Post-studentship report

During my placement in Professor Sam Hay's group, I undertook a computational biology project. The primary aim was to give me an informative and guided introduction to the use of empirical and quantum simulations within biochemical systems. My assignment focused on studying Toll/interleukin-1 receptor (TIR) domains, which are intracellular signalling domains found in receptor proteins, like human SARM1 [1]. TIR domains cleave NAD+ which is a cofactor in many metabolic pathways within the cell. Adenosine diphosphate ribose (ADPR) is a product of this reaction, which is a part of second messenger systems[2].

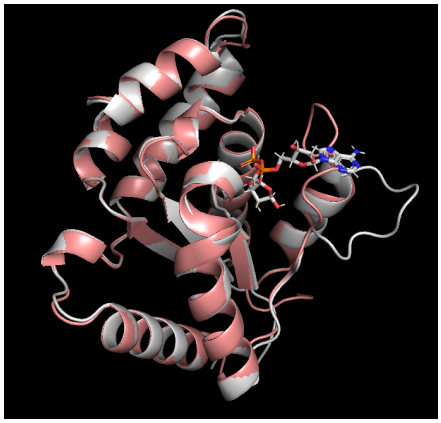

Figure 1: PDB: 7XOZ (white) aligned with PDB: 5TEB (pink).

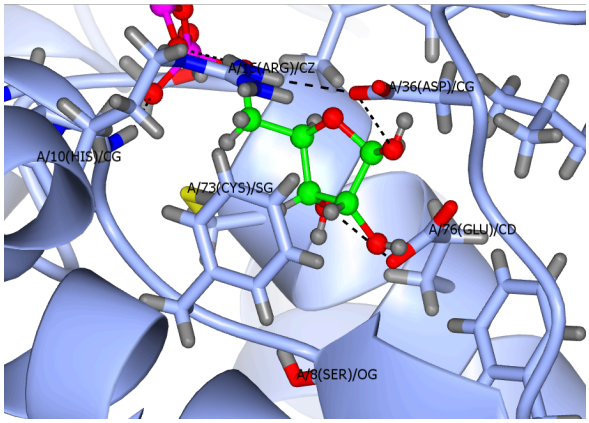

Figure 2: Active site of TIR domain with ADPR bound. PDB: 7XOZ.

The goal of my project was to construct a computational study of this reaction, with the theoretical goal being a full mechanistic study of NAD+ cleavage within a TIR monomer. An additional aspirational goal was to compare head-to-tail and head-to-head dimers of TIR domains and observe how these arrangements affect the mechanism. While parts of the mechanism, such as it being SN1 rather than SN2, had been theorised, a complete mechanism was not yet found in the literature. This project therefore delved into a novel area of biochemistry. A more thorough mechanistic knowledge of these receptors will impact an extensive area of biology, since TIR receptors are found in archaea, bacteria, animals and plants. In addition to this, it will have a great impact on the enzymology community, due to their role in catalysis of NAD+ cleavage.

Initially, relevant crystal structures (PDB IDs: 5TEB, 7XOZ) were selected for comparison. These are identical TIR domains except 7XOZ has ADPR bound in the active site while 5TEB does not. Using PyMol, I simplified and aligned these structures, which revealed a conformational change (the active site "opens") upon binding (Figure 1). The rest of the structure remained mostly unaffected. Upon closer inspection of the bound structure, the product ADPR appears to have the same stereochemistry as NAD, validating the proposed idea of the cleavage being an SN1 type mechanism.

The next step was to identify the key residues present in the active site of the TIR domain. Initially, I simply selected residues near the ADPR. Then, to reduce it further, comparison to the lysozyme mechanism revealed which residues may be catalytic: the Asp36 (PDB: 7XOZ) (Figure 2) and the Glu76 appeared especially vital. The serine and cysteine were too far from the reaction to have much impact, thus ruling out the classic catalytic triad hydrolase mechanism. Selecting these residues is necessary for docking as well as creating the cluster model.

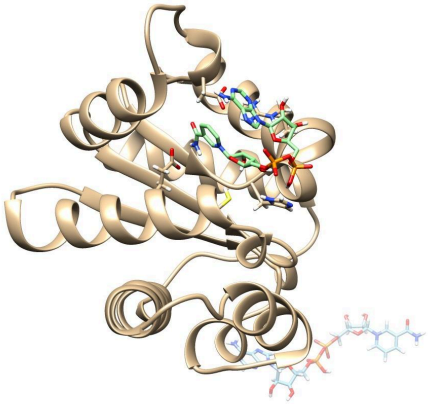

Figure 3: NAD docked into TIR active site (PDB: 7XOZ) with substrate removed.

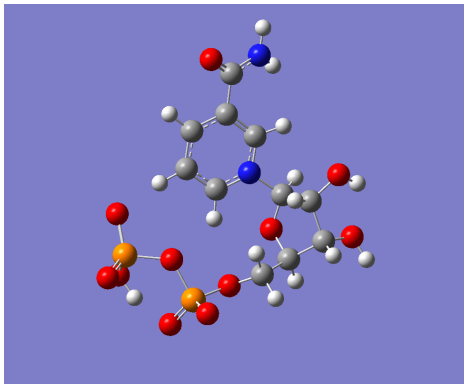

Figure 4: NAD after geometry optimisation.

Next, I edited the 7XOZ file to remove the substrate and docked NAD into the active site (Figure 3). This was aligned to the crystal structure to confirm that any differences were minimal. This docking provided a solid preliminary structure for future geometry optimization.

To familiarise myself with DFT (density functional theory) calculations, I began by creating and geometry optimising a solvated NAD model (Figure 4). Once this successfully ran, I made a cluster model of ADP bound to the active site, whittling it down from ~500 atoms to ~300. For all future calculations, the active site was reduced to a hydrophobic box (with key structural, mainly aromatic, residues) as well as the catalytic residues. Initially I just used empirical methods (PM6), before transitioning to higher levels of theory (B3LYP/6-31G(d.p)). I also created and submitted a second cluster model with docked NAD for optimization.

While these extensive jobs were running, I performed some bioinformatics analyses to compare the residues found in the active sites of TIR domains across different proteins. This allowed me to identify potential variations in active site composition and gain insight into the catalytic mechanisms these active sites might be involved in. In general, I was looking for evidence to back up our theory of it undergoing a lysozyme type mechanism[3]. The glutamic acid (Figure 2) appears to be vital for NAD cleavage, and mutation of such abolishes NAD cleavage activities[4].

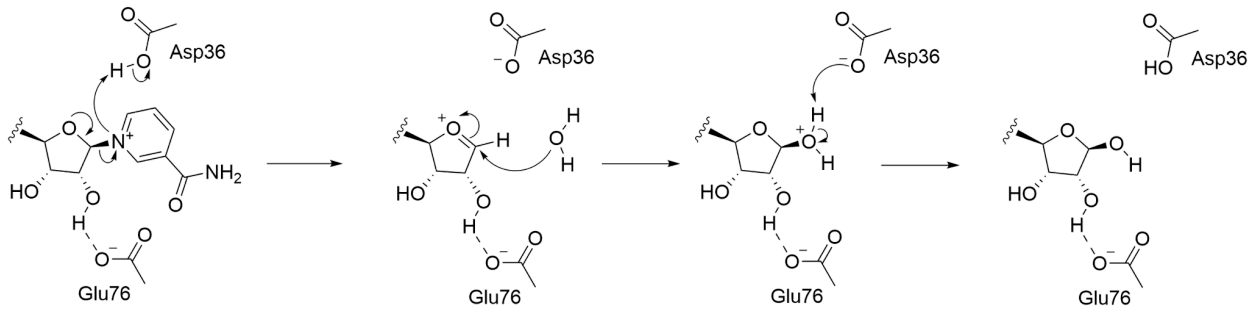

Figure 5: Proposed mechanism for the cleavage of NAD.

In order to begin simulating the mechanism, the system was protonated to observe the leaving of the water on the ribose ring. This is essentially forcing the mechanism backwards (the forward reaction being the nicotinamide leaving, and water adding on). By approaching the mechanism from both the forward and reverse direction the aim was to facilitate the identification of the key transition state. This resulted in the Asp36 abstracting a hydrogen (rather than the water leaving), this therefore suggested the aspartate protonated the hydroxyl group.

Various methods were next used to probe the mechanism: increasing the bond length of the C-OH on the ribose ring, removing the water and letting it optimise somewhere in the pocket, and finally creating a potential energy surface of the transition state, extending the ribose-nicotinamide bond in NAD. The latter yielded the most productive results; lengthening the C-N bond in small increments let the program draw a PES. This was not completed during my internship, but will be picked up by future students.

Though my project remains incomplete, we are now in a position to probe a mechanism (Figure 5), which will be built upon by future students and researchers. I gained many skills: using software for docking like chimera and protein visualisation using PyMol and ChemCraft, and learning how to use the Computational Shared Facility (CSF) for running large jobs on Gaussian. I would like to say thank you to Professor Sam Hay for his invaluable support, guidance, and patience throughout this project. I am also very grateful for the researchers at the MIB for their friendliness and helpfulness, which made this work possible.

References

1 D. W. Summers, D. A. Gibson, A. DiAntonio and J. Milbrandt, Proc. Natl. Acad. Sci. U.S.A., , DOI:10.1073/pnas.1601506113.

2 R. Fliegert, A. Gasser and A. H. Guse, Biochem Soc Trans, 2007, **35**, 109–114.

3 A. J. Kirby, Nat Struct Mol Biol, 2001, **8**, 737–739.

4 M. Chang, M. Clinton, F. Liu and Z. Q. Fu, Trends in Plant Science, 2019, **24**, 1069–1072.