



Pichia 2014

Protein Expression Conference
Catamaran Resort Hotel & Spa
March 2 - 5, 2014 | San Diego, CA USA

Organized by:



Production of Proteins using the Yeast *Pichia pastoris*: Interfacing Fermentation and Radial Flow Bed IMAC Primary Capture

Maria Livanos
UCL Cancer Institute,
London,
United Kingdom

Gaurav Bhavsar¹, Gabriela Nagy², Andreas Plückthun², Berend Tolner¹ and Kerry Chester¹

¹UCL Cancer Institute, London, United Kingdom

²University of Zurich, Zurich, Switzerland

Abstract: Production of vast amounts of recombinant protein inherently requires processing of large volumes of feedstock with high biomass. Consequently, primary capture of the target protein is challenging; entailing elaborate upfront clarification by centrifugation, tangential flow or depth filtration.

Here we show how recombinant proteins secreted by *Pichia pastoris* can be readily isolated from unpurified feedstock in a procedure that yields clinical grade product. We exemplify the process with Designed Ankyrin Repeat Proteins (DARPin) which are non-immunoglobulin scaffold proteins.

To this end, we engineered a (His Glu)₃ tag (HE tag) to the proteins. The target protein was directly captured from feedstock by immobilized metal ion affinity chromatography (IMAC) using radial flow bed adsorption. IMAC facilitates initial fast capture and isolation, yielding concentrated target protein in a small volume. The described procedure simplifies and significantly reduces cost (time and materials) of primary capture and downstream processing. Subsequent use of anion exchange followed by a desalting step, yielded fully functional, unglycosylated protein, with *P. pastoris* host cell protein contamination and endotoxin levels less than <0.0005% and 0.5 EU / mg, respectively.

This is the first report showing feasibility of cGMP manufacture of DARPins in *P. pastoris* utilizing radial flow technology for direct capture.