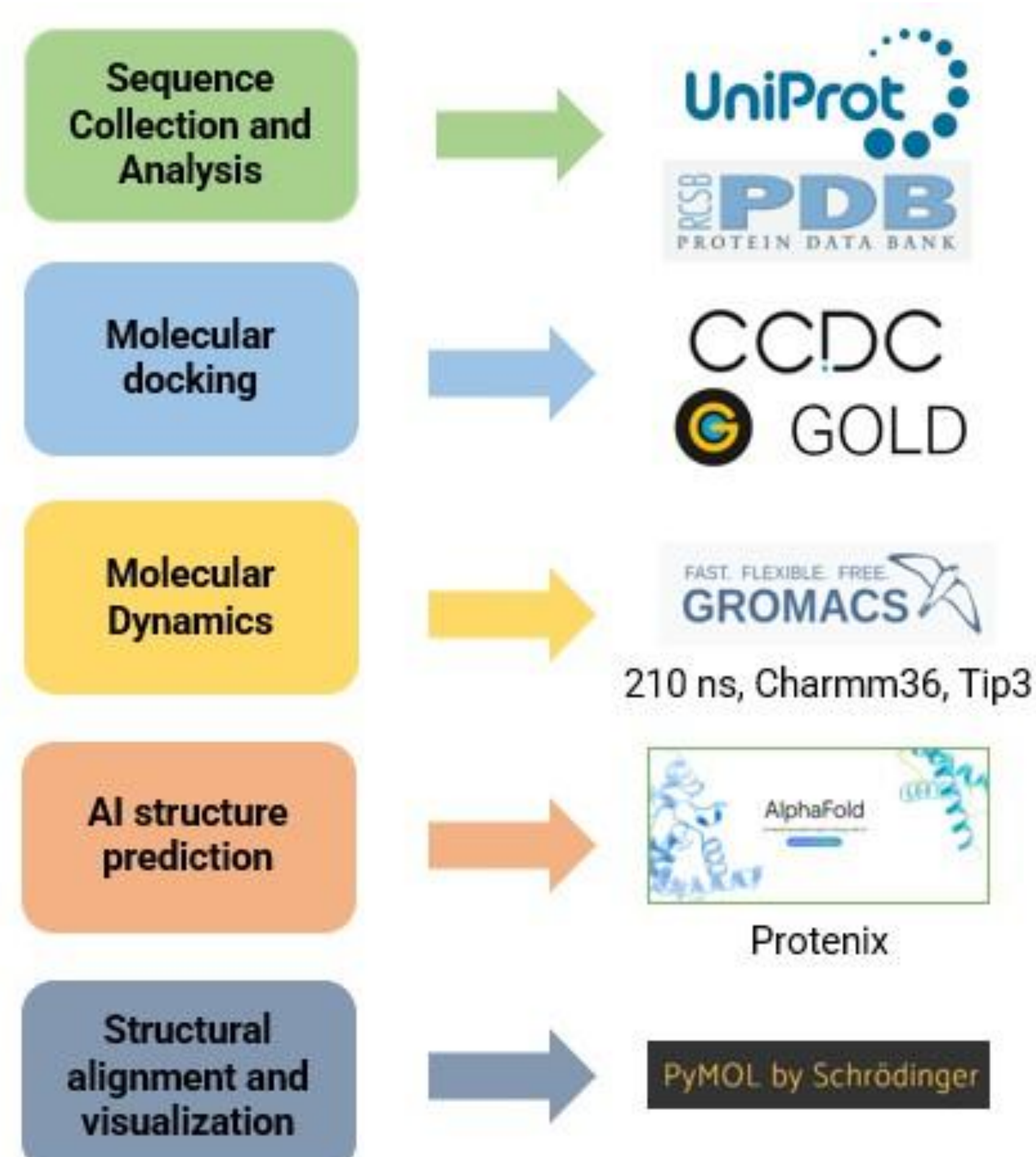


INTRODUCTION

Microtubules (MT) are major components of the eukaryotic cytoskeleton, found in all eukaryotic cells and involved in mitosis, cell motility, intracellular transport, and the maintenance of cell shape. MTs are composed of α - and β -tubulin subunits assembled into linear protofilaments. By targeting plant α -tubulin, derivatives of 2,6-dinitroanilines (e.g., trifluralin, ethalfluralin, pendimethalin, oryzalin, etc.) disrupt microtubule assembly, inhibiting cell division and growth.

In addition to their use as herbicides, dinitroanilines show promise as antiprotozoal agents for treating parasites such as *Toxoplasma gondii*, *Trypanosoma cruzi*, *Leishmania major* and *Plasmodium falciparum*. Since these compounds do not bind to vertebrate tubulin, they exhibit low toxicity to humans, paving the way for the development of safe and effective next-generation antimalarial drugs. In light of new data on the mechanism of ligand binding in *T. gondii*, the objective of this work is a comprehensive *in silico* study of dinitroaniline compound binding with *Plasmodium* α -tubulins across different species and ecotypes.

METHODS



RESULTS

Several models for dinitroaniline binding to tubulin have been proposed over the last decades. However, none of these models adequately explained all known resistance mutations. A new ligand-induced dinitroaniline binding model, similar to that of pironetin (PDB: 5FNV and 5LA6), was recently proposed for *T. gondii* by Aguayo-Ortiz and Dominguez (2022) and reasonably explains the basis of the main dinitroaniline resistance mutations.

The interaction with Apicomplexan α -tubulin and its absence in vertebrates render dinitroanilines promising antiprotozoal candidates. Consequently, the challenge of this work was to study their binding to *Plasmodium* α -tubulins across different species and ecotypes.

Based on data from UniProtKB and the RCSB Protein Data Bank, 101 full-length α -tubulin amino acid sequences of *Plasmodium* spp. were selected. The key amino acid residues of the binding site "pocket" were identified based on structural modelling, multiple alignment of sequences and structures.

RESULTS

The clustering of selected α -tubulins resulted in 37 unique sequences, while the analysis of the binding site revealed three variations in the ligand-binding pocket (Figure 1). These variations in site determinate three types of tubulin variants that were selected for structural modeling and protein–ligand binding analysis.

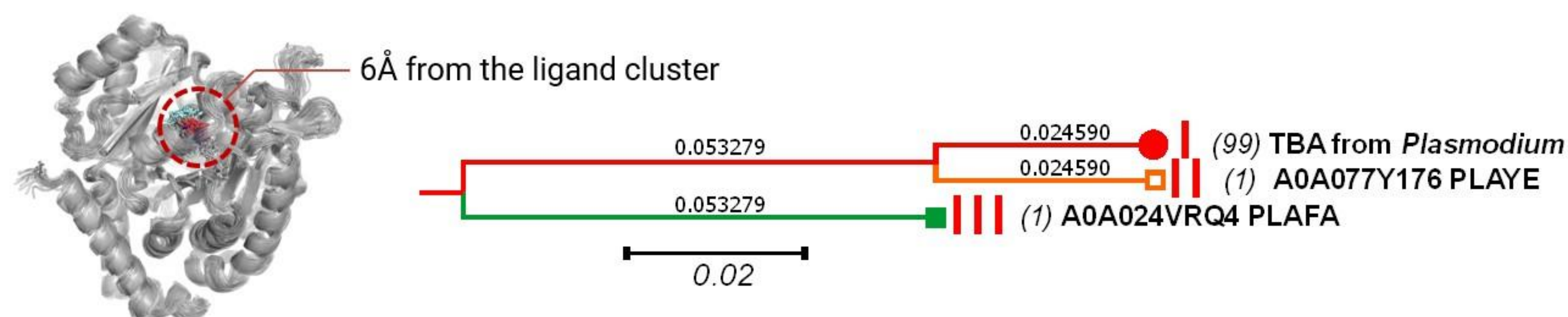


Figure 1. Three representative types of *Plasmodium* α -tubulin, that were specified based on a.a. variations of the binding site, and selected for structural modelling.

Using AlphaFold3-based Protenix service, the complexes of three *Plasmodium* α -tubulin variants with dinitroanilines (trifluralin, oryzalin, pendimethalin, etalfluralin) and amyprophos-methyl were predicted (Figure 2).

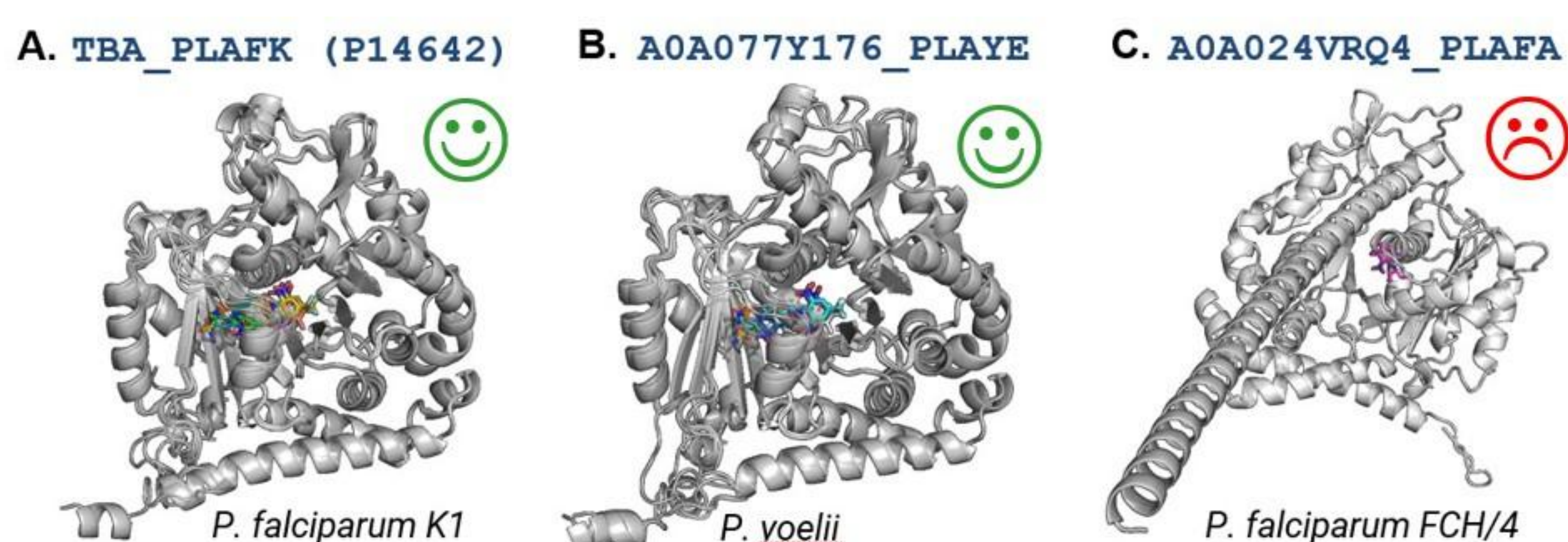


Figure 2. AI-predictions of ligand-protein complexes of *Plasmodium* α -tubulins with dinitroaniline derivatives.

Only TBA_PLAFK and A0A077Y176 yielded high scores (pLDDT, pTM and ipTM), coherent structures with well-defined ligand-binding sites. The A0A024VRQ4 model exhibited serious structural anomalies (incorrect folding, aberrant secondary structure), leading to the exclusion of this isotype from further docking and molecular dynamics studies.

The Protenix / AlphaFold3 predictions confirmed ligand poses similar to previous model proposed for *T. gondii* by Aguayo-Ortiz and Dominguez (2022). Also, structural superposition of AI-predicted complexes, revealed nearly identical ligands orientations for both types of ligand-binding pocket. The high conservatism of the binding site and the stability of the complexes mark dinitroaniline derivatives as promising candidates for antimalarial drugs that affect the parasite's microtubules with minimal impact on human tubulin (Figure 3). The results of molecular dynamics simulations (210 ns) proved the stability of all studied dinitroaniline complexes (APM - amyprophos-methyl, ORY - oryzalin, TFL - trifluralin, PEN - pendimethalin, ETH ethylfluralin) with α -tubulin isotypes known at the time of the study for *Plasmodium* sp. (Figure 4).

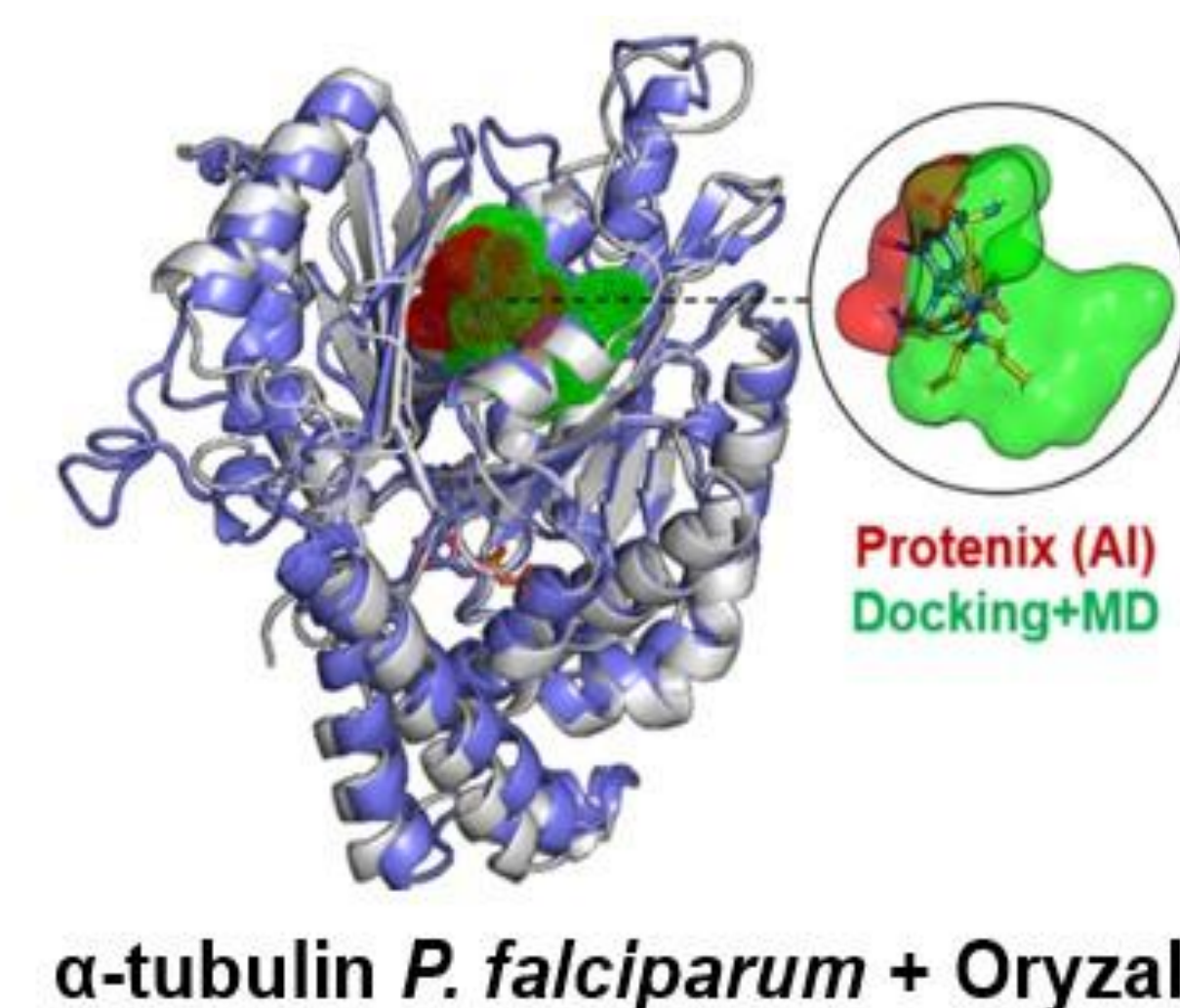


Figure 3. Comparison of *de novo* predicted classically derived binding modes of oryzalin to α -tubulin.

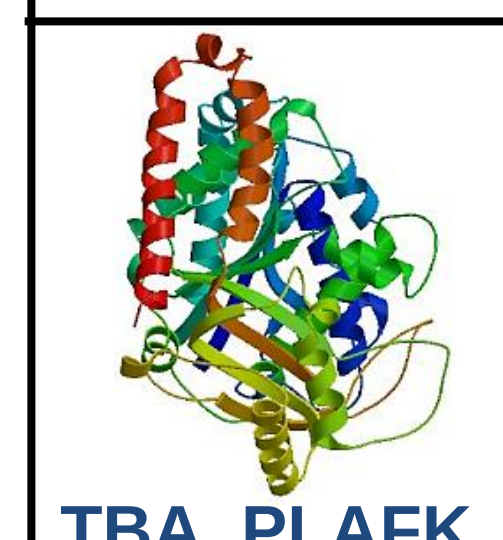
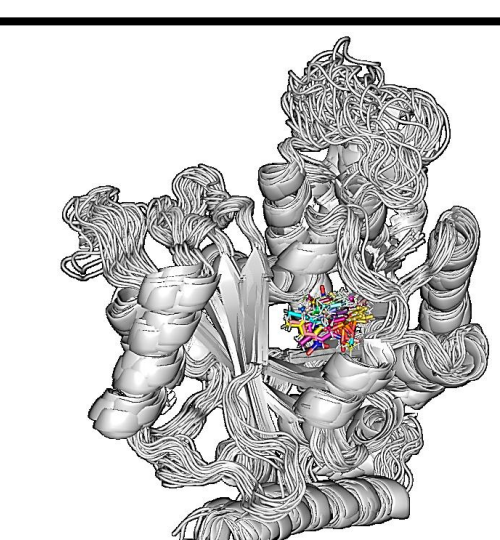
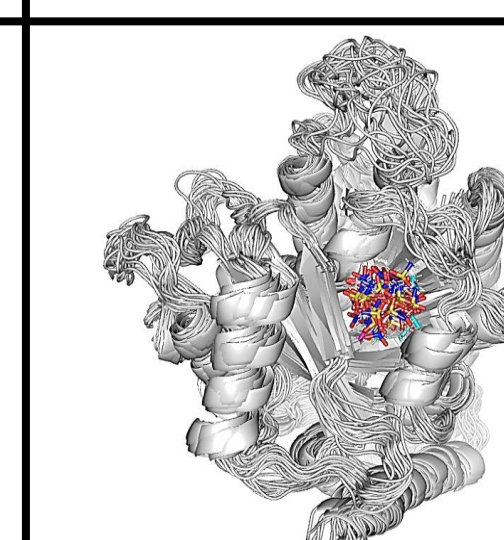
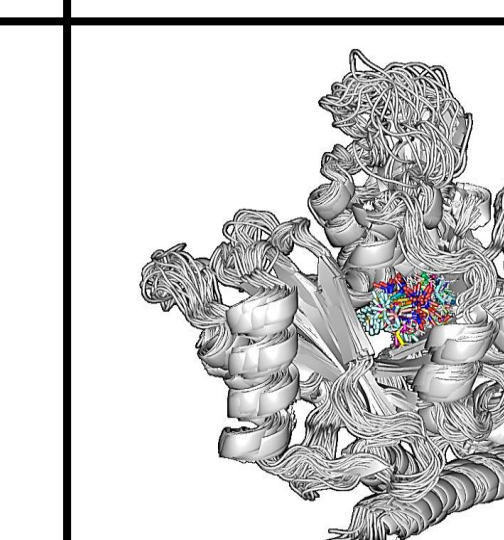
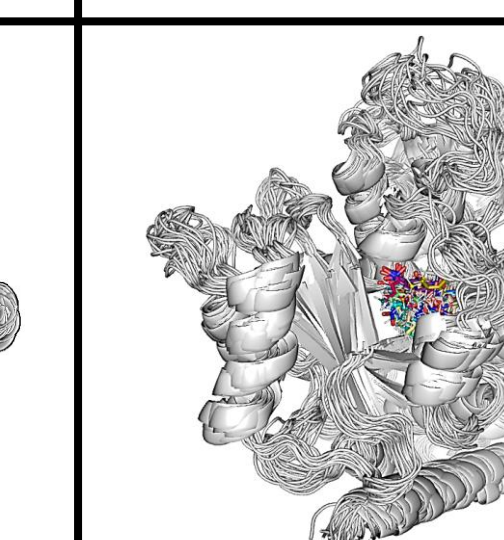
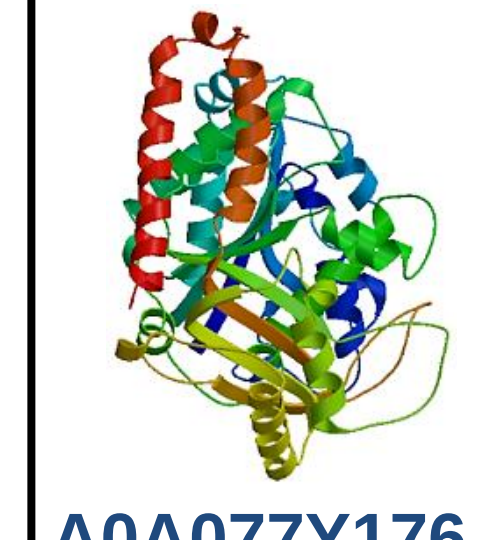
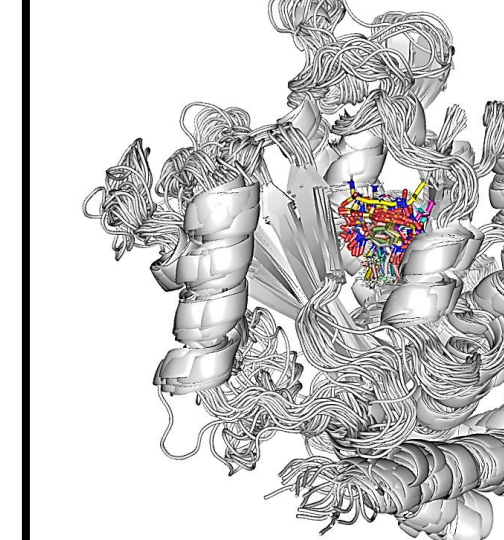
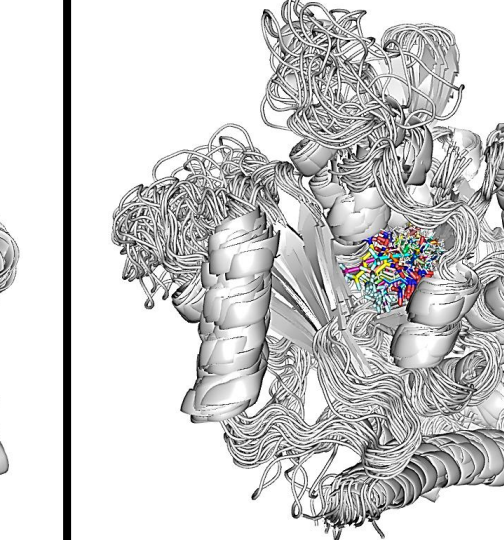
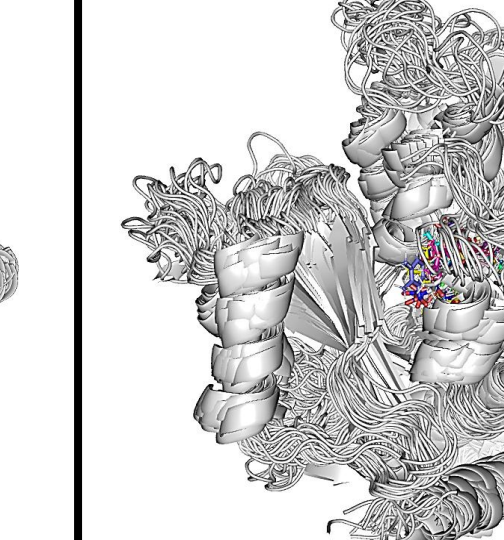
	APM	ORY	TFL	PEN	ETH
TBA_PLAFK					
A0A077Y176					

Figure 4. Structural alignment of MD frames ensembles sampled from the stable region of molecular dynamics (210 ns MD in Gromacs with Charmm36 and Tip3).

CONCLUSIONS

Research has demonstrated that dinitroaniline derivatives bind to α -tubulin in *Plasmodium* species through a ligand-induced mechanism similar to that observed in *Toxoplasma gondii*. An examination of available α -tubulin sequences identified two variations in the amino acid composition of the dinitroaniline binding pockets within the genus. Nevertheless, all known α -tubulin isoforms in *Plasmodium* species exhibit an equal capacity to interact with these compounds, as corroborated by molecular docking, molecular dynamics, and molecular complex reconstruction using artificial intelligence tools (AlphaFold3 / Protenix + Gromacs).

ACKNOWLEDGEMENTS

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