Supplementary Information

Redacted supplementary tables for bioRxiv submission

Manuscript: Biophysical Derisking of Non-ATP Ligand Engagement in FGR Tyrosine Kinase: A Computational Strategy for Cancer-Relevant Kinase Discovery

Redaction policy: This supplementary file reports non-ligand-identifying processed summaries only. Ligand-identifying chemical information, atomistic ligand figures, raw ligand-containing trajectories, reconstruction-enabling files, exact ligand-site reconstruction maps, raw internal residue-index tables, and private implementation details are intentionally omitted.

Table S1. Redacted holo and apo system audit.

Item	Public-facing summary	Interpretation	Redaction boundary
Holo system	Human FGR tyrosine kinase with structurally undisclosed probe ligand UNL/Analog 7	Used to evaluate ligand-site classification and local biophysical remodeling	Ligand chemistry and reconstruction-enabling files withheld
Apo system	Ligand-free FGR comparator	Used as baseline for groove behavior, hydration, mobility, and exposure	Detailed file names and private preparation metadata omitted
Holo simulation length	100 ns trajectory	Sufficient for trajectory-level derisking in this case	Not treated as ensemble convergence
Apo simulation length	50 ns trajectory	Matched ligand-free comparator	Not treated as exhaustive apo conformational sampling
Frame handling	Different frame spacing was accounted for where residence-style comparisons were made	Reduces frame-spacing bias in apparent dwell analysis	Raw trajectory file names omitted
Residue mapping	Local analysis used corrected biological-to-trajectory mapping	Prevented wrong-region interpretation	Exact raw residue-index table omitted
Overall audit call	Systems were suitable for matched apo-holo comparison	Supports downstream analysis integrity	Quality control only, not ligand-engagement evidence

Table S2. Redacted ATP-site exclusion and competing-site control summary.

Analysis component	Public-facing result	Derisking interpretation	Boundary
ATP-site reference	FGR-specific crystallographic ATP-site ligand reference used as competing-site control	Tests whether the probe samples the canonical ATP-site region	Reference-state dependent
Mapping approach	Sequence-aware local structural mapping used rather than direct same-number residue matching	Reduces construct-numbering artifacts	Full operational mapping details omitted
Transfer quality	Local reference transfer was stable and acceptable for site-classification use	Supports use of transferred ATP-site reference	Detailed local-core tables omitted
ATP-site proximity	Ligand remained more than 16 Angstrom from the ATP-site reference across the analyzed trajectory	Supports ATP-site exclusion in this trajectory	Exact value differs slightly between internal mapping outputs; public threshold-safe wording retained
Threshold check	Zero frames were within 8 Angstrom of the ATP-site reference	Supports non-ATP site classification	Not universal ATP-site exclusion
Functional-site landmarks	No close approach to tested canonical-site landmarks	Independent negative-control layer	Supporting control only
Final call	ATP-site exclusion supported for the analyzed FGR trajectory	Reduces risk of canonical-site misassignment	Not inhibition, noncompetitive behavior, allostery, or ligandability

Table S3. Redacted local engagement summary.

Analysis component	Public-facing result	Interpretation	Boundary
Initial state	Ligand was initially separated from the protein	Frame 0 was not treated as bound-state evidence	Avoids starting-pose overclaim
Contact onset	Direct protein and local-region contact occurred after ligand approach	Engagement inferred from trajectory behavior	Not a binding-rate measurement
Local-region persistence	Ligand showed recurrent proximity to a predefined non-ATP region across most analyzed frames	Supports reproducible local engagement	Not affinity, potency, inhibition, or allostery

Analysis component	Public-facing result	Interpretation	Boundary
Regional distribution	Contacts were concentrated within the predefined analysis region rather than interpreted as nonspecific whole-protein retention	Supports local-site interpretation	Exact residue-level site map omitted
Central/peripheral grouping	Grouped local-region interpretation used for hydration, mobility, and HDX-mimic analyses	Supports region-level follow-up logic	Full residue reconstruction not disclosed
Final call	Persistent local engagement supported	Hypothesis merits downstream derisking layers	Not validated ligandability

Table S4. Redacted apo-holo groove comparison summary.

Comparison layer	Public-facing result	Interpretation	Boundary
Holo selected-frame pocket mapping	Selected holo frames showed intermittent local groove organization	Supports ligand-associated local groove organization	Snapshot support only
Holo strict overlap class	Strict local overlap observed in 3 of 7 selected holo frames	Strongest selected-frame support for local organization	Does not prove full-trajectory pocket persistence
Holo relaxed overlap class	Relaxed local overlap observed in 2 of 7 selected holo frames	Supports weaker or shifted local groove signal	Interpreted cautiously
Holo no-clear class	No clear local overlap observed in 2 of 7 selected holo frames	Shows pocket organization is not uniform across selected frames	Prevents overclaiming
Apo selected-frame pocket mapping	Related local-groove signal detected in selected apo frames	Indicates the groove was pre-existing rather than newly formed	Rules out strict cryptic-pocket emergence
Apo pocket character	Apo local groove was weak by pocket-scoring criteria	Supports weak pre-existing groove interpretation	Not proof or disproof of ligandability
Final call	Ligand-associated organization of a pre-existing weak non-ATP groove	Supports experimental prioritization	Not cryptic-pocket emergence or allostery

Table S5. Redacted local hydration and apparent residence summary.

Analysis layer	Apo comparator	Holo ligand-associated state	Interpretation	Boundary
Mean local water occupancy	56.7 waters	36.9 waters	Lower local water occupancy in holo ligand-associated states	Geometric count, not free energy
Holo-minus-apo occupancy difference	Reference	Approximately -19.8 waters	Supports local hydration remodeling	Not thermodynamic water displacement
Mean apparent residence	36.5 ps	41.7 ps	Holo waters were not shorter-lived	No accelerated water-release claim
Residence interpretation	Local dwell proxy	Local dwell proxy	Remaining waters show no shorter residence signal in holo	Not rigorous water kinetics
Final hydration call	Higher occupancy baseline	Lower occupancy with no faster escape signal	Supports reduced local water occupancy	Not ligandability, inhibition, or allostery

Table S6. Redacted RMSF and SASA regional summary.

Region-level comparison	Mobility result	Solvent-exposure result	Interpretation	Boundary
Peripheral local region	Lower holo C-alpha mobility	Lower holo solvent exposure	Strongest protection-like simulation signature	Not measured HDX protection
Central local region	Lower holo C-alpha mobility	Exposure behavior mixed or higher	Dynamics-stabilized but not uniformly shielded	Prevents broad central-protection claim
Full local region	Lower holo mobility overall	Near-neutral or mixed net exposure behavior	Supports local stabilization with exposure heterogeneity	Simulation-derived descriptor only
Final RMSF/SASA call	Holo ligand-associated states show reduced regional mobility	Exposure changes are region-dependent	Supports experimental prioritization	Not affinity, allostery, or ligandability

Table S7. Redacted HDX-mimic prioritization summary.

HDX-mimic layer	Public-facing result	Experimental implication	Boundary
Peripheral peptide window	Strongest predicted protection-like signature	Highest-priority region for possible HDX-MS follow-up	Computational prioritization only

HDX-mimic layer	Public-facing result	Experimental implication	Boundary
Central peptide windows	Mixed behavior due to lower mobility but non-uniform exposure	Include as secondary regions if peptide coverage allows	Do not expect uniform protection
Full local region	Mixed regional behavior	Use as regional context, not as a single protected unit	Not measured deuterium uptake
Final HDX-mimic call	Prioritizes candidate peptide regions for experimental footprinting	Guides HDX-MS design	Does not replace HDX-MS

Table S8. Redacted exploratory state-classification summary.

State-analysis layer	Public-facing result	Interpretation	Boundary
Analysis window	Post-approach holo sampling used	Reduces separation of early approach from ligand-associated behavior	Not proof of equilibrium sampling
Input class	Standardized local-groove geometry descriptors	Tests recurrent groove organization	Exact features and loadings omitted
Annotations	Ligand proximity, hydration, pocket behavior, and ATP-site separation used after clustering	Supports interpretation of states	Not used here as full operational recipe
Clustering outcome	Two recurrent descriptive holo substates identified	Supports state-resolved local groove organization	Not validated metastability
Hydration annotation	States showed similar ligand proximity but different hydration behavior	Supports hydration-linked substates	Not kinetic water mechanism
MSM feasibility	Kinetic interpretation was not retained	Clustering-only interpretation is safer	No validated MSM kinetics
Final state call	Exploratory state classification supported	Adds descriptive state-resolution layer	Not allosteric-state modeling

Table S9. Interpretation boundaries and unsupported claims.

Unsupported claim	Reason it is not supported	Safer public wording
UNL/Analog 7 is an FGR inhibitor	No kinase activity, ATP-competition, or cellular phosphorylation data	Structurally undisclosed probe ligand showing trajectory-supported local engagement
UNL/Analog 7 is an allosteric modulator	No functional coupling or activity modulation shown	Relevant to non-ATP/allosteric discovery, but allostery remains untested
The local groove is newly cryptic	Apo comparison showed related groove signal	Ligand-associated organization of a pre-existing weak groove
Hydration proves water displacement	Water counts and apparent residence are descriptive	Lower local water occupancy, not thermodynamic water displacement
HDX-mimic proves protection	No experimental deuterium uptake measured	HDX-mimic prioritizes regions for future HDX-MS
tICA proves metastable states	Kinetic validation was not retained	Exploratory state classification only
The site is validated ligandable	No binding, structural, or functional validation	Experimental prioritization, not validated ligandability

Table S10. Recommended experimental follow-up.

Validation layer	Purpose	Positive result would support	Boundary
Low-assumption binding assay	Test whether the probe measurably associates with purified FGR	Ligand-protein association outside the simulation environment	Does not localize the site
HDX-MS footprinting	Test whether the predicted local region shows ligand-associated exchange changes	Site-local protection or altered exchange	Requires peptide coverage and experimental optimization
Mutational testing	Assess whether the local region contributes to ligand positioning or binding	Change or loss of binding/footprint after targeted mutation	Mutations can destabilize protein and require controls
Kinase activity assay	Test functional consequence after binding or footprint evidence	Reproducible activity modulation	Required before inhibitor or allostery claims
ATP-competition format	Distinguish non-ATP engagement from canonical-site competition	Noncompetitive behavior if validated experimentally	Simulation ATP-site exclusion alone is not enough
Structural biology	Attempt direct site confirmation if binding and footprinting are positive	Structural evidence of site occupancy	May be difficult for shallow dynamic grooves
Analogue design	Proceed only after orthogonal support	Improved site engagement or clarified SAR	Premature without experimental signal

End of redacted supplementary information.