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Final Report: DURIP: Fluorescence Correlation Micropcopy for Characterizing the Structural Dynamics of Biochemical Nanosystems

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5c. PROGRAM ELEMENT NUMBER
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6. AUTHORS

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
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Approved for public release; distribution is unlimited.

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The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Jinglin Fu
UU	UU	UU	UU		19b. TELEPHONE NUMBER 856-225-6612

RPPR Final Report
as of 08-May-2019

Agency Code:

Proposal Number: 68428LSRIP **Agreement Number: W911NF-16-1-0220**
INVESTIGATOR(S):

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Organization: **Rutgers, The State University of New Jersey - Camden**
Address: 311 North 5th Street, Camden, NJ 081021405
Country: USA

DUNS Number: 625216556 EIN: 226001086
Report Date: 17-Dec-2016 Date Received: 11-Feb-2019
Final Report for Period Beginning 18-Apr-2016 and Ending 17-Sep-2016
Title: DURIP: Fluorescence Correlation Micropcopy for Characterizing the Structural Dynamics of Biochemical Nanosystems
Begin Performance Period: 18-Apr-2016 **End Performance Period:** 17-Sep-2016
Report Term: 0-Other
Submitted By: Jinglin Fu Email: jinglin.fu@rutgers.edu
Phone: (856) 225-6612
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 2 **STEM Participants:** 5

Major Goals: We propose to develop a confocal fluorescence correlation spectroscopy (FCS) to support research programs in the Department of Chemistry and Center for Computational and Integrative Biology at Rutgers University-Camden. The proposed FCS will be an upgrade of an existing confocal laser scanning microscope. FCS is a single-molecule detection method that measures fluctuations of fluorescence intensity in a sub-femtoliter volume, which detects kinetic and structural parameters, such as the diffusion time, number of molecules and dark states of fluorescently labeled molecules. Several research projects will be enhanced by the FCS that are either supported by Army or are relevant to DoD goals, including: (1) Characterization of the assembly of switchable DNA nanostructures for developing regulatory biochemical reaction circuits; (2) Develop an enzyme-powered nanomotor propelled by chemical fuels; (3) Nanoparticle-mediated photoporation of cell membranes and the optical injection of exogenous matter; (4) Study of protein and peptide aggregation; (5) Structure and dynamics of protein unfolding in aqueous ionic liquid solutions.
The proposed research projects with FCS will provide students opportunities to learn and practice the skills that are required in scientific research. This helps to maintain a sustainable future for STEM programs that develop students' interests and skills for the future participation in scientific research. The program will also educate students about the Army's involvement of supporting fundamental science and engineering research as well as educational opportunities for students through the Army Educational Outreach Program (AEOP) and Department of Defense Graduate Fellowships. It will also provide research opportunities for under-represented/low-income high school students and undergraduates in the Camden area.

Accomplishments: (1)Installation of Leica TCS SP8 Fluorescence Correlation Spectroscopy: We successfully installed the Single Molecule Detection Platform on Leica TCS SP8 confocal microscopy for Fluorescence Correlation Spectroscopy.

(2) Characterization of small-molecule dyes and dye-labelled DNA.
(3) Characterization of DNA nanostructures: We tested FCS for evaluating the diffusion of DNA nanostructure and their sizes distribution.
(4) FCS for characterizing DNA-crowed enzyme complexes.
(5) Characterization of peptide-protein aggregation: We tested FCS for measuring peptide-protein binding and aggregations.
(6) Leica confocal imaging for characterizing protein/polysaccharides bio-composite.

For detailed information, please see attached technical report.

Training Opportunities: High-School Apprenticeship Program (HSAP), Undergraduate Research Apprenticeship Program (URAP) in 2016.

1. Total number of undergraduate bachelor degrees awarded: 2
Gina Disalvo, Gabriele Stankeviciute
2. Total number of Master's degree awarded: 0
3. Other graduate students: 2
John Collins, Sung Won Oh
4. Total number of high school students sponsored for summer research: 1
Olivia Zapfe
5. Total Number of URAP student: 1
Adriana Pereira

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: 1. Jinglin Fu, John Collins and Ting Zhang "DNA-crowded Enzyme Complex with Controlled Spatial Confinements" US Provisional Patent. App. No. 62/482,882.

2. Jinglin Fu, Sung Won Oh and Adriana Pereira "DNA Logic-gated Proximity Assembly Circuit for Biochemical Sensing" U.S. Patent Application No. 62/647,014.

PARTICIPANTS:

Participant Type: PD/PI
Participant: Jinglin Fu
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

DISSERTATIONS:

RPPR Final Report
as of 08-May-2019

Publication Type: Thesis or Dissertation
Institution: Rutgers University-Camden
Date Received: 09-Feb-2019 Completion Date: 10/2/17 1:25AM
Title: EXPLORING GLOBAL AND MICRO-ENVIRONMENT CONDITIONS FOR AFFECTING ENZYME ACTIVITIES AND FUNCTIONAL TRANSITIONS
Authors: John Collins
Acknowledged Federal Support: **Y**

PATENTS:

Intellectual Property Type: Patent Date Received: **09-Feb-2019**
Patent Title: DNA-CROWDED ENZYME COMPLEX WITH CONTROLLED SPATIAL CONFINEMENT
Patent Abstract: This application provides nucleotide-crowded macromolecular complex compositions and methc
Patent Number: 62/482,882
Patent Country: USA
Application Date: 08-Apr-2017 Application Status: 1
Date Issued:

Intellectual Property Type: Patent Date Received: **09-Feb-2019**
Patent Title: DNA-CROWDED ENZYME COMPLEX WITH CONTROLLED SPATIAL CONFINEMENT
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Patent Number: 62/482,882
Patent Country: USA
Application Date: 08-Apr-2017 Application Status: 1
Date Issued:

Intellectual Property Type: Patent Date Received: **11-Feb-2019**
Patent Title: DNA Logic-gated Proximity Assembly Circuit for Biochemical Sensing
Patent Abstract: This application relates to nucleic acid-based sensors, kits that include such
Patent Number: 62/647,014
Patent Country: USA
Application Date: 24-Mar-2018 Application Status: 1
Date Issued:

DURIP: Fluorescence Correlation Microscopy for Characterizing the Structural Dynamics of Biochemical Nanosystems

Principal Investigator: Jinglin Fu, Rutgers University-Camden

Reporting period: 4/12/16-7/31/16
Proposal Number: 65330LSYIP
Agreement Number: W911NF1610220

I. Scientific and Technical Objectives

We propose to develop a confocal fluorescence correlation spectroscopy (FCS) to support research programs in the Department of Chemistry and Center for Computational and Integrative Biology at Rutgers University-Camden. The proposed FCS will be an upgrade of an existing confocal laser scanning microscope. FCS is a single-molecule detection method that measures fluctuations of fluorescence intensity in a sub-femtoliter volume, which detects kinetic and structural parameters, such as the diffusion time, number of molecules and dark states of fluorescently labeled molecules. Several research projects will be enhanced by the FCS that are either supported by Army or are relevant to DoD goals, including: (1) Characterization of the assembly of switchable DNA nanostructures for developing regulatory biochemical reaction circuits; (2) Develop an enzyme-powered nanomotor propelled by chemical fuels; (3) Nanoparticle-mediated photoporation of cell membranes and the optical injection of exogenous matter; (4) Study of protein and peptide aggregation; (5) Structure and dynamics of protein unfolding in aqueous ionic liquid solutions.

The proposed research projects with FCS will provide students opportunities to learn and practice the skills that are required in scientific research. This helps to maintain a sustainable future for STEM programs that develop students' interests and skills for the future participation in scientific research. The program will also educate students about the Army's involvement of supporting fundamental science and engineering research as well as educational opportunities for students through the Army Educational Outreach Program (AEOP) and Department of Defense Graduate Fellowships. It will also provide research opportunities for under-represented/low-income high school students and undergraduates in the Camden area.

II. Concise Accomplishments

(1) Installation of Leica TCS SP8 Fluorescence Correlation Spectroscopy: We successfully installed the Single Molecule Detection Platform on Leica TCS SP8 confocal microscopy for Fluorescence Correlation Spectroscopy.
(2) Characterization of small-molecule dyes and dye-labelled DNA.
(3) Characterization of DNA nanostructures: We tested FCS for evaluating the diffusion of DNA nanostructure and their sizes distribution.
(4) FCS for characterizing DNA-crowed enzyme complexes.
(5) Characterization of peptide-protein aggregation: We tested FCS for measuring peptide-protein binding and aggregations.
(6) Leica confocal imaging for characterizing protein/polysaccharides bio-composite.

III. Expanded Accomplishments

III-(1). Installation of Leica TCS SP8 Fluorescence Correlation Spectroscopy

Instrument location: Rutgers University-Camden, Center for Computational and Integrative Biology, Department of Chemistry, Science Building B-32.
Instrument type/code: An upgraded single-molecule fluorescence correlation on an existing Leica TCS SP8 Confocal Laser Scanning Microscopes.
Installation technician: Jonathan Body and Ghevant Hovsepian from Leica.
Date: 6/29/2016

We have installed the Single Molecule Detection Platform on Leica TCS SP8 confocal microscopy for Fluorescence Correlation Spectroscopy. The installation and upgrade include: (1) *Leica patented Acousto-Optical Beamsplitter (AOBS)* that provides optimal spectral flexibility with up to 8 laser lines simultaneously. It provides advantages of highly efficient spectral separation, equal brightness between channels due to W-shaped slit design, highly synergistic in combination with prism and Leica SP detector. (2) *SMD Electronic and detector package* that includes a SMD HyD working in single photon counting mode for detection of the SMDsignal. The detector is built into the SP module. The spectral detection range can be selected by software-controlled mirror sliders. (3) SMD Software Package FCS 64 bit: A package consisting of software and specific hardware for acquisition and fitting analysis of FCS data.

III-(2). FCS for characterizing the small-molecule dyes and DNA

After the installation, we first tested FCS for characterizing the diffusion kinetics of dye-labelled molecules. As shown in **Figure 1**, **a** small organic dye of Alexa 488 showed very fast fluorescence correlation decay with a fitted diffusion coefficient ~ 285 $\mu m^2/s$. In contrast, a Cy5-labelled ssDNA showed a slower correlation decay with a fitted diffusion coefficient ~ 86 $\mu m^2/s$. Using FCS, we can analyze the purity of dye-DNA conjugates by their fluorescence correlation functions. Leica also provides a fitting program for analyzing the fluorescence correlation functions, given parameters of multi-component distribution, concentration and diffusion constants. The multi-component FCS fitting can be used to analyze the bioconjugation of dye-DNA or cofactor-DNA molecules, and the purity of sample.

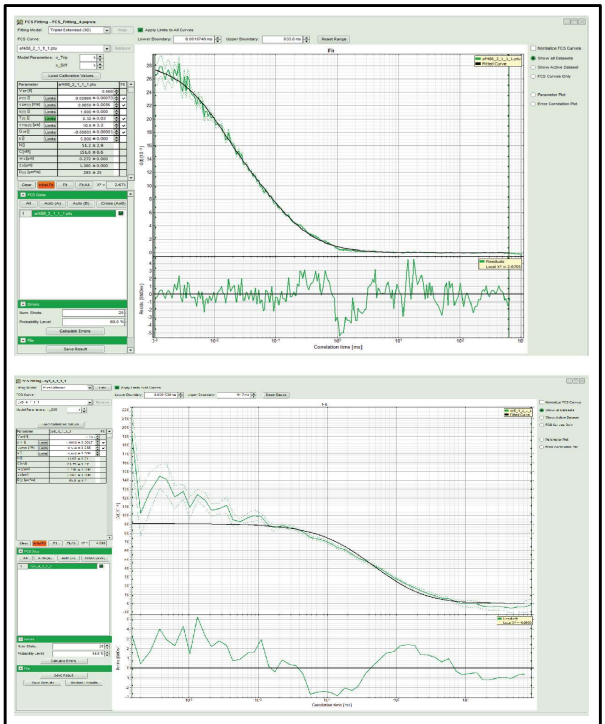

Figure 1. FCS for characterizing the small organic dye of Alexa 488 (top) and Cy5-labeled ssDNA (bottom), and fitting of diffusion coefficients.

III-(3). FCS for characterizing the diffusion of DNA nanostructures.

We used FCS to investigate how diffusion correlates to the shape of DNA nanostructures. With DNA nanotechnology, we were able to define precisely the shapes for 1-D, 2-D and 3-D nanostructures. This provides a unique way to study diffusion theory and modeling. As shown in **Figure 2**, FCS were used to measure the diffusion coefficients for different DNA origami nanostructures with the same molecular weight (~ 4.8 million Dalton). FCS results showed that the diffusion of linear DNA nanotube (6-HB), 2D triangular (TRI) and rectangular origami (REC) were slower than 3D DNA nanocage (CAGE) and compact cube (CUBE). These difference in diffusion coefficients can be attributed to the fact that the structures with large surface-to-volume (e.g. nanotube, or 2D structures) ratio experienced more resistance in solution, resulting in a slower diffusion.

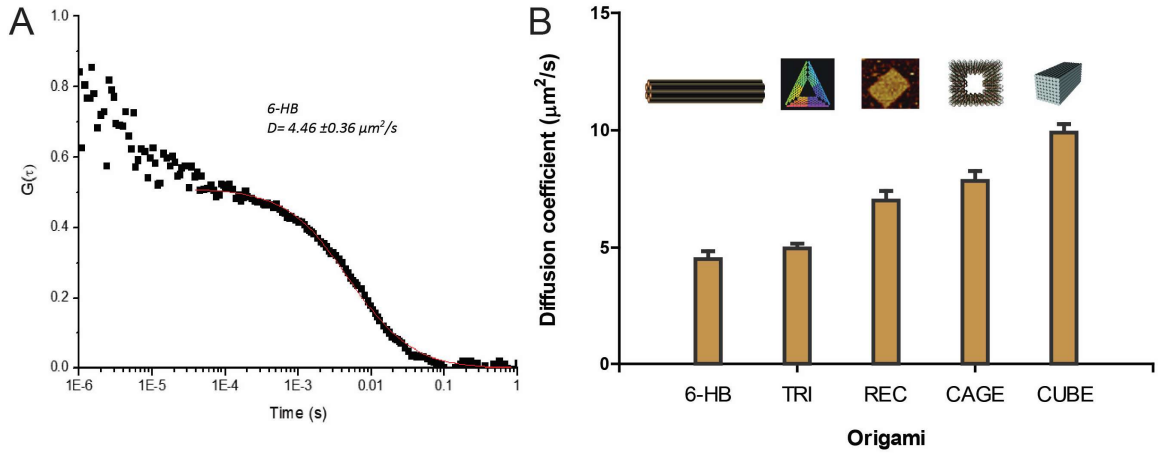

Figure 2. (A) FCS for characterizing 6-helices bundle DNA nanotubes (6-HB). (B) Measured diffusion coefficients for DNA origami structures of 6-HB, REC, CAGE and CUBE. The results show that the compact 3D structures of CAGE and CUBE diffuse faster than 1D nanotube and 2D structures.

III-(4) FCS for characterizing DNA-crowed enzyme complexes.

As shown in **Figure 3A**, the construction of DNA-crowded enzyme complex involves two steps: (1) the conjugation of an initiator strand to an enzyme (enzyme-I) and (2) the growth of double-strand DNA (dsDNA) on the enzyme surface by the initiator-triggered hybridization chain reaction (HCR). One initiator on the enzyme surface can trigger the growth of a long dsDNA with the alternative hybridization of hairpin 1 and 2. The relative length of dsDNA was determined by the initiator-to-hairpin ratio, where excess hairpins resulted in longer dsDNA by HCR. The long HCR duplex on the enzyme surface behaves as a flexible 'DNA hair' to crowd the enzyme (Non-nick dsDNA's persistence length is ~ 50 nm. But a HCR duplex has many nick points that make it easier to bend). As shown in **Figure 3B**, the gel electrophoresis was used to characterize the growth of dsDNA on the enzyme surface, where larger dsDNA with slower mobility was observed with the addition of more hairpins. The fluorescent imaging of Cy3-labelled enzyme showed that the enzyme was migrated together with the large dsDNA. As shown in **Figure 3C**, dynamic light scattering (DLS) was used to characterize the hydrodynamic diameters of multiple enzyme-HCR complexes in solution phase. An enzyme-initiator conjugate showed a peak diameter ~ 7 nm. The diameter of enzyme-HCR complexes was increased from ~ 8 nm to ~ 30 nm with the addition of hairpins from 1-fold to 16-fold excess. We also used atomic force microscopy (AFM) to directly

visualize DNA-crowded enzymes on mica surface, where long DNA "hairs" were observed with the attachment of bright spots of proteins.

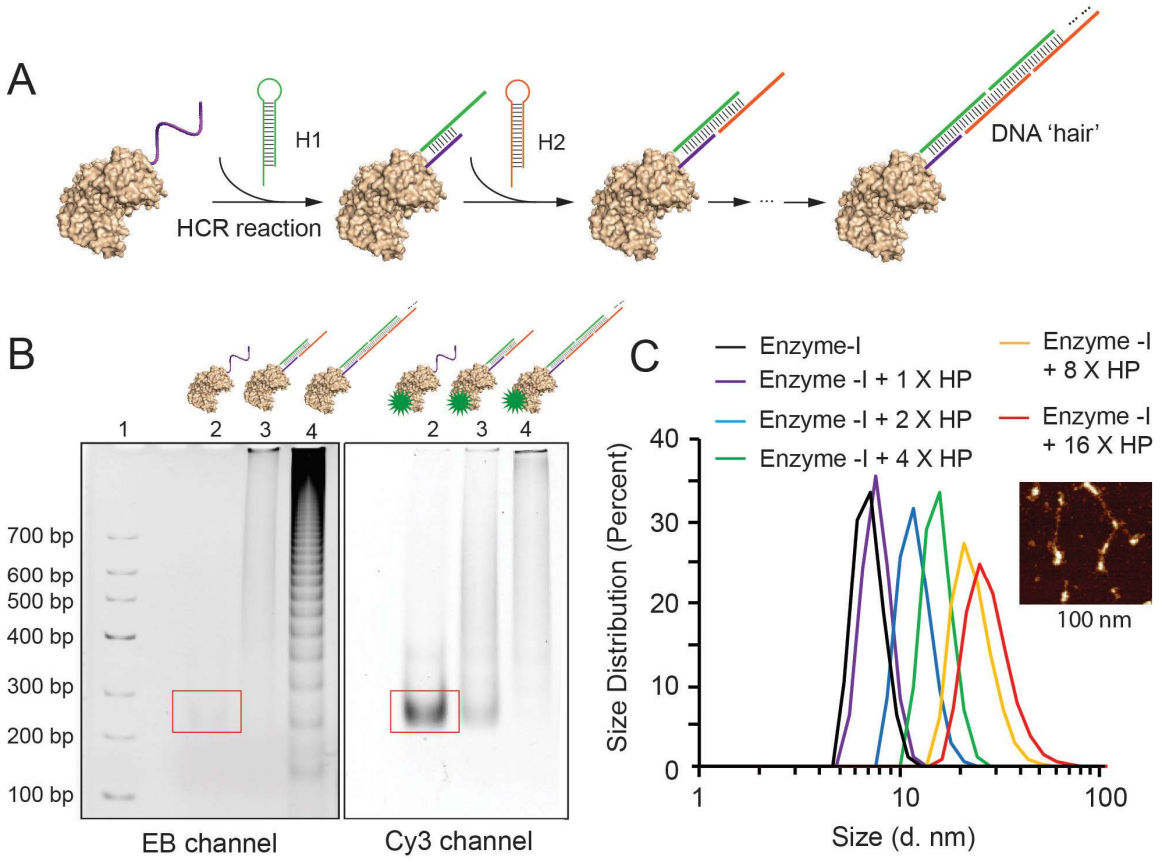

Figure 3. The design and characterization of DNA-crowded enzymes. (A) The construction of long DNA duplex on an enzyme surface by HCR. (B) (3%) PAGE characterization of DNA-crowded enzymes with EB staining (left) of DNA and fluorescent imaging (right) of Cy3-labelled G6PDH. Lane 1: DNA ladder; Lane 2: 1 μM initiator-conjugated G6PDH (G6PDH-I); Lane 3: 1 μM G6PDH-I + hairpins (1×); Lane 4: 1 μM G6PDH-I + hairpins (10 ×). (C) DLS analysis of DNA-crowded enzyme sample with the titration of HPs-to-enzyme ratios. Inset: AFM imaging of DNA-crowded enzyme complex.

As shown in **Figure 4**, FCS was used to characterize the formation of DNA-crowded enzyme complexes at the single-molecule level by measuring the diffusion coefficient of nanostructures. A ssDNA initiator showed a fast diffusion of ~ 140 $\mu m^2/s$. As the initiator triggered HCR reaction on the enzyme surface which subsequently grew into a long

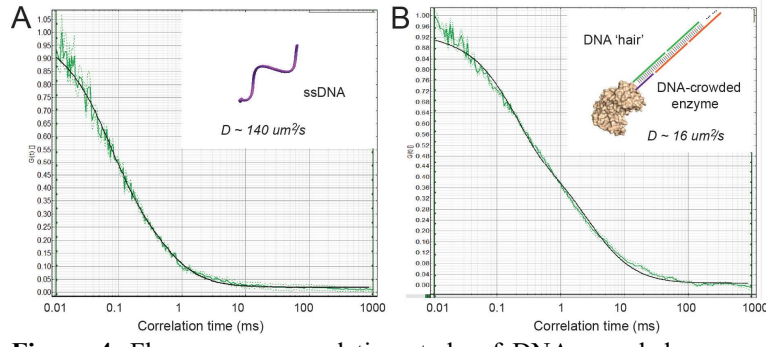

Figure 4. Fluorescence correlation study of DNA-crowded enzyme complex with (A) a ssDNA initiator and (B) a DNA-crowded enzyme.

dsDNA 'hair', the diffusion of this DNA-protein complex significantly slowed down to ~ 16 $\mu m^2/s$.

III-(5) Fluorescence Correlation for Studying Protein and Peptide Aggregation

We have used FCS to study the aggregation of peptides with proteins at nanomolar protein concentrations. **Figure 5A** shows the autocorrelation curves derived from monitoring the fluctuations in dye molecules moving in and out of a small volume of liquid. Small molecules like free Alexa 555 dyes move in and out of the volume rapidly and thus their autocorrelation decays completely within 2 ms (their resident time in the small volume was less than 2 ms). In contrast, the Alexa 555-labeled β-Gal (3 nM enzyme) gave rise to an autocorrelation trace that extended out to roughly 100 ms, which was consistent with its much larger size. When 20 μM peptide 1 was added to the enzyme solution, causing the strong inhibition of β-Gal, the correlation time increased dramatically (it is difficult to measure, but was on the order of 60 s), indicating the formation of very large particles with long resident times in the confocal volume. This is shown in **Figure 5B**, right panel, where one can see large, long-lived fluctuations in the fluorescence from the confocal volume as a function of time, representing the slow movement of large particles in and out of the volume. The dye molecules and labeled enzymes themselves do not show this behavior.

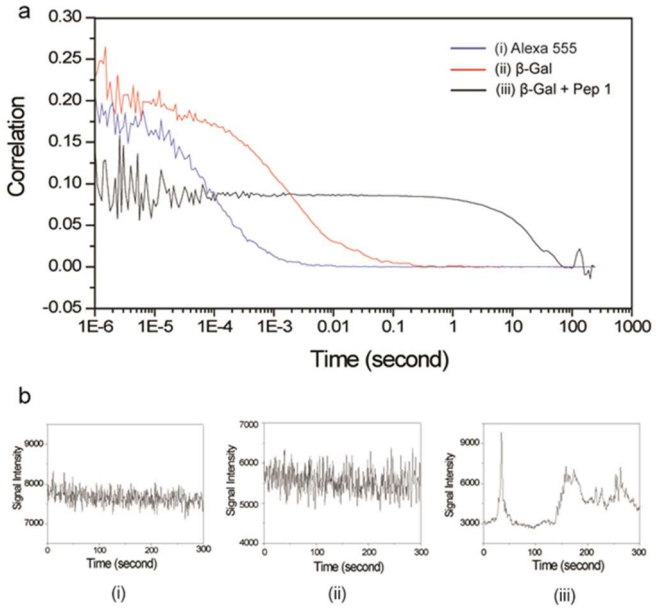

Figure 5. Fluorescence correlation study of β-Gal/peptide aggregation. (A) Fluorescence correlation decay of (i) Free Alexa 555 (Blue line), (ii) β-Gal (Red line) and (iii) β-Gal/peptide 1 complexes (Black line). (B) Time-dependent signals from monitoring fluorescence fluctuations in the confocal volume of a microscope set up for single molecule detection.

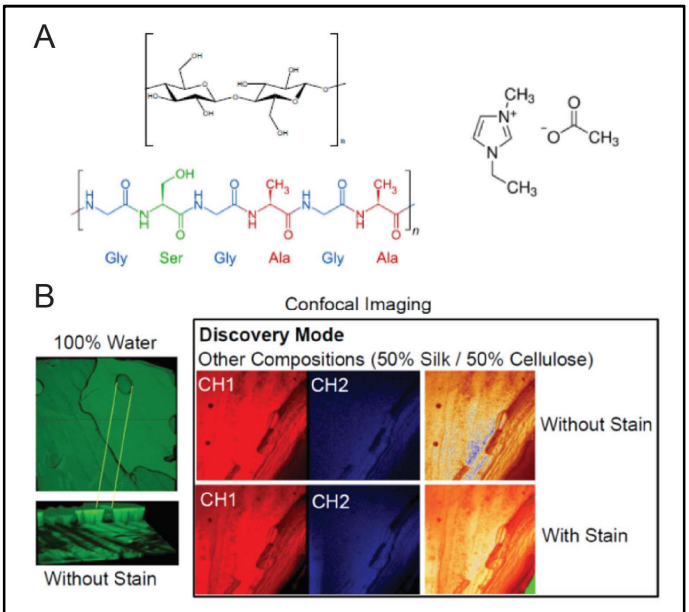

Figure 6. (A) Regeneration of Silk/Cellulose bio-composite using ionic liquids. (B) Confocal imaging of the bio-composite materials with color staining.

III-(6) Leica confocal imaging for protein/polysaccharides bio-composite characterization

Prof. David Salas from Chemistry Department at Rutgers University-Camden used the upgraded Leica confocal-FCS microscope to characterize

the protein-polysaccharides bio-composite. The main objective was to regenerate the hybrid material using ionic liquids and to understand the material physicochemical properties as a function of three polar coagulation agents: water (H2O), ethanol (EtOH), and hydrogen peroxide (H_2O_2). As shown in **Figure 6**, the Leica SP8 Confocal Microscope was used with the HyD detectors and the UV laser of 448nm. XYλ was initially conducted to determine the ranges of detection for the stain. The detectors were set in the range of 450 nm to 550nm for the HyD 2 and HyD 3 was set to be from 550nm to 650nm with the laser at 10% power. The images were taken at 1024 x1024 at 600Hz using the XYZ Acquisition mode to allow for 3D images to be generated. The images were taken on the x10 lens to get an overall view of the sample. The confocal image showed detailed structures of bio-composites with the multi-color staining.

IV. Future Directions:

a. Development of synthetic compartments with controlled geometry and confined local environments. We will design DNA nanocages with controlled cavity sizes and shapes. DNA nanocages will be outfitted with synthetic lipids to generate an artificial vesicle. Fluorescent correlation spectroscopy will be used to evaluate the permeability of vesicles.

b. Enzyme-Powered Nanomotor Propelled by Chemical Fuels. We will exploit the phenomena of the catalysis-driven diffusion for propelling the movement of nanodevices by chemical fuels. Fluorescence Correlation Spectroscopy will enhance the ability to accurately characterize the diffusion kinetics of nanomotors.

c. Biomolecular interaction and aggregation. FCS is an extremely valuable tool for characterizing the aggregation pathway of protein. FCS measurements can be performed directly in solution and allow to monitor the dynamic progress of aggregation in real time, such as the formation of amyloid fiber.

V. Publications and presentations from this reporting period:

V.1. Total number of papers published in peer reviewed journals: 3

1. Sung Won Oh, Adriana Pereira, Ting Zhang and **Jinglin Fu*** "DNA-Regulated Proximity Assembly Circuit for Actuating Biochemical Reactions", *Angewandte Chemie* **2018**, 57, 13086-13090. doi: 10.1002/anie.201806749.
2. John Collins, Ting Zhang, Sung Won Oh, Robert Maloney and **Jinglin Fu*** "DNA-Crowded Enzyme Nanoparticles with Enhanced Activities and Stabilities", *Chem Comm.* **2017**, 53, 13059–13062.
3. **Jinglin Fu**, Luca Larini, Anthony J. Cooper, John W. Whittaker, Azka Ahmed, Junhao Dong, Minyoung Lee and Ting Zhang "Computational and Experimental Analysis of Short Peptide Motifs for Enzyme Inhibition", *PLoS ONE* **2017**, https://doi.org/10.1371/journal.pone.0182847.

V.2. Total numbers of manuscript under journal consideration or in preparation: 3

1. Ting Zhang, Jia Nong, Nouf Alzahrani, Zhicheng Wang, Tristan Meier, Dong Gyu Yang, Yonggang Ke, Yinghui Zhong and **Jinglin Fu*** "Self-assembly of DNA-Minocycline Complexes by Metal Ions with Controlled Drug Release" *ACS Applied Materials & Interfaces*, under peer review.

2. **Jinglin Fu***, Sung Won Oh, Kristin Monckton, Georgia Arbuckle-Keil, Yonggang Ke and Ting Zhang "Bio-mimetic Compartments Scaffolded by Nucleic Acid Nanostructures" *Small*, under peer review.

3. **Jinglin Fu**, Ting Zhang and Neal W. Woodbury "Peptide-induced enzyme aggregation with inhibited activities" manuscript in preparation.

V.3. Total number of presentations (Presented by Dr. Fu): 4

1. "Spatially Interactive Biomolecular Networks Organized by Nucleic Acids Nanostructures", invited talk, East Lake International Forum for Outstanding Overseas Young Scholars **2016**, Wuhan, China.
2. "DNA-crowded enzyme complexes with improved activity and stability", oral presentation, 252nd ACS National Meeting **2016**, Philadelphia.
3. "Assembly of multi-enzyme complexes on DNA nanoscaffolds", oral presentation, 252nd ACS National Meeting **2016**, Philadelphia.
4. "Spatially Interactive Biomolecular Networks Organized by DNA Architectures", invited talk, National Center for Nanoscience and Technology at Beijing, China, May 20, **2016**.

VI. Honors and awards: N/Z

VII. Patents disclosed during the reporting period: 2

1. **Jinglin Fu**, Sung Won Oh and Adriana Pereira "DNA Logic-gated Proximity Assembly Circuit for Biochemical Sensing" U.S. Patent Application No. 62/647,014.
2. **Jinglin Fu**, John Collins and Ting Zhang "DNA-crowded Enzyme Complex with Controlled Spatial Confinements" US Provisional Patent. App. No. 62/482,882.

VIII. Students Training

1. Total number of undergraduate bachelor's degrees awarded: 2

Gina Disalvo, Gabriele Stankeviciute

2. Total number of Master's degree awarded: 0

3. Other graduate students: 2

Ariel Lane, Tianran Li

4. Total number of high school students sponsored for summer research: 1

Olivia Zapfe,

5. Total Number of URAP students: 1

Adriana Pereira

IX Dissemination

N/Z