HALO®

Protein Separations Using Large Pore Superficially Porous Particles:

Method Development Strategy for Reversed-Phase LC

Benjamin Libert
Associate Scientist, R&D
Advanced Materials Technology, Inc.
Wilmington, Delaware, USA
blibert@advanced-materials-tech.com

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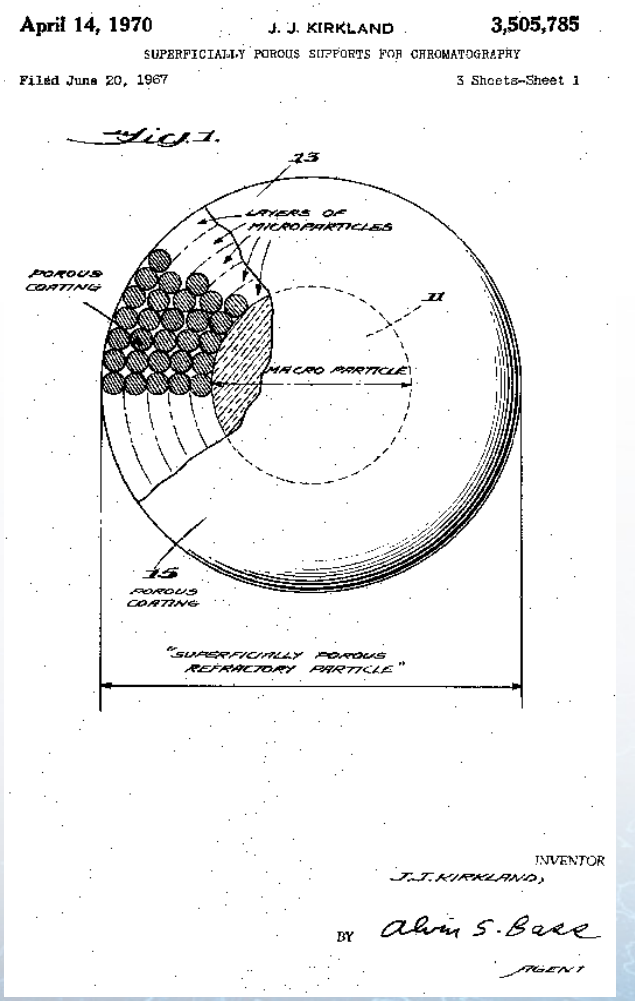
The Early Days -Conceptual


April 14, 1970 J. J. KIRKLAND 3,505,785

SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY

Filed June 20, 1967 3 Sheets-Sheet 1

Fig. 1.

INVENTOR
J. J. KIRKLAND,
BY Alvin S. Barr
AGENT

3,505,785

SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY

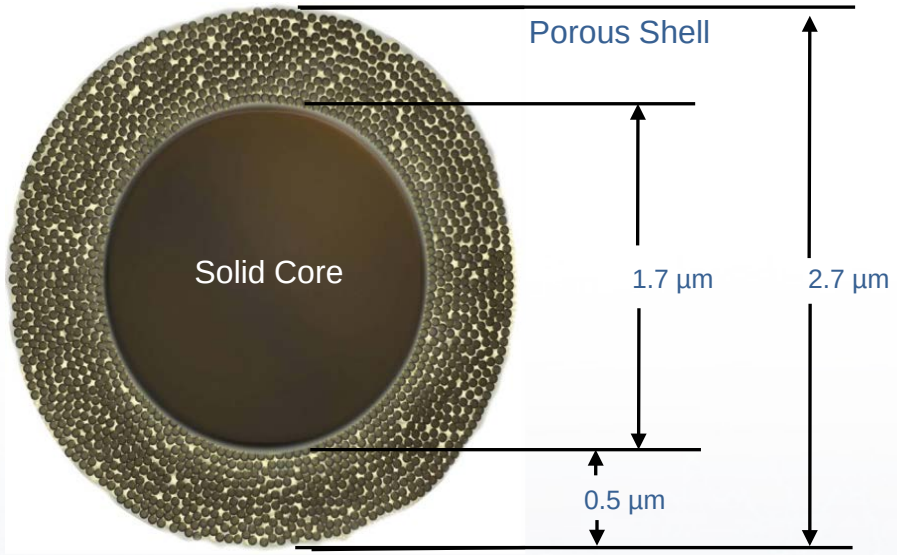
Joseph J. Kirkland, Wilmington, Del., assignor to E. I. du Pont de Nemours and Company, Wilmington, Del., a corporation of Delaware

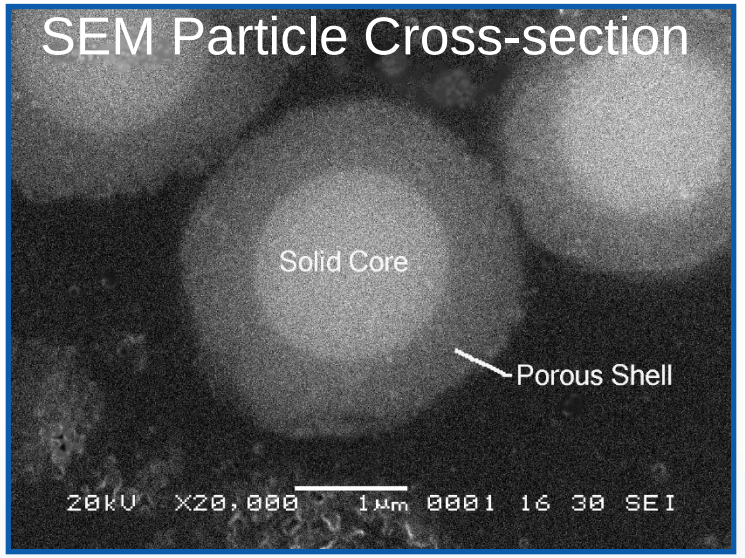
Filed June 20, 1967, Ser. No. 647,506
Int. Cl. B01d *15/08*
U.S. Cl. 55—67 8 Claims

ABSTRACT OF THE DISCLOSURE

This invention relates to an improvement in chromatography and chromatographic columns. A novel packing of superficially porous refractory particles for use in chromatography has been prepared consisting of a plurality of discrete macroparticles with impervious cores and having irreversibly joined thereto a coating of a series of sequentially adsorbed like monolayers of like colloidal inorganic microparticles. The coating is characterized by being uniform and of predetermined thickness. In preferred embodiments, the cores would be ceramics, preferably glass spheres, and the coating would consist of monolayers of colloidal refractory particles, preferably silica, in a structure of predetermined thickness and porosity.

Superficially Porous Particles (SPP-90 Å): 2006

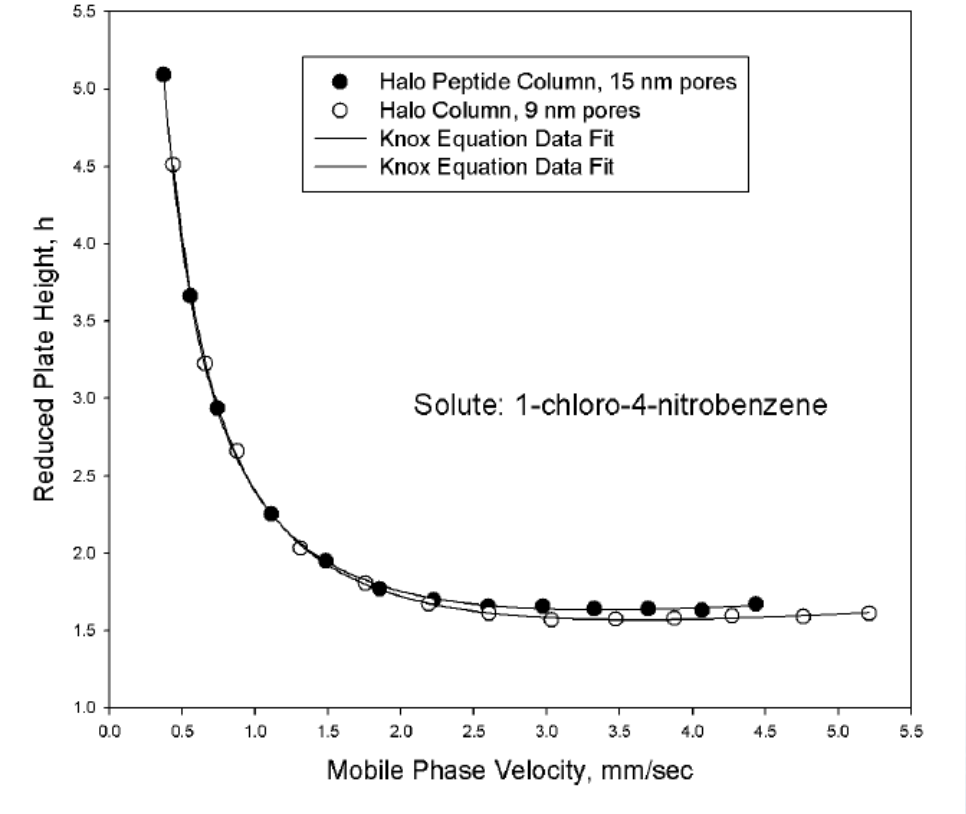




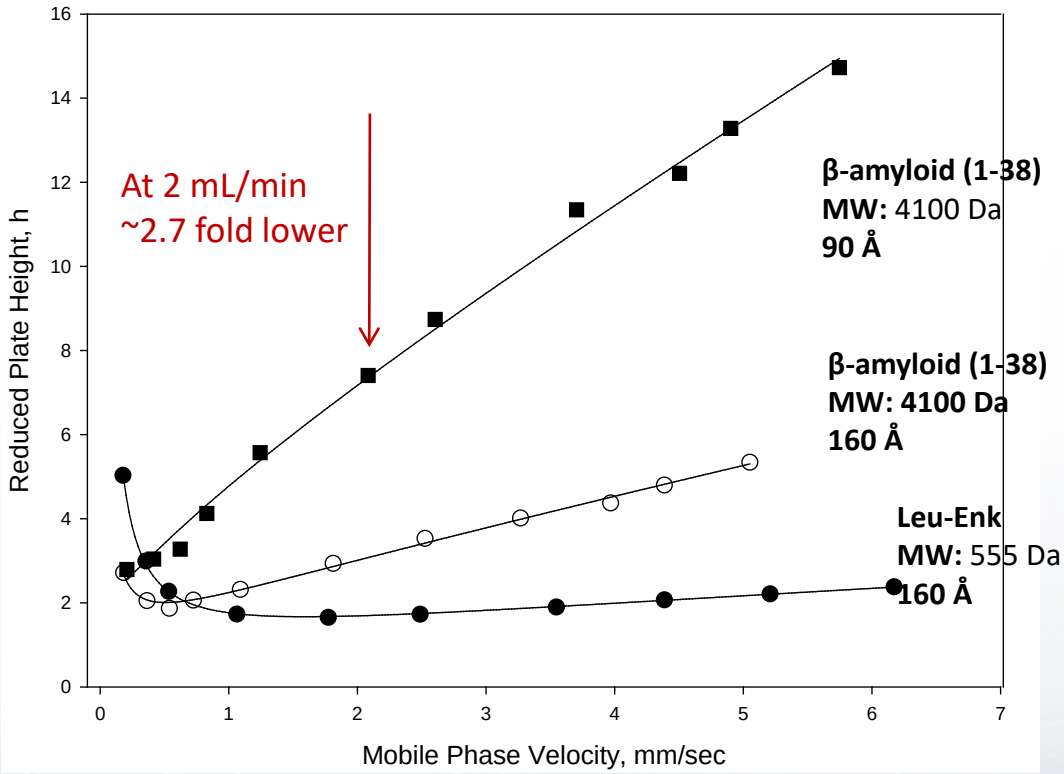
- Low back pressure due to the particle design (solid core with a porous shell)
- No need for specialized HPLC equipment
- Not necessary to filter samples and mobile phase since frits are not as small as needed for sub-2-µm
- High resolution is maintained at high flow rates (flat C-term in van Deemter plot)

J.J. Kirkland, T. Langlois, J. DeStefano, Fused core particles for HPLC columns, Am. Lab. 39 (2007) 18–21.

HALO Peptide Column Efficiency



Columns: 4.6 x 100 mm; Particle size: 2.7 µm
Mobile Phase: 50% ACN/50% water/0.1% TFA



Columns: 4.6 x 100 mm; Particle size: 2.7 µm;
Mobile Phase: Leu-Enk: 21% ACN/79% Water/0.1% TFA
ß-amyloid (1-38) 160 Å : 29% ACN/71% Water/0.1% TFA
ß-amyloid (1-38) 90 Å : 27% ACN/73% Water/0.1% TFA

S.A. Schuster, B.M. Wagner, B.E. Boyes, Kirkland, J.J Wider pore superficially porous particles for peptide separations by HPLC, J. Chromatogr. Sci. 48 (2010) 566–571.

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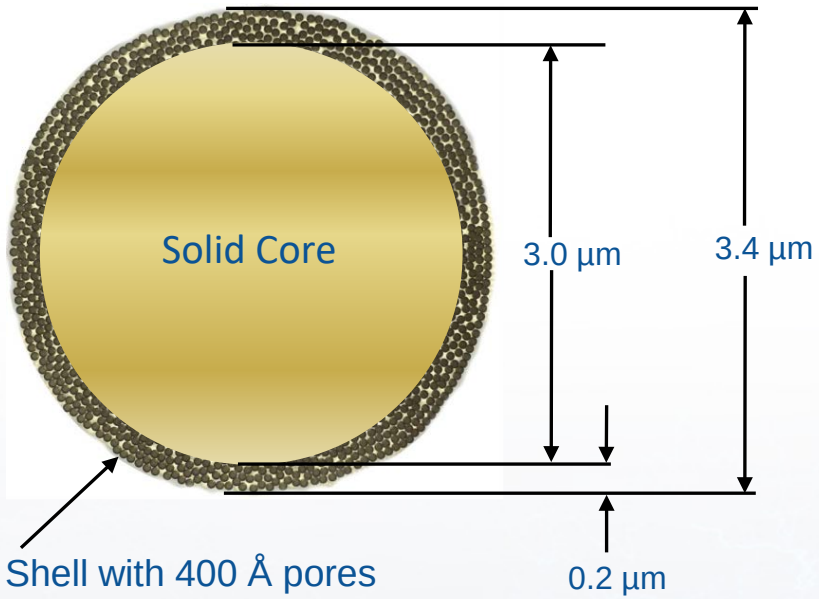
Wide Pore SPP Can Fit the Needs for Protein Science

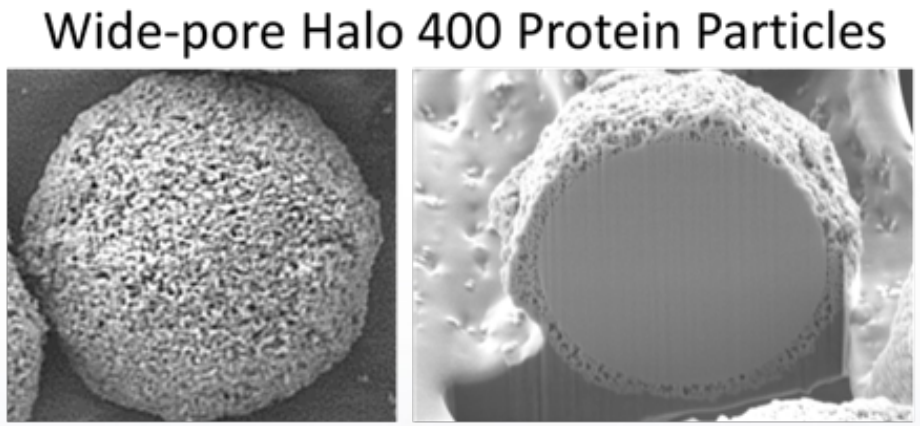
What is needed for high performance separations of larger (Bio) molecules?

- **Pore size must "fit" molecule size**
  - Restricted diffusion limits efficiency and load capacity
  - Peak capacity effects by kinetic and retention limitations
- **Particle morphology must optimize surface area/volume**
  - Shell thickness determines diffusion path and surface area
  - Must have "Right" size and desirable particle distribution
- **Surface chemistry appropriate to samples**

Very Large Pore SPP **Surface Chemistry Options**

Superficially Porous (Fused-Core®) Wide Pore Particles: 400 Å

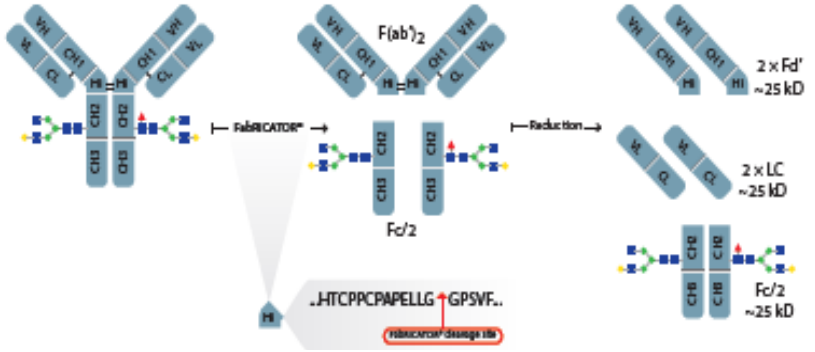




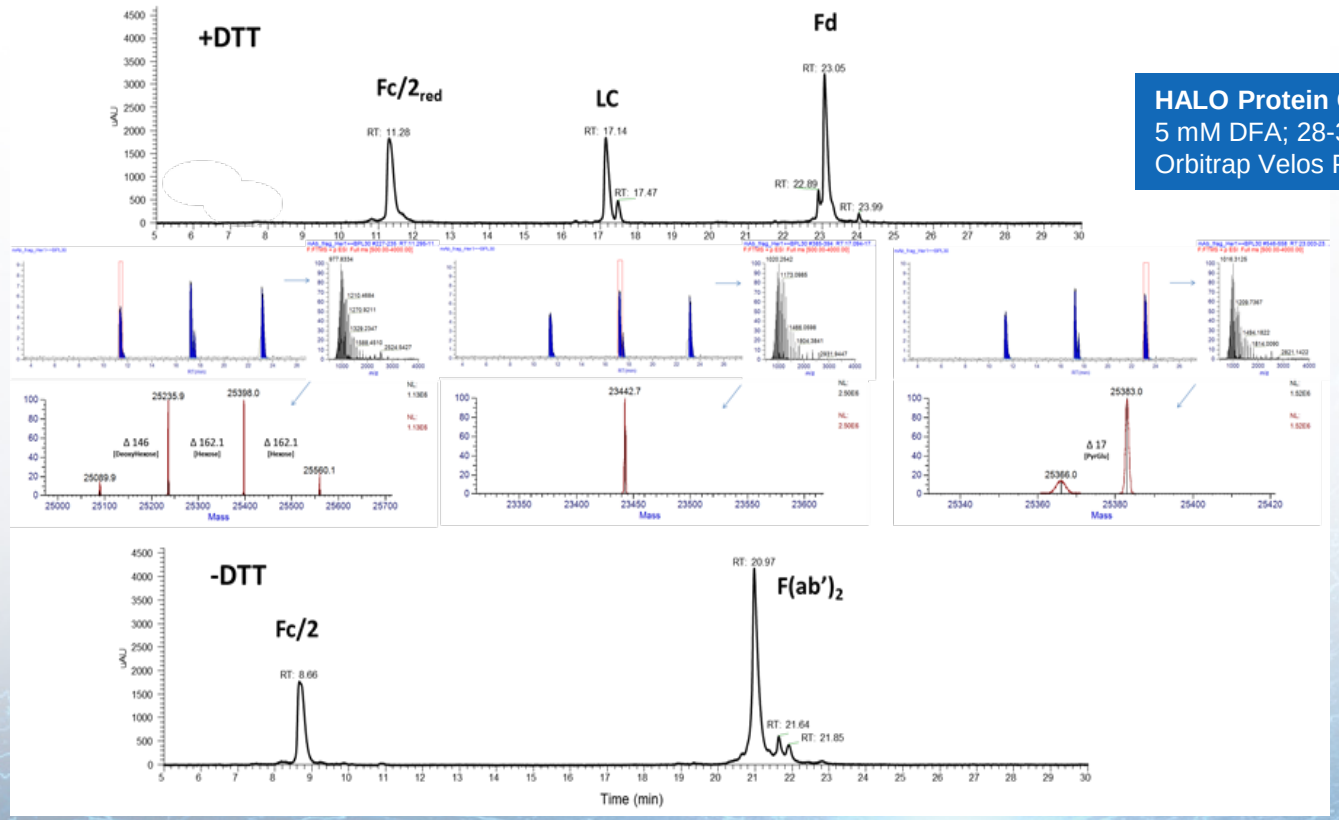
- Example above is 3.4 µm particle/400 Å pore size
- Many variations in shell thickness, pore size and particle size have been studied
- Theory to support “best properties” is complex, with limited tests using proteins, particularly with larger proteins
- Look for compromise in diffusion path for high MW molecules (to maintain small C-term), load tolerance, usability, speed and efficiency

S.A. Schuster, B.M. Wagner, B.E. Boyes, J.J. Kirkland, Optimized superficially porous particles for protein separations, J. Chromatogr. A 1315 (2013)118–126.

Fragments for mAb Structure: IdeS Digest



An, Zhang, Mueller, Shameem & Chen (2014) A new tool for monoclonal antibody analysis, mAbs, 6:4, 879-893, DOI: 10.4161/mabs.28762



HALO Protein C4 400 Å, 2.1 mm ID x 150 mm;
5 mM DFA; 28-38% AcN in 20 min; 0.35 mL/min, 80 ºC;
Orbitrap Velos Pro (30,000 Res) 500-4000 m/z, +3.8 kV, 275 ºC capillary

High Resolution Separations for Protein LC/MS. ASMS 2016 556
B Boyes, B Libert, S Schuster, B Wagner, W Miles, J Kirkland

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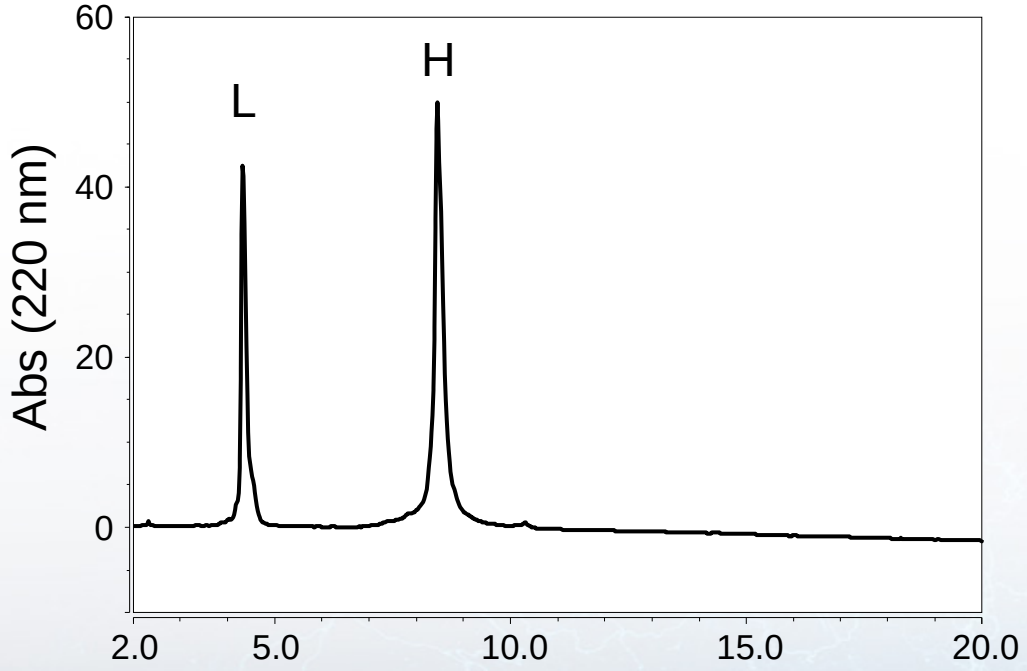
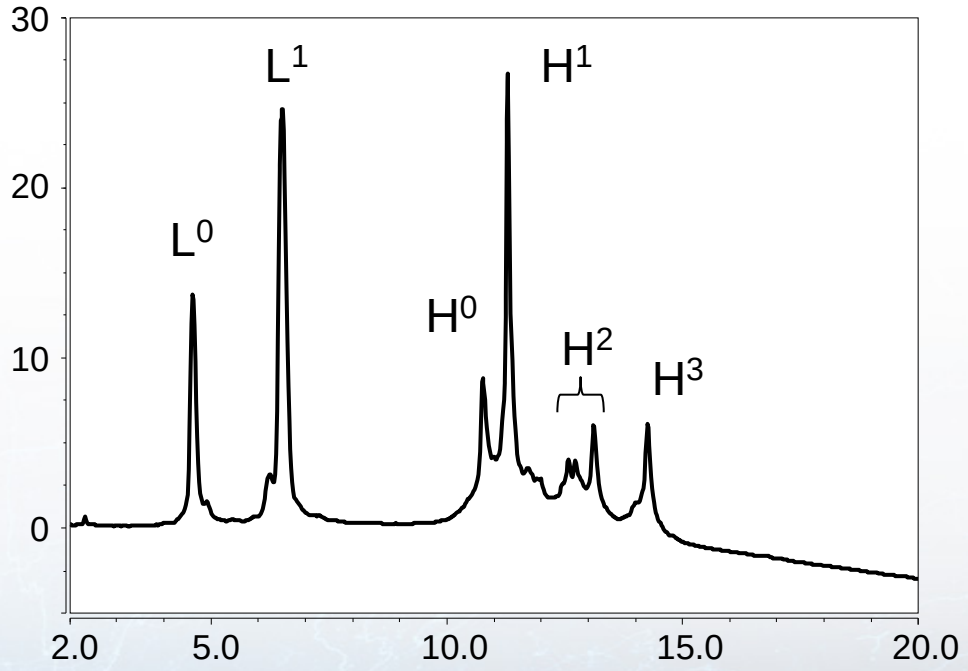
IgG H and L Chain Separations

Column: HALO 400 Å C4, 2.1 x 150 mm; Flow rate: 0.4 mL/min; Temp: 75 °C
Mobile Phase A: water/10 mM DFA; Mobile Phase B: AcN/ 10 mM DFA;
Gradient: 28.5-31.2%B 8 min; 31.2-45.8% in 12min
Instrument: Shimadzu Nexera/Abs (220nm); Orbitrap Velos Pro, 15k Res, ESI 3.8 kV
Injection Volume: 10 µL of mAb (5 µG) in 0.1% TFA Reduced and IAm alkylated Cys

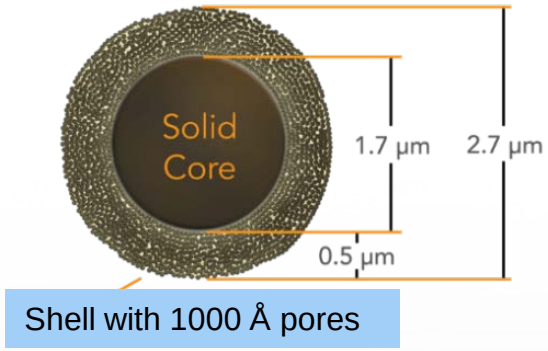


Trastuzumab
L – 23,728 Da
H – 49,997 Da + Glycans (G_o, G_oF, G_1F, G_2F)

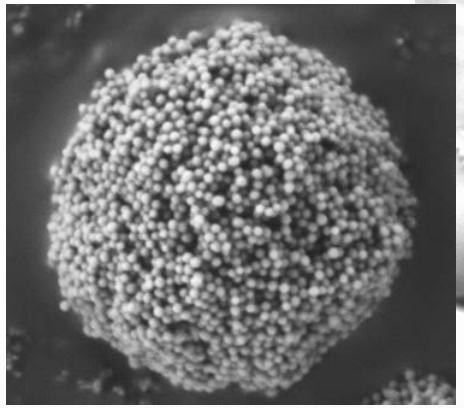


S/M MSQ8 ADC Mimic
L^0 – 23,284 Da; $L1^1$ – 23,895
H^n – 49,585 Da + Glycans (G_0F, G_1F) + n(611 Da)

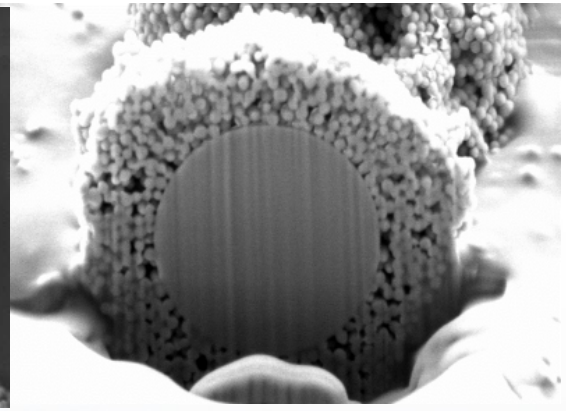
Superficially Porous (Fused-Core®) Wide Pore Particles: 1000 Å



Shell with 1000 Å pores



SEM



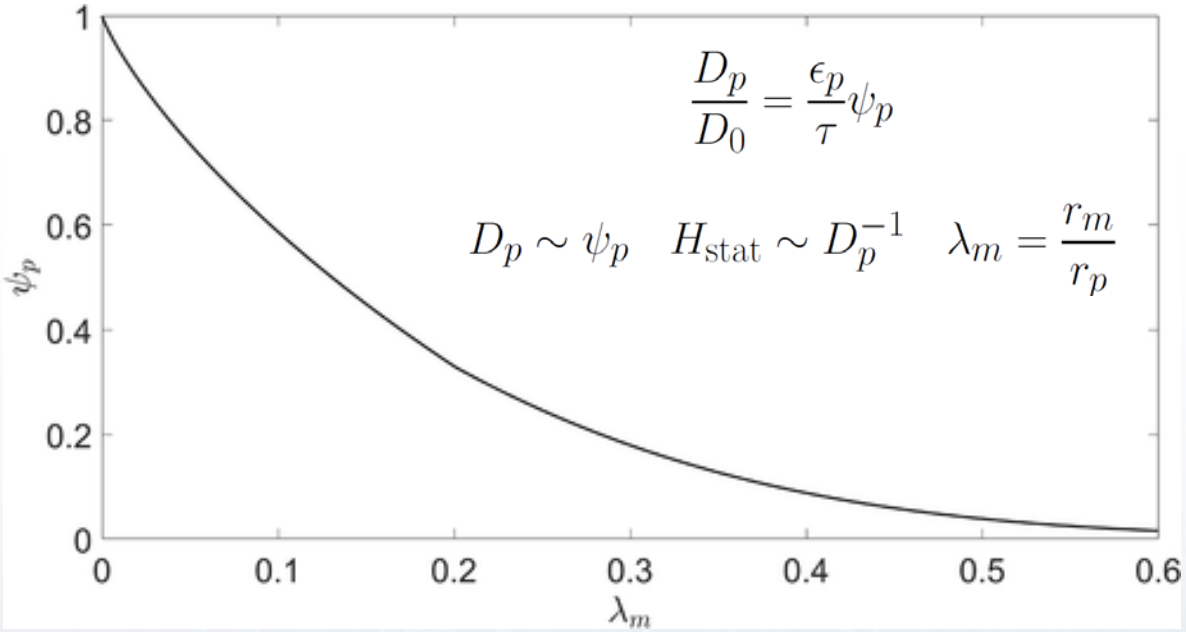
Section analysis by FIB-SEM

- 2.7 µm particle with 0.5 µm thick shell and 1000 Å pores
- Surface area ~ 22 m^2/g
- Designed for larger proteins
- Densely bonded C4 phase with end-capping
- High temperature and low pH stable

Wagner, Schuster, Boyes, Shields, Miles, Haynes, Kirkland, and Schure.
Superficially porous particles with 1000 Å pores for large biomolecule high performance liquid chromatography and polymer size exclusion chromatography
J. Chromatogr. A 1485 (2017) 75–85.

Restricted diffusion: Why you need larger pores than the size of the solute

It has long been recognized that diffusion of molecules in pores is slower than diffusion in bulk liquid. This leads to more zone broadening through the resistance to mass transport within the stationary phase term of the plate height equation.



Theories have been developed which account for this effect in idealized pore shapes (cylinders, slabs) as shown to the left.[1]

Diffusion in more realistic particle geometries shows a similar effect.[2]

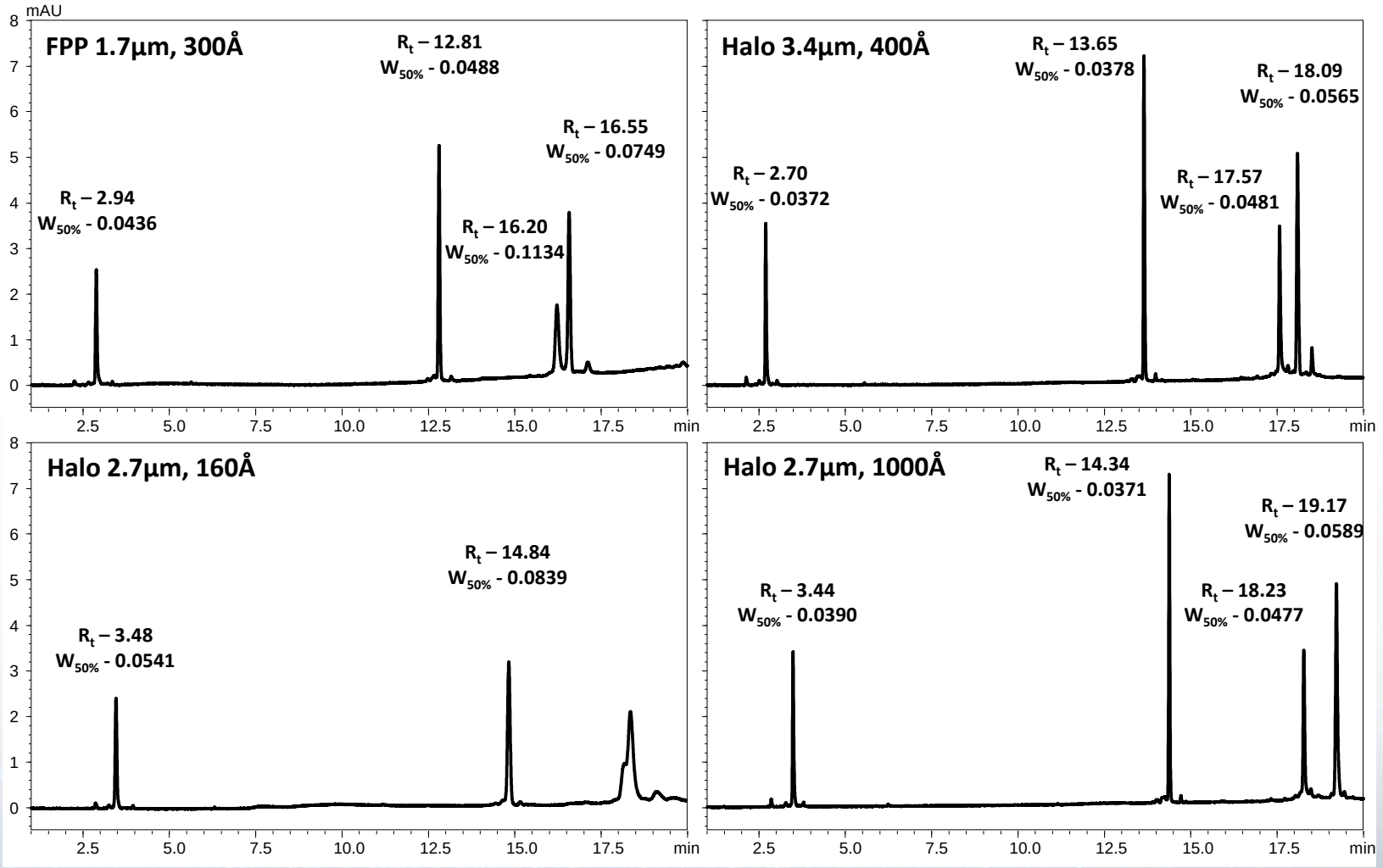
Ongoing efforts examine the fluid mechanics and transport properties of SPPs in packed beds.[3]

[1]P. Dechadilok, W.M. Deen, Hindrance factors for diffusion and convection in pores, Ind. Eng. Chem. Res. 45 (2006) 6953–6959.
[2]R. S. Maier, M. R. Schure, Transport properties and size exclusion effects in wide-pore superficially porous particles, Chem. Eng. Sci. 185 (2018) 243-255.
[3]M. R. Schure, R. S. Maier, T. J. Shields, C. M. Wunder, B. M. Wagner, Intraparticle and interstitial flow in wide-pore superficially porous and fully porous particles, Chem. Eng. Sci. 174 445–458 (2017).

Protein Separation on Wide Pore SPP vs FPP

2.1 mm ID x 150 mm C4 columns
20-50% AcN/0.1% DFA in 24 min
Flow: 0.5 mL/min
Temp: 60°C
1.5 µL (0.15-0.2 ug each)

1. RNase A 13.7 kDa
2. α-Lactalbumin 14.2 kDa
3. Enolase 93.1 kDa
4. Carbonic Anhydrase 30.0 kDa



- Improvement in peak width and retention with larger pore SPP
- As protein size increases, peak widths decrease with increasing pore size
- Similar results in TFA and DFA as mobile phase acidic modifiers

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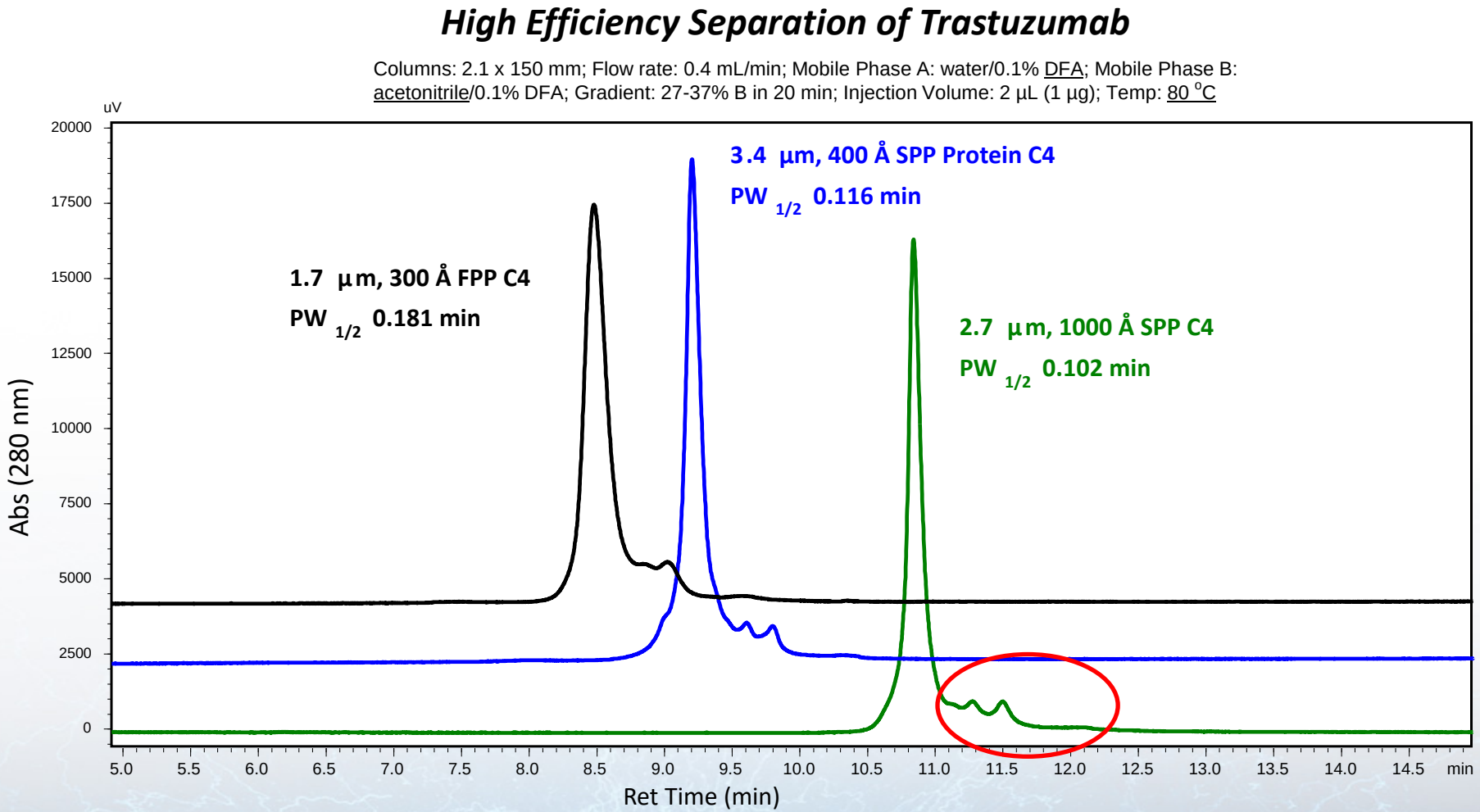
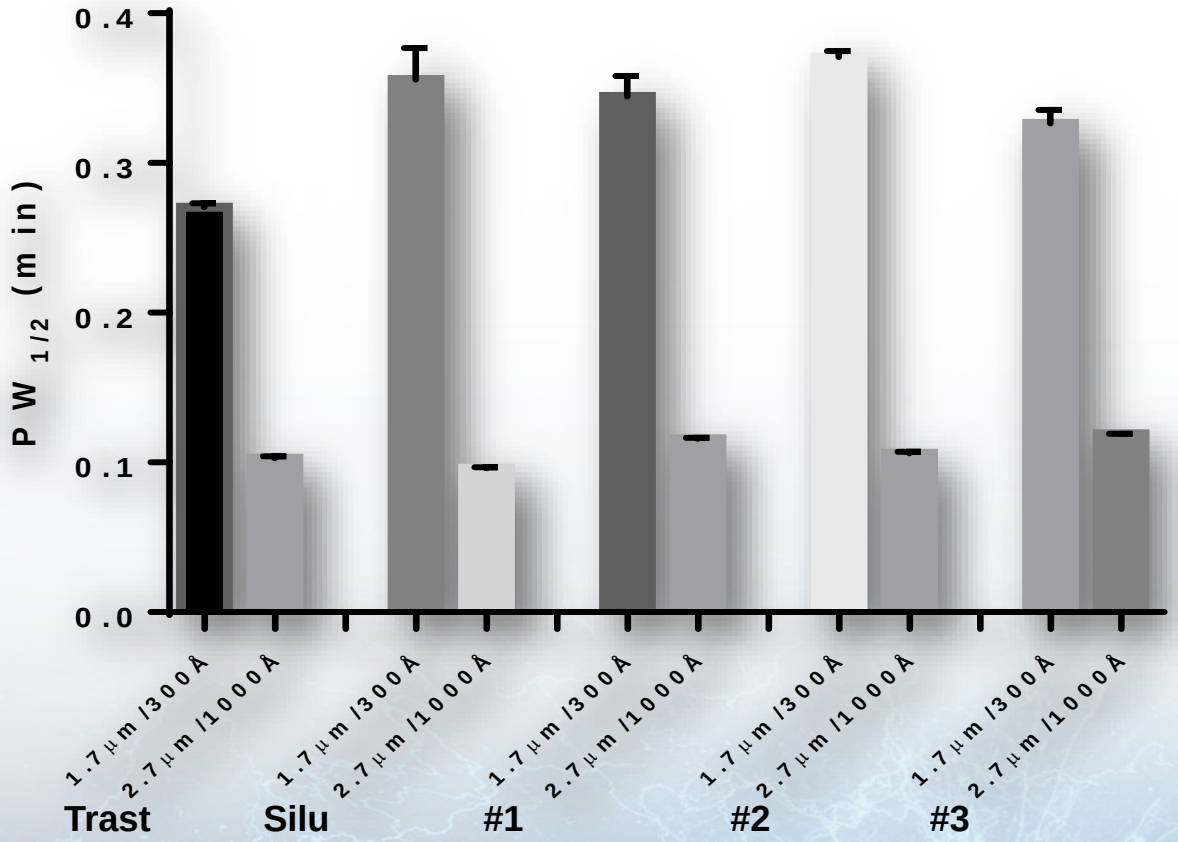
mAb IgG Separation on Wide Pore SPP vs FPP



- Large improvement in peak width and ***increased*** retention with pore size for SPP, additional improvement in peak width with 1000 Å pores

mAb IgGs Separation on Wide Pore SPP vs FPP

Columns: 2.1 x 150 mm; Flow rate: 0.4 mL/min; Mobile Phase A: water/0.1% DFA; Mobile Phase B: acetonitrile/0.1% DFA; Gradient: 27-37% B in 20 min; Injection Volume: 2 µL (1 µg); Temp: 80 °C



FPP : 0.334 min
SPP : 0.108 min

- The SPP advantage is not unique to one mAb.
- Advantage is greater at higher flow rates.

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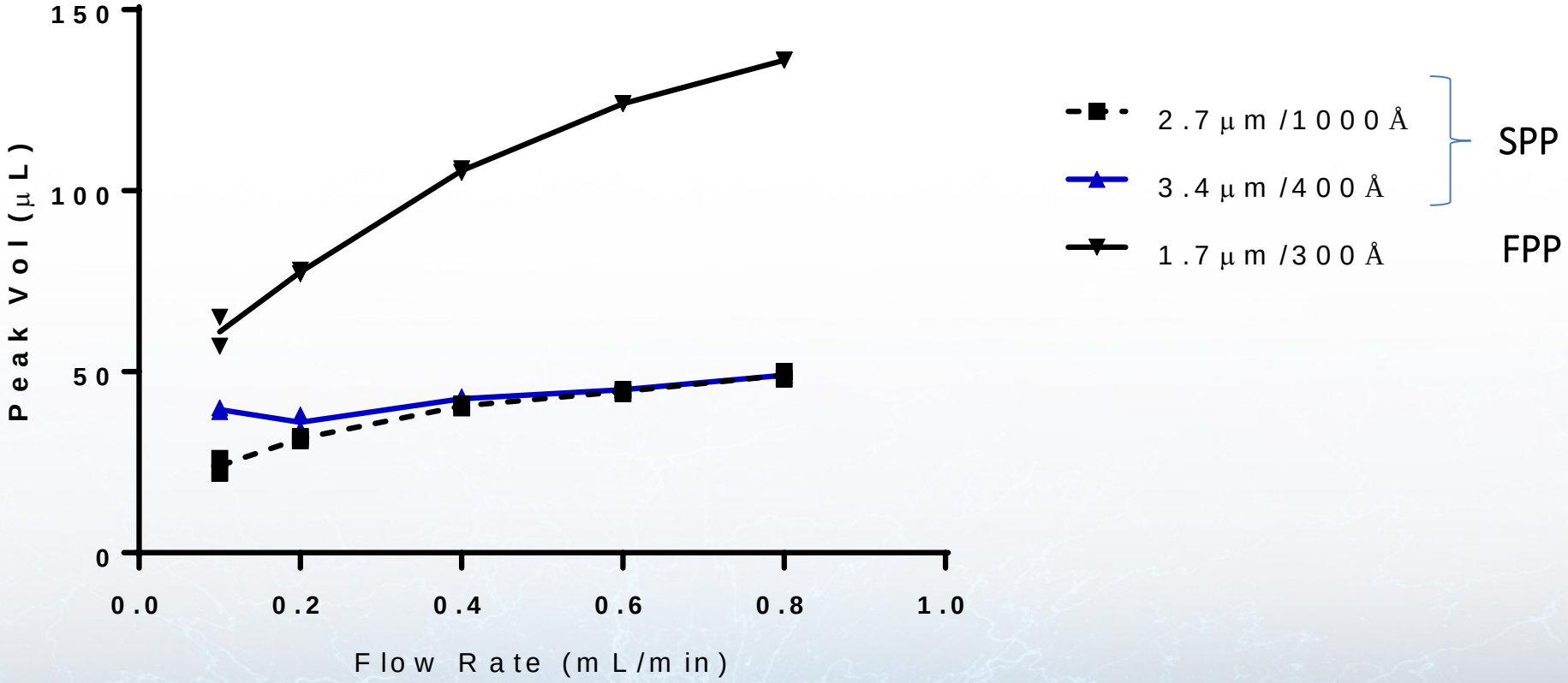
Flow Rate Effects on Peak Volume for mAb IgG

Fixed Volume Gradient Conditions (4.8 mL); Peak Volume = $PW_{1/2}$ x Flow Rate
Trastuzumab 0.5 µg; 29-35% AcN in 0.1% DFA; 80°C;



- Mass transfer is improved for the large pore SPP particles with higher MW protein.
- Trastuzumab and Silumab exhibited similar results

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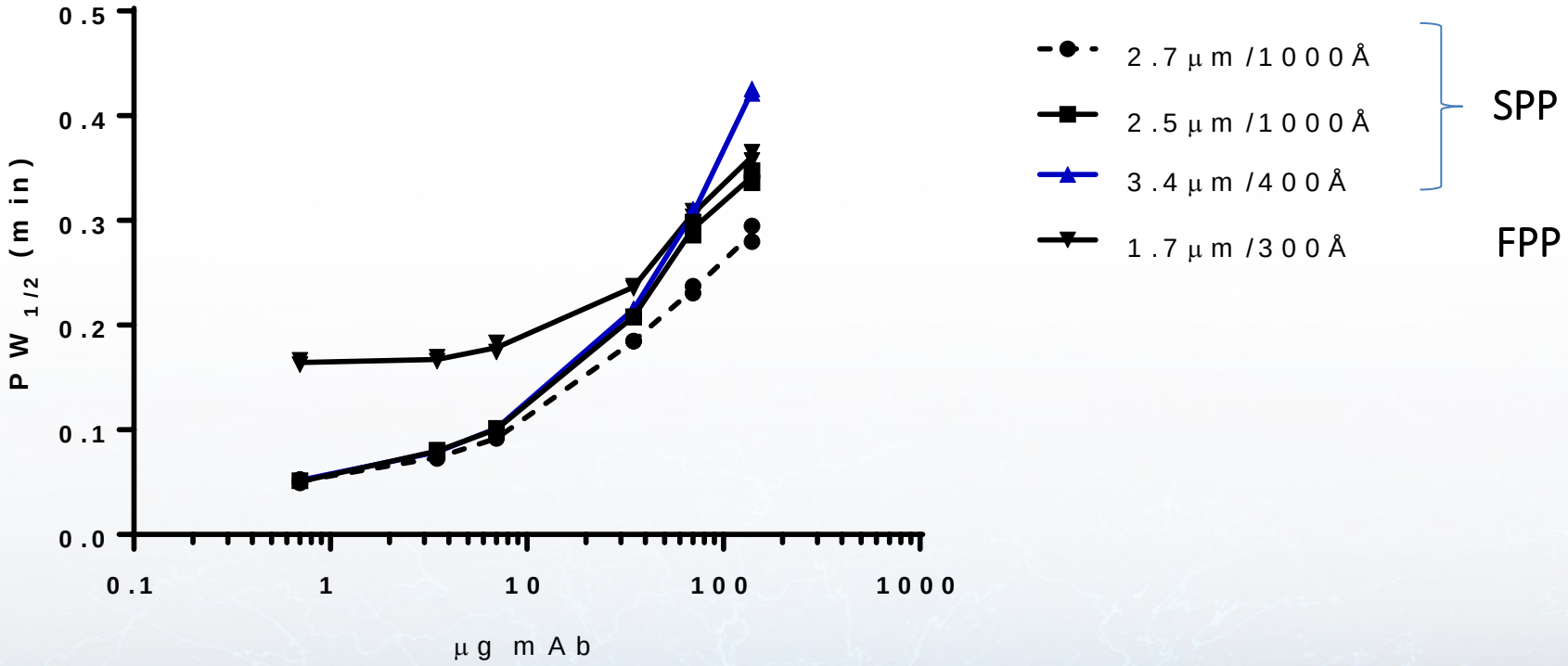
Load Effects on Peak Width for SPP and FPP for mAb IgG

2.1 mm ID x 150 mm C4 columns; Trastuzumab 0.7 – 140 µg;
27-37% AcN (0.1% DFA) in 10 min; 80°C



- For larger molecules, large pore SPP particles tolerate large sample masses effectively.
- Performance loss is progressive, occurring around 20-50 µg on column
- At all load levels 1000Å pore size SPP performed best for this mAb

Schemes for Improving Protein Separations by Reversed Phase

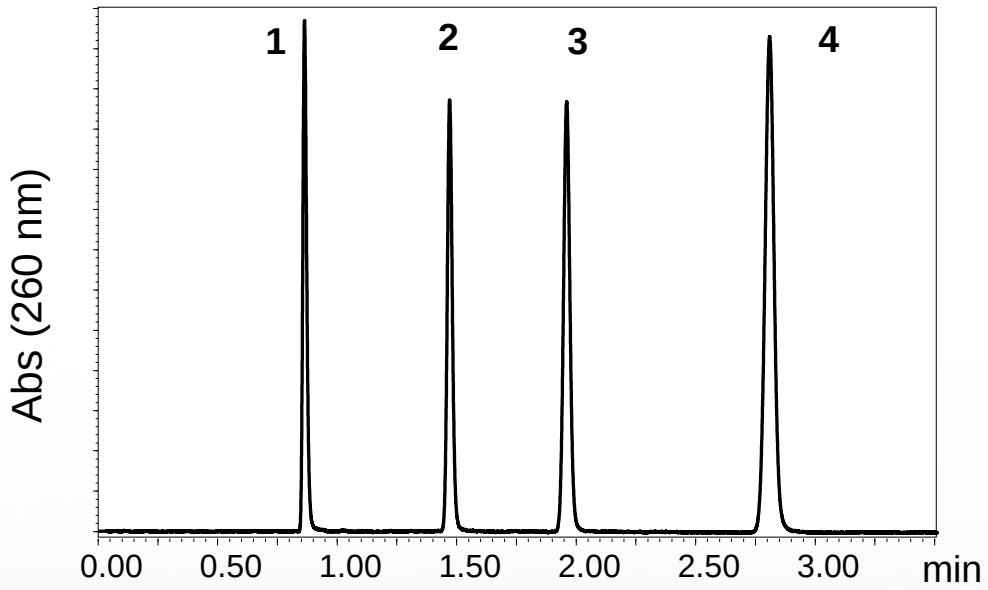
Limited choices of favorable conditions: low pH, elevated T_{col}, UV transparent, productive for ESI-MS detection, high recovery of proteins of varying natures

Many biomolecules of interest are inherently heterogeneous, and RP will not resolve all variants (eg., glycosylation)

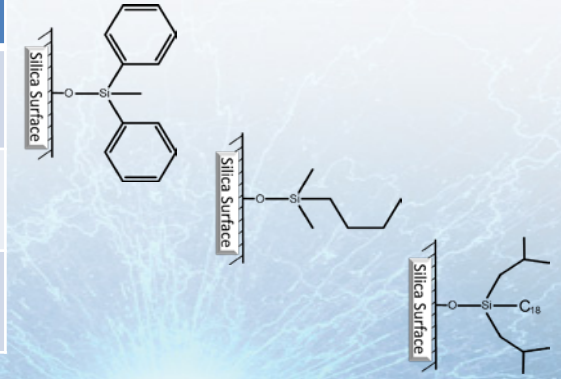
Parameters readily altered for RP selectivity/recovery optimization

- **Bonded Phase Manipulations**
  - **C4 is not the only option**
- **Mobile Phase Manipulations**
  - **Acids (FA, TFA, DFA) and Organic Modifiers (AcN, short chain alcohols)**
- **Operational Temperatures**
  - **40-90°C is a reasonable window**

Effect of Bonded Phase on RP Separations of Small Probes using HALO 1000 Å SPP (2.7 µm; 0.5 µm shell)



Bonded Phase	Retention Factor k'_4	Selectivity $\alpha_{4,3}$
Diphenyl	2.25	1.77
C4	4.95	2.13
ES-C18	17.92	2.81



Columns: 2.1 x 150 mm; Flow rate: 0.4 mL/min; Mobile Phase H_2O/AcN; 50/50 Instrument: Injection Volume: 2 µL; Detection: 260 nm; 25°C

1 - Uracil 60 µg/ml
2 - Hexanophenone 130 µg/ml
3 - Octanophenone 200 µg/ml
4 - Decanophenone 360 µg/ml

- Highly similar N, Tf, different k' and α
- Retention for small molecules is: DP<C4<<C18

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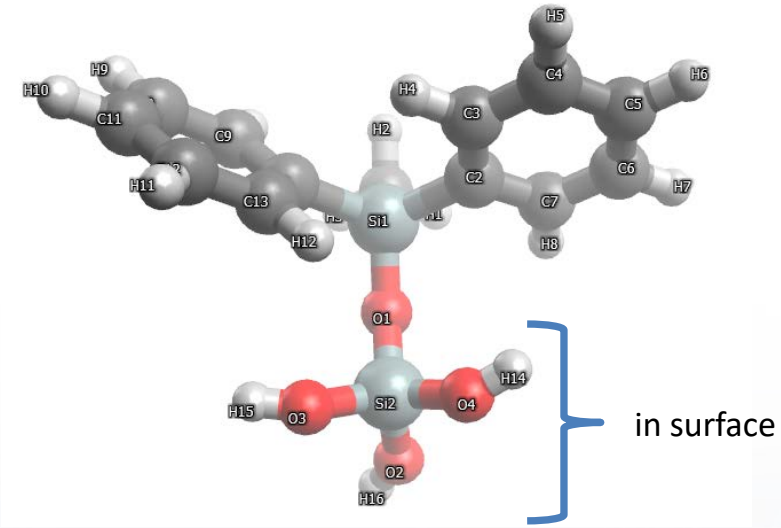
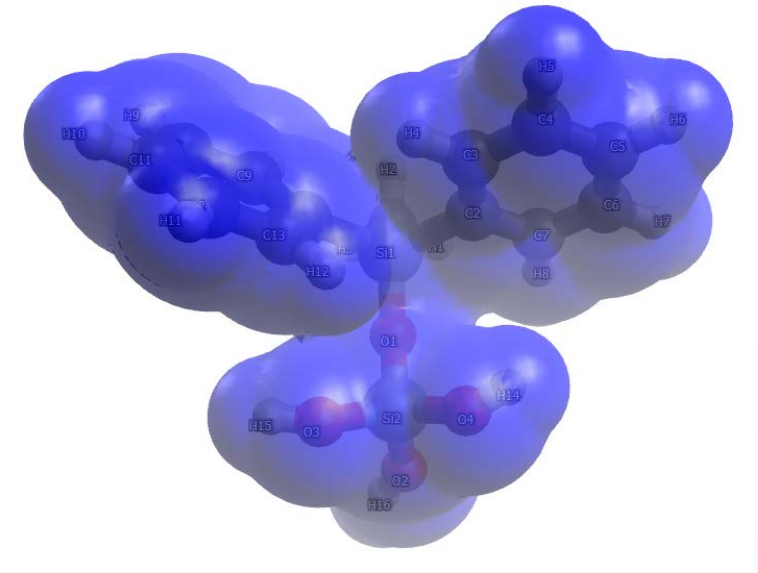
Diphenyl methyl sil(ane)oxane



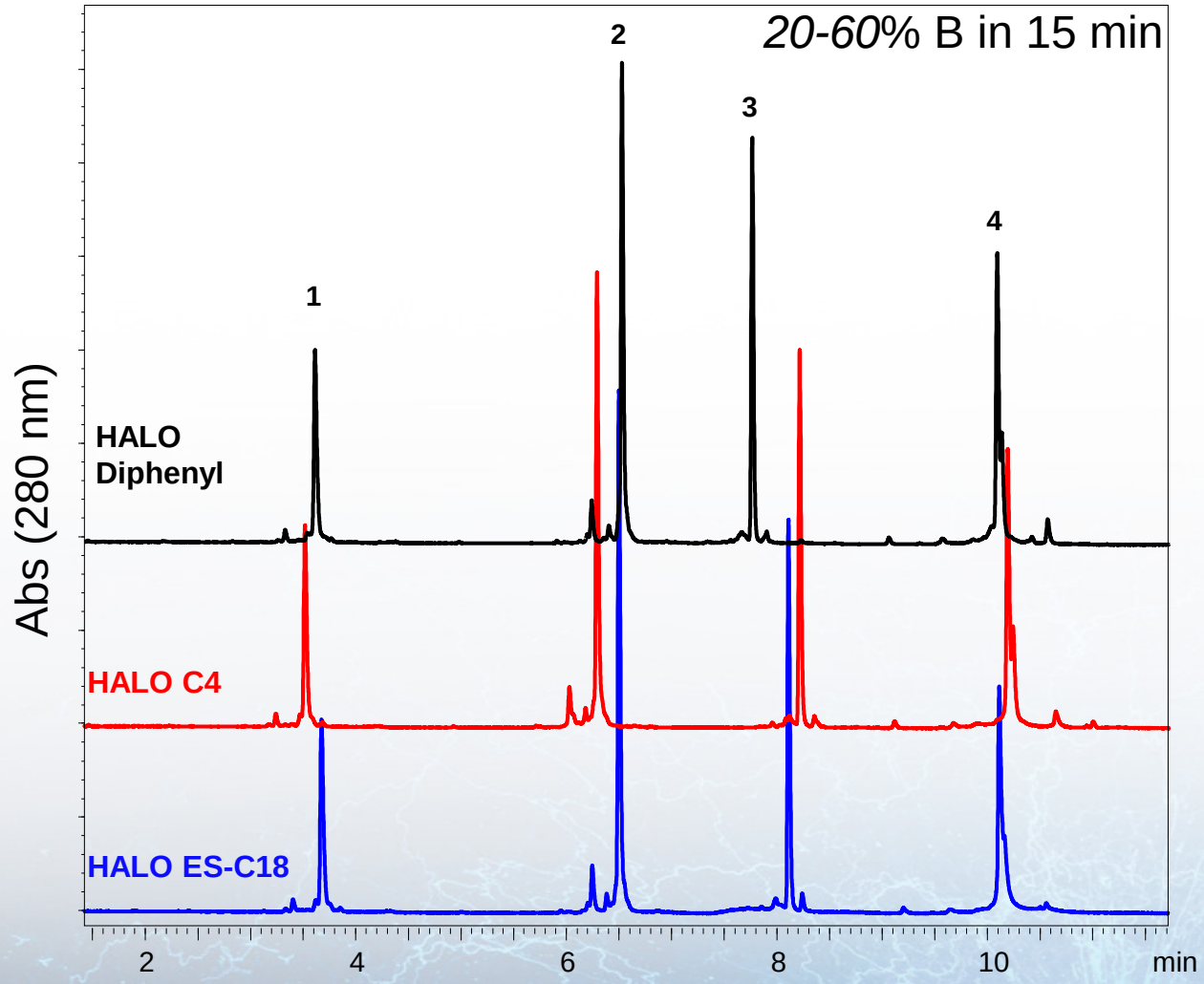
Ball and stick atom model



With van der Waals surface

- Phenyl groups are not coplanar
- Phenyl groups form a nice van der Waals surface (a pocket) for large molecule interaction.
- Rotation around C2-Si1 and C8-Si1 bonds accommodate large molecule fragments.
  - These rotations, when not sterically crowded, don't cost much energy.
  - As with C_{18}, these groups will accommodate the solute through bending and rotation.
- 1000 Å HALO SPP surface reaction is 2.7 µmol/m^2 (5.4 phenyl)

Effect of Bonded Phase on Protein Separations using HALO 1000 Å SPP



Columns: 2.1 x 150 mm; Flow rate: 0.4 mL/min; Mobile Phase A: H_2O/0.1% TFA; Mobile Phase B: ACN/0.1% TFA; Gradient: 20-60 %B in 15 min; Instrument: Shimadzu Nexera; Injection Volume: 2 µL; Detection: 280 nm; Temp: 80 ^{o}C

1. Ribonuclease A
2. Lysozyme
3. α-Lactalbumin
4. Enolase

- Retention of proteins across bonded phase columns:
  - not correlated to small molecule retention
  - not a global pattern
- Selectivity differences allow separations choices.
- Similar peak widths for these proteins with each bonded phase

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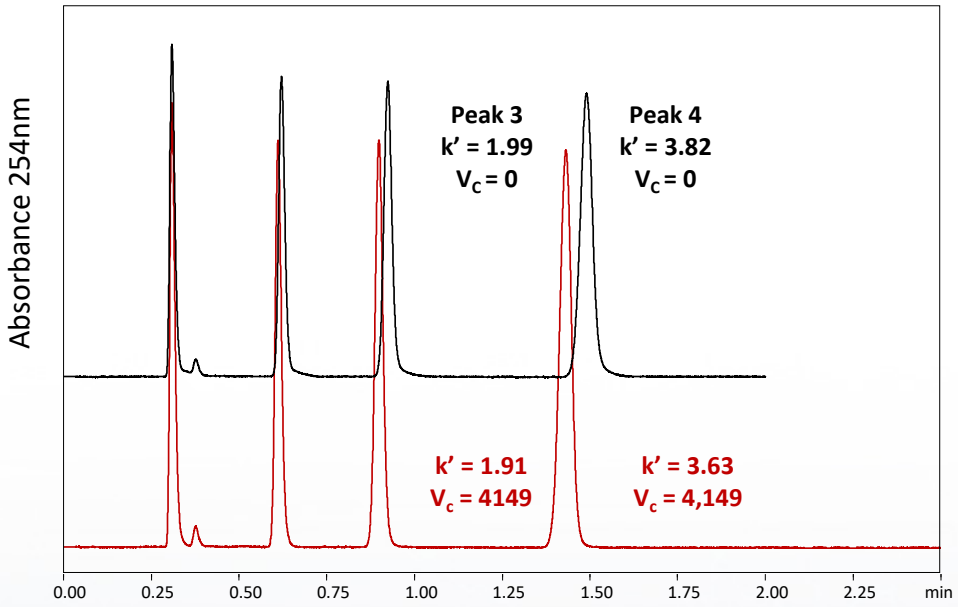
Acidic Stability of HALO 1000 Å Diphenyl Bonded Phase



Retention Results of Stability Test

25 °C, isocratic 45/55 ACN/H_2O, 100 Hz, 0.2µL inj

Stability Testing Regime

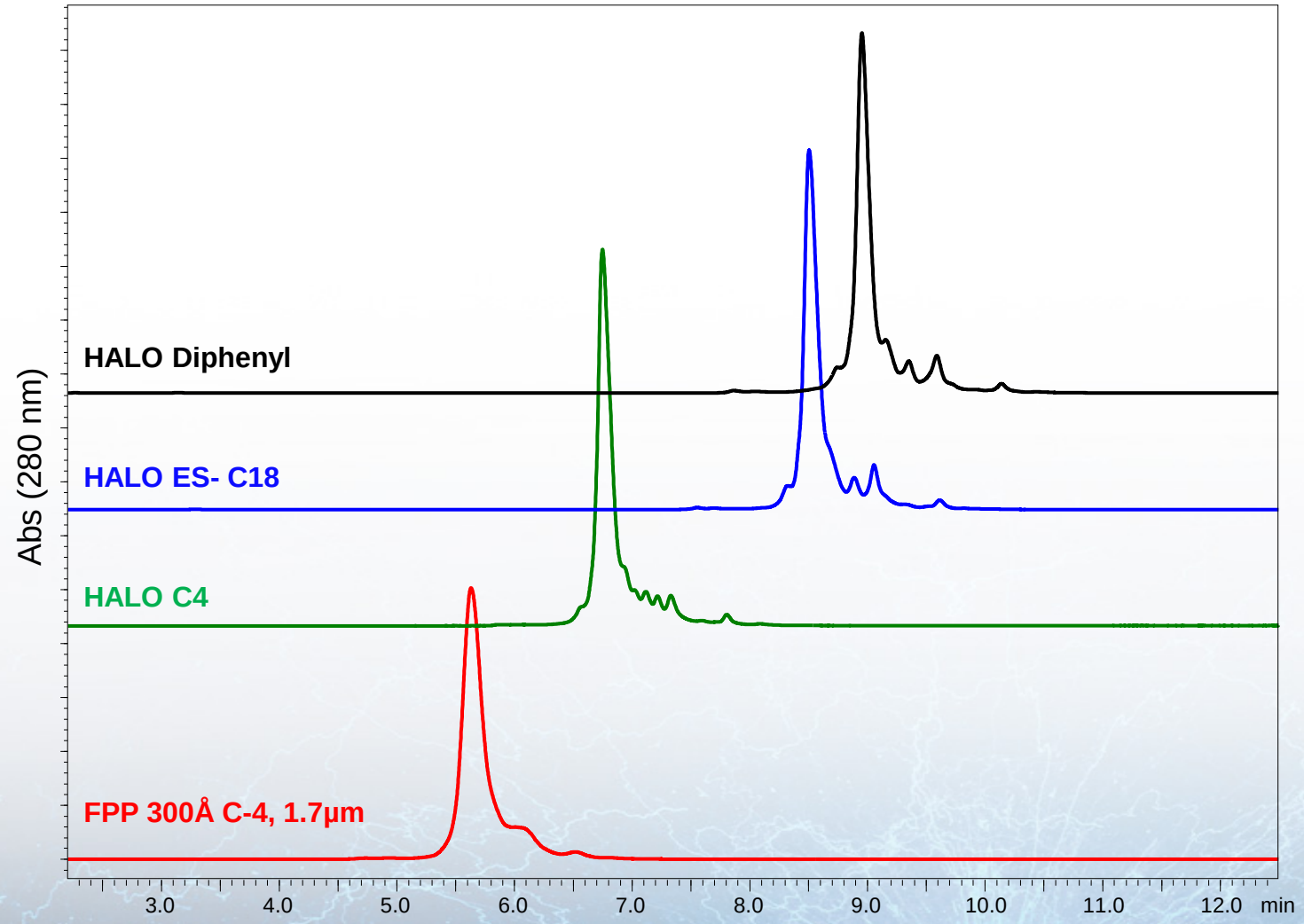
- Temp. – 90 °C
- Flow Rate – 0.70 mL/min
- Mobile Phase A – aq. 0.2% TFA *(pH = 1.60)*
- Mobile Phase B – ACN + 0.2% TFA
- Gradient - 5-40% B in 10 min + 5 min equil.

Simulates aggressive gradient method

Blank gradients were followed by small molecule retention measurement following every 10 injections. Retention and peak shape changes were minimal during the course of this column challenge of low pH and high temperature.

A decrease of 5% in the retention factor for small molecules was observed for the Diphenyl after 4,000 column volumes using highly aggressive stability challenge (high T and low pH). This result supports that the Diphenyl phase shows increased resistance to hydrolytic attack at the siloxane bond to the silica surface.

Effect of Bonded Phase on mAb Separations using HALO 1000 Å: Trastuzumab



Columns: 2.1 x 150 mm; Flow rate: 0.4 mL/min; Mobile Phase A: H2O/0.1% TFA; Mobile Phase B: ACN/0.1% TFA; Gradient: 32-40 %B in 16 min; Instrument: Shimadzu Nexera; Injection Volume: 2 µL; Detection: 280 nm; Temp: 80°C

- Retention of mAb is often:
  DP>C18>C4
- Selectivity differences observed with variants
- Similar peak widths for this mAb with each bonded phase

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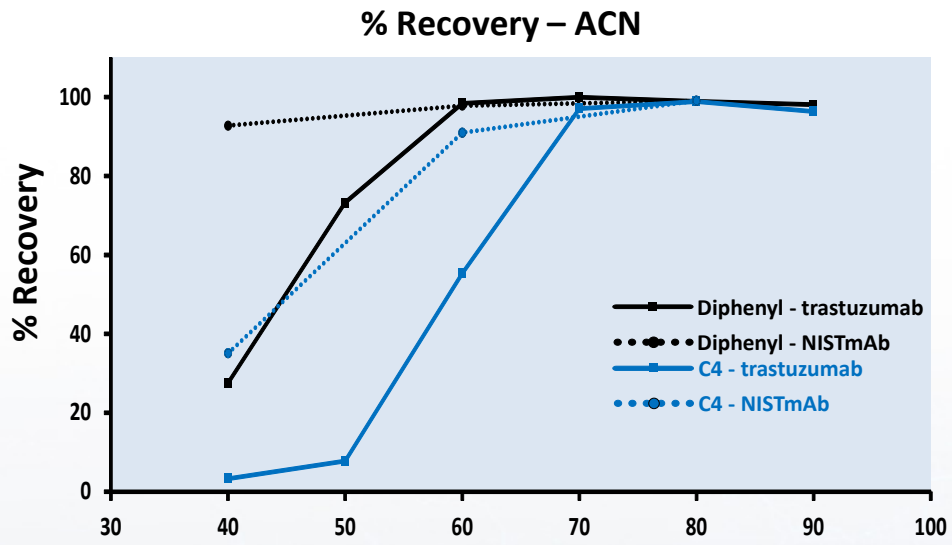
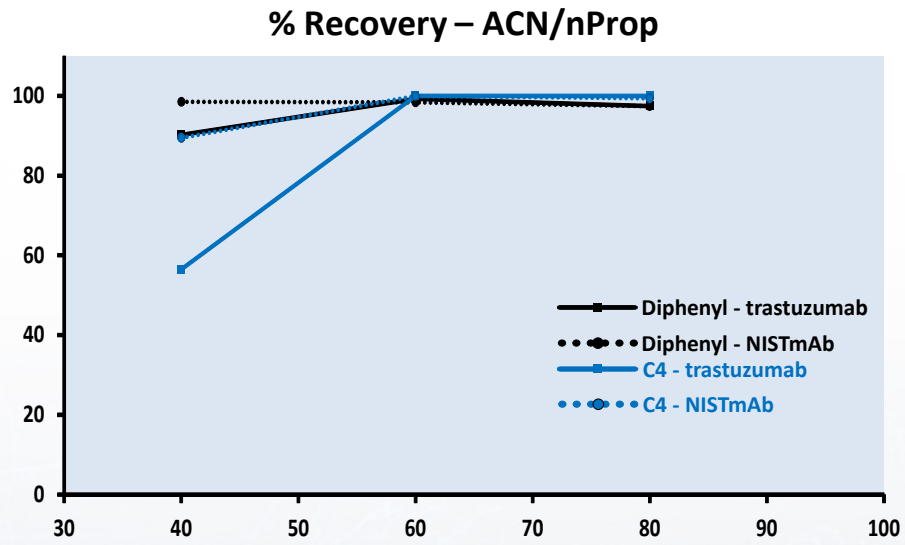
Temperature-dependent Recovery of mAbs: Bonded Phase/Mobile Phase Effects

Columns: 2.1 x 150 mm; Flow rate: 0.4 mL/min, Recovery as %Maximum Area

A – H_2O/0.1% TFA, B – ACN/0.1% TFA: 30-45%B in15min
4 µL at 2 mg/mL (8 µg)

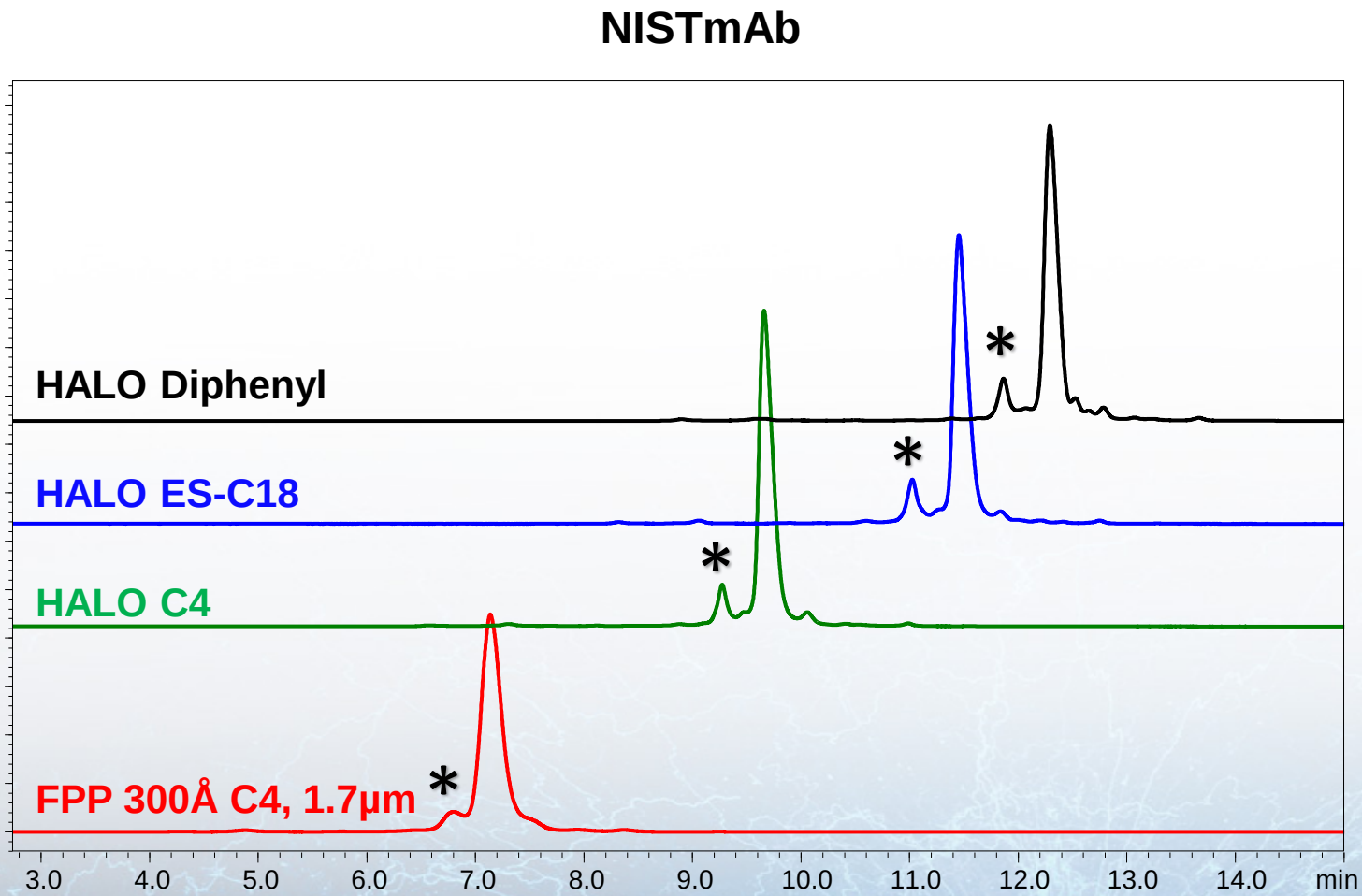


A – H_2O/0.1% TFA, B – (50/50 ACN/nPropanol)/0.1% TFA: 28-43%B in15min
4 µL at 2 mg/mL (8 µg)



- ES-C18 yields similar results to C4 bonded phase for recoveries; at maximum recovery many columns show similar area counts
- Diphenyl exhibits a lower temperature for full recovery of many mAbs
- Not all mAbs require T>70° for highest recovery in AcN, with alkyl bonded phases
- Many mAbs show lower T for high recovery using the AcN/n-Propanol mixture
- Mixtures of propanol (i- and n-) and AcN between 80/20 and 20/80 have not shown large effects
- Similar patterns of recovery are observed for a couple of IgG2 mAb examined to date

Effect of Temperature on mAb Separation: DANGER



Columns: 2.1 x 150 mm; Flow rate: 0.4 mL/min; Mobile Phase A: H_2O/0.1% TFA; Mobile Phase B: ACN/0.1% TFA; Gradient: 34-42 %B in 16 min; Instrument: Shimadzu Nexera; Injection Volume: 2 µL; Detection: 280 nm; Temp: 80°C

- * denotes a high temperature artifact
- Reinjection of main peak generates this; the artifact remains a single peak
- Is absent at or below 60°C
- Forms at higher temperature, with all columns and mobile phases

Improved Protein LC/MS Mobile Phases: Properties That May Help

Volatility

- **Necessary but not sufficient for additives. Must NOT plug our ESI interface and capillary ion entrance path!**
- **Henry's Law Coefficients (Hcc): A higher value of the coefficient indicates ease of transfer of the protonated acid from the idealized aqueous phase of the mobile phase mixture. Not readily available, and not certain to predict partitioning from organic aqueous mixtures.**

Low pKa

- **Low pH and dissociation of acid; sufficient ionic strength appears beneficial for separation needs, while effect on ESI suppression must be managed**

Favor Peptide and Protein Solubility

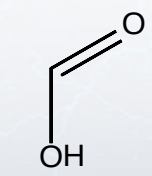
- **Acidic (usually). Fluorinated? Polar? Chaotropic?**

Improved Protein LC/MS Mobile Phases: Properties That May Help

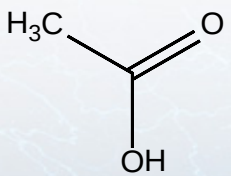
Initial selection and testing indicated some candidates with promise:

Share required features of volatility, lower pKa, but variable protein solubility

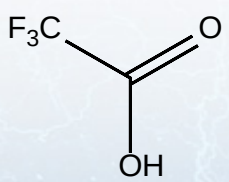
	FA	HOAc	TFA	DFA	3FPA
pK_a	3.8	4.8	0.3-1.6	1.3-1.4	3.1



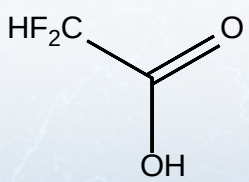
formic acid



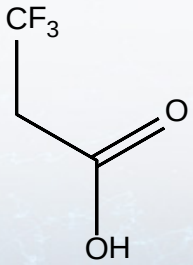
acetic acid



2,2,2-trifluoroacetic acid



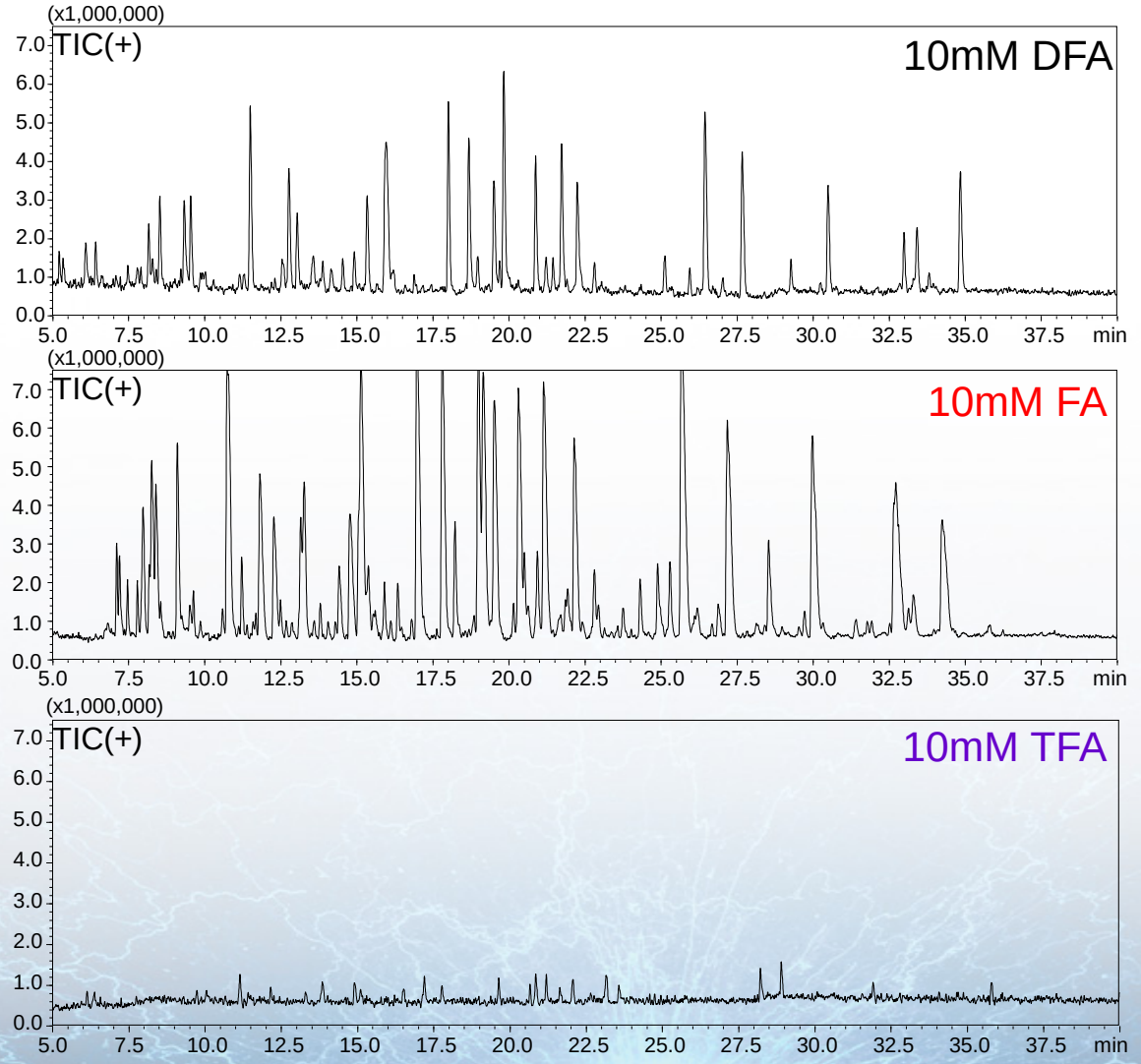
2,2-difluoroacetic acid



3,3,3-trifluoropropanoic acid

Tryptic Digest Peptide Mixture LC/MS in Several Acidic Modifiers

10 mM acid in mobile phases; 2.1 x 150 mm HALO 160 Å ES-C18, 0.3mL/min, 2-47%AcN in 40min, 60°C, 300-1800m/z, 4kV, 0.33s



50pmol Reduced and Alkylated Enolase Digest Peptides

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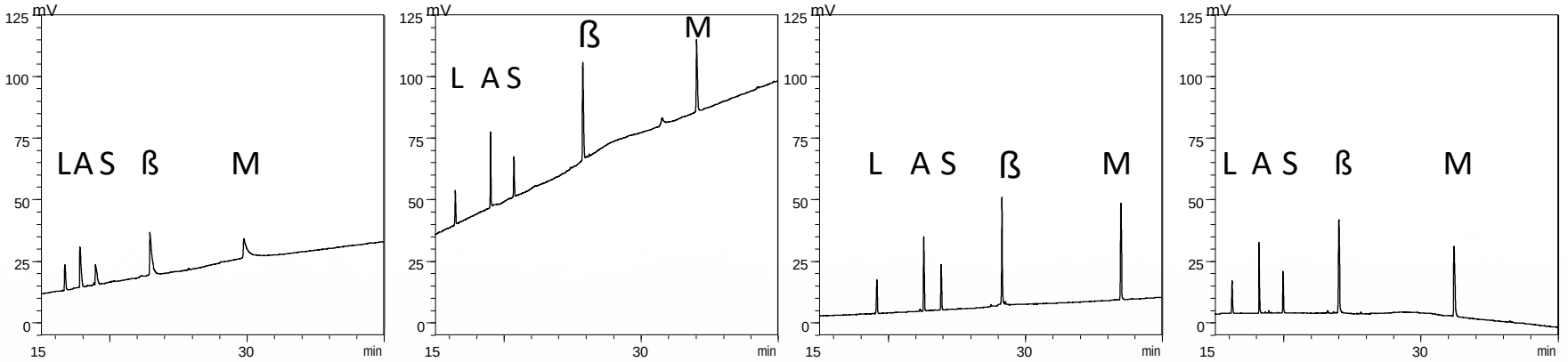
Synthetic Peptide Mixture LC/MS in Several Acidic Modifiers

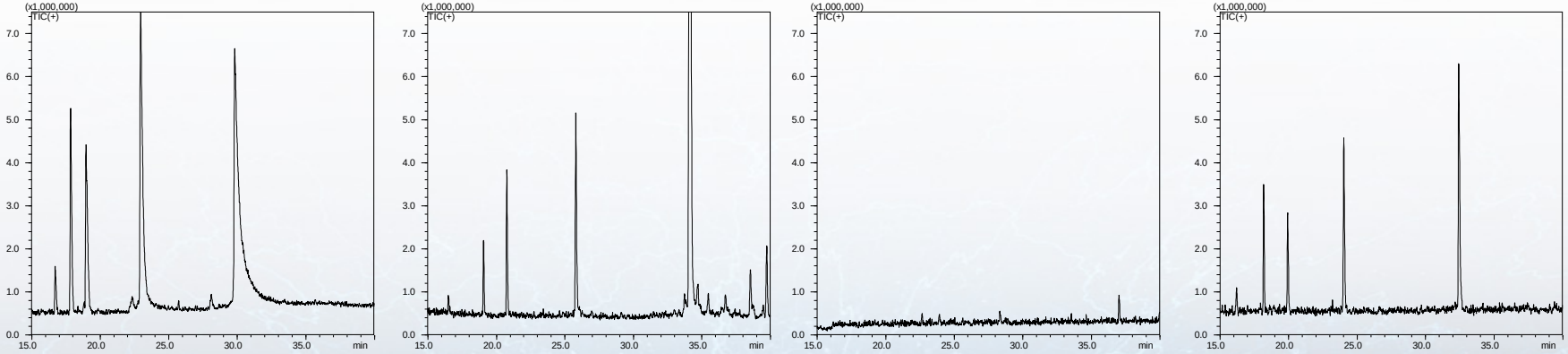
10 mM Acid; 50pmol5 peptide mix

2.1 x 150 mm HALO 160 Å C18, 0.3mL/min, 2 -47%AcN in 40min,60°C, 300-1800m/z, 4kV, 0.33s

A_{220}



TIC



Peptide	Abbrev.	MW
[Leu5]-enkephalin	L	555.6
angiotensin I, human acetate hydrate	A	1297
substance P acetate salt hydrate	S	1348
Melittin, honey bee venom	M	2847
beta-endorphin, human	ß	3465

Mobile Phases for Improved Protein LC/MS

2.1 x 100 mm
Protein 400 Å C4

15-55% AcN 30 min

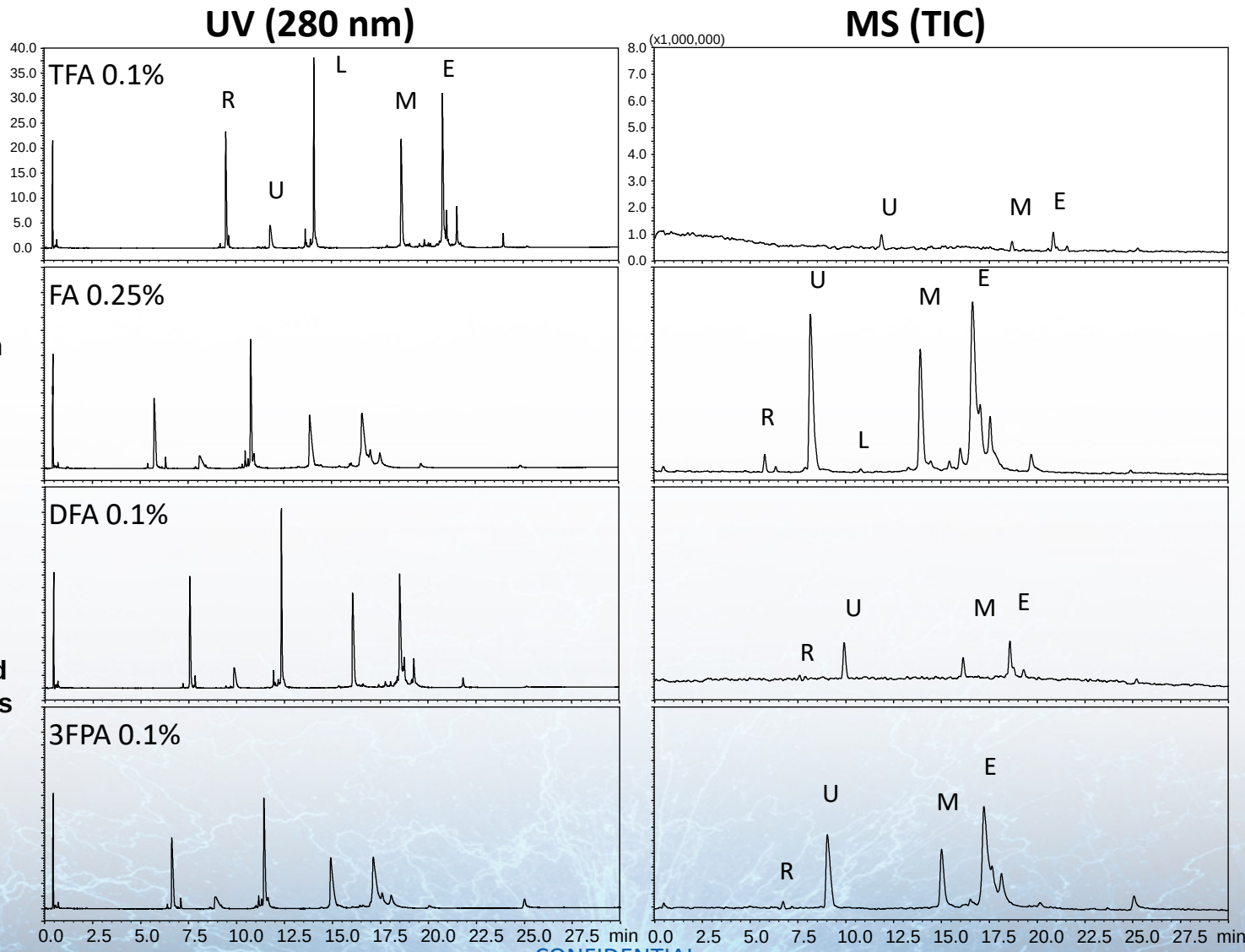
0.35 mL/min; 50ºC

25 pmol each protein

R – Ribonuclease
U – rec. Ubiqutin
L – Lysozyme
M – apo-Myoglobin
E – Enolase

Nexera LC system

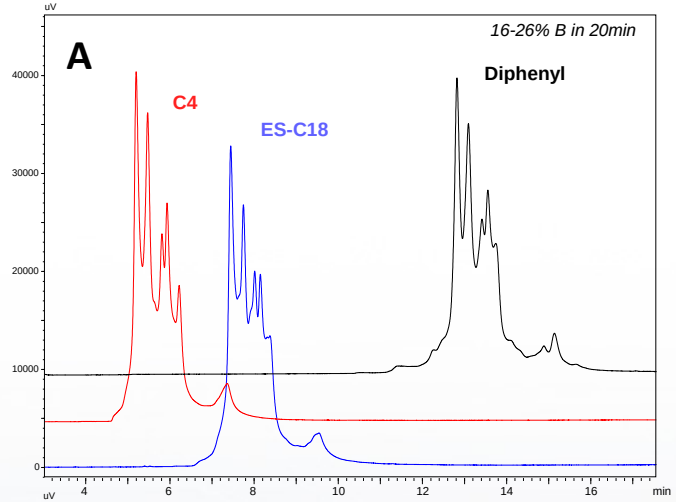
MS-2020 Single Quad
400 – 2000 m/z 3 pps
3.8 kV ESI



Method Development Approaches: BP, MP, T

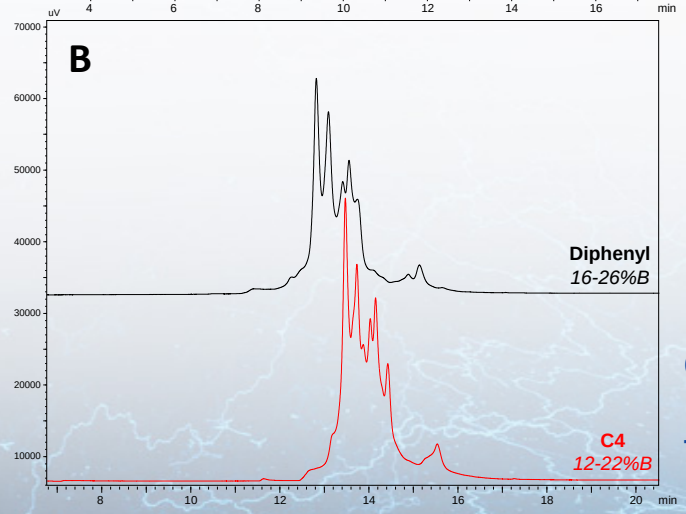
Column Based Approach

Columns: 2.1 x 150 mm; Flow rate: 0.2 mL/min; Mobile Phase A: 88/10/2 H_2O/ACN/nProp + 0.1% DFA; Mobile Phase B: 70/20/10 nProp/ACN/H_2O + 0.1% DFA; Gradient: 16-26 %B in 20 min; Instrument: Shimadzu Nexera; Injection Volume: 2 µL; Detection: 280 nm; Temp: 80 °C



BP compared

Hi/Low T scan



Rt adjusted

C4 selected

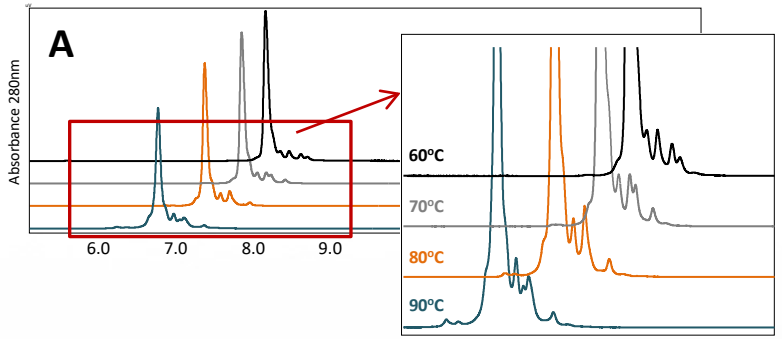
T and ΔG adjusted

T and MP Composition

Columns: 2.1 x 150 mm HALO 1000 Diphenyl; Flow rate: 0.4 mL/min; A H_2O/0.1% TFA; B: ACN/0.1% TFA; Gradient: 30-45 %B in 15 min; Instrument: Shimadzu Nexera; Injection Volume: 2 µL; Detection: 280 nm;

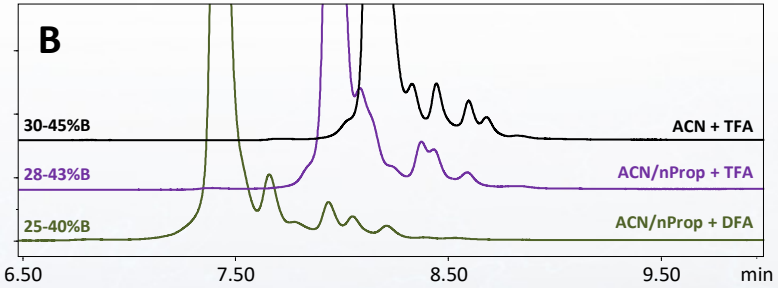
BP fixed

T Compared



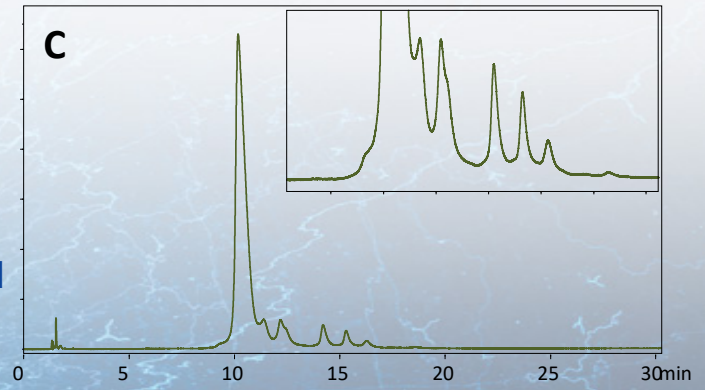
T selected (60)

MP Compared (acid, Organic)

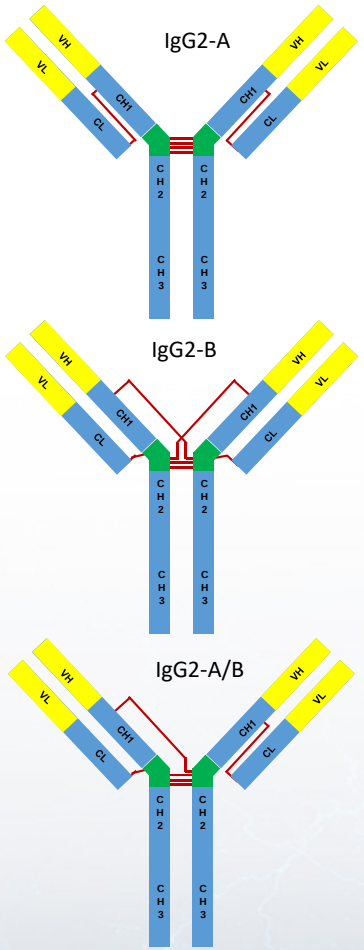


MP selected AcN/n-Prop/DFA

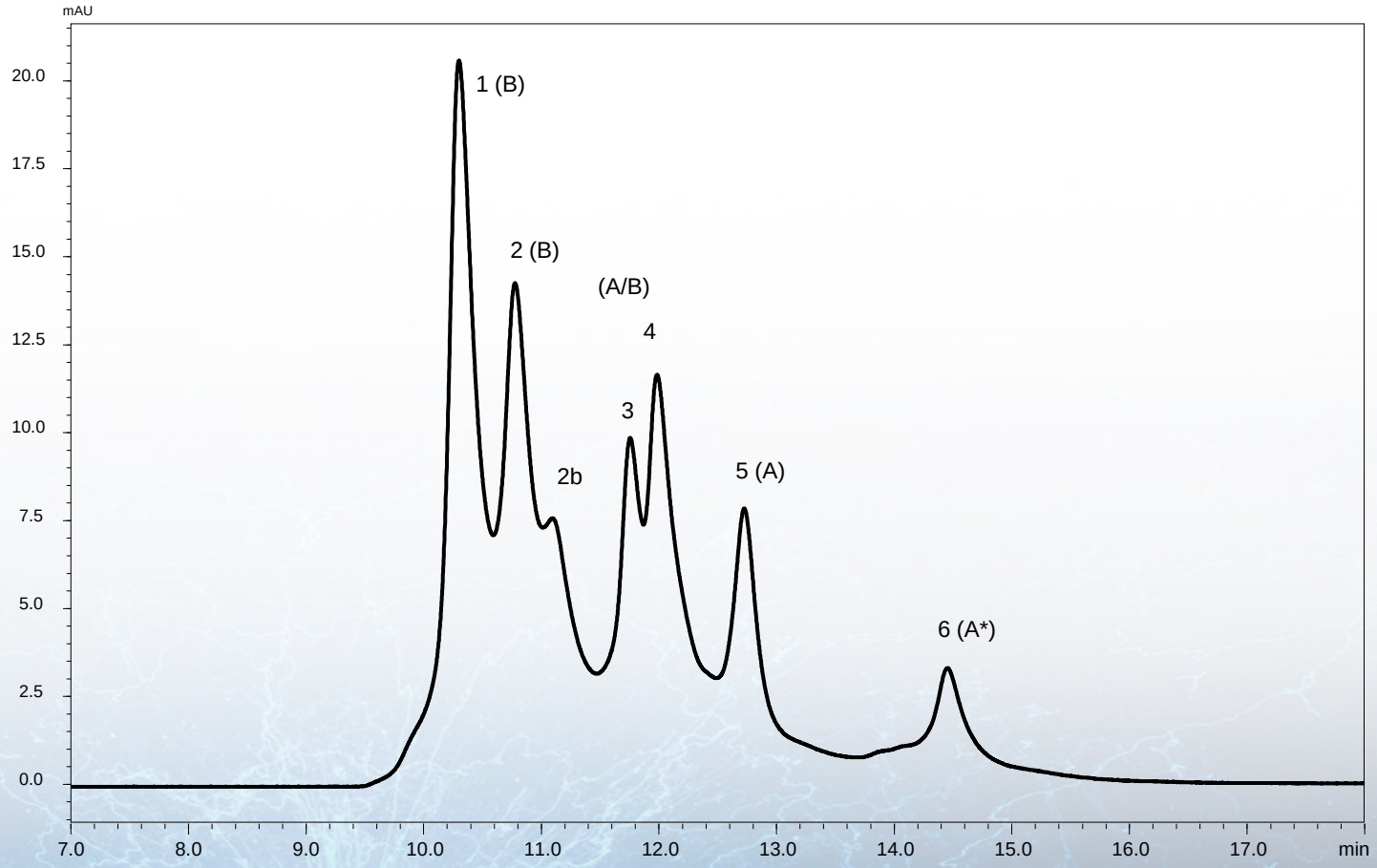
ΔG, flow and Time adjusted



IgG2 Disulfide Bridge Variant Separation



Column: HALO 1000 Å C4, 2.1 x 150 mm; Flow rate: 0.2 mL/min; Temp: 60 °C
Mobile Phase A: 88/10/2 water/AcN/n-propanol/0.1% TFA; Mobile Phase B: 70/20/10 n-propanol/AcN/water/0.1% TFA; Gradient: 20-28% B in 32 min; Instrument: Shimadzu Nexera; Injection Volume: 2 µL of 2 mg/mL denosumab in 0.1% TFA; Detection: 280 nm; Temp: 60 °C



Wypych, et al., J. Biol. Chem. 283 (2008) 16194–205.

Dillon, et al., J. Biol. Chem. 283 (2008) 16206-205.

Wei, Zhang, Boyes, and Zhang. J. Chromatogr. A 1526 (2017) 104-111.

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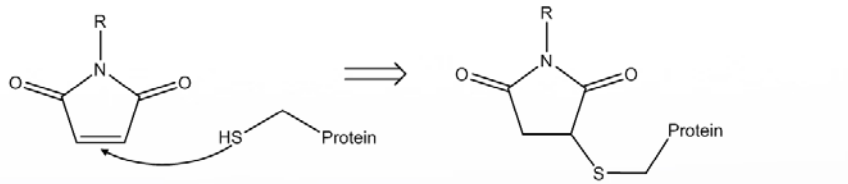
IgG Disulfide Bridge and Free Thiol Variant Analysis

Many proteins possess disulfide bridges and may also have free thiol groups (R-SH) present. In IgG1, these may be considered problematic, or at least must be monitored during bioprocessing, and in therapeutic formulations. In the case of IgG2, free thiols are formed during conversion of IgG2 disulfide variants - A, B, A/B isoforms. Conversions of R-S-S-R to $(R\text{-}HS)_2$ can occur, but exhibit a small mass shift, and are challenging for analysis.

Maleimides are convenient reagents for attaching a label at free thiols: R groups used can be manipulated to effect greater retention shift.



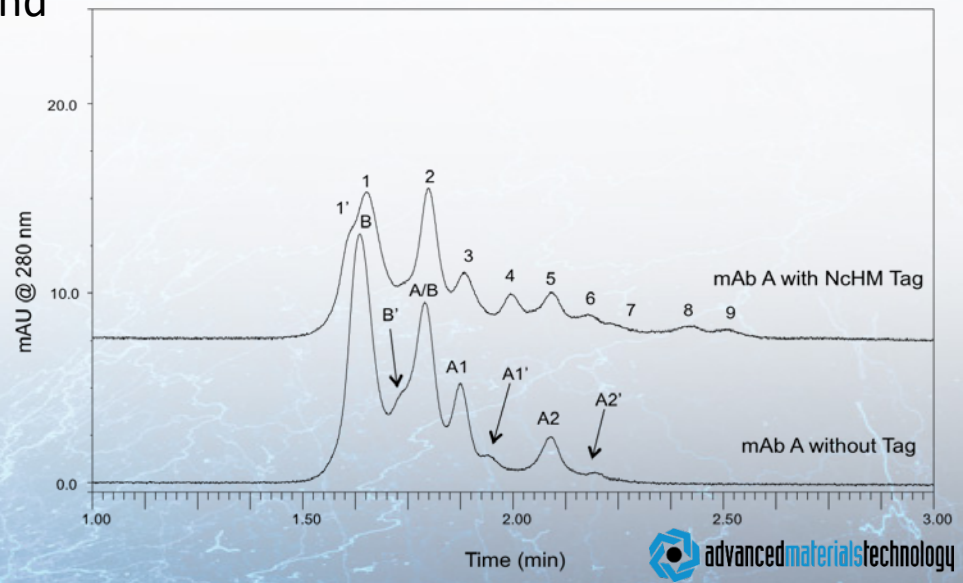
Maleimide Free thiol protein Maleimide protein conjugate

This approach has recently been described for IgG1, IgG1 constructs, and IgG2 mAbs recently by a number of investigators.

Zhang, Zhang, Hewitt, Tran, Gao, Qiu, Tejada, Gazzano-Santoro, and Kao. Identification and Characterization of Buried Unpaired Cysteines in a Recombinant Monoclonal IgG1 Antibody. Anal Chem. 84 (2012) 7112–7123.

Wei, Zhang, Boyes, and Zhang. Reversed-phase chromatography with large pore superficially porous particles for high throughput immunoglobulin G2 disulfide isoform separation. J. Chromatogr. A 1526 (2017) 104-111.

Welch, Dong, Hewitt, Irwin, McCarty, Tsai, and Baginski. Facile quantitation of free thiols in a recombinant monoclonal antibody by reversed-phase high performance liquid chromatography with hydrophobicity-tailored thiol derivatization. J Chromatogr. B 1092 (2018) 158-167.



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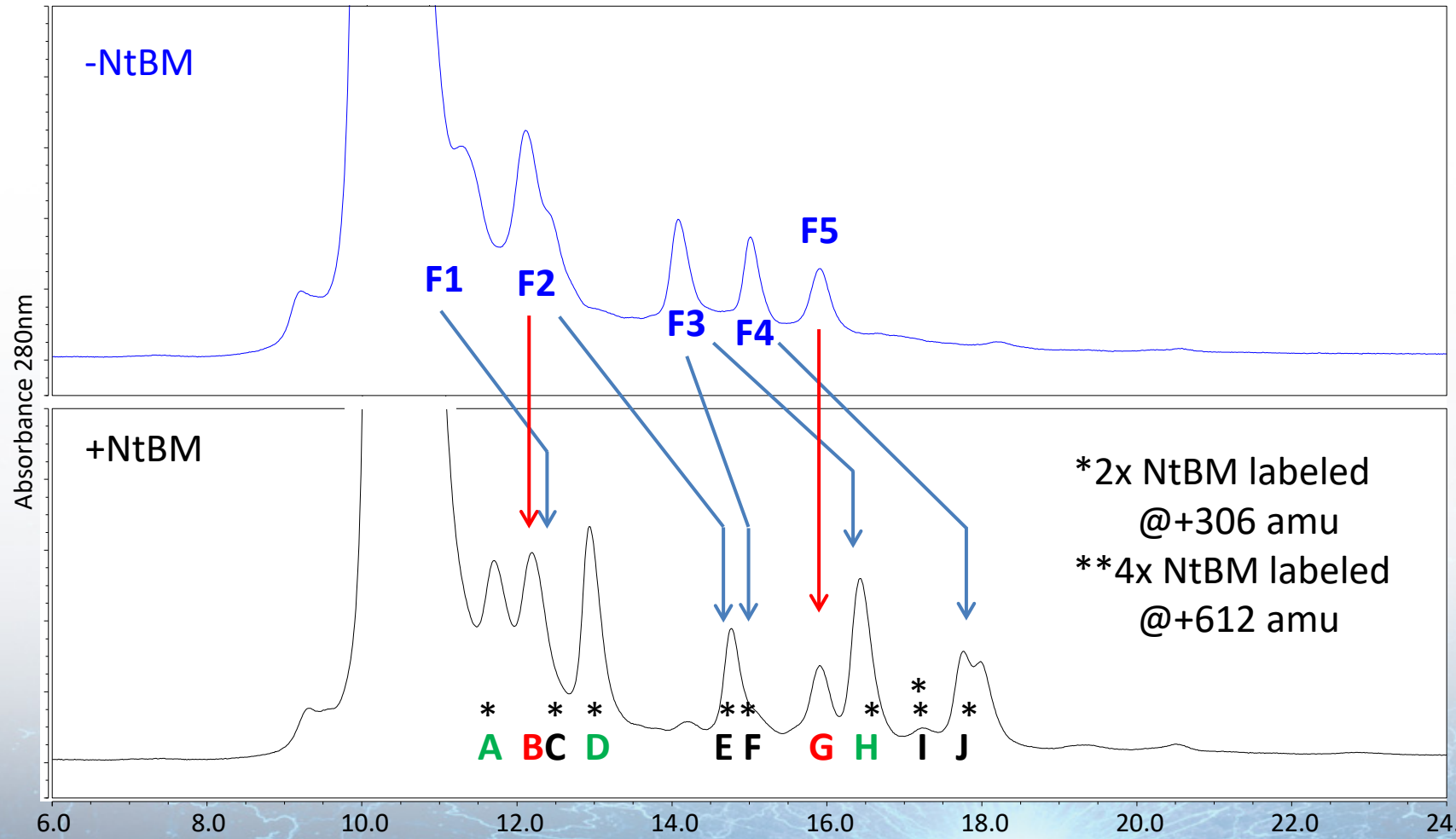
Resolution Has it Costs: What are those Resolved Peaks (trastuzumab)?

Column: 2.1 x 150 mm HALO 1000 Diphenyl; Flow rate: 0.25 mL/min; A H_2O/0.1% DFA; B: ACN/nPropanol (50/50)/0.1% DFA; Gradient: 29-33 %B in 30 min; 60°C;
Injection Volume: 2 µL; Detection: 280 nm; MS Analysis in Orbitrap Velos Pro, 15,000 Rs, 3.8 kV, 275°C; NtBM labeling in 3.6 M GuHCl/100 mM NaOAc (pH 5.3)



Literature suggests resolved "late peaks" are free thiols. This is mostly confirmed.

Direct LC/MS reveals "late peaks" are within 3-11 amu of native IgG1 (PNGase +/-).

NtBM labeling was conducted on whole IgG1, and resolved fractions.

Two of the components (B/G) are not NtBM labeled, and assumed to not have free thiols. ↓

Main peak shows no free thiols after fractionation.

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Summary and Future Work

- Improving protein separations is both particle and chemistry (SP and MP).
- Superficially porous particle silica packing materials have met the promise of supplying superior separations. Fused-Core with enlarged pore sizes (400 and 1000 Å) have particular utility for protein analyses, are robust, and routinely allow *faster* protein separations with *higher* efficiency.
- Subtle, but useful, differences in selectivity are available with additional bonded phases available on 1000 Å pore size materials (C4, ES-C18, DP).
- For protein analysis, temperature optimization is crucial to maximize recovery and selectivity, but diligence is required to avoid artifacts.
- Work continues on optimizing pore size and geometry for silica SPP, using novel materials science approaches. The end points will be defined by careful analysis of resolved protein samples, with limited guidance guaranteed from small molecule analysis.
- The more resolution gained with these newer RP materials, the greater detail that can be obtained on subtle structure variations in WCBPs.

Acknowledgements

- Joe Destefano, Tim Langlois, Conner McHale, and Bob Moran, Ben Libert, Stephanie Schuster (AMT), Joe Glajch (Momenta), Prof. Ronald Orlando and Marla Popov (UGA, Glycoscientific) are thanked for advice, technical assistance and samples. Dr. Wei (Genentech), Dr. Zhang (Celgene) and Dr. Liu (Nektar) have been valued collaborators on mAb analysis.
- AMT management for ongoing support on protein analytical science.
- This work was supported in part by National Institute of General Medical Sciences, [GM116224 and GM108122 to BEB]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health.

Thank you for your Attention