feature

Drug target residence time: a misleading concept

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Since the importance of drug target residence time was first highlighted more 10 years ago, slow binding kinetics has received much attention in the drug discovery literature, and indeed within pharmaceutical research. However, the residence concept as presented in most papers is supported by rather misleading simulations and arguments, and by examples where compounds are taken out of their pharmacokinetic context. Moreover, fast association is typically more desirable than slow, and advantages of long residence time, notably a potential disconnect between pharmacodynamics (PD) and pharmacokinetics (PK), would be partially or completely offset by slow on-rate. Therefore, plain potency is likely a better predictor of drug development success than is residence time.

Introduction

Increasingly often, I come across long residence time being used to denote the mode of action of a certain inhibitor or lead compound, or the suggestion that optimizing residence time can be a valuable drug discovery strategy. By contrast, researchers rarely claim that high affinity would be a successful approach for their project, or that high potency will be the desired mode of action for their inhibitors. However, more often than not, potent compounds will have long residence times, and there is little difference between desiring long residence time and pursuing high affinity. It is apparent that the introduction of the residence time concept [1] has led many drug discovery scientists to believe that long residence time offers something extra that plain potent compounds do not.

Here, I bring some nuance to the residence time literature by critically examining and discussing the published simulations and examples in relation to the claims that they were meant to support. I outline that there are no examples of, and only very limited mathematical support for, the often-heard claim that long residence time can cause PD effects to outlast PK. I hope to convince the reader that both on- and off-rates are important in drug discovery and, if one were to choose only one parameter, good-old potency is still one's best bet.

Binding kinetics terminology

Several mechanisms of ligand–receptor interactions can be described, the simplest being a one-step association and dissociation process (Eq. (1)):

$$R + L \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} RL \qquad (1)$$

where R and L are receptor and ligand, respectively, RL is their binary complex, and k_{on} and k_{off} are the association and dissociation rate constants, respectively. The equilibrium dissociation constant K_d is defined as k_{off}/k_{on}. A useful way of looking at these rate constants is as follows: k_{off} describes the fit or complementarity of the compound to the target in the bound form. The tighter and more productive the interaction, the longer k_{off} will be. As such, it is independent of target or ligand concentration, and its unit is s^{-1}. By contrast, k_{on} is a measure of the fit of compound to the target when both are still in the unbound form. If a significant conformational change is required for binding, in either the compound or protein, the on-rate will become slower. If no such changes need to happen, the on-rate will be faster, and essentially limited only by diffusion. Here, concentrations do matter, because receptor and ligand need to come close enough to each other for binding to happen and, therefore, k_{on} is a second-order rate constant, with the unit $M^{-1}\ s^{-1}$.

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This one-step model is sufficient to explain the value of residence time, which is simply defined as $1/k_{off}$. However, most residence time literature focuses on a two-step, induced fit mechanism, probably because this implicitly involves a slow isomerization step, which helps to understand where slow off-rates can originate from (Eq. (2)):

$$R + L \underset{k_2}{\overset{k_1}{\rightleftarrows}} RL \underset{k_4}{\overset{k_3}{\rightleftarrows}} RL*, \quad (2)$$

Here, RL* denotes a 'final' high-affinity complex formed after the receptor is isomerized upon ligand binding. The math becomes more complicated, because the off-rate now includes the forward k_3, and both backward k_2 and k_4 rates (Eq. (3) [2]):

$$k_{off} = k_2 k_4/(k_2 + k_3 + k_4) \quad (3)$$

An equilibrium dissociation constant K_d* can be defined that describes the equilibrium between the free receptor and ligand and the total ligand bound to receptor, as Equation 4:

$$K_d* = k_2 k_4/(k_1(k_3 + k_4)). \quad (4)$$

While this constant depends on four individual rate constants, it is still an equilibrium constant that can be used, for example, to calculate the fractional occupancy of the receptor using a classical Langmuir isotherm (Eq. (5)):

$$\text{occupancy} = [L]/(K_d* + [L]). \quad (5)$$

If L is an inhibitor, the target will be inhibited in both the RL and RL* states, which underpins the relevance of this isotherm.

Many medicinal chemists, enzymologists, and drug discovery scientists will have appreciated the value of a slow off-rate for decades. Nevertheless, slow kinetics has received increasing interest in recent years, following the publication by Robert Copeland of a perspective article on this topic, where he also introduced the term 'residence time' [1]. Drug target residence time is now referred to as a model or concept, and it was presented in the article as 'an alternative perspective on drug optimization'. Essentially, the concept as presented can be summarized with two premises: (i) for *in vivo* situations, the efficacy of a ligand is not well described by the dissociation constant, but critically depends on the off-rate of the receptor–ligand complex; and (ii) a slow off-rate will result in sustained inhibition, even when the drug has been almost completely eliminated from the serum and/or system.

Published examples do not show that k_{off} is a better metric than K_d

To support the first statement, the residence time literature offers many examples [3–5], including the following: 'Maschera et al. [6] measured the kinetics of the anti-retroviral drug saquinavir across a panel of different HIV-1 mutant proteins. Significant differences in IC50 were found across the mutants. Interestingly, the values of k_{on} for saquinavir varied only two-fold across all mutant proteins studied, while k_{off} (and therefore residence time) varied over several orders of magnitude, and correlated well with IC_{50} values for viral replication' [4].

What exactly does this example show? It is not too surprising that the on-rates are similar; in fact, the opposite would be more difficult to rationalize for one compound against a series of mutant proteins. Moreover, since $K_d = k_{off}/k_{on}$, if the on-rate is constant between a series of compounds (or mutant proteins), then the affinity constant K_d would be just as good a measure for efficacy as the residence time.

It would be more interesting to look at examples where the on-rate varies over a range of compounds, and then study whether the off-rate is a better predictor of efficacy than either K_d or K_i, in an *in vivo* setting. Unfortunately, such studies are difficult to do, because *in vivo* efficacy depends on a wider variety of parameters than only binding kinetics, such as clearance, absorption, distribution, plasma protein binding, and so on. One often-cited example in this context is the study by Lu and co-workers [7], who tested various analogs of the prevalent antibacterial triclosan in a 10-day mouse model of infection. They showed a stunningly linear correlation between residence time of the five compounds tested and the percentage survival rate of mice after the 10 days, which was better than the correlation of the mouse survival rate with K_i. Although this could be interpreted as confirmation of a residence time concept, there are several aspects to this observation that require highlighting. First, percentage survival rate after 10 days is an arbitrary parameter, and there is no reason why one would expect a linear correlation with any other observable. If the study had been for 7 or 14 days, the correlations would have looked different. Median survival would have been more appropriate. Second, animals were given a high dose once daily, which was frequent enough to secure a benefit. The spread in residence time of the five inhibitors was 20–140 min. Therefore, one would

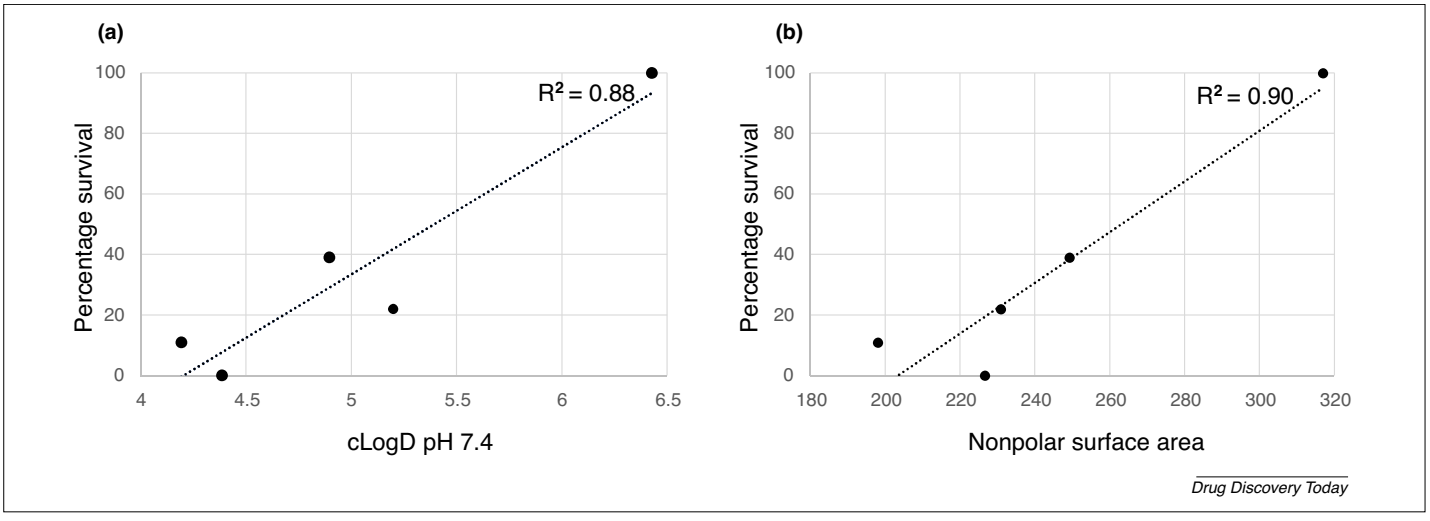

FIGURE 1

Correlation plots of two compound properties versus percentage survival in the mouse infection model published by Lu et al. [7]. These were survival rates at 10 days after the mice were infected with the bacterium *Francisella tularensis*. cLogD (a) was calculated with ACD/Labs version 12, and nonpolar surface areas (b) with the AstraZeneca in-house program Selma (Sherbukin and Olsson, unpublished data, 2000).

expect at least a dozen binding events to happen on each target molecule in between the doses even for the longest residence time compound, and it is not obvious *a priori* why the off-rate would be more important for those than the on-rate. Most importantly, the authors did not describe any other properties of these compounds and there were no PK or physicochemical data. We do not know how quickly they were cleared, how well they were absorbed, and so on. Those are pivotal parameters in an *in vivo* setting and, without these, we cannot draw any meaningful conclusions. To illustrate the complexity, Fig. 1 shows how calculated LogD and nonpolar surface area for these inhibitors correlated with percentage survival rate in that same study. These parameters are important for absorption, plasma protein binding, distribution in the body, bioavailability, and so on. Therefore, it is likely that PK is what truly differentiates these compounds in terms of their biological effect, rather than the modest differences in their residence times.

In the absence of good *in vivo* examples showing the superiority of k_{off} over K_d, it is interesting to look at an *in vitro* study by Shuman et al. [8] who measured the kinetics of 37 HIV-1 protease inhibitors, and correlated that to cell culture efficacy. They found a spread of almost three orders of magnitude in the on-rates between these 37 compounds, rendering it a rich data set for this purpose. Their work clearly showed that the individual association and dissociation rate constants alone showed weak correlation with efficacy, whereas K_d correlated much better [reproduced in Fig. 2 for the 28 compounds that had a determined cell culture efficacy (ED_{50})]. Clearly, efficacy depends on affinity more than on the individual kinetic parameters, confirming that the on-rate is also an important factor.

No evidence for sustained effect

The second claim, that long off-rates drive sustained effect even after the ligand has been almost cleared from the system, is often substantiated by a diagram similar to that detailed in Fig. 3. The diagram shows fractional occupancy as a function of time after the dosing of four compounds with different kinetics. The individual on- and off-rates are the same as those used by Copeland [1], and the corresponding K_d* values are shown in Fig. 3 alongside the four occupancy curves. It is important to realize that these are steady-state kinetics calculations. The curves represent plain Langmuir isotherms, that is, a plot of fractional occupancy as a function of ligand concentration for four compounds with different affinities. Figure 3 is misleading for two reasons. First, it is used in the context of a concept specifically introduced for open systems (*in vivo*), whereas the data shown in the figure were calculated from an equilibrium situation. Second, by plotting the occupancy as a function of drug concentration that reflects a typical PK time course, and focusing the discussion on the time aspect (notably the later time points), the reader is made to believe that the slow off-rate causes hysteresis with respect to receptor binding. However, what happens here is that even at the far right of Fig. 2, the plasma concentration (2.5 nM) is still (much) higher than the dissociation constants of the two most potent compounds (99 pM and 0.4 nM). Simple steady-state binding kinetics, in a closed system, informs that the receptor will be occupied to 96% and 89%, respectively, as shown in Fig. 3. There is no sustained effect and no PK/PD disconnect. These are simply potent compounds at high enough concentrations to saturate the receptor.

The triclosan study mentioned earlier exemplifies a common issue with the residence time literature, namely that binding kinetics is discussed in isolation from other parameters relevant for drug effectiveness. A successful drug molecule needs to be in the sweet spot of a complex multidimensional grid of many factors (potency, stability, clearance, toxicity, solubility, bioavailability, distribution, etc). To try and explain the success of one compound over the other by only looking at residence time is a serious oversimplification of drug discovery that will likely lead to biased or flawed conclusions. Tiotropium, an often-cited example of the residence time concept, illustrates this well. Tiotropium is a muscarinic receptor antagonist bronchodilator that is used in the treatment of chronic obstructive pulmonary disease (COPD). Different values of its dissociation half-life have been reported [9], ranging from 46 min to 35 h, but the consensus is that tiotropium has a longer residence time than its predecessor medication ipratropium (mainly but not totally driven by potency). Ipratropium requires four-times daily dosing, while tiotropium is once daily, and the common conclusion in the residence time literature is that the slower off-rate of tiotropium gives rise to its longer *in vivo* duration of effect. However, target dissociation rates are only one of many parameters that affect the duration of the effects of inhaled drugs. Other properties that influence include permeability, dissolution rate, tissue affinity, and

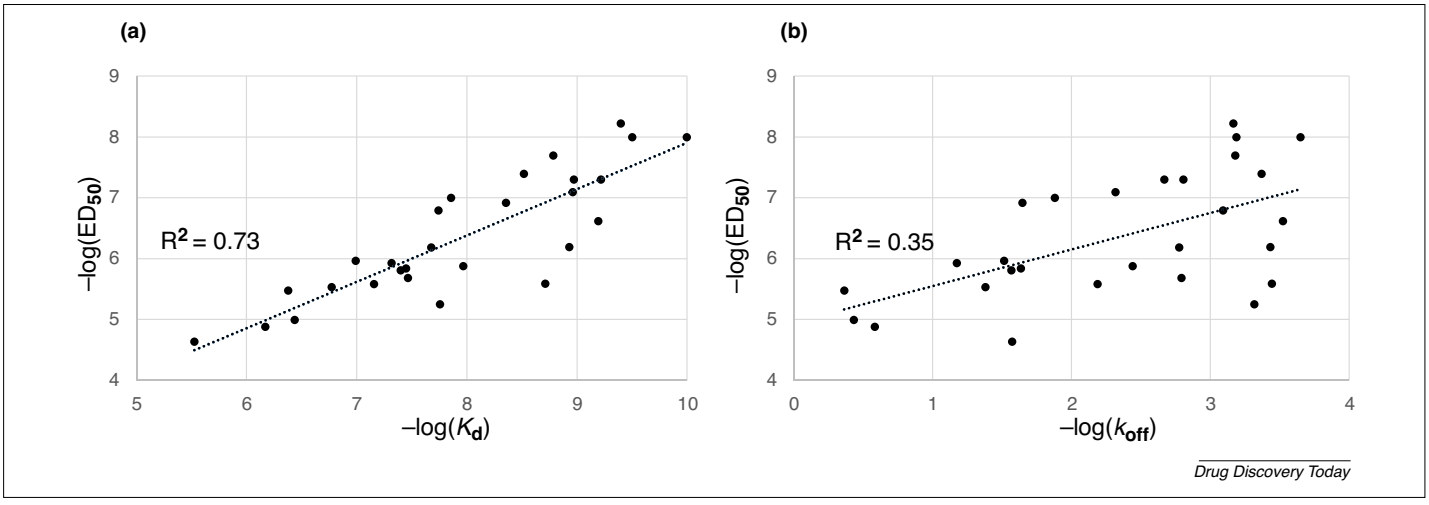

FIGURE 2

Correlation plots of biological effect (ED_{50}) versus (a) affinity constant and (b) off-rate for 28 HIV-1 protease inhibitors. These compounds span nearly three orders of magnitude in their on-rate. Data from Table 1 in Ref. [8]; only compounds for which a finite ED_{50} could be measured were included.

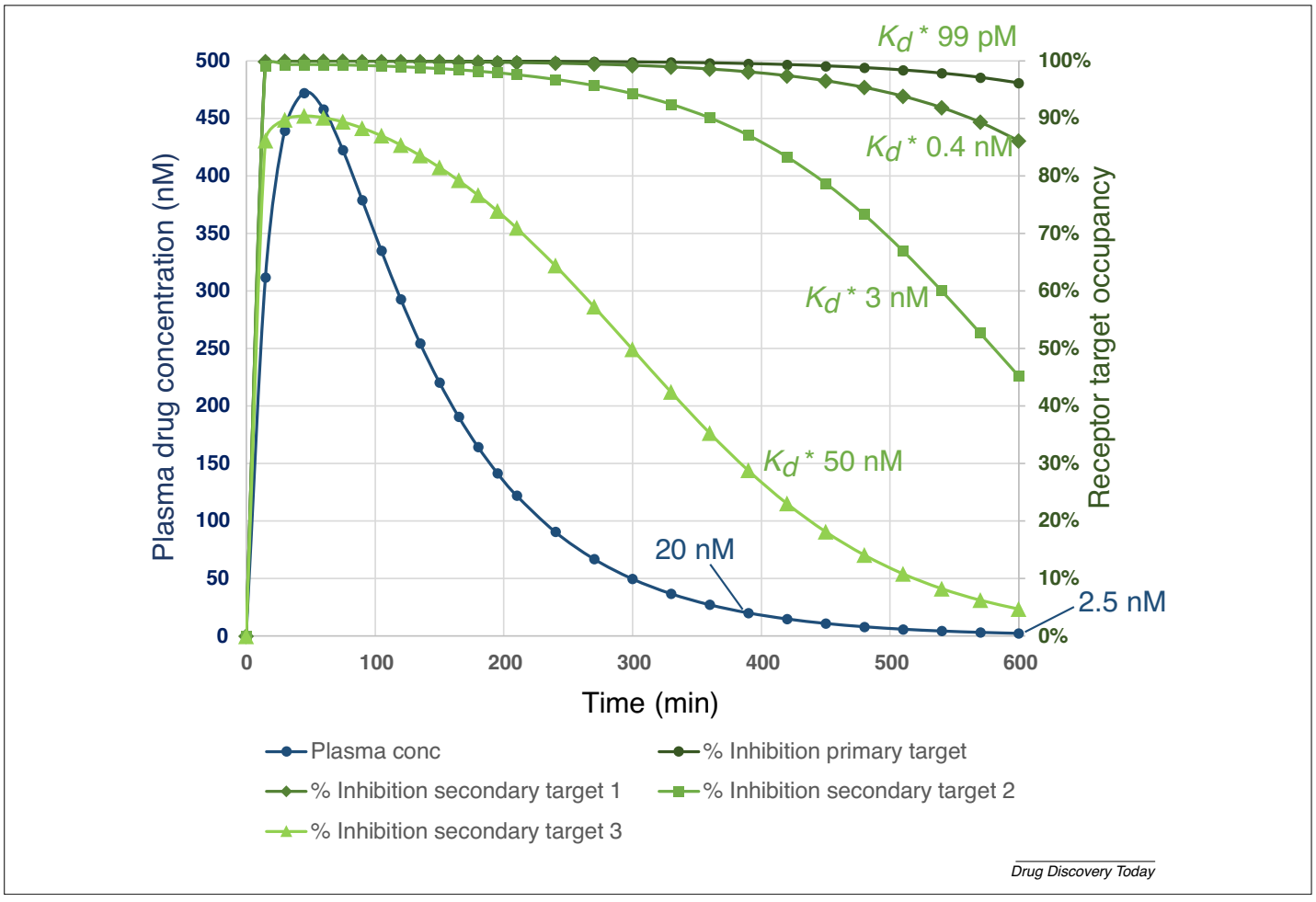

FIGURE 3

Plot of receptor fractional occupancy (green curves, right axis) as a function of plasma concentration (in blue, left axis), for four dissociation constants K_d*. These represent a primary target (highest affinity), and three secondary targets (lower affinity). Numbers in blue indicate that concentrations at the trailing end of the plasma curve are still significant in relation to the affinity constants of the receptor–ligand interaction. The drug concentration curve is arbitrarily set to $\left(1-e^{-0.03t}\right)\left(e^{-0.01t}\right) \times 1000$ nM, with t in minutes.

basicity. Unfortunately, the direct effects in the lung of each individual property are difficult to measure. Consequently, only limited information exists about how these parameters differ between ipratropium and tiotropium, and how they each contribute to the efficacy of the drugs. However, we do know that we generally want fast on-rates for topically administrated drugs: if the on-rate is slow, the drug molecule may diffuse out of the target tissue before engaging with the intended receptor. Thus, it is unlikely that tiotropium is more effective than ipratropium as a result of a slowing down of the overall kinetics, but rather this is a result of the superior potency of tiotropium combined with a favorable overall property profile.

Tiotropium is also frequently mentioned as an example drug whose PD effect outlasts the PK, together with drugs such as candesartan, desloratine, and granisetron [10]. However, for all four compounds, the dissociation half-life is five–ten times shorter than the terminal plasma half-life [11]. In fact, when Dahl and Åkerud looked at 34 drugs or candidate drugs for which they could find both binding kinetic and PK data, it was clear that the vast majority had longer plasma half-lives than target residence times [11]. In that paper, the authors also demonstrated mathematically (using a non-steady-state, 'open' model) that residence time only dictates occupancy if it exceeds the clearance. Even then, there are limitations to how slow the kinetics can be if the on-rate is to be fast enough to cause significant receptor occupancy before the ligand is being cleared from the system [11]. To my knowledge, more than 10 years after publication of the first residence time papers, there are still no examples where slow kinetics convincingly decouples PD from PK (with the obvious exception of covalent ligands).

Slow on-rates are generally not desirable

The role of the association rate (k_{on}) is not often discussed in the residence time literature. This is unfortunate, because a better understanding of the on-rate highlights a key downside of the residence time concept: if one is aiming to drive down the off-rate without simply increasing the potency of a ligand, one would need to slow down the on-rate. Not only is this considered difficult to achieve in a rational manner – indeed, a recent survey of binding kinetics data indicated that on-rates do not differ systematically between discovery compounds and (candidate) drugs [12]. But also, and more fundamentally, slow association is usually not desirable in the first place. A very slow on-rate will not give appreciable binding at low doses before the compound is cleared from the system (the on-rate also depends on the ligand concentration, *vide supra*). Although modeling data have been

presented showing that multiple dosing can overcome low target occupancy caused by slow kinetics [11,13], these simulations do not take into account protein turnover (degradation and synthesis) and, therefore, overestimate day-to-day build-up of target occupancy. Protein half-live *in vivo* is broadly on the same time scale as drug dosing, typically a couple of hours up to a day [14]. This means that target occupancy will not necessarily increase upon multiday dosing for compounds that are extremely slow on/slow off. Therefore, slow-on drugs need to be dosed at high concentrations if they are required to be effective quickly [11], which offsets the advantage that slow off-rates would generally allow for low doses and decreased off-target toxicity.

Moreover, fast on-rate will increase rebinding, which is the situation where a ligand, after target dissociation, binds to the target anew as opposed to diffusing away from it. Evidence is emerging that rebinding increases the *in vivo* duration of a drug, compared with its *in vitro* kinetic data [12,15–18]. A slow on-rate would reduce rebinding, which offsets the advantage that long residence times offer in an *in vivo*, open setting. Indeed, recent PK/PD modeling studies show that increasing the on-rate of a compound decreases the rate of decline of target occupancy (i.e., closely produces the same outcome as decreasing the off-rate [12,13]).

In other words, it is hard to envisage situations where one would purposely design slow association in a compound. The best compounds are fast on, slow off (ignoring special cases, such as on-target toxicity and desired short duration of effect). Ergo, the best compounds are potent compounds. The recent analysis by De Witte and co-workers, who compared a collection of drugs and/or drug candidates with a large set of preclinical compounds (Fig. 4 in Ref. [12]) further corroborates this point. If there were a residence time concept that goes beyond just increasing affinity, one would expect the distribution of on-rates of advanced compounds to be slower than that of discovery compounds. However, the analysis by De Witte et al. shows that these distributions of on-rates are comparable and that, overall, drugs are simply more potent than preclinical compounds.

Concluding remarks

To conclude, binding kinetics is undeniably an important element in drug discovery, and knowing the individual off- and on-rates may help understand modes of action, structure–activity relations, differentiation between ligand classes, importance of rebinding and so on, and also in relation to other compound properties, notably PK. However, the current trend to single out the residence time provides a limited view of binding kinetics and affinity, and is a suboptimal way to drive drug discovery programs.

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