EEF1A2 D91N supplementary methods and measurements

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Simulation composition and sampling

WT, D91N and the D252H comparator each had three 25 ns trajectories in the Mg-free series. The systems shared the backbone of the WT seed-23 structural prediction, with one solvent box and protonation assignment per condition. Within a condition, velocities, thermostat and barostat initialization used seeds 11, 23 and 47. Each seed had its own minimization and equilibration.

Table S1. Composition of the three neutral, Mg-free systems. Solute includes EEF1A2, the capped EEF1B2 fragment and GDP.

Condition	Total atoms	Solute atoms	Waters	Na^+ ions	Cl^- ions
WT	85,623	9,027	25,485	69	72
D91N	84,747	9,029	25,192	69	73
D252H	85,221	9,032	25,349	69	73

GDP used the GDP template in charmm36_2024.xml and carried charge −3e. His95 was protonated at NE2. The D252H comparator used His252 protonated at ND1. The production systems contained the CHARMM TIP3P solvent model, 0.15 M added NaCl and charge-neutralizing ions.

Measurements and denominators

Production time was 0.000004 ns × production step. Each run yielded 12,501 scalar observations spaced 2 ps apart, 2,501 solute frames at 10 ps and 251 full-system frames at 100 ps, including step zero. The inclusive 5–25 ns interval comprised 10,001, 2,001 and 201 observations per run, respectively. Means and sample standard deviations across the three run means describe variation within each prepared condition.

Distance calculations used the closest of 27 periodic images around the nearest fractional cell. For RMSD, bonded molecules were made whole and aligned by one proper rigid fit using the 120 specified EEF1A2 Cα atoms. The fit reference preceded protonation and minimization. The EEF1B2 fragment was evaluated after that fit without refitting the partner.

WT seed 11 prompted revision of the trajectory acceptance rule. Of its 12,500 positive-time samples, 63 had a maximum relative constraint residual above 10^{-5}, reaching 1.46×10^{-5}. All nine runs had such samples (33–67 per run), and all met the revised 10^{-4} ceiling. The integrator tolerance remained 10^{-5}.

Source data. The nine scalar files and endpoint specification are in data/scalars/. Each run's atom map, bonds and alignment mask are in data/raw/. File paths here and below are relative to the research package.

Preparation checkpoints and atom identity

D91N was built by fitting the donor Asn91 N, Cα and C onto WT and transferring its CB, CG, OD1 and ND2 atoms. The protein retained the WT backbone. D91N ND2 came from donor ND2. The WT Asp91 OD2 atom was removed with the WT side chain.

The preparation used a harmonic restraint on each nonhydrogen solute atom, including protein, cap and GDP. During whole-system minimization the spring constant was 1000 kJ mol^{-1} nm^{-2}. Equilibration comprised 100 ps NVT at 1000, followed by 200, 200 and 100 ps NPT at 1000, 100 and 0 kJ mol^{-1} nm^{-2}, respectively. Production began at the end of that final unrestrained stage.

Table S2. Minimum residue-91–GDP heavy-atom distances at saved preparation stages. Production-zero values are shown separately for each velocity seed.

Checkpoint	WT (Å)	D91N (Å)	D252H (Å)
After construction	4.813	4.855	4.813
After solvation	4.813	4.855	4.813
After minimization	5.748	4.841	5.778
Production zero, seed 11	7.809	5.193	6.244
Production zero, seed 23	6.400	4.709	6.968
Production zero, seed 47	6.592	4.731	6.859

At saved precision, the three postminimization solute configurations coincided within each condition. During equilibration, all three D91N distances fell below all three WT distances at 296 of 300 scalar sampling times. The ranges overlapped at 72, 380, 486 and 492 ps. The retained scalar records do not contain the winning atom identities. Solute coordinates at minimization and at production zero bracket the unrecorded coordinate changes within equilibration.

Identifying the nearest pair

At each of 37 coordinate stages, eight residue-91 heavy atoms and 28 GDP heavy atoms define 224 distances. Pairs within 0.001 Å of the smallest unrounded distance were treated as indistinguishable. On minimization, WT switched from Asp91 OD2–GDP O2B (4.813 Å) to OD1–O2B (5.748 Å). D91N retained Asn91 ND2–O2B (4.855 to 4.841 Å). D252H also switched from Asp91 OD2 to OD1 against O2B (4.813 to 5.778 Å). A window mean averages per-frame minima across potentially different pairs.

Source data. The 37 minima and runner-up gaps are in analysis/atom_pairs/nearest_pairs.csv. All 8,288 ranked distances are in pair_ranks.csv in the same directory.

Water bridges and partner reference deviation

A direct hydrogen bond required donor–acceptor separation ≤3.5 Å and a donor–H–acceptor angle ≥150°. A one-water bridge required a single water molecule to satisfy this geometry at both the residue-91 terminal group and GDP in the same full-system frame. If water donated on both legs, the two donations had to use different hydrogens. The residue-91 terminal group comprised OD1/OD2 in Asp or OD1/ND2 in Asn. GDP O2B supplied the nucleotide contact in the qualifying bridges.

Table S3. Sampled residue-91–water–GDP bridges and nearby sodium by run. Entries are counts of frames with at least one qualifying event.

Condition	Seed	Bridge, 5–25 ns (of 201)	Bridge, 0–25 ns (of 251)	Na^+ near GDP β, 0–25 ns (of 251)
WT	11	0	0	131
WT	23	0	0	193
WT	47	0	0	188
D91N	11	97	123	185
D91N	23	122	155	128
D91N	47	0	5	0
D252H	11	0	0	134
D252H	23	0	0	238
D252H	47	0	0	80

The sodium criterion was ≤3.0 Å to any GDP β-phosphate oxygen. At 100 ps sampling, the counts characterize snapshot occupancy. They cannot establish continuous water or ion residence, ion substitution, stabilization energy or exchange rate.

Partition of partner displacement

For each aligned frame, the mean squared deviation of the 114 partner Cα atoms from the fixed reference equals the sum of the segment mean squared deviations multiplied by 24/114 (B112–135) or 90/114 (B136–225). We first averaged the squared quantities over frames, then compared D91N and WT at equal numerical seed labels. Subtracting the time-averaged RMSDs would yield a different quantity. Per-residue RMSF was calculated about the run-mean aligned position and includes motion relative to EEF1A2.

Source data. In data/coordinate_analysis/reference_tables.zip, hbond/per_frame.csv contains the hydrogen-bond observations, hbond/contact_motifs.csv distinguishes donor and acceptor roles, and fragment/squared_displacement_contributions.csv gives the segment contributions. Scalar and coordinate-based RMSDs agreed to within 4.5×10^{-7} Å at 22,509 shared timestamps.

Deposited structural comparators

Fitting and symmetry expansion were unnecessary for distances within an asymmetric-unit copy. For each first deposited coordinate model, the selection retained shared atoms and the residue conformer with the highest mean positive alternate-site occupancy, with alphabetical tie breaking. The reported sites had unique shared coordinates. 4C0S chains A and B are two copies from the same rabbit structure.

Table S4. Exchange-factor Lys205 NZ–eEF1A Ser21 OG distances and Asp91 χ1 angles in the reference coordinates. The modeled component can differ from the nucleotide used experimentally.

PDB / copy	Experimental condition	Modeled nucleotide	NZ–OG (Å)	Asp91 χ1 (°)
1F60	Nucleotide-free yeast	None	2.819	−179.5
1G7C	Yeast, GDPNP	5GP	2.861	+176.3
1IJE	Yeast, GDP	GDP	2.785	+180.0
1IJF	Yeast, GDP/Mg	GDP	2.944	−179.5
4C0S / A	Rabbit, GDP/Mg	GDP	No partner	−146.0
4C0S / B	Rabbit, GDP/Mg	GDP	No partner	−141.7

Human EEF1B2 Lys224 corresponds to yeast Lys205 (author residue B1205). Human EEF1A2 Asp252 corresponds to yeast A250, and EEF1B2 Lys147 to yeast B1128. Neither yeast GDP entry contains PB, O1B, O2B or O3B coordinates. 1G7C contains 5GP, guanosine 5′-monophosphate. Neither the absence of a modeled β group nor the modeled protein distances imply a catalytic distance cutoff.

Scope of reference selection

5O8W re-refines the 1F60 diffraction data. It gives 2.834 Å for NZ–OG and was considered separately. Yeast entries 2B7B and 2B7C replace exchange-factor Lys205 with alanine and lack the target NZ atom. Human 9A9S is an integrative model of an assembly containing EEF1A1 and EEF1B2. These entries provide context for selecting the four original yeast models with the target lysine. Model-to-crystal comparisons are local geometric comparisons across different species and chemical states.

Structural records. Andersen et al., Mol Cell (2000), doi:10.1016/S1097-2765(00)00122-2; Andersen et al., Nat Struct Biol (2001), doi:10.1038/88598; Pittman et al., J Biol Chem (2006), doi:10.1074/jbc.M601076200; Crépin et al., Nucleic Acids Res (2014), doi:10.1093/nar/gku974; Jank et al., J Biol Chem (2017), doi:10.1074/jbc.M117.801035. PDB entry 9A9S, doi:10.2210/pdb9A9S/pdb.

Production trajectories and averaging windows

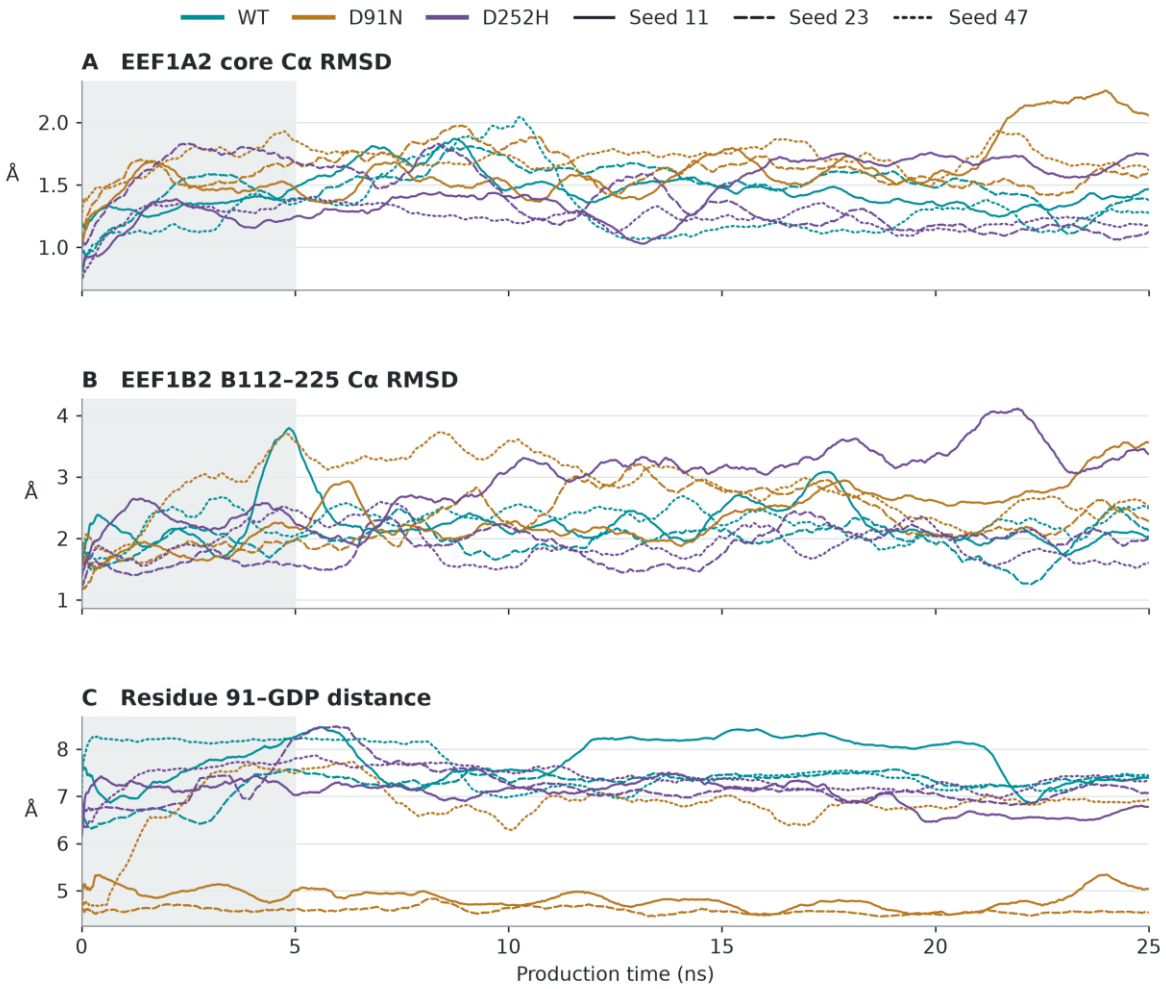


Figure S4. Trailing 1 ns means of EEF1A2 core RMSD (A), EEF1B2 fragment RMSD (B) and the residue-91–GDP heavy-atom minimum (C). At each time t the window is (t − 1 ns, t], limited to available samples at the start of production. Gray shading marks the first 5 ns. Color identifies condition and line style identifies velocity seed. Each curve is one trajectory.

Window sensitivity

For fragment RMSD in 5–25 ns, the D91N minus WT difference (+0.452 Å) exceeded the larger of the two within-condition ranges (0.412 Å). Over 0–25 ns, the difference (+0.359 Å) was smaller than the larger range (0.533 Å). In both windows the three D91N means lay above all three WT means.

The [0, 0.1) ns core RMSD contrast was +0.245 Å using 50 rows per run. Including the 0.100 ns row gives +0.247 Å from 51 rows. For early windows the opening endpoint is included and the closing endpoint is excluded. The three longer windows include their final 25 ns row.

Source data. The running means use all 12,501 production scalar observations per run in data/scalars/. No interpolation or frame-level hypothesis test was used.