Analysis of spatio-temporal dynamics by artificial and real FRAP data

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1. Introduction

In recent years the interest in noninvasive methods to observe and analyse molecular mobility and interactions in a cell increased dramatically [1, 3]. Fluorescence recovery after photobleaching (FRAP) is one of the techniques widely used for this purpose.
FRAP curves enables us to analyse binding and diffusion of fluorescent molecules. Already published analytical solutions which describe these FRAP curves for several cases only deal with diffusion of unbounded molecules [4]. Therefore, we derived the so far missing Laplace transformed solution which allows diffusion of all particles. Making use of derived analytical solutions we propose to develop a robust, inverse method to infer binding and diffusion coefficients from FRAP data.

2. Methods & Materials

FRAP experiment:

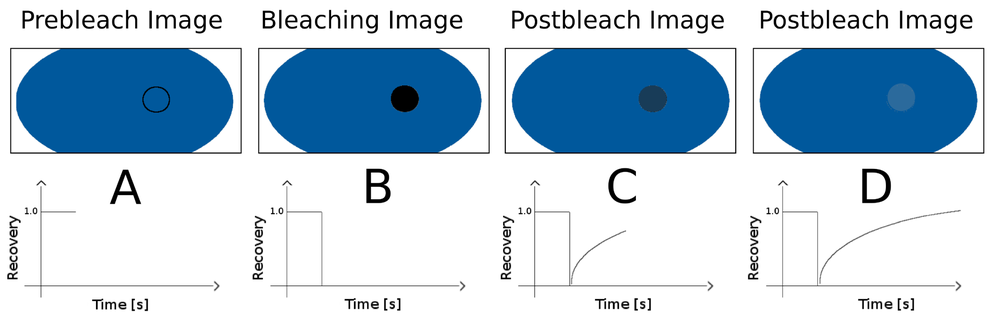

Fig. 1: Concept of FRAP experiments

Model functions:

n vacant binding sites:

$$F + S_\mathsf{i} \underset{k_{\mathsf{off}_\mathsf{i}}}{\overset{k_{\mathsf{on}_\mathsf{i}}}{\rightleftharpoons}} B_\mathsf{i} \quad , \ \mathsf{i} = 1 \dots n \tag{1}$$

where F represents the unbound (free) fraction, S_i the vacant binding sites and B_i the bound fraction.

M1 Reaction Dominant Model (n BS)[4]

M2 Reaction Diffusion Model with Single Diffusion (n BS)[4]

M3 Reaction Diffusion Model with Multiple Diffusion (n BS)[2]

Datasets:

(1) Artificial FRAP Datasets

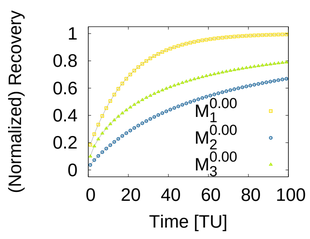

Fig. 2: no noise signal

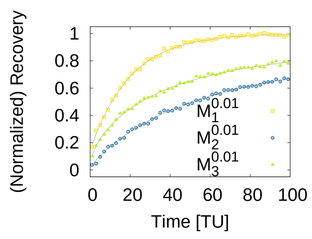

Fig. 3: low noise signal

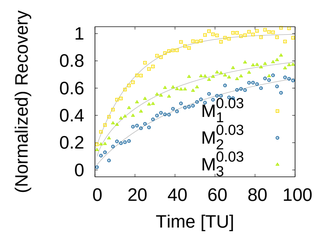

Fig. 4: high noise signal

(2) Real FRAP Dataset

- FRAP dataset on yellow protein labelled aryl hydrocarbon receptor which was transiently transfected into mouse hepatoma cells: FRAP experiments inside nucleus (51 samples)

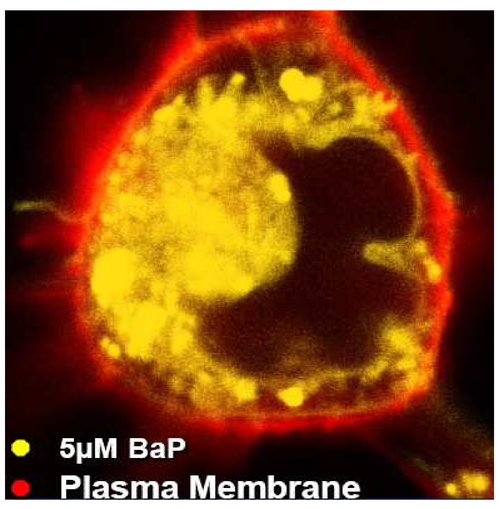

Fig. 5: cell before FRAP treated with BaP

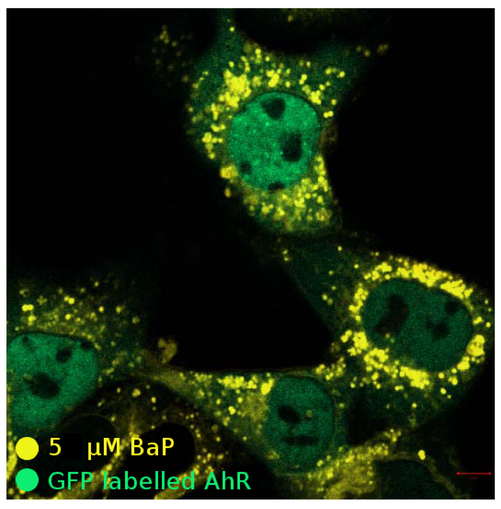

Fig. 6: cell before FRAP with labelled AhR and BaP treatment

3. Results

Artificial Datasets

- for every dataset the correct model was identified (Tab. 1)
- analysis of histograms of error values of 500 Simulated Annealing (SA) runs (Fig. 7)
- correct diffusion coefficients and reaction rates were determined
- to analyse robustness of estimated parameters we analysed mean and variance of the parameters fitted by the best 100 SA runs (Fig. 8)

	Model 1	Model 2	Model 3
$M_1^{0.00}$	**0.00000**	1.92217	2.07131
$M_1^{0.01}$	**0.78581**	2.09649	2.24620
$M_1^{0.03}$	**2.11161**	3.03402	3.16262
$M_2^{0.00}$	1.50488	**0.00885**	0.06056
$M_2^{0.01}$	1.70500	**0.78243**	0.78933
$M_2^{0.03}$	2.87615	**2.23449**	2.35603
$M_3^{0.00}$	2.17175	0.03437	**0.00092**
$M_3^{0.01}$	2.26306	0.77783	**0.77610**
$M_3^{0.03}$	2.97956	2.48055	**2.45850**

Tab. 1: Error function values of fitting artificial datasets

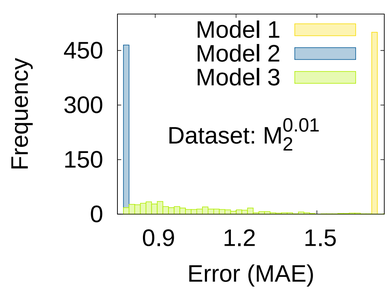

Fig. 7: Histogram of error values of 500 Simulated Annealing fitting runs

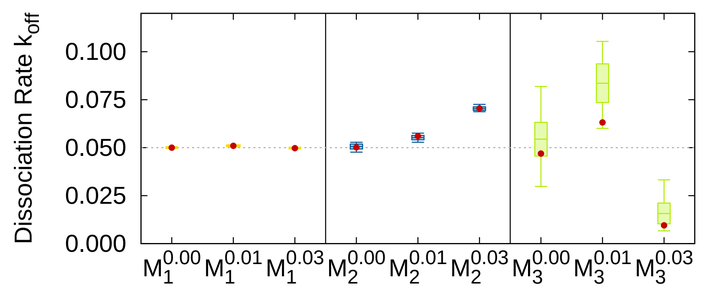

Fig. 8: Robustness of estimated dissociation rate k_{off}

Real Datasets

- different model functions fitted to real FRAP dataset
- best model type: *Reaction Diffusion Model with Multiple Diffusion ($n = 2$)* (Fig. 9)
- best parameter values:
  $D_F = 1.035 \mu m^2 s^{-1}$
  $D_{B_1} = 0.0469 \mu m^2 s^{-1}$
  $D_{B_2} = 22.18 \mu m^2 s^{-1}$
  $k_{\mathsf{on}_1} = 0.633 s^{-1}$, $k_{\mathsf{off}_1} = 0.017 s^{-1}$
  $k_{\mathsf{on}_2} = 0.482 s^{-1}$, $k_{\mathsf{off}_2} = 0.005 s^{-1}$

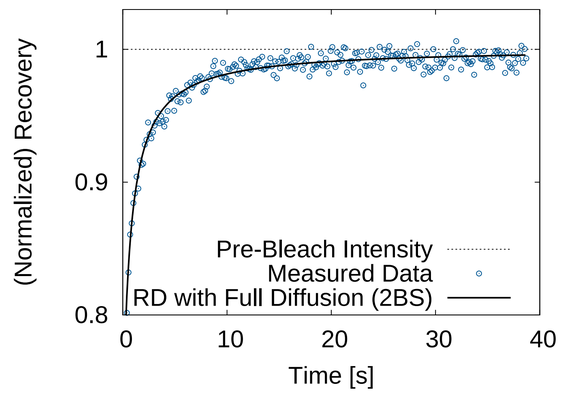

Fig. 9: FRAP measurement inside Nucleus (dots) and best fitted model function

4. Summary

- introduction of novel approach to analyse FRAP data
- presentation of missing (semi-) analytical solution for a multiple diffusion problem with reaction component
- assessment of performance by fitting analytical solutions to artificial FRAP datasets
- relevance of model which allows for multiple diffusion and reaction shown by fitting real FRAP datasets

References

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[2] J. Mai, S. Trump, G. Hager, T. Karpova, J. G. McNally, and S. Attinger, "Analysis of spatio-temporal dynamics by frap," submitted to Biophysical Journal.

[3] B. L. Sprague and J. G. McNally, "Frap analysis of binding: proper and fitting," *Trends in Cell Biology*, vol. 15, no. 2, pp. 84–91, February 2005.

[4] B. L. Sprague, R. L. Pego, D. A. Stavreva, and J. G. McNally, "Analysis of binding reaction by fluorescence recovery after photobleaching," *Biophysical Journal*, vol. 86, pp. 3473–3495, June 2004.

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