Supporting Information

Indirect Readout in Protein-Peptide Recognition: A Different Story from Classical Biomolecular Recognition

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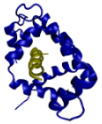
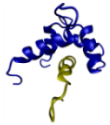
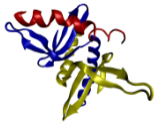
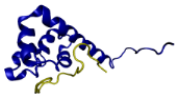
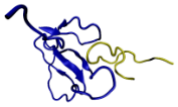
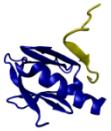
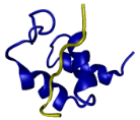
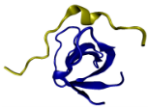
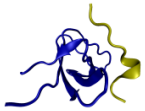
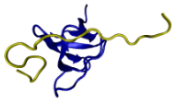
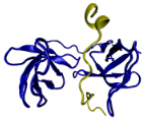
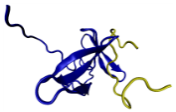
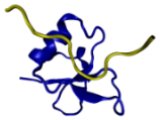
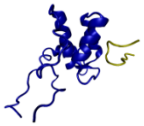
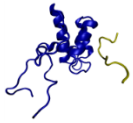
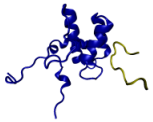
Table S1. Structures of 20 Protein-Peptide Complexes and Their Experimental Conditions and Assay Methods of Dissociation Constants (K_d).

PDB code	Structure	Experimental conditions and method of K_d				
		T (°C)	pH	Buffer	Concentration	Assay method
1BXL		NS	NS	NS	NS	Fluorescence
1SY9		RT	7.3	50 mM tris-Cl, 150 mM NaCl and 0.5 mM $CaCl_2$	3.3 nM dansyl-CaM	Fluorescence
2K2I		30	7.4	50 mM Mops, 100 mM NaCl and 1 mM $CaCl_2$	Peptide was 10 times more concentrated than centrin	ITC
2PHE		20	6.2	20 mM potassium phosphate, 50 mM KCl, 0.2 mM PMSF, 1 mM DTT, 0.2 µg/µL BSA	0.005 µg/µL GST-VP16, the indicated amounts of PC4	GST-pull-down assay
1HAA		NS	7.4	10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% polysorbate 20	10 µg/mL α-bungarotoxin, concentrations of peptides range from 6.5 to 0.06 µM	SPR
1JGN		25	7.4	10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% Tween 20	NS	SPR

1KC4		RT	7.4	NS	2.5 nM Bgtx, a wide range of concentrations of peptide	Competition experiment
2ASQ		30	7.4	20 mM phosphate, 1mM 2-mercaptoethanol	~ 0.4 mM peptide, ~ 0.03 mM SUMO-1	ITC
2K00		20	6.5	50 mM potassium phosphate, 100 mM NaCl	2 -16 μM talin F3	Fluorescence spectroscopy
2KHH		25	7.5	50 mM Tris-buffer containing 150 nM NaCl, 4% glycerol	100 to 4000 nM peptide, 400 nM UBA-Mex67	Fluorescence titration
1JEG		NS	7.2	20 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 5mM EDTA, 2 mM ADP and 0.02% NaN_3	NS	Fluorescence titration
1H3H		NS	NS	NS	NS	Fluorescence polarization
1WA7		18	NS	PBS, 1 mM DTT	5 mg/mL peptide, 16.7 μg/mL GST-SH3 domain fusion proteins	Fluorescence spectroscopy
1WLP		25	7.0	20 mM sodium phosphate	3.0 μM protein ($p47^{phox}$ -(151–286))	Fluorescence titration
2KXQ		20	7.2	40 mM Na_2HPO_4, 20 mM NaCl, 1 mM fresh β-mercaptoethanol	NS	Fluorescence

1ZSG		23	NS	NS	1 μM SH3, 2 mM peptide	Fluorescence spectroscopy
1L2Z		25	7.4	5 mM sodium phosphate, 150 mM NaCl	NS	NMR titration
2KFH		25	NS	NS	100 μM EH-domain	NMR titration
2KFG		25	NS	NS	100 μM EH-domain	NMR titration
2KFF		25	NS	NS	100 μM EH-domain	NMR titration

Abbreviations: RT, room temperature; NS, not stated; DTT, dithiothreitol; PBS, phosphate-buffered saline; CaM, calmodulin; GST, glutathion S-transferase; SH3, src-homology domain 3; VP16, herpes simplex virion protein 16; PC4, human positive cofactor 4; Bgtx, α-bungarotoxin; SUMO, small ubiquitin-like modifier; UBA-Mex67, ubiquitin-associated domain of the principal saccharomyces cerevisiae mRNA nuclear export factor; EH-domain, Eps15 homology domain; ITC, isothermal titration calorimetry; SPR, surface plasmon resonance; NMR, nuclear magnetic resonance.

Table S2. Detailed Results of All the Energetic Components.

		1BXL		1SY9		2K2I		2PHE	
		Average	Err. Est.	Average	Err. Est.	Average	Err. Est.	Average	Err. Est.
Complex simulation	E_{ele}	-1508.8	33	-9478.53	72	-1057.16	33	-4963.01	250
	E_{van}	-431.426	1.8	-664.645	5.3	-324.043	2.7	-313.332	7.1
	$E_{non\text{-}bonded}$	-1940.226	–	-10143.175	–	-1381.203	–	-5276.342	–
	G_{polar}(complex)	-12781.5	65	-23715.8	110	-10435.5	67	-10371.9	130
	$G_{non\text{-}polar}$(complex)	166.995	1.2	152.262	1.5	125.31	0.81	160.466	0.97
	G_{polar}(protein)	-12304.4	100	-30437.3	71	-10080	45	-9989.89	140
	$G_{non\text{-}polar}$(protein)	170.186	1.2	167.516	1.3	117.595	0.52	146.669	1.8
	G_{polar}(peptide)	-2056.35	16	-2877.07	19	-1524.11	35	-5442.44	85
	$G_{non\text{-}polar}$(peptide)	44.9235	0.23	61.1037	0.62	49.5407	0.61	65.7351	0.69
	ΔG_{solv}	1531.1355	–	9522.2123	–	1126.7843	–	5008.4919	–
Peptide simulation	S_{pep}(bound)	4155.43	–	4711.85	–	5553.32	–	7124.22	–
	E_{pep}(bound)	-2001.26	3.9	-2075.1	4.9	-899.355	5.4	-1132.17	15
	S_{pep}(unbound)	4813.36	–	7159.25	–	6126.25	–	7901.85	–
	E_{pep}(unbound)	-2042.98	8.1	-2123.35	7.6	-990.92	5.5	-1230.49	5.6
Result	ΔG_{dir}	-409.0905	–	-620.9627	–	-254.4187	–	-267.8501	–
	ΔG_{indir}	197.379	–	734.22	–	171.879	–	233.289	–
	$\Delta G_{binding}$	-211.7115	–	113.2573	–	-82.5397	–	-34.5611	–
	ΔE_{deform}	41.72	–	48.25	–	91.565	–	98.32	–

Energies are in kJ/mol, except S_{pep}(bound) and S_{pep}(unbound) in J/(mol K). T = 300 K.

		1HAA		1JGN		1KC4		2K00	
		Average	Err. Est.	Average	Err. Est.	Average	Err. Est.	Average	Err. Est.
Complex simulation	E_{ele}	-1274.64	16	-1672.48	65	-641.601	59	-976.651	75
	E_{van}	-315.882	9.1	-406.315	5	-207.396	6.1	-274.381	1.4
	$E_{non\text{-}bonded}$	-1590.522	–	-2078.795	–	-848.997	–	-1251.032	–
	G_{polar}(complex)	-3365.64	35	-5402.7	79	-4538.58	71	-7628.27	140
	$G_{non\text{-}polar}$(complex)	81.3076	0.52	132.556	0.58	110.032	1.2	118.599	0.75
	G_{polar}(protein)	-3255.14	11	-5362.93	51	-3538.91	19	-6853.08	41
	$G_{non\text{-}polar}$(protein)	80.651	0.64	128.911	0.48	95.3207	0.44	111.946	0.45
	G_{polar}(peptide)	-1451.87	12	-1917.01	27	-1732.78	25	-1754.61	37
	$G_{non\text{-}polar}$(peptide)	36.939	0.38	49.3436	0.24	40.9286	0.12	44.6013	0.86
	ΔG_{solv}	1305.0876	–	1831.5414	–	706.8927	–	941.4717	–
Peptide simulation	S_{pep}(bound)	3285.33	–	6205.38	–	4747.98	–	4064.62	–
	E_{pep}(bound)	-782.187	2.6	121.259	5.8	-738.913	8.9	-1305.64	9.1
	S_{pep}(unbound)	3803.93	–	6355.73	–	5158.67	–	4699.52	–
	E_{pep}(unbound)	-786.933	2.5	-1.00744	12	-824.878	13	-1371.76	7.5
Result	ΔG_{dir}	-285.4344	–	-247.2536	–	-142.1043	–	-309.5603	–
	ΔG_{indir}	155.58	–	45.105	–	123.207	–	190.47	–
	$\Delta G_{binding}$	-129.8544	–	-202.1486	–	-18.8973	–	-119.0903	–
	ΔE_{deform}	4.746	–	122.26644	–	85.965	–	66.12	–

Energies are in kJ/mol, except S_{pep}(bound) and S_{pep}(unbound) in J/(mol K). T = 300 K.

		1WA7		1WLP		2KXQ		1ZSG	
		Average	Err. Est.	Average	Err. Est.	Average	Err. Est.	Average	Err. Est.
Complex simulation	E_{ele}	-707.306	23	-5098.47	93	-414.661	20	-1106.59	58
	E_{van}	-293.79	5.9	-434.188	2.7	-361.938	3.2	-261.594	7.6
	$E_{non\text{-}bonded}$	-1001.096	–	-5532.658	–	-776.599	–	-1368.184	–
	G_{polar}(complex)	-4641.43	36	-13653.8	100	-6175.1	120	-4731.95	55
	$G_{non\text{-}polar}$(complex)	88.5263	1.2	166.14	2.1	117.176	1.9	103.242	1.2
	G_{polar}(protein)	-3884.65	8.4	-15888.9	41	-4670.72	97	-4279.3	38
	$G_{non\text{-}polar}$(protein)	73.0413	0.38	152.219	1.4	111.192	2	84.7969	1.7
	G_{polar}(peptide)	-1525.85	34	-2972.86	31	-2046.03	60	-1640.1	19
	$G_{non\text{-}polar}$(peptide)	54.1428	0.096	65.5662	0.52	39.4553	0.53	57.8167	0.35
	ΔG_{solv}	730.4122	–	5156.3148	–	508.1787	–	1148.0784	–
Peptide simulation	S_{pep}(bound)	6117.4	–	7020.62	–	5013.29	–	6824.7	–
	E_{pep}(bound)	-516.662	7.1	-156.798	5.2	-298.236	7.6	-439.517	3.8
	S_{pep}(unbound)	6892.16	–	7944.44	–	5556.32	–	7375.99	–
	E_{pep}(unbound)	-563.24	5.3	-241.245	4.2	-384.419	4.4	-585.026	5.6
Result	ΔG_{dir}	-270.6838	–	-376.3432	–	-268.4203	–	-220.1056	–
	ΔG_{indir}	232.428	–	277.146	–	162.909	–	165.387	–
	$\Delta G_{binding}$	-38.2558	–	-99.1972	–	-105.5113	–	-54.7186	–
	ΔE_{deform}	46.578	–	84.447	–	86.183	–	145.509	–

Energies are in kJ/mol, except S_{pep}(bound) and S_{pep}(unbound) in J/(mol K). T = 300 K.

		1L2Z		2KFH		2KFG		2KFF	
		Average	Err. Est.	Average	Err. Est.	Average	Err. Est.	Average	Err. Est.
Complex simulation	E_{ele}	-1207.2	38	-224.294	32	-396.678	32	-321.04	20
	E_{van}	-197.908	5	-197.278	1.2	-195.317	3.5	-230.793	2
	$E_{non\text{-}bonded}$	-1405.108	–	-421.572	–	-591.995	–	-551.833	–
	G_{polar}(complex)	-4039.59	51	-7438.81	100	-7726.72	38	-7922.85	58
	$G_{non\text{-}polar}$(complex)	85.4218	0.74	122.836	0.57	128.162	0.9	125.668	2.4
	G_{polar}(protein)	-4106.58	38	-6642.33	67	-7287.92	52	-7306.7	54
	$G_{non\text{-}polar}$(protein)	74.5583	0.65	111.167	0.62	117.526	1	119.06	2.3
	G_{polar}(peptide)	-1165.64	3.5	-1100.41	12	-930.421	41	-1022.28	6.7
	$G_{non\text{-}polar}$(peptide)	35.9721	0.22	34.8178	0.065	32.2171	0.82	33.7898	0.46
	ΔG_{solv}	1207.5214	–	280.7812	–	470.0399	–	378.9482	–
Peptide simulation	S_{pep}(bound)	3241.09	–	3261.76	–	3829.5	–	3323.35	–
	E_{pep}(bound)	-499.641	1.1	-490.215	1.7	-712.42	2.4	-698.271	4.5
	S_{pep}(unbound)	3605.57	–	3797.53	–	3991.11	–	3642.34	–
	E_{pep}(unbound)	-517.205	2.3	-496.682	7.1	-752.219	3.9	-718.221	4.3
Result	ΔG_{dir}	-197.5866	–	-140.7908	–	-121.9551	–	-172.8848	–
	ΔG_{indir}	109.344	–	160.731	–	48.483	–	95.697	–
	$\Delta G_{binding}$	-88.2426	–	19.9402	–	-73.4721	–	-77.1878	–
	ΔE_{deform}	17.564	–	6.467	–	39.799	–	19.95	–

Energies are in kJ/mol, except S_{pep}(bound) and S_{pep}(unbound) in J/(mol K). T = 300 K.

		1JEG		2ASQ		2ASQ-10res	
		Average	Err. Est.	Average	Err. Est.	Average	Err. Est.
Complex simulation	E_{ele}	-1211.22	58	-2014.91	130	-2009.32	36
	E_{van}	-320.404	3.5	-236.364	4.3	-244.894	3.7
	$E_{non\text{-}bonded}$	-1531.624	–	-2251.274	–	-2254.214	–
	G_{polar}(complex)	-5091.79	63	-6991.52	120	-6633.43	61
	$G_{non\text{-}polar}$(complex)	93.5408	0.97	109.722	0.98	106.34	0.86
	G_{polar}(protein)	-3593.04	21	-6122.02	87	-6583.68	43
	$G_{non\text{-}polar}$(protein)	83.6373	0.47	99.3637	0.97	102.656	1
	G_{polar}(peptide)	-2767.13	16	-2969.02	21	-2126.86	7.9
	$G_{non\text{-}polar}$(peptide)	52.129	0.28	43.684	0.37	36.0361	0.062
	ΔG_{solv}	1226.1545	–	2066.1943	–	2044.7579	–
Peptide simulation	S_{pep}(bound)	5055.54	–	5021.97	–	3608.51	–
	E_{pep}(bound)	-528.09	0.76	-730.315	5.6	-550.949	3
	S_{pep}(unbound)	5413.21	–	5471.66	–	3712.8	–
	E_{pep}(unbound)	-609.819	4.8	-820.797	7.3	-626.749	7.4
Result	ΔG_{dir}	-305.4695	–	-185.0797	–	-209.4561	–
	ΔG_{indir}	107.301	–	134.907	–	31.287	–
	$\Delta G_{binding}$	-198.1685	–	-50.1727	–	-178.1691	–
	ΔE_{deform}	81.729	–	90.482	–	75.8	–

Energies are in kJ/mol, except S_{pep}(bound) and S_{pep}(unbound) in J/(mol K). T = 300 K.

		1H3H		1H3H-model-3		1H3H-model-18	
		Average	Err. Est.	Average	Err. Est.	Average	Err. Est.
Complex simulation	E_{ele}	-1510.2	48	-1339.56	35	-1821.95	5.2
	E_{van}	-190.117	1.4	-193.212	3.4	-148.494	1.5
	$E_{non\text{-}bonded}$	-1700.317	–	-1532.772	–	-1970.444	–
	G_{polar}(complex)	-4162.1	30	-4146.84	64	-4159.96	39
	$G_{non\text{-}polar}$(complex)	87.1324	0.8	85.771	0.58	87.4552	1.1
	G_{polar}(protein)	-4481.2	51	-4290.5	54	-4606.36	39
	$G_{non\text{-}polar}$(protein)	84.0285	0.9	81.4208	0.69	81.4505	1.1
	G_{polar}(peptide)	-1209.36	7.2	-1233.81	8.1	-1244.63	1.3
	$G_{non\text{-}polar}$(peptide)	32.3553	0.066	32.591	0.31	31.2955	0.013
	ΔG_{solv}	1499.2086	–	1349.2292	–	1665.7392	–
Peptide simulation	S_{pep}(bound)	2622.35	–	2876.44	–	2675.08	–
	E_{pep}(bound)	-582.276	3.3	-574.725	2.8	-548.226	1.3
	S_{pep}(unbound)	3184.25	–	3214.07	–	3554.83	–
	E_{pep}(unbound)	-599.592	2.7	-642.985	2.4	-616.478	5.2
Result	ΔG_{dir}	-201.1084	–	-183.5428	–	-304.7048	–
	ΔG_{indir}	168.57	–	101.289	–	263.925	–
	$\Delta G_{binding}$	-32.5384	–	-82.2538	–	-40.7798	–
	ΔE_{deform}	17.316	–	68.26	–	68.252	–
	Z-value	0.8382	–	0.5519	–	0.8662	–
	W-value	0.0861	–	0.3719	–	0.2240	–

Energies are in kJ/mol, except S_{pep}(bound) and S_{pep}(unbound) in J/(mol K). T = 300 K.

		2KHH		2KHH-model-12		2KHH-model-20	
		Average	Err. Est.	Average	Err. Est.	Average	Err. Est.
Complex simulation	E_{ele}	-250.428	19	-334.509	68	-451.052	26
	E_{van}	-193.379	3	-187.64	9.6	-165.483	2.9
	$E_{non\text{-}bonded}$	-443.807	–	-522.149	–	-616.535	–
	G_{polar}(complex)	-3566.51	18	-3964.75	70	-3839.35	30
	$G_{non\text{-}polar}$(complex)	85.1991	1.6	89.6406	1.9	89.5081	1.4
	G_{polar}(protein)	-2950.38	9.3	-3453.62	45	-3332.54	33
	$G_{non\text{-}polar}$(protein)	78.3117	1.5	83.9942	0.8	82.7981	1.4
	G_{polar}(peptide)	-906.81	5.2	-894.215	23	-968.773	15
	$G_{non\text{-}polar}$(peptide)	32.8018	0.18	33.2622	0.7	34.9995	0.27
	ΔG_{solv}	264.7656	–	355.4692	–	433.6735	–
Peptide simulation	S_{pep}(bound)	2824.75	–	2878.96	–	2931.2	–
	E_{pep}(bound)	-263.827	1.7	-249.612	3.3	-236.277	2.7
	S_{pep}(unbound)	3174.22	–	3205.03	–	3351.5	–
	E_{pep}(unbound)	-298.701	3.8	-299.15	7.5	-290.823	6.9
Result	ΔG_{dir}	-179.0414	–	-166.6798	–	-182.8615	–
	ΔG_{indir}	104.841	–	97.821	–	126.09	–
	$\Delta G_{binding}$	-74.2004	–	-68.8588	–	-56.7715	–
	ΔE_{deform}	34.874	–	49.538	–	54.546	–
	Z-value	0.5856	–	0.5869	–	0.6895	–
	W-value	0.1948	–	0.2972	–	0.2983	–

Energies are in kJ/mol, except S_{pep}(bound) and S_{pep}(unbound) in J/(mol K). T = 300 K.

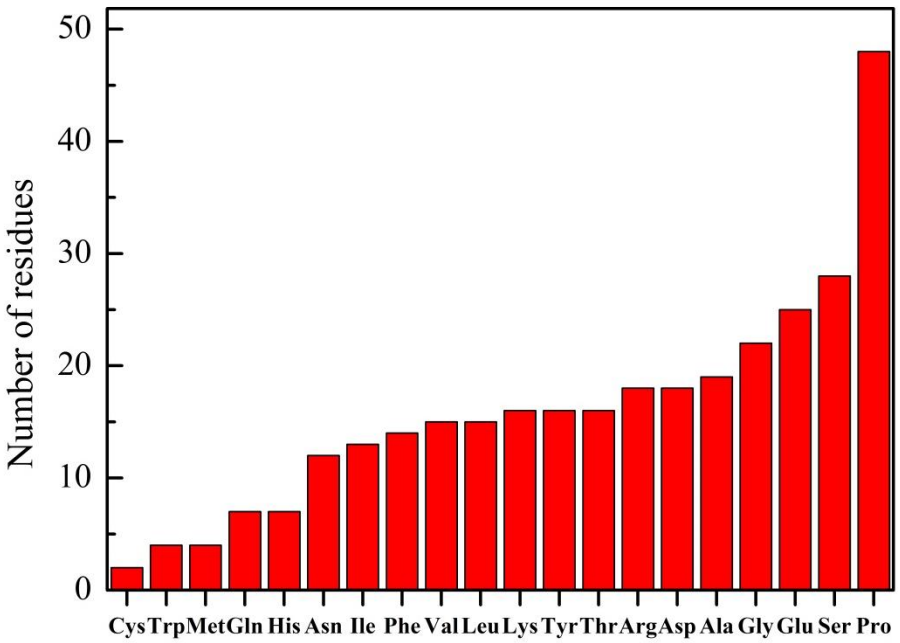
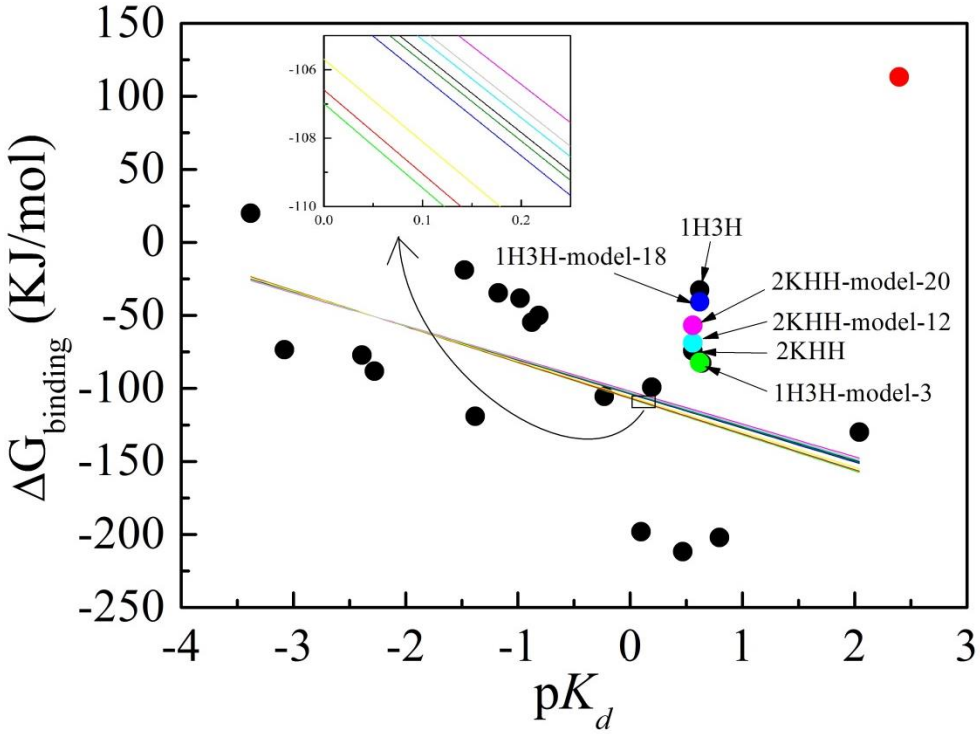
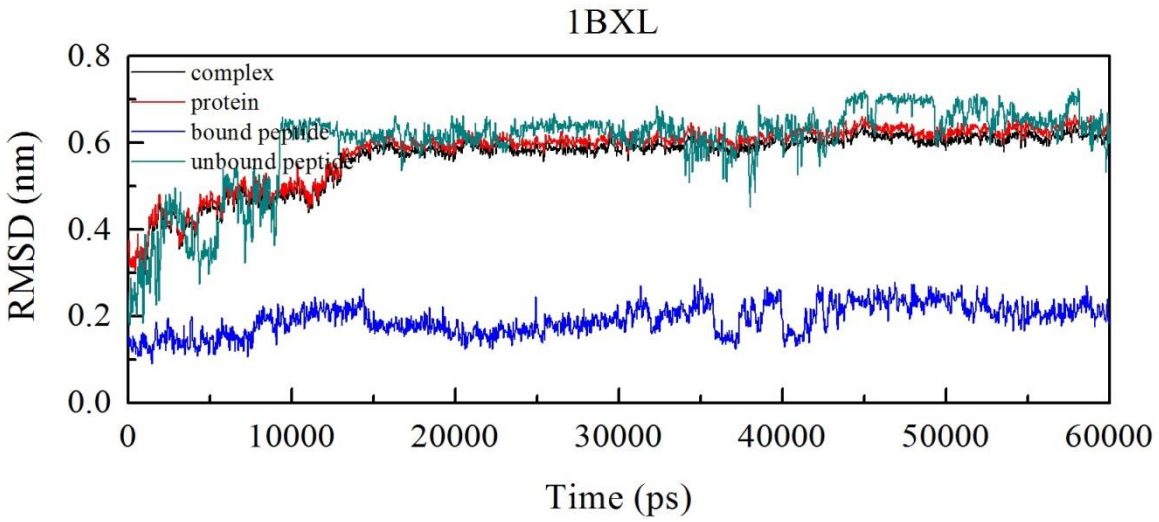


Figure S1. Statistical numbers of various amino acids in peptides of the data set.



r model / model	2KHH	2KHH-model-12	2KHH-model-20
1H3H	-0.527 (black line)	-0.522 (cyan line)	-0.511 (magenta line)
1H3H-model-3	-0.578 (green line)	-0.574 (red line)	-0.562 (yellow line)
1H3H-model-18	-0.536 (blue line)	-0.531 (olive line)	-0.520 (light gray line)

Figure S2. Influence of different models on the correlation analysis. Correlation coefficient was calculated with various combinations of models of complex 1H3H and 2KHH while other complexes remain unchanged (black dots unlabeled in the figure). The red dot represents the discarded 1SY9 complex.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protein
bound peptide
unbound peptide
RMSD (nm)
Time (ps)
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0.6
0.8
0
10000
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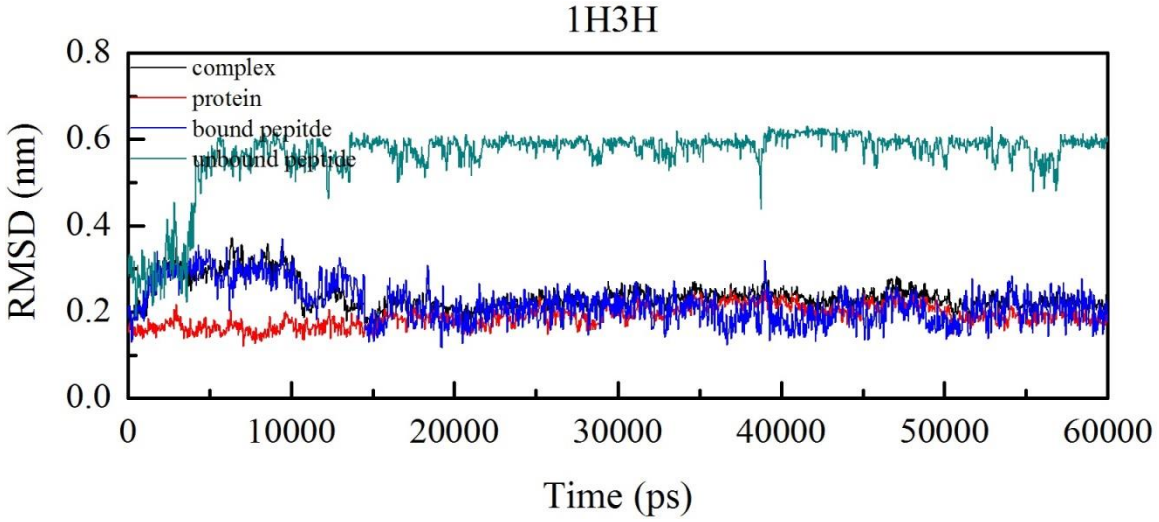
30000
40000
50000
60000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protein
bound pepitde
unbound peptide
RMSD (nm)
Time (ps)
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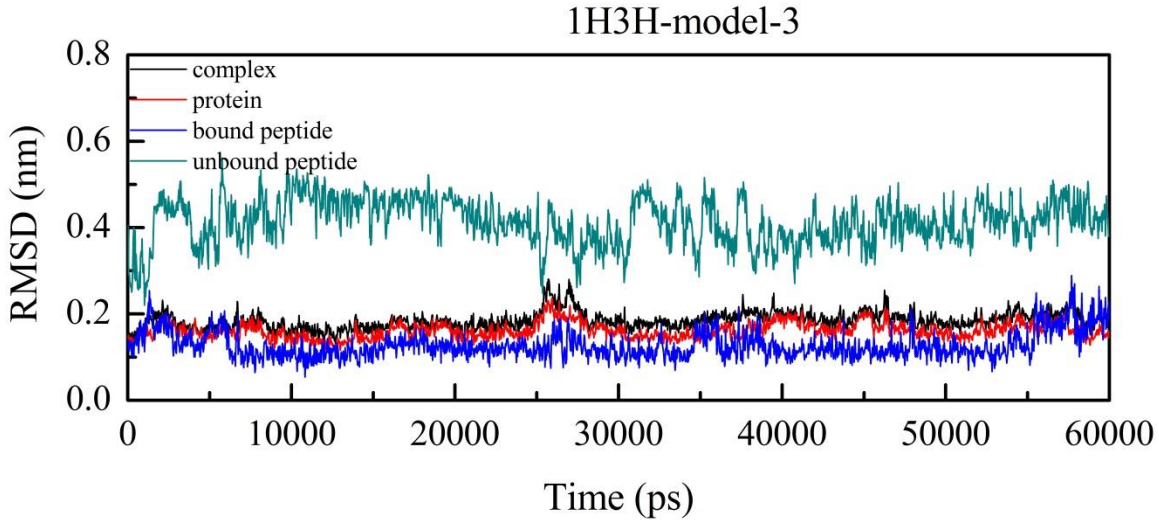
40000
50000
60000


1H3H-model-3
complex
protein
bound peptide
unbound peptide
RMSD (nm)
Time (ps)
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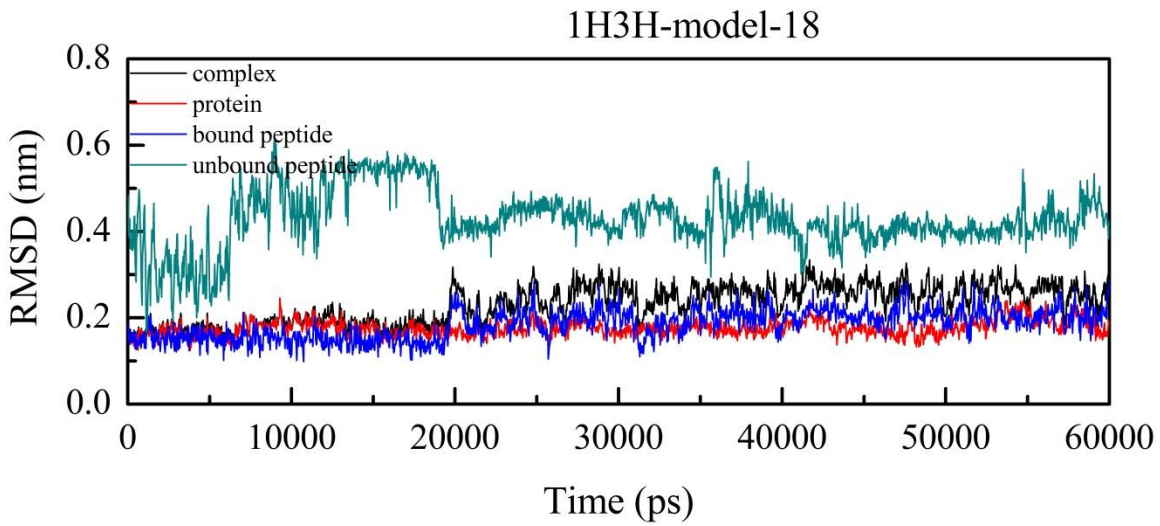
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bound peptide
unbound peptide
RMSD (nm)
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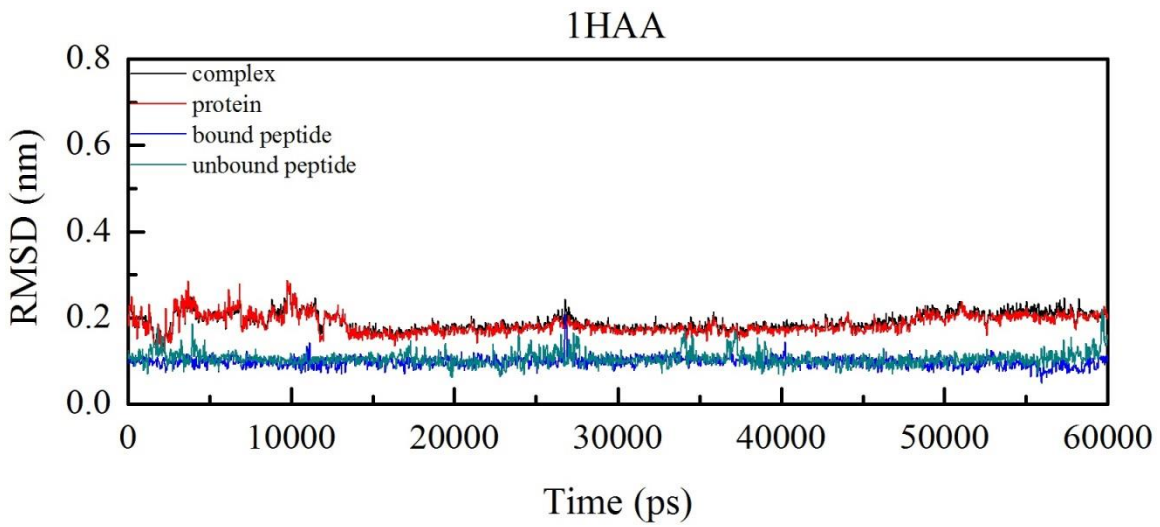
50000
60000
Time (ps)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unbound peptide
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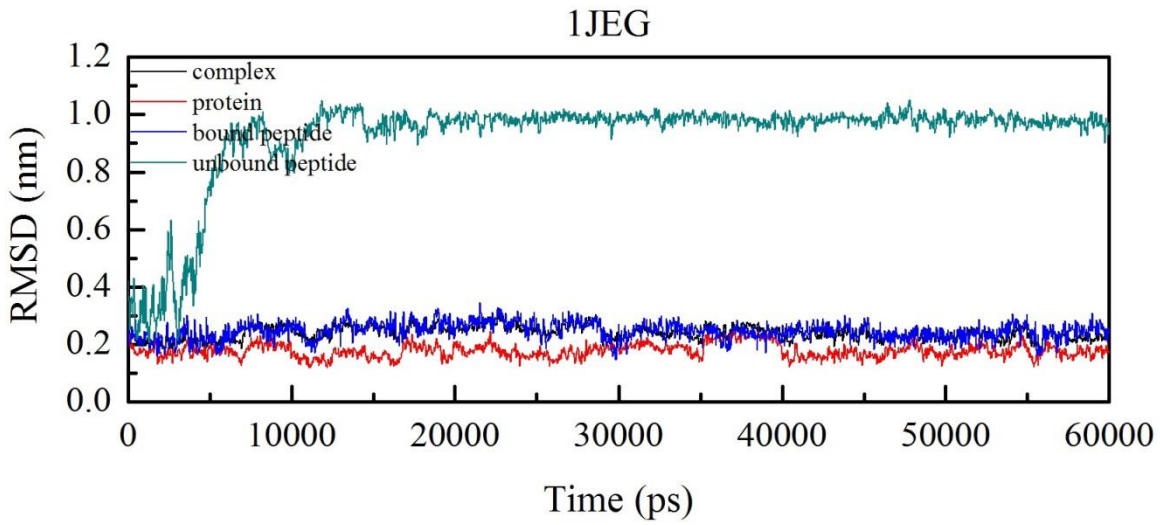
Time (ps)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protein
bound peptide
unbound peptide
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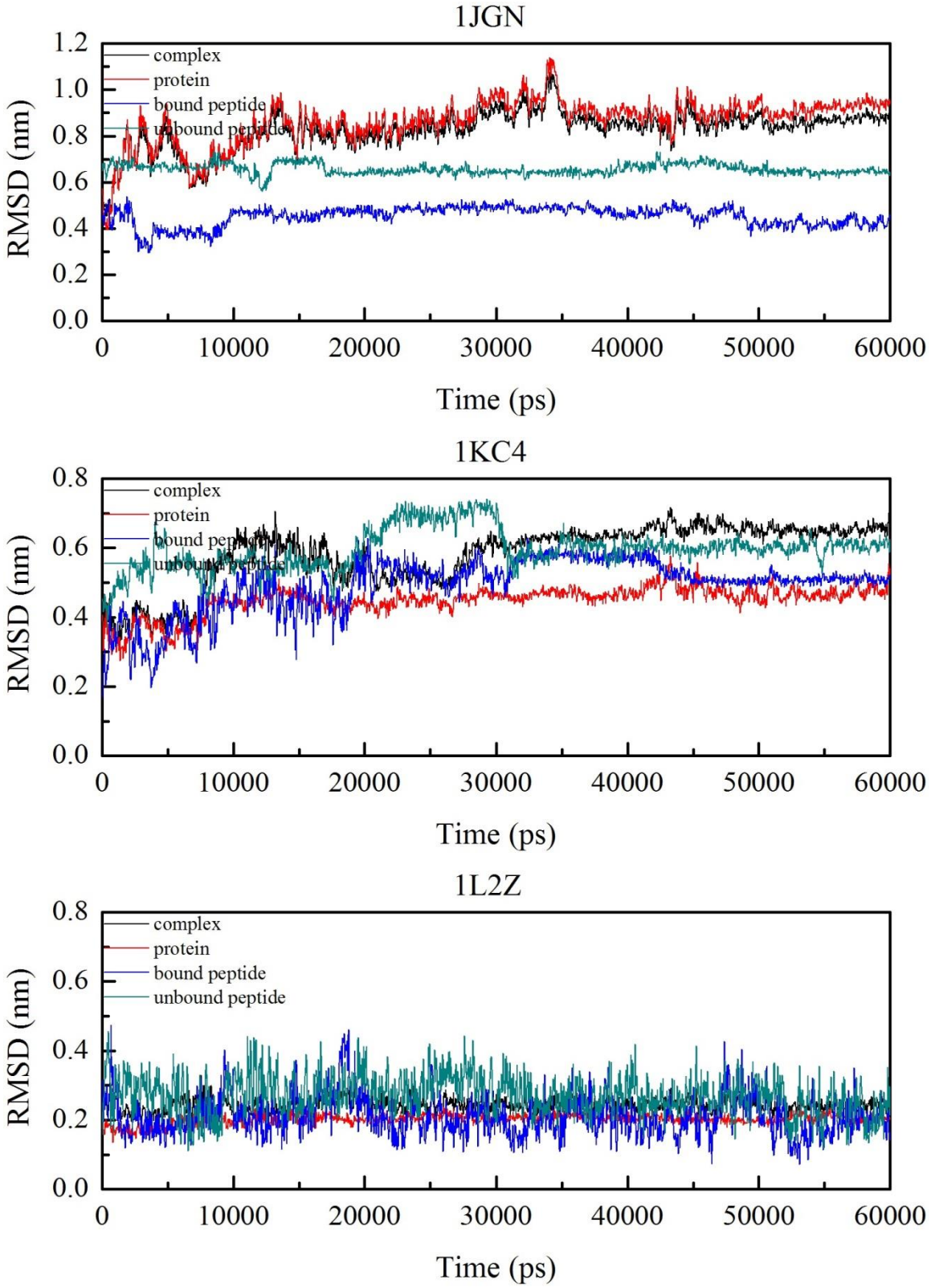
60000
Time (ps)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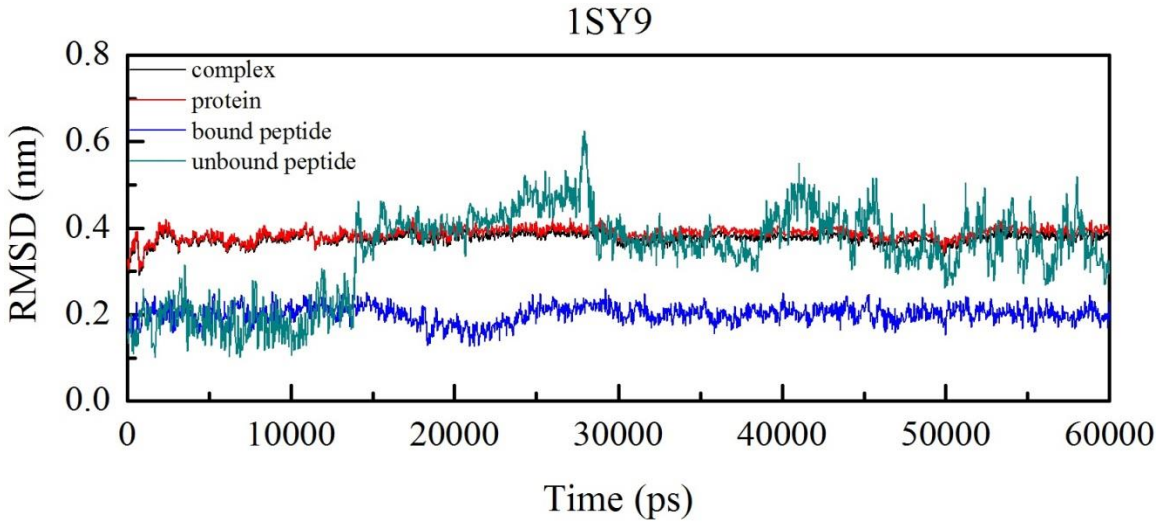
protein
bound peptide
unbound peptide
RMSD (nm)
Time (ps)
1KC4
complex
protein
bound peptide
unbound peptide
RMSD (nm)
Time (ps)
1L2Z
complex
protein
bound peptide
unbound peptide
RMSD (nm)
Time (ps)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bound peptide
unbound peptide
RMSD (nm)
Time (ps)
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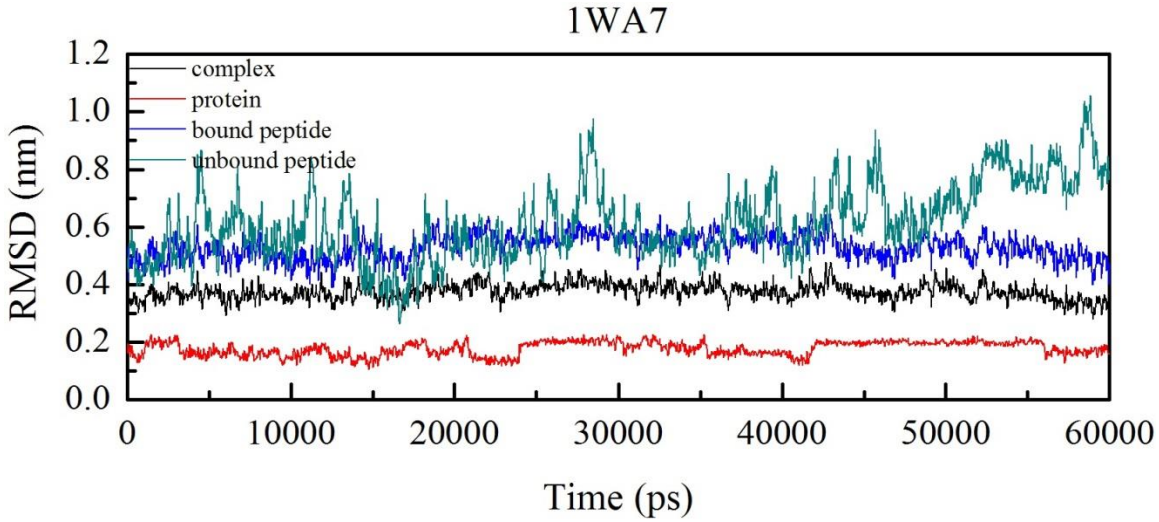
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unbound peptide
RMSD (nm)
Time (ps)
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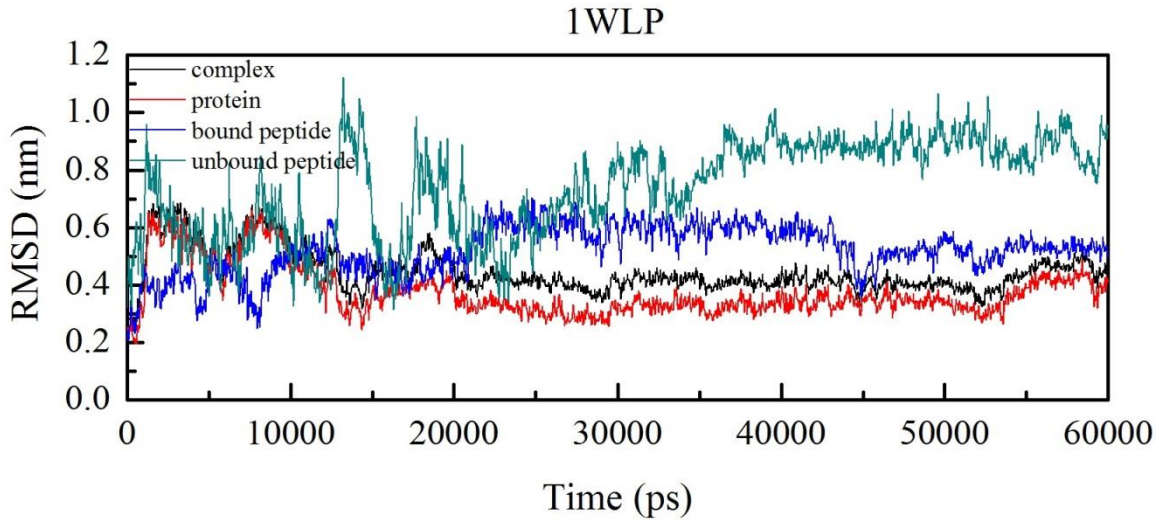
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unbound peptide
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Time (ps)
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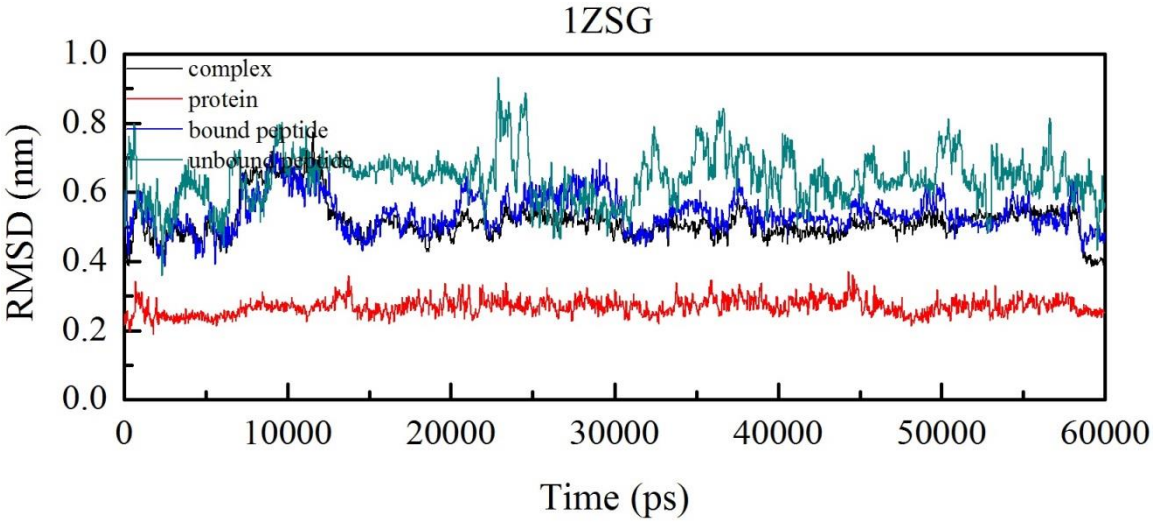
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unbound peptide
RMSD (nm)
Time (ps)
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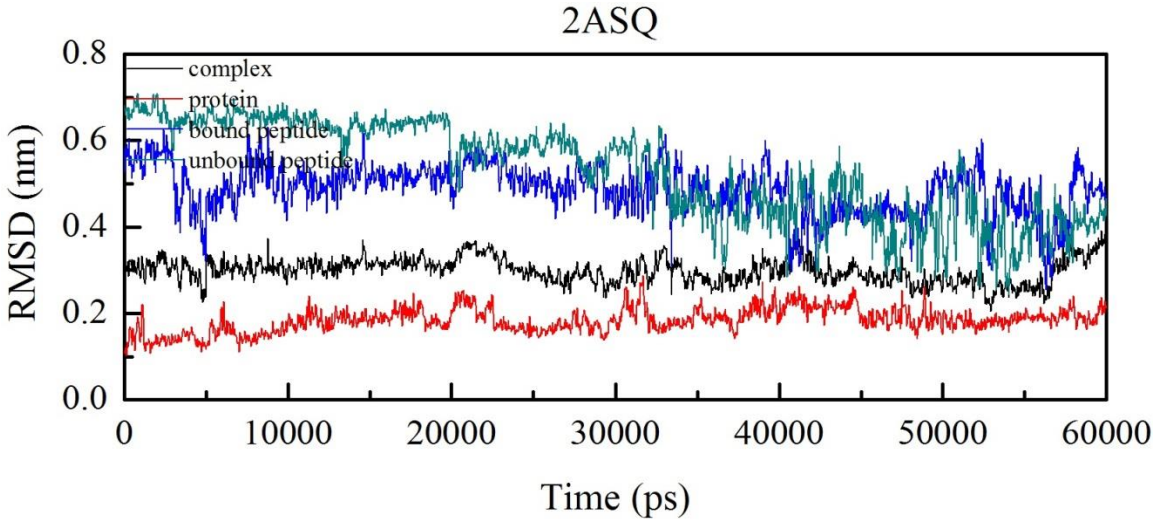
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protein
bound peptide
unbound peptide
RMSD (nm)
Time (ps)
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40000
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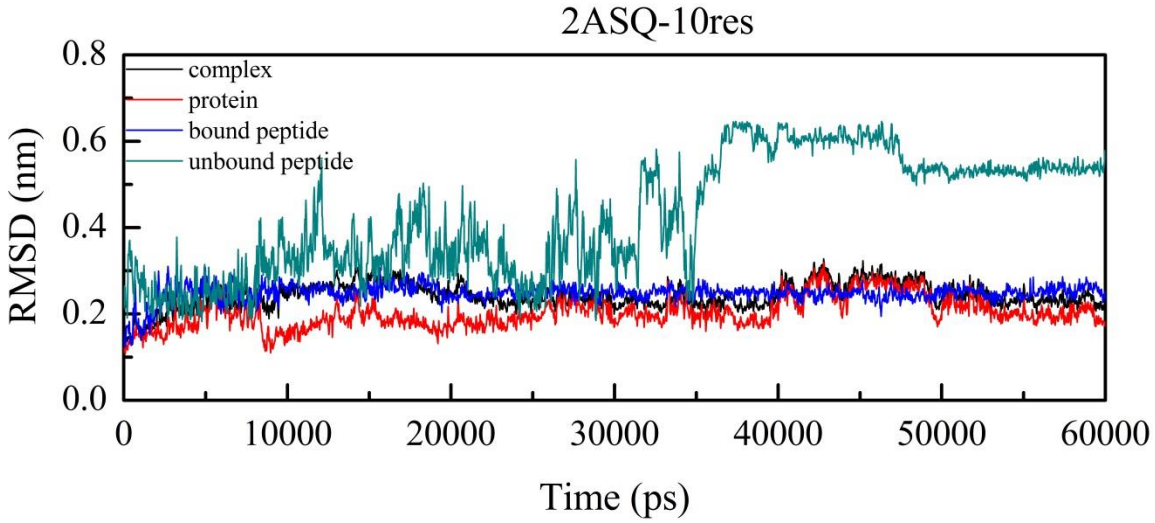
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RMSD (nm)
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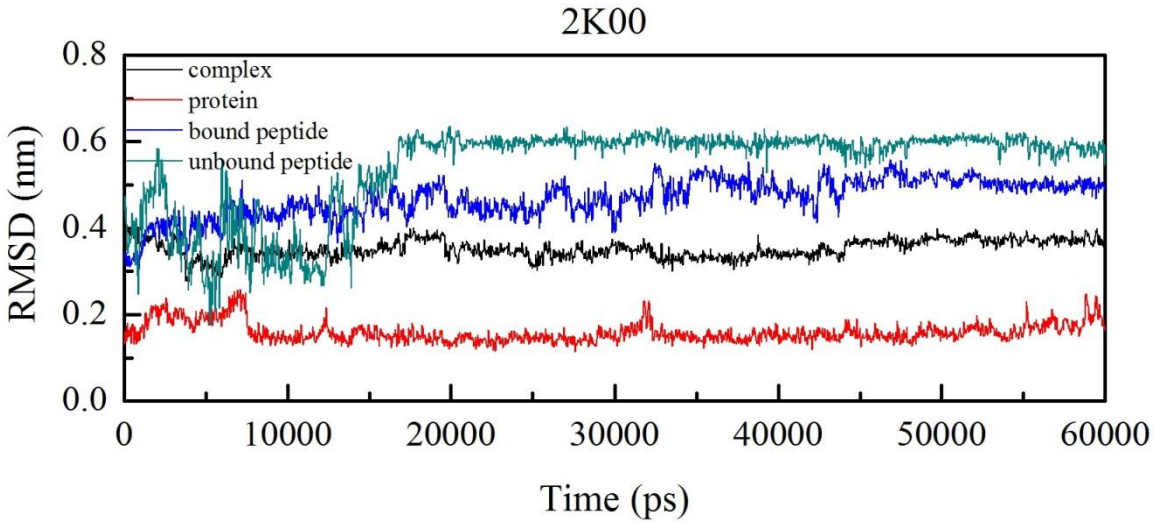
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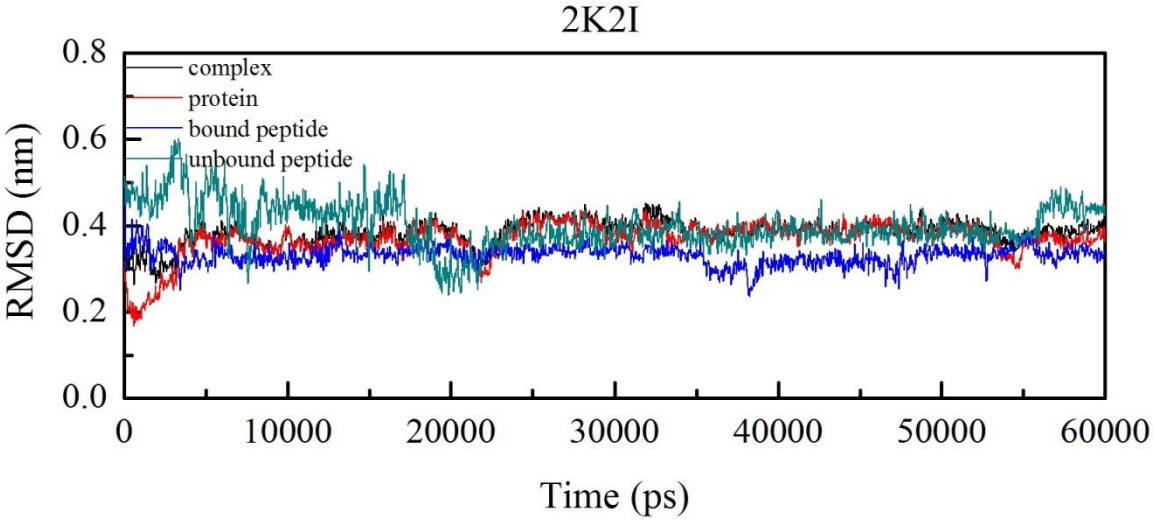
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bound peptide
unbound peptide
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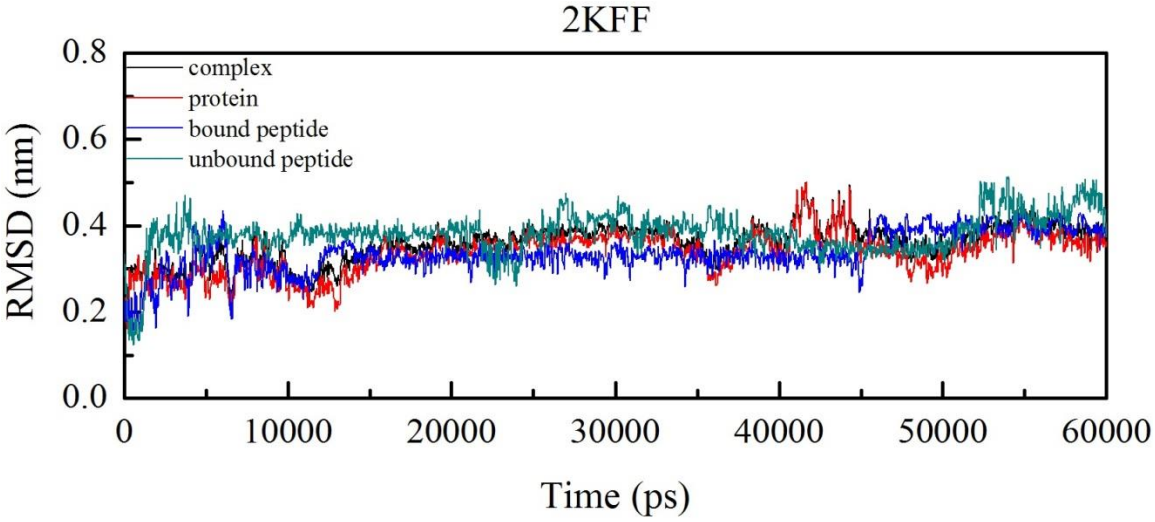
Time (ps)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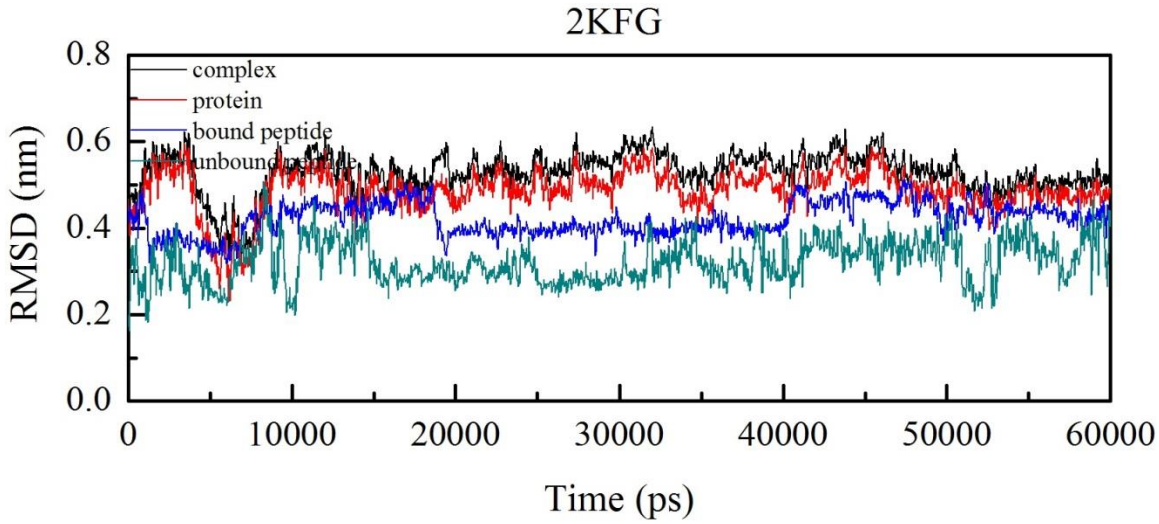
Time (ps)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unbound peptide
RMSD (nm)
Time (ps)
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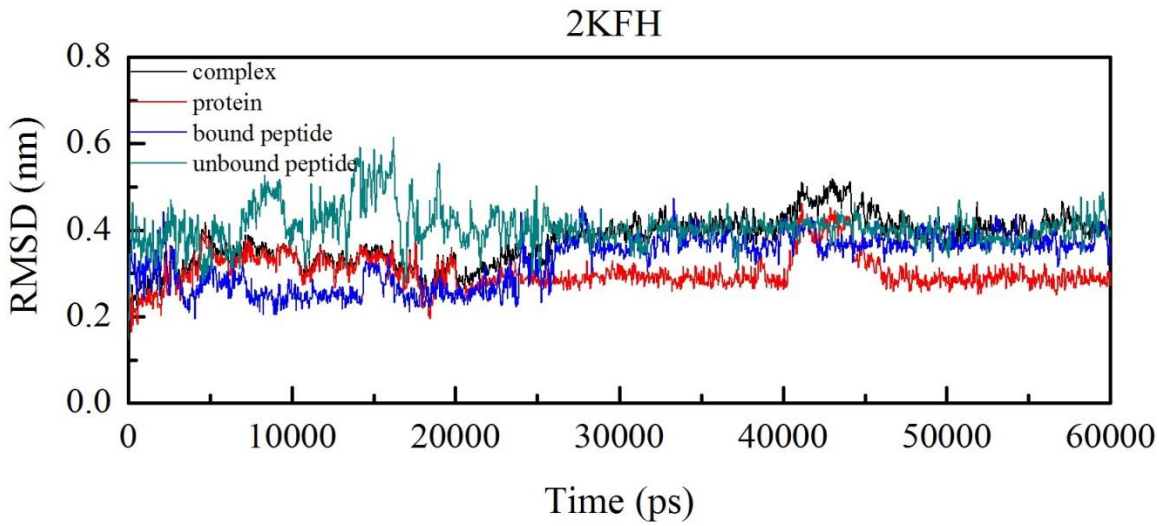
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RMSD (nm)
Time (ps)
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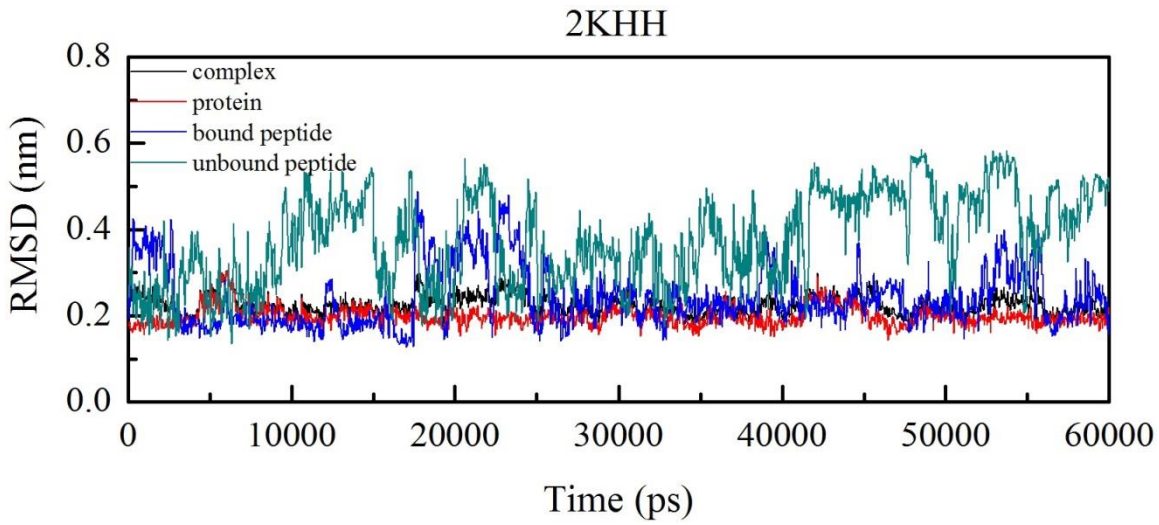
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unbound peptide
RMSD (nm)
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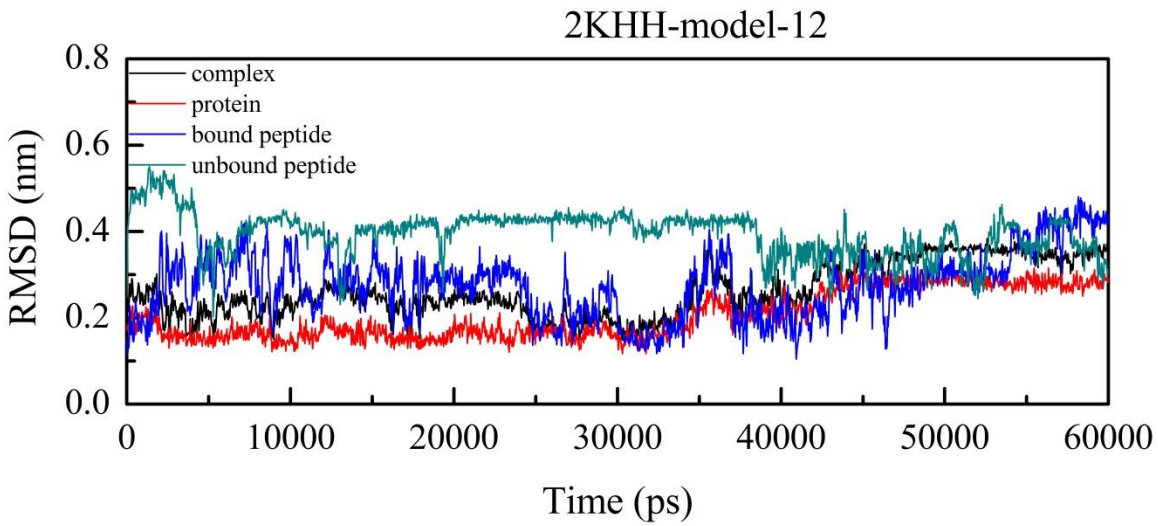
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protein
bound peptide
unbound peptide
RMSD (nm)
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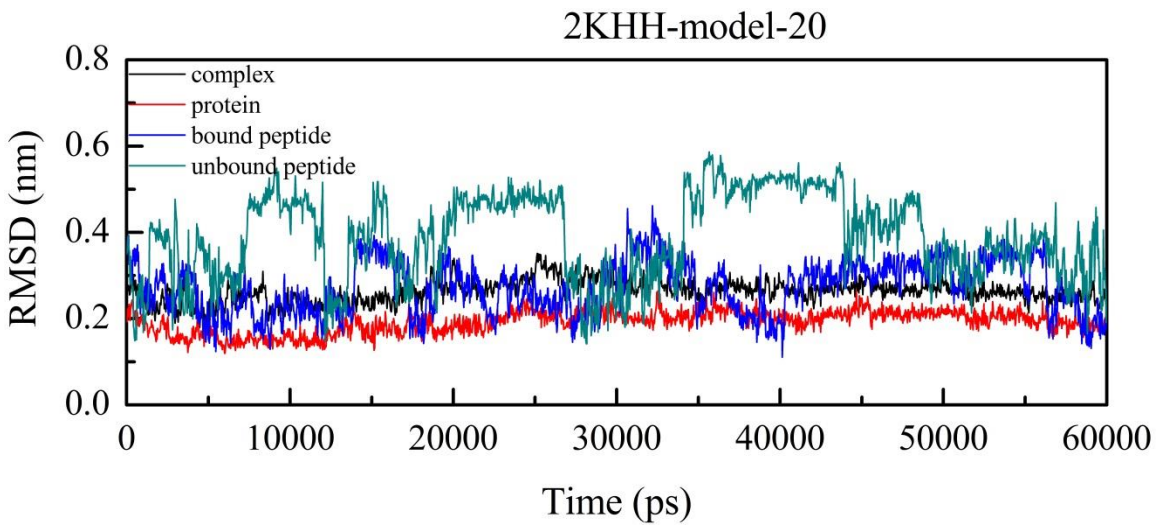
Time (ps)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bound peptide
unbound peptide
RMSD (nm)
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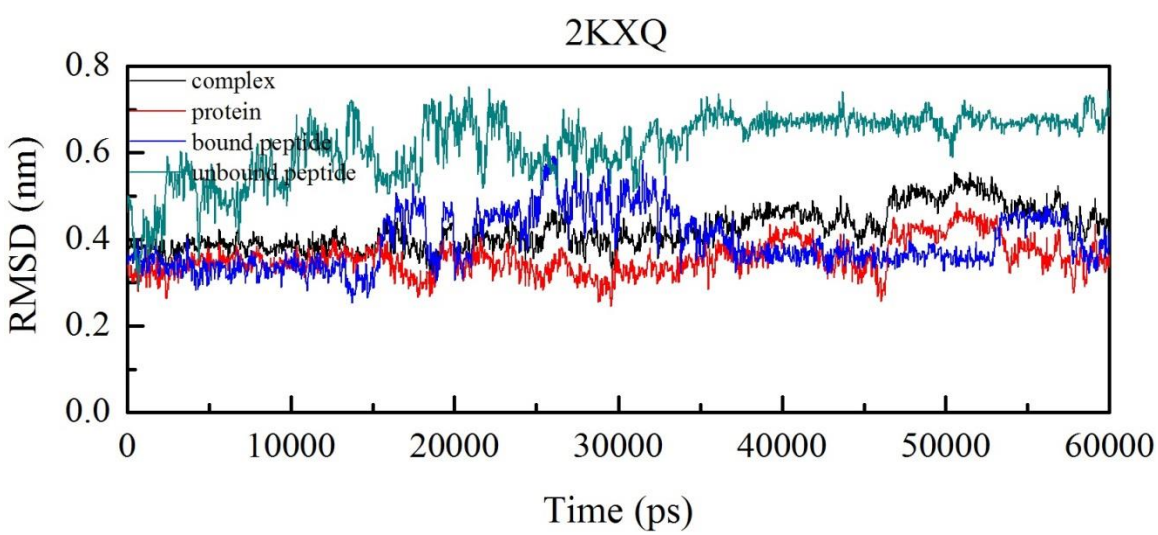
Time (ps)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protein
bound peptide
unbound peptide
RMSD (nm)
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Time (ps)

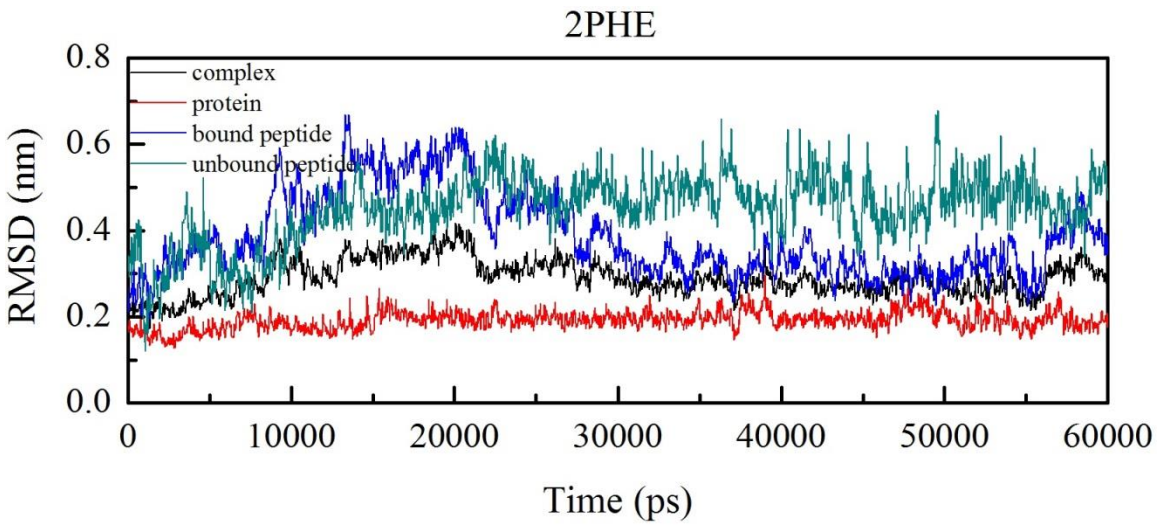
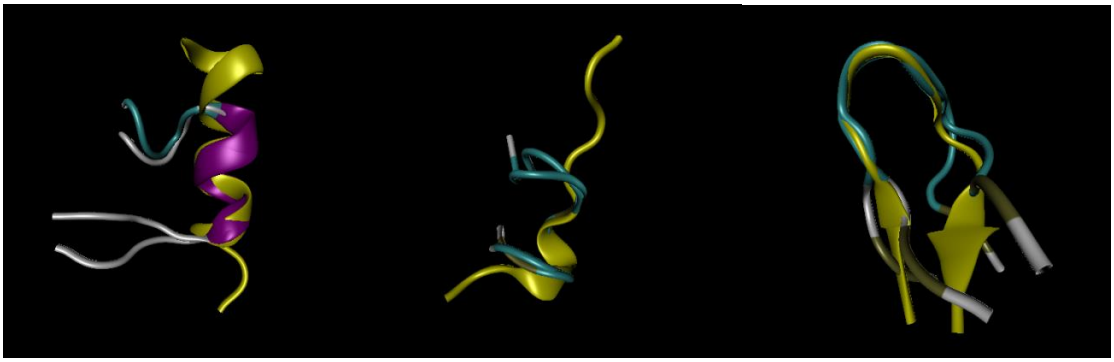
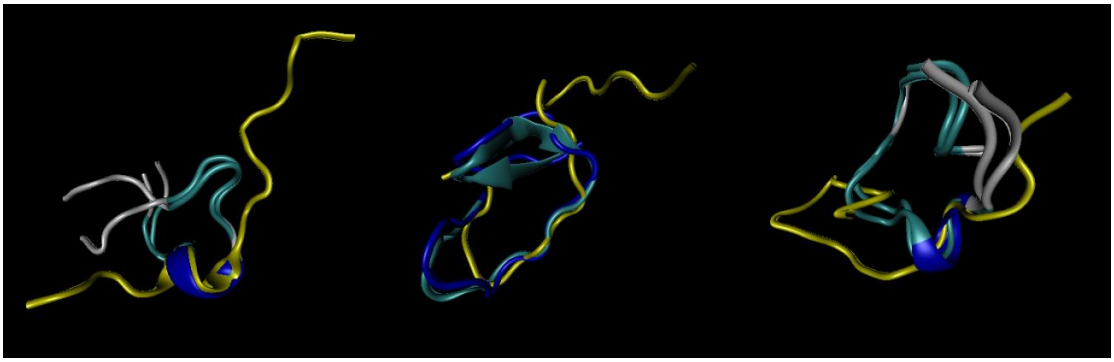


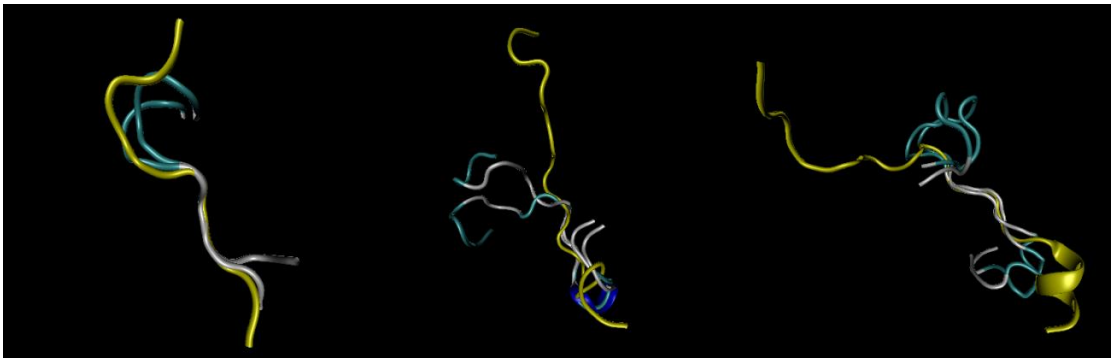
Figure S3. Root-mean-square deviation (RMSD) of all 25 complexes and 25 unbound peptides**.** Black represents the backbone RMSD of complex; red, the backbone RMSD of protein in complex; blue, the backbone RMSD of bound peptide in complex; cyan, the backbone RMSD of unbound peptide.



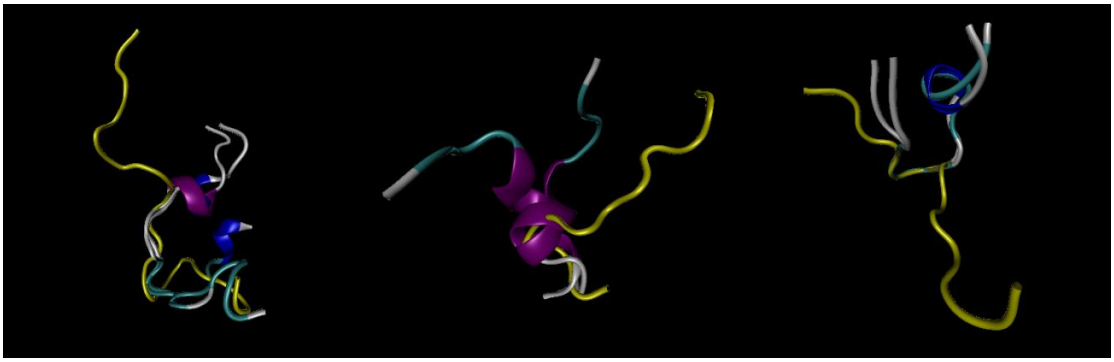
1BXL 1H3H 1HAA



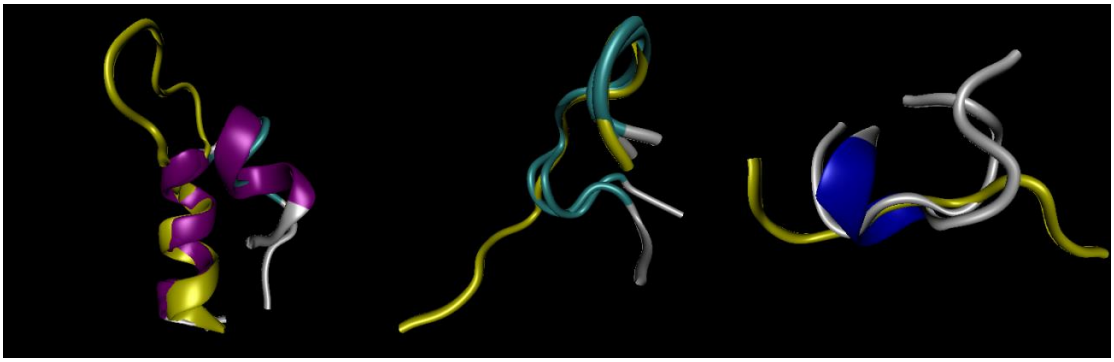
1JEG 1JGN 1KC4



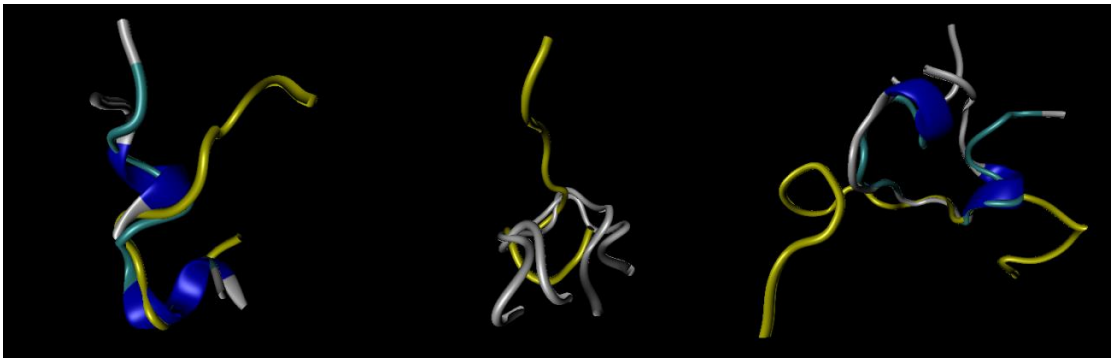
1L2Z 1WA7 1WLP



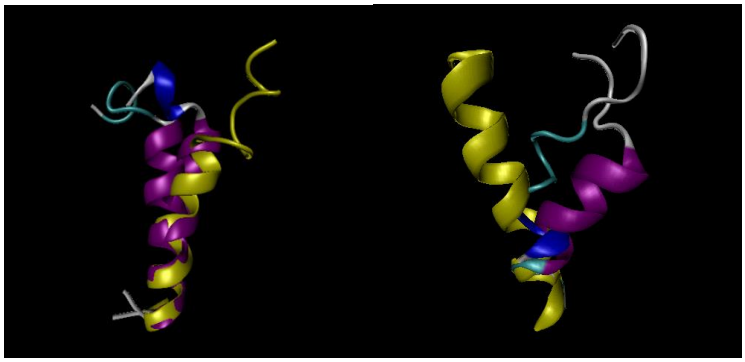
1ZSG 2ASQ 2K00



2K2I 2KFF 2KHH

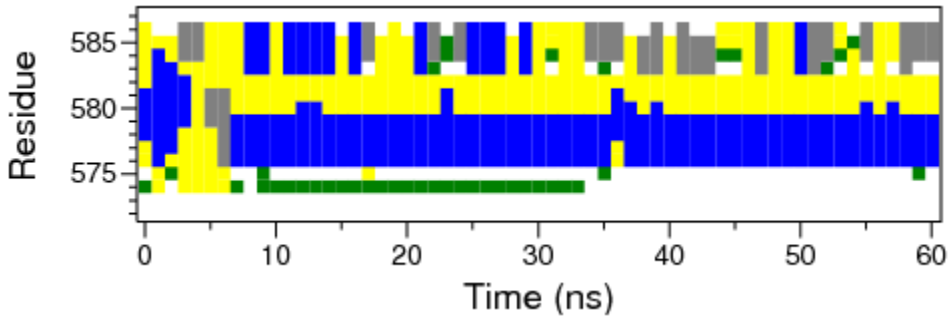


2KFG 2KFH 2KXQ

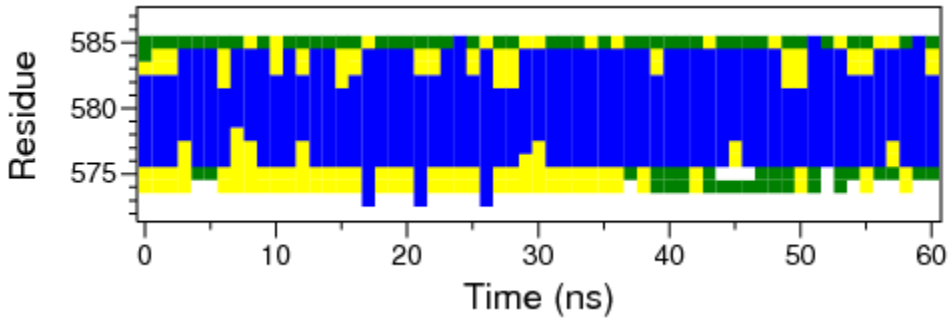


2PHE 1SY9

Figure S4. The aligned unbound peptide structures. Yellow represents the starting unbound peptide structure; the other two conformations come from frames of 50 ns and 60 ns selected from production simulation. Conformations were aligned using 3-7 residues that adopt similar structure in all these three conformations.

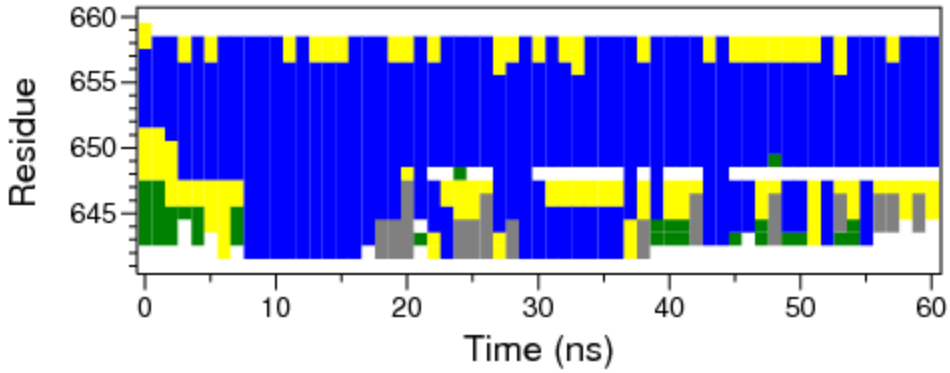


1BXL: unbound peptide

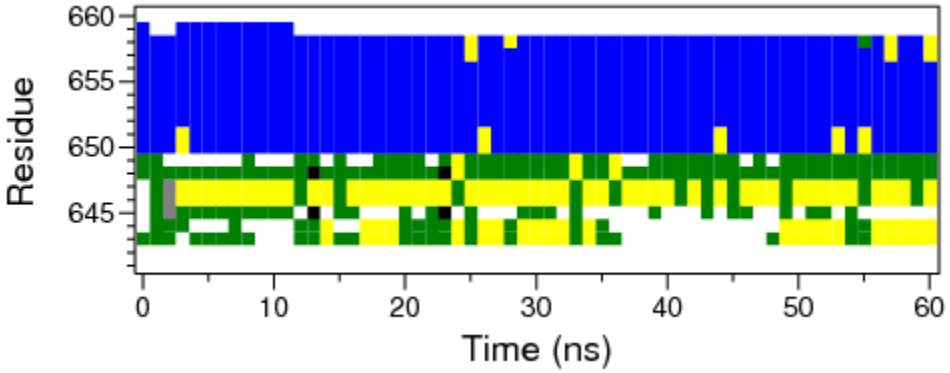


1BXL: bound peptide

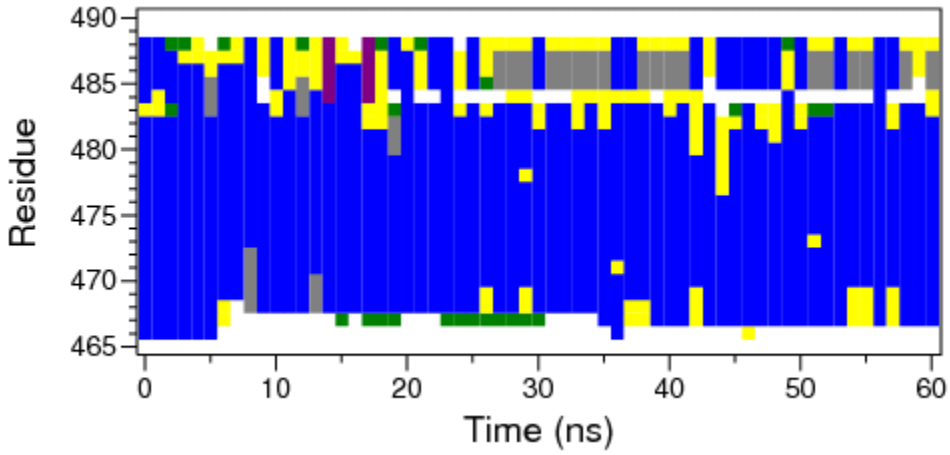
Coil Bend Turn A-Helix 3-Helix



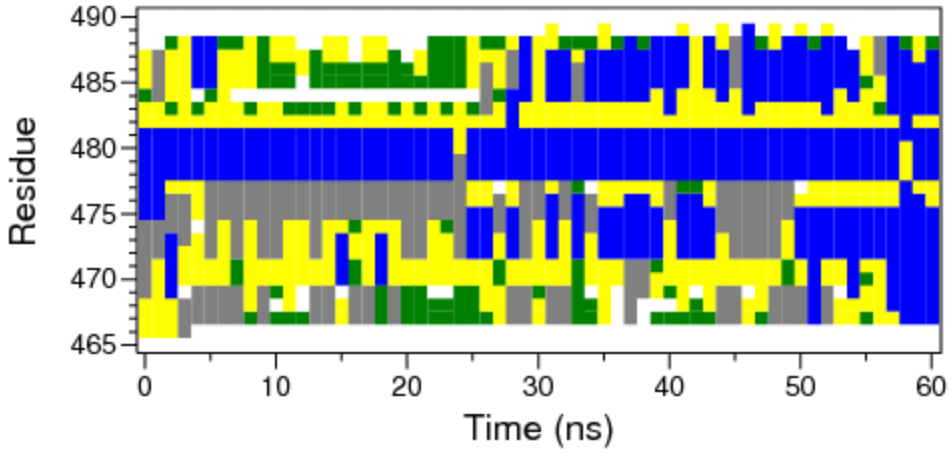
2K2I: unbound peptide



2K2I: bound peptide



2PHE: unbound peptide



2PHE: bound peptide

Figure S5. Secondary structure evolution of the unbound and bound peptides during the course of 60 ns production simulations of 1BXL, 2K2I, and 2PHE. Residues are numbered as what they are when we downloaded them from the PDB.