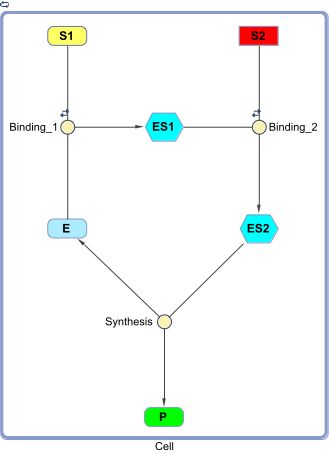
# **Simulation and Analysis of Enzymatic Reactions.**

****The simulation and analysis of enzymatic reactions is based on the same principles and tools used for the simulation of binding reactions. Consider as an example the sequential binding of two substrates to an enzyme to produce a single product: this is defined as a *sequential bi-uni reaction*.

The simulation of this reaction can be carried out by executing the cells in the script:

../TOOLBOXES/ENZYME\_KINETICS/

Bi\_Uni\_Michaelis\_Menten\_Global\_Fit.m

The following are the three reactions involved:

S1 + E <-> ES1

ES2 -> P + E

S2 + ES1 <-> ES2

which correspond to the following ordinary differential equations (ODE):

ODEs:

d(S1)/dt = 1/Cell\*(-ReactionFlux1)

d(E)/dt = 1/Cell\*(-ReactionFlux1 + ReactionFlux2)

d(ES1)/dt = 1/Cell\*(ReactionFlux1 - ReactionFlux3)

d(P)/dt = 1/Cell\*(ReactionFlux2)

d(S2)/dt = 1/Cell\*(-ReactionFlux3)

d(ES2)/dt = 1/Cell\*(-ReactionFlux2 + ReactionFlux3)

Fluxes:

ReactionFlux1 = (kon1\*S1\*E)\*Cell-(koff1\*ES1)\*Cell

ReactionFlux2 = (kcat\*ES2)\*Cell

ReactionFlux3 = (kon2\*S2\*ES1)\*Cell-(koff2\*ES2)\*Cell

Parameter Values:

koff1 = 200 1/second

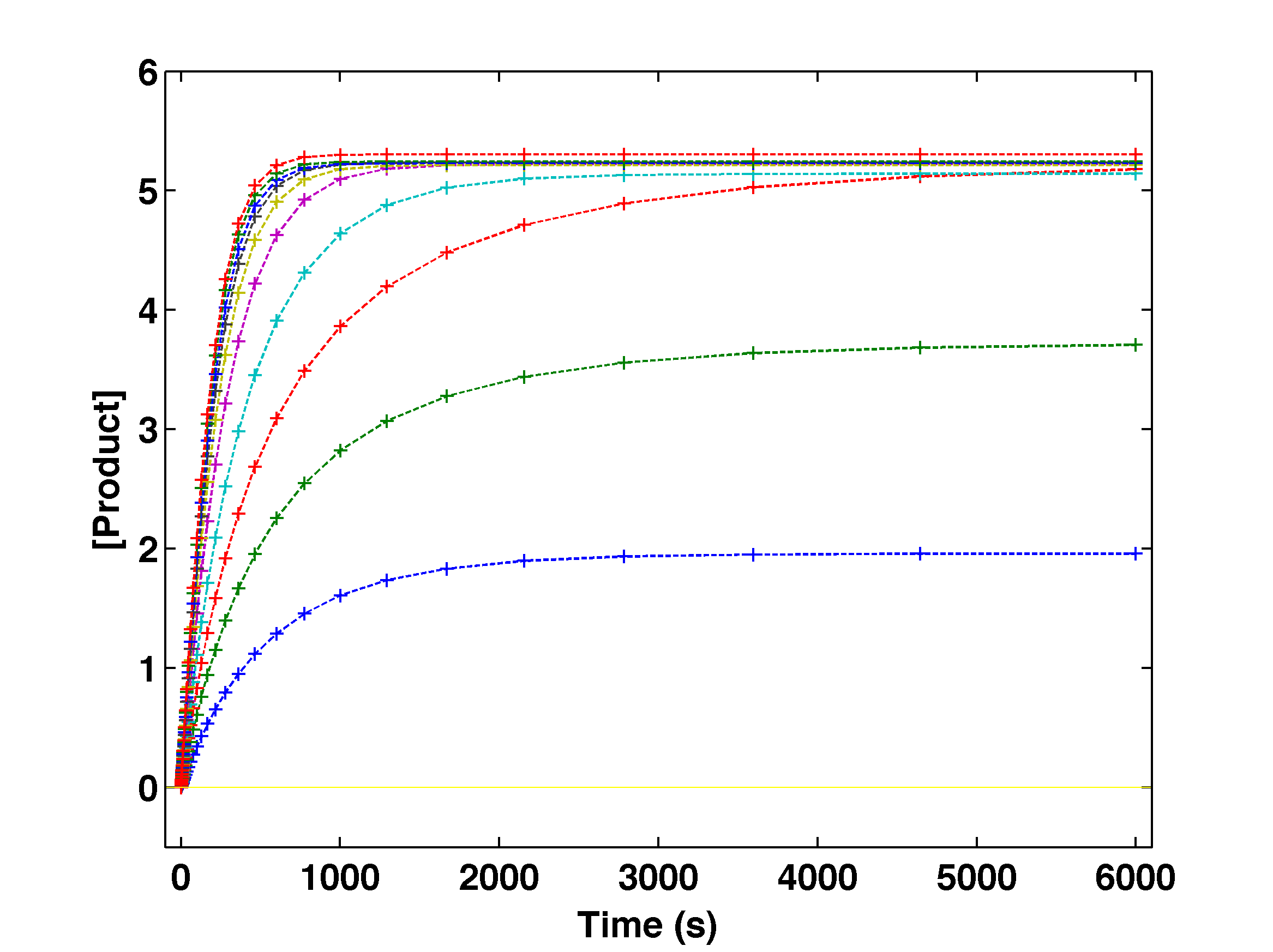
kon1 = 10 1/(micromolarity\*second)

kcat = 0.05 1/second

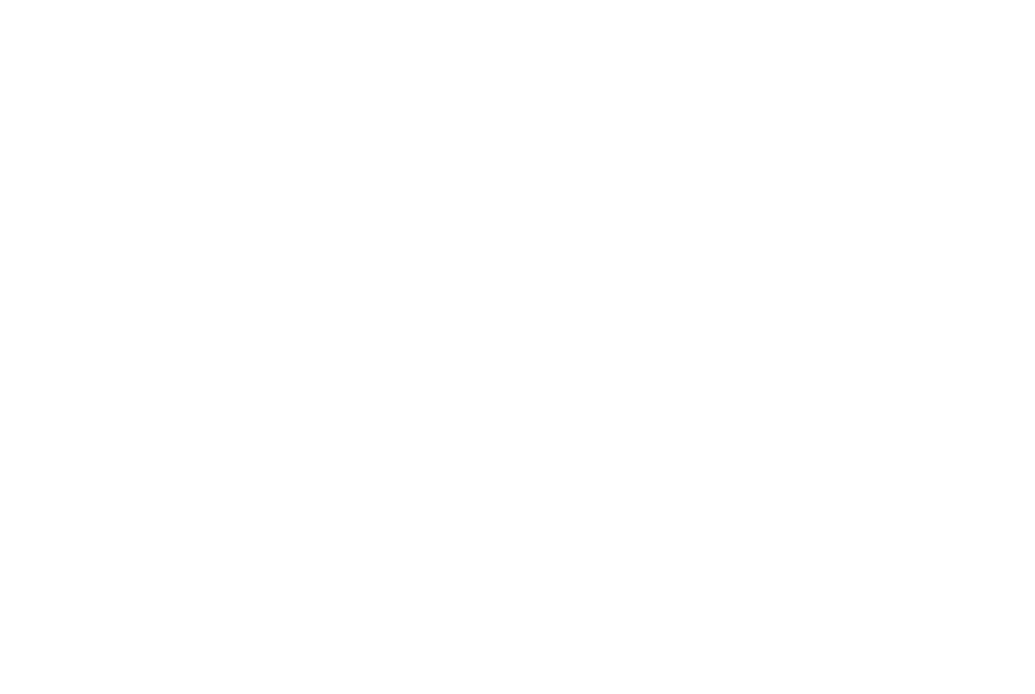
kon2 = 10 1/(micromolarity\*second)

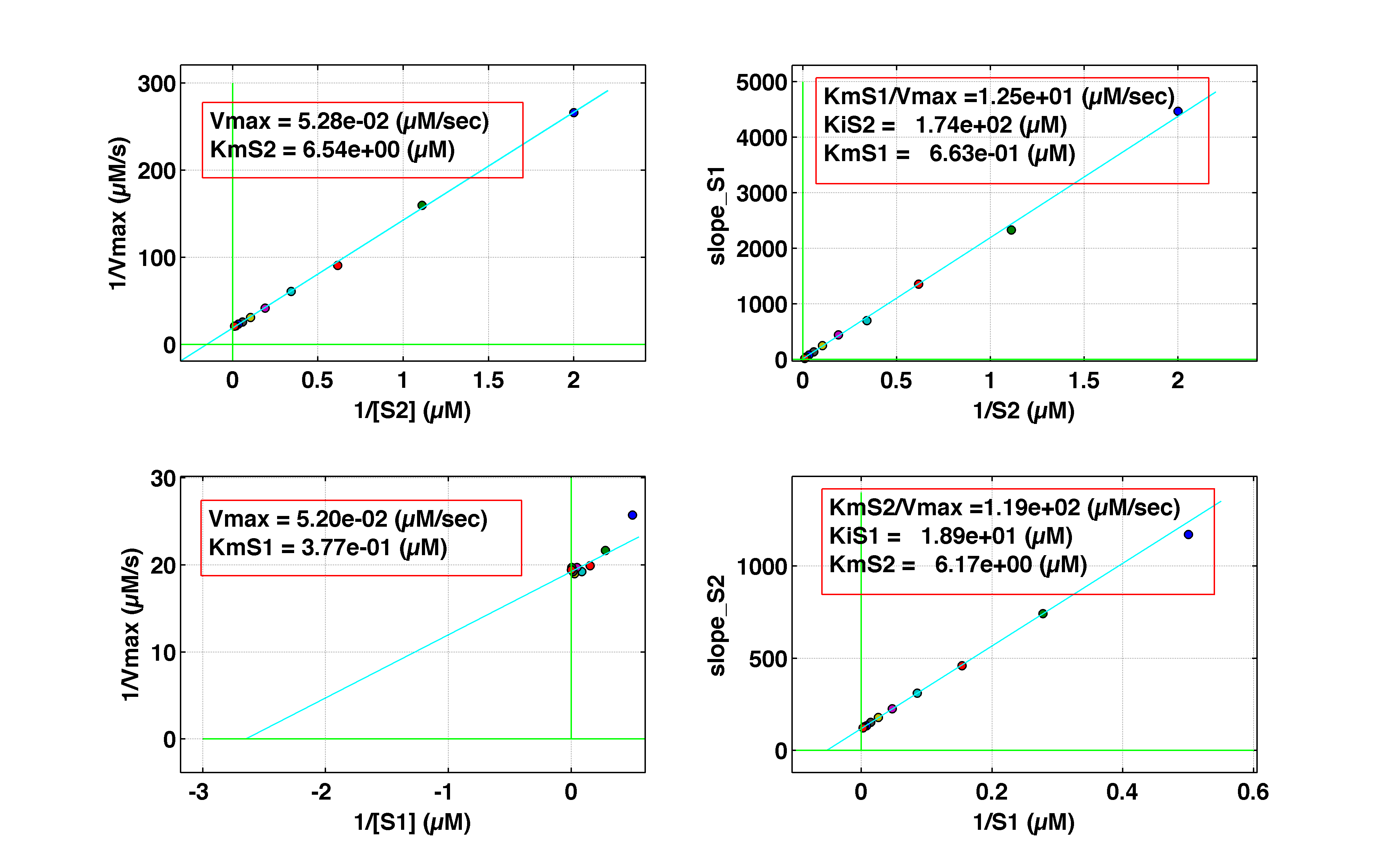
koff2 = 50 1/second

Cell = 1 milliliter

In order to reproduce an experimental kinetic analysis of this reaction we need to simulate progress curves at different combinations of the two substrates. We notice that *K*d1 = *k*off1/*k*on1 = 200/10 = 20 μM, so we can try a concentration range (spaced logarithmically) from 1/10 to 20 times the *K*d (2 to 400 μM). Since *K*d2 = *k*off2/*k*on2 = 50/10 = 5 μM, we can try a concentration range (also spaced logarithmically) from 0.5 to 100 μM. We also choose 35 time points spaced logarithmically between 1 and 6000 seconds with an additional 0 time point as the reference reaction start.

After simulating the reactions using all the different combinations of substrates concentrations we can choose various ways of analyzing the data. We start by plotting the initial velocity of the reaction for each initial concentration of the 1st substrate at the different concentration of the 2nd substrate. We also derive two different Lineweaver-Burke plot in which the reciprocal of the initial velocity is plotted against the reciprocal of the 1st substrate concentration at different concentrations of the 2nd substrate, and against the reciprocal of the 2nd substrate concentration at different concentrations of the 1st substrate.

The *V*max and *K*m's for S1 and S2 can be obtained directly from these two plots, although for better accuracy it is usually recommended to use 4 different replots of the points from the LB plots.



From these replots we can obtain all the kinetic parameters for this enzymatic reaction:

*kcat* = 0.052 s-1

*K*m\_S1 = 0.52 μM

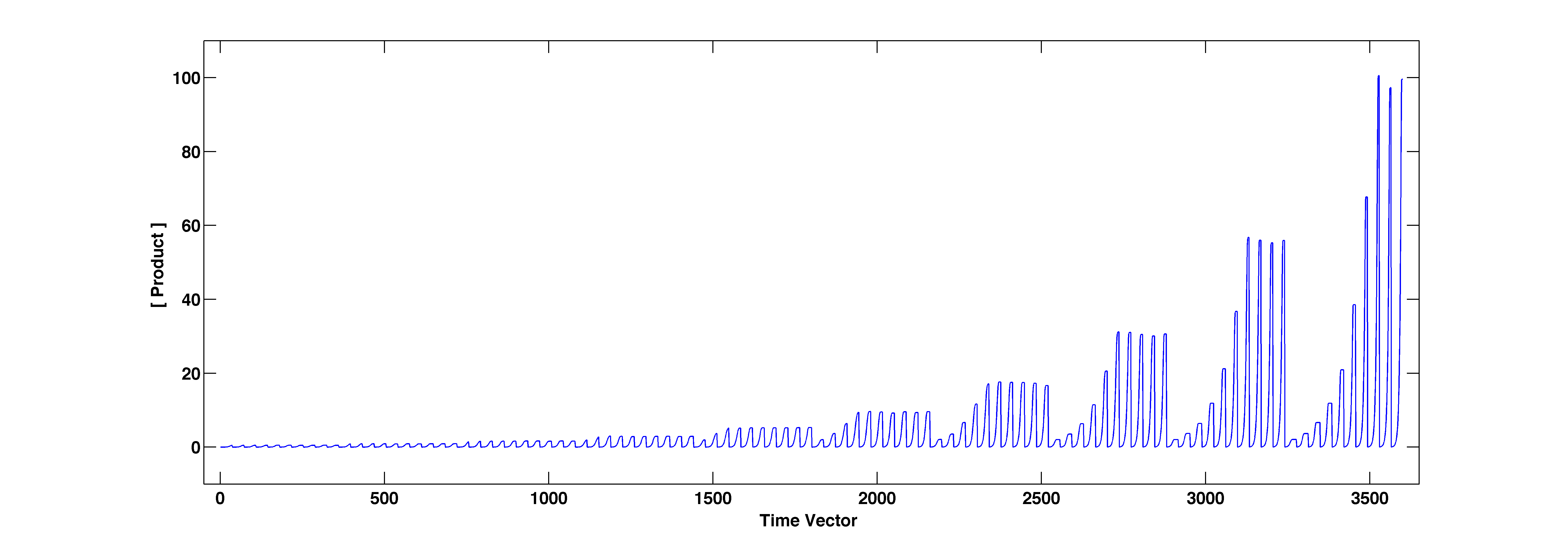
*K*d\_S1 = 18.89 μM

*K*m\_S2 ≈ *K*d\_S2 = 6.35 μM

*k*off\_S1 = 189 s-1

*k*off\_S2 = 63.5 s-1

Alternatively we can fit globally all the progress curves with a single set of parameters. For this purpose we first convert all the progress curves into a single consecutive progress curve.

To understand what this means we plot one set (i.e. set 5) of progress curves at a fixed concentration of S2, and also the vector containing all the concatenated progress curve. The 5th set of curves starts around 1500 in the concatenated vector.

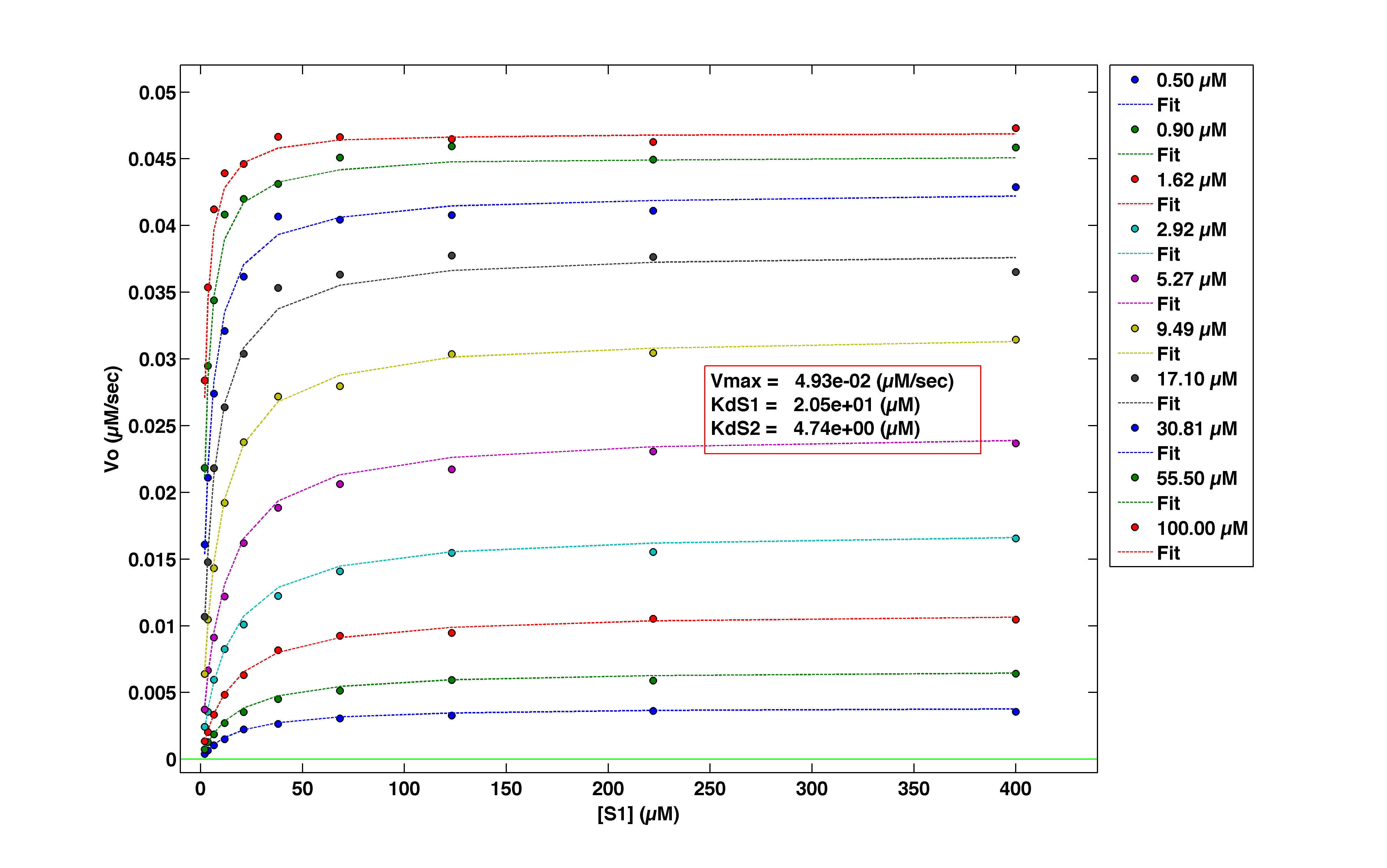
We will refine *k*off\_S1, *k*cat, and *k*off\_S2: the initial values are taken from the previous fit.

Next, we use the *nlinfit* function from the Statistics Toolbox (or *lsqcurvefit* from the Optimization Toolbox) as we did for the global fit of the progress curves originated from the binding of a ligand to two cooperative binding site (see CHAPTER 14). The function called by *nlinfit* at each optimization cycle uses the current value of the parameters to calculate a new concatenated set of progress curves. Refinement of the kinetic parameters is driven by minimization of the residual between the concatenated vector calculated at each refinement cycle and the concatenated vector derived from the observed (or synthetic) progress curves. In this case the refined parameters are:

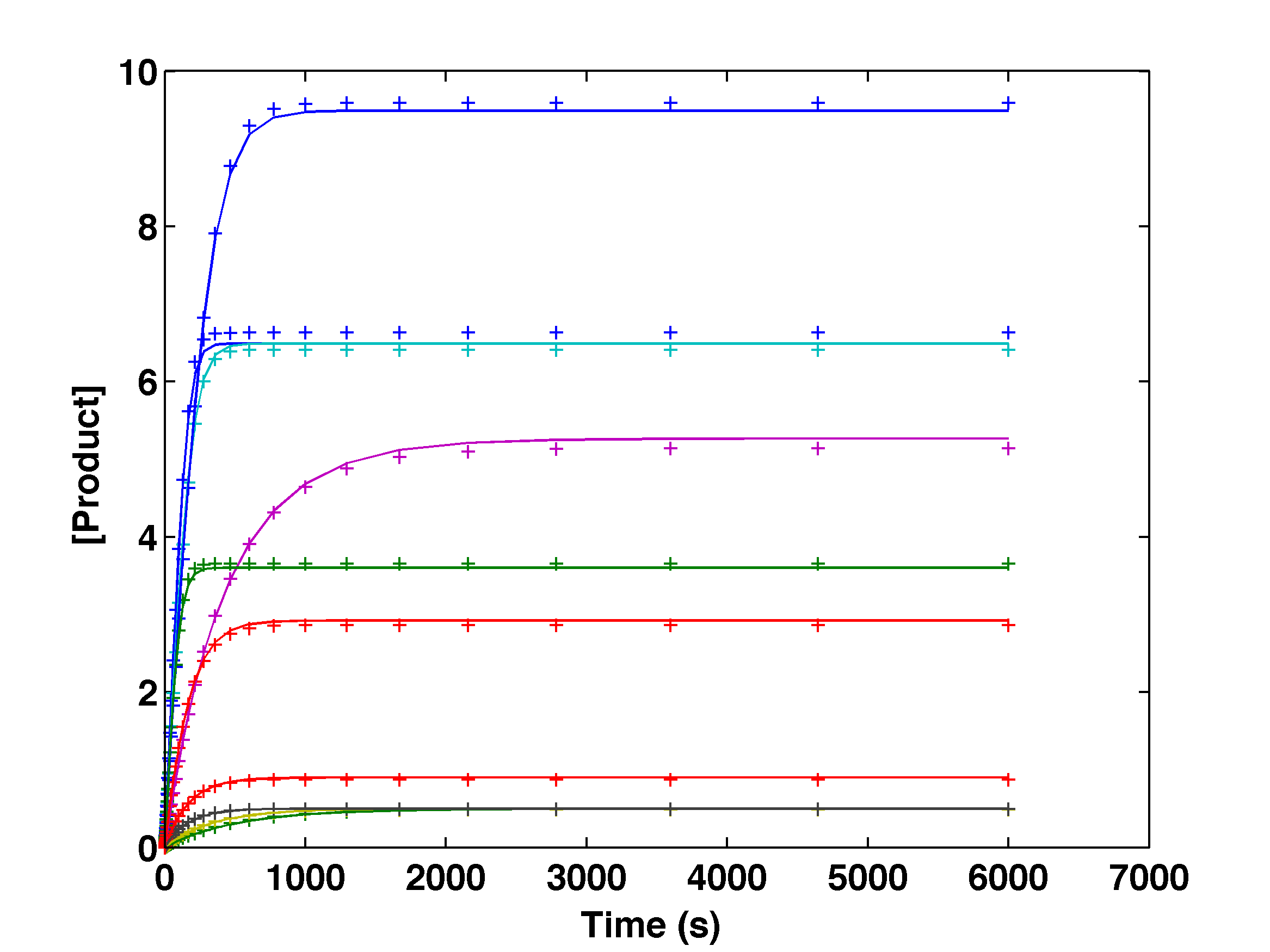
*kcat* = 0.0495 s-1

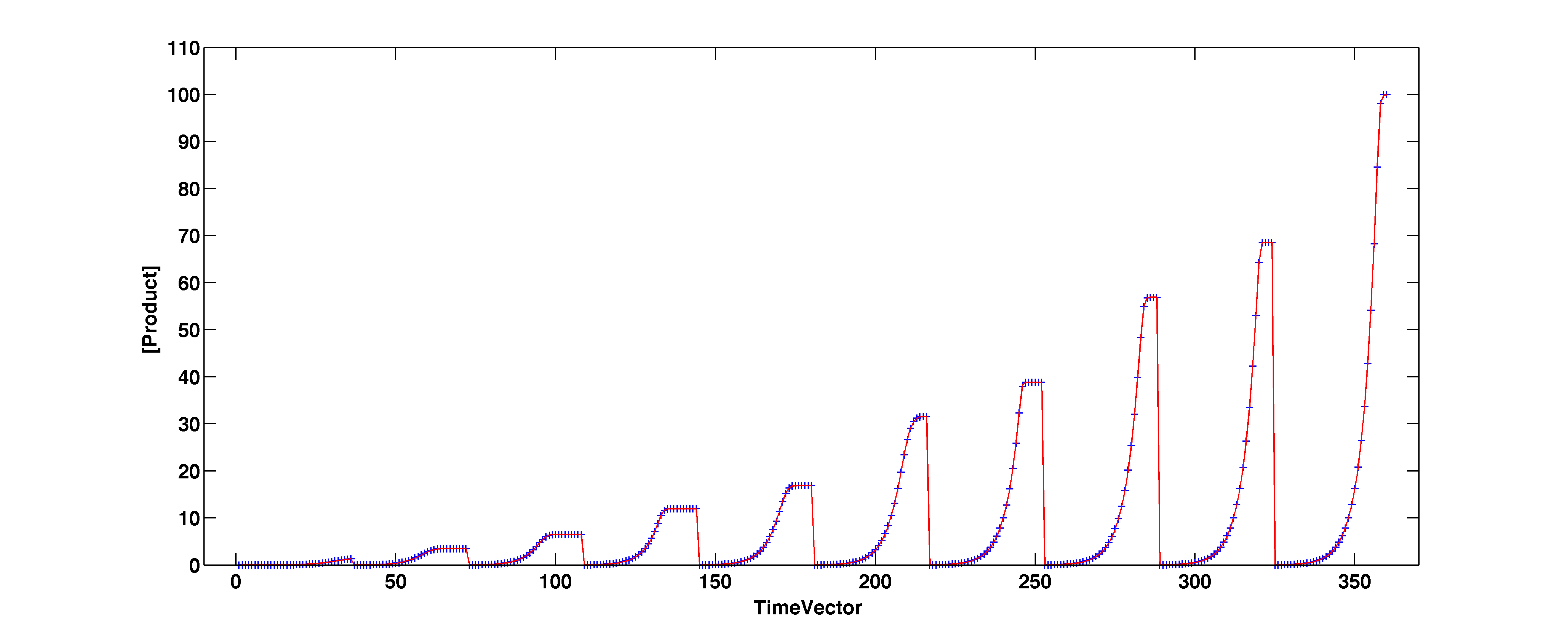
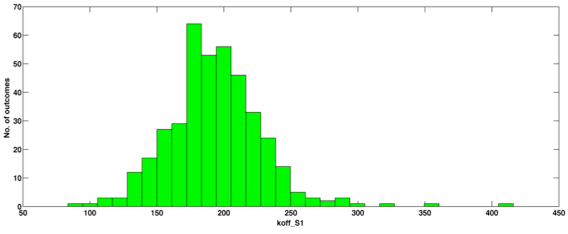
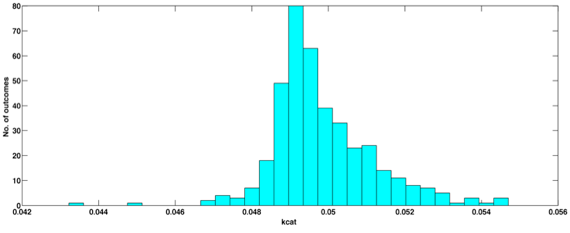
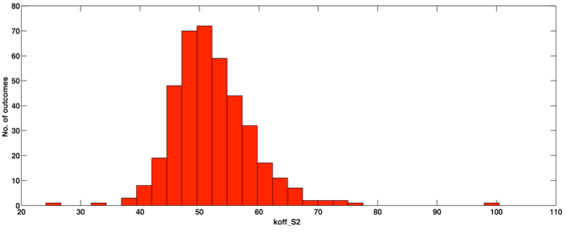
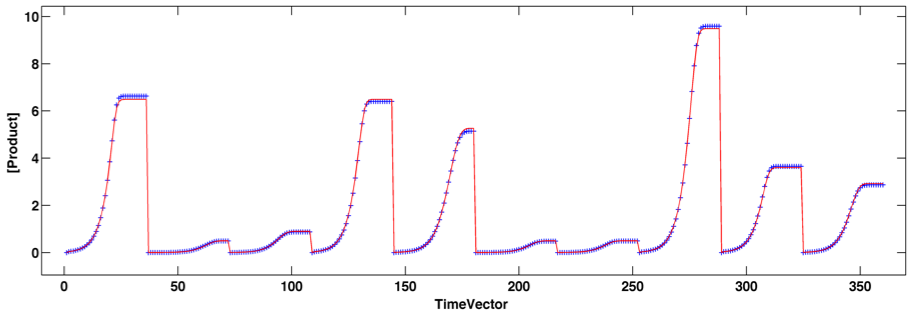
*k*off\_S1 = 191.7 s-1

*k*off\_S2 = 50.7 s-1



At the end of the refinement we can compare the initial velocities of all the progress curves in the synthetic data with those calculated from the refined parameters.

While global refinement of the kinetic parameters against all the progress curves provides more accurate values for these parameters if used as shown, it does not decrease the amount of experimental data that needs to be collected. Fortunately, in most cases even just 10-20 progress curves contain sufficient information to drive the refinement. We can check this by selecting randomly 10 progress curves from different combinations of S1 and S2 concentrations, and by repeating the parameter refinement using only those curves. For the curves shown in the figures above (with the regular or concatenated vector), the refinement yields kinetic parameters values that are within 10% of the true values. However, not any random choice will give acceptable values. This can be easily ascertained by repeating the refinement several hundred times with random sets of 10 curves extracted from the original data. While the distributions of the refined parameters are centered around the true values, the tails of the distributions extend to values that are clearly incorrect. In general, it is advisable to choose combinations of the values of S1 and S2 that provide a good distribution of the amount of product formed over the entire available range. For example, in the concatenated vector shown in the earlier figure only a small range of product concentrations (up to less than 10 μM) was sampled. A much better distribution is shown in the following concatenated vector, which produced improved values (within 5% of the true values) for two of the three parameters.

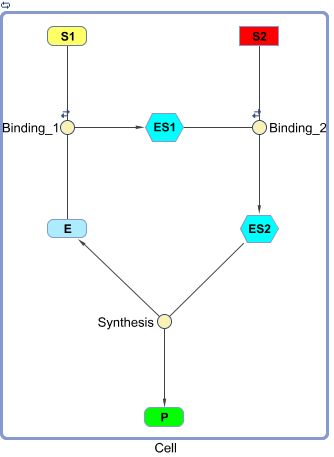


Since the analysis of only 10 progress curves is much faster, also substrate concentrations can be refined in addition to the kinetic parameters. For example, in the case of the concatenated vector shown above we would refine 3 rate constants and 20 substrates concentrations (2 for each progress curve).

**Conclusion**

Global fit of all the progress curves with a single set of kinetic parameters can be used effectively either to obtain *highly accurate* values for the kinetic parameters of the reaction under study using the same complete set of progress curves employed with traditional initial velocity studies, or to obtain *reasonably accurate* values for the same kinetic parameters, using only a small number of progress curves.

**PRACTICE**



Additional MATLAB scripts allowing the simulation of different types of enzyme mechanisms are provided in the directory:

../TOOLBOXES/ENZYME\_KINETICS

Global fit of enzyme reactions progress curves is a type of non-linear least-squares. Practice the generation of synthetic data and its global analysis running the scripts:

Simple\_Michaelis\_Menten\_Global\_Fit

Comp\_Inhib\_Michaelis\_Menten\_Global\_Fit

Bi\_Uni\_Michaelis\_Menten\_Global\_Fit

Using as examples these scripts and the MATLAB program for non-linear least-squares analyzed in CHAPTER 8, write a program for the global fit of experimental kinetic data obtained with an enzyme that catalyzes a reaction of the Bi-Uni type (shown on the side).

For this purpose, from the same directory load:

1. The enzyme model contained in the SimBiology project:

Bi\_Uni\_Michaelis\_Menten

1. The experimental data (Times, Substrate Concentrations, Product Concentrations) in the files:

Bi\_Uni\_Michaelis\_Menten\_Time\_mat.txt

Bi\_Uni\_Michaelis\_Menten\_Substrate\_mat.txt

Bi\_Uni\_Michaelis\_Menten\_Product\_mat.txt

1. Using the same experimental data, show that the global fit is much poorer if you use a model of the enzyme (Bi\_Uni\_Michaelis\_Menten\_Switch\_Substrates) in which the order of binding of the substrates is inverted.