Supplementary Methods

MBP-MutS-H6 control binding experiment. Results presented in Supplementary Figure #2.

The following primers were used to generate hetero/homo duplex DNA with covalently attached fluorophores.

#1 40mer GTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAG
#2 40mer Cy5-CTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATTAAC
#3 39mer Fl-CTTCACCCTCTCCACTGACGAAAATTTGTGCCCATTAAC

Oligos #1 and #2 anneal to provide a homoduplex labeled with Cy5 fluorophore. Oligos #1 and #3 anneal to provide a heteroduplex labeled with fluorescein fluorophore with a deletion mutation located at position 20. 10 μM of each oligo was annealed in 10 mM Tris-HCl pH=7.8 + 50 mM NaCl by heating for 5 min. at 95 °C followed by cooling 0.1 °C/sec. to 25 °C. Binding reactions with MBP-MutS-H6 were carried out in 1x binding buffer (20 mM Tris-HCl pH=7.8, 5 mM $MgCl_2$, 5 mM NaCl, 1 mM DTT, 0.8% glycerol). Both hetero- and homoduplex DNA were present at a concentration of 50 nM. MBP-MutS-H6 dimers were added to a final concentration of ~150 nM. Negative control reactions were carried out by adding protein storage buffer alone in place of MBP-MutS-H6 in storage buffer. Binding reactions were allowed to incubate at room temperature for 10 min. prior to incubating with an equal volume of amylose resin pre-equilibrated in 1x binding buffer for 30 min. Amylose resin was pelleted using low-speed centrifugation and washed 2x for 5 min. at room temperature using an equal volume of 1x binding buffer. Protein/DNA complexes were eluted 2x by incubating amylose resin with an equal volume of 1x binding buffer + 10 mM maltose for 5 min. at room temperature. 100 μL samples were collected at each stage for fluorescence spectroscopy. Fluorescence spectroscopy was carried out in a 96-well plate format using a Perkin-Elmer Wallac Envision 2100 multilabel reader. Excitation and emission filters for each fluorophore were as follows: Cy5, excitation 'Cy5 620', emission 'APC 665'; Fluorescein, excitation 'FITC 485', emission 'FITC 535.'