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Final Report: DURIP: Nanoparticle Tracking Analysis for Characterizing the Structural Dynamics of Biochemical Nanosystems

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5c. PROGRAM ELEMENT NUMBER
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14. ABSTRACT

15. SUBJECT TERMS

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Jinglin Fu
UU	UU	UU	UU		19b. TELEPHONE NUMBER 856-225-6612

RPPR Final Report
as of 27-Jan-2022

Agency Code: 21XD

Proposal Number: 75708LSRIP **Agreement Number: W911NF-20-1-0107**
INVESTIGATOR(S):

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DUNS Number: 625216556 EIN: 226001086

Report Date: 31-Jul-2021 Date Received: 27-Jan-2022
Final Report for Period Beginning 20-Apr-2020 and Ending 30-Apr-2021
Title: DURIP: Nanoparticle Tracking Analysis for Characterizing the Structural Dynamics of Biochemical Nanosystems
Begin Performance Period: 20-Apr-2020 **End Performance Period:** 30-Apr-2021
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:** 6

Major Goals: We propose to acquire a nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) instrumentation to support multidisciplinary research programs in Chemistry, Physics and Center for Computational and Integrative Biology at Rutgers University-Camden. DLS is a non-invasive technique for measuring the size distribution of particles in solution, based on the analysis of scattering intensity fluctuations. NTA can directly visualize and track the scattering traces of individual particles, which can determine the diffusion coefficient, particle sizes and their absolute concentrations. Multiple research projects are proposed wherein the acquisition of a NTA/DLS will enable DoD-supported and relevant fundamental research, including: (1) characterization of DNA nanostructure assembly; (2) Synthetic cellular compartments with controlled spatial confinements and activities; (3) Develop an enzyme-powered nanomotor propelled by chemical fuels; (4) Synthesis of nanoalloys and their application as photosensitizers within polymersomes; (5) Self-assembly of DNA-small molecule complexes by metal ions; (6) Effects of ionic liquids on lipid vesicles: sizes, stabilities, and fusion mechanisms and (7) Study of aggregation of proteins and peptides, and bio-fibril materials.

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

RPPR Final Report
as of 27-Jan-2022

Accomplishments: Concise Accomplishments

1) We completed the purchase of a Zetasizer Pro (DLS) and a Nanoparticle tracking analysis (NTA) of Nanosight300 from Malvern Instruments, and the initial installation and training were completed in September 2020.

2) The instrument has enhanced and benefited multiple research projects funded by DoD and other federal agents.

3) We have trained undergraduates, graduate students and postdoc for using the instrument.

4) The instrument is also open to the external users for research, e.g. Rowan University.

5) The instrument is used to generate data for publications and presentations.

Detailed information is described in the report attachment.

Training Opportunities: Training: The instrument has provided training opportunities for undergraduates, graduate students, postdoc and faculty members to use it for research.

Faculty: 2
Jinglin Fu, Rutgers-Camden
Rachel Riley, Rowan University

Postdoc: 2
Dr. Sung Won Oh in Fu lab at Rutgers-Camden
Dr. Pedro Urquiza in Solesio lab at Rutgers-Camden

Graduate Students: 4
Karena Joy, Master in Biology, Rutgers-Camden
Kaitlyn Nguyen, Master in Chemistry, Rutgers-Camden
Sam Hofbauer, Ph.D in Biomedical Engineering, Rowan University
Ezry St. Iago-Mcrae, Ph.D in CCIB, Rutgers-Camden

Undergraduates: 2
Akshay Shah, Rutgers-Camden
Omri Bar, Rutgers-Camden

Results Dissemination: We have published paper with data generated by the instrument funded by DURIP, as well as conference presentations.

Honors and Awards: Chancellor's Award for Outstanding Research and Creative Activity, Rutgers-Camden, 2020

Protocol Activity Status:

Technology Transfer: Nothing to Report

ARTICLES:

RPPR Final Report
as of 27-Jan-2022

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Topics in Current Chemistry
Publication Identifier Type: DOI Publication Identifier: 10.1007/s41061-020-0299-3
Volume: 378 Issue: 3 First Page #: 38
Date Submitted: 1/25/22 12:00AM Date Published: 4/1/20 4:00AM
Publication Location:
Article Title: DNA-Scaffolded Proximity Assembly and Confinement of Multienzyme Reactions
Authors: Jinglin Fu, Zhicheng Wang, Xiao Hua Liang, Sung Won Oh, Ezry St. Iago-McRae, Ting Zhang
Keywords: Biomimetic systems · DNA nanotechnology · DNA scafolded assembly · Enzyme encapsulation · Enzyme immobilization · Enzyme regulation · Multienzyme cascade · Synthetic reactors
Abstract: Cellular functions rely on a series of organized and regulated multienzyme cascade reactions. The catalytic efciencies of these cascades depend on the precise spatial organization of the constituent enzymes, which is optimized to facilitate substrate transport and regulate activities. Mimicry of this organization in a non-living, artifcial system would be very useful in a broad range of applications with impacts on both the scientifc community and society at large. Self-assembled DNA nanostructures are promising applications to organize biomolecular components into prescribed, multidimensional patterns. In this review, we focus on recent progress in the feld of DNA-scafolded assembly and confnement of multienzyme reactions. DNA self-assembly is exploited to build spatially organized multienzyme cascades with control over their relative distance, substrate difusion paths, compartmentalization and activity actuation.
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: ACS Applied Bio Materials
Publication Identifier Type: DOI Publication Identifier: 10.1021/acsabm.1c01128
Volume: Issue: First Page #:
Date Submitted: 1/25/22 12:00AM Date Published: 1/1/22 5:00AM
Publication Location:
Article Title: Reduction of Promiscuous Peptides-Enzyme Inhibition and Aggregation by Negatively Charged Biopolymers
Authors: Jinglin Fu, Kaitlyn Nguyen
Keywords: peptide promiscuous inhibitor enzyme aggregation biopolymers activity recovery ?-galactosidase
Abstract: In this work, peptides selected from a microarray were found to inhibit ?-gal with promiscuous mechanisms. Peptides inhibited the enzyme in a noncompetitive kinetics, and the inhibition of enzyme activities was reduced under high enzyme concentrations and the addition of detergent. Dynamic light scattering and atomic force microscope revealed that peptide/enzyme aggregation was related to inhibited enzyme activities. Positively charged residues of arginine and lysine were critical for the enzyme inhibition. The preincubation of peptide inhibitors with negatively charged biopolymers of polyphosphates, ssDNA, and low pI peptides could increase the residual activity of peptide-inhibited enzyme, possibly due to the disruption of the electrostatic interaction between positively charged peptide residues and the ?-gal surface. Further, negative biopolymers were able to recover the activity of the aggregated peptide/?-gal complex. Negatively charged biopolymers could be used in high-throughput
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

DISSERTATIONS:

RPPR Final Report
as of 27-Jan-2022

Publication Type: Thesis or Dissertation
Institution: Rutgers University-Camden
Date Received: 27-Jan-2022 Completion Date: 1/1/21 10:35PM
Title: DEVELOPING DNA-MEDIATED PROXIMITY ASSEMBLY CIRCUIT FOR ACTUATING BIOCHEMICAL REACTIONS AND MOLECULAR SENSING
Authors: Sung Won Oh
Acknowledged Federal Support: **Y**

Partners

,

I certify that the information in the report is complete and accurate:
Signature: Jinglin Fu
Signature Date: 1/27/22 5:40PM

FINAL PROGRESS REPORT

DURIP : Nanoparticle Tracking Analysis for Characterizing the Structural Dynamics of Biochemical Nanosystems

Principal Investigator: Jinglin Fu
Rutgers University-Camden

Reporting period: 4/20/2020-4/30/2021
Proposal number: 75708LSRIP
Agreement number: W911NF2010107

I. Scientific and Technical Objectives and DoD impact.

We propose to acquire a nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) instrumentation to support multidisciplinary research programs in Chemistry, Physics and Center for Computational and Integrative Biology at Rutgers University-Camden. DLS is a non-invasive technique for measuring the size distribution of particles in solution, based on the analysis of scattering intensity fluctuations. NTA can directly visualize and track the scattering traces of individual particles, which can determine the diffusion coefficient, particle sizes and their absolute concentrations. Multiple research projects are proposed wherein the acquisition of a NTA/DLS will enable DoD-supported and relevant fundamental research, including: (1) characterization of DNA nanostructure assembly; (2) Synthetic cellular compartments with controlled spatial confinements and activities; (3) Develop an enzyme-powered nanomotor propelled by chemical fuels; (4) Synthesis of nanoalloys and their application as photosensitizers within polymersomes; (5) Self-assembly of DNA-small molecule complexes by metal ions; (6) Effects of ionic liquids on lipid vesicles: sizes, stabilities, and fusion mechanisms and (7) Study of aggregation of proteins and peptides, and bio-fibril materials.

The proposed research will provide opportunities for graduate training and outreach to graduates, undergraduates, high school students and teachers. Research within the Center for Computational and Integrative Biology at Rutgers University-Camden exposes students to a highly interdisciplinary research environment which involves people from different backgrounds including chemistry, biology, physics, computer and mathematics. The proposed research will provide research opportunities for under-represented/low-income undergraduates. Camden is a national low-income area. Rutgers-Camden is committed to the improvement of the community and better education for young people in the local area. The PI's lab has participated in mentoring undergraduates and high school students to expose them to a wide variety of STEM concepts and techniques. We anticipate that the interdisciplinary training opportunity made possible by the NTA/DLS will encourage more creative thinking and inspire a greater interest in science.

DoD impact: These project outcomes provide the scientific foundations of biochemistry studies that will aid the development of adaptable platforms on which biocatalysis, biomaterials and biosensor devices can be integrated, activated and regulated, harnessing new capabilities that enhance warfighter protection and performance. An insightful understanding of nanoparticle formation and biomaterials assembly will enable the development in novel material design, energy

transfer structures, nanomedicine and diagnosis with potential applications related to DoD mission. The proposed NTA/DLS instrumentation will assist the Chemistry and Physics Department to establish a material science center for studying various nanoparticles and bio-selfassembly from single-molecule level to bulk level. These research projects 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. The PI will make a survey of the undergraduates/high school students who are willing to pursue future studies and careers relevant to DoD after taking these research programs, for example, 21st Century Skills Assessment developed by AEOP.

II. Concise Accomplishments

1) We completed the purchase of a Zetasizer Pro (DLS) and a Nanoparticle tracking analysis (NTA) of Nanosight300 from Malvern Instruments, and the initial installation and training were completed in September 2020.

2) The instrument has enhanced and benefited multiple research projects funded by DoD and other federal agents.

3) We have trained undergraduates, graduate students and postdoc for using the instrument.

4) The instrument is also open to the external users for research, e.g. Rowan University.

5) The instrument is used to generate data for publications and presentations.

III. Expanded Accomplishments

III-(1) The final equipment cost of NS300/Zetasizer Pro package from Malvern Panalytical INC.

On June 17th, 2020, Dr. Fu placed an order request of a NS300/Zetasizer Pro package instruments through Rutgers-University-Camden. The final cost is $154,240; the detailed items are listed below:

Ref.: Q-28539-4

Item	Quant.	Product Code	Description	List Price ($)	Total List Price ($)
1	1	NTA3301	NS300 NTA Instrument USB	41,190.00	41,190.00
2	1	NTA1003	USB High Sensitivity Camera Option	18,560.00	18,560.00
3	1	NTA2001	NS300 2 LASER SYSTEM KIT	714	714
4	1	NTA1301	NS300 488nm Blue Laser Module	25,440.00	25,440.00
5	1	NTA1302	NS300 532nm Green Laser Module	16,230.00	16,230.00
6	1	NTA1330	motorised filter wheel	4,620.00	4,620.00
7	1	NTA4181	NS300 488nm FLUORESCENCE KIT	3,050.00	3,050.00
8	1	NTA4182	NS300 532nm FLUORESCENCE KIT	2,040.00	2,040.00
9	1	NTA1010	NTA Syringe Pump	4,740.00	4,740.00
10	1	NTA4212	NS300 Syringe Pump Installation Kit	220	220
11	1	CPH3180US	64-bit Windows 10 NTA PC	2,540.00	2,540.00
12	1	ZSU5800P	Zetasizer Pro plus outer carton	56,400.00	56,400.00
13	1	VIS5001	SV-10 Viscometer Package	8,650.00	8,650.00
14	1	ZEN2112	Low-volume quartz batch cuvette	921.00	921.00
Discount					32,645.00
Net Price					152,670.00
Freight					1,570.00
Grand Total					154,240.00

III-(2) Installation and training of the instrument: The instrument was received and installed on September 4th, 2020. Due to the COVID-19 pandemic, the external engineer and scientist required the approval from the Rutgers University-Camden, and they needed to wear masks when entering Rutgers building. The training was limited for less than 5 people, and was completed on September 4th, 2020.

III-(3) Research Project impacted by the acquisition of the NS300/Zetasizer Pro instrument.

The acquisition of the instrument package has enhanced multidisciplinary programs, including DoD-supported and relevant fundamental research in self-assembled DNA nanostructure circuit, bio-mimetic compartments, nanoparticle-biosystem interaction and protein/peptide aggregations, as well as benefit other users in the local area. The instrument is also used to enhance STEM education for high school students, undergraduates and graduate students.

a. **Characterization of the DNA Nanostructures Assembly (funded by ARO PECASE, DoD I-Corps) :** The instrument is used to characterize the structure size, heterogeneous assembly and concentration of DNA nanostructures, which will enhance the precise design and control of DNA-templated biochemical circuits and their regulatory functions.

b. **Synthesis of DNA-conjugated Gold nanoalloys and their application as Photosensitizers within Polymersomes (funded by Rutgers and NSF):** The instrument

is used to (1) provide precise particle sizes of the initial colloidal solutions, as well as DNA-conjugated nanoparticles. This will allow more precise control of the molar ratio in the produced nanoalloys. (2) it can monitor the size and concentration of particle group evolution during irradiation, which will provide further insight into the fragmentation/melting processes occurring during PLI. Additionally, in the light-triggered release of polymersomes, the effective pore size on the membrane will be correlated with the particle population inserted into the membrane.

c. **NTA/DLS for Studying Protein and Peptide Aggregation (funded by ARO PECASE):** The Fu lab has used DLS to study the aggregation of peptides with proteins in bulk solution. Zetasizer Pro was used to analyze the particle sizes of peptides and proteins, which revealed a unique mechanism of peptide-induced protein aggregation and fiber formation. The results have been published on ACS Applied Biomaterials in the beginning of 2022.

d. **The formation of gold and lipid nanoparticles**: The Riley lab from Rowan University engineer gold and lipid nanoparticles for drug delivery to treat cancer and pregnancy-related diseases. This instrument is used to characterize our nanomaterials for size, dispersity, and surface charge.

e. **Enhancement of AEOP-sponsored High School/Undergraduate Research Apprenticeship:** The Fu lab has participated in multiple AEOP-sponsored programs since 2015, including High-school Research Apprenticeship Program (HSAP), Undergraduate Research Apprenticeship Program (URAP) and Research and Engineering Apprenticeship Program (REAP). The nanoparticle tracking analysis instrument will enhance the study and characterization of biochemical nanostructures, which provides students more 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.

IV. Publications and presentations from this reporting period:

IV.1. Total number of presentations: 4

1. "DNA- Scaffolded Proximity Assembly for Biomimetic Systems and Molecular Sensing", invited speaker, Web Symposium/Session on Biomaterials and Biodevices, IAAM, February 5th, **2021**.
2. "DNA-Guided Proximity Assembly and Confinement of Biochemical Reactions", invited speaker, Army Research Office Life Sciences Division, Program Review/Seminar Series on Innovations in Bioscience Sensing and Signaling, October **2020**.
3. "Develop nucleic acid assembly circuits for disease diagnosis", invited talk, the Biology Club and American Medical Student Association Chapter at Rutgers University- Camden, September **2020**.

4. "Rapid and reliable molecular test for COVID-19 diagnosis", invited presentation, RCAF webinar series, Rutgers University, **May 2020**.

IV.2. Publications: 4

1. **Jinglin Fu***, Zhicheng Wang, Xiaohua Anna Liang, Sung Won Oh and Ting Zhang "DNA-Scaffolded Proximity Assembly and Confinement of Multienzyme Reactions" *Topics in Current Chemistry* **2020**, published online, DOI: 10.1007/s41061-020-0299-3.
2. **Jinglin Fu** and Kaitlyn Nguyen "Reduction of Promiscuous Peptides-Enzyme Inhibition and Aggregation by Negatively Charged Biopolymers", *ACS Applied Bio Materials* **2022**, **https://doi.org/10.1021/acsabm.1c01128**
3. Ezry Santiago-McRae, Sung Won Oh, Anthony Monte Carlo, Omri Bar, Emily Guan, Doris Zheng, Catherine Grgicak and **Jinglin Fu** "Rapid Nucleic Acid Reaction Circuits for Point-Of-Care Diseases Diagnosis", *Current Topics in Medicinal Chemistry*, acceptance for publication.
4. Sung Won Oh, Zhicheng Wang and **Jinglin Fu*** "DNA Nano-scaffolds for Multienzyme Systems Assembly" *Methods in Molecular Biology*, acceptance for publication.

V. Honors and awards:

Jinglin Fu, Chancellor's Award for Outstanding Research and Creative Activity, Rutgers-Camden, 2020

V. Total number of students being trained: 9

Postdoc: 3
Dr. Sung Won Oh in Fu lab at Rutgers-Camden
Dr. Zhicheng Wang in Fu lab at Rutgers-Camden
Dr. Pedro Urquiza in Solesio lab at Rutgers-Camden

Graduate Students: 4
Karena Joy, Master in Biology, Rutgers-Camden
Kaitlyn Nguyen, Master in Chemistry, Rutgers-Camden
Sam Hofbauer, Ph.D in Biomedical Engineering, Rowan University
Ezry St. Iago-Mcrae, Ph.D in CCIB, Rutgers-Camden

Undergraduates: 2
Akshay Shah, Rutgers-Camden
Omri Bar, Rutgers-Camden