EEF1A2 D91N: a matched two-seed structural pilot

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Six retained computational models: WT, D91N and D252H at seeds 11 and 23. One sample per condition/seed. All planned seed 23 predictions succeeded; source-bound technical geometry checks were completed separately from biological interpretation.

Results summary

D91N: one primary difference retains its sign and the other does not. This is a mixed result; selecting the agreeing distance would not establish a reproducible combined local rearrangement.

D252H: both primary mutant-minus-WT differences retain their signs across the two seeds. This is limited descriptive consistency of these specific distances, not a validated effect size or biological mechanism.

Primary distances and signed variant contrasts

All distances are Å. The two atom pairs were specified before seed 23: A Cα20-A Cα91 and A backbone O91-B backbone N224. A is EEF1A2 and B is EEF1B2. Values are measured without a cutoff; all twelve required observations are available. No model was selected or excluded based on the result.

Model	Cα20-Cα91 (Å)	O91-N224 (Å)
WT11	6.723	2.661
WT23	6.644	2.900
D91N11	7.479	3.869
D91N23	6.922	2.498
D252H11	6.428	2.696
D252H23	6.454	2.997

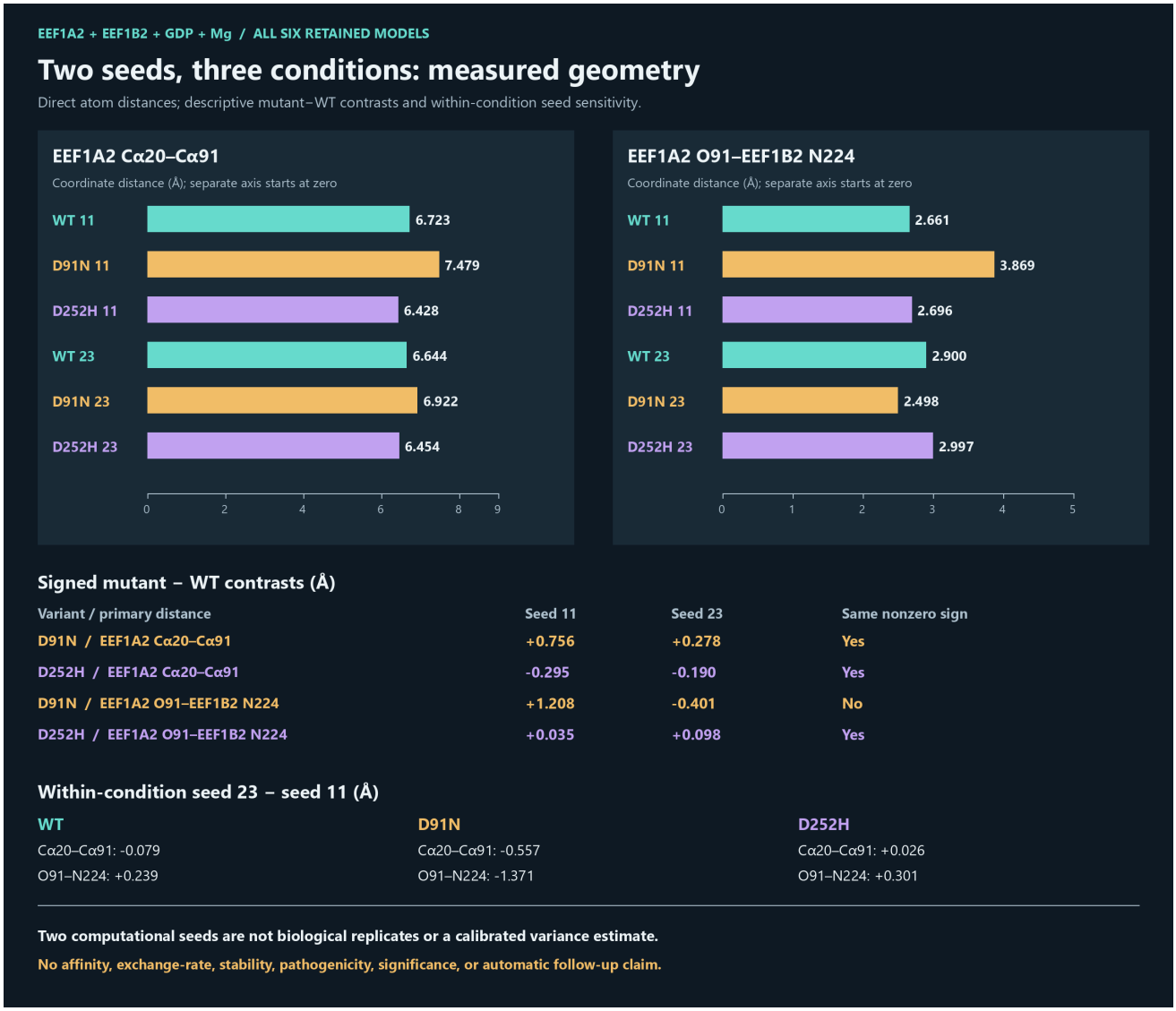
Variant / distance	Mutant−WT, seed 11 (Å)	Mutant−WT, seed 23 (Å)	Same nonzero sign
D91N / EEF1A2 Cα20-Cα91	+0.756	+0.278	Yes
D252H / EEF1A2 Cα20-Cα91	-0.295	-0.190	Yes

Variant / distance	Mutant−WT, seed 11 (Å)	Mutant−WT, seed 23 (Å)	Same nonzero sign
D91N / EEF1A2 O91-EEF1B2 N224	+1.208	-0.401	No
D252H / EEF1A2 O91-EEF1B2 N224	+0.035	+0.098	Yes

Within-condition seed sensitivity

Condition / distance	Seed23−seed 11 (Å)	Matched seed 23 variant contrast (Å)
WT / EEF1A2 Cα20-Cα91	-0.079	WT reference
D91N / EEF1A2 Cα20-Cα91	-0.557	+0.278
D252H / EEF1A2 Cα20-Cα91	+0.026	-0.190
WT / EEF1A2 O91-EEF1B2 N224	+0.239	WT reference
D91N / EEF1A2 O91-EEF1B2 N224	-1.371	-0.401
D252H / EEF1A2 O91-EEF1B2 N224	+0.301	+0.098

These changes describe predictor sensitivity. Two seeds do not estimate a calibrated variance distribution, and are not biological replicates. Comparisons above retain both metrics, including reversals and null contrasts; no posthoc magnitude threshold is used.



Six measured models, signed contrasts and within-condition changes

Both plotted axes begin at zero and have separate labeled scales. No error bars, statistical significance, affinity score or molecular illustration is inferred.

Seven fixed-core comparisons

Mobile → reference	Core RMSD, 120 Cα (Å)	Local85-110 RMSD, 26 Cα (Å)	Reuse
D252H11 → WT11	0.883	1.274	saved
D252H23 → D252H11	0.738	1.070	new
D252H23 → WT23	0.460	0.309	new
D91N11 → WT11	0.928	1.561	saved
D91N23 → D91N11	1.247	1.297	saved
D91N23 → WT23	0.818	0.663	saved
WT23 → WT11	0.766	0.430	saved

All fits use the original unweighted 120-position core once. All 26 local Cα positions are evaluated under that transform, without local refitting, trimming or confidence weighting. A-chain and B-chain evaluations and the transform itself are preserved. RMSD is a nonnegative magnitude without a direction-of-effect interpretation.

Confidence and proximity context

Model	Site91 pLDDT	Site252 pLDDT	Local pLDDT	PAE A→B (Å)	PAE B→A (Å)	≤4 Å atom / residue pairs
WT11	72.94	93.35	78.22	13.87	15.04	461 / 125
WT23	71.77	93.49	77.72	13.21	14.31	451 / 118
D91N11	73.79	93.05	75.82	16.46	17.66	365 / 106
D91N23	81.29	93.63	80.14	16.46	17.60	452 / 133
D252H11	74.33	92.49	78.43	14.60	15.79	454 / 138
D252H23	72.59	91.43	78.64	16.85	17.88	374 / 116

pLDDT is on the 0-100 scale. PAE direction is row/frame → column/position; both directions are retained. Confidence is separate from geometry and does not turn a contact count into binding affinity. Native frame-validity masks were not independently established and GDP/Mg frame PAE is not interpreted.

Native chemistry and technical geometry

Model	Native site91-GDP minimum (Å)	Native site91-Mg minimum (Å)	Native site91-B minimum (Å)	GDP-Mg minimum (Å)
WT11	4.776	3.904	2.661	1.857
WT23	4.813	4.071	2.900	1.949
D91N11	6.084	5.519	2.721	2.077
D91N23	4.969	4.730	2.498	2.074
D252H11	4.654	4.028	2.635	1.911
D252H23	4.653	3.802	2.924	1.941

Native ASP/ASN/HIS chemistry remains distinct: ASP OD2 is never equated with ASN ND2 or a histidine ring atom. These minima are secondary chemistry-specific observations, not equivalent charged-group measurements.

D252H23 identity: 5,316 coordinate atoms, A463/B225 complete sequence correspondence, 688 complete protein residues, GDP28 and Mg1. The full confidence outputs map to 717 native tokens; pLDDT and full PAE/PDE arrays passed finite/shape/order checks. HIS252 is the intended only D252H sequence change. All automatic technical checks passed: **True**.

D252H23 has 0 cross-entity heavy-atom approaches below 1.5 Å. Its maximum absolute declared GDP bond-length deviation from the retained CCD ideal is 0.111 Å. Mg neighbors within 3.2 Å are listed below; this includes any nearby carbon/phosphorus atoms and is not a coordination number.

Neighbor atom	Element	Mg distance (Å)
A:SER21:CB	C	3.136
A:SER21:OG	O	2.042
C:GDP1:PB	P	3.158
C:GDP1:O1B	O	1.941

These checks do not establish full topology, stereochemistry, hydrogen bonding or physiological Mg coordination/solvation. The generic multi-entity chemistry hook is unsupported and is not counted as a pass.

Discussion

D91N: one primary difference retains its sign and the other does not. This is a mixed result; selecting the agreeing distance would not establish a reproducible combined local rearrangement.

D252H: both primary mutant-minus-WT differences retain their signs across the two seeds. This is limited descriptive consistency of these specific distances, not a validated effect size or biological mechanism.

Within-condition changes must be considered alongside each variant contrast. Where seed sensitivity is comparable to the contrast, predictor variation remains a plausible explanation. No consistent or inconsistent geometric outcome establishes normal function, pathogenicity, affinity, stability, or slower/faster nucleotide exchange. D252H remains an uncalibrated comparator; its cellular phenotype does not validate a contact-count metric.

The technical suitability decision is independent of the hypothesis outcome. No additional prediction or MD result is included in this release; no alternative mechanism is inferred from a mixed result.

Methods

The final adapter composes preserved model identity, canonical heavy-atom, native confidence, GDP bond and geometry helpers. Five per-model analyses and five existing fits are source-bound and reused unchanged. Only D252H23 and its two predeclared fits are calculated in this stage. The original CIFs are preserved, and the fitting routine checks unchanged in-memory coordinates.

Core mask SHA256: 22612cf2d9f33a9f3eceaf6dfa87aa67b662da938769a2f5d28927a7c49f4933. D252H23 is fit onto WT23 and onto D252H11 with all 120 core and 26 local identities. Primary atom distances are measured without a contact cutoff; unavailable or ambiguous identities cannot become zero or a claimed lost contact. All twelve observations are available here.

Protocols, planned/copied inputs, raw alignments, terminal receipts, actual successful queue state, checkpoint/image/settings and native output identities are checked before calculation. The current D252H23 audit must explicitly prove successful stopped ownership, absent runner, stable file inventories and all receipt hashes. Both predecessor gates/source inventories and the saved seed 11 D252H analysis are bound to their preserved evidence.

WT11 used the earlier raw MSA. Normalized WT equality is audited only for native MSA arrays and scoped downstream features, not raw bytes or full stochastic-feature equality. Identical seeds across conditions do not imply identical random paths or molecular reference features.

CPU analysis used Python 3.12.3 (main, Aug 31 2026, 10:18:26) [GCC 13.3.0], NumPy 1.26.4, Gemmi 0.6.5, and one numerical thread. Recorded analysis time: 42.149641 seconds, separate from inference and final artifact writing. Rendering uses installed Pillow 12.3.0; no new package/download/inference is involved.

Detailed evidence

SUBJECTS.json binds all six public subject identifiers to original model and confidence hashes. 03_results/ contains every retained numeric table in the completed round and seven fitted transformations; local operational identifiers were removed without changing measurements. 06_methods/core-mask.json supplies the explicit residue correspondence. The exact source models and confidence arrays retain their original bytes.

The historical seed-11 report and literature review are retained under 01_report/historical-seed11/, explicitly as an earlier snapshot. They motivated the subsequent primary-distance check; their one-seed interpretation must be read alongside this mixed two-seed result. The v1.0.0 website release is preserved

without replacement.

Literature and experimental follow-up

Human sequence sources are UniProt Q05639 and UniProt P24534. Experimental reference structures are 4C0S, 1F60, 1IJE and 1IJF. Their species, constructs, missing atoms and modifications remain distinct from the predicted human complexes.

The retained literature review includes rabbit eEF1A2 structural work, EEF1A2 variant experiments, Human Molecular Genetics 29:1592, and additional EEF1A2 experimental literature. Full bibliographic entries, source attribution and scope are preserved in the historical report and 01_report/historical-seed11/sources/references.bib; these publications do not validate our predicted D91N mechanism.

A useful proposed experiment is whether D91N alters eEF1B2-stimulated nucleotide exchange after accounting for soluble, competent eEF1A2 abundance. Intrinsic nucleotide behavior, partner association/release, folding and turnover should remain separable explanations. This is a proposed experiment, not an observation from the models. No MD trajectory, biochemical experiment, affinity measurement or clinical assessment was performed here.

Attribution and reproducibility

Filippo D. Galbo is the sole human author. Boltz-2 generated the predictions; ExoMBridge supplied the local orchestration, retained-source identity and analysis workflow. AI tools assisted software, literature discovery, analysis checks, visualization and writing. Automated validation is not independent human scientific review. Third-party sources retain their own terms; no blanket package license or DOI is claimed.

See README.md, tools-ledger.md, THIRD_PARTY_NOTICES.md, MANIFEST.json and the read-only verification helpers. These reproduce saved-coordinate checks, not inference or a private application installation. Canonical study: https://www.filippogalbo.com/research/eef1a2-d91n .