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REVIEW ARTICLE

The translational value of ligand-receptor binding kinetics in drug discovery

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Abstract

The translation of in vitro potency of a candidate drug, as determined by traditional pharmacology metrics (such as EC_{50}/IC_{50} and K_D/K_i values), to in vivo efficacy and safety is challenging. Residence time, which represents the duration of drug-target interaction, can be part of a more comprehensive understanding of the dynamic nature of drug-target interactions in vivo, thereby enabling better prediction of drug efficacy and safety. As a consequence, a prolonged residence time may help in achieving sustained pharmacological activity, while transient interactions with shorter residence times may be favourable for targets associated with side effects. Therefore, integration of residence time into the early stages of drug discovery and development has yielded a number of clinical candidates with promising in vivo efficacy and safety profiles. Insights from residence time research thus contribute to the translation of in vitro potency to in vivo efficacy and safety. Further research and advances in measuring and optimizing residence time will bring a much-needed addition to the drug discovery process and the development of safer and more effective drugs. In this review, we summarize recent research progress on residence time, highlighting its importance from a translational perspective.

KEYWORDS
binding kinetics, drug-target interaction, k_{off}, residence time, translation

1 | INTRODUCTION

Pharmacodynamic effects, whether desired therapeutic effects or untoward side effects depend critically on the binding of the drug to its target. Traditionally, equilibrium binding parameters, namely the concentration needed for half-maximal effect (EC_{50} or IC_{50}) and the inhibition constant (K_i), are commonly used to evaluate drug-target interactions. However, these indicators are measured under equilibrium conditions and the concentrations of drug molecule and target are considered constant throughout the experiment. Such a condition is not applicable in the context of the open, non-equilibrium settings in vivo, due to various pharmacokinetic processes, such as absorption, distribution, metabolism and excretion (ADME) (Copeland, 2016). Consequently, despite significant efforts invested in this area, the success rate of in vitro to in vivo translation has been disappointingly low. Many candidate compounds that display favourable in vitro

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; ADPKD, autosomal dominant polycystic kidney disease; Ang II, angiotensin II; CLL, chronic lymphocytic leukemia; eEF1A, eukaryotic elongation factor-1α; EPS, extrapyramidal symptoms; KW/BW, kidney weight/body weight; MD, molecular dynamics; PAE, post-antibiotic effect; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetics; REM, rapid eye movement; RT, residence time; sEH, soluble epoxide hydrolase; SPR, surface plasmon resonance; V2R, V2 receptor.

parameters, such as a high affinity for the intended target, fail to exhibit the desired in vivo efficacy (Harrison, 2016). How to decrease the translational attrition from in vitro metrics to in vivo efficacy remains therefore challenging in the early stages of drug discovery.

Copeland and colleagues found that the effectiveness and duration of drug action depend critically on the duration of drug-target interaction by retrospectively analysing the kinetic profiles of marketed drugs (Knockenhauer & Copeland, 2023). The term 'residence time' was defined as an experimentally measurable representation of the lifetime of the drug-target binary complex, which is the reciprocal of the dissociation rate constant (k_{off}) (Copeland, 2005; Copeland, 2016; Tummino & Copeland, 2008). In the past decades, the residence time parameter has grown from its infancy and developed into an indispensable metric in the early phase of drug design and discovery. More and more proof-of-mechanism studies support the proposition that the drug-target residence time parameter provides a more accurate prediction of in vivo efficacy and safety (Berger et al., 2021; Ma et al., 2019; Zhang, Yan, et al., 2022). Slow dissociation of the drug-target complex is an important factor for sustained pharmacological activity in vivo (Copeland, 2016), while a transient ligand-receptor interaction might be favourable for targets that are involved in (on-target) side effects. Therefore, there has been an increasing interest in integrating residence time into drug research that led to the identification of clinical candidates with optimal in vivo efficacy and safety. Several examples are given in Table 1, including drugs for **D_2 receptor**s, **acetylcholinesterase**, **M_3 receptor**s and **H_1 receptor**s. Apparently, compounds with long or short residence time displayed distinct in vivo efficacy and safety profiles.

In this review we summarize the recent research progress on residence time, highlighting its importance from a translational perspective. The existing challenges around residence time and insights into future directions are also discussed.

2 | RESIDENCE TIME FROM A PHARMACOLOGICAL PERSPECTIVE

Although factors such as affinity and drug distribution have been extensively studied to explain differences in drug efficacy and side effects, they are often inadequate. There are variations in drug efficacy or resistance that cannot be explained solely by comparing affinity and other similar pharmacological parameters (Karaman et al., 2020; Zhang, Yan, et al., 2022). Taking residence time into account can provide a new perspective to better comprehend the mechanisms of drug effects and side effects. We have summarized the relationship between residence time and drug effect, from a pharmacological aspect, in Figure 1.

2.1 | Duration of drug-target interaction

Drugs exhibit activity only upon binding to their respective targets, and the pharmacological effect(s) persists as long as the target remains sufficiently occupied by the drug. Moreover, drugs with longer residence times can produce higher local drug concentrations near the target (Copeland, 2016; Vauquelin, 2010). Therefore, the duration of

TABLE 1 Representative compounds with different receptor residence times and their respective clinical efficacies. Examples of a drug with long or short residence time are also given for each target.

Target	Compd.	Residence time	Clinical indications	The clinical effects of compounds with long or short residence time	Status	Ref.
D_2 receptor	JNJ-37822681	9 s	Schizophrenia	JNJ-37822681 has a relatively lower incidence of extrapyramidal symptoms than haloperidol.	Phase II	(Langlois et al., 2012)
	Haloperidol	104 s			Marketed	(Kapur et al., 2000; Langlois et al., 2012)
Acetylcholinesterase	Compound 12	134 s	Alzheimer's disease	Compound 12 significantly ameliorated the cognitive impairments with a lower effective dose than donepezil.	Phase I	(Qian et al., 2023; Zhou et al., 2021)
	Donepezil	34 s			Marketed	(Zhou et al., 2021)
M_3 receptor	Tiotropium	38 h	Chronic obstructive pulmonary disease	Tiotropium produced sustained bronchodilation and reduced the number of administrations compared with ipratropium.	Marketed	(Casarosa et al., 2009)
	Ipratropium	0.32 h			Marketed	(Casarosa et al., 2009)
H_1 receptor	Bilastine	73 min	Allergic rhinitis	Bilastine prolonged H_1 receptor antagonism compared to diphenhydramine.	Marketed	(Bosma et al., 2018)
	Diphenhydramine	0.41 min			Marketed	(Bosma et al., 2018)

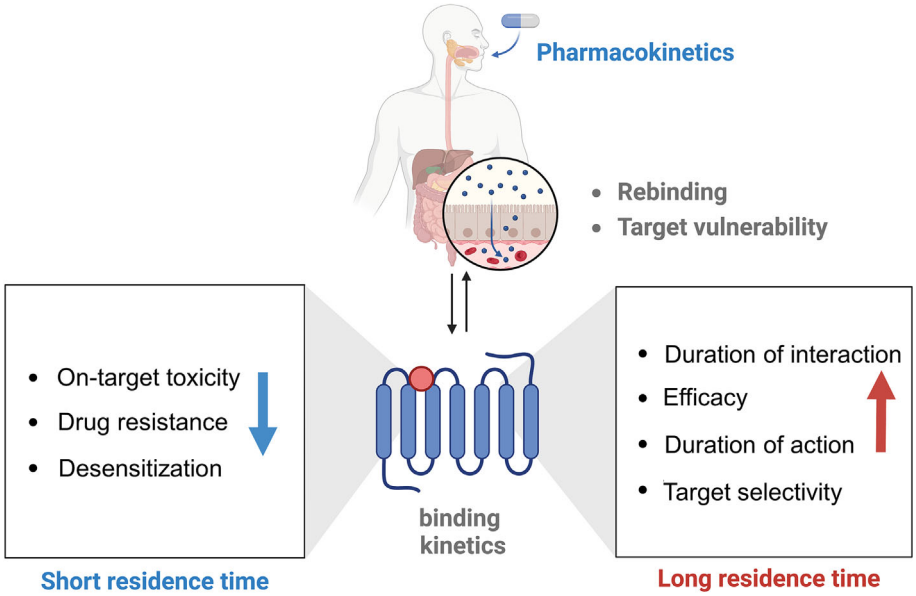

FIGURE 1 Summary of residence time within a pharmacological perspective. The content in the right-hand box indicates that increasing the residence time is beneficial for the corresponding pharmacological effects (a G protein-coupled receptor shown as a typical target). The content in the left-hand box indicates that shortening the residence time is beneficial by reducing on-target toxicity and desensitization, and is associated with the development of drug resistance. Systemic pharmacokinetics as well as rebinding and target vulnerability in the 'micro-compartment' where the drug-target interaction takes place should also be taken into consideration in the translation of in vitro binding kinetics to in vivo effects. The figure was created with BioRender.com.

target occupancy can affect the duration of interaction between the drug and its target, thereby influencing the duration and intensity of drug efficacy. For example, to examine the correlation between in vitro residence time and the duration of the drug-target interaction in vivo, Lee et al. (2019) selected **soluble epoxide hydrolase** (sEH) as a model target and identified a series of sEH inhibitors with similar K_i but different residence times on sEH. They demonstrated that residence time influences the duration of the in vivo drug-target interaction.

2.2 | Target vulnerability

The translation of target occupancy into drug pharmacodynamics relies on the relationship between occupancy and effect, which, in turn, is determined by target vulnerability, a concept defined and elaborated on by Tonge and his colleagues. Target vulnerability is a critical aspect of drug development, as it can directly influence the amount of drug exposure required to achieve the intended pharmacological outcome. Low vulnerability targets necessitate high levels of occupancy to produce the desired physiological outcome, whereas high vulnerability targets require much lower occupancy to achieve the desired effect (Tonge, 2018). Residence time is correlated with target vulnerability and can be used to provide an assessment of such vulnerability. Davoodi et al. discovered that the correlation between residence time and post-antibiotic effect (PAE) could be used to define the vulnerability of bacterial targets. In the case of macrolides, there was a strong correlation between residence time on the *Escherichia coli* ribosome and the PAE, indicating the ribosome is a highly vulnerable drug target. Moreover, they found that increased residence time led to prolonged PAE (Davoodi et al., 2020). However, one should bear in mind that the rate of target turnover might reduce the advantages of kinetic selectivity even on a high vulnerability target. For instance, Basu et al. found that the rapid turnover of the enzyme EcLpxC in wild type *E. coli* reduced the PAE of its inhibitors. However, when levels of EcLpxC were stabilized, PAE was enhanced again. This study highlights the pivotal role of protein turnover in controlling the translation of sustained target occupancy to prolonged drug activity (Basu et al., 2021).

2.3 | Pharmacokinetics (PK) and residence time

Target occupancy is also influenced by the pharmacokinetic half-life of the drug in the systemic circulation. It has been argued that PK is the main driver of drug efficacy. Dahl and Akerud examined a simple model that takes both PK and target binding kinetics into account and concluded that residence time is influential only when the dissociation is slower than the drug's elimination process. If the drug-target residence time is shorter or comparable to the pharmacokinetic half-life, the temporal receptor occupancy ratio by the drug will mirror that of the systemic drug concentration and pharmacodynamics will not be prolonged. (Dahl & Akerud, 2013). There are many examples of drugs, however, that show a sustained pharmacodynamic effect even after most of the drug has been eliminated from the body (Copeland, 2016; Tummino & Copeland, 2008). This may be due to prolonged target binding kinetics, but unfortunately, this has not always been documented.

2.4 | Effect of residence time on drug efficacy

Many studies have presented convincing evidence that the in vivo pharmacological activity of drugs is predominantly influenced by their residence time with the target rather than their binding affinity (Cusack et al., 2015; Martella et al., 2017; Zhang, Yan, et al., 2022; Zhou et al., 2021). For example, Zhang et al. developed 10 vasopressin **V_2 receptor** antagonists that spanned a range of K_i values and residence time for this receptor and investigated their effects on autosomal dominant polycystic kidney disease (ADPKD) (Zhang, Yan, et al., 2022). They used kidney-selective *Pkd1* knockout mice to study the in vivo activity of the V_2 receptor antagonists. The results showed that the inhibitory effects on renal cyst development and growth were correlated with the residence time of the compounds, rather than their affinity (Figure 2). Similarly, Liu and colleagues discovered a new-generation acetylcholinesterase inhibitor (fluoropezil) that possesses a fourfold longer drug-target residence time than **donepezil**, a first-line drug for Alzheimer's disease (Zhou et al., 2021). As a result, fluoropezil needed a much lower effective dose than donepezil in different mouse models of cognitive impairment. These studies indicate that slowing the dissociation of a drug from its target may improve its efficacy.

2.5 | Duration of drug action

Duration of drug action refers to the length of time during which a drug produces its therapeutic effects in the body. The duration of drug action is a significant pharmacological parameter, as it directly affects the efficacy and persistence of therapeutic effects in vivo. For example, in a study on histamine receptor antagonists (Bosma et al., 2018) the properties of **bilastine** and **diphenhydramine** were compared and analysed. Although these compounds have similar H_1 receptor binding affinities, there was a more than 100-fold difference in their dissociation rate constants. Bilastine exhibited a drug target residence time of 73 ± 5 min on the H_1 receptor, which was much longer than that of diphenhydramine (0.41 ± 0.1 min), resulting in prolonged H_1 receptor antagonist action even after the unbound H_1 receptor antagonist was washed out. Therefore, the long residence time of bilastine in vitro could explain the long duration of its actions in vivo. Furthermore, the importance of residence time in pharmacodynamics has been highlighted in a study on the **5-HT_7 receptor** (Penna et al., 2022). The authors conducted a comparative analysis of two 5-HT_7 receptor agonists with comparable receptor binding affinities but various residence times to stimulate 5-HT_7 receptor-mediated neurite outgrowth. The effect of **LP-211**, characterized by a longer residence time (24 min), started after 2 h of stimulation and persisted for 4 h, then gradually diminishing thereafter. Compared with LP-211, the effect of 5-HT_7 receptor agonists with a shorter residence time (8 min) on stimulating neurite outgrowth started earlier (30 min) and ended more rapidly (2 h). These findings demonstrate the significance of considering residence time in drug development and translation, as it can alter the efficacy and duration of drug action.

2.6 | Rebinding

The in vivo duration of drug action is also affected by drug rebinding, that is, the continuous binding of dissociated drug molecule to the

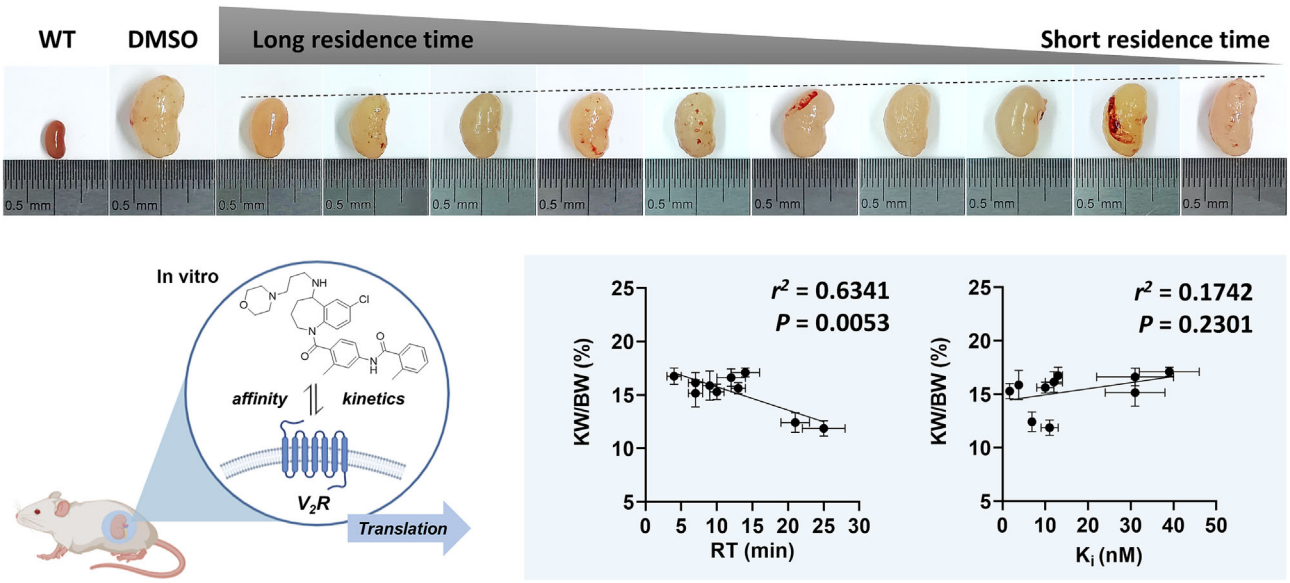

FIGURE 2 The inhibitory effects on renal cyst development and growth (shown as kidney weight/body weight; KW/BW) were correlated with the target residence time (RT), but not with the affinity (K_i), of 10 antagonists of the V_2 receptor (V_2R). The figure was adapted from Zhang, Yan, et al. (2022).

original target and/or nearby targets. Rebinding provides an additional chance for prolonging the duration of drug action. For example, Wang et al. (2022) identified SR-A3, a potential anti-cancer drug that acts on eukaryotic elongation factor-1α (eEF1A), which exhibited enhanced residence time and rebinding kinetics, resulting in long-lasting inhibitory effects after transient exposure of cells. SR-A3 monotherapy significantly prolonged the survival of tumour-bearing mice in a dose-dependent manner and was well-tolerated. Similarly, Petrov et al. conducted a study on the pharmacokinetics of C-547 in rats, examining its duration of action using a rodent model of myasthenia gravis (Petrov et al., 2018). C-547 exhibited enduring effects on the extensor digitorum longus muscle for over 96 hours due to its prolonged residence time on AChE. This allowed for micro-pharmacodynamics to take place in the constrained environment of the neuromuscular junction, leading to subsequent re-binding of C-547 and slowing its clearance from the muscle. In comparison to other cholinesterase inhibitors employed for palliative therapy of myasthenia gravis, the high selectivity and long duration of C-547 make it a promising drug candidate. Collectively, these results suggest that rebinding can prolong the clinical efficacy of a drug.

2.7 | Target selectivity

Preclinical drug optimization requires a critical consideration of target selectivity. Target selectivity is generally assessed by calculating the ratio of a drug's IC_{50} or K_D values for an off-target to those for the primary target of interest (Tummino & Copeland, 2008). This definition of selectivity refers to thermodynamic selectivity, which is derived from equilibrium binding assays. It is important to note that thermodynamic selectivity may not necessarily correlate with kinetic selectivity, and kinetic selectivity can still be present even in the absence of thermodynamic selectivity (Tonge, 2018). Furthermore, thermodynamic selectivity does not reflect the temporal changes in occupancy of primary and collateral targets that arise in an open system. Thus, to obtain a more accurate measure of target selectivity, it is necessary to consider the occupancy of both primary and collateral receptors over time (Tummino & Copeland, 2008). In other words, a drug exhibits a prolonged residence time for its primary target and shorter residence times for collateral receptors would have a high level of target selectivity during dosing. Conversely, a drug with a long residence time on a collateral receptor may potentially give rise to increased safety concerns (Copeland, 2016; Tummino & Copeland, 2008). The kinetic selectivity analysis of the kinase inhibitor **sorafenib** is a typical example (Neumann et al., 2011). Neumann et al. analysed the residence time of sorafenib on 15 potential kinase targets. It was found that the binding activity of sorafenib to **CDK8/CycC** and **DDR1** was similar, with K_D values of 30 nM and 72 nM, respectively. Although sorafenib was not significantly selective for both targets from a thermodynamic (K_D) point of view, the residence times of sorafenib for CDK8/CycC (residence time = 576 min) and DDR1 (residence time = 24 min) were significantly different from a kinetic point of view. The inhibitory effect in vivo showed that after 7 hours, the fast-dissociated DDR1 was no longer inhibited by sorafenib at all, while the activity of the slow-dissociated CDK8/CycC remained 90% inhibited by sorafenib, showing significant kinetic selectivity. Moreover, Berger et al. reported a structural rationale for kinetic selectivity between two structurally highly homologous kinases **FAK** and **PYK2** (Berger et al., 2021). They found that prolonging the residence time of the compound on FAK through inducing a helical structure at the DFG motif of FAK, but not PYK2, enhanced the selectivity of the compound for FAK, thereby avoiding off-target toxicity. Similarly, **tiotropium**, a drug for the treatment of chronic obstructive pulmonary disease, displayed highly divergent dissociation rates, being slow for muscarinic M_3 ($t_{1/2}$ = 27 hr) and faster for **M_2 receptors** ($t_{1/2}$ = 2.6 h), while it has similar affinities for the two highly homologous receptors (M_2, $pK_i = 10.7$; M_3, $pK_i = 11.0$) (Casarosa et al., 2009). Subsequent studies showed that amino acid $K523^{7.32}$, which is not conserved between the subtypes, and extracellular loop flexibility are the main factors for the kinetic selectivity between M_2 and M_3 receptors (Tautermann et al., 2013). Therefore, optimizing the target selectivity of drugs from a kinetic point of view such as residence time can more effectively improve their efficacy and safety.

2.8 | On-target toxicity

Drugs with long residence time can prolong the occupancy of the intended target, improve efficacy, and reduce off-target side effects. However, a longer residence time may have unfavourable or even harmful consequences. Such intrinsic adverse events are known as on-target toxicity or mechanism-based toxicity. The extrapyramidal symptoms (EPS) caused by antipsychotic drugs acting on the dopamine D_2 receptor provide a clear example of this effect. **Clozapine**, **quetiapine**, **olanzapine** and **haloperidol** are dopamine D_2 receptor antagonists with different residence times. Many studies have shown that due to the shorter residence time of clozapine, quetiapine and olanzapine on the dopamine D_2 receptor (residence time = 19.6, 43.5, 55.6 s, respectively), the incidence of EPS of clozapine-like antagonists is significantly lower, compared with haloperidol (residence time = 100 s) (Kapur & Seeman, 2000; Langlois et al., 2012; Sahlholm et al., 2016). Therefore, it was proposed that for on-target toxicity drugs, rapid dissociation with shorter residence time is preferred. However, residence time might not be the only factor for clozapine to cause little, if any, relevant EPS. A recent study by Sykes et al. highlighted that association rates of the antipsychotics, but not their dissociation rates, were positively correlated with their incidence of EPS (Sykes et al., 2017). In addition, the binding to receptors other than D_2 is thought to be another reason for the low incidence of EPS with drugs such as clozapine and quetiapine (Meltzer, 2000). Their low occupancy of the D_2 receptor, compared with the **5-HT_{2A} receptor**, may present another reason their low incidence of EPS

(Langlois et al., 2012). In comparison, drugs with a higher incidence of EPS, such as haloperidol, specifically bind to the D_2 receptor, but not to the 5-HT_{2A} receptor.

2.9 | Drug resistance

A decreased residence time is associated with drug resistance. Lyczek et al. proposed that target mutations can cause drug resistance by elevating ligand dissociation rates, consequently leading to decreased drug residence times and diminished target inhibition (Lyczek et al., 2021). In particular, they identified three mutations (N368S, V299L and G251E) causing resistance to **imatinib** in the **Abl receptor**, for which imatinib was found to have similar binding affinity to both the wild type and mutant forms of Abl, but it dissociates more rapidly from all the three mutant forms. Considering the importance of residence time in drug efficacy, mutations that alter binding kinetics could potentially lead to drug resistance.

2.10 | Desensitization

Studies have shown that ligand-receptor interaction kinetics are determinants of desensitization (Duarte et al., 2022). The **AT_1 receptor** agonist **angiotensin II** (Ang II) is an agonist causing substantial desensitization. Repeated administration of Ang II results in a dramatic reduction in responsiveness in different smooth muscles. Duarte et al. examined the role of different dissociation kinetics of the ligand in AT_1 receptor desensitization, using a bioluminescence resonance energy transfer assay (Duarte et al., 2022). They found that the prolonged residence time of Ang II at AT_1 receptors was a critical factor leading to desensitization. The longer residence time (characterized by a low dissociation rate) of Ang II results in sustained activation and prolonged internalization of AT_1 receptors, further preventing receptor responses. Consequently, receptor recycling to the plasma membrane slowed down, with fewer receptors re-sensitized, ultimately resulting in desensitization and poor drug efficacy. Ang II analogues with short residence time allow a more transient receptor activation and internalization. The subsequent early recycling of the receptor to the plasma membrane allows further receptor responses, which in turn reduces desensitization. Therefore, understanding the kinetic properties of agonist ligand-receptor interactions and optimizing the residence time of such ligands can have significant implications to mitigate desensitization and improve the efficacy of drug therapies.

3 | RESIDENCE TIME FROM A CHEMICAL PERSPECTIVE

With the growing focus on residence time, kinetics-directed structural optimization of new chemical entities is frequently adopted in the hit-to-lead process (Basak et al., 2022; Berger et al., 2021; Cao et al., 2023; Liu et al., 2022; Voss et al., 2021). These studies together demonstrated that many factors may affect the residence time during the hit-to-lead optimizations. These include structural modifications of the compound, binding stability between the ligand and target, energy barriers, and other related factors (Du & Wang, 2020; Soethoudt et al., 2018; Tian et al., 2021; Veber et al., 2002). We will discuss some of these factors individually, while acknowledging that, for example, changing 'binding stability' will also affect the energy barriers between unbound and bound target states.

3.1 | The effects of energy barriers on drug-target residence time

Dissociation of a ligand from a protein requires overcoming a sizable energy barrier. This energy barrier is mainly enthalpic, as dissociation necessitates enhanced mobility of both the protein and ligand, thereby contributing favourably to entropy contribution. Consequently, augmenting the enthalpic contributions of protein-ligand interactions will be likely to promote slower dissociation, leading to a longer drug-target residence time (Tummino & Copeland, 2008). Based on this principle, the residence time of a drug with its target can be modulated by manipulating the magnitude of the energy barrier between the ground and transition states along the binding reaction coordinate. For example, Tian et al. found a new **COX-2** inhibitor with a long residence time by stabilizing the ground state to improve thermodynamic efficiency or destabilizing the transition state to increase the internal energy barrier (Tian et al., 2021). Similarly, Spagnuolo et al. found that a triazole-containing diphenyl ether had a longer residence time on InhA (the FabI enoyl-ACP reductase from *Mycobacterium tuberculosis*) due to destabilization of the transition state (Spagnuolo et al., 2017). They subsequently developed a triazole-based inhibitor that increased the energy barrier by adding bulky side chains to introduce steric hindrance, resulting in a prolonged residence time on InhA. Moreover, Schuetz et al. found that introducing polar substituents to those parts of the ligand pointing towards a hydrophobic binding pocket increased ligand desolvation barriers. As a result, this slowed down the on-rate of compounds en route to the hydrophobic pocket of **heat shock protein 90** (Schuetz et al., 2018). As the reverse process of ligand association, the introduced desolvation barrier resulted in a nine-fold prolongation of the ligand dissociation process. Taken together, such studies suggest that the energy barriers play a crucial role in drug-target residence time. Enhancing the enthalpic contributions of ligand-receptor interactions can promote slower dissociation and result in a longer residence time.

3.2 | The effects of binding stability on drug-target residence time

In addition to the energetic aspect, binding stability between ligand and target is also important for residence time (Du & Wang, 2020;

Suchankova et al., 2022; Zhang, Han, et al., 2022). Structural changes upon the binding of the drug molecule that further strengthen the interactions of the molecule with the binding pocket will increase its target residence time (Copeland, 2016). For example, Zhang et al. identified key structural motifs of phenoxyphenol derivatives as direct inhibitors of InhA through molecular dynamics simulations. They found that the cyano group of the PT119 and PT506 inhibitors forms a hydrogen bond with the hydroxyl group of the pyrophosphate moiety of NADH, such that the inhibitor needs a longer time to overcome the hydrogen bond during its dissociation from the target (Zhang, Han, et al., 2022). In another study, Suchankova et al. discovered an adenosine receptor antagonist having a long residence time after the introduction of a 3-phenyl group, together with a 7-benzylamino and 1-methyl group at the pyrazolopyridazine scaffold. This compound forms a π-π interaction with F171 and a hydrogen bond with N254 to stabilize the receptor, resulting in high affinity and long residence time for the **adenosine A_1 receptor** (Suchankova et al., 2022). In summary, binding stability between drug and target, achieved through formation of favourable interactions, is a critical factor influencing residence time and can be strategically optimized in drug design and development.

3.3 | The effects of subtle structural changes on residence time

Subtle changes in the molecular structure of a ligand, such as alterations of the position and physicochemical properties of the substituents, may lead to significant kinetic changes. For example, a structure-kinetics relationship study of a set of arylpiperazine-based 5-HT_7 receptor ligands demonstrated that the position of polar groups on the aryl moiety linked to the piperazine ring can influence drug-target residence time and efficacy (Penna et al., 2022). In another study, Soethoudt et al. synthesized 24 compounds using the structural scaffold of a **CB_2 receptor** partial agonist (LEI101). The authors systematically investigated the effect of the physicochemical properties of substituents on the binding kinetics of CB_2 receptor agonists. They found that there is a lipophilic binding domain in the CB_2 receptor, and addressing that pocket prolonged the residence time of the compound (Soethoudt et al., 2018). In another study, Cao et al. synthesized and analysed the structure-kinetics relationship of a series of benzodiazepine derivatives as V_2 receptor antagonists. Their results show that subtle structural modifications could significantly alter the binding kinetics of the compounds, without significantly affecting their binding affinity. For example, the introduction of an electron-withdrawing group at the *meta*-position of the phenoxy group in the compounds extends the residence time. The preferred bulky aromatic substituent on the benzamide group was linked to a longer residence time as well (Cao et al., 2023). These examples provide insights into the effects of physicochemical properties of specific substituents on the binding kinetics of the drug molecules and their pharmacology, which facilitate the design and development of drugs with desired binding kinetics.

4 | GAPS IN THE TRANSLATION BETWEEN IN VITRO KINETICS TO IN VIVO EFFICACY

4.1 | Integrating drug-target residence time into drug efficacy prediction

Residence time is a vital aspect of the efficacy of drugs in vivo. However, there is currently a lack of tools to integrate drug-target residence times into drug efficacy prediction, hindering the integration of drug-target kinetics into the drug discovery cascade. To address this challenge, de Witte and colleagues developed kinetics-integrated models to predict the duration of target occupancy for binding in both plasma and tissues (de Witte et al., 2016). The authors emphasized the inclusion of target saturation in the analysis of the effect of drug–target binding kinetics on target occupancy, as the duration of target occupancy may not be solely determined by k_{off} when the target approaches saturation. (de Witte et al., 2018). In another study, Walkup et al. developed a PK-PD model that incorporated drug-target kinetic parameters (Walkup et al., 2015). Their findings demonstrate that the model can accurately predict time-dependent antimicrobial activity at the whole-cell level by measuring the PAE of a series of compounds. Interestingly, the PAE was found to be correlated with the off-rate of inhibitor dissociation from target, rather than the thermodynamic affinity of the compound for the target. More recently, physiologically based pharmacokinetic (PBPK) modelling has emerged as a powerful technique for accurately estimating the dynamic concentration-time profiles of drugs in blood and tissues. Combining residence time with PBPK modelling is an effective approach, as exemplified in a retrospective study of **topiroxostat** (Luo et al., 2020). A PBPK model was combined with a drug-target residence time model to predict the PK and PD of topiroxostat in humans, providing a comprehensive understanding of drug absorption and disposition following oral administration. Additionally, the authors revealed that the long residence time of topiroxostat on **xanthine oxidoreductase** plays a crucial role in its long-lasting pharmacodynamics in vivo.

4.2 | Detection methods and models for residence time

Currently, there are two classes of methods for determining/predicting the ligand-receptor residence time. The first involves the 'wet' experiment, such as radioligand binding assays, fluorescent ligand-based technologies and surface plasmon resonance approaches (SPR). The second class involves computational simulations, such as molecular dynamics simulations and machine learning methods. Because there are previous reviews that have extensively documented the methods or models for kinetic measurements (Guo et al., 2014; Hoffmann et al., 2015; Tautermann, 2016), we provide, here, only some background regarding the methods and models for calculating/predicting residence time.

For wet experiments, one of the classic methods for detecting residence time is the radioligand binding assay. Briefly, an excess of

unlabelled ligand is used to compete for binding of the radiolabelled ligand to the receptor. The dissociation rate constant is then determined by measuring the change in concentration of remaining radiolabelled ligand in the complex over time (Guo et al., 2018). Similarly, the ligand can be labelled with a fluorophore. By monitoring the fluorescence signal in the complex over time, the dissociation rate of the ligand from the receptor can be inferred (Hill & Kilpatrick, 2023). Similar methods also allow the determination of the kinetic behaviour of unlabelled ligands. In addition, SPR has been widely used in recent years (de Jong et al., 2005), but it needs the availability of immobilized protein, which is not always self-evident with membrane-bound protein targets, due to their fragility.

The second group of methods for predicting drug-target residence time are computational methods. Molecular dynamics (MD) simulations can provide atomistic details of the unbinding process, which can be used prospectively to predict residence time (Huang et al., 2020; Kokh et al., 2018). Furthermore, machine learning methods can efficiently process and analyse large datasets. This can also be used to calculate the residence time. For example, Lamim Ribeiro et al. conducted a study on the unbinding kinetics of two classical drugs with different residence times using a combination of two machine learning techniques: automatic mutual information noise omission and reweighted auto encoded variational Bayes for enhanced sampling (Lamim Ribeiro et al., 2020). The study demonstrates the usefulness of machine learning in predicting residence time. Overall, the integration of various detection methods and computational approaches holds great promise in advancing our understanding of residence time and, more generally, target binding kinetics.

4.3 | The contributions of k_{on} to residence time need to be more systematically studied

The association rate constant k_{on} has not received much attention in previous studies of residence time due to physicochemical and pharmacological limitations (Copeland, 2016). However, k_{on} is associated with the onset of drug action and duration of target occupancy in vivo. Recently, IJzerman and Guo summarized the evidence that association kinetic parameters warrant attention, highlighting the importance of comprehensive analysis of binding kinetics in the early stages of the drug discovery process (IJzerman & Guo, 2019). de Witte et al. used mathematical models for drug-target binding and showed that a high association rate constant could raise the (local) drug concentration, which delayed the rate of decline of target occupancy (de Witte et al., 2016). In another study, Vauquelin et al. demonstrated that the association and dissociation rate constants of a drug and its target have comparable effects on the duration of target occupancy when rebinding takes place (Vauquelin, 2016). Additionally, fast k_{on} values can promote the rapid onset of action. For example, **indacaterol**, a bronchodilator used for the treatment of chronic obstructive pulmonary disease (Beier et al., 2007), exhibits a relatively fast binding rate to **β_2 adrenoceptors** (Sykes & Charlton, 2012), resulting in improved lung function in asthma as soon as 5 min post-administration (Beeh et al., 2007). Moreover, k_{on} influences the affinity of ligand binding to the target. For example, Guo et al. evaluated the activity and binding kinetics between three adenosine receptor subtypes (A_1, **A_{2A}**, **A_3**) and six antagonists by radioligand displacement and competition association assays (Guo et al., 2016). The results showed that k_{on}, rather than k_{off}, control affinity values. Similarly, Doornbos et al. synthesized a series of 7-aryl-1,2,4-triazolo[4,3-a] pyridines with potent positive allosteric modulator activity and high affinity for **mGlu2 receptors**. The authors found a strong correlation between affinity and k_{on}, but not residence time and affinity. Interestingly, longer $mGlu_2$ receptor residence times appeared to prolong and intensify the effects on rapid eye movement (REM) sleep in vivo (Doornbos et al., 2017). Furthermore, computational methods to derive and assess k_{on} values are being developed, which may trigger further attention to this somewhat undervalued parameter (Dror et al., 2011).

5 | FUTURE PERSPECTIVES

5.1 | Clinical therapy and prediction of drug efficacy in vivo

In the previous sections, we discussed the relevance of residence time for drug efficacy and summarized recent research progress in the practical application of aspects of residence time. These findings provide new insights for drug discovery, design, evaluation and application, particularly in clinical treatment. The optimization of residence time not only has the potential to lead to the discovery of new drugs with desired efficacy and fewer side effects or toxicities, but it may also widen the therapeutic window, which is a major concern in clinical practice. For example, traditional opioid receptor antagonists are known to have a narrow therapeutic window and are associated with drug resistance, off-target side effects and potential addiction. By optimizing the residence time on **μ-opioid receptors**, it may be possible to design modified analgesics that produce sufficient and durable effects with fewer doses, thereby reducing the risk of drug addiction caused by overdose. As an example, Yassen et al. performed mechanism-based pharmacokinetic-pharmacodynamic modelling of **buprenorphine**'s anti-nociceptive effect in healthy volunteers. The authors found that a combined biophase equilibration/receptor association-dissociation model with a linear transduction function best described the time course of buprenorphine's anti-nociceptive effects. The model provided precise estimation of the parameters characterizing hysteresis and the relation between relative receptor occupancy and anti-nociceptive effect (Yassen et al., 2006).

5.2 | Covalent drugs

Covalent inhibitors with 'infinite' residence times are a next, but not undisputed, step in increasing drug efficacy. For example,

clopidogrel, interacting covalently and irreversibly with the $P2Y_{12}$ receptor, maintains its effect for up to 7 days, even though most of it is cleared from the system within 6 h of dosing. As a result, clopidogrel is often administered with a high-loading dose, followed by a much lower maintenance dose (Danese et al., 2016). However, it is important to note that this favourable effect may not apply to all covalent inhibitors, and this may have to do with the effect of target turnover (Barf et al., 2017; Daryaee & Tonge, 2019; Peletier, 2022). Supposing the target has a rapid turnover rate, target occupancy by the inhibitor will decrease significantly over time as the target itself disappears, necessitating repeated dosing. For example, acalabrutinib is a covalent BTK inhibitor for the treatment of chronic lymphocytic leukaemia (CLL). The differences in BTK occupancy in CLL patients by acalabrutinib were related to the variation in the rate of BTK (re)synthesis (Alsadhan et al., 2018). As a result, a dosing regimen of 100 mg twice daily instead of 200 mg once daily was preferred to provide sufficient BTK occupancy (Sun et al., 2020). Because irreversible binding can also make the drug binding uncontrollable and toxic, increasing attention has been focused on the development of reversible covalent inhibitors (Reja et al., 2022), as they can minimize off-target reactions and avoid permanent modifications to target proteins. In a study of cysteine-targeted covalent inhibitors, researchers identified reversible covalent inhibitors with residence times spanning from a few minutes to a few days, facilitating their use not only in therapeutic applications necessitating prolonged target engagement but also in situations where rapid target disengagement is desired (Bradshaw et al., 2015). To enable better development and application of covalent inhibitors, further systematic research is still needed to understand their properties in drug development projects.

5.3 | Other aspects

In translational drug research, a close connection between molecular mechanism modelling, pharmacokinetics, and pharmacodynamics is necessary (Petrov et al., 2018). However, the predictive capacity for estimating the magnitude and duration of pharmacodynamic effects of a drug in humans is constrained by the reductionist nature of preclinical research. Therefore, preclinical drug optimization must always consider multiple factors, as human physiology is complex (Copeland, 2021). While residence time plays a significant role in translational studies, the prediction and evaluation of clinical efficacy is a multifactorial problem. Effective and appropriate translational strategies need to be developed, and in this process, studies of pharmacokinetics and pharmacodynamics could provide insights into the relationship between drug exposure and clinical response, which may be an important aspect to consider. Furthermore, in order to better integrate residence time with other disciplines, to address gaps in scientific knowledge and to pave the way for future advancements, systematic studies on residence time are still necessary.

6 | CONCLUSIONS

Target-binding kinetics has emerged as a valuable pharmacological parameter that provides a more comprehensive understanding of the dynamic nature of the drug-target interaction in vivo, enabling better prediction of drug efficacy. The incorporation of residence time as a parameter in drug discovery and development has the potential to significantly affect the pharmacology of a given compound, leading to the design of safer, more effective and more durable drugs. It also has significant implications for the chemical aspects, guiding the optimization of target binding properties. Therefore, considering residence time as a critical parameter in clinical therapy and drug efficacy prediction provides valuable insights for the advancement of drug discovery and the development of more effective and safer drugs in the future.

6.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in https://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMCOLOGY 2021/22 (Alexander, Christopoulos et al., 2021; Alexander, Fabbro et al., 2021a, 2021b; Alexander, Kelly, et al., 2021).

AUTHOR CONTRIBUTIONS

H. Liu: Formal analysis (equal); visualization (equal); writing—original draft (lead); writing—review and editing (supporting). **H. Zhang:** Formal analysis (equal); visualization (equal); writing—original draft (equal). **A. P. IJzerman:** Conceptualization (equal); supervision (equal); writing—original draft (supporting); writing—review and editing (equal). **D. Guo:** Conceptualization (equal); funding acquisition (lead); visualization (equal); writing—original draft (equal); writing—review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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