GENERALISED MATHEMATICAL MODEL FOR INTERPRETATION OF FRAP EXPERIMENTS

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Abstract

A novel mathematical approach for quantitative investigation of protein dynamics in living cells with fluorescence recovery after photobleaching (FRAP) experiments has been developed. Existing FRAP fitting models are based on the specific chemical reaction equations and often exhausting for parameters fitting. Our model allows to study different biomolecular processes and can be used as a non-fitting procedure for quantitative FRAP analysis. The mathematical formalism is based on the theory of stochastic processes. The derived model was validated on the examples of binding and actin polymerization reactions.

1 Introduction

Fluorescence recovery after photobleaching (FRAP) is a microscopy technique for quantification of molecular dynamics [3]. Fluorescent labels, such as green fluorescent protein (GFP) are bleached in a small region under exposure of high intensity laser light. The recovery of fluorescence intensity occurs due to biochemical processes, which regulate the exchange of bleached and non-bleached molecules from other cellular regions. Existing models adequately reproduce experimental data for simple intracellular interactions and become computationally inefficient or even non-applicable for more complex systems, such as actin polymerisation assay. Thus, there is a need for new proper mathematical issues, which can be used to estimate parameters of underlying processes from FRAP curves.

In this work we present and verify a novel general mathematical model for analysis of biochemical reactions studied by the FRAP experiments. These interactions usually affect the mobility of proteins, making them either freely diffusing or fixed on (bound to) some cellular structures. Processing of experimental data with our procedure will provide the distribution of the residence times of proteins in a bound state. Consequently, the FRAP signal can be analysed by a non-fitting computational procedure.

2 Methodology

The residence time is the only actual physical characteristic of a labelled protein that is measured by the quantitative FRAP experiment. Models for fitting of other parameters, such as reaction rate constants, turnover time, equilibrium concentrations of proteins in different states, are usually based on the *a priori* assumptions concerning

underlying processes. Our aim is to reduce the problem of FRAP recovery simulation to calculation of residence time distribution.

We assume the following: i) system is in the steady state; ii) bound molecules are immobile, free molecules diffuse rapidly; iii) the bleached molecules have tiny chance for re-entering the relatively small bleached spot; iv) FRAP recovery is quantified as the average fluorescence intensity inside the bleached spot for each scanned picture.

As it follows from our formalisation, bleached molecules have to become bound at time t_+ and get unbound at time t_-, such as $t_+ \leq t_b \leq t_-$, where t_b - the bleach moment. The value $\tau = t_- - t_+$ is a residence time of the molecule in bound state; $\tau_f = t_- - t_b$ is the time after bleach moment when a molecule contributes to the fluorescence recovery. Because binding and unbinding of molecules are stochastic processes, τ and τ_f are dependent random variables. For stationary processes the conditional probability density function $f(\tau_f|\tau)$ is

$$f(\tau_f|\tau) = \begin{cases} \frac{1}{\tau} & \text{for } 0 < \tau_f \leq \tau, \\ 0 & \text{for } \tau_f \leq 0,\, \tau_f > \tau \end{cases} \tag{1}$$

Probability to get the time interval of the length τ covering the bleach event is proportional to the length of this interval and the probability of the molecule to have the residence time τ. The corresponding probability density function (PDF) is

$$f_1(\tau) = \frac{\tau f(\tau)}{\int_0^\infty \tau f(\tau)\, d\tau} = \frac{\tau f(\tau)}{m_\tau} \qquad \tau > 0, \tag{2}$$

where $f(\tau)$ - PDF for the residence time distribution of fluorescently labelled molecules in the bound state; m_τ - mean residence time in the bound state. The PDF for the random variable τ_f is calculated as

$$f_f(\tau_f) = \int_{-\infty}^{\infty} f_1(\tau) f(\tau_f|\tau)\, d\tau = \frac{1}{m_\tau} \int_{\tau_f}^{\infty} f(\tau)\, d\tau = \frac{1 - F(\tau_f)}{m_\tau}, \tag{3}$$

where $F(\tau)$ - the cumulative distribution function (CDF) of the residence times τ.

Finally, the FRAP intensity at the time moment t_f after the bleach could be estimated as the probability that $\tau_f < t_f$, i.e.

$$FRAP(t_f) = \int_0^{t_f} f_f(\tau_f)\, d\tau_f = \frac{1}{m_\tau} \int_0^{t_f} (1 - F(\tau_f))\, d\tau_f \tag{4}$$

3 Results and Discussion

3.1 Binding Interactions

First we show that the presented theory works well for simple binding/unbinding reactions of proteins with immobile binding sites [4]. The PDF for the residence time is defined by the chemical master equation:

$$\frac{df(\tau)}{d\tau} = -k_{off} \cdot f(\tau) \tag{5}$$

where k_{off} - the unbinding reaction rate constant. Solving the Eq. (5) and substituting the result into Eq. (4) provide the PDF, CDF, and the FRAP expression (Fig. 1a):

$$f(\tau) = k_{off} \cdot e^{-k_{off} \cdot \tau}, \qquad F(\tau) = 1 - e^{-k_{off} \cdot \tau}, \qquad FRAP(t_f) = 1 - e^{-k_{off} \cdot t_f} \tag{6}$$

A similar result in a less general contest was obtained earlier [4]. Surprisingly, the random variables τ and τ_f have the same CDFs. It appears because the probability to register unbinding event of a molecule is proportional to the observation period despite of the time moment when it was bound. Next, we show an actin polymerization process as an example where the variables τ and τ_f may have distinct distributions.

3.2 Actin Polymerisation

Actin polymerisation is an important mechanism in many intracellular processes, including cell morphogenesis and disease. Structural dynamical properties of actin in living cells are frequently studied using confocal microscopy and, particularly, FRAP experiments [2]. Basic actin polymerisation reactions include association and dissociation of actin monomers at the ends of linear polymers, so-called filaments. Here we focus on the simplified system, in which actin monomers associate only at the barbed ends and disassociate only at the pointed ends of filaments. A set of parameters determine the actin turnover and the FRAP kinetics [1]: L - the average filament length; v - the polymerisation rate; D - the diffusivity coefficient of actins in filaments. When $(D \approx 0)$, all actins have the same residence times in filaments $\tau = \frac{L}{v}$, e.g.the equations for PDF of residence times distribution and FRAP kinetics are:

$$f(\tau) = \delta(\tau - \frac{L}{v}), \qquad FRAP(t_f) = \begin{cases} \frac{v}{L} t_f & \text{for } t_f < \frac{L}{v}, \\ 1 & \text{for } t_f \geq \frac{L}{v} \end{cases} \tag{7}$$

where $\delta(u)$ - Dirac delta function. The constant residence time results in the linear regime of the fluorescence recovery (Fig. 1b) as it was predicted elsewhere [1]. In this regime one parameter, that is the ratio $\frac{L}{v}$, can be estimated from the FRAP data.

To account for the diversity of the residence times we use the Gaussian distribution as some generalisation of the Dirac delta function. Then the characteristics of the residence time and the FRAP curve are (see an example in Fig. 1c):

$$f(\tau) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{\left(\tau - \frac{L}{v}\right)^2}{2\sigma^2}}, \quad F(\tau) = \Phi\left(\frac{\tau - \frac{L}{v}}{\sigma}\right), \quad FRAP(t_f) = \sigma \frac{v}{L} \int_{\frac{L - v t_f}{v\sigma}}^{\frac{L}{v\sigma}} \Phi(u)\, du, \tag{8}$$

where $\Phi(u)$ - the CDF for the standard normal distribution; σ - the standard deviation of the residence time. Proper accounting for actin diffusivity in filaments with boundary conditions gives the following equation for $F(\tau)$ [1]:

$$F(\tau) = \Phi\left(\frac{v\tau - L}{\sqrt{2D\tau}}\right) + e^{-\frac{vL}{D}} \Phi\left(\frac{-v\tau - L}{\sqrt{2D\tau}}\right) \tag{9}$$

The exponential term in Eq. (9) is close to 0 for typical reaction rate constants and $L > 1\mu m$. Variable τ in the denominator of the first term can be replaced by its

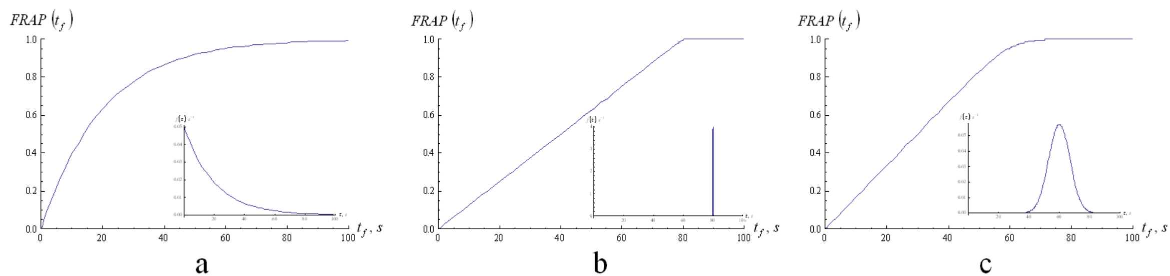

Figure 1: The simulated FRAP curves: a) binding reactions ($k_{off} = 0.05s^{-1}$); b) actin treadmilling along filaments for simplified case $v \neq 0$, $D \approx 0$; c) the same as in (b) for $v \neq 0$, $D \neq 0$. Insets represent corresponding PDFs for residence time distributions.

average value $\frac{L}{v}$ for low the diffusivity coefficients. Result of these simplifications will be similar to the Eq. (8) with the parameter $\sigma = \sqrt{\frac{2DL}{v^3}}$. Therefore two independent parameters can be estimated from the FRAP data in this regime.

4 Conclusions

Overall, here we introduce the novel methodology for deriving mathematical functional equations for analysis of the FRAP experiments. The presented mathematical theory outperforms recently reported models [1, 4] because: i) it does not require accounting for the concentrations of bleached/unbleached molecules; ii) it associates the residence time distribution directly to the FRAP data; iii) our approach can be used for determination of parameters or parameter combinations estimated independently from measured recovery curves. In the future, the model will be extended for studying complex biomolecular reactions and protein diffusion in steady-state and non-steady-state conditions.

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