Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

How exascale computing can shape drug design: A perspective from multiscale QM/MM molecular dynamics simulations and machine learning-aided enhanced sampling algorithms

Giulia Rossetti[1,2,3] and Davide Mandelli[1]

Abstract

Molecular simulations are an essential asset in the first steps of drug design campaigns. However, the requirement of high-throughput limits applications mainly to qualitative approaches with low computational cost, but also low accuracy. Unlocking the potential of more rigorous quantum mechanical/molecular mechanics (QM/MM) models combined with molecular dynamics-based free energy techniques could have a tremendous impact. Indeed, these two relatively old techniques are emerging as promising methods in the field. This has been favored by the exponential growth of computer power and the proliferation of powerful data-driven methods. Here, we briefly review recent advances and applications, and give our perspective on the impact that QM/MM and free-energy methods combined with machine learning-aided algorithms can have on drug design.

Addresses

[1] Computational Biomedicine, Institute of Advanced Simulations IAS-5/Institute for Neuroscience and Medicine INM-9, Forschungszentrum Jülich GmbH, Jülich 52428, Germany

[2] Department of Neurology, University Hospital Aachen (UKA), RWTH Aachen University, Aachen, Germany

[3] Jülich Supercomputing Centre (JSC), Forschungszentrum Jülich GmbH, Jülich 52428, Germany

Corresponding author: Mandelli, Davide (d.mandelli@fz-juelich.de)
(Rossetti G.)

Current Opinion in Structural Biology 2024, **86**:102814

This review comes from a themed issue on **Theory and Simulation/Computational Methods (2024)**

Edited by **Paolo Carloni** and **Karissa Y. Sanbonmatsu**

For complete overview of the section, please refer the article collection - Theory and Simulation/Computational Methods (2024)

Available online 16 April 2024

https://doi.org/10.1016/j.sbi.2024.102814

Introduction

Computational approaches are progressively shaping drug design in both academia and pharma [1]. However, the complexity of many protein–ligand interactions stands against the accuracy and efficiency of historically used "fast" approaches such as classical molecular simulations and bioinformatics methods based on (semi-) empirical force fields (FFs). Two methods have emerged as key in drug design and discovery for such challenging cases: free-energy algorithms and hybrid quantum mechanics/molecular mechanics (QM/MM) simulations, as well as a combination of the two.

The advantage of free energy methods is in their ability to accelerate by orders of magnitudes the exploration of the configurational ensemble, in particular when kinetic bottlenecks are present, which is the typical scenario encountered in most applications. They allow reconstructing free energy profiles along the most relevant (slow) degrees of freedom, computing binding affinities, as well as extracting kinetics rates [2,3].

The advantage of using accurate ab-initio QM models (based, e.g., on density functional theory (DFT)) is evident whenever charge transfer and polarization effects are dominant. This encompasses many important cases: (i) proteins in which metals are involved in the binding process [4]; (ii) hydrophobic or stacking interactions, where dispersion forces play a significant role [5]; (iii) ligand binding involving the formation of covalent bonds with a protein reactive residues [6]; (iv) ligand binding involving proton transfer, tautomerization events or polarization effects [7].

Historically, QM/MM calculations have been designed specifically to model chemical reactions in biological systems and are traditionally used in the design of transition state-like inhibitors for enzymatic reactions involved in drug metabolism [8]. Free-energy methods, on the other hand, already find widespread use for the estimate of binding affinities [2]. However, both techniques have not yet achieved a broad impact on rational drug design mostly due to the complex and system-dependent set-up, as well as their high computational cost.

Quantum mechanics/molecular mechanics simulations

In a recent perspective, Bolnhyk et al. [9] highlighted the role that High Performance Computing (HPC) can

have in expanding the boundaries of ligand−target modeling. This can be achieved by developing dedicated software able to exploit the unprecedented computational power currently offered by HPC infrastructures. In the following, we briefly review how recent advancements in hardware and software have impacted the performance of QM/MM MD simulations and present applications of this method both in virtual screening campaigns and in drug design.

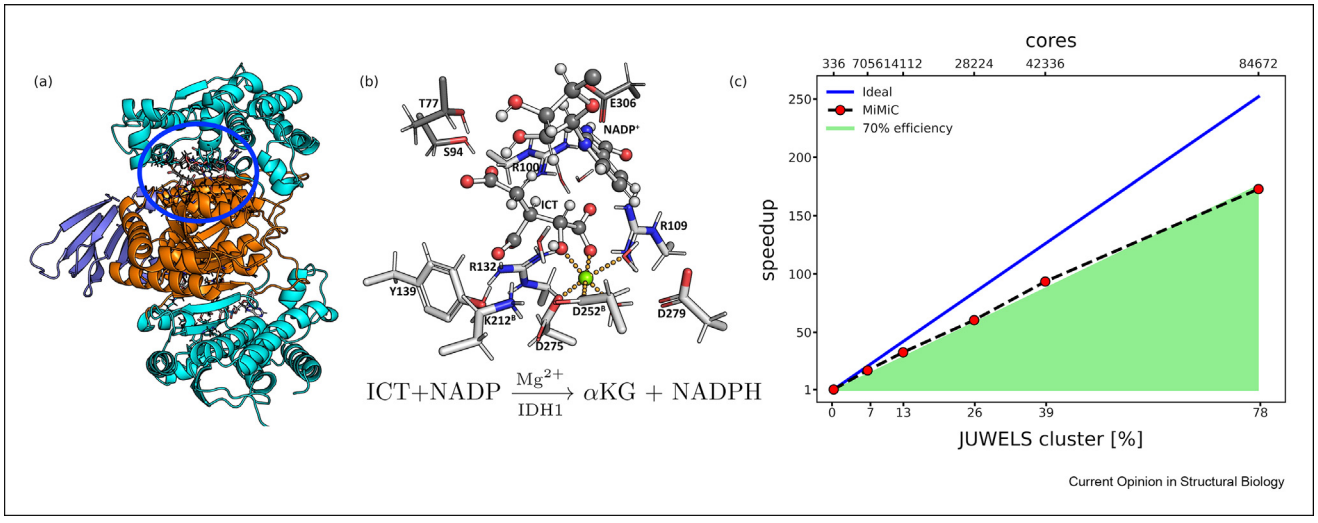
Hardware and software developments

Modern supercomputers are based on thousands of interconnected compute nodes. Exascale machines reach unprecedented computational power via heterogeneous architectures, where nodes feature standard CPUs equipped with accelerators (e.g., Graphical Processing Units (GPUs)) and are coupled through extremely fast interconnects [10]. Fully exploiting this technology thus requires designing dedicated software able to scale on thousands of (accelerated) nodes. Recent advances in QM/MM software development have demonstrated efficient scaling up to >80 kcores, allowing performing ∼0.7 ps/day of QM/MM MD at the B3LYP [11] level of the IDH1 enzyme, a target for the early diagnosis and treatment of brain cancer [12] (see Figure 1). This was made possible by the use of an efficient framework, based on a multiple program multiple data approach, that interfaces existing QM and MM software, minimizing communication and preserving the performance of the QM layer, which ultimately dictates the scaling [13,14]. The TeraChem [15] and Quick [16] QM/MM codes recently demonstrated the enormous impact GPUs can have on throughput. Benchmarks showed performances up to ∼2 ps/day at the B3LYP level running on as few as 2 GPUs (see Table 1). Indeed, it has already been shown how exploiting GPU-based hardware makes it feasible to perform virtual screening of a refined chemical library at quantum mechanical level to identify lead compounds with improved accuracy [17].

QM/MM simulations for virtual screening and drug design

QM/MM simulations approaches with relatively high-throughput (mainly point energy calculations and structural optimizations) can improve the success rate of virtual screening campaigns by refining the starting geometries for docking, building more accurate charge models, and improving the accuracy of scoring functions [18]. In recent contributions, Borbulevych et al. successfully included an automated approach for macromolecular refinement of X-ray structures based on a two layer QM/MM ONIOM model able to yield superior quality protein:ligand complex structures compared to conventional methods [19]. Molani et al. developed a protocol to obtain effective protein-ligand binding free energy predictions using QM/MM simulations to substitute atomic charges of FFs with quantum-mechanically recalculated ones [20]. Jin et al. combined NMR chemical shifts calculations from the

Figure 1



(**a**) The model wild type isocitrate dehydrogenase (wtIDH1) enzyme studied via QM/MM MD simulations in Ref. [12]. The circle indicates one of the two catalytic sites used as the QM region. (**b**) Details of the QM region including the isocitrate (ICT) substrate and the NADP cofactor that participate in the reaction leading to the production of alpha keto-glutarate (alphaKG) and NADPH. The presence of a magnesium ion makes the use of accurate *ab initio* QM methods essential. (**c**) Strong scaling of the MiMiC QM/MM interface on the pre-exascale JUWELS cluster at the Jülich Supercomputing Center, showing parallel efficiency above 70% up to 84,672 cores, corresponding to ~80% of the total resources of that machine.

Table 1

Performances of the MiMiC [12], Terachem Protocol Buffer (TCBP) [15] and QUICK/AMBER [16] interfaces running QM/MM MD simulations using density functional theory at the B3LYP [11] level using QM regions of comparable size. IDH1: isocitrate dehydrogenase enzyme. PYP: photoactive yellow protein. We report the published peak performances and the corresponding optimal computational resources in terms of number of standard CPU nodes (for MiMiC) and number of GPUs (for TCPB and QUICK/AMBER).

QM code	MM code	Interface	GPU	System	QM atoms	Theory	CPU Nodes* or GPUs**	ps/day
CPMD	GROMACS	MiMiC	NO	IDH1	141	B3LYP PW	1764*	0.74
Terachem	AMBER	TCPB	YES	PYP	159	B3LYP/def2-SVP	2**	1.83
QUICK	AMBER	QUICK/AMBER	YES	PYP	159	B3LYP/def2-SVP	4**	1.13

automated fragmentation QM/MM method within a scoring function that can distinguish the native ligand pose from decoy structures, suggesting that NMR information can provide an accurate and efficient platform for protein-ligand binding structure prediction [21]. Notably, highlighting the impact that data-driven methods can have at different levels of the pipeline, Gupta and Zhou designed a machine learning-enabled workflow for large-scale virtual drug screening that combines clustering algorithms with docking based on a neuronal network-based pseudo-QM/MM potential trained on QM DFT data [22]. QM/MM MD simulations, on the other hand, are being successfully applied in drug design projects where sampling is needed to obtain detailed mechanistic insights. A number of recent publications showcased the increasing role that these methods (predominantly based on the umbrella sampling technique [23]) can have in the design of drug binding to metalloenzyme, covalent drugs, TS-like inhibitors, and to study the effects of drug-resistant mutations [24–29].

Molecular dynamics-based free energy methods

Drug–protein unbinding processes occur on long time scales, typically ranging from millisecond to hours, depending on the nature and the strength of the interaction between the ligand and target. Being able to fast and reliably predict free energy changes using numerical simulations has long been extremely attractive for drug design. Indeed, free-energy calculations can be used to support a variety of tasks in drug design, not only guiding positional analogue scanning and validating binding mode hypotheses, which are key in virtual screening workflows; but free-energy calculations can also be used in other tasks of the drug design process, like scaffold hopping or fragments-to-leads optimization.

Different enhanced sampling techniques have been proposed to predict free-energy barriers and uncover the kinetics of ligand-binding interaction [2,30]. These include free-energy perturbation, umbrella sampling, replica exchange, metadynamics, steered MD, accelerated MD, milestoning, transition-path sampling, and several combinations among them [23,31–38].

Here we will discuss two examples of how Machine Learning speeds up relative and absolute binding free-energy approaches, respectively.

Relative binding free-energy (RBFE) approaches and machine learning

Iteratively training automated ML (AutoML) models with a limited number of RBFE calculations has been suggested as an alternative to the computationally intensive traditional RBFE approaches. This strategy was for instance implemented in Gusev et al. [39], where an iterative Amber GPU- RBFE calculations were conducted for a small number of SARS-CoV-2 papain-like protease binders: RBFE were only calculated for the 10% of the compounds and used as dependent variables of an AutoML workflow that was then implemented for the remaining molecules, demonstrating the utility of the approach for the rapid exploration of large chemical spaces.

Also, a collaboration between Google Research and Relay Therapeutics showed how an exhaustive study of RBFE calculations on 10,000 congeneric tyrosine kinase 2 (TYK2) inhibitors can be conducted using RDKit Morgan fingerprints as descriptors and deploying different active learning (AL) strategies [40]. Independently of the specific ML algorithm used with this strategy, only 6% of the 10,000 TYK2 inhibitors was explicitly calculated with an RBFE protocol, while the remaining compounds were predicted using the AL approach identifying 75 of the top 100 scorers.

Absolute binding free-energy and machine learning

A key factor for most absolute binding free-energy approaches is the identification of a collective variable (CV), representing a physical pathway, that allows the calculation of the free energy profile. Hence, correct identification of appropriate CVs becomes a problem, with very few practical ways to build them properly. To automate this process, a number of procedures have

been developed, many of them based on machine learning (ML) approaches [41]. For instance Deep Linear Discriminant Analysis (Deep-LDA) [42] rather than finding the ideal CVs, focused on sampling the transitions between different metastable basins. Autoencoders [43,44] approaches, instead obtain a good set of CVs using general coordinates of a system in dimensionality reduction schemes. The network is optimized to represent the configurational state of a system in a lower dimensional space. Recently, Deep-LDA was used to investigate the non-covalent interactions between a calixarene host and different guest molecules: not only protein-ligand interactions were considered, but also the role of water molecules was taken into account to identify the CVs and calculate the binding free energies [44].

Another challenge of binding free-energy is the complex relationship with the expected efficacy in vitro/vivo. Namely, free energy dictates the strength of interactions between a ligand and its target, but it does not provide any information of the pharmacological activity of the drug in *vitro/in vivo* experiments. Such a relationship becomes even more challenging in the presence of allosteric compounds that impact on the efficacy of the main/orthosteric substrate, while binding at a distal site. In this framework, ML was exploited to identify the links between different degrees of allosteric inhibition of the ATPase function in the molecular chaperone TRAP1 and local dynamic patterns of the corresponding allosteric ligand over ns-μs -long MD simulations [45]. The authors were able to prove that it is possible to discriminate and predict the functional effect of allosteric ligands on a given target by combining MD and ML, thus complementing affinity data.

Kinetics calculations and machine learning

A recent paradigm shift in drug design highlighted the importance of modulating residence time as a key objective in addition to strong binding affinity [46]. Free-energy approaches were already used in the past to predict the ligand−protein unbinding kinetics: for instance metadynamics was implemented for predicting the k_{off} of p38 MAP kinase bound to type II inhibitors, but depending on the set of CVs chosen, different values for k_{off} were obtained [47]. It was also shown later on that combining metadynamics with QM/MM simulations allowed a more accurate prediction of the unbinding kinetics [48], but the dependence on the CVs was not solved. In this respect, ML approaches were recently combined with free-energy calculations to solve this issue: specifically, it was shown how a supervised ML approach can be implemented using as inputs unbiased "downhill" trajectories initiated near the transition state (TS) ensemble of the unbinding path. The model was then exploited to identify key ligand−protein interactions driving the system through the TS [49] and the free energy barrier for the ligand unbinding process, therefore providing quantitative information about the residence time of a specific ligand. The key role of ML is in allowing a combination of automated iterative addition and removal of the collective variables determining an unbinding trajectory to identify the relevant interactions during the unbinding process. This allows the methods to provide consistent free energy barriers, despite unbinding trajectories showing different paths between different replicas for the same system.

Perspectives

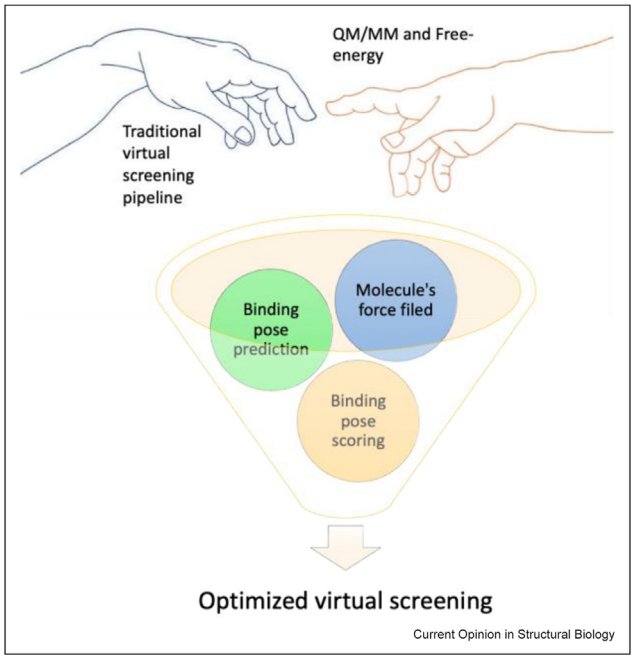
Absolute biding free energies from apo-structures

Machine learning approaches to automatize CV selection have brought significant improvements to CV-based methods for free-energy calculations. However, especially for deep-buried binding pockets, they still need training on the ligand−protein complex, i.e. holo structure of the target protein. As such, they might not perform well when only the native unbound conformation (or apo) is available and/or the bound one is obtained by docking of the ligand on an apo-structure, which is known to be less reliable [50]. Enhanced sampling of pocket shape was proposed as a viable solution by Vargiu and co-worker [51], with remarkable results for a chosen ensemble of proteins undergoing different extents of conformational changes upon ligand binding. More generally, it would be very interesting to explore the possibility to develop ML approaches trained on dedicated data-sets of apo-proteins [52] to develop CVs able to capture (un)binding processes when no holo-structures are available.

Kinetics predictions and machine learning/molecular mechanics potential

Making QM/MM MD and free energy methods a standard for pharmacology would represent a breakthrough under many aspects (see Figure 2). For virtual screening campaigns, by substantially increasing the efficiency in discriminating true from false positives. For rational drug design, by providing high quality structural information and microscopic insights for the engineering of novel ligands. Furthermore, using transferable *ab initio* DFT QM/MM would avoid the burden of re-parametrization needed by semi-empirical and classical FFs when moving to new protein/ligand complexes. Of particular appeal is the possibility to predict with QM accuracy ligands' residence times, which have been shown to strongly correlate to drug efficacy [53]. In this respect, we currently witness a gap between the methodological advancement in the field of MD-based enhanced sampling methods (helped by ML) [3,54] and the limitations of QM/MM MD simulations. In fact, in absence of well-established adaptive QM/MM algorithms, large QM regions are needed to describe ligand unbinding, especially from deep-buried pockets. Exascale machines and ML can be leveraged to overcome this obstacle. On one

Figure 2



The advent of the exascale era brings an opportunity to develop high-throughput *in silico* pipelines combining traditional approaches and data-driven methods with more rigorous QM/MM MD-based free energy calculations for optimized drug design.

hand, coupling general QM/MM frameworks like MiMiC with highly scalable DFT codes that can run on thousands of GPUs [55–57] can enable large scale simulations including thousands of atoms in the QM region. On the other hand, the development of hybrid ML/MM potentials [21,58–63] trained over such data can significantly extend the time scale of simulations to improve statistical accuracy. Finally, data-driven methods applied to free energy perturbation calculations [64,65] can be used to correct for the loss of accuracy of ML/MM potentials. At the moment, proof-of-concepts of these approaches have been already published and real world applications can be foreseen in the near future.

Applications to nucleic acids

DNA and RNA play essential roles in many biological processes and represent an important class of drug targets [66]. Small molecules interact with nucleic acids using different mechanisms: intercalation, cross-linkage, strand-cleavage, and reading-molecules. Unfortunately, if from a structural point of view nucleic acids result in being suitable for target-based drug discovery, the high prevalence of charges and metal ions poses a major challenge: In solution, the negative phosphates are normally neutralized by counterions as Sodium ions (Na+). Their mobile nature is difficult to handle with traditional *in silico* drug design approaches like molecular docking. Also, the presence of the phosphate negative charges brings along a multitude of metal ions and water molecules strongly interacting to each other. A particular challenge is represented by the water polarization effect caused by the frequent presence of charges and ions, which might reflect their effect at several layers of distances. Therefore, to disrupt the hydration shell ligands need high polarity when considering the polyanionic character of the nucleic acid molecules. All these issues impact a number of factors that are not limited to wrong FF parameters. QM methods are theoretically exact, capturing the underlying physics of the system and accounting for all missing effects in FFs (such as electronic polarization, covalent-bond formation, and coupling among terms). In this respect the results obtained using QM approaches are very encouraging, but still different sources of error should be addressed in order to improve accuracy and predictability of these methods: (i) they are still system-dependent; thus, further validation and benchmarking are needed; (ii) in spite of the progress in computational speed, most QM applications to drug discovery cannot still be used in industrial settings, highlighting the need for further optimized codes, especially those using GPUs and exascale.

Applications to covalent ligands

Covalent drugs are currently showing great promise for systems that are normally difficult to target with small molecules therapies [6]. This renewed interest has spurred the refinement of existing computational methods as well as the design of new ones, expanding the toolbox for discovery and optimization of selective and effective covalent inhibitors. Current covalent docking methods mostly focus on modeling the conformation of covalent inhibitors in the bound covalent complex but they ignore the energetics of covalent bond formation, enforcing an idealized bond and scoring docked complexes primarily on the noncovalent interactions [67]. Retrospective comparisons of such docking methods indeed show that they are effective in reproducing observed covalent complexes about half of the time and that the success is system-dependent, i.e. it depends on the size and flexibility of the ligands, as well as the accessibility and flexibility of the target amino acid side chain, with significant differences between different types of reactive group [68,69]. Methods such as QM/MM would be able to quantitatively characterize the entire process of binding and covalent attachment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data were used for the research described in the article.

Acknowledgements

DM and GR acknowledges support from the Helmholtz European Partnering program ("Innovative high-performance computing approaches for molecular neuromedicine") and from the European Union's HORIZON MSCA Doctoral Networks programme, under Grant Agreement No. 101072344, project AQTIVATE (Advanced computing, QuanTum algorIthms and data-driVen Approaches for science, Technology and Engineering). Open Access publication fee funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 491111487. GR acknoledge the Federal Ministry of Education and Research (BMBF) and the state of North Rhine-Westphalia as part of the NHR Program.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. ** Sadybekov AV, Katritch V: **Computational approaches streamlining drug discovery**. *Nature* Apr. 2023, **616**:673–685, https://doi.org/10.1038/s41586-023-05905-z.

Sadybekov and Katritch review recent advances in drug discovery technologies, highlighting the role that synergistic computational approaches exploiting modern accelerated computing and data-driven methods can play in the cost-effective development of safer and more effective small molecule treatments.

2. Decherchi S, Cavalli A: **Thermodynamics and kinetics of drug-target binding by molecular simulation**. *Chem Rev* 2020, **120**: 12788–12833, https://doi.org/10.1021/acs.chemrev.0c00534.

3. Ahmad K, Rizzi A, Capelli R, Mandelli D, Lyu W, Carloni P: **Enhanced-sampling simulations for the estimation of ligand binding kinetics: current status and perspective**. *Front Mol Biosci* 2022, **9**:1–17, https://doi.org/10.3389/fmolb.2022.899805.

4. Riccardi L, Genna V, De Vivo M: **Metal–ligand interactions in drug design**. *Nat Rev Chem* 2018, **2**:100–112, https://doi.org/10.1038/s41570-018-0018-6.

5. Crespo A, Rodriguez-Granillo A, Lim VT: **Quantum-mechanics methodologies in drug discovery: applications of docking and scoring in lead optimization**. *Curr Top Med Chem* 2017, **17**: 2663–2680, https://doi.org/10.2174/1568026617666170707120609.

6. Mihalovits LM, Ferenczy GG, Keserű GM: **The role of quantum chemistry in covalent inhibitor design**. *Int J Quant Chem* 2022, **122**:1–17, https://doi.org/10.1002/qua.26768.

7. Capelli R, *et al.*: **Accuracy of molecular simulation-based predictions of k off values: a metadynamics study**. *J Phys Chem Lett* 2020, **11**:6373–6381, https://doi.org/10.1021/acs.jpclett.0c00999.

8. P450 enzymes: their structure, reactivity, and selectivity – modeled by QM/MM calculations | Chem Rev." Accessed: October. 13, 2023. [Online]. Available: https://pubs.acs.org/doi/10.1021/cr900121s.

9. Bolnykh V, Rossetti G, Rothlisberger U, Carloni P: **Expanding the boundaries of ligand–target modeling by exascale calculations**. *WIREs Comput Mol Sci* 2021, **11**:1–17, https://doi.org/10.1002/wcms.1535.

10. Schneider D: **The exascale era is upon us: the frontier supercomputer may be the first to reach 1,000,000,000,000,000,000 operations per second**. *IEEE Spectr* 2022, **59**:34–35, https://doi.org/10.1109/MSPEC.2022.9676353.

11. Becke AD: **Density-functional thermochemistry. II. The effect of the Perdew–Wang generalized-gradient correlation correction**. *J Chem Phys* 1992, **97**:9173–9177, https://doi.org/10.1063/1.463343.

12. * Raghavan B, *et al.*: **Drug design in the exascale era: a perspective from massively parallel QM/MM simulations**. *J Chem Inf Model* 2023, **63**:3647–3658, https://doi.org/10.1021/acs.jcim.3c00557.

The work of Raghavan and co-authors reports the most scalable DFT QM/MM molecular dynamics simulation of a biological system documented so far, running on more than 80 thousands standard cores with a parallel efficiency above 70%, using the MiMiC QM/MM interface [13,14]. The paper investigates enzymes of pharmacological relevance to showcase how extremely scalable software – able to exploit massively parallel architectures – combined with enhanced-sampling techniques can be leveraged to extend the time-scale of QM/MM MD for applications in drug design.

13. Olsen JMH, *et al.*: **MiMiC: a novel framework for multiscale modeling in computational chemistry**. *J Chem Theor Comput* Jun. 2019, **15**:3810–3823, https://doi.org/10.1021/acs.jctc.9b00093.

14. Bolnykh V, *et al.*: **Extreme scalability of DFT-based QM/MM MD simulations using MiMiC**. *J Chem Theor Comput* 2019, **15**: 5601–5613, https://doi.org/10.1021/acs.jctc.9b00093.

15. * Cruzeiro VWD, Wang Y, Pieri E, Hohenstein EG, Martínez TJ: **TeraChem protocol buffers (TCPB): accelerating QM and QM/MM simulations with a client–server model**. *J Chem Phys* 2023, **158**:44801, https://doi.org/10.1063/5.0130886.

The work of Cruzeiro et al. reports benchmark QM/MM molecular dynamics simulations using the TeraChem protocol buffer interface, a state-of-the-art GPU-ready QM/MM software able to deliver up to few ps/day of performance when running highly accurate DFT-level simulations on as few as two GPUs. The paper demonstrates the impact accelerators like GPUs can have in boosting the performance of software running QM/MM molecular dynamics of complex biological systems.

16. * Manathunga M, Aktulga HM, Götz AW, Merz KM: **Quantum mechanics/molecular mechanics simulations on NVIDIA and AMD graphics processing units**. *J Chem Inf Model* Feb. 2023, **63**:711–717, https://doi.org/10.1021/acs.jcim.2c01505.

The work of Manathunga et al. reports benchmark QM/MM molecular dynamics simulations using the QUICK/AMBER interface, a state-of-the-art GPU-ready QM/MM software able to deliver up to few ps/day of performance when running highly accurate DFT-level simulations on as few as four GPUs. The paper demonstrates the impact accelerators like GPUs can have in boosting the performance of software running QM/MM molecular dynamics of complex biological systems.

17. Shi M, Xu D, Zeng J: **GPU accelerated quantum virtual screening: application for the natural inhibitors of New Delhi metalloprotein (NDM-1)**. *Front Chem* 2018, **6** [Online]. Available: https://www.frontiersin.org/articles/10.3389/fchem.2018.00564. Accessed 13 October 2023.

18. Kar RK: **Benefits of hybrid QM/MM over traditional classical mechanics in pharmaceutical systems**. *Drug Discov Today* 2023, **28**:103374, https://doi.org/10.1016/j.drudis.2022.103374.

19. Borbulevych OY, Martin RI, Westerhoff LM: **The critical role of QM/MM X-ray refinement and accurate tautomer/protomer determination in structure-based drug design**. *J Comput Aided Mol Des* 2021, **35**:433–451, https://doi.org/10.1007/s10822-020-00354-6.

20. Molani F, Webb S, Cho AE: **Combining QM/MM calculations with classical mining minima to predict protein–ligand binding free energy**. *J Chem Inf Model* 2023, **63**:2728–2734, https://doi.org/10.1021/acs.jcim.2c01637.

21. Jin X, Zhu T, Zhang JZH, He X: **Automated fragmentation QM/MM calculation of NMR chemical shifts for protein-ligand complexes**. *Front Chem* 2018, **6**:1–11, https://doi.org/10.3389/fchem.2018.00150.

22. * Gupta A, Zhou H-X: **Machine learning-enabled pipeline for large-scale virtual drug screening**. *J Chem Inf Model* Sep. 2021, **61**:4236–4244, https://doi.org/10.1021/acs.jcim.1c00710.

The work of Gupta and Zhou demonstrates the use of data-driven methods at different levels of a drug design pipeline to deliver a large-scale virtual drug screening workflow. These include machine learning algorithms to trim a large library of ligands, the use of machine learning/molecular mechanics potentials for improved estimates of ligand-RMSD and MM/PBSA binding free energies, and a dense neural network trained for discriminating true and false positives.

23. Torrie GM, Valleau JP: **Nonphysical sampling distributions in Monte Carlo free-energy estimation: umbrella sampling**.

J Comput Phys Feb. 1977, **23**:187–199, https://doi.org/10.1016/0021-9991(77)90121-8.

24. McGregor NGS, *et al.*: **Cysteine nucleophiles in glycosidase catalysis: application of a covalent β-l-arabinofuranosidase inhibitor**. *Angew Chem Int Ed* 2021, **60**:5754–5758, https://doi.org/10.1002/anie.202013920.

25. Arafet K, *et al.*: **Mechanism of inhibition of SARS-CoV-2 Mpro by N3 peptidyl Michael acceptor explained by QM/MM simulations and design of new derivatives with tunable chemical reactivity**. *Chem Sci* 2021, **12**:1433–1444, https://doi.org/10.1039/D0SC06195F.

26. Martí S, Arafet K, Lodola A, Mulholland AJ, Świderek K, Moliner V: **Impact of warhead modulations on the covalent inhibition of SARS-CoV-2 Mpro explored by QM/MM simulations**. *ACS Catal* 2022, **12**:698–708, https://doi.org/10.1021/acscatal.1c04661.

27. Grigorenko BL, *et al.*: **Multiscale simulations of the covalent inhibition of the SARS-CoV-2 main protease: four compounds and three reaction mechanisms**. *J Am Chem Soc* 2023, **145**:13204–13214, https://doi.org/10.1021/jacs.3c02229.

28. Taguchi M, Oyama R, Kaneso M, Hayashi S: **Hybrid QM/MM free-energy evaluation of drug-resistant mutational effect on the binding of an inhibitor indinavir to HIV-1 protease**. *J Chem Inf Model* Mar. 2022, **62**:1328–1344, https://doi.org/10.1021/acs.jcim.1c01193.

29. Dos Santos AM, *et al.*: **Assessment of reversibility for covalent cysteine protease inhibitors using quantum mechanics/molecular mechanics free energy surfaces**. *J Chem Inf Model* 2022, **62**:4083–4094, https://doi.org/10.1021/acs.jcim.2c00466.

30. Nunes-Alves A, Kokh DB, Wade RC: **Recent progress in molecular simulation methods for drug binding kinetics**. *Curr Opin Struct Biol* 2020, **64**:126–133, https://doi.org/10.1016/j.sbi.2020.06.022.

31. Jorgensen WL, Thomas LL: **Perspective on free-energy perturbation calculations for chemical equilibria**. *J Chem Theor Comput* 2008, **4**:869–876, https://doi.org/10.1021/ct800011m.

32. Jorgensen WL, Ravimohan C: **Monte Carlo simulation of differences in free energies of hydration**. *J Chem Phys* 1985, **83**:3050–3054, https://doi.org/10.1063/1.449208.

33. Sugita Y, Okamoto Y: **Replica-exchange molecular dynamics method for protein folding**. *Chem Phys Lett* 1999, **314**:141–151, https://doi.org/10.1016/S0009-2614(99)01123-9.

34. Grubmüller H, Heymann B, Tavan P: **Ligand binding: molecular mechanics calculation of the streptavidin-biotin rupture force**. *Science* 1996, **271**:997–999, https://doi.org/10.1126/science.271.5251.997.

35. Isralewitz B, Gao M, Schulten K: **Steered molecular dynamics and mechanical functions of proteins**. *Curr Opin Struct Biol* 2001, **11**:224–230, https://doi.org/10.1016/S0959-440X(00)00194-9.

36. Hamelberg D, Mongan J, McCammon JA: **Accelerated molecular dynamics: a promising and efficient simulation method for biomolecules**. *J Chem Phys* 2004, **120**:11919–11929, https://doi.org/10.1063/1.1755656.

37. Faradjian AK, Elber R: **Computing time scales from reaction coordinates by milestoning**. *J Chem Phys* 2004, **120**:10880–10889, https://doi.org/10.1063/1.1738640.

38. Bolhuis PG, Chandler D, Dellago C, Geissler PL: **Transition path sampling: throwing ropes over rough mountain passes, in the dark**. *Annu Rev Phys Chem* Oct. 2002, **53**:291–318, https://doi.org/10.1146/annurev.physchem.53.082301.113146.

39. Gusev F, Gutkin E, Kurnikova MG, Isayev O: **Active learning guided drug design lead optimization based on relative binding free energy modeling**. *ChemRxiv* 2022, https://doi.org/10.26434/chemrxiv-2022-krs1t.

40. Thompson J, *et al.*: **Optimizing active learning for free energy calculations**. *Artif Intell Life Sci* 2022, **2**:100050, https://doi.org/10.1016/j.ailsci.2022.100050.

41. Application of machine learning algorithms to metadynamics for the elucidation of the binding modes and free energy landscape of drug/target interactions: a case study Siddiqui Chem Eur J." Accessed: October. 13, 2023. [Online]. Available: https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/chem.202302375.

42. Bonati L, Rizzi V, Parrinello M: **Data-driven collective variables for enhanced sampling**. *J Phys Chem Lett* 2020, **11**:2998–3004, https://doi.org/10.1021/acs.jpclett.0c00535.

43. Chen W, Tan AR, Ferguson AL: **Collective variable discovery and enhanced sampling using autoencoders: innovations in network architecture and error function design**. *J Chem Phys* May 2018, **149**:72312, https://doi.org/10.1063/1.5023804.

44. Bonati L, Piccini G, Parrinello M: **Deep learning the slow modes for rare events sampling**. *Proc Natl Acad Sci USA* 2021, **118**, e2113533118, https://doi.org/10.1073/pnas.2113533118.

45. Marchetti F, Moroni E, Pandini A, Colombo G: **Machine learning prediction of allosteric drug activity from molecular dynamics**. *J Phys Chem Lett* 2021, **12**:3724–3732, https://doi.org/10.1021/acs.jpclett.1c00045.

46]. Biomolecular simulations: from dynamics and mechanisms to computational assays of biological activity – Huggins – 2019 – WIREs Computational Molecular Science – Wiley Online Library." Accessed: October. 13, 2023. [Online]. Available: https://wires.onlinelibrary.wiley.com/doi/10.1002/wcms.1393.

47. Casasnovas R, Limongelli V, Tiwary P, Carloni P, Parrinello M: **Unbinding kinetics of a p38 MAP kinase type II inhibitor from metadynamics simulations**. *J Am Chem Soc* 2017, **139**:4780–4788, https://doi.org/10.1021/jacs.6b12950.

48. Haldar S, *et al.*: **SI: a multiscale simulation approach to modeling drug–protein binding kinetics**. *J Chem Theor Comput* Nov. 2018, **14**:6093–6101, https://doi.org/10.1021/acs.jctc.8b00687.

49. Badaoui M, *et al.*: **Combined free-energy calculation and machine learning methods for understanding ligand unbinding kinetics**. *J Chem Theor Comput* 2022, **18**:2543–2555, https://doi.org/10.1021/acs.jctc.1c00924.

50. Seeliger D, De Groot BL: **Conformational transitions upon ligand binding: holo-structure prediction from apo conformations**. *PLoS Comput Biol* 2010, **6**, https://doi.org/10.1371/journal.pcbi.1000634.

51. Basciu A, Malloci G, Pietrucci F, Bonvin AMJJ, Vargiu AV: **Holo-like and druggable protein conformations from enhanced sampling of binding pocket volume and shape**. *J Chem Inf Model* 2019, **59**:1515–1528, https://doi.org/10.1021/acs.jcim.8b00730.

52. Aggarwal R, Gupta A, Priyakumar UD: *APObind: a dataset of ligand unbound protein conformations for machine learning applications in de novo drug design*. 2021 [Online]. Available: http://arxiv.org/abs/2108.09926.

53. Pan AC, Borhani DW, Dror RO, Shaw DE: **Molecular determinants of drug–receptor binding kinetics**. *Drug Discov Today* 2013, **18**:667–673, https://doi.org/10.1016/j.drudis.2013.02.007.

54. Ray D, Parrinello M: **Kinetics from metadynamics: principles, applications, and outlook**. *J Chem Theor Comput* Aug. 2023, https://doi.org/10.1021/acs.jctc.3c00660.

55. Motamarri P, Das S, Rudraraju S, Ghosh K, Davydov D, Gavini V: **DFT-FE – a massively parallel adaptive finite-element code for large-scale density functional theory calculations**. *Comput Phys Commun* Jan. 2020, **246**:106853, https://doi.org/10.1016/j.cpc.2019.07.016.

56. Das S, Motamarri P, Subramanian V, Rogers DM, Gavini V: *DFT-FE 1.0: a massively parallel hybrid CPU-GPU density functional theory code using finite-element discretization*. 2022:1–55, https://doi.org/10.1016/j.cpc.2022.108473.

57. Schade R, *et al.*: **Towards electronic structure-based ab-initio molecular dynamics simulations with hundreds of millions of atoms**. *Parallel Comput* 2022, **111**:102920, https://doi.org/10.1016/j.parco.2022.102920.

58. Gastegger M, Schütt KT, Müller K-R: **Machine learning of solvent effects on molecular spectra and reactions**. *Chem Sci* 2021, **12**:11473–11483, https://doi.org/10.1039/D1SC02742E.

59. Galvelis R, *et al.*: **NNP/MM: accelerating molecular dynamics simulations with machine learning potentials and molecular mechanics**. *J Chem Inf Model* 2023, **63**:5701–5708, https://doi.org/10.1021/acs.jcim.3c00773.

60. Lier B, Poliak P, Marquetand P, Westermayr J, Oostenbrink C: **BuRNN: buffer region neural network approach for polarizable-embedding neural network/molecular mechanics simulations**. *J Phys Chem Lett* 2022, **13**:3812–3818, https://doi.org/10.1021/acs.jpclett.2c00654.

61. Hofstetter A, Böselt L, Riniker S: **Graph-convolutional neural networks for (QM)ML/MM molecular dynamics simulations**. *Phys Chem Chem Phys* 2022, **24**:22497–22512, https://doi.org/10.1039/D2CP02931F.

62. Rufa DA, *et al.*: **Towards chemical accuracy for alchemical free energy calculations with hybrid physics-based machine learning/molecular mechanics potentials**. *bioRxiv* 2020, **0**, https://doi.org/10.1101/2020.07.29.227959. 2020.07.29.227959.

63. Shen L, Yang W: **Molecular dynamics simulations with quantum mechanics/molecular mechanics and adaptive neural networks**. *J Chem Theor Comput* 2018, **14**:1442–1455, https://doi.org/10.1021/acs.jctc.7b01195.

64. Rizzi A, Carloni P, Parrinello M: **Targeted free energy perturbation revisited: accurate free energies from mapped reference potentials**. *J Phys Chem Lett* Oct. 2021, **12**:9449–9454, https://doi.org/10.1021/acs.jpclett.1c02135.

65. Rizzi A, Carloni P, Parrinello M: *Multimap targeted free energy estimation*. 2023 [Online]. Available: http://arxiv.org/abs/2302.07683.

66. Warner KD, Hajdin CE, Weeks KM: **Principles for targeting RNA with drug-like small molecules**. *Nat Rev Drug Discov* 2018, **17**, https://doi.org/10.1038/nrd.2018.93. 8.

67. Bianco G, Goodsell DS, Forli S: **Selective and effective: current progress in computational structure-based drug discovery of targeted covalent inhibitors**. *Trends Pharmacol Sci* 2020, **41**:1038–1049, https://doi.org/10.1016/j.tips.2020.10.005.

68. Wen C, *et al.*: **Systematic studies on the protocol and criteria for selecting a covalent docking tool**. *Molecules* 2019, **24**, https://doi.org/10.3390/molecules24112183. 11.

69. Scarpino A, Ferenczy GG, Keserű GM: **Comparative evaluation of covalent docking tools**. *J Chem Inf Model* 2018, **58**:1441–1458, https://doi.org/10.1021/acs.jcim.8b00228.