It frequently happens that measurements of a response as a function of time say, or concentration, etc., are made in order to measure an initial rate, a lag time, an asymptotic steady state rate, a half saturation point, or a horizontal asymptote. Examples could be the initial rate of an enzyme catalyzed reaction, the transport of labeled solute out of loaded erythrocytes, or extent of muscle contraction in response to an agonist.

The models required to perform these operations are available from the main SIMFIT menu after first choosing [A/Z] then opening program **inrate**.

Theory

Stated in equations we have the responses given by a deterministic component plus a random error

$$y_i = f(t_i) + \epsilon_i, \; i = 1, 2, \ldots, m$$

and it is wished to measure the limiting values

$$\begin{aligned}
\text{the initial rate} &= \frac{df}{dt} \text{ at } t = 0\\
\text{the asymptotic slope} &= \frac{df}{dt} \text{ as } t \to \infty\\
\text{the half saturation point} &= t_{1/2} \text{ where } f(t_{1/2}) = f(0)/2\\
\text{the final asymptote} &= f \text{ as } t \to \infty.
\end{aligned}$$

There are numerous ways to make such estimates in SIMFIT and the method adopted depends critically on the type of experiment. Choosing the wrong technique can lead to biased estimates, so you should be quite clear which is the correct method for your particular requirements. In particular, is $f(0) = C = 0$ required ?

The models used by program **inrate** are

$$\begin{aligned}
f_1 &= Bt + C\\
f_2 &= At^2 + Bt + C\\
f_3 &= \alpha[1 - \exp(-\beta t)] + C\\
f_4 &= \frac{V_{max}t^n}{K_m^n + t^n} + C\\
f_5 &= Pt + Q[1 - \exp(-Rt)] + C
\end{aligned}$$

and there are test files to illustrate each of these that can be selected from the SIMFIT file selection control after using the [Demo] button. It is usual to assume that $f(t)$ is an increasing function of t with $f(0) = 0$, which is easily arranged by suitably transforming any initial rate data. For instance, if you have measured efflux of an isotope from vesicles you would analyze the rate of appearance in the external solute, that is, express your results as

$$f(t) = \text{initial counts - counts at time } t$$

so that $f(t)$ increase from zero at time $t = 0$. All you need to remember is that, for any constant K,

$$\frac{d}{dt}\{K - f(t)\} = -\frac{df}{dt}.$$

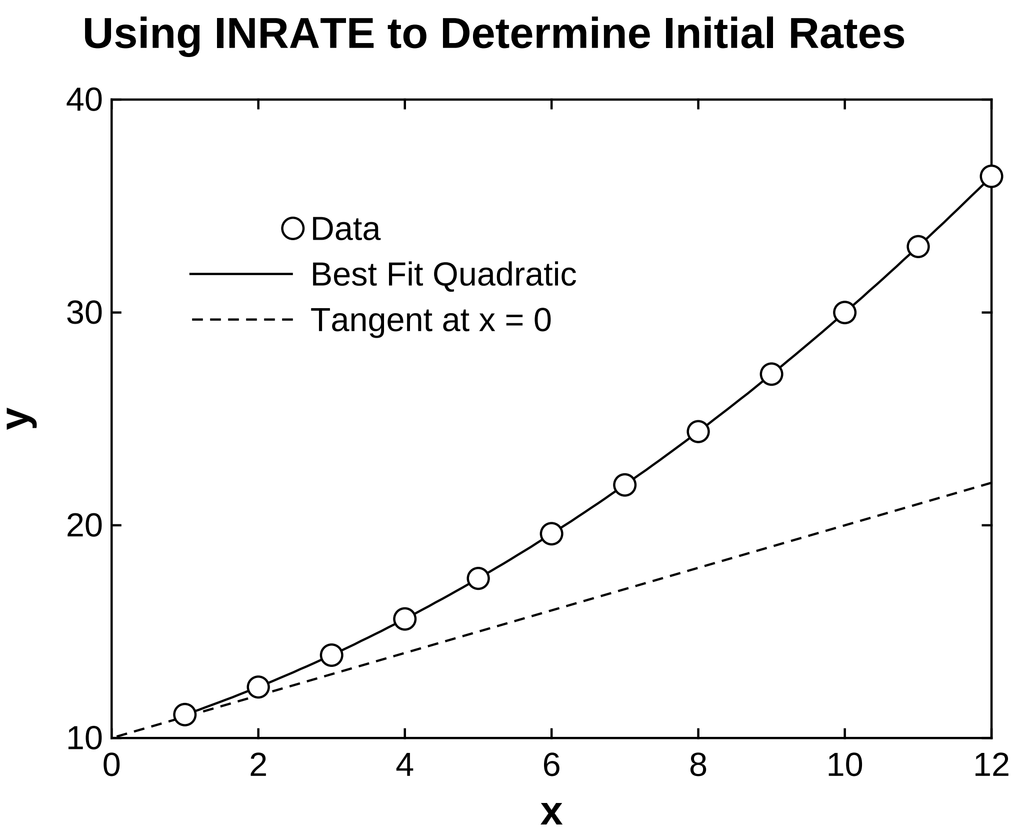
However it is sometimes difficult to know exactly when $t = 0$, e.g., if the experiment involves quenching, so there exists an option to force the best fit curve to pass through the origin with some of the models if this is essential. The models available will now be summarized.

1. f_1: This is used when the data are very close to a straight line and it can only measure initial rates.
2. f_2: This adds a quadratic correction and is used when the data suggest only a slight curvature. Like the previous model it can only estimate initial rates.
3. f_3: This model is used when the data rapidly bend to a horizontal asymptote in an exponential manner. It can be used to estimate initial rates, half-times ($\log(2)/\beta$), and final horizontal asymptotes.
4. f_4: This model can be used with n fixed (e.g., $n = 1$) for the Michaelis-Menten equation, or with n varied (the Hill equation). It is not used for initial rates but is sometimes better for estimating half saturation points (K_m), or final horizontal asymptotes than the previous model.
5. f_5: This is the progress curve equation used in transient enzyme kinetics. It is used when the data have an initial lag phase followed by an asymptotic final steady state. It is not used to estimate initial rates, final horizontal asymptotes, or AUC. However, it is very useful for experiments with cells or vesicles which require a certain time before attaining a steady state, and where it is wished to estimate both the length of lag phase and the final steady state rate.

To understand these issues, see what happens the test files. These are, models f_1 and f_2 with `inrate.tf1`, model f_3 with `inrate.tf2`, model f_4 with `inrate.tf3` and model f_5 using `inrate.tf4`.

Example 1: Using $f_2(t)$ to estimate initial rates

A useful method to estimate initial rates when the true deterministic equation is unknown is to fit the quadratic $f_2(t) = At^2 + Bt + C$, in order to avoid the bias that would inevitably result from fitting a line to nonlinear data. Use **inrate** to fit the test file `inrate.tf1`, and note that, when the model has been fitted, it also estimates the slope at the origin. The reason for displaying the tangent in this way, as in the figure, is to give you some idea of what is involved in extrapolating the best fit curve to the origin, so that you will not accept the estimated initial rate uncritically.



Example 2: Using $f_3(t)$ to estimate half times, initial rates, and horizontal asymptotes

This model,

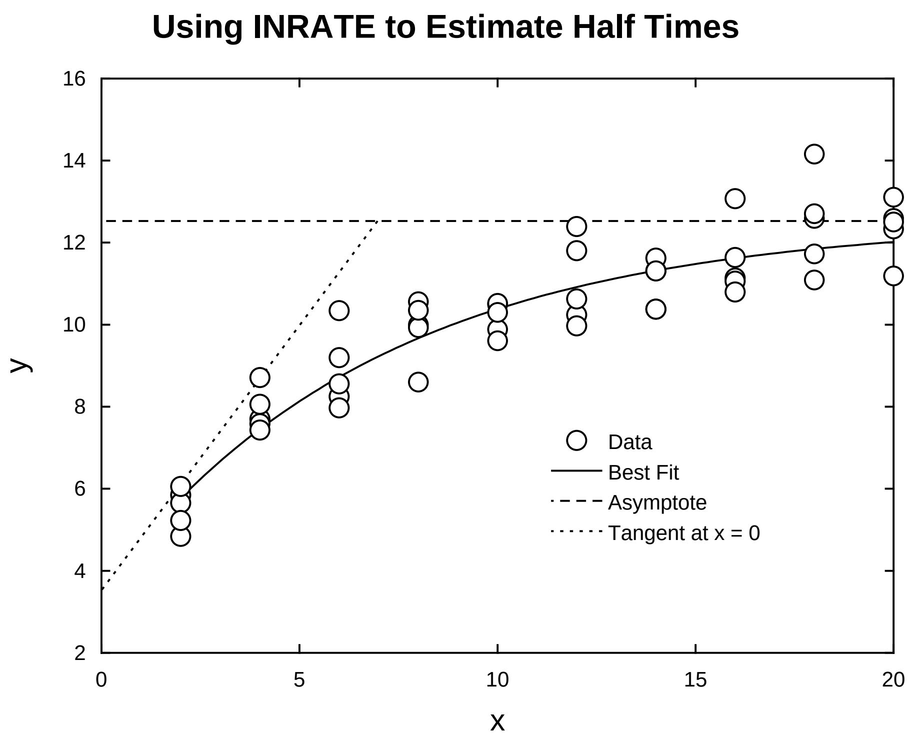
$$f_3(t) = \alpha[1 - \exp(-\beta t)] + C,$$

sometimes referred to as the monomolecular model, is useful for characterizing time-dependent processes that approach a horizontal asymptote.

For instance, program **adderr** was used to generate 5 replicates with 7.5% relative error added to the exact data in test file `inrate.tf2` to simulate experimental error with standard errors estimated from the replicates, and the monomolecular equation was fitted to yield the following results.

Parameter	Value	Std. error	Lower95%cl	Upper95%cl	p
α	9.0018	0.39481	8.2075	9.7960	0.0000
β	0.14346	0.023000	0.097186	0.18973	0.0000
C	3.5251	0.48988	2.5396	4.5106	0.0000

Estimated initial value i.e. $f(t = 0)$ = 3.5251
Estimated initial rate $df/dt(t = 0)$ = 1.2914
At t = -2.3035, $f = 0$ and df/dt = 1.7971 ... extrapolated value
Estimated final asymptote $f(\infty)$ = 12.527
Estimated half time (i.e. $\log(2)/\beta$) = 4.8318



Note that the estimate referred to as the half time is defined by

$$\hat{t}_{1/2} = \log(2)/\hat{\beta}$$

which will only be the time to reach half the maximal value $(\hat{\alpha} + \hat{C})$ when the model is fitted using the option to set $C = 0$. Often it will be sensible to use the option $f(0) = 0$, i.e. fixing $C = 0$ when using **inrate**.

Example 3: Using $f_4(t)$ to estimate half saturation points and horizontal asymptotes

The Hill equation given by

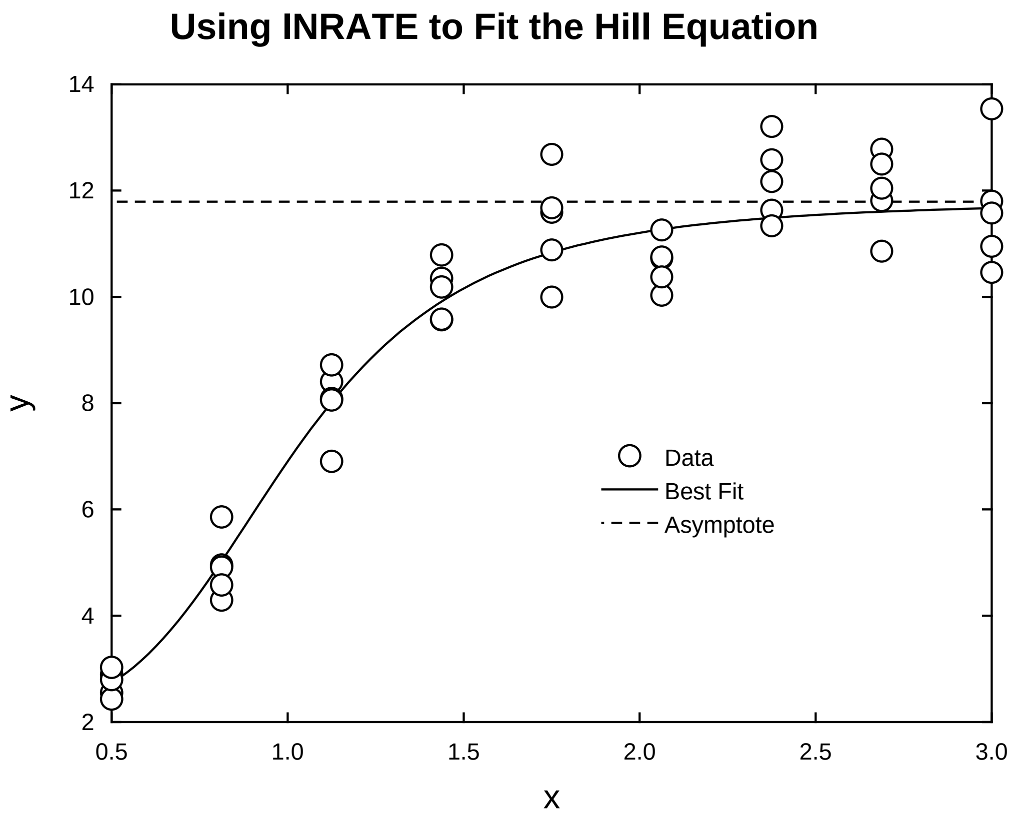
$$f_4(t) = \frac{V_{max} t^n}{K_m^n + t^n} + C$$

only models a meaningful process when $n = 1$, as the cases with $n < 1$ and n non-integer have no sensible interpretation, while those with integer $n > 1$ are only coarse approximations to receptor saturation. Nevertheless, this equation is widely used as an empirical model to fit data in order to estimate the origin C, half saturation point K_m, and horizontal asymptote $V_{max} + C$. Note that program **inrate** allows the exponent n to be fixed. which is preferred, or varied as a parameter, which is not usually recommended as it can lead to an ill-defined best-fit model.

For instance, program **adderr** was used to generate 5 replicates with 7.5% relative error added to the exact data in test file `inrate.tf3` to simulate experimental error with standard errors estimated from the replicates, and the Hill equation with $n = 4$ was fitted to yield the following results.

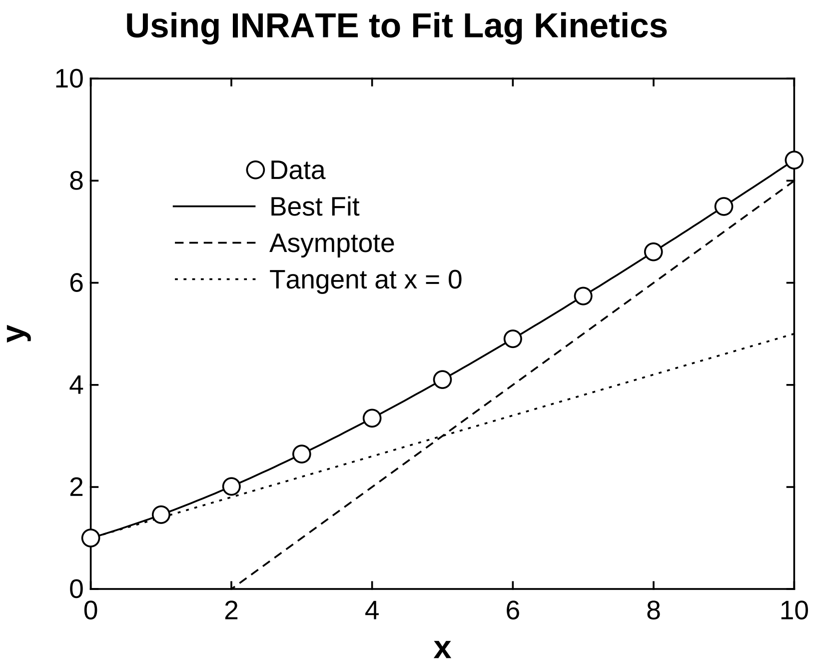
Parameter	Value	Std. error	Lower95%cl	Upper95%cl	p
V_{max}	9.6039	0.21213	9.1758	10.032	0.0000
K_m	1.0090	0.034222	0.93999	1.0781	0.0000
C	2.1875	0.16332	1.8579	2.5171	0.0000
n	4.0000	0.0000	4.0000	4.0000	Fixed

Estimated initial value i.e. $f(t = 0) = 2.1875$
Estimated initial rate $df/dt(t = 0) = 0.0000$
Estimated final asymptote $f(\infty) = 11.91$
The exponent was fixed at the value $n = 4$



Example 4: Using $f_5(t)$ to estimate lag times and asymptotic steady states

Use **inrate** to fit $f_5(t) = Pt + Q[1 - \exp(-Rt)] + C$ to data in test file `inrate.tf4`, and observe that the asymptotic line is displayed in addition to the tangent at the origin, as in the next figure.



However, sometimes a burst phase is appropriate, rather than lag phase, as shown next.