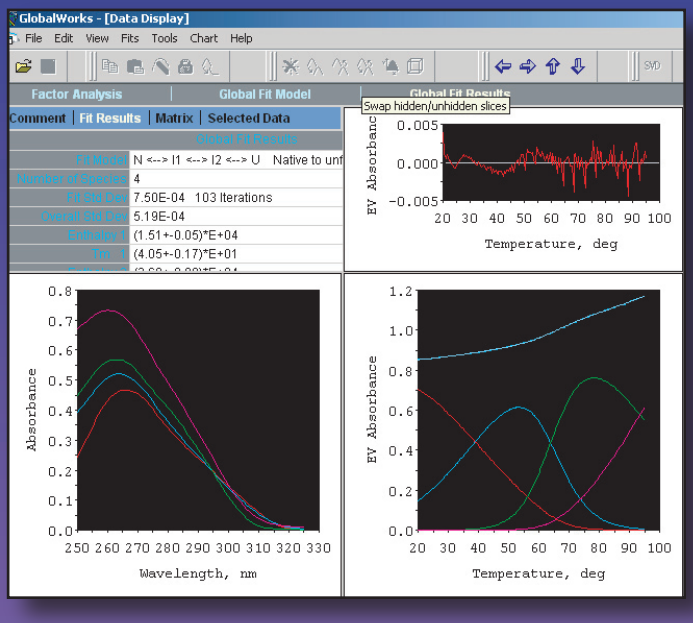
Olis GlobalWorks 3D Analysis Software



Post-collection software with a modern emphasis on multiple wavelength -- Global -- data analysis and handling.

Global analysis works better, providing more accurate rate constants and definitive spectral reconstruction.

Single wavelength analysis is often incomplete and incorrect.[1] But analyzing large **multiple wavelength** data sets is beyond the scope of most software packages or too slow to be practical.

In the early 1990s, after introducing a rapid-scanning spectrophotometer which produces 1 MB of data per second,[2] Olis programmers were charged with developing fast and effective **multiple wavelength** data analysis software. Today's Olis GlobalWorks software is the extraordinary result of more than 14 years of implementing and improving algorithms for 3D analysis.

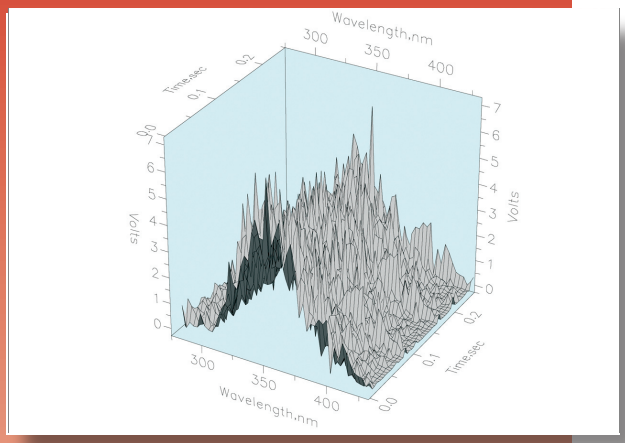
Start with **multiple wavelength** data (spectra) acquired as a function of time, temperature, or other process. Apply SVD. Instantly, the spectral species are found and separated from the noise in the raw data.

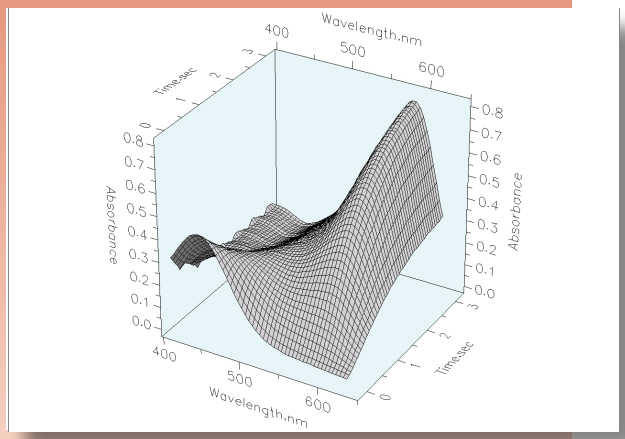
Now, using precise graphic and numeric parameters returned by SVD to conclude how many species are present, choose a chemical model to define the reaction (e.g., A↔B→C) . Moments elapse from raw 3D data entry to fitted results.

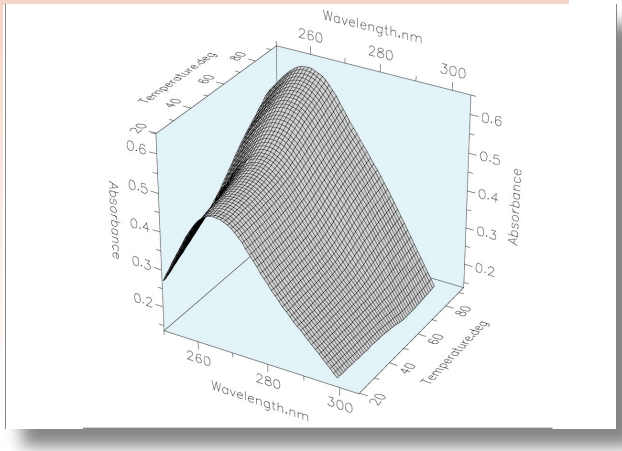
Do not let insufficient data or frustrating software limit you to single wavelength data analysis. Move to Olis GlobalWorks. More than 70 kinetic and equilibrium cases are supported for files containing ten to thousands of scans.

[1] Methods in Enzymology, 1992; 210:37-54. [2] The Olis RSM 1000 acquires 1,000 scans per second.

Step 1: Time, Temperature, or Other Process Dependent 3D Data

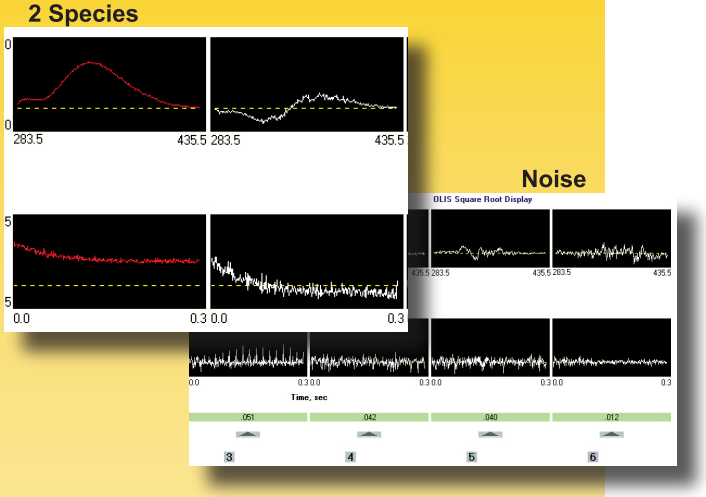




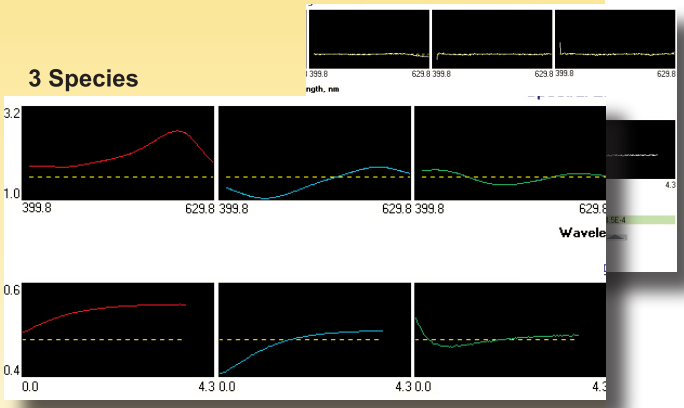


Step 2: SVD Separates Noise and Suggests Number of Species

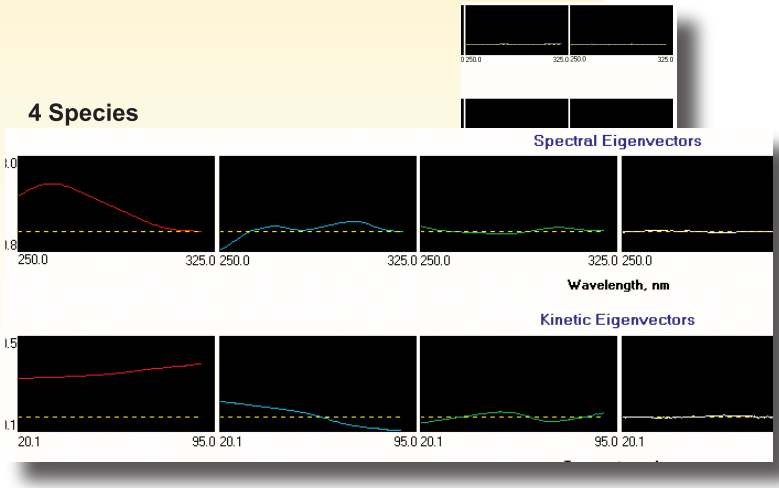
2 Species



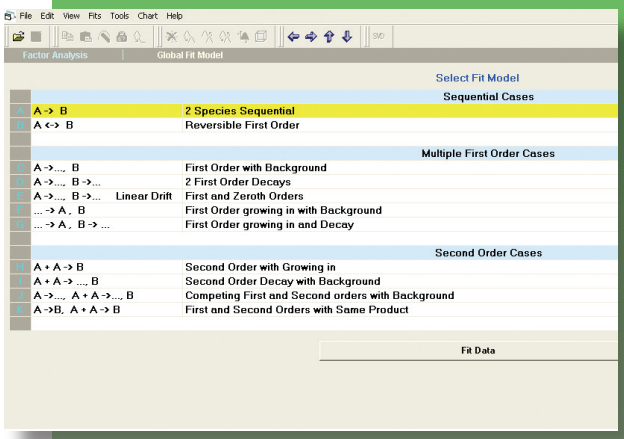
3 Species

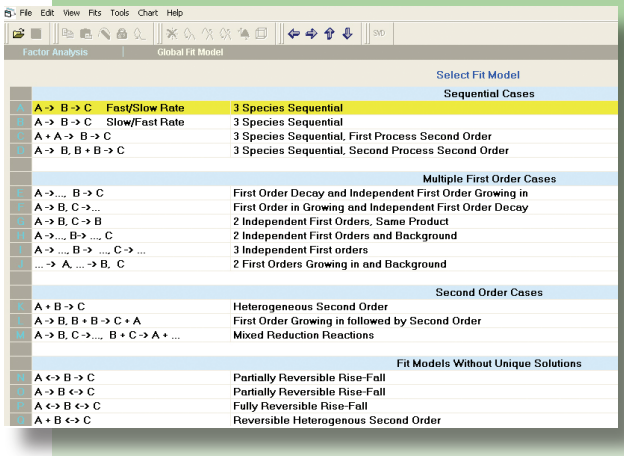


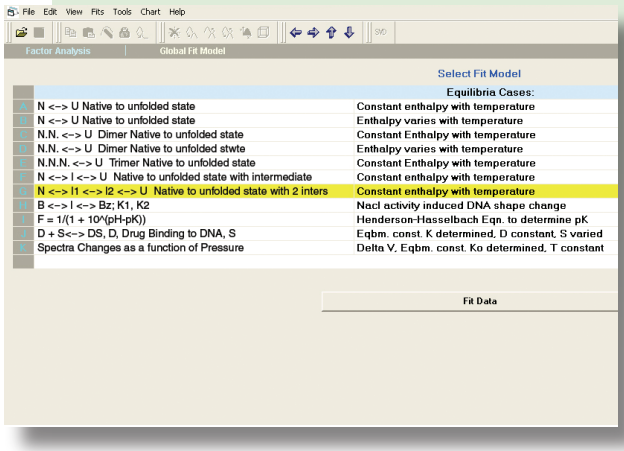
4 Species



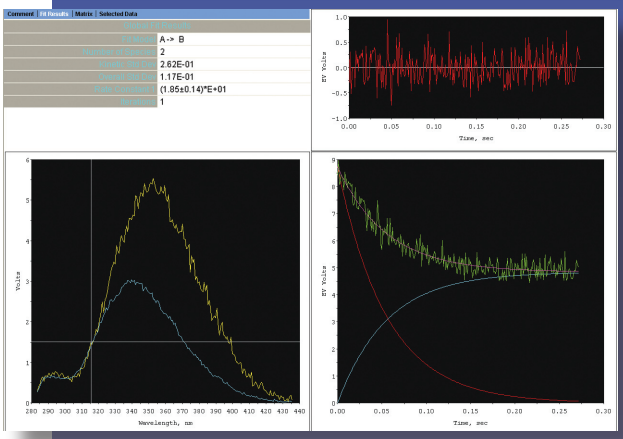
Step 3: Chemical Models for Chosen Number of Species

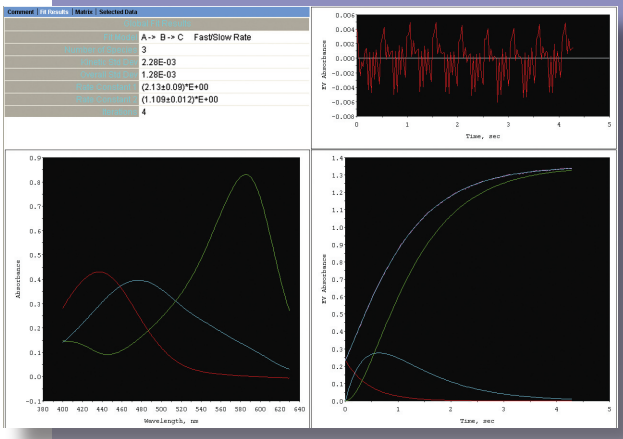


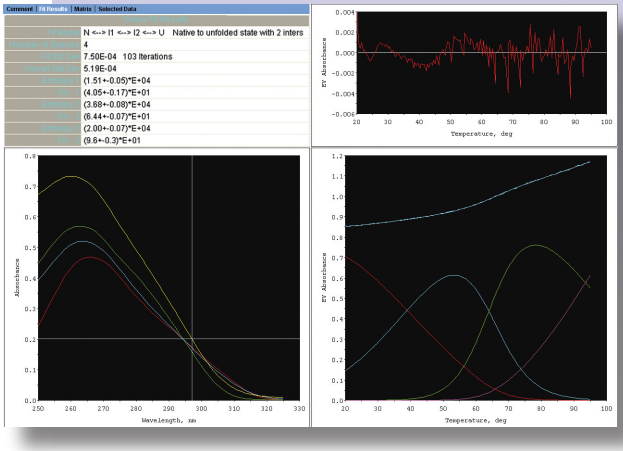




Answer: Numeric Results, and plotted Residuals, Kinetics and Spectra.

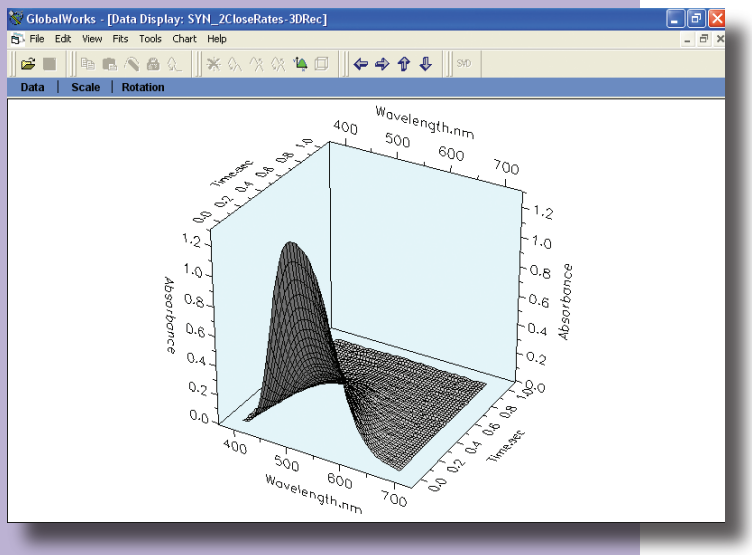






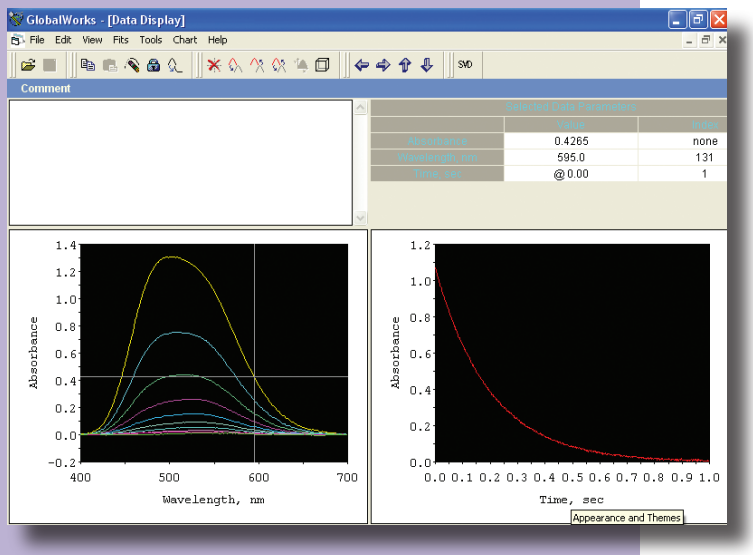
2D is Insufficient

Seminal example[1] of 'simple' two rate reaction requiring multiple wavelength data for correct analysis.



Synthetic 3D Data

A multiple wavelength data set containing two species, A and B, with associated rate constants of 5 and 6, was synthesized; a modest amount of noise was added.



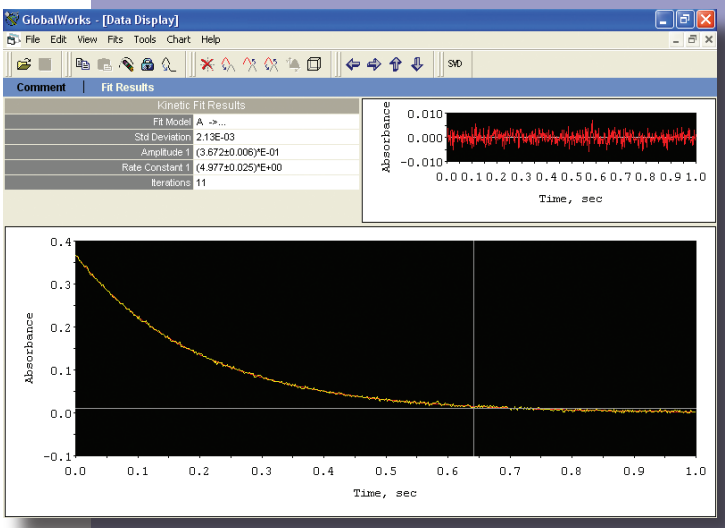
Fixed Wavelength Trace

Looking only at the kinetic trace at a given wavelength, there is no clear suggestion of two rates. This looks like a single exponential. And any one wavelength analyzed is insufficient and even misleading, as we see on the facing page.

[1]Knutson JR., 1992 Methods Enzymol. 210 p 357-74. Maeder, M., and Zuberbuhler, A.D., 1990 Anal. Chem. 62 p 2220.

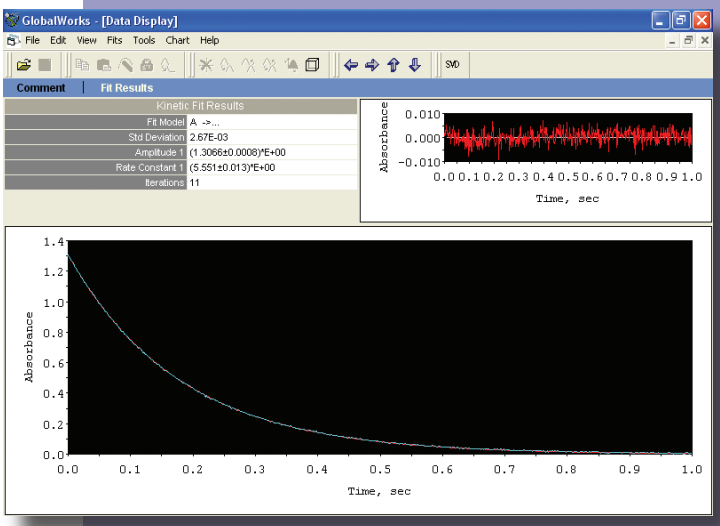
Fit at 600 nm

When one considers data extracted at 600 nm - a completely reasonable wavelength to choose - the single rate returned is 5 s^{-1}. The residuals give no clue that the fit is more complicated than we show.



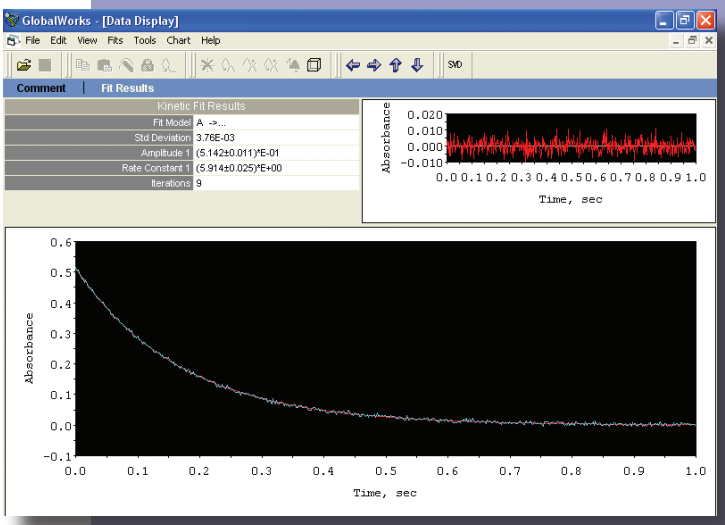
Fit at 500 nm

When one considers data extracted at 500 nm - again, a completely reasonable wavelength to choose - the single rate returned is 5.5 s^{-1}. Again, with no other information, we would confidently declare this is one exponential.



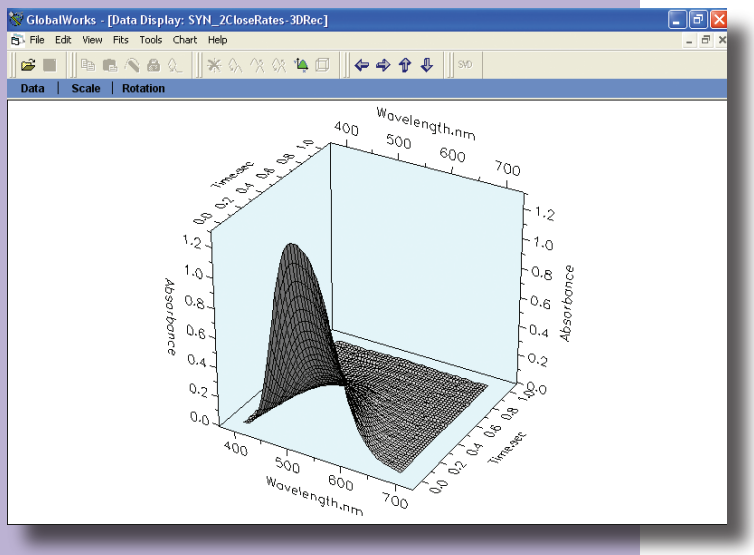
Fit at 450 nm

This time, your alert should go up (but would it?) because the rate constant is not within the 5–5.5 s^{-1} range that the other fits calculated, but is now at 6 s^{-1}! What is wrong? The fit in all three cases - if considered individually - would seem conclusive. The rate is 5 s^{-1}. Or 5.5 s^{-1}. Or 6 s^{-1}. But when they are put together, and if a knowledgeable kineticist is interpreting the results, the conclusion would have to be made that “More experiments need to be done!” But, oops. The enzyme is all gone. Or, the student has graduated. Or...?



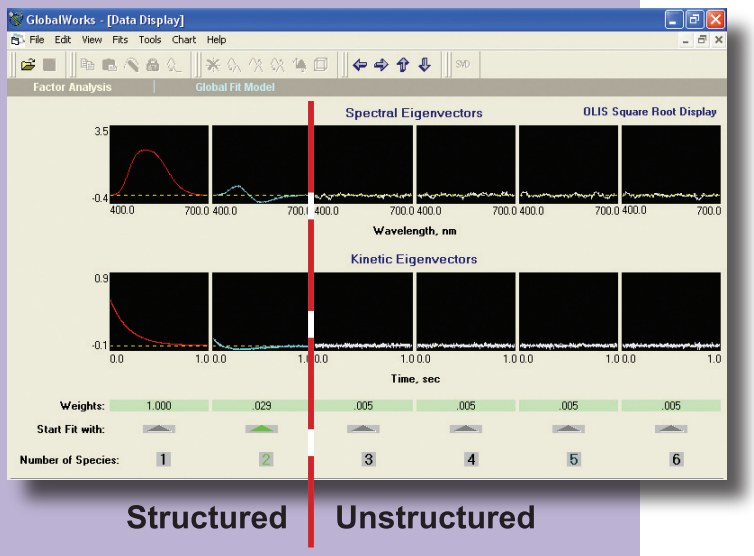
3D is Required & Successful

Continuing with the previous example, see how 3D global analysis easily finds two rates with rate contants of 5 and 6.



Synthetic 3D file

The synthetic data set was created to have 1000 multiple wavelength data points, exactly as would be produced by an Olis RSM 1000 rapid-scanning spectrophotometer (which collects 1,000 scans per second).

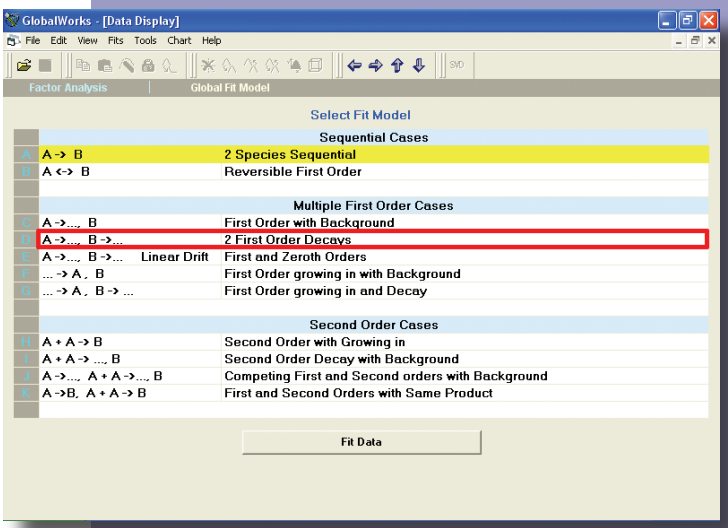


Eigenvector display

Hand all or part of the raw data to the OLIS Global Fitting scheme. The first step is singular value decomposition (SVD), or 'factor analysis.' Factor analysis finds things with some shape ("spectra") which are changing at some rate. The results are shown in "eigenvectors," graphical representations of shapes. The upper row shows spectral eigenvectors; the lower row shows kinetic eigenvectors. Eigenvectors without structure are "noise." Thus, noise contributions are isolated from the useful information and can be excluded from subsequent data handling.

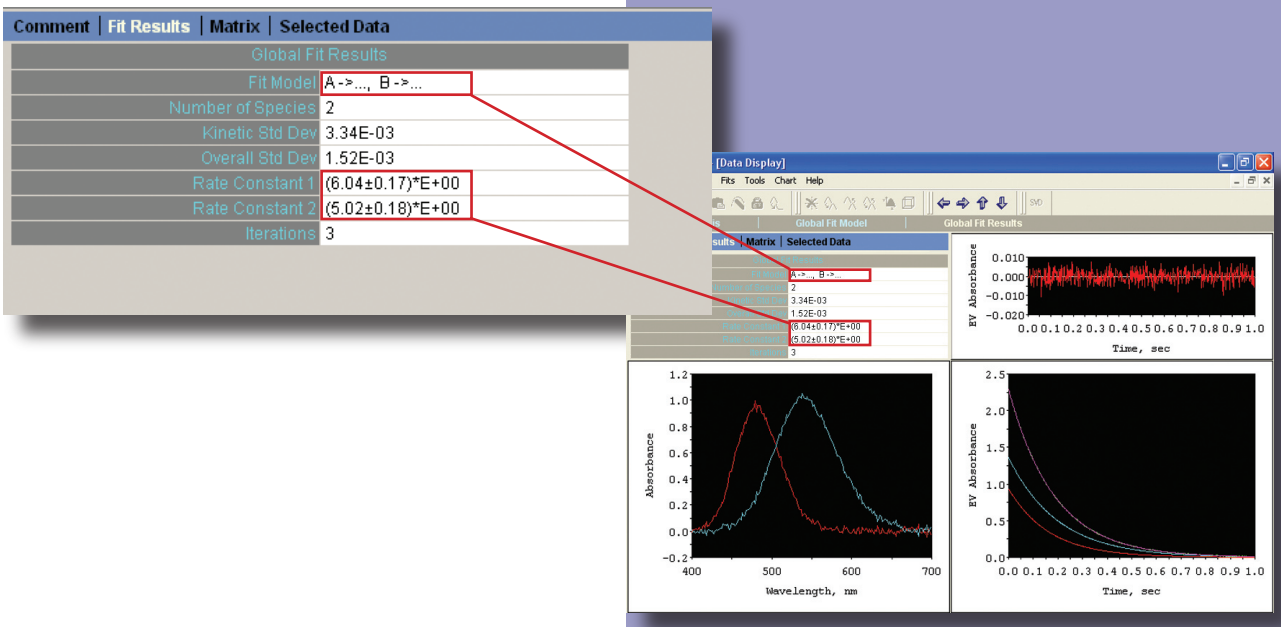
Possible Chemical Equations

With the proposed number of species selected, one moves to defining how these species are related. Do we have one form of A changing into another form (as happens in a protein-fold reaction)? Or do we have a bleaching reaction? Are the species related sequentially (A → B)? These are questions we cannot answer without spectra.



The Answer: 2 Species, 2 Rates

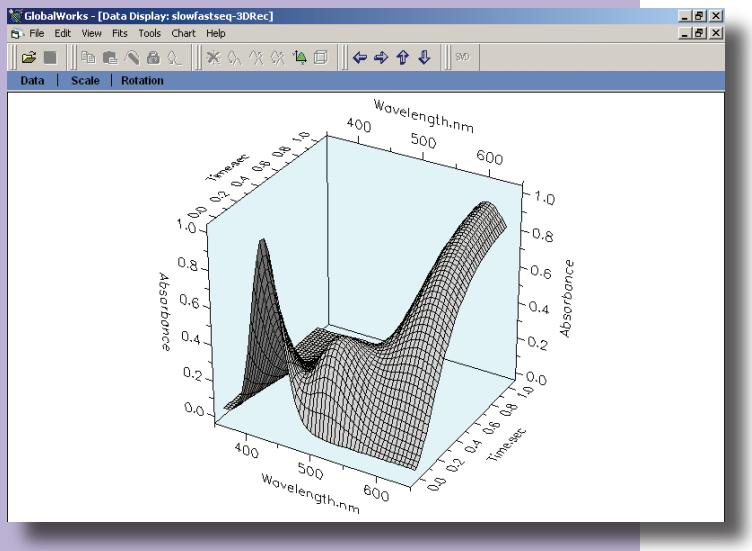
The data were fitted to selection D: (A →..., B →) .... Since these are synthetic data, we know the answer. The known rates of 5.0 and 6.0 are found exactly by Olis GlobalWorks.



The answer: Colored A going to colorless product, colored B going to colorless product, i.e., two sequential bleaching processes with similar rate constants

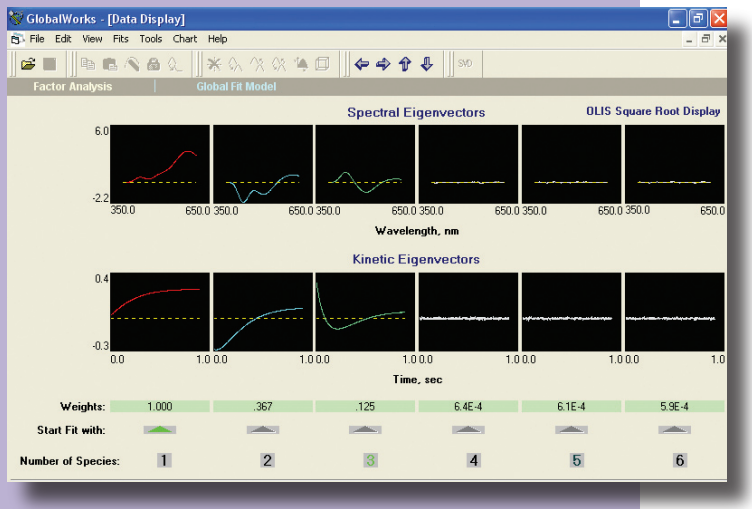
3D Data Supports Determination of the Order of Rate Constants

With single wavelength data, the order of two rate constants in a simple sequential mechanism cannot be determined; with multiple wavelength data, it can!



Raw 3D Data file

The synthetic data set was created to have 1000 multiple wavelength data points. Rate constants of 5 sec^{-1} and 7 sec^{-1} were used for this A → B → C case.



Results of SVD

Eigenvectors suggest (clearly) three species undergoing changes, and noise.

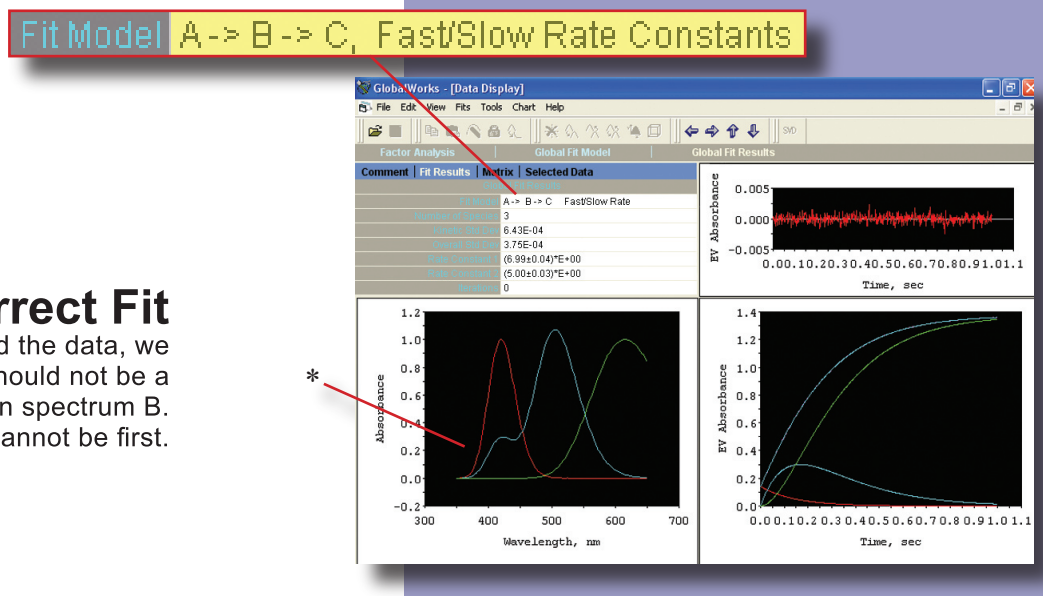
Chemical models

Fit options available when there are three species and two or three rates.

Select Fit Model		
	Sequential Cases	
A	A -> B -> C Fast/Slow Rate	3 Species Sequential
B	A -> B -> C Slow/Fast Rate	3 Species Sequential
C	A + A -> B -> C	3 Species Sequential, First Process Second Order
D	A -> B, B + B -> C	3 Species Sequential, Second Process Second Order
	Multiple First Order Cases	
E	A ->..., B -> C	First Order Decay and Independent First Order Growing in
F	A -> B, C ->...	First Order in Growing and Independent First Order Decay
G	A -> B, C -> B	2 Independent First Orders, Same Product
H	A ->..., B-> ..., C	2 Independent First Orders and Background
I	A -> ..., B -> ..., C -> ...	3 Independent First orders
J	... -> A, ... -> B, C	2 First Orders Growing in and Background
	Second Order Cases	
K	A + B -> C	Heterogeneous Second Order
L	A -> B, B + B -> C + A	First Order Growing in followed by Second Order
M	A -> B, C ->..., B + C -> A + ...	Mixed Reduction Reactions
	Fit Models Without Unique Solutions	

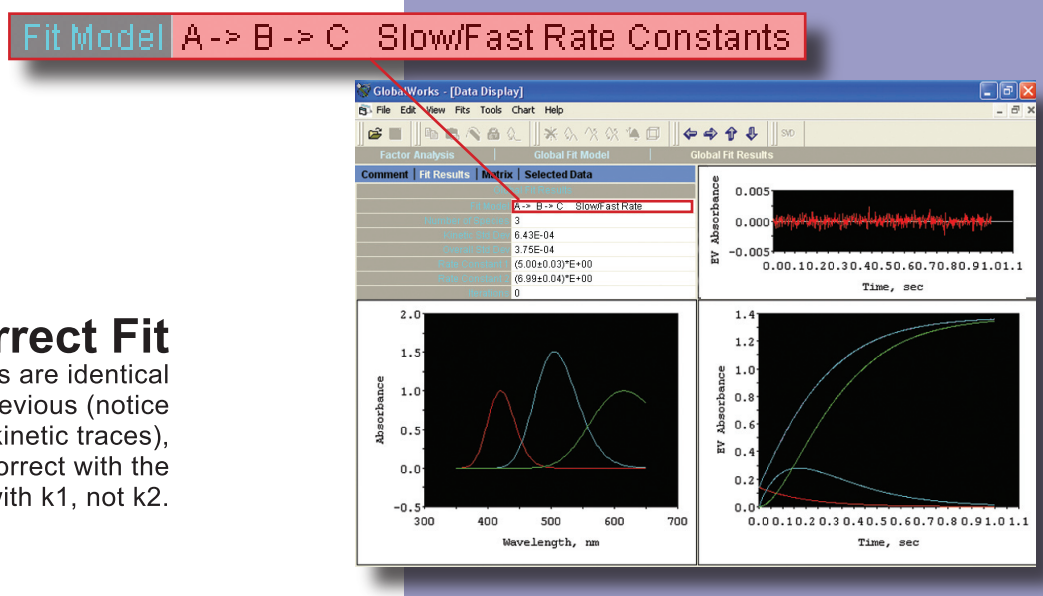
Incorrect Fit

Since we synthesized the data, we know that there should not be a shoulder at 400 nm in spectrum B. Thus, the fast rate cannot be first.

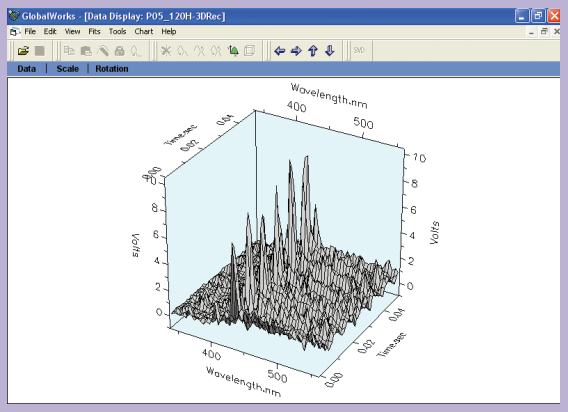


Correct Fit

While the kinetic results are identical with this fit and the previous (notice the residuals and the kinetic traces), the spectra are now correct with the slow rate associated with k1, not k2.

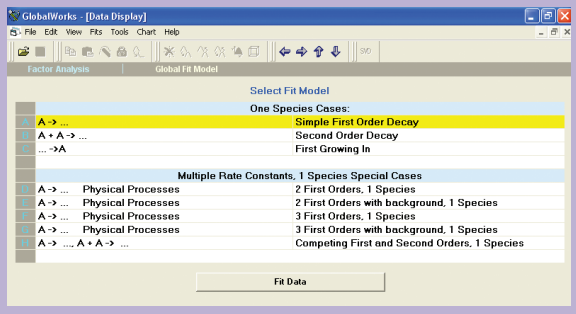


Collect Data

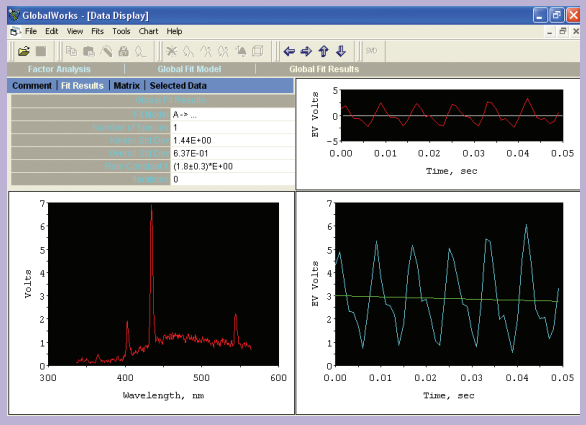


Apply SVD

Choose Fit From One Species Mechanisms



Evaluate Results



Proving the Absolute Absence of Bias in GlobalWorks

Using the Olis RSM 1000 in its fluorescence mode, 50 scans were collected in 0.05 seconds from light emitted by overhead fluorescent lamps. The mercury lines and the 120 Hz modulation of the lamp jump from in the eigenvectors (second panel) and the answer (final graphs).

SVD identified the changes in the light caused by the lamp's on/off operation at 60 Hz, and the spectrum of the light (a continuum with sharp Hg emission lines). SVD acts with no bias about the source of the data, chemical, kinetic, thermal, or other process. And, since user starting values are only used for the most complicated cases, bias cannot be entered during the fit, either.

Collection: 50 scans collected in 0.05 seconds of light emitted by overhead fluorescent lamps

SVD: Single species

Kinetic Fit: Select 1 species and then fit single rate so as to construct the spectrum

Mechanism: Not a kinetic process

Comments: Fluorescent lights switching on and off at 120 Hz, demonstration of a particular shape (the spectrum) varying in a particular way (sinusoidal). The eigenvectors are presented in the way shown to emphasize that the kinetic and spectral eigenvectors are related. One reads the display by noting that the kinetic eigenvectors show a particular time course and that it is the corresponding spectral eigenvector which varies in that way.

[1]These data, as with all data shown in this brochure, are provided for pedagogical, demonstration, and testing purposes in the Olis GlobalWorks software.

Temperature Dependent Data

Using an Olis DSM CD spectrophotometer with automatic temperature regulation, 38 CD scans were collected over 50 degrees.

SVD suggests two species, which, are one form of a protein turning into another form of the protein in a (potentially) reversible manner.

As a thermal study, numeric results returned include the transition (“melting”) temperature and the enthalpy value(s).

With single wavelength results only, one could say nothing about the substance undergoing the thermal denaturation. With multiple wavelength results, one has the correct spectra of the native and unfolded form of the human serum albumin in addition to the more accurate determination of the transition temperature. How much more satisfying this full result is!

Collection: 38 CD scans collected as a function of temperature with an OLIS DSM CD spectrometer.

SVD: Shows clearly that two species are involved. Note that the X- axis is temperature, not time.

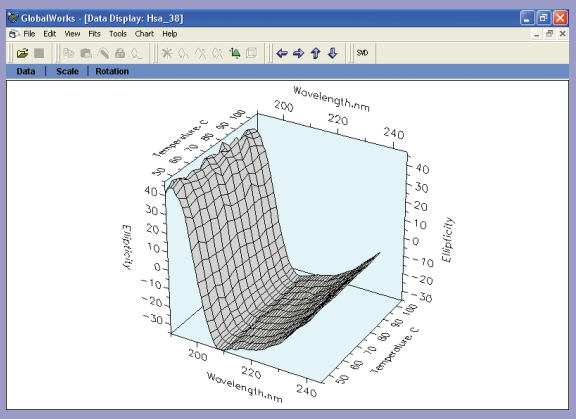
Thermodynamic Fit: Calculate thermodynamic properties (e.g. melting temperature and enthalpy).

Mechanism: N↔U

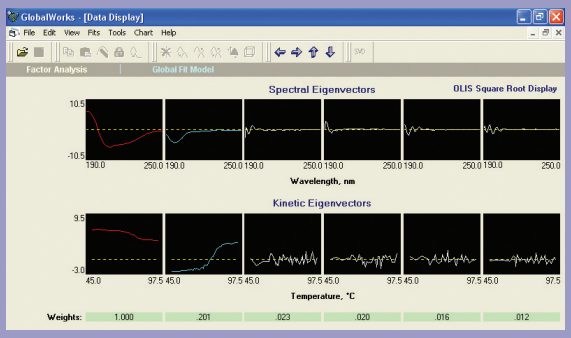
Comments: These data show that the protein changed as the temperature was increased. A fit to these data will give a value for the ‘melting temperature’ or transition temperature. Spectral reconstruction produced the spectrum of the native (folded) and denatured (unfolded or melted) forms.

[1]These data, as with all data shown in this brochure, are provided for pedagogical, demonstration, and testing purposes in the Olis GlobalWorks software.

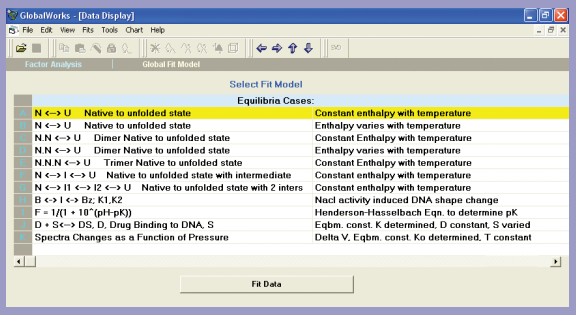
Collect Data



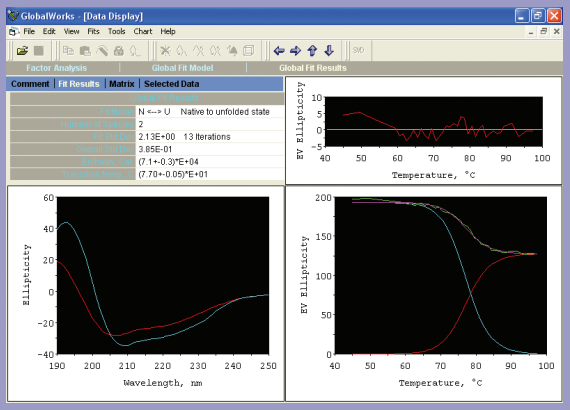
Apply SVD



Choose Fit From Equilibrium Mechanisms



Evaluate Results



Currently Supported Mechanisms

Equilibrium Mechanisms		
N→U	Native to Unfolded State	Constant Enthalpy with Temperature
N→U	Native to Unfolded State	Enthalpy Varies with Temperature
N→U	CD=Cdo[V(V+Vo)] Returns Vo	CD Titration Curve as function of Added Volume
N⇔U	Native to Unfolded State	Constant Enthalpy with Temperature
N⇔U	Native to Unfolded State	Enthalpy Varies with Temperature
N.N⇔U	Dimer Native to Unfolded State	Constant Enthalpy with Temperature
N.N⇔U	Dimer Native to Unfolded State	Enthalpy Varies with Temperature
N.N.N⇔U	Trimer Native to Unfolded State	Constant Enthalpy with Temperature
N⇔I⇔U	Native to Unfolded State with Intermediate	Constant Enthalpy with Temperature
N⇔I1⇔I2⇔U	Native to Unfolded State with 2 Intermediates	Constant Enthalpy with Temperature
CD=CDo*[V/(V+Vo)]	Returns Vo	CD Titration Curve as Function of Added Volume
F = 1/(1+10^(pH-pK))		Henderson-Hasselbach Eqn. to Determine pK
N+D⇔U	Denatruration Process	Eqbm. Const. Ko Determiined, N Constant, D Varied
Spectral Changes as a Function of Pressure		Delta V, Eqbm. Const. Ko Determined, T Constant
One Species Mechanisms		
Sequential Cases		
A→...		Simple First Order Decay
A + A→...		Second Order Decay
...→A		Growing First In
Two Species Mechanisms		
Sequential Cases		
A→B		First Order with Growing In
A⇔B		Reversible Reaction
Multiple First Order Cases		
A→..., B		First Order with Background
A→..., B→...		2 First Order Decays
A→..., B→...	Linear Drift	First and Zeroth Orders
...→A, B		First Order Growing In with Background
...→A, B→...		First Order Growing In and Decay
Second Order Cases		
A+A→B		Second Order with Growing In
A+A→...B		Second Order Decay with Background
A→..., A+A→...B		Competing First and Second Orders with Background
A→B, A+A→B		Competing First and Second Orders with Same Product
Three Species Mechanisms		
Sequential Cases		
A→B→C	Fast/Slow Rate Constants	3 Species Sequential
A→B→C	Slow/Fast Rate Constants	Special 3 Species Sequential
A+A→B→C		3 Species Sequential, First Process Second Order
A→B, B+B→C		3 Species Sequential, Second Process Second Order
Multiple First Order Cases		
A→..., B→C		First Order Decay & Independent First Order Growing In
A→B, C→...		First Order & Independent First Order Decay
A→B, C→B		2 Independent First Orders, Same Product
A→..., B→..., C		2 Independent First Orders and Background
A→..., B→..., C→...		3 Independent Fisrt Orders
...→A, ...→B, C		2 First Orders Growing In and Background

Currently Supported Mechanisms

Second Order Cases	
A+B→C	Heterogeneous Second Order
A→B, B+B→C+A	First Order In followed by Second Order
A→B, C→..., B+C→A...	Mixed Reduction Reactions
Fit Models Without Unique Solutions	
A↔B→C	Partially Reversible Rise-Fall
A→B↔C	Partially Reversible Rise-Fall
Four Species Mechanisms	
Sequential Cases	
A→B→C→C	4 Species Sequential
Multiple First Order Cases	
A→..., B→C	First Order Decay & Independent First Order Growing In
A→B, C→...	First Order In Growing & Independent First Order Decay
Second Order and Enzyme Cases	
A→..., B→..., C→..., D	Pulse Radiolysis Case
E+S→ES→E+P	Irreversible Enzyme Case
Fit Models Without Unique Solutions: *Reversible Sequential Cases*	
A↔B→C→D	Sequential with Step 1 Reversible
A↔B↔C↔D	Fully Reversible Sequential
A↔B, B↔C, B↔D	Branching Reaction
Fit Models Without Unique Solutions: *Reversible Enzyme Cases*	
E+S↔ES→E+P	Partially Reversible Enzyme Reaction
E+S↔ES↔E+P	Fully Reversible Enzyme Reaction
Five Species Mechanisms	
Sequential Cases	
A→B→C→D→E	5 Species Sequential
Multiple First Order Cases	
A→..., B→..., C→..., D→..., E	4 Independent First Orders and Background
A→..., B→..., C→..., D→..., E→...	First Order In Growing & Independent First Order Decay
Enzyme Case	
E+S→ES→EP→E+P	Enzyme Reaction, 2 Enzyme Complexes
Fit Models Without Unique Solutions: *Reversible Sequential Cases*	
A↔B↔C↔D↔E	Fully Reversible Sequential
Fit Models Without Unique Solutions: *Reversible Enzyme Cases*	
E+S↔ES→EP→E+P	Partially Reversible Enzyme Reaction, 2 Enzyme Complexes
E+S↔ES↔EP↔E+P	Fully Reversible Enzyme Reaction, 2 Enzyme Complexes
Six Species Mechanisms	
Sequential Cases	
A→B→C→D→E→F	6 Species Sequential
Multiple First Order Cases	
A→..., B→..., C→..., D→..., E→..., F	5 Independent First Orders and Background
A→..., B→..., C→..., D→..., E→..., F→	6 Independent First Orders
Fit Models Without Unique Solutions: *Reversible Case*	
A↔B↔C↔D↔E↔F	Fully Reversible Sequential

This supported mechanism list continues to grow as people ask for additional equations. If you do not see the mechanism which may apply to your multiple wavelength data, provide us the equation and we will incorporate it in Olis GlobalWorks at no cost to you. **If you can express your equation algebraically, we can create a fit for it.**

Numeric Results Returned

Global Fit Results	
Fit Model	A -> B -> C
Overall Std Dev	1.29e-3
Kinetic Std Dev	2.3e-3
k1	(2.14±0.09)*E+00
k2	(1.107±0.025)*E+00
Number of Iterations	20

= *User Chosen Model to Fit*

Global Fit Results	
Fit Model	A -> ..., B -> ...
Overall Std Dev	1.52e-3
Kinetic Std Dev	3.34e-3
k1	(6.0±0.5)*E+00
k2	(5.0±0.6)*E+00
Number of Iterations	13

= *Standard Deviation of Spectral Surface*

Global Fit Results	
Fit Model	A -> B
Overall Std Dev	1.17e-1
Kinetic Std Dev	2.62e-1
k1	(1.84±0.14)*E+01
Number of Iterations	10

= *Standard Deviation of Kinetic Trace(s)*

Global Fit Results	
Fit Model	A -> ...
Overall Std Dev	5.8e-1
Kinetic Std Dev	1.44e+0
k1	(2.8±0.3)*E+00
Number of Iterations	10

= *Rate Constant Associated with 1st Step*

Global Fit Results	
Fit Model	A -> B -> C Slow/Fast Rate Constants
Overall Std Dev	3.74e-4
Kinetic Std Dev	6.42e-4
k1	(4.99±0.03)*E+00
k2	(7.00±0.04)*E+00
Number of Iterations	10

= *Rate Constant Associated with 2nd Step*

Global Fit Results	
Fit Model	N -> U Native to Unfolded State
Overall Std Dev	5.37e-1
Kinetic Std Dev	1.13e+0
Enthalpy	(6.5±0.3)*E+04
Trans Temperature	(7.69±0.03)*E+01
Number of Iterations	26

No fit merit is implied by this number of items

Quintessentially New Global Fitting Software Uses Simplex Method and Matrix Exponentiation

Analyzing data in a multidimensional form is superior to analyzing 2D data. The new GlobalWorks algorithms by Olis make analyzing multidimensional data easy and fast. Algorithms for data as a function of time, temperature, pressure, concentration, and angle are now supported; additional models can be added.

Matrix Exponential can be extended to very complicated cases, including reversible cases for which solutions are algebraically intractable. The limit to fit complexity is not computational, but the data (the more rates to be determined, the higher the S/N of the data must be).

There is no longer justification to limit one's analyses to single wavelength data. Use multiple wavelength data and Olis GlobalWorks for better rate constants, digital noise reduction, and chemical mechanism testing. Results are so much more satisfying and defensible!

Multidimensional data provide a much stronger basis for accurate conclusions.

GlobalWorks provides the best algorithms extant for instantaneous and correct analysis of multidimensional data.

Correct Results the Multidimensional Way

Olis GlobalWorks differs from all other kinetic fitting software in three ways. Our SVD is 1300 fold faster than the original algorithm. Instead of Levenberg-Marquardt, we use Downhill Simplex. And instead of numeric integration routines such as Runge-Kutta, we use Matrix Exponentiation.

Matrix Exponential can be extended to very complicated cases, including reversible cases for which solutions are algebraically intractable. Consider the simple case of A → B, such as defines a protein unfold experiment. By applying the Law of Mass Action, a matrix containing only rate constants and zeros is produced. The columns represent the concentrations and the rows their derivatives with respect to time. The matrix is exponentiated to provide the solution for A → B.

The Matrix for $A \xrightarrow{k_1} B$:

	A	B
dA/dt	-k1	0
dB/dt	+k1	0

The Matrix for $A \xrightarrow{k_1} B \xrightarrow{k_2} C$:

	A	B	C
dA/dt	-k1	0	0
dB/dt	k1	-k2	0
dC/dt	0	k2	0

For additional details on how Olis GlobalWorks makes 3D analysis fast, easy, and indispensable, see Methods in Enzymology, volume 384, 2004, chapters 1, 2, and 3. The three data sets used in this document (protein unfolding, Xylenol reacting with iron, and nucleic acids undergoing thermal denaturation) are some of the demonstration files provided with the software.

Use the software today. A 30 day free trial version is available at http://www.olisweb.com/software/

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