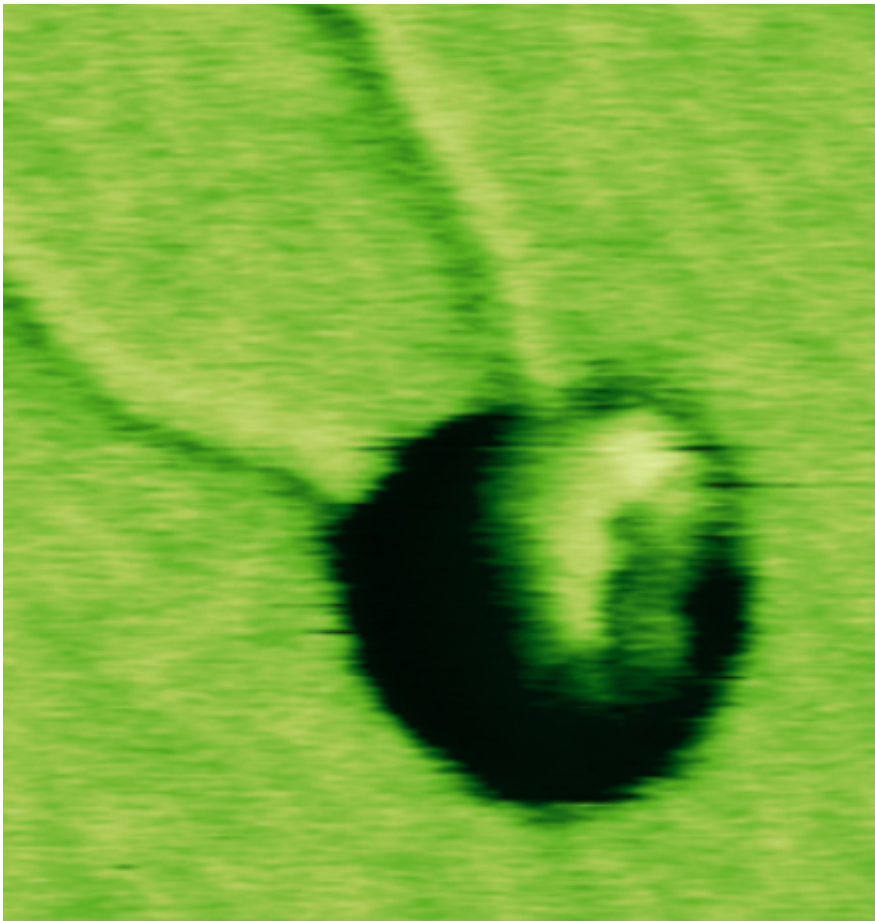


# New imaging technique shows how DNA is protected at chromosomes' ends

February 11 2016, by Tracey Peake

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A DNA T-loop (light green) protrudes from a TRF2 protein complex (dark area).

A new imaging technique has allowed researchers at North Carolina

State University, the University of North Carolina at Chapel Hill, and the University of Pittsburgh to see how DNA loops around a protein that aids in the formation of a special structure in telomeres. The work provides new insights into the structure of telomeres and how they are maintained.

Telomeres are essentially caps on the ends of linear chromosomes, which are the structures inside our cells that contain DNA with our genetic information. In terms of function, telomeres are like the plastic coating (aglet) on the ends of shoelaces that prevents the laces from unraveling. In healthy cells, telomeres protect the chromosome by tucking away any overhanging ends of DNA strands to form a lasso-like structure known as a T-loop. Without telomeres, the cell's DNA repair proteins would read the overhanging ends as a break to be mended and attempt to either bind chromosomes together or send special proteins to digest them away.

Researchers know that a protein called telomeric repeat-binding factor 2 (TRF2) is key to telomeric structural integrity due to the role it plays in forming the T-loop. But researchers didn't know the mechanics behind the DNA compaction and T-loop formation by TRF2.

"TRF2 can compact DNA, which is important for T-loop formation," says NC State physicist Hong Wang, lead author of a paper describing the research. "But prior to this work, researchers did not know where the DNA was going or how TRF2 compacted it—we could only see the DNA strand going into and out of TRF2 complexes, but couldn't see the DNA in the complexes. This is because we were using traditional atomic force microscopy (AFM) techniques, in which the protein-DNA shows up as a single blob, and the DNA path information is missing."

The breakthrough came with a new imaging technique, dual-resonance-frequency-enhanced electrostatic force microscopy (DREEM), which was developed by University of North Carolina at Chapel Hill chemist

and co-author Dorothy Erie, former UNC and NC State postdoctoral researchers Dong Wu and Parminder Kaur, and was featured earlier this year in [Molecular Cell](#). The technique utilizes the fact that DNA is negatively charged along its backbone. By applying DC and AC biases between the AFM probe and sample surface, DREEM can detect very weak electrostatic interaction differences when it scans over protein versus DNA regions. In this way, DREEM enables direct visualization of DNA wrapping outside histone proteins.

"DREEM allowed us to see the DNA's path through the TRF2 complex," says Wang. "Based on the DREEM images that we got, we now think there may be two orders of DNA compaction within the telomere - first, DNA wraps around a TRF2 protein in the interior of the complex. Then, multiple TRF2 molecules come together and create DNA loops that stick out from the TRF2 proteins.

"We think that this protruding loop provides the entering site for the telomere overhangs to tuck in to form the T-loop structure. This process ultimately helps to maintain the protective structure that prevents fusion of chromosomes or the slow erosion of telomere DNA. Our future work will try to determine if this is indeed the case."

The researchers' work appears in *Nature Scientific Reports*. The work was supported by grants from the National Institutes of Health and a pilot grant from CHHE at NC State. NC State physicist Robert Riehn, postdoc Jianguo Lin, graduate student Preston Countryman and the Opresko lab at the University of Pittsburgh also contributed to the work.

**More information:** Parminder Kaur et al. Enhanced electrostatic force microscopy reveals higher-order DNA looping mediated by the telomeric protein TRF2, *Scientific Reports* (2016). [DOI: 10.1038/srep20513](https://doi.org/10.1038/srep20513)

Provided by North Carolina State University

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