PURIFICATION OF ANTIBODY FRAGMENTS WITH AMSPHERE™ A3 PROTEIN A RESIN

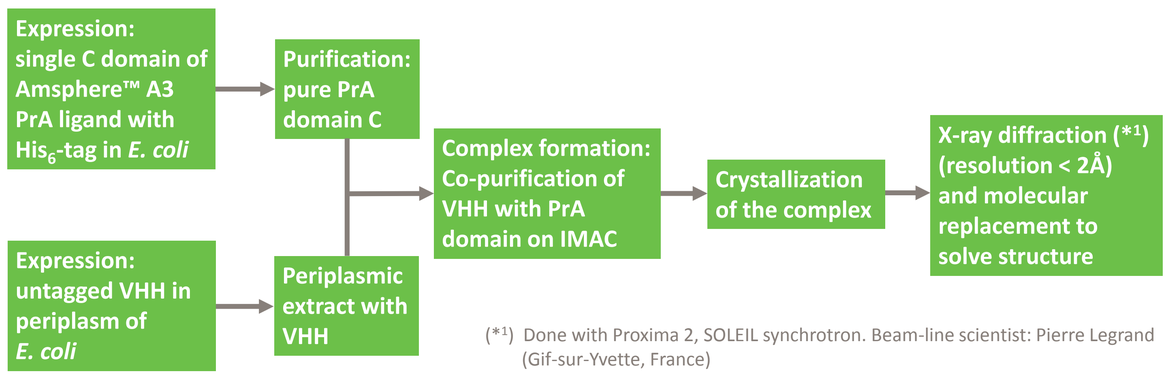
Platteau, Gerald[1]; Van der Jeugt, Bob [1]; Lissens, Geert [1]; Ströhlein, Guido [1];
Gaspariunaite, Vaiva[2]; Vincke, Cécile[2]; Sterckx, Yann[2]; Muyldermans, Serge[2]

[1] JSR Life Sciences – JSR Micro NV, Technologielaan 8, 3001 Leuven, Belgium
[2] Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel (VUB) – Pleinlaan 2, 1050 Elsene, Belgium

SUMMARY

The binding mechanism between the engineered C domain of the Amsphere™ A3 protein A (PrA) ligand and a VHH single domain antibody (sdAb) was revealed. Binding sites in the PrA ligand in helices 2 and 3 and in framework regions 1 and 3 of the VHH were confirmed. Identified VHH residues are not involved in antigen recognition. Overlap with a human VH showed the same interaction sites.
These results provide insight on why Amsphere™ A3 is a suitable tool for antibody fragment purification, with the known benefits of high process robustness, selectivity and caustic stability. Amsphere™ A3 has the same capacity for sdAbs as the current non-PrA affinity ligand resins (20-30 g/L).

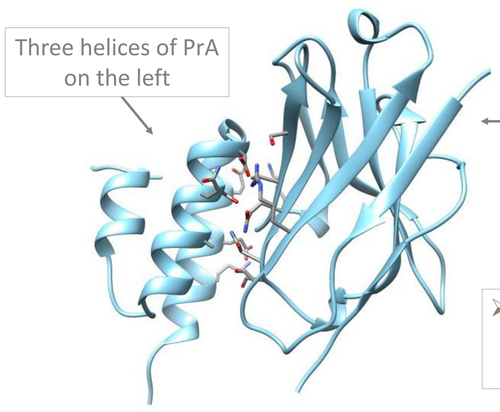
MATERIALS AND METHODS



(*1) Done with Proxima 2, SOLEIL synchrotron. Beam-line scientist: Pierre Legrand (Gif-sur-Yvette, France)

RESULTS

Structure of PrA-VHH complex



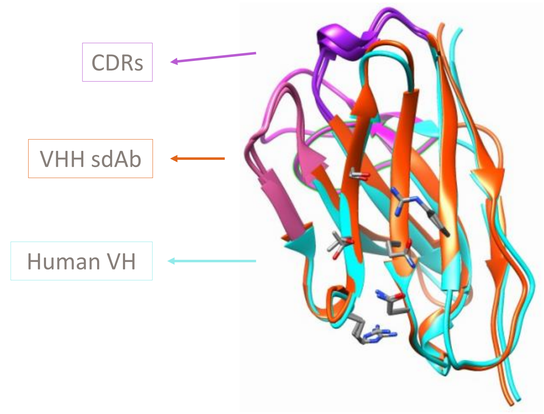
β-stranded structure of VHH on the right
- 2 sheets of 4 and 5 β-strands respectively
- 4 β-stranded sheet interacts with the PrA
- Sheet with 5 β-strands corresponds to side in a VH that interacts with VL in a Fab
- CDRs are the loops on the top

- Side chains of amino acids involved in PrA and VHH association are shown in stick representation and colored by element (N in blue, O in red, C in grey)

Binding sites in protein A ligand:
- Situated in helices 2 and 3 of the PrA ligand
- No overlap with Fc binding

Interaction sites in VHH sdAb:
- Located in framework regions 1 and 3 (4 β-stranded-sheet)
- Do not participate at all in antigen recognition
- Solvent exposed; mainly polar and/or charged amino acids
- Ser17, Arg19, Lys65, Thr69, Ser71, Gln82, Asn84 and Ser85 of the VHH face the PrA domain.

Comparing interaction with PrA of VHH and a human VH



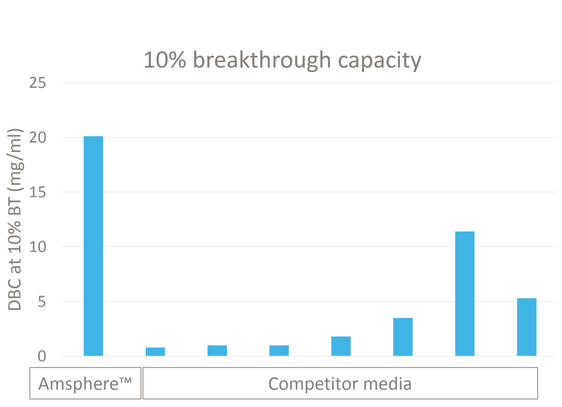
- PrA's-eye view of the overlay of VHH (orange) and a human VH (cyan)
- CDRs located on top (magenta colored)
- Side chains of amino acids that interact with PrA are shown in stick representation and colored by element (N in blue, O in red, C in grey)

- Perfect overlap between interaction side chains of the VHH with a published (*2) human VH
- Binding region for PrA in the VH does not interact with the VL domain in a classical antibody
- Also in VH, the interacting site is not involved in antigen binding

Contact residues are highly conserved in human VH3 antibodies, but not in other subfamilies.
In almost every case, reduced or eliminated binding can be correlated with variations in residues in contact with PrA. Since those residues are in framework regions and thus not involved in antigen binding, mutating non-PrA binding VHHs into PrA binders will be tested.

(*2) Graille et al. (2000) Proc. Natl. Acad. Sci. 97, 5399-5404

DISCUSSION

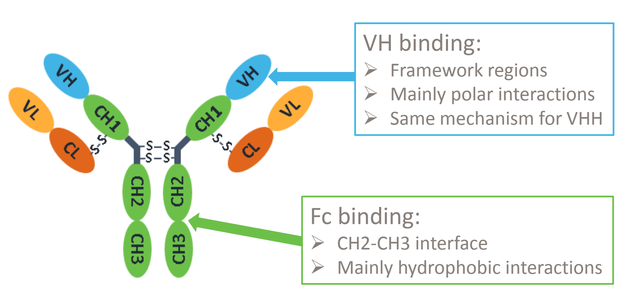


DBC experiments:
DBC of Amsphere™ for the VHH used for the complex formation was compared to other commercial affinity resins.
- Load: periplasmic extract containing VHH
- Residence time: 1 min
- Column: 5mm I.D.; 50mm bed height (1mL)

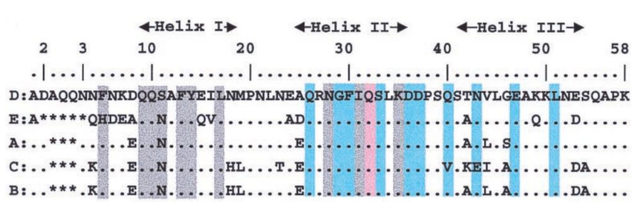
Interpreting capacity values:
- Capacity for monovalent and bivalent VHHs: 20-30 mg/ml → 2-3 times lower than for a full size IgG
  - However: MW of full IgG = 150 kDa, while MW of a monovalent VHH = 13 kDa
- Molar DBC values provide more accurate comparison of resin capacity for different molecule types.
  - showing how many molecules are bound instead of the total mass bound

It is better to take into account the molecular weight and size of the molecule of interest instead of only looking at DBC values in units of mass.
Not only the affinity between ligand and antibody fragment plays a role. As the results above show only 1 VH(H) can bind for each PrA domain, molar DBC values show that spatial limitations are also determining how many target molecules can be bound. For Amsphere™ A3, the amount of molecules bound per multimeric protein A ligand is clearly higher for the VHHs than for full size IgGs.

Antibody formats suitable for capture with Amsphere™ A3:



- Extensive data set for binding of VHH to Amsphere™ A3
- Binding of several Fab and scFv formats also confirmed
- Amsphere™ A3 has highest capacity for VHH and VH molecules or constructs



- Alignment of the amino acid sequences of the five SpA domains (*2).
  - Blue: involved in interaction with Fab
  - Gray: mediating Fc binding
  - Pink: Gln-32 is the only amino acid involved in both Fc and Fab binding

CONTACT DETAILS

JSR Life Sciences

Gerald Platteau
Application Development Engineer Bioprocess
gerald.platteau@jsrlifesciences.com
+32 (0)479 32 72 86