Calibrating Mechanical Amplifiers of DNA Bend Dynamics

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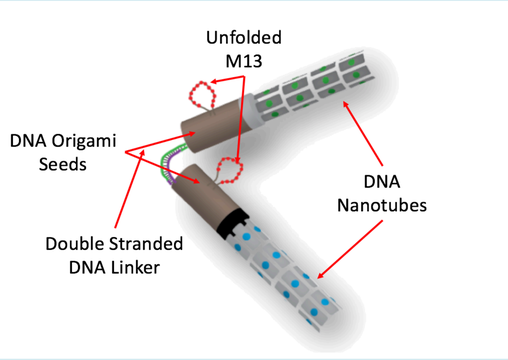
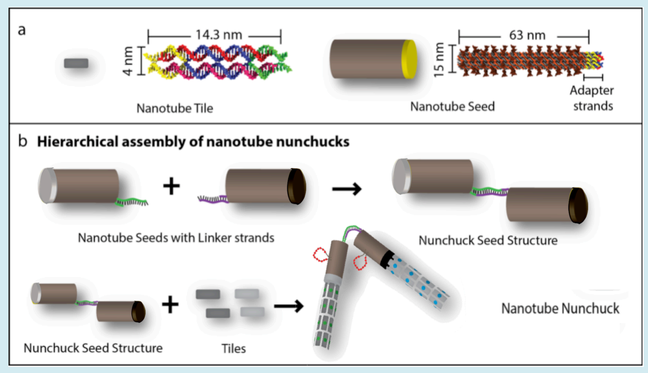
Abstract

The bending of DNA is essential to the expression of genes. Average bends are widely studied, but little is known about how they fluctuate in time. To observe DNA bending dynamics, we are developing a tool called a "nunchuck"[1], a double stranded piece of DNA that links a pair of DNA nanotubes endwise so that the nanotubes magnify the strand's bend angle. Essential to the construction of this tool is a pair of nanotube "seeds"[2] made of gel-purified DNA origami, but the gel-purification process has a highly variable yield. To simplify the process of producing nunchucks with good yield, we calibrated a fluorescence-based assay for seed concentration against microscopy. We found that higher Qubit readings correlated with greater seeds concentrations that could be added in smaller amounts to maintain tube length, whereas lower Qubit readings indicated lower seed concentration that required being added in greater amounts to boost yield. Qubit measurements provide a quick and easy way to achieve consistent nunchuck yields given the variability of origami seed concentration.

How to make and quantify DNA origami seeds

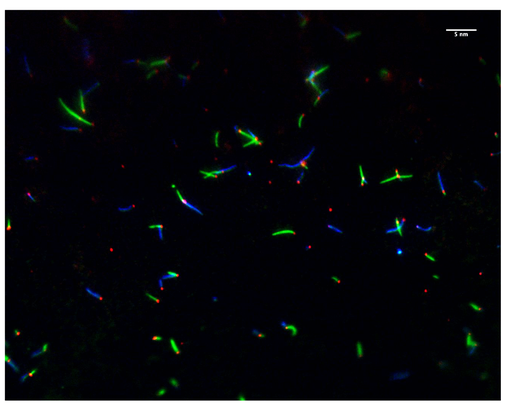
- Added staple strands and linker strands to single stranded M13 DNA; annealed to fold
- Mixed seeds with corresponding linkers and left overnight to dimerize
- Purified seeds with gel electrophoresis and measured the concentration of double stranded DNA with a Qubit Fluorometer 3.0
- Annealed tile strands, which are labeled with two green or one blue and one green fluorophore
- Added seeds in 0, 1, 2, 3, or 5 microliter amounts to allow tiles to nucleate off the seeds
- Analyzed each condition for average number of seeds and nunchucks to determine what percent of seeds formed nunchucks (nunchuck yield) for two different batches of seeds



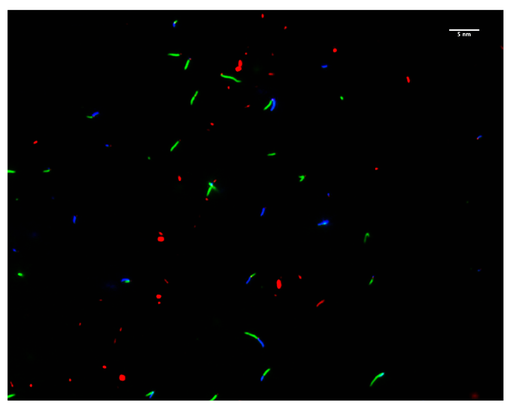


Analyzing Nunchuck Yield with Fluorescent Microscopy

1 uL of Seeds Added

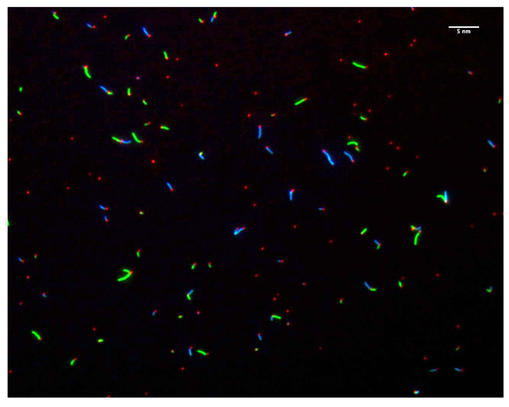


Batch 1: 352 ng/mL of double stranded DNA resulting in a 26% yield

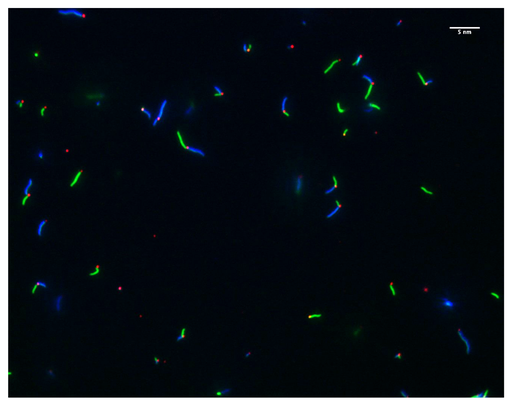


Batch 2: 203 ng/mL of double stranded DNA resulting in a 6% yield

3 uL of Seeds Added



Batch 1: 352 ng/mL of double stranded DNA resulting in a 8% yield



Batch 2: 203 ng/mL of double stranded DNA resulting in a 34% yield

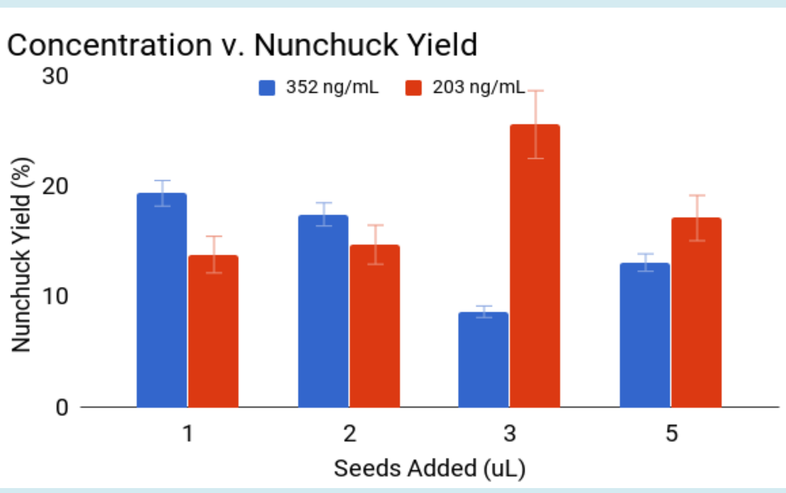


Figure 1. Nunchuck yield of two seed batches, one with a Qubit reading of 352 ng/mL of double stranded DNA and the other with a reading of 203 ng/mL.

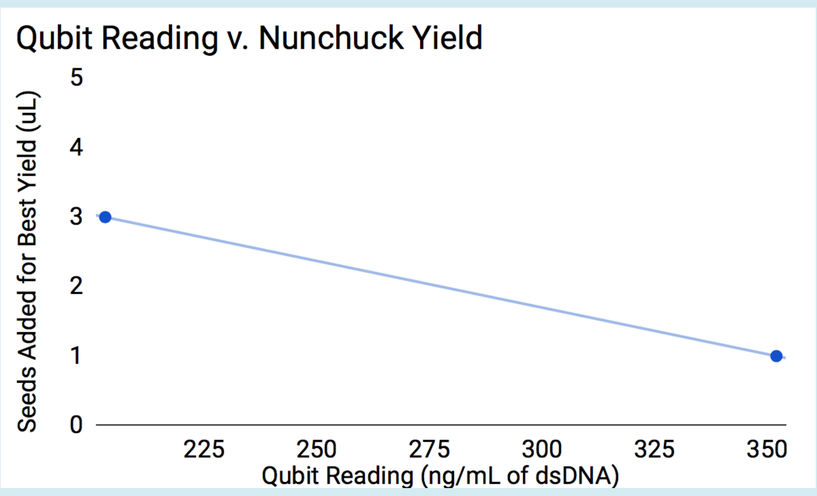


Figure 2. Optimal amount of seeds to add (uL) for highest nunchuck yield based on Qubit reading of the amount of double stranded DNA present in sample (ng/mL).

The sweet spot for seeding

An optimal amount of seeds must be added to the tiles to achieve a high yield of nunchucks with long enough arms. Adding too few seeds resulted in low yields, but adding an excess of seeds creates competition for the limited amount of tiles, resulting in arms too short to be visualized. The higher Qubit reading of seeds, 352 ng/mL of double stranded DNA, produced the highest yields when seeds were added at the 1 uL amount. The lower Qubit reading of seeds, 203 ng/mL, produced the highest yields when added at the 3uL amount.

Discussion

Lower Qubit readings indicated less double stranded DNA was present, and therefore a lower seed concentration; higher Qubit readings indicated more dsDNA and a higher seed concentration. To boost nunchuck yield, seeds with lower Qubit readings should be added in larger amounts, while seeds with higher Qubit readings should be added in smaller amounts to maintain arm length. By calibrating the Qubit, more consistent nunchuck yields can be achieved in spite of the variability of DNA origami, streamlining future experiments involving the nunchucks.

Citations

1.Mohammed, A. M. *et al.* Self-assembly of precisely defined DNA nanotube superstructures using DNA origami seeds. *Nanoscale* 9, 522–526 (2017).
2.Mohammed, A. M and Schulman.. *Nanoletters* 13, 4006– (2013).

Acknowledgments

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