Supplementary Information for:

Redesigning an antibody H3 loop by virtual screening of a small library of human germline-derived sequences

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Supplementary Tables and Figures

Table S1. Backbone RMSD vs Stem Residue Position for 248 Antibody:Antigen complexes. The results have been broken down by ranges of loop length. 7 to 24 corresponds to all loops in the dataset. Loop length 7 is separated out as there are only two distinct loop conformations according to North *et al.*[1] and therefore no loop search is needed. 8 to 11 corresponds to loops on the shorter side of the germline library (25%). 12 to 13 correspond to medium size loops in the germline library (27%) where the loop tip to be searched is 7 to 8 residues long and overall good prediction would be expected with published loop methods. 14 to 16 corresponds to the longest loop in the germline library (46%) and corresponds to loop tips of 9-11 residues which may pose some difficulty for most loop prediction methods. Cells in orange correspond to RMSD values greater than 0.75Å.

Loop Length		BMRSD Å (N, CA, C, O)						
min	max	H93	H93 + 1	H93 + 2	H102 - 3	H102 -2	H102 - 1	H102
7	24	0.30	0.48	1.40	1.06	0.63	0.56	0.41
7	7	0.28	0.42	0.40	0.48	0.37	0.44	0.40
8	11	0.33	0.49	1.45	1.25	0.73	0.58	0.43
12	13	0.29	0.51	1.45	0.91	0.56	0.52	0.40
14	16	0.29	0.38	1.37	0.82	0.56	0.62	0.41

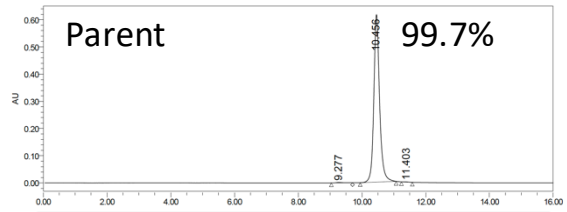
Table S2. Number of CDR-H3 loop sequence with Z-Scores lower than –1.5.

	N (Z-Score <= –1.5)	
H3 Length	By H3 Length[a]	Complete[b]
7	2	0
8	3	0
9	5	0
10	6	0
11	3	0
12	3	0
13	13	7
14	22	33
15	20	36
16	11	37

[a] The Z-Score is calculated using only sequences of a given H3 loop length.
[b] The Z-Score is calculated using sequences of all H3 loop lengths.

Table S3. Comparison of CDR loop prediction accuracy between using the parent 3BDY crystal structure versus using the cognate crystal structure for the surrounding residues.

Design	3BDY as Starting Template			Crystal Structure as Template		
	Top 1	**5 kcal/mol**	**Closest**	**Top 1**	**5 kcal/mol**	**Closest**
Parent	1.47	1.22	1.22	1.47	1.22	1.22
16_0325	3.21	2.74	2.55	3.69	2.80	2.12
14_0112	3.12	2.76	1.44	0.92	0.59	0.59
14_0472	5.95	5.93	5.47	1.43	1.35	1.35
13_0346	2.58	2.25	2.24	2.65	1.97	1.97
Avg of Designs	*3.71*	*3.42*	*2.93*	*2.17*	*1.68*	*1.51*
Avg for 14-16 CDR-H3 Length				2.26	1.66	1.56
BRMSD						
Design	**3BDY as Starting Template**			**Crystal Structure as Template**		
	Top 1	**5 kcal/mol**	**Closest**	**Top 1**	**5 kcal/mol**	**Closest**
Parent	0.83	0.61	0.61	0.83	0.61	0.61
16_0325	3.04	2.32	2.12	3.36	2.67	2.03
14_0112	2.61	2.49	1.01	0.70	0.46	0.46
14_0472	3.43	3.40	3.34	0.59	0.48	0.48
13_0346	*2.13*	1.80	1.78	2.24	1.52	1.52
Avg of Designs	*2.80*	*2.50*	*2.06*	*1.72*	*1.28*	*1.12*
Avg for 14-16 CDR-H3 Length				1.48	1.02	0.92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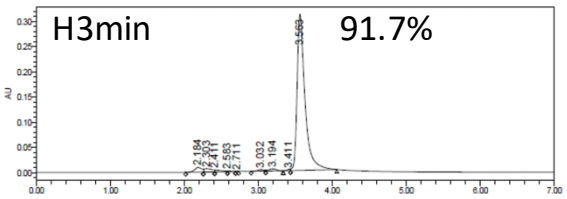
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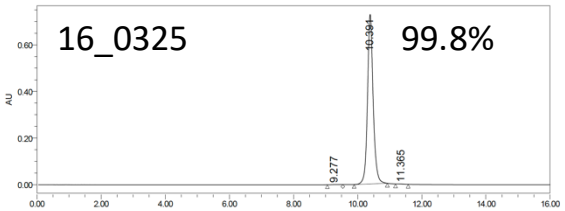
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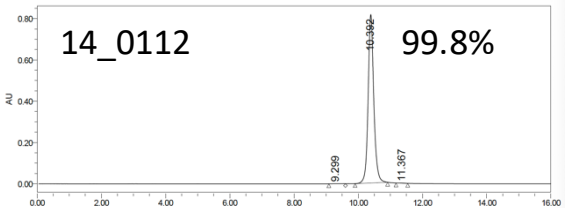
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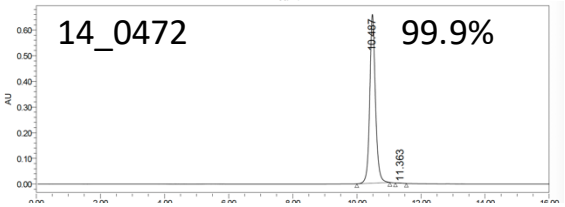
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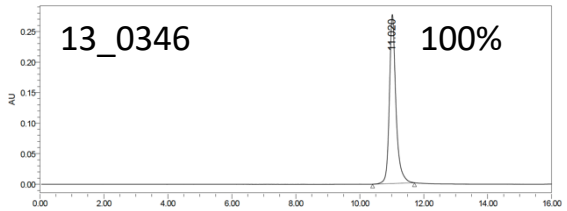
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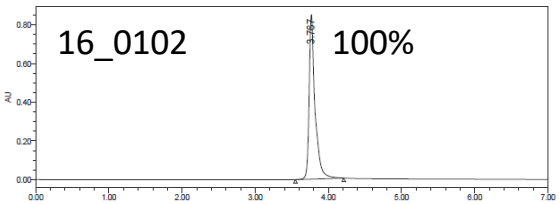
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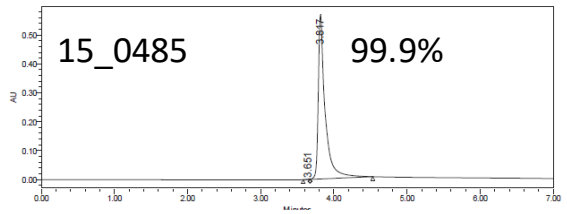
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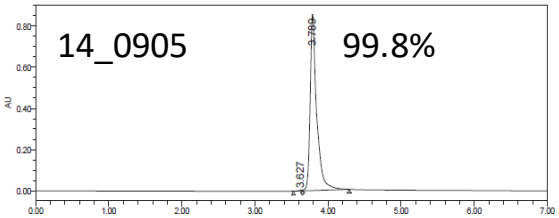
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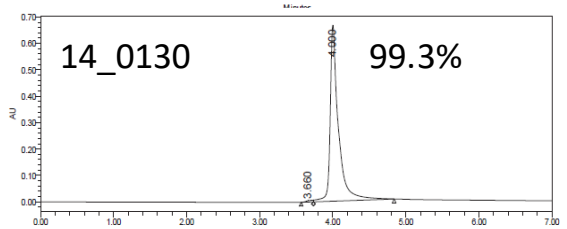
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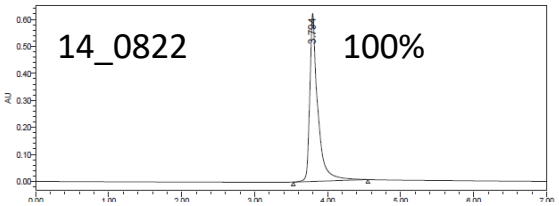
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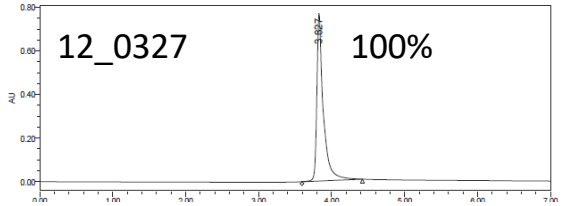
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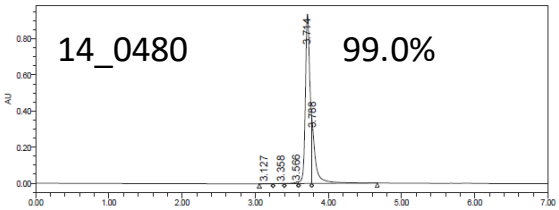
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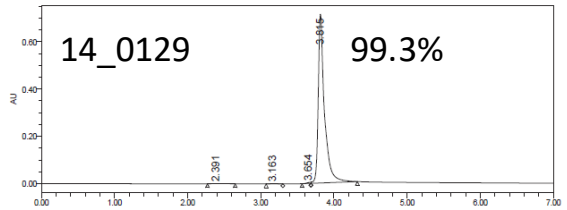
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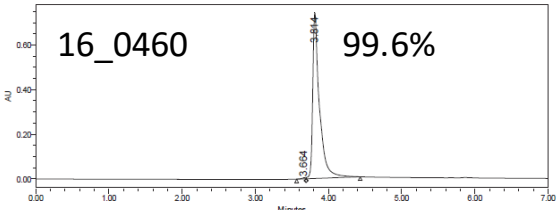
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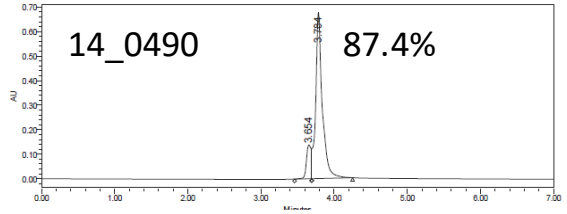
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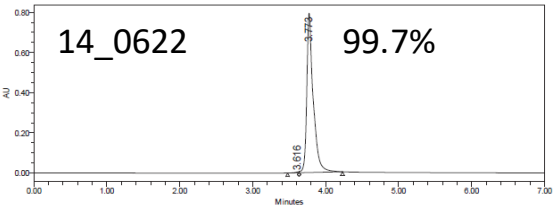
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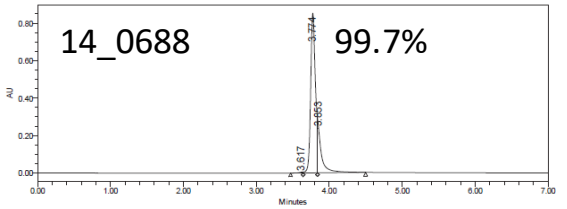
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Figure S1. UPLC-SEC chromatograms of the parental and H3-redesigned Fab variants after IMAC purification (see Materials and Methods section). Main peak contribution is indicated.

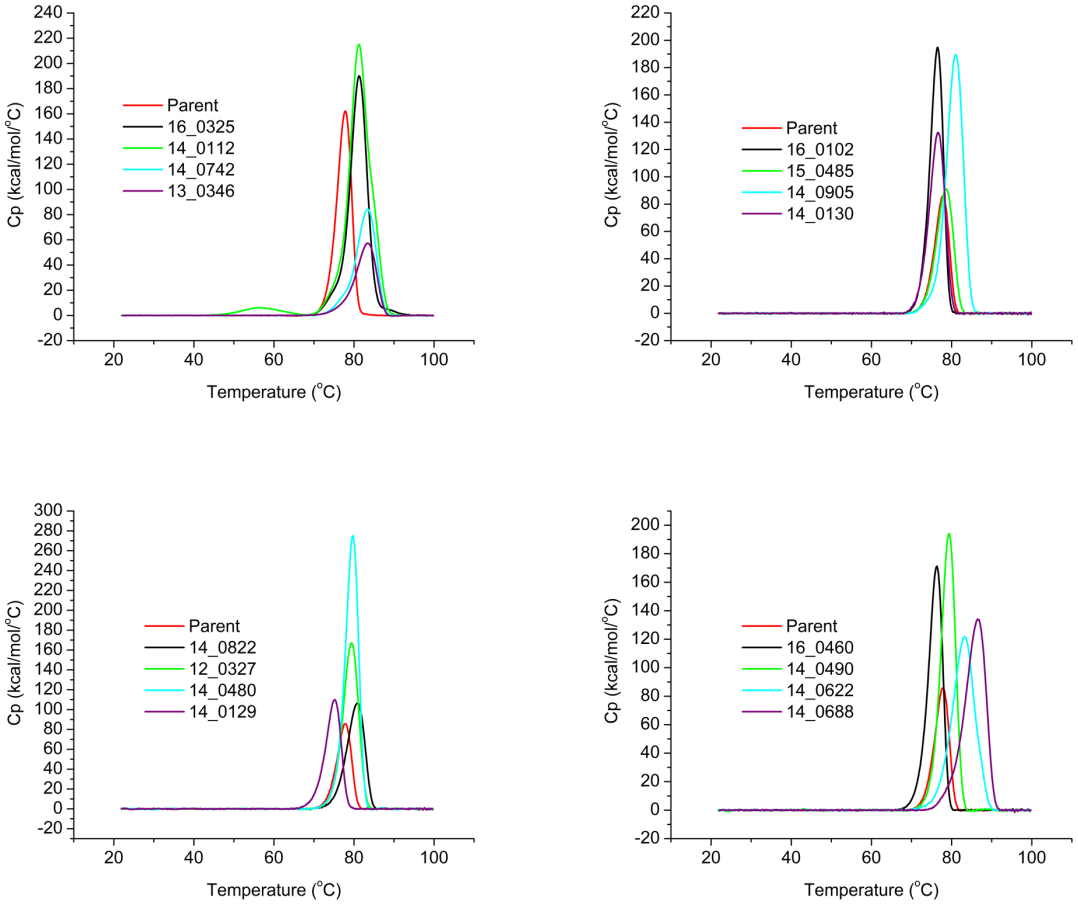

Figure S2. DSC thermograms of the variants relative to the parental Fab. See the methods for experimental details.

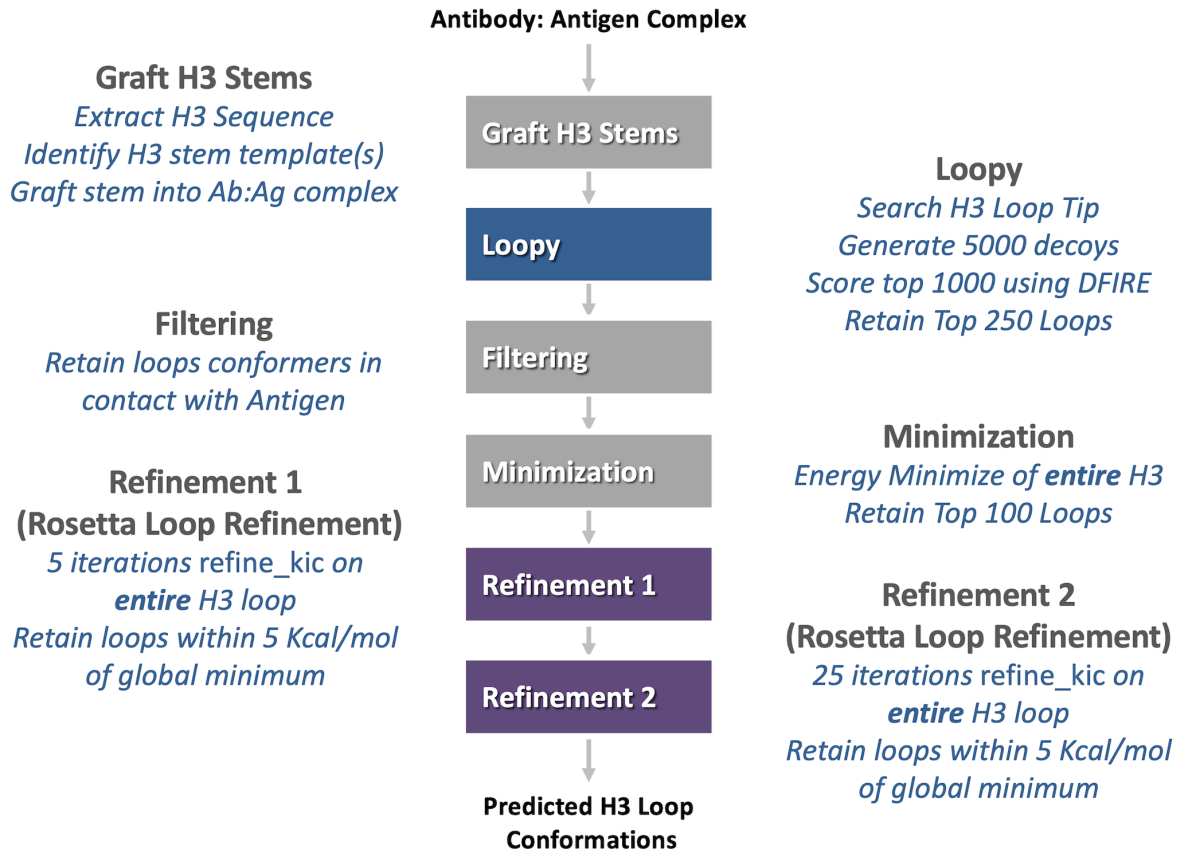

Figure S3. Conformational sampling of grafted H3 loops. With the stem regions grafted into the parent antibody-antigen structure, a hierarchal approach to conformational sampling of the inner tip of the loop was created. If the H3 sequence fits into multiple stem templates, both stems are included in the search protocol. Initially, Loopy is used to quickly generate inner-tip conformations, which are then rescored using DFIRE and filtered to identify loop conformation which are in contact with the antigen. These loops are then energy-minimized with AMBER and go through a two-stage refinement using Rosetta KIC method to generate a dense ensemble around the best-scoring conformation for further affinity scoring.

References

1. North, B.; Lehmann, A.; Dunbrack, R. L., A New Clustering of Antibody CDR Loop Conformations. *J. Mol. Biol.* **2011,** *406* (2), 228-256.