Rapid Development of anti-SARS CoV-2 Spike S1 Affimer® Reagents for Diagnostic Applications

POS038

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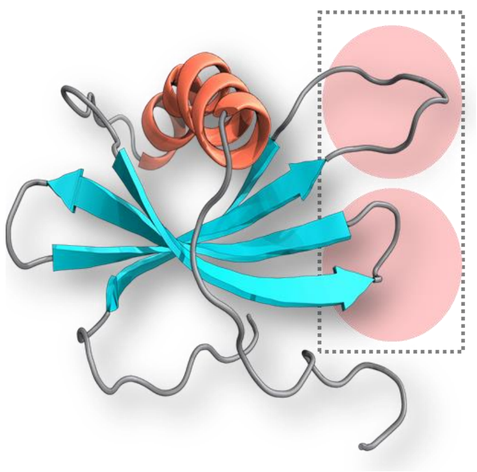
Project Aims

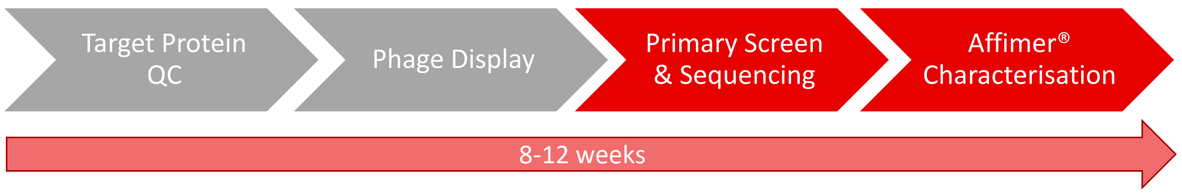
To generate Affimer® affinity pairs to S1 protein as a rapid response to the SARS CoV-2 Pandemic, to support the global effort to develop an accurate and sensitive point-of-care *in vitro* diagnostic (IVD). The S glycoprotein is synthesized as a precursor protein consisting of ~1,300 amino acids that is cleaved into an amino (N)-terminal S1 subunit (~700 amino acids) and a carboxyl (C)-terminal S2 subunit (~600 amino acids). The S1 region contains a receptor-binding domain (RBD) which binds to ACE2 on the host cell and an N-terminal domain, while the S2 region contains a hydrophobic fusion peptide and two heptad repeat regions which the virus uses to gain access to the host cell.

The Affimer® Scaffold



- Based on naturally occurring proteins (cystatins).
- Currently operate with 2 scaffolds.
- Randomised binding loops (2x9aa) give rise to unique binding surfaces.
- Use phage display library (10^{10} – 10^{11}) to identify binders.
- Single domain proteins 1/10^{th} size of antibody (14 KDa).
- Simple structure means production is rapid.
- Affimer® reagents are easily engineered.

Affimer® Reagent Discovery and Characterisation



Target Protein QC: SARS CoV-2 S1 target protein was obtained from 3 vendors, and MERS S1, SARS S1 as well as other coronavirus S1 proteins were biotinylated and QC'd by WB. Binding of S1 to ACE2 was tested by ELISA prior to initiating phage display.

Phage Display: 3-rounds of phage display were performed against biotinylated SARS CoV-2 S1 protein and deselected against the MERS S1 / SARS S1 proteins.

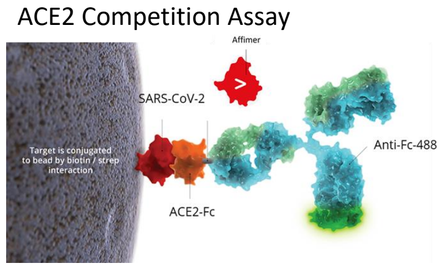
Primary Screening and Sequencing: 1152 Affimer® candidates were picked, sequenced, and 1 mL cultures were expressed and purified. Affimer® candidates were normalized where possible to 2.5 µg/mL.

Affimer® Characterisation: Primary screening data was used to rank the clones and determine how the clones bind to S1 protein, giving information into potential Affimer® pairs. Lead Affimer® candidates were used in a quantitative sandwich ELISA. AffiDX® SARS CoV-2 rapid antigen test developed as a CE-marked IVD (product code 11711125) for Professional Use Only.

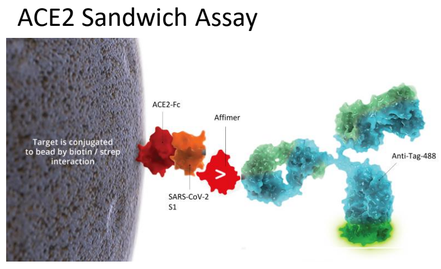
Primary Screening Strategy

1. Cross-reactivity Assay (iQue)
   SARS-CoV-2 S1 (from 3 suppliers), plus
   SARS/MERS/229E S1 proteins immobilised to beads.
   Multiplexed assay determines specificity to SARS CoV-2 S1 for Affimer® detection with anti-tag-488 antibody.
2. ACE2 Competition Assay (iQue)
   ACE2-Fc & Anti-Fc-488 detection of SARS-CoV-2 S1 immobilised to beads. Assayed for signal reduction caused by 40x excess of Affimer® reagent.
3. ACE2 Sandwich Assay (iQue)
   ACE2 immobilised to beads, capturing unlabelled SARS-CoV-2 S1. Assayed for Affimer® detection with anti-tag-488 antibody.
4. Affimer® capture assay (iQue)
   Anti-tag immobilisation of Affimer® reagent to beads. Assayed for capture of biotinylated SARS-CoV-2 S1, with streptavidin-488 detection.
5. Affimer® capture assay (ELISA)
   Affimer® reagents passively adsorbed to plates, assayed for capture of biotinylated SARS-CoV-2 S1 or SARS S1 (with streptavidin-polyHRP detection)

ACE2 Competition Assay



ACE2 Sandwich Assay



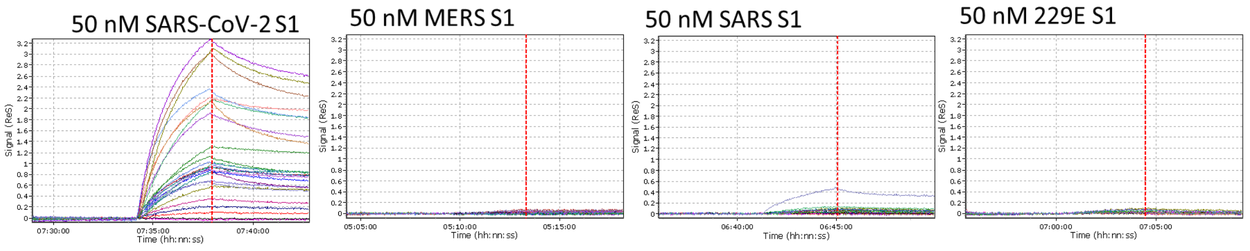
Primary Screening Results

Working ID	GXII Conc. (ng/µl)	GXII Purity	Conc. (ng/µl)	SARS-CoV-2 (Sino) (CoV2S1_03_1728-06-B) FL1-A of QSH01	No Target FL1-A of QSH30	Anti-FLAG FL1-A of QSH07	SARS-CoV-2 S1 (Sino) (CoV2S1_03_1728-06-B) FL1-A of QSH05	SARS-CoV-2 S1 (Nac) (CoV2S1_36_1715-03-B) FL1-A of QSH07	SARS-CoV-2 S1 (ACRO) (CoV2S1_03_1718-07-B) FL1-A of QSH04	MERS S1 (MERS1_15_1716-06-B) FL1-A of QSH17	SARS S1 (SARS1_15_1714-07-B) FL1-A of QSH21	HCoV-229E (229ES1_15_1734-03-B) FL1-A of QSH30	ACE2-Fc-AVI (BACEFc_03_1747-02-B) FL1-A of QSH01	mIgG2b (mIgG2b_18_1129-08-B) FL1-A of QSH08	No Target FL1-A of QSH27	ACE2-Fc-AVI (BACEFc_03_1747-02-B) FL1-A of QSH01	mIgG2b (mIgG2b_18_1129-08-B) FL1-A of QSH08	No Target FL1-A of QSH27	OD450nm-OD630nm (Target) Biotinylated CoV2S1_03_1728 (0.5ug/ml)	OD450nm-OD630nm (Negative Control 1) Biotinylated SARS1_15_1714 (0.5ug/ml)	OD450nm-OD630nm (Negative Control 2) NO TARGET
620_825594	375.0	100	2.5	62918	1336	1581	182598	20203	176590	1144	2138	1125	1758	985	1136	70661	908	1093	1.32	0.08	0.01
620_825543	392.3	100	2.5	51286	1276	1292	92502	42195	122931	1056	2059	1087	1747	954	1062	17059	860	963	1.38	0.06	0.01
620_826257	71.4	100	2.5	34052	1080	1314	83107	33359	97353	1147	1986	1054	1918	931	1081	17983	940	962	1.43	0.07	0.02
620_825489	379.7	100	2.5	62081	1139	942	58928	33157	100773	1148	1995	1041	1626	943	991	7914	965	1015	1.46	0.06	0.01
620_825329	279.4	90	2.5	40605	1094	1153	64092	17226	35882	1049	1963	998	1682	948	1031	11484	914	862	1.11	0.12	0.01
620_826101	61.5	100	2.5	45681	1118	1219	22345	7856	19746	1015	1829	931	1645	966	949	7216	952	988	0.74	0.12	0.01
620_825265	556.1	97	2.5	49211	1161	1136	20597	18730	80718	975	1734	999	1473	871	895	3468	815	877	0.36	0.09	0.01
620_825302	332.6	95	2.5	59639	1061	884	7923	41288	135770	1108	1977	1045	1648	982	1024	4060	993	1132	0.92	0.15	0.01
620_825358	321.1	100	2.5	46279	1156	1108	16422	13400	35082	1092	1821	944	1699	866	852	5384	790	1085	1.64	0.17	0.01
620_825456	417.5	100	2.5	25701	1281	4002	150305	12654	144615	1160	2065	1056	1644	926	1002	1524	909	1095	1.53	0.07	0.01
620_825425	346.8	100	2.5	5141	1125	3285	142431	12480	135198	1135	2075	1066	1575	1023	1015	1573	859	1012	1.27	0.06	0.01
620_825478	249.2	100	2.5	16047	1228	2597	136679	10761	132275	970	1918	1085	1513	908	977	1486	877	1068	1.58	0.05	0.01
620_825317	530.8	96	2.5	5698	1143	1858	107641	21525	120145	1036	1873	957	1337	882	929	1860	1006	1243	1.61	0.11	0.01
620_825392	254.0	98	2.5	11465	1104	2320	108829	14283	112749	1238	1859	1146	1396	1028	1027	1590	906	1133	0.97	0.06	0.01
620_825607	481.3	100	2.5	2739	1216	2209	72121	11688	65001	1121	2642	1089	1361	906	975	1677	837	1069	1.87	0.59	0.01
620_825511	475.6	100	2.5	5521	1160	1299	61793	7237	64993	1077	1866	1140	1501	803	1007	1445	907	961	1.34	0.05	0.01
620_825336	541.7	98	2.5	3505	1181	1696	39022	9152	43571	1044	1773	1079	1342	815	995	1493	906	961	1.02	0.07	0.01
620_826005	84.7	100	2.5	10409	1150	1309	29591	3988	32008	1268	1988	1110	1628	1048	998	1485	922	999	1.53	0.09	0.01
620_825436	380.4	72	2.5	8462	1187	2852	55737	6951	52089	1066	2072	1069	1447	1055	1043	1578	875	983	1.55	0.05	0.01
620_825349	542.6	99	2.5	47198	1094	1022	48429	10715	41955	1135	4243	1037	3161	1065	1014	28179	1177	1091	0.39	0.21	0.01
620_825411	323.7	94	2.5	63591	1160	1256	149368	34410	96600	1259	2095	1189	1528	1049	1105	18297	1053	1139	1.25	0.23	0.02
620_826209	235.5	100	2.5	55422	1160	1240	89074	25249	99137	1159	2174	1098	1647	994	1032	11422	987	948	1.23	0.53	0.01
620_825529	258.7	100	2.5	44659	1200	986	60696	52196	143822	1161	11957	1078	1918	928	1079	10845	945	1025	0.77	1.64	0.02
620_825322	600.8	99	2.5	39763	1181	945	47636	20156	44106	1065	10673	1025	1989	883	986	8581	875	1002	1.12	1.43	0.02

- An example data set of 24 unique Affimer® candidates. Affimer® binding characteristics highlighted as green (ACE2 pairing); light red (ACE2 competing); and blue (SARS cross-reactive).
- Affimer® pairs were identified by their ability to bind S1 in the presence of ACE2.

Kinetic evaluation of selected Affimer® clones

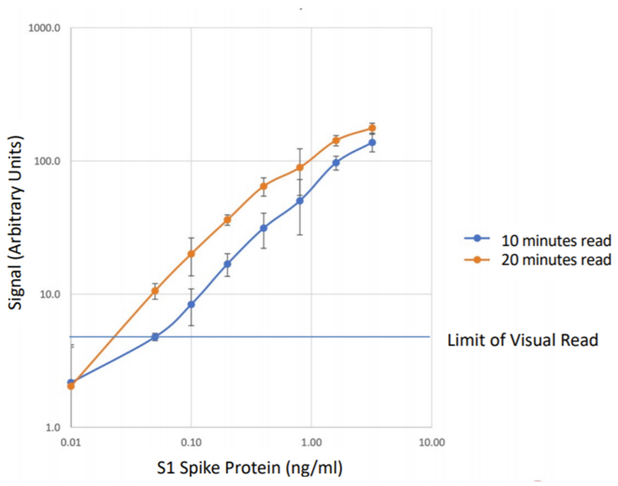
	Cross-reactivity profiling summary					Kinetic Summary	
Clone ID	Binds SARS S1?	Binds MERS S1?	Binds 229E S1?	Binds SARS-CoV-2 S1?	K_a/ M^{-1} s^{-1}	K_a/ M^{-1} s^{-1}	K_D
620_825543	N	N	N	y	7.7e4	1.34e5	3.72 nM
620_825594	N	N	N	y	1.64e5	1.74e5	3.98 nM
620_825489	N	N	N	y	1.36e5	2.07e5 (Poor fit)	5.69 nM (Poor fit)
620_826257	N	N	N	y	4.7e4	9.15e4	2.20 nM
620_825265	N	N	N	y (low signal)	*7.4e4*	Signal too low	
620_825302	N	N	N	y (low signal)	6.84e4	Signal too low	
620_825329	Y (v low signal)	N	Y (v low signal)	Y	6.8e4	1.15e5	"6.72 nM" (Only 3 curves)
620_825358	Y (v low signal)	N	N	Y	1.00e5	1.10e5	7.37 nM
620_825586	N	N	N	Y	1.45e5	2.09e5	4.68 nM
620_825601	N	N	N	Y	1.99e5	3.25e5 (Poor fit)	5.60 nM (Poor fit)
620_826110	N	N	N	Y	1.39e5	Signal too low	
620_826284	N	N	N	Y	1.01e5	Signal too low	
620_825425	N	N	N	Y	2.89e5	2.28e5 (Poor fit)	3.76 nM (Poor fit)
620_825436	N	N	N	Y	2.01e5	2.82e5 (Poor fit)	5.27 nM (Poor fit)
620_825456	N	N	N	Y	1.92e5	2.62e5 (Poor fit)	4.32 nM (poor fit)
620_825478	N	N	N	Y	2.05e5	3.54e5 (poor fit)	4.29 nM (poor fit)
620_825392	N	N	N	Y	1.34e5	1.41e5 (poor fit)	7.61 nM (poor fit)
620_826005	N	N	N	Y	3.5e4	8.9e4	10.3 nM (Only 3 curves)
620_825317	Y (v low signal)	Y (v low signal)	N	Y	1.47e5	1.37e5	4.61 nM (Only 3 curves)
620_825336	N	N	N	Y	1.31e5	1.42e5	6.66 nM (Only 3 curves)
620_826209	Y (v low signal)	N	N	Y	1.32e5	2.59e5 (Poor fit)	3.93 nM (Poor fit, only 3 curves)
620_825411	Y (v low signal)	N	Y (v low signal)	Y	7.55e4	1.09e5	8.55 nM
620_825322	Y	N	N	Y	2.18e5	2.11e5	5.48 nM (Only 3 curves)
620_826285	Y (v low signal)	N	N	Y	1.23e5	Signal too low	



The Affimer® candidates were analysed in a label-free surface plasmon resonance imaging (SPRi) system (HORIBA XelPlex). Each Affimer® reagent was printed onto an Epoxy-activated chip and were exposed to varying concentrations of SARS-CoV-2 S1, and the binding responses evaluated. Between each injection, undissociated SARS-CoV-2 S1 was removed from the surface using Glycine pH2 elution. The kinetics were fitted using a 1:1 Langmuir model using the software Scrubber. Cross-reactivity was also evaluated at fixed 50 nM injections against SARS CoV-2 S1, MERS S1, SARS S1 and 229E S1.

Analytical Performance of AffiDX® SARS CoV-2 Rapid Antigen Lateral Flow Test

Analytical Sensitivity Using Negative Nasal Swab Eluate Spiked with S1 Protein



Analytical Sensitivity:

- Can detect S1 protein concentration to <50 pg/mL.
- Analytical Limit of Detection (LoD): 6 x 10^2 pfu/mL

Analytical Specificity:

- No cross-reactivity to S1 proteins of other coronaviruses.
- Able to detect all SARS CoV-2 variants of concern tested
- Alpha, Beta and Delta variants validated (awaiting Gamma variant reference material).

Conclusion

Here we demonstrate the rapid development and characterisation of Affimer® reagents against SARS CoV-2 S1 protein and demonstrate the analytical performance of the AffiDX® SARS CoV-2 rapid antigen lateral flow test (product code 11711125 – For Professional Use Only). This study shows how powerful the Affimer® platform is in generating binders that can be used in IVD's to respond quickly to this and future Pandemics.