AFRL-AFOSR-VA-TR-2022-0056

Understanding the 'Mission Versatility' of Membrane Proteins via Nanoscopic Imaging

Chen, Qian
UNIVERSITY OF ILLINOIS
352 HENRY ADMINISTRATION BLDG
URBANA, IL, 61801
USA

09/30/2021
Final Technical Report

Air Force Research Laboratory
Air Force Office of Scientific Research
Arlington, Virginia 22203
Air Force Materiel Command

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
30-09-2021	Final	15 May 2017 - 14 May 2021

4. TITLE AND SUBTITLE
Understanding the 'Mission Versatility' of Membrane Proteins via Nanoscopic Imaging

5a. CONTRACT NUMBER

5b. GRANT NUMBER
FA9550-17-1-0296

5c. PROGRAM ELEMENT NUMBER
61102F

6. AUTHOR(S)
Qian Chen

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF ILLINOIS
352 HENRY ADMINISTRATION BLDG
URBANA, IL 61801
USA

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AF Office of Scientific Research
875 N. Randolph St. Room 3112
Arlington, VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL/AFOSR RTB2

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-AFOSR-VA-TR-2022-0056

12. DISTRIBUTION/AVAILABILITY STATEMENT
A Distribution Unlimited: PB Public Release

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of this AFOSR Young Investigator Program grant is to investigate the nanoscale structural and functional dynamics of membrane proteins in their native liquid environment using liquid phase
transmission electron microscopy (TEM). Over the project time period, we optimized the self-assembly and purification of nanodiscs—membrane proteins in a “single-particle” form amenable to TEM
observation—containing the protein cytochrome p450 2J2 (CYP-2J2) and then developed a graphene liquid sandwich to encapsulate CYP- 2J2 nanodiscs in their native buffer conditions for imaging.

Using the samples, we optimized our encapsulation and imaging protocols to enhance contrast, resolution, and stability for nanodisc imaging and prepared several more nanodisc structures, with variable size and shape, and were able to probe their diffusion, rotation, structural fluctuation, and other dynamics with direct, nanometer resolution imaging. This served as key validation of the potential of liquid-phase TEM to interrogate diverse, nanoscale biomolecular dynamics, and our continued development of new video analysis and morphometry tools under this project has enabled us to quantify these dynamics with unprecedented detail. Lastly, besides amassing more data on the dynamics of diverse membrane protein nanodiscs, we have expanded our experimental and analytical toolbox still further in an effort to provide greater insight and reveal quantities with true biophysical significance. In particular, incorporating elements of molecular dynamics simulation, electron microscopy theory, and biophysical theory now allow us to (a) infer much greater structural detail from otherwise low signal-to-noise movies, (b) quantitatively understand the molecular mechanics and lipid–protein interactions involved in nanodisc fluctuations, and (c) push to understanding membrane protein functions in more complex chemical and physical environments. In parallel, we have developed and incorporated other imaging modules such as electron tomography to account for 3D nanomorphology, nanobeam electron diffraction to delineate local molecular structure, and spectroscopy to map composition, all at nanometer resolution, which are demonstrated using membrane protein nanodiscs and also systems beyond, such as the model system
of polyamide films, microtubules, and amyloid beta fibrils. In summary, we have achieved significant progress in each of our proposed efforts: protein sample preparation, liquid-phase TEM imaging, and quantitative video and biophysical analysis to establish liquid-phase TEM as a new and powerful biophysics imaging tool.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			SOFI BIN-SALAMON
U	U	U	UU	7	19b. TELEPHONE NUMBER *(Include area code)* 426-8411

Standard Form 298 (Rev.8/98)
Prescribed by ANSI Std. Z39.18

Abstract

The goal of this AFOSR Young Investigator Program grant is to investigate the nanoscale structural and functional dynamics of membrane proteins in their native liquid environment using liquid-phase transmission electron microscopy (TEM). Over the project time period, we optimized the self-assembly and purification of nanodiscs—membrane proteins in a "single-particle" form amenable to TEM observation—containing the protein cytochrome p450 2J2 (CYP-2J2) and then developed a graphene liquid sandwich to encapsulate CYP-2J2 nanodiscs in their native buffer conditions for imaging. Using the samples, we optimized our encapsulation and imaging protocols to enhance contrast, resolution, and stability for nanodisc imaging and prepared several more nanodisc structures, with variable size and shape, and were able to probe their diffusion, rotation, structural fluctuation, and other dynamics with direct, nanometer resolution imaging. This served as key validation of the potential of liquid-phase TEM to interrogate diverse, nanoscale biomolecular dynamics, and our continued development of new video analysis and morphometry tools under this project has enabled us to quantify these dynamics with unprecedented detail. Lastly, besides amassing more data on the dynamics of diverse membrane protein nanodiscs, we have expanded our experimental and analytical toolbox still further in an effort to provide greater insight and reveal quantities with true biophysical significance. In particular, incorporating elements of molecular dynamics simulation, electron microscopy theory, and biophysical theory now allow us to (a) infer much greater structural detail from otherwise low signal-to-noise movies, (b) quantitatively understand the molecular mechanics and lipid–protein interactions involved in nanodisc fluctuations, and (c) push to understanding membrane protein functions in more complex chemical and physical environments. In parallel, we have developed and incorporated other imaging modules such as electron tomography to account for 3D nanomorphology, nanobeam electron diffraction to delineate local molecular structure, and spectroscopy to map composition, all at nanometer resolution, which are demonstrated using membrane protein nanodiscs and also systems beyond, such as the model system of polyamide films, microtubules, and amyloid beta fibrils. In summary, we have achieved significant progress in each of our proposed efforts: protein sample preparation, liquid-phase TEM imaging, and quantitative video and biophysical analysis to establish liquid-phase TEM as a new and powerful biophysics imaging tool.

Final Performance Report (2017–2021) for Project FA9550-17-1-0296

Project Title: Understanding the "Mission Versatility" of Membrane Proteins via Nanoscopic Imaging (2017 Air Force Young Investigator Award)

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 Proposed Project Goals and Objectives:

The overarching goal of this project is to develop and implement imaging tools to investigate the nanoscale structural and conformational dynamics of biomolecules, in particular using electron microscopy (EM). While existing EM techniques, particularly cryo-EM, have brought atomic or near-atomic resolution insight to structural biology, they generally require immobilization of the specimen to be compatible with the vacuum of an electron microscope. As a consequence, structural dynamics—which play a critical role in any biomolecule's function—cannot be interrogated with the same resolution using the same tools. To overcome this limitation, this project aims to harness recent developments in *liquid-phase* EM, which uses vacuum-tight, electron-transparent "nanoaquariums" to preserve solutions within an electron microscope, as a tool for nanoscale biophysics. Given their size and the importance of their conformational dynamics in bioelectricity, human performance and protection, signal transduction, etc., our focus is on membrane proteins. These nanoscale functionalities of membrane proteins may also suggest design and operation principles which can address the emerging needs of Air Force for modernized, adaptive, and multi-functional weapons and materials. Specifically, the objectives of this work include: (i) resolving the nanoscale structure of membrane proteins in their native liquid environment; (ii) imaging membrane protein conformational dynamics upon introduction of chemical stimuli; and (iii) capturing movies of the nanoscale morphology dynamics of ion channel membrane proteins in response to electric field stimulation.

2. Major Achievements and Technical Deliverables

2.1. Accomplished systematically membrane protein sample preparation, nanodiscs for cytochrome p450 reductase with different membrane scaffold proteins (MSPs)

The implementation of liquid phase TEM as a biophysical imaging tool is new and unprecedented, so we have first optimized the sample preparation protocols for a diversity of nanodiscs for comparative studies. The making of nanodiscs is a technology developed at UIUC first by Prof. Stephen Sligar, which include three components—the membrane protein in the middle, wrapped by rafts of lipid bilayers much like how the proteins sit through a cellular membrane, and then with membrane scaffold protein (MSP) to circle the lipid bilayer. This nanodisc architecture can ensure membrane proteins are stable, staying in native lipid

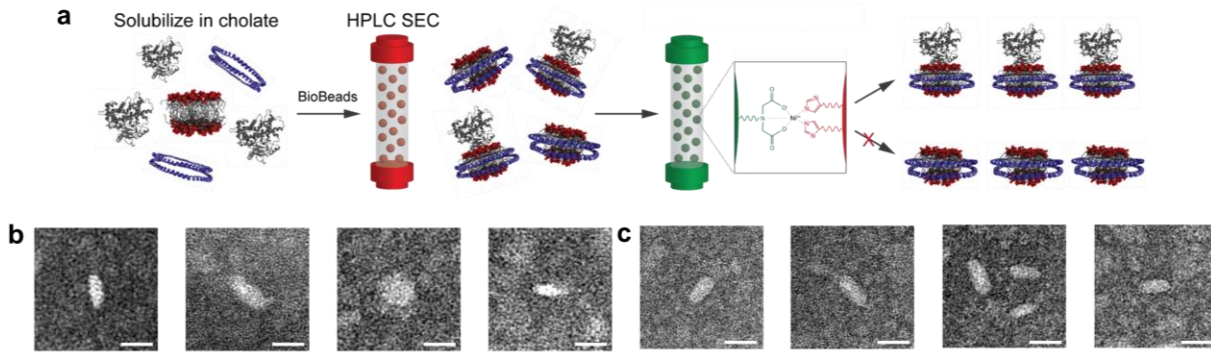

Figure 1: (a) Schematics and TEM image of CYP2J2 nanodiscs. (b, c) Negative-stain electron microscopy of MSP 2N2–CYP2J2 nanodiscs ("large, filled") (b) and MSP 1E3D1–CYP2J2 nanodiscs ("small filled") from the same preparations used for liquid-phase TEM characterization. Scale bar: 20 nm

environment, and easily processible in the form of a particle dispersion. In particular, we focus on the membrane protein cytochrome p450 reductase (CYP2J2) to develop sample preparation protocols. CYP2J2 is a cardiac cytochrome P450 involved in arachidonic acid metabolism and a representative peripheral membrane protein. First, we express and purify recombinant CYP2J2 (about 58 kDa) with functional tags facilitating later purification. Next, we disperse them with lipids and MPS, in which they spontaneously assemble into either filled or empty nanodiscs due to hydrophobic attraction as shown in **Fig. 1a**. Finally, we use several liquid chromatography columns to purify and collect different portions of nanodiscs for imaging. In addition to liquid chromatography spectra which confirm the existence of the nanodiscs, we performed TEM imaging, with the solution negatively stained (**Fig. 1b**). The whitish portion in the TEM image corresponds to standing nanodiscs, with sizes consistent with the periphery enclosed by MSP.

This method allows for high tunability and generalizability. First, multiple copies of membrane proteins can be wrapped up within a single nanodisc, which is controlled through stoichiometry of the nanodisc components. For example, given the same MSP concentration, fewer lipids and more membrane protein can wrap up more copies of membrane protein inside. Second, the diameter of the nanodisc is determined by the length of MSP as it is the periphery of the circle. In order to simultaneously validate and expand our experimental observations, we prepared nanodiscs that have different shapes and sizes. To do so, we first expressed MSPs that have different sizes, which lead to nanodiscs that have larger or smaller diameters. In particular, in our initial experiments, we used one of the more conventional MSPs, known as MSP1E3D1, which produces nanodiscs ~13 nm in diameter. To make larger nanodiscs, we expressed and purified MSP2N2, which can be used to make nanodiscs that have a larger diameter. We proceeded to prepare nanodiscs with MSP1E3D1 or MSP2N2, and with or without the insertion of CYP-2J2 (**Fig. 1b,c**), hypothesizing that changes in the MSP or the protein content would affect not only the size of the nanodiscs, but also the structural fluctuations we observed using liquid-phase TEM.

2.2. Optimized a customized graphene sandwiching method for low-dose TEM imaging of the nanodisc fluctuation

To image the prepared nanodisc solution using liquid-phase TEM, we customized a special graphene sandwiching method that uses a combination of one flat graphene sheet and one sheet that is free to fold or deform. The free-standing graphene is flexible (**Fig. 2**), and can readily encapsulate a large liquid droplet, whereas traditional graphene liquid cell configuration was unsuccessful in yielding adequate liquid chambers, even after considerable effort. Based on this method, we obtained the first liquid-phase TEM movie of a nanodisc preserved in a liquid environment (its native buffer, 0.1 M potassium phosphate), without staining. With these improvements, we can now achieve highly reproducible liquid pockets which are larger in scale, have much cleaner backgrounds, and therefore provide significantly improved imaging contrast and resolution. We have found that the "channels" of liquid that are produced are also much more stable, and thus allow for longer movies (and therefore more dynamics) to be acquired.

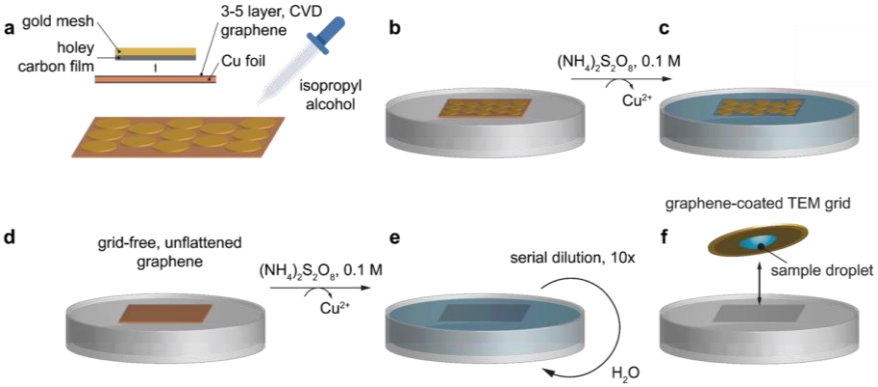

Figure 2: (a) Gold-supported TEM grids were adhered CVD graphene on copper foil. (b–c) Grids adhered to the copper foil were etched and then rinsed in water baths before use. (d–e) CVD graphene without grids was etched on 0.1 M ammonium persulfate. (f) Graphene coated grids from (c) were loaded with ~0.1 µL nanodisc suspension and inverted onto the rinsed, freely floating graphene.

2.3. Dynamic liquid-phase electron microscopy imaging and inferring of molecular coordinates of membrane protein nanodiscs.

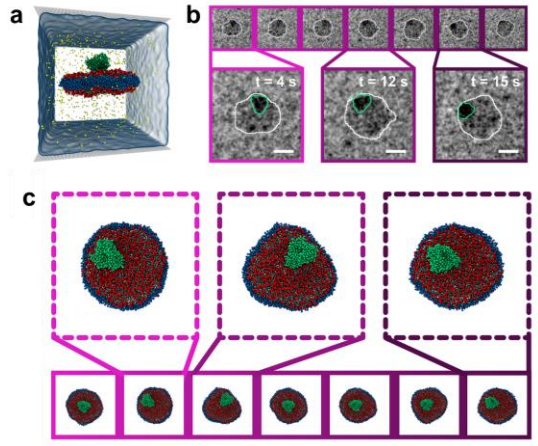

Figure 3. (a) Schematics of a nanodisc in a graphene liquid cell. (b) Continuous shape fluctuations of individual nanodiscs were captured for the first time. In some cases, interior contrast features allowed for interpretation of the molecular arrangement in great detail. (c) Synthetic liquid-phase TEM images based on molecular dynamics simulation. Scale bars: 10 nm.

Using the special graphene sandwiching method noted above, we obtained a series of first liquid-phase TEM movies of nanodiscs preserved in a liquid environment, without staining (**Fig. 3a–b**). From these movies we successfully tracked fluctuating outlines, and measured features like the local curvature of the boundary through time. By optimizing imaging conditions such as dose rate and the level of defocus, we endeavored to make the most of the experimentally achievable contrast. In particular, we developed an approach to generate hypotheses for distinct atomic arrangements corresponding to intensity distributions in low signal-to-noise TEM snapshots. This methodology uses a combination of molecular dynamics simulations to generate a large library of nanodisc structures "expected" at equilibrium, and these coordinates are then combined with electron microscopy theory to predict corresponding "expected" images (**Fig. 3c**). Our eventual aim is to match these hypothetical images with experimental ones in a feedback loop, to match contrast variation with molecular structure variation. We have also used this framework to validate the intensity distributions observed experimentally and show that, for example, the position of CYP2J2 in contrast-rich snapshots can be precisely determined.

2.4. Extracted biophysically meaningful quantities from nanoscopic, single-nanodisc fluctuations to understand lipid–protein interactions

We sought to use some of the quantitative biophysical models from this field of liquid vesicle dynamics to understand the molecular-scale processes involved in nanodisc fluctuations. For example, using our contour analysis within the framework of Helfrich's theory for the mechanics of lipid vesicles, we were able to measure the effective tension and bending rigidity of the membrane scaffold proteins at the nanodisc periphery (**Fig. 4**). Contour undulations were also used to understand the types of mass transport involved in nanodisc shape transformations, which is directly related to the fluid-like diffusion of lipid molecules encircled by the scaffold proteins. The power of these analytical tools lies in the variable space that is now at hand. Through systematic variation of factors like the lipid composition, we can move forward to understand how changes in lipid–protein interactions, as well as lipid phase behavior, manifest in distinct fluctuation properties. In some cases our real-time monitoring of nanodisc dynamics revealed extensive structural rearrangements not predicted by molecular dynamics simulations. While the origin of these drastic

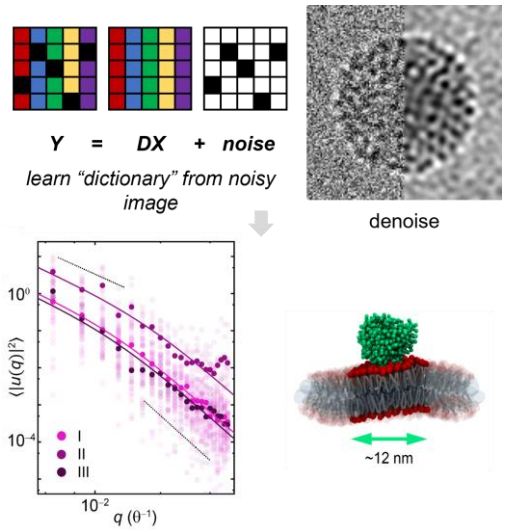

Figure 4. (Top) Machine learning based denoising to analyze nanodisc fluctuations. (Bottom) The spectrum analysis of the fluctuations to elucidate a long-range lipid–protein interaction.

fluctuations is still under investigation, we developed various analysis tools, based on both experiment and simulation, to understand the role of lipid–protein interactions involved in such events. Our results show that such lipid–protein interactions can define domains which have an altered local structure, with tighter lipid packing, lower membrane thickness, and perhaps other modified properties. These structural effects come together to stabilize nanodiscs against the observed extensive fluctuations and promote "recovery." In contrast, nanodiscs that don't have membrane proteins are not stabilized, and only disintegrate when they undergo extensive stretching. We see future experiments with different lipid compositions, and therefore different lipid–protein interactions, incorporated with machine learning based analysis methods will shed greater light on and lend broader scientific relevance to these stabilization effects.

2.5. Developed electron tomography and cellular morphometry analysis for 3D structures

We have customized low-dose electron tomography to our model system of polyamide membranes, which present an easier-to-handle model than proteins, yet include all the features of proteins, such as being non-crystalline, irregular, spatially heterogeneous, and with functionally relevant interior features (**Fig. 5a–b**). To minimize the beam damage, the tilt TEM image series are taken at a low dose rate of 7.4 e^{-} $Å^{-2}$ s^{-1}. Successful reconstruction is realized at a voxel resolution of 6.8 Å, which reveals nanoscopic features that are not available by other means, such as surface area and interconnectivity of single crumples. Quantitative analysis of local surface curvature groups the seemingly unrelated crumples into three distinct classes, which influence the performance of the membrane by changing the affinity and diffusion length of ions inside the membranes. This imaging and analysis as we apply at the next stage to protein molecules can complement the projection-based analysis of protein imaging achieved in liquid-phase TEM with 3D information.

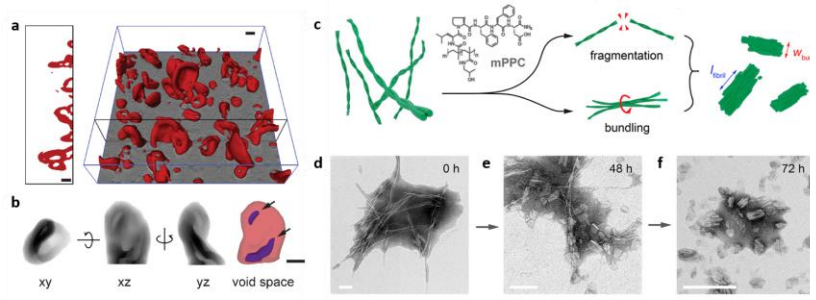

Figure 5. (a) 2D slice and 3D view of the polyamide membrane after reconstruction. (b) Individual crumples segmented from the 3D dataset resolving various projections and interior void (purple). Scale bars: 50 nm. (c–f) Overview of mPPC-induced $A\beta_{40}$ disassembly. (c) Schematic representation of the joint effects of fragmentation and bundling, induced by the introduction of mPPCs, to produce bundles of amyloid segments. (d–f) Representative time-lapse TEM images of disassembly 0, 48, and 72 h after the addition of mPPC. Scale bars: 50 nm (a–b); 200 nm (d–f).

2.6. Map the diassembly kinetics and pathways of $A\beta_{40}$ amyloid fibrils governed by multi-protein interactions at the nanoscale

Besides the single protein level fluctuation dynamics we focused on in the system of nanodiscs, we extended to time-course TEM studies of multi-protein interactions, particularly the polymer assisted disassembly pathways of $A\beta_{40}$ amyloid fibrils (**Fig. 5c–f**) which are associated with neurodegenerative diseases. We utilize electron microscopy-based approaches combined with quantitative statistical analysis to show how a recently developed class of amyloid modulators—multivalent polymer–peptide conjugates (mPPCs)—can be applied to control the structure and stability of amyloid fibrils. As shown in **Fig. 5d–f**, mPPCs are able to convert $A\beta_{40}$ fibrils into ordered nanostructures through a combination of fragmentation and bundling. Through statistical analysis, we show that fragmentation is consistent with a model where the rate constant of fragmentation is independent of the fibril length, suggesting a local and specific interaction between fibrils and mPPCs. Subsequent bundling, which was previously not observed, leads to the formation of sheetlike nanostructures that are surprisingly much more uniform than the original fibrils. This effort reveals a quantitative and nanoscopic understanding of how mPPCs can be applied to control the

structure and stability of amyloid. The approach of time-course TEM images and kinetic analysis elucidates the potential to study other multi-protein interaction involved kinetic processes, especially in the presence of functional macromolecules and other modulators.

2.7. Extension of liquid-phase TEM imaging to new biomolecular dynamics

We have also begun to pursue the use of liquid-phase TEM capabilities that we have realized in this project to investigate other kinds of biomolecular dynamics, such as the conformational transitions of motor proteins as they move along microtubules or actin and drive intracellular transport.

3. Relevant Public Presentations of the Supported Work

3.1. Peer-reviewed journal publications

Based on the achievements described above, the following manuscripts have been published 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.
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.
3. Jiang, X.; Halmes, A. J.; Licari, G.; Smith, J. W.; Song, Y.; Moore, E. G.; Chen, Q.; Tajkhorshid, E.; Rienstra, C. M.; Moore, J. S. Multivalent Polymer–Peptide Conjugates: A General Platform for Inhibiting Amyloid Beta Peptide Aggregation. *ACS Macro Lett.* **2019**, *8*, 1365–1371.
4. Smith, J. W.; Jiang, X.; An, H.; Barclay, A. M.; Licari, G.; Tajkhorshid, E.; Moore, E. G.; Rienstra, C. M.; Moore, J. S.; Chen, Q. Polymer−Peptide Conjugates Convert Amyloid into Protein Nanobundles through Fragmentation and Lateral Association. *ACS Appl. Nano Mater.* **2020**, *3*, 937–945.
    - Selected as the front cover of the issue.
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 Synthesis Conditions. *Mol. Syst. Des. Eng.* **2020**, *5*, 102–109.
    - Selected as the front cover of the issue.
    - Selected as a HOT MSDE article by the editor.
6. Smith, J. W.; Chen, Q. Liquid-phase Electron Microscopy Imaging of Cellular and Biomolecular Systems. *J. Mater. Chem. B* **2020**, 8, 8490–8506.

3.2. Public presentations of the work

Work supported by this grant has been presented in the following conferences:

1. J. W. Smith, “Capturing Dynamics of Proteins on Film Using ‘Nanoaquariums’ and Electron Microscopy,” Center for the Physics of Living Cells and Center for Bioinformatics and Quantitative Biology Graduate Student Seminar, Urbana, IL, November 15, 2019
2. H. An, J. W. Smith, W. Chen, N. J. Zaluzec, M. A. Kulzick, and Q. Chen “3D Analysis of Nanostructured Polyamide Membranes Using Quantitative Electron Tomography,” American Institute of Chemical Engineers Annual Meeting, November 13, 2019
3. J. W. Smith, “Capturing Dynamics of Proteins on Film Using ‘Nanoaquariums’ and Electron Microscopy,” Beckman Institute for Advanced Science and Technology Graduate Student Seminar, Urbana, IL, October 8, 2019

4. J. W. Smith, L. N. Carnevale, A. Das, and Q. Chen, "Real-time Electron Microscopy of Protein Nanodiscs using Graphene Liquid Cells," Microscopy & Microanalysis 2019 Meeting, Portland, Oregon, August 4–8, 2019
5. J. W. Smith, S. Shukla, P. R. Selvin, and Q. Chen, "Imaging Nanoscale Protein Dynamics with Liquid-phase Electron Microscopy" (poster) Center for the Physics of Living Cells, University of Illinois at Urbana-Champaign, April 17–18, 2019.
6. Smith, J. W.; Carnevale, L. N.; Das, A.; Chen, Q. "Imaging Nanodisc Dynamics with Liquid-phase Transmission Electron Microscopy," Third Coast Workshop on Biological Cryo-EM, Chicago, IL, May 19, 2018.
7. Smith, J. W.; Chen, Q. "'Softening' new electron microscopies: three strategies, three techniques, three stories," MRS Spring Meeting 2018, Phoenix, AZ, April 2–6, 2018.
8. Smith, J. W.; Chen, Q. "Imaging the Dynamics and Self-assembly of Biomolecules Using Liquid-phase Transmission Electron Microscopy," Midwest Thermodynamics and Statistical Mechanics Conference, University of Notre Dame, June 4–6, 2017.

The PI has given **33** invited talks in national conferences (Gordon Research Conferences, MRS, M&M) and universities (Penn State, Harvard, MIT, University of Michigan, etc.) based on the work supported by the AFOSR YIP, and 2 invited plenary talks (one at the AIChE Annual Meeting in Orlando, 2019 and the other at the 92nd ACS Colloid & Surface Science Symposium).

3.3. Awards and honors

Prestigious awards for the PI based on the work supported by the AFOSR YIP:

- ❑ Tenured with Racheff Faculty Scholar (2021), College of Engineering, University of Illinois at Urbana-Champaign, to recognize outstanding achievements during tenure-track.
- ❑ Dean's Award for Excellence in Research (2020), College of Engineering, University of Illinois at Urbana-Champaign
- ❑ Unilever Award (2018), Division of Colloid and Surface Science, American Chemical Society (**one per year worldwide**)
- ❑ Alfred P. Sloan Research Fellow in Chemistry. See the highlight in ***New York Times***: https://sloan.org/storage/app/media/programs/SRF/2018-SRF-NYTimes_Ad.pdf
- ❑ **NSF CAREER award** by Division of Materials Research on the platform developed partially by AFOSR which extended its use to colloidal nanoparticles.

Awards of the PhD student John W. Smith on the AFOSR YIP project:

- ❑ **3M Graduate Fellowship** with $30,000 cash award (2018).
- ❑ National Science Foundation Graduate Research Fellowship, Honorable Mention (2018)

Award of the PhD student Lauren N. Carnevale partially supported by the AFOSR YIP project:

- ❑ Best Speaker Award on Vetmed research day (2018)

Award of the postdoctoral researcher Hyosung An partially supported by the AFOSR YIP project:

- ❑ Will start as a tenure-track assistant professor at Chonnam National University of South Korea (August, 2021).
- ❑ **Hanwha Travel Award** at the Emerging Junior Investigator Open Innovation Forum in the 2019 AIChE Annual Meeting (2019).