Estimating Diffusion Parameters, the Effects of Dimensionality

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Abstract.

Measurement of spatial and temporal evolution of intracellular protein transport provides valuable information about the internal architecture and dynamics of cellular systems. Flourescence Recovery After Photobleaching (FRAP) has been used since the 1970s to measure the kinetic properties of macromolecules within cell membranes, and more recently it has been used to describe protein motion and interaction with immobile structures inside a cell nucleus. The mathematical models thus far presented to deal with FRAP have led to widely conflicting results. We look at the cause of this discrepancy and show that certain approximations to the diffusion based reactions lead to wide margins of error.

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1. Introduction

Simple diffusive motion, while a purely physical phenomena, is ubiquitous in molecular biophysics. It is the primary, if not sole, method of kinetic transport for cellular proteins[**?**]. In describing diffusive motion, the kinetic parameter used to measure the rate of movement, is the diffusion coeficient, and reflects the mean squared displacement explored by the proteins through a random walk over time. Since the most simple diffusion equation does not account for interactions that these proteins are subject to, the measurement obtained via experimental techniques has been more appropriately termed the effective diffusion coefficient or apparent diffusion coefficient, which indicates the overall measure of motion, with many other factors 'hidden' within the parameter[**?**]. Nevertheless, the ability to obtain consistent and accurate effective diffusion coefficients from experimental data is essential to the development of more complex models which explicitly account for binding and interactions with a complex intracellular structure.

One of the most useful experimental protocols for the quantitative determination of intracellular kinetic parameters is Flourescence Recovery After Photobleaching (FRAP). FRAP has recently produced an abundance of data on the kinetic motions of intracellular proteins[**?**], however the mathematical models implemented to extract the parameters lead to strikingly conflicting results. For example, green flourescent protein (GFP), which is used to tag proteins of interest in FRAP, has a reported effective diffusion coefficient ranging from 62.08 $\mu m^2 s^{-1}$[10] to 3.2 $\mu m^2 s^{-1}$[2]. This extreme disparity points to flaws in the modeling process which must be resolved before more complex models can be tested and verified.

FRAP has been extensively applied to diffusion of membrane bound proteins, and researchers have directly adopted various modeling schemes based on this for the more recent application of FRAP to intracellular diffusion. Many of these models utilize a dimensional simplficiation of the 3-dimensional motion intracellular proteins undergo, reducing the model to 1- or 2 spatial dimensions[**?**]. While a 2-dimensional model has been shown accurate for membrane proteins, it is only an approximation to the actual kinetics in the case of intracellular proteins. Various corrections to these reduced dimensional models have been successfully applied to yield agreement with the 3-dimensional case, but they must be applied on a case-by-case basis and are highly dependent on the experimental conditions[**?**]. This is further evidence that an improved 'base-line' model is a dire necessity.

Still more compelling, diffusion theory states as a consequence of the Einstein-Smoluchowski relation diffusion of a substance is highly dependent on the Euclidiean dimension, d, of the medium in which it occurs[16]. This points again to the unsuitability of 1- or 2-dimensional approximations to the diffusion equation for estimating FRAP data from intracellular kinetics.

Some of the proposed models were based solely on empirical observations [14], while others have a firm theoretical footing in diffusion theory[10, 1, 11]. Derivations from the simple diffusion equation, $\nabla^2 c(\boldsymbol{r}, t) = \frac{1}{D}\frac{\partial c(\boldsymbol{r},t)}{\partial t}$, are most abundant and successful. Various researchers have solved and implemented diffusion based models to fit FRAP data based on this equation. Absent from the literature however is the presence of a full 3-dimensional model. In this paper we present such a model after showing that 1- and 2- dimensional approximations to 3-dimensional diffusion are unable to account for the motion of intracellular proteins. Data from previous

FRAP experiments involving GFP[10, 14], p53[10], and the several of the RAD proteins[14] is analyzed, and correct effective diffusion coefficients are presented. A fitting protocol for general intracellular FRAP curves, with parameters easily defined by the experimenter, is outlined.

2. Theoretical Considerations

Diffusion theory has been widely studied, and much information on the topic has been well-documented. Intracellular FRAP experiments have been extensively modeled and successfully reproduce the data. However, these various models show little to no agreement with each other, indicating that the predictive power of these models is suspect. The suitability of applying models based on simple diffusive motion to FRAP data has been challenged by numerous other techniques. While it is very apparent that simple diffusion is a vast oversimplification of the intracellular process we are studying, it has been noted that the FRAP protocol only provides enough information to estimate a single parameter uniquely, and that both rate and diffusion coefficients cannot be determined effectively unless one or the other is previously known[17]. For this reason it is absolutely essential we have a valid and complete single-parameter model to describe FRAP. The diffusion coefficient is arguably the most fundamental quantification of a diffusive process.

Dimensionality plays a crucial role in many diffusion-dependant mechanisms in physical systems. Diffusion theory is based primarily on mass-action laws which are mean-field approximations since they evaluate local reaction rates on the basis of average values over a large spatial domain. Numerous diffusion-driven cellular reactions occur in two-dimensional (2d) membranes, and many tools have been presented to study 2d diffusion. One-dimensional diffusion is also an acceptable model for diffusive processes in some cases, such as FRAP experiments in highly elongated bacterial cells []. However, models which seek to represent a 3-dimensional process (intracellular diffusion) with a two dimensional approximation cannot do so directly because diffusion is highly dependant on the dimension.

Consider that in dimension $\gamma > 2$, only a very small portion of the accessible volume is explored by the diffusing particle. On average, the molecule will always escape its initial position[16]. In contrast, for dimension $\gamma < 2$, the molecule primarily remains localized in the vicinity of its initial position[3]. Hence, diffusion is not a perfectly mixing process in low dimension ($\gamma < 2$) because the diffusing molecule will eventually return to its initial position with probability 1, whereas, for $\gamma > 2$, there is a significant probability that the diffusing molecule will never return to its origin[7]. This has important consequences on the mean-squared displacement ($MSD = \langle \boldsymbol{r}(t) - \boldsymbol{r}(0) \rangle^2$) of the molecule, and since the diffusion coefficient can be expressed by the Einstein-Smoluchowski relation, namely, $MSD = 2\gamma Dt$ where γ is a proportionality constant which is dependent on dimension[**?**, ?] The direct proportionality indicates we can expect dimensionality of our models to have a large effect of the D_{eff} extracted from the models. Consider then that the diffusion coefficient may be expressed as:

$$D = \frac{MSD}{\alpha_\gamma t} \tag{1}$$

where $\alpha_\gamma = 2\gamma$ and γ is the number of dimensions. Then in the context of a FRAP experiment, we are indirectly, through the intensity of the fluorescence 'recovered'

in the bleaching region, measuring the MSD of GFP (or GFP tagged) molecules over a finite time interval. Hence the FRAP protocol always measures 3d MSD, so we will be overestimating the diffusion coefficient by a factor of at least 3 when approximating a 3d process by a 1d equation. Note that this direct relation assumes ideal diffusion which is almost certainly not the case, so the quantitative relation may in fact differ slightly from the theoretical values of α_i given here, though the principle itself remains inviolate. Particularly, modifications to the theory have been made to account for anomalous diffusion which will in fact further reduce the value of the diffusion coefficient[].

The effects of dimensionality in diffusion-driven processes have also been explored to some extent in a biophysical context. It has been shown that target-search processes such as specific DNA sequence search by transcription factors in cell nucleus, are dramatically accelerated when diffusion is at least partly two-dimensional (as opposed to a 3-dim. search process) [9]. We can expect then, that in estimating diffusion coefficients from experimental values that reduction of model dimension will yield an (often vast) overestimate of the diffusion coefficient.

3. Data and Methods

As mentioned in the introduction, FRAP has recently seen extensive use in the measurement and determination of kinetic parameters for intracellular diffusive motion. FRAP can be performed using a variety of bleaching geometies, but regardless of the geometry, it has been shown that the same value for D_{eff} should result if the model is correct. Here we focus on previous experiments which have utilized 'strip' FRAP, where the geometry of the bleaching region is depicted in figure 1. To obtain identical values, it is obvious that boundary conditions other than those imposed in equation 2 would be required, however the method of solution is generally the same.

Essential to the photobleaching protocol is the use of GFP as a marker molecule which is tagged to a protein of interest. As such, quantifying the diffusive motion of GFP is a natural first step in building an accurate model. GFP does not exhibit binding interactions in a cellular environment, and hence is known to be 'freely diffusing'[**?**, ?] It should therefore be possible to derive meaningful diffusion coefficients for GFP from FRAP data modeled on simple diffusion. As previously mentioned however, there a large discrepancies among values of the diffusion coefficient presented in the literature. Several of these values from recent publications are shown in table 1 along with the corresponding model used to fit the FRAP data.

Data from previous FRAP experiments [10, 14] was obtained, and fitting of the theoretical recovery curves was done with the Mathematica statistics package using the nonlinear regression function. Series expansions for the recovery functions were computed to 50 terms in all three cases.

4. Modeling

If we denote $c(\boldsymbol{r}, t)$ the concentration of unbleached particles at position $\boldsymbol{r}$ and time t, the differential equation of motion is:

$$\nabla^2 c(\boldsymbol{r}, t) = \frac{1}{D} \frac{\partial c(\boldsymbol{r}, t)}{\partial t} \tag{2}$$

where $\boldsymbol{r}$ is the position vector in 1,2, or 3 dimensions and D is the diffusion coefficient. We will approximate the cell and bleaching region as a rectangular box with experimental parameters defining the monitoring region as in figure 2, which leads to the homogeneous von Neumann boundary conditions:

$$\frac{\partial c(0,t)}{\partial x} = \frac{\partial c(\alpha,t)}{\partial x} = 0 \tag{3}$$

where here α represents the edges of the rectangular region in any dimension. The cellular volume is approximated as a finite rectangular domain of length $x = l$, width $y = w$ and height $z - h$. Initial conditions,

$$c(\boldsymbol{r},0) = \left\{ \theta \ , \mid x - c \mid \leq h_x \qquad\qquad 1, \mid x - c \mid > h_x \right. \tag{4}$$

are applied as in [10], and account for diffusion into and out of the bleach region during bleaching. Separation of variables followed by application of a fourier transform to obtain the coefficients leads to a series solution which can be integrated over the region of interest to obtain a time dependent recovery function. Our solution method for the 3-dimensional case is outlined in the appendix, and both the one and two dimensional cases are easily obtained by the same method. The 3-dimensional recovery function is then:

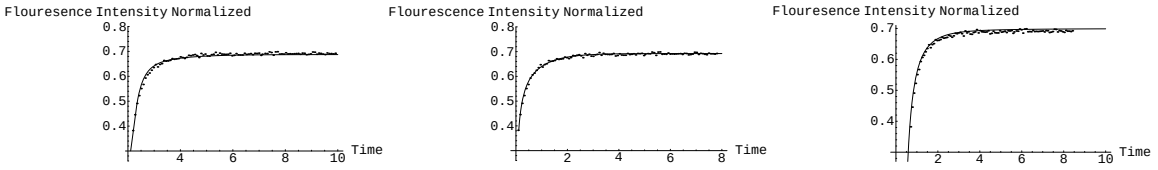
$$F_3(t;D) = \tag{5}$$

5. Results

We solved equation (2) in 1,2, and 3 dimensions, and fit the solution curves to FRAP data sets described above. In all cases we were able to obtain curves which fit the data sets very well (figure 3). While the simple diffusion equation may only be a rough approximation to kinetic motion in an intracellular environment, the derived values of D_{eff} from the simple diffusion model in several dimensions are in excellent agreement with those from previous work. In fact, the considerable range of reported D_{eff} for GFP is covered by consideration of simple diffusion in 1,2, and 3-dimensions indicating the discrepancy between effective diffusion coefficients obtained in the literature is accounted for entirely by the dimension of the imposed model. Clearly then, the dimensionality of the model used to extract D_{eff} is of primary importance, and models which use dimensional simplification are incomplete, and potentially provide misleading information about protein mobility.

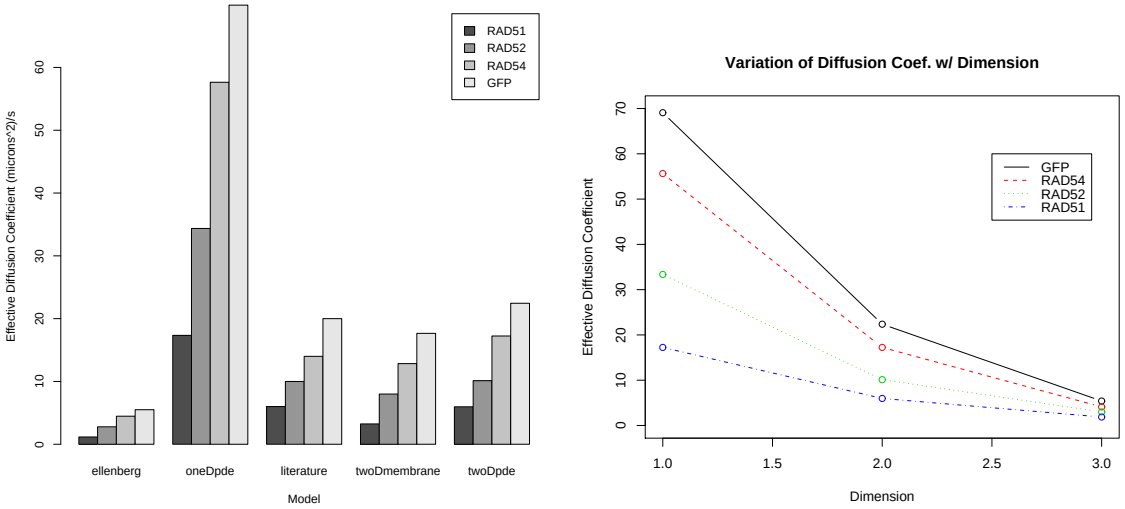
While previous work has led to the correct determination of D_{eff} for proteins such as GFP, the mathematical models used to obtain these values were flawed. The numerical approximations of D_{eff} based on dimensionally simplified models are suspect, but as we argued above, should be off only by a constant scaling factor. Hence we should observe identical qualitiative approximations from models in any dimension. That is, if GFP is found to diffuse faster than, say, RAD51, regardless of the model used we should find this to be the case. Figure 4(a) is a histogram of the various models from which it is apparent that this is in fact the case.

For further verification of our technique, and the effect of dimensionality on D_{eff}, FRAP data for several other intranuclear proteins was fitted using equation 3. RAD51 and p53 both have very low mobility ($D_{eff} =$ and $D_{eff} =$, respectively) while RAD54 and GFP have much higher mobility ($D_{eff} =$ and $D_{eff} =$, respectively). Qualitatively, these findings are in agreement with previous studies on the intranuclear



(a) (b) (c)

Figure 1: Best fit curves to GFP data. (a) is the 1 dimensional diffusion equation with $D_{eff} = 62.08$. (b) is the 2 dimensional diffusion equation with $D_{eff} = 22.7$. (c) is the 3 dimensional equation with $D_{eff} = 4.73$ (note this is preliminary, still have an issue with my solution).



(a) (b)

Figure 2: Variation between models. A histogram of the effective diffusion coefficients for GFP and the RAD proteins is shown in (A). In (B) the dependence on the dimensionality is shown (NOTE, right now this is REDI values for the 3d case.

mobility of these several proteins. Quantitatively, though our model provides an identical fit to the experimental data sets, the estimated values of D_{eff} are significantly lower (figure 4(b)). This is precisely what we expected based on physical diffusion theory and the Einstein-Smolcowski relation.

6. Ending remarks

More on FRAP/diffusion

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