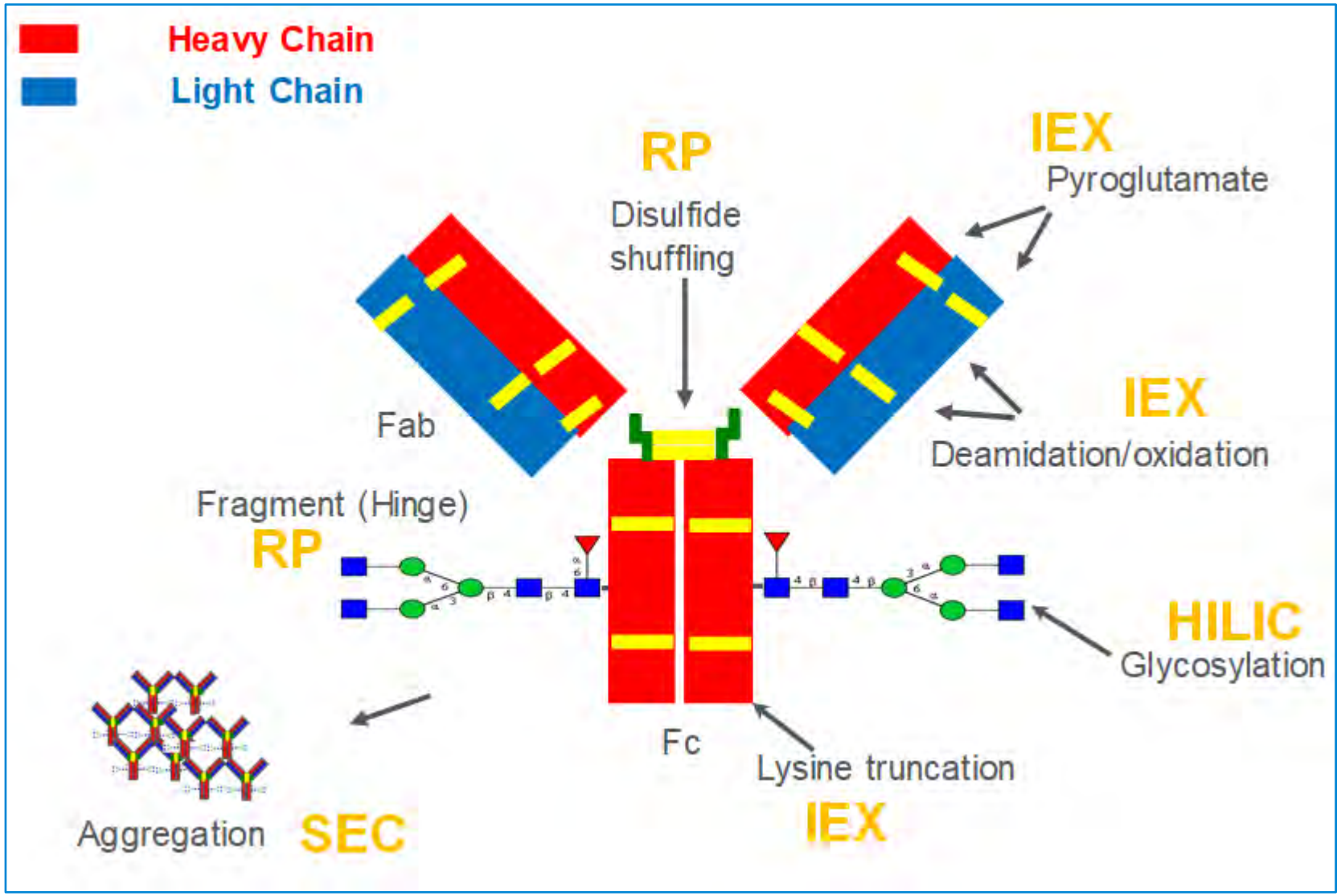
Ion-pair RP or SAX

Delivers robust, high-efficiency separations for DNA/RNA



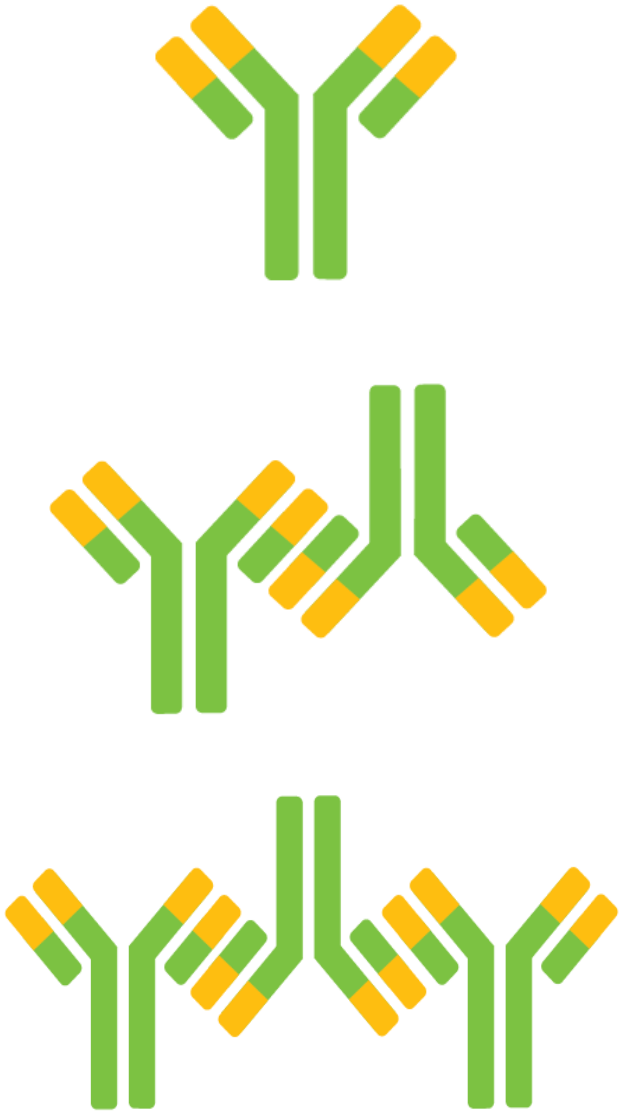
Key Attributes and Testing Methods

mAb analysis and characterization



Aggregation

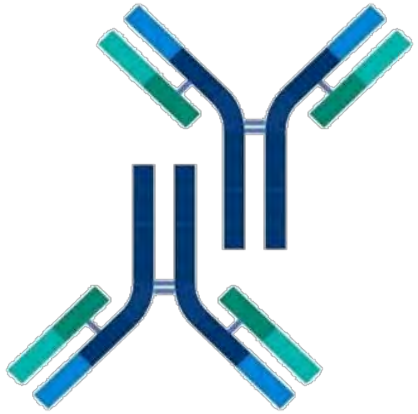
- Aggregation is a key critical quality attribute throughout the whole drug development process
- Aggregates can exist as small dimers, fragments, and progress towards larger structures
- Aggregation can affect biological activities, potency, and efficacy. It can also form during storage, shipping, freezing and thawing cycles.
- Aggregate analysis can be carried out through different analytical methods and detection tools
- Need for a robust start-to-end solution that is optimized, sensitive, and reproducible

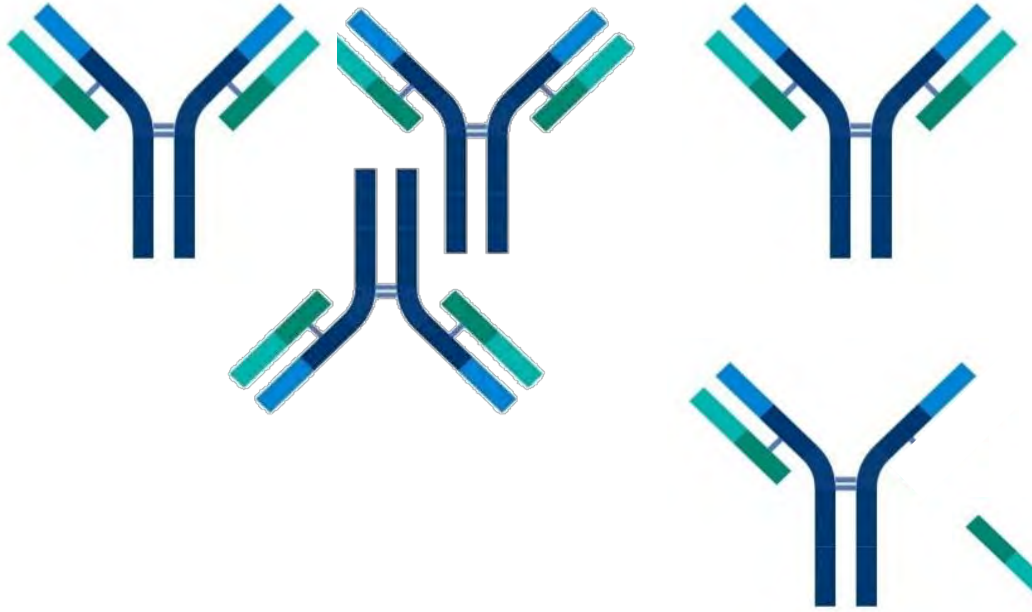


Protein Characterization

Importance of aggregates and fragments

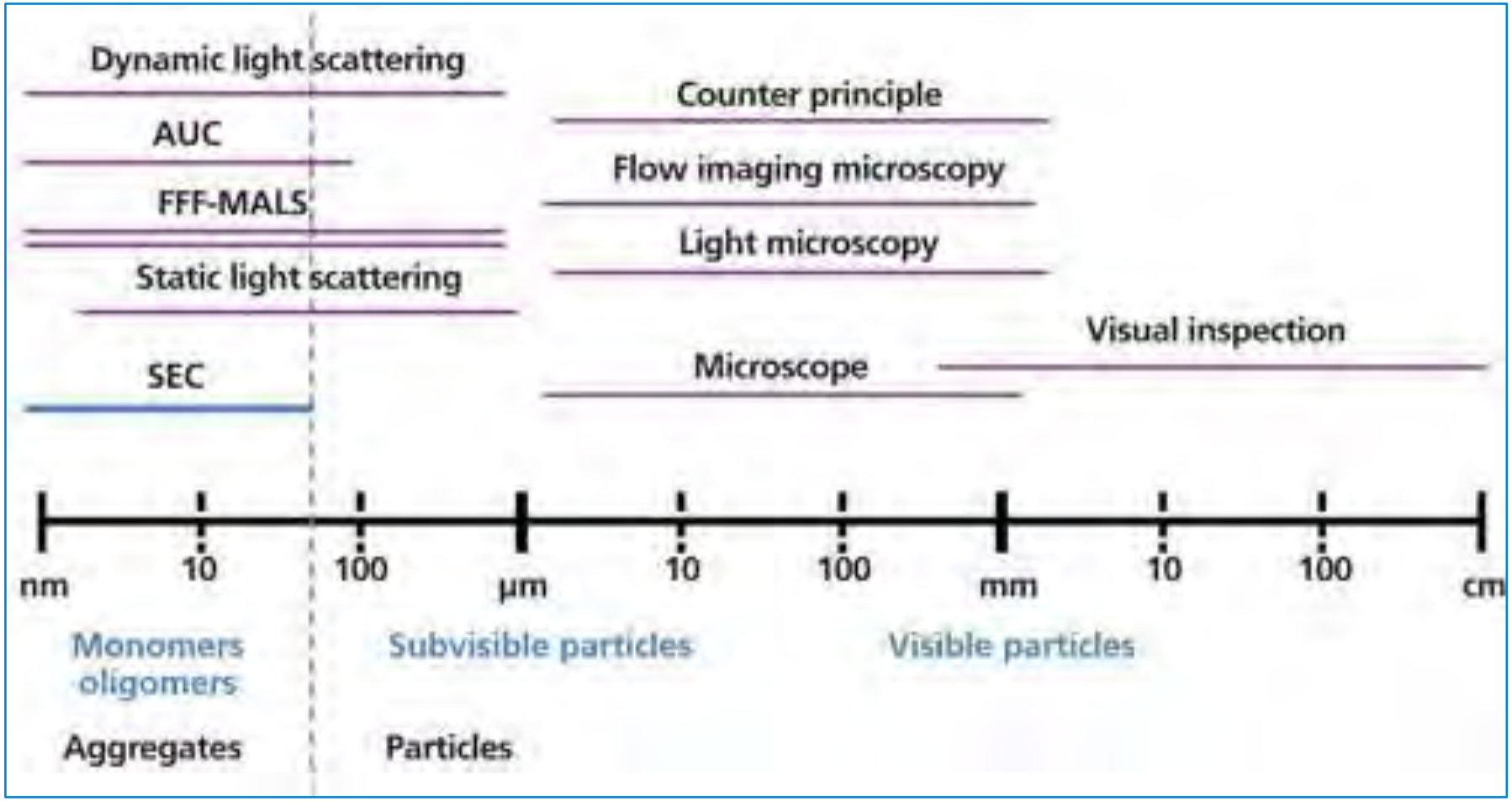
- Aggregation is not only highly risky – possibly triggering an immunogenic response – it is very common
- Aggregation is a common response when a protein is exposed to stress conditions:
  - Upstream or downstream processes
  - Changes in concentration
  - Changes in pH or temperature
  - Exposure to surfaces or shear forces
- Proteins are relatively unstable
- Do not always adopt native conformation





Analytical Methods for Aggregation Analysis

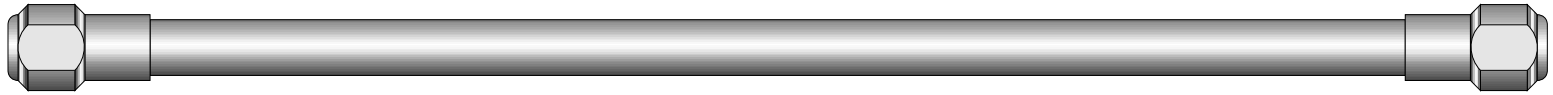
Separating and nonseparation techniques



Reference publication: LCGC Supplements, Special Issues-04-01-2016, Volume 34, Issue 4, Pages: 28–36

SEC Nomenclatures

- Column volume
- Exclusion limit/void volume
- Interstitial volume
- Pore volume
- Total permeation
- Nonspecific interaction



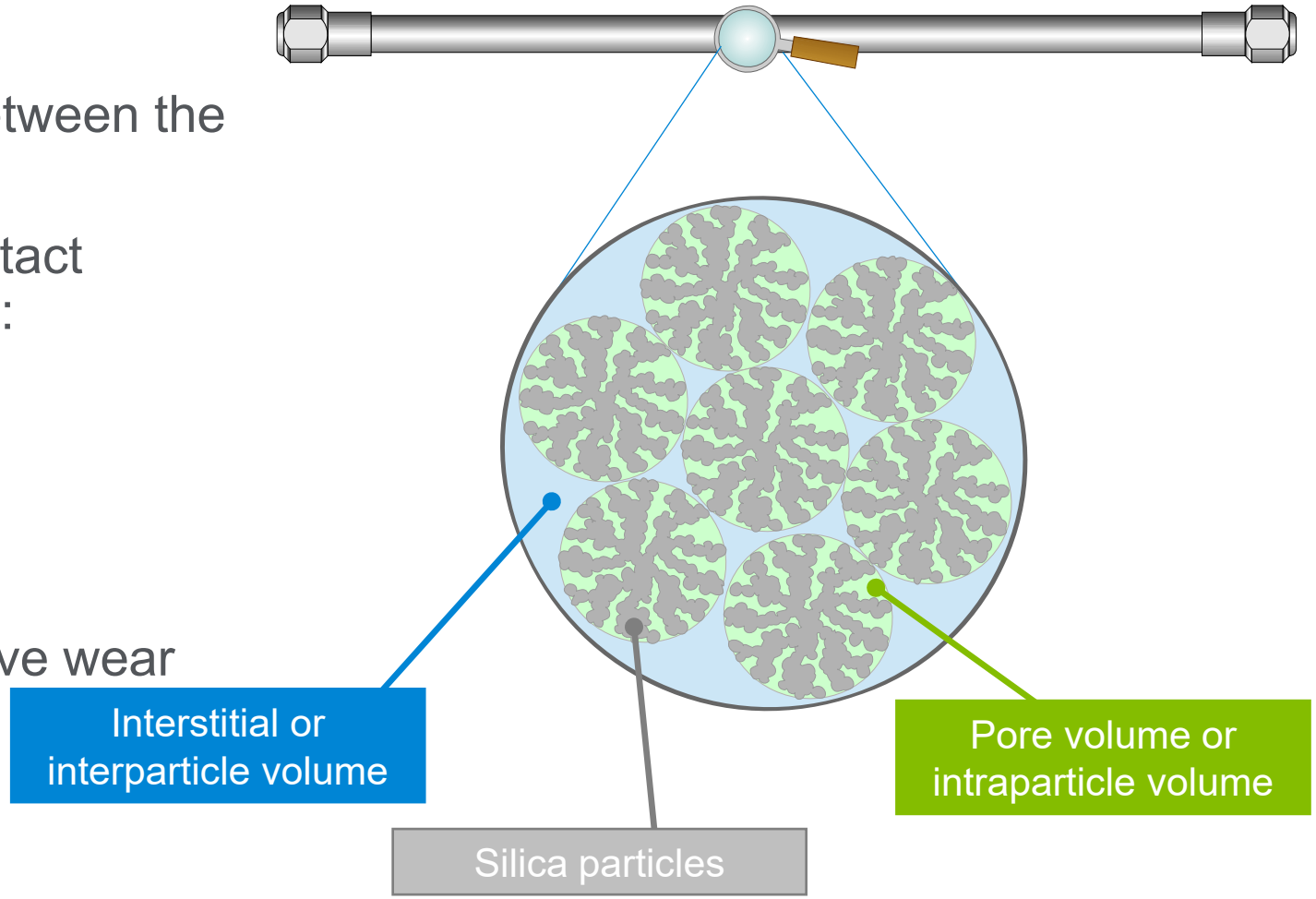
Column dimensions: 7.8 x 300 mm
Column volume = 14.3 mL

Column dimensions: 4.6 x 300 mm
Column volume = 4.98 mL

Size Exclusion Chromatography (SEC)

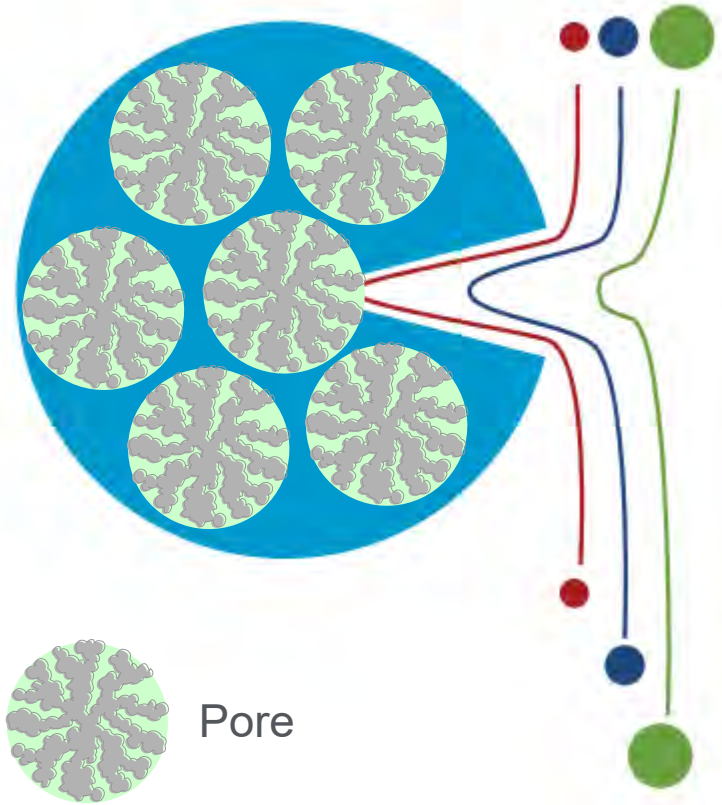
Separation of biomolecules based on size

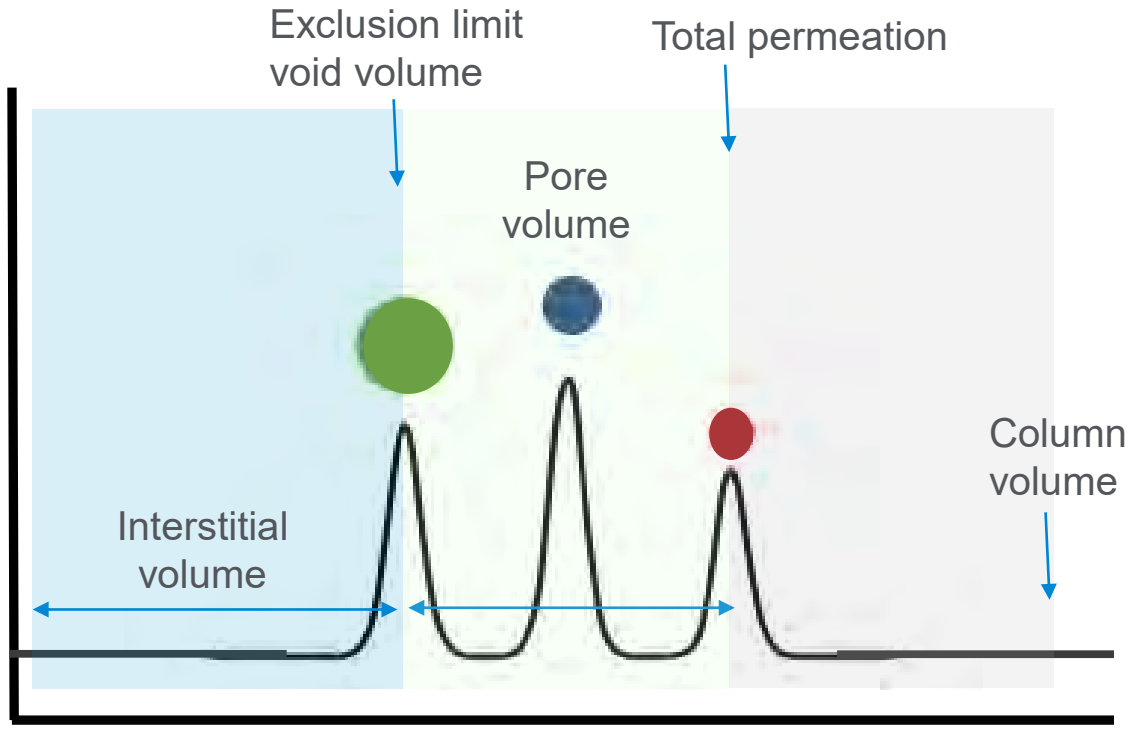
- Separation by size under native (nondenaturing) mode
- Relies on absence of interactions between the analyte and the stationary phase
- Ideal for separating and analyzing intact proteins from contaminants, such as:
  - Aggregates
  - Excipients
  - Cell debris
  - Degradation impurities
- High salt concentration puts excessive wear on instrument parts



Size Exclusion Chromatography (SEC)

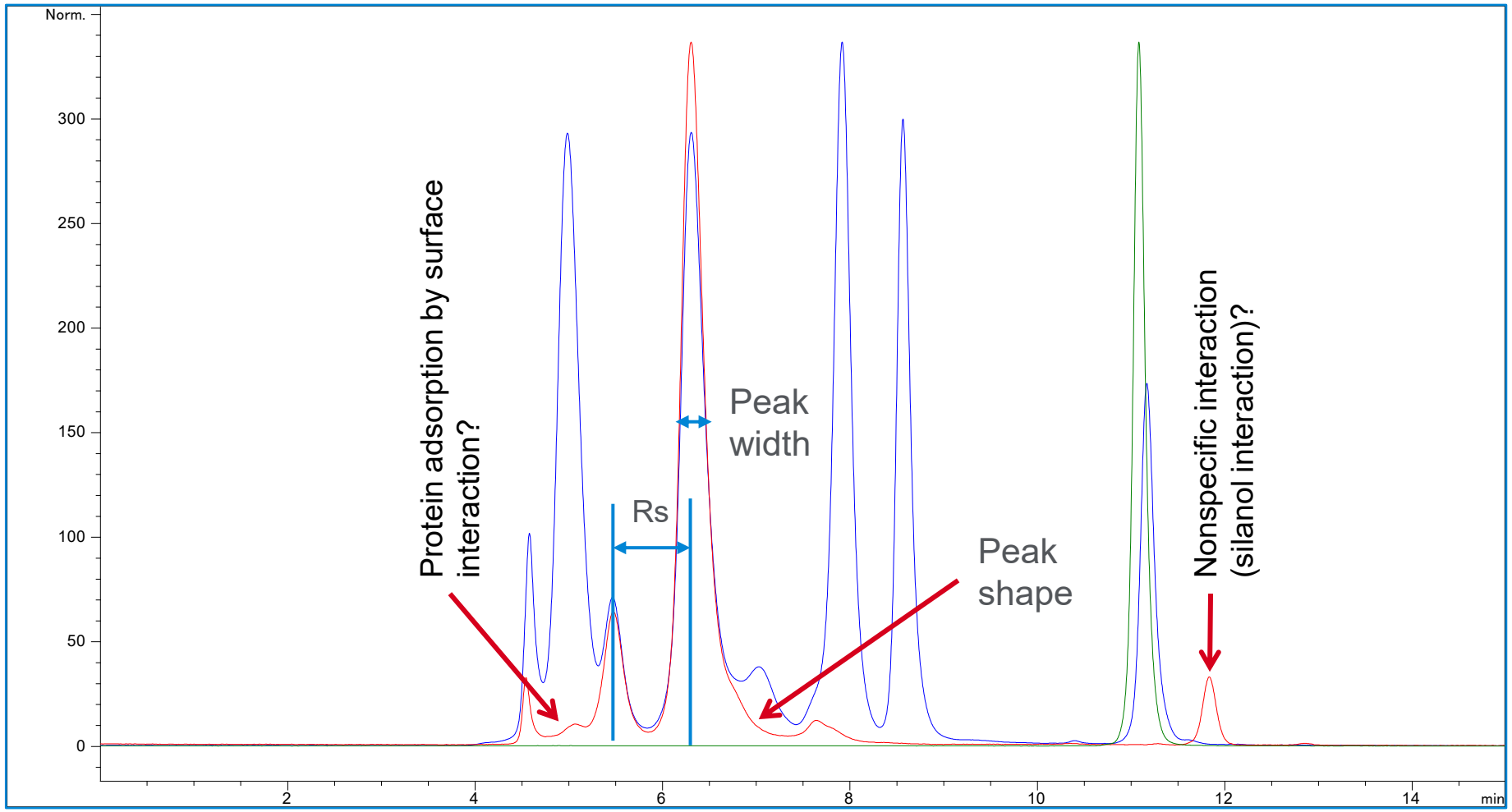
Separation by size under native (nondenaturing) mode





- Smaller molecules spend longer in the pores and elute later
- Larger molecules spend less time in the pores and elute sooner

How to Evaluate and Compare Column Performance



- Peak width and resolution depend on particle morphology (particle size, pore size, and pore volume)
- Peak shape, protein adsorption, and nonspecific interaction are dependent on bonding chemistry (hydrophilic group, surface coverage)

Column Selection Criteria for SEC

- Pore size
  - Sample MW range
  - Exclusion limit
  - Maximize pore volume
- Particle size
  - Smaller particles → higher resolution
- Number of columns and length
  - Resolution versus analysis time
  - Shorter column length → higher throughput
  - Longer column length → improved resolution
- Column id
  - smaller column id → reduced solvent consumption, smaller injection volume, or improved sensitivity

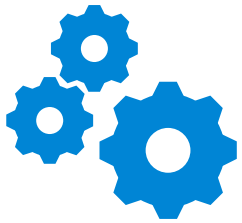
Column Selection Criteria for SEC

Common challenges

- Limited resolution – insufficient/incorrect pore sizes can reduce resolution
- Nonspecific interactions contribute to loss of sample, lead to inconsistent results, rework
- Long analysis times – SEC is typically slow
- Poor pressure stability creates rework and increases cost
- Consistent and reproducible results are desirable
- High salt conditions puts excessive wear on instrument, parts

How Do We Approach SEC for Aggregate Analysis ?

Possible parameters for optimization



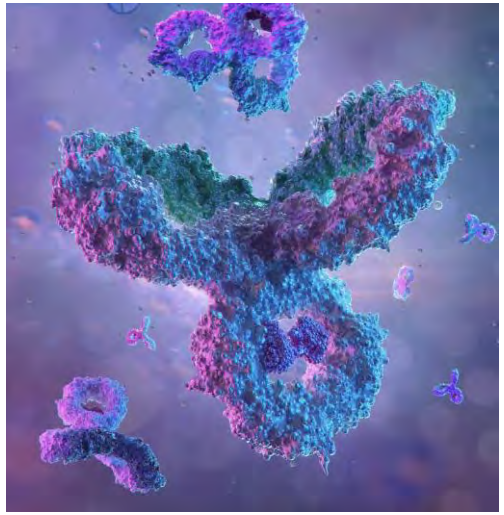
Instrument setup and configuration

- Choosing the right pore size
- High-resolution columns
- Minimal system dispersion

Method development parameters

- Mobile phase composition
- Temperature
- Flow rate

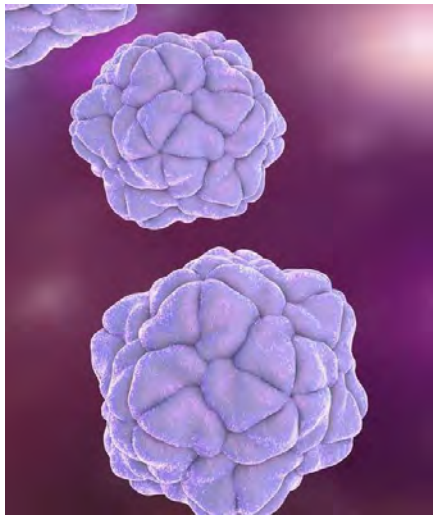
Biotherapeutics Size?



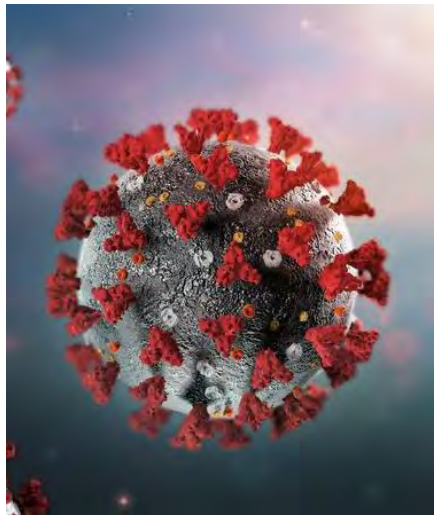
mAb
- ~150 kDa
- 5 nm hydrodynamic radius

mRNA
- ~ 1000 – 5000 nt
- ~ 320 – 1,600 kDa
- 300 – 1,500 nm chain length
- 100 – 200 nm diameter LNP

AAV
- ~ 3,800 kDa
- VP1:VP2:VP3 (5:5:50)
- ~ 25 – 30 nm diameter



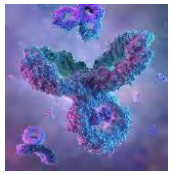
Viruses
- ~ 250 – 400 nm diameter



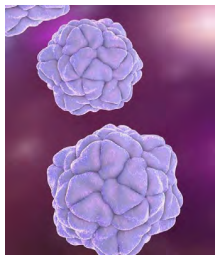
Pore Size Selection (SEC)

Suggested guidelines

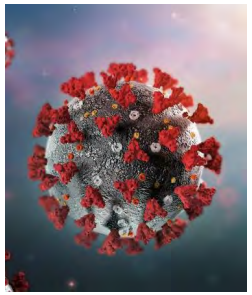
The pore size should be 3x larger than the **diameter** of the molecule you are interested in



- mAb (hydrodynamic radius ~ 5 nm); optimum pore size is about 3 x 10 nm
- 300 Å



- AAV (diameter ~ 25 to 30 nm); optimum pore size is about 3 x 30 nm
- 500 – 1000 Å

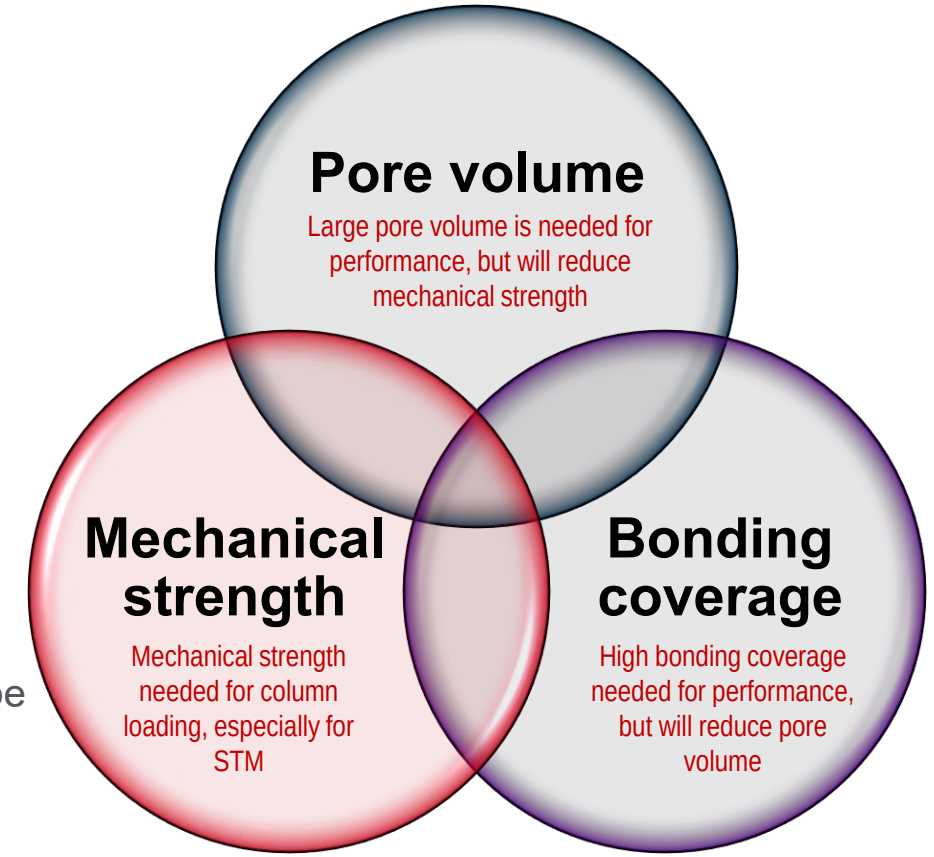


- LNP (diameter ~ 100 to 200 nm); optimum pore size is about 3 x 200 nm; **6000 Å**
- VLP (diameter ~ 250 to 400 nm); optimum pore size is about 3 x 400 nm; **1.2 µm**

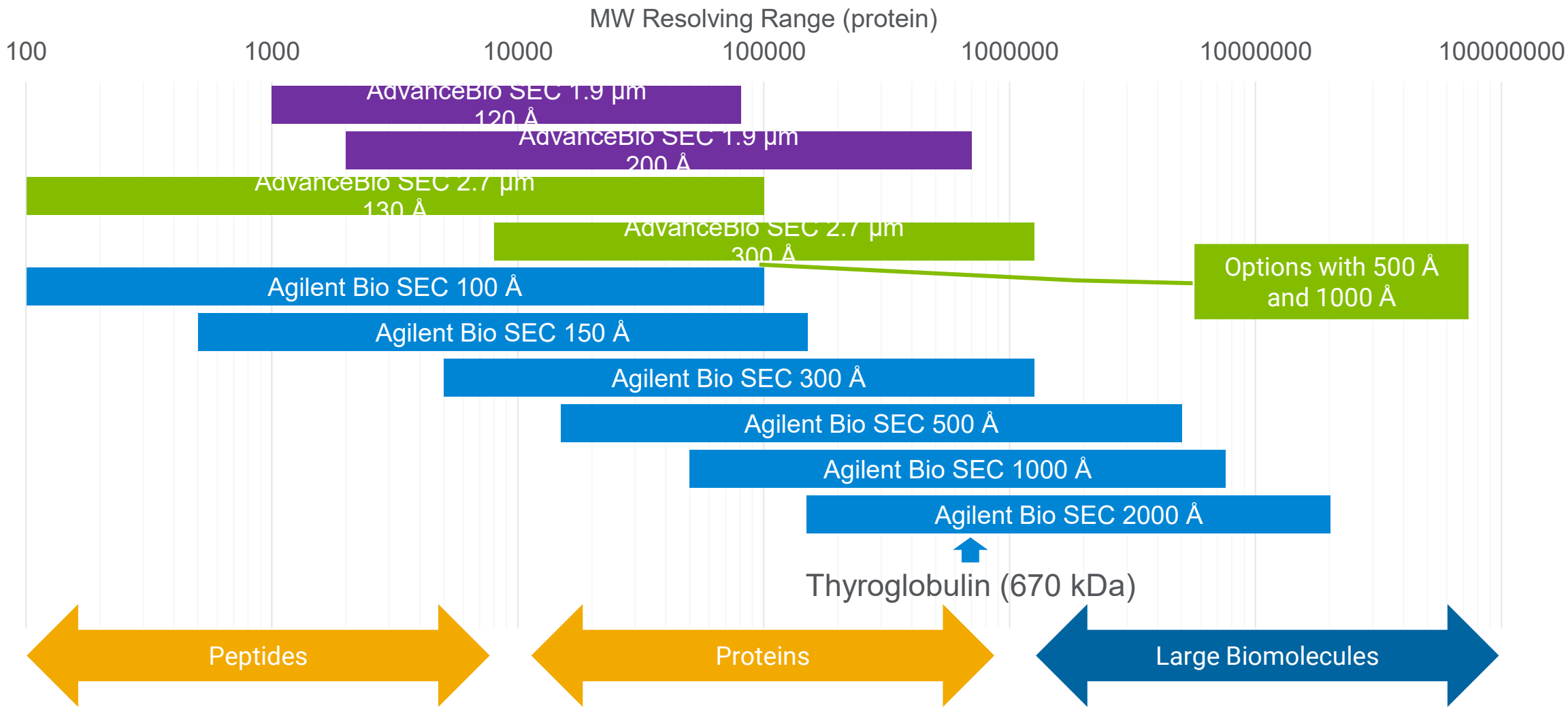
Characteristics of SEC Column

For high resolution and high throughput SEC columns

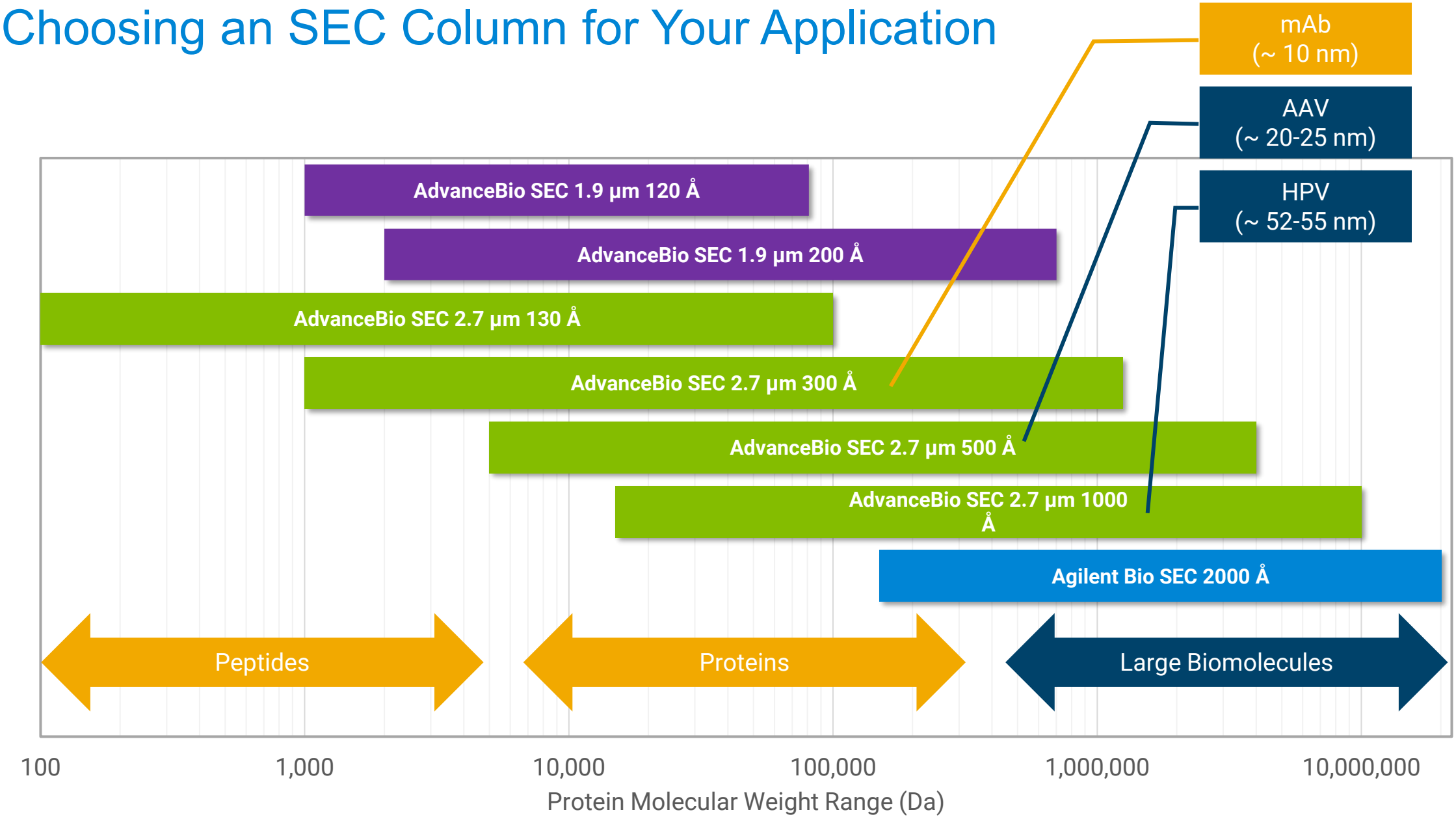
- Particle requirements:
  - Right pore size and narrow pore size distribution
    - The most effective pores at the linear range of the target molecules
  - Large pore volume
    - Large separation window and higher resolution
  - Smaller particles
    - Less mass transfer distance and higher efficiency
- Chemistry requirements:
  - Hydrophilic surface and inertness
    - No surface silanol interaction and hydrophobic interaction
  - Stable chemistry
    - Multiple injections → no shift in retention time and changes in peak shape
- Column loading requirements:
  - Strong particles
    - Column can be loaded at higher pressure
  - Stable bed for mechanical lifetime
    - No shifting of stationary phase over time



Choosing the Correct Pore Size: MW Resolving Ranges (SEC)



Choosing an SEC Column for Your Application



Agilent Size Exclusion Columns

AdvanceBio SEC	AdvanceBio SEC	Agilent Bio SEC-3	Agilent Bio SEC-5	ProSEC 300S	ZORBAX GF-250 and GF-450
1.9 µm	2.7 µm	3 µm	5 µm	5 µm	4 µm, 6 µm
200 Å, 120 Å	130 Å, 300 Å, 500 Å, 1000 Å	100 Å, 150 Å, 300 Å	100 Å, 150 Å, 300 Å, 500 Å, 1000 Å, 2000 Å	Nominal 300 Å (linear resolving range)	150 Å, 300 Å
Coated silica (USP L59)	Coated silica (USP L59)	Coated silica (USP L59)	Coated silica (USP L59)	Silica diol (USP L20)	Zirconium stabilized silica diol (USP L35)
mAb and ADC analysis • Dimer/monomer • LMW mAb fragments **Small proteins and peptides**	**mAb and ADC analysis** • Higher-order aggregates • Dimer/monomer **Small proteins and peptides**	• Polypeptide to small proteins • MS capable separations	• Broadest range of pore sizes for wide variety of biomolecules	• Unique linear resolving range • 30 cm and 60 cm column lengths	• Legacy product • Larger column dimensions • Ideal for GF-450 and GF-250 in series

Buffer Preparation
Good practices

- Use HPLC grade water or Milli Q DI water
- Use HPLC grade reagents, including salts and base and acid modifiers
- Use HPLC grade organic mobile phase modifiers
- Always rinse pH electrode thoroughly when measuring/adjusting pH of mobile phase
- Prepare fresh buffers to avoid contaminants from the growth of bacteria or algae
- Do not top off your mobile phase bottle. Replace with a clean bottle
- Consider using amber solvent bottles to decrease light exposure
- Filter your mobile phase buffer with 0.2 µm or 0.45 µm filter before use
- Solvent filters installed at the end of solvent lines should be replaced periodically

Solvent Filtration

Buffer Advisor - Simplifies SEC Workflows

An Independent Utility Software Tool

- Calculates pump timetables for SEC chromatography with G5654A (Bio-inert pump)
- Provides the foundation for automated mixing of acid, base, water and salt in SEC
- Is capable to calculate the selected buffer concentration and pH with high accuracy
- Suggests for the most suitable stock solutions and provides recipes
- Has included more than 50 different buffers and more than 20 were experimentally validated
- Is capable to correct automatically for pH shifts during salt gradients
- Provides the option to include user defined buffers
- Generates an xml file as output which can be imported into the method of the Bio-inert pump by a button implemented in the driver of G5654A

Agilent Buffer Advisor supports DoE workflows

Designed-In PEEK Lined Column Hardware for Biocompatibility

- UHPLC PEEK lined columns eliminate potential issues such as stainless-steel column related artefacts (oxidation, metal interaction etc.).
- Particularly suited to MS detection … narrower column IDs for lower flow rates and higher sensitivity.
- Improved recovery of proteins and peptides (consistent peak areas, reduced carryover).
- Combined with high performance stationary phases for maximum performance, including native mode SEC (or SEC-MS), and reversed phase chromatography of intact, fragmented, or digested proteins.

UHP-FF connector

PEEK-lined column

Bioinert and Biocompatible

The **Necessity** of Bio Instrumentation

BIO — The materials in the sample flow path do **not** contain **any** iron, **only** very **noble** materials

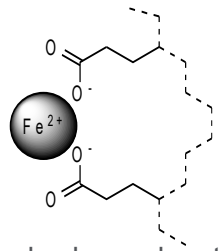
BIO INERT — The materials in the sample flow path do **not** contain **any** metals

Why is it necessary

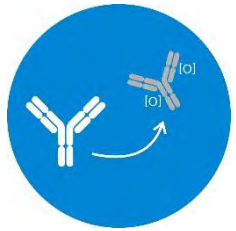
- **Behavior of some biomolecules:**
  - Chelation – interaction of the biomolecule with iron
  - Oxidation
- **Harsh application conditions typical for bio applications**
  - High salt concentration
  - High or low pH

→ *“avoid rusty instruments”*

- **Another effect: Photo degradation** (high light intensity) – or *“frying eggs”*,
  Agilent’s solution: the “Max Light Cartridge **LSS** DAD flow cell” with a neutral grey filter!



Positively charged metal ions can attract negatively charged biomolecules through **chelation**



Iron can also cause **oxidation** of some proteins

Agilent 1260 Infinity III Bio-SEC Solution – Aggregate Analysis

A brief introduction

A solution for:

- Sizing and aggregation studies
- Sample: proteins and other large biomolecules
- Method: Size Exclusion Chromatography (SEC)

True Bio-inert sample flow path + UHPLC power range:

- The sample never touches metal surface
- No stainless steel in the mobile phase flow path

The combined Static and Dynamic Light Scattering Detector simultaneously determines:

- absolute Molecular Weight (Mw)
- size of the molecule (Rh)

Agilent Bio-SEC Software:

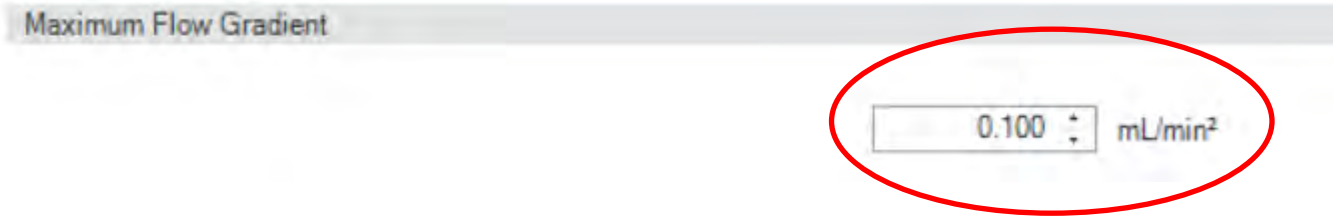
- a dedicated Chromatography Data System (CDS)
- for an easy, yet powerful, workflow-based solution

Considerations for LC Instrument

Best practices to keep in mind

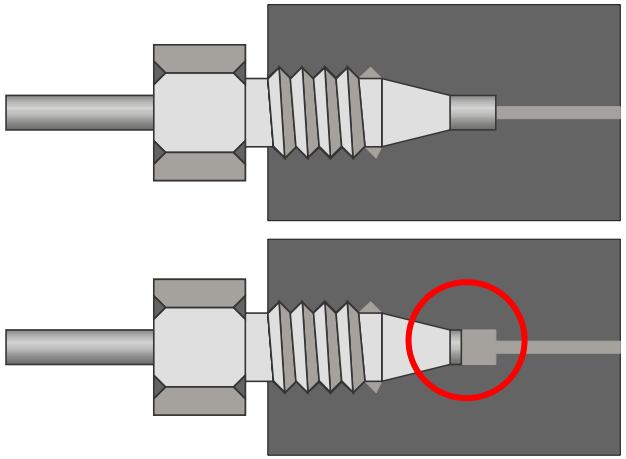
Low dispersion LC
Minimized tubing id and length to reduce extra column volume and band broadening (shortest possible 0.12 mm id red tubing).

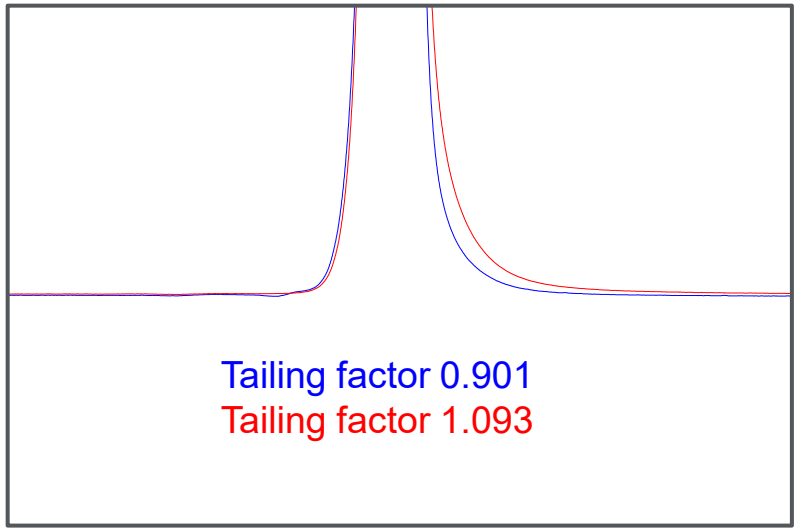
Start at a low flow rate, such as 0.1 mL/min, and gradually increase the flow in no more than 0.1 mL/min increments until you reach the intended operating flow rate.



Best Practices to Keep in Mind

Avoid bad connections
Ensure column connections do not leave dead spots





Use high data collection rates
Data collection rates of 10 to 20 Hz could result in 4 to 5% reduction in column efficiency compared to 40 or 80 Hz.

Common LC consumables

Quick connect and quick turn fittings

Correct connection every time

- Spring-loaded design
- Easy, no tools needed
- Works for all column types
- Reusable
- Consistent ZDV connection

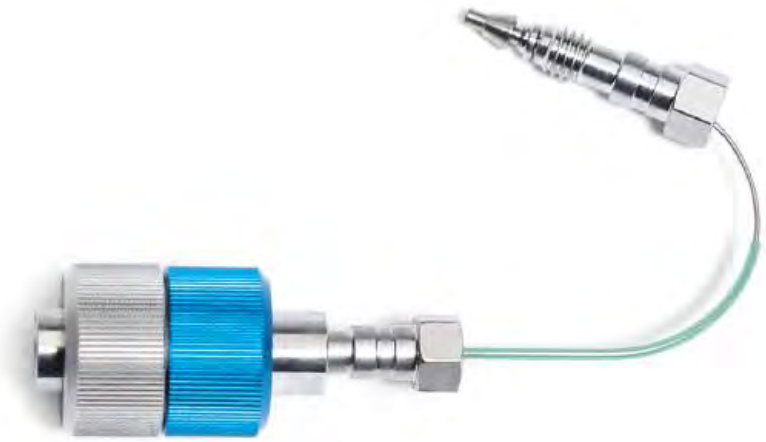
Quick Connect Fitting
- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever

Quick Turn Fitting
- Finger tight up to 400 bar
- Up to 1300 bar with a wrench
- Compact design

Common LC Consumables

Inline filters

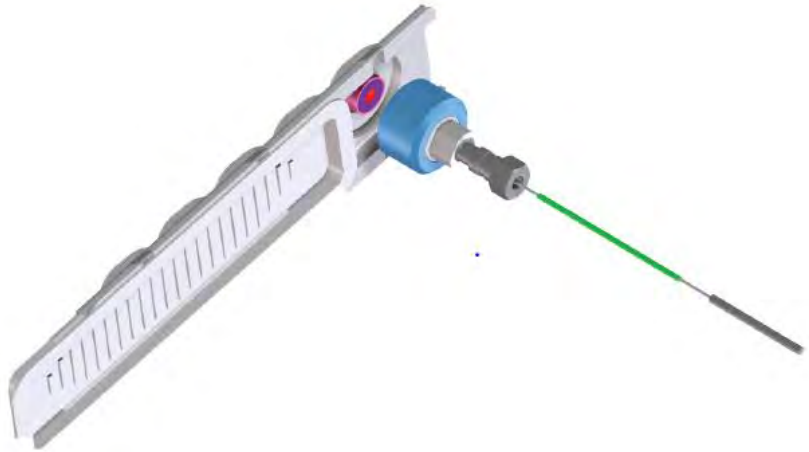
Agilent InfinityLab Quick Change inline filter



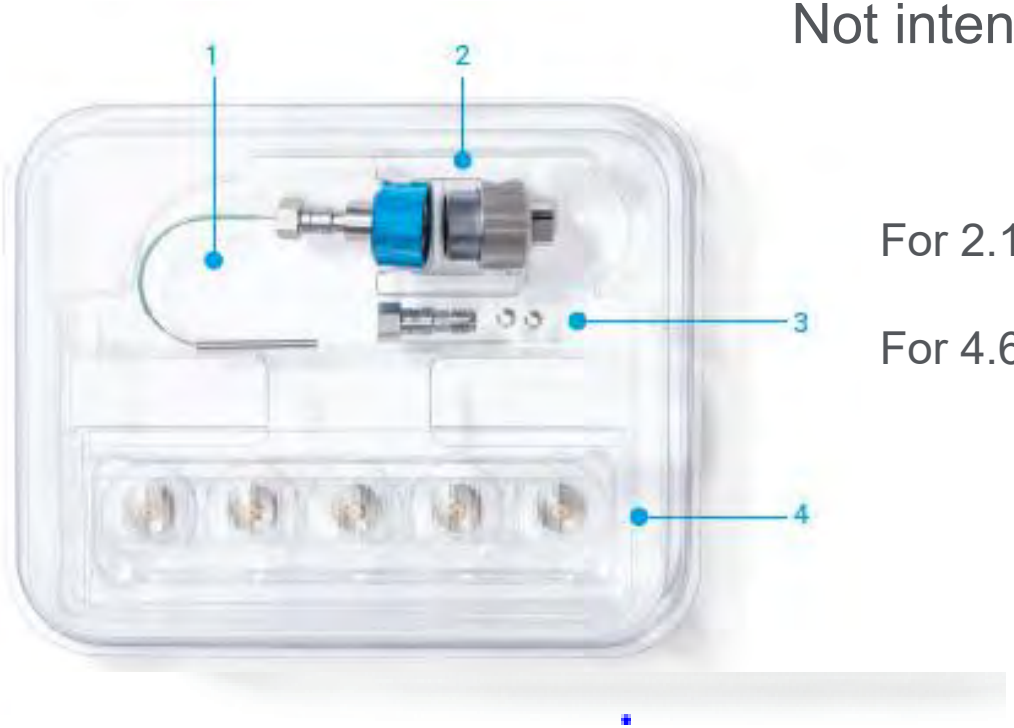
Touchless packaging

Dimensions and porosities of filter discs

Touchless insertion of filter disc to housing



InfinityLab Quick Change Inline Filter Assembly



1. Capillary, SST, 90 mm length
2. Filter housing (two parts)
3. Loose fitting for non-swaged end of capillary
4. Filter discs in touchless packaging, 5/pk

Inline filters can help extend the life of your column
Not intended to be a replacement for good sample cleanup

For 2.1 mm id – comes with 0.2 µm filter discs, 0.12 x 90 mm SS capillary

For 4.6mm id – comes with 0.5 µm filter discs, 0.17 x 90 mm SS capillary

Parts available to purchase separately

InfinityLab Quick Change Inline Filters (Agilent.com)
Publication number: 5994-3028EN

Common LC Consumables
Solvent inlet filters

Use a solvent inlet filter

- Glass solvent inlet filter (20 µm), 5041-2168
- Stainless steel solvent inlet filter, 01018-60028
- Stainless inlet filter recommended for LC/MS

Not designed to take the place of good mobile phase hygiene

Aggregation and Fragment Analysis (SEC)

Column selection parameters

Choose initial columns and conditions for size-based separation of biomolecules, aggregation analysis—peptides, polypeptides, and proteins

Peptides, polypeptides, proteins
Mol Wt >0.1–1,250 kda

Peptides, polypeptides, proteins
Mol Wt >0.1–10,000 kda

Select column based on molecular weight range and pore size

AdvanceBio SEC (2.7 µm)

Pore size	Mol Wt range, kDa
130 Å	0.1–120
300 Å	5–1,250

Bio SEC-3 (3 µm)

Pore size	Mol Wt range, kDa
100 Å	0.1–100
150 Å	0.5–150
300 Å	5–1,250

Bio SEC-5 (5 µm)

Particle size, µm	Flow rate, mL/min
100 Å	0.1–100
150 Å	0.5–150
300 Å	5–1,250
500 Å	15–5,000
1000 Å	50–7,500
2000 Å	>10,000

Aggregation and Fragment Analysis (SEC)

Recommended initial separation conditions

Columns:	AdvanceBio SEC Bio SEC (3 µm and 5 µm)
Mobile phase:	Phosphate buffer 150 mM, pH 7.0*
Gradient:	Isocratic in 15 to 60 min range
Temperature:	Recommended 10 to 30 °C, maximum 80 °C
Flow rate:	0.1 to 0.4 mL/min for 4.6 mm id columns 0.1 to 1.25 mL/min for 7.8 mm id columns 1.0 to 10.0 mL/min for 21.2 mm id columns
Sample size:	≤5% of total column volume

* Other aqueous buffers with high and low salt can be used

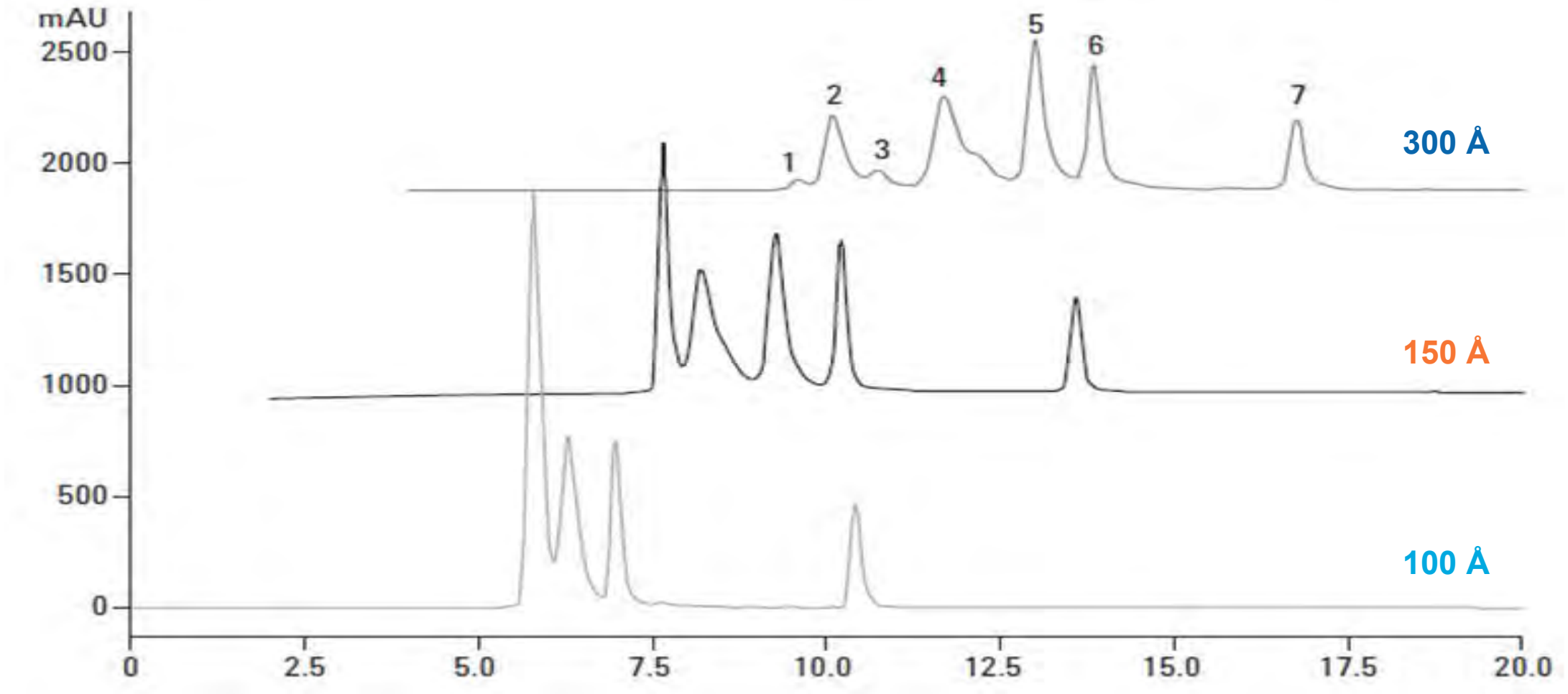
For additional information, see:
Resolve Protein Aggregates and Degradants With Speed and Confidence (publication 5991-2898EN)
www.agilent.com/search

Aggregation and Fragment Analysis (SEC)

Mobile phase selection

- Minimize secondary interactions by adjusting the mobile phase composition
  - pH
  - Ionic strength
  - Organic modifiers
- Increase salt concentration
  - 100 to 150 mM sodium chloride in 50 mM sodium phosphate, pH 7.0
  - 100 to 150 mM sodium sulfate in 50 mM sodium phosphate, pH 7.0
  - 50 to 100 mM urea in 50 mM sodium phosphate, pH 7.0
- Make a fresh batch, filter, and promptly use the mobile phase
- Buffers should maintain constant pH but be wary of methods that state the pH can be +/- 0.2 pH units.
- Avoid sudden changes in operating pressure (either as a result of changing flow rate, or as a result of changing viscosity).

Effect of Pore Size on Resolution (SEC)



Column used was AdvanceBio SEC 3.0 µm, 4.6 x 300 mm, mobile phase: 100 mM sodium phosphate with 150 mM sodium chloride, pH 6.8, 0.35 mL/min, gradient: 10 to 58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B, DAD: 220 nm.
Agilent publication: 5994-0974EN

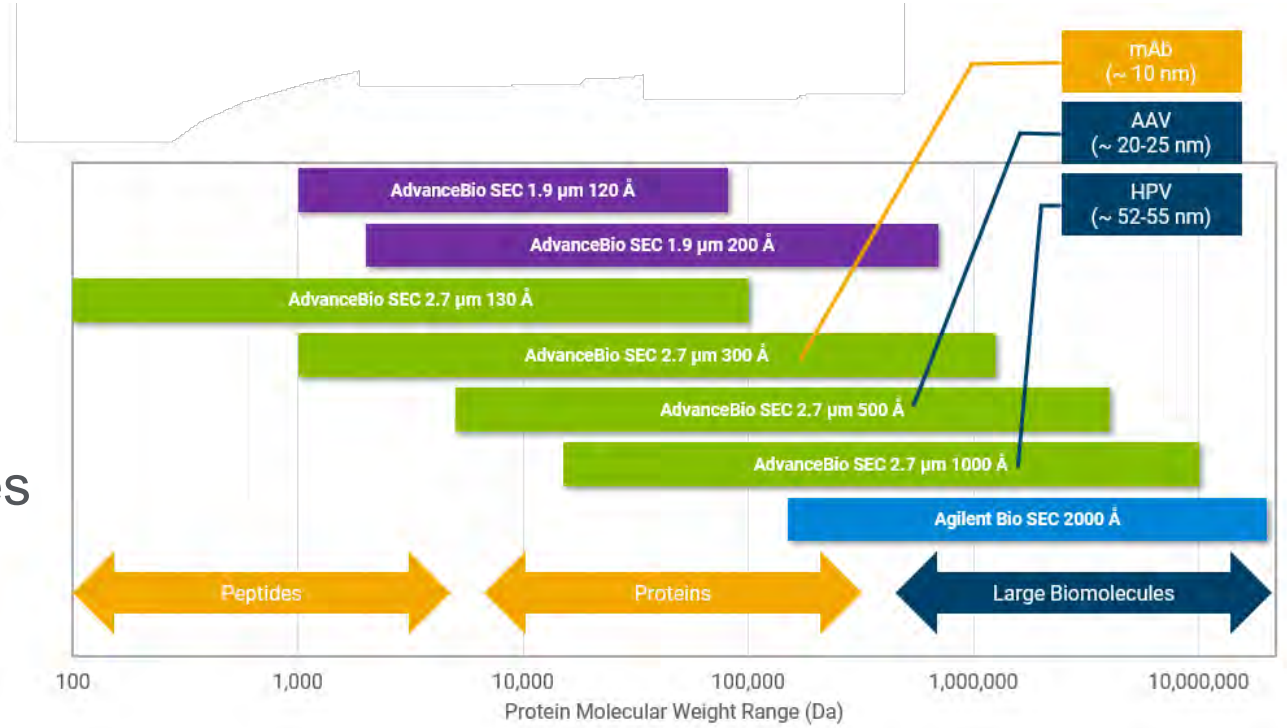
Agilent Columns for Aggregate Analysis

AdvanceBio SEC

- Unique hydrophilic coating for minimal secondary interactions
- Large pore volume for maximum separation opportunity
- Excellent peak shape and resolution for challenging samples like ADCs with simple phosphate buffer
- 120 Å, 130 Å, 300 Å, 500 Å, and 1000 Å pore options
  - 300 Å ideal for mAbs

Agilent Bio SEC

- MS compatibility
- Pore sizes ranging from 100 Å for peptides and small proteins to 2000 Å for VLPs



Column Dimensions, Flow Rates, and Instrumentation

I need to implement high-throughput solutions !

I need to monitor aggregate profile throughout the manufacturing process

4.6 or 7.8 x 150 mm for higher throughput, faster separations

7.8 x 300 mm for higher resolution
4.6 x 300 mm for higher sensitivity

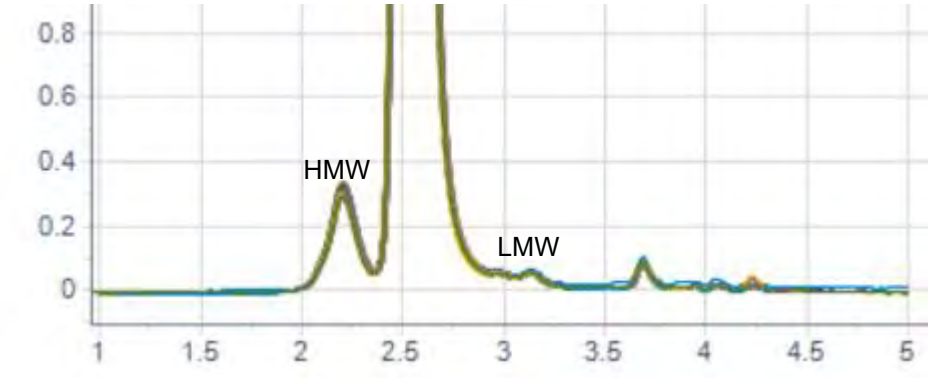
I work with small sample quantities

We test all samples to ensure it is within specification before we can do a lot release.

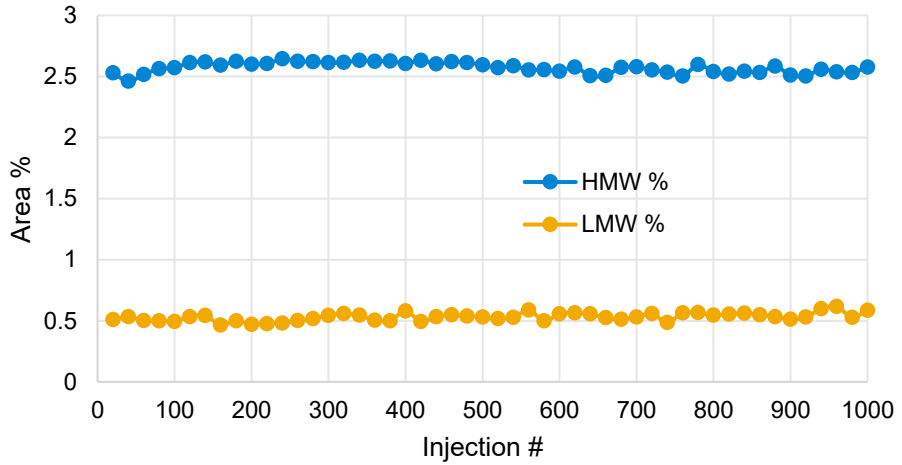
Lifetime (SEC)

AdvanceBio SEC 200 Å 1.9 µm, 4.6 x 150 mm, flow rate: 0.5 mL/min
1000 continuous injections of bovine IgG and SigmaMAb

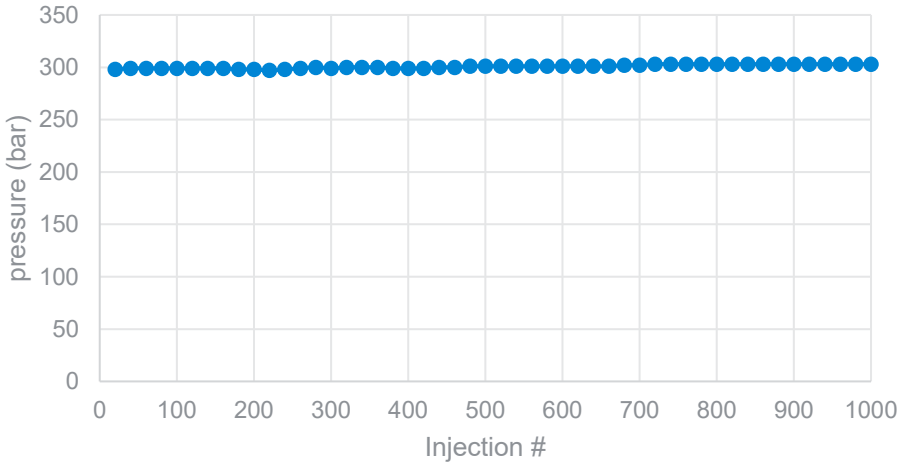
Overlay of 50 mAb injections throughout 1000 injection sequence



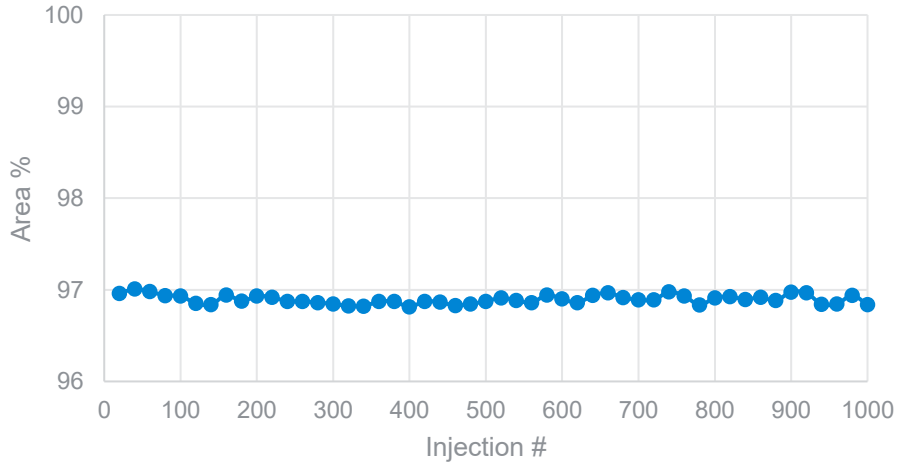
HMW and LMW area %



Column back pressure

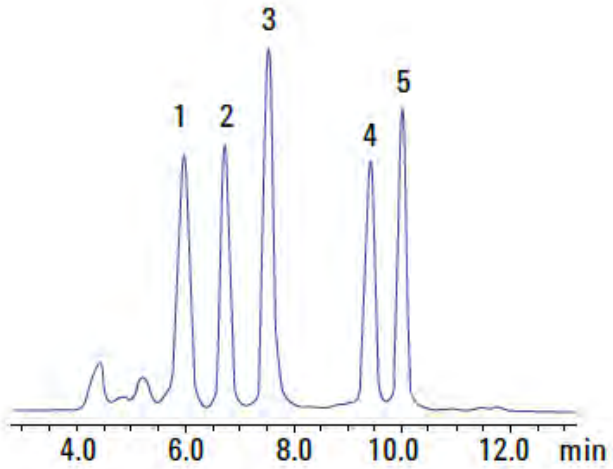


Monomer Area %

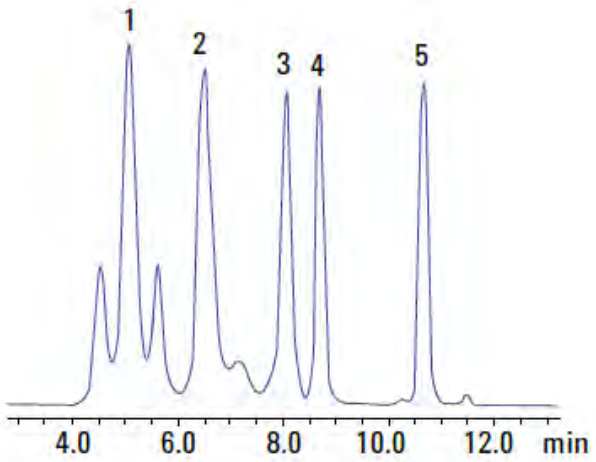


Robustness over 1000 injections with reproducible mAb area % for HMW, monomer, and LMW peaks and stable column back pressure.

AdvanceBio SEC Protein Standards



AdvanceBio SEC 130Å Protein Standard separation on AdvanceBio SEC 130Å column



AdvanceBio SEC 300Å Protein Standard separation on AdvanceBio SEC 300Å column

AdvanceBio SEC 130Å Protein Standard p/n 5190-9416, 1.5 mL vial)	
Analyte	**MW**
1. Ovalbumin	45,000
2. Myoglobin	17,000
3. Aprotinin	6,700
4. Neurotensin	1,700
5. Angiotensin II	1,000

AdvanceBio SEC 300Å Protein Standard (p/n 5190-9417, 1.5 mL vial)	
Analyte	**MW**
1. Thyroglobulin	670,000
2. γ-globulin	150,000
3. Ovalbumin	45,000
4. Myoglobin	17,000
5. Angiotensin II	1,000

Target Workflow – Aggregation Analysis (UV quantitation)

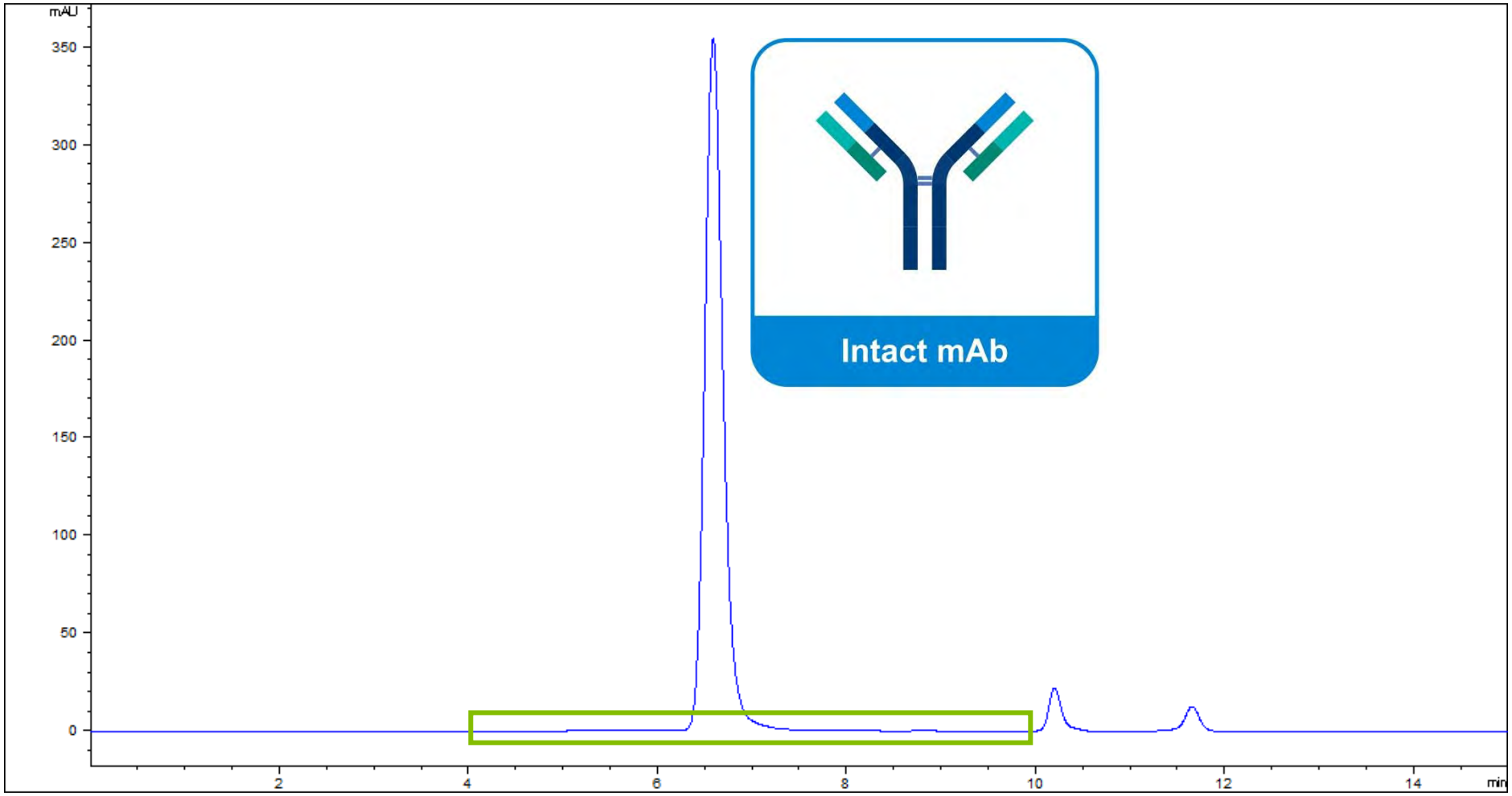
Objective: quantitation of protein aggregates

Analyte: monoclonal antibodies, other recombinant proteins

Analytical platform: 1260 Infinity II Bio-inert LC, OpenLab CDS

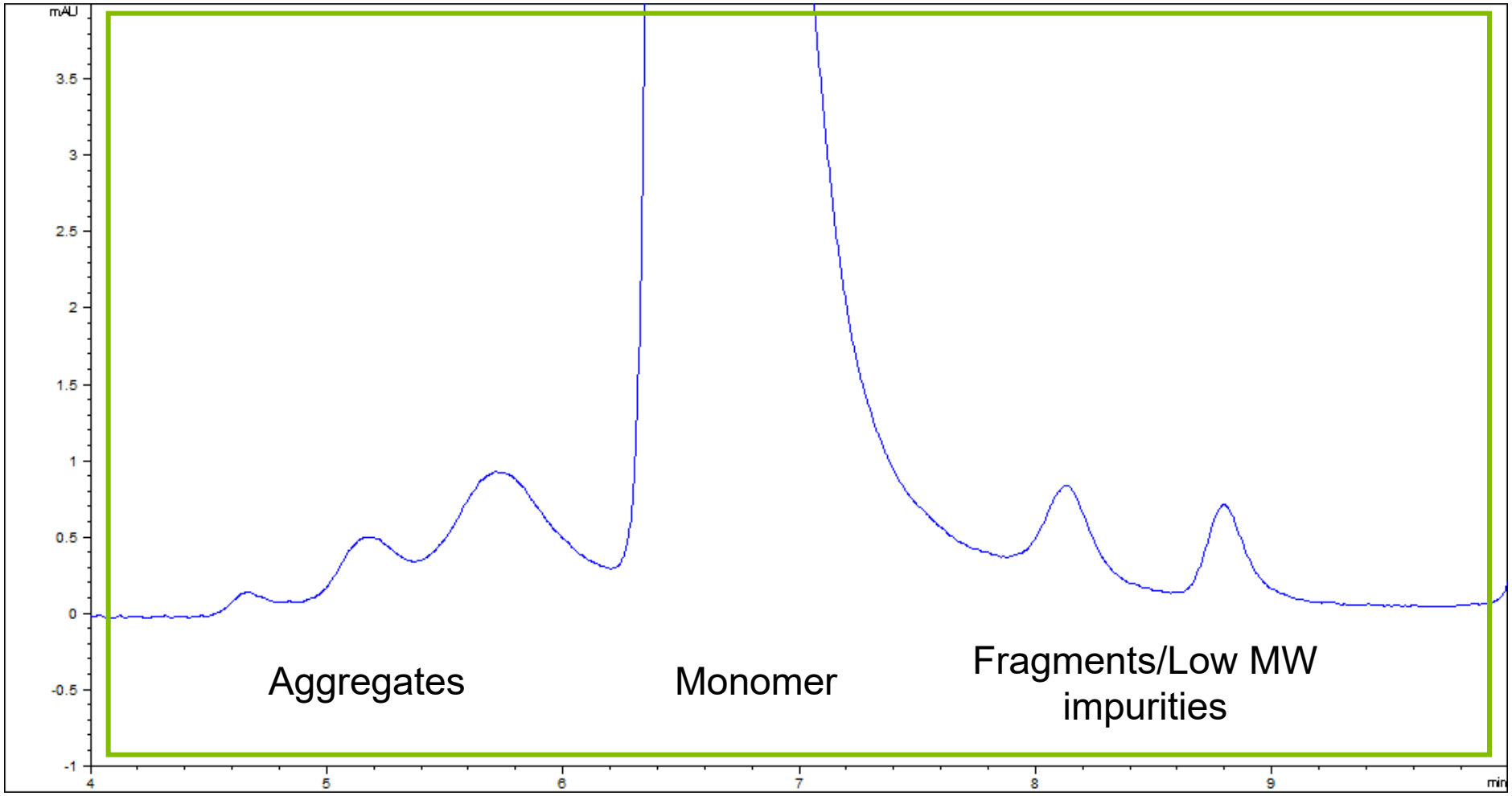
Detecting and Quantifying mAb Aggregation

AdvanceBio SEC 300 Å
150 mM phosphate, pH 7



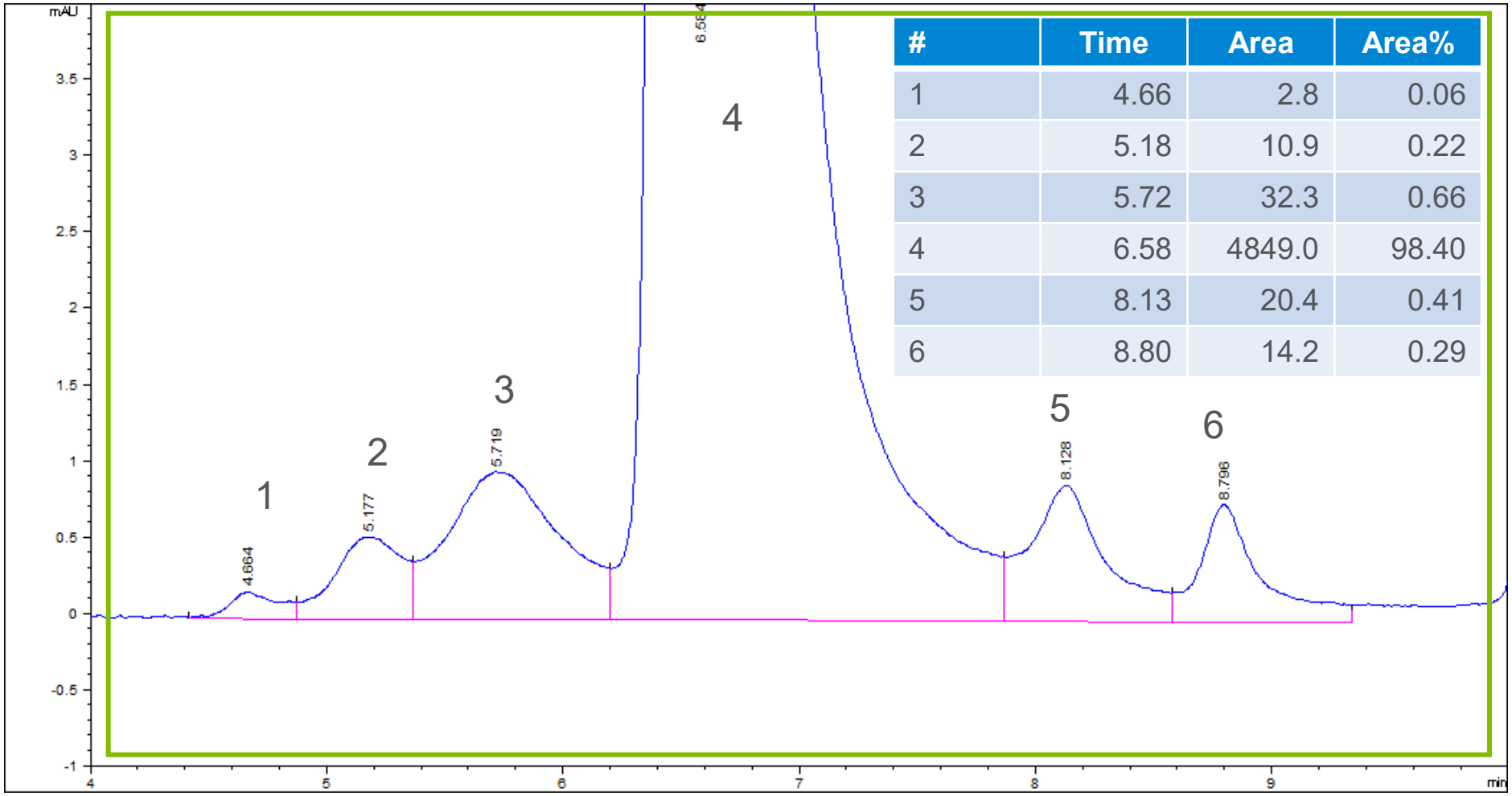
Detecting and Quantifying mAb Aggregation

AdvanceBio SEC 300 Å
150 mM phosphate, pH 7



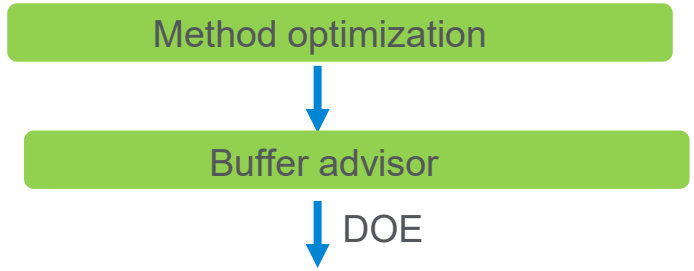
Detecting and Quantifying mAb Aggregation

AdvanceBio SEC 300 Å
150 mM phosphate, pH 7



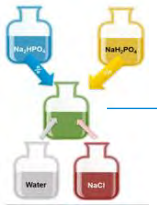
#	Time	Area	Area%
1	4.66	2.8	0.06
2	5.18	10.9	0.22
3	5.72	32.3	0.66
4	6.58	4849.0	98.40
5	8.13	20.4	0.41
6	8.80	14.2	0.29

BufferAdvisor Simplifies SEC Method Development



Parameter	Settings
Mobile phase	Optimization
Column	AdvanceBio SEC 2.7 µm 300 Å 7.8 × 300 mm
TCC	25 °C
Run time	20 mins
Inj volume	6 µL
Flow rate	0.8 mL/min
DAD	280 nm

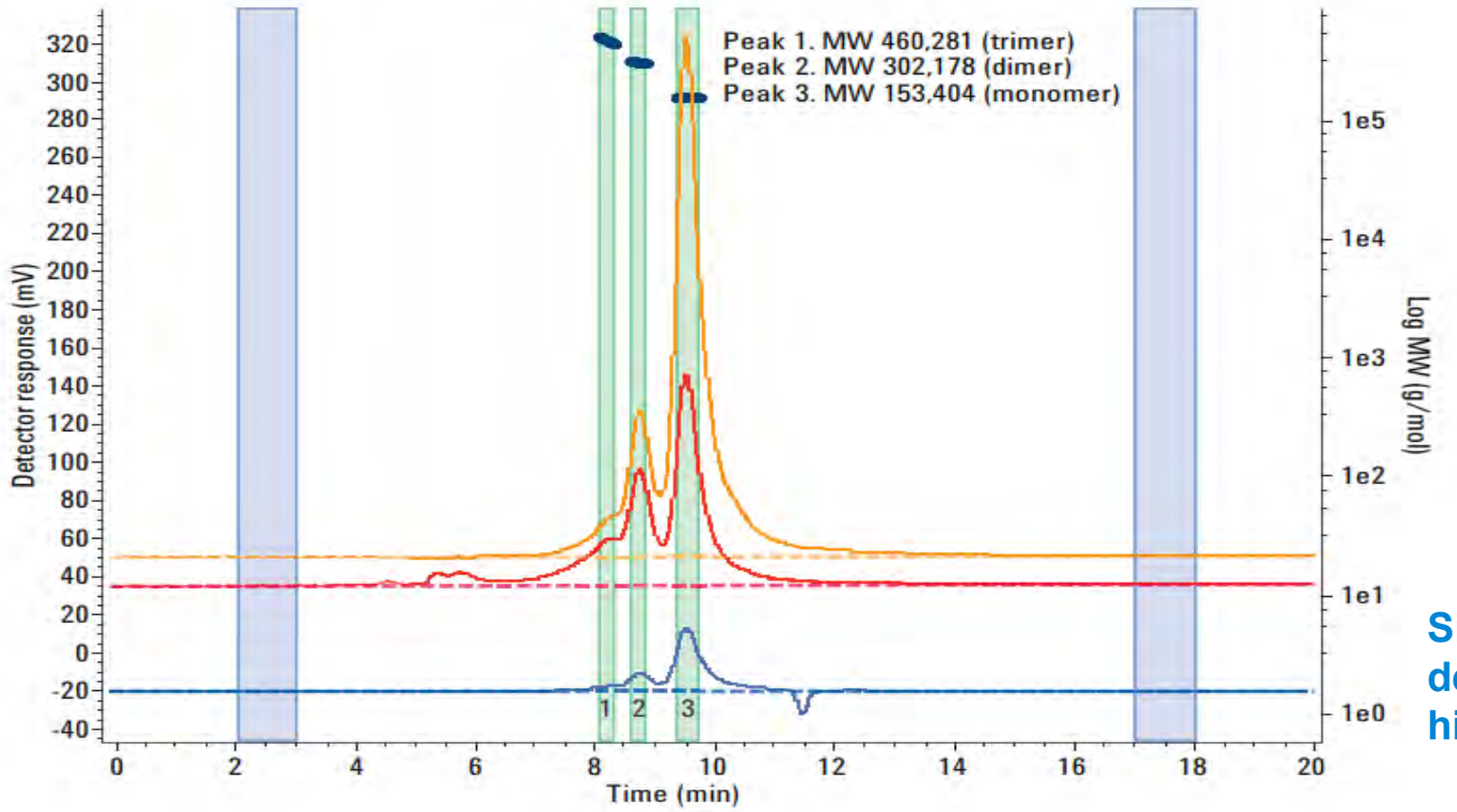
A: Water
B: 1000 mM NaCl
C: 200 mM NaH_2PO_4
D: 400 mM Na_2HPO_4



Expt	pH	Buffer (mM)	NaCl (mM)	Total Concentration (mM)	%A	%B	%C	%D
1	6.2	150	0	150	34	0	57	9
2	6.4	150	0	150	37.5	0	50.1	12.4
3	6.6	150	0	150	41.4	0	42.3	16.3
4	6.8	150	0	150	45.5	0	34.1	20.4
5	7.0	150	0	150	49.4	0	26.3	24.3
6	7.2	150	0	150	52.8	0	19.4	27.8
7	7.4	150	0	150	55.6	0	13.8	30.6
8	7.6	150	0	150	57.8	0	9.5	32.7
9	7.6	100	0	100	71.7	0	6.6	21.7
10	7.6	200	0	200	43.7	0	12.7	43.6
11	7.6	300	0	300	14.8	0	20.4	64.8
12	7.6	200	50	200	39	5	12.1	43.9
13	7.6	200	100	300	34.2	10	11.6	44.2
14	7.6	200	200	400	24.7	20	10.6	44.7
15	7.6	200	300	500	15.1	30	9.8	45.1
16	7.6	200	400	600	5.5	40	9.1	45.4

Multidetector Approach

Light scattering detector in combination with a concentration detector, such as UV or RI



- UV 280 nm
- Light scattering 90°
- Refractive index

Signal from the light scattering detector was more responsive to higher molecular weight molecules

Column used was Agilent Bio SEC-5, 7.8 x 300 mm, stainless steel; A: 50 mM sodium phosphate, 250 mM NaCl, pH 7.0, isocratic elution, 1.0 mL/min, 30 °C, 1.0, 2.0, and 4.0 mg/mL of Bovine Y-globulin. Agilent publication: 5991-1400EN

Agilent Biomolecule Columns Portfolio

Protein Therapeutics									Oligonucleotides		Vector Therapeutics		
Titer Determination	Aggregate Analysis	Intact Purity & PTM Analysis		Peptide Mapping & PTM Analysis	Charge Variant Analysis	Glycan Analysis	Amino Acid / Cell Culture Media Analysis		Purification & Impurity Analysis		Aggregation	Empty/Full	Capsid Identity
Affinity	Size exclusion	Reversed Phase > 150 Å	Hydrophobic Interaction	Reversed Phase < 150 Å	Ion Exchange	Hydrophilic Interaction	Reversed Phase < 150 Å	Hydrophilic Interaction	Reversed Phase	Ion Exchange	Size exclusion	Anion Exchange	Reversed Phase
Bio-Monolith rProtein A	AdvanceBio SEC 1.9 µm	PLRP-S 1000Å 5 µm	AdvanceBio HIC	AdvanceBio EC-C18	Bio mAb / Bio IEX 5 µm	AdvanceBio Glycan Mapping	AdvanceBio Amino Acid Analysis	AdvanceBio MS Spent Media	AdvanceBio Oligonucleotide	PL-SAX	Bio SEC-5	Bio SAX	ZORBAX RRHD 300Å, 1.8 µm
Bio-Monolith Protein A	AdvanceBio SEC 1.9 µm	PLRP-S		AdvanceBio Peptide Mapping	Bio mAb (WCX)		ZORBAX Eclipse AAA 3.5 µm		PLRP-S	Bio SAX		Bio SAX	
Bio-Monolith Protein G	AdvanceBio SEC 2.7 µm	AdvanceBio RP mAb 450Å		AdvanceBio Peptide Plus	Bio IEX (SAX, WAX, SCX, WCX)					Bio SAX			
	Bio SEC-3	ZORBAX RRHD 300Å, 1.8 µm		ZORBAX RRHD 300 Å, 1.8 µm	PL SCX, SAX								
	Bio SEC-5	ZORBAX 300SB 3.5, 5 & 7 µm			Bio-Monolith (QA, DEAE, SO3)								
	ZORBAX GF250 and GF450	Poroshell 300 5 µm											

Stainless steel (SS) column hardware

Solid PEEK or PEEK-lined SS bioinert column hardware

Agilent Biomolecule Columns Portfolio

Protein Therapeutics									Oligonucleotides		Vector Therapeutics		
Titer Determination	Aggregate Analysis	Intact Purity and PTM Analysis		Peptide Mapping and PTM Analysis	Charge Variant Analysis	Glycan Analysis	Amino Acid/Cell Culture Media Analysis		Purification and Impurity Analysis		Aggregation	Empty/Full	Capsid Identity
Affinity	Size exclusion	Reversed phase > 150 Å	Hydrophobic interaction	Reversed phase < 150 Å	Ion exchange	Hydrophilic interaction	Reversed phase < 150 Å	Hydrophilic interaction	Reversed phase	Ion exchange	Size exclusion	Anion exchange	Reversed phase
Bio-Monolith rProtein A	AdvanceBio SEC 1.9 µm	PLRP-S 1000 Å 5 µm	AdvanceBio HIC	AdvanceBio EC-C18	Bio mAb/Bio IEX 5 µm	AdvanceBio Glycan Mapping	AdvanceBio Amino Acid Analysis	AdvanceBio MS Spent Media	AdvanceBio Oligonucleotide	PL-SAX	Bio SEC-5	Bio SAX	ZORBAX RRHD 300 Å, 1.8 µm
Bio-Monolith Protein A	AdvanceBio SEC 1.9 µm	PLRP-S		AdvanceBio Peptide Mapping	Bio mAb (WCX)		ZORBAX Eclipse AAA 3.5 µm		PLRP-S	Bio SAX		Bio SAX	
Bio-Monolith Protein G	AdvanceBio SEC 2.7 µm	AdvanceBio RP mAb 450 Å		AdvanceBio Peptide Plus	Bio IEX (SAX, WAX, SCX, WCX)					Bio SAX			
	Bio SEC-3	ZORBAX RRHD 300 Å, 1.8 µm		ZORBAX RRHD 300 Å, 1.8 µm	PL SCX, SAX								
	Bio SEC-5	ZORBAX 300SB 3.5, 5 and 7 µm			Bio-Monolith (QA, DEAE, SO3)								
	ZORBAX GF250 and GF450	Poroshell 300 5 µm										Stainless steel (SS) column hardware	Solid PEEK or PEEK-lined SS bioinert column hardware

Characteristics and User Guide

Parameter	Value
Columns	AdvanceBio SEC 130 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5350) AdvanceBio SEC 300 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5301) AdvanceBio SEC 500 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5325) AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5302) AdvanceBio SEC 120 Å, 1.9 µm, 4.6 × 300 mm (PL1580-5250) AdvanceBio SEC 200 Å, 1.9 µm, 4.6 × 300 mm (PL1580-5201)
Flow Rate	0.35 mL/min
Mobile Phase	150 mM sodium phosphate, pH 7.0
Wavelength	220 nm
Injection Volume	1 to 5 µL

AdvanceBio 2.7 µm SEC columns are available in four pore sizes:
130 Å – peptides and small therapeutic proteins
300 Å – mAbs, ADCs, and other proteins
500 Å – AAVs and other large biotherapeutic proteins and oligonucleotides
1000 Å – VLPs and other large biotherapeutics, such as oligonucleotides.

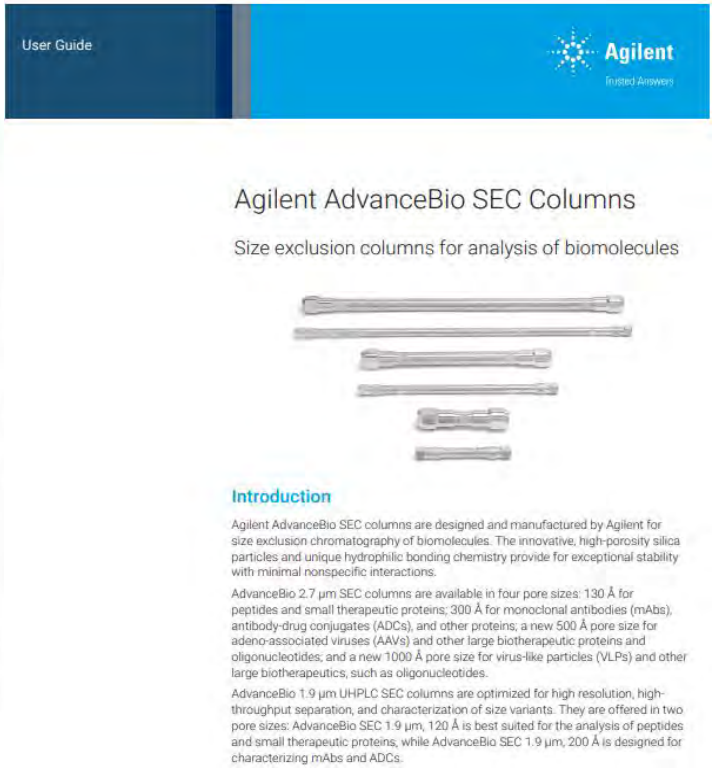
AdvanceBio 1.9 µm SEC columns are available in two pore sizes:
120 Å – peptides and small therapeutic proteins
200 Å – mAbs and ADCs

- The columns must first be flushed into the mobile phase required for your separation. **Ramp up the flow rate slowly from 0.0 mL/min to the intended operating flow rate over a period of several minutes. If possible, the maximum flow gradient should be set at 0.1 mL/min/min.**

Operating parameters

Parameter	Value
Mobile Phase Compatibility	Aqueous buffers with high and low salt can be used. Mixtures of water and organic solvent can be used **with careful attention to solubility of buffer components and system pressure**.
pH Stability	2 to 8.5
Operating Temperature	20 to 30 °C (recommended), 80 °C (maximum)
Maximum Pressure	400 bar (5,800 psi) for 2.7 µm columns 620 bar (9,000 psi) for 1.9 µm columns
Recommended Flow Rates	0.1 to 2.0 mL/min for 7.8 mm id columns 0.1 to 0.7 mL/min for 4.6 mm id columns 0.05 to 0.1 mL/min for 2.1 mm id columns For two columns in series, lower flow rates may be necessary to ensure that maximum pressure is not exceeded.
Injection Volume	1 to 10 µL (recommended) Maximum 1% column volume

Note: Working at extremes of the operating parameters may reduce column lifetime.


User Guide

Agilent

Agilent AdvanceBio SEC Columns

Size exclusion columns for analysis of biomolecules

Introduction

Agilent AdvanceBio SEC columns are designed and manufactured by Agilent for size exclusion chromatography of biomolecules. The innovative, high-porosity silica particles and unique hydrophilic bonding chemistry provide for exceptional stability with minimal nonspecific interactions.

AdvanceBio 2.7 µm SEC columns are available in four pore sizes: 130 Å for peptides and small therapeutic proteins; 300 Å for monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and other proteins; a new 500 Å pore size for adeno-associated viruses (AAVs) and other large biotherapeutic proteins and oligonucleotides; and a new 1000 Å pore size for virus-like particles (VLPs) and other large biotherapeutics, such as oligonucleotides.

AdvanceBio 1.9 µm UHPLC SEC columns are optimized for high resolution, high-throughput separation, and characterization of size variants. They are offered in two pore sizes: AdvanceBio SEC 1.9 µm, 120 Å is best suited for the analysis of peptides and small therapeutic proteins, while AdvanceBio SEC 1.9 µm, 200 Å is designed for characterizing mAbs and ADCs.

Be Agilent Sure of Your Biologic's Critical Quality Attributes

The importance of understanding the attributes of a biologic molecule, and the processes used to create it, cannot be understated.

www.agilent.com/chem/advancebio-columns

Agilent InfinityLab

Agilent

DE-004149

Biochromatography

Biomolecules come in different shapes and sizes, with different surface characteristics…

… so do Agilent Biocolumns

Resources for Support

Technical support www.agilent.com/chem/techsupport

Agilent product catalogs, www.agilent.com/en/promotions/catalog

- InfinityLab BioHPLC Supplies catalog (5994-6123EN)

Resource page www.agilent.com/chem/agilentresources

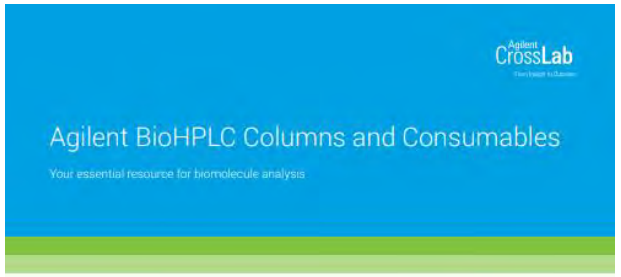
- Quick reference guides
- Catalogs, column user guides
- Online selection tools, how-to videos

Agilent University http://www.agilent.com/crosslab/university

YouTube – Agilent Channel

Your local Field Service Engineer and Specialists

Agilent service contracts



Contact Agilent Chemistries and Supplies Technical Support

Available in the U.S. and Canada, 8am to 5pm all time zones

Web chat: Product pages of Agilent.com

1-800-227-9770 option 3, option 3:

- Option 1 – GC and GC/MS columns and supplies
  gc-column-support@agilent.com
- Option 2 – for LC and LC/MS columns and supplies
  lc-column-support@agilent.com
- Option 3 – for sample preparation, filtration, and QuEChERS
  spp-support@agilent.com
- Option 4 – for spectroscopy supplies
  spectro-supplies-support@agilent.com
- Option 5 – for standards
  chem-standards-support@agilent.com
- Option 6 for ProZyme products
  pzi.info@agilent.com

Thank you!