Previous Research Experience

My previous research experience as a graduate student with Professor Carlos Simmerling in the Chemistry Department at Stony Brook University focused on using molecular dynamics to understand the mechanism of DNA repair. Fpg is a bacterial glycosylase which recognizes and excises the 8-oxoguanine (8OG) lesion from DNA, an especially difficult task since 8OG, a product of oxidative stress on the genome, is only a two atom difference from a guanine base. To understand the mechanism by which 8OG is recognized and subsequently flipped out of the duplex into Fpg's active site, I developed a novel implementation of the nudged elastic band (NEB) method in the AMBER Molecular Dynamics program called partial NEB (PNEB). Unlike the original NEB method, in PNEB the path can be calculated for a subset of the system's coordinates, allowing use of explicit solvent (which is extremely important for simulations of nucleic acids) as well as targeted conformational changes.[1] Using PNEB I generated the minimum energy path between the recognition complex and the pre-excision Fpg-DNA complex with 8OG bound in the active site (Figure 1). The PNEB minimum energy path helped select reaction coordinates for calculating the potential of mean force, equivalent to the free energy of the transition. We showed that processivity of 8OG through the DNA major groove was more energetically favorable than through the minor groove, identifying key components of this base excision repair mechanism, whose roles have since been confirmed by experiment using point mutations.[2,3] I went on to probe the dynamics of recognition by mutating

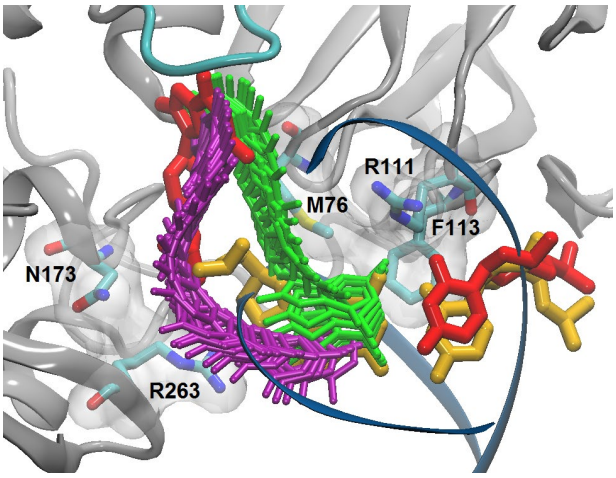

Figure 1. Base eversion paths from PNEB calculations (Ref 2). Major groove path shown in purple and minor groove path in green. Intrahelical 8OG:C base pair is shown in yellow, extrahelical 8OG:C base pair in red, and catalytic loop in cyan.

a key phenylalanine wedge residue in Fpg, a common feature in other base excision repair proteins.[4] Together with experimental collaborators Prof. Grollman, Prof. de los Santos, and Dr. Zharkov, we found that insertion of the wedge actively destabilizes the DNA helix.[5] This represents significant proof the wedge residue plays an important role in lesion recognition, providing a hypothesis for a common mechanism between other DNA glycosylases containing an aromatic wedge.

Current Research Experience

As a post-doctoral researcher with Professor Thomas Cheatham in the Department of Medicinal Chemistry at the University of Utah I have delved further into the world of nucleic acid structure and dynamics by researching RNA conformational dynamics. The high charge and high conformational variability in RNA makes it particularly difficult to parameterize for nucleic acid force fields. However, when assessing molecular mechanics force fields it is often difficult to divorce problems resulting from bad parameters from those resulting from limited sampling. To overcome the limited sampling problem, I have developed a biasing potential for Hamiltonian replica exchange MD simulations and coupled it with temperature replica exchange in a Multi-dimensional replica exchange (M-REMD) protocol which more efficiently searches conformational space.[6] Initial M-REMD simulations were performed on tetranucleotides, simple systems which show high conformational variability, and for which we have NMR data. Further, I used M-REMD simulations to converge the ensembles of tetraloops, a significantly more complex motif. In doing so, I have made extensive use of XSEDE and NCSA Blue Waters as computational resources. Simulation results help us identify problems [7] (such as the imbalance between solute-solute and solute-solvent interactions) and potential areas of improvement.[8]

Additionally, I have worked on understanding the dynamics of a Mg^{2+} dependent

stem-loop in the Varkud Satellite Ribozyme. Stem-loop V adopts significantly different structures in the presence and absence of Mg^{2+}. By using a combination of fluorescence experiments by our collaborator Professor Hall and MD simulations, we have shown that the role of the Mg^{2+} ions is to limit the conformational ensemble of the loop, allowing the bases of the loop to form Watson-Crick interactions with residues of its cognate stem-loop I, orienting the substrate in the active site of the ribozyme (Figure 2).[9] In doing so, we have defined the characteristics of a potential subset of ion dependent RNA hairpin loop structures. Further, we have shown that the Mg^{2+} ion dependent conformational shift can be captured with current ion parameters, validating the use of common 12-6 Lennard Jones potentials and assessing the limitations of newer 12-6-4 Lennard Jones potential modifications.[10]

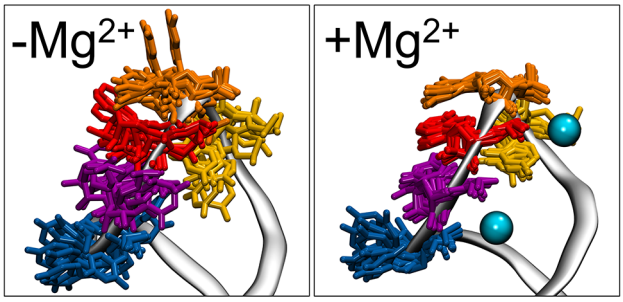

Figure 2. Mg^{2+} ions limit the conformational ensemble of Varkud Satellite Ribozyme Stem Loop V (Ref 9).

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