enzymlogic

KINETICfinder®: better by design

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The new 'Gold Standard' in kinetics

- A High-Throughput Screening platform that delivers all key binding kinetics (k_{on}, k_{off}, residence time) and affinity (K_d) parameters for reversible binders.
- Highly optimized patented TR-FRET assay.
- Quickly and at scale.
- Designed to enable kinetic parameters to be factored in to H2L and LO decision making.
- Offering all the advantages of the 'workhorse' SPR platform without the significant drawbacks.

Benefits

- HTS
- Accurate.
- Robust
- Reproducible.
- Sensitive.
- Broad dynamic range.
- Activated and non-activated targets.
- Rapid turnaround.

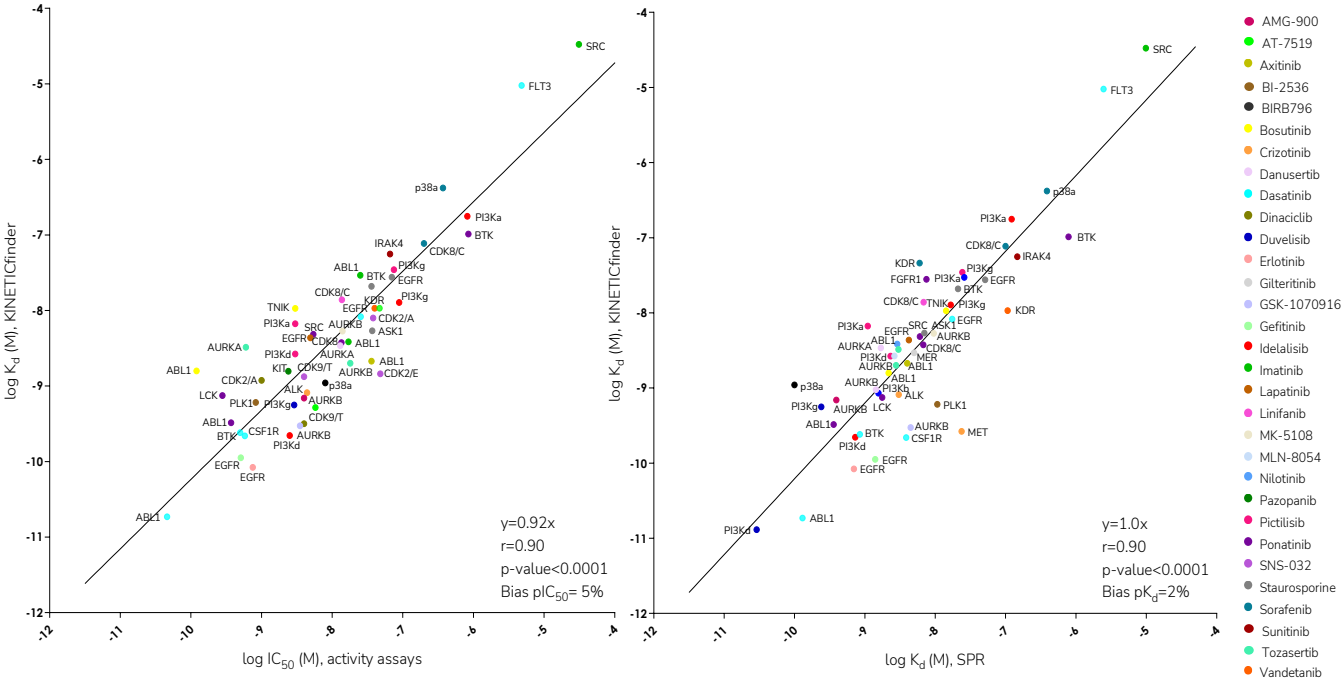
Applications

- Modify the on- and off-target kinetics (k_{on}, k_{off}, residence time and K_d).
- Speed Med-Chem iterations.
- Modulate the therapeutic index and safety profile.
- Understand PK/PD disconnects.
- Differentiate between similar therapies.

Reliable

In depth studies demonstrate a very high correlation between the K_d, k_{on} and k_{off} values obtained from our assays and the IC_{50} and kinetics values obtained from activity assays and SPR (Fig.1).

Good correlation with IC_{50} and SPR affinity measurements



Good correlation with SPR k_{on} and k_{off} measurements

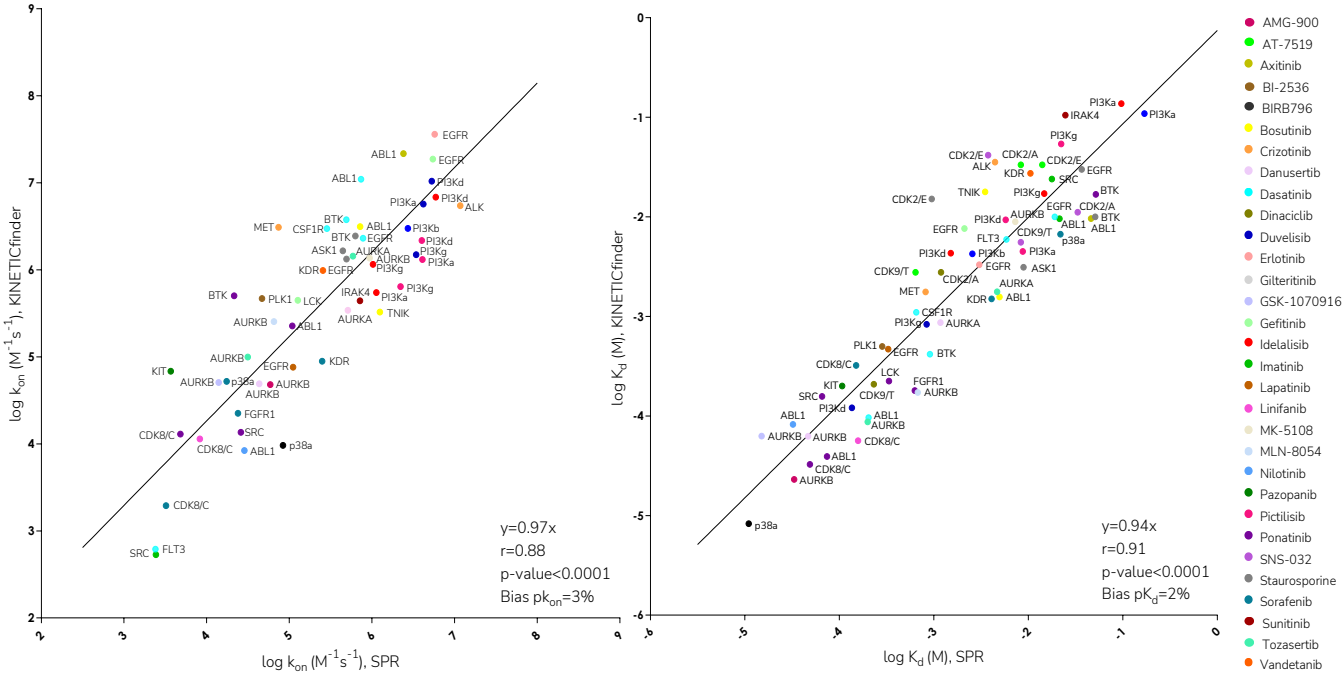


Figure 1. **KINETICfinder® is a reliable method.** Correlation between the K_d and kinetic constants of 31 inhibitors and 30 kinases obtained with KINETICfinder® and those obtained from end-point activity and SPR assays reported in the literature. The plots display the Pearson correlation coefficient (r), p-value and Bland Altman analysis (Bias).

Accurate

Large dynamic range for accurate affinity, k_{on} and k_{off} measurements

All our assays go through a rigorous establishment and validation process prior to use and ongoing assay performance is closely monitored to ensure they meet the highest of standards. KINETICfinder® provides precise and reproducible readouts, regardless of the magnitude of the values, in just a single assay (Fig.2).

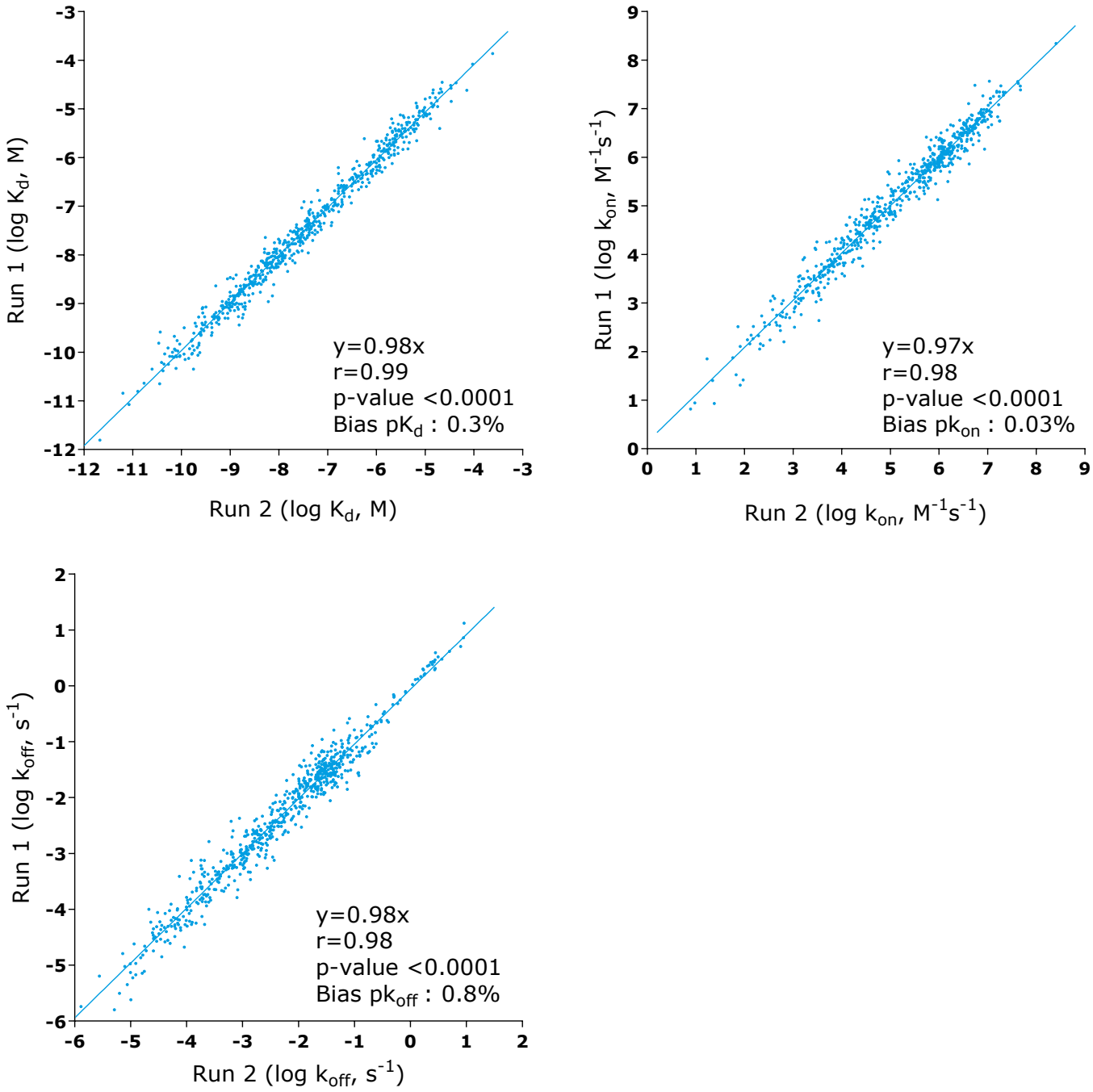


Figure 2. **KineticFinder® provides accurate results with a wide dynamic range**. k_d, k_{on} and k_{off} values of 110 inhibitors and 110 different kinases from two independent experiments (720 interactions total). The plots display the Pearson correlation coefficient (r), p-value and Bland Altman analysis (Bias).

Robust and reproducible

The high and sustained stability of KINETICfinder® guarantees superior quality results over time.

- Enables data to be interpreted with confidence (Fig.3).
- Outstanding reproducibility between plates and days (Fig.4).
- High Z-values ensure low false positive/negative rates.

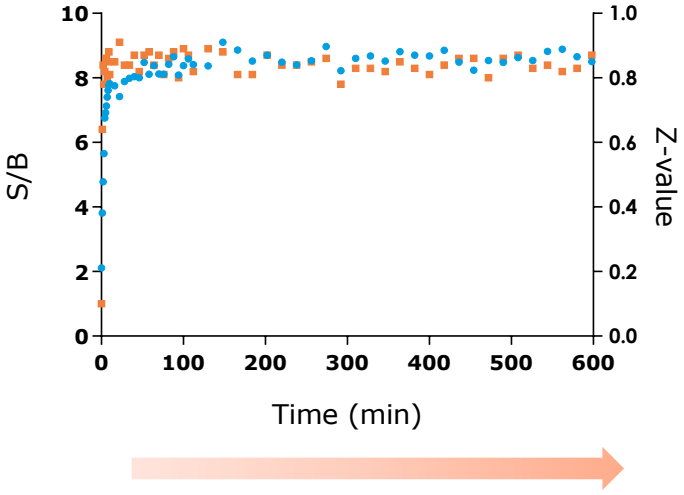


Figure 3. **Sustained TR-FRET signal stability enables data to be interpreted with confidence.** KINETICfinder® S/B (blue) and Z-values (orange) were calculated based on 32 control wells over 10 h.

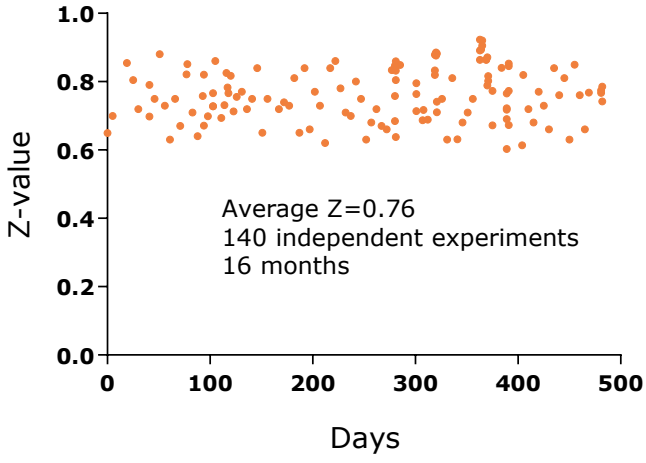
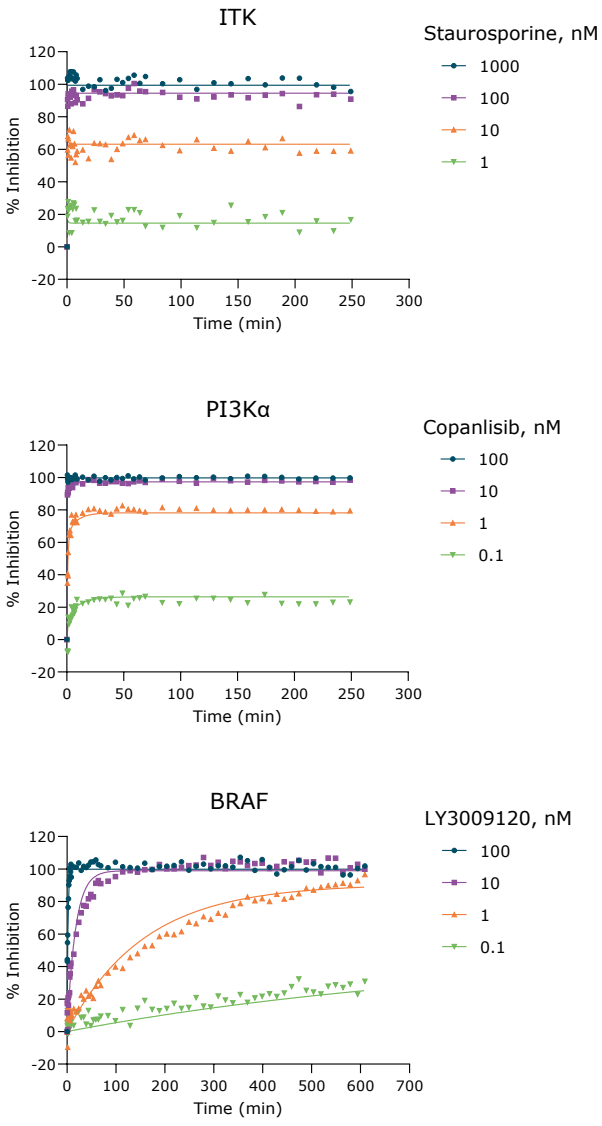


Figure 4. **Robust results over time.** KINETICfinder® shows outstanding robustness and reproducibility between plates over time.

Sensitive

KINETICfinder® use very low protein concentrations, precise TR-FRET readouts and continuous measurements that afford a broad dynamic range (Fig.5):

- K_d: mM-pM (8 log).
- k_{on}: 10^{1}-6×10^{7} $M^{-1}s^{-1}$ (7 log).
- k_{off}: 10^{-6}-0.5 s^{-1} (7 log).



	Staurosporine	Copanlisib	LY3009120
Target	**ITK**	**PI3Kα**	**BRAF**
k_{on} ($M^{-1}s^{-1}$)	$2.02\times10^{7} \pm 6.17\times10^{6}$	$2.89\times10^{7} \pm 9.53\times10^{5}$	$2.35\times10^{5} \pm 6.99\times10^{3}$
SE k_{on} (%)	30	3	3
k_{off} (s^{-1})	$4.64\times10^{-2} \pm 1.36\times10^{-2}$	$1.22\times10^{-3} \pm 5.33\times10^{-5}$	$9.85\times10^{-6} \pm 2.01\times10^{-6}$
SE k_{off} (%)	29	4	20
Residence time (min)	0.36	14	1692
K_d (M)	$2.29\times10^{-9} \pm 6.67\times10^{-11}$	$4.23\times10^{-11} \pm 9.66\times10^{-13}$	$4.18\times10^{-11} \pm 7.78\times10^{-12}$
SE K_d (%)	3	2	19

Figure 5. **KINETICfinder® can cover a wide dynamic range while keeping the standard error at a low level.** The table shows the low standard errors (SE) of fast associating drugs (Staurosporine, and Copansilib) and very fast (Staurosporine) or extremely slow (LY3009120) dissociating drugs. In all cases, affinities are accurately calculated with SE<20%.

Without complications

KINETICfinder® assays are performed in solution, benefiting clients who have experienced limitations when using biosensing technologies like SPR, stemming from complications due to target immobilization on the sensor chip (Table 1).

	KINETICfinder®	SPR
Suitable for	Soluble targets, multiprotein complexes, membranes or cells	Soluble targets
Method	Solution-based	Immobilization-based
Target activity	No issues	Possible loss
Target conformation	No issues	Possible change
Target stability	No issues	Potential limitation
Surface heterogeneity	No issues	Data hard to interpret
Nonspecific binding	Low	Potential limitation
Mass transport	No issues	Potential limitation
Sample handling	No issues	Subsequent re-runs
Sensitivity	High	Dependent on Molecular weight
Affinity range	0.001 nM–1 mM	0.1 nM–500 µM
Kinetic resolution	k_{on}: $6x10^{7}$-$1x10^{1}$ $M^{-1}s^{-1}$	k_{on}: $1x10^{6}$-$1x10^{3}$ $M^{-1}s^{-1}$
	k_{off}: $5x10^{-1}$-$1x10^{-6}$ s^{-1}	k_{off}: $1x10^{-1}$-$1x10^{-4}$ s^{-1}
Throughput	High	Medium
Protein demand	Low	High

Table 1. Comparison of KINETICfinder® and SPR methods for the determination of binding kinetics.

Rapid turnaround and cost effective

Quality data delivered on schedule

We are laser focused on rapid and reliable data delivery, with a keen eye on timelines and budgets.

- Standard data turnaround for study requests is 10 business days from compound receipt.
- Expedited turnaround is available for regularly scheduled ongoing screening programs.

We offer flexible solutions

Together we will define the best way to collaborate through:

- Ready-to-use or user-defined assays.
- User-defined screening concentrations.
- Bespoke assay development.
- Flexible service models to support your discovery goals and maximize program success rates.