430e Circumventing the Effects of High Binding Immobilized Metal Affinity Chromatography Contaminants

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Using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectroscopy, we have been characterizing the *Eschericia coli* contaminant pool associated with Co^{+2} Immobilized Metal Affinity Chromatography (IMAC) in an effort to determine binding affinity and metabolic function. Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) mass spectroscopy analyses of samples excised from two-dimensional gels has revealed that two contaminant proteins strongly adsorb during the loading of the column, and also elute at high concentrations of imidazole typically used to elute proteins tagged with commonly employed histidine affinity tails. Adsorption and stringent elution behavior of these two proteins when compared to the target protein therefore (i) reduces column capacity, (ii) complicates gradient elution, and finally (iii) requires additional purification steps. Based on this information we have taken a systems approach to improved bioseparation by working backwards to develop an improved host strain and expression system. Specific to this presentation will be a discussion regarding the development of a novel deletion strain useful for Co^{+2} IMAC, the use of this strain for target protein expression and isolation, and the continued improvement by identifying characteristics associated with the next generation of IMAC affinity tails.