Development, High-Throughput Profiling, and Biopanning of a Large Phage Display Single-Domain Antibody Library

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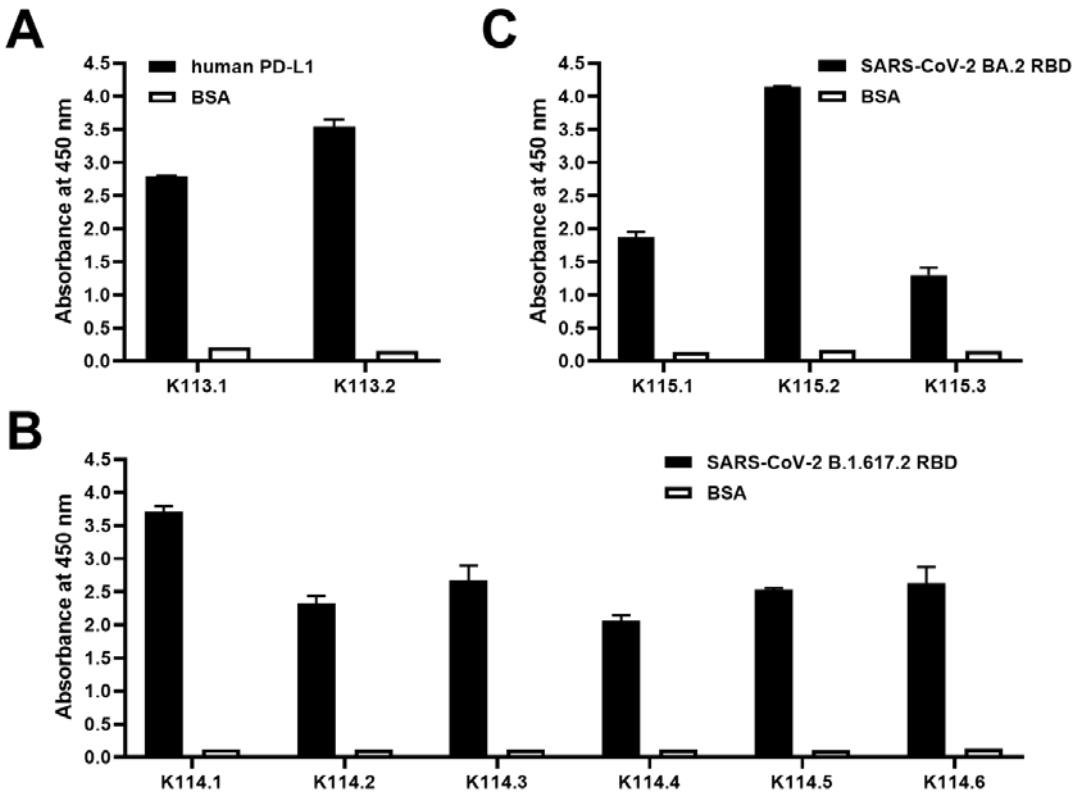

Supplementary Figure S1. Specific binding of the selected VHHs to target antigens. Phage ELISA was performed to verify the reactivity of the selected VHHs to human PD-L1 (A), SARS-CoV-2 BA.2 RBD (B), and SARS-CoV-2 B.1.617.2 RBD (C). Bovine serum albumin (BSA) was used as a negative control.

Supplementary Table S1. Primers used for the construction of the single domain antibody library.

Primer name	5′ to 3′ Sequence
Alp-Vh-F1 Sfi I	ACTGTGGCCCAGGCGGCCCAGKTGCAGCTCGTGGAGTCNGGNGG
AlpVhh-R1 Sfi I	ATGACTCGCGGCCGGCCTGGCCTCGTGGGGGTCTTCGCTGTGGTGCG
AlpVhh-R2 Sfi I	ATGACTCGCGGCCGGCCTGGCCTCGCCTTGTGGTTTTGGTGTCTTGGG

Supplementary Table S2. The percentage of pairings between the 63 individual IGHV gene segments and the seven IGHJ gene families in the constructed library.

	IGHJ1	IGHJ2	IGHJ3	IGHJ4	IGHJ5	IGHJ6	IGHJ7
IGHV1-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3-2	0.000	0.008	0.002	0.139	0.000	0.028	0.026
IGHV3-3	0.000	0.028	0.015	5.149	0.002	0.455	0.195
IGHV3S1	0.000	0.017	0.029	0.475	0.000	0.112	0.135
IGHV3S10	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S12	0.000	0.001	0.000	0.009	0.000	0.003	0.008
IGHV3S14	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S17	0.000	0.001	0.000	0.010	0.000	0.002	0.001
IGHV3S18	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S2	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S20	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S22	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S24	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S25	0.000	0.005	0.008	0.508	0.015	0.119	0.062
IGHV3S26	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S27	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S28	0.000	0.002	0.006	0.429	0.001	0.061	0.050
IGHV3S29	0.000	0.000	0.004	0.010	0.000	0.001	0.001
IGHV3S30	0.000	0.007	0.028	1.420	0.003	0.628	0.141
IGHV3S31	0.000	0.000	0.000	0.001	0.000	0.000	0.000
IGHV3S32	0.000	0.000	0.000	0.001	0.000	0.000	0.000
IGHV3S33	0.000	0.000	0.000	0.010	0.000	0.007	0.004
IGHV3S34	0.000	0.001	0.000	0.025	0.000	0.031	0.001
IGHV3S35	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S36	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S37	0.000	0.001	0.031	0.095	0.000	0.014	0.005
IGHV3S38	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S39	0.000	0.045	0.043	0.931	0.001	0.213	0.213
IGHV3S40	0.000	0.000	0.000	0.007	0.000	0.002	0.000
IGHV3S41	0.000	0.031	0.112	2.533	0.002	0.368	0.203
IGHV3S42	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S43	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S44	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S45	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S5	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S53	0.003	1.369	0.566	41.481	0.670	5.979	4.217
IGHV3S54	0.000	0.017	0.007	0.052	0.005	0.016	0.003
IGHV3S55	0.000	0.000	0.000	0.044	0.000	0.013	0.002
IGHV3S56	0.000	0.000	0.000	0.074	0.000	0.012	0.000
IGHV3S57	0.000	0.000	0.000	0.008	0.000	0.001	0.001
IGHV3S58	0.000	0.000	0.000	0.007	0.000	0.000	0.000
IGHV3S59	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S6	0.000	0.000	0.000	0.046	0.000	0.004	0.014
IGHV3S60	0.000	0.001	0.000	0.494	0.000	0.016	0.010
IGHV3S61	0.000	0.177	0.108	5.684	0.004	2.363	1.259
IGHV3S62	0.000	0.002	0.001	0.088	0.000	0.035	0.017
IGHV3S63	0.000	0.001	0.001	0.107	0.000	0.016	0.022

IGHV3S64	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S65	0.001	0.315	0.114	11.964	0.022	3.663	2.535
IGHV3S66	0.000	0.000	0.000	0.019	0.000	0.004	0.017
IGHV3S67	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV3S68	0.000	0.000	0.000	0.019	0.000	0.000	0.000
IGHV3S7	0.000	0.001	0.001	0.036	0.000	0.006	0.004
IGHV3S8	0.000	0.000	0.000	0.022	0.000	0.009	0.018
IGHV3S9	0.000	0.001	0.000	0.083	0.000	0.091	0.012
IGHV4S1	0.000	0.024	0.017	0.238	0.000	0.078	0.112
IGHV4S11	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV4S2	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV4S5	0.000	0.014	0.001	0.153	0.000	0.048	0.039
IGHV4S8	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IGHV4S9	0.000	0.000	0.000	0.000	0.000	0.000	0.000

The percentages of all the pairing of germline V and J gene segments with a cut-off ≥ 0.001 are shown. Pairing with a of the gene segment cut-off < 0.001 are expressed as 0.000.

Supplementary Table S3. The amino acid composition of CDR1, CDR2, and CDR3 in the constructed library, expressed as a percentage.

	Amino acid composition (%)																			
	G	**A**	**L**	**M**	**F**	**W**	**K**	**Q**	**E**	**S**	**P**	**V**	**I**	**C**	**Y**	**H**	**R**	**N**	**D**	**T**
CDR1	13.0	6.7	5.1	0.8	12.5	0.4	0.7	0.5	1.3	13.3	1.2	3.2	7.7	0.1	9.5	1.3	4.3	4.8	5.0	8.7
CDR2	18.4	3.2	1.4	0.9	1.0	0.7	1.1	0.3	1.0	18.5	1.6	2.0	13.1	0.2	0.8	0.6	5.5	4.4	6.0	19.5
CDR3	9.7	10.6	5.4	1.3	2.8	2.1	1.8	1.9	4.0	7.3	4.5	5.0	2.5	1.9	9.9	2.0	8.3	5.8	8.0	5.4

Supplementary Table S4. The amino acid frequency at each position in CDRs of the constructed library, expressed as a percentage.

Position	G	A	L	M	F	W	K	Q	E	S	P	V	I	C	Y	H	R	N	D	T
27	85.38	2.06	0.18	0.03	0.01	0.01	0.61	0.23	3.16	0.55	0.14	0.83	0.15	0.01	0.00	0.00	5.24	0.03	0.48	0.89
28	3.33	1.07	4.84	1.06	35.69	0.18	0.49	0.10	0.21	25.78	0.81	2.98	6.52	0.33	0.80	0.48	6.72	3.66	1.71	3.23
29	1.18	3.98	2.22	1.29	2.43	0.05	0.28	0.14	0.10	9.88	2.20	3.07	23.53	0.02	2.12	0.21	2.33	4.83	1.94	38.20
30	0.78	1.07	25.18	0.70	50.65	0.58	0.17	0.09	0.38	6.61	0.77	2.81	4.37	0.14	1.37	0.41	0.76	0.43	1.70	1.04
31	1.81	1.63	4.05	0.09	21.21	0.67	0.37	0.49	0.51	15.80	0.86	1.15	4.39	0.18	0.63	0.25	1.09	27.85	3.47	13.51
32	3.57	3.66	2.52	0.39	2.35	0.02	0.08	0.03	2.05	5.10	0.65	37.56	21.86	0.05	1.39	0.16	4.80	0.67	2.55	10.55
33	0.34	0.75	1.86	0.38	0.14	0.02	0.16	0.07	0.12	4.13	0.42	1.05	4.81	0.02	2.95	1.20	0.88	58.66	0.84	21.21
34	0.83	3.68	7.23	0.20	42.03	4.95	0.14	0.02	0.77	3.24	1.27	1.54	5.80	0.18	3.78	0.40	1.10	0.74	0.66	21.41
35	6.90	1.86	0.30	0.53	0.76	0.12	2.02	0.23	4.64	41.82	0.44	1.41	0.82	0.15	0.63	1.04	9.21	5.98	18.47	2.68
36	2.09	2.40	5.78	2.39	4.65	0.56	0.66	2.33	0.53	10.29	1.82	3.17	23.47	0.11	12.53	2.21	6.77	7.33	5.49	5.42
37	1.45	1.77	1.86	0.19	3.92	0.78	0.91	0.58	0.58	5.32	0.59	1.37	0.82	0.17	57.30	3.74	1.31	12.49	2.90	1.96
38	3.73	39.66	0.89	0.19	0.76	0.81	0.36	0.18	0.60	6.66	2.72	10.06	1.71	0.15	1.88	2.05	2.57	2.33	7.70	15.01
56	0.04	0.79	3.38	3.03	2.02	0.05	0.33	0.13	0.08	1.69	0.04	3.94	81.84	0.08	0.09	0.12	0.73	0.25	0.06	1.31
57	3.74	2.84	1.17	0.85	1.21	0.28	0.77	0.50	0.41	29.22	1.08	1.69	2.93	0.18	0.74	0.60	4.52	8.71	3.69	34.88
58	7.21	4.32	1.27	0.63	2.14	4.24	1.94	0.51	1.16	39.77	2.48	1.15	1.67	0.26	1.18	0.89	10.21	8.02	2.37	8.57
59	26.32	5.85	1.62	0.41	0.52	0.11	0.82	0.20	1.15	23.45	3.17	1.76	1.26	0.18	0.74	0.50	7.89	5.13	12.18	6.74
60	14.30	16.39	1.25	0.43	1.39	0.19	0.38	0.59	0.92	19.78	1.01	1.36	2.08	0.12	0.23	0.79	4.01	2.99	17.79	14.00
61	21.13	4.12	0.11	1.41	0.10	0.04	0.78	0.06	20.46	32.47	0.20	1.32	0.41	0.10	0.20	0.35	5.81	3.43	3.42	4.06
62	53.02	3.05	0.18	0.19	0.22	0.02	0.13	0.14	2.59	4.52	0.22	1.12	0.26	0.22	0.47	0.28	1.44	2.10	27.52	2.31
63	67.40	1.99	0.64	0.22	0.24	0.07	0.28	0.26	1.89	6.11	0.21	1.55	0.75	0.27	0.67	0.81	5.10	1.36	7.94	2.25
64	5.52	2.38	1.20	1.22	0.96	0.34	1.95	0.43	0.90	34.16	0.61	3.79	4.91	0.10	2.01	1.13	9.60	8.52	4.21	16.08
65	0.19	3.87	0.96	0.20	0.13	0.02	2.19	0.16	0.54	2.72	4.15	0.97	5.38	0.00	0.05	0.02	2.31	0.33	0.12	75.69
105	1.50	35.60	0.81	0.15	0.26	0.06	3.26	0.36	0.28	2.55	0.07	1.58	0.21	0.10	3.96	3.87	1.45	41.51	0.39	2.03
106	2.74	55.08	2.55	0.44	0.97	0.11	2.82	0.50	0.78	2.55	1.30	7.19	3.01	0.26	0.76	0.21	8.07	0.88	0.44	9.33
107	9.02	6.20	3.69	0.71	1.07	1.92	3.35	4.52	8.14	3.03	4.54	6.15	1.93	0.28	2.10	3.75	14.77	3.41	18.62	2.79
108	12.47	6.62	8.25	0.73	2.34	2.80	1.69	1.82	4.99	7.97	6.73	6.00	4.62	0.72	5.77	1.73	14.32	2.37	3.15	4.91
109	12.51	4.93	6.55	1.21	2.93	2.98	1.44	2.71	2.64	10.49	5.48	6.22	3.50	0.61	5.89	1.31	13.97	2.05	4.96	7.62
110	12.72	4.54	8.35	1.00	2.79	2.94	0.95	0.95	1.94	9.47	5.63	9.07	3.70	1.89	6.93	1.62	11.33	2.00	5.31	6.86
111	12.49	4.20	7.15	1.06	2.84	3.63	2.13	1.73	1.85	9.66	5.76	6.09	3.17	4.16	8.09	1.59	8.27	3.77	5.18	7.18
111.1	11.89	6.76	5.11	0.64	2.04	2.73	1.51	1.81	2.37	11.76	5.70	5.16	3.42	7.56	6.29	1.19	6.08	2.01	4.98	10.98
111.2	12.15	4.74	6.60	0.98	1.95	2.24	1.28	1.35	1.95	10.33	5.29	7.10	3.31	7.43	6.71	1.24	8.18	2.05	8.65	6.48
H111.3	9.86	4.73	11.05	1.80	2.27	4.17	1.89	2.80	2.29	11.36	3.78	8.41	2.16	5.55	8.84	0.91	4.51	2.70	4.18	6.73
111.4	9.85	11.06	3.93	1.72	5.18	1.05	1.37	2.42	2.90	18.06	3.51	5.72	1.65	4.96	6.44	0.89	3.57	3.27	7.94	4.51
111.5	4.93	5.70	3.43	3.39	2.91	1.01	1.28	22.63	3.28	6.27	3.13	9.28	2.33	10.41	6.60	0.69	4.62	0.65	4.45	3.03
111.6	7.06	4.25	3.06	1.47	0.57	0.37	0.58	1.74	0.90	4.93	2.74	4.33	2.28	2.00	4.21	1.83	2.32	42.53	3.23	9.60
111.7	76.84	2.31	1.34	0.56	0.16	0.14	0.17	2.30	0.32	1.10	0.95	1.79	1.35	1.13	0.87	0.28	3.03	0.57	3.62	1.17
111.8	71.07	0.24	0.51	0.17	0.07	0.40	0.06	0.36	21.06	0.36	0.14	1.27	0.16	0.12	0.14	0.31	0.31	0.10	1.63	1.52
111.9	4.20	4.05	0.15	2.40	3.60	0.30	1.65	47.90	0.30	4.35	4.50	18.02	0.45	0.15	0.60	0.45	4.50	0.15	0.90	1.35
112.9	5.60	2.44	5.75	2.09	8.50	0.76	1.53	24.03	1.07	4.84	1.73	19.20	1.02	0.36	0.76	0.05	7.28	2.39	4.63	5.96

112.8	2.05	1.45	0.60	0.16	0.33	0.05	0.06	0.66	0.43	0.75	0.58	0.85	0.16	0.44	0.54	0.77	87.07	0.09	0.52	2.44
112.7	4.84	3.47	53.66	1.03	0.93	3.29	0.78	0.81	0.47	5.51	1.08	2.41	8.23	3.16	2.96	0.58	1.55	0.79	2.22	2.25
112.6	30.54	13.08	3.99	2.13	1.39	0.70	1.15	1.46	1.43	4.60	11.34	4.06	0.88	5.45	4.03	1.58	1.90	0.89	5.40	4.02
112.5	4.71	4.13	15.58	4.28	1.28	1.17	1.10	3.15	1.95	7.74	4.36	8.11	3.53	5.20	5.56	0.70	5.06	8.74	3.94	9.71
112.4	13.00	4.51	6.10	3.32	1.16	2.82	1.04	1.61	7.09	6.80	3.45	7.17	1.68	10.46	6.30	1.33	4.10	5.08	5.05	7.94
112.3	9.07	5.90	8.06	3.83	2.02	5.55	1.19	1.50	2.32	8.12	7.02	5.10	3.70	9.73	8.60	2.01	4.02	1.89	3.68	6.69
112.2	11.38	5.81	11.93	1.65	2.35	3.24	3.04	1.08	4.70	8.15	6.26	4.02	2.95	8.42	4.76	1.50	6.75	2.34	4.12	5.56
112.1	9.62	5.44	9.14	2.99	2.17	2.54	1.72	2.51	3.78	8.82	7.44	4.72	2.25	2.50	5.34	1.90	8.22	3.23	7.54	8.12
112	12.86	8.51	6.07	1.57	2.38	3.14	1.75	2.03	4.28	8.79	12.19	4.41	2.93	1.26	6.05	1.99	7.55	2.28	4.55	5.41
113	14.71	5.91	4.37	0.83	1.87	3.95	1.81	1.78	3.94	9.49	4.07	3.82	2.29	0.34	9.99	2.54	11.16	3.59	7.45	6.09
114	17.38	5.96	6.58	1.15	3.00	1.45	1.78	4.30	11.61	5.30	6.21	2.96	2.55	0.12	2.94	1.50	8.47	2.50	9.57	4.66
115	5.10	2.65	5.40	4.78	12.04	1.73	1.61	1.59	2.83	3.96	3.00	5.00	1.86	0.20	25.85	1.31	11.80	3.20	2.96	3.12
116	11.15	3.06	1.13	0.35	0.39	0.51	0.85	1.13	9.55	4.20	1.52	2.27	0.91	0.15	1.20	2.24	2.52	9.99	43.08	3.82
117	0.54	3.15	2.75	0.15	5.87	0.61	0.10	0.19	0.17	12.86	0.95	3.50	1.37	0.51	56.67	3.59	0.67	1.99	2.13	2.22

The amino acid frequency at each position in CDRs with a cut-off ≥ 0.01 are represented. Amino acid frequencies with a cut-off < 0.01 are expressed as 0.00.

Supplementary Table S5. Length of complementarity-determining regions (CDR1, CDR2, CDR3) in selected VHHs.

Antigen	Antibody (VHH)	Length		
		CDR1	CDR2	CDR3
Human PD-L1	K113.1	8	8	8
	K113.2	8	8	8
SARS-CoV-2 B.1.617.2 RBD	K114.1	8	8	19
	K114.2	8	8	19
	K114.3	8	8	9
	K114.4	8	8	9
	K114.5	8	8	13
	K114.6	8	8	9
SARS-CoV-2 BA.2 RBD	K115.1	8	8	12
	K115.2	8	8	17
	K115.3	8	7	12

Supplementary Table S6. Primers used for amplification of VHH sequence.

Primer name	5′ to 3′ Sequence
Forward	TGGCTGGTTTCGCTACC
Reverse	TGATGGTGATGGTGCTGG

Supplementary Table S7. Index adapters used for enrichment adapter-ligated product.

Index Adapter	5′ to 3′ Sequence
Index 1 (i7)	GATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG
Index 2 (i5)	AATGATACGGCGACCACCGAGATCTACACNNNNNNNNACACTCTTTCCCTACACG