Supplementary file 3

pMHC interdomain interactions analyzed by PDBePISA software

(Supplementary files 3A-to-D)

Legend to Supplementary files 3A, 3B, 3C, and 3D

The pMHC-I structures were analyzed on-line by PDBePISA software (https://www.ebi.ac.uk/msd-srv/prot_int/pistart.html), using the functions "Interfaces" and "Details". The software provided a list of "Interfacing residues" together with their Accessible Surface Area (ASA) in Å², Buried Surface Area (BSA) in Å², Solvation energy effect $\Delta^{i}G$ in kcal/mol, and predictions of hydrogen bonds (H) and salt bridges (S); for shark pUAA domains these values are indicated in Supplementary files 3A and 3C, except that instead of the ASA value the BSA/ASA percentage value is given. For the pMHC-I structures carp UAA (PDB 5Y91), frog UAA (PDB 6A2B), chicken BF2*0401 (PDB 4E0R), and HLA-A2 (PDB 3PWN), in the same files only the "Interfacing residues" are shown together with information on hydrogen bond and salt bridge formation. Residues shown in Supplementary files 3A and 3C that are not part of the interdomain interface, unlike some of their counterparts in the other compared pMHC-I structures, are shown in gray-shaded small Italic font and are followed by a "-" symbol; non-shark pMHC-I residues that do form part of the interdomain interface are followed by a "+" symbol. Except for gray, the residue shading colors used in Supplementary files 3A and 3C match with those used in Supplementary files 2A and 2B and relate to conservation patterns.

The software also provided a more detailed summary of the predicted hydrogen bonds and salt bridges, which is shown in Supplementary files 3B and 3D; in these tables the interacting pairs of residues that are common among pMHC-I structures are indicated with colors.

(3A) Residues that are part of the interface between pMHC-I α1α2 and β_2-m domains. The numbers 8, 9, and 10 in the "pa9-pl." column refer to the top residues ("9") and lower ridge residues ("8" and "10") of a pa9 pleat and are named after their respective constituent residues at α1 domain positions 8, 9, and 10. As explained in the main text, in pMHC-I structures the α1α2 domain pa9 pleat forms a major part of the α1α2 domain interaction with β_2-m, including a direct interaction with the pleat top residues at the "9" positions.

(3B) Summary of predicted hydrogen bonds and salt bridges between pMHC-I α1α2 and β_2-m domains.

(3C) Residues that are part of the interface between pMHC-I α3 and β_2-m domains. Note that also β_2-m residues C-terminal of the IgSF-exon-encoded stretch (positions 95a-to-d) are included (below the dashed line).

(3D) Summary of predicted hydrogen bonds and salt bridges between pMHC-I α3 and β_2-m domains.

Supplementary file 3A

Residues that are part of the interface between pMHC-I α1α2 and β_2-m domains.
HC and β_2-m residues are numbered as shown in Supplementary file 2A

		Shark UAA pa9- BSA				For comparison: MHC-I			
HC	pl.	% / Å^2	H/S	Δ^iG	Carp	Frog	Chick	HLA-A2
6		r-			K+	R+	r-	R+
8	8	F 79% / 46		0.72	V+	Y+	I+	F
9	9	F 21% / 2		-0.02	Y+	Y+	R+	F+
10	10	T 97% / 40		0.11	T+	T+(H)	T+	T+
12		S 47% / 11		-0.08	V+	V+	M+	V+
14		--			--	d-	D+(HS)	r-
15		a-			R+	r-	P+	p-
16		g-			G+	A+	G+	g-
17		S 16% / 19		-0.15	I+(H)	F+	P+	R+
18		G 5% / 4		0.07	D+(HS)	G+	g-	g-
19		I 75% / 79		1.27	F+(H)	L+	Q+(H)	e-
21		e-			e-	e-	w-	R+
23	10	V 82% / 33		0.49	T+	s-	V+	I+
25	8	V 97% / 32		0.51	V+	V+	V+	V+
27		Y 51% / 29		-0.10	M+	Y+(H)	Y+	Y+(H)
32		Q 32% / 16	H	-0.25	Q+	Q+	L+	Q+(H)
35	8	Q 55% / 9		0.03	Y+	R+(HS)	H+	R+(HS)
48		R 14% / 18	HS	-0.43	K+(S)	a-	R+(HS)	R+(HS)
87		q-			Q+	q-	q-	Q+
91		g-			g-	g-	g-	G+
92		I 44% / 69		0.94	V+	T+	S+	S+
93		H 3% / 0.5		0.01	H+	h-	H+	H+
94		T 92% / 27		-0.13	T+	S+(H)	T+	T+
96	10	Q 100% / 62	H	-0.36	Q+(H)	Q+(H)	Q+(H)	Q+(H)
97	9	L 69% / 2		-0.00	N+	V+	W+	R+
98	8	M 81% / 57		1.72	M+	M+	M+	M+
113		f-			F+	Y+	y-	y-
115	8	Q 34% / 26		-0.06	Q+	Q+	Q+	Q+
116	9	H 39% / 4		-0.00	Y+	Y+	S+	Y+
117	10	A 99% / 18		0.29	A+	G+	A+	A+
119		D 48% / 22	H	0.07	D+(S)	D+	D+	D+
120		S 83% / 70	H	0.15	G+	G+	G+	G+
121		T 31% / 24		0.39	E+	R+	R+	K+
122	10	D 22% / 23	H	-0.21	D+(H)	E+	D+(H)	D+(H)

	Shark UAA BSA				For comparison: MHC-I			
b2-m	% / Å^2	H/S	Δ^iG	Carp	Frog	Chick	HLA-A2
B3	s-			s-	s-	l-	R+
B31	S 66% / 14		0.19	H+(S)	H+(H)	H+	H+(H)
B32	P 70% / 34		0.54	P+	P+	P+	P+
B33	P 96% / 52		0.48	P+	P+	P+	S+
B34	N 65% / 52		0.29	D+(H)	R+	K+(HS)	D+
B35	I 53% / 14		-0.14	I+(H)	L+	I+	i-
B36	k-			s-	E+	s-	e-
B51	Q 17% / 11		-0.13	Q	q-	E+	H+
B52	S 1% / 0.5		0.01	T+	T+	S+	S+
B53	D 81% / 105	HS	-0.26	D+(S)	D+(HS)	D+(HS)	D+(HS)
B54	L 93% / 87		0.65	L+	P+	M+	L+
B55	S 91% / 42		0.09	A+	S+	S+	S+
B56	F 97% / 103		1.21	F+	F+	F+	F+
B57	E 4% / 4		0.04	E+	Q+	n-	S+
B58	S 27% / 31		0.18	K+	H+	D+	K+
B59	D 4% / 4		-0.04	G+	N+	D+	D+
B60	W 95% / 173	H	2.04	W+(H)	W+(H)	W+(H)	W+(H)
B61	S 5% / 0.7		0.01	q-	K+	t-	s-
B62	F 99% / 37		0.60	F+	Y+(H)	F+	F+
B63	K 15% / 10		-0.35	H+	Y+(H)	q-	Y+(H)
B64	L 84% / 7		0.11	L+	t-	R+(H)	l-
B67	y-			s-	s-	h-	Y+
B83	t-			R+(HS)	s-	e-	n-
B85	N+ 7% / 6		-0.06	M+	n-	E+	v-

Supplementary file 3B

Summary of predicted hydrogen bonds and salt bridges between pMHC-I α1α2 and β_2-m domains. *HC (left of the distance in ångström) and β_2-m residues (right) are numbered as shown in Supplementary file 2A*

In Shark UAA:

Hydrogen bonds

Q32	[NE2]	2.76	A	DB53	[OD2]
R48	[NE]	2.88	A	DB53	[OD2]
R48	[NH1]	3.03	A	D53	[OD1]
Q96	[NE2]	3.02	A	WB60	[O]
S120	[OG]	3.67	A	WB60	[O]
D122	[OD1]	2.83	A	WB60	[NE1]

Salt bridges

R48	[NE]	3.81	A	DB53	[OD1]
R48	[NE]	2.88	A	DB53	[OD2]
R48	[NH1]	3.03	A	DB53	[OD1]
R48	[NH1]	3.59	A	DB53	[OD2]

In Carp UAA:

Hydrogen bonds

I17	[N]	3.50	A	DB34	[OD1]
D18	[OD1]	2.96	A	IB35	[N]
D18	[OD1]	3.48	A	RB83	[NH2]
F19	[N]	3.85	A	DB34	[OD2]
Q96	[NE2]	2.84	A	WB60	[O]
D122	[OD1]	2.64	A	WB60	[NE1]

Salt bridges

D18	[OD1]	3.48	A	RB83	[NH2]
K48	[NZ]	3.99	A	DB53	[OD2]
D119	[OD1]	4.00	A	HB31	[NE2]

In Frog UAA:

Hydrogen bonds

T10	[OG1]	2.78	A	YB62	[OH]
Y27	[OH]	2.65	A	YB63	[OH]
R35	[NH1]	3.49	A	DB53	[OD1]
S94	[OG]	3.49	A	HB31	[NE2]
Q96	[OE1]	3.39	A	HB31	[NE2]
Q96	[NE2]	3.09	A	WB60	[O]

Salt bridges

R35	[NH1]	3.49	A	DB53	[OD1]

In Chicken BF2*0401

Hydrogen bonds

D14	[OD2]	2.85	A	KB34	[NZ]
Q19	[OE1]	2.90	A	RB64	[NH2]
R48	[NH1]	3.05	A	DB53	[OD1]
R48	[NH2]	2.77	A	DB53	[OD2]
Q96	[NE2]	2.92	A	WB60	[O]
D122	[OD1]	2.59	A	WB60	[NE1]

Salt bridges

D14	[OD2]	2.85	A	KB34	[NZ]
D14	[OD1]	3.70	A	KB34	[NZ]
R48	[NH1]	3.05	A	DB53	[OD1]
R48	[NH2]	3.17	A	DB53	[OD1]
R48	[NH2]	2.77	A	DB53	[OD2]

In HLA-A2:

Hydrogen bonds

Y27	[OH]	3.30	A	YB63	[OH]
Q32	[NE2]	2.79	A	DB53	[OD1]
R35	[NH1]	2.99	A	DB53	[OD2]
R48	[NE]	2.84	A	DB53	[OD1]
Q96	[OE1]	2.83	A	HB31	[NE2]
Q96	[NE2]	2.83	A	WB60	[O]
D122	[OD2]	2.71	A	WB60	[NE1]

Salt bridges

R35	[NH1]	3.92	A	DB53	[OD1]
R35	[NH1]	2.99	A	DB53	[OD2]
R48	[NE]	2.84	A	DB53	[OD1]
R48	[NH1]	3.42	A	DB53	[OD1]
R48	[NH1]	3.77	A	DB53	[OD2]

Supplementary file 3C

Residues that are part of the interface between pMHC-I α3 and β_2-m domains.
HC and β_2-m residues are numbered as shown in Supplementary file 2A

	Shark UAA BSA				For comparison: MHC-I			
HC		% / Å^2	H/S	Δ^iG	Carp	Frog	Chick	HLA-A2
183	v-				v-	v-	E+	d-
186	s-				Q+	h-	E+	k-
187	v-				v-	v-	V+	t-
188	T	17% / 10		0.16	S+	R+	R+(H)	H+
190	T	8% / 4		-0.02	L+	s-	W+(H)	T+(H)
191	s-				Q+(H)	d-	g-	h-
192	V	68% / 35		0.57	K+(HS)	H+	K+(S)	H+(H)
193	R	51% / 93	HS	-1.37	d-	q-	e-	a-
202	S	92% / 10	H	0.07	T+	R+	S+	R+(H)
204	V	29% / 9		0.15	H+(H)	H+	R+	W+(H)
206	T	70% / 34	H	0.32	T+(H)	Y+	H+(H)	L+
207	G	43% / 12		0.20	G+	g-	G+	s-
231	T	38% / 27	H	-0.08	g-	k-	G+(H)	V+
232	G	12% / 3		0.05	E+(HS)	E+(HS)	G+	E+(H)
233	V	16% / 10		-0.11	L+	I+	I+	T+
234	R	82% / 79		-0.66	L+	L+	V+	R+(H)
235	P	71% / 72	H	0.70	P+(H)	P+(H)	P+(H)	P+(H)
236	N	82% / 37		-0.07	N+	N+	N+(H)	A+(H)
237	H	70% /122		0.95	E+(HS)	P+	G+	G+
238	D	11% / 10		-0.17	D+	D+	D+(HS)	D+
240	S	11% / 1		-0.01	S+	s-	T+(H)	t-
242	Q	98% / 31		-0.36	Q+(H)	Q+	H+	Q+(H)
244	H	54% / 30	H	-0.83	M+	R+(HS)	W+	W+
246	S	19% / 4		0.02	t-	t-	t-	a-
279	d--				I+	--	--	--
280	e--				T+(H)	--	--	--
281	f--				N+(H)	--	--	--
282	g--				F+	--	--	--

	Shark UAA BSA				For comparison: MHC-I			
b2-m		% / Å^2	H/S	Δ^iG	Carp	Frog	Chick	HLA-A2
B6	n-				K+(HS)	V+	k-	K+
B8	Q	70% / 64	H	-0.68	Q+(H)	K+(S)	Q+(H)	Q+(H)
B9	V	7% / 0.9		-0.01	v-	V+	V+	V+
B10	Y	90% / 77	H	0.76	Y+(H)	T+(H)	Y+(H)	Y+(H)
B11	T	38% / 9		-0.10	S+(H)	T+	S+(H)	S+(H)
B12	Y	64% /108	H	0.71	H+(HS)	A+	R+(HS)	R+(H)
B13	K	8% / 9		0.15	Y+	E+	F+	H+
B14	L	21% / 25		0.40	P+	P+	P+	P+
B15	i-				G+(H)	v-	A+(H)	a-
B16	k-				E+	d-	S+(H)	e-
B17	e-				Y+(H)	f-	a-	n-
B22	V	71% / 26		0.41	T+(H)	E+	V+	f-
B24	L	100% / 29		0.47	I+	I+	N+(H)	N+(H)
B26	H	69% / 26		0.14	Y+	Y+	H+	Y+
B28	K	29% / 22		-0.76	s-	Y+(H)	a-	S+
B50	t-				Q+	K+	q-	e-
B52	s-				t-	T+	s-	s-
B55	s-				a-	s-	s-	s-
B57	e-				e-	q-	n-	S+
B63	k-				h-	T+	Q+	Y+
B65	T	68% / 17		0.08	T+	S+	L+	L+
B67	Y	48% / 34		0.34	S+	h-	H+	Y+
B69	E	6% / 8		-0.07	s-	h-	d-	e-
B74	s-				K+	k-	s-	e-
B85	n-				m-	n-	e-	v-
B95	l-				w-	D+(HS)	W+	w-
B95a	D	23% / 27	HS	-0.33	E+(HS)	D+(HS)	D+(HS)	d-
B95b	R	9% / 8	H	-0.10	S+	--	P+(H)	r-
B95c	Y	67% /158	H	1.42	N+(H)	--	E+	D+(H)
B95d	--				M+(H)	--	--	M+(H)

Supplementary file 3D

Summary of predicted hydrogen bonds and salt bridges between pMHC-I α3 and β_2-m domains. *β_2-m (left of the distance in ångström) and HC residues (right) are numbered as shown in Supplementary file 2A*

In shark UAA:

Hydrogen bonds

QB8	[NE2]	3.54	A	T231	[O]
YB10	[OH]	2.66	A	P235	[O]
YB12	[O]	3.06	A	T206	[OG1]
DB95a	[OD1]	2.99	A	R193	[NH2]
DB95a	[OD2]	3.35	A	R193	[NH1]
RB95b	[O]	3.57	A	R193	[OG]
YB95c	[OH]	3.19	A	S202	[OG]
YB95c	[OH]	2.78	A	H244	[ND1]

Salt bridges

DB95a	[OD1]	2.99	A	R193	[NH2]
DB95a	[OD1]	3.91	A	R193	[NH1]
DB95a	[OD2]	3.54	A	R193	[NH2]
DB95a	[OD2]	3.35	A	R193	[NH1]

In carp UAA:

Hydrogen bonds

KB6	[NZ]	2.93	A	E232	[OE1]
KB6	[NZ]	3.22	A	E232	[OE2]
QB8	[NE2]	3.42	A	E232	[OE2]
YB10	[OH]	2.66	A	P235	[O]
SB11	[O]	2.97	A	Q242	[NE2]
HB12	[OD1]	2.85	A	E237	[OE2]
HB12	[OD2]	3.62	A	H204	[NE2]
HB12	[O]	2.80	A	T206	[OG1]
HB12	[OH]	3.53	A	Q242	[NE2]
GB15	[OH]	2.96	A	N280	[ND2]
YB17	[OH]	3.20	A	N280	[OD1]
TB22	[OH]	3.77	A	E237	[OE1]
EB95a	[OE2]	2.89	A	Q191	[NZ]
NB95c	[O]	3.63	A	Q191	[N]
NB95c	[O]	3.62	A	K192	[N]
MB95d	[O]	2.98	A	T279	[OG1]
MB95d	[O]	2.83	A	N280	[N]
MB95d	[OXT]	2.79	A	K192	[NZ]

Salt bridges

KB6	[NZ]	2.93	A	ibE53	[OE1]
KB6	[NZ]	3.22	A	ibE53	[OE2]
HB12	[ND1]	3.40	A	ibE58	[OE1]
HB12	[ND1]	2.85	A	ibE58	[OE2]
EB95a	[OE2]	2.89	A	ibK12	[NZ]

In Frog UAA:

Hydrogen bonds

YB10	[OH]	2.65	A	P235	[O]
YB28	[OH]	2.56	A	E232	[OE2]
DB95a	[OD1]	3.68	A	R243	[NH1]

Salt bridges

KB8	[NZ]	3.99	A	E232	[OE2]
DB95a	[OD1]	3.68	A	R243	[NH1]

In chicken BF2*0401:

Hydrogen bonds

QB8	[NE2]	2.83	A	G231	[O]
YB10	[OH]	2.66	A	P235	[O]
SB11	[O]	2.94	A	H206	[ND1]
RB12	[NE]	2.86	A	D238	[OD2]
RB12	[NE]	3.51	A	T240	[OG1]
RB12	[O]	3.37	A	H206	[ND1]
AB15	[O]	2.68	A	R188	[NH1]
AB15	[O]	2.78	A	R188	[NH2]
SB16	[OG]	3.79	A	R188	[NH2]
NB24	[ND2]	3.01	A	N236	[OD1]
DB95a	[OD2]	3.90	A	W190	[NE1]
PB95b	[O]	3.69	A	R188	[NH1]

Salt bridges

RB12	[NE]	3.51	A	D238	[OD1]
RB12	[NE]	2.86	A	D238	[OD2]
RB12	[NH2]	3.33	A	D238	[OD2]
DB95a	[OD1]	3.97	A	K192	[NE]

In HLA-A2:

Hydrogen bonds

QB8	[NE2]	2.91	A	E232	[O]
QB8	[OE1]	2.91	A	R234	[NH1]
YB10	[OH]	2.60	A	P235	[O]
SB11	[O]	3.79	A	Q242	[NE2]
RB12	[NE]	3.03	A	A236	[O]
RB12	[O]	3.04	A	Q242	[NE2]
NB24	[ND2]	3.89	A	P235	[O]
NB24	[ND2]	3.00	A	A236	[O]
DB95c	[O]	3.20	A	H192	[NE2]
MB95d	[O]	3.00	A	R202	[NH1]
MB95d	[O]	2.74	A	T190	[OG1]
MB95d	[O]	2.98	A	W204	[NE1]

No salt bridges found