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advancedmaterialstechnology

Understanding and Overcoming Separation Challenges in the Biological Drug Development Process

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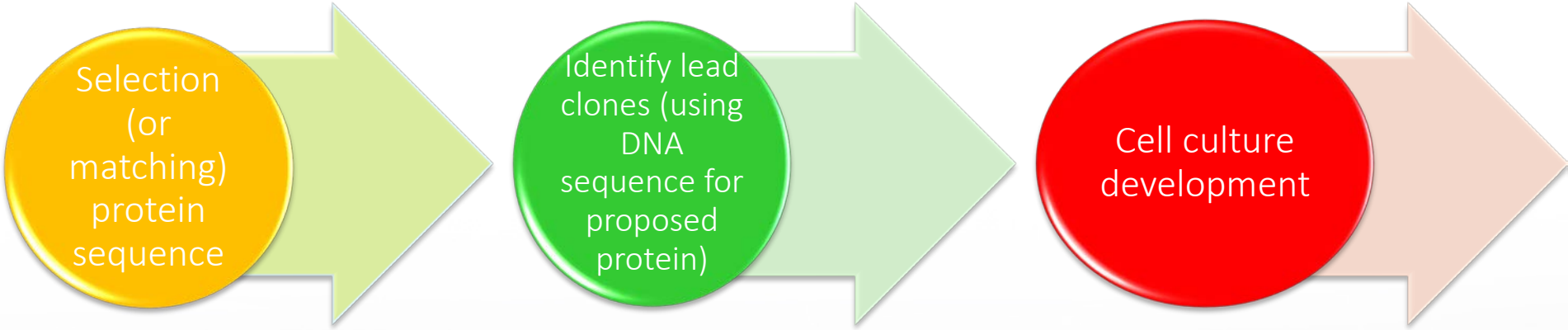
Outline

- Overview of the Biological Drug development stages
  - Background on methodologies and techniques used
- What are the advantages of using Fused-Core particle technology for biologics?
  - When SPP is helpful
  - How to get the most from tailored solutions for large molecules
- Proposed strategies for versatile platform methods
  - Process, Characterization, Final Release/QC

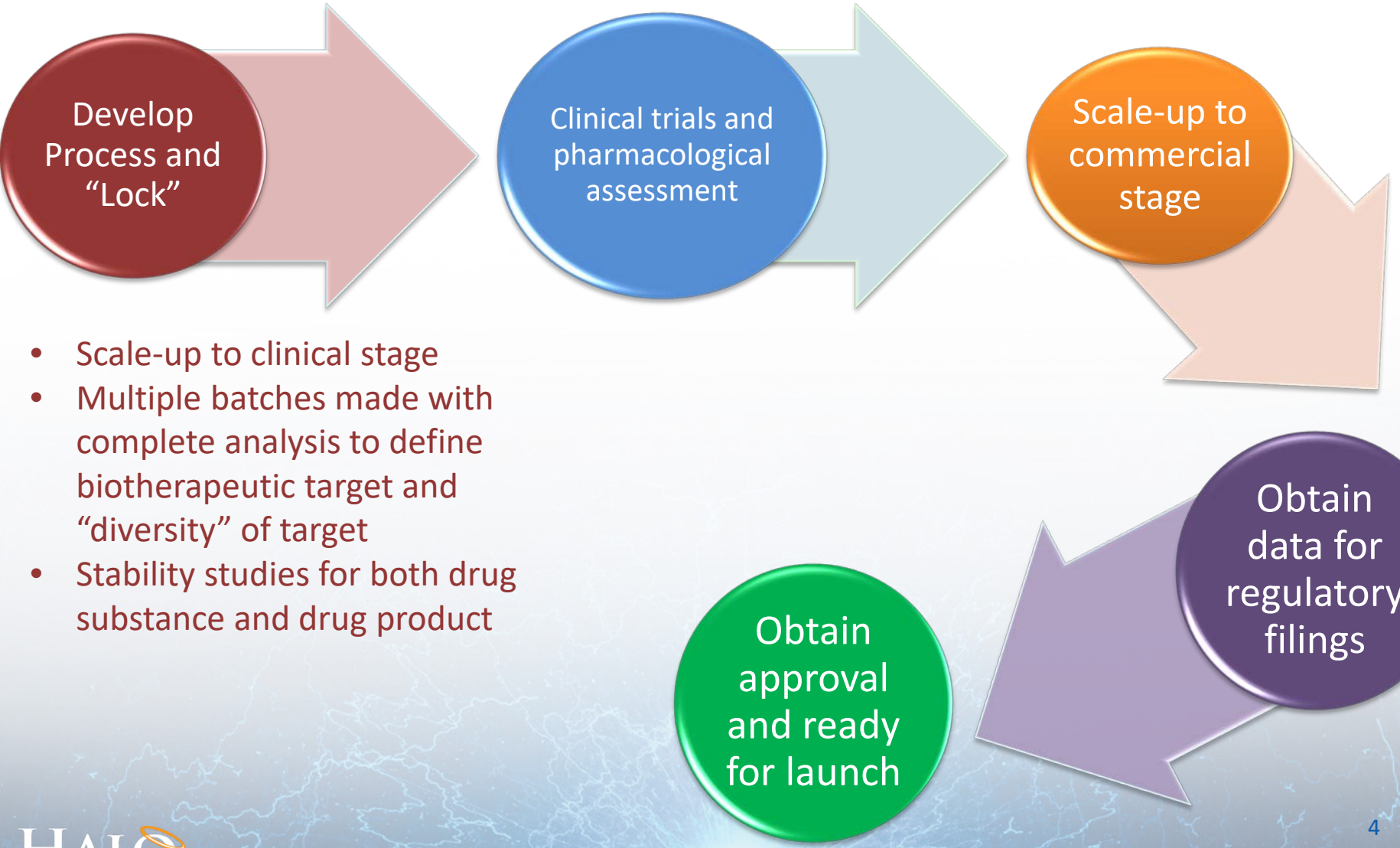
Development Stages for Biotherapeutic



- Clonal pool
- Individual lead clones (usually 2-3)
- Selection of final clone (based on exactness of match, titer, and stability)
- Generation of Master Cell Bank (MCB) and Working Cell Bank (WCB)
- **Primary analytical needs are titer (protein concentration/run) and sequence verification**

- Develop feed times, cycles, days per run, etc.
- Primary analytical needs are titer, IEX for charge states, SEC for monomer and aggregates

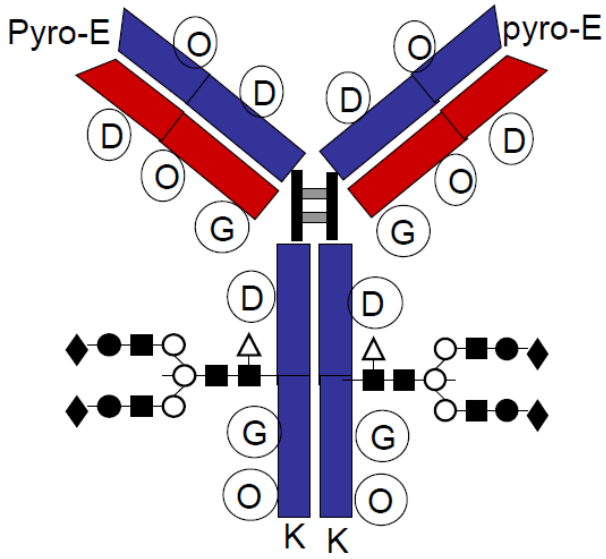
Development Stages of Biotherapeutic



- Scale-up to clinical stage
- Multiple batches made with complete analysis to define biotherapeutic target and "diversity" of target
- Stability studies for both drug substance and drug product

Complexity of Monoclonal Antibody

Attributes & Combinatorics



- Pyro-Glu (2)
- Deamidation (3x2x2)
- Methionine oxidation (3x2)
- Glycation (2x2)
- High mannose, Fucosylation G0, G1, G1, G2 (10)
- Sialylation (+5)
- C-term Lys (2)

- $(16,920)^2 \approx$ 285 million
- 2 x 12 x 6 x 4 x (10+5) x 2 = 16,920

Note: Taken from Kozlowski, FDA/PQRI Conference on Evolving Quality, Sept. 2015

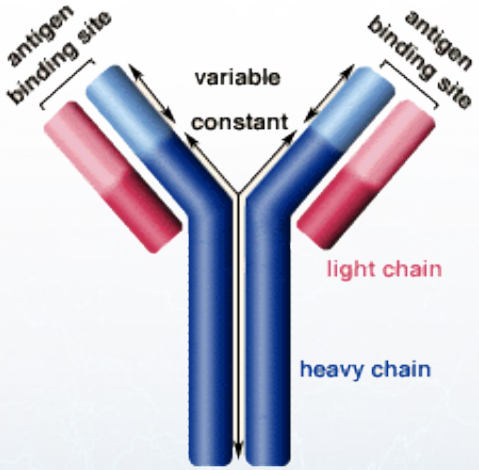
Overview of some methods used for physicochemical characterization

Protein Backbone
- Amino acid sequence
- Molecular weight
- Amino acid composition
- Charge profile distribution

PTMs Glycosylation
- Galactosylation
- Galactose-α-1,3 galactose
- Sialylation
- N-glycolylneurominic acid
- Core fucosylation
- High mannose structure
- Low abundance glycan species
- Aglycosylation

Protein Backbone Modifications
- N-terminal variation
- C-terminal variation
- Deamidation
- Oxidation
- C-terminal amidation
- Glycation

Higher Order Structure
- Protein Folding
- Disulfide connectivity
- Free cysteine
- Enthalpy of unfolding
- Tertiary structure
- Spectroscopic properties



Aggregation
- Percent Monomer
- Aggregates
- Fragmentation
- Sub-visible particles
- Hydrodynamic radius

Formulation and DP properties
- Protein extinction coefficient
- Protein concentration
- Solution properties
- Formulation Components
- Container Closure Components
- Process Impurities
- Leachables and Extractables

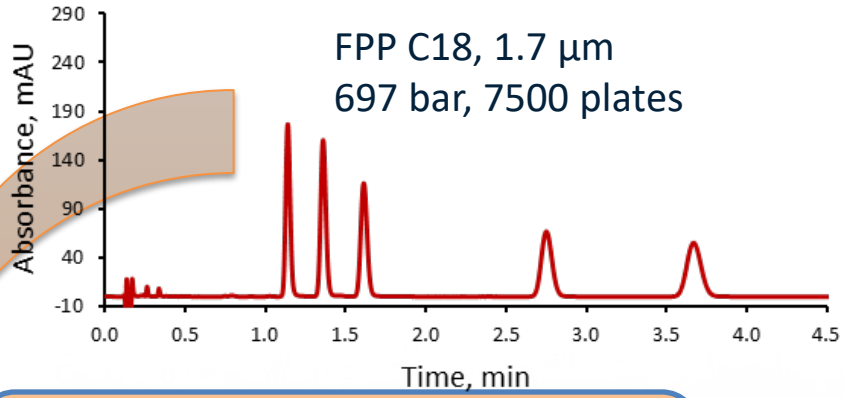
Stability Profile
- Comparative stress stability
- Stress stability
- Long term stability studies

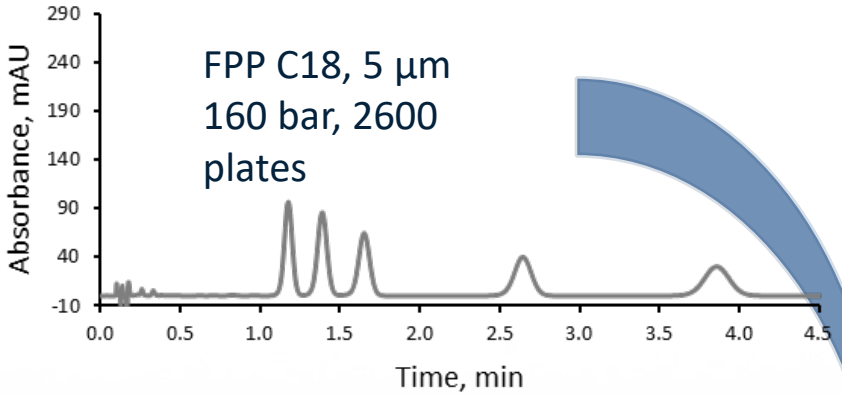
Host Derived Impurities
- Host Cell Protein
- Host Cell DNA

Separations needs in Biopharmaceutical Development

- Most separations can be done by any type of modern column
  - Fully porous 3-5 µm particles (typically with HPLC)
  - Fully porous sub 2 µm particles (typically with UPLC)
  - Superficially porous 2-5 µm particles (either UPLC or HPLC)
- Major advantage of superficially porous particles (SPP) is ability to generate high efficiency separations (plate number) in reasonable time
  - Couple the low back pressure with high efficiency of SPP

Comparison of Fully Porous and Superficially Porous Particle Columns



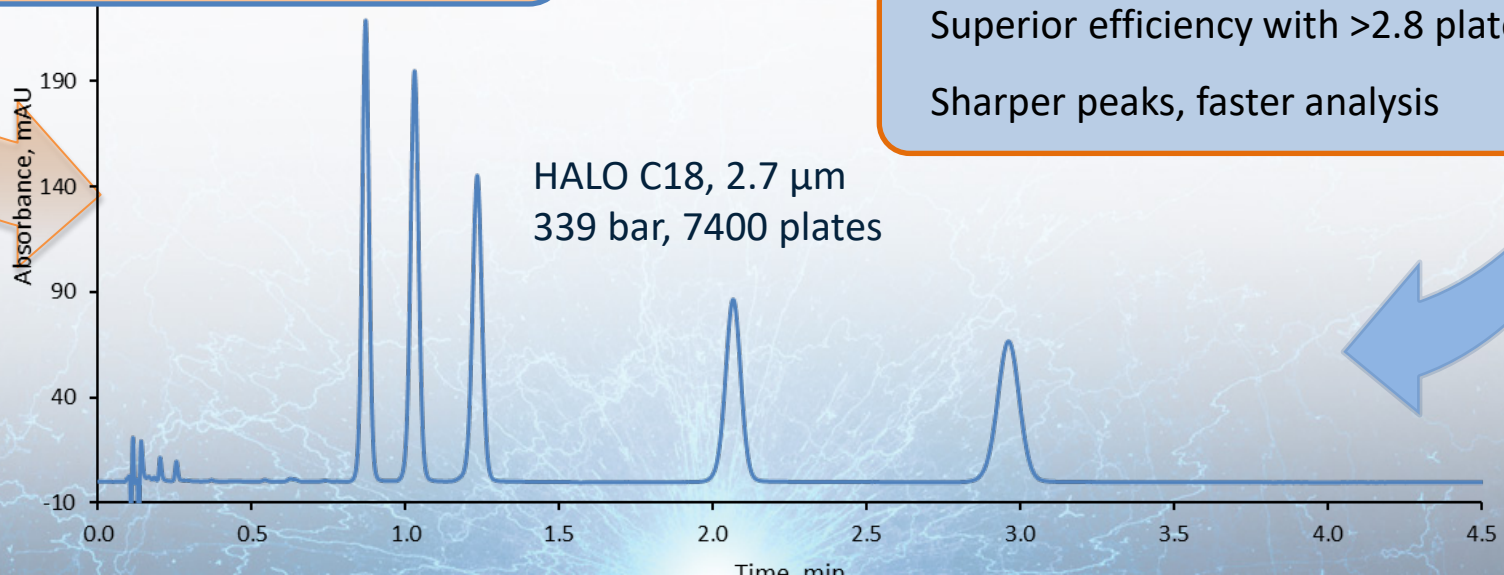


High performance with <1/2 the back pressure

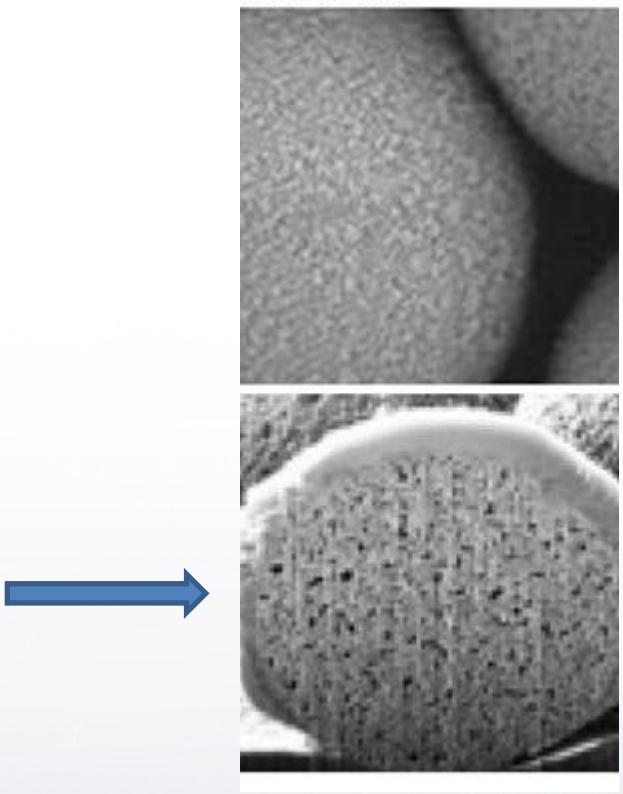
Faster analysis

Superior efficiency with >2.8 plates!

Sharper peaks, faster analysis

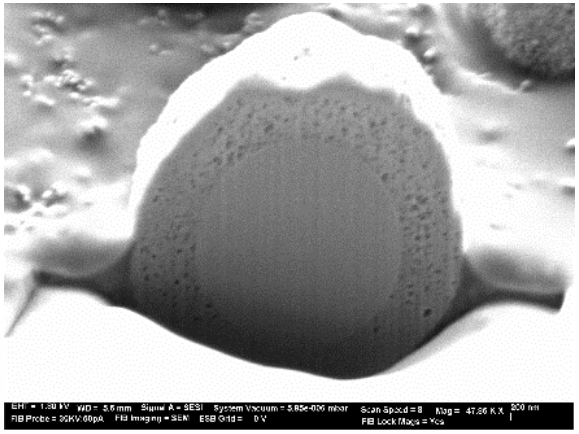


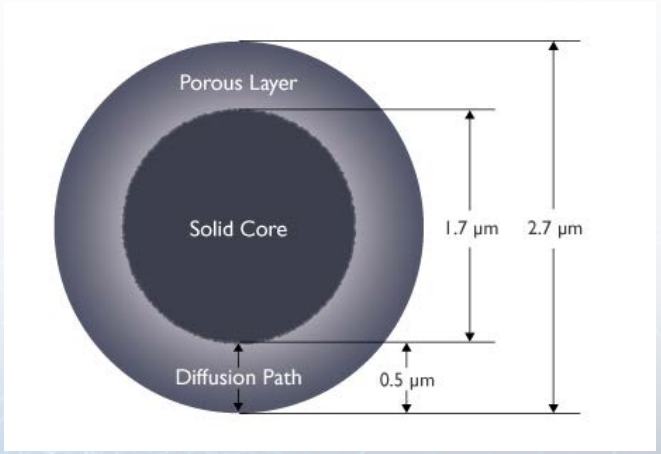
What is SPP Technology?



Fully Porous Particle (FPP)

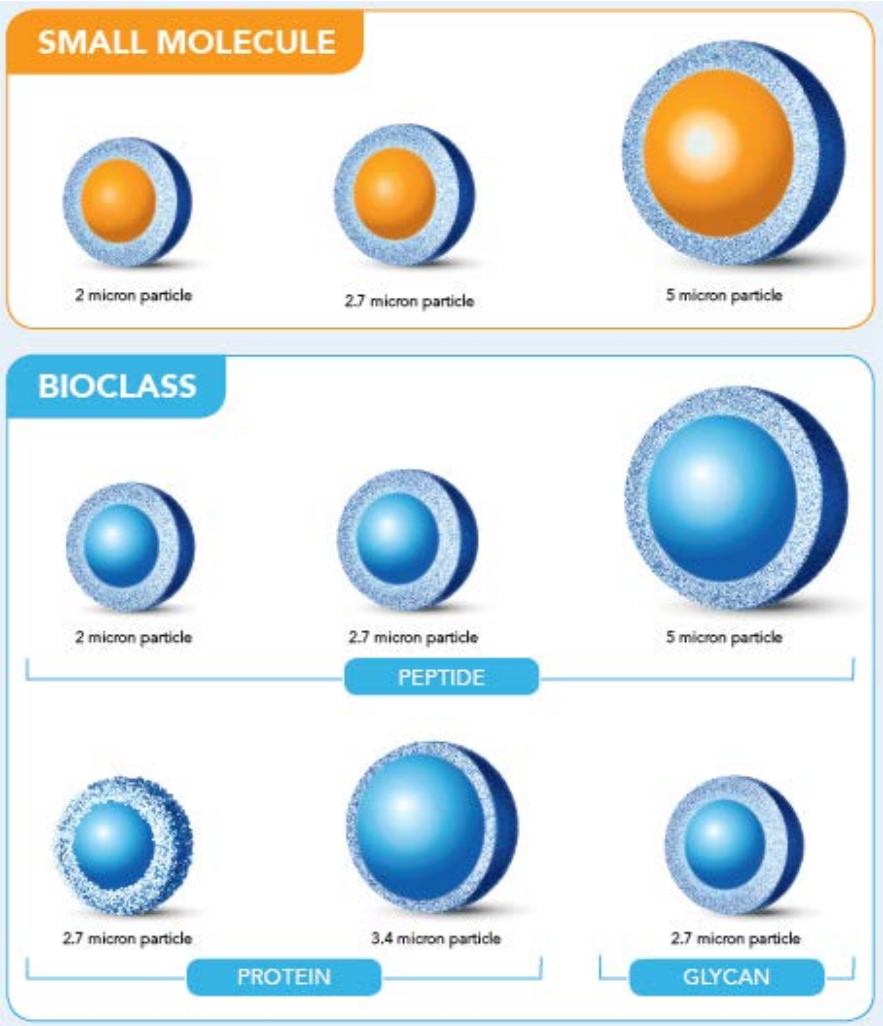
HALO 90 Å, 2.7 µm

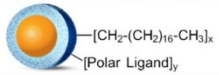
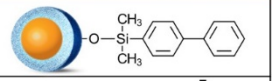
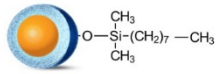
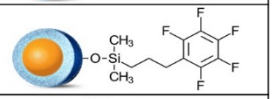
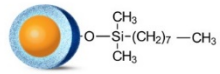
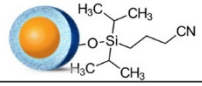
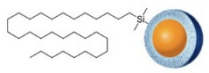
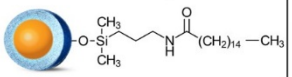
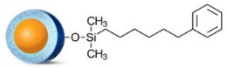
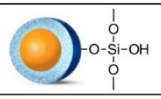
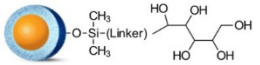


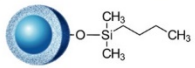
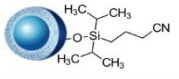
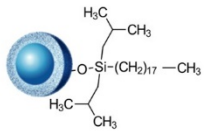
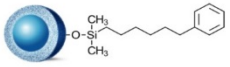
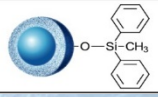
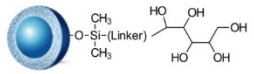


Superficially Porous Particle (SPP)

HALO® Fused-Core® Particles

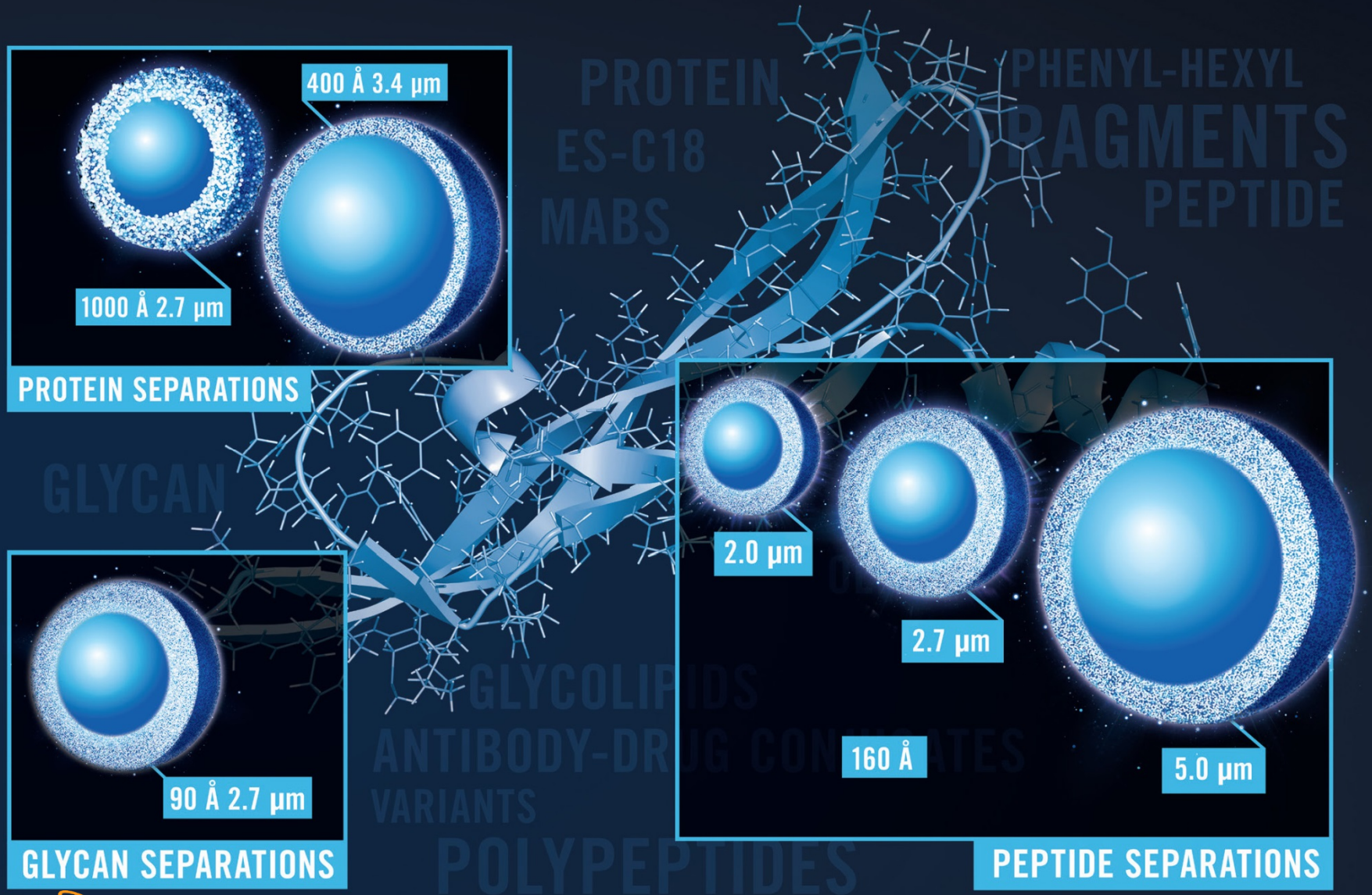


Phase	Structure	Phase	Structure
AQ-C18		Biphenyl	
C8		PFP	
C18		ES-CN	
C30		RP-Amide	
Phenyl-Hexyl		HILIC	
		Penta-HILIC	

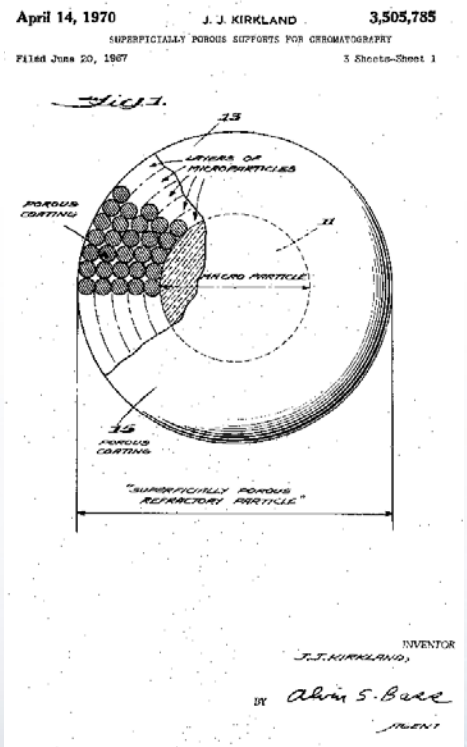
Phase	Structure	Phase	Structure
C4		ES-CN	
ES-C18		Phenyl-Hexyl	
Diphenyl		Glycan	

Portfolio of products with varying particle morphologies designed for a broad range of applications

Large Molecule Options: Bioclass



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.

13

LAYERS OF MICROPARTICLES

POROUS COATING

11

MACRO PARTICLE

15

POROUS COATING

"SUPERFICIALLY POROUS REFRACTORY PARTICLE"

INVENTOR

J. J. KIRKLAND,

BY Alvin S. Burr

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.

Wide Pore SPP 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

Higher Overall Efficiency (Maximum N)

- If time is not a constraint
  - Lower back pressure of highly efficient porous shell columns can produce high efficiency (N)
- Comparison of different columns and maximum plates (theoretical calculations for small molecule at 50% ACN/water)

Column type	Length (mm)	Flow rate (mL/min)	Plates (N)	Pressure (bar)	Plates per pressure	Plates at 400 bar	Plates at 700 bar
5 µm porous	150	0.6	14,600	100	146	**58,400**	102,200
3 µm porous	150	0.6	24,200	309	78	**31,327**	54,822
1.8 µm porous	150	0.6	30,840	771	40	**16,000**	28,000
5 µm porous shell	150	0.6	28,300	78	363	**145,128**	253,974
2.7 µm porous shell	150	0.6	38,300	284	135	**53,944**	94,401

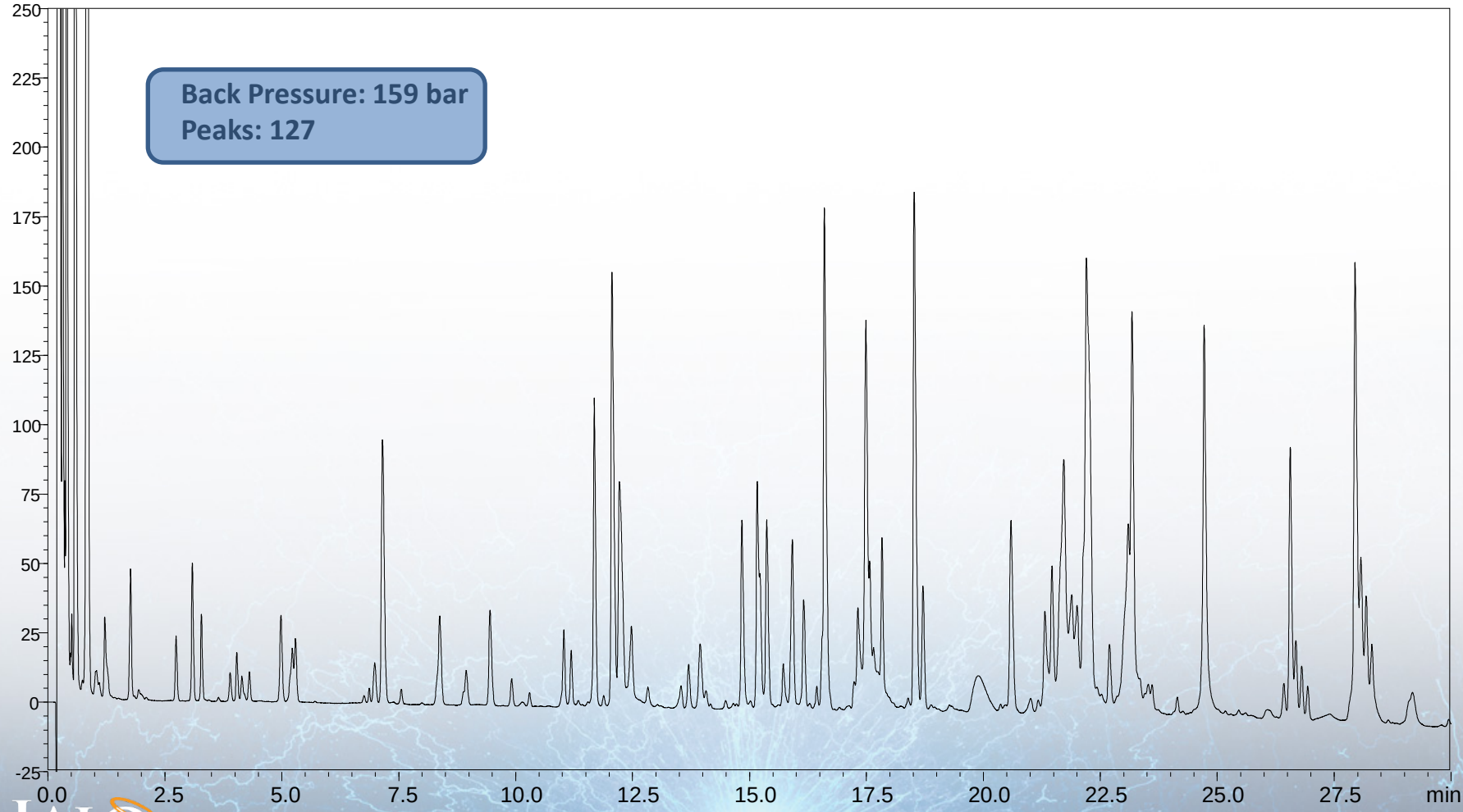
Demonstration of Utility for Peptide Mapping

- Peptide mapping is widely used and needed for both characterization and release of biological molecules
- Typically digest a protein with an enzyme (Trypsin, Chymotrypsin, Lys-C, etc.) and separate the peptides generated
- Generate sample with many peaks (50-100 or more)
- Need for high efficiency separation

Comparison of Complex Peptide Map

30 min gradient

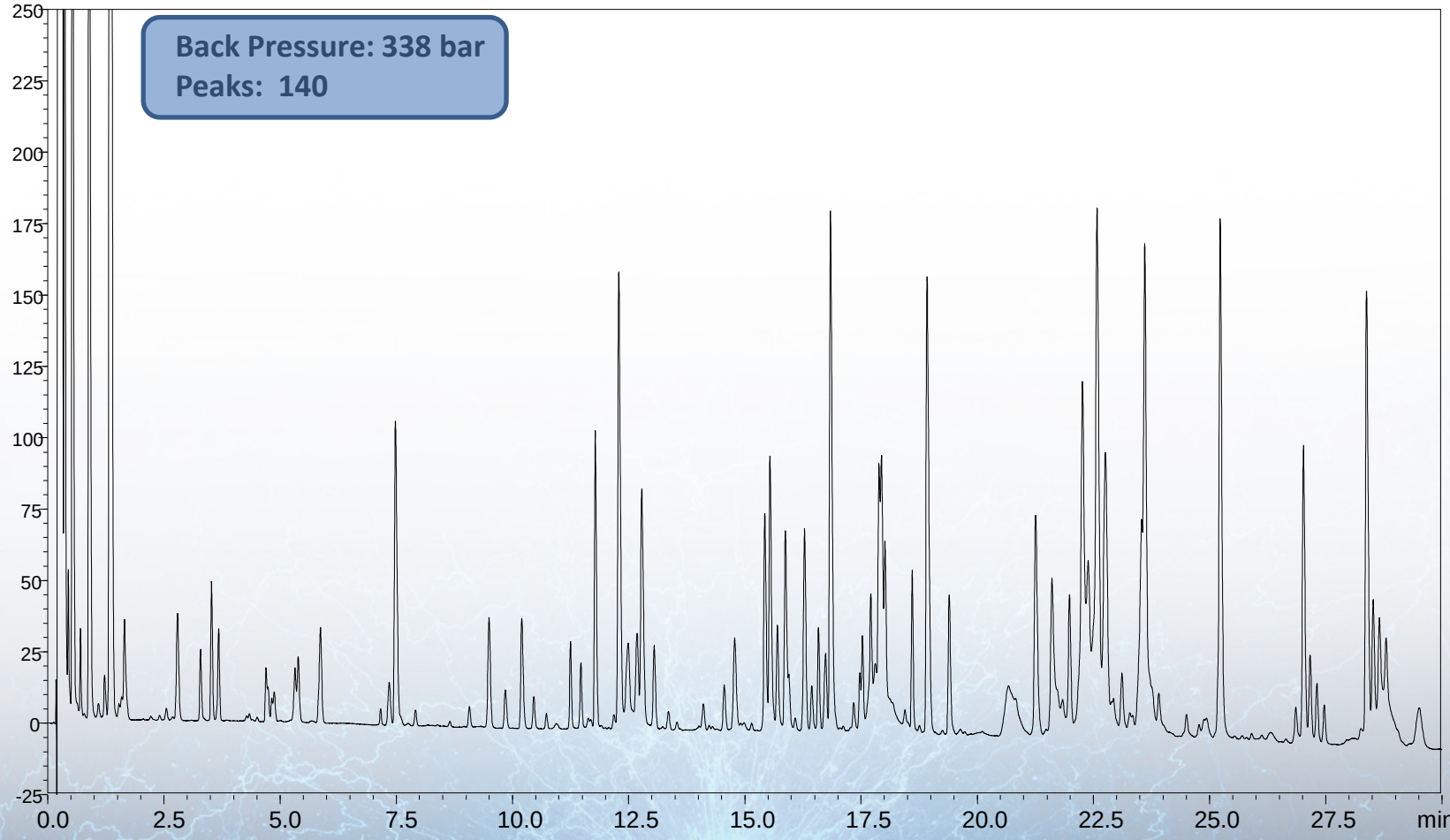
HALO 160 Å ES-C18, 2.7 µm, 2.1x50 mm



Comparison of Complex Peptide Map

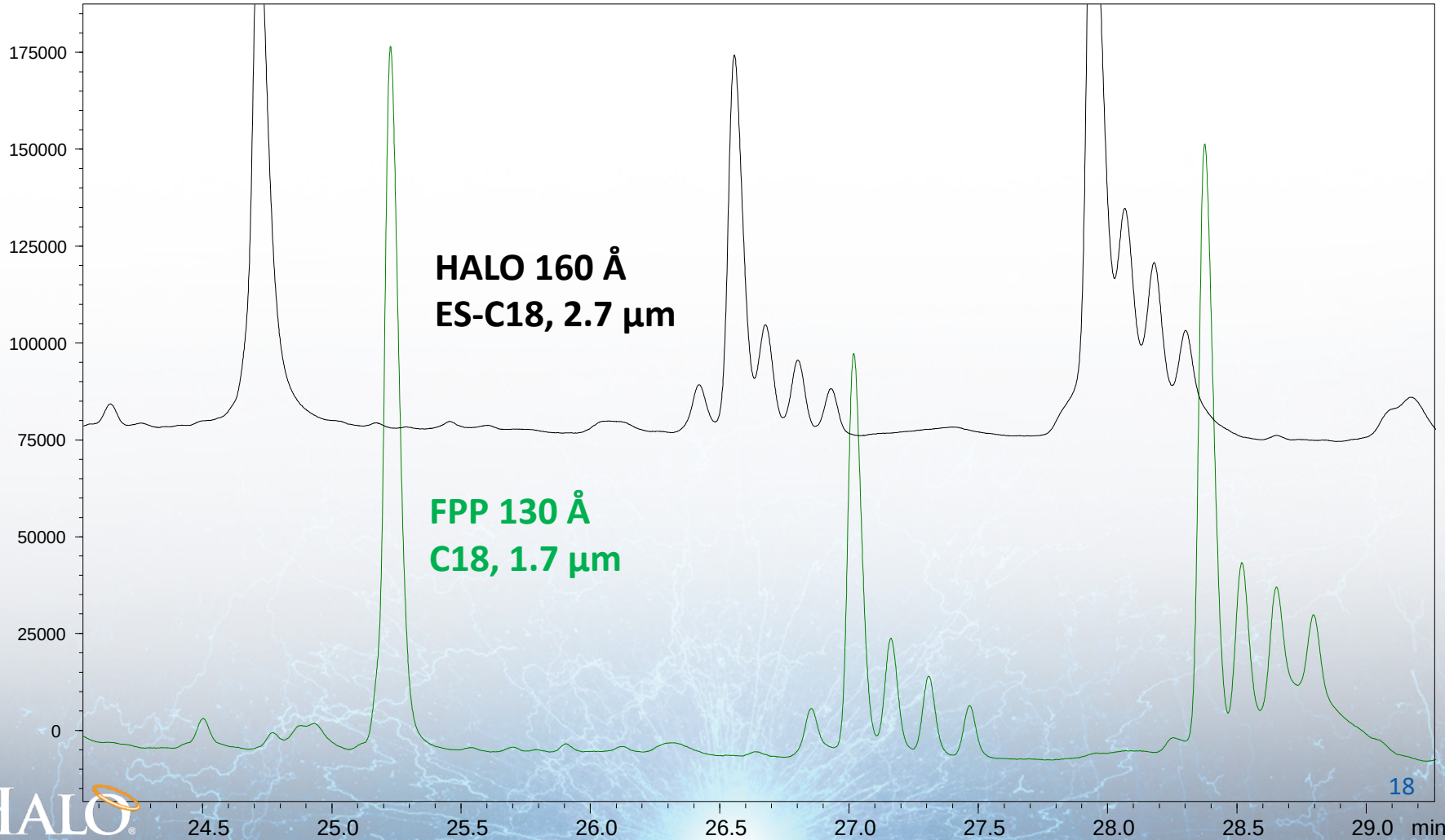
30 min gradient

Competitor C18 (FPP) 130 Å C18, 1.7 µm, 2.1x50 mm



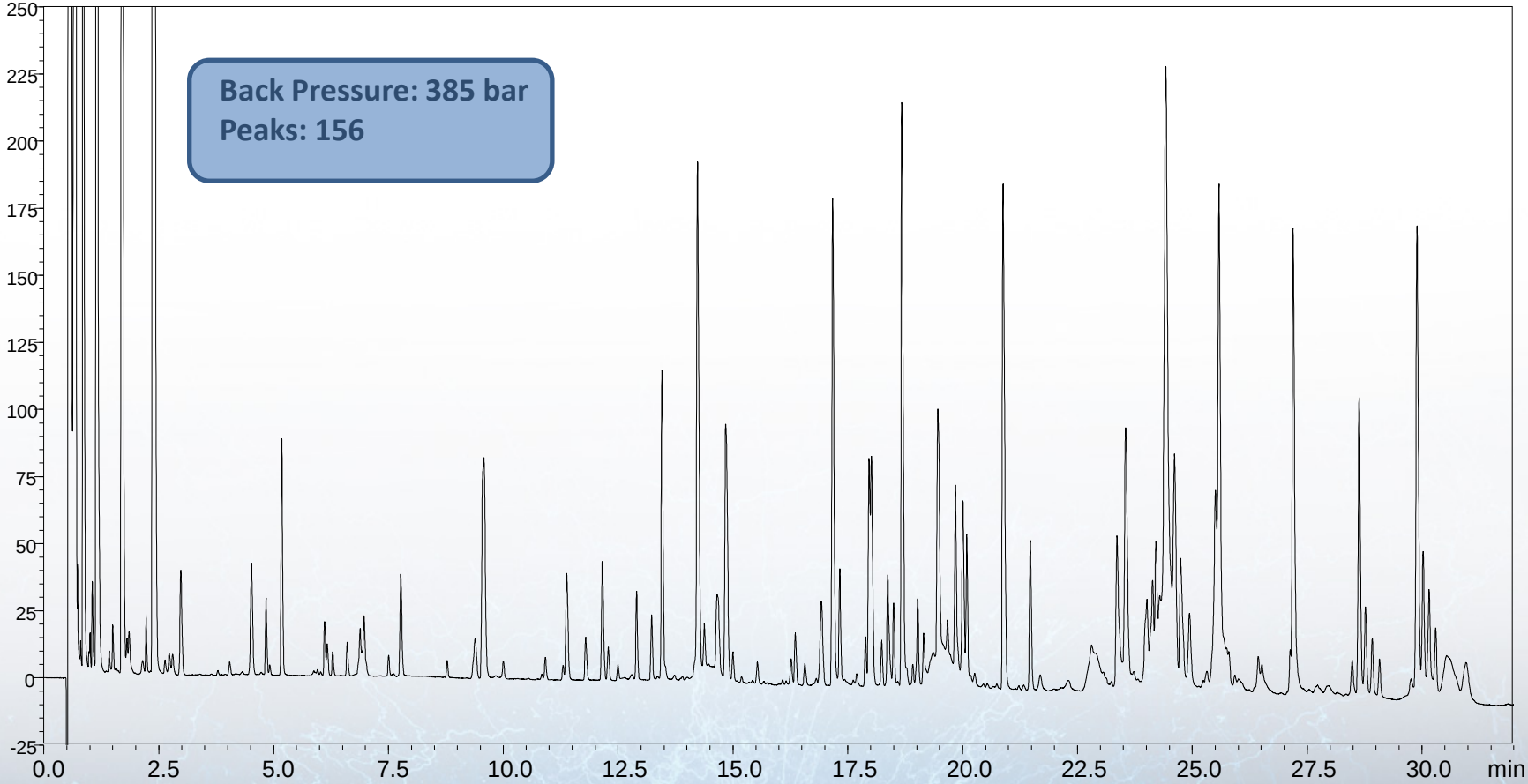
Comparison of Complex Peptide Map

SPP vs. FPP Comparison, 2.1x50 mm
30 min gradient, 24-29min segment



Improvement Potential of Peptide Map with SPP

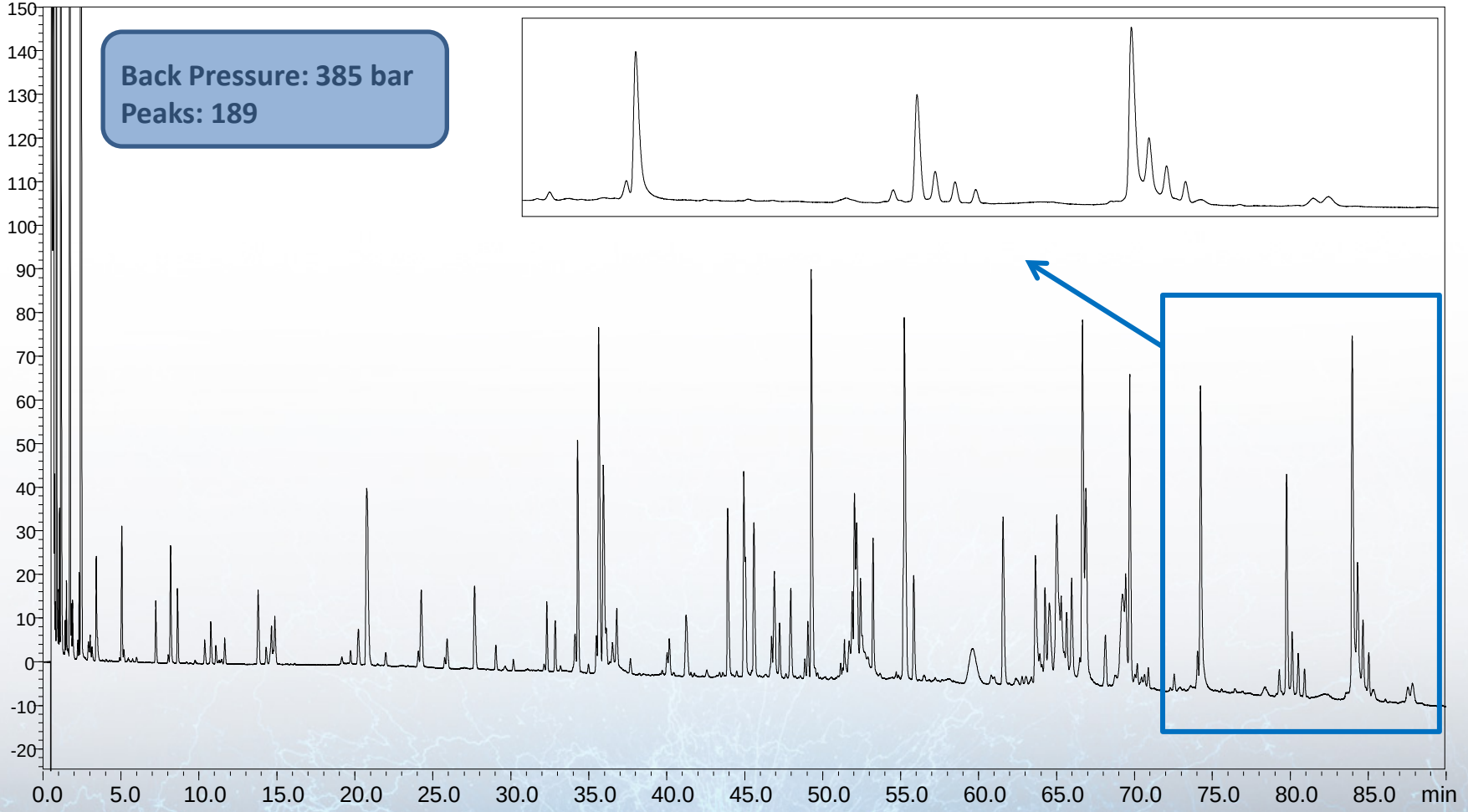
30 min gradient



HALO 160 Å ES-C18, 2.7 µm, 2.1x150 mm

35% more peaks with SPP vs. FPP and less than 400 bar back pressure!

90 min gradient



HALO 160 Å ES-C18, 2.7 µm, 2.1x150 mm

Three main areas for separations in development

Process Support

- Titer, glycans, or other post-translational modifications
- Can be rapid and lower resolution

Characterization

- Methods should be high resolution, can afford extra time for analysis since characterization is done only on limited lots

Final release/QC

- Methods should be fast and need not be high efficiency

Proposed Strategy for Versatile, Platform Methods

1

- Develop high resolution methods using long SPP columns (150 mm or longer) with either 2.7 µm or 5 µm particles

2

- Use these methods for characterization

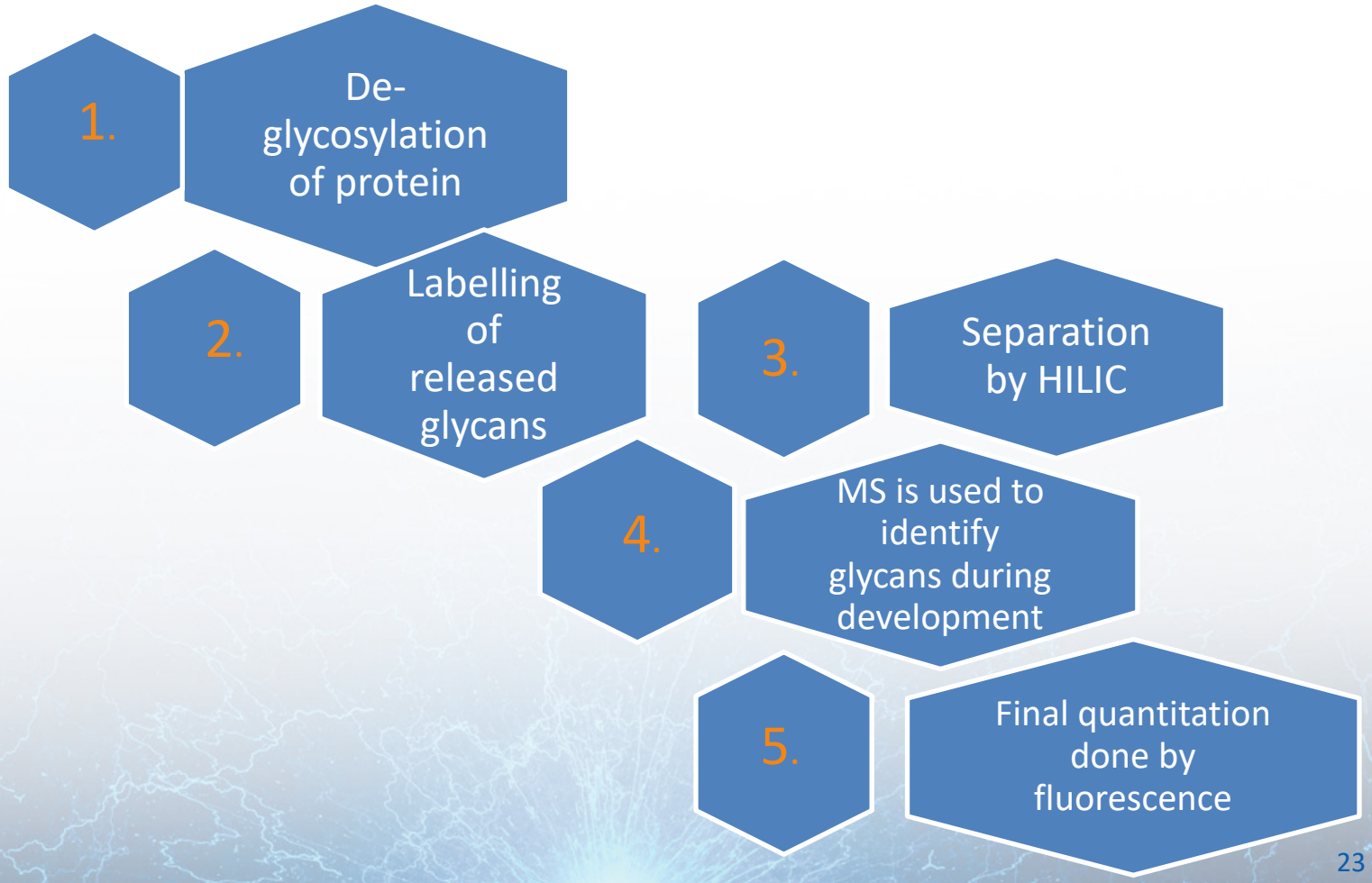
3

- Modify methods by shortening column length and/or increasing flow rate for process support and QC work

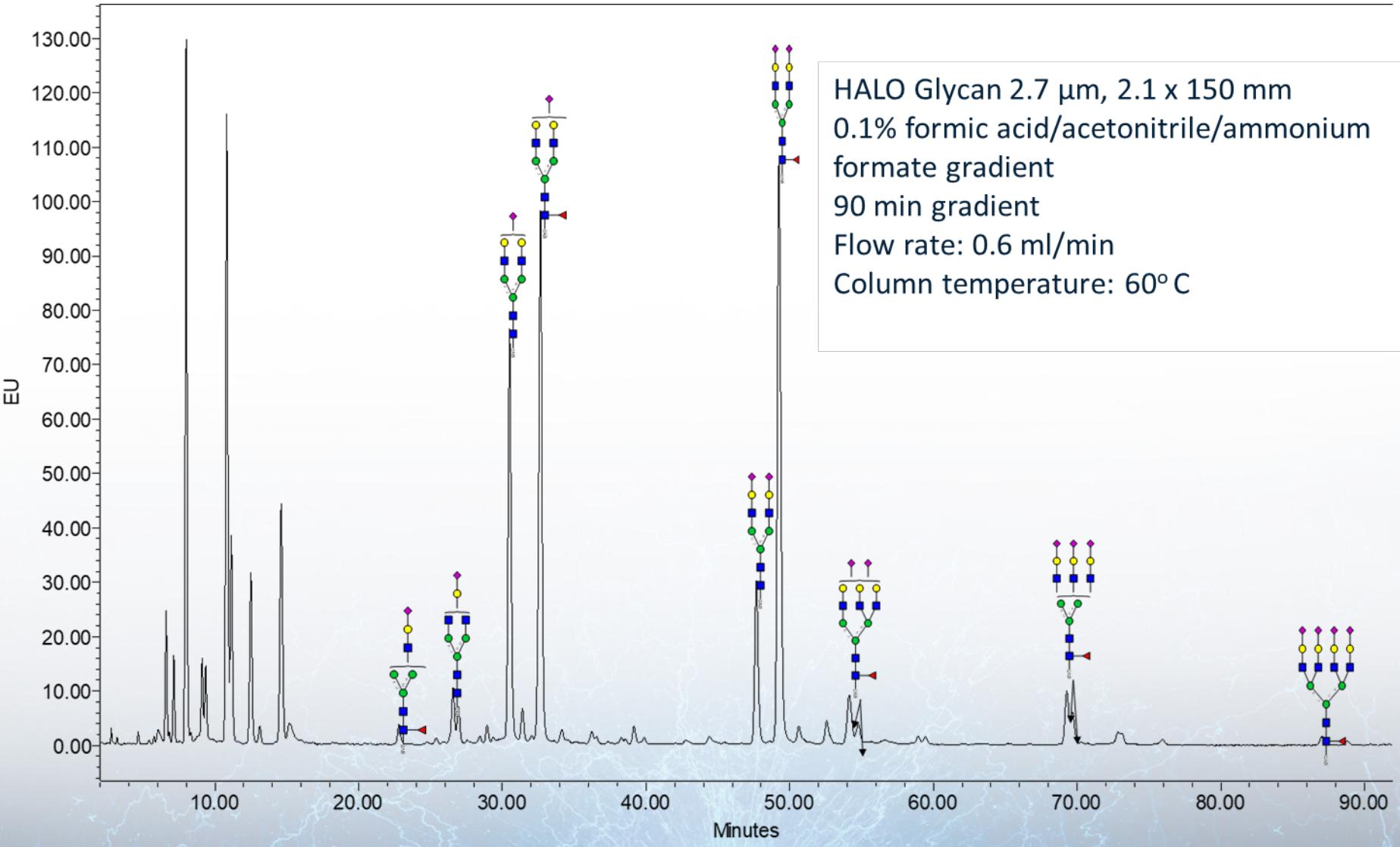
- Advantage of this approach
  - Same columns used for multiple purposes and translation from one method to another is easy

Glycan Analysis by HPLC/(MS)

Analysis of glycosylation:



HILIC Analysis (Typical Extended Gradient) of a Highly Sialylated Protein



Initial results of HILIC method

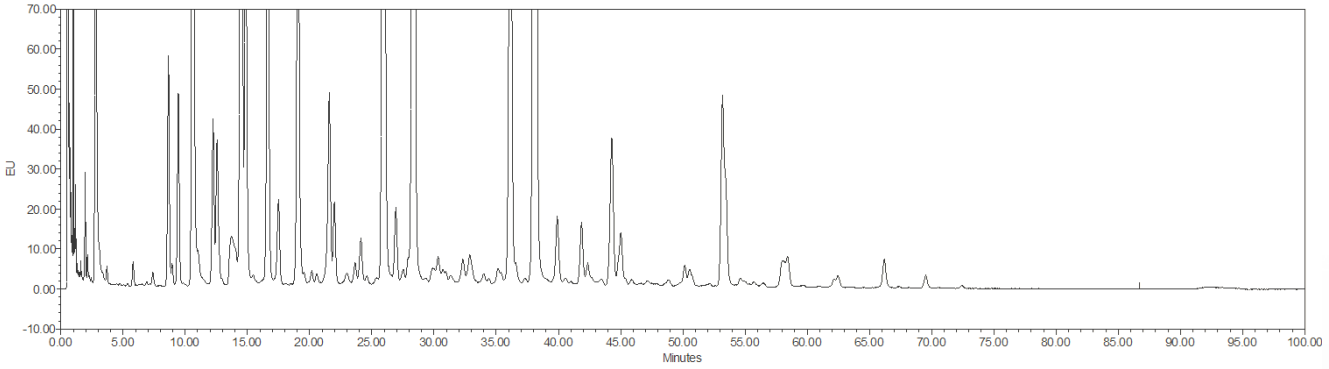
- Able to separate > 70 glycan species
  - Peak capacity was about 200
- Quantify most of those ≥ 0.2%
- Quantitation by fluorescence; identification by MS
- Only 1 µL of sample could be injected; limited sensitivity
- Was gradient really optimized? Look at k* calculation

$$k^* = \frac{0.87 * tGF}{Vm * \Delta\%B * 4}$$

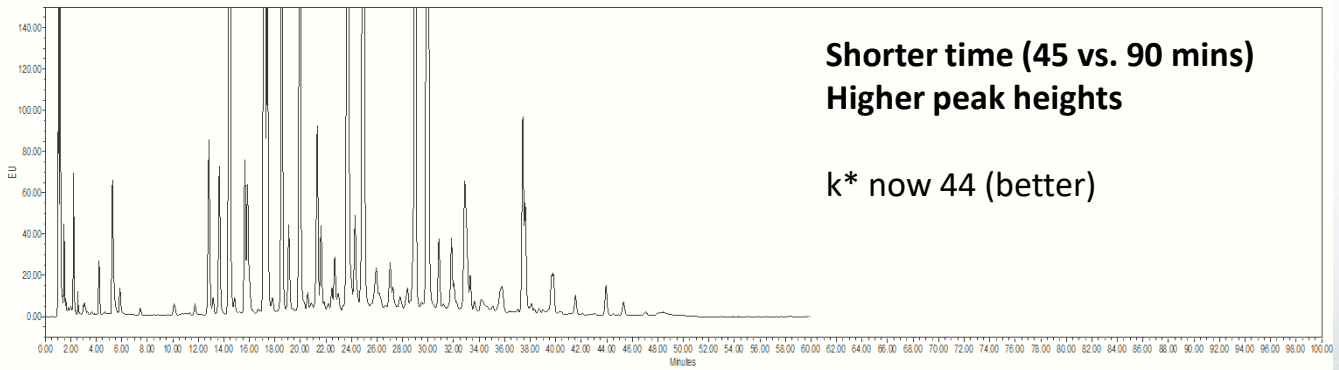
- Best k* is usually between 5-10
- Initial condition; k* =178!
- How to reduce? Lower t_G (faster gradient); lower flow rate

Comparison of Initial vs. Final Results

0.6 mL/min; 90 min gradient; 150 mm column



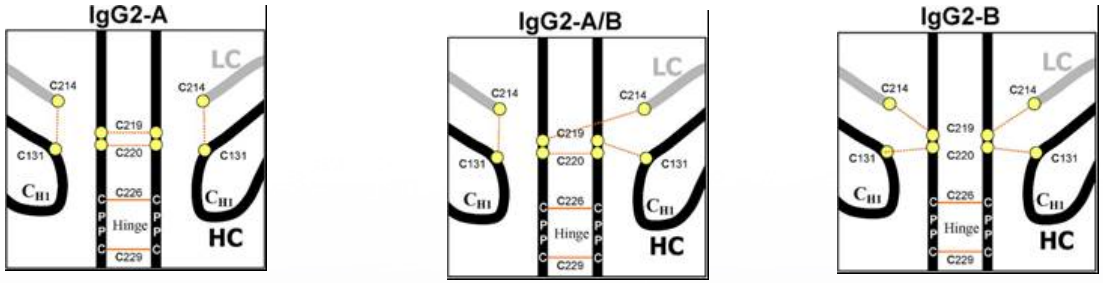
0.3 mL/min; 45 min gradient; 150 mm column



Shorter time (45 vs. 90 mins)
Higher peak heights

k* now 44 (better)

Wide-Pore Columns for Difficult “Isomer” Separations
Separation of IgG2 isoforms

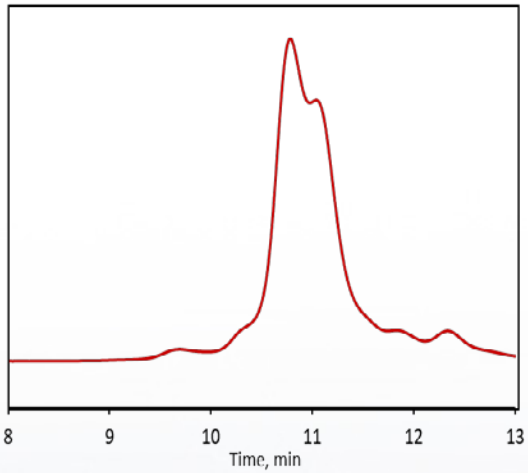


Hinge disulfides in IgG2 molecules can exist in three different forms in the native state: A, A/B and B.
Figure is from Dillon et al. (2008).

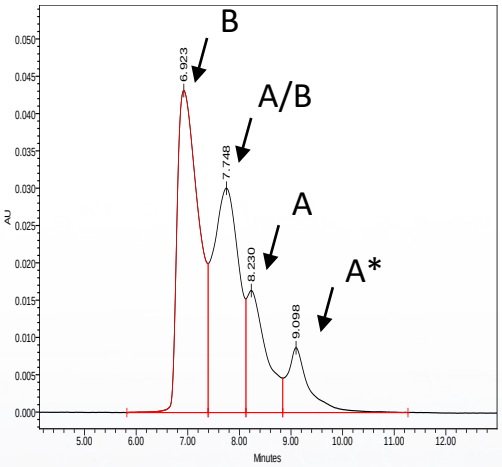
Dillon, T.M., Ricci, M.S., Vezina, C., Flynn, G.C., Liu, Y.D., Rehder, D.S., Plant, M., Henkle, B., Li, Y., Deechongkit, S., Varnum, B., Wypych, J., Balland, A., Bondarenko, P.V. (2008) Structural and Functional Characterization of Disulfide Isoforms of the Human IgG2 Subclass, *J. Biol. Chem*., 283, 16206-16215.

HALO 1000 Å C4 Column vs. Competitor Protein Columns (IgG2 Antibody X)

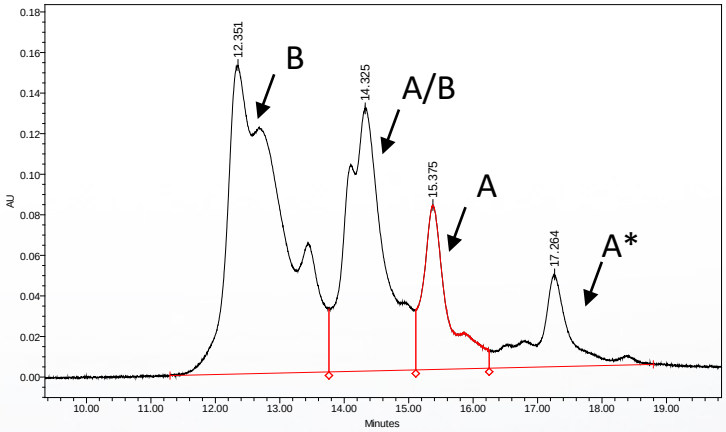
Competitor Fully Porous 1.8 µm 300 Å C4

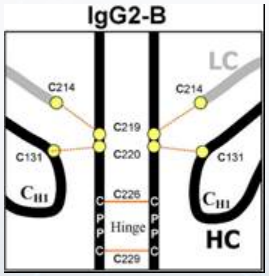


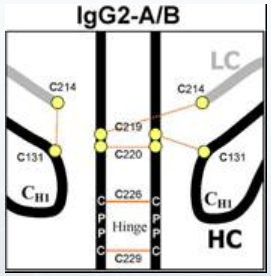
Competitor Superficially Porous 300 Å C8

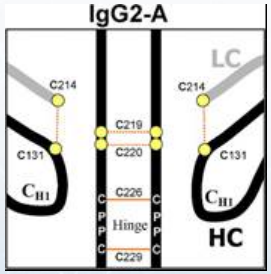


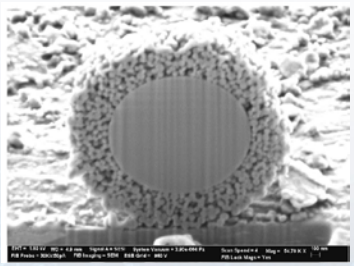
HALO 1000Å C4











Summary and Conclusions

- Biological Drug Development requires testing at many stages during and after the process
- Separations, especially HPLC, are very important in this testing
- Process, Characterization, and Final Release/QC all have specific requirements
- Use of column with Superficially Porous Particles (SPP) can be beneficial in all stages to maximize analytical efficiency

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- Mac-Mod Analytical