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Methods

Details of the spectroscopic apparatus and data analysis methods are described elsewhere[14]. Improvements in the experimental signal-to-noise ratio have allowed us to make full use of the best <20 ns time resolution of the experiment. In our previous work, the fast phase was visible in unaveraged raw data with poor signal to noise. Although briefly discussed[8], it was averaged out in block-averages over individual fluorescence transient to allow more reliable least squares fitting of the slower kinetic component. The fast phase also appears to disappear at higher temperatures.

For all experiments, 200–400 μM ApoMb samples were prepared in a 10 mM acetate buffer at pH 5.2–5.9, unless otherwise noted. CD spectra were taken of all samples to confirm cold denaturation. The pH 4 eq-apoMb molten globule state was also shown to cold denature, eliminating it as a likely structure for the cold denatured protein at higher pH[8]. The lyophilized Met 131→Ala mutant was used without further purification.

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picture story

A fork in the works

In *Escherichia coli*, replication of the genome is initiated at Ori and two replication forks travel in opposite directions around the circular chromosome. Replication comes to a halt at asymmetric replication termination (*Ter*) sites, found diametrically opposite Ori, which bind the terminus utilizing substance (Tus protein)—but termination only occurs if the *Ter* sites are in the right orientation.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The structure of the Tus–*Ter* complex pictured here, from Katsuhiko Kamada and colleagues *Nature* **383** (598–603), indicates how the protein–DNA complex may selectively trap a replication fork travelling in one direction while ignoring one moving in the opposite direction.

The Tus protein blocks the replication machinery, and specifically DNA helicases, by acting like the buffers at the end of a railway track. Fork-blocking is known to occur at the top of the complex, as shown in the figure. The buffers that the helicases would run up against are formed by helices αVII, αVI αIV and αV, which almost encircle the DNA at the top of the figure. The buffers would also protect the protein's tight, anchor-like grip of the DNA (mediated by the interdomain β-sheet, centre, behind the DNA) from the interfering attention of the replication machinery. Replication forks travelling in the other direction (from the bottom up) would not encounter the helical buffers and—as the polar interactions of Tus with the phosphate backbone are biased to one strand—could easily peel off the exposed DNA strand and thereby eject Tus from the chromosome. The fork would not normally be able to whirl around the chromosome forever, though—as soon as it encounters the other blocked fork it too would stall, bring replication to a halt. **GR**