

## Supplementary equations

A guide for the use of the program and more details about the formulas applied are available in the **TReCCA Analyser user manual**:

[www.uni-heidelberg.de/fakultaeten/biowissenschaften/ipmb/biologie/woelfl/Research.html](http://www.uni-heidelberg.de/fakultaeten/biowissenschaften/ipmb/biologie/woelfl/Research.html).

Two tutorials including the data used for this publication are also available at this address:

- **Tutorial 1** - Sensor correction, Normalisation, Averaging and IC50 determination
- **Tutorial 2** - Smoothing and slope calculation

### Sensor correction: recalibration

Equation A: The mean of each sensor  $m_s$  is the average of the logarithmic data  $z$  of each sensor  $s$  for  $n$  calibration time points and a calibration time frame ranging from  $t_1$  to  $t_n$ .

$$m_s = \frac{1}{n} \sum_{i=0}^{n-1} z_{t_1+i, s}$$

Equation B: The mean of the whole plate  $m_p$  is the average of the mean of each sensor  $m_s$  for  $s$  number of sensors.

$$m_p = \frac{1}{s} \sum_{i=1}^s m_i$$

### Sensor correction: normalisation

Equation C: The normalised data  $y_{t,s}$  for each time point  $t$  and each sensor  $s$  is the data  $x_{t,s}$  divided by the average of each calibrated sensor  $mc$  (Equation A in S1 File) and multiplied by the target value  $T$ .

$$y_{t,s} = \frac{x_{t,s} \cdot T}{mc_s}$$

### Normalisation

Equation D: The normalised data  $y_t$  for each time point  $t$  is the data  $x_t$  divided by the average of the normalisation condition over time  $m_t$  and multiplied by the target value  $M$ .

$$y_t = \frac{x_t \cdot M}{m_t}$$

### Average and standard deviation

Equation E: Standard deviation  $sd(x)$ , where  $\bar{x}$  is the average and  $n$  the number of conditions averaged.

$$sd(x) = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2}$$

### Smoothing

Equation F: The smoothed data point  $y_t$  at the time point  $t$  is the average of the data point  $x_t$  and  $n$  neighbouring data points.

$$y_t = \frac{1}{n} \sum_{i=t-\frac{n-1}{2}}^{t+\frac{n-1}{2}} x_i$$

### Slope calculation

Equation G: The slope  $m$  is determined by applying a linear fit function to each smoothed data point  $y_t$  and  $n$  smoothed data points on either side for their corresponding time points  $t$ .

$$(y_{x-n}, \dots, y_{x+n}) = m \cdot (t_{x-n}, \dots, t_{x+n}) + c$$

### Time-resolved IC<sub>50</sub>

Equations H, I and J: Two, three and five log-logistic model functions respectively.  $b$  is the slope of the hill,  $c$  and  $d$  are the upper and lower plateau respectively,  $e$  is the IC<sub>50</sub> value and  $f$  differs from 1 if the function is asymmetric.

$$f(x, (b, e)) = \frac{1}{1 + e^{(b(\log(x) - \log(e)))}}$$
$$f(x, (b, d, e)) = \frac{d}{1 + e^{(b(\log(x) - \log(e)))}}$$
$$f(x, (b, c, d, e, f)) = \frac{d - c}{(1 + e^{(b(\log(x) - \log(e)))})^f} + c$$