Development and evaluation of a novel high-capacity protein A chromatography resin with significantly increased alkaline stability

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Abstract

This poster presents the development and evaluation of a novel protein A chromatography resin. The significantly increased capacity and alkaline stability of the resin is enabled by base matrix optimization and protein engineering. Resin performance data, that is, yield and purity, in a mAb purification step is also presented.

Background

Sensitive amino acids and/or exposed protein surfaces were mutated to increase alkaline stability (Fig 1). More than 400 constructs were screened using the Biacore™ surface plasmon resonance (SPR) system. The MabSelect SuRe™ ligand was used as reference. The most alkaline-stable constructs were selected and coupled to a base matrix for further evaluation in packed columns. A new base matrix was also developed and optimized towards the new ligand (Fig 2). To increase binding capacity, base matrix properties such as particle size, matrix volume, and pore size were optimized in combination with ligand length and density (Fig 3).

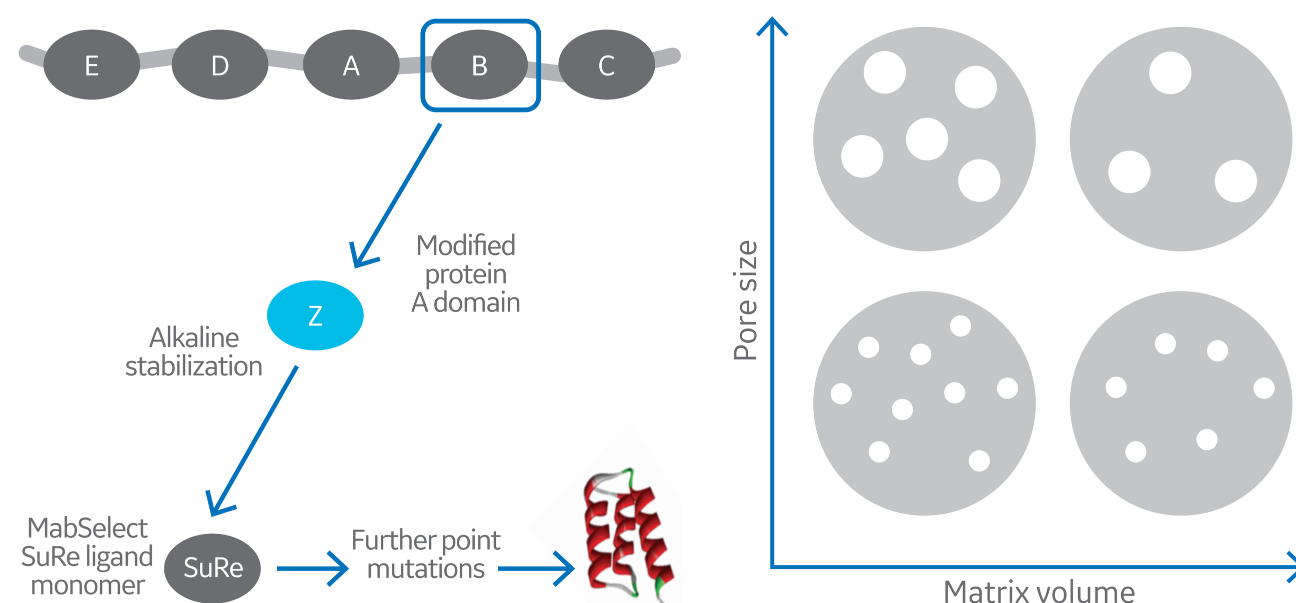

Fig 1. Further development of MabSelect SuRe protein A ligand by point mutation of sensitive amino acids.

Fig 2. Development of base matrix by optimizing pore size and matrix volume.

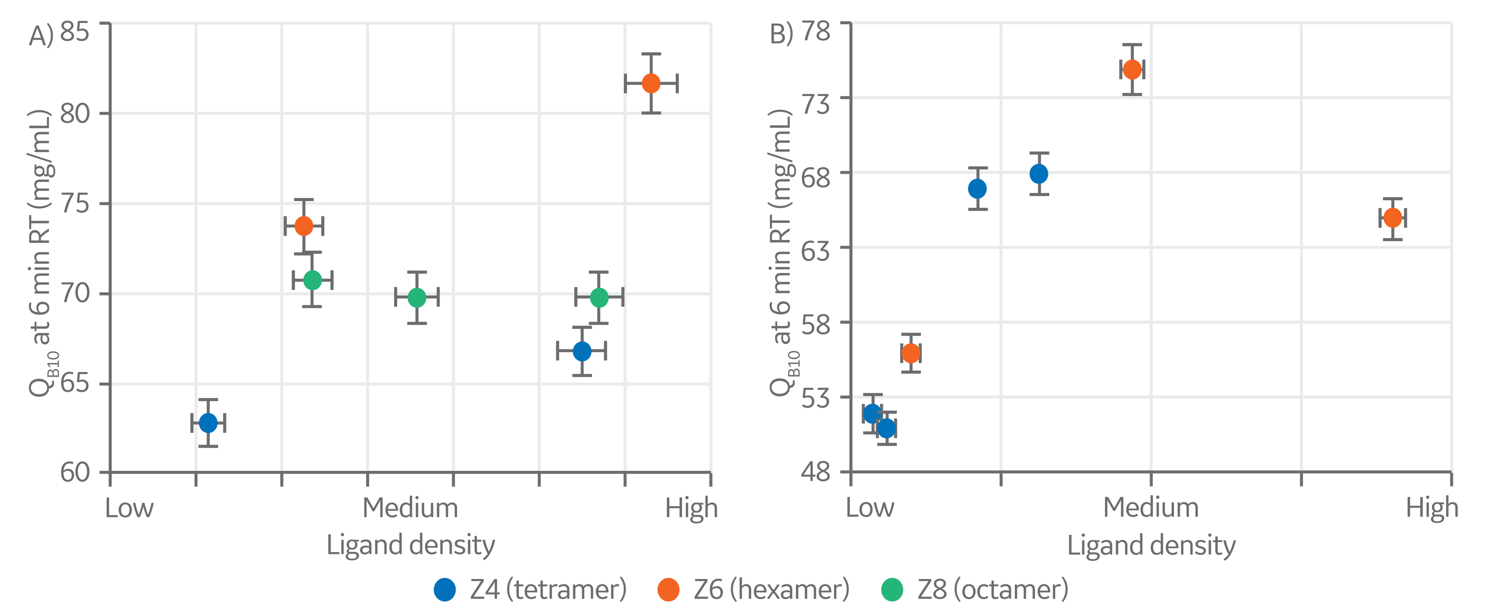

Fig 3. Evaluation of the combination of different ligand densities with **(A)** base matrix A (larger pores) and **(B)** base matrix B (smaller pores) for optimal binding capacity.

Results

Dynamic binding capacities

Dynamic binding capacities (DBC) were determined by frontal analysis. All resins were packed in 2 mL columns. Polyclonal IgG was applied on the columns at different flow rates, yielding residence times (RT) of 1, 2.4, 4, 6, and 10 min.

Compared with MabSelect SuRe LX, the results displayed in Figure 4 show that MabSelect™ PrismA offers:

- Up to 40% increased DBC at 2.4 min RT.
- Up to 30% estimated increased DBC at 4 min RT.
- Up to 25% increased DBC at 6 min RT.

Alkaline stability

Repetitive purification cycles were performed with buffers only using 0.5 or 1.0 M NaOH as cleaning agent for 15 min/cycle. The capacity was evaluated every 25th cycle with polyclonal IgG. The results show that the newly developed MabSelect PrismA resin has significantly higher alkaline stability than its predecessors MabSelect SuRe LX and MabSelect SuRe resins (Fig 5).

MabSelect PrismA has:

- Less than 10% loss of capacity after 300 cycles with 0.5 M NaOH.
- Less than 20% loss of capacity after 150 cycles with 1.0 M NaOH.

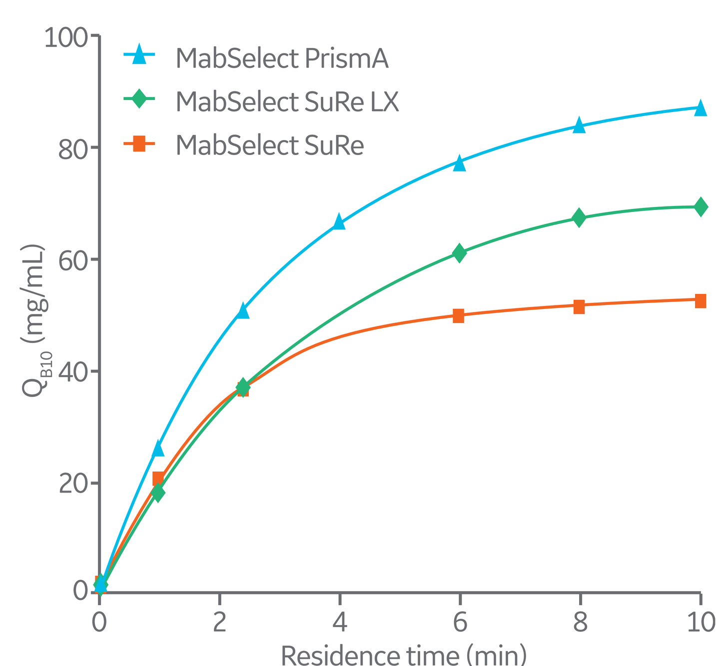

Fig 4. Dynamic binding capacity at 10% breakthrough (Q_{B10}) for polyclonal IgG at different residence times.

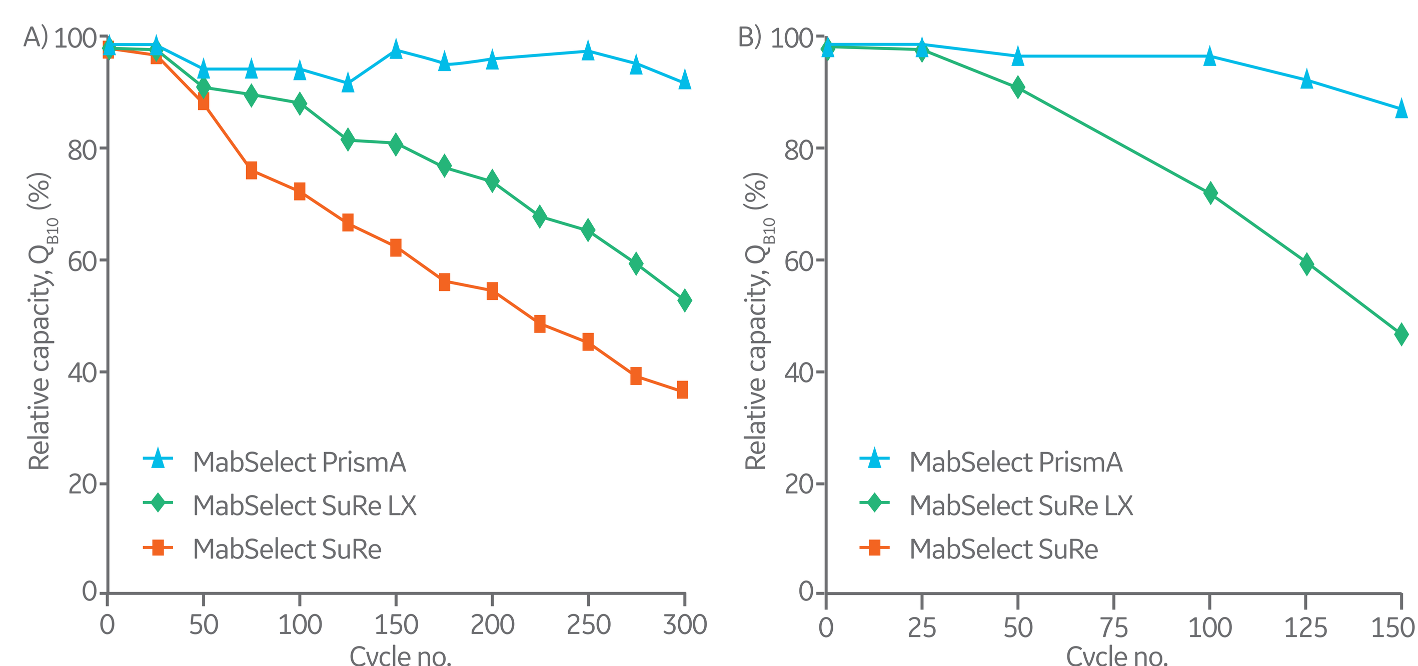

Fig 5. Relative remaining dynamic binding capacity at 10% breakthrough (Q_{B10}) using **(A)** 0.5 M NaOH or **(B)** 1.0 M NaOH as cleaning agents with a contact time of 15 min in each purification cycle.

mAb purification

Purification performance of MabSelect PrismA was compared with MabSelect SuRe and MabSelect SuRe LX using the same mAb. The sample load was 80% of Q_{B10} (63 mg/mL for MabSelect PrismA; 43 mg/mL for MabSelect SuRe LX; and 36 mg/mL for MabSelect SuRe). The chromatogram for MabSelect PrismA is shown in Figure 6. The results summarized in Table 1 show that yield and purity were comparable for the different protein A resins.

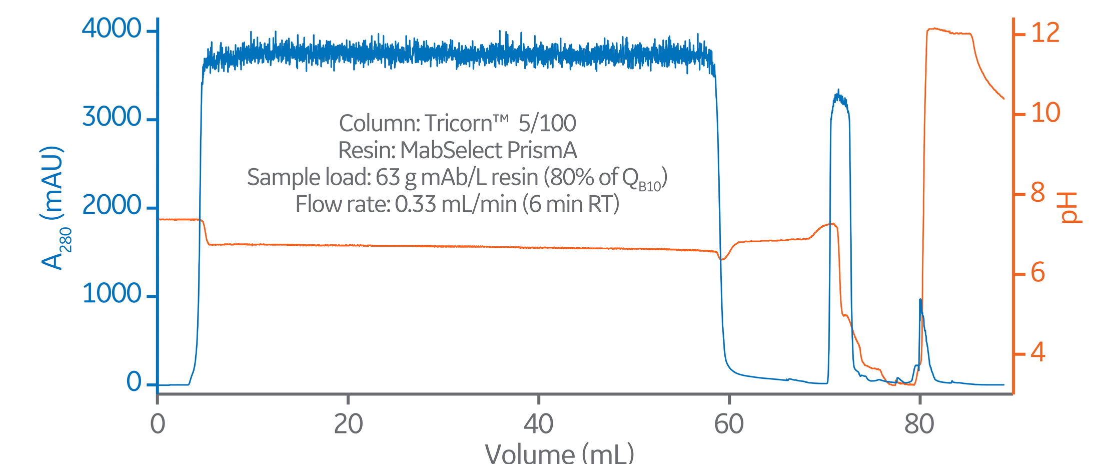

Fig 6. Purification of a monoclonal antibody from a mammalian cell culture supernatant.

Table 1. Summary of purification data from a capture step on the different protein A resins (host cell protein at start: 5.7×10^7 ppm, host cell DNA at start: 6785 ppm)

	MabSelect PrismA	MabSelect SuRe LX	MabSelect SuRe
Load mAb (g/L resin)	63	43	36
Yield (%)	97	100	100
HCP (ppm)	1107	1230	1130
Aggregates (%)	0.8	0.4	0.5
DNA (ppm)	4	3.8	5.5
Pool volume (CV)	1.4	1.3	1.2
Leached protein A (ppm)	19	9	9

Conclusions

- New optimized ligand with better alkaline stability.
- New base matrix with optimized particle size, pore size, and porosity.
- The new base matrix combined with a longer ligand increases the dynamic binding capacity at all studied residence times.
- Performance in terms of recovery and purity is similar to reference protein A resins.
- The improved alkaline stability enables efficient cleaning of the resin using 0.5 to 1.0 M NaOH.
- The use of 1.0 M NaOH for sanitization will improve bioburden control.

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