Affimer®

Discovery, characterisation and pharmacokinetics of Serum Albumin Binding Affimer proteins that can be used for half-life extension

POS018

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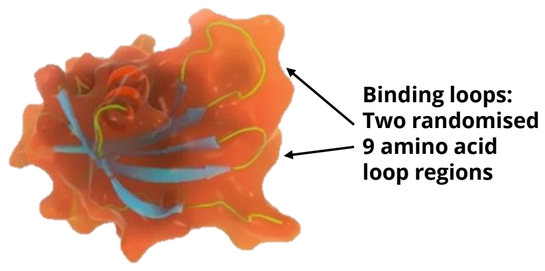
Affimer Protein Library



- The Affimer biotherapeutic scaffold is based on human Stefin A (12 kDa)
- 9 amino acids in two loops with equal diversity at each position (no cys, met or stop)
- Library size of 10^{11}
- Binding to serum albumin used to extend the *in vivo* half-life

Serum Albumin Binder Discovery (AVA03

Phage Selections

- Biopanning on captured Human (HSA) or Mouse Serum Albumin (MSA)
- Solution selection on biotinylated HSA or MSA
- 3 rounds of selection on either HSA or MSA, including cross-selection between species
- Enrichment monitored by output size and polyclonal Phage ELISA

Primary Screening

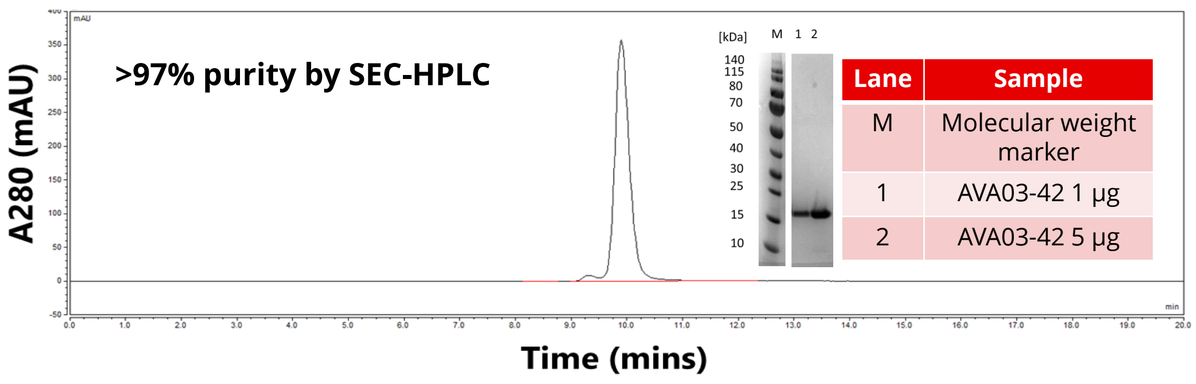
- Monoclonal Phage ELISA against captured HSA and MSA
- 109 binders, 47 unique sequences identified

Secondary Screening

- ELISA on HSA and MSA at pH 6.0 and 7.4
- Kinetic screening by biolayer interferometry (Octet) on HSA, MSA and Cynomolgus Serum Albumin (CSA)

Affimer Protein Production

- Affimer proteins were expressed from *E. coli* and purified using IMAC, IEX and SEC
- Expression of clone AVA03-42 was >200mg/L, >97% purity (shown as example in Figure 1)

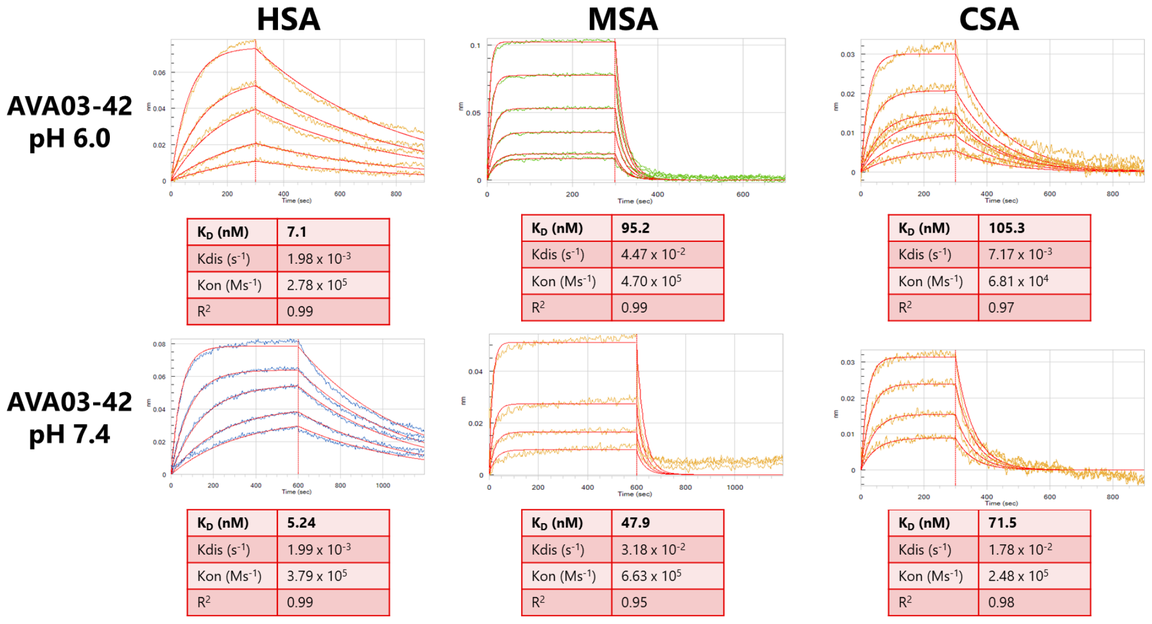


Lane	Sample
M	Molecular weight marker
1	AVA03-42 1 µg
2	AVA03-42 5 µg

Figure 1. Purity of the Affimer protein AVA03-42 following expression and purification

Lead Characterisation

- Binding affinities of purified Affimer proteins were assessed by biolayer interferometry (Octet). HSA binding affinities ranged from 7.1 to 135.5 nM at pH 6 (example shown in Figure 2)



K_D (nM)	7.1
Kdis (s^{-1})	1.98 x 10^{-3}
Kon (Ms^{-1})	2.78 x 10^{5}
R^2	0.99

K_D (nM)	95.2
Kdis (s^{-1})	4.47 x 10^{-2}
Kon (Ms^{-1})	4.70 x 10^{5}
R^2	0.99

K_D (nM)	105.3
Kdis (s^{-1})	7.17 x 10^{-3}
Kon (Ms^{-1})	6.81 x 10^{4}
R^2	0.97

K_D (nM)	5.24
Kdis (s^{-1})	1.99 x 10^{-3}
Kon (Ms^{-1})	3.79 x 10^{5}
R^2	0.99

K_D (nM)	47.9
Kdis (s^{-1})	3.18 x 10^{-2}
Kon (Ms^{-1})	6.63 x 10^{5}
R^2	0.95

K_D (nM)	71.5
Kdis (s^{-1})	1.78 x 10^{-2}
Kon (Ms^{-1})	2.48 x 10^{5}
R^2	0.98

Figure 2. Analysis of Affimer protein AVA03-42 by BioLayer interferometry (Octet). K_D values for binding to human, mouse and cynomolgus serum albumin protein at pH 6 and 7.4. Association constant (Kon (Ms^{-1})); Dissociation constant (Kdis (s^{-1})).

- Cross reactivity was measured for 7 lead Affimer proteins by BioLayer Interferometry (Octet) for MSA, CSA and HSA at pH 6 and 7.4 (Table 1)
- 5 Affimer proteins with a range of different affinity, association and dissociation constant for MSA were selected to be tested *in vivo* (Figure 4 and Table 2)

Clone	MSA		CSA		HSA	
	pH 6.0	pH 7.4	pH 6.0	pH 7.4	pH 6.0	pH 7.4
AVA03-19	618.2	981.1	68.7	106.5	14.9	36.1
AVA03-21	511.4	622.3	133	212.6	23.4	40.5
AVA03-32	3.7	3.3	18.5	83	21.1	15.3
AVA03-37	435	243.6	1140	2600	132.2	135.8
AVA03-42	95.2	47.9	105.3	71.5	7.1	5.2
AVA03-23	334	-	146	347.9	44.7	152.3
AVA03-38	833.7	-	231.3	221.4	135.5	113.1

Table 1. K_D values for Affimer proteins binding to human, mouse and cynomolgus serum albumin protein at pH 6 and 7.4.

- Affimer proteins with the highest affinity for HSA were also analysed for cross reactivity to rat, canine and pig serum albumin by ELISA (Figure 3)

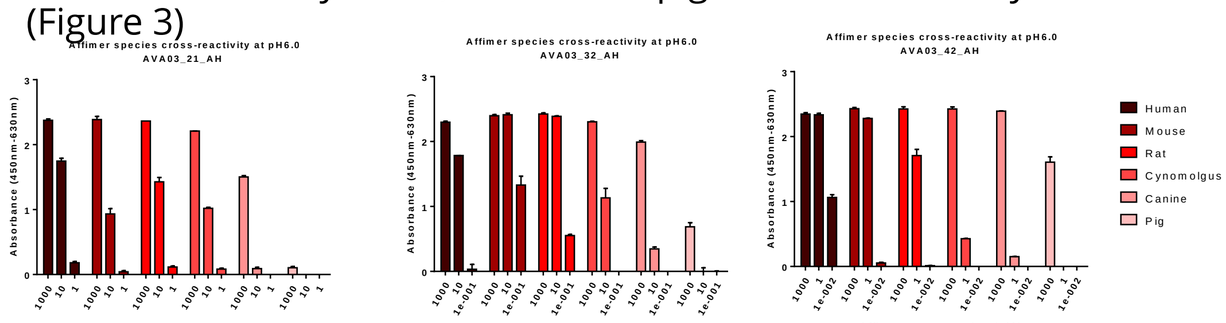


Figure 3. Species cross reactivity of lead clones AVA03-21, -32 and -42 measured by ELISA at pH 6

Pharmacokinetic Study in Mice

- Affimer proteins were radiolabelled using I-125 and dosed at 10 mg/kg as a bolus IV injection, 3 mice per time point
- Serum concentration of Affimer proteins was determined over 7 days by measurement of radioactivity (Figure 3, Table 2)
- All Affimer proteins tested were well tolerated *in vivo*

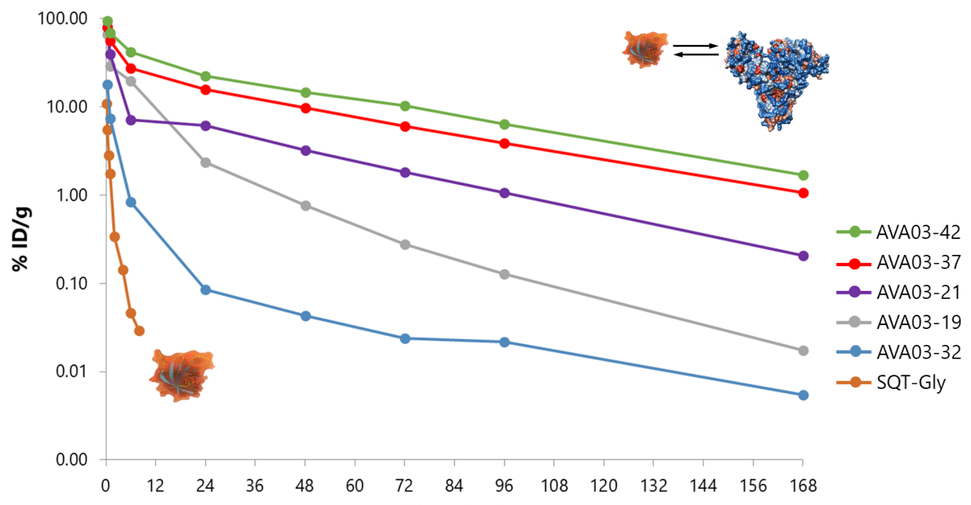


Figure 4. PK profiles of SQT-Gly and AVA03 clones in mouse. Affimer proteins were labelled with I-125 and dosed at 10 mg/kg via the IV route

Table 2. Pharmacokinetic parameters in mouse of the Affimer proteins: (half-life, $t_{1/2}$) and exposure (AUC 0-t) in a non-compartmental analysis

Clone	$t_{1/2}$ (hrs)	AUC 0-t h*µg/mL
AVA03-42	38.2	5,670
AVA03-37	37.7	3,435
AVA03-21	30.6	1,401
AVA03-19	24.3	1,059
AVA03-32	29.0	112
SQT-Gly	1.6	18.1

Conclusion

- We have demonstrated the proof-of-principle that the serum half-life of Affimer proteins can be significantly increased by binding to albumin in mice
- The resulting *in vivo* half-life extension of the Affimer proteins is similar to other scaffold technologies
- The next steps will include generating AVA03-fusions to demonstrate half-life extension in mouse and cynomolgus

For further information please contact affimers@avacta.com or visit www.avacta.com