A Single Molecule Investigation of Structure and Accessibility of Long Telomeric Overhangs

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Telomeric Overhang mixed with Cy5-PNA Binding of Cy5-PNA to different parts of overhang results in different FRET levels

Laser Beam in TIR Mode

Human Telomeric Overhang

• Human telomeres contain 50-300 nt G-overhang

• Telomeres shorter than a critical length ⇒ senescence or apoptosis

• How does telomere accessibility change with telomere length?
DNA-PAINT (Point Accumulation for Imaging in Nanoscale Topography) adapted for studying telomeric overhang.

Telomeric Overhang mixed with Cy5-PNA

Binding of Cy5-PNA to different parts of overhang results in different FRET levels

Telomere: TTAGGGTTA repeats
Cy5-PNA: TAACCCTT-Cy5

Laser Beam in TIR Mode

Intensity (a.u.)

Time (s)

FRET-E

Dwell Time
Repeating Folding Pattern

Telomeric Overhang mixed with Cy5-PNA

Binding of Cy5-PNA to different parts of overhang results in different FRET levels

NG-Tract: \((TTAGGG)_N TTAG-3'\)
4G-Tract: \((TTAGGG)_4 TTAG\)
24G-Tract: \((TTAGGG)_{24} TTAG\)

- \([4n]\)G-Tract distributions broadest
- \([4n+2]\)G-Tract distributions narrowest

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S = \sum_{i} p_i \log p_i
\]
Dwell Time Analysis

- Differences in $\tau$ not statistically significant for binding to different segments of an overhang
- There is no systematic pattern in $\tau$ across overhangs of different lengths
Frequency Analysis

Binding frequency increases with overhang length

Binding frequency per G-Tract decreases with telomere length.

Telomeres less accessible at longer lengths

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