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Isolation of Glyco- & Phosphopeptides via ERLIC*

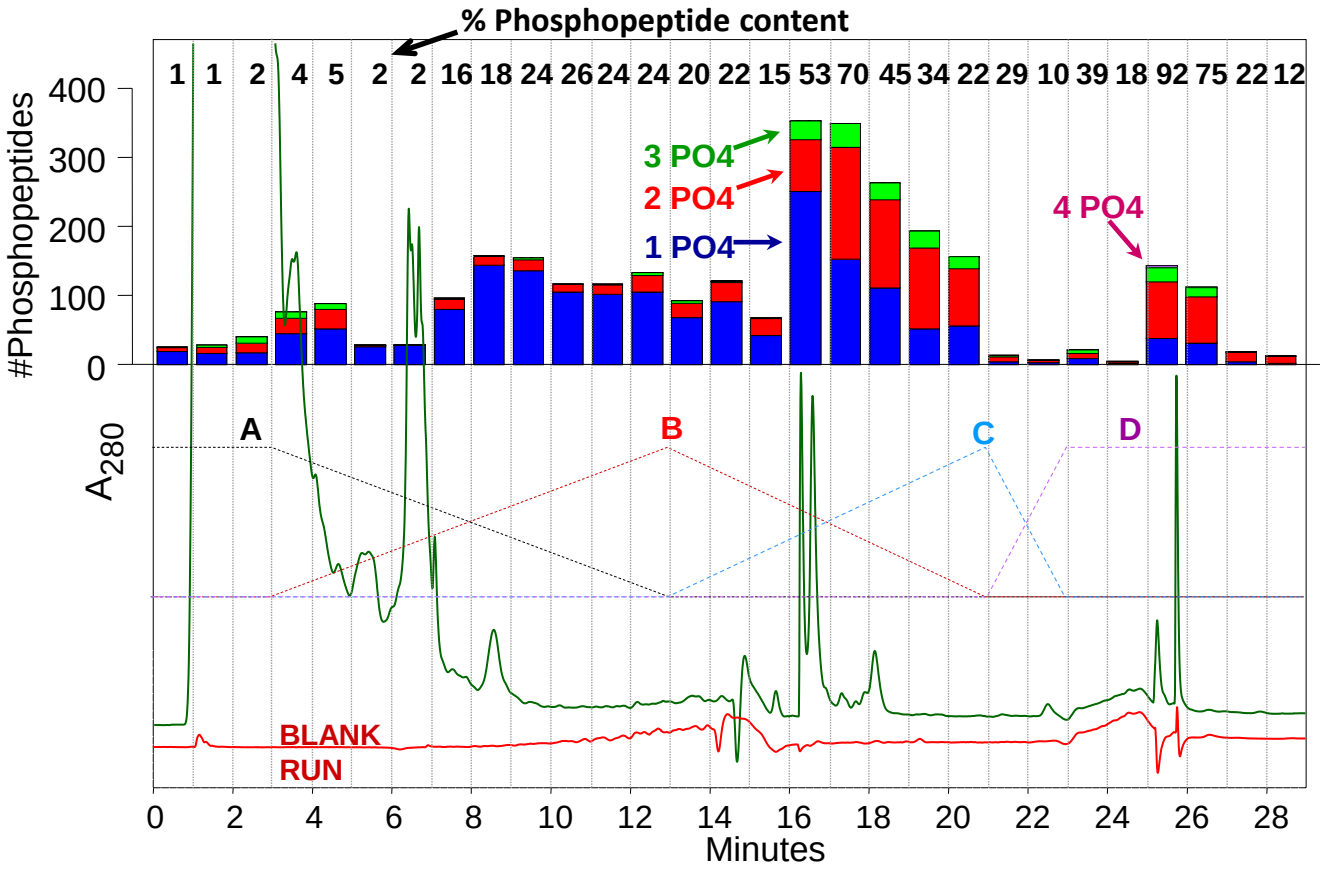
*(Electrostatic Repulsion-Hydrophilic Interaction Chromatography) March 2010

ERLIC is a new, general-purpose mode of chromatography; a column of the same electrostatic charge as the solutes is run in the HILIC mode. Both **sialylated glycopeptides and phosphopeptides** (as well as some nonsialylated glycopeptides) can be isolated selectively from tryptic digests via ERLIC.

At pH 2.0, peptides with phosphate and sialic acid residues retain some negative charge. This does not permit their isolation from tryptic digests by **anion-exchange chromatography (AEX)**, since the electrostatic attraction is not sufficient to overcome the electrostatic repulsion from the N-terminus and the C-terminal Lys/Arg residue. When an AEX column is run in the **ERLIC mode**, though, then the combination of electrostatic attraction and hydrophilic interaction suffices to pull singly phosphorylated tryptic peptides and many glycopeptides away from the unmodified peptides. Unlike the situation with high-affinity media such as IMAC or titania, phospho- and glycopeptides can be well-resolved from each other in ERLIC. This permits their convenient separation into numerous fractions, an important tool in **proteomics** for identifying thousands of modified peptides from a single sample. Peptides with multiple phosphate or sialyl- groups are retained so strongly that gradient elution is necessary.

Example 1: ERLIC of HeLa Cell Lysate Tryptic Digest

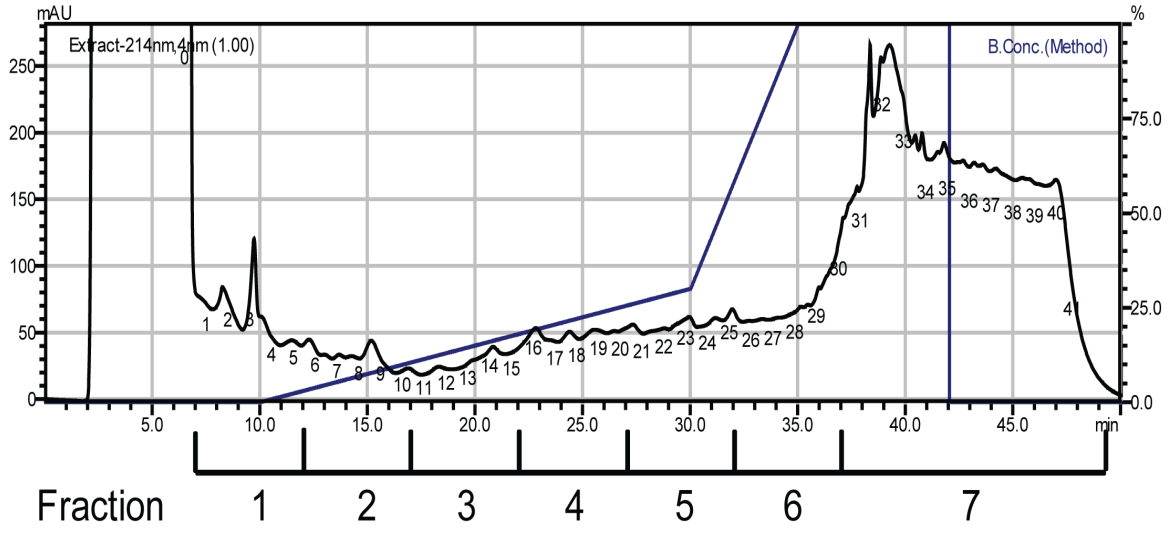
A 100x4.6-mm column of PolyWAX LP® (5-µm, 300-Å) was used (item# 104WX0503). Over 3000 unique tryptic phosphopeptides were identified. Solvents through Fraction 20 were volatile.

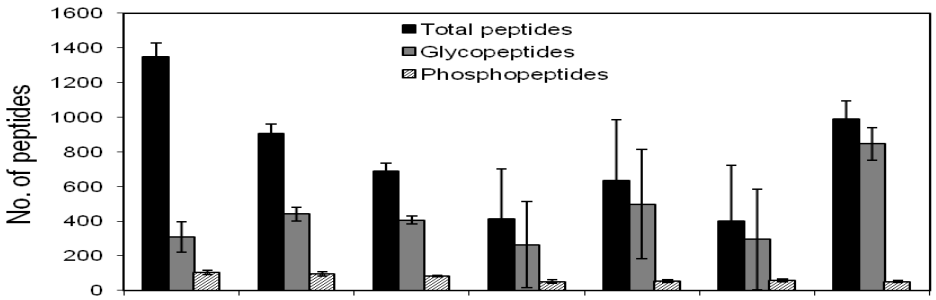


Example 2: Isolation of tryptic phospho- and glycopeptides

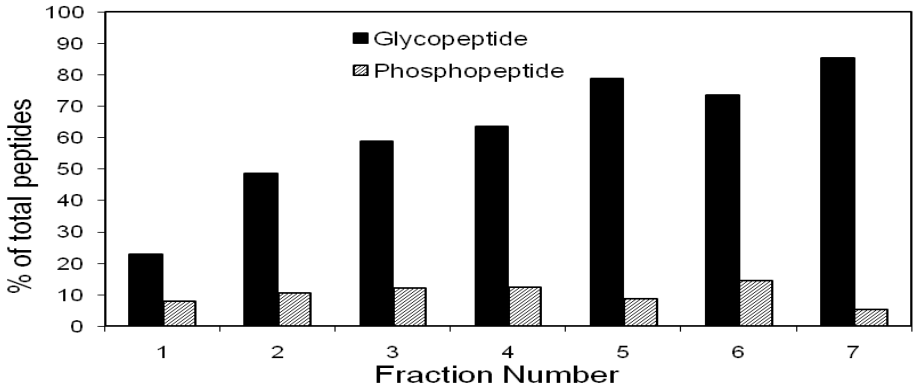
Column: 204WX0503 Flow rate: 1 ml/min. Detection: 214 nm
Gradient: 0-10': 0%B; 10-35': 0-30% B; 35-40': 30-100%B; 40-50': 100%B
MP A: 10 mM Na-methylphosphonate, pH 2.0, with 70% ACN
MP B: 200 mM TEA-phosphate, pH 2.0, with 25% ACN
(data courtesy of S.K. Sze, Nanyang Technol. Univ.)

Mouse Brain Extract (1 mg)





Phospho- and glycopeptide content is ~ 30-90% in fractions 1-7



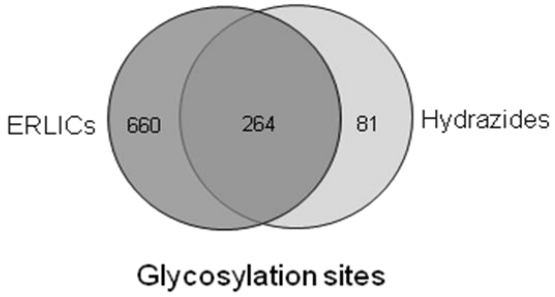
NOTE: Glycosylation sites were found in 41% of phospho-proteins id'd here

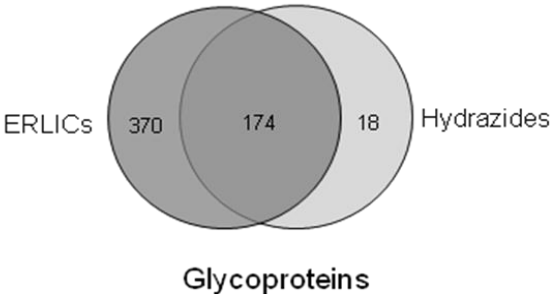
Isolation of N-linked Glycopeptides:
Comparison of ERLIC with Hydrazide Covalent Chromatography

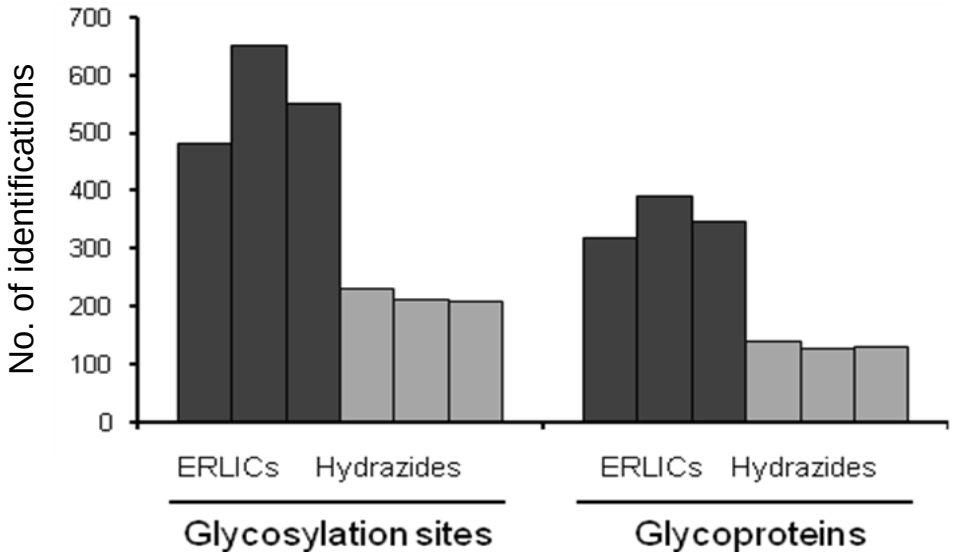
ERLIC: 50-minute run (same sample & conditions as in Example 2).

Hydrazide Method: 1) Oxidation with NaIO4; 2) Incubation overnight with Hydrazide gel; 3) Incubation overnight with PNGase F.

All fractions and isolates were analyzed via C18-MS (LTQ-FT).



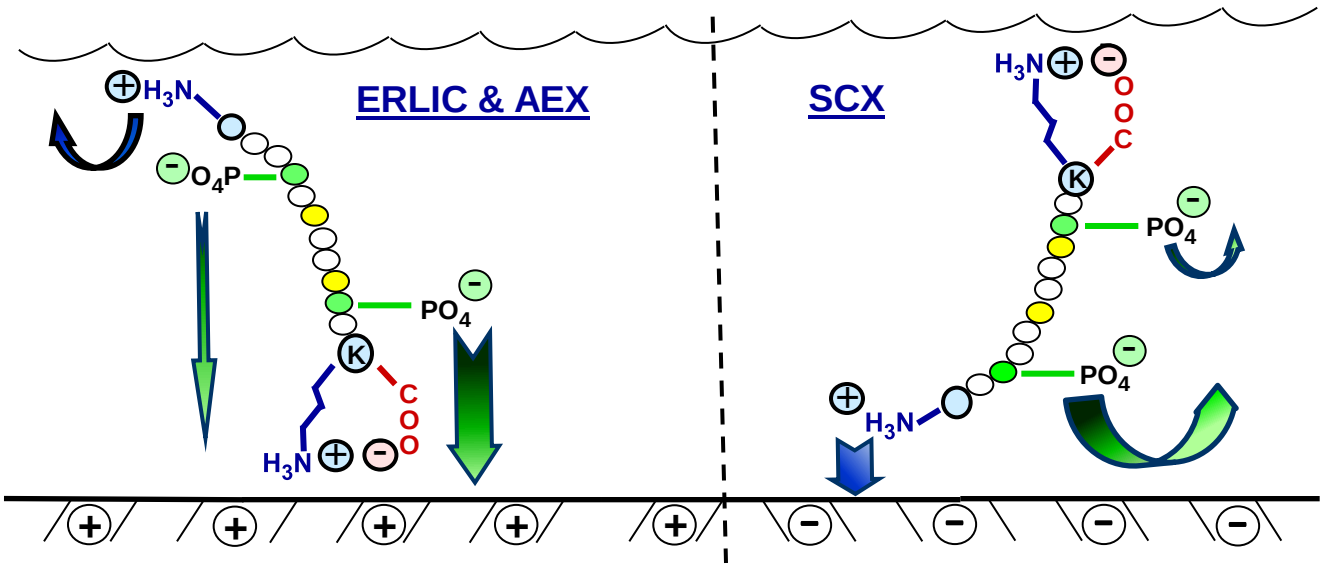




∴ ERLIC is much more effective and convenient than the hydrazide method. In addition, glycopeptides elute in ERLIC with the glycan still attached, which is not true of the hydrazide method.

Applications of ERLIC in Proteomics:

1) ORIENTATION OF TRYPTIC PHOSPHOPEPTIDES



Peptides are frequently highly oriented in their migration through ion-exchange columns of all kinds. This affects the degree to which a charged group interacts with the surface and accounts for the separation of peptides of the same net charge. Ask for our paper on the subject.

2) FRACTIONATION OF TRYPTIC PEPTIDES IN GENERAL

ERLIC seems to be superior to SCX in some ways as a first dimension of chromatography for distribution of tryptic peptides into fractions. See our separate bulletin on the subject.

3) FRACTIONATION OF PHOSPHOPEPTIDES VIA SPE-ERLIC

Please ask for examples.

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Buying products: Contact The Nest Group, Inc., 800-347-6378, www.nestgrp.com

PolyWAX LP column, 100 x 4.6mm, 5µm, 300Å (item# P104WX0503)
PolyWAX LP column, 200 x 4.6mm (item# P204WX0503)

Other sizes of PolyWAX LP columns are available. TopTips ® are available for SPE of samples 1-10 µl, 10-200 µl, and 200-1000 µl. Regular SPE cartridges (0.5-5 ml) of PolyWAX LP are also available. Please consult The Nest Group (800-347-6378) www.nestgrp.com.