Supporting Information

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SI Text

SI Materials and Methods. ***Proteins and DNA*** RecQ concentration was determined using an extinction coefficient of 1.48×10^4 M^{-1} cm^{-1} at 280 nm (1). Oligonucleotides were purchased from Integrated DNA technologies (IDT) and purified by denaturing polyacrylamide (12%) gel electrophoresis using 8 M urea, followed by gel extraction and ethanol precipitation. Oligonucleotide concentrations were determined using an extinction coefficient of 9,600 M^{-1} cm^{-1} at 267 nm (2). Poly dT (Sigma-Aldrich) was resuspended in 10 mM Tris HCl (pH 7.5) and 1 mM EDTA; according to the manufacturer, the average length was 4,000 nucleotides. Concentration (in nucleotides) was determined using an extinction coefficient of 8,520 M^{-1} cm^{-1} at 260 nm (2).

Reagents. 7-diethylamino-3-((((2-maleimidyl) ethyl)-amino) carbonyl) coumarin (MDCC) was purchased from Molecular Probes. The dry powder was resuspended in DMSO to a concentration of 50 mM and kept at −20 °. 7-methylguanosine (MEG) was purchased from Sigma-Aldrich, resuspended in Nanopure water and stored at −20°. Heparin (Sigma-Aldrich) was resuspended in 10 mM Tris HCl (pH 8.0) and 1 mM EDTA (TE buffer). The solution was then dialyzed extensively against TE buffer to remove any contaminants.

Labeling reaction. The concentration of *E. coli* Phosphate binding protein (PBP), containing the A197C mutation, was determined using an extinction coefficient of 60,880 M^{-1} cm^{-1} at 280 nm (3). The PBP was labeled with 7-diethylamino-3-((((2-maleimidyl) ethyl)-amino) carbonyl) coumarin (MDCC) as described (3). The concentration and the amount of labeling were calculated as described (3–5). The degree of labeling was calculated by taking $A_{280,\text{corrected}}/A_{430}$, where $A_{280,\text{corrected}}$ is the absorbance of PBP corrected for the contribution of MDCC. The degree of labeling for the two preparations used in this study was 2.0 and 1.8, indicating that some protein was unlabeled (4, 5). Tests were conducted on each preparation to determine the stoichiometry of P_i binding and characterize the linearity of the fluorescence change. Accordingly, we find that approximately 70–75% of MDCC-PBP is responsive to inorganic phosphate, which is due to unlabeled PBP, as well as labeled but unresponsive MDCC-PBP (5).

Equations and data analysis. The n-step kinetic mechanism assumes that RecQ, bound to ssDNA with a binding site size, d, in nucleotides, undergoes multiple kinetic steps on ssDNA, with a forward kinetic rate constant, k_t (6). At each step, RecQ either moves forward or dissociates with a dissociation rate constant, k_d. During each kinetic step, RecQ binds and hydrolyzes ATP, followed by release of ADP and P_i. At saturating concentrations of ATP, this process occurs much faster than the translocation rate and these enzymatic steps are folded into one step in which RecQ couples the hydrolysis of c ATP molecules, where c is defined as the coupling efficiency (6). Each kinetic step leads to translocation by RecQ over a number of bases. The kinetic step size, m, describes the number of nucleotides RecQ translocates in one kinetic step. Multiplying m by k_t gives the translocation rate of RecQ in nucleotides per second. Translocation coupled to hydrolysis occurs until RecQ reaches the end of the lattice, and dissociates from the end with a dissociation rate constant, k_{end}.

The processivity is defined as the probability for RecQ to moving forward as opposed to dissociating from the ssDNA. Processivity, P, is given by Eq. **S1** (6, 7)

$$P = \frac{mk_t}{mk_t + k_d} \quad \textbf{[S1]}$$

where m, k_t, and k_d are as described before. A more intuitive value is the average number of nucleotides translocated by RecQ in a single round of translocation or N_{av} (Eq. **S2**).

$$N_{\text{av}} = \frac{1}{1-P} \quad \textbf{[S2]}$$

The kinetic traces measuring phosphate release were fit to Eq. **S3** using GraphPad Prism v5.0:

$$\frac{[P_i]}{[\text{RecQ}]} = A(1 - e^{-tk_{\text{obs}}}) + k_{\text{ss}}t \quad \textbf{[S3]}$$

where A is the amplitude of phosphate released, k_{obs} is the observe rate constant, and k_{ss} the rate constant for steady state ATP hydrolysis.

The kinetic traces for the dissociation of RecQ from poly dT (6) were fit to Eq. **S4** using GraphPad Prism v5.0:

$$f(t) = A(1 - e^{-k_{\text{obs}}t}) \quad \textbf{[S4]}$$

where A is the amplitude of the fluorescence change and k_{obs} is the observed rate of dissociation of RecQ from internal DNA regions.

The dependence of the observed dissociation rate constants on heparin concentration were fit to Eq. **S5** (6) using GraphPad Prism v5.0:

$$k_{\text{obs}} = k_d + k_{d,\text{heparin}}[\text{heparin}] \quad \textbf{[S5]}$$

where k_{obs} is the observed rate constant, k_d is the intrinsic dissociation rate constant from internal DNA sites, $k_{d,\text{heparin}}$ is the dissociation rate constant in the presence of heparin.

The kinetic traces for dissociation of RecQ from oligonucleotides were fit to Eq. **S6** (6).

$$F(t) = \frac{A}{(1+nr)}\mathcal{L}^{-1}\left(\frac{1}{s}\left(\frac{k_d r(n(k_d+s)+k_t((\frac{k_t}{k_t+k_d+s})^n-1)}{(k_d+s)^2} + k_{\text{end}}\frac{1}{s+k_{\text{end}}}\left(1+\frac{k_t r}{k_d+s}\left(1-\left(\frac{k_t}{k_t+k_d+s}\right)^n\right)\right)\right)\right) \quad \textbf{[S6]}$$

In this equation, the fluorescence $F(t)$ is given as a function of the several kinetic parameters mentioned above (k_t, k_d, and k_{end}) as well as r, the probability or ratio of the helicase binding internal DNA sites versus the ends of DNA, n, the number of kinetic step, and s, the Laplace variable (6). The value of the amplitude, A, is equal to the fluorescence change associated with tryptophan quenching. The step size, m, of the helicase is determined by monitoring presteady state translocation on increasing DNA lengths, globally fitting the solution for Scheme 1 and determining the number of kinetic steps, n, required to translocate on each DNA length. The software program Scientist version 3.0 (Micromath) was used to fit the n-step model to the dissociation traces

obtained with oligonucleotides using the inverse Laplace function and nonlinear least squares (NLLS) algorithm.

Using Eq. **S6**, we fit three sets of kinetic data. In each case, a dataset consisting of eight traces, dT_{35}-dT_{80}, was globally fit to Eq. **S6**. For each fit, k_t, k_{end}, and r, k_d were global parameters, while A and n were allowed to float for each trace. To simplify the fitting, we constrained k_d to be the value determined from the dissociation experiments with poly dT. Despite this constraint, converging to a solution was still difficult. To aid in the fitting of the model, a subset of the data, dT_{40}-dT_{60}, was initially fit to Eq. **S6**. Once parameters for k_t, k_{end}, and r were obtained, the larger dataset was fit (dT_{35}-dT_{80}) with k_d, k_{end} and r held fixed, and only k_t allowed to fit globally. Values reported in table 1 are the mean of the three datasets and the error is the standard deviation of the three values.

The kinetic step size was determined by a linear-least squares analysis of the data to Eq. **S7** using GraphPad Prism v5.0 (6).

$$n = \frac{(L-d)}{m} \qquad \textbf{[S7]}$$

In this equation, L is the DNA length in nucleotides, d is the binding site size of the helicase, and m is the kinetic step size.

The dependence of the amplitudes of the rapid phase of phosphate release were fit using to Eq. **S8** (6),

$$\frac{[ADP]_{rapid}}{[RecQ]} = \left(\frac{rcP(n(1-P)+P(P^n-1))}{(1+nr)(P-1)^2}\right) \qquad \textbf{[S8]}$$

where the parameters r, n, are described above and the processivity, P, is defined as $P = \frac{k_t}{k_t+k_d}$. Eq. **S8** is a simplification of time-dependent production of ADP from the n-step kinetic model at infinite time where c is the coupling efficiency (the number of ATP molecules hydrolyzed per kinetic step) (6). GraphPad Prism v.5 was used to fit Eq. **S8** using NLLS by holding the values for step size m and r constant, and letting the processivity, P, coupling efficiency, c, and site size, d, be fitted parameters. We found that although the value of c itself was not well constrained in the fitting, the ratio, m/c, was well defined as determined by varying the fitting parameters and constraints. The values reported in Table 1 are the best-fit value and the standard deviation.

Equilibrium binding experiments. Spectra for equilibrium binding of RecQ to poly dT were collected on a SLM fluorimeter controlled by Vinci Software (ISS). Excitation and emission wavelengths and slit-widths were set to 8 mm. Binding of poly dT to RecQ was carried out in 25 mM TrisOAc (pH 7.5) and 25 mM NaCl. This solution (350 μL) was added to a 700 μL quartz cuvette (Starna Cells), and RecQ was added to a final concentration of 0.5 μM. The solution was incubated at 25° for 2 min at each titration point before data acquisition was started. The native tryptophan residues were excited at 280 nm and the fluorescence emission was observed at 340 nm. Fluorescence emission was observed for 10 s, and the mean intensity was recorded for each titration point.

To monitor binding of inorganic phosphate to MDCC-PBP, titrations were carried out in solution containing 25 mM TrisOAc (pH 7.5) and 1 mM DTT. The reaction solution (350 μL) was added to a 700 μL quartz cuvette, along with MDCC-PBP to a final concentration of 3 μM. The fluorophore was excited at 430 nm and the fluorescence emission was observed at 465 nm. NaH_2PO_4 at the indicated concentrations was added and the solution was incubated for 2 min prior to taking a reading. The activity of the inorganic phosphate sensor was determined as described (3).

Stopped-flow assays. Stopped-flow experiments were conducted on an SX.18MV-R stopped-flow reaction analyzer (Applied Photophysics). All concentrations reported below are prior to mixing and the final concentrations are half of these values. Traces consisted of 1,000 points and each trace presented is an average of 5–8 traces. The experiments were repeated several times on different days to obtain a standard deviation for the amplitude and observed rate.

Dissociation assays monitored by tryptophan fluorescence. All reactions were carried out in a solution containing 25 mM TrisOAc (pH 7.5) and 0.1 mM DTT (SF buffer). Syringe 1 in the stopped-flow apparatus contained 0.2 μM RecQ, 0.6 μM molecules of thymine oligonucleotide (dT_n $n = 30, 35, 40, 45, 50, 55, 60, 70, 75, 80$) or 40 μM in nucleotides of poly dT. Syringe 2 contained 1 mM ATP, 1 mM $Mg(OAc)_2$ and 1 mg/mL heparin unless noted. Reaction solutions were allowed to incubate in their syringes, at 20° for 2 min before starting the experiment. Five reactions were shot through the flow cell to fill the apparatus with the chemistry. The excitation wavelength was set to 280 nm while fluorescence emission was filtered with a 320 nm cutoff filter. Between each experiment, the syringes were washed with Nanopure water and SF buffer prior to loading the reaction.

Phosphate release assays. All reactions were carried out in SF buffer, which included a P_i mop consisting of 0.01 Units/mL of bacterial purine nucleoside phosphorylase (PNPase (Sigma-Aldrich); resuspended in 20 mM TrisOAc (pH 7.5), and stored at −80 °C and 200 μM MEG to eliminate contaminating, free phosphate. Syringe 1 in the stopped-flow apparatus contained additionally 0.2 μM RecQ, 0.6 μM molecules dT_n ($n = 30, 35, 40, 45, 50, 55, 60, 70, 75, 80$). Syringe 2 contained 6 μM MDCC-PBP, 1 mM ATP, 1 mM $Mg(OAc)_2$ and 1 mg/mL heparin unless otherwise noted. The solutions were allowed to incubate at 20° for 2 min prior to mixing. The excitation wavelength used for MDCC-PBP was 430 nm, while the emission wavelength was monitored using a 455 nm cutoff filter. Between each experiment, the syringes were washed with Nanopure water, and then filled with SF buffer containing 0.1 Units/mL PNPase and 200 μM 7-MEG for 2 min to remove free phosphate. The syringes were then washed with Nanopure water and then filled with SF buffer without any mop.

A known, standard concentration of NaH_2PO_4 was used to generate a standard curve relating a change in photo-multiplier tube voltage to concentration of P_i for each day by mixing the inorganic phosphate against the solution in syringe 2. Heparin is known to affect the kinetics and affinity of binding of MDCC-PBP (8); hence 0.5 mg/mL heparin was included in the solution during calibration. The fluorescence change for MDCC-PBP was determined to be linear up to 2 μM NaH_2PO_4. This experimental range was appropriate as the phosphate release experiments with RecQ only required measuring at most 1.5 μM P_i.

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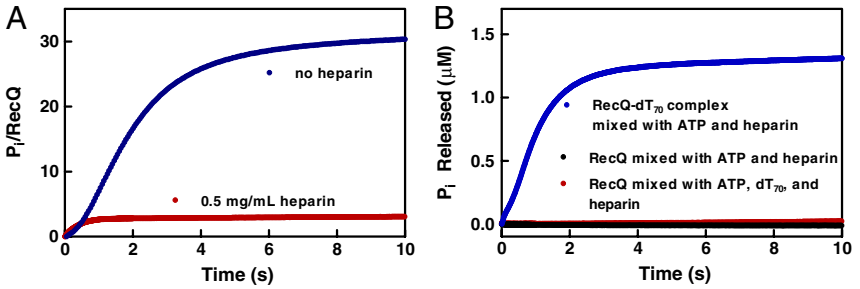

Fig. S1. Heparin acts to trap free RecQ in solution. (*A*) Traces show the kinetics of phosphate release by RecQ (100 nM) bound to dT_{45} (0.3 μM molecules) in the presence (red trace) or absence of heparin (blue trace). In the presence of the free-protein trap, the helicase undergoes only one round of translocation and is prevented from rebinding to the DNA. (*B*) To demonstrate the effectiveness of heparin as a trap, RecQ (100 nM) prebound to dT_{70} (0.3 μM molecules) was rapidly mixed with ATP in the presence of heparin and MDCC-PBP (blue trace). For comparison, RecQ alone was mixed rapidly with heparin and ATP, either in the presence or absence of dT_{70} (red and black traces, respectively). The initial rapid production of P_i was only observed when RecQ is prebound to dT_{70}.

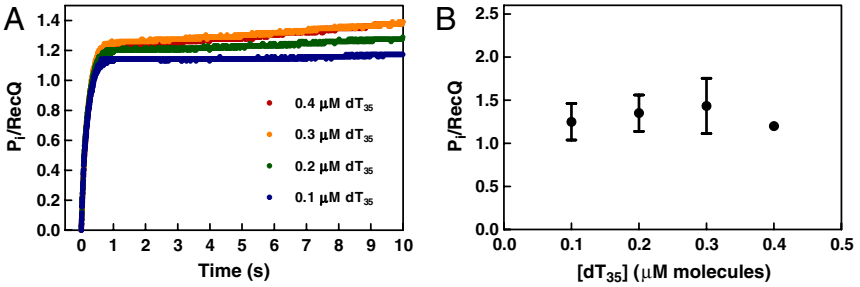

Fig. S2. Increasing the concentration of ssDNA has no effect on the amplitude of phosphate release. (*A*) RecQ (100 nM) was preincubated with increasing concentrations of dT_{35} and then rapidly mixed with ATP in the presence of heparin and MDCC-PBP. Traces were converted from raw fluorescence values to the concentration of inorganic phosphate using a calibration curve. (*B*) The amplitudes of the rapid phase of phosphate release did not change with varying concentration of DNA.

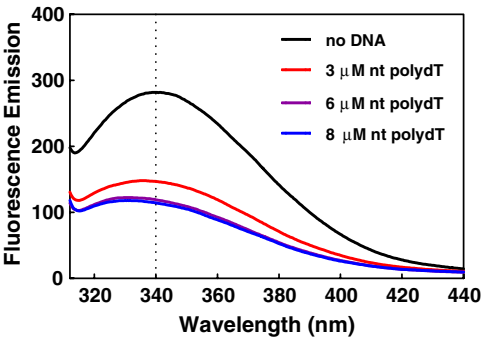

Fig. S3. The intrinsic tryptophan fluorescence of RecQ is quenched upon binding poly dT. The fluorescence emission spectra of RecQ (1 μM) using an excitation wavelength of 280 nm are plotted. Upon addition of ssDNA, the fluorescence peak at 340 nm decreases. The fluorescence intensity at 380 nm, where the emission from tyrosine residues is negligible, was also quenched by DNA, implying that tryptophan residues are being quenched upon binding of DNA by RecQ.

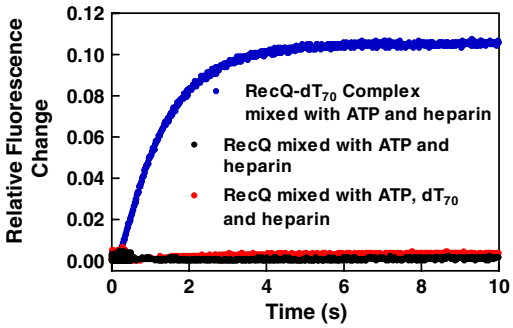

Fig. S4. The fluorescence of native tryptophan residues within RecQ does not change upon binding to heparin. Dissociation was monitored by mixing RecQ (100 nM) prebound to dT_{70} (0.3 μM molecules) with ATP in the presence of heparin (blue trace). RecQ alone was also mixed rapidly with heparin and ATP, either in the presence or absence of dT_{70} (black and red traces, respectively). The initial rapid increase in fluorescence was only observed when RecQ is prebound to dT_{70}.

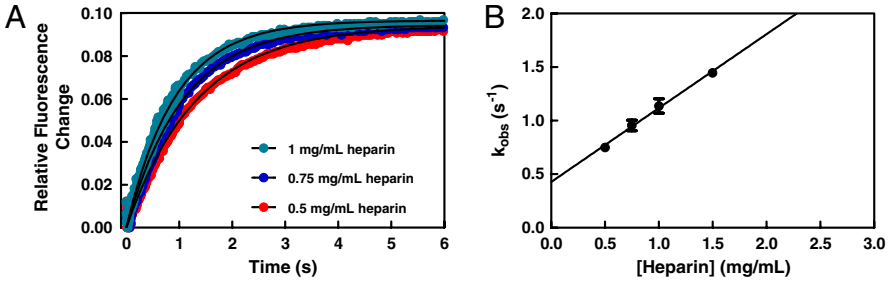

Fig. S5. The dissociation rate constant from internal DNA sites (k_d) is dependent on the heparin concentration. (*A*) RecQ (100 nM) prebound to poly dT (20 μM in nucleotides) was rapidly mixed with ATP, and increasing concentrations of heparin. Dissociation from the DNA was monitored by an increase in the fluorescence of native fluorescence. Traces are the best-fit curves to Eq. **S4** (solid lines). (*B*) The observed rate constant for dissociation, k_{obs}, increases linearly with the concentration of heparin. The data were fit to Eq. **S5** (solid line), resulting in a slope of 0.69 ± 0.04 (mg/mL) heparin • s^{-1} and Y-intercept of 0.43 ± 0.04 s^{-1}. The Y-intercept is taken to be the rate of dissociation of RecQ from DNA in the absence of heparin.