MATERIALS AND METHODS (SUPPLEMENT)

Reagents – Unless otherwise stated, all reagents were from Sigma-Aldrich Co. ATP was from Roche Applied Science, and mdATP from Jena Bioscience. P_i standard solution was from Merck. m13mp18 ssDNA isolation was carried out as in (1).

Oligonucleotides were from Invitrogen and VBC-Biotech (DNA_54mer: 5’-CGT TGC GCA ATT AAG CTC TAA GCC ATC GTC CTG AAC AAT GAC CTC TTA TCA AAA GGA-3’; DNA_1: 3’-FLU-TCC TTT TGA TAA GAG GT C ATT TTT GCG GAT GGC TTA GAG CTT AAT TGC GCA ACG-FLU-3’; DNA_2: 5’-HEX-DNA_54mer).

Heparin (Sigma H3393) was dissolved in sterile distilled water at 50 mg/ml and dialyzed against sterile distilled water and then SF-50 buffer in MWCO 3500 dialysis tubing (Serva 44183).

Plasmids – The coding region of the BLM construct comprising amino acids 642-1290 (“helicase module” (BLMHM) comprising the N- and C-core, RecQCt and HRDC domains) was amplified by PCR from pLK621 (full-length BLM in pYES2 (2), kindly provided by Lumir Krejci (Masaryk Univ., Brno, Czech Rep.)), and subcloned between the NcoI and SapI sites of pTXB3 (New England Biolabs). Subcloning was carried out in two steps due to an additional NcoI site within the BLM coding region. The final DNA construct was verified by DNA sequencing.

Protein purification – BLM_HM was expressed and purified as described previously (3), with the exception that we used 5x1 ml HiTrap CM FF columns. Fractions containing BLM_HM were pooled and dialyzed against Storage Buffer (50 mM Tris-HCl pH 7.5, 200 mM NaCl, 1 mM DTT, 10 (v/v)% glycerol) overnight at 4°C. Typically, 1-2 mg pure (> 95 % by SDS-PAGE), untagged BLM_HM could be produced per liter culture. Coumarin-labeled P_i binding protein (MDCC-PBP) was produced as described earlier (4).

MDCC-PBP fluorescence calibration – To establish correspondence between MDCC-PBP fluorescence change amplitudes and released P_i concentration, the system was calibrated by rapidly mixing 2µM MDCC-PBP (pre-incubated with a “P_i mop” described in Materials and Methods) with P_i standard solutions of different concentrations in the stopped-flow. The amplitudes of MDCC-PBP fluorescence transients depended linearly on P_i concentration in the observed range (0-2µM) (Fig. S2).
SUPPLEMENT TO FIG. 3B: EQUATION FOR THE BINDING OF BLM$^{\text{HM}}$ TO OLIGO-DT

Amplitude = $A_0 + \Delta A \frac{c_B + nc_{dT} + K_d - \sqrt{(c_B + nc_{dT} + K_d)^2 - 4c_B nc_{dT}}}{2c_B}$

where $A_0$ and $(A_0 + \Delta A)$ are amplitudes at zero and saturating oligo-dT concentrations, respectively; $c_B$ and $c_{dT}$ are concentrations of BLM$^{\text{HM}}$ and oligo-dT, respectively; $n$ is the binding stoichiometry (mol BLM$^{\text{HM}}$/mol oligo-dT), and $K_d$ is the dissociation constant.

SUPPLEMENT TO FIG. 4B: EQUATION FOR ssDNA LENGTH-DEPENDENCE OF ATP CONSUMPTION UPON SINGLE-ROUND TRANSLOCATION

$A_{\text{rand}}(L) = \frac{P}{1-P} \left( \frac{1}{1 - \frac{1}{C(L-b)+1}} \right)$

where $A_{\text{rand}}(L)$ is the amplitude of ATP consumption in single-round translocation conditions (expressed as mol ATP/mol enzyme), assuming random initial start site (cf. Fig. S1A-B), $P$ is processivity (expressed as the probability of taking the next step of translocation), $L$ is the length of the track (in nucleotides), $b$ is the binding site size (in nucleotides), and $C$ is the coupling stoichiometry (mol ATP hydrolyzed/mol enzyme/nucleotide traveled). Parameters $P$, $b$ and $C$ were left to float in the fit. Note that this equation can be interpreted only for $L \geq b$, and a y offset of 1 was applied in the fit shown in Fig. 4B to account for the single-turnover burst in $P_i$ production that was present even in the absence of translocation (Fig. 4A inset). Deduction and applicability of the equation is detailed in (5).

SUPPLEMENT TO FIG. 4C: EQUATION FOR DEPENDENCE OF PROCESSIVITY ON HEPARIN CONCENTRATION

$P([T]) = \frac{K_d' + [T]}{P_0 + [T]}$

where $P$ is processivity (expressed as the probability of taking the next step of translocation), $[T]$ is heparin (protein trap) concentration, $K_d'$ is the apparent dissociation constant for heparin binding to BLM$^{\text{HM}}$ during translocation; and $P_0$ and $P_T$ are processivity values at zero and saturating heparin concentrations, respectively. Parameters $K_d'$, $P_0$ and $P_T$ were left to float in the fit. Deduction and applicability of the equation is detailed in (5).
SUPPLEMENT TO FIG. 5B: EQUATION FOR ssDNA LENGTH-DEPENDENCE OF THE STEADY-STATE ATPase $k_{\text{cat}}$ OF BLM$^\text{HM}$

The overall steady-state ATP hydrolysis rate constant ($k_{\text{cat}}$) of BLM$^\text{HM}$ is dictated by two types of ATP hydrolysis cycles occurring at different turnover rates. Upon binding to ssDNA, BLM$^\text{HM}$ performs a mean number of $(L-b)/2s$ ATPase cycles at rate constant $k_{\text{trans}}$ before reaching the 5'-end (Fig. 1A). At the end, it may undergo ATPase cycling at rate constant $k_{\text{end}}$ or, alternatively, it may dissociate from the terminus at $k_{\text{off, end}}$. The mean number of ATPase cycles performed at a rate $k_{\text{end}}$ will thus be $k_{\text{end}}/k_{\text{off, end}}$. At saturating ssDNA concentration (>100*K$^\text{DNA}$), rebinding to a new ssDNA molecule occurs rapidly and does not influence the cycling rate. Neither does the relatively infrequent dissociation of BLM$^\text{HM}$ from internal sites of ssDNA ($k_{\text{off, int}}$). Thus, at ssDNA lengths greater than the BLM$^\text{HM}$ binding site size, the overall ATPase cycle time (1/$k_{\text{cat}}$) will be the weighted average of the 1/$k_{\text{trans}}$ and 1/$k_{\text{end}}$ cycle times, hence

$$k_{\text{cat}} = \left( \frac{L-b}{2s} \cdot \frac{1}{k_{\text{trans}}} + \frac{k_{\text{end}}}{k_{\text{off, end}}} \cdot \frac{1}{k_{\text{end}}} \right)^{-1}$$

In the fit, we fixed $s = 1$ (Fig. 4B) and $b = 14$ (Fig. 3B, 4B, 5A; or $b = 12$ to account for the point obtained using dT$_{12}$, yielding quasi-identical results), and floated the other parameters.
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**SUPPLEMENTARY FIGURES**

**Figure S1: ssDNA and mdATP binding to BLM\(^{HM}\)**

*A.* Fluorescence titration of 40 nM 3’-FLU-labeled ssDNA (DNA\(_1\), solid symbols) and 5’-HEX-labeled ssDNA (DNA\(_2\), open symbols) with BLM\(^{HM}\). Solid lines show hyperbolic fits to the datasets based on the equation \(F = F_0 + \Delta F \cdot c_{BLM}/(c_{BLM} + K_{d,app})\) where \(F\) is the fluorescence intensity at a given BLM\(^{HM}\) concentration \((c_{BLM})\); \(F_0\) and \((F_0 + \Delta F)\) are fluorescence intensities of the labeled DNA substrates in the BLM-free and BLM-bound states, respectively; and \(K_{d,app}\) is the apparent dissociation constant of BLM\(^{HM}\) binding to the DNA substrate assuming a binding stoichiometry of 1:1. The fits yielded \(K_{d,app}\) values of 240 ± 40 nM and 150 ± 30 nM for DNA\(_1\) and DNA\(_2\), respectively. To assess the effect of binding stoichiometry on \(K_{d,app}\), we also performed fits to the datasets based on the quadratic binding equation

\[
\text{Fluorescence} = F_0 + \Delta F \left(\frac{c_B + nc_{oligo}}{2nc_{oligo}} + K_d - \sqrt{\left(\frac{c_B + nc_{oligo} + K_d}{2nc_{oligo}}\right)^2 - 4c_Bnc_{oligo}}\right)
\]
where $F_0$ and $(F_0 + \Delta F)$ are fluorescence levels at zero and saturating BLM$^{\text{HM}}$ concentrations, respectively; $c_B$ and $c_{\text{oligo}}$ are concentrations of BLM$^{\text{HM}}$ and oligonucleotide, respectively; $n$ is the binding stoichiometry (mol BLM$^{\text{HM}}$/mol oligonucleotide), and $K_d$ is the dissociation constant. Fits using a fixed $n = 1$ yielded $K_d$ values $210 \pm 30$ nM and $100 \pm 20$ nM for DNA$_{\text{1}}$ and DNA$_{\text{2}}$, respectively (curves were indistinguishable from those of hyperbolic fits). When using $n = 4$ (dotted lines), the resulting $K_d$ values were $100 \pm 20$ nM and $27 \pm 16$ nM for DNA$_{\text{1}}$ and DNA$_{\text{2}}$, respectively. The similar $K_d$ values obtained with the two different DNA substrates confirmed the hypothesis that BLM$^{\text{HM}}$ binds randomly along ssDNA. The moderate differences between the $K_d$ values for DNA$_{\text{1}}$ and DNA$_{\text{2}}$ may arise from the possibility that different fluorophores may sense protein binding over different distances (as was observed in (6)). The obtained $K_d$ values represent averages over several possible binding modes (at oligonucleotide ends and middle regions). 

B, Normalized fluorescence levels of 400 nM 5’-HEX-labeled oligo-dT substrates of different length (dT$_{\text{20}},$ dT$_{\text{35}},$ dT$_{\text{50}},$ dT$_{\text{65}},$ dT$_{\text{79}}$) upon addition of 100 or 400 nM BLM$^{\text{HM}}$. (Fluorescence levels of BLM$^{\text{HM}}$-free 5’-HEX-oligo-dT substrates were taken as unity.) BLM$^{\text{HM}}$:oligo-dT molar ratios are indicated in the figure. Fluorescence levels were strongly dependent on the BLM$^{\text{HM}}$ :ssDNA molar ratio in dT$_{\text{20}},$ whereas they showed a tendency toward unity as the oligo-dT length increased. This behavior corroborates random binding of BLM$^{\text{HM}}$ along oligo-dT. 

C, Kinetics of mdATP binding to BLM$^{\text{HM}},$ measured by rapidly mixing 0.5 µM BLM$^{\text{HM}}$ in the absence (closed squares) and in the presence (open circles) of 1 µM dT$_{\text{18}}$ with 0-20 µM mdATP in the stopped-flow, monitoring mdATP fluorescence. The applied dT$_{\text{18}}$ concentration can be regarded as saturating based on the data of Figs. S1A, 3B and 5A, indicating that the $K_{\text{DNA}}$ or $K_d$ values are well below the micromolar range for both dT$_{\text{18}}$ and dT$_{\text{54}},$ and the overall DNA-binding properties of BLM$^{\text{HM}}$ are independent of DNA length above the binding site size ($b = 14$ nt). Dependence of observed rate constants ($k_{\text{obs}}$) of single exponential fits to experimental traces (inset) on mdATP concentration is shown. Slopes of linear fits to the datasets delineated mdATP binding rate constants of $5.5 \pm 1.7$ µM$^{-1}$s$^{-1}$ (no DNA) and $6.9 \pm 0.7$ µM$^{-1}$s$^{-1}$ (in DNA; $k_1$ in Fig. 1B, Table 1), whereas the intercepts (mdATP dissociation rate constants) were $140 \pm 20$ s$^{-1}$ (no DNA) and $120 \pm 10$ s$^{-1}$ (in DNA; $k_{-1}$ in Fig. 1B, Table 1).
Figure S2: $P_i$ calibration of MDCC-PBP fluorescence amplitudes

Shown is the $P_i$ concentration dependence of the amplitude of MDCC-PBP fluorescence changes obtained upon rapidly mixing 2 µM MDCC-PBP with different concentrations (0-1 µM) of $P_i$ standard (Merck) in the stopped-flow. MDCC-PBP was preincubated with a $P_i$ mop. The slope of the linear fit to the plot (1.46 V/µM $P_i$ in the experiment shown) was used to calculate the concentration of released $P_i$ in MDCC-PBP fluorescence experiments. The positive $y$ intercept is indicative of the presence of a small amount of $P_i$ in the buffer in the absence of the $P_i$ mop. (The $P_i$ mop was present in all syringes in all other experiments.) Calibration was performed in each experiment session.
Figure S3: dmADP binding to and dissociation from BLM$^{\text{HM}}$ in the absence of DNA

Stopped-low record of 1 µM BLM$^{\text{HM}}$ mixed with 1 µM dmADP (light gray squares). The solid line is a best-fit double-exponential approximation to the data, yielding the rate constants $k_{\text{fast}} = 31 \pm 1 \text{ s}^{-1}$, and $k_{\text{slow}} = 0.37 \pm 0.11 \text{ s}^{-1}$ with fractional amplitudes $A_{\text{slow}}/A_{\text{fast}} = 0.07$. The presence of two phases in the binding reaction even with a slow second phase corroborates that BLM$^{\text{HM}}$ distributes between two conformational states in the ADP-bound form, and the isomerization between the two states is a slow process. The dmADP dissociation transient with the corresponding fit (dark gray squares and solid black line) from Fig. 3A is shown to aid comparison of the slow phases in the ADP binding and release reactions.
Figure S4: Effect of heparin on the ATPase activity of DNA-free BLM\textsuperscript{HM}

The ATPase activity of 0.5 µM BLM\textsuperscript{HM} was measured using a PK/LDH coupled assay in the absence and in the presence of heparin (0.5-4.5 mg/ml). 0.5 mg/ml heparin increased the ATPase activity of BLM\textsuperscript{HM} about 2.5-fold (from 0.09 s\textsuperscript{-1} to 0.22 s\textsuperscript{-1}). No further activation was observed at higher heparin concentrations. Heparin-induced activation of the BLM\textsuperscript{HM} ATPase is negligible compared to the >200-fold acceleration of the ATPase activity by ssDNA substrates (Fig. 2B, Fig. 2D, Fig. 5B).
Figure S5: ATP consumption during single-round translocation of BLM<sup>HM</sup> on m13mp18 circular ssDNA

50 nM BLM<sup>HM</sup> was preincubated with 10 nM m13mp18 and was rapidly mixed with 0.5 mM ATP plus 4 mg/ml heparin in the stopped-flow, and P<sub>i</sub> production from ATP was followed using MDCC-PBP (2 µM in all syringes). The amplitude of the rapid P<sub>i</sub> production phase corresponding to single-round translocation (14 mol P<sub>i</sub>/mol BLM<sup>HM</sup>) was in good agreement with the processivity value of P = 0.92 at this heparin concentration (cf. Fig. 4B-C). Conversion of MDCC-PBP fluorescence to mol P<sub>i</sub>/mol BLM<sup>HM</sup> ratio was based on a calibration curve (Fig. S2).
Figure S6: Trp fluorescence experiments monitoring dissociation of BLM\textsuperscript{HM} from ssDNA

\textbf{A}, Trp fluorescence profiles recorded upon rapidly mixing 1\,μM BLM\textsuperscript{HM} plus 1\,μM dT\textsubscript{36} or dT\textsubscript{54} with 500 \,μM ATP in the presence and absence of heparin (HEP; 4\,mg/ml). Traces are offset for clarity. The absence of the Trp fluorescence increase in the absence of heparin (-HEP) confirmed that the fluorescence enhancement reports dissociation of BLM\textsuperscript{HM} from oligo-dT after completing a single round of translocation. Solid lines indicate the results of global kinetic simulations in which BLM\textsuperscript{HM} molecules (initially randomly distributed along oligo-dT) perform single-round translocation and then dissociate from ssDNA (as sensed by Trp fluorescence) to bind to the heparin trap, governed by parameters in Table 1. Initial and final Trp fluorescence levels, corresponding to those of ssDNA-bound and heparin-bound BLM\textsuperscript{HM}, respectively, were left free to float. The simulated profiles of dissociation closely matched the Trp fluorescence time courses.

\textbf{B}, Trp fluorescence profiles recorded upon rapidly mixing 1 \,μM BLM\textsuperscript{HM} plus 10 nM m13mp18 circular ssDNA in the presence and absence of 0.5 \,mg/ml heparin. In the presence of heparin, dissociation of BLM\textsuperscript{HM} from DNA occurred with a $k_{\text{obs}}$ of 0.18 ± 0.04 \,s\textsuperscript{-1} (Table 1). This value is expected to be close to the rate constant of BLM\textsuperscript{HM} dissociation from ssDNA during translocation ($k_{\text{off, int}}$ in Fig. 1B, Table 1). The ssDNA dissociation rate constant in the nucleotide-bound states of BLM\textsuperscript{HM} is probably similar to this value, as ssDNA did not affect BLM\textsuperscript{HM}'s ATP affinity (Fig. S1C), and BLM\textsuperscript{HM} bound ssDNA with high affinity also in the ADP-bound state (Fig. 3B).

The paradoxical phenomenon that BLM\textsuperscript{HM} dissociation from oligo-dT substrates resulted in a Trp fluorescence increase (panel \textbf{A}) while that from m13mp18 substrate resulted in a fluorescence decrease (panel \textbf{B}) is likely a structure- and isoform-specific property of circular ssDNA-bound BLM\textsuperscript{HM}, as these reactions resulted in a fluorescence increase in all DNA substrates when we used \textit{E. coli} RecQ in place of BLM\textsuperscript{HM}.
Table S1: Sequence alignments of SF1 and SF2 helicase regions containing the Trp residue implicated in hydrophobic stacking-based translocation mechanism

| Superfamily | Enzyme  | Sequence        |
|-------------|---------|----------------|
| SF1         | UvrD    | SIYG<sup>256</sup>WRG |
|            | PcrA    | SIYR<sup>259</sup>WRG |
|            | Rep     | SIYS<sup>259</sup>WRG |
| SF2         | RecQ    | 154<sup>W</sup>GHDFR |
|            | BLM     | 803<sup>W</sup>GHDFR |
|            | WRN     | 676<sup>W</sup>GHDFR |
|            | RecQL1  | 227<sup>W</sup>GHDFR |
|            | RecQL4  | 613<sup>W</sup>SHNFR |
|            | RecQL5  | 165<sup>W</sup>GHDFR |
|            | Sgs1    | 816<sup>W</sup>GHDFR |
|            | dmBLM   | 873<sup>W</sup>GHDFR |