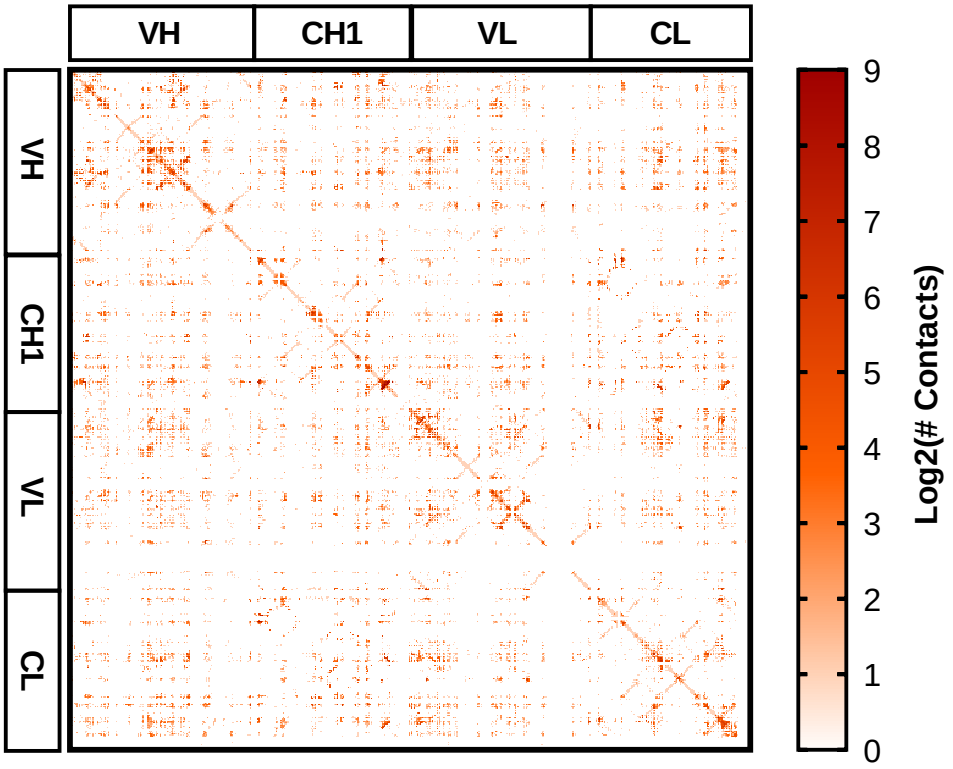
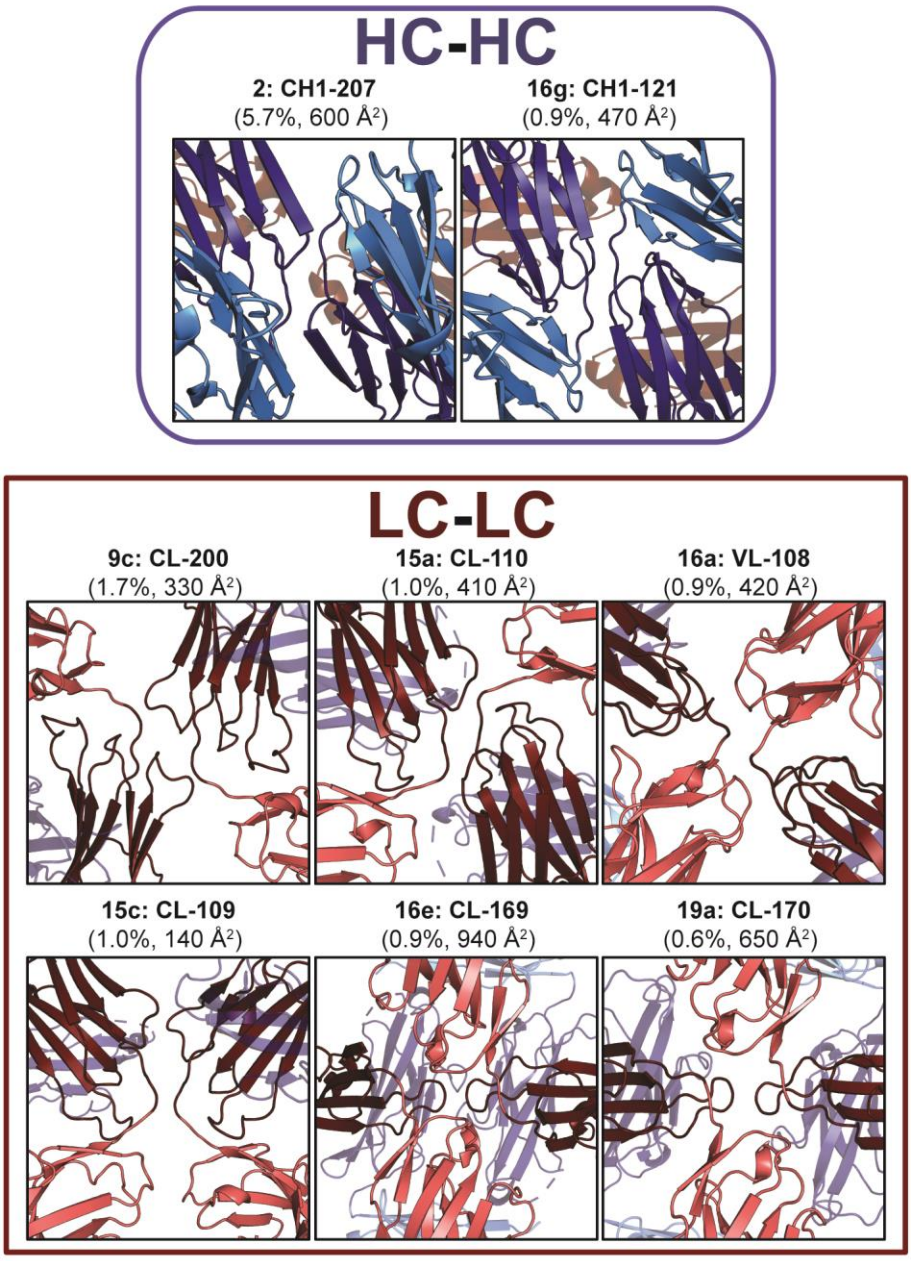
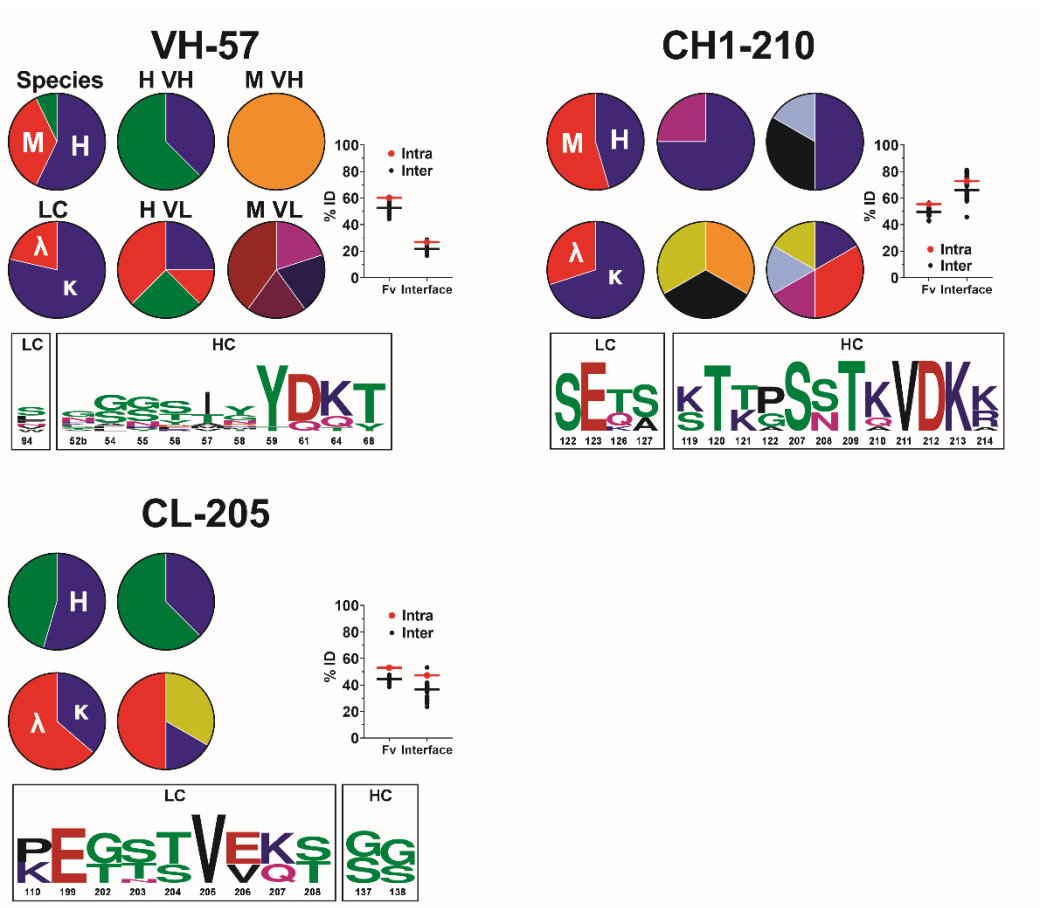


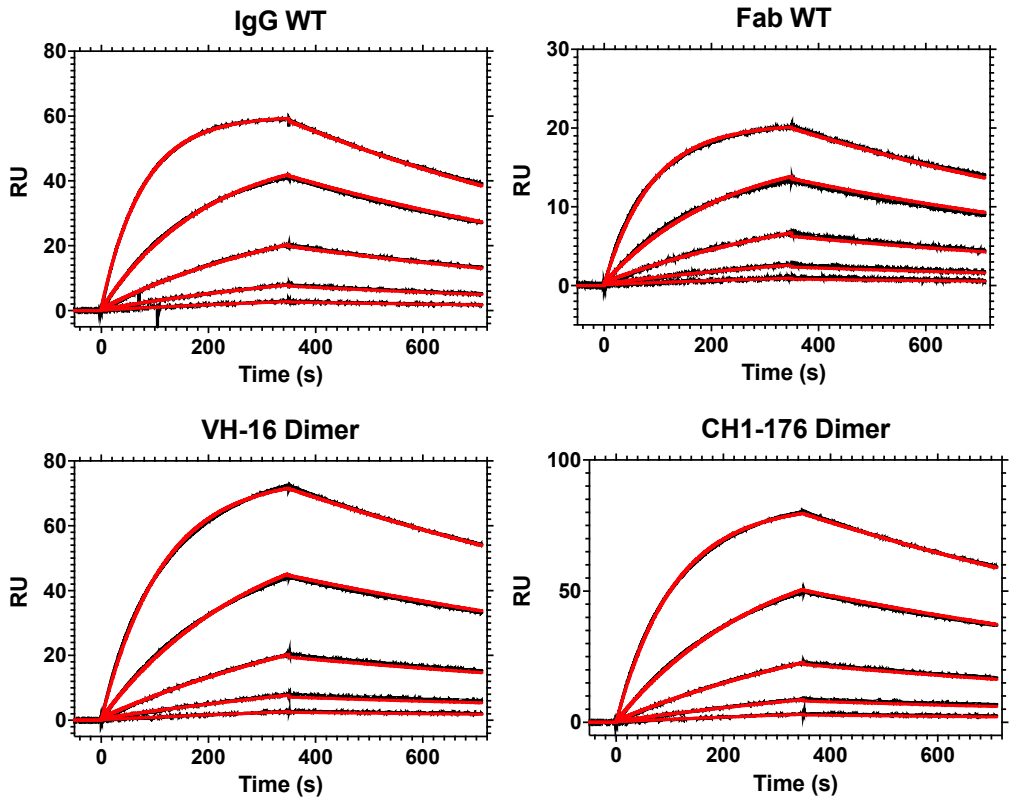
Supplementary Fig. S1. 4 Å contact map of inter-Fab residue pairs. Heavy and light chains are arbitrarily represented by concatenation from left to right and top to bottom. Map coloring on log2 scale reflects the number of times a contact is observed in the lattices of Fab structures, with 0 being the minimum and 512 being the maximum count.



Supplementary Fig. S2. Fab dimers mediated through VH-CH1 and VL-CL elbow regions. Structural similarity between elbow region Fab oligomers that interact as HC-HC (*top*) or LC-LC (*bottom*) dimers. Domains are colored as follows: VH (light blue), CH1 (dark blue), VL (pink), CL (dark red). The labels for each interface include the cluster rank, interface name, nonredundant prevalence percentage, and mean buried surface area.



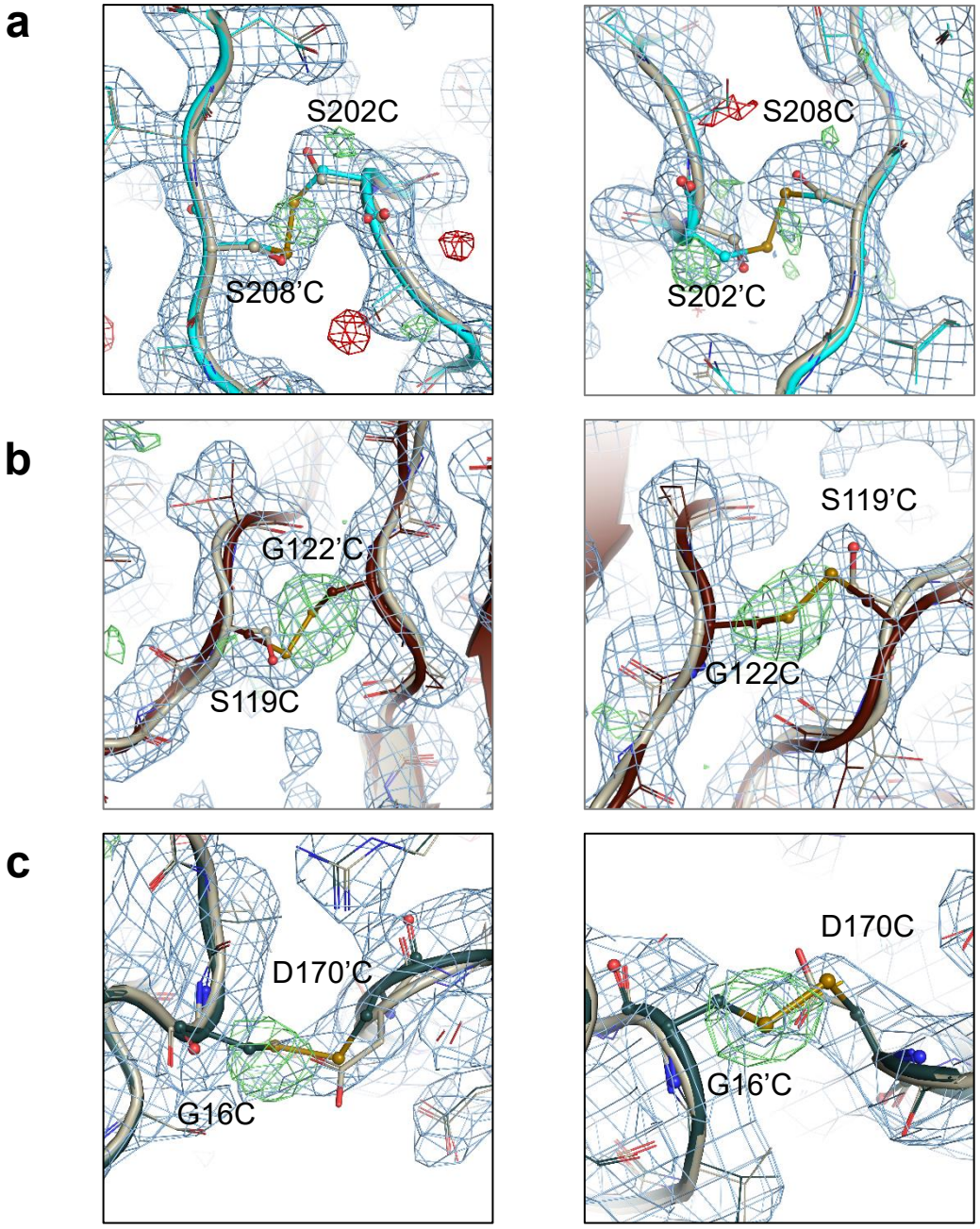
Supplementary Fig. S3. Interface profiles of three β-sheet dimers VH-57, CH1-210, and CL-205. For all interfaces, the upper left-most pie chart provides the sequence distribution of cluster members based on species: human (blue, H), mouse (red, M), and other (e.g., rat, rabbit, etc., green). The lower left-most pie chart provides the sequence distribution of cluster members based on light chain type: kappa (blue, κ), and lambda (red, λ). The four right-most pie charts provide the sequence distribution of human VH (H VH), human VL (H VL), mouse VH (M VH), and mouse VL (M VL) subgroups. The labels above the pie charts in the upper left profile VH-57 are not repeated in the other charts for visual simplicity. The %ID plot on the right provides the intra (red) versus inter (black) cluster sequence identity for both the entire variable region (Fv) as well as only those residues at the interface as shown in the sequence logo and Supplementary Table 2. For the Fv, these values reflect the mean pairwise identities for all VH and VL sequences within the cluster (intra) versus the mean pairwise identities between each member of the cluster and all other members of all other clusters (inter). A similar comparison is made for each interface, where %ID reflects the mean pairwise identity of the residues at the interface of a given cluster aligned with those same residues for each member of the cluster (intra) versus all other members of all other clusters (inter). The sequence logo at the bottom provides weighted sequence composition at interface residues for members of the designated cluster, with numbering according to Kabat and EU conventions for the Fv and constant regions, respectively



	Wild-type		VH-16	CH1-176
	IgG	Fab	Dimer	
k_a (x10^5, s^{-1})	1.2	1.0	0.81	0.84
k_d (x10^{-3}, s^{-1} M^{-1})	1.2	1.1	0.78	0.83
K_D (nM)	9.9	10	9.7	9.9
Normalized Analyte Binding*	0.40	0.48	0.52	0.57

*R_{max} normalized to ligand capture level

Supplementary Fig. S4. OX40 binding by WT IgG and Fab, VH-16 dimer, and CH1-176 dimer by SPR. All antibodies contain the Fv region of the anti-OX40 antibody 3C8. VH-16 represents the Fab dimer of cysteine variant VH(S113C) / CH1(G178C), and CH-176 represents the Fab dimer of cysteine variant VH(P14C) / CL(D151C). Shown are the sensorgrams (*top*) and kinetic fit values and normalized analyte binding (*bottom*). The dimers have slightly slower on/off rates but have the same K_D as the WT Fab and IgG. The normalized binding data are consistent across Fab monomer (WT) and dimer. The value is slightly lower for IgG, but that is expected due to the extra mass of the Fc on the capture ligand.



Supplementary Fig. S5. Electron density at engineered Cys locations. 2Fo-Fc density at 1 sigma contour (blue) and Fo-Fc difference density at +/- 3 sigma contour (green/red) are shown in the vicinity of the targeted cysteine sites. Maps shown were calculated at the initial stage of refinement before introducing the engineered cysteines such that the Fab model (ivory) still had its native sequence. Positive difference density (green mesh) and the contiguous blue mesh between sites thus are consistent with the introduction of the expected cysteine-mediated disulfide bonds, as shown in the overlaid final refined models for (a) CL-205 (final model cyan), (b) CH1-207 (brown), and (c) VL-108 (teal).

Rank	Interface (Kabat, EU)	Total Size (of 1,456)	NR Size (of 981)	NR Prevalence	Area (Å^2)	H-Bonds
1	CH1-211	166	133	13.6%	668 ± 134	10.4 ± 3.6
2	CH1-207	61	56	5.7%	596 ± 129	12.9 ± 3.6
3	CH1-209	82	36	3.7%	397 ± 53	6.6 ± 2.6
4	CL-211	34	33	3.4%	678 ± 204	10.7 ± 4.7
5	VL-11	37	29	3.0%	768 ± 112	14.5 ± 4.3
6	CH1-212	59	27	2.8%	580 ± 220	8.9 ± 3.1
7	VL-107	22	19	1.9%	594 ± 58	7.5 ± 1.3
8	VH-16	20	18	1.8%	861 ± 291	5.9 ± 4.1
9a	CL-187	24	17	1.7%	804 ± 180	20.5 ± 8
9b	CH1-176	20	17	1.7%	646 ± 81	12.3 ± 3.5
9c	CL-200	17	17	1.7%	335 ± 47	6.9 ± 3
10	VL-59	15	15	1.5%	364 ± 126	5.9 ± 2.3
11a	VH-82b	26	14	1.4%	685 ± 159	8.8 ± 3.4
11b	VL-13	15	14	1.4%	416 ± 92	7.2 ± 3.1
11c	VH-57	14	14	1.4%	524 ± 140	9.2 ± 4.4
12a	CL-145	16	13	1.3%	1092 ± 119	5.4 ± 3.1
12b	CH1-161	15	13	1.3%	1269 ± 145	13.4 ± 2.8
13a	CH1-188	13	12	1.2%	1397 ± 166	12.8 ± 3.2
13b	VH-82a	12	12	1.2%	306 ± 111	8.6 ± 2.3
14a	CH1-210	14	11	1.1%	672 ± 149	7.4 ± 3.6
14b	VL-74	13	11	1.1%	402 ± 27	3.2 ± 1.8
14c	CL-205	13	11	1.1%	439 ± 89	9.7 ± 3.6
15a	CL-110	15	10	1.0%	414 ± 79	3.8 ± 1.7
15b	CH1-206	13	10	1.0%	647 ± 138	10.8 ± 3.5
15c	CL-109	10	10	1.0%	139 ± 76	0.9 ± 0.6
15d	CH1-197	10	10	1.0%	421 ± 105	4.3 ± 3.3
16a	VL-108	23	9	0.9%	417 ± 29	5 ± 3.1
16b	VH-54	17	9	0.9%	113 ± 59	0.2 ± 0.6
16c	VL-7	14	9	0.9%	630 ± 197	8.8 ± 5
16d	CH1-139	12	9	0.9%	556 ± 132	8.8 ± 2.3
16e	CL-169	11	9	0.9%	945 ± 233	9.7 ± 3.6
16f	CL-151	11	9	0.9%	227 ± 52	4.1 ± 1.5
16g	CH1-121	11	9	0.9%	467 ± 42	10.5 ± 3.6
16h	CH1-194	10	9	0.9%	240 ± 55	0.8 ± 1.4
17a	VL-162	15	8	0.8%	216 ± 45	4.8 ± 0.9
17b	CH1-165	11	8	0.8%	1472 ± 168	14 ± 3.9
18a	VL-61	11	7	0.7%	526 ± 94	9.9 ± 2.8
18b	VH-8	10	7	0.7%	852 ± 112	10.8 ± 2.3
19a	CL-170	10	6	0.6%	646 ± 107	1.5 ± 1.1
19b	VL-25	10	6	0.6%	728 ± 111	9.4 ± 4.4
20a	VH-83	12	5	0.5%	885 ± 112	13.2 ± 2.9
20b	VH-25	10	5	0.5%	355 ± 97	8.8 ± 5

Supplementary Table S1. Prevalence and structural parameters for the 42 most prevalent interface clusters.

Rank	Interface	Positions (VL / CL / VH / CH1)
1	CH1-211	CL(122,123,126) / CH1(119,120,121,122,207,208,209,210,211,212,213,214)
2	CH1-207	VH(11,12,13,23,25,72,74,75,112,113) / CH1(118,119,120,206,207,208,209,210)
3	CH1-209	CL(126,127) / CH1(119,207,208,209,210,211,212,213)
4	CL-211	CL(109,110,112,123,126,202,203,204,205,206,207,208) / CH1(119,120,121,136,137,207,208,209,210,211,212,213,214)
5	VL-11	VL(5,6,7,8,9,10,11,12,13,14,17,18,19,20,22,24,70,107) / CL(147,154,155,156)
6	CH1-212	CL(151,152,153,154,155,156,157,158) / CH1(160,176,210,211,212,213,214)
7	VL-107	VL(14,107) / CL(108,110,111,112,140,141,160,199,200,201,202) / VH(112,113) / CH1(118,119,150,174,175,176,177,178,179,180)
8	VH-16	VL(1,3,5,6,7,9,24,26) / CL(160) / VH(13,14,15,16,41,43,84,85,113) / CH1(174,175,176,177,178)
9a	CL-187	CL(155,157,182,184,185,187,188,189) / CH1(159,162,163,165,188,189,192,193,196,197,198)
9b	CH1-176	CL(151,152,153,154,155,185,188,189) / VH(14,82c,83,84,85,113) / CH1(118,119,176,178,179)
9c	CL-200	VL(107) / CL(108,109,110,140,198,199,200,201,202,203)
10	VL-59	VL(59,60,61,76,77,79,80,81)
11a	VH-82b	VH(14,15,61,62,63,64,65,66,82a,82b,82c,83,84,85)
11b	VL-13	VL(8,11,12,13,14,17,18,19,20,107) / CL(199)
11c	VH-57	VL(94) / VH(52b,54,55,56,57,58,59,61,64,68)
12a	CL-145	VL(3,4,5,7,8,9,10,11,12,24,26,27,98,99,100) / CL(143,145,149,151,152,190,191,195,197,199,202,203,204,206,208,210)
12b	CH1-161	CL(138) / VH(1,3,5,7,23,24,25,26,75,76,77,105) / CH1(138,139,157,160,161,162,165,166,187,188,189,191,192,195,196,205,210)
13a	CH1-188	VH(42,84,85,87,110,111,112) / CH1(132,135,136,137,138,139,150,152,165,171,172,173,174,175,176,180,187,188,189,190,191,192,193,194,195,196)
13b	VH-82a	VH(15,16,17,65,66,68,81,82a,82b,82c)
14a	CH1-210	CL(122,123,126,127) / CH1(119,120,121,122,207,208,209,210,211,212,213,214)
14b	VL-74	VL(17,18,20,52,63,64,65,67,74,76)
14c	CL-205	CL(110,199,202,203,204,205,206,207,208) / CH1(137,138)

Supplementary Table S2 (Part 1).

Rank	Interface	Positions (VL / CL / VH / CH1)
15a	CL-110	VL(107) / CL(108,109,110,112,140,199,200,201,202,203)
15b	CH1-206	VH(3,11,12,13,16,24,25,74,75,76,112,113) / CH1(118,119,120,205,206,207,208,209,210)
15c	CL-109	CL(108,109,110)
15d	CH1-197	CL(157,159,160) / VH(84,85) / CH1(160,161,174,175,176,194,195,197)
16a	VL-108	VL(14,15,16,17,107) / CL(108,109,169,170,172)
16b	VH-54	VH(52b,54)
16c	VL-7	VL(5,6,7,8,9,10,22,24,67,70) / CL(143)
16d	CH1-139	CL(108,109,114,138,169,170,171,172) / CH1(137,138,139,191,192,193,195)
16e	CL-169	VL(15,61,79,80,81) / CL(108,109,168,169,170,171) / VH(1,3,4,5,105) / CH1(137,155,157,160,161,162,189,190,191)
16f	CL-151	CL(149,151,152,188,189,190,191)
16g	CH1-121	VH(13) / CH1(119,120,121,122,123,124,205,206,207,208,209,210,211,212)
16h	CH1-194	CH1(194,195,197,217,218,219)
17a	VL-162	VL(60,61) / CH1(161,162,163,164,165)
17b	CH1-165	CL(108,170) / VH(1,3,5,7,21,23,24,25,75,76,105) / CH1(138,139,155,157,160,161,162,165,189,191,192,203,205,210,212)
18a	VL-61	VL(15,18,52,54,55,56,57,58,59,60,61,62,63,64,65,74,76,77,79)
18b	VH-8	VH(7,8,9,10,13,16,17,19,21,30,52a,54,71,72,73,75,77,79,81,82a,107,108) / CH1(205,206,208)
19a	CL-170	VL(15,16,56,57,58,59,60,80) / CL(108,109,168,169,170) / CH1(138,189,191)
19b	VL-25	VL(1,3,25,26,27,67,68,69,70) / CL(144,145,159,160,161,163) / CH1(173,174,175,176)
20a	VH-83	CL(154,155,156,157,158,160,181,188) / VH(14,15,61,62,64,65,66,68,82b,82c,83,113) / CH1(174,175,176,177)
20b	VH-25	VH(1,25,26,27,28,29,73,74,76)

Supplementary Table S2 (Part 2). Residue positions of the 42 most prevalent interfaces. Positions are numbered according to Kabat for the VL/VH and EU for the CL/CH1.

Rank	Interface	Variant	% Monomer	% Dimer	% Higher Order
1	CH1-211	CH1(N208C) / CH1(K214C)	74	24	
1	CH1-211	CH1(K210C) / CH1(D212C)	59	39	
2	CH1-207	VH(L11C) / CH1(N208C)	84	16	
2	CH1-207	CH1(S119C) / CH1(G122C)	53	47	
2	CH1-207	CH1(S119C) / CH1(S207C)	96	4	
2	CH1-207	CH1(S119C) / CH1(T209C)	70	29	
2	CH1-207	CH1(T120C)	99	1	
2	CH1-207	CH1(T120C) / CH1(S207C)	98	2	
3	CH1-209	CH1(N208C) / CH1(D212C)	60	39	
3	CH1-209	CH1(K210C)	99		
4	CL-211	CH1(S119C) / CH1(S190C)	80	19	
8	VH-16	VH(P14C) / CH1(L174C)	90	10	
8	VH-16	VH(S113C) / CH1(G178C)	62	25	13
8	VH-16	VH(Q13C) / CH1(S176C)	83	16	
10	VL-59	VH(E1C)	95		4
11a	VH-82b	VH(P14C) / VH(K64C)	50	47	3
11a	VH-82b	VH(P14C) / VH(G65C)	39	60	
11a	VH-82b	VH(D61C) / VH(A84C)	70	30	
11a	VH-82b	VH(N82aC) / VH(S82bC)	67	31	
11a	VH-82b	VH(S82bC)	67	28	
11a	VH-82b	VH(G65C) / VH(R83C)	49	50	
11a	VH-82b	VH(G15C) / VH(G65C)	9	84	
11c	VH-57	VH(T57C)	99		
11c	VH-57	VH(N54C) / VH(K64C)	28	32	38
12b	CH1-161	VH(V5C) / CH1(S192C)	68	22	9
12b	CH1-161	VH(S7C) / CH1(T195C)	79	19	
12b	CH1-161	VH(A23C) / CH1(P189C)	90	10	
12b	CH1-161	VH(A23C) / CH1(S191C)	95	5	
12b	CH1-161	VH(A23C) / CH1(S192C)	66	32	
12b	CH1-161	VH(S25C) / CH1(T139C)	89	11	
12b	CH1-161	VH(S25C) / CH1(P189C)	88	11	
12b	CH1-161	VH(S25C) / CH1(G138C)	80	10	9
12b	CH1-161	VH(K75C) / CH1(S191C)	91	9	
12b	CH1-161	CH1(T155C) / CH1(A162C)	90	10	
12b	CH1-161	CH1(S157C) / CH1(G161C)	88	12	
12b	CH1-161	CH1(S160C) / CH1(N201C)	73	23	4
12b	CH1-161	VH(T77C) / CH1(S191C)	97	3	
12b	CH1-161	CH1(G161C)	93	7	

Supplementary Table S3 (Part 1).

Rank	Interface	Variant	% Monomer	% Dimer	% Higher Order
13a	CH1-188	VH(P41C) / CH1(S190C)	38	45	17
13a	CH1-188	VH(P41C) / CH1(S191C)	41	56	3
13a	CH1-188	VH(G42C) / CH1(L193C)	78		21
13a	CH1-188	VH(T110C) / CH1(G138C)	89		11
13a	CH1-188	VH(S112C) / CH1(G137C)	82	9	8
13a	CH1-188	CH1(L174C) / CH1(P189C)	95	5	
13a	CH1-188	CH1(S165C) / CH1(S176C)	45	55	
13b	VH-82a	VH(G15C)	86	14	
13b	VH-82a	VH(S17C) / VH(S82bC)	90	10	
13b	VH-82a	VH(R66C) / VH(N82aC)	85	14	
15d	CH1-197	VH(E85C) / CH1(G161C)	99		
16d	CH1-139	CH1(G138C) / CH1(T139C)	93	7	
16d	CH1-139	CH1(T139C)	94	6	
16e	CL-169	VH(Q3C) / CH1(S191C)	90	10	
16e	CL-169	CH1(S160C)	96	4	
16g	CH1-121	CH1(T120C) / CH1(G122C)	80	19	
16g	CH1-121	CH1(K121C)	99		
16h	CH1-194	CH1(G194C)	95	5	
16h	CH1-194	CH1(G194C) / CH1(T195C)	92	8	
17a	CH1-162	VH(G99C) / CH1(T195C)	65	32	
17b	CH1-165	VH(A23C) / CH1(G138C)	84	11	4
17b	CH1-165	VH(N76C) / CH1(G138C)	87	12	
17b	CH1-165	VH(Q105C) / CH1(S165C)	99	1	
17b	CH1-165	CH1(S160C) / CH1(N203C)	92	7	
17b	CH1-165	VH(S7C) / CH1(P189C)	93	7	
17b	CH1-165	CH1(A162C) / CH1(N203C)	98	2	
18b	VH-8	VH(G9C) / VH(Y79C)	91	9	
18b	VH-8	VH(G10C) / VH(D72C)	93	7	
18b	VH-8	VH(S17C)	82	18	
18b	VH-8	VH(R19C)	98	2	
18b	VH-8	VH(T73C) / CH1(K205C)	97	3	
20a	VH-83	VH(G15C) / CH1(S176C)	16	51	29
20a	VH-83	VH(G15C) / CH1(S177C)	27	67	6
20a	VH-83	VH(S82bC) / CH1(S176C)	34	45	18
20a	VH-83	VH(R83C) / CH1(S176C)	89	11	
20a	VH-83	VH(S113C)	67	29	4
20b	VH-25	VH(S25C)	97	2	
20b	VH-25	VH(S25C) / VH(N76C)	84	15	
20b	VH-25	VH(G26C) / VH(N76C)	96	4	
WT	WT	WT	97	3	
WT	WT	WT	99	1	

Supplementary Table S3 (Part 2). Cysteine variants at heavy chain / heavy chain (HCHC) interfaces. Data represent normalized integrated peaks of monomer, dimer, and higher order (> dimer) species from analytical SEC. Due to the nature of the peak integration the results do not necessarily add to precisely 100%. Variants are labeled according to Kabat for the VH and EU for the CH1.

Rank	Interface	Variant	% Monomer	% Dimer	% Higher Order
4	CL-211	CL(T109C) / CL(K126C)	90	3	6
5	VL-11	VL(S7C) / VL(D17C)	67	24	10
5	VL-11	VL(S7C) / VL(R18C)	66	26	
5	VL-11	VL(P8C) / VL(L11C)	89	11	
5	VL-11	VL(P8C) / VL(A13C)	82	11	
5	VL-11	VL(S9C) / VL(K107C)	83	7	8
5	VL-11	VL(S10C) / VL(S12C)	76	22	12
5	VL-11	VL(L11C)	87	12	
7	VL-107	VL(P8C) / CL(S156C)	58	34	5
7	VL-107	VL(P8C) / CL(G157C)	84	8	8
9c	CL-200	CL(V110C) / CL(S202C)	75	19	4
11b	VL-13	VL(R18C)	87	11	
12a	CL-145	VL(Q3C) / CL(K190C)	84	7	8
12a	CL-145	VL(Q3C) / CL(V191C)	89	10	
12a	CL-145	VL(S7C) / CL(T206C)	72	21	4
12a	CL-145	VL(P8C) / CL(S203C)	85	3	11
12a	CL-145	VL(S26C) / CL(K190C)	83	8	7
12a	CL-145	VL(Q100C) / CL(N152C)	88	2	8
12a	CL-145	CL(Q199C)	88	11	
12a	CL-145	VL(S9C) / CL(P204C)	88	11	
14a	CH1-210	CL(S127C)	88	11	
14a	CH1-210	CL(G128C)	88	11	
14b	VL-74	VL(R18C) / VL(S63C)	84	10	
14b	VL-74	VL(R18C) / VL(S65C)	80	18	
14c	CL-205	CL(S202C) / CL(S208C)	62	28	4
14c	CL-205	CL(P204C) / CL(T206C)	86	4	9
15a	CL-110	CL(T109C) / CL(A112C)	57	31	6
15a	CL-110	CL(T109C) / CL(G200C)	83	11	5
15a	CL-110	CL(T109C) / CL(L201C)	78	12	7
15a	CL-110	CL(V110C) / CL(G200C)	87	12	

Supplementary Table S4 (Part 1).

Rank	Interface	Variant	% Monomer	% Dimer	% Higher Order
15c	CL-109	CL(T109C)	89	11	
16a	VL-108	VL(G16C) / CL(K169C)	54	38	7
16a	VL-108	VL(G16C) / CL(D170C)	45	50	5
16c	VL-7	VL(S7C)	78	18	3
16c	VL-7	VL(S7C) / VL(R24C)	79	17	
16c	VL-7	VL(P8C) / VL(D70C)	89	10	
16c	VL-7	VL(S67C) / CL(E143C)	85	14	
16c	VL-7	VL(G68C) / CL(E143C)	75	18	5
16e	CL-169	VL(P80C) / CL(D170C)	89	10	
16e	CL-169	CL(S168C) / CL(K169C)	92	8	
17b	CH1-165	CL(D151C)	54	41	5
17b	CH1-165	CL(D151C) / CL(K190C)	78	16	4
17b	CH1-165	CL(D151C) / CL(V191C)	81	17	
17b	CH1-165	CL(N152C)	88	10	
18a	VL-61	VL(R18C) / VL(S52C)	81	17	
18a	VL-61	VL(S60C)	79	15	5
18a	VL-61	VL(S60C) / VL(R61C)	89	11	
18a	VL-61	VL(S63C) / VL(S76C)	87	12	
19a	CL-170	VL(P80C) / CL(K169C)	90	10	
19a	CL-170	VL(G16C) / CL(T109C)	52	37	7
19b	VL-25	VL(Q3C) / CL(S156C)	66	22	10
19b	VL-25	VL(T5C) / CL(G157C)	73	5	21
19b	VL-25	VL(S26C) / CL(S159C)	88	11	
19b	VL-25	VL(Q27C) / CL(E161C)	88	12	
WT	WT	WT	99		
WT	WT	WT	99		

Supplementary Table S4 (Part 2). Cysteine variants at light chain / light chain (LCLC) interfaces. Data represent normalized integrated peaks of monomer, dimer, and higher order (> dimer) species from analytical SEC. Due to the nature of the peak integration the results do not necessarily add to precisely 100%. Variants are labeled according to Kabat for the VL and EU for the CL.

Rank	Interface	Variant	% Monomer	% Dimer	% Higher Order
3	CH1-209	CH1(S119C) / CL(K126C)	62	36	
4	CL-211	CH1(K213C) / CL(S203C)	89	7	
4	CL-211	CH1(D212C) / CL(P204C)	97		
4	CL-211	CH1(K210C) / CL(T206C)	96		
4	CL-211	CH1(N208C) / CL(S208C)	85	14	
6	CH1-212	CH1(K210C) / CL(A153C)	86	11	
6	CH1-212	CH1(D212C) / CL(A153C)	87	12	
6	CH1-212	CH1(K213C) / CL(S156C)	66	29	
6	CH1-212	CH1(K214C) / CL(N158C)	91	5	
6	CH1-212	CH1(S160C) / CL(K188C)	90	9	
6	CH1-212	CH1(T197C) / CL(S182C)	94	4	2
6	CH1-212	CH1(K214C) / CL(G157C)	90	4	5
7	VL-107	CH1(L174C) / VL(S14C)	94		
7	VL-107	CH1(L174C) / VL(K107C)	95		
7	VL-107	CH1(A118C) / CL(V110C)	92		
7	VL-107	CH1(G178C) / CL(V110C)	95		
7	VL-107	VH(S113C) / CL(A112C)	67	29	
7	VL-107	CH1(A118C) / CL(A112C)	92		
7	VL-107	CH1(G178C) / CL(P141C)	n.a.		
7	VL-107	CH1(S119C) / CL(S202C)	72	19	
7	VL-107	CH1(A118C) / CL(G200C)	92		
9a	CL-187	CH1(A162C) / CL(S182C)	91		
9b	CH1-176	VH(P14C) / CL(D151C)	65	33	
9b	CH1-176	VH(R83C) / CL(A153C)	85	7	7
9b	CH1-176	VH(R83C) / CL(N152C)	87	6	6
9b	CH1-176	VH(A84C) / CL(A153C)	89	4	6

Supplementary Table S5 (Part 1).

Rank	Interface	Variant	% Monomer	% Dimer	% Higher Order
13a	CH1-188	CH1(T164C) / CL(G157C)	74	11	14
13a	CH1-188	CH1(T195C) / CL(E165C)	91		
14c	CL-205	CH1(T135C) / CL(V110C)	94		
15d	CH1-197	CH1(L193C) / CL(G157C)	92		
15d	CH1-197	CH1(G194C) / CL(G157C)	88	5	6
16d	CH1-139	CH1(G138C) / CL(S114C)	89	11	
16d	CH1-139	CH1(S191C) / CL(A111C)	94		
16d	CH1-139	CH1(S191C) / CL(N138C)	95		
16d	CH1-139	CH1(S191C) / CL(D170C)	61	39	
16d	CH1-139	CH1(S192C) / CL(D170C)	61	36	
16d	CH1-139	CH1(G194C) / CL(T109C)	95		
16d	CH1-139	CH1(T195C) / CL(D170C)	76	21	
16d	CH1-139	CH1(G137C) / CL(S114C)	97		
16d	CH1-139	CH1(S191C) / CL(T172C)	97		
17a	CH1-162	CH1(A162C) / VL(P59C)	92		
17b	CH1-165	VH(E1C) / CL(A111C)	87	5	7
19a	CL-170	CH1(G138C) / VL(P59C)	96		
19b	VL-25	CH1(V173C) / VL(D28C)	95		
19b	VL-25	CH1(L174C) / VL(G68C)	92		
19b	VL-25	CH1(Q175C) / VL(T69C)	97		
20a	VH-83	VH(G65C) / CL(Q155C)	96		
20a	VH-83	VH(G65C) / CL(N158C)	90	9	
20a	VH-83	VH(R66C) / CL(G157C)	91		
WT	WT	WT	99		

Supplementary Table S5 (Part 2). Cysteine variants at heavy chain / light chain (HCLC) interfaces. Data represent normalized integrated peaks of monomer, dimer, and higher order (> dimer) species from analytical SEC. Due to the nature of the peak integration the results do not necessarily add to precisely 100%. Variants are labeled according to Kabat for the VH/VL and EU for the CH1/CL.

Rank	Interface	HC Variant	LC Variant	Expression (%)			In vitro oxidation (%)		
				Mon	Dim	HO	Mon	Dim	HO
2	CH1-207	CH1(S119C) / CH1(G122C)		53	47	0	65	35	0
11a	VH-82b	VH(G15C) / VH(G65C)		9	84	0	34	50	17
12b	CH1-161	VH(A23C) / CH1(S192C)		66	32	0	70	29	1
20a	VH-83	VH(G15C) / CH1(S176C)		16	51	29	35	63	2
5	VL-11		VL(S7C) / VL(D17C)	67	24	8	59	41	0
14c	CL-205		CL(S202C) / CL(S208C)	62	28	4	45	52	4
15a	CL-110		CL(T109C) / CL(A112C)	57	31	6	52	48	0
16a	VL-108		VL(G16C) / CL(D170C)	45	50	5	52	48	0
19a	CL-170		VL(G16C) / CL(T109C)	52	38	10	43	54	3
9b	CH1-176	VH(P14C)	CL(D151C)	65	33	1	52	37	11
16d	CH1-139	CH1(S191C)	CL(D170C)	61	39	0	24	68	8
	MM1	VH(P14C)	CL(D170C)	90	9	1	51	32	17
	MM2	CH1(S191C)	CL(D151C)	14	71	15	8	26	66
	MM3	CH1(S119C)	CL(D170C)	64	29	7	44	32	24
	MM4	CH1(S191C)	CL(K126C)	64	29	7	30	34	37

Supplementary Table S6. In vitro assembly of select cysteine variants. Data represent normalized integrated peaks of monomer (Mon), dimer (Dim), and higher order (HO, > dimer) species from both expression and in vitro oxidation. Due to the nature of the peak integration the results do not necessarily add to precisely 100%. Control mismatched (MM) variants represent in vitro assembled heavy and light chains cysteine variants that were not designed based on the discovered interfaces. Variants are labeled according to Kabat for the VH/VL and EU for the CH1/CL.

	CH1-207 (7T97)	VL-108 (7T98)	CL-205 (7T99)
Wavelength	0.9795	0.9999	0.9792
Resolution range	75.69 - 2.144 (2.297 - 2.144)	67.01 - 2.906 (3.01 - 2.906)	61.35 - 2.651 (2.746 - 2651)
Space group	C 1 2 1	P 31 2 1	P 1
Unit cell	151.377 141.075 118.605 90 90.323 90	99.634 99.634 212.675 90 90 120	52.824 62.451 124.018 89.992 98.374 89.893
Anisotropic Data Analysis	STARANISO	STARANISO	N/A
Diffraction limits	2.998 2.146 2.144	3.548 3.548 2.817	-
Eigenvector-1	0.999 0.000 -0.040	1 0 0	-
Eigenvector-2	0.000 1.000 0.000	0 1 0	-
Eigenvector-3	0.040 0.000 0.999	0 0 1	-
Direction-1	0.999 _a_*-0.036 _c_*	0.894 _a_*-0.447 _b_*	-
Direction-2	_b_*	_b_*	-
Direction-3	0.052 _a_*+0.999 _c_*	_c_*	-
Total reflections	313557 (16196)	177656 (8545)	69121 (7639)
Unique reflections	90662 (4534)	18037 (903)	37752 (4287)
Multiplicity	3.5 (3.6)	9.8 (9.5)	1.8 (31.9.5)
Completeness (spherical, %)	66.59 (17.9)	69.3 (16.0)	95.55 (9424)
Completeness (ellipsoidal, %)	92.5 (64.8)	93.2 (69.5)	-
Mean I/sigma(I)	7.8 (1.5)	7.9 (1.6)	15.45 (4.68)
Wilson B-factor	32.54	65.36	62.95
R-merge	0.126 (0.921)	0.319 (1.46)	0.02947 (0.1667)
R-meas	0.149 (1.085)	0.337 (1.544)	0.04165 (0.2357)
R-pim	0.080 (0.570)	0.107 (0.498)	0.02942 (0.1665)
CC1/2	0.995 (0.502)	0.992 (0.609)	0.999 (0.974)
CC*	0.998 (0.614)	0.997 (0.647)	1 (0.993)
Reflections used in refinement	90617 (1731)	18568 (166)	43501 (4285)
Reflections used for R-free	4655 (110)	923 (6)	1668 (164)
R-work	0.2057 (0.2914)	0.1955 (0.1878)	0.2114 (0.2247)
R-free	0.2370 (0.3377)	0.2463 (0.1192)	0.2797 (0.3476)
CC(work)	0.932 (0.652)	0.840 (0.613)	0.924 (0.658)
CC(free)	0.902 (0.541)	0.781 (0.888)	0.937 (0.639)
Number of non-hydrogen atoms	13931	66334	13261
macromolecules	13191	6590	13180
ligands	0	0	10
solvent	740	44	71
Protein residues	1729	866	1728
RMS(bonds)	0.011	0.017	0.015
RMS(angles)	1.63	2.25	2.14
Ramachandran favored (%)	97.14	88.46	89.37
Ramachandran allowed (%)	2.69	9.32	8.29
Ramachandran outliers (%)	0.18	2.21	2.34
Rotamer outliers (%)	3.15	18.87	12.84
Clashscore	3.42	14.9	10.48
Average B-factor	35.98	84.38	69.03
macromolecules	35.77	84.8	69.17
solvent	39.81	22.41	45.74
TLS groups	None	4	8

Supplementary Table S7. Crystallographic data collection and refinement statistics. Statistics for the highest resolution shell are shown in parentheses. The first two column datasets were treated with anisotropic data inclusion criteria, for which parameters of the ellipsoid are described.