



Discovery of a benzo-fused macrolactone dual IDO1/TDO2 inhibitor from *Asparagus nelsii* via integrative Cheminformatics and Molecular Dynamics Simulations

Simeon I Ambuga^a, Lamech Mwapagha^b, Marius K Mutorwa^b

^{a,b} Faculty of Health, Natural Resources and Applied Sciences, Namibia University of Science and Technology, Namibia.

CONTACT

Simeon I Ambuga
Department of Biology, Chemistry, Physics
Email: sambuga@nust.na
Phone: +264 81 379 0509



ABSTRACT

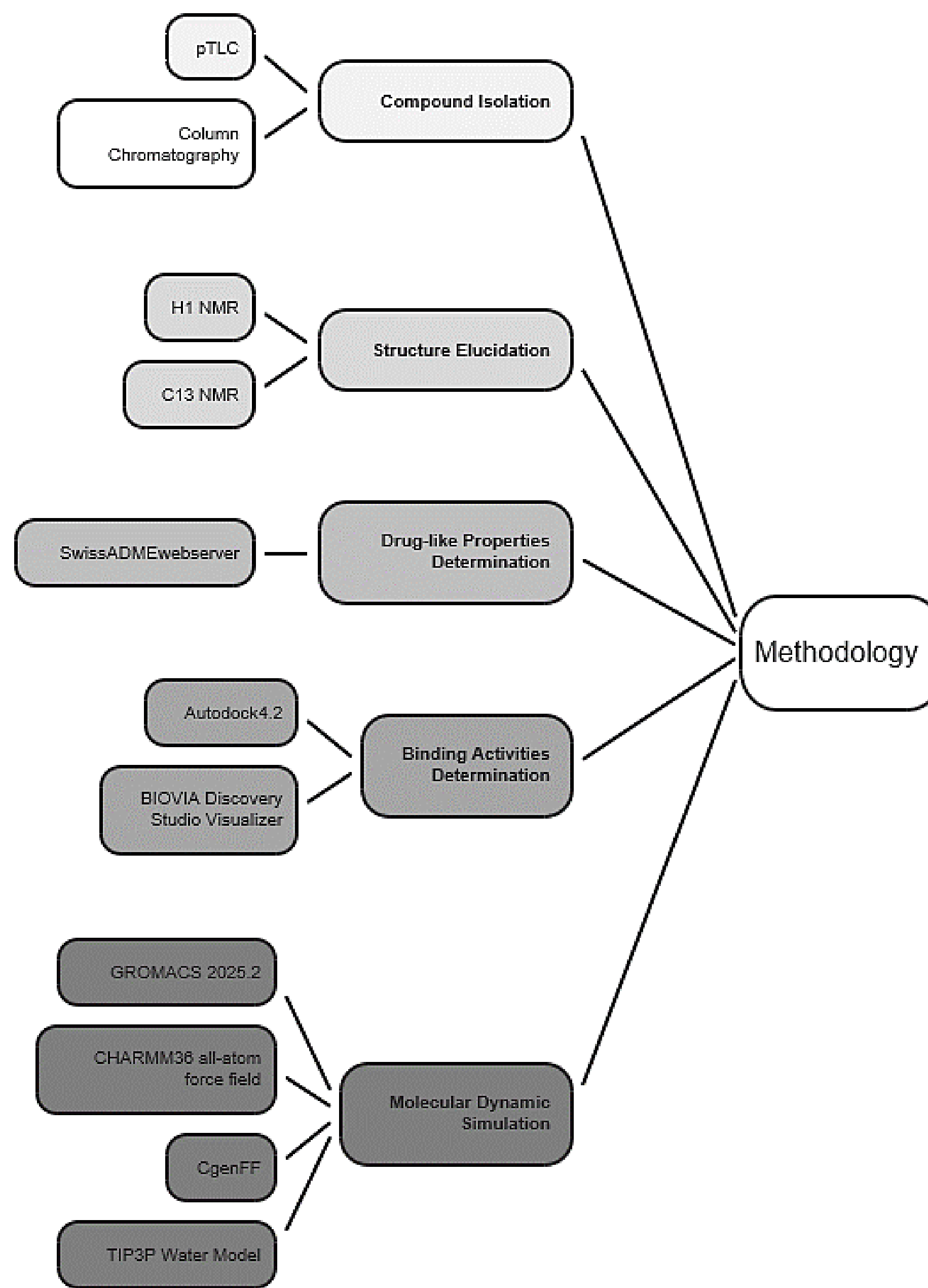
The identification of bioactive natural products remains a cornerstone of medicinal chemistry. In this study, compounds isolated from the roots of *Asparagus nelsii*, a plant endemic to Southern Africa, were evaluated for their potential as dual inhibitors of indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase 2 (TDO2). Their inhibition represents a promising chemical strategy to modulate immune suppression pathways associated with cancer progression. The isolated compound, a benzo-fused macrolactone, which displayed favourable drug-like properties, was subjected to molecular docking using AutoDock against the holo forms of IDO1 (PDB ID: 2D0T) and TDO2 (PDB ID: 8R5R). The compound exhibited strong predicted binding affinities to both. Key interactions included hydrogen bonds, and π - π stacking. All atom molecular dynamics simulations (100 ns, CHARMM36 force field, GROMACS) demonstrated stable binding, with backbone RMSD < 2.0 Å and sustained active site contacts (occupancy > 80%).

INTRODUCTION

Plants remain a prolific but under-explored source of biologically active compounds, with only ~15% of higher plants screened for bioactivity despite widespread traditional use. Members of the genus *Asparagus* produce diverse secondary metabolites with antioxidant, anti-inflammatory and cytotoxic activities; several species (e.g. *A. racemosus*, *A. officinalis*) have shown apoptosis induction and growth inhibition across multiple cancer cell lines.

IDO1 expression was to be significantly upregulated in various cancers, including melanoma, lung cancer, pancreatic cancer, and breast carcinoma. The correlation between elevated IDO1/TDO2 expression and poor clinical outcomes across multiple cancer types highlighted the therapeutic potential of targeting this pathway, spurring extensive drug discovery efforts in both academic and pharmaceutical settings.

METHODOLOGY



RESULTS AND DISCUSSION

1. Drug likeness

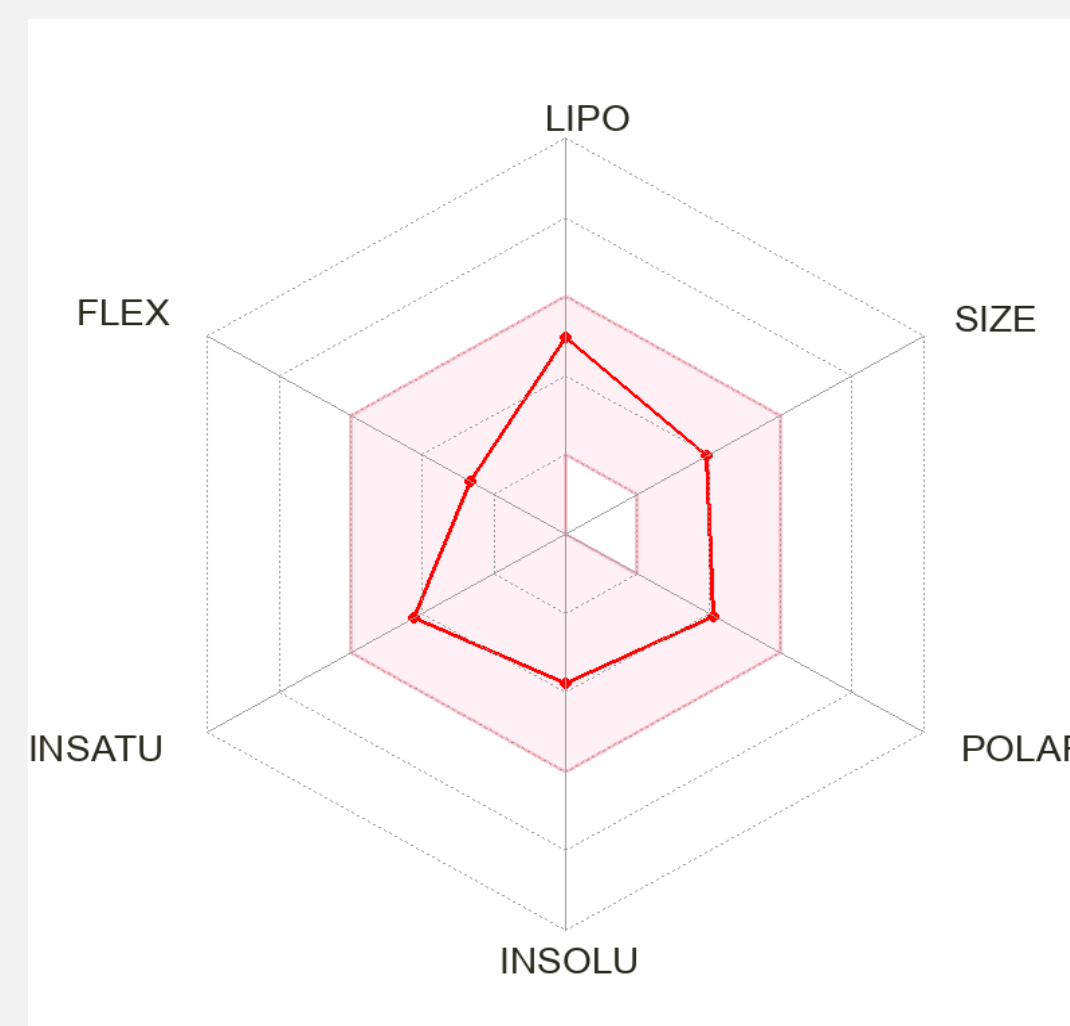
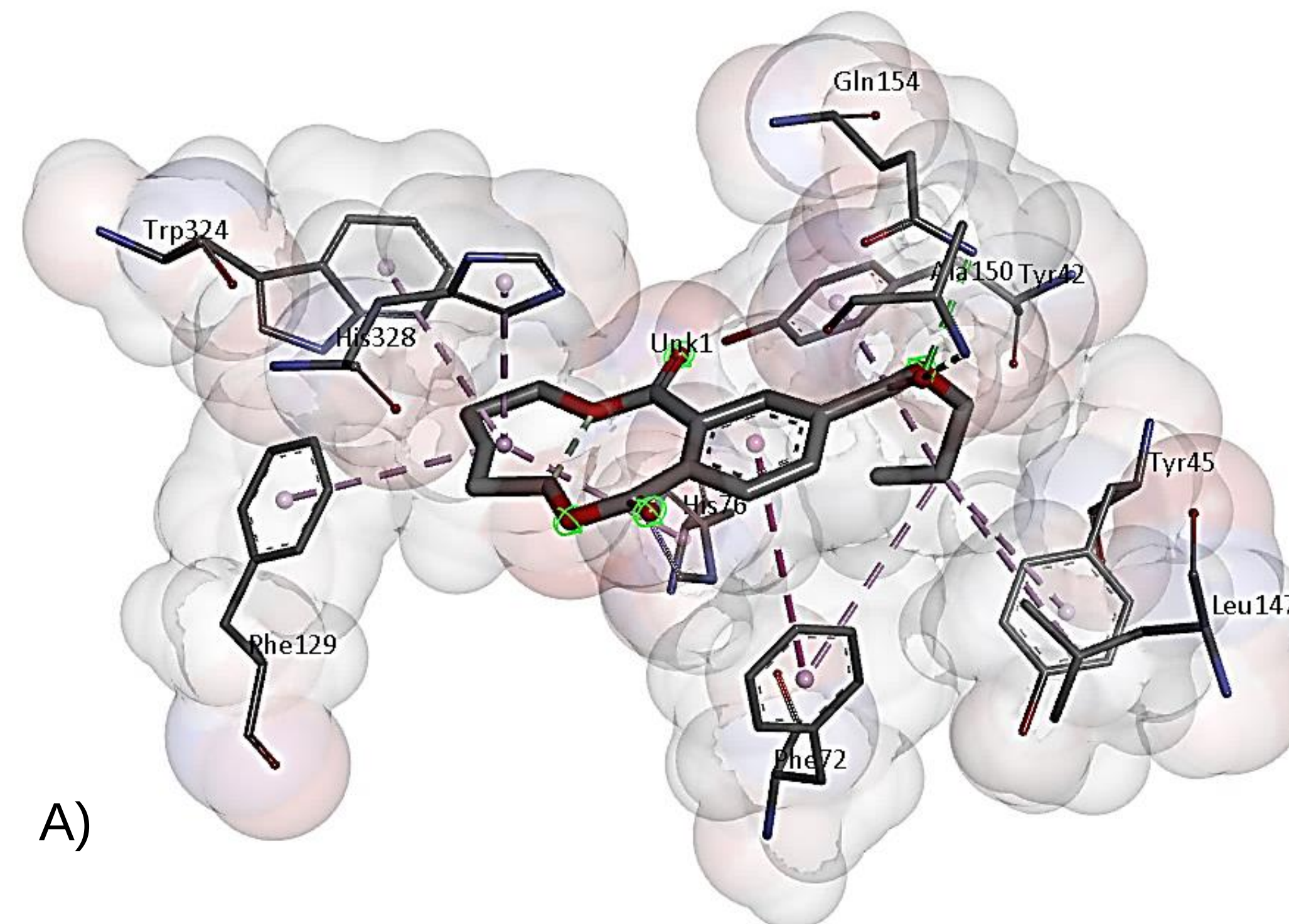


Figure 1: Bioavailability radar displaying drug-likeness compared to the optimal range

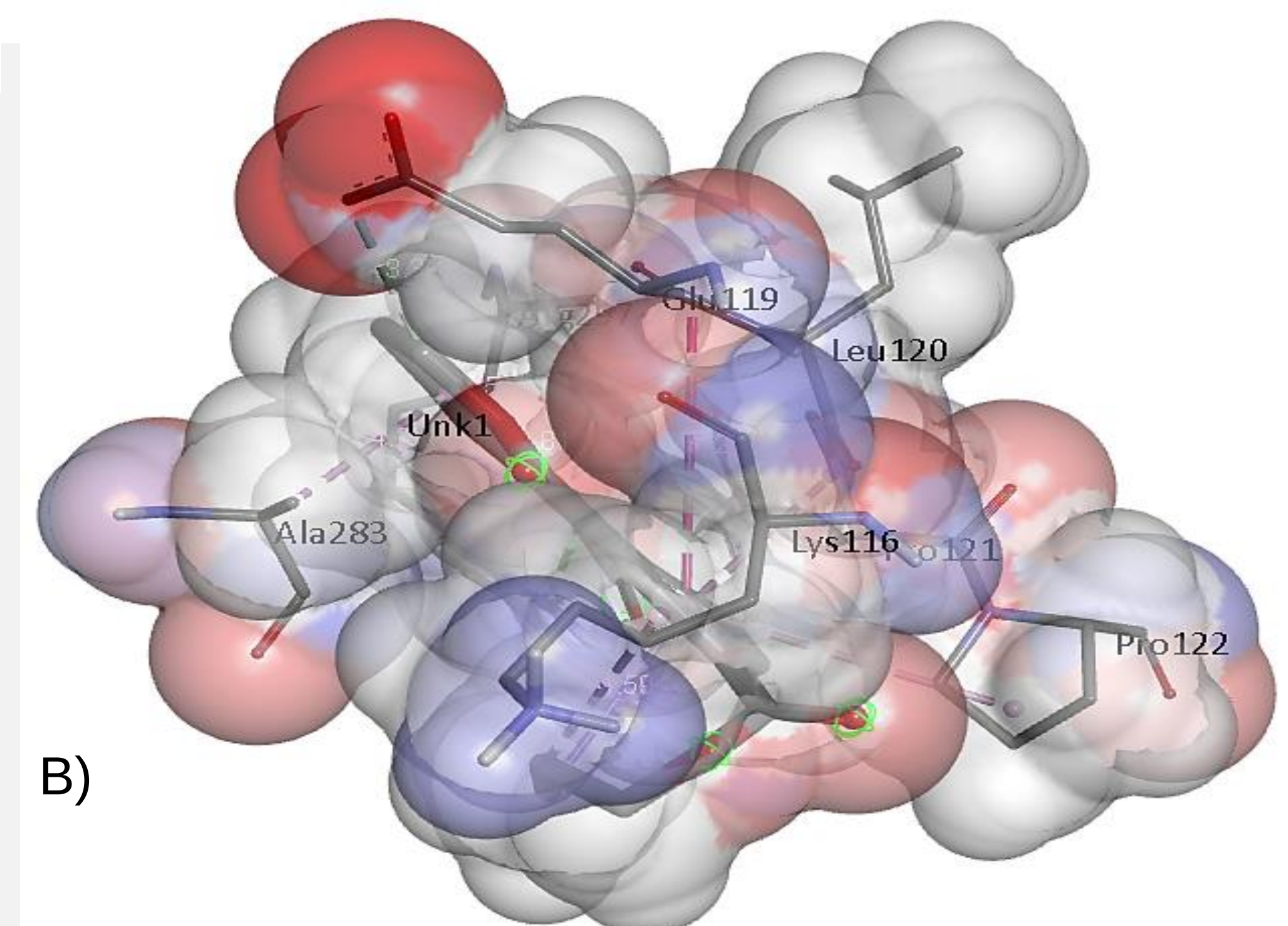
2. Molecular docking

Table 1: Binding affinities of the compounds from *A. nelsii* extract with targeted proteins

	ΔG (kcal/mol)	Ki value (μ mol)	Interactions
TDO2 (PDB ID: 8r5r)	-9.62	0.0892	HB, Alkyl, Pi-Pi T-shaped
IDO1 (PDB ID: 2d0t)	-10.31	0.0278	HB, VDW, Pi-Pi stack, Alkyl



A)



B)

Figure 2: A) Interactions in binding pocket of compound with TDO2. B) Interactions in binding pocket of compound with IDO1.

- IDO1 ($\Delta G = -10.31$ kcal·mol⁻¹) is predicted to bind more tightly than TDO2 ($\Delta G = -9.62$ kcal·mol⁻¹), although both of them show relatively good binding energies.
- IDO1: docking suggests hydrogen bonding, van der Waals packing, a π - π stacking interaction and alkyl contacts. The combination of directional H-bonds plus aromatic stacking is typical of a stable, specific pose and supports a favorable enthalpic contribution to binding.
- TDO2: predicted interactions include hydrogen bonds, alkyl contacts and a π - π T-shaped contact.
- Both targets show chemically sensible poses: polar groups form H-bonds while hydrophobic/alkyl surfaces make complementary van der Waals contacts. The docking scores indicate both ligands are likely to bind, with IDO1 predicted as the stronger binder.

3. Molecular dynamic simulations

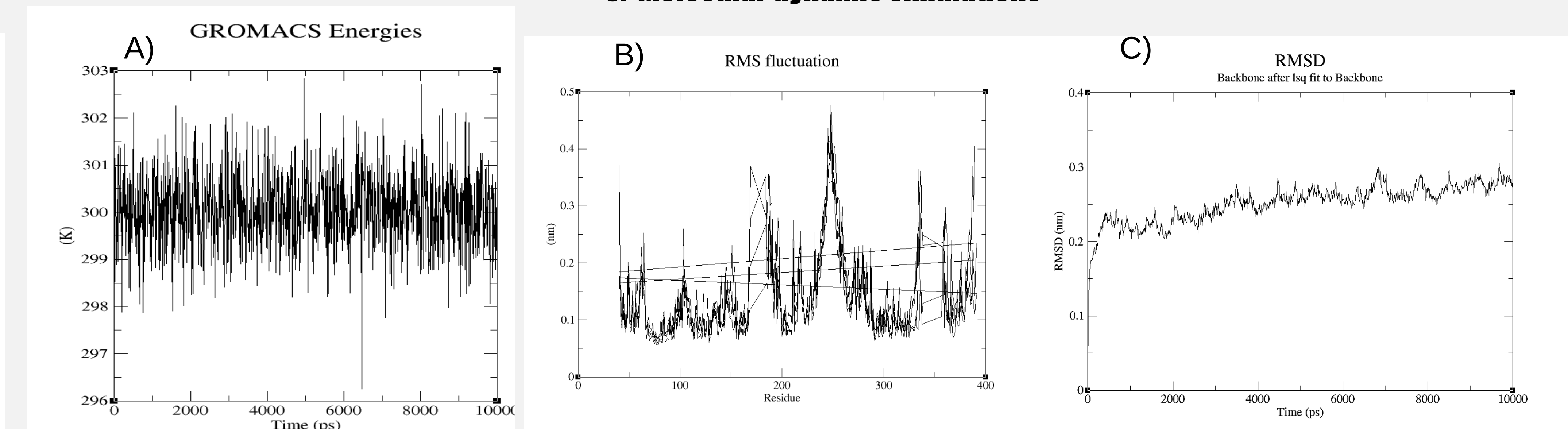


Figure 3: The RMSD, RMSF and temperature visualisation of the target protein-ligand complexes (TDO2 in complex with ligand. A) Temperature plot, B) RMSF plot, C) RMSD plot.

- The pressure fluctuates widely (from roughly -300 to +350 bar) around the 0-1 bar region, with no obvious long-term trend. Crucially, there is no sustained drift.
- The temperature plot shows that the system's kinetic temperature remained tightly clustered around the 300 K set-point.
- The backbone RMSD appears to approach a plateau (~0.28-0.30 nm) by ~10 ns, consistent with reaching a pseudo-equilibrium. This is similar to reported cases where well-folded proteins stabilize around 0.3 nm after ~10 ns of MD.
- The majority of residues fluctuate <0.3 nm (3 Å) which is typical for a stable protein core.
- Most hydrogen bonds fall well below the standard cutoff (≤ 0.35 nm) used to define an H-bond, indicating predominantly strong, well-defined bonding. The prominent peak near 0.27 nm corresponds to particularly strong, nearly linear hydrogen bonds

CONCLUSION

Docking predicts a sensible binding geometry for the ligand in both enzymes, with IDO1 showing the stronger predicted affinity ($\Delta G = -10.31$ kcal·mol⁻¹ vs -9.62 kcal·mol⁻¹ for TDO2). The predicted interaction patterns (H-bonds + aromatic packing + hydrophobic contacts) are exactly the kind that stabilize ligand binding. The 10 ns explicit-solvent MD run supports the docking hypothesis. Together these indicate the docked pose is dynamically stable on the 10 ns timescale. Combining docking and short MD increases confidence that the ligand binds in the predicted pose and that IDO1 is the more promising target computationally. However, docking scores are approximate and 10 ns of MD is modest sampling; thus these results are supportive but not definitive and need to be supported by biological tests *in vitro* and *in vivo* to help support SDG 3.