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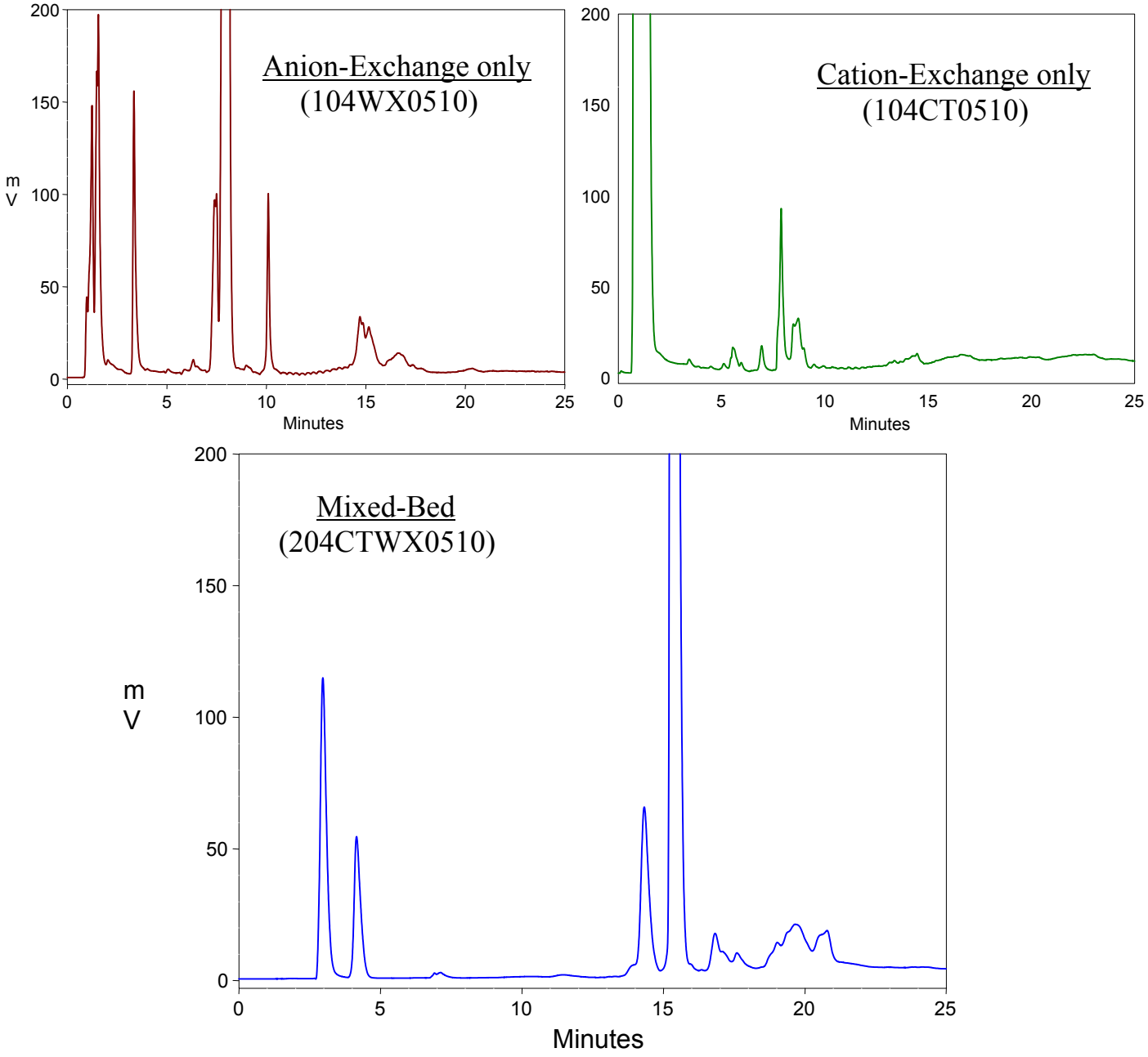
Mixed-Bed Ion-Exchange of Proteins

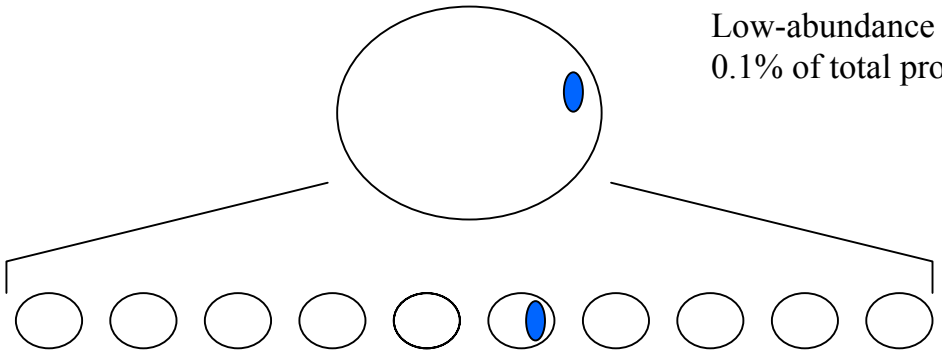
With complex protein mixtures like lysates or serum, some protein will elute in the void volume from any single ion-exchange column. With a mixed-bed column, though, almost all proteins are retained, per the following example (a yeast lysate with a NaCl gradient in MES buffer, pH 6):



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.

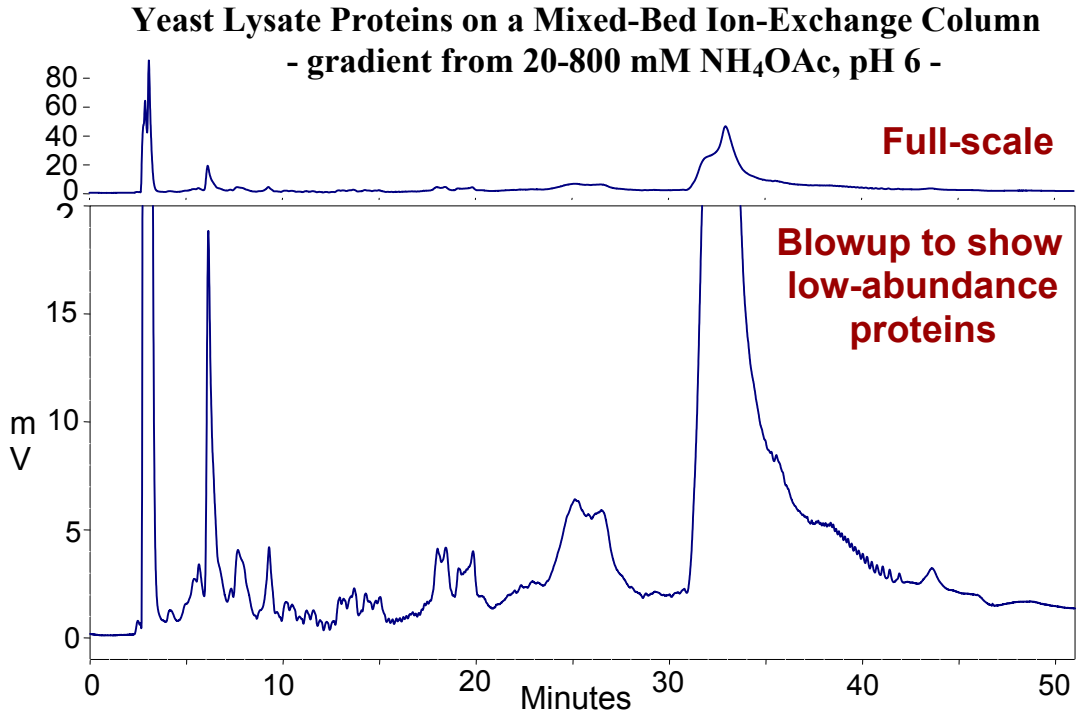
Why fractionate intact proteins for proteomics? The schematic below rationalizes the increase in protein identifications that result:

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



Now **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 unfractionated 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:



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The mixed-bed column shown here, item# 204CTWX0510, costs $ 540. Other column sizes are available. PolyCAT A and PolyWAX LP are trademarks of PolyLC Inc.

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