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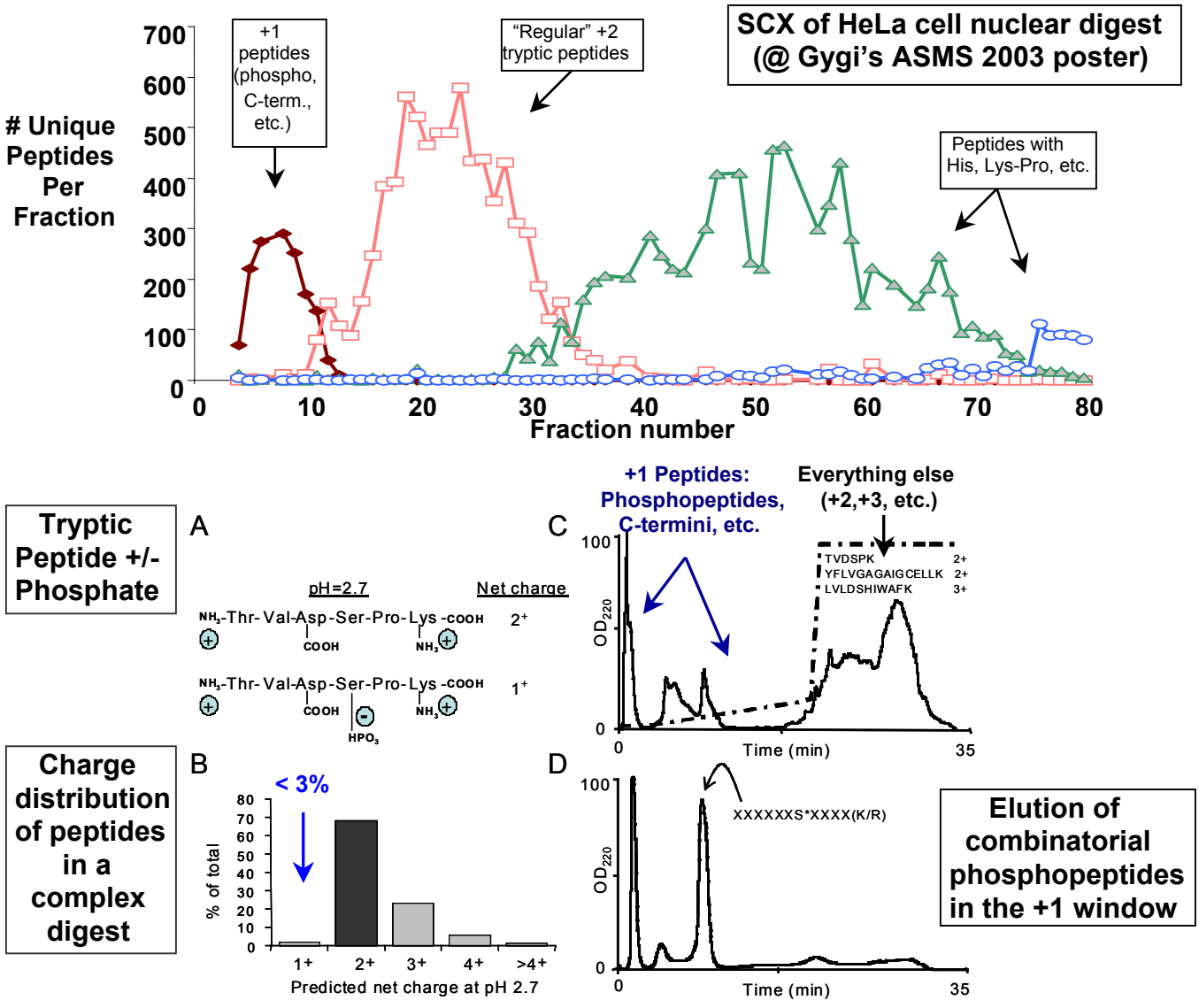
Proteomics Update 2005

SELECTIVE ISOLATION OF PHOSPHOPEPTIDES BY CHARGE

At pH 2.7, a typical tryptic peptide has a net charge of +2. Attachment of a phosphate group lowers the net charge to +1. You can make a clean separation of the +1 and +2 peptide windows with the 200-Å pore version of our PolySULFOETHYL A material (which has a higher surface area than the 300-Å material usually used for the SCX-RPC sequence in bottom-up proteomics). Steve Gygi and his colleagues have found that the +1 fractions (< 3% of complex tryptic digests) are indeed greatly enriched in phosphopeptides. Getting rid of the other 97+% of the digest makes identification of the phosphopeptides comparatively easy; Gygi *et al.* have identified > 2000 phosphopeptides from HeLa cell nuclear proteins.

Ref.: 1) S.A Beausoleil *et al.*, *PNAS 101* (2004) 12130-12135.
2) B.A. Ballif *et al.*, *Mol. Cell. Proteomics 3* (2004) 1093-1101.

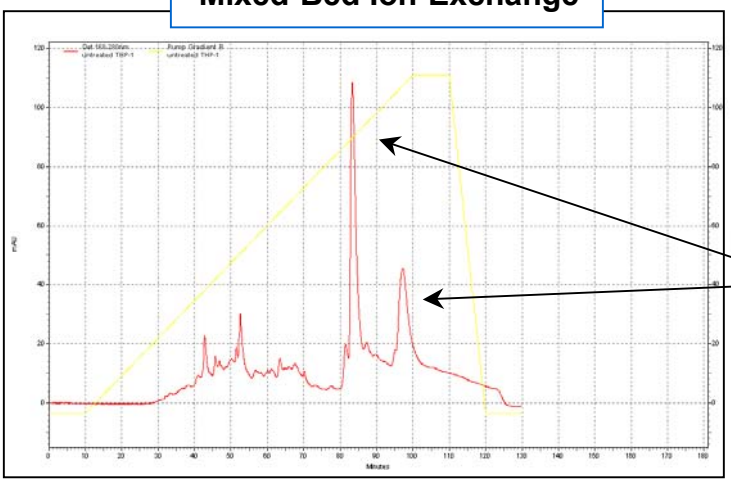


- data courtesy of Steven Gygi, Harvard Medical School -

Dig *Deep*! Fractionate the Intact Proteins before Trypsinizing

Fractionating your proteins prior to bottom-up proteomics will significantly increase identification of proteins of low abundance. The reason is the same that accounted for the success of the Gygi group in identifying phosphopeptides: Distributing the low-abundance proteins into smaller sets will make it easier to identify their peptides, especially if they're separated from the proteins of high abundance. If the resulting increase in the number of fractions creates problems with throughput, then collect less fractions at the SCX step after trypsinization and more fractions prior to trypsinization. The following are examples of how one might implement this approach:

Mixed-Bed Ion-Exchange

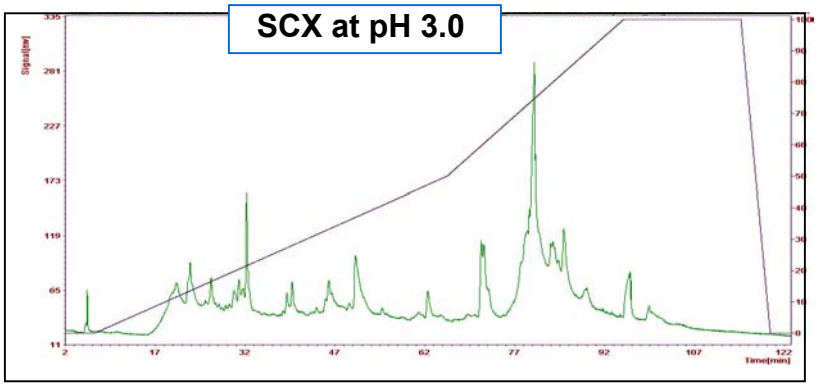


SAMPLE: Lysate of THP-1 Monocytes
COLUMN: Anion- and cation-exchangers in series (PolyWAX LP & PolyCAT A; items# 104WX0510 & 104CT0510; 1000-Å).

$NaClO_4$ gradient, pH 6.

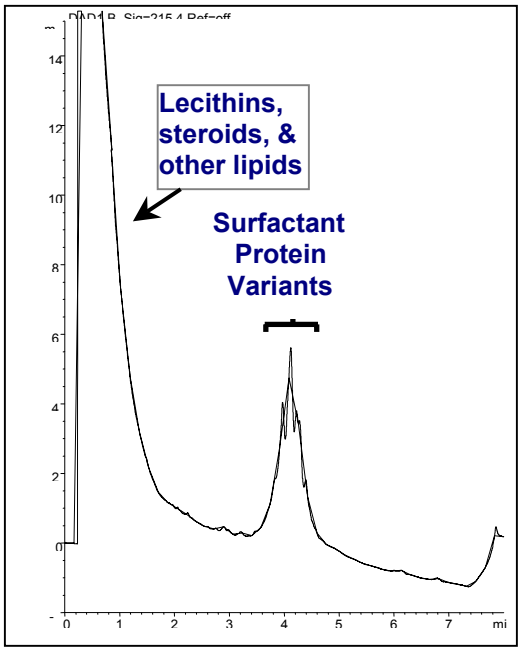
With mixed-bed ion-exchangers in series, all proteins are retained.
Two proteins are present in very high abundance. These should be collected in their own fractions so that their peptides don't mask those from low-abundance proteins. NOTE: Affinity columns designed for plasma proteins won't help here!

SCX at pH 3.0



SAMPLE: Liver homogenate
COLUMN: PolySULFOETHYL A, item# 204SE0510 (1000-Å)
NaCl gradient, pH 3, with 20% ACN/PrOH

An intriguing alternative. Like peptides, proteins have a net + charge at pH 3 and are retained in SCX. Be sure to use 1000-Å pore material; proteins are big molecules!



SCX in organic solvent

SAMPLE: Lung surfactant protein in an emulsion with 500 parts lipid.
COLUMN: PolySULFOETHYL A, item# 204SE0510 (1000- Å)

$NaClO_4$ gradient in 70% ACN, pH 3.

The same approach also works for water-insoluble proteins. It is necessary to add appreciable organic solvent and use chaotropes such as $NaClO_4$ and HFIP. Under these conditions, the column operates in a IEX-HILIC mixed mode. Neither lipids nor detergents are retained, but proteins are.