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Current Opinion in Chemical Biology

Structure–kinetic relationships that control the residence time of drug–target complexes: insights from molecular structure and dynamics

Hao Lu[1], James N Iuliano[2] and Peter J Tonge[2,3]

Time-dependent target occupancy is a function of both the thermodynamics and kinetics of drug–target interactions. However, while the optimization of thermodynamic affinity through approaches such as structure-based drug design is now relatively straight forward, less is understood about the molecular interactions that control the kinetics of drug complex formation and breakdown since this depends on both the ground and transition state energies on the binding reaction coordinate. In this opinion we highlight several recent examples that shed light on current approaches that are elucidating the factors that control the life-time of the drug–target complex.

Addresses
[1] EMD Serono Research & Development Institute, Inc., Billerica, Massachusetts, USA
[2] Department of Chemistry, Institute for Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, New York, USA
[3] Department of Radiology, Stony Brook University School of Medicine, Stony Brook, New York, USA

Current Opinion in Chemical Biology 2018, **44**:101–109

This review comes from a themed issue on **Next generation therapeutics**

Edited by **Adrian Whitty** and **Peter J Tonge**

https://doi.org/10.1016/j.cbpa.2018.06.002

Introduction

Drug discovery is a complex and expensive process with a high risk of failure. An analysis of 7372 independent clinical trials from 2003 to 2011 revealed that only about 10% of the drug candidates were eventually approved by the FDA [1]. Two major contributors to the high attrition rate were lack of efficacy and unacceptable safety, both of which often result from unpredicted mechanisms of action (MOA) such as poor engagement with the primary target(s) and/or undesired binding to off-target proteins [2,3]. Since hit identification and lead optimization are early but critical steps that generate and select quality candidates for clinical development, new approaches at this stage of discovery, including activity-based profiling and parallel structure activity and liability relationship (SAR/SLR) screening, are now being used to better understand MOA [4,5]. Whilst these techniques will likely improve the success rate of new drug approvals, there is still a heavy reliance on drug–target binding affinities determined under conditions where drug and target are at equilibrium [6], and which therefore cannot fully cannot for drug–target engagement in the non-equilibrium environment of the human body. Thus, there is now increasing emphasis on strategies that include both the thermodynamics and kinetics of drug–target interactions so that the generation and selection of clinical candidates can be better informed and the rate of attrition further reduced [7••,8••,9,10••,11••,12].

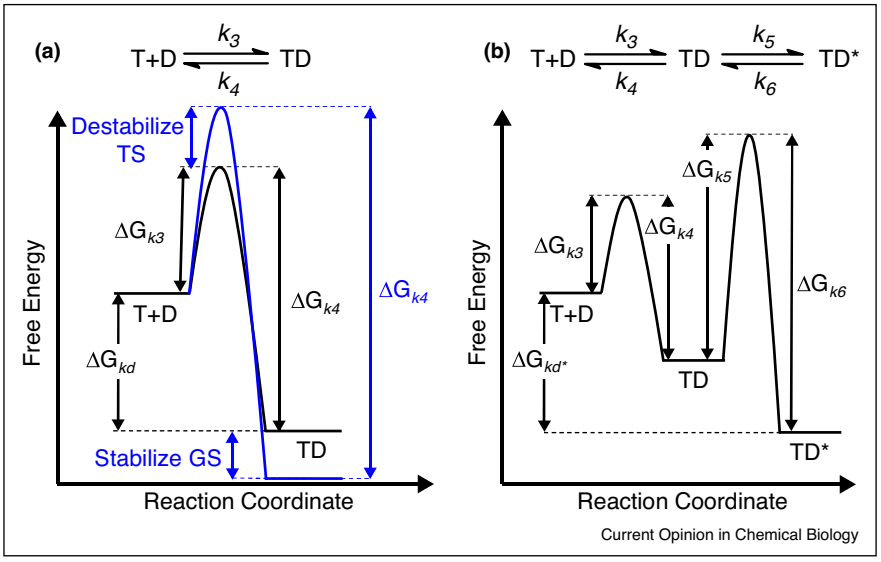
Residence time (t_R), the reciprocal of the rate at which the drug dissociates from the target to generate free (active) target ($1/k_{off}$), is a non-equilibrium intrinsic parameter that quantitatively measures the lifetime of the drug–target complex [8••]. In general, increasing drug–target residence time will be a valuable strategy for increasing the therapeutic window when the desired pharmacological outcome results from prolonged target occupancy, and provided that the drug dissociates rapidly from off-target proteins. The utility of residence time for the discovery and development of new drugs depends on several factors including drug pharmacokinetics, which can impact the benefits of kinetic selectivity, as well as target vulnerability and target turnover [11••]. In this opinion we highlight some of the molecular factors that are known to influence residence time, in order to serve as guidance for the ultimate goal of rationally controlling the lifetime of the drug–target complex.

Kinetic mechanisms for prolonged residence time

Several kinetic schemes can give rise to prolonged target occupancy including a simple one-step binding mechanism as well as a two-step induced-fit binding mechanism where the rapid formation of the initial drug–target complex (TD) is followed by a slow step leading to the final complex (TD*) [13] (Figure 1). Importantly, the on and off rates for formation and breakdown of the drug–target complex are controlled by the difference in free energy between the relevant ground and transition states on the binding reaction coordinate.

Consideration of the precepts behind the reaction coordinate diagram lead to several key points including,

Figure 1



Reaction coordinate for drug–target complex formation **(a)**. One-step binding mechanism showing that an increase in residence time ($1/k_4$) can occur either by stabilization of the ground state (GS) and/or destabilization of the transition state (TS). The on-rate for drug–target complex formation ($k_{on} = k_3$) is second order and thus will depend on drug concentration. **(b)** Two-step induced-fit binding mechanism in which the rapid formation of the initial drug–target complex (TD) is followed by a slow step leading to the final complex (TD*). The forward rate for formation of TD* is k_5 and is thus first order.

firstly, that stabilization of the drug–target complex ground state may or may not affect the off-rate depending on whether the stability of the transition state is also altered, secondly, that a compound can bind to two targets with the same thermodynamic affinity but with different on and off-rates, thereby displaying kinetic but not thermodynamic selectivity, thirdly, that a drug with a slow on rate will always have a slow-off rate, and fourthly, that a drug with a slow-off rate may or may not bind rapidly to the target. The first point is particularly important given the almost exclusive focus in drug discovery campaigns on increasing the stability of the drug–target complex.

Since technological advances have now reached a point where robust kinetic data can be generated in high-throughput mode, one might wonder why structure–kinetic relationships (SKRs) have not become part of the paradigm in early drug discovery to actively hunt for small molecules with long residence times. The biggest hurdle is the lack of specific information to guide medicinal chemistry campaigns explicitly aimed at rationally modifying residence time. Structural tools, such as X-ray crystallography and NMR spectroscopy, can readily capture ground state structures and thus be used to elucidate molecular interactions that are important for optimizing binding affinity. However, transition states are short-lived and, with the exception of approaches pioneered by Schramm and coworkers [14•], there is generally very limited structural information available to rationalize how to alter their stability. Efforts to unravel the molecular basis for residence time include the analysis of molecular properties of drugs that correlate with residence time, and the investigation of the conformational changes in the target linked to residence time using X-ray structural data, and thus focused on drug–target ground states, in some cases supplemented with computational approaches to provide insight into the structure of both ground and transition states on the binding reaction coordinate. Table 1 summarizes data for a number of targets in order to provide insight into diversity of approaches and mechanisms that have been uncovered, and in the subsequent discussion we highlight a few target classes to exemplify the current state of knowledge.

Bacterial enoyl-ACP reductase — reorganization of the substrate binding loop

The bacterial enoyl-ACP reductase FabI is a target for the development of new antibacterial agents. Previous studies on the *Francisella tularensis* FabI (ftFabI) demonstrated that residence time of diphenyl ether-based transition state analogs directly correlated with *in vivo* efficacy and was a better indicator of preclinical antibacterial activity than thermodynamic affinity [36]. Subsequently, structure-guided inhibitor discovery led to the synthesis of diphenyl ethers that are slow tight binding inhibitors of the FabI from *Staphylococcus aureus* (saFabI) with residence times ranging from 2 to 750 min [15]. SKR studies revealed that hydrophobic substituents at the 5-position