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

RPPR Final Report
as of 26-Jan-2022

Agency Code:

Proposal Number: 74130SDICR

Agreement Number: W911NF-19-1-0414

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EIN: 226001086

Report Date: 31-Oct-2021

Date Received: 25-Jan-2022

Final Report for Period Beginning 01-Aug-2019 and Ending 31-Jul-2021

Title: DNA-Mediated Proximity Assembly Circuits for Point-of-Care Diagnosis

Begin Performance Period: 01-Aug-2019

End Performance Period: 31-Jul-2021

Report Term: 0-Other

Submitted By: Jinglin Fu

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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 1

STEM Participants: 1

Major Goals: We proposed to commercialize a DNA nanodevice product that can be adopted for POC testing. The nanodevice uses a mechanism of DNA-mediated proximity assembly circuit (DPAC) that contains a limited number of oligonucleotides and a simple catalytic system. Target molecules can be identified by visible color change. It does not require any additional steps to separate detectable components from the assay background, and can be applied to at-home use or diagnosis at diverse locations. Our developed DPAC sensors have the advantages: (1) simplified operation (e.g. One-pot assay) by non-specialists at diverse locations; (2) easy-to-read signals; (3) reliable and rapid assay; (4) low-cost for the test. As proof-of-concept, Dr. Fu's team has developed and tested a DPAC sensor to detect a microRNA biomarker for prostate cancer at concentration as low as 0.1 nM. Similar sensors can be readily redesigned for the detection of other disease-relevant nucleic acids, proteins and small molecules that have broad use in healthcare, environmental monitoring and safety of soldiers at ground warfare. The target market includes: POC testing for a variety of disease-relevant nucleic acids, small molecules and proteins; metal detection in water; detection of reactional drug metabolites and explosive compounds sensing.

The goal of this project is to utilize the opportunities provided by the DoD towards commercializing the results of fundamental research conducted in our lab supported by current and previous ARO grants. Being a part of the program would enable our lab members to receive entrepreneurial education and gain knowledge necessary for attracting start-up funding. Sponsored by the I-Corps curriculum, we first plan to demonstrate the DPAC sensor to potential customers and partners that will be given by the ELs. There will be a real-time demonstration of

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technology during visits and the customers could also test it on their own, using the demonstration product that we provide. Then, we will use these meetings to figure out potential customers' needs to help us in formulating the best value proposition, strategy towards creating a start-up, its business plan, funding pitch and determining what additional R&D work is required. In line with DoD applications, the product can be used to improve the warfighter protection by detecting water contaminations (metal ions), recreational drugs or drug abuse, and explosive compounds.

Accomplishments: Concise Accomplishments

(1) NSF I-Corps@Philadelphia: We have participated the 10-week NSF national I-Corp at Philadelphia. A dNANOSISTM team is formed who aims to develop DNA Nanosensor products for Point-of-Care Diagnosis of Disease and Infection. The primary goal of I-Corps is to identify the marketplace and to conduct interviews with potential customers or related people for understanding the market need. Our team (dNanosis) have interviewed professionals in primary care, urgent care and ER, as well as medical insurance agents.

(2) 100 + customer interviewers: We have performed 113 customer interviews, including 108 in-person interviews with health professionals and insurance agents.

(3) Application of NSF PFI program: Toward commercialization, the dNANOSISTM team has applied to a NSF Partnerships for Innovation (PFI) for Technology Translation. The NSF PFI is designed to increase the impact of their NSF-funded research discoveries by developing their technology into a prototype or proof-of-concept. Due to the DoD sponsored participation of NSF I-Corps, our team is eligible to apply for NSF PFI for the technology translation activity. Our application is recommended by the PFI program director, and is pending on the administrative approval.

Please see the attachment report for the detailed information.

Training Opportunities: VII. Total number of graduate students awarded: 1
Sung Won Oh (CCIB, Ph.D)

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Results Dissemination: 1. We have participated in NSF I-Corps to present our technology.
2. The team has performed 113 customer interviews, and introduced our team and technology.
3. The team has made 11 conference presentations, and 3 publications.

Total number of presentations: 11

1. "DNA-Scaffolded Nanoreactors for Biomimetic Catalysis and Molecular Sensing", invited speaker, NanoBoston 2021.
2. "Bottom-up Fabrication of Bio-mimetic Materials by Nucleic Acids Self-assembly", invited speaker, Yong Scholar Forum, Wuhan University of Science and Technology, 2021.
3. "Bottom-up Fabrication of Bio-mimetic Materials with Nanometer Precision by Nucleic Acids Self-assembly", invited speaker, WCMNM 2021 (Sep.).
4. "DNA- Scaffolded Proximity Assembly for Biomimetic Systems and Molecular Sensing", invited speaker, Web Symposium/Session on Biomaterials and Biodevices, IAAM, February 5th, 2021.
5. "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.
6. "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.
7. "Rapid and reliable molecular test for COVID-19 diagnosis", invited presentation, RCAF webinar series, Rutgers University, May 2020.
8. "DNA-Scaffolded Proximity Assembly of Biochemical Reactions", invited presentation, ACS Philadelphia Section, April 2020.
9. "DNA-Scaffolded Proximity Assembly and Confinement of Biochemical Reactions", invited talk, Department of Chemistry and Biochemistry, Temple University, 2020.
10. "DNA Nanostructures-Templated Proximity Assembly and Confinement of Biochemical Reactions", invited talk, Department of Chemistry and Biochemistry, Rutgers University-New Brunswick, 2019.
11. "DNA-mediated proximity assembly circuit for actuating biochemical reactions", the 7th international meeting on Quadruplex Nucleic Acids, Changchun, September, 2019.

Publications: 3

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. 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.
3. Sung Won Oh, Zhicheng Wang and Jinglin Fu* "DNA Nano-scaffolds for Multienzyme Systems Assembly" Methods in Molecular Biology, acceptance for publication.

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

Protocol Activity Status:

Technology Transfer: A US Patent No. 10,961,565B2 was granted on March 30, 2021: "DNA LOGIC-GATED PROXIMITY ASSEMBLY CIRCUIT FOR BIOCHEMICAL SENSING". This application relates to nucleic acid-based sensors, kits that include such sensors, and methods for making and using such sensors. The sensors permit detection of a broad array of target agents, such as nucleic acids (e.g., DNA and RNA), proteins, cells, and small molecules (e.g., toxins and metals).

PARTICIPANTS:

Participant Type: PD/PI

Participant: Jinglin Fu

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

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Participant Type: Graduate Student (research assistant)

Participant: Sung Won Oh

Person Months Worked: 2.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Consultant

Participant: Bo Wang

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

ARTICLES:

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Journal: Topics in Current Chemistry

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Date Submitted: 1/25/22 12:00AM

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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 scaffolded 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 efficiencies 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, artificial system would be very useful in a broad range of applications—with impacts on both the scientific 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 field of DNA-scaffolded assembly and confinement of multienzyme reactions. DNA self-assembly is exploited to build spatially organized multienzyme cascades with control over their relative distance, substrate diffusion paths, compartmentalization and activity actuation.

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

PATENTS:

Intellectual Property Type: Patent

Date Received: **25-Jan-2022**

Patent Title: DNA LOGIC-GATED PROXIMITY ASSEMBLY CIRCUIT FOR BIOCHEMICAL SENSING

Patent Abstract:

RPPR Final Report

as of 26-Jan-2022

Patent Number: 10,961,565B2

Patent Country: USA

Application Date: 23-Mar-2018

Date Issued: 30-Mar-2021

Application Status: 3

Partners

,

I certify that the information in the report is complete and accurate:

Signature: Jinglin Fu

Signature Date: 1/25/22 7:26PM

FINAL PROGRESS REPORT: DNA-Mediated Proximity Assembly Circuits for Point-of-Care Diagnosis

Principal Investigator: Jinglin Fu
Rutgers University-Camden

Reporting period: 8/01/2019-7/31/2021
Proposal Number: 74130-SD-ICR
Agreement Number: W911NF-19-1-0414

I. Scientific and Technical Objectives

We proposed to commercialize a DNA nanodevice product that can be adopted for POC testing. The nanodevice uses a mechanism of DNA-mediated proximity assembly circuit (DPAC) that contains a limited number of oligonucleotides and a simple catalytic system. Target molecules can be identified by visible color change. It does not require any additional steps to separate detectable components from the assay background, and can be applied to at-home use or diagnosis at diverse locations. Our developed DPAC sensors have the advantages: (1) simplified operation (e.g. One-pot assay) by non-specialists at diverse locations; (2) easy-to-read signals; (3) reliable and rapid assay; (4) low-cost for the test. As proof-of-concept, Dr. Fu's team has developed and tested a DPAC sensor to detect a microRNA biomarker for prostate cancer at concentration as low as 0.1 nM. Similar sensors can be readily redesigned for the detection of other disease-relevant nucleic acids, proteins and small molecules that have broad use in healthcare, environmental monitoring and safety of soldiers at ground warfare. The target market includes: POC testing for a variety of disease-relevant nucleic acids, small molecules and proteins; metal detection in water; detection of reactional drug metabolites and explosive compounds sensing.

The goal of this project is to utilize the opportunities provided by the DoD towards commercializing the results of fundamental research conducted in our lab supported by current and previous ARO grants. Being a part of the program would enable our lab members to receive entrepreneurial education and gain knowledge necessary for attracting start-up funding. Sponsored by the I-Corps curriculum, we first plan to demonstrate the DPAC sensor to potential customers and partners that will be given by the ELs. There will be a real-time demonstration of technology during visits and the customers could also test it on their own, using the demonstration product that we provide. Then, we will use these meetings to figure out potential customers' needs to help us in formulating the best value proposition, strategy towards creating a start-up, its business plan, funding pitch and determining what additional R&D work is required. In line with DoD applications, the product can be used to improve the warfighter protection by detecting water contaminations (metal ions), recreational drugs or drug abuse, and explosive compounds.

II. Concise Accomplishments

- (1) NSF I-Corps@Philadelphia: We have participated the 10-week NSF national I-Corps at Philadelphia. A dNANOSISTM team is formed who aims to develop DNA Nanosensor products for Point-of-Care Diagnosis of Disease and Infection. The primary goal of I-Corps is to identify the marketplace and to conduct interviews with potential customers or related people for understanding the market need. Our team (dNanosis) have interviewed professionals in primary care, urgent care and ER, as well as medical insurance agents.

- (2) 100 + customer interviews: We have performed 113 customer interviews, including 108 in-person interviews with health professionals and insurance agents.
- (3) Application of NSF PFI program: Toward commercialization, the dNANOSIS™ team has applied to a NSF Partnerships for Innovation (PFI) for Technology Translation. The NSF PFI is designed to increase the impact of their NSF-funded research discoveries by developing their technology into a prototype or proof-of-concept. Due to the DoD sponsored participation of NSF I-Corps, our team is eligible to apply for NSF PFI for the technology translation activity. Our application is recommended by the PFI program director, and is pending on the administrative approval.

III. Expanded Accomplishments

III-(1) NSF I-Corps@Philadelphia:

The dNANOSIS™ team includes an Entrepreneurial Lead, Sung Won Oh who currently is a 5th year PhD student in the PI's lab; a Technical Lead (Prof. Jinglin Fu) who is the PI on the ARO YIP grant that have funded the technology development; and a Industrial Mentor, Dr. Bo Wang who is a Managing Director of Apex Lab Solutions and a Laboratory Director of Delaware Diagnostic Labs. Team aims to develop DNA Nanosensor products for Point-of-Care Diagnosis of Disease and Infection. During the 10-week NSF I-Corps, the team has identified an unmet market need and problem-solution fit, developed a business canvas, and performed 100+ interviews with potential customer segments.

The Unmet Market need is the rapid and reliable nucleic acid tests for point-of-care diagnosis of diseases, especially for MRSA, HCV and COVID-19. Our proposed solution of DNA-mediated proximity assembly circuit (DPAC) addresses this unmet need by providing a the rapid and reliable nucleic acid test that is required for screening of viral infection. The product handles various sample type with a simple procedure and fast read-out within 1 -2 hours, which can be easily operated without any specialized expensive equipment, and is affordable.

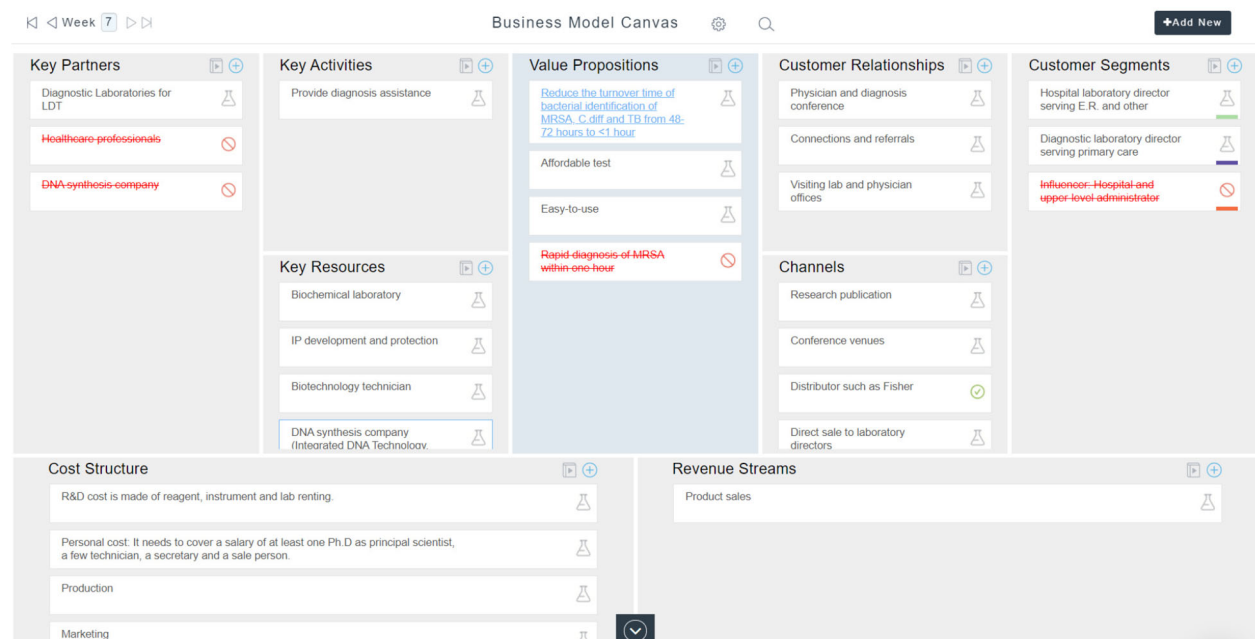


Figure 1. Business Model Canvas developed by NSF I-Corps@ Philadelphia.

As shown in **Figure 1**, the Business Model Canvas include a key partners of diagnostic laboratory and service, key activity of provide diagnosis assistance, key resources, value propositions, customer relationships, sale channel and customer segments, as well as cost structure and revenue streams. The value proposition of our product include (1) a rapid assay within 2 hours, (2) a reliable and confirmative assay for detecting viral RNA/DNA, (3) a simple paper trip for fluorescence read-out and (4) affordable cost.

III-(2) 100 + customer interviewers:

In the 10-week I-Corps, the team has performed a total of 113 interviews: 108 in-person interview, 4 phone interview and 1 online interview. The team interviewed potential customer segments national widely, including Arizona, Nevada, Texas, Ohio, Pennsylvania, New Jersey, Delaware, Maryland and New York.

	Interview Count			
New	15	15	0	0
Total	113	108	4	1

Figure 2. Interview summary of dNANOSIS team at NSF I-Corps from October 2nd to November 15th, 2019.

III-(3) Application of grants for supporting the continuous commercialization effort:

Toward commercialization, the dNANOSISTM team has applied to a NSF Partnerships for Innovation (PFI) for Technology Translation. The NSF PFI is designed to increase the impact of their NSF-funded research discoveries by developing their technology into a prototype or proof-of-concept. Due to the DoD sponsored participation of NSF I-Corps, our team is eligible to apply for NSF PFI for the technology translation activity. Our application is recommended by the PFI program director, and is pending on the administrative approval.

Overview of NSF PFI proposal: An urgent need exists for a rapid, cost-effective, facile and reliable nucleic acid assays for mass screening to control and prevent the spread of emerging pandemic diseases such as COVID-19. This urgent need is not fully met by current diagnostic tools. Based on the unmet need, this PFI-TT project proposes to develop a rapid and reliable nucleic acid assay for confirming the infection status of diseases and variants. The assay will use a mechanism of DNA-mediated proximity assembly circuit (DPAC), in which a DNA structural switch is used to trigger the co-assembly of an enzyme/cofactor system in response to the input of specific nucleic acid sequences. A dNANOSIS (referred as DNA-NANO-Diagnosis) assay will integrate RNA extraction, isothermal amplification, and DPAC sensor. The proposed dNANOSIS aims to rapidly detect viral RNA from collected samples of swab eluent, saliva or blood within 20-

60 mins, with a sensitivity and specificity comparable to PCR. The proposed assay can be automated or be integrated into a user-friendly paper-based detection and lateral flow device for point-of-care use. **Value proposition:** (1) a sensitive and reliable assay for confirming virus infection status; (2) rapid results within 1 hour; (3) a simple operation supporting facile use; (4) an affordable cost for disposable use; (5) analytical and statistical models for improved diagnosis. **Translational outcome:** The successful completion of the proposed research will demonstrate a minimum viable product (MVP) prototype that can be used as is, or developed further for simplicity of operation. The proposed MVP is affordable, easily-operated and, therefore, can be used for rapid point-of-care and home-based testing/diagnostics to prevent the transmission of infectious diseases, such as COVID-19, as well as improving the use of viable organs in transplant. **NSF Lineage:** This PFI-TT project is based on the previous participation of NSF I-Corps in Fall 2019 at Philadelphia site (dNANOSIS team#1682). The dNANOSIS team has completed >100 custom interviews related to the molecular diagnosis market. Although the team was supported by DoD I-Corps (Contract# W911NF1910414; I-Corps@DoD: DNA-Mediated Proximity Assembly Circuits for Point-of-Care Diagnosis), the PFI program officers have confirmed the eligibility of NSF Lineage.

Intellectual Merit: The proposed DPAC is a novel mechanism of engineering smart molecular devices that integrate molecular computation with specific target recognition and signal amplification on the nanoscale. The proposed research will employ a multidisciplinary approach that combines computational design, DNA assembly, bio-conjugation and biochemical assays. The proposed research promises to deliver several technical breakthroughs that will enable rapid and reliable POC detection.

Broader impact: The proposed development will improve health outcomes by supporting mass screening surveillance to identify early infected individuals for quarantine to prevent the spread of diseases, and to initiate early medical treatment. The proposed training of “Innovator Bootcamp” will teach students the pathways of translating discoveries from the benchtop experiments to commercialization products. The project will recruit undergraduate and graduate students from different backgrounds, including women and minorities. This PFI-TT project can be leveraged with other on-going apprenticeship programs in Fu lab, including high-school apprenticeship program and undergraduate apprenticeship sponsored by Army Research Education Outreach, Rutgers–Camden MARC program (Research experience for junior and senior, multiple students) sponsored by NIH. Industrial interns are also available for students to participate the translational development between the research lab and the Delaware Diagnostic Lab.

IV. Publications and presentations from this reporting period:

IV.1. Total number of presentations: 11

1. “DNA-Scaffolded Nanoreactors for Biomimetic Catalysis and Molecular Sensing”, invited speaker, NanoBoston **2021**.
2. “Bottom-up Fabrication of Bio-mimetic Materials by Nucleic Acids Self-assembly”, invited speaker, Yong Scholar Forum, Wuhan University of Science and Technology, **2021**.
3. “Bottom-up Fabrication of Bio-mimetic Materials with Nanometer Precision by Nucleic Acids Self-assembly”, invited speaker, WCMNM **2021 (Sep.)**.

4. “DNA- Scaffolded Proximity Assembly for Biomimetic Systems and Molecular Sensing”, invited speaker, Web Symposium/Session on Biomaterials and Biodevices, IAAM, February 5th, **2021**.
5. “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**.
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8. “DNA-Scaffolded Proximity Assembly of Biochemical Reactions”, invited presentation, ACS Philadelphia Section, **April 2020**.
9. “DNA-Scaffolded Proximity Assembly and Confinement of Biochemical Reactions”, invited talk, Department of Chemistry and Biochemistry, Temple University, 2020.
10. “DNA Nanostructures-Templated Proximity Assembly and Confinement of Biochemical Reactions”, invited talk, Department of Chemistry and Biochemistry, Rutgers University-New Brunswick, 2019.
11. “DNA-mediated proximity assembly circuit for actuating biochemical reactions”, the 7th international meeting on Quadruplex Nucleic Acids, Changchun, September, 2019.

IV.2. Publications: 3

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. 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.
3. 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

VI. Patents disclosed during the reporting period: 1

A US Patent No. 10,961,565B2 was granted on March 30, 2021: “DNA LOGIC-GATED PROXIMITY ASSEMBLY CIRCUIT FOR BIOCHEMICAL SENSING”. This application relates to nucleic acid-based sensors, kits that include such sensors, and methods for making and using such sensors. The sensors permit detection of a broad array of target agents, such as nucleic acids (e.g., DNA and RNA), proteins, cells, and small molecules (e.g., toxins and metals).

VII. Total number of graduate students awarded: 1

Sung Won Oh (CCIB, Ph.D)