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Dynamic Biomolecular Interaction Analysis
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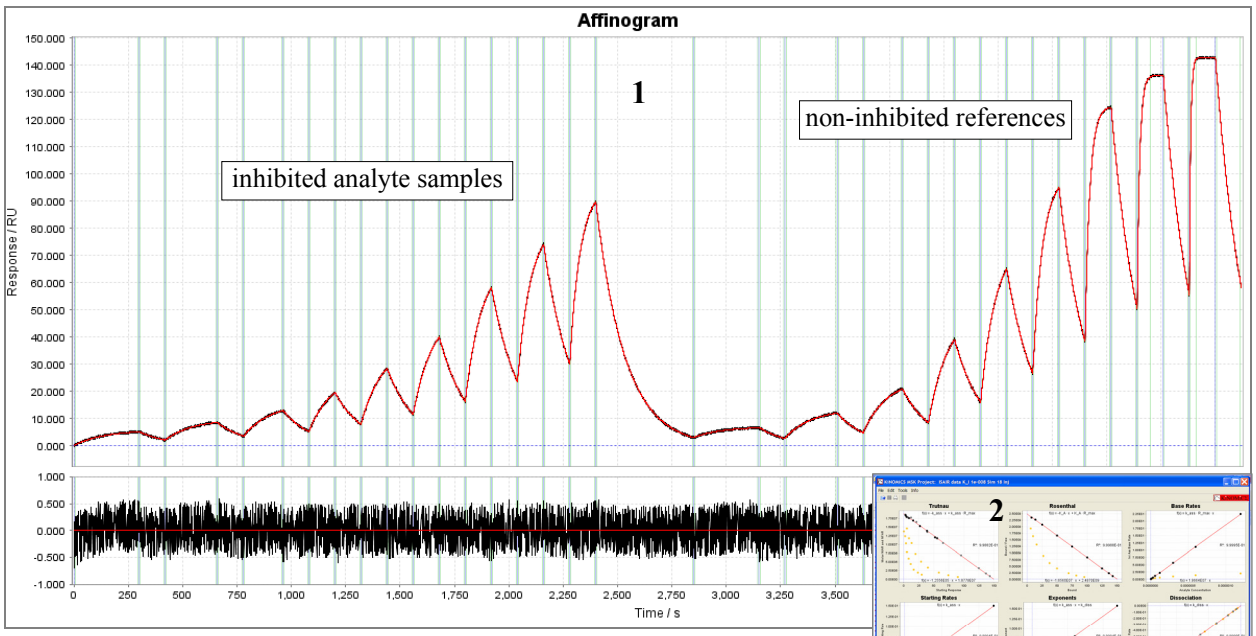
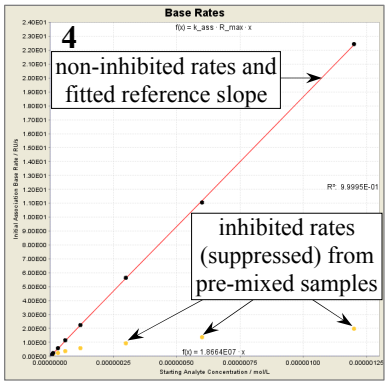
Kinomics MSK ISA Application Note

Inhibition-in-Solution Assay (ISA) utilizing Multi-Step Kinetics (MSK) on Biacore Systems



Determination of the inhibition constant K_I (*in solution*) between analyte A and inhibitor I that are mixed *prior* to the MSK study on a Biacore system where the equilibrated solutions are checked for the free analyte A against ligand L (immobilized on the sensor) with regard to non-inhibited analyte references in order to identify inhibitors of interest. A data simulation.



- Mix analyte:inhibitor (A:I = 1:1 reaction type) at different (≥5) concentrations; especially in biosensor capture assays, prepare non-inhibited analyte samples.
- Run MSK (w/o intermediate regenerations) with L immobilized (or captured):
- Vary contact times (allow low analyte concentrations to bind longer; see 1).
- Inject the ≥5 pre-mixed inhibited samples, each followed by a dissociation.
- In the same way, inject non-inhibited reference samples (right in 1); the # of these may be less or, in case the assay is highly reproducible, skipped at all.
- Non-linear fitting and evaluation of data with MSK software incl cross-checks:
- Import affinogram (black curves in 1) and fit by a single click within 2 s (red).
- Exclude inhibited samples from evaluation (*even so, they **are kept** calculated*; shown yellow in 2) and, if indicated, omit outliers of non-inhibited references.
- Evaluated references yield K_D of A:L (here $6 \cdot 10^{-8}$ mol L^{-1}) incl kinetics; the green traffic lights (3) indicate an excellently cross-checked reference result.
- K_I from initial association rates (*non-linearly* extrapolated back to baseline):
- This *true* initial base rate is *directly proportional* to the analyte concentration.
  In MSK from Kinomics, *this rate is readily available* for each single step!
- The fit of the non-inhibited samples' rate yields the reference slope (red in 4).
- From the suppressed rate of the inhibited pre-mixes (yellow dots in 4), their actual free A concentration is calculated with regard to the reference slope.
- Thus, the equilibrium concentration (c_{eq}) of relevant compounds (A, I, AI) in the pre-mixes are known: K_I is calculated according to the mass action law (5).
- *Intuitive*: The 50 % inhibition position of A (in 4) indicates the K_I at a glance or, in other words, strong inhibitors suppress the rates more strongly.
- In comparison with others, identify the inhibitor(s) of your interest.

Done. Fast, easy, and intuitive. It's unique – it's from Kinomics.

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Suppressed initial rate of inhibited pre-mixes with regard to reference

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Free analyte concentration $c_{eq}(A)$ and all other equilibrium concentrations of pre-mixes plus mass action law:
$K_I = c_{eq}(A) \cdot c_{eq}(I) / c_{eq}(AI)$

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Inhibition constant K_I of pre-mixes:
Mean $K_I = 9.8 \cdot 10^{-9}$ mol L^{-1}
StD = ± $8.9 \cdot 10^{-10}$ mol L^{-1}

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Select inhibitor(s) of your choice

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FAST, EASY, INTUITIVE:

Weak inhibitors suppress less A!

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