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Supporting Material

Conformational change in MSH2-MSH6 upon binding DNA coupled to ATPase activity

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Supplementary material for

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Detailed simulation methodology

We have carried out two MD simulations on human MSH2-MSH6 starting from a complete model for residues 1-855 (MSH2) and 362-1335 (MSH6), respectively, based on the crystal structure 2O8B[1]. In order to complete residues that are missing in the crystal structure, the fragments involving residues 550-552, 651-653, 718-728, 933-935, 991-993, 1098-1105, 1122-1126, 1178-1188, and 1270-1284 in MSH6 as well as fragments involving residues 108-112, 139-145, 243-247, 316-321, and 713-723 in MSH2 were built with the loop modeling function[2] in MODELLER[3]. The complete MSH2 and MSH6 sequences contain 934 and 1360 amino acids, respectively, but except for an NMR structures of the PWWP domain in the N-terminal part of MSH6 (residues 68-201)[4], there is not sufficient structural information to construct a complete model for the missing N- and C-terminal parts. However, the MSH6 sequence with known structure (residues 362-1335) extends beyond the sequence of the aligned MutS crystal structures[5] while MutS and MSH2-MSH6 are presumed to share a conserved functional mechanism. *In vitro* studies have shown that a human MSH6Δ341 mutant retains mismatch repair activity comparable to the full-length protein[1]. According to secondary structure prediction servers, residues 341-361 are disordered and although they are missing from the crystal structure and our model, we assume that they are not essential for function. The missing residues at the MSH2 and MSH6 C-termini align with part of the 50 C-terminal residues in MutS which are also missing in the MutS crystal structure. This domain appears to be important for tetramer formation but the truncation mutant MutSΔC800 in *E. coli* was also found to retain ATPase activity and mismatch recognition functions similar to wild type[6]. Therefore, the truncated MSH2-MSH6 complex used here is believed to contain all of the essential components for DNA binding, mismatch recognition, and initiation of repair. One simulation has the protein bound to a mismatch DNA and the other is in absence of the DNA. Bound ADP molecules were removed from the two nucleotide binding sites of chain MSH2 and MSH6 in both simulations.

The CHARMM-27/CMAP force-field was used to described molecular interactions [7, 8] and simulations were run with NAMD2.6 [9]. MSH2-MSH6 in both the presence and absence of DNA was solvated in a rectangular TIP3P solvent box with Na^+ counter-ions to neutralize the system. The dimensions of the final solvated systems were 156 x 131 x 93 $Å^3$ and 159 x 131 x 93 $Å^3$ with 190,270 and 195,878 atoms for the DNA bound and DNA-free systems, respectively. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) technique. A non-bonded cut-off of 10 Å was applied to the Lennard-Jones potential and the direct sum of the Ewald calculation. A 2 fs time step was used for all MD simulations.

The systems were initially minimized and equilibrated using CHARMM under harmonic restraints of 10 kcal/mol/$Å^2$ on the heavy atoms of the protein and the DNA allowing only the water molecules to reorient themselves during 10,000 steps of adopted-basis Newton-Raphson minimization. The minimized structures were then heated in three steps with short simulations over 10,000 steps at 100K, 250K and 300K while still maintaining the restraints on the protein and the DNA heavy atoms. The simulation at 300K was then continued while slowly releasing the restraints from 10 kcal/mol/$Å^2$ to 0 over ten simulations, each for 10ps. The final structure was then continued with NAMD for 170ns and 140ns for the protein in the absence and presence of the DNA, respectively. All simulations were carried out in the NPT ensemble.

1. Warren, J.J., T.J. Pohlhaus, A. Changela, R.R. Iyer, P. Modrich, and L.S. Beese, *Structure of the human MutSa-DNA lesion recognition complex.* Mol. Cell, 2007. **26**: 579-592.
2. Fiser, A., M. Feig, C.L. Brooks, III, and A. Sali, *Evolution and Physics in Comparative Protein Structure Modeling.* Accounts Chem. Res., 2002. **35**: 413-421.
3. Sali, A. and T.L. Blundell, *Comparative protein modeling by satisfaction of spatial restraints.* J. Mol. Biol., 1993. **234**: 779-815.
4. Laguri, C., I. Duband-Goulet, N. Friedrich, M. Axt, P. Belin, I. Callebaut, B. Gilquin, S. Zinn-Justin, and J. Couprie, *Human mismatch repair protein MSH6 contains a PWWP domain that targets double stranded DNA.* Biochemistry, 2008. **47**(23): 6199-6207.
5. Sixma, T.K., *DNA mismatch repair: MutS structures bound to mismatches.* Curr. Opin. Struct. Biol., 2001. **11**: 47-52.

6. Lamers, M.H., A. Perrakis, J.H. Enzlin, H.H.K. Winterwerp, N. de Wind, and T.K. Sixma, *The crystal structure of DNA mismatch repair protein MutS binding to a G.T mismatch.* Nature, 2000. **407**: 711-717.
7. MacKerell, A.D., D. Bashford, M. Bellott, R.L. Dunbrack, J.D. Evanseck, M.J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F.T.K. Lau, C. Mattos, S. Michnick, T. Ngo, D.T. Nguyen, B. Prodhom, W.E. Reiher, B. Roux, M. Schlenkrich, J.C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiorkiewicz-Kuczera, D. Yin, and M. Karplus, *All-atom empirical potential for molecular modeling and dynamics studies of proteins.* Journal of Physical Chemistry B, 1998. **102**(18): 3586-3616.
8. Mackerell, A.D., M. Feig, and C.L. Brooks, *Extending the treatment of backbone energetics in protein force fields: Limitations of gas-phase quantum mechanics in reproducing protein conformational distributions in molecular dynamics simulations.* Journal of Computational Chemistry, 2004. **25**(11): 1400-1415.
9. Phillips, J.C., R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R.D. Skeel, L. Kale, and K. Schulten, *Scalable molecular dynamics with NAMD.* J. Comput. Chem., 2005. **26**: 1781-1802.