<u>Supporting Information:</u> OptMAVEn-2.0: *De novo* design of variable antibody regions against targeted antigen epitopes

Ratul Chowdhury[1,§], Matthew F. Allan[2,§], & Costas D. Maranas[1,*]

[1]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802. USA.
[2]Department of Computational and Systems Biology, Massachusetts Institute of Technology, Cambridge, MA 02139. USA.

[§]These authors contributed equally to this work

* Corresponding author

Table S1. The Spearman rank correlation coefficient (ρ) for each MAPs part category at each gap penalty g. ρ is independent of g for the J parts because the J parts do not have gaps.

g	HV	HCDR3	HJ	LV	LCDR3	LJ	KV	KCDR3	KJ
4	0.932	0.804	0.982	0.987	0.818	1.000	0.934	0.910	0.996
6	0.935	0.831	0.982	0.988	0.838	1.000	0.935	0.922	0.996
8	0.939	0.855	0.982	0.987	0.852	1.000	0.939	0.931	0.996
10	0.935	0.774	0.982	0.986	0.839	1.000	0.921	0.894	0.996
12	0.948	0.891	0.982	0.987	0.875	1.000	0.941	0.946	0.996

Table S2. The root mean squared error (*RMSE*) for each MAPs part category at each gap penalty g. *RMSE* is independent of g for the J parts because the J parts do not have gaps.

g	HV	HCDR3	HJ	LV	LCDR3	LJ	KV	KCDR3	KJ
4	26.313	15.441	0.593	13.005	9.833	0.321	21.669	8.484	1.124
6	26.793	15.133	0.593	13.146	9.585	0.321	21.983	8.508	1.124
8	27.179	15.306	0.593	13.244	9.585	0.321	21.906	8.574	1.124
10	27.360	15.902	0.593	13.513	10.096	0.321	22.877	9.551	1.124
12	27.404	15.439	0.593	13.620	9.674	0.321	22.468	8.699	1.124

Table S3. The antigen chain, heavy chain, light chain, and epitope residues from each of the 64 antigens used for testing OptMAVEn-2.0. The ten antigens used for benchmarking are tabulated first.

PDB	Antigen Chain	Heavy Chain	Light Chain	Epitope
1NSN	S	H	L	57, 60, 61, 64, 68, 70, 93, 95, 96, 97, 98, 105, 106, 120, 121, 123, 124, 127
2IGF	P	H	L	70, 71, 72, 73, 74, 75
2R0W	Q	H	L	3, 4, 5, 6, 7, 8
2VXQ	A	H	L	32, 34, 39, 40, 41, 43, 67, 68, 69, 74, 75, 76, 77, 78, 79, 80
2ZUQ	A	C	B	95, 96, 97, 98, 99, 100, 101, 132, 133, 134, 141

3BKY	P	H	L	169, 170, 171, 172, 173, 174, 175
3FFD	P	A	B	16, 17, 19, 20, 21, 23, 24, 26, 27, 28, 30, 31
3G5V	C	B	A	288, 289, 293, 296, 297, 298, 299, 300, 301, 302
3L5W	I	H	L	14, 15, 101, 104, 105, 107, 108, 109
3MLS	P	H	L	3, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17, 18
1ACY	P	H	L	316, 319, 320, 321, 322, 323, 324
1CE1	P	H	L	0, 1, 2, 3, 4, 5, 6, 7
1CFT	C	B	A	1, 2, 3, 4, 5
1DZB	X	A	A	20, 21, 23, 62, 63, 73, 75, 96, 97, 98, 100, 101, 102, 103, 104, 106, 112, 116
1EGJ	A	H	L	362, 363, 364, 365, 366, 367, 395, 416, 417, 418, 419, 421
1F90	E	H	L	1, 2, 3, 4, 5, 6, 7, 8
1FPT	P	H	L	96, 97, 98, 99, 100, 101, 102, 103
1HH6	C	B	A	2, 3, 4, 5, 6, 7, 8, 9, 10, 11
1I8I	C	B	A	502, 503, 505, 506, 507, 508, 509
1JHL	A	H	L	21, 22, 23, 102, 103, 104, 106, 111, 112, 113, 116, 117, 118, 119, 121
1JRH	I	H	L	47, 49, 50, 51, 52, 53, 54, 55, 56, 76, 78, 79, 80, 82, 84, 98, 99
1KC5	P	H	L	1, 2, 3, 4, 5, 6, 7
1KIQ	C	B	A	18, 19, 22, 23, 24, 25, 27, 102, 103, 116, 117, 118, 119, 120, 121, 124, 125
1MLC	E	B	A	41, 43, 45, 46, 47, 48, 49, 50, 51, 53, 66, 67, 68, 70, 79, 81, 84
1N64	P	H	L	26, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38
1NAK	P	H	L	313, 314, 315, 316, 319, 320, 321, 322
1OBE	P	H	L	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
1ORS	C	B	A	111, 112, 113, 114, 115, 116, 117, 119, 120, 123
1PZ5	C	B	A	1, 2, 3, 4, 5, 6, 7, 8
1QNZ	P	H	L	233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 248
1SM3	P	H	L	2, 3, 4, 5, 6, 7, 8, 9, 10
1TQB	A	B	C	127, 128, 158, 159, 185, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199
1V7M	V	H	L	57, 58, 61, 68, 71, 75, 98, 101, 102, 105, 106, 109, 110, 111, 112, 113, 114
1XGY	P	H	L	1, 2, 3, 4, 5, 6
1ZA3	R	H	L	25, 26, 27, 34, 36, 37, 38, 53, 54, 56, 57, 58, 59, 61, 62
2A6I	P	B	A	3, 4, 5, 6, 7, 8, 9, 10
2BDN	A	H	L	28, 30, 31, 32, 34, 37, 38, 39, 40, 41, 55, 56, 61, 64, 65, 68, 69
2DQJ	Y	H	L	14, 15, 16, 19, 20, 21, 62, 63, 73, 74, 75, 77, 93, 96, 97, 98, 100, 101, 102, 103
2FJH	V	H	L	16, 17, 18, 19, 21, 22, 23, 25, 61, 66, 101, 104
2H1P	P	H	L	602, 603, 604, 605, 606, 608, 609, 610, 611, 612
2HH0	P	H	L	2, 3, 4, 5, 6, 7, 8, 9, 10
2HRP	P	H	L	36, 37, 38, 39, 40, 41, 42, 43, 44, 45

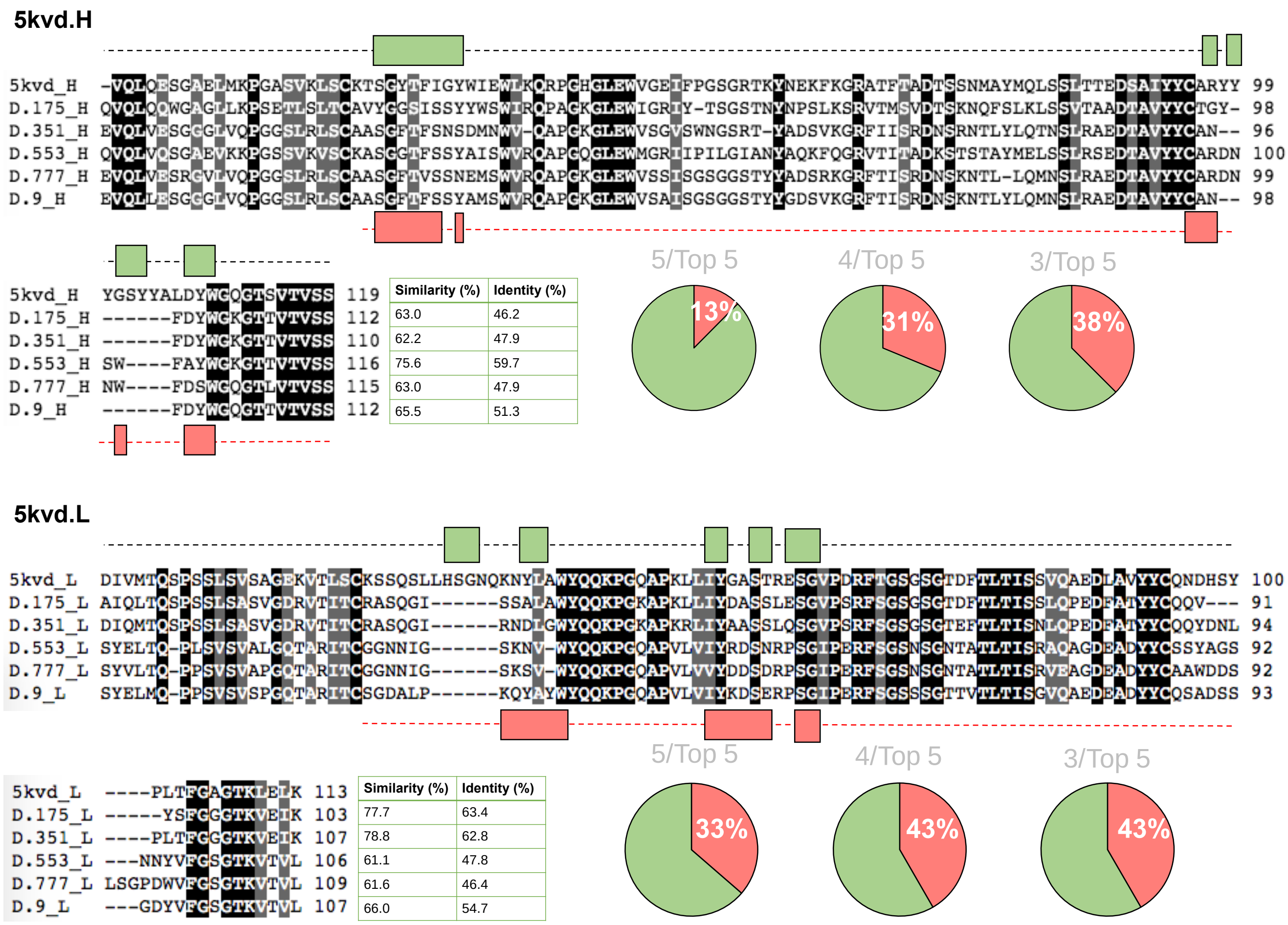
2IFF	Y	H	L	41, 43, 45, 46, 47, 48, 49, 51, 53, 67, 68, 69, 70, 81, 84
2JEL	P	H	L	1, 2, 3, 4, 34, 36, 41, 64, 66, 67, 68, 70, 71, 72, 75, 76
2OR9	P	H	L	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
2QHR	P	H	L	405, 406, 407, 408, 409, 410, 411, 412, 413, 414
2R29	A	H	L	306, 307, 308, 309, 310, 311, 312, 325, 362, 363, 364, 387, 388, 389, 390, 391
3AB0	A	B	C	102, 104, 113, 119, 120, 121, 122, 123, 125, 154, 156, 157, 158, 186, 188, 190, 192
3BDY	V	H	L	48, 81, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93
3CVH	C	H	L	4, 5, 6, 7, 8
3D85	C	B	A	82, 86, 87, 88, 91, 92, 93, 94, 95, 106, 110, 133, 134, 135, 136, 137, 139, 140
3E8U	P	H	L	4, 5, 6, 7, 8, 9, 10, 11, 13
3ETB	J	H	L	649, 651, 652, 653, 654, 655, 657, 680, 681, 682, 683, 684, 685, 686, 687, 716, 718
3F58	P	H	L	315, 316, 319, 320, 321, 322, 323, 324, 10, 11
3G6D	A	H	L	5, 6, 14, 18, 19, 21, 22, 23, 24, 97, 100, 101, 104, 105, 107, 108, 109
3GHB	P	H	L	306, 307, 308, 309, 312, 313, 314, 315
3GHE	P	H	L	304, 305, 306, 307, 308, 309, 312, 313, 314, 315, 316
3HR5	R	H	L	7, 8, 9, 10, 11, 12, 13, 14, 15
3KS0	B	H	L	30, 63, 64, 65, 66, 67, 68, 69, 70, 72, 74
3MLX	P	H	L	305, 306, 307, 308, 309, 312, 313, 314, 315, 316, 317, 318, 319
3NFP	I	H	L	1, 2, 3, 4, 5, 6, 25, 27, 43, 45, 116, 118, 120, 149, 152, 153, 154, 155
3P30	A	H	L	640, 643, 647, 650, 651, 653, 654, 657, 658, 661
3QG6	C	H	L	3, 4, 5, 6, 7, 8
3RKD	A	H	L	476, 477, 479, 484, 485, 496, 497, 498, 499, 508, 510, 512, 513, 514, 515, 534, 572, 573, 574, 575, 576, 577, 578, 592

Table S4. The antigen chains and epitope residues of the designs used in the test cases.

PDB	Antibody	Chain	Epitope residues
5GZN	Z3L1	A	46, 47, 52, 136, 138, 140, 156, 158, 159, 166, 168, 276, 277, 278, 279, 280, 281, 283
5KVD	ZV-2	E	301, 315, 316, 317, 318, 319, 320, 321, 322, 327, 329, 362, 364, 365, 366, 367, 372, 373, 374, 375, 377
5KVE	ZV-48	E	307, 340, 342, 343, 344, 347, 348, 350, 351, 352, 353, 354, 355, 358, 384, 386
5KVF	ZV-64	E	307, 340, 342, 343, 344, 347, 348, 350, 351, 352, 353, 354, 355, 358, 391
5KVG	ZV-67	E	309, 310, 311, 312, 313, 314, 331, 332, 333, 334, 335, 336, 337, 368, 370, 371, 393, 394, 395, 396, 397
1BVK	N/A	C	18, 19, 22, 23, 24, 27, 102, 103, 116, 117, 118, 119, 120, 121, 124, 125, 129
4PGJ	N/A	B	35, 46, 47, 48, 49, 52, 57, 59, 61, 62, 63, 70, 107, 108, 109, 110, 112
4TSB	N/A	A	21, 22, 23, 102, 103, 104, 106, 109, 111, 112, 113, 114, 116, 117, 118

Supplementary Figure 1:.

(a)



5kvd.H

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5kvd_H   -VQLQESGAELMKPGASVKLSCKTSGYTFIGYWIEWLKQRPGHGLEWVGEIFPGSGRTKYNEKFKGRATFTADTSSNMAYMQLSSLTTEDSAIYYCARYY 99
D.175_H  QVQLQQWGAGLLKPSETLSLTCAVYGGSISSYYWSWIRQPAGKGLEWIGRIY-TSGSTNYNPSLKSRVTMSVDTSKNQFSLKLSSVTAADTAVYYCTGY- 98
D.351_H  EVQLVESGGGLVQPGGSLRLSCAASGFTFSNSDMNWV-QAPGKGLEWVSGVSWNGSRT-YADSVKGRFIISRDNSRNTLYLQTNSLRAEDTAVYYCAN-- 96
D.553_H  QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARDN 100
D.777_H  EVQLVESRGVLVQPGGSLRLSCAASGFTVSSNEMSWVRQAPGKGLEWVSSISGSGGSTYYADSRKGRFTISRDNSKNTL-LQMNSLRAEDTAVYYCARDN 99
D.9_H    EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYGDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAN-- 98

5kvd_H   YGSYYALDYWGQGTSVTVSS 119
D.175_H  ------FDYWGKGTTVTVSS 112
D.351_H  ------FDYWGKGTTVTVSS 110
D.553_H  SW----FAYWGKGTTVTVSS 116
D.777_H  NW----FDSWGQGTLVTVSS 115
D.9_H    ------FDYWGQGTTVTVSS 112
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Similarity (%)	Identity (%)
63.0	46.2
62.2	47.9
75.6	59.7
63.0	47.9
65.5	51.3

5kvd.L

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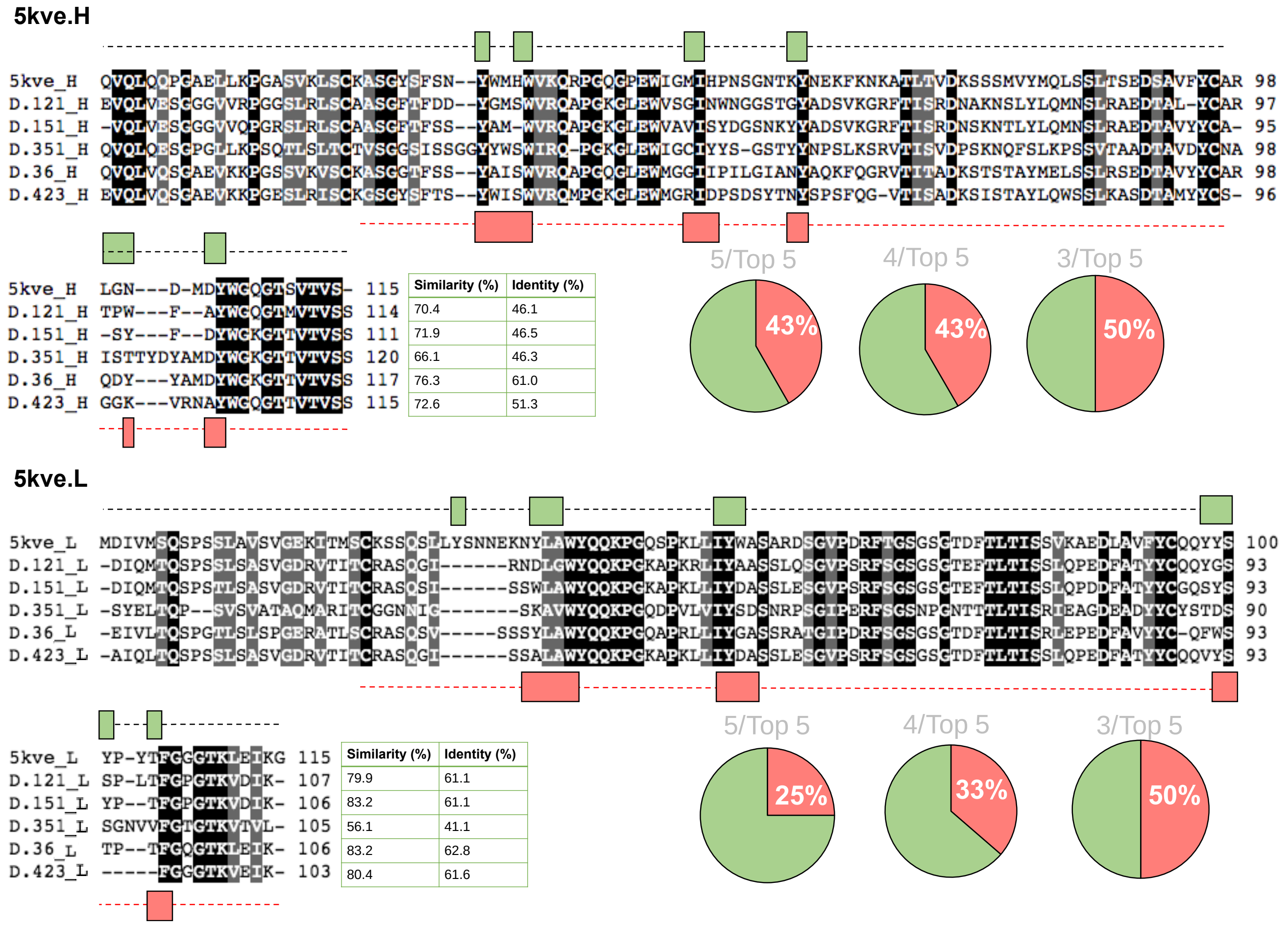
5kvd_L   DIVMTQSPSSLSVSAGEKVTLSCKSSQSLLHSGNQKNYLAWYQQKPGQAPKLLIYGASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDHSY 100
D.175_L  AIQLTQSPSSLSASVGDRVTITCRASQGI------SSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQV--- 91
D.351_L  DIQMTQSPSSLSASVGDRVTITCRASQGI------RNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISNLQPEDFATYYCQQYDNL 94
D.553_L  SYELTQ-PLSVSVALGQTARITCGGNNIG------SKNV-WYQQKPGQAPVLVIYRDSNRPSGIPERFSGSNSGNTATLTISRAQAGDEADYYCSSYAGS 92
D.777_L  SYVLTQ-PPSVSVAPGQTARITCGGNNIG------SKSV-WYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCAAWDDS 92
D.9_L    SYELMQ-PPSVSVSPGQTARITCSGDALP------KQYAYWYQQKPGQAPVLVIYKDSERPSGIPERFSGSSSGTTVTLTISGVQAEDEADYYCQSADSS 93

5kvd_L   ----PLTFGAGTKLELK 113
D.175_L  -----YSFGGGTKVEIK 103
D.351_L  ----PLTFGGGTKVEIK 107
D.553_L  ---NNYVFGSGTKVTVL 106
D.777_L  LSGPDWVFGSGTKVTVL 109
D.9_L    ---GDYVFGSGTKVTVL 107
```

Similarity (%)	Identity (%)
77.7	63.4
78.8	62.8
61.1	47.8
61.6	46.4
66.0	54.7

(b)



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5kve_H  QVQLQQPGAELLKPGASVKLSCKASGYSFSN--YWMHWVKQRPGQGPEWIGMIHPNSGNTKYNEKFKNKATLTVDKSSSMVYMQLSSLTSEDSAVFYCAR 98
D.121_H EVQLVESGGGVVRPGGSLRLSCAASGFTFDD--YGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL-YCAR 97
D.151_H -VQLVESGGGVVQPGRSLRLSCAASGFTFSS--YAM-WVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA- 95
D.351_H QVQLQESGPGLLKPSQTLSLTCTVSGGSISSGGYYWSWIRQ-PGKGLEWIGCIYYS-GSTYYNPSLKSRVTISVDPSKNQFSLKPSSVTAADTAVDYCNA 98
D.36_H  QVQLVQSGAEVKKPGSSVKVSCKASGGTFSS--YAISWVRQAPGQGLEWMGGIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAR 98
D.423_H EVQLVQSGAEVKKPGESLRISCKGSGYSFTS--YWISWVRQMPGKGLEWMGRIDPSDSYTNYSPSFQG-VTISADKSISTAYLQWSSLKASDTAMYYCS- 96

5kve_H  LGN---D-MDYWGQGTSVTVS- 115
D.121_H TPW---F--AYWGQGTMVTVSS 114
D.151_H -SY---F--DYWGKGTTVTVSS 111
D.351_H ISTTYDYAMDYWGKGTTVTVSS 120
D.36_H  QDY---YAMDYWGKGTTVTVSS 117
D.423_H GGK---VRNAYWGQGTTVTVSS 115
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Similarity (%)	Identity (%)
70.4	46.1
71.9	46.5
66.1	46.3
76.3	61.0
72.6	51.3

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5kve_L  MDIVMSQSPSSLAVSVGEKITMSCKSSQSLLYSNNEKNYLAWYQQKPGQSPKLLIYWASARDSGVPDRFTGSGSGTDFTLTISSVKAEDLAVFYCQQYYS 100
D.121_L -DIQMTQSPSSLSASVGDRVTITCRASQGI------RNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYGS 93
D.151_L -DIQMTQSPSTLSASVGDRVTITCRASQSI------SSWLAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCGQSYS 93
D.351_L -SYELTQP--SVSVATAQMARITCGGNNIG-------SKAVWYQQKPGQDPVLVIYSDSNRPSGIPERFSGSNPGNTTTLTISRIEAGDEADYYCYSTDS 90
D.36_L  -EIVLTQSPGTLSLSPGERATLSCRASQSV-----SSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC-QFWS 93
D.423_L -AIQLTQSPSSLSASVGDRVTITCRASQGI------SSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVYS 93

5kve_L  YP-YTFGGGTKLEIKG 115
D.121_L SP-LTFGPGTKVDIK- 107
D.151_L YP--TFGPGTKVDIK- 106
D.351_L SGNVVFGTGTKVTVL- 105
D.36_L  TP--TFGQGTKLEIK- 106
D.423_L -----FGGGTKVEIK- 103
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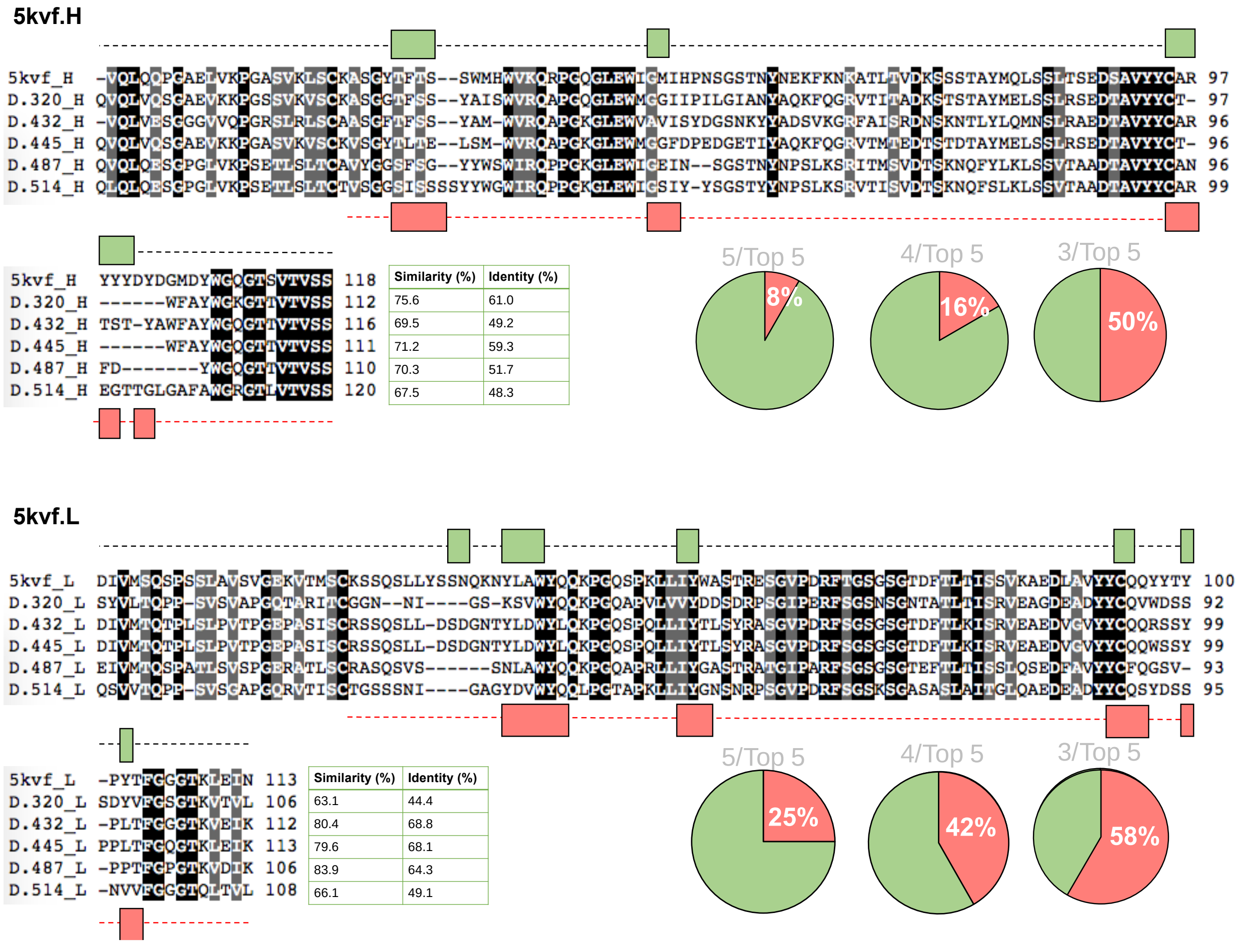
Similarity (%)	Identity (%)
79.9	61.1
83.2	61.1
56.1	41.1
83.2	62.8
80.4	61.6

(c)



5kvf.H

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5kvf_H  -VQLQQPGAELVKPGASVKLSCKASGYTFTS--SWMHWVKQRPGQGLEWIGMIHPNSGSTNYNEKFKNKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAR 97
D.320_H QVQLVQSGAEVKKPGSSVKVSCKASGGTFSS--YAISWVRQAPGQGLEWMGGIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCT- 97
D.432_H -VQLVESGGGVVQPGRSLRLSCAASGFTFSS--YAM-WVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFAISRDNSKNTLYLQMNSLRAEDTAVYYCAR 96
D.445_H QVQLVQSGAEVKKPGASVKVSCKVSGYTLTE--LSM-WVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCT- 96
D.487_H QVQLQESGPGLVKPSETLSLTCAVYGGSFSG--YYWSWIRQPPGKGLEWIGEIN--SGSTNYNPSLKSRITMSVDTSKNQFYLKLSSVTAADTAVYYCAN 96
D.514_H QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGSIY-YSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR 99

5kvf_H  YYYDYDGMDYWGQGTSVTVSS 118
D.320_H ------WFAYWGKGTTVTVSS 112
D.432_H TST-YAWFAYWGQGTTVTVSS 116
D.445_H ------WFAYWGQGTTVTVSS 111
D.487_H FD-------YWGQGTTVTVSS 110
D.514_H EGTTGLGAFAWGRGTLVTVSS 120
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Similarity (%)	Identity (%)
75.6	61.0
69.5	49.2
71.2	59.3
70.3	51.7
67.5	48.3

5kvf.L

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5kvf_L  DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYTY 100
D.320_L SYVLTQPP-SVSVAPGQTARITCGGN--NI----GS-KSVWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSS 92
D.432_L DIVMTQTPLSLPVTPGEPASISCRSSQSLL-DSDGNTYLDWYLQKPGQSPQLLIYTLSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQRSSY 99
D.445_L DIVMTQTPLSLPVTPGEPASISCRSSQSLL-DSDGNTYLDWYLQKPGQSPQLLIYTLSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQWSSY 99
D.487_L EIVMTQSPATLSVSPGERATLSCRASQSVS------SNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCFQGSV- 93
D.514_L QSVVTQPP-SVSGAPGQRVTISCTGSSSNI----GAGYDVWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGASASLAITGLQAEDEADYYCQSYDSS 95

5kvf_L  -PYTFGGGTKLEIN 113
D.320_L SDYVFGSGTKVTVL 106
D.432_L -PLTFGGGTKVEIK 112
D.445_L PPLTFGQGTKLEIK 113
D.487_L -PPTFGPGTKVDIK 106
D.514_L -NVVFGGGTQLTVL 108
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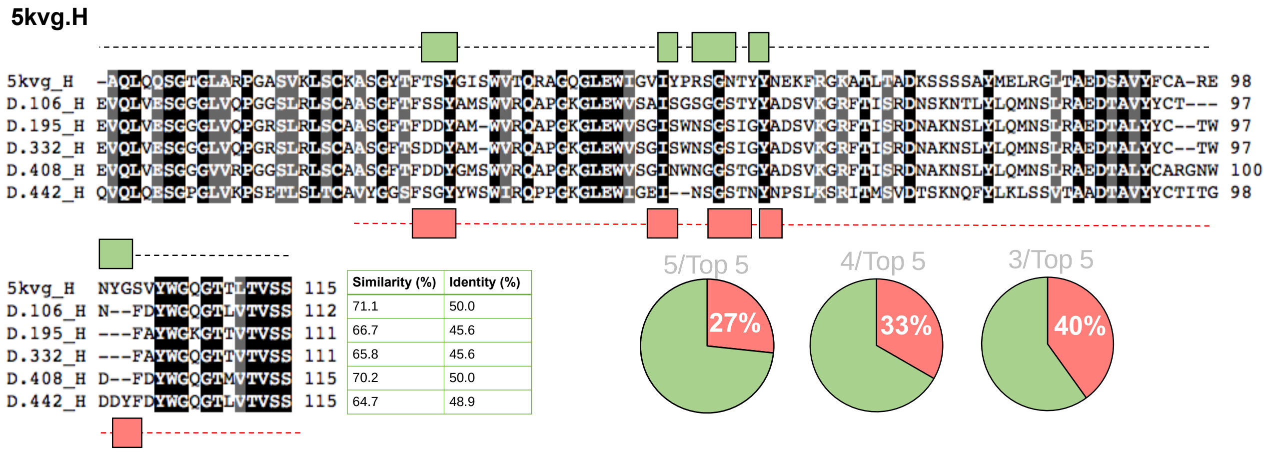
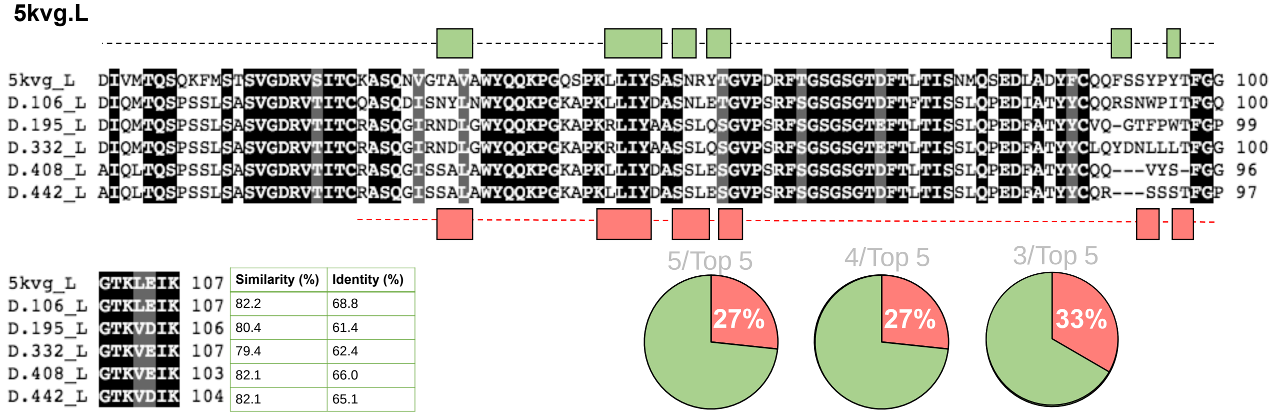
Similarity (%)	Identity (%)
63.1	44.4
80.4	68.8
79.6	68.1
83.9	64.3
66.1	49.1

(d)



Similarity (%)	Identity (%)
71.1	50.0
66.7	45.6
65.8	45.6
70.2	50.0
64.7	48.9

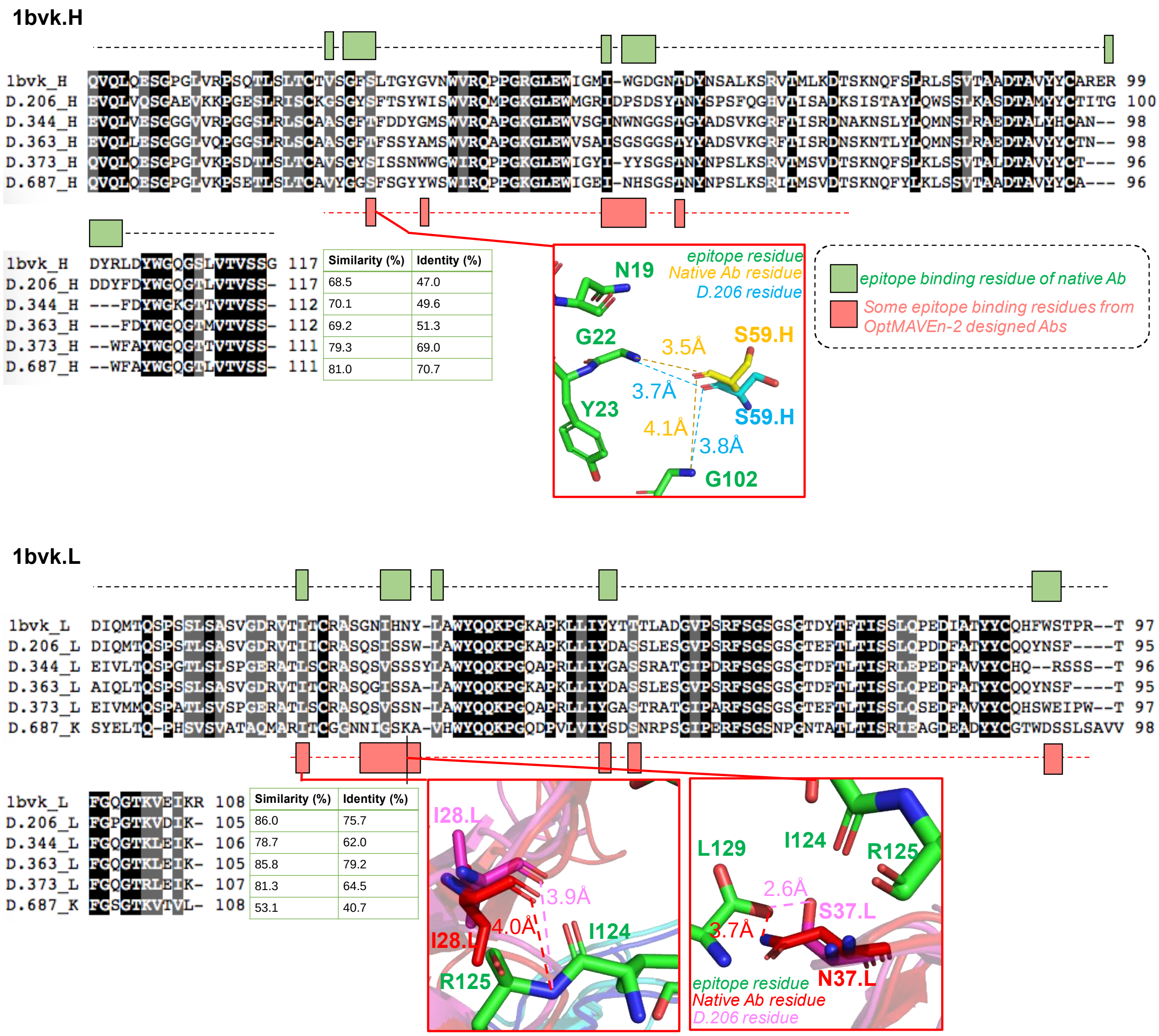


Similarity (%)	Identity (%)
82.2	68.8
80.4	61.4
79.4	62.4
82.1	66.0
82.1	65.1

Figure S1 (**a**–**d**). Sequence alignments of the native heavy and light chain antibody sequences with the top five de novo designed sequences for (a) 5KVD, (b) 5KVE, (c) 5KVF, and (d) 5KVG respectively, have been represented. The green blocks represent native antibody residues which interact with the antigen epitope. The red blocks represent the antigen binding residues that are either seen in all the top five de novo designed variable domains or contribute significantly to the antigen binding affinity in at least one of the top five de novo designs. The three pie charts show the percentage of recovery of the native epitope binding interactions in (i) all five, (ii) any four, and (iii) any three of the top five de novo designed sequences.

Supplementary Figure 2:

(a)



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1bvk_H  QVQLQESGPGLVRPSQTLSLTCTVSGFSLTGYGVNWVRQPPGRGLEWIGMI-WGDGNTDYNSALKSRVTMLKDTSKNQFSLRLSSVTAADTAVYYCARER 99
D.206_H EVQLVQSGAEVKKPGESLRISCKGSGYSFTSYWISWVRQMPGKGLEWMGRIDPSDSYTNYSPSFQGHVTISADKSISTAYLQWSSLKASDTAMYYCTITG 100
D.344_H EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYHCAN-- 98
D.363_H EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTN-- 98
D.373_H QVQLQESGPGLVKPSDTLSLTCAVSGYSISSNWWGWIRQPPGKGLEWIGYI-YYSGSTNYNPSLKSRVTMSVDTSKNQFSLKLSSVTALDTAVYYCT--- 96
D.687_H QVQLQESGPGLVKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEI-NHSGSTNYNPSLKSRITMSVDTSKNQFYLKLSSVTAADTAVYYCA--- 96

1bvk_H  DYRLDYWGQGSLVTVSSG 117
D.206_H DDYFDYWGQGTLVTVSS- 117
D.344_H ---FDYWGKGTTVTVSS- 112
D.363_H ---FDYWGQGTMVTVSS- 112
D.373_H --WFAYWGQGTTVTVSS- 111
D.687_H --WFAYWGQGTLVTVSS- 111
```

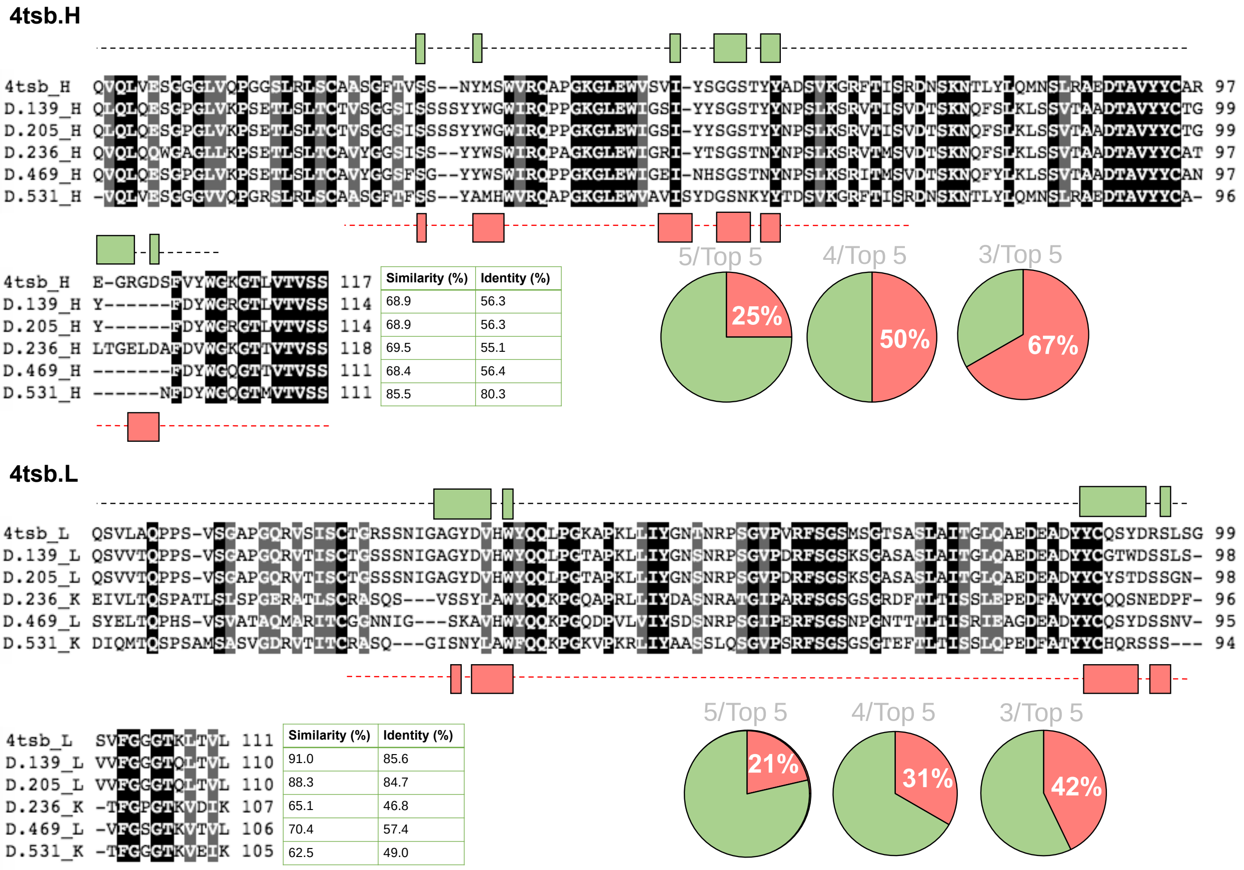
Similarity (%)	Identity (%)
68.5	47.0
70.1	49.6
69.2	51.3
79.3	69.0
81.0	70.7

```
1bvk_L  DIQMTQSPSSLSASVGDRVTITCRASGNIHNY-LAWYQQKPGKAPKLLIYYTTTLADGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHFWSTPR--T 97
D.206_L DIQMTQSPSTLSASVGDRVTIICRASQSISSW-LAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSF----T 95
D.344_L EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQ--RSSS--T 96
D.363_L AIQLTQSPSSLSASVGDRVTITCRASQGISSA-LAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSF----T 95
D.373_L EIVMMQSPATLSVSPGERATLSCRASQSVSSN-LAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQHSWEIPW--T 97
D.687_K SYELTQ-PHSVSVATAQMARITCGGNNIGSKA-VHWYQQKPGQDPVLVIYSDSNRPSGIPERFSGSNPGNTATLTISRIEAGDEADYYCGTWDSSLSAVV 98

1bvk_L  FGQGTKVEIKR 108
D.206_L FGPGTKVDIK- 105
D.344_L FGQGTKLEIK- 106
D.363_L FGQGTKLEIK- 105
D.373_L FGQGTRLEIK- 107
D.687_K FGSGTKVTVL- 108
```

Similarity (%)	Identity (%)
86.0	75.7
78.7	62.0
85.8	79.2
81.3	64.5
53.1	40.7

(b)



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4tsb_H  QVQLVESGGGLVQPGGSLRLSCAASGFTVSS--NYMSWVRQAPGKGLEWVSVI-YSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR 97
D.139_H QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGSI-YYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCTG 99
D.205_H QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGSI-YYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCTG 99
D.236_H QVQLQQWGAGLLKPSETLSLTCAVYGGSISS--YYWSWIRQPAGKGLEWIGRI-YTSGSTNYNPSLKSRVTMSVDTSKNQFSLKLSSVTAADTAVYYCAT 97
D.469_H QVQLQESGPGLVKPSETLSLTCAVYGGSFSG--YYWSWIRQPPGKGLEWIGEI-NHSGSTNYNPSLKSRITMSVDTSKNQFYLKLSSVTAADTAVYYCAN 97
D.531_H -VQLVESGGGVVQPGRSLRLSCAASGFTFSS--YAMHWVRQAPGKGLEWVAVISYDGSNKYYTDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA- 96

4tsb_H  E-GRGDSFVYWGKGTLVTVSS 117
D.139_H Y------FDYWGRGTLVTVSS 114
D.205_H Y------FDYWGRGTLVTVSS 114
D.236_H LTGELDAFDVWGKGTTVTVSS 118
D.469_H -------FDYWGQGTTVTVSS 111
D.531_H ------NFDYWGQGTMVTVSS 111
```

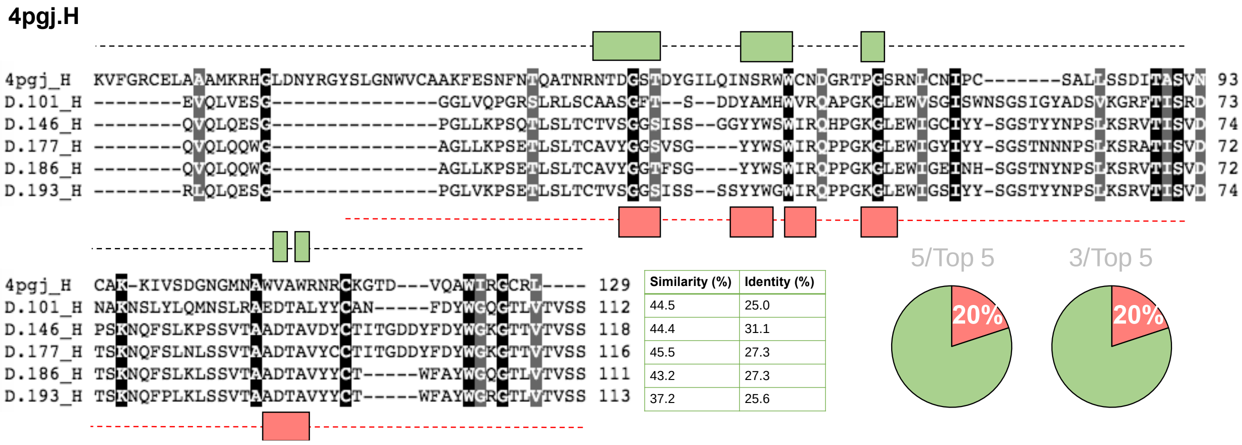
Similarity (%)	Identity (%)
68.9	56.3
68.9	56.3
69.5	55.1
68.4	56.4
85.5	80.3

```
4tsb_L  QSVLAQPPS-VSGAPGQRVSISCTGRSSNIGAGYDVHWYQQLPGKAPKLLIYGNTNRPSGVPVRFSGSMSGTSASLAITGLQAEDEADYYCQSYDRSLSG 99
D.139_L QSVVTQPPS-VSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGASASLAITGLQAEDEADYYCGTWDSSLS- 98
D.205_L QSVVTQPPS-VSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGASASLAITGLQAEDEADYYCYSTDSSGN- 98
D.236_K EIVLTQSPATLSLSPGERATLSCRASQS---VSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGRDFTLTISSLEPEDFAVYYCQQSNEDPF- 96
D.469_L SYELTQPHS-VSVATAQMARITCGGNNIG---SKAVHWYQQKPGQDPVLVIYSDSNRPSGIPERFSGSNPGNTTTLTISRIEAGDEADYYCQSYDSSNV- 95
D.531_K DIQMTQSPSAMSASVGDRVTITCRASQ---GISNYLAWFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCHQRSSS--- 94

4tsb_L  SVFGGGTKLTVL 111
D.139_L VVFGGGTQLTVL 110
D.205_L VVFGGGTQLTVL 110
D.236_K -TFGPGTKVDIK 107
D.469_L -VFGSGTKVTVL 106
D.531_K -TFGGGTKVEIK 105
```

Similarity (%)	Identity (%)
91.0	85.6
88.3	84.7
65.1	46.8
70.4	57.4
62.5	49.0

(c)



```
4pgj_H  KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPC--------SALLSSDITASVN 93
D.101_H --------EVQLVESG---------------GGLVQPGRSLRLSCAASGFT--S--DDYAMHWVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRD 73
D.146_H --------QVQLQESG---------------PGLLKPSQTLSLTCTVSGGSISS--GGYYWSWIRQHPGKGLEWIGCIYY-SGSTYYNPSLKSRVTISVD 74
D.177_H --------QVQLQQWG---------------AGLLKPSETLSLTCAVYGGSVSG----YYWSWIRQPPGKGLEWIGYIYY-SGSTNNNPSLKSRATISVD 72
D.186_H --------QVQLQQWG---------------AGLLKPSETLSLTCAVYGGTFSG----YYWSWIRQPPGKGLEWIGEINH-SGSTNYNPSLKSRVTISVD 72
D.193_H --------RLQLQESG---------------PGLVKPSETLSLTCTVSGGSISS--SSYYWGWIRQPPGKGLEWIGSIYY-SGSTYYNPSLKSRVTISVD 74

4pgj_H  CAK-KIVSDGNGMNAWVAWRNRCKGTD---VQAWIRGCRL---- 129
D.101_H NAKNSLYLQMNSLRAEDTALYYCAN-----FDYWGQGTLVTVSS 112
D.146_H PSKNQFSLKPSSVTAADTAVDYCTITGDDYFDYWGKGTTVTVSS 118
D.177_H TSKNQFSLNLSSVTAADTAVYCCTITGDDYFDYWGKGTTVTVSS 116
D.186_H TSKNQFSLKLSSVTAADTAVYYCT-----WFAYWGQGTLVTVSS 111
D.193_H TSKNQFPLKLSSVTAADTAVYYCT-----WFAYWGRGTLVTVSS 113
```

Similarity (%)	Identity (%)
44.5	25.0
44.4	31.1
45.5	27.3
43.2	27.3
37.2	25.6

Figure S2 (a–c). Sequence alignments of the native heavy and light chain antibody sequences with the top five *de novo* designed sequences for (a) 1BVK, (b) 4TSB, and (c) 4PGJ have been represented. The insets in 2(a) show comparison of epitope interactions between the native antibody and the D.206 design. The green blocks represent native antibody residues which interact with the antigen epitope. The red blocks represent the epitope binding residues that are either seen in all the top five *de novo* designed variable domains or contribute significantly to the antigen binding affinity in at least one of the top five *de novo* designs.