

## scanMODE™

### Gain structural insights from biochemistry

#### Kinases: Conformationally Dynamic Proteins

Kinase conformational equilibria are often governed by the phosphorylation of key residues in regulatory elements, including the activation loop. Activation loop phosphorylation can shift the equilibrium to favor a “catalytically active-like” state characterized by a “DFG-in” structure at the loop’s N-terminus. Importantly, inhibitor binding can be conformation-specific and thus affected by the activation/phosphorylation state. For example, the ABL inhibitor Imatinib, which recognizes an “inactive DFG-out” kinase conformation, binds with high affinity to nonphosphorylated ABL but with reduced affinity to activated ABL phosphorylated on the activation loop.

#### Inhibitor Classification and Binding Mode

The majority of ATP-competitive kinase inhibitors are classified as either Type I or Type II. Although both Type I and II inhibitors generally contact the ATP binding site, only Type II inhibitors access an “allosteric site” unmasked in the inactive-DFG-out conformation. Consequently, Type II inhibitor binding can be significantly more activation state-dependent than Type I inhibitor binding. Examples of Type I and Type II inhibitors are listed in Table 1.

**Table 1. Select Type I and Type II Kinase Inhibitors**

Drug	Inhibitor Type	Primary Target	Status
Imatinib	II	ABL1	FDA-Approved
Nilotinib	II	ABL1	FDA-Approved
Dasatinib	I	ABL1	FDA-Approved
Sorafenib	II	VEGFR2	FDA-Approved
Gefitinib	I	EGFR	FDA-Approved
Erlotinib	I	EGFR	FDA-Approved

#### Characterization of Compound Binding Mode

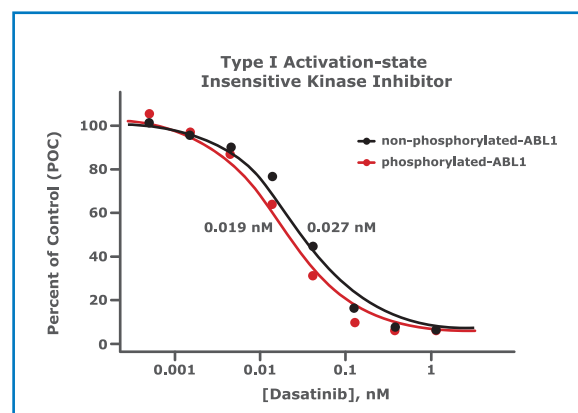
Activation state-sensitive (e.g. Type II) and insensitive inhibitors (e.g. Type I) are embodiments of related but distinct paradigms for ATP-competitive kinase inhibition. The binding mode can impact several key parameters in drug discovery, including enzyme inhibition kinetics, offsets between *in vitro* and cellular potency, nearest neighbor & kinome-wide selectivity, on target residence time & pharmacodynamics, interactions with upstream and downstream signaling molecules, and intellectual property position. Since the optimal inhibition paradigm is likely to be target-specific, it is essential to understand the binding mode of multiple leads at

program outset and during optimization. Furthermore, if the optimal binding mode is unknown *a priori*, a strategy to pursue two lead series with distinct binding modes can de-risk early lead selection decision making. However, binding mode determination can be difficult, time consuming, and expensive, often requiring the use of x-ray crystallography or *in silico* modeling.

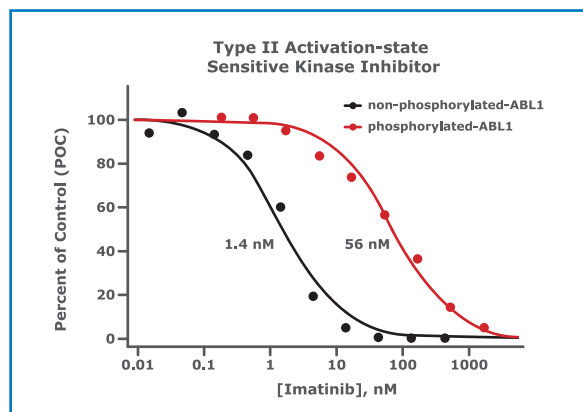
KINOMEScan now offers scanMODE, a novel biochemical tool that can simplify inhibitor binding mode elucidation. Comprised of a panel of phosphorylated/nonphosphorylated ABL assay pairs, scanMODE capitalizes on two key observations, which taken together, enable the use of ABL assay pairs to serve as surrogates to study inhibitor binding mode.

- An inhibitor’s binding mode is maintained across kinases (e.g. Imatinib is a Type II ABL inhibitor and a Type II LCK inhibitor).
- A significant fraction of kinase inhibitors have off-target affinity for ABL and/or clinically relevant ABL mutants.

#### Using scanMODE to explore inhibitor binding mode by measuring phosphorylation state-dependent affinity changes



**Figure 1.** Binding constant (Kd) determinations were measured for interactions between dasatinib, a known Type I inhibitor and ABL preparations differentially phosphorylated on the A-loop. Dasatinib exhibited no affinity preference for either nonphosphorylated state (Kd = 0.027 nM) or the phosphorylated state (Kd = 0.019 nM).



**Figure 2.** Binding constant (Kd) determinations were measured for interactions between imatinib, a known Type II inhibitor, and ABL preparations differentially phosphorylated on the A-loop. Imatinib exhibited a 30-fold affinity preference for the nonphosphorylated state (Kd = 1.4 nM) relative to the phosphorylated state (Kd = 56 nM).

#### Key references

- Liu, Y. and Gray, N. S. (2006). Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.* 2, 358-364
- Wodicka, L. et al. (2010). Activation State-Dependent Binding of Small Molecule Kinase Inhibitors: Structural Insights from Biochemistry. *Chem. Biol.* 17, 1241-9

**Table 2. scanMODE Panel (12 Kinases)**

KINOMEScan Gene Symbol	Entrez Gene Symbol	Accession Number
ABL1-nonphosphorylated	ABL1	NP_005148.2
ABL1-phosphorylated	ABL1	NP_005148.2
ABL1(F317I)-nonphosphorylated	ABL1	NP_005148.2
ABL1(F317I)-phosphorylated	ABL1	NP_005148.2
ABL1(F317L)-nonphosphorylated	ABL1	NP_005148.2
ABL1(F317L)-phosphorylated	ABL1	NP_005148.2
ABL1(H396P)-nonphosphorylated	ABL1	NP_005148.2
ABL1(H396P)-phosphorylated	ABL1	NP_005148.2
ABL1(Q252H)-nonphosphorylated	ABL1	NP_005148.2
ABL1(Q252H)-phosphorylated	ABL1	NP_005148.2
ABL1(T315I)-nonphosphorylated	ABL1	NP_005148.2
ABL1(T315I)-phosphorylated	ABL1	NP_005148.2

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