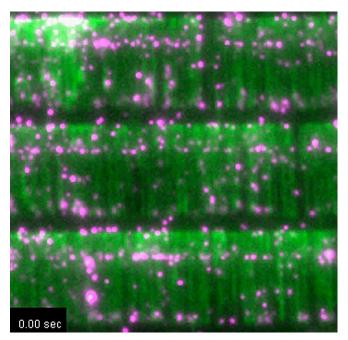
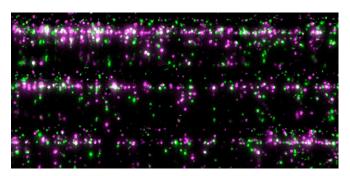
Supporting Information

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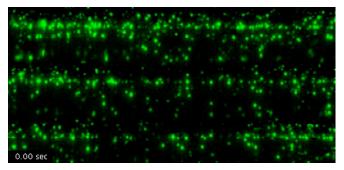
Movie S1. Mismatch-binding by $MutS\alpha$ on single-tethered DNA curtains. Example of mismatch-bound $MutS\alpha$ and is the same field as presented in Fig. 1*B*. The proteins are shown in magenta, the DNA is labeled with YOYO1 and is shown in green. As is shown here (and in Fig. 1 *B* and *D*), $MutS\alpha$ binds preferentially to the lesions. When flow is paused the proteins disappear because the DNA to which they are bound diffuses out of the evanescent field. This control verifies the proteins are on the DNA.

Movie S1



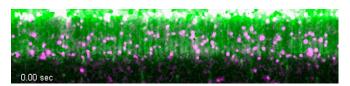
Movie 52. ATP-triggered mismatch release of MutSα on a double-tethered DNA molecule. This movie demonstrates the behavior of lesion-bound MutSα upon the injection of 1 mM ATP, along with the corresponding particle tracking data, which is used to quantitatively analyze the behavior of the protein. The protein is shown as a white spot in the movie, the DNA is doubletethered and unlabeled, *Flow* indicates when buffer flow is initiated for the ATP injection, and *ATP* indicates when ATP actually enters the sample chamber.

Movie S2



Movie S3. Spontaneous escape and return of mismatch-bound MutS α . This movie shows an example of MutS α bound to a mismatch in the presence of 1 mM ADP, along with the corresponding particle tracking data. The protein is shown as a white spot in the movie, the DNA is double-tethered and unlabeled. In this example, the protein makes 3 spontaneous excursions away from the lesion, and in each case returns to rebinds the lesion.

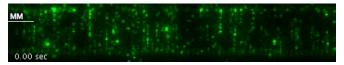
Movie S3



Movie S4. The 1D diffusion of $MutL\alpha$ on a double-tethered DNA curtain. This movie shows the behavior of QD-tagged $MutL\alpha$ on double-tethered DNA curtains in the absence of buffer flow. The DNA is stained with YOYO1 and shown in green, and $MutL\alpha$ in magenta. As shown here, $MutL\alpha$ diffuses rapidly along the DNA. Additional examples of $MutL\alpha$ diffusion and a detailed analysis of the diffusive behavior of $MutL\alpha$ on DNA can be found in ref. 1.

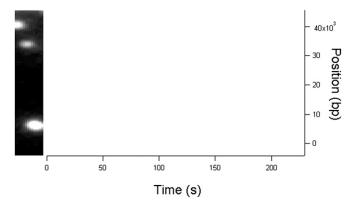
Movie S4

1. Gorman J, Fazio T, Wang F, Wind S, Greene E (2010) Nanofabricated racks of aligned and anchored DNA substrates for single-molecule imaging. Langmuir 26:1372–1379.



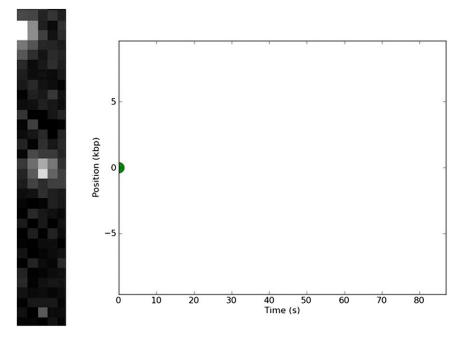
Movie S5. MutL α diffusion past lesions in the absence of MutS α . This movie shows the diffusion of QD-tagged MutL α (green) on double-tethered DNA curtains comprised of lesionbearing substrates (unlabeled). There is no buffer flow and the location of the lesion is indicated. As shown here, MutL α diffuses rapidly along the DNA and does not stop at the lesions.

Movie S5



Movie S6. "Lines" of $MutS\alpha$ and $MutL\alpha$ on lesions-bearing single-tethered DNA curtains. This movie was collected from the same field of view as shown in Fig. 4A. The DNA is unlabeled, $MutS\alpha$ is in magenta, $MutL\alpha$ is green, and the predominant location of the two proteins is revealed as the three lines spanning the field of movie; these line are highlight as "MM" in Fig. 4A. Both proteins remain bound to the lesions, and when buffer flow is transiently terminated the proteins disappear.

Movie S6



Movie 57. "Lines" of $MutL\alpha$ on lesions-bearing single-tethered DNA curtains. This movie was collected from the same field of view as shown in Movie S6 and Fig. 4A, but only the "green" signal corresponding to the QD-tagged $MutL\alpha$ is shown. $MutS\alpha$ is not shown to clearly demonstrate the location of $MutL\alpha$ on the DNA.

Movie S7

Other Supporting Information Files

SI Appendix (PDF)