enzymlogic

Exploiting the temporal dimension of GPCR signaling

Exploiting the temporal dimension of GPCR signaling

Drug binding and signaling at G-Protein-Coupled Receptors (GPCRs) are dynamic events that vary dramatically over time[1-7]. Many reports link the kinetics of drug binding and unbinding to GPCRs to the effectiveness and safety of a variety of drugs[8-17].

Profiling the drug binding kinetics with KINETICfinder® is key to:

- **Optimize in vivo receptor occupancy, ensuring therapeutic efficacy and avoiding drug-induced side effects.**
- **Understand the impact of binding kinetics on the sustained signaling from internalized receptors.** Drugs with different on/off binding kinetics have been shown to differentially promote receptor internalization and persistent signaling from internalized receptors.
- **Exploit ligand bias.** Differences in GPCR-drug binding kinetics contribute to the diverse biological effects in biased agonism. This kinetic bias offers the opportunity to design pathway-specific drugs while avoiding on-target side effects.
- **Design conformation-specific drugs.** GPCRs undergo conformational changes upon ligand binding to communicate signaling information across the plasma membrane of cells. Receptor dynamics can greatly influence ligand dissociation, and thus, kinetic profiling can provide new insights to facilitate conformation-specific drug design.

Avoid missing promising compounds using KINETICfinder®

Features and benefits

- Accurate.
- Robust.
- Reproducible.
- Sensitive.
- Broad dynamic range.
- Activated and non-activated targets.
- HTS.
- Rapid turnaround.

Applications

- Modify the on- and off-target kinetics (k_{on}, k_{off}, residence time and K_d).
- Predict clinical efficacy.
- Modulate the therapeutic index and safety profile.
- Understand PK/PD disconnects.
- Build better PK/PD models.
- Differentiate between similar therapies.

How KINETICfinder® assays work

KINETICfinder® is a highly tuned TR-FRET Kinetic Assay. Real-time binding of an active-site directed fluorescent probe is detected using a labeled anti-tag antibody, which binds to the target of interest. The binding of the probe and antibody to the target increases the TR-FET signal, whereas the displacement of the probe with a compound decreases the TR-FRET signal.

Assay process

1. **Performance:**
    - 384 microplates containing the target of interest, a fluorescent probe and labelled antibody.
    - 4-point 10-fold serial dilutions of test compounds.
    - A reference compound.
    - Up to 32 total binding and non-specific binding controls.
2. **Detection:** Reaction is monitored over time at room temperature.
3. **Analysis:** Specific TR-FRET signals are fitted to the Motulsky-Mahan equation. Association (k_{on}) and dissociation (k_{off}) rate constants of test and reference compounds are determined and the K_d and residence time (τ) values are calculated:

$$K_d = \frac{k_{off}}{k_{on}} \qquad \tau = \frac{1}{k_{off}}$$

4. **Quality control:** S/B, Z-value and MSR (within and between-run variability) for each assay.

KINETICfinder® provides reliable binding kinetics of mu-opioid receptor antagonists and agonists

Opioid drugs are the gold standard for the management of pain, but their use is limited by dangerous side effects. All clinically available opioid analgesics bind to and activate the mu-opioid receptor, a G-protein-coupled receptor, to produce analgesia.

In this study, we determine the binding kinetics of the clinically relevant mu-opioid receptor buprenorphine and naloxone using KINETICfinder® in living cells.

- We employ intact cells to preserve the intracellular proteins that interact with GPCRs and may stabilise different conformations of the receptors, affecting the binding kinetics of the ligands.
- The high and sustained stability of our GPCR assay guarantees superior quality results, enabling kinetic data to be interpreted with confidence (Fig. 2).

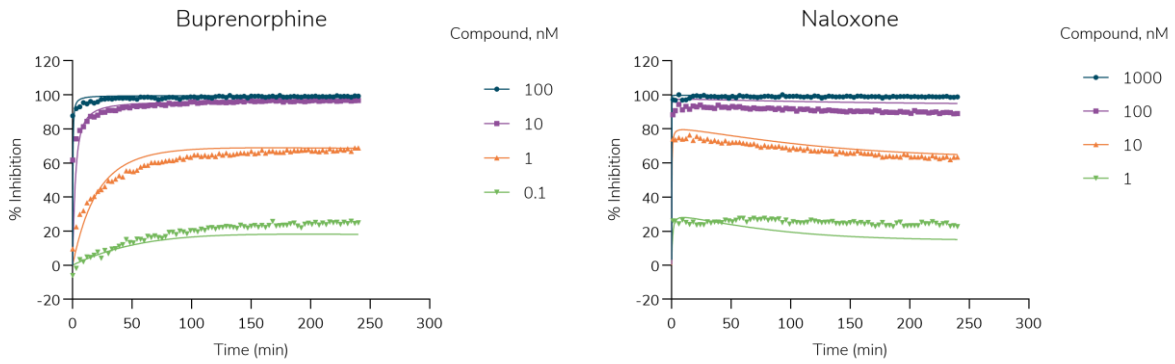

Figure 1. Binding kinetic profile of buprenorphine and naloxone against mu-opioid receptor using KINETICfinder®.

- The affinity and binding kinetic values obtained for the mu-opioid receptor agonists and antagonists with KINETICfinder® are in line with previous studies (Fig. 2).[18-21]
- Buprenorphine is a FDA-approved partial agonist for chronic pain and opioid dependence.
- Buprenorphine has high-affinity binding to the mu-opioid receptors (0.2 nM) and slow-dissociation kinetics ($2.0x10^{-4}$ s^{-1}). In this way, it differs from other full-opioid agonists like morphine and fentanyl, allowing respiratory depressant effect to be milder.
- Naloxone is a FDA-approved antagonist for the use in an opioid overdose and the reversal of respiratory depression associated with opioid use. It has 13-fold lower affinity binding (2.3 nM) for the mu-opioid receptors and rapid-dissociation kinetics ($2.4x10^{-2}$ s^{-1}).

	Buprenorphine	Naloxone
k_{on} ($M^{-1}s^{-1}$)	$1.1x10^{6}$	$1.1x10^{7}$
k_{off} (s^{-1})	$2.0x10^{-4}$	$2.4x10^{-2}$
τ (min)	82	0.7
$T_{1/2}$ (min)	56	0.5
K_d (nM)	0.2	2.3
IC_{50} (nM) [18-20]	0.5	2.5
$T_{1/2}$ (min) [21]	68	1.4

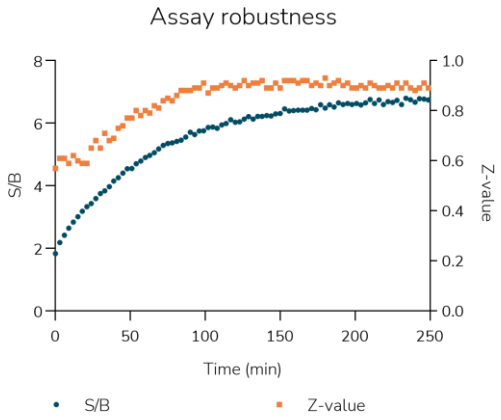

Figure 2. Graph: Signal-to-background and Z-values were calculated based on 32 control wells over 4 hours. **Table:** Association rate (k_{on}), dissociation rate (k_{off}), residence time (τ), half-life time ($T_{1/2}$) and affinity values obtained with KINETICfinder® and IC_{50} and $T_{1/2}$ values from literature.

References

1. Van der Velden W.J.C. Et al (2020) Perspective: Implications of Ligand-Receptor Binding Kinetics for Therapeutic Targeting of G Protein-Coupled Receptors. ACS Pharmacol Transl Sci. 3(2):179-189.
2. Nederpelt I. et al. (2017) From receptor binding kinetics to signal transduction; a missing link in predicting in vivo drug-action. Sci Rep. 26;7(1):14169.
3. Guo D. et al. (2018) Molecular Basis of Ligand Dissociation from G Protein-Coupled Receptors and Predicting Residence Time. Methods Mol Biol. 1705:197-206.
4. Manuel Grundmann M. et al. (2017) Temporal Bias: Time-Encoded Dynamic GPCR Signaling. Trends Pharmacol Sci. 38(12):1110-1124.
5. Lane R.L. et al (2017) A kinetic view of GPCR allostery and biased agonism. Nat Chem Biol. (9):929-937.
6. Herenbrink C.K. et al (2016) The role of kinetic context in apparent biased agonism at GPCRs. Nat Commun. 7:10842.
7. Hothersall D.J. et al (2016) Can residence time offer a useful strategy to target agonist drugs for sustained GPCR responses? Drug Discov Today 21(1):90-96.

8. Nordskov Gabe M.B. et al. (2020) Enhanced agonist residence time, internalization rate and signalling of the GIP receptor variant [E354Q] facilitate receptor desensitization and long-term impairment of the GIP system. Basic Clin Pharmacol Toxicol. 126 Suppl 6(Suppl 6):122-132.
9. Ramos I. et al. (2018) Abediterol (LAS100977), an inhaled long-acting β 2-adrenoceptor agonist, has a fast association rate and long residence time at receptor. Eur J Pharmacol. 819:89-97
10. Doornbos M.L.J. et al. (2017) Discovery and Kinetic Profiling of 7-Aryl-1,2,4-triazolo[4,3-a]pyridines: Positive Allosteric Modulators of the Metabotropic Glutamate Receptor 2. J Med Chem. 10;60(15):6704-6720.
11. Xia L. et al. (2017) Structure–Affinity Relationships and Structure–Kinetics Relationships of Pyrido[2,1-f]purine-2,4-dione Derivatives as Human Adenosine A3 Receptor Antagonists. J Med Chem. 14;60(17):7555-7568.
12. Hothersall J.D. et al. (2017) Structure-Activity Relationships of the Sustained Effects of Adenosine A2A Receptor Agonists Driven by Slow Dissociation Kinetics. Mol Pharmacol. 91(1):25-38.
13. Bosma R. et al. (2017) The Target Residence Time of Antihistamines Determines Their Antagonism of the G Protein-Coupled Histamine H1 Receptor. Front Pharmacol. 25;8:667.
14. Rosethorne E.M. et al (2016) Long Receptor Residence Time of C26 Contributes to Super Agonist Activity at the Human β2 Adrenoceptor. Mol Pharmacol. 89(4):467-75.
15. Louvel J. et al (2015) Kinetics of human cannabinoid 1 (CB1) receptor antagonists: Structure-kinetics relationships (SKR) and implications for insurmountable antagonism. Eur J Med Chem. 28;101:681-91.
16. Vilums M. et al (2015) Evaluation of (4-Arylpiperidin-1-yl)cyclopentanecarboxamides As High-Affinity and Long-Residence-Time Antagonists for the CCR2 Receptor. ChemMedChem. 10(7):1249-58.
17. Swinney D.C. et al. (2014) A study of the molecular mechanism of binding kinetics and long residence times of human CCR5 receptor small molecule allosteric ligands. Br J Pharmacol. 171(14):3364-75.
18. Huang P. et al. (2001) Comparison of pharmacological activities of buprenorphine and norbuprenorphine: norbuprenorphine is a potent opioid agonist. J Pharmacol Exp Ther. 297(2):688-95.
19. DeWire S.M. et al (2013) A G protein-biased ligand at the μ-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. J Pharmacol Exp Ther. 344(3):708-17.
20. Livingston K.E. et al. (2014) Disruption of the Na+ ion binding site as a mechanism for positive allosteric modulation of the mu-opioid receptor. Proc Natl Acad Sci USA. 111(51):18369-74.
21. Yassen A. et al (2007) Mechanism-based PK/PD Modeling of the Respiratory Depressant Effect of Buprenorphine and Fentanyl in Healthy Volunteers. Clin Pharmacol Ther. 81(1):50-8.