



Research article

Halogen substitution strengthens Z944 binding to the human Cav3.1 calcium channel pore domain: Insights from molecular dynamics simulations

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Table S1. Residue boundaries of the pore-domain structural elements and the Z944-binding pocket in human Cav3.1. Residue ranges corresponding to the S5 helix, pore helix 1 (PH1), selectivity filter (SF), pore helix 2 (PH2), and S6 helix are listed for each homologous domain (DI–DIV). Residue ranges corresponding to unresolved regions in the cryo-EM structures are also indicated. Residues lining the Z944-binding pocket are listed at the bottom.

	Residue number			
	Domain I	Domain II	Domain III	Domain IV
S5	210–233	864–886	1389–1410	1716–1742
S5–PH1 Loop	234–339	887–907	1411–1447	1743–1764
PH1	340–350	908–918	1448–1458	1765–1775
SF	351–357	919–926	1459–1466	1776–1782
PH2	358–368	927–937	1467–1477	1783–1793
S6	370–397	939–968	1489–1516	1802–1832
Unresolved residues	290–312	894–899	None	1792–1798
Z944-binding pocket	384, 387, 388, 391, 868, 872, 875, 876, 916, 917, 918, 920, 921, 922, 948, 950, 951, 952, 953, 955, 956, 957, 959, 960, 1816, 1819, 1820, 1823, 1824, 1462, 1495, 1498, 1499, 1502, 1505, 1506, 1509, 1510			

Table S2. Summary of the molecular dynamics simulation systems used in this study. The table lists the Cav3.1 pore-domain models (apo, Z944-bound, and mZ944-bound), system compositions, total numbers of atoms, simulation box dimensions, simulation lengths, and numbers of independent simulation replicas.

System	Composition (molecules) POPC: H ₂ O: Na ⁺ : Cl [−]	Total atoms	Dimension (Å ³)	Replicas: Time (ns) × run
Apo	560: 58016: 119: 109	257394	154 × 154 × 105	3: 600 × 1, 500 × 2
mZ944	560: 57792: 118: 108	256773	154 × 154 × 105	3: 600 × 1, 500 × 2
Z944	560: 57792: 118: 108	256773	154 × 154 × 105	3: 600 × 1, 500 × 2

Table S3. Multi-stage molecular dynamics simulation protocol for the Cav3.1 pore-domain systems. The table summarizes the sequential relaxation, equilibration, and production stages, the restrained structural components, the relative restraint scaling factors, and the duration of each stage.

Stage	Restraints	Scaling	Length (ns)
Relaxation 1	Protein + Lipid + Ligand	1	0.1
Relaxation 2	Protein + Ligand	1	0.2
Relaxation 3	Backbone + Ligand + Ca ²⁺	1	0.6
Relaxation 4	Termini + Ca ²⁺	1	0.4
Equilibration	Termini	1	2
Production	Termini	0.5	500–600

Table S4. Mean RMSD (Å) of the transmembrane (TM) domain, S6 helices, selectivity filter, binding pocket, whole protein, and DZR (Z944 or mZ944) ligand from three independent MD simulations of the apo, mZ944-bound, and Z944-bound Cav3.1 systems. Values represent the mean $\pm$ SD over the analyzed trajectory interval for each replica after structural alignment using the pore-domain transmembrane-region C α atoms as defined in Section 2.4.1 of the main text. Ligand RMSD was evaluated only for the ligand-bound systems.

System	Run	Time analyzed	Mean RMSD $\pm$ SD (Å)					
			TM domain	S6	Selectivity filter	Binding pocket	Whole protein	DZR
Apo	run1	0.0–500.3 ns	1.29 $\pm$ 0.13	0.98 $\pm$ 0.09	1.25 $\pm$ 0.16	0.94 $\pm$ 0.10	3.00 $\pm$ 0.48	—
Apo	run2	0.0–499.8 ns	1.08 $\pm$ 0.10	0.97 $\pm$ 0.11	0.99 $\pm$ 0.12	0.83 $\pm$ 0.12	1.93 $\pm$ 0.29	—
Apo	run3	0.0–499.8 ns	1.22 $\pm$ 0.11	1.13 $\pm$ 0.11	1.15 $\pm$ 0.11	0.94 $\pm$ 0.09	2.37 $\pm$ 0.29	—
mZ944	run1	0.0–592.0 ns	1.27 $\pm$ 0.13	0.98 $\pm$ 0.10	1.08 $\pm$ 0.14	0.73 $\pm$ 0.07	2.59 $\pm$ 0.47	5.36 $\pm$ 0.57
mZ944	run2	0.0–499.8 ns	1.39 $\pm$ 0.13	1.11 $\pm$ 0.09	1.19 $\pm$ 0.19	0.96 $\pm$ 0.16	2.42 $\pm$ 0.28	2.78 $\pm$ 0.52
mZ944	run3	0.0–499.8 ns	1.32 $\pm$ 0.15	1.05 $\pm$ 0.09	1.06 $\pm$ 0.13	0.86 $\pm$ 0.09	2.62 $\pm$ 0.43	3.04 $\pm$ 0.73
Z944	run1	50.0–649.9 ns	1.20 $\pm$ 0.14	1.00 $\pm$ 0.10	0.90 $\pm$ 0.14	0.90 $\pm$ 0.11	2.51 $\pm$ 0.53	2.72 $\pm$ 0.58
Z944	run2	0.0–494.6 ns	1.22 $\pm$ 0.20	1.06 $\pm$ 0.20	1.02 $\pm$ 0.19	0.91 $\pm$ 0.13	2.55 $\pm$ 0.45	2.71 $\pm$ 0.81
Z944	run3	0.0–494.6 ns	1.22 $\pm$ 0.14	0.93 $\pm$ 0.11	1.29 $\pm$ 0.11	0.85 $\pm$ 0.09	2.88 $\pm$ 0.48	3.21 $\pm$ 0.50

Table S5a. Residue-specific protein–ligand interactions identified in three independent MD simulations of the Z944-bound Cav3.1 complex. Residues with overall contact occupancies $\geq 20\%$ are listed together with their structural domain, total contact occupancy, and dominant interaction type(s) with corresponding occupancies. These data provide the quantitative replica analysis supporting the representative interaction analyses shown in Figure 4A–D.

Run	Residue	Domain	Any contact (%)	Dominant interaction(s) and occupancy
1	Phe956	DII	100	Hydrophobic 100.0%; VdW 72.9%
1	Phe917	DII	95.3	Hydrophobic 95.3%; VdW 55.1%
1	Leu920	DII	92.5	VdW 90.7%; H-bond donor 52.3%; hydrophobic 43.0%
1	Thr921	DII	87.9	VdW 87.9%; cation– π 10.3%
1	Asn952	DII	68.2	Hydrophobic 58.9%; VdW 34.6%
1	Gln922	DII	64.5	VdW 53.3%; hydrophobic 25.2%
1	Leu1499	DIII	60.7	VdW 59.8%
1	Lys1462	DIII	46.7	VdW 44.9%; H-bond acceptor 12.1%; hydrophobic 11.2%
2	Phe956	DII	96.4	Hydrophobic 92.9%; VdW 71.4%
2	Asn952	DII	92.9	VdW 82.1%; H-bond donor 76.2%; hydrophobic 59.5%
2	Leu920	DII	86.9	Hydrophobic 83.3%; VdW 60.7%
2	Thr921	DII	67.9	VdW 67.9%
2	Gly951	DII	58.3	VdW 58.3%
2	Gln922	DII	52.4	VdW 35.7%; hydrophobic 29.8%
2	Phe917	DII	50	Hydrophobic 46.4%; VdW 21.4%
2	Leu872	DII	40.5	VdW 39.3%; hydrophobic 13.1%
2	Lys1462	DIII	33.3	VdW 29.8%; H-bond acceptor 13.1%; hydrophobic 10.7%
3	Asn952	DII	97.6	VdW 97.6%; H-bond acceptor 73.8%; hydrophobic 13.1%
3	Phe956	DII	92.9	Hydrophobic 78.6%; VdW 56.0%; π -stacking 19.0%
3	Lys1462	DIII	82.1	VdW 73.8%; hydrophobic 39.3%
3	Thr921	DII	56	VdW 44.0%; cation– π 34.5%
3	Asn388	DI	44	VdW 44.0%; H-bond acceptor 23.8%
3	Tyr953	DII	36.9	Hydrophobic 27.4%; VdW 13.1%
3	Leu920	DII	35.7	Hydrophobic 27.4%; VdW 20.2%
3	Val1823	DIV	29.8	VdW 29.8%

Table S5b. Residue-specific protein–ligand interactions identified in three independent MD simulations of the mZ944-bound Cav3.1 complex. Residues with overall contact occupancies $\geq 20\%$ are listed together with their structural domain, total contact occupancy, and dominant interaction type(s) with corresponding occupancies. These data provide the quantitative replica analysis supporting the representative interaction analyses shown in Figure 4A–D.

Run	Residue	Domain	Any contact (%)	Dominant interaction(s) and occupancy
1	Lys1462	DIII	100	VdW 98.0%; H-bond acceptor 95.0%; hydrophobic 15.8%
1	Thr921	DII	93.1	VdW 91.1%; cation– π 14.9%
1	Phe956	DII	74.3	Hydrophobic 73.3%
1	Leu1498	DIII	43.6	Hydrophobic 43.6%; VdW 10.9%
1	Gln922	DII	42.6	VdW 40.6%
1	Leu920	DII	39.6	Hydrophobic 33.7%; VdW 12.9%
1	Leu353	DI	35.6	VdW 35.6%
1	Phe384	DI	29.7	VdW 29.7%
2	Thr921	DII	97.6	VdW 91.7%; H-bond acceptor 51.2%; cation– π 33.3%
2	Phe956	DII	96.4	Hydrophobic 91.7%; VdW 61.9%; π -stacking 25.0%
2	Leu920	DII	90.5	Hydrophobic 89.3%; VdW 47.6%
2	Asn952	DII	85.7	VdW 79.8%; H-bond donor 47.6%; hydrophobic 28.6%
2	Phe917	DII	70.2	Hydrophobic 70.2%; VdW 15.5%
2	Asn388	DI	58.3	VdW 58.3%; H-bond acceptor 20.2%
2	Lys1462	DIII	58.3	VdW 58.3%; H-bond acceptor 48.8%
2	Gln922	DII	45.2	VdW 33.3%; hydrophobic 16.7%
3	Lys1462	DIII	84.5	VdW 69.0%; hydrophobic 52.4%; H-bond acceptor 14.3%
3	Phe956	DII	79.8	Hydrophobic 71.4%; VdW 36.9%
3	Thr921	DII	78.6	VdW 77.4%; cation– π 19.0%
3	Asn952	DII	54.8	Hydrophobic 42.9%; VdW 41.7%; H-bond donor 29.8%
3	Phe917	DII	53.6	Hydrophobic 53.6%
3	Leu1498	DIII	46.4	Hydrophobic 44.0%; VdW 17.9%
3	Gln922	DII	40.5	VdW 40.5%
3	Leu920	DII	35.7	VdW 26.2%; hydrophobic 22.6%

Table S6. Water-mediated protein–Z944 interaction pathways identified in three independent MD simulations of the Z944-bound Cav3.1 system. For each residue, the occurrence (%) of water-bridge interactions is reported for runs 1–3 together with the principal protein–water–Z944 interaction pathway. Percentages represent the fraction of analyzed trajectory frames in which the corresponding water-mediated interaction was observed.

Domain:Residue	Structural location	Run1	Run2	Run3	Principal protein–water–Z944 pathway
DI: SER383	S6	42.90%	62.38%	33.51%	SER383 O/OG–water–Z944 O1; occasional N3
DII: ASN957	S6, pocket	52.29%	27.59%	18.51%	ASN957 OD1–water–Z944 N3, N1 or O1
DII: THR921	Selectivity filter, pocket	0.81%	35.06%	49.60%	THR921 O/OG1–water–Z944 O2 or N2
DIII: LYS1462	Selectivity filter, pocket	41.19%	39.70%	24.23%	LYS1462 NZ–water–Z944 O2, N1 or N2
DIV: GLN1816	S6, pocket	3.81%	0.13%	29.81%	GLN1816 OE1/NE2–water–Z944 O1
DII: ASN952	S6, pocket	21.84%	14.60%	0.47%	ASN952 O/OD1–water–Z944 N3 or N2
DII: GLN922	Selectivity filter, pocket	11.26%	16.08%	6.39%	GLN922 NE2/OE1–water–Z944 O1, O2, N1 or N2
DI: ASN388	S6, pocket	0.16%	5.65%	11.04%	ASN388 ND2/OD1–water–Z944 O1, N3 or N1
DII: LEU920	Selectivity filter, pocket	1.32%	10.09%	0.27%	LEU920 backbone O–water–Z944 N2
DIV: THR1777	Selectivity filter	0.00%	0.20%	5.25%	THR1777 O/OG1–water–Z944 O1 or N3

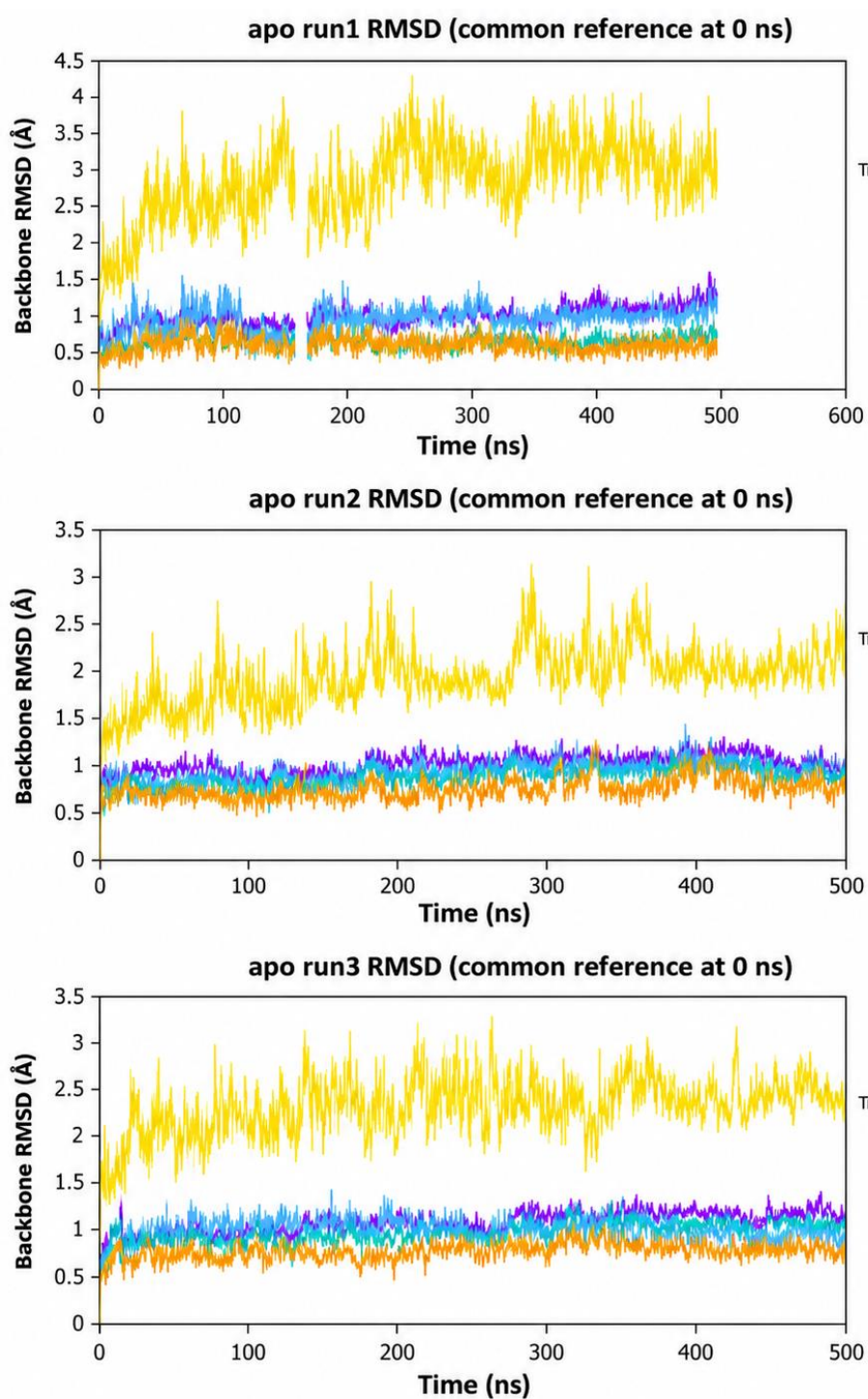


Figure S1. RMSD time series of the apo Cav3.1 system from three independent MD simulations (run1–run3). RMSDs of the whole protein, transmembrane (TM) domain, S6 helices, selectivity filter (SF), and blocker-binding pocket are shown after structural alignment using the pore-domain transmembrane-region C α atoms.

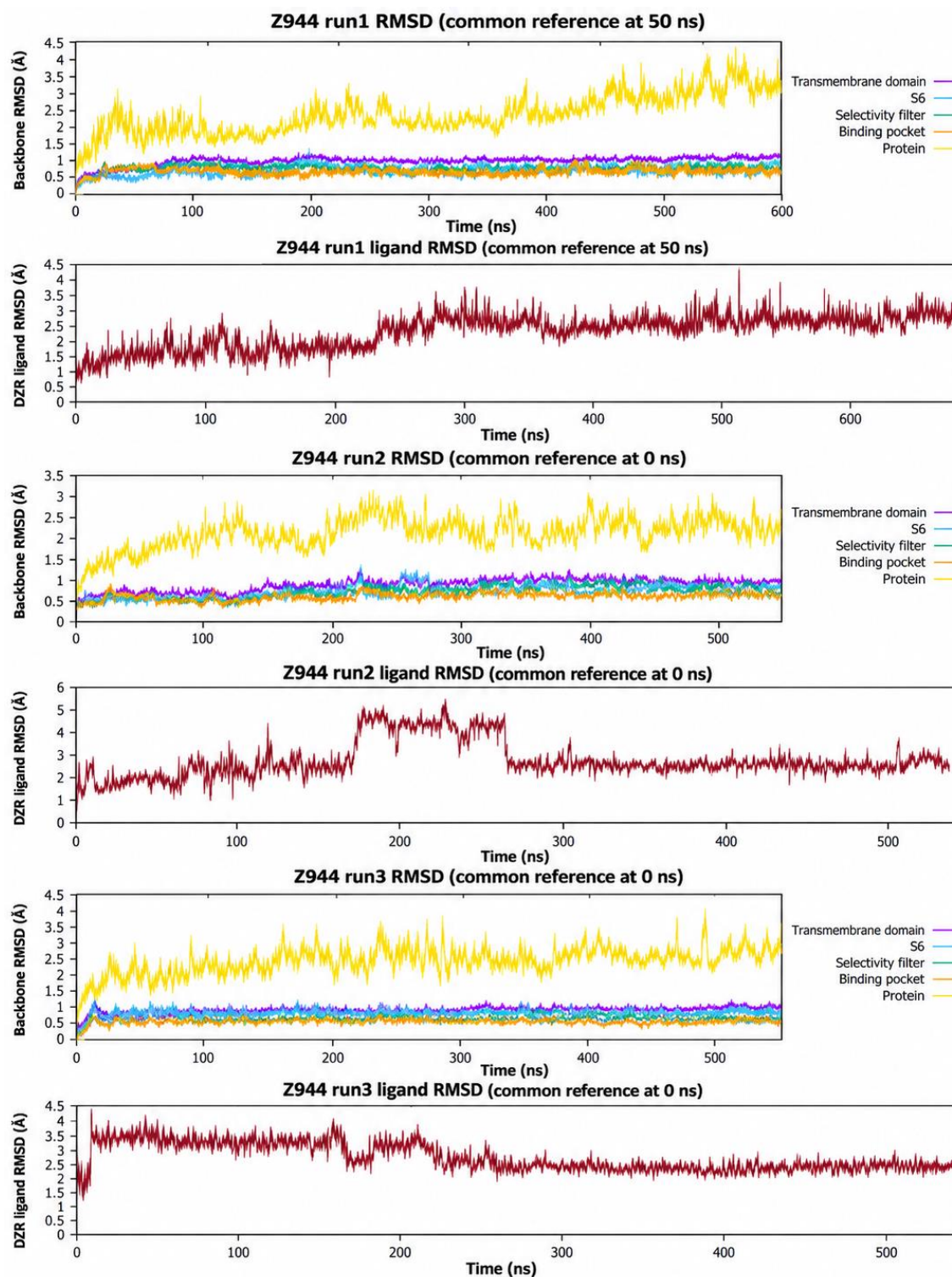


Figure S2. RMSD time series of the Z944-bound Cav3.1 system from three independent MD simulations (run1–run3). RMSDs of the whole protein, transmembrane (TM) domain, S6 helices, selectivity filter (SF), blocker-binding pocket, and Z944 ligand (DZR) are shown after structural alignment using the pore-domain transmembrane-region C α atoms.

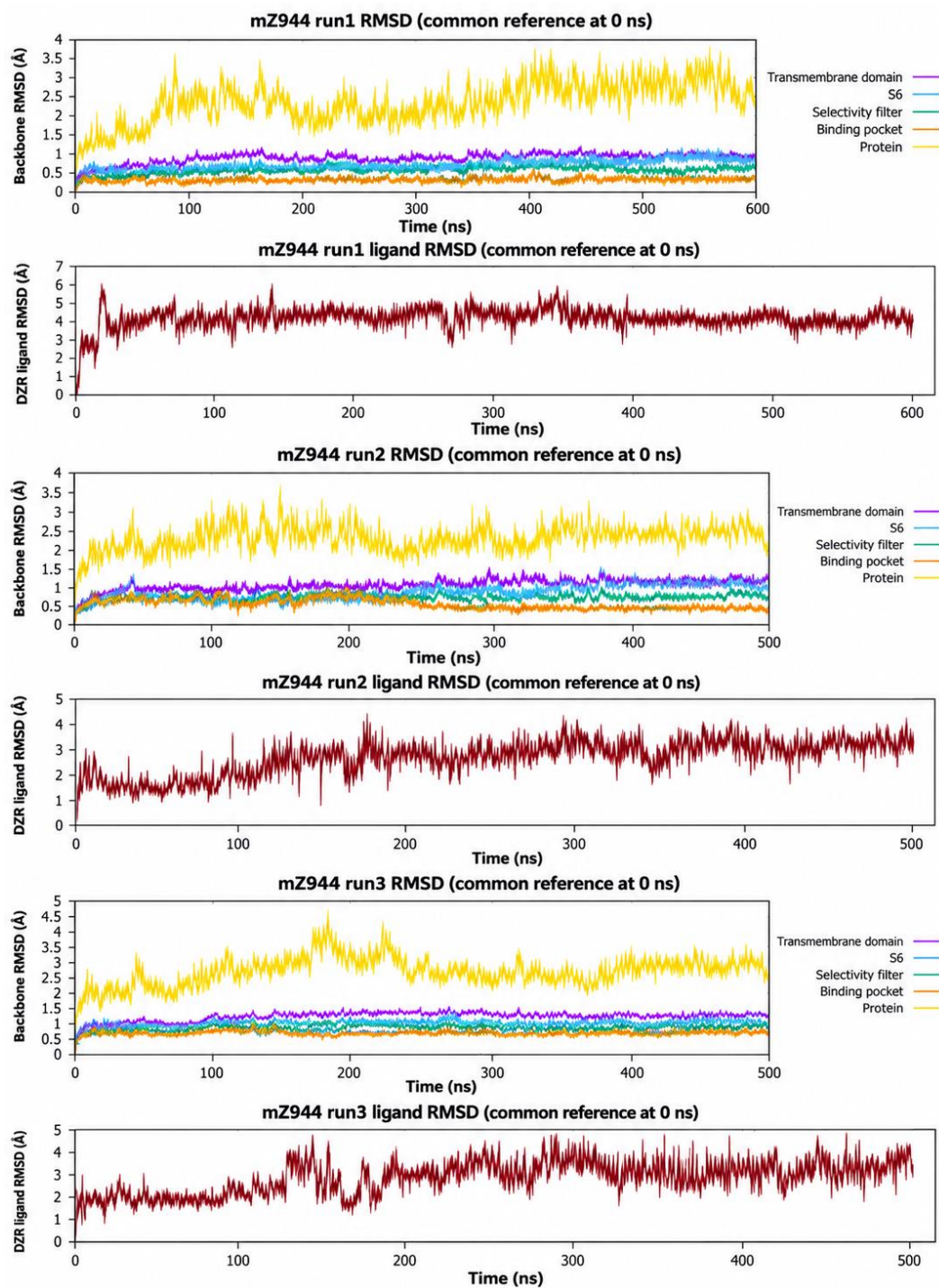


Figure S3. RMSD time series of the mZ944-bound Cav3.1 system from three independent MD simulations (run1–run3). RMSDs of the whole protein, transmembrane (TM) domain, S6 helices, selectivity filter (SF), blocker-binding pocket, and mZ944 ligand (DZR) are shown after structural alignment using the pore-domain transmembrane-region C α atoms.

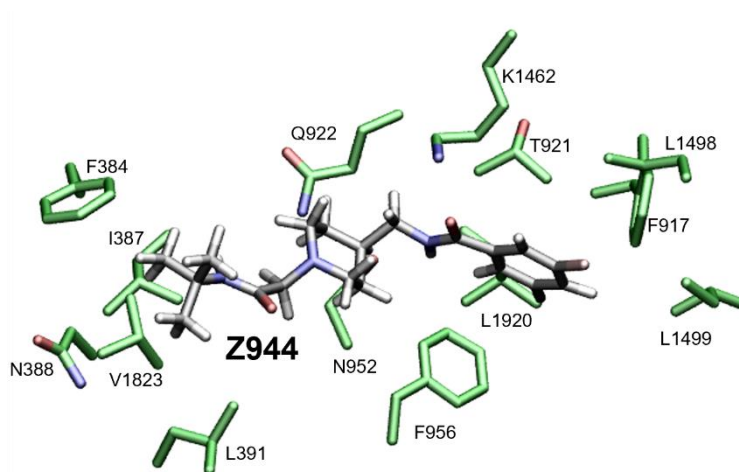
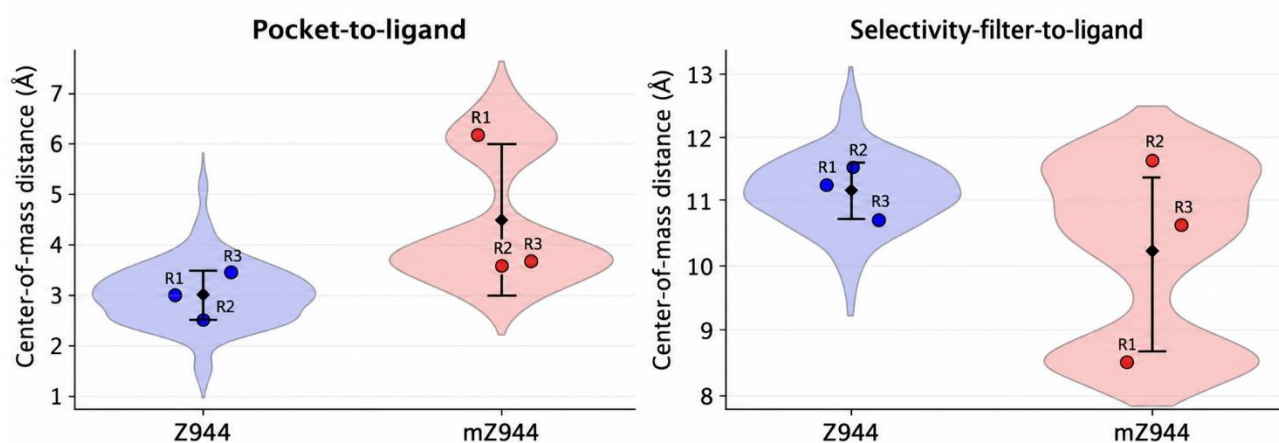


Figure S4. Representative binding pose of Z944 within the Cav3.1 blocker-binding cavity from the representative production trajectory (run1). Z944 is shown as gray sticks, and surrounding interacting residues identified by the ProLIF contact analysis are shown as green sticks and labeled. The snapshot illustrates the spatial arrangement of the conserved Domain II interaction core (Leu920, Thr921, Gln922, Asn952, and Phe956), together with additional interacting residues from Domains I, III, and IV that contribute to stabilization of the ligand within the blocker-binding cavity.



Violins: 1,800 correlated frames per ligand (descriptive only); colored points: trajectory means; black error bars: mean $\pm$ SD across trajectory means.

Figure S5. Replica-resolved center-of-mass distances between Z944 or mZ944 and the Cav3.1 blocker-binding pocket (left) or selectivity filter (right). Violin plots show equal-weight pooled distributions obtained from 600 snapshots sampled every 0.5 ns over the final 300 ns of each of three independent simulations (1,800 frames per ligand). Colored circles indicate the mean values from the three individual simulations (R1–R3), and black diamonds and error bars indicate the mean $\pm$ SD calculated from the three trajectory means. Because frames within individual trajectories are temporally correlated, the pooled violin distributions are presented only as descriptive representations of the sampled conformational ensembles; the three independent simulations constitute the independent sampling units.

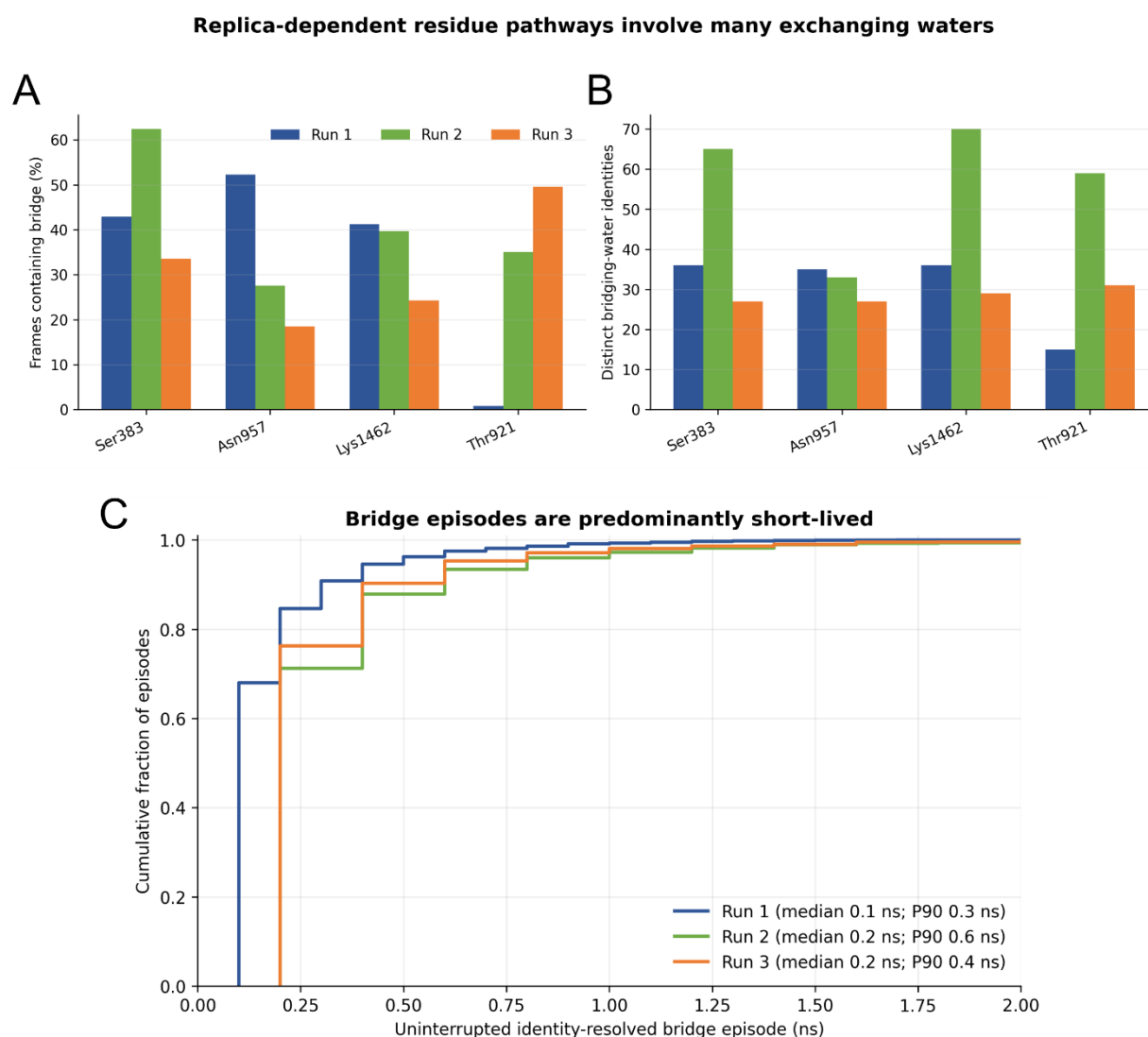


Figure S6. Replica-resolved water-bridge pathways and water turnover in the Cav3.1–Z944 complex. (A) Residue-level water-bridge occupancy. (B) Number of distinct bridging-water identities contributing to each residue pathway. (C) Cumulative distributions of uninterrupted, identity-resolved bridge-episode durations. High residue occupancy can arise from repeated short-lived bridges involving different water molecules rather than persistence of a single water molecule.

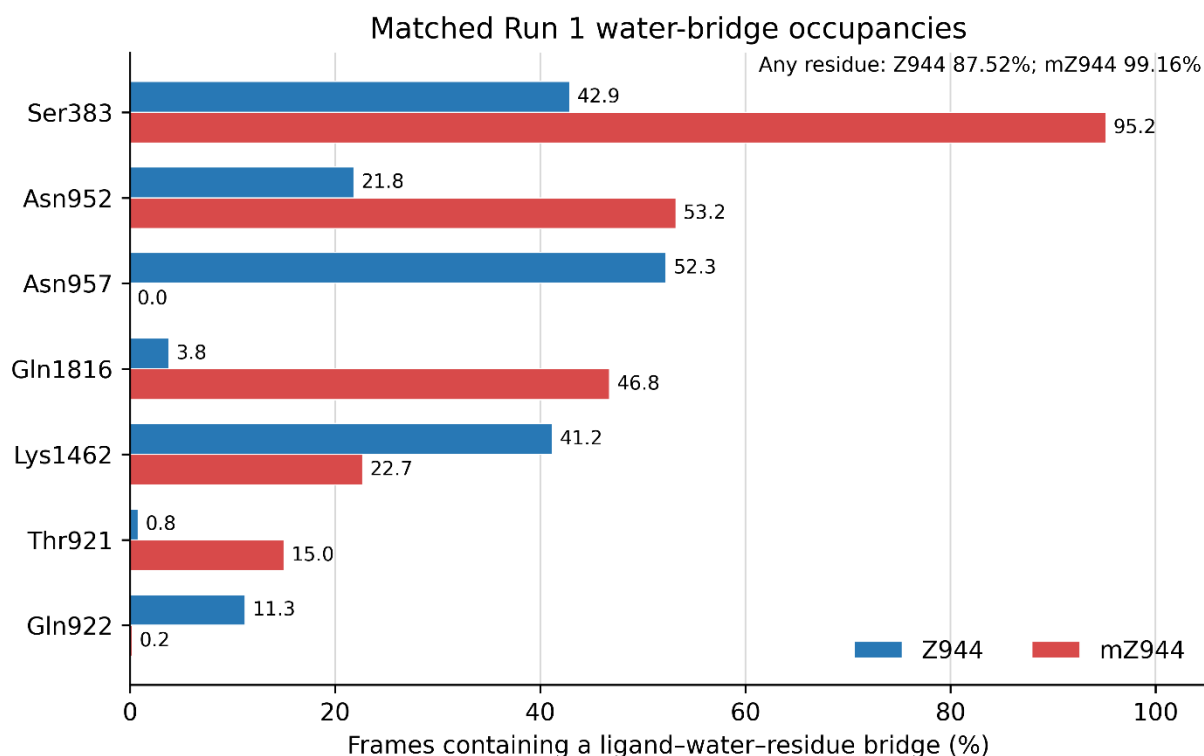


Figure S7. Matched Run 1 comparison of water-mediated protein–ligand interactions for Z944 and mZ944. Residue-level water-bridge occupancies represent the percentage of analyzed frames containing at least one single-water bridge between the ligand and the indicated Cav3.1 residue. At least one protein–water–ligand bridge involving any residue was observed in 87.52% of Z944 frames and 99.16% of mZ944 frames. The comparison shows differences in the sampled residue-level water-mediated pathways between Z944 and mZ944. Because the comparison is based on matched Run 1 trajectories, the differences are interpreted descriptively and are not assumed to be reproduced across independent simulations.

Ligand dihedral-angle dynamics during the final 300 ns

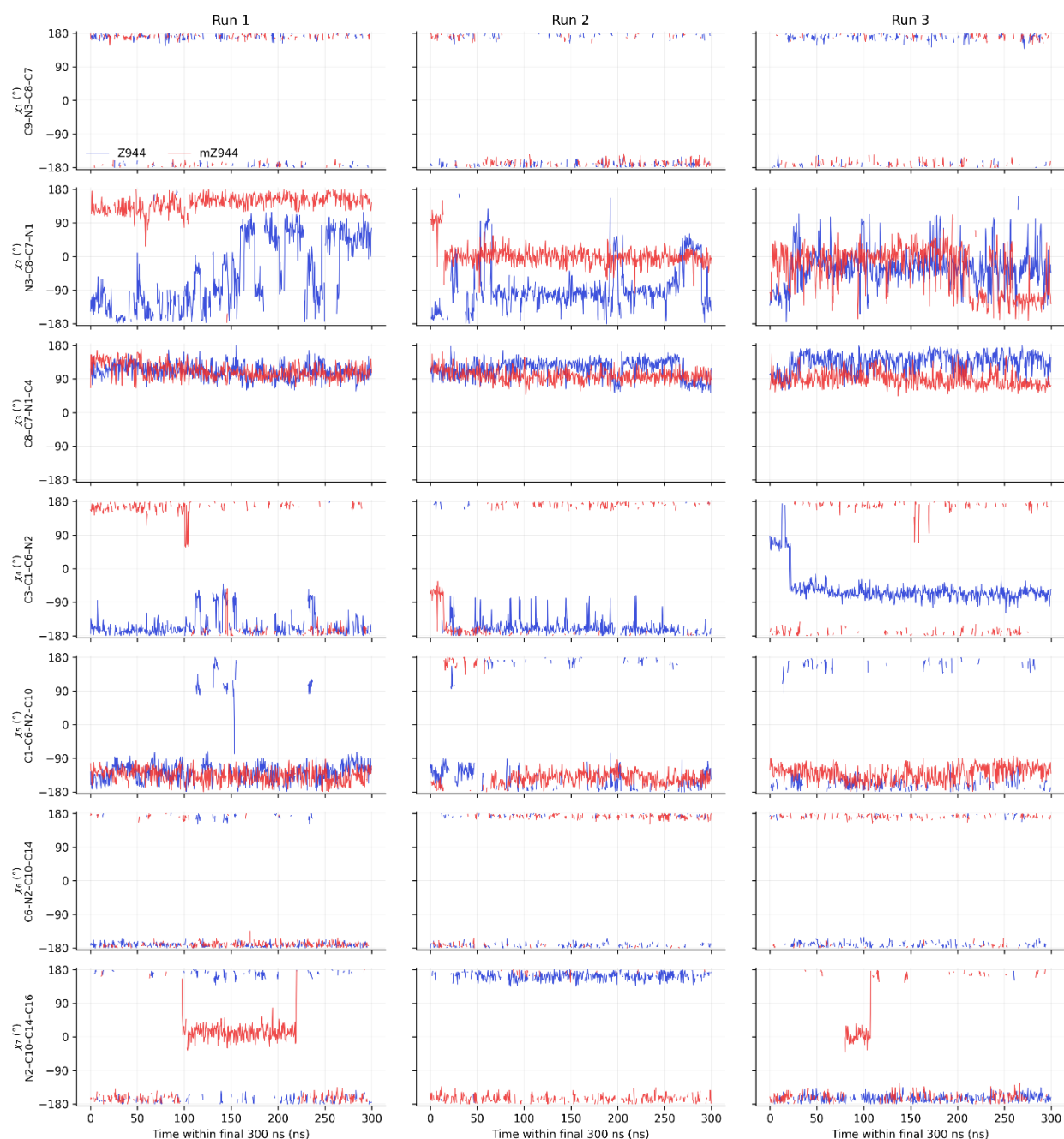


Figure S8. Time evolution of the seven principal ligand dihedral angles (χ_1 – χ_7) during the final 300 ns of three independent MD simulations. Dihedral-angle trajectories for Z944 (blue) and mZ944 (red) are shown for runs 1–3. These trajectories provide the underlying conformational sampling used to generate the three-replica pooled probability distributions shown in Figure 5.

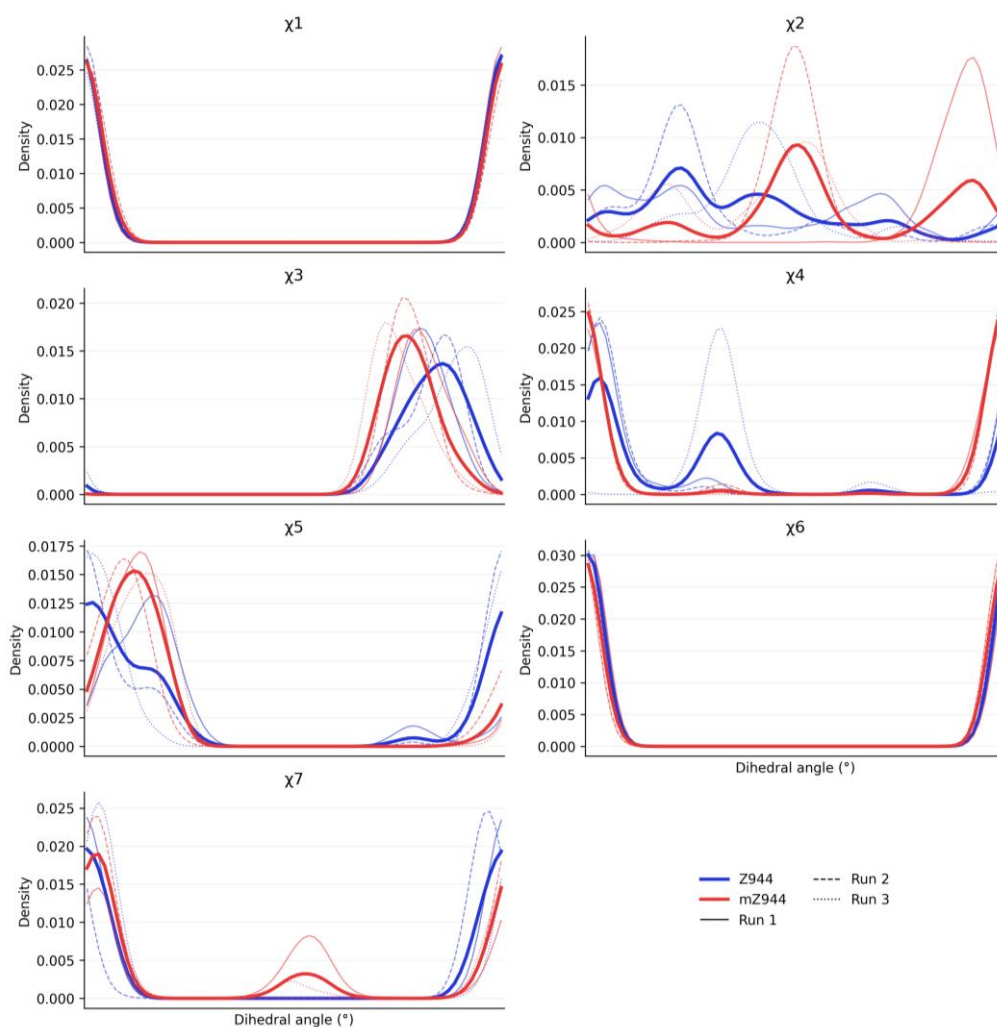


Figure S9. Pooled and replica-resolved ligand dihedral distributions. Thick curves show equal-weight pooled probability densities for Z944 and mZ944, with each independent simulation contributing 600 snapshots from the final 300 ns. Thin solid, dashed, and dotted curves show the corresponding distributions for Runs 1–3, respectively. The pooled distributions are descriptive ensemble summaries; the three trajectories represent the independent simulation replicates.



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