Developing a Structural Model of the Msh2-Msh6-Holliday Junction Interaction

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Introduction

Previous studies show that Msh2-Msh6 both stabilizes the open conformation of Holliday Junction (HJ) DNA and binds HJ with high affinity similar to mismatched DNA substrates (Marsischky et al. 1999). More recently, former members in our lab have used fluorescent intensity and anisotropy experiments to characterize the binding affinity and stoichiometry of *S. cerevisiae* Msh2-Msh6 with the J3 junction, a non-migrating junction, but the exact structural details of this interaction between Msh2-Msh6 and HJ DNA has yet to be elucidated.

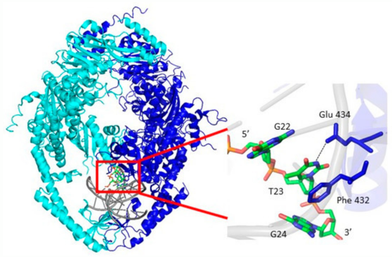


Figure 1. Schematic of the Phe-X-Glu motif of human Msh6's mismatch binding domain interacting with the DNA's G:T mismatch (Li et al. 2019).

Crystal Structure of Human Msh2-Msh6

Human Msh2-Msh6 was co-crystallized with 15bp duplex DNA containing a G:T mismatch. For our preliminary studies, we use this structure to model interactions between the DNA binding domain and the Holliday Junction.

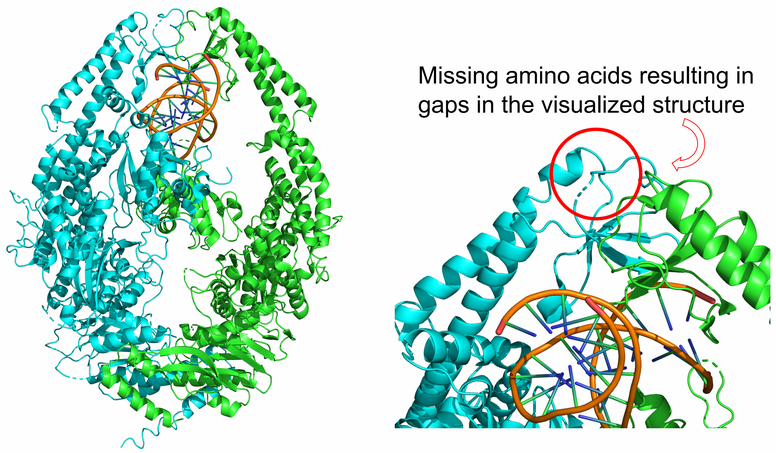


Figure 2. This figure shows Msh2-Msh6 at 2.8Å resolution, co-crystallized with 15bp duplex DNA containing a G:T mismatch (PDB file 2O8B). Msh2 is green, and Msh6 is teal. The crystal structure of human Msh-Msh6 contains the full length of Msh2. 23 amino acid residues are missing from the N terminal and 24 amino acids are missing from the C terminal of Msh6. Both chains contain missing amino acid residues that were modelled in using the program Phyre2. The right image shows a zoomed in view of a loop near the DNA binding domain that contains missing residues.

Structural Alignments in PyMol

The alignment results indicate strong structural similarity between the starting crystal structure of human Msh2-Msh6 and the complete structure of Msh2-Msh6 generated by Phyre2. Missing amino acid residues modelled in appear to create slight deviations in structure among local secondary structures and loops.

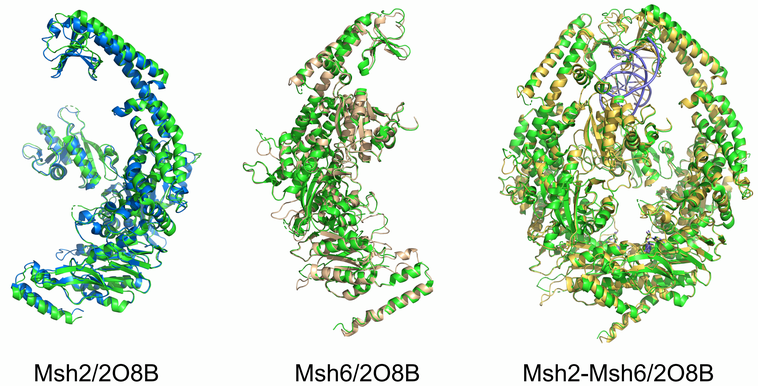


Figure 3. Structural alignment of human Msh2-Msh6's crystal structure (shown in green) with predicted Msh2 structure (blue), predicted Msh6 structure (gold), and predicted Msh2-Msh6 structure containing mismatched DNA, Mg^{2+}, and ADP (yellow). Structural visualization and alignments were performed in PyMol.

Root Mean Square Deviations from 2O8B

The RMSD values among C-alpha atoms were calculated following each of the structural alignments. After rejecting outliers caused by adding in missing amino acid residues, the low RMSD values (<1Å) indicate high similarity between the newly generated structure and the crystal structure. All outliers reported are consistent with the missing amino acids modeled in. Alignment of 2O8B with MutS was included to provide an example of a protein that, despite being a homolog, demonstrates significant distance from the original crystal structure and would not be an ideal model system or starting point.

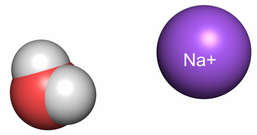
Root Mean Square Deviations of Structural Alignments (Å)

	MutS	2O8B	Msh2_Ph	Msh6_Ph	Msh2-Mshuyl.6
2O8B	3.652 (13.250)	0.000 (0.000)	**0.396** (1.766)	**0.567** (2.683)	**0.428** (2.208)

Table 1. RMSD values were calculated based on structural alignments in PyMol. 2O8B is the human Msh2-Msh6 crystal structure, MutS is E. Coli MutS protein (a prokaryotic homolog of Msh2-Msh6), Msh2_Ph represents the Phyre2 predicted structure of Msh2, Msh6_Ph is the Phyre2 predicted structure of Msh6, and Msh2-Msh6 is the complete Phyre2 predicted structure super-positioned to include DNA, Mg^{2+}, and ADP from the original crystal structure. RMSD values calculated prior to rejecting outliers are reported in parentheses.

Setting Up Msh2-Msh6 for MD

The AMBER16 suite of programs, PyMol, and VMD were used to check and set up the system to be used in MD simulations. The ff14SB force field was used and special features, ADP and Mg^{2+}, were parametrized. The protein was solvated in a 12Å octahedron box with TIP3P water molecules. Electroneutrality was achieved through adding Na^{+} counterions.



Yeast and Human Msh2-Msh6 Alignments

Sequence alignments performed reveal 41% identity between human and yeast Msh2 sequences and 33% identity between human and yeast Msh6 sequences. Interestingly, the ATPase domain was the most highly identical, whereas the DNA binding regions of both chains (shown below) demonstrate some variability in the amino acid sequences. This may be because only specific interactive regions of this domain need their sequences to be identical (such as the Phe-X-Glu motif), whereas other sections of this domain only need to maintain their structure.

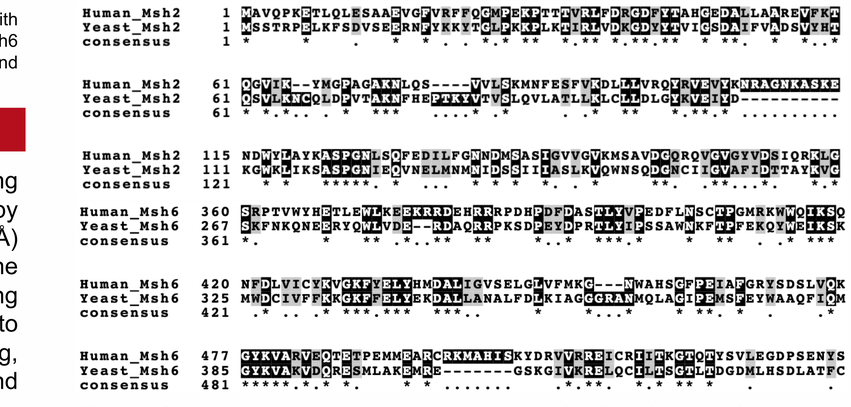


Figure 4. Sequence alignments between human and yeast Msh2-Msh6 were performed using ClustalW and BoxShade. The alignment section chosen for this figure are based on the DNA binding domains of human Msh2 and human Msh6.

Future Directions

1. Following energy minimization, heating, and equilibration of the above system, we will perform MD simulations of the protein-mismatch interaction and, in the future, dock Holliday Junction DNA onto our model of Msh2-Msh6.

2. We aim to develop a model of *S. cerevisiae* Msh2-Msh6 for MD simulations to perform MD simulations in conjunction with *in vitro* experiments to study yeast Msh2-Msh6's interaction with HJ DNA.

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Acknowledgements

I'd like to thank Ishita Mukerji, Kelly Thayer, Abhilash Jayaraj, Anastasia Saar, and Mark Han for their constant encouragement and guidance. Thank you to Henk Meij for technical assistance with Wesleyan's high-performance computing cluster, and to all the members of Thayer and Mukerji Lab for their support. I am grateful for the Department of Molecular Biology & Biochemistry and the College of Integrative Sciences for providing the funds that made this research possible.

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