Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Material

for

RNA Model Evaluation Based on MD Simulation of Four tRNA Analogs

Anna Grzybkowska,[a] Dominika Jedrzejczyk,[b] Michał Rostkowski,[a] Arkadiusz Chworos,[b,*] and Agnieszka Dybala-Defratyka[a,*]

[a] Institute of Applied Radiation Chemistry, Faculty of Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924, Lodz, Poland

[b] Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland

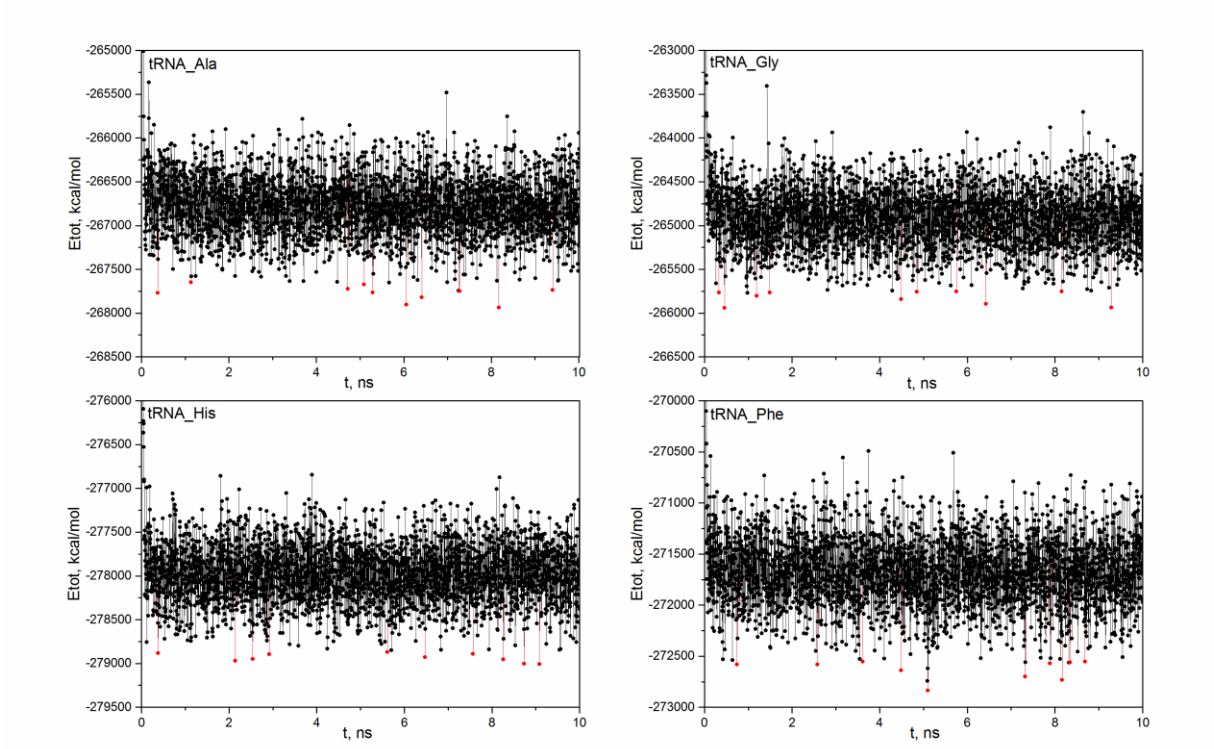

Fig. S1 Total energy as function of time for the first 10 ns of simulation for studied systems. Red circles indicate chosen structures (example plots for one trajectory for each analog).

Table S1. Sequences and secondary structure interactions for four mitochondrial tRNAanalogs (tRNA^{Ala}, tRNA^{Gly}, tRNA^{His} and tRNA^{Phe}). Color code represents WC pairing fragments for amino-acyl (orange), D-loop (lime) anticodon (blue) and T-loop (pink) stems respectively. The sequence numbering is based on the well-accepted numbering of phenylalanine tRNA from yeast, following the rules proposed by the participants of the *Cold Spring Harbor Meeting on tRNA* in 1978.

	-4-3	1	8	10		22	26	27	32	39	44	49		61	66	73
tRNA^{Ala}	GGG	AAGGGCU	UA	GCUU	AAUUA	AAGU	G	GCUGA	UU**UGC**GU	UCAGU	UGAU	GCAGA	GUGGGGU	UUUGC	AGUCCUU	A
tRNA^{Gly}	GGG	ACUCUUU	UA	GUAU	AAAUA	GUAC	C	GUUAA	CU**UCC**AA	UUAAC	UAGU	UUUGA	CAACAU	UCAAA	AAAGAGU	A
tRNA^{His}	GGGA	GUAAAUA	UA	GUUU	AACCA	AAAC	A	UCAGA	UU**GUG**AA	UCUGA	CAAC	AGAGG	CUUACGA	CCCCU	UAUUUAC	C
tRNA^{Phe}	GGGA	GUUUAUG	UA	GCUU	ACCUCCUCA	AAGC	A	AUACA	CU**GAA**AA	UGUUU	AGAC	GGGCU	CACAU	CACCC	CAUAAAC	A

Table S2. Average backbone heavy atom and all atoms RMSD for tRNA molecules and backbone heavy atom and all atoms) RMSD for anticodon stem loops along their standard deviations.

	Backbone	Backbone – anticodon loop	All atoms	All atoms – anticodon loop
Ala1	6.24 ± 2.02	2.44 ± 0.68	6.12 ± 1.92	3.02 + 0.66
Ala2	6.97 ± 1.86	2.07 ± 0.38	6.67 ± 1.75	3.21 ± 0.62
Ala3	4.80 ± 0.87	1.83 ± 0.43	4.73 ± 0.81	2.05 ± 0.32
Average	**6.00 ± 1.89**	**2.11 ± 0.57**	**5.84 ± 1.77**	**2.76 ± 0.75**
Gly1	7.61 ± 2.11	2.33 ± 0.30	7.44 ± 1.87	3.91 ± 0.47
Gly2	7.59 ± 1.58	3.81 ± 0.94	7.34 ± 1.48	4.49 ± 0.83
Gly3	8.57 ± 1.96	2.05 ± 0.32	8.42 ± 1.74	3.36 ± 0.34
Average	**7.92 ± 1.95**	**2.73 ± 0.98**	**7.73 ± 1.77**	**3.92 ± 0.74**
His1	8.22 ± 2.97	1.58 ± 0.21	7.92 ± 2.82	2.19 ± 0.31
His2	7.10 ± 0.91	1.68 ± 0.26	6.73 ± 0.26	2.34 ± 0.31
His3	10.31 ± 1.76	1.64 ± 0.20	9.63 ± 1.60	2.34 ± 0.27
Average	**8.54 ± 2.45**	**1.63 ± 0.23**	**8.09 ± 2.27**	**2.29 ± 0.31**
Phe1	5.35 ± 0.93	2.23 ± 0.46	5.51 ± 0.84	2.72 ± 0.50

Phe2	6.05 ± 0.98	2.30 ± 0.40	6.21 ± 0.96	2.80 ± 0.49
Phe3	6.70 ± 1.27	2.11 ±0.36	6.58 ± 1.20	2.61 ± 0.43
Average	**6.03 ± 1.21**	**2.21 ± 0.42**	**6.10 ± 1.10**	**2.71 ± 0.48**

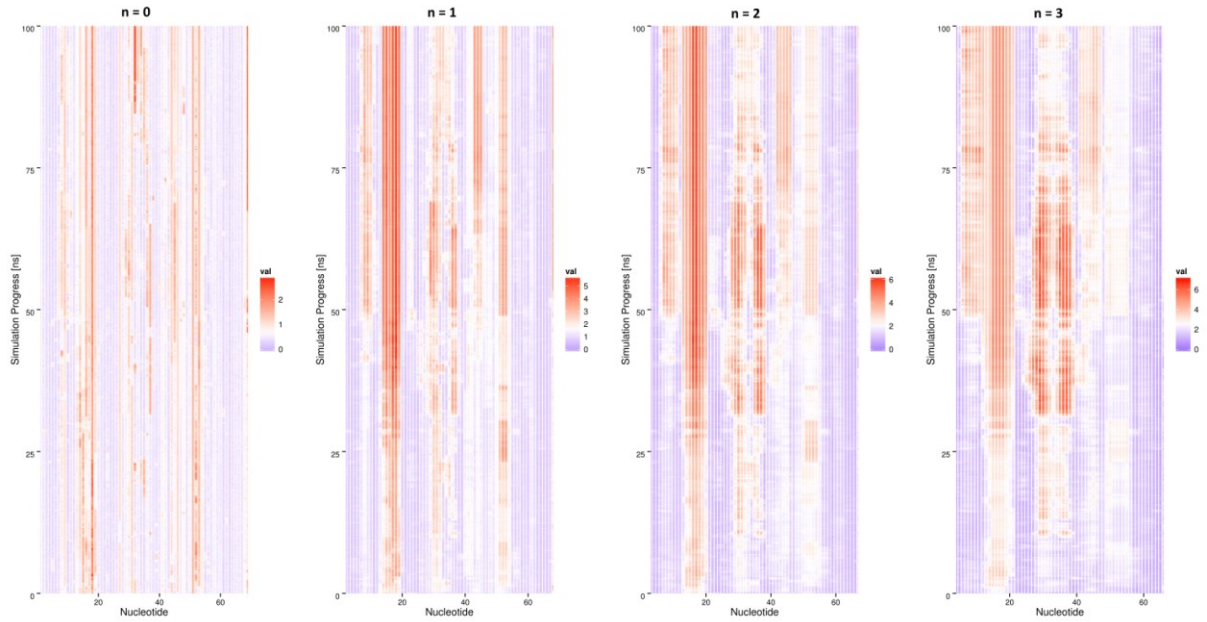

Fig. S2a Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{Ala}$, where each residue was considered together with 0, 1, 2 and 3 former and subsequent in the sequence neighboring residues, respectively.

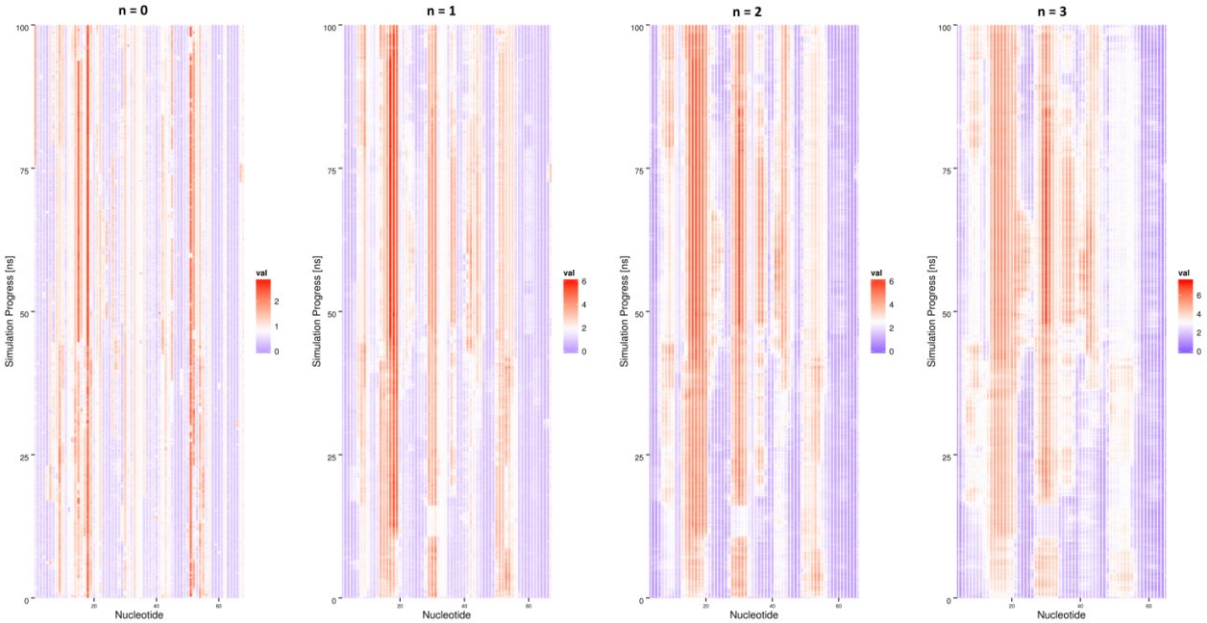

Fig. S2b Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{Gly}$, where each residue was considered together with 0, 1, 2 and 3 former and subsequent in the sequence neighboring residues, respectively.

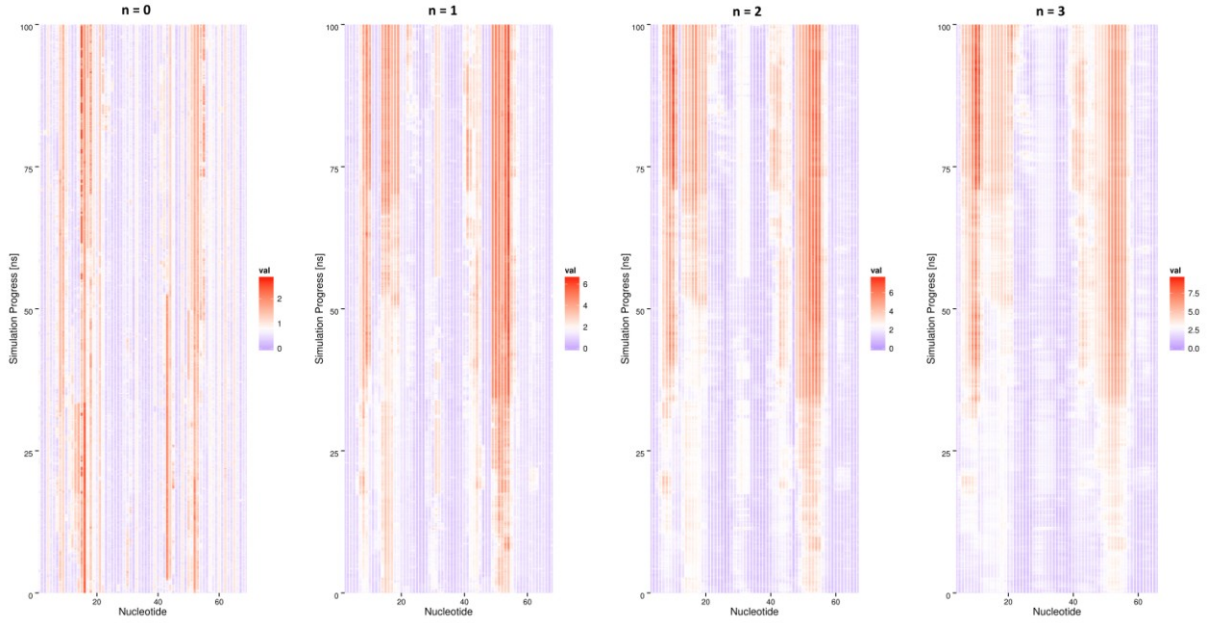

Fig. S2c Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{His}$, where each residue was considered together with 0, 1, 2 and 3 former and subsequent in the sequence neighboring residues, respectively.

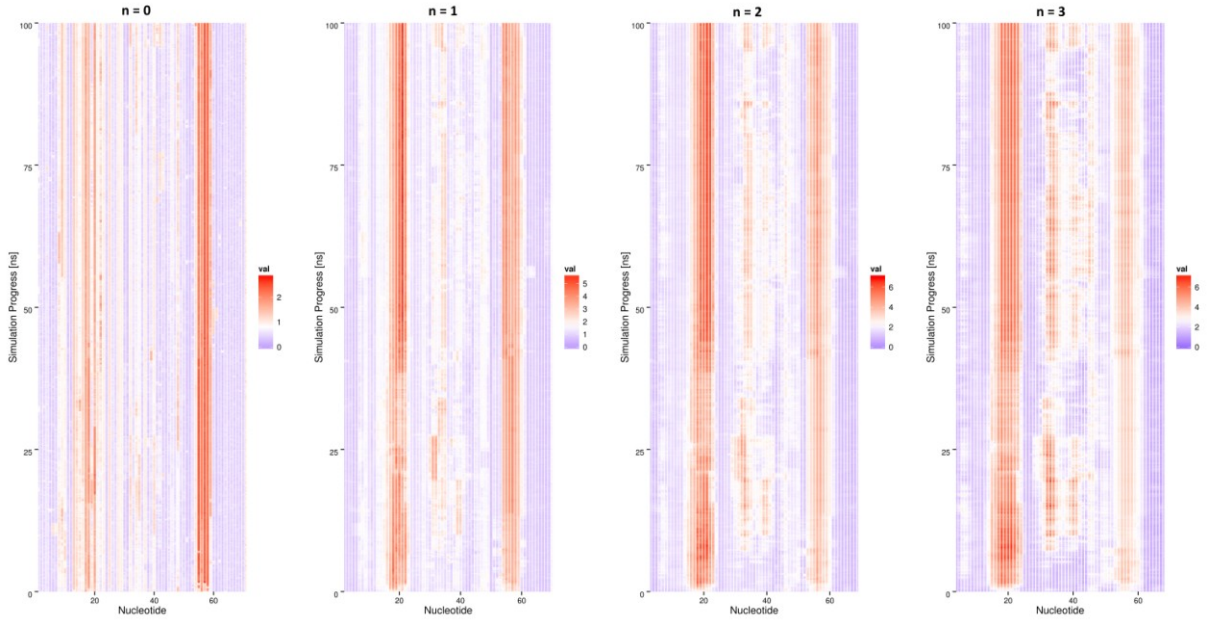

Fig. S2d Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{Phe}$, where each residue was considered together with 0, 1, 2 and 3 former and subsequent in the sequence neighboring residues, respectively.

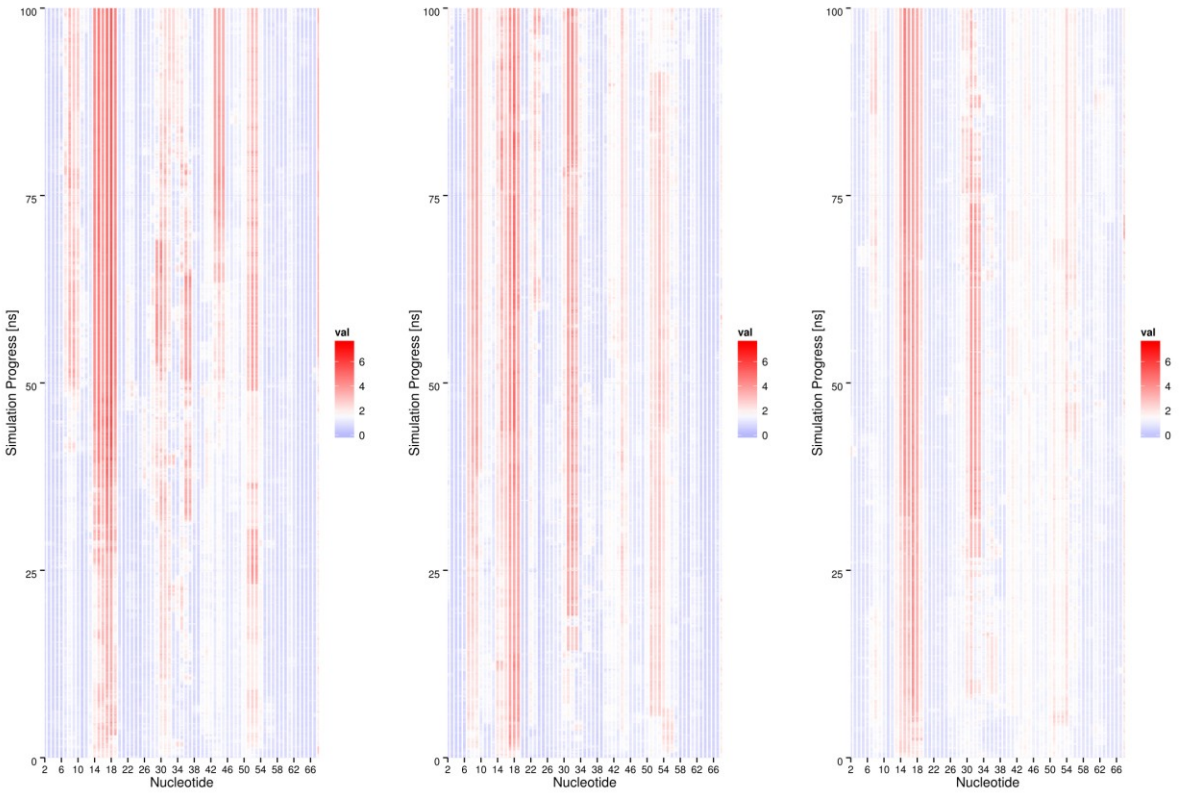

Fig. S3a Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{Ala}$, where each residue was considered together with 1 former and subsequent neighboring residues, for three independent 100 ns simulations

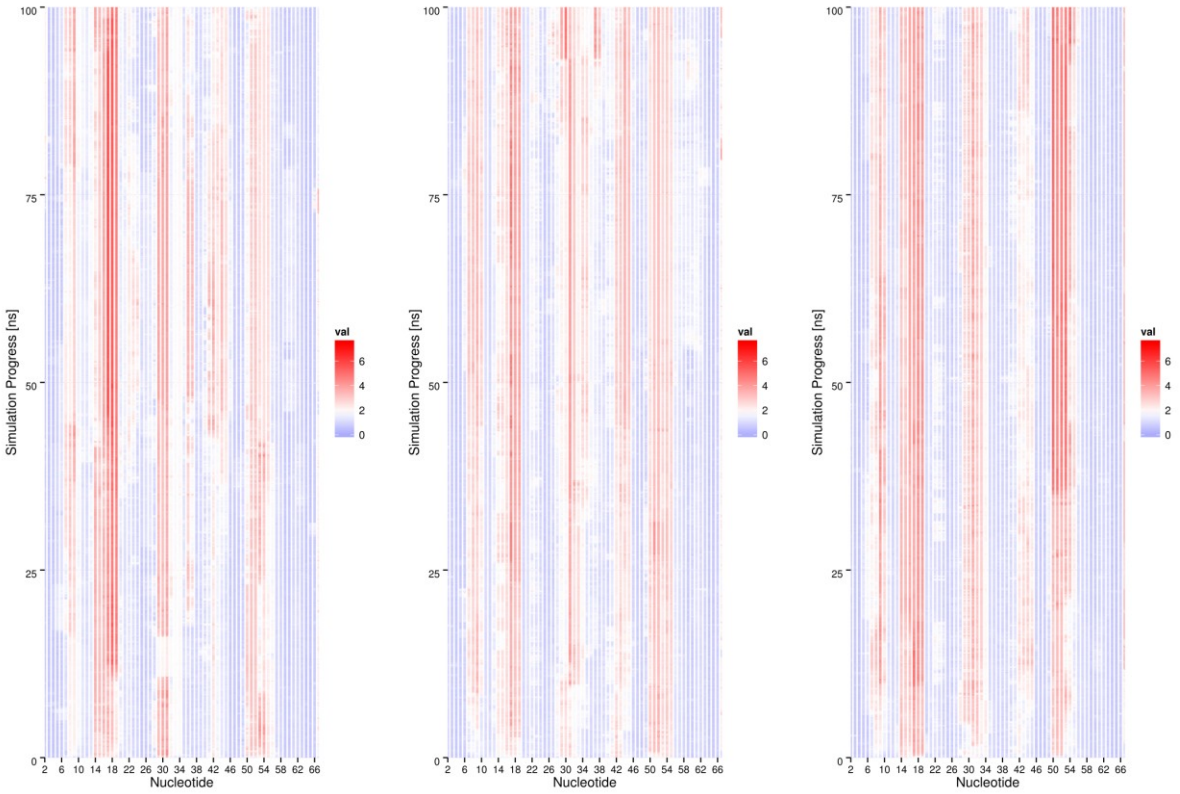

Fig. S3b Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{Gly}$, where each residue was considered together with 1 former and subsequent neighboring residues, for three independent 100 ns simulations

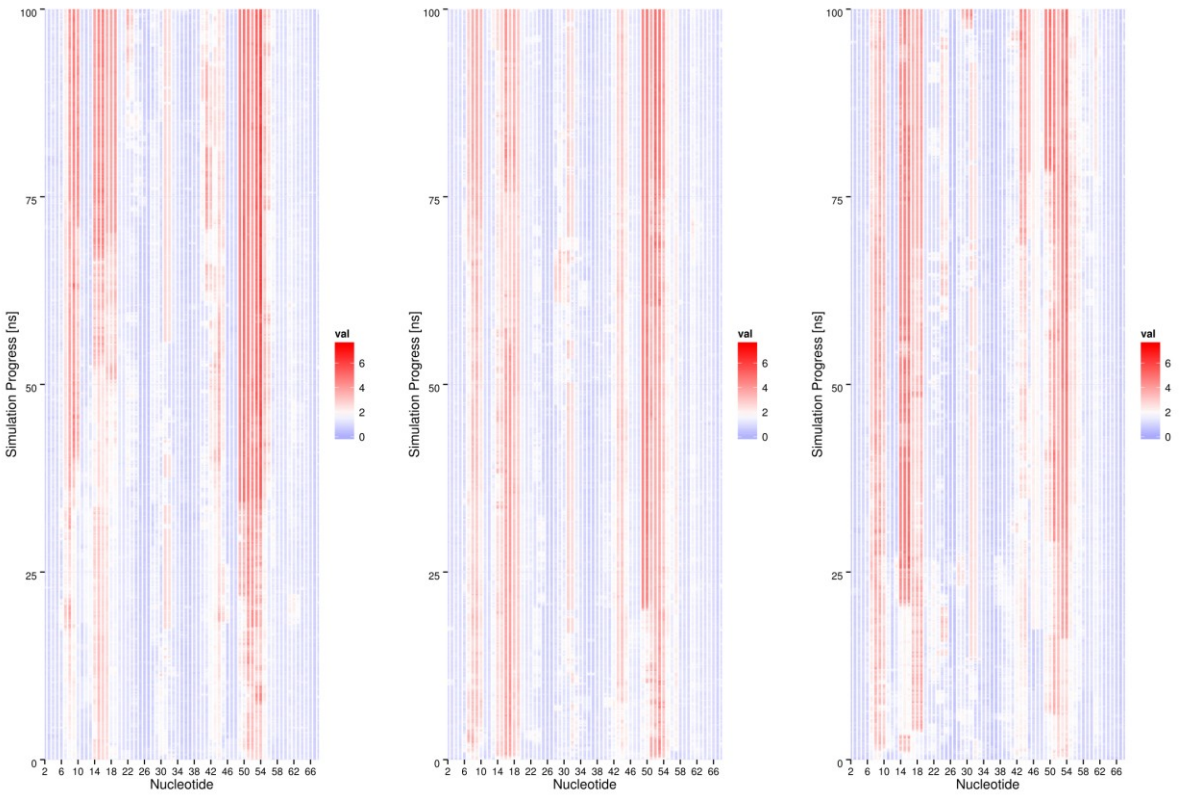

Fig. S3c Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{His}$, where each residue was considered together with 1 former and subsequent neighboring residues, for three independent 100 ns simulations

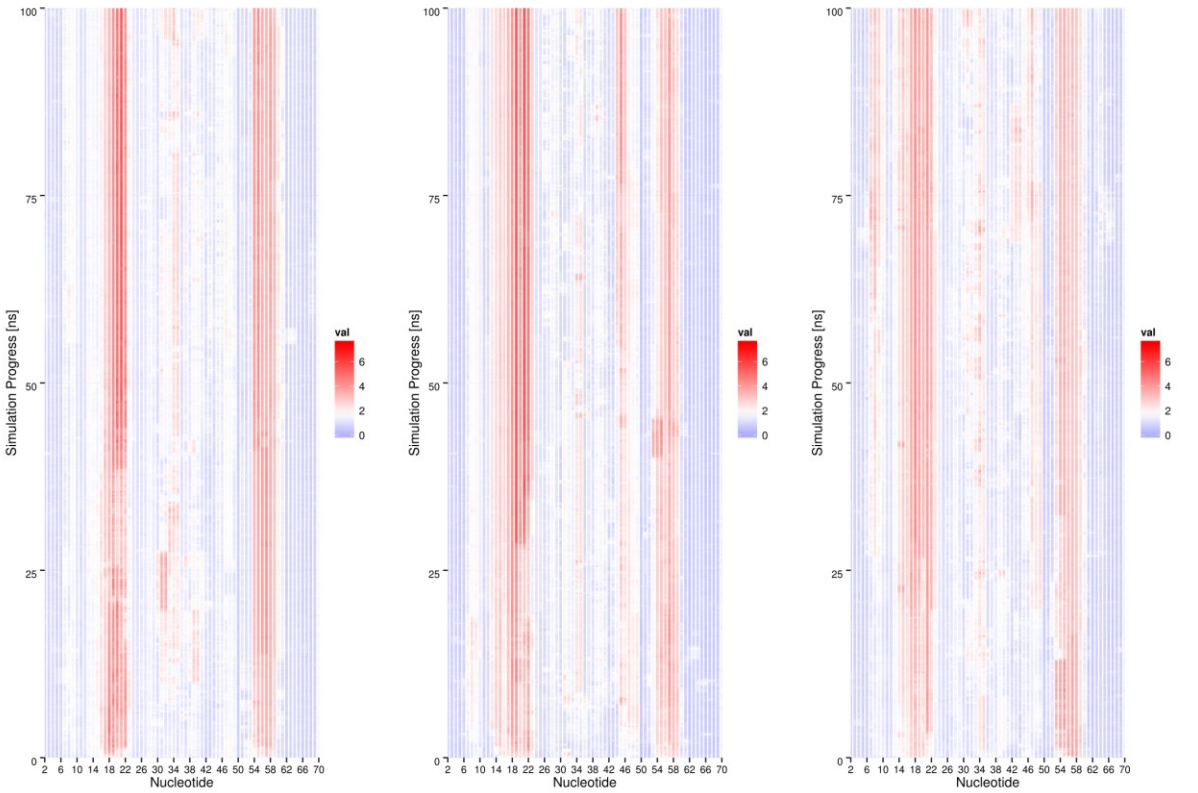

Fig. S3d Local RMSD (values on the color scales on the right-side of each panel) analysis for $tRNA^{Phe}$, where each residue was considered together with 1 former and subsequent neighboring residues, for three independent 100 ns simulations

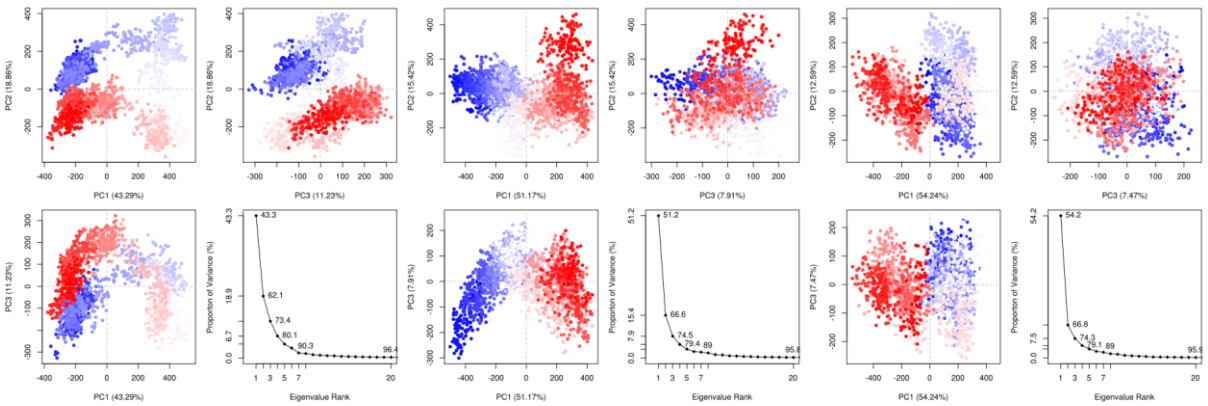

Fig. S4a Principal components analysis for three individual 100 ns simulations of $tRNA^{Ala}$, respectively. Three colored subplots for each tRNA analog represent molecular trajectories projected onto the three first principal components, colored from blue to red in a function of time. The fourth plot presents percentage of the total variance of atom positional fluctuations for each principal component.

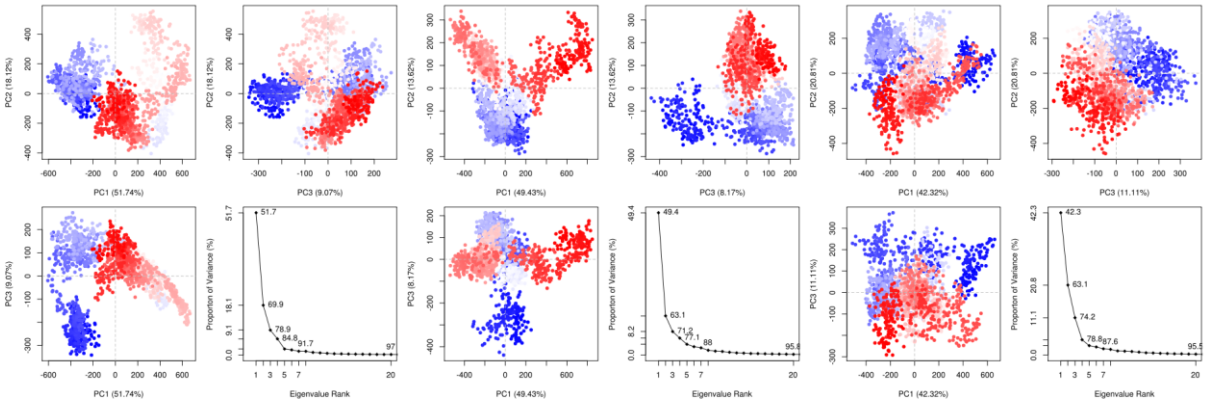

Fig. S4b Principal components analysis for three individual 100 ns simulations of $tRNA^{Gly}$, respectively. Three colored subplots for each tRNA analog represent molecular trajectories projected onto the three first principal components, colored from blue to red in a function of time. The fourth plot presents percentage of the total variance of atom positional fluctuations for each principal component.

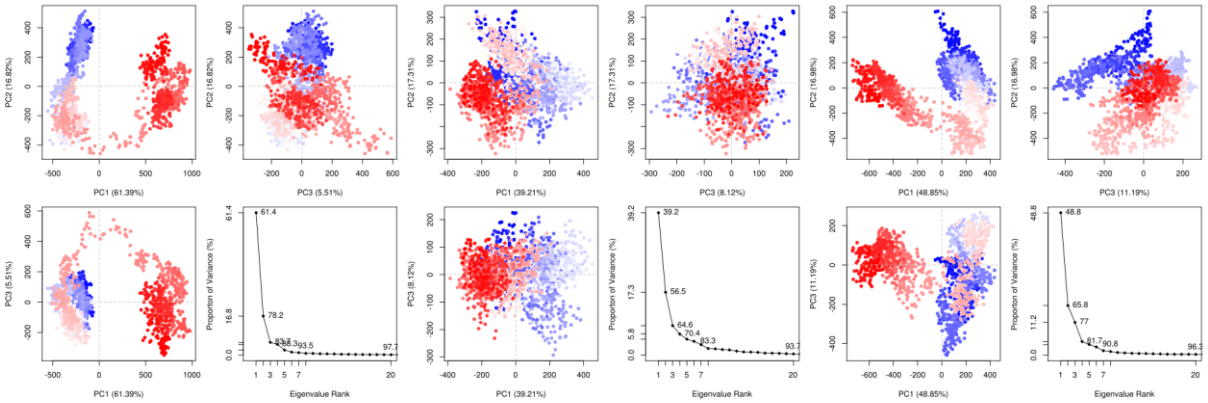

Fig. S4c Principal components analysis for three individual 100 ns simulations of $tRNA^{His}$, respectively. Three colored subplots for each tRNA analog represent molecular trajectories projected onto the three first principal components, colored from blue to red in a function of time. The fourth plot presents percentage of the total variance of atom positional fluctuations for each principal component.

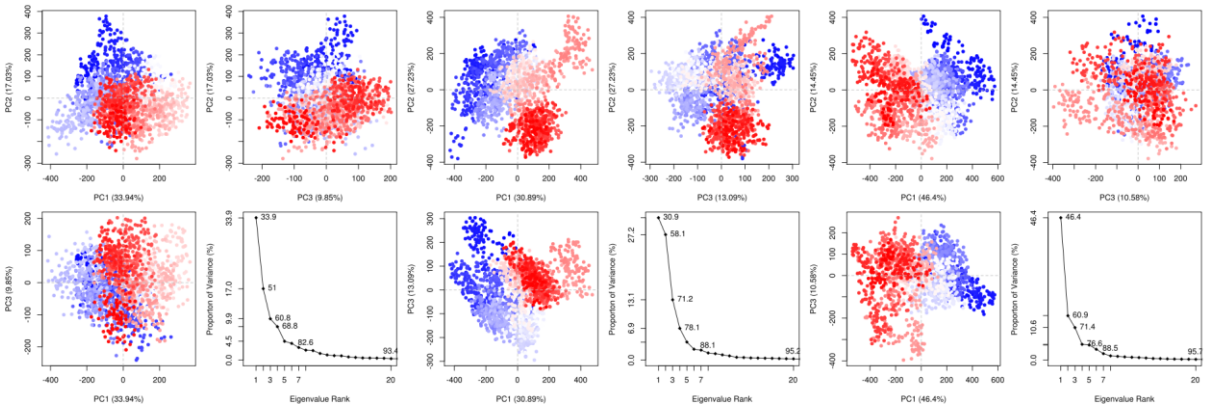

Fig. S4d Principal components analysis for three individual 100 ns simulations of $tRNA^{Phe}$, respectively. Three colored subplots for each tRNA analog represent molecular trajectories projected onto the three first principal components, colored from blue to red in a function of time. The fourth plot presents percentage of the total variance of atom positional fluctuations for each principal component.

Table S3 RMSD, INF (Interaction Network Fidelity) and DI (Deformation Index) values for 100 selected conformations having the lowest total energy.

	$tRNA^{Ala}$			$tRNA^{Gly}$			$tRNA^{His}$			$tRNA^{Phe}$		
structure	RMSD	INF	DI	RMSD	INF	DI	RMSD	INF	DI	RMSD	INF	DI
1	3.33	0.81	4.11	1.78	0.83	2.14	3.45	0.80	4.29	3.92	0.76	5.15
2	3.76	0.80	4.71	3.99	0.79	5.04	3.39	0.79	4.28	4.20	0.71	5.91
3	3.31	0.73	4.57	4.05	0.74	5.51	3.45	0.73	4.71	4.08	0.68	5.98
4	3.80	0.79	4.81	4.13	0.75	5.51	3.77	0.78	4.83	5.37	0.68	7.94
5	3.26	0.77	4.21	4.60	0.83	5.54	4.19	0.82	5.13	4.52	0.74	6.11
6	4.33	0.79	5.50	4.51	0.76	5.91	4.05	0.79	5.11	5.51	0.73	7.54
7	4.09	0.76	5.40	6.37	0.76	8.35	4.06	0.81	5.04	4.97	0.70	7.08
8	3.32	0.79	4.18	4.96	0.80	6.23	4.79	0.78	6.10	5.47	0.69	7.92
9	3.94	0.75	5.24	4.68	0.77	6.06	4.40	0.79	5.58	4.52	0.71	6.36
10	3.90	0.78	4.97	4.81	0.76	6.34	5.07	0.80	6.32	4.98	0.69	7.18
11	3.41	0.78	4.39	4.55	0.76	5.97	3.83	0.73	5.23	6.29	0.70	9.02
12	4.53	0.81	5.61	4.37	0.76	5.73	4.23	0.74	5.71	5.86	0.72	8.20
13	4.10	0.86	4.74	4.65	0.82	5.66	4.21	0.84	5.00	5.69	0.77	7.43
14	4.03	0.77	5.23	4.65	0.81	5.75	4.55	0.75	6.10	5.85	0.67	8.74
15	3.97	0.78	5.11	4.47	0.79	5.65	4.32	0.70	6.15	5.17	0.73	7.12
16	4.49	0.77	5.83	5.01	0.74	6.73	4.14	0.76	5.44	4.72	0.70	6.73
17	4.30	0.78	5.55	7.09	0.77	9.16	4.19	0.78	5.39	4.49	0.72	6.27
18	3.54	0.80	4.45	7.29	0.79	9.28	4.42	0.76	5.80	5.24	0.68	7.67
19	3.57	0.77	4.61	6.45	0.79	8.22	5.38	0.82	6.57	4.73	0.68	6.98
20	3.69	0.73	5.03	5.70	0.83	6.87	6.12	0.77	7.91	5.20	0.69	7.55
21	3.59	0.74	4.83	5.75	0.79	7.29	6.41	0.81	7.94	5.22	0.72	7.29
22	3.52	0.78	4.50	6.33	0.83	7.63	5.03	0.75	6.74	5.54	0.71	7.79
23	3.16	0.72	4.36	6.30	0.77	8.22	5.15	0.76	6.74	4.76	0.75	6.38
24	3.35	0.78	4.29	5.70	0.80	7.17	4.93	0.85	5.76	4.95	0.76	6.51
25	3.33	0.79	4.21	5.90	0.80	7.38	4.60	0.76	6.02	4.67	0.69	6.74
26	3.29	0.76	4.32	7.51	0.76	9.91	4.54	0.76	6.00	4.36	0.71	6.13
27	4.08	0.78	5.25	8.26	0.78	10.60	4.98	0.79	6.32	4.91	0.75	6.58
28	4.36	0.72	6.05	7.09	0.79	9.00	4.95	0.80	6.21	4.97	0.76	6.54
29	5.60	0.72	7.82	7.51	0.80	9.35	4.57	0.81	5.65	4.56	0.77	5.96
30	5.20	0.72	7.17	6.35	0.80	7.90	4.84	0.76	6.33	4.75	0.74	6.42
31	4.82	0.71	6.83	7.27	0.81	9.02	5.85	0.79	7.44	3.70	0.69	5.40
32	4.09	0.66	6.17	7.34	0.73	10.04	5.79	0.78	7.42	3.94	0.76	5.21
33	6.54	0.66	9.98	7.58	0.73	10.42	7.46	0.74	10.07	4.36	0.78	5.59
34	7.61	0.68	11.16	7.93	0.77	10.26	6.87	0.75	9.15	3.80	0.77	4.96
35	6.22	0.75	8.26	7.21	0.81	8.88	6.39	0.77	8.30	3.88	0.71	5.46
36	6.71	0.67	9.95	6.40	0.79	8.12	6.99	0.74	9.43	4.90	0.75	6.57
37	9.12	0.70	13.10	6.25	0.76	8.25	7.76	0.73	10.67	4.49	0.69	6.51
38	9.53	0.69	13.88	6.56	0.77	8.51	7.66	0.77	9.91	4.04	0.68	5.97
39	7.99	0.66	12.04	7.85	0.77	10.24	7.23	0.76	9.54	4.06	0.75	5.41
40	9.33	0.67	13.97	7.19	0.75	9.54	7.32	0.76	9.61	3.70	0.73	5.06
41	8.69	0.68	12.82	7.14	0.73	9.74	7.94	0.80	9.98	4.76	0.74	6.43
42	8.40	0.74	11.29	6.85	0.78	8.82	8.79	0.78	11.23	6.10	0.70	8.66

43	7.90	0.72	11.03	8.32	0.68	12.27	8.77	0.76	11.61	5.79	0.72	8.02
44	8.87	0.61	14.52	9.87	0.72	13.65	8.88	0.76	11.71	6.04	0.72	8.36
45	8.57	0.72	11.97	10.02	0.73	13.70	8.39	0.74	11.33	5.97	0.77	7.80
46	8.12	0.80	10.12	9.31	0.78	11.90	8.02	0.78	10.30	5.53	0.76	7.32
47	8.63	0.69	12.56	9.40	0.74	12.63	7.77	0.78	9.97	5.95	0.73	8.14
48	8.70	0.69	12.57	9.74	0.69	14.15	8.04	0.76	10.57	6.98	0.70	9.95
49	10.42	0.71	14.76	9.34	0.72	13.04	9.74	0.77	12.74	5.15	0.73	7.04
50	9.57	0.72	13.28	10.10	0.72	13.97	9.28	0.76	12.20	5.90	0.75	7.92
51	8.93	0.72	12.32	9.61	0.79	12.24	7.72	0.77	10.08	6.34	0.70	9.09
52	8.13	0.69	11.84	9.05	0.74	12.19	7.49	0.78	9.57	5.61	0.71	7.90
53	7.07	0.71	9.95	9.43	0.74	12.68	7.55	0.80	9.45	6.51	0.72	9.10
54	7.82	0.66	11.80	8.53	0.71	12.00	8.09	0.80	10.07	4.70	0.66	7.07
55	7.57	0.67	11.25	8.64	0.78	11.13	8.07	0.77	10.55	5.47	0.72	7.65
56	7.75	0.69	11.29	9.78	0.73	13.40	8.26	0.76	10.94	6.38	0.73	8.79
57	7.85	0.77	10.18	9.81	0.83	11.79	7.63	0.81	9.46	7.55	0.74	10.20
58	7.49	0.70	10.75	9.85	0.74	13.33	8.22	0.77	10.75	6.65	0.70	9.55
59	7.95	0.72	11.04	10.73	0.74	14.45	8.04	0.74	10.84	6.35	0.70	9.06
60	7.93	0.69	11.53	10.41	0.72	14.54	8.32	0.77	10.74	6.92	0.73	9.54
61	7.67	0.70	11.01	10.71	0.69	15.58	8.51	0.77	11.11	6.99	0.70	10.06
62	9.60	0.68	14.17	11.11	0.69	16.06	6.95	0.74	9.38	6.53	0.69	9.47
63	9.07	0.66	13.69	11.84	0.74	16.10	6.80	0.74	9.17	6.65	0.69	9.64
64	8.01	0.73	10.90	11.24	0.75	15.06	7.11	0.74	9.65	6.87	0.71	9.72
65	8.54	0.73	11.70	11.24	0.69	16.36	6.79	0.80	8.51	7.02	0.69	10.15
66	7.85	0.73	10.76	9.99	0.70	14.30	6.57	0.75	8.76	6.99	0.70	10.02
67	6.61	0.73	8.99	10.02	0.70	14.23	8.42	0.73	11.59	6.74	0.68	9.91
68	6.94	0.79	8.83	10.06	0.80	12.56	9.10	0.78	11.69	5.76	0.71	8.15
69	6.50	0.72	9.09	8.85	0.73	12.17	8.23	0.76	10.83	6.01	0.73	8.23
70	6.64	0.72	9.29	8.59	0.74	11.66	8.93	0.77	11.62	6.88	0.73	9.47
71	6.16	0.72	8.61	7.89	0.70	11.19	9.02	0.73	12.37	4.55	0.73	6.22
72	6.45	0.75	8.63	7.63	0.74	10.33	10.83	0.76	14.21	5.68	0.76	7.53
73	6.67	0.74	8.96	7.05	0.79	8.88	11.49	0.78	14.81	4.96	0.68	7.31
74	6.06	0.75	8.04	7.92	0.71	11.14	12.96	0.78	16.52	4.91	0.73	6.71
75	6.46	0.75	8.64	6.54	0.73	8.93	12.00	0.76	15.83	5.22	0.73	7.18
76	6.27	0.71	8.82	6.72	0.75	8.95	12.97	0.75	17.28	5.36	0.73	7.39
77	5.41	0.74	7.31	6.77	0.67	10.07	12.45	0.78	15.98	5.72	0.76	7.52
78	5.14	0.71	7.28	7.06	0.71	9.98	11.67	0.79	14.73	5.75	0.72	7.98
79	5.92	0.74	8.01	7.33	0.79	9.23	10.52	0.79	13.25	5.46	0.73	7.47
80	7.70	0.70	11.05	7.33	0.77	9.52	11.96	0.83	14.50	4.74	0.73	6.49
81	6.28	0.69	9.15	6.47	0.75	8.61	11.99	0.74	16.25	5.15	0.70	7.35
82	5.25	0.75	7.05	6.48	0.70	9.24	12.50	0.74	16.96	5.55	0.73	7.59
83	5.33	0.73	7.26	7.47	0.75	9.91	12.58	0.72	17.41	5.42	0.70	7.78
84	4.99	0.74	6.71	7.34	0.76	9.65	12.74	0.73	17.40	5.07	0.71	7.12
85	6.28	0.70	8.95	7.16	0.77	9.25	12.76	0.72	17.80	4.50	0.70	6.39
86	5.63	0.69	8.20	6.70	0.73	9.20	11.66	0.72	16.15	6.05	0.70	8.68
87	5.88	0.70	8.38	5.88	0.76	7.79	11.73	0.77	15.21	5.28	0.72	7.36
88	4.97	0.72	6.85	5.50	0.73	7.58	11.50	0.76	15.22	5.30	0.71	7.44

89	4.54	0.72	6.31	5.43	0.75	7.26	11.33	0.75	15.19	5.28	0.72	7.33
90	4.70	0.79	5.97	5.25	0.78	6.73	10.71	0.80	13.37	4.22	0.70	6.07
91	4.43	0.69	6.45	5.42	0.74	7.37	10.46	0.76	13.72	4.47	0.73	6.11
92	4.53	0.74	6.14	6.48	0.79	8.26	11.37	0.78	14.64	5.50	0.71	7.80
93	4.99	0.72	6.88	6.12	0.75	8.11	12.10	0.76	15.90	5.42	0.75	7.22
94	4.55	0.72	6.31	6.69	0.76	8.86	11.24	0.76	14.87	5.61	0.74	7.62
95	4.71	0.75	6.28	6.78	0.73	9.24	10.23	0.78	13.17	5.85	0.70	8.33
96	5.47	0.73	7.50	8.13	0.70	11.59	10.13	0.76	13.33	5.92	0.70	8.45
97	4.75	0.68	6.96	7.14	0.75	9.57	10.33	0.75	13.76	5.37	0.69	7.78
98	5.07	0.71	7.18	7.03	0.70	10.01	9.92	0.75	13.29	5.52	0.73	7.56
99	5.04	0.69	7.34	6.71	0.76	8.86	12.01	0.74	16.20	5.95	0.66	9.05
100	4.77	0.69	6.89	6.24	0.72	8.70	12.59	0.75	16.78	5.81	0.69	8.42

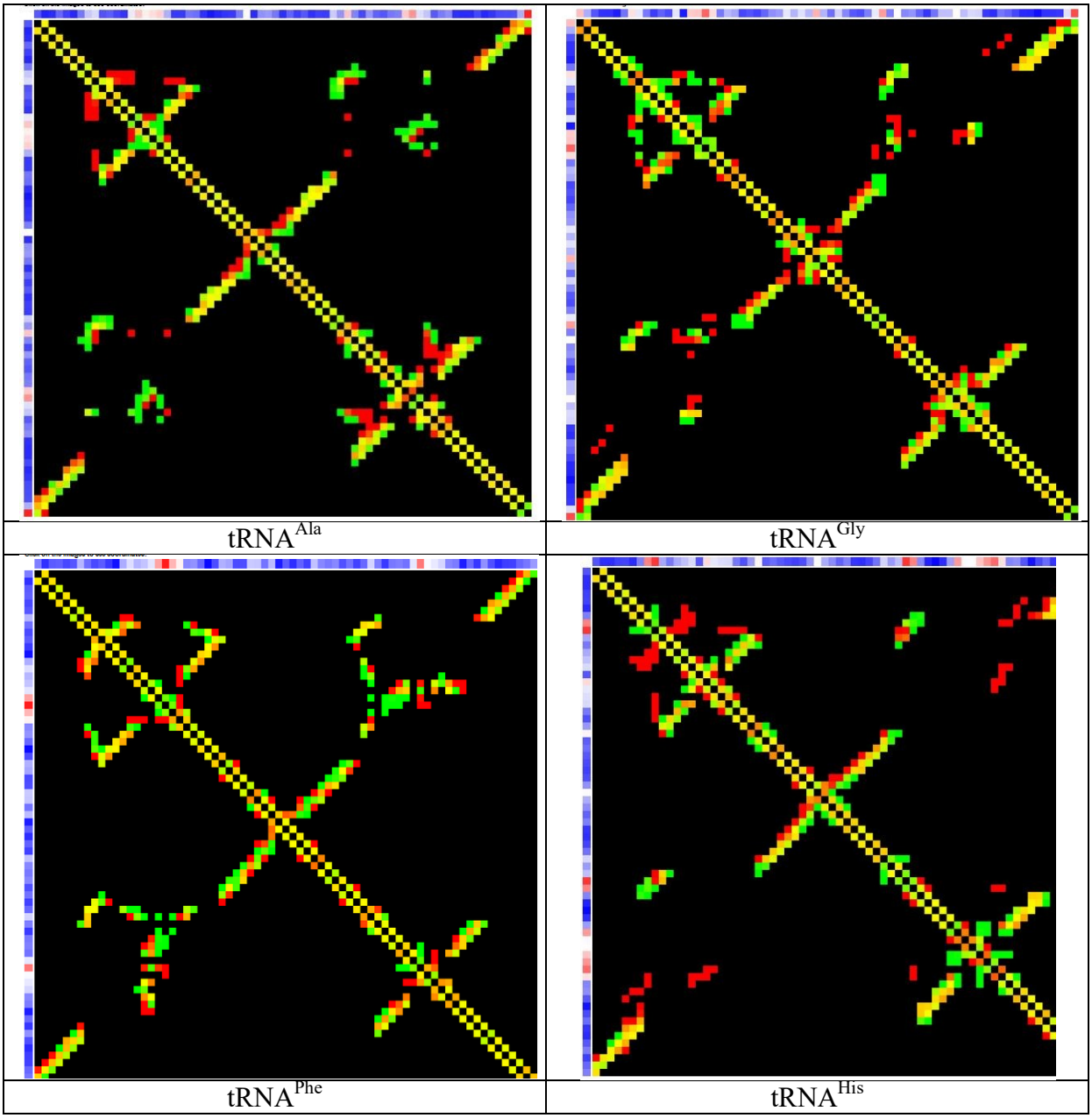

Fig. S5 Superimposed contact maps of A–A contacts for studied analogs of tRNA. Red areas on both axes represent bad contacts between model and reference structure. Red-yellow-green coloring scheme represents contact presence: red (in reference structure only), yellow (in both), and green (in model structure only).

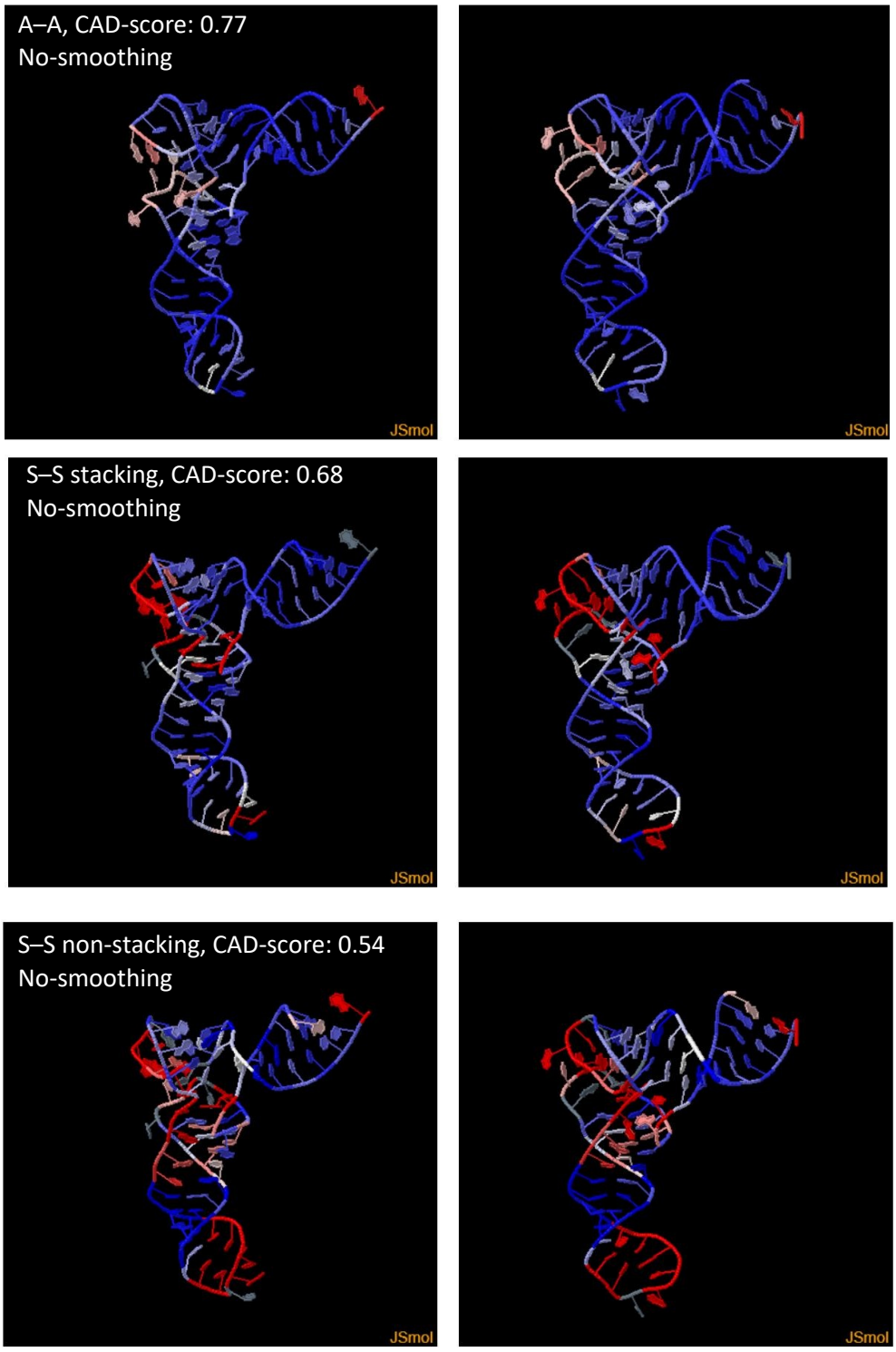

Fig. S6a Local differences between the reference structure (left) and the final conformation from 100 ns simulation for $tRNA^{Ala}$ (right).

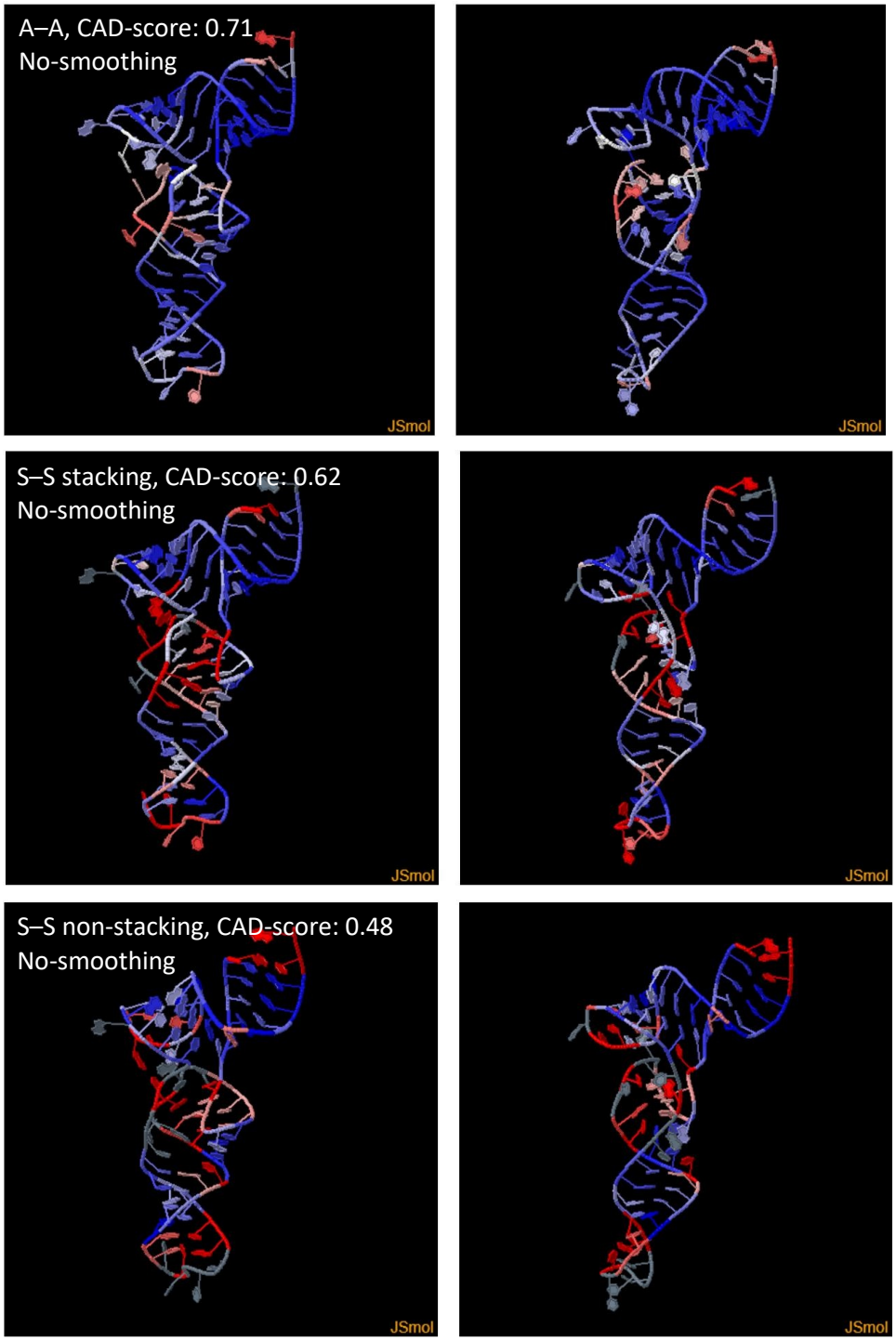

Fig. S6b Local differences between the reference structure (left) and the final conformation from 100 ns simulation for $tRNA^{Gly}$ (right).

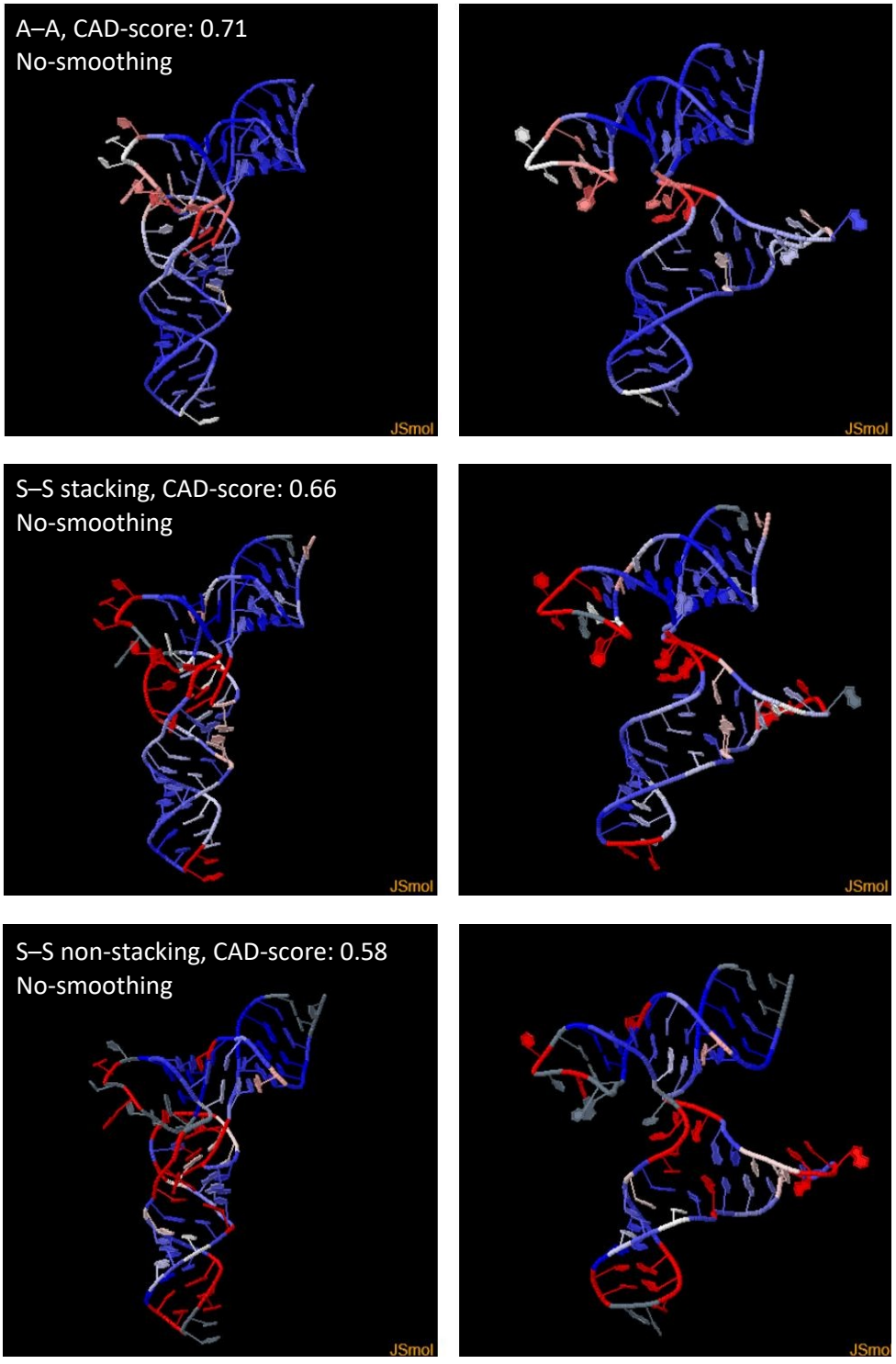

Fig. S6c Local differences between the reference structure (left) and the final conformation from 100 ns simulation for $tRNA^{His}$ (right).

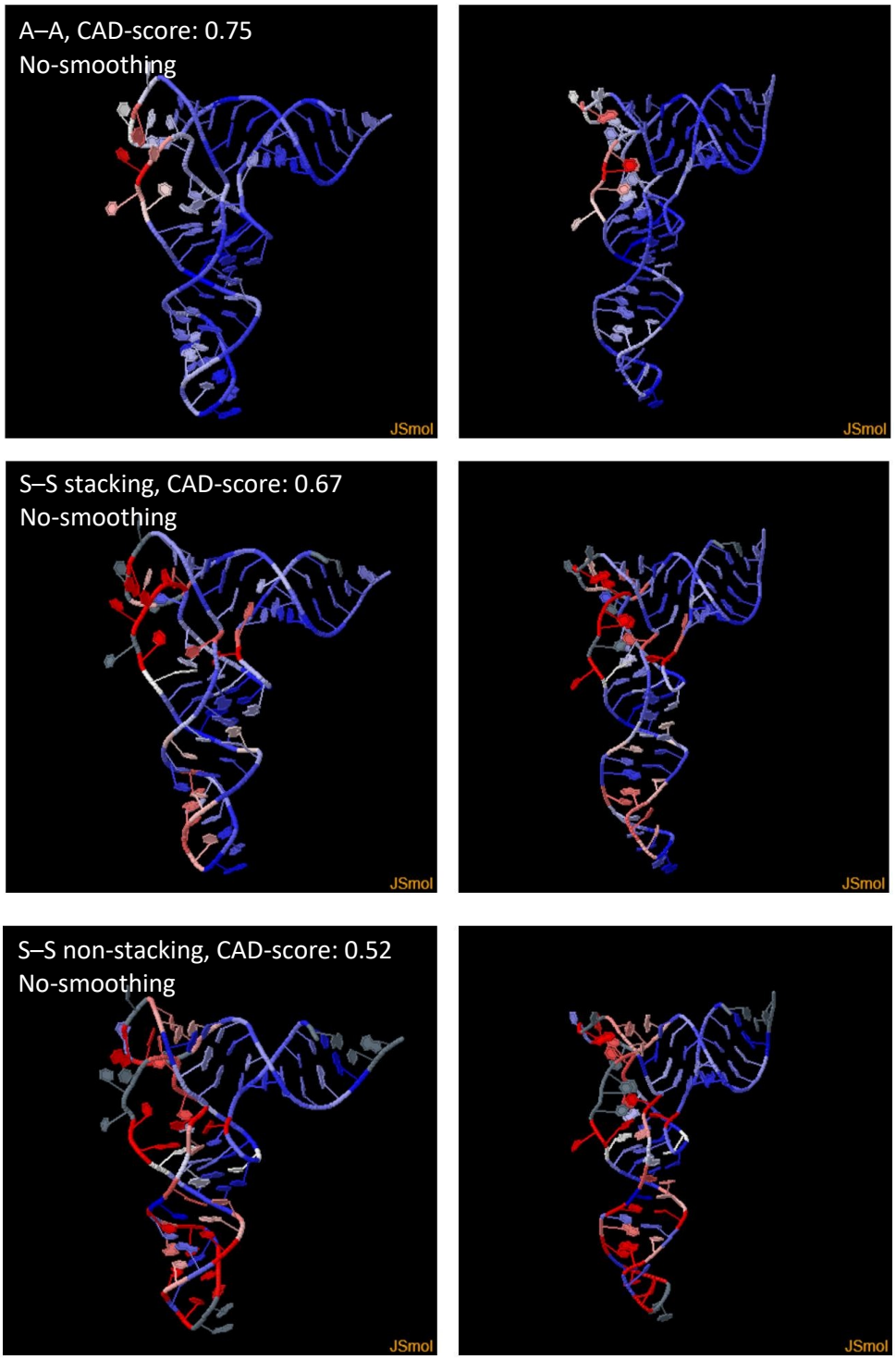

Fig. S6d Local differences between the reference structure (left) and the final conformation from 100 ns simulation for $tRNA^{Phe}$ (right).

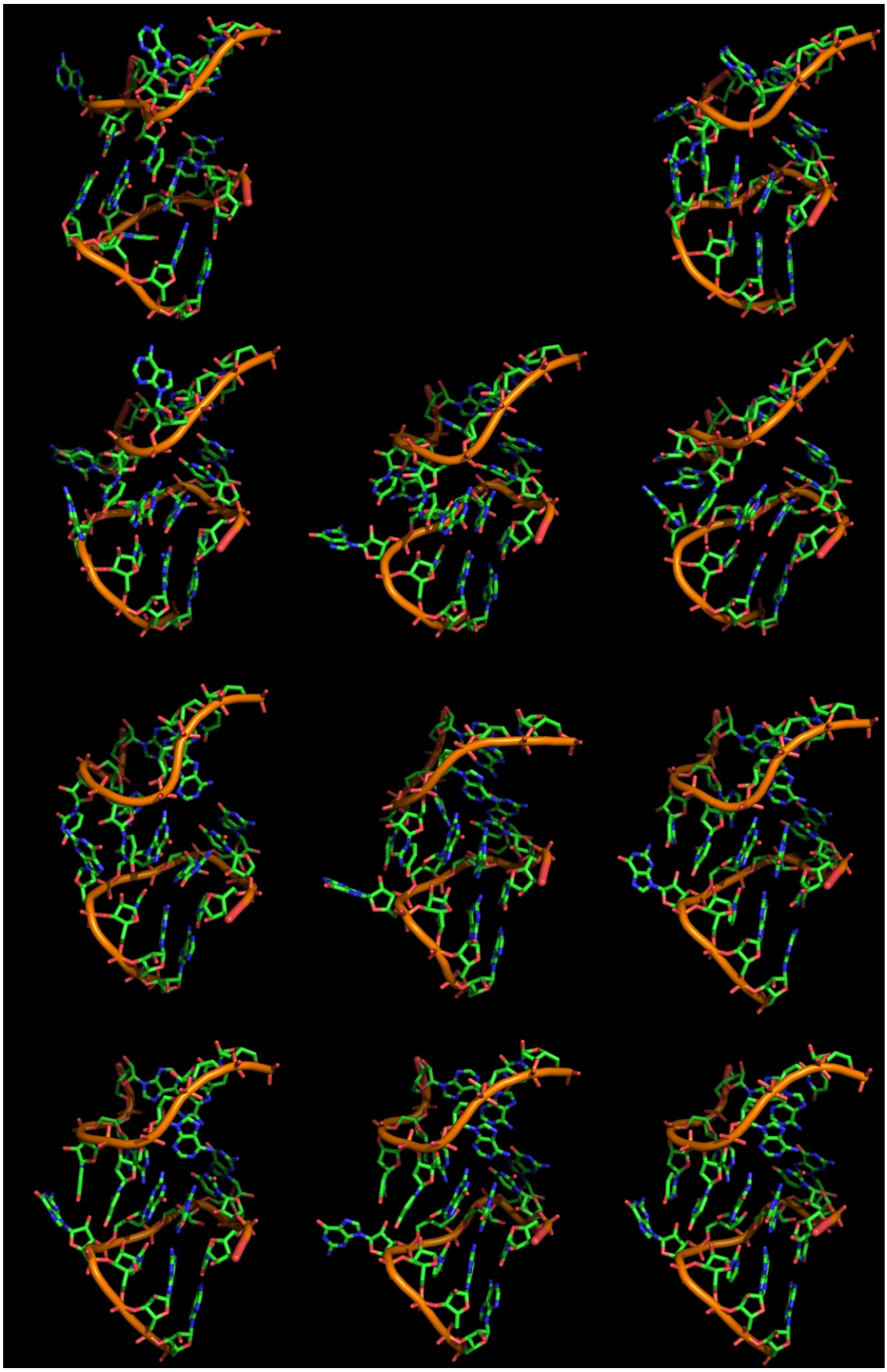

Fig. S7a The 3D model of D-T loop-loop interaction for $tRNA^{Ala}$ analog extracted from the simulation at 0ns (reference), 9ns, 17ns, 28ns, 33ns, 46ns, 58ns, 70ns, 77ns, 81ns, 92ns respectively.

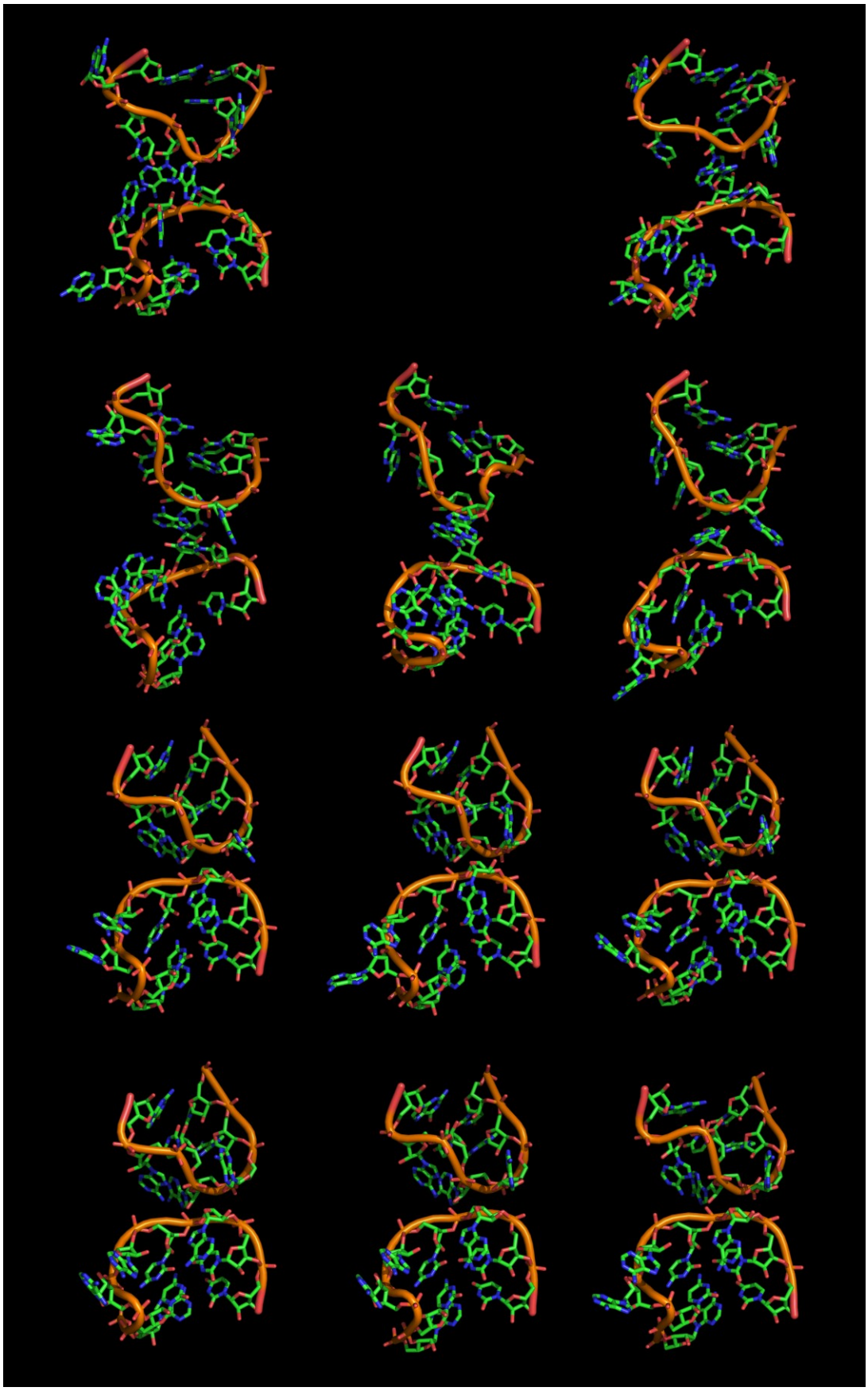

Fig. S7b The 3D model of D-T loop-loop interaction for $tRNA^{Gly}$ analog extracted from the simulation at 0ns (reference), 2ns, 11ns, 27ns, 37ns, 48ns, 54ns, 63ns, 74ns, 89ns, 93ns respectively.

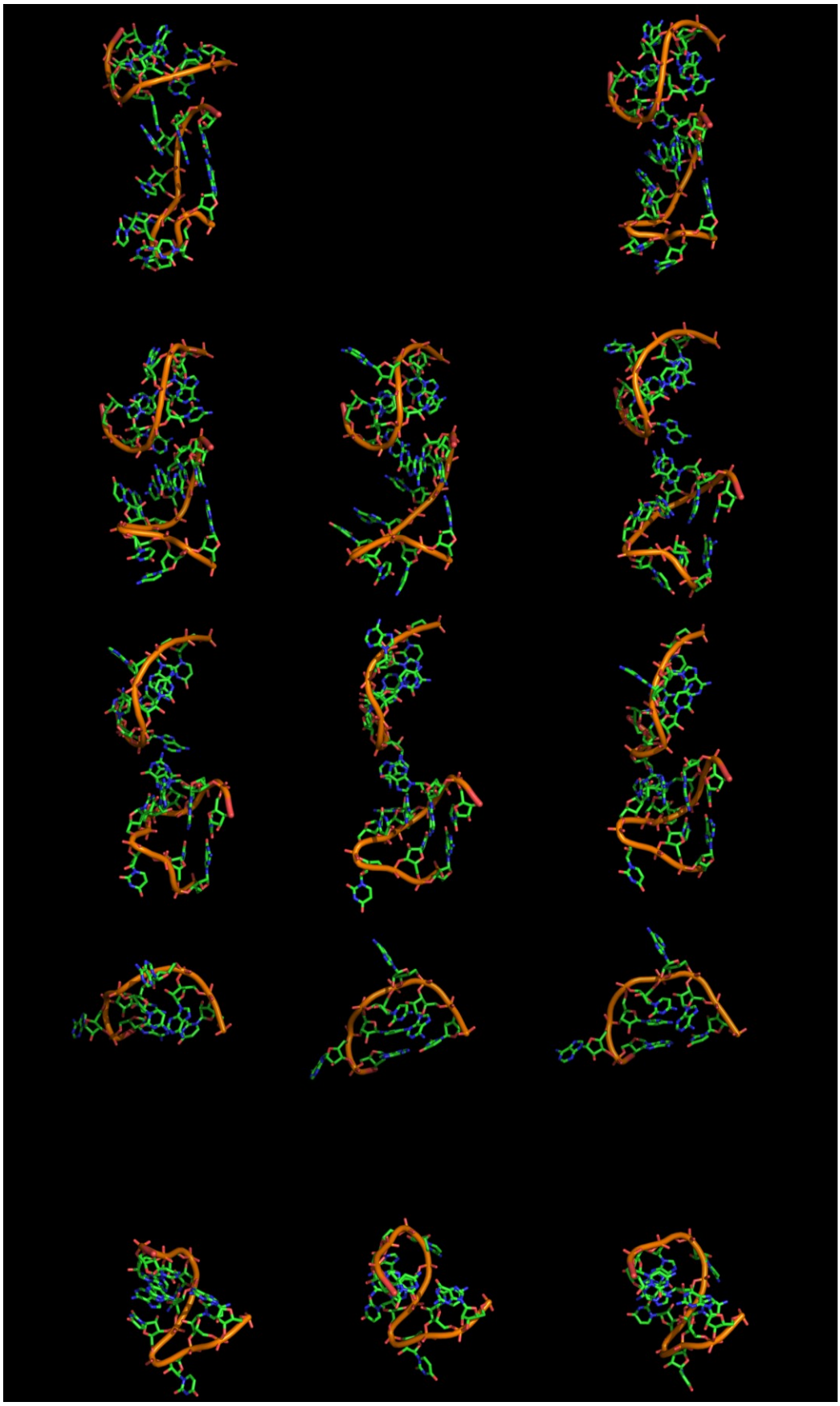

Fig. S7c The 3D model of D-T loop-loop interaction for $tRNA^{His}$ analog extracted from the simulation at 0ns (reference), 10ns, 14ns, 29ns, 35ns, 47ns, 57ns, 63ns, 79ns, 90ns, 92ns respectively.

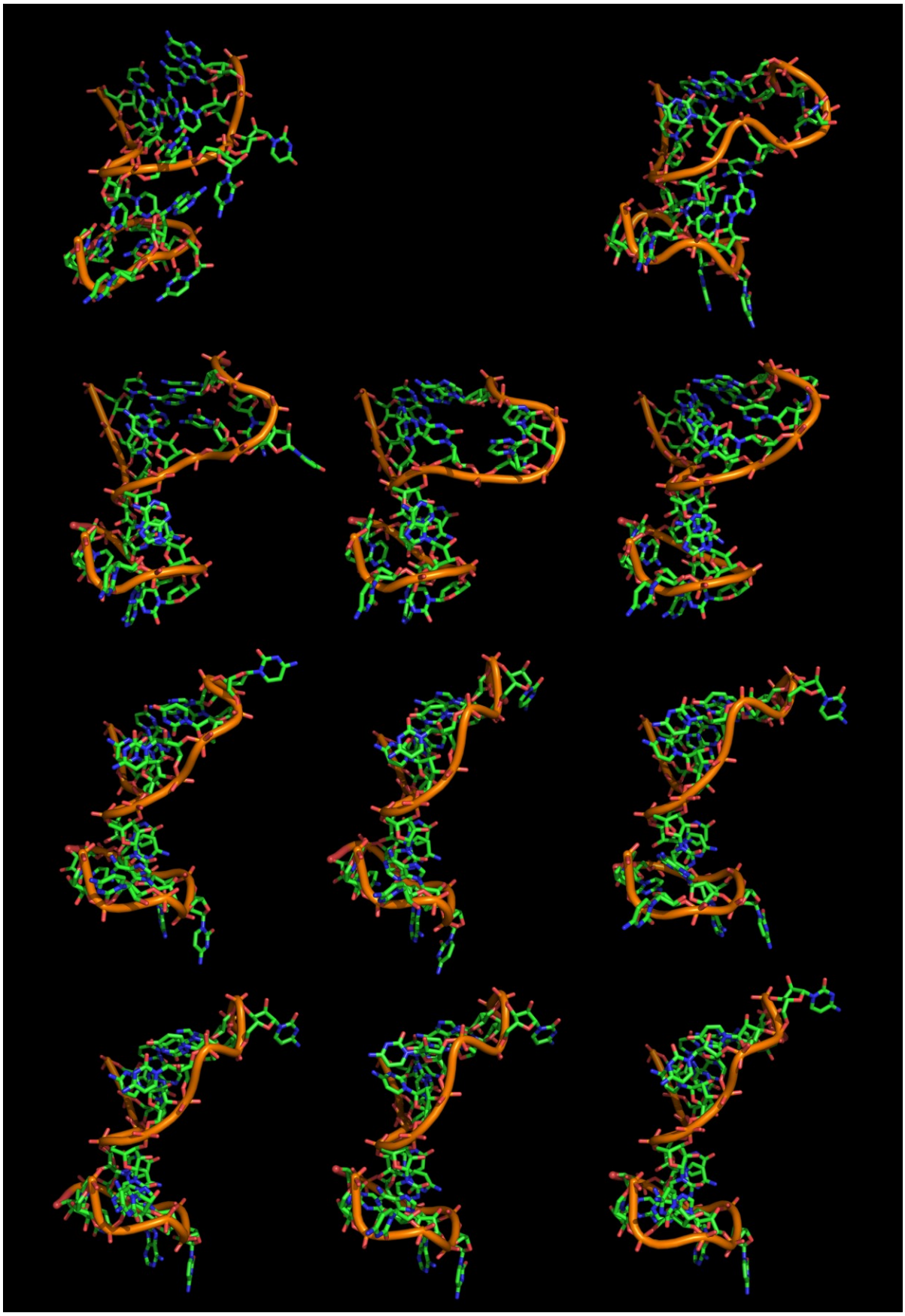

Fig. S7d The 3D model of D-T loop-loop interaction for $tRNA^{Phe}$ analog extracted from the simulation at 0ns (reference), 5ns, 19ns, 25ns, 37ns, 44ns, 54ns, 70ns, 75ns, 89ns, 92ns respectively.

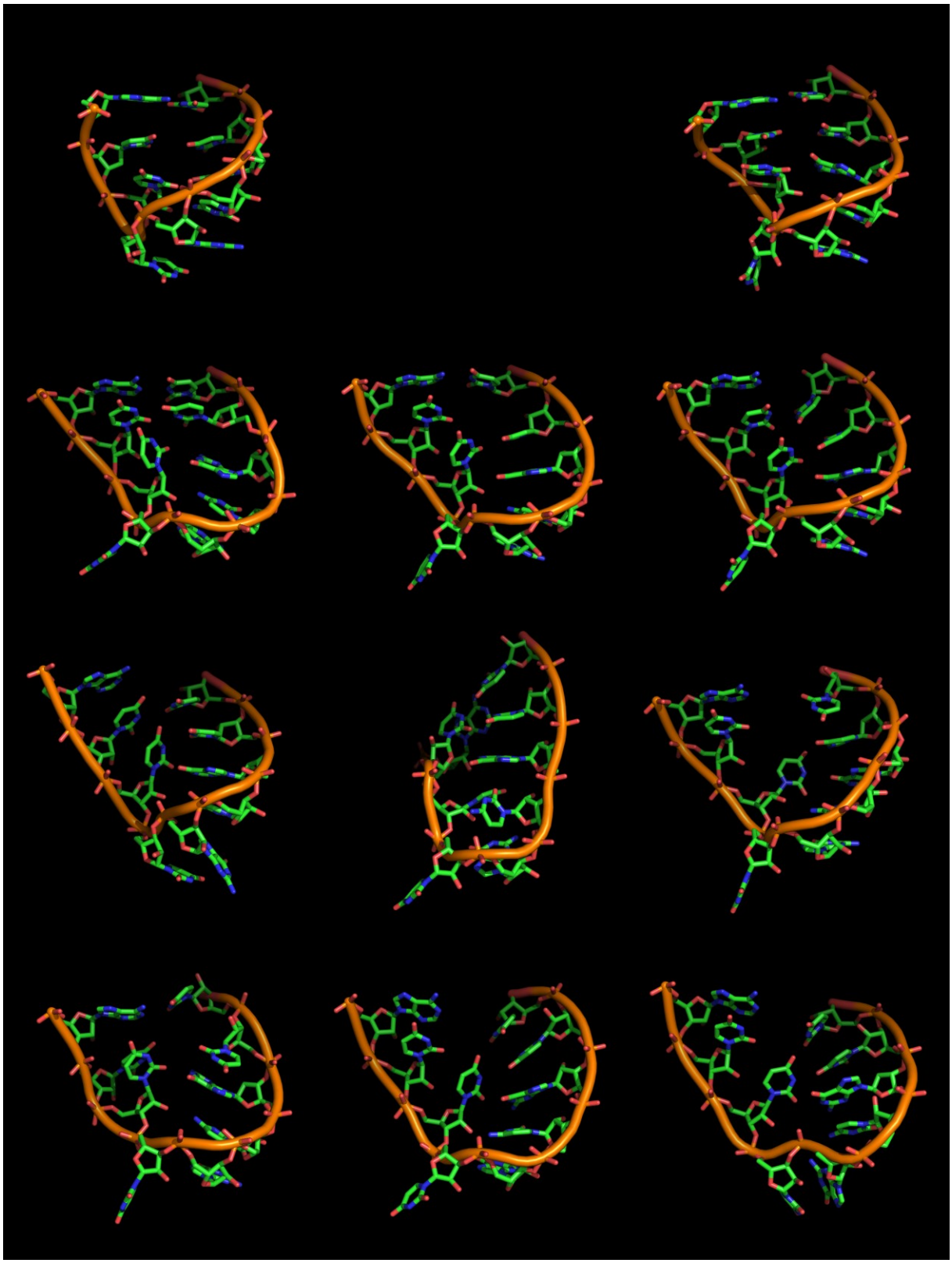

Fig. S8a The 3D model of anticodon loop for $tRNA^{Ala}$ analog extracted from the simulation at 0ns (reference), 9ns, 17ns, 28ns, 33ns, 46ns, 58ns, 70ns, 77ns, 81ns, 92ns respectively.

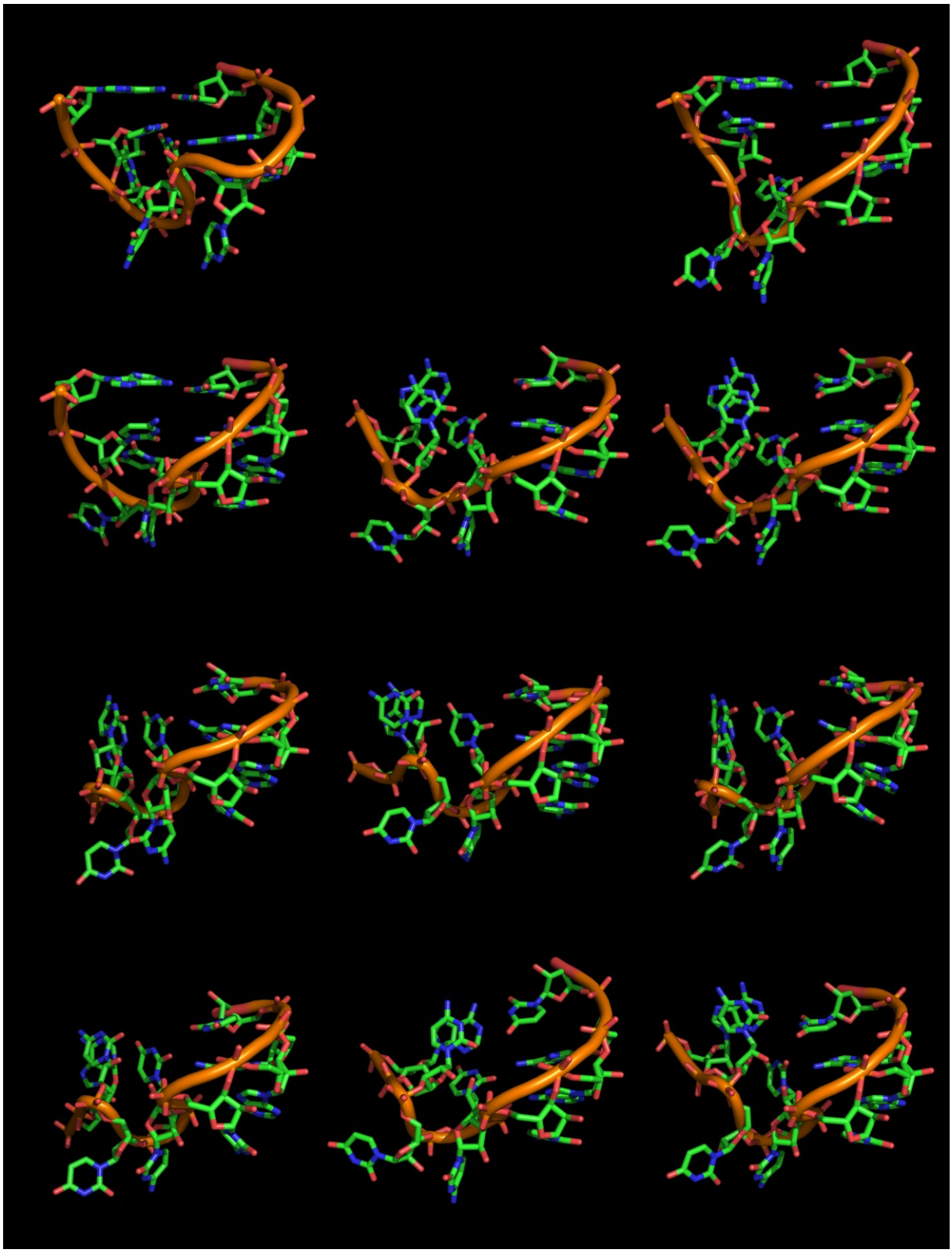

Fig. S8b The 3D model of anticodon loop for $tRNA^{Gly}$ analog extracted from the simulation at 0ns (reference), 2ns, 11ns, 27ns, 37ns, 48ns, 54ns, 63ns, 74ns, 89ns, 93ns respectively.

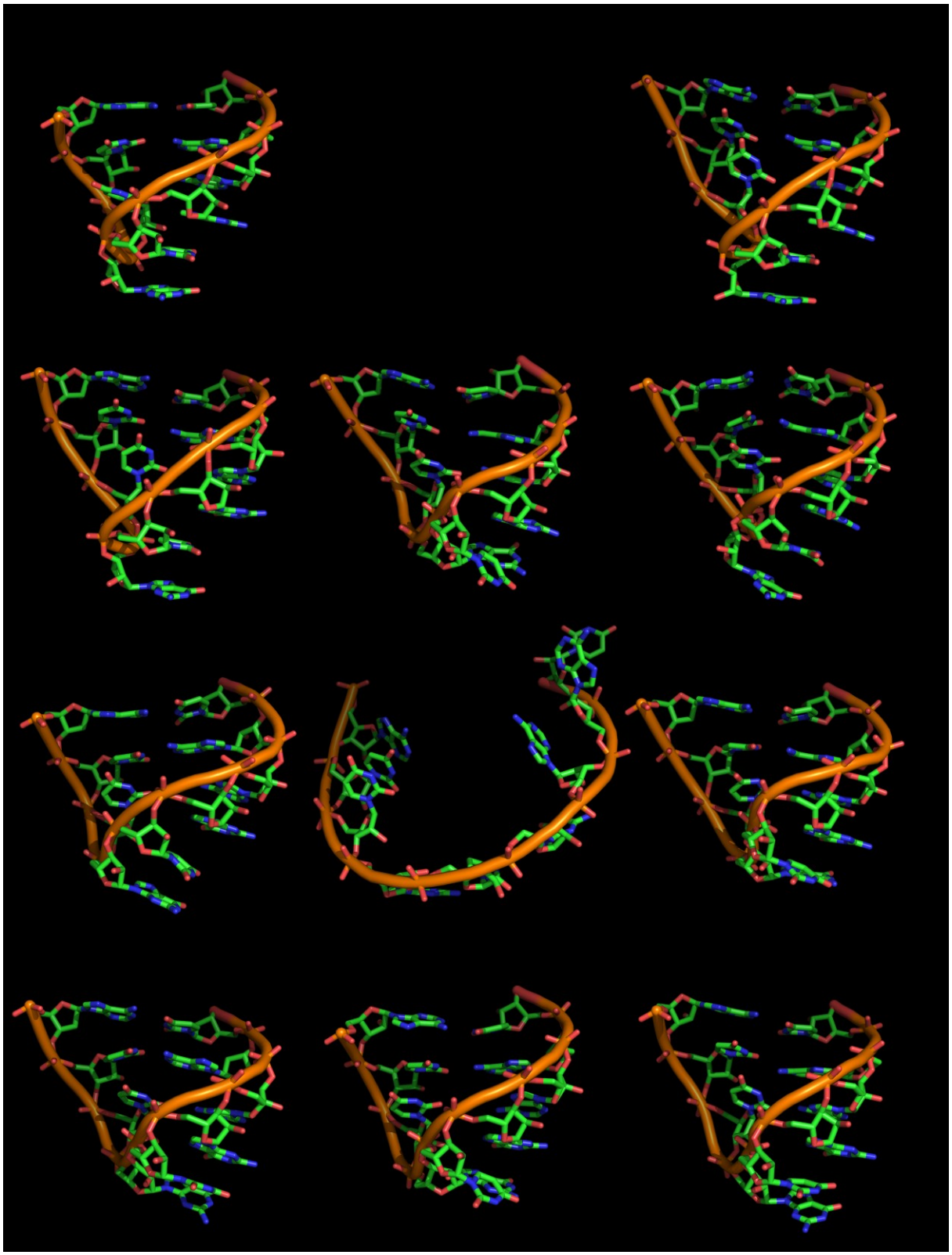

Fig. S8c The 3D model of anticodon loop for $tRNA^{His}$ analog extracted from the simulation at 0ns (reference), 10ns, 14ns, 29ns, 35ns, 47ns, 57ns, 63ns, 79ns, 90ns, 92ns respectively.

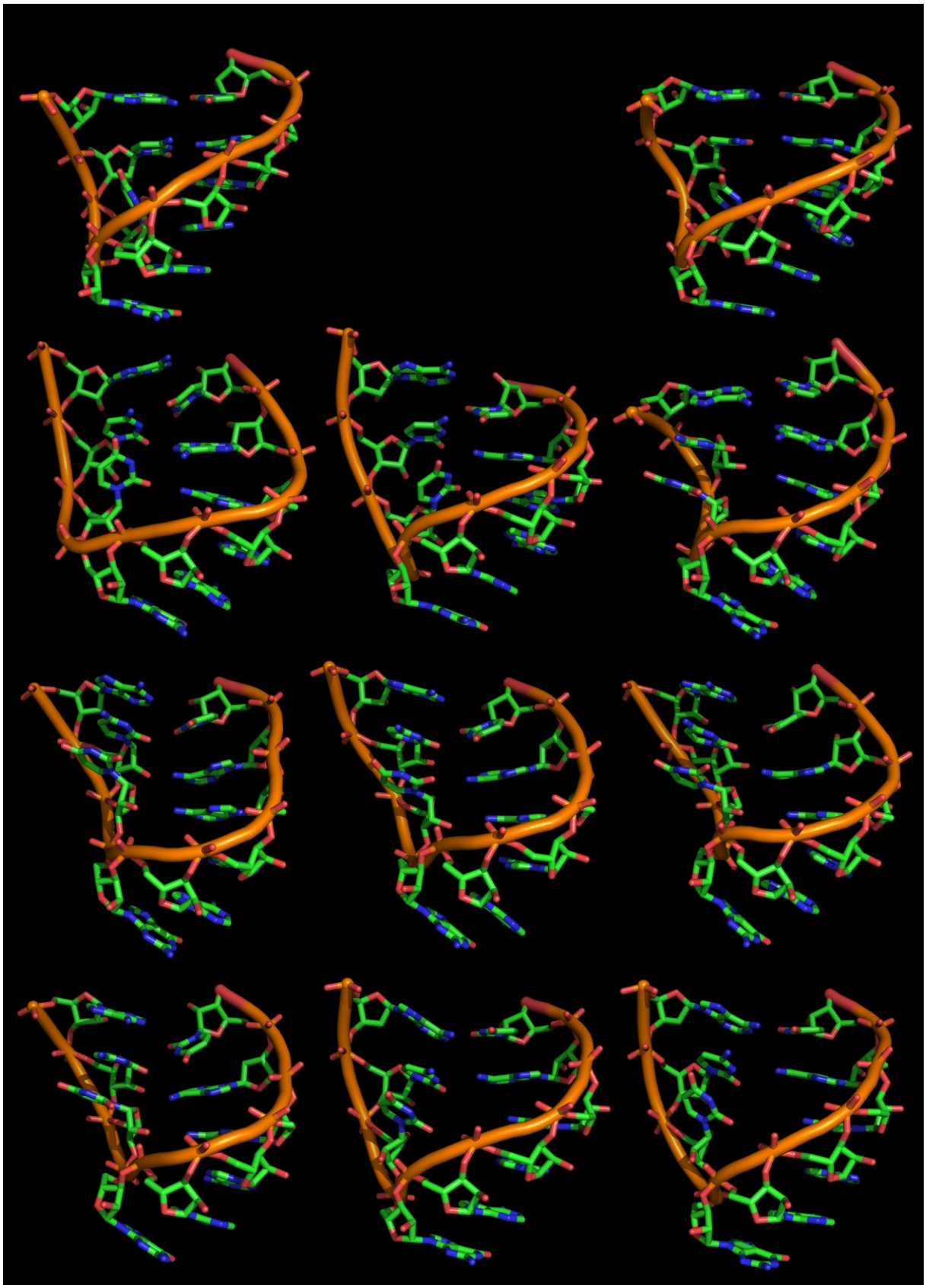

Fig. S8d The 3D model of anticodon loop for $tRNA^{Phe}$ analog extracted from the simulation at 0ns (reference), 5ns, 19ns, 25ns, 37ns, 44ns, 54ns, 70ns, 75ns, 89ns, 92ns respectively.

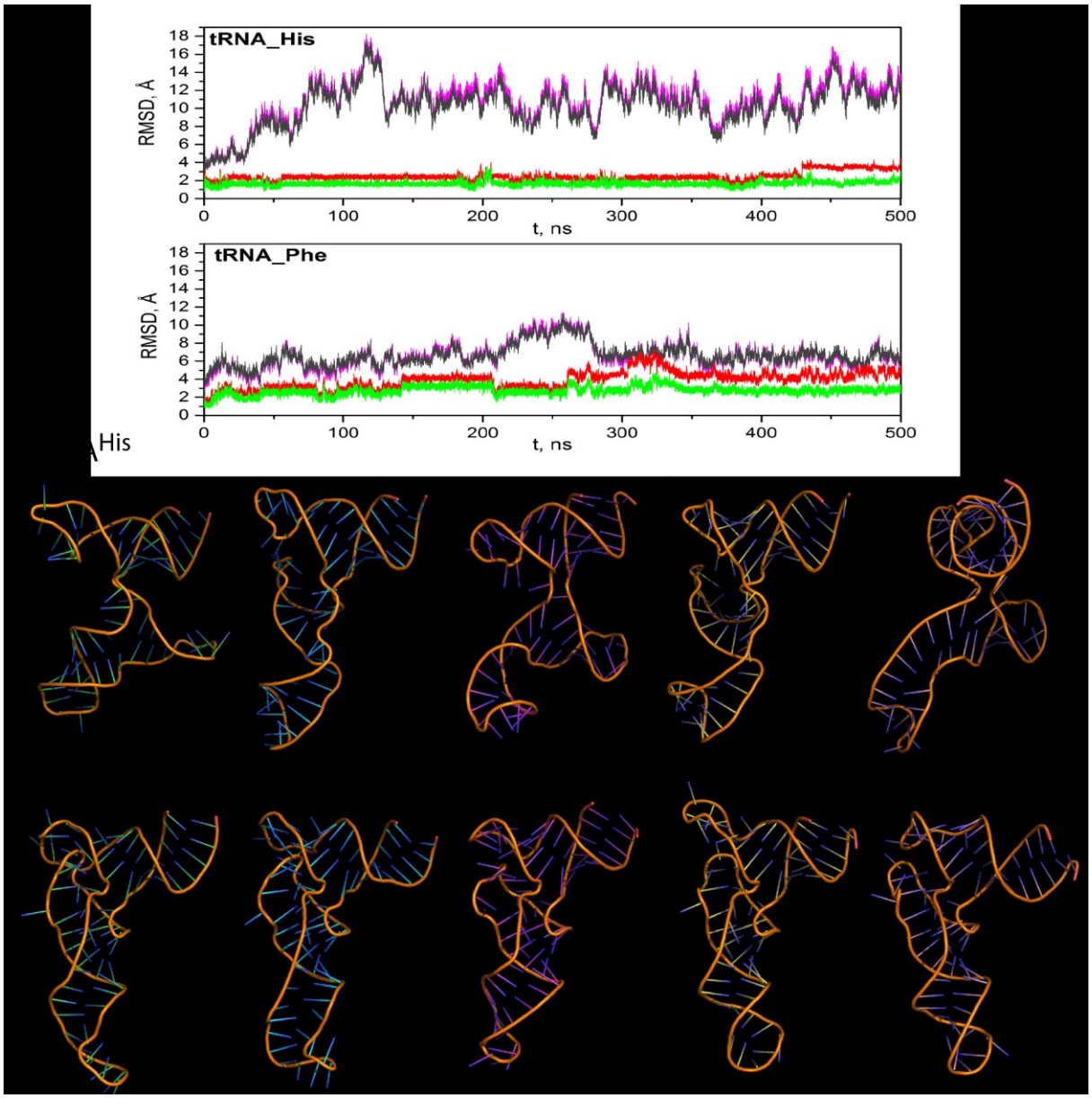

Fig. S9 All atoms RMSD values for $tRNA^{His}$, $tRNA^{Phe}$ trajectories in the course of 500 ns calculation and structure snapshots for every 100 ns.