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Molecular Dynamics Simulation and Free Energy Landscape Analysis of Next-Generation KRAS G12D Inhibitors

Riis I[1*], Golob P[2] and Wojcik T[3]

[1]*Department of Medicinal Chemistry, University of Copenhagen, Copenhagen, Denmark*
[2]*Department of Bioinformatics, University of Maribor, Maribor, Slovenia*
[3]*Department of Pharmacology, Jagiellonian University, Krakow, Poland*

ABSTRACT

Background: MRTX1133 and the family of noncovalent, salt-bridge-forming chemotypes that followed it have turned KRAS G12D from a textbook 'undruggable' target into an active medicinal chemistry problem, but the biophysical reasoning behind why these molecules work, and why closely related analogues sometimes fail, lives almost entirely in the molecular dynamics (MD) and free energy literature rather than in static crystal structures.

Objective: This review reads that literature specifically for its methodology and mechanistic claims about switch I/switch II conformational behaviour, deliberately taking a distinct angle from a companion virtual-screening-focused review published previously in this journal.

Scope: We examine force-field and simulation protocol choices used across KRAS G12D MD studies, the application of MM-GBSA/MM-PBSA, alchemical free energy perturbation, umbrella sampling, and metadynamics to G12D-directed binders, Markov state model reconstructions of MRTX1133 binding pathways, comparative free energy landscapes across G12 mutants, and the still-thin literature on resistance-mutation dynamics.

Conclusions: Across heterogeneous force fields, simulation lengths, and free energy estimators, a broadly consistent physical picture emerges in which G12D substitution destabilizes the closed switch conformation enough to expose a transient, druggable pocket that MRTX1133-class chemistry locks via an Asp12 salt bridge network. Quantitative free energy estimates disagree considerably between studies, alchemical FEP remains underused relative to its apparent suitability for this scaffold, and atomistic simulation of resistance-associated substitutions lags well behind the discovery-side literature, representing the most consequential gap for future work.

Keywords: *KRAS G12D; molecular dynamics; free energy landscape; MRTX1133; switch I/II conformational dynamics*

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Corresponding Author: *Ida Riis*

E-mail Address: *ida.riis@universityofcopenhagen.edu*

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Abstract

Background: MRTX1133 and the wave of noncovalent, salt-bridge-forming chemotypes that followed it have turned KRAS G12D from an "undruggable" target into an active medicinal chemistry problem, but the biophysical reasoning behind why these molecules work — and why some analogues fail — sits almost entirely in molecular dynamics (MD) and free energy literature rather than in the crystal structures themselves.

Objective: This review reads that literature specifically for its methodology and its mechanistic claims about switch I/switch II conformational behaviour, rather than repeating a virtual-screening-campaign narrative.

Scope: We cover force-field choices and simulation protocols used across KRAS G12D studies, the application of MM-GBSA/MM-PBSA, alchemical free energy perturbation, umbrella sampling, and metadynamics to G12D-directed binders, the reported free energy landscapes distinguishing GDP- and GTP-bound switch states, Markov state model reconstructions of MRTX1133 binding pathways, and the emerging MD literature on resistance-associated substitutions.

Conclusions: Across a genuinely heterogeneous set of force fields, simulation lengths, and free energy estimators, a fairly consistent physical picture emerges: G12D substitution reshapes the switch I/switch II interface into a transient pocket whose opening is coupled to Asp12 side-chain reorientation, and MRTX1133-class

inhibitors work by locking that geometry through a bidentate salt bridge network rather than by simple steric occlusion. We flag where the computational estimates disagree with each other or with experimental affinities, and where resistance-mutation dynamics remain thin, as the areas most in need of further simulation work.

Introduction

It has been a little over a decade since the original wave of Ras "undruggability" reviews conceded that the GTPase's flat, largely featureless surface left few obvious pockets for a small molecule to grab onto [1]. That assessment held up remarkably well until crystallographic and computational work on Cys12-directed covalent chemistry showed that a transient switch II pocket could be trapped and exploited, and it held up less well after Mirati's disclosure of MRTX1133, a noncovalent compound that reaches nanomolar affinity for KRAS G12D by forming a direct salt bridge with the mutant aspartate itself [2,3]. What followed was not simply a burst of new chemical matter — it was a burst of computational papers trying to explain, at the level of switch-region conformational dynamics and free energy, why this particular pocket opens up for G12D and why this particular scaffold sits in it so well.

That second body of work is the subject of this review. A companion review in this journal already surveyed structure-based virtual screening campaigns against the G12D allosteric pocket; here we deliberately narrow the lens to the molecular dynamics and free energy calculation side of the literature — the papers asking how the switch I and switch II loops actually move, what the underlying free energy surface looks like for GDP- versus GTP-loaded and mutant versus wild-type KRAS, and what quantitative binding free energy methods say about MRTX1133 and its relatives once a docking pose has already been generated. This is a narrower question than "can we screen for hits," and the answer is less settled than the growing pipeline of preclinical candidates might suggest.

Three things make this an unusually productive niche for MD and free energy work. First, KRAS is small enough (roughly 170 residues in the catalytic domain) that microsecond-scale all-atom simulation is computationally tractable on a modern GPU cluster, so groups have been willing to run the long trajectories that switch-loop transitions actually require. Second, the G12D mutation introduces a negatively charged side chain exactly where MRTX1133's chemotype was designed to form a direct ionic interaction, which gives simulators an unusually clean and interpretable hypothesis to test against trajectory data. Third, because Mirati and academic groups both deposited GDP- and GTP-bound, ligand-free and ligand-bound structures in relatively short order, there is now enough starting-structure diversity to actually compare simulation protocols against each other rather than relying on a single crystal form, as earlier RAS MD work often had to.

KRAS G12D Structural Biology and the Switch I/Switch II Conformational Landscape

The switch I (residues ~25–40) and switch II (residues ~60–76) loops are the parts of KRAS that physically respond to whether GDP or GTP occupies the nucleotide pocket, and they are also the loops that most MD studies of G12D inhibitors track as their principal reaction coordinate. Early accelerated MD work on wild-type and oncogenic Ras already established that switch I and switch II sample multiple conformational substates on the microsecond timescale, well beyond what static crystallography captures, and that nucleotide state biases which substates are populated without fully determining the outcome [4]. That basic finding — conformational heterogeneity rather than a two-state on/off switch — has held up in essentially every subsequent simulation study of KRAS mutants, G12D included.

What G12 mutations specifically do to this landscape has been mapped more directly using free energy profile reconstructions along switch-domain collinear coordinates. Chen and colleagues built free energy profiles for GTP-bound wild-type KRAS alongside G12C, G12D, and G12R variants and found that all three oncogenic substitutions raise the population of higher-energy, more disordered switch conformations relative to wild-type, with the specific residue introduced at position 12 determining which substates are favoured and how deep the associated free energy wells are [5]. This is a genuinely useful result for inhibitor design because it means the "druggable" switch I/switch II pocket exploited by MRTX1133 is not a static structural feature present in the apo protein — it is a state that has to be populated, at some free energy cost, before a ligand can bind it, and that cost differs by G12 substitution. A closely related and more recent free energy barrier analysis extended this comparison across the G12 mutant panel using microsecond-scale trajectories and explicitly mapped drugging pockets onto the resulting energy landscape, reporting that G12D shifts the switch I/II interface toward a geometry more compatible with the MRTX1133 binding mode than several of the other oncogenic substitutions examined, which is a computational rationale for why G12D-selective chemistry has proven more tractable than, say, G12V-directed noncovalent design [6].

The switch II pocket itself has an independent structural biology literature worth folding in here, because monobody-based structural work has shown that this pocket's precise shape is mutant-selective at a finer level than "G12D versus G12C": Akkapeddi and colleagues solved structures of mutant-selective monobodies bound to the switch II pocket of KRAS(G12D) and showed conformational differences in pocket geometry relative to G12C-bound monobodies that track with each mutant's local electrostatic environment [7]. That structural granularity matters for MD work because it means a simulation protocol validated against one G12-mutant switch II pocket cannot simply be assumed to transfer to another without re-parameterizing the starting geometry and re-checking the sampled ensemble.

Molecular Dynamics Simulation Methodology Applied to KRAS G12D

The methodological toolkit used across this literature is fairly conventional classical MD, but the specific choices — force field, water model, simulation length, ion treatment around the highly charged nucleotide-binding site — vary enough between papers that direct numerical comparison across studies is often not really valid, a point worth making explicit before summarizing what the free energy numbers say.

Most recent G12D-focused papers run GROMACS as the simulation engine, largely because of its throughput on commodity GPU hardware and its long track record in the biomolecular simulation community [8]. Protein force field choice splits fairly evenly between AMBER-family parameter sets, particularly ff14SB, which corrected several backbone and side-chain torsion issues present in earlier ff99SB parameterizations that had shown up as artifactual secondary structure propensities in longer

simulations [9], and CHARMM36, whose validation against NMR-derived order parameters and scalar couplings gave it a reputation for accurately reproducing loop flexibility — a property that matters directly for switch I/II work, since these are exactly the flexible, solvent-exposed loops CHARMM36 was benchmarked against [10]. Neither force field family has emerged as clearly superior for RAS systems specifically; the choice in most papers appears to track lab tradition and prior in-house validation more than a documented head-to-head comparison on this particular protein.

Simulation length has grown substantially over the past decade, tracking GPU hardware improvements rather than any methodological breakthrough. Where early Ras accelerated MD work ran on the order of tens to low hundreds of nanoseconds per trajectory, current MRTX1133-focused papers routinely report multiple independent runs in the 500 ns to several-microsecond range per system, sometimes aggregated into tens of microseconds of total sampling across replicate trajectories used to build Markov state models [11,12]. That aggregate sampling strategy — many shorter parallel trajectories combined via kinetic clustering rather than one very long continuous run — has become close to a de facto standard for RAS conformational transition studies specifically because switch-loop transitions are rare events on the timescale of a single unbiased trajectory, and Markov state modelling gives a principled way to stitch together transition kinetics from an ensemble of shorter, more computationally tractable simulations.

Ligand-bound systems bring their own parameterization headache: MRTX1133 and its analogues are moderately large, conformationally flexible small molecules with several rotatable bonds around a piperazine-pyrimidine-quinazoline-type core, and partial charge assignment for the ligand — typically via RESP fitting under general AMBER force field (GAFF) conventions, or CHARMM general force field (CGenFF) parameters when the protein side uses CHARMM36 — is a nontrivial source of variance between independently run studies that is rarely discussed explicitly in methods sections but almost certainly contributes to some of the free energy discrepancies noted below.

Free Energy Landscape Mapping: MM-GBSA/MM-PBSA, FEP, Umbrella Sampling, and Metadynamics

Four broad free energy strategies recur across this literature, and they answer genuinely different questions, which is easy to lose track of when papers are read only for their headline binding energy numbers.

End-point methods — MM-GBSA and MM-PBSA — dominate numerically simply because they are cheap: both post-process a set of MD snapshots (often the last tens of nanoseconds of a single trajectory) by decomposing the ligand-protein interaction into molecular mechanics energy terms plus an implicit solvation correction, either generalized Born (GB) or Poisson-Boltzmann (PB) electrostatics, without running any additional biased or alchemical simulation [13]. Nearly every G12D-focused MD paper we reviewed reports an MM-GBSA or MM-PBSA estimate somewhere, usually to rank a small set of analogues or to decompose per-residue contributions and confirm that the Asp12 salt bridge dominates the binding energy budget. The trouble, well known in the wider MM-GBSA literature and visible here too, is that absolute binding free energies from these methods routinely miss experimental values by several kcal/mol, and the ranking of closely related analogues is not always reliable — useful for confirming a binding hypothesis (does the salt bridge contribute what we think it does?) but a weaker tool for genuinely prospective potency prediction.

Alchemical free energy perturbation (FEP) sits at the opposite end of the cost-accuracy spectrum. The modern FEP+ protocol, built around a carefully validated OPLS-family force field, replica-exchange sampling with solute tempering, and a curated set of perturbation cycles, was benchmarked broadly across pharmaceutical targets with reported accuracy within roughly 1 kcal/mol of experiment for well-behaved series [14]. We found comparatively few published FEP studies specifically on KRAS G12D chemotypes relative to the volume of MM-GBSA work — a real gap given how well suited a rigid, well-defined salt bridge interaction ought to be to alchemical perturbation of substituents around that anchor point. Where relative binding free energy calculations have been applied to KRAS ligand series more broadly, the method has performed credibly on ranking analogues that share a common binding mode, which is exactly the situation an MRTX1133 backup-series optimization campaign would present, so this reads as an underused rather than an unsuitable tool for this specific problem.

Umbrella sampling and metadynamics address a different question again: not "what is the binding free energy of this fixed pose" but "what is the free energy as a function of some structural coordinate as it varies continuously," which is exactly what is needed to characterize switch I/II loop opening rather than ligand binding per se. Umbrella sampling constructs a free energy profile along a chosen reaction coordinate by running a series of biased simulations restrained at different coordinate values and then unbiasing the combined histogram, typically via weighted histogram analysis; metadynamics instead builds up a history-dependent bias on the fly that discourages resampling already-visited regions of the coordinate space, which is often more efficient for coordinates whose relevant range is not known in advance. Combined slice-sampling protocols that use metadynamics to identify a coarse reaction pathway and umbrella sampling to refine free energy profiles along the resulting slices have been developed for exactly this kind of multi-well conformational problem [15], and the logic of that combined approach maps naturally onto switch-loop opening, where the relevant collective variable is not obvious a priori and the free energy surface is expected to have multiple local minima corresponding to different switch conformational substates. The free energy barrier mapping of G12-mutant KRAS discussed earlier used exactly this kind of enhanced-sampling reconstruction to characterize the barrier separating the closed and druggable-pocket-open switch states, rather than relying on unbiased sampling alone to observe the transition often enough to characterize it statistically [6].

Markov state models deserve separate mention as a fifth, increasingly common strategy that is not strictly a free energy method but is usually reported alongside one. By clustering large ensembles of shorter trajectories into kinetically distinct conformational states and estimating transition probabilities between them, MSMs recover both the relative free energies of metastable states (via their equilibrium populations) and the kinetics connecting them — information neither umbrella sampling nor MM-GBSA provides on its own. Two independent MSM studies of MRTX1133 binding to KRAS G12D, run by different groups with somewhat different clustering and featurization choices, converge on a broadly similar mechanistic picture: the ligand does not simply diffuse into a pre-formed pocket but

instead engages first through peripheral contacts before a slower, rate-limiting conformational rearrangement of switch I completes pocket closure around the bound ligand [11,12]. That two independently constructed MSMs from separate labs land on a similar binding pathway topology is one of the more reassuring points of convergence in this literature, even though the specific rate constants and intermediate-state populations they report differ enough that neither should be treated as quantitatively definitive on its own.

Binding Free Energy Findings for MRTX1133 and Related G12D-Directed Chemotypes

Turning to what the free energy calculations actually say about specific molecules: MM-GBSA decomposition studies on MRTX1133 consistently attribute a large share of the total binding free energy to the salt bridge and associated hydrogen-bond network with Asp12, echoing the mutational and structural rationale from the original discovery papers [2,16], but the simulations add a dynamic dimension the static structures cannot: per-residue energy decomposition across a trajectory shows that this salt bridge is not a fixed, rigid contact but fluctuates in occupancy over the simulation, occasionally breaking and reforming, with overall binding stability maintained by a broader shell of hydrophobic and van der Waals contacts from switch II residues that compensate during the moments the salt bridge is transiently disrupted.

Microsecond-scale comparative simulations pitting MRTX1133 against a repurposed kinase inhibitor scaffold (trametinib, examined here purely as a computational drug-repurposing exercise rather than a clinically validated G12D strategy) found substantially lower and less stable binding free energy for the repurposed compound, consistent with its lack of the salt-bridge-forming moiety, and used RMSF and hydrogen-bond occupancy analysis across the trajectory to argue that MRTX1133's higher rigidity within the bound pocket, not merely its static binding pose, accounts for much of its potency advantage [17]. Steered MD work pulling GDP out of the nucleotide pocket across wild-type and multiple G12 mutants, including G12D with and without MRTX1133 bound, found that ligand occupancy of the adjacent allosteric pocket measurably alters the force profile required to displace GDP, a computational demonstration that MRTX1133 binding is allosterically coupled to nucleotide pocket stability rather than acting as a purely local, spatially isolated perturbation [18]. That kind of mechanical-coupling result is difficult to obtain from equilibrium MD or end-point free energy methods alone and is one of the clearer examples in this literature of enhanced sampling adding mechanistic information beyond a binding affinity number.

Independent structure-based virtual screening efforts that used MD and MM-GBSA rescoring as a post-docking filter, rather than as the primary discovery tool, reported that a meaningful fraction of docking-favoured poses failed to maintain stable protein-ligand contacts once relaxed under explicit-solvent MD, underscoring a point several groups in this literature make explicitly: docking scores and even single-snapshot MM-GBSA values from a minimized pose correlate only loosely with the free energy estimates recovered once a candidate is actually equilibrated over tens to hundreds of nanoseconds, which is the practical argument for treating MD-based refinement as a required filter rather than an optional validation step in this kind of pipeline [19].

Comparison of Free Energy Methods and Reported Binding Energetics Across Cited Studies

Free energy calculation methods applied to KRAS G12D and MRTX1133-class inhibitors in the cited literature, with representative reported binding energetics and the mechanistic question each method addresses.

Method	Typical system / study	Representative reported value or finding	Primary question addressed	Key reference
MM-GBSA / MM-PBSA (end-point)	MRTX1133-KRAS G12D complex, post-MD rescoring	Asp12 salt bridge identified as dominant per-residue contribution; absolute values typically deviate several kcal/mol from experiment	Ranking poses/analogues; decomposing binding hotspots	Genheden & Ryde 2015; Wang et al. 2022
Markov state modeling of unbiased MD ensembles	MRTX1133 binding pathway reconstruction	Two-step mechanism: peripheral engagement followed by rate-limiting switch I closure	Binding kinetics and pathway/intermediate identification	Liang et al. 2023; Tu et al. 2024
Umbrella sampling / metadynamics (enhanced sampling)	Switch I/II conformational transition, GTP-bound G12 mutants	G12D lowers the free energy barrier to a druggable, pocket-open switch conformation relative to several other G12 substitutions	Free energy surface mapping along conformational reaction coordinates	Awasthi et al. 2016; Hu et al. 2026
Alchemical free energy perturbation (FEP/FEP+)	Congeneric ligand series bound to a shared pocket (general RAS/drug-discovery benchmark protocol)	Reported accuracy within ~1 kcal/mol of experiment for well-behaved series; comparatively underused specifically on G12D chemotypes	Relative binding free energy ranking for lead optimization	Wang et al. 2015
Steered molecular dynamics	GDP displacement from KRAS WT/G12C/G12D/G12R/G12V ± MRTX1133	Ligand occupancy of the allosteric pocket alters the force profile required to displace GDP, indicating allosteric coupling	Mechanical/allosteric coupling between ligand site and nucleotide pocket	Alamri et al. 2026

Method	Typical system / study	Representative reported value or finding	Primary question addressed	Key reference
Free energy profile reconstruction along switch-domain coordinates	GTP-bound wild-type vs. G12C/G12D/G12R KRAS	All three oncogenic substitutions raise population of disordered switch conformations relative to wild-type, with substitution-specific well depths	Comparative mutant-vs-wild-type conformational free energy landscape	Chen et al. 2022

(see accompanying table)

Resistance Mutation Dynamics and the Structural Basis of Escape

The MD literature specifically addressing acquired resistance to G12D-directed inhibitors is thinner than the discovery-side literature, which is itself informative: most of what currently exists on resistance mechanisms comes from cellular and biochemical mutagenesis screening rather than from atomistic simulation of the resistant complexes themselves. Site-directed mutagenesis screens across the KRAS(G12D) background have identified a set of secondary substitutions, several clustered around the switch I/II interface and the nucleotide-binding loop, that reduce MRTX1133 sensitivity in cellular assays, and the pattern of hits — concentrated near the ligand contact surface rather than distributed randomly across the protein — is consistent with direct steric or electrostatic disruption of the binding pocket rather than a purely allosteric resistance mechanism, though the mutagenesis data alone cannot distinguish those mechanisms with certainty. This is precisely the kind of question atomistic MD and free energy recalculation on the resistant mutant backgrounds could answer directly, by testing whether the same switch-domain free energy landscape used to rationalize G12D druggability collapses, shifts, or simply becomes sterically incompatible with the existing chemotype once a resistance-associated substitution is introduced — and it is a gap we think the field should prioritize, because right now the mechanistic interpretation of these resistance hits rests more on structural inference from static models than on the dynamic free energy evidence this review has otherwise relied on throughout.

The broader clinical and cell-biological literature on KRAS inhibitor resistance, encompassing both G12C and G12D programs, documents a recurring theme of pathway reactivation — reactivated RTK/SOS1/RAS-GTP signalling, amplification of the mutant allele, or bypass through parallel effector pathways — running alongside a smaller but growing set of on-target secondary mutations of the kind just described. Where MD studies exist for the covalent G12C inhibitor class, they have been more directly applied to characterizing how secondary switch II pocket mutations alter covalent warhead accessibility and pocket electrostatics; the analogous simulation work for noncovalent G12D binders and their resistance mutations is, at the time of this review, still largely unpublished or in early preprint form, which we regard as the single most consequential open question this literature needs to address before MRTX1133-class chemistry can be considered mechanistically as well understood as its G12C covalent counterparts.

Discussion and Future Directions

Reading this body of work end to end, the most striking thing is not disagreement about the basic mechanism — essentially every paper we reviewed converges on the same qualitative story, in which G12D substitution destabilizes the closed switch conformation just enough to make a transient, druggable pocket accessible, and MRTX1133-class chemistry exploits that pocket through an Asp12 salt bridge — but disagreement about the numbers attached to that story. MM-GBSA binding free energies for the same or closely related complexes reported by different groups routinely differ by several kcal/mol, which is not surprising given the force field, water model, ligand parameterization, and trajectory-length differences catalogued above, but it does mean that the field currently lacks anything close to a standardized computational benchmark for this target, in the way that, say, the SAMPL blind prediction challenges have provided for other protein-ligand systems. A community effort to run a common set of MRTX1133-class ligands through several force fields and free energy protocols against a shared starting structure would do more to resolve open questions about method reliability here than another round of single-lab discovery papers.

Enhanced sampling methods — metadynamics and umbrella sampling specifically — remain underused relative to their apparent suitability for this problem. Switch loop opening is exactly the kind of rare-event, multi-well conformational transition these methods were built for, and yet the majority of papers we found still rely on unbiased MD plus Markov state model reconstruction to characterize it, an approach that works but requires an order of magnitude more aggregate sampling to achieve comparable statistical confidence in the resulting free energy estimates. We would also flag alchemical FEP as underused specifically for backup-series optimization around the MRTX1133 salt bridge scaffold, where the well-defined, largely rigid anchor interaction is close to the ideal use case the method was validated against.

The resistance-mutation gap identified above deserves to be treated as a priority rather than a footnote. Given how quickly acquired resistance emerged clinically for the covalent G12C inhibitors, it would be surprising if noncovalent G12D chemistry proved immune to the same evolutionary pressure once these compounds see broader clinical use, and the field currently has very little atomistic simulation data to say in advance which resistance substitutions will most severely disrupt binding versus which might be tolerated by second-generation chemotypes still under development.

Finally, essentially all of the simulation work reviewed here treats KRAS as a soluble catalytic domain in isolation, despite the fact that the protein's biology, and arguably some of its conformational preferences, are shaped by membrane anchoring through the C-terminal hypervariable region. Membrane-embedded simulations of oncogenic Ras have shown that lipid contact can bias orientation and, in principle, switch-region accessibility, but essentially none of the current MRTX1133-focused free energy literature incorporates a membrane environment. Whether that omission meaningfully changes the free energy landscape relevant to inhibitor binding, or whether the soluble-domain approximation is adequate for this particular question, is itself an open methodological question this literature has not yet addressed directly.

Conclusion

The molecular dynamics and free energy literature built up around MRTX1133 and its relatives over the past four years has done real explanatory work: it has turned "this molecule forms a salt bridge with Asp12" from a static structural observation into a dynamic, quantitative account of switch I/II conformational behaviour, pocket formation kinetics, and binding energetics, using tools ranging from cheap MM-GBSA rescoring through Markov state modelling to steered and enhanced-sampling simulation. That account is reasonably convergent on the qualitative mechanism and considerably less convergent on the numbers, and it remains substantially thinner on the resistance-mutation side than the discovery side. Closing those two gaps — standardizing free energy protocols enough to make cross-study comparison meaningful, and extending the same dynamic, energetic reasoning that explained G12D druggability to the mutations most likely to defeat it — looks like the most useful direction for this specific subfield over the next several years.

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