

Seeing the (previously) invisible: using ¹⁹F NMR to probe a dynamic DNA structure

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Biology DNA replication & repair Triplet repeat diseases



Kenneth Marians

DNA three way junctions

Nanotechnology

DNA scaffolds Charge/excitation transfer guides/switches



Guests for small molecules





Previous Structures of TWJs





Perfectly complementary TWJs?







What complementary techniques tell us

Ensemble FRET



Gel mobility Crosslinking/ligation



H-arm



MD

SAXS



Single molecule measurements allow more precise distance measurements





A fully complementary TWJ model





So why can't NMR see what's going on in a TWJ?





Well – actually it might if you look in the right way

$R1\rho$ Relaxation Dispersion



Kimsey at al. Nature 519, 315–320



So why can't NMR see what's going on in a TWJ?





But is there a cheaper, less ambiguous way?

$$v = \frac{\gamma}{2\pi} B_0 (1 - \sigma)$$

Nucleus	γ (10 ⁶ rad s ⁻¹ T ⁻¹)	γ /2 π (MHz T ⁻¹)
<u>¹H</u>	267.513	42.577
² H	41.065	6.536
¹³ C	67.262	10.705
¹⁵ N	-27.116	-4.316
¹⁹ F	251.662	40.052
³¹ P	108.291	17.235



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¹⁹F NMR in nucleic acids?





5F-U





5F-dU





5F-(d)C



Puffer et al. NAR 37, 7728-7740



Back to the TWJ – sequence dependence





GC rich TWJ shows multiple conformations





Major population looks like less-GC rich TWJ





Major population looks like less-GC rich TWJ

Not enough info on the minor conformer





5¹⁹F-dC GC-rich TWJ





5¹⁹F-dC ssDNA





5¹⁹F-dC GC-rich TWJ





5¹⁹F-dC secondary isotope effect







5¹⁹F-dC GC rich dsDNA





5¹⁹F-dC GC-rich TWJ





What conformation is responsible for the shift?





What are the exchange parameters?

TEDDY (Dynamic NMR) in Topspin

• How fast does it exchange?





Future?



- ¹⁹F ¹H heteronuclear NOEs?
- Other TWJ sequences
- 19F-dU to complement 19F-dC?
- Try ¹³C CEST etc to correlate
- TWJ chelators
- More biologically relevant DNA structures



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