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Acquisition of a Nanoscopic Imaging and Analysis Platform for Understanding

Qian Chen
UNIVERSITY OF ILLINOIS

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Final Report

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Chen, Qian (PI)
Smith, John, W. (PhD student)

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This DURIP grant has provided support on the purchase and establishment of a nanoscopic imaging and analysis platform that is required for the execution of the PI's funded AFOSR YIP project. We have developed and implemented a new nanoscopic biophysics imaging tool where the near-atomic resolution imaging capabilities of electron microscopy—which is conventionally limited to static, dried or flash-frozen specimens—are harnessed to study dynamic biological samples preserved in their native liquid environment. The membrane protein system we study plays a central role in myriad life processes, including virtually all those relevant to the Air Force's mission to augment human performance and protection.

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Abstract

This DURIP grant has provided financial support towards the purchase and establishment of a nanoscopic imaging and analysis platform that is required for the execution of my funded AFOSR YIP project (initiated from May, 2017). The scientific aim of the AFOSR YIP project is to develop and implement a new nanoscopic biophysics imaging tool where the near-atomic resolution imaging capabilities of electron microscopy—which is conventionally limited to static, dried or flash-frozen specimens—are harnessed to study dynamic biological samples preserved in their native liquid environment. Cell membrane proteins are the system of interest; due to their size and the fact that their functions depend on configurational dynamics, they are an ideal system in which to develop this imaging technology. Moreover, membrane proteins play a central role in myriad life processes, including virtually all those relevant to the Air Force's mission to augment human performance and protection, and to the Air Force Biophysics Program's mission to better understand bioelectricity and electromagnetic stimulation below the diffraction limit. Additionally, membrane proteins' responsive structures suggest design and operation principles which can address the emerging needs of the Air Force for modernized, adaptive, and multi-functional weapons and materials. Current state-of-the art biophysics tools, however, are unable to directly observe the nanoscale structural dynamics of membrane proteins, as they have to compromise between high-resolution imaging and preserving the liquid environment in which membrane protein dynamics occur. Our goal is to bridge this gap with a novel implementation of liquid-phase transmission electron microscopy, which utilizes an electron-transparent "nano-aquarium," composed of atomically thin graphene or silicon nitride windows, to observe individual membrane proteins in real time and at unmatched resolution. Liquid-phase transmission electron microscopy of membrane proteins is an uncharted field, and developing it to its full potential requires the budgeted instrumentation we have acquired by the DURIP grant. The contributing instrumentation to the platform falls into three categories: (i) equipment for membrane protein sample preparation and purification (including a powerful centrifuge and an advanced spectrophotometer), (ii) computational workstations devoted to extracting high-resolution structural and dynamics information from electron microscopy movies, and (iii) electron microscopy equipment required to apply and quantify *in situ* the effects of stimuli, including temperature and electrical stimulation, on membrane protein dynamics. For this membrane protein project, we have four peer-reviewed journal papers published acknowledging the AFOSR-YIP grant, two of which also acknowledging the DURIP grants because the new capabilities have been crucial to these two papers. In addition, we have submitted 1 peer-reviewed journal paper (acknowledging both the AFOSR-YIP and the DURIP grants) and 1 more peer-reviewed journal papers to be submitted supported by both grants. The acquired instrumentation enables the characterization of the structural response of membrane proteins to electrical signals with nanometer detail and in their native functional environment, lending new insight into bioelectricity at the single molecule level. We expect these tools to open new opportunities for membrane protein studies, new frontiers in probing nanoscale biophysics in a liquid environment, and opportunities for transformational progress in biomimetic materials design.

Final Report for Project FA9550-18-1-0393

Title: Acquisition of a Nanoscopic Imaging and Analysis Platform for Understanding the "Mission Versatility" of Membrane Proteins

Program officer:

Dr. Sofi Bin-Salamon, Biophysics Program, Air Force Office of Scientific Research

Principal Investigator: Qian (Alice) Chen, University of Illinois at Urbana-Champaign

1. Overview of the Project Goals:

This DURIP grant provides financial support for the purchase of a nanoscopic imaging and analysis platform that are used in the execution of the PI's AFOSR YIP project. This YIP project is a highly interdisciplinary venture, with an overarching goal of developing a new nanoscopic bioimaging technique, but developing this novel tool to its full potential requires some equipment to which the PI did not have direct access, which we have now established through the DURIP grant. Specifically, the scientific aim of this YIP project is to resolve and understand the fundamental structuring and working mechanisms of membrane proteins at the nanoscale, in various biological states and in an ever-changing surrounding liquid media. The computational and experimental toolkits that biophysicists have developed, although successful for bulk soluble proteins, all face one key challenge: membrane proteins function in the presence of lipid membranes and dynamic membrane potentials. The PI's approach thus has been to develop and implement a new biophysics imaging tool which is compatible with both the native, dynamic liquid environment of membrane proteins and the near-atomic resolution imaging capabilities of transmission electron microscopy (TEM).

This essential combination of capabilities is missing from the experimental techniques currently available to biophysicists, since current state-of-the-art methods must compromise between high-resolution imaging and preserving the native liquid environment in which membrane protein dynamics occur. Consequently, such a tool would greatly advance: (i) the fundamental science of membrane proteins, promising new insights into their function, (ii) their configurational dynamics, their response to stimuli at the single-molecule level, and (iii) even the roles that these phenomena play in myriad higher-order processes, from cell motility to electrophysiology. These processes are central to virtually all physiological phenomena relevant to the Air Force's mission to augment human performance and protection.

At the heart of our innovation is the utilization of low-dose liquid-phase TEM, which can be used to image protein dynamics with nanometer and millisecond resolution concurrently with *in situ* control of the surrounding liquid environment. In liquid-phase TEM, samples in solution can be kept between thin layers of graphene or in a SiN_x-windowed chamber and sealed against vacuum, which allows them to stay in native liquid environment during imaging. Changes of the liquid environment can be facilitated in the SiN_x-windowed chamber configuration by flowing other solutions and applying electric field. This technique recently has shown great impact in materials sciences by revealing inorganic nanoparticle growth and dynamics and discovering the mechanisms at the nanoscale. We foresee expansion and applicability of its power to answer long-standing questions in biophysics. By combining these capabilities into a single imaging modality with the power to image the dynamics of individual membrane proteins in a tunable environment, our work will provide a powerful, generalizable tool for biophysical investigations and, in the long-

run, bioinspired complex materials design. The equipment we acquired via this grant enables us to develop and implement this cutting-edge methodology to its full potential, with the capability to quantify the dynamics of membrane proteins in response to diverse, physiologically relevant stimuli. The principles of basic membrane protein science learned from this work will help address the emerging needs of the Air Force for bio-inspired, adaptive, multi-functional weapons and materials.

2. Specific Objectives

The specific objectives of this project and the corresponding instrumentation associated with these objectives are detailed in the following sections. The current platform is shown in Figure 1.

Objective 1: Resolve the three-dimensional (3D) structure of a membrane protein nanodisc in a liquid environment using liquid-phase TEM.

Relevant Instrumentation Acquired and Established: Fulfilling this objective requires setting up a complete nanoscopic imaging and analysis platform based on three types of equipment that were not available in the PI's lab but we have now acquired, with an **estimated lifetime of 20 years**: (i) equipment for the purification of membrane proteins and their insertion into nanodiscs, (ii) computing hardware, which is necessary to extract high-resolution 3D structures from many different 2D projections of a nanodisc tumbling about in a liquid-phase TEM graphene cell and (iii) a liquid-phase TEM temperature control module to maintain membrane protein nanodisc specimens at the proper temperatures during imaging. The specific sample preparation instrumentation includes a high-power centrifuge with temperature control, which is necessary in many steps in the extraction of membrane proteins such as membrane protein cytochrome p450 2J2 (CYP-2J2) from their native tissues, and a high-power, high-throughput spectrophotometer, which is involved in such steps as verifying successful purification of a membrane protein of interest, measuring the concentration of membrane proteins in solution (carefully controlled protein—lipid—scaffold stoichiometries are critical for successful production of nanodiscs), and other spectroscopic assays. The specific hardware we acquired for this objective includes a multi-core precision workstation devoted to advanced TEM movie analysis and the extraction of dynamics data, and a graphics processing-optimized system exclusively for performing high-resolution 3D structure calculations. These two types of computing power fulfill distinct but critical roles in extracting quantitative information from large liquid-phase TEM datasets, which can contain several millions of images each. The heating module and potentiostat will interface directly with the existing liquid-phase TEM holder to enable concurrent imaging and stimulation.

Objective 2: Image dynamic structural transformations due to chemical signaling

Relevant Instrumentation Acquired and Established: Completing this objective requires some of the instrumentation detailed in **Objective 1**, including an ultracentrifuge and spectroscopy equipment for the extraction and purification of membrane proteins which we have now fully established. Additional equipment allowing us to probe other stimuli to which membrane protein structural dynamics respond include: (i) the liquid-phase TEM temperature control module to quantify how membrane protein configurations and dynamics are sensitive to temperatures above or below physiological temperatures and (ii) a Clark electrode, which monitors the concentration of oxygen in solution, to examine how oxygen-sensitive membrane proteins modulate their activity and conformation. Collectively, these tools make liquid-phase TEM a highly versatile technique for studying membrane proteins, capable of probing physiologically relevant dynamics in a variety of systems.

Objective 3: *In situ* electrical stimulation of membrane proteins

Relevant Instrumentation Acquired and Established:

Just as in **Objective 2**, fulfilling **Objective 3** requires the same equipment to purify and prepare membrane protein samples, as well as the liquid-phase TEM temperature control holder to maintain the relevant temperature conditions during imaging. We already have access to a state-of-the-art electrical stimulation chamber, but utilizing it to its full potential requires a potentiostat which we have now acquired and established. A potentiostat is a device used to perform electrochemical measurements in the liquid chamber, and allows us to quantitatively characterize the voltage response of individual membrane proteins in the form of I-V (current-voltage) curves. By imaging membrane proteins at the same time, we are able to map specific opening and closing transformation events to specific electrochemical conditions.

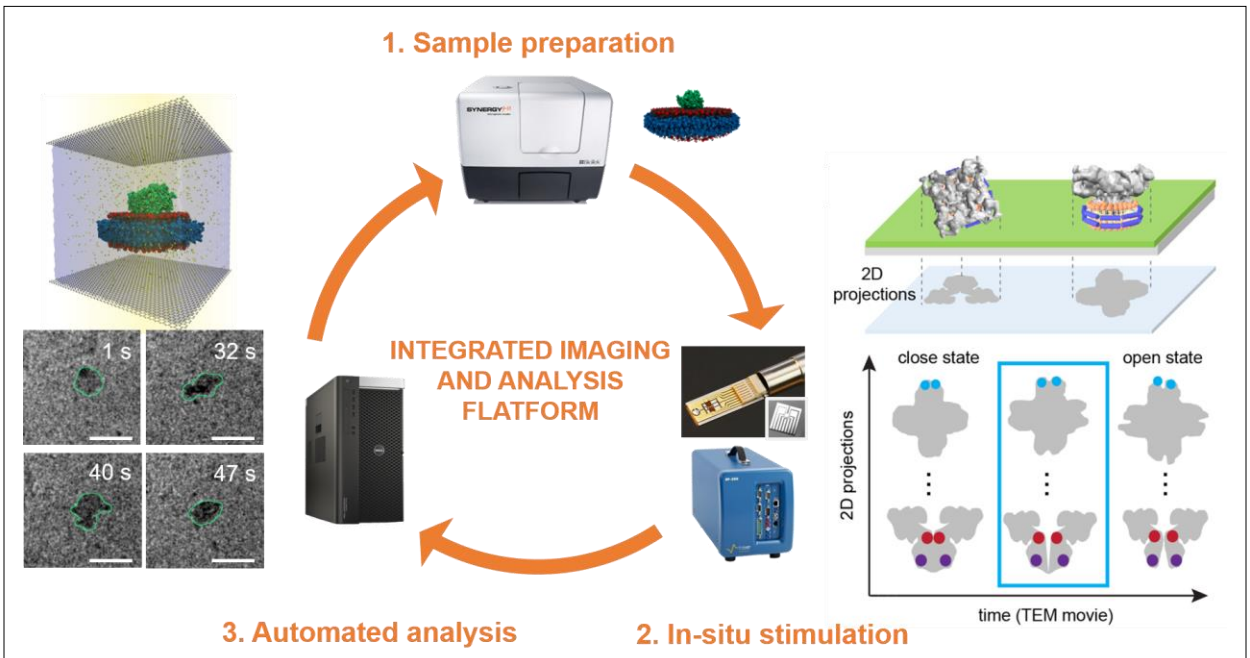

Fig. 1. Schematics of the established integrated imaging and analysis platform. 1: Instrument for sample preparation. A plate reader (middle) is shown with CYP-2J2-nanodiscs. 2: *In situ* stimulation. A liquid holder with the heating module and a potentiostat are shown on the left. The top panel shows two nanodiscs attached on the SiN_x chip and corresponding 2D projected shape from TEM imaging, and the bottom panel is 2D projection-time curve with predicted 2D projected images showing transition from close state to open state during the imaging. Colored dots are tracked positions, implying kinetic pathways. Highlighted in blue shows an intermediate state. 3: Automated analysis via workstation. Our data on the real-time fluctuation dynamics of nanodiscs analyzed using the analysis platform (published and to be submitted, Chen group). Scale bars: 20 nm

3. Relevant Public Presentations of the Supported Work

3.1. Peer-reviewed journal publications.

The following manuscripts have been published or have been accepted in peer-reviewed journals:

1. Smith, J. W.*; Song, X.*; Kim, J.; Zaluzec, N. J.; Chen, W.; An, H.; Dennison, J. M.; Cahill, D. G.; Kulzick, M. A.; Chen, Q. Unraveling the Morphology–Function Relationships of Polyamide Membranes Using Quantitative Electron Tomography. *ACS Appl. Mater. Interfaces* **2019**, *11*, 8517–8526. [Acknowledging the AFOSR-YIP grant]
2. Smith, J. W.; Carnevale, L. N.; Das, A.; Chen, Q. Real-time Electron Microscopy of Protein Nanodiscs using Graphene Liquid Cells. *Microsc. Microanal.* **2019** *25 (S2)*, 1498–1499. [Acknowledging the AFOSR-YIP grant]
3. J. W. Smith, X. Jiang, H. An, A. M. Barclay, G. Licari, E. Tajkhorshid, E. G. Moore, C. M. Rienstra, J. S. Moore, and Q. Chen, Polymer−Peptide Conjugates Convert Amyloid into Protein Nanobundles through Fragmentation and Lateral Association. *ACS Appl. Nano Mater*. **2019**, in press; DOI: 10.1021/acsanm.9b01331. [Acknowledging both the AFOSR-YIP grant and the DURIP grant.]
4. X. Jiang, A. Halmes, G. Licari, J. W. Smith, Y. Song, E. G. Moore, Q. Chen, E. Tajkhorshid, C. M. Rienstra, and J. S. Moore Multivalent Polymer–Peptide Conjugates—A General Platform for Inhibiting Amyloid Beta Peptide Aggregation. *ACS Macro Lett*. **2019**, accepted. [Acknowledging both the AFOSR-YIP grant and the DURIP grant.]

The following manuscript has been submitted:

5. An, H.; Smith, J. W.; Chen, W.; Ou, Z.; Chen, Q. Charting the Quantitative Relationship between Two-Dimensional Morphology Parameters of Polyamide Membranes and Molecular Reaction Conditions. *Mol. Syst. Des. Eng.* **2019**. Submitted. [Acknowledging both the AFOSR-YIP grant and the DURIP grant.]

The following manuscript is under preparation.

1. Smith, J. W.; Carnevale, L. N.; Das, A.; Chen, Q. Fluctuations and Fingering Dynamics of Individual Protein Nanodiscs Imaged by Graphene Liquid Cell Electron Microscopy. 2019. To be submitted. [Acknowledging both the AFOSR-YIP grant and the DURIP grant.]

3.2. Public presentations of the work.

This work has also been presented at the following conferences (since July 2018).

For the PhD student John W. Smith:

1. John W. Smith, Lauren N. Carnevale, Aditi Das, and Qian Chen, "Real-time Electron Microscopy of Protein Nanodiscs using Graphene Liquid Cells," Microscopy & Microanalysis 2019 Meeting, Portland, Oregon, August 4–8, 2019.
2. John W. Smith "Capturing the Dynamics of Proteins on Film Using 'Nanoaquariums' and Electron Microscopy," Beckman Institute Graduate Student Seminar, University of Illinois at Urbana-Champaign, Urbana, Illinois, October 2, 2019.

The PI has given or scheduled **22** invited talks since the initiation of the DURIP grant in national conferences (Gordon Research Conferences, MRS, ACS, APS) and universities (MIT, Harvard, etc.) based on the work supported by this grant:

1. Invited Talk, Session on "Advances in Colloidal Crystal Engineering", **2020 Pacifichem Conference**, Honolulu, HI (Dec 2020).
2. Invited Talk, Symposium on "NM02: Colloidal Nanoparticles—From Synthesis to

Applications", **MRS Spring Meeting 2020**, Phoenix, AZ (Apr 2020).

3. Invited Talk, Symposium on "CT08 – Crystallization via Nonclassical Pathways in Synthetic, Biogenic and Geologic Environments", **MRS Spring Meeting 2020**, Phoenix, AZ (Apr 2020).
4. Invited Talk, Department of Chemistry, University of Chicago, Chicago, IL (Mar 20, 2020).
5. Invited Talk, Department of Chemistry, Penn State University, State College, PA (Feb 26, 2020).
6. Invited Talk, **Liquid Phase Electron Microscopy Gordon Research Conference**, Lucca, Italy (Jan 2020).
7. Invited Talk, Symposium on "Building Advanced Materials via Particle-Based Crystallization and Self-assembly of Molecules with Aggregation-Induced Emission," **MRS Fall Meeting 2019**, Boston, MA (Dec 2019).
8. Invited Talk, Department of Chemical Engineering, University of Michigan, Ann Arbor, MI (Nov 5, 2019).
9. Plenary Talk, Area 1C "Interfacial Phenomena," **AIChE Annual Meeting**, Orlando, FL (Nov 2019).
10. Invited Talk, PPG Seminar, PPG Industries, Pittsburgh, PA (Sep 24, 2019).
11. Invited Talk, Symposium on "P01 – In situ TEM Characterization of Dynamic Processes During Materials Synthesis and Processing," **Microscopy & Microanalysis 2019 Meeting**, Portland, OR (Aug 6, 2019).
12. Invited Talk, Plenary Lecture on "Nano Assembly 2040", Shanghai, China (Aug 3, 2019)
13. Invited Talk, Track C: "Colloidal & Surface Interactions", 93rd ACS Colloid & Surface Science Symposium, Atlanta, GA (Jun 18, 2019).
14. Invited Talk, Colloid & Interface Symposium, Hong Kong SAR, China (Jun 13, 2019)
15. Invited Talk, Dow Discussion Group on Interface Science, Dow Chemical Company, Midland, MI (May 13, 2019)
16. Invited Talk, Symposium on "QN08 – Colloidal Nanoparticles—From Synthesis to Applications," **MRS Spring Meeting 2019**, Phoenix, AZ (Apr 9, 2019).
17. Invited Talk, GSOFT Short Course on "Structures and Order in Soft Matter Physics," **2019 APS March Meeting**, Denver, CO (Mar 3, 2019).
18. Invited Talk, Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA (Mar 13, 2019).
19. Invited Talk, Topics in Bioengineering Seminar, School of Engineering and Applied Science, Harvard University, Cambridge, MA (Feb 7, 2019).
20. Invited Talk, International Centre for Advanced Materials in BP Incorporation (Dec 7, 2018).
21. Invited Talk, Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX (Oct 12, 2018).
22. Invited Talk, Symposium on "Advances in colloid & surface chemistry enabled by cryogenic and in situ liquid-cell electron microscopy," **2018 ACS national meetin**g, Boston, MA (Aug 19, 2018).

3.3. Awards and honors. The PhD student John W. Smith on the AFOSR YIP project and utilized the instrumentation provided by the DURIP grant has been awarded with a 3M graduate fellowship with ***$30,000*** cash award (2018).