

Supplementary Note 1. P2-seq modification state occupancies enable thermodynamic analysis of topology-dependent structural ensembles.

Permanganate treatment was applied to DNA populations that have been pre-equilibrated for 2 h at 37°C prior to modification. This equilibration period is orders of magnitude longer than the seconds-to-minutes timescale reported for cruciform extrusion under comparable conditions^{1,2}. Consequently, the distribution of modification patterns across sequencing reads is expected to approximate a Boltzmann-weighted snapshot of the structural ensemble at the time of treatment. Under these assumptions, the fraction of reads assigned to a given structural class estimates its equilibrium occupancy. P2-seq can therefore provide direct access to occupancies of topology-dependent structural states, enabling thermodynamic analysis based on sequencing-derived state populations.

As a proof-of-principle example, we estimated the effective free-energy balance between cruciform-containing and cruciform-negative ensembles in purified topoisomers. For this thermodynamic analysis, reads were conservatively classified as cruciform-positive (fully extruded cruciform states only) or cruciform-negative (all remaining states, including folding intermediates, partial melting states, and dsDNA; Supplementary Fig. 4E), after exclusion of nicked DNA. Folding intermediates were deliberately included in the cruciform-negative ensemble because they represent molecules that have not yet committed to full extrusion and therefore were not considered folded for purposes of calculating an effective equilibrium constant.

The effective equilibrium constant was defined as:

$$K_{eff} = \frac{cruciform + reads}{cruciform - reads}$$

and the corresponding effective free energy was calculated as:

$$\Delta G^{eff} = -RT \ln(K_{eff})$$

where $R = 1.987 \times 10^{-3} \text{ kcal mol}^{-1} \text{ K}^{-1}$ and $T = 310 \text{ K}$.

<i>Topoisomer</i>	<i>Cruciform + reads</i>	<i>Cruciform - reads</i>	<i>ΔG^{eff} (kcal/mol)</i>
$\Delta Lk = -22$	22,935	28,452	+0.13
$\Delta Lk = -23$	51,405	34,304	-0.25

The change in effective free energy upon addition of one negative superhelical turn was estimated as:

$$\Delta \Delta G^{eff} = \Delta G^{eff}(\Delta Lk = -23) - \Delta G^{eff}(\Delta Lk = -22) = -0.38 \text{ kcal/mol}$$

The shift in ΔG^{eff} from positive at $\Delta Lk = -22$ to negative at $\Delta Lk = -23$ is consistent with a shift in the thermodynamic balance towards cruciform-containing ensembles, mirroring the occupancy redistribution observed in Fig. 4. Under this interpretation, addition of a single negative superhelical turn moves the system across a narrow energetic regime in which fully extruded cruciform states become favored over competing structural configurations.

Importantly, ΔG^{eff} should not be interpreted as the intrinsic free energy of cruciform formation in isolation. The cruciform-negative ensemble is not a single unfolded reference state but rather comprises a heterogeneous population of molecules in which torsional stress is accommodated through alternative structural transitions, including FUSE-proximal melting, DUE melting, cruciform folding intermediates, distributed duplex destabilization, and writhe^{3,4}. Consequently, the corresponding partition function includes contributions from multiple competing torsional sinks³. K_{eff} therefore reflects the effective thermodynamic balance between competing structural ensembles within a constrained topological domain rather than a simple two-state folding equilibrium.

This effective free energy, rather than the intrinsic free energy of cruciform formation in isolation, may be particularly relevant to genomic contexts because it explicitly incorporates competition among alternative torsional sinks⁵. More broadly, this example illustrates how P2-seq-derived occupancies can be converted to effective free energies for topology-dependent structural transitions. Applied systematically across superhelical densities and sequence contexts, this framework could enable quantitative reconstruction of the energetic landscapes governing topology-dependent DNA structural ensembles and their competition for torsional stress.

References

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