Detecting DNA damage with single molecule resolution using Atomic Force Microscopy

Tobi Firth[b,c], Matthew Newton[a], Helen Bryant[b], Alice Pyne[c]

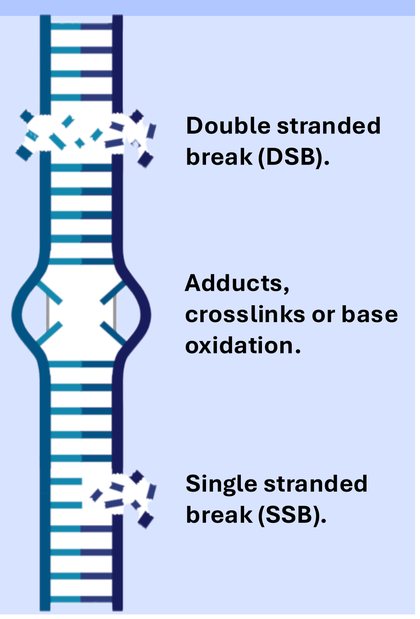
[a] *School of Biosciences,* University of Sheffield, S10 2TN.
[B] *School of Medicine and Population Health*, University of Sheffield, S10 2RX.
[c] *School of Chemical, Materials and Biological Engineering*, University of Sheffield, S1 3JD.

1. DNA damage – a hallmark of cancer and a cancer treatment

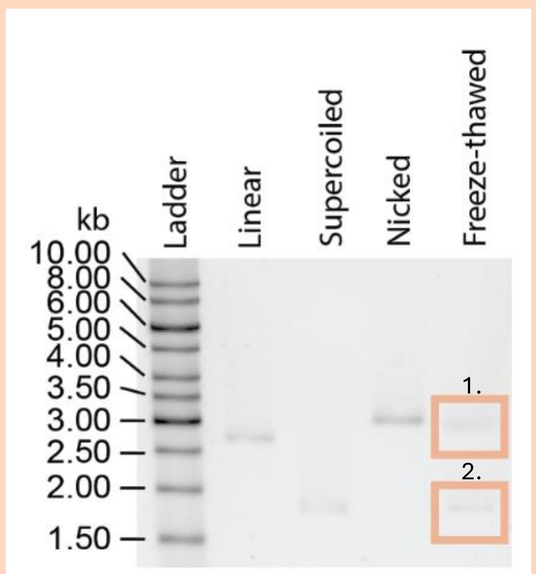
DNA damage can lead to mutations that promote uncontrolled cell proliferation, invasion, metastasis and the ability to resist apoptosis - these are hallmarks of cancer[1].

DNA damage is also the most effective way of treating cancer. By inflicting additional, irreparable DNA damage we can induce cell death.

The most common way of inducing DNA damage is chemotherapy and radiation, but these treatments are not well tolerated by patients. Understanding DNA damage better will help us design new and more efficacious treatments.



2. Observing DNA damage at the population level



1% agarose gel of pUC19 in different conformations.
1. Nicked. 2. Supercoiled.

To observe damage in a model system we used the DNA plasmid, pUC19 (2686 bp).

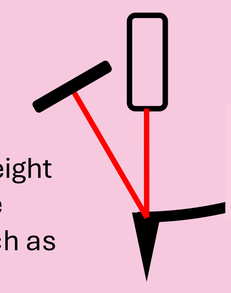
The aim of this experiment was to compare a damage model (freeze-thawed) against known conformations of pUC19 including linear (DSB), supercoiled and nicked (SSB).

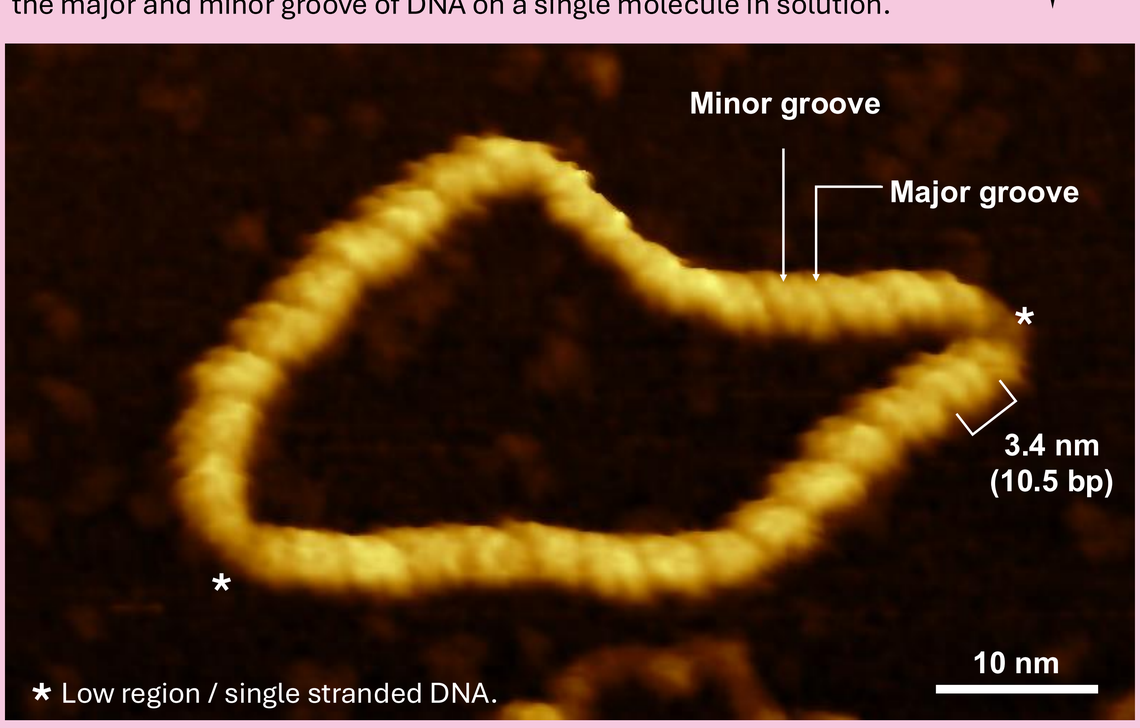
- The freeze-thaw damaged plasmid gave us a mixed population of nicked and supercoiled DNA.
- The damage in the freeze-thaw sample is equivalent to nicking.
- The resolution of agarose gel electrophoresis is limited.

3. Atomic Force Microscopy

Unlike agarose gel electrophoresis, which is best described as an 'ensemble averaging technique', **A**tomic **F**orce **M**icroscopy (AFM) is a single molecule technique.

AFM uses a sharp cantilever to 'feel' a surface in liquid. Changes in height are measured by the deflection of a laser, reflected off the back of the cantilever, into a photodiode. This technique can resolve features such as the major and minor groove of DNA on a single molecule in solution.





High-resolution image of a DNA minicircle (339 bp).
Height scale: 0 to +4 nm. Scale bar: 10 nm.

4. Observing DNA damage at the single molecule level

Single molecule imaging of individual DNA populations reveals conformation heterogeneity not seen by agarose gel electrophoresis.

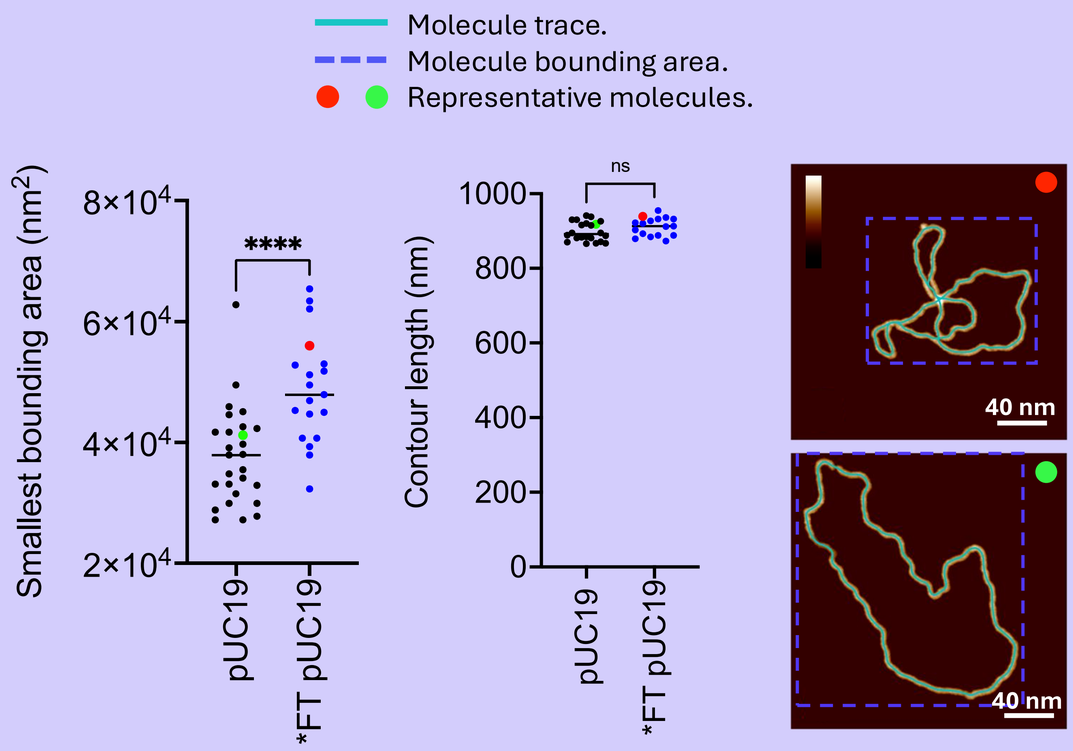
- Linear plasmids have a **DSB** with **two free ends**.
- Supercoiled plasmids are more writhed than nicked plasmids. **Writhe** is an instance of **two (or more) DNA strands crossing**. In the image panel, the upper supercoiled plasmid has 2 crossings and the lower has 3.
- Nicked and freeze-thawed plasmids are less writhed than supercoiled plasmids and have **low regions** indicative of **single stranded DNA**. These plasmids show variable levels of damage.

AFM imaging reveals conformational changes in DNA plasmids subjected to freeze-thaw cycles.

Representative AFM images of linear, supercoiled, nicked and freeze-thaw damaged pUC19 plasmid as illustrated by agarose gel in panel 2. Height scale -2 to +4 nm. pUC19 was adhered to the mica using divalent cations.

5. Quantifying changes in plasmid conformation

AFM allows us to capture images of DNA molecules – TopoStats gives us the tools to quantify the structural heterogeneity we can see[3].



Quantifying AFM images of pUC19 species shows populations of DNA which vary in their conformation but have the same contour length as expected.

- Supercoiled pUC19 has a smaller bounding area indicating a more compact conformation. Nicked plasmids have a bigger bounding area indicative of a more open conformation.
- The plasmid contour length (how long the plasmid is) remains constant at 913.24 nm.

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Email: TFirth2@Sheffield.ac.uk

[1] Lhannahan, D. *et al.* (2022). Hallmarks of Cancer: New Dimensions.
[2] Haynes, P. *et al.,* (2020). Atomic Force Microscopy of DNA and DNA-Protein Interactions.
[3] Pyne, A. *et al.,* (2021). TopoStats – A program for automated tracing of biomolecules from AFM images.
Panel 1: Image – BioRender.