

Enabling custom chromatography solutions for downstream processing

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Bioprocessing

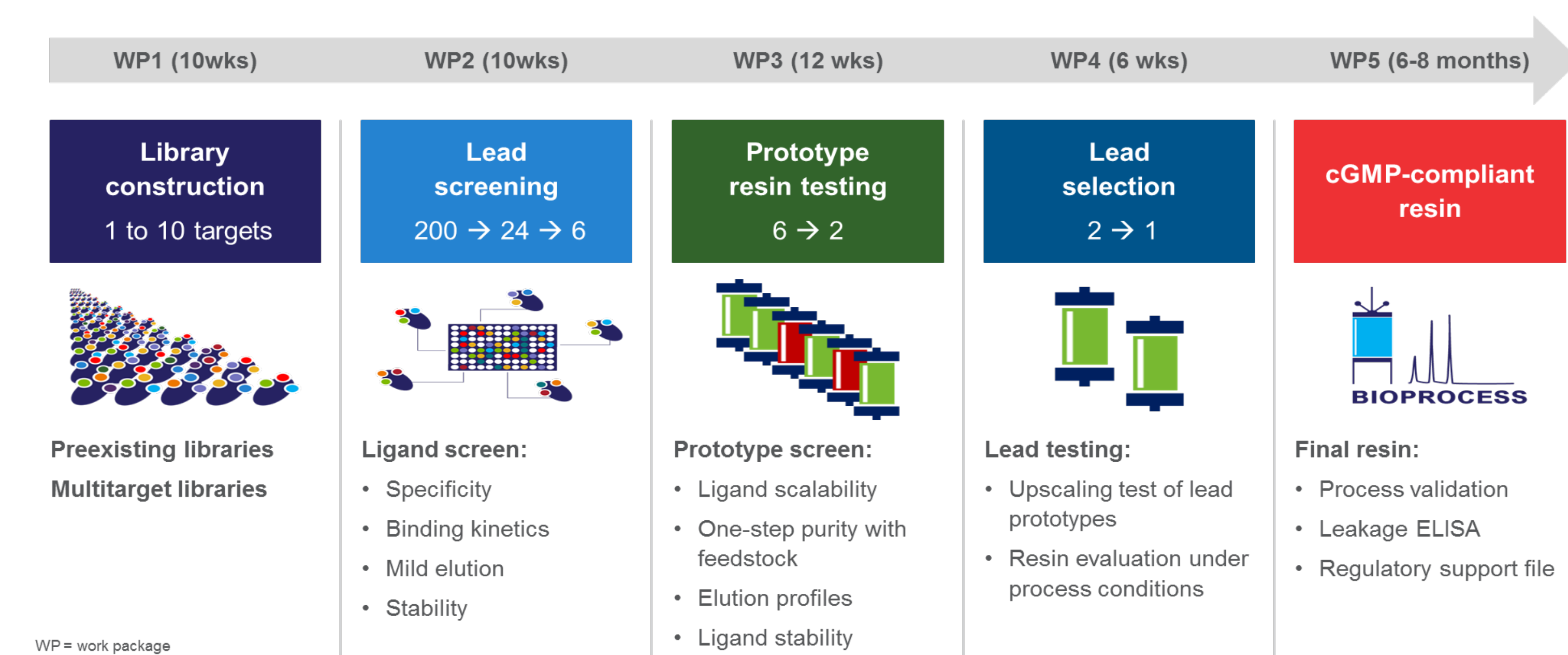
INTRODUCTION

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 Camelid single domain [V_HH] antibody fragments (Fig 1.) – produced in an animal origin free production process (*Saccharomyces cerevisiae*)
- Unique milestone-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

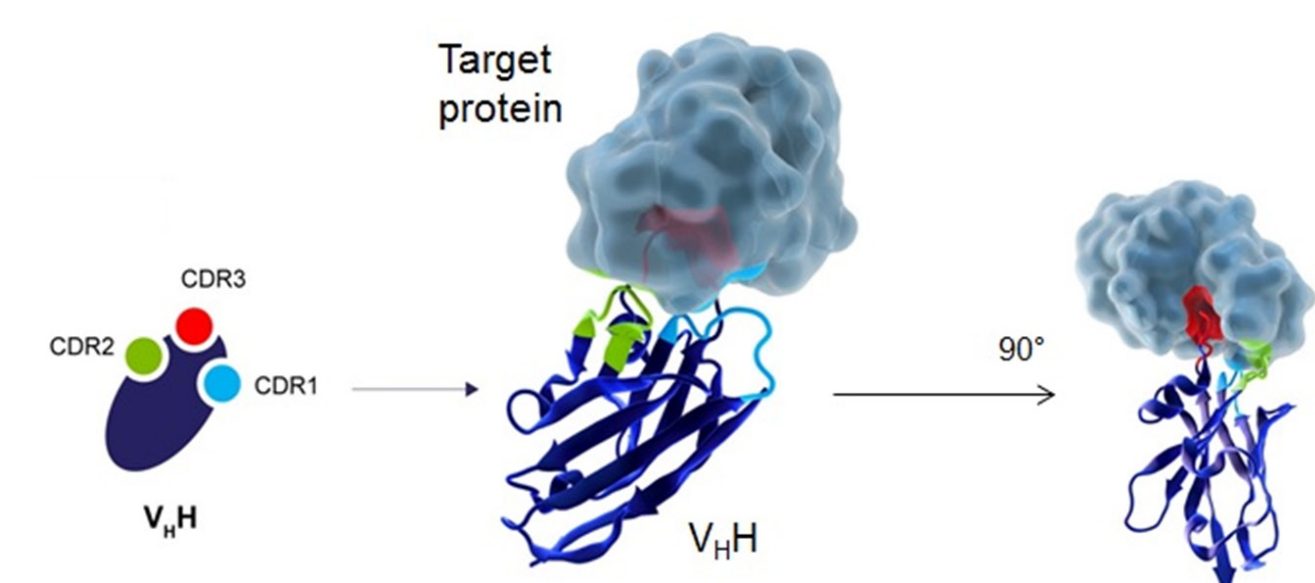


Fig.1 CaptureSelect ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecule.

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)

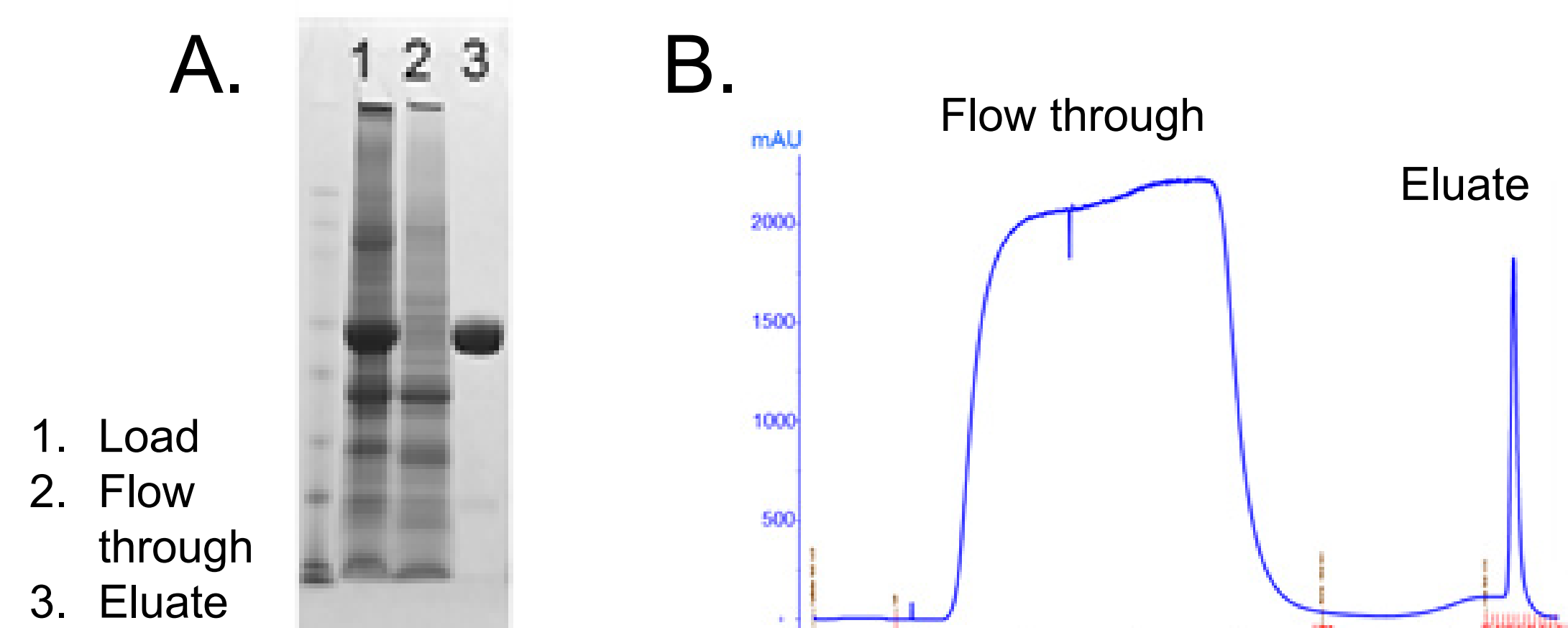


Fig.2 Analysis of the CaptureSelect Prothrombin Affinity resin

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 from further development of a product from our development pipeline.

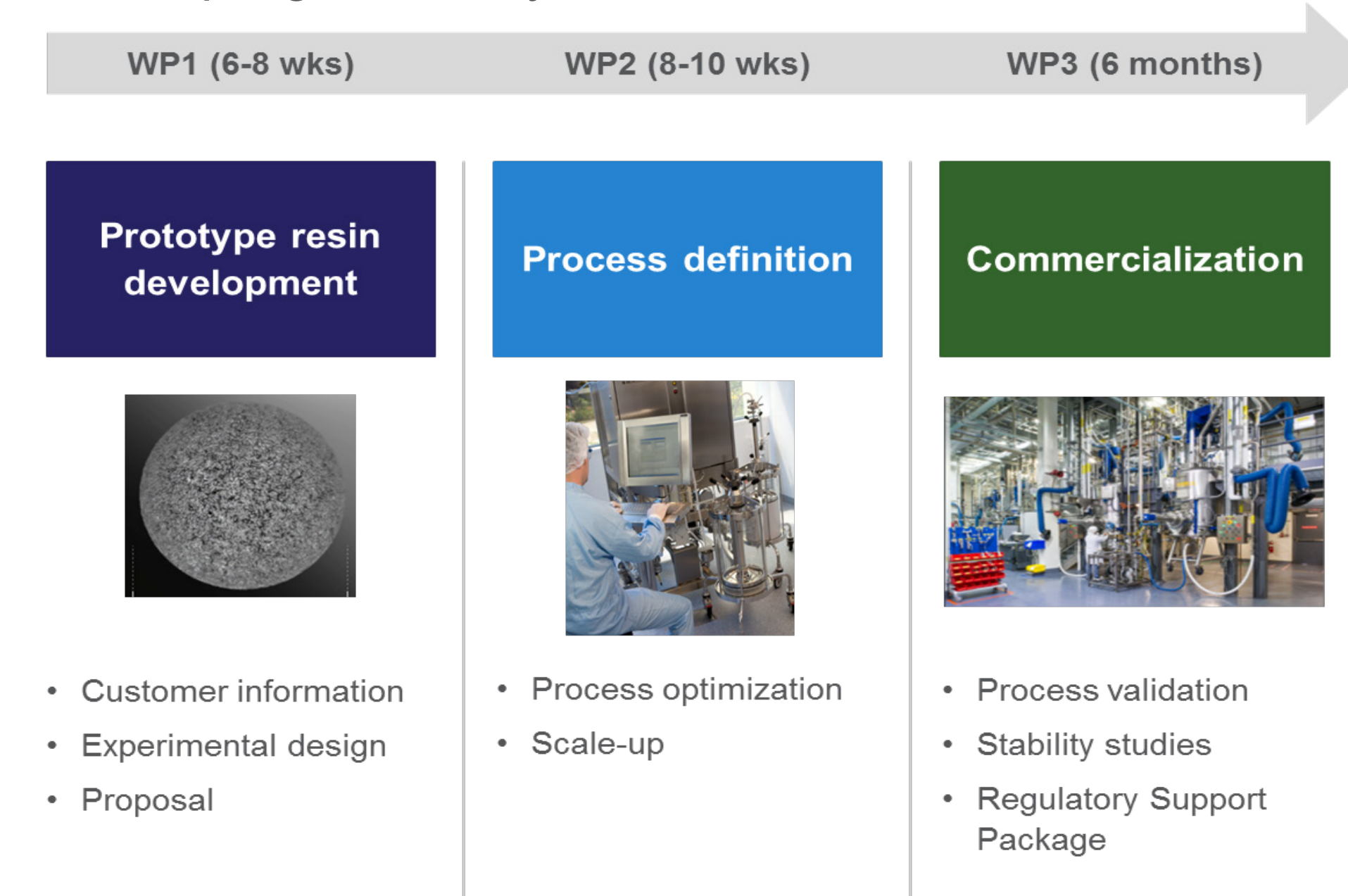
| Product Stage | Therapeutic proteins | Viruses & Vaccines | Antibody types |
|---|---|-------------------------------------|---|
| Developed RUO resin (research use only) | Antithrombin III, Fibrinogen, Transferrin, ApoH, C1-inhibitor, GM-CSF, Factor X | Adenovirus 5 | IgA (Fc), IgA-CH1, IgM, IgE, Bovine IgA, Rabbit IgG |
| Lead Selection | Prothrombin, DNase | Influenza (HA), Baculovirus | |
| Prototype screen | Protein C | Lentivirus (VSV-G), Exosomes (CD81) | Mouse IgG, IgG2 |
| Lead screening | IFN α/β , hIL2, FV, FXI, FXII, FXIII, FH, EPO | | scFv/VH, Rat IgG, Mouse IgG3, IgY |

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.

DoE screening includes:

- Optimal pore size of the beads
- Ligand density
- Coupling chemistry



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

TARGET MOLECULES

- Enzymes
- Polysaccharides
- Oligos
- Peptides
- Antibodies
- ...

✓ MEETING THE NEEDS FOR EVEN THE MOST DEMANDING BIO-THERAPEUTIC PURIFICATION PROCESSES

POROS PROTOTYPE RESIN DEVELOPMENT

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

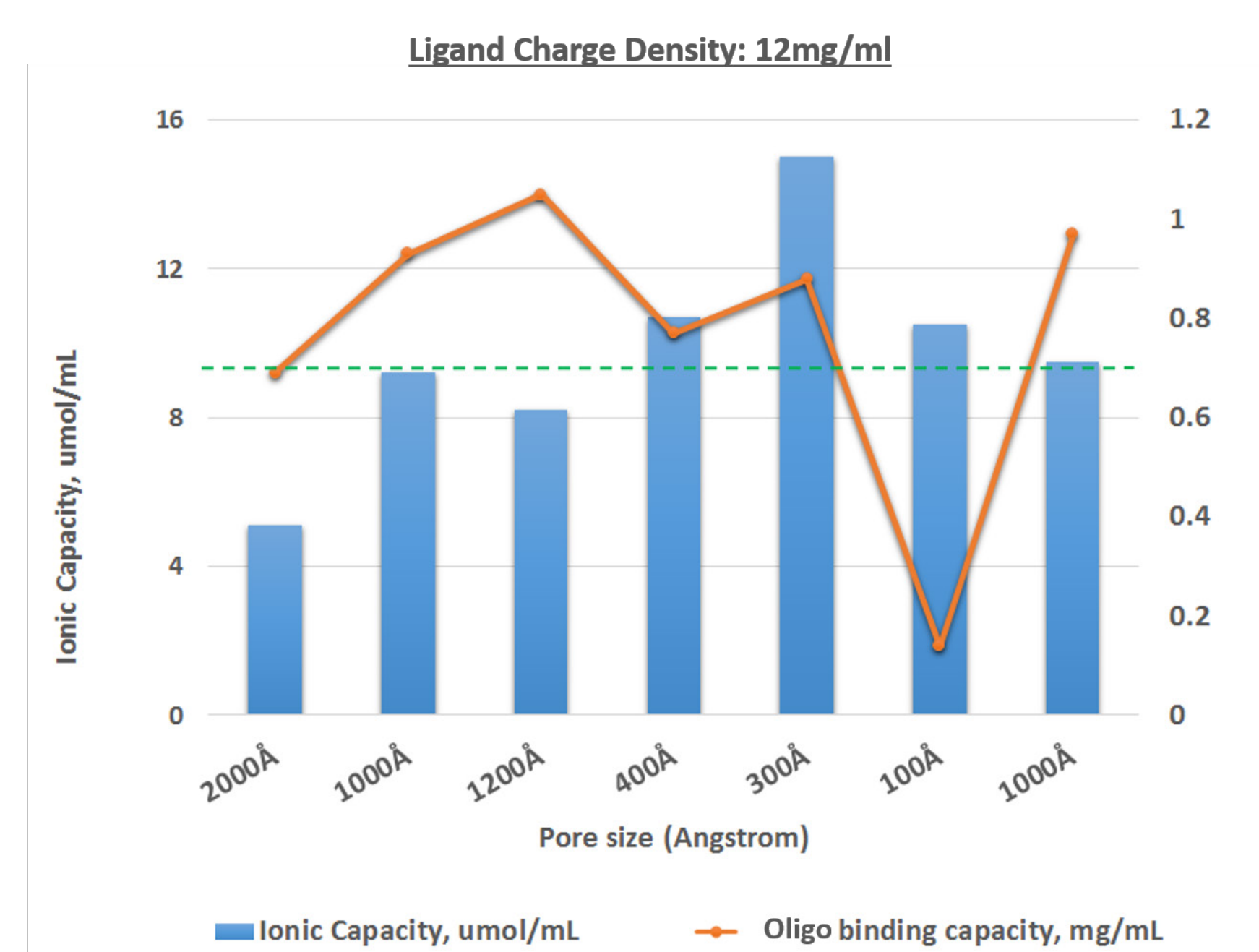


Fig. 3 Influence of pore size on ionic and binding capacity

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.

CAPABILITIES AND EXPERIENCE

Partnering with us will provide you:

- ✓ **A Custom resin that helps solving 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

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