Irreversible Inhibition Kinetics

Automation and Simulation

Petr Kuzmič, Ph.D.
BioKin, Ltd.

1. **Automate** the determination of biochemical parameters
2. PK/PD **simulations** with multiple injections

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EGFR inhibition by covalent drugs

Schwartz, P.; Kuzmic, P. *et al*. (2014)

"Covalent EGFR inhibitor analysis reveals
importance of reversible interactions
to potency and mechanisms of drug resistance"

Proc. Natl. Acad. Sci. USA. **111**, 173-178. Issue 1, January 7

PRACTICAL CHALLENGES:

Outlier rejection

Certain "defective" progress curves were **manually** excluded from analysis.

Initial estimates

Suitable initial estimates of rate constants were discovered by **trial and error**.

This "manual" method is not ideally suited for routine production environment.

Full automation: Five passes through raw data

Piecewise linear fit:
Eliminate "defective" progress curves

↓

"Local" algebraic fit of reaction progress:
Determine offsets and initial rates

↓

Algebraic fit of initial rates:
Determine $K_i^{(app)}$ for initial non-covalent complex

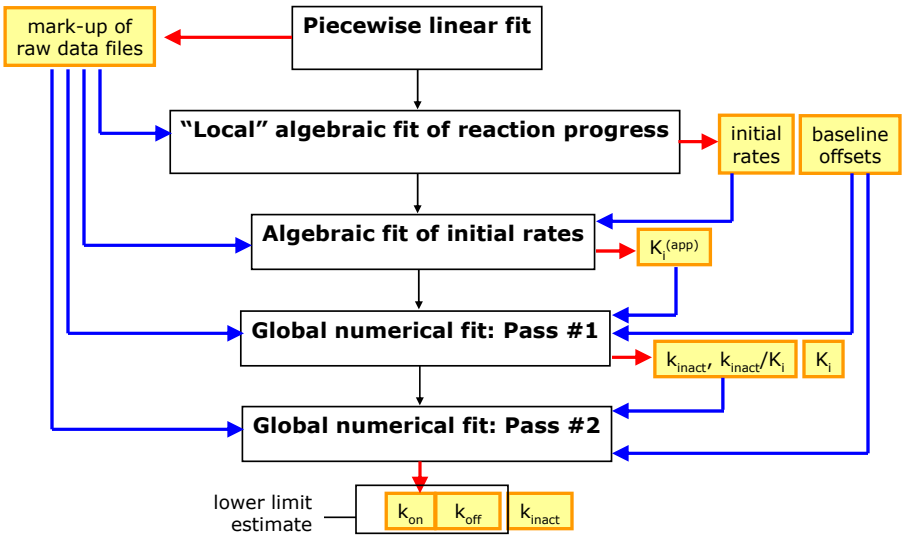
↓

Global numerical fit of reaction progress: Pass #1
Determine k_{inact}, K_i, and k_{inact}/K_i under rapid-equilibrium approximation

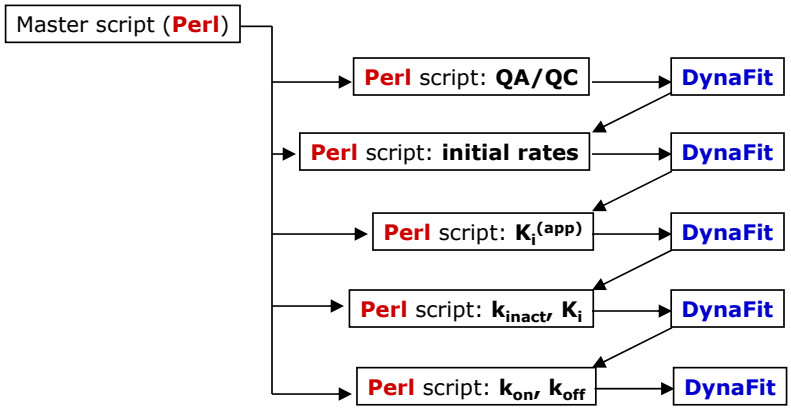
↓

Global numerical fit of reaction progress: Pass #2
Estimate lower limits for k_{on} and k_{off} under steady-state approximation

Full automation: Sharing of intermediate results



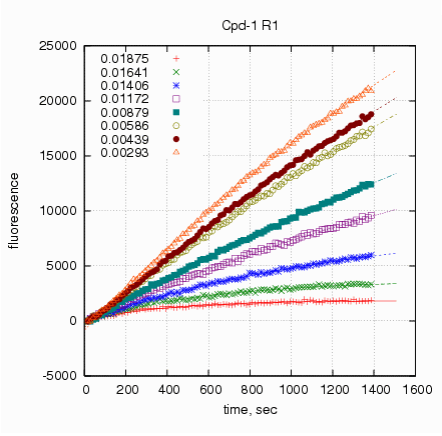
Full automation: Implementation - *Scripting*

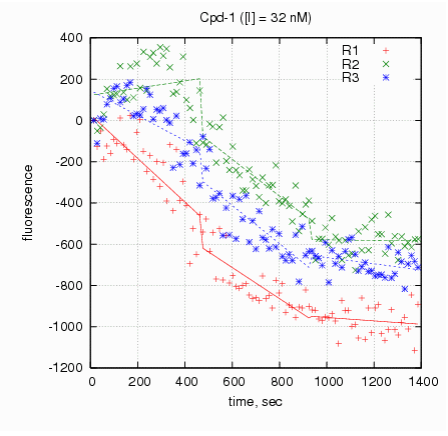


Quality control of raw data: Piecewise linear fit - Method

1. Fit progress curves to three linear segments.
2. Examine the linear slopes in each segment.
3. If the slope in either the second or the third segment is negative reject the entire progress curve.
4. Reject also corresponding curves from remaining replicates.

Quality control of raw data: Piecewise linear fit - Results





Accept

Reject

Quality control of raw data: Piecewise linear fit - Summary

Compound	I_{12}	I_{11}	I_{10}	I_9	I_8	I_7	I_6	I_5	I_4	I_3	I_2	I_1
Afatinib	.	.	.	.	.	.	.	.	.	.	.	.
CI-1033	X	X	.	.	.	.	.	.	.	.	.	.
CL-387785	X	.	.	.	.	.	.	.	.	.	.	.
Cpd-1	X	X	X	.	.	.	.	.	.	.	.	.
Cpd-2	X	X	.	.	.	.	.	.	.	.	.	.
Cpd-3	.	.	.	.	.	.	.	.	.	.	.	.
Cpd-4	.	.	.	.	.	.	.	.	.	.	.	.
Cpd-5	.	.	.	.	.	.	.	.	.	.	.	.
Dacomitinib	X	.	.	.	.	.	.	.	.	.	.	.
Neratinib	.	.	.	.	.	.	.	.	.	.	.	.
WZ-4002	X	.	.	.	.	.	.	.	.	.	.	.

Table 2.1: Acceptance results for inhibitor concentrations. "X" means that the given concentration was rejected for all three replicates. For detailed explanation see text.

NOTE: Each assay will require its own of set of heuristic QA/QC rules!

Local algebraic fit to determine initial rates - Method

Fit fluorescence vs. time to an exponential equation

$$F = F_0 + r_P[P]$$

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

F … fluorescence signal at time *t*
F_0 … instrument baseline
r_P … concentration-to-signal scaling parameter
[P] … product concentration at time *t*

t … time
v_i … initial reaction rate
k_{obs} … first-order rate constant

Reused in subsequent steps of the fully automated system

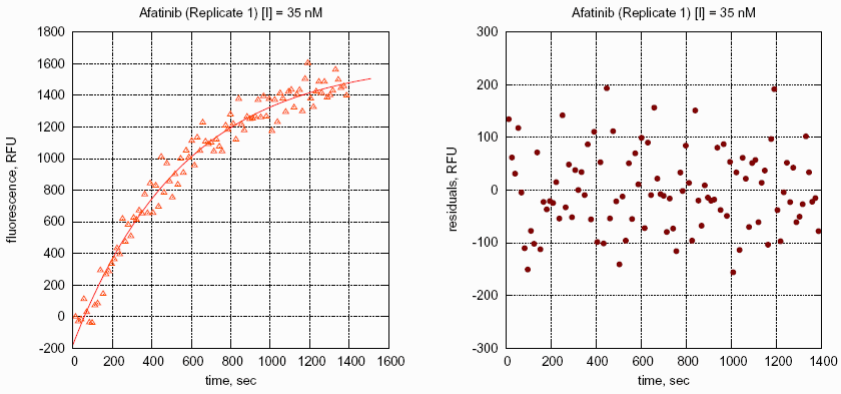
Local algebraic fit to determine initial rates - Results

reused

$$F_0 = (-179 \pm 31) \quad \text{RFU}$$
$$v_\text{i} = (3.23 \pm 0.18) \quad \text{RFU/sec}$$

ignored

$$k_\text{obs} = (0.0018 \pm 0.0001) \quad 1/sec$$



Algebraic fit of initial rates - Method

"Morrison equation" for tight-binding enzyme inhibition:

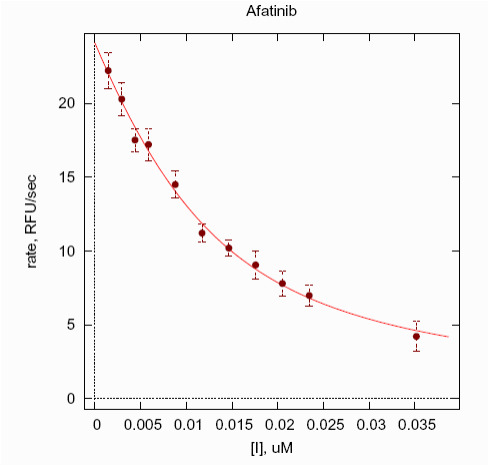
$$v_\text{i} = V_0 \frac{[E]_0 - [I]_0 - K_\text{i} + \sqrt{([E]_0 - [I]_0 - K_\text{i})^2 + 4\,[E]_0\,K_\text{i}}}{2\,[E]_0} \qquad (4.5)$$

symbol	unit	significance	note
v_i	RFU/sec	observed initial rate	dependent variable
V_0	RFU/sec	initial rate at $[I] = 0$	adjustable parameter
$[E]_0$	M	enzyme concentration	adjustable parameter
K_i	M	apparent binding affinity	adjustable parameter
$[I]_0$	M	inhibitor concentration	independent variable

A little twist:

Optimize $[E]_0$ but only within a narrow range (up to $[E]_{nominal}$).
See Kuzmic P., *et al.* (2000) *Anal. Biochem.* **286**, 45-50.

Algebraic fit of initial rates - Results



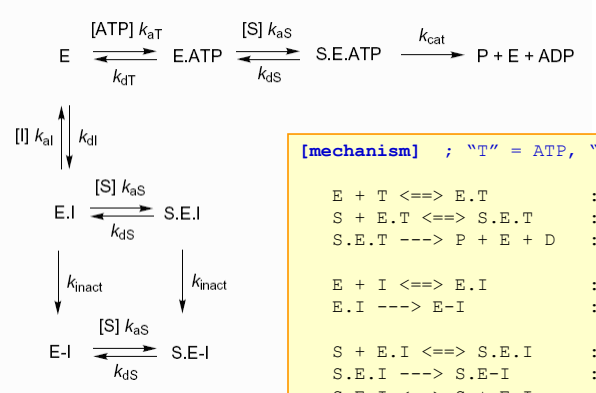
$K_i^{(app)}$ = (6.3 ± 0.8) nM

Used to make the initial estimate of **$k_{(off)}$** in global fit of progress curves

$k_{(off)} = K_i^{(app)} \times k_{(on)}$

Global fit of reaction progress - Method

"Generalized mechanism" (no longer simplified "Hit-and-Run" model):

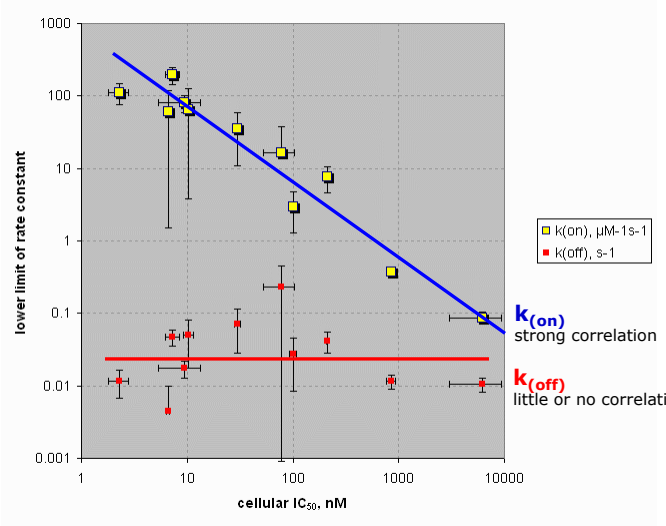



```
[mechanism]  ; "T" = ATP, "D" = ADP

   E + T <==> E.T          :      kaT    kdT
   S + E.T <==> S.E.T      :      kaS    kdS
   S.E.T ---> P + E + D    :      kcat

   E + I <==> E.I          :      kaI    kdI
   E.I ---> E-I            :      kinact

   S + E.I <==> S.E.I      :      kaS    kdS
   S.E.I ---> S.E-I        :      kinact
   S.E-I <==> S + E-I      :      kdS    kaS
```

DynaFit notation

Global fit of reaction progress - Results

Correlation of **biochemical** rate constants with **cellular** potency



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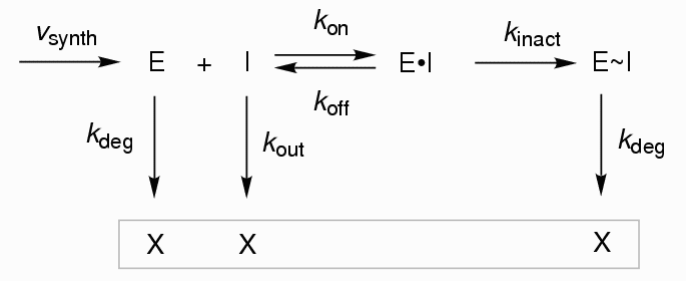
Possible cellular mechanism

REALISTIC PK/PD MODEL MUST ACCOUNT FOR METABOLISM OF PROTEIN AND DRUG MOLECULES

protein re-synthesis



protein degradation drug elimination protein degradation

Possible cellular mechanism in DynaFit software

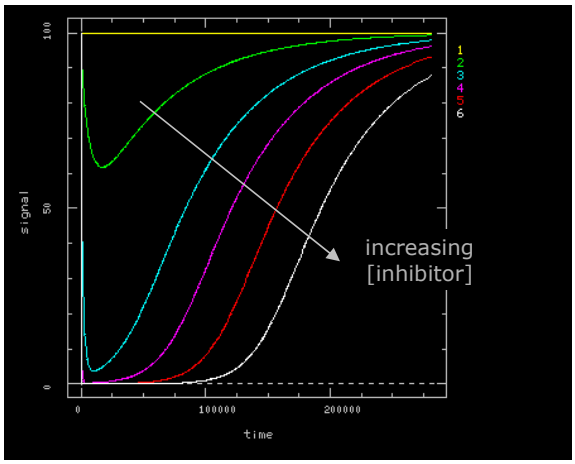
DYNAFIT USES "SYMBOLIC" REPRESENTATION OF ARBITRARY MOLECULAR MECHANISM

Example DynaFit input:

```
[task]

   task = simulate
   data = progress

[mechanism]

   E + I <==> E.I    :    kon      koff
     E.I ---> E~I    :    kinact

       I ---> X      :    kout
         ---> E      :    ksyn
       E ---> X      :    kdeg
     E~I ---> X      :    kdeg

...
```

DynaFit simulation output: Afatinib – strong inhibitor

target concentration, %



Afatinib:

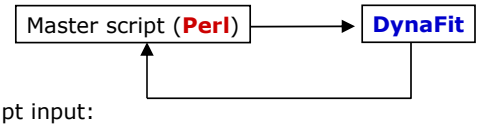
```
kon    = 18
koff   = 0.044
kinact = 0.0024
```

time, seconds (total = 72 hours)

Simulate multiple injections - Method

1. Set initial concentrations of [Enzyme] and [Inhibitor]
2. Run a DynaFit simulation for one injection
3. Record concentrations at the end of the run
4. Increase [Inhibitor] concentration by next injection amount
5. Set initial concentrations to the final values (after adjusting [I])
6. Go to step #2 above

Multiple injections: Implementation - *Scripting*

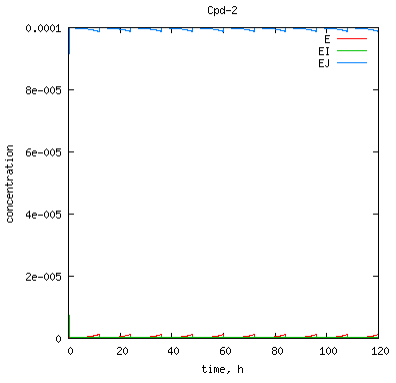


Master script input:

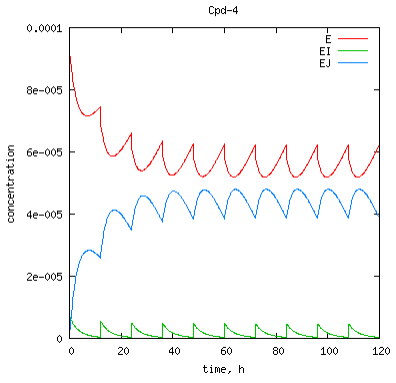
```
kon    = 198.954            ; binding
koff   = 0.0472361          ; dissociation
kinact = 0.0016792          ; covalent inactivation
kelim  = 0.0000641803       ; 3 h drug half-life
kpsyn  = 0.000000001605     ; 0.0001 uM per 12 h * ln(2)
kpdeg  = 0.00001605         ; 12 h protein half-life

E  = 0.0001
EI = 0
EJ = 0
I  = 0.01
ReinjectI = 0.01

Mesh        = linear from 0 to 43200 step 600  ; 12 hours total
Injections  = 10
...
```


Multiple injections: Results

simulate 10 injections @ 12 hours each:



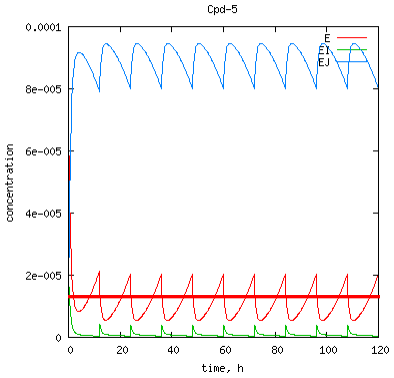
Compound 2:
strong inhibitor



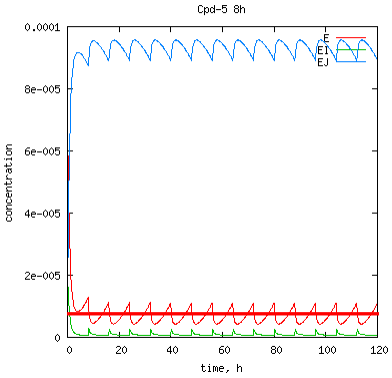
Compound 4:
weak inhibitor

Multiple injections: Results – Increase injection frequency

Compound 5:
intermediate inhibitor



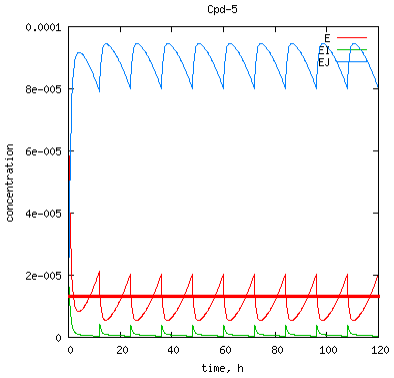
inject every **12 hours**



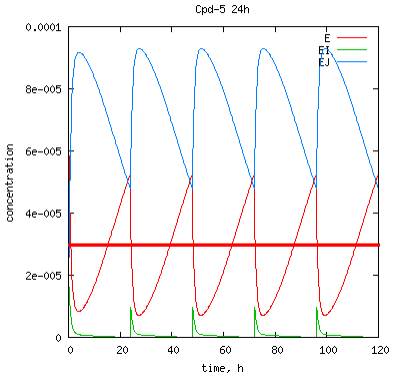
inject every **8 hours**

Multiple injections: Results – Decrease injection frequency

Compound 5:
intermediate inhibitor



inject every **12 hours**



inject every **24 hours**

Simulating multiple injections: Summary and conclusions

IMPLEMENTATION:

- DynaFit does not have to be enhanced or modified to do PK/PD simulations
- PK/PD module can be implemented as a simple **Perl script**
- Perl scripts are simple text files: can be modified by any programmer

RESULTS (not shown):

- Association ("on") rate constants are very important for PK/PD outcome
- Dissociation ("off", "residence time") rate constants appear less important

CAVEAT: Highly reliable values for "on" / "off" rate constants are needed!