Supporting Information

Mg^{2+}-Dependent Methyl Transfer by a Knotted Protein: A Molecular Dynamics Simulation and Quantum Mechanics Study

Agata P. Perlinska[1,2], Marcin Kalek[1]*, Thomas Christian[3], Ya-Ming Hou[3]* and Joanna I. Sulkowska[1,4]*

[1] Centre of New Technologies, University of Warsaw, Poland
[2] College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Poland
[3] Department of Biochemistry and Molecular Biology, Thomas Jefferson University, USA
[4] Faculty of Chemistry, University of Warsaw, Poland

* Corresponding authors: m.kalek@cent.uw.edu.pl, ya-ming.hou@jefferson.edu and jsulkowska@cent.uw.edu.pl

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1 Molecular Dynamics simulations

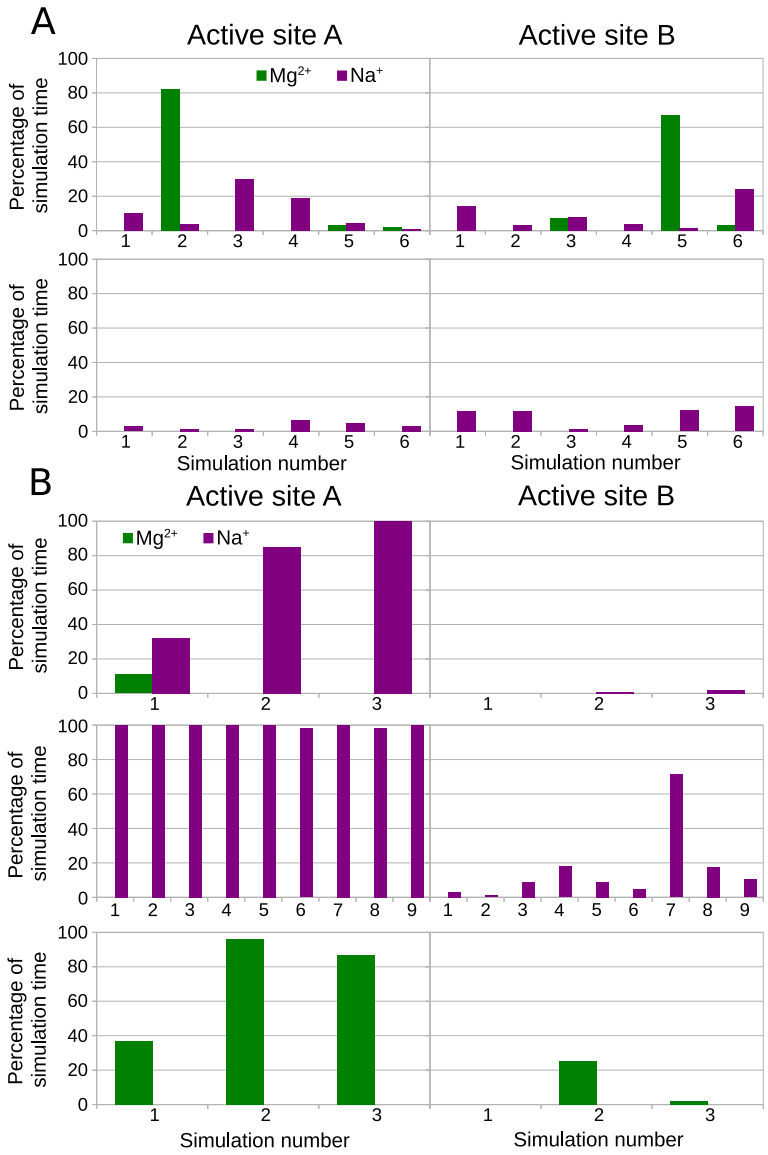


Figure S1: Frequency of ions occurrence in the negatively-charged pocket in the active site. A. holoenzyme TrmD; upper panel – both Mg^{2+} and Na^{+} ions present; lower panel – only Na^{+} ions present, B. holoenzyme TrmD with tRNA bound; upper panel – both Mg^{2+} and Na^{+} ions present; middle panel – only Na^{+} ions present; lower panel – only Mg^{2+} ions present.

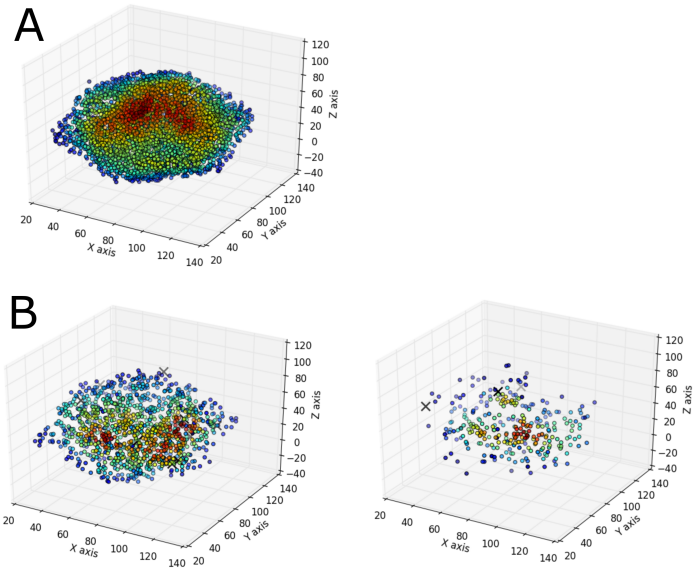


Figure S2: Examples of ion distribution in holoenzyme TrmD simulations with ions randomly distributed in the solvent colored by density. A. only Na^+ ions present, B. both Mg^{2+} (right) and Na^+ (left) ions present. Position of each ion is plotted using its Cartesian coordinates. Each trajectory was sampled every nanosecond.

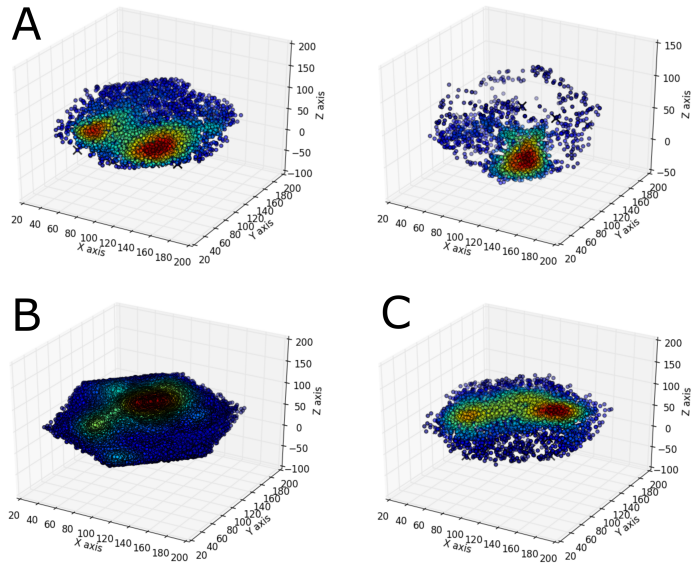


Figure S3: Examples of ion distribution in holoenzyme with tRNA TrmD simulations with ions randomly distributed in the solvent colored by density. A. both Mg^{2+} (right) and Na^+ (left) ions present, B. only Na^+ ions present, C. only Mg^{2+} ions present. Position of each ion is plotted using its Cartesian coordinates. Each trajectory was sampled every nanosecond.

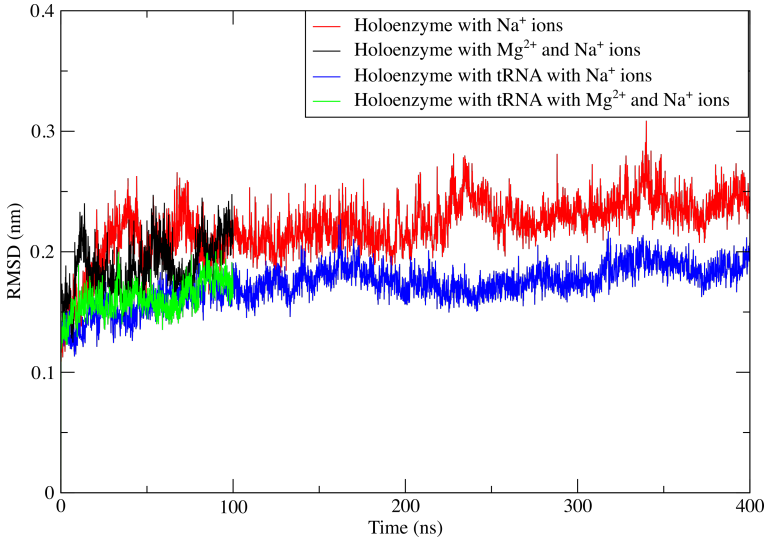


Figure S4: Cα RMSD of example trajectories of TrmD with and without tRNA bound.

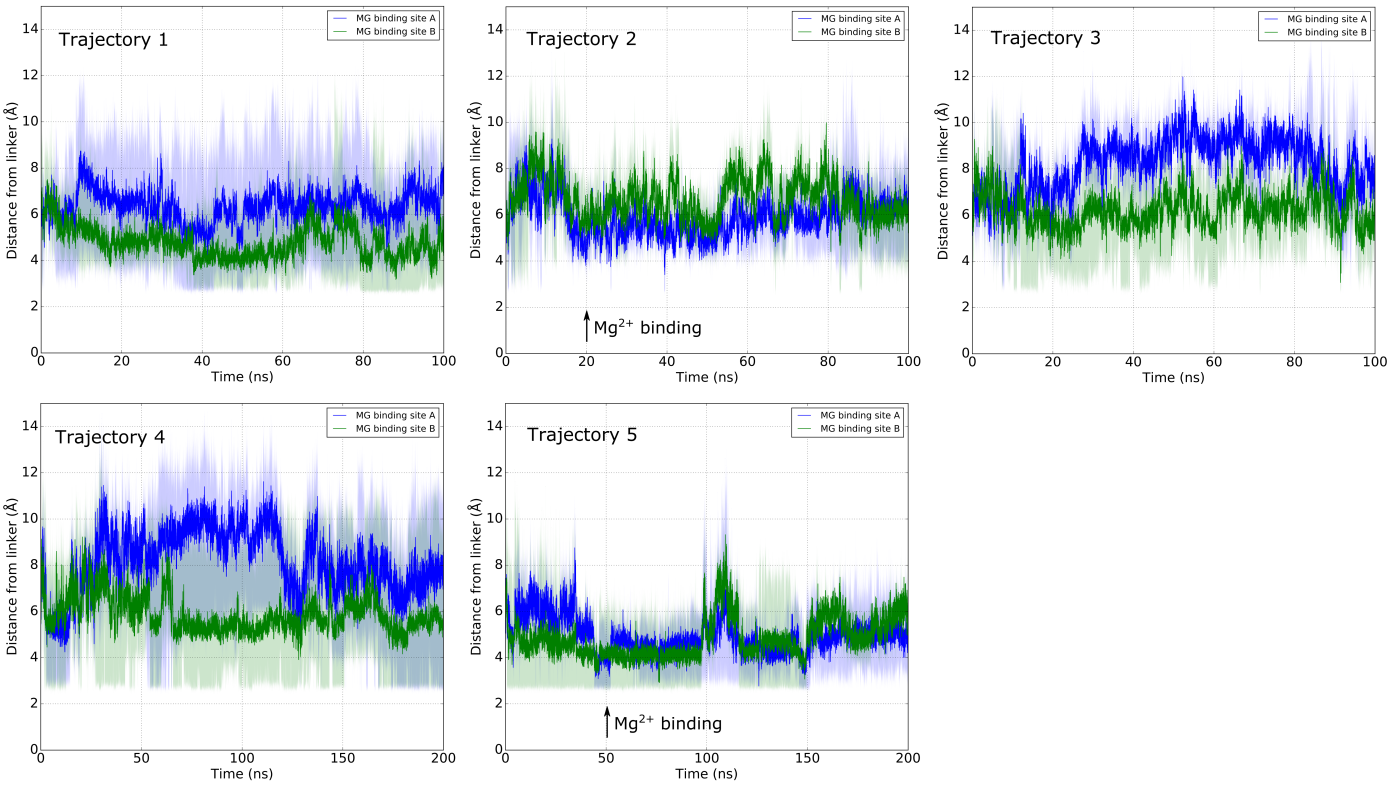


Figure S5: Average minimal distance between the heavy atoms of three residues forming the ion binding site (E116, D177 and SAM) and the interdomain linker (residues 160-170). The shaded area shows the span between the longest and the shortest of those distances. The trajectories show holoenzyme TrmD with Mg^{2+} and Na^{+} ions. Mg^{2+} binds only in trajectories 2 and 5 (in each to one binding site). This binding influences the position and flexibility of both linkers. Specifically, the linker interacting with an empty ion binding site blocks the access to it.

Table S1: Type and duration of MD simulations of TrmD holoenzyme or in complex with tRNA.

		Simulation time [ns]	Type
Ions randomly distributed in the solvent			
TrmD holoenzyme (Mg^{2+} and Na^{+} ions)	1	100	MD
	2	100	MD
	3	100	MD
	4	200	MD
	5	200	MD
	6	200	MD
TrmD holoenzyme (Na^{+} ions)	1	400	MD
	2	400	MD
	3	400	MD
	4	400	MD
	5	400	MD
	6	400	MD
TrmD holoenzyme with tRNA (Mg^{2+} ions)	1	100	MD
	2	100	MD
	3	100	MD
	4	23 x 50	REMD
	5	23 x 50	REMD
TrmD holoenzyme with tRNA (Mg^{2+} and Na^{+} ions)	1	100	MD
	2	100	MD
	3	100	MD
	4	22 x 50	REMD
	5	23 x 50	REMD
	6	23 x 50	REMD
	7	23 x 50	REMD
TrmD holoenzyme with tRNA (Na^{+} ions)	1	400	MD
	2	400	MD
	3	263	MD
	4	301	MD
	5	400	MD
	6	400	MD
	7	400	MD
	8	400	MD
	9	625	MD
Single ions placed in certain locations			
TrmD holoenzyme with tRNA (Mg^{2+} and Na^{+} ions) Mg^{2+} near G37	1	100	MD
	2	100	MD
	3	100	MD
	4	100	MD
	5	100	MD
	6	100	MD
	7	100	MD
	8	100	MD
	9	100	MD
	10	100	MD
TrmD holoenzyme with tRNA (Mg^{2+} and Na^{+} ions) Mg^{2+} in the negatively-charged pocket	1	100	MD
	2	100	MD
	3	100	MD
	4	100	MD
	5	100	MD
	6	100	MD
	7	100	MD
	8	100	MD
	9	100	MD
	10	100	MD

2 Identification of putative ions in crystal structures

We performed an analysis of all water molecules present in biological assemblies of crystal structures of TrmD. We found putative ions in TrmD structures among water molecules based on their coordination geometry. In 18 cases water molecules have a coordination number exceeding 4 (Table S2). Because it is difficult to distinguish Mg^{2+} (or other ions

frequently present in protein's surroundings) from a water molecule based on electron density, the observed waters are likely to be hidden ions. In four structures the water-represented ions are placed in the active site, corresponding to localization of the Mg^{2+} ion in the simulations. All four structures have resolution smaller than 2Å and their putative ions' B-factors are smaller than $55Å^2$, indicating that their positions are likely meaningful.

Table S2: All of the water molecules in TrmD crystal structures that have more than 4 polar interactions.

PDB code	Chain	ResID	Coordination Number	Localization	B-Factor
1uaj	A	1040	5	knot's vicinity	29.88
1uaj	B	1040	5	knot's vicinity	29.88
1ual	A	1083	5	surface	32.81
1ual	A	1145	5	surface	53.26
1ual	A	1152	5	surface	28.74
1ual	B	1083	5	surface	32.81
1ual	B	1145	5	surface	53.26
1ual	B	1152	5	surface	28.74
4mcb	A	438	5	dimer interface	26.10
4mcb	A	547	5	**active site**	53.72
4mcb	A	620	5	dimer interface	50.81
4mcc	B	604	5	dimer interface	41.58
4yvg	A	557	5	dimer interface	23.15
4yvg	B	557	5	dimer interface	23.15
4yq0	A	407	5	**active site**	39.89
4yq6	A	401	5	**active site**	39.83
4yqg	A	434	6	vicinity of the active site	48.28
4yqo	A	404	5	**active site**	35.24

3 Impact on the structure

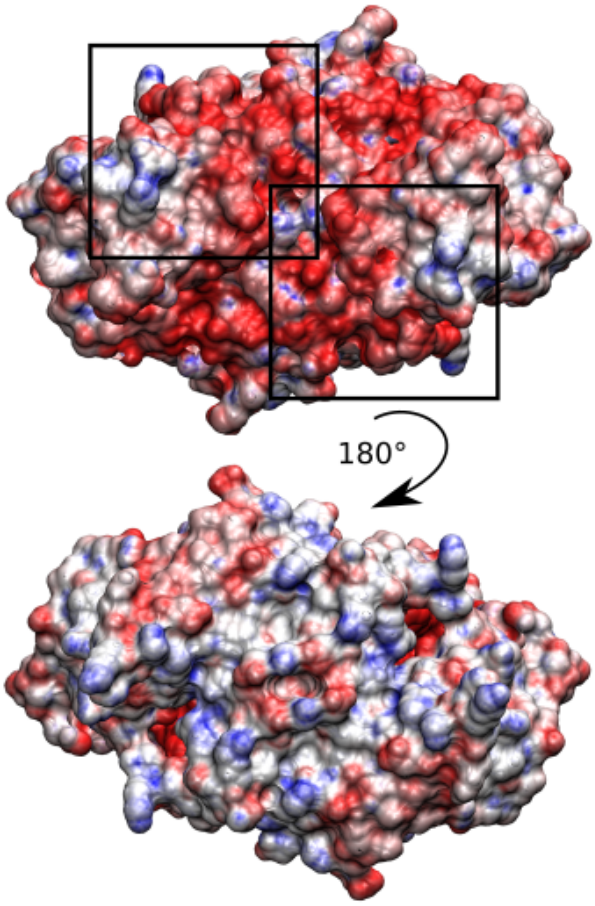


Figure S6: Structure of holoenzyme TrmD in surface representation shown from different angles. The position of the squares indicates the surface of the active sites. The surface is colored by the electrostatic potential ranging from -10kBT/e (in red) to 10 kBT/e (in blue).

Table S3: RMSD of SAM and the number of hydrogen (H) bonds between SAM and protein. SAM A and SAM B represent ligands from chain A and B, respectively. Cutoffs used for H-bond calculations are 3.2Å for donor-acceptor distance and 50^o for the angle.

Simulation	Trajectory	RMSD (SAM A)	RMSD (SAM B)	H-Bond (SAM A)	H-Bond (SAM B)
TrmD holoenzyme (Na^+ ions)	1	2.00 ± 0.36 Å	1.90 ± 0.32 Å	6.79 ± 1.42	5.90 ± 1.46
	2	1.87 ± 0.27 Å	2.01 ± 0.37 Å	5.40 ± 1.2	6.53 ± 1.47
	3	1.79 ± 0.24 Å	1.86 ± 0.33 Å	7.43 ± 1.52	5.71 ± 1.22
	4	1.90 ± 0.52 Å	2.20 ± 0.22 Å	5.58 ± 1.37	4.26 ± 1.02
	5	2.12 ± 0.24 Å	1.05 ± 0.54 Å	5.82 ± 1.37	7.13 ± 1.53
	6	1.14 ± 0.52 Å	1.85 ± 0.32 Å	6.49 ± 1.27	6.36 ± 1.47
TrmD holoenzyme (Mg^{2+} and Na^+ ions)	1	2.18 ± 0.34 Å	2.32 ± 0.35 Å	6.09 ± 1.48	5.63 ± 1.79
	2	1.15 ± 0.58 Å	1.36 ± 0.28 Å	5.71 ± 1.24	5.72 ± 1.16
	3	1.56 ± 0.40 Å	0.85 ± 0.06 Å	6.28 ± 1.08	5.86 ± 1.04
	4	0.77 ± 0.05 Å	1.38 ± 0.32 Å	6.12 ± 1.11	6.22 ± 1.36
	5	0.86 ± 0.27 Å	1.34 ± 0.26 Å	6.18 ± 1.13	6.48 ± 1.20
	6	2.06 ± 0.42 Å	0.99 ± 0.08 Å	6.38 ± 1.29	6.32 ± 1.12

4 Quantum chemical models

Table S4: Energies for all models (relative to respective initial states). The best models are indicated with underline.

	ΔE (kcal/mol)			
	TS 1	Intermediate	TS 2	Final state
Mg^{2+} in the negatively-charged pocket				
Model 1	8.5	9.9	25.3	-21.2
Model 2	7.6	26.3	30.9	-7.2
Model 3	14.0	13.0	30.9	-9.2
Model 4	22.1	23.7	45.6	-0.9
Model 5	21.9	26.7	39.6	-0.4
Model 6	20.8	19.9	42.2	-8.5
Model 7	13.6	19.9	40.3	7.2
Model 8	21.3	20.2	51.4	2.9
Mg^{2+} near G37				
Model 9	6.2	2.3	35.6	-8.6
Model 10	7.7	7.9	40.2	-16.7
Two Mg^{2+} ions				
Model 11	2.3	5.0	27.5	8.1
Model 12	4.8	6.8	32.9	1.3
Model 13	2.4	1.8	48.1	-5.0
Na^{+} in the negatively-charged pocket				
Model 14	12.0	13.2	37.5	2.2
Model 15	28.6	19.1	38.4	4.8

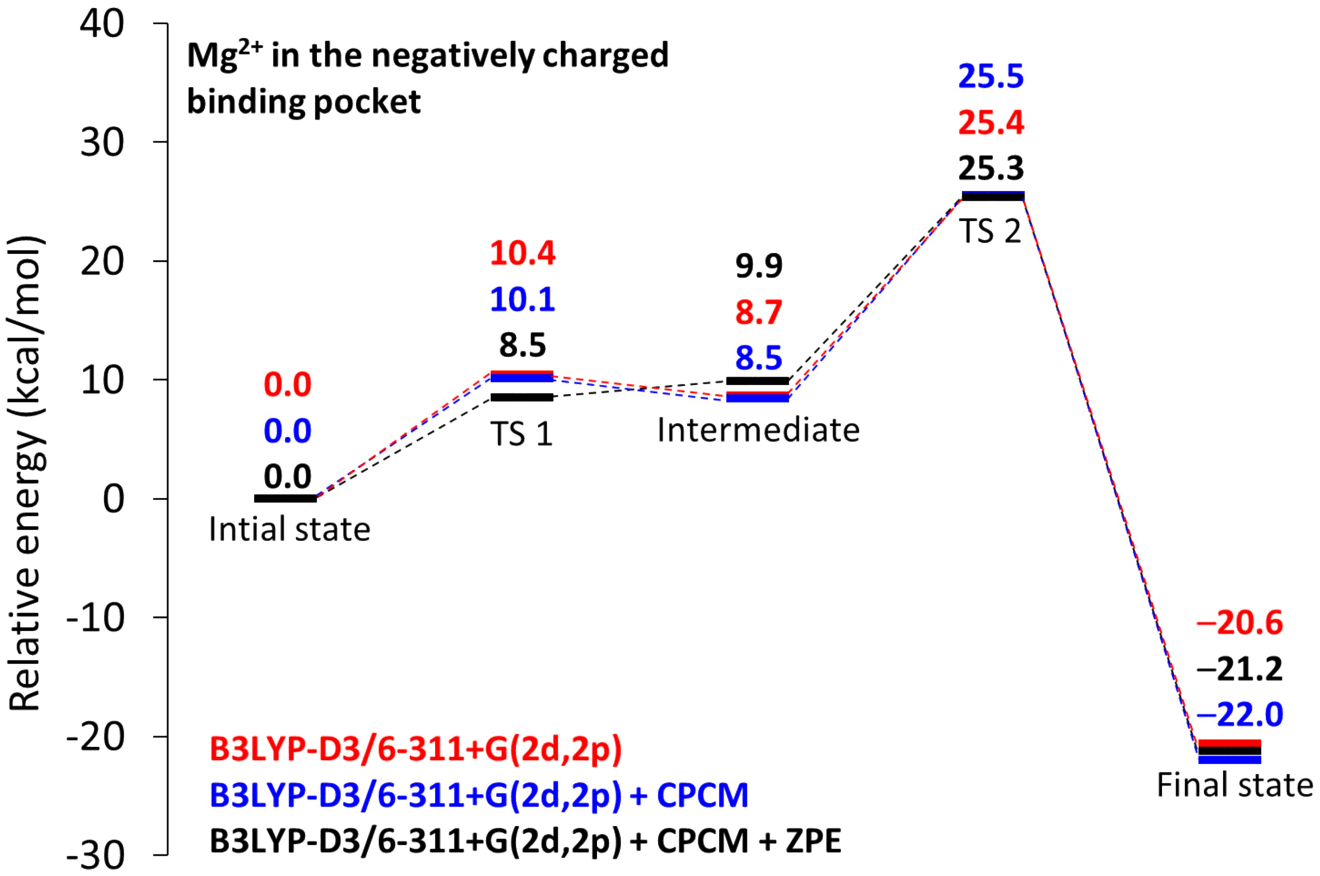


Figure S7: Energy profiles (electronic, electronic with solvation, and electronic with solvation and ZPE) for the methyl transfer reaction in Model 1.

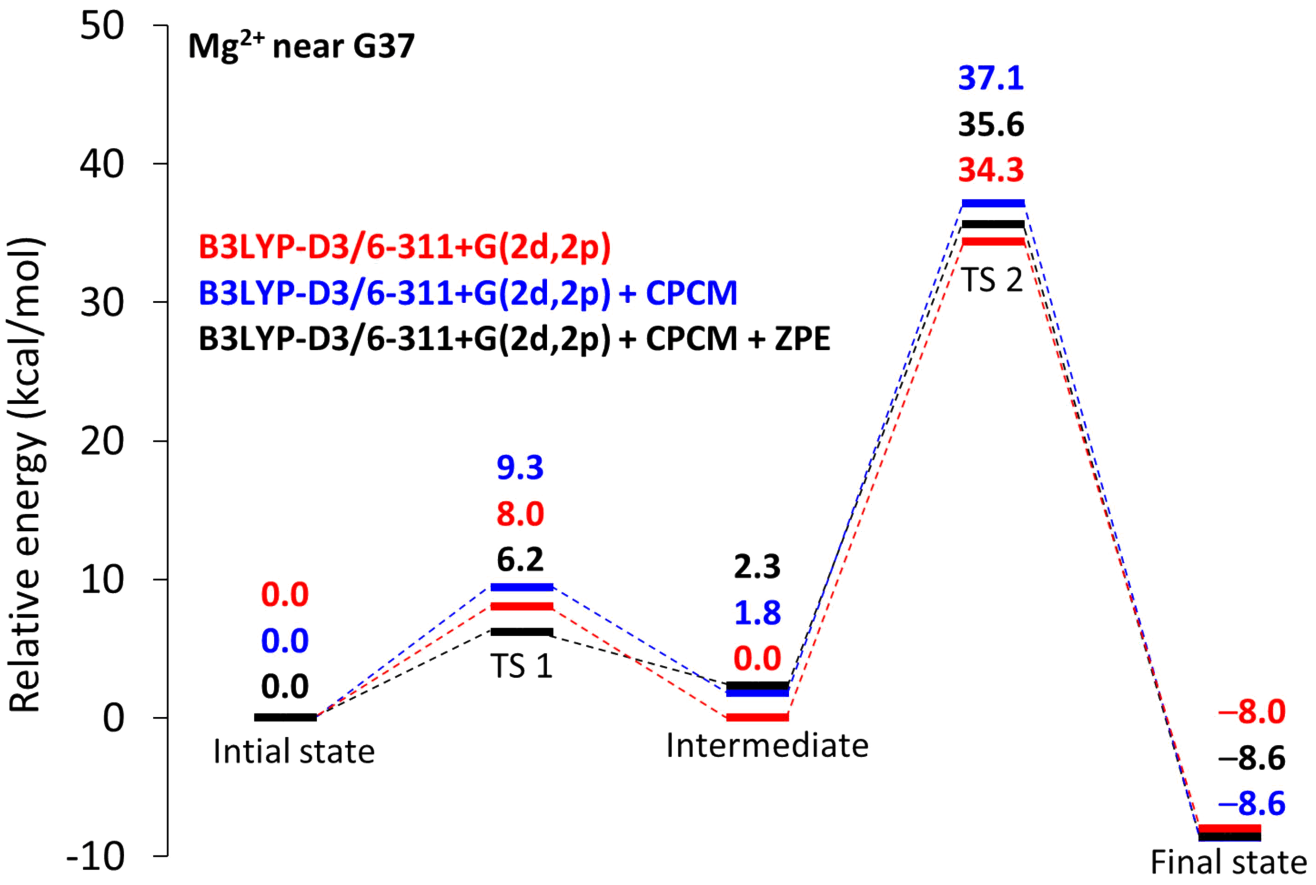


Figure S8: Energy profiles (electronic, electronic with solvation, and electronic with solvation and ZPE) for the methyl transfer reaction in Model 9.

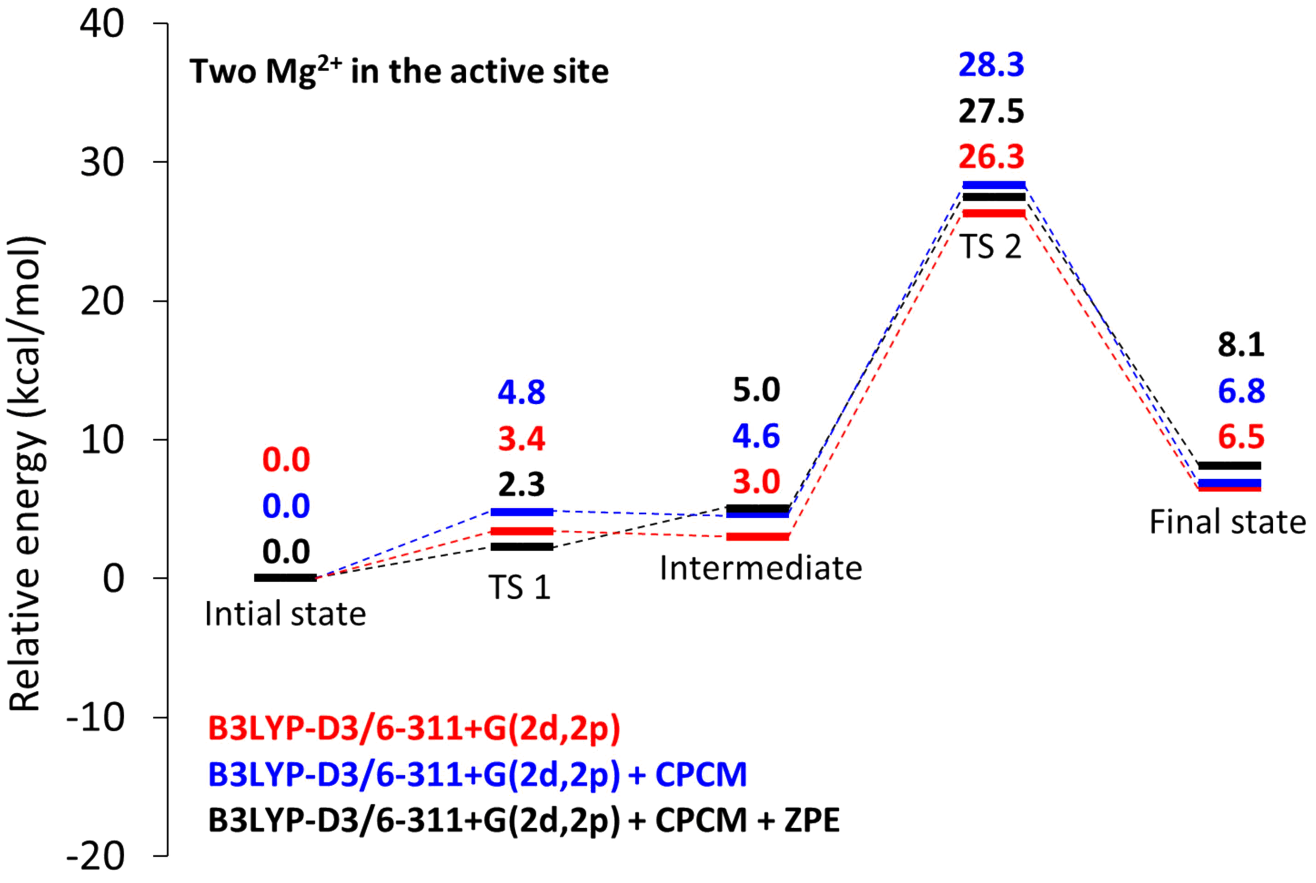


Figure S9: Energy profiles (electronic, electronic with solvation, and electronic with solvation and ZPE) for the methyl transfer reaction in Model 11.

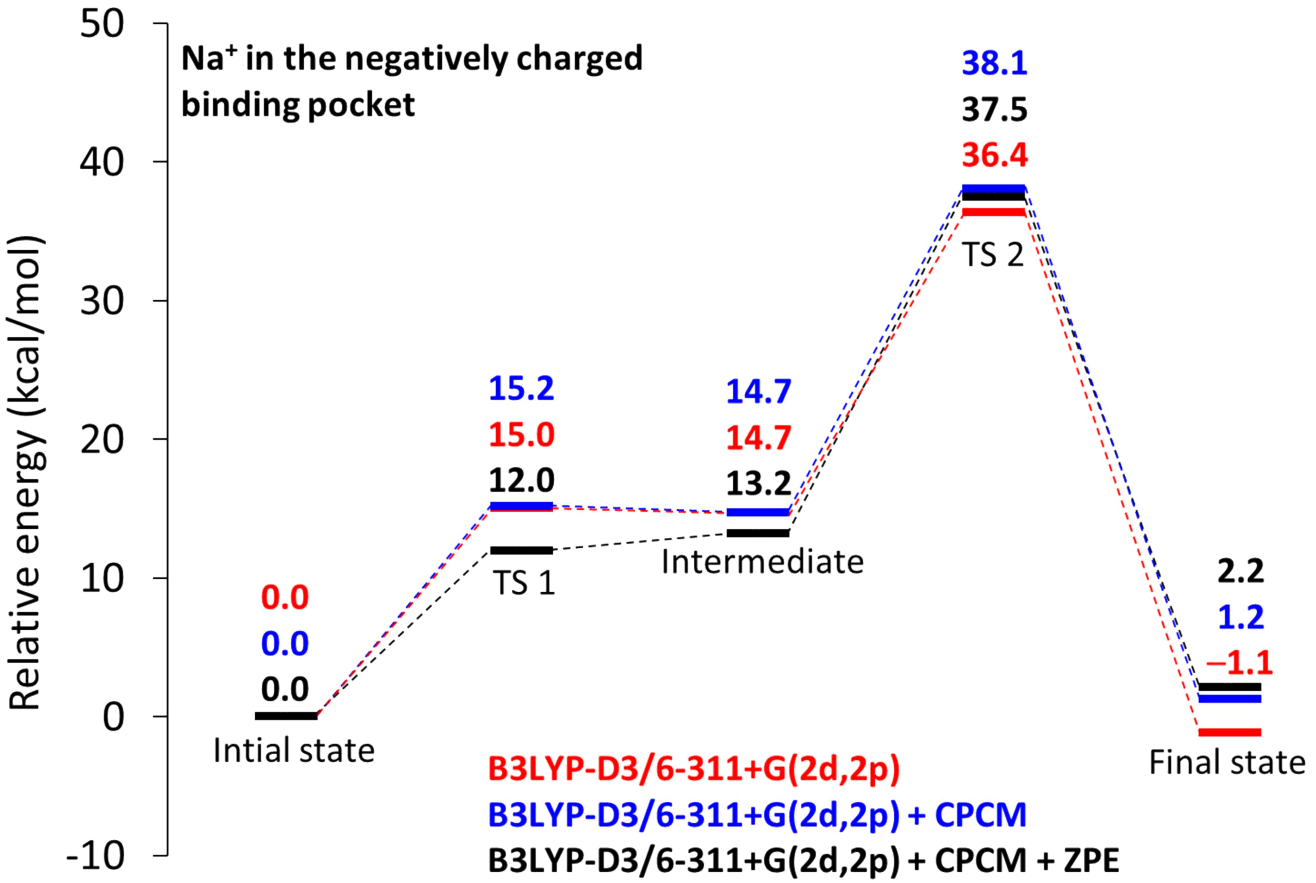


Figure S10: Energy profiles (electronic, electronic with solvation, and electronic with solvation and ZPE) for the methyl transfer reaction in Model 14.

Table S5: Calculated energies and energy corrections for all stationary points in the best models using B3LYP functional (in atomic units).

	E opt (6–31G(d,p))	E bb (6–311+G(2d,2p))	E solv (CPCM)	ZPE
Mg^{2+} in the negatively-charged pocket – Model 1				
Initial state	-5176.364754	-5177.790054	-5176.439093	1.641279
TS 1	-5176.345336	-5177.773513	-5176.420154	1.638787
Intermediate	-5176.348241	-5177.776245	-5176.422926	1.643546
TS 2	-5176.321042	-5177.749532	-5176.395290	1.641054
Final state	-5176.395747	-5177.822921	-5176.472287	1.642540
s^6-G37, Mg^{2+}				
Initial state	-5499.330698	-5500.760093	-5499.409096	1.642393
TS 2	-5499.280651	-5500.712037	-5499.359793	1.638774
s^6-G37, Co^{2+}				
Initial state	-5444.141037	-5445.664165	-5444.219037	1.642843
TS 2	-5444.094363	-5445.619108	-5444.173185	1.636747
WT G37, Co^{2+}				
Initial state	-5121.174810	-5122.698976	-5121.253793	1.642305
TS 2	-5121.135248	-5122.655790	-5121.210395	1.641737
Mg^{2+} near G37 – Model 9				
Initial state	-5397.153440	-5398.668162	-5397.237162	1.785401
TS 1	-5397.142421	-5398.655357	-5397.223999	1.780303
Intermediate	-5397.154555	-5398.668125	-5397.235444	1.786240
TS 2	-5397.099828	-5398.613431	-5397.179108	1.783007
Final state	-5397.164084	-5398.680932	-5397.248807	1.785424
s^6-G37, Mg^{2+}				
Initial state	-5720.117031	-5721.637086	-5720.200429	1.782230
TS 2	-5720.060269	-5721.580219	-5720.138694	1.779264
s^6-G37, Co^{2+}				
Initial state	-5665.024070	-6904.204417	-5665.106616	1.782286
TS 2	-5664.969442	-6904.154010	-5665.046837	1.779312
WT G37, Co^{2+}				
Initial state	-5342.062446	-6581.242218	-5342.144937	1.785289
TS 2	-5342.009608	-6581.187906	-5342.087976	1.783145
Two Mg^{2+} ions – Model 11				
Initial state	-7203.841782	-7205.767767	-7204.001187	2.133037
TS 1	-7203.838977	-7205.762423	-7203.996158	2.129098
Intermediate	-7203.840070	-7205.762992	-7203.996902	2.133691
TS 2	-7203.797304	-7205.725851	-7203.953546	2.131750
Final state	-7203.834890	-7205.757356	-7203.993815	2.134998
s^6-G37, Mg^{2+}				
Initial state	-7526.680835	-7526.680836	-7526.840002	2.130857
TS 2	-7526.640495	-7528.686830	-7526.795520	2.129142
s^6-G37, Co^{2+}				
Initial state	-7416.481860	-7418.541478	-7416.641768	2.129321
TS 2	-7416.444681	-7418.506536	-7416.598830	2.128475
WT G37, Co^{2+}				
Initial state	-7093.527037	-7095.582240	-7093.684289	2.132342
TS 2	-7093.484475	-7095.533907	-7093.639816	2.130990
Na^{+} in the negatively-charged pocket – Model 14				
Initial state	-5669.290544	-5670.893989	-5669.349873	1.785462
TS 1	-5669.274355	-5670.870046	-5669.333424	1.780370
Intermediate	-5669.274435	-5670.870556	-5669.333761	1.783081
TS 2	-5669.239188	-5670.836079	-5669.295738	1.784524
Final state	-5669.303811	-5670.895804	-5669.359344	1.786904

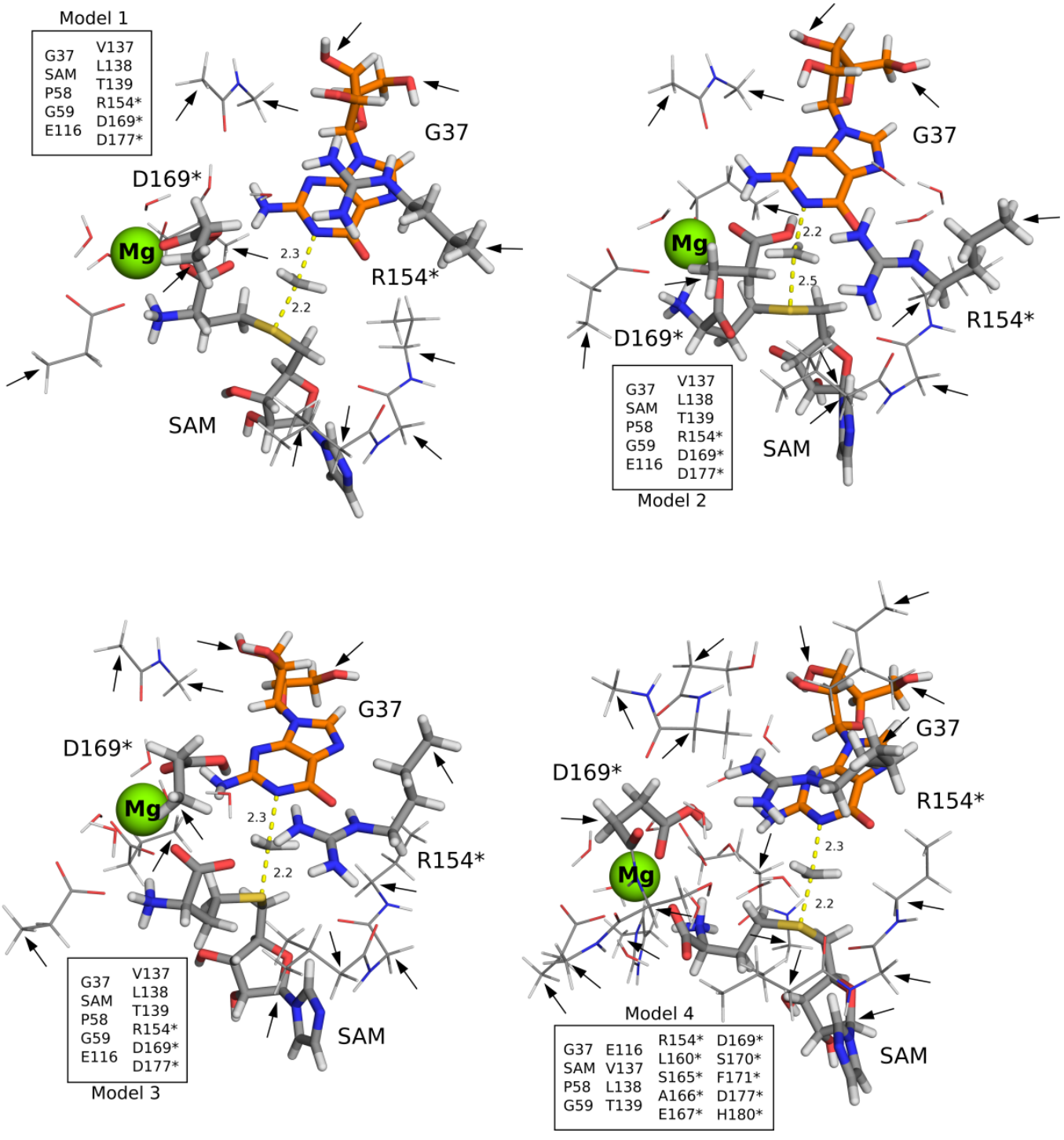


Figure S11: Optimized structures of the transition state of methyl transfer for the constructed models, with magnesium located in the vicinity of E116, D169*, D177*, and SAM (Models 1-4). Key residues are shown with sticks. Yellow dotted lines represent the distances (in Å) from the methyl group to the sulfur atom and the N1 of G37. Arrows indicate atoms that were kept fixed during geometry optimizations.

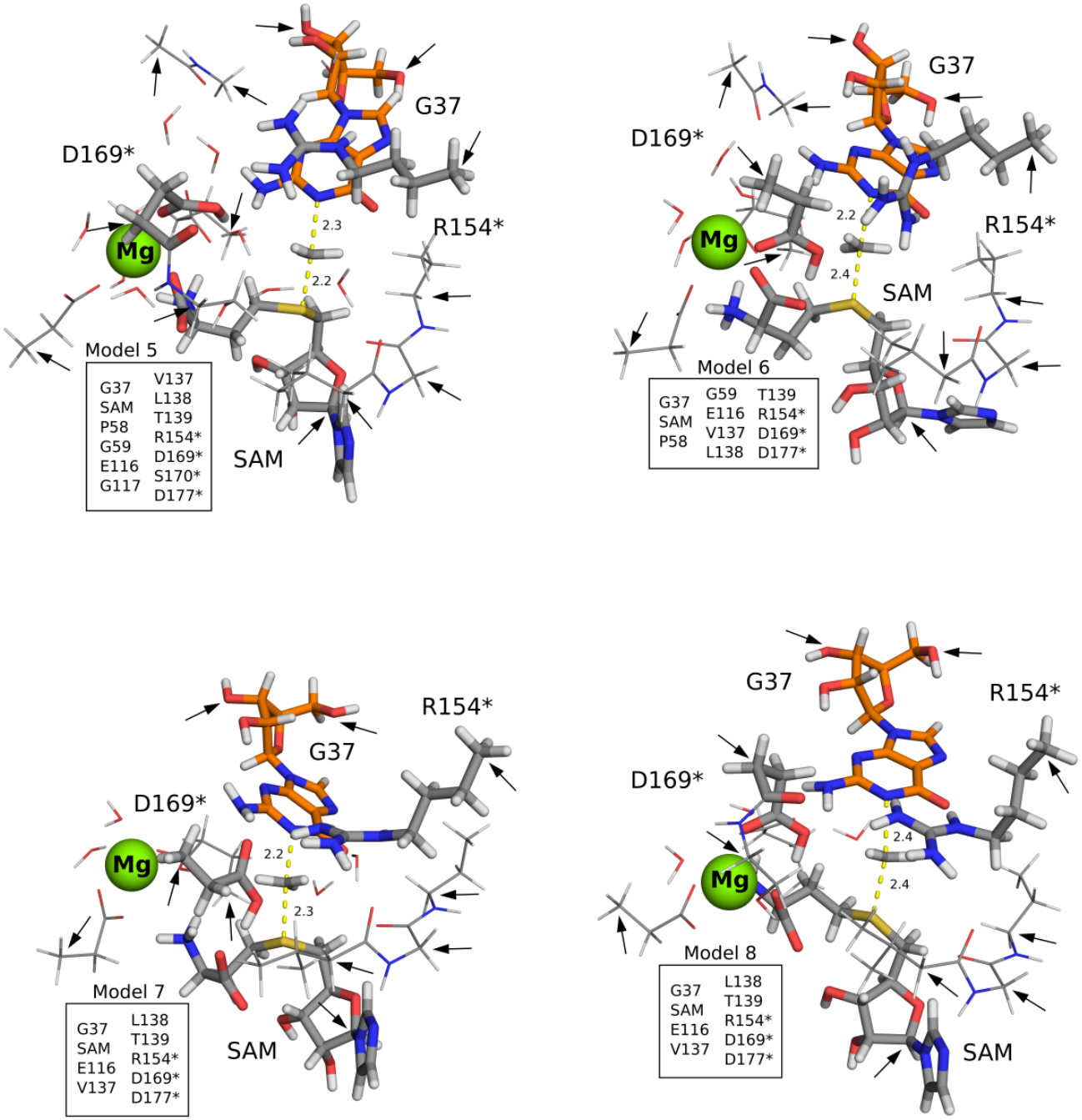


Figure S12: Optimized structures of the transition state of methyl transfer for the constructed models, with Mg^{2+} located in the negatively-charged binding pocket (Models 5-8). Key residues are shown with sticks. Yellow dotted lines represent the distances (in Å) from the methyl group to the sulfur and the N1 atom of G37. Arrows indicate atoms that were kept fixed during geometry optimizations.

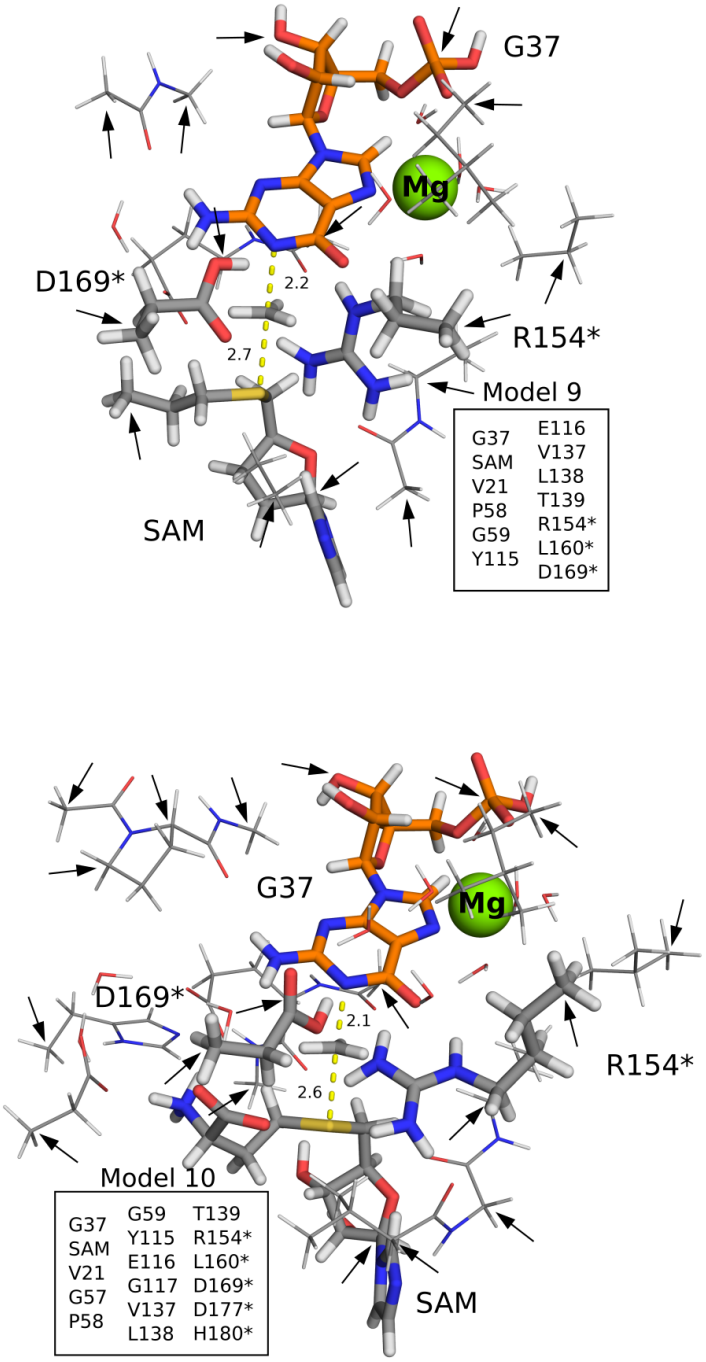


Figure S13: Optimized structures of the transition state of methyl transfer for the constructed models, with magnesium located near the G37 (Models 9-10). Key residues are shown with sticks. Yellow dotted lines represent the distance (in Å) from the methyl group to the sulfur and the N1 of G37. Arrows indicate atoms that were kept fixed during geometry optimizations.

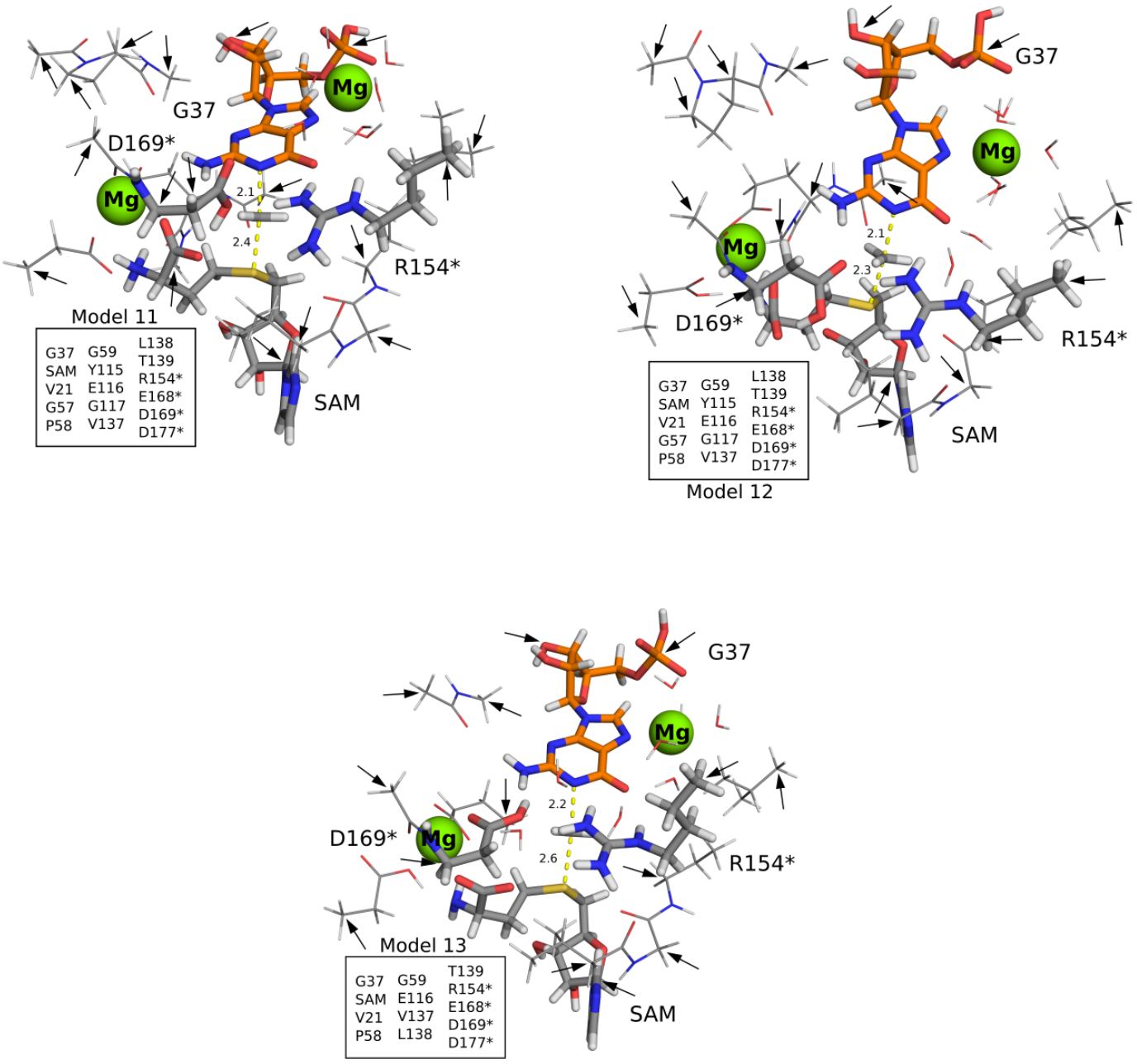
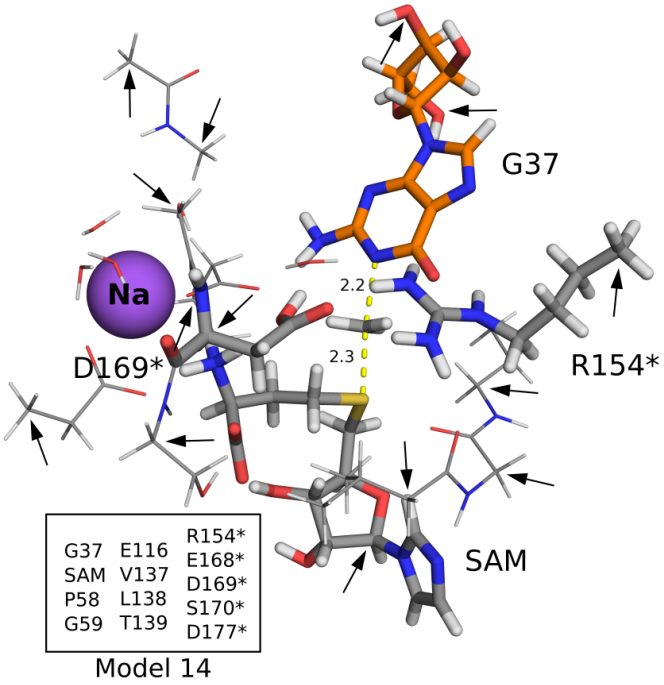


Figure S14: Optimized structures of the transition state of methyl transfer for the constructed models, with two magnesiums located in the active site (Models 11-13). One ion is coordinated by the N7 and five water molecules, while the other one by E116, D169*, D177*, SAM, and E168*. Key residues are shown with sticks. Yellow dotted lines represent the distance (in Å) from the methyl group to the sulfur and the N1 of G37. Arrows indicate atoms that were kept fixed during geometry optimizations.



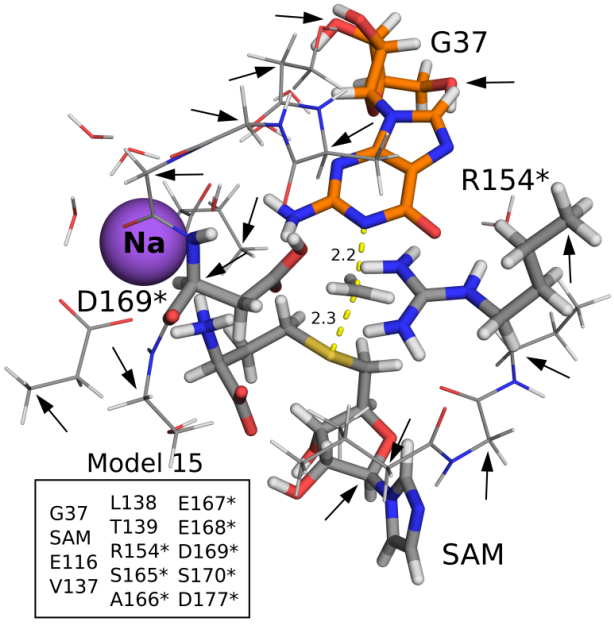


Figure S15: Optimized structures of the transition state of methyl transfer for the constructed models, with sodium located in the vicinity of E116, D169*, D177*, and SAM (Models 14-15). Key residues are shown with sticks. Yellow dotted lines represent the distance (in Å) from the methyl group to the sulfur and the N1 of G37. Arrows indicate atoms that were kept fixed during geometry optimizations.

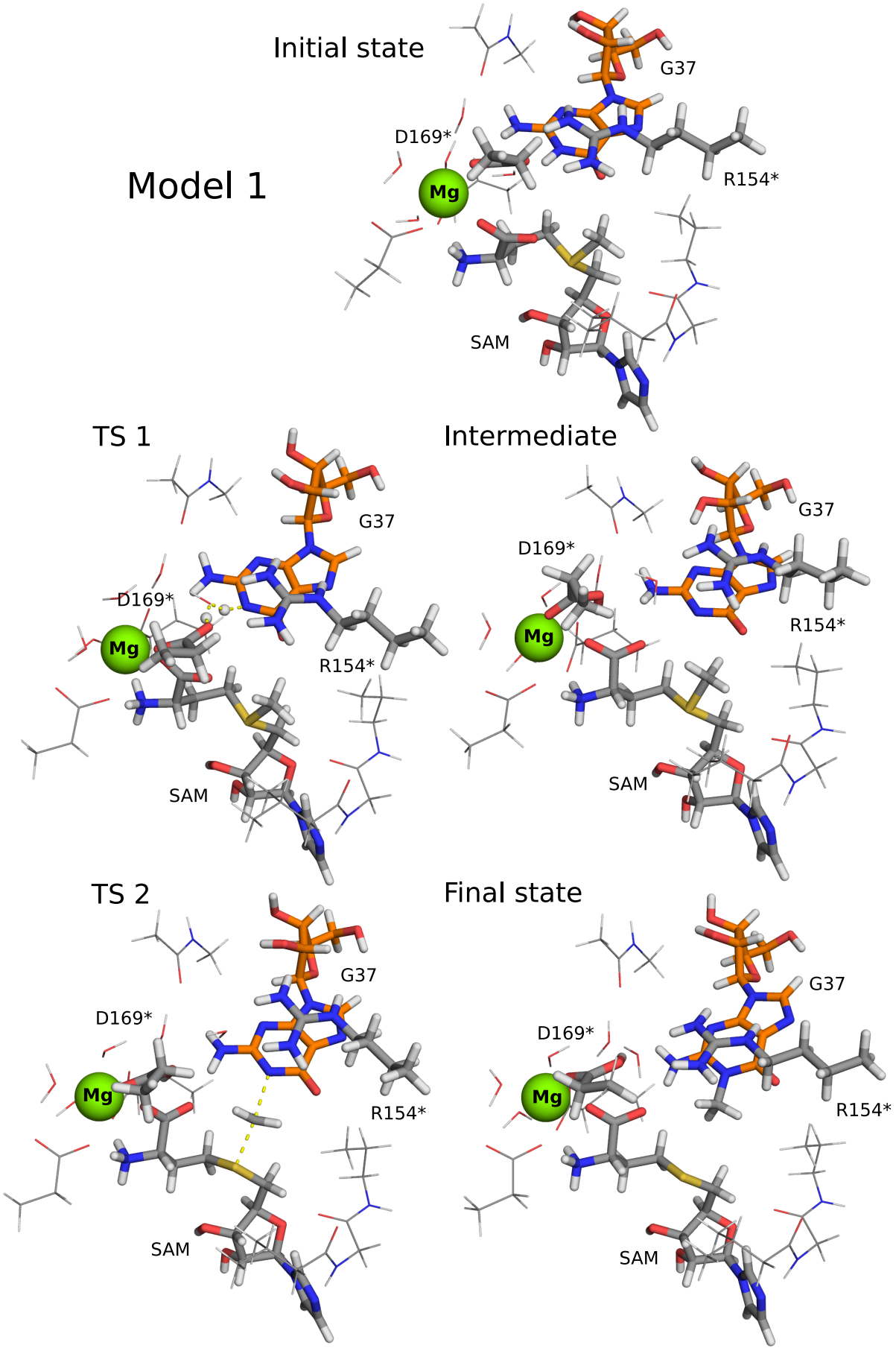


Figure S16: Optimized structures of all of the stationary points in the lowest energy barrier model with Mg^{2+} located in the negatively-charged binding pocket. Key residues are shown with sticks.

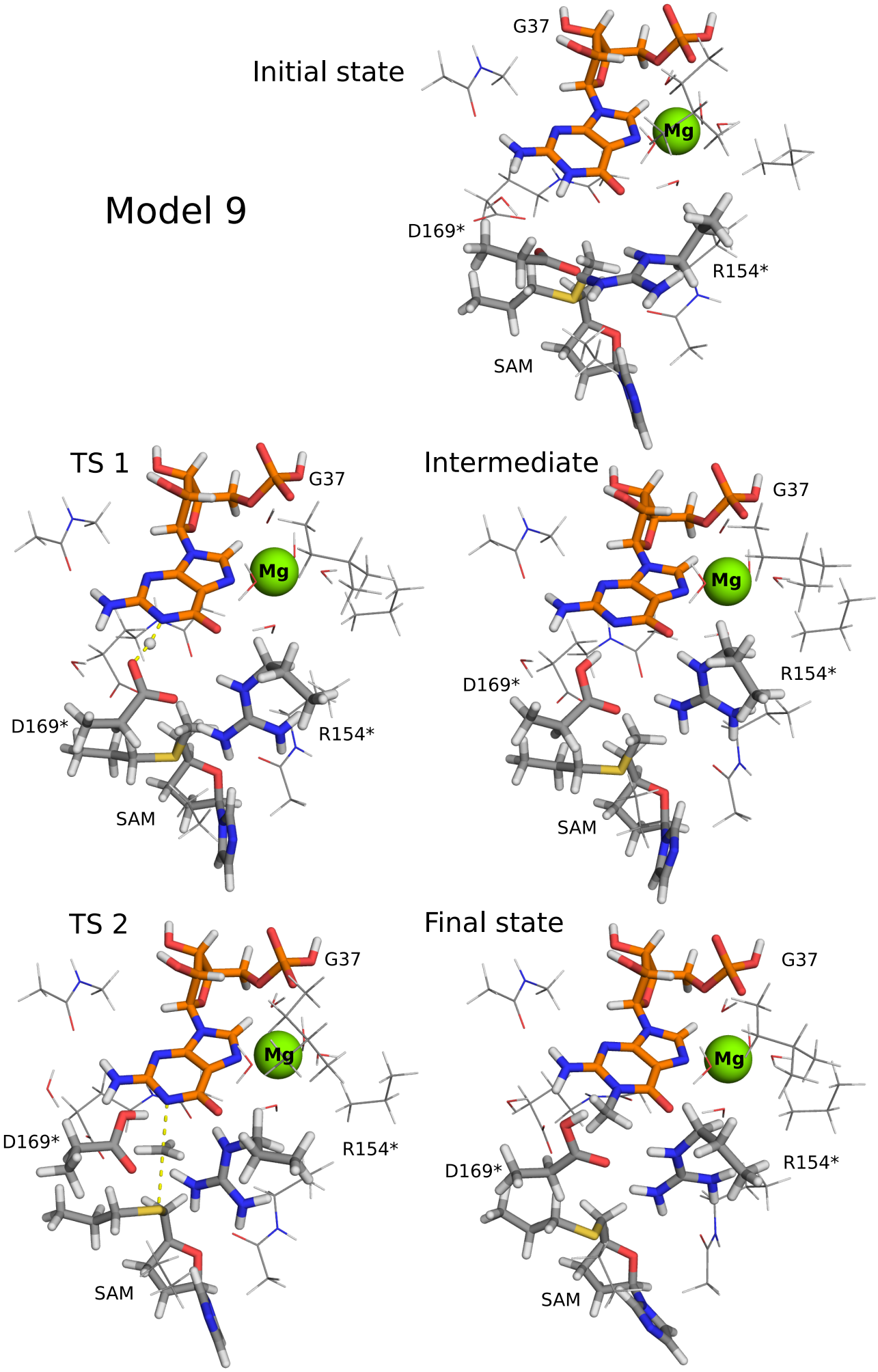


Figure S17: Optimized structures of all of the stationary points in the lowest energy barrier model with Mg^{2+} located near G37. Key residues are shown with sticks.

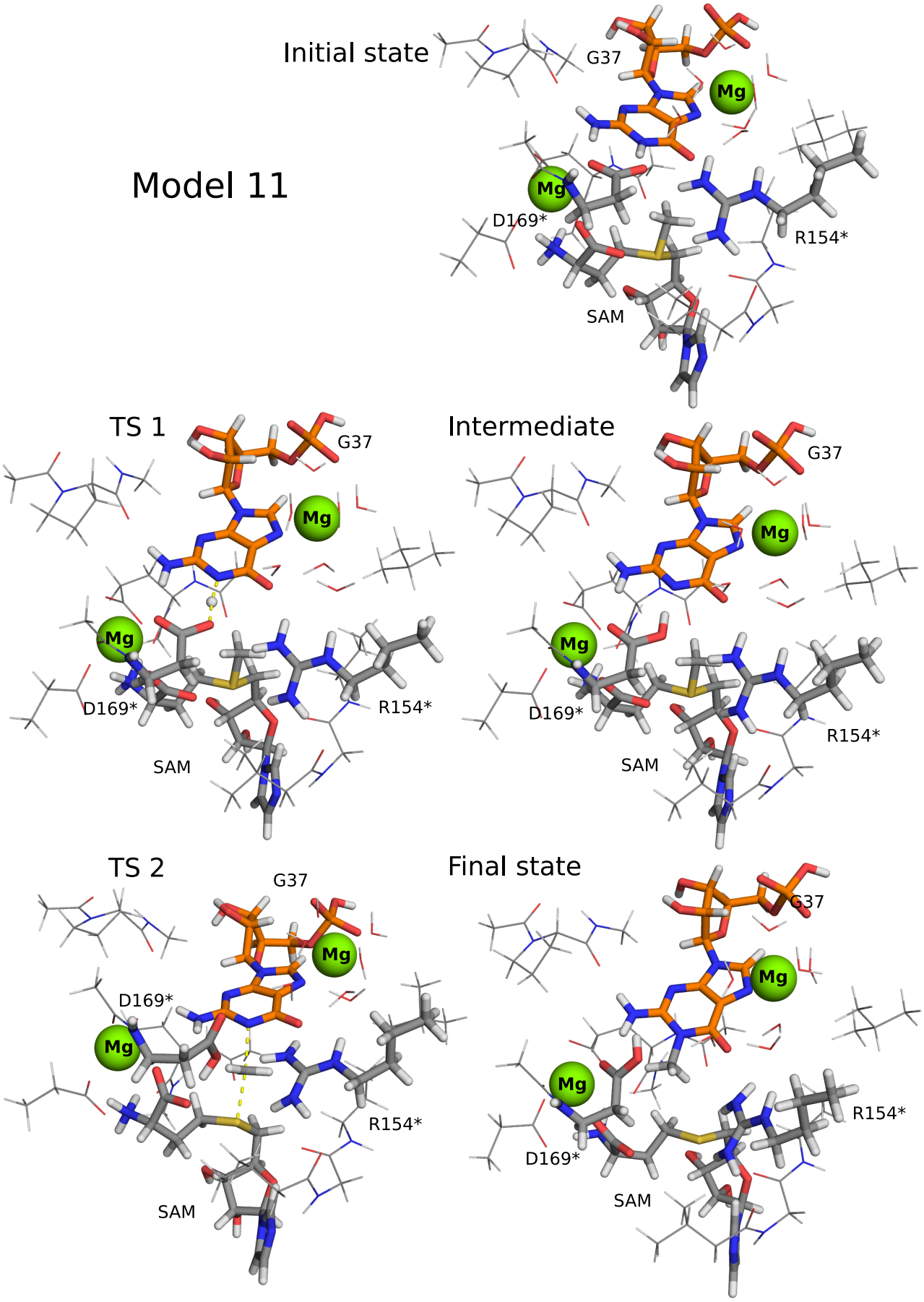


Figure S18: Optimized structures of all of the stationary points in the lowest energy barrier model two Mg^{2+} located in the active site, one coordinated with N^7 and five water molecules, while the other one with E116, D169*, D177*, SAM and E168*. Key residues are shown with sticks.

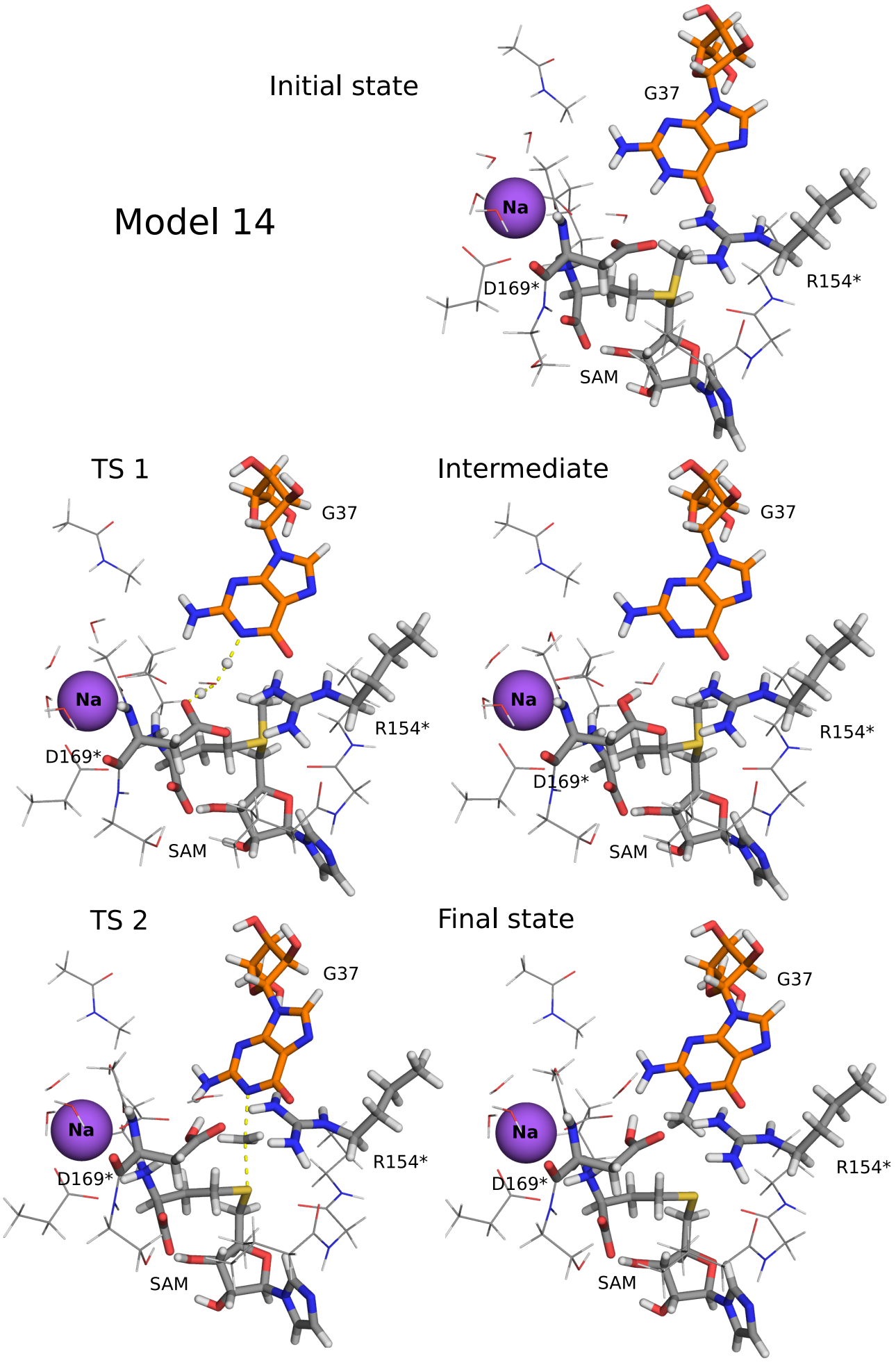


Figure S19: Optimized structures of all of the stationary points in the lowest energy barrier model with Na^+ located in the negatively-charged binding pocket. Key residues are shown with sticks.