Supplementary Information

Regulation of UvrD Helicase Activity by MutL

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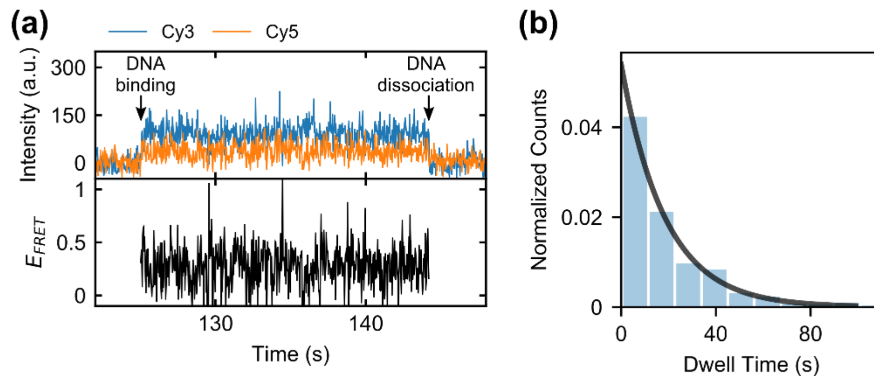
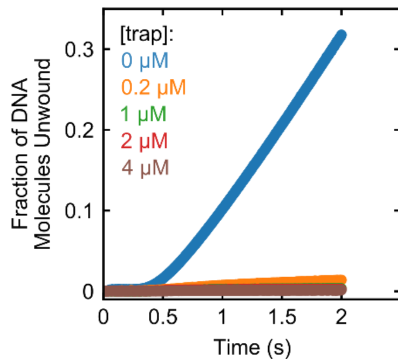
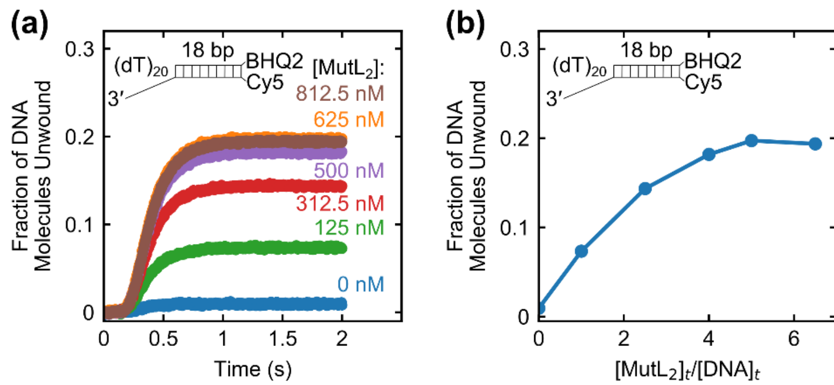


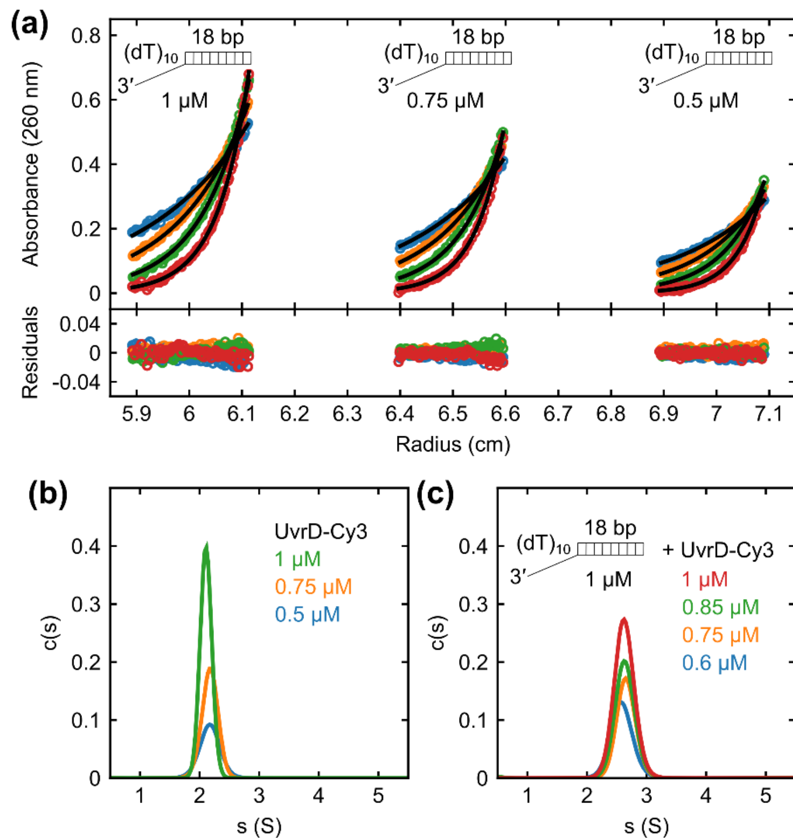
Supplementary Fig. 1. DNA binding to and dissociation from surface immobilized biotin-UvrD monomers. (a) Example of a time trace for binding to and dissociation of DNA (1 nM) from a surface-immobilized UvrD monomer at 5 μM ATP in imaging buffer at 25 °C. (b) Histogram of the dwell times of DNA bound to UvrD and a single exponential fit (black line) indicating a dissociation rate constant of 0.054 ± 0.002 s^{-1}.



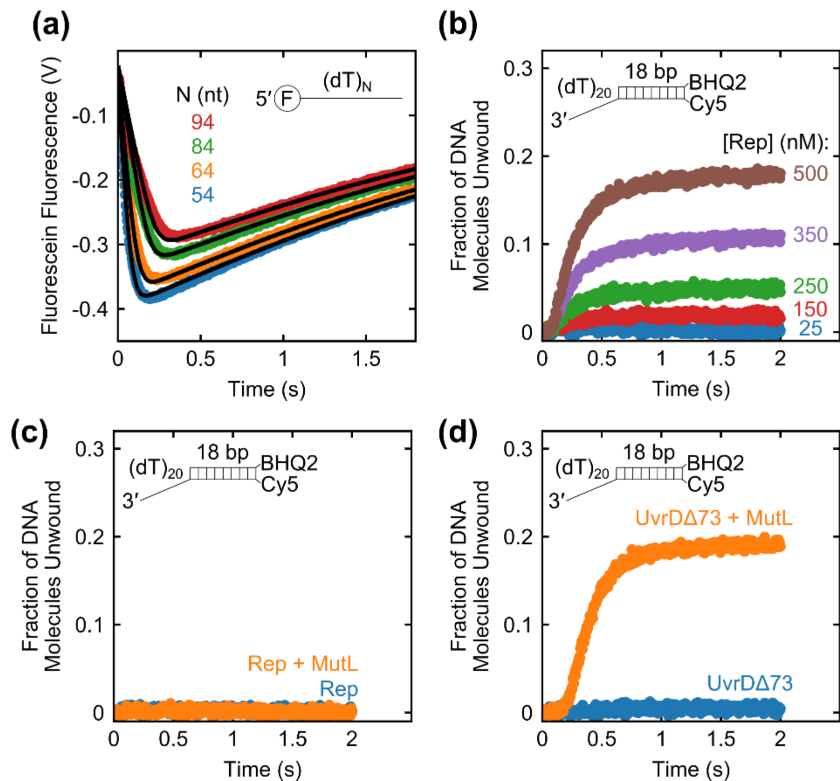
Supplementary Fig. 2. Protein trap test. Stopped-flow experiments were performed with 160 nM UvrD and 250 nM MutL dimer in one syringe and mixing vs. 50 nM 3′-$(dT)_{20}$-ds18-BHQ2/Cy5, 1 mM ATP, 2 mM $MgCl_2$, plus varying concentrations of 10-bp DNA hairpin with a 3′-$(dT)_{40}$ ssDNA tail (protein trap) (0 μM (blue); 0.2 μM (orange); 1 μM (green); 2 μM (red); 4 μM (brown)) in buffer T at 25 °C.



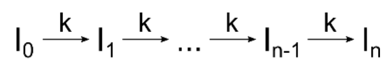
Supplementary Fig. 3. Effects of MutL concentration on UvrD activation. (a) Stopped-flow DNA unwinding time courses performed with 125 nM 3′-$(dT)_{20}$-ds18-BHQ2/Cy5 and 50 nM UvrD as a function of MutL dimer concentration (0 nM (blue); 125 nM (green); 312.5 nM (red); 500 nM (purple); 625 nM (orange); 812.5 nM (brown)) in buffer T at 25 °C. (b) Fraction of DNA molecules unwound plotted as a function of $[MutL_2]_t/[DNA]_t$.



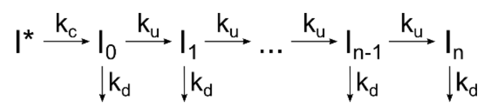
Supplementary Fig. 4. UvrD-Cy3 binds to 3′-$(dT)_{10}$-ds18 as a monomer in buffer M20/20. (a) Sedimentation equilibrium experiments of 3′-$(dT)_{10}$-ds18 (0.5 μM, 0.75 μM, 1 μM) were performed in buffer M20/20 at 25 °C. Global analysis of absorbance profiles at 260 nm shows a single species at four rotor speeds (18k rpm (blue); 22k rpm (orange); 27k rpm (green); 33k rpm (red)). Smooth curves are simulations using best fit parameters with residuals shown below the plots. (b,c) Sedimentation velocity experiments monitoring the Cy3 absorbance of UvrD-Cy3 at 555 nm were performed at 42k rpm in buffer M20/20 at 25 °C. (b) Continuous sedimentation coefficient distributions $c(s)$ of UvrD-Cy3 at three concentrations (0.5 μM (blue); 0.75 μM (orange); 1 μM (green)) show a single peak at 2.15 ± 0.02 S. (c) $c(s)$ distributions of UvrD-Cy3 at four concentrations (0.6 μM (blue); 0.75 μM (orange); 0.85 μM (green); 1 μM (red)) in complex with 3′-$(dT)_{10}$-ds18 (1 μM) show a single peak at 2.63 ± 0.02 S.



Supplementary Fig. 5. MutL specifically stimulates UvrD helicase. (a) Time courses for stopped-flow ssDNA translocation experiments performed with Rep (50 nM) and a series of 5′-F-$(dT)_N$ (N = 54, 64, 84, 94 nt) (100 nM) to ensure that no more than one Rep monomer is bound per DNA. The macroscopic translocation rate (mk_t) of a Rep monomer determined from NLLS analysis (see Methods) is 293±16 nt/s. (b,c,d) Stopped-flow DNA unwinding time courses for experiments performed with 3′-$(dT)_{20}$-ds18-BHQ2/Cy5 in buffer T at 25 °C (b) DNA (50 nM) was pre-incubated with Rep at varying concentrations (25 nM (blue); 150 nM (red); 250 nM (green); 350 nM (purple); 500 nM (brown)). Helicase activity is observed only for [Rep] >> [DNA]. (c) DNA (50 nM) was pre-incubated with 25 nM Rep (blue) or 25 nM Rep plus 250 nM MutL dimer (orange), favoring Rep monomer bound to the DNA. (d) DNA was pre-incubated with 25 nM UvrDΔ73 (blue) or 25 nM UvrDΔ73 plus 250 nM MutL dimer (orange).



Supplementary Scheme 1



Supplementary Scheme 2

Supplementary Table 1. Hydrodynamic properties of 3′-$(dT)_{10}$-ds18, UvrD-Cy3, $MutL_2$, and their complexes in buffer M20/20

Species	M (kg/mol)	$\bar{v}_{calc}$[b] (ml/g)	$s_{20,w}$ (S)	f/f_0	$M_{predicted}$ (kg/mol)	$\bar{v}_{exp}$[c] (ml/g)
3′-$(dT)_{10}$-ds18	14.044	-	-	-	-	0.563 ± 0.002
UvrD-Cy3	84.672	0.7308	4.66 ± 0.05	1.46 ± 0.02	82.3 ± 1.2	0.737±0.003
$MutL_2$[a]	135.824	0.7414	4.88 ± 0.04	1.88 ± 0.02	130.2 ± 6.9	0.750 ± 0.010
UvrD-DNA	98.717	0.712	5.63 ± 0.03	1.40 ± 0.01	92.4 ± 1.4	0.727 ± 0.003
$MutL_2$-UvrD-DNA	234.540	0.734	-	-	235 ± 6	0.734 ± 0.005

[a] Niedziela-Majka *et al.*[1]
[b] Calculated from the amino acid composition using SEDNTERP[2] for UvrD-Cy3 and $MutL_2$ as well as weight-average partial specific volumes for UvrD-DNA and $MutL_2$-UvrD-DNA complexes according to Eq. (7)
[c] Estimated from sedimentation equilibrium analysis by constraining to the known molecular mass of the species

Supplementary Table 2. DNA substrate sequences

dsDNA pairs	Length (nt)	Oligonucleotide sequence
I	19	5′-(Y) GCC CTG CTG CCG ACC AAC-3′
I	39	5′-(X) GTT GGT CGG CAG CAG GGC $(dT)_{20}$-3′
II	19	5′-GCC CTG CTG CCG ACC AAC (Q)-3′
II	19 + N	5′-(Y) GTT GGT CGG CAG CAG GGC $(dT)_{N}$-3′
III	22	5′-GCC CTG CTG CCG ACC AAC GAT (Q)-3′
III	42	5′-(Y) ATC GTT GGT CGG CAG CAG GGC $(dT)_{20}$-3′
IV	26	5′-GCC CTG CTG CCG ACC AAC GAT GGT T (Q)-3′
IV	26 + N	5′-(Y) AAC CAT CGT TGG TCG GCA GCA GGG C $(dT)_{N}$-3′
V	33	5′-GCC CTG CTG CCG ACC AAC GAT GGT TAC ATT CC (Q)-3′
V	33 + N	5′-(Y) GGA ATG TAA CCA TCG TTG GTC GGC AGC AGG GC $(dT)_{N}$-3′
VI	41	5′-GCC CTG CTG CCG ACC AAC GAT GGT TAC ATT CCC GCT GCT G (Q)-3′
VI	41 + N	5′-(Y) CAG CAG CGG GAA TGT AAC CAT CGT TGG TCG GCA GCA GGG C $(dT)_{N}$-3′
VII	51	5′-GCC CTG CTG CCG ACC AAC GAT GGT TAC ATT CCC GCT GCT GCT AGT GCA GG(Q)-3′
VII	51 + N	5′-(Y) CCT GCA CTA GCA GCA GCG GGA ATG TAA CCA TCG TTG GTC GGC AGC AGG GC $(dT)_{N}$-3′
VIII	81	5′-GCC CTG CTG CCG ACC AAC GAT GGT TAC ATT CCC GCT GCT GCT AGT CGA GGT AGC GTT CAC GTC GCA TTC GAC GCT CGA CG (Q)-3′
VIII	95	5′ -(Y) CGT CGA GCG TCG AAT GCG ACG TGA ACG CTA CCT CGA CTA GCA GCA GCG GGA ATG TAA CCA TCG TTG GTC GGC AGC AGG GC $(dT)_{14}$-3′
IX	18	5′-GCC CTG CTG CCG ACC AAC-3′
IX	28	5′-GTT GGT CGG CAG CAG GGC $(dT)_{10}$-3′
X	65	5′-GCC TCG CTG CTT TTT GCA GCG AGG C $(dT)_{40}$-3′

X = Cy3; Y = Cy5; Q = BHQ2

1. Niedziela-Majka, A., Maluf, N.K., Antony, E. & Lohman, T.M. Self-assembly of Escherichia coli MutL and its complexes with DNA. *Biochemistry* **50**, 7868-80 (2011).
2. Laue, T.M., Shah, B.D, Ridgeway, T.M. and Pelletier, S.L. *Computer-aided interpretation of analytical sedimentation data for proteins.*, 90-125 (Royal Society of Chemistry, Cambridge, UK, 1992).