ChiSurf Documentation

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Apr 20, 2020

CONTENTS

ChiSurf is a software-suite dedicated to the analysis of fluorescence data. Its main purpose is the interpretation of fluorescence measurements by structural models and the analysis time-resolved fluorescence measurements.

Currently, ChiSurf is capable to analyze TCSPC, FCS and stopped-flow data. Its open frame-work and API allows to extend its capabilities relatively easy.

ChiSurf supports Windows, Linux and MacOS. Pre-assembled packages are available for Windows and MacOS. Currently, ChiSurf is developed for Windows.

CHAPTER

ONE

INSTALLATION

conda install

run from bash

chisurf

or run tools directly, e.g,

clsm_pixel_select

CHAPTER

TWO

USER-DOCUMENTATION

2.1 Tutorials

2.1.1 TCSPC

Gaussian

Under our measurement conditions we have a mixture of monomeric and dimeric hGBP1. Thus all donor fluorescence decays FD(t) were fitted with the decays of at least two molecular species, FD(0)(t) for the Donor-only species and FD(A)(t) for the dimeric FRET species:

$$`F(t) = (1 - x_{\mathrm{DOnly}})F_{\mathrm{D(A)}}(t) + x_{\mathrm{DOnly}}F_{\mathrm{D(0)}}(t) + c`$$

where xDOnly corresponds to the fraction of Donor-only molecules and c is a constant offset. Due to local quenching the fluorescence decay of the donor in the absence of FRET is tri-exponential with the individual species fractions $x_{\mathrm{D}}^{(i)}$ and fluorescence lifetimes $tau_{\mathrm{D(0)}}^{(i)}$ (see Table S3)

Thus, the time resolved fluorescence intensity decays of donor-/acceptor-labeled protein-complex (FRET-sample) were fitted globally with the decays of the donor-/unlabeled protein-complexes (donor only sample, DOnly). Generally it is reasonable to assume that the radiative lifetime of the donor is not affected by quenching. Hence, the FRET-rate constant (kFRET) is only determined by the donor-acceptor distance and their relative orientation [Ref: Kalinin, S.,& Johansson, L.B.-Å. Energy Migration and Transfer Rates Are Invariant to Modeling the Fluorescence Relaxation by Discrete and Continuous Distributions of Lifetimes. (2004) J. Phys. Chem. B 108, 3092-3097]. Expressing the FRET-rate constant in terms of distances the donor-fluorescence in presence of acceptor is given by:

$$`F_{\mathrm{D(0)}}(t) = \sum_i x_{\mathrm{D}}^{(i)} \exp(-t/\tau_{\mathrm{D(0)}}^{(i)})`$$

Whereas p(RDA) is a FRET-rate distribution expressed as distance and R0 is the Forster-radius (in this case R0 =52 Å) and $k_0 = 1/\tau_0$ is the radiative rate the unquenched dye. The flurophores are attached to the biomolecule by long flexible linkers. Hence, a donor-acceptor distance distribution is expected which is not averaged during the fluorescence lifetime of the dyes [Sindbert, S., Kalinin, S., Nguyen, H., Kienzler, A., Clima, L., Bannwarth, W., Appel, B., Müller, S. & Seidel, C.A.M. (2011) J. Am. Chem. Soc. 33, 2463-2480.] and the fluorescence decay FD(A) has to be expressed as by an donor-acceptor distance distribution p(RDA) with a non-zero width. Here the experimental time-resolved fluorescence intensities were either fitted by a Gaussian distribution of donor-acceptor distances (p(RDA)) with a mean inter dye distance RDA and a width wDA (Eq. 4) or, analog to the Tikhonov regularization, p(RDA) was determined by deconvolution of the fluorescence intensity decays by using the maximum-entropy method (MEM) [Livesey, A. K.; Skilling J. Maximum Entropy Theory. Acta Crystallogr. Sect. A 1985, 41, 113-122. Ref. Brochon, J. C. Methods Enzymol. 1994, 240, 262-311.].

$$`F_{\mathrm{D(A)}}(t) = F_{D(0)} \cdot \int_{R_{\mathrm{DA}}} p(R_{\mathrm{DA}}) \cdot \exp\left(-t \cdot k_0 \cdot (R_0/R_{\mathrm{DA}})^6\right) dR_{\mathrm{DA}}`$$

The width of the Gaussian donor-acceptor distance distribution wDA should not be misinterpreted as the experimental/statistical-error but it describes a real physical property of the donor-acceptor pair. The experimental fluorescence decays presented below are described by combining the above formulas and were fitted by custom software written in Python.

$$F_{\mathrm{D(A)}}(t) = F_{D(0)} \cdot \int_{R_{\mathrm{DA}}} \frac{1}{w_{\mathrm{DA}}\sqrt{\pi/2}} \exp\left(-2\left[\frac{R_{\mathrm{DA}}-\langle R_{\mathrm{DA}}\rangle}{w_{DA}}\right]^2\right) \exp\left(-t\cdot k_0\left[1+(R_0/R_{\mathrm{DA}})^6\right]\right) dR_{\mathrm{DA}}$$

2.1.2 Stopped Flow

In the reaction system model a system of reactions has to be defined by the user. Given the user-defined reaction system and initial values of the reacting species the time-evolution of the species is calculated by numerical integration of the differential equations. Below the theoretical frame-work is described followed by a description how to define custom reaction models and fit experimental data. Given a set of species and a number of elementary reactions with associated rate constants the reaction system can be described by the following chemical reactions:

$$\begin{array}{ccc}
e_{11}X_1 + e_{21}X_2 + \ldots + e_{N1}X_N & \xrightarrow{k1} & p_{11}X_1 + p_{21}X_2 + \ldots + p_{N1}X_N \\
\vdots & \vdots & \vdots \\
e_{1j}X_1 + e_{2j}X_2 + \ldots + e_{Nj}X_N & \xrightarrow{kj} & p_{1j}X_1 + p_{2j}X_2 + \ldots + p_{Nj}X_N \\
\vdots & \vdots & \vdots \\
e_{1R}X_1 + e_{2R}X_2 + \ldots + e_{NR}X_N & \xrightarrow{kR} & p_{1R}X_1 + p_{2R}X_2 + \ldots + p_{NR}X_N
\end{array}$$

Using the law of mass action the flux of molecules per time and unit-volume is given by:

$$f_j\left(\vec{c}\right) = k_j \prod_{i=1}^{N} c(X_i)^{e_i}$$

$$\frac{dc\left(X_i\right)}{dt} = \sum_{j=1}^{R} p_{ij} f_j\left(\vec{c}\right) - e_{ij} f_j\left(\vec{c}\right)$$

$$I(t) = s \cdot \langle \vec{c}(t), \vec{q} \rangle$$

$$I'(t) = \frac{\int D(t)dt}{\int I(t)dt} \cdot I(t)$$

$$\begin{array}{ccc}
A & \xrightarrow{kf} & B \\
B & \xrightarrow{kb} & A \\
B & \xrightarrow{kR} & C
\end{array}$$

2.2 Command line tools

2.2.1 Decay histogram

CHAPTER

THREE

INDICES AND TABLES

- genindex
- modindex
- search

CHAPTER

FOUR

FEEDBACK

The best way to report a bug or request is to contact me personally. Just pass by in my office and we can try to reproduce the bug. Right now ChiSURF is not stable. Therefore it is not officially released in the internet.

If you want to contribute the best thing you can do is writing user-documentations, examples and tutorials.