

**BEAMLINE**  
X28C

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## Kinetics of Association Between the Integration Host Factor and DNA in *Escherichia coli*

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*Scientists at the Albert Einstein College of Medicine in New York and Yale University in New Haven, Connecticut, have conducted the first analysis by synchrotron footprinting of the fast kinetics of a protein-DNA interaction. The work was conducted at NSLS beamline X28C, and provided a detailed, single base-pair resolution of the exact points of contact of a protein called Integration Host Factor on the DNA surface. The scientists have shown that when protein and DNA samples are mixed together, proteins capable of binding to DNA in a sequence-specific manner yield protections against hydroxyl radical attack along the nucleic acid surface. Two distinct phases are also observed in the association process: an initial "burst" phase, followed by a slower one.*

Integration Host Factor (IHF) is a sequence-specific DNA binding protein that bends DNA and recognizes several sites in the *Escherichia coli* and lambda phage genomes. IHF's ability to bend lambda phage DNA makes it an important architectural protein in the formation of a nucleoprotein complex that precedes the integration of the phage into the bacterial genome, as well as in several other biological processes.

The kinetics of IHF-DNA complex

formation affects the overall rate of IHF-dependent biological process. In our work we have determined the site-specific association kinetics of IHF binding to one of its sites from the lambda phage genome, the H site. A key discovery from our kinetic experiments is that IHF binding and bending of DNA happens in a concerted manner.

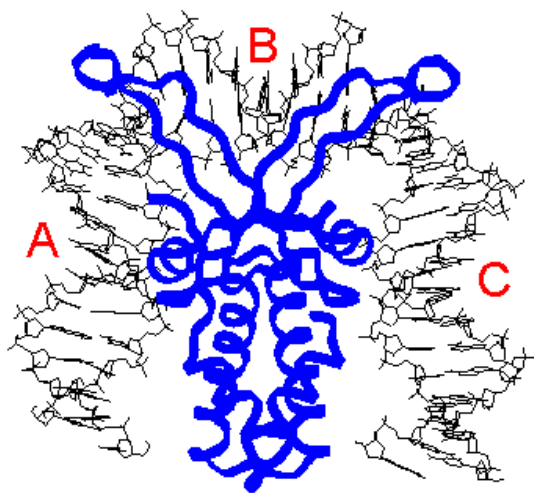
The IHF-DNA crystal structure (**Figure 1**) was solved in 1996 by University of Chicago's biochemist

Phoebe A. Rice and her collaborators. We conducted our time-resolved x-ray footprinting experiments on this complex. Phosphorus(32)-radiolabeled DNA was mixed with IHF, the solution was incubated, and later exposed to NSLS-generated x-ray beams.

The cleaved fragments of DNA were run on a denaturing gel, as shown in **Figure 2**. The three protections along the



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**Figure 1.** The IHF-DNA crystal structure is shown with the three sequence protein-DNA contact surfaces observed in our kinetic study labeled A, B and C.

DNA correspond to the three contacts made by a single molecule of IHF labeled A, B and C respectively. A kinetic fit to the protections calculated for each site relative to a control (unbound) DNA sequence shows that the three 'footprints' upon the DNA occur with similar kinetics (**Figure 3**).

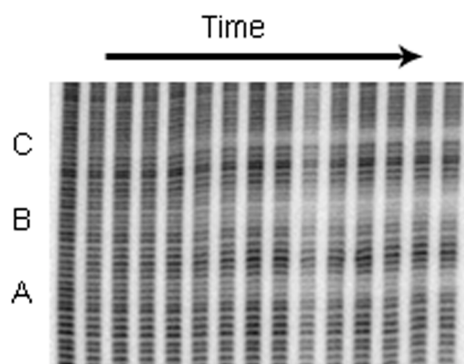
All three DNA binding sites are bound by IHF with comparable kinetic profiles, indicating that they are contacted concertedly in the last step of IHF-DNA complex formation.

The best fit of the data to a bi-exponential equation is characterized

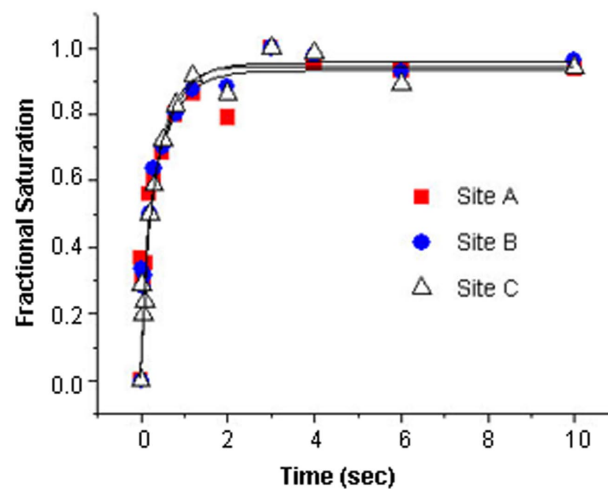
by an initial burst phase in binding that displays rates faster than the speed of conventional diffusion (around  $10^9 \text{ M}^{-1} \text{ sec}^{-1}$ ) followed by a slower phase in binding corresponding to a bimolecular association rate around  $10^8 \text{ M}^{-1} \text{ sec}^{-1}$ .

The time-resolved x-ray footprinting assay has also proved extremely useful in determining site-specific rates of RNA folding in another nucleic acids kinetic study. We envision that the set-up at our committed beamline at the NSLS

will be instrumental in determining specific kinetics for individual molecules in multi-protein-nucleic acid complexes.



**Figure 2.** The image of the gel shown contained separated DNA fragments during complex formation for each time point (each column). The developing protection patterns as a function of time are labeled A, B and C and correspond to the appropriate contacts identified in the crystal structure.



**Figure 3.** The kinetic curves obtained for each protection pattern are shown as a least squares fit to a bi-exponential equation.