Supporting Material

Kinetics of fast changing intramolecular distance distributions obtained by combined analysis of FRET efficiency kinetics and time resolved FRET equilibrium measurements

E. Lerner and T. Orevi, E. Ben Ishay, D. Amir, E. Haas*

The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel

* Corresponding author Elisha.Haas@biu.ac.il

Applicability of the model –simulation setup

Mean FRET efficiency kinetics data $\langle E\rangle(t)$, were simulated and tested assuming time independent R_o values using *Eq. 4*. The input for preparation of simulated transfer efficiency kinetics data were initial and final distance distributions assuming several combinations of the parameters a, b and R_0, and folding rate constant, k_F , of 0.2 s^{-1}. Gaussian noise profile was added to the simulated curves with standard deviation similar to that known for the experimental stopped flow apparatus used in our lab (1), typically 1%. The simulated curves were then analyzed using the fitting of a theoretical curve obtained by computation of $<E>(t)_{calc}$ with input of the simulated $P_F(r)$ (fixed), determined from equilibrium trFRET measurements; $P(r)_{c,calc}$ and $k_{F,calc}$, the free parameters, were then recovered.

The extent of deviation of the fitted $P(r)_{c,calc}$ from the simulated $P(r)_{c,simul}$ was then assessed by the following parameters: their overlap area, the relative deviation of their peak, FWHM and k_F values, and most importantly, the 95% confidence interval error ranges of peak, FWHM of $P(r)_{c,calc}$ and of k_F. The simulated and best fit parameters as well as the comparison parameters, for a total of 720 combinations of simulated distance distributions, rate constant, and R_o, are summarized in Supporting spreadsheet S1. The conclusions of the simulation results are reported in the main text and are based on the improvements in error ranges while gradually changing the simulated parameter sets that were free parameters in model fitting.

Validity of the model –simulation setup

To assess the limits of the two-state approximation, in realms that may deviate from such a scenario, we synthesized average FRET efficiency kinetics for various types of such deviations from two-state transition and then analyzed the resulting kinetics, after Gaussian noise profile addition, using either a mono-exponential function or the our two-state model (*Eq. 3+4)*. The deviations from a simple two state scenario that were implemented were:

1. An underlying three state transition.
2. Two state transition with a time-dependent R_o.
3. Two state transition with a mixture of different rate constants at different fluxes
    a. Central distribution of rate constants
    b. Two discrete rate constants with two relative fluxes
4. Unimodal (one state) transition
5. Two state transition with the initial-state having high intramolecular flexibility

A three state model is described in Eqs. S1-S4. The resulting FRET kinetics curves were fit to our two-state model, described in *Eq. 4*. Tests of the quality of fit yielded three-state parameter ranges at which the two-state approximation fails; the resulting conclusions are reported in the main text. The Supporting spreadsheet S1 shows the simulated FRET kinetics goodness-of-fit to a two-state model.

In the case of a two state model with R_o changing throughout the transition, average FRET efficiency kinetic curves were simulated by the use of *Eq. 3* and fit to a mono-exponential function. Tests of the quality of fit yielded criteria for R_o values for which the mono-exponential approximation fails. Typical R_o value changes (up to 2 Å) were used. The Supporting spreadsheet S1 shows the quality of the fit. As reported in the main text, this possibility does not introduce changes in the mono-exponential trends of the transitions.

Two state model average FRET efficiency kinetic traces with either two discrete rate constants or a central distribution of rate constants, were synthesized with values in the range of 0.1-1.0 s^{-1}. These, in turn, were fitted to a mono-exponential function. In the case of two discrete rate constants, for a 0.5:0.5 flux ratio, the curves deviated from the mono-exponential function with a rate constant difference of 0.1 s^{-1}. The higher the flux ratio, the higher the allowed rate constant difference. At a 0.95:0.05 flux ratio, the maximal allowed rate constant difference was 0.4 s^{-1}. In the case of a central distribution of rate constants, the maximal allowed standard deviation was 0.4 s^{-1}.

A unimodal transition is indicated by a gradual change of conformation (2-4). A unimodal FRET efficiency detected transition was simulated as a gradual exponential change not of the population fraction of the two states, *f(t),* but of both PEAK and FWHM values of the transient distance distribution, between $P_C(r)$ and $P_F(r)$. Examples of such average efficiency kinetics in comparison to their two-state counterparts are shown in Fig. S1. One can see that average transfer efficiency kinetics of unimodal transitions are far from exponential.

We used the Haas-Steinberg (5, 6) equations to produce donor fluorescence decay curves corresponding to a given distance distribution with intramolecular diffusion coefficients ranging from 0 to 20 $Å^2$/ns (a high value reported for unfolded proteins) and then checked the extent to which raising the flexibility changed average transfer efficiency kinetics. The maximal increase was 5% which is within the error ranges.

Treatment for Three-State Processes

For the following three-state transition, , $R \to I \to P$ the results of the states' kinetics laws are shown in Eqs. S1

$$\begin{aligned}
&[R](t) = C_2 e^{-k_I t} \\
&[I](t) = C_1 e^{-k_P t} - C_2\left[k_I/(k_I - k_P)\right]e^{-k_I t} \\
&[P](t) = N - [I](t) - [R](t) \\
&N = Const.
\end{aligned} \quad \text{(S1)}$$

where *N*, is the total amount of molecules, which is constant (conserved); *[R]*, *[I]* and *[P]*, are the time dependent concentrations of the Reactants-initial, Intermediate and Product-final state species respectively; k_I. and k_P, are the rate constants of the first and second transitions, respectively; and C_1 and C_2, are kinetic coefficients.

Assuming that at t=0 there are non-negligible occupancies of only states *R* and *I*, we find that the coefficients, C_1 and C_2, of Eq. S1 are shown in Eq. S2.

$$\begin{aligned}
&C_1 = N + \frac{k_P}{k_I - k_P} \cdot U(t=0) \\
&C_2 = R(t=0)
\end{aligned} \quad \text{(S2)}$$

Substitution of the coefficients to the kinetic Eq. S1 and division by the total amount of matter, *N*, obtain kinetic traces of the states' fractions as is shown in Eqs. S3

$$\begin{aligned}
&\alpha_R(t) = \alpha_R(0)e^{-k_I t} \\
&\alpha_I(t) = e^{-k_P t} + \left[\alpha_R(0)/(k_I - k_P)\right]\left(k_P e^{-k_P t} - k_I e^{-k_I t}\right) \\
&\alpha_P(t) = 1 - \alpha_I(t) - \alpha_R(t)
\end{aligned} \quad \text{(S3)}$$

having α_R, α_I and α_P as the initial, intermediate and final state occupancies.

Then, if each state, *i*, can be characterized by a three-dimensional radial distance distribution, $P_i(r)$, as in Eqs. S4, the joint distance distribution at each time point, $P(r,t)$, should be a superposition of the three, weighted by their occupancies, as shown in Eqs. S4.

$$\begin{aligned} P(r,t) &= \sum_i \alpha_i(t) p_i(r); i \in \{R, I, P\} \\ p_i(r) &= 4\pi r^2 e^{-b_i (r-a_i)^2} \end{aligned} \qquad \text{(S4)}$$

Using Eqs. S3 and S4 in the framework of the model described by Eq. 3, as a fitting model, while knowing the folded state characteristics (therefore holding them constant), one can retrieve the state characteristics of *R* and I, the two rate constants and the *R*↔*I* equilibrium, through the *R* state fraction at equilibrium.

Maintaining both *R* and *P* distributions as free parameters is assumed to yield large error ranges. Nevertheless, knowledge of the initial- and final-state distributions, *R* and *P*, can reduce error ranges of the rate constants and the intermediate distributions.

In order to assess the limits of the applicability of the three state transition model, we modeled average FRET efficiency kinetics using *Eqs. S1-S4* and *Eq. 4*, added noise and then fitted the resulting kinetic data back to our three-state model, having the initial and final state distributions as constant constraints. Tests of the quality of fit yielded intermediate state distributions and rate constant parameter ranges, for which the proposed three-state model can yield acceptable results. The extent of deviation of the fitted $P(r)_{I,calc}$ from the simulated $P(r)_{I,simul}$ was then assessed by the following parameters: the 95% confidence interval error ranges of peak, and FWHM of $P(r)_{I,calc}$ and of k_I and k_P. The simulated and best fit parameters, as well as the comparison parameters, for a total of 5400 combinations of simulated distributions, rate constants, and R_o, are summarized in the Supporting spreadsheet S1.

Time dependent R_o

Tryptophan fluorescence spectrum and quantum yield is strongly solvent dependent (7-11). Additional changes can be caused by solvent relaxation (12-14) in the excited state (14-17). An initial change of the Trp emission spectrum, intensity and lifetime occurs upon dilution of the denaturant. This is followed by specific conformation-related effects such as interactions with main chain and side chain atoms that enhance or reduce the decay of the emission. In the stopped flow apparatus used in the current study, Trp emission was selected by a band-pass filter, which covers the entire range of AK Trp fluorescence emission spectra, 330-370 nm (BP 357/44 nm).

We can therefore conclude that the changes in fluorescence intensity of the DO mutant, after the mixing dead-time of the Stopped-Flow apparatus, is mostly due to quantum yield changes. The value of the donor quantum yield for calculation of the time dependent R_o values, $R_o(t)$, can be obtained from the ratio of the Trp emission intensity at any time to its final value after completion of the folding transition, as shown in Eq. S5.

$$\frac{R_0(t)}{R_0(t \to \infty)} = \left[\frac{\varphi_D(t)}{\varphi_D(t \to \infty)}\right]^{1/6} = \left[\frac{I_{DO}(t)}{I_{DO}(t \to \infty)}\right]^{1/6} \quad \text{(S5)}$$

where R_0, φ_D and I_{DO} are the Förster critical distance, donor fluorescence quantum yield, and intensity in the absence of an acceptor.

A R_o time-vector was therefore calculated and implanted in the model (Fig. 2, *B*). The simulations showed no deviation of the transfer efficiency change kinetics from mono-exponential trend.

Calculation of the kinetics of changes of transfer efficiency in the combined analysis of the donor emission intensities and the equilibrium trFRET data

We calculated the kinetics of changes of the mean transfer efficiency, *<E>(t)*, as a ratio of donor fluorescence intensities, $I_{i,}$ as in Eq. S6

$$\langle E\rangle(t) = 1 - l\, I_{DA}(t) / I_{DO}(t) \quad \text{(S6)}$$

where *i* is for either DO or DA measurements. The l parameter accounts for extensive possible differences between DO and DA fluorescence intensities, such as different concentrations. l is calculated from the trFRET efficiencies in the final state at equilibrium as in Eq. S7

$$\langle E\rangle(t \to \infty) = 1 - l\, I_{DA}(t \to \infty) / I_{DO}(t \to \infty) = 1 - \langle \tau \rangle_{DA} / \langle \tau \rangle_{DO} \quad \text{(S7)}$$

where $\langle \tau \rangle_{DA}$ and $\langle \tau \rangle_{DO}$ are the average donor fluorescence lifetimes of DA and DO in the final-state at equilibrium.

Supporting Spreadsheet

The full dataset of simulated parameters that were used to synthesize the transfer efficiency kinetics together with the best model fitting results and statistical and model comparison parameters, are reported in a Supporting Excel Spreadsheet S1. Supporting spreadsheet S1 is organized in several tabs, each summarizing a different type of simulation: tab '2state', in which the applicability of the proposed model in Eq. 4 was checked against a given set of distributions and a rate constant parameter; tab ‘2 states approximation on 3 states', in which the validity of the two-state proposed model in Eq. 4 was checked against an underlying three-state scenario, produced using *Eqs. S1-S4*; tab 'exponentiality when R_0 changes', in which the validity of the model proposed in *Eq. 4* is checked against cases with a time change of the Förster Radius, R_0; tab '3state', in which the applicability of the model proposed in *Eqs. S1-S4* and *Eq. 4* was checked against a given set of intermediate state IDDs, rate constants and R_o values. Each column in each tab of spreadsheet S1 is described in comments of the headers. One can go through different possible realms by filtering columns of desired parameters.

Supporting Matlab files

1. 'model_implementation.m' – shows how to basically implement the model proposed in this work
2. 'pop2_kinetics_fit_simple.m' – a function that calculates FRET kinetics assuming a constant time invariant R0 value throughout kinetics
3. 'pop2_kinetics_fit_R0.m' - a function that calculates FRET kinetics assuming a R0 value changing throughout kinetics
4. 'time_variable_R0.m' – a function that calculates the R0 kinetics from Donor-Only fluorescence intensity kinetics from Eq. S5
5. ' FRET_kinetics.m' – a function that calculates the mean FRET efficiency, <E>, kinetics out of donor fluorescence intensity kinetics and fixing it to the <E> value at infinite time known from equilibrium trFRET measurements
6. ' SkewedGaussian.m' - a function that calculates a normalized skewed Gaussian distance distribution and its PEAK and FWHM values
7. ' exponential_kinetics.m' – a function used for fitting intensity kinetics with as mono-exponential

Supporting Figures

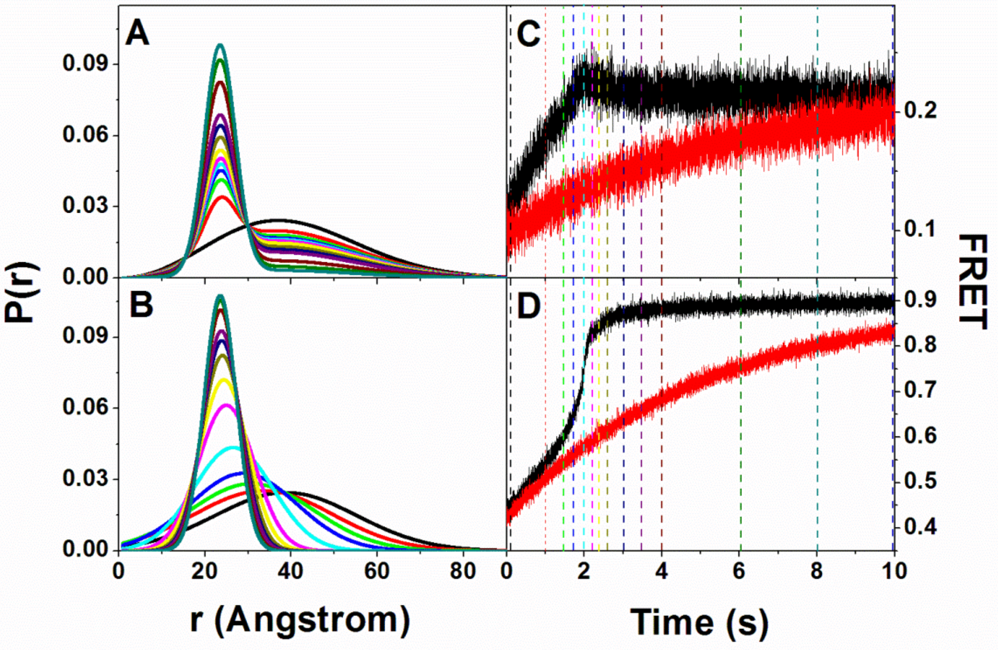


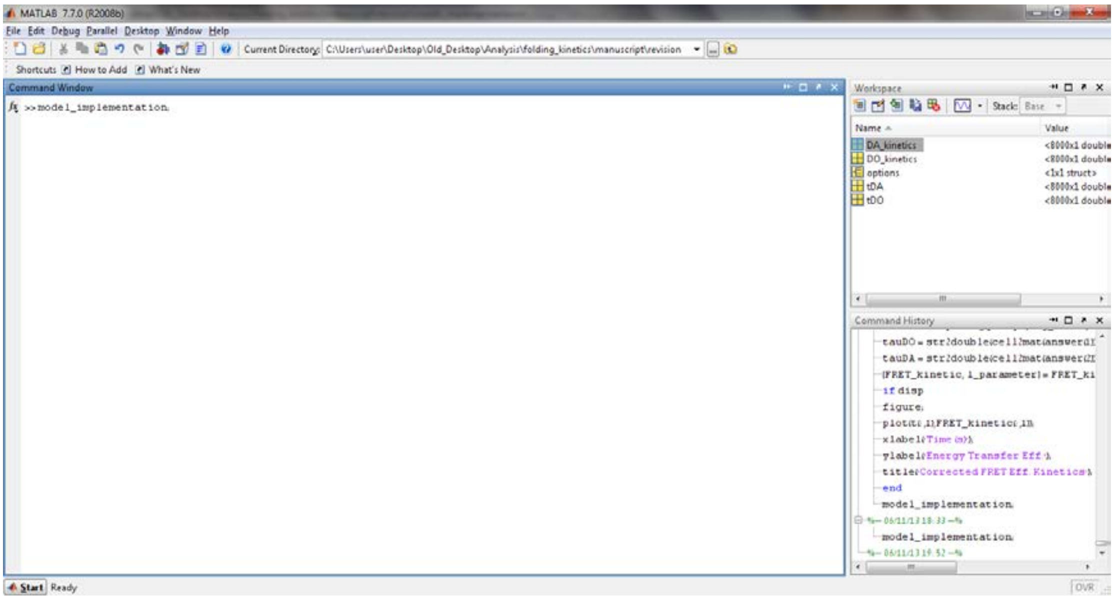
Figure S1: FRET kinetics of one- and two-state transitions. Temporal distance distribution time change of a two- (*A*) and one-state (*B*) transition, and comparison between the expected resulting steady-state FRET kinetics of two- (*red*) and one-state (*black*) transitions at R_0 values 15 (*C*) and 35 Å (*D*). Vertical dashed lines indicate the time points at which the distance distributions are taken in panels A and B.

Analysis of FRET Eff. Kinetics – program manual

Input time vector of kinetic traces in '*tDO*' (for DO experiment) and '*tDA*' (for DA experiment) and intensity vector of kinetic traces in '*DO_kinetics*' (for DO experiment) and in '*DA_kinetics*' (for DA experiment). Together with an list of variables important for the analysis, '*options*', all these variables are found (as an example) in the file '*matlabStart.mat*'.

Work in a directory that includes all *.m* files.

Start analysis by entering '*model_implementation;*' command in the Matlab workspace.

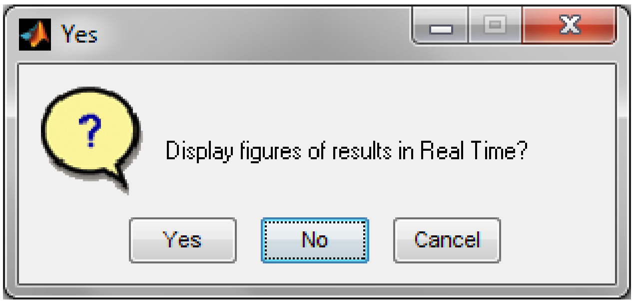


```
>>model_implementation
```

Name	Value
DA_kinetics	<8000x1 double
DO_kinetics	<8000x1 double
options	<1x1 struct>
tDA	<8000x1 double
tDO	<8000x1 double

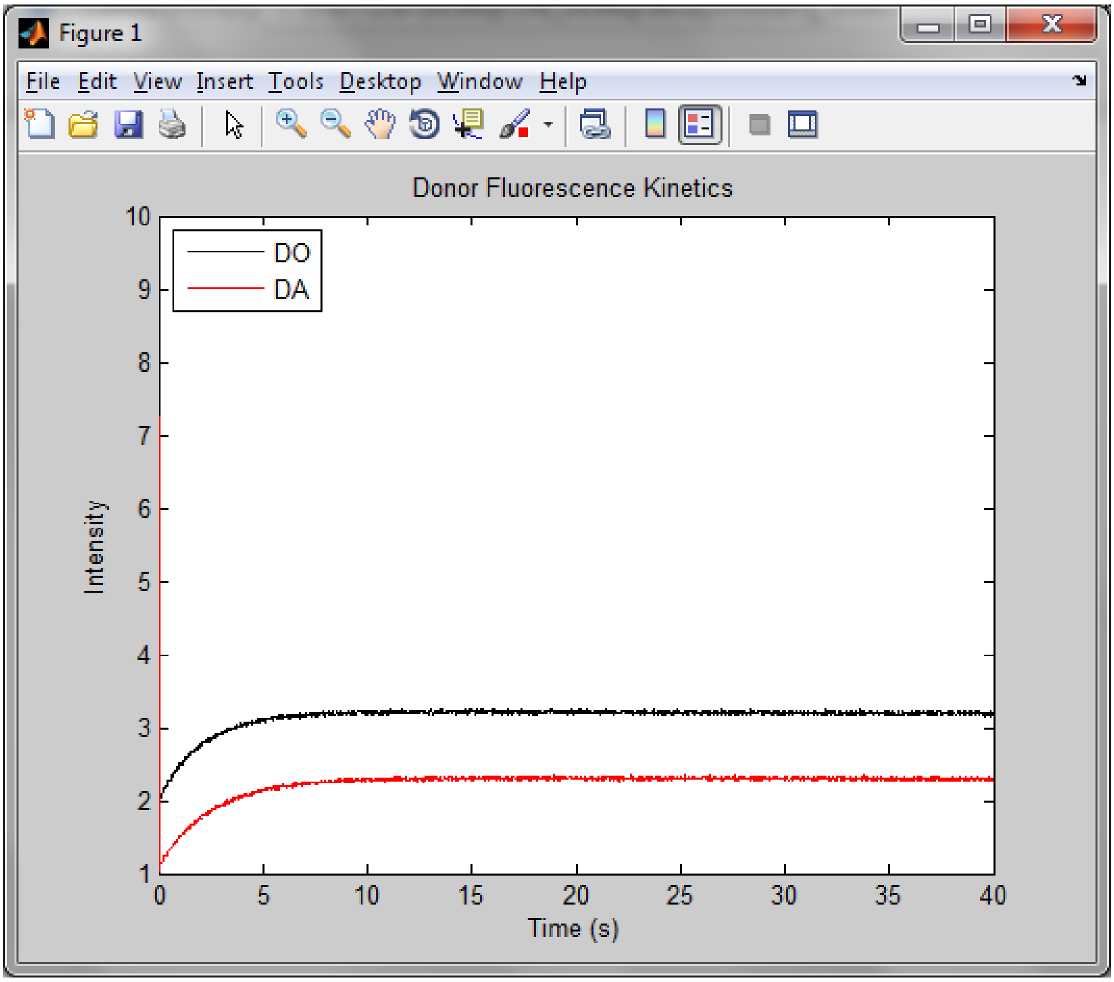
```
tauDO = str2double(cell2mat(answer(1
tauDA = str2double(cell2mat(answer(2
[FRET_kinetic, l_parameter] = FRET_ki
if disp
figure;
plot(t(:,1),FRET_kinetic(:,1));
xlabel('Time (s)');
ylabel('Energy Transfer Eff.');
title('Corrected FRET Eff. Kinetics');
end
model_implementation;
%-- 06/11/13 18:33 --%
model_implementation;
%-- 06/11/13 19:52 --%
```

A question pops up asking '*Display figures of results in Real Time?*'. If you want figures that portrait results of the stages of the analysis choose '*Yes*'. Otherwise choose '*No*'.



Display figures of results in Real Time?

After Choosing '*Yes*' the next result shows the raw data of the DO and DA donor fluorescence kinetic traces.



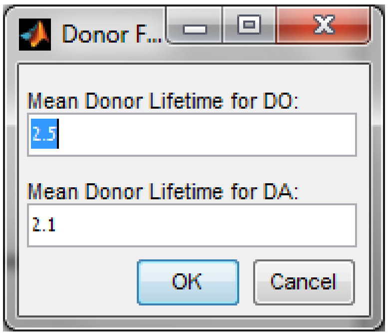
Next pops a dialogue box that asks for the donor fluorescence mean lifetimes for DO and DA ('*Mean Donor Lifetime for DO*' and '*Mean Donor Lifetime for DA*', respectively) experiments that were measured in Equilibrium for the Final-state. Change the default values to the values relevant to your analysis, then press '*OK*' to proceed with the analysis.

This step is important for the calculation of the FRET Eff. Kinetic trace. The FRET Eff. Kinetics is corrected for possible concentration differences between DO and DA measurements. The correction is performed by using the equation (Eqs S6, S7 from the article):

$$\langle E\rangle_{Final} = 1 - \frac{\tau_{DA}}{\tau_{DO}} = \lim_{t\to\infty}\langle E\rangle(t)$$

$$\langle E\rangle(t) = 1 - l\frac{I_{DA}(t)}{I_{DO}(t)}$$

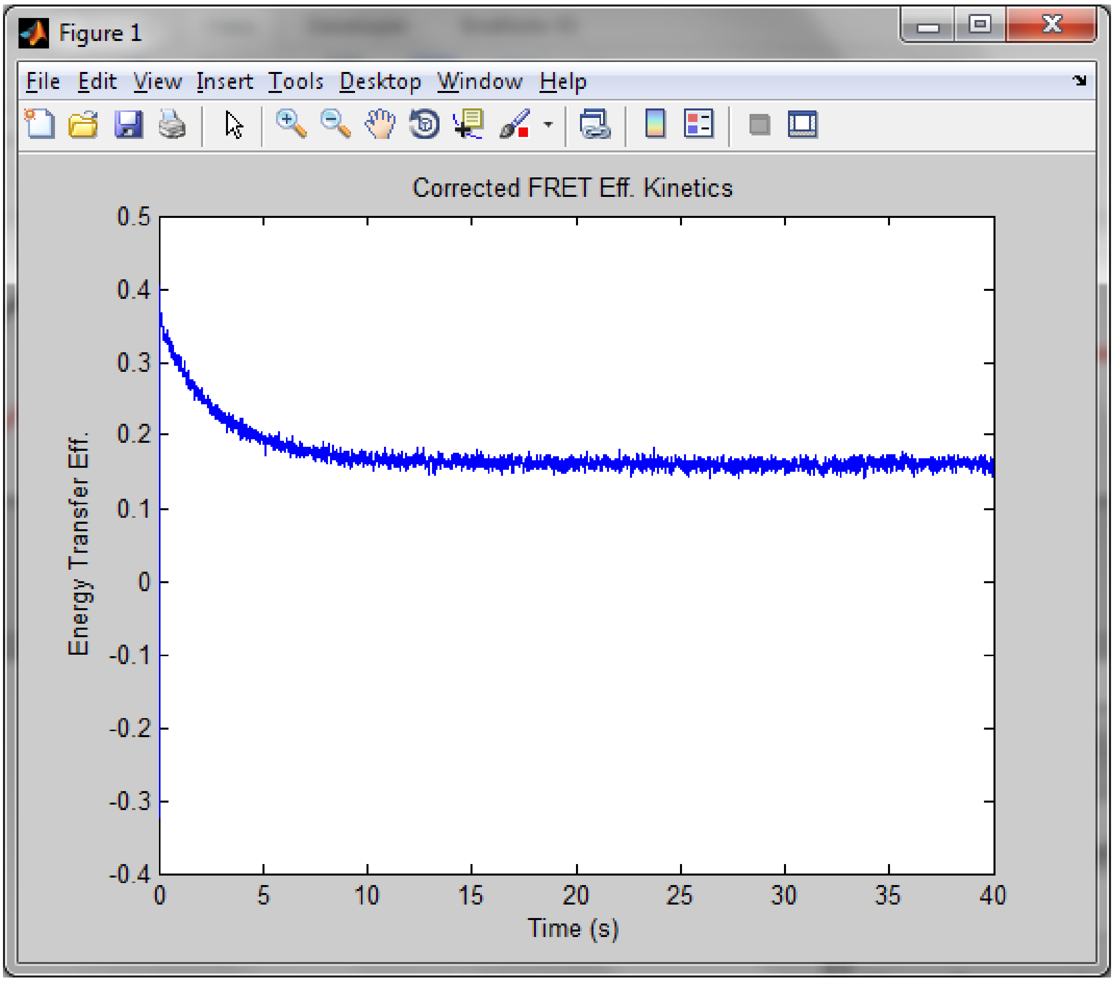
Therefore, the l correction parameter is found so that the FRET Eff. at the end of the kinetic trace will equal that of the final-state equilibrium out of trFRET.



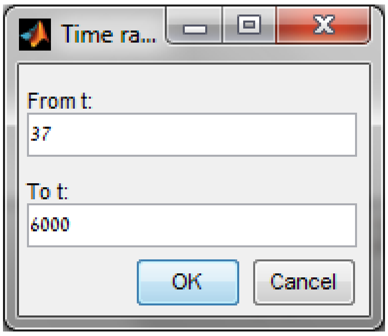
Mean Donor Lifetime for DO: 2.5

Mean Donor Lifetime for DA: 2.1

Choose '*OK*'. Afterwards, the corrected FRET Eff. Kinetic trace will be shown.



Then a question will pop up. The question asks the user for the time range for fitting (from '*From t*' to '*To t*'). It is not in time units of time but rather in units of numbers of cells in the time vector. After choosing the time range suitable for fitting, click '*OK*'.



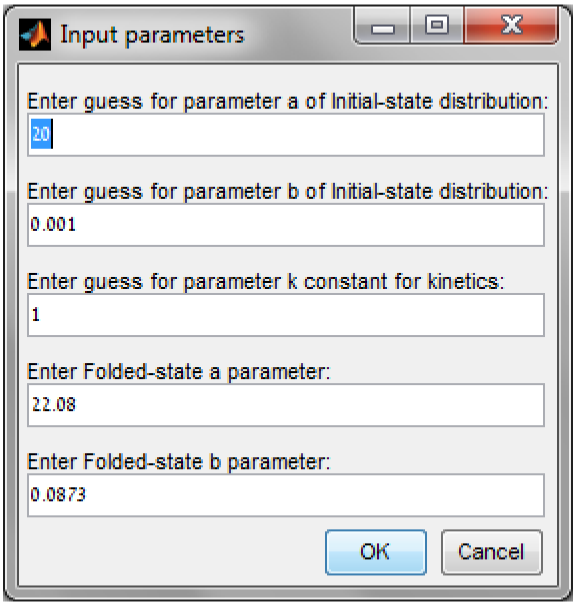
From t: 37

To t: 6000

Then a dialogue box '*Input parameters*' pops up. It asks for initial guesses of the values of the free parameters, as well as the known Final-state distance distribution parameters:

1. '*Enter guess for parameter a of initial-state distribution*' – The initial-state distance distribution if of the form $Norm \cdot r^2 e^{-b(r-a)^2}$. This is for the unknown a parameter to be optimized by the analysis.
2. '*Enter guess for parameter b of initial-state distribution*' – The initial-state distance distribution if of the form $Norm \cdot r^2 e^{-b(r-a)^2}$. This is for the unknown b parameter to be optimized by the analysis.
3. '*Enter guess for parameter k constant for kinetics*' – This is the unknown two-state rate constant to be optimized by the analysis.
4. '*Enter Folded-state a parameter*' - The final-state distance distribution if of the form $Norm \cdot r^2 e^{-b(r-a)^2}$. This is for the a parameter already found out of trFRET analysis of the equilibrium of final-state.
5. '*Enter Folded-state b parameter*' - The final-state distance distribution if of the form $Norm \cdot r^2 e^{-b(r-a)^2}$. This is for the b parameter already found out of trFRET analysis of the equilibrium of final-state.

Next choose '*OK*'.



Input parameters

Enter guess for parameter a of Initial-state distribution: 20

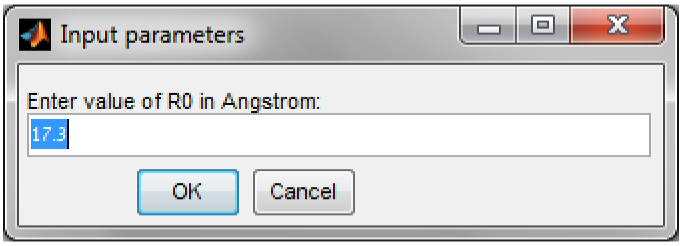
Enter guess for parameter b of Initial-state distribution: 0.001

Enter guess for parameter k constant for kinetics: 1

Enter Folded-state a parameter: 22.08

Enter Folded-state b parameter: 0.0873

Then a dialogue box '*Input parameters*' pops up. It asks for the value of R_0 found (by steady-state fluorescence measurements) in the equilibrium of the final-state. After entering its value choose '*OK*'.



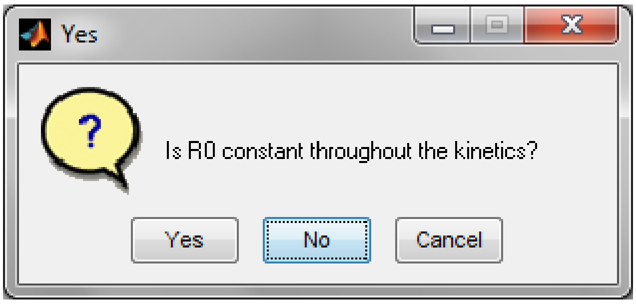
Input parameters

Enter value of R0 in Angstrom: 17.3

Now pops a question '*Is R0 constant throughout the kinetics?*'.

If DO kinetic trace is not changing throughout time, press '*Yes*'. Then the analysis will use Eq. 4 from the article.

If DO kinetic trace is changing throughout time press, press '*No*'. Then the analysis will use Eq. 3 from the article.



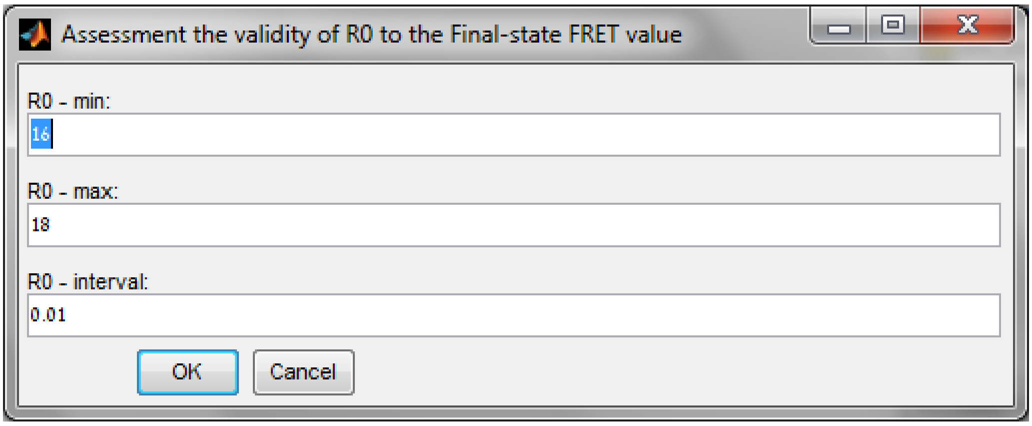
Yes

Is R0 constant throughout the kinetics?

After a R_0 value have been input characterizing the value in the Equilibrium of the final-state, a test of its validity to the final FRET Eff. Will be performed according to:

$$\langle E \rangle = \int_{r_{min}}^{r_{max}} \frac{p(r)}{1 + \left(\frac{r}{R_0}\right)^6}$$

A dialogue box '*Assessment the validity of R0 to the Final-state FRET value*' will appear. It will ask for a range, (minimal – '*R0-min*', maximal – '*R0 max*' and a given interval – '*R0 – interval*') of possible R_0 values. Press '*OK*' to proceed with the assessment.



Assessment the validity of R0 to the Final-state FRET value

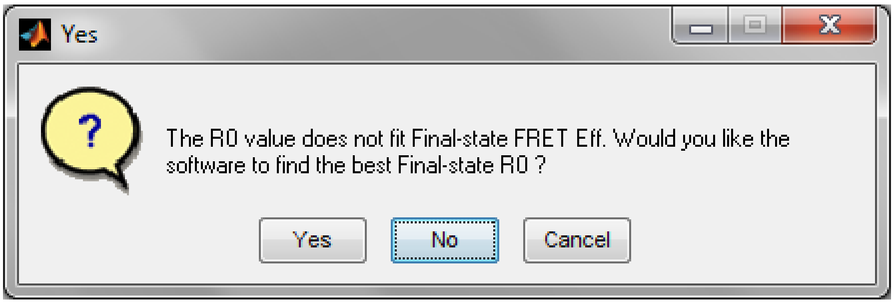
R0 - min:
16

R0 - max:
18

R0 - interval:
0.01

If the input R_0 value, together with the input folded-state distribution parameters, yields a FRET Eff. Which is within 1% error from the values at the end of the FRET Eff. Kinetic trace, the analysis will proceed. On the other hand, if the error is larger than 1%, the program will notify that '*The R0 value does not fit Final-state FRET Eff.*' Then it will ask '*Would you like the software to find the best Final-state R0 ?*'

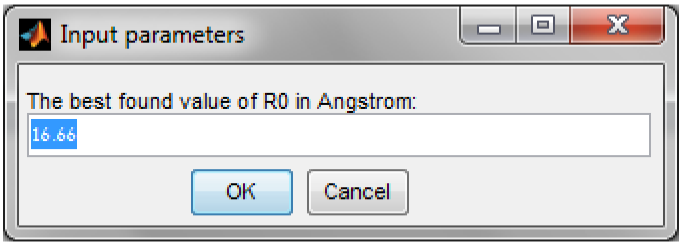
Pressing '*No*' will end the analysis. Pressing '*Yes*' will allow the software to scope over the given range of R0 values to find the one which yields a FRET Eff. that deviates from the values at the end of the FRET Eff. kinetics in less than 1%.



Yes

The R0 value does not fit Final-state FRET Eff. Would you like the software to find the best Final-state R0 ?

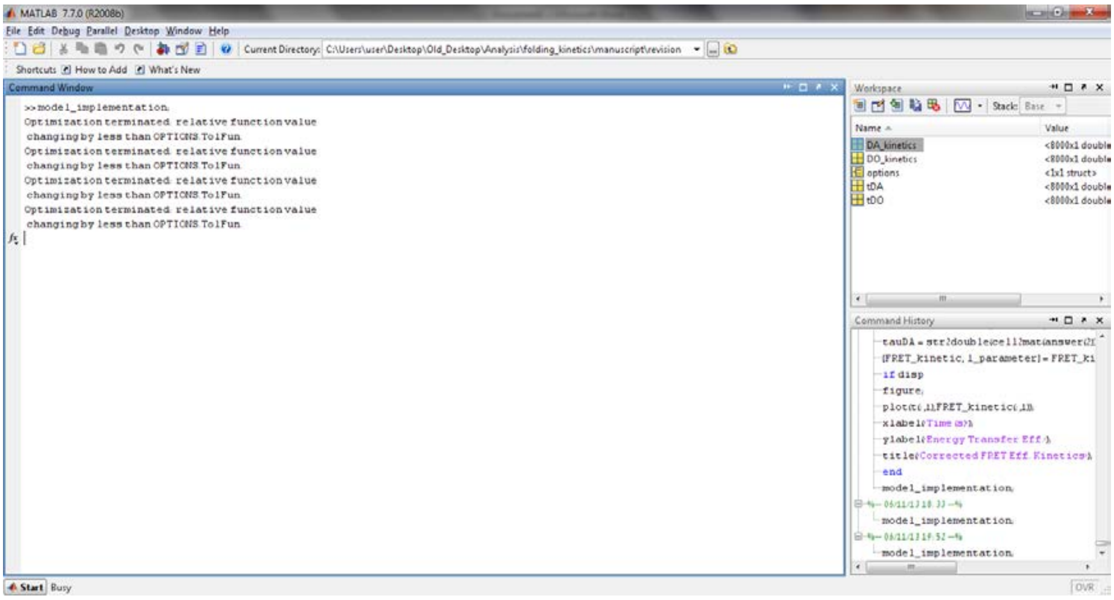
A dialogue box will show the best value for R_0 ('*The best found value of R0 in Angstrom*'). If the new value does not deviate too much from the one input by the user (the user decides)press '*OK*'. Pressing '*Cancel*' terminates the analisys.



Input parameters

The best found value of R0 in Angstrom:
16.66

Then the actual optimization will start…

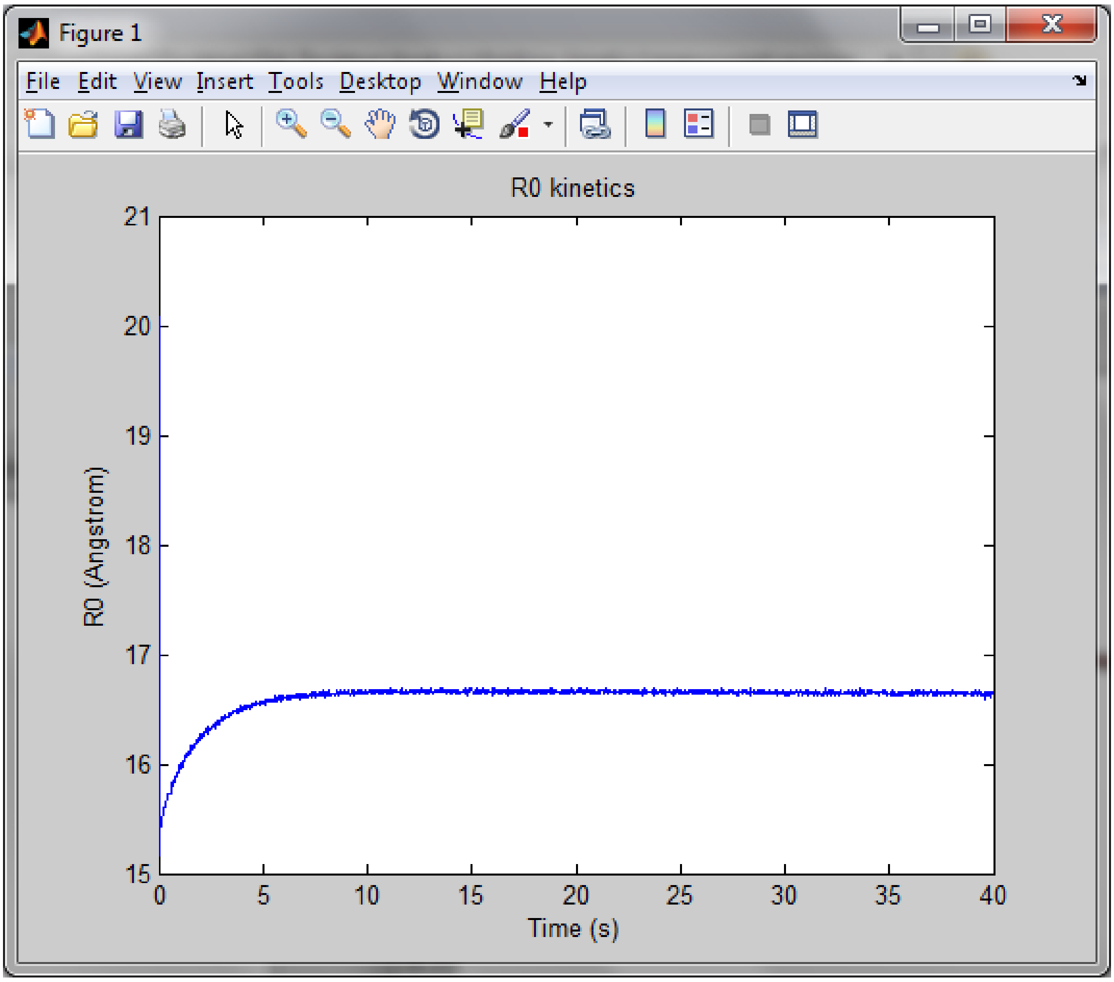


```
>> model_implementation
Optimization terminated: relative function value
 changing by less than OPTIONS.TolFun
Optimization terminated: relative function value
 changing by less than OPTIONS.TolFun
Optimization terminated: relative function value
 changing by less than OPTIONS.TolFun
Optimization terminated: relative function value
 changing by less than OPTIONS.TolFun
fx |
```

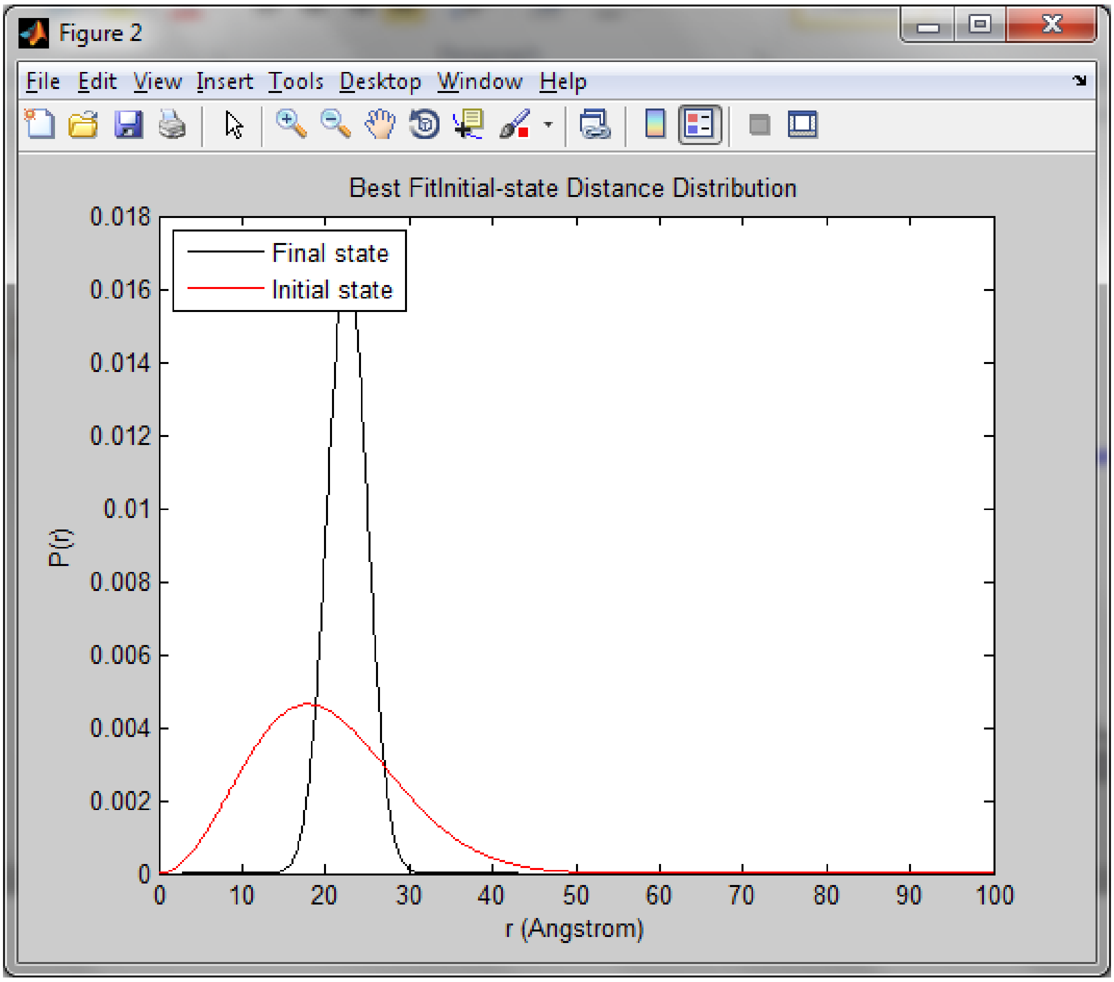
Name	Value
DA_kinetics	<8000x1 double
DO_kinetics	<8000x1 double
options	<1x1 struct>
tDA	<8000x1 double
tDO	<8000x1 double

At the end of the optimization process three more figures will appear (if 'display figures' option was chosen in the beginning of the procedure):

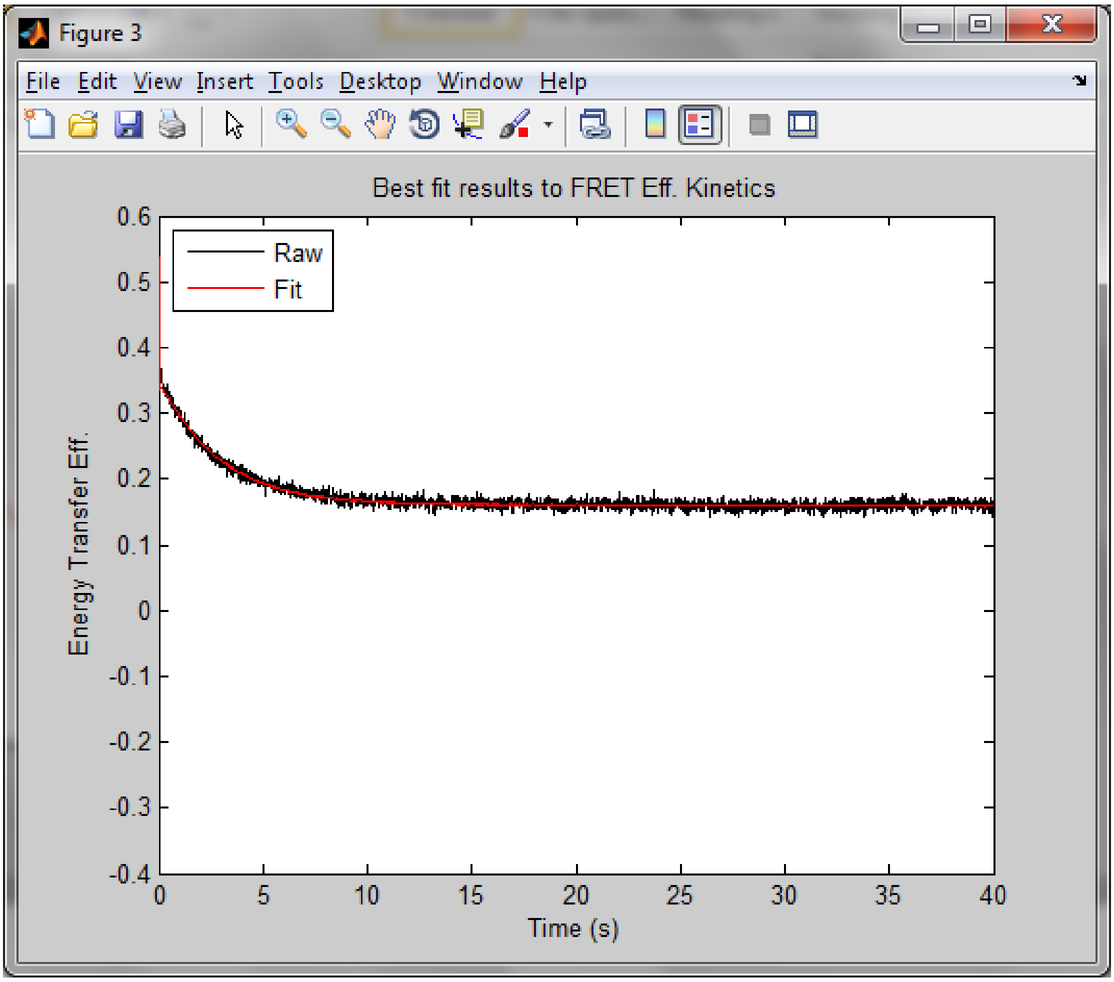
1. The resulting '*R0 kinetics*' – the change of R_0 values due to changes in donor fluorescence quantum yields, calculated using the DO kinetic trace (Eq. S5 from the article)



2. Both the known '*Final state*' and the optimized '*Initial state*' distance distributions



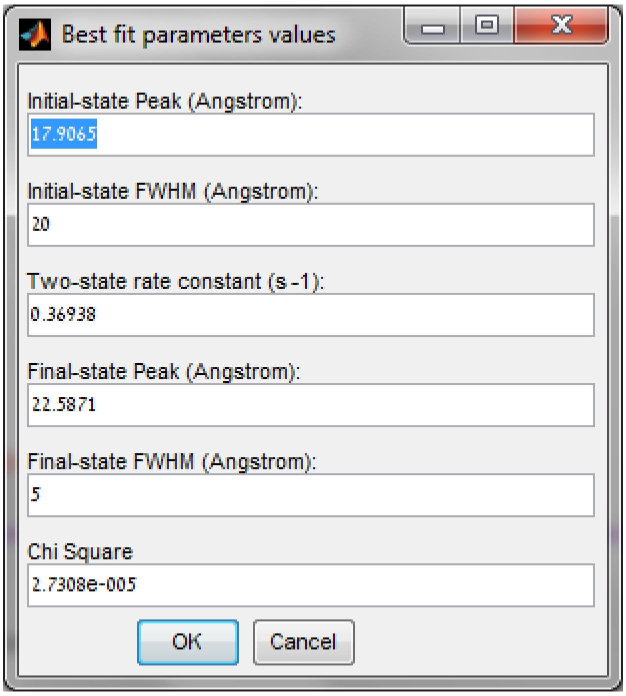
3. '*Best fit results to FRET Eff. Kinetics*' – will show the raw (corrected) FRET Eff. kinetic trace ('*Raw*') and the best fit calculated kinetic trace ('*Fit*').



And the best fit results of the parameters will be shown:

1. '*Initial-state Peak (Angstrom)*' – The most probable distance of the initial-state
2. '*Initial-state FWHM (Angstrom)*' – The width of the distance distribution of the initial-state
3. '*Two-state rate constant (s -1)*' – The rate constant of the transition from the initial- to the final-state assuming a two-state transition
4. '*Final-state Peak (Angstrom)*' - The most probable distance of the final-state, known from Equilibrium trFRET
5. '*Final-state FWHM (Angstrom)*' - The width of the distance distribution of the final-state, known from Equilibrium trFRET
6. '*Chi Square*' – the value of the fitting χ^2value calculated from $\chi^2 = \frac{\sum_{i=t_{min}}^{t_{max}} (\langle E\rangle(t)_{Raw} - \langle E\rangle(t)_{Fit})^2}{DOF}$ $DOF = t_{max} - t_{min} - 3$ where t_{min} and t_{max} are given not in time units but rather in units of numbers of cells in the time vector

The user can then copy&paste the values and save all figures. To proceed with the analysis, press '*OK*'.


Best fit parameters values

Initial-state Peak (Angstrom): 17.9065

Initial-state FWHM (Angstrom): 20

Two-state rate constant (s -1): 0.36938

Final-state Peak (Angstrom): 22.5871

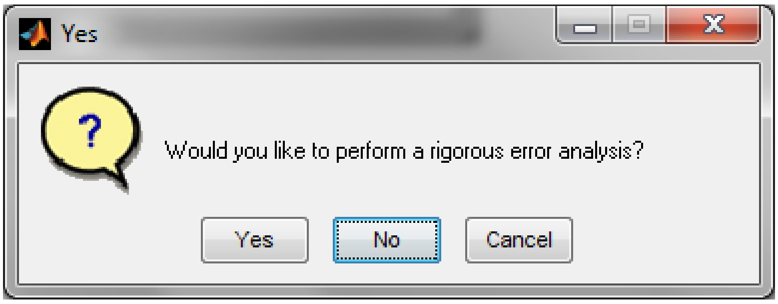
Final-state FWHM (Angstrom): 5

Chi Square: 2.7308e-005

Now, after the optimization has been finished and the best-fit values have been reached, the user can choose to find also the error ranges for the optimized parameters. The user will decide whether to proceed to error analysis judging by the quality of the fit.

Accordingly, a question '*Would you like to perform a rigorous error analysis*' will appear.

Pressing '*No*' will terminate the analysis procedure. To proceed to error analysis press '*Yes*'.

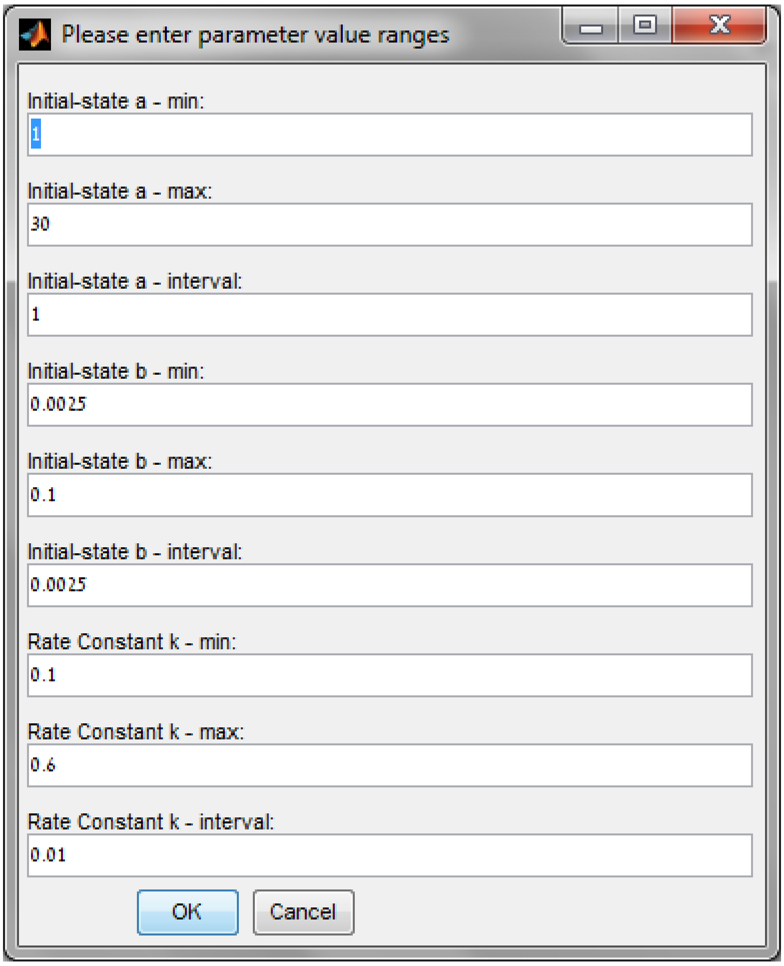

Yes

Would you like to perform a rigorous error analysis?

A dialogue box will appear '*Please enter parameter value ranges*'. According to the best fit parameter values, the user will decide upon the value ranges and the increments to be assessed:

1. Initial-state *a* parameter:
    a. '*Initial-state a – min*'
    b. '*Initial-state a – max*'
    c. '*Initial-state a – interval*' – the increment between each assessed *a* value in the range.
2. Initial-state *b* parameter:
    a. '*Initial-state b – min*'
    b. '*Initial-state b – max*'
    c. '*Initial-state b – interval*'
3. The two-state transition Rate Constant *k* parameter:
    a. '*Rate Constant k – min*'
    b. '*Rate Constant k – max*'
    c. '*Rate Constant k – min*'

To proceed with the error analysis, press '*OK*'.



Initial-state a - min:
1

Initial-state a - max:
30

Initial-state a - interval:
1

Initial-state b - min:
0.0025

Initial-state b - max:
0.1

Initial-state b - interval:
0.0025

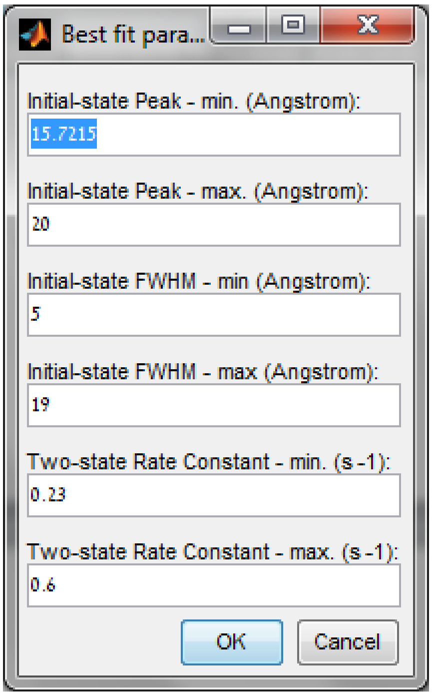
Rate Constant k - min:
0.1

Rate Constant k - max:
0.6

Rate Constant k - interval:
0.01

The error analysis can take a while…

After the error analysis is finished, the resulting minimal and maximal values, that are accepted within 95% confidence will be shown in the dialogue box '*Best fit parameters values*'.



Initial-state Peak - min. (Angstrom):
15.7215

Initial-state Peak - max. (Angstrom):
20

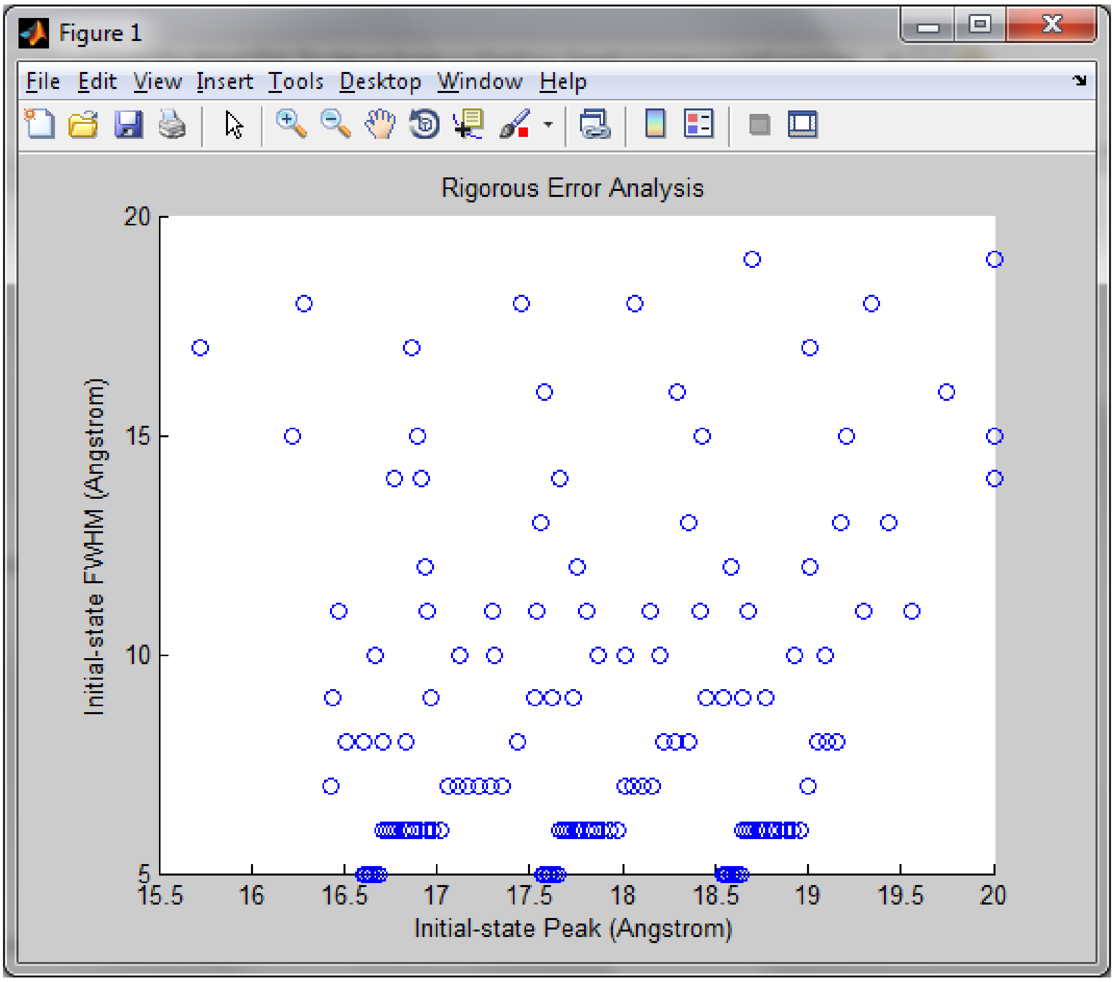
Initial-state FWHM - min (Angstrom):
5

Initial-state FWHM - max (Angstrom):
19

Two-state Rate Constant - min. (s-1):
0.23

Two-state Rate Constant - max. (s-1):
0.6

and three scatter plots of the Initial-state Peak, FWHM and two-state Rate Constant acceptable values will be shown.



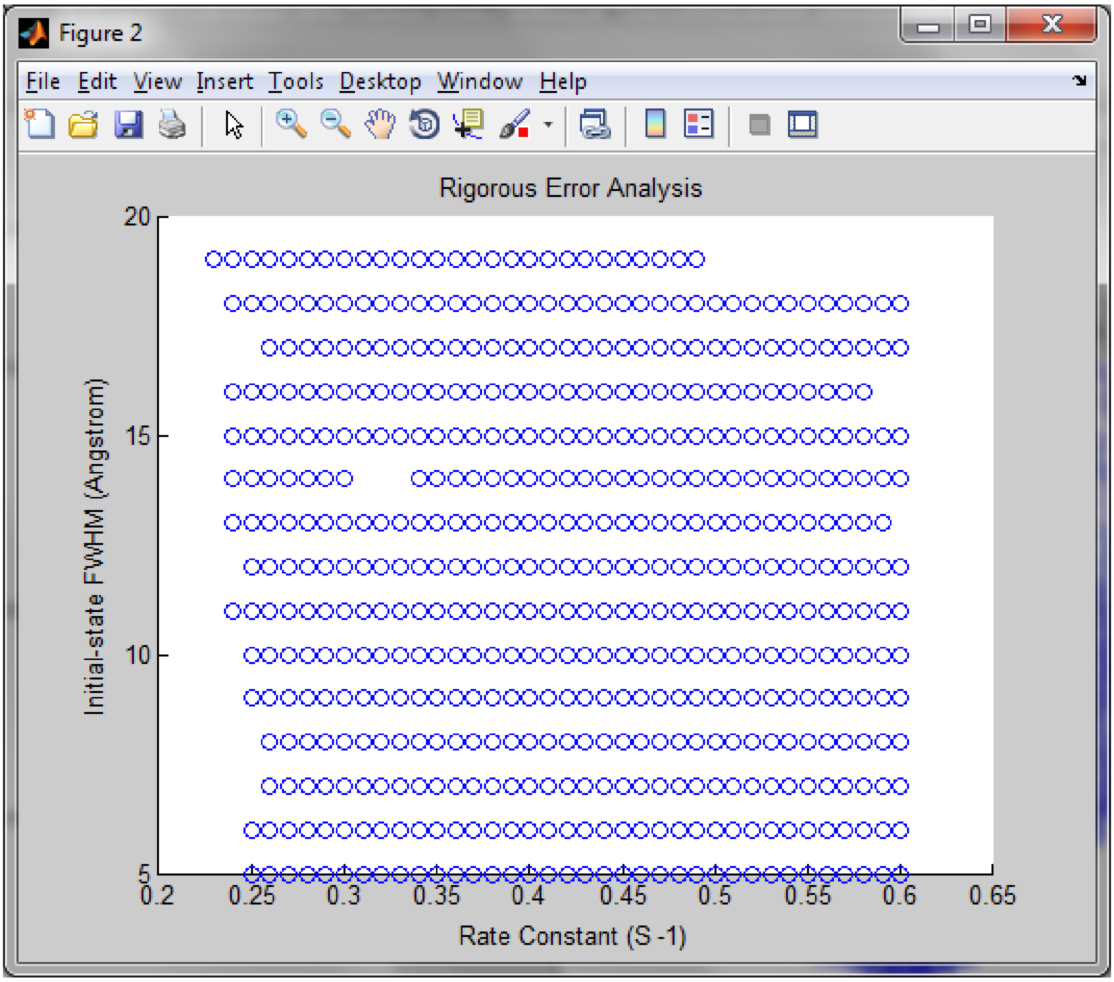


Figure 2
File Edit View Insert Tools Desktop Window Help
Rigorous Error Analysis
Initial-state FWHM (Angstrom)
20
15
10
5
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
Rate Constant (S -1)

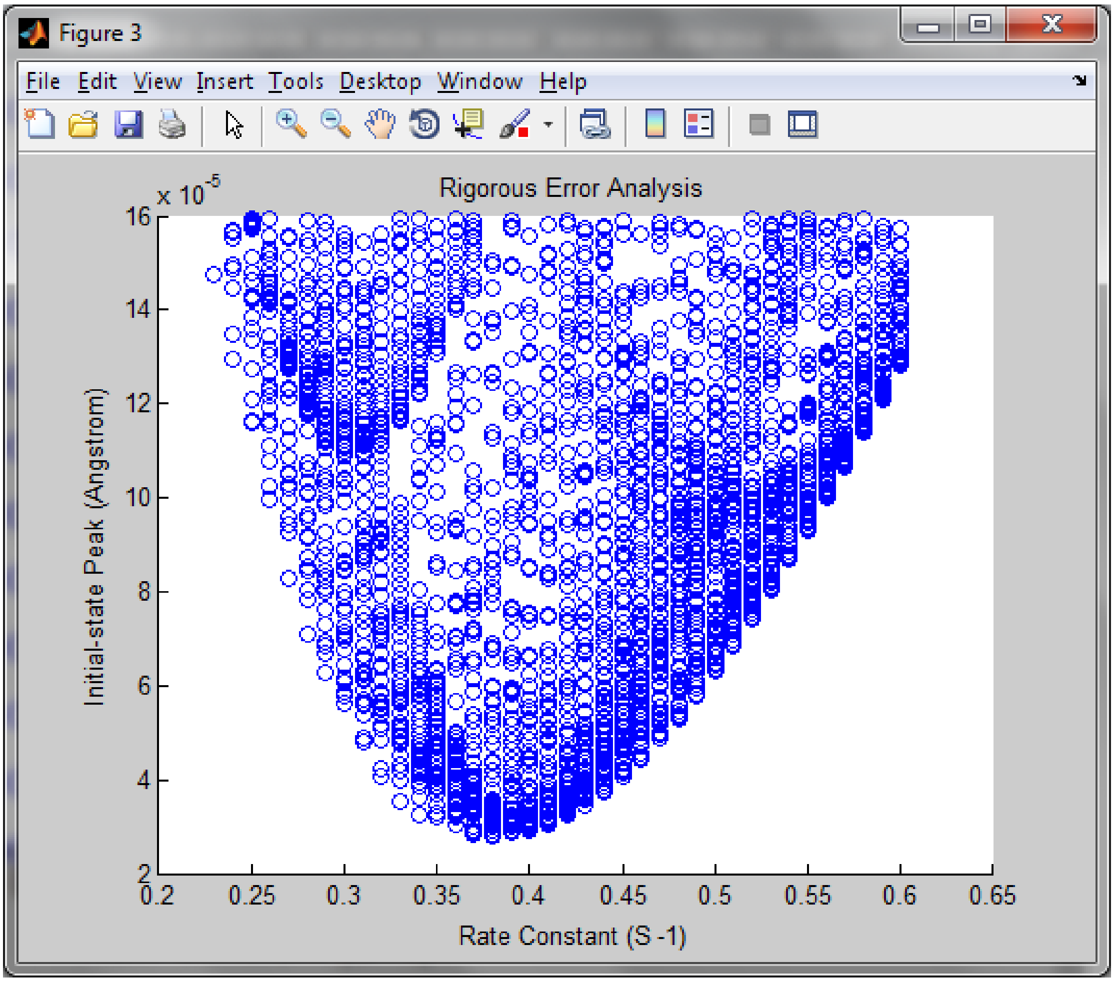


Figure 3
File Edit View Insert Tools Desktop Window Help
Rigorous Error Analysis
x 10^-5
Initial-state Peak (Angstrom)
16
14
12
10
8
6
4
2
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
Rate Constant (S -1)

The output variables of this specific example are given in the file '*matlabFinish.mat*'.

Workspace parameters:

Parameter Name	Description
tauDO	The average donor fluorescence lifetime in the absence of acceptor (DO), input by the user
tauDA	The average donor fluorescence lifetime in the presence of acceptor (DA), input by the user
tDO	Time vector of DO fluorescence kinetic trace
tDA	Time vector of DO fluorescence kinetic trace
DO_kinetics	Intensity vector of DO fluorescence kinetic trace
DA_kinetics	Intensity vector of DA fluorescence kinetic trace
t	Time vector of FRET Eff. kinetic trace
min_time	The minimal cell number in the time vector to which the model will be fit
max_time	The maximal cell number in the time vector to which the model will be fit
FRET_kinetic	The corrected experimental FRET Eff. kinetic trace
FRET_kinetic_fit	The best fit calculated FRET Eff. kinetic trace
l	The FRET Eff. correction factor
a_start	An array with parameter initial guesses
a_start(1)	The initial-state 'a' parameter guess
a_start(2)	The initial-state 'b' parameter guess
a_start(3)	The two-state transition rate constant parameter guess (s^{-1})
a_start(4)	The final-state 'a' parameter known from trFRET in equilibrium
a_start(5)	The final-state 'b' parameter known from trFRET in equilibrium
a_start(6)	(optional): The time-constant R_0 value. Is being used if time-constant-R_0 analysis is chosen
a_min	An array of parameter lower boundaries.
a_min(1)	The initial-state 'a' parameter lower boundary
a_min(2)	The initial-state 'b' parameter lower boundary
a_min(3)	The two-state transition rate constant parameter lower boundary (s^{-1})
a_min(4)	The final-state 'a' parameter lower boundary – is equated to *a_start(4)*0.999* since this parameter is Const. in the analysis
a_min(5)	The final-state 'b' parameter lower boundary – is equated to *a_start(5)*0.999* since this parameter is Const. in the analysis
a_min(6)	(optional): The time-constant R_0 lower boundary. Is being used if time-constant-R_0 analysis is chosen. – is equated to *a_start(6)*0.999* since this parameter is Const. in the analysis
a_max	An array of parameter upper boundaries.
a_max(1)	The initial-state 'a' parameter upper boundary
a_max(2)	The initial-state 'b' parameter upper boundary
a_max(3)	The two-state transition rate constant parameter upper boundary (s^{-1})
a_max(4)	The final-state 'a' parameter upper boundary – is equated to *a_start(4)*1.001* since this parameter is Const. in the analysis
a_max(5)	The final-state 'b' parameter upper boundary – is equated to *a_start(5)*1.001* since this parameter is Const. in the analysis
a_max(6)	(optional): The time-constant R_0 upper boundary. Is being used if time-constant-R_0 analysis is chosen. – is equated to *a_start(6)*1.001* since this parameter is Const. in the analysis
x	An array with parameter optimized values
x(1)	The initial-state 'a' parameter optimized value
x(2)	The initial-state 'b' parameter optimized value
x(3)	The two-state transition rate constant parameter optimized value (s^{-1})
x(4)	The final-state 'a' parameter known from trFRET in equilibrium

	– Const.
x(5)	The final-state 'b' parameter known from trFRET in equilibrium – Const.
x(6)	(optional): The time-constant R_0 value. Is being used if time-constant-R_0 analysis is chosen – Const.
PEAK_F	Most probable distance of known final-state distance distribution (Å)
FWHM_F	Full Width at Half Maximum of known final-state distance distribution (Å)
PEAK_I	Most probable distance of optimized initial-state distance distribution (Å)
FWHM_I	Full Width at Half Maximum of optimized initial-state distance distribution (Å)
PEAK_min	The lower boundary value of the most probable distance of initial-state distance distribution (Å)
PEAK_max	The upper boundary value of the most probable distance of initial-state distance distribution (Å)
FWHM_min	The lower boundary value of the Full Width at Half Maximum of initial-state distance distribution (Å)
FWHM_max	The upper boundary value of the Full Width at Half Maximum of initial-state distance distribution (Å)
rigorous	A value of '1' if the user chooses the perform a rigorous error analysis and avalue of '0' if not.
rigorous_array	An array that includes the fitting parameter values which yield a χ^2 value lower or equal to the 95% Confidence limit.
rigorous_array(:,1)	Initial-state 'a' parameter
rigorous_array(:,2)	Initial-state 'b' parameter
rigorous_array(:,3)	Two-state transition Rate Constant (s^{-1})
rigorous_array(:,4)	Initial-state Most probable distance (Å)
rigorous_array(:,5)	Initial-state Full Width at Half Maximum (Å)
rigorous_array(:,6)	χ^2 value
R	Distance vector
PF	Known final-state distance distribution
PI	Optimized initial-state distance distribution
chi2	Value of Best fit χ^2 value
chi2limit_alpha_0_05	95% Confidence limit for χ^2 values
chi2limit_alpha_0_01	99% Confidence limit for χ^2 values
chi2limit_alpha_0_1	90% Confidence limit for χ^2 values
chi2limit_alpha_0_33	67% Confidence limit for χ^2 values
R0constant	A Boolean variable with a value of 1 if time-constant-R_0 analysis is chosen and a value of 0 if time-dependent-R_0 analysis is chosen
R0_final_equilibrium	The value input by the user of R_0 (Å) in the case of a time-dependent- R_0 analysis
R0vec	The vector of R_0 values in the case of a time-dependent- R_0 analysis
R0_test	The value of R_0 input by the user
Proposed_R0	The value of R_0 proposed by the script, that best fits the final-state FRET Eff. and distance distribution
Find_R0	A Boolean variable with a value of 1 the R_0 value input by the user deviates from the optimized one and the user chooses to let the script find the optimal value of R_0 that best fits the final-state FRET Eff. and distance distribution
options	A list of variables important for the optimization procedure. Should always be present in the analysis
options.MaxFunEvals	The maximal number of function evaluations in the optimization. The default value used here is 5000.
options.MaxIter	The maximal number of optimization iterations for each function evaluation step. The default value used here is 5000

options.TolFun	The functional Tolerance value. The default value used here is 1E-25
options.TolX	The iterational Tolerance value. The default value used here is 1E-25
options.DiffMinChange	The minimal change that proceeds the optimization procedure to the next step. The default value used here is 1E-5

Supporting References

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