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Molecular Docking Using PyRx: A Practical Guide to Virtual Screening and Binding Affinity Analysis

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SUMMARY

Molecular docking is an established computational technique used to predict the preferred orientation and binding affinity of a small molecule (ligand) within the active site of a macromolecular target (receptor). PyRx, an open-source virtual screening platform, integrates Auto Dock Vina and Open Babel to provide a user-friendly graphical interface that streamlines the entire docking pipelinefrom structure preparation to result visualization. This article outlines a step-by-step workflow for performing molecular docking using PyRx, covering protein and ligand preparation, grid box configuration, docking parameter optimization, and interpretation of binding free energy (ΔG) scores. Emphasis is placed on practical considerations such as removal of crystallographic water molecules, assignment of Gasteiger charges, and selection of exhaustiveness parameters. The article further discusses validation strategies, common pitfalls, and downstream interaction profiling using PyMOL and LigPlot+. Understanding this workflow equips researchers with the skills to efficiently prioritize lead compounds before committing to resource-intensive wet-lab assays.

INTRODUCTION

The rational design of bioactive molecules is central to modern drug discovery, agrochemical development, and the study of host–pathogen interactions. Traditional high-throughput screening of large compound libraries is expensive and time-consuming; consequently, computational methods such as molecular docking have become indispensable tools for narrowing the chemical search space. Molecular docking algorithms evaluate the thermodynamic feasibility of non-covalent interactions between a candidate ligand and a biological receptor, generating ranked binding poses alongside predicted free energies of binding.Auto Dock Vina (Trott and Olson 2010) is among the most widely cited docking engines, offering a gradient-based scoring function that balances accuracy with computational speed.

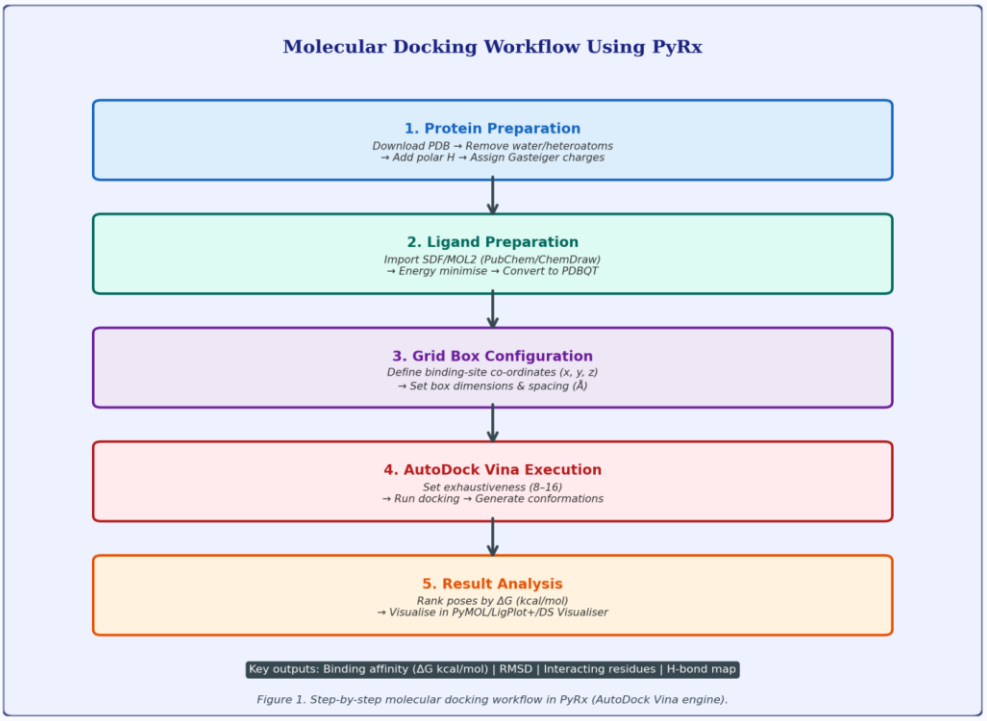

Figure 1. Step-by-step molecular docking workflow in PyRx, from protein/ligand preparation through Auto Dock Vina execution to result interpretation.

PyRx (Dallakyan and Olson 2015) wraps Auto Dock Vina and Open Babel within a cross-platform graphical interface, lowering the barrier to entry for experimentalists unfamiliar with command-line tools. This makes PyRx particularly well-suited for students and researchers in plant pathology, agricultural science, and related disciplines.

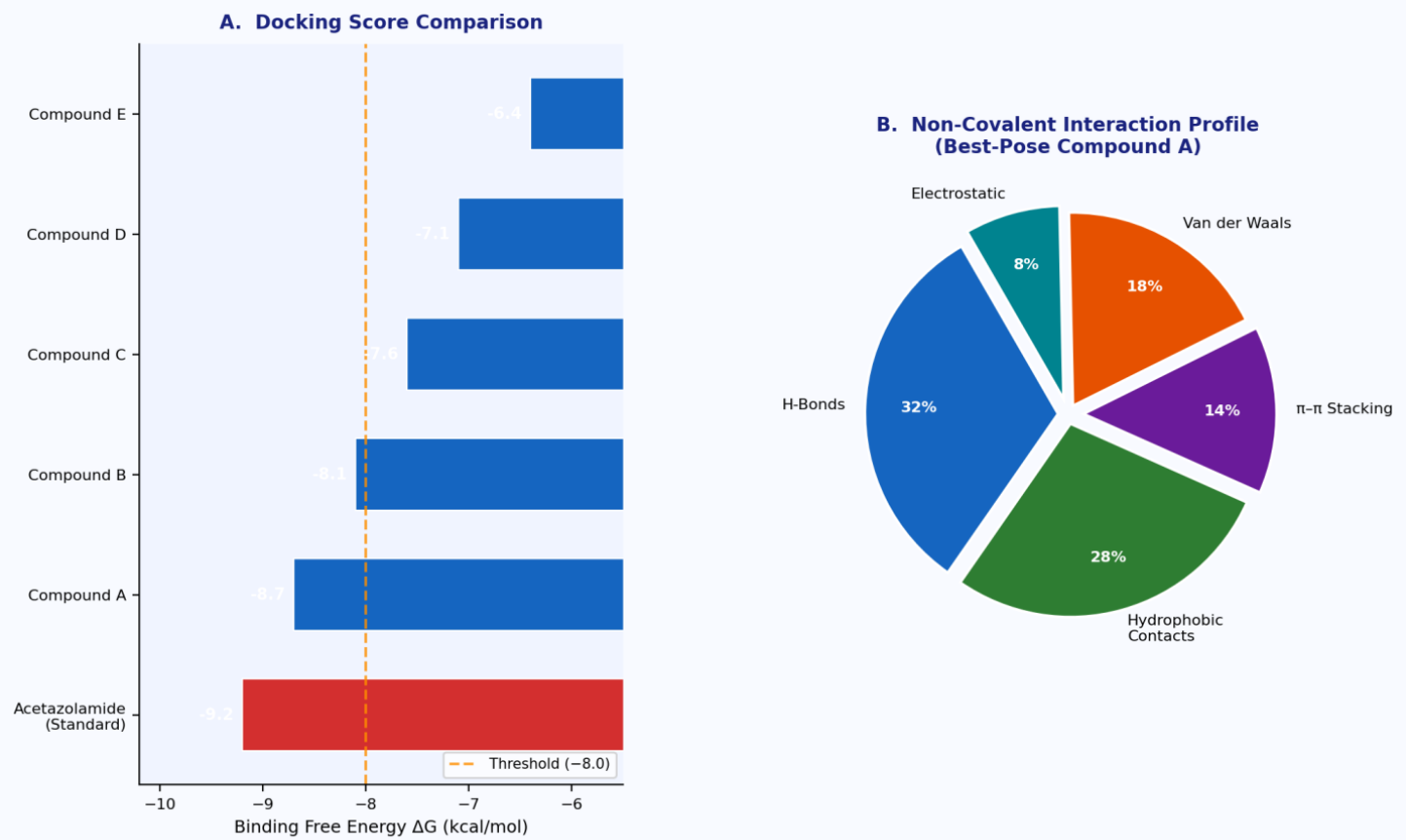

Figure 2. (A) Comparative docking binding affinities for six ligands against Carbonic Anhydrase II (PDB: 1CA2). (B) Distribution of non-covalent interaction types observed in the highest-affinity pose of Compound A.

Figure 2. (A) Comparative binding free energies (ΔG, kcal/mol) for six ligands docked against Carbonic Anhydrase II (PDB: 1CA2). (B) Non-covalent interaction type distribution in the best-ranked pose of Compound A.

Software Installation and Data Acquisition

PyRx is freely available at https://pyrx.sourceforge.io and runs on Windows, Linux, and macOS. Following installation, two categories of structural data must be acquired: (i) three-dimensional protein co-ordinates in PDB format from the RCSB Protein Data Bank (www.rcsb.org); and (ii) ligand structures in SDF or MOL2 format from PubChem (pubchem.ncbi.nlm.nih.gov) or ChemSpider. A high-resolution crystal structure (< 2.5 Å) with a co-crystallized ligand is recommended, as the bound ligand provides an experimentally validated reference for grid box placement.

Protein Preparation

Raw PDB files contain crystallographic water molecules, heteroatoms, and co-factors that must be removed prior to docking. Essential preparatory steps are: (1) deletion of irrelevant HETATM records; (2) addition of polar hydrogen atoms; and (3) assignment of Gasteiger partial charges using AutoDockTools. The prepared protein is saved in PDBQT format, which encodes partial charges alongside atomic co-ordinates. Correct protein preparation is the single most influential determinant of docking reliability.

Ligand Preparation

Ligands are subjected to a two-stage procedure within PyRx. First, three-dimensional structures are generated and energy-minimized using the Universal Force Field (UFF) embedded in Open Babel. Second, the minimized ligands are converted to PDBQT format via the Auto Dock Vina Wizard, which automates charge assignment and defines rotatable bonds governing conformational sampling. For high-throughput virtual screening, a compound library in SDF format can be batch-processed using the Run Auto Dock Vina function, which parallelizes calculations across available CPU cores.

Grid Box Configuration

The grid box defines the three-dimensional search space in which Auto Dock Vina samples ligand poses. Its center should coincide with the known or predicted binding site, typically guided by the position of the co-crystallized ligand. Grid dimensions of 25 × 25 × 25 Å are generally sufficient to accommodate drug-like molecules. An exhaustiveness of 8–16 is appropriate for standard runs; flexible receptor studies may require higher values. Grid spacing defaults to 0.375 Å, which balances sampling resolution against computation time.

Docking Execution and Result Interpretation

Once protein, ligands, and grid box are configured, docking is initiated via the Vina Wizard panel. PyRx returns a ranked list of binding poses with predicted binding free energies (ΔG, kcal/mol); more negative values indicate stronger predicted binding. The top-ranked pose for each ligand is subjected to detailed interaction analysis. Standard thresholds for promising leads range from −7.0 to −10.0 kcal/mol, benchmarked against the co-crystallized control (Acetazolamide: $\Delta G \approx -9.2$ kcal/mol). Interaction profiling is conducted in PyMOL or Bio via Discovery Studio Visualizer, while LigPlot+ generates compact two-dimensional diagrams suitable for publication. Root means square deviation (RMSD) < 2.0 Å between the top-ranked pose and the co-crystallized ligand is the accepted validation criterion.

Validation and Common Pitfalls

Protocol validation through re-docking the co-crystallized ligand is mandatory before any screening results are interpreted. Common pitfalls include: (i) failure to remove all water molecules, which can artificially favour hydrophilic contacts; (ii) incorrect protonation state assignment at histidine residues; (iii) use of a 2D ligand structure without prior 3D conversion and energy minimization; and (iv) over-reliance on ΔG scores without considering ADMET (absorption, distribution, metabolism, excretion, toxicity) properties. Complementary tools such as SwissADME and pkCSM should be used alongside docking to evaluate drug-likeness.

Applications in Agricultural and Plant Sciences

Molecular docking has found increasing application in agricultural research, including identification of inhibitors targeting viral replicase enzymes (e.g., Citrus tristeza virus RNA-dependent RNA polymerase), fungal cytochrome P450 lanosterol demethylases implicated in fungicide resistance, and bacterial type III secretion system effectors. For plant virologists, docking can guide rational design of antiviral compounds that disrupt capsid assembly or replication complex formation, providing computational hypotheses testable through bioassays, field trials, and molecular diagnostics.

CONCLUSIONS

Molecular docking using PyRx provides an accessible entry point into structure-based virtual screening applicable across pharmacology, plant science, and agrochemistry. The integrated AutoDock Vina engine delivers a robust scoring function, while Open Babel automates ligand preparation. Success depends critically on rigorous protein and ligand preparation, judicious grid box placement, and systematic validation. When combined with ADMET profiling and interaction visualization, PyRx enables researchers to prioritize promising lead compounds efficiently before experimental validation. As structural databases expand and computational resources become more affordable, in silico screening workflows such as that offered by PyRx will remain central to cost-effective drug and agrochemical discovery pipelines.

REFERENCES

Dallakyan S and Olson AJ (2015) Small-molecule library screening by docking with PyRx. Methods in Molecular Biology 1263: 243–250.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS and Olson AJ (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry 30(16): 2785–2791.

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T and Hutchison GR (2011) Open Babel: An open chemical toolbox. Journal of Cheminformatics 3(1): 33.

Trott O and Olson AJ (2010) AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimisation, and multithreading. Journal of Computational Chemistry 31(2): 455–461.

Laskowski RA and Swindells MB (2011) LigPlot+: Multiple ligand–protein interaction diagrams for drug discovery. Journal of Chemical Information and Modeling 51(10): 2778–2786.

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN and Bourne PE (2000) The Protein Data Bank. Nucleic Acids Research 28(1): 235–242.

Daina A, Michielin O and Zoete V (2017) SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports 7: 42717.