The Applications of Empore™ Membrane SPE for Proteomics

May 2020

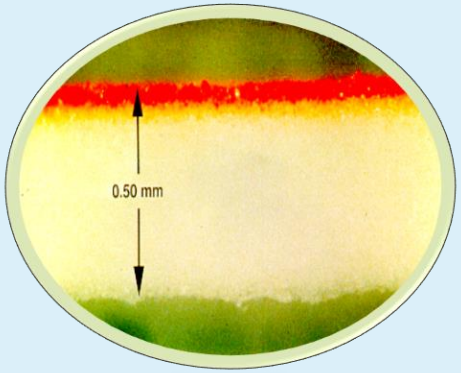
CDS Analytical

Outline

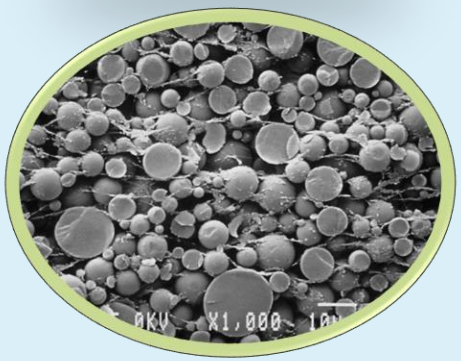
1. Empore SPE Introduction
2. Application in Proteomics

Empore™ Technology Highlights

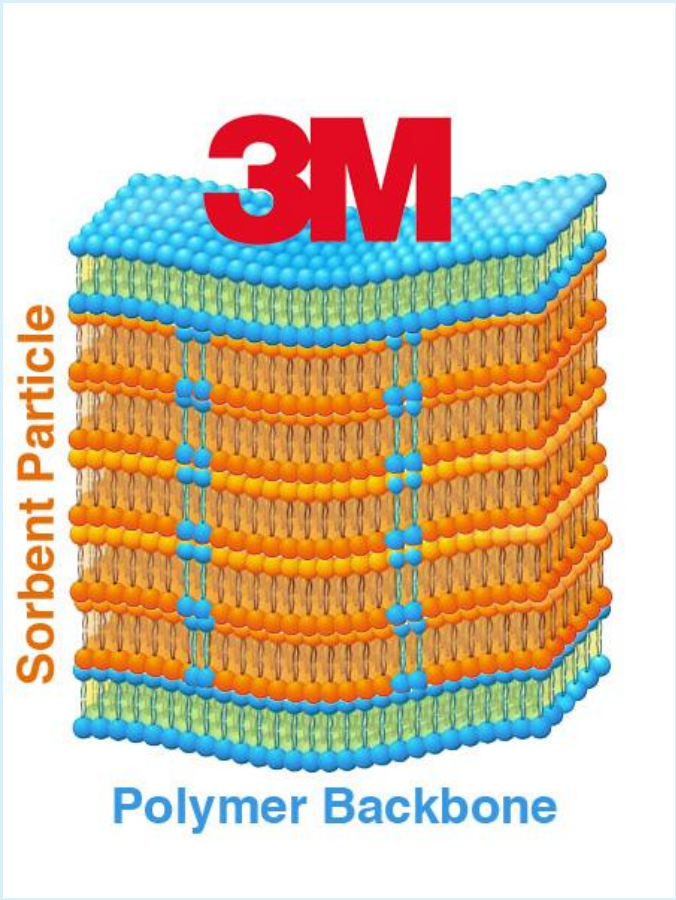
- 3M™ patented SPE particle-loaded membrane

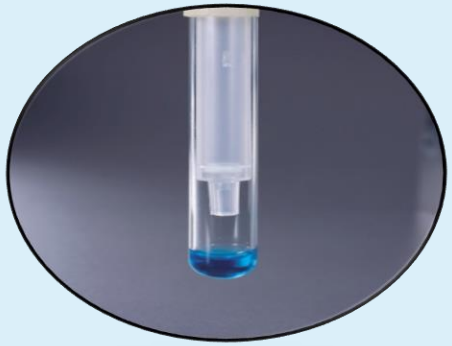


- Best Uniformity

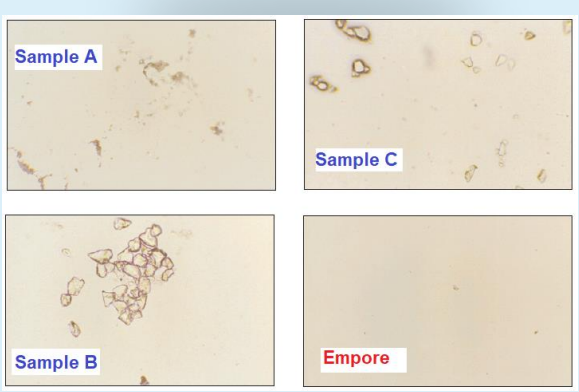


- High Density



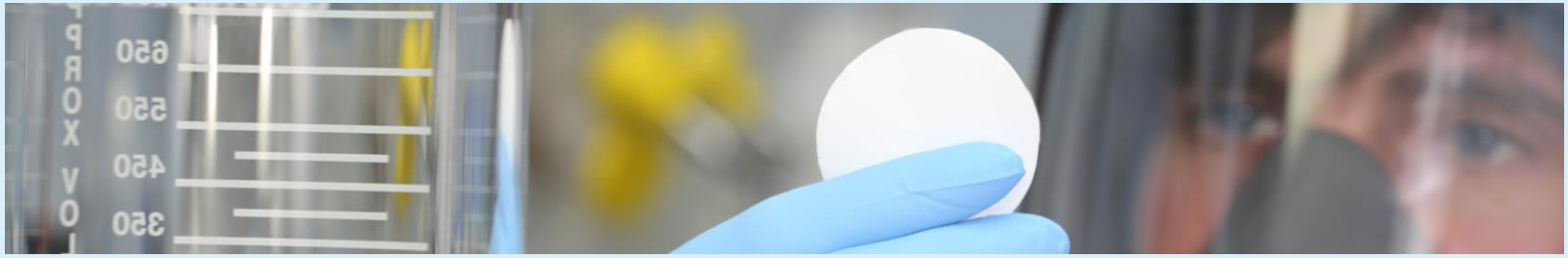


- Least Elution Volume



- Reduce Particle Fines

Empore™ Portfolio



- **Empore™ by Package Style**
  - **Disks**
  - **Cartridges**
  - **Plates**
  - **StageTips**

- **Empore™ by Sorbent Style**

Reverses Phases: C8, C18, UR, SDB-XC, Activated Carbon, Oil & Grease

Mixed Phases: MPC, UR, SDB-RPS

Ion Exchange: Cation, Anion, Chelating, SDB-RPS

Empore™ Unique Features

- **Ultra-fast flow rate up to 700ml/min – 2X faster than other leading brands**
- **Least elution volume – 1/3 of other leading brands and 1/10 of loose-packed SPE**
- **Highest consistency & reproducibility – 10-15% higher than Waters Oasis plates**
- **Least particle fines in eluates -1/10 of other leading brands to increase efficiency (reducing tube clogging & system downtime)**

Recommended in Dozens of EPA Methods:

1664 (Rev. A)	- N- Hexane Extrachable Material (HEM; Oil and Grease)
506	- Phthalate and Adipate Esters in Drinking Water
507	- Nitrogen- and Phosphorous-Containing Pesticides in Water
508.1	- Chlorinated Pesticides, Herbicides, and Organohalides in Water
512.2	- Chlorinated Acids in Water
525.3	- Organic Compounds in Drinking Water
549.1	- Diquat and Paraquat in Drinking Water
550.1	- Polycyclic Aromatic Hydrocarbons in Drinking Water
552.1	- Haloacetic Acids and Dalapon in Drinking Water
553	- Benzidines and Nitrogen-Containing Pesticides in Water
1613 (Rev. B)	- Tetra-Through Octa- Chlorinated Dioxins and Furans by Isotope Dilution e.g. in Water
SW846 method 3535	– Test Methods for TCLP Leachates
QTM	– Aqueous Phases Quick Turnaround Methods
	- PAH
	- Phenols
	- Pesticides & PCBs

Typical Customers:

- **Environmental**

- **Food and Agricultural**

- **Pharmaceutical/Clinical**

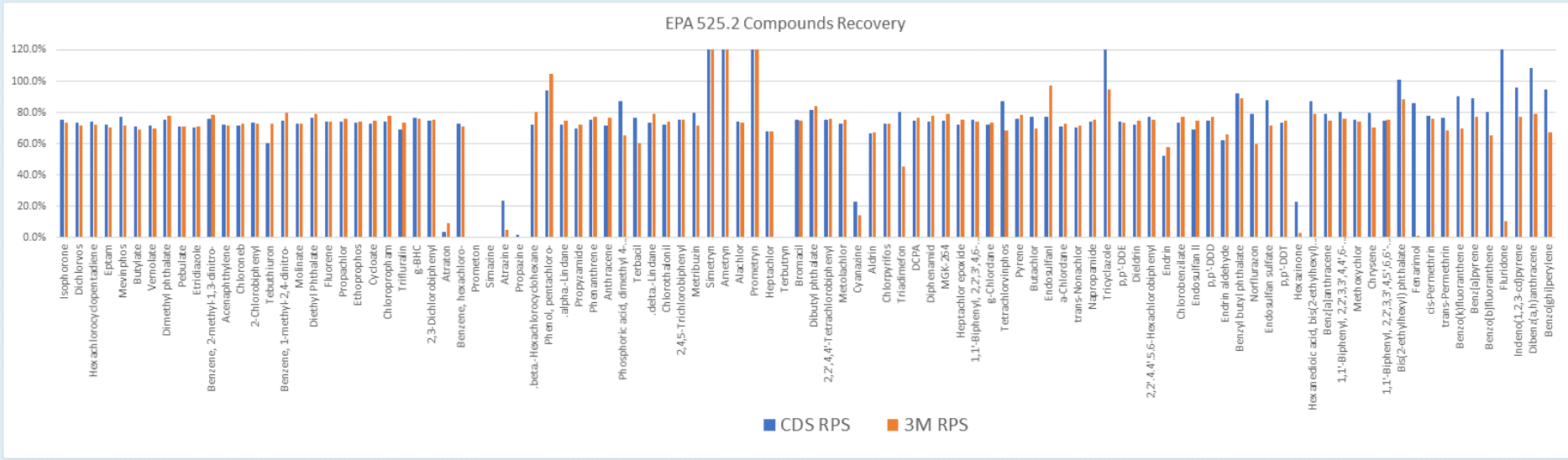
- **Research**

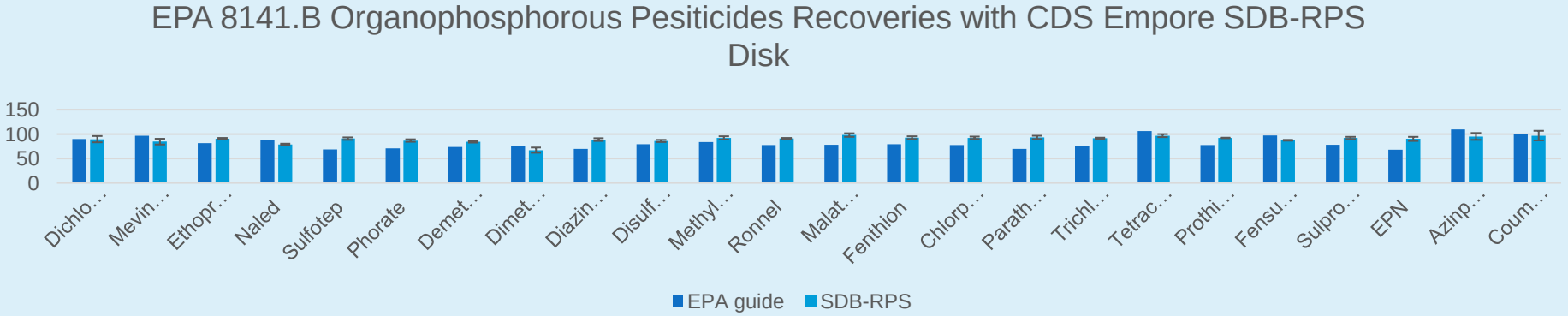
CDS has rebuilt Empore production line in a new-construct, GMP-compliant, clean room facility at Oxford, PA.

Empore production line at 3M

Empore new production line at CDS

Empore Disks with Improved Quality at CDS than at 3M





Part 2 Empore Disks' Applications in Proteomics

- 1. Empore StageTips Basics
- 2. Applications in Proteomics
- 3. High-pH Fractionation for Global Proteomics

Empore StageTips Basics

Preparation of Single-Disk Stop and Go Extraction Tips (StageTips)

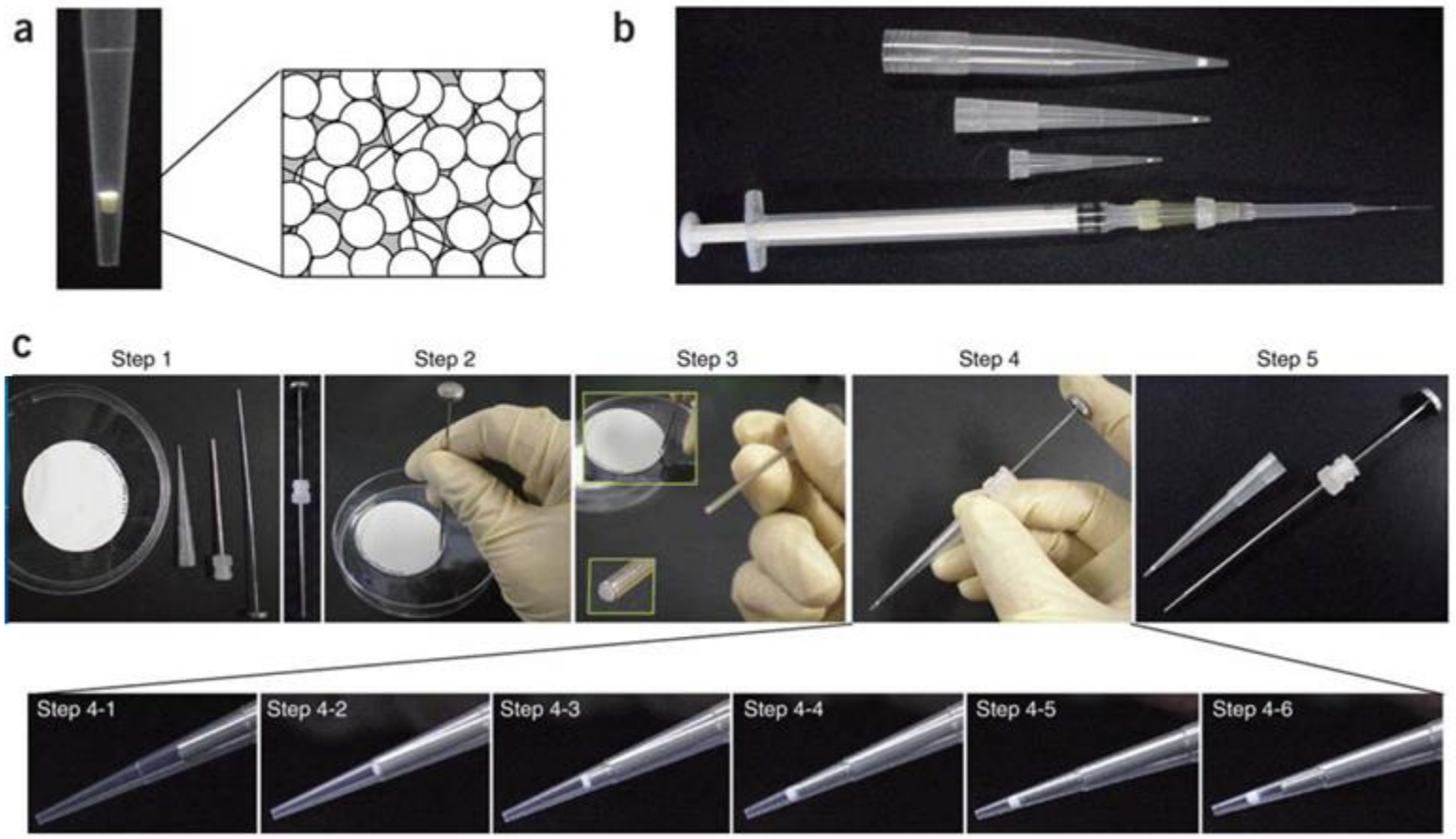
- Empore™ 47 mm disk for various functionality
  - *C18*
  - *C8*
  - *Cation exchange*
  - *Anion exchange*
  - *SDB-XC*
- Self-made tool for customization
- Ease-of-use
- Good recovery
- Reproducible and robust

Rappsilber, J., Mann, M., and Ishihama, Y. "Protocol for Micro-purification, Enrichment, Pre-fractionation and Storage of Peptides for Proteomics using StageTips." *Nature Protocols* **2**, 1896-1906 (2007) Published online: 2 August 2007

StageTips Preparation

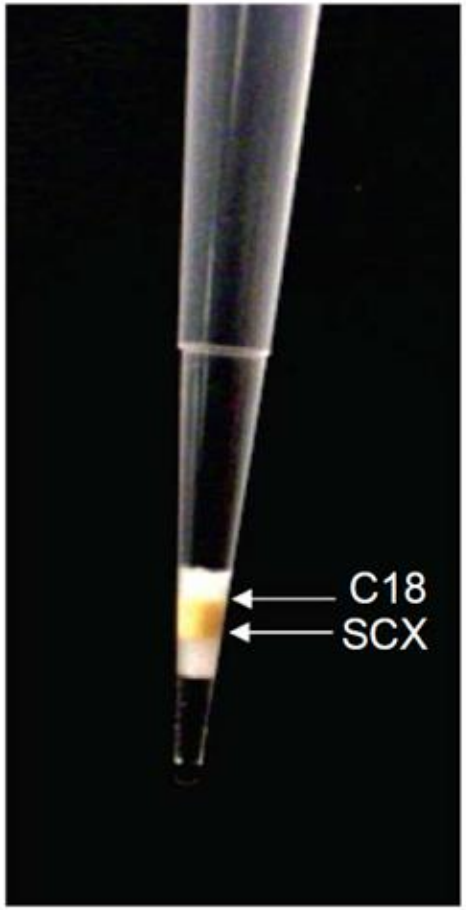
- Small disk is stamped out using a blunt-ended syringe needle (cutter)
  - *Particles held together for easy handling*
  - *Size of disk adjusted by using different gauge needle*
- Place the cutter inside a pipette tip and release the disk using a correct size needle plunger
- Press the disk gently into place using the plunger
- Remove cutter
- Additional disks can be layered to provide multiple functionality

Stage Tip Preparation



Multiple Layers for Additional Separating Ability

- C18 allows for desalting of the protein
- Fractionation done on the cation exchange disk.
- Can be followed up by another desalting step with C18
- Very clean sample for mass spec analysis

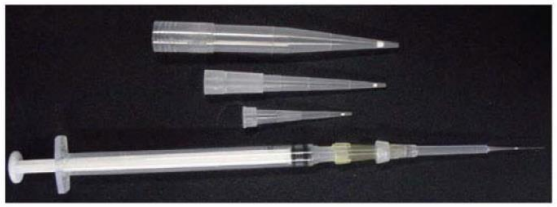


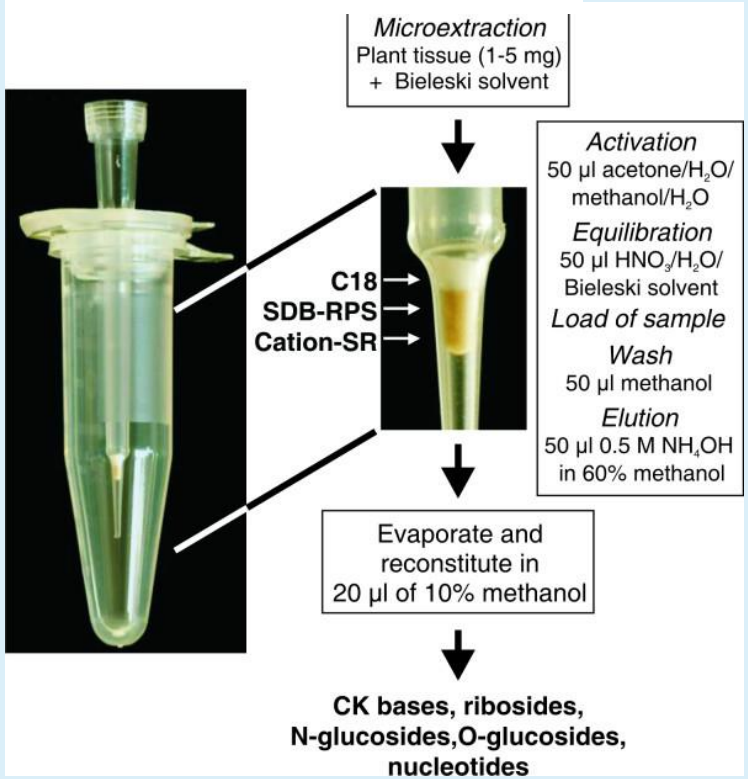
Ishihama, Y., Rappsilber, J., and Mann, M., "Modular Stop and Go Extraction Tips with Stacked disks for Parallel and Multidimensional Peptide Fractionation in Proteomics." *J. Proteome Res.*, **2006**, 5 (4), pp 988–994
Publication Date (Web): March 07, 2006 (Technical Note)

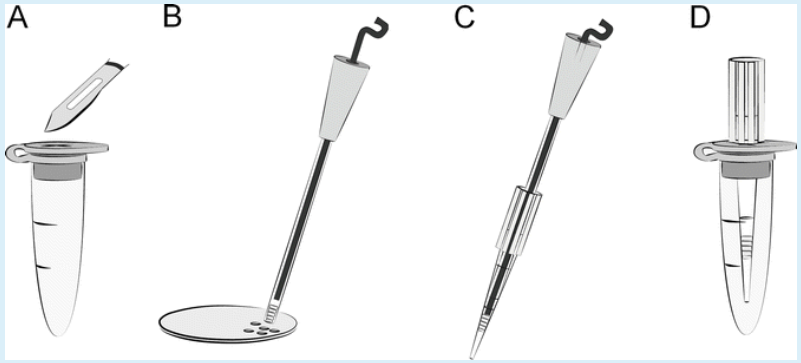
Why Desalting is Important for LCMS Based Proteomics?

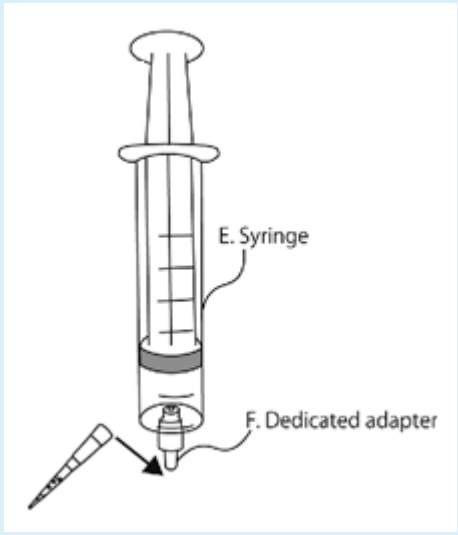
Salt crystals can damage switching valves in LC system, cause instable backpressure and potential sample loss!

Manual Push, NO!









Automatable StageTips

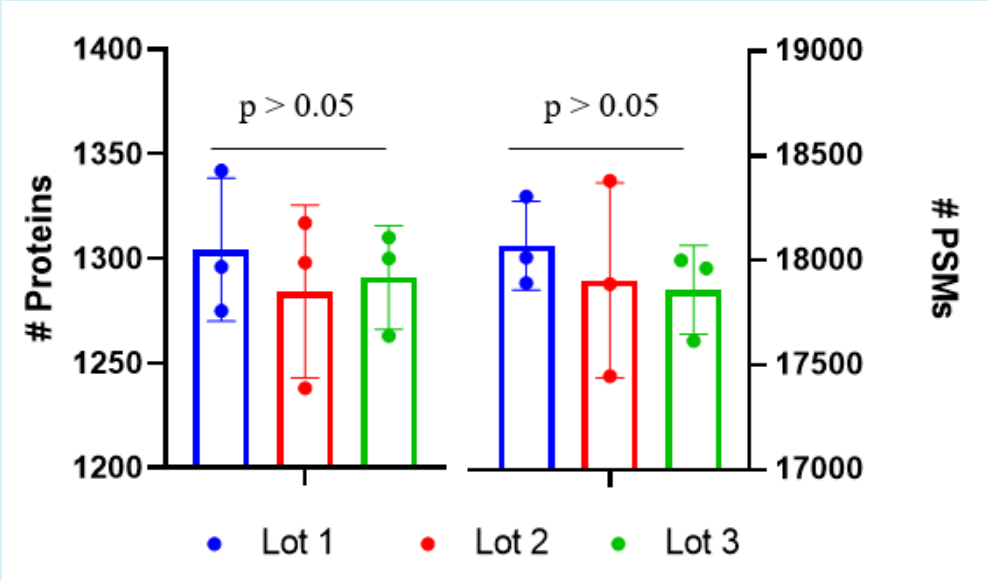
Pipette tip adaptors for spinable StageTipping!
Hands-free, scalable, high throughput!

Empore StageTips QA/QC: SDB-RPS StageTips Lot-to-Lot Consistency

Identification Rate

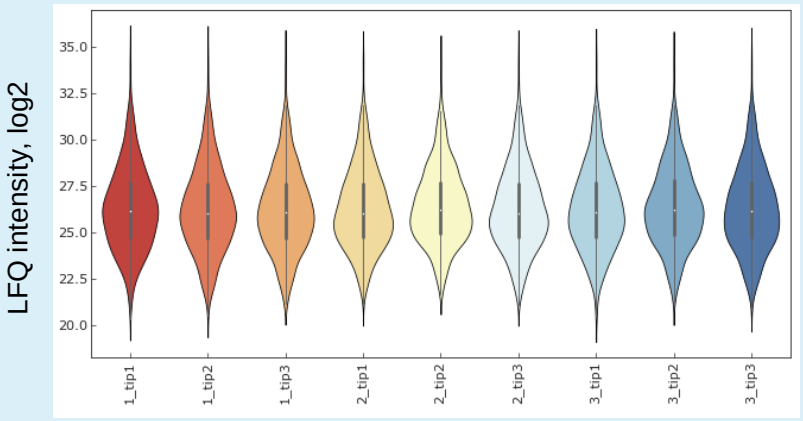
	Tip	# Proteins	#PSMs
Lot1 (760179D)	Tip1	1,296	17,893
	Tip2	1,342	18,014
	Tip3	1,275	18,306
Lot2 (760214D)	Tip1	1,298	17,448
	Tip2	1,238	17,888
	Tip3	1,317	18,380
Lot3 (760002C)	Tip1	1,310	18,001
	Tip2	1,263	17,615
	Tip3	1,300	17,963

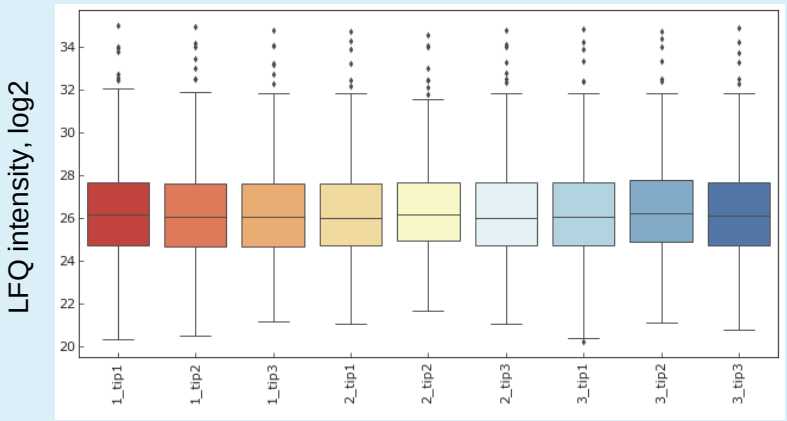
Cell lysate: BMDM cells.
Digestion: STrap method
Desalting: SDB tips
LCMS: Q Exactive (150-min)



No significant variations were found between different tips and different lots, suggestion excellent reliability and reproducibility of our manufacturing procedure.

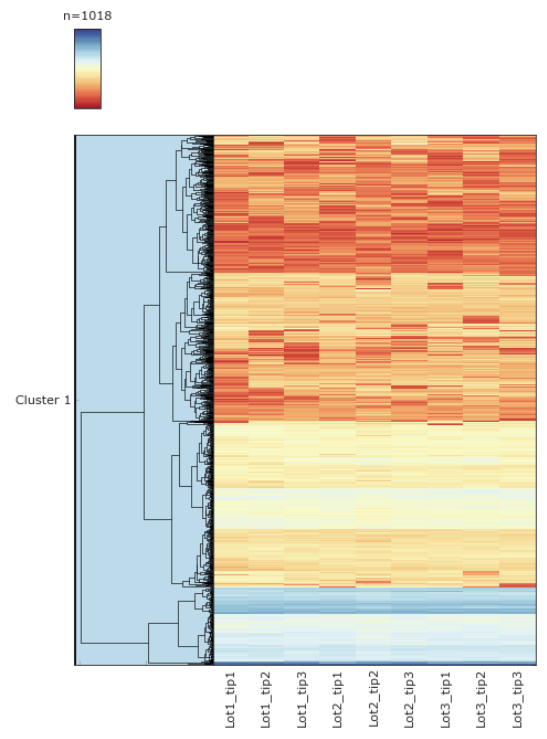
Quantitation Performance

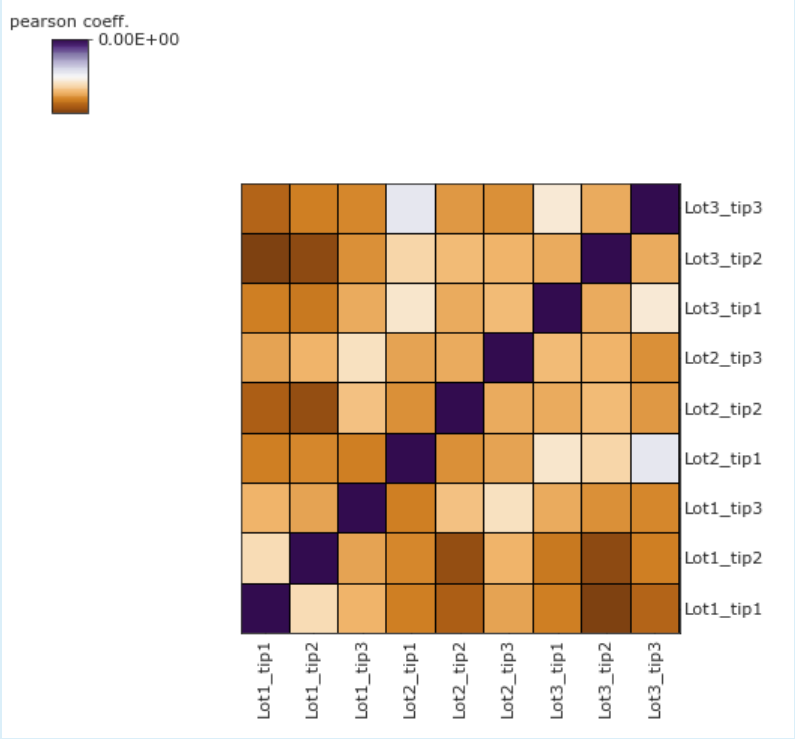




Proteins showed highly similar intensity distribution, suggesting excellent quantatitation performance!

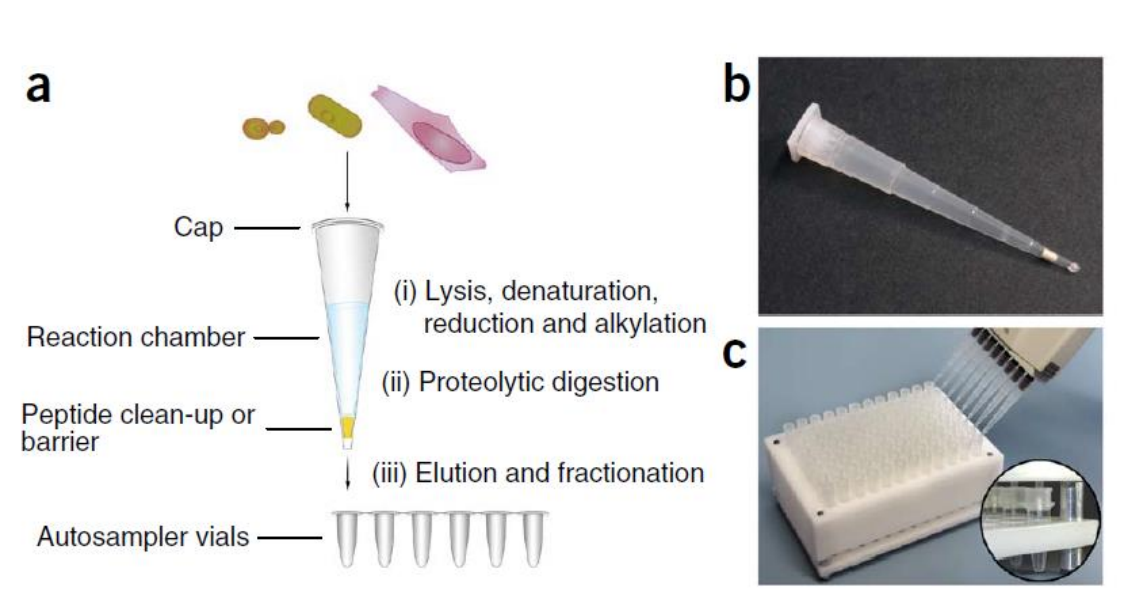
Heatmap and Correlation



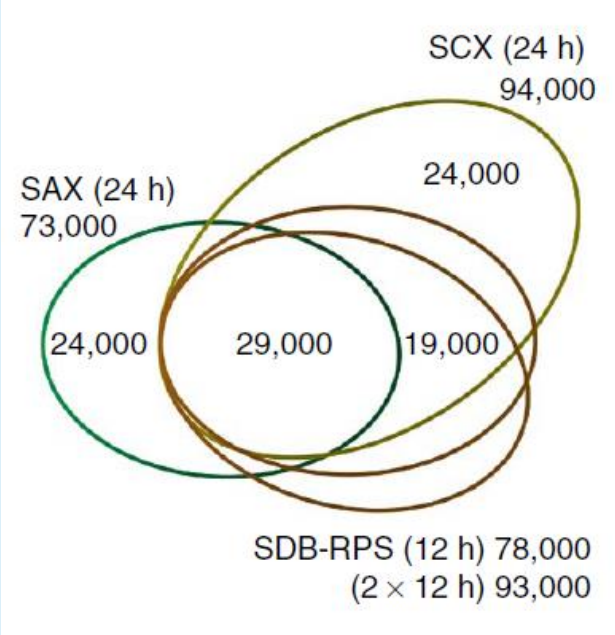


Quantified proteins showed high correlation (Pearson r > 0.93) between different tips and different lots, suggesting again excellent quantitation performance!

2. Applications in Proteomics

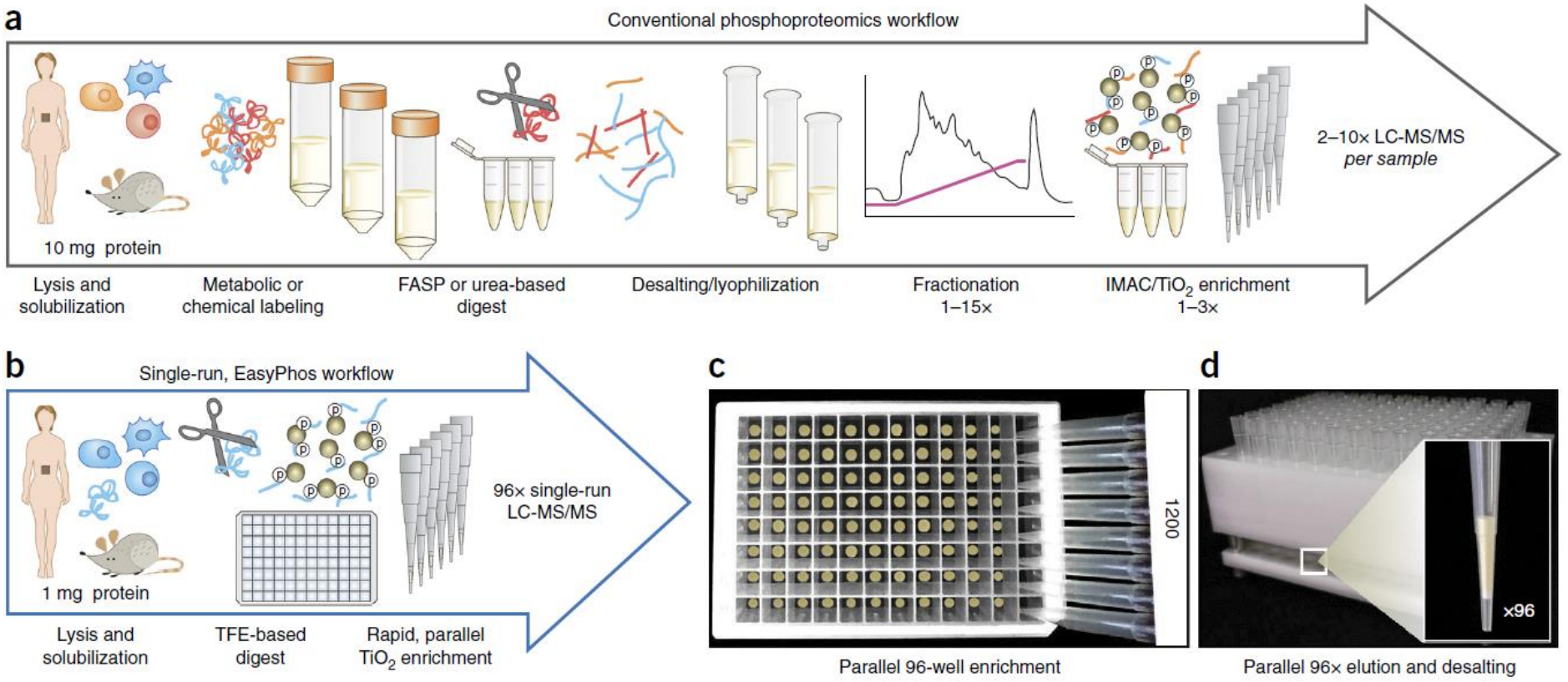


HeLa Cells



Peptide identifications from 20 µg starting material fractionated by SAX, SCX and SDB-RPS StageTips.

Nils A Kulak, Garwin Pichler, Igor Paron, Nagarjuna Nagaraj & Matthias Mann, Nature Method, 2014, 11, 319-326.



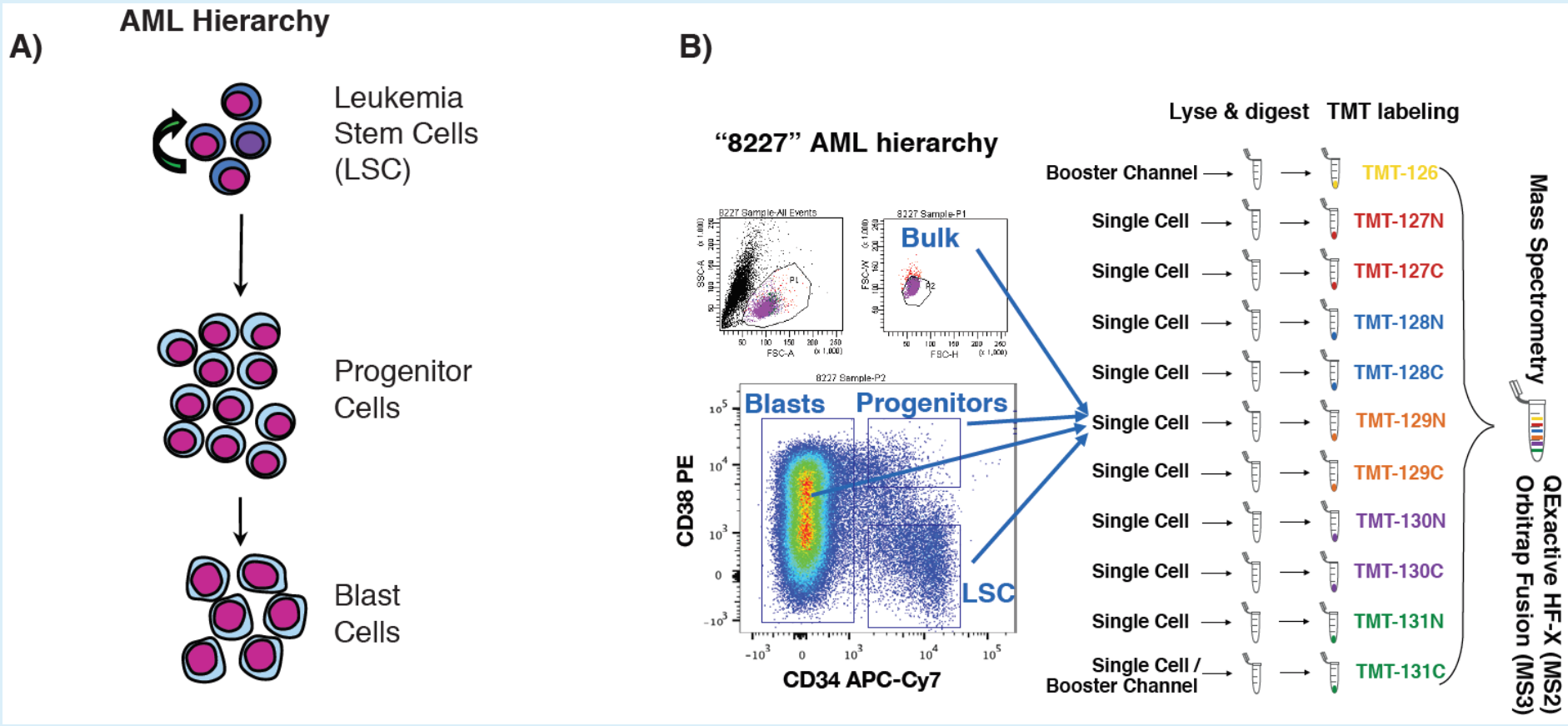
High-throughput phosphoproteomics: HePa 1-6 cells

StageTips V. S. Traditional method:

10 times less starting material;
3 times less measurement time;
identifying 3 times of the quantified sites.

Sean J Humphrey, S Babak Azimifar & Matthias Mann, Nature Biotech., 2015, 33, 990.

Single-cell Proteomics for AML Hierarchy



StageTips in sample preparation to help desalting and peptide purification.

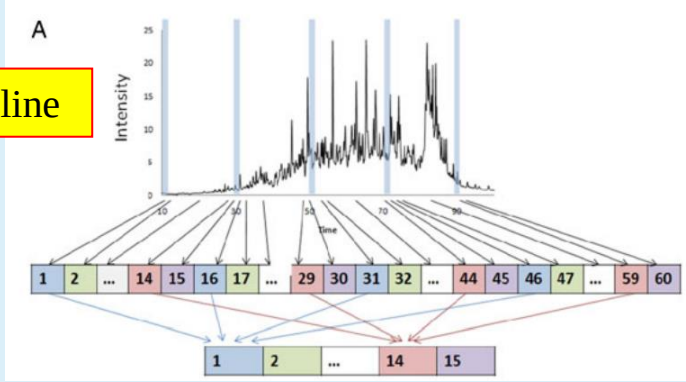
Erwin M. Schoolf, Nicolas Rapin, Simonas Savickas, Coline Gentil, Eric Lechman, James Seymour Haile, Ulrich Keller, John E. Dick, Bo T. Porse. bioRxiv preprint. doi: https://doi.org/10.1101/745679

3. High-pH Fractionation for Global Proteomics

Current High pH Fractionation Approaches

Proteomics **2011**, 11, 2019–2026

Pump-based off-line

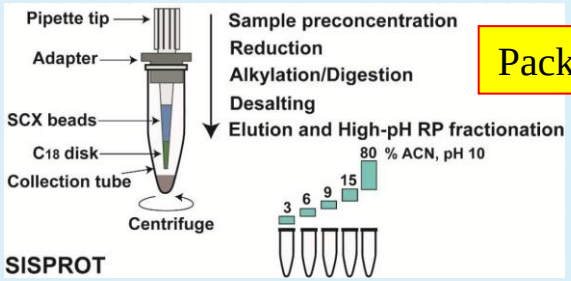


1. Pump-based approach (e.g., using off-line HPLC) is sophisticated and expensive.
2. Beads-based approaches are unreliable (e.g., need to weight certain amount of beads material and do packing every time).

Our approach, C18 membrane tip based, is ***simple, reliable and robust.***

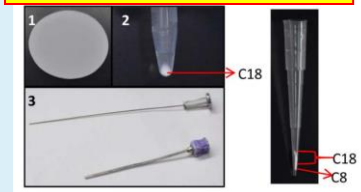
Anal. Chem., **2016**, *88* (9), pp 4864–4871

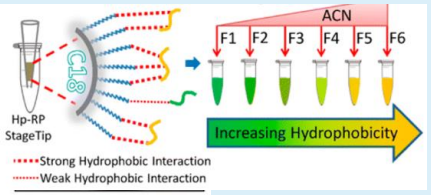
Packing SCX beads



Anal. Chem., **2015**, *87* (24), pp 12016–12023

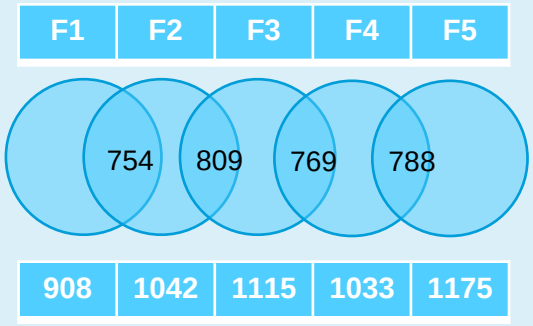
Packing C18 beads

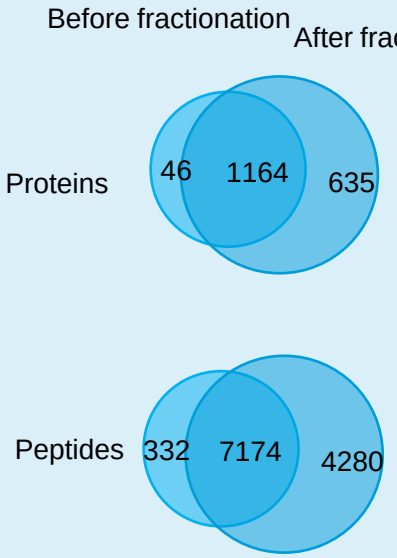




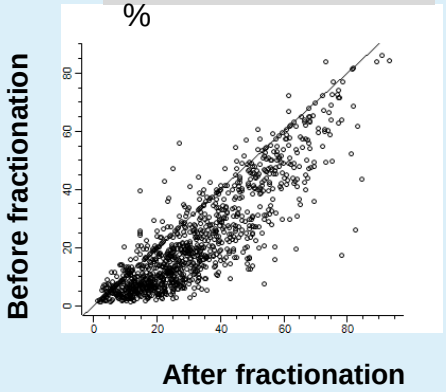
Comparison among Fractions

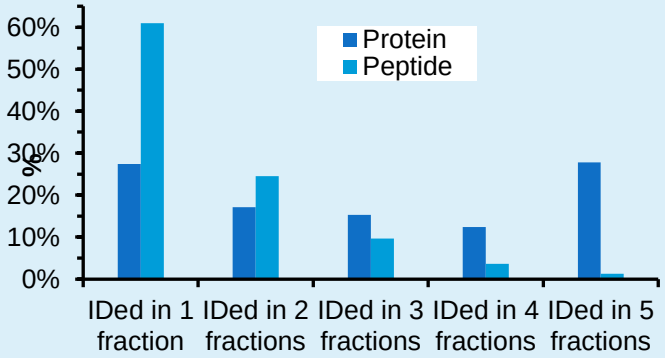
HeLa cell lysate:



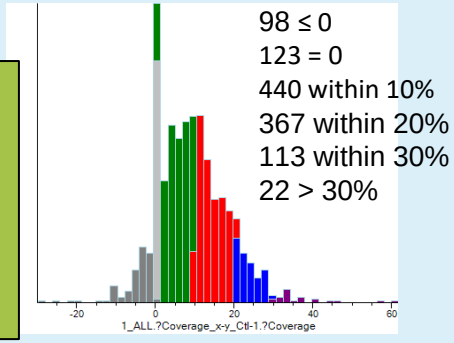


Sequence coverage, %





Fractionation significantly increases identification rate on both protein and peptide levels.



Conclusions of Empore C18 StageTips for High-pH Fractionation

1. High pH fractionation increased the number of protein identification by > 50%.
2. The sequence coverage of most of the identified proteins increased > 10%.
3. At least five or six fractions should be applied (5%, 10%, 15%/25%, 40%, 80% acetonitrile in 10 mM NH_4F, pH=10).
4. C18 StageTip-based high pH fractionation is convenient, easy-to-manipulate and pump-free.
5. Current high pH fractionation methods usually apply off-line HPLC pump, or pack C18 resins into tips; C18 StageTip is much simple and cost-effective.
6. This method has not been reported for global proteomics before.

Summary

- 1. Empore StageTips is one of the most important methods for protein desalting and peptide purification in Proteomics research.

- 2. Empore C18 StageTips is a much simpler, cost-effective and efficient sample preparation method in high-pH fractionation in proteomics than off-line HPLC pump and packed C18 resins into tips.