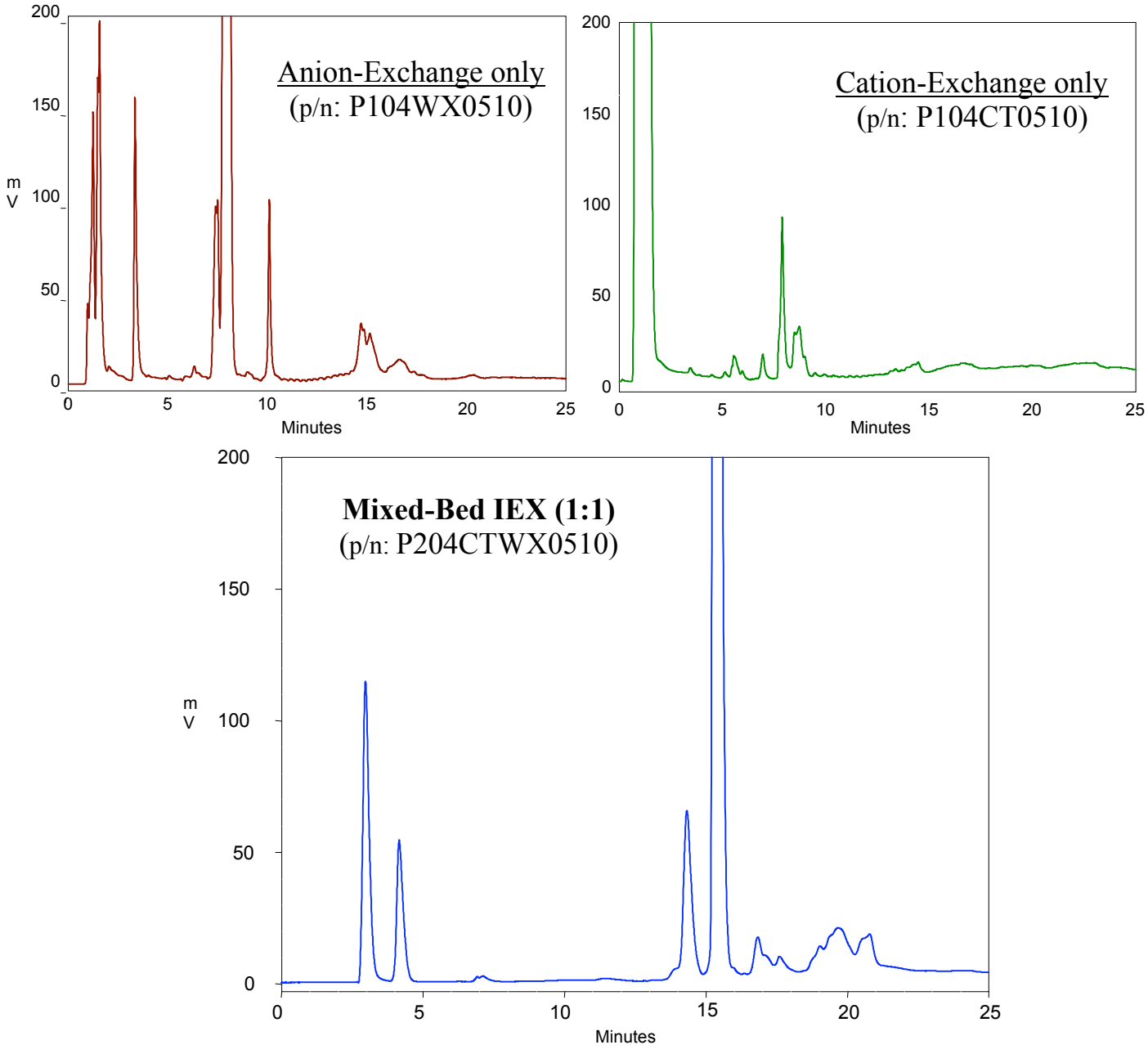
Proteomics: Pre-Fractionation of Proteins
Using Mixed-Bed Ion-Exchangers

With complex protein mixtures from lysates or serum, some protein will elute in the void volume from any single type of ion-exchange column. However, with a mixed-bed ion-exchange column, almost all proteins are retained, as shown for a yeast lysate (NaCl gradient in MES buffer, pH 6) below:



The 200x4.6-mm mixed-bed contains the cation-exchange (PolyCAT A™) and anion-exchange (PolyWAX LP™) materials in equal amounts. Particle diameter: 5 µm. Pore diameter: 1000 Å.

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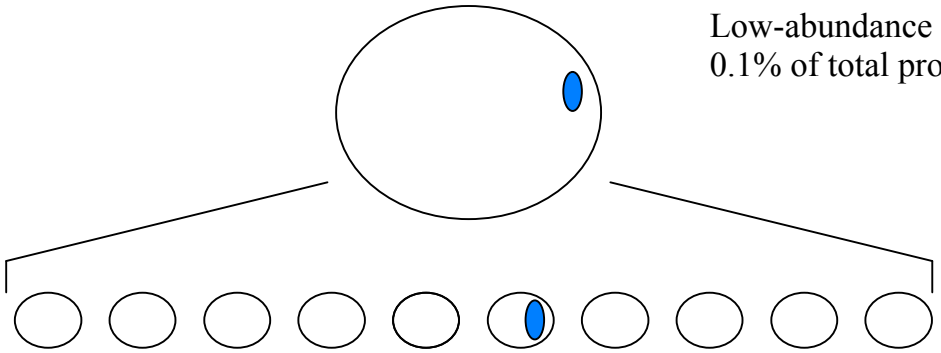
Why fractionate intact proteins for proteomics? The schematic below rationalizes the increase in protein identifications that result:

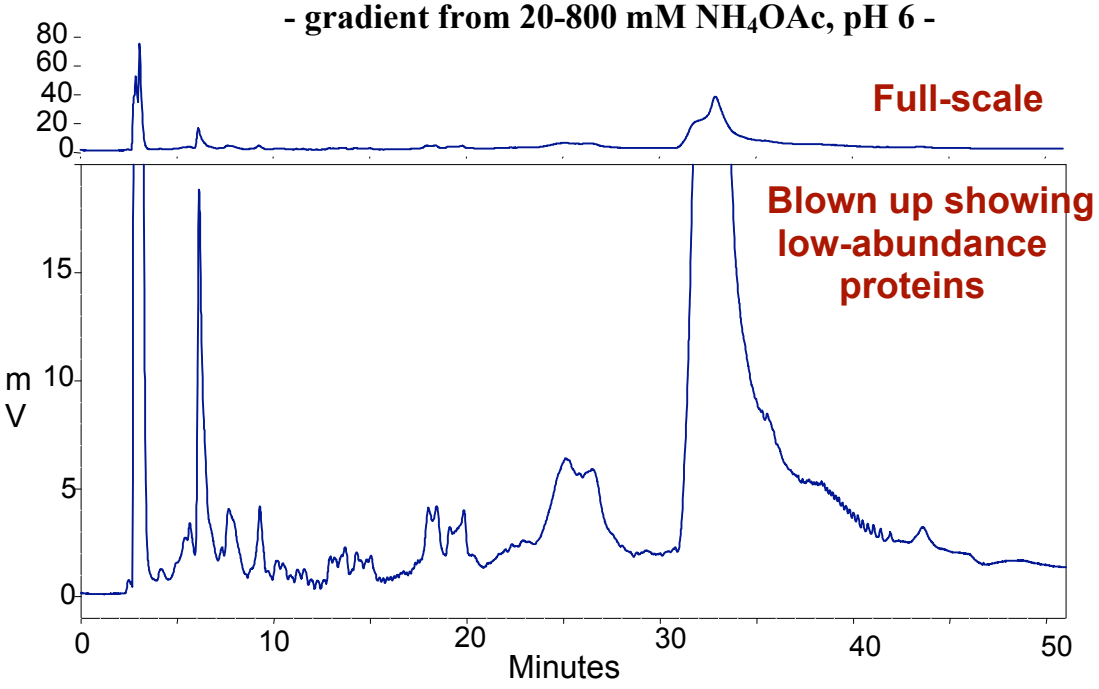
Fractionating Intact Proteins Increases Detection of Peptides from Proteins of Low Abundance



If **Protein X** is 1.0 % of total protein in Fraction #6, after digestion, its peptides will be 10x higher a percentage of the total in that fraction than would have been true in a digest of the un-fractionated mixture. That greatly increases the chances of identifying Protein X through 2-3 of its fragments rather than just one.

It is also possible to use volatile mobile phases for protein ion-exchange:

Yeast Lysate Proteins on a Mixed-Bed Ion-Exchange Column
- gradient from 20-800 mM NH_4OAc, pH 6 -



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