AFRL-AFOSR-VA-TR-2023-0299

PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors

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PI: Rebecca Taylor, Carnegie Mellon University
Year 4 Period of Performance: 8/15/21 – 8/14/22

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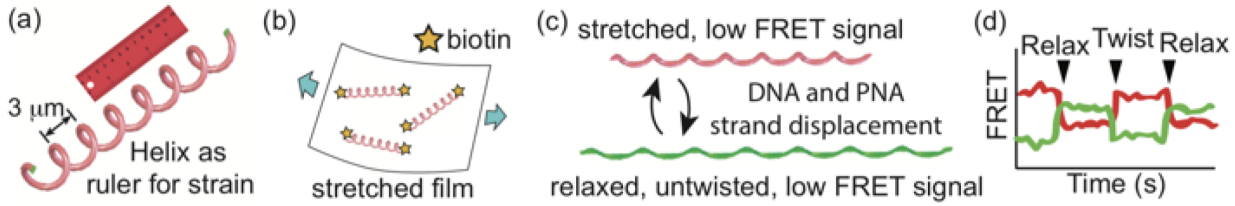

Figure 1. This YIP proposal aims to utilize (a) functionalized nucleic-acid based nanosprings that can be (b) incorporated into hydrated soft materials and surfaces. (c) Conformation-sensitive fluorescent labeling is needed to allow these nanosprings to (d) report stretch and actuation fluorescently.

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This additional year of support is allowing us to computationally and experimentally characterize our nanoscale 3D lever sensor while working to increase the water-solubility of our PNA nanofiber structures.

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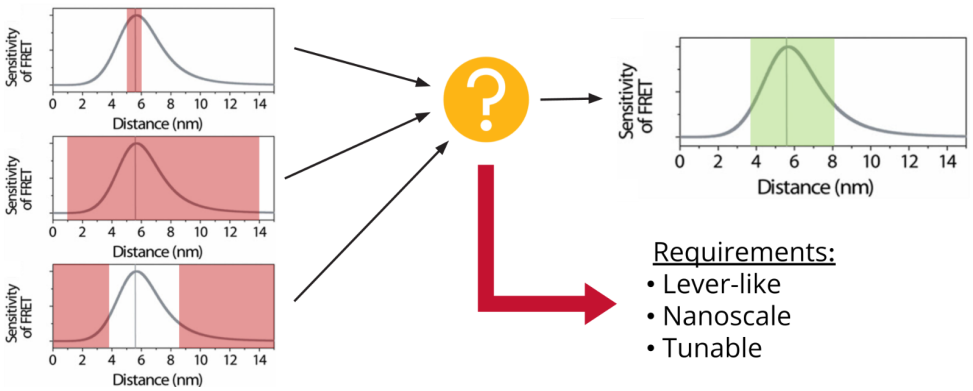

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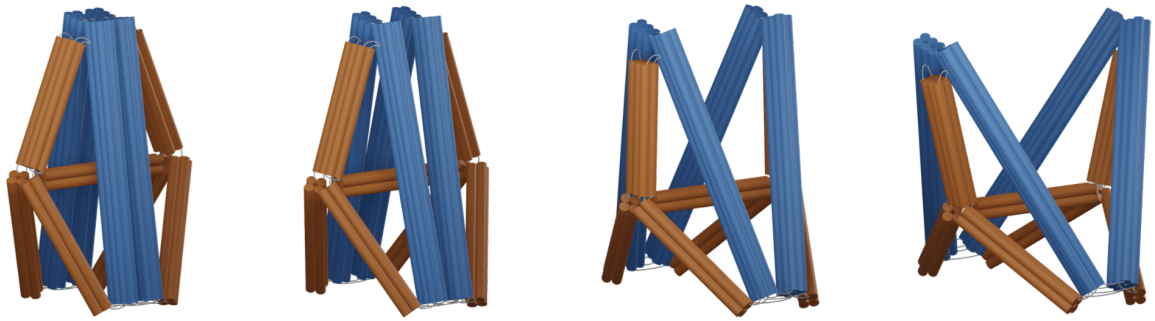

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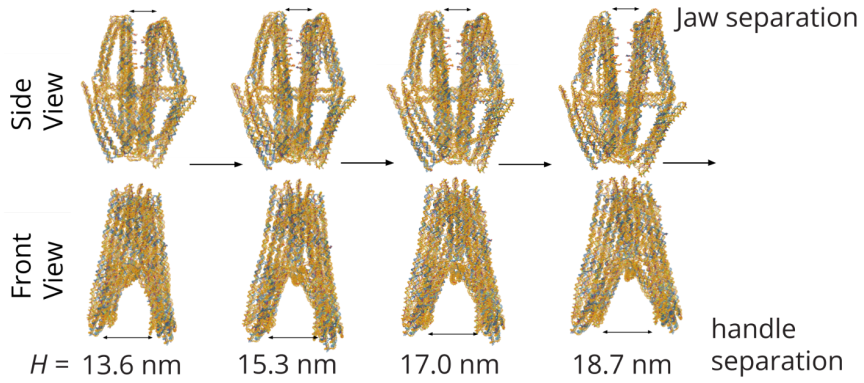

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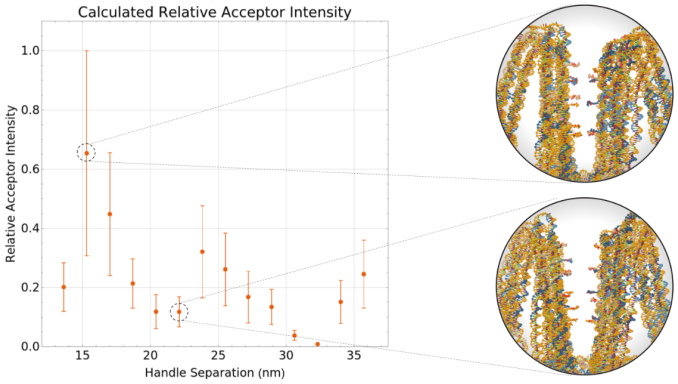
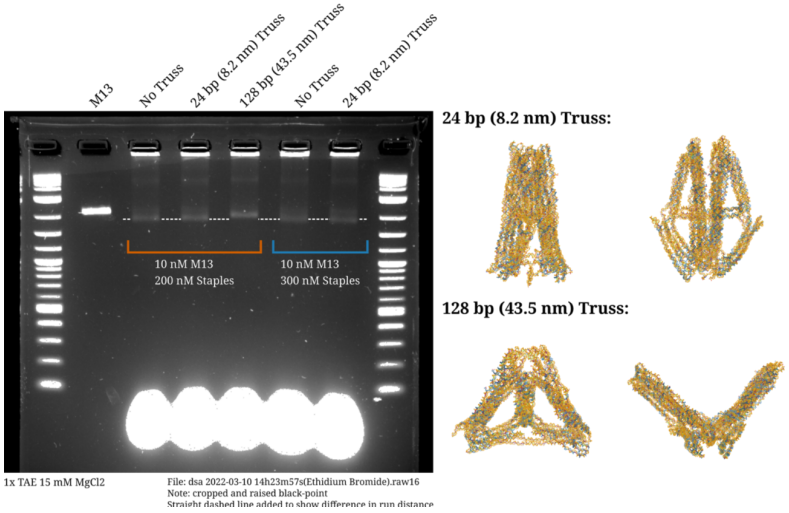


Figure 5. Coarse-grained simulations were used to find the mean separations of FRET pair fluorophores and the relative acceptor intensity we expect to see in upcoming experimental characterization of this device.

Fluorophore separations were extracted from those mean configurations and expected relative fluorescences values were calculated (**Fig. 5**).

With confidence that fluorescence changes should be visible as devices are actuated, we finalized our design and have built this second iteration. Our preliminary gel studies of this device indicate substantial device aggregation, which is common for wireframe structures. However sharp bands are also visible, indicating that a fraction of the origami are forming distinct structures. Trussed devices formed with constrained handle separations also had bands at different positions, suggesting that the trusses successfully changed the configuration and thus run speed of the nanoscale oriceps devices (**Fig. 6**).



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2.2 Year 4 Subaim 2: Map the analytical design space for DNA origami-based "Oriceps" sensors to create a simple tool that facilitates future kinematic synthesis of these sensors. This tool will return structure dimensions needed to attain desired deflection-conversion performance.

The design of these 3D levers is dependent on four independent variables (a,b,c, and α), which means that to attain a given mapping between jaw and gripper minima and maxima, we have an underdetermined system, which may result in there being numerous possible designs that will achieve the desired behavior. To address this issue and create a design resource, we mapped the analytical design space, identifying *all possible solutions for all possible jaw-handle separation constraints that will enable high sensitivity FRET*. We can then evaluate our options and select the best one for our application (for example which design deviates the least from the analytical model in coarse-grained simulations).

Our mapping demonstrated that designs exist for nearly all desirable starting and ending handle separations (**Fig. 7**). We have created a database of solutions and a software interface that quickly provides the design parameters of multiple designs that meet the desired handle separations. An SVG of a dimensioned flat design is also generated so that users can print their designs, cut them out, and fold models of their DNA origami oriceps designs.

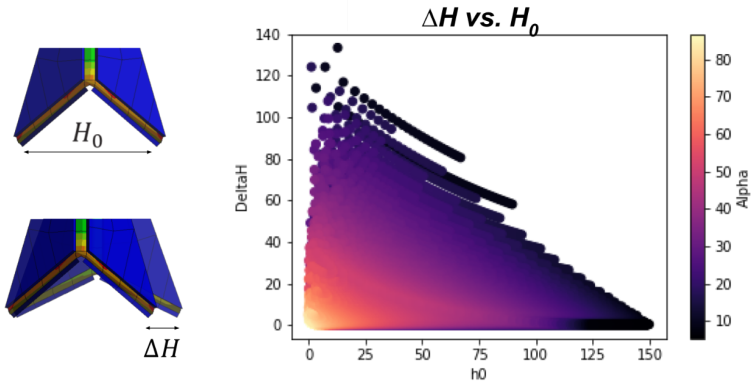

Figure 7. To determine the generalizable capability of this nanosensor, we mapped the design space. For a given application a user will want an initial distance (H_0) for a high FRET signal and a desired maximum displacement (ΔH) for a low FRET signal. Our plot of ΔH *vs.* H_0 shows that device dimension solutions exist for nearly all desired combinations.

2.3 Year 4 Subaim 3: Explore actuation of PNA-based nanofibers using strand displacement to drive twist, bending and stiffening, and develop nanofiber decoration strategy to attach Oriceps to PNA-based nanostructures.

In previous years we performed visualizations of hybrid nanostructures using Python and UCSF Chimera molecular visualization, but this approach was limited to modeling gPNA duplexes as simple cylinders. In 2021 a new tool called oxView was released, and it enables a major advance for the design of gPNA nanostructures (Poppleton et al. in Nucleic Acids Research https://doi.org/10.1093/nar/gkaa417). Our group has created custom functions to drive the creation of nucleic acid structures that mimic the helicity of gPNA-gPNA as well as gPNA-DNA duplexes (see **Fig. 8A**).

Using this tool we developed (and modeled in oxView) a novel structural motif for strand-minimal 3-helix nanofibers constructed from just three 12-base gPNA oligomers (see **Fig. 8B and 8C**). Our new 3-helix nanofibers will be used to study nanofiber solubility in water-based solutions, which is essential for enabling the interfacing of DNA oriceps sensors with gammaPNA nanofibers. Previous aggregations of nanofibers in water-based solutions limited their use as discrete nanostructures, while the surfactant plus DMSO solutions that

provided discrete nanofibers are incompatible with most DNA nanostructures (only the most stable DNA nanostructures can resist denaturation in a 75% DMSO solution).

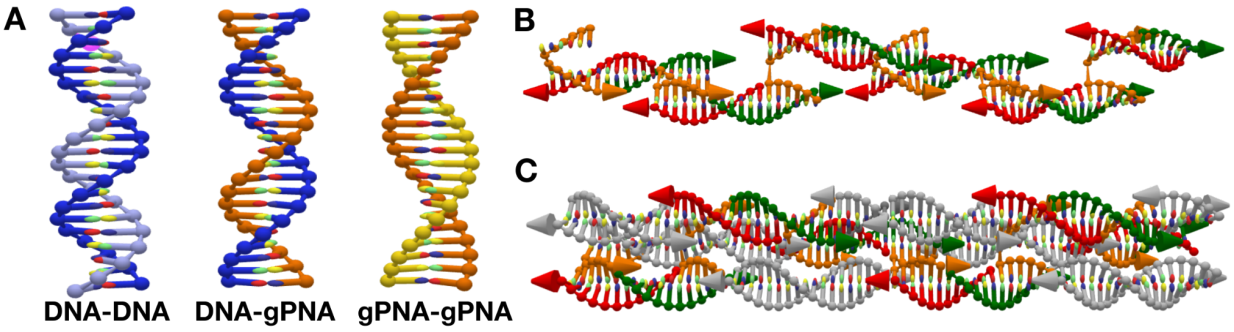

Figure 8. Custom scripted function in oxView enable visualization and design of novel PNA nanostructures. Design of PNA structural motifs must take into account (A) altered helicity of γPNA duplexes must be taken into account in the (B and C) design of structural motifs for γPNA interweaving.

2.4 Year 4 Subaim 4: Utilize Oriceps to measure displacements that are sub 2nm or greater than 8nm, which demonstrates that this tool extends the range of displacements measurable using a molecular ruler technology like FRET.

We have verified successful formation of our structures using atomic force microscopy, and in our next steps we will characterize the FRET using proof-of-principle studies of potassium responsive g-quadruplexes. These studies will cyclically actuate the oriceps through buffer exchange experiments. These experiments will allow us to examine the responsiveness of g-quadruplexes to ion concentration and, from an alternative perspective, demonstrate the capabilities of oriceps as a fluorescence based distance sensor that is in theory not limited to traditional FRET pair measurement limitations.

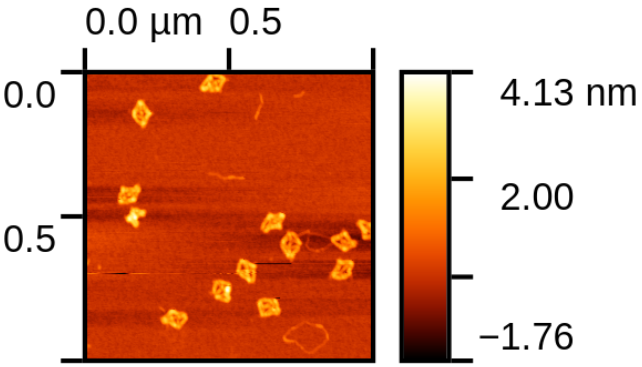

Figure 9. Successful formation of oriceps DNA origami nanolever structures has been verified by atomic force microscopy imaging.

Section 3. Synergistic activities

The NM&S community has been instrumental as a network of colleagues and collaborators. This group and the AFOSR in general has been extremely generous and creative in connecting me with resources and researchers with complementary research interests and capabilities.

- Our group is actively collaborating with Nicholas Stephanopoulos' team at the Biodesign Institute at Arizona State University on the application of PNA-peptides for self-assembly applications. The NMS program was the venue in which our teams connected and started collaborations.
- Our group is also collaborating with fellow YIP awardee, Stacy Copp from UCIrvine. We are investigating the application of PNAs for the stabilization of fluorescent silver

clusters. Our labs have another synergistic connection in that one of Stacy's former undergraduate researchers from her time at LANL is now a Ph.D. candidate in my research lab.

- We were recently awarded a DURIP grant with Co-PI Philip LeDuc (Mechanical Engineering, CMU) entitled "Peptide Nucleic Acid-Based Nanostructures at Biotic and Abiotic Interfaces". To quote from the DURIP proposal "This instrument will primarily be used to characterize the morphology, mechanics, and surface charge distribution of γPNA-containing nanostructures as part of an AFOSR Young Investigator Program (YIP) award entitled "PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors." This instrument will be used to perform label-free time-course visualization of dynamic structural changes in neurons that are part of a synthetic gut-brain-axis as part of Co-PI Phil LeDuc's AFOSR award "Synthetically Tuned Gut-Brain-Axis Communication." The supported research under this grant will be described in greater detail below. Taylor also anticipates leveraging the liquid imaging capabilities of this equipment in nascent research in the following two areas: (1) Characterization into the morphology and mechanics of γPNA nanostructures in a range organic solvent solutions, and (2) research on nucleic acid nanostructures and sensors anchored to the delicate surface of the cell membrane and glycocalyx."

Section 4. Relevant Publications

This year our lab has multiple papers in preparation, and in the upcoming year we expect to prepare manuscripts with both of our NMS collaborations.

[1] Gupta, M.K; Pearse, A.; Armitage, B.; Taylor, R.E. Terminal lysine decoration improves solubility of strand-minimal micron-scale gammaPNA nanofibers. (in preparation)

[2] Arias, D.S.; Taylor, R.E. Nanoscape DNA linkages for fluorescent measurement of arbitrary distances and displacements. (in preparation)

[3] Wang, W.; Hayes, P.; Ren, X.; Taylor, R.E. Synthetic cell armor made of DNA origami. (submitted)

[4] Goodwin-Schoen, C.; Taylor, R.E. Modular, articulated models of DNA and peptide nucleic acids for nanotechnology education (submitted)

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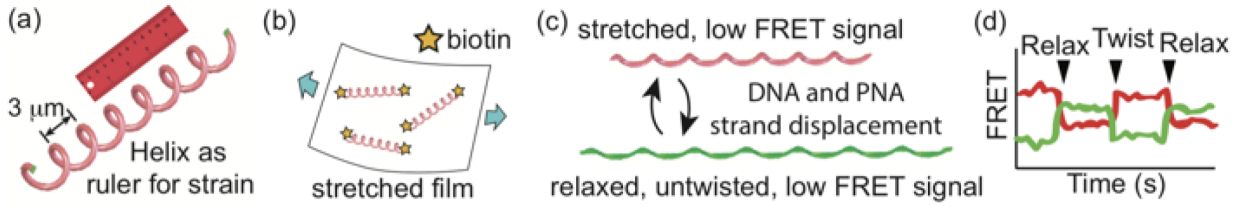

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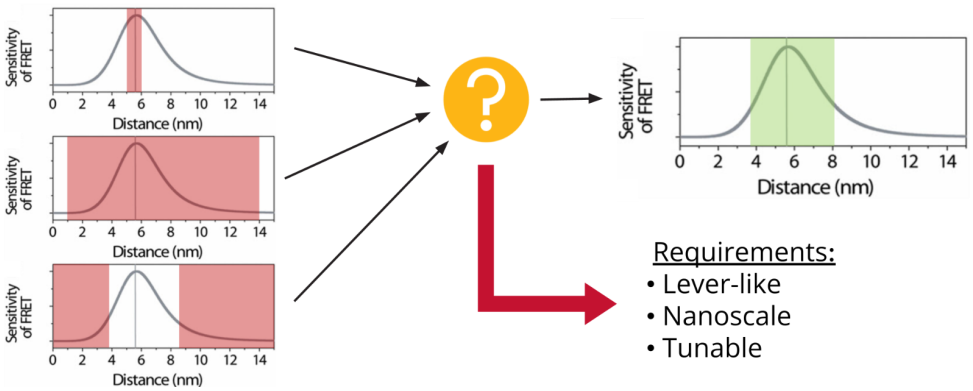

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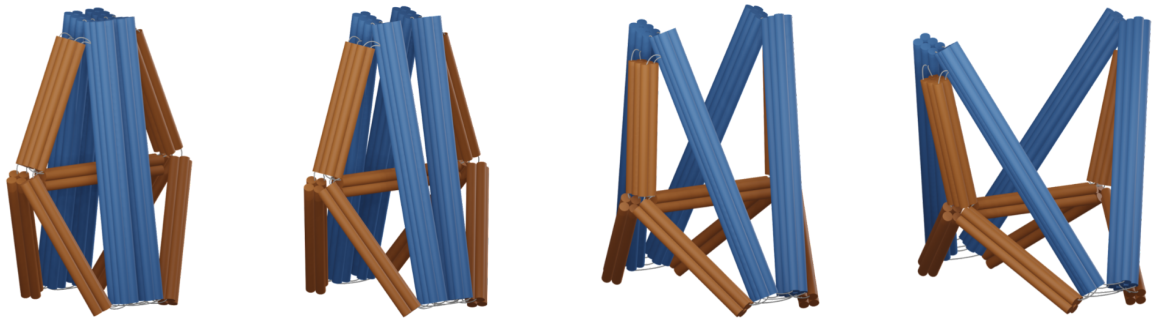

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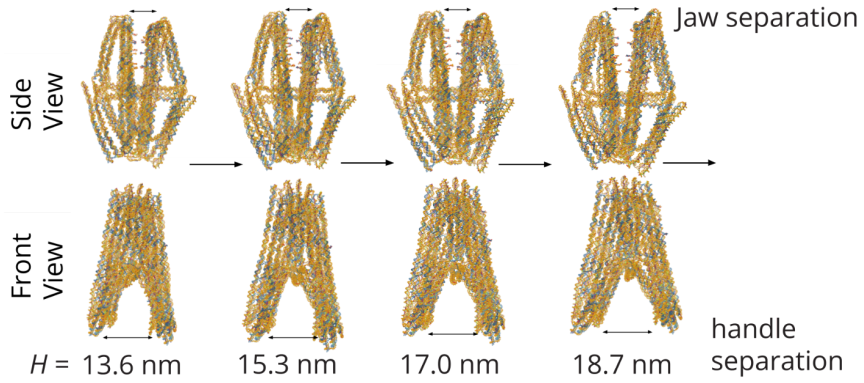

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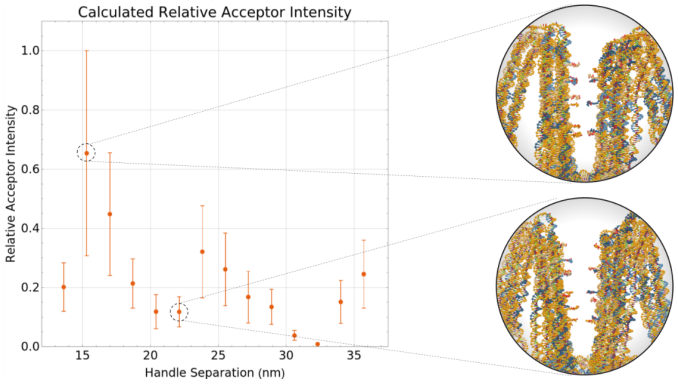

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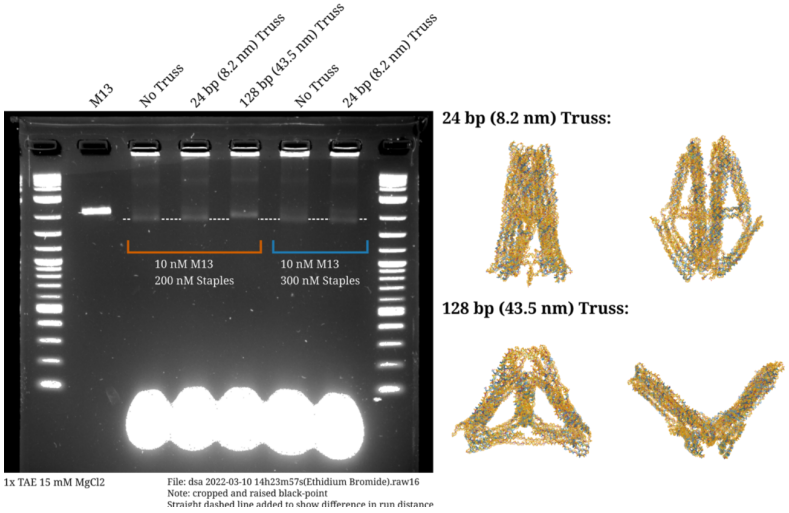

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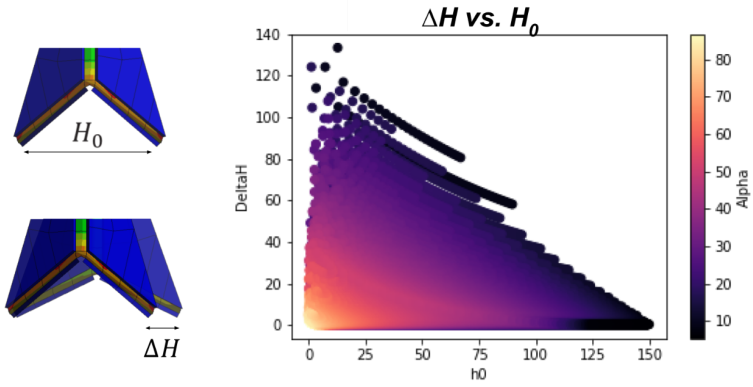

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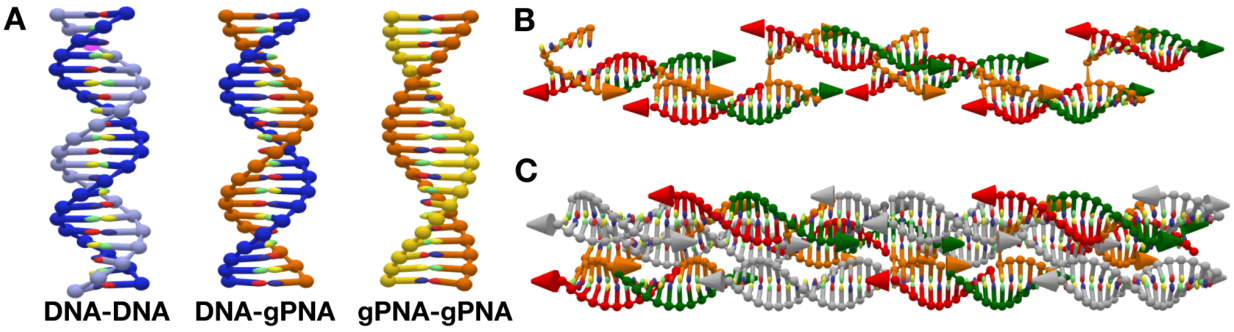

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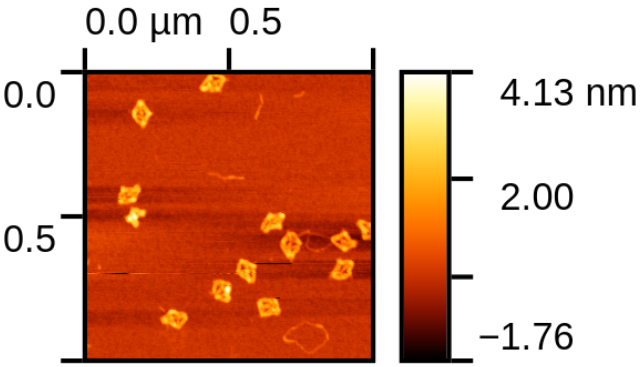

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AFOSR YIP award #FA9550-18-1-0199 (Year 1-3 Period of Performance: 5/15/18 - 5/14/21)
PI: Rebecca Taylor, Carnegie Mellon University
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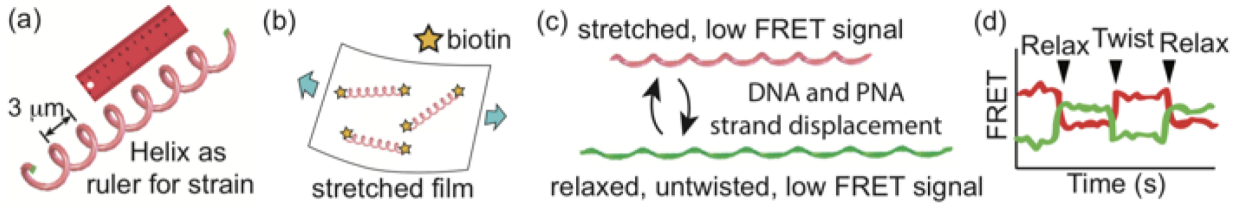

Figure 1. This YIP proposal aims to utilize (a) functionalized nucleic-acid based nanosprings that can be (b) incorporated into hydrated soft materials and surfaces. (c) Conformation-sensitive fluorescent labeling is needed to allow these nanosprings to (d) report stretch and actuation fluorescently.

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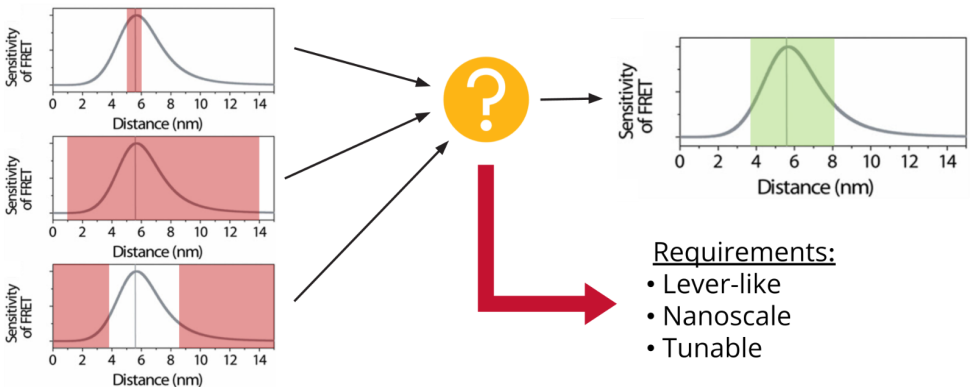
This additional year of support is allowing us to computationally and experimentally characterize our nanoscale 3D lever sensor while working to increase the water-solubility of our PNA nanofiber structures.

2.1 Year 4 Subaim 1: Synthesize, purify, and characterize first demonstrations of "Oriceps" displacement sensors, and measure sensor FRET efficiency as a function of handle separation to generate calibration curves for 3D nanolevers.

Briefly, fluorescent strain measurements to date are limited to measuring distances from 3 to 8nm. Structural DNA nanotechnology approaches have shown the utility of lever- or caliper-based systems for measuring larger and smaller nanoscale distances, but simple levers are unable to sensitively measure arbitrary displacements from arbitrary starting separations, because the start and end positions must be multiples of FRET measurable distances, like 3n to 8n. For example, to date it has not been possible to measure small displacements of just a couple nanometers from initial wide separations in the tens of nanometers range, because caliper systems linearly scale the sensitivity of a given FRET pair. A lever with a wide starting separation (3n) is accordingly only sensitive to large displacements (8n-3n). Our DNA origami oriceps design was created to directly address this problem, enabling the high sensitivity measurement of arbitrary start and end positions (**Fig. 2**).



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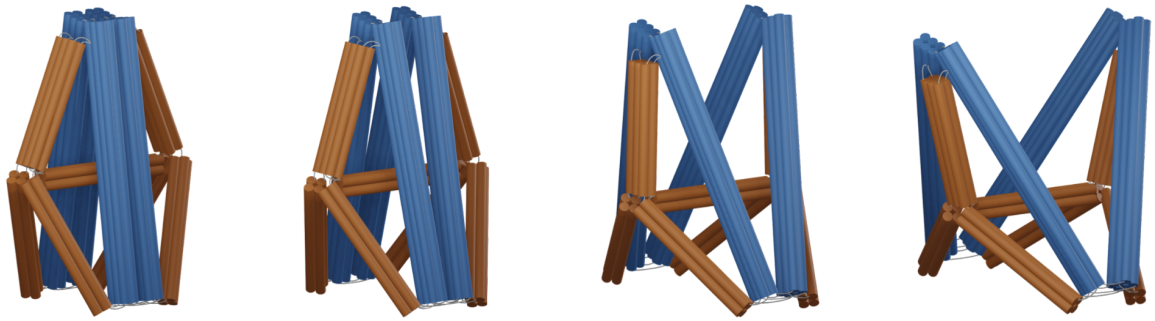
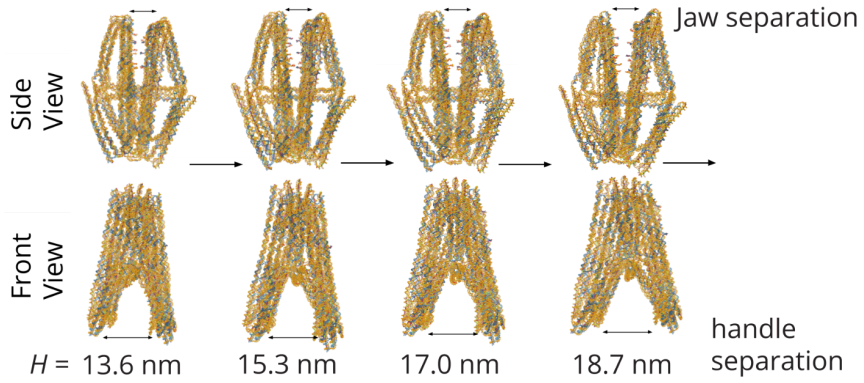


Figure 3. Cartoon representations of this sensor from closed (left) to open (right) configurations.

Our current oriceps design has the following critical dimensions: a = 26.2 nm, b = 14.3 nm, and c = 25.8 nm. Our analytical model of the system predicts peak FRET when the starting handle separation, H0, equals 18.9 nm and the change of handle separation, dH, equals 8.61 nm. Our lever is designed to operate in the most sensitive range of the FRET pairs, reporting peak FRET at 18.9 nm and minimum FRET at 27.5 nm. This is unlike a linear-scaling lever, which would need to scale the distances up 6.3 times to have PEAK FRET at 18.9 nm. However a displacement of (8*6.3-3*6.3) about 31.5 nm to 50.4 nm would be required to have minimal FRET. In practice, therefore, a linear-scaling lever would operate in just a fraction (in this case about half) of the FRET-sensitive range, substantially reducing the signal to noise ratio for measurements.

Through multiple rounds of coarse-grained simulation in oxDNA we tuned the design of these oriceps. Coarse-grained molecular dynamics simulations were performed to determine the mean configurations at equilibrium when the handles were held at specific displacements simulated potential wells and double-stranded truss connectors (**Fig. 4**).



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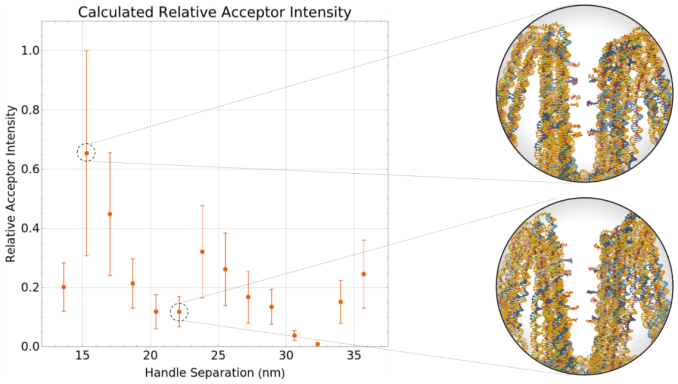
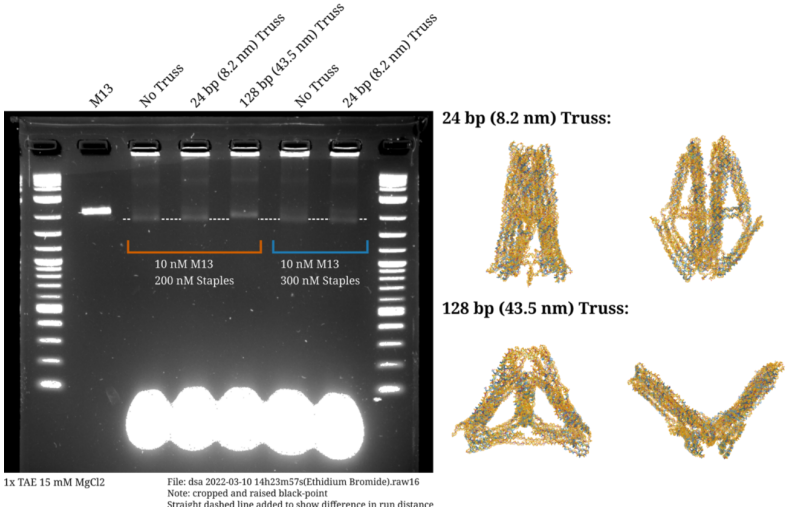


Figure 5. Coarse-grained simulations were used to find the mean separations of FRET pair fluorophores and the relative acceptor intensity we expect to see in upcoming experimental characterization of this device.

Fluorophore separations were extracted from those mean configurations and expected relative fluorescences values were calculated (**Fig. 5**).

With confidence that fluorescence changes should be visible as devices are actuated, we finalized our design and have built this second iteration. Our preliminary gel studies of this device indicate substantial device aggregation, which is common for wireframe structures. However sharp bands are also visible, indicating that a fraction of the origami are forming distinct structures. Trussed devices formed with constrained handle separations also had bands at different positions, suggesting that the trusses successfully changed the configuration and thus run speed of the nanoscale oriceps devices (**Fig. 6**).



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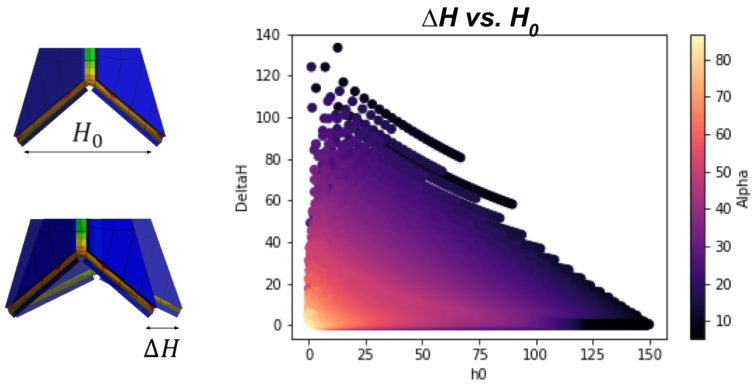

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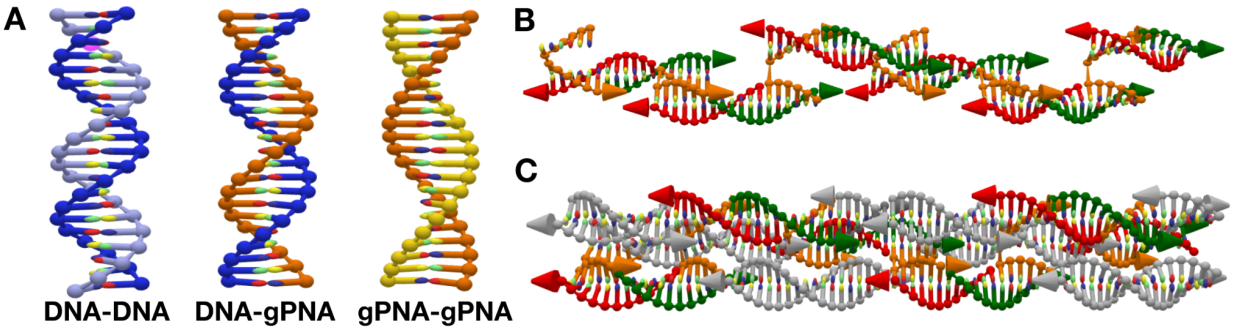

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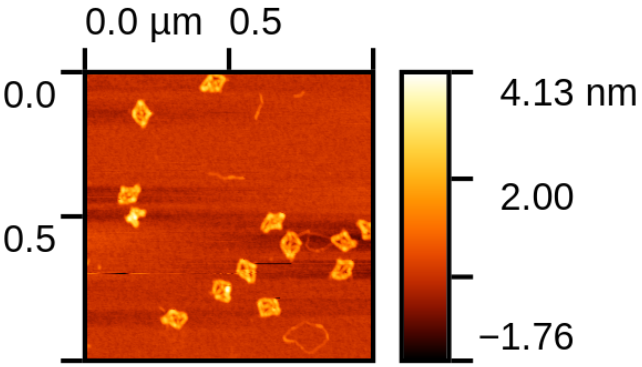

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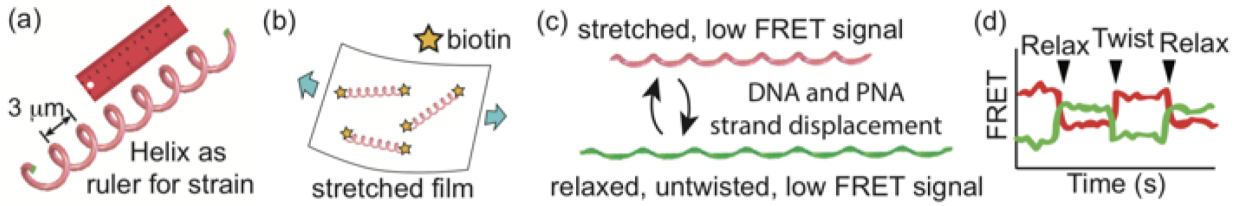

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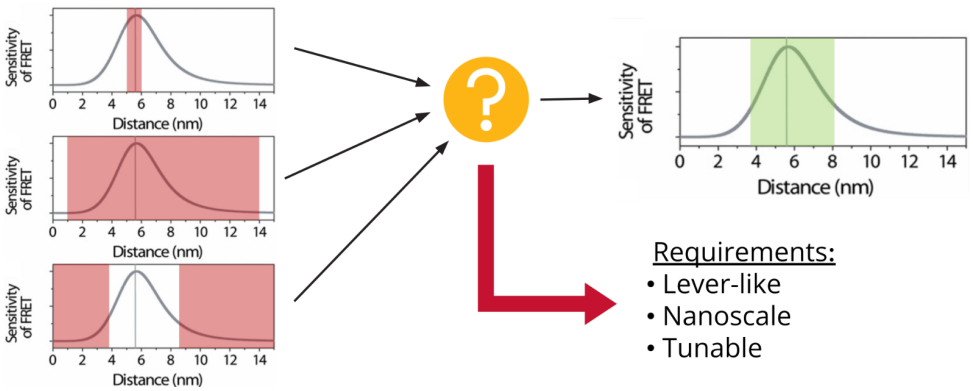

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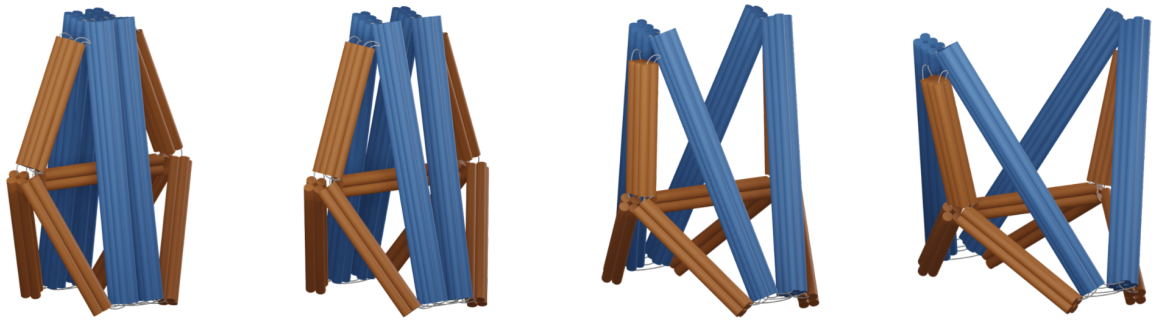

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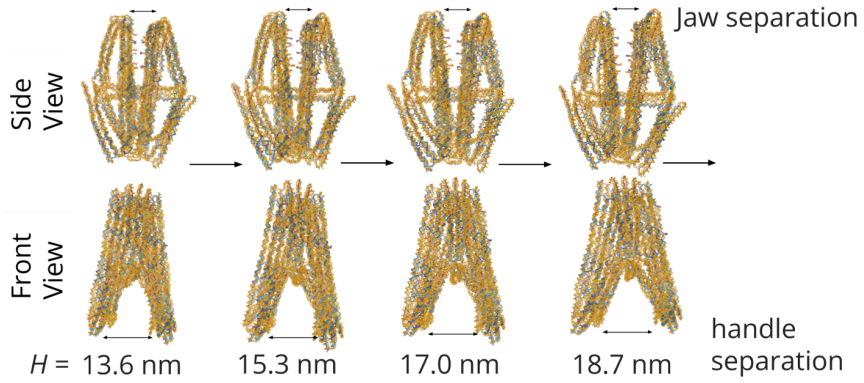

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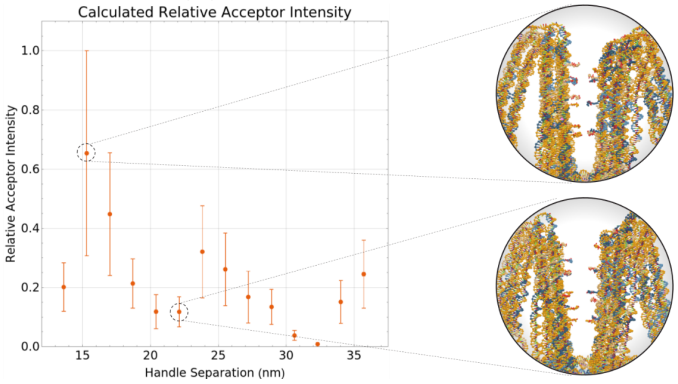

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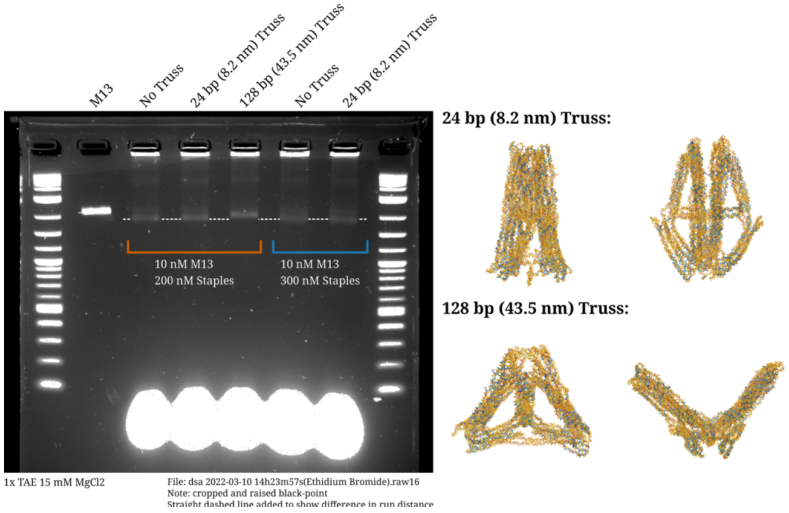

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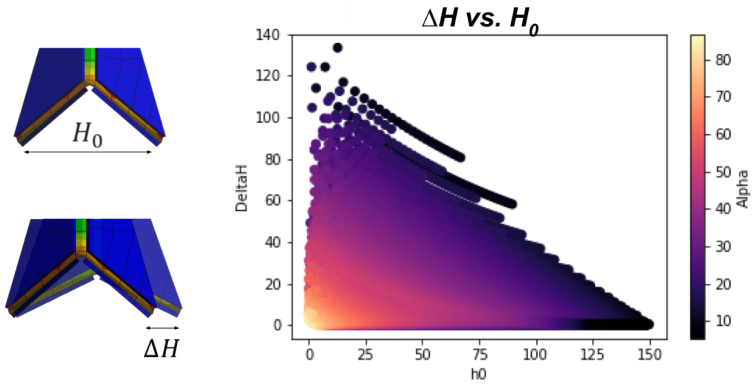

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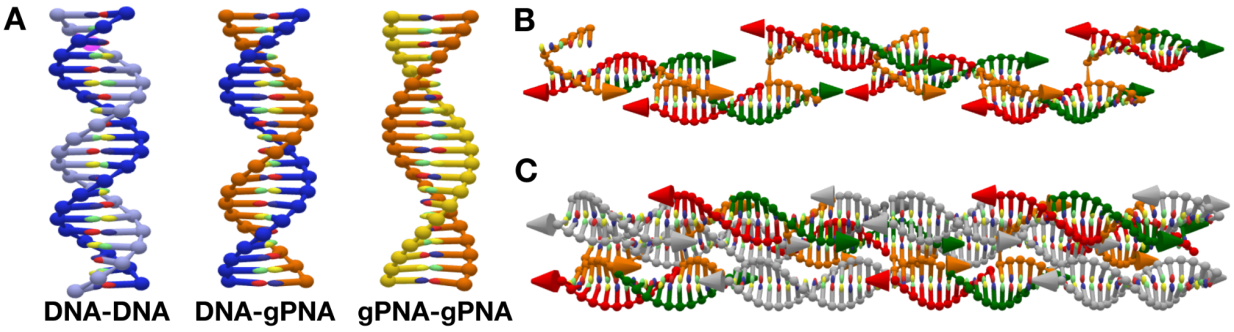

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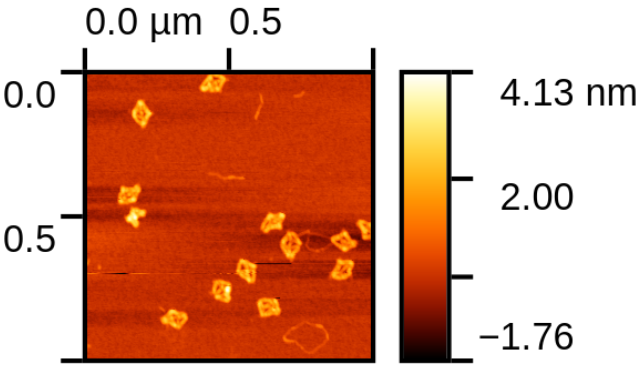

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Program Officer Name

Dr. Bennett Ibey

Principal Investigator Name

Dr. Rebecca Taylor

Project Title

PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors

ABSTRACT

The Year 4 extension of this project allowed us to investigate the actuation of novel gPNA-based nanofibers and characterization of 3D lever system. This 4th year extension has the following four subaims: (1) First demonstration of nanoscale "Oriceps" sensor; (2) Create design tool for oriceps by mapping the design space; (3) Explore actuatable PNA systems and attach oriceps; (4) Use oriceps to measure sub 2nm or greater than 8nm separations.

Year 4 of "PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors"
AFOSR YIP award #FA9550-18-1-0199 (Year 1-3 Period of Performance: 5/15/18 - 5/14/21)
PI: Rebecca Taylor, Carnegie Mellon University
Year 4 Period of Performance: 8/15/21 – 8/14/22

1. Years 4 aims

The Year 4 extension of this project is enabling the experimental validation of the 3D lever concept, involving the synthesis and characterization of the device and a deeper exploration of the design space available for similar structures. This year of support is allowing us to investigate the actuation of novel gPNA-based nanofibers, and finally the integration of the 3D lever with the DNA nanosprings would enable the major endpoint of the initial YIP: the demonstration of a conformation-reporting fluorescent nanospring construct. These activities are captured in the following subaims. To achieve this goal, the 4th year extension has the following four subaims: (1) First demonstration of nanoscale "Oriceps" sensor; (2) Create design tool for oriceps by mapping the design space; (3) Explore actuatable PNA systems and attach oriceps; (4) Use oriceps to measure sub 2nm or greater than 8nm separations.

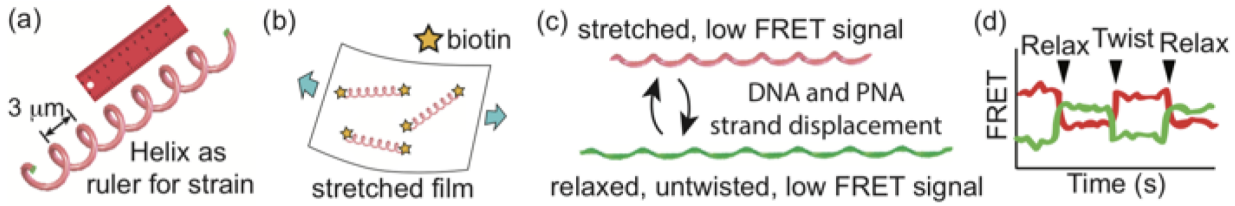


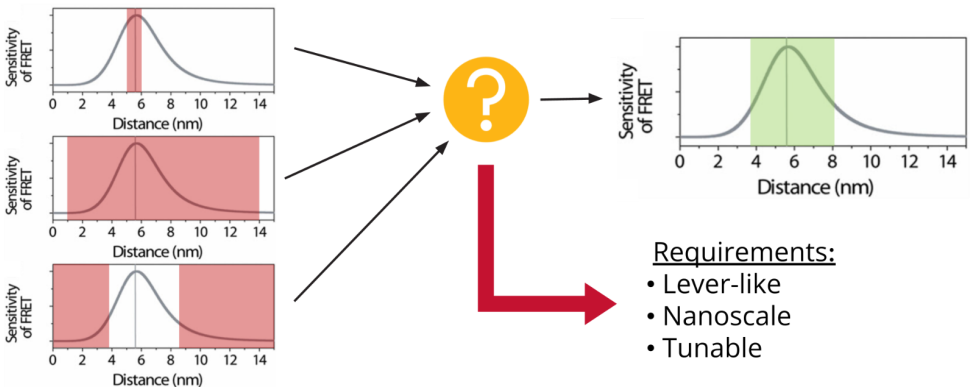
Figure 1. This YIP proposal aims to utilize (a) functionalized nucleic-acid based nanosprings that can be (b) incorporated into hydrated soft materials and surfaces. (c) Conformation-sensitive fluorescent labeling is needed to allow these nanosprings to (d) report stretch and actuation fluorescently.

2. Progress in Year 4

This additional year of support is allowing us to computationally and experimentally characterize our nanoscale 3D lever sensor while working to increase the water-solubility of our PNA nanofiber structures.

2.1 Year 4 Subaim 1: Synthesize, purify, and characterize first demonstrations of "Oriceps" displacement sensors, and measure sensor FRET efficiency as a function of handle separation to generate calibration curves for 3D nanolevers.

Briefly, fluorescent strain measurements to date are limited to measuring distances from 3 to 8nm. Structural DNA nanotechnology approaches have shown the utility of lever- or caliper-based systems for measuring larger and smaller nanoscale distances, but simple levers are unable to sensitively measure arbitrary displacements from arbitrary starting separations, because the start and end positions must be multiples of FRET measurable distances, like 3n to 8n. For example, to date it has not been possible to measure small displacements of just a couple nanometers from initial wide separations in the tens of nanometers range, because caliper systems linearly scale the sensitivity of a given FRET pair. A lever with a wide starting separation (3n) is accordingly only sensitive to large displacements (8n-3n). Our DNA origami oriceps design was created to directly address this problem, enabling the high sensitivity measurement of arbitrary start and end positions (**Fig. 2**).



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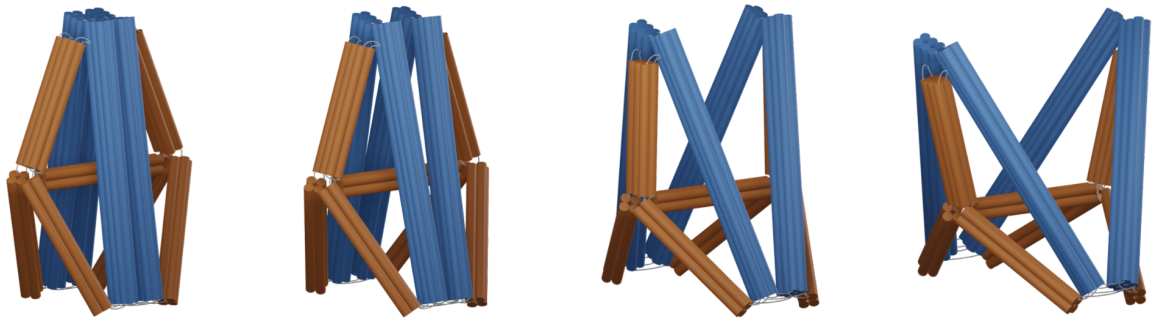

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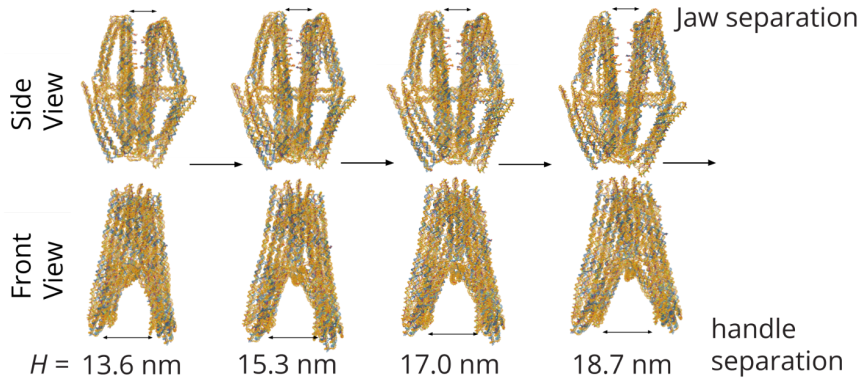

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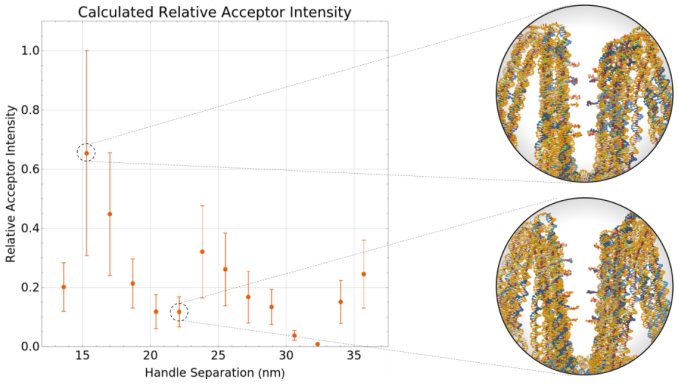

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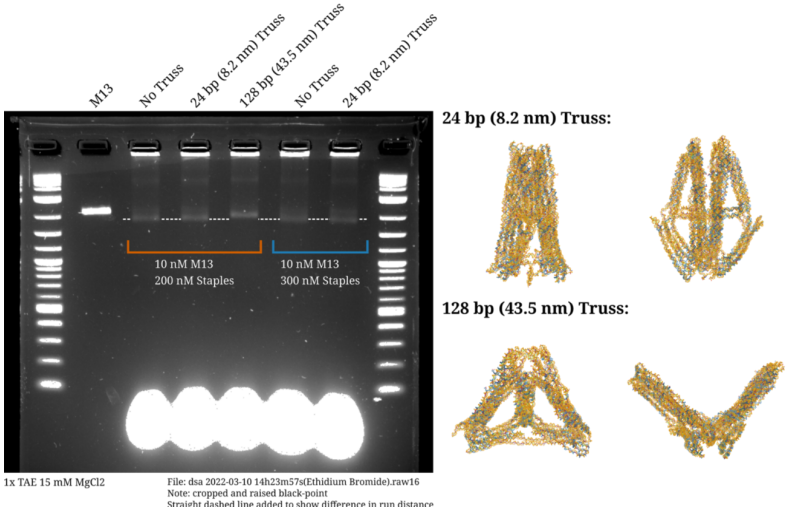

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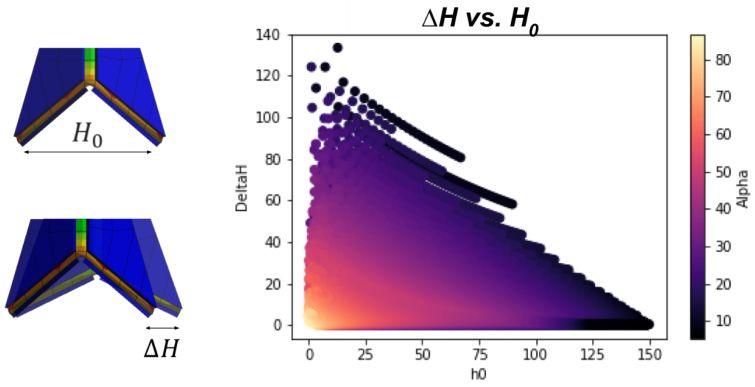

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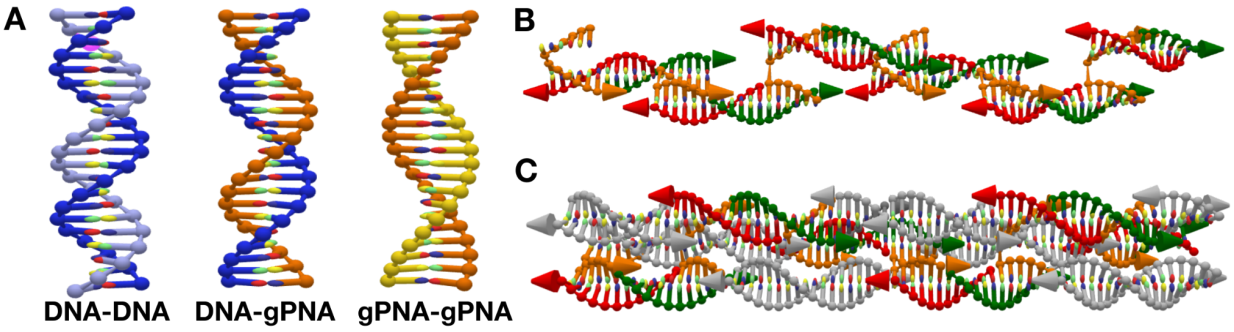

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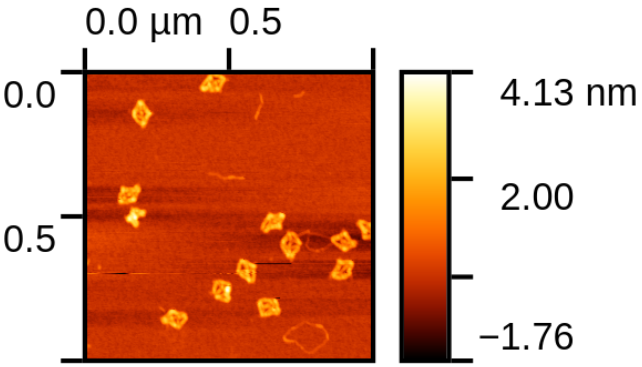

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Program Officer Name

Dr. Bennett Ibey

Principal Investigator Name

Dr. Rebecca Taylor

Project Title

PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors

ABSTRACT

The Year 4 extension of this project allowed us to investigate the actuation of novel gPNA-based nanofibers and characterization of 3D lever system. This 4th year extension has the following four subaims: (1) First demonstration of nanoscale "Oriceps" sensor; (2) Create design tool for oriceps by mapping the design space; (3) Explore actuatable PNA systems and attach oriceps; (4) Use oriceps to measure sub 2nm or greater than 8nm separations.

Year 4 of "PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors"
AFOSR YIP award #FA9550-18-1-0199 (Year 1-3 Period of Performance: 5/15/18 - 5/14/21)
PI: Rebecca Taylor, Carnegie Mellon University
Year 4 Period of Performance: 8/15/21 – 8/14/22

1. Years 4 aims

The Year 4 extension of this project is enabling the experimental validation of the 3D lever concept, involving the synthesis and characterization of the device and a deeper exploration of the design space available for similar structures. This year of support is allowing us to investigate the actuation of novel gPNA-based nanofibers, and finally the integration of the 3D lever with the DNA nanosprings would enable the major endpoint of the initial YIP: the demonstration of a conformation-reporting fluorescent nanospring construct. These activities are captured in the following subaims. To achieve this goal, the 4th year extension has the following four subaims: (1) First demonstration of nanoscale "Oriceps" sensor; (2) Create design tool for oriceps by mapping the design space; (3) Explore actuatable PNA systems and attach oriceps; (4) Use oriceps to measure sub 2nm or greater than 8nm separations.

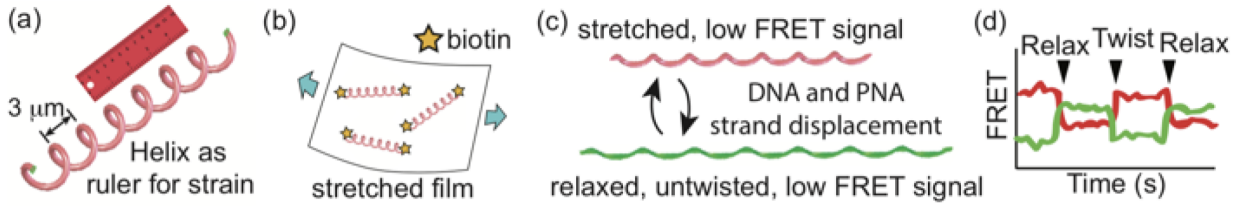

Figure 1. This YIP proposal aims to utilize (a) functionalized nucleic-acid based nanosprings that can be (b) incorporated into hydrated soft materials and surfaces. (c) Conformation-sensitive fluorescent labeling is needed to allow these nanosprings to (d) report stretch and actuation fluorescently.

2. Progress in Year 4

This additional year of support is allowing us to computationally and experimentally characterize our nanoscale 3D lever sensor while working to increase the water-solubility of our PNA nanofiber structures.

2.1 Year 4 Subaim 1: Synthesize, purify, and characterize first demonstrations of "Oriceps" displacement sensors, and measure sensor FRET efficiency as a function of handle separation to generate calibration curves for 3D nanolevers.

Briefly, fluorescent strain measurements to date are limited to measuring distances from 3 to 8nm. Structural DNA nanotechnology approaches have shown the utility of lever- or caliper-based systems for measuring larger and smaller nanoscale distances, but simple levers are unable to sensitively measure arbitrary displacements from arbitrary starting separations, because the start and end positions must be multiples of FRET measurable distances, like 3n to 8n. For example, to date it has not been possible to measure small displacements of just a couple nanometers from initial wide separations in the tens of nanometers range, because caliper systems linearly scale the sensitivity of a given FRET pair. A lever with a wide starting separation (3n) is accordingly only sensitive to large displacements (8n-3n). Our DNA origami oriceps design was created to directly address this problem, enabling the high sensitivity measurement of arbitrary start and end positions (**Fig. 2**).

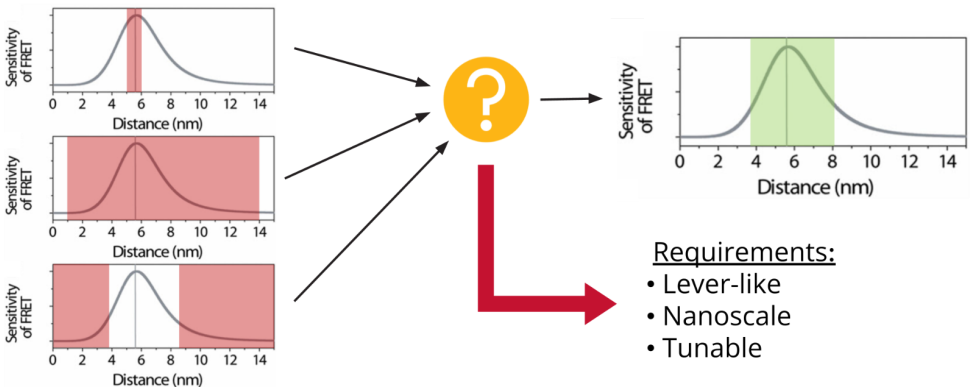

Figure 2. Device requirements for this fluorescent strain / displacement sensor.

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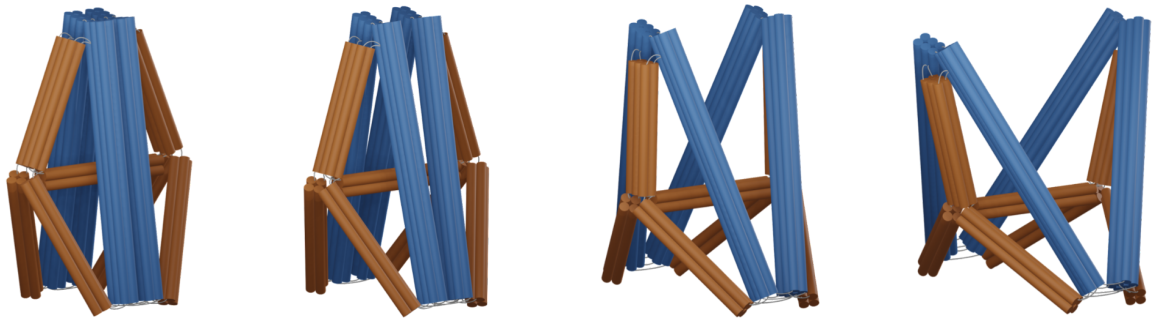

Figure 3. Cartoon representations of this sensor from closed (left) to open (right) configurations.

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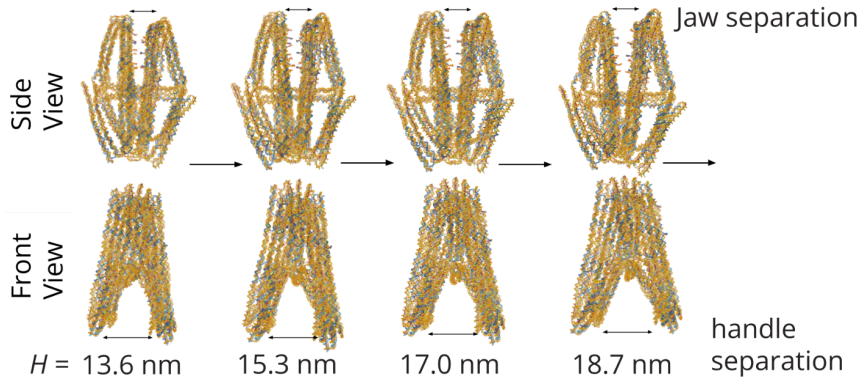

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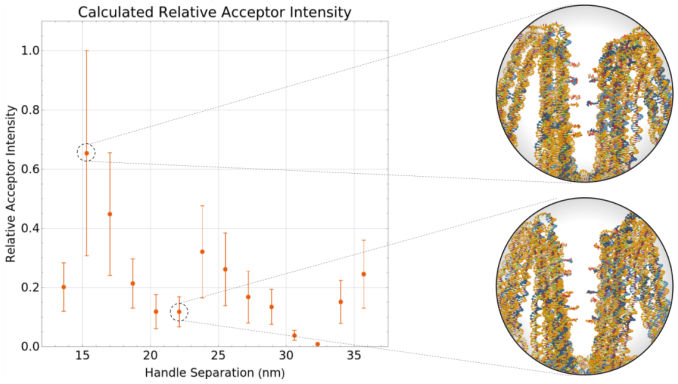

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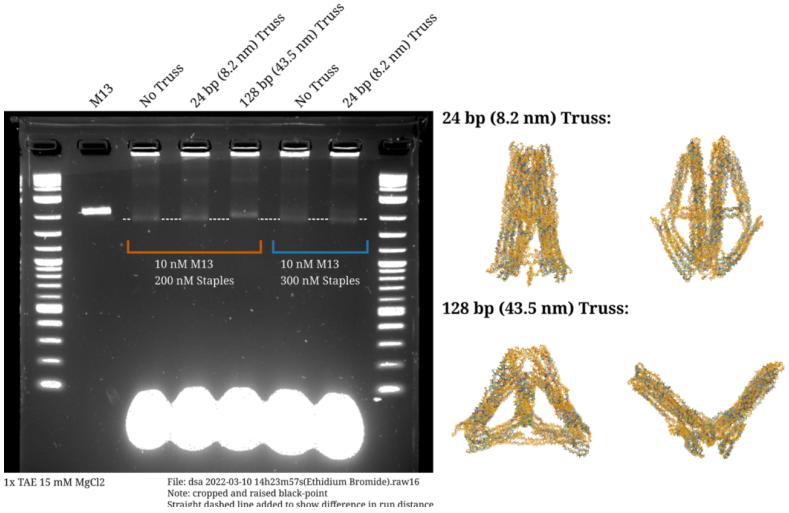

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2.2 Year 4 Subaim 2: Map the analytical design space for DNA origami-based "Oriceps" sensors to create a simple tool that facilitates future kinematic synthesis of these sensors. This tool will return structure dimensions needed to attain desired deflection-conversion performance.

The design of these 3D levers is dependent on four independent variables (a,b,c, and α), which means that to attain a given mapping between jaw and gripper minima and maxima, we have an underdetermined system, which may result in there being numerous possible designs that will achieve the desired behavior. To address this issue and create a design resource, we mapped the analytical design space, identifying *all possible solutions for all possible jaw-handle separation constraints that will enable high sensitivity FRET*. We can then evaluate our options and select the best one for our application (for example which design deviates the least from the analytical model in coarse-grained simulations).

Our mapping demonstrated that designs exist for nearly all desirable starting and ending handle separations (**Fig. 7**). We have created a database of solutions and a software interface that quickly provides the design parameters of multiple designs that meet the desired handle separations. An SVG of a dimensioned flat design is also generated so that users can print their designs, cut them out, and fold models of their DNA origami oriceps designs.

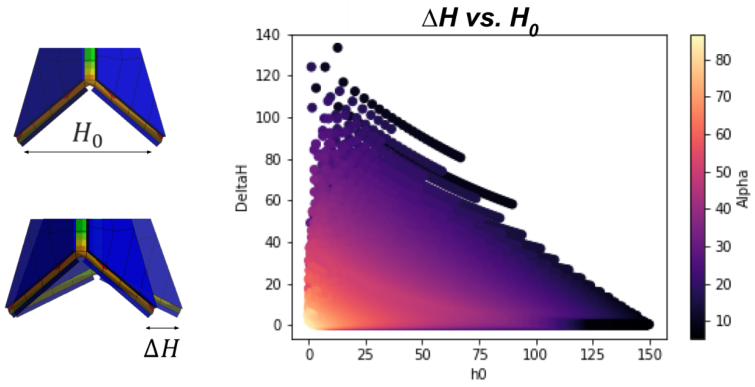

Figure 7. To determine the generalizable capability of this nanosensor, we mapped the design space. For a given application a user will want an initial distance (H_0) for a high FRET signal and a desired maximum displacement (ΔH) for a low FRET signal. Our plot of ΔH *vs.* H_0 shows that device dimension solutions exist for nearly all desired combinations.

2.3 Year 4 Subaim 3: Explore actuation of PNA-based nanofibers using strand displacement to drive twist, bending and stiffening, and develop nanofiber decoration strategy to attach Oriceps to PNA-based nanostructures.

In previous years we performed visualizations of hybrid nanostructures using Python and UCSF Chimera molecular visualization, but this approach was limited to modeling gPNA duplexes as simple cylinders. In 2021 a new tool called oxView was released, and it enables a major advance for the design of gPNA nanostructures (Poppleton et al. in Nucleic Acids Research https://doi.org/10.1093/nar/gkaa417). Our group has created custom functions to drive the creation of nucleic acid structures that mimic the helicity of gPNA-gPNA as well as gPNA-DNA duplexes (see **Fig. 8A**).

Using this tool we developed (and modeled in oxView) a novel structural motif for strand-minimal 3-helix nanofibers constructed from just three 12-base gPNA oligomers (see **Fig. 8B and 8C**). Our new 3-helix nanofibers will be used to study nanofiber solubility in water-based solutions, which is essential for enabling the interfacing of DNA oriceps sensors with gammaPNA nanofibers. Previous aggregations of nanofibers in water-based solutions limited their use as discrete nanostructures, while the surfactant plus DMSO solutions that

provided discrete nanofibers are incompatible with most DNA nanostructures (only the most stable DNA nanostructures can resist denaturation in a 75% DMSO solution).

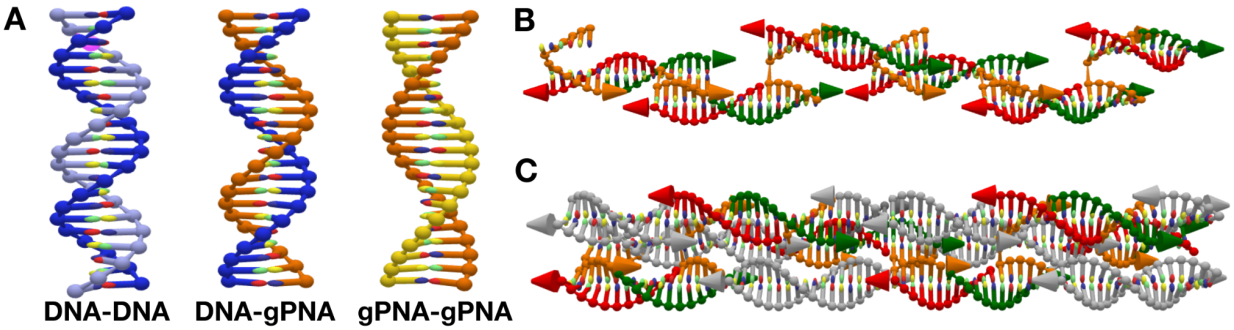

Figure 8. Custom scripted function in oxView enable visualization and design of novel PNA nanostructures. Design of PNA structural motifs must take into account (A) altered helicity of γPNA duplexes must be taken into account in the (B and C) design of structural motifs for γPNA interweaving.

2.4 Year 4 Subaim 4: Utilize Oriceps to measure displacements that are sub 2nm or greater than 8nm, which demonstrates that this tool extends the range of displacements measurable using a molecular ruler technology like FRET.

We have verified successful formation of our structures using atomic force microscopy, and in our next steps we will characterize the FRET using proof-of-principle studies of potassium responsive g-quadruplexes. These studies will cyclically actuate the oriceps through buffer exchange experiments. These experiments will allow us to examine the responsiveness of g-quadruplexes to ion concentration and, from an alternative perspective, demonstrate the capabilities of oriceps as a fluorescence based distance sensor that is in theory not limited to traditional FRET pair measurement limitations.

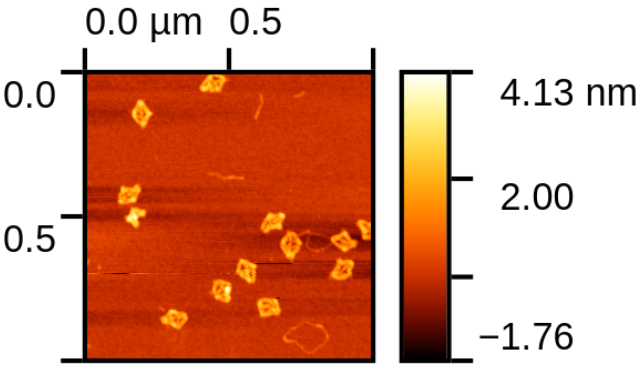

Figure 9. Successful formation of oriceps DNA origami nanolever structures has been verified by atomic force microscopy imaging.

Section 3. Synergistic activities

The NM&S community has been instrumental as a network of colleagues and collaborators. This group and the AFOSR in general has been extremely generous and creative in connecting me with resources and researchers with complementary research interests and capabilities.

- Our group is actively collaborating with Nicholas Stephanopoulos' team at the Biodesign Institute at Arizona State University on the application of PNA-peptides for self-assembly applications. The NMS program was the venue in which our teams connected and started collaborations.
- Our group is also collaborating with fellow YIP awardee, Stacy Copp from UCIrvine. We are investigating the application of PNAs for the stabilization of fluorescent silver

clusters. Our labs have another synergistic connection in that one of Stacy's former undergraduate researchers from her time at LANL is now a Ph.D. candidate in my research lab.

- We were recently awarded a DURIP grant with Co-PI Philip LeDuc (Mechanical Engineering, CMU) entitled "Peptide Nucleic Acid-Based Nanostructures at Biotic and Abiotic Interfaces". To quote from the DURIP proposal "This instrument will primarily be used to characterize the morphology, mechanics, and surface charge distribution of γPNA-containing nanostructures as part of an AFOSR Young Investigator Program (YIP) award entitled "PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors." This instrument will be used to perform label-free time-course visualization of dynamic structural changes in neurons that are part of a synthetic gut-brain-axis as part of Co-PI Phil LeDuc's AFOSR award "Synthetically Tuned Gut-Brain-Axis Communication." The supported research under this grant will be described in greater detail below. Taylor also anticipates leveraging the liquid imaging capabilities of this equipment in nascent research in the following two areas: (1) Characterization into the morphology and mechanics of γPNA nanostructures in a range organic solvent solutions, and (2) research on nucleic acid nanostructures and sensors anchored to the delicate surface of the cell membrane and glycocalyx."

Section 4. Relevant Publications

This year our lab has multiple papers in preparation, and in the upcoming year we expect to prepare manuscripts with both of our NMS collaborations.

[1] Gupta, M.K; Pearse, A.; Armitage, B.; Taylor, R.E. Terminal lysine decoration improves solubility of strand-minimal micron-scale gammaPNA nanofibers. (in preparation)

[2] Arias, D.S.; Taylor, R.E. Nanoscape DNA linkages for fluorescent measurement of arbitrary distances and displacements. (in preparation)

[3] Wang, W.; Hayes, P.; Ren, X.; Taylor, R.E. Synthetic cell armor made of DNA origami. (submitted)

[4] Goodwin-Schoen, C.; Taylor, R.E. Modular, articulated models of DNA and peptide nucleic acids for nanotechnology education (submitted)

Report Coversheet Template

Award Number/Project Number

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Final

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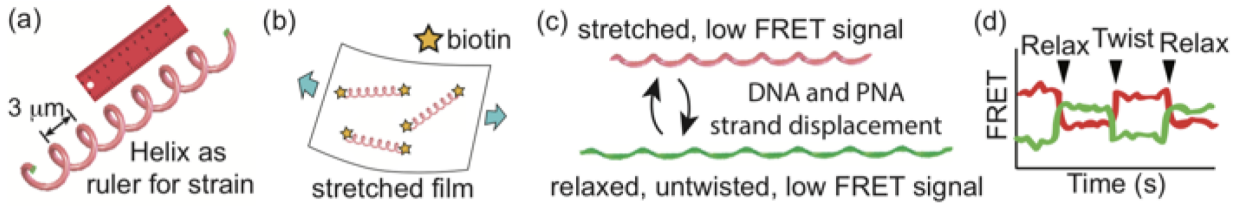

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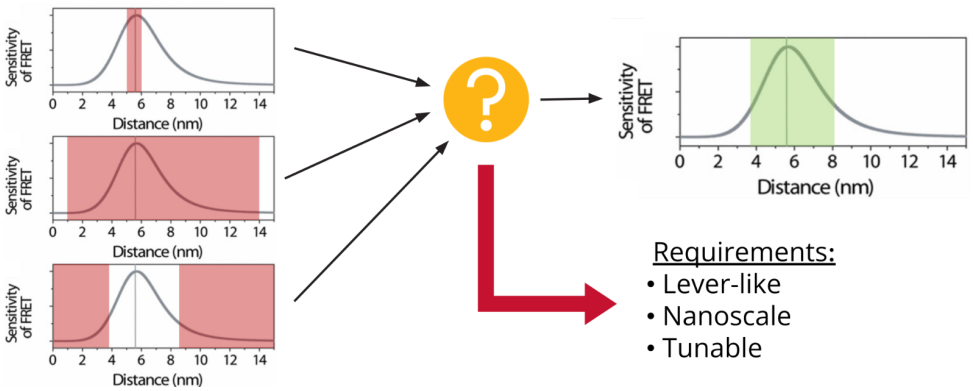

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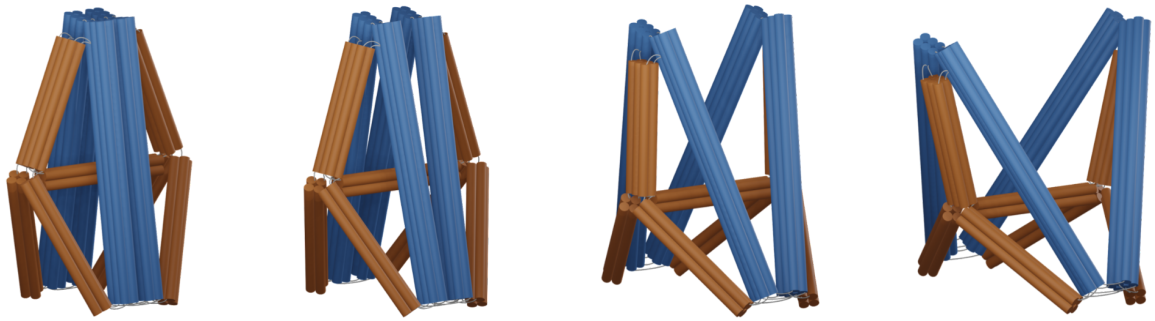

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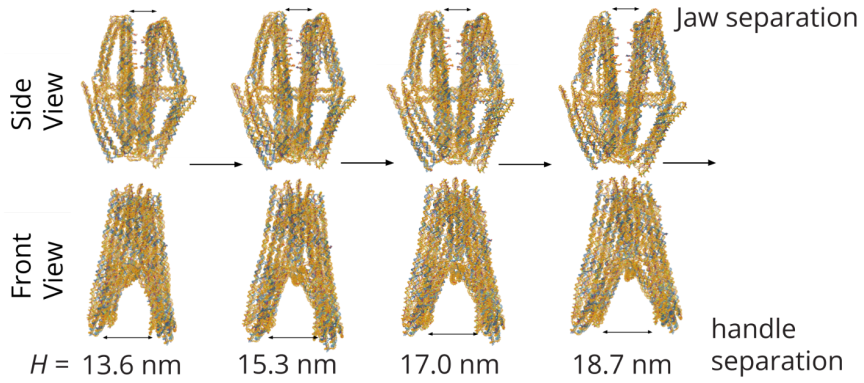

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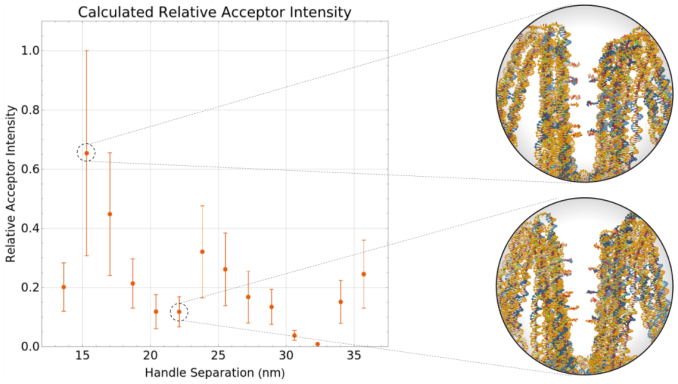

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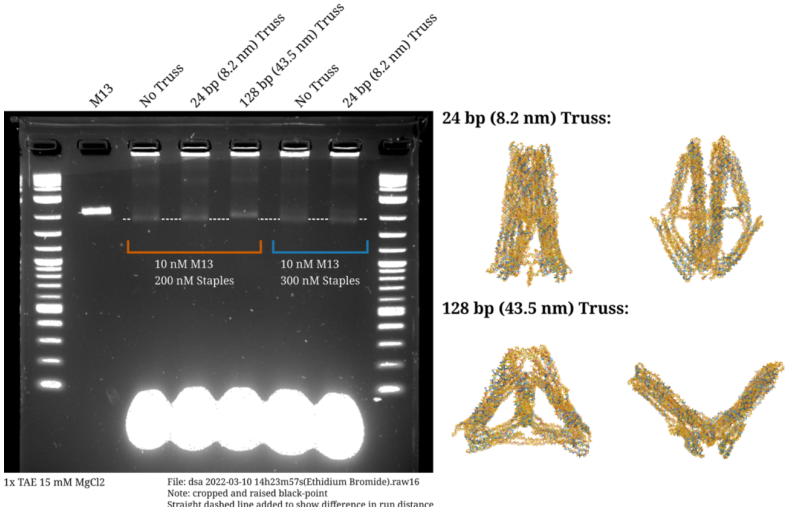

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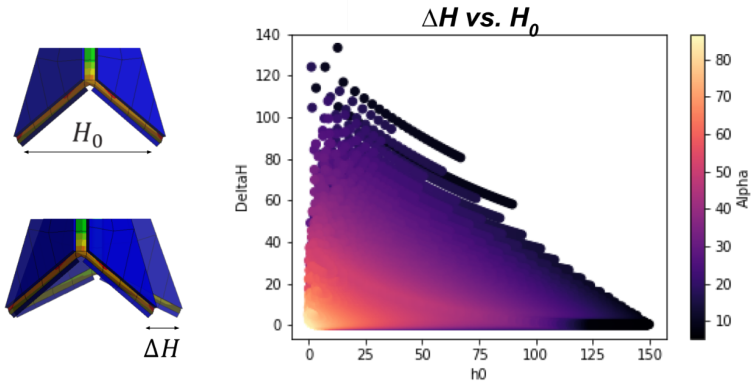

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provided discrete nanofibers are incompatible with most DNA nanostructures (only the most stable DNA nanostructures can resist denaturation in a 75% DMSO solution).

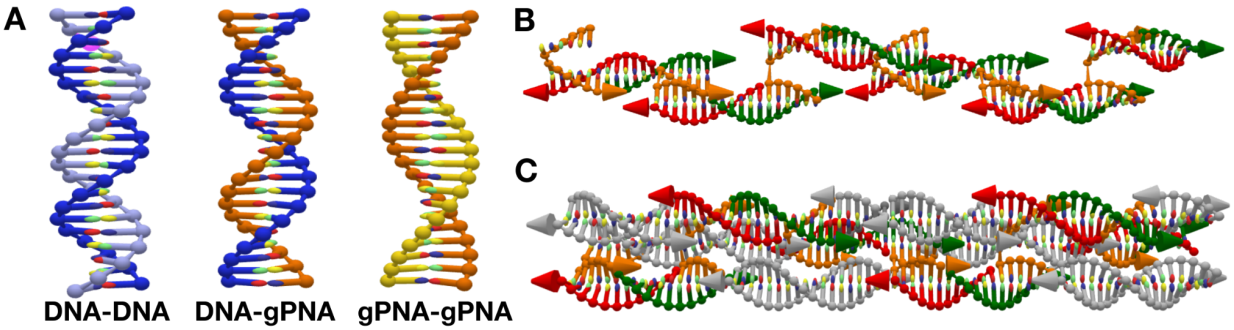

Figure 8. Custom scripted function in oxView enable visualization and design of novel PNA nanostructures. Design of PNA structural motifs must take into account (A) altered helicity of γPNA duplexes must be taken into account in the (B and C) design of structural motifs for γPNA interweaving.

2.4 Year 4 Subaim 4: Utilize Oriceps to measure displacements that are sub 2nm or greater than 8nm, which demonstrates that this tool extends the range of displacements measurable using a molecular ruler technology like FRET.

We have verified successful formation of our structures using atomic force microscopy, and in our next steps we will characterize the FRET using proof-of-principle studies of potassium responsive g-quadruplexes. These studies will cyclically actuate the oriceps through buffer exchange experiments. These experiments will allow us to examine the responsiveness of g-quadruplexes to ion concentration and, from an alternative perspective, demonstrate the capabilities of oriceps as a fluorescence based distance sensor that is in theory not limited to traditional FRET pair measurement limitations.

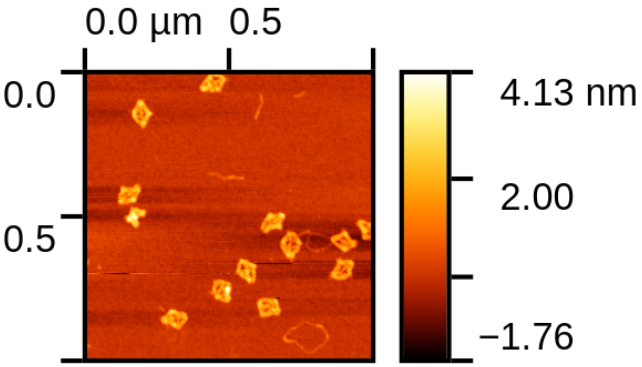

Figure 9. Successful formation of oriceps DNA origami nanolever structures has been verified by atomic force microscopy imaging.

Section 3. Synergistic activities

The NM&S community has been instrumental as a network of colleagues and collaborators. This group and the AFOSR in general has been extremely generous and creative in connecting me with resources and researchers with complementary research interests and capabilities.

- Our group is actively collaborating with Nicholas Stephanopoulos' team at the Biodesign Institute at Arizona State University on the application of PNA-peptides for self-assembly applications. The NMS program was the venue in which our teams connected and started collaborations.
- Our group is also collaborating with fellow YIP awardee, Stacy Copp from UCIrvine. We are investigating the application of PNAs for the stabilization of fluorescent silver

clusters. Our labs have another synergistic connection in that one of Stacy's former undergraduate researchers from her time at LANL is now a Ph.D. candidate in my research lab.

- We were recently awarded a DURIP grant with Co-PI Philip LeDuc (Mechanical Engineering, CMU) entitled "Peptide Nucleic Acid-Based Nanostructures at Biotic and Abiotic Interfaces". To quote from the DURIP proposal "This instrument will primarily be used to characterize the morphology, mechanics, and surface charge distribution of γPNA-containing nanostructures as part of an AFOSR Young Investigator Program (YIP) award entitled "PNA-Driven Remote Actuation of DNA Nanospring Strain Sensors." This instrument will be used to perform label-free time-course visualization of dynamic structural changes in neurons that are part of a synthetic gut-brain-axis as part of Co-PI Phil LeDuc's AFOSR award "Synthetically Tuned Gut-Brain-Axis Communication." The supported research under this grant will be described in greater detail below. Taylor also anticipates leveraging the liquid imaging capabilities of this equipment in nascent research in the following two areas: (1) Characterization into the morphology and mechanics of γPNA nanostructures in a range organic solvent solutions, and (2) research on nucleic acid nanostructures and sensors anchored to the delicate surface of the cell membrane and glycocalyx."

Section 4. Relevant Publications

This year our lab has multiple papers in preparation, and in the upcoming year we expect to prepare manuscripts with both of our NMS collaborations.

[1] Gupta, M.K; Pearse, A.; Armitage, B.; Taylor, R.E. Terminal lysine decoration improves solubility of strand-minimal micron-scale gammaPNA nanofibers. (in preparation)

[2] Arias, D.S.; Taylor, R.E. Nanoscape DNA linkages for fluorescent measurement of arbitrary distances and displacements. (in preparation)

[3] Wang, W.; Hayes, P.; Ren, X.; Taylor, R.E. Synthetic cell armor made of DNA origami. (submitted)

[4] Goodwin-Schoen, C.; Taylor, R.E. Modular, articulated models of DNA and peptide nucleic acids for nanotechnology education (submitted)