Dissecting protein-induced DNA looping dynamics in real time

Supplementary Information

Authors

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Thresholding Kinetics

As is seen in figure 1, the hypothesis of the protein association being the only concentration dependant rate is confirmed. Also, the curvature of both the loop formation and breakage rate at high D79A concentration is a well understood; when a tether spends relatively little time in one of the two states (looped or unlooped), the distribution of the RMS and the threshold value will shift. For example, when a tether spends most of its time in the looped state, a lower threshold value will be obtained. This in turn will lead to a perceived lowering of the loop formation rate and an increased loop breakage rate.

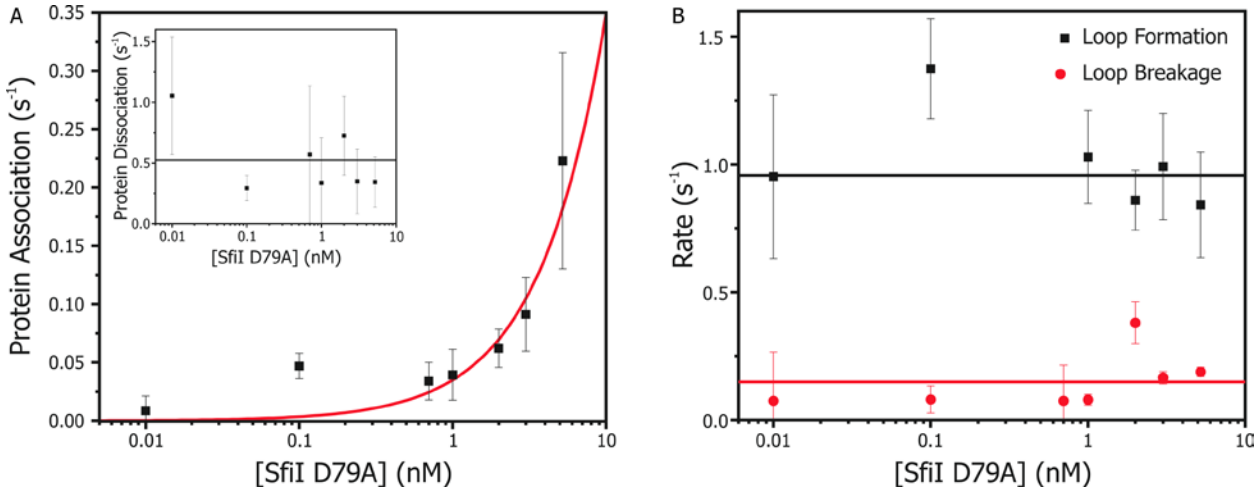

Figure 1 Thresholding reaction rates. The panels show all the measured reaction rates as a function of protein concentration. Each data point shows the average fit value of multiple tethers and the standard error in these values. **A** The protein association rates. These values are obtained from a double exponential fit of the *loop formation times*. The rate is fitted with a linear function that is forced through the origin resulting in a value of $3.5\pm 0.6\cdot 10^{8}$ M^{-1} s^{-1}. The inset shows the rate of proteins dissociating from one recognition site. The weighted average is shown as a straight line (0.5 ± 0.1 s^{-1}). **B** The black squares show the *loop formation rates* and the weighted average as a black straight line (0.9 ± 0.1 s^{-1}). The red circles are the measured *loop breakage rates*, and the weighted average value is drawn as a red line (0.12 ± 0.02 s^{-1}).

Video of DNA cleavage

The video shows fragments from the cleavage experiments done with 0.01nM SfiI WT in the Mg^{2+} buffer, the actual time-span of the movie is around 3 hours of measurements. The movie starts by flowing in the enzyme around 3s extending the tethers in the flow. Around 10 seconds in the movie we re-centre the ROIs. After 15 seconds the movie jumps forward towards the last frames of the movie to show that all tethers are released.