High Resolution Protein and Peptide Separations on a Nanoengineered Analytical IMAC Column

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ABSTRACT

Immobilized metal affinity chromatography (IMAC) is used as a tool for protein/peptide purifications in a wide variety of application areas. Because fractions collected from IMAC cartridges usually contain impurities, there is a need for a high resolution IMAC-HPLC column. To achieve this, we used living/controlled radical polymerization and polymer chain collapse to engineer the surface of 10 µm, nonporous, polymeric beads with isolated, metal-containing nanoparticles that act as IMAC interaction sites. Surface-bound nanoscale particles were visible in transmission electron microscope (TEM) images of bead cross-sections. TEM was also used to visualize individual ferritin molecules interacting with nanoparticles on the substrate surface. In the copper mode, the resolving power of the column was tested by injecting a mixture from a library of prion-related peptides. As expected, the column was capable of separating prion peptides differing in number of octapeptide repeat units (PHGGGWGQ), $(PHGGGWGQ)_2$, and $(PHGGGWGQ)_4$. Unexpectedly, the column was also able to separate prion-related peptide mixtures where the peptides contained the same number of copper binding sites but differed only in presence of a hydrophilic tail or Q → A mutation. The IMAC column was also used in the immobilized copper mode for separation of His-tagged protein aggregation variants. Aggregation number was confirmed by on-line light scattering. Phosphopeptide enrichment experiments were performed using the IMAC column in the immobilized iron mode. Reversed phase was used to measure phosphopeptide recovery and linearity.

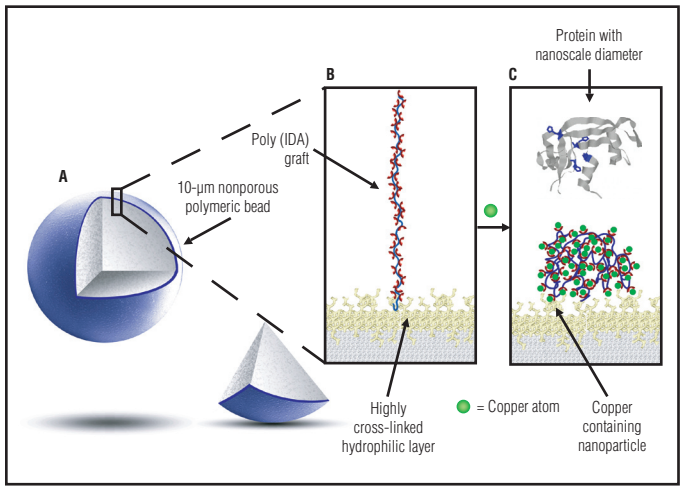


Figure 1. Illustration of ProPac® IMAC-10 surface. A. Nonporous polystyrene-divinylbenzene substrate. B. Hydrophilic layer coated and flexible metal chelating poly (iminodiacetic acid) grafted resin. C. Collapse of graft in the presence of copper sulphate.

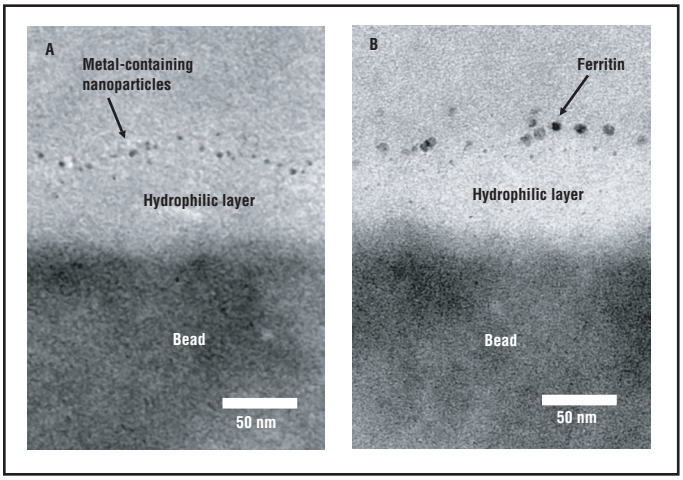


Figure 2. Transmission electron microscope images of: A. cross-section of ProPac IMAC-10 resin loaded with copper. B. ProPac IMAC-10 resin loaded with copper and washed with ferritin.

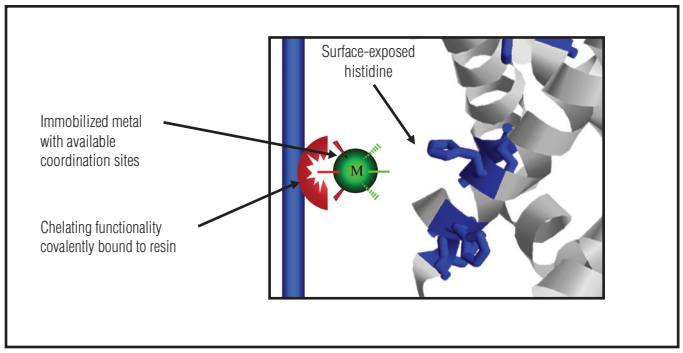


Figure 3. The mechanism of protein separation in IMAC is coordination. Copper exposed on the resin surface provides coordination acceptor sites capable of binding surface-exposed histidine residues on the protein.

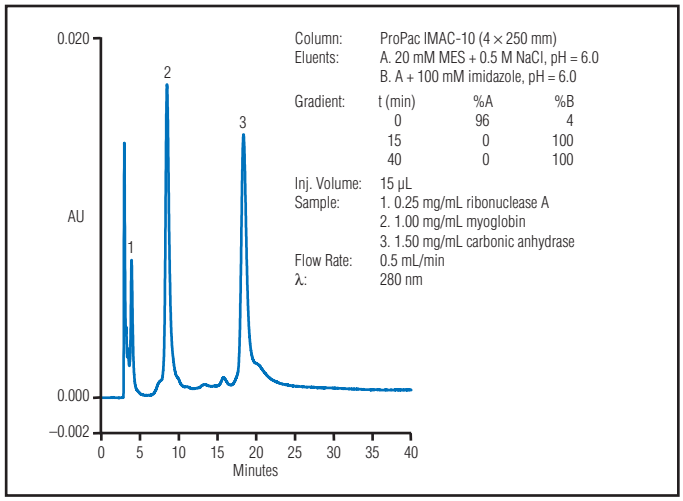


Figure 4. Separation of standard proteins in the order of surface exposed histidines.

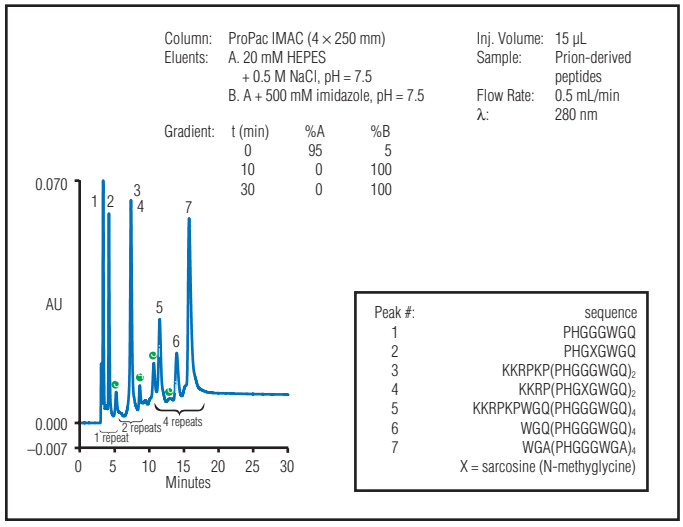


Figure 5. Separation of prion-derived peptides.

HIS-TAGGED PROTEINS

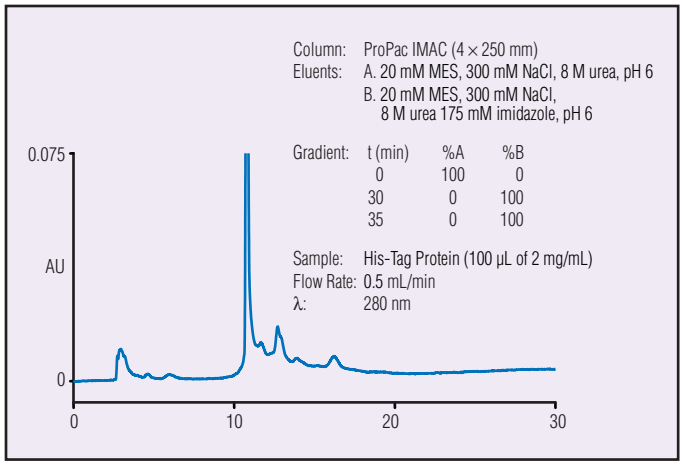


Figure 6. Separation of His-tagged protein that was previously purified by an IMAC cartridge. This trace shows that ultra-pure His-tagged protein can be obtained by removing residual unwanted impurities.

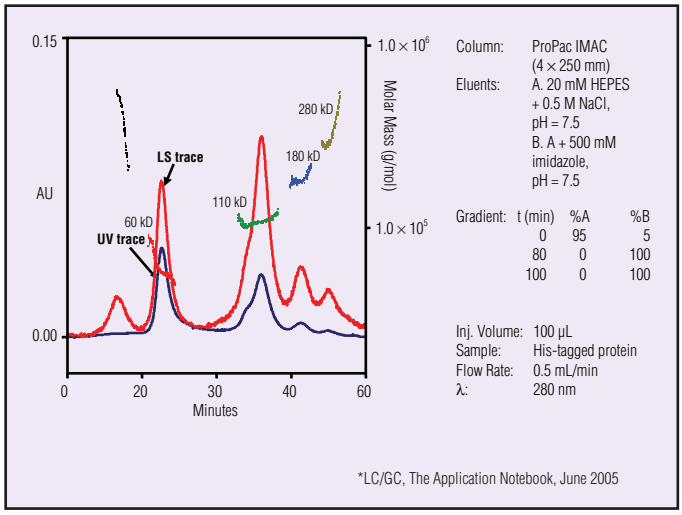


Figure 7. The IMAC purification example demonstrates the advantage of analytical IMAC for detection and fractionation of His-tagged protein aggregation variants. (Reference 1).

MONOCLONAL ANTIBODIES

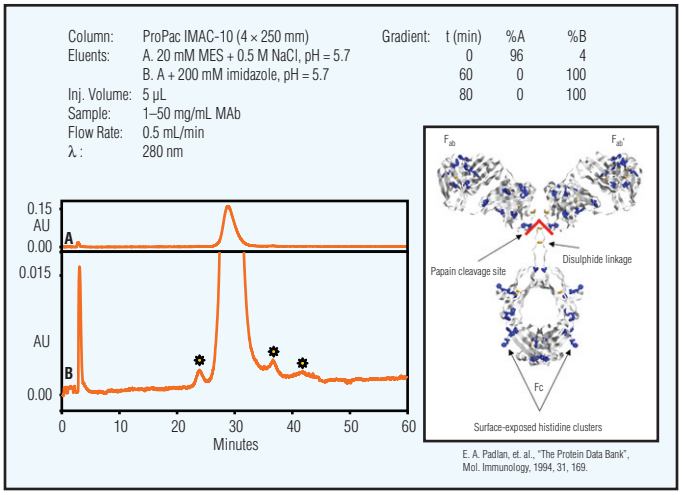
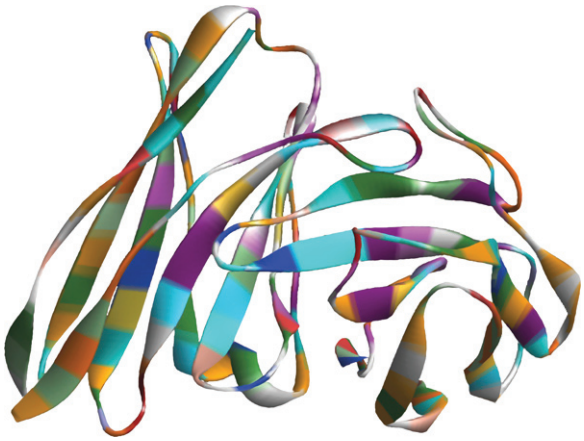


Figure 8. A. Separation of pure MAb (DX-1). B. 10x magnification of the separation to visualize unknown variants resolved from major MAb peak.



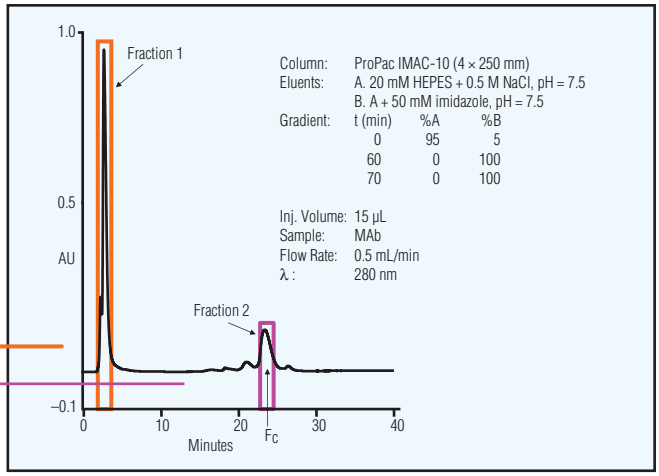


Figure 9. Fractionation MAb papain digest fragments; Fab and Fc by ProPac IMAC-10.

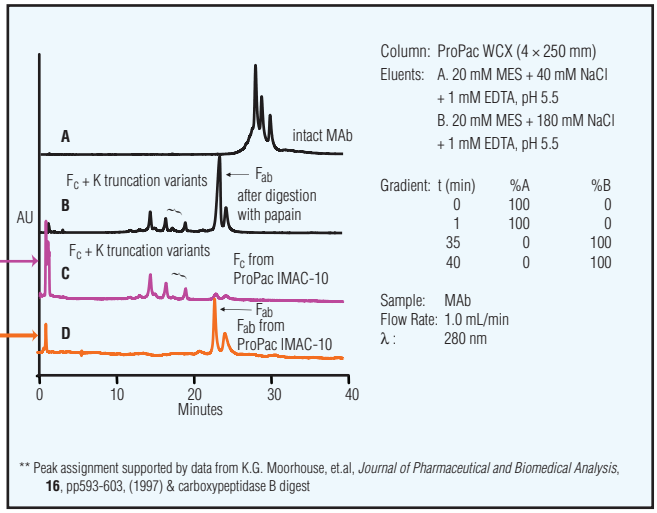


Figure 10. ProPac WCX-10 separation of ProPac IMAC-10 fractions from MAb papain digest. A. Intact MAb. B. After digestion with papain. C. ProPac IMAC-10 captured fraction (Fc is the predominant species in the captured fraction). D. ProPac IMAC-10 flow through fraction (Fab is the predominant species in the flow-through fraction).

PHOSPHOPEPTIDES

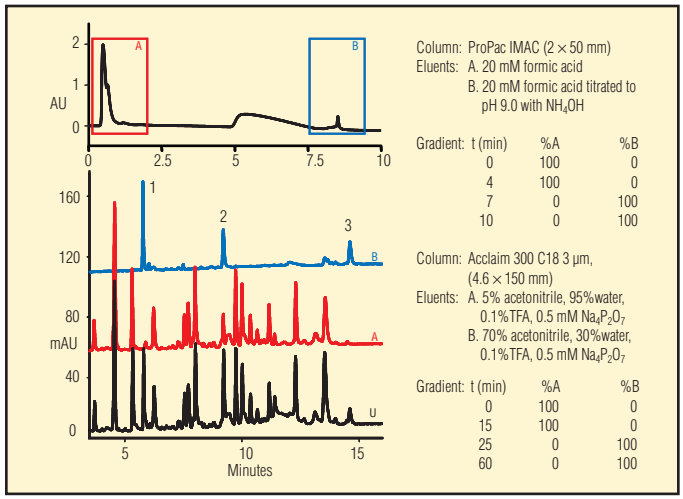


Figure 11. Fractionation of phophopeptides from β-casein tryptic digest (top trace) on ProPac IMAC-10. Reversed phase separations of: A. ProPac IMAC-10 flow through (red), B. ProPac IMAC-10 retained fraction (blue) and, U. Un-fractionated β-casein digest (black).

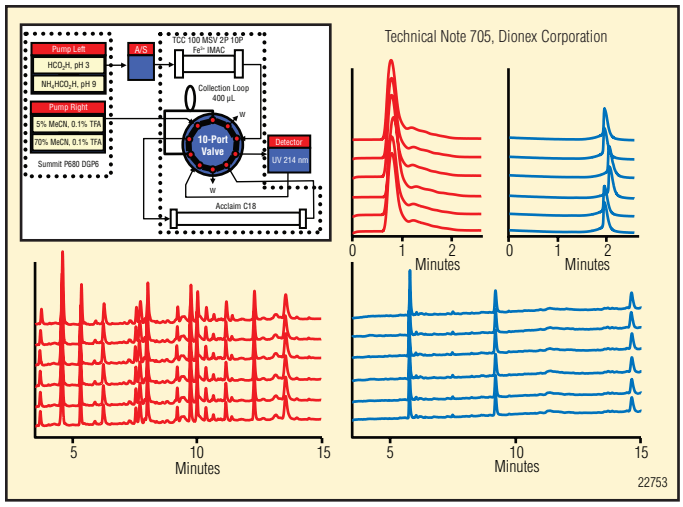


Figure 12. Automated fractionation and analysis of digests containing phosphopeptides. (Reference 3).

CONCLUSION

- ProPac IMAC was used to:
  1. Separate proteins based on number of surface exposed histidine residues (Figure 4).
  2. Separate prion-derived peptides (Figure 5).
  3. Obtain ultra-pure His-tagged protein (Figure 6).
  4. Separate His-tagged protein aggregation variants (Figure 7).
  5. Resolve several unknown monoclonal antibody variants (Figure 8).
  6. Fractionate monoclonal antibody Fc and Fab (Figures 9–10).
  7. Enrich and analyse phosphopetides from tryptic digests (Figures 11–12).

- ProPac IMAC-10:
  1. Has high efficiency and resolution for analytical gradient separations of proteins and peptides.
  2. Is shipped free from metal ions and can be charged with the metal most appropriate for the application.
  3. Is stable at high pressure, supports multiple injections without recharging, is easily automatable, and can be recharged with metal for extended use.
  4. Is offered in several formats to serve both HPLC and FPLC users for analytical and semi-preparative applications.

REFERENCE

1. P. McCarthy, A. Heckenberg, C. Pohl. Separation of His-tag Protein Aggregation Variants on the New Analytical ProPac IMAC-10 Column. *LC/GC, The Application Notebook*, June 2005.
2. E. A. Padlan, et. al., "The Protein Data Bank", *Mol. Immunology*, **1994**, *31*, 169.
3. *Automated Enrichment and Determination of Phosphopeptides Using Immobilized Metal Affinity and Reversed-Phase Chromatography with Column Switching*. Technical Note 705, Dionex Corporation, Sunnyvale, CA.

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