BIOGRAPHICAL SKETCH

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NAME: Mickolajczyk, Keith J.

eRA COMMONS USER NAME (credential, e.g., agency login): KEITH_MICKOLAJCZYK

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Lehigh University, Bethlehem, PA	B.S.	05/2011	Bioengineering
Princeton University, Princeton, NJ	Postbac	07/2013	Biophysics
Penn State University, University Park, PA	Ph.D.	08/2018	Bioengineering
The Rockefeller University, New York, NY	Postdoc	09/2022	Biophysics

A. Personal Statement

I am a biophysicist interested in the mechanisms by which mechanoenzymes convert chemical energy into mechanical work to drive critical biological processes. My career has focused on developing a combination of single-molecule biophysical techniques (PhD training with Will Hancock at Penn State University) and small molecule inhibitors (postdoc training with Tarun Kapoor at Rockefeller University) to investigate enzyme mechanochemistry. My current research involves integrating biophysical techniques, inhibitor development, and biochemical reconstitution to study the molecular mechanisms of ribosome biogenesis by force-producing enzymes. Ribosome biogenesis is deeply tied to cellular metabolism, and its dysregulation is a hallmark of several disease states (ribosomopathies) as well as of proliferative cancers. Discoveries on the enzymology of ribosome biogenesis are essential for understanding and developing new therapies to treat these diseases. My lab is well situated for drug discovery, screening, and mechanism of action studies.

My lab currently houses customized and home-built single-molecule instruments including optical tweezers and TIRF plus dark field microscopes. Both instruments have suitable resolution (sub-nanometer, >1 kHz) to carry out the experiments set forth in this proposal. These instruments are fully owned by my lab, and there are no time-sharing restrictions that will limit the proposed work. My lab has experience in expressing and purifying AAA and dozens of other enzymes/proteins, as well as in designing and carrying out single-molecule assays. We are ideally suited to introduce single-molecule biophysical approaches to the ribosome biogenesis field.

Ongoing and recently completed projects that I would like to highlight include:

Private contract / Services Agreement 12/16/2024 – 06/15/2026, renewable 12/16/2025
Pfizer Inc. - Oncology Biochemistry & Screening
The goal of this project is to develop single-molecule enzymatic assays to differentiate candidate anticancer compounds and assess viability for clinic

1R35GM157075-01 (PI: Mickolajczyk) 01/01/2025 – 12/31/2029
National Institutes of Health / National Institute for General Medical Sciences
Title: Molecular mechanisms of ribosome biogenesis by force-producing enzymes
The goal of this project is to characterize the mechanisms of large ribosomal subunit assembly by ATPase mechanoenzymes

Busch Biomedical Grant 604141 (PI: Mickolajczyk) 10/01/2024-09/30/2026
Rutgers Office for Research
Title: Mechanisms of ribosomal RNA processing by the helicase Mtr4
The goal of this project is to develop single-molecule techniques to study helicases involved in rRNA processing.

2R01GM057720-53A1 (PI: Dubnau and Neiditch; Collaborator: Mickolajczyk) 07/01/2024-06/30/2028
National Institutes of Health / National Institute for General Medical Sciences
Title: Understanding the mechanism of genetic transformation
The goal of this project is to study the molecular mechanisms of natural transformation in gram positive bacteria.

Research Grant PC 114-23 (PI: Mickolajczyk) 02/15/2023-02/14/2024
New Jersey Health Foundation
Title: Towards developing novel anticancer therapeutics that target ribosome biogenesis
The major goal of this project is to synthesize human ribosome biogenesis AAA protein genes and to develop compound screening assays for drug discovery.

5 K00 CA223018 (PI: Mickolajczyk) 08/13/2018-07/31/2022
NIH/NCI
Title: A biophysical approach to elucidating the molecular mechanisms of mitotic inhibitor targets
The goal of this project was to study the mechanisms of mitotic mechanoenzymes using single-molecule methods.

Key recent publications:

1. Mickolajczyk KJ, Olinares PDB, Chait BT, Liu S, Kapoor TM. The MIDAS domain of AAA mechanoenzyme Mdn1 forms catch bonds with two different substrates. *Elife*. 2022 Feb 11;11 PubMed Central PMCID: PMC8837202.
2. Mickolajczyk KJ, Shelton PMM, Grasso M, Cao X, Warrington SE, Aher A, Liu S, Kapoor TM. Force-dependent stimulation of RNA unwinding by SARS-CoV-2 nsp13 helicase. *Biophys J.* 2021 Mar 16;120(6):1020-1030. PubMed Central PMCID: PMC7837305.
3. Mickolajczyk KJ, Olinares PDB, Niu Y, Chen N, Warrington SE, Sasaki Y, Walz T, Chait BT, Kapoor TM. Long-range intramolecular allostery and regulation in the dynein-like AAA protein Mdn1. *Proc Natl Acad Sci U S A*. 2020 Aug 4;117(31):18459-18469. PubMed Central PMCID: PMC7414173.
4. Mickolajczyk KJ, Geyer EA, Kim T, Rice LM, Hancock WO. Direct observation of individual tubulin dimers binding to growing microtubules. *Proc Natl Acad Sci U S A.* 2019 Apr 9;116(15):7314-7322. PubMed Central PMCID: PMC6462098.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2024 - 2026	Co-Chair, Single-molecule Forces, Manipulation and Visualization Subgroup, Biophysical Society Annual Meeting
2022 -	Assistant Professor, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ
2021 -	Early Career Reviewer, eLife
2018 - 2022	Postdoctoral Associate, The Rockefeller University, New York, NY
2015 - 2017	Lead organizer, Penn State University College of Engineering Research Symposium, University Park, PA
2013 -	Member, Biophysical Society
2013 -	Member, American Society for Cell Biology
2013 - 2018	Graduate Fellow, Penn State University, University Park, PA
2011 - 2013	Research Specialist, Princeton University, Princeton, NJ
2009 - 2009	Research Technician, Lehigh University, Bethlehem, PA

Honors

2021	Promotional Research Highlight at Annual Meeting, Biophysical Society

2018 - 2022	K00 Predoctoral to Postdoctoral Fellow Transition Award, NIH/NCI
2018	Alumni Association Dissertation Award, Penn State Alumni Society
2017 - 2018	F99 Predoctoral to Postdoctoral Fellow Transition Award, NIH/NCI
2017	1st Place in Poster Competition, Biophysical Society Central PA Regional Networking Meeting
2016	Student Travel Grant, Biophysical Society Biomolecular Motors Thematic Meeting, IUPAB
2016	Education Travel Grant, Biophysical Society 2016 Annual Meeting, Biophysical Society
2015	Student Leadership Scholarship, Penn State Union and Student Activities
2015	Leighton Reiss Graduate Fellowship, Penn State University Alumni Relations
2013-2014	Distinguished University Fellowship, Penn State University Graduate College
2010	2nd Place in Poster Competition, Lehigh University Undergraduate Research Symposium
2007	Edward J. Bloustein Distinguished Scholar, State of New Jersey

C. Contributions to Science

1. Investigating the mechanisms and mechanoregulation of ATP-consuming mechanoenzymes. Mechanoenzymes do not only produce force, but they also respond to forces. Mechanoregulation is increasingly recognized as important mode of modulating context-specific protein-protein interactions, much akin to post-translational modifications. To assess the role of mechanical forces in regulating the enzymatic activity of mechanoenzymes, I have developed and applied optical tweezers-based force spectroscopy techniques. In one study, I used single-molecule optical tweezers to measure the RNA-unwinding capabilities of the SARS-Cov-2 replicative helicase nsp13 at different applied loads. Interestingly, applied forces increase the catalytic efficiency of nsp13 more than fifty-fold, indicating that changes in the local mechanical environment are sufficient for turning the replication machinery on and off. This work was published in a special issue of the *Biophysical Journal*, included in the promotional materials for the Biophysical Society 2021 Meeting, and was selected for invited seminars at both the Biophysical Society 2021 Annual Meeting and the Helicases 2021 Annual Meeting. Additionally, this work was disseminated to the broader public through invited interviews with news outlets including the Academic Times. I followed this study up with a collaborative project using “nanopore tweezers” to study the mechanism of action of nsp13 inhibitors under applied force. More recently, I developed a single-molecule optical tweezers “force jump” assay to show that the AAA mechanoenzyme Mdn1 forms force-dependent catch bonds with its substrates, providing evidence that mechanoregulation plays an important during ribosome biogenesis. I was invited to present on this work, along with my new work as an independent PI, at the Biophysical Society 2023 Annual Meeting.
    a. **Mickolajczyk, K. J.***, Shelton, P. M.*, Grasso, M.*, Cao, X., Warrington, S. E., Aher, A., Liu, S., & Kapoor, T. M. (2021). Force-dependent stimulation of RNA unwinding by SARS-CoV-2 nsp13 helicase. *Biophysical Journal*, 120(6), 1020-1030. PMC7837305.
    b. **Mickolajczyk, K. J.**, Olinares, P. D. B., Chait, B. T., Liu, S., & Kapoor, T. M. (2022). The MIDAS domain of AAA mechanoenzyme Mdn1 forms catch bonds with two different substrates. *eLife*, 11, e73534. PMC8837202.
    c. Marx, S. K., **Mickolajczyk, K. J.**, Craig, J. M., Thomas, C. A., Pfeffer, A. M., Abell, S. J., Carrasco, J. D., Franzi, M. C., Huang, J. R., Kim, H. C. Brinkerhoff, H.D., Kapoor, T. M., Gundlach, J. H., & Laszlo, A. H. (2023). Observing inhibition of the SARS-CoV-2 helicase at single-nucleotide resolution. *Nucleic Acids Research*, gkad660. PMC10516658.
    d. **Mickolajczyk, K. J.** Intramolecular tension and catch bonds play key roles in the conformational cycle of the dynein-like AAA mechanoenzyme Mdn1. Biophysical Society 67th Annual Meeting. February 2023 (Platform: Ribosomes and Translation).

2. Determining the mechanisms of action for small molecule inhibitors of AAA mechanoenzymes. AAA ATPases are mechanoenzymes involved in dynamic cellular processes including intracellular transport, protein degradation, and ribosome biogenesis. Small molecule probes for AAA proteins have the potential to be useful tools for acute inhibition, as needed to study the AAA-driven processes on the timescales of minutes and seconds. They can also lock these mechanoenzymes into conformational states that are otherwise highly transient, enabling mechanistic studies. Longer term, AAA-targeting chemical probes may be developed into small molecule anticancer therapeutics. I have used a combination of biophysical and chemical biology approaches to study how new and existing small molecule probes bind to and inhibit AAA proteins in the context of their ATPase cycles. Specifically, I have studied the mechanisms of action of small molecules on

the AAA proteins Mdn1 and dynein**.** A particularly interesting paradigm that spans these studies is that effective small molecule inhibitors of AAA proteins are often noncompetitve; rather than competing for the ATP-binding pocket, they serve to bias allosterically-regulated conformational changes in the AAA domains towards enzymatically inactive states.

a. **Mickolajczyk, K. J.**, Olinares, P. D. B., Niu, Y., Chen, N., Warrington, S. E., Sasaki, Y., Walz, T., Chait, B. T., & Kapoor, T. M. (2020). Long-range intramolecular allostery and regulation in the dynein-like AAA protein Mdn1. *Proceedings of the National Academy of Sciences*, 117(31), 18459-18469. PMC7414173.
b. Santarossa, C. C., **Mickolajczyk, K. J.**, Steinman, J. B., Urnavicius, L., Chen, N., Hirata, Y., ... & Kapoor, T. M. (2021). Targeting allostery in the Dynein motor domain with small molecule inhibitors. *Cell Chemical Biology*, 28(10), 1460-1473. PMC8542630.

3. Developing new technologies for high-resolution single-molecule microscopy. In order to answer questions about mechanoenzymes in biology, I have worked to develop new high-resolution single-molecule imaging tools. A common problem in single-molecule work is low signal to noise. For example, a single GFP molecule emits only a limited number of photons per second, limiting the camera frame rate that can be used to successfully localize it. One way I have worked to get more photons per second from single molecules is to use scattering-based methods. I have custom-built interferometric scattering (iSCAT) and total-internal reflection dark field microscopes, and have written two book chapters providing building instructions, notes on troubleshooting, and example applications. A second way to get more photons in to increase collection efficiency. Towards this end, I have designed and built dielectric planar waveguides for enhanced total internal reflection fluorescence microscopy. My work on waveguides led to a 2017 US patent.
    a. Andrecka, J., Takagi, Y., **Mickolajczyk, K. J.**, Lippert, L. G., Sellers, J. R., Hancock, W. O., Goldman, Y. E., & Kukura, P. (2016). Interferometric scattering microscopy for the study of molecular motors. In *Methods in enzymology* (Vol. 581, pp. 517-539). Academic Press. PMC5098560
    b. **Mickolajczyk, K.**, Yan, Y., Gong, Y., Li, H., Giebink, N. C., Jackson, T., & Hancock, W. (2017). *U.S. Patent No. 9,733,465*. Washington, DC: U.S. Patent and Trademark Office.
    c. Pisupati, A., **Mickolajczyk, K. J.**, Horton, W., van Rossum, D. B., Anishkin, A., Chintapalli, S. V., Li, X., Chu-Luo, J., Busey, G., & Jegla, T. (2018). The S6 gate in regulatory Kv6 subunits restricts heteromeric K+ channel stoichiometry. *Journal of General Physiology*, 150(12), 1702-1721. PMC6279357.
    d. **Mickolajczyk, K. J.**, & Hancock, W. O. (2018). High-resolution single-molecule kinesin assays at kHz frame rates. In *Molecular Motors* (pp. 123-138). Humana Press, New York, NY. PMC7851763.

4. Measuring the kinetic and mechanical properties of microtubule polymers. Microtubules are common chemotherapeutic targets, but much is still unknown about how they grow and how they mechanically contribute to mitosis. To measure microtubule assembly kinetics, I used iSCAT microscopy to track the fates of individual gold-labeled recombinant tubulin subunits as they reversibly bound to growing microtubules. This approach yielded the first direct measurements of tubulin on- and off-rates. I presented these results in poster form at the 2018 ASCB/NCI Subcellular to Cellular Cancer Imaging Workshop, and it was later published in *PNAS*. My paper was selected for the online cover of its issue, and was co-published with a commentary written by the reviewers. I have also worked to develop methods for measuring the mechanical properties of microtubules from curvatures measurable in fluorescence images. Using the curvature technique, we found that the chemotherapy drug paclitaxel reduces the persistence length of microtubules, leading to “softer” polymers.
    a. **Mickolajczyk, K. J.**, Geyer, E. A., Kim, T., Rice, L., and Hancock, W. O. 2018. Tracking the fates of individual tubulin dimers in dynamic microtubule tips using interferometric scattering microscopy. Poster. ASCB/NCI Subcellular to Cellular Cancer Imaging Workshop, Bethesda, MD.
    b. **Mickolajczyk, K. J.**, Geyer, E. A., Kim, T., Rice, L. M., & Hancock, W. O. (2019). Direct observation of individual tubulin dimers binding to growing microtubules. *Proceedings of the National Academy of Sciences*, 116(15), 7314-7322. PMC6462098.
    c. Wisanpitayakorn P., **Mickolajczyk K. J.**, Hancock W. O., Vidali L, Tüzel E. (2022) Measurement of the Persistence Length of Cytoskeletal Filaments using Curvature Distributions. *Biophysical Journal*, 121(10), 1813-1822. PMC9199094.

5. Uncovering the structural kinetics underlying kinesin stepping. As a main part of my graduate school research, I developed and applied advanced single-molecule microscopy techniques to measure kinesin stepping patterns. This work is critical for understanding how molecular motors transduce chemical energy into mechanical work, and serves as the basis for understanding motor-driven emergent subcellular behaviors. Using iSCAT microscopy, I made the first published observation of a structural intermediate in the kinesin stepping cycle, leading to a first author *PNAS* paper. Based on this work, I was awarded a travel grant to attend the Biophysical Society 2016 Annual Meeting and give a Platform Presentation in the Cytoskeletal Motors session. I also won a second travel grant to present a poster at the Biophysical Society 2016 Biomolecular Motors Thematic Meeting (Vancouver). Further, In October 2016, I was invited back to my alma mater Lehigh University to give a Platform Talk at the Biophysical Society Pennsylvania Network Meeting. Also in 2016, my *PNAS* paper was recommended by F1000 Prime faculty as being of special significance to its field. Following up on this success, I applied high-resolution tracking and other single-molecule and computational methods to determine how differences in the stepping patterns lead to differences in the mechanical properties of kinesins -1 and -2. Most recently, I used high-resolution tracking to show definitively that certain kinesin-14 isoforms, previously thought to be non-processive, are capable of processive stepping.
    a. **Mickolajczyk, K. J.**, Deffenbaugh, N. C., Arroyo, J. O., Andrecka, J., Kukura, P., & Hancock, W. O. (2015). Kinetics of nucleotide-dependent structural transitions in the kinesin-1 hydrolysis cycle. *Proceedings of the National Academy of Sciences*, *112*(52), E7186-E7193. PMC4702989
    b. **Mickolajczyk, K. J.**, & Hancock, W. O. (2017). Kinesin processivity is determined by a kinetic race from a vulnerable one-head-bound state. *Biophysical Journal*, *112*(12), 2615-2623. PMC5479115
    c. **Mickolajczyk, K. J.***, Cook, A. S.*, Jevtha, J. P., Fricks, J., & Hancock, W. O. (2019). Insights into kinesin-1 stepping from simulations and tracking of gold nanoparticle-labeled motors. *Biophysical Journal*, 117(2), 331-345. PMC6700715
    d. Tseng, K. F*., **Mickolajczyk, K. J.***, Feng, G., Feng, Q., Kwok, E. S., Howe, J., Barbar, E. J., Dawson, S. C., Hancock, W. O., & Qiu, W. (2020). The Tail of Kinesin-14a in Giardia Is a Dual Regulator of Motility. *Current Biology*, 30(18):3664-3671. PMC7511442

*Denotes equal contribution

For full list of publications: https://www.ncbi.nlm.nih.gov/myncbi/1t5mUTnZ7aakB/bibliography/public/
ORCID: http://orcid.org/0000-0001-9445-0325

BIOGRAPHICAL SKETCH

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NAME: Heonhwa Choi

eRA COMMONS USER NAME (credential, e.g., agency login): CHOIHEONHWA

POSITION TITLE: Postdoctoral Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Konkuk University	B.S	02/2007	Physics
Graduate School of Konkuk University	M.S	02/2009	Physics
Korea National University of Science and Technology	Ph.D.	02/2017	Nano science

A. Personal Statement

I am a physicist and structural biologist with a deep interest in applying principles of low-temperature physics and nano mechanics to study biological macromolecules. My research journey began with the development of ultra-sensitive cantilever magnetometry techniques to study superconducting micro rings. During my Ph.D. and postdoctoral work at KRISS, I specialized in quantum metrology, low-temperature NMR, and nanoscale force measurements. Since transitioning to structural biology, I have focused on single-particle cryoEM and cryo-FIB/SEM techniques to study the mechanical properties of vitreous ice and the conformational landscapes of complex proteins and viral vectors. At Rutgers, I have contributed to structural studies of AAV vectors and large AAA+ ATPases like MDN1, as well as the implementation of beam-induced motion analysis and recombinant protein purification workflows. My diverse training across physics and structural biology allows me to bridge instrumentation development and biological discovery. I am excited to continue advancing cryoEM methodology and using it to answer fundamental questions about biomolecular structure and function.

B. Positions, Scientific Appointments, and Honors

Positions and Appointments:

- Postdoctoral Research Associate, Rutgers University, Piscataway, NJ, USA (Apr. 2025 – Present)
  Advisor: Dr. Keith Mickolajczyk
- Postdoctoral Research Associate, Rutgers University, Piscataway, NJ, USA (Jan. 2022 – Jan. 2025)
  Advisor: Dr. Jason T. Kaelber
- Postdoctoral Research Associate, KRISS, Daejeon, Korea (Jun. 2017 – Dec. 2021)
  Advisor: Dr. Jae-Hyuk Choi

Honors and Presentations:

- Microscopy & Microanalysis 2023, Oral Presentation: "Direct Measurement of Mechanical Properties of Vitreous Ice by Cryo-FIB"
- CPEM 2018, Paris, Invited Poster: “Progress toward quantum-based force standard in KRISS”

- MMM 2016, USA, Poster: “A Novel Mechanical Magnetometry of a Micron-sized Superconducting Ring”

C. Contributions to Science

1. Quantum Metrology at Cryogenic Temperatures
   I developed a high-resolution cantilever magnetometry system for detecting single fluxoids in superconducting microrings and contributed to the understanding of nanoscale magnetic phenomena.
   - H. Choi et al., Precise determination of magnetic moment of a fluxoid quantum, Phys. Rev. B, 2017.

2. CryoEM-Based Structural Biology of Viral Vectors and AAA+ ATPases
   I contributed to structural investigations of genome-packaged AAV vectors and ATPase proteins such as MDN1 and RIG-I. My work included 3D classification, symmetry expansion, and atomic modeling to elucidate nucleotide binding and conformational heterogeneity.
   - Work in preparation for publication.

3. Beam-Induced Motion and Vitreous Ice Mechanics
   I pioneered methods for measuring the elastic modulus of vitreous ice using cryo-FIB-fabricated cantilevers, revealing how substrate mechanics influence beam-induced motion in cryoEM.
   - H. Choi et al., Direct Measurement of Mechanical Properties of Vitreous Ice by Cryo-FIB, M&M 2023.