

Enabling custom chromatography solutions for downstream processing

ThermoFisher
SCIENTIFIC

Frank Detmers¹, Pim Hermans¹, Al De Leon², Mike Coleman², David Humphries², Gregor Richter¹, Dirk van der Steen¹

1. Thermo Fisher Scientific, Leiden, the Netherlands 2. Thermo Fisher Scientific, Bedford, MA

Bioprocessing

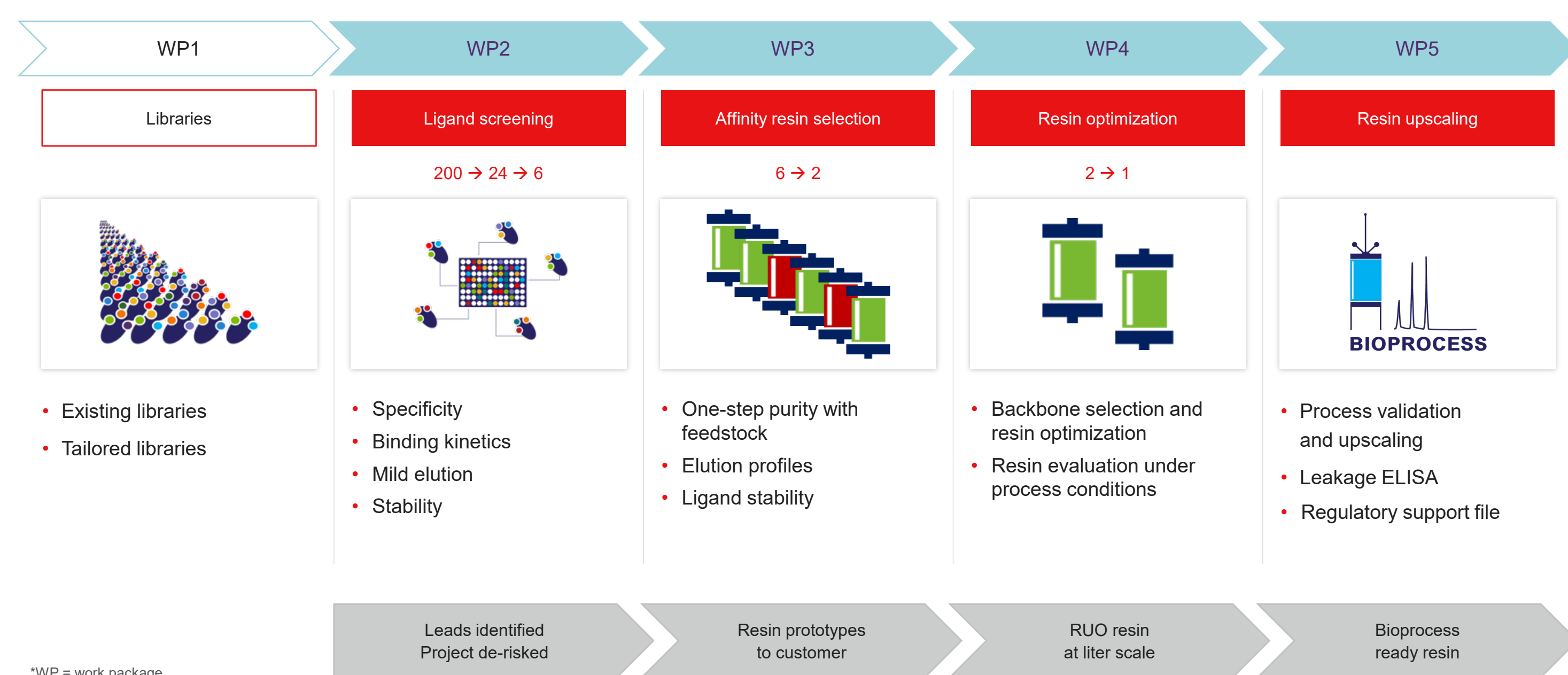
INTRODUCTION

The manufacture of complex bio-therapeutics, 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.

CAPTURESELECT CUSTOM AFFINITY RESIN DEVELOPMENT

- Affinity resins based on single domain [V_HH] antibody fragments (Fig 1.) – produced in an animal origin free production process (*Saccharomyces cerevisiae*)
- Dedicated stage-based service for the development of affinity solutions tailored to a specific target protein
- Custom ligand can be immobilized on a variety of backbones, including our high performing POROS™ beads
- Affinity ligands are screened for binding specificity, mild elution properties and stability



✓ COMBINING ANTIBODY-BASED SELECTIVITY AND PROCESS ROBUSTNESS IN A UNIQUE CUSTOM AFFINITY RESIN

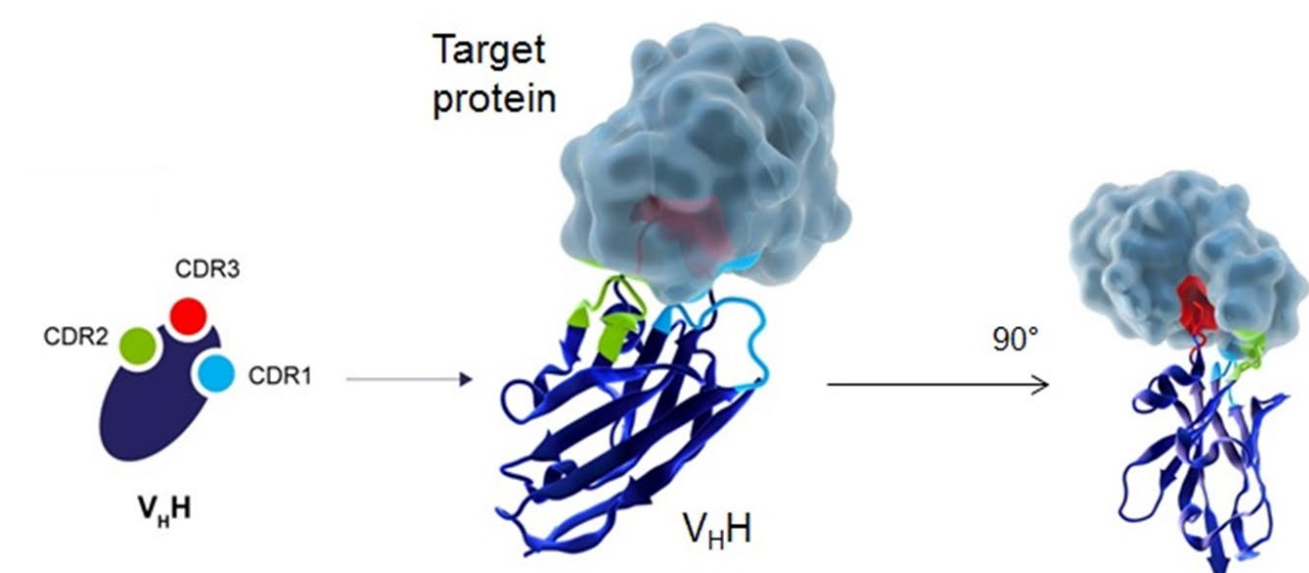


Fig.1 CaptureSelect ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecule.

The small size of V_HH fragments (15kD) allows binding at difficult to reach epitopes. Overall, V_HH fragments offer high specificity, affinity and stability.

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)

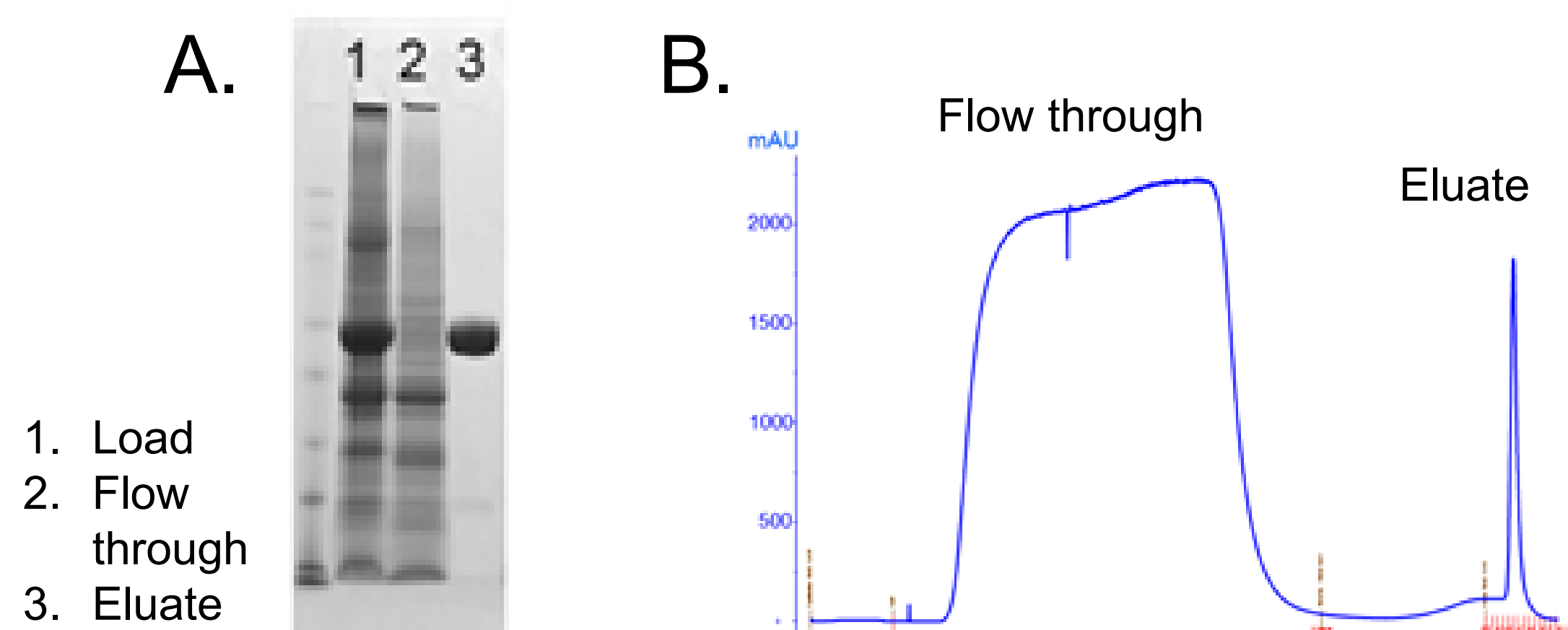


Fig.2 Analysis of the CaptureSelect Prothrombin Affinity resin

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

Comparison between anion exchange (old process) and affinity chromatography

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

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

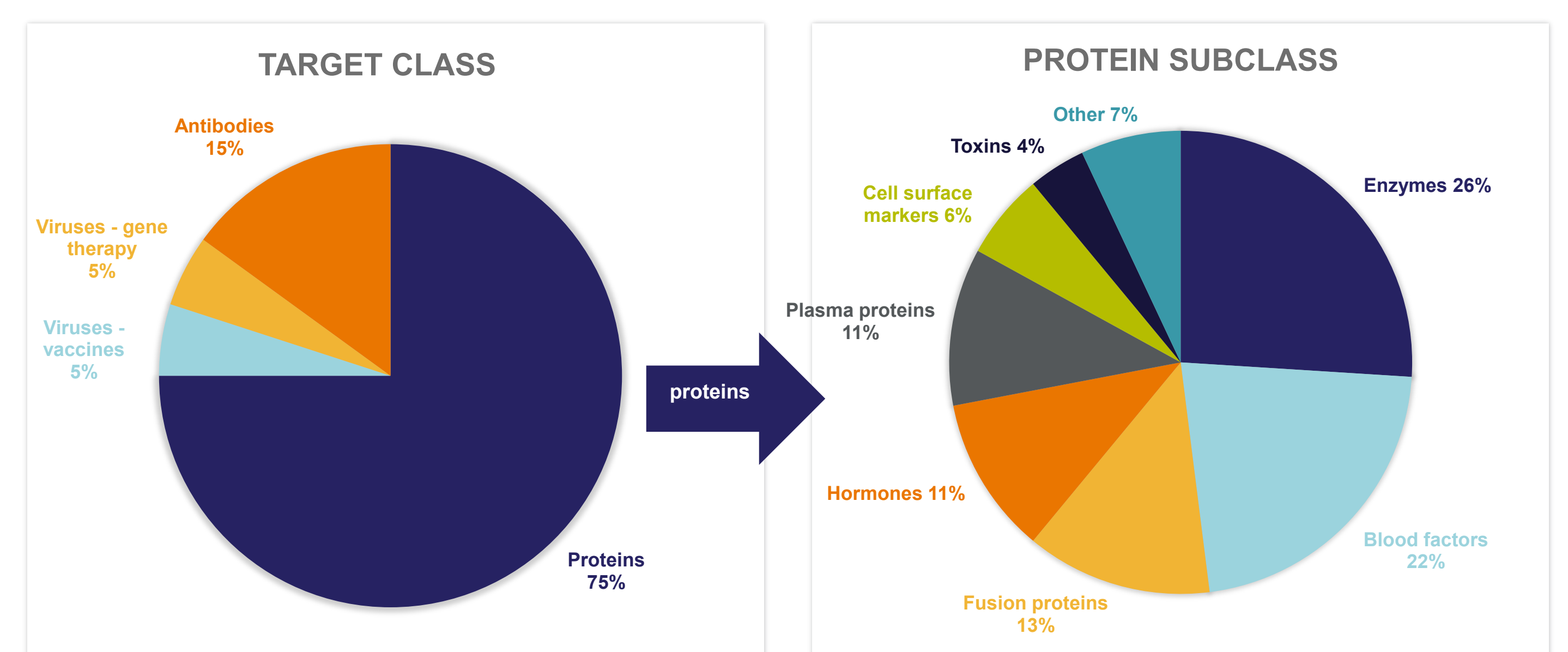
START DEVELOPMENT FROM EXISTING CANDIDATES IN OUR PIPELINE

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

Product Stage	Therapeutic proteins	Antibody Types	Viruses & Vaccines
RUO products (research use only)	Antithrombin III (AT III), Fibrinogen (POROS FibXL), Prothrombin, GM-CSF, Factor II(a), Factor H	IgM-XL, IgA-XL	Adenovirus (POROS Adv5), Baculovirus removal (BacuClear) Lenti VSVG, Exosomes (CD81)
Lead resin	HSA-XL, EPO / Darbepoetin Protein C	IgY-Fc	
Prototype resin	Factor V, Factor XI, Factor XIII IL15, CRM197 CHO-HCPs: Legumain, PLBL2	Mouse & Rat IgG-Fc	GP64, dsRNA Exosomes (CD9) SARS-CoV2
Ligand screening	Factor VIII, Factor XII, Tenecteplase Hyaluronidase, Haptoglobin, IFN α / β , IL2, IL2R, CHO-Lipases: LPL, LAL, LPLA2	Dog and Cat IgG-Fc	Exosomes (CD63) Plasmid DNA, Oncolytic viruses (HSV / VSV / Vaccinia)

TRACK RECORD – CAPTURESELECT CUSTOM SERVICES

- Custom programs conducted for more than 60 therapeutic target molecules since 2003
- High success rate (>90%) in identifying lead candidates meeting pre-defined requirements



POROS CUSTOM RESIN DEVELOPMENT

POROS resins help maintain performance and add flexibility to your downstream process. Combine high resolution and high capacity in a custom chromatography resin, tailored to your specific process requirements for both:

- **AFFINITY** - CaptureSelect or any other customer-defined ligand
- **NON-AFFINITY** - multiple surface chemistries

POROS PROTOTYPE RESIN DEVELOPMENT EXAMPLE

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
 - pore size, ligand density, coupling chemistry, spacer length)
3. Evaluation of bead morphology impact in resin performance

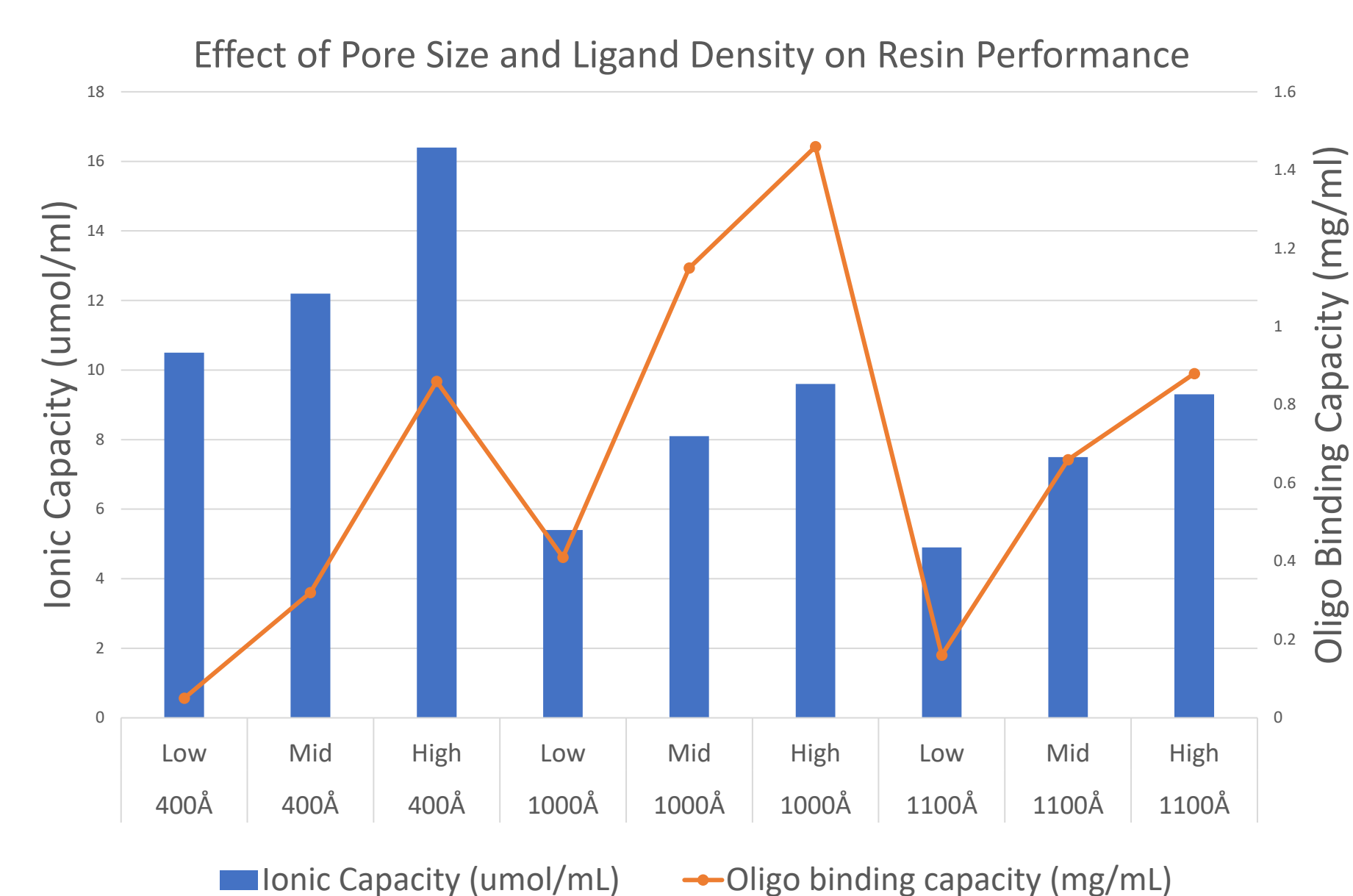


Fig. 3 Influence of pore size and ligand density on ionic and binding capacity

Resins of large pore size (1100Å) revealed lower ionic and binding capacity, due to their lower surface area. Resins with smaller pore sizes (400Å) show high ionic but lower binding capacity.

As expected, there is increased binding with ligand density (Low, Mid, High).

Prototypes were then further evaluated for specific mRNA binding capacity and recovery.

CAPABILITIES AND EXPERIENCE

Collaborating with us will provide you:

- ✓ **A Custom resin that helps solve your manufacturing challenges**
- ✓ **Scale-up and commercial production of the customized resin to lot sizes of 250L**
- ✓ **Regulatory support documentation for your custom resin used in regulated environments**

TRADEMARKS/LICENSING

© 2024 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. POROS™ chromatography resins: Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only. CaptureSelect™ chromatography resins: For Research Use or Further Manufacturing. Not for use in diagnostic procedures.