Simple off-rate measurements

LanthaScreen® Eu Kinase Binding Assay

The vast majority of small-molecule drug discovery is currently driven by enzymatic assays or binding assays aimed at equilibrium binding measurements (e.g., IC_{50} and K_i values). However, the kinetics of compound binding, which are not addressed in typical equilibrium binding experiments, can have a profound impact on *in vivo* efficacy [1]. In particular, low k_{off} rates can contribute to longer residence times (compound bound to target), and this slow dissociation can lead to prolonged effects of the drug. With respect to kinase inhibitors, a slow k_{off} for Tykerb® as compared to the related EGFR inhibitors Tarceva® and Iressa™ has been proposed as an explanation for the longer-lasting effects of Tykerb® in cell culture.

invitrogen™

Currently, off rates for kinases can be measured by surface plasmon resonance (SPR)-based instruments (e.g., Biacore™) or by detailed enzyme kinetics experiments. While SPR yields highly quantitative data, it is a low-throughput platform and depends on costly instrumentation. Furthermore, it often involves covalent conjugation of protein targets to surfaces, which can lead to loss of protein function. Methods for obtaining k_{off} measurements using classical enzyme kinetics methods are established but are fairly labor-intensive.

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To address the need for a simple compound dissociation assay, we have investigated the utility of a TR-FRET binding assay platform to measure off rates (Figure 1). The basis of the LanthaScreen® Eu Kinase Binding Assay is an ATP-competitive tracer, which binds to the kinase of interest and is detected by a europium-labeled anti-tag antibody, also bound to the kinase of interest. When both tracer and antibody are bound, there is a high TR-FRET signal. When the tracer is displaced by a kinase inhibitor, there is a loss of the TR-FRET signal. Unlike enzyme activity assays, such measurements report binding events in real time.

Dissociation measurements

While the LanthaScreen® Eu Kinase Binding Assay has been used extensively for IC_{50} measurements, we sought to configure the assay in a manner that would allow us to observe dissociation of compounds followed by binding of the tracers. EGFR was chosen as a test case for which a slowly dissociating compound was already identified (Tykerb®) and for which a covalent (i.e., non-dissociating) compound was available (CL-387,785). While not necessary to determine off-rate kinetics, a covalent compound can serve as a useful reference or control. These experiments are carried out by first incubating the kinase with a saturating amount of inhibitor (Figure 2). This kinase/inhibitor complex is then diluted with a saturating concentration of tracer. Following dilution, the samples are read kinetically to monitor the change in signal over time. As the inhibitor dissociates and the tracer binds, the TR-FRET signal increases. Corresponding to literature reports [2], Tykerb® dissociated approximately 10 times more slowly than Iressa™ and Tarceva®, while staurosporine dissociated much more rapidly (Figure 3). As expected, the control covalent compound CL-387,785 remained bound to EGFR over the time course.

Conclusions

These studies demonstrate a rapid and simple means of measuring kinase inhibitor dissociation using the LanthaScreen® Eu Kinase Binding Assay.

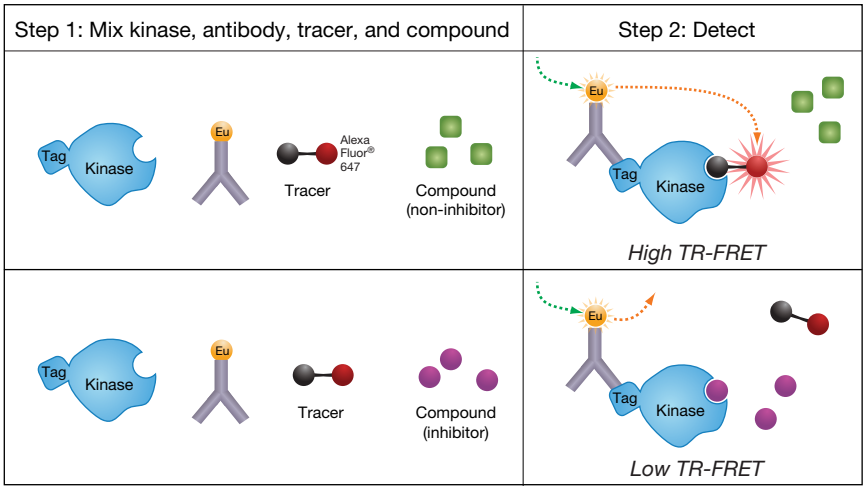


Figure 1. LanthaScreen® Eu Kinase Binding Assay schematic.

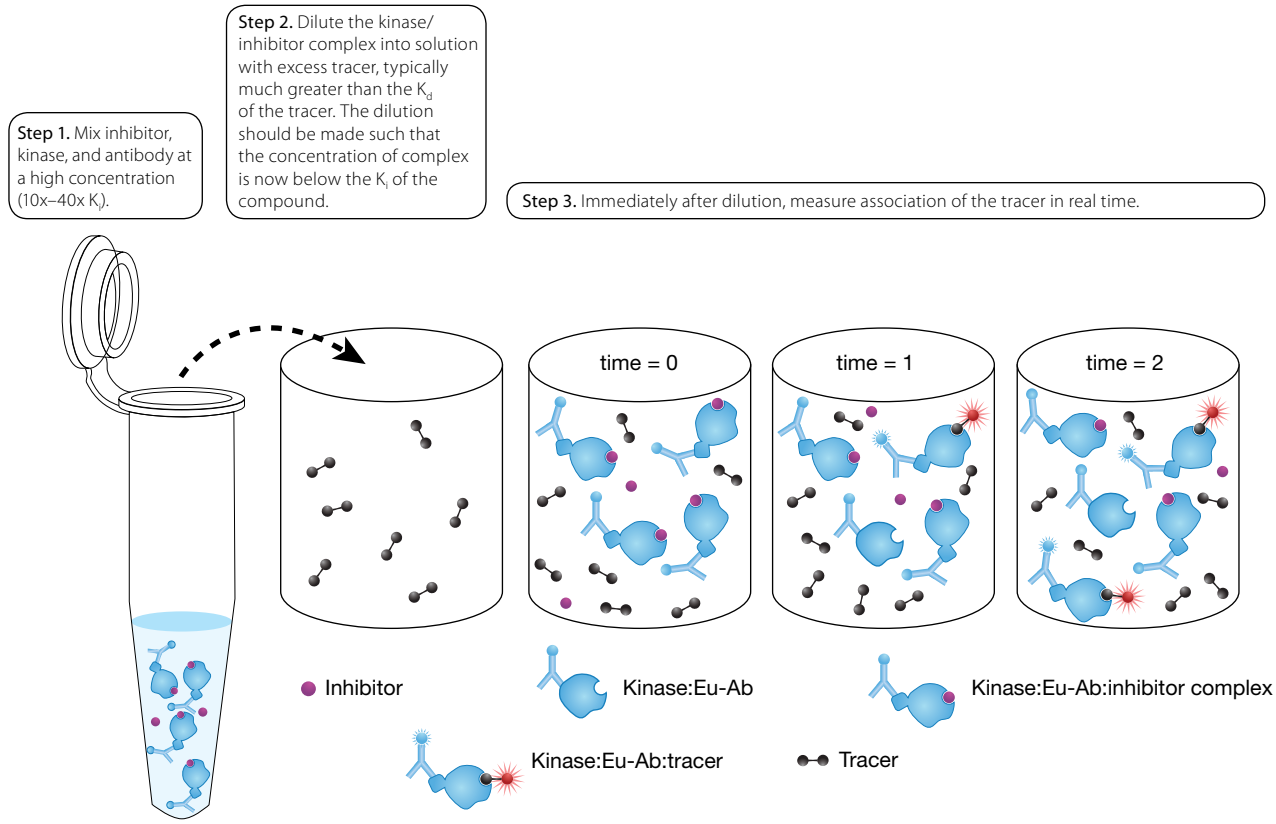
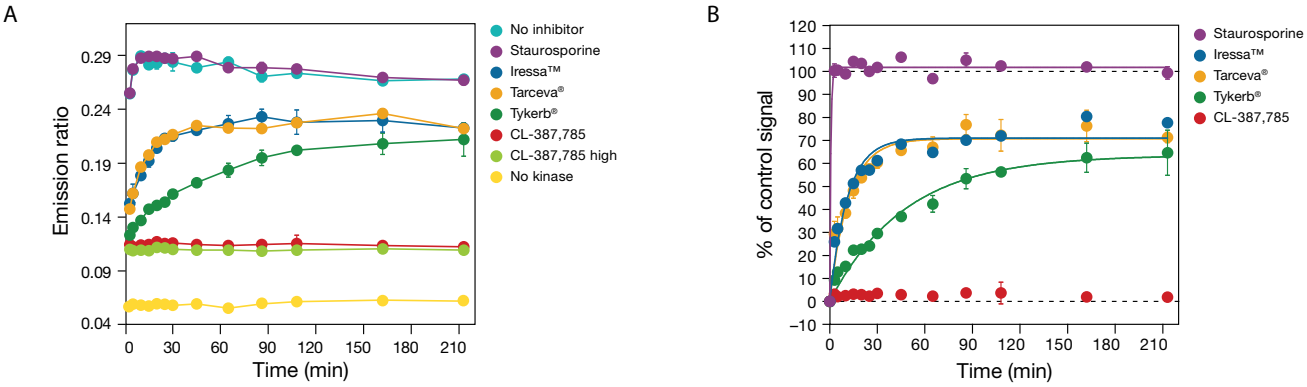


Figure 2. Basic steps in an off-rate experiment using the LanthaScreen® Eu Kinase Binding Assay.



Inhibitor	$t_{1/2}$ (min)	Apparent k_{off} (min^{-1})	Literature k_{off} (min^{-1})
Staurosporine	<3	>0.2	Not determined
Iressa™	5	0.14	>0.069
Tykerb®	49	0.014	0.0023
CL-387,785	Irreversible		Not determined

Figure 3. Off-rate measurements of EGFR. Several well-characterized inhibitors of EGFR were preincubated with the kinase for 30 min at concentrations such that they would provide >90% saturation of the kinase, or at a concentration equal to the concentration of the kinase, whichever was greater. The reactions were then diluted 33-fold in a saturating concentration of tracer and monitored over time. Raw data (**A**) were converted into percent of control signal and re-plotted (**B**). Apparent k_{off} values were determined, and they correlated well with literature values [2].

Ordering information

Product	Quantity	Cat. No.
5X Kinase Buffer A	4 mL	PV3189
Kinase Tracer 178	25 µL	PV5593
Kinase Tracer 199	25 µL	PV5830
Kinase Tracer 236	25 µL	PV5592
LanthaScreen® Eu-Anti-GST Antibody	25 µg	PV5594
LanthaScreen® Eu-Anti-His Antibody	25 µg	PV5596
LanthaScreen® Eu-Anti-DYKDDDDK Antibody	25 µg	PV6026
LanthaScreen® Eu-Streptavidin	25 µg	PV5899

To learn more about the LanthaScreen® Eu Kinase Binding Assay, visit www.invitrogen.com/bindingassay.

References

1. Copeland RA, Pompliano DL, Meek TD (2006) Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov* 5(9):730–739.
2. Wood ER, Truesdale AT, McDonald OB et al. (2004) A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res* 64(18):6652–6659.