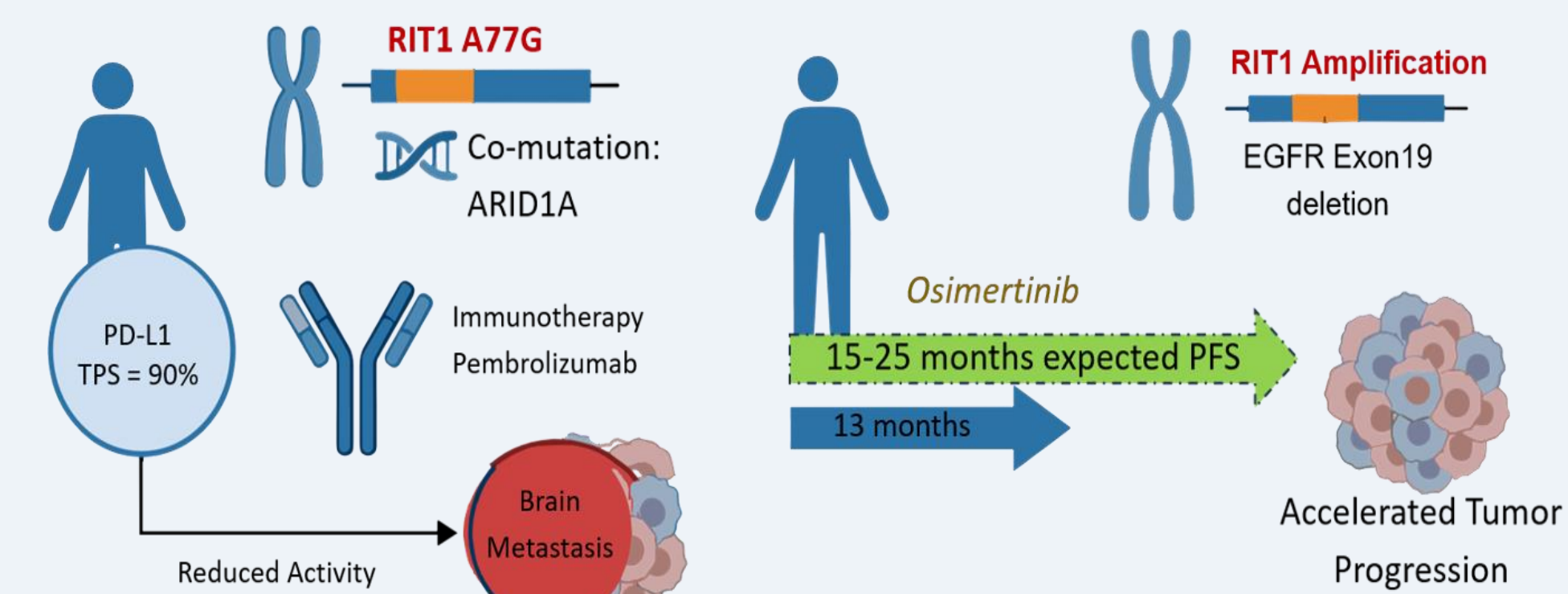


Introduction

- RIT1 (Ras-like without CAAX) is a RAS-family small GTPase.
- RIT1 is amplified in ~14% of NSCLC cases, while mutations occur in ~2% of cases and mutually exclusive with EGFR/KRAS/ALK genomic aberrations.
- Brisbane cases reported with RIT1-related treatment resistance.



- Unlike KRAS, RIT1 has no direct small-molecule inhibitors.

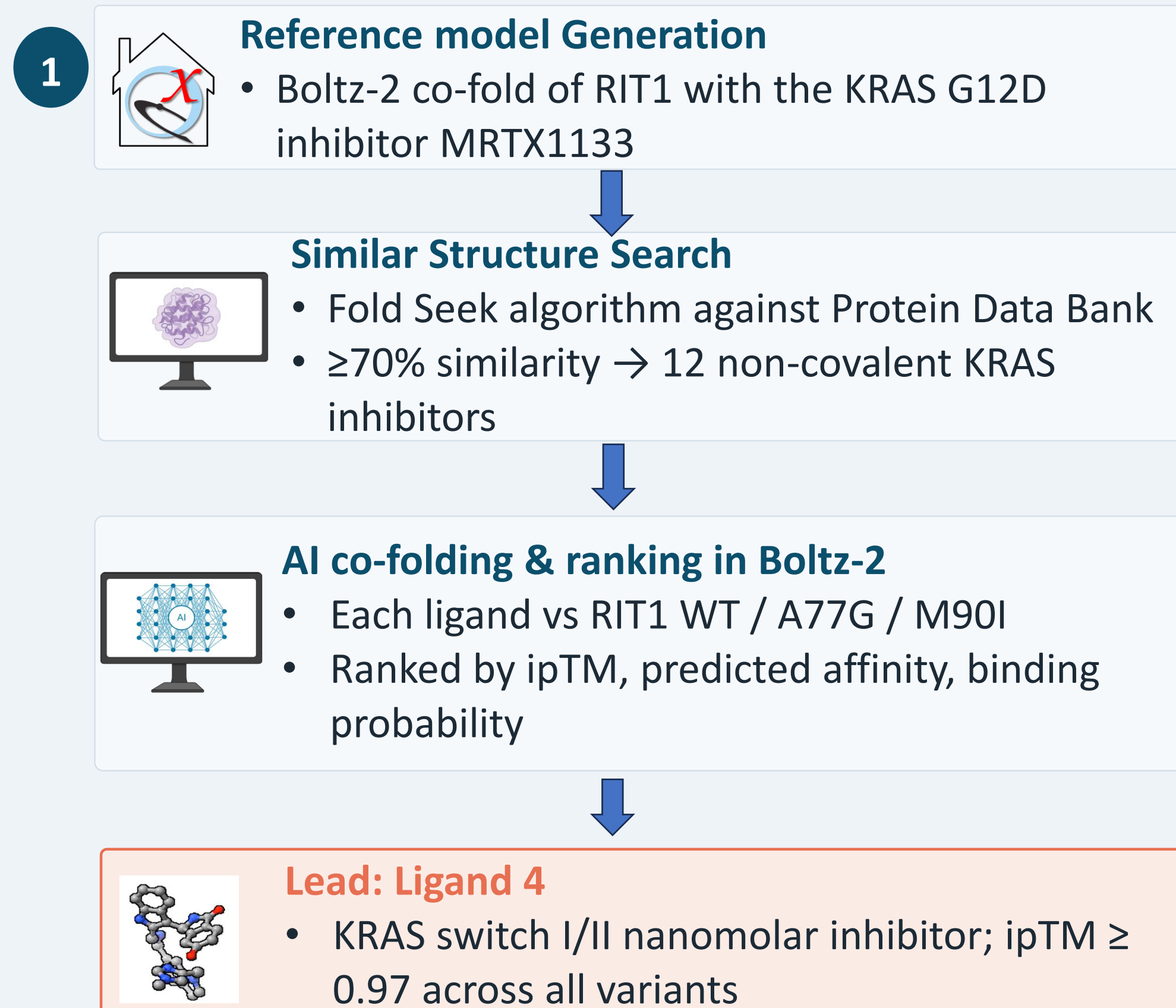


KRAS (wheat) on RIT1 (rainbow) ground our repurposing rationale to target RIT1.

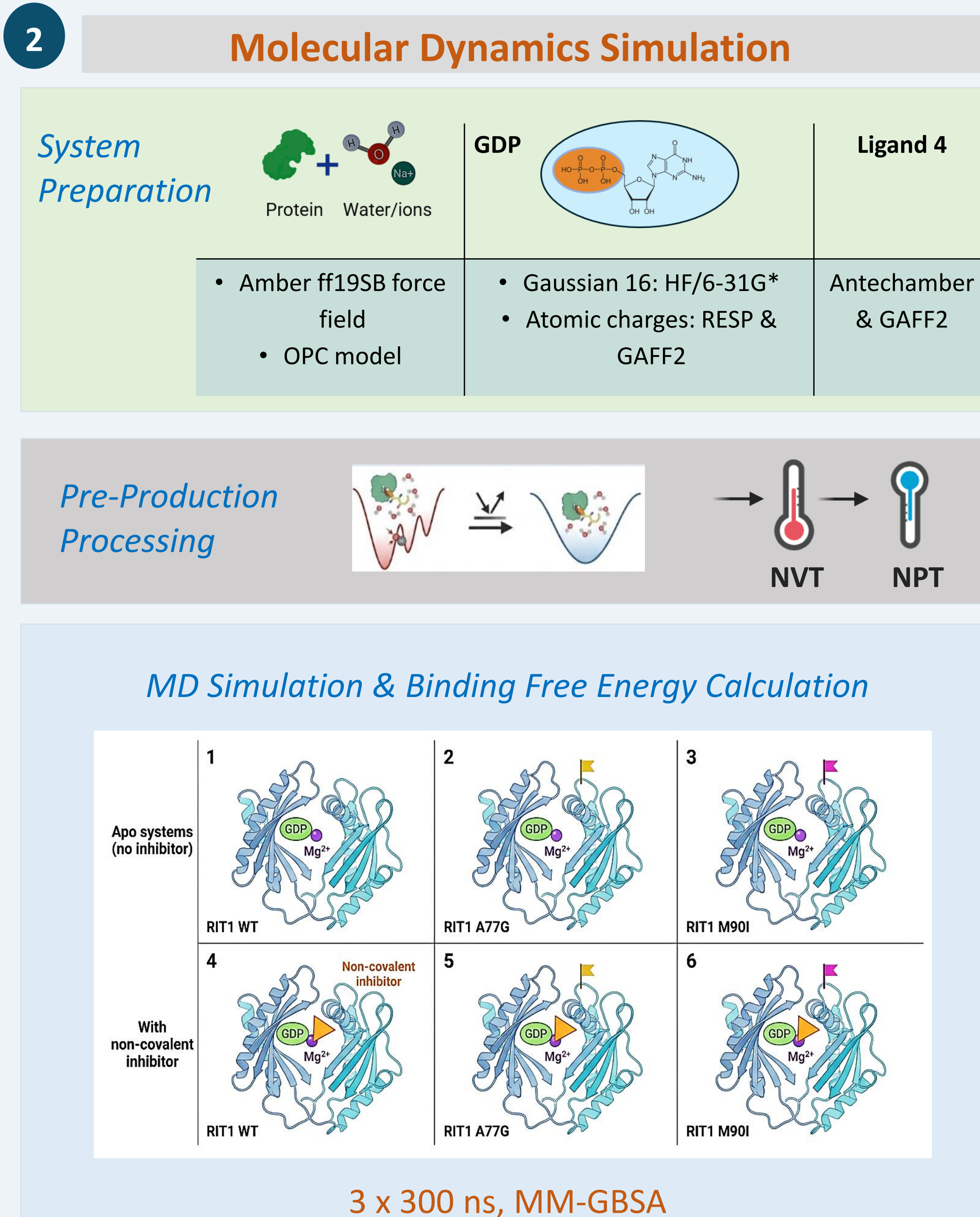
Aims

- To explore how RIT1 switch mutations impact protein dynamics.
- To identify druggable pockets in RIT1 and investigate whether KRAS inhibitors can be computationally repurposed as candidate RIT1 binders.

Methods



Methods



Results

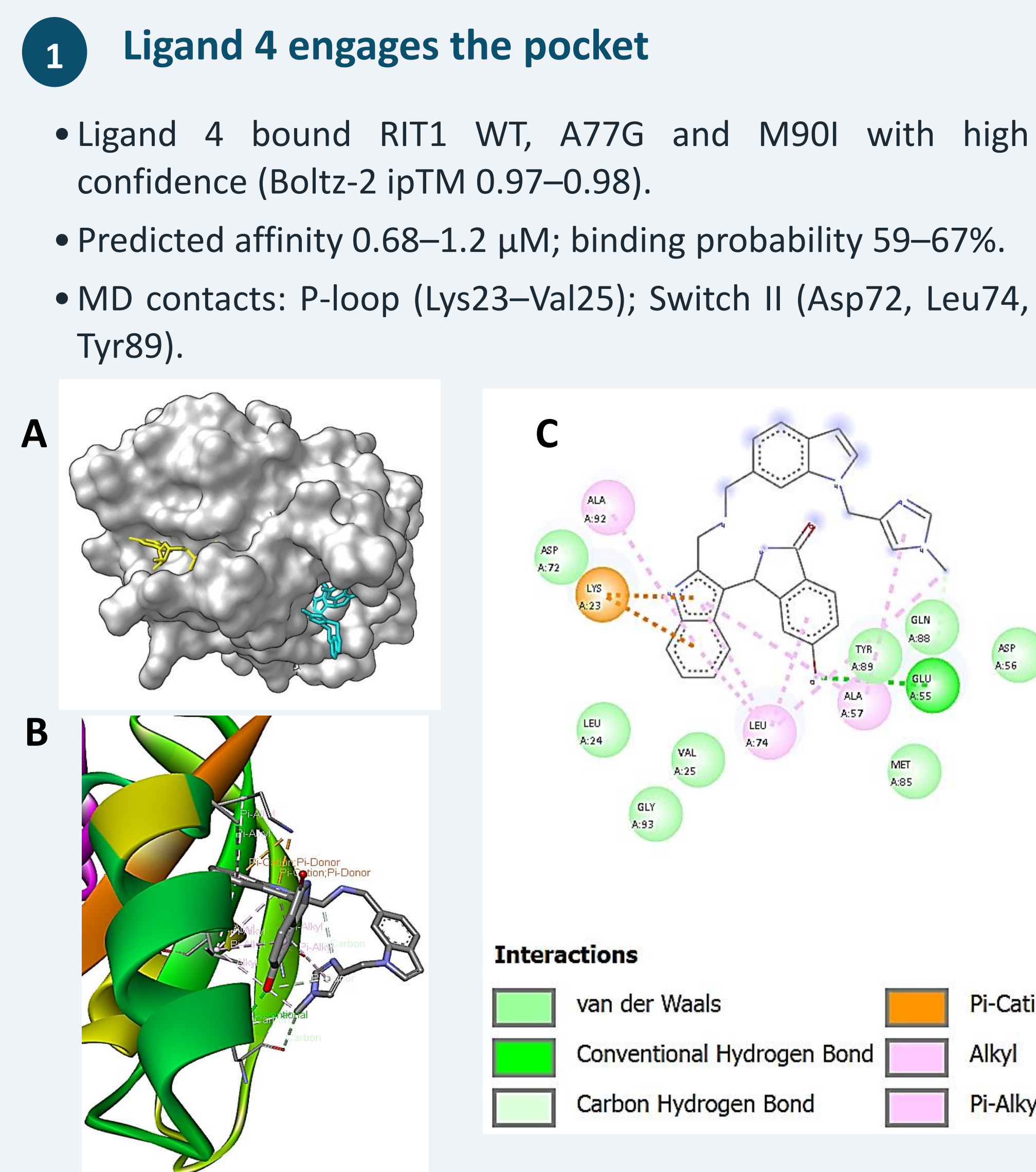


Fig 1: Ligand 4 bound to RIT1 Wild-type. (A) Surface (Ligand 4 cyan, GDP yellow); (B) 3D site, key residues; (C) 2D interaction map.

Results

2 Binding energetics (MM-GBSA)

- Binding free energy ≈ -40 kcal·mol⁻¹ for all three variants.
- Van der Waals-dominated, stable across replicates.
- Top ΔG drivers: Leu74, Tyr89, Lys23.

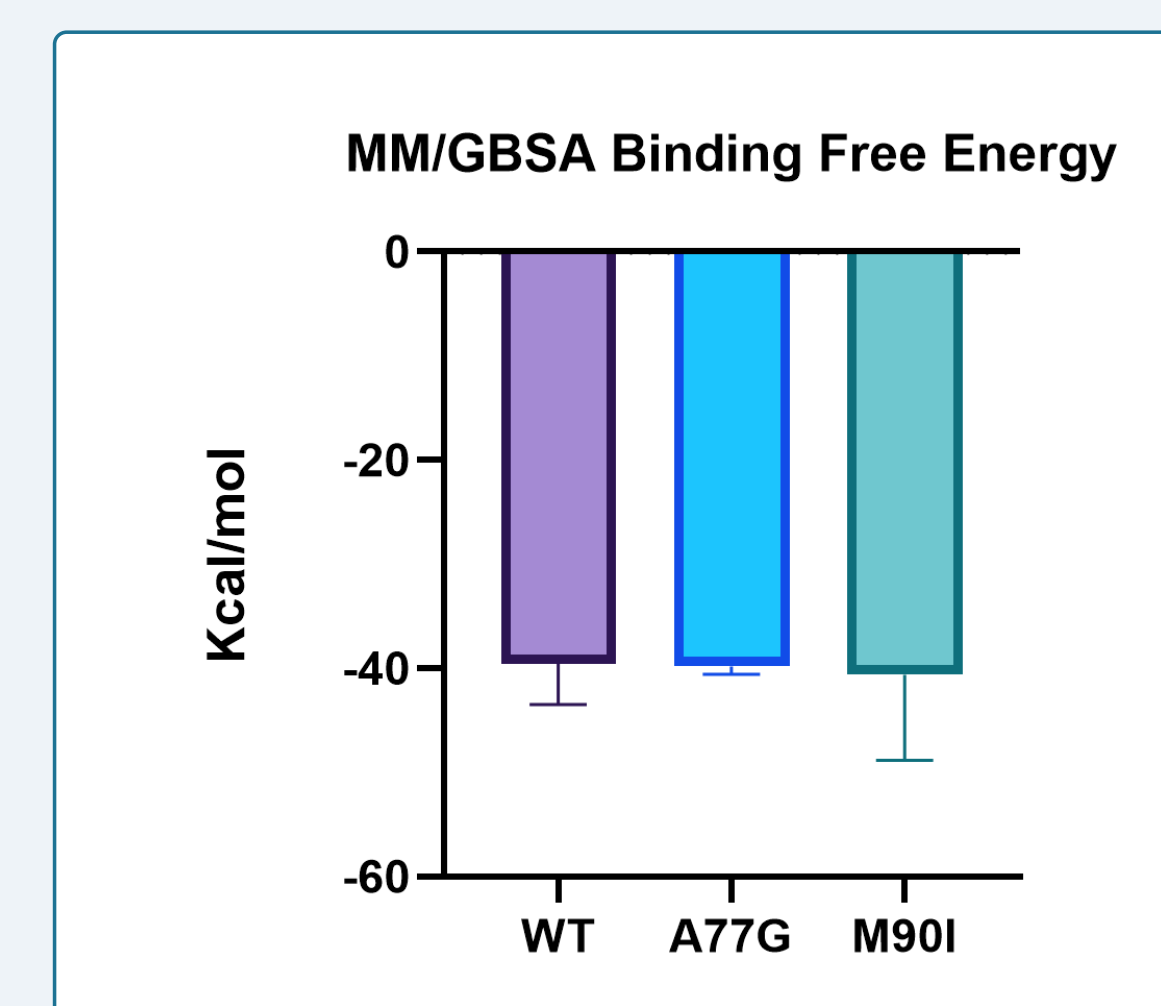


Fig 2: Binding Free Energy Calculated by MM/GBSA

3 Mutations reshape dynamics (apo)

- Ligand-free MD reveals mutation-specific flexibility.
- WT most flexible; M90I rigidifies both switch regions; A77G intermediate.

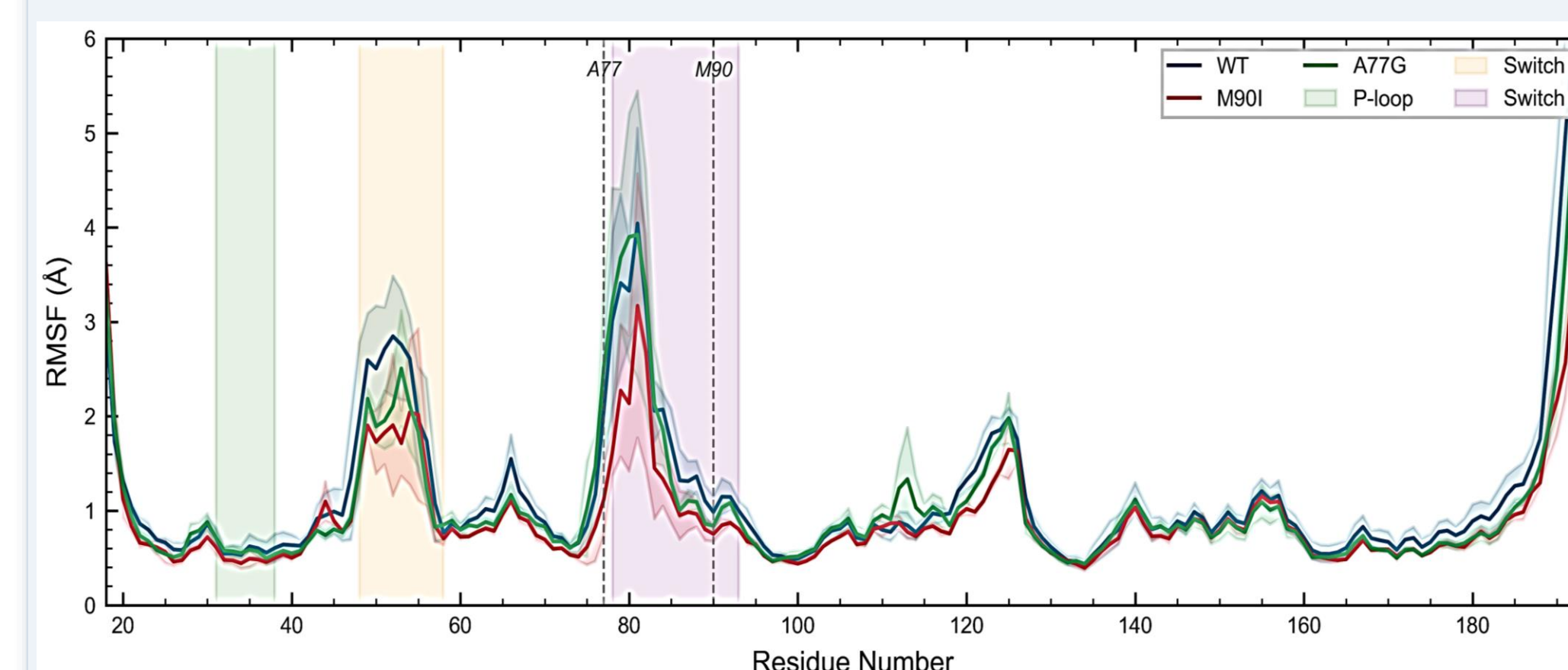


Fig 3a : RMSF (apo). Structural stability and overall conformational changes within RIT1 WT (blue), M90I (red) and A77G (green) mutants

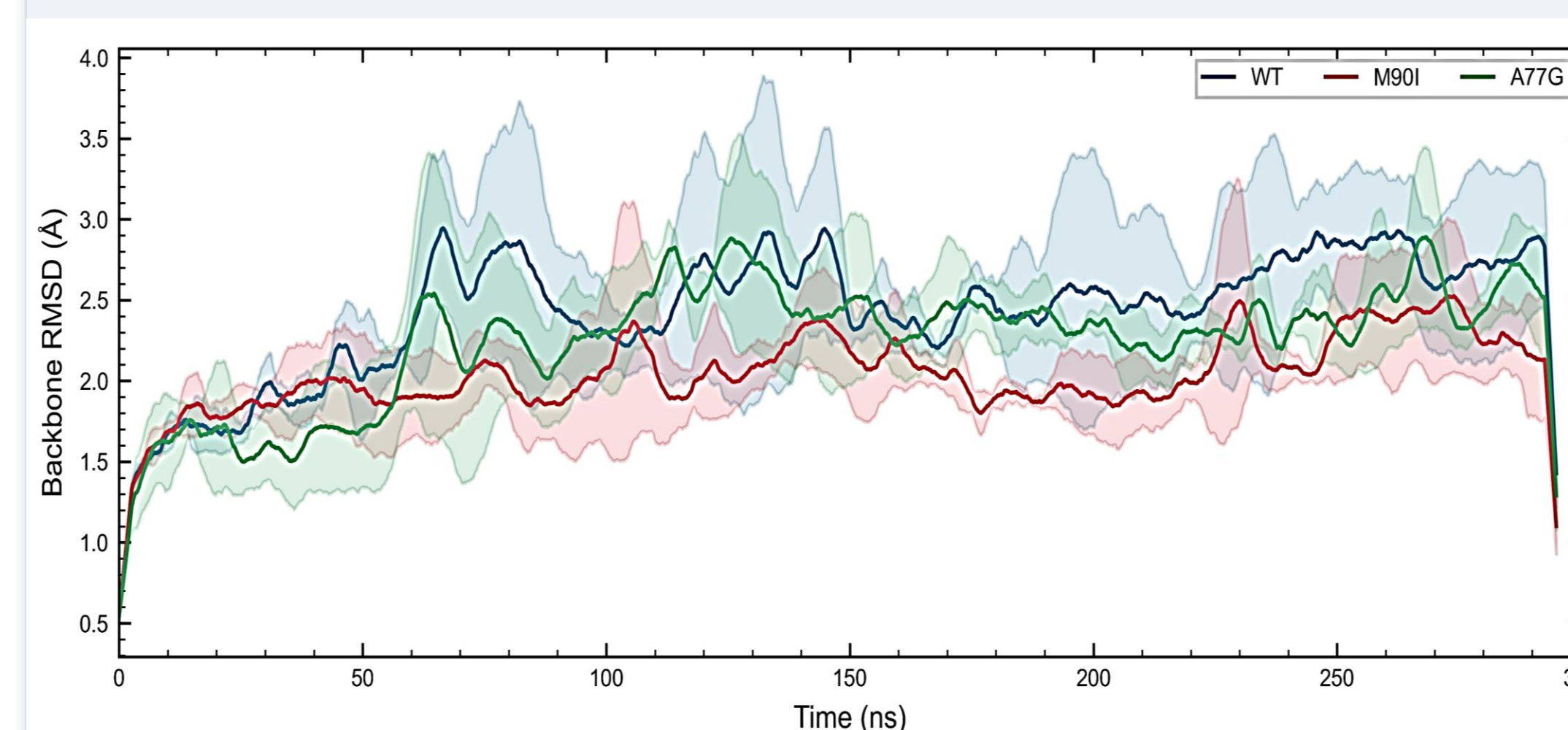


Fig 3b: RMSD (apo). Local flexibility and internal mobility of RIT1 WT (blue), M90I (red) and A77G (green) mutants.

Results

4 Convergence & engagement

- Ligand 4 reduced flexibility and converged the three variants toward a common bound state.

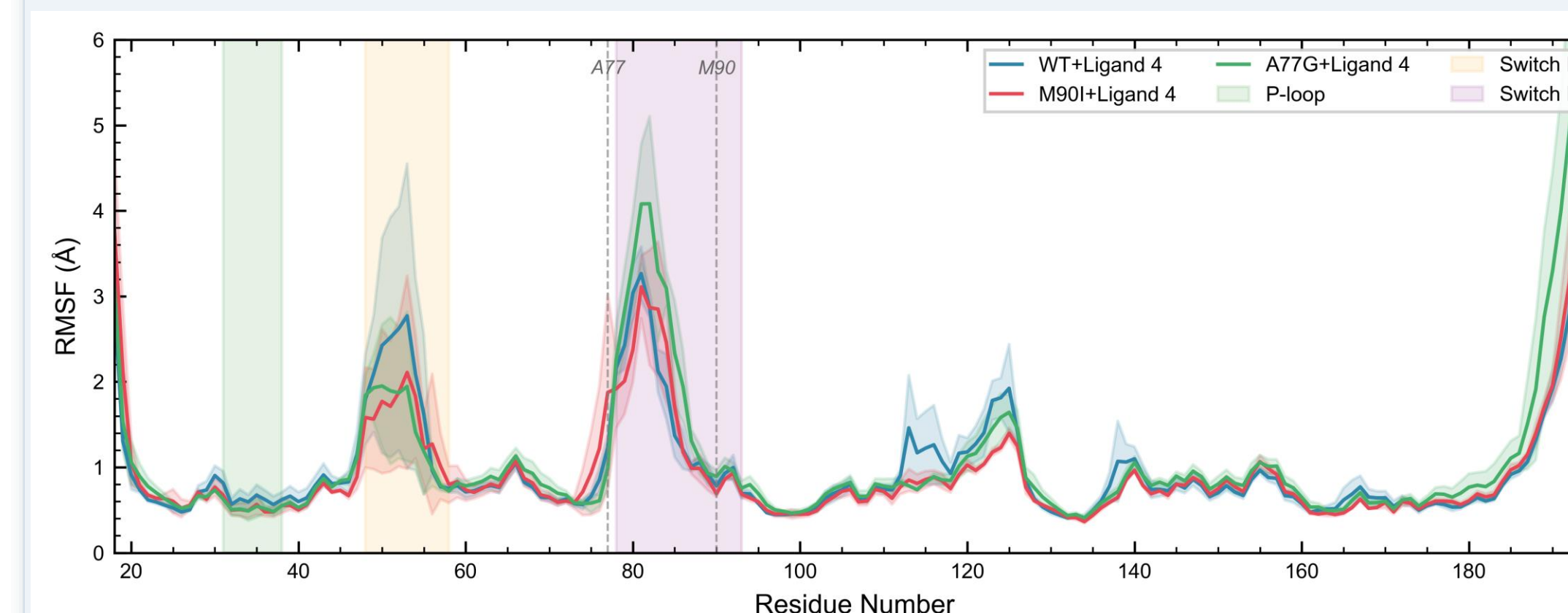


Fig 4a: RMSF (Ligand 4 bound)

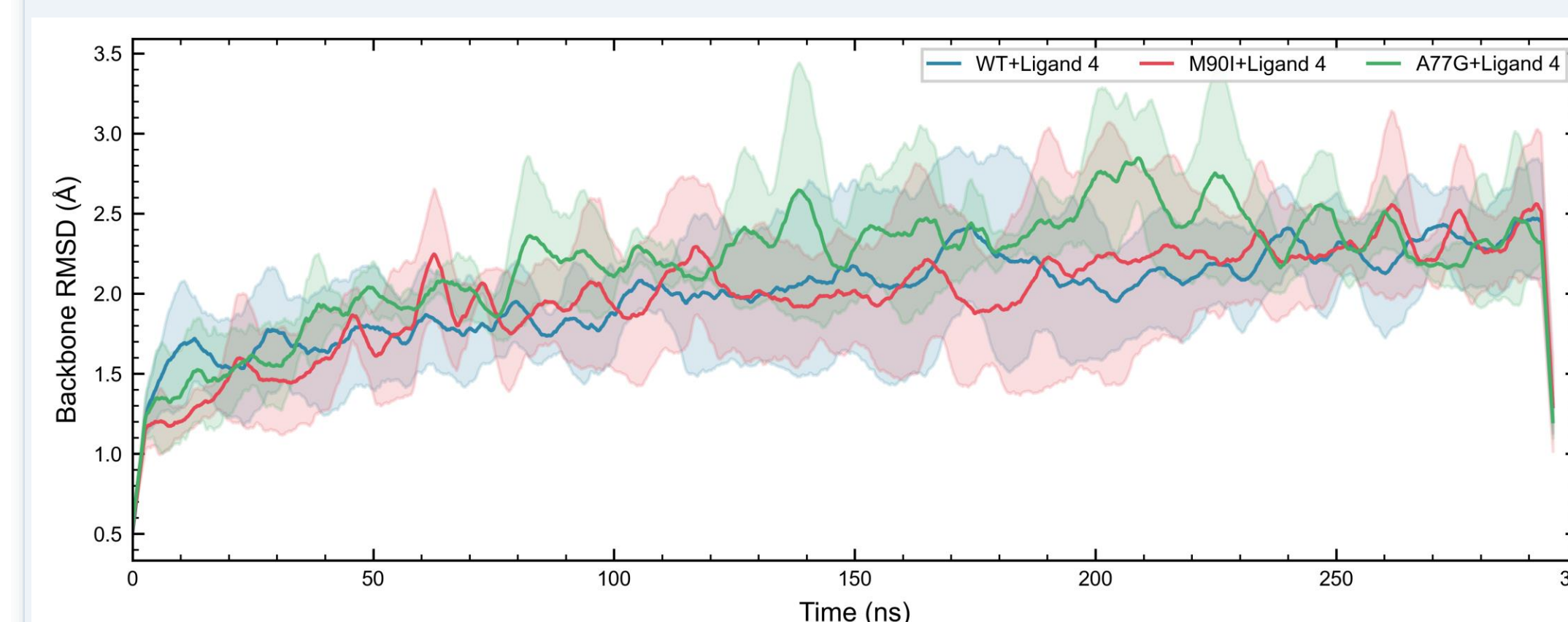


Fig 4b: RMSD (Ligand 4 bound)

A77G - incomplete engagement with Ligand 4 and retains Switch II flexibility when bound, unlike RIT1 WT and M90I.

5 Cellular validation

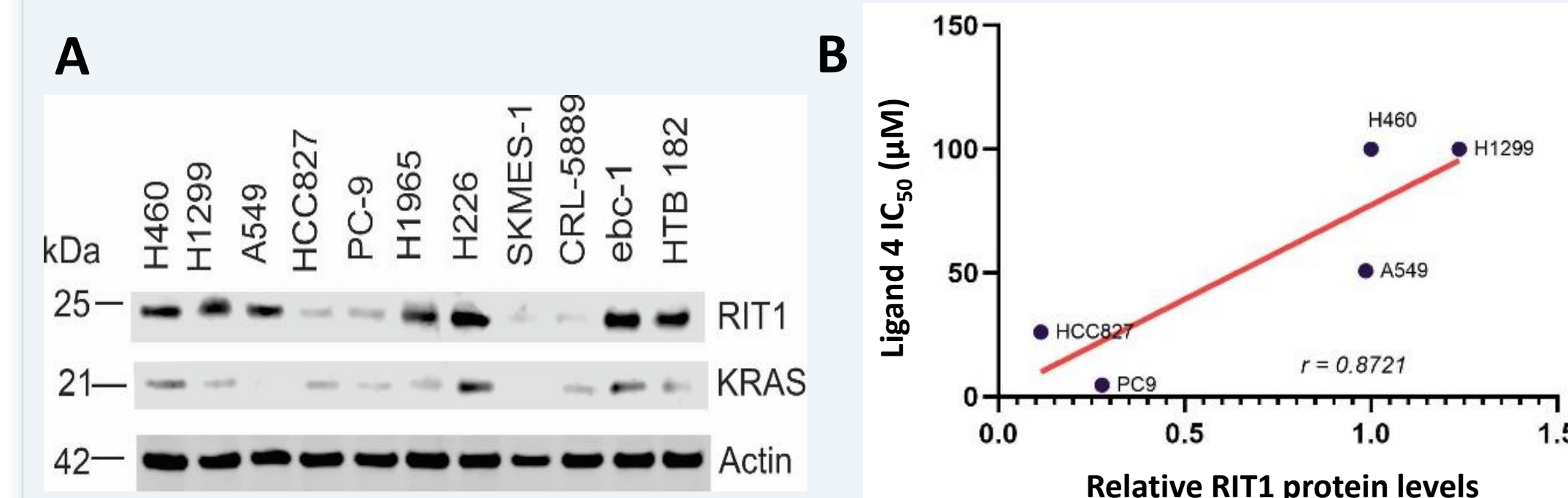


Fig 5: Ligand 4 sensitivity vs endogenous RIT1. (A) RIT1 and KRAS western blot analysis across NSCLC lines (actin control). (B) Correlation of RIT1 protein with Ligand 4 sensitivity ($r = 0.87$).

Conclusion and Future Plan

- Pairing AI structure prediction (Boltz-2) with all-atom MD nominated Ligand 4 as a binder for the undrugged GTPase RIT1.
- Ligand 4 engages the GDP-bound pocket across WT and mutants ($\Delta G \approx -40$ kcal/mol); A77G shows incomplete engagement.
- A starting point for structure-guided RIT1 inhibitor design.
- Next: pharmacophore & large-library (ZINC) screening; refined MD; cytotoxicity assays.

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