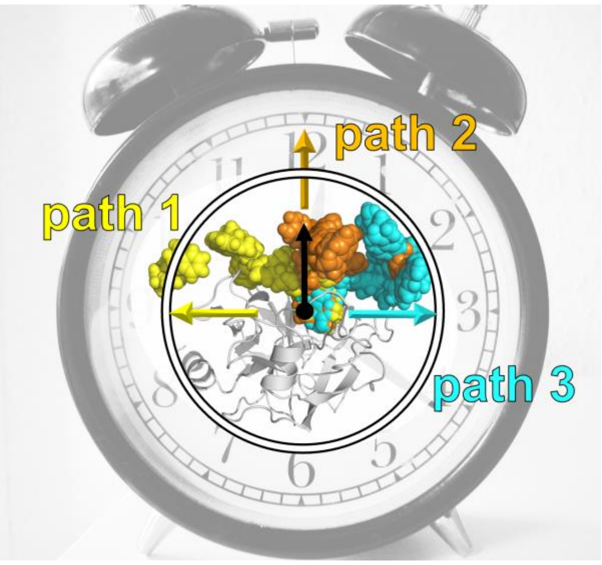
Predicting and understanding drug-target binding kinetics via molecular simulations



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CECAM-HQ-EPFL, Lausanne, Switzerland

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1. Description

The prediction of drug-target binding kinetics is a growing topic in computational biophysics of high relevance for biomedical research: about 15 years ago, it was realised that such kinetics often correlate better with drug efficacies than drug-target protein binding affinities[1-4]. This insight poses a significant challenge for molecular dynamics (MD) simulation techniques, which can typically only access the lower microsecond range, as the unbinding of drugs from their protein receptors typically occurs on time scales extending to many hours. Attempts to bridge the time scale gap have led to the development of novel approaches in MD simulations[5-12]. These approaches are aimed at, on the one hand, improving our understanding of the – often complex – mechanistic determinants of drug-target binding kinetics and, on the other hand, providing reliable and user-friendly tools to predict binding kinetic parameters that can be applied in the drug design pipeline[13-15]. For example, biased or enhanced sampling MD simulation approaches enable the acceleration of binding and unbinding processes to investigate their mechanisms[16-22] and to provide a fast scoring of compounds according to their (un)binding characteristics[23-25]. Further approaches that have been shown to hold promise are structural coarse-graining methods[26] and machine learning-based approaches[27-30]. Compared to the much more established field of binding free energy calculations[31], binding kinetics calculations face significantly larger challenges as regards adequate sampling, binding/unbinding path dependencies, force field accuracy for intermediate binding states, and the small available number of experimental datasets for computational benchmarks[32]. Following the insights of Copeland and others, comprehensive actions were undertaken to bundle theoretical as well as experimental[33-36] method development elucidating molecular kinetics. For example, the “Kinetics for Drug Discovery” IMI brought together researchers from both academia and industry to develop methods to measure and compute drug binding kinetic properties[37].

While the work on understanding (un)binding kinetics on a molecular level has continued and is present as sub-topics on computational conferences and workshops, the research of different groups has diverged, and no generally agreed-on “quality control” procedures exist for predictions of drug-target (un)binding kinetics. One critical cause is that no dedicated and repeated venue exists to bring together researchers of the field to discuss its state-of-the-art in depth, to identify current problems, to assess the problems that need to be solved in the future and to define “gold standards” for benchmark prediction methods. In this regard, it is indispensable to include experimentalists in these discussions for the generation of reliable experimental data sets on (un)binding characteristics of well-selected protein-ligand systems. Furthermore, connecting researchers from academia and industry is important to identify common goals and evaluate the applicability of currently existing approaches in drug discovery. Therefore, we propose a workshop that shall serve to gather the major players in the field of computational prediction of kinetics to focus the goals of the community, assess the current state-of-the-art and generate a generally agreed-on set of benchmark systems.

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2. Program

Day 1 - Monday March 10th 2025

- 12:00 to 13:50 – Registration
- 13:50 to 14:00 - Welcome & Introduction

Symposium I: Method development

- 14:00 to 14:40 - **Paolo Carloni**
  Towards calculating free energies with quantum accuracy
- 14:40 to 15:20 - **Yinglong Miao**
  Predicting biomolecular binding kinetics for therapeutic design through Selective Gaussian accelerated Molecular Dynamics
- 15:20 to 15:50 - Coffee break
- 15:50 to 16:30 - **György M. Keserű**
  The role of binding kinetics on the development of fluorescent GPCR tracers
- 16:30 to 17:10 - **Edina Rosta**
  Enhanced sampling simulations of biomolecular systems
- 17:10 to 17:30 - **Federica Moraca**
  Funnel metadynamics-driven insights into ligand-protein binding to inhibit the LIF/LIFR interaction
- 17:30 to 21:00 - Poster session & aperitif

Day 2 - Tuesday March 11th 2025

- 08:50 to 09:00 - Welcome from CECAM director Andrea Cavalli

Symposium II: Applications and new developments I

- 09:00 to 09:40 - **Chris de Graaf**
  Computational prediction of binding kinetics for GPCR structure-based drug discovery
- 09:40 to 10:20 - **Christa Elisabeth Müller**
  Determination of drug residence time – will its prediction become a reality?
- 10:20 to 10:50 - Coffee break *brought to you by* Schrödinger
- 10:50 to 11:10 - **Rebecca Wade**
  Some challenges in computing protein binding kinetics
- 11:10 to 11:30 - **Giulia D'Arrigo**
  Let it go: exploring and learning from unbinding pathways
- 11:30 to 11:50 - **Felipe Bravo**
  Computational insights into binding kinetics: unraveling the residence time of Roniciclib derivatives in CDK2
- 11:50 to 12:10 - **Ariane Nunes-Alves**

  Investigation of ligand unbinding from [NiFe] hydrogenase using molecular dynamics simulations and machine learning
- 12:15 to 14:00 - Lunch

Symposium III: Model building

- 14:00 to 14:40 - **Chia-en chang**
  Binding kinetics toolkit (BKiT) for analyzing transient molecular conformations and computing free energy landscapes
- 14:40 to 15:20 - **Pratyush Tiwary**
  Physics driven ai approaches for enhancing and understanding rare events
- 15:20 to 17:00 - Coffee break *brought to you by* Schrödinger
  and Roundtable discussion I
- 17:00 to 19:00 - Poster session II
- 19:00 to 21:30 - Social dinner

Day 3 - Wednesday March 12th 2025

Symposium IV: Applications and new developments II

- 09:00 to 09:40 - **Bizan N. Balzer**
  Adhesion and friction studied by stereographic force spectroscopy
- 09:40 to 10:00 - **Steffen Wolf**
  Calculating drug (un)binding kinetics via dissipation-corrected targeted MD and Langevin simulations
- 10:00 to 10:20 - **Niels Kristian Kjærgård Madsen**
  Residence-time scoring for high-throughput computer-aided drug design
- 10:20 to 10:50 - Coffee break
- 10:50 to 11:10 - **Sabina Podlewska**
  Elucidating key residues of serotonin receptor ligands for prolonged residence time via molecular dynamics simulations
- 11:10 to 11:30 - **Piotr Chyży**
  Exploring ligand binding through enhanced sampling techniques
- 11:30 to 11:50 - **Sina Safaei**
  Drug permeation through phospholipid membranes: predicting exact kinetics via path sampling methodology
- 12:00 to 14:00 - Lunch

Symposium V: Applications and new developments III

- 14:00 to 14:40 - **Lane Votapka**
  Predicting residence times of drug-target systems with the multiscale simulation method SEEKR
- 14:40 to 15:00 - **Giovanni Bottegoni**
  Adiabatic - bias molecular dynamics simulations reveal the impact of mutations on muscarinic antagonist unbinding kinetics
- 15:00 to 17:00 - Coffee break, Roundtable discussion II and Wrap-up

3. Abstracts

Adhesion and friction studied by stereographic force spectroscopy

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Atomic force microscopy (AFM)-based stereographic force spectroscopy offers the means to study the adhesion and friction of single polymers and single bonds under liquid conditions using a combination of vertical and lateral pulling (i.e., angle-dependent pulling). [1-5]
In order to study the dynamics of (bio)molecular interactions dynamic force spectroscopy is applied, varying the force loading rate at which AFM-based pulling is performed. Using the Bell-Evans [6,7] and the Dudko-Hummer-Szabo models,[8] parameters describing the unbinding kinetics of a molecular system are obtained.
Here, the directional anisotropy of single bonds and the underlying kinetics are investigated for different molecular interactions, such as covalent bonds, coordination bonds and receptor-ligand-protein complexes. [3,5] The presented methods are further applied to the unfolding of protein motifs of complex biomolecules, such as mucins.[9]

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Adiabatic - bias molecular dynamics simulations reveal the impact of mutations on muscarinic antagonist unbinding kinetics

Giovanni Bottegoni[1], Adriana Coricello[1], Anna Lisa Chiaravalle[1], Maria Musgaard[2], Benjamin G. Tehan[2], Gian Marco Elisi[1]
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Ligand-target dissociation rates (koff) strongly correlate with efficacy and safety profiles as well as with the therapeutic effect of drugs. As a prototypical example, muscarinic receptor antagonists used as bronchodilators show similar affinity profiles towards the muscarinic M3 receptors (M3R) and M2 receptors (M2R), whereas their kinetic selectivity towards M3R avoids the adverse effects that a prolonged inhibition of M2R would induce at the cardiac level. Previous studies on the dissociation kinetics of human M3R showed that the residence time and binding affinity of muscarinic antagonists are deeply affected by the presence of specific mutations. The aim of our work was to reproduce the rankings of these experimental kinetic rates through an approach based on the application of adiabatic-bias molecular dynamics (ABMD) simulations using Path Collective Variables (PCVs), PCV-ABMD. Employing this methodology, we simulated the translocation of tiotropium, a long-acting bronchodilator targeting M3R, from the orthosteric site to the extracellular vestibule, without considering the whole unbinding process. The estimated times necessary for translocation displayed a strong correlation against the experimental pkoff values. Moreover, a thorough analysis of protein-ligand contacts provided deeper insights into the mechanism of unbinding of muscarinic antagonists. The newly described PCV-ABMD protocol captured relevant metastable states and offered a reliable approach for the prediction of kinetic selectivity in sets of mutants.

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Binding kinetics toolkit (BKiT) for analyzing transient molecular conformations and computing free energy landscapes

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Examining drug-protein binding free energy landscape, which reveals both binding kinetics and thermodynamics, is crucial for estimating efficacy and selectivity of drug candidates. Consideration of partially bound intermediate states also provides a detailed explanation for drug binding residence time, as well as understanding of binding mechanisms. Here, we present a Binding Kinetics Toolkit (BKiT) that includes several utilities to analyze trajectories and compute binding free energy and kinetics profile. The ligand-protein unbinding pathways were generated by all atom explicit solvent molecular dynamics (MD) simulations with enhanced sampling technique such as GaMD or metadynamics. BKiT then uses principal component analysis (PCA) to reduce all-atom (3N-6) dimensions to 2 or 3 dimensions to generate descriptions/indexes for drug unbinding or protein conformational transition, which can more accurately describe and easily visualize the molecular motions. The adapted milestoning theory is used to construct the transition matrix and then free energy profile along the unbinding indexes. Using model systems such as cyclin-dependent kinase 8 (CDK8) with cyclin C (CDK8/CycC) and pyrazolourea ligands and HIV protease (HIVp) with market drugs, we discussed detailed drug unbinding. We also demonstrated our design strategies to develop drug candidates with preferred binding kinetics.

Calculating drug (un)binding kinetics via dissipation-corrected targeted MD and Langevin simulations

Steffen Wolf, Miriam Jäger, Victor Tänzel, Simon Bray, Benjamin Lickert, Matthias Post, Gerhard Stock
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To compute drug (un)binding dynamics and the related affinity constants, here we present a combination of dissipation-corrected targeted MD [1] and temperature-boosted Langevin equation simulations [2]. Using simulations of a few nanoseconds individual length, the fully atomistic data is rationalized in the form of only two profiles, which are the free energy ΔG(x) and friction Gamma(x). Using these profiles in Langevin equation-based simulations allows to access dynamics on time scales of seconds to hours, which is several magnitudes beyond the capabilities of atomistic MD approaches. Analyzing the kinetics-defining transition states ΔG_NEQ(x) and Gamma(x) for a range of pharmaceutically relevant target-drug complexes such as Hsp90 or GPCRs, we find that the transition barrier height usually is related to the rupture of enthalpic interactions such as salt bridges and hydrogen bonds, while friction corresponds to the solvation of a ligand and the protein binding site. Electrostatic interaction can both accelerate and slow down unbinding, depending on the individual chemical scaffold of a drug.

Furthermore, we investigate the pathways drugs take in and out of the binding site. In MD simulation data, paths are usually not observable per se, but need to be inferred from simulation trajectories. Here we present novel approaches to cluster trajectories according to similarities. These approaches include neighbor-nets allowing to correct for input data ambiguity [3] and an unsupervised learning approach employing only a single free parameter [4,5]. We demonstrate how such clusters of trajectories correspond to pathways, and how the approaches help in the identification of reaction coordinates for a considered process [5]. Last, we present a theoretical framework how potentials of mean force can be calculated for individual pathways, and how these potentials and kinetics along paths can be combined into a comprehensive complete free energy profile and process kinetics [6].

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Computational insights into binding kinetics: unraveling the residence time of Roniciclib derivatives in CDK2

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Binding kinetics, including k_{on} (association rate), k_{off} (dissociation rate), and residence time constant (= $1/k_{off}$), have emerged as essential parameters in the field of drug discovery programs. Compared with thermodynamics parameters, binding kinetics have proven to assess better the functional efficacy of drugs on the target protein in the body. The constant dissociation rate (k_{off}) and residence time () have shown a strong correlation, in *in-vivo* conditions, with the functional efficacy of drugs. The residence time can be studied using experimental methods such as Surface Plasmon Resonance (SPR) and computational methods such as classical molecular dynamics (cMD) simulations and enhanced sampling techniques.

Here, using computational methods, particularly cMD and Metadynamics, we studied the binding kinetics for Roniciclib and its derivatives in complex with CDK2 kinase, intending to characterize the unbinding process, measure the relative values in nanosecond time scale, and give details of the critical residues and molecular features that influence drugs binding kinetics. In this sense, changes in the R^5 position on the aminopyrimidine core of Roniciclib by larger substituents enhanced the experimental residence time notably, which correlated with the observed computationally values in a nanosecond time scale, with an R^2=0.83.

The cMD simulations and Metadynamics, revealed the importance of Phe80, Lys33, and Asp145 as critical residues for the stability of drug-target binding and residence time on CDK2. Our analysis revealed critical structural features and hydration network rearrangement on binding, which could explain the longer values observed as size substituents increase. In addition, we depicted the role of Asp145 as an essential residue for the binding due to the high electrostatic contribution on the different derivatives. We explored the compound's hydration network and hydrogen bond with the residues of the CDK2 active site, and we characterized the unbinding path from CDK2 by two main routes.

Computational prediction of binding kinetics for GPCR structure-based drug discovery

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Determination of drug residence time – will its prediction become a reality?

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The kinetics of a drug's dissociation from a target protein is a critical factor affecting drug efficacy. Drug−target residence time (RT), calculated from the dissociation rate constant koff (RT = 1/koff) has been recognized as a crucial parameter for the clinical efficacy of a drug. Drugs with relatively long residence times (e.g. of minutes to hours at 37°C) often show prolonged pharmacodynamic effects and, in some cases, reduced toxicity, which is beneficial for designing more effective therapeutics. In many cases, a long residence time is desired, e.g. for cancer therapy. Recently, there has even been an upsurge in the development of covalent drugs for cancer, infections, and other indications, showing infinite residence time. A long residence time means that a drug is still active even if it is no longer measurably in body fluids.

On the other hand, a drug with a short residence time may in some cases offer advantages such as a reduced risk of persistent side effects, easy dosage adjustment, and fast reversibility.

Our medicinal chemistry projects are focused on protein targets, mainly involved in purinergic signaling, for cancer (immuno)therapy. We have determined RTs of a range of compounds for different targets, including adenosine A2B receptors(unpublished), Gq proteins [1-3], and ecto5'-nucleotidase (CD73, unpublished), trying to analyze structure-RT relationships. Small changes in a compound's structure can result in a major change in RT. Computational approaches are required to rationalize the observed data, and to predict this important property for the design of future drugs [4].

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Drug permeation through phospholipid membranes: predicting exact kinetics via path sampling methodology

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The study of drug permeation through phospholipid bilayers is critical to understanding biological processes and advancing pharmacological applications. Similar to (un)binding kinetics, permeation events can be complex when involving multiple pathways and intermediate states. Moreover, permeation events are often rare events, occurring infrequently on timescales that are inaccessible to conventional molecular dynamics (MD) simulations. To address this computational challenge, replica exchange transition interface sampling (RETIS) has been developed as a path sampling methodology to quantify the exact kinetics of rare events while significantly reducing computational cost [1].
RETIS generates unbiased trajectories that can be either reactive or non-reactive, using the Metropolis-Hastings algorithm, providing both qualitative and quantitative insights into rare events. The ∞RETIS extension advances this further by introducing infinite swapping and asynchronous Monte Carlo moves, dramatically enhancing computational efficiency without compromising accuracy [2]. This innovative approach enables the study of the kinetics of rare events on time scales ranging from microseconds to seconds, overcoming challenges posed by high energy barriers and computational limitations.
Previous permeation work involved a small permeant, i.e.\ molecular oxygen permeating through a phospholipid bilayer, using RETIS [3]. In this study, we report a demonstration of how the new algorithm of ∞RETIS provides permeation kinetics of a larger permeant that is clinically relevant for cancer treatment. Specifically, the permeation kinetics of 5-aminolevulinic acid (5-ALA) across a dipalmitoylphosphatidylcholine (DPPC) bilayer is studied with ∞RETIS. 5-ALA plays a vital role in photodynamic therapy by acting as a precursor to a photosensitizer, making it critical to understand its transport properties to optimize therapeutic delivery. The permeation of 5-ALA involves overcoming significant energy barriers, which exemplifies the complexity of such processes.\\
The ∞RETIS approach effectively addresses the complexity of ALA-5's permeation by leveraging exceptional parallelizability of the algorithm, in contrast to traditional Monte Carlo methods that are inherently serial. Our work shows that the groundbreaking advancement of ∞RETIS opens the door to a wide range of future realistic applications, including addressing long-standing challenges such as drug (un)binding [4].

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Elucidating key residues of serotonin receptor ligands for prolonged residence time via molecular dynamics simulations

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Despite the exponential growth of knowledge regarding factors influencing the biological activity of compounds, the precise determinants of ligand-protein binding kinetics and their translation into in vivo effects remain not fully understood. In this project, we aim to comprehensively investigate the molecular determinants governing the formation and stability of ligand-protein complexes, focusing on ligand residence time for ligands of serotonin receptors 5-HT2A and 5-HT7. Although Paul Ehrlich's doctrine Corpora non agunt nisi fixate was proposed in the 19th century [1], the direct relationship between compound residence time and its pharmacological effects is still not fully elucidated [1].

Our approach integrates in silico and in vitro studies to identify the molecular factors influencing ligand binding and the time it remains bound to the target. In the initial phase, we concentrated on well-characterized reference ligands, such as ritanserin and risperidone for 5-HT2AR and 5-CT, LP-211, DMT, and 5-MeOT in the case of 5-HT7R. Furthermore, we synthesized derivatives of selected 5-HT2AR reference compounds and carried out examinations for selected 5-HT7R ligands previously developed by our team with structures based on 5-aryl-1-alkylimidazole core [2].

To understand ligand behaviour in their complexes with respective receptors, we conducted molecular dynamics simulations on the microsecond scale under varying temperature conditions. By analyzing ligand-protein interaction statistics and the evolution of atom-pair distances in relation to kinetic parameters (k_on, k_off/residence time), we identified factors potentially associated with prolonged ligand occupancy of 5-HT2AR and 5-HT7R. These findings together with the outcome of our previous studies [3] will guide further in-depth computational and experimental investigations into the kinetics of serotonin receptor ligands.

Acknowledgments

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Enhanced sampling simulations of biomolecular systems

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Important biological functions of biomolecular systems, such as catalysis, large-scale conformational changes, or ligand unbinding are often out of reach of atomistic molecular simulations. Enhanced sampling algorithms proved essential to mapping the pathway for these processes. However, the selection of collective variables is generally highly challenging.

Here, we present algorithms [1,2] that enable the identification of ligand unbinding pathways and pave the way for the calculation of unbinding free energy profiles. Without *a priori* defining CVs, we can identify useful atomic distances to map the unbinding pathway. Subsequently, the finite-temperature string method is used to calculate the free energy barrier and obtain an estimate of the off rate.

However, the key variables that determine the transition state (TS) are not necessarily obtained. To enable the identification of the most important CVs that play a major role at the TS, we developed a general machine learning (ML) approach, applicable to any molecular processes. We generate short downhill trajectories initiated near the TS and train ML networks to predict the trajectory outcomes. These trained networks are subsequently used to pinpoint the most relevant CVs at the TSs.

Our calculations are applied to drugs targeting CDK2 [1] and the M3 muscarinic acetylcholine receptor [3]. We hope to provide key molecular features that help design inhibitors based on their allosteric and kinetic properties.

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Exploring ligand binding through enhanced sampling techniques

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Riboswitches are messenger RNA (mRNA) fragments located before the coding part of a gene. Recent reports have shown that we can impact their structure and control gene regulation by binding appropriate metabolites to riboswitches. It suggests that riboswitches can be a modern approach to combating antibiotic resistance focusing on using mRNA segments to control the synthesis of essential proteins for bacterial life.

Nevertheless, designing riboswitches is tough. The fact that the ligand binds to RNA does not mean it will work as expected [1]. Moreover, it was shown that even a single-point mutation of N1 riboswitch affects gene regulation [2]. Among them, the A17G mutation reduces 6-fold the translation regulation efficiency and 14-fold the binding affinity [2]. Unfortunately, this mutant is highly flexible, making it impossible to synthesize and explain how the A17G mutation so drastically affects gene regulation. Thus, we performed a computational study to determine sequence-structure-activity relationships and answer how the structure of N1 changes during ligand association [3,4].

Unfortunately, molecular dynamics (MD) simulations are insufficient - they cannot overcome high-energy barriers on the rugged free-energy landscape, so ligand associations/dissociations are rare. Therefore, I developed MD simulations in multidimensional space using enhanced sampling techniques [3,4]. We expanded the conformational space by simulating the complex in parallel at different temperature ranges using solute tempering [3,4] and applying biasing potentials to observe distance-dependent dynamics along the ligand-RNA distance range and reveal the kinetic properties [4].

The conformational analysis, performed with a modified Dijkstra algorithm, indicated the key structures and steps that control the dynamics and kinetics of the ligand-N1 complex [4]. Such structures (extracted from a specified position in the energy landscape), combined with the obtained free energy profiles, allowed us to define a two-step binding model consisting of conformational selection and induced fit [4]. Initially, the ligand waits for the proper folding of the RNA structural motifs, followed by its recognition and entry into the binding site [4]. After binding, we observed the RNA backbone rearrangement that determines the regulatory activity of N1 [4]. Surprisingly, we also captured how short-range interactions within RNA between conformational selection and induced fit become crucial for ligand binding [4]. Finally, we assessed the thermodynamic and kinetic properties along the proposed binding model using Markov state models [4], which agreed with experimental data [2] and fluorescence measurements [5].

Overall, we described a two-step binding mechanism, consisting of conformational selection and induced fit, in atomistic detail that agrees with the fluorescence assays [5]. In addition, our multidimensional molecular dynamics simulations of ligand and RNA along their association paths, combined with the Markov State Model analysis, explained different activities of the mutants considering not only the unbound and bound end states but also the events occurring during ligand binding [4].

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Funnel metadynamics-driven insights into ligand-protein binding to inhibit the LIF/LIFR interaction

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Understanding the binding/unbinding processes of protein-ligand complexes at atomistic level is considered a significant approach for the design of ligands with a better *in vivo* efficacy thanks to an accurate estimation of the binding affinity [1]. Unfortunately, in many protein-ligand systems, ligand unbinding processes are strongly coupled to protein conformational changes that hamper the binding sampling over these degrees of freedom [2]. This is the case of the Leukemia Inhibitory Factor Receptor (LIFR), a heterodimeric receptor whose binding with Interleukin (IL)-6 family cytokine LIF, activate the LIF/LIFR axis promoting tumor growth, metastasis and therapy resistance. X-Ray experiments revealed that, among the five extracellular domains (D1-D5) of LIFR, LIF binds in a saddle-shaped region of the

D3-D4 domains defined by three loops (L1-L3) that represent a putative binding site for promising LIF/LIFR antagonists. In its apo form, L3-L2 loops of LIFR represent a gate by undergoing to deep opening/closing conformational changes that hamper the ligand recognition. For this reason, in order to design more potent LIF/LIFR antagonists by taking into account the loop flexibility, we have used the Well-Tempered Funnel-Metadynamics (fun-MetaD) [3][4] on the most promising steroidal derivative lead compound. Microseconds long fun-MetaD allowed us to achieve several binding/unbinding recrossing events that provided precious insight for the rational improvement of the lead compound to design a novel derivative with increased capability of LIF/LIFR inhibition [5].

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Investigation of ligand unbinding from [NiFe] hydrogenase using molecular dynamics simulations and machine learning

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Hydrogenases are important enzymes in biotechnology due to their ability to act as efficient catalysts for hydrogen oxidation and biofuel production. However, some [NiFe] hydrogenases are inhibited by O_2 and CO. A possible strategy to obtain resistant enzymes is to block the access of inhibitors to the catalytic site by mutation. Using the [NiFe] hydrogenase from *Desulfovibrio fructosovorans* and 10 different mutants as a model system, we employed molecular dynamics (MD) simulations and the enhanced sampling method τRAMD (τ-Random Accelerated Molecular Dynamics) to study substrate (H_2) and inhibitor (O_2 and CO) unbinding [1]. We found a high correlation between the relative residence times computed from simulations for CO unbinding and the experimental ones. We computed pathway probabilities for the unbinding of different gas molecules from the wild type and mutant forms of [NiFe] hydrogenase and we observe that, while the most probable pathways are the same for different gas molecules and different mutants, the secondary pathways are different.
Another challenge in the investigation of protein-ligand unbinding is data analysis, especially the identification of unbinding pathways in multiple trajectories. In this talk, I will present PathInHydro, a set of supervised machine learning models developed for automated identification of unbinding paths in MD simulations [2]. The mechanistic insights and methods obtained in this work could be exploited in the engineering of inhibitor-tolerant [NiFe] hydrogenases.

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Let it go: exploring and learning from unbinding pathways

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The estimation of drug-target residence time has been widely adopted in drug discovery and lead optimization campaigns as a metric to control and modulate *in vivo* drug efficacy. Over the years, several computational approaches have been developed to simulate unbinding kinetics and calculate dissociation rates. In addition to accurately predicting residence time, understanding the molecular basis of the unbinding event is crucial to support and drive the design of drugs with optimized kinetic profiles. Here, we present the application of the unbinding kinetics workflow developed by Schrödinger to accurately predict the residence time and to study the unbinding mechanism of a set of drug-target systems. We applied the presented approach to different target classes and modalities, looking at the details of the dissociation process and understanding the determinants of such an event. Overall, the results demonstrate the applicability of the workflow in assisting drug design with minimal human intervention and a computational cost compatible with drug design cycle timeline.

Physics driven ai approaches for enhancing and understanding rare events

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Naive AI models are only as good as the dataset they are trained on, and generally do not extrapolate out of distribution. For rare events such as drug unbinding, this becomes a problem. At the same time, the simplest Physics models tend to need very limited training data and extrapolate far beyond the dataset, but often do so with oversimplified assumptions that break down in complex, high-dimensional systems. A key advantage of AI, however, is its ability to automatically learn intricate correlations (though rarely causations) and capture subtle, high-dimensional dependencies that traditional Physics-based models might overlook. I will discuss some methods from my group that use different ideas from Physics, especially classical thermodynamics and statistical mechanics, to come up with Physics-driven AI approaches that combine the best of both worlds. I will show some applications such as calculating protein-ligand residence times and others.

Predicting biomolecular binding kinetics for therapeutic design through selective gaussian accelerated molecular dynamics

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It is critical to predict binding thermodynamics and kinetics of biomolecules for effective therapeutic design1. However, such task has proven challenging for particularly conventional Molecular Dynamics (MD), due to limited simulation timescales. We aim to address this challenge through powerful enhanced sampling simulations. Building on a robust Gaussian accelerated MD (GaMD) technique, we have developed novel “Selective GaMD” algorithms, including Ligand GaMD (LiGaMD)2-4, Peptide GaMD (Pep-GaMD)5 and Protein-Protein Interaction GaMD (PPI-GaMD)6. All-atom microsecond simulations performed using these new methods have unprecedentedly captured repetitive dissociation and binding of small-molecule ligands, flexible peptides and proteins. The simulations have uncovered binding pathways and mechanisms of the biomolecules. They have also allowed us to calculate the ligand/peptide/protein binding free energies and kinetic rates accurately and efficiently. These are critical parameters in therapeutic design of small-molecule drugs, peptides and antibodies.

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Predicting residence times of drug-target systems with the multiscale simulation method seekr

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TBA

Residence-time scoring for high-throughput computer-aided drug design

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Drug-target residence time is commonly put forward as a key indicator of drug efficacy and a valuable metric for assessing features such as selectivity, toxicity, and dosage. Nevertheless, computational tools for predicting residence times have still not been widely adopted into real-world computer-aided drug design projects. While a variety of accurate and efficient molecular dynamics (MD) based approaches are under active development, their applicability within a high-throughput setting is still limited by the large differences in time scales between (un)binding events and integration step size. We have developed *koffee*: a fully physics-based tool for scoring ligands or protein variations according to residence time. *koffee* requires no training data or target-specific calibration and reduces the required number of function evaluations by 2-3 orders of magnitude compared to corresponding MD-based approaches. The accuracy of the tool has been demonstrated on a variety of drug targets and use cases including ranking of ligands for HSP90 and prediction of the effect of binding-pocket mutations on the M_3/tiotropium complex.

Some challenges in computing protein binding kinetics

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The role of binding kinetics on the development of fluorescent GPCR tracers

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G protein-coupled receptors (GPCRs) are integral to numerous physiological processes and represent key targets of many drugs. Fluorescent ligands play a pivotal role in the investigation of their binding and functional properties, organization, and regulation that is essential for advancing therapeutic strategies. Special emphasis is placed on challenges related to the design of fluorescently labeled small molecules. Over the years, extensive research has been conducted on developing fluorescent probes, resulting in a wide array of designs optimized for diverse applications. Despite significant progress, a standardized framework for their development remains lacking. This challenge stems from the fact that conjugating a fluorophore to a ligand can form a novel pharmacological entity. The addition of fluorescent labels to ligands leads to structural modifications that complicate the accurate prediction of their pharmacological properties such as binding affinity and kinetics, selectivity, functional activity. Binding kinetics characterization of the probes is of primary importance since it has a major impact on the potential applications. In our case study we discuss the development of fluorescent tracers for dopamine D3 receptors. Binding kinetics characterization of the potential ligands and the corresponding tracers complemented by molecular simulations helped us to identify a tracer suitable for super-resolution and ultrasensitive imaging of D3 receptors in living cells, tissues and animals.

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Towards calculating free energies with quantum accuracy

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We will present a machine-learning-assisted simulation approach which attempts to improve the calculations of free energies using QM and QM/MM potentials [1]. The straightforward application of the method is the calculation of the affinity. The approach is very general and eventually could be used also to predict residence times.

4. Posters

A theoretical framework for random acceleration molecular dynamics

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Random Acceleration Molecular Dynamics (RAMD) is an enhanced sampling technique that accelerates the dissociation of a bound ligand from its receptor by applying an external random force to the ligand. While the magnitude of the external force FRAMD remains constant throughout the simulation, its direction is randomly chosen and maintained as long as the ligand covers a distance greater than a selected threshold during a predefined number of steps. Otherwise, a new direction of the force is randomly selected. The external force facilitates rapid displacements of the ligand until it escapes from its binding site at a time defined as the computational unbinding time. The outcome of RAMD depends on two key parameters: the lag time τ, the time after which the force direction is changed randomly, and the magnitude of the applied random force.

The RAMD approach is commonly used in drug design, as it can provide relative estimates of dissociation rate constants. However, the theoretical basis of RAMD remains essentially unexplored, posing challenges for a quantitative implementation of simulation results as well as the most optimal selection of parameters. Moreover, an open question is whether absolute physical unbinding times can be directly derived from RAMD simulation.

In this work, we established a fundamental comprehensive theoretical framework for RAMD, starting with simplified mathematical models and progressing to realistic biological systems of higher complexity. We investigated RAMD's underpinning from the point of view of statistical mechanics: using a Kramers-Moyal expansion of the first two moments of the ligand displacement for a time lag τ, we formulated a Smoluchowski-like diffusion equation with an effective temperature and diffusion constant that captures the effect of the random external force on the dynamics. Conceptually, this analysis shows that RAMD corresponds to a stochastic process model that effectively increases the diffusion constant.

The unique capacity of RAMD to increase the diffusion constant set it apart from other enhanced sampling techniques, such as rescale-MD. Moreover, we derived a relationship that links the effective diffusion coefficients and the unbinding time. This relationship can be used to determine escape times in the absence of the random force and the height of the free energy barrier.

The one-dimensional simulations of this model are supported by atomistic RAMD simulations of the protein-ligand complex Trypsin-Benzamidine. By performing RAMD simulations with different force magnitudes, the relationship between the effective diffusion coefficient and the computational unbinding time can be derived and used to estimate the absolute physical unbinding time.

Overall, our work investigates the theoretical basis of RAMD, demonstrating its potential not only for ranking similar compounds but also for estimating absolute physical unbinding times and the height of the free energy barrier associated with the process.

Adiabatic bias md and path collective variables elucidate dissociation kinetics of m3 receptor mutants

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Ligand-target dissociation rates are critical for determining drug efficacy, safety, and therapeutic outcomes [1]. For treatments targeting muscarinic M3 receptors (M3R) in the airways, such as bronchodilators for chronic obstructive pulmonary disease, dissociation rates distinguish short-acting drugs for mild symptoms from long-acting drugs used in maintenance therapy [2]. Despite similar affinity profiles toward M2 and M3 receptors, the kinetic selectivity toward M3R mitigates adverse effects at the cardiac level that are caused by prolonged M2 inhibition. Therefore, understanding the molecular determinants at the basis of the kinetic selectivity has clear repercussions on drug discovery and the therapeutic relevance of this class of bronchodilators.

As a prototypical case study, mutations in M3R significantly affect the residence time and binding affinity of muscarinic antagonists like tiotropium [3]. This study involves the application of adiabatic bias MD [4] to predict dissociation kinetics of muscarinic antagonists at various receptor mutants. Unbinding simulations employed path collective variables [5] to simulate the translocation of tiotropium from the orthosteric binding site to the extracellular vestibule, which is thought to represent the main barrier associated with the unbinding process [6].

Our method offered a good correlation with experimental ranking within reasonable simulation times that are compatible with the constraints of drug discovery programs [7]. Moreover, extensive analysis of protein-ligand contacts [8] during simulations and finetuned exploration of transition states guaranteed by path collective variables remark the different ECL2 conformational flexibility as the basis for the kinetic selectivity over M2 receptor.

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Computational insights into binding kinetics: unraveling the residence time of Roniciclib derivatives in CDK2

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Binding kinetics, including k_{on} (association rate), k_{off} (dissociation rate), and residence time constant (= 1/k_{off}), have emerged as essential parameters in the field of drug discovery programs. Compared with thermodynamics parameters, binding kinetics have proven to assess better the functional efficacy of drugs on the target protein in the body. The constant dissociation rate (k_{off}) and residence time ( ) have shown a strong correlation, in *in-vivo* conditions, with the functional efficacy of drugs. The residence time can be studied using experimental methods such as Surface Plasmon Resonance (SPR) and computational methods such as classical molecular dynamics (cMD) simulations and enhanced sampling techniques.

Here, using computational methods, particularly cMD and Metadynamics, we studied the binding kinetics for Roniciclib and its derivatives in complex with CDK2 kinase, intending to characterize the unbinding process, measure the relative values in nanosecond time scale, and give details of the critical residues and molecular features that influence drugs binding kinetics. In this sense, changes in the R^5 position on the aminopyrimidine core of Roniciclib by larger substituents enhanced the experimental residence time notably, which correlated with the observed computationally values in a nanosecond time scale, with an R^2=0.83.

The cMD simulations and Metadynamics, revealed the importance of Phe80, Lys33, and Asp145 as critical residues for the stability of drug-target binding and residence time on CDK2. Our analysis revealed critical structural features and hydration network rearrangement on binding, which could explain the longer values observed as size substituents increase. In addition, we depicted the role of Asp145 as an essential residue for the binding due to the high electrostatic contribution on the different derivatives. We explored the compound’s hydration network and hydrogen bond with the residues of the CDK2 active site, and we characterized the unbinding path from CDK2 by two main routes.

Dancing towards death, interactions between Bcl2 proteins.

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The Bcl2 family of similarly folded proteins is responsible for apoptotic activation and regulation. Its members contain conserved canonical binding grooves and Bcl2 homology domains. This protein family consists of apoptotic effectors, inhibitors and activators. Its regulatory network arises from intra-family protein-protein interactions, dictated by their kinetics and relative binding free energies (1). Here, we aim to characterize differences between the intra-family interactions. To this end, we probe the binding mechanisms of a minimal protein network consisting of Bcl-xL (inhibitor), Bax (effector) and BH3 helices from Bid or Bim (activator) with biased enhanced sampling molecular dynamics simulations. Preliminary results reveal differences in encounter complexes of Bid BH3 to Bcl-xL and Bax and highlight the allosteric mechanism of Bim during Bax activation (2). Further, we aim to recover the interaction network between Bcl-xL, Bax and Bid/Bim BH3 helices by estimating the binding energies between interaction partners.

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Drug permeation through phospholipid membranes: predicting exact kinetics via path sampling methodology

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The study of drug permeation through phospholipid bilayers is critical to understanding biological processes and advancing pharmacological applications. Similar to (un)binding kinetics, permeation events can be complex when involving multiple pathways and intermediate states. Moreover, permeation events are often rare events, occurring infrequently on timescales that are inaccessible to conventional molecular dynamics (MD) simulations. To address this computational challenge, replica exchange transition interface sampling (RETIS) has been developed as a path sampling methodology to quantify the exact kinetics of rare events while significantly reducing computational cost [1].
RETIS generates unbiased trajectories that can be either reactive or non-reactive, using the Metropolis-Hastings algorithm, providing both qualitative and quantitative insights into rare events. The ∞RETIS extension advances this further by introducing infinite swapping and asynchronous Monte Carlo moves, dramatically enhancing computational efficiency without compromising accuracy [2]. This innovative approach enables the study of the kinetics of rare events on time scales ranging from microseconds to seconds, overcoming challenges posed by high energy barriers and computational limitations.
Previous permeation work involved a small permeant, i.e.\ molecular oxygen permeating through a phospholipid bilayer, using RETIS [3]. In this study, we report a demonstration of how the new algorithm of ∞RETIS provides permeation kinetics of a larger permeant that is clinically relevant for cancer treatment. Specifically, the permeation kinetics of 5-aminolevulinic acid (5-ALA) across a dipalmitoylphosphatidylcholine (DPPC) bilayer is studied with ∞RETIS. 5-ALA plays a vital role in photodynamic therapy by acting as a precursor to a photosensitizer, making it critical to understand its transport properties to optimize therapeutic delivery. The permeation of 5-ALA involves overcoming significant energy barriers, which exemplifies the complexity of such processes.\\
The ∞RETIS approach effectively addresses the complexity of ALA-5's permeation by leveraging exceptional parallelizability of the algorithm, in contrast to traditional Monte Carlo methods that are inherently serial. Our work shows that the groundbreaking advancement of ∞RETIS opens the door to a wide range of future realistic applications, including addressing long-standing challenges such as drug (un)binding [4].

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Elucidating key residues of serotonin receptor ligands for prolonged residence time via molecular dynamics simulations

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Despite the exponential growth of knowledge regarding factors influencing the biological activity of compounds, the precise determinants of ligand-protein binding kinetics and their translation into in vivo effects remain not fully understood. In this project, we aim to comprehensively investigate the molecular determinants governing the formation and stability of ligand-protein complexes, focusing on ligand residence time for ligands of serotonin receptors 5-HT2A and 5-HT7. Although Paul Ehrlich's doctrine Corpora non agunt nisi fixate was proposed in the 19th century [1], the direct relationship between compound residence time and its pharmacological effects is still not fully elucidated [1].

Our approach integrates in silico and in vitro studies to identify the molecular factors influencing ligand binding and the time it remains bound to the target. In the initial phase, we concentrated on well-characterized reference ligands, such as ritanserin and risperidone for 5-HT2AR and 5-CT, LP-211, DMT, and 5-MeOT in the case of 5-HT7R. Furthermore, we synthesized derivatives of selected 5-HT2AR reference compounds and carried out examinations for selected 5-HT7R ligands previously developed by our team with structures based on 5-aryl-1-alkylimidazole core [2].

To understand ligand behaviour in their complexes with respective receptors, we conducted molecular dynamics simulations on the microsecond scale under varying temperature conditions. By analyzing ligand-protein interaction statistics and the evolution of atom-pair distances in relation to kinetic parameters (k_on, k_off/residence time), we identified factors potentially associated with prolonged ligand occupancy of 5-HT2AR and 5-HT7R. These findings together with the outcome of our previous studies [3] will guide further in-depth computational and experimental investigations into the kinetics of serotonin receptor ligands.

Acknowledgments

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Exploring ligand binding through enhanced sampling techniques

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Riboswitches are messenger RNA (mRNA) fragments located before the coding part of a gene. Recent reports have shown that we can impact their structure and control gene regulation by binding appropriate metabolites to riboswitches. It suggests that riboswitches can be a modern approach to combating antibiotic resistance focusing on using mRNA segments to control the synthesis of essential proteins for bacterial life.

Nevertheless, designing riboswitches is tough. The fact that the ligand binds to RNA does not mean it will work as expected [1]. Moreover, it was shown that even a single-point mutation of N1 riboswitch affects gene regulation [2]. Among them, the A17G mutation reduces 6-fold the translation regulation efficiency and 14-fold the binding affinity [2]. Unfortunately, this mutant is highly flexible, making it impossible to synthesize and explain how the A17G mutation so drastically affects gene regulation. Thus, we performed a computational study to determine sequence-structure-activity relationships and answer how the structure of N1 changes during ligand association [3,4].

Unfortunately, molecular dynamics (MD) simulations are insufficient - they cannot overcome high-energy barriers on the rugged free-energy landscape, so ligand associations/dissociations are rare. Therefore, I developed MD simulations in multidimensional space using enhanced sampling techniques [3,4]. We expanded the conformational space by simulating the complex in parallel at different temperature ranges using solute tempering [3,4] and applying biasing potentials to observe distance-dependent dynamics along the ligand-RNA distance range and reveal the kinetic properties [4].

The conformational analysis, performed with a modified Dijkstra algorithm, indicated the key structures and steps that control the dynamics and kinetics of the ligand-N1 complex [4]. Such structures (extracted from a specified position in the energy landscape), combined with the obtained free energy profiles, allowed us to define a two-step binding model consisting of conformational selection and induced fit [4]. Initially, the ligand waits for the proper folding of the RNA structural motifs, followed by its recognition and entry into the binding site [4]. After binding, we observed the RNA backbone rearrangement that determines the regulatory activity of N1 [4]. Surprisingly, we also captured how short-range interactions within RNA between conformational selection and induced fit become crucial for ligand binding [4]. Finally, we assessed the thermodynamic and kinetic properties along the proposed binding model using Markov state models [4], which agreed with experimental data [2] and fluorescence measurements [5].

Overall, we described a two-step binding mechanism, consisting of conformational selection and induced fit, in atomistic detail that agrees with the fluorescence assays [5]. In addition, our multidimensional molecular dynamics simulations of ligand and RNA along their association paths, combined with the Markov State Model analysis, explained different activities of the mutants considering not only the unbound and bound end states but also the events occurring during ligand binding [4].

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Exposing unseen passages for ligand transport through proteins with high-throughput simulations

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Active sites of approximately 50 % of enzymes are deeply buried in their structure and connected to the bulk solvent through molecular transport pathways called tunnels. Tunnel anatomy, physicochemical properties, and dynamics often determine substrates and products' access and release rates [1], making them essential targets for drug discovery [2] and protein engineering [3]. However, understanding transport processes through these tunnels remains challenging due to their complex network topologies and the presence of molecular gates, which make both tunnel opening and molecular transport rare events [4].

Accurate analyses of tunnel conformations and explicit ligand migrations from very long and/or highly parallel or enhanced simulations are required to uncover the details of these elusive transport mechanisms [5, 6]. To alleviate this often rather daunting task, we have developed the TransportTools library to perform effective quantitative analyses of voids in protein interiors and couple them with explicit assignment of ligands utilizing those voids without compromising the level of details involved [7]. Using this toolbox, we analyzed water migration paths in 250 simulations of five hydrolases, in total 25 µs of simulation time [8]. We have tracked nearly 400,000 water transport events through more than 80 million distinct tunnel conformations to determine the minimal requirements for transporting water molecules. Surprisingly, these tunnels are more prevalent than previously believed, revealing a significant gap in our understanding of structure-dynamics-function relationships in enzymes with buried active sites [8].

Given water's crucial role in facilitating ligand dissociation and active site desolvation prior to ligand binding, we investigated how different water models affect their transport through enzyme tunnels. Our comparison of TIP3P, TIP4P-Ew, and OPC water models revealed significant differences in water entry and release rates to/from active sites [9]. Preliminary data suggests that protein-ligand complex stability correlates with these differences, highlighting the importance of considering the models used when comparing the calculated residence times of small molecules in buried binding sites.

Finally, we have selected a set of 40 diverse enzymes from five EC classes and four SCOPE classes and performed adaptive sampling simulations to generate 1.6 µs simulated time per enzyme. By analyzing tunnel networks (over 90 million tunnels) and water migration (2.4 million transport events) in each enzyme, we are starting to get insights into the localization of transiently open tunnels, their prevalence, forming residues, and their functional roles, hopefully enabling the development of predictive models of functional tunnels.

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Funnel metadynamics-driven insights into ligand-protein binding to inhibit the LIF/LIFR interaction

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Understanding the binding/unbinding processes of protein-ligand complexes at atomistic level is considered a significant approach for the design of ligands with a better *in vivo* efficacy thanks to an accurate estimation of the binding affinity [1]. Unfortunately, in many protein-ligand systems, ligand unbinding processes are strongly coupled to protein conformational changes that hamper the binding sampling over these degrees of freedom [2]. This is the case of the Leukemia Inhibitory Factor Receptor (LIFR), a heterodimeric receptor whose binding with Interleukin (IL)-6 family cytokine LIF, activate the LIF/LIFR axis promoting tumor growth, metastasis and therapy resistance. X-Ray experiments revealed that, among the five extracellular domains (D1-D5) of LIFR, LIF binds in a saddle-shaped region of the D3-D4 domains defined by three loops (L1-L3) that represent a putative binding site for promising LIF/LIFR antagonists. In its apo form, L3-L2 loops of LIFR represent a gate by undergoing to deep opening/closing conformational changes that hamper the ligand recognition. For this reason, in order to design more potent LIF/LIFR antagonists by taking into account the loop flexibility, we have used the Well-Tempered Funnel-Metadynamics (fun-MetaD) [3][4] on the most promising steroidal derivative lead compound. Microseconds long fun-MetaD allowed us to achieve several binding/unbinding recrossing events that provided precious insight for the rational improvement of the lead compound to design a novel derivative with increased capability of LIF/LIFR inhibition [5].

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High throughput determination of relative binding kinetics of covalent inhibitors for cyclin-dependent kinase 12

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The development of targeted covalent inhibitors, molecules that form covalent bonds with their target proteins, has traditionally been considered a risky endeavour owing to the possibility of off-target toxicity. The elucidation of previously unknown mechanisms of covalently acting drugs, particularly aspirin and penicillin, as well as the more recent development and approval of successful targeted covalent inhibitors, has led to a resurgence in the field. High throughput virtual screening is a common practice in the development of traditional, non-covalent inhibitors, which allows rapid assessment of large numbers of lead molecules through various computational methods such as docking and free energy perturbation calculations. The kinetics of covalent inhibitors renders the use of computationally predicted IC50 values unreliable, and instead, one should compare efficacies of covalent inhibitors by comparison of the apparent second-order rate constant, kinact/KI. In this work, the nature of this rate constant will be discussed, along with the underlying theory regarding high throughput virtual screening of targeted covalent inhibitors. Furthermore, the current state of a modified version of a high-throughput virtual screening protocol for the determination of relative kinact/KI values, originally proposed by Yu et. al.1, will be discussed.

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Investigation of the bottlenecks and pathways for ligand binding to [NiFe] hydrogenases using simulations and machine learning

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Hydrogenases are efficient catalysts for hydrogen oxidation and can be used for biofuel production. However, some [NiFe] hydrogenases are inhibited by O_2 and CO, limiting their application in biofuel production cells. By investigating the unbinding pathways of the substrate, H_2, and the inhibitors from the wild type *Desulfovibrio fructosovorans* (Df) [NiFe] hydrogenase plus 10 different mutations using τ-Random Accelerated Molecular Dynamics (τRAMD) simulations, we found key features governing the access of gas molecules to the catalytic site (1). We found that there is a bottleneck between residues 74 and 122 that strongly modulates the residence times of CO, and that different gas molecules and mutants display different secondary unbinding paths. We also investigated the binding of H_2 to the catalytic site of Df [NiFe] hydrogenase using ~20 µs of unbiased MD simulations. Understanding the (un)binding pathways of the inhibitors and substrate gives us the opportunity to design mutations that can block the access of inhibitors to the catalytic site, which may lead to inhibitor-tolerant enzymes.
We developed supervised machine learning (ML) models, namely PathInHydro, capable of classifying the unbinding pathways based on pairwise contacts between the gas molecules and all residues in the enzyme (2). The ML models generalize to systems absent in the training set, such as O_2's unbinding trajectories and another enzyme, the [NiFe] hydrogenase from *Megalodesulfovibrio gigas*. PathInHydro is a proof-of-concept of the application of supervised ML models to automate the identification of protein-ligand unbinding paths in MD simulations.

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Investigation of the effect of loop motion on the residence times of histamine-1-receptor (H1R) antagonists by τramd

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Drug-target dissociation rate (k_{off}) or its inverse residence time (τ) has been shown, in some cases, to be more relevant than binding affinity as a predictor of drug efficacy in non-equilibrium *in vivo* conditions [1]. However, computational prediction of drug-target residence time is challenging due to dissociation occurring over a wide range of timescales that generally extend beyond those accessible by conventional molecular dynamics (MD) simulations. This is why many methods for computing residence time use an enhanced sampling procedure. One of these methods is τRAMD, which uses Random Acceleration Molecular Dynamics (RAMD) simulations for computing the relative residence times of ligand-protein complexes [2-3]. RAMD employs a randomly-oriented force to accelerate ligand egress from the protein binding pocket. Histamine-1-receptor (H_1R) is a G protein-coupled receptor (GPCR) that is a common target for treating various allergic disorders. In H_1R-drug molecule complexes, flexible extracellular loops (ECL) line the dissociation routes. We modified the τRAMD protocol to account for the motion of ECL2 by separating loop conformations into open and closed conformations and used the τRAMD protocol to compute the relative residence times of compounds binding to H_1R. After validation on a small set of well-studied antagonists, we applied τRAMD to a set of compounds made to probe H_1R binding site subpockets [4]. We find that the modified τRAMD protocol gives a better correlation of the computed residence times with experimental values when the ECL2 conformation adopts a closed state. Moreover, τRAMD values are in good agreement with experiment for a further set of three compounds found to have longer residence times on average compared to all other compounds in study. The τRAMD calculations provide mechanistic insights into the H_1R-ligand dissociation kinetics and highlight the importance of considering loop motion in residence time calculations.

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Let it go: exploring and learning from unbinding pathways

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The estimation of drug-target residence time has been widely adopted in drug discovery and lead optimization campaigns as a metric to control and modulate *in vivo* drug efficacy. Over the years, several computational approaches have been developed to simulate unbinding kinetics and calculate dissociation rates. In addition to accurately predicting residence time, understanding the molecular basis of the unbinding event is crucial to support and drive the design of drugs with optimized kinetic profiles. Here, we present the application of the unbinding kinetics workflow developed by Schrödinger to accurately predict the residence time and to study the unbinding mechanism of a set of drug-target systems. We applied the presented approach to different target classes and modalities, looking at the details of the dissociation process and understanding the determinants of such an event. Overall, the results demonstrate the applicability of the workflow in assisting drug design with minimal human intervention and a computational cost compatible with drug design cycle timeline.

Machine learning of ligand unbinding pathways via community detection

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N-glycosylation-induced pathologic protein conformations as a tool to guide the selection of biologically active small molecules

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In the case of ER chaperone protein Grp94, glycosylation is linked to disease in some breast cancer subtypes. Designing small molecules to selectively target malfunctioning Grp94 could block the pathological glycosylated form without affecting the normal one. This study aims to develop structure-dynamics relationships to rationalize the activity of compounds against N-glycosylated Grp94 using computational approaches.
Previous findings show N62-glycosylation favors a semi-open state of the NTD; specific active ligands binds specifically and contribute to sustain such receptor conformation, providing a tailored chemical tool able to target only Grp94's pathological-mutant. Through unbinding simulations on HAC and HANC compounds we were able to prove that HAC compounds have slower unbinding times from NTD semi-open state, hence justifying their cytotoxicity. Also, thanks to QSAR and EPM analysis we were able to pinpoint which HAC chemical groups were responsible for the cytotoxicity and how they interact within the binding pocket, providing further explanation to their cytotoxic effects.

Residence-time scoring for high-throughput computer-aided drug design

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Drug-target residence time is commonly put forward as a key indicator of drug efficacy and a valuable metric for assessing features such as selectivity, toxicity, and dosage. Nevertheless, computational tools for predicting residence times have still not been widely adopted into real-world computer-aided drug design projects. While a variety of accurate and efficient molecular dynamics (MD) based approaches are under active development, their applicability within a high-throughput setting is still limited by the large differences in time scales between (un)binding events and integration step size. We have developed *koffee*: a fully physics-based tool for scoring ligands or protein variations according to residence time. *koffee* requires no training data or target-specific calibration and reduces the required number of function evaluations by 2-3 orders of magnitude compared to corresponding MD-based approaches. The accuracy of the tool has been demonstrated on a variety of drug targets and use cases including ranking of ligands for HSP90 and prediction of the effect of binding-pocket mutations on the M_3/tiotropium complex.

Towards automated physics-based absolute drug residence time predictions.

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The residence time (τ) of a drug bound to a receptor target is increasingly recognized as a key property to control during ligand-optimization campaigns and provides a useful dimension for modulating compound profiles in addition to binding affinity. For this reason, many computational approaches have been developed to predict this quantity. Several methods employ an empirical correlation between the residence time and the measured simulation time for a ligand to escape the binding pocket during biased molecular dynamics (MD), while others rely on more formal approaches that require a substantially larger computational effort and/or setup times often impractical in a fast-paced drug-discovery setting. Here we propose a new scheme to calculate absolute residence times by using two enhanced sampling approaches, consisting of an exploration phase followed by an exploitation phase that estimates the residence time: Random Acceleration Molecular Dynamics (RAMD)1 to harvest plausible egress pathways, and then Infrequent Metadynamics (iMetaD)2 to estimate residence time. This protocol caters to drug discovery programs, where a key aspect is the compromise between accuracy, throughput, and ease of use. We benchmark this approach by computing residence times for a congeneric series of ligands binding to several diverse drug targets and show that we can achieve good accuracy (RMSE of 1.22 and R^2 of 0.80 in $\log_{10}(\tau)$) without manually tuning the enhanced sampling parameters.

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