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a. REPORT	b. ABSTRACT	c. THIS PAGE			Michael Norton
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RPPR Final Report
as of 25-Jul-2018

Agency Code:

Proposal Number: 55114EL **Agreement Number: W911NF-09-1-0218**
INVESTIGATOR(S):

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Country: USA
DUNS Number: 036156615 EIN: 550683361
Report Date: 21-May-2018 Date Received: 20-May-2018
Final Report for Period Beginning 22-Jun-2009 and Ending 21-Feb-2018
Title: Transcription as Sequencing - TAS
Begin Performance Period: 22-Jun-2009 **End Performance Period:** 21-Feb-2018
Report Term: 0-Other
Submitted By: Michael Norton Email: norton@marshall.edu
Phone: (304) 696-3489
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 4 **STEM Participants:** 7

Major Goals: The objective of this program of research is the development of an inexpensive, self-assembling, mass producible platform for the rapid, sensitive and parallel sequencing of DNA via a sequencing by synthesis approach using optical output. There are multiple component goals for this project, or three major subobjectives: 1. Development of the polymerase design necessary for this project and 2. Development of methods for the generation of a self-assembling dipole antenna system appropriate for increasing the intensity of the output signal from single fluorophores and 3. Contribute to the human scientific research infrastructure by providing in lab experience to students.

The first objective has several components, including design and cloning of polymerases compatible with localization on origami, origami platform optimization, optimizing protein production and purification, confirming activity of the free and bound protein, development of techniques for the characterization of the bound protein, evaluating activity of the bound protein, developing single molecule fluorescence monitoring techniques for individual proteins, characterizing individual protein activity and characterizing individual base incorporation into polymers by localized, surface bound protein molecules.

The second sub-objective requires development of both the understanding of plasmonic interactions with fluorophores and development of methods for metal (gold) nanoparticle modification, purification, isolation, precision placement on origami with respect to emitters and characterization of the assemblies.

The third, educational objective is to be achieved by incorporation, in funded or unfunded roles, students of all ages to participate in the research enterprise and learn research objectives of the DOD and the ARO in particular.

Accomplishments: This is a summary. Many of these points are discussed in greater detail in the uploaded report since this component of the report is text only.

1. Development of the polymerase design necessary for this project:
This first objective has several components, including design and cloning of polymerases compatible with localization on origami, origami platform optimization, optimizing protein production and purification, confirming activity of the free and bound protein, development of techniques for the characterization of the bound protein, evaluating activity of the bound protein, developing single molecule fluorescence monitoring techniques for individual proteins, characterizing individual protein activity and characterizing individual base incorporation into polymers by localized, surface bound protein molecules.

We have designed and cloned T7RNAP, a polymerase molecule made compatible with localization on origami by including in the design a biotinylation site, which enables immobilization of the polymerase on the origami through a biotin-streptavidin-biotin bridge to a component “staple” on the origami construct. We have developed a highly reproducible “cross shaped” origami, derived from the original design of Ned Seeman’s lab, however with sequences optimized for assembly into 1D arrays as might be desired for the intended application. The generation of this protein in E-coli has been optimized by determining the peak time after induction to harvest the protein with minimal degradation products. The polymerase was also designed with a his-tag, to enable ease of protein purification. We have performed several different tests, each confirming activity of the free modified protein. Tests were also designed and performed to determine the activity of the protein bound to streptavidin. While those tests did demonstrate activity, it is difficult to prove that the activity is from bound protein and not from unbound protein. We have not demonstrated activity of the enzyme bound to origami unambiguously. Our best single molecule observation technique, AFM, may denature the enzyme. In some cases AFM imaging disrupts the origami over prolonged imaging series. The polymerase may be sensitive to an undetectably small amount of tip induced damage. In parallel, we have developed single molecule fluorescence monitoring techniques for individual fluorophores. Because of the lack of unambiguous polymerase activity on origami, we have not been able to characterize individual protein activity and therefore we have not been able to characterize individual base incorporation into polymers by localized, surface bound protein molecules. We are seeking other sources of funding in order to continue all three components of this effort, polymerase development, plasmonic nanoantenna development and human infrastructure development.

2. Development of methods for the generation of a self-assembling dipole antenna system appropriate for increasing the intensity of the output signal from single fluorophores:
This second sub-objective required development of both the understanding of plasmonic interactions with fluorophores and development of methods for metal (gold) nanoparticle modification, purification, isolation, precision placement on origami with respect to emitters and characterization of the assemblies.
The state of the art in this area has developed rapidly, indicating that this is the first time in history the tools have been available to address the multiple challenges associated with plasmonic amplification of single fluorophore emission in laboratories not focused on “standard” lithographic methods. Two of the most relevant publications (not from this lab) are:
Synergistic Combination of Unquenching and Plasmonic Fluorescence Enhancement in Fluorogenic Nucleic Acid Hybridization Probes, authors: Carolin Vietz, Birka Lalkens, Guillermo P. Acuna, and Philip Tinnefeld. DOI: 10.1021/acs.nanolett.7b03844 Nano Lett. 2017, 17, 6496−6500
And
Broadband Fluorescence Enhancement with Self-Assembled Silver Nanoparticle Optical Antennas authored by Carolin Vietz, Izabela Kaminska, Maria Sanz Paz, Philip Tinnefeld, and Guillermo P. Acuna,
DOI:10.1021/acsnano.7b01621 ACS Nano 2017, 11, 4969−4975.
While the approach employed by this group is an excellent demonstration of the joining of origami and plasmonics and, while it did not involve any polymerases, it may be amenable to modification to enable single molecule sequencing. However it would be our position that an approach based on the more planar, rather than “tower like” structure employed in these publications, could be produced more reproducibly and reliably. We have developed methods for metal (gold) nanoparticle modification, enabling immobilization on DNA origami, purification of these particles modified with DNA, isolation of these modified particles. While we have placed particles on origami, for the large (ca 100 nm long, 20 nm diameter) rods we have employed, we have not achieved the precision of placement (nor even the precision of particle length and diameter) of these particles on origami with respect to emitters that we sought. We have, however, developed the tools required for characterization of the assemblies and for characterization of the emitter fluorescent properties. We have also advanced designs for the placement of the emitters at the appropriate z coordinate for the “hot spots” of the rod shaped nanoantenna assemblies.
In parallel with the development of the origami as a substrate, we have developed sapphire as a new substrate for origami constructs. Sapphire can be generated in a form with near atomically flat terraces on the surface sufficient for ideal AFM characterization. Sapphire is also optically transparent, therefore offering the potential for parallel optical and AFM studies, both for characterization and for technical implementation of this and related technologies.

3. Contribute to the human scientific research infrastructure by providing in lab experience to students.:
This third, educational objective was achieved by incorporation, in funded or unfunded roles, students of all ages to participate in the research enterprise and learn research objectives of the DOD and of the ARO in particular. Five postdocs were trained, three graduate students performed research, they were supported by the efforts of three technicians. Four undergraduates participated directly in the research. However, because this was a central project in the lab, with the requirements of the project requiring expertise and input from students on other projects, all students in the lab who participated in our weekly group meeting would have become well informed not only

about this research, but also about the breadth of ARO technical challenges requiring the efforts of scientists and engineers to overcome. This lab participated in the URAP program to support undergraduate research participation over the summer, in the HSAP program to support high school students performing research over the summer and is currently participating in the REAP program, encouraging high school students in the sciences to join ARO based researchers by supporting their research during the summer.

Training Opportunities: In addition to graduate students and postdocs supported by this project, the laboratory has regularly participated in the REAP program for the last two year, and the HSAP program for ~ 4 prior years. All levels of students have participated in design and fabrication elements of the research. The particular design and biological production of the enzyme RNAP, has been the highest level barrier to progress, and has only been addressed by a postdoc with a background in biochemistry.

Results Dissemination: Published papers are reported in the Products section. We have annually participated in the FNANO conference, held in Salt Lake City. This international conference enables us, through either poster or platform presentation, to disseminate our results to a large fraction of the DNA nanostrucutres community of the world. Three platform presentations were delivered over the course of this project.

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Technician
Participant: David Neff
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Technician
Participant: Jennifer Markiewicz
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Manjira Ghosh Kumar
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: PD/PI
Participant: Michael Louis Norton
Person Months Worked: 4.00 **Funding Support:**

Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Kathryn Pitton
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Technician
Participant: Thunla Banjopporn
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Zachary Andrews
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Robert Jackson
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Xiaoning Zhang
Person Months Worked: 8.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Tanner Bakhshi

Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Weidong Zhang
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Anshu Mangalum
Person Months Worked: 8.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Masudur Rahman
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Samantha Cotsmire
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Anuradha Rajulapati
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)

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as of 25-Jul-2018

Participant: Wallace Kunin
Person Months Worked: 7.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

ARTICLES:

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Journal: International Journal of Molecular Sciences
Publication Identifier Type: DOI Publication Identifier: 10.3390/ijms13067149
Volume: 13 Issue: 6 First Page #: 7149
Date Submitted: Date Published:
Publication Location:
Article Title: Structural DNA Nanotechnology: From Design to Applications
Authors:
Keywords: DNA nanotechnology; self-assembly; nanostructures; DNA origami
Abstract: The exploitation of DNA for the production of nanoscale architectures presents a young yet paradigm breaking approach, which addresses many of the barriers to the self-assembly of small molecules into highly-ordered nanostructures via construct addressability. There are two major methods to construct DNA nanostructures, and in the current review we will discuss the principles and some examples of applications of both the tile-based and DNA origami methods. The tile-based approach is an older method that provides a good tool to construct small and simple structures, usually with multiply repeated domains. In contrast, the origami method, at this time, would appear to be more appropriate for the construction of bigger, more sophisticated and exactly defined structures.
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Acknowledged Federal Support:

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Chemical Physics
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.chemphys.2013.08.015
Volume: 425 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Sub-THz spectroscopic characterization of vibrational modes in artificially designed DNA monocrystal
Authors:
Keywords: Terahertz; Spectroscopy; Molecular crystal; Vibrational modes; High resolution; Experiment; Modeling
Abstract: Sub-terahertz (sub-THz) vibrational spectroscopy is a new spectroscopic branch for characterizing biological macromolecules. In this work, highly resolved sub-THz resonance spectroscopy is used for characterizing engineered molecular structures, an artificially designed DNA monocrystal, built from a short DNA sequence. Using a recently developed frequency domain spectroscopic instrument operating at room temperature with high spectral and spatial resolution, we demonstrated very intense and specific spectral lines from a DNA crystal in general agreement with a computational molecular dynamics (MD) simulation of a short double stranded DNA fragment. The spectroscopic signature measured in the frequency range between 310 and 490 GHz is rich in well resolved and reproducible spectral features thus demonstrating the capability of THz resonance spectroscopy to be used for characterizing custom macromolecules and structures designed and implemented via nanotechnology for a wide variety of ap
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support:

RPPR Final Report
as of 25-Jul-2018

CONFERENCE PAPERS:

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: SPIE BiOS
Date Received: 13-Aug-2017 Conference Date: 21-Jan-2012 Date Published: 01-Mar-2012
Conference Location: San Francisco, California
Paper Title: Concentration methods for high-resolution THz spectroscopy of nucleic-acid biomolecules and crystals
Authors: Brown, E. R.; Zhang, W.; Mendoza, E. A.; Kuznetsova, Y.; Brueck, S. R. J.; Rahman, M.; Norton, M. L.
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 2014 International Workshop on Computational Electronics (IWCE)
Date Received: 13-Aug-2017 Conference Date: 03-Jun-2014 Date Published: 28-Jul-2014
Conference Location: Paris, France
Paper Title: Study of electric field caused by semiconductor quantum dots in close proximity to DNA origami
Authors: Ke Xu ; Xenia Meshik ; Min Choi ; Mitra Dutta ; Michael Stroscio ; Tsai-Chin Wu ; Masudur Rahman
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Foundations of Nanoscience Meeting (FNANO 2017)
Date Received: 13-Aug-2017 Conference Date: 09-Apr-2017 Date Published: 09-Apr-2017
Conference Location: Snowbird, UT Snowbird Cliff Lodge
Paper Title: The Sapphire (0001) Surface: A Transparent Substitute for Mica for DNA Nanostructure Imaging
Authors: Masudur Rahman, David Neff and Michael Norton
Acknowledged Federal Support: **N**

DISSERTATIONS:

Publication Type: Thesis or Dissertation
Institution:
Date Received: 31-Jul-2011 Completion Date:
Title: DETERMINING THE RATE OF TRANSCRIPTION OF T7 RNA POLYMERASE USING SINGLE MOLECULE FLUORESCENCE IMAGING
Authors:
Acknowledged Federal Support:

Title: Transcription as Sequencing – TAS Report Date: 5 20 2018

Contract Number: W911NF0910218
Recipient of Award

Organization
MARSHALL UNIVERSITY RESEARCH CORP
Huntington, 257012218, USA

Investigator:

Dr. Norton, Michael
Principal: Yes

The overall objective of the award was development of a DNA origami based platform for sequencing by synthesis. The origami was to organize the polymerase into arrays, and as individual labeled fluorophores were integrated into the DNA polymer, these fluorophores would be registered. To enhance the sensitivityof the system, plasmonic structures would also be organized by the same origami construct "hosting" the polymerase molecule. The project then had three thrusts, 1) development of the polymerase, the hosting Origami structure and capabilities to image single fluorophores, 2) development of the plasmonic and optical environment for observation and 3) develop the human infrastructure required to support this line of research.

1. Development of the polymerase design necessary for this project: This first objective has several components, including design and cloning of polymerases compatible with localization on origami, origami platform optimization, optimizing protein production and purification, confirming activity of the free and bound protein, development of techniques for the characterization of the bound protein,evaluating activity of the bound protein, developing single molecule fluorescence monitoring techniques for individual proteins, characterizing individual protein activity and characterizing individual base incorporation into polymers by localized, surface bound protein molecules.

The targeted behavior of this system is illustrated in Figure 1 below, in which the immobilized polymerase (green), interacts with a DNA molecule (yellow) and "copies" this sequence producing the pink elongated strand. Either the product strand can be labeled (blue stars in figure 1) or optimally, the incorporated fluorophores report their incorporation and are immediately removed, to enable the next fluorophore to be registered.

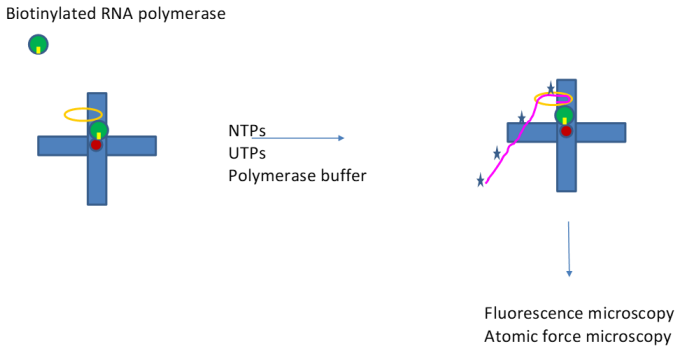


Figure 1 Schematic representation of the function of the polymerase in sequencing by synthesis project.

We have designed and cloned T7RNAP, a polymerase molecule made compatible with localization on origami by including in the design a biotinylation site, which enables immobilization of the polymerase on the origami through a biotin-streptavidin-biotin bridge to a component "staple" on the origami construct. We attempted, over an extended time, to utilize an alternative, covalent method to bind the polymerase to a DNA strand, to enable incorporation into the origami structure, however all attempts were stymied by modifications presumably introduced by the bacteria during reproduction of the plasmid. This "sortase" line of inquiry was therefore dropped. We have developed a highly reproducible "cross shaped" origami, derived from the original design of Ned Seeman's lab, however with sequences optimized for assembly into 1D arrays as might be desired for the intended application. The generation of this protein in E-coli has been optimized by determining the peak time after induction to harvest the protein with minimal degradation products. The polymerase was also designed with a his-tag, to enable ease of protein purification. We have performed several different tests, each confirming activity of the free modified protein. An example is shown in Figure 2.

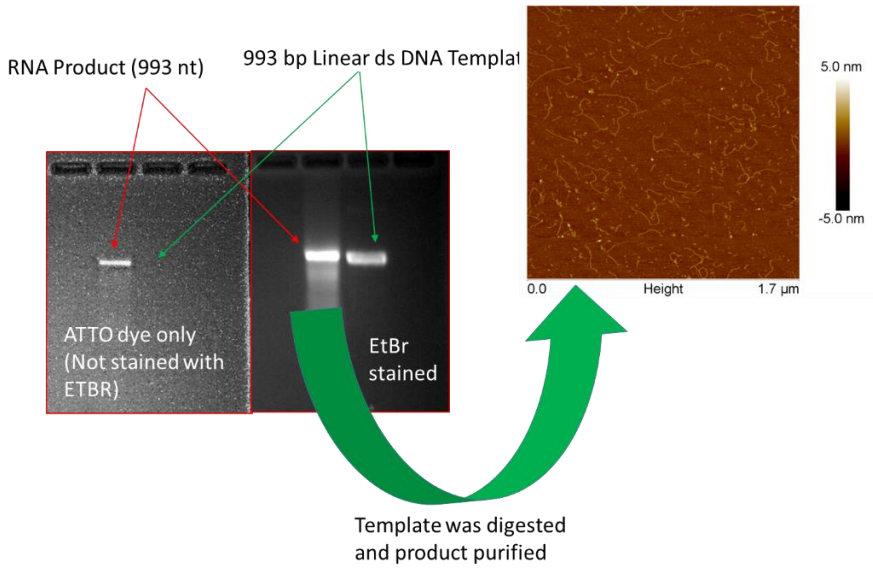


Figure 2 993 nt RNA product of genetically modified (his tag and biotin) RNAP. Left gel image band (red arrow) is fluorescent because of incorporated ATTO dye molecules. Right gel image compares size of RNA and template DNA. AFM image on right side presents purified (all DNA template has been digested) reflecting production of RNA observable via AFM.

Tests were also designed and performed to determine the activity of the protein bound to streptavidin. While those tests did demonstrate activity, it is difficult to prove that the activity is from bound protein and not from unbound protein. A gel demonstrating activity of the streptavidin conjugated RNAP is shown in Figure 3. We have not demonstrated activity of the enzyme bound to origami unambiguously. Our best single molecule observation technique, AFM, may denature the enzyme and over long time periods may disrupt the origami itself. This is particularly probable in solution where dissociated origami components can diffuse to other locations on the substrate. In some cases it has been clear that AFM imaging disrupts the origami over the course of a prolonged imaging series. The polymerase may be sensitive to an undetectably small amount of tip induced damage. An example of an experiment for which the results are ambiguous because of this potential degradation of the origami substrate is

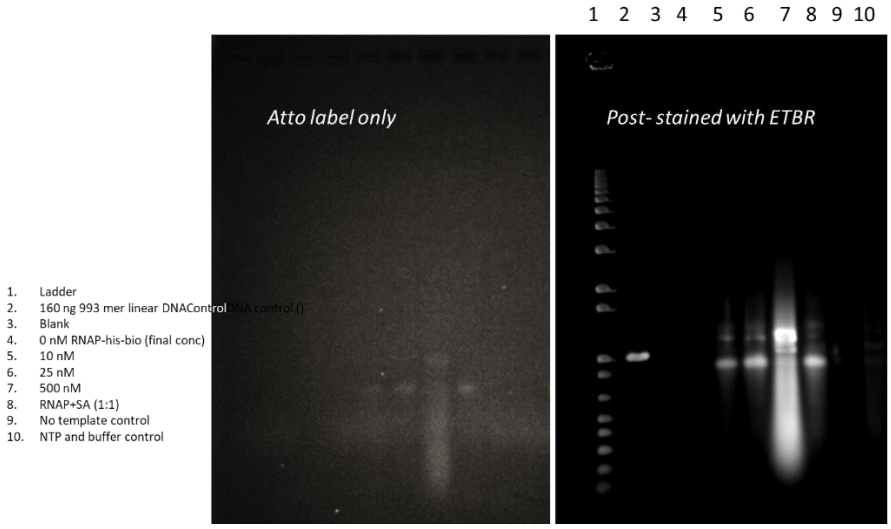


Figure 3 Gel Electrophoresis of RNA labeled with (Left) Atto dye via incorporation of the fluorophore into the growing RNA molecule. (Right) after staining with ethidium bromide. Lane 6 intensity reflects RNA produced using 25nM RNAP enzyme with biotin modification and Lane 8 intensity reflects RNA produced using 25nM RNAP enzyme with biotin modification after reaction with streptavidin (immobilizing moiety). Similar activity indicates that the activity of the streptavidin bound enzyme is the same as the unbound protein.

presented in Figure 4. The AFM images represent a time series of images taken under solution, in which: a->b the RNAP-Streptavidin complex is added to the origami, b->c the DNA template and the other reagents necessary for RNA production are added; c->g additional polymeric materials appear in the images. Areas being imaged have been changed in order to view fresh origami, due to tip damage to the origami. Accumulation is difficult to evaluate because during imaging the solution is being "stirred" by the rapid oscillations of the imaging AFM tip. The last image (h) presents a dry AFM image, where the accumulation of polymeric material is apparent. The exact nature of this material cannot be determined via AFM.

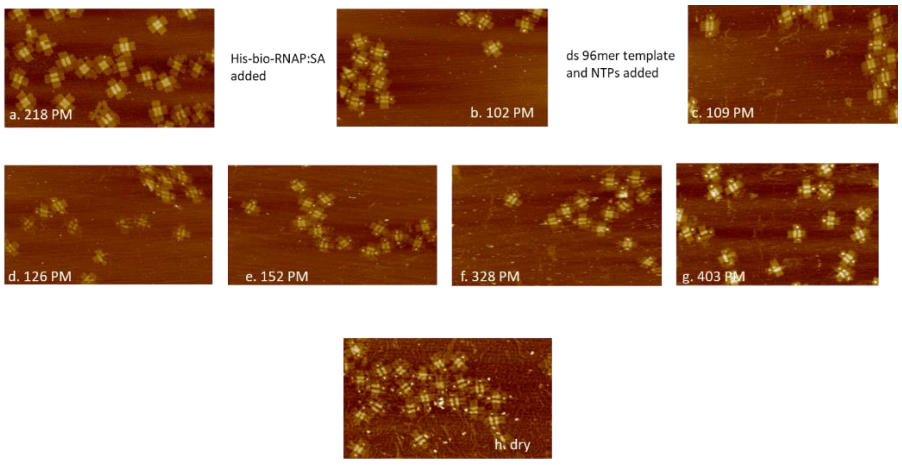


Figure 4 Time series of solution phase AFM images presenting the accumulation of polymeric material as a function of time. Individual transitions described in the text.

In parallel, we have developed single molecule fluorescence monitoring techniques for individual fluorophores. Because of the lack of unambiguous polymerase activity on origami, we have not been able to characterize individual protein activity and therefore we have not been able to characterize individual base incorporation into polymers by localized, surface bound protein molecules. We are seeking other sources of funding in order to continue all three components of this effort, polymerase development, plasmonic nanoantenna development and human infrastructure development.

2. Development of methods for the generation of a self-assembling dipole antenna system appropriate for increasing the intensity of the output signal from single fluorophores: This second sub-objective required development of both the understanding of plasmonic interactions with fluorophores and development of methods for metal (gold) nanoparticle modification, purification, isolation, precision placement on origami with respect to emitters and characterization of the assemblies.

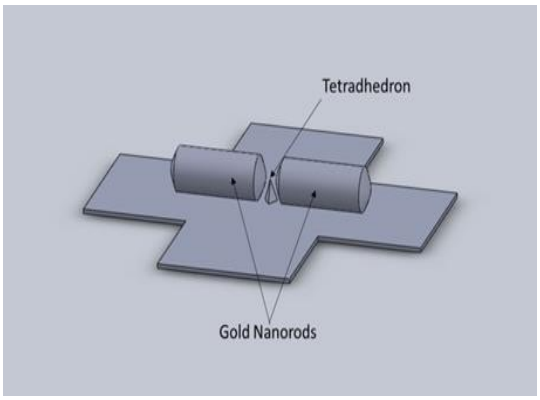
The state of the art in this area has developed rapidly, indicating that this is the first time in history the tools have been available to address the multiple challenges associated with plasmonic amplification of single fluorophore emission in laboratories not utilizing "standard" or top-down lithographic methods. Two of the most relevant publications (not from this lab) are:

Synergistic Combination of Unquenching and Plasmonic Fluorescence Enhancement in Fluorogenic Nucleic Acid Hybridization Probes, authors: Carolin Vietz, Birka Lalkens, Guillermo P. Acuna, and Philip Tinnefeld. DOI:10.1021/acs.nanolett.7b03844 Nano Lett. 2017, 17, 6496−6500

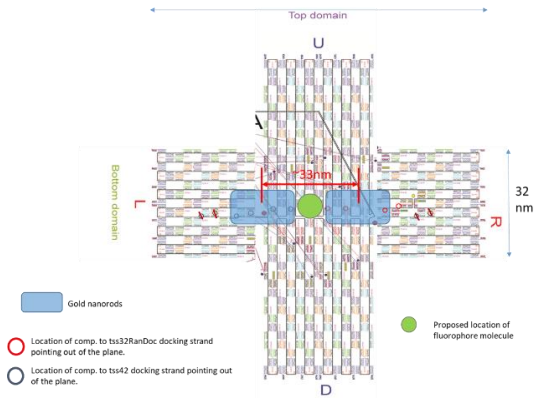
And

Broadband Fluorescence Enhancement with Self-Assembled Silver Nanoparticle Optical Antennas authored by Carolin Vietz, Izabela Kaminska, Maria Sanz Paz, Philip Tinnefeld, and Guillermo P. Acuna, DOI:10.1021/acsnano.7b01621 ACS Nano 2017, 11, 4969−4975.

While the approach employed by this group is an excellent demonstration of the joining of origami and plasmonics and, while it did not involve any polymerases, this approach may be amenable to modification to enable single molecule sequencing. However it would be our position that an approach based on the more planar, rather than the "tower like" structure employed in these publications, could be produced more reproducibly and reliably. We (and others) have developed methods for metal (gold) nanoparticle modification, enabling immobilization on DNA origami, purification of these particles modified with DNA and isolation of these modified particles. We have developed methods for placement of these particles on origami. A schematic diagram of the design of a construct with two nanorods as plasmonic subsystems and a representation of the DNA origami based construct are both presented in Figure 5.



A



B

Figure 5 A) Schematic representation of the two nanorod plasmonic structure with tetrahedral platform modification for positioning fluorescent molecules at the optimal position for electric field enhancement. B) DNA based structure used to implement dual gold nanorod positioning experiments.

For the large (ca 20 nm long, 10 nm diameter) rods we have employed, we have not achieved the precision of placement (nor even the precision of particle length and diameter) of these particles on origami with respect to emitters that we sought. The TEM image provided in Figure 6 suggests wide variation in the dimensions of the particles and in their mutual orientations.

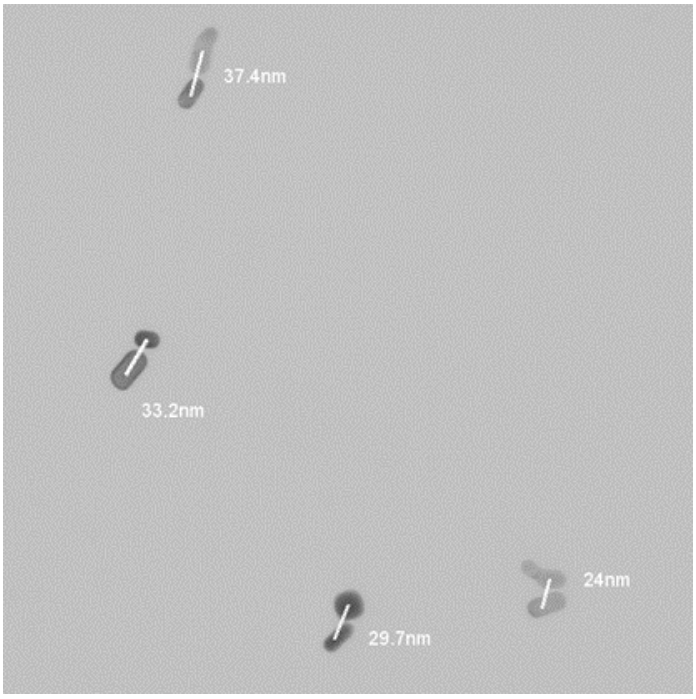


Figure 6 Transmission Electron Microscope image of particles potentially arranged and aligned using DNA origami platforms. Sample was drop deposited on formvar/C grid and imaged at 75kV. Because the DNA was not stained, it cannot be observed. Therefore the organization of these particles into pairs of nanorods cannot be unambiguously attributed to the origami based structures. The dark spots are the particles while the white lines are used to measure center to center distances (shown in the image).

While the TEM image provided in Figure 6 would seem to suggest that a high fraction of nanoparticles occur as pairs after interaction with the DNA platforms, these results are not consistent with our AFM observations. The yield of single particles on origami is relatively high (32/82 –> 39%), however the yield of two particles on origami is vanishingly small (9/82–> 11%). Due to the symmetry of the constructs, it is difficult to ascertain whether one of the nanorods is binding significantly less than the other, studies are in progress to resolve this question. Figure 7 provides an AFM image showing origami mostly occupied by single particles.

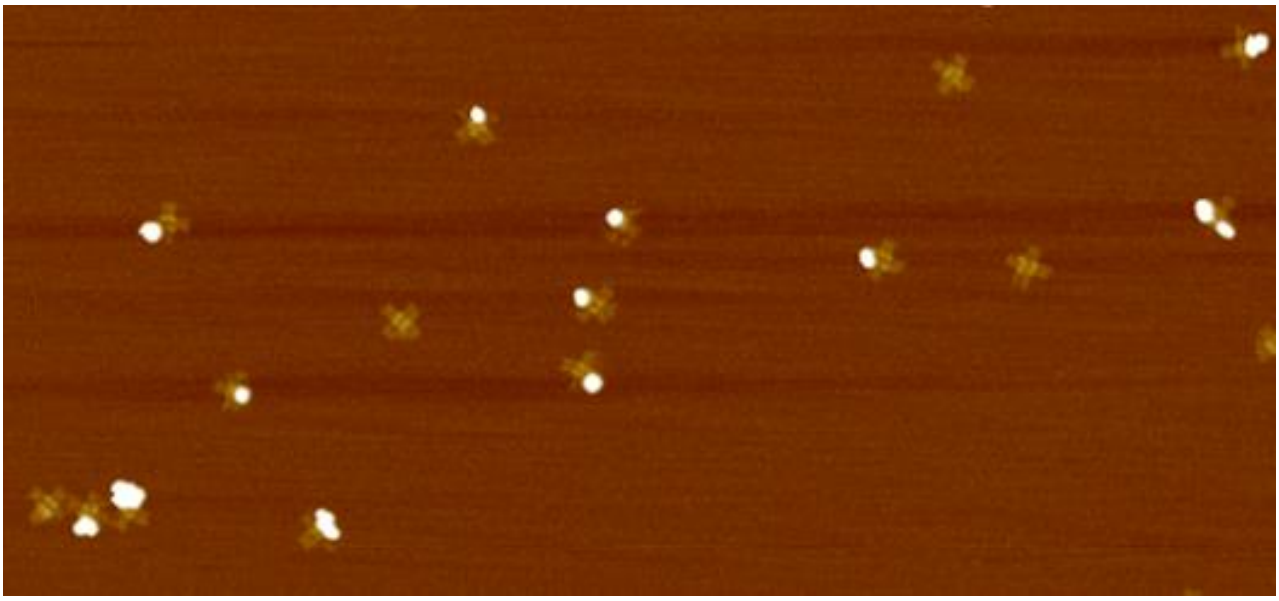


Figure 7 AFM image of region with higher than average fractional occupation of one site on origami.

Figure 8 Provides AFM images of three different structures occupied by two particles.

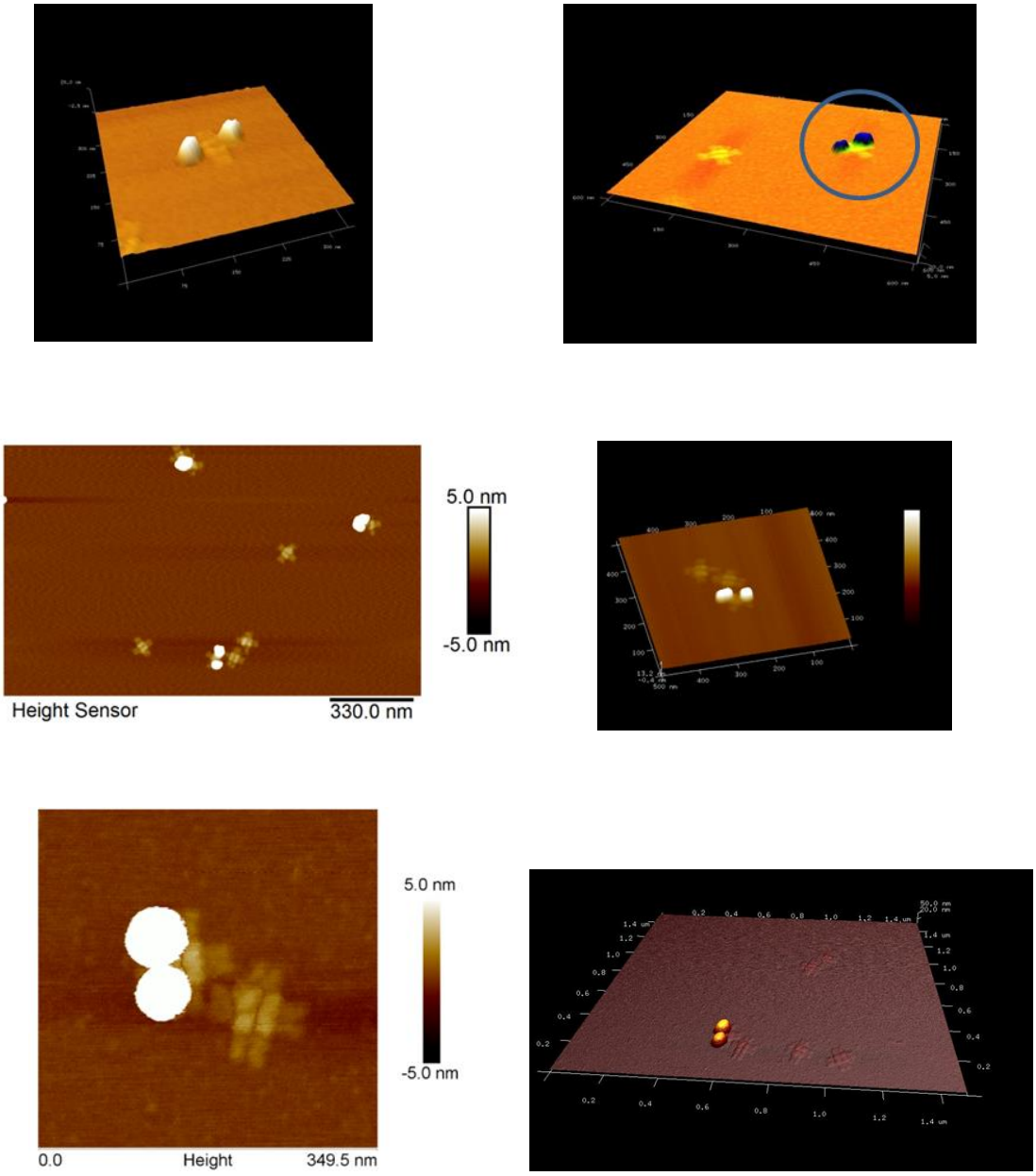


Figure 8 Two views (left topographic and right perspective) of three different origami constructs which are each decorated with two nanoparticles. Both the offset and the round structure of the nanoparticles can be reasonably attributed to the convolution of the tip shape with the particle shape, examples of the problems associated with scanning probe microscopy of “large” particles.

We have, however, developed the tools required for characterization of the assemblies and for characterization of the emitter fluorescent properties. We have also advanced designs for the placement of the emitters at the appropriate z coordinate for the “hot spots” of the rod shaped

nanoantenna assemblies, at the top of the 5 nm tall tetrahedron shown schematically in Figure 5A above. The design and sequence for this "tripod" structure is shown in Figure 9 below. An AFM image of a prototype structure is shown in Figure 10.

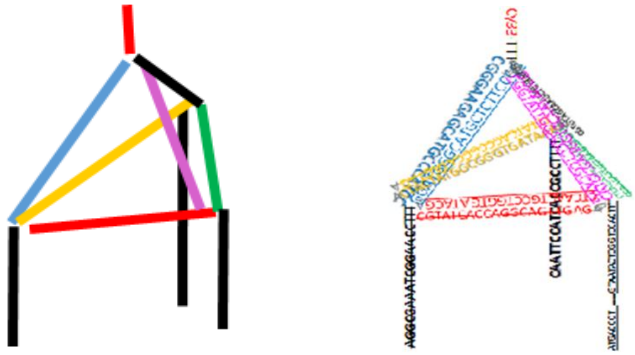


Figure 9. Design (left) and DNA sequence (right) for origami based tripod to position test molecule in plasmonic "hot spot".

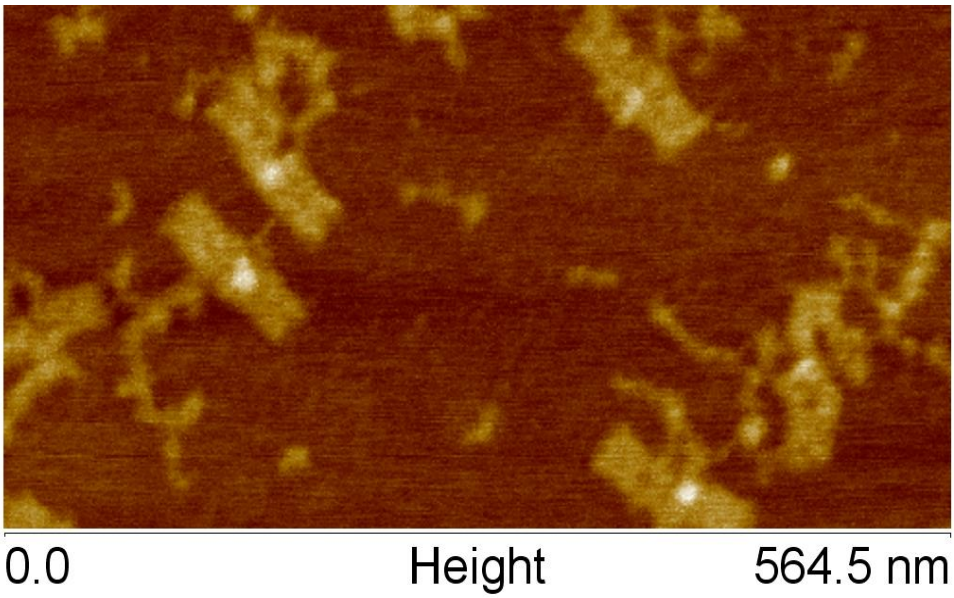


Figure 10. AFM image of DNA origami "planks" (half cross structures) with tetrahedra in place (white spots at center of planks.

In parallel with the development of the origami as a substrate, we have developed sapphire as a new substrate for origami constructs. Sapphire can be generated in a form with near atomically flat terraces on the surface sufficient for ideal AFM characterization. An example image of origami imaged on sapphire is provided in Figure 11 below. Sapphire is also optically transparent, therefore offering the

potential for parallel optical and AFM studies, both for characterization and for technical implementation of this and related technologies. An example of preliminary correlative AFM/Optical nanoscopy is provided in Figure 12. Figure 13 provides a manually aligned overlay of the optical image and the AFM image. While significant progress has been made in combining information from these two modalities, improvements in both sample design and preparation and in image analysis remain to be made.

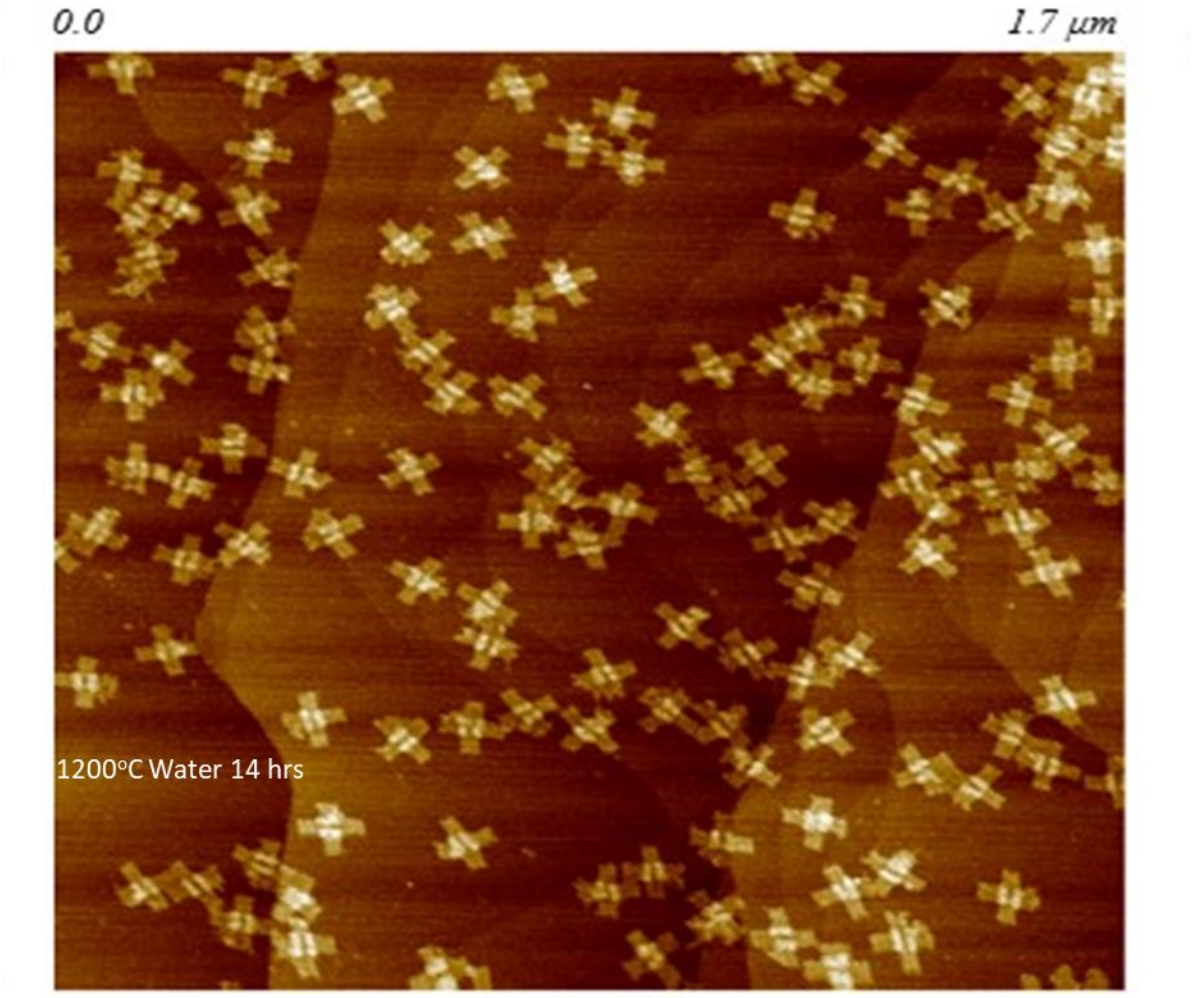


Figure 11. Cross shaped origami imaged in air on heat treated single crystal sapphire substrate. As a marker, the dark stripe at the center of the structures is approximately 0.3 nm deep. The structures themselves are ~ 100 nm wide X 100 nm tall. Total origami structure thickness is less than 3 nm.

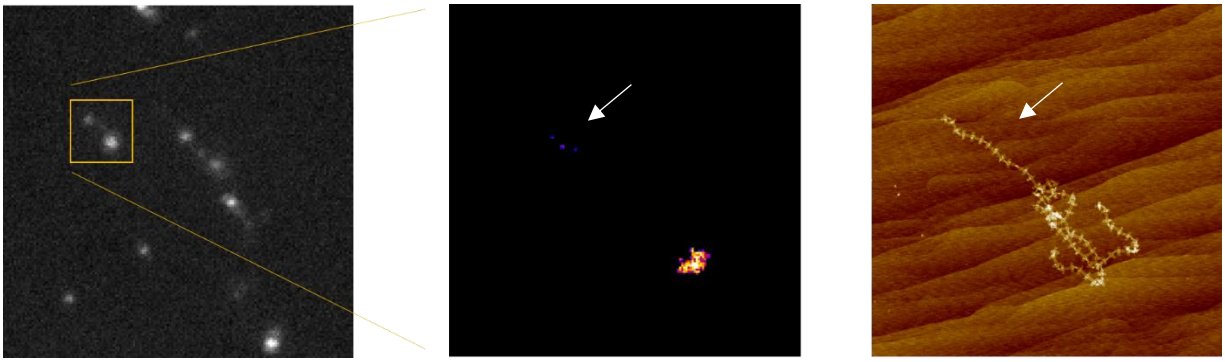


Figure 12. Optical (20 X 20 μm^2 area), super-resolved (3.5 X 3.5 μm^2 area) and AFM (3.5 X 3.5 μm^2 area) images of the same one dimensional origami array, decorated with fluorescent molecules (one fluorescent molecule per cross).

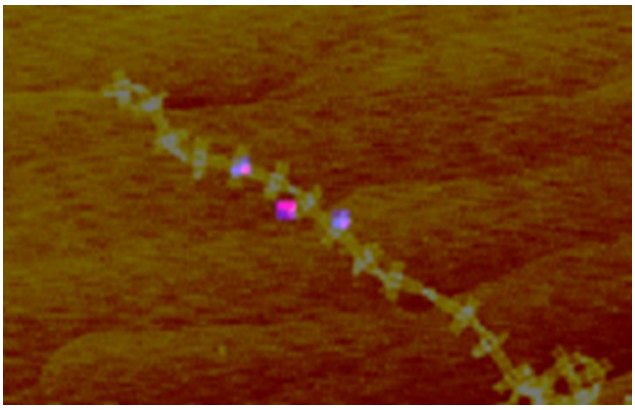


Figure 13. Manual overlay of optical image and AFM image to provide multimodal information. The three pink spots represent clusters of individual localizations within a range on the order of 20 -30 nm. Faint green spots are an artifact of the overlay process.

3. Contribute to the human scientific research infrastructure by providing in lab experience to students.: This third, educational objective was achieved by incorporation, in funded or unfunded roles, students of all ages to participate in the research enterprise and learn research objectives of the DOD and of the ARO in particular. Five postdocs were trained, three graduate students performed research, they were supported by the efforts of three technicians. Four undergraduates participated directly in the research. However, because this was a central project in the lab, with the requirements of the project requiring expertise and input from students on other projects, all students in the lab who participated in our weekly group meeting would have become well informed not only about this research, but also about the breadth of ARO technical challenges requiring the efforts of scientists and engineers to overcome. This lab participated in the URAP program to support undergraduate research participation over the summer, in the HSAP program to support high school students performing research over the summer and is currently participating in the REAP program, encouraging high school students in the sciences to join ARO based researchers by supporting their research during the summer.