Double-helical DNA is particularly resistant to bending and twisting deformations. This property has important implications for the packaging and function of DNA in living cells. Among the outstanding questions in the field of DNA biophysics are the underlying origin of DNA stiffness and the mechanisms by which DNA stiffness is overcome within cells. Exploring these questions requires experimental methods to quantitatively measure DNA bending and twisting stiffness both in vitro and in vivo. Using the techniques of T4 DNA ligase-mediated DNA cyclization kinetics and atomic force microscopy to address the origin and management of local DNA stiffness, we show how each can be applied to quantitate biophysical parameters that describe the DNA polymer.

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