Fig. S1. KMC simulations of the alternative sequential dissociation model. (A) Schematic illustration of a hybrid model invoking components of both accumulation and sequential eviction. In this example, three proteins accumulate ($N = 3$) before dissociation. Additional details of this model are presented in Materials and Methods. (B) Pause duration distributions ($\langle \Delta t \rangle$) for simulations involving differential numbers of accumulated proteins ($N$) for each different array size as indicated. We fit the data for $n = 5$ with the linear and exponential equations. The sums of squared residuals for best fit curves are 0.2 (seconds$^2$) for the linear equation and 221.9 (seconds$^2$) for the exponential equation, suggesting that the linear equation fits better than the exponential equation. (C) Experimental schematic and kymograph showing that RecBCD (unlabeled) can push two molecules of differentially labeled EcoRI$^{E111Q}$. 
Fig. S2. KMC simulations to predict translocase movement through protein arrays. (A) Representative kymographs derived from the KMC simulations of the accumulation, sequential, and spontaneous models. The white lines represent the movement of the translocase along a DNA molecule, pausing on encountering the protein array. (B) Distributions of pause durations ($\Delta t$). Colored lines represent the distribution derived from simulations using the 5–50x protein arrays as indicated. Each distribution was calculated from a total of 1,000 simulation trajectories.
Fig. S3. Engineered 5x, 10x, 30x, and 50x arrays of EcoRI binding sites on λ-DNA. (A) A schematic of the λ-DNA and a schematic of the expected fragment sizes for the different DNA array lengths when digested with SalI and Eagl. (B) A 1% agarose gel showing that the length of engineered sites is as expected. In this assay, 5-μL aliquots of the engineered λ-DNA were incubated with 1 μL of SalI-HF (R3138S; New England BioLabs) and Eagl-HF (R3505S; New England BioLabs) in 38 μL water supplemented with 5 μL of 5× CutSmart buffer (B7204S; New England BioLabs) for 2 h. The reaction was mixed with 10 μL of 6× loading dye (B7024S; New England BioLabs) and run on the gel for 1 h at 100 V; a 1-kb DNA ladder (N3232S; New England BioLabs) was also run. The magenta arrow highlights the 3.3-kb native DNA fragment [SalI (33.4 kb) to Eagl (36.7 kb)] present before cloning in the array fragments. The green arrows highlight the array-containing fragments for each of the different substrates. MW, molecular weight.
Fig. S4. Translocation properties of Qdot-tagged RecBCD in the absence of EcoRI\textsuperscript{E111Q}. (A) An example of a kymograph showing the movement of Qdot-tagged RecBCD along an unlabeled DNA molecule in the absence of EcoRI\textsuperscript{E111Q}. RecBCD was prebound to the DNA, and translocation was initiated by the injection of ATP (white arrow). (B) Velocity distribution for Qdot-tagged RecBCD ($n = 269$). (C) Scatter plot of RecBCD velocities before and after encountering the EcoRI binding site array for control experiments conducted in the absence of any EcoRI\textsuperscript{E111Q} ($n = 269$). The $R$ represents the Pearson correlation coefficient.
The saturation of the λ-DNA substrate with EcoRI\textsuperscript{E111Q}. (A) An example of the bulk biochemical assay used to establish conditions for saturating the EcoRI binding site arrays with EcoRI\textsuperscript{E111Q}. In this example, the λ-DNA substrate bearing a 5x binding site array was preincubated with varying concentrations of EcoRI\textsuperscript{E111Q} for 30 min at room temperature. The reactions were then challenged by the addition of WT EcoRI for 15 min at 37 °C. DNA products were then resolved on a 1% agarose gel. (B) Position distribution histogram from a DNA curtain assay with Qdot-tagged EcoRI\textsuperscript{E111Q} bound to a λ-DNA harboring a 5x array. The location of the array is indicated along with the four peaks corresponding to the native EcoRI binding sites. The height of the 5x peak is similar to the 1x peak heights, because the data are not corrected for signal intensity.
**Fig. S6.** The collision of unlabeled RecBCD with an unlabeled 5× EcoRI array. (A) Representative kymographs from experiments where unlabeled RecBCD translocates on YoYo-1–stained DNA containing the 5× EcoRI binding site array (position indicated by red arrows) in the absence (Upper) and presence (Lower) of unlabeled EcoRI<sup>E111Q</sup>. The yellow arrows in Lower indicate the beginning and ending of the RecBCD pause when EcoRI<sup>E111Q</sup> is bound to the DNA. Note that the protein-bound EcoRI arrays routinely fluoresce more brightly with YoYo-1 compared with the naked flanking DNA. (B) Pause time distribution for unlabeled in the presence of unlabeled EcoRI<sup>E111Q</sup>. The dataset is fitted by a Gaussian distribution to derive the average pause duration (9.5 ± 0.4 s).
Fig. 57. Digestion of DNA oligonucleotides by WT EcoRI in the presence and absence of RecBCD. (A) Schematic of the oligonucleotide substrates containing proximal and distal EcoRI binding sites. The EcoRI binding sites are shown in blue, and the RecBCD binding site is shown in red. (B) An example denaturing gel showing a time course of EcoRI digestion in the presence of (static) RecBCD. (C) The normalized signal intensity of digested fraction at 30 min is plotted for each substrate in the presence and absence of RecBCD. Error bars represent SD calculated from three independent experiments. In the cartoon depictions, the DNA is black, EcoRI binding sites are red, EcoRI is magenta, and the RecBCD molecules are green.