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A class of exact solutions for biomacromolecule diffusion–reaction in live cells

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ABSTRACT

A class of novel explicit analytic solutions for a system of $n+1$ coupled partial differential equations governing biomolecular mass transfer and reaction in living organisms are proposed, evaluated, and analyzed. The solution process uses Laplace and Hankel transforms and results in a recursive convolution of an exponentially scaled Gaussian with modified Bessel functions. The solution is developed for wide range of biomolecular binding kinetics from pure diffusion to multiple binding reactions. The proposed approach provides solutions for both Dirac and Gaussian laser beam (or fluorescence-labeled biomacromolecule) profiles during the course of a Fluorescence Recovery After Photobleaching (FRAP) experiment. We demonstrate that previous models are simplified forms of our theory for special cases. Model analysis indicates that at the early stages of the transport process, biomolecular dynamics is governed by pure diffusion. At large times, the dominant mass transfer process is effective diffusion. Analysis of the sensitivity equations, derived analytically and verified by finite difference differentiation, indicates that experimental biologists should use full space–time profile (instead of the averaged time series) obtained at the early stages of the fluorescence microscopy experiments to extract meaningful physiological information from the protocol. Such a small time frame requires improved bioinstrumentation relative to that in use today. Our mathematical analysis highlights several limitations of the FRAP protocol and provides strategies to improve it. The proposed model can be used to study biomolecular dynamics in molecular biology, targeted drug delivery in normal and cancerous tissues, motor-driven axonal transport in normal and abnormal nervous systems, kinetics of diffusion-controlled reactions between enzyme and substrate, and to validate numerical simulators of biological mass transport processes *in vivo*.

1. Introduction

Systems biology focuses on the systematic study of the interactions between different components of a biological system and how these interactions control the function and behavior of the biosystem (Snoep and Westerhoff, 2005). Some consider systems biology as an integration paradigm (instead of the so-called *reductionist paradigm*) that requires rigorous ways of thinking about integration programs in the analysis of biological systems (Sauer et al., 2007). Others view systems biology as a methodology consisting of theory, mathematical/computational modeling to formulate specific testable hypotheses about biological systems, experimental validation of the model or theory, and then using the newly gathered data to refine the model or theory (Kholodenko et al., 2005).

Biological mass transport phenomena are at the core of the systematic study of biosystems. They have key roles in biological processes that take place in different units of bioenvironmental systems across structural scales from the cellular domain (e.g. cell division, cell motility, axonal growth, etc.) to regional levels (e.g. endemic, epidemic, epizootic, pandemic, etc.). They determine the behavior and function of biosystems and regulate the interactions between drugs and recipient targets (Sadegh Zadeh et al., 2007a). Biotransport phenomena are crucial elements in the design and use of biosensors and are critical in the removal of toxins from the blood (Truskey et al., 2005). They also play critical roles in the remediation of impaired water bodies (sources of water borne diseases) and bioremediation and phytoremediation of contaminated lands. In bioenvironmental systems, transport processes are important to understand, simulate, predict, analyze, and prevent point and non-point source pollution. They regulate the delivery of nutrients and water to plants and control the movement of pesticides, viral, and bacterial agents through the landscape (Sadegh Zadeh, 2006; Sadegh Zadeh et al., 2007b).

One of the goals of system biology is to develop cellular scale process-based mathematical models to study, understand, predict, and control biological mass transfer processes. Despite a growing body of analysis of biotransport processes (Berk et al.,

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1997; Broady, 2000, 2002; Coscoy et al., 2007; Kaufman and Jain, 1990; Lubkin and Wan, 2006; Maly, 2002; Misteli et al., 2000; Phair and Misteli, 2000; Stenoien et al., 2001; Tardy et al., 1995; Tsay and Jacobsen, 1991; Zhang et al., 2008), accurate prediction of biomolecular and cellular dynamics remains a major challenge in real heterogeneous and complex biological systems which exhibit remarkable spatial and temporal variability. The variability stems from local scale heterogeneity of the biological systems, diffusion coefficient, and *localized equilibrium* reaction rate parameters. The implication of this viewpoint is that the mathematics of biomacromolecule transport and binding may be better described using the random process theory. This kind of treatment, which is termed *stochastic modeling*, may lead to a comprehensive insight into the dynamics of biomacromolecules in systems biology perspective. However, this approach is beyond the scope of this paper and will be addressed in future contributions. Instead, we will adapt a deterministic approach with multiple-binding kinetics, to model biomolecular binding reactions, combined with diffusion and circular geometries. Considering multiple binding kinetics is crucial in analyzing cellular and biomolecular dynamics *in vivo*, as the previous studies have assumed that biomolecular affinity to different binding sites inside highly heterogeneous and complex biological systems is similar (the same *on* and *off* rate constants for all different binding sites). A close-to-real world approach would be considering different association and dissociation (*on* and *off*) rate constants for different binding sites inside biosystem under study. The circular geometry is important for analyzing the results of fluorescence microscopy experiments (for instance Fluorescence Recovery After Photobleaching (FRAP)) where a circular laser beam is used by experimental biologists to locally inactivate fluorophores (see Berk et al., 1997; Bretscher and Rafe, 1975; Dunder et al., 2002; Dundr and Misteli, 2002; Ediden et al., 1976; Kalaidzidis, 2008; Lever et al., 2000; Liebman and Entine, 1974; Poo and Cone, 1974; Schlessinger et al., 1976; Tsibidis and Ripoll, 2008; Sadegh Zadeh et al., 2007c, 2007d for a thorough discussion). The FRAP protocol is used to study the mobility and binding of biomacromolecules in different units of organisms. Mathematical modeling of the relevant biotransport processes can guide experimental biologists to identify when to sample the experiments for maximum sensitivity, to evaluate the asymptotic behavior of the biological systems, to analyze targeted drug delivery in normal and cancerous tissues, to understand intramembrane ligand diffusion, to study motor-driven axonal transport in normal and abnormal nervous systems (Sadegh Zadeh and Shah, 2010), to simulate kinetics of diffusion-controlled reactions between enzyme and substrate (Chou, 1976; Chou and Forsen, 1980; Chou and Jiang, 1974; Chou et al., 1980, 1981; Chou and Zhou, 1982; Zhou et al., 1983, 1981; Zhou and Zhong, 1982), and to verify numerical codes in molecular biology.

Assuming a Gaussian laser beam profile and neglecting binding interactions in the FRAP experiment, Axelrod et al. (1976) presented a closed form solution for *pure isotropic diffusion* and *pure advection* of biomolecules in cells. Soumpasis (1983) simplified the solution by assuming a uniform circular laser beam profile but also neglected biomolecular binding reactions. He also suggested that movement of some biomacromolecules may be better described by two diffusing transported entities, a slow and a fast population (mobile-immobile concept) (Soumpasis, 1983). Sprague et al. (2006, 2004) developed semi-analytic solutions for biomolecular diffusion–reaction, but the solutions are in Laplace space and requires numerical inversion to return to real time. Some real space–time solutions for transport with one binding site (or two transport regions) have been developed in other branches of science and engineering (Fusco and Manganaro, 1996; Goldstein, 1951; Lapidus and Amundson, 1952; Lindstrom and Narasimhan, 1973; McNabb, 1985; Uflyand, 1988; Walker, 1987) and may be adaptable to cellular processes. However, they generally consider a linear geometry that differs from the radial nature of the FRAP experiments. The closed form solution presented by Hill (1981) and extended by Lee and Hill (1982) for a system of two reaction–diffusion equations, and the solution of Montas (2003) for a three-equation system are also possibilities for representing FRAP dynamics, except for the geometry of the system. A solution presented recently by Lele et al. (2004), properly respects bleach spot geometry, assumes that the bleach spot is at the center of a circular cell, and neglects possible transport of fluorophores across the cell membrane. However, it has the form of a Fourier–Bessel series that can be demanding to evaluate due to the Gibbs phenomenon (ringing artifacts). This may make the application of this solution to parameter estimation less desirable if bleach spot walls (where Gibbs ringing is expected to concentrate) are sensitive response points. Kang and Kenworthy (2008) present explicit analytic solutions for FRAP where the bound complex can diffuse under uniform circular and/or Gaussian laser profiles. Their solutions, however, use only single-binding kinetics. Using a reaction and diffusion model, Dushek et al. (2008) provide theoretical descriptions of how and when Fluorescence Recovery After Photobleaching (FRAP) experiments can be used to quantify binding reaction rates.

The goal of this paper is to develop and analyze a class of closed form analytic solutions for a system of $n+1$ coupled partial differential equations governing biomacromolecule mass transport and binding in living organisms during the course of a fluorescence microscopy experiment. The plan of the paper is as follow: In Section 2 we present the system of coupled partial differential equations. Section 3 presents the strategy used to obtain exact solutions of the system. Explicit forms of the solutions, for up to three binding reactions, are presented in Section 4, followed by analysis of the behavior of the solution for small and large times in Section 5. Sensitivity equations are developed in Section 6 and used to identify strong control points and to evaluate the effects of averaging on parameter sensitivity. We end the article with a summary and concluding remarks in Section 7.

2. Biomolecular diffusion–reaction equation

The proposed system of partial differential equations governing biomolecular mobility and binding inside living organisms and the corresponding analytical solutions are based on the following assumptions (Sadegh Zadeh, 2006; Sadegh Zadeh et al., 2006): (1) Two-dimensional diffusion takes place in the plane of focus during the FRAP experiment. This is a legitimate assumption when the bleaching area creates a cylindrical path through the cell, which is the case for a circular bleach spot with reasonable spot size. (2) The Peclet number ($P = Lv/D$, where v is velocity and L is a characteristic length) is small such that advection is not a significant mass transfer process, which is the case in nuclear protein transport and for DNA-binding proteins. (3) The effect of heating (caused by the absorption of the laser beam by the sample and fluorophore) on biomacromolecule mobility is negligible (Kaufman and Jain, 1990). (4) The bound complex is relatively immobile. (5) The biological system is in the state of local equilibrium before photobleaching and it remains so over the time course of the FRAP experiment.

Denoting concentration of free biomacromolecule by F, number of binding sites by n, concentration of the i th vacant binding site by S_i, concentration of the i th bound complex by C_i, free biomolecule-vacant binding site association rate constant for site i by K_{ai}, and dissociation rate constant for site i by K_{di}; we propose a multiple-binding state equation to describe