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Introducing ERLIC:

Electrostatic Repulsion-Hydrophilic Interaction Chromatography

ERLIC is a new, general-purpose mode of chromatography. With ERLIC,

1) Many gradient separations become **isocratic** separations;
2) **Phosphopeptides** can be isolated selectively from tryptic digests and separated with high resolution.

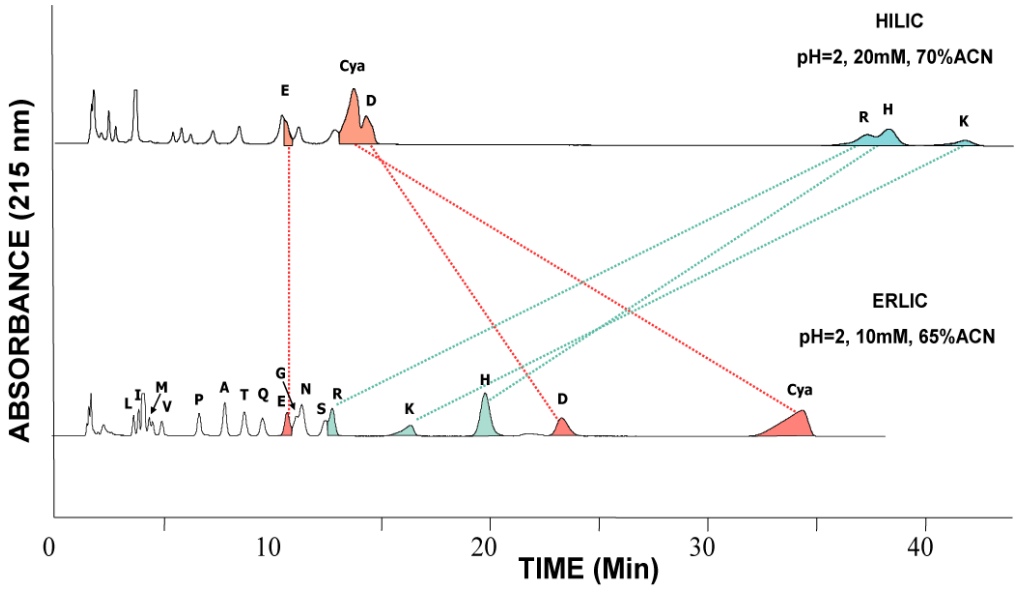
Biochemicals of high charge are frequently much better retained in chromatography than compounds of low charge. Example: ATP vs. AMP. This is true in both ion-exchange and hydrophilic interaction chromatography (HILIC). A gradient is necesssary to make both types of compounds elute in the same time frame. However, if HILIC is performed using an ion-exchange column of the same charge as the most highly-charged compounds, then their retention is selectively antagonized by **electrostatic repulsion**. This permits their elution in the same time frame as less highly-charged compounds using isocratic conditions.

A. Isocratic Separations

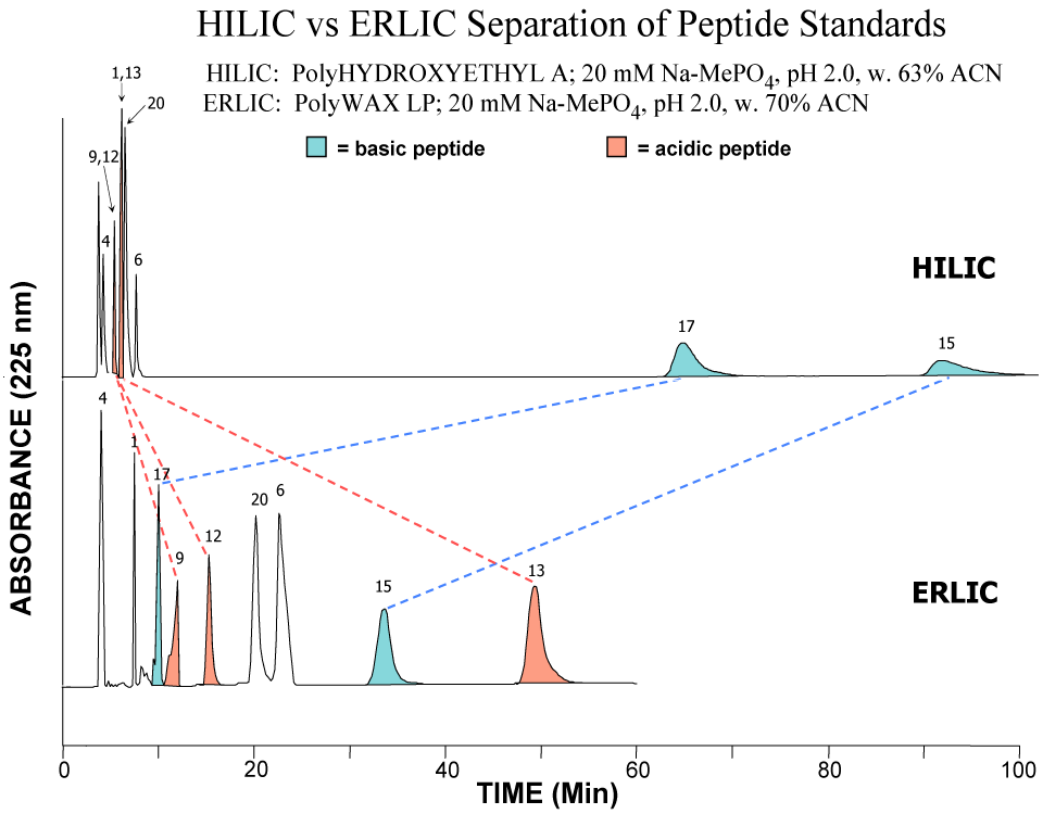
1) **Amino acids, peptides and proteins:** The most polar groups are basic residues. ERLIC of these compounds is performed with an anion-exchange column (*e.g.*, our PolyWAX LP™) at pH ~ 2.0, low enough for carboxyl- groups to lose their negative charge. Amino acids, peptides and proteins will have a net + charge and will all experience some degree of electrostatic repulsion. The most basic compounds, normally the best-retained, experience the most repulsion.

2) **Nucleotides and nucleic acids:** ERLIC is performed with a cation-exchange column (our PolySULFOETHYL A™) at a pH < 3.5, low enough for all phosphate groups to have a single negative charge (repulsion is too great at higher pH, where phosphate groups acquire a second negative charge).

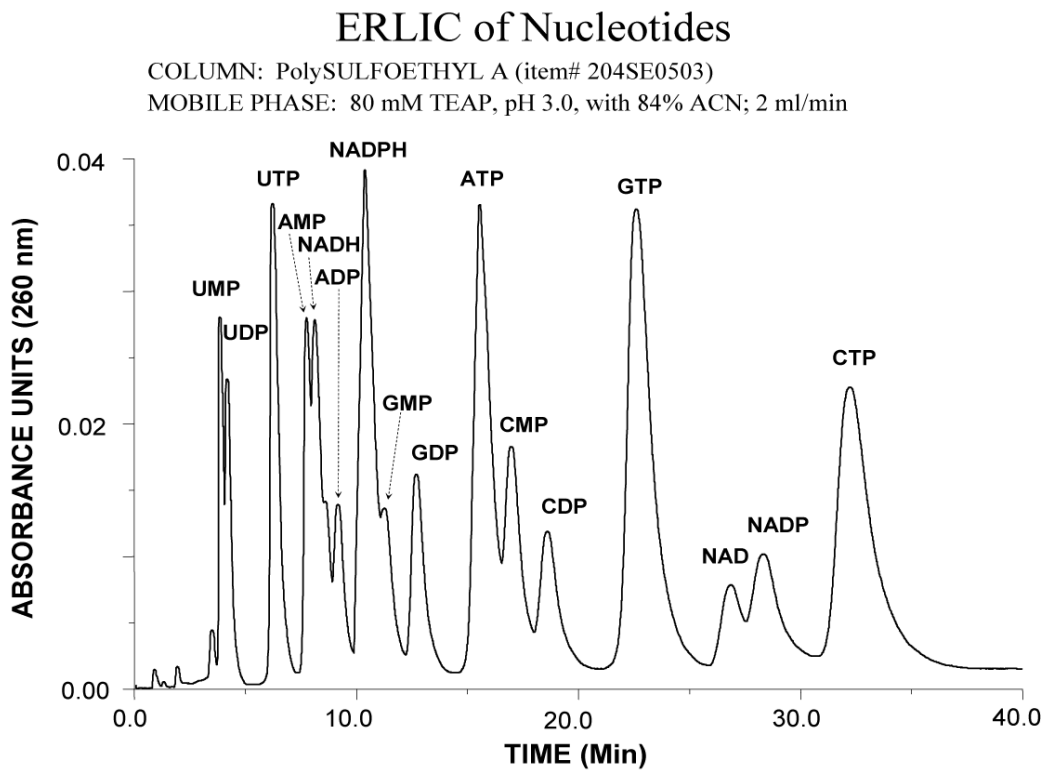
Example 1: ERLIC vs. HILIC of Amino Acids (100-Å pore columns):



Example 2: ERLIC vs. HILIC of Acidic, Basic, and Neutral Peptides (300-Å pore columns):



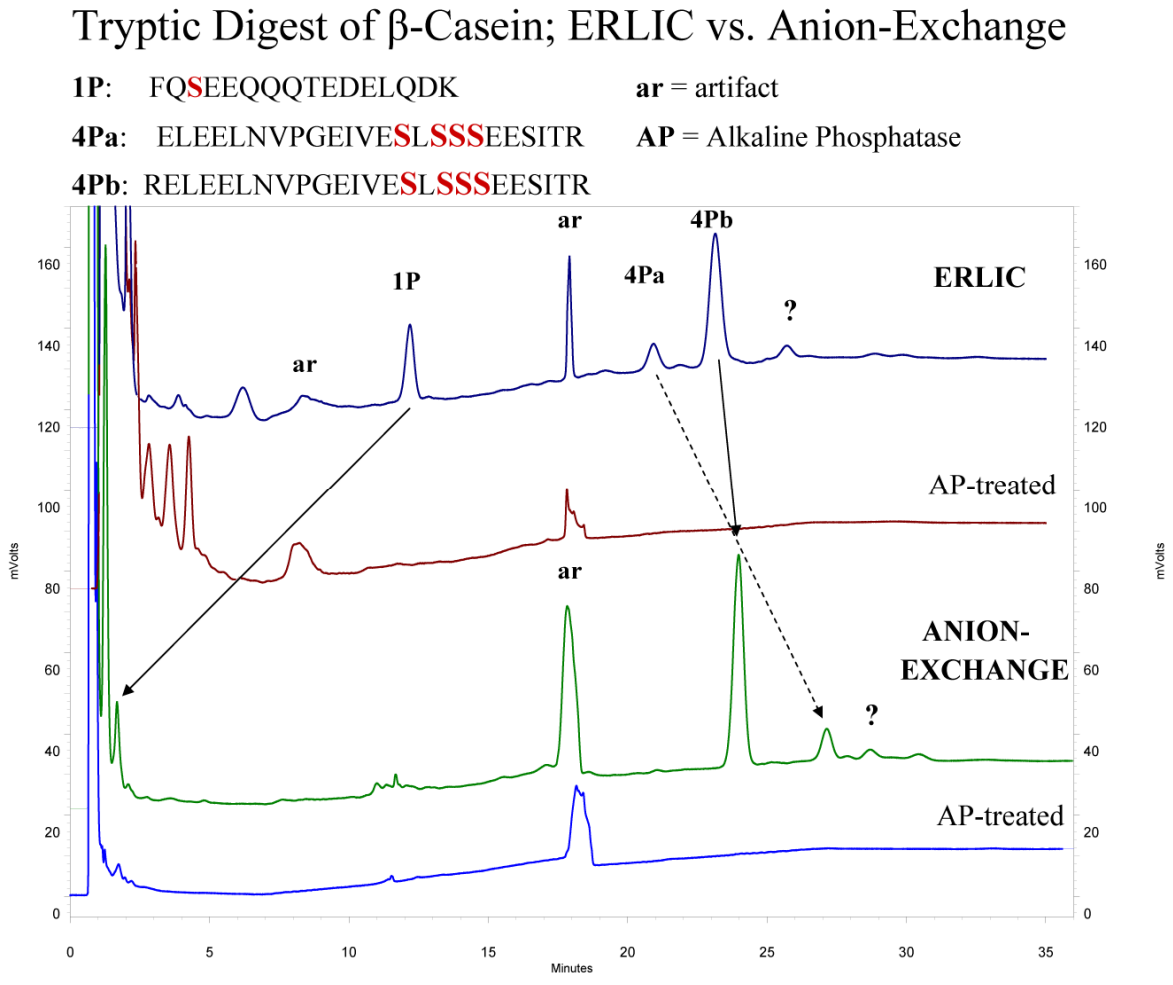
Example 3: ERLIC of Nucleotides:



B. Isolation of Phosphopeptides

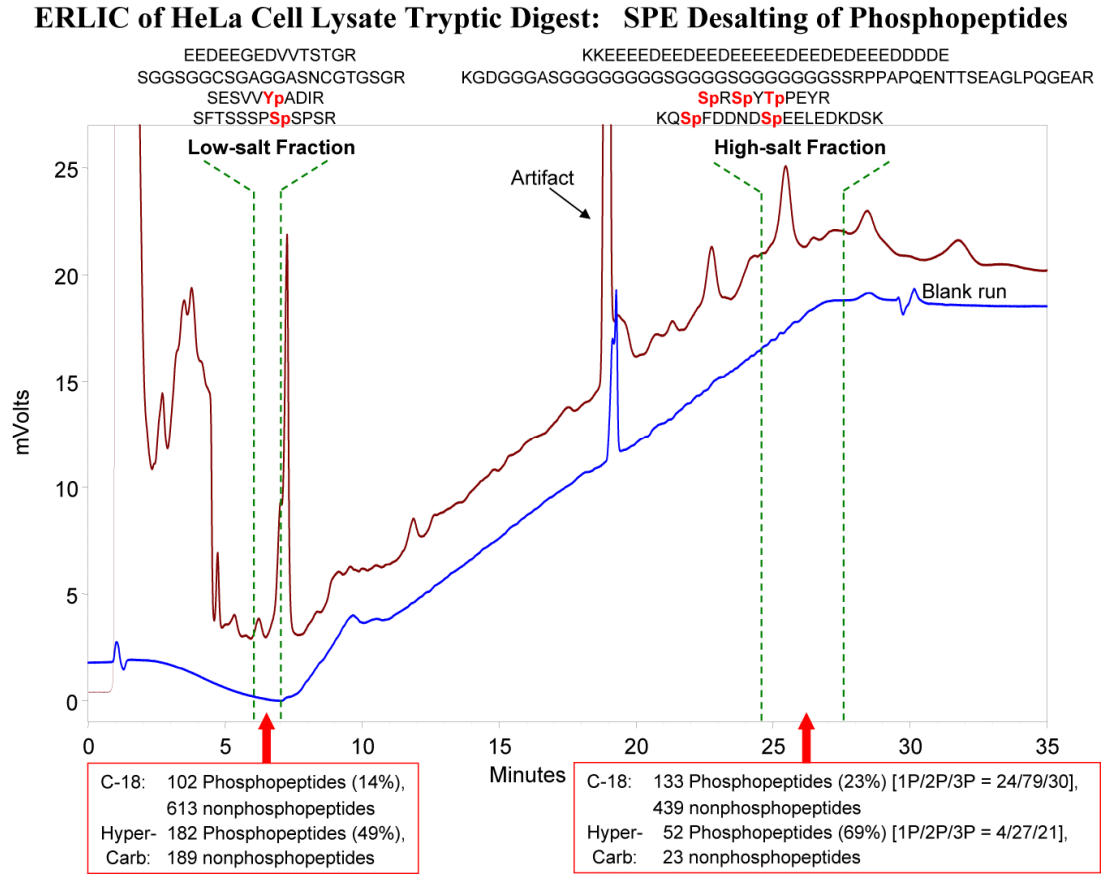
At pH 2.0, phosphate groups in peptides retain some of their negative charge. This does not permit the isolation by *anion-exchange chromatography* of singly phosphorylated peptides from tryptic digests, since the electrostatic attraction is not sufficient to overcome the electrostatic repulsion from the N-terminus and the C-terminal Lys- or Arg- residue. However, phosphate residues are quite hydrophilic. In the ERLIC mode, the combination of electrostatic attraction and hydrophilic interaction does suffice to pull singly phosphorylated peptides away from the nonphosphorylated peptides in tryptic digests. Also, unlike the situation with high-affinity media such as IMAC or titania, the phosphopeptides are well-resolved from each other. This permits their convenient separation into numerous fractions, an important tool in **phosphoproteomics** for identifying the sequences of thousands of phosphopeptides from a single sample. Peptides with multiple phosphate groups are retained so strongly that a salt gradient is necessary for elution.

Example 1: Tryptic Digest of Beta-Casein: Separation with the Same Column in the ERLIC and Anion-Exchange Modes:



A 100x4.6-mm column of PolyWAX LP (5-µm, 300-Å) was used (item# 104WX0503). Note the poor retention of the singly phosphorylated fragment in the anion-exchange mode.

Example 2: Fractionation of the Tryptic Digest of HeLa Cell Lysate:



Again, PolyWAX LP column# 104WX0503 was used here. It should be noted that recovery of phosphopeptides is highest if low-salt fractions are desalted using HyperCarb®; high-salt fractions, using C-18 silica.

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Ordering information for the ERLIC columns used in these examples:

Amino acids:PolyWAX LP column, item# 204WX0501 (200x4.6-mm; 5µm, 100-Å); $ 540.
Peptides: PolyWAX LP column, item# 204WX0503 (200x4.6-mm; 5µm, 300-Å); $ 540.
Phosphopeptides: PolyWAX LP column, item# 104WX0503 (100x4.6-mm; 5µm, 300-Å); $ 455.
Nucleotides: PolySULFOETHYL A column, item# 204SE0503 (200x4.6-mm; 5µm, 300-Å): $575.

Please see our Web site for other columns dimensions or pore and particle diameters.

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