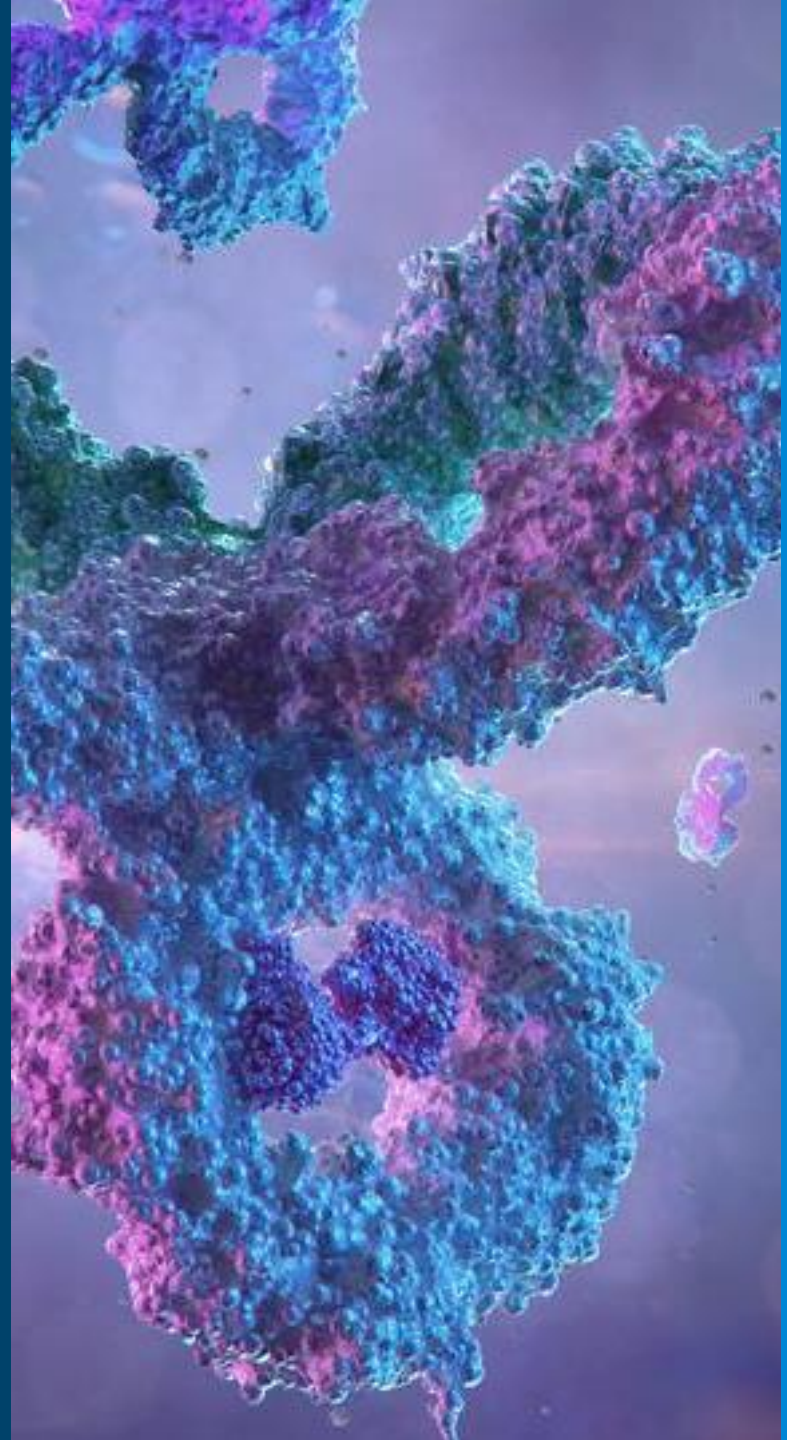
Reversed-Phase for Biomolecules:

From Column Selection to Troubleshooting

Melissa Goodlad, PhD
Columns and Supplies Applications Engineer
October 5, 2023



Agilent
Trusted Answers

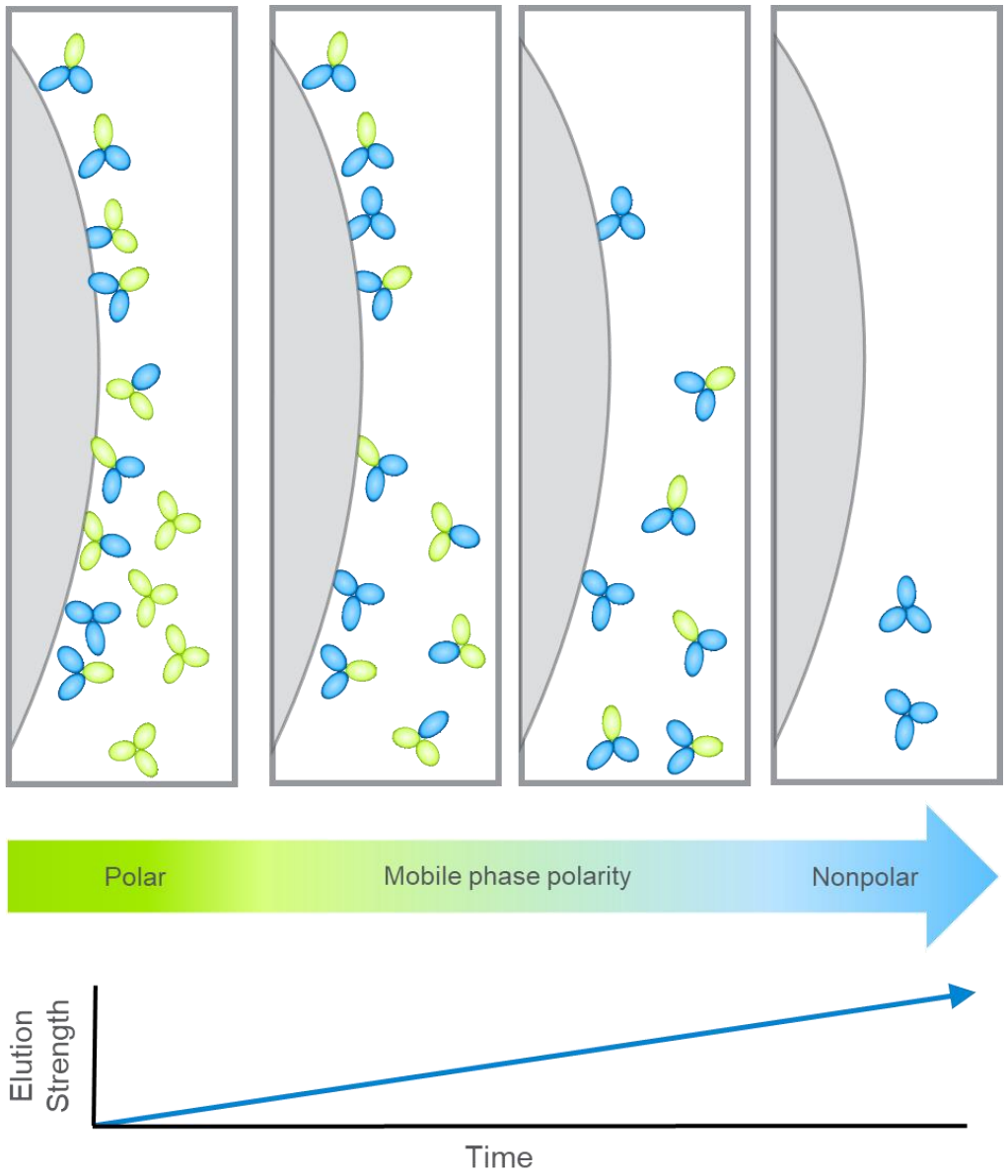
Agenda

- Introduction to reversed-phase chromatography
- Column selection
- Protein separations
- Peptide separations
- Oligonucleotide separations

Introduction

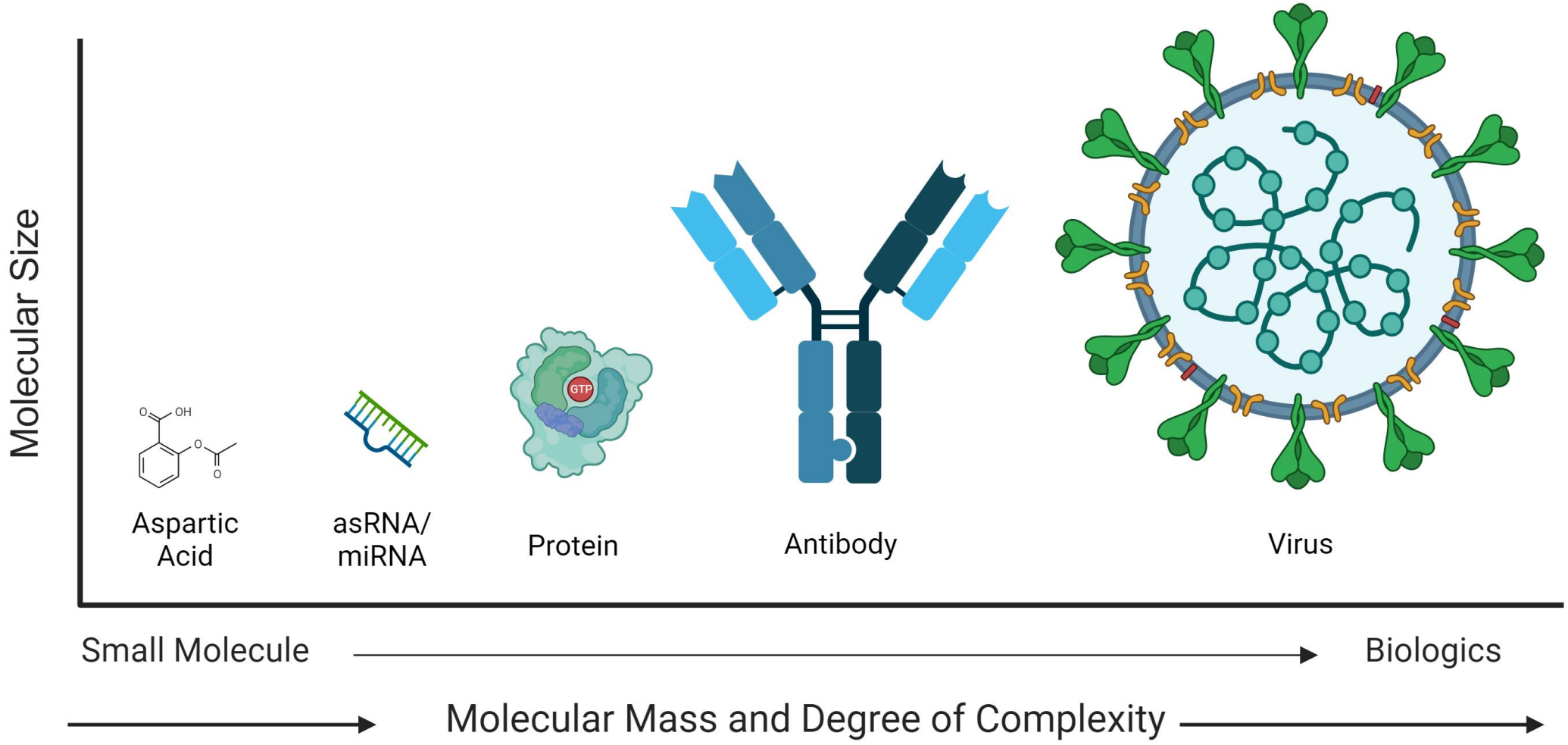
What is reversed phase liquid chromatography?

Reversed-Phase LC	
Polarity	Non-polar stationary phase (e.g., C18)
Mobile Phase	Polar mobile phase H_2O/CH_3OH, H_2O/CH_3CN
Gradient	Decrease retention by decreasing polarity of mobile phase ddH2O ↓ = retention ↑ CH_3CN ↑ = retention ↓
Elution Order	Polar to non-polar



Introduction

RP for biomolecules

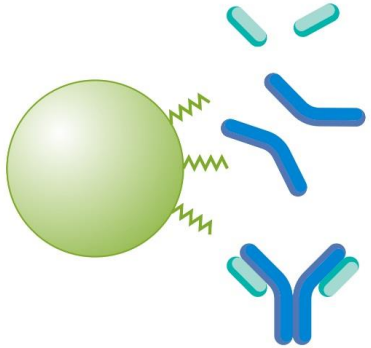


Introduction

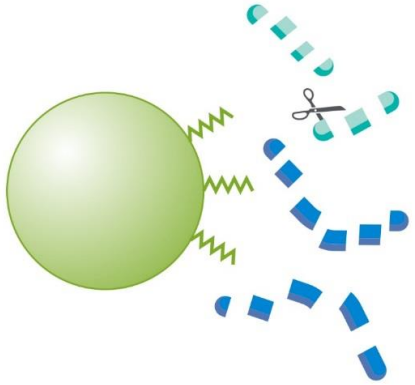
Critical quality attributes (CQA)

"A CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. Desired product quality includes clinically safe and efficacious product." – ICH Q8 (R2)

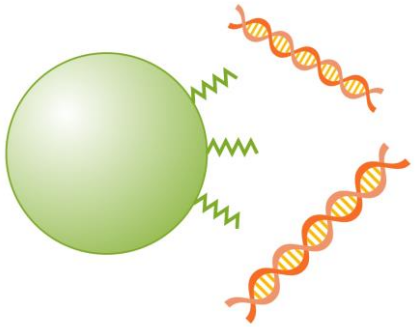
- Any feature of the biotherapeutic, formulation, or packaging that impacts the safety or effectiveness of the medication
- Not every attribute is a critical quality attribute



Intact & Subunit Purity



Peptide Mapping

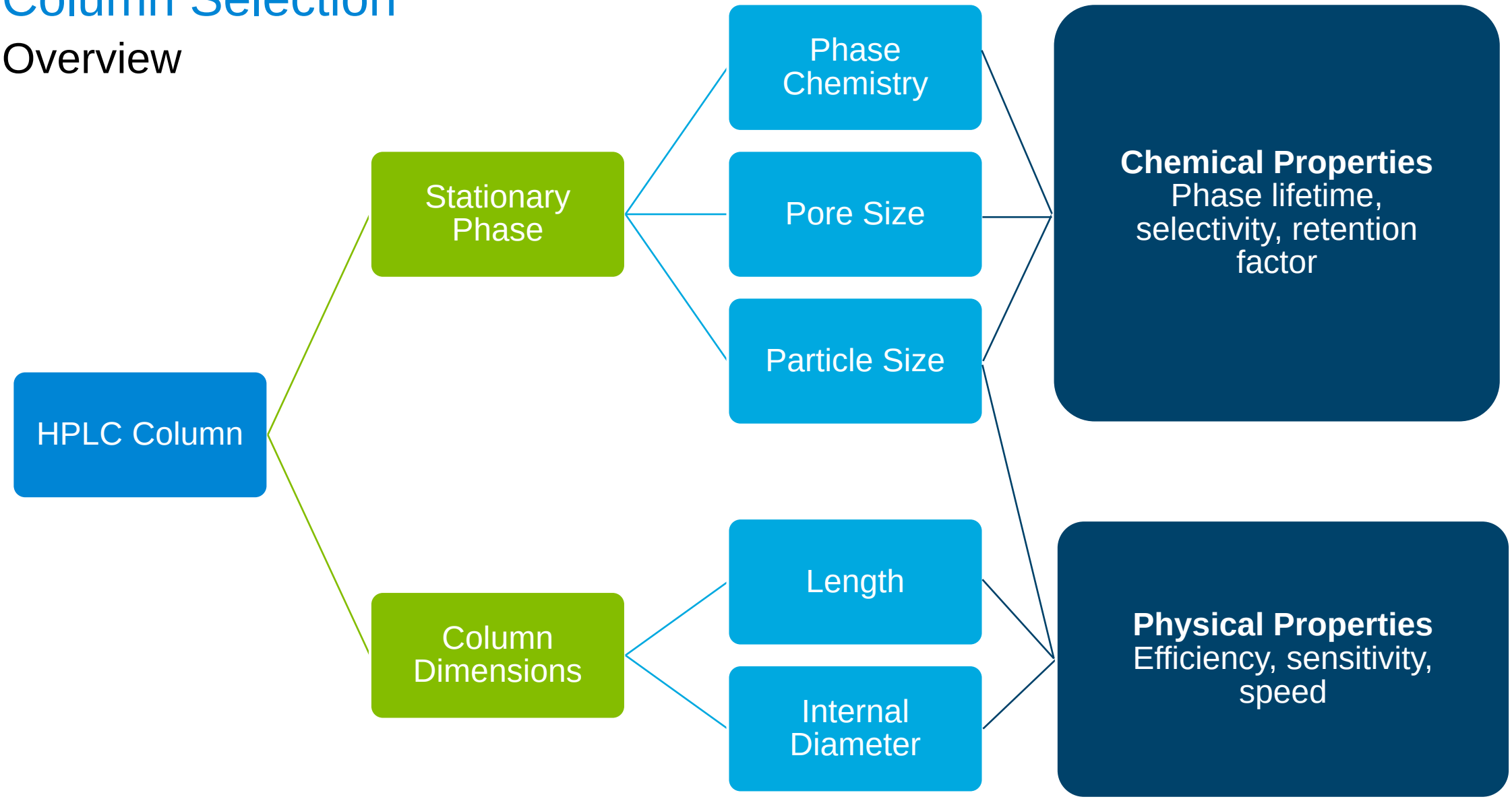


Oligonucleotide Analysis

Column Selection

Column Selection

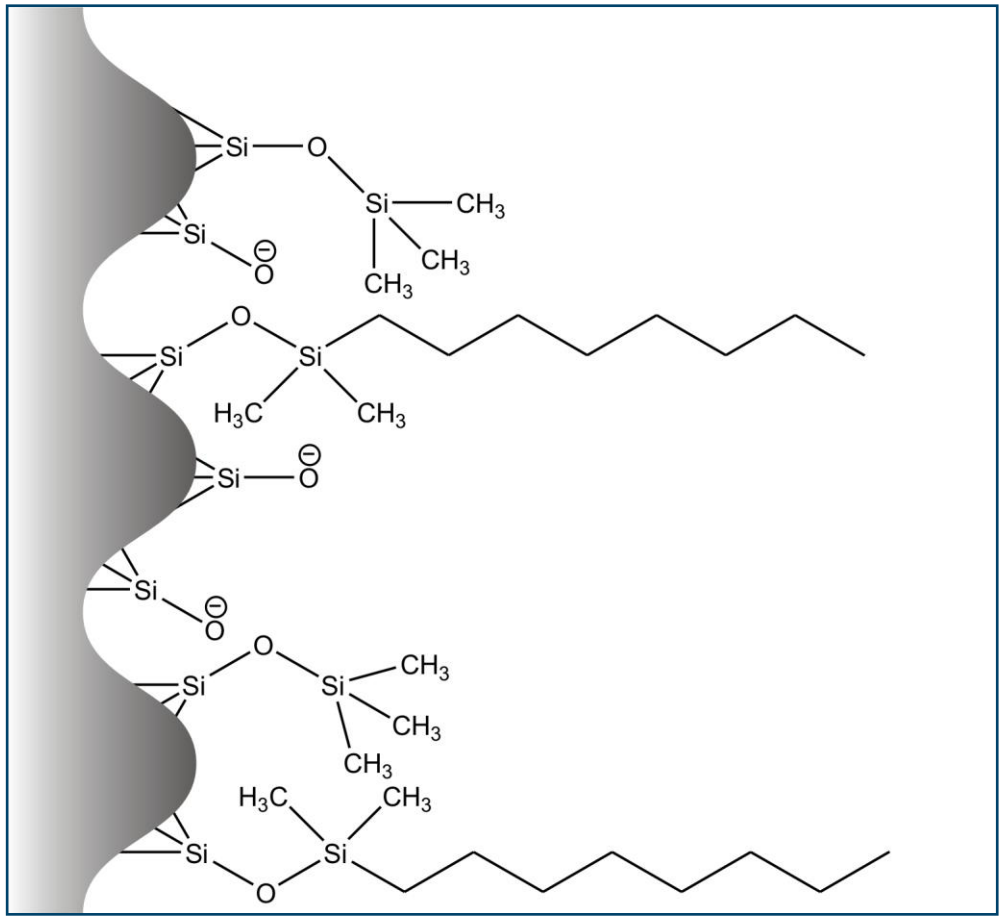
Overview



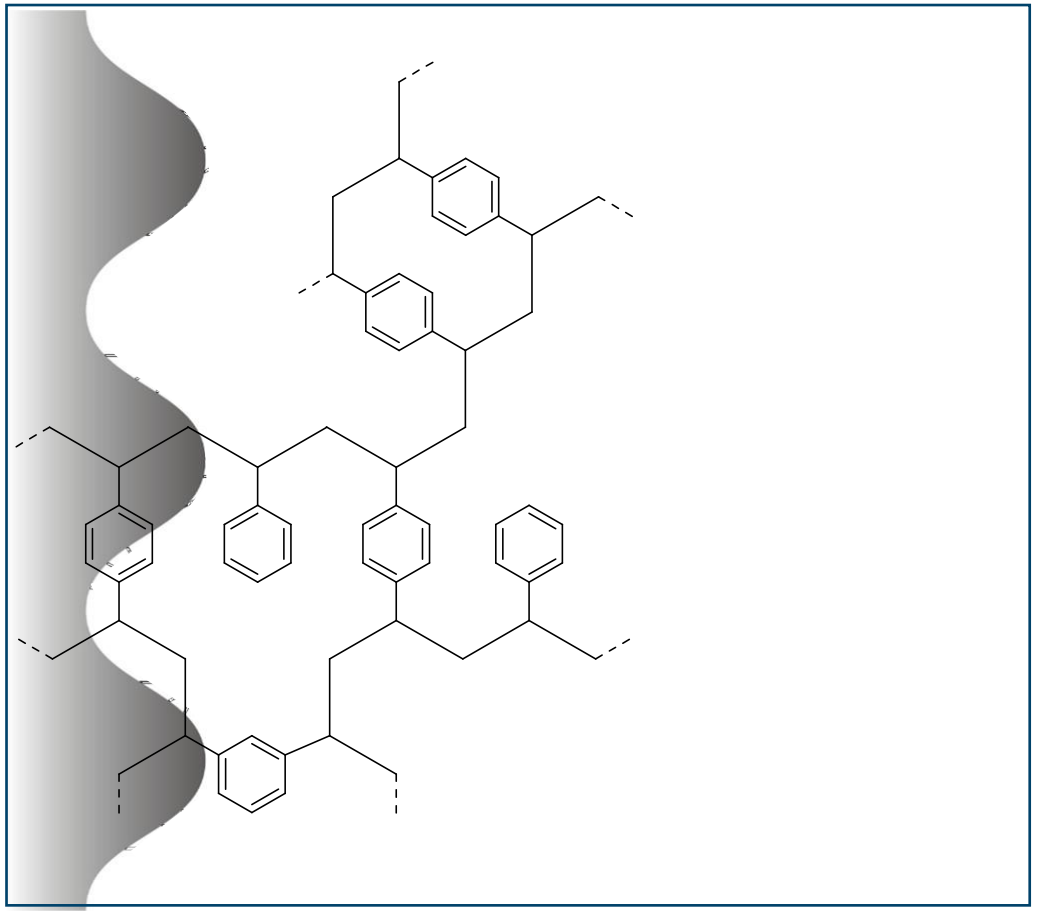
Column Selection

Base chemistry

Silica



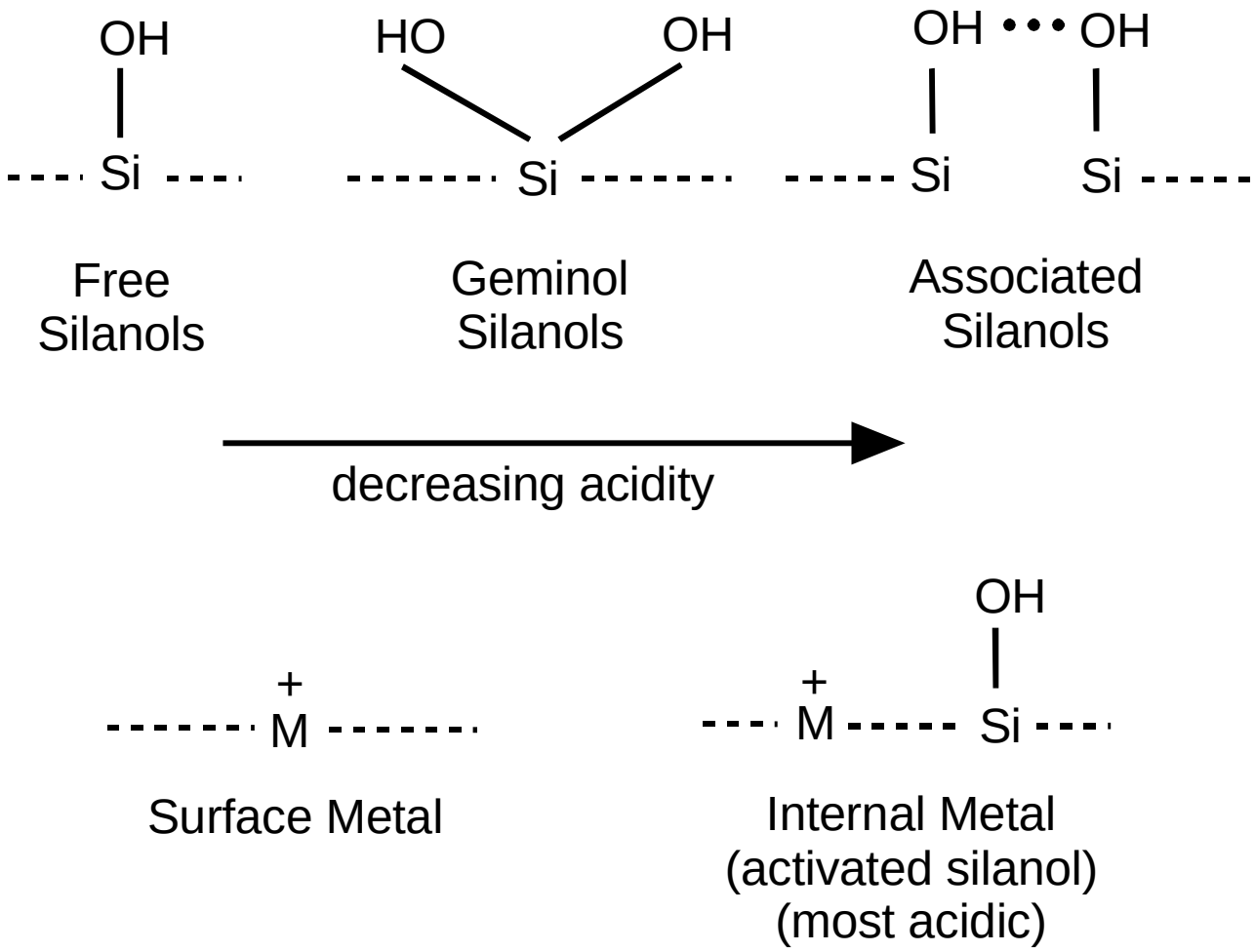
Polymeric



Column Selection

Base chemistry: silica

- Bonding is never complete, a significant amount of the silica surface is still bare
- Free silanols on the surface have varying acidity depending on their structure
- Metal impurities introduce the strongest acidic sites

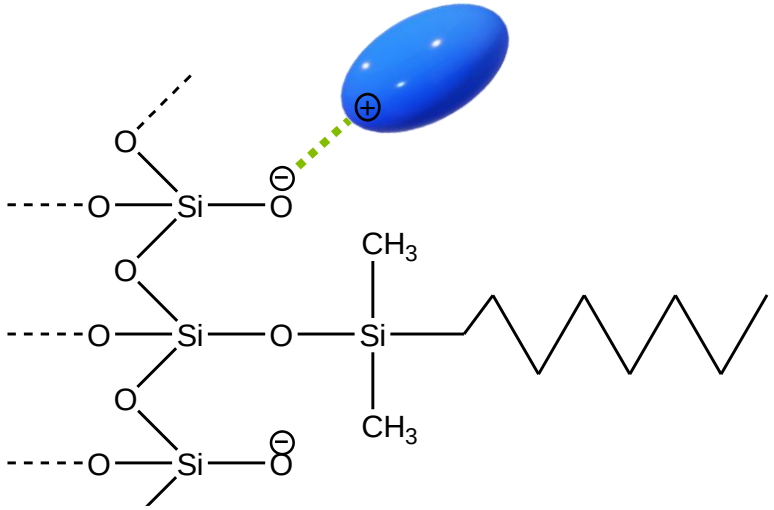


Column Selection

Silica: Potential secondary interactions

Ion-exchange

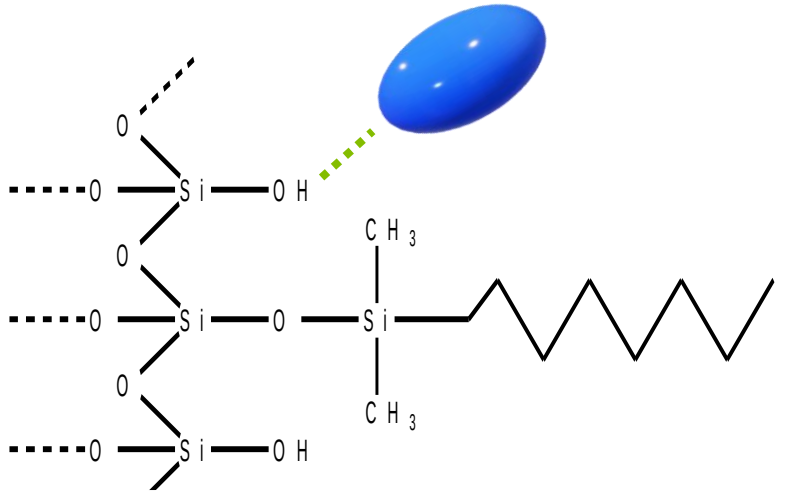
$$SiO^- Na^+ + R_3NH^+ \rightleftharpoons SiO^- N^+R_3 + Na^+$$



Ionized silanols (SiO^-) will ion-exchange with protonated bases (R_3NH^+) which can cause tailing and method variability. This occurs most often at mid pH where silanols are ionized.

Hydrogen bonding

$$-SiOH + RCOO^- \rightleftharpoons -SiO^- \ldots H^+ \ldots {}^-OOCR$$



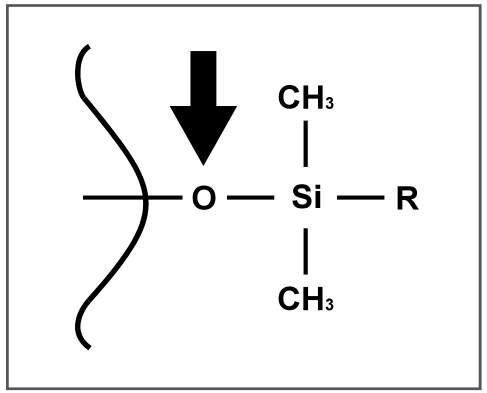
Unprotonated acids can compete for H^+ with protonated silanols. This can occur at low pH.

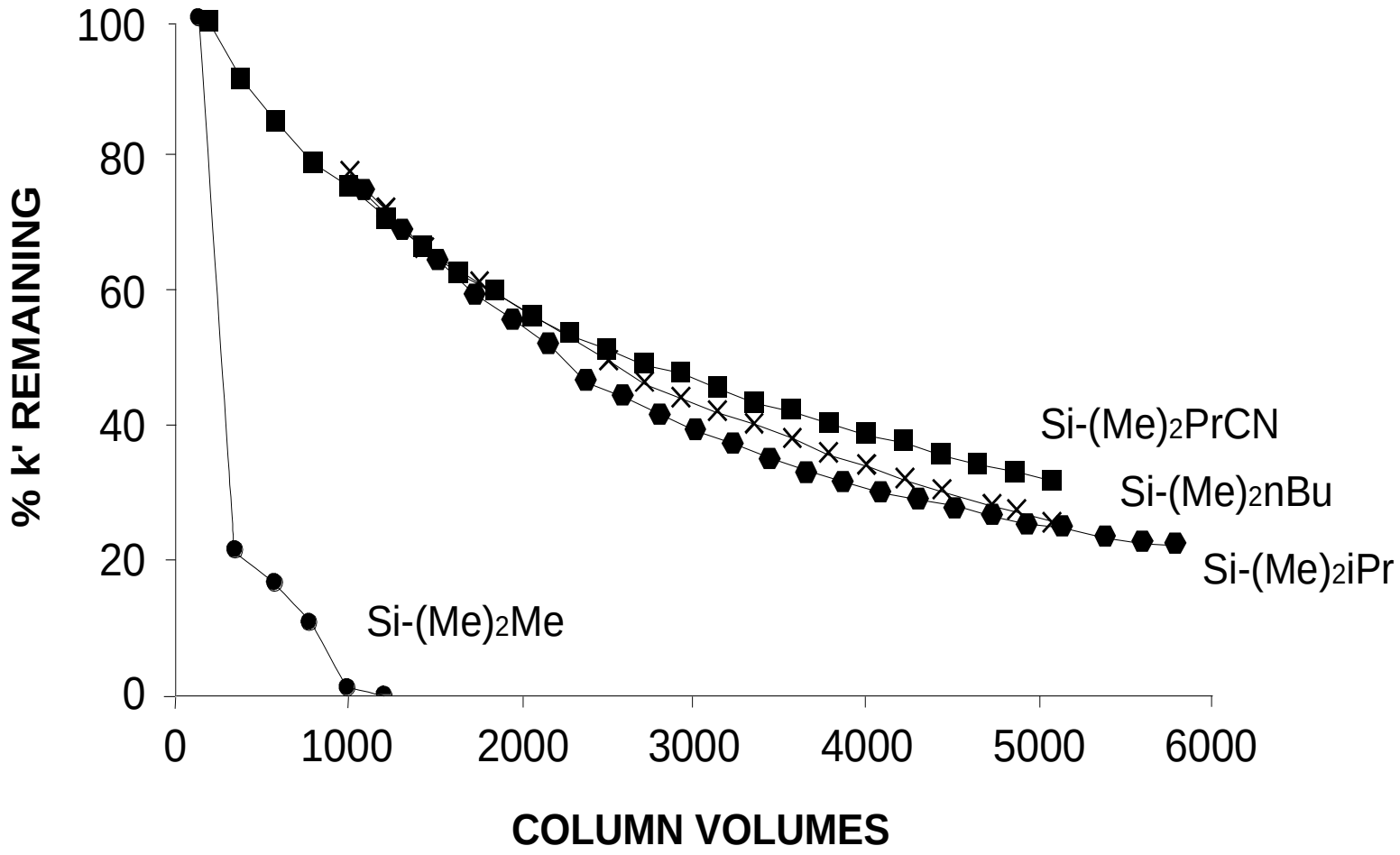
Column Selection

Column lifetime

Low pH methods

- Breaking of siloxane bond reduces column lifetime, especially with short chain alkyl ligands





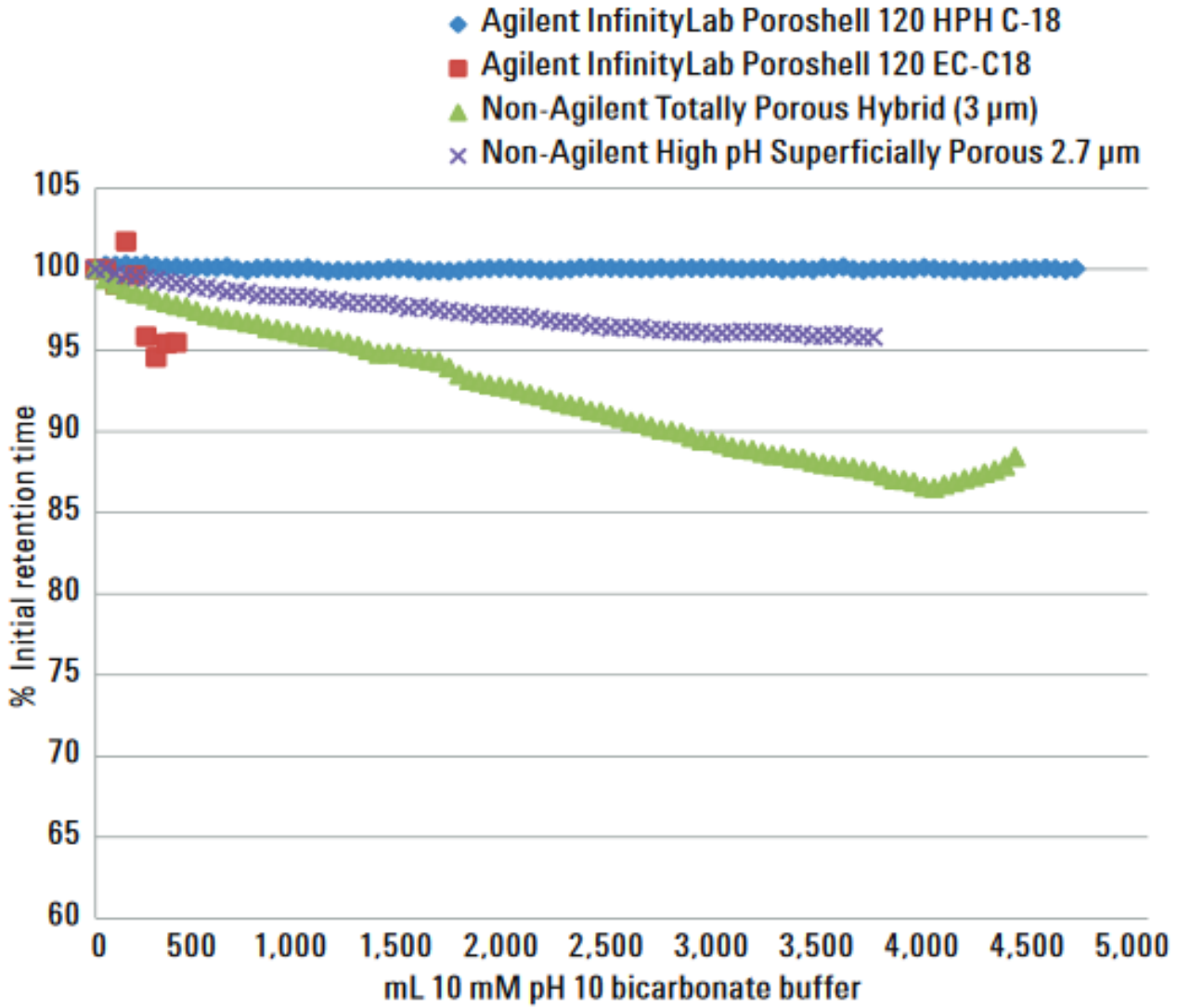
Kirkland, J.J., J.L. Glajch, and R.D. Farlee, *Analytical Chemistry* (1989), 61, 2.

Column Selection

Column lifetime

High pH methods

High pH methods can lead to the dissolution of the silica stationary phase

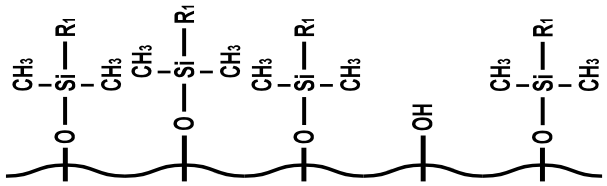


Column Selection

Silica modifications

Conventional Silica

Hydrolytically unstable, free silanols

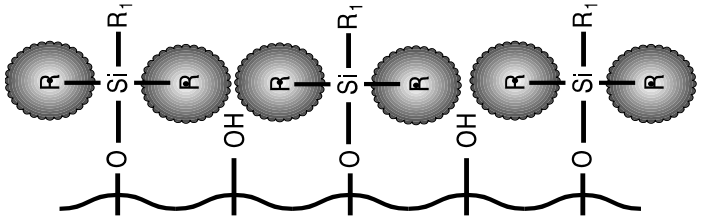


Sterically Protected

Alkyl groups sterically block silanols and protect siloxane bond

pH range: 1.0-8.0

Columns: Zorbax 300 Stable Bond (SB), AdvanceBio RP-mAb (SB)

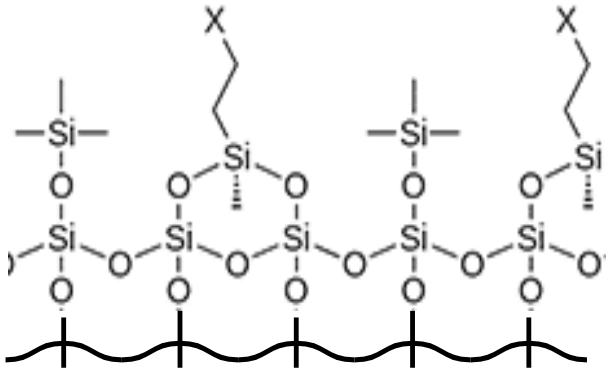


Extensive Endcapping

Double endcapping and/or bidentate bonding improves peak shape of basic compounds and high pH stability

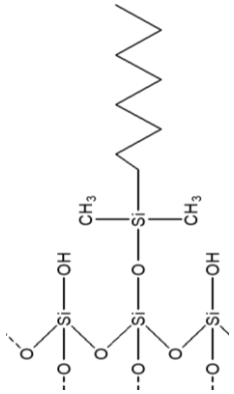
pH ranges: 2.0-11.5, 3.0-11.0

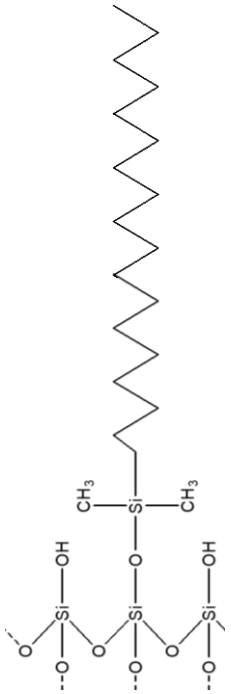
Columns: Zorbax 300 Extend, AdvanceBio Oligonucleotide



Column Selection

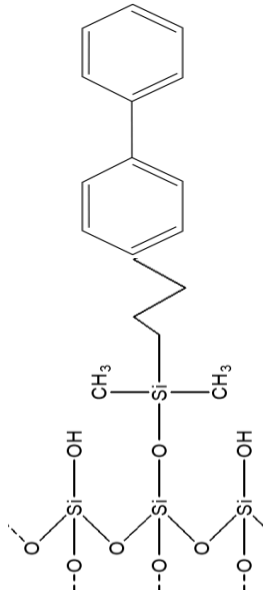
Basic phase chemistry





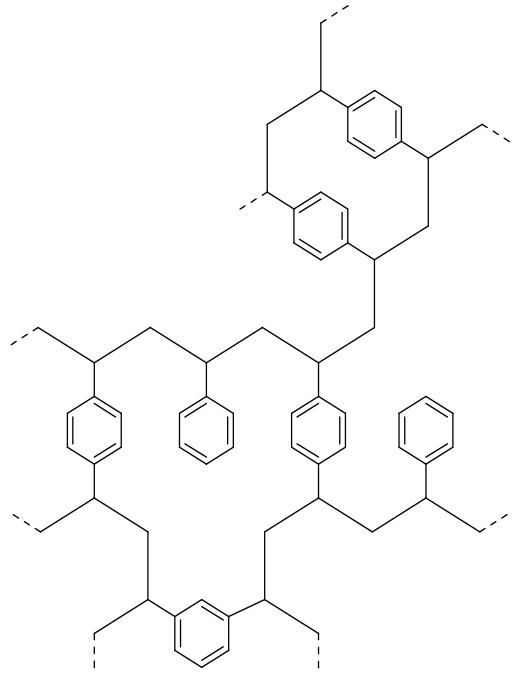
C3 | **C8** | **C18**

Alkyl ligands are most common RP stationary phase.
Hydrophobicity increases with chain length.
Van der Waal (VDW) interactions



Diphenyl

Alternative selectivity to alkyl phases.
π-π, aromatic, VDW interactions



Polymeric

Styrene-divinylbenzene copolymer.
VDW interactions

Column Selection

Pore size

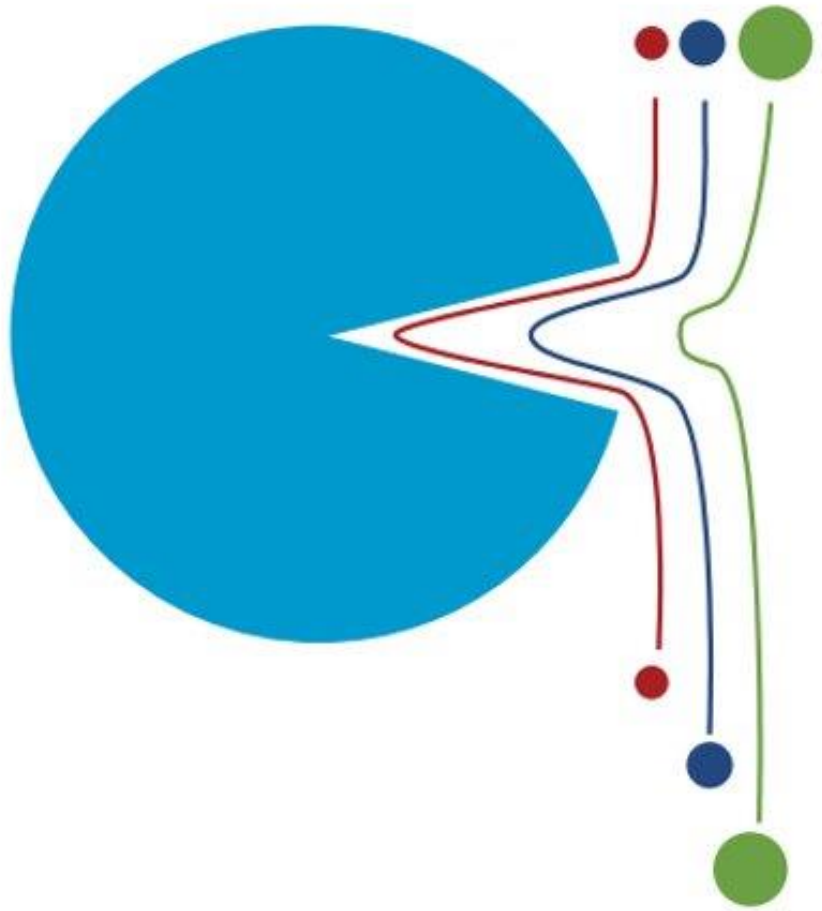
As a general rule, the pore size should be 3X the hydrodynamic radius of your analyte

Small molecules

- 80 – 120 Å
- Maximizes loading and retention

Peptides, proteins, other large biomolecules

- 120 Å (Peptides, small oligonucleotides)
- 300 Å to 450 Å (Proteins, mAb)
- 1000 Å (Larger proteins, larger oligonucleotides)
- 4000 Å (mRNA, pDNA, VLP)
- Maintain high efficiency

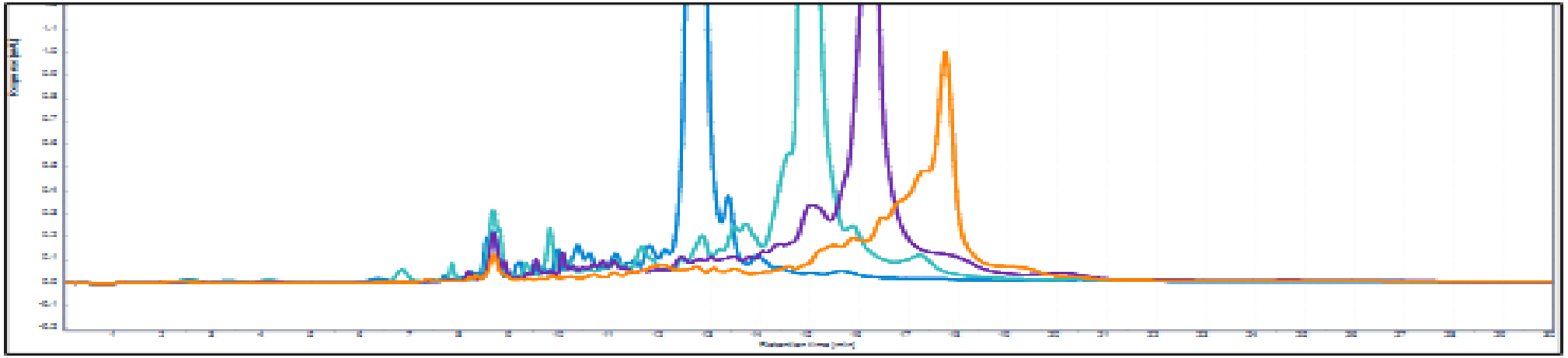


Column Selection

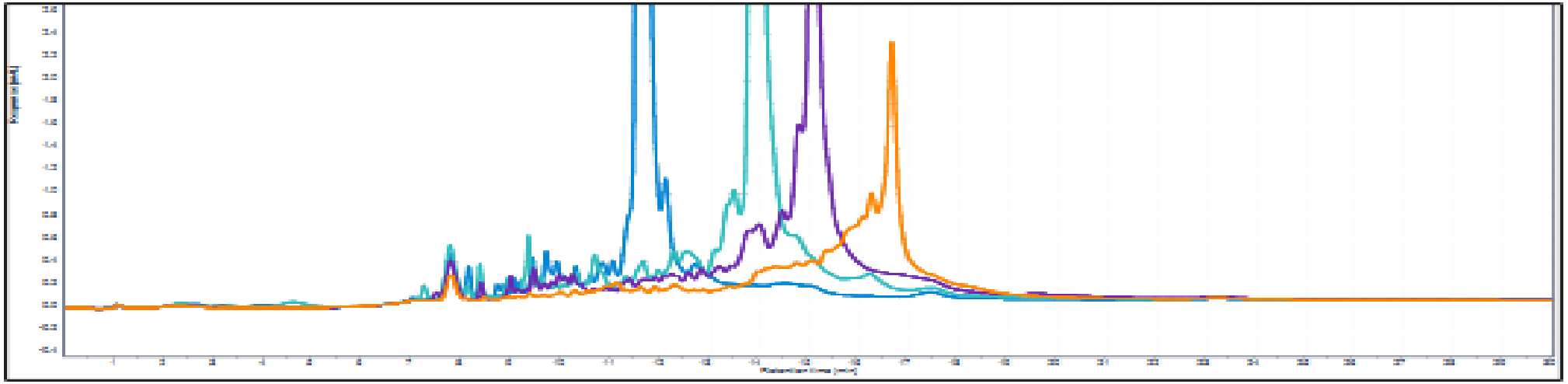
Pore size

25 mer, 50 mer, 75 mer, 100 mer

PLRP-S
100Å



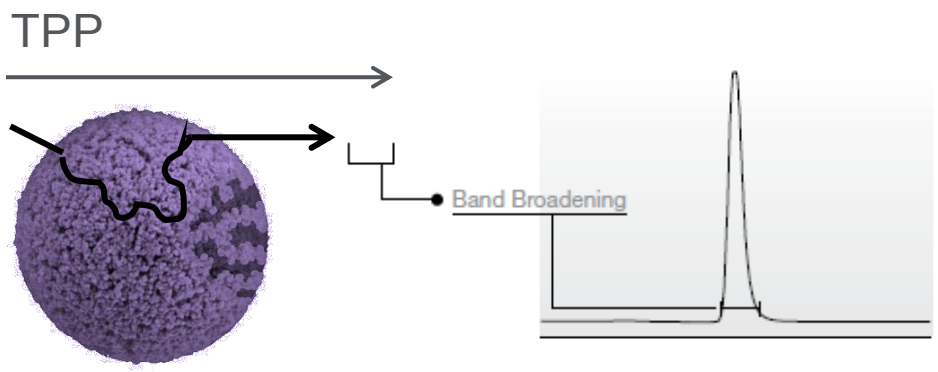
PLRP-S
1000Å

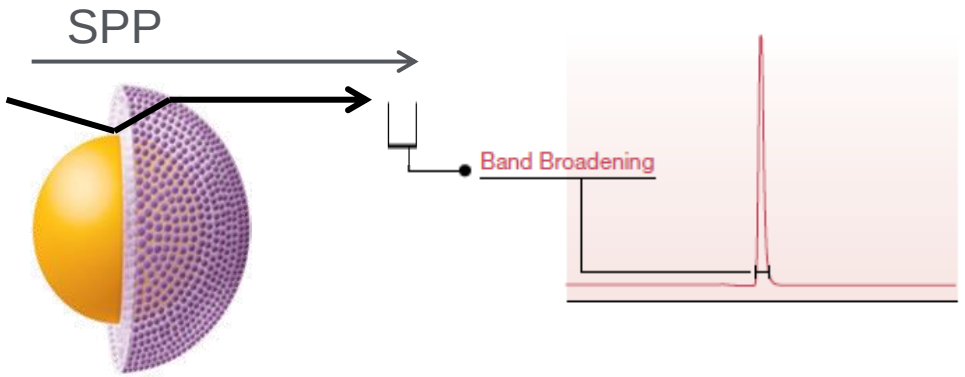


Column Selection

Totally porous particles (TPP) vs. superficially porous particles (SPP)

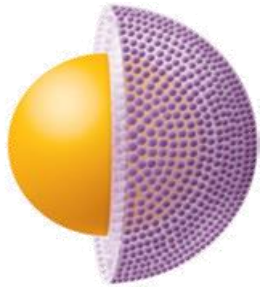
- Analytes travel though the particle more efficiently
- High efficiency allows you to use a larger SPP (i.e., 2.7um) for nearly equivalent performance to a smaller sub-2 um (STM) TPP column
- Using a larger particle allows for lower backpressure than comparably efficient totally porous STM columns and flexible use on HPLC or UHPLC systems





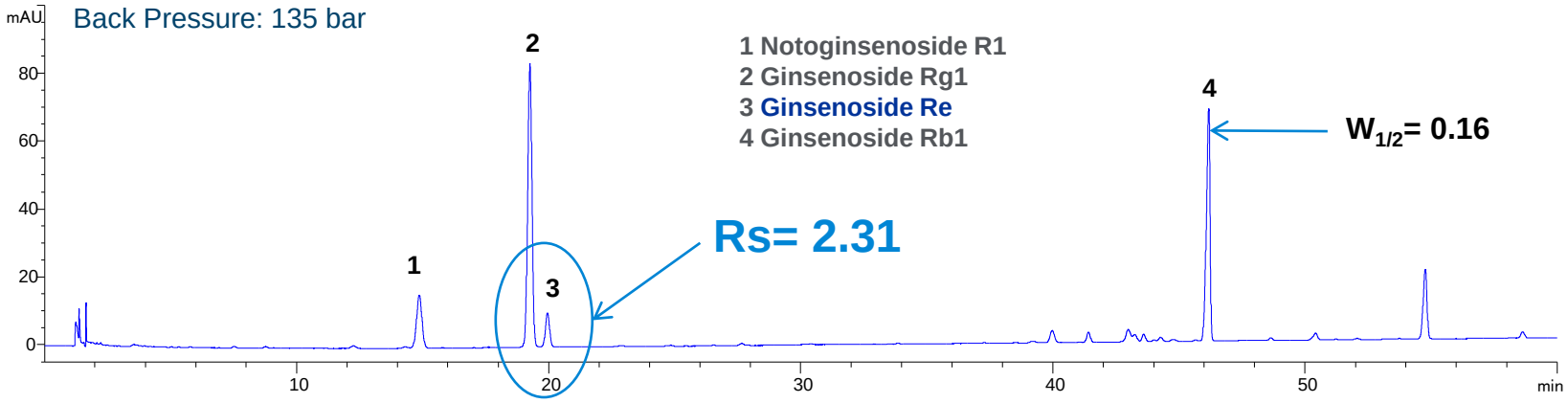
Column Selection

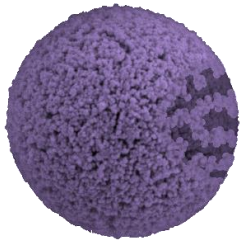
TPP vs. SPP



Poroshell 120 EC-C18, 4.6 x 150 mm, 4 µm

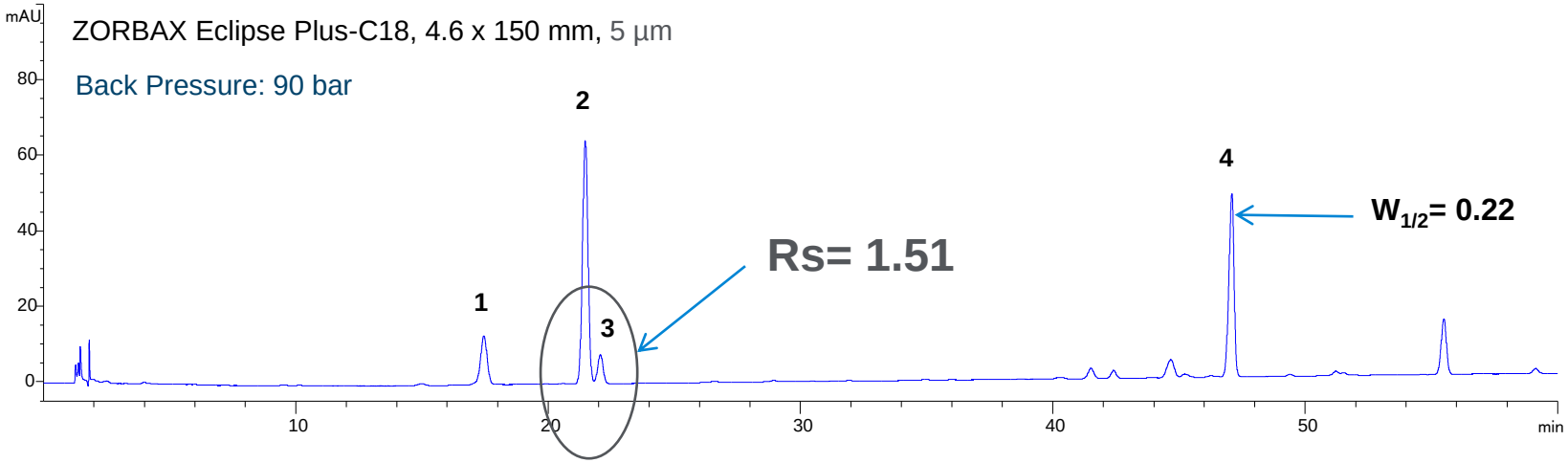
Back Pressure: 135 bar





ZORBAX Eclipse Plus-C18, 4.6 x 150 mm, 5 µm

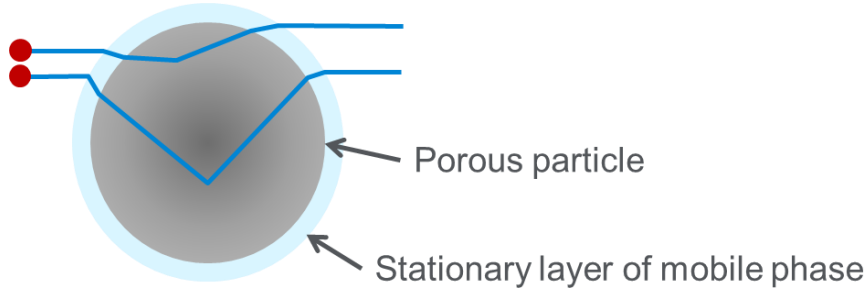
Back Pressure: 90 bar

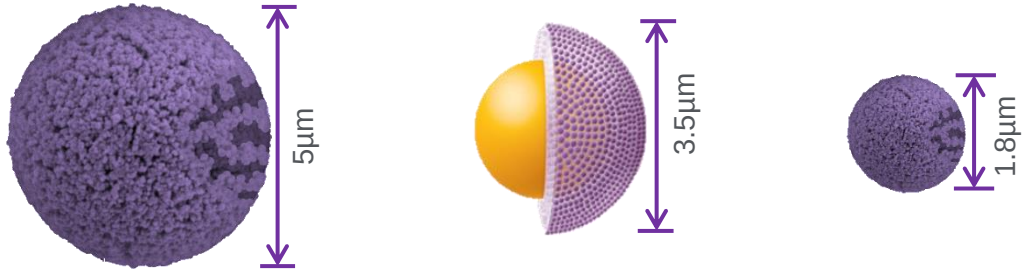


Column Selection

Particle size

- Stationary phase mass transfer is the diffusion of
- Smaller particles have short, more efficient paths





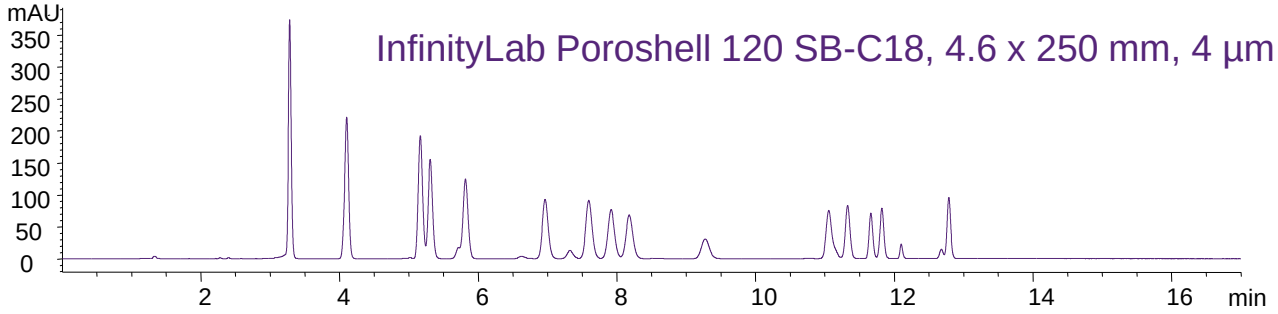
Particle diameter (d_p)

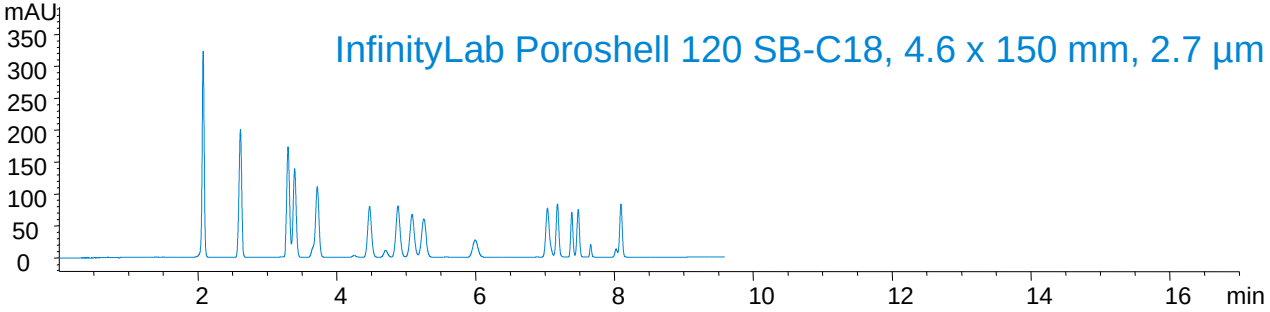
$$\Delta P \propto \frac{1}{\Delta d_p^2}$$

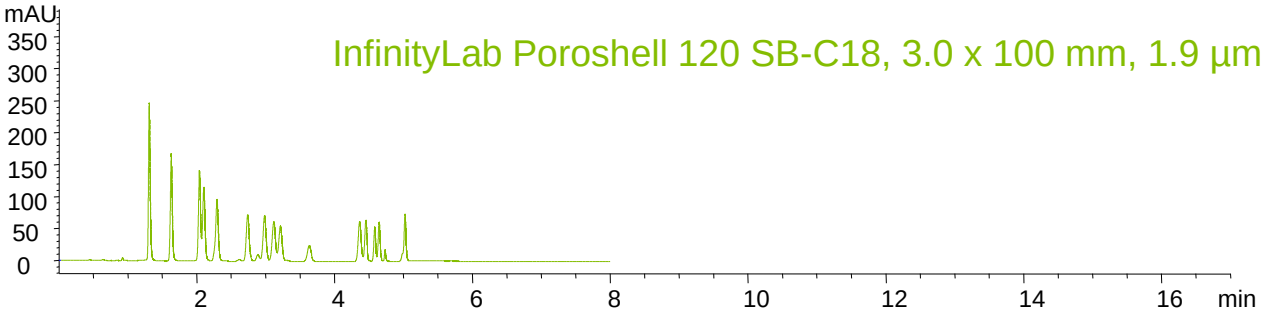
Increasing resolution, pressure

Column Selection

Particle Size







HPLC (4 µm)	Value	Difference
Run time	14 min	--
Response / injection volume	80 mAU / µl	--
Solvent consumption	21 mL	--
Samples per 8 h day	**24**	**--**

UHPLC (2.7 µm)	Value	Difference
Run time	8.75 min	- 37.5%
Response / injection volume	113 mAU / µl	+ 41%
Solvent consumption	13.1 mL	- 37.5%
Samples per 8 h day	**48**	**+24**

LD UHPLC (1.9 µm)	Value	Difference
Run time	5.25 min	- 62.5%
Response / injection volume	295 mAU / µl	+ 269 %
Solvent consumption	3.36 mL	- 84 %
Samples per 8 h day	**80**	**+56**

Column Selection

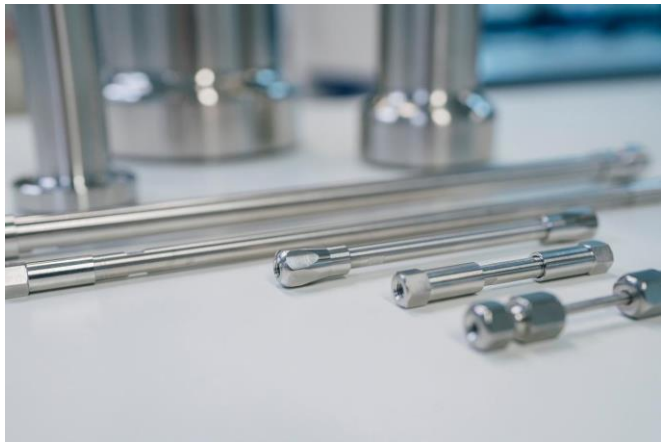
Column dimensions

Inner diameter (ID)

ID	Optimum Flow Rate	Recommended Use
4.6 mm	1.00-1.25 mL/min	Legacy methods
3.0 mm	0.8-1.0 mL/min	Lower solvent use
2.1 mm	0.4-0.5 mL/min	MS applications, lowest solvent use

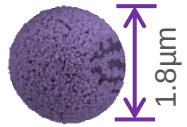
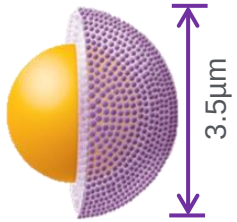
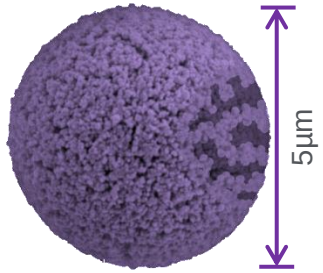
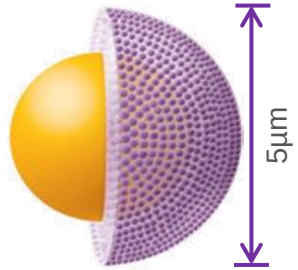
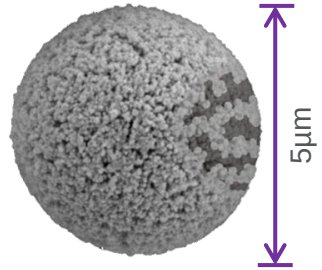
Column length

Column Length	Recommended Use
50 mm	High throughput
100 mm	High resolution
≥150 mm	Ultra-high resolution



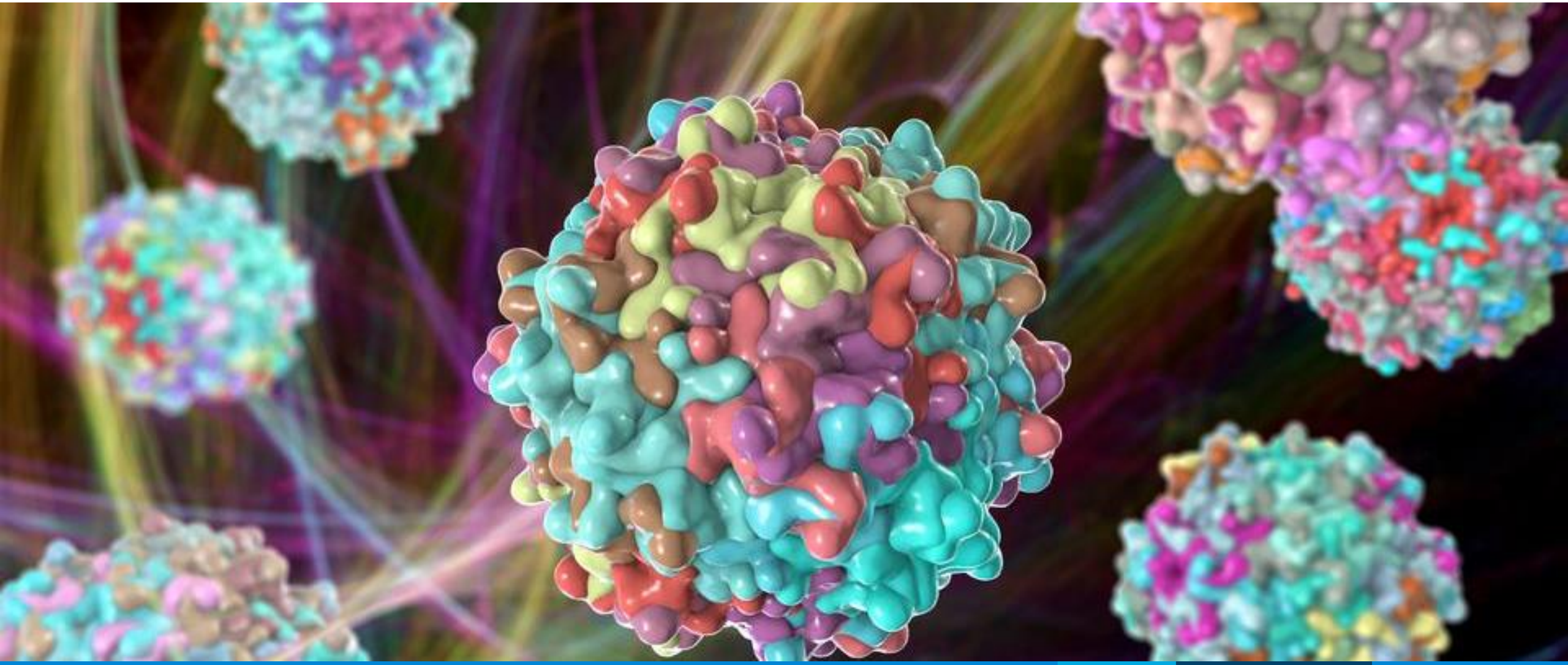
Column Selection

Biocolumns for reversed-phase separations

	Zorbax RRHD SB 300	AdvanceBio RP-mAb	ZORBAX SB300	Poroshell 300	PLRP-S
					
Phase Options	SB-C3, SB-C8, Diphenyl	C4, SB-C8, Diphenyl	SB-C3, SB-C8, Diphenyl	SB-C3, SB-C8	Polymeric
Pore Size	300 Å	450 Å	300 Å	300 Å	1000 Å
HPLC		●	●	●	●
UHPLC	●				
Strengths	• Maximum resolution • Unique diphenyl chemistry	• Designed for mAbs • Unique diphenyl chemistry	• Robust workhorse • Unique diphenyl chemistry	• Smaller globular proteins • Higher throughput update to Zorbax 300SB methods	• Recommended for MS – excellent peak shape with both FA & TFA • PEEK-lined SS hardware option

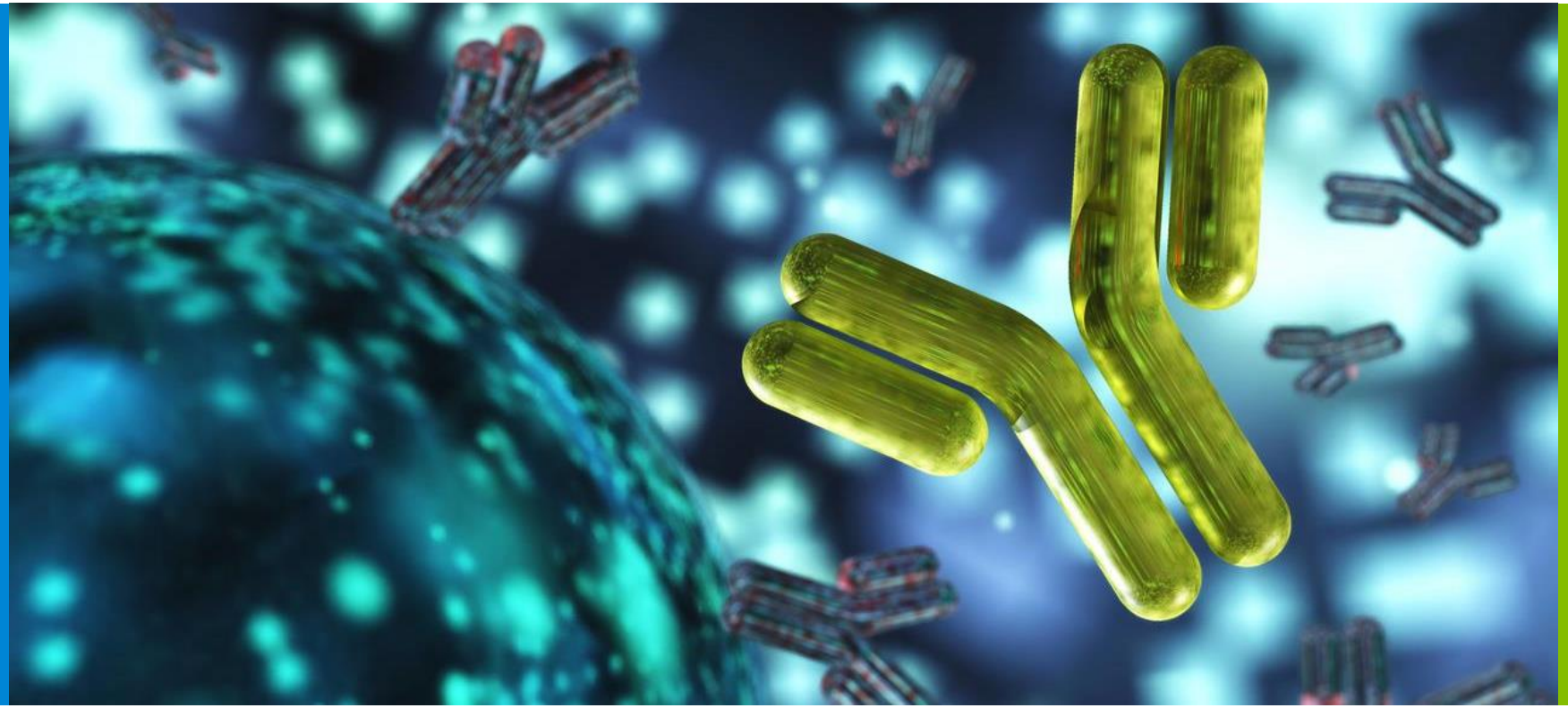
Bioseparations

Proteins, peptides, and oligonucleotides



Bioseparations

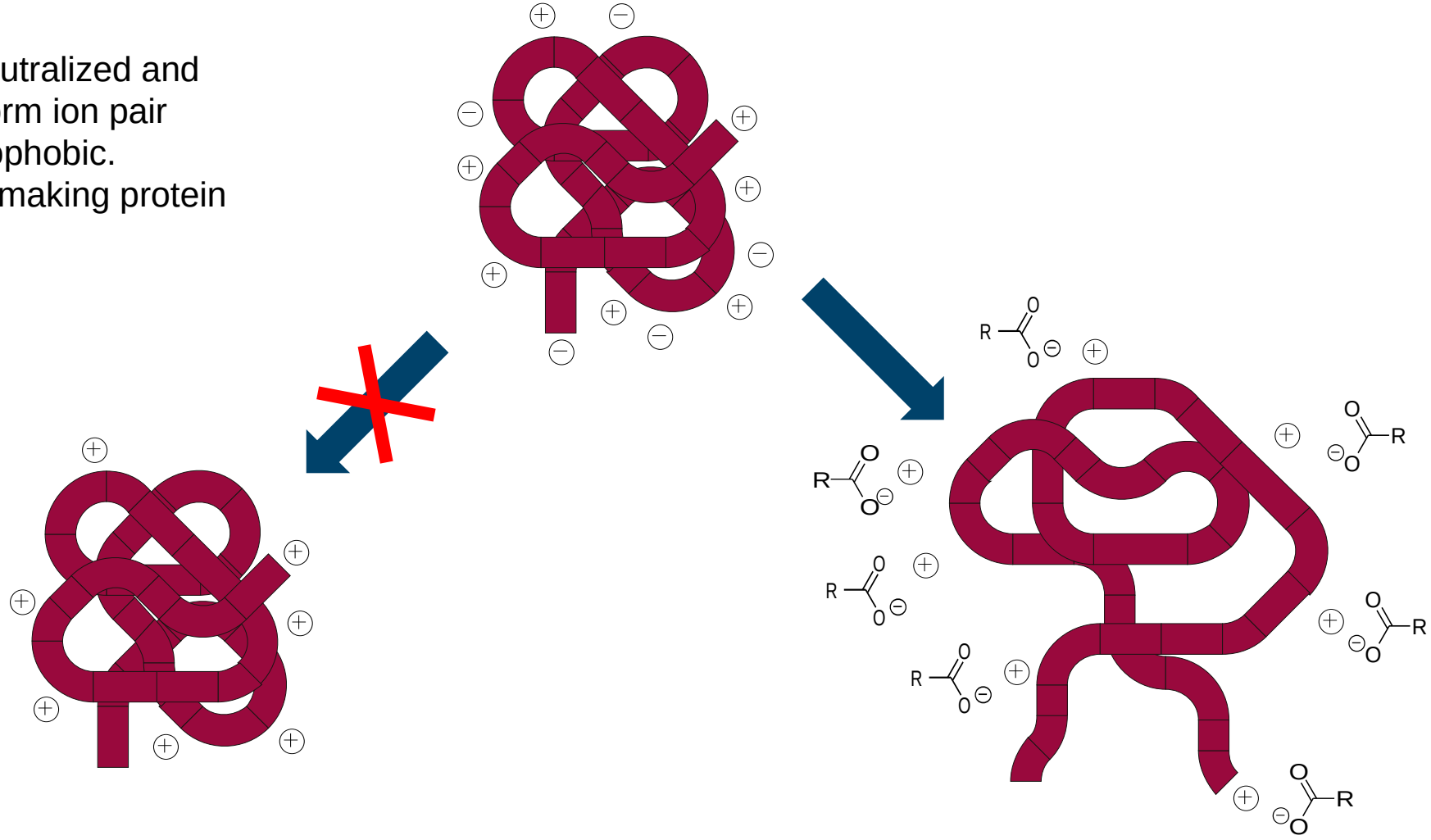
Proteins



Proteins

Ion-pair effect on proteins

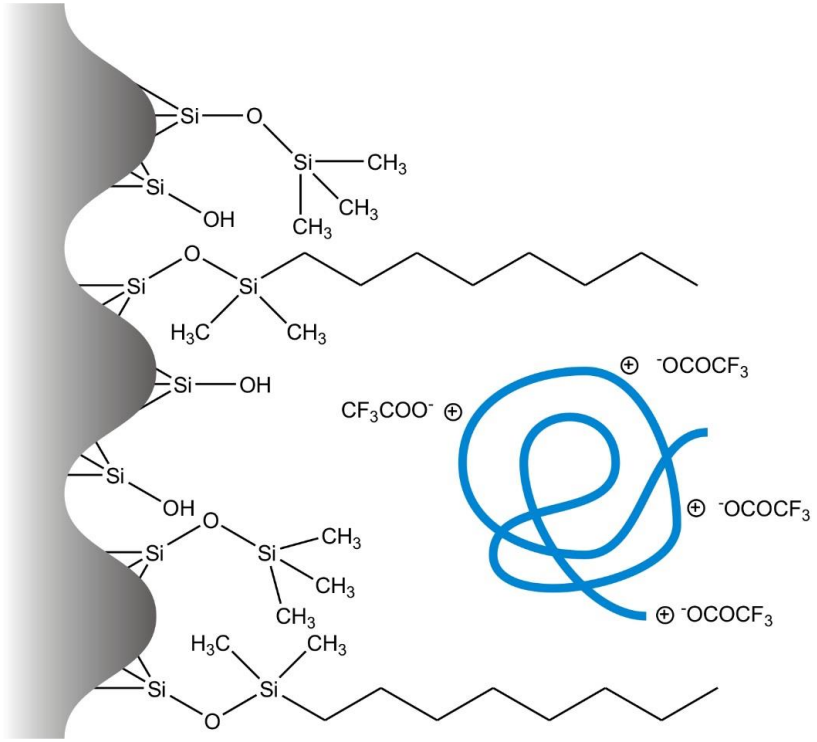
Carboxylate groups are neutralized and protonated basic groups form ion pair making protein more hydrophobic. Conditions are denaturing making protein larger in solution.



Proteins

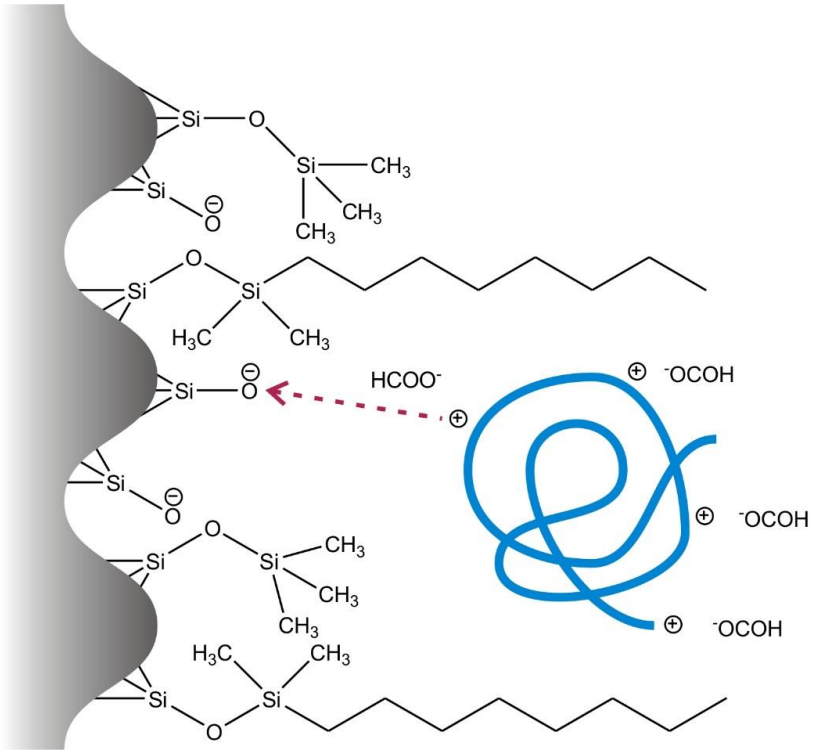
Mobile phase modifiers

Trifluoroacetic Acid (TFA), UV analysis



TFA blocks silanol interaction, enabling good peak shape.

Formic Acid, MS Analysis

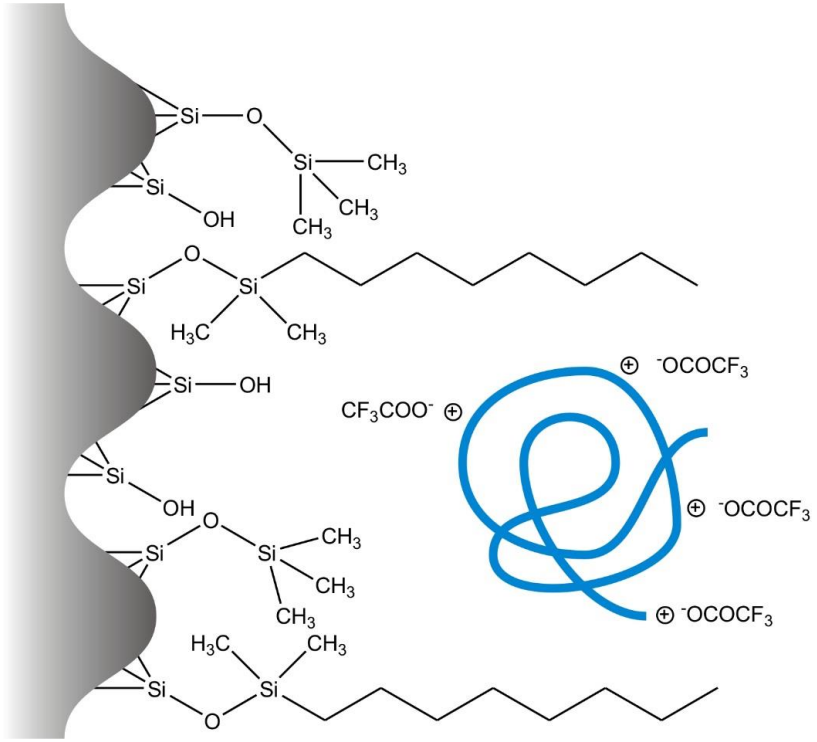


Formic acid weakens but doesn't block silanol interaction.

Proteins

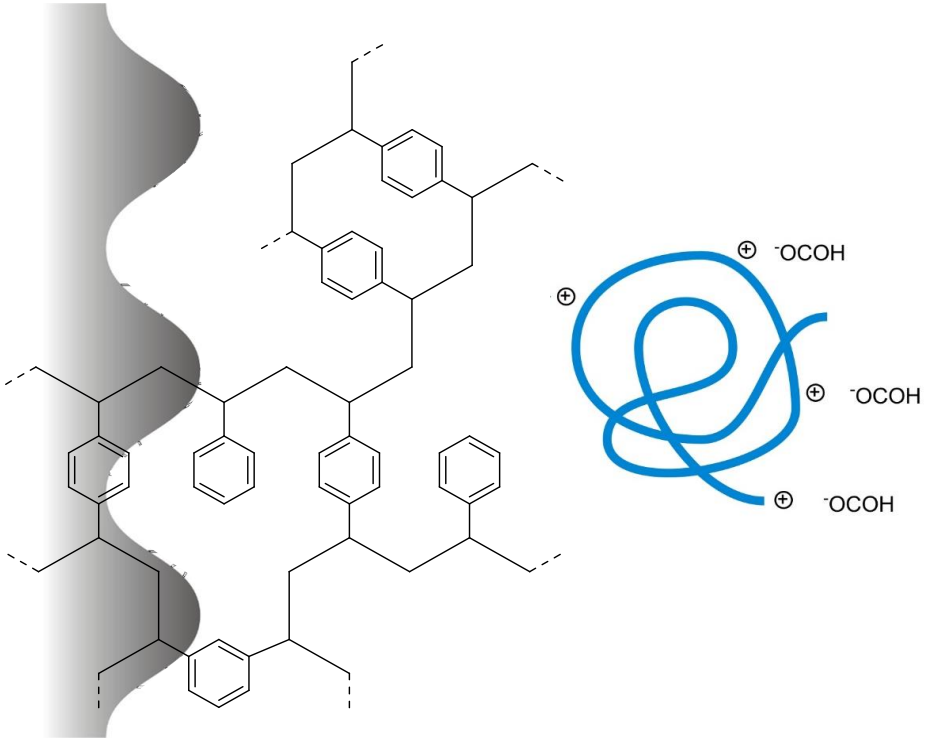
Mobile phase modifiers

Trifluoroacetic Acid (TFA), UV analysis



TFA blocks silanol interaction, enabling good peak shape.

Formic Acid, MS Analysis



Excellent peak shape with formic acid

Proteins

Column selection

Base particle chemistry – polymer or silica?

- PLRP-S has excellent peak shape with both formic acid and TFA.
- Stable Bond silica phases for longer column lifetime at low pH

Bonded phase chemistry – C3, C4, C8, or diphenyl?

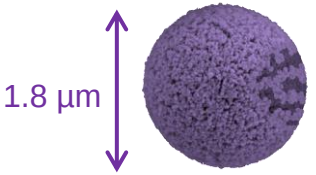
- The larger the protein, the shorter the alkyl chain should be
- Diphenyl offers a unique selectivity option

Particle size – < 2µm to 5µm or higher

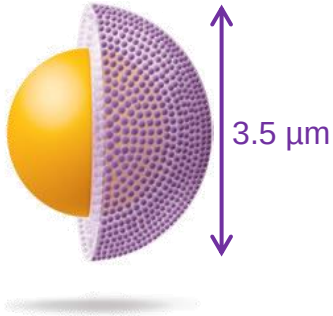
- Smaller particles give high resolution, but also higher back pressure

Pore size – anywhere from 300-1000Å!

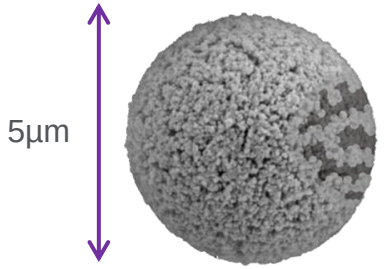
- Pores should be ≥ 3x the hydrodynamic radius of the protein
- 300-500Å most common, but 1000Å increasing in popularity



ZORBAX RRHD 300SB
300Å pore size



RP mAb
450Å pore size



PLRP-S
100Å, 300Å, 1000Å pore sizes

Proteins

Initial column selection

Peptides/ polypeptides
MW <10 kDa

First choice:
AdvanceBio (AB)
Peptide Mapping

Also consider:
Zorbax 300 Å SB-C18

Proteins
MW >50 kDa

First choice:
Zorbax 300 Å SB-C8

Also consider:
PLRP-S
Zorbax 300 Å SB-C18
Zorbax 300 Å SB-C3
Poroshell 300 SB-C18
Poroshell 300 SB-C8
Poroshell 300 SB-C3

Reduced/fragmented
MW <50 kDa

First choice:
AB RP-mAb SB-C8

Also consider:
PLRP-S
AB RP-mAb SB-C4
AB RP-mAb diphenyl
Zorbax 300 Å diphenyl
Zorbax 300 Å SB-C8
Zorbax 300 Å SB-C3

Intact
MW >150 kDa

First choice:
AB RP-mAb C4

Also consider:
PLRP-S
AB RP-mAb SB-C8
AB RP-mAb diphenyl
Zorbax 300 Å diphenyl
Zorbax 300 Å SB-C8
Zorbax 300 Å SB-C3

Proteins

Initial method conditions

- Start at low pH with simple aqueous/organic gradient
- Typically, a water: acetonitrile with 0.1% trifluoroacetic acid (TFA) gradient is used to elute all components of interest.
- A typical high-resolution gradient on a 300 Å pore size column requires 30 to 50 min.
- For LC/MS methods, TFA can reduce detector sensitivity and is often replaced with ammonium formate/formic acid.

Proteins

Initial separation conditions

Peptides/ polypeptides
MW <10 kDa

Mobile phase:
A: 95% H2O:5% ACN with 0.1% TFA
B: 5% H2O:95% ACN with 0.085% TFA

Gradient: 3 to 60% in 30 min

Temperature: 40 °C

Proteins
MW >50 kDa

Mobile phase:
A: 95% H2O:5% ACN with 0.1% TFA
B: 5% H2O:95% ACN with 0.085% TFA

Gradient: 5 to 70% in 20 min

Temperature: 80 °C

Reduced/fragmented
MW <50 kDa

Mobile phase:
A: 100% H2O with 0.1% TFA
B: 10% H2O:10% ACN:80% n-PA with 0.08% TFA

Gradient: 5 to 40% in 10 min

Temperature: 60 °C

Intact
MW >150 kDa

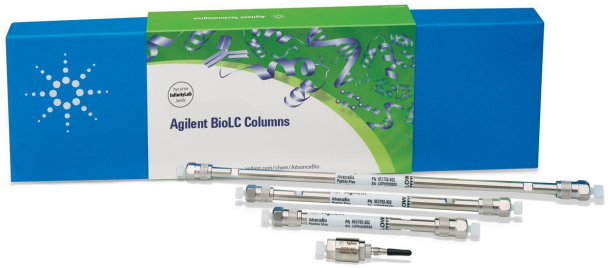
Mobile phase:
A: 98% H2O:2% IPA with 0.1% TFA
B: 10% H2O:0% ACN:70% IPA with 0.08% TFA

Gradient: 10 to 60% B in 5 min

Temperature: 80 °C

Proteins

Method optimization



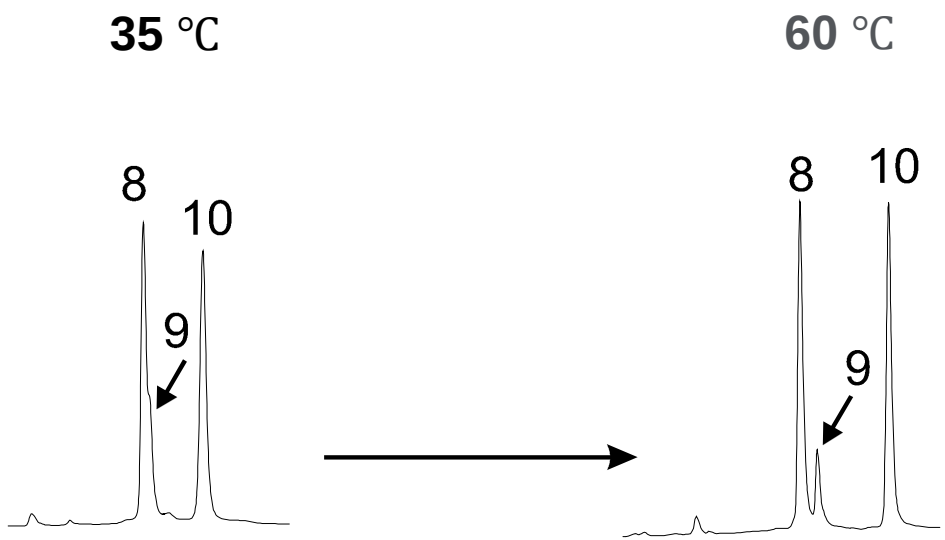
Method Optimization

Temperature	• Increase temperature reduces mobile phase viscosity and improves peak shape
Gradient Steepness	• A steeper gradient can shorten a long method • A shallower gradient improves resolution
Mobile Phase	• Methanol and isopropyl alcohol (IPA) are alternative to ACN • RP mAb often has improved resolution with some IPA • Change concentration of ion-pair reagent • Changing to a more hydrophobic ion-pair reagent
Particles	• Smaller particles or SPP particles can improve resolution

Proteins

Improving resolution: temperature

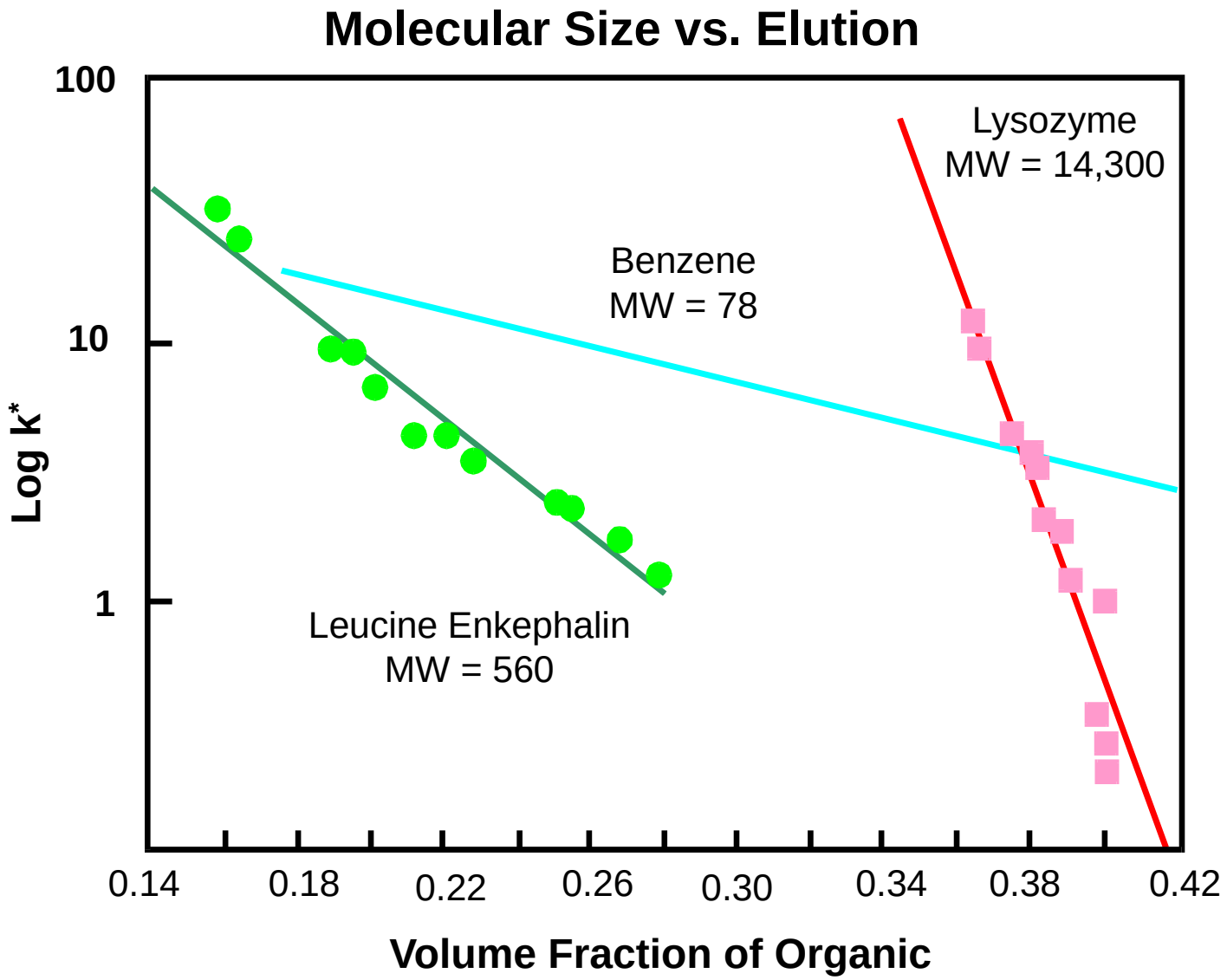
- Increasing the method temperature can sometimes improve resolution



Column: Zorbax 300 SB-C3
8. Myoglobin
9. Calmodulin
10. Carbonic Anhydrase

Proteins

Improving resolution: gradient

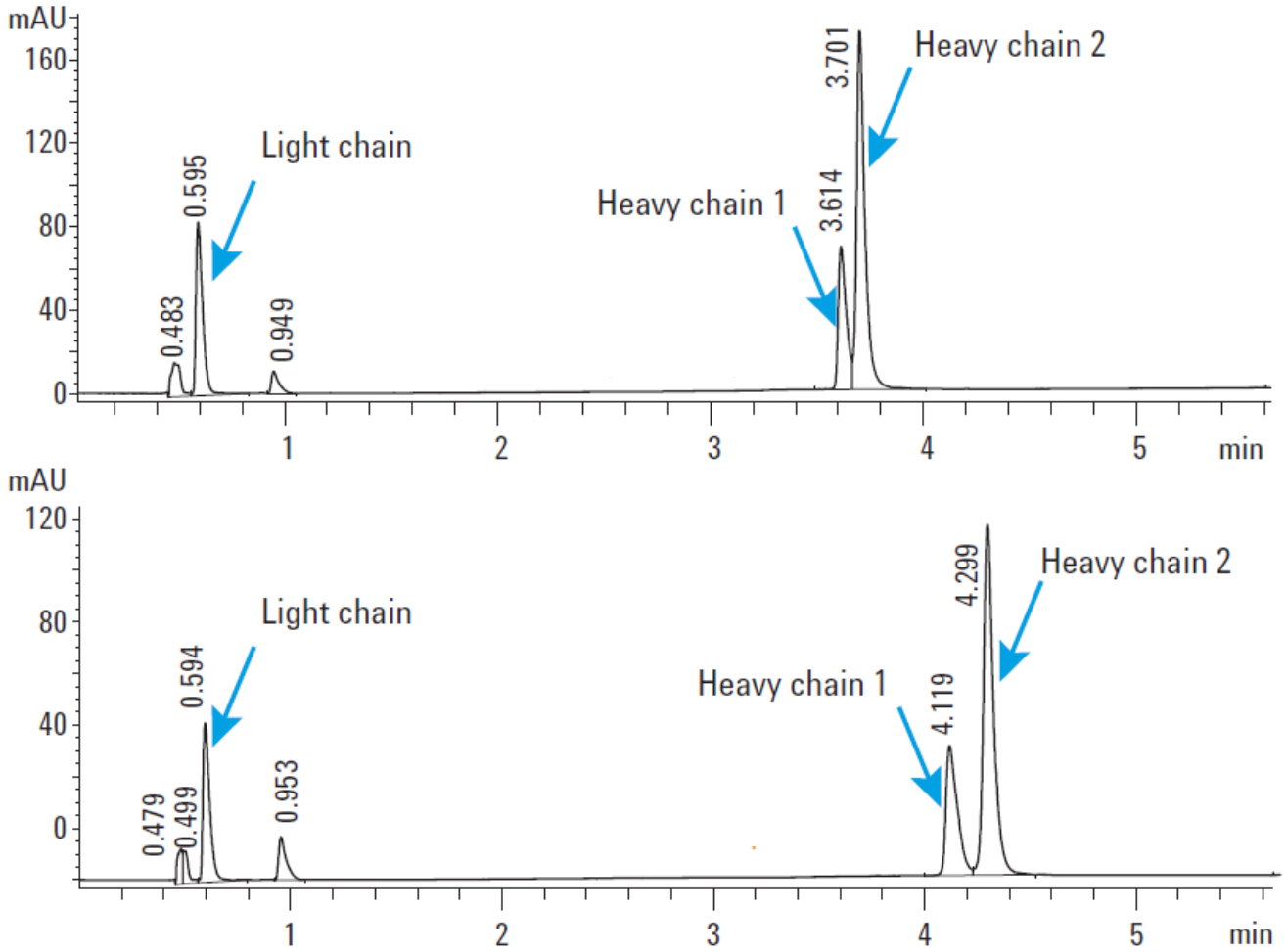


Proteins

Improving resolution: gradient

Column: ZORBAX RRHD 300-Diphenyl

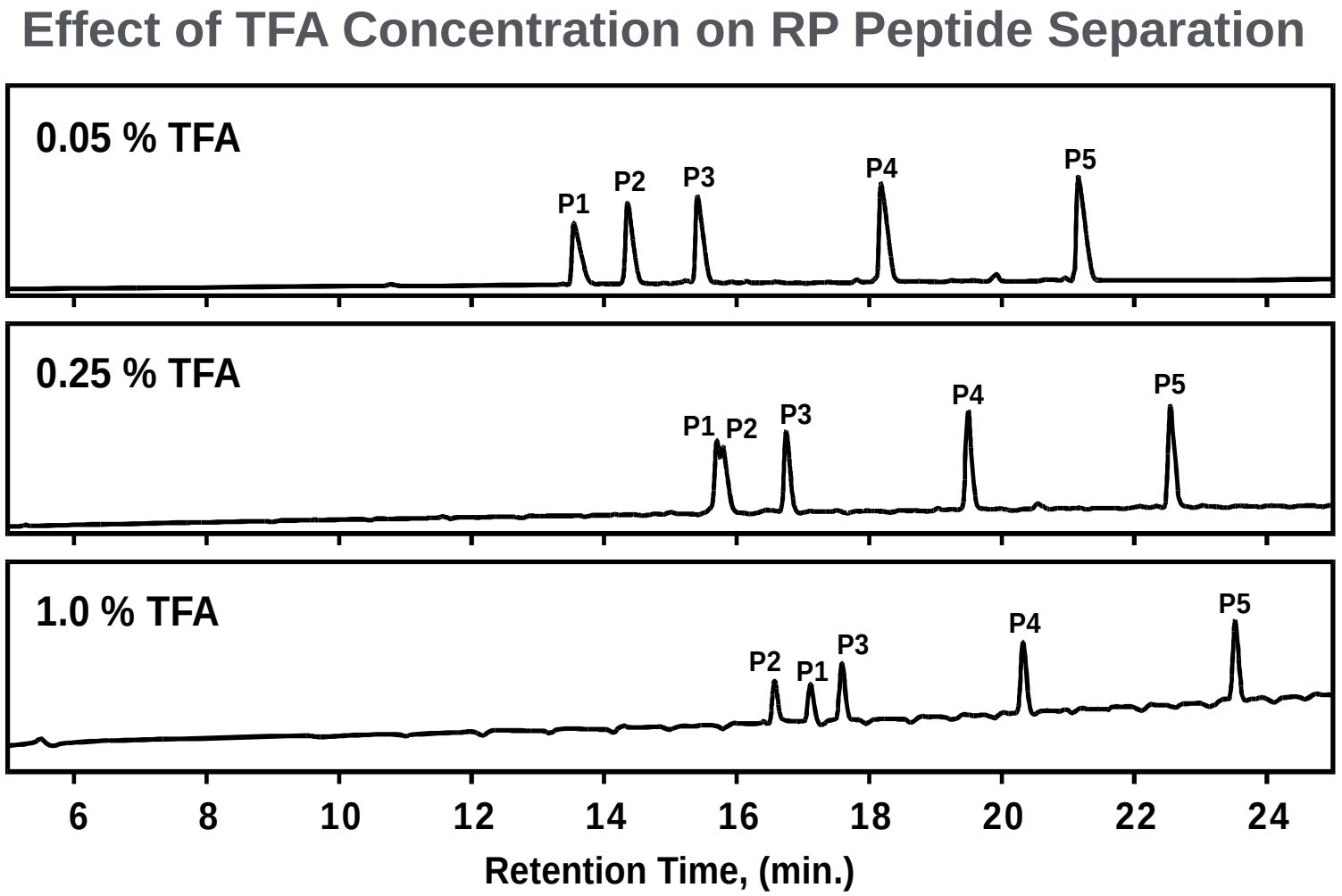
Time (min)	Gradient 1 %B	Gradient 2 % B
0	1	1
2	20	20
5	70	50



Gradient 2: Heavy chain peaks are more resolved.

Proteins

Improving resolution: change mobile phase



Conditions

Column:
Zorbax 300SB-C8, 4.6 x 150 mm

Mobile Phase:
A= H_2O and TFA,
B= ACN and TFA

Gradient: 0-30% B in 30 min.

Flow: 1 mL/min.

Temp.: 40°C

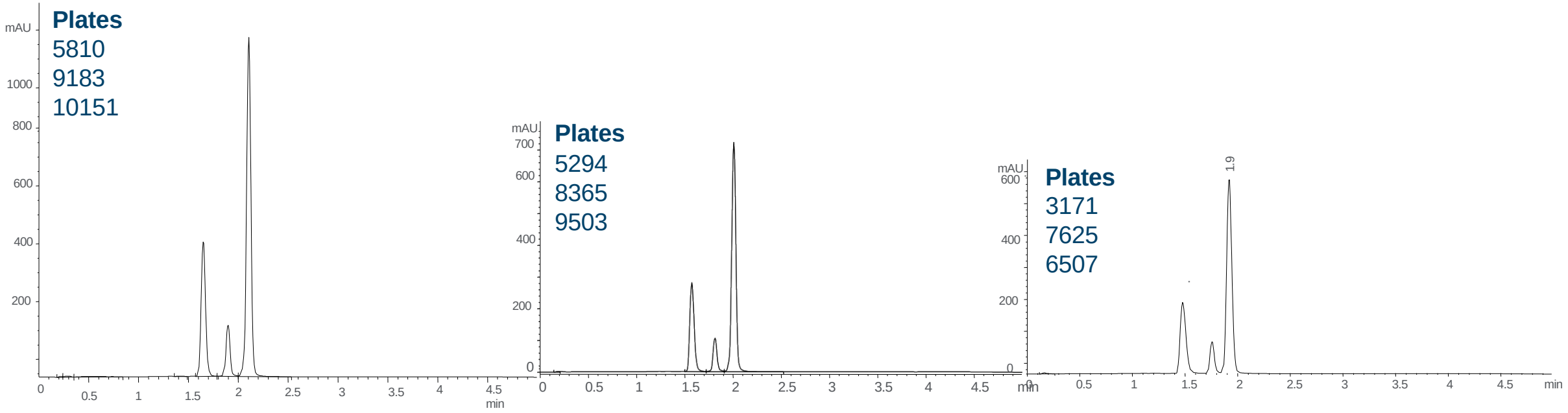
Proteins

Improving resolution: particle size

1.8 µm, 2.1 x 50 mm

3.5 µm, 2.1 x 50 mm

5 µm, 2.1 x 75 mm



Column: ZORBAX 300 SB-C18

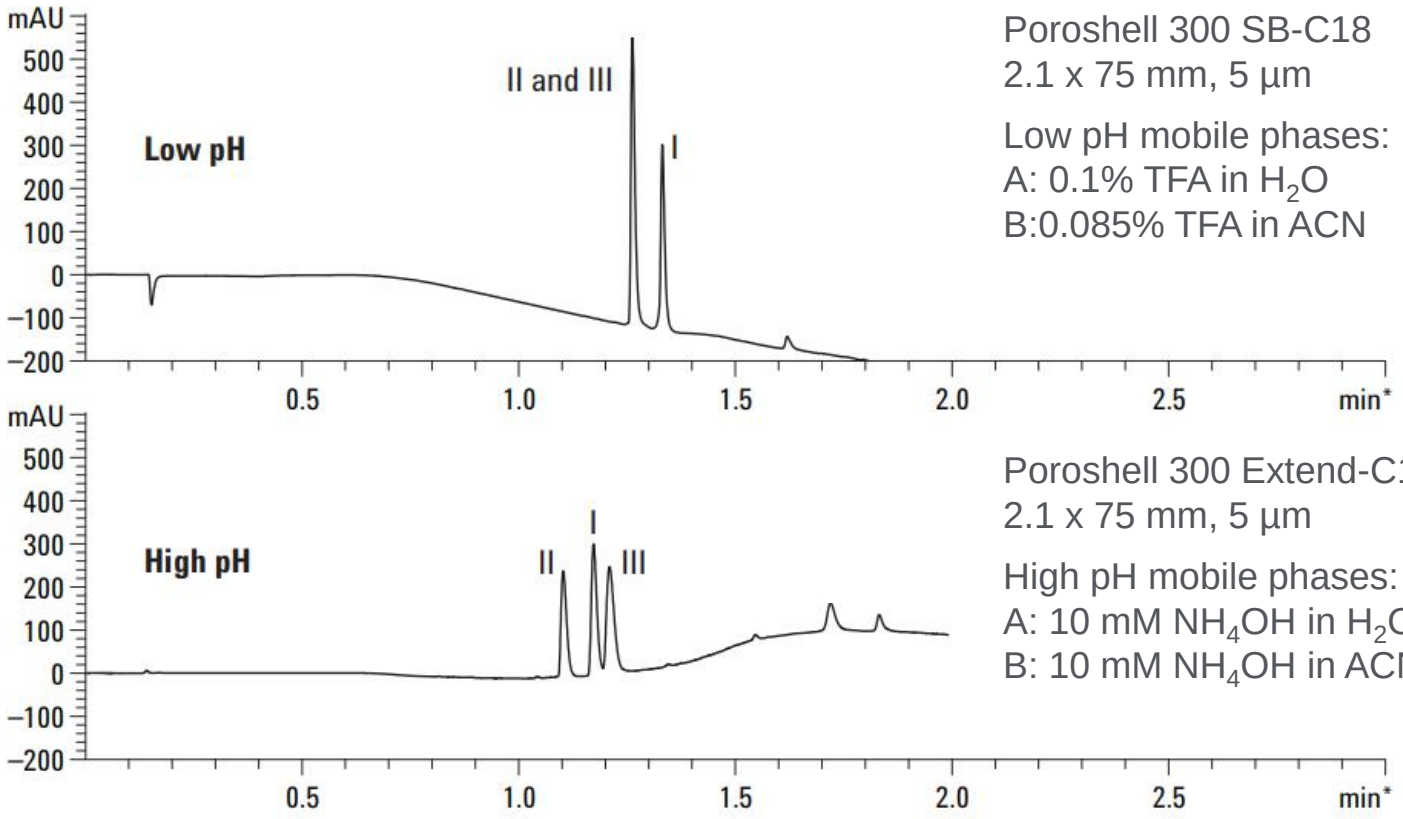
Flow Rate: 1.0 mL/min

Proteins

Mid and high pH low pH methods

- If an optimized, low pH method does not provide an ideal separation, then mid or high pH mobile phase can be used.
- At high pH, selectivity is often very different because acidic amino acids become negatively charged and some basic amino acids may lose their charge.

Separation of Angiotensin I, II and III at Low and High pH



Poroshell 300 SB-C18
2.1 x 75 mm, 5 µm

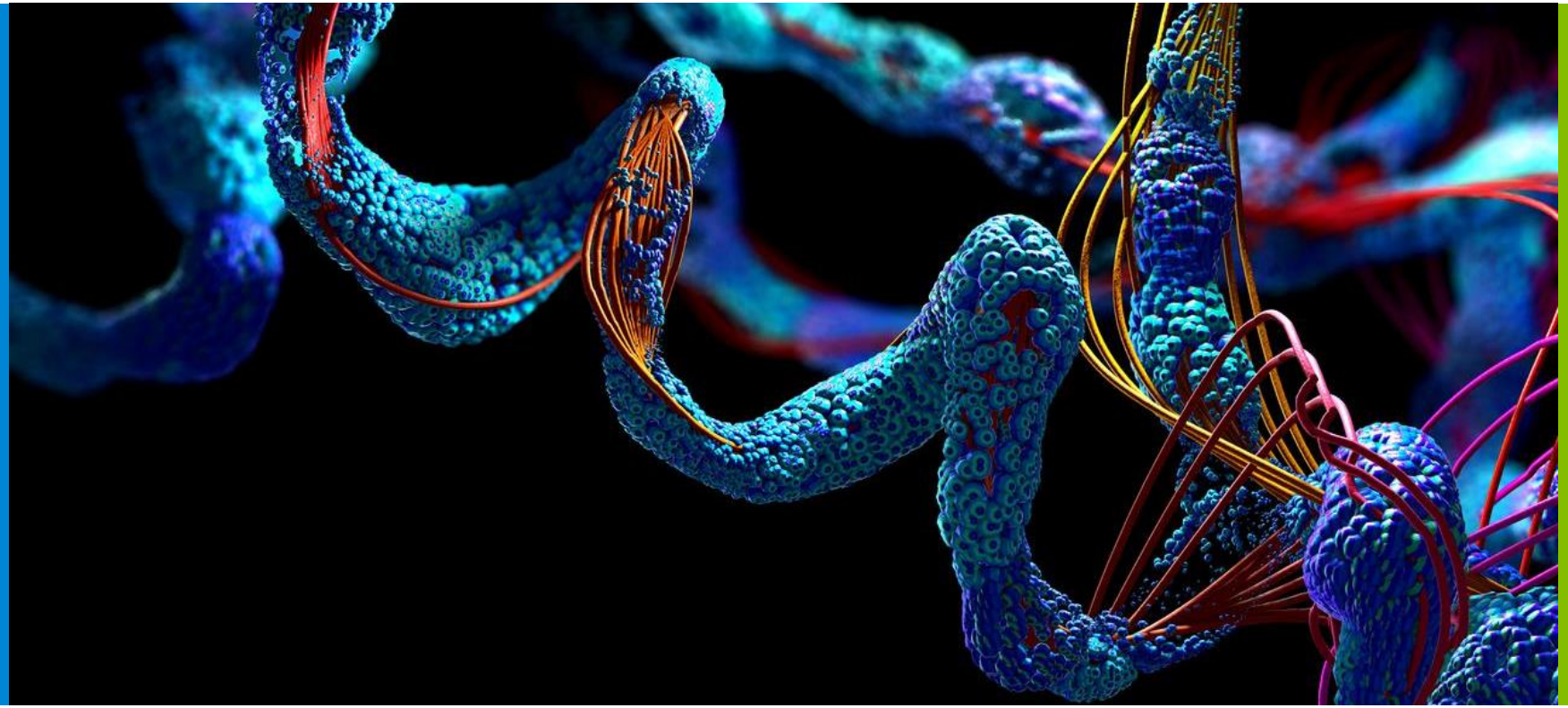
Low pH mobile phases:
A: 0.1% TFA in H_2O
B:0.085% TFA in ACN

Poroshell 300 Extend-C18
2.1 x 75 mm, 5 µm

High pH mobile phases:
A: 10 mM NH_4OH in H_2O
B: 10 mM NH_4OH in ACN

Bioseparations

Peptide mapping



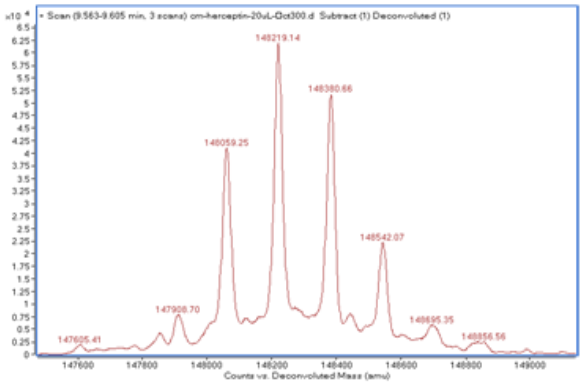
Peptide Mapping

CQAs

Peptide mapping:

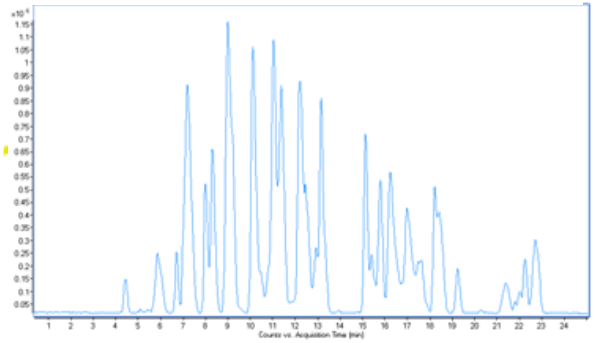
- Confirms primary structure & detects alterations in structure
- Demonstrates process consistency
- Stability studies
- Used to indentify multiple CQA

Intact mAb



Enzymatic Digestion

Digested Peptides



Peptide Mapping

Column selection

Instrument capabilities and requirements

- UV vs MS detection *or both*
- Pressure capabilities

Mobile phase requirements

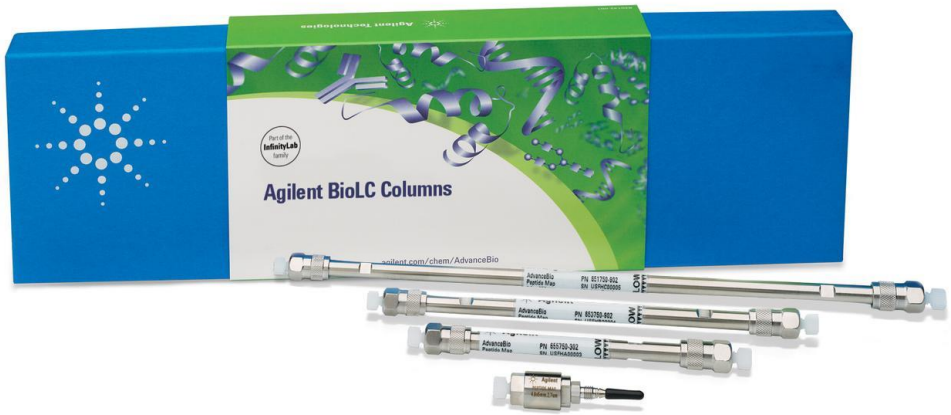
- High pH required for sample?
- TFA vs Formic acid

Sample

- Hydrophilic vs hydrophobic peptides
- Larger polypeptides present

Column dimensions

- Generally, prefer longer columns, especially for more complicated maps
  - 50, 150, and 250 mm lengths available
- Use 2.1 mm ID. for MS-sensitivity
  - 3.0 and 4.6 mm ID also available
- Smaller pore sizes with higher surface area are ideal, usually 100-150 Å

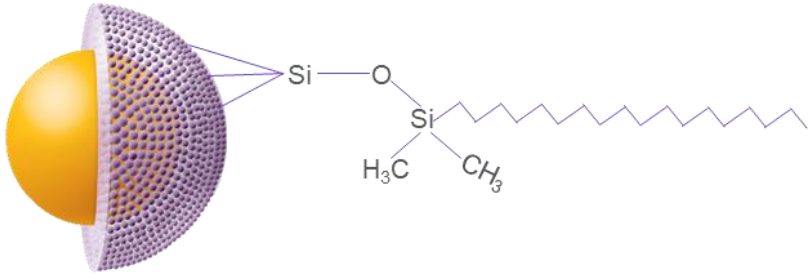


Peptide Mapping

Column Selection

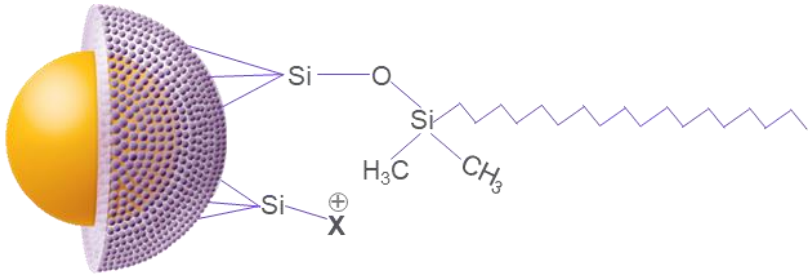
AdvanceBio Peptide Mapping

- Traditional C18 chemistry
- Good retention of small, hydrophilic peptides → most things retained longer
- Solid all-around option
- BioInert hardware option with AdvanceBio EC-C18 in PEEK-lined stainless steel



AdvanceBio Peptide Plus

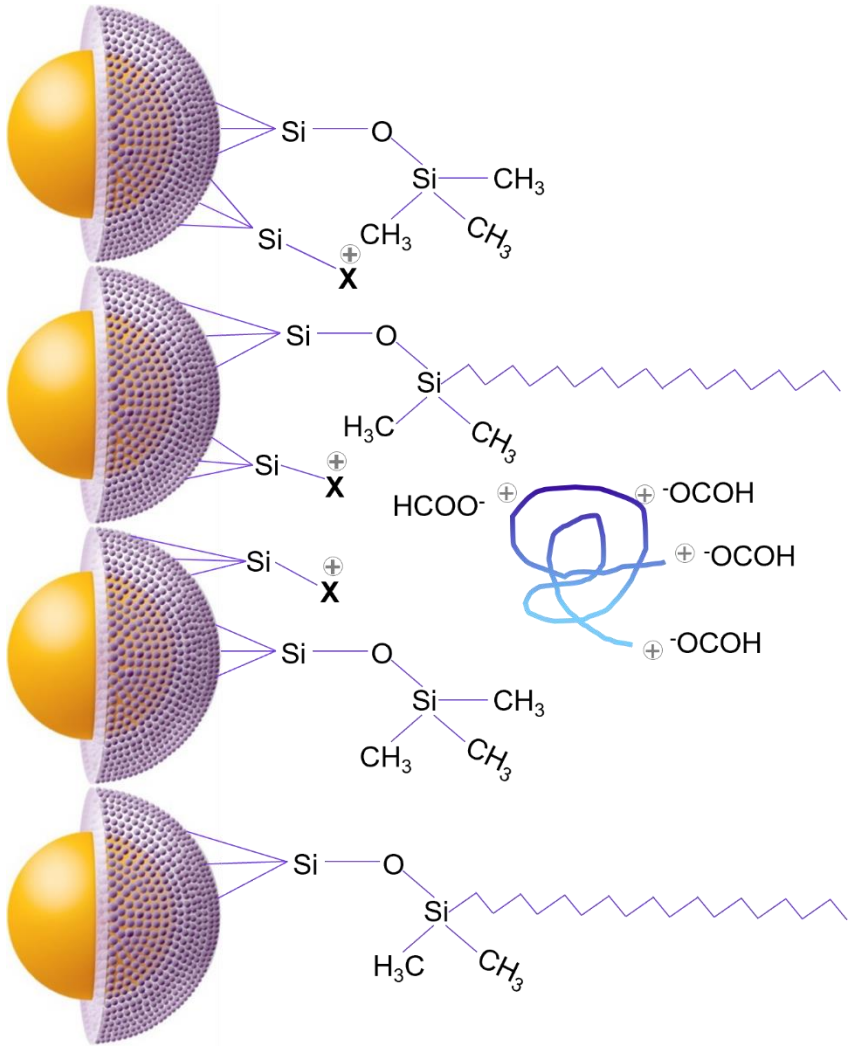
- Charged surface C18 chemistry
- Good elution of larger, hydrophobic peptides → most things elute earlier
- Alternate selectivity, especially for deamidated peptides
- Excellent peak shape with formic acid mobile phase and under high loading conditions
- Helps identify low abundance peptides, such as from host cell proteins or impurities



Peptide Mapping

Charged surface stationary phase

The small amount of positive charge enables formic acid as mobile phase and improves MS sensitivity



Agilent AdvanceBio Peptide Plus (CS C18)

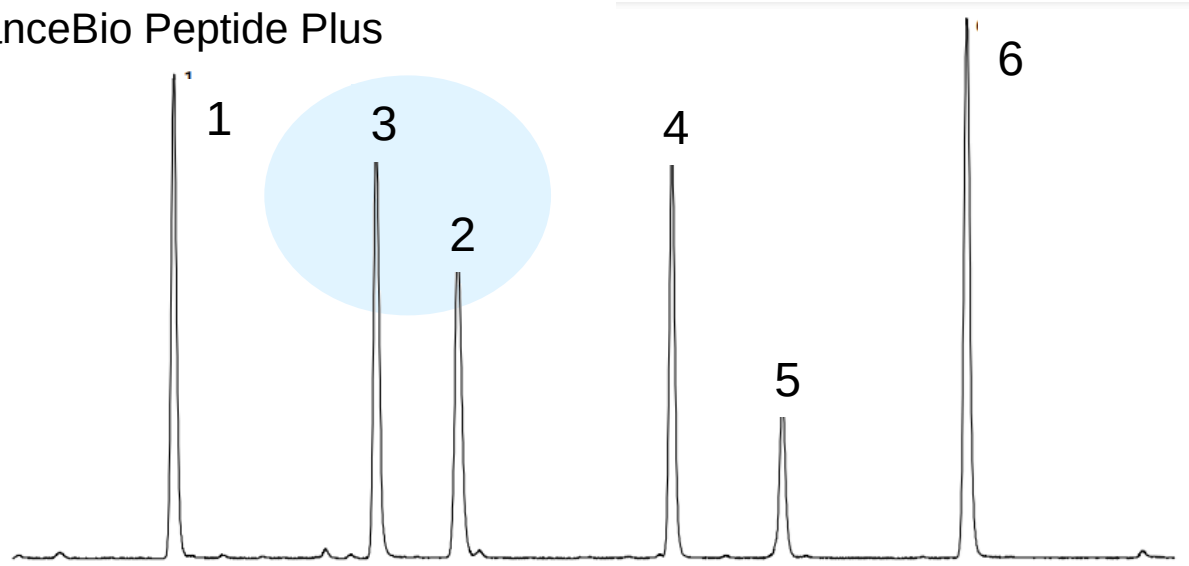
Peptide Mapping

Selectivity

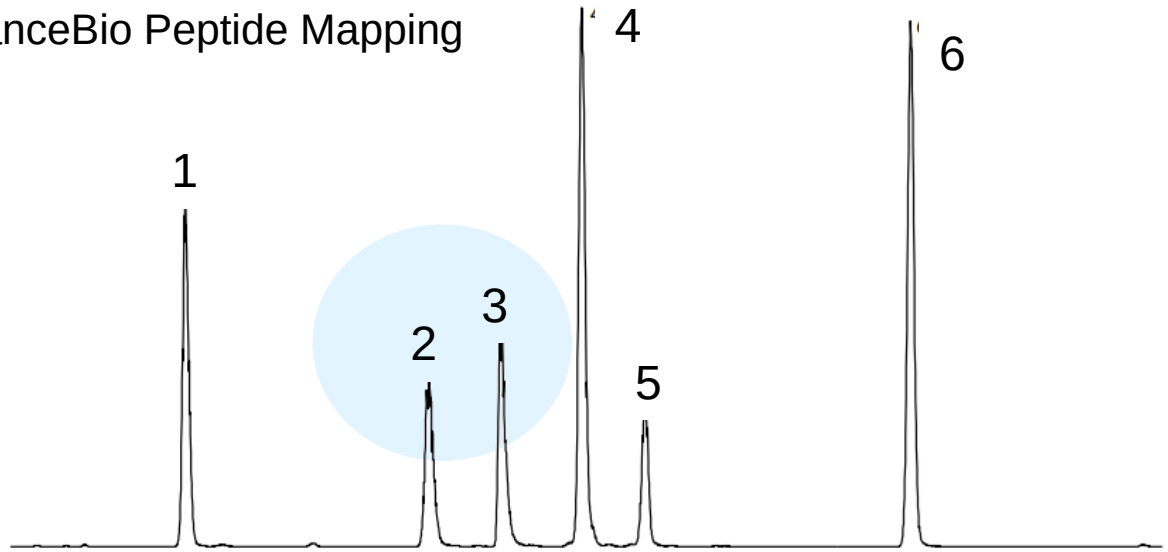
Peptides elute in different order (sometimes with better resolution too) due to different column chemistries.

Peak	Peptide Sequence
1	RPPGFSPFR
2	Glp-LYENKPRRPYIL
3	DRVYIHPFHL
4	GLILVGGYGTR
5	GILFVGSGVSGGEEGAR
6	LTILEELR

AdvanceBio Peptide Plus

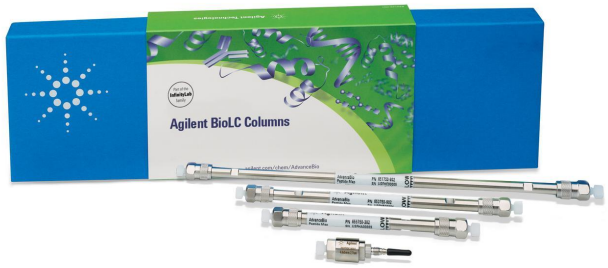


AdvanceBio Peptide Mapping



Peptide Mapping

Starting conditions

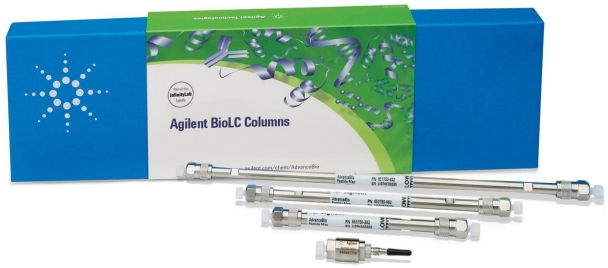


Starting Conditions

Mobile Phases	A: 0.1% TFA in H_2O B: 0.85% TFA in ACN	• Low pH suppresses ionization of silanols to reduce peak tailing • Helps denature peptide fragments, improving resolution • ACN allows for low UV detection
Gradient	0-60% B in 45 minutes	• Most peptides elute with less than 60% ACN
Temperature	30-50 ℃	• A slightly elevated temperature improves mobile phase viscosity

Peptide Mapping

Method Optimization



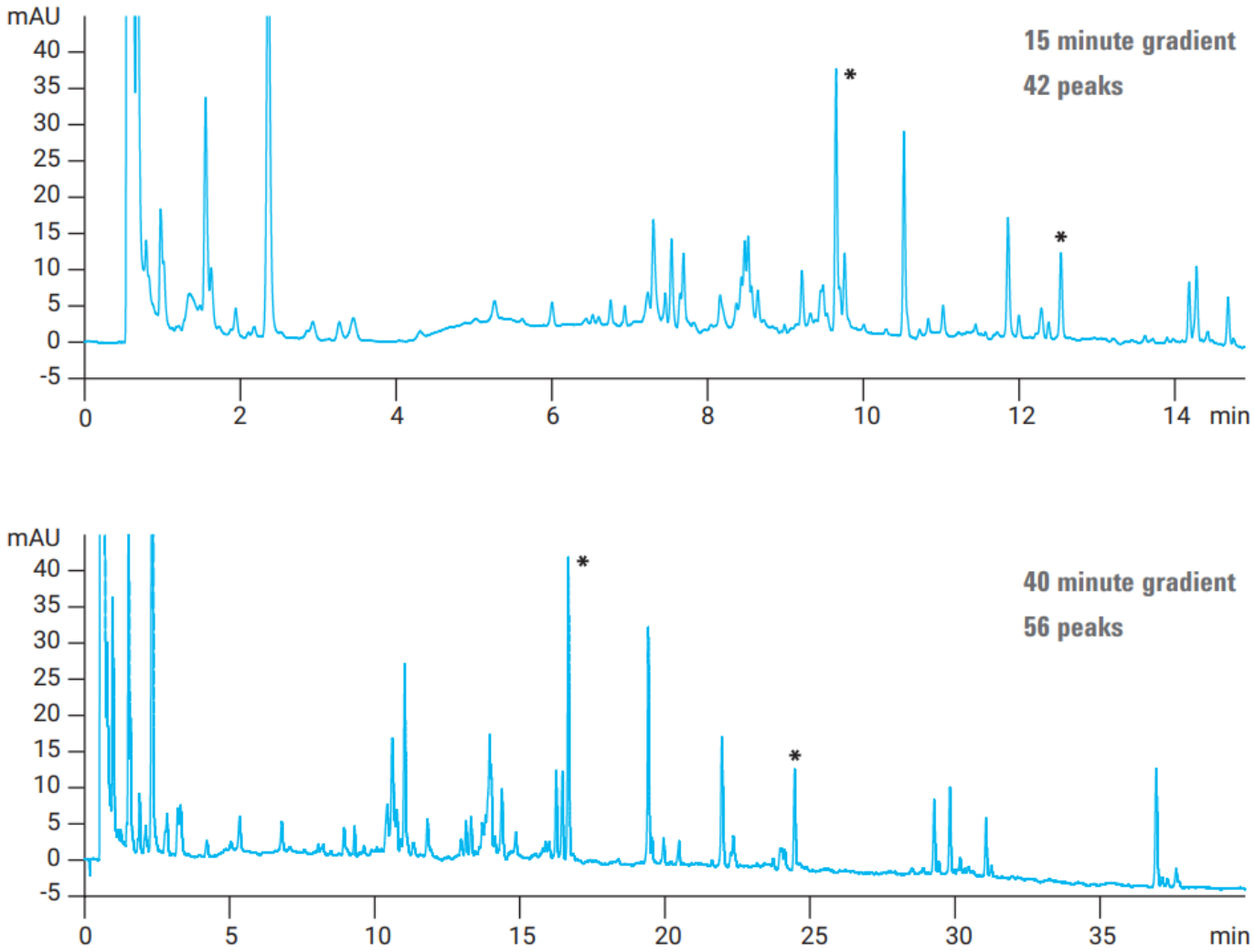
Method Optimization

Temperature	• Increase temperature → narrower peaks • Some hydrophobic peptides may need temperatures 60-80 ℃ for maximum recovery
Gradient Steepness	• A steeper gradient can shorten method • A shallower gradient improves resolution
Flow Rate	• Reducing flow rate can improve resolution • Increasing flow rate can shorten a long method
Column Length	• Longer columns can increase resolution

Peptide Mapping

Method optimization

Gradient Steepness



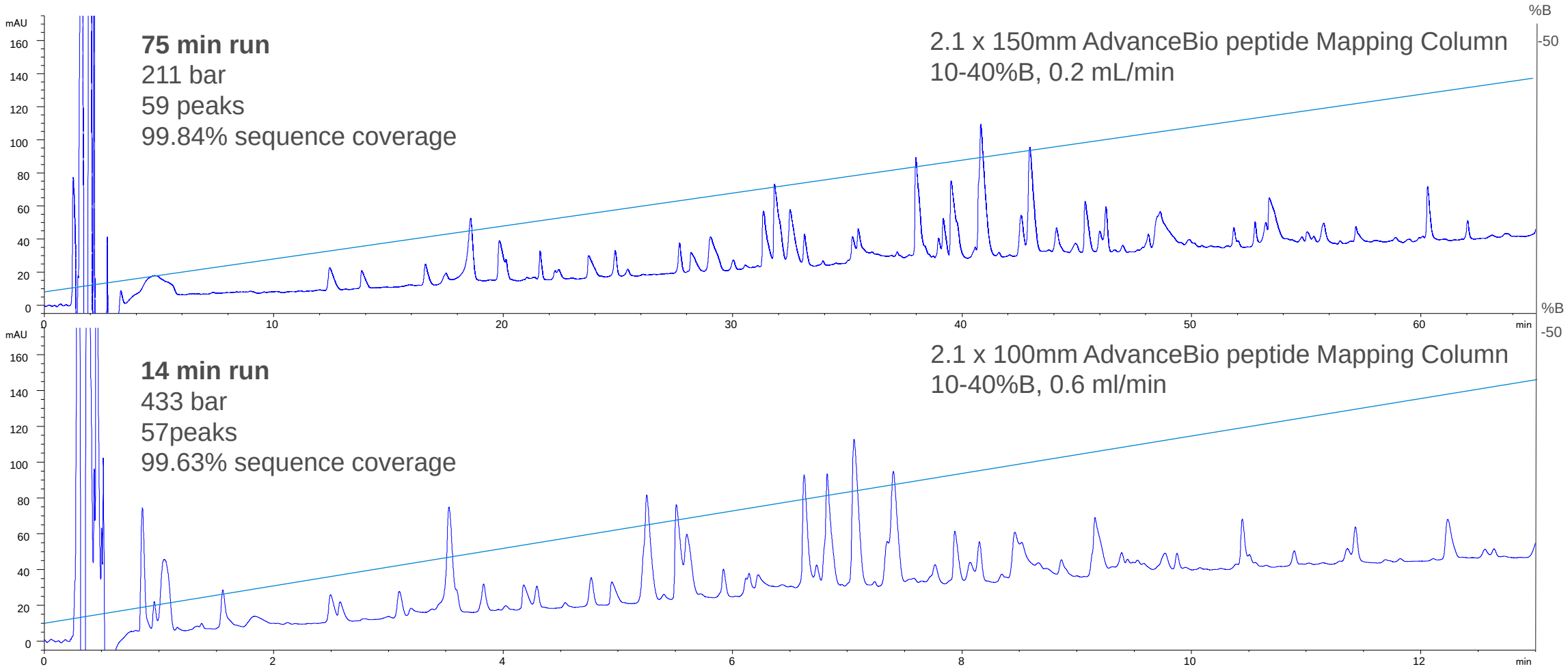
Myoglobin tryptic digest

Column: AB Peptide Mapping, 2.1 x 150 mm

Peptide Mapping

Method optimization

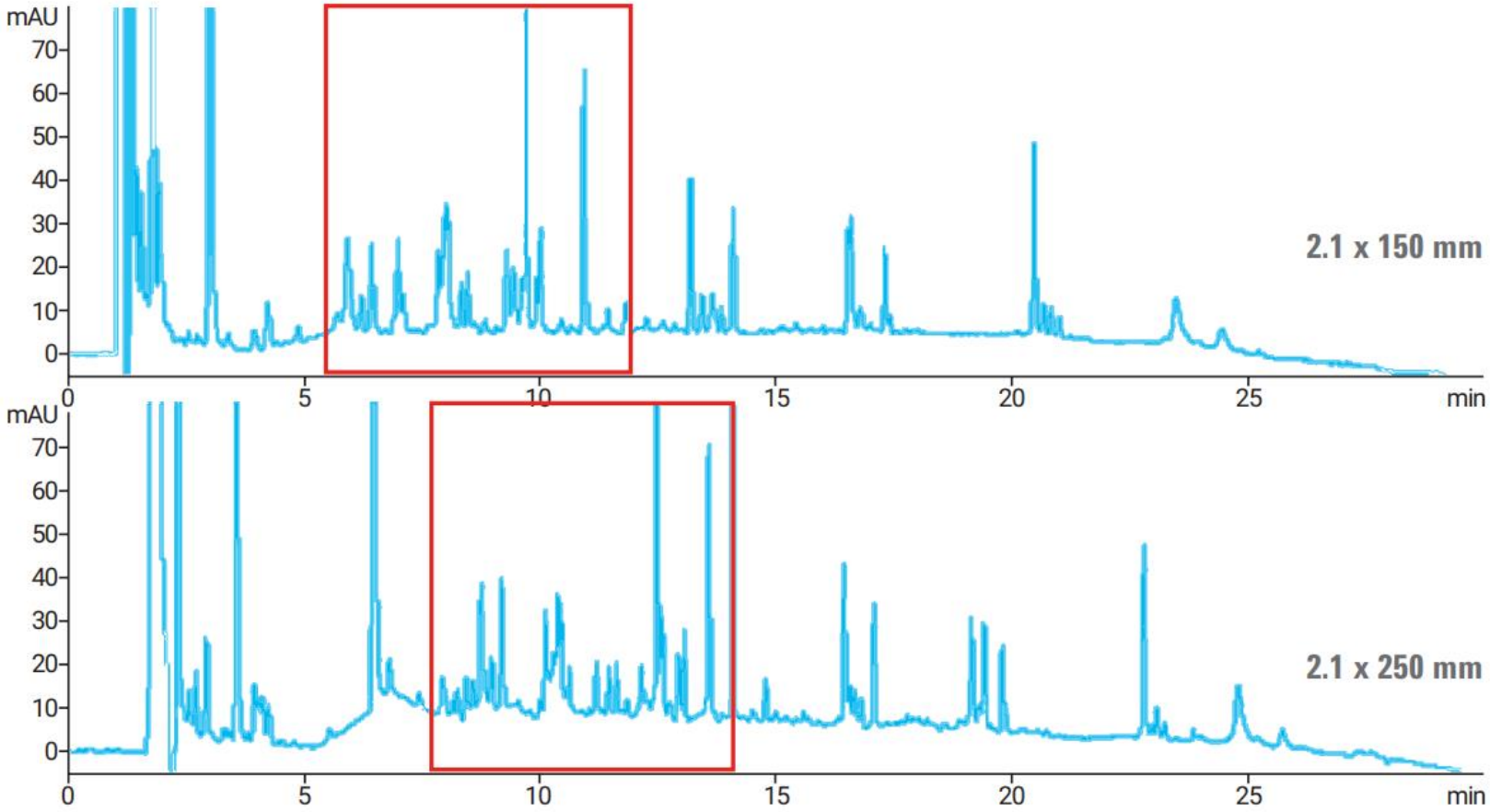
Flowrate



Peptide Mapping

Method optimization

Column Length

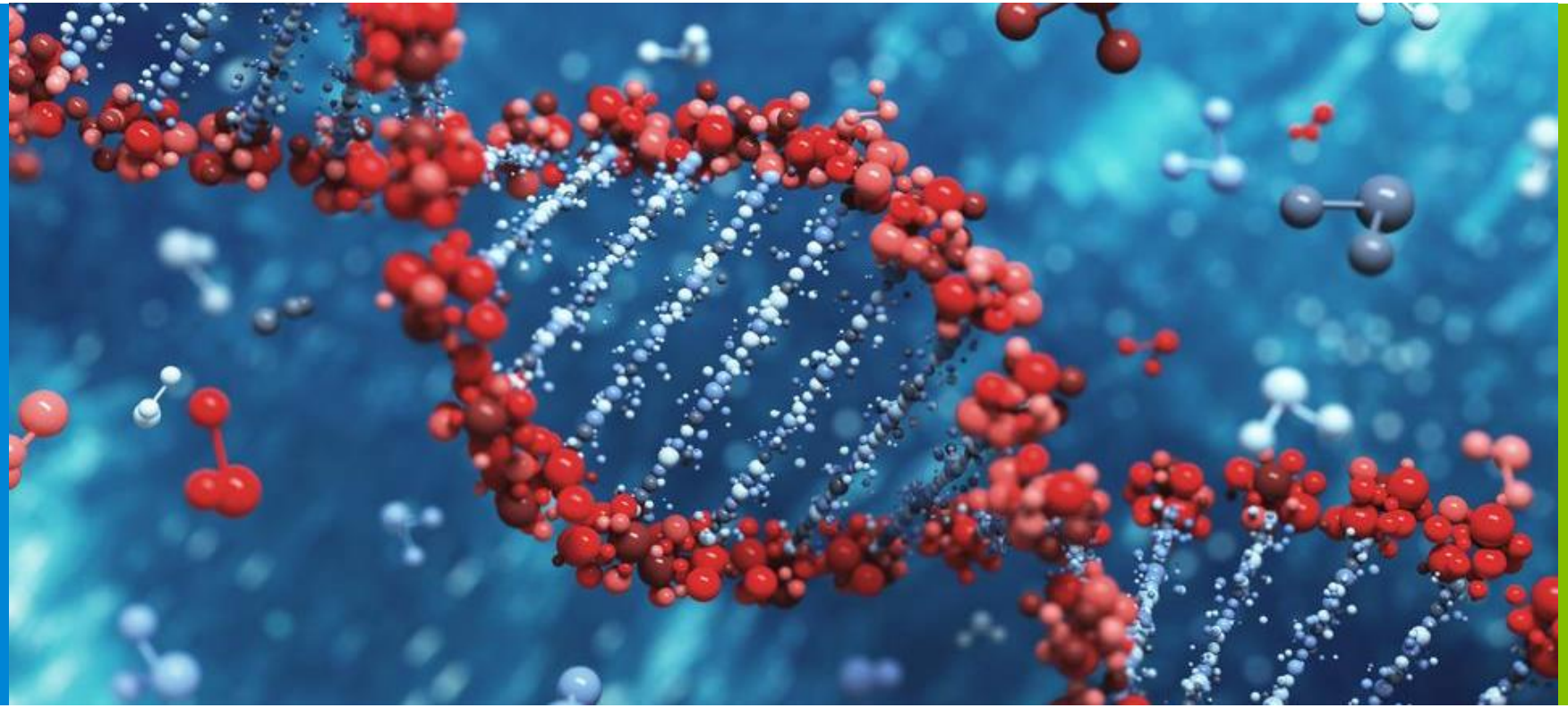


Myoglobin tryptic digest

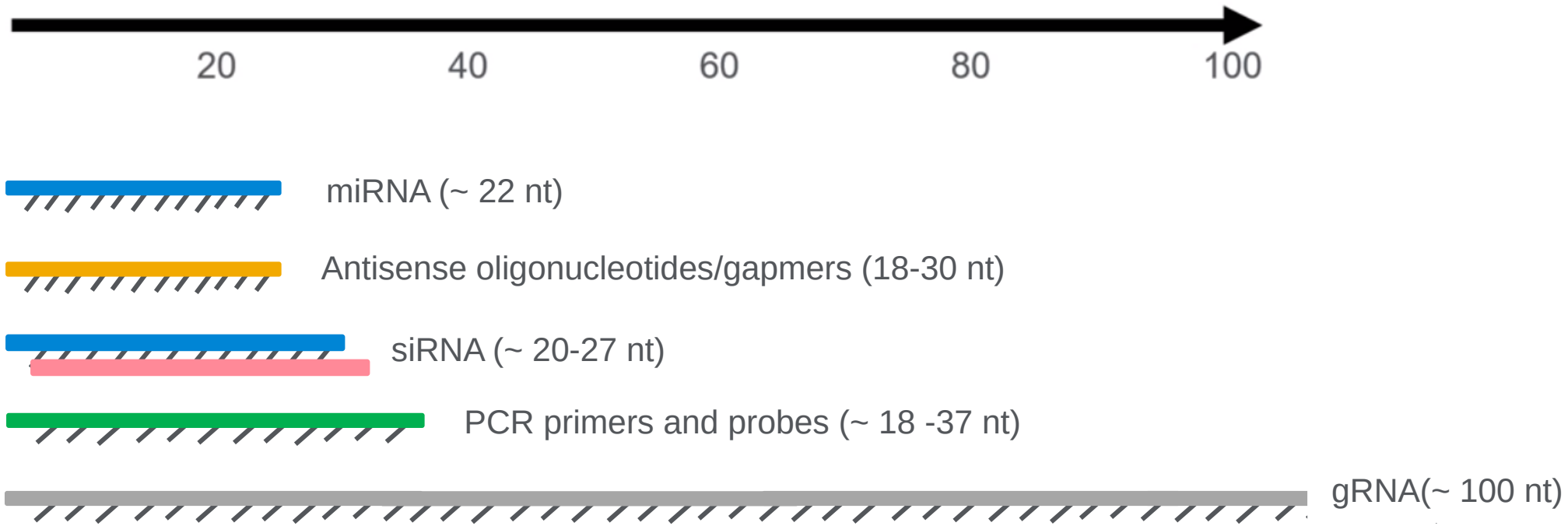
Column: AB Peptide Mapping,
2.1 x 150 mm &
2.1 x 250 mm

Bioseparations

Oligonucleotides



Oligonucleotides represent a diverse set of therapeutics

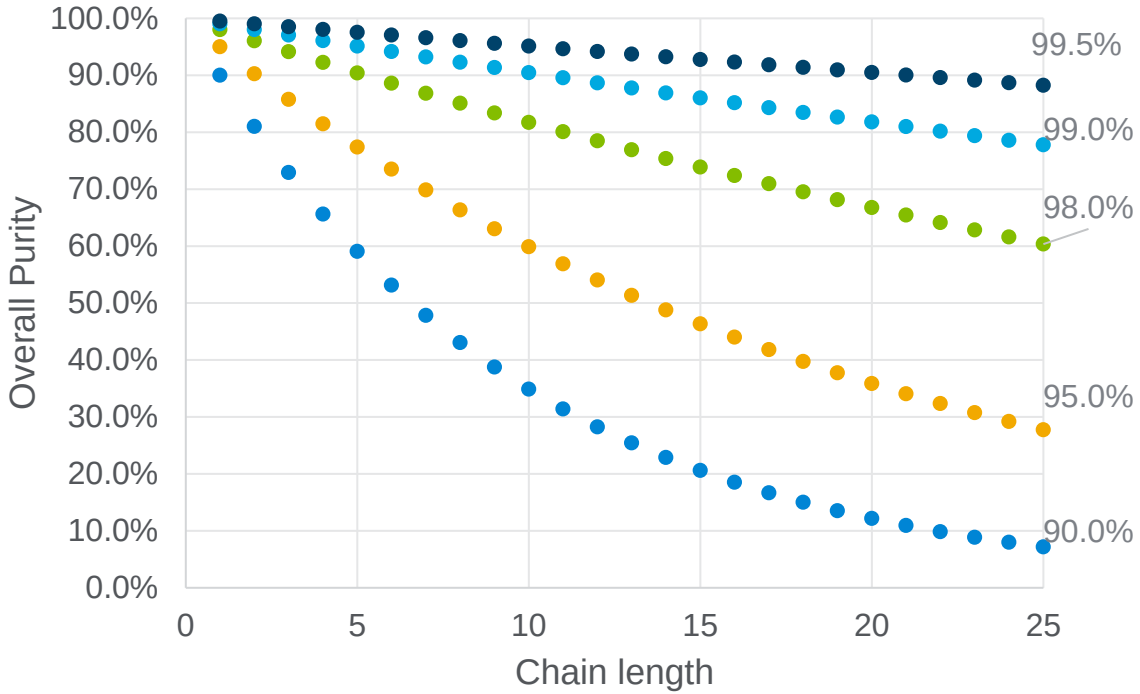


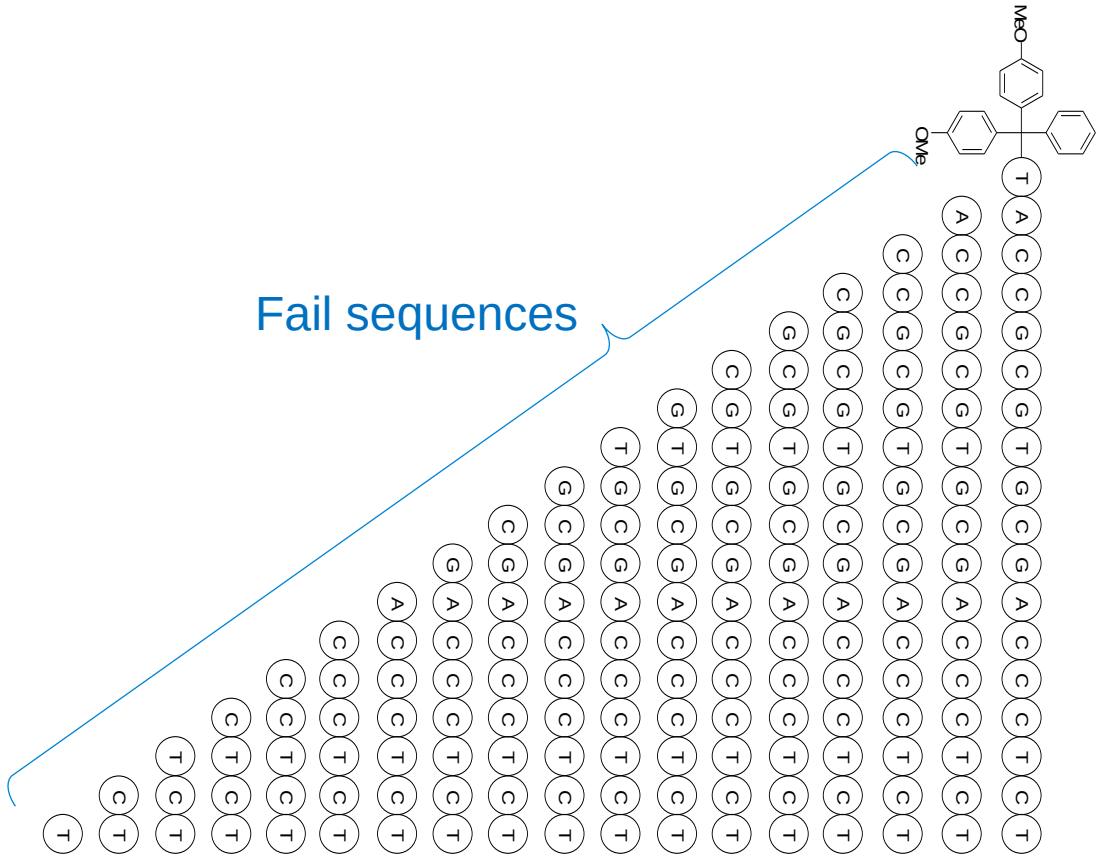
Oligonucleotides come in many shapes and sizes ! …

… the linear length of 1,000 nt mRNA would be ~ 300 nm.

Oligonucleotides

CQAs

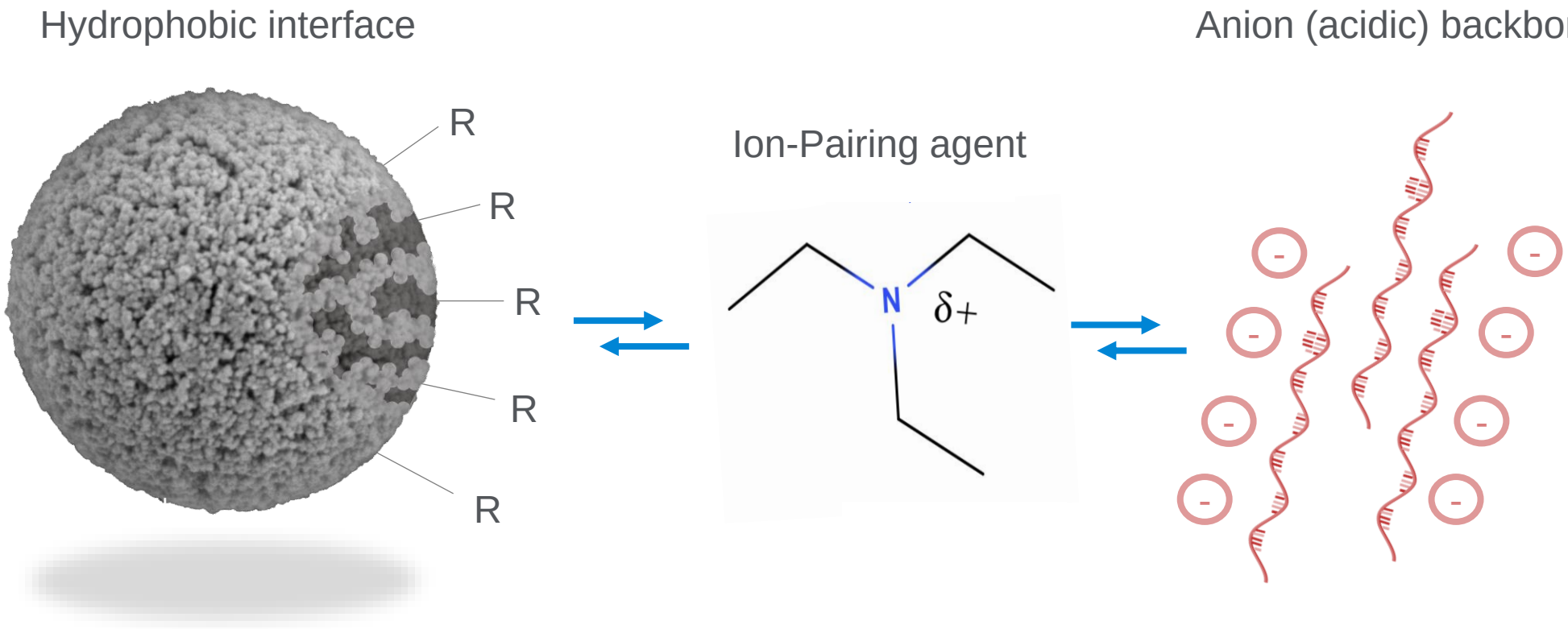




- As many oligos are synthesized, failed sequences in addition to synthesis-related impurities ranging from acetylation byproducts to depurination must be separated from the target sequence.
- Therapeutic oligos can range from short single or double strand oligos (13-25 nucleotides, or bases) to mRNA with thousands of nucleotides.

Oligonucleotides

Ion-paired reversed-phase (IP-RP) chromatography



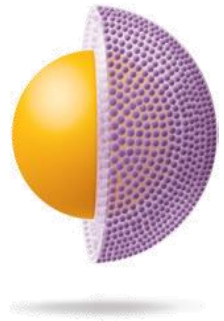
Anionic phosphate backbone has no interaction with the hydrophobic reverse phase media on its own. Introduction of alkylamine ion-pairing agent in the mobile phase facilitates the interaction and retention of oligos with the hydrophobic RP stationary phase.

Oligonucleotides

Ion-paired reversed-phase chromatography

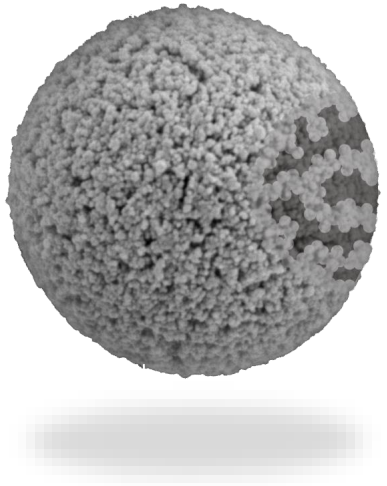
- Ion-paired reversed-phased (IP-RP) HPLC uses ion-pairing reagents to increase hydrophobicity of anionic oligonucleotides
- Method conditions tend to have neutral mobile phases and an elevated temperatures

AdvanceBio Oligonucleotide
2.7 µm, Pore Size 120 Å



pH range: 3-11
Maximum Temp: 65 ℃

PLRP-S 1000Å
5.0 µm, Pore Size 1000Å



pH range: 1-14
Maximum Temp: 200 ℃

Other particle sizes:	10µm, 15-20µm, 30µm
Other pore sizes:	100Å, 300Å, 4000Å

Oligonucleotides

Column selection

Base particle chemistry – polymer or silica?

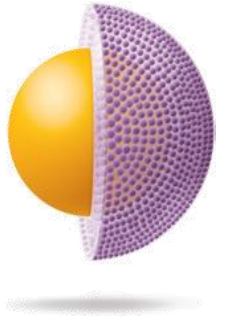
- PLRP-S is stable at high temperatures and pressures
- AB Oligonucleotide stationary phase has proprietary endcapping to increase pH stability

Pore size – anywhere from 100-4000Å!

- Pores should be ≥ 3x the hydrodynamic radius of the oligo

Particle size – < 2µm to 5µm or higher

- Smaller particles give high resolution, but also higher back pressure
- Larger particle sizes can increase binding capacity for particles with large pores



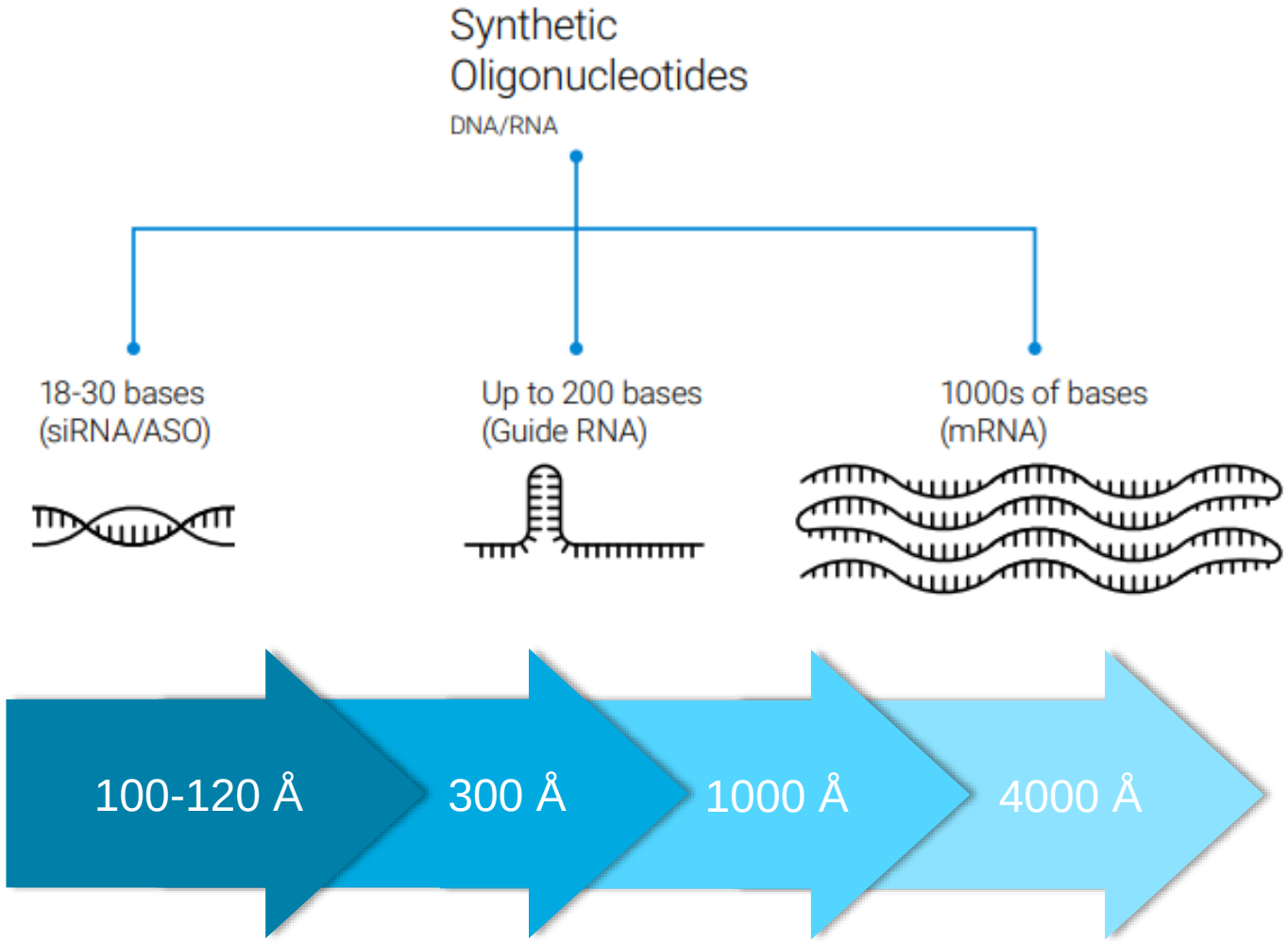
AdvanceBio Oligonucleotide
2.7 µm, Pore Size 120 Å

PLRP-S 1000Å
5.0 µm, Pore Size 1000Å

Oligonucleotides

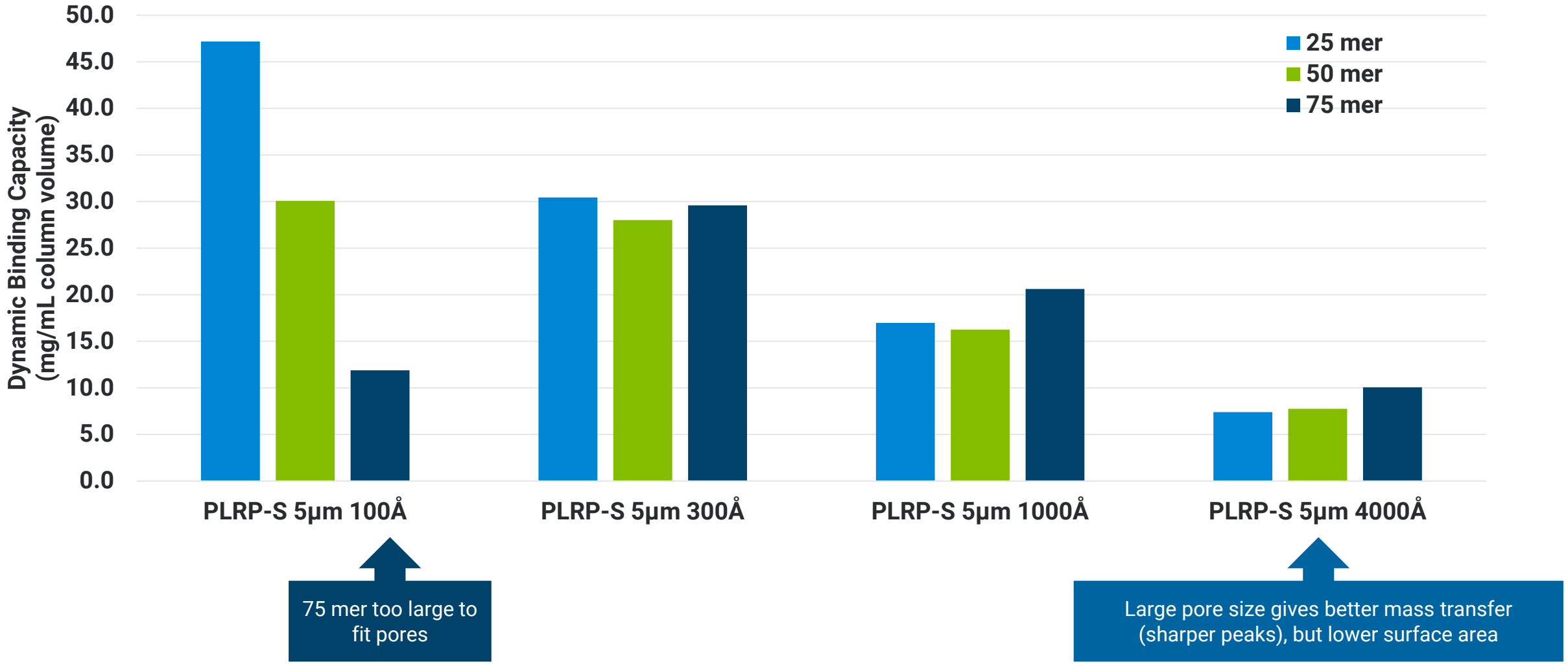
Pore size

- Purification requires determination of the optimal pore size to ensure effective mass transfer of an oligonucleotide in solution.
- Determining the balance between pore size and binding capacity is important for ensuring the highest resolution and yield.



Oligonucleotide

Effect of pore size on oligonucleotide binding capacity



Oligonucleotides

Starting conditions

Starting Conditions	
Mobile Phases (UV)	A: 100 mM TEAA in H_2O B: ACN
Mobile Phases (MS)	A: 15 mM TEA & 400 mM HFIP in H_2O B: Methanol
Gradient	0 to 1 min, 10% B 1 to 10 min, 10 to 40% B 10 to 11 min, 40 to 95% B
Temperature	60 ℃

Oligonucleotides

Method optimization

Method Optimization

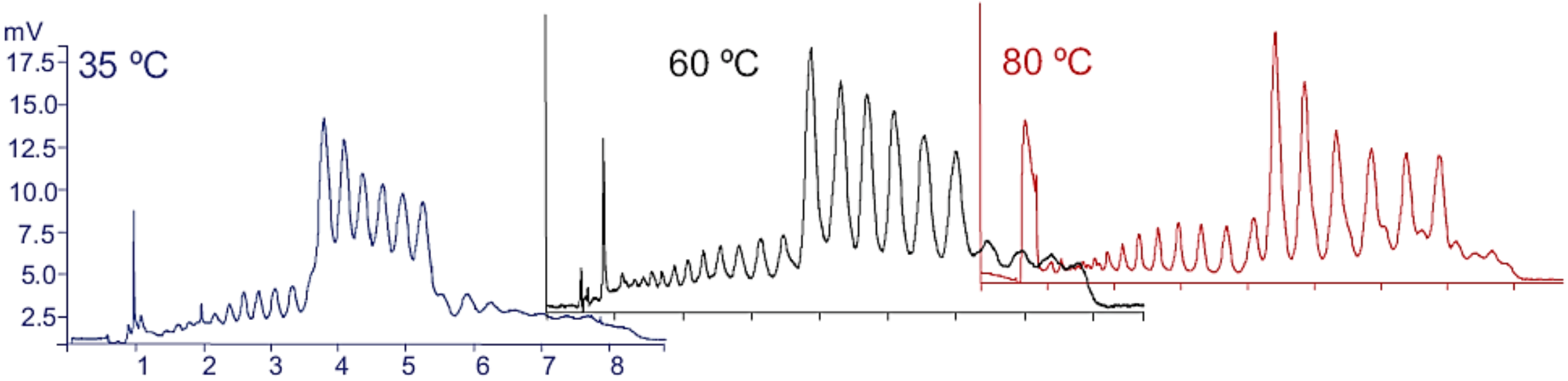
Temperature	• Increase temperature reduces secondary oligo structures • Reduces mobile phase viscosity and secondary column interactions→improved peak shape
Gradient Steepness	• A steeper gradient can shorten method • A shallower gradient improves resolution
Ion-Pairing Reagent	• Amines with longer alkyl chains can increase oligo hydrophobicity and increase retention • Increasing the concentration of ion-pairing buffer can increase retention
Column Length	• Longer columns can increase resolution

Oligonucleotides

Method optimization

Temperature

Sharper peaks are obtained by running at a higher temperature to denature the oligos



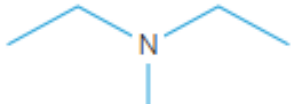
Poly dT 19-24 ladder,
PLRP-S 50 x 4.6mm, 3um, 100A
5%B per minute over 6 mins.
A: 100mM TEAA
B: 100mM TEAA 75:25 Water: ACN

Oligonucleotides

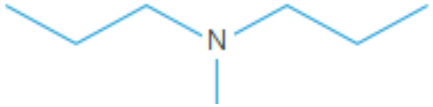
Method optimization

Ion Pairing Reagent

Triethylamine (TEA)

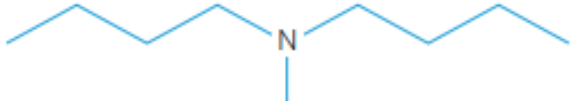


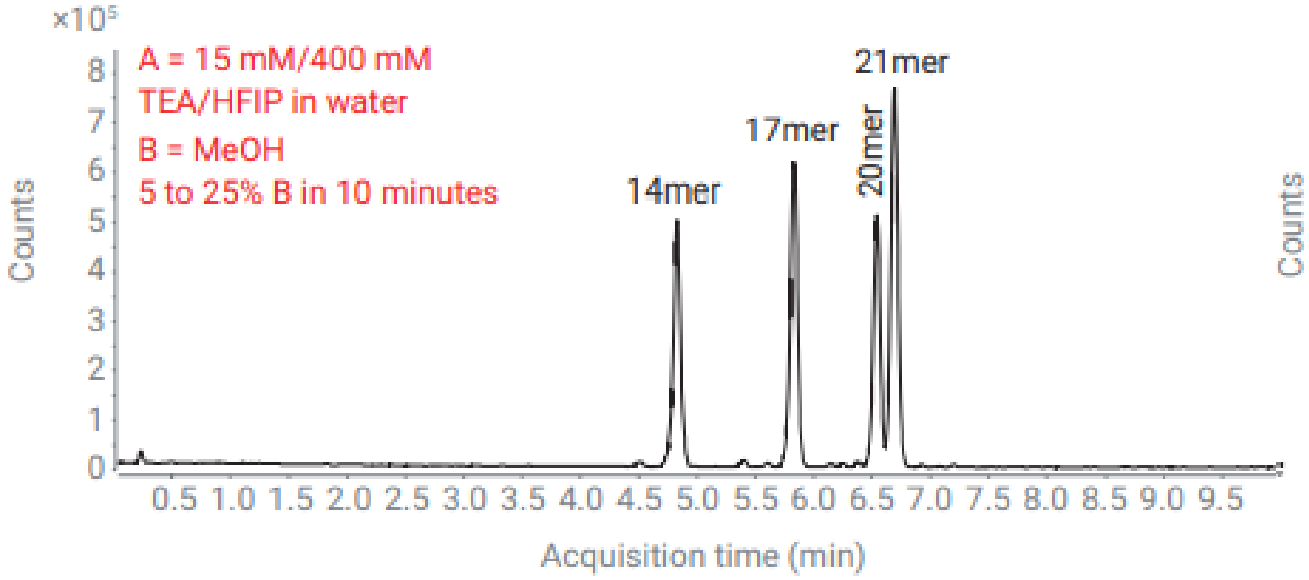
Tripropylamine (TPA)

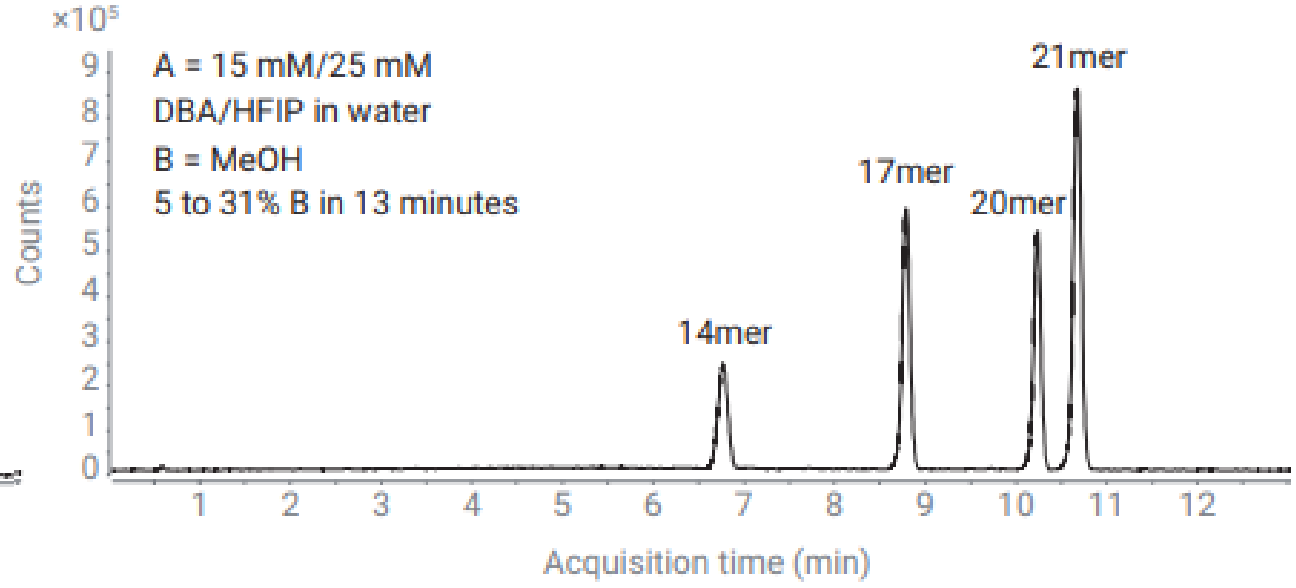


Hexylamine (HA)

Dibutylamine (DBA)







Thank you!

Biocolumn Resources

Biocolumn Catalog: Agilent BioHPLC Columns and Consumables – Your Resource for Biomolecule Analysis

Biocolumn CQA Application Compendiums:

- Agilent Biocolumns Application Compendium - Critical Quality Attributes
- Agilent Biocolumns Application Compendium - Intact and Subunit Purity
- Agilent-NISTmAb_Application-Compendium-Intact Analysis
- Agilent-NISTmAb_Application-Compendium-Variant-Analysis
- Agilent Biocolumns Application Compendium - Aggregate/Fragment Analysis
- Agilent Biocolumns Application Compendium - Titer Determination

Biocolumn User Guides: Bio LC Column User Guides | Agilent

Agilent Resources for Support

- Resource page http://www.agilent.com/chem/agilentresources
  - Quick reference guides, product catalogs
  - Online selection tools, “How-to” videos
  - Column user guides - https://www.agilent.com/en-us/support/liquid-chromatography/kb005965
- Tech support: http://www.agilent.com/chem/techsupport
- InfinityLab LC Supplies catalog (5991-8031EN)
- Agilent University http://www.agilent.com/crosslab/university
- YouTube – Agilent Channel
- Your local product specialists
- Subscribe to Agilent Peak Tales podcasts at peaktales.libsyn.com

Contact Agilent Chemistries and Supplies Technical Support

Available in the USA and Canada 8-5 all time zones

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

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

Questions?

Agilent
Trusted Answers

Agilent

Trusted Answers