Conformational adaptation in drug–target interactions and residence time

Although drug–target interactions are commonly illustrated in terms of structurally static binding and dissociation events, such descriptions are inadequate to explain the impact of conformational dynamics on these processes. For high-affinity interactions, both the association and dissociation of drug molecules to and from their targets are often controlled by conformational changes of the target. Conformational adaptation can greatly influence the residence time of a drug on its target (i.e., the lifetime of the binary drug–target complex); long residence time can lead to sustained pharmacology and may also mitigate off-target toxicity. In this perspective, the kinetics of drug–target association and dissociation reactions are explored, with particular emphasis on the impact of conformational adaptation on drug–target residence time.

The pharmacologic basis of drug action almost always involves modulation of the physiologic activity of macromolecules (e.g., enzymes, receptors and ribosomes) by binding of drug molecules (e.g., small organic molecules and biologics) to these targets. Thus, pharmacology is based on the formation of a drug–target complex and, in turn, the duration of pharmacologic effect is often dictated by the temporal persistence of target occupancy by the drug. Although drug–target interactions are commonly illustrated in terms of structurally static binding and dissociation events, in which the conformation of the drug molecule and that of its target macromolecule are fixed, such a description is inadequate to explain the impact of conformational dynamics on drug–target interactions. Both the association phase of drug binding to a target, and the subsequent dissociation of the binary drug–target complex, are often controlled by conformational changes, especially involving structural changes in the immediate vicinity of the drug-binding pocket [1–3].

Historically, the effectiveness of a drug’s interaction with a target has been quantified by measuring the concentration of drug required to achieve a specific level of target occupancy under equilibrium conditions (e.g., the K_\text{d} or IC_{50} value). In recent years, however, there has been increased recognition that drug–target interactions in vivo are not defined by equilibrium conditions. In particular, the importance of stabilizing the binary drug–target complex in vivo for sustained pharmacologic effect has been highlighted, and the term residence time has been coined to describe the temporal duration of the drug–target complex under different conditions [4,5]. In addition, a differentially long target residence time provides a mechanism for temporal target selectivity, hence a cogent approach to the mitigation of off-target based toxicity in vivo; this topic has been covered in considerable detail in previous reports [4–6].

Residence time (\(\tau\)) is commonly quantified through experimental measurements of the reciprocal of the dissociation rate constant (\(\tau = 1/k_{\text{off}}\)) or the dissociative half-life (\(t_{1/2} = 0.693/k_{\text{off}}\)), and it has been argued that the residence time provides an important metric for compound optimization through medicinal chemistry. The residence time of the drug–target complex is very clearly dependent on the conformational stabilization of the drug–target complex; hence, conformational adaptation plays a key role in drug binding and stabilization of the final structure of the drug–target complex, as will be described later.

While the concept of conformational adaptation in drug–target interactions has been presented previously [1–7], it is not widely appreciated throughout the drug-discovery and medicinal chemistry communities. In this brief perspective, we review some aspects of conformational adaptation in drug–target interactions as they relate to drug-discovery efforts.

The static view of drug–target interactions

The conventional view of drug–target interactions was first formulated by H Emil Fischer, to describe enzyme–substrate interactions and has been dubbed the lock-and-key model [8].

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Temporal target selectivity: Refers to the degree of target occupancy, relative to occupancy of collateral off-target proteins, by a drug as a function of time over the course of in vivo dosing. The temporal target selectivity can be quantified as the ratio of residence times for the off-target protein and that for the target protein.

In this model, the target macromolecule contains a binding pocket that is complementary to the drug molecule (or other ligand), in both steric and electronic ways, such that a network of favorable interactions between the drug and recognition elements within the binding pocket is established upon binding; thus the binary drug–target complex is stabilized relative to the free reactants (i.e., receptor and ligand) [2,3]. This conventional view further considers the recognition elements of the binding pocket to be held static in the most complementary arrangement with respect to ligand interactions. Hence, drug association and dissociation each occur in a single kinetic step, and the efficiency of interaction may be quantified by familiar, mathematically related parameters such as IC₅₀ values, Kᵰ values and ΔG(binding) (Figure 1) [3]. Although Kᵰ and ΔG(binding) are thermodynamic constants, they can be readily related to the kinetic rate constants for drug association and dissociation as follows [2].

\[ Kᵰ = \frac{k_{on}}{k_{off}} \]

**Equation 1**

\[ ΔG_{binding} = RT\ln(Kᵰ) = RT\ln\left(\frac{k_{on}}{k_{off}}\right) \]

**Equation 2**

For weak binding interactions (i.e., Kᵰ values in the μM to mM range), association and dissociation are usually rapid, with half-lives on the µs timescale. This rapidity of binding and dissociation can be important for physiological reactions, such as enzymes binding to substrates and cofactors. As binding affinity increases, however, it is often the case that the rate of association and, especially, of dissociation slow down to timescales of seconds, minutes and sometimes longer; hence, these reactions may be conveniently measured in vitro by a number of biochemical and biophysical methods [1–4,9].

Using convenient experimental methods, one can mix a macromolecular target (let us refer to these universally as receptors and use the symbol R to represent them) with a drug or other ligand (we will use the symbol L to universally represent these molecules) and measure the amount of binary complex (RL) formed as a function of time after mixing. In most experimental approaches to measuring receptor–ligand binding, the receptor concentration is held constant at a very low, limiting concentration relative to that of the ligand. Under these conditions, binding of ligand to the receptor follows pseudo-first order kinetics [3] and the approach to equilibrium can therefore be described by a pseudo-first order rate constant kₜₐₙ⁻¹.

The value of kₜₐₙ depends on the concentration of ligand in characteristic ways that can be mechanistically informative [1,5]. For the simple, static binding mechanism illustrated in Figure 1, the value of kₜₐₙ is a linear function of ligand concentration for which the slope is equal to the value of kₜₐₙ and the intercept is equal to the value of kₜₐₙ [5].

There are indeed examples of drug–target complexes for which this type of binding measurement yields a linear plot of kₜₐₙ as a function of ligand concentration. Hence, in these cases the experimental data are consistent with single-step binding and dissociation, and therefore a static drug binding pocket. These cases are not common, however, and often it turns out that there are conformational adjustments to the binding pocket that attend ligand binding. In these cases the thermodynamic stability of the protein conformers is similar and therefore interconversion among conformers occurs too rapidly to be observed in standard binding experiments. This was the case, for example, in studies of piperidine inhibitors of the aspartyl protease pepsin [10].

Conformational adaptation in drug–target interactions

Despite being commonly found in textbooks, the static model of drug–target interactions (see earlier) is seldom adequate to describe fully the association and dissociation of high-affinity drugs with their targets. For the majority of drugs that bind with nanomolar or lower Kᵰ values, it is common to find conformation adjustments of the drug binding pocket that attend complex formation [2–6,11]. This type of conformational adaptation can result from two
kinetic pathways for drug association that have been referred to as the conformational selection and induced-fit models of binding (Figure 2) [1].

In the conformational selection model the receptor exists in an ensemble of conformers in the absence of ligand; only some of these conformers are capable of binding ligand. For simplicity let us say that the ensemble of conformers is composed of only two states that are in equilibrium with one another: a state that is unable to bind ligand (R) and an alternative conformer that does bind ligand (R*). In the absence of ligand, the equilibrium strongly favors the R state over the R* state, and interconversion between these states is relatively slow. Upon addition of ligand, those receptor molecules in the R* conformer will bind ligand (R*L) and therefore be removed from the equilibrium between the free forms R and R*. This leads to a shift in the equilibrium position to favor formation of more R*, which can then bind more ligand until, at infinite ligand concentration, the entire system has shifted to the R*L state (Figure 2). In this model the rate limiting step in binding is assumed to be interconversion between the two free forms R and R*; once formed, R* binds ligand rapidly.

The induced-fit model (Figure 2) results in the same final form of the drug–target complex, R*L, but arrives at this state through a different kinetic pathway. Here the unbound receptor exists in a single conformational state, R, that is capable of binding ligand to form an encounter complex, RL. The recognition elements within the binding pocket are not optimally complementary to ligand in the RL state. The act of ligand binding causes a conformational readjustment of the target to form a new conformation (R*L) in which optimal complementarity between ligand and binding pocket is achieved. In this model, ligand binding to the initial encounter state, R, is considered rapid and the rate-limiting step is a slow conformational transition (i.e., isomerization) from the RL state to the final R*L state.

An important point to bear in mind is that, for both models, each target conformer (R, R*, RL and R*L) represents an ensemble of
conformational microstates that may interconvert through vibrational, rotational and translational excursions, depending on the energy barrier to interconversion. Hence, stabilization of a particular state, such as a ligand-bound state, depends on populating a deep, narrow potential energy well that creates a substantial energy barrier to escape and thus to interconversion. This concept is pictorially illustrated in Figure 2B. It is also important to realize that the conformational selection and induced-fit models merely represent opposite poles of a continuum of conformational adaptation mechanisms that nature may use to promote molecular recognition between binding partners [12,13]. A final point that is worth mention is that the conformational selection and induced-fit models are thermodynamically indistinguishable. That is, the overall binding affinity in both models will depend on the free energy difference between the starting and ending states of the system. By what kinetic process the system arrives at the final state is irrelevant from a thermodynamic perspective (i.e., the free energy of binding is a path-independent parameter).

The conformational selection and induced-fit models may be experimentally distinguished by measurements of the pseudo-first order rate constant for approach to equilibrium as a function of ligand concentration [1–5,12]. Equations describing the dependence of $k_{obs}$ on ligand concentration for the two mechanisms have been independently derived by multiple investigators and are well established in the biochemical literature. One finds that the quantitative value of $k_{obs}$ varies with ligand concentration in opposing fashions for the two mechanisms [3,5,14,15]. For the conformational selection, mechanism $k_{obs}$ depends on ligand concentration as described by the following equation:

$$k_{obs} = k_{ss} + k_{on} \frac{K_d}{K_d + [L]}$$

Equation 3

where $k_{ss}$ is the rate constant for forward isomerization from state R to $R^*$ and $k_{on}$ is the rate constant for the reverse isomerization from $R^*$ to R (Figure 2A). We can define the limits of $k_{obs}$ at zero and infinite ligand concentrations by inspection of Equation 3. When [L] is zero, $k_{obs}$ reduces to $(k_{ss} + k_{on})$, and when [L] is infinite, $k_{obs}$ reduces to $k_{on}$. Thus the value of $k_{obs}$ decreases curvilinearly with increasing ligand concentration from an intercept value of $(k_{ss} + k_{on})$ to a final value of $k_{on}$ at infinite ligand concentration (Figure 3).

For the induced-fit model, $k_{obs}$ depends on ligand concentration as follows:

$$k_{obs} = k_{ss} \frac{[L]}{K_d + [L]} + k_{on}$$

Equation 4

The limits at zero and infinite concentration from Equation 4 are $k_{ss}$ and $(k_{ss} + k_{on})$, respectively. Thus, for the induced-fit model, $k_{obs}$ is a saturable, hyperbolic function of ligand concentration, increasing from an intercept value of $k_{ss}$ to a final value of $(k_{ss} + k_{on})$ at infinite ligand concentration (Figure 3).

This type of experiment provides a clear and unambiguous basis for defining the mechanism of interaction that is germane to a specific drug–target pair. In this manner, the two mechanisms of conformational adaptation in drug binding are readily distinguished from one another.

Most high-affinity drugs bind to their targets through a conformational adaptation mechanism [11]. Hence, one may ask which of the two conformational adaptation models presented above is most germane for drugs binding to their macromolecular targets. In fact, examples of both mechanisms can be found in the literature, based on the type of kinetic analysis just described. Table I provides examples of drugs or drug-related compounds for which either a conformational selection or induced-fit model may be invoked on the basis of kinetic data. Note that the entries in Table I for conformational selection represent all examples of this mechanism that are known to the author from survey of the literature. In contrast, the entries for induced fit represent a sampling of a much larger pool of known examples of this mechanism.

Reviewing the information summarized in Table I, all of the examples of conformational selection are for enzymes, mainly binding to natural substrates or cofactors. In the overwhelming majority of cases of a drug molecule binding to a macromolecular target, the binding appears to conform to an induced-fit mechanism. Thus, while both mechanisms appear to be applicable to receptor-ligand binding in general, pharmacologic modulation of targets appears to often involve an induced-fit mechanism of conformational adaptation.

**Structural changes associated with conformational adaptation**

The difference in affinity between the states RL and $R^*$L can be quite significant for some drug–target pairs; it is not uncommon to see the $K_d$
value change from micromolar to nanomolar or picomolar during this transition [16]. A common question raised in such cases is what are the structural alterations to the target that result in such dramatic changes in compound affinity? Recently, Garvey reviewed this topic and concluded that three broad mechanisms account for the affinity changes observed during conformational adaptation [16]: protein conformational changes, covalent adduct formation and compound ionization. Of the three, protein conformational changes are perhaps of the most interest to medicinal chemists and others focused on lead optimization. In his survey of the literature, Garvey found many cases in which the conformational changes that attend two-step binding of compounds to targets were quite subtle in nature [16]. Garvey concluded that most of the recognition elements that resulted in high-affinity interactions between compound and target are formed within the context of the initial encounter complex (RL) and these are reinforced through small conformational adjustments that lead to the final binary complex state (R*L).

The conclusions of Garvey are based largely on comparison of crystallographic structures of the ligand-free target with that of compound-bound forms of the target. In fact, there is a dearth of structural information in the literature comparing different ligand-bound conformers of targets (e.g., RL and R*L) as a basis for formulating a structural hypothesis for explaining affinity differences. Certainly, there are many exceptions to the generalization that structural differences between RL and R*L states are small. Among the proteins kinases, for example, there are several examples of significant loop movement and other structural rearrangements that attend inhibitor binding [17,18]. Likewise, in the case of the aspartyl proteases, closure of a flap region to occlude the enzyme active site from bulk solvent is a common feature of substrate and inhibitor binding [19].

Structural changes that stabilize the R*L state also lead to extended residence times for the overall drug–target complex. Indeed, while thermodynamic affinity (i.e., $K_d$ for the R*L state) and residence time can be independent parameters, it is often the case that the same structural elements of recognition are involved in optimization of both. In many cases, the optimization of $K_d$ is actually achieved by inadvertent optimization of residence time [4–6]. Hence, understanding structure–activity relationships (SARs) with a view towards maximizing the stability of the R*L state should be an important goal of lead optimization activities [11].

Although, as discussed, the literature is scant on this topic, some generalization can nevertheless be made with respect to SARs. First, conformational changes that attend the RL to R*L transition tend to lead to greater occlusion of the binding pocket from bulk solvent; hence, hydrophobic–hydrophobic interactions favor stabilization of the R*L state. Often, this occlusion involves ordering of loops and other structural elements of proteins to form a ‘lid’ over the drug binding site [19]. Second, while similar recognition elements tend to be engaged in the RL and R*L states (e.g., hydrogen bonds and salt bridges), these tend to be strengthened in the R*L state. Finally, the R*L state can provide additional recognition elements for compound engagement that are not available in the RL state. Examples of this include engagement of hydrogen bond networks between bound inhibitors and flap elements in aspartyl protease inhibitors [2,19], ‘back pocket’ engagement by ATP-competitive inhibitors of protein kinases [18], and ‘side-pocket’ engagement by selective inhibitors of cyclooxygenase-2 [2]. A particularly common mechanism of binding pocket occlusion for...
targets that display induce-fit inhibitor binding is the folding (or ordering) of unstructured, flexible loops within the polypeptide sequence, over the solvent-exposed surface of the binding site [19]. In this manner, proteins form ‘lids’ over the inhibitor-bound pocket to block the escape trajectory of ligands from the protein (Figure 4B; vide infra). This mechanism is seen, for example, upon potent inhibitor binding to a variety of kinases, HIV protease, HIV integrase [20], methionine adenosyltransferase [21], ribulose-bisphosphate carboxylase [22], Hepatitis NS3 protease [23], enoyl-ACP reductase [24] and many other protein targets. Given the commonality of this mechanism, it seems reasonable to suggest medicinal chemistry efforts focused on engaging specific intermolecular interactions between drug molecules and recognition elements within such flexible loop ‘lids’ as a concrete approach to systematic optimization of both overall target affinity and drug-target residence time.

A retrograde induce-fit model of drug–target complex dissociation

Regarding drug interactions with pharmacologic targets, it seems clear that the induced-fit mechanism is relevant to a large number of medically important systems. As described previously, formation of the drug–target binary complex is a bimolecular process that can be mediated by the induced-fit mechanism, the conformational selection mechanism or other mechanisms that incorporate features of both extreme models. Regardless of the sequence of events that lead to drug–target binary complex formation, durable pharmacologic action is determined by the residence time of drug occupancy on the receptor. We [4–6] and others [25–27] have made the case that in vivo, the duration of drug–target occupancy is determined mainly by the rate of drug dissociation (i.e., dissociative half-life and residence time [4]). Drug dissociation from the binary drug–target complex is kinetically a unimolecular process (i.e., the observed rate constant for the process is dependent only on the concentration of binary complex and not on the concentrations of total [or free] receptor and ligand). Thus, any conformational changes that must accompany drug dissociation most likely occur through the equivalent of a retrograde induced-fit mechanism (i.e., operating in the reverse sequence of conformational events leading to association).

As described above, formation of the final R*L state likely includes conformational changes that occlude the drug binding site (hence the drug)

### Table 1. Some examples of receptor–ligand binding interactions for which a conformational selection or induced-fit mechanism has been demonstrated.

<table>
<thead>
<tr>
<th>Target</th>
<th>Ligand(s)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human glucokinase</td>
<td>Glucose</td>
<td>[32]</td>
</tr>
<tr>
<td>Rat liver glucokinase</td>
<td>Glucose</td>
<td>[33]</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>Proflavin</td>
<td>[34]</td>
</tr>
<tr>
<td>Escherichia coli alkaline phosphatase</td>
<td>2,4-dinitrophenyl phosphate</td>
<td>[35]</td>
</tr>
<tr>
<td>Ribonuclease T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Guanosine 3'-GMP</td>
<td>[36]</td>
</tr>
<tr>
<td>Protein kinase A</td>
<td>PLN&lt;sub&gt;1,20&lt;/sub&gt;</td>
<td>[37]</td>
</tr>
</tbody>
</table>

### Induced-fit

<table>
<thead>
<tr>
<th>Target</th>
<th>Ligand(s)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclooxygenase-2</td>
<td>DuP697 NS-398</td>
<td>[38]</td>
</tr>
<tr>
<td>Cyclooxygenase-1</td>
<td>Indomethacin</td>
<td>[39]</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase</td>
<td>DADMe-ImM</td>
<td>[40]</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Allopurinol</td>
<td>[41]</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis enoyl reductase</td>
<td>Isoniazid</td>
<td>[42]</td>
</tr>
<tr>
<td>Dihydrofolate reductase</td>
<td>Methotrexate</td>
<td>[43]</td>
</tr>
<tr>
<td>Hepatitis C virus NS3 protease</td>
<td>ITMN-191 VX-950</td>
<td>[44,45]</td>
</tr>
<tr>
<td>HIV-1 protease</td>
<td>Darunavir</td>
<td>[46]</td>
</tr>
<tr>
<td>Prostate-specific antigen</td>
<td>Phosphoramidate peptidomimetics</td>
<td>[47]</td>
</tr>
<tr>
<td>Hsp90</td>
<td>Geldanamycin</td>
<td>[48]</td>
</tr>
<tr>
<td>Bacterial β-ketoacyl-acetyl carrier protein synthases</td>
<td>Thiolactomycin</td>
<td>[49]</td>
</tr>
<tr>
<td>HIV-1 Integrase</td>
<td>Elitegravir</td>
<td>[50]</td>
</tr>
<tr>
<td>Aurora B</td>
<td>GSK1070916</td>
<td>[51]</td>
</tr>
<tr>
<td>AKT</td>
<td>GSK690693</td>
<td>[52]</td>
</tr>
<tr>
<td>Steroid 5α-reductase</td>
<td>Finasteride</td>
<td>[53]</td>
</tr>
<tr>
<td>Bacterial ribosome</td>
<td>Erythromycin</td>
<td>[54]</td>
</tr>
<tr>
<td>HIV reverse transcriptase</td>
<td>Efavirenz</td>
<td>[55]</td>
</tr>
<tr>
<td>Glu-tRNA&lt;sup&gt;GS&lt;/sup&gt; amidotransferase</td>
<td>Boronate peptidomimetics</td>
<td>[56]</td>
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<tr>
<td>Polypeptide deformylase</td>
<td>Actinonin</td>
<td>[57]</td>
</tr>
<tr>
<td>Kinesin motor protein</td>
<td>Ispinesib</td>
<td>[58]</td>
</tr>
<tr>
<td>Bacterial deacetylase LpxC</td>
<td>Ciprofloxacin</td>
<td>[59]</td>
</tr>
<tr>
<td>HMG CoA reductase</td>
<td>Rosuvastatin</td>
<td>[60]</td>
</tr>
<tr>
<td>Lipoxigenase-1</td>
<td>Amidrazine</td>
<td>[61]</td>
</tr>
<tr>
<td>Calcineurin</td>
<td>L-732531</td>
<td>[62]</td>
</tr>
<tr>
<td>Xylanase</td>
<td>ABT1</td>
<td>[63]</td>
</tr>
<tr>
<td>Nitric oxide synthase</td>
<td>1400W</td>
<td>[64]</td>
</tr>
<tr>
<td>BACE</td>
<td>Statine peptidomimetic</td>
<td>[65]</td>
</tr>
<tr>
<td>p38 MAP kinase</td>
<td>BIRB796</td>
<td>[66]</td>
</tr>
<tr>
<td>Dialkylglycine decarboxylase</td>
<td>Aminophosphonates</td>
<td>[67]</td>
</tr>
</tbody>
</table>
from exposure to bulk solvent [19]. A well-known example of such a conformational change is the flap closing that occurs after ligand binding to the active site of aspartyl proteases, such as the HIV protease. It is difficult to imagine how a drug molecule could escape from such an occluded binding pocket without first opening up an escape trajectory by displacement of the occluding flap region. Hence, a retrograde induced-fit mechanism seems likely to be a necessary component of drug dissociation in cases such as this. Thermodynamically, an energetically equal path to ligand dissociation is afforded by the reverse trajectory associated with the conformational selection model, as illustrated in Figure 2A. From a physical structure perspective, however, this latter dissociation path would require the ligand to diffuse out of the binding pocket through the occlusion barrier imposed by the protein lid, flap, or other conformation transitions resulting in the R*L state. Ligand ‘tunneling’ through proteins has been invoked to describe the diffusion of protons and diatomic gaseous ligands (e.g., O$_2$, CO and NO) out of metalloproteins (e.g., heme proteins) [28], but this seems a very unlikely possibility for a large, organic compound, such as a drug molecule.

The retrograde induced-fit mechanism requires the conversion of the R*L complex back to the RL complex before dissociation of the drug and recovery of the free R state of the receptor. As illustrated in Figure 4, both of these conversions require the system to surmount a significant energy barrier to transiently attain two sequential transition states: R*L‡ and RL‡. Once the system has reached the RL state, it can again surmount the R*L‡ transition state to return to the R*L state or surmount the RL transition state to complete the ligand escape process. Thus, the residence time of a drug–target complex relates directly to the relative stabilities of the R*L and RL states, which in turn relate directly to the depth of the potential energy wells associated with each state of the system and the energetic height of the accompanying transition states [27]. The value of the free energy differences between the states R, RL and R*L are experimentally measurable through a variety of biochemical and biophysical methods, as described previously [2]. For example, among the drug–target pairs summarized in Table 1, the ratio of K/I/K* vary from 3.5-fold to more than 2300-fold, representing differences in binding free energy (ΔG$_{binding}$) between R*L and RL of 0.7 to more than 4.6 kcal/mol [15]. Consideration of this type of retrograde induced-fit mechanism provides a useful framework for drug optimization.
activities. Thus, improvements in overall compound affinity and residence time may, in some cases, be achieved by optimization of compound interactions with both states RL and R*L. In other cases, destabilization of the RL state that is accompanied by stabilization of the R*L state may be most optimal for prolonged residence time. Ultimately, it is the overall stabilization of the R*L state that has the greatest impact on affinity and residence time.

Future perspective

There is growing appreciation for the importance of understanding the kinetics of drug interactions with their macromolecular targets. In particular, the drug-discovery community has begun to consider drug–target residence time as an important factor for sustained pharmacologic impact in patients. Hence, there is growing interest in measuring dissociation rates of compound–target complexes during lead optimization activities, to identify clinical candidates that may demonstrate long residence time in vivo. This is a significant change from the exclusive reliance on thermodynamic parameters (e.g., IC₅₀) that has dominated drug-discovery efforts for much of the 19th and 20th Centuries. Yet, treating residence time as a phenomenological measurement is unsatisfying in the context of hypothesis-driven SAR. Hence, medicinal chemists rightly ask questions about the elements of molecular recognition that bear on prolonged residence time and how these recognition elements may be most optimally engaged by small-molecule drugs.

The key theme of this article has been that recognition elements are not static, and that conformational adaptation is an important aspect of drug–target interactions that must be considered carefully during lead optimization. We have seen how conformational adjustments can lead to changes in drug–target affinity that contribute directly to prolongation of residence time. We have introduced the concept of a retrograde induced-fit mechanism for drug dissociation in the common situation of two-step, conformationally gated interactions between drugs and their targets. This concept highlights the importance of conformational adaptation for enhanced residence time and the need to take this into consideration in drug discovery. While not stated explicitly above, it is clear that failure to properly consider the role of binding kinetics and conformational adaptation in the evaluation of drug–target interactions can lead to significant errors in SAR that can mislead medicinal chemistry efforts. For example, failure to account for slow compound association and/or dissociation during binding assays can grossly underestimate the affinity and residence time of a compound [2]. An excellent example of this is provided by the evaluation of Hsp90 inhibitors, such as geldenamycin [29]. For some time researchers were puzzled by the low affinity of such compounds, determined by in vitro Hsp90 binding assays (IC₅₀ ~1 µM), when contrasted to the nanomolar effects of such compounds in cellular assays. This apparent discordance was resolved by Gooljarsingh et al. by carefully measuring the time required to reach full equilibrium in the binding assays [29]. Geldenamycin and similar compounds turn out to be slow binding and very slow dissociating compounds with nanomolar affinity for Hsp90. The true affinity was not previously realized because the binding assays failed to account fully for the kinetics of compound association and dissociation. Surely, there are many other unknown examples of such misinformed SAR due to a failure to properly account for the kinetics of drug–target interactions.

The residence time concept is now fairly well established within the medicinal chemistry and pharmacology communities. In many, but certainly not all cases, prolonged residence time is seen as a cogent mechanistic underpinning for durable pharmacology and mitigation of off-target mediated toxicity for drugs in vivo (see [4–6] however, for examples where long residence time is contraindicated). What remains to be developed over the next 5 to 10 years, is a detailed understanding of the structural determinants that mediate prolonged drug–target residence time. In this article we have made the general statement that longer residence time is facilitated by stabilization of the more closed, solvent occluded R*L state (see earlier), within the context of the retrograde induced-fit mechanism of drug–target dissociation. We have further suggested that stabilization of the R*L state might be optimized by engagement of recognition elements within flexible loops of the target macromolecule, that form lid-like gates to compound exodus. Yet, these generalizations provide little direction to medicinal chemists in their efforts to optimize residence time.

Overall, this article should be viewed as a call-to-action for the medicinal chemistry, biochemistry and structural biology communities. As these topics have not yet received the experimental efforts that they deserve, we have not been able to address the questions of residence time SAR in any systemic fashion. This remains a challenge for the drug-discovery community to address in...
a prospective way. Recently, a number of more detailed reports of residence time SAR, coupled with crystallographic data, have been submitted to various journals for publication. This is an encouraging development, and it is hoped that more such reports will begin to appear in the scientific literature. Basic questions also remain to be answered regarding the relative contributions of enthalpic and entropic forces in drug binding and release, and in surmounting the multiple transition states associated with retrograde induced-fit drug dissociation. Similarly, the question of how heat capacity differences among conformational states of the target might influence drug dissociation remains to be addressed systematically. Again, some preliminary reports, based largely on calorimetric studies, are beginning to appear in the literature to address these questions. A final area for future exploration is the influence of auxiliary proteins and other intracellular binding partners of targets, on drug–target residence time. It is reasonable to consider that binding partners could influence the conformational dynamics of targets and in this way indirectly influence drug residence time. For example, in a recent set of studies, Anderson et al., demonstrated that substrate and inhibitor binding to Aurora kinases could be significantly influenced by the presents of auxiliary proteins, such as TPX2 and INCEPE [30,31].

As additional work in all of these areas continues and begins to populate the literature, it is hoped that more definitive answers to these important questions may soon emerge. As more clear descriptions of the structural determinants of drug–target residence time arise, the pharmaceutical community will be in a much better position to fully exploit the residence time concept for improved development of safe, long-lasting therapies against currently unmet medical needs.

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Executive summary
- Conformational dynamics of target macromolecules significantly affect the binding and dissociation of drug molecules.
- It is the lifetime of the drug–target complex, rather than the affinity, that determines the duration of pharmacologic effect of drugs 
  _in vivo_. Long target residence time can also provide a mechanism for mitigating off-target mediated toxicity by limiting systemic exposure of drugs.
- Many potent drugs bind their targets through a two-step, induced-fit mechanism.
- Drug–target dissociation is often mediated by a retrograde induced-fit mechanism, requiring surmounting of multiple energy barriers along the drug escape trajectory.
- Stabilization of the final drug–target complex state provides a mechanism for prolonging drug–target residence time.

Bibliography


46 Dieryckyn I, De Wit M, Gustin E et al. Binding kinetics of darunavir to human immunodeficiency virus type 1 protease.


