# *Pichia pastoris GMP* Protein Production: Interfacing Fermentation & Radial Flow Bed IMAC Primary Capture

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## Introduction

**Challenge** Production 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.

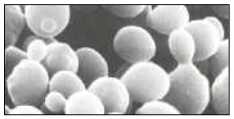
**Solution** We show how recombinant proteins secreted by *Pichia pastoris* can be readily isolated from unpurified feedstock in a procedure that yields clinical grade product.

We exemplify the process with Designed Ankyrin Repeat Proteins (DARPin) which are non-immunoglobulin scaffold proteins.

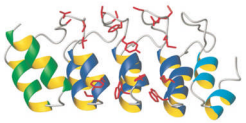
**Method** We engineered a Histidine tag to the proteins. The target protein was directly captured from feedstock by 'Immobilized Metal-ion Affinity Chromatography' (IMAC) using radial flow bed adsorption. IMAC facilitates initial fast capture and isolation, yielding concentrated target protein in a small volume. Subsequent use of anion exchange followed by a desalting and endotoxin removal 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.

## Expression



*Pichia pastoris*

DARPin

## Production process

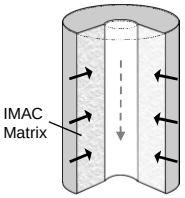
Bioreactor
- generate biomass: glycerol
- generate product: methanol

### Radial flow bed IMAC primary capture

125 ml column Cellthru BigBead

resin charged with $Ni^{2+}$ or $Cu^{2+}$

apply feedstock (10L)




Concentration & dialysis


FPLC SEC

FPLC IEX

FPLC desalting ②

Detox column ③

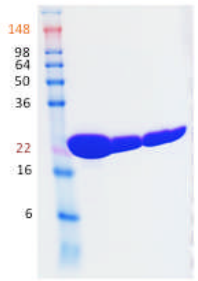
Aim for first fill: August 2014

## Data

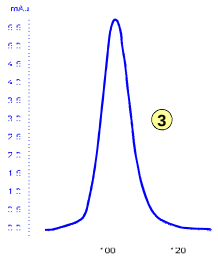
- yield approximately 20 mg/litre (final product)
- single peak purity
- *P. pastoris* host cell protein: less than <0.0005%
- endotoxin levels less than 0.5 EU / mg
- affinity for antigen similar to *E. coli* produced product

### *Economics*

- scalable process
- simplification and significant cost reduction (time and materials) of primary capture




**1, post FPLC SEC;**
**2, post FPLC desalting**
**3, post detox column**




FPLC final product

## Summary

### *We developed*

A production process for GMP grade recombinant protein based on interfacing *P. pastoris* fermentation and soluble expression of recombinant proteins with capture of secreted proteins directly from fermentation broth using radial flow IMAC.

The process was exemplified using DARPins for imaging studies.

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