Supporting Information

Molecular determinants of binding to the *Plasmodium* subtilisin-like protease 1

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Table S1. Binding free energies of PfSUB1-peptide complexes calculated for different parameters.

	$\Delta G_{GB\text{-}Eff}$				$\Delta G_{PB\text{-}Eff}$
Internal dielectric constant	e_{in} = 1	e_{in} = 2	e_{in} = 3	e_{in} = 4	e_{in} = 2
Native sequences					
LVSAD↓NIDIS	-81.5	-89.6	-92.3	-93.7	-63.21
KITAQ↓DDEES	-66.4	-79.1	-83.3	-85.5	-61.71
EIKAE↓TEDDD	-67.5	-77.8	-81.2	-82.9	-60.2
KVKAQ↓DDFNP	-77.2	-91.8	-96.6	-99.0	-73.1
VVTGE↓AISVT	-84.4	-95.3	-99.0	-100.8	-76.17
Prime side variations					
KITAQ↓ADEES		-78.9			-62.62
KITAQ↓ADAES		-89.1			-73.34
KITAQ↓ADDES		-76.4			-63.84
KITAQ↓AAAAA		-68.3			-54.78
KITAQ↓D		-56.5			-46.53

All values are given in kcal/mol; calculated for trajectory range 10-50 ns. Downward-pointing arrow denotes the scissile bond.

$\Delta G_{GB/PB\text{-}Eff}$ = sum of the gas-phase interaction energy and the electrostatic and non-polar contributions to desolvation upon peptide binding ($\Delta G_{GB\text{-}Eff} = \Delta G_{MM} + \Delta G_{GB} + \Delta G_{SA;}$ $\Delta G_{PB\text{-}Eff} = \Delta G_{MM} + \Delta G_{PB} + \Delta G_{SA}$)

Table S2: Per-residue contribution for residues of substrate peptides bound to the PfSUB1 binding site

	P5	P4	P3	P2	P1	P1´	P2´	P3´	P4´	P5´
LVSAD↓NIDIS	-4.01	-7.68	-2.68	-3.78	-4.99	-4.70	-4.27	-2.77	-0.86	-2.62
KITAQ↓DDEES	-2.72	-8.73	-3.13	-4.60	-7.33	-3.56	-1.87	-0.52	-1.63	-0.30
EIKAE↓TEDDD	-2.61	-7.73	-3.08	-4.45	-4.96	-5.47	-2.49	-0.49	-0.47	-0.28
KVKAQ↓DDFNP	-3.93	-8.37	-4.82	-4.39	-8.16	-3.51	-2.41	-0.65	-4.80	-0.57
VVTGE↓AISVT	-4.66	-8.45	-4.95	-2.86	-6.60	-4.36	-5.61	-1.55	-3.59	-1.30
Mean	-3.6	-8.2	-3.7	-4.0	-6.4	-4.3	-3.3	-1.2	-2.3	-1.0
STD	0.9	0.5	1.1	0.7	1.4	0.8	1.6	1.0	1.9	1.0

All values are given in kcal/mol; calculated for trajectory range 10-50 ns and using $e_{in} = 2$.

Table S3: Individual energy contributions for binding free energy of the LVSAD↓NIDIS peptide.

	P5	P4	P3	P2	P1	P1´	P2´	P3´	P4´	P5´
MM-ELE	-0.66	-1.82	-2.46	-2.50	-19.04	-7.34	-0.03	-14.92	0.99	0.42
MM-VDW	-3.94	-6.64	-3.29	-3.59	-5.78	-4.75	-3.80	-1.70	-1.16	-3.09
MM-INT	0	0	0	0	0	0	0	0	0	0
GAS	-4.59	-8.46	-5.75	-6.09	-24.82	-12.09	-3.83	-16.62	-0.17	-2.67
GBSUR	-0.61	-1.09	-0.35	-0.53	-1.11	-0.58	-0.72	-0.41	-0.13	-0.64
GB	1.20	1.88	3.42	2.84	20.94	7.96	0.28	14.26	-0.56	0.68
GBSOL	0.59	0.78	3.07	2.31	19.83	7.38	-0.44	13.85	-0.69	0.04
GBELE	0.54	0.06	0.96	0.34	1.90	0.62	0.25	-0.66	0.43	1.10
ΔGBELE	0.28	0.06	0.52	0.2	1.27	0.41	0.12	-0.08	0.22	0.56
GB-Eff	-4.01	-7.68	-2.68	-3.78	-4.99	-4.70	-4.27	-2.77	-0.86	-2.62

All values are given in kcal/mol; calculated for trajectory range 10-50 ns and using e_{in} = 2.

MM-ELE = electrostatic energy as calculated by the molecular mechanics force field

MM-VDW = van der Waals contribution from the molecular mechanics force field

MM-INT = internal energy in the molecular mechanics force field

GAS = sum of MM-ELE, MM-VDW and MM-INT (total gas phase energy)

GBSUR = non-polar contribution to the solvation free energy

GB = electrostatic contribution to the solvation free energy

GBSOL = sum of GBSUR and GB

GBELE = sum of MM-ELE and GB

ΔGBELE = Difference of GBELE using e_{in} = 2 and GBELE using e_{in} = 4

GB-Eff = sum of GAS and GBSOL

Table S4: Individual energy contributions for binding free energy of the KITAQ↓DDEES peptide.

	P5	P4	P3	P2	P1	P1′	P2′	P3′	P4′	P5′
MM-ELE	-6.12	-1.79	-1.48	-2.96	-6.20	-22.09	-16.60	-17.81	-25.85	-0.10
MM-VDW	-3.46	-7.47	-3.59	-4.05	-7.81	-4.05	-2.27	-0.82	-1.81	-0.56
MM-INT	0	0	0	0	0	0	0	0	0	0
GAS	-9.58	-9.26	-5.07	-7.01	-14.01	-26.14	-18.87	-18.63	-27.67	-0.66
GBSUR	-0.47	-1.28	-0.36	-0.63	-1.10	-0.72	-0.41	-0.21	-0.60	-0.10
GB	7.33	1.81	2.30	3.04	7.79	23.30	17.41	18.32	26.64	0.46
GBSOL	6.86	0.53	1.94	2.41	6.69	22.58	16.99	18.11	26.04	0.36
GBELE	1.21	0.02	0.82	0.08	1.59	1.21	0.81	0.51	0.79	0.36
ΔGBELE	0.62	0.04	0.44	0.07	0.89	0.95	0.64	0.51	0.75	0.19
GB-Eff	-2.72	-8.73	-3.13	-4.60	-7.33	-3.56	-1.87	-0.52	-1.63	-0.30

All values are given in kcal/mol; calculated for trajectory range 10-50 ns and using e_{in} = 2.

Table S5: Individual energy contributions for binding free energy of the EIKAE↓TEDDD peptide.

	P5	P4	P3	P2	P1	P1′	P2′	P3′	P4′	P5′
MM-ELE	11.23	-1.99	-10.83	-2.10	0.10	-3.66	-16.82	-6.50	-11.58	-5.43
MM-VDW	-3.58	-6.54	-4.02	-3.86	-6.82	-3.44	-3.41	-1.34	-0.83	-0.58
MM-INT	0	0	0	0	0	0	0	0	0	0
GAS	7.65	-8.52	-14.85	-5.96	-6.72	-7.10	-20.23	-7.84	-12.41	-6.01
GBSUR	-0.61	-1.16	-0.50	-0.54	-1.03	-0.73	-0.83	-0.23	-0.22	-0.14
GB	-9.66	1.95	12.26	2.06	2.79	2.36	18.57	7.58	12.15	5.86
GBSOL	-10.27	0.79	11.77	1.51	1.76	1.63	17.74	7.35	11.93	5.73
GBELE	1.57	-0.04	1.43	-0.04	2.89	-1.30	1.75	1.08	0.57	0.43
ΔGBELE	0.8	0	0.77	0.01	1.57	-0.62	1.15	0.7	0.47	0.33
GB-Eff	-2.61	-7.73	-3.08	-4.45	-4.96	-5.47	-2.49	-0.49	-0.47	-0.28

All values are given in kcal/mol; calculated for trajectory range 10-50 ns and using e_{in} = 2.

Table S6: Individual energy contributions for binding free energy of the KVKAQ↓DDFNP peptide.

	P5	P4	P3	P2	P1	P1´	P2´	P3´	P4´	P5´
MM-ELE	-17.25	-2.17	-14.81	-2.63	-5.09	-11.30	-9.68	-0.59	-4.15	-0.19
MM-VDW	-4.46	-7.18	-4.73	-4.12	-8.82	-3.78	-4.08	-1.26	-4.60	-0.62
MM-INT	0	0	0	0	0	0	0	0	0	0
GAS	-21.72	-9.35	-19.54	-6.74	-13.91	-15.07	-13.76	-1.86	-8.75	-0.81
GBSUR	-0.67	-1.14	-0.59	-0.58	-1.26	-0.74	-0.72	-0.04	-0.92	-0.03
GB	18.46	2.11	15.31	2.93	7.01	12.30	12.07	1.25	4.87	0.27
GBSOL	17.79	0.98	14.72	2.35	5.75	11.56	11.35	1.20	3.94	0.24
GBELE	1.21	-0.06	0.50	0.30	1.92	1.00	2.39	0.66	0.72	0.08
ΔGBELE	0.71	0	0.35	0.18	1.04	0.74	1.39	0.35	0.42	0.05
GB-Eff	-3.93	-8.37	-4.82	-4.39	-8.16	-3.51	-2.41	-0.65	-4.80	-0.57

All values are given in kcal/mol; calculated for trajectory range 10-50 ns and using e_{in} = 2.

Table S7: Individual energy contributions for binding free energy of the VVTGE↓AISVT peptide.

	P5	P4	P3	P2	P1	P1´	P2´	P3´	P4´	P5´
MM-ELE	-0.84	-1.60	-3.40	-3.40	-14.09	-3.56	-1.94	-0.64	-0.42	-1.73
MM-VDW	-4.81	-7.54	-4.68	-2.89	-7.95	-3.62	-4.64	-2.33	-3.64	-1.44
MM-INT	0	0	0	0	0	0	0	0	0	0
GAS	-5.64	-9.13	-8.08	-6.29	-22.03	-7.19	-6.58	-2.98	-4.06	-3.17
GBSUR	-0.68	-1.11	-0.61	-0.46	-1.13	-0.66	-0.69	-0.41	-0.57	-0.30
GB	1.67	1.79	3.74	3.89	16.56	3.48	1.66	1.84	1.05	2.17
GBSOL	0.98	0.68	3.13	3.43	15.43	2.83	0.97	1.43	0.47	1.87
GBELE	0.83	0.19	0.34	0.49	2.47	-0.08	-0.28	1.20	0.63	0.44
ΔGBELE	0.44	0.12	0.21	0.29	1.49	0	-0.12	0.62	0.33	0.24
GB-Eff	-4.66	-8.45	-4.95	-2.86	-6.60	-4.36	-5.61	-1.55	-3.59	-1.30

All values are given in kcal/mol; calculated for trajectory range 10-50 ns and using e_{in} = 2.

Table S8: Individual energy contributions for binding free energy.

	LVSAD↓ NIDIS	KITAQ↓ DDEES	EIKAE↓ TEDDD	KVKAQ↓ DDFNP	VVTGE↓ AISVT
MM-ELE	-99.7 ± 0.3	-208.3 ± 0.7	-101.9 ± 0.9	-140.3 ± 0.3	-70.0 ± 0.2
MM-VDW	-82.2 ± 0.1	-75.2 ± 0.2	-72.8 ± 0.2	-90.6 ± 0.1	91.6 ± 0.1
MM-INT	0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0 ± 0
GAS	-181.9 ± 0.3	-283.5 ± 0.8	-174.7 ± 1.0	-230.9 ± 0.4	-161.6 ± 0.2
GBSUR	-12.4 ± 0	-11.1 ± 0	-11.3 ± 0	-12.3 ± 0	-12.6 ± 0
GB	104.7 ± 0.3	215.4 ± 0.7	108.2 ± 0.9	151.5 ± 0.3	78.8 ± 0.2
GBSOL	92.3 ± 0.2	204.4 ± 0.7	96.9 ± 0.9	139.2 ± 0.3	66.2 ± 0.2
GBELE	5.0 ± 0.1	7.2 ± 0.1	6.3 ± 0.1	11.2 ± 0.1	8.8 ± 0.1
GB-Eff	-89.6 ± 0.1	-79.1 ± 0.2	-77.8 ± 0.2	-91.8 ± 0.1	-95.3 ± 0.1

All values are given in kcal/mol (± standard error of the mean); calculated for trajectory range 10-50 ns using e_{in} = 2.

Table S9: Per-residue contribution for residues of the PfSUB1 binding site bound to different peptide substrates.

	LVSAD↓ NIDIS	KITAQ↓ DDEES	EIKAE↓ TEDDD	KVKAQ↓ DDFNP	VVTGE↓ AISVT
Tyr427	-2.02	-	-	-	-
Lys465	**-4.18**	**-4.41**	-2.66	-3.43	-2.73
Leu466	-2.91	-3.19	-3.73	-3.28	-3.01
Arg468	-	-	**-4.22**	-	-
Leu469	-2.18	-2.53	-2.18	-	-2.78
Phe491	**-4.04**	-3.21	-3.00	-2.82	-3.05
Ser492	-3.38	-	-	-3.33	-3.08
Phe493	-2.03	-	-	-2.92	-3.31
Asn520	-	-2.35	-	-2.51	-2.96
Lys601	-	-	-	-	-2.04
Leu602	-	-	-	-2.02	-2.98
Asn603	-	-2.71	-2.99	**-5.29**	-2.86
Ser606	-	-	-2.16	-	-

All values are given in kcal/mol; calculated for trajectory range 10-50 ns and using $e_{in} = 2$. Effective free energies are only listed if $\Delta G_{Eff} \leq -2$ kcal/mol and depicted in bold if $\Delta G_{Eff} \leq -4$ kcal/mol.

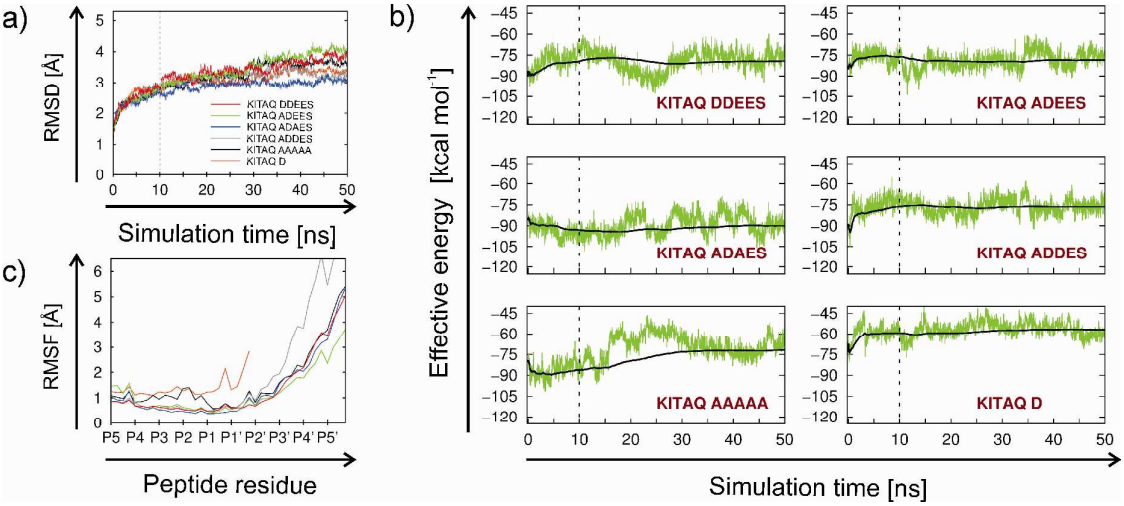

Figure S1: Convergence and stability examination for MD simulations of PfSUB1-peptide complex structures (with sequence variations at the prime side of the KITAQ↓DDEES peptide). (a) Time series of RMSD values of PfSUB1-peptide complexes are shown with respect to structures obtained at the end of the equilibration procedure. (b) Effective binding free energies (green) are shown together with accumulated mean values (red) for PfSUB1-peptide complexes. (c) Atomic fluctuations (RMSF) of peptide backbone atoms during MD trajectories of the respective PfSUB1-peptide complex structure. Color-coding of PfSUB1-peptide complexes is as in (a). Vertical lines in (a) and (b) indicate the time after which snapshots were extracted for further analysis.

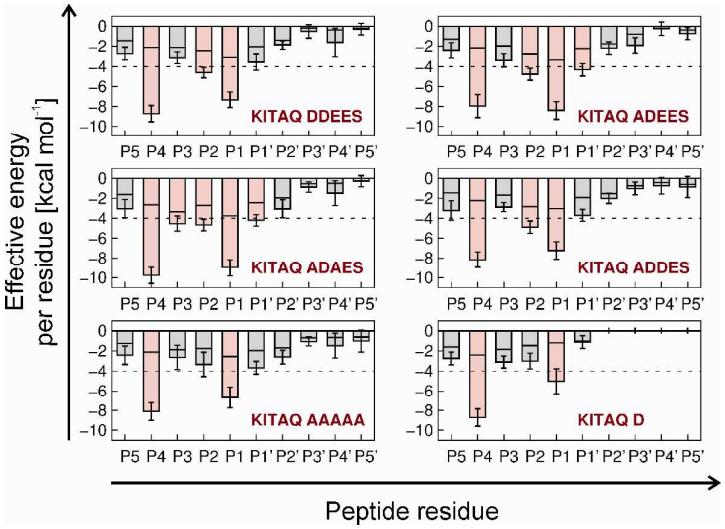

Figure S2: Per-residue contribution to the binding effective energy of PfSUB1-peptide complexes (with sequence variations at the prime side of the KITAQ↓DDEES peptide) are depicted as bar plots. Per-residue contributions were calculated by the MM-GBSA decomposition method. Residues whose contributions to the effective energy $\Delta G_{Eff} \leq$ -4 kcal/mol are depicted in red. The backbone and side-chain contributions to the effective free energy are indicated by partitioning the bar plots. The areas at the top of the bar plots correspond to the backbone and the areas at the bottom correspond to the side chain contributions, respectively. The per-residue contributions were calculated by applying the MM-GBSA decomposition approach to MD trajectories of PfSUB1 in complex with the KITAQ↓DDEES peptide and sequence variations at the prime side of this peptide.

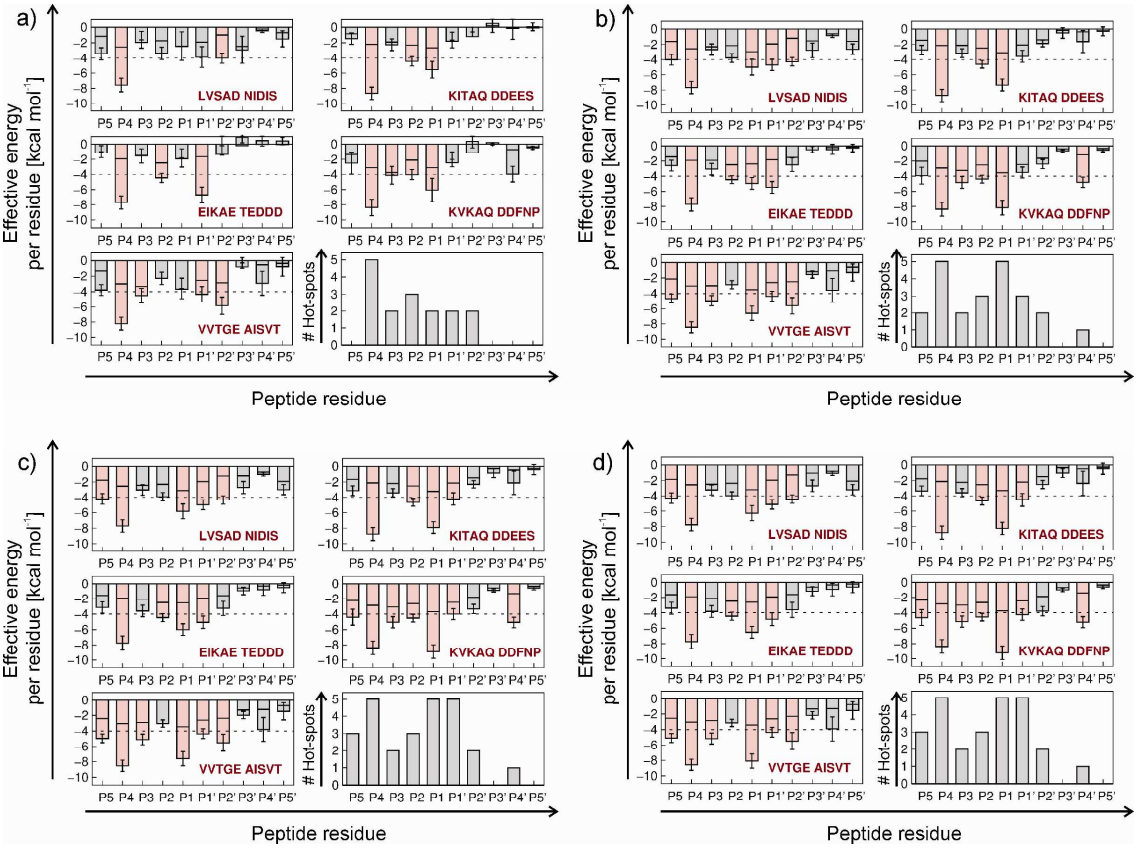

Figure S3: Per-residue contribution to the binding effective energy of PfSUB1-peptide complexes (calculated by using different internal dielectric constants e_{in}) are depicted as bar plots. Per-residue contributions were calculated by the MM-GBSA decomposition method by using a) e_{in} = 1, b) e_{in} = 2, c) e_{in} = 3, and d) e_{in} = 4. Residues whose contributions to the effective energy $\Delta G_{Eff} \leq$ -4 kcal/mol are depicted in red. The backbone and side-chain contributions to the effective free energy are indicated by partitioning the bar plots. The areas at the top of the bar plots correspond to the backbone and the areas at the bottom correspond to the side-chain contributions, respectively. The per-residue contributions were calculated by applying the MM-GBSA decomposition approach to MD trajectories of PfSUB1 in complex with five substrate peptides (red labels). The distribution of hot spot residues (chosen cut-off: $\Delta G_{Eff} \leq$ -4 kcal/mol) along the investigated substrates is shown in the lower right panel, respectively.