Implementation of the King-Altman method in DynaFit

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Abstract

The DynaFit software package was updated to enable the automatic derivation of Michaelis constants, turnover numbers, and inhibition constants for reagents (substrate and products) as well as external ligands (inhibitors and activators). An illustrative example includes the derivation of kinetic constants for the "Ordered Bi Bi" kinetic mechanism [Segel, I. (1975) *Enzyme Kinetics*, p. 560-564].

Key words: steady-state; enzyme kinetics; mathematics; bisubstrate mechanisms; King-Altman method

Contents

1. Introduction

The King-Altman method [1] for the derivation of steady-state initial rate equation in enzyme kinetics is useful specifically for the study of enzymes that are characterized by chemical steps that are relatively rapid, compared to the dissociation rates of ligands (substrate, products, and modifiers).

However, manually performing the necessary derivations is tedious and error prone. Thus, for example, the initial rate equation for the "Random Bi Bi" mechanism [2, p. 649] contains 48 terms in the denominator, some of which consist of up to 16 rate constants grouped in complicated ways. To address this complexity, Cornish-Bowden [3] devised a computer algorithm that can be used to derive steady-state initial rate equations fully automatically.

Cornish-Bowden's automatic method [3] produces a rate equation which is formulated in terms of the *microscopic rate constants* that appear in the detailed reaction mechanism. It has been conventional, whenever possible, to rearrange such rate equations into an equivalent algebraic form, formulated in terms of "kinetic constants" such as Michaelis constants and turnover numbers. This second step of the overall derivation has now been enabled in the software DynaFit [4, 5].

2. Example: "Ordered Bi Bi" mechanism

The main purpose of this example is to demonstrate the correctness of the automatic algebraic derivations performed by DynaFit [4, 5]. The derived rate equation is not necessarily very useful in practice, because it assumes that the enzyme-catalyzed reaction can be experimentally observed to proceed in either direction (forward and reverse). There seem to be only few enzyme reactions for which full reversibility can be easily achieved.

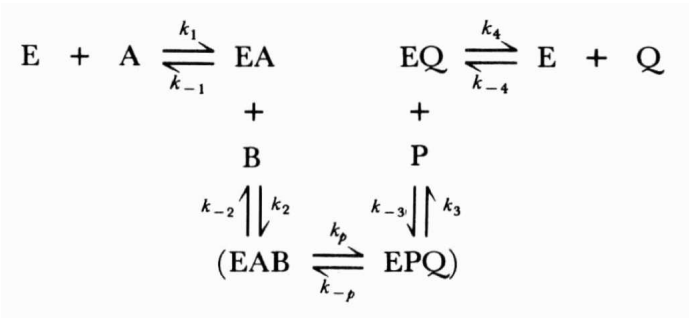

Figure 1: "Ordered Bi Bi" kinetic mechanism [2, p. 560].

With that caveat, *Figure 1* shows the "Ordered Bi Bi" mechanism as presented by Segel [2]. We wish to simulate a family of dose-response curves, as follows:

- All 10 microscopic rate constants in *Figure 1* are set to unity.
- The enzyme concentration is 1 nM.
- Substrate "A" dilution series starts at 10 μM, dilution factor 2/3.
- Substrate "B" dilution series starts at 8 μM, dilution factor 1/2.

The full listing of DynaFit script that can be used to accomplish this task is shown in Appendix A.1. The [mechanism] section follows the usual conventions for representing individual reaction steps, as illustrated in *Figure 2*,

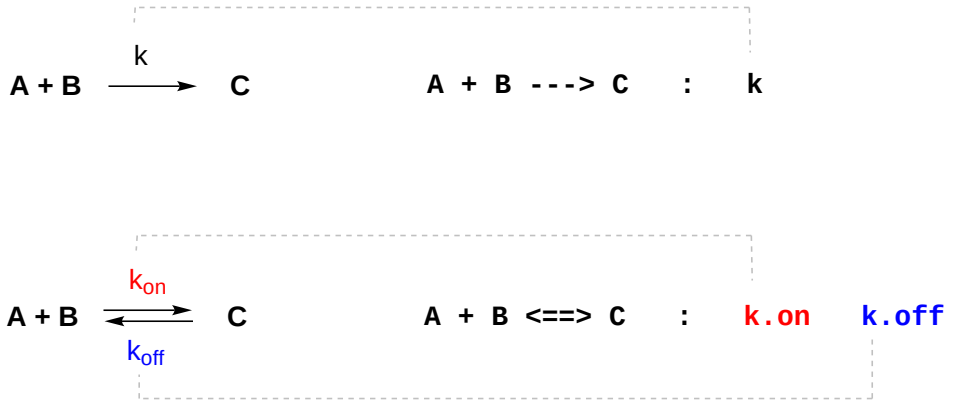

Figure 2: Representing reaction irreversible (top) and reversible (bottom) reaction steps in DynaFit script files.

However, in the specific case of the steady-state initial rate models, the script must also contain the special `reaction` line:

```
[mechanism]
   reaction A + B ---> P + Q
   ...
   ...
```

To derive the initial rate equation *and* the "kinetic constants" (K_m, V_{max}, *etc*.) corresponding to the "Ordered Bi Bi" mechanism, follow these steps.[1]

1. Start DynaFit, if it is not already running.
2. Select menu File ... Open.
3. Navigate to subdirectory ./TN/2015-03/sim.
4. Open script file sim-001.txt.
5. Select menu File ... Run.
6. Click on the **Model** link on the main output page.

The **Model** output page will display the following algebraic model automatically generated by DynaFit. In the auto-generated equations below, c_A – c_Q represent the total or analytic concentrations of reagents A through Q, respectively.

[1] These instructions assume that the user has downloaded and installed the requisite *sample data set* as described at `www.biokin.com/TN/2015/03/`.

Rate Equation

$$v = c_{\mathrm{E}}^{(0)} \frac{N}{D} = \frac{\mathrm{d}c_{\mathrm{P}}}{\mathrm{d}t} = +k_3 c_{\mathrm{EPQ}} - k_{-3} c_{\mathrm{EQ}} c_{\mathrm{P}} \tag{1}$$

Numerator

$$N = n_1 c_{\mathrm{P}} c_{\mathrm{Q}} + n_2 c_{\mathrm{A}} c_{\mathrm{B}} \tag{2}$$

$$n_1 = \frac{-k_{-2} k_{-\mathrm{p}} k_{-3} k_{-4}}{k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{3}$$

$$n_2 = \frac{k_1 k_2 k_{\mathrm{p}} k_3}{k_{-1} \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{4}$$

Denominator

$$\begin{aligned} D = {} & d_1 + d_2 c_{\mathrm{Q}} + d_3 c_{\mathrm{P}} + d_4 c_{\mathrm{B}} + d_5 c_{\mathrm{A}} + d_6 c_{\mathrm{P}} c_{\mathrm{Q}} + d_7 c_{\mathrm{B}} c_{\mathrm{Q}} \\ & + d_8 c_{\mathrm{A}} c_{\mathrm{P}} + d_9 c_{\mathrm{A}} c_{\mathrm{B}} + d_{10} c_{\mathrm{B}} c_{\mathrm{P}} c_{\mathrm{Q}} + d_{11} c_{\mathrm{A}} c_{\mathrm{B}} c_{\mathrm{P}} \end{aligned} \tag{5}$$

where

$$d_1 = 1 \tag{6}$$

$$d_2 = \frac{k_{-4}}{k_4} \tag{7}$$

$$d_3 = \frac{k_{-2} k_{-\mathrm{p}} k_{-3}}{k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{8}$$

$$d_4 = \frac{k_2 k_{\mathrm{p}} k_3}{k_{-1} \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{9}$$

$$d_5 = \frac{k_1}{k_{-1}} \tag{10}$$

$$d_6 = \frac{k_{-3} k_{-4} \left(k_{-2} k_{-\mathrm{p}} + k_{-1} k_{-\mathrm{p}} + k_{-1} k_{-2} + k_{-1} k_{\mathrm{p}} \right)}{k_{-1} k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{11}$$

$$d_7 = \frac{k_2 k_{\mathrm{p}} k_3 k_{-4}}{k_{-1} k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{12}$$

$$d_8 = \frac{k_1 k_{-2} k_{-\mathrm{p}} k_{-3}}{k_{-1} k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{13}$$

$$d_9 = \frac{k_1 k_2 \left(k_{-\mathrm{p}} k_4 + k_3 k_4 + k_{\mathrm{p}} k_4 + k_{\mathrm{p}} k_3 \right)}{k_{-1} k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3 \right)} \tag{14}$$

$$d_{10} = \frac{k_2 k_{-3} k_{-4} \left(k_{-\mathrm{p}} + k_{\mathrm{p}}\right)}{k_{-1} k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3\right)} \tag{15}$$

$$d_{11} = \frac{k_1 k_2 k_{-3} \left(k_{-\mathrm{p}} + k_{\mathrm{p}}\right)}{k_{-1} k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3\right)} \tag{16}$$

Kinetic Constants

Turnover numbers:

$$k_{\mathrm{cat(f)}} = \frac{n_2}{d_9} = \frac{k_{\mathrm{p}} k_3 k_4}{k_{-\mathrm{p}} k_4 + k_3 k_4 + k_{\mathrm{p}} k_4 + k_{\mathrm{p}} k_3} \tag{17}$$

$$k_{\mathrm{cat(r)}} = \frac{n_1}{d_6} = \frac{-k_{-1} k_{-2} k_{-\mathrm{p}}}{k_{-2} k_{-\mathrm{p}} + k_{-1} k_{-\mathrm{p}} + k_{-1} k_{-2} + k_{-1} k_{\mathrm{p}}} \tag{18}$$

Michaelis constants:

$$K_{\mathrm{m(A)}} = \frac{d_4}{d_9} = \frac{k_{\mathrm{p}} k_3 k_4}{k_1 \left(k_{-\mathrm{p}} k_4 + k_3 k_4 + k_{\mathrm{p}} k_4 + k_{\mathrm{p}} k_3\right)} \tag{19}$$

$$K_{\mathrm{m(B)}} = \frac{d_5}{d_9} = \frac{k_4 \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3\right)}{k_2 \left(k_{-\mathrm{p}} k_4 + k_3 k_4 + k_{\mathrm{p}} k_4 + k_{\mathrm{p}} k_3\right)} \tag{20}$$

$$K_{\mathrm{m(P)}} = \frac{d_2}{d_6} = \frac{k_{-1} \left(k_{-2} k_{-\mathrm{p}} + k_{-2} k_3 + k_{\mathrm{p}} k_3\right)}{k_{-3} \left(k_{-2} k_{-\mathrm{p}} + k_{-1} k_{-\mathrm{p}} + k_{-1} k_{-2} + k_{-1} k_{\mathrm{p}}\right)} \tag{21}$$

$$K_{\mathrm{m(Q)}} = \frac{d_3}{d_6} = \frac{k_{-1} k_{-2} k_{-\mathrm{p}}}{k_{-4} \left(k_{-2} k_{-\mathrm{p}} + k_{-1} k_{-\mathrm{p}} + k_{-1} k_{-2} + k_{-1} k_{\mathrm{p}}\right)} \tag{22}$$

Inhibition constants:

$$K_{\mathrm{i(A)}} = \frac{d_1}{d_5} = \frac{k_{-1}}{k_1} \tag{23}$$

$$K_{\mathrm{i(B,P,Q)}} = \frac{d_6}{d_{10}} = \frac{k_{-2} k_{-\mathrm{p}} + k_{-1} k_{-\mathrm{p}} + k_{-1} k_{-2} + k_{-1} k_{\mathrm{p}}}{k_2 \left(k_{-\mathrm{p}} + k_{\mathrm{p}}\right)} \tag{24}$$

$$K_{\mathrm{i(P,A,B)}} = \frac{d_9}{d_{11}} = \frac{k_{-\mathrm{p}} k_4 + k_3 k_4 + k_{\mathrm{p}} k_4 + k_{\mathrm{p}} k_3}{k_{-3} \left(k_{-\mathrm{p}} + k_{\mathrm{p}}\right)} \tag{25}$$

$$K_{\mathrm{i(Q)}} = \frac{d_1}{d_2} = \frac{k_4}{k_{-4}} \tag{26}$$

It can be verified by inspection that the auto-generated algebraic equations listed above are in fact identical to the algebraic model as presented by Segel (see *Figure 3* and ref. [2, p. 564]). Similar verifications were performed for numerous other steady-state kinetic models presented in

Table IX-2 Definition of Kinetic Constants for an Ordered Bi Bi System

$K_{ia} = \dfrac{k_{-1}}{k_1}$	$K_{iq} = \dfrac{k_4}{k_{-4}}$
$K_{m_A} = \dfrac{k_3k_4k_p}{k_1(k_3k_4 + k_3k_p + k_4k_p + k_4k_{-p})}$	$K_{m_Q} = \dfrac{k_{-1}k_{-2}k_{-p}}{k_{-4}(k_{-1}k_{-2} + k_{-1}k_p + k_{-1}k_{-p} + k_{-2}k_{-p})}$
$K_{ib} = \dfrac{k_{-1}k_{-2} + k_{-1}k_p + k_{-1}k_{-p} + k_{-2}k_{-p}}{k_2(k_p + k_{-p})}$	$K_{ip} = \dfrac{k_3k_4 + k_3k_p + k_4k_p + k_4k_{-p}}{k_{-3}(k_p + k_{-p})}$
$K_{m_B} = \dfrac{k_4(k_{-2}k_3 + k_{-2}k_{-p} + k_3k_p)}{k_2(k_3k_4 + k_3k_p + k_4k_p + k_4k_{-p})}$	$K_{m_P} = \dfrac{k_{-1}(k_{-2}k_3 + k_{-2}k_{-p} + k_3k_p)}{k_{-3}(k_{-1}k_{-2} + k_{-1}k_p + k_{-1}k_{-p} + k_{-2}k_{-p})}$
$\dfrac{V_{\max_f}}{[E]_t} = \dfrac{k_3k_4k_p}{k_3k_4 + k_3k_p + k_4k_p + k_4k_{-p}}$	$\dfrac{V_{\max_r}}{[E]_t} = \dfrac{k_{-1}k_{-2}k_{-p}}{k_{-1}k_{-2} + k_{-1}k_p + k_{-1}k_{-p} + k_{-2}k_{-p}}$

Figure 3: Kinetic constants for "Ordered Bi Bi" kinetic mechanism published by Segel [2, p. 564].

Segel's textbook [2, Chap. 9] and no discrepancies were found. This completes the verification that the DynaFit software package [4] does correctly perform algebraic derivations of steady-state initial rate equations *and* "kinetic constants", such as turnover numbers and Michaelis constants.

Note that for *branched* mechanisms such as "Random Bi Bi" or "Random Bi Uni", no kinetic constants can be derived as a matter of principle [2, p. 647]. The DynaFit software will recognize such unfavorable situations and will issue an appropriate message in the **Model** output page.

3. DynaFit notation

This section summarizes the specialized notation that is required in DynaFit scripts, in order to work with the King-Altman method.

3.1. The `approximation` *line in the* `[task]` *section*

The script file must contain the following pair of text lines in the `[task]` section of the script:

```
[task]
   ...
   data = rates
   approximation = king-altman
```

This notation can be used both for simulations and for fitting of actual experimental data.

3.2. The `reaction` *line in the* `[mechanism]` *section*

The `[mechanism]` section of the DynaFit script will contain a representation of the reaction mechanism, using the usual DynaFit notation (see the *Scripting Manual* for details). However,

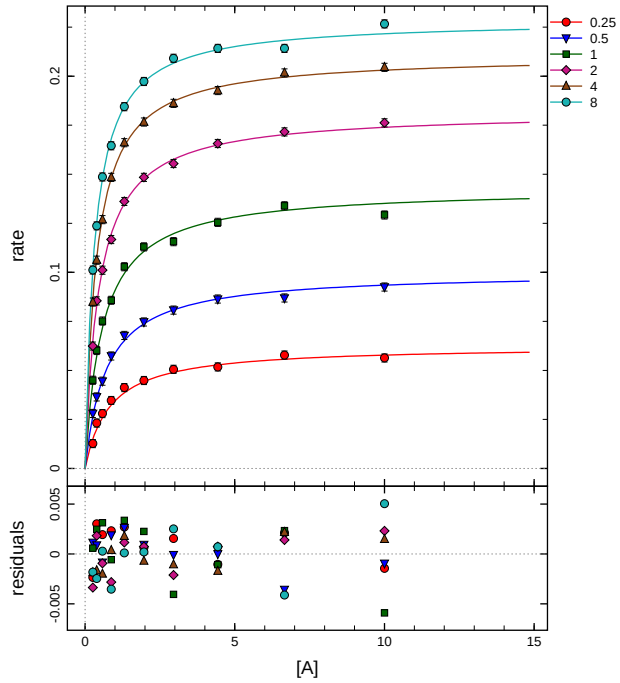

Figure 4: Pseudo-experimental data simulated by the DynaFit software package [4, 5] using the input script listed in the Appendix. The smooth model curves are drawn by using Eqn (1) derived automatically. The legend represents the concentration of substrate B in arbitrary units.

the script must must also include a special line beginning with the key word `reaction`, followed by a representation of the overall reaction stoichiometry.

For example, let us assume that the overall reaction catalyzed by the enzyme involves two substrates (A, B) and two products (P, Q). In that case the `[mechanism]` section of the script must contain the following line:

```
[mechanism]
   reaction A + B ---> P + Q
   ...
```

3.3. The `modifiers` *line in the* `[mechanism]` *section*

If the reaction mechanism involves any *modifiers* (i.e., inhibitors or activators) in addition to reactants (i.e., substrates and products), the `[mechanism]` section must name those modifiers using a notation similar to the following:

```
[mechanism]
```

```
reaction ...
modifiers I
```

In this example, the symbol `I` stands for an inhibitor that participates in the kinetic mechanism.

3.4. The optional `enzyme` *line in the* `[mechanism]` *section*

DynaFit will assume that in any reaction mechanism that is to be investigated by using the King-Altman method the free (unbound) enzyme species is named `E`, for "enzyme". This is a departure from the usual approach in DynaFit scripting, where molecular species can have any arbitrary names.

However, in certain special cases it might be convenient to use a different label or name for the (effectively) free enzyme species. For example, Riera *et al.* [6] proposed for the kinetics of inosine-5'-phosphate dehydrogenase (IMPDH) from *C. parvum* a reaction scheme shown in *Figure 5*.

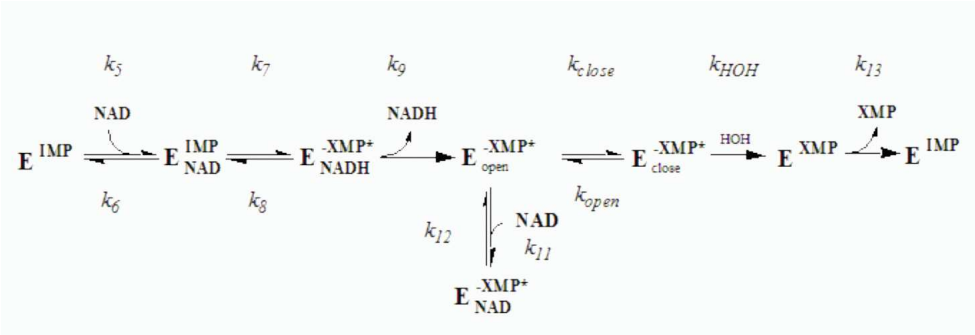

Figure 5: A postulated kinetic mechanism for the inosine-5'-phosphate dehydrogenase (IMPDH) from *C. parvum* [6, Scheme S1].

Importantly, even though IMPDH is a bi-substrate enzyme, the particular set of kinetic experiment was conducted under the conditions where one of the two substrates (IMP) was always present at saturating concentrations ([IMP] >> $K_{m,IMP}$). Consequently the enzyme was always fully saturated with either IMP or the reaction product, XMP (xanthosine-5'-monophosphate).

Under these circumstances the complex `E.IMP` *effectively* plays the role of the "free enzyme" species that catalyzes a biochemical reaction involving only one substrate (NAD) and one reaction product (NADH). To capture that particular idea, we could represent the reaction scheme shown in *Figure 5* as follows:

```
[mechanism]
   reaction NAD ---> NADH
   enzyme   E.IMP             ; <== "free enzyme" species

   E.IMP + NAD <==> E.IMP.NAD            :  k5        k6
```

```
E.IMP.NAD <==> E-XMP*.NADH             :  k7        k8
E-XMP*.NADH ---> (E-XMP*)open + NADH   :  k9
(E-XMP*)open + NAD <==> (E-XMP*).NAD   :  k11       k12
(E-XMP*)open <==> (E-XMP*)close        :  k.close   k.open
(E-XMP*)close ---> E.XMP               :  k.HOH
E.XMP ---> E.IMP                       :  k13
```

Please note especially the line `enzyme E.IMP`, signifying that in the automatic derivation of the King-Altman rate equation and kinetic constants (K_m, etc.) the species `E.IMP` should be considered to be the "free enzyme" species. Also note that the actual product of the reaction (XMP) does not appear as a stand-alone species in the `[mechanism]` section, similar to the substrate `IMP`.

References

[1] E. L. King, C. Altman, A schematic method of deriving the rate laws for enzyme-catalyzed reactions, J. Phys. Chem. 60 (1956) 1375–1378.

[2] I. H. Segel, Enzyme Kinetics, Wiley, New York, 1975.

[3] A. Cornish-Bowden, An automatic method for deriving steady-state rate equations, Biochem. J. 165 (1977) 55–59.

[4] P. Kuzmič, Program DYNAFIT for the analysis of enzyme kinetic data: Application to HIV proteinase, Anal. Biochem. 237 (1996) 260–273.

[5] P. Kuzmič, DynaFit - A software package for enzymology, Meth. Enzymol. 467 (2009) 247–280.

[6] T. V. Riera, W. Wang, H. R. Josephine, L. Hedstrom, A kinetic alignment of orthologous inosine-5'-monophosphate dehydrogenases, Biochemistry 47 (2008) 8689–8696.

Appendix

A. DynaFit scripts

A.1. Simulation of "Ordered Bi-Bi" mechanism

```
Simulate a family of substrate saturation curves for the
"Ordered Bi Bi" kinetic mechanism.
;------------------------------------------------------------
[task]
   task = simulate
   data = rates
   approximation = king-altman

[mechanism]

   ; Reaction scheme on p. 560 of Segel's "Enzyme Kinetics" (1975)

   reaction A + B ---> P + Q

   E + A <==> EA      :     k1   k-1
   EA + B <==> EAB    :     k2   k-2
   EAB <==> EPQ       :     kp   k-p
   EPQ <==> EQ + P    :     k3   k-3
   EQ <==> E + Q      :     k4   k-4

[constants]

   k1 = 1,   k-1 = 1
   k2 = 1,   k-2 = 1
   kp = 1,   k-p = 1
   k3 = 1,   k-3 = 1
   k4 = 1,   k-4 = 1

[concentrations]

   E = 0.001

[responses]

   P = 1000

[data]

   variable   A
   mesh       logarithmic from 10 to 0.2 step 0.666
   error      constant 1 percent
   directory ./TN/2015-03/simul/ordered-bi-bi/data/sim-001
   extension txt

   file      d01 | conc B = 0.25 | label 0.25
```

```
   file      d02 | conc B = 0.5  | label 0.5
   file      d03 | conc B = 1    | label 1
   file      d04 | conc B = 2    | label 2
   file      d05 | conc B = 4    | label 4
   file      d06 | conc B = 8    | label 8

[output]
   directory ./TN/2015-03/simul/ordered-bi-bi/output/sim-001

[settings]
{Output}
   WriteTeX = y
   WriteEPS = y

[end]
```