

## **ATOMIC FORCE MICROSCOPY STUDIES OF THREE-WAY DNA JUNCTIONS**

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Three-way DNA junctions, the simplest branched DNA molecules, can arise as intermediates in homologous recombination or form transiently during DNA replication. We applied atomic force microscopy (AFM) to look directly at dynamics of the three-way DNA junctions and their global geometry. Three-way junctions were obtained by hybridizing a plasmid vector fragment with homologous fragments containing inverted repeats of different length, purified from gels, and examined with AFM. Heteroduplex fragments are clearly seen as three-arm molecules with one short arm and two flexible long arms. The AFM analysis revealed two important features of three-way DNA junctions. First, the three-way junctions are very dynamic structures. This conclusion is supported by a high variability of the inter-arm angle detected on dried samples, as well as by the mobility of the junction arms observed by a direct sample imaging in liquid (AFM in situ). Second, the three-way junction shape is not flat but is rather pyramid-like. This can be implied from a smaller value of the angle between the long arms if the short arm becomes relatively long and can adhere to the surface thereby flattening the junction. A non-flatness of the junction should be taken into account in analysis of the AFM data.