thermo scientific

Enabling custom solutions for downstream processing

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Introduction

The manufacture of complex biotherapeutics, such as activated proteins or closely related product forms, requires novel purification strategies, which may not always exist. Our custom ligand and resin development platforms enable the development of innovative purification resins, providing a solution for challenging downstream processes.

Here we present custom downstream processing solutions, developed in close collaboration with our customers, including a case study covering the single step purification of prothrombin. Custom resin production can start with affinity ligand development or with existing affinity ligands provided by the customer.

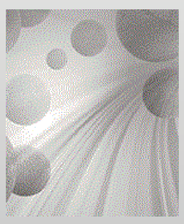
Custom POROS™ resin development

A customer-defined or custom developed affinity ligand, can be immobilized through surface chemistries that are aligned with application needs, on the POROS backbone.

POROS Chromatography Resins are rigid 50 micron polymeric beads characterized by the addition of large “throughpores”. The large pore structure makes these resins ideally suited for the capture of large biomolecules.

In addition, other resins such as ion exchange or reversed phase resins can be developed specific to your down stream processing needs.

Prototype resin development



- Experimental design
- Customer evaluation

Process definition

- Process optimization
- Scale up

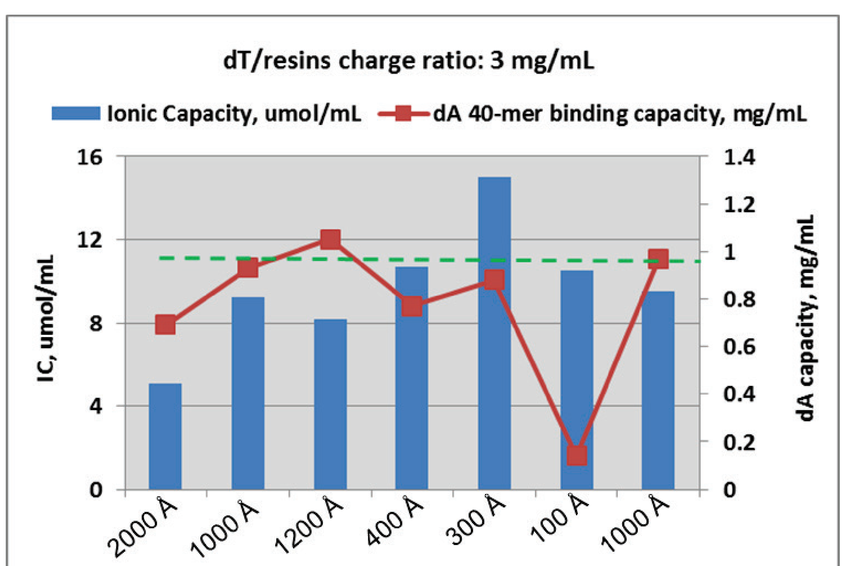
Commercialization

- Process validation
- Stability studies
- Regulatory support file

Custom POROS resins are developed in close collaboration with the customer to meet the needs for even the most demanding biotherapeutic purification processes.

Prototype resin development – Experimental design

1. **Investigation of ligand immobilization to offer superior performance as it relates to capacity**
   - Understand correlation between coupling yields and ionic capacity
   - Determine ligand charge /resin ratio.
2. **Screening DoE study to further optimize coupling conditions**
   - Understand which critical inputs during the coupling reaction impact resin performance
   - Determine coupling conditions.
3. **Evaluating bead morphology impact in resin performance**



Influence of pore size on ionic and binding capacity

Resins of large pore size revealed lower ionic and binding capacity, due to their lower surface area.

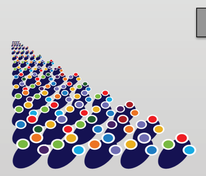
Resins with smaller pore sizes show high ionic but lower binding capacity.

Resins from mid range pore size seem to be best candidate for the defined coupling conditions as binding capacity is higher.

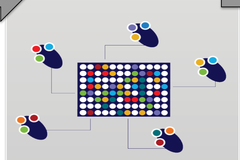
CaptureSelect™ custom ligand development

We offer a unique, milestone-based service for the development of product- and/or process-specific affinity solutions tailored to a target protein and its specific requirements. The custom ligand can be further developed into an affinity resin, which can be used in large-scale processing of biopharmaceuticals. CaptureSelect ligands offer a unique affinity purification solution based on Camelid-derived single domain [V_HH] antibody fragments.

Library Construction
1 to 10 targets

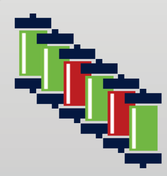


Lead Screening
200 → 24 → 6



Ligand Screen:
- Specificity
- Binding Kinetics
- Mild elution
- Stability

Prototype Resin testing
6 → 2



Prototype Screen:
- Ligand Scalability
- One-step purity with feedstock
- Elution profiles
- Ligand Stability

Lead Selection
2 → 1

Lead Testing:
- Upscaling test of lead prototypes
- Resin evaluation under process conditions

cGMP suitable resin

Final resin:
- Process validation
- Leakage ELISA
- Regulatory support file

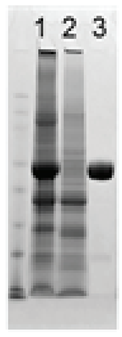
CaptureSelect ligands are designed to meet the most challenging purification needs, such as closely related product forms or activated proteins.

Prothrombin – case study

Customer need:

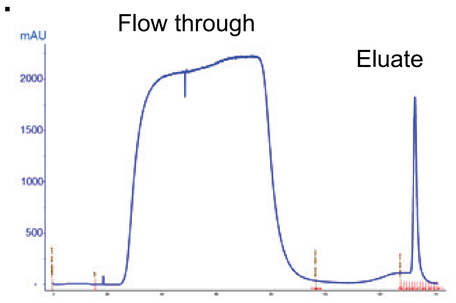
- Reduction of chromatography steps in the purification process of prothrombin
- Mild elution conditions to maintain protein activity
- Selectivity for the most biologically active form of prothrombin (fully carboxylated form)

A.



1. Load
2. Flow through
3. Eluate

B.



A. SDS-PAGE analysis shows high selectivity of the affinity resin for prothrombin in a single-step purification.
B. Chromatogram showing a high resolution elution peak under mild elution conditions: 25 mM EDTA

During the anion exchange chromatography process, the uncarboxylated variants of prothrombin elute in the pre-peak (AEC-HPLC). Comparison between anion exchange (QFF) and V_HH affinity chromatography shows the V_HH affinity resin has a very high selectivity for the most biologically active form of phrothrombin. The single-step purification results in significant lower host cell protein content in the eluate, without compromising purity.

Parameter	Units	Feedstock	QFF	V_HH affinity (CaptureSelect)
Host cell protein content	ng/mg	1,453,955	19,336	3,109
AEC-HPLC pre-peak	%	-	30.5	7.5
Purity	%	-	94.9	97.6

CaptureSelect development pipeline

Custom production of CaptureSelect affinity resins can start from library construction all the way to a cGMP suitable resin or from further development of a product from our development pipeline.

Product Stage	Therapeutic proteins& *viruses*	Antibody types
Research Use Only products	Human Growth Hormone (hGH), C-tagXL, Tissue plasminogen activator (tPA), Antithrombin III, Fibrinogen (Fib), Transferrin, Apolipoprotein H (ApoH), C1-inhibitor (C1-Inh), Granulocyte-macrophage colony-stimulating factor (GM-CSF)	IgA, IgM CH1-XL
Lead Development	Prothrombin, Erythropoietin (EPO) *Adeno-Associated Virus multi serotype (AAVX), Adenovirus (Adv5)*	IgE
Lead Selection	Insulin, Thyroid-Stimulating Hormone (TSH) *Influenza (HA),* Deoxyribonuclease (DNAse)	Free LC-kappa
Lead screening	Interferon a and b (IFNa/b), Interleukin 2 (hIL2), Factor V, FX, FXI, FXII, FXIII, and FH, *Lentivirus (VSV-G)*	Rabbit IgG

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