Probing DNA Conformation: A Multi-Technique Approach

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The determination of distances between specific points in nucleic acids is essential to understanding their behaviour at the molecular level. The ability to measure distances of 2–10 nm is particularly important: deformations arising from protein binding commonly fall within this range, but the reliable measurement of such distances for a conformational ensemble remains a significant challenge. We show that MD simulations are a robust tool to interpret electron paramagnetic resonance (EPR) measurements of oligonucleotides spin-labelled with triazole-appended nitroxides at the 2' position. For two nitroxide spin-labels attached to B-form DNA duplexes, we present results from EPR spectroscopy, MD simulations, X-ray crystallography, and NMR spectroscopy. These four methods are mutually supportive and pinpoint the locations of the spin labels on the duplexes. In doing so, this work establishes 2'-alkynyl nitroxide spin-labelling as a minimally perturbing method for probing DNA conformation.

[1] J. S. Hardwick, M. M. Haugland, A. H. El-Sagheer, D. Ptchelkine, F. R. Beierlein, A. N. Lane, T. Brown, J. E. Lovett, E. A. Anderson, *Nucleic Acids Res.* **2020**, *48*, 2830-2840. (DOI: 10.1093/nar/gkaa086)

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