Dearth, Damage and Desiccation: How DNA origami can help us create drought resistant crops

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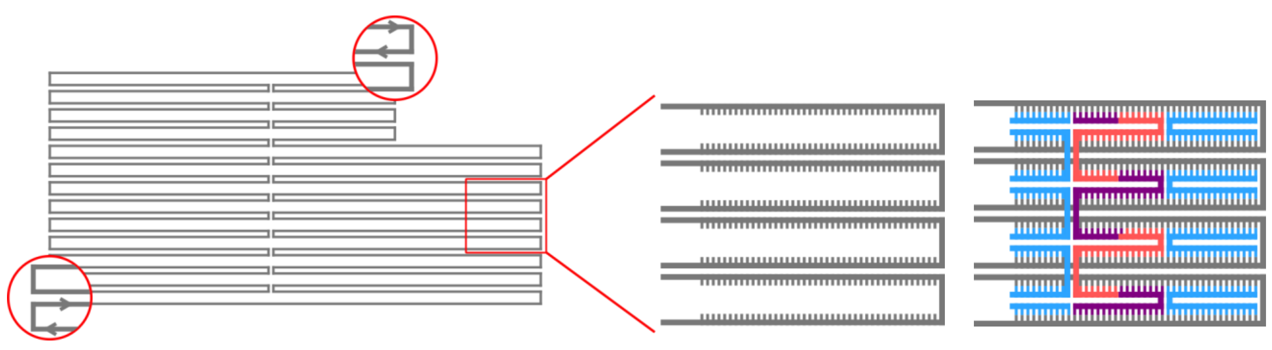
Introduction

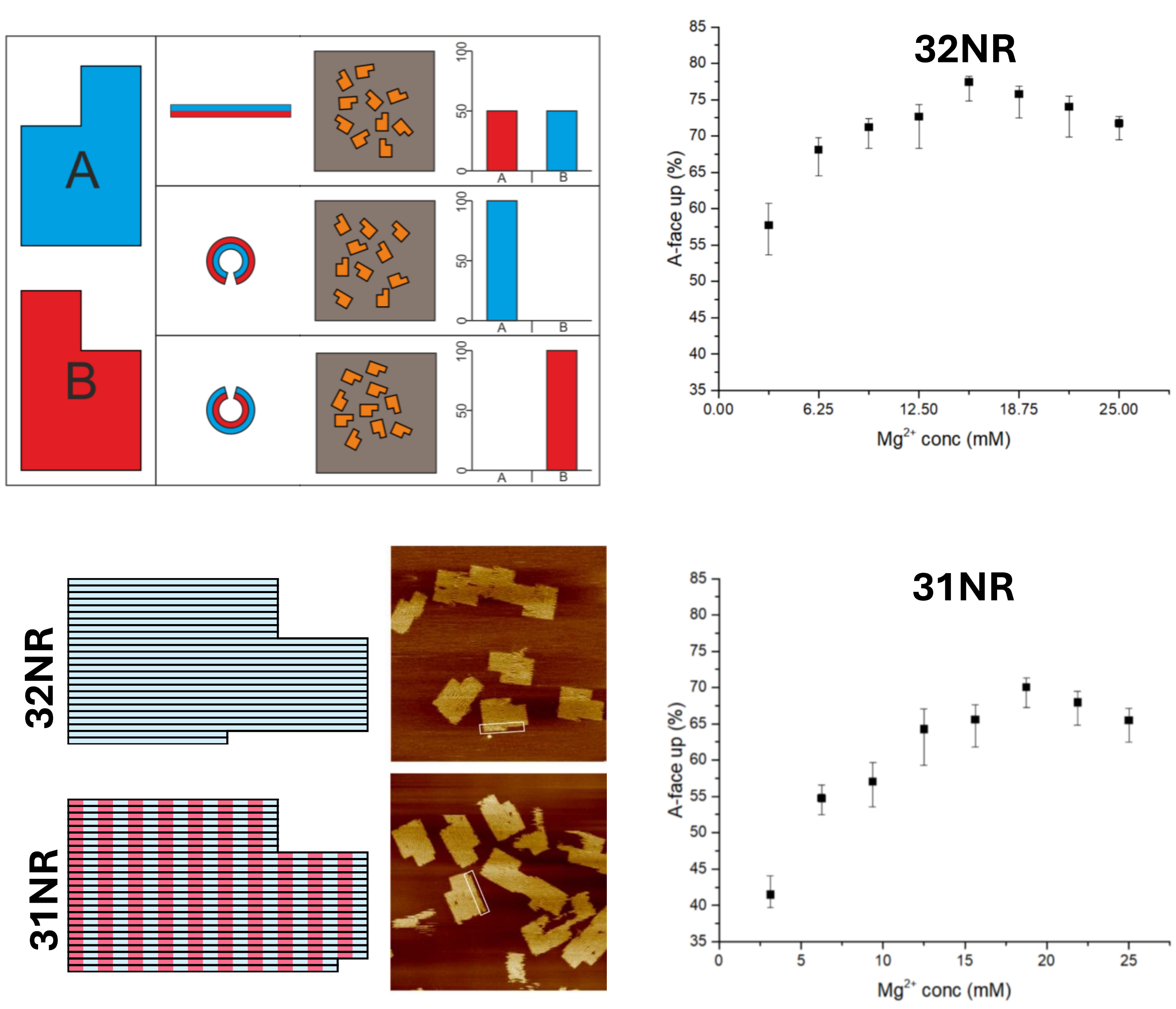
The ability to survive desiccation is an impressive act, found in extremophiles across kingdoms of life. This remarkable survival strategy prolongs viability from years to millennia. However, dehydration results in high levels of genomic damage, which itself can limit the survival of desiccated organisms. By leveraging the rational design of nanoscale shapes constructed out of DNA itself, we aim to probe the variety of factors that both influence damage and help protect DNA during drying. We will also explore rehydration of these model DNA systems, to see which is more damaging. We anticipate that by uncovering this mechanism, it will allow us to develop methods for more efficient ways of engineering drought resistant crops.

DNA origami

Predictable and reliable Watson-Crick base pairing to direct the folding pathway of long ssDNA results in reproducible nanostructures. The dsDNA helices are held together with immobile DNA junctions (cross-overs) akin to Holliday junctions. Spacings between cross-overs are limited by helical pitch of DNA and physical offset between helices.

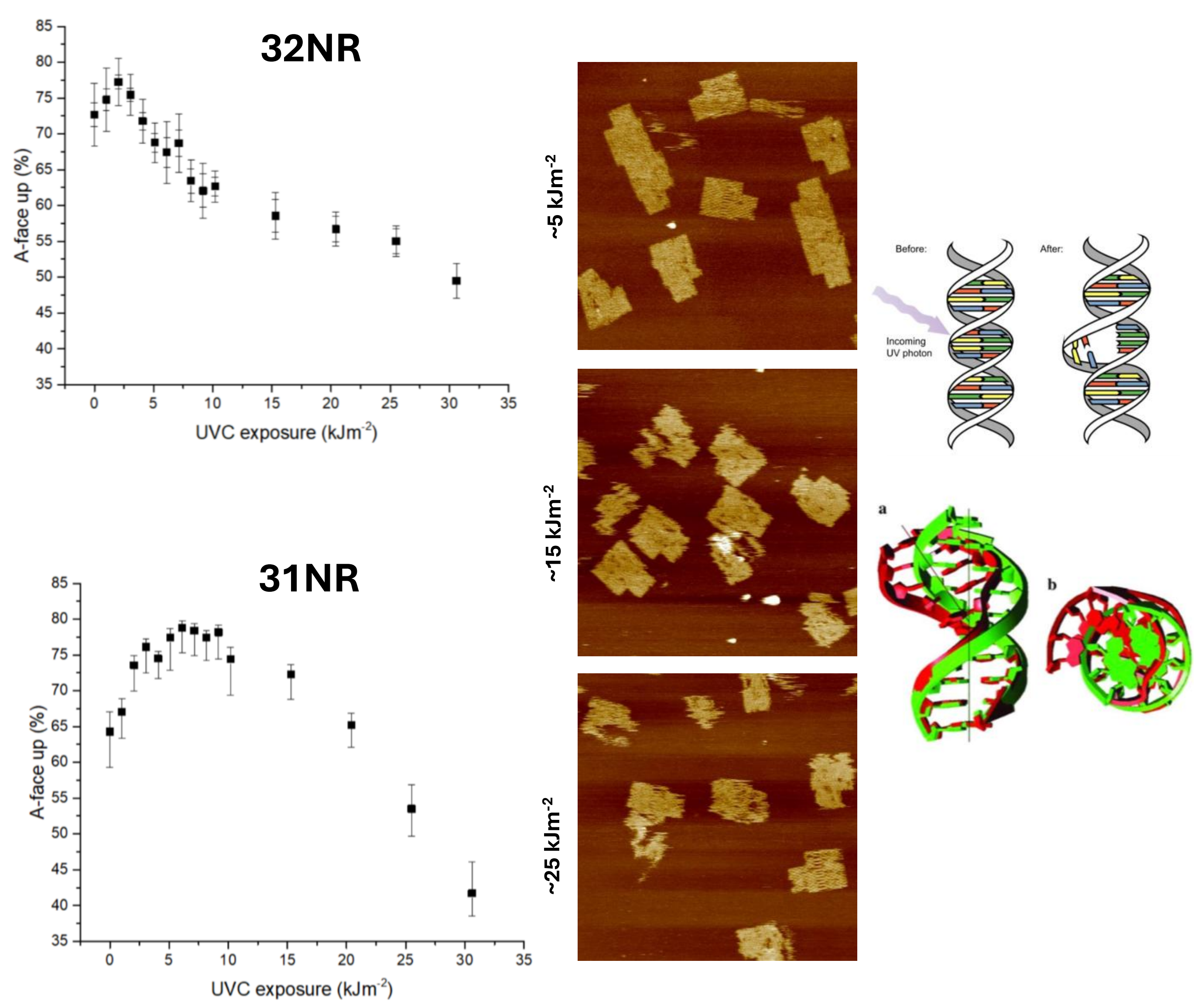


Shape of DNA origami



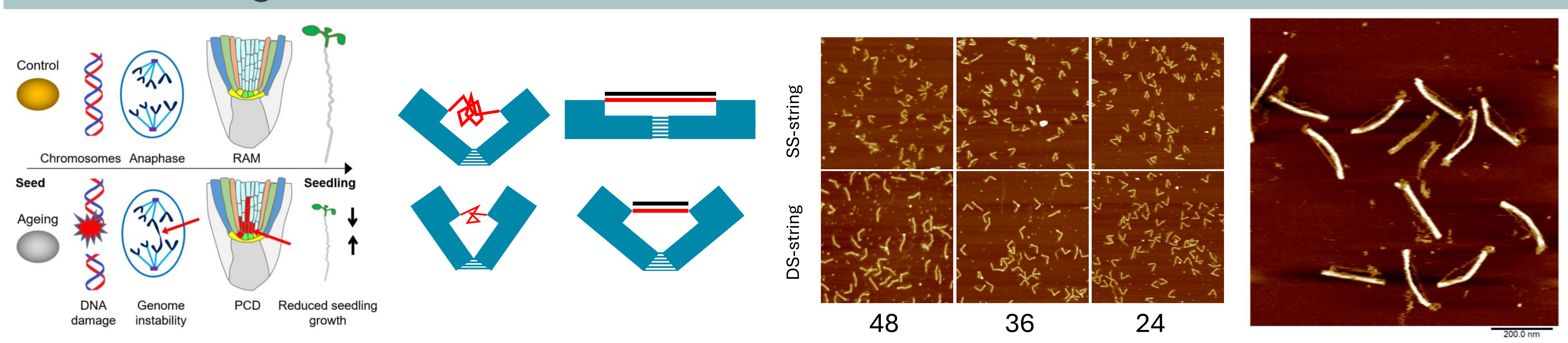
AFM is a surface-based technique, constraining DNA origami to a flat substrate, preventing direct observation of the global solution-structure. As single-layer origami exhibit some degree of intrinsic out-of-plane bending, they have adsorption orientation bias. Asymmetric notched-rectangular (NR) designs enable surface orientation to be quantified, allowing solution structure to be inferred indirectly; however, monomeric constructs do not permit direct measurement of the twist element. Here, two designs differing in cross-over spacing — 32 bp, which overestimates planarity, and 31 bp, which underestimates it — show that Mg^{2+} alters the orientation bias on mica under liquid AFM. The decrease in bias is associated with increased electrostatic repulsion with a fully stable helix.

UVC Damage



UVC short wavelength radiation induces direct DNA damage through cyclobutane pyrimidine dimer (CPD) formation, in which adjacent pyrimidine bases link covalently, causing local bending and underwinding of the helices. Following UVC exposure, 32NR shows reduced surface-orientation bias, whereas 31NR initially shows increased bias. This opposing response is consistent with CPD-induced underwinding partially correcting the 32NR overestimation of planarity while further exaggerating the 31NR underestimation. At higher UVC doses, bias decreases in both designs, which is attributed to strand breakage becoming dominant after CPD formation approaches saturation at available pyrimidine sites. This leads to lower mechanical integrity of the origami as observed by AFM imaging.

Tensioning DNA



Chromatin packaging in plant nuclei imposes non-uniform mechanical constraints on genomic DNA. Linker DNA between nucleosomes may become locally tensioned through nucleosome positioning, chromatin compaction along with higher-order nuclear structures. These strained regions may be more susceptible to strand breakage, particularly during dehydration or exposure to damaging agents, such as UV radiation. To investigate this directly, a DNA origami "violin bow" was designed as a controllable model of tensioned DNA. The structure contains a relatively rigid helical-bundle backbone and a variable-length DNA string. The un-tensioned design uses a 48-turn (504 nt) ssDNA string, which can be shortened to increase tension across the bow. Complementary oligonucleotides can convert the string into dsDNA, allowing damage susceptibility to be compared between ssDNA and dsDNA under defined mechanical constraints. The string behaves as an entropic spring: shorter strings increase tension, but overly short strings may become overstretched and inhibit oligo binding. Initial optimisation therefore focuses on less extreme designs, with 42–45 turn strings forming bow-like structures without sharp backbone bending (far-right AFM image). This platform will enable DNA damage to be measured as a function of applied tension, testing whether mechanically strained chromatin-like DNA is intrinsically more break-prone, especially under dehydrating conditions.

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