Investigating the influence of tracer kinetics on competition-kinetic association assays; identifying the optimal conditions for kinetic fragment screening

D. A. Sykes, S. J. Charlton. Cell Signalling Group, The University of Nottingham, Nottingham, UNITED KINGDOM.

Introduction

The importance of optimising drug-binding kinetics has led to an increase in the development and utilisation of assay-systems for measuring the kinetics of unlabelled compounds. One popular approach is the competition-association kinetic binding approach, first described by Motulsky and Mahan[1]. It is now accepted that a tracers kinetic characteristics can greatly effect the reliability of estimated kinetic parameters,[2] an obstacle to successfully introducing kinetic assays earlier in the drug discovery screening-cascade. Using a simulation approach we have identified the optimal tracer characteristics for determining the kinetics of unlabeled ligands typically encountered during the different stages of a drug discovery program (i.e. rapidly-dissociating eg. k_{off} = 100min^{-1} low-affinity "hits" through to slowly-dissociating eg. k_{off} = 0.01min^{-1} high-affinity "candidates").

Method

Monte Carlo simulations (200 per condition with an associated error of 1 SD) using an association kinetic binding model were performed in GraphPad Prism 6.0, with four model tracers, with off-rates ranging from 10-0.01 min^{-1}. For simulation purposes assay read start-time was fixed at either 1 sec to mimic online addition of membranes via injectors, or 30 sec to mimic the delay in time to read following offline addition. Read interval-time (i.e. the time between well-reads) was varied between 1-60 secs. Further simulations were performed using the competition-association kinetic binding model to assess our ability to determine the kinetics of unlabeled compounds in competition with the model tracers.

Results

For more rapidly dissociating unlabeled ligands (eg. k_{off} = 100min^{-1}) the key to obtaining accurate kinetic parameters is to employ a tracer with a relatively fast off-rate (eg. 10min^{-1}), utilizing online addition and a short read interval-time. Table 1 compares kinetic data obtained using online and offline addition protocols. Online addition also proved crucial for accurate parameter estimation of the most rapidly dissociating tracer examined (10 min^{-1}). The potential to impose strict timing constraints is largely governed by sample injection capability and the method of detection employed (eg. TR-FRET versus radiometric).

Table 1. *Summary of kinetic input and output parameters for a fragment-like compound using a rapidly-dissociating tracer (k_{on} 3E7M^{-1}min^{-1} and k_{off} 10min^{-1}) at a fixed read interval-time of 5sec.*

Assay start time (sec)	No of ambiguous fits : outliers per 200 stimulations	Input k_{on} (output k_{on}) M^{-1} min^{-1}	% CV	Input k_{off} (output k_{off}) : min^{-1}	%CV
1	0 : 0	1E5 (1.04E5)	20.5	100 (103.86)	20.6
30	191 : 5	1E5 (3.01E8)	209.6	100 (3.01E5)	209.6

Conclusion

The insight into tracer binding presented has consequences for experimental design strategy and provides a framework for the identification and testing of tracers necessary for profiling rapidly dissociating low-affinity competitors, e.g. fragments.

References

1. Motulsky and Mahan (1984). *Mol Pharmacol*. 25(1):1-9.

2. Klein-Herenbrink C *et al* (2016). *Nature Communications* 7, 10842.