Supplementary Information

The model for cadmium binding to multiple, independent binding sites of equal affinity on Msh2-Msh6, following which the protein undergoes conformational change that alters it intrinsic fluorescence and ATPase activity, as well as *gfit*, the program used for global analysis of the data, can be downloaded from http://gfit.sourceforge.net.

Supplementary Methods

ATP and ATPγS binding to Msh2-Msh6 was measured by nitrocellulose membrane binding assays (Antony and Hingorani, 2003). Membranes were treated with 0.5 N NaOH for 2 minutes, washed with water and equilibrated in Buffer B (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 5 % glycerol). In 15 μl reactions Msh2-Msh6 (1 μM) was incubated for 10 minutes in Buffer B with α^{32}P-ATP (0 – 150 μM) at 4 °C or with ^{35}S-ATPγS (0 – 200 μM) at 25 °C, in the presence of $CdCl_2$ (0 – 300 μM), then 10 μl of each reaction was filtered through a membrane, followed by 150 μl buffer. Bound nucleotide on the membranes was quantified by PhosphorImager and plotted *versus* total nucleotide, and the data were fit to a quadratic equation.

Increase in turbidity (630 nm) in the presence of $CdCl_2$ was measured over time in a stopped-flow instrument by mixing 60 μl of Msh2-Msh6 (2 μM) or MutS (2 μM) with 60 μl of 400 μM – 4 mM $CdCl_2$ in Buffer A (final concentrations: 1 μM Msh2-Msh6 or MutS, 200 μM – 2 mM $CdCl_2$).

Supplementary Figure Legends

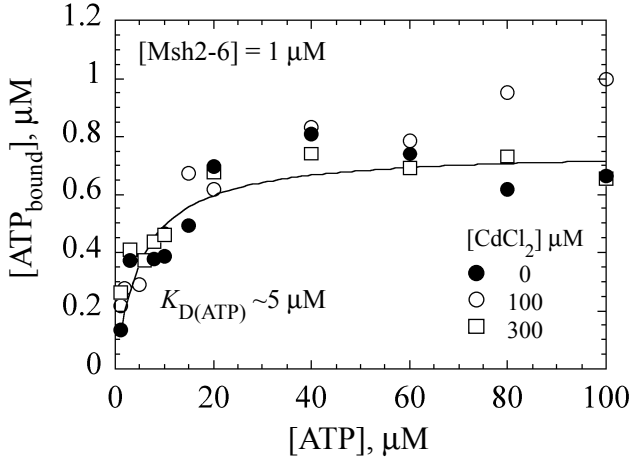
FIGURE S1: Nucleotide binding to Msh2-Msh6. (A) Nitrocellulose membrane filtration assays show 0.8 – 1 μM α^{32}P-ATP binding 1 μM Msh2-Msh6 with apparent K_D of 3 – 8 μM at 0 (●) 100 (○) and 300 μM (□) $CdCl_2$, suggesting that ATP binding is not as susceptible to cadmium-mediated inhibition; a maximum stoichiometry of 1 ATP per Msh2-Msh6 with $K_D \sim 7$ μM, has been reported previously using this assay (Antony and Hingorani, 2003). (B) ^{35}S-ATPγS binding saturates at ~2 molecules per Msh2-Msh6 (1 μM) in the absence of $CdCl_2$ (red), consistent with previous reports (Antony and Hingorani, 2003). In the presence of 200 μM $CdCl_2$, however, ATPγS binding does not saturate even at >12 molecules per Msh2-Msh6 (green), which suggests excess ATPγS binding to the multiple Cd^{2+} ions coordinated by ligands on Msh2-Msh6.

FIGURE S2: Mixing of Msh2-Msh6 with $CdCl_2$ eventually leads to increase in the turbidity of the solution (630 nm), indicating protein aggregation at very high $CdCl_2$ concentration of 1 mM whereas *T. aquaticus* MutS remains unchanged even at 2 mM $CdCl_2$.

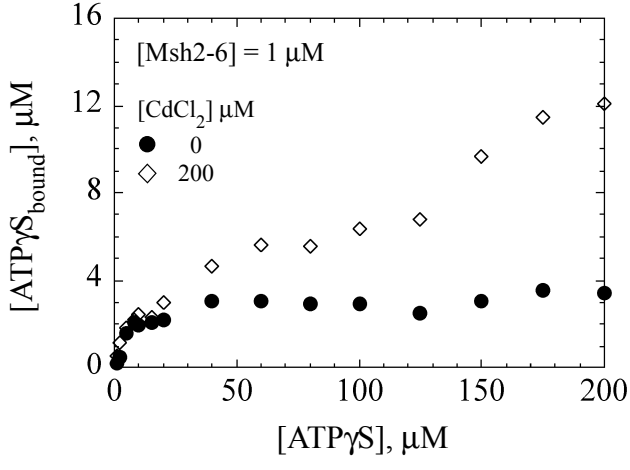
Reference

Antony, E., and Hingorani, M.M. (2003). Mismatch recognition-coupled stabilization of Msh2-Msh6 in an ATP-bound state at the initiation of DNA repair. Biochemistry *42*, 7682-7693.

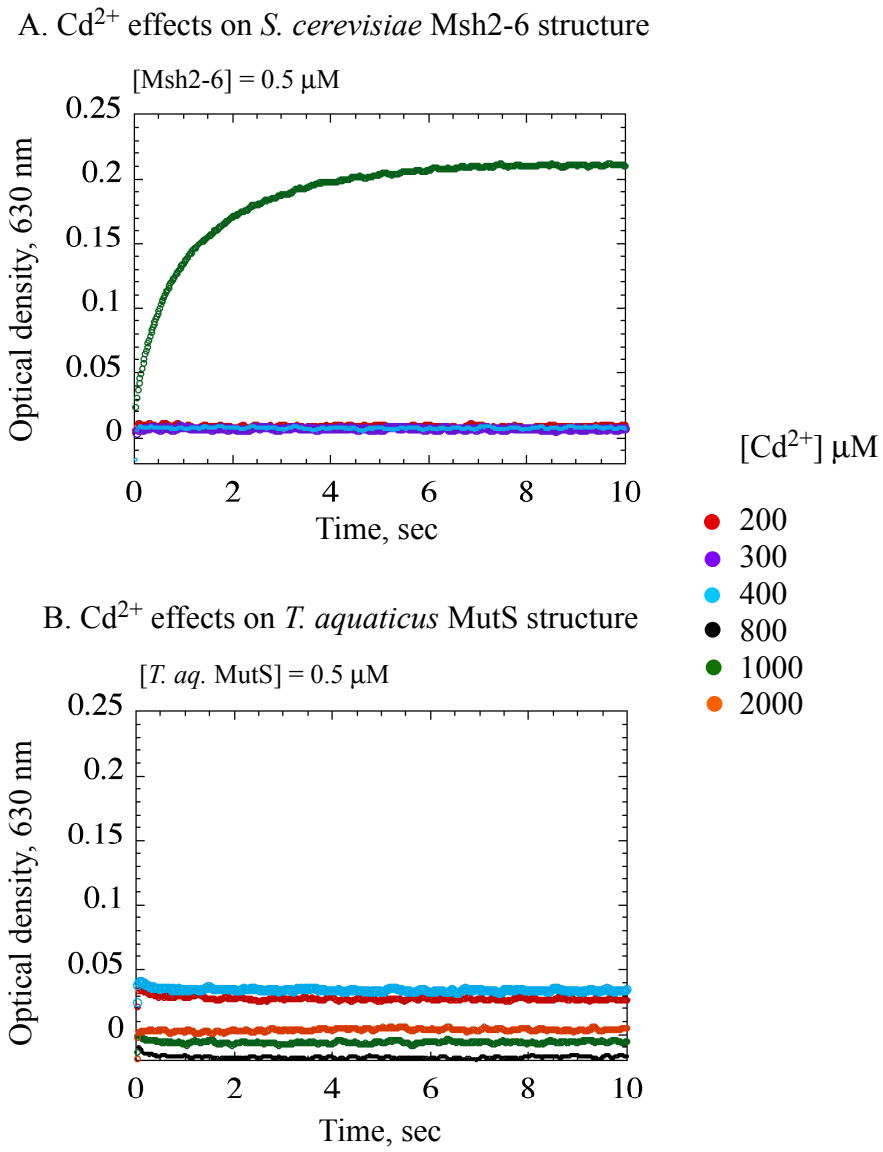
A. Effect of Cd^{2+} on ATP binding by Msh2-6



B. Effect of Cd^{2+} on ATPγS binding by Msh2-6



Supplementary Figure 1



Supplementary Figure 2