Manuscript
Click here to view linked References

Kinetics for Drug Discovery – An industry driven effort to target drug residence time

Doris A. Schuetz[1], Wilhelmus Egbertus Arnout de Witte[2], Yin Cheong Wong[2], Bernhard Knasmueller[1], Lars Richter[1], Daria B. Kokh[3], S. Kashif Sadiq[3], Reggie Bosma[4], Indira Nederpelt[5], Elena Segala[6], Marta Amaral[7,10], Dong Guo[5], Dorothee Andres[8], Leigh A. Stoddart[9], Steve Hill[9], Robert M. Cooke[6], Chris De Graaf[4] ,Rob Leurs[4], Matthias Frech[7], Rebecca C. Wade[3,11,12], Elizabeth Cunera Maria de Lange[2], Adriaan P. IJzerman[5], Anke Müller-Fahrnow[8], Gerhard F. Ecker[1]

[1]Department of Pharmaceutical Chemistry, University of Vienna, UZA 2, Althanstrasse 14, 1090 Vienna, Austria

[2]Division of Pharmacology, Leiden Academic Centre for Drug Research, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands

[3]Molecular and Cellular Modeling Group, Heidelberg Institute for Theoretical Studies (HITS), Schloß-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany

[4]Department of Chemistry and Pharmaceutical Sciences, Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, PO Box 7161, 1007 MC Amsterdam, The Netherlands

[5]Division of Medicinal Chemistry, Leiden Academic Centre for Drug Research (LACDR), Leiden University, P.O. Box 9502, Leiden, Einsteinweg 55, Leiden, 2300RA, The Netherlands

[6]Heptares Therapeutics, Biopark, Broadwater Road, Welwyn Garden City, Hertfordshire AL7 3AX, UK

[7]Discovery Technologies, Merck KGaA, Frankfurter Straße 250 , 64293 Darmstadt, Germany

[8]Bayer Pharma AG, Drug Discovery, Lead Discovery Berlin, Müllerstr. 178, 13342 Berlin, Germany

[9]School of Life Sciences, University of Nottingham, Nottingham, NG7 2UH, UK

[10]Instituto de Biologia Experimental e Tecnológica, Avenida da República, Estação Agronómica Nacional, 2780-157 Oeiras, Portugal

[11]Zentrum für Molekulare Biologie der Universität Heidelberg, DKFZ-ZMBH Alliance, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

[12]Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Im Neuenheimer Feld 205, 69120 Heidelberg, Germany

Keywords: binding kinetics, residence time, K4DD, structure-kinetics relationships

1. Introduction

Drug discovery and development programs are still driven by optimizing the target binding affinity and selectivity of the respective candidate. However, although

tremendous technological and methodological progress has been made, both in experimental and computational techniques, attrition rates remain disappointingly high. Besides unexpected toxicity, we often observe a lack of *in vivo* efficacy for many compounds. Such compounds appear promising in early drug discovery programs, but fail in later clinical trials. One of the reasons for this is the increasing evidence that kinetic parameters seem to correlate much better with efficacy than affinity does [1–4]. Considering that a considerable amount of all approved drugs exhibit non-equilibrium characteristics, it has been advocated that drug residence time may be more important for *in vivo* efficacy than *in vitro* equilibrium binding affinity. Furthermore, there is an increasing number of reports linking drug selectivity to the kinetic profile of the compound. Besides the well-known example of Tiotropium and the subtype selectivity profile at the five different subtypes of the muscarinic receptor [5,6], there was a recent report outlining the role of on-kinetics for the SERT/DAT selectivity of methylphenidate [7]. A correlation of *in vivo* efficacy with residence time has already been demonstrated for selected GPCRs, such as CCR5 [8], the β-2-adrenergic receptor [9], and the A_{2A} adenosine receptor [10]. In the kinase family, the dual tyrosine kinase inhibitor Lapatinib showed a long residence time that could be correlated with efficacy [11]. Another example is the ABL inhibitor Nilotinib [12].

Data such as these prompted David Swinney to state “These observations indicate that for the majority of drug targets, mass action driven equilibrium binding alone is not sufficient for maximal therapeutic utility” [13]. For most targets, a long residence time is desired. Certain proteins, however, show on-target toxicity with longer duration of the molecule bound to the receptor. For the D2 receptor (D2R), compounds possessing fast off-rates are ideal, as side effects increase drastically when residence time is prolonged [14,15]. Thus, the role and influence of on- and off-kinetics should be analyzed on a case-by-case basis.

1.1. The Drug Residence Time Concept

Since it was introduced in the scientific community in 2006, the drug-target residence time concept has gained in popularity [16]. In traditional *in vitro* methods, drug–target interactions have mostly been treated in terms of affinity measures or by means of static crystal structures of the bound complex [17]. The residence time concept, however, also takes into account the conformational dynamics of the protein, which affect drug binding and unbinding. Thus, it considers the residence time of the drug-target complex rather than the binding affinity *per se* as the major contribution to *in vivo* pharmacological activity [16].

The binding kinetics of a drug on its protein target is characterized by the bimolecular association rate constant (k_{on}), which is the rate the drug binds, and the dissociation rate constant (k_{off}), which is the rate of unbinding. The sum of many effects determine these two rate constants: (i) ligand specific induced fit [18], (ii) a

conformational adaptation by the system [19], (iii) water rearrangements and changes in water networks [20,21], and (iv) shielded hydrogen bonds [22].

Furthermore, apart from the pharmacokinetic half-life of the drug, receptor degradation can have an important effect on residence time in the system [23].

Most of the work pursued so far has focused on the dissociation kinetics of the ligands. However, there is increasing evidence, that the association rate should also be considered. A recent study of all data uploaded so far into the K4DD database revealed that only 0,4% of the compounds show a diffusion controlled on-rate, when defining diffusion control by a K_{on} value greater than 10^7 $M^{-1}s^{-1}$ [24,25]. K_{on} plays an important role for the *in vivo* translation, especially in terms of the phenomena of drug rebinding [26]. There are also studies on how to boost the drug-target association rate, e.g. by introducing polar moieties into a ligand [27].

2. K4DD – a Public Private Partnership

The fundamental hypothesis behind the drug residence time concept is very appealing: A detailed understanding of the kinetics of association and dissociation of a target-ligand complex can provide crucial insight into the molecular mechanism of action of a compound. This deeper understanding might help to improve decision making in drug discovery, thus leading to a better selection of interesting compounds to be profiled further. When an initial core group of scientists from pharmaceutical companies decided to further explore the concept, it was soon obvious that quite a number of open questions needed to be addressed. These comprise the important aspect of small molecule optimization by analyzing molecular aspects of drug binding kinetics, by providing data-driven guidelines for future drug discovery, and by enabling rapid and robust generation of structure-kinetic data in the design-make-test-analyze (DMTA) cycle. As these tasks go across all pharmaceutical companies which might consider the drug residence time concept relevant for their daily work, it perfectly fits the precompetitive collaboration concept of the Innovative Medicines Initiative (IMI) [28]. With this idea in mind, an IMI project was initiated: K4DD (Kinetics for Drug Discovery, www.k4dd.eu). The 5 year project with a budget of 21M€ started in November 2012. 20 partners (9 academic institutes, 7 large pharmaceutical companies and 4 SMEs) from 6 European countries work closely together on targets that have been selected by the consortium. The approach is truly collaborative: Several partners contribute to the work on each target and we share our results in regular meetings including bi-annual meetings of the entire consortium. K4DD focuses on how drug binding kinetics can be influenced and how therefore compounds can be optimized in terms of residence time (Figure 1).

From the outset of K4DD, it was decided to work on three scientific work packages (WPs) in parallel. The combination of the three formed the integrated framework and

starting point for the research in this IMI consortium. When the proposal was drafted, it was realized that target binding kinetics are not considered sufficiently in the current drug discovery process, stemming from a lack of knowledge in three domains, which were subsequently redefined as the work packages driving the K4DD consortium. WP1 is aimed at gaining a molecular understanding of kinetic characteristics to aid the development of predictive kinetic analyses. Partners in WP2 evaluate and develop technologies to enable the rapid and robust assessment of compounds' kinetic characteristics. The WP3 team sees to the translation of *in vitro* data (as in WP1 and WP2) to *in vivo* effects, moving from intact cells to whole animals and men. In all cases, the consortium's focus is on both membrane-bound and soluble drug targets, particularly G protein-coupled receptors, and kinases and proteases, respectively. The consortium has defined a target list at the very beginning of the five years program to ensure synergy between multiple partners that have actively exchanged methods and materials to speed up the research. Finding overall guidelines of how target interaction kinetics can be altered in the drug discovery process, will lead to a different approach - moving away from affinity driven strategies towards implementing kinetic studies at an early stage of drug discovery.

3. New Experimental Approaches

Reproducible and accurate experiments constitute the basis for hypothesis-driven research. There is a broad range of biological and biophysical assays available for assessing the affinity of a ligand to a macromolecule. The data generated quite often serve as input for computational approaches, which have become an indispensable tool in drug discovery and development. With the increasing amount of data available in the public domain, data quality, standardization, and comparability across different assay types have become an issue [30]. In the case of binding kinetics, several assays have been established and are routinely used across industry and academia. However, due to the need for time-resolved data collection, the assay panel available is limited. Furthermore, as most of the drug targets of current interest are membrane-bound, this poses an additional layer of complexity. Thus, one of the aims of K4DD is to develop new assays and to assess their reproducibility and comparability across different laboratories.

3.1. Brief Overview of Existing Methods

Although kinetic binding rate constants are increasingly accepted as important drug-optimization metrics, respective experimental implementation has not been trivial. In contrast to IC_{50} values and other affinity based metrics, the kinetic binding rate constants can only be determined by measuring across multiple incubation times. Conventional binding measurement techniques are often not suitable for doing this in an efficient way, since only a single time-point is quantified per binding reaction. Therefore, most methods described below focus on the continuous measurement of binding.

Over the years, Surface Plasmon Resonance (SPR) has proven to be a powerful approach to analyze kinetic binding rate constants of soluble drug targets. However, throughput is still limited, since ligands are mostly measured in series. Moreover, the need for purified protein, which has to be stably bound to a chip, can sometimes be limiting for the applicability of this approach. Especially for membrane-bound drug targets, purification out of the lipid environment is often difficult, making SPR analysis impossible. For GPCR targets, low-throughput radioligand binding techniques have therefore been the method of choice. Below, new advances in obtaining stably purified proteins and alternative methods for the continuous measurement of ligand binding are discussed and compared. Moreover, techniques are discussed in terms of usability for drug discovery.

3.2. The Challenge of Membrane Proteins - GPCRs as a Use Case

G-protein coupled receptors (GPCRs) represent a diverse group of membrane receptors that play a very important role as therapeutic targets. They are involved in a broad range of diseases, including diabetes, cancer, inflammation, obesity, central nervous system disorders and cardiovascular and respiratory diseases [31]. Although around 30% of all marketed drugs are directed towards GPCRs [31], there is still a strong need for new molecules. Especially targeting “undrugable” receptors, which represent valuable GPCR targets for which it has not been possible to discover drug candidates [32], and “orphan” receptors, whose ligands and biology are as yet uncharacterized [33], is of high interest. However, measurement of the binding kinetics of ligands to GPCRs is hampered by the difficulty of obtaining pure and active membrane-free receptors.

Surface Plasmon Resonance (SPR) is a label-free technique, which requires a low amount of protein and is able to generate kinetics data in real time. SPR has been used with detergent solubilized GPCRs for kinetic profiling of compounds and for screening fragment libraries [34,35]. However, it is now possible to produce membrane proteins in reconstituted high density lipoprotein (rHDL) particles. rHDLs are a new technology that enables the reconstitution of membrane proteins into a lipidic environment close to the cell membrane making the reconstituted receptor very stable and highly soluble [36].

Within the K4DD consortium two groups have successfully reconstituted the thermo-stabilized adenosine A2A receptor into rHDL particles and immobilized it on a SPR sensor chip [37,38]. Both groups compared the SPR kinetics results for the receptor reconstituted into rHDLs with the receptor solubilized in detergent or embedded in its native membrane. Bocquet et al. [38] tested different immobilization procedures whereas Segala and colleagues [37] focused on a large set of ligands with affinities ranging from 50 pM to 2 µM. These two successful studies showed that this new approach represents a valuable opportunity to obtain affinities and kinetics from low molecular weight compounds binding to GPCRs. It thus allows the characterization of the interaction in a detergent-free environment avoiding problems of ligands

partitioning into detergent micelles. In addition, this system can be used to study the binding of ligands to GPCRs in the presence of other components such as G proteins.

One of the challenges of studying binding kinetics for membrane-bound proteins is that it requires the removal of the proteins from their native membrane environment. For membrane proteins like GPCRs, the lipids within the membrane and adaptor proteins can have allosteric effects on the affinity and efficacy of selected ligands and therefore their kinetics may also be influenced [39]. Radioligand binding studies do not require removal of the protein from the membrane but can be time consuming to execute. Therefore, researchers have been looking towards new technologies to enable the kinetics of GPCRs to be studied in a higher throughput manner. The basis of these new technologies has been the development of fluorescent ligands for many different GPCRs and technologies that utilize them [40]. Kinetics have been studied either by directly monitoring the binding of the fluorescent ligand using confocal microscopy or by using an energy transfer based technique such as bioluminescence energy transfer (BRET) or time resolved fluorescence energy transfer (TR-FRET). Microscopy based techniques are also time consuming but do have the multiple advantages of using living cells, allowing dissociation kinetics to be measured under conditions of infinite dilution and uniquely at the single cell level. Binding kinetics of fluorescent ligands and allosteric influences of receptor-receptor interactions have been studied at the adenosine A1 and A3 receptors [41,42] and β-1-adrenoceptor [43] using confocal microscopy. A recently described BRET based assay utilizing a luciferase from a deep sea shrimp, NanoLuc, and fluorescent ligands has been used to investigate kinetics of a fluorescent ligand in live cells expressing the adenosine A1 receptor [44] and in membranes expressing free fatty acid receptor 1 [45]. Although to date the kinetics of unlabeled ligand have not been studied, this method can be used to study kinetics in living cells and membranes and has the potential to be a useful addition to the tool box of assays to measure binding kinetics of GPCRs. The TR-FRET based assay has been successfully applied to measure the kinetics of both labelled and unlabeled ligands at the histamine H1 receptor [46], gonadotropin-releasing hormone (GnRH) receptor [47] and the dopamine D2 receptor (D2R) [48]. The study on the GnRH receptor is discussed in detail below (Section 3.3). For the D2R, kinetic studies using the TR-FRET assay were central in demonstrating that the kinetic profile of ligands may be the basis of bias in intracellular signaling observed [48]. The use of fluorescent ligands for GPCRs is thus an important new development for understanding the role of kinetics in receptor-drug interactions and intracellular signaling.

In line with their importance, the structural biology of GPCRs has also been quite useful to visualize receptor-ligand interactions and to boost drug discovery. However, tThe structural basis of binding kinetics is difficult to understand and predict. An industry-academia collaboration within the K4DD consortium pursued by Heptares Therapeutics and Leiden University has given insights into why some ligands have a

long residence time at the A2A receptor [49]. This recent study used crystallography and computational chemistry to investigate the dissociation rates of ligands from the adenosine A2A receptor. Five ligands with high affinities for the human A2A, but a 100-fold range of dissociation rate constants were studied [50]. Five high resolution structures of the receptor in complex with each of these antagonists were generated, with a resolution ranging from 1.7 Å to 2.2 Å. Superposition of the five structures shows that they are identical except for a different interaction with His264 in the third extracellular loop (Figure 2). This histidine is involved in a salt bridge with Glu169. Molecular dynamics and metadynamics simulations revealed that the residence time of the ligands correlate with the energy required to break the salt bridge His264 - Glu169. Long residence time ligands appear to stabilize the Glu-His ionic interaction, while fast off-rate ligands were shown to destabilize this salt bridge. These results highlight a key determinant of the ligand-receptor binding that can be used to optimize receptor residence time.

3.3. Comparing Different Methods - A Case Study

The gonadotropin-releasing hormone (GnRH) receptor is involved in numerous hormone-dependent diseases and multiple drugs are on the market to treat e.g. prostate cancer [51]. The GnRH receptor is one of the targets which was accepted by the K4DD consortium, it served for a comparison of three different assays for binding kinetics. Firstly, equilibrium and kinetic binding parameters of 12 well-known GnRH peptide agonists were determined using a radiolabeled GnRH analogue displacement assay (Figure 3A) [47]. Affinity (Ki) values ranged from 13 nM for GnRH to 0.1 nM for Buserelin. Kinetic binding parameters were equally diverse with association rate constants ranging from 0.02 nM^{-1} min^{-1} for Fertirelin to 0.8 nM^{-1} min^{-1} for Nafarelin and dissociation rate constants ranging from 0.009 min^{-1} for Buserelin to 0.2 min^{-1} for Goserelin. Secondly, these results were compared to data obtained with a novel time-resolved fluorescence energy transfer (TR-FRET) assay (Figure 3B) and both the affinity and dissociation rate constants were highly correlated between both assays ($R2 = 0.5$, $P < 0.05$ for pKi values and $R2 = 0.7$, $P < 0.0005$ for pk_{off} values) [47]. Lastly, the kinetic binding parameters from both assays were translated to functional effects *in vitro* using a label-free morphological assay [52]. The activation profiles of endogenous GnRH (a fast dissociating agonist), and a well-known marketed analogue Buserelin (a slow dissociating agonist) were examined. It was shown that Buserelin had a much higher potency than GnRH, i.e. 0.46 nM vs 17 nM respectively. Interestingly, persistent GnRH receptor activation was observed for both agonists. Wash-out experiments (Figure 3C) resulted in more than 70% loss of signal for fast dissociating agonist GnRH, while for slow dissociating Buserelin less than 30% of the original response was abolished [52].

The use of these different protocols allowed for the first time to compare three diverse assays investigating qualitative and quantitative binding kinetics of a set of compounds to one receptor. Radioligand binding studies and TR-FRET assays

provided highly comparable kinetic data which in turn could be translated to wash-out resistant receptor responses.

3.4. Kinetic Probe Competition Assay

Indirect kinetic binding assays measure, in real time, the competition/displacement of labelled tracer compounds by unlabeled test compounds in solution. The binding kinetic constants of the tracer have to be previously determined in direct kinetic measurements so that the kinetic rate constants, k_{on} and k_{off}, of the unlabeled compounds can be calculated with a suitable mathematical model [53]. In the course of the project, the group of Amaury Fernandez-Montalvan at Bayer Pharma AG developed a time-resolved fluorescence energy transfer (TR-FRET) based homogenous kinetic probe competition assay (kPCA) that uses proteins and tracers labelled with TR-FRET donors and acceptors, respectively [46]. It uses microtiter plate readers equipped with the necessary optics to measure TR-FRET, and a pump-based injection system to enable fast sample mixing and immediate signal acquisition. If a suitable tracer is available, k_{on} and k_{off} parameters can be obtained for many compounds with high throughput and kinetic resolution at relatively low costs. The principle is applicable to many target classes, including kinases and GPCRs [46,47].

3.5. Transition State Analysis of the Drug-Target Binding Process

One of the major challenges associated with predicting binding kinetics is the molecular understanding of the interactions between the drug and the receptor in the transition state, which has the highest free energy and is therefore difficult to characterize. One of the methods applied to study the transition state energies of the binding event is the temperature dependence of drug-target association and dissociation rate constants. By applying the linear Eyring equation (Equation 1), this method gives the entropic and the enthalpic contribution to the activation energy of the transition state and therefore constructs a detailed thermodynamic signature for the binding of drugs to their targets.

There have been relatively few studies describing this type of analysis. Klein et al. have studied the influence of the DFG flip in FGFR1 kinase on the binding kinetics of selected type I (PDA) and type II (Ponatinib) inhibitors [54]. Kinetic analysis revealed that although both inhibitors have comparable binding affinities (K_D= 7.9 nM for Ponatinib and K_D = 5.7 nM for PDA), Ponatinib has extremely slow association and dissociation rates in contrast to the fast kinetic rate constants of PDA. In addition, the thermodynamic signatures for both inhibitors were very dissimilar, with the binding of PDA being mostly enthalpically driven, in contrast to the highly entropically driven interaction for Ponatinib. By measuring the temperature dependence of the kinetic association and dissociation rate constants for PDA and Ponatinib and applying the Eyring equation, the authors were able to dissect the thermodynamic signature of the binding of both inhibitors to FGFR1. Their structural and dynamic approaches suggest that the key factor for the strikingly different binding modes between type I

and type II inhibitors on FGFR1 might be due to the significantly high free-energy barrier between DFG-in and DFG-out states.

Kwon et al. reported a transition state analysis on the biotin and bio-5´-AMP dissociation from *E. coli* biotin holoenzyme synthetase [55]. Mutations in the "glycine-rich" loop region resulted in impairment of dissociation rates of the complexes. Analysis of the temperature-dependence of the rate of biotin dissociation by the Eyring method revealed that for the G115S mutant the decrease in the residence time was mainly due to a reduction of 6.8 kcal/mol in the enthalpic barrier to dissociation compensated by an increase of 3.0 kcal/mol in the entropy change. In contrast, the drop of the residence time for the R118G mutant is primarily due to a reduction of the transition state entropy.

The studies described here highlight that a deep understanding of the free energy landscape for the binding/unbinding process between a drug and a receptor is central to the rational optimization of drug binding kinetics.

4. Standardizing Data – the K4DD Database

One of the tasks of K4DD is to develop predictive *in silico* models for binding kinetics. This requires sets of standardized high quality data. When looking into the public domain, only small amounts of data on binding kinetics are available. Furthermore, mostly only k_{on}, k_{off} and K_D values are provided without structured information on the respective assay. As outlined above, several different experimental methods (assay types) to measure drug target kinetics are available. While all of them reveal values for k_{on}, k_{off} and K_D, there are also method-specific outputs for each of those methods, which need to be considered when using them for modelling. The consortium thus agreed to set up a database, which is accessible to all project members via a web-based interface (https://db.k4dd.eu). Adding new kinetic data to the database is a two-step process. At first, a web form is used to enter the experimental conditions and properties of the assay itself (e.g. temperature or pH). In a second step, experimentally measured bioactivity endpoints based on that assay are entered into a standardized spreadsheet template and then uploaded to the web server. This upload also has to include the structures of the chemical compounds that were investigated in the experiment (either in SDF or SMILES format). Several processing steps transform the uploaded structures into a chemically normalized form. This is necessary because the uploaded data is automatically linked to already existing compounds and targets in the database. Also, this allows sophisticated search queries and makes it easier to integrate data into existing third-party data stores.

For this procedure to work smoothly, standardized upload formats were established for a variety of assay types. Until now, such formats have been developed for SPR, ATR-FTIR, ITC, radioligand binding, kPCA and enzyme activity assays in close collaboration with the respective experimentalists. This is definitely a major step

forward in standardizing kinetic assays, since those are the first data models for kinetic assays agreed on and used both by public and private institutions. Also, this allows the direct comparison of experimental results from different sources within the consortium. Currently, the K4DD database contains more than 8000 individual endpoints.

Since the project focuses on a small number (less than 20) of target proteins, the database holds many data points per target. This allows the influence of specific experimental factors on kinetics to be studied. For example, it is possible to compare the differences in kinetics resulting from the same target being measured at two different temperatures or using different chemical buffers. For some targets, the database contains experimental data for the same compounds obtained from different assay types (e.g. SPR and radioligand binding). This is interesting if someone wants to compare different assay types with each other.

In funded projects, one of the major concerns regarding data management is the sustainability of the data. There are numerous examples of databases which were set up with grant money and then disappeared once the funding was finished. The K4DD consortium decided that integration into an already established and actively maintained bioactivity database such as ChEMBL [56] would be the best option to guarantee sustainability. ChEMBL is an open, large-scale bioactivity database containing millions of bioactivity data points for thousands of targets. ChEMBL also constitutes the main source for compound-pharmacology associations in the Open PHACTS Drug Discovery Platform (https://www.openphacts.org) [29]. Although its focus is currently not on kinetic data, the underlying database schema is, after small adaptions, suitable for such data points. Thus, the data format has been extended for some method-specific experimental parameters that are reported and otherwise could not be modelled. Since a prefilled version of ChEMBL was used as a basis for the K4DD database, most of the relevant targets are already correctly annotated. Therefore, it is possible to effortlessly link kinetic data with other structural and bioactivity data from ChEMBL. At the end of the project, the data in K4DD will be made publicly available, most probably via donation to ChEMBL.

5. Structure Kinetic Relationships

With the increasing knowledge about the importance of binding kinetics for the drug discovery and development process, attempts to develop computational models for predicting k_{on} and k_{off} values have also been initiated. The first thorough analysis of kinetic parameters of small, drug like molecules was undertaken by Miller et al. in 2012 [57]. He provided distributional statistics of physicochemical properties of slow/fast compounds by examining kinetic data from the public domain, and an unpublished internal dataset of Pfizer. Apart from this, matched pair analysis for understanding structure kinetic relationships of drug-like molecules have been

reported [22,94,95]. These matched pairs studies showed that small changes, like introducing methylene linkers [94], halogens [95], bulky hydrophobic groups [96] or simple methyl groups [22,97], can drastically change the kinetic profile of an inhibitor. Nonetheless, no pattern could be generalized for future guidance from these findings. The role of hydrophobicity was recently strengthened by Gaspari et al. [58].

With respect to Quantitative Structure Kinetic Relationship (QSKR) first insights were obtained from analyzing the influence of structural variations of peptides on kinetic parameters. In 2006, Andersson et al. established predictive QSKR models for dissociation and association rate constants for peptides binding to the recombinant antibody Fab 57P isolated from TMVP (tobacco mosaic virus protein) [59,92]. Clear differences between the linear regression models for k_{on} and k_{off} values could be observed using physicochemical descriptors like hydrophobicity, size and electronic characteristics. As the equations for k_{on} and k_{off} differed significantly in descriptor contributions, they hypothesized that different driving forces are involved in formation of the ligand receptor complex and in the unbinding of the ligand from its binding site. Furthermore, they highlighted the necessity of considering kinetic parameters apart from affinity to trigger biological response. Another study on therapeutic peptides was conducted by Magotti et al in 2009 (ref). They analyzed the kinetic variations of Compstatin analogues binding Complement component 3 (C3). By modifying certain residues in the Compstatin peptide, complex stability was improved. They identified that increased hydrophobicity of a particular residue was the main contributing factor to prolonged dissociation rate. Nevertheless, Magotti et al. noticed that individual increases in association rate were often compensated by increased dissociation rate. In this context, they pointed to the widely known phenomenon of entropy-enthalpy compensation that was supported by their biophysical measurements [93].

In a recent study, Vilums et al. reported a 56-fold increase in residence time within a congeneric series of cyclopentylamines inhibiting chemokine receptor 2 (CCR2) [98]. The selection of structural variations was led by observed structure kinetic relationships. In addition, they discovered that changes in chirality had a big impact on kinetic rates.

Despite these recent activities, knowledge is restricted to small congeneric series or matched pairs on certain targets. The scientific community is lacking thoroughly validated, predictive QSKR models for prospective design of drug-like molecules in terms of kinetic parameters. One contribution to this current scenario might be the absence of appropriate kinetic datasets for the deployment of high level QSKR methodologies. The K4DD consortium noticed this gap and is generating appropriate kinetic datasets for relevant drug targets. Computational partners within the consortium conduct QSKR studies on these datasets in order to identify general relationships between structural and/or physicochemical properties and kinetic parameters. The resulting QSKR models should guide the medicinal chemist in their quest of optimizing kinetic parameters.

6. Mechanistic Simulation Approaches to drug binding kinetics

Another approach to computing drug-protein binding kinetics, recently reviewed in [62], is to use molecular dynamics (MD) and Brownian dynamics (BD) simulation techniques.

Recent advances in computer technology, such as the use of programmable graphics processor units (GPUs), volunteer distributed computing initiatives [63] and dedicated computing architectures [64], have increased the speed of conventional MD simulations with classical all-atom models into the millisecond timescale and contributed to the growing feasibility of 'brute-force' simulation of the binding of low molecular weight compounds to a macromolecular target [65]. Statistical counting of association events can be used to estimate k_{on} values [20]. Simulation of ligand unbinding is more computationally demanding and has only been achieved with conventional MD for weak binders (0.2 and 20 mM) [66].

Accurate drug-protein binding/unbinding kinetic constants can be formally obtained by the construction of kinetic network models, also called Markov state models (MSMs), of the kinetically meaningful metastable states and their transition probabilities during the binding process [67]. This approach requires simulations of both binding and unbinding events and has only been demonstrated for small molecules with relatively fast k_{on} (> 10^7 $M^{-1}s^{-1}$) and k_{off} (> 10^2 s^{-1}) values [63,67] and for targets whose binding site can practically be considered rigid. There are several reasons for this. Firstly, the aggregate simulation time required to estimate association events is at least the timescale of the corresponding process under standard conditions. Therefore, given that a small-sized MD simulation system consisting of one small globular protein, for example HIV-1 protease, and one ligand in a solvated cubic box of 55x55x55 Å³ (50,000 atoms) has a concentration of ~0.01 M, a single binding event with k_{on} ~ 10^6 M^{-1} s^{-1} would only be expected to be observed after a time of about τ = 100 µs. The generation of multiple events from which a k_{on} value could be statistically estimated would require at least one order of magnitude greater sampling (τ =1 ms).

This problem can be partially overcome by adaptive sampling methods by which the MSM is iteratively computed with re-initiation of simulations into less well explored regions of the collective variable (CV) space [65]. This leads to more efficient sampling of the space and thus faster convergence of the computed kinetic parameters. Similarly, identification of the CV space corresponding to slow timescale motions [68] can reduce the required simulation time, taken together, by an order of magnitude. However, simulation timescales still remain computationally prohibitive for single drug-protein calculations let alone multiple drug screens. Moreover, increasing system size, as required when simulating larger proteins, such as kinases, or membrane proteins, such as GPCRs [69], substantially increases computational demand. This problem is compounded by the fact that such targets are not rigid, exhibit significant slow conformational fluctuations that can modulate

both the binding kinetics and the shape of the active site [70] and often themselves require sizeable drugs with substantially greater degrees of freedom (more rotamers, increased conformational flexibility) than so far simulated by conventional MD to compute kinetic parameters.

Given that such targets are predominant in drug discovery and relevant binders have k_{on} values that are often in the range of 10^4 – 10^5 $M^{-1}s^{-1}$ [71], conventional MD methods are still several orders of magnitude away from routine calculation of drug-binding kinetics for clinically relevant inhibitors; rather they remain more suited to high throughput fragment screens (Figure 4) [72].

Brownian dynamics (BD) simulations provide an alternative path to calculating the kinetics of drug-protein association by offering a number of simplifying assumptions. The drug binding process can be considered in terms of two sub-processes: the formation of a metastable diffusional encounter complex followed by an induced fit of both receptor and ligand conformations towards the final bound state. When the reaction is diffusion-limited [74], simulating the first step is sufficient to compute k_{on} [62]. In this regime, the internal motion of the solutes can often be neglected and the solutes treated as rigid bodies diffusing in implicit solvent (IS). The k_{on} value can be computed by performing a large number of simulations of receptor-ligand association to calculate the probability that an encounter complex is formed [75]. Furthermore, by exploring the spatial distribution of the ligand around the receptor, BD simulations can be used to estimate the relative residence time of encounter, which gives insight into the binding mechanism and the determinants of the ligand residence time [76].

Such approaches offer a computationally efficient way to calculate even slow association rates but major challenges in BD approaches are how to define formation of encounter complexes and how to treat the effects of internal motion. Furthermore, specific interactions by individual water molecules and/or ions can also influence binding kinetics [22,77,78], and are not accounted for in IS models.

In order to bridge the gap between the current capabilities of all-atom MD and slower kinetic regimes, several methods are being explored.

6.1. Multiscale Methods Coupling BD to MD

Combining rigid-body BD with flexible all-atom MD is a promising approach to explore slow conformational gating. Early work by Luty et al. [79] imposed coupling at small ligand-receptor (L-R) distances, whereas more recent approaches assume discrete conformational changes of either L or R [80,81], whilst the 'BDflex' algorithm [82] separates internal and external ligand regions but uses a coarse-grained, CG, representation of molecules to achieve computational feasibility. Another approach, based on the original spatial separation of Luty et al., couples BD to an MD region that is further partitioned according to 'milestoning' theory [83]. Milestoning is an

alternative to MSMs for calculating transition probabilities [84]. The first hitting point distribution rather than the equilibrium distribution is used as the starting phase of spatial points for the milestoning trajectories. This approach is promising but so far has only been applied to idealized small systems like sodium ion binding [83]. Furthermore, the milestone surfaces are rather simple – usually concentric spheres, and their compatibility with the complexity of ligand structure and dynamics remains to be determined.

6.2. Enhanced Sampling Techniques

Enhanced sampling techniques aim to reduce computational effort by accelerating sampling over the relevant degrees of freedom. Methods include metadynamics [85], conformational flooding [86], accelerated MD [69,87], and potential-scaled MD [88]. All such methods impose a potential bias on the system enabling it to visit less energetically favorable states with higher frequency. Whilst the free energy landscape can be recovered, such methods distort time thus preventing direct retrieval of the kinetic parameters. However, recently a method has been implemented that determines the acceleration factor [89], thus enabling recovery of kinetics. One challenge is that care has to be taken when recovering the kinetics because deposited potentials close to the transition barrier can adversely affect the dynamics and thus the transition probabilities. Another drawback of this class of methods is that knowledge of the relevant CV subspace is required in advance, in order to apply the biasing potential(s). If the CV subspace is not known, it still has to be chosen - then *a priori*, there is no guarantee that the chosen subspace captures the relevant kinetic transitions. Recently, however, a CV-independent method has been put forward based on potential-scaled MD simulations [90]. Introducing the software Biki (www.bikitech.com), the method is aimed at prioritizing compounds according to their residence time using multiple replica simulations and statistical analysis. Its application is, however, limited to targets whose binding site can be considered as rigid, since the protein motion is restrained in order to prevent protein unfolding. Recently, Cavalli et al have applied potential-scaled MD simulations to a series of glucokinase activators. Results indicate that the ligand shape might influence induced fit and thereby have an impact on the residence time. Additionally, specific residues influencing residence time were identified [91].

A combination of data-based approaches using machine learning techniques with structure-based mechanistic modelling might provide a new strategy for prediction of drug binding kinetic rates. MD-based methods can reveal particular protein residues and protein-ligand contacts that affect association and dissociation barriers, and thereby provide kinetics-specific descriptors for QSKR models. Furthermore, the development of reliable mechanistic simulation methods might extend the variety of compounds used for training of QSKR models.

7. Translating *in vitro* to *in vivo*

A detailed study that highlights the complexity of binding kinetics and links them to *in vivo* effects was recently published by Ayaz and colleagues. They analyzed the binding of a series of Roniciclib analogues to different CDKs to assess their structure-kinetics relationships [60]. Variation of the substituent at the 5-position of the pyrimidine scaffold resulted in changes of up to three orders of magnitude in the drug–target residence time (Figure 5). Trifluoromethyl substituted compounds show three times longer residence times on CDK2/Cyclin A than on CDK9/Cyclin T in the range of hours. X-ray crystal structures revealed that the introduction of the apolar trifluoromethyl group into the aminopyrimidine scaffold induces a rearrangement of the hydration network. This seems to be accompanied by a conformational adaption of the DFG loop, likely giving rise to the prolonged pCDK2/cyclin A residence time of Roniciclib which is almost one order of magnitude greater than that of the 5-bromo analogue. The trifluoromethyl substituted compounds show superior efficacy in tumor growth inhibition relative to the corresponding 5-bromo analogues despite their similar *in vitro* kinase inhibition activity and cell proliferation IC50 values [61]. In tumor cells, the prolonged residence time of Roniciclib on CDK2 is reflected in a sustained inhibitory effect on retinoblastoma protein phosphorylation, indicating that the target residence time on CDK2 may contribute to sustained target engagement and antitumor efficacy. Hence, it appears likely that for antitumor efficacy driven by CDK inhibition, an increased target residence time on CDK2 and CDK9 positively contributes to efficacy by sustained inhibition of CDK signaling.

However, to understand the impact of *in vivo* drug-target binding kinetics on the time-course of target occupancy and drug effect, one should realize that drug-target binding is only one aspect of the causal chain from drug dosing to drug effect [99]. Other aspects include:

- The concentration profile of the free drug in plasma and at the target site (pharmacokinetics, rebinding) [26,100]
- Non-specific binding in plasma and target tissue [101]
- The concentration of the target [26]
- Competition between drug and endogenous ligand binding [14,15]
- Target turnover [23], [102]
- Signal transduction [103]

These factors can all influence the ultimate importance of drug-target binding kinetics, thus putting the binding kinetics in the *in vivo* context. Therefore, these factors need to be taken into account in the translation from *in vitro* to *in vivo* target binding [104].

The best way to get information on the relevant factors in the *in vivo* context is to perform *in vivo* experiments. Not all of the factors summarized above can be

determined this way but *in vivo* experiments allow for the measurement of free and total drug concentration-time profiles in plasma and in target tissue. This is especially important when the target is located in a tissue protected by restrictive barriers, such as the blood-brain barrier. The distinction between total drug concentration in a reference tissue without the drug target and the respective target tissue provides information on the specific target binding. To that end, experiments should include the measurement of drug concentration at steady state conditions (extent of distribution) and at different equilibration times (rate of distribution). Microdialysis is the best possible technique to measure the free concentration-time profiles at the target tissue in individual animals (or humans; except for brain), while post-mortem tissue homogenate (at multiple time points, using multiple animals) can be used to measure total tissue concentration (being the sum of specific target binding and non-specific binding to other cellular components). Quantification of drug concentrations can then be performed with radioactivity-based or non-radiolabeled, LC-MS based methods [104]. The data obtained from the *in vivo* experiments can be further used in mathematical modelling to derive the target occupancy as a function of time. The group of Liesbeth de Lange at Leiden University has applied this methodology to unravel the relationship between pharmacokinetics, drug-target binding kinetics and target occupancy of non-radiolabeled dopamine D2 antagonists and agonists (Figure 6). Based on the *in vivo* drug concentration-time profiles in different brain compartments and *in vitro* k_{on} and k_{off} values at the D2 receptor, a mechanistic model incorporating these three factors is required to assess the impact of binding kinetics on the brain D2 receptor occupancy-time profile.

Another approach is to get insight into the binding kinetics use modelling in an *in vivo* context is to use mathematical modelling and simulations. Especially the insight into the rate limiting step in the time-course of *in vivo* target occupancy is of high value. Predictions of *in vivo* target occupancy made on the basis of such simulations should then be validated by performing specific *in vivo* experiments. Deviations from predictions can be used to improve the model and the insight. For example, in a simplified situation with only elimination, distribution and target binding of the free drug, target binding of the free drug, the duration of target occupancy is determined by elimination rate of the drug from plasma, the distribution of the drug from tissue to plasma or by drug-target dissociation rate. When the rate of all these processes is known, the duration of target occupancy can be predicted by taking into account that the slowest of these processes is most influential in determining the duration of target occupancy.

As commonly acknowledged, the time course of target occupancy is influenced by the free plasma concentration, as it drives drug-target binding. However, drug-target association also decreases the free plasma concentration, whereas drug-target dissociation increases the free plasma concentration. This influence of binding on free drug concentrations in plasma has been described for many biologics and a few small molecules as so-called “Target-Mediated Drug Disposition models” [105,106] ,

and has been clearly illustrated for a series of small-molecule HSP90 inhibitors [107]. The influence of this mutual interaction between target binding and free concentrations is influenced by:

- the amount of target available for binding
- the drug-target binding kinetics (k_{on}, k_{off}) and affinity (K_D)
- the elimination rate of the drug from the plasma

Further analysis of this interaction reveals an important role for the value of k_{on} to determine both the duration of target occupancy and the concentration profile of free drug near the target. This influence of k_{on} for high affinity drugs can be clearly seen in the simulations in Figure 7, where k_{on} is the only changing parameter between the different simulations (line colors).

If target binding occurs in a separate (sub)tissue, the interaction between target binding and free drug concentrations in the tissue leads, for high values of k_{on}, to a slower decrease of drug concentrations in the tissue, compared to the decrease of plasma concentrations. A longer duration of target occupancy, also for fast dissociating drugs, should be expected for drugs with high values of k_{on}. This influence of target binding on the free drug concentration near the target has also been described in terms of “rebinding” [26]. An integrated analysis of the influence of drug-target binding kinetics, plasma pharmacokinetics and local drug concentrations has recently revealed the role of binding kinetics on target occupancy duration from a comprehensive analysis of the rate-limiting step in this system [108]. This analysis shows both the need and the opportunities for an integrated analysis of the relevant determinants of target occupancy and effect, including drug-target binding kinetics.

8. Residence Time as a Decision Criterion

It is often necessary to measure for long periods of time to determine the residence time of drugs, which substantially reduces throughput. However, it may not be necessary to determine the exact residence time of drugs. In addition, experimental assay formats that do not attempt to measure the exact residence time but rather the relative residence time of ligands have much higher throughput. By doing this in a pseudo-quantitative way, this could still give a lot of information. Therefore it might be useful to change the assay format by establishing a cut off value and divide drug candidates into fast and slow dissociating ligands. In terms of prolonged occupancy, which is considered a benefit of long residence drugs, the limiting factor will be that at some point drugs stay bound for the entire lifespan of a protein. Re-synthesis of unbound receptor will therefore determine how long the drug has an effect after elimination of the unbound drug from the body [23].

As discussed, there is often limited throughput in which ligand binding kinetics for a drug target can be measured (as mentioned in section 3.1). A popular approach to

deal with low throughput is to use a limited set of time points [109,110]. As a trade-off for this increased throughput, the exact dissociation rate can no longer be determined and instead arbitrary metrics are used to quantify the relative differences in binding kinetics. Therefore, the comparison with other datasets is limited and this can only be partially solved by including reference compounds in the test-set to obtain comparative data.

For example, more than 1800 antagonists were evaluated for their relative dissociation rates from the D2R [110]. To do so, membranes expressing the receptor were pre-incubated with antagonist. Consecutively, membranes were separated from unbound ligands using filtration and membranes were then incubated with radioligand. The rate of dissociation from the D2R of the unlabeled ligands determines the available receptors for binding the radioligand, which is evaluated after a 5 min incubation time. The amount of radioligand is therefore an indirect measure, distinguishing between unlabeled ligands with various degrees of dissociation within this 5 min incubation time. However, theoretically, the incubation time could be easily tailored to reflect the differences in residence time for any relevant timespan.

A major drawback of such an approach is the required knowledge of the optimal drug-target residence time. If it was known what drug-target residence time would be required to elicit an *in vivo* response, it would be an excellent incubation time in the above example for ranking the relative drug-target residence times. However, research describing the effects of the respective drug-target residence time *in vivo* is lacking, making it often unclear what the desired residence time would be.

A popular rationale for increasing the residence time in the literature is to retain a prolonged receptor occupancy after the clearance of unbound drug [2,3,111]. As mentioned above, a long duration of drug-action *in vivo* is not just dependent on the drug-target residence time. However, in cases in which drug-target residence time could increase the therapeutic window, effectiveness would also depend on the rate at which new unbound drug target is synthesized and degraded, subsequently lowering the occupancy of the total receptor population. For example, it was shown *in vitro* and *in vivo* that the inhibitor of BTK1, despite its 167 hours residence time had a >50% reduction in occupancy within a day due to re-synthesis of the kinase [112]. Increasing the drug-target residence time far beyond the time needed for re-synthesis of the target will therefore not lead to an increasing therapeutic window.

Another example where drug-target residence time could make a difference is for drug-targets that can be antagonized in an insurmountable fashion. This can occur when the presence of the agonist is transient (e.g. neuronal signaling), enabling the long-residence time antagonist to outlast the presence of the agonist while bound to the target [15]. In this way, signaling will be blocked by the antagonist even when there are very high concentrations of agonist. However, once an antagonist would already have a full and insurmountable inhibition of the agonist *in vivo* by outlasting

the pulse of agonist exposure, a further increase in residence time will not improve the *in vivo* activity, unless the agonist pulse is repetitive and the elimination of unbound drug is faster than the dissociation. Pinpointing the required residence time is not just dependent on the timing and frequency of the agonist pulse but also on the number of receptors and transduction efficiency, which will be cell type dependent [113]. Therefore, determining the minimum residence time for complete insurmountable antagonism might not be as straightforward.

In conclusion, long residence time drugs might have a kinetic advantage *in vivo*, but if this advantage is at some point limited by the biological system, a further increase of the residence time would be worthless. Hence, the relation between the kinetics of drugtarget binding and its imposed effect *in vivo* requires much more attention. Breakthroughs here will not only help in establishing selection criteria in early drug discovery, but as discussed it could also enhance the throughput with which information can be obtained by using arbitrary metrics describing the underlying drug-target binding rate constants. This would also make it easier to use functional assays for drug optimization in which it is difficult to quantitatively determine the binding rate constants of drugs, but easier to measure the relative effects on signaling [52,114]. Moreover, when using functional assays to measure the duration of action of a test-set of ligands, this could already capture some of the biological limits, which arguably could be more valuable information for drug optimization then just the drug-target residence time.

Summary and Outlook

For decades, drug discovery and development has been focused on optimizing binding affinity while essentially neglecting drug binding kinetics. In recent years, it has become evident that *in vitro* information on drug target binding kinetics is of utmost importance in candidate compound selection. However, *in vitro* information should be accompanied by information on the *in vivo* context in which the drug needs to exert its ultimate effect. While target degradation/internalization, the concentration of the target, and endogenous competition might be difficult to assess, important insights into the role of binding kinetics can be obtained by investigating *in vitro* binding kinetics (k_{on}, k_{off}). This can be done in conjunction with measuring the free and total concentration-time profile of the drug in plasma and (sub)tissue(s) the binding rate constants can identify the rate limiting step in the target occupancy profile. Due to the complexity of the factors determining drug binding kinetics, a multilevel approach is necessary with both *in vitro* and *in vivo* experiments as well as computational modelling. Public-private partnerships, such as the K4DD project, are well suited to targeting drug binding kinetics in a holistic way and to providing new insights which will allow better decision making for selecting drug candidates.

Acknowledgements

The authors are part of the K4DD consortium, which is supported by the Innovative Medicines Initiative Joint Undertaking (IMI JU) under grant agreement no 115366. The IMI JU is a project supported by the EU's Seventh Framework Programme (FP7/2007– 2013) and the European Federation of Pharmaceutical Industries and Associations (EFPIA). DBK, SKS and RCW gratefully acknowledge the support of the Klaus Tschira Foundation.

References

[1] Pan AC, Borhani DW, Dror RO, Shaw DE. Molecular determinants of drug-receptor binding kinetics. Drug Discov Today 2013;18:667–73. doi:10.1016/j.drudis.2013.02.007.
[2] Copeland RA, Pompliano DL, Meek TD. Drug-target residence time and its implications for lead optimization. Nat Rev Drug Discov 2006;5:730–9. doi:10.1038/nrd2082.
[3] Zhang R, Monsma F. The importance of drug-target residence time. Curr Opin Drug Discov Devel 2009;12:488–96.
[4] Swinney DC. The role of binding kinetics in therapeutically useful drug action. Curr Opin Drug Discov Devel 2009;12:31–9.
[5] Barnes PJ. The pharmacological properties of tiotropium. Chest 2000;117:63S–6S.
[6] Kruse AC, Hu J, Pan AC, Arlow DH, Rosenbaum DM, Rosemond E, et al. Structure and dynamics of the M3 muscarinic acetylcholine receptor. Nature 2012;482:552–6. doi:10.1038/nature10867.
[7] Hasenhuetl PS, Schicker K, Koenig X, Li Y, Sarker S, Stockner T, et al. Ligand Selectivity among the Dopamine and Serotonin Transporters Specified by the Forward Binding Reaction. Mol Pharmacol 2015;88:12–8. doi:10.1124/mol.115.099036.
[8] Watson C, Jenkinson S, Kazmierski W, Kenakin T. The CCR5 receptor-based mechanism of action of 873140, a potent allosteric noncompetitive HIV entry inhibitor. Mol Pharmacol 2005;67:1268–82. doi:10.1124/mol.104.008565.
[9] Tee AKH, Koh MS, Gibson PG, Lasserson TJ, Wilson AJ, Irving LB. Long-acting beta2-agonists versus theophylline for maintenance treatment of asthma. Cochrane Database Syst Rev 2007:CD001281. doi:10.1002/14651858.CD001281.pub2.
[10] Guo D, Mulder-Krieger T, IJzerman AP, Heitman LH. Functional efficacy of adenosine A2A receptor agonists is positively correlated to their receptor residence time. Br J Pharmacol 2012;166:1846–59. doi:10.1111/j.1476-5381.2012.01897.x.
[11] Puttini M, Redaelli S, Moretti L, Brussolo S, Gunby RH, Mologni L, et al. Characterization of compound 584, an Abl kinase inhibitor with lasting effects. Haematologica 2008;93:653–61. doi:10.3324/haematol.12212.
[12] Wood ER, Truesdale AT, McDonald OB, Yuan D, Hassell A, Dickerson SH, et al. A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer Res 2004;64:6652–9. doi:10.1158/0008-5472.CAN-04-1168.
[13] Swinney DC. Biochemical mechanisms of New Molecular Entities (NMEs) approved by United States FDA during 2001-2004: mechanisms leading to optimal efficacy and safety. Curr Top Med Chem 2006;6:461–78.
[14] Kapur S, Seeman P. Does fast dissociation from the dopamine d(2) receptor explain the action of atypical antipsychotics?: A new hypothesis. Am J Psychiatry 2001;158:360–9. doi:10.1176/appi.ajp.158.3.360.
[15] Vauquelin G, Bostoen S, Vanderheyden P, Seeman P. Clozapine, atypical antipsychotics, and the benefits of fast-off D2 dopamine receptor antagonism. Naunyn Schmiedebergs Arch Pharmacol 2012;385:337–72. doi:10.1007/s00210-012-0734-2.

[16] Copeland RA. The drug-target residence time model: a 10-year retrospective. Nat Rev Drug Discov 2016;15:87–95. doi:10.1038/nrd.2015.18.
[17] Swinney DC. Biochemical mechanisms of drug action: what does it take for success? Nat Rev Drug Discov 2004;3:801–8. doi:10.1038/nrd1500.
[18] McDonnell null. The Molecular Pharmacology of SERMs. Trends Endocrinol Metab TEM 1999;10:301–11.
[19] Copeland RA. Conformational adaptation in drug-target interactions and residence time. Future Med Chem 2011;3:1491–501. doi:10.4155/fmc.11.112.
[20] Shan Y, Kim ET, Eastwood MP, Dror RO, Seeliger MA, Shaw DE. How does a drug molecule find its target binding site? J Am Chem Soc 2011;133:9181–3. doi:10.1021/ja202726y.
[21] Englert L, Biela A, Zayed M, Heine A, Hangauer D, Klebe G. Displacement of disordered water molecules from hydrophobic pocket creates enthalpic signature: binding of phosphonamidate to the S_1'-pocket of thermolysin. Biochim Biophys Acta 2010;1800:1192–202. doi:10.1016/j.bbagen.2010.06.009.
[22] Schmidtke P, Luque FJ, Murray JB, Barril X. Shielded hydrogen bonds as structural determinants of binding kinetics: application in drug design. J Am Chem Soc 2011;133:18903–10. doi:10.1021/ja207494u.
[23] Hong Y, Gengo FM, Rainka MM, Bates VE, Mager DE. Population pharmacodynamic modelling of aspirin- and Ibuprofen-induced inhibition of platelet aggregation in healthy subjects. Clin Pharmacokinet 2008;47:129–37. doi:10.2165/00003088-200847020-00006.
[24] Leach A, Jhoti H. Structure-Based Drug Discovery. Dordrecht: Springer Netherlands; 2007.
[25] Richter L, Ecker GF. Comprehensive analysis of a large multiple target kinetic dataset (K4DD database). (2015, June) Poster presented at the NovAliX Conference, Strasbourg, France.
[26] Vauquelin G. Rebinding: or why drugs may act longer in vivo than expected from their in vitro target residence time. Expert Opin Drug Discov 2010;5:927–41. doi:10.1517/17460441.2010.512037.
[27] Doris A. Schuetz, Richter L, Amaral M, Frech M, Ecker GF. Analysis of molecular features influencing HSP90 binding kinetics. (2015, July) Poster presented at the Gordon Research Conference, Mount Snow, West Dover, VT.
[28] Description of work; Proposal: K4DD K4DD; Grant Agreement number: 115366; Title: Kinetics for Drug Discovery; Description of work version 1.0 20120720 2012.
[29] Williams AJ, Harland L, Groth P, Pettifer S, Chichester C, Willighagen EL, et al. Open PHACTS: semantic interoperability for drug discovery. Drug Discov Today 2012;17:1188–98. doi:10.1016/j.drudis.2012.05.016.
[30] Zdrazil B, Pinto M, Vasanthanathan P, Williams AJ, Balderud LZ, Engkvist O, et al. Annotating Human P-Glycoprotein Bioassay Data. Mol Inform 2012;31:599–609. doi:10.1002/minf.201200059.
[31] Congreve M, Dias JM, Marshall FH. Structure-based drug design for G protein-coupled receptors. Prog Med Chem 2014;53:1–63. doi:10.1016/B978-0-444-63380-4.00001-9.
[32] Ballesteros J, Ransom J. In-Target versus Off-Target allosteric modulators of GPCRs. Drug Discov Today Ther Strateg 2006;Vol. 3.
[33] Tang X, Wang Y, Li D, Luo J, Liu M. Orphan G protein-coupled receptors (GPCRs): biological functions and potential drug targets. Acta Pharmacol Sin 2012;33:363–71. doi:10.1038/aps.2011.210.
[34] Christopher JA, Brown J, Doré AS, Errey JC, Koglin M, Marshall FH, et al. Biophysical fragment screening of the β1-adrenergic receptor: identification of high affinity arylpiperazine leads using structure-based drug design. J Med Chem 2013;56:3446–55. doi:10.1021/jm400140q.
[35] Aristotelous T, Ahn S, Shukla AK, Gawron S, Sassano MF, Kahsai AW, et al. Discovery of β2 Adrenergic Receptor Ligands Using Biosensor Fragment Screening of Tagged Wild-Type Receptor. ACS Med Chem Lett 2013;4:1005–10. doi:10.1021/ml400312j.

[36] Bayburt TH, Sligar SG. Membrane protein assembly into Nanodiscs. FEBS Lett 2010;584:1721–7. doi:10.1016/j.febslet.2009.10.024.
[37] Segala E, Errey JC, Fiez-Vandal C, Zhukov A, Cooke RM. Biosensor-based affinities and binding kinetics of small molecule antagonists to the adenosine A2A receptor reconstituted in HDL like particles. FEBS Lett 2015;589:1399–405. doi:10.1016/j.febslet.2015.04.030.
[38] Bocquet N, Kohler J, Hug MN, Kusznir EA, Rufer AC, Dawson RJ, et al. Real-time monitoring of binding events on a thermostabilized human A2A receptor embedded in a lipid bilayer by surface plasmon resonance. Biochim Biophys Acta BBA - Biomembr 2015;1848:1224–33. doi:10.1016/j.bbamem.2015.02.014.
[39] Christopoulos A. Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. Nat Rev Drug Discov 2002;1:198–210. doi:10.1038/nrd746.
[40] Stoddart LA, White CW, Nguyen K, Hill SJ, Pfleger KDG. Fluorescence- and bioluminescence-based approaches to study GPCR ligand binding. Br J Pharmacol 2015:n/a-n/a. doi:10.1111/bph.13316.
[41] May LT, Self TJ, Briddon SJ, Hill SJ. The effect of allosteric modulators on the kinetics of agonist-G protein-coupled receptor interactions in single living cells. Mol Pharmacol 2010;78:511–23. doi:10.1124/mol.110.064493.
[42] May LT, Bridge LJ, Stoddart LA, Briddon SJ, Hill SJ. Allosteric interactions across native adenosine-A3 receptor homodimers: quantification using single-cell ligand-binding kinetics. FASEB J Off Publ Fed Am Soc Exp Biol 2011;25:3465–76. doi:10.1096/fj.11-186296.
[43] Gherbi K, May LT, Baker JG, Briddon SJ, Hill SJ. Negative cooperativity across β1-adrenoceptor homodimers provides insights into the nature of the secondary low-affinity CGP 12177 β1-adrenoceptor binding conformation. FASEB J Off Publ Fed Am Soc Exp Biol 2015;29:2859–71. doi:10.1096/fj.14-265199.
[44] Stoddart LA, Johnstone EKM, Wheal AJ, Goulding J, Robers MB, Machleidt T, et al. Application of BRET to monitor ligand binding to GPCRs. Nat Methods 2015;12:661–3. doi:10.1038/nmeth.3398.
[45] Christiansen E, Hudson BD, Hansen AH, Milligan G, Ulven T. Development and Characterization of a Potent Free Fatty Acid Receptor 1 (FFA1) Fluorescent Tracer. J Med Chem 2016;59:4849–58. doi:10.1021/acs.jmedchem.6b00202.
[46] Schiele F, Ayaz P, Fernández-Montalván A. A universal homogeneous assay for high-throughput determination of binding kinetics. Anal Biochem 2015;468:42–9. doi:10.1016/j.ab.2014.09.007.
[47] Nederpelt I, Georgi V, Schiele F, Nowak-Reppel K, Fernández-Montalván AE, IJzerman AP, et al. Characterization of 12 GnRH peptide agonists - a kinetic perspective. Br J Pharmacol 2016;173:128–41. doi:10.1111/bph.13342.
[48] Klein Herenbrink C, Sykes DA, Donthamsetti P, Canals M, Coudrat T, Shonberg J, et al. The role of kinetic context in apparent biased agonism at GPCRs. Nat Commun 2016;7:10842. doi:10.1038/ncomms10842.
[49] Segala E, Guo D, Cheng RKY, Bortolato A, Deflorian F, Doré AS, et al. Controlling the Dissociation of Ligands from the Adenosine A2A Receptor through Modulation of Salt Bridge Strength. J Med Chem 2016;59:6470–9. doi:10.1021/acs.jmedchem.6b00653.
[50] Guo D, Xia L, van Veldhoven JPD, Hazeu M, Mocking T, Brussee J, et al. Binding Kinetics of ZM241385 Derivatives at the Human Adenosine A2A Receptor. ChemMedChem 2014;9:752–61. doi:10.1002/cmdc.201300474.
[51] Labrie F. GnRH agonists and the rapidly increasing use of combined androgen blockade in prostate cancer. Endocr Relat Cancer 2014;21:R301-317. doi:10.1530/ERC-13-0165.
[52] Nederpelt I, Vergroesen RD, IJzerman AP, Heitman LH. Persistent GnRH receptor activation in pituitary αT3-1 cells analyzed with a label-free technology. Biosens Bioelectron 2016;79:721–7. doi:10.1016/j.bios.2015.12.066.
[53] Motulsky HJ, Mahan LC. The kinetics of competitive radioligand binding predicted by the law of mass action. Mol Pharmacol 1984;25:1–9.

[54] Klein T, Vajpai N, Phillips JJ, Davies G, Holdgate GA, Phillips C, et al. Structural and dynamic insights into the energetics of activation loop rearrangement in FGFR1 kinase. Nat Commun 2015;6:7877. doi:10.1038/ncomms8877.
[55] Kwon K, Streaker ED, Beckett D. Binding specificity and the ligand dissociation process in the E. coli biotin holoenzyme synthetase. Protein Sci Publ Protein Soc 2002;11:558–70. doi:10.1110/ps.33502.
[56] ChEMBL: a large-scale bioactivity database for drug discovery n.d. http://nar.oxfordjournals.org/content/early/2011/09/22/nar.gkr777.abstract?ct=ct (accessed August 8, 2016).
[57] Miller DC, Lunn G, Jones P, Sabnis Y, Davies NL, Driscoll P. Investigation of the effect of molecular properties on the binding kinetics of a ligand to its biological target. MedChemComm 2012;3:449–52. doi:10.1039/C2MD00270A.
[58] Gaspari R, Rechlin C, Heine A, Bottegoni G, Rocchia W, Schwarz D, et al. Kinetic and Structural Insights into the Mechanism of Binding of Sulfonamides to Human Carbonic Anhydrase by Computational and Experimental Studies. J Med Chem 2016;59:4245–56. doi:10.1021/acs.jmedchem.5b01643.
[59] Andersson K, Hämäläinen MD. Replacing affinity with binding kinetics in QSAR studies resolves otherwise confounded effects. J Chemom 2006;20:370–5. doi:10.1002/cem.1010.
[60] Ayaz P, Andres D, Kwiatkowski DA, Kolbe C-C, Lienau P, Siemeister G, et al. Conformational Adaption May Explain the Slow Dissociation Kinetics of Roniciclib (BAY 1000394), a Type I CDK Inhibitor with Kinetic Selectivity for CDK2 and CDK9. ACS Chem Biol 2016;11:1710–9. doi:10.1021/acschembio.6b00074.
[61] Siemeister G, Lücking U, Wengner AM, Lienau P, Steinke W, Schatz C, et al. BAY 1000394, a novel cyclin-dependent kinase inhibitor, with potent antitumor activity in mono- and in combination treatment upon oral application. Mol Cancer Ther 2012;11:2265–73. doi:10.1158/1535-7163.MCT-12-0286.
[62] Romanowska J, Kokh DB, Fuller JC, Wade RC. Computational Approaches for Studying Drug Binding Kinetics. In: Keserü GM, Swinney DC, editors. Thermodyn. Kinet. Drug Bind., Wiley-VCH Verlag GmbH & Co. KGaA; 2015, p. 211–35.
[63] Buch I, Giorgino T, De Fabritiis G. Complete reconstruction of an enzyme-inhibitor binding process by molecular dynamics simulations. Proc Natl Acad Sci U S A 2011;108:10184–9. doi:10.1073/pnas.1103547108.
[64] Shaw DE, Maragakis P, Lindorff-Larsen K, Piana S, Dror RO, Eastwood MP, et al. Atomic-level characterization of the structural dynamics of proteins. Science 2010;330:341–6. doi:10.1126/science.1187409.
[65] Doerr S, De Fabritiis G. On-the-Fly Learning and Sampling of Ligand Binding by High-Throughput Molecular Simulations. J Chem Theory Comput 2014;10:2064–9. doi:10.1021/ct400919u.
[66] Huang, D.; Caflisch, A. The Free Energy Landscape of Small Molecule Unbinding. PLOS Computational Biology: 2011, 7 (2). doi: 10.1371/journal.pcbi.1002002
[67] Plattner N, Noé F. Protein conformational plasticity and complex ligand-binding kinetics explored by atomistic simulations and Markov models. Nat Commun 2015;6:7653. doi:10.1038/ncomms8653.
[68] Pérez-Hernández G, Paul F, Giorgino T, De Fabritiis G, Noé F. Identification of slow molecular order parameters for Markov model construction. J Chem Phys 2013;139:15102. doi:10.1063/1.4811489.
[69] Kappel K, Miao Y, McCammon JA. Accelerated molecular dynamics simulations of ligand binding to a muscarinic G-protein-coupled receptor. Q Rev Biophys 2015;48:479–87. doi:10.1017/S0033583515000153.
[70] Stank A, Kokh DB, Fuller JC, Wade RC. Protein Binding Pocket Dynamics. Acc Chem Res 2016;49:809–15. doi:10.1021/acs.accounts.5b00516.
[71] Markgren P-O, Schaal W, Hämäläinen M, Karlén A, Hallberg A, Samuelsson B, et al. Relationships between structure and interaction kinetics for HIV-1 protease inhibitors. J Med Chem 2002;45:5430–9.

[72] Lauro G, Ferruz N, Fulle S, Harvey MJ, Finn PW, De Fabritiis G. Reranking docking poses using molecular simulations and approximate free energy methods. J Chem Inf Model 2014;54:2185–9. doi:10.1021/ci500309a.
[73] Ferruz N, Harvey MJ, Mestres J, De Fabritiis G. Insights from Fragment Hit Binding Assays by Molecular Simulations. J Chem Inf Model 2015:2200–5. doi:10.1021/acs.jcim.5b00453.
[74] Raman CS, Jemmerson R, Nall BT, Allen MJ. Diffusion-limited rates for monoclonal antibody binding to cytochrome c. Biochemistry (Mosc) 1992;31:10370–9.
[75] Northrup SH, Allison SA, McCammon JA. Brownian dynamics simulation of diffusion-influenced bimolecular reactions. J Chem Phys 1984;80:1517–24. doi:10.1063/1.446900.
[76] ElSawy KM, Twarock R, Lane DP, Verma CS, Caves LSD. Characterization of the Ligand Receptor Encounter Complex and Its Potential for in Silico Kinetics-Based Drug Development. J Chem Theory Comput 2012;8:314–21. doi:10.1021/ct200560w.
[77] Mondal J, Friesner RA, Berne BJ. Role of Desolvation in Thermodynamics and Kinetics of Ligand Binding to a Kinase. J Chem Theory Comput 2014;10:5696–705. doi:10.1021/ct500584n.
[78] Mondal J, Morrone JA, Berne BJ. How hydrophobic drying forces impact the kinetics of molecular recognition. Proc Natl Acad Sci 2013;110:13277–82. doi:10.1073/pnas.1312529110.
[79] Luty BA, El Amrani S, McCammon JA. Simulation of the bimolecular reaction between superoxide and superoxide dismutase: synthesis of the encounter and reaction steps. J Am Chem Soc 1993;115:11874–7. doi:10.1021/ja00078a027.
[80] Berezhkovskii AM, Szabo A, Zhou H-X. Diffusion-influenced ligand binding to buried sites in macromolecules and transmembrane channels. J Chem Phys 2011;135:75103. doi:10.1063/1.3609973.
[81] Cai L, Zhou H-X. Theory and simulation on the kinetics of protein-ligand binding coupled to conformational change. J Chem Phys 2011;134:105101. doi:10.1063/1.3561694.
[82] Greives N, Zhou H-X. BDflex: a method for efficient treatment of molecular flexibility in calculating protein-ligand binding rate constants from brownian dynamics simulations. J Chem Phys 2012;137:135105. doi:10.1063/1.4756913.
[83] Votapka LW, Amaro RE. Multiscale Estimation of Binding Kinetics Using Brownian Dynamics, Molecular Dynamics and Milestoning. PLoS Comput Biol 2015;11. doi:10.1371/journal.pcbi.1004381.
[84] Vanden-Eijnden E, Tal FA. Transition state theory: Variational formulation, dynamical corrections, and error estimates. J Chem Phys 2005;123:184103. doi:10.1063/1.2102898.
[85] Laio A, Parrinello M. Escaping free-energy minima. Proc Natl Acad Sci U S A 2002;99:12562–6. doi:10.1073/pnas.202427399.
[86] Grubmüller H. Predicting slow structural transitions in macromolecular systems: Conformational flooding. Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Top 1995;52:2893–906.
[87] Hamelberg D, Mongan J, McCammon JA. Accelerated molecular dynamics: a promising and efficient simulation method for biomolecules. J Chem Phys 2004;120:11919–29. doi:10.1063/1.1755656.
[88] Frank AT, Andricioaei I. Reaction Coordinate-Free Approach to Recovering Kinetics from Potential-Scaled Simulations: Application of Kramers' Rate Theory. J Phys Chem B 2016. doi:10.1021/acs.jpcb.6b02654.
[89] Tiwary P, Limongelli V, Salvalaglio M, Parrinello M. Kinetics of protein-ligand unbinding: Predicting pathways, rates, and rate-limiting steps. Proc Natl Acad Sci U S A 2015;112:E386-391. doi:10.1073/pnas.1424461112.
[90] Mollica L, Decherchi S, Zia SR, Gaspari R, Cavalli A, Rocchia W. Kinetics of protein-ligand unbinding via smoothed potential molecular dynamics simulations. Sci Rep 2015;5:11539. doi:10.1038/srep11539.

[91] Mollica L, Theret I, Antoine M, Perron-Sierra F, Charton Y, Fourquez J-M, et al. Molecular Dynamics Simulations and Kinetic Measurements to Estimate and Predict Protein–Ligand Residence Times. J Med Chem 2016;59:7167–76. doi:10.1021/acs.jmedchem.6b00632.
[92] Choulier L, Andersson K, Hämäläinen MD, Regenmortel MHV van, Malmqvist M, Altschuh D. QSAR studies applied to the prediction of antigen–antibody interaction kinetics as measured by BIACORE. Protein Eng 2002;15:373–82. doi:10.1093/protein/15.5.373.
[93] Magotti P, Ricklin D, Qu H, Wu Y-Q, Kaznessis YN, Lambris JD. Structure-kinetic relationship analysis of the therapeutic complement inhibitor compstatin. J Mol Recognit 2009;22:495–505. doi:10.1002/jmr.972.
[94] Basavapathruni A, Jin L, Daigle SR, Majer CRA, Therkelsen CA, Wigle TJ, et al. Conformational adaptation drives potent, selective and durable inhibition of the human protein methyltransferase DOT1L. Chem Biol Drug Des 2012;80:971–80. doi:10.1111/cbdd.12050.
[95] Miller DC, Klute W, Brown AD. Discovery of potent, metabolically stable purine CRF-1 antagonists with differentiated binding kinetic profiles. Bioorg Med Chem Lett 2011;21:6108–11. doi:10.1016/j.bmcl.2011.08.040.
[96] Jin M, Petronella BA, Cooke A, Kadalbajoo M, Siu KW, Kleinberg A, et al. Discovery of novel insulin-like growth factor-1 receptor inhibitors with unique time-dependent binding kinetics. ACS Med Chem Lett 2013;4:627–31. doi:10.1021/ml400160a.
[97] Regan J, Pargellis CA, Cirillo PF, Gilmore T, Hickey ER, Peet GW, et al. The kinetics of binding to p38MAP kinase by analogues of BIRB 796. Bioorg Med Chem Lett 2003;13:3101–4.
[98] Vilums M, Zweemer AJM, Yu Z, de Vries H, Hillger JM, Wapenaar H, et al. Structure-kinetic relationships--an overlooked parameter in hit-to-lead optimization: a case of cyclopentylamines as chemokine receptor 2 antagonists. J Med Chem 2013;56:7706–14. doi:10.1021/jm4011737.
[99] Danhof M, de Jongh J, De Lange ECM, Della Pasqua O, Ploeger BA, Voskuyl RA. Mechanism-based pharmacokinetic-pharmacodynamic modeling: biophase distribution, receptor theory, and dynamical systems analysis. Annu Rev Pharmacol Toxicol 2007;47:357–400. doi:10.1146/annurev.pharmtox.47.120505.105154.
[100] Dahl G, Akerud T. Pharmacokinetics and the drug-target residence time concept. Drug Discov Today 2013;18:697–707. doi:10.1016/j.drudis.2013.02.010.
[101] Peletier LA, Benson N, van der Graaf PH. Impact of protein binding on receptor occupancy: a two-compartment model. J Theor Biol 2010;265:657–71. doi:10.1016/j.jtbi.2010.05.035.
[102] Katashima M, Yamamoto K, Tokuma Y, Hata T, Sawada Y, Iga T. Comparative pharmacokinetic/pharmacodynamic analysis of proton pump inhibitors omeprazole, lansoprazole and pantoprazole, in humans. Eur J Drug Metab Pharmacokinet 1998;23:19–26.
[103] Yin N, Pei J, Lai L. A comprehensive analysis of the influence of drug binding kinetics on drug action at molecular and systems levels. Mol Biosyst 2013;9:1381–9. doi:10.1039/c3mb25471b.
[104] de Witte WEA, Wong YC, Nederpelt I, Heitman LH, Danhof M, van der Graaf PH, et al. Mechanistic models enable the rational use of in vitro drug-target binding kinetics for better drug effects in patients. Expert Opin Drug Discov 2016;11:45–63. doi:10.1517/17460441.2016.1100163.
[105] Levy G. Pharmacologic target-mediated drug disposition. Clin Pharmacol Ther 1994;56:248–52.
[106] Dua P, Hawkins E, van der Graaf P. A Tutorial on Target-Mediated Drug Disposition (TMDD) Models. CPT Pharmacomet Syst Pharmacol 2015;4:324–37. doi:10.1002/psp4.41.

[107]Yamazaki S, Shen Z, Jiang Y, Smith BJ, Vicini P. Application of target-mediated drug disposition model to small molecule heat shock protein 90 inhibitors. Drug Metab Dispos Biol Fate Chem 2013;41:1285–94. doi:10.1124/dmd.113.051490.
[108]de Witte WEA, Danhof M, van der Graaf PH, de Lange ECM. In vivo Target Residence Time and Kinetic Selectivity: The Association Rate Constant as Determinant. Trends Pharmacol Sci 2016. doi:10.1016/j.tips.2016.06.008.
[109]Guo D, van Dorp EJH, Mulder-Krieger T, van Veldhoven JPD, Brussee J, Ijzerman AP, et al. Dual-point competition association assay: a fast and high-throughput kinetic screening method for assessing ligand-receptor binding kinetics. J Biomol Screen 2013;18:309–20. doi:10.1177/1087057112464776.
[110]Tresadern G, Bartolome JM, Macdonald GJ, Langlois X. Molecular properties affecting fast dissociation from the D2 receptor. Bioorg Med Chem 2011;19:2231–41. doi:10.1016/j.bmc.2011.02.033.
[111]Lu H, Tonge PJ. Drug-target residence time: critical information for lead optimization. Curr Opin Chem Biol 2010;14:467–74. doi:10.1016/j.cbpa.2010.06.176.
[112]Bradshaw JM, McFarland JM, Paavilainen VO, Bisconte A, Tam D, Phan VT, et al. Prolonged and tunable residence time using reversible covalent kinase inhibitors. Nat Chem Biol 2015;11:525–31. doi:10.1038/nchembio.1817.
[113]Kenakin T, Jenkinson S, Watson C. Determining the potency and molecular mechanism of action of insurmountable antagonists. J Pharmacol Exp Ther 2006;319:710–23. doi:10.1124/jpet.106.107375.
[114]Sykes DA, Dowling MR, Leighton-Davies J, Kent TC, Fawcett L, Renard E, et al. The Influence of receptor kinetics on the onset and duration of action and the therapeutic index of NVA237 and tiotropium. J Pharmacol Exp Ther 2012;343:520–8. doi:10.1124/jpet.112.194456.

Weblinks:

www.k4dd.eu – last accessed September, 28th, 2016

www.openphacts.org - last accessed September, 28th, 2016

www.bikitech.com - last accessed September, 28th, 2016

Figure 1: A combination of experimental and computational approaches are used in the in the K4DD consortium to study drug binding kinetics and learn about structure-kinetics relationships [28]

Figure 2: View of five antagonists in the binding site of the A2AR receptor. ZM241385 and compound 12x have a long residence time at the A2A receptor and stabilize the salt bridge His264-Glu169 (Adapted from [49] Segala J. Med. Chem. 2016)

Figure 3: Schematic representation of the three kinetic assays. **A)** radioligand binding studies. Assay requirements are cell membrane preparations and a high affinity radiolabeled tracer. Over time, the unlabeled ligand of interest will displace the radiolabeled tracer and from this the k_{on}, k_{off} and residence time (RT) values of the unlabeled ligand can be calculated. **B**) TR-FRET™ assay. Assay requirements are whole cells with a SNAP-tagged receptor and a high affinity fluorescent tracer. When the fluorescent tracer and tagged receptor are in close proximity, a FRET signal can be detected, over time the unlabeled ligand of interest will displace the fluorescent tracer and from this the k_{on}, k_{off} and RT values of the unlabeled ligand can be calculated. **C)** label-free xCELLigence assay. Assay requirements are whole cells, no tracer or labeling necessary. Receptor activation can be followed over time by monitoring the cell morphology through impedance. Purple circle is a fast dissociating ligand, pink circle is a slowly dissociating agonist

Equation 1: Linear Eyring equation, where h and k_B are the Planck and Boltzmann constants, k is either the association rate constant (k_{on}) or the dissociation rate constant (k_{off}). $\Delta H^{\#}$ and $\Delta S^{\#}$ are the changes in enthalpy and entropy of the transition state, respectively. T is the absolute temperature and R is the gas constant

Figure 4: A) Kinetic landscape showing the timescale gap between computationally accessible (blue) small molecules (based on conventional molecular dynamics of fragment binding to Trypsin [73]) and clinically relevant (red) compounds (in this case, several FDA approved and trial inhibitors of HIV-1 protease [71]). **B)** Different strategies required for improvement of K_D, k_{on}, and k_{off} values (yellow, orange, and red arrows, respectively)

Figure 5: Residence time of 5-substituted Roniciclib analogues; **a)** Modification of the R5-group on the sulphonamides. **b)** Residence time (T) of compounds 6-10 on pCDK2/cyclin A and pCDK2/cyclin E (SPR experiment at 25°C). **c)** Substitution at the 5-position on the aminopyridine (van der Waals volumes with corresponding topological nonpolar surface areas (TNSA))

Reprinted from "Conformational Adaption May Explain the Slow Dissociation Kinetics of Roniciclib (BAY 1000394), a Type I CDK Inhibitor with Kinetic Selectivity for CDK2 and

CDK9", ACS Chem Biol 2016, 11, 1710−1719; Ayaz P, Andres D, Kwiatkowski DA, Kolbe C-C, Lienau P, Siemeister G, et al.; With permission of ACS.

Figure 6: LC-MS based approach for exploring *in vivo* target occupancy-time profile, using dopamine D_2 receptor ligands as paradigm compounds. After dosing the animal with the ligand, plasma, tissues and microdialysate ligand concentrations are quantified by LC-MS. The difference in ligand concentrations between target tissue and reference tissue represents the specific ligand-target binding. Alternatively, target occupancy could also be estimated from the unbound ligand concentration at the target site (obtained from continuous microdialysis sampling) and the k_{on} and k_{off} values obtained from *in vitro* studies. A mechanistic computational model can then be constructed to predict the impact of binding kinetics and other factors (e.g. ligand dosing regimen) on target occupancy-time profile. Assessment of target pharmacokinetics and occupancy using LC-MS based approach offers several advantages over radioligand-based imaging approaches like PET and SPECT [104]. Most importantly, it provides higher throughput for drug screening and preclinical development, and the interferences due to radioactive metabolites and the anesthetic procedures during imaging could be avoided. PK, pharmacokinetics; TO, target occupancy

Figure 7: Model simulations demonstrating that increasing affinities result in higher free drug concentrations (lower panel) and longer target occupancies (upper panel), while k_{off} is constant. In these simulations, the first-order elimination rate constant k_{el} = 1 h^{-1}, k_{off} = 36 h^{-1} and the target concentration is 1 nM. The simulated drug dose is relative to the K_D. Right: schematic representation of the applied model structure

Figure

Click here to download high resolution image

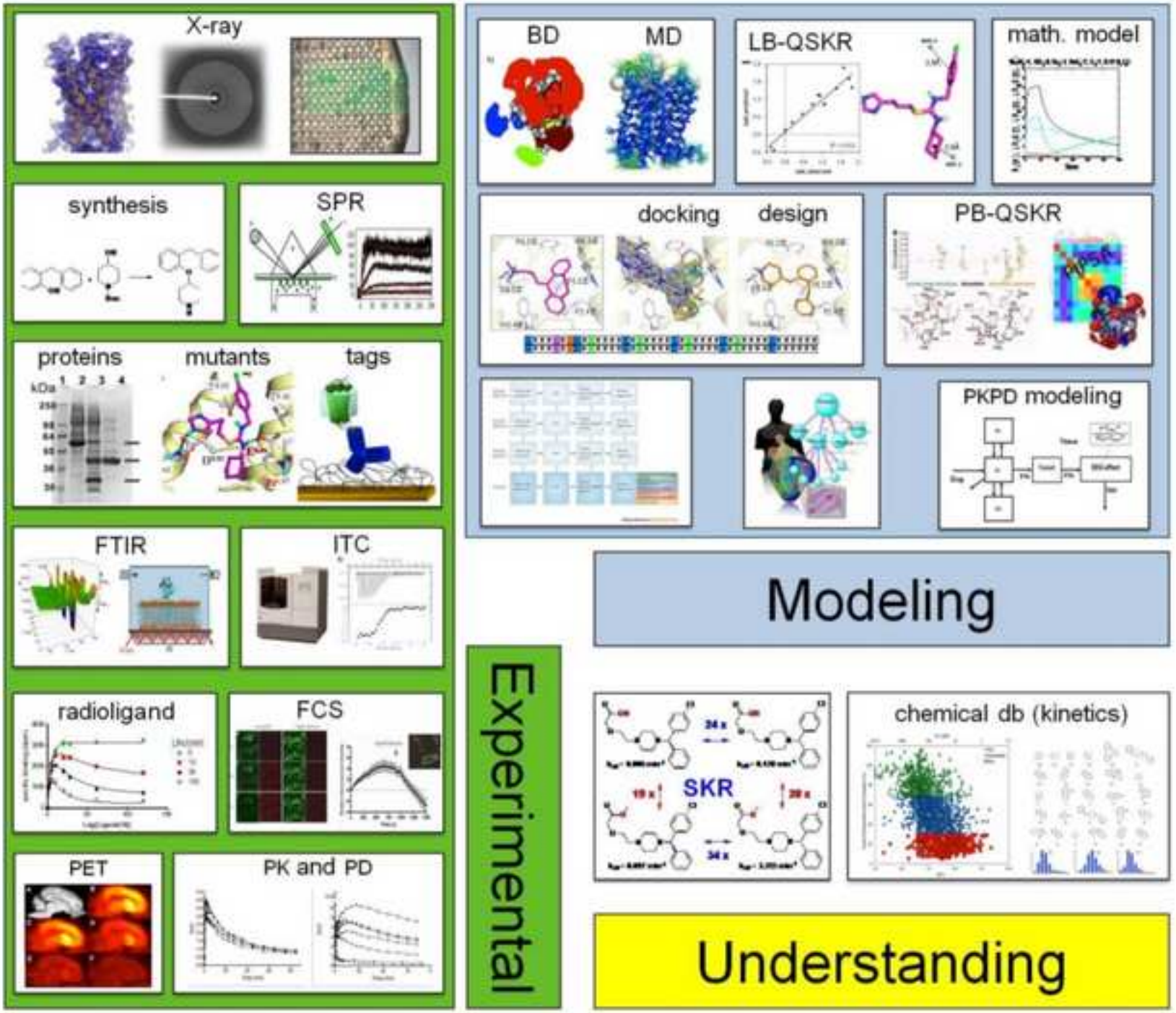


Figure 2

Click here to download high resolution image

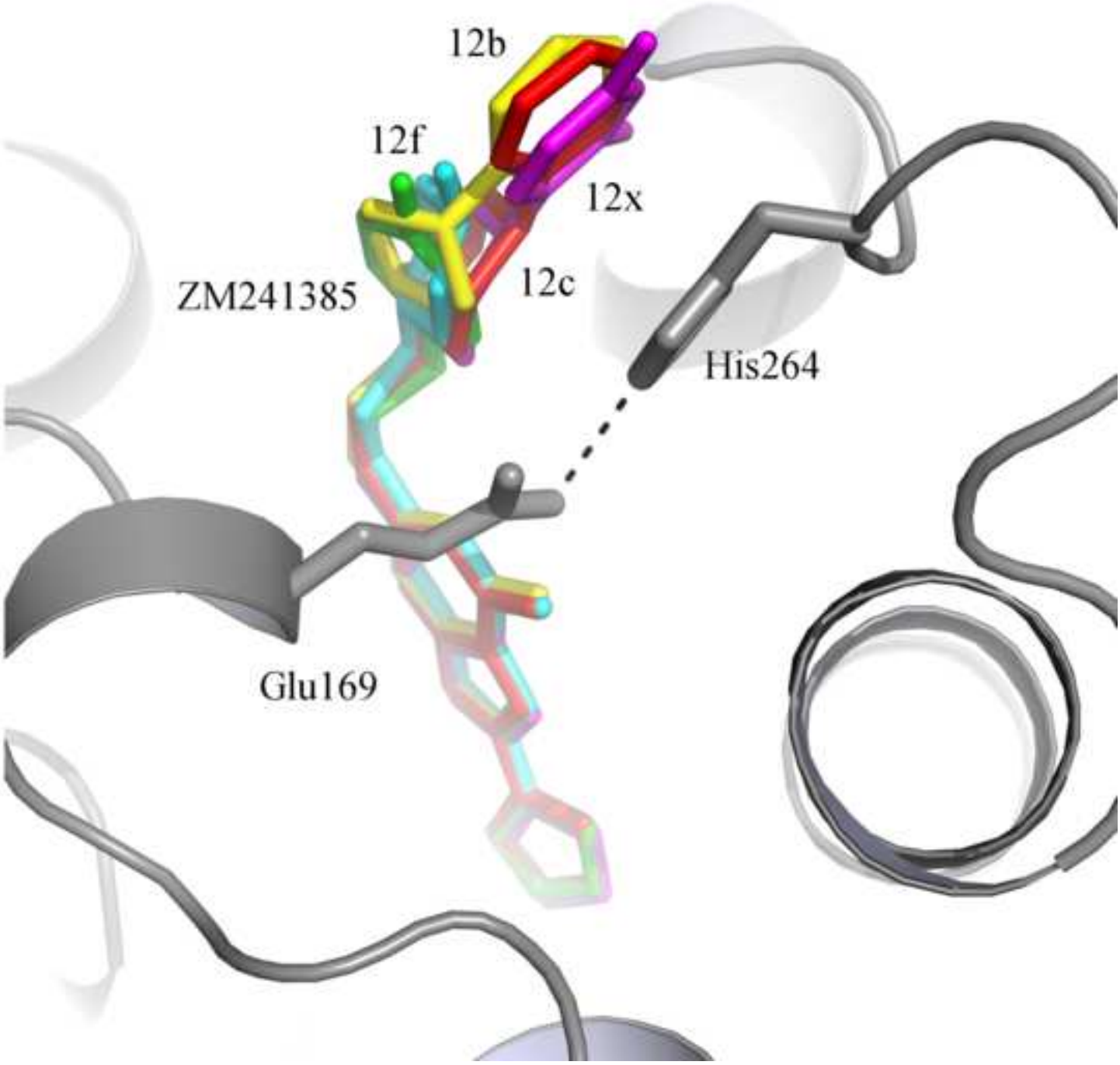


Figure 3

Click here to download high resolution image

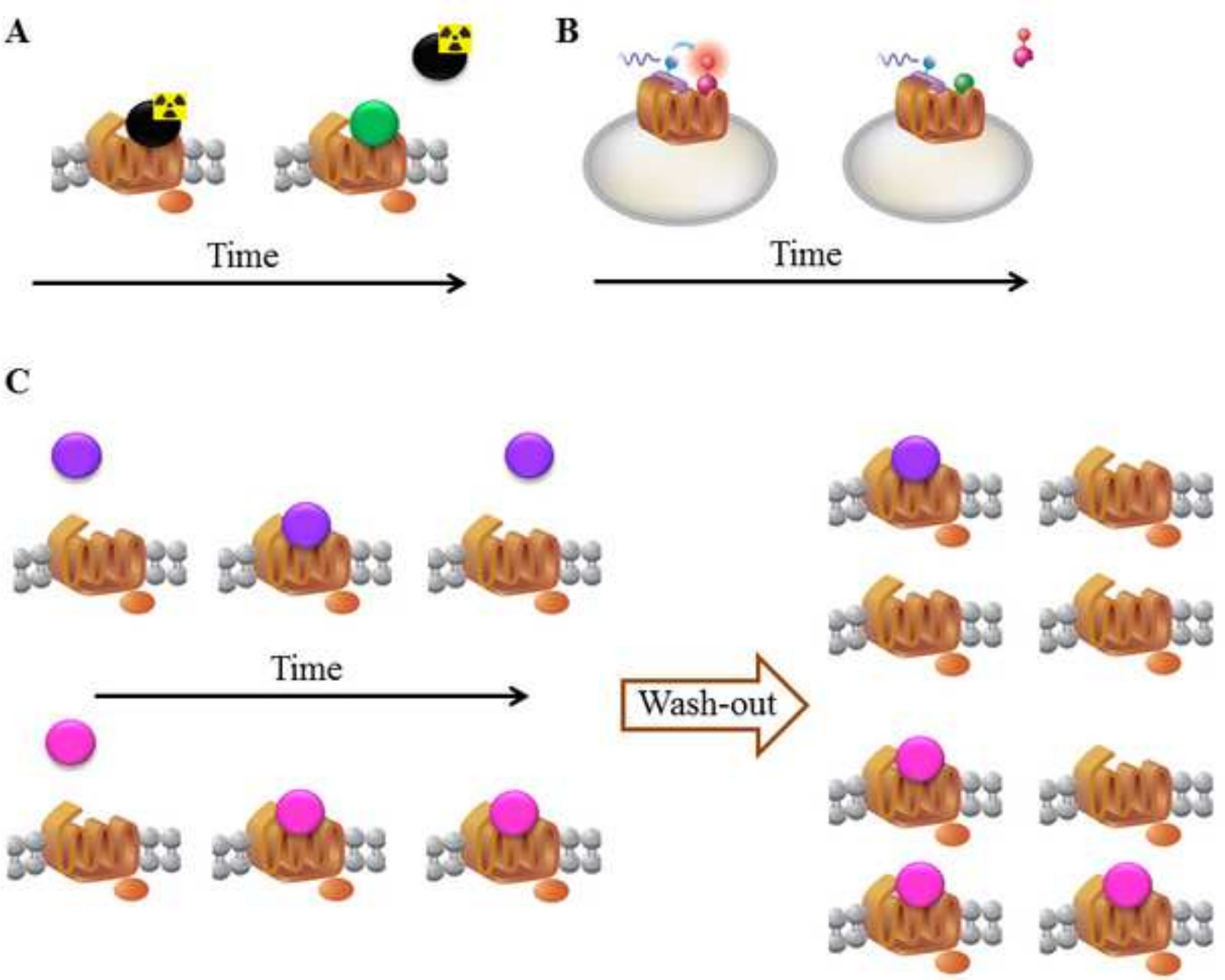


Figure 4

Click here to download high resolution image

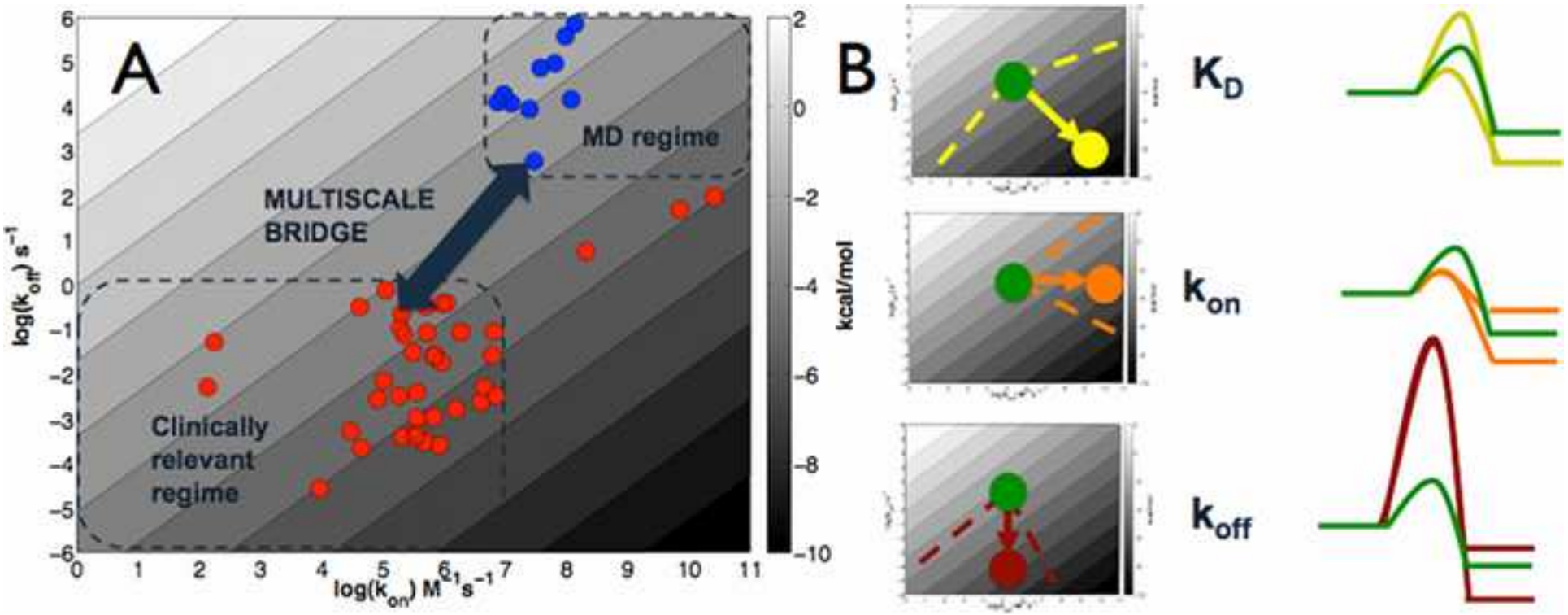


Figure 5

Click here to download high resolution image

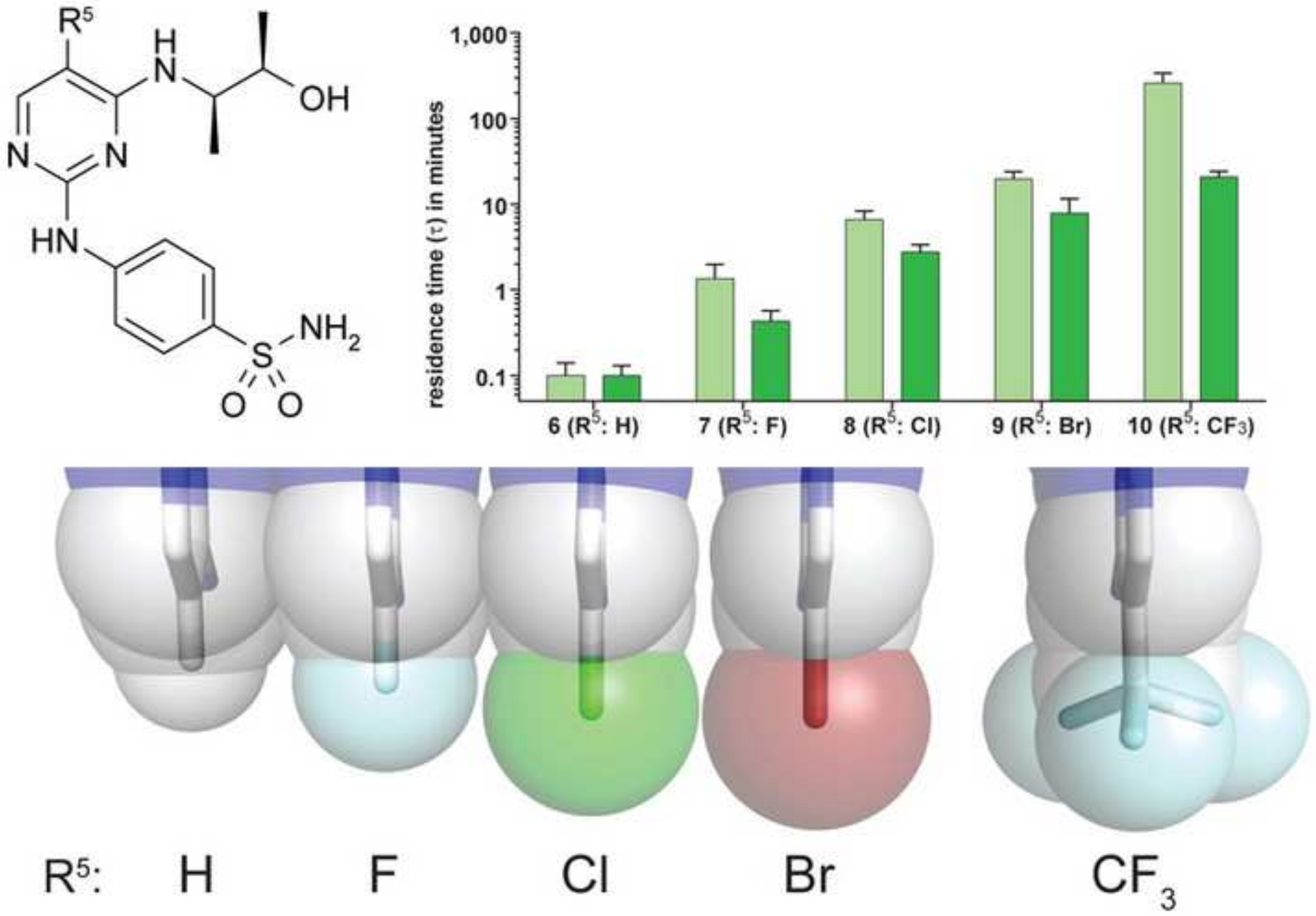


Figure 6

Click here to download high resolution image

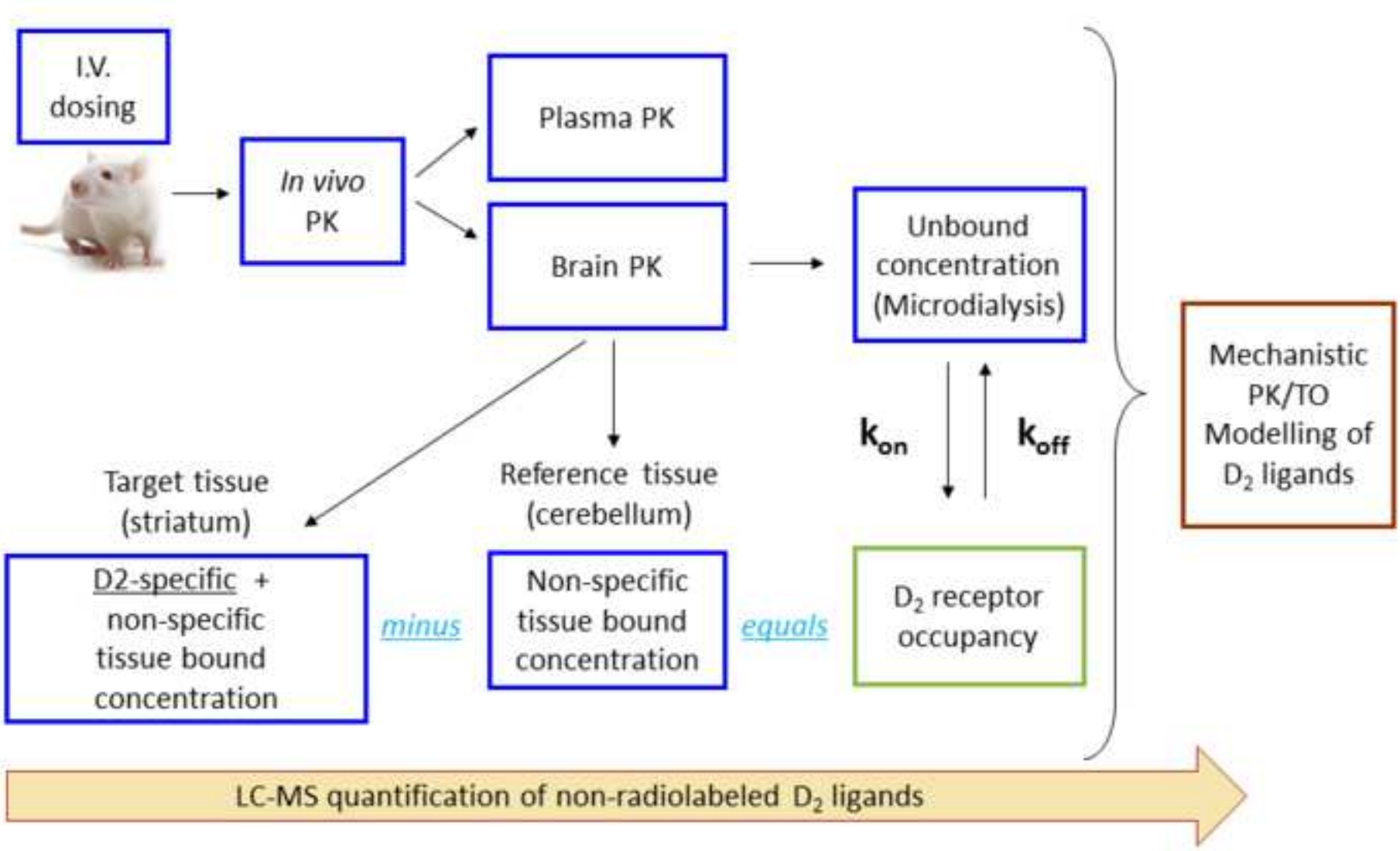
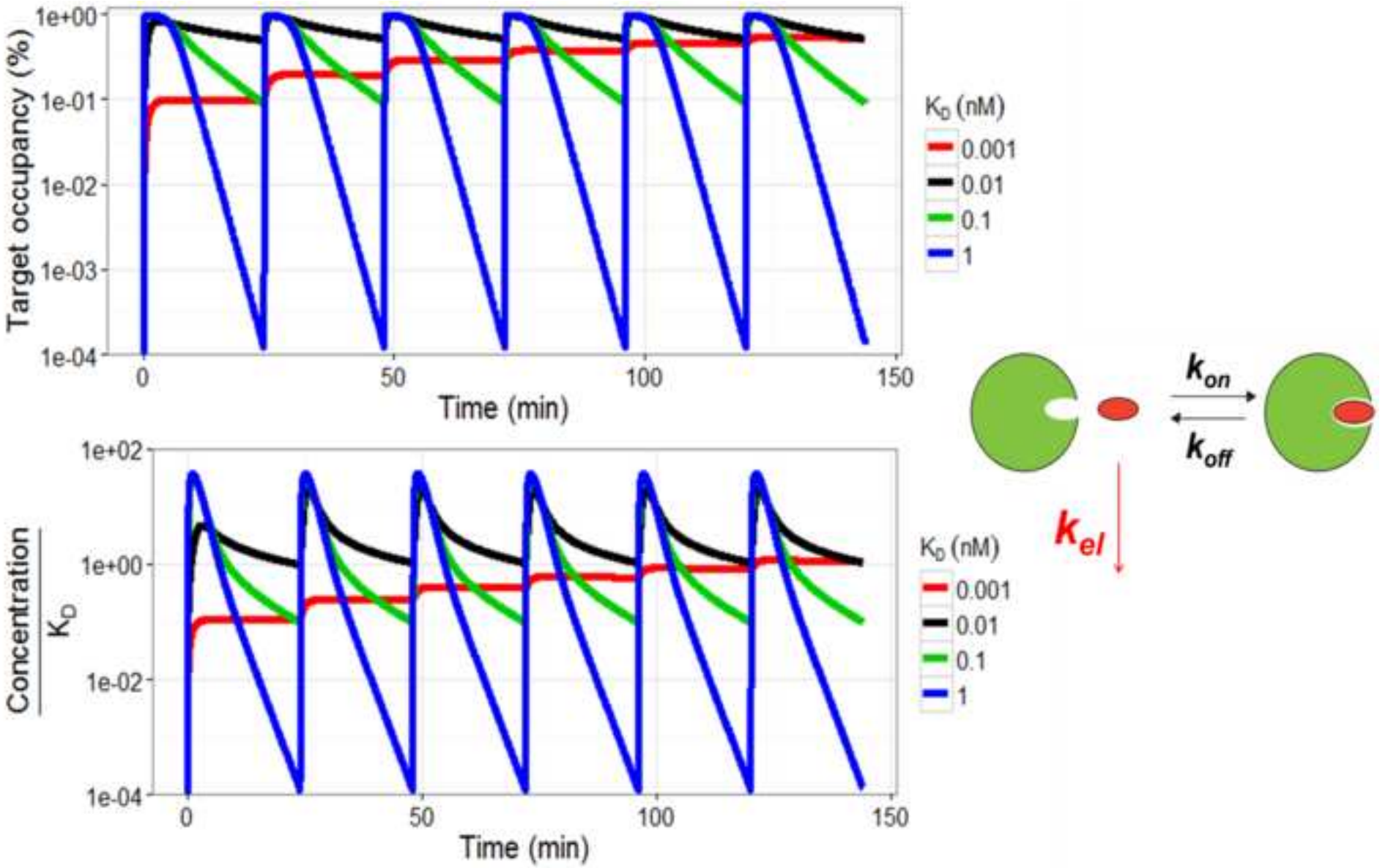


Figure 7

Click here to download high resolution image



Equation 1

$$\ln\left(\frac{kh}{k_B T}\right) = -\frac{\Delta H^{\#}}{RT} + \frac{\Delta S^{\#}}{R}$$

Kinetics for Drug Discovery – An industry driven effort to target drug residence time

Schütz et al.

Highlights

- An industry driven private-public partnership targets drug binding kinetics in a multilevel approach
- New experimental approaches for measuring drug residence time are presented
- Standardized data formats will guarantee sustainability of the data generated
- Progress in quantitative structure-kinetics relationships as well as mechanistic simulation approaches are discussed
- Transition from in vitro to in vivo is key for adoption of the residence time as decision criterion in drug development