Visualization of Long Human Telomere Mimics by Single-Molecule Fluorescence Imaging

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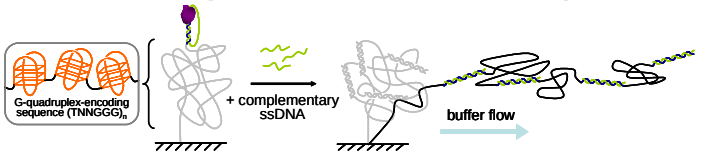
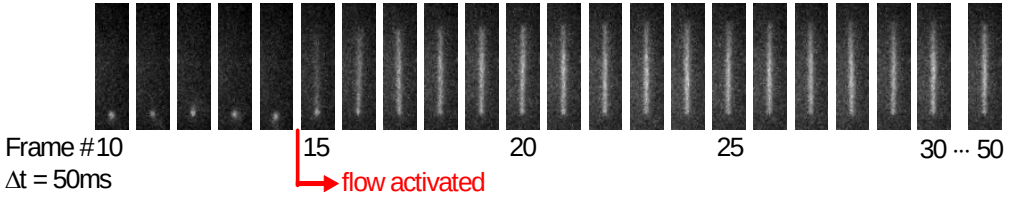
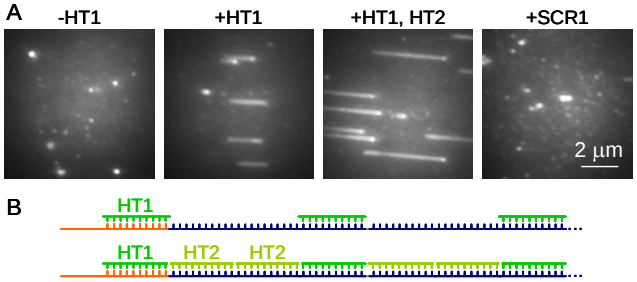
Study of long single-stranded telomeric DNA is important for a variety of basic science and biotechnological applications, yet few methods exist for preparation and observation of single copies of this DNA in solution at biologically relevant length scales (~200 nt to >1000 nt) necessary for assessment of heterogeneity in its structure and behavior.

We synthesized kilobase-long single-stranded human telomere mimics *in situ* by rolling circle replication (RCR) on a coverslip and visualized individual strands by staining with SYBR Gold. Under buffer flow, these telomere-mimicking DNA sequences were observed at the single-molecule level in real time (below right, 4 μm vertical scale).

Schematic diagram of flow-stretched RCR products

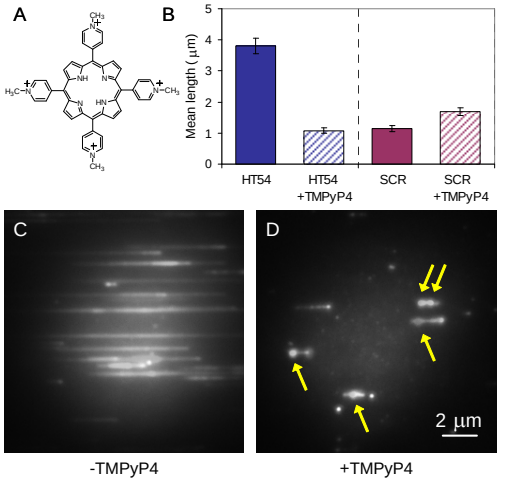






Using this flow-stretching procedure, we detected striking differences in the extensibility of individual telomere mimics in the presence and absence of short ssDNA complements.

We also apply this new mode of ssDNA characterization to probe the interaction of long telomere mimics with the small-molecule G-quadruplex-binding agent TMPyP4.



With our SM flow-stretching experiments, we have qualitatively characterized a naturally-derived DNA repeat at previously unexplored length scales, while demonstrating the potential for interrogating real-time DNA dynamics that are difficult or impossible to access via conventional methods of probing surface-immobilized ssDNA (e.g., bulk fluorescence, AFM).