

## **“Band-by-Band” Densitometric Analysis of Synchrotron X-ray Footprinting Autoradiograms**

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A nucleotide-by-nucleotide density fitting procedure has been successfully developed and has been applied to an analysis of a domain of the *Tetrahymena* ribozyme and a TBP-DNA interaction. This procedure is described in a paper that has been submitted for publication [Pastor, N., Weinstein, H., Jamison, E. & Brenowitz, M. A Detailed Interpretation of OH Radical Footprints in a TBP-DNA Complex Reveals the Role of Dynamics in the Mechanism of Sequence-Specific Binding.] In this paper a detailed comparison of the •OH footprint of a TBP-DNA complex is compared to the solvent accessibility of the DNA calculated from co-crystal structures and structures derived from molecular dynamics simulations.

The hydroxyl radical footprint of the TATA Binding Protein (TBP) bound to the high affinity sequence TATAAAAG of the Adenovirus 2 Major Late Promoter has been quantitatively compared to a 2-ns molecular dynamics simulation of the complex in aqueous solution at room temperature using the CHARMM23 potential. The nucleotide by nucleotide analysis of the TBP-TATA hydroxyl radical footprint correlates with the solvent accessible surface calculated from the dynamics simulation. The results suggest that local reactivity towards •OH radicals results from the interplay between the local DNA geometry imposed by TBP binding, and the dynamics of the side chains contacting the sugar hydrogens. Analysis of the dynamics suggests that, over time, TBP forms stable interactions with the sugar-phosphate backbone through multiple contacts to different partners. This mechanism results in an enthalpic advantage to complex formation at a low entropic cost. The results of these studies of the TBP-DNA complex provide a foundation for further investigations into the kinetics of assembly of transcription pre-initiation complexes.