Supplemental Data

A Nonuniform Stepping Mechanism for E. coli UvrD Monomer Translocation along Single-Stranded DNA

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Supplemental Results and Discussion

Fluorescent Labeling of Phosphate Binding Protein

PBP was fluorescently labeled with the coumarin derivative, MDCC, as previously described (Brune et al., 1994), with the following modifications. A HiTrap Heparin column (10 mL pre-packed) (GE Healthcare, Piscataway, NJ), equilibrated with 10 mM Tris-HCl pH 7.5 (25°C), was used to separate unlabeled PBP from PBP-MDCC. The column was eluted with a 200 mL linear gradient (0 – 200 mM NaCl) (10 mM Tris-HCl pH 7.5 (25°C)). PBP-MDCC elutes in the second peak (Figure S1) and was dialyzed into PBP-MDCC buffer at 4°C, concentrated to ~0.5 mM by ultrafiltration and stored at -80°C. An $A_{280\text{corr}}/A_{430} = ~1.47$, indicated > 99 % singly labeled PBP-MDCC (Brune et al., 1994).

Optimization of the Fluorescence Assay for Inorganic Phosphate

ATP hydrolysis during UvrD translocation was measured using a fluorescent assay that monitors the production of inorganic phosphate, $P_i$ (Brune et al., 1994; Brune et al., 1998; Dillingham et al., 2000). This assay was modified in that an excess of the polyanion heparin was included in the ATP solution to serve as a trap for free UvrD protein to insure that these are “single round” experiments, which greatly simplifies the analysis (Fischer and Lohman, 2004). We showed previously (Fischer et al., 2004) that
although heparin actively dissociates some fraction of the UvrD during translocation (i.e., increases the apparent dissociation rate constant, $k_d$), heparin does not affect the translocation rate constant, $k_t$ or the kinetic step-size, $m$. The additional advantage of heparin is that it does not stimulate the ATPase activity of UvrD and thus heparin bound UvrD does not contribute to the production of $P_i$.

**Effectiveness of heparin as a trap for free UvrD.** All ssDNA used in these experiments were deoxyribothymidylates ((dT)$_L$ or poly(dT)) to avoid intramolecular base pair formation. The concentrations of UvrD monomer, ssDNA, heparin, and PBP-MDCC reported are the final concentrations after mixing. Figure S2A shows the effects of heparin on the time courses for $P_i$ production resulting from mixing a pre-formed UvrD-(dT)$_{124}$ complex with solutions containing ATP, Mg$^{2+}$ and PBP-MDC in buffer T$_{20}$ at 25°C. Both time courses show an increase in PBP-MDCC fluorescence due to the hydrolysis of ATP and the concomitant production of $P_i$. In the absence of heparin, a non-linear increase in PBP-MDCC fluorescence is observed with no clear “burst” phase, but in the presence of heparin the fluorescence reaches a plateau value after $\sim$ 0.5 sec. In the presence of heparin, the increase in fluorescence results only from ATP hydrolysis by UvrD that is initially bound to the ssDNA ((dT)$_{124}$). UvrD monomers that dissociate from the (dT)$_{124}$ are trapped by heparin and prevented from rebinding ssDNA.

Figure S2B shows that at several heparin concentrations a small steady state increase in fluorescence occurs after the burst phase, the slope of which decreases with increasing heparin concentration (data not shown). The slopes of the steady state phase are identical to the slopes obtained in control experiments (dashed lines in Figure S2B) in which free UvrD was mixed in a stopped-flow experiment vs. a mixture of heparin and
dT_{124}, in buffer T_{20} containing ATP, MgCl_{2}, and PBP-MDCC. This control shows that
the heparin serves as an adequate trap for free UvrD. Although a small fraction of free
UvrD is able to rebind (dT)_{124} and hydrolyze ATP even in the presence of heparin, this
steady state phase can be quantitatively separated from the burst phase amplitude. In all
subsequent experiments we used a final heparin concentration of 4 mg/mL to maximize
the signal observed in the burst phase. Figure S2B also shows that the burst amplitude
decreases with increasing heparin concentration due to the fact that heparin not only acts
as a trap, but also can actively displace some UvrD monomers from ssDNA (Fischer et
al., 2004). However, with independent knowledge of the kinetic parameters for UvrD
monomer translocation along ssDNA (rate constant, \(k_t\), step size, \(m\), and processivity, \(P\))
and the rate constant, \(k_d\), for dissociation of UvrD monomer from ssDNA, we can account
quantitatively for the amount of UvrD that dissociates from the ssDNA during
translocation due to heparin-induced dissociation and thus can calculate the average
amount of ATP hydrolyzed per nucleotide translocated by each UvrD monomer.

**Determination of optimal ssDNA concentrations.** To ensure that all of the UvrD is
initially bound to ssDNA, we determined the ssDNA (\((dT)_{L}\)) concentration needed to
obtain maximum P_i production for a given UvrD concentration. Figure S2C shows time
courses for P_i production during UvrD monomer translocation on (dT)_{54}, although
experiments were also performed with (dT)_{10} since UvrD affinity for ssDNA is affected
by ssDNA length. The burst amplitudes (Figure S2D) saturate at \(~500\ nM\ (dT)_{10}\) and \(~
100\ nM\ (dT)_{54}\), whereas the rate of ATP hydrolysis in the steady state phase, although
slow, increases with increasing (dT)_{10} and (dT)_{54} concentrations (data not shown),
consistent with this phase being due to a small amount of rebinding of UvrD to the ssDNA.

**Effect of heparin on the kinetics of phosphate binding to PBP-MDCC and calibration of the PBP-MDCC fluorescence signal.** The enhancement of PBP-MDCC fluorescence was calibrated to obtain the extent of ATP hydrolysis (P$_i$ production). Stocks of NaH$_2$PO$_4$ (0.125 to 1.0 μM) in buffer T$_{20}$ were mixed in the stopped-flow with PBP-MDCC (10 μM), heparin (4 mg/ml), ATP (0.5 mM), and MgCl$_2$ (2.0 mM) also in buffer T$_{20}$ (all concentrations are after mixing, see Experimental Procedures). The time courses for P$_i$ binding to PBP-MDCC are well described by a single exponential function (Figure S3A) and the increase in PBP-MDCC fluorescence is directly proportional to [NaH$_2$PO$_4$] up to at least 2.0 μM (Figure S3B), which is sufficient since the longest ssDNA in our studies (124 nts) results in the release of ≤ 1.5 μM P$_i$.

The rate of P$_i$ binding to PBP-MDCC is slower than observed in previous studies (Brune et al., 1998) (Figure S3A), hence we examined the kinetics of P$_i$ binding to PBP-MDCC (0.10 μM) under our solution conditions at [heparin] from 2 mg/ml to 8 mg/ml (Figures S4 and S5). In the absence of heparin, P$_i$ binding to PBP-MDCC occurs with an apparent bimolecular rate constant of ~$1.2 \times 10^8$ M$^{-1}$ s$^{-1}$, consistent with previous estimates performed under different solution conditions (Brune et al., 1998). However, in the presence of heparin (2 to 8 mg/mL), this was reduced to ~$1.3 \times 10^7$ M$^{-1}$sec$^{-1}$ (Figure S5C). Direct titrations of PBP-MDCC with NaH$_2$PO$_4$ performed under our conditions also show that PBP-MDCC bound to heparin has reduced affinity for P$_i$ (Figure S6). Since the binding of PBP-MDCC to heparin results in reduced phosphate affinity and slower phosphate binding kinetics, it is possible that the rate of P$_i$ detection can be slower
than the actual rate of ATP hydrolysis and thus limit the observed rate of ATP hydrolysis. However, even if this is the case, one can still obtain an accurate measure of the total amount of $P_i$ produced as long as the PBP-MDCC fluorescence signal is calibrated in the presence of heparin. As a result, we only analyze the total amount of $P_i$ produced during UvrD translocation to determine the ATP coupling stoichiometry, rather than the time course of $P_i$ production. However, this does not result in any loss of information, since we show in the main text that the overall rate of ATP hydrolysis by a translocating UvrD monomer is limited by a kinetic step that is repeated every four nucleotides translocated and this step is much slower than the rate of ATP hydrolysis. Hence, information about the kinetics of ATP hydrolysis during UvrD translocation is not obtainable in any case.

Although the rate and affinity of $P_i$ binding to PBP-MDCC are reduced by heparin binding under the conditions used here (10 mM Tris-HCl pH 8.3, 20 mM NaCl, 20% (v/v) glycerol, 2 mM MgCl$_2$, 0.5 mM ATP), we note that heparin does not interfere with $P_i$ binding to PBP-MDCC if the [Mg$^{2+}$] is increased to 10 mM (Colin Wu, and EJT, unpublished observations). In practice, one always should examine whether heparin affects PBP-MDCC binding to $P_i$ under the particular solution conditions used.

In light of the above observations, we compared the kinetics of $P_i$ production using the fluorescent PBP-MDCC stopped-flow assay with the kinetics of ADP production using a standard quenched-flow assay with radioactive ($\alpha^{32}$P)-ATP. We compared the two assays at 25 $\mu$M ATP since the signal to noise ratio is too low to perform the radioactive assay at 500 $\mu$M ATP. The total amounts of ATP hydrolyzed, as measured by ADP production (quenched-flow), or $P_i$ production (stopped-flow) agree well (Figure S3C), hence the stopped-flow fluorescence assay provides an accurate measure of the
amount of P\textsubscript{i} produced (ATP hydrolyzed) during UvrD monomer translocation along ssDNA.

*Effects of using a “MOP” to reduce the background of inorganic phosphate.* In the stopped-flow experiments monitoring P\textsubscript{i} production in the presence of heparin, both the control and experimental time courses show an initial decrease in PBP-MDCC fluorescence within the first 50 msec, which is not observed in the absence of heparin (Figures S7A and B). Control experiments indicate that this decrease is not dependent on UvrD, but is due to the presence of contaminating P\textsubscript{i} that is initially bound to the PBP-MDCC, some of which dissociates upon addition of heparin due to the fact that PBP-MDCC bound to heparin has a lower affinity for P\textsubscript{i} (see above). To investigate this further we examined the effects of including a “MOP” (purine nucleoside phosphorylase with 7-methylguanosine (Nixon et al., 1998)) for P\textsubscript{i} that removes P\textsubscript{i} from solution by sequestering it in the form of ribose-1 phosphate. In the presence of the MOP, the initial decrease in PBP-MDCC fluorescence was eliminated (Figure S8A). However, after subtraction of control time courses performed in the absence of MOP and ssDNA, the normalized time courses for P\textsubscript{i} release during UvrD monomer translocation are independent of the presence of the MOP (S8B). Therefore, we did not include a MOP for P\textsubscript{i} in our experiments although we pre-treated the stopped-flow lines with MOP prior to a set of experiments to remove contaminating phosphate from the instrument.

**Supplemental Experimental Procedures**

**Buffers and Reagents**

Storage buffer is 20 mM Tris-HCl (pH 8.3 at 25° C), 200 mM NaCl, 50 %\(\text{v/v}\) glycerol (enzyme grade), 1 mM EDTA, 0.5 mM EGTA, and 25 mM 2-mercaptoethanol.
Storage minimal buffer is 20 mM Tris-HCl (pH 8.3 at 25°C), 200 mM NaCl, and 50 % (v/v) glycerol (enzyme grade). ssDNA buffer is 10 mM Tris-HCl (pH 8.3 at 25°C) and 50 mM NaCl. Heparin buffer is 19 mM Tris-HCl (pH 8.3 at 25°C), 40 mM NaCl, 2 mM MgCl₂, and 40 % (v/v) glycerol (enzyme grade). PBP-MDCC buffer is 10 mM Tris-HCl (pH 8.0 at 25°C) and 1 mM MgCl₂. PBS buffer is 10 mM Potassium Phosphate buffer (pH 7.0), 100 mM NaCl. MgCl₂ stock concentrations were determined by refractive index (Wolf, 1974).

Heparin (sodium salt) (catalog no. H-3393) stocks were dialyzed vs. Heparin buffer at 4°C and concentrations determined as described (Mascotti and Lohman, 1995). 5'-adenosine triphosphate (ATP) stocks in 50 mM NaOH (pH 7.0) were stored at -20°C and concentrations determined in PBS buffer (ε₂₆₀nm = 15340 M⁻¹ cm⁻¹)(Gray et al., 1995). 7-Methylguanosine (7-MEG) stocks in Milli-Q water were stored at -20°C and concentrations determined spectrophotometrically (ε₂₅₆ nm = 13300 M⁻¹ cm⁻¹)(Dunn, 1968). Nucleoside phosphorylase (PNPase) stocks (500-units/mL) were flash-frozen with liquid N₂ and stored at -80°C. 7-diethylamino-3(((2-maleimidyl)-ethyl)amino)carbonyl)coumarin (MDCC) (Invitrogen, Carlsbad, CA) was dissolved in N,N-dimethylformamide and concentrations determined spectrophotometrically in methanol (ε₄₁₉ nm = 49300 M⁻¹ cm⁻¹).

**Analysis of Translocation Time courses**

**Kinetics of inorganic phosphate production.** The total amount of ATP hydrolyzed (Pᵢ produced) per UvrD monomer was determined for each (dT)L by fitting the time course for Pᵢ production to eq. (4), without including the time points within the first 50
ms (see text and Supplemental Data) to obtain the rate constants for the burst phase, $k_{\text{obs}}$, and steady-state phase, $k_{\text{ss}}$.

$$
\frac{P}{UvrD} = A\left(1 - \exp^{-k_{\text{obs}}t}\right) + k_{\text{ss}}t
$$

The burst phase amplitude, $A$ (P$_i$ per UvrD monomer), was plotted vs. ssDNA length, $L$, and fit to eq. (2) to determine $c$ and $k_d$ using Scientist (Micromath, St. Louis, MO), while the parameters $k_t$, $k_d$, $k_{\text{end}}$, $r$, $m$, and $d$ were constrained to the values determined from the translocation experiments.

Kinetics of UvrD monomer translocation. The multiple time courses for UvrD translocation determined with 5'-Cy3-(dT)$_L$ and 5-F-(dT)$_L$ were analyzed globally using eq. (3) to obtain fluorophore independent estimates of the translocation kinetic parameters using NLLS analysis as described (Fischer et al., 2004). In this analysis, $k_t$, $r$, and $n$ were floated as global parameters (i.e., constrained to be the same for all ssDNA lengths and independent of fluorophore), and $k_d$ was constrained to the value determined independently from poly(dT) ($k_d = 0.81 \text{ s}^{-1}$). The kinetic parameters $k_c$, $k_{\text{end}}$, $f^*_{\text{end}}/f_{\text{end}}$, and $f_i/f_{\text{end}}$, were also assumed to be the same for all ssDNA lengths, but dependent on the type of fluorophore (Cy3 vs. fluorescein). The parameter, $A$, was allowed to float for each ssDNA length. The uncertainties in the fitted parameters (68% confidence limits) were determined by performing a 100 cycle Monte Carlo (Straume and Johnson, 1992) simulation routine in Conlin. Kinetic parameters within 2-5% of the values reported in Table 1 were also obtained from two independent determinations.
**Kinetics of UvrD monomer dissociation from ssDNA.** To determine $k_d$, time courses of dissociation of UvrD monomers from Poly-(dT) during translocation were analyzed by NLLS analysis using a single exponential equation, where $k_{obs} = k_d$.

To determine $k_{end}$, time courses of dissociation of UvrD monomers from a series of unlabeled (dT)$_L$ in the presence of ATP (i.e., during translocation) were analyzed by global NLLS analysis using eq. (5), based on Scheme 1, where $f(t)$ is the time dependent

$$f(t) = \frac{A}{(1+n r)} \left[ \frac{1}{s} \left( \frac{k_d r \left( n \left( k_d + s + k_i \left( \frac{k_i}{k_i + k_d + s} \right)^n - 1 \right) \right)}{(k_d + s)^2} \right) + \frac{k_{end}}{s + k_{end}} \left( 1 + \frac{k_r}{s + k_d} \left( 1 - \left( \frac{k_i}{s + k_i + k_d} \right)^n \right) \right) \right]$$

fluorescence signal for free UvrD ($P_f$) (Fischer and Lohman, 2004). For this, $k_i$, $k_d$, $r$ and $n$ were constrained to the values determined from analysis of the translocation experiments conducted with fluorescently labeled ssDNA, while $k_{end}$ was assumed to be the same for each (dT)$_L$ and obtained as a fitting parameter. The parameter $A$ was allowed to float for each (dT)$_L$.

**Phosphate Binding Experiments**

**Equilibrium binding of phosphate to PBP-MDCC.** Equilibrium titrations of PBP-MDCC with phosphate were monitored by the increase in PBP-MDCC fluorescence at 25°C using a QM-4 fluorometer (Photon Technology International, Lawrenceville, NJ). Samples were excited at 430 nm and the emission was scanned from 455 nm to 480 nm (0.5 mm slit widths). Titrations of PBP-MDCC (10 µM) with Na$_2$HPO$_4$ were carried in a
3 mL quartz cuvette (1 cm pathlength) in buffer T20 plus 2 mM MgCl2 in the presence and absence of heparin (2 mg/ml to 6 mg/ml). Prior to each titration, cuvettes were treated with MOP (300 μM 7-MEG, 0.2-units/mL PNPase, 10 mM Tris-HCl, pH 8.0) to remove contaminating phosphate. The total fluorescence for each addition of phosphate was obtained by integrating the emission spectrum. The fluorescence signal associated with zero phosphate was obtained after the titration by adding excess MOP (400 μM 7-MEG, 1.5-units/mL PNPase) to remove all phosphate from PBP-MDCC.

*Phosphate binding kinetics.* The kinetics of phosphate binding to PBP-MDCC (0.1 μM, final) was measured in the stopped-flow in buffer T20 with 2 mM MgCl2 at 25°C in the presence and absence of heparin (2-4mg/ml, final). Na2HPO4 stock solutions in buffer T20 plus 2 mM MgCl2 were mixed with PBP-MDCC in buffer T20 plus 2 mM MgCl2 in the presence and absence of heparin (λex =430 nm, 9.3 nm bandwidth; λem > 450 nm; long-pass filter (Oriel Cat. # 57347)). Concentrations of Na2HPO4 stocks were determined by refractive index (Wolf, 1974).

**Chemical Quenched-flow Experiments**

*Quenched-flow assay for ATP hydrolysis during ssDNA translocation.* The kinetics of ATP hydrolysis during UvrD translocation along (dT)124 was also measured using a radioactive assay to compare with the stopped-flow fluorescence assay. Quenched-flow experiments were carried out in buffer T20 without PBP-MDCC using an RQF-3 quenched-flow instrument (KinTek Instruments, University Park, PA). UvrD (25 nM, final concentration) was pre-incubated with (dT)124 (300 nM final concentration) on ice for 5 min, then loaded into one syringe and incubated for an additional 5 min at 25°C. The solution containing ATP, α-32P-ATP (GE healthcare, Piscataway, NJ), heparin, and
MgCl₂ was loaded into the other sample syringe (final concentrations of 25 μM, 3.3 nM, 4 mg/ml, and 2 mM, respectively) and incubated for 5 min at 25°C. These solutions were mixed rapidly and the reaction allowed to proceed for a set time interval, Δt, after which the reaction was quenched by mixing with either 1 M HCl or 0.4 M Na₃EDTA. This experiment was repeated with varied time intervals to generate a full time course of ATP hydrolysis. The quenched samples were mixed by vortexing and neutralized (for the acid quench) by adding approximately 70 μL of neutralization buffer (2.5 M NaOH, 0.5 M Tris-Base, 0.5 M EDTA). The amounts of α⁻³²P-ATP and α⁻³²P-ADP in each sample were analyzed by thin layer chromatography on PEI-cellulose plates (P.J. Cobert Associates, Inc., St. Louis, MO) developed in 0.6 M KPO₄ buffer, pH 3.4, at 25°C (Pedersen and Catterall, 1979). The plates were dried, and the amounts of α⁻³²P-ATP and α⁻³²P-ADP determined using a STORM phosphorimager and ImageQuant Software (GE Healthcare, Piscataway, NJ). To determine the total amount of ATP hydrolyzed per UvrD monomer the fraction of ATP hydrolyzed (α⁻³²P-ADP/(α⁻³²P-ATP + α⁻³²P-ADP)) was multiplied by the total ATP concentration of 25 μM after correcting for background hydrolysis of α⁻³²P-ATP.
Figure S1. A Heparin Column can separate labeled PBP-MDCC from unlabeled PBP. Two different PBP-MDCC preparations (prep1 (A$_{280}$: blue curve, A$_{430}$: orange curve) and prep2 (A$_{280}$: green curve, A$_{430}$: red curve)) were applied to a HiTrap heparin column (10ml pre-packed, GE Healthcare) equilibrated in 10 mM Tris-HCl pH 7.5 (25°C) and eluted with a linear NaCl gradient (0-200 mM, 200 mL). MDCC conjugated to PBP absorbs at 430 nm. Unlabeled PBP (second peak) can be separated from PBP-MDCC (third peak). The first peak has an A$_{280}$:A$_{430}$ ratio consistent with PBP bound with a higher stoichiometry of MDCC.
Figure S2. Effect of heparin and ssDNA concentration on time courses of ATP hydrolysis as measured by $P_i$ production during UvrD translocation. (A)-Stopped-flow experiments were performed by pre-incubating UvrD monomer (25 nM, final concentration) with excess (dT)$_{124}$ (300 nM, final concentration) in buffer T$_{20}$ at 25°C and initiating the reaction by the addition of 500 μM ATP, 2 mM Mg$^{2+}$, 10 μM PBP-MDCC in the presence or absence of heparin to final concentrations 4-8 mg/ml (all concentrations are after mixing). Control experiments were carried out as above, but without (dT)$_{124}$ to correct for the signal due to contaminating phosphate in the buffers. Time course of PBP-MDCC fluorescence measuring $P_i$ production in the absence and presence of heparin (4 mg/mL). (B)-Corrected fluorescence time courses obtained in the
presence of 4, 6, and 8 mg/ml heparin. Dashed curves result from mixing UvrD in one syringe with ATP, Mg\(^{2+}\), heparin (4, 6 or 8 mg/mL), (dT)\(_{124}\), and PBP-MDCC in the other syringe at the same concentrations as above. (C)- Stopped-flow experiments were performed by pre-incubating UvrD monomer (25 nM) with (dT)\(_{54}\) (25-400 nM) (final concentrations) in buffer T\(_{20}\) at 25\(^{\circ}\)C and initiating the reaction by the addition of ATP, Mg\(^{2+}\), PBP-MDCC and heparin to final concentrations of 500 \(\mu\)M, 2 mM, 10 \(\mu\)M, and 4 mg/ml, respectively. Fluorescence time courses were corrected for contaminating phosphate (see Experimental Procedures) and the fluorescence signal was converted to moles of P\(_i\) produced using the calibration (Figure S3B). (D)- The burst phase amplitude of P\(_i\) produced as a function of [(dT)\(_{54}\)] (circles) or [(dT)\(_{10}\)] (triangles).
Figure S3. Calibration of PBP-MDCC fluorescence signal. (A)- Stopped-flow time courses of PBP-MDCC fluorescence due to phosphate binding, after correcting for contaminating phosphate (buffer T20 at 25°C, 500 μM ATP, 2 mM MgCl2, 4 mg/ml heparin, and 10 μM PBP-MDCC). (B)- The corrected PBP-MDCC fluorescence intensity change plotted as a function of the NaH2PO4 concentration. Error bars reflect the standard deviation from an average of 12 traces. (C)- Time courses of ATP hydrolysis were measured by the fluorescent stopped-flow P_i production assay and a radioactive quenched-flow assay. The final conditions in both assays were buffer T20, 2 mM MgCl2, 25 μM ATP, 4 mg/ml heparin, 25 nM UvrD, 300 nM (dT)_{124}, (and 10 μM PBP-MDCC in stopped-flow assay). The thick curve shows the time course of P_i.
production, while the thin curves represent the standard deviation from an average of 12 traces. In the quenched-flow assay, ((α-[^32]P)-ATP/ ATP) reactions were quenched at each time point using either EDTA (squares) or HCl (circles) and the time course of (α-[^32]P)-ADP production is plotted (see Supplemental Experimental Procedures). The error bars represent the standard deviation of an average of three determinations.
**Figure S4.** Stopped-flow time courses of phosphate binding to PBP-MDCC in the presence and absence of heparin in buffer T\textsubscript{20}, 2 mM MgCl\textsubscript{2}, at 25°C. Phosphate (NaH\textsubscript{2}PO\textsubscript{4}) solutions of known concentration in buffer T\textsubscript{20} at 25°C were mixed in the stopped-flow instrument with PBP-MDCC and MgCl\textsubscript{2} to final concentrations of 0.1 μM and 2 mM, respectively, in the absence and presence of heparin (2-8 mg/ml, final concentration). Control time courses obtained in the absence of any added NaH\textsubscript{2}PO\textsubscript{4} were subtracted from each time course. (A) - Fluorescence time courses obtained in the absence of heparin. Continuous curves are single exponential fits. At 10 μM NaH\textsubscript{2}PO\textsubscript{4}, 90% of the amplitude is lost in the dead time of the instrument (~1 msec). (B) - Fluorescence time courses obtained in the presence of 4mg/ml heparin. The time courses display biphasic kinetics.
Figure S5. Heparin reduces the apparent bimolecular rate constant for phosphate binding to PBP-MDCC in buffer T20, 2 mM MgCl2, at 25°C. (A) Dependence on NaH2PO4 of the observed rate for the fast kinetic phase in the absence (circle) and presence of 2mg/ml (square), 4 mg/ml (diamond), and 8 mg/ml (triangle) heparin. (B) Dependence on NaH2PO4 the observed rate for the slow kinetic phase observed in the presence of 2 mg/ml (square), 4 mg/ml (diamond) and 8 mg/ml (triangle) heparin. (C) Effect of heparin on the apparent bimolecular rate constant for phosphate binding to PBP-MDCC, obtained from the slopes of the lines in panel (A).
Figure S6. Heparin reduces the affinity of phosphate for PBP-MDCC in buffer T$_{20}$, 2 mM MgCl$_2$, at 25°C. PBP-MDCC (10 μM) was titrated with NaH$_2$PO$_4$ in the absence (circle) and presence of 2 mg/ml (square), 4 mg/ml (cross), and 8 mg/ml (diamond) heparin while monitoring the MDCC fluorescence emission intensity (excitation at 430 nm, emission: 455-485 nm). The signal corresponding to zero phosphate was obtained after each titration by adding MOP (400 μM 7-MEG, 1.5 units PNPase) to remove all phosphate from PBP-MDCC.
**Figure S7.** Effect of heparin on time courses of P$_i$-released during UvrD translocation. Stopped-flow experiments were performed by pre-incubating UvrD monomer (25 nM post mix) with excess (dT)$_{124}$ (300 nM post mix) in buffer T$_{20}$ at 25°C and initiating the reaction by the addition of ATP, Mg$^{2+}$, PBP-MDCC and with or without heparin to final concentrations of 500 μM, 2 mM, 10 μM, and 4-8 mg/ml, respectively. Control experiments were carried out as above, but leaving out (dT)$_{124}$, to correct for contaminating phosphate in the reaction. A, Fluorescence time courses obtained in the absence of heparin. B, Fluorescence time courses obtained in the presence of 4 mg/ml heparin.
Figure S8. A phosphate MOP is not required in single round phosphate release experiments. UvrD (25 nM post mix) was pre-incubated with (dT)_{124} (300 nM, post mix) in buffer T\textsubscript{20} at 25°C and translocation initiated by the addition of ATP, Mg\textsuperscript{2+}, heparin, and PBP-MDCC to final concentrations of 500 μM, 2 mM, 4 mg/ml, and 10 μM, respectively. In experiments with the phosphate MOP, 7-MEG and PNPass were included in both the UvrD:ssDNA solution and the solution containing ATP, Mg\textsuperscript{2+}, heparin, and PBP-MDCC, to final concentrations of 150 μM and 0.1 units/mL, respectively. Control experiments to correct for contaminating phosphate in the reaction were performed, but without (dT)\textsubscript{124}. (A)- Fluorescence time courses obtained in the presence (red solid curve) and absence (blue dashed curve) of the phosphate MOP. The control time courses are shown in green and orange, respectively. (B)- Normalized fluorescence time courses after subtracting the control time courses.
Supplemental References


