High throughput drug residence time screening using "jump dilution" kinetics

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Abstract

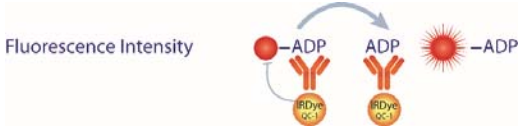
In drug discovery, in vitro assessment of compound affinity is often quantified by endpoint parameters such as IC_{50}. However, drug efficacy is also influenced by duration of the molecule binding to the target, or residence time. An increased residence time can confer longer pharmacological effect, a lower required efficacious dose, and potentially mitigate off-target risks. While surface plasmon resonance provides highly quantitative measurements of residence time, its low throughput is not amenable to profiling large sets of compounds. Here, we present the use of a "jump dilution" kinetic read that has been scaled up to characterize hundreds of molecules in a single screen. In this experiment, compounds are pre-incubated at concentrations of inhibitor and enzyme that favor drug-target binding. The reaction is then diluted to dissociate the drug from the target. Using the ADP² Assay with a fluorescence intensity readout, production of ADP can be monitored in real time, which directly correlates to recovery of kinase activity following a rapid dilution. These data can be used to triage compounds, estimate residence time, and select the most promising inhibitors for follow-up studies. Traditional jump dilution experiments rely on previously generated affinity data to dose each inhibitor. Here, we introduce a method to profile compounds at a single concentration by increasing the factor by which the enzyme is diluted. This type of experiment and the Transcreener® ADP² Assay's versatility generate a high value metric for lead molecule optimization.

Introduction

Jump dilution experiments are a widely utilized method for determining inhibitor reversibility and estimating off-rates, but traditional protocols involve manipulating drug concentration by a factor of its IC50. This is predicated on having prior endpoint data available for the study. In these experiments, we instead adjusted the enzyme concentration to cover a wide range of inhibitor affinities while keeping inhibitor concentration constant.

The Labcyte® Echo 555 instrument enables rapid and low volume transfer of aqueous solutions. By pre-incubating the enzyme and inhibitor reaction in an Echo-qualified source plate, a small volume from each well can be transferred to an assay plate. The enzyme-inhibitor complex is diluted 1000X and recovery of enzyme activity is observed by reading fluorescence intensity in kinetic mode. Using this method, over 350 inhibitors can be profiled in a single experiment.

Overview

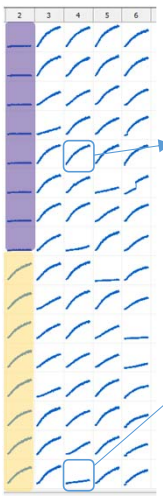


As ADP is generated in the reaction, a fluorescent tracer is displaced from a dye-conjugated monoclonal antibody. A fluorescence intensity readout enables rapid, real time detection of activity for any ADP-generating enzyme. Here, we studied potential inhibitors of a serine/threonine kinase.

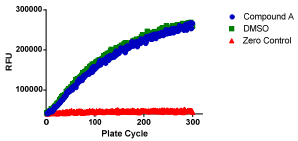
Binding
- 5 uL Enzyme-Inhibitor pre-incubation at 100x [I] and 1000x [E]

Dilution
- 20 nL aqueous transfer to assay plate by acoustic dispense

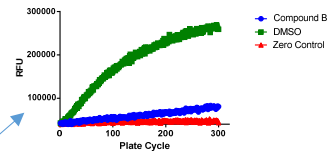
Read
- Dilute with buffer containing detection reagents and read plate



No enzyme control (purple) and DMSO only (yellow) are used as standards to quantify recovery of kinase activity.



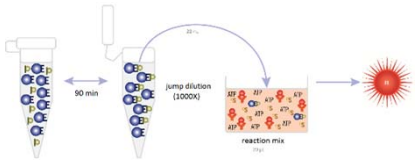
Kinase inhibitors with fast off-rates mirror the recovery rate of DMSO control.

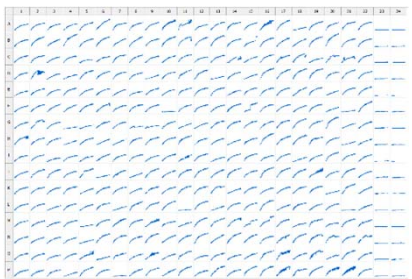


Several inhibitors with potentially long residence time are identified in the screen. Follow up studies can confirm and generate more sensitive measurements.

Methods

- Compounds from internal library were plated at a single concentration in an Echo® qualified source plate.
- Buffer solution with 1000X screening concentration of enzyme was added to all wells with automated liquid handler and incubated for 90 minutes,
- 20 nL of enzyme-inhibitor solution was replicated from the source plate to a 384-well assay plate.
- A buffer solution containing ATP, peptide substrate, DTT, cofactor, and Transcreener® ADP² reagents was added using an automated liquid handler, diluting the 1000X enzyme concentration down to 1X.
- The plate was read immediately in kinetic mode.
- Raw data was converted to a time course measurement. Potentially slowly dissociating inhibitors were identified with a formula that compares each compound's kinetics to control wells. Outputted well selections were mapped to internal compound name and verified manually.





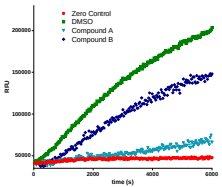
A full 384-well plate timecourse with controls

Results

For each inhibitor showing slow recovery of enzymatic activity, the rate of return after dilution was fit to the following equation:

$$[P] = v_s t + \frac{v_i - v_s}{k_{obs}}[1 - \exp(-k_{obs} t)]$$

where v_i and v_s are the initial and steady-state rates of the reaction in the presence of inhibitor, *k*obs is the apparent first order rate constant for the transition from v_i to v_s, and *t* is time.



Under the experimental conditions, *k*obs approximates the dissociation rate constant (k_{off}) of the enzyme–inhibitor complex, and therefore allows us to estimate the inhibitor residence time as $1/k_{obs}$

	IC_{50} (nM)	K_{obs} (1/s)	Residence time (min)
Compound A	13	0.0006	28
Compound B	13	> 0.1	< 1

Conclusions

- Residence time is a valuable metric in drug discovery for optimization of molecules
- Jump dilution experiments present a method to determine mode of action and estimate residence time for large sets of compounds.
- The Transcreener® ADP² FI assay is a highly sensitive readout for detecting ADP. Its continuous, real time capability enables kinetic reads, making it amenable to jump dilution analysis.
- Studies with additional targets and new methods for automating data treatment are in progress.

References

Copeland, Robert A. 2013. *Evaluation of Enzyme Inhibitors in Drug Discovery*. Second Edition. Hoboken, NJ: John Wiley & Sons, Inc.