Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Residue-Specific Binding Mechanisms of PD-L1 to Its Monoclonal Antibodies by Computational Alanine Scanning

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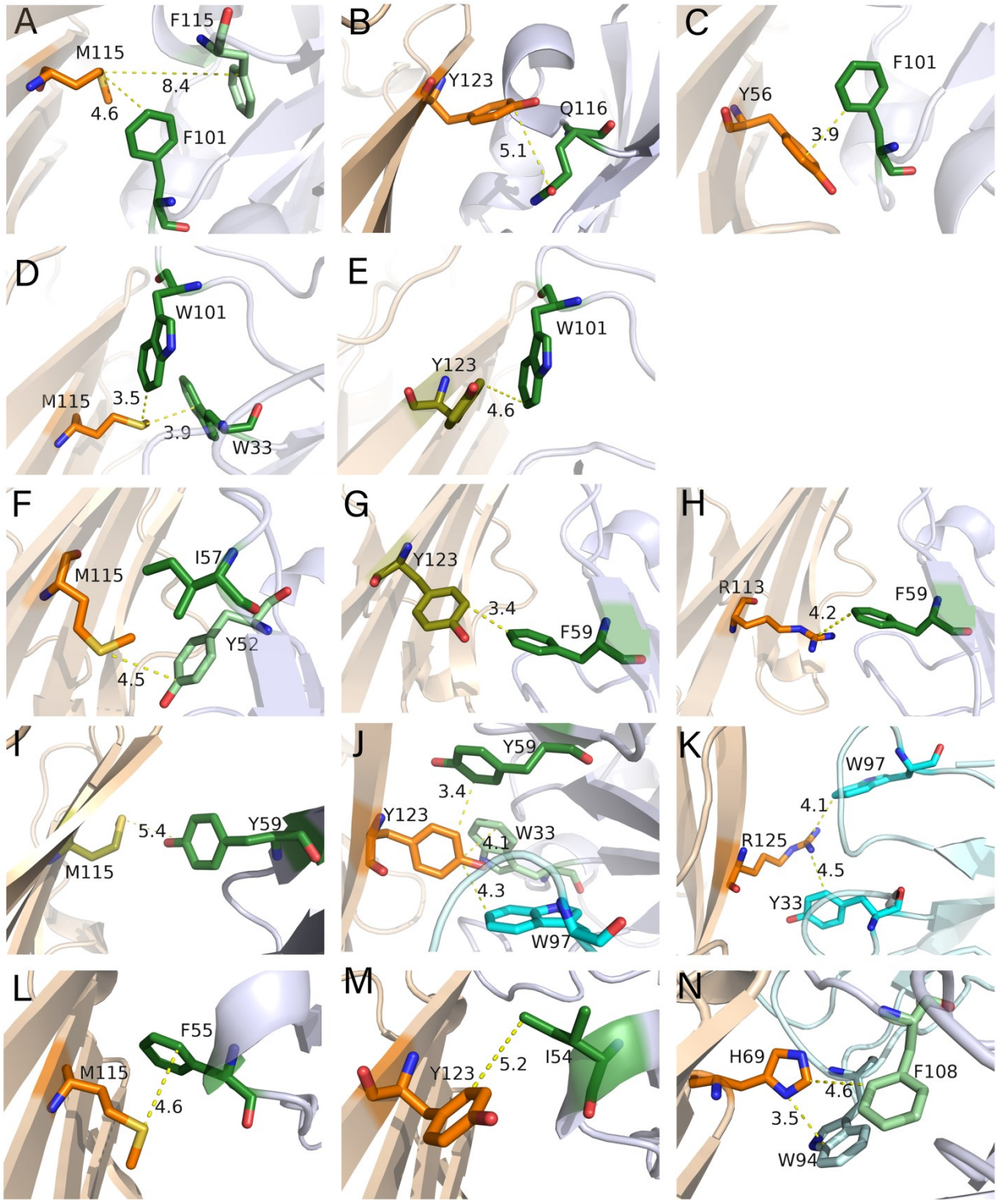

Figure S1. Detailed interactions of the hotspots of PD-L1 in 5 PD-L1/mAb systems. The chains of PD-L1 are colored in wheat. The heavy chains of mAb are colored in light purple and light chain in cyan. Residues are colored according to their location: orange, on PD-L1; green, on heavy chain of mAb; cyan, on light chain of mAb. Residues are also colored by ΔΔG values: darker color for hotspots (typically defined as > 2 kcal/mol); lighter color for warm spots, 1 to 2 kcal/mol. The atom coloring style is red for oxygen and blue for nitrogen and yellow for sulfur. For clarity, the color of warm spot in PD-L1 is changed from light orange to dark olive. The shortest distance between the atoms in aromatic-

aromatic, sulfur-aromatic and cation-aromatic binding residues are marked and presented by yellow dash lines. A-C, D-E, F-H, I-K and L-N are respectively for PD-L1/KN035, PD-L1/atezolizumab, PD-L1/avelumab, PD-L1/durvalumab and PD-L1/BMS-936559.

Table S1. Prediction on hotspots and warm spots in PD-L1/avelumab system (PDB code 5GRJ).

Chain	Mutation	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta G_{gb}$	$\Delta\Delta G_{np}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$	Sd[a]
(A) PD-L1									
A	**R113A**	**5.24**	**-11.43**	**11.35**	**0.51**	**5.66**	**-1.20**	**4.47**	**0.73**
A	**Y56A**	**3.78**	**2.05**	**-1.30**	**0.05**	**4.58**	**-0.74**	**3.84**	**1.07**
A	**D61A**	**-0.55**	**34.74**	**-28.77**	**0.22**	**5.65**	**-2.98**	**2.67**	**0.43**
A	**Q66A**	**2.53**	**1.70**	**-1.32**	**0.03**	**2.95**	**-0.40**	**2.55**	**0.60**
A	**I54A**	**2.59**	**-0.26**	**-0.09**	**0.04**	**2.28**	**-0.21**	**2.07**	**0.23**
A	**M115A**	**2.82**	**-0.44**	**0.00**	**-0.06**	**2.32**	**-0.3**	**2.02**	**0.34**
A	H78A	1.80	0.78	-0.67	0.29	2.20	-0.36	1.84	0.28
A	V76A	2.74	-0.04	-0.85	0.08	1.92	-0.19	1.73	0.69
A	N63A	1.90	-0.20	-0.05	-0.02	1.64	-0.07	1.57	0.34
A	K75A	2.61	-12.07	11.42	0.41	2.37	-1.02	1.35	0.60
A	R125	1.83	-4.51	4.89	0.36	2.57	-1.34	1.23	0.67
A	Y123A	1.63	0.05	-0.64	0.22	1.26	-0.09	1.18	0.23
A	E58A	0.70	19.21	-17.18	-0.06	2.67	-1.60	1.07	1.52
A	E60A	1.42	16.93	-16.58	0.11	1.88	-0.86	1.02	0.71
(B) avelumab									
H	**I57A**	**5.71**	**0.14**	**-1.04**	**0.18**	**4.99**	**-0.57**	**4.42**	**0.12**
L	**Y93A**	**4.39**	**1.29**	**-1.77**	**0.04**	**3.95**	**-0.42**	**3.53**	**0.14**
L	**Y34A**	**3.66**	**4.96**	**-5.27**	**0.56**	**3.91**	**-0.73**	**3.18**	**0.67**
H	**F59A**	**4.07**	**2.73**	**-3.53**	**0.34**	**3.61**	**-0.67**	**2.94**	**0.44**
H	**V104A**	**3.23**	**0.35**	**-0.02**	**0.01**	**3.58**	**-1.04**	**2.54**	**1.00**
L	**R99A**	**0.38**	**18.76**	**-16.12**	**-0.02**	**3.01**	**-0.62**	**2.38**	**0.42**
H	**I33A**	**2.06**	**0.27**	**-0.12**	**0.02**	**2.23**	**-0.10**	**2.14**	**0.25**
H	Y52A	1.81	4.95	-3.91	0.08	2.93	-1.00	1.93	0.69
H	T103A	1.50	-0.90	1.26	0.03	1.90	-0.02	1.88	0.21
H	L101A	2.57	-0.24	-0.38	-0.06	1.89	-0.18	1.71	0.18
L	S97A	0.43	3.68	-2.42	0.02	1.72	-0.49	1.23	0.57

[a]Standard deviation of $\Delta\Delta G$.

Table S2. Prediction on hotspots and warm spots in PD-L1/durvalumab system (PDB code 5X8M).

Chain	Mutation	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta G_{gb}$	$\Delta\Delta G_{np}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$	Sd[a]
(A) PD-L1									
A	**R125A**	**4.53**	**10.79**	**-8.17**	**0.61**	**7.76**	**-2.98**	**4.78**	**0.68**
A	**Y123A**	**5.33**	**0.46**	**-1.07**	**0.27**	**4.99**	**-0.58**	**4.41**	**0.19**
A	**R113A**	**1.64**	**2.34**	**1.17**	**0.01**	**5.16**	**-2.67**	**2.49**	**0.54**
A	D122A	1.35	8.07	-7.44	0.04	2.02	-0.29	1.73	0.06
A	E58A	0.68	13.62	-11.41	0.11	3.00	-1.32	1.68	0.32
A	K124A	3.19	-7.25	6.45	0.20	2.59	-1.01	1.58	0.21
A	V111A	2.15	0.33	-0.97	0.04	1.55	-0.32	1.22	0.30
A	E60A	1.54	8.51	-8.36	0.12	1.81	-0.67	1.15	0.21
A	M115A	1.90	1.86	-2.00	0.07	1.82	-0.68	1.14	0.17
(B) durvalumab									
B	**F103A**	**7.23**	**1.72**	**-4.37**	**0.53**	**7.11**	**-0.77**	**6.35**	**0.64**
B	**Y59A**	**4.05**	**-0.91**	**0.91**	**0.33**	**4.36**	**-0.36**	**4.00**	**0.16**
C	**W97A**	**2.24**	**3.55**	**-1.21**	**0.02**	**4.61**	**-1.32**	**3.29**	**1.16**
C	**Y33A**	**2.85**	**0.86**	**-1.22**	**0.22**	**2.71**	**-0.32**	**2.39**	**0.40**
B	K52A	1.26	11.09	-9.68	0.22	2.88	-1.25	1.63	0.20
B	W33A	3.05	7.55	-7.36	-0.03	3.20	-1.60	1.60	0.93
C	L95A	2.20	0.55	-0.94	-0.03	1.78	-0.29	1.49	0.18
B	E105A	1.64	-0.55	0.73	0.03	1.84	-0.52	1.32	0.17

[a]Standard deviation of $\Delta\Delta G$.

Table S3. Prediction on hotspots and warm spots in PD-L1/BMS-936559 system (PDB code 5GGT).

Chain	Mutation	$\Delta\Delta E_{vdw}$	$\Delta\Delta E_{ele}$	$\Delta\Delta G_{gb}$	$\Delta\Delta G_{np}$	$\Delta\Delta H$	$-T\Delta\Delta S$	$\Delta\Delta G$	Sd[a]
(A) PD-L1									
A	**H69A**	**4.09**	**0.91**	**-1.28**	**0.34**	**4.06**	**-0.79**	**3.27**	**0.49**
A	**I54A**	**3.44**	**-0.25**	**-0.33**	**0.12**	**2.97**	**-0.34**	**2.63**	**0.20**
A	**M115A**	**2.70**	**-1.03**	**0.93**	**0.11**	**2.12**	**-0.50**	**2.22**	**0.68**
A	**Y123A**	**2.62**	**-0.22**	**-0.33**	**0.28**	**2.35**	**-0.22**	**2.13**	**0.19**
A	Y56A	1.88	0.69	-0.91	0.20	1.86	-0.34	1.52	0.82
A	E58A	0.26	16.63	-15.58	0.06	1.37	-0.00	1.37	0.39
A	V68A	1.47	-0.07	-0.18	0.00	1.22	-0.14	1.08	0.32
A	Q66A	0.77	4.41	-4.17	0.12	1.13	-0.08	1.05	0.24
(B) BMS-936559									
H	**F55A**	**5.63**	**0.99**	**-2.39**	**0.44**	**6.67**	**-0.78**	**5.90**	**0.31**
H	**H59A**	**3.02**	**0.93**	**-1.28**	**0.36**	**3.04**	**-0.42**	**2.62**	**0.39**
H	**I54A**	**3.41**	**0.08**	**-0.84**	**0.10**	**2.75**	**-0.48**	**2.27**	**0.84**
H	F108A	1.55	-0.03	0.22	-0.04	1.70	-0.15	1.55	0.37
H	I52A	2.38	0.05	-0.71	-0.07	1.65	-0.16	1.49	0.24
L	W94A	2.39	2.70	-3.11	0.20	2.18	-0.88	1.30	1.22
H	K57A	1.77	10.84	-10.33	0.53	2.81	-1.56	1.25	0.57

[a]Standard deviation of $\Delta\Delta G$.

Table S4. Comparation of hotspots and warm spots in PD-L1/KN035 (PDB code 5JDS) and PD-L1/atezolizumab (PDB code 5XXY) by ASIE, m-CSM and Fold-X methods. The data with **ΔΔG** value greater than 2 kcal/mol are bold, and the hotspots in the column of "Mutation" are bold. All values are in kcal/mol.

Chain	Mutation	$\Delta\Delta G_{ASIE}$	$\Delta\Delta G_{m\text{-}CSM}$	$\Delta\Delta G_{Fold\text{-}X}$	$\Delta\Delta G_{exp}$[a]
(A) PD-L1 in PD-L1/KN035					
A	**Y56A**	**4.62**	**3.64**	**5.92**	**3.57**
A	**R113A**	**4.59**	-0.06	**2.35**	**3.07**
A	**Q66A**	**3.97**	1.24	**2.54**	**3.02**
A	**M115A**	**3.20**	0.51	**3.95**	1.73
A	**Y123A**	**3.12**	1.27	0.38	1.58
A	**I54A**	**2.72**	0.67	**3.24**	**2.60**
A	**E58A**	**2.14**	1.62	1.49	**2.31**
A	N63A	1.46	0.36	-0.55	1.21
A	D73A	1.33	0.37	-1.02	ND[c]
A	V68A	1.08	0.54	2.33	1.31
A	S117A	0.77	0.33	-1.32	0.85
A	D61A	0.42	1.91	1.60	1.12
MAE[b]		**0.76**	**1.10**	**1.26**	
R[d]		**0.88**	**0.42**	**0.73**	
(B) KN035					
B	**F101A**	**7.25**	**4.63**	**4.07**	ND[c]
B	**Q116A**	**4.39**	1.54	-0.96	ND[c]
B	T105A	1.50	**2.14**	0.79	ND[c]
B	V109A	1.25	1.00	1.44	ND[c]
B	L108A	1.16	0.53	1.15	ND[c]
B	S111A	1.16	1.39	-0.74	ND[c]
B	F115A	1.13	0.70	**3.44**	ND[c]
B	R32A	0.96	1.30	1.78	ND[c]
(C) PD-L1 in PD-L1/atezolizumab					
A	**M115A**	**4.07**	0.52	**3.04**	0.90
A	**R113A**	**3.61**	1.68	-2.06	1.27
A	**Y56A**	**2.50**	0.49	**2.61**	0.59
A	**E58A**	**2.10**	**3.22**	-0.36	1.72
A	I54A	1.69	0.32	**2.12**	0.69
A	Y123A	1.65	**2.63**	0.77	0.92
A	R125A	1.58	**2.20**	0.19	1.06
A	D61A	1.19	0.58	-0.97	0.00
A	N63A	0.89	0.63	-2.51	0.31
A	Q66A	0.61	0.62	-1.96	-0.82
MAE[b]		**1.33**	**0.80**	**1.70**	
R[d]		**0.6**	**0.67**	**0.28**	
(D) atezolizumab					
H	**Y54A**	**6.36**	**4.17**	-0.44	ND[c]
H	**W33A**	**3.59**	**3.44**	1.62	ND[c]
H	**W50A**	**2.68**	0.73	1.91	ND[c]
H	**R99A**	**2.62**	**2.60**	-3.05	ND[c]
H	**W101A**	**2.38**	**3.82**	**2.76**	ND[c]

L	L92A	1.55	1.40	0.93	ND[c]
H	Y59A	1.50	1.04	1.84	ND[c]
L	H94A	1.40	1.40	0.91	ND[c]

[a]The experimental data are from Tables 1 and 2.

[b]Mean absolute error.

[c]No data from experiment.

[d]Correlation coefficient.

Table S5. The stabilizing residues (SR) which are important for the folding of mAbs are predicted by SRide method. 0 denotes null spot, and 1 denotes SR, BR or KR detected by the SRide and ASIE method.

Residue	SR[a]	BR[b]	KR[c]
(A) PD-L1/KN035			
$_{KN035}$W36	**1**	**0**	**0**
(B) PD-L1/atezolizumab			
$_{H}$W36	**1**	**0**	**0**
$_{H}$V126	1	0	0
$_{H}$C145	1	0	0
$_{H}$Y150	1	0	0
$_{H}$Y181	1	0	0
$_{H}$S182	1	0	0
$_{H}$C201	1	0	0
$_{L}$W35	1	0	0
$_{L}$V115	1	0	0
(C) PD-L1/avelumab			
ND[d]	ND[d]	ND[d]	ND[d]
(D) PD-L1/durvalumab			
$_{B}$W36	**1**	**0**	**0**
$_{B}$V129	1	0	0
$_{B}$C148	1	0	0
$_{B}$V150	1	0	0
$_{B}$Y153	1	0	0
$_{B}$Y184	1	0	0
$_{B}$C204	1	0	0
$_{C}$L34	1	0	0
$_{C}$W36	1	0	0
$_{C}$I49	1	0	0
$_{C}$Q90	1	0	0
$_{C}$Q91	1	0	0
$_{C}$C135	1	0	0
(E) PD-L1/BMS-936559			
$_{H}$W36	**1**	**0**	**0**
$_{H}$M48	1	0	0
$_{H}$R98	1	0	0
$_{H}$V131	1	0	0
$_{H}$C150	1	0	0
$_{H}$V152	1	0	0
$_{H}$Y186	1	0	0
$_{H}$S187	1	0	0
$_{H}$C206	1	0	0
$_{L}$W35	1	0	0
$_{L}$V114	1	0	0

[a]Stabilizing residues.

[b]Binding residues.

[c]Key residues which are the intersection of SR and BR.

[d]No SR is detected.