

Description of Additional Supplementary Files

File name: Supplementary Data 1

Description: Source data for all data-containing figures

File name: Supplementary Data 2

Description: A list of the core engineered features implemented in the current version of DeepTRACE used in this study. Additional features undergoing development and testing will be incorporated in future releases.

File name: Supplementary Movie 1

Description: Synchronized fluorescence video and model annotation corresponding to the reversible two state diffusion simulation shown in Fig. 3b. The left panel shows the raw fluorescence sequence of the tracked molecule; the centre panel shows the reconstructed track coloured by the ground truth state; and the right panel shows the track coloured by predictions made by a DeepTRACE model trained on 147 independent tracks. The dynamic range is normalised independently for each frame.

File name: Supplementary Movie 2

Description: Synchronized fluorescence video and model annotation corresponding to the periplasmic transport simulation shown in Fig. 4a. The left panel shows the raw fluorescence sequence of the tracked molecule; the centre panel shows the reconstructed track coloured by the ground truth state; and the right panel shows the track coloured by DeepTRACE model predictions trained on 1,298 independent tracks from a separate simulation. The video displays the full sequence of frames, whereas Fig. 4a shows a cropped segment centred on the transport event for visual clarity. Video dynamic range is normalised globally across all frames.

File name: Supplementary Movie 3

Description: Synchronized fluorescence video and model annotation corresponding to the polar plasmid binding simulation shown in Fig. 5a. The left panel shows the raw fluorescence sequence of the tracked molecule; the centre panel shows the reconstructed track coloured by the ground truth state; and the right panel shows the track coloured by DeepTRACE model predictions trained on 1,061 independent tracks from a separate simulation. Video dynamic range is normalised globally across all frames.

File name: Supplementary Movie 4

Description: Synchronized fluorescence video and annotations corresponding to the single-molecule DNA repair event shown in Fig. 3d (PolI experimental data). The left panel shows the raw fluorescence sequence of the tracked molecule overlaid on an inverted brightfield reference image; the centre panel shows the reconstructed track coloured by independent human annotation; and the right panel shows the trajectory coloured by DeepTRACE model predictions using a model trained on 1,098 tracks from a two state

reversible diffusion simulation. Video dynamic range is normalised globally across the video. Dashed white lines indicate the segmented cell boundary.

File name: Supplementary Movie 5

Description: Synchronized fluorescence video and model annotation corresponding to the uniform binding simulation shown in Fig. 3f. The left panel shows the raw fluorescence sequence of the tracked molecule; the centre panel shows the reconstructed track coloured by the ground truth state; and the right panel shows the track coloured by DeepTRACE model predictions trained on 1,048 independent simulated tracks undergoing uniformly-distributed binding events. Video dynamic range is normalised globally across all frames.

File name: Supplementary Movie 6

Description: Synchronized fluorescence video and model annotation corresponding to the polar binding simulation shown in Fig. 3f. The left panel shows the raw fluorescence sequence of the tracked molecule; the centre panel shows the reconstructed track coloured by the ground truth state; and the right panel shows the track coloured by DeepTRACE model predictions trained on 1,048 independent simulated tracks undergoing uniformly-distributed binding events. Video dynamic range is normalised globally across all frames.