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wREFERASS: Rate Equations for Enzyme Reactions at Steady State under MS-Windows

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Abstract

The derivation of steady-state equations is necessary for the interpretation of enzyme kinetic studies. We have implemented a C++ library and its graphical interface for MS-Windows, named wREFERASS, to facilitate the study of complex reaction mechanisms at the strict steady-state. The C++ library is freely available and could be used directly in any program language as a library function call. We have tested the program with very complex enzyme-catalyzed reaction mechanisms and found its performance to be satisfactory.

The program wREFERASS along with instructions and many examples, can be downloaded from http://oretano.iele-ab.uclm.es/~fgarcia/wREFERASS/

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Introduction

Some years ago, after considering the available algorithms and computer programs [1]-[3] we developed the REFERASS program [4]. This program, which runs under MS-DOS, uses the algorithm published by Varon *et. al.* [5] to derive the strict steady-state rate equations for enzyme-catalyzed reaction mechanisms of arbitrary complexity. The program can produce full rate equations in terms of the individual rate constants or when one or more of the reversible steps are in rapid equilibrium. Nevertheless, REFERASS program is not open source and we have found that it does not run on personal computers with operating systems newer than MS-Windows 2000, which makes it unusable in many cases.

Although other related programs have been published since 1997 (e.g. [6]), we have rewritten the program from scratch and divided it in two modules. One is a C++ library that makes all necessary computations to obtain the desired steady-state equations and the second module, written in Delphi, acts only as a graphical interface between the user and the C++ library.

The core of the C++ library implements the algorithm described by Varon *et. al.* [4]. All the functions and structures have been redesigned to obtain a more portable source code, so the communication with the library (input and output) is made via plain text files. By default, the number of enzyme species in the enzyme-catalyzed reaction mechanism has been limited to 255 and these can be connected by up to 255 reaction steps. These numbers well exceed what are likely to occur in real systems but can be increased easily by the user at compilation time, although a great amount of memory will be needed during execution of the program.

In order to facilitate the use of the C++ library, we also have developed a simple graphic interface for MS-Windows, named wREFERASS (Fig. 1), written in Delphi, which calls our dynamic link library (DLL) compiled with MS-Visual C++. In this program, for compatibility reasons, we have maintained the same syntax that used in the previous MS-DOS version of REFERASS. Thus, mechanism files already written for the previous version may be used without modification.

Figure 1. The wREFERASS user interface.

wREFERASS software has been tested exhaustively with many real enzyme systems (such as those described in [1] and [7]) which can be obtained from the same web page already mentioned above.

Implementation

The computer program wREFERASS represents a considerable improvement of a previous program written by us for MS-DOS [4]. The main characteristics of the present version are:

a) It runs properly under all MS-Windows systems like 2000, XP, Vista and Seven (32 and 64 bits).

b) It admits reaction mechanisms up to 255 enzyme species and up to 255 reaction steps subject to limits imposed by the physical and virtual memory available on the computer. These numbers can be increased easily if necessary (see Results and Discussion section).

c) The computation time is very short, even for complex mechanisms. Obviously, this time depend on the computer used. Thus when a 1.5 GHz Intel Centrino was used, the computer time was less than 10 s for all examples.

d) The input of the data is very easy and versatile.

e) It can furnish simultaneously the steady-state concentration equation of one or more (even all) of the involved enzyme species and/or the steady-state rate of one or more (even all) of the involved ligand species.

f) It provides the results in the most simplified form.

Input Syntax and Structure

The interaction between the program and the user is made either by creating a text file or by writing it directly on a memo field, and having the following syntax and structure:

There are five possible types of text lines:

- Comment lines
- Species notation lines
- Rate constants notation lines
- Rapid Equilibrium steps lines
- User option lines

Comment lines: To enter a comment, type a number sign (#) in the first column, then type the comment to the right of the number sign. If the comment requires more than one line, repeat the number sign on each line before continuing the comment. E.g.:

```
#Title: The mechanism under study
#User: The one which writes the file
#...
```

Species notation lines: For each enzyme species type X in the first column, followed by a index number between 1 and 255 which must be different for each one; then write the equal symbol (=) and finally the original notation of the corresponding enzyme species used in the reaction scheme. X1 must always correspond with the free enzyme. The numbering of the other enzyme species is arbitrary. E.g.:

```
X1 = E
X2 = EM
X3 = ES
X4 = EP
X5 = EMP
X6 = MES
```

An alternative notation of the enzyme species is to type the letter n, then the equal symbol and finally the number of enzyme species; e.g. n=6 instead of the above six lines. In this case the enzyme species will be shown in the output as X_1 to X_n. If both syntaxes are used simultaneously, then the second form (i.e. where the n-value is entered) takes precedence.

Rate constants notation lines: You must type only the non-null Ki,j's. For each first or pseudo-first order rate constant $K_{i,j}$ type a capital K in the first column, followed by the numbers i and j separated by a comma (e.g. K2,7); then write the equal symbol (=) and finally the expression of the corresponding rate constant. Any expression for the rate constant of a reaction step consists of a lowercase k (or k') followed by a plus or a minus sign and the subindex (one or more characters). The plus sign can be omitted. If the rate constant is of pseudo-first order, then the notation of the corresponding ligand species must be written in square brackets. The two rate constants involved in the same reversible reaction step must be denoted using the same subindex (one of them preceded necessarily by the minus sign) and the two expressions must begin with either k or k'. E.g.:

```
K1,2 = k+1[S]
K1,3 = k+3[M]
K2,4 = k+4[M]
K3,1 = k-3
K3,4 = k+5[S]
K4,2 = k-4
K5,6 = k'9[A]
K6,5 = k'-9
K7,8 = k'-12[Q]
```

In those cases in which two or more parallel steps exist between two enzyme species Xi and Xj, the rate constant Ki,j corresponding to each step must be distinguished writing at the right side of the Ki,j a different arbitrary number (1..255) between brackets for each step. E.g.:

```
K2,1(1) = k-1
K2,1(2) = k+2
K4,3(1) = k-5
K4,3(2) = k+6
```

If there is an irreversible step, parallel or not, in which one or more ligand species are released then you must write on the right of the corresponding Ki,j the symbol for implication (=>) followed by the expression(s) used in the reaction mechanism for the ligand species released. If more than one ligand species are released then their expressions must be separated by at least one blank space. E.g.:

```
K2,1(2) = k+2 => P
K9,1    = k-5 => Q S
K4,3(1) = k+6 => P
```

Rapid equilibrium step lines: If there are one or more different pairs of enzyme species connected by at least one reversible reaction step in rapid equilibrium, then type, for each of the above pairs, RE in the first two columns of the file followed by one or more blank spaces and finally the index numbers corresponding to each enzyme species notation in rapid equilibrium, separated by a comma. E.g., if the pairs of enzyme species X7 and X8 and X3 and X4 are in rapid equilibrium, then this would be specified:

```
RE 7,8
RE 3,4
```

User option lines: You can select which results you want to obtain and the form of the output file. To do this there are four option lines, all of them beginning with a dot. These options are:

1) If you want to obtain the steady state concentration of one or more of the enzyme species then type *.Species* = followed by the notation used for the corresponding species in the reaction scheme separated by one or more blank spaces. If you want the steady state concentration of all of the enzyme species,

then you can either write all notations or an asterisk on the right hand side of the equal symbol. E.g.:

```
.Species = E EM
.Species = *
```

2) If you want to obtain the steady state rate of one or more of the ligand species then type *.Ligands* = followed by the notation used for the corresponding ligand species in the reaction scheme separated by one or more blank spaces. If you want the steady state rate of all of the ligand species then you can either write all notations or an asterisk on the right hand side of the equal symbol. E.g.:

```
.Ligands = S P
.Ligands = *
```

3) In those cases in which one or more pairs of enzyme species are in rapid equilibrium, there are two possible forms to give the results: as a function of only the individual rate constants or including the corresponding equilibrium constants. If you choose the second form, type

```
.Equilibrium Constants
```

or its abbreviation:

```
.EC
```

at the beginning of any line. By default, i.e., if you omit this line, the expressions are printed using the individual rate constants. If you enter this command when there are no steps at rapid equilibrium, then this line is ignored by the program.

4) By default, the program sets the width of the output file to 80 columns. If you want another width, you can use the option line *.Width* = followed by a number (greater than 49) indicating the number of columns you want. If you write a number less than 50, the program changes it to 50. In complex mechanisms some terms may be too long to fit it in the width chosen. In these cases the program uses automatically the width necessary for the term and the highest width used is given at the end of the output file.

The .Species, .Ligands and .Width parameters in the option lines may be replaced by .S, .L and .W, respectively.

Output of the results

When the input file has been written, as described above, then press the *"Run"* button and the program creates automatically an ASCII file with the same name as that you chose for the input file with the extension *.lis*, e.g., if your input file is named *foo.in*, then the output file is named by the program as *foo.lis*. We select an ASCII file to present the results, because of the facility for display and printing, compatibility with word processors, etc.

This file contains the expressions of the steady state concentrations of the enzyme species selected and/or those of the steady state rate of the ligand species selected in the most simplified possible form. In those cases in which are one or more reversible steps in rapid equilibrium, the results are printed in the form you selected, i.e., containing or not equilibrium rate constants.

To unify the form of the steady-state equations derived by the program and contained in the file created, these equations are printed as:

$$[X_i] = N(X_i)[E]_0/Den \quad (1)$$

$$v(Y_s) = M(Y_s)[E]_0/Den \quad (2)$$

and then expressions of $N(X_i)$, $M(Y_s)$ and Den are given.

As described in [4] and [5], the quotient $N(X_i)/Den$ coincides, with the corresponding quotient G_i/G, H_i/H or L_i/L after making all possible simplifications, thus no common ligand species concentration appears in the terms of the denominator Den. Likewise, quotient $M(Y_s)/Den$ coincides, with one of the quotients G_{Ys}/G, H_{Ys}/H and L_{Ys}/L in the most simplified form.

In most cases, as indicated in eqns. (1) and (2), the denominator of the corresponding eqn. (1) coincides with the denominator of eqn. (2), Den. Nevertheless, the program foresees the possibility that in some reaction schemes the denominator, resulting after simplification of the corresponding quotient H_{Ys}/H and L_{Ys}/L, does not coincide with Den. In theses cases, the denominator Den is not modified, but the numerator of the expression is divided by the

appropriate factor given by the program.

Obviously, the concentrations of an enzyme species or/and the rate of a ligand species can be zero at the steady-state, being these cases also foreseen by the program, which yields 0 for the corresponding concentration or rate (see some examples below).

Expressions such as [S]^2, [I]^3, etc. in the resulting equations must be interpreted as $[S]^2$, $[I]^3$, etc.

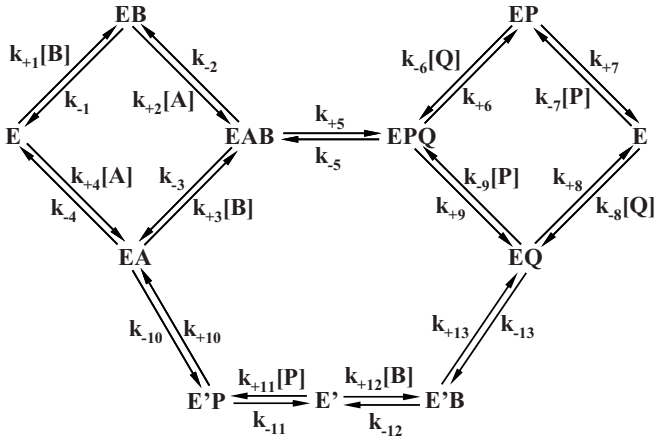
Error messages.

During the execution of the program any error in the input data is detected and the corresponding error message is displayed on the screen for its correction and then the program stops its execution. Once the error in the input file has been corrected you only need to press the *"Run"* button again.

Examples

In the following we apply the computer program developed by us to three reaction mechanisms of different complexity. In the web site are available many other reaction schemes which are solved with wREFERASS.

Example 1: *General Mechanism for Two-Substrate Enzyme Systems*



Scheme 1

As a first example we considered the reaction mechanism in Scheme 1 in which we assume that the steps involving binding and dissociation of the reactants are in rapid equilibrium. Let us derive the concentrations of all of the enzyme species and the rate of the ligand species P at the steady-state as a function of the equilibrium constants of the reversible steps at rapid equilibrium and of the individual rate constants of the remaining steps.

INPUT FILE

```
#Title: General Mechanism for Two-Substrate System
#Enzyme Species notation
X1  = E
X2  = EB
X3  = EA
X4  = EAB
X5  = EPQ
X6  = EP
X7  = EQ
X8  = E'P
X9  = E'
X10 = E'B

#Expressions of the rate constants
K1,2 = k+1[B]
K1,3 = k+4[A]
K1,6 = k-7[P]
K1,7 = k-8[Q]
K2,1 = k-1
K2,4 = k+2[A]
K3,1 = k-4
K3,4 = k+3[B]
K3,8 = k-10
K4,2 = k-2
K4,3 = k-3
K4,5 = k+5
```

```
K5,4 = k-5
K5,6 = k+6
K5,7 = k+9
K6,1 = k+7
K6,5 = k-6[Q]
K7,1 = k+8
K7,5 = k-9[P]
K7,10 = k-13
K8,3  = k+10
K8,9  = k-11
K9,8  = k+11[P]
K9,10 = k+12[B]
K10,7 = k+13
K10,9 = k-12

#Pairs of enzyme species connected by at least one
#reversible step in rapid equilibrium
RE     1,2
RE     1,3
RE     1,6
RE     1,7
RE     2,4
RE     3,4
RE     5,6
RE     5,7
RE     8,9
RE     9,10
.Width = 80
.Equilibrium Constants
.Concentrations = *
.Ligands = P
```

OUTPUT FILE

Concentrations:

[E] = N(E)[E]o/Den

[EB] = N(EB)[E]o/Den

[EA] = N(EA)[E]o/Den

[EAB] = N(EAB)[E]o/Den

[EPQ] = N(EPQ)[E]o/Den

[EP] = N(EP)[E]o/Den

[EQ] = N(EQ)[E]o/Den

[E'P] = N(E'P)[E]o/Den

[E'] = N(E')[E]o/Den

[E'B] = N(E'B)[E]o/Den

Ligands:

v(P) = M(P)[E]o/Den

Where:

N(E) = K1K2K9(k+10)K12[P] + K1K2K9K11(k+13)[B]

N(EB) = K2K9(k+10)K12[B][P] + K2K9K11(k+13)[B]^2

N(EA) = K3K9(k+10)K12[A][P] + K3K9K11(k+13)[A][B]

N(EAB) = K9(k+10)K12[A][B][P] + K9K11(k+13)[A][B]^2

N(EPQ) = K7K1K2K6K9(k+10)K12[P]^2[Q] + K7K1K2K6K9K11(k+13)[B][P][Q]

N(EP) = K7K1K2K9(k+10)K12[P]^2 + K7K1K2K9K11(k+13)[B][P]

N(EQ) = K7K1K2K6(k+10)K12[P][Q] + K7K1K2K6K11(k+13)[B][Q]

N(E'P) = (k-10)K3K9K12[A][P] + K7K1K2K6(k-13)K12[P][Q]

N(E') = (k-10)K3K9K11K12[A] + K7K1K2K6(k-13)K11K12[Q]

N(E'B) = (k-10)K3K9K11[A][B] + K7K1K2K6(k-13)K11[B][Q]

M(P) = (k-10)K3K9K11(k+13)[A][B] - K7K1K2K6(k-13)(k+10)K12[P][Q] -
K7K1K2(k-5)K6K9(k+10)K12[P]^2[Q] - K7K1K2(k-5)K6K9K11(k+13)[B][P][Q] +
(k+5)K9(k+10)K12[A][B][P] + (k+5)K9K11(k+13)[A][B]^2

Den = (k-10)K3K9K11K12[A] + K7K1K2K6(k-13)K11K12[Q] + K1K2K9(k+10)K12[P] +
K1K2K9K11(k+13)[B] + K3K9K11{(k-10) + (k+13)}[A][B] + K3K9K12{(k-10) +
(k+10)}[A][P] + K2K9{(k+10)K12 + K7K1K11(k+13)}[B][P] + K2K9K11(k+13)[B]^2 +
K7K1K2K9(k+10)K12[P]^2 + K7K1K2K6K11{(k-13) + (k+13)}[B][Q] +
K7K1K2K6K12{(k-13) + (k+10)}[P][Q] + K9(k+10)K12[A][B][P] +
K9K11(k+13)[A][B]^2 + K7K1K2K6K9(k+10)K12[P]^2[Q] +
K7K1K2K6K9K11(k+13)[B][P][Q]

Relationships between the equilibrium constants arising from the application

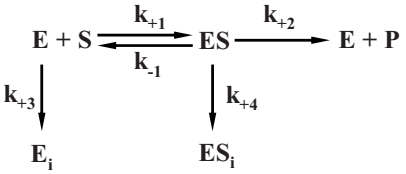
```
of the mass-action law to the reversible steps in the alpha-loops:

       K3K4 = K1K2

       K7K6 = K9K8

Ki  = (k+i)/(k-i)   (i = 1, 4, 2, 3, 6, 9, 7, 8, 11, 12)
```

Example 2: *Michaelis-Menten Mechanism in which the Enzyme Species are Unstable*



Scheme 2

As a second example we considered the reaction mechanism in Scheme 2 above under strict steady-state assumptions. Let us derive the concentrations of all of the enzyme species and the rate of the ligand species P and S at the steady-state as a function of the individual rate constants.

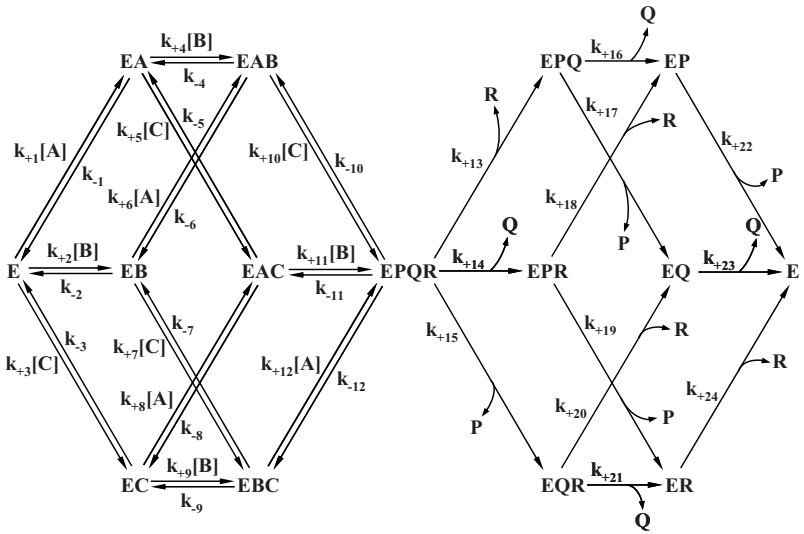
INPUT FILE

```
#Title: Michaelis-Menten Mechanism in which
#       the enzyme species are unstable
#Enzyme Species notation
n = 4
#Expressions of the rate constants
K1,2    = k+1[S]
K1,3    = k+3
K2,1(1) = k-1
K2,1(2) = k+2    => P
K2,4    = k+4
.W = 80
.S = *
.L = *
```

OUTPUT FILE

```
Concentrations:
                [X1] = 0
                [X2] = 0
                [X3] = N(X3)[E]o/Den
                [X4] = N(X4)[E]o/Den
Ligands:
        v(S) = 0
        v(P) = 0
Where:
N(X3) =   (k+3){(k-1) + (k+2) + (k+4)}
N(X4) =   (k+1)(k+4)[S]
Den =   (k+3){(k-1) + (k+2) + (k+4)} + (k+1)(k+4)[S]
```

Example 3: *Random Ter-Ter Mechanism*



Scheme 3

Example 3 corresponds to the random Ter-Ter mechanism, a very complex scheme proposed by Fromm [8].

Assumptions with regard to reversible steps:

All reversible steps are in rapid equilibrium.

Steady-state equations wanted:

Rate equation of the product P, involving the equilibrium constants of the reversible steps.

INPUT FILE

```
#Random Ter-Ter
X1  = E
X2  = EA
X3  = EB
X4  = EC
X5  = EAB
X6  = EAC
X7  = EBC
X8  = EPQR
X9  = EPQ
X10 = EPR
X11 = EQR
X12 = EP
X13 = EQ
X14 = ER

K1,2 = k+1[A]
K1,3 = k+2[B]
K1,4 = k+3[C]
K2,1 = k-1
K2,5 = k+4[B]
K2,6 = k+5[C]
K3,1 = k-2
K3,5 = k+6[A]
K3,7 = k+7[C]
K4,1 = k-3
```

```
K4,6 = k+8[A]
K4,7 = k+9[B]
K5,2 = k-4
K5,3 = k-6
K5,8 = k+10[C]
K6,2 = k-5
K6,4 = k-8
K6,8 = k+11[B]
K7,3 = k-7
K7,4 = k-9
K7,8 = k+12[A]
K8,5   = k-10
K8,6   = k-11
K8,7   = k-12
K8,9   = k+13            => R
K8,10  = k+14            => Q
K8,11  = k+15            => P
K9,12  = k+16            => Q
K9,13  = k+17            => P
K10,12 = k+18            => R
K10,14 = k+19            => P
K11,13 = k+20            => R
K11,14 = k+21            => Q
K12,1  = k+22            => P
K13,1  = k+23            => Q
K14,1  = k+24            => R

RE     1,2
RE     1,3
RE     1,4
RE     2,5
RE     2,6
RE     3,5
```

```
RE      3,7
RE      4,6
RE      4,7
RE      5,8
RE      6,8
RE      7,8

.W = 132
.L = P
.EC
```

OUTPUT FILE

Ligands:

$$v(P) = M(P)[E]o/Den$$

Where:

$$M(P) = (k+22)(k+23)(k+24)\{(k+15)(k+16)(k+18)(k+20) + (k+15)(k+16)(k+18)(k+21) + (k+15)(k+16)(k+19)(k+20) + (k+15)(k+16)(k+19)(k+21) + (k+15)(k+17)(k+18)(k+20) + (k+15)(k+17)(k+18)(k+21) + (k+15)(k+17)(k+19)(k+20) + (k+15)(k+17)(k+19)(k+21) + (k+13)(k+17)(k+18)(k+20) + (k+13)(k+17)(k+18)(k+21) + (k+13)(k+17)(k+19)(k+20) + (k+13)(k+17)(k+19)(k+21) + (k+14)(k+16)(k+19)(k+20) + (k+14)(k+16)(k+19)(k+21) + (k+14)(k+17)(k+19)(k+20) + (k+14)(k+17)(k+19)(k+21) + (k+13)(k+16)(k+18)(k+20) + (k+13)(k+16)(k+18)(k+21) + (k+13)(k+16)(k+19)(k+20) + (k+13)(k+16)(k+19)(k+21) + (k+14)(k+16)(k+18)(k+20) + (k+14)(k+16)(k+18)(k+21) + (k+14)(k+17)(k+18)(k+20) + (k+14)(k+17)(k+18)(k+21)\}[A][B][C]$$

$$Den = K1K4K10(k+22)(k+23)(k+24)\{(k+16)(k+18)(k+20) + (k+16)(k+18)(k+21) + (k+16)(k+19)(k+20) + (k+16)(k+19)(k+21) + (k+17)(k+18)(k+20) + (k+17)(k+18)(k+21) + (k+17)(k+19)(k+20) + (k+17)(k+19)(k+21)\} + K8K11(k+22)(k+23)(k+24)\{(k+16)(k+18)(k+20) + (k+16)(k+18)(k+21) + (k+16)(k+19)(k+20) + (k+16)(k+19)(k+21) + (k+17)(k+18)(k+20) + (k+17)(k+18)(k+21) + (k+17)(k+19)(k+20) + (k+17)(k+19)(k+21)\}[C] + K6K10(k+22)(k+23)(k+24)\{(k+16)(k+18)(k+20) + (k+16)(k+18)(k+21) + (k+16)(k+19)(k+20) + (k+16)(k+19)(k+21) + (k+17)(k+18)(k+20) + (k+17)(k+18)(k+21) + (k+17)(k+19)(k+20) + (k+17)(k+19)(k+21)\}[B] + K4K10(k+22)(k+23)(k+24)\{(k+16)(k+18)(k+20) + (k+16)(k+18)(k+21) + (k+16)(k+19)(k+20) + (k+16)(k+19)(k+21) + (k+17)(k+18)(k+20) + (k+17)(k+18)(k+21) + (k+17)(k+19)(k+20) + (k+17)(k+19)(k+21)\}[A] + K12(k+22)(k+23)(k+24)\{(k+16)(k+18)(k+20) + (k+16)(k+18)(k+21) +$$

```
        (k+16)(k+19)(k+20) + (k+16)(k+19)(k+21) + (k+17)(k+18)(k+20) +
        (k+17)(k+18)(k+21) + (k+17)(k+19)(k+20) + (k+17)(k+19)(k+21)}[B][C] +
         K11(k+22)(k+23)(k+24){(k+16)(k+18)(k+20) + (k+16)(k+18)(k+21) +
        (k+16)(k+19)(k+20) + (k+16)(k+19)(k+21) + (k+17)(k+18)(k+20) +
        (k+17)(k+18)(k+21) + (k+17)(k+19)(k+20) + (k+17)(k+19)(k+21)}[A][C] +
         K10(k+22)(k+23)(k+24){(k+16)(k+18)(k+20) + (k+16)(k+18)(k+21) +
        (k+16)(k+19)(k+20) + (k+16)(k+19)(k+21) + (k+17)(k+18)(k+20) +
        (k+17)(k+18)(k+21) + (k+17)(k+19)(k+20) + (k+17)(k+19)(k+21)}[A][B] +
       {(k+13)(k+16)(k+18)(k+20)(k+23)(k+24) + (k+13)(k+16)(k+18)(k+21)(k+23)(k+24) +
        (k+13)(k+16)(k+19)(k+20)(k+23)(k+24) + (k+13)(k+16)(k+19)(k+21)(k+23)(k+24) +
        (k+13)(k+17)(k+18)(k+20)(k+22)(k+24) + (k+13)(k+17)(k+18)(k+21)(k+22)(k+24) +
        (k+13)(k+17)(k+19)(k+20)(k+22)(k+24) + (k+13)(k+17)(k+19)(k+21)(k+22)(k+24) +
        (k+13)(k+18)(k+20)(k+22)(k+23)(k+24) + (k+13)(k+18)(k+21)(k+22)(k+23)(k+24) +
        (k+13)(k+19)(k+20)(k+22)(k+23)(k+24) + (k+13)(k+19)(k+21)(k+22)(k+23)(k+24) +
        (k+14)(k+16)(k+18)(k+20)(k+23)(k+24) + (k+14)(k+16)(k+18)(k+21)(k+23)(k+24) +
        (k+14)(k+16)(k+19)(k+20)(k+22)(k+23) + (k+14)(k+16)(k+19)(k+21)(k+22)(k+23) +
        (k+14)(k+16)(k+20)(k+22)(k+23)(k+24) + (k+14)(k+16)(k+21)(k+22)(k+23)(k+24) +
        (k+14)(k+17)(k+18)(k+20)(k+23)(k+24) + (k+14)(k+17)(k+18)(k+21)(k+23)(k+24) +
        (k+14)(k+17)(k+19)(k+20)(k+22)(k+23) + (k+14)(k+17)(k+19)(k+21)(k+22)(k+23) +
        (k+14)(k+17)(k+20)(k+22)(k+23)(k+24) + (k+14)(k+17)(k+21)(k+22)(k+23)(k+24) +
        (k+15)(k+16)(k+18)(k+20)(k+22)(k+24) + (k+15)(k+16)(k+18)(k+21)(k+22)(k+23) +
        (k+15)(k+16)(k+18)(k+22)(k+23)(k+24) + (k+15)(k+16)(k+19)(k+20)(k+22)(k+24) +
        (k+15)(k+16)(k+19)(k+21)(k+22)(k+23) + (k+15)(k+16)(k+19)(k+22)(k+23)(k+24) +
        (k+15)(k+17)(k+18)(k+20)(k+22)(k+24) + (k+15)(k+17)(k+18)(k+21)(k+22)(k+23) +
        (k+15)(k+17)(k+18)(k+22)(k+23)(k+24) + (k+15)(k+17)(k+19)(k+20)(k+22)(k+24) +
        (k+15)(k+17)(k+19)(k+21)(k+22)(k+23) + (k+15)(k+17)(k+19)(k+22)(k+23)(k+24) +
        (k+16)(k+18)(k+20)(k+22)(k+23)(k+24) + (k+16)(k+18)(k+21)(k+22)(k+23)(k+24) +
        (k+16)(k+19)(k+20)(k+22)(k+23)(k+24) + (k+16)(k+19)(k+21)(k+22)(k+23)(k+24) +
        (k+17)(k+18)(k+20)(k+22)(k+23)(k+24) + (k+17)(k+18)(k+21)(k+22)(k+23)(k+24) +
        (k+17)(k+19)(k+20)(k+22)(k+23)(k+24) +
        (k+17)(k+19)(k+21)(k+22)(k+23)(k+24)}[A][B][C]


Relationships between the equilibrium constants arising from the application

of the mass-action law to the reversible steps in the alpha-loops:

       K6K2 = K1K4

       K8K3 = K1K5

       K11K5 = K4K10

       K12K7 = K6K10

       K12K9 = K8K11

Ki  = (k+i)/(k-i)   for i = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
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