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Simulation Study of Binding Chemistry in Crowded Conditions Using Two- and Three-Dimensional Stochastic Off-Lattice Models

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Molecular crowding is one of crucial properties that distinguish the intracellular environment from the well-mixed and diluted *in vitro* environment. Crowding can significantly alter the rates and equilibria of biochemical reactions, potentially either enhancing or inhibiting a reaction system depending on numerous physical parameters of that system. We have developed stochastic off-lattice models for two and three dimensional spaces based on Green's function reaction dynamics to better simulate binding chemistry in crowded conditions and to provide a platform for investigating how the crowding effect is influenced by various model assumptions. We have examined a simple homodimerization system to test dependence on seven system parameters: the total concentration of particles; the binding probability between two reactant monomers upon a collision; the mean time until a dissociation event (the inverse of the rate constant); the diffusion coefficient; the ratio of dimer area to monomer area (volume in 3D); the ratio of inert particle area to reactant monomer area (volume in 3D); and the threshold distance, defined as the maximum interaction range between two particles. Results from a two-dimensional variant show that the first four parameters act essentially independently and their joint influences can be accurately modeled by a simple polynomial regression function. The remaining three parameters show more complicated cross-dependencies. Early studies with a three-dimensional model are showing qualitatively similar behaviors to those of the two-dimensional model. Continuing work is aimed at quantitatively accounting for cross-dependent parameters and extending the full results to three-dimensional models. It is hoped that this work will lead to improved corrections for crowding effects in models too large in time or space scale to permit detailed crowding models and ultimately to more realistic general models of assembly chemistry in the cellular environment.

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Modeling and Mutation of NHERF1 Dimerization Domains

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Na/H Exchange Regulatory Factor-1 (NHERF1) is a cytoplasmic scaffolding protein that regulates parathyroid hormone receptor (PTHR) ligand bias, trafficking, and signaling. Mice lacking NHERF1 present a constellation of mineral ion abnormalities and are osteopenic. NHERF1 consists of 2 tandem PDZ domains and a MERM domain that itself terminates with a PDZ ligand (EB). Recently, 3 NHERF1 mutations were described (N Engl J Med 359: 1128-1135, 2008) in patients who exhibited pathologies similar to the mice. Two mutations (R153Q, E225K) are in PDZ2, and the third, L110V is in the linker region between PDZ1 and PDZ2. In earlier work we showed that the PTHR binds productively to either PDZ1 or PDZ2. Therefore, we hypothesized that the described mutations interfere with NHERF1 dimerization. To probe such interactions we performed computational mutations using thermodynamic integration method with atomistic Molecular Dynamics (MD) simulations to quantitatively assess these predictions. We investigated interactions of EB with PDZ2, R153Q PDZ2 or E225K PDZ2 mutant. For the PDZ2-EB system it was assumed that the C-terminus LFSNL (L) of EB interacts with PDZ2. We confirmed that EB has a higher affinity to R153Q-PDZ2 than to PDZ2 or E225K-PDZ2. In the simulations the complex of EB with the mutated R153Q-PDZ2 is predicted to be more stable than that with the wild type PDZ2 by approximately 2 kcal/mol. Molecular biological methods show that wild-type NHERF1 dimerizes. NHERF1-R153Q abolishes dimerization; NHERF1-E225K reduces dimerization; and NHERF1-L110V dimerizes normally. Dimerization is enhanced in the presence of the PTHR carboxy terminus, which includes the PDZ ligand. Importantly, NHERF1 mutations do not disrupt interaction with the PTH1R. We conclude that NHERF1-R153Q and NHERF1-E225K may undermine PTHR action because of compromised dimerization. NHERF1-L110V interferes with PTHR action through a different mechanism.

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Conformational Plasticity of the Adenylyl Cyclase CyaA from Bordetella Pertussis

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Conformational plasticity of the adenylyl cyclase CyaA from *Bordetella pertussis*

The toxin CyaA from *Bordetella pertussis* is activated by calmodulin (CaM), and catalyses the production of cAMP in an uncontrolled way, that will damage the host immune system. The crystallographic structure of the complex between CyaA and the C terminal domain of calmodulin (C-CaM) was determined recently (Guo et al, 2005), the structures of isolated CyaA and of CyaA/CaM being undetermined up to date. As other adenylyl cyclases (Drum et al, 2002; Laine et al, 2008), CyaA/CaM is a good example of interaction associated to conformational transitions. Besides, CyaA represents a good target for the understanding of the *B. pertussis* action and for the search of whopping cough inhibitors.

The conformational tendencies of the isolated CyaA and of the CyaA bound to C-CaM in the presence and in the absence of Calcium, were monitored along molecular dynamics trajectories. The isolated CyaA shows an important conformational drift, in agreement with independent hydrodynamic measures. Besides, the principal component analysis of the three system dynamics, reveals a permanent kink motion of CyaA around the catalytic site, this motion being only amplified in the absence of Calcium or C-CaM. The energetic influences between complex domains (Laine et al, 2009) concern only the domains Switch A and C-CaM.

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Dynamics of Telomere Clustering in the Nucleus:

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For yet unclear reasons, telomeres form small but dynamical clusters. We study here the clustering of 32 independent telomeres modeled as Brownian particles moving inside the restricted cellular domain.

When the average number of telomere clusters is 5, the dissociation constant of a telomere packet, we find that regardless of its size in 3D is around 20s. By comparing several different models, we suggest that in a cluster, telomeres should all be attached to a unique docking zone. Finally, we study the dynamics of two independent telomeres and show that at equilibrium, when the mean number of clusters is constant, two individual telomeres are highly dynamic and we obtain the law of intermittent switching between clusters.

We conclude that telomere clustering is a highly dynamical process in Yeast {S. cerevisiae} and we suggest similar results for Plasmodium falciparum telomeres.

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Molecular Architecture of the Collagen Based Extra-Cellular Matrix

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The fibrous collagens are the fundamental constituents of the Extracellular Matrix (ECM) of animals, forming the structural basis of all known mammalian connective tissues and organ systems (1). Yet, despite the fundamental biological importance of collagen, many of us are perplexed by the complexity of the assemblies that the collagens form. This is particularly true at what may be the most significant aspect of collagen structure from a cellular point of view, at the intermediate sub-fibrillar and at fibril surface levels (i.e. collagens molecular packing) where many important biological processes occur in growth, development and disease (1, 2). These include but are not limited to: fibrillogenesis, tissue remolding and in forming the scaffolding upon which organ systems, bones, cartilage, etc., i.e. the animal body, are built upon. Clearly, obtaining an unambiguous and contextualized visualization of collagen molecules would be of significant value to the scientific community. We have recently determined the structure of the type I collagen microfibril (3) and fibril (2) at the molecular level from whole intact rat-tail tendons and produced an initial one dimensional structure for type II collagen from lamprey notochord. Using these data, it is possible to map the amino acid chemistry, ligand binding data and other observations onto the defining shape modality of the fibrillar collagen ECM. In so doing, we have been able to propose the first fibrillar based mechanism of collagenolysis and provide a number of illuminating observations regarding other collagen fibril - ligand interactions involving cell adhesion and matrix organization.

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