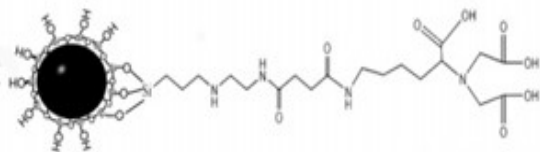


**Reference:** Yi-Cheng Li, Ya-Shiuan Lin, Pei-Jane Tsai, Cheng-Tai Chen, Wei-Yu Chen, and Yu-Chie Chen. "Nitrilotriacetic Acid-Coated Magnetic Nanoparticles as Affinity Probes for Enrichment of Histidine-Tagged Proteins and Phosphorylated Peptides." *Anal. Chem.* **2007**, 79, 7519-7525.

**Bioanalysis and Sample:** The authors report the synthesis of superparamagnetic iron oxide nanoparticles that have been conjugated to a chelating agent (Nitrilotriacetic Acid) coordinated to a metal ion. This allows the separation of proteins that contain the proper motif from a mixture. The authors used the Ni(II) ion to coordinate proteins bearing a His tag (at least 6 His residues in sequence). They also used the Zr(IV) and Ga(III) ions to separate phosphorylated proteins from a mixture. Separation is achieved by using a magnet to trap the nanoparticles containing the conjugated protein of interest. Identification of the samples is performed using MALDI-MS. Samples analyzed include streptopain, bradykinin,  $\alpha$  and  $\beta$  albumin, and other protein mixtures.

**Importance:** Separating, isolating, and identifying a protein from a complex mixture, such as a cell lysate, is an arduous task. New methods that cut down on analysis time are highly desired. The methods presented in this paper allow for the complete separation, enrichment, and characterization of a protein from a complex mixture in less than ten minutes when coupled to MALDI-MS.

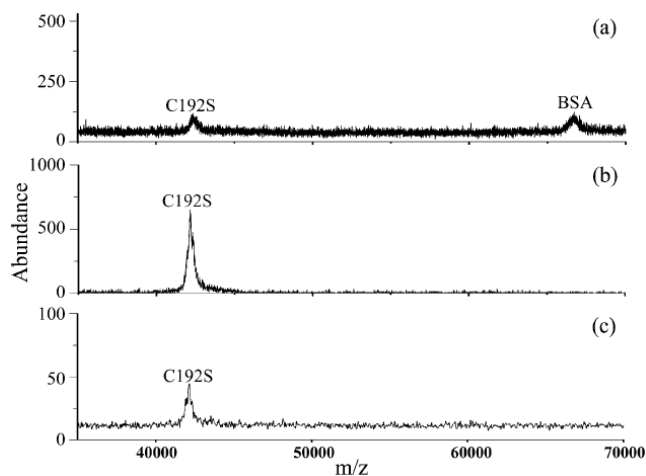
**Technique:** The authors demonstrated the synthesis of iron oxide nanoparticles coordinated to three different metal ions (see figure below). Mixing a solution of the nanoparticles with the protein mixture



allows the desired proteins to coordinate to the nanoparticles. Simply holding a magnet to the side of the reaction vessel fixates the nanoparticles so other debris can be washed away. MALDI MS analysis can be used while the proteins are still attached to the nanoparticles

by subjecting them to a tryptic digest. Coupling this separation with mass spec allows rapid characterization of a protein based upon molecular fragmentation in the mass spectrometer. This technique has the two fold advantage of not only isolating a peptide from a mixture but also enriching or concentrating that peptide in the sample.

**Example of Results:** The figure at the right shows three separate mass spectra of a mixture of two proteins, the C192S mutant of streptopain and bovine serum albumin (BSA). The streptopain protein contains a His tag while the BSA does not. Figure (a) shows the mass trace of the mixture itself. In (b) the mixture has been subjected to the Ni(II) nanoparticles which coordinate the His tag of the C192S and allow its separation from BSA. The disappearance of the BSA peak unequivocally demonstrates that the nanoparticles are selective at isolating only the His tagged protein while at the same time concentrating the peptide, as evidenced by an increase in abundance. Figure (c) shows a cell lysate mixture from which the C192S streptopain protein has been isolated upon nanoparticle treatment.



**Opinion:** This technique allows very rapid isolation and characterization of proteins from mixtures. It is certainly a tool worth implementing for protein isolation when the circumstance presents itself. The limitation of this technique is that it will only work for His tagged proteins and phosphorylated proteins.