Metabolic Channeling and Spatial Effects of Bifunctional Enzymes

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***Short Abstract* — Metabolic channeling is expected to be a major advantage for both synthetic and natural multi-enzymes complexes. Yet this mechanism is not yet fully characterized. In this study, we compare the advantages of bifunctional enzymes (BIFE) with monofunctional enzymes (MOFE) arising from spatial effects using both steady-state analysis and simulations. We find and characterize two kinds of spatial effects, and show their dependence on molecule size. Lastly, we discuss the effects of having multiple copies of active sites. This study may contribute to a better understanding of spatial organization of metabolism, as well as of other protein interaction networks.**

***Keywords* — Synthetic biology, spatial organization, diffusion, metabolic channeling, bifunctional enzyme.**

I. Introduction

Spatial organization of metabolism on multiple scales, from microbial consortia to proteins, is drawing increasing attention as an additional dimension to optimize for synthetic biologists [1]. Synthetic multi-enzymes complexes (MECs) hold promise to increase metabolic fluxes and reduce undesirable and hard-to-characterize contextual interactions with host systems [2,3]. MECs are also widely found in diverse natural systems [2]. A major advantage of MECs in metabolism is referred to as metabolic channeling [4,5] wherein the product of one enzyme reaction is channeled to the active site of the subsequent enzyme in the MECs. Yet this mechanism is not very well characterized. In this study, we take two sequential metabolic reactions as our minimal model. We explore the advantages of metabolic channeling by comparing bifunctional enzymes (BIFE) and its corresponding monofunctional enzymes (MOFE) with same enzymatic parameters via both steady-state analyses and simulations. This study may also provide insights on the spatial organization of other protein-protein interaction networks [1].

II. Methods and Results

We develop a compartmentalized model of successive enzyme kinetics that allows for analytical solutions. We assume that a "vicinity volume" surrounds each enzyme, such that the intermediate produced by the first enzyme can only react with the second enzyme inside the vicinity volume. Intermediates are assumed to be well-mixed both inside the vicinity and in the bulk solution. For BIFE, the intermediate is produced inside the vicinity, while for MOFE, it is produced in the bulk solution. Both theoretical analysis and simulations are utilized to explore the advantages of BIFE compared to MOFE. The theoretical analyses are based on an integration of classic Michaelis Menton Kinetics, the diffusion-controlled reaction rate and the first exit time approaches formulated in [6]. Smoldyn is used for simulations [7].

A. The governing dimensionless group α

We find that whether metabolic channeling is an significant advantage or not depends upon a governing dimensionless group, α, which is the ratio between the timescales of the diffusion of the intermediate and its binding reaction to the subsequent enzyme.

B. Spatial effects

As α increases, BIFE leads to a faster turnover of the intermediate and has a smaller lag time for the appearance of the first product molecules. These two spatial effects become increasingly significant with the increase in α.

C. Effects of increases in enzyme size

If the BIFE is larger than the corresponding MOFEs in size, its diffusion coefficient will decrease. We find that the smaller the diffusion coefficient of the BIFE, the larger the two spatial effects.

D. Effects of multiple copies of BIFE

With multiple copies of active sites in the vicinity, the size effects become larger. This also increases the binding reaction rate, making the effective α larger, and thereby leading to stronger spatial effects.

III. Conclusion

Based on analytical calculations and stochastic simulation results, we study the spatial effects that lead to metabolic channeling. Increases in enzyme size, and increasing the copy number of the BIFE in the vicinity volume are found to be able to enhance these two effects.

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