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Measuring disorder at DNA junctions

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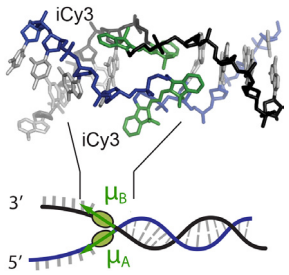
Spectroscopic measurements of chromophore probes reveal changes in the sugar-phosphate backbone structure of DNA at single-stranded - double-stranded junctions.



When regulatory proteins manipulate DNA to carry out essential biological functions like replication, the DNA double helix must open to expose its genetic coding information. Such proteins typically operate at single-stranded – double-stranded (ss-ds) DNA junctions, where the DNA shifts from the typical Watson-Crick double helix into a single-stranded form. However, the structure of these junctions is not well understood.

Heussman et al. studied structural disorder at ss-ds DNA junctions using a combination of absorbance and circular dichroism techniques and two-dimensional fluorescence spectroscopy. This disorder must be structurally characterized, as fluctuations often create the intermediate conformations that are identified and captured by proteins.

“We put dyes inside model DNA constructs at certain locations relative to the ss-ds DNA junction to probe what happens in sugar-phosphate backbones at different positions,” said author Andrew Marcus.

Because the dyes, or chromophore probes, are rigidly inserted into the sugar-phosphate backbones, they are sensitive to changes in backbone structure. The team developed models to explain their spectra and characterize these structures.

They used this information to explore the disorder at the junctions, which reflects an existing distribution of available structures. The distribution was more ordered than expected at room temperature, as the sugar-phosphate backbones tended to follow one structure in the double-stranded segment and another as the DNA transitioned into single-stranded sequences. At different temperatures, the distribution of structures broadened.

The researchers are now conducting single molecule versions of these experiments. The ultimate goal is to understand structure and disorder at the biologically significant junctions using fluorescent backbone and base analogue probes.

Source: “Temperature-dependent local conformations and conformational distributions of cyanine dimer labeled single-stranded - double-stranded DNA junctions by 2D fluorescence spectroscopy,” by Dylan Heussman, Justin Kittell, Peter H. von Hippel, and Andrew H. Marcus, *Journal of Chemical Physics* (2022). The article can be accessed at https://doi.org/10.1063/5.0076261.

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