NANOSCIENCE COLLOQUIUM

Tracking and tracing complex DNA structures critical to human health

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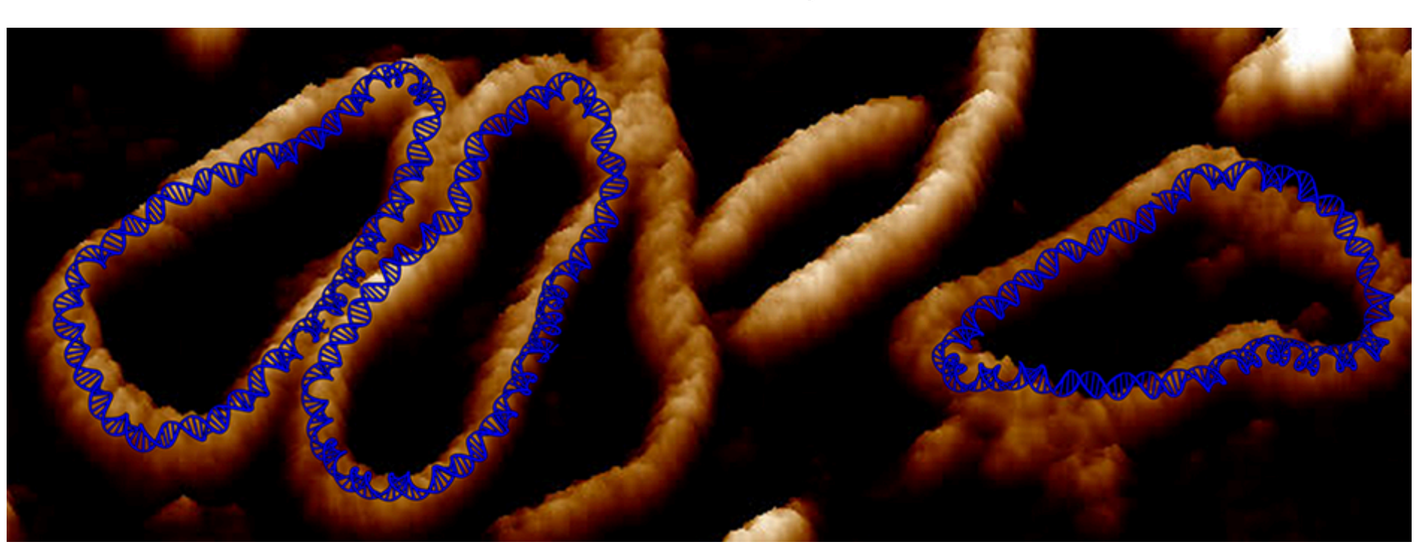
ABSTRACT: Complexity in DNA structures, from defects and damage to the formation of alternative structures, plays a critical role in maintaining, regulating and disrupting the flow of cellular information within the genome. We have developed a single-molecule pipeline combining atomic force microscopy and deep-learning image analysis to quantify complex DNA formation with nanometre resolution.

Our pipeline can trace the structure of replication intermediates from Xenopus egg extracts and determine the extent of replication progression. We identify stalled complexes as theta structures, separating and quantifying the length of replicated and unreplicated DNA. In ~7% of complexes, we observe reversed forks of length ~20 nm, providing new insights into the likelihood of complex DNA structures forming in response to replication stress.

Beyond replication, we can also assess the effect of complex DNA structures on transcription. We determine that single nick sites can drive up to a 150-fold increase in R-loops, which appear in a range of conformations, depending on the presence of defects in the template strand. Finally, we extend our pipeline by incorporating multiclass U-Nets to understand the effect of complex DNA structures on protein recognition, spanning Cas-9, topoisomerase and Shelterin activity.

We have developed a single-molecule pipeline combining AFM and deep-learning to quantify complex DNA formation with nanometre resolution. We trace the structure of replication intermediates from Xenopus egg extracts, identifying stalled complexes as theta structures, separating and quantifying the length of replicated and unreplicated DNA with 7% containing reversed forks. For transcription, we determine that single nick sites can drive up to a 150-fold increase in R-loops, which appear in a range of conformations, depending on defects in the template strand. Finally, we probe the effect of complex DNA structures on protein recognition, spanning Cas-9, topoisomerase and Shelterin activity.



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