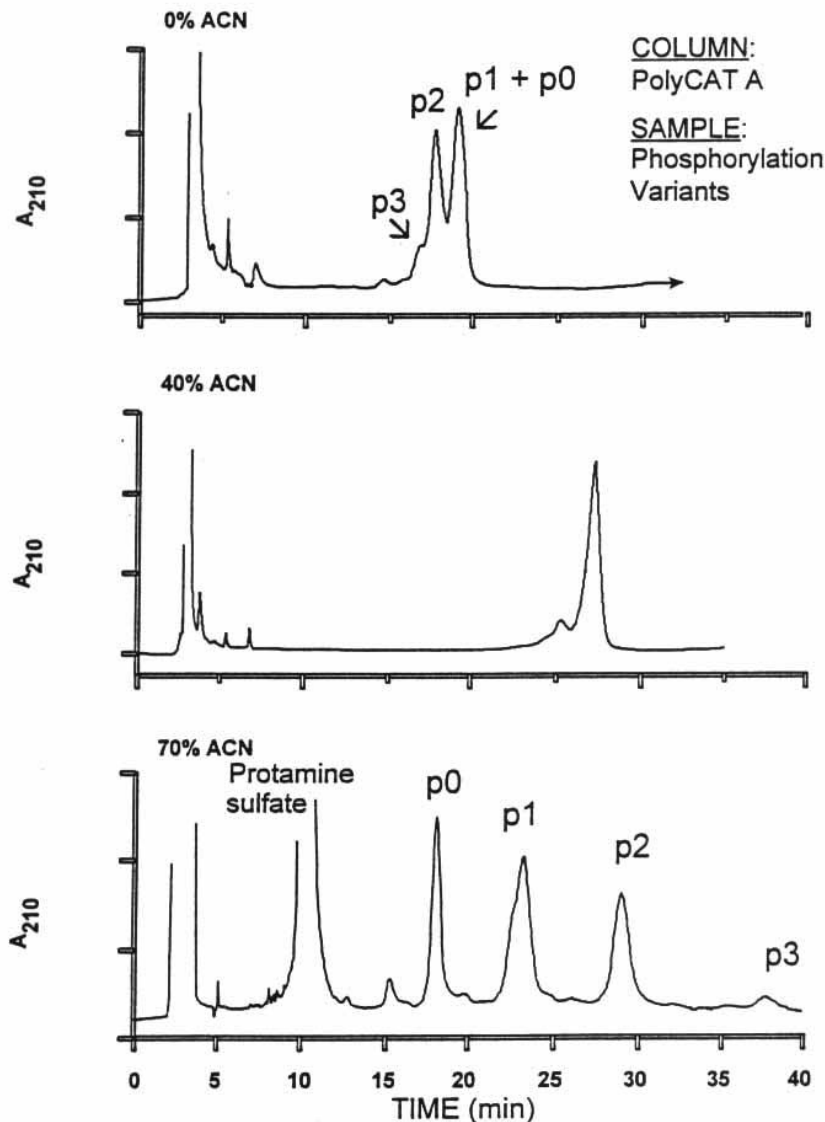


Phosphorylation Variants of Histone H1.1 Via CEX-HILIC



(TOP: pure cation-exchange) With a regular salt gradient, the forms with more phosphate elute earlier, due to electrostatic repulsion of the stationary phase.

(BOTTOM: cation-exchange + HILIC) With 70% acetonitrile (ACN) in both mobile phases, hydrophilic interactions dominate the chromatography. While electrostatic repulsion is still present, the polarity of phosphate groups is great enough to cause a net increase in retention. This affords an extraordinarily good separation, with inversion of the order of elution.

(MIDDLE) An intermediate case. With 40% ACN, electrostatic repulsion and hydrophilic interaction are balanced, and all forms coincide.

Data courtesy of H. Lindner (Univ. of Innsbruck)