High content biochemical screens, kinetic FRET assays with the Neonatal Fc Receptor

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Introduction: UCB has been working with the University of Nottingham to develop assays to simultaneously detect competition whilst measuring rate constants, for both the receptor-ligand and receptor-inhibitor interactions. This was achieved with using time-resolved FRET (TrFRET) and equations derived by Motulsky and Mahan, describing the effect of competitive inhibitors on the simultaneous equilibria of receptors and their ligands (1).

The aim is to increase throughput of kinetic measurements relative to SPR. In instances where interference is non-time dependent, such as fluorescent quenching, it is hoped that such assays may lower the incidence of false positives.

The Neonatal Fc Receptor (FcRn) was used as a model system. FcRn exhibits a pH specific interaction with IgG for which well characterised peptide inhibitors exist (2). These were profiled by kinetic FRET and compared to Biacore, as the current SPR gold standard. A small library of Pan Assay Interference Compounds (PAINS) were selected and used to probe the false positive rate (3).

Method: A TrFRET assay was created as follows: the extra cellular domain of FcRn was labelled with terbium chelate and human IgG1 was labelled with AlexaFluor 647 dye. FcRn was added to a 384-well plate, containing mixture of IgG and inhibitor. Time resolved FRET signal was measured kinetically, using a PHERAstar FS plate reader.

Results: Table 1. Comparison of kinetic FRET and SPR rate constants

	Kinetic FRET ***n=3 ± S.E.M***	**Biacore** ***n=1***
Ligand: Receptor kon (M^{-1} min^{-1})	1.67E+07 ± 4.18E+05	2.46E+07
Ligand: Receptor koff (min^{-1})	21.68 ± 0.48	40.8
Ligand: Receptor KD (µM)	1.30± 0.268	1.66
Peptide:Receptor kon (M^{-1} min^{-1})	4.97E+08 ± 0.38E+08	5.16E+07
Peptide:Receptor koff (min^{-1})	45.91 ± 5.27	2.187
Peptide:Receptor KD (nM)	92.1nM + 4.4nM	42.0

Conclusion: For FcRn, kinetic FRET provides a surrogate for SPR for deriving compound and protein kinetics in solution. The assay affords significantly higher throughput than SPR, with a sampling time

of 20 seconds per well. Our initial experiments with known PAINS indicate a low incidence of false positives, with only one compound giving a plausible inhibition profile.

References:

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3. Baell, J. B. & Holloway, G. A. (2010) J. Med. Chem. 53, 2719-2740.