31/12/2022 r.

Abstract

Whole-cell simulations have been identified as the grand challenge of XXIst-century. Since even in the exascale computing era, first-principle atomistic whole-cell simulations are computationally prohibitive, coarse-grained reaction-diffusion models appear as essential and feasible alternatives. In such models, it is crucial to adequately describe the diffusion and reaction properties of all biologically-relevant molecules. However, as it has been known for decades, the cell interior is crowded with macromolecules occupying 20 to 40% of the cell volume. Clearly, diffusion and reactions under such crowding conditions are not the same as in test tubes. To understand these differences, in this Thesis, we explore the generic effects of how crowding affects macromolecular diffusion, chemical equilibria, and reaction kinetics.

In many simulation studies, hard spherical particles are used to model macromolecules composing the crowded intracellular milieu. However, the macromolecules inside living cells have various shapes and softness. We show with Brownian dynamics (BD) simulations that these factors can considerably affect macromolecular diffusion. For instance, we find that elongated crowders slow down diffusion more substantially than spherical ones, while soft crowders slow down diffusion less effectively than hard ones, but not in the case of elongated diffusing particles.

To minimize computational costs, many studies consider hydrodynamic interactions using the (generalized) Rotne-Prager-Yamakawa (RPY, or far-field) approximation, neglecting near-field lubrication effects. Here, we provide an open-source computer program (`pyBrown`) that includes both near-field and far-field hydrodynamic interactions. Using this program, we demonstrate that the popular RPY approximation visibly overestimates the diffusion coefficients of macromolecules in crowded environments. We find that the RPY approximation may provide even less accurate results than simulations without hydrodynamic interactions at all.

A living cell is a non-equilibrium system where many reactions continuously occur. Thus, another critical aspect of cellular modeling is to account for the coupling of diffusion and reactions. With BD simulations, we explore the effect of crowding on a catalysis-induced enhancement of enzyme diffusion, reported recently for dilute systems (*i.e.*, without crowders). We consider a model enzyme that shrinks upon binding a substrate and find that such enzymes exhibit enhanced diffusion also in crowded environments. The enhanced diffusion increases when crowding is due to enzymes but decreases when it is due to passive particles. The catalytically-active enzymes enhance the diffusion of passive crowders as well. One possibility to study such

catalysis-induced diffusion enhancements experimentally is microfluidic H-cell measurement. We perform the rational design of an H-cell experiment and investigate under which conditions such experiments are feasible to study the enhanced enzyme diffusion.

Since the seminal work by Minton, it has been known that crowding affects chemical equilibria and reaction kinetics. However, our understanding of how reactions proceed under crowding is still limited. Herein, we study the kinetics of reactions catalyzed by conformation-changing enzymes and how crowding affects them. Using scaled particle theory (SPT), we derive an expression relating the crowding-dependent reaction rates and geometrical factors describing how an enzyme changes its shape during catalytic turnover. We test these results with BD simulations of a model enzyme consisting of two beads changing their separation upon binding a substrate.

Multivalent binding reactions play an essential role in many life-sustaining and life-threatening processes, from antibody-antigen binding in the immune response to amyloid aggregation. Although such reactions often occur under crowding conditions, the effect of crowding on their equilibria is not fully understood. In this Thesis, we study the cooperativity of divalent binding – an important measure describing how the binding of one site affects the binding affinity of other sites – using SPT and combined Monte Carlo and BD simulations. We show that crowding can enhance or reduce cooperativity depending on how the conformation of a molecule changes upon subsequent binding events. In particular, we find that non-cooperative reactions can become cooperative under crowding and *vice versa*.