Determination of k_{inact} and K_{i} for covalent inhibition using the Omnia® assay

BioKin Technical Note TN-2015-02

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Abstract

This document is an accompaniment to a published report [Schwartz *et al.* (2014) *Proc. Natl. Acad. Sci. USA* **111**, 173–178] on covalent inhibition the EGFR kinase. It describes in detail the raw experimental data that were used to generate the list of K_{i} and k_{inact} values specifically for the double mutant EGFR-L858R/T790M enzyme. The document also provides links to all theoretical model description files utilized within the DynaFit software package to analyze the raw experimental data. The data and the DynaFit "script" (i.e., model description) files are made part of a fully automated demonstration package.

Key words: enzyme kinetics; mathematics; covalent inhibition; EGFR kinase

Contents

1. Introduction

1.1. Background: covalent enzyme inhibition

Many if not most medicines in use today are enzyme inhibitors. Therapeutic enzyme inhibitors can be classified into two fundamental classes, noncovalent (reversible) and covalent (irreversible). Proper understanding of detailed molecular mechanisms governing the *in vitro* kinetic properties of inhibitors, both covalent and non-covalent, is essential for predicting and interpreting their physiological and pharmacological effects.

Covalent enzyme inhibitors, including widely used drugs such as Aspirin, achieve their overall inhibitory effect in a sequence of two separate steps. In the first reversible step the inhibitor binds non-covalently, forming an initial non-covalent complex. The overall strength of the binding affinity is usually measured by the *inhibition constant*, K_i, which could be thought of as the equilibrium dissociation constant of this initial complex. In the second irreversible step the inhibitor forms a covalent chemical bond with the target enzyme, resulting in a covalent conjugate. The chemical reactivity of the inhibitor is usually measured by the *inactivation rate constant*, k_inact, which is the rate constant for the conversion of the initial complex to the final covalent complex.

For various technical reasons that are beyond the scope of this brief note, researchers involved in the discovery and evaluation of therapeutic enzyme inhibitors frequently rely for guidance mainly on the *ratio* of the above fundamental characteristics, $k_\mathrm{inact}/K_\mathrm{i}$. Indeed more highly reactive inhibitors (higher k_inact) or more tightly bound inhibitors (lower K_i) will produce higher $k_\mathrm{inact}/K_\mathrm{i}$. However, the subject of this note is the determination of k_inact and K_i as distinct biochemical characteristics. In full generality, this is a mathematically challenging problem if the inhibitors are "tight binding" under the condition of the assay [1–3].

This document is intended as an accompaniment to ref. [4], which describes – among other results – the determination of inhibition constants (K_i) and inactivation rate constants (k_inact) for a series of covalent inhibitors of the epidermal growth factor receptor (EGFR) kinase enzyme. The original report is available for download from PubMed Central[1]. An extensive Supporting Information document is available from the same source or alternatvely from BioKin Ltd[2].

1.2. About this document

The main purpose of this Note is to provide three distinct types of information to all interested students and researchers in enzyme kinetics:

1. **Raw data.** This document provides a web link to the raw experimental data that were utilized to generate the list of K_i and k_inact published in Table 1 (p. 175) of the published report [4].
2. **Theoretical model.** We also provide a detailed definition of the mathematical model that was utilized for the kinetic analysis, as well as relevant model description files ("script" files) for the software package DynaFit [5, 6].
3. **Certified model parameters.** A detailed list of all best-fit model parameters for each EGFR inhibitor in the compound collection is provided. This will allow interested parties to compare the DynaFit results reported here with results possibly obtained by using alternate software packages.

```
X:\...\dynafit-TN201502
+---proj
|   \---EGFR
|       \---L858R-T790M
|           +---inhib
|           |   +---Afatinib
|           |   |   +---data
|           |   |   +---R1
|           |   |   |   \---data        <=== RAW DATA
...         .   .   .
|           +---scripts
|           |   +---01-plot-raw-data
|           |   |   +---results
|           |   |   \---templates
|           |   +---02-exclude-outliers
|           |   |   +---results
|           |   |   \---templates
...         .   .   .
|           |   \---system
|           |       \---html
|           \---raw-data               <=== RAW DATA: ARCHIVED
\---system
    +---DynaFit
    |   \---template
    |       +---eps
    |       +---gnuplot
    |       +---html
    |       +---tex
    |       \---txt
    \---style
```

Figure 1: Layout of subdirectories in the distributed software-plus-data bundle.

The raw experimental data associated with ref. [4] were made a part of a larger software-plus-data package including DynaFit. This package allows all interested parties to reproduce the published kinetic analysis starting from the raw data and proceeding (through a series of intermediary steps) to a final list of k_{inact} and K_{i} values for all 11 kinase inhibitors mentioned in Table 1 of the original report [4].

DOWNLOADING INSTRUCTIONS

To download the entire data-plus-software package, follow these steps:

1. Point your www browser to `http://www.biokin.com/TN/2015/02/`.
2. Follow the downloading and archive-extraction information found there.

The data-plus-software bundle distributed here in principle allows all interested parties to analyze their own inhibition data. The only requirement to accomplish this is to modify the

[1] `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3890870/`
[2] `http://www.biokin.com/publications/technotes/pdf/Schw1473-SI.pdf`

"settings" files distributed with the demonstration package. Detailed instructions on how to accomplish this modification are available from the author.

After extracting the downloaded ZIP archive file, to top-level extracted directory will be named DynaFit-TN201502. The layout of subdirectories is shown in *Figure 1*. The annotated raw experimental are located in the subdirectory inhib. Another archive copy of the raw experimental data is located in the subdirectory raw-data.

2. Raw experimental data

The raw experimental data are being made available specifically for enzyme kinetic experiments concerning the EGFR-L858R/T790M double mutant enzyme. The experimental conditions were as follows.

> Fluorometric assays were performed by using the Omnia® continuous fluorometric kinase assay system (Invitrogen, Carlsbad, California) using peptide Y-12, a fluorogenic tyrosine phosphoacceptor peptide modified with a chelation-enhanced fluorophore (cSx) coupled to a cysteine residue, Ac-EEEEYI(cSx)IV-NH_2. Phosphopeptide formation was monitored in 50 μL reactions in 96-well plates with a Tecan Safire II microplate reader in fluorescence mode using 360 nm excitation and 485 nm emission wavelengths. Reactions were comprised of 12 mM free $MgCl_2$, 0.2 mM TCEP, 13 μM peptide-cSx, 800 μM ATP, 150 mM NaCl, and 0.01% Tween-20 in 50 mM HEPES pH 7.5. The reaction mixture contained various concentrations of the inhibitor described in ref. [4]. Reactions were initiated by the addition of 20 nM EGFR L858R/T790M (final concentration).

2.1. Files and directories

This data set consists of kinetic traces recording changes of fluorescence intensity over time, recorded in biochemical assays of 11 EGFR kinase inhibitors. Each inhibitor was assayed in three separate replicated experiments, labeled "R1" through "R3" below. Each replicated experiment utilized a separate 96-well microtiter plate with only one row (12 wells) filled, while the remaining 84 wells remained empty.

The plate-reader output files were exported into the plain-text (ASCII) format files and subsequently annotated (see below). All data files, uniformly named sheet.txt, were placed into a subdirectory uniquely named according to the particular compound and replicate. This directory and file organization is schematically depicted below. See also *Figure 2*.

```
[-] Afatinib
   [-] R1
       [-] data
              sheet.txt
   [-] R2
       [+] data
              sheet.txt
   [-] R3
       [+] data
              sheet.txt
[+] CI-1033
[+] CL-387785
... etc. ...
```

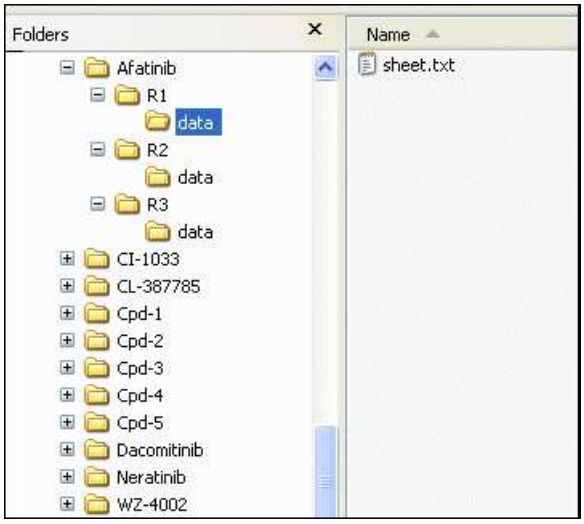


Figure 2: Organization of directories and files. Subdirectories R1 through R3 correspond to separate replicates. Data for each compound and replicate are contained in the text file named sheet.txt.

2.2. Annotations

The raw data files in plain-text (ASCII) format are annotated according to the example shown in *Figure 3*. The leftmost column contains time in seconds. Columns 2–13 contain recordings of fluorescence intensity corresponding to the phosphorylation of the fluorogenic peptide substrate.

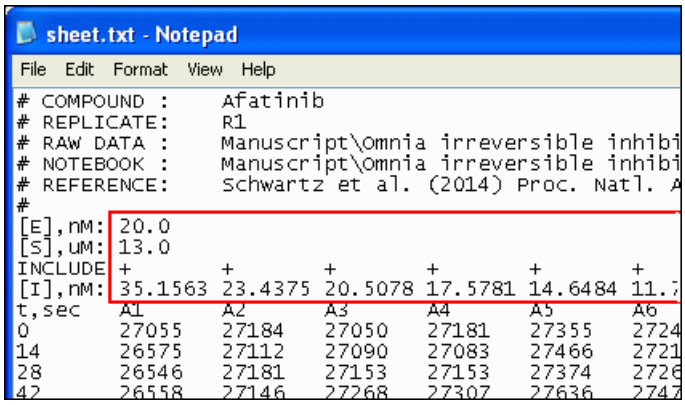


Figure 3: Example of raw data files annotation. Entries enclosed in the red rectangle are mandatory for automatic processing. Symbol uM stands for μM.

In the automated processing using the DynaFit software package [5, 6], the the lines of text starting with the "hash" character (#) are ignored entirely. DynaFit utilizes only the text starting with the zero in the first column. The Perl automation scripts referred to in this Note utilize the lines of text enclosed in the red rectangle. The meaning of those particular annotations is self-explanatory, except perhaps the INCLUDE line.

The INCLUDE line contains either "+" (plus) or "-" (minus), depending on whether or not the particular data column labeled A1 through A12 should be included in the regression analysis. Columns labeled with "-" are considered *outliers* and will be excluded from analysis. As distributed initially the data set contains the "+" sign in every column. The Perl automation script exclude-outliers.pl (see section 5 for details) rewrites the INCLUDE line based on a particular algorithm for the exclusion of outliers.

2.3. Exclusion of outliers

This data set consists from 11 inhibitors assayed in three replicates, with each replicate consisting of 12 reaction progress curves recorded at different inhibitor concentrations. Thus the complete data set contains $11 \times 3 \times 12 = 396$ reaction progress curves. Approximately 20 progress curves (five percent of the total) are unsuitable for detailed kinetic analysis, because they display gross deviations from the theoretically expected shape. An example is shown in *Figure 4*.

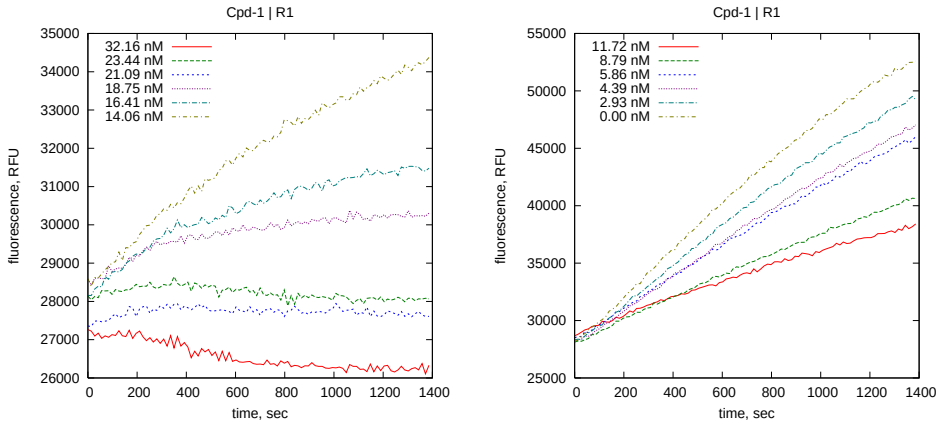


Figure 4: Example of anomalous (outlier) progress curve: Compound **1**, Replicate 1, $[I]_0$ = 32.16 nM (bottom curve in the left-hand panel).

In the Omnia® protein kinase assay the fluorescence intensity is expected to increase monotonously over time. However, the bottom progress curve in the left-hand panel of *Figure 4* ($[I]_0$ = 32.16 nM) shows an overall *decrease* in fluorescence intensity, which is clearly a gross anomaly. It is not clear why such anomalies occasionally occur in the assay, but experience shows that the detailed kinetic analysis would be negatively impacted if clear *outliers* were included.

The exclusion of outliers is best performed in a fully automated setting, rather than relying on the subjective judgment of any particular investigator or data manager. Each project where outlier curves are seen will call for a different automated outlier exclusion algorithm. In this particular case, the algorithm is as follows:

1. Divide the entire progress curve into three equal-length segments (S1, S2, S3).
2. Perform linear if of the individual segments. Examine the corresponding linear slopes.
3. If the slopes are negative in segments S1 *and* S2, mark the given curve as an outlier.

The identification of outlier curves conforming to the particular pattern described above was fully automated by using the DynaFit [5, 6] software package. A sample input file (a DynaFit "script") is displayed in *Listing 1*.

Listing 1

```
[task]
   data = piecewise-linear
   task = fit
[data]
   directory ./data
   sheet     sheet.txt
   column    2
[output]
   directory ./output/fit-progress-linear
[settings]
{PieceWiseLinearFit}
   Segments = 3
[end]
```

An automated procedure is described in section 5. *Table 1* summarizes the results of automatic outlier exclusion. Thus, for example, no progress curves were excluded for Afatinib. In the case of CI-1033, one progress curve corresponding to the single highest inhibitor concentration was excluded for replicate R1 and two highest inhibitor concentrations were excluded for replicate R2.

Compound	Replicate R1	Replicate R2	Replicate R3
Afatinib	.	.	.
CI-1033	1	2	.
CL-387785	1	1	1
Cpd-1	3	2	2
Cpd-2	2	2	1
Cpd-3	.	.	.
Cpd-4	.	.	.
Cpd-5	.	.	.
Dacomitinib	.	.	.
Neratinib	.	.	.
WZ-4002	1	1	1

Table 1: Exclusion of outliers. Each numerical entry indicates the number of highest inhibitor concentrations that were excluded from analysis.

3. The theoretical model

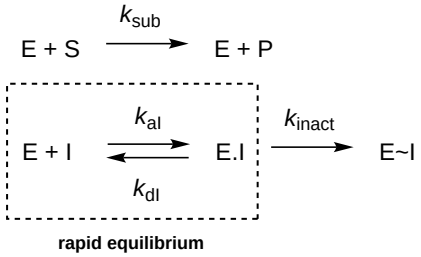
The traditional mathematical approach to the analysis of covalent inhibition data [7] relies on the following assumptions:

1. In the absence of inhibitors, the reaction progress curve – i.e. a plot of product concentration over time - is strictly linear (no substrate depletion).
2. The mole fraction of the inhibitor bound to the enzyme is negligibly small (no inhibitor depletion, no "tight binding" [1–3]).

If both simplifying assumptions are satisfied sufficiently well, one can use a simple *algebraic formula* [7, Chap. 9] to analyze the reaction progress and determine the kinetic parameters k_{inact} and K_{i}. However, in our specific case neither assumption is valid and therefore the we used a fully general kinetic model, based on numerically solving systems of simultaneous first order ordinary *differential equations* (ODE).

3.1. Differential equation system

The assumed molecular mechanism is shown in *Scheme 1*, where k_{sub} is the second-order rate constant for substrate conversion to product; k_{aI} is the second-order rate constant for for formation of the initial non-covalent complex; k_{dI} is the first-order rate constant for for dissociation of the initial non-covalent complex into its constituent components; and k_{inact} is the first-order rate constant for the formation of the covalent conjugate



Scheme 1

The differential equation system corresponding to *Scheme 1* is shown in Eqn (1) – Eqn (6).

$$\mathrm{d[E]}/\mathrm{d}t = -k_{\mathrm{aI}}[\mathrm{E}][\mathrm{I}] + k_{\mathrm{dI}}[\mathrm{E.I}] \quad (1)$$

$$\mathrm{d[S]}/\mathrm{d}t = -k_{\mathrm{sub}}[\mathrm{E}][\mathrm{S}] \quad (2)$$

$$\mathrm{d[P]}/\mathrm{d}t = +k_{\mathrm{sub}}[\mathrm{E}][\mathrm{S}] \quad (3)$$

$$\mathrm{d[I]}/\mathrm{d}t = -k_{\mathrm{aI}}[\mathrm{E}][\mathrm{I}] + k_{\mathrm{dI}}[\mathrm{E.I}] \quad (4)$$

$$\mathrm{d[E.I]}/\mathrm{d}t = +k_{\mathrm{aI}}[\mathrm{E}][\mathrm{I}] - k_{\mathrm{dI}}[\mathrm{E.I}] - k_{\mathrm{inact}}[\mathrm{E.I}] \quad (5)$$

$$\mathrm{d[E{\sim}I]}/\mathrm{d}t = +k_{\mathrm{inact}}[\mathrm{E.I}] \quad (6)$$

Based on the preliminary examination of the data (see Supporting Information for ref. [4] for details) we invoked the "rapid equilibrium" approximation for inhibitor binding, meaning that the the noncovalent complex is assumed to form instantaneously, during the mixing-delay time.

In practical terms we assumed that by the time the instrument recorded the first time point, the initial noncovalent complex was already fully formed.

This "rapid equilibrium" assumption was expressed by assigning a sufficiently high arbitrary value to the association rate constant, $k_{aI} = 10^7\ \text{M}^{-1}\text{s}^{-1}$. The association rate constant k_{aI} was then treated as a *fixed* model parameter. In contrast, the remaining microscopic rate constants k_{sub}, k_{dI}, and k_{inact} were treated as *adjustable* model parameters.

3.2. The model equation for fluorescence changes

Solving the initial value problem represented by the ODE system Eqn (1) – Eqn (6) is a prerequisite for constructing a suitable mathematical model for the observed experimental data, such as those that are shown in *Figure 4*, but it is not sufficient. What remains to be done is to link the the concentrations of species, obtained by the numerical solution, to the experimental signal.

Figure 4 shows that the overall fluorescence intensity increases approximately two-fold, from approximately 28,000 relative fluorescence units (RFU) to to approximately 55,000 RFU. However, the initial fluorescence signal (at time zero) varies form one experimental progress curve to the next. At least in part, this variation is due to the fact that the plate-reader detection system itself introduces a certain amount of experimental uncertainty. Secondly, a very small amount of substrate can already be consumed during the short mixing-delay time.

Based on these practical considerations we had decided to construct the mathematical model not for the actual experimental signal (raw RFU values) but rather for the fluorescence *changes* over time, ΔF. In other words, the fluorescence intensity F observed at time zero ($t = 0$) was arbitrarily set to zero and was then subtracted from all the remaining fluorescence intensities associated with the given progress curve. Thus the mathematical model must account for the fluorescence changes, ΔF, rather than fluorescence readings as such.

However, there is no justifiable reason why the best-fit model curve should pass *exactly* through the first recorded time point. Therefore we must allow for an adjustable model parameter that allows the entire progress curve model to "float" on the vertical axis. This parameter is the "offset" on the signal axis, F_0, and is specific for each individual progress curve.

Finally, it remains to be decided which molecular species is to be treated as observable in the fluorescence experiment. Strictly speaking, both the fluorogenic substrate S and the final reaction product P appearing in *Scheme 1* can be detected by the instrument. However, we have decided to build a model for fluorescence *changes* over time, ΔF, rather than for the fluorescence readings proper, F. Therefore we are free to assign zero fluorescence intensity to the substrate, S, and non-zero fluorescence intensity to the reaction product, P.

The above considerations lead to the theoretical model for each individual reaction progress curve defined by Eqn (7), where $\Delta F(t)$ is the increase in fluorescence intensity observed at the reaction time t; F_0 is the offset on the signal axis treated as an adjustable model parameter; r_P is the molar response coefficient of the reaction product P; and $[\text{P}](t)$ is the concentration of the reaction product P at time t. The later quantity is obtained by solving the initial value problem defined by Eqn (1) – Eqn (6).

$$\Delta F(t) = F_0 + r_P\ [\text{P}](t) \tag{7}$$

3.3. Global fit of multiple combined curves

In order to determine the binding affinities (K_i) and chemical reactivities (k_{inact}) of covalent EGFR inhibitors, we had performed *global analysis* [8] of multiple combined kinetic traces.

That is, rather then analyzing individual reaction time courses associated with each particular inhibitor concentrations, all twelve reaction progress curve associated with each given plate were combined into a single global "superset" of experimental data and analyzed together. In this treatment the optimized model parameters break down in the two subcategories:

1. **Globally optimized parameters.** These are adjustable model parameters that apply *jointly* to all reaction progress curves collected in the global "superset" of data.
   - *Rate constants.* The microscopic rate constants k_{sub}, k_{dI}, and k_{inact} were always treated as globally optimized model parameters.
   - *Molar responses.* The molar response coefficient r_{P} was also globally optimized for all compounds.
   - *Enzyme concentration.* For some, but not for all, compounds in our collection the active (as opposed to nominal) enzyme concentration was treated as a globally optimized model parameter. See section 4 for details.
2. **Locally optimized parameters.** These are adjustable model parameters that apply *selectively* to each individual reaction progress curve.
   - *Instrument offsets.* The offset parameter F_0 in Eqn (7) was always treated as locally optimized parameter for all progress curves and all compounds.
   - *Inhibitor concentrations.* Each global data set consisted of up to 12 reaction progress curves[3], including the control ($[\text{I}]_0 = 0$). The six highest inhibitor concentrations were treated as locally optimized, whereas the remaining five nonzero inhibitor concentrations were treated as fixed parameters.

4. The model parameters

4.1. Initial estimates

Nonlinear regression analysis always requires that the investigator supplies suitable initial estimates of all fitting parameters. In many cases the initial estimate must be quite close to the "true" or best-fit value, which is of course unknown at the outset. To address this significant challenge the case of covalent inhibition kinetics, we had implemented a series of automatic parameter-estimation algorithm, as described in the Supporting Information document that is a part of ref. [4].

Dissociation rate constants k_{dI}

The initial estimates of k_{dI} were made separately for each inhibitor on the basis of the relationship

$$k_{\text{dI}} = \frac{K_{\text{i}}^{*}}{k_{\text{aI}}} \quad ,$$

where K_{i}^{*} is the apparent inhibition constant determined from the initial reaction rates [4] and k_{aI} is the fixed value of the association rate constant set to 10^7 $\text{M}^{-1}\text{s}^{-1}$. The initial estimates of k_{dI} for each inhibitor are listed in the Appendix (column labeled **initial** in parameter tables).

[3] For certain specific compounds and replicates, up to three reaction progress curve had to be excluded. See section 2.3 for details.

Inactivation rate constant k_{inact}

The initial estimate of the inactivation rate constant k_{inact} was set to 0.01 s^{-1} on the basis of preliminary analyses of the raw data ("trial and error" method).

Molar response coefficient r_P

The molar response coefficient of the fluorescent product *P* was estimated to be approximately 6000 RFU/μM, again on the basis of preliminary analyses. In particular, the control reaction progress curves observed in the absence of inhibitors were fit the first-order exponential model and the molar response of the reaction product was computed from the exponential amplitude.

Active enzyme concentration [E]

In preliminary analyses we found that the active enzyme concentration for sufficiently "tight binding" [1] inhibitors needed to be treated as an adjustable parameter in regression analysis. In contrast, for "weak binding" inhibitors the enzyme concentration needed to be treated as a fixed constant, otherwise the model would become over-parameterized. This situation is similar to what we previously observed in the analysis of initial reaction rates [9]. The initial estimate of the active enzyme concentration was set to the nominal value, [E] = 20 nM.

Inhibitor concentrations [I]

A close examination of the data plots collected in the Appendix will easily reveal that the inhibitor concentrations were affected by nonzero titration error. This is normal in particular in the microtiter plate-reader format that was deployed for this kinetic study. However, experience shows that it is *impossible to optimize all inhibitor concentrations* in the given global "superset" of experimental data. In this case we have chosen to optimize the six highest inhibitor concentrations that were included in the global data set.

Offset on the signal axis

The offset parameter F_0 was locally optimized for all progress curves included in the global data set. The initial estimate was set to the arbitrary value F_0 = -300.

4.2. Best-fit values

The final best-fit values of all nonlinear model parameters (11 compounds, 3 replicates each) are collected in the Appendix.

5. Automated analysis using DynaFit software

This section describes a procedure that can be used to reproduce the DynaFit analysis of EGFR covalent inhibition as described in ref. [4]. The automation algorithms are embodied in a collection of Perl script that are included with the package. Follow these steps to perform the complete kinetic analysis of covalent EGFR inhibition.

1. Point your browser to the following URL:
   `http://www.biokin.com/publications/technotes/data/TN201502-data.zip`
2. Download the ZIP archive file to any location on a computer running MS Windows.
3. Extract the ZIP archive. This will create a directory named dynafit-TN201502.

4. Navigate to the following subdirectory:
   ./dynafit-TN201502/proj/EGFR/L858R-T790M/scripts/
5. Double-click on the executable file complete-analysis.exe.[4]

The executable file complete-analysis.exe is a compiled (binary) version of the Perl script complete-analysis.pl located in the same directory. Upon execution the script will perform the following tasks in sequence:

- Exclusion of outliers, by annotating the raw experimental data as described in sections 2.2 and 2.3 above.
- Determination of initial reaction rates by "local" exponential fit, as described in the Supporting Information document in ref. [4].
- Determination of apparent inhibition constants, as described in the Supporting Information document in ref. [4].
- Nonlinear "global" fit of combined progress curves to determined k_{inact} and K_{i}.
- Correlation analysis: linear fit (in double logarithmic coordinates) of biochemical parameters vs. the corresponding cellular IC_{50} values.

6. Summary and conclusions: Correlation analysis

Upon executing the sequence of automated analyses described in section 5, we obtain the final results that can be summarized as follows.

6.1. Cellular IC_{50} vs. inactivation rate constant

The linear regression analysis (in double-logarithmic coordinates) of cellular IC_{50} vs. the inactivation rate constant k_{inact} is shown in *Figure 5*. The slope of the regression line is -0.308 and the coefficient of determination is $R^2 = 0.446$. These results suggest only a *weak link* between cellular potency and the chemical reactivity of these covalent EGFR inhibitors.

6.2. Cellular IC_{50} vs. initial binding affinity

The linear regression analysis (in double-logarithmic coordinates) of cellular IC_{50} vs. the initial binding affinity K_{i} is shown in *Figure 6*. The slope of the regression line is 0.963 and the coefficient of determination is $R^2 = 0.884$. These results suggest a *strong link* between cellular potency and the initial binding affinity in the noncovalent enzyme–inhibitor complex.

6.3. Cellular IC_{50} vs. $k_{\mathrm{inact}}/K_{\mathrm{i}}$

The linear regression analysis (in double-logarithmic coordinates) of cellular IC_{50} vs. the second-order rate constant $k_{\mathrm{inact}}/K_{\mathrm{i}}$ is shown in *Figure 7*. The slope of the regression line is -1.279 and the coefficient of determination is $R^2 = 0.939$. These results suggest the *strongest link* between cellular potency and the lower limit of the bimolecular association rate constant k_{aI}.[5]

[4] Note that the directory contains files named complete-analysis.exe, complete-analysis.pl, and complete-analysis.ini. To distinguish these files it is important to disable the MS Windows default setting "Hide extensions of known file types."

[5] The second-order rate constant $k_{\mathrm{inact}}/K_{\mathrm{i}}$ indeed represents the lowest feasible value of k_{aI}, as will be shown in a separate forthcoming report.

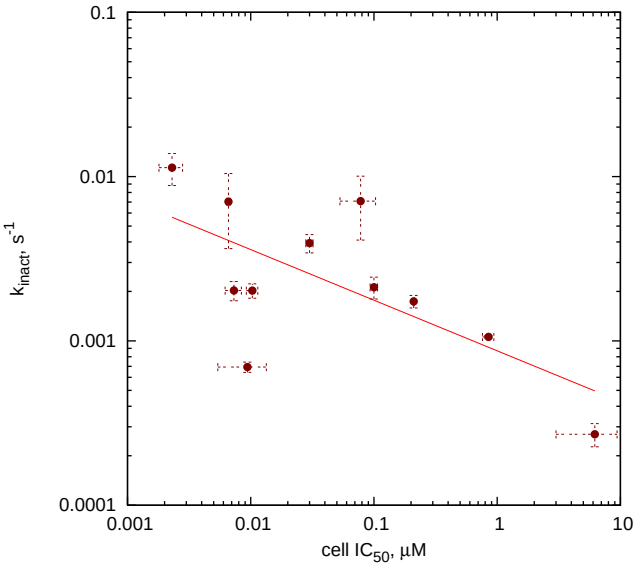


Figure 5: Correlation of cellular IC_{50} vs. inactivation rate constant k_{inact}.

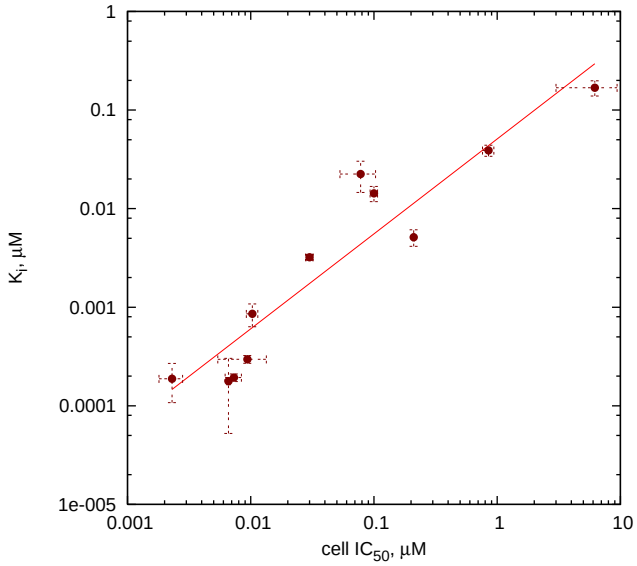


Figure 6: Correlation of cellular IC_{50} vs. inhibition constant I_i.

Bibliographic Information

How to Cite this Publication:

Kuzmič, P. (2015) *Automated determination of k_{inact} and K_i for covalent inhibition using*

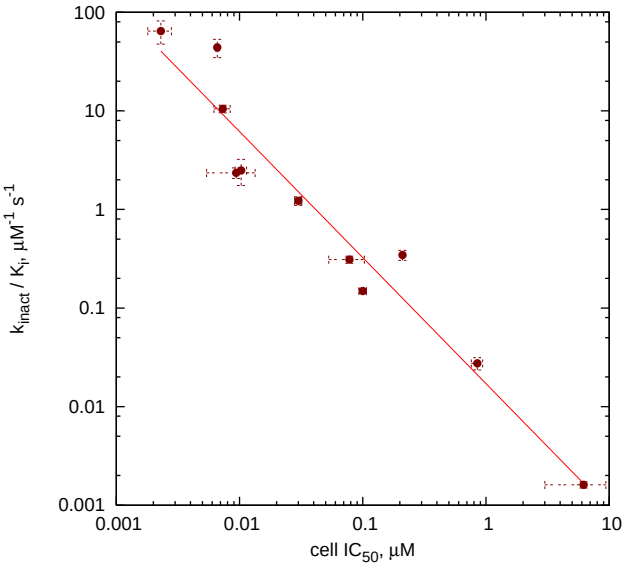


Figure 7: Correlation of cellular IC_{50} vs. second-order rate constant $k_{\text{inact}}/K_{\text{i}}$.

the Omnia® assay, BioKin Technical Note TN-2015-02, BioKin Ltd., Watertown MA, [Online] `www.biokin.com/TN/2015/02`

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[9] P. Kuzmic, K. C. Elrod, L. M. Cregar, S. Sideris, R. Rai, J. W. Janc, High-throughput screening of enzyme inhibitors: Simultaneous determination of tight-binding inhibition constants and enzyme concentration, Anal. Biochem. 286 (2000) 45–50.

Appendix

A. Results of fit

This section contains the initial and "certified" values of model parameters associated with each compound and replicate. Also included are DynaFit [6] script files that were actually utilized to generate this Technical Note. The DynaFit scripts are included to ultimately clarify which concentrations were utilized for which compound and replicate, as well as clarify the initial estimates of microscopic rate constants and other model parameters.

In the tables below, an asterisk ("*") in the **set** column identifies a *globally* optimized parameter. A numerical value in the **set** column identifies a *locally* optimized parameter, indicating the data set number with which the given parameter is associated.

The **initial** column lists the initial estimate of each globally or locally optimized nonlinear parameter. In the case of inhibitor concentration ([I]) and enzyme concentrations ([E]) the initial estimates are the nominal values. The **fit** column lists the best-fit values generate by unweighted least-squares fit of the global "superset" of fluorescence changes over time to Eqn (7).

The **std. error** column lists the formal standard error from nonlinear regression. Note that this value was ignored for the purpose of correlation analysis, linking biochemical kinetic parameters with cellular potency [4]. The true uncertainty of biochemical parameters was determined as the standard deviation from three independent replicates.

Figure legends below show inhibitor concentrations in nanomolar units (nM). However, the tables list all concentrations in micromolar units (μM). Note that the inhibitor concentration ([I]) associated with data set "1" is always the *highest* inhibitor concentration that actually utilized for the analysis. Also note that only the following five "tight binding" [1] inhibitors had their enzyme concentrations optimized:

- Afatinib
- CI-1033
- Compound **1**
- Dacomitinib
- Neratinib

For the remaining compounds in this collection the enzyme concentration was held constant.

A.1. Afatinib

A.1.1. Replicate R1

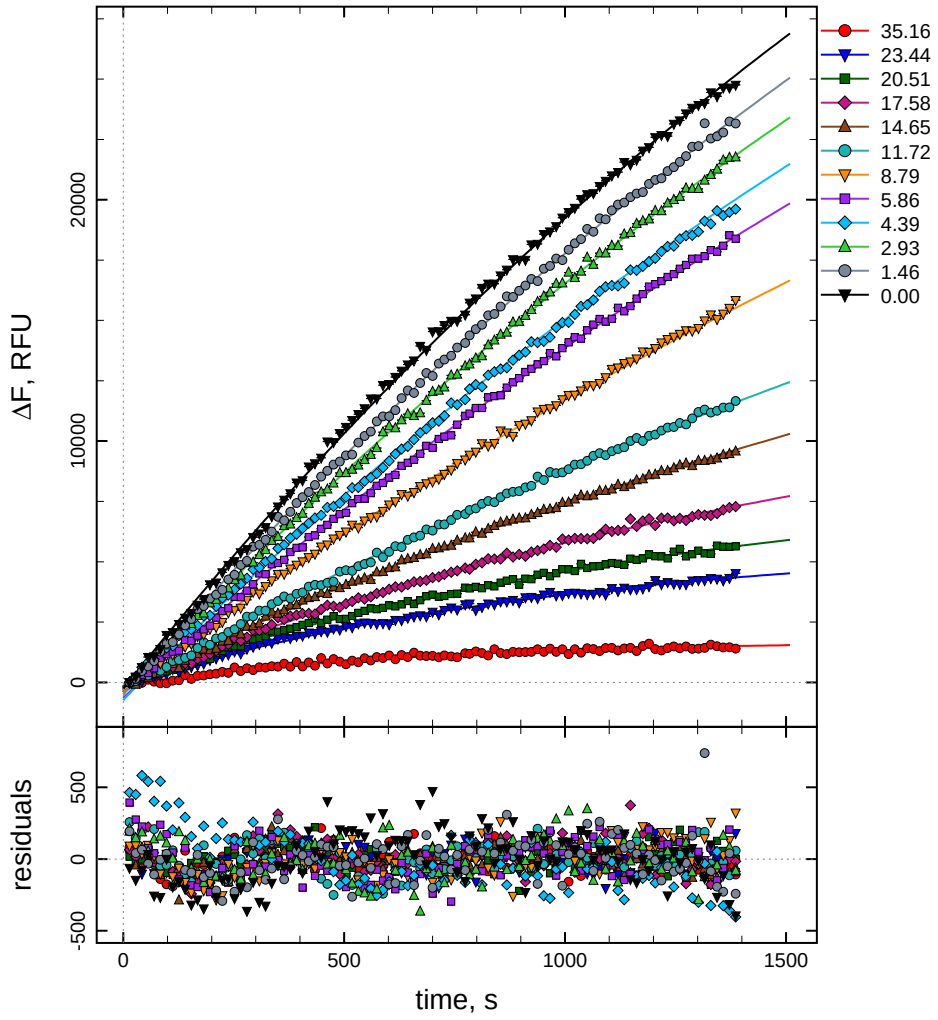
Afatinib, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.06722 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Afatinib/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   3 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   4 | offset = -300 ?  | conc I = 0.0205078 ? | label 20.51
   column   5 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   6 | offset = -300 ?  | conc I = 0.0146484 ? | label 14.65
   column   7 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   8 | offset = -300 ?  | conc I = 0.00878906 | label 8.79
   column   9 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  10 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  11 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  12 | offset = -300 ?  | conc I = 0.00146484 | label 1.46
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Afatinib/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Afatinib, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0185227	0.000402215
k_{dI}, s^{-1}	*	0.06722	0.0277886	0.00222109
k_{inact}, s^{-1}	*	0.01	0.00181292	0.000150989
[E], μM	*	0.02	0.01835	0.000236779
r_P, RFU/μM	*	6000	5204.92	74.0966
[I], μM	1	0.0351563	0.0363392	0.00238772
[I], μM	2	0.0234375	0.0217037	0.000643511
[I], μM	3	0.0205078	0.0192588	0.000428727
[I], μM	4	0.0175781	0.0168698	0.000265057
[I], μM	5	0.0146484	0.0141541	0.000142397
[I], μM	6	0.0117188	0.0121869	9.06704e-005
offset, RFU	1	-300	-181.295	42.0584
offset, RFU	2	-300	-186.482	37.1089
offset, RFU	3	-300	-184.83	33.5009
offset, RFU	4	-300	-247.075	31.0757
offset, RFU	5	-300	-390.877	30.3118
offset, RFU	6	-300	-471.479	30.0341
offset, RFU	7	-300	-332.593	27.2327
offset, RFU	8	-300	-659.441	20.762
offset, RFU	9	-300	-742.7	21.0756
offset, RFU	10	-300	-503.868	22.5477
offset, RFU	11	-300	-500.562	24.7701
offset, RFU	12	-300	-260.034	27.6333

Afatinib, Replicate R1



A.1.2. Replicate R2

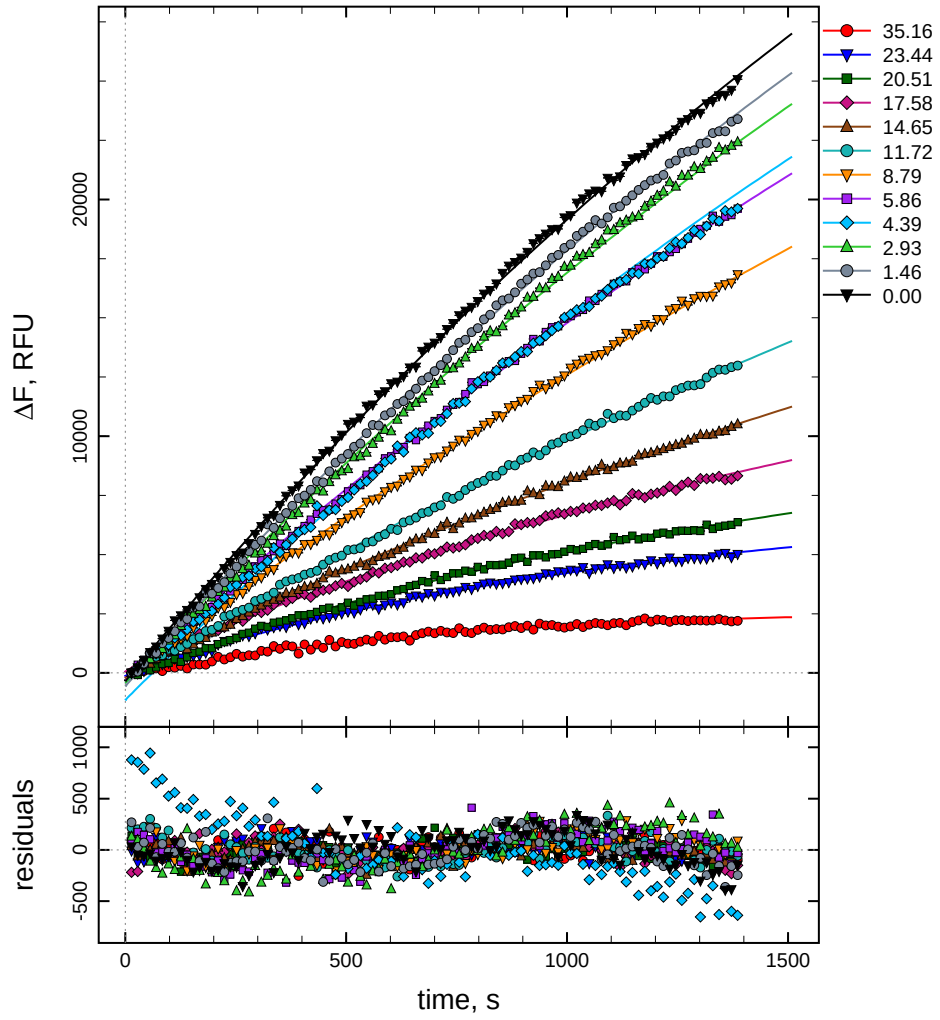
Afatinib, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.06722 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Afatinib/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   3 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   4 | offset = -300 ?  | conc I = 0.0205078 ? | label 20.51
   column   5 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   6 | offset = -300 ?  | conc I = 0.0146484 ? | label 14.65
   column   7 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   8 | offset = -300 ?  | conc I = 0.00878906 | label 8.79
   column   9 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  10 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  11 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  12 | offset = -300 ?  | conc I = 0.00146484 | label 1.46
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Afatinib/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Afatinib, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0145395	0.000398672
k_{dI}, s^{-1}	*	0.06722	0.0322867	0.00306037
k_{inact}, s^{-1}	*	0.01	0.00193406	0.000210134
[E], μM	*	0.02	0.0210946	0.000312815
r_P, RFU/μM	*	6000	5680.03	107.685
[I], μM	1	0.0351563	0.0325858	0.00201304
[I], μM	2	0.0234375	0.0228864	0.00071958
[I], μM	3	0.0205078	0.0203149	0.00047392
[I], μM	4	0.0175781	0.0179563	0.00030725
[I], μM	5	0.0146484	0.0152739	0.000183247
[I], μM	6	0.0117188	0.012409	0.000110085
offset, RFU	1	-300	-207.523	53.3806
offset, RFU	2	-300	-146.653	43.5233
offset, RFU	3	-300	-305.023	39.46
offset, RFU	4	-300	38.965	37.631
offset, RFU	5	-300	-233.945	36.9153
offset, RFU	6	-300	-429.805	36.2223
offset, RFU	7	-300	-321.011	31.9064
offset, RFU	8	-300	-331.945	25.4463
offset, RFU	9	-300	-1155.18	25.6659
offset, RFU	10	-300	-420.963	27.3666
offset, RFU	11	-300	-571.13	30.0789
offset, RFU	12	-300	-349.024	33.5262

Afatinib, Replicate R2



A.1.3. Replicate R3

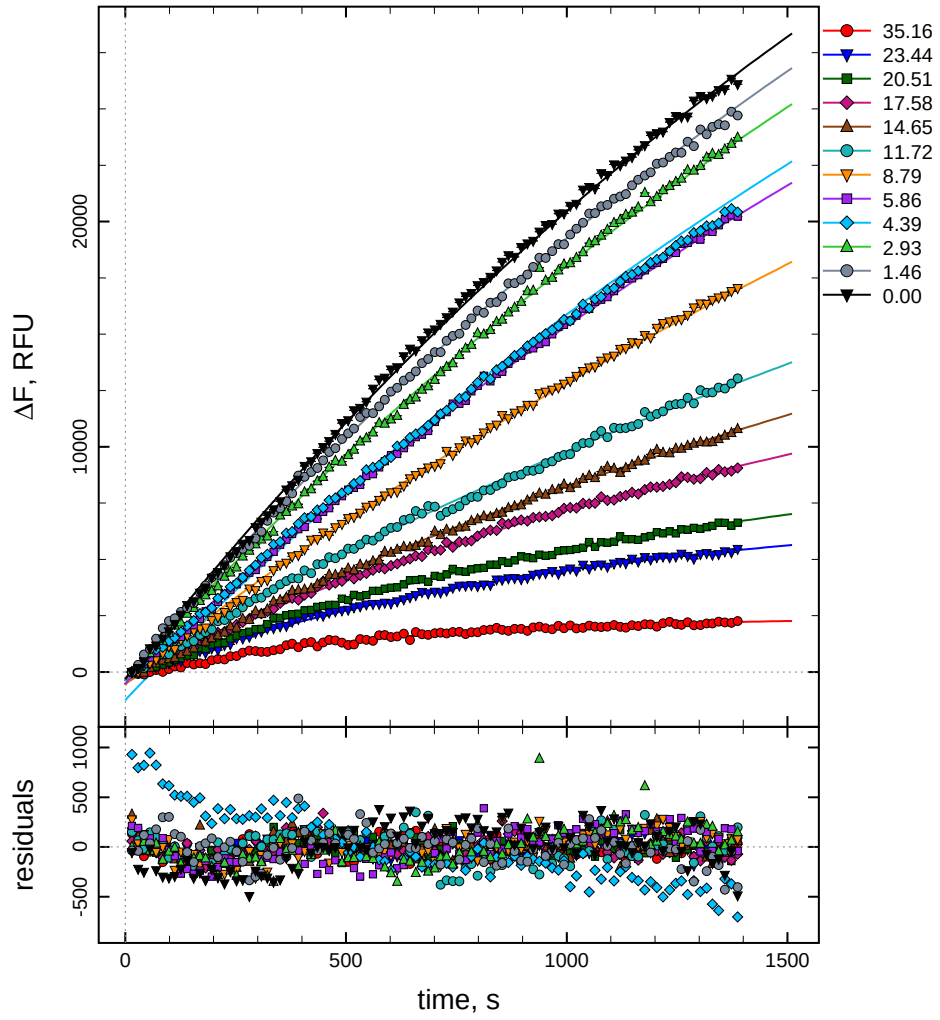
Afatinib, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.06722 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Afatinib/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   3 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   4 | offset = -300 ?  | conc I = 0.0205078 ? | label 20.51
   column   5 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   6 | offset = -300 ?  | conc I = 0.0146484 ? | label 14.65
   column   7 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   8 | offset = -300 ?  | conc I = 0.00878906 | label 8.79
   column   9 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  10 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  11 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  12 | offset = -300 ?  | conc I = 0.00146484 | label 1.46
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Afatinib/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Afatinib, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0197661	0.000468416
k_{dI}, s^{-1}	*	0.06722	0.0326739	0.00245528
k_{inact}, s^{-1}	*	0.01	0.0023321	0.000236542
[E], μM	*	0.02	0.0195494	0.000250141
r_P, RFU/μM	*	6000	4988.09	74.0575
[I], μM	1	0.0351563	0.0309552	0.00171592
[I], μM	2	0.0234375	0.0208344	0.000534672
[I], μM	3	0.0205078	0.0189444	0.000379638
[I], μM	4	0.0175781	0.0162423	0.000220742
[I], μM	5	0.0146484	0.0143914	0.000151946
[I], μM	6	0.0117188	0.0125979	0.000109344
offset, RFU	1	-300	-268.615	59.1664
offset, RFU	2	-300	-341.563	47.3114
offset, RFU	3	-300	-367.816	43.5336
offset, RFU	4	-300	-276.815	40.7105
offset, RFU	5	-300	-559.152	39.9355
offset, RFU	6	-300	-397.686	39.3727
offset, RFU	7	-300	-557.258	34.6026
offset, RFU	8	-300	-521.488	27.5974
offset, RFU	9	-300	-1254.16	27.8869
offset, RFU	10	-300	-344.188	29.8231
offset, RFU	11	-300	-323.247	32.8465
offset, RFU	12	-300	-320.915	36.6028

Afatinib, Replicate R3



A.2. CI-1033

A.2.1. Replicate R1

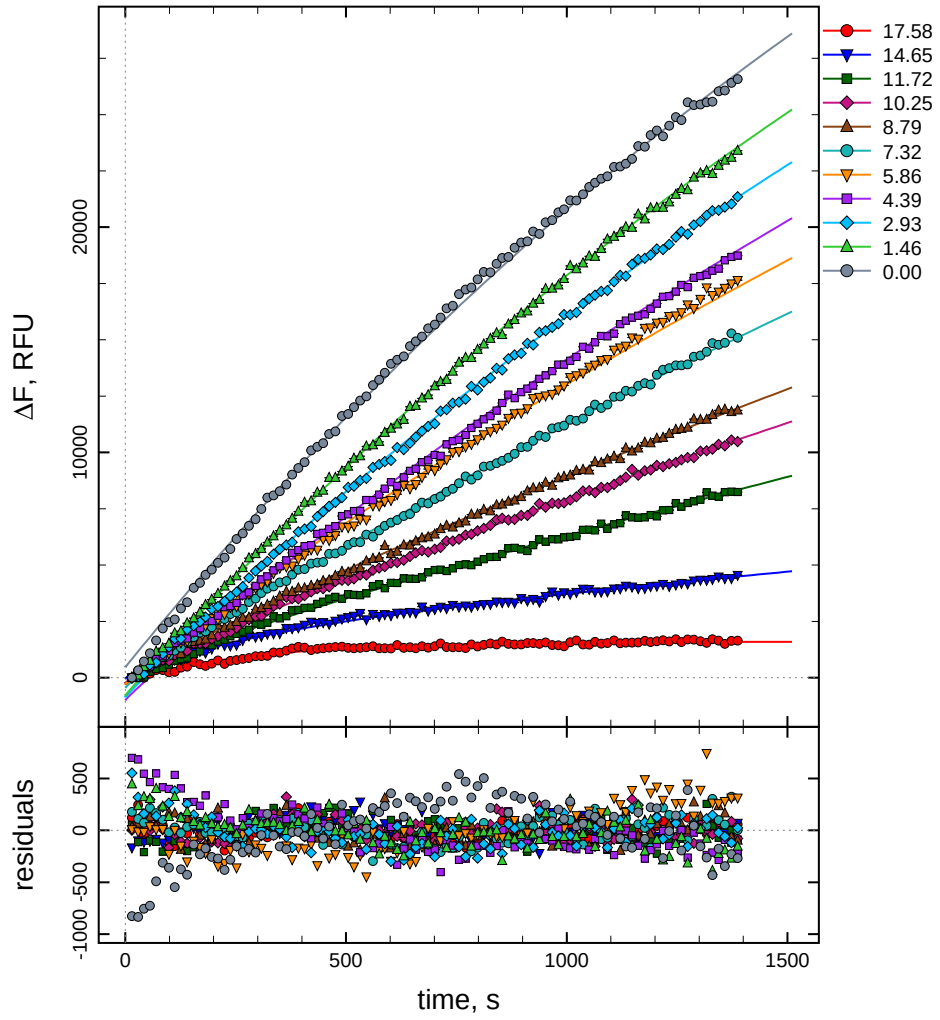
CI-1033, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.0446 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/CI-1033/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   4 | offset = -300 ?  | conc I = 0.0146484 ? | label 14.65
   column   5 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   6 | offset = -300 ?  | conc I = 0.0102539 ? | label 10.25
   column   7 | offset = -300 ?  | conc I = 0.00878906 ? | label 8.79
   column   8 | offset = -300 ?  | conc I = 0.00732422 ? | label 7.32
   column   9 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  10 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  11 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  12 | offset = -300 ?  | conc I = 0.00146484 | label 1.46
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/CI-1033/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

CI-1033, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0251265	0.000651517
k_{dI}, s^{-1}	*	0.0446	0.0186077	0.00411259
k_{inact}, s^{-1}	*	0.01	0.00846711	0.00165741
[E], μM	*	0.02	0.0146386	0.000108491
r_P, RFU/μM	*	6000	5078.39	84.2315
[I], μM	1	0.0175781	0.016053	0.000331163
[I], μM	2	0.0146484	0.0134413	0.000123036
[I], μM	3	0.0117188	0.011073	8.13742e-005
[I], μM	4	0.0102539	0.00984709	6.93094e-005
[I], μM	5	0.00878906	0.00903431	6.2665e-005
[I], μM	6	0.00732422	0.00711792	5.03602e-005
offset, RFU	1	-300	-314.177	65.4955
offset, RFU	2	-300	-48.1496	52.1237
offset, RFU	3	-300	-286.751	46.4705
offset, RFU	4	-300	-247.834	43.654
offset, RFU	5	-300	-285.216	42.0712
offset, RFU	6	-300	-453.281	39.5661
offset, RFU	7	-300	-298.565	33.4906
offset, RFU	8	-300	-1005.25	28.8594
offset, RFU	9	-300	-875.528	29.07
offset, RFU	10	-300	-787.773	32.9365
offset, RFU	11	-300	462.841	38.6186

CI-1033, Replicate R1



A.2.2. Replicate R2

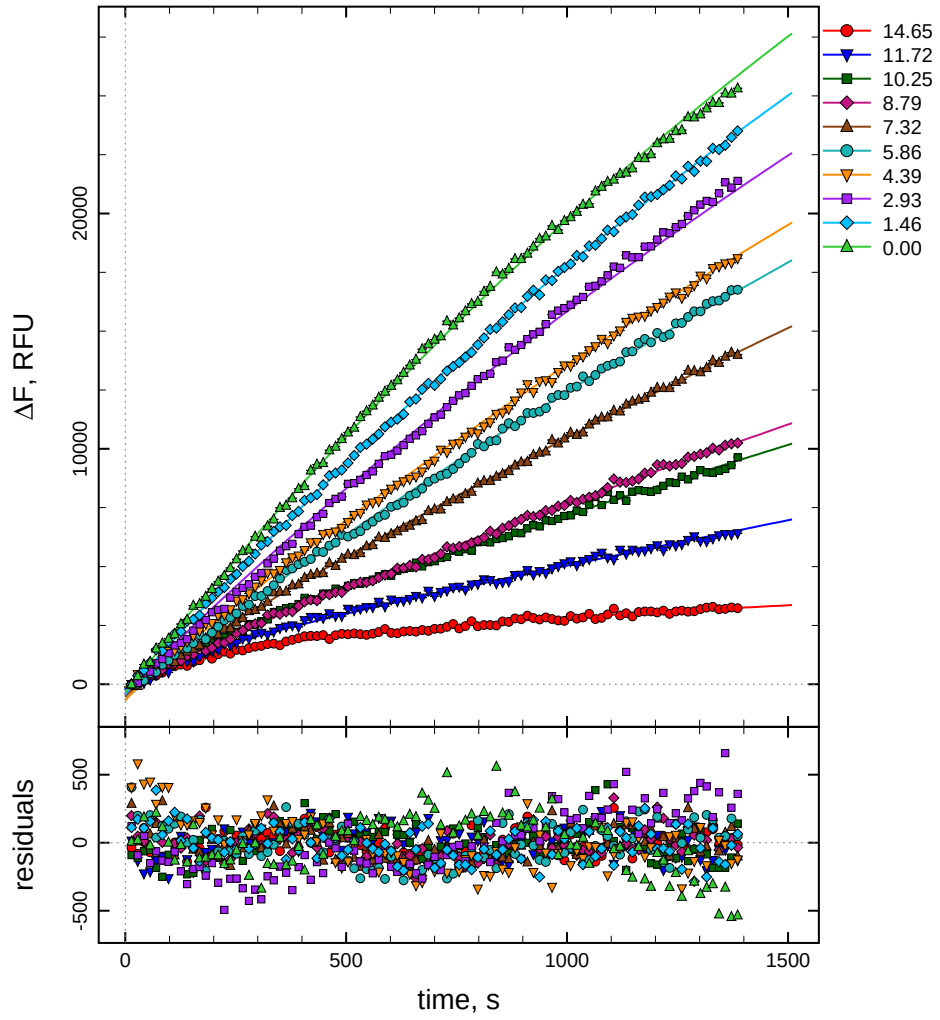
CI-1033, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.0446 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/CI-1033/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   4 | offset = -300 ?  | conc I = 0.0146484 ? | label 14.65
   column   5 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   6 | offset = -300 ?  | conc I = 0.0102539 ? | label 10.25
   column   7 | offset = -300 ?  | conc I = 0.00878906 ? | label 8.79
   column   8 | offset = -300 ?  | conc I = 0.00732422 ? | label 7.32
   column   9 | offset = -300 ?  | conc I = 0.00585938 ? | label 5.86
   column  10 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  11 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  12 | offset = -300 ?  | conc I = 0.00146484 | label 1.46
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/CI-1033/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

CI-1033, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0254712	0.000711254
k_{dI}, s^{-1}	*	0.0446	0.0276664	0.00911993
k_{inact}, s^{-1}	*	0.01	0.0131143	0.0033932
[E], μM	*	0.02	0.0131001	0.000115752
r_P, RFU/μM	*	6000	5442.35	97.9867
[I], μM	1	0.0146484	0.0126494	0.000133809
[I], μM	2	0.0117188	0.0107634	9.52207e-005
[I], μM	3	0.0102539	0.00930429	7.79594e-005
[I], μM	4	0.00878906	0.00875723	7.22944e-005
[I], μM	5	0.00732422	0.0067203	5.3907e-005
[I], μM	6	0.00585938	0.00523697	4.35215e-005
offset, RFU	1	-300	-164.64	57.9675
offset, RFU	2	-300	-231.78	53.3661
offset, RFU	3	-300	-138.927	48.6599
offset, RFU	4	-300	-433.255	46.8775
offset, RFU	5	-300	-540.798	41.1289
offset, RFU	6	-300	-677.165	38.4552
offset, RFU	7	-300	-685.174	34.0303
offset, RFU	8	-300	-417.371	28.7136
offset, RFU	9	-300	-424.101	30.8333
offset, RFU	10	-300	-337.85	37.8289

CI-1033, Replicate R2



A.2.3. Replicate R3

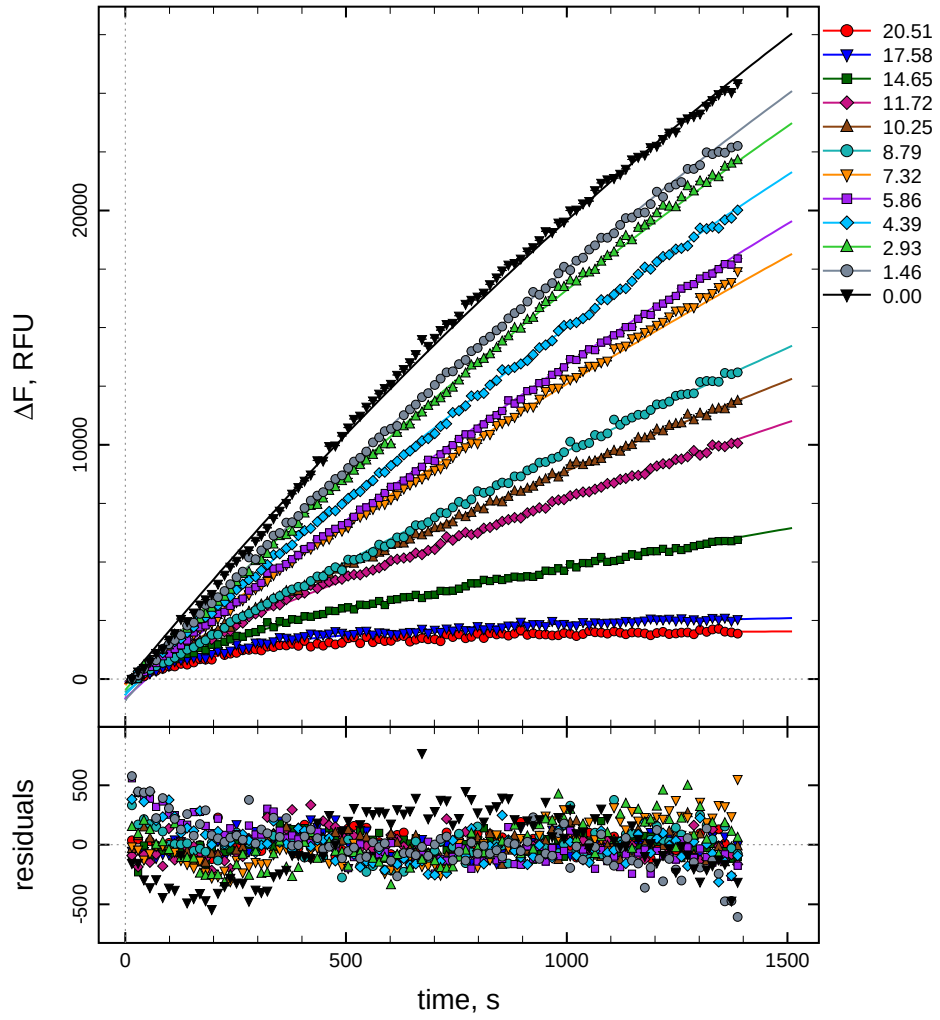
CI-1033, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.0446 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/CI-1033/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.0205078 ? | label 20.51
   column   3 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   4 | offset = -300 ?  | conc I = 0.0146484 ? | label 14.65
   column   5 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   6 | offset = -300 ?  | conc I = 0.0102539 ? | label 10.25
   column   7 | offset = -300 ?  | conc I = 0.00878906 ? | label 8.79
   column   8 | offset = -300 ?  | conc I = 0.00732422 | label 7.32
   column   9 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  10 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  11 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  12 | offset = -300 ?  | conc I = 0.00146484 | label 1.46
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/CI-1033/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

CI-1033, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0166336	0.000439602
k_{dI}, s^{-1}	*	0.0446	0.0440826	0.0117478
k_{inact}, s^{-1}	*	0.01	0.0123922	0.00268753
[E], μM	*	0.02	0.0183128	0.000118779
r_P, RFU/μM	*	6000	5787.48	106.308
[I], μM	1	0.0205078	0.019327	0.000271903
[I], μM	2	0.0175781	0.0185822	0.000210768
[I], μM	3	0.0146484	0.0154458	0.000108292
[I], μM	4	0.0117188	0.0123593	7.77606e-005
[I], μM	5	0.0102539	0.0111008	6.9052e-005
[I], μM	6	0.00878906	0.00987593	6.19095e-005
offset, RFU	1	-300	-218.944	60.1661
offset, RFU	2	-300	-134.886	56.7922
offset, RFU	3	-300	-112.042	51.2076
offset, RFU	4	-300	-137.449	45.1328
offset, RFU	5	-300	-203.645	42.7071
offset, RFU	6	-300	-580.399	40.6205
offset, RFU	7	-300	-255.124	32.0695
offset, RFU	8	-300	-842.465	27.7362
offset, RFU	9	-300	-679.568	25.9934
offset, RFU	10	-300	-462.087	26.8422
offset, RFU	11	-300	-904.781	29.6416
offset, RFU	12	-300	-189.007	33.5818

CI-1033, Replicate R3



A.3. CL-387785

A.3.1. Replicate R1

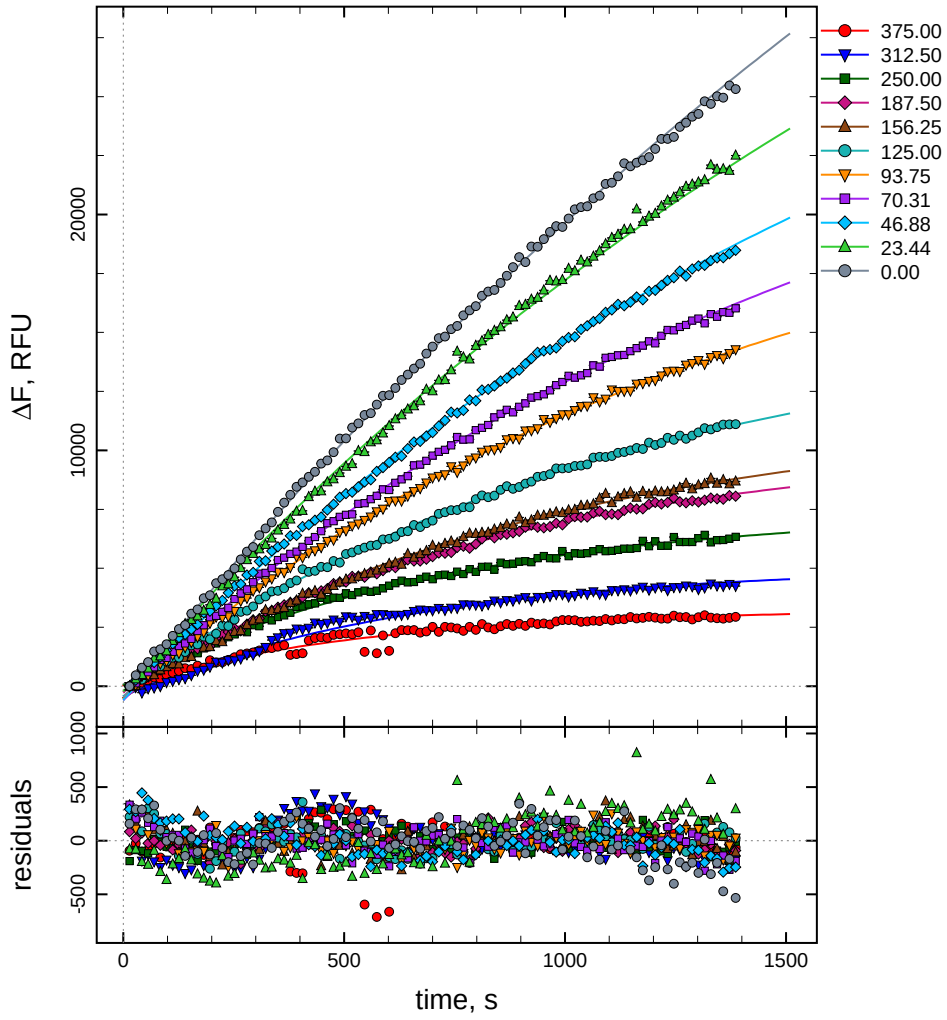
CL-387785, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 1.564 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/CL-387785/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.375 ? | label 375.00
   column   4 | offset = -300 ?  | conc I = 0.3125 ? | label 312.50
   column   5 | offset = -300 ?  | conc I = 0.25 ? | label 250.00
   column   6 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   7 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   8 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   9 | offset = -300 ?  | conc I = 0.09375 | label 93.75
   column  10 | offset = -300 ?  | conc I = 0.0703125 | label 70.31
   column  11 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  12 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/CL-387785/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

CL-387785, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0161067	0.000417381
k_{dI}, s^{-1}	*	1.564	2.48936	0.0605632
k_{inact}, s^{-1}	*	0.01	0.00235865	6.45914e-005
r_P, RFU/μM	*	6000	5626.76	115.802
[I], μM	1	0.375	0.761661	0.0158411
[I], μM	2	0.3125	0.448608	0.00634211
[I], μM	3	0.25	0.332146	0.00393436
[I], μM	4	0.1875	0.231282	0.00232305
[I], μM	5	0.15625	0.201606	0.00193031
[I], μM	6	0.125	0.147152	0.00130279
offset, RFU	1	-300	10.4362	41.2625
offset, RFU	2	-300	-433.557	40.2033
offset, RFU	3	-300	45.7995	39.3017
offset, RFU	4	-300	-258.683	37.7828
offset, RFU	5	-300	-516.494	37.0995
offset, RFU	6	-300	-433.599	35.4578
offset, RFU	7	-300	-555.248	26.5552
offset, RFU	8	-300	-596.295	25.6726
offset, RFU	9	-300	-573.292	25.5396
offset, RFU	10	-300	-222.357	28.7403
offset, RFU	11	-300	-487.545	39.0339

CL-387785, Replicate R1



A.3.2. Replicate R2

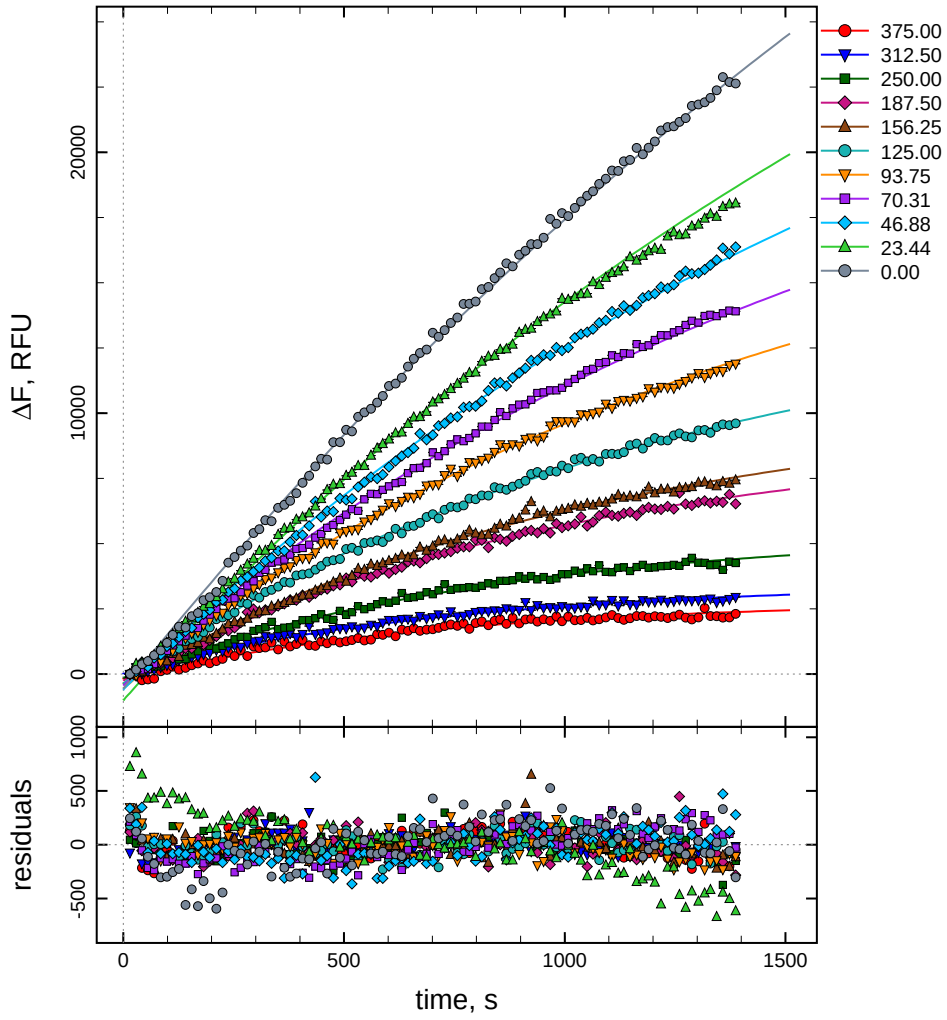
CL-387785, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 1.564 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/CL-387785/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.375 ? | label 375.00
   column   4 | offset = -300 ?  | conc I = 0.3125 ? | label 312.50
   column   5 | offset = -300 ?  | conc I = 0.25 ? | label 250.00
   column   6 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   7 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   8 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   9 | offset = -300 ?  | conc I = 0.09375 | label 93.75
   column  10 | offset = -300 ?  | conc I = 0.0703125 | label 70.31
   column  11 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  12 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/CL-387785/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

CL-387785, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0164697	0.000516856
k_{dI}, s^{-1}	*	1.564	1.82088	0.0498604
k_{inact}, s^{-1}	*	0.01	0.00175375	5.86048e-005
r_P, RFU/μM	*	6000	4928.79	121.914
[I], μM	1	0.375	0.732476	0.0184341
[I], μM	2	0.3125	0.630355	0.0141075
[I], μM	3	0.25	0.389402	0.00624832
[I], μM	4	0.1875	0.228161	0.00279037
[I], μM	5	0.15625	0.195912	0.00226436
[I], μM	6	0.125	0.139013	0.00146623
offset, RFU	1	-300	-197.994	41.7067
offset, RFU	2	-300	1.27184	41.6165
offset, RFU	3	-300	-150.422	41.2196
offset, RFU	4	-300	-262.059	40.0614
offset, RFU	5	-300	-381.252	39.5256
offset, RFU	6	-300	-364.442	38.0689
offset, RFU	7	-300	-562.424	28.4379
offset, RFU	8	-300	-448.636	27.4471
offset, RFU	9	-300	-595.738	27.0487
offset, RFU	10	-300	-1007.56	30.37
offset, RFU	11	-300	-561.355	42.6478

CL-387785, Replicate R2



A.3.3. Replicate R3

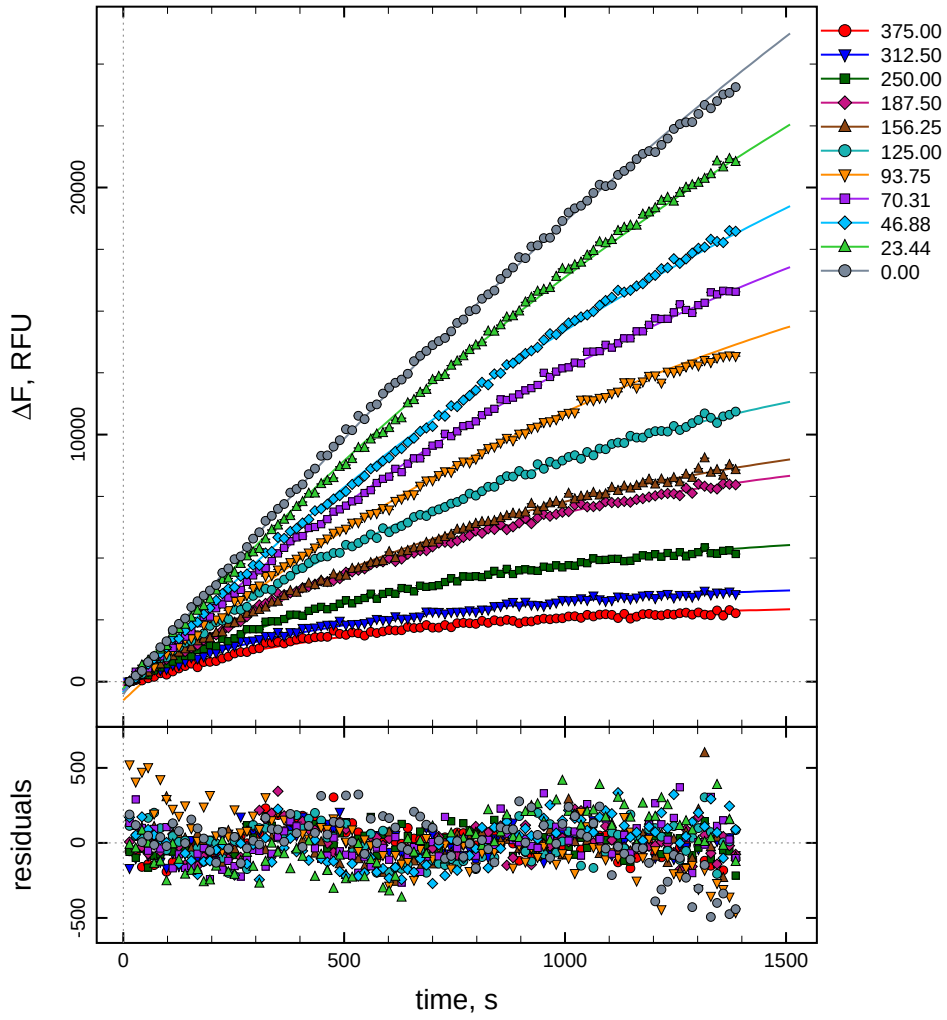
CL-387785, Replicate R3

```
;-------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 1.564 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/CL-387785/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.375 ? | label 375.00
   column   4 | offset = -300 ?  | conc I = 0.3125 ? | label 312.50
   column   5 | offset = -300 ?  | conc I = 0.25 ? | label 250.00
   column   6 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   7 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   8 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   9 | offset = -300 ?  | conc I = 0.09375 | label 93.75
   column  10 | offset = -300 ?  | conc I = 0.0703125 | label 70.31
   column  11 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  12 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/CL-387785/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

CL-387785, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0160942	0.000390508
k_{dI}, s^{-1}	*	1.564	2.54627	0.0586534
k_{inact}, s^{-1}	*	0.01	0.00224965	5.95248e-005
r_P, RFU/μM	*	6000	5345.48	103.023
[I], μM	1	0.375	0.793165	0.0154426
[I], μM	2	0.3125	0.637716	0.0105219
[I], μM	3	0.25	0.392362	0.00472487
[I], μM	4	0.1875	0.235026	0.00223493
[I], μM	5	0.15625	0.207251	0.00189122
[I], μM	6	0.125	0.149955	0.00126868
offset, RFU	1	-300	-22.1655	36.8551
offset, RFU	2	-300	75.6492	36.5744
offset, RFU	3	-300	-66.2621	35.6808
offset, RFU	4	-300	-174.489	33.9543
offset, RFU	5	-300	-348.86	33.4054
offset, RFU	6	-300	-314.633	31.9108
offset, RFU	7	-300	-756.584	24.0007
offset, RFU	8	-300	-382.993	23.099
offset, RFU	9	-300	-437.839	22.9228
offset, RFU	10	-300	-277.004	25.779
offset, RFU	11	-300	-502.326	34.8637

CL-387785, Replicate R3



A.4. Cpd-1

A.4.1. Replicate R1

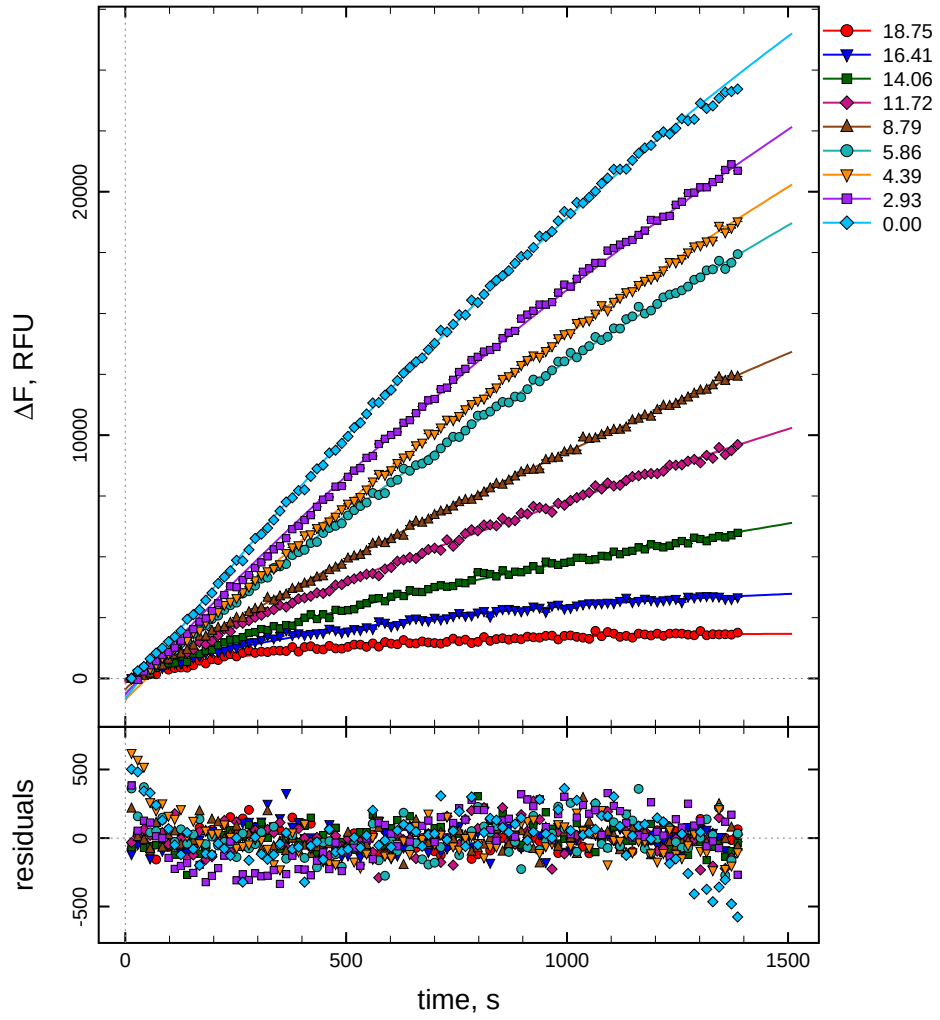
Cpd-1, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.0359 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-1/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   5 | offset = -300 ?  | conc I = 0.01875 ? | label 18.75
   column   6 | offset = -300 ?  | conc I = 0.0164063 ? | label 16.41
   column   7 | offset = -300 ?  | conc I = 0.0140625 ? | label 14.06
   column   8 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   9 | offset = -300 ?  | conc I = 0.00878906 ? | label 8.79
   column  10 | offset = -300 ?  | conc I = 0.00585938 ? | label 5.86
   column  11 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-1/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-1, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0239061	0.000624254
k_{dI}, s^{-1}	*	0.0359	0.0121234	0.00104045
k_{inact}, s^{-1}	*	0.01	0.00379312	0.00043789
[E], μM	*	0.02	0.015435	0.000155042
r_P, RFU/μM	*	6000	4925.06	76.9044
[I], μM	1	0.01875	0.0183561	0.000568436
[I], μM	2	0.0164063	0.0156232	0.000284306
[I], μM	3	0.0140625	0.0133569	0.000160648
[I], μM	4	0.0117188	0.0109796	0.00010845
[I], μM	5	0.00878906	0.00898448	8.15136e-005
[I], μM	6	0.00585938	0.0056111	4.94233e-005
offset, RFU	1	-300	-81.7419	47.8859
offset, RFU	2	-300	-61.7323	38.5328
offset, RFU	3	-300	-130.632	33.7259
offset, RFU	4	-300	-184.297	31.3963
offset, RFU	5	-300	-449.933	30.0251
offset, RFU	6	-300	-628.07	30.3297
offset, RFU	7	-300	-899.403	27.8867
offset, RFU	8	-300	-679.142	25.9495
offset, RFU	9	-300	-832.285	36.9804

Cpd-1, Replicate R1



A.4.2. Replicate R2

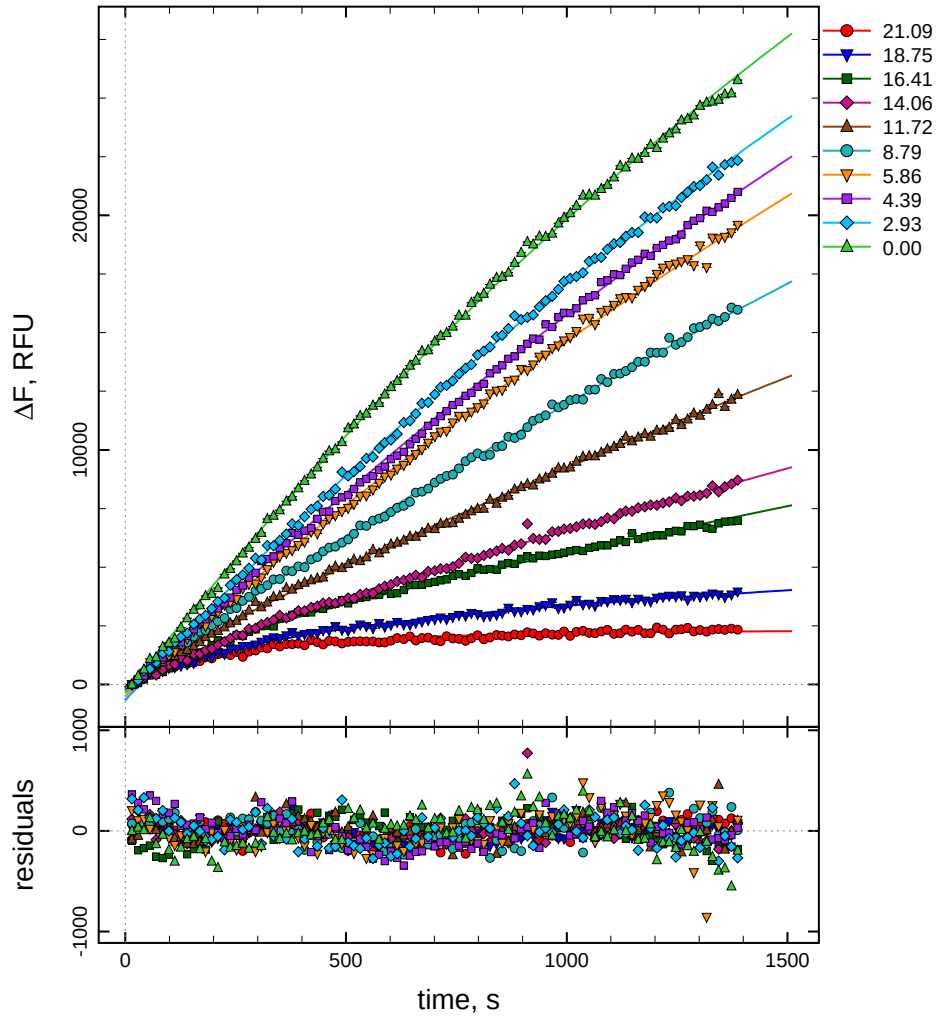
Cpd-1, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.0359 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-1/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   4 | offset = -300 ?  | conc I = 0.0210938 ? | label 21.09
   column   5 | offset = -300 ?  | conc I = 0.01875 ? | label 18.75
   column   6 | offset = -300 ?  | conc I = 0.0164063 ? | label 16.41
   column   7 | offset = -300 ?  | conc I = 0.0140625 ? | label 14.06
   column   8 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   9 | offset = -300 ?  | conc I = 0.00878906 ? | label 8.79
   column  10 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  11 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-1/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-1, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0173922	0.000439551
k_{dI}, s^{-1}	*	0.0359	0.0507615	0.00923105
k_{inact}, s^{-1}	*	0.01	0.0105624	0.00161588
[E], μM	*	0.02	0.0197861	0.000179127
r_P, RFU/μM	*	6000	5334.73	83.5198
[I], μM	1	0.0210938	0.0216772	0.000343602
[I], μM	2	0.01875	0.0189775	0.000203757
[I], μM	3	0.0164063	0.0161658	0.00014722
[I], μM	4	0.0140625	0.014823	0.000129623
[I], μM	5	0.0117188	0.012113	9.97169e-005
[I], μM	6	0.00878906	0.00896818	7.11603e-005
offset, RFU	1	-300	-96.3796	54.0056
offset, RFU	2	-300	-224.312	46.3446
offset, RFU	3	-300	-115.266	42.7099
offset, RFU	4	-300	-341.191	40.7824
offset, RFU	5	-300	-161.731	36.8931
offset, RFU	6	-300	-343.733	33.5868
offset, RFU	7	-300	-502.791	28.1319
offset, RFU	8	-300	-673.609	25.1751
offset, RFU	9	-300	-645.374	26.0197
offset, RFU	10	-300	-357.197	35.4583

Cpd-1, Replicate R2



A.4.3. Replicate R3

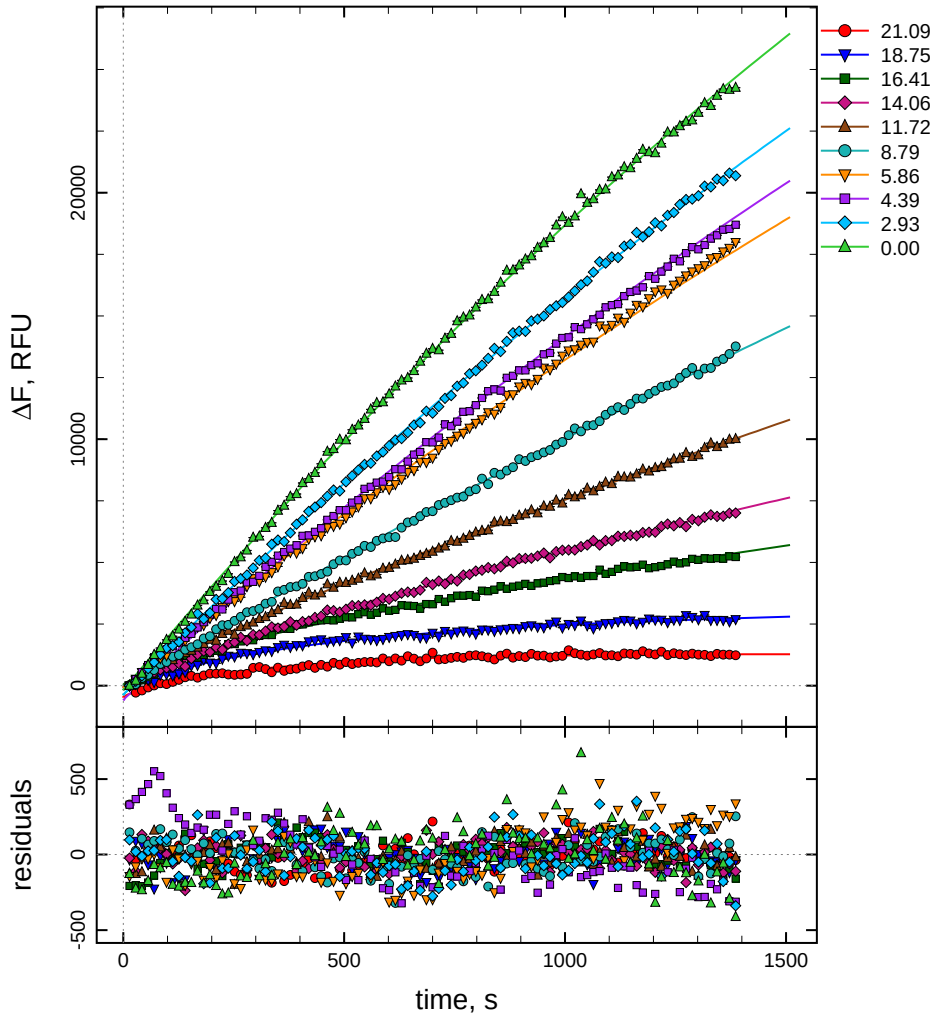
Cpd-1, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.0359 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-1/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   4 | offset = -300 ?  | conc I = 0.0210938 ? | label 21.09
   column   5 | offset = -300 ?  | conc I = 0.01875 ? | label 18.75
   column   6 | offset = -300 ?  | conc I = 0.0164063 ? | label 16.41
   column   7 | offset = -300 ?  | conc I = 0.0140625 ? | label 14.06
   column   8 | offset = -300 ?  | conc I = 0.0117188 ? | label 11.72
   column   9 | offset = -300 ?  | conc I = 0.00878906 ? | label 8.79
   column  10 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  11 | offset = -300 ?  | conc I = 0.00439453 | label 4.39
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-1/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-1, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0157331	0.000490659
k_{dI}, s^{-1}	*	0.0359	0.0223698	0.00299648
k_{inact}, s^{-1}	*	0.01	0.00675752	0.000898283
[E], μM	*	0.02	0.0175236	0.000139199
r_P, RFU/μM	*	6000	6018.33	132.358
[I], μM	1	0.0210938	0.0198684	0.000419786
[I], μM	2	0.01875	0.0176317	0.000228721
[I], μM	3	0.0164063	0.0152396	0.00013561
[I], μM	4	0.0140625	0.0137372	0.000110367
[I], μM	5	0.0117188	0.011647	8.62029e-005
[I], μM	6	0.00878906	0.00883807	6.21659e-005
offset, RFU	1	-300	-481.012	49.0341
offset, RFU	2	-300	-128.398	41.1858
offset, RFU	3	-300	32.031	36.5461
offset, RFU	4	-300	-162.118	34.6542
offset, RFU	5	-300	-73.9444	32.2246
offset, RFU	6	-300	-369.029	30.2344
offset, RFU	7	-300	-103.161	25.7616
offset, RFU	8	-300	-590.578	23.5817
offset, RFU	9	-300	-370.036	24.9926
offset, RFU	10	-300	-172.933	34.3552

Cpd-1, Replicate R3



A.5. Cpd-2

A.5.1. Replicate R1

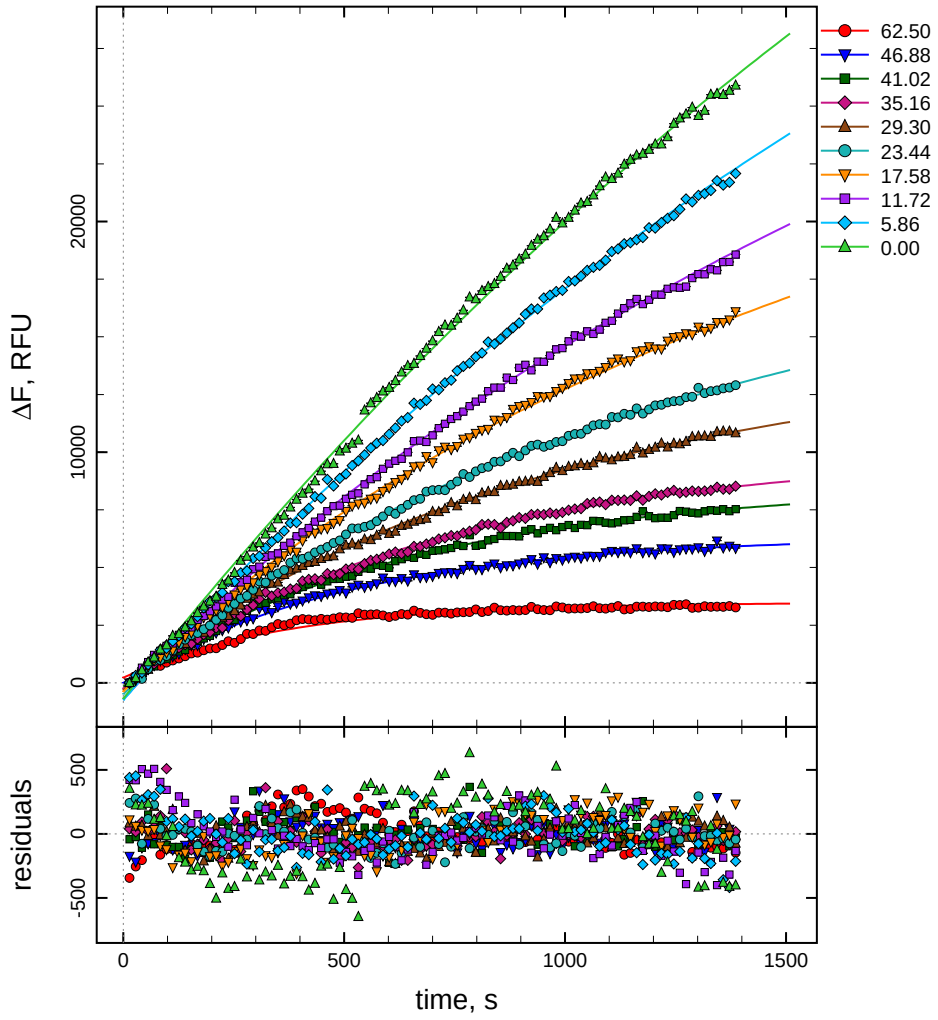
Cpd-2, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.457 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-2/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   4 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   5 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   6 | offset = -300 ?  | conc I = 0.0410156 ? | label 41.02
   column   7 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   8 | offset = -300 ?  | conc I = 0.0292969 ? | label 29.30
   column   9 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column  10 | offset = -300 ?  | conc I = 0.0175781 | label 17.58
   column  11 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  12 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-2/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-2, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0166537	0.000454751
k_{dI}, s^{-1}	*	0.457	0.528859	0.0221253
k_{inact}, s^{-1}	*	0.01	0.00449937	0.000167957
r_P, RFU/μM	*	6000	5615.78	120.901
[I], μM	1	0.0625	0.102185	0.00203765
[I], μM	2	0.046875	0.0579744	0.000730724
[I], μM	3	0.0410156	0.0449616	0.000485521
[I], μM	4	0.0351563	0.0395712	0.000399607
[I], μM	5	0.0292969	0.0301322	0.000269952
[I], μM	6	0.0234375	0.0238471	0.000198796
offset, RFU	1	-300	209.951	49.1915
offset, RFU	2	-300	-13.7793	44.6855
offset, RFU	3	-300	-166.469	42.1712
offset, RFU	4	-300	-257.592	40.8488
offset, RFU	5	-300	-322.764	38.2024
offset, RFU	6	-300	-499.627	36.3975
offset, RFU	7	-300	-387.335	31.5062
offset, RFU	8	-300	-716.046	28.6572
offset, RFU	9	-300	-756.591	32.0907
offset, RFU	10	-300	-692.973	42.0028

Cpd-2, Replicate R1



A.5.2. Replicate R2

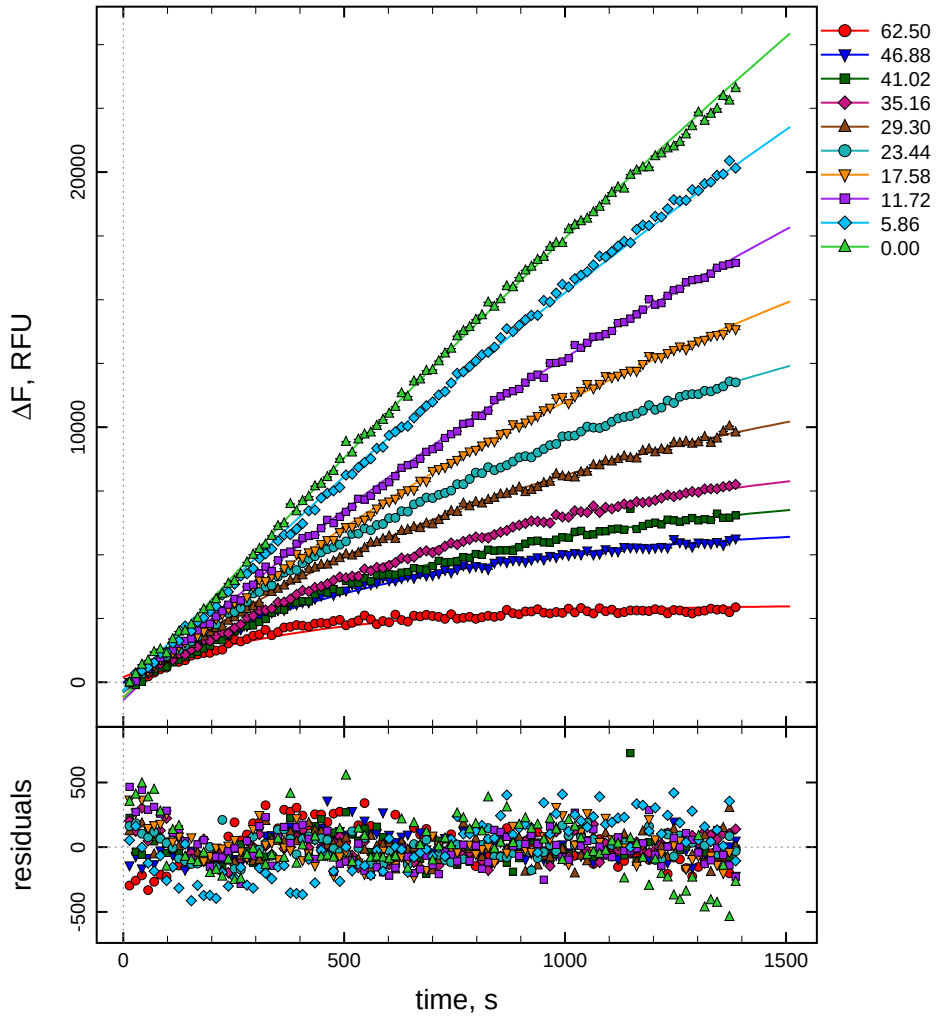
Cpd-2, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.457 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-2/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   4 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   5 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   6 | offset = -300 ?  | conc I = 0.0410156 ? | label 41.02
   column   7 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   8 | offset = -300 ?  | conc I = 0.0292969 ? | label 29.30
   column   9 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column  10 | offset = -300 ?  | conc I = 0.0175781 | label 17.58
   column  11 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  12 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-2/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-2, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.00899226	0.000446403
k_{dI}, s^{-1}	*	0.457	0.534739	0.0213622
k_{inact}, s^{-1}	*	0.01	0.00374546	0.00014022
r_P, RFU/μM	*	6000	8433.84	369.018
[I], μM	1	0.0625	0.114342	0.00237305
[I], μM	2	0.046875	0.0586633	0.000729134
[I], μM	3	0.0410156	0.0471991	0.000519422
[I], μM	4	0.0351563	0.0406681	0.000416653
[I], μM	5	0.0292969	0.0310068	0.000285692
[I], μM	6	0.0234375	0.0241292	0.000208213
offset, RFU	1	-300	195.073	42.4804
offset, RFU	2	-300	-9.03789	38.4916
offset, RFU	3	-300	-393.067	36.7668
offset, RFU	4	-300	-379.963	35.53
offset, RFU	5	-300	-318.675	33.386
offset, RFU	6	-300	-371.471	31.8154
offset, RFU	7	-300	-591.132	27.4023
offset, RFU	8	-300	-705.852	25.2501
offset, RFU	9	-300	-310.196	28.058
offset, RFU	10	-300	-627.312	36.8419

Cpd-2, Replicate R2



A.5.3. Replicate R3

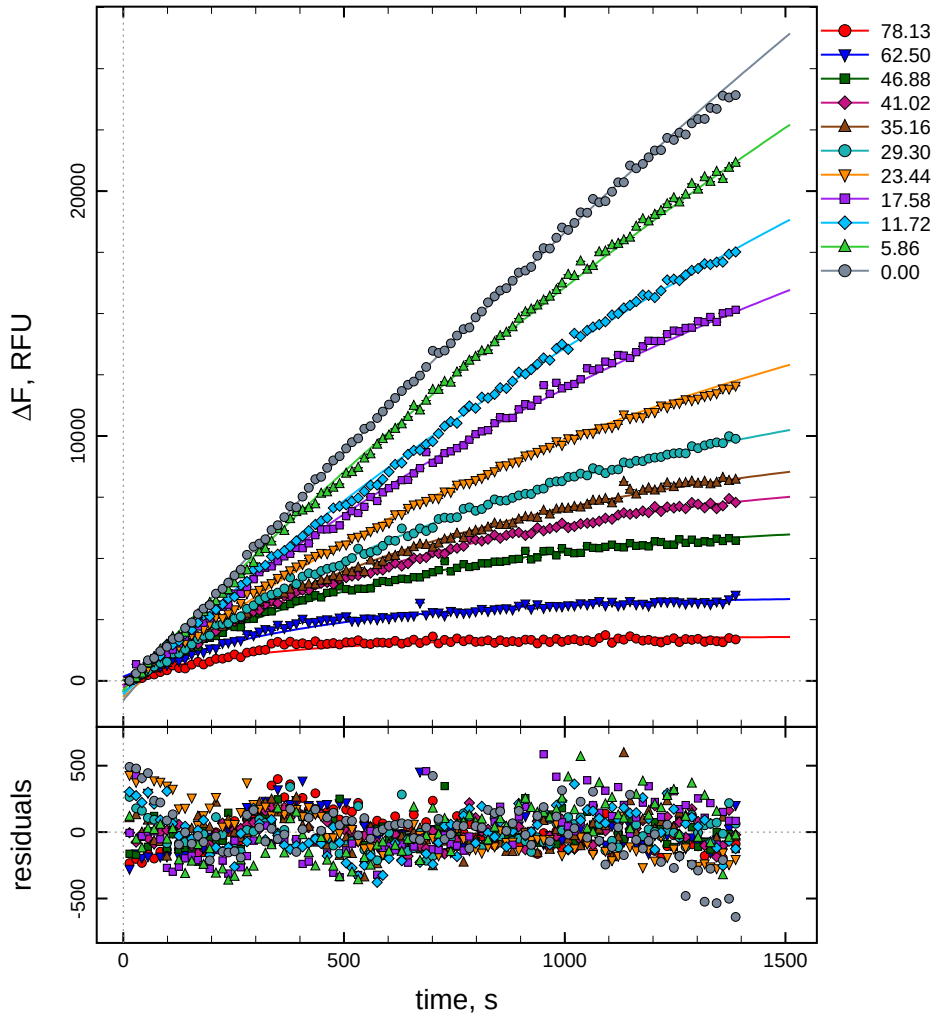
Cpd-2, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.457 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-2/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.078125 ? | label 78.13
   column   4 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   5 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   6 | offset = -300 ?  | conc I = 0.0410156 ? | label 41.02
   column   7 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   8 | offset = -300 ?  | conc I = 0.0292969 ? | label 29.30
   column   9 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  10 | offset = -300 ?  | conc I = 0.0175781 | label 17.58
   column  11 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  12 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-2/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-2, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.011808	0.000460559
k_{dI}, s^{-1}	*	0.457	0.479009	0.018027
k_{inact}, s^{-1}	*	0.01	0.00355036	0.000126047
r_P, RFU/μM	*	6000	6981.35	230.654
[I], μM	1	0.078125	0.192377	0.00688626
[I], μM	2	0.0625	0.103499	0.00191747
[I], μM	3	0.046875	0.0576614	0.000643645
[I], μM	4	0.0410156	0.0451516	0.00043064
[I], μM	5	0.0351563	0.0392165	0.000347288
[I], μM	6	0.0292969	0.0317126	0.000257328
offset, RFU	1	-300	163.365	47.9769
offset, RFU	2	-300	165.507	45.0087
offset, RFU	3	-300	4.54318	41.2669
offset, RFU	4	-300	-173.724	39.2729
offset, RFU	5	-300	-294.082	38.0843
offset, RFU	6	-300	-469.363	36.3579
offset, RFU	7	-300	-654.03	28.9123
offset, RFU	8	-300	-234.379	26.7583
offset, RFU	9	-300	-561.109	26.4948
offset, RFU	10	-300	-393.861	30.5843
offset, RFU	11	-300	-790.311	38.9272

Cpd-2, Replicate R3



A.6. Cpd-3

A.6.1. Replicate R1

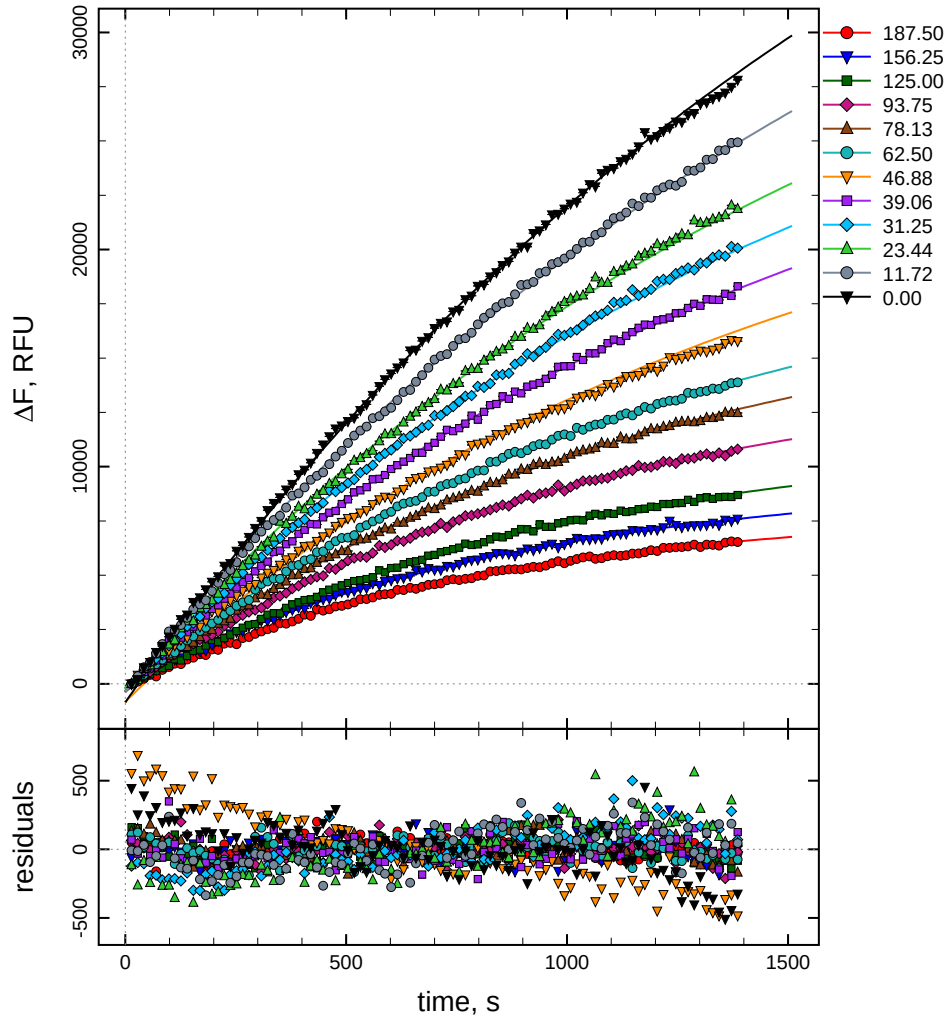
Cpd-3, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.7596 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-3/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   3 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   4 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   5 | offset = -300 ?  | conc I = 0.09375 ? | label 93.75
   column   6 | offset = -300 ?  | conc I = 0.078125 ? | label 78.13
   column   7 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   8 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column   9 | offset = -300 ?  | conc I = 0.0390625 | label 39.06
   column  10 | offset = -300 ?  | conc I = 0.03125 | label 31.25
   column  11 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  12 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-3/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-3, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.025377	0.000365296
k_{dI}, s^{-1}	*	0.7596	0.918107	0.0181323
k_{inact}, s^{-1}	*	0.01	0.00182026	4.01686e-005
r_P, RFU/μM	*	6000	4414.69	43.87
[I], μM	1	0.1875	0.179829	0.00183509
[I], μM	2	0.15625	0.154198	0.0014515
[I], μM	3	0.125	0.124998	0.00107052
[I], μM	4	0.09375	0.09633	0.000752163
[I], μM	5	0.078125	0.0763414	0.000563316
[I], μM	6	0.0625	0.0653923	0.000471296
offset, RFU	1	-300	-203.081	37.2789
offset, RFU	2	-300	-108.501	36.9522
offset, RFU	3	-300	-348.248	36.3601
offset, RFU	4	-300	-281.351	35.3935
offset, RFU	5	-300	-373.252	34.43
offset, RFU	6	-300	-363.881	33.8097
offset, RFU	7	-300	-842.206	24.6438
offset, RFU	8	-300	-372.699	23.6816
offset, RFU	9	-300	-197.577	23.0842
offset, RFU	10	-300	-222.435	23.4981
offset, RFU	11	-300	-362.834	27.8377
offset, RFU	12	-300	-853.486	37.8179

Cpd-3, Replicate R1



A.6.2. Replicate R2

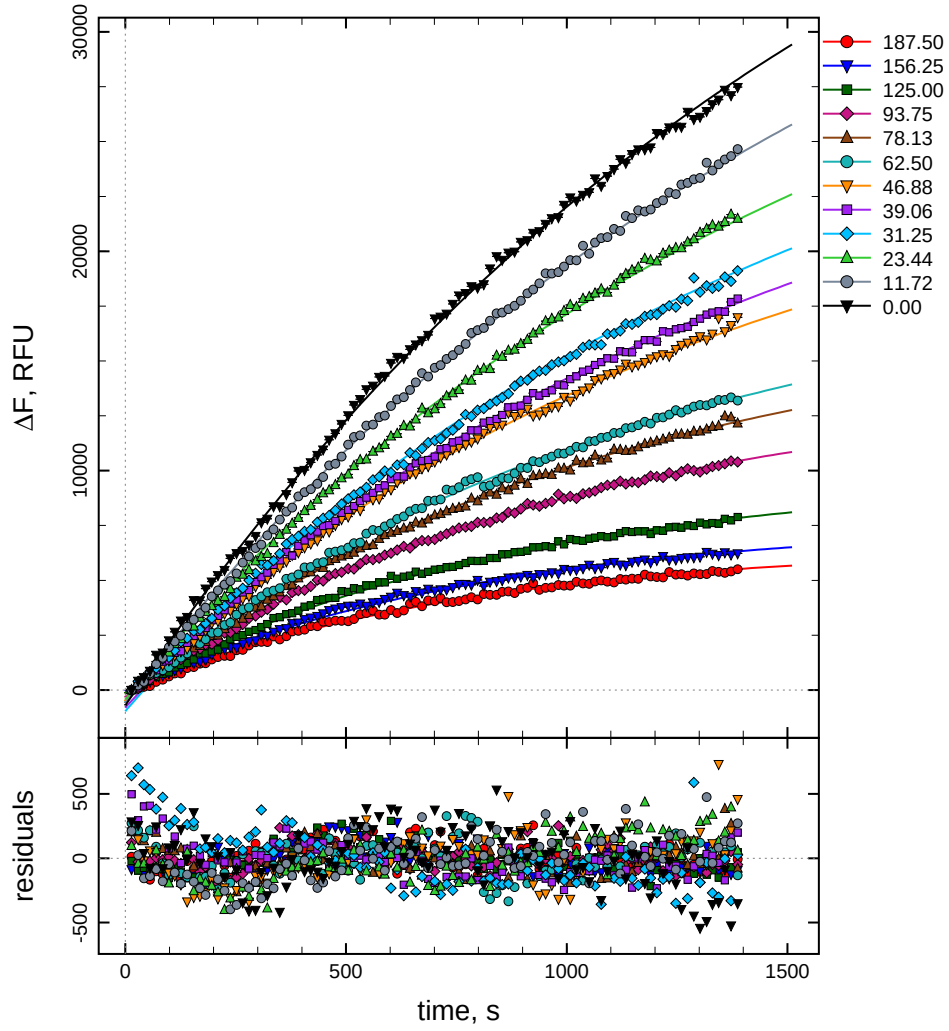
Cpd-3, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.7596 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-3/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   3 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   4 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   5 | offset = -300 ?  | conc I = 0.09375 ? | label 93.75
   column   6 | offset = -300 ?  | conc I = 0.078125 ? | label 78.13
   column   7 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   8 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column   9 | offset = -300 ?  | conc I = 0.0390625 | label 39.06
   column  10 | offset = -300 ?  | conc I = 0.03125 | label 31.25
   column  11 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  12 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-3/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-3, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0290507	0.000407372
k_{dI}, s^{-1}	*	0.7596	0.900672	0.0195506
k_{inact}, s^{-1}	*	0.01	0.00183454	4.38406e-005
r_P, RFU/μM	*	6000	3967.48	36.3044
[I], μM	1	0.1875	0.220236	0.00279863
[I], μM	2	0.15625	0.192226	0.0022514
[I], μM	3	0.125	0.147354	0.00150316
[I], μM	4	0.09375	0.100255	0.000880891
[I], μM	5	0.078125	0.0792198	0.000655131
[I], μM	6	0.0625	0.0687932	0.00055528
offset, RFU	1	-300	-144.69	41.4567
offset, RFU	2	-300	-59.7351	41.2558
offset, RFU	3	-300	-153.132	40.6979
offset, RFU	4	-300	-337.843	39.3666
offset, RFU	5	-300	-443.868	38.3287
offset, RFU	6	-300	-528.945	37.706
offset, RFU	7	-300	-502.414	27.3968
offset, RFU	8	-300	-807.518	26.2695
offset, RFU	9	-300	-970.032	25.5509
offset, RFU	10	-300	-442.886	25.98
offset, RFU	11	-300	-592.597	30.7871
offset, RFU	12	-300	-703.586	41.7203

Cpd-3, Replicate R2



A.6.3. Replicate R3

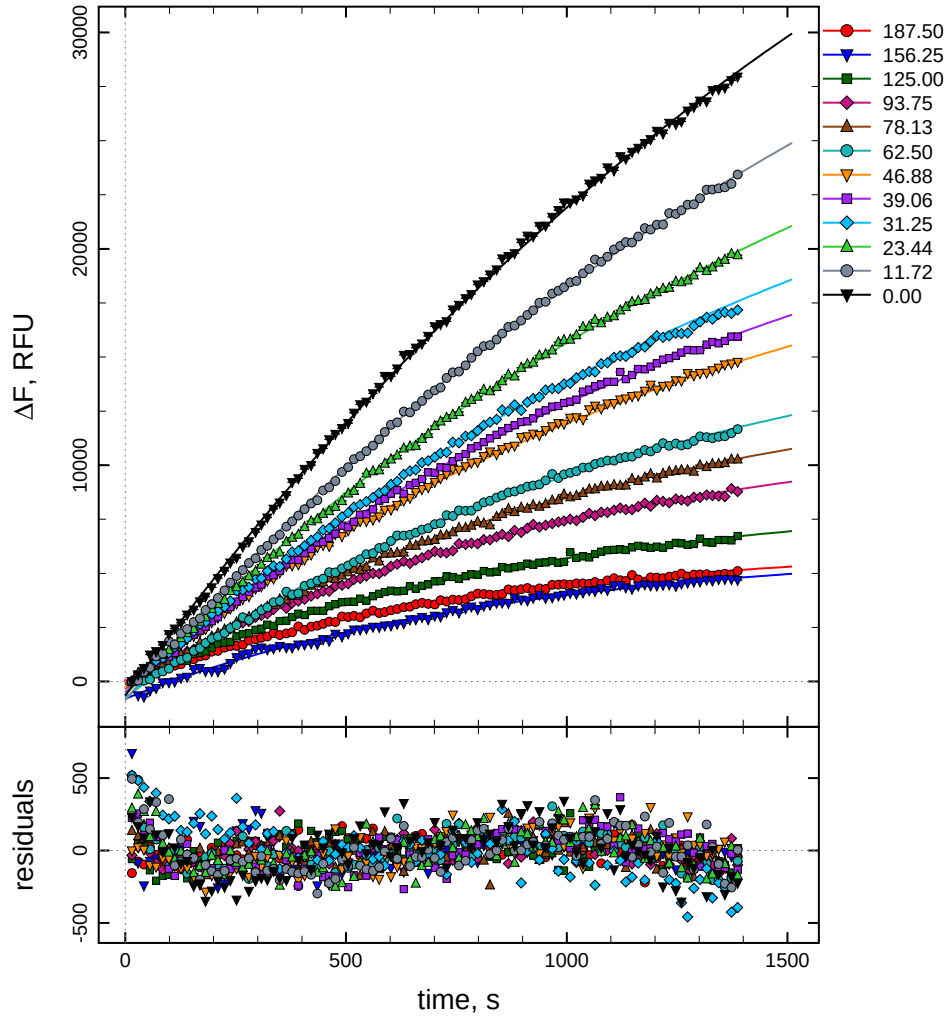
Cpd-3, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.7596 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-3/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   3 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   4 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   5 | offset = -300 ?  | conc I = 0.09375 ? | label 93.75
   column   6 | offset = -300 ?  | conc I = 0.078125 ? | label 78.13
   column   7 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   8 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column   9 | offset = -300 ?  | conc I = 0.0390625 | label 39.06
   column  10 | offset = -300 ?  | conc I = 0.03125 | label 31.25
   column  11 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  12 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-3/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-3, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0232375	0.000350573
k_{dI}, s^{-1}	*	0.7596	0.638717	0.0110989
k_{inact}, s^{-1}	*	0.01	0.00156245	3.17145e-005
r_P, RFU/μM	*	6000	4668.08	50.2201
[I], μM	1	0.1875	0.188475	0.0020332
[I], μM	2	0.15625	0.170788	0.00172169
[I], μM	3	0.125	0.137167	0.00120432
[I], μM	4	0.09375	0.0978411	0.000721911
[I], μM	5	0.078125	0.0791385	0.0005373
[I], μM	6	0.0625	0.0631775	0.000402497
offset, RFU	1	-300	35.1125	33.1378
offset, RFU	2	-300	-802.804	33.0561
offset, RFU	3	-300	-92.9366	32.8081
offset, RFU	4	-300	-156.2	32.1674
offset, RFU	5	-300	-348.58	31.5837
offset, RFU	6	-300	-756.717	30.8827
offset, RFU	7	-300	-270.146	22.2928
offset, RFU	8	-300	-514.115	21.5584
offset, RFU	9	-300	-828.181	20.9774
offset, RFU	10	-300	-621.949	21.1897
offset, RFU	11	-300	-866.668	25.1506
offset, RFU	12	-300	-653.024	34.9825

Cpd-3, Replicate R3



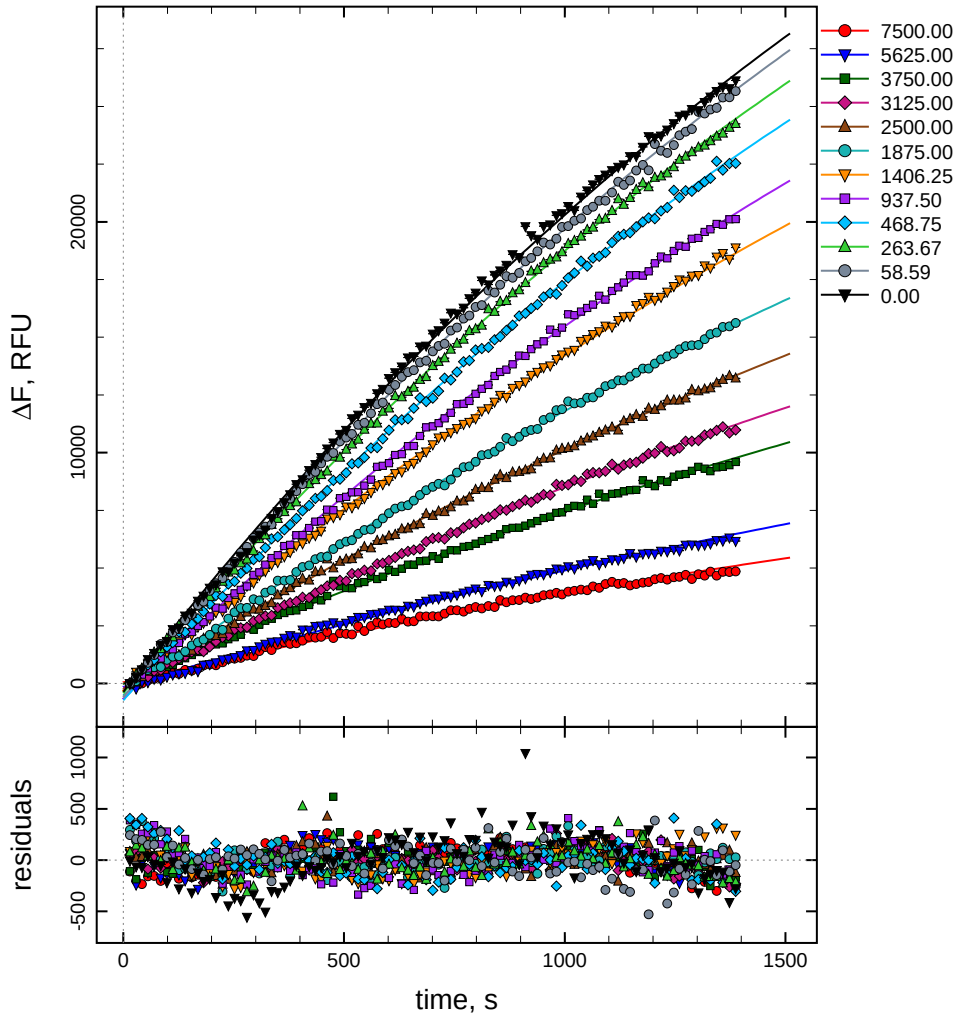
Cpd-4, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 18.34 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-4/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 7.5 ? | label 7500.00
   column   3 | offset = -300 ?  | conc I = 5.625 ? | label 5625.00
   column   4 | offset = -300 ?  | conc I = 3.75 ? | label 3750.00
   column   5 | offset = -300 ?  | conc I = 3.125 ? | label 3125.00
   column   6 | offset = -300 ?  | conc I = 2.5 ? | label 2500.00
   column   7 | offset = -300 ?  | conc I = 1.875 ? | label 1875.00
   column   8 | offset = -300 ?  | conc I = 1.40625 | label 1406.25
   column   9 | offset = -300 ?  | conc I = 0.9375 | label 937.50
   column  10 | offset = -300 ?  | conc I = 0.46875 | label 468.75
   column  11 | offset = -300 ?  | conc I = 0.263672 | label 263.67
   column  12 | offset = -300 ?  | conc I = 0.0585938 | label 58.59
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-4/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-4, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0191162	0.00030457
k_{dI}, s^{-1}	*	18.34	31.6275	0.560064
k_{inact}, s^{-1}	*	0.01	0.00029864	2.05702e-005
r_P, RFU/μM	*	6000	5002.65	60.1331
[I], μM	1	7.5	14.4395	0.214449
[I], μM	2	5.625	10.2268	0.132934
[I], μM	3	3.75	5.86286	0.0665217
[I], μM	4	3.125	4.56112	0.0499837
[I], μM	5	2.5	3.29115	0.0352145
[I], μM	6	1.875	2.2607	0.0242659
offset, RFU	1	-300	43.8894	32.9168
offset, RFU	2	-300	-154.626	33.6195
offset, RFU	3	-300	-20.4509	34.6722
offset, RFU	4	-300	-203.005	34.9458
offset, RFU	5	-300	-262.41	35.0136
offset, RFU	6	-300	-512.73	34.747
offset, RFU	7	-300	-344.782	28.4145
offset, RFU	8	-300	-674.542	25.0291
offset, RFU	9	-300	-730.409	23.2546
offset, RFU	10	-300	-418.893	24.8343
offset, RFU	11	-300	-609.163	29.155
offset, RFU	12	-300	-365.33	30.9938

Cpd-4, Replicate R1



A.7.2. Replicate R2

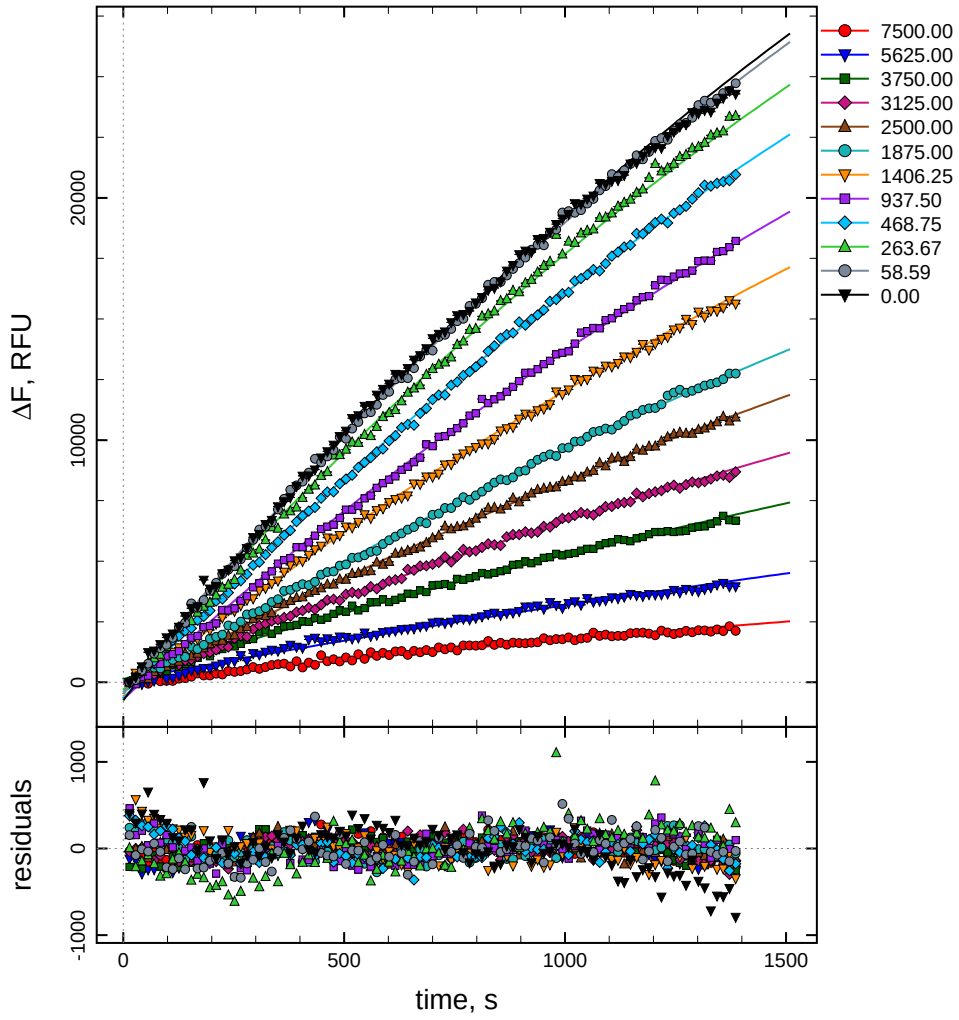
Cpd-4, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 18.34 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-4/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 7.5 ? | label 7500.00
   column   3 | offset = -300 ?  | conc I = 5.625 ? | label 5625.00
   column   4 | offset = -300 ?  | conc I = 3.75 ? | label 3750.00
   column   5 | offset = -300 ?  | conc I = 3.125 ? | label 3125.00
   column   6 | offset = -300 ?  | conc I = 2.5 ? | label 2500.00
   column   7 | offset = -300 ?  | conc I = 1.875 ? | label 1875.00
   column   8 | offset = -300 ?  | conc I = 1.40625 | label 1406.25
   column   9 | offset = -300 ?  | conc I = 0.9375 | label 937.50
   column  10 | offset = -300 ?  | conc I = 0.46875 | label 468.75
   column  11 | offset = -300 ?  | conc I = 0.263672 | label 263.67
   column  12 | offset = -300 ?  | conc I = 0.0585938 | label 58.59
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-4/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-4, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0188405	0.00037798
k_{dI}, s^{-1}	*	18.34	22.2006	0.450561
k_{inact}, s^{-1}	*	0.01	0.000220093	2.35168e-005
r_P, RFU/μM	*	6000	4881.66	74.206
[I], μM	1	7.5	24.7081	0.693031
[I], μM	2	5.625	12.9388	0.236284
[I], μM	3	3.75	6.93952	0.0958055
[I], μM	4	3.125	4.71928	0.0585552
[I], μM	5	2.5	3.18397	0.0370832
[I], μM	6	1.875	2.3882	0.0272882
offset, RFU	1	-300	3.11697	35.2474
offset, RFU	2	-300	69.8729	36.1827
offset, RFU	3	-300	131.357	37.667
offset, RFU	4	-300	-73.0556	38.6079
offset, RFU	5	-300	-291.474	39.2661
offset, RFU	6	-300	-400.683	39.4026
offset, RFU	7	-300	-585.232	31.9546
offset, RFU	8	-300	-699.02	28.7403
offset, RFU	9	-300	-654.115	26.1152
offset, RFU	10	-300	-303.565	27.6408
offset, RFU	11	-300	-480.173	33.3542
offset, RFU	12	-300	-740.531	36.0178

Cpd-4, Replicate R2



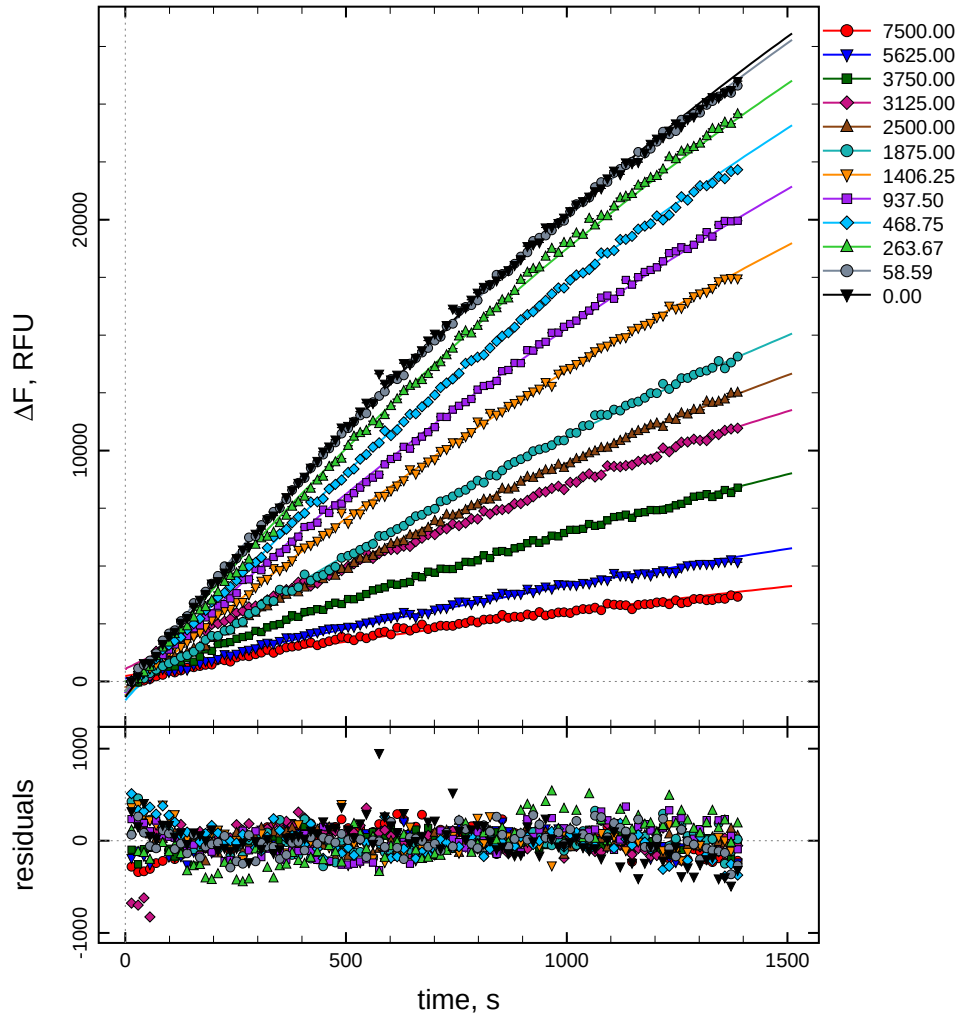
Cpd-4, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 18.34 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-4/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 7.5 ? | label 7500.00
   column   3 | offset = -300 ?  | conc I = 5.625 ? | label 5625.00
   column   4 | offset = -300 ?  | conc I = 3.75 ? | label 3750.00
   column   5 | offset = -300 ?  | conc I = 3.125 ? | label 3125.00
   column   6 | offset = -300 ?  | conc I = 2.5 ? | label 2500.00
   column   7 | offset = -300 ?  | conc I = 1.875 ? | label 1875.00
   column   8 | offset = -300 ?  | conc I = 1.40625 | label 1406.25
   column   9 | offset = -300 ?  | conc I = 0.9375 | label 937.50
   column  10 | offset = -300 ?  | conc I = 0.46875 | label 468.75
   column  11 | offset = -300 ?  | conc I = 0.263672 | label 263.67
   column  12 | offset = -300 ?  | conc I = 0.0585938 | label 58.59
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-4/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-4, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0202295	0.000332268
k_{dI}, s^{-1}	*	18.34	26.9047	0.498127
k_{inact}, s^{-1}	*	0.01	0.000291777	2.17556e-005
r_P, RFU/μM	*	6000	4834.38	58.8779
[I], μM	1	7.5	18.629	0.354106
[I], μM	2	5.625	11.9865	0.180147
[I], μM	3	3.75	6.44045	0.0780514
[I], μM	4	3.125	4.62796	0.0525061
[I], μM	5	2.5	3.30792	0.0361371
[I], μM	6	1.875	2.48628	0.0268903
offset, RFU	1	-300	237.117	34.4245
offset, RFU	2	-300	123.67	35.2358
offset, RFU	3	-300	-6.91519	36.6576
offset, RFU	4	-300	546.409	37.2505
offset, RFU	5	-300	-263.681	37.5571
offset, RFU	6	-300	-610.959	37.5157
offset, RFU	7	-300	-596.388	30.4617
offset, RFU	8	-300	-498.132	27.0684
offset, RFU	9	-300	-814.261	24.8871
offset, RFU	10	-300	-437.552	26.5018
offset, RFU	11	-300	-412.939	31.5015
offset, RFU	12	-300	-674.408	33.7102

Cpd-4, Replicate R3



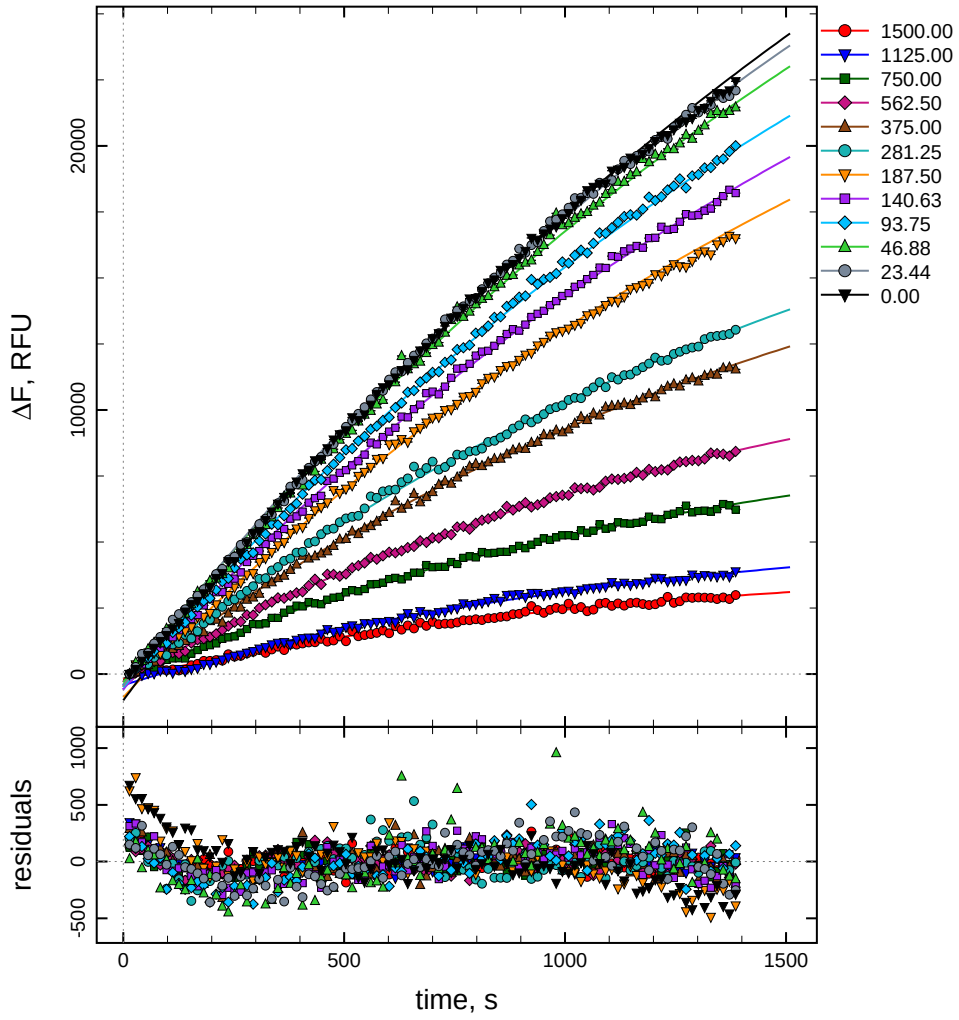
Cpd-5, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 4.279 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-5/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 1.5 ? | label 1500.00
   column   3 | offset = -300 ?  | conc I = 1.125 ? | label 1125.00
   column   4 | offset = -300 ?  | conc I = 0.75 ? | label 750.00
   column   5 | offset = -300 ?  | conc I = 0.5625 ? | label 562.50
   column   6 | offset = -300 ?  | conc I = 0.375 ? | label 375.00
   column   7 | offset = -300 ?  | conc I = 0.28125 ? | label 281.25
   column   8 | offset = -300 ?  | conc I = 0.1875 | label 187.50
   column   9 | offset = -300 ?  | conc I = 0.140625 | label 140.63
   column  10 | offset = -300 ?  | conc I = 0.09375 | label 93.75
   column  11 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  12 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-5/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-5, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0207495	0.000358694
k_{dI}, s^{-1}	*	4.279	7.07448	0.169541
k_{inact}, s^{-1}	*	0.01	0.00103473	3.97066e-005
r_P, RFU/μM	*	6000	4172	53.3542
[I], μM	1	1.5	3.2956	0.074631
[I], μM	2	1.125	2.31976	0.0437174
[I], μM	3	0.75	1.3229	0.0203203
[I], μM	4	0.5625	0.900674	0.0126859
[I], μM	5	0.375	0.508106	0.00668443
[I], μM	6	0.28125	0.414752	0.00541407
offset, RFU	1	-300	-185.87	37.8921
offset, RFU	2	-300	-420.01	38.341
offset, RFU	3	-300	-234.242	38.927
offset, RFU	4	-300	-290.263	38.798
offset, RFU	5	-300	-471.777	37.4559
offset, RFU	6	-300	-400.532	36.7858
offset, RFU	7	-300	-876.123	28.4408
offset, RFU	8	-300	-575.986	26.0539
offset, RFU	9	-300	-499.288	24.7672
offset, RFU	10	-300	-317.188	26.5274
offset, RFU	11	-300	-453.703	29.2437
offset, RFU	12	-300	-989.825	33.4199

Cpd-5, Replicate R1



A.8.2. Replicate R2

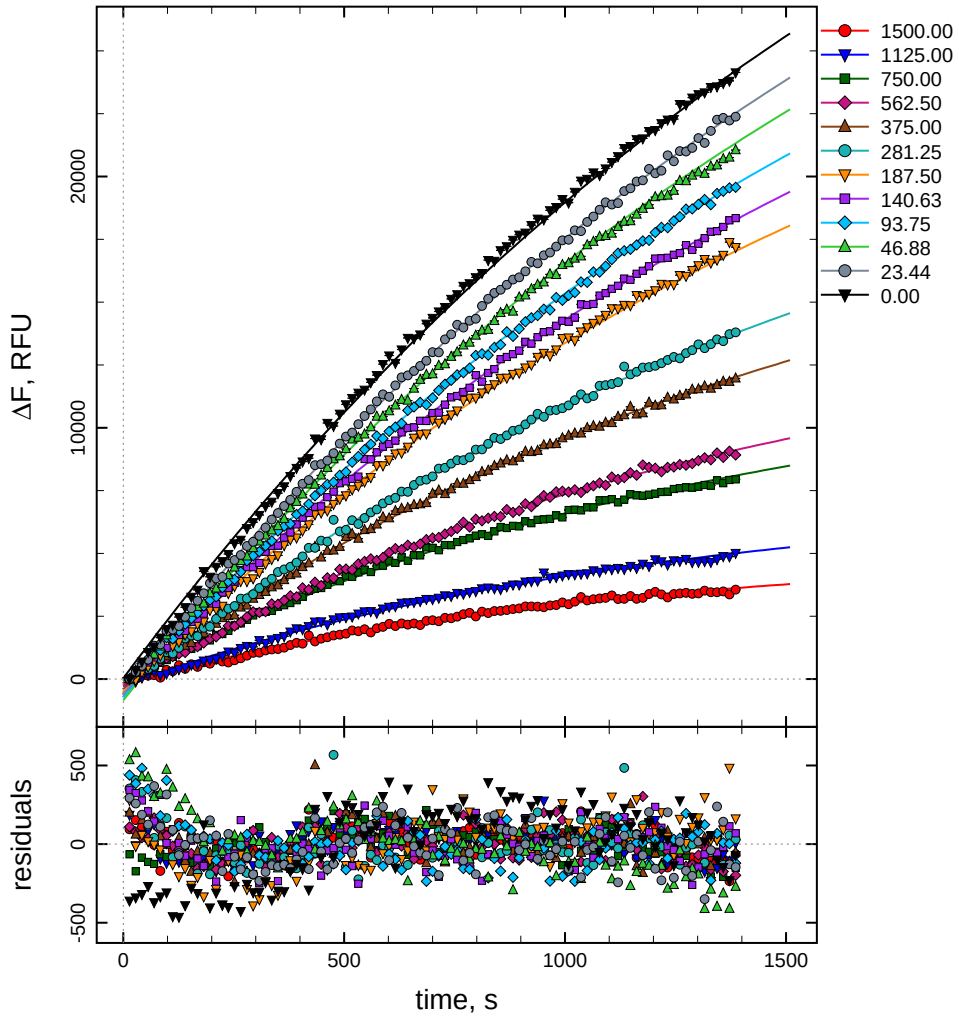
Cpd-5, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 4.279 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-5/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 1.5 ? | label 1500.00
   column   3 | offset = -300 ?  | conc I = 1.125 ? | label 1125.00
   column   4 | offset = -300 ?  | conc I = 0.75 ? | label 750.00
   column   5 | offset = -300 ?  | conc I = 0.5625 ? | label 562.50
   column   6 | offset = -300 ?  | conc I = 0.375 ? | label 375.00
   column   7 | offset = -300 ?  | conc I = 0.28125 ? | label 281.25
   column   8 | offset = -300 ?  | conc I = 0.1875 | label 187.50
   column   9 | offset = -300 ?  | conc I = 0.140625 | label 140.63
   column  10 | offset = -300 ?  | conc I = 0.09375 | label 93.75
   column  11 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  12 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-5/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-5, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0234935	0.000344772
k_{dI}, s^{-1}	*	4.279	6.14331	0.132557
k_{inact}, s^{-1}	*	0.01	0.00106875	3.57477e-005
r_P, RFU/μM	*	6000	3886.43	40.4346
[I], μM	1	1.5	2.42181	0.0448949
[I], μM	2	1.125	1.62328	0.0249086
[I], μM	3	0.75	0.910015	0.0116807
[I], μM	4	0.5625	0.739539	0.00910483
[I], μM	5	0.375	0.45374	0.00527594
[I], μM	6	0.28125	0.333554	0.00385538
offset, RFU	1	-300	-161.848	36.16
offset, RFU	2	-300	-278.072	36.6054
offset, RFU	3	-300	-70.8797	36.8135
offset, RFU	4	-300	-262.206	36.6036
offset, RFU	5	-300	-394.461	35.4971
offset, RFU	6	-300	-570.765	34.5929
offset, RFU	7	-300	-535.729	26.9592
offset, RFU	8	-300	-616.525	24.7432
offset, RFU	9	-300	-728.542	23.4914
offset, RFU	10	-300	-843.522	25.2589
offset, RFU	11	-300	-613.372	28.0582
offset, RFU	12	-300	28.9378	32.4168

Cpd-5, Replicate R2



A.8.3. Replicate R3

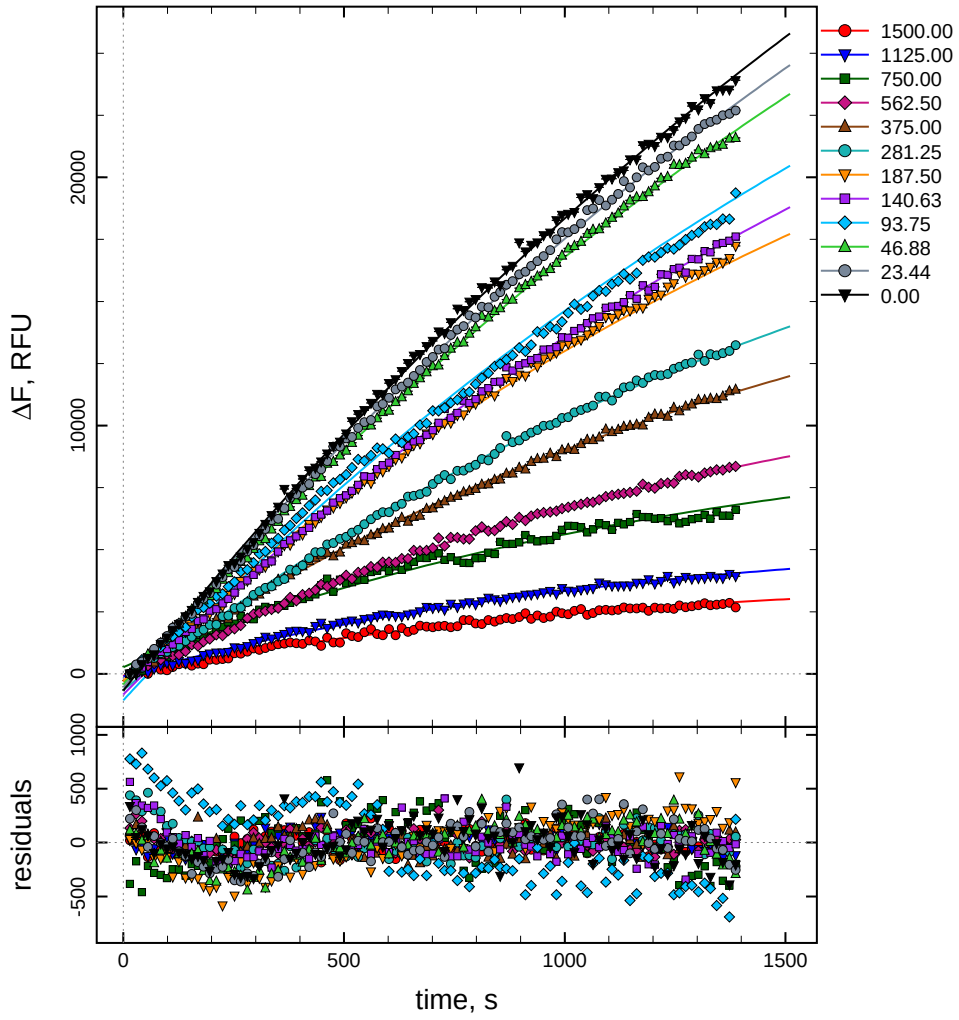
Cpd-5, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 4.279 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-5/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 1.5 ? | label 1500.00
   column   3 | offset = -300 ?  | conc I = 1.125 ? | label 1125.00
   column   4 | offset = -300 ?  | conc I = 0.75 ? | label 750.00
   column   5 | offset = -300 ?  | conc I = 0.5625 ? | label 562.50
   column   6 | offset = -300 ?  | conc I = 0.375 ? | label 375.00
   column   7 | offset = -300 ?  | conc I = 0.28125 ? | label 281.25
   column   8 | offset = -300 ?  | conc I = 0.1875 | label 187.50
   column   9 | offset = -300 ?  | conc I = 0.140625 | label 140.63
   column  10 | offset = -300 ?  | conc I = 0.09375 | label 93.75
   column  11 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  12 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Cpd-5/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Cpd-5, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0172448	0.000446555
k_{dI}, s^{-1}	*	4.279	5.49057	0.152953
k_{inact}, s^{-1}	*	0.01	0.00107057	4.82948e-005
r_P, RFU/μM	*	6000	5015.01	101.185
[I], μM	1	1.5	2.70694	0.0744752
[I], μM	2	1.125	1.84886	0.0402454
[I], μM	3	0.75	1.05593	0.017854
[I], μM	4	0.5625	0.721175	0.0108948
[I], μM	5	0.375	0.442455	0.00615402
[I], μM	6	0.28125	0.314578	0.00429676
offset, RFU	1	-300	-78.512	46.3509
offset, RFU	2	-300	-101.134	46.8085
offset, RFU	3	-300	267.897	47.3511
offset, RFU	4	-300	-282.485	47.1132
offset, RFU	5	-300	-295.237	45.714
offset, RFU	6	-300	-655.952	44.2946
offset, RFU	7	-300	-285.004	34.18
offset, RFU	8	-300	-833.426	31.6988
offset, RFU	9	-300	-1065.59	30.1319
offset, RFU	10	-300	-424.205	32.266
offset, RFU	11	-300	-545.468	35.9792
offset, RFU	12	-300	-675.5	42.027

Cpd-5, Replicate R3



A.9. Dacomitinib

A.9.1. Replicate R1

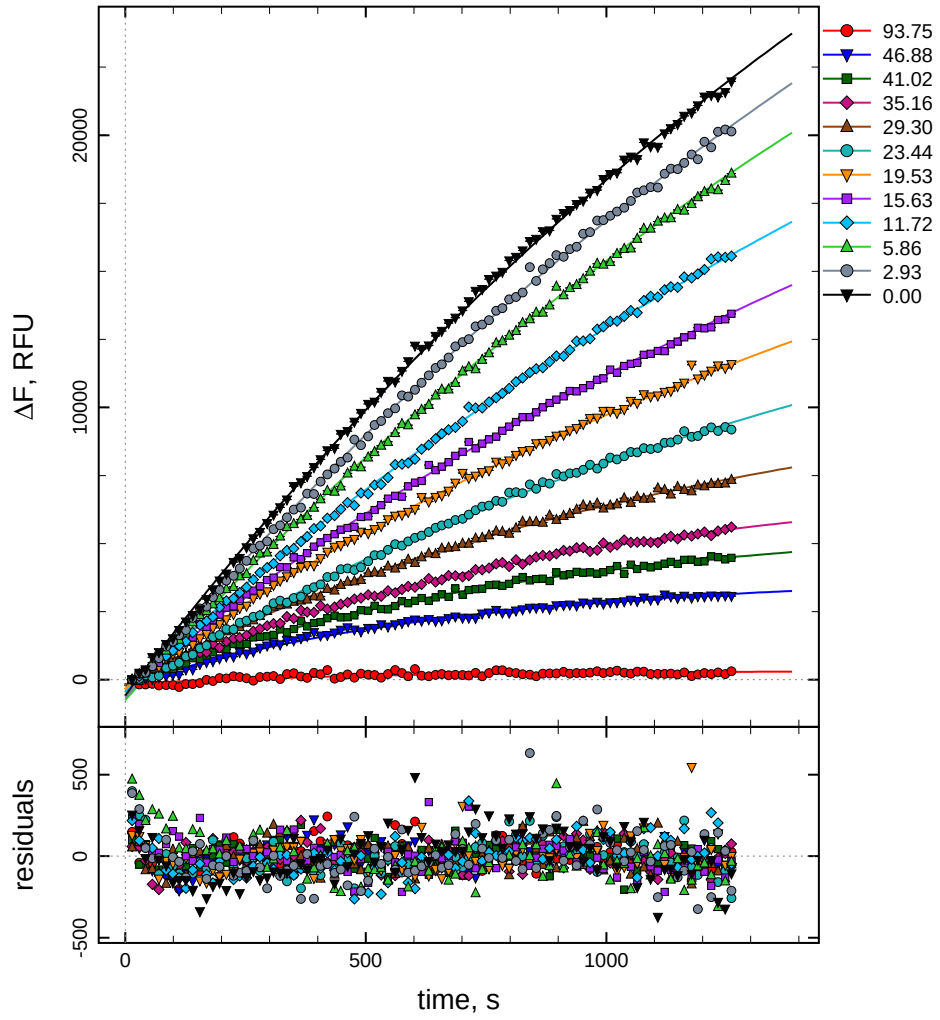
Dacomitinib, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.1642 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Dacomitinib/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.09375 ? | label 93.75
   column   3 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   4 | offset = -300 ?  | conc I = 0.0410156 ? | label 41.02
   column   5 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   6 | offset = -300 ?  | conc I = 0.0292969 ? | label 29.30
   column   7 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   8 | offset = -300 ?  | conc I = 0.0195313 | label 19.53
   column   9 | offset = -300 ?  | conc I = 0.015625 | label 15.63
   column  10 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  11 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Dacomitinib/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Dacomitinib, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0162091	0.000403746
k_{dI}, s^{-1}	*	0.1642	0.113631	0.00517692
k_{inact}, s^{-1}	*	0.01	0.00201075	8.3159e-005
[E], μM	*	0.02	0.0266869	0.000523611
r_P, RFU/μM	*	6000	4148.38	53.7147
[I], μM	1	0.09375	0.293108	0.030365
[I], μM	2	0.046875	0.0551912	0.00158922
[I], μM	3	0.0410156	0.0432169	0.000928115
[I], μM	4	0.0351563	0.037558	0.000653282
[I], μM	5	0.0292969	0.0302532	0.000354799
[I], μM	6	0.0234375	0.0236771	0.00016875
offset, RFU	1	-300	-169.375	32.3666
offset, RFU	2	-300	-227.685	31.8363
offset, RFU	3	-300	-230.798	30.6171
offset, RFU	4	-300	-205.45	29.5232
offset, RFU	5	-300	-223.437	27.869
offset, RFU	6	-300	-593.539	26.8792
offset, RFU	7	-300	-341.826	25.9149
offset, RFU	8	-300	-463.579	19.6543
offset, RFU	9	-300	-470.554	18.6807
offset, RFU	10	-300	-756.772	21.0181
offset, RFU	11	-300	-692.37	22.9025
offset, RFU	12	-300	-580.51	25.9764

Dacomitinib, Replicate R1



A.9.2. Replicate R2

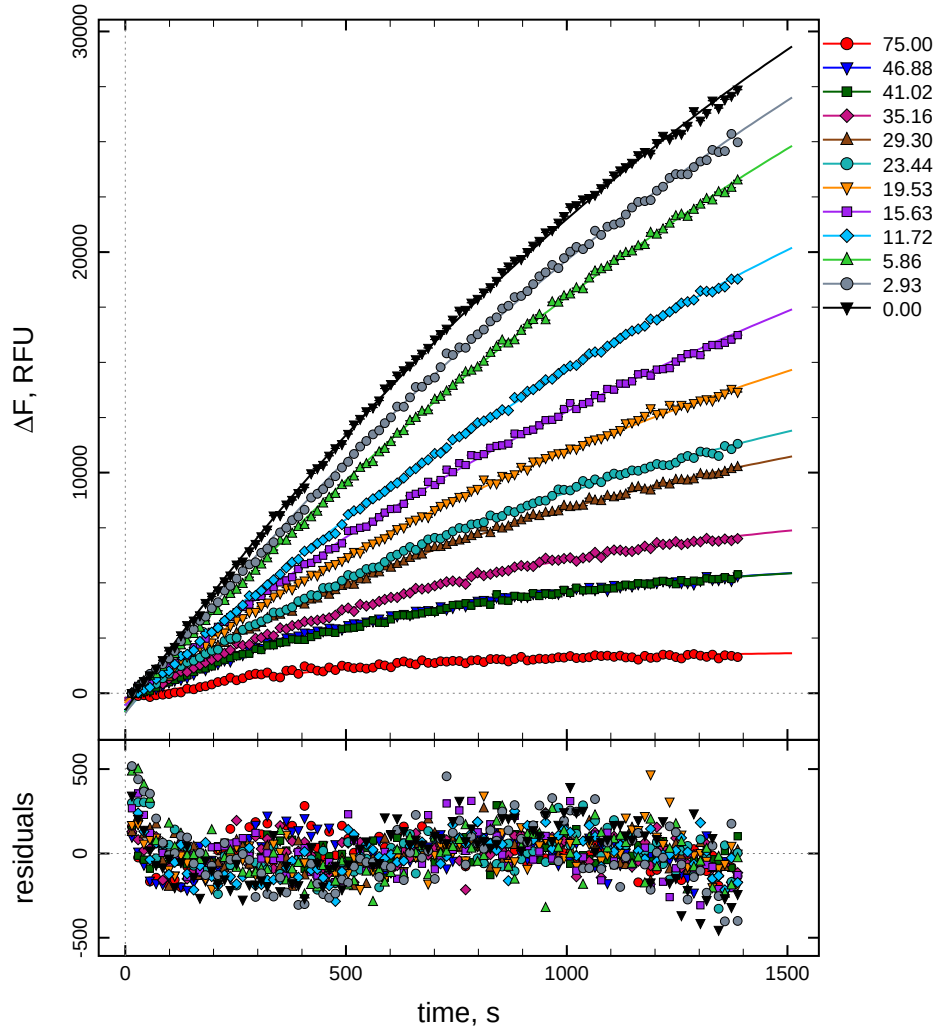
Dacomitinib, Replicate R2

```
;-------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.1642 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Dacomitinib/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.075 ? | label 75.00
   column   3 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   4 | offset = -300 ?  | conc I = 0.0410156 ? | label 41.02
   column   5 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   6 | offset = -300 ?  | conc I = 0.0292969 ? | label 29.30
   column   7 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   8 | offset = -300 ?  | conc I = 0.0195313 | label 19.53
   column   9 | offset = -300 ?  | conc I = 0.015625 | label 15.63
   column  10 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  11 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Dacomitinib/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Dacomitinib, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0196895	0.000375613
k_{dI}, s^{-1}	*	0.1642	0.119642	0.00397005
k_{inact}, s^{-1}	*	0.01	0.00223258	7.10883e-005
[E], μM	*	0.02	0.0245515	0.00036916
r_P, RFU/μM	*	6000	4465.93	39.7571
[I], μM	1	0.075	0.0915181	0.00321861
[I], μM	2	0.046875	0.0424854	0.000723155
[I], μM	3	0.0410156	0.0423041	0.00071601
[I], μM	4	0.0351563	0.0343473	0.000425549
[I], μM	5	0.0292969	0.0260863	0.00019183
[I], μM	6	0.0234375	0.0236388	0.000143235
offset, RFU	1	-300	-245.678	36.8828
offset, RFU	2	-300	-266.458	34.5876
offset, RFU	3	-300	-310.615	34.554
offset, RFU	4	-300	-325.249	32.6509
offset, RFU	5	-300	-360.924	30.3999
offset, RFU	6	-300	-545.723	29.9632
offset, RFU	7	-300	-437.102	28.5403
offset, RFU	8	-300	-561.776	22.0244
offset, RFU	9	-300	-826.019	20.8708
offset, RFU	10	-300	-853.032	23.4286
offset, RFU	11	-300	-910.473	25.6383
offset, RFU	12	-300	-762.531	29.1193

Dacomitinib, Replicate R2



A.9.3. Replicate R3

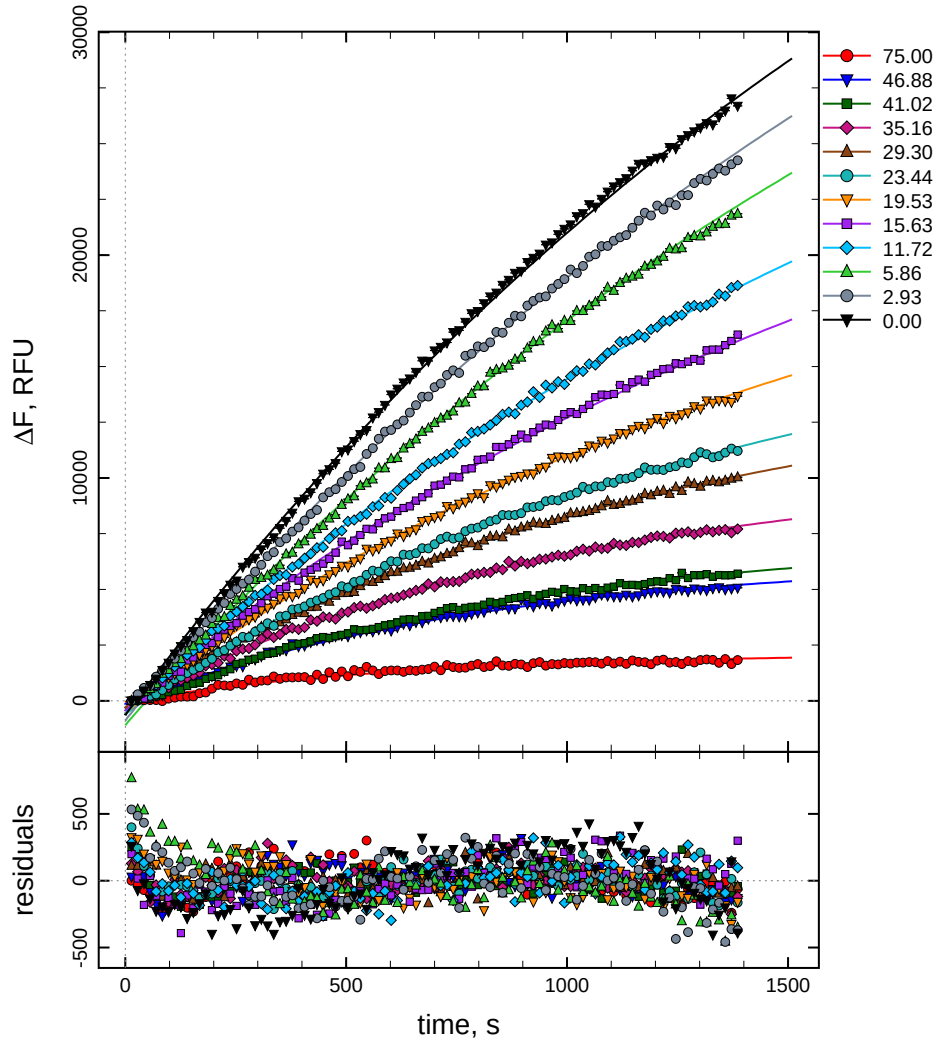
Dacomitinib, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.1642 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Dacomitinib/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.075 ? | label 75.00
   column   3 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   4 | offset = -300 ?  | conc I = 0.0410156 ? | label 41.02
   column   5 | offset = -300 ?  | conc I = 0.0351563 ? | label 35.16
   column   6 | offset = -300 ?  | conc I = 0.0292969 ? | label 29.30
   column   7 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   8 | offset = -300 ?  | conc I = 0.0195313 | label 19.53
   column   9 | offset = -300 ?  | conc I = 0.015625 | label 15.63
   column  10 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  11 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Dacomitinib/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Dacomitinib, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0228974	0.00073584
k_{dI}, s^{-1}	*	0.1642	0.178036	0.00624505
k_{inact}, s^{-1}	*	0.01	0.00182656	5.60717e-005
[E], μM	*	0.02	0.0194452	0.000582814
r_P, RFU/μM	*	6000	4630.96	53.8423
[I], μM	1	0.075	0.136062	0.00550089
[I], μM	2	0.046875	0.0544073	0.00118913
[I], μM	3	0.0410156	0.0480473	0.00093825
[I], μM	4	0.0351563	0.0369798	0.000543492
[I], μM	5	0.0292969	0.0289059	0.000304793
[I], μM	6	0.0234375	0.0246496	0.000205627
offset, RFU	1	-300	-63.1429	38.821
offset, RFU	2	-300	-169.13	37.7684
offset, RFU	3	-300	-399.84	37.2964
offset, RFU	4	-300	-310.139	35.8633
offset, RFU	5	-300	-320.741	34.341
offset, RFU	6	-300	-624.542	33.5502
offset, RFU	7	-300	-573.864	31.1683
offset, RFU	8	-300	-466.864	24.8245
offset, RFU	9	-300	-576.187	23.3449
offset, RFU	10	-300	-1097	26.2146
offset, RFU	11	-300	-883.505	28.6897
offset, RFU	12	-300	-634.78	33.6882

Dacomitinib, Replicate R3



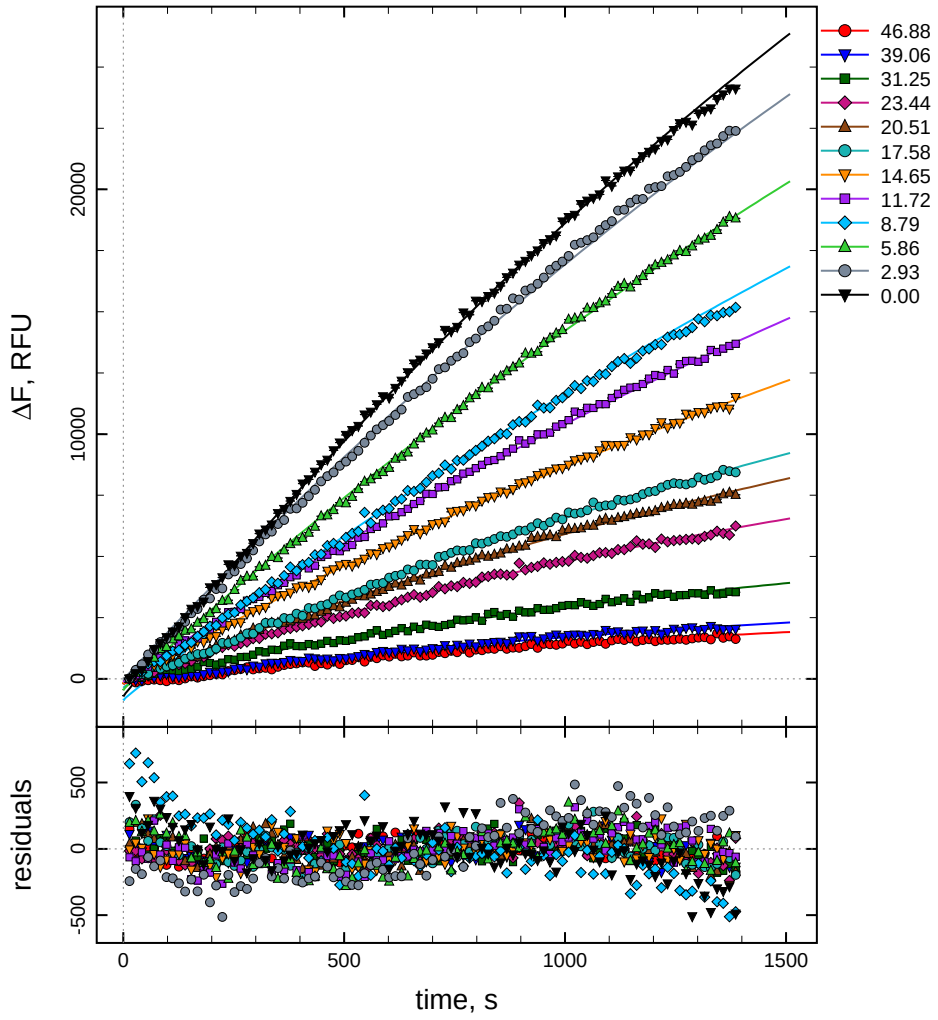
Neratinib, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.04565 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Neratinib/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   3 | offset = -300 ?  | conc I = 0.0390625 ? | label 39.06
   column   4 | offset = -300 ?  | conc I = 0.03125 ? | label 31.25
   column   5 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   6 | offset = -300 ?  | conc I = 0.0205078 ? | label 20.51
   column   7 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   8 | offset = -300 ?  | conc I = 0.0146484 | label 14.65
   column   9 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  10 | offset = -300 ?  | conc I = 0.00878906 | label 8.79
   column  11 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Neratinib/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Neratinib, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0185613	0.000674135
k_{dI}, s^{-1}	*	0.04565	0.0466497	0.00230418
k_{inact}, s^{-1}	*	0.01	0.000636741	3.9299e-005
[E], μM	*	0.02	0.016798	0.000375886
r_P, RFU/μM	*	6000	5550.37	111.775
[I], μM	1	0.046875	0.0609216	0.00244845
[I], μM	2	0.0390625	0.0536901	0.00196016
[I], μM	3	0.03125	0.0368233	0.000956164
[I], μM	4	0.0234375	0.025106	0.000393636
[I], μM	5	0.0205078	0.0209606	0.000239854
[I], μM	6	0.0175781	0.018733	0.000172982
offset, RFU	1	-300	-213.909	33.0332
offset, RFU	2	-300	-182.701	33.137
offset, RFU	3	-300	-103.092	33.4737
offset, RFU	4	-300	-126.375	33.2746
offset, RFU	5	-300	-198.514	32.8121
offset, RFU	6	-300	-373.076	32.4624
offset, RFU	7	-300	-189.572	30.2469
offset, RFU	8	-300	-151.504	23.1266
offset, RFU	9	-300	-878.406	22.7935
offset, RFU	10	-300	-456.625	24.687
offset, RFU	11	-300	-42.9159	27.6089
offset, RFU	12	-300	-711.351	35.6467

Neratinib, Replicate R1



A.10.2. Replicate R2

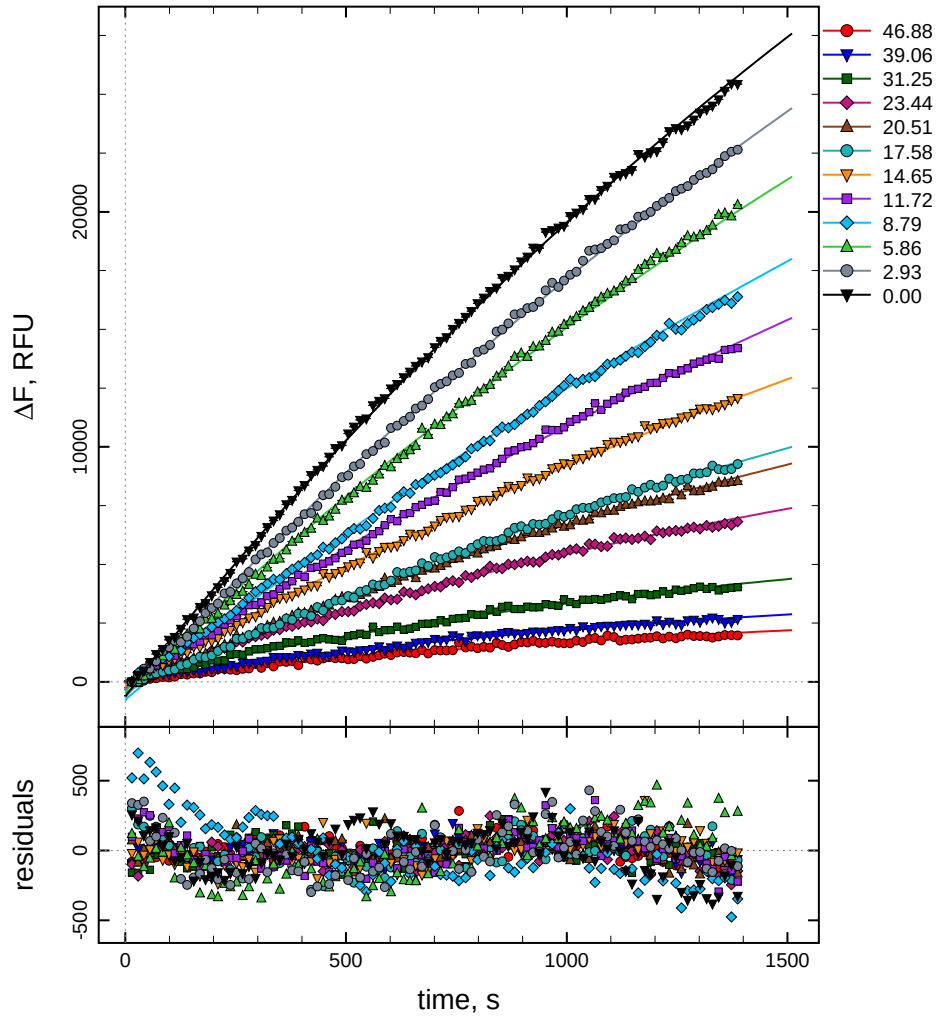
Neratinib, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.04565 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Neratinib/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   3 | offset = -300 ?  | conc I = 0.0390625 ? | label 39.06
   column   4 | offset = -300 ?  | conc I = 0.03125 ? | label 31.25
   column   5 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   6 | offset = -300 ?  | conc I = 0.0205078 ? | label 20.51
   column   7 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   8 | offset = -300 ?  | conc I = 0.0146484 | label 14.65
   column   9 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  10 | offset = -300 ?  | conc I = 0.00878906 | label 8.79
   column  11 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Neratinib/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Neratinib, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0177743	0.000569001
k_{dI}, s^{-1}	*	0.04565	0.0436048	0.00221097
k_{inact}, s^{-1}	*	0.01	0.000732102	4.16032e-005
[E], μM	*	0.02	0.0180521	0.000354708
r_P, RFU/μM	*	6000	5648.09	100.93
[I], μM	1	0.046875	0.0575187	0.00229341
[I], μM	2	0.0390625	0.0466609	0.00156464
[I], μM	3	0.03125	0.0345157	0.0008463
[I], μM	4	0.0234375	0.0235619	0.000321969
[I], μM	5	0.0205078	0.0195533	0.000183252
[I], μM	6	0.0175781	0.0180924	0.000143806
offset, RFU	1	-300	7.38841	32.7234
offset, RFU	2	-300	3.74201	32.9042
offset, RFU	3	-300	37.8538	33.0606
offset, RFU	4	-300	-65.2842	32.3121
offset, RFU	5	-300	-259.262	31.5953
offset, RFU	6	-300	-497.335	31.3134
offset, RFU	7	-300	-198.95	29.6151
offset, RFU	8	-300	-339.264	22.2738
offset, RFU	9	-300	-784.097	22.0737
offset, RFU	10	-300	-410.461	23.9087
offset, RFU	11	-300	-660.423	26.779
offset, RFU	12	-300	-611.297	34.0302

Neratinib, Replicate R2



A.10.3. Replicate R3

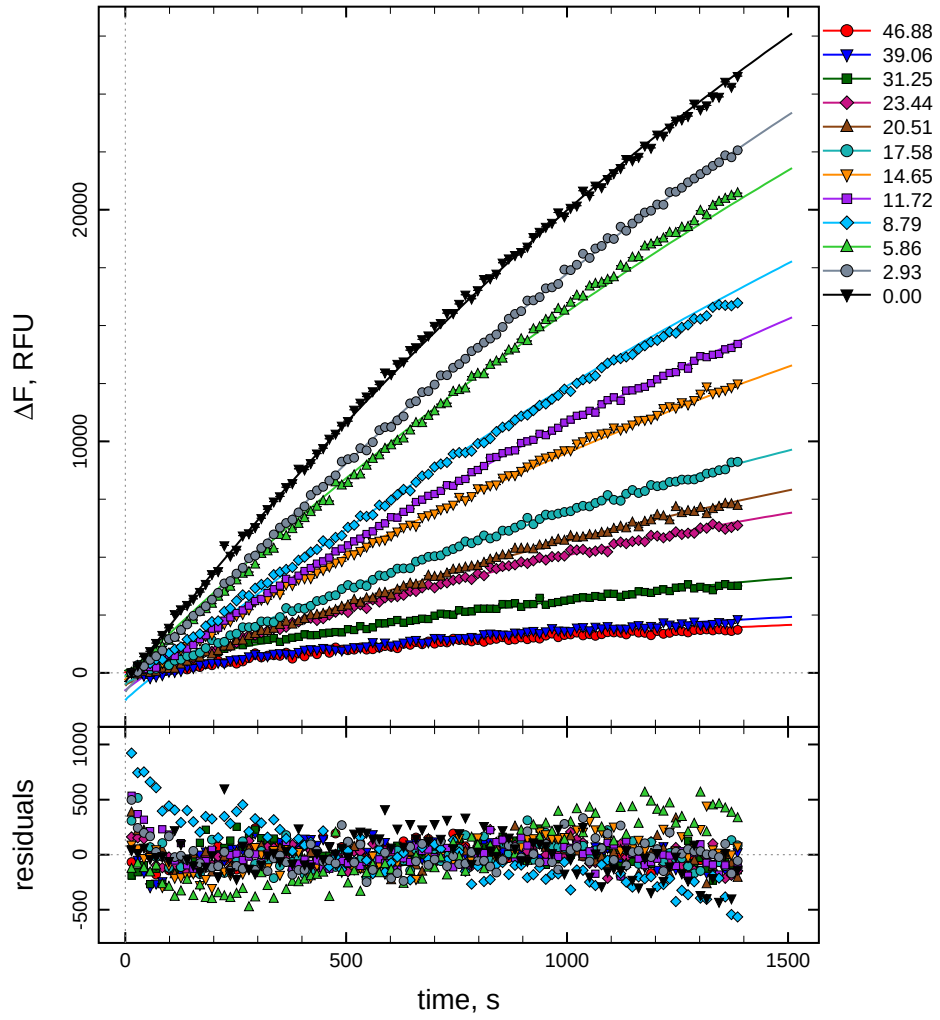
Neratinib, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 0.04565 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02 ?
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/Neratinib/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   2 | offset = -300 ?  | conc I = 0.046875 ? | label 46.88
   column   3 | offset = -300 ?  | conc I = 0.0390625 ? | label 39.06
   column   4 | offset = -300 ?  | conc I = 0.03125 ? | label 31.25
   column   5 | offset = -300 ?  | conc I = 0.0234375 ? | label 23.44
   column   6 | offset = -300 ?  | conc I = 0.0205078 ? | label 20.51
   column   7 | offset = -300 ?  | conc I = 0.0175781 ? | label 17.58
   column   8 | offset = -300 ?  | conc I = 0.0146484 | label 14.65
   column   9 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  10 | offset = -300 ?  | conc I = 0.00878906 | label 8.79
   column  11 | offset = -300 ?  | conc I = 0.00585938 | label 5.86
   column  12 | offset = -300 ?  | conc I = 0.00292969 | label 2.93
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/Neratinib/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

Neratinib, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.023675	0.000865102
k_{dI}, s^{-1}	*	0.04565	0.0520252	0.00288345
k_{inact}, s^{-1}	*	0.01	0.000709967	4.40033e-005
[E], μM	*	0.02	0.0169229	0.000450637
r_P, RFU/μM	*	6000	4748.43	77.2451
[I], μM	1	0.046875	0.070587	0.00344605
[I], μM	2	0.0390625	0.059621	0.00259164
[I], μM	3	0.03125	0.0410716	0.00131984
[I], μM	4	0.0234375	0.0259878	0.000478022
[I], μM	5	0.0205078	0.0229183	0.0003426
[I], μM	6	0.0175781	0.019651	0.000221305
offset, RFU	1	-300	5.24914	38.5864
offset, RFU	2	-300	-113.026	38.758
offset, RFU	3	-300	84.2668	39.2206
offset, RFU	4	-300	-314.846	38.9938
offset, RFU	5	-300	-552.037	38.6187
offset, RFU	6	-300	-494.421	38.0646
offset, RFU	7	-300	-307.822	35.6797
offset, RFU	8	-300	-774.049	27.0521
offset, RFU	9	-300	-1182.95	26.5132
offset, RFU	10	-300	-159.784	28.7357
offset, RFU	11	-300	-811.989	32.1735
offset, RFU	12	-300	-393.589	41.3065

Neratinib, Replicate R3



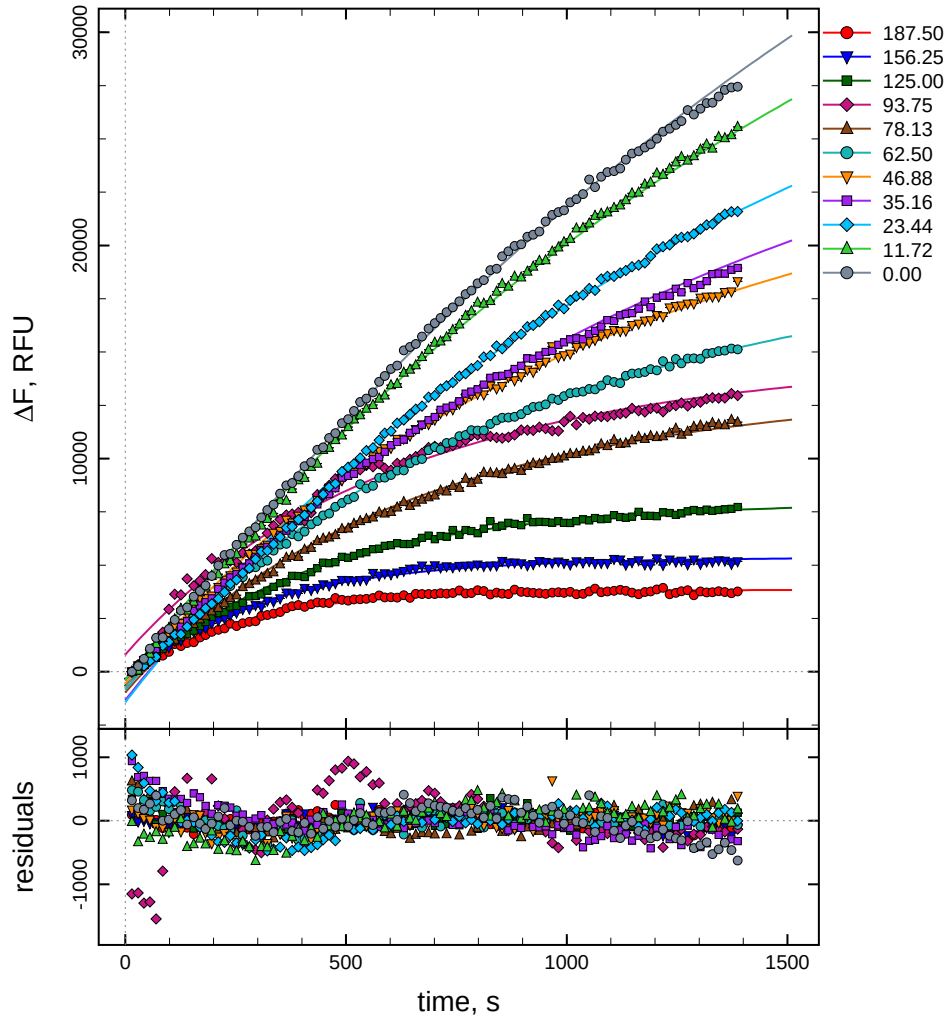
WZ-4002, Replicate R1

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 2.685 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/WZ-4002/R1/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   4 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   5 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   6 | offset = -300 ?  | conc I = 0.09375 ? | label 93.75
   column   7 | offset = -300 ?  | conc I = 0.078125 ? | label 78.13
   column   8 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   9 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  10 | offset = -300 ?  | conc I = 0.0351563 | label 35.16
   column  11 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  12 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/WZ-4002/R1/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

WZ-4002, Replicate R1

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.023168	0.000525427
k_{dI}, s^{-1}	*	2.685	4.70863	0.359693
k_{inact}, s^{-1}	*	0.01	0.00991078	0.000748018
r_P, RFU/μM	*	6000	4675.8	76.3137
[I], μM	1	0.1875	0.323241	0.00803746
[I], μM	2	0.15625	0.236107	0.0045148
[I], μM	3	0.125	0.156833	0.00228122
[I], μM	4	0.09375	0.0958432	0.00114292
[I], μM	5	0.078125	0.0934796	0.00110786
[I], μM	6	0.0625	0.0627414	0.000709121
offset, RFU	1	-300	-339.229	77.3704
offset, RFU	2	-300	-368.988	71.9657
offset, RFU	3	-300	-660.005	64.2823
offset, RFU	4	-300	803.37	55.3398
offset, RFU	5	-300	-971.734	54.9524
offset, RFU	6	-300	-852.371	50.0672
offset, RFU	7	-300	-555.295	40.6818
offset, RFU	8	-300	-1332.01	38.9191
offset, RFU	9	-300	-1433.37	39.2835
offset, RFU	10	-300	-391.874	44.2068
offset, RFU	11	-300	-743.25	55.242

WZ-4002, Replicate R1



A.11.2. Replicate R2

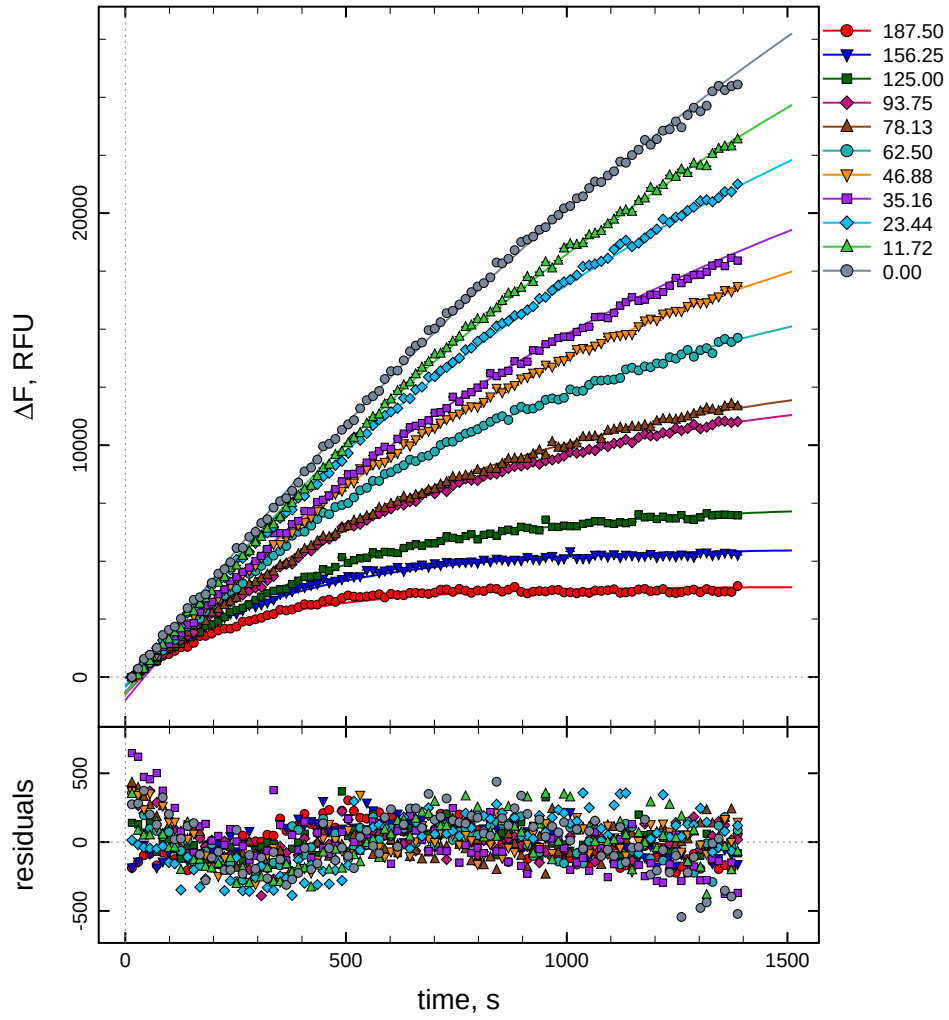
WZ-4002, Replicate R2

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 2.685 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/WZ-4002/R2/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   4 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   5 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   6 | offset = -300 ?  | conc I = 0.09375 ? | label 93.75
   column   7 | offset = -300 ?  | conc I = 0.078125 ? | label 78.13
   column   8 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   9 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  10 | offset = -300 ?  | conc I = 0.0351563 | label 35.16
   column  11 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  12 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/WZ-4002/R2/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

WZ-4002, Replicate R2

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.021991	0.000382255
k_{dI}, s^{-1}	*	2.685	3.82794	0.175075
k_{inact}, s^{-1}	*	0.01	0.00736986	0.000335275
r_P, RFU/μM	*	6000	4502.48	57.3032
[I], μM	1	0.1875	0.345148	0.00628932
[I], μM	2	0.15625	0.24125	0.00332814
[I], μM	3	0.125	0.171835	0.00192069
[I], μM	4	0.09375	0.0970917	0.000875701
[I], μM	5	0.078125	0.0898661	0.000797725
[I], μM	6	0.0625	0.0627153	0.000540296
offset, RFU	1	-300	-13.9928	51.0067
offset, RFU	2	-300	-51.4857	47.5359
offset, RFU	3	-300	-405.537	43.7791
offset, RFU	4	-300	-712.43	37.367
offset, RFU	5	-300	-744.457	36.6309
offset, RFU	6	-300	-688.493	34.0115
offset, RFU	7	-300	-712.275	27.7201
offset, RFU	8	-300	-998.871	26.4451
offset, RFU	9	-300	-377.334	26.6642
offset, RFU	10	-300	-723.145	29.9665
offset, RFU	11	-300	-660.146	37.4756

WZ-4002, Replicate R2



A.11.3. Replicate R3

WZ-4002, Replicate R3

```
;------------------------------------------------------------

[task]
   data = progress
   task = fit

[mechanism]
   E + S ---> E + P    :      ksub
   E + I <==> E.I      :      kaI    kdI
   E.I ---> E-I        :      kinact

[constants]
   ksub = 0.02 ?

   kaI = 10
   kdI = 2.685 ?
   kinact = 0.01 ?

[concentrations]
   E = 0.02
   S = 13

[responses]
   P = 6000 ?

[data]
   directory ./proj/EGFR/L858R-T790M/inhib/WZ-4002/R3/data
   sheet     sheet.txt
   monitor   E, E.I, E-I

   column   3 | offset = -300 ?  | conc I = 0.1875 ? | label 187.50
   column   4 | offset = -300 ?  | conc I = 0.15625 ? | label 156.25
   column   5 | offset = -300 ?  | conc I = 0.125 ? | label 125.00
   column   6 | offset = -300 ?  | conc I = 0.09375 ? | label 93.75
   column   7 | offset = -300 ?  | conc I = 0.078125 ? | label 78.13
   column   8 | offset = -300 ?  | conc I = 0.0625 ? | label 62.50
   column   9 | offset = -300 ?  | conc I = 0.046875 | label 46.88
   column  10 | offset = -300 ?  | conc I = 0.0351563 | label 35.16
   column  11 | offset = -300 ?  | conc I = 0.0234375 | label 23.44
   column  12 | offset = -300 ?  | conc I = 0.0117188 | label 11.72
   column  13 | offset = -300 ?  | conc I = 0 | label 0.00

[output]
   directory ./proj/EGFR/L858R-T790M/inhib/WZ-4002/R3/output/fit-progress-global-HR

[settings]
{Filter}
   TimeMin = 1
   ZeroBaselineSignal = y
{Output}
   WriteEPS = y
   XAxisLabel = time, s
   YAxisLabel = {/Symbol D}F, RFU

[end]
```

WZ-4002, Replicate R3

parameter	set	initial	fit	std. error
k_{sub}, $\mu M^{-1}s^{-1}$	*	0.02	0.0217238	0.000425844
k_{dI}, s^{-1}	*	2.685	2.22968	0.0769263
k_{inact}, s^{-1}	*	0.01	0.00397873	0.000139404
r_P, RFU/μM	*	6000	4710.56	67.6772
[I], μM	1	0.1875	0.334425	0.00609489
[I], μM	2	0.15625	0.193758	0.00250687
[I], μM	3	0.125	0.126331	0.00136856
[I], μM	4	0.09375	0.097866	0.000991301
[I], μM	5	0.078125	0.0971255	0.000982407
[I], μM	6	0.0625	0.0671616	0.000650083
offset, RFU	1	-300	-242.677	51.9334
offset, RFU	2	-300	-210.128	48.2849
offset, RFU	3	-300	-474.543	44.3955
offset, RFU	4	-300	-459.607	41.9926
offset, RFU	5	-300	-718.496	41.9262
offset, RFU	6	-300	-498.14	39.1307
offset, RFU	7	-300	-677.194	31.2213
offset, RFU	8	-300	-1242.33	29.6344
offset, RFU	9	-300	-743.98	29.7692
offset, RFU	10	-300	-496.136	33.7812
offset, RFU	11	-300	-818.789	43.0682

WZ-4002, Replicate R3