DNA Structure/Conformation

The structure and conformation of DNA are important considerations in studying the interactions of DNA with proteins and other DNA ligands. Techniques for determining DNA structure and conformation include X-ray crystallography, circular dichroism (CD), fluorescence, and UV-vis absorbance spectroscopy. With the exception of X-ray crystallography, all these techniques are supported by Olis instrumentation.

**Circular Dichroism:** DNA exhibits a distinctive CD spectrum in the 200-250 nm region that is particularly sensitive to DNA structure. CD spectra can be utilized to probe the DNA structure as perturbants such as increased temperature or DNA ligands are added. A typical experiment involves collection of spectra while the temperature is increasing or ligand is added to the sample.

*All Olis CD models* DSM 17, DSM 20, DSM 1000, and Protein Machine support CD measurements of DNA, and include accessories such as the CD 250 Peltier temperature controlled cell holder, Automatic Titrator for ligand binding, and Stopped-Flow for kinetic studies.

**Fluorescence:** whether originating from the DNA molecule directly, or an external probe, is a valuable technique to elucidate DNA structural information. Emission maxima and fluorescence intensity changes reveal information about the DNA structural integrity. Intercalating fluorescence probes exhibit enormous increases in fluorescence intensity upon binding DNA and are also useful probes of DNA structure. Fluorescence energy transfer (FRET) in which two probes with overlapping emission and excitation spectra are attached to the same DNA molecule. The energy transferred from the donor dye to the acceptor dye is a function of the distance between the two. FRET probes can therefore be valuable tools in monitoring the structural changes undergone by an appropriately labeled DNA molecule.

*All Olis Fluorescence models* (link to new Fluorescence landing page) DM 45, DM 245, SLM 8000, SPF-500, RSM 1000F, and Cary Fluorescence systems are ideally configured to support fluorescence studies of DNA. The Protein Machine is configured to measure both fluorescence and CD for important complementary information about the DNA molecule. The DSM 17, DSM 20, and DSM 1000 can also be configured for both CD and fluorescence studies.

**Fluorescence Anisotropy:** is a measure of the mobility of the fluorescence probe, can also be used to observe DNA structure and conformation. In this technique, the fluorescence probe is excited with vertically polarized light, the amount of polarization of the emission is related to the mobility of the fluorescence probe during the fluorescence lifetime. Fast moving probes will be completely depolarized (anisotropy
is zero) while slower probes, such as those bound to DNA, will remain partially polarized (non-zero anisotropy).

The Olis Polarization Toolbox accessory adds anisotropy capability to any Olis fluorimeter (except the SLM series). Other useful accessories include the TLC 50 Peltier cell holder, 4-cell Peltier turret, Automatic Titrator, and Stopped-Flow.

UV Absorbance: UV absorbance spectra of DNA also reveal important information about DNA structure. While changes due to structural differences are typically subtle, Olis fitting algorithms in GlobalWorks can pull out even the smallest of spectral changes.

The RSM 1000, Olis 14/17 and HPDA 8452 support the collection of UV-Vis absorbance spectra to probe DNA structure. Useful accessories include TLC 40 Peltier cell holder and Stopped-Flow.

Links to client publications:

Download a PDF of client publications related to DNA structure/conformation here

Links to lab websites/ databases/ on-line information about this application:

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