Kinetics fast track

1 Importance of binding kinetics in compound profiling and drug discovery

Equilibrium versus real life: why binding kinetics is important

Measuring target *affinity* is central to compound profiling, and is described by the dissociation constant **Kd** (**Box 1**) at equilibrium – in other words, when the concentration of bound drug-target complexes reaches steady state.

In practice equilibrium is rarely approached in *in vivo* systems. The concentration of unbound drug in the local target vicinity is continually changing, dependent on pharmacokinetic properties regulating plasma concentration and distribution to local tissue reservoirs. The drug may be in competition with native messengers or substrates for the target, whose concentrations – for example at a neuronal synapse – can alter greatly over a short space of time. In these common dynamic systems, individual association and dissociation rate constants (k_{on}, k_{off}; **Box 1**) become key compound optimisation parameters.

Box 1 Ligand binding parameters

Interactions between a ligand (L) and a receptor (R) are governed by the law of mass action. Rates of association and dissociation are related to the participant concentrations via constants k_{on} and k_{off}.

$$L + R \rightleftarrows LR$$

Association rate = k_{on}.[L].[R]

Dissociation rate = k_{off}.[LR]

- K_d, the **equilibrium dissociation constant**, is calculated as k_{off} / k_{on}. K_d is an inverse measure of ligand affinity, and is the concentration of free ligand required to occupy 50 % of the receptor population *at equilibrium*.
- k_{off}, the **dissociation rate constant**, represents the proportion of ligand-receptor complex that dissociates in unit time, in the absence of free ligand. Compounds with slow off rate (small k_{off}) exhibit prolonged receptor binding, and have potential for an insurmountable mode of action.
- **Residence time** ($1/k_{off}$) and **half-life** ($0.693/k_{off}$) are further descriptors of dissociation kinetics.
- k_{on}, the **association rate constant**, describes the ligand on rate at the receptor. Differences in k_{on} may particularly influence mode of action under conditions which enable compound rebinding, for example when free ligand diffusion is restricted within a tissue reservoir.

Slow off rates: a way to increase compound duration of action

An important effect of kinetic parameters is on duration of action (Figure 1). Slow dissociating drugs (low k_{off}) can extend the duration of effects *in vivo*, in tandem with pharmacokinetic optimisation[1]. Inhaled long acting muscarinic receptor antagonists for asthma, such as tiotropium[2,3] and biologics such as abciximab[4] demonstrate clinical benefits of this approach. Drugs with slow receptor dissociation have also been suggested to offer better selectivity, lower toxicity and a broader therapeutic window[1,2].

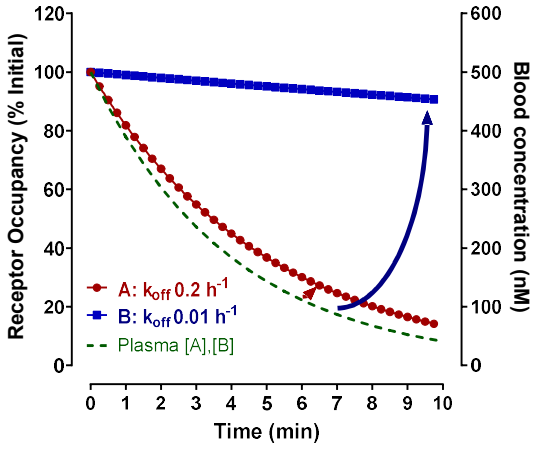

Figure 1 Slow off rates extend duration of action. Receptor occupancy of drugs A and B to a target receptor population is shown after a single dose and equilibration to steady state. A and B share the same affinity (K_d), and are eliminated from the plasma with the same half-life (dotted line). As B has a much slower dissociation rate constant (k_{off}), its occupancy of the target is sustained compared to A, despite an equivalent fall in plasma concentration over for the two drugs.

Mode of action: the benefits of insurmountable inhibition

Most receptor antagonists reversibly compete for the messenger binding site. Rapidly dissociating ligands display surmountable properties (Figure 2), in which high levels of messenger can overcome the desired inhibitory effect of the drug. In contrast, slowly dissociating antagonists show prolonged levels of target occupancy, and this leads to non-surmountable inhibition that is resistant to variations in stimulating agonist concentrations[5]. Optimising for slow **k_{off}** and increased functional insurmountability can be advantageous for effective antagonist design, in microenvironments where the stimulating messenger concentration is high - such as tumours or neuronal synapses[6,7].

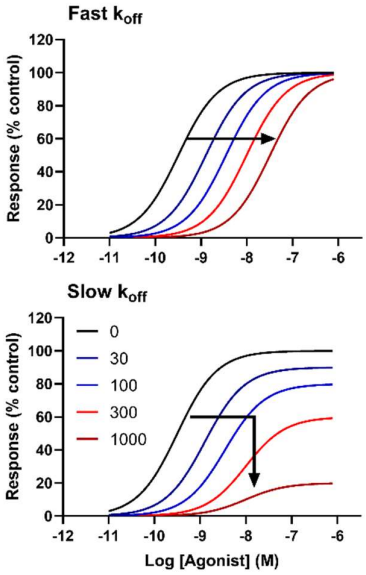

Figure 2 Slow dissociation kinetics can lead to an insurmountable mode of antagonist action. Receptor occupancy by fast dissociating competitive reversible antagonists rapidly re-equilibrates as the agonist concentration changes. Thus, these ligands display a classic surmountable mode of action (*top panel*): agonist potency reduces in the presence of increasing antagonist concentrations, but the maximum response is unaffected. However, antagonists with a slow k_{off} (*bottom panel*) display prolonged occupancy of the receptor population, which limits agonist competition and activation. This leads to insurmountable properties, and inhibition of the maximum agonist response, even if present at high concentration.

Association matters: rebinding

The importance of ligand association rates (**k_{on}**) for compound pharmacology has only recently become apparent. When the target is within a region of restricted diffusion, such as neuronal or neuromuscular synapses, drug molecules may not escape to the bulk flow after dissociation. Instead they re-associate with the same or nearby receptor molecules, known as **rebinding**[1]. More rapid association rates (**k_{on}**) promote increased compound rebinding and elevate the local effective drug concentration, and so provide a second kinetic mechanism to prolong target occupancy and duration of action[1,8].

Binding kinetics: PKPD models

Integrating the influence of binding kinetics on PKPD interactions helps predict target occupancy *in vivo*. For example, some duration effects (Figure 1) become relevant when the drug k_{off} is slower than its plasma elimination rate[1]. Equally drugs with a slow association rate (k_{on}), combined with a short plasma half-life, may undershoot the desired receptor occupancy during the dosing time window[9]. In these circumstances increasing k_{on} and decreasing k_{off} may both impact positively on target engagement over time, dependent on the context of the pharmacokinetic properties. New PKPD models consider this dynamic interplay, and can better inform compound profiling when provided with accurate binding kinetic data[9].

References: 1. Vauquelin G & Charlton SJ (2010) Br J Pharmacol *161*: 48. 2. Sykes DA et al (2012) J Pharmacol Exp Ther 343:520. 3. Dowling MR & Charlton SJ (2006) Br J Pharmacol 148:97. 4. Schneider DJ & Aggarwal A (2004) Expert Rev Cardiovasc Ther. 2:903. 5. Sykes DA et al (2016) Mol Pharmacol 89: 593. 6. Murray IA et al (2014) Nat Rev Cancer 14: 801. 7. Scimemi A & Beato M (2009) Mol Neurobiol 40: 289. 8. Sykes DA et al (2017) Nat Commun 8: 763. 9. Witte WEA (2016) Trends Pharmacol Sci 37: 831–842