Analysis of spatio-temporal dynamics by FRAP data

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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 unbound molecules [4]. Here we present the so far missing Laplace transformed solution which allows diffusion of all molecular fractions involved. Making use of the derived analytical solutions we developed a robust, inverse method to infer binding and diffusion coefficients from FRAP data.

Methods & Materials

FRAP experiment:

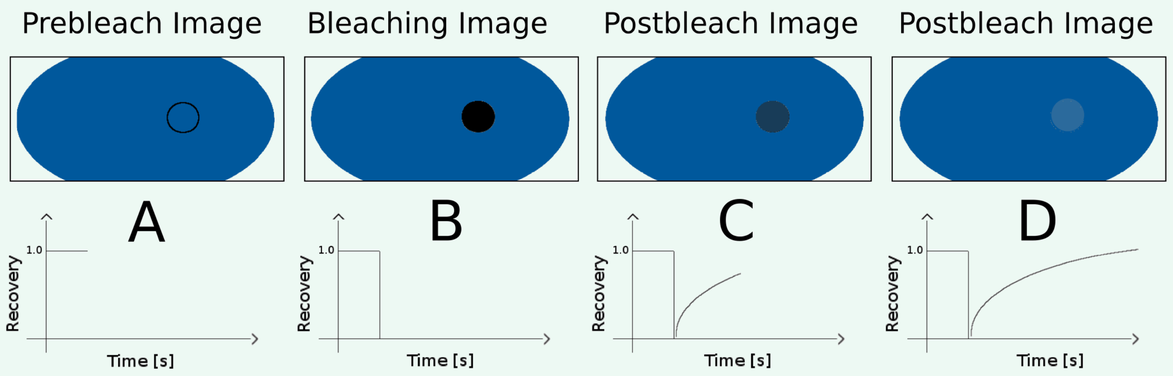


Fig. 1: Concept of FRAP experiments

Model functions:

n vacant binding sites:

$$F + S_i \underset{k_{off_i}}{\overset{k_{on_i}}{\rightleftharpoons}} B_i \quad , \; i = 1 \dots n$$

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]

(1) Artificial Datasets

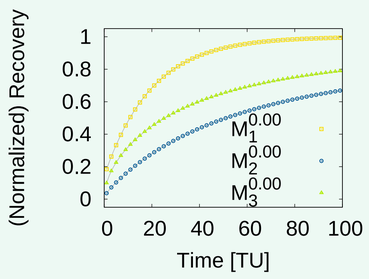


Fig. 2: No noise signal

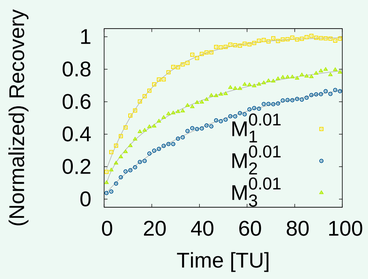


Fig. 3: Low noise signal

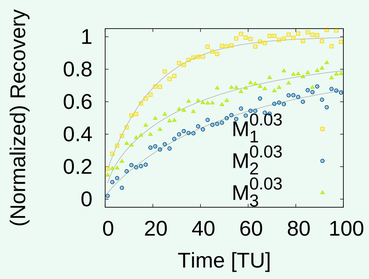


Fig. 4: High noise signal

(2) Real Datasets

Mouse hepatoma cells stably transfected with green fluorescent protein tagged aryl hydrocarbon receptor (AhR) and treated with 50nM BaP were used for nuclear FRAPs (50 datasets).

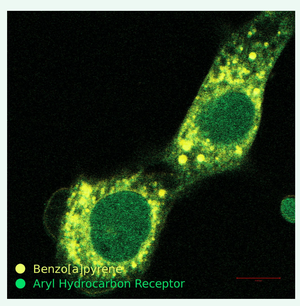


Fig. 5: Distribution of AhR and BaP 15 min after treatment with BaP

Results

(1) Artificial Datasets

- correct range for number of binding sites n^* was pre-estimated using Prony's method
- for every dataset the correct model was identified

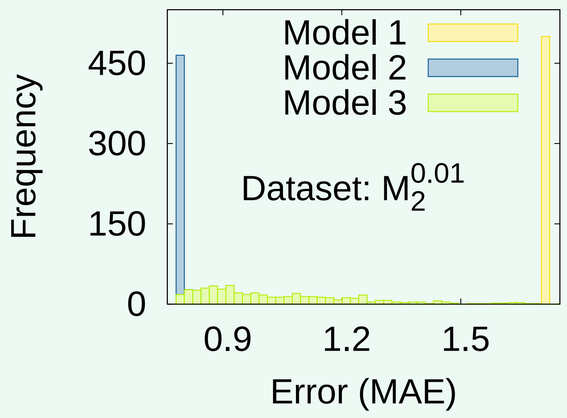


Fig. 6: Histogram of error values of 500 Simulated Annealing runs

- analysis of histograms of error values of 500 Simulated Annealing (SA) runs (Fig. 6)
- correct diffusion coefficients and reaction rates were determined
- robustness test of estimated parameters: analysis of mean and variance of parameters fitted by the best 100 SA runs (Fig. 7)

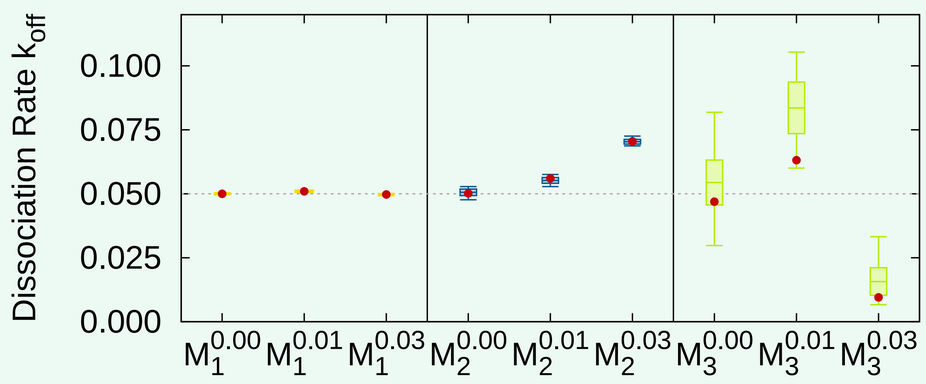
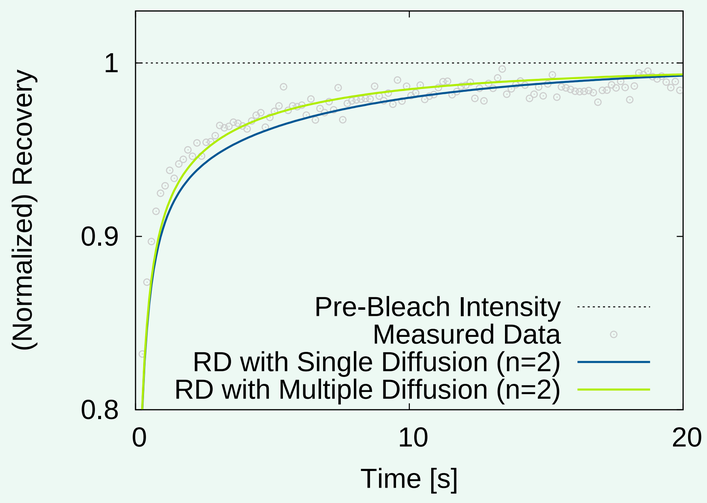


Fig. 7: Robustness of estimated dissociation rate k_{off}. Red dots represent parameters which gives least error function value.

(2) Real Datasets



Different model functions were fitted to real FRAP data (50nM BaP, 15 min treatment). The Reaction Diffusion Model with Multiple Diffusion performs best (Fig. 8).

Fig. 8: Comparison of real FRAP data 15 min after treatment (dots) with models for single and multiple diffusion

Summary

- application of a pre-processing algorithm to estimate number of binding sites
- presentation of missing (semi-) analytical solution for a multiple diffusion problem with reaction component
- performance tested based on simulated and real FRAP data
- multiple diffusion model performs best, suggesting that the real system consists of at least two diffusing components

References

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