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Supplementary Methods

Computational design information: Rosetta command lines and input files

Fold From Loops

fold_from_loops.linuxiccrelease -database minirosetta_database -s target_topology.pdb -loops:loop_file input.loop -loops:frag_sizes 9 3 1 -in:file:frag9 aa*****09_05.200_v1_3 -in:file:frag3 aa*****03_05.200_v1_3 -out:nstruct int_number_of_designs -out:file:silent -fold_from_loops:swap_loops mota_loop.pdb -fold_from_loops:add_relax_cycles 2 -abinitio:increase_cycles 10 -mute all -in:file:psipred_ss2 *****.psipred_ss2 -fold_from_loops:ca_rmsd_cutoff 5 -out:file:silent_struct_type binary -fold_from_loops:native_ca_cst -fold_from_loops:ca_csts_dev 3.0 -fold_from_loops:res_design_bs 67 70 74 80 84 -fold_from_loops:loop_mov_nterm 2 -fold_from_loops:loop_mov_cterm 2

Loop File:

#LOOP start end cutpoint skip-rate extend

LOOP 65 86 0 0.0 X

Fixed-backbone design

fixbb.linuxiccrelease -database minirosetta_database -s input_structure -nstruct 50 -resfile resfile -ex1 -ex2 -packing:extrachi_cutoff 0 -pdb_gz -linmem_ig 10

Resfile (used for resurfacing):

NATRO
start
1 A PIKAA G
2 A PIKAA S
4 A PIKAA S
5 A PIKAA D
7 A POLAR
8 A PIKAA K
9 A PIKAA D
11 A PIKAA E
13 A PIKAA R
15 A PIKAA D
16 A PIKAA K

19 A PIKAA E
20 A PIKAA A
22 A PIKAA K
23 A PIKAA N
24 A PIKAA K
26 A PIKAA D
27 A PIKAA K
29 A PIKAA K
30 A PIKAA A
31 A PIKAA A
33 A PIKAA R
34 A PIKAA K
39 A PIKAA E
40 A PIKAA E
41 A PIKAA R
43 A PIKAA K
44 A PIKAA D
47 A PIKAA K
50 A PIKAA R
52 A PIKAA E
54 A PIKAA E
55 A PIKAA Q
57 A PIKAA R
59 A PIKAA A
61 A PIKAA R
62 A PIKAA N
70 A PIKAA K
87 A POLAR
90 A PIKAA K
92 A POLAR
93 A PIKAA A
97 A PIKAA K
98 A PIKAA K
100 A PIKAA E
101 A PIKAA A
104 A PIKAA A
105 A PIKAA D
107 A PIKAA E
106 A PIKAA A
111 A PIKAA T
112 A PIKAA Q

Full-atom relaxation

relax.linuxiccrelease -database minirosetta_database -s input.pdb -in:file:fullatom -in:ignore_unrecognized_res -out:nstruct 5 -out:file:silent silent_output -in:file:native input.pdb -out:file:silent_struct_type binary -out:file:fullatom -mute all -ex1 -ex2 -packing:extrachi_cutoff 0

Experimental Methods

Crystallography

FFL_005

Crystals of FFL_005 purified by size exclusion chromatography were grown by the hanging-drop vapor-diffusion method at 4°C. The protein solution, at a concentration of 7 mg/ml, was mixed 1:1 with a reservoir solution of 0.2 M ammonium citrate tribasic (pH = 6.5) and 22% w/w PEG 3350. Diffraction-quality crystals grew in approximately one week and were cryopreserved in mother liquor consisting of reservoir solution plus 15% v/v glycerol. Diffraction data were collected on beamline 5.0.1 at the Advanced Light Source (Lawrence Berkeley National Laboratory, Berkeley, CA) and were reduced with HKL-2000 [1]. Initial phase information was determined by molecular replacement, using the FFL_005 computational model structure with residues 34-39 and 78-89 deleted as the search model, with the program PHASER [2] as implemented in the CCP4i program suite [3]. Initial solutions were refined as rigid bodies with REFMAC [4], yielding initial crystallographic R-values (R_{cryst} = 53.3%, R_{free} = 54.3%). Phases were improved by subsequent rounds of model building and refinement using COOT [5] and REFMAC [4]. Structure validation was performed through the MolProbity [6] (overall score: 100th percentile) and PDB ADIT servers [7]. Crystallographic statistics are reported in Supplementary Table 6.

FFL_001+Motavizumab complex

Fab/scaffold complexes were isolated by size exclusion chromatography in PBS, concentrated to 8.5 mg/ml and crystallized by multiple rounds of macro-seeding, with seeds isolated from crushed intergrown crystals grown by vapor diffusion at room temperature over well solutions of 100 mM Li_2SO_4, 100 mM phosphate-citrate (pH = 4.2), 2% v/v glycerol and 25% w/w PEG 1000. Diffraction-quality crystals were grown by vapor diffusion by seeding into 2 µl drops of 2.5 to 5 mg/ml protein solution mixed with 2 µl of a well solution consisting of 100 mM Li_2SO_4, 40 mM phosphate-citrate (pH = 4.2), 2% v/v glycerol and 18 to 23% w/w PEG 1000. Crystals were cryopreserved in 100 mM Li_2SO_4, 40 mM phosphate-citrate (pH = 4.2), 10% v/v glycerol and 30% w/w PEG 1000. Diffraction data were collected on beamline 5.0.1 at the Advanced Light Source (Lawrence Berkeley National Laboratory, Berkeley, CA) and were reduced with HKL-2000 [1]. Initial phase information was determined by molecular replacement, using the 3QWO.pdb [8] and 3LHP.pdb [9] structures as search models for the Fab and scaffold, respectively, with the program PHASER [2] as implemented in the CCP4i program suite [3]. Initial solutions were refined as rigid bodies with REFMAC [4], yielding reasonable initial

crystallographic R-values (R_{cryst} = 33.7%, R_{free} = 40.4%). Iterative rounds of alternating positional refinement and model building, using the programs REFMAC [4] and COOT [5], including placing ordered solvent molecules and the ordered portion of a single PEG molecule co-crystallized with the complex, were followed by a final round of TLS refinement[10]. Residues or side-chains that did not exhibit $2F_{obs}$-F_{calc} electron density when contoured at 0.7σ were removed or truncated to the Cβ atom. Structure validation was performed using PROCHECK [11] and MolProbity [6] (overall score: 99th percentile). Crystallographic statistics are reported in Supplementary Table 6.

FFL_001+31-HG7 complex

Fab/scaffold complexes were isolated by size exclusion chromatography in HEPES buffered saline, concentrated to 8.1 mg/ml and screened for crystallizability at room temperature by vapor diffusion in 0.2 μl sitting drops (1:1 protein solution:well solution). Initial crystals were observed in Qiagen's JCSG+ condition #43 (0.2 M Li_2SO_4, 0.1 M Tris (pH = 8.5), 40% w/w PEG 400). Optimization in microliter-scale hanging drops yielded 100 to 200 μm crystals which grew within three days over a well solution of 0.24 M Li_2SO_4, 0.1 M Tris (pH = 8.8), 37.5% w/w PEG 400. Diffraction data were collected on beamline 8.2.2 at the Advanced Light Source (Lawrence Berkeley National Laboratory, Berkeley, CA) and were reduced with d*TREK [12]; poor spot shape was accommodated by the use of a 30x30 μm collimator setting. Initial phase information was determined by molecular replacement with the program PHASER [2] as implemented in the CCP4i program suite [3], using the FFL_001 structure and a Motavizumab-aligned composite Fab model with scaffold epitope and Fab CDR residues excised as sequentially placed search models. The composite Fab model used the heavy chains of PDB entries 3Q6G and 3QZH for N and C terminal domains, and the light chains of entries 3EO9 and 3SOB; Fab subdomains were selected on the basis of interdomain sequence identity from high resolution PDB entries. Initial solutions were refined as rigid bodies with REFMAC5 [4], yielding reasonable initial crystallographic R-values for this resolution (R_{cryst} = 38.3%, R_{free} = 38.5%). Fourier syntheses calculated with the initial rigid-body refined phases showed continuous electron density features consistent with the CDR and epitope loops excluded from the search models. These loops were modeled by fitting substructures from locally homologous Fab structures and the epitope-bearing loop from the FFL_001 structure in the higher-resolution Motavizumab complex into these density features (Supplementary Fig. 24), allowing the overall docking of Fab onto scaffold to be determined. However, subsequent attempts to refine this model failed to resolve geometric and steric issues introduced at the interfaces between the MR model and placed loops. Applicable crystallographic statistics are reported in Supplementary Table 6.

FFL_001+17HD9 complex

Fab/scaffold complexes were isolated by size exclusion chromatography in HEPES buffered saline, concentrated to 9.5 mg/ml and screened for crystallizability at room temperature by vapor diffusion in 0.2 mL sitting drops (1:1 protein solution:well solution). Initial crystals were observed after approximately three months in the Qiagen PEGs II screen condition #54 (0.2M $(NH_4)_2SO_4$, 0.1M sodium acetate (pH = 5.6), and 12% w/w PEG-4000). Subsequent optimization yielded diffraction-quality crystals which grew in approximately 24 hours by vapor diffusion (2 uL total drop volume, 1 mL total well volume) with the concentrated stock used for the initial crystals mixed with an equal volume of 0.2M $(NH_4)_2SO_4$, 0.1M sodium acetate (pH = 5.6), and 14% w/w PEG-4000. Diffraction data were collected on beamline 5.0.2 at the Advanced Light Source (Lawrence Berkeley National Laboratory, Berkeley, CA) and were reduced with d*TREK[12]; subsequent molecular replacement was performed using the same model as for 31HG7, with the scaffold failing to generate a successful placement. Iterative rounds of alternating positional refinement and model building, using the programs REFMAC5[4] and COOT[5], were conducted using non-crystallographic symmetry restraints between the pairs of complexes related by near-perfect dyad axes; TLS refinement[10] and the placement of solvent molecules followed the placement of all protein residues. Structure validation was performed using MolProbity[6] (overall score: 96th percentile). Crystallographic statistics are reported in Supplementary Table 6.

Microneutralization assay

The RSV microneutralization assay was performed in 96-well microplates using HEp-2 cells and the RSV A2 strain. Cells were seeded at a concentration of $5x10^3$ cells/well in 100 μL/well of EMEM (Quality Biological, Inc., Gaithersburg, MD, USA) supplemented with 10% FBS, 1% Pen/Strep, 1% L-glutamine and incubated overnight at 37°C/5%CO_2. Sera derived from immunized animals were serially diluted in 1xPBS/5% FBS and mixed with equal volume of virus diluted in 1xPBS/5% FBS. The dilution amount of virus was selected to yield 80-120 syncytia/well in the absence of neutralizing serum. The virus/serum mix was incubated for 2 hours at 37°C/5%CO_2. Medium was removed from the seeded cells, 25 μL/well of the virus/serum mixture was added, in triplicate, to the cells and incubated for 2 hours at 37°C/5%CO_2. The virus/serum mix was removed from the seeded cells and 100 μL/well of

0.75% methylcellulose in EMEM supplemented with 10% FBS, 1% Pen/Strep, 1% L-glutamine was added. The plates were incubated for 48 hours (+/-8 hours) at 37°C/5%CO_2. To detect and enumerate infected cells, medium was removed, cells were fixed with 100 µL/well of 10% buffered formalin (Sigma-Aldrich, St. Louis, MO, USA) for 1.5 hours at room temperature and permeabilized with 100 µL/well of 1xPBS/2.5% FBS, 0.5% saponin, 0.1% sodium azide for 1.5 hours at room temperature. The infected cells were incubated with a mouse monoclonal antibody to RSV F (Abcam, Inc., Cambridge, MA, USA) for 1 hour at room temperature. Bound antibody was detected by incubating with peroxidase-labeled goat anti-mouse IgG (Vector, Burlingame, CA, USA) for 1 hour at room temperature followed by staining with True Blue Peroxidase Substrate (KPL, Gaithersburg, MD, USA). The next day, blue foci (represent infected cells) were counted using a light microscope (Fisher Scientific, Hampton, NH, USA). The number of infected cells representing 50% reduction in the virus count was calculated for each plate on the basis of the average infected cell count in the diluent plus virus control wells. Fifty percent neutralization titer was defined as the serum dilution factor yielding 50% reduction in the virus count and was calculated by linear regression interpolation between the 2 dilutions with wells yielding average infected cell counts above and below the number of infected cells presenting 50% reduction in the virus count. The microneutralization assay was also used to measure neutralization activities of NHP_D39 isolated monoclonal antibodies. The neutralizing activity of the antibodies was measured in pg/µL with titers being calculated by linear regression interpolation.

References

1 Otwinowski, Z. & Minor, W. in *Meth. Enzymol.* Vol. 276 eds C.W. Carter Jr. & R.M. Sweet) 307-326 (Academic Press, 1997).

2 McCoy, A. J. *et al.* Phaser crystallographic software. *Journal of Applied Crystallography* **40**, 658-674 (2007).

3 Potterton, E., Briggs, P., Turkenburg, M. & Dodson, E. A graphical user interface to the CCP4 program suite. *Acta Crystallogr D Biol Crystallogr* **59**, 1131-1137 (2003).

4 Murshudov, G. N., Vagin, A. A. & Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D Biol Crystallogr* **53**, 240-255 (1997).

5 Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* **60**, 2126-2132 (2004).

6 Davis, I. W. *et al.* MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res* **35**, W375-383 (2007).

7 Berman, H. M. *et al.* The Protein Data Bank. *Nucleic Acids Res* **28**, 235-242 (2000).

8 McLellan, J. S. *et al.* Design and characterization of epitope-scaffold immunogens that present the motavizumab epitope from respiratory syncytial virus. *J Mol Biol* **409**, 853-866 (2011).

9 Correia, B. E. *et al.* Computational design of epitope-scaffolds allows induction of antibodies specific for a poorly immunogenic HIV vaccine epitope. *Structure* **18**, 1116-1126 (2010).

10 Winn, M. D., Isupov, M. N. & Murshudov, G. N. Use of TLS parameters to model anisotropic displacements in macromolecular refinement. *Acta Crystallogr D Biol Crystallogr* **57**, 122-133 (2001).

11 Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography* **26**, 283-291 (1993).

12 Pflugrath, J. W. The finer things in X-ray diffraction data collection. *Acta Crystallogr D Biol Crystallogr* **55**, 1718-1725 (1999).

Supplementary Table 1. ELISA endpoint titers for NHP sera binding to whole RSV lysate (white columns) and recombinant RSV F protein (gray columns).

Group	Animal	W0		W2		W4		W8		W12		W16		W18		W20		W24		W28		W32	
FFL_001	C012	0	160	0	-	200	178	800	2.7E+4	1600	9.1E+4	3200	7.5E+4	1600	4.6E+4	3200	8.2E+4	800	-	800	-	400	2.5E+4
	D027	0	99	0	-	0	99	0	1476	200	1.1E+4	3200	8131	800	6057	200	1.7E+4	0	-	0	-	0	2644
	D052	0	99	0	-	200	470	100	6927	200	3.7E+4	200	2.5E+4	200	2.1E+4	400	6.3E+4	200	-	0	-	0	1.0E+4
	D172	0	99	0	-	0	292	100	1.5E+4	200	7.3E+4	200	3.0E+4	200	2.4E+4	200	6.8E+4	200	-	100	-	0	8295
AVE		0	1.4	0	-	100	260	250	1.2E+4	550	5.3E+4	1700	3.4E+4	700	2.4E+4	100	5.7+E4	300	-	225	-	100	1.2E+4
SD			30			116	161	370	1.1+4	700	3.6E+4	1732	2.8E+4	663	1.7E+4	1470	2.8E+4	346		386		200	9698
HBcAG-FFL_001	C004	0	167	100	-	200	688	400	1.3E+4	800	5.6E+4	1600	6.3E+4	3200	4.8E+4	3200	2.1E+5	800	-	400	-	400	2.9E+4
	D039	0	99	0	-	100	703	800	2.4E+4	3200	9.4E+4	6400	7.0E+4	3200	5.3E+4	12800	1.5E+5	1280 0	-	6400	-	160 0	3.0E+4
	D130	0	99	0	-	100	943	200	2.7E+4	400	6.2E+4	400	5.6E+4	400	5.9E+4	400	8.6E+4	400	-	100	-	0	6080
	D184	0	99	0	-	0	262	100	1.0E+4	400	6.4E+4	800	4.1E+4	400	2.8E+4	800	7.2E+4	200	-	100	-	0	9429
AVE		0	116	25	-	100	649	374	1.9E+4	1200	6.9E+4	2300	57E+4	1800	4.7E+4	4300	1.3E+5	3550	-	1750	-	500	1.8E+4
SD			34	50		82	284	310	8180	1347	1.7E+4	2778	12E+4	1617	1.3E+4	5800	6.4E+5	6172		3103		757	1.2E+4
FFL_005	D032	0	99	0	-	0	326	0	2.9E+4	0	8.9E+4	0	6.1E+4	200	2.9E+4	0	1.2E+5	0	-	0	-	0	1.4E+4
	D104	0	99	0	-	0	633	0	4.1E+4	100	1.0E+5	400	7.7E+4	200	6.8E+4	200	1.3E+5	100	-	0	-	0	2.7E+4
	D180	0	99	0	-	0	738	100	8.2E+4	400	2.4E+5	1600	1.2E+5	400	9.5E+4	1600	2.9E+5	400	-	800	-	100	8.0E+4
	D190	0	103	0	-	0	437	0	2.0E+4	0	1.3E+5	100	3.7E+4	0	2.8E+4	200	1.2E+5	0	-	0	-	0	1.1E+4
AVE		0	100	0	-	0	533	25	4.3E+4	125	1.4E+5	525	7.3E+4	200	5.5E+4	500	1.7E+5	3550	-	1750	-	500	3.3E+4
SD			1.9				186	50	2.7E+4	189	7.2E+5	737	3.3E+4	163	3.2E+4	739	8.6E+4	6172		3103		757	3.2E+4
FFL_007	C010	0	99	0	-	100	99	0	3663	400	5.3E+4	400	6.3E+4	800	2.7E+4	800	1.4E+5	200	-	200	-	100	1.9E+4
	D030	0	99	0	-	400	369	200	2.0E+4	800	2.0E+5	1600	1.3E+5	1600	9.6E+4	800	2.2E+5	400	-	400	-	200	5.1E+4
	D087	0	99	0	-	0	99	0	5928	0	2.0E+4	0	7465	0	6223	0	2.4E+4	0	-	0	-	0	6428
	D227	0	99	0	-	0	99	0	1617	0	6.0E+4	200	5.2E+4	0	2.8E+4	100	7.3E+4	0	-	0	-	0	2.2E+4
AVE		0	99	0	-	125	167	50	7839	300	8.3E+4	550	6.4E+5	600	4.0E+4	425	1.1E+5	150	-	150	-	75	2.5E+4
SD			0			189	137	100	8394	383	8.1E+4	719	5.3E+4	766	3.9E+4	435	5.5E+5	192		192		98	1.9E+4

Supplementary Table 2. RSV neutralization titers expressed as the serum dilution producing 50% neutralization, from a plaque reduction assay (white columns) or a microneutralization assay (gray columns).

Group	Animal	W0		W2		W4		W8		W12		W16		W18		W20		W28		W32	
FFL_001	C012	0	-	0	-	0	-	0	71	0	-	23	-	0	-	156	801	-	237	25	-
	D027	0	-	0	-	0	-	0	50	24	-	19	-	0	-	31	302	-	127	0	-
	D052	0	-	0	-	0	-	0	84	0	-	0	-	21	-	44	296	-	161	238	-
	D172	0	-	0	-	0	-	0	25	0	-	0	-	0	-	0	25	-	25	0	-
AVE		0	-	0	-	0	-	0	57.5	6	-	10.5	-	5.25	-	57.75	326	-	137.5	65.75	-
SD									25.8	12		12.23		10.5		68.1	323.57		88	115.4	
HBcAG-FFL_001	C004	0	-	0	-	0	-	0	84	48	-	47	-	44	-	249	2052	-	839	53	-
	D039	0	-	0	-	0	-	61	374	772	-	195	-	263	-	757	3289	-	1367	24	-
	D130	0	-	0	-	0	-	0	53	0	-	20	-	24	-	47	342	-	118	0	-
	D184	0	-	0	-	0	-	0	58	23	-	0	-	20	-	23	260	-	138	0	-
AVE		0	-	0	-	0	-	15.3	142.3	210.8	-	65.5	-	87.8	-	269	1485.8	-	615.5	19.3	-
SD								30.5	155.1	374.7		88.5		117.3		340.8	1458.6		602.3	25.2	
FFL_005	D032	0	-	0	-	0	-	0	25	0	-	0	-	0	-	0	83	-	25	0	-
	D104	0	-	0	-	0	-	0	54	0	-	0	-	0	-	34	97	-	25	0	-
	D180	0	-	0	-	0	-	0	25	26	-	47	-	27	-	40	212	-	383	0	-
	D190	0	-	0	-	0	-	0	25	23	-	0	-	20	-	23	61	-	71	0	-
AVE		0	-	0	-	0	-	0	32.3	12.3	-	11.8	-	11.8	-	24.3	113.3	-	126	0	-
SD									14.5	14.2		23.5		13.9		17.6	67.5		172.7	0	
FFL_007	C010	0	-	0	-	0	-	0	25	57	-	21	-	0	-	72	1235	-	157	0	-
	D030	0	-	0	-	0	-	0	68	176	-	67	-	62	-	99	669	-	356	0	-
	D087	0	-	0	-	0	-	0	25	0	-	0	-	0	-	0	25	-	25	0	-
	D227	0	-	0	-	0	-	0	25	0	-	0	-	0	-	0	25	-	75	0	-
AVE		0	-	0	-	0	-	0	35.8	58.3	-	22	-	15.5	-	42.75	488.5	-	153.3	0	-
SD									21.5	83		31.6		31		50.6	582.95		145.7	0	

Supplementary Table 3. Solvent accessibility of epitope residues in the complexes of Mota+peptide (PDBid: 3ixt) and 17HD9+peptide (PDBid: 4N9G). Solvent accessible surface areas were computed using NACCESS. Accessibilities are presented as the percentage of total area (backbone+sidechain) of a given residue accessible to solvent. The residue burial is the percentage area buried by antibody binding, and was computed as: burial = unbound accessibility - bound accessibility.

		Motavizumab					17-HD9(chain HLY)			17-HD9(chain ABC)		
AA	RES	Antibody bound Accessibility	Unbound Accessibility	Residue burial	AA	RES	Antibody bound accessibility	Unbound accessibility	Residue burial	Antibody bound accessibility	Unbound accessibility	Residue burial
					ASN	62	104.3	104.3	0			
					ARG	63	32.6	32.6	0	50.9	50.9	0
ASN	254	86.7	86.7	0	LEU	64	45	45	0	37.3	37.3	0
SER	255	64.5	84.7	20.2	SER	65	50.7	54.1	3.4	61.8	71.5	9.7
GLU	256	65.8	65.8	0	GLU	66	50.7	50.7	0	58.5	58.5	0
LEU	257	50	50	0	LEU	67	52.9	52.9	0	43.9	43.9	0
LEU	258	8.8	50.6	41.8	LEU	68	8.8	27.2	18.4	8.8	26.7	17.9
SER	259	26.3	49.6	23.3	SER	69	24.9	44.6	19.7	24.8	53	28.2
LEU	260	61.8	61.8	0	LYS	70	55.4	55.4	0	54.4	54.4	0
ILE	261	11	12.7	1.7	ILE	71	8.5	9	0.5	4.9	5	0.1
ASN	262	4.7	68.5	63.8	ASN	72	0.1	76	75.9	0	67.6	67.6
ASP	263	67	84.2	17.2	ASP	73	11.6	81	69.4	7.5	73	65.5
MET	264	34	34.8	0.8	MET	74	39.3	40.6	1.3	28.8	40.5	11.7
PRO	265	97.6	97.6	0	PRO	75	63.7	100.5	36.8	61.1	95.8	34.7
ILE	266	30.3	30.3	0	ILE	76	22.3	32.4	10.1	29.3	37.2	7.9
THR	267	54.6	59.2	4.6	THR	77	21.4	49.6	28.2	26.6	58.9	32.3
ASN	268	7.2	82.4	75.2	ASN	78	0	85.2	85.2	0	87	87
ASP	269	23.6	77.7	54.1	ASP	79	33	63	30	41.1	69.8	28.7
GLN	270	44.9	44.9	0	GLN	80	46	46	0	43.6	43.6	0
LYS	271	8.7	24.2	15.5	LYS	81	0	18.9	18.9	0	19.9	19.9
LYS	272	1.2	65.7	64.5	LYS	82	39.6	76.3	36.7	14.1	26.4	12.3
LEU	273	58.1	66.4	8.3	LEU	83	69.5	69.5	0	50.9	50.9	0
MET	274	42.8	42.8	0	MET	84	33.2	33.2	0	39.5	39.5	0
SER	275	13.5	46.4	32.9	SER	85	3.3	24.8	21.5	7.7	22.8	15.1
ASN	276	53.7	76.4	22.7	ASN	86	53.7	53.7	0	61.8	61.8	0
ASN	277	106.6	106.6	0	ASP	87	61	61	0	40.1	40.1	0
					VAL	88	11	11	0	15.4	15.4	0
					LEU	89	35.6	41.8	6.2	43.7	44.5	0.8
					LYS	90	54.4	54.4	0	54.9	54.9	0
					PHE	91	76.5	76.5	0	74.2	74.2	0
					ALA	92	17	17	0	21.8	21.8	0
					ALA	93	51.5	51.5	0	52.9	52.9	0
					GLU	94	71.1	71.1	0	68.5	68.5	0
					ALA	95	82.8	82.8	0	81.6	81.6	0
					GLU	96	76.2	76.2	0	85.4	85.4	0
					LYS	97	63.5	63.5	0	60	60	0

Supplementary Table 4. Pairs of residues making van der Waals contacts across the antibody-epitope interfaces in the Mota+peptide complex and the 17-HD9+peptide complexes. Atomic contacts were assessed using the contact application in CCP4.

Epitope Residue (Mota+peptide)	Epitope Residue (17HD9+peptide)	Motavizumab (chain HLP)	17-HD9 (chain HLY)	17-HD9_ABC (chain ABC)
SER255	-	ALA32H		
LEU68	LEU68			MET99H
SER69	SER69		MET99H	
ASN262	ASN72	ASP54H TRP53H	TYR33H ILE97H VAL98H VAL100H ARG100EH	TYR33H ILE97H VAL98H VAL100H ARG100EH
	ASP73		TYR33H TYR56H ASN58H	TYR33H TYR56H
	MET74			TYR94L
	ILE76		TYR94L	TYR94L
	THR77		ASN92L SER93L	ASN92L SER93L
ASN268	ASN78	GLY91L SER92L TYR94L PHE96L PHE100H	TYR32L HIS91L ASN92L PHE100AH ARG100EH	TYR32L HIS91L ASN92L PHE100AH ARG100EH
ASP269	ASP79	GLY31L SER92L	SER30L	SER30L
LYS272	LYS82	ASP50L ILE97H PHE98H	ASP100C	

Supplementary Table 5. Hydrogen bonds across the antibody-epitope interfaces in the Mota+peptide complex and the 17-HD9+peptide complexes. Hydrogen bonds were assessed using the angle application implemented in CCP4. Each hydrogen bond is given with the donor-acceptor distance and the donor-H-acceptor angle. NA indicates that the angle calculation could not be made due to missing atoms.

Epitope Residue (Mota+peptide)	Epitope Residue (17HD9+peptide)	Motavizumab (chain HLP)	17-HD9 (chain HLY)	17-HD9_ABC (chain ABC)
ASN262-O	ASN72-O	LYS56H-NZ (2.60 A, NA)	TYR33H–OH (3.17,111.5) ARG100EH-NH2 (3.02,178.1)	TYR33H–OH (3.45,108.8) ARG100EH–NH2 (3.38,128.7)
ASN262-ND2	ASN72-ND2	ASP54H-OD2 (2.70,116.11)	VAL98H-O (2.78,104.5) ILE97H-O (3.09,133.5)	VAL98H-O (3.38,107.9) ILE97H-O (2.96,136.6)
ASN262-OD1	ASN72-OD1		ARG100EH-NE (2.75,142.3)	
	ASP73-N		TYR33H-OH (3.28,144.7)	TYR33H-OH (3.01,140.0)
	ASP73-O		ASN58H-ND2 (2.73,NA)	ASN58H-ND2 (2.79,NA)
	ASP73-OD1		TYR33H-OH (2.67,109.5) ASN52H-ND2 (2.86,NA)	ASN52H-ND2 (3.40,NA)
	ASP73-OD2		ASN52H-ND2 (3.57,NA)	
	ASN78-N		ASN92L-OD1 (2.88,132.6) ASN92L-O (2.81,134.4)	ASN92L-OD1 (2.80,134.7) ASN92L-O (3.22,115.1)
ASN268-ND2	ASN78-ND2	GLY91L-O (2.96,147.5)	ARG100EH-O (2.94,119.7) HIS91L-O (3.25,159.0)	ARG100EH-O (2.88,134.7) HIS91L-O (2.82,142.8)
	ASN78-OD1			HIS91L-O (3.59,149.9)
	ASP79-N		ASN92L-OD1 (3.21,156.0)	ASN92L-OD1 (3.08,151.3)
ASP79-OD1			SER30L-OG (2.57,128.6)	
LYS81-NZ			TYR94L-OH (3.02,123.2)	ARG100EH-NH1 (3.48,130.1) TYR94L-OH (3.12,117.3)
LYS272-NZ	LYS82-NZ	ASP50L-OD1 (2.82,133.9)	TYR50L-OH (3.15,119.4)	
SER275-OG		ILE97H-O (2.89,103.5)		
ASN276-ND2		PHE98H-O (2.80,143.2)		
ASN276-OD1		PHE98H-O (2.64,110.8)		