Mechanistic Analysis of the Oligomerization States of *Dm*Gen *in vitro* Using Atomic Force Microscopy

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Background

- DNA damage is implicated in many disease pathologies. One form of damage is double strand breaks (DSBs). DSB can occur in the genome due to ionizing agents as well as in endogenous processes like meiotic recombination.
- One important intermediate in the DSB repair pathway (Figure 1) elucidated by Szostak and colleagues include Holliday Junctions (HJs)[1].
- Resolvases that act on this complex intermediate were elucidated in eukaryotes such as Mus81 and GEN1 in humans and mice. There is more susceptibility to DSB damage and lethality with Mus81 KOs than GEN1, in humans[2,3].

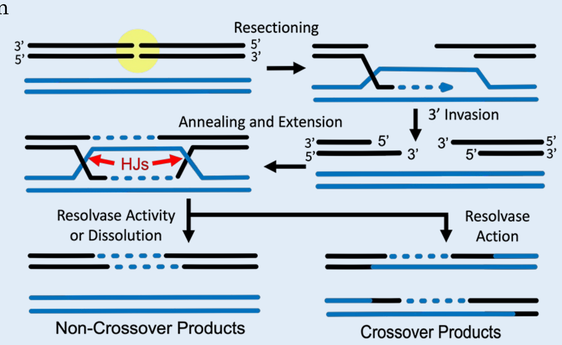

Figure 1. Summary of DSB Repair pathway.

Current Findings

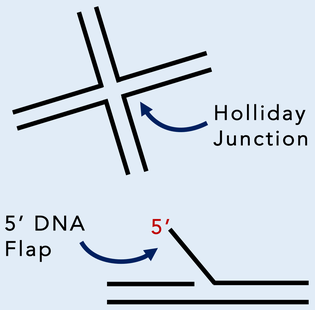

Figure 2. Example substrates

- Compared to other analogous eukaryotic HJ resolving endonucleases such as MUS81 in humans and mice, *Dm*Gen has more lethality when eliminated than dmMus81 and acts directly on HJs in the DSB repair pathway [4,5].
- Faster activity of this resolvase on 5' flaps (seen in Figure 2) suggesting the possible importance of this substrate in DSB repair processes[4].
- Possible dimerization of this protein on the 5' Flap substrate at high concentrations (60 nM) of protein[4].
- Activity of a truncated protein of *Dm*Gen from 1-518 AA identical to full length protein (726AA) [4]. Truncated *Dm*Gen wasused in the following study.

Methods

- **Nuclease activity assays** on denaturing gels to ensure protein activity and understand how rapid cleavage is occurring on the DNA substrate
- **Atomic force microscopy** to investigate the binding volume of the protein on 5' flap DNA substrate (~2700 bp in length) in relation to oligomerization state and bending angle properties.

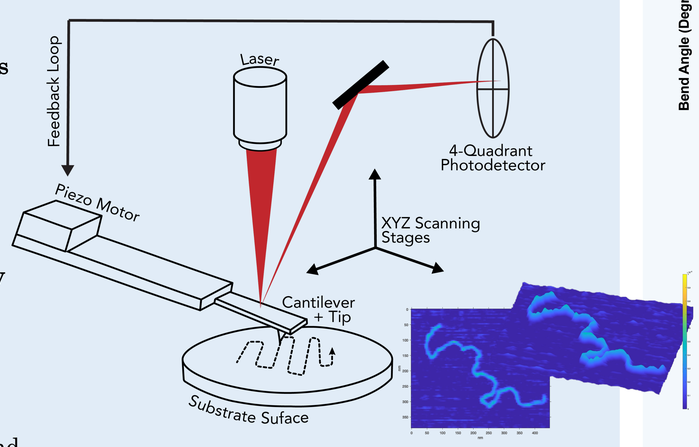

Figure 3. General diagram of an AFM setup

Results

Volume and Oligomerization

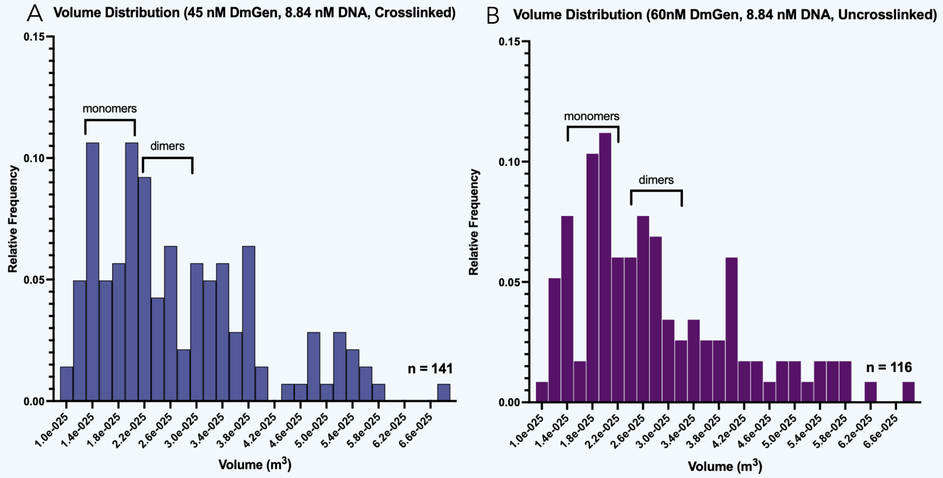

Figure 4. (A) Volume distribution of the 45 nM *Dm*Gen with 8.84 nM 5' Flap substrate crosslinked deposition in *Dm*Gen binding buffer (Figure 6D). (B) Volume distribution of the 60 nM dmGen with 8.84 nM 5' Flap substrate un-crosslinked deposition in *Dm*Gen binding buffer (Figure 6C).

- There appears to be a general distribution of both the dimer and monomer state that are not significantly different from each other in these conditions (Figure 4).
- No clear preference between the dimer or monomer state.

Volume and Bend Angle

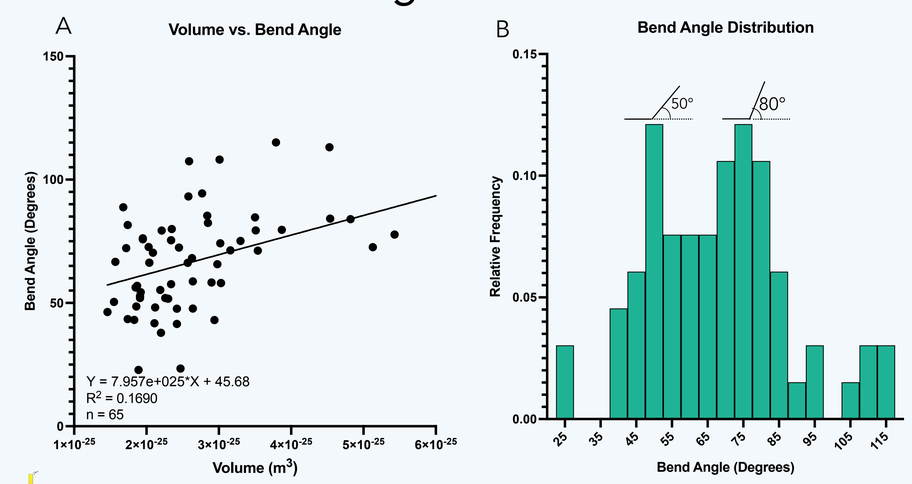

Figure 5. (A) Angle measurements and volume distribution derived from the 45nM *Dm*Gen with 8.84 nM 5' Flap substrate DNA deposition (Figure 6C). The angles were measured as the distance from 180 degrees. (B) Distribution of the angles sampled in this deposition.

- From the above distribution there is a slight bimodal distributions of the bending angle at 50° and 80° from the 180° standard as well as a general positive linear relationship between volume and bend angle (Figure 5).
- Suggest possibility of distinct pathways with the oligomeric state.

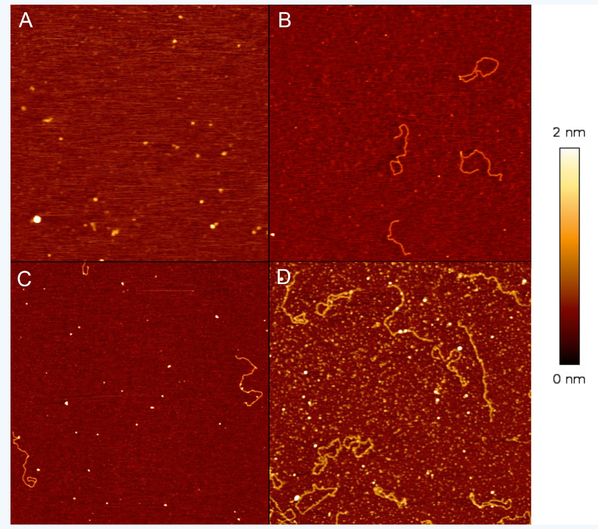

Figure 6. Array of the processed images on all depositions used in this study. (a) 1 µm x 1 µm 10 nM *Dm*Gen deposition in low salt buffer (b) 2 µm x 2 µm image of 5.30 nM 5' Flap DNA substrate deposition in dmGen binding buffer. (c) 2 µm x 2 µm image of 8.84 nM 5' Flap DNA substrate with 45 nM *Dm*Gen glutaraldehyde crosslinked deposition in dmGen binding buffer. (d) 2 µm x 2 µm image of 8.84 nM 5' Flap DNA substrate with 60 nM *Dm*Gen un-crosslinked deposition in dmGen binding buffer.

Conclusions

- At the conditions tested in low salt buffer, both monomer and dimers are readily visible which confirmed prior findings.
- There appears to be a positive relationship between the bending angle and the volume bound which shows specific angle for the monomer and dimer states aligning with distinct resolution paths based of oligomeric states.
- These results align with the possibility that D*m*Gen may have evolved multiple pathways for resolution of 5' flap substrates and could be a prominent intermediate in the DSB repair processes

Future Directions

- Test effects of gradually increasing volume in the determined AFM conditions by increasing protein concentration
- See how *Dm*Gen oligomerizes on other DNA substrates of interest with AFM
- Monitor actions of *Dm*Gen using other techniques such as FRET or in-solution AFM imaging

Acknowledgements

Funding for this work was provided by NIH Grant MIRA 5110139
Thank you to my mentors Emily, Corey, Dr. Dorothy Erie and the rest of the group

References

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