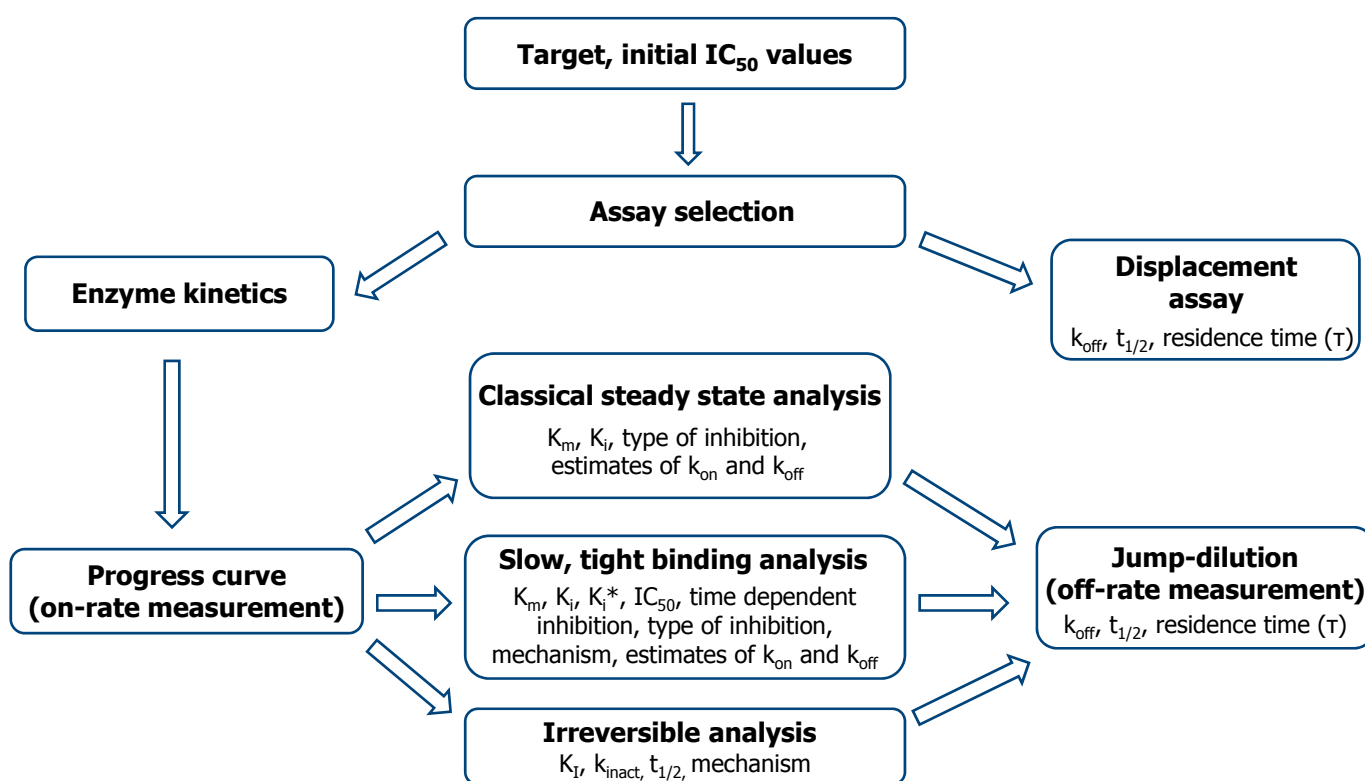


In vitro Pharmacology: Enzyme kinetics

- ❑ Beside potency data (IC_{50} or K_i) obtained from screening experiments, data obtained from kinetic studies are very important for appropriate understanding and interpretation of drug-target interaction.
- ❑ During lead optimization campaigns increased affinity of the compounds is very often driven by slow dissociation of the compounds from the target protein and consequently long residence time.
- ❑ Classification of compounds as a fast or slow reversible or irreversible compounds contributes to improvement of pharmacodynamics effects and mitigation of toxicity.
- ❑ At Fidelta, we are using **continuous enzymatic assays** for studying enzyme kinetics of various protein families.
- ❑ With measurement of both, **on-** and **off-rates**, we are able to determine various parameters such as **type of inhibition, reversibility, binding kinetics, k_{on} , k_{off} , $t_{1/2}$, residence time (τ), K_i and IC_{50} .**
- ❑ When it is technically feasible we can also develop **displacement assays** for determination of kinetic parameters (k_{off} , $t_{1/2}$, residence time (τ)).

Compound evaluation flowchart



Literature

- Copeland RA, Evaluation of enzyme inhibitors in drug discovery, 2nd ed., Wiley & Sons, 2013
- Swinney DC, Biochemical mechanisms of drug action: what does it take for success, Nat Rev Drug Discov, 2004, 3(9):801-808
- Copeland RA et al, Impact of enzyme concentration and residence time on apparent activity recovery in jump dilution analysis, Anal Biochem, 2011, 416:206-210

In vitro Pharmacology: Enzyme kinetics

Determination of kinetic parameters - examples

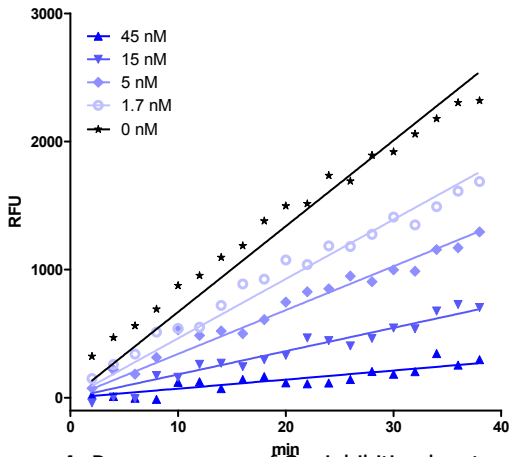


Figure 1. Progress curve of Src inhibition by staurosporine (rapid equilibrium kinetics)

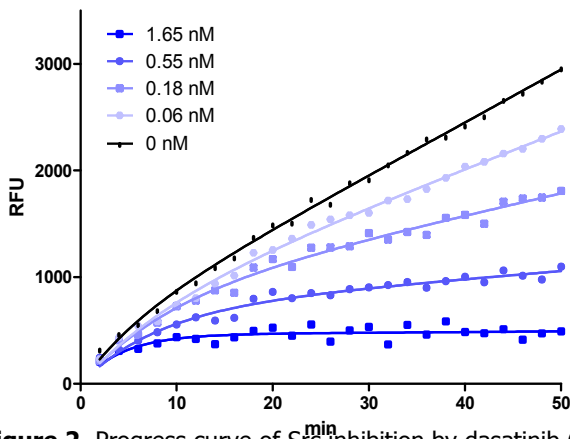


Figure 2. Progress curve of Src inhibition by dasatinib (slow binding kinetics)

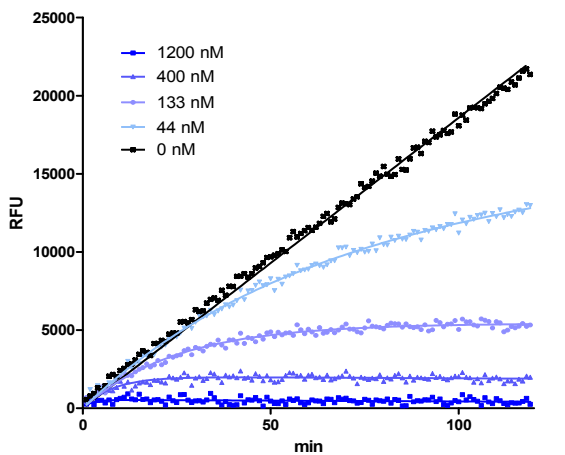


Figure 3. Progress curve of human leukocyte elastase inhibition by irreversible inhibitor

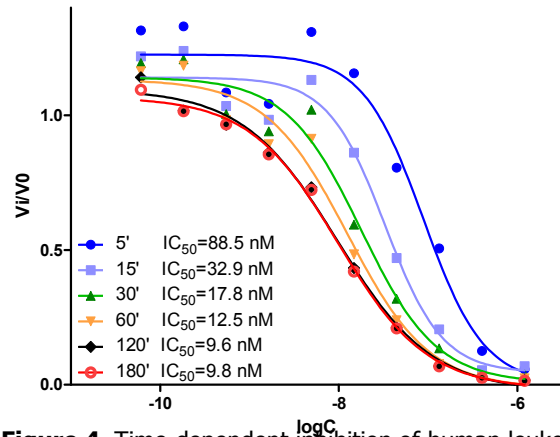


Figure 4. Time-dependent inhibition of human leukocyte elastase by ONO-5046

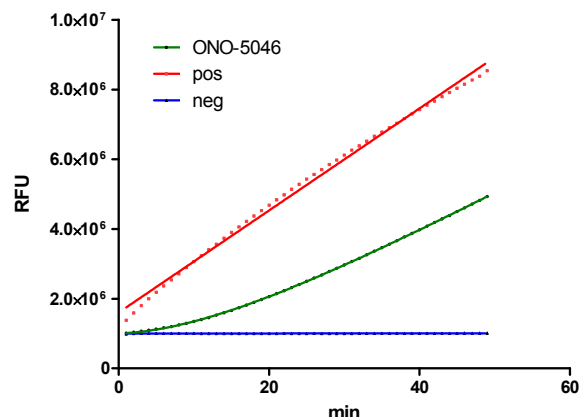


Figure 5. k_{off} determination for ONO-5046 with human leukocyte elastase using jump dilution analysis

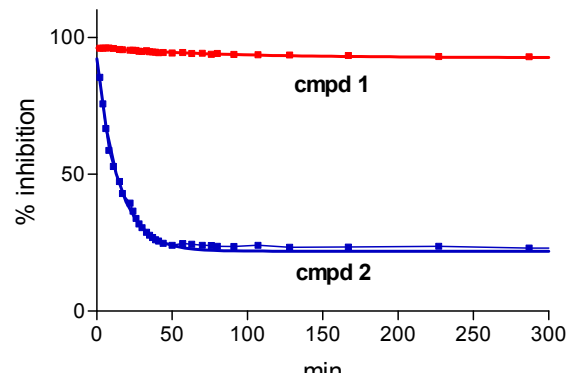


Figure 6. Displacement assay for p38a kinase with SKF-86002 as a tracer