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Dynamics of Protein-DNA Interactions probed with Laser Temperature-Jump and Time-Resolved FRET Measurements. SERGUEI KUZNETSOV, UIC, PAULA VIVAS, UIC, SAWAKO SUGIMURA, Yale, DONALD CROTHERS, Yale, ANJUM ANSARI, UIC — In many protein-DNA complexes, the DNA is often bent or sharply kinked. In order to elucidate the energetics of the binding mechanism it is necessary to study the dynamics of the binding and bending of DNA. Previous kinetics measurements failed to resolve DNA bending on the millisecond time-scales of stopped-flow techniques. Here we report measurements on the binding of IHF, an architectural protein from *E. coli*, to its native H' substrate end-labeled with FRET pair to monitor the DNA bending. Stopped-flow measurements show relaxation kinetics that become concentration independent at high IHF concentrations, suggesting that under these conditions, the DNA bending becomes rate-limiting. To test this interpretation, we use a laser temperature-jump to perturb the IHF-H' complex, and to probe the dynamics with submillisecond time-resolution. These measurements support a sequential model for DNA binding and bending to IHF. The time-scales for DNA bending, when in complex with the protein, are not inconsistent with thermal fluctuations that can spontaneously bend DNA.

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