199f Fluorescence Relaxation in 3d from a Diffraction-Limited Sink of Egfp or Source of Pagfp in Live Cho Cells

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We report the experimental study and mathematical modeling of intracellular protein diffusion. Specifically, the fluorescence relaxation from a brief, small sink of photobleached EGFP or source of photoactivatable GFP (PAGFP) was monitored by rapid confocal "*x-t*" scanning at 488 nm along a line passing through the photoactivation locus. The source was created by multiphoton activation with 100 μs flashes of 820 nm light from a pulsed Ti-Sapphire laser focused to the diffraction limit. The method was applied to purified PAGFP in solutions of varied viscosity, and to PAGFP expressed in spherical (passed) CHO cells. A model of 3D diffusion in an isotropic sphere incorporating the measured shape of the photoactivation pulse and (in the case of CHO cells) the no-flux boundary condition was developed and applied to the data to estimate the diffusion coefficients. In CHO cells $D_{PAGFP} = 20 \pm 3$ $\mu m^2\ s^{-1}$. Experiments with purified PAGFP in solutions of known viscosity (η) yielded the expected linear (Stokes-Einstein) relationship between D and η^{-1} and the estimate $D_{PAGFP,aq} = 87\ \mu m^2\ s^{-1}$ in good agreement with previous determinations.

The mathematical model used to compare computed spatial profiles as a function of time with experimental spatial profiles is based on the scalar, isotropic diffusion equation in spherical coordinates. The photoactivation of the PAGFP produced a two-state (nonactivated and activated protein) system with concentrations $b(r,\theta,\varphi,t)$ and $c(r,\theta,\varphi,t)$, respectively, given by the solution of the PDEs

$$\frac{\partial b}{\partial t} - D\left[\frac{\partial^2 b}{\partial r^2} + \frac{2}{r}\frac{\partial b}{\partial r} + \frac{1}{r^2}\left(\frac{\partial^2 b}{\partial \theta^2} + \frac{\cos\theta}{\sin\theta}\frac{\partial b}{\partial \theta}\right) + \frac{1}{r^2 \sin\theta}\frac{\partial^2 b}{\partial \varphi^2}\right] = -Q_s \tag{1.1}$$

$$\frac{\partial c}{\partial t} = D\left[\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r} + \frac{1}{r^2}\left(\frac{\partial^2 c}{\partial \theta^2} + \frac{\cos\theta}{\sin\theta}\frac{\partial c}{\partial \theta}\right) + \frac{1}{r^2 \sin\theta}\frac{\partial^2 c}{\partial \varphi^2}\right] + Q_s \tag{1.2}$$

where Q_s is a source term for the multiphoton transition point spread function (psf) taken as a Gaussian ellipsoid. Thus,

$$Q_s = a_m \gamma I^m b, \quad 0 \le t \le \Delta T \tag{2a}$$

and

$$I^m(r,\theta) = I_0 e^{-\frac{1}{2}\left[\frac{(r\sin\theta)^2}{\sigma_x^2} + \frac{(r\cos\theta)^2}{\sigma_z^2}\right]} \tag{2b}$$

In (2) a_m is the cross section for a multiphoton transition with m photons, g the quantum efficiency of the process and I_0 the photon flux density at the center of the psf. The solution of eqs. (1) and (2) was computed by the numerical method of lines (MOL). A particular challenge was the regularization of the singularities in the term $\frac{1}{r^2}\left(\frac{\cos\theta}{\sin\theta}\frac{\partial c}{\partial \theta}\right)$ at $r = 0, \theta = 0$. This regularization was accomplished by switching from the spherical coordinates of eqs. (1) to Cartesian coordinates at $r = 0$. Spherical

coordinates were retained every where else in the r,θ domain, and in particular, at the cell boundary ($r = r_0$) where no-flux (impermeable membrane) BCs were imposed

$$\frac{\partial b(r = r_0, \theta, \varphi, t)}{\partial r} = 0 \quad , \quad \frac{\partial c(r = r_0, \theta, \varphi, t)}{\partial r} = 0 \qquad (3.1),(3.2)$$

In addition to the test for spatial convergence by varying the number of radial and angular grid points in the MOL solution, mass conservation of the numerical solution was checked by computing the mass balance integral

$$M_{spherical}(t) = \int_0^{r_0} \int_0^{\pi} \int_0^{2\pi} c(r, \theta, \varphi, t)(dr)(rd\theta)(r \sin\theta d\varphi) \qquad (4.1)$$

which has the initial value

$$M_{spherical}(t = 0) = 2\pi \int_0^{r_{cell}} \int_0^{\pi} e^{-\frac{1}{2}\left[\frac{(r\sin\theta - r_{cent})^2}{\sigma_r^2} + \frac{(r\cos\theta)^2}{\sigma_z^2}\right]} r^2 \sin\theta d\theta dr = (2\pi)^{3/2} \sigma_r^2 \sigma_z \qquad (4.2)$$

The numerical value of the integral in eq. (4.1) was computed by a 2D quadrature; its value agreed with the value given by eq. (4.2) to four figures throughout the entire time, t ,of the numerical solutions. This mass balance calculation was a stringent test for the no flux BCs, eqs. (3).

We will report some of the details of: (1) the experimental facility, (2) the numerical analysis applied to eqs. (1) to (4), and (3) a comparison of the computed and spatial PAGFP profiles, and associated diffusivities.