PolyLC INC.
9151 Rumsey Road, Suite 180 • Columbia, MD 21045 USA
Phone: (410) 992-5400 • FAX: (410) 730-8340 • e-mail: POLYLC@aol.com

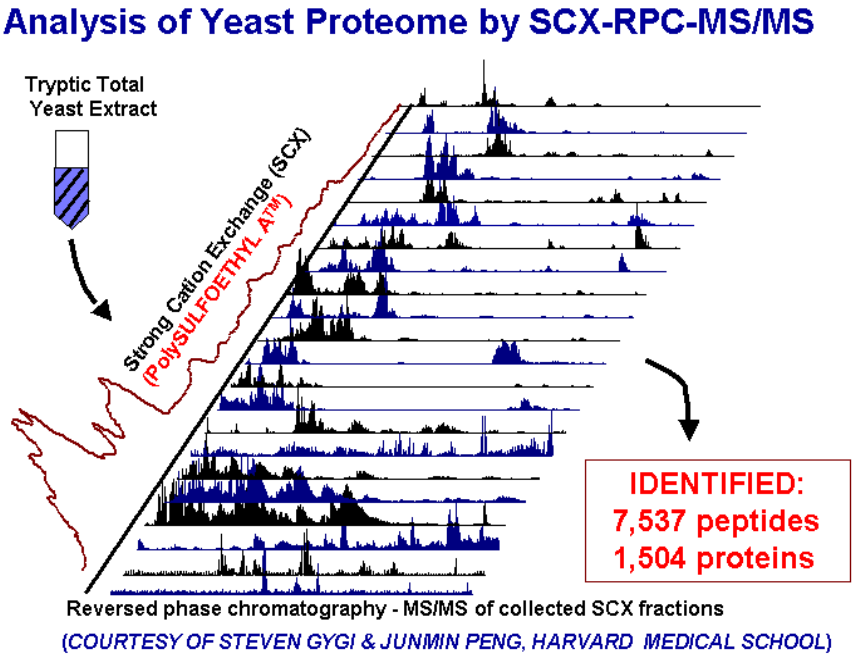
Proteomics Update 2004

IF YOU WANT TO IDENTIFY 10,000 PEPTIDES

No single analytical method suffices for large collections of proteins. They must be fractionated using two complementary methods. 2-D electrophoresis is often used for this. Extraction of the proteins is inconvenient, though, and this method doesn't work well for proteins of low abundance or from membranes. A better alternative is 2-D chromatography, per the following sequence:

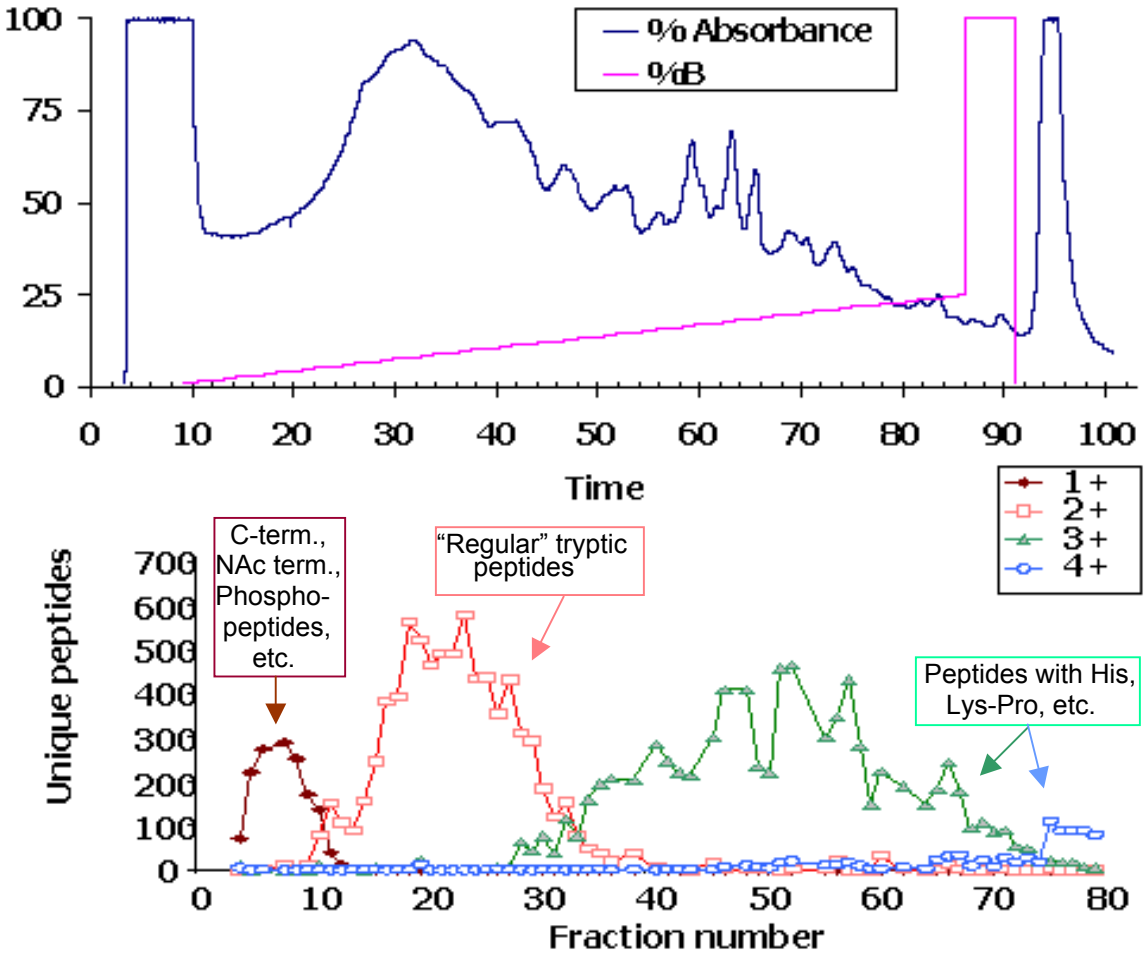
1) Tryptic digestion of the entire complex.
2) Use of PolyLC's **PolySULFOETHYL Aspartamide™** SCX material to trap all peptides on the basis of *charge* (at pH 2.7-3.0, they're all + charged). The peptides are separated into as many as 100 sets of increasingly positive charge with a salt gradient, linear or step.
3) Each set is further fractionated by *polarity* on a capillary of a reversed-phase material.
4) The eluting peptides are identified by MS/MS analysis.
5) The peptide sequences are matched to their parent proteins by searching the genome with a suitable algorithm (*e.g.*, SEQUEST).

Currently this method has been used to identify > 10,000 peptides per run.



Above is an example of the raw data generated by this method: A number of reversed-phase HPLC chromatograms, each one obtained from one of the fractions (100 in all) eluted from the SCX material by a salt step gradient.

Tryptic Digest of HeLa Cell Nuclear Proteins: Charge vs. Retention in SCX



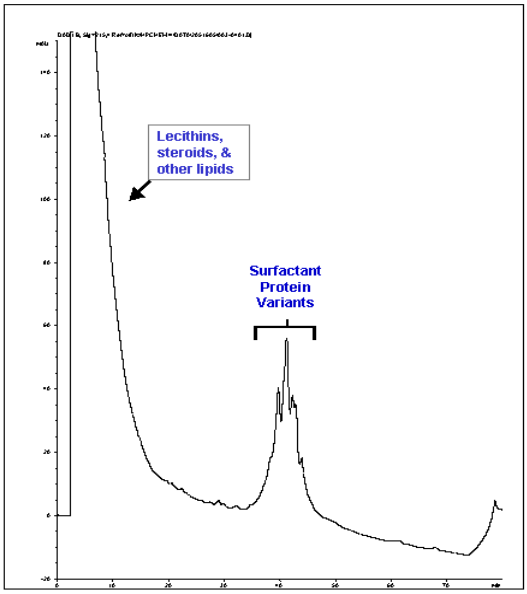
Column: PolySULFOETHYL A, item# 202SE0502
(*Courtesy of Steven Gygi, Harvard Medical. School*)

PolySULFOETHYL A is capable of pulling the +2 peptides away from the +1 peptides. This makes it possible to collect and identify peptides in the latter group, which is enriched in phosphopeptides, C-terminal fragments, and other interesting peptides.

NOTE: There are actually far more tryptic peptides in the +2 region than in the others, the impression of the bottom graph notwithstanding. The **percentage** of peptides successfully identified by sequence is higher in fractions that contain fewer peptides.

∴ Break up the mixture into smaller subsets by collecting more fractions.
Result: More successful identifications, especially of peptides of lower abundance.

Multiply your Separation Power: Fractionate the *INTACT* Proteins Prior to Trypsinization (or even prior to a 2-D gel)!



LUNG SURFACTANT PROTEIN:
SCX FROM AN EMULSION
WITH 500 PARTS LIPID

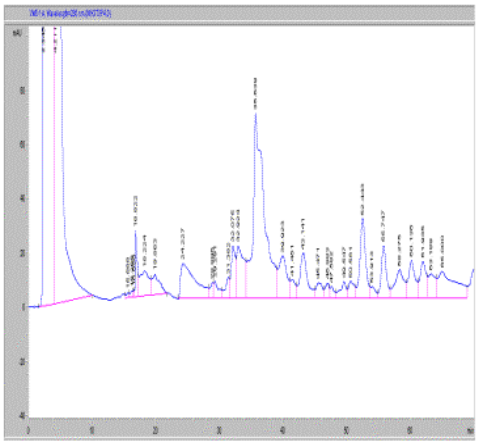
This is a particularly difficult sample, analogous to a membrane protein prep.
Column: 204SE0510

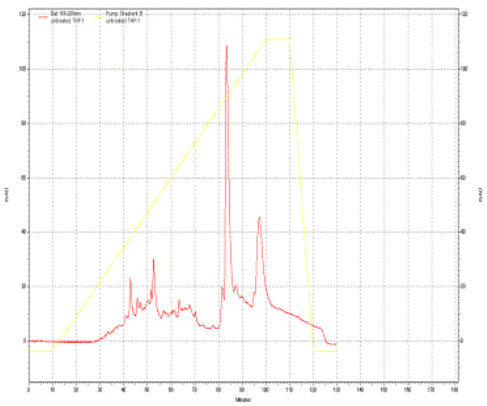
Gradient: $NaClO_4$ (pH 3) in 70% ACN

Points to consider:
1) The 1000-Å pore diameter insures facile diffusion of proteins into the pores.

2) At pH 3 all proteins, of any pI, have a net positive charge and will be retained.

3) In the HILIC-SCX mode used here, lipids and detergents are not retained and elute in the void volume. The salt gradient subsequently elutes the proteins in sharp peaks (here, variants of the surfactant protein).





CRUDE CELL LYSATES

TOP: Anion-exchange of mouse fibroblast line (NIH3T3)
Column: PolyWAX LP

Gradient: NH_4OAc in 20% ACN, pH ~ 7
(*Courtesy of Henrik Molina, Johns Hopkins Med. School*)

BOTTOM: THP-1 monocyte cell lysate in mixed-bed IEX
Column: PolyCAT A™ and PolyWAX LP™ in series

Gradient: $NaClO_4$ in 40% PrOH & 30% ACN
with 0.1% HFIP
(*Courtesy of Leticia Cano, City of Hope*)

NOTE: Both profiles feature several proteins of much greater abundance than the others. Fractionation at the intact protein stage allows you to lock those up in their own fractions so their peptides don't mask peptides from proteins of lower abundance.

Selected PolyLC Products Useful for Proteomics

(Prices effective February 2004. All prices in US dollars)

We offer columns from capillary to process-scale and bulk material with various pore and particle diameters. The following combinations are especially popular for proteomics applications:

Capillaries & Columns: Used for high-resolution separations of peptides.

Category	Dimensions	Material	Part number	Price each
Capillary	50 x 0.30mm	PolySULFOETHYL A, 5µ, 300Å	050.30SE0503	$450.00
Capillary	100 x 0.30mm	PolySULFOETHYL A, 5µ, 300Å	100.30SE0503	$500.00
Capillary	150 x 0.30mm	PolySULFOETHYL A, 5µ, 300Å	150.30SE0503	$550.00
Microbore Column	50 x 1.0mm	PolySULFOETHYL A, 5µ, 300Å	051SE0503	$445.00
Microbore Column	150 x 1.0mm	PolySULFOETHYL A, 5µ, 300Å	151SE0503	$575.00
Narrow Bore Column	100 x 2.1mm	PolySULFOETHYL A, 5µ, 300Å	102SE0503	$475.00
Narrow Bore Column	200 x 2.1mm	PolySULFOETHYL A, 5µ, 300Å	202SE0503	$545.00
Analytical Column	100 x 4.6mm	PolySULFOETHYL A, 5µ, 300Å	104SE0503	$475.00
Analytical Column	200 x 4.6mm	PolySULFOETHYL A, 5µ, 300Å	204SE0503	$545.00

Guard Cartridges: Used to protect the corresponding column.

Category	Dimensions	Material	Part number	Price each
Javelin guard cartridge	10 x 1.0mm	PolySULFOETHYL A, 5µ, 300Å	J11GCSE0503	$75.00
Javelin guard cartridge	10 x 2.0mm	PolySULFOETHYL A, 5µ, 300Å	J22GCSE0503	$75.00
Javelin guard cartridge	10 x 4.0mm	PolySULFOETHYL A, 5µ, 300Å	JGCSE0503	$75.00

Bulk Material: If you want to pack capillaries yourself.

Category	Dimensions	Material	Part number	Price/gram
Bulk Material	per gram	PolySULFOETHYL A, 3µ, 300Å	BMSE0303	$100.00
Bulk Material	per gram	PolySULFOETHYL A, 5µ, 200Å	BMSE0502	$60.00
Bulk Material	per gram	PolySULFOETHYL A, 5µ, 300Å	BMSE0503	$60.00

Columns for Ion-Exchange of Intact Proteins.

Category	Dimensions	Material	Part number	Price each
Cation-exchange, pH 3.0	200 x 4.6mm	PolySULFOETHYL A, 5µ, 1000Å	204SE0510	$545.00
Cation-exchange, pH 4-7	100 x 2.1mm	PolyCAT A, 5µ, 1000Å	102CT0510	$430.00
Cation-exchange, pH 4-7	100 x 4.6mm	PolyCAT A, 5µ, 1000Å	104CT0510	$430.00
Cation-exchange, pH 4-7	200 x 4.6mm	PolyCAT A, 5µ, 1000Å	204CT0510	$510.00
Anion-exchange, pH 5-7.5	100 x 2.1mm	PolyWAX LP, 5µ, 1000Å	102WX0510	$430.00
Anion-exchange, pH 5-7.5	100 x 4.6mm	PolyWAX LP, 5µ, 1000Å	104WX0510	$430.00
Anion-exchange, pH 5-7.5	200 x 4.6mm	PolyWAX LP, 5µ, 1000Å	204WX0510	$510.00

NOTES:

1) BMSE0502 is about 15-25% more retentive for peptides than is BMSE0503. Use the former if you're particularly interested in the most weakly-retained peptides.
2) If you want a combination of column dimension, pore or particle diameter not listed above, ask.
3) Guard cartridges are available for the columns listed above for ion-exchange of intact proteins.
4) Contact us to get details of the following:
   a) Solubilizing proteins in membranes or whole cell pellets.
   b) Getting rid of the resulting lipid and keeping all proteins in solution during ion-exchange HPLC.