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Biophysical journal

"Multi-scale simulations of diffusion-influenced reactions"

0.1 Abstract

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Biochemical reaction networks connect molecular signaling events with cellular function. It has been suggested that subset of these reactions are strongly influenced by diffusion "barriers" arising from impenetrable cellular structures and macromolecules, as well as interactions between the reaction substrate, its environment and its target enzyme. For these diffusion-influenced reactions, the spatial organization of diffusion barriers, specific and non-specific binding kinetics, and molecular composition may strongly control the temporal and spatial reaction dynamics. To this end we have developed simulation tools to determine how densely-packed diffusion obstacles, like proteins, and cellular structures give rise to compartments within which substrate signals are decoupled, and how this decoupling depends on obstacle distribution and composition. A key advance of this approach is the use of homogenization theory to coarse-grain hindrance of diffusion due to substrate/obstacle interactions.

0.2 Introduction

Coupled, diffusion-influenced reactions are commonplace in biology. An example includes the creatine kinase shuttle, which enables the rapid exchange of nucleotides between the cell cytosol and mitochondrial matrix, where adenosine triphosphate (ATP) is hydrolyzed and synthesized, respectively [1]. This rapid exchange is in part dependent on the fast conductance of creatine through the mitochondrial outer membrane (MOM) by the voltage-dependent anion carrier (in a cation-selective mode) [2], relative to hindered nucleotide transport. The MOM thus acts as a diffusional barrier to nucleotides and anions, giving rise to compartments of distinct nucleotide pools [2]; such compartmentalization can be shown to dampen fluctuations in the nucleotide concentration within the mi-

tochondrium relative to the cytosol. Interestingly, a similar diffusional barrier has been proposed to exist directly adjacent to the cell membrane, which has been hypothesized to ensure a stable nucleotide supply to sarcolemmal ATPases [3], and may be implicated in elevated ion distributions near the phospholipid membrane (REF). Unlike the mitochondrial outer membrane that imposes an obvious diffusion barrier, the composition of the subsarcolemmal diffusional restriction is unknown. Nevertheless, it is known that diffusion is hindered in dense porous media (REF), and is further influenced by interactions between the substrate and surrounding media (REF), thus it is plausible that the dense arrangement of cystolic proteins could give rise to the apparent diffusional restriction. By characterizing a range of diffusion constants based on physiological protein distributions and charges in the cytosol, the extent to which cytosolic crowding accounts for the experimental evidence of compartmentalized reaction dynamics can be assessed.

The notion of diffusion barriers further raises the possibility that the dynamics of biochemical reactions exhibiting oscillatory behavior, such as in glycolysis (REF) and transcriptional regulation (REF), may be in part controlled by the configuration of the cellular environment. Interestingly, recently a circadian clock that arises from the interactions of three phosphorylatable proteins was identified (REF). In that study, the amplitude and frequency of substrate concentration oscillations were exquisitely controlled by relative rates of phosphorylation. Since these proteins must either directly associate or indirectly interact via a shared substrate, it is conceivable that diffusional restrictions could tune the inherent oscillations of that system. As a simple example, a tenfold reduction in a substrate's diffusion rate in the cytosol relative to the bulk (REF) due to crowding would suppress a reaction association rate by the same factor (REF). However, common models for reaction kinetics in cellular systems commonly assume a well-mixed cytosol, which obscures the impact of spatial organization and diffusional barriers on determining the system's inherent oscillations. Investigation of the effects of compartmentalization on oscillatory reactions could therefore delineate the cell's capacity to tune signaling based on

the localization of participating proteins.

Thus, we investigated the hypothesis that a diffusional barrier arising from densely packed, charged proteins is sufficient to support compartmentalized reactant pools with differing reaction dynamics. We illustrated this for a simple equilibrium reaction (A $\leftrightarrow$ B) distributed across a diffusional barrier, subject to a periodic source term acting on A within one compartment. Our data support evidence suggesting that fluctuations in substrate concentrations are strongly controlled by the diffusional environment. We then expanded this system by developing a model of an oscillatory reaction, whose periodicity arises due to negative inhibition by a synthesized substrate. Our results for this system indicate that diffusional restrictions imposed on the inhibitory substrate can tune the frequency of oscillations. These findings implicate the strong coupling of environment, spatial distribution and enzyme kinetics in shaping the dynamics of biochemical reactions. This knowledge could provide important constraints in quantifying energy metabolism, as well as a range of biochemical reactions occurring with the cell cytosol.

0.3 Materials and methods

0.3.1 Homogenization

(Put in JCP details) We consider a lattice of globular proteins (of radius 13 Ang) that are evenly distributed. The spacing between proteins is varied such that the accessible volume fraction of of the lattice varies from 0.5 to 0.95. We further assume a net negative charge on the protein and a series of ligands whose unit charge varies between -1 and 1. Using our homogenization model [4](In review), we predict the normalized effective diffusion constants that represent the diffusional hindrance imposed by the charged lattice of proteins.

0.3.2 Finite element method

(Put in JCP details)

0.3.3 Compartments and Reactions

- We consider three compartments (1, 2 and 3) that are sequentially-linked. By altering the transport coefficients for compartment 1 into 2 and compartment 3 into 2 (and vice versa), we simulate the effect of a diffusional barrier between compartments 1 and 3, such that the substrate pools in compartment 1 exchange with compartment 3 by diffusion through compartment 2.

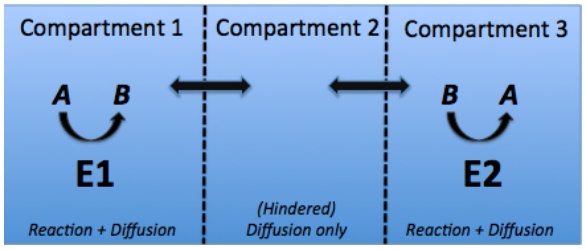

Figure 1: Reaction-diffusion compartments. Reactions are generally restricted to compartments 1 and 3. Diffusion through compartment 2 may be fast (unrestricted) or slow (hindered).

- Two reaction system: We consider the reaction, $A \leftrightarrow B$, catalyzed by an enzyme E2, with the forward rate of A into B is defined as kf and kb is the reverse rate. A second enzyme, E1, generates A in a sinusoidal manner. Enzymes E1 and E2 are distributed in compartment 1, no enzymes are present in compartment 2 or 3.

$$E1 \rightarrow A \in \Omega_1 \tag{1}$$

$$E2 + A \leftrightarrow E2 + B \in \Omega_1 \tag{2}$$

- Three reaction system: The Goodwin oscillator is represented by the reactions

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$$E1 \rightarrow A \in \Omega_1 \tag{3}$$

$$E2 + A \rightarrow E2 + B \in \Omega_1 \tag{4}$$

$$E3 + B \rightarrow E3 + C \in \Omega_3 \tag{5}$$

$$C - X \rightarrow E1 \tag{6}$$

In our example, we restrict enzymes E1 and E2 into compartment 1 and enzyme E3 in compartment 3. As with the previous example, no reactions, only substrate diffusion occurs in compartment 2.

0.3.4 ODE

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0.4 Results

Homogenizaton predicts a range/upper bound of diffusivities for protein In a previous study, we used homogenization theory to predict the effective diffusion constants for diffusion of a charged substrate (-1, 0, or 1) through a densely-packed lattice of charged globular proteins. The results in Fig. 2 indicate that decreasing accessible volume fraction (ϕ) tends to reduce the effective diffusion coefficient from its normalized bulk value of 1.0. Repulsive interactions between substrate and the lattice yield the smallest diffusion constants, while attractive interactions yield the fastest constants. We utilize these effective diffusion constants in the reaction systems illustrated below.

Diffusion barrier can depress fluctuations In the figures below, we report the populations of A and B in each of the three compartments separated by diffusion barriers. In Fig. 3, we consider the first reaction ($A \leftrightarrow B$), but use large diffusion constants that permit rapid diffusion across compartment 2, which is tantamount to the absence of a diffusion barrier. We observe that

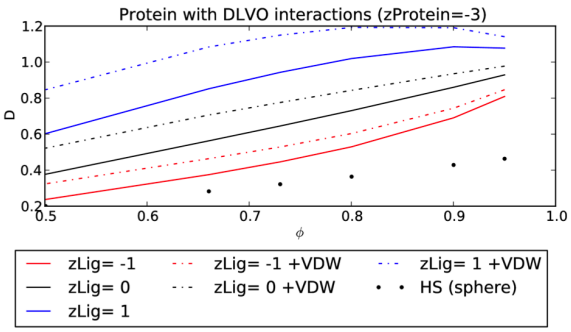

Figure 2: Effective diffusion constant, D, for coions (red), counterions (blue) and neutral diffusers (black) for a unit cell with a 12.5 [Å] negatively-charged, spherical protein. Attractive counte- rion/protein interactions have faster diffusion relative to neutral and negatively charged diffusers. Inclusion of attractive van der Waals interactions (+VDW) through the homogenized Smoluchowski equation with a DLVO poten- tial increases the effective diffusion constants.

A and B approach equilibrium values of approximately 0.25 and 1.0, respectively. Furthermore, the fluctuation amplitudes of A due to the periodic source in compartment 1 are identical in all compartments. In contrast, Fig. 4 we show distinct differences in compartments 1 and 2 when small diffusion constants are used. Namely, the fluctuations in compartment 3 are a small fraction of those observed in compartment 1. In Fig. 5, we quantify the suppression of fluctuations in A in compartment 3 by the diffusional barrier (through varying the diffusion rate of A and B). Namely, we compare the covariance of A in compartments 1 and 3, normalized by the auto covariance of A in compartment 1. Values approaching zero correspond to a complete suppression of fluctuations in compartment 3, while values approaching 1 indicate that the fluctuations are identical in compartments 1 and 3. We observe that the fluctuations are strongly suppressed for diffusion constants less than 1, while suppression is minimized as D approaches a large rate corresponding to the absence of a diffusion barrier.

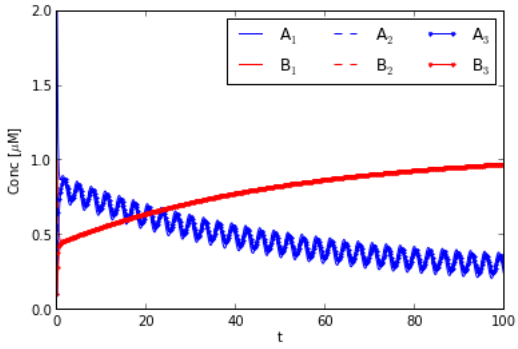

Figure 3: Concentrations of A (blue) and B (red) in compartments 1, 2 and three for the reaction $A \leftrightarrow B$ in the absence of a diffusion barrier.

Diffusion barrier can alter reaction frequency In the figures below, we demonstrate how changes in the diffusion barrier imposed on a negative inhibitor impacts the frequency of a prototypical oscillatory model (Goodwin model). In Fig. 6 we demonstrate that the oscillator yields periodically-varying concentrations of species A (blue) and C (black,C is the inhibitor). In this case, the diffusion barriers are negligible given that a very large diffusion constant for C was used. Fig. 7, we reduce the diffusion constants to 1.0 for species A and B, while we vary DC between 0.1 and 10.0. We observe that frequency of the A and C oscillations is largest when DC is large and decreases for smaller values of DC. Fig. 8 we compare the change in frequency over a range of diffusion constants and find that very small and very large diffusion constants can change the baseline frequency (1.0) by -80% and +40%, respectively.

Spatial considerations In the previous section, we explored effects of compartmentalization through disparate, well-mixed domains. Here we explicitly examine how the coupling between the diffusional barrier height, distance and spatial extent influence compartmentalization and oscillations. We do so by replacing the diffusional compartment from the previous section with an explicit domain within which we solve a spatially-dependent diffusion model described

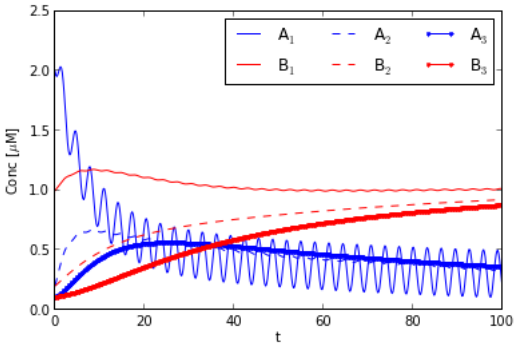

Figure 4: Same as Fig. 3, except in the presence of a diffusion barrier created by imposeing reduced diffusion constants into compartment 2.

by a partial differential equation.

Preliminary:

0.5 Discussion

0.6 Conclusions

A crowded cellular environment represented by a lattice of charged proteins can significantly vary the diffusion rate of substrates. We demonstrate that the dynamics of substrate pools can vary significantly between compartments that are separated by diffusional barriers typical of those suggested by our lattice model. Furthermore, for oscillatory biochemical reactions, the presence of diffusion barriers can substantially shift baseline periods to lower frequencies. These findings provide insight into the ability of the cellular environment to tune biochemical reaction dynamics.

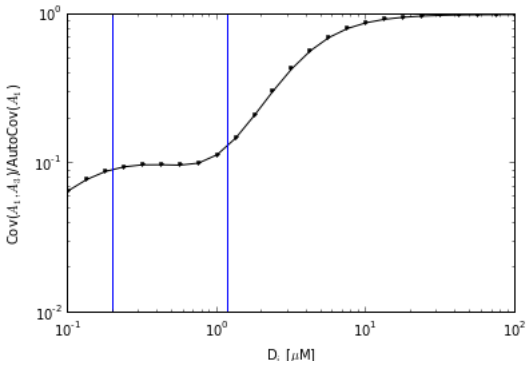

Figure 5: Degree of suppression of A fluctuations in compartment 3 by varying the diffusion barrier based on values from Fig. 2. Values decreasing from 1.0 indicate increasing degree of amplitude supression.

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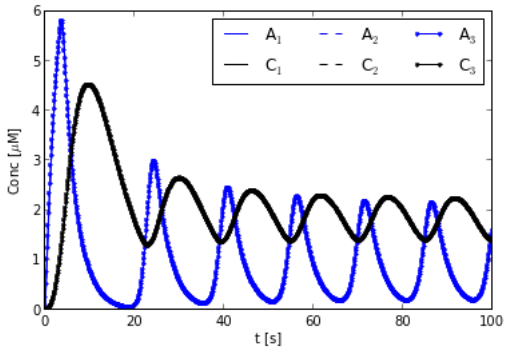

Figure 6: Concentrations of A and C (the inhibitory substrate) for compartments 1-3 in the absence of a diffusion barrier.

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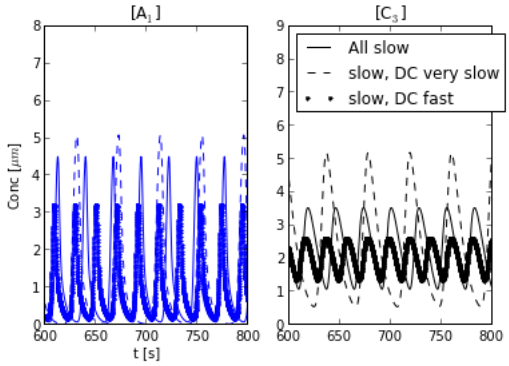

Figure 7: Same as Fig. 6, but using $D_A = D_B = D_C = 1.0$ (All slow), $D_A = D_B = 1.0,\ D_C = 0.1$ (slow, DC slow) and $D_A = D_B = 1.0,\ D_C = 10$ (slow, DC fast).

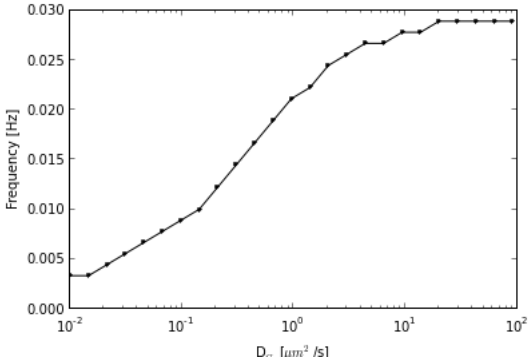

Figure 8: Change C oscillation frequency with respect to $D_C = 1.0$ by varying D_C (mislabeled as Dz).

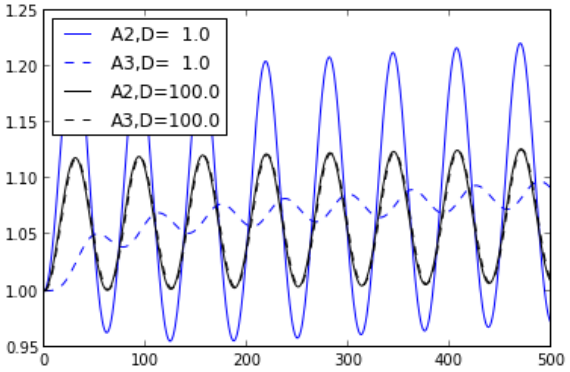

Figure 9: Diffusional barrier PDE. A2 corresponds to the concentration of substrate A in compartment 2 (the species w a periodic source). Compartment 1 (not plotted) is the PDE domain that links compartments 2 and 3. Note that with a large diffusion constant (D=100), that the fluctuations in A are identical in both compartments. Smaller diffusion constants show differences in the concentrations of A.