ISSN 0972-8406
The NEHU Journal Vol. XXI, No. 1&2, 2023 (2025), pp. 12-31

Computational Advances in Drug Design: An overview of structure-based and AI-Driven Approaches

Rik Ganguly, Shashi Kumar Yadav, Angneh Ngoruh, Prosperwell Ingty, Atanu Bhattacharjee*

Department of Biotechnology and Bioinformatics,

North-Eastern Hill University, Shillong

**Corresponding author: atanubioinfo@gmail.com*

Abstract

Computational approaches have radically improved in the field of drug discovery. The conventional method often takes more time and is non-economical and uncertain. Computer-aided drug discovery (CADD) has emerged as a powerful tool in pharmaceutical research, which is further classified into structure-based drug design (SBDD) and ligand-based drug design (LBDD). The target proteins are obtained through X-ray crystallography, cryo-electron microscopy, or NMR spectroscopy to design molecules with high binding affinity and specificity. Techniques like pharmacophore modeling, quantitative structure-activity relationships (QSARs) and artificial intelligence (AI) have improved drug screening and optimization and accelerated early-stage in drug discovery. Integration of AI further enhances the toxicity prediction of lead, target identification, and de novo drug design making drug design more efficient. This review highlights the application of SBDD and LBDD, emphasizing their importance in modern-day drug discovery and their potential to facilitate the development of novel therapeutic medicines.

Keywords: Docking, machine learning, MD simulation, structure-based drug design, virtual screening.

Introduction

Over the last few decades, the application of computational approaches in drug discovery has been consolidated. The lack of approved drugs or vaccines continues to be a challenge and further necessitates the discovery of new therapeutic molecules. Small molecule designing with drug-like properties remains a challenge in both fundamental and biopharmaceutical

research. Traditional drug development methods are known for being time-consuming, expensive and less efficient, often taking around a decade. This method of drug development process follows a pipeline consisting of target identification, lead discovery, pre-clinical testing, clinical trials and regulatory approval which leads to many errors (Kiriiri *et al.* 2020).

The use of CADD techniques in preliminary studies by leading pharmaceutical companies and research groups has helped to expedite the drug discovery and development process minimizing the costs and failures in the final stage. CADD has several success stories and continues to play a vital role in the drug discovery process. This approach has been utilized in proposing drug candidates against coronavirus disease 2019 (COVID-19) (Tarighi *et al.* 2021). The advancement of computational biology is very evident from a comprehensive review that covers MERS-CoV, covering epidemiology, genome analysis, pathogenesis, diagnostics, vaccine development and predictive modelling (Ganesh *et al.* 2021; Chakrabartty *et al.* 2022). CADD can be broadly divided into structure-based and ligand-based drug design approaches, both have been widely used in the drug discovery process in the identification of suitable lead molecules (Gurung *et al.* 2021; Mouchlis *et al.* 2021; Isert *et al.* 2023).

Structure-based drug design (SBDD)

SBDD is a computational approach that uses protein 3D structures to predict potential drug molecules (Pant *et al.* 2022). It aims to design small-ligand molecules that bind with high affinity and specificity to pre-determined protein targets. Understanding the principles by which small molecules recognize and interact with macromolecules is of great importance in pharmaceutical research and development (Gohlke *et al.* 2002). SBDD systematically uses the structural data, such as macromolecular targets or receptors, gained by experimentation or computational homology modeling (Bajad *et al.* 2021). Recent advances in geometric deep learning, especially in modeling 3D structures of biomolecules, provide a promising direction for SBDD. Despite significant advances in the application of deep learning as surrogate docking models, the deep learning-based design of ligands that bind to target proteins remains a major difficulty in molecular modeling (Sumathi *et al.* 2023). By utilizing structural data, which is acquired by X-ray crystallography, cryo-electron microscopy, or NMR spectroscopy, SBDD makes it possible to generate compounds that selectively interact with the target protein's active region (Bajad *et al.* 2021; Cebi *et al.* 2024). The rational

design of inhibitors, modulators, or activators with enhanced efficacy and selectivity is made easier by this approach which is essential to contemporary drug discovery. SBDD makes extensive use of methods including molecular docking, molecular dynamics simulation and free energy computations to forecast binding affinities and maximize drug-like characteristics (Anwar *et al.* 2021; Rakshit *et al.* 2022).

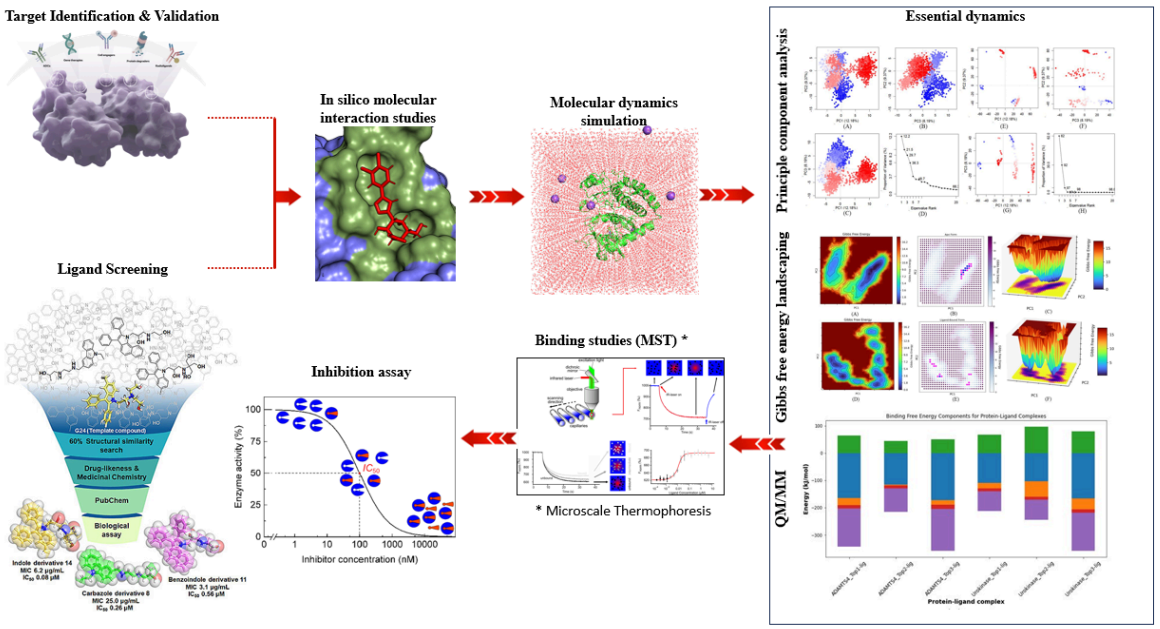

Fig. 1. Showing the general pipeline of structure-based drug discovery.
(Picture courtesy: Adapted from Smirnovienė *et al.* 2021; Jerabek-Willemsen *et al.*2014; Pakamwong *et al.* 2024).

Ligand-based drug design (LBDD)

LBDD is another widely used approach in computer-aided drug discovery and is employed when the three-dimensional structure of the target protein is not available (Ajjarapu *et al.* 2022; Yadav *et al.* 2022). Information from a set of active compounds against a specific target protein receptor reveals key structural properties linked to biological function based on their similarities(Yadav *et al.* 2022). This approach helps in screening virtual compound libraries, optimising lead compounds and accelerating the early stages of drug discovery. Some common techniques used in ligand-based virtual screening approach include pharmacophore modeling, quantitative structure-activity relationships (QSARs) and artificial intelligence (AI) (Murugan *et al.* 2022). QSAR methods help to evaluate the activity of a large number of compounds virtually, reducing the time and labor costs required for the chemical synthesis and experimental determination. This method increases the efficacy of

drug discovery (Wang *et al.* 2021). Integration of AI in drug discovery has become a prominent part of modern pharmaceutical research. It helps to automate, assure quality, improve drug efficacy polypharmacology and personalized manufacturing with the least detected error. Furthermore, AI has a wide range of applications in drug discovery, including prediction of protein folding, protein-protein interaction, virtual screening, QSAR, evaluation of ADMET characteristics, and de novo drug design (Gupta *et al.* 2021). By using the capabilities of AI models and large databases, researchers are expediting the prediction, identification and validation of potential drug targets.

Virtual screening

Virtual screening is a computer-based approach developed for comparative analysis of multiple leads, evaluating chemical, biological features and protein-ligand interactions to determine which compounds have the greatest binding to a target protein (Lavecchia 2013). Virtual screens are often done utilizing libraries of substances that can be purchased cheaply and do not require specialised synthesis (Irwin *et al.* 2020). Using computer models of complexes, structure-based virtual screening seeks to identify compounds that generate favourable interactions with biological macromolecules (Carlsson and Luttens 2024). A virtual screening is a fast in silico approach that combines scoring and ranking methods to screen massive database compounds against a biological target. (Giordano *et al.* 2022). High-throughput screening is a widely used technique for identifying potential medication candidates (Maia *et al.* 2020). However, filtering millions of molecules still requires a significant amount of time and energy.

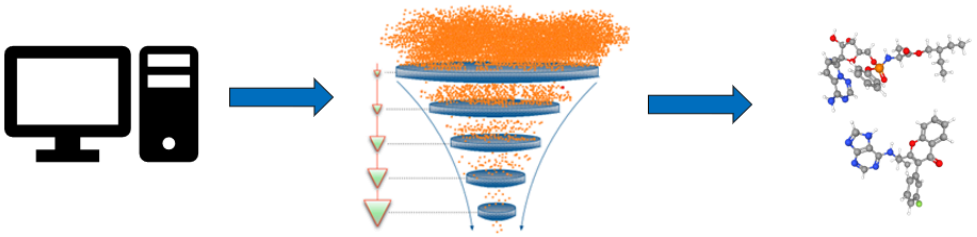

Fig. 2. Showing the different stages of virtual screening of compounds

(Picture courtesy:Adapted from Pyzer-Knapp *et al.* 2015).

An automated screening of compounds using cellular assays, typically carried out robotically, is what distinguishes high-throughput screening from virtual screening (Maia *et al.* 2020). A high-throughput screening is more expensive than a virtual screening (Maia *et al.* 2020). Although virtual screening holds promise for streamlining the drug discovery process, it has

its drawbacks. False positives can occasionally emerge from virtual screenings, and the various technologies available for virtual screenings can yield disparate findings using the same data (Maia *et al.* 2020). Nonetheless, virtual screenings are currently being used extensively because of the anticipated time and efficiency savings in the drug discovery process. Acute toxicity, carcinogenicity, hepatotoxicity, Lipinski's rule of five, ADME and blood-brain barrier (BBB) penetration are some of the filtration criteria used to eliminate molecules with undesirable biological availability from the database of ligands (Yamashita and Hashida 2004).

Molecular Docking

Molecular docking usually serves to predict the energy and geometry of receptor-ligand binding by simulating the interaction between the ligand and the receptor's active region (Peluso *et al.* 2019). Molecular docking of small molecules to a biological target involves a creative sampling of potential ligand poses in the designated pocket or groove of the target candidate to determine the best binding shape (Mukesh and Rakesh2011; Guedes *et al.* 2014; Agarwal *et al.* 2015; Seeliger and de Groot 2010). This can be done with the docking software's user-defined fitness or score feature. AutoDock (Morris *et al.* 1998), GLIDE (Friesner *et al.* 2004), Dock (Allen *et al.* 2015) and GOLD (Verdonk *et al.* 2003) are popular programs for docking.

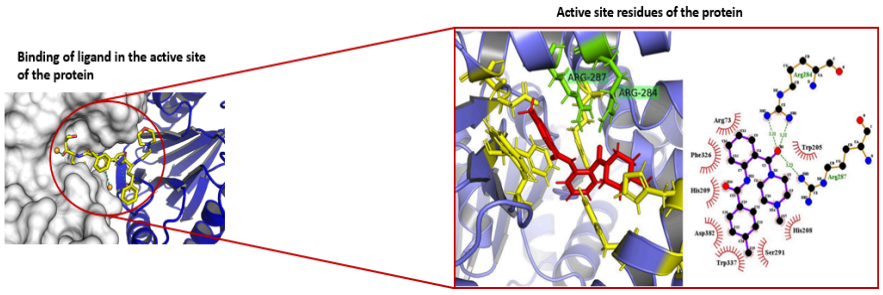

Fig.3. Showing the molecular interaction between the target protein and ligand (Picture courtesy:Adapted from Ferreira *et al.* 2015; Ganguly *et al.*2023).

Conformational search using different algorithms and scoring or ranking of the docked poses are the two main processes in a docking procedure. Scoring functions take as input a candidate binding pose and score the energetic favourability of the ligand binding to the target at that pose. Most docking software use empirical scoring functions (Eldridge *et al.*1997). Empirical scoring functions are made up of words that represent various

protein-ligand interactions that are known to be essential for determining binding energy. Scoring functions are designed to maximise the accuracy with which they predict binding postures, binding energies, or the best binders from a set of compounds (Adeshina *et al.* 2020; Li *et al.* 2019). Depending on the goals of the docking simulations, there are many types of molecular docking processes that use either flexible or stiff ligand/target combinations. Flexible ligand docking, which includes the target as a hard molecule. This is the most typical method for docking. stiff body docking involves keeping both the target and ligand molecules stiff. Flexible docking involves both interacting molecules (Guedes *et al.* 2014; Shoichet *et al.* 2002; Gschwend *et al.* 1996). The highest-ranked complex by docking is carried for MD simulation, which explores the complex in detail from a dynamic perspective (Salmaso and Moro 2018).

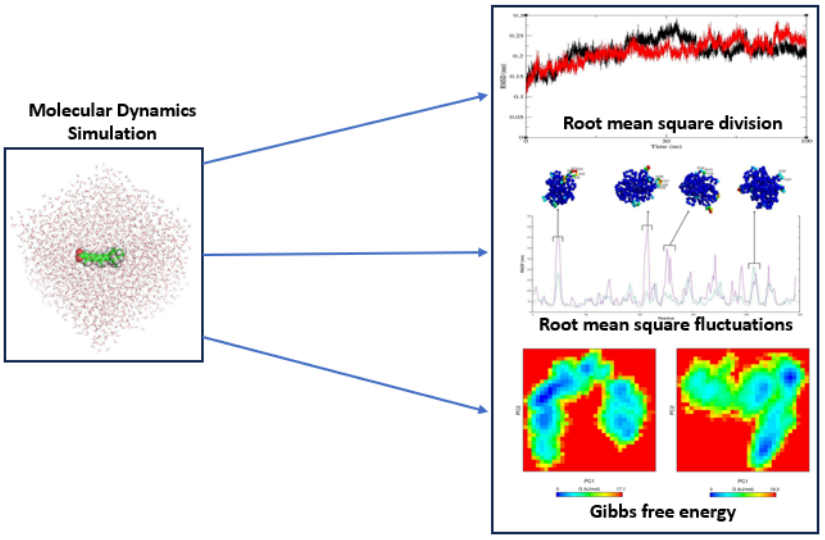

Fig. 4. Depicting the MD simulation between the target protein and ligand and its analysis (Picture courtesy:Böselt *et al.* 2021;Ganguly *et al.* 2022).

Molecular dynamics (MD) simulations

MD is a simulation that demonstrates how molecules move, vibrate, diffuse, and interact over time, inside a sufficiently simulation box, where their movements are governed by traditional Newton's laws of motion (Salo-Ahen *et al.* 2020). Several computer programs have been made available, and today regularly used programs for MD simulations include AMBER (Case *et al.* 2005), CHARMM (Brooks *et al.* 2009), GROMACS (Hess *et al.* 2008), and NAMD (Phillips *et al.* 2020). The MD algorithm incorporates Newton's equations of motion

to mimic the motions of atoms over time, and common integration methods include the verlet and leapfrog algorithms (de Oliveira *et al.* 2008). The verlet algorithm is popular because it is simple and time-reversible, despite the fact that it does not explicitly calculate velocities, which can be obtained separately. The leapfrog algorithm, as an alternative, estimates velocities in half-time steps, enhancing velocity calculation accuracy. Choosing an appropriate time step is critical to balance accuracy and computing economy; higher time steps increase sampling efficiency, but can cause errors if too large (González 2011; Hollingsworth and Dror 2018). The force field is a set of equations and constants that represent the potential energy of a system, often split into bound interactions including bond stretching, angle bending, and dihedral torsions and non-bonded interactions such as van der Waals and electrostatic forces (Edeling *et al.* 2024; González 2011).

Hardware advancements, such as specialised supercomputers and graphics processing units (GPUs), have enabled microsecond to millisecond simulations of protein folding, conformational changes, and ligand binding (Durrant and McCammon 2011, Lazim *et al.* 2020). Molecular dynamics (MD) simulations have become essential for researching protein movements at the atomic level, yet they have numerous significant drawbacks. One key challenge is the accuracy of force fields, which are the mathematical models used to represent the forces between atoms; imperfections in force field parameters can lead to large errors in the simulated protein conformations and dynamics (Hollingsworth and Dror 2018; Ormeño and General 2024). The computing expense of MD simulations remains a considerable barrier, especially for long-timescale simulations aimed at capturing physiologically important events happening on millisecond to second durations. Although developments in hardware, such as the use of GPUs and specialised supercomputers, have permitted lengthier simulations, they still demand large resources and typically give restricted sampling due to the enormous configurational space of proteins (Lazim *et al.* 2020; Rácz *et al.* 2022)

Validation of protein-ligand interactions and structural determination

To confirm and characterize protein-ligand interactions, a range of biophysical, structural, and functional techniques are utilized. These methods help determine binding affinity, kinetics, conformational changes, and the biological impact of ligand binding. Each technique provides unique insights, and together, they offer a comprehensive understanding of molecular interactions (Arumugam *et al.* 2024).

Biophysical Techniques for Protein-Ligand Binding Analysis

Microscale Thermophoresis (MST)

MST is a powerful and versatile method used to quantify binding affinity between proteins and ligands by measuring changes in molecular movement within a temperature gradient. When a ligand binds to a protein, it alters the molecule's hydration shell, charge distribution, and size, affecting its thermophoretic mobility. This change is detected using fluorescence, enabling precise determination of dissociation constants (Kd). MST is particularly advantageous due to its low sample consumption, ability to work in native buffer conditions, and capacity to detect weak to strong interactions. Unlike immobilization-based techniques, MST allows interactions to be studied in free solution, preserving the native environment of biomolecules (Picchi 2023; Brunner 2020).

Surface Plasmon Resonance (SPR)

SPR provides real-time insights into binding interactions by detecting changes in the refractive index when a ligand interacts with an immobilized protein on a sensor chip. This technique enables the determination of crucial binding kinetics parameters, including the association rate (ka), dissociation rate (kd), and equilibrium dissociation constant (Kd). SPR is particularly valuable in studying protein-protein interactions, antibody-antigen binding, and small-molecule drug interactions (Ritzefeld *et al.* 2012; Puiu, 2016). It is highly sensitive, allowing the detection of transient and high-affinity interactions. The real-time nature of SPR makes it one of the most effective tools for understanding dynamic binding events, making it indispensable in drug discovery and biomolecular research.

Circular dichroism (CD) spectroscopy

CD Spectroscopy provides valuable information on the secondary structure and conformational stability of proteins upon ligand binding.CD measures the differential absorption of left- and right-circularly polarized light, generating spectral signatures characteristic of α-helices, β-sheets, and random coils. Ligand-induced structural changes can lead to shifts in the CD spectrum, allowing researchers to assess protein folding, stability, and ligand-induced conformational alterations (Pelton *et al.* 2020; Micsonai *et al.* 2022). This technique is particularly useful for evaluating how small molecules, peptides, or mutations affect protein integrity and stability. It plays a significant role in drug discovery, especially when screening compounds that induce conformational changes in target proteins.

Structural determination techniques

X-ray crystallography (XRD)

X-ray crystallography remains the gold standard for high-resolution structural determination of protein-ligand complexes (Srivastava *et al.* 2018**;** Majorek *et al.* 2020). This technique involves crystallizing the protein-ligand complex and using X-ray diffraction patterns to elucidate atomic-level details of molecular interactions. XRD provides precise insights into binding pockets, hydrogen bonding, steric interactions and overall molecular architecture. However, successful crystallization can be challenging, requiring optimization of conditions specific to each protein-ligand system. Despite its limitations, XRD is crucial for structure-based drug design, as it allows for the rational modification of ligands to enhance binding affinity and specificity.

Cryo-electron microscopy (Cryo-EM)

Cryo-EM has revolutionized structural biology by enabling the determination of near-atomic resolution structures of large, dynamic protein-ligand complexes without the need for crystallization. In this technique, biomolecules are rapidly frozen in a thin layer of vitreous ice, preserving their native conformations (Haymaker, 2024). Electron beams are then used to capture multiple projections of the complex, which are computationally reconstructed into three-dimensional structures. Cryo-EM is particularly advantageous for studying flexible proteins, large macromolecular assemblies, and transient interactions that are difficult to crystallize. This method has become an essential tool for visualizing conformational changes upon ligand binding and understanding protein dynamics at the molecular level.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy provides structural information for proteins in solution, offering insights into their dynamics and conformational flexibility. However, conventional solution-state NMR is generally limited to proteins smaller than 30 kDa due to signal overlap and spectral complexity (Ikeya *et al.* 2018). Recent advancements, such as solid-state NMR and small-angle X-ray scattering (SAXS), have expanded its applicability to larger biological macromolecules. By integrating SAXS data as constraints, researchers have successfully solved the structures of proteins beyond 20 kDa, improving resolution and accuracy (Delhommel *et al.* 2020).

Functional and mutagenesis studies

Enzyme inhibition assays

Beyond structural techniques, functional assays such as enzyme inhibition assays are crucial for assessing the biological activity of protein-ligand interactions (Riccardi*et al.* 2018; Khan*et al.* 2025). These assays measure whether ligand binding modulates enzymatic function, providing insights into inhibitory potential and mechanism of action. Enzyme activity can be assessed using various methods, including colorimetric assays that detect changes in substrate or product concentration, fluorescence-based assays that utilize fluorogenic substrates, and radioactive assays that offer high sensitivity. Key parameters such as the half-maximal inhibitory concentration (IC_{50}) and inhibition constant (Ki) are determined to evaluate the effectiveness of potential inhibitors. These assays are indispensable in drug discovery, particularly for identifying compounds that target key enzymatic pathways (Fienberg 2017; Tanwar *et al.* 2024).

Mutagenesis studies

Mutagenesis studies further contribute to the validation of protein-ligand interactions by identifying key residues involved in binding. Site-directed mutagenesis, where specific amino acids are substituted, allows researchers to determine the functional significance of individual residues within the binding pocket (Anand *et al.* 2014). Alanine scanning mutagenesis, a commonly used approach, systematically replaces amino acids with alanine to assess their contribution to binding affinity and stability (Moreira *et al.* 2017). More advanced techniques, such as deep mutational scanning, involve the simultaneous screening of multiple mutations to map interaction hotspots comprehensively (Verkhivker *et al.* 2023). These studies are instrumental in designing improved therapeutic proteins, enhancing ligand specificity, and understanding resistance mechanisms in drug-target interactions.

AI and machine learning in structural biology

AI and machine learning have significantly accelerated protein structure determination and ligand binding analysis. In X-ray crystallography, AI-driven algorithms assist in interpreting electron density maps, automating atomic placement, and refining structural models **(**Vollmar*et al.* 2021). In NMR spectroscopy, AI enhances the assignment of resonance peaks to specific atoms, improving the accuracy of chemical shift predictions and protein folding analysis (Shukla *et al.* 2023). Cryo-EM has particularly benefited from deep learning

methods that improve image processing, particle detection, and three-dimensional reconstruction (Vilas *et al.* 2022). Convolutional neural networks (CNNs) have been instrumental in automating key steps such as particle classification and heterogeneity analysis, while generative models have been developed to reconstruct high-resolution 3D structures from heterogeneous samples (Da Wang *et al.* 2021).

The integration of AlphaFold's predictive models with experimental data exemplifies how AI can complement traditional structural biology techniques. AlphaFold has provided highly accurate models of protein structures, which researchers have used as starting points for further refinement through experimental methods (Jumper *et al.* 2021). Moreover, AI-driven approaches are now being applied to solve inverse problems in structural biology, such as refining cryo-EM density maps into atomic models using diffusion-based techniques. The combination of physics-based models with generative learning has proven superior to traditional posterior sampling methods, marking a significant advancement in structural refinement **(**Tiwary *et al.* 2024; Sil *et al.* 2024**)**.

Conclusion

Computational methods have revolutionized drug discovery by reducing costs and increasing efficiency. SBDD utilizes 3D protein structures to design highly specific molecules, enhanced by advances in structural biology and deep learning. LBDD identifies bioactive compounds through structural and physicochemical similarities, aiding virtual screening and lead optimization. Virtual screening rapidly evaluates large compound libraries, while molecular docking predicts ligand-receptor interactions, and molecular dynamics simulations provide insights into molecular stability and conformational changes. Despite challenges like false positives and computational costs, these techniques are essential in modern drug discovery, evolving with AI and high-performance computing. Biophysical methods (MST, SPR, CD spectroscopy) assess binding affinity, while high-resolution techniques (XRD, Cryo-EM) reveal atomic structures. Functional assays confirm biological relevance. AI-driven structural biology further enhances precision, creating a robust framework for drug discovery and targeted therapeutics.

References

Adeshina, Y. O., Deeds, E. J. and Karanicolas, J. 2020. 'Machine learning classification can

reduce false positives in structure-based virtual screening',*Proceedings of the National Academy of Sciences*, 117(31): 18477-18488.

Agarwal, S., Chadha, D. and Mehrotra, R. 2015. 'Molecular modeling and spectroscopic studies of semustine binding with DNA and its comparison with lomustine–DNA adduct formation',*Journal of Biomolecular Structure and Dynamics,* 33(8): 1653-1668.

Ajjarapu, S. M., Tiwari, A., Ramteke, P. W., Singh, D. B. and Kumar, S. 2022. 'Ligand-based drug designing. In *Bioinformatics*, Academic Press: 233-252.

Allen, W. J., Balius, T. E., Mukherjee, S., Brozell, S. R., Moustakas, D. T., Lang, P. T. and Rizzo, R. C. 2015. 'DOCK 6: Impact of new features and current docking performance',*Journal of Computational Chemistry,* 36(15): 1132-1156.

Anand, P., Nagarajan, D., Mukherjee, S. and Chandra, N. 2014. 'ABS–Scan: In silico alanine scanning mutagenesis for binding site residues in protein-ligand complex',*F1000Research*, 3.

Anwar, T., Kumar, P. and Khan, A. U. 2021. 'Modern tools and techniques in computer-aided drug design', In *Molecular Docking for Computer-Aided Drug Design,* Academic Press: 1-30.

Arumugam, S., Muthuvel, R., Anjugam, C., Kulanthaivel, L. and Subbaraj, G. K. 2024. 'Challenges and opportunities for analyzing protein-ligand interactions',*Biochemical Techniques for Analyzing Protein-Lipid Interactions*, 1-20.

Bajad, N. G., Rayala, S., Gutti, G., Sharma, A., Singh, M., Kumar, A. and Singh, S. K. 2021. 'Systematic review on role of structure-based drug design (SBDD) in the identification of anti-viral leads against SARS-Cov-2',*Current Research in Pharmacology and Drug Discovery*, 2:100026.

Böselt, L., Thürlemann, M. and Riniker, S. 2021. 'Machine learning in QM/MM molecular dynamics simulations of condensed-phase systems',*Journal of Chemical Theory and Computation,*17(5): 2641-2658.

Brooks, B. R., Brooks III, C. L., Mackerell Jr, A. D., Nilsson, L., Petrella, R. J., Roux, B., ... and Karplus, M. 2009. 'CHARMM: the biomolecular simulation program',*Journal of Computational Chemistry,* 30(10): 1545-1614.

Brunner, C. G. 2020. *Comparative Perspectives on Protein-Protein Interactions Investigated with Biophysical Methods* (Doctoral dissertation, ETH Zurich).

Carlsson, J. and Luttens, A. 2024. 'Structure-based virtual screening of vast chemical space as a starting point for drug discovery', *Current Opinion in Structural Biology,* 87: 102829.

Case, D. A., Cheatham III, T. E., Darden, T., Gohlke, H., Luo, R., Merz Jr, K. M., ... and Woods, R. J. 2005. 'The Amber biomolecular simulation programs',*Journal of Computational Chemistry,* 26(16): 1668-1688.

Cebi, E., Lee, J., Subramani, V. K., Bak, N., Oh, C. and Kim, K. K. 202. 'Cryo-electron microscopy-based drug design',*Frontiers in Molecular Biosciences*, 11: 1342179.

Chakrabartty, I., Khan, M., Mahanta, S., Chopra, H., Dhawan, M., Choudhary, O. P. and Emran, T. B. 2022. 'Comparative overview of emerging RNA viruses: Epidemiology, pathogenesis, diagnosis and current treatment',*Annals of Medicine and Surgery*, 79: 103985.

Cheng, Y. 2018. 'Single-particle cryo-EM—How did it get here and where will it go', *Science*, 361(6405): 876-880.

Da Wang, Y., Blunt, M. J., Armstrong, R. T. and Mostaghimi, P. 2021. 'Deep learning in pore scale imaging and modeling',*Earth-Science Reviews*, 215: 103555.

De la Rosa-Trevin, J. M., Quintana, A., Del Cano, L., Zaldívar, A., Foche, I., Gutiérrez, J. Carazo, J. M. 2016. 'Scipion: A software framework toward integration, reproducibility and validation in 3D electron microscopy', *Journal of Structural Biology*, 195(1), 93-99.

de Oliveira, C. A. F., Hamelberg, D. and McCammon, J. A. 2008. 'Coupling accelerated molecular dynamics methods with thermodynamic integration simulations',*Journal of Chemical Theory and Computation,* 4(9): 1516-1525.

Delhommel, F., Gabel, F. and Sattler, M. 2020. 'Current approaches for integrating solution NMR spectroscopy and small-angle scattering to study the structure and dynamics of biomolecular complexes',*Journal of Molecular Biology*, 432(9): 2890-2912.

dos Santos Nascimento, I. J. and de Moura, R. O. 2023. 'Ligand and structure-based drug design (lbdd and sbdd): promising approaches to discover new drugs',*Applied Computer-Aided Drug Design: Models and Methods*, 1.

Durrant, J. D. and McCammon, J. A. 2011. 'Molecular dynamics simulations and drug discovery',*BMC Biology,* 9: 1-9.

Edeling, W., Vassaux, M., Yang, Y., Wan, S., Guillas, S. and Coveney, P. V. 2024. 'Global ranking of the sensitivity of interaction potential contributions within classical molecular dynamics force fields',*npj Computational Materials,* 10(1): 87.

Eldridge, M. D., Murray, C. W., Auton, T. R., Paolini, G. V. and Mee, R. P. 1997. 'Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes',*Journal of Computer-Aided Molecular Design*, 11: 425-445.

Ferreira, L. G., Dos Santos, R. N., Oliva, G. and Andricopulo, A. D. 2015. 'Molecular docking and structure-based drug design strategies',*Molecules*, 20(7): 13384-13421.

Fienberg, S. 2017. 'Development of N-domain selective Angiotensin-I Converting Enzyme (ACE) inhibitors using Computer Aided Drug Discovery (CADD)',University of Cape Town.

Friesner, R. A., Banks, J. L., Murphy, R. B., Halgren, T. A., Klicic, J. J., Mainz, D. T. and Shenkin, P. S. 2004. 'Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy',*Journal of Medicinal Chemistry,* 47(7): 1739-1749.

Ganesh, B., Rajakumar, T., Malathi, M., Manikandan, N., Nagaraj, J., Santhakumar, A. and Malik, Y. S. 2021. 'Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: An updated overview of current knowledge and future perspectives',*Clinical Epidemiology and Global Health*, 10: 100694.

Ganguly, R., Mylliemngap, B. J. and Bhattacharjee, A. 2023. 'Discovery of a novel inhibitor against urokinase-type plasminogen activator, a potential enzyme with a role in atherosclerotic plaque instability',*Journal of Biomolecular Structure and Dynamics*, 41(8): 3485-3495.

Ganguly, R., Ngoruh, A., Ingty, P., Yadav, S. K. and Bhattacharjee, A. 2023. 'Identification of an inhibitor for atherosclerotic enzyme NOX-1 to inhibit ROS production',*Future Journal of Pharmaceutical Sciences*, 9(1): 24.

Gauto, D. F., Estrozi, L. F., Schwieters, C. D., Effantin, G., Macek, P., Sounier, R., ... and Boisbouvier, J. 2019. Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex. *Nature Communications*, 10(1): 2697.

Giordano, D., Biancaniello, C., Argenio, M. A. and Facchiano, A. 2022. Drug design by pharmacophore and virtual screening approach. *Pharmaceuticals,* 15(5): 646.

Gishie, A., Guo, L., Irving, T. and Bax, A. 2010. 'Improved fitting of solution X-ray scattering data to macromolecular structures and structural ensembles by explicit water modeling', *Journal of the American Chemical Society*, 132(44): 15484-15486.

Gohlke, H. and Klebe, G. 2002. 'Approaches to the description and prediction of the binding affinity of small‐molecule ligands to macromolecular receptors',*Angewandte Chemie International Edition*, 41(15): 2644-2676.

González, M. A. 2011. 'Force fields and molecular dynamics simulations',*École thématique de la Société Française de la Neutronique,* 12: 169-200.

Grigorieff, N. and Harrison, S. C. 2011. 'Near-atomic resolution reconstructions of icosahedral viruses from electron cryo-microscopy', *Current Opinion in Structural Biology*, 21(2): 265-273.

Gschwend, D. A., Good, A. C. and Kuntz, I. D. 1996. 'Molecular docking towards drug discovery',*Journal of Molecular Recognition: An Interdisciplinary Journal,* 9(2): 175-186.

Guedes, I. A., de Magalhães, C. S. and Dardenne, L. E. 2014. 'Receptor–ligand molecular docking',*Biophysical Reviews,* 6: 75-87.

Gupta, H., McCann, M. T., Donati, L. and Unser, M. 2021. 'CryoGAN: A new reconstruction paradigm for single-particle cryo-EM via deep adversarial learning', *IEEE Transactions on Computational Imaging*, 7: 759-774.

Gurung, A. B., Ali, M. A., Lee, J., Farah, M. A. and Al-Anazi, K. M. 2021. 'An updated review of computer‐aided drug design and its application to COVID‐19',*BioMed Research International*, 2021(1): 8853056.

Haymaker, A. 2024. *Electron Microscopy: A Study in Applications and Methods for Structural Biology* (Doctoral dissertation, Arizona State University).

Hess, B., Kutzner, C., Van Der Spoel, D. and Lindahl, E. 2008. 'GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation',*Journal of Chemical Theory and Computation,* 4(3): 435-447.

Hollingsworth, S. A. and Dror, R. O. 2018. 'Molecular dynamics simulation for all',*Neuron,* 99(6): 1129-1143.

Ikeya, T., Ban, D., Lee, D., Ito, Y., Kato, K. and Griesinger, C. 2018. 'Solution NMR views of dynamical ordering of biomacromolecules',*Biochimica et Biophysica Acta (BBA)-General Subjects*, 1862(2): 287-306.

Irwin, J. J., Tang, K. G., Young, J., Dandarchuluun, C., Wong, B. R., Khurelbaatar, M. and Sayle, R. A. 2020. 'ZINC20—a free ultra large-scale chemical database for ligand discovery',*Journal of Chemical Information and Modeling*, 60(12): 6065-6073.

Isert, C., Atz, K. and Schneider, G. 2023. 'Structure-based drug design with geometric deep learning',*Current Opinion in Structural Biology*, 79: 102548.

Jerabek-Willemsen, M., André, T., Wanner, R., Roth, H. M., Duhr, S., Baaske, P. and Breitsprecher, D. 2014. 'MicroScale thermophoresis: interaction analysis and beyond', *Journal of Molecular Structure*, 1077: 101-113.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O. and Hassabis, D. 2021. 'Highly accurate protein structure prediction with AlphaFold',*Nature*, 596(7873): 583-589.

Khan, S., Anwar, S., Choudhury, A., Mohammad, T. and Hassan, M. I. 2025. 'Validation of drug targets using molecular methodologies and enzymatic activity assays for validation of inhibitory potential', In *Bacterial Enzymes as Targets for Drug Discovery,* Academic Press: 91-112.

Kiriiri, G. K., Njogu, P. M. and Mwangi, A. N. 2020 'Exploring different approaches to improve the success of drug discovery and development projects: a review',*Future Journal of Pharmaceutical Sciences*, 6: 1-12.

Klukowski, P., Riek, R. and Güntert, P. 2022. 'Rapid protein assignments and structures from raw NMR spectra with the deep learning technique ARTINA', *Nature Communications*, 13(1): 6151.

Kühlbrandt, W. 2014. 'The resolution revolution', *Science*, 343(6178): 1443-1444.

Lavecchia, A. and Di Giovanni, C. 2013. 'Virtual screening strategies in drug discovery: a critical review',*Current Medicinal Chemistry,* 20(23): 2839-2860.

Lazim, R., Suh, D. and Choi, S. 2020. 'Advances in molecular dynamics simulations and enhanced sampling methods for the study of protein systems',*International Journal of Molecular Sciences,* 21(17): 6339.

Lengyel, J., Hnath, E., Storms, M. and Wohlfarth, T. 2014. 'Towards an integrative structural biology approach: combining Cryo-TEM, X-ray crystallography and NMR', *Journal of Structural and Functional Genomics*, 15: 117-124.

Levy, A., Chan, E. R., Fridovich-Keil, S., Poitevin, F., Zhong, E. D. and Wetzstein, G. 2024. 'Solving Inverse Problems in Protein Space Using Diffusion-Based Priors', *arXiv preprint arXiv:2406.04239*.

Li, J., Fu, A. and Zhang, L. 2019. 'An overview of scoring functions used for protein–ligand interactions in molecular docking',*Interdisciplinary Sciences: Computational Life Sciences*, 11: 320-328.

Maia, E. H. B., Assis, L. C., De Oliveira, T. A., Da Silva, A. M. and Taranto, A. G. 2020. Structure-based virtual screening: from classical to artificial intelligence. *Frontiers in Chemistry,* 8: 343.

Majorek, K. A., Zimmerman, M. D., Grabowski, M., Shabalin, I. G., Zheng, H. and Minor, W. 2020. 'Assessment of crystallographic structure quality and protein–ligand complex structure validation',*Structural Biology in Drug Discovery: Methods, Techniques, and Practices*, 253-275.

Micsonai, A., Moussong, E., Wien, F., Boros, E., Vadászi, H., Murvai, N. and Kardos, J. 2022. 'BeStSel: a web server for secondary structure and fold prediction for protein CD spectroscopy',*Nucleic Acids Research*, 50(W1): W90-W98.

Moreira, I. S., Fernandes, P. A. and Ramos, M. J. 2007. 'Computational alanine scanning mutagenesis—an improved methodological approach',*Journal of Computational Chemistry*, 28(3): 644-654.

Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K. and Olson, A. J. 1998. 'Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function',*Journal of Computational Chemistry,* 19(14): 1639-1662.

Mouchlis, V. D., Afantitis, A., Serra, A., Fratello, M., Papadiamantis, A. G., Aidinis, V. and Melagraki, G. 2021. 'Advances in de novo drug design: from conventional to machine learning methods',*International Journal of Molecular Sciences*, 22(4): 1676.

Mukesh, B. and Rakesh, K. 2011. 'Molecular docking: a review',*International Journal of Research in Ayurveda Pharmacy*, 2(6): 1746-51.

Murugan, N. A., Priya, G. R., Sastry, G. N. and Markidis, S. 2022. 'Artificial intelligence in virtual screening: Models versus experiments',*Drug Discovery Today*, 27(7): 1913-1923.

Ormeño, F. and General, I. J. 2024. Convergence and equilibrium in molecular dynamics simulations. *Communications Chemistry,* 7(1): 26.

Pakamwong, B., Thongdee, P., Kamsri, B., Phusi, N., Taveepanich, S., Chayajarus, K. and Pungpo, P. 2024. 'Ligand-based virtual screening for discovery of indole derivatives

as potent DNA gyrase ATPase inhibitors active against Mycobacterium tuberculosis and hit validation by biological assays',*Journal of Chemical Information and Modeling*, 64(15): 5991-6002.

Pant, S., Verma, S., Pathak, R. K. and Singh, D. B. 2022. 'Structure-based drug designing', In *Bioinformatics,* Academic Press: 219-231.

Papageorgiou, A. C., Poudel, N. and Mattsson, J. 2021. 'Protein structure analysis and validation with X-ray crystallography', *Protein Downstream Processing: Design, Development and Application of High and Low-Resolution Methods*, 377-404.Pelton, J. T. and McLean, L. R. 2000. 'Spectroscopic methods for analysis of protein secondary structure',*Analytical Biochemistry*, 277(2): 167-176.

Peluso, P., Dessì, A., Dallocchio, R., Mamane, V. and Cossu, S. 2019. 'Recent studies of docking and molecular dynamics simulation for liquid‐phase enantioseparations',*Electrophoresis,* 40(15): 1881-1896.

Phillips, J. C., Hardy, D. J., Maia, J. D., Stone, J. E., Ribeiro, J. V., Bernardi, R. C. and Tajkhorshid, E. 2020. 'Scalable molecular dynamics on CPU and GPU architectures with NAMD',*The Journal of Chemical Physics*, 153(4).

Picchi, E. (2023). 'The role of microscale thermophoresis (MST) in drug discovery of protein kinase inhibitors', https://hdl/handle.net/1889/5355.

Puiu, M. and Bala, C. 2016. 'SPR and SPR imaging: recent trends in developing nanodevices for detection and real-time monitoring of biomolecular events',*Sensors*, 16(6): 870.

Punjani, A., Rubinstein, J. L., Fleet, D. J. and Brubaker, M. A. 2017. 'cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination', *Nature Methods*, 14(3): 290-296.

Pyzer-Knapp, E. O., Suh, C., Gómez-Bombarelli, R., Aguilera-Iparraguirre, J. and Aspuru-Guzik, A. 2015. 'What is high-throughput virtual screening? A perspective from organic materials discovery',*Annual Review of Materials Research*, 45(1): 195-216.

Rácz, A., Mihalovits, L. M., Bajusz, D., Héberger, K. and Miranda-Quintana, R. A. 2022. 'Molecular dynamics simulations and diversity selection by extended continuous similarity indices',*Journal of Chemical Information and Modeling,* 62(14): 3415-3425.

Rakshit, G., Murtuja, S., Kumar, B. K., Murugesan, S. and Jayaprakash, V. 2022. 'Structure-based drug design (SBDD)', In *Computer Aided Drug Design (CADD): from ligand-based methods to structure-based approaches,* Elsevier: 181-229.

Rawat, W. and Wang, Z. 2017. 'Deep convolutional neural networks for image classification: A comprehensive review', *Neural Computation*, 29(9): 2352-2449.

Riccardi, L., Genna, V. and De Vivo, M. 2018. 'Metal–ligand interactions in drug design',*Nature Reviews Chemistry*, 2(7): 100-112.

Ritzefeld, M. and Sewald, N. 2012. 'Real‐time analysis of specific protein‐DNA interactions with surface plasmon resonance', *Journal of Amino Acids*, 2012(1): 816032.

Salmaso, V., & Moro, S. (2018). Bridging molecular docking to molecular dynamics in exploring ligand-protein recognition process: An overview. *Frontiers in Pharmacology*, 9, 923.

Salo-Ahen, O. M., Alanko, I., Bhadane, R., Bonvin, A. M., Honorato, R. V., Hossain, S. and Vanmeert, M. 2020. 'Molecular dynamics simulations in drug discovery and pharmaceutical development*', Processes, 9*(1): 71.

Sanchez-Garcia, R., Gomez-Blanco, J., Cuervo, A., Carazo, J. M., Sorzano, C. O. S. and Vargas, J. 2021. 'DeepEMhancer: a deep learning solution for cryo-EM volume post-processing', *Communications Biology*, 4(1): 874.

Sarkar, C., Das, B., Rawat, V. S., Wahlang, J. B., Nongpiur, A., Tiewsoh, I. and Sony, H. T. 2023. 'Artificial intelligence and machine learning technology driven modern drug discovery and development',*International Journal of Molecular Sciences*, 24(3): 2026.

Seeliger, D. and de Groot, B. L. 2010. 'Ligand docking and binding site analysis with PyMOL and Autodock/Vina',*Journal of Computer-aided Molecular Design,* 24(5): 417-422.

Shoichet, B. K., McGovern, S. L., Wei, B. and Irwin, J. J. 2002. 'Lead discovery using molecular docking',*Current Opinion in Chemical Biology,* 6(4): 439-446.

Shukla, V. K., Heller, G. T. and Hansen, D. F. 2023. 'Biomolecular NMR spectroscopy in the era of artificial intelligence',*Structure*, 31(11): 1360-1374.

Sil, S., Datta, I. and Basu, S. 2024. 'Use of AI-methods over MD simulations in the sampling of Conformational Ensembles in IDPs', *Frontier in Molecular Biosciences*, 12: https://doi.org/10.3389/fmolb.2025.1542267

Smirnovienė, J., Baranauskienė, L., Zubrienė, A. and Matulis, D. 2021. 'A standard operating procedure for an enzymatic activity inhibition assay',*European Biophysics Journal*, 50: 345-352.

Srivastava, A., Nagai, T., Srivastava, A., Miyashita, O. and Tama, F. 2018. Role of computational methods in going beyond X-ray crystallography to explore protein structure and dynamics. *International journal of molecular sciences*, 19(11): 3401.

Sumathi, S., Suganya, K., Swathi, K., Sudha, B., Poornima, A., Varghese, C. A. and Aswathy, R. 2023. 'A review on deep learning-driven drug discovery: strategies, tools and applications',*Current Pharmaceutical Design*, 29(13): 1013-1025.

Tanwar, A. K., Sengar, N., Mase, N. and Singh, I. P. 2024. 'Tetrahydroisoquinolines–an updated patent review for cancer treatment (2016–present)',*Expert Opinion on Therapeutic Patents*, 34(10): 873-906.

Tarighi, P., Eftekhari, S., Chizari, M., Sabernavaei, M., Jafari, D. and Mirzabeigi, P. 2021. 'A review of potential suggested drugs for coronavirus disease (COVID-19) treatment',*European Journal of Pharmacology*, *895*: 173890.

Tiwary, P., Herron, L., John, R., Lee, S., Sanwal, D. and Wang, R. 2024. 'Generative artificial intelligence for computational chemistry: a roadmap to predicting emergent phenomena',*arXiv preprint arXiv:2409.03118*.

Verdonk, M. L., Cole, J. C., Hartshorn, M. J., Murray, C. W. and Taylor, R. D. 2003. 'Improved protein–ligand docking using GOLD',*Proteins: Structure, Function, and Bioinformatics*, 52(4): 609-623.

Verkhivker, G., Alshahrani, M., Gupta, G., Xiao, S. and Tao, P. 2023. 'From deep mutational mapping of allosteric protein landscapes to deep learning of allostery and hidden allosteric sites: zooming in on "allosteric intersection" of biochemical and big data approaches',*International Journal of Molecular Sciences*, 24(9): 7747.

Vilas, J. L., Carazo, J. M. and Sorzano, C. O. S. 2022. 'Emerging themes in CryoEM─ Single particle analysis image processing',*Chemical Reviews*:122(17): 13915-13951.

Vollmar, M. and Evans, G. 2021. 'Machine learning applications in macromolecular X-ray crystallography', *Crystallography Reviews*, 27(2): 54-101.

Wang, Y. L., Wang, F., Shi, X. X., Jia, C. Y., Wu, F. X., Hao, G. F., and Yang, G. F. 2021. 'Cloud 3D-QSAR: a web tool for the development of quantitative structure–activity relationship models in drug discovery',*Briefings in Bioinformatics*, 22(4): bbaa276.

Yadav, V. and Tonk, R. K. 2022. Ligand-based drug design (LBDD). In *Computer Aided Drug Design (CADD): From Ligand-Based Methods to Structure-Based Approaches* Elsevier: pp. 57-99.

Yamashita, F. and Hashida, M. 2004. '*In silico* approaches for predicting ADME properties of drugs',*Drug Metabolism and Pharmacokinetics,* 19(5): 327-338.