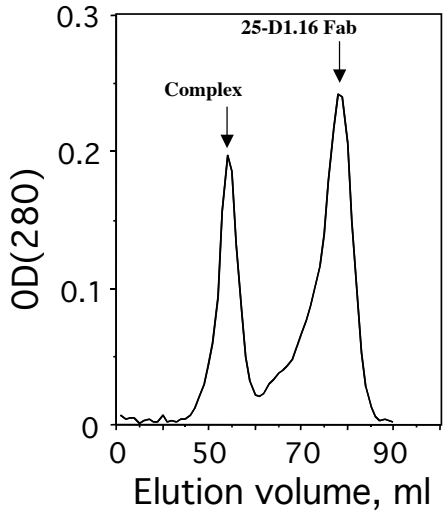
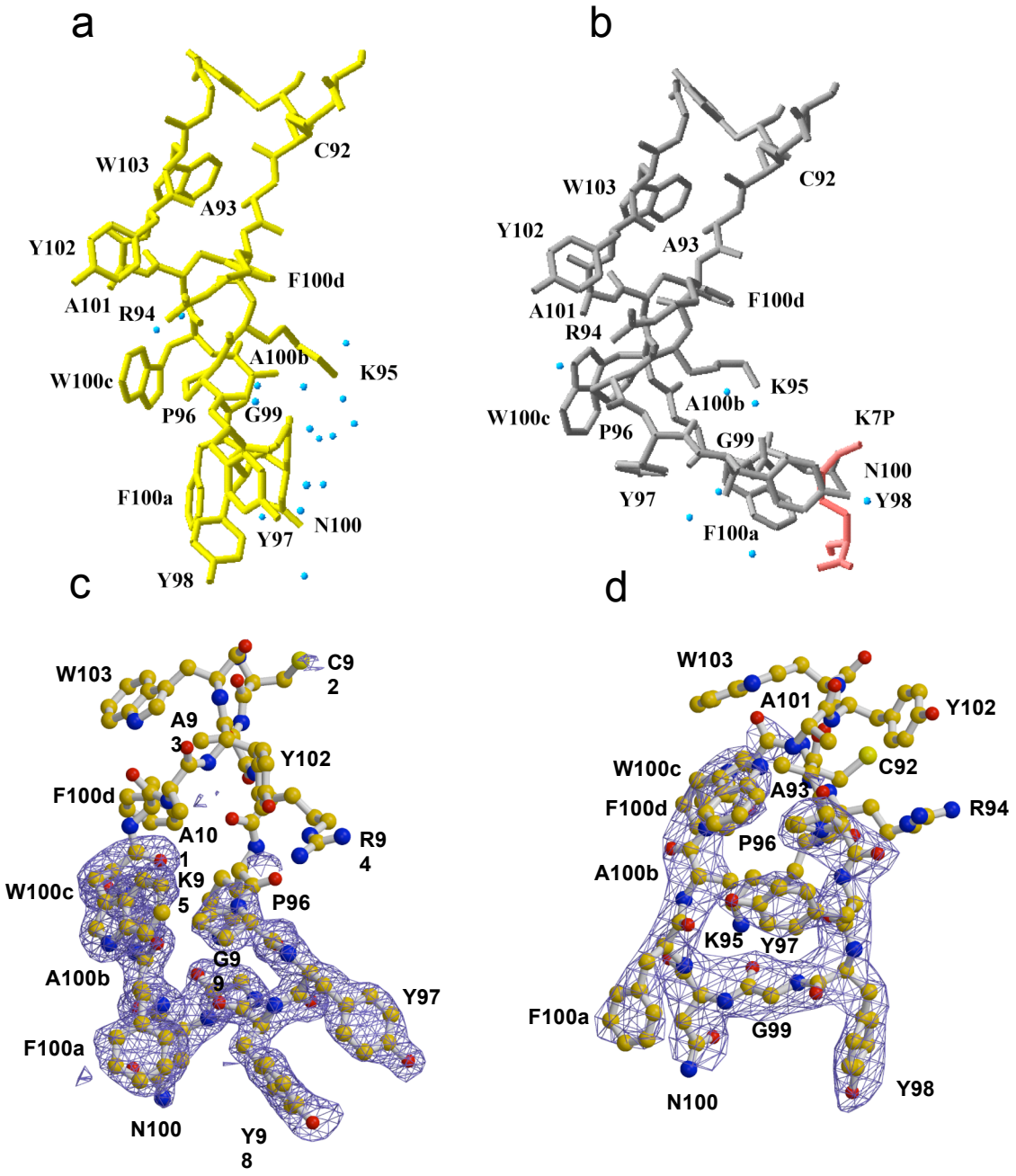
SUPPLEMENTARY MATERIAL



Supplemental Figure 1. Purification of 25-D1.16-pOV8-K^b complex.
312 μl of purified 25-D1.16 Fab at 2 mg/ml was combined with 200 μl soluble pOV8-K^b complex at 1 mg/ml in 50 mM HEPES, pH7.2, containing 50 mM NaCl. The Mixture was incubated at room temperature for 1 hour and was loaded on Superose S200HR column (16x75 cm) equilibrated with the same buffer. Analysis of the chromatographic profile shown demonstrates that there was no detectable level of unbound pOV8-K^b in the reaction mixture and that excess of the Fab was clearly separated from the Fab-pMHC complex. The purified complex was concentrated to 10 mg/ml by ultrafiltration.



Supplemental Figure 2. Conformational changes in CDR-H3 of 25-D1.16 induced by interactions with pOV8-K^b ligand

The CDR-H3 loop of intact 25-D1.16 Fab (a) is stabilized by 6 direct hydrogen bonds. The loop incorporates 16 water molecules (shown in blue) that form 34 water-bridged contacts within the loop, which are necessary to increase stability of the loop when it is exposed to the solvent. Upon binding of 25-D1.16 Fab to pOV8-K^b protein, the CDR-H3 loop undergoes significant conformational changes, resulting in the displacement of 7 water molecules (b). TyrH97, which was exposed to the solvent in the intact 25-D1.16 molecule, swings around by 124^o to form a direct hydrogen bond with the carbonyl oxygen of PheH100A. LysH95 forms a hydrogen bond with the carbonyl oxygen of GlyH99. These shifts move ProH96 to the vicinity of TrpH100C, resulting in the formation of multiple van der Waals' interactions. Two additional hydrogen bonds between ArgH97 and TyrH102 and AlaH101 are also

formed. As a result of these structural rearrangements, the CDRH3 loop is compacted, which then allows for the CDR1 and CDR2 loops of the Fab to reach K^b helices. Side chains of TyrH98, GlyH99 and AsnH100 wrap around the side chain of the LysP7 (pink). Simulated annealing omit F_o–F_c electron density map around CDR-H3 of intact (c) and bound (d) 25-D1.16 Fab contoured at 3.0 sigma. Residues TyrH97-AlaH100B were omitted in the simulated annealing omit calculation. Residues CysH92-TrpH103 are shown in ball-and-stick diagram. Electron density for omitted residues is unambiguous.

Supplemental Table 1. Contacts between 25-D1.16 and pOV8 peptide

25-D1.16 element	25-D1.16 contact atom	Peptide contact atom	Distance, Å	Type of interaction[1)]
Direct contacts				
CDR-H1	AsnH33 ND2	LysP7 NZ	3.03	HB
CDR-H2	AspH50 OD2	LysP7 NZ	3.94	SB
CDR-H3	TyrH98 OH	LeuP8 O	2.94	HB
	TyrH98 CZ	LysP7 CD	3.49	VDW
	GlyH99 N	LysP7 CD	3.83	VDW
	AsnH100 ND2	PheP5 O	3.26	HB
	AsnH100 N	GluP6 OE1	2.94	HB
	AsnH100 OD1	LysP7 CE	2.95	VDW
	PheH100A CZ	AsnP4 O	3.29	VDW
	PheH100A CZ	PheP5 O	3.20	VDW
	PheH100A CE2	GluP6 N	3.97	VDW
CDR-L3	SerL93 OG	AsnP4 ND2	3.82	HB
	ThrL94 N	AsnP4 ND2	3.67	HB

Water-bridged contacts				
25-D1.16 element	25-D1.16 contact atom	Water molecule	Peptide contact atom	Distance, Å
CDR-H3	TyrH98 O	Wat 336	GluP6 OE2	3.22/3.24
	PheH100A O	Wat 184	GluP6 OE2	2.72/3.14
	PheH100A N	Wat 184	GluP6 OE2	2.77/3.14
	AsnH100 N	Wat 184	GluP6 OE2	3.24/3.14

[1)]HB, hydrogen bond; SB, salt bridge; VDW, van der Waals' interaction.

Supplemental Table 2. Contacts between 25-D1.16 and K^b heavy chain

25-D1.16 element	25-D1.16 contact atom	K^b element	K^b contact atom	Distance, Å	Type of interaction[1)]
Direct contacts					
CDR-H2	AsnH54 OD1	α1 helix	ValB76 CG1	3.53	VDW
	AsnH54 O	α1 helix	ArgB79 NH1	2.67	HB
	AsnH54 ND2	α1 helix	ThrB80 OG1	3.50	HB
	GlyH56 N	α1 helix	ArgB79 NH1	3.76	HB
	ThrH57 N	α1 helix	ValB76 CG2	3.83	VDW
	TyrH59 N	α1 helix	GlnB72 NE2	2.69	HB
	GlnH61 CG	α1 helix	GlnB65 OE1	3.15	VDW
CDR-H3	TyrH97 CD2	α2 helix	GlnB149 O	3.91	VDW
	TyrH97 OH	α2 helix	AlaB150 O	3.93	HB
	TyrH97 CE1	α2 helix	AlaB150 CB	3.38	VDW
	TyrH97 CD1	α2 helix	AlaB150 CB	3.47	VDW
	TyrH98 CD2	α2 helix	LysB146 CD	3.35	VDW
	AsnH100 ND2	α1 helix	SerB73 OG	3.17	HB
	PheH100A CE2	α2 helix	ArgB155 CZ	3.36	VDW
CDR-L1	TyrL30 CE1	α2 helix	AlaB158 O	3.65	VDW
	TyrL30 OH	α2 helix	ThrB163 N	3.33	HB
	ArgL32 NH2	α2 helix	GluB154 O	3.63	HB
	ArgL32 NH2	α2 helix	ArgB155 N	3.54	HB
	GluL27 OE1	α1 helix	ArgB62 NH1	2.84	SB
CDR-L3	TrpL92 CZ2	α2 helix	ThrB163 OG1	3.89	VDW
	TrpL92 CZ3	α2 helix	ArgB155 CG	3.87	VDW
	TrpL92 CZ3	α2 helix	AlaB158 CB	3.80	VDW
	TrpL92 CH2	α2 helix	AlaB158 CB	3.54	VDW

Water-bridged contacts				
25-D1.16 element	25-D1.16 contact atom	Water molecule	K^b contact atom	Distance, Å
CDR-H2	AsnH52 OD1, AsnH53 ND2, TyrH98 OH	Wat 204	ThrB80 CG2 (α1 helix) LysB146 NZ (α2 helix)	3.3, 3.6, 3.3/3.1, 3.3
	ThrH57 O	Wat 142	GlnB72 O (α1 helix)	2.8/2.86
	ThrH57 OG1,	Wat 30	GlnB72 NE2 (α1 helix)	3.0/3.3
	GlnH61 NE2	Wat 267	GlyB69 N, GlnB72 OE1 (α1 helix)	2.7/3.0, 2.7
CDR-H3	TyrH98 O, TyrH98 N	Wat 104	LysB146 O, (α2 helix)	2.75, 3.3/ 3.0
CDR-L1	AspL28 OD1	Wat 327	GluB166 OE1, GluB166 OE2 (α2 helix)	2.63/2.7, 2.8
	TyrL30 CE1	Wat 129	Glu166 OE1 (α2 helix)	3.3/3.1
CDR-L3	TrpL92 O	Wat 178	Arg155 NH1 (α2 helix)	2.7/2.8

[1)]see Supplemental Table 1.