Affimer® reagents facilitate affinity chromatography purification of IgG

POS013

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Affimer reagents in affinity resins

Affimer reagents are proteins which present high affinity binding surfaces for specific interactions with a wide range of targets. The scaffold is based on the cystatin fold and permits the presentation of two variable binding loops (**Fig 1**). The Affimer reagents are small (~12-14 kDa), easy to manufacture in bacterial expression systems and biophysically stable over a wide range of pH conditions.

As both the scaffold and the randomised binding loops are engineered to lack cysteines, the introduction of this residue using basic molecular biology methods provides a straightforward route for immobilising the protein to surfaces in a defined orientation *via* thiol chemistry (**Fig 1**).

Affimer clones that bind different regions of IgG molecules were immobilised on highly cross-linked 4 % agarose beads *via* iodoacetyl chemistry and the resins packed on columns (bed height 31 mm, column volume 0.6 mL). The characteristics of the affinity resins were studied for target specificity, binding capacity, reproducibility and stability.

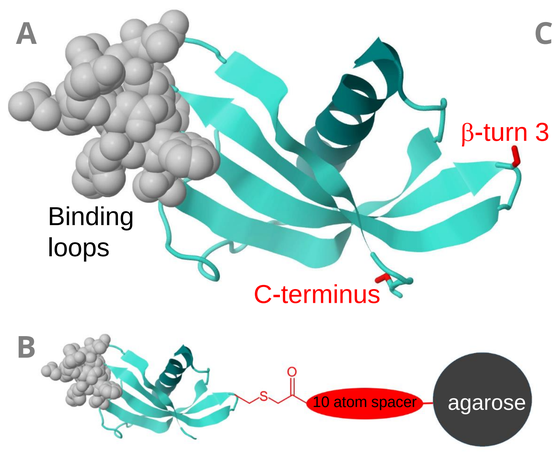

Figure 1A. Structure of the Affimer scaffold protein with the two regions containing variable amino acid loops in grey. **1B.** Schematic showing conjugation of Affimer protein to agarose resin. **1C.** Column loaded with affinity resin.

Specific target binding and elution

Affimer D11 binds the F_C domain of human IgG (hIgG) and exhibits high target specificity in the presence of complex mixtures and when challenged with mammalian homologues (**Fig 2**). High specificity has enabled the use of this resin for pharmacokinetic studies of hIgG-drug conjugate stability in mouse plasma (data not shown).

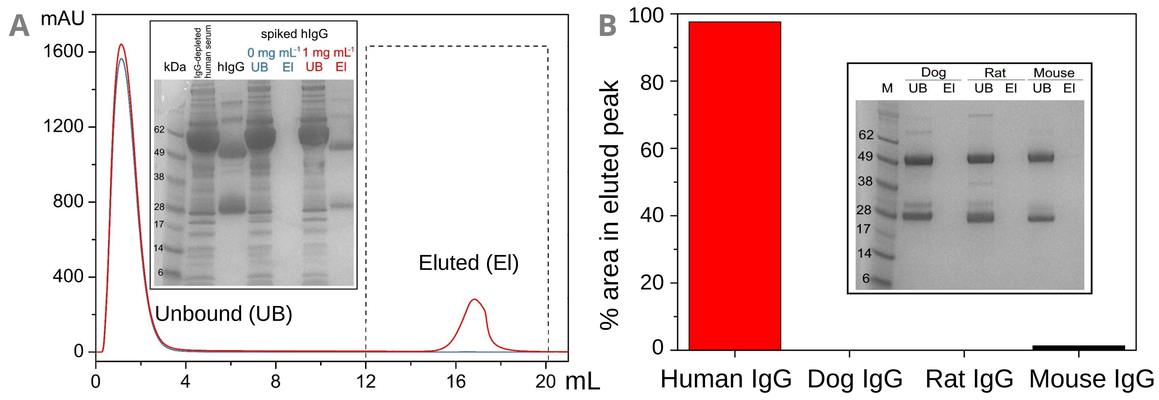

Figure 2A. Traces produced upon loading IgG-depleted human serum spiked with known concentrations of hIgG onto Affimer D11 resin. The wash buffer used is PBS (pH 7.4) and elution buffer is glycine, NaCl (pH 3.0). **Inset:** SDS-PAGE (under reducing conditions) confirms specificity of binding and elution. **2B.** % Area of elution peak after injection of 1 mg of pure immunoglobulins.

Binding capacity

Affimer D11 was engineered to include extra residues (Gly-Gly-Ser) that extend the length of linker to the agarose bead. The dynamic binding capacities of columns packed with Affimer resin containing standard and extended linkers were measured. Extension of the linker produced a resin with increased binding capacity (**Fig 3**).

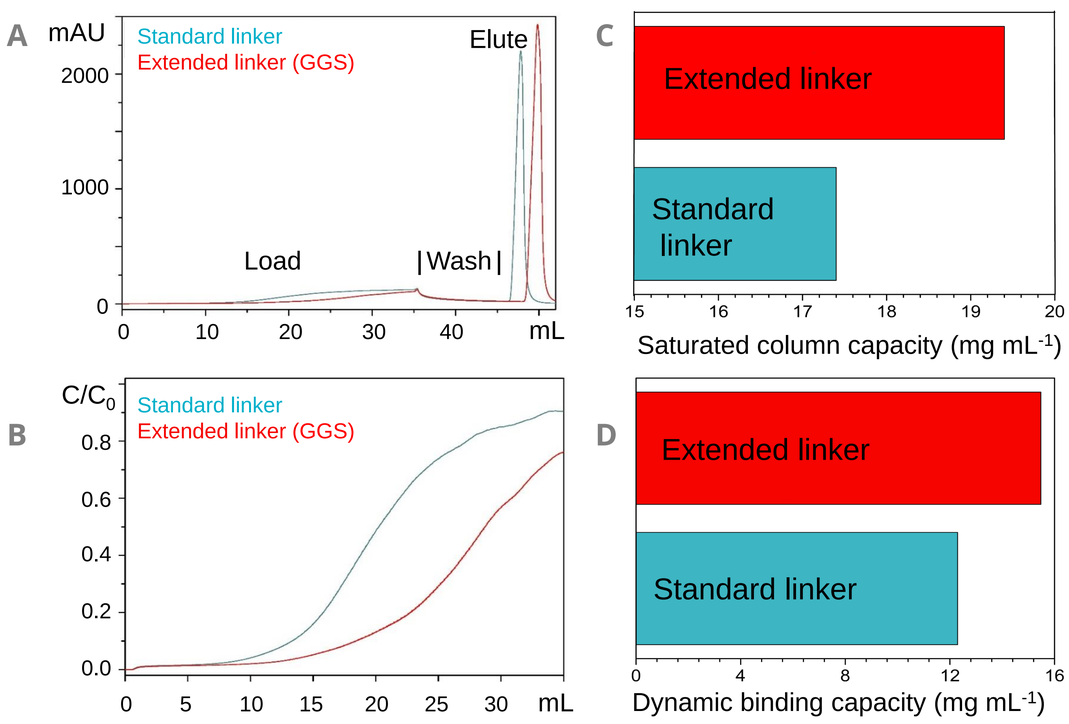

Figure 3. Dynamic binding during continual loading of 0.5 mg mL^{-1} pure hIgG over Affimer D11 resins with a column residence time of 3 min. **3A.** Full traces showing load, wash and elution phases. **3B.** Normalised breakthrough curves. **3C.** The amount of target protein recovered during elution, normalised to column volume. **3D.** The dynamic binding capacity at 10 % breakthrough, normalised to column volume.

Stability and reproducibility

The reproducibility and stability of an Affimer-based resin was measured by repeated injection and elution of pure hIgG (81 runs), including 19 clean-in-place (CIP) cycles (100 mM NaOH, 10 min). The data indicate consistent capture performance over the course of this study (**Fig 4A**). No leaching from Affimer columns was detected by western blotting of concentrated elution fractions (**Fig 4B**).

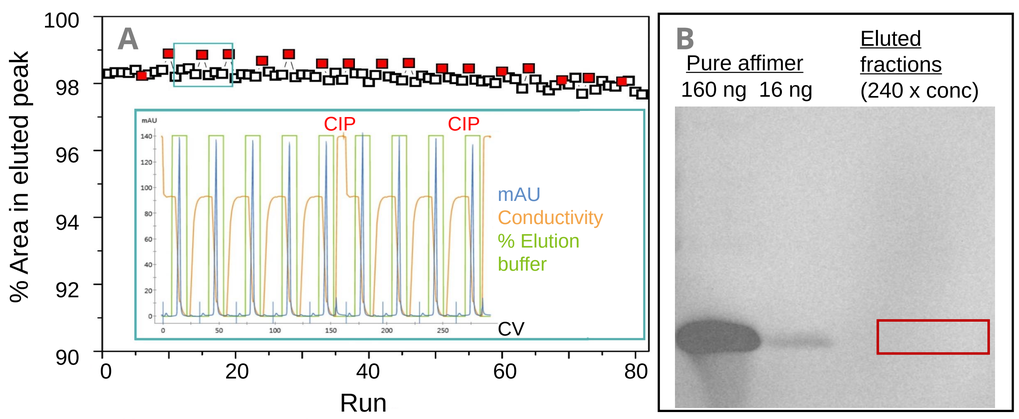

Figure 4A. An Affimer D11 column demonstrated high levels of reproducibility over 81 consecutive purification cycles. **Inset:** Example traces for a section of the experiment. **4B.** Example western blot indicating no detectable leaching during elution, pure Affimer protein is included for reference.

Fragment Binder

Affimer H2 binds intact F_{ab} domains allowing purification of whole IgGs and F_{ab}s from contaminants such as free light and heavy chains (**Fig 5**). The novel ability of Affimer H2 resin to bind intact F_{ab} suggests it could be useful in bioprocessing and analytical applications where discrimination of intact F_{ab}s from free light chains (LCs) is required. In this respect the Affimer-based resin would be complementary to protein L, which bind kappa LCs. Indeed it displays more efficient elution of targets at pH 3 than commercially available protein L resins.

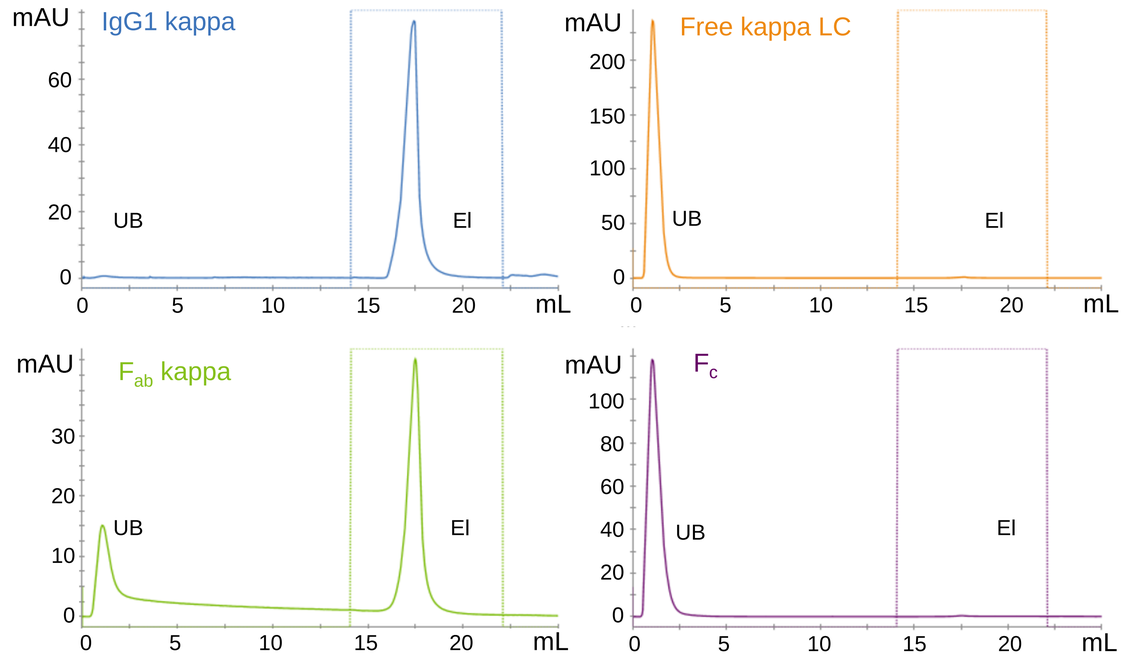

Figure 5. Example traces produced upon loading pure IgG species across Affimer H2 resin, suggesting this Affimer clone is capable of recognizing and enriching assembled IgGs and F_{ab} fragments.

Summary

- The high specificity achieved by Affimer protein-based affinity resins suggests potential applications in pharmacokinetic studies and bioprocessing platforms.
- Flexible reformatting provides a route to further optimisation - improvements in performance may be possible by development of the resin, cysteine location, multimer options, linker lengths and coupling methods.
- Affimer resins are robust to a wide range of pH conditions enabling a high level of reproducibility and ability to endure cleaning routines.