BIOGRAPHICAL SKETCH

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NAME: TAYLOR, KENNETH ALLEN

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

POSITION TITLE: Postdoctoral Scientist

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)	END DATE MM/YYYY	FIELD OF STUDY
North Carolina State University	BS	05/1969	Textile Chemistry
North Carolina State University	BS	08/1969	Chemistry
North Carolina State University	MS	08/1971	Physical Chemistry
University of California at Berkeley, Berkeley, CA	PHD	05/1975	Biophysics
University of California at Berkeley, Berkeley, CA	Postdoctoral Fellow	09/1976	
MRC Laboratory of Molecular Biology, Cambridge	Postdoctoral Fellow	06/1980	

A. Personal Statement

My laboratory's primary experimental tool is 3-D cryoelectron microscopy (cryoEM). One of our major projects is the 3D imaging of muscle in different structural states. The asynchronous flight muscles of insects are the best ordered muscles in nature and are thus ideal for structural studies. I began my work on the 3-D imaging of insect flight muscle with the late Prof. Michael Reedy (d. 6/18/2019) at the beginning of my first academic appointment at Duke University Medical Center, a collaboration that continued up to his recent death. Before that, I started the cryoEM field as a graduate student in Prof. Robert Glaeser's laboratory at U. C. Berkeley. I was the first to demonstrate that molecular images could be obtained from specimens embedded in ice. I then spent almost four years at the MRC Laboratory of Molecular Biology in Cambridge, U.K. where I learned 3-D image reconstruction with the late Dr. L. A. Amos and began my career in muscle research with the late Dr. H. E. Huxley, which continued taking my first academic position at Duke University Medical Center and after my relocation to the Florida State University, Institute of Molecular Biophysics, in 1995. My research on muscle since my postdoctoral days at the MRC-LMB led to the first 3-D images of the muscle sarcomere in rigor in 1984 using electron crystallography methods on the well-ordered asynchronous flight muscle of Lethocerus indicus. We developed Tony Crowther's oblique section reconstruction method to reconstruct several additional biochemical states before adopting electron tomography as our method of choice for muscle imaging. Our tomography work required software development, including the best marker-free tilt series alignment method and ways of classifying and averaging subtomograms. I believe that my research program is the only one that has been able to image myosin head conformations within the muscle lattice during actual contraction using fast frozen, freeze substituted and sectioned muscle. Our current research is focused on the atomic structure of striated muscle thick filaments. We produced the first subnanometer resolution structure of a striated muscle thick filament, from the flight muscle of Lethocerus indicus, followed by structures from the fruit fly, Drosophila melanogaster and the Asian bumble bee, Bombus ignitus. We produced the highest resolution reconstruction at 4.2 A resolution from which we built an atomic model which revealed some surprising features of the myosin coiled coil. My first NIH review assignment occurred in 1983. I have participated in 81 Study Sections over a 40 year period. I also served on the NIGMS Council Advisory Committee for the PSI:Biology program in 2013, on the NIGMS Council Advisory Committee for the Structural Biology of AIDS Program from 2014-2018, the NIAID Board of Scientific Councilors Ad Hoc member in 2016 and am currently an Advisor to the NIH Common Fund cryoEM Centers. I serve on the External Advisory Committees of the BioCAT beam line at Argonne National Lab since 2007 and Dr. Tamir Gonin's MEDIC P41 grant, serve on the Editorial Board of the Journal of Structural biology since its founding in 1990 and as Associate Editor since October 2018. I chaired a Gordon Research Conference on 3-D Electron Microscopy of Macromolecules in 2003. I am co-PI of an NIGMS funded cryoEM consortium grant entitled the SouthEast Center for Microscopy of Macromolecular Machines (SECM4). I was the faculty director of FSU's Biological Science Imaging Resource from 1996-2021, growing it from a facility with a JEOL 1200EX TEM and a JEOL

JSM 840 SEM to what it is today with a CM120 BioTwin TEM, recently installed Hitachi HT7800 and a ThermoFisher Titan Krios, the latter with a pair of direct electron detectors, a Direct Electron Ltd. Apollo and a K3 mounted on a BioQuantum imaging filter, as well as a Volta Phase Plate. Since 1995, I have mentored 16 PhD students through graduation (I currently have 6), 1 MS student, 14 postdoctoral students, one of whom remains with me. One of my former graduate students, Deborah Kelly, is now a full professor at Penn State University and Director of that institution's Center for Structural Oncology, the former MS student is a Lecturer at Perimeter College's Alpharetta Campus of Georgia State University, two of my former postdocs, Profs. Yifan Cheng (UCSF) and Jun Liu (Yale), are among the most successful practitioners of cryoelectron microscopy in the world.

1. Rahmani H, Ma W, Hu Z, Daneshparvar N, Taylor DW, McCammon JA, Irving TC, Edwards RJ, Taylor KA. The myosin II coiled-coil domain atomic structure in its native environment. Proc Natl Acad Sci U S A. 2021 Apr 6;118(14) PubMed Central PMCID: PMC8040620.
2. Wu S, Liu J, Reedy MC, Winkler H, Reedy MK, Taylor KA. Methods for identifying and averaging variable molecular conformations in tomograms of actively contracting insect flight muscle. J Struct Biol. 2009 Dec;168(3):485-502. PubMed Central PMCID: PMC2805068.
3. Winkler H, Zhu P, Liu J, Ye F, Roux KH, Taylor KA. Tomographic subvolume alignment and subvolume classification applied to myosin V and SIV envelope spikes. J Struct Biol. 2009 Feb;165(2):64-77. PubMed Central PMCID: PMC2656979.
4. Winkler H, Taylor KA. Accurate marker-free alignment with simultaneous geometry determination and reconstruction of tilt series in electron tomography. Ultramicroscopy. 2006 Feb;106(3):240-54. PubMed PMID: 16137829.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

1995 -	Professor, FLORIDA STATE UNIVERSITY
1980 - 1985	Assistant Medical Research Professor, Department of Anatomy, Duke University Medical Center, Durham, NC
1980 - 1995	Research Associate Professor, DUKE UNIVERSITY
1976 - 1980	Postdoctoral Scientist, MRC Laboratory of Molecular Biology, Division of Structural Studies, Cambridge
1975 - 1976	Postdoctoral, University of California, Lawrence Berkeley Laboratory, Berkeley, CA

Honors

2022	Distinguished Scientist in Biology, Microscopy Society of America
2016	Fellow, Microscopy Society of America
2006	Donald L. D. Caspar Professor of Biological Science, Florida State University
2003	Chair, Gordon Conference on 3-D Electron Microscopy of Macromolecules
2002	Distinguished Research Professor, Florida State University
1994	James A. Shannon Director's Award, NIH
1984	Established Investigator , American Heart Association
1979	Postdoctoral Fellowship, Muscular Dystrophy Fellowship
1978	Postdoctoral Fellowship, NATO
1976	Postdoctoral Fellowship, Jane Coffin Childs Memorial Fund for Medical Research

C. Contribution to Science

1. My first contribution to science was the development of cryoEM which constituted my PhD dissertation research under the direction of Prof. Robert M. Glaeser. For this work, I built (myself) a cold stage, devices for the introduction of prefrozen specimens into the electron microscope and learned how to freeze a thin aqueous film. As we planned to use this method for electron crystallography, at the time single particle image reconstruction being a distant dream, I confined my effort to crystalline specimens from which I was able to show that the crystallinity of a protein crystal, catalase, was preserved after freezing in liquid nitrogen, and that the contrast was quite good even given the fact

that there was no negative stain applied. One paper in particular published in the *Journal of Microscopy* in 1978 was the inspiration for Jacques Dubochet to learn how to freeze thin aqueous specimens into a vitreous ice film from which he was awarded the Nobel Prize. This image showed the kind of detail that a typical frozen-hydrated specimen, not just a crystal, can show in cryoEM. Prof. Robert M. Glaeser and I wrote a retrospective/prospective on this work in 2008, which pointed out the need to prevent protein molecules from aggregating on the air-water interface.

a. Taylor KA, Glaeser RM. Retrospective on the early development of cryoelectron microscopy of macromolecules and a prospective on opportunities for the future. J Struct Biol. 2008 Sep;163(3):214-
23. PubMed Central PMCID: PMC3291472.
b. Taylor KA. Structure determination of frozen, hydrated, crystalline biological specimens. J Microsc. 1978 Jan;112(1):115-25. PubMed PMID: 641983.
c. Taylor KA, Glaeser RM. Electron microscopy of frozen hydrated biological specimens. J Ultrastruct Res. 1976 Jun;55(3):448-56. PubMed PMID: 933264.
d. Taylor KA, Glaeser RM. Electron diffraction of frozen, hydrated protein crystals. Science. 1974 Dec 13;186(4168):1036-7. PubMed PMID: 4469695.

2. For my entire academic career, I have worked on the 3D imaging of asynchronous insect flight muscle (IFM) from the large waterbug Lethocerus indicus. The project continues to this day. Initially, the 3D reconstruction techniques developed for 2-D crystalline arrays were applied resulting in the first 3D image of a muscle A-band, published in *Nature* in 1984. This was the only method at that time that could produce a 3-D image of this type of specimen. Although a major advance, it was clear to me that we needed to find a solution to the missing cone problem which we solved initially by adding in data obtained from thick longitudinal and transverse sections. A better solution was application of Tony Crowther's oblique section reconstruction method. We developed this method to its highest level and were able to obtain 3D images of the entire unit cell, with no missing data, and of multiple states. However, this was still not enough because the myosin crossbridges were spatially averaged reducing the resolution below where individual myosin heads and actin subunits could be resolved. The solution to this was ET, which produces a 3D image without spatial averaging. We applied ET to several states and for the first time to muscle rapidly frozen while producing active tension. This result was published in *Cell* in 1999 and utilized a limited spatial averaging technique called column averaging. After developing software to apply multivariate data analysis to individual unit cells, we were able to identify multiple, sometimes completely novel, actin-myosin interactions in a rapidly frozen muscle generating active tension in an isometric contraction and following a stretch and a release. All this work is summarized in a recent review article. With the development of FIB milling of frozen cells and tissue samples for electron tomography, this approach could be productively exploited now thereby avoiding the problems associated with fixation, embedding, staining and sectioning. Through the talents and drive of a graduate student, we succeeded in obtain a FIB milling result from relaxed human cardiac muscle without the use of drugs to artificially order the myosin heads. The result showed an unexpected pattern of ordered and partially ordered heads from which we were able to propose an apparently novel interpretation for how cardiac muscle works.

a. Taylor KA, Rahmani H, Edwards RJ, Reedy MK. Insights into Actin-Myosin Interactions within Muscle from 3D Electron Microscopy. Int J Mol Sci. 2019 Apr 5;20(7) PubMed Central PMCID: PMC6479483.
b. Arakelian C, Warrington A, Winkler H, Perz-Edwards RJ, Reedy MK, Taylor KA. Myosin S2 origins track evolution of strong binding on actin by azimuthal rolling of motor domain. Biophys J. 2015 Mar 24;108(6):1495-1502. PubMed Central PMCID: PMC4375447.
c. Wu S, Liu J, Reedy MC, Tregear RT, Winkler H, Franzini-Armstrong C, Sasaki H, Lucaveche C, Goldman YE, Reedy MK, Taylor KA. Electron tomography of cryofixed, isometrically contracting insect flight muscle reveals novel actin-myosin interactions. PLoS One. 2010 Sep 9;5(9) PubMed Central PMCID: PMC2936580.
d. Chen L, Liu J, Rastegarpouyani H, Janssen PML, Pinto JR, Taylor KA. Structure of mavacamten-free human cardiac thick filaments within the sarcomere by cryoelectron tomography. Proc Natl

Acad Sci U S A. 2024;121(9):e2311883121. Epub 20240222. doi: 10.1073/pnas.2311883121. PubMed PMID: 38386705; PMCID: PMC10907299.

3. In our studies of flight muscle structure, we had to develop methods that enabled us to progress with our

structure determinations. One of the first problems was how to utilize the new information coming out of crystallography and cryoEM of the atomic structures of F-actin and the myosin head. We teamed up with a crystallographer at FSU, Prof. Michael Chapman, to adapt his real space refinement technique for modifying the atomic structures from crystals into our 3-D images of muscle. To eliminate the missing cone in our reconstructions, we developed the oblique section reconstruction method which produced an average structure of the entire unit cell with no missing cone. With the advent of electron tomography, we learned to use multivariate data analysis as a device for classifying the heterogeneous structures in our tomograms. We developed a suite of programs, designed around the requirements of our flight muscle work, that have proven useful in a number of other studies.

e. Winkler H, Taylor KA. Marker-free dual-axis tilt series alignment. J Struct Biol. 2013 May;182(2):117-24. PubMed Central PMCID: PMC4098971.

f. Wu S, Liu J, Reedy MC, Winkler H, Reedy MK, Taylor KA. Methods for identifying and averaging variable molecular conformations in tomograms of actively contracting insect flight muscle. J Struct Biol. 2009 Dec;168(3):485-502. PubMed Central PMCID: PMC2805068.

g. Winkler H, Zhu P, Liu J, Ye F, Roux KH, Taylor KA. Tomographic subvolume alignment and subvolume classification applied to myosin V and SIV envelope spikes. J Struct Biol. 2009 Feb;165(2):64-77. PubMed Central PMCID: PMC2656979.

h. Chen LF, Blanc E, Chapman MS, Taylor KA. Real space refinement of acto-myosin structures from sectioned muscle. J Struct Biol. 2001 Feb-Mar;133(2-3):221-32. PubMed PMID: 11472093.

3. Our work on muscle has not entirely involved 3-D image reconstruction of sectioned muscle. One of our laboratories signature techniques is "lipid monolayer crystallization". This technique was originally demonstrated by Roger Kornberg and colleagues. However, we have gone them one better in that we have produced 3D structures of unstained, frozen hydrated 2-D crystalline arrays, 6 times in fact. With one of these, we obtained a unique and apparently universal structure for myosin heads in relaxed muscle. We later obtained a structure showing the same head-head conformation in the 10S conformation of full length smooth muscle myosin II. Originally found for smooth muscle myosin II, where the "relaxed" state is accompanied by instability of the filaments themselves. The asymmetric interaction between myosin heads was subsequently demonstrated by Roger Craig and colleagues for striated muscle. While on a sabbatical with Charles Brooks III, Florence Tama and myself carried out a modelling study which suggested that the formation of the inhibited state of smooth muscle myosin II was accompanied by torsional motions that are propagated through the long alpha-helical coiled-coil rod domain which forms the thick filament backbone and that these torsional motions contribute in part to the destabilization of the filaments themselves. We are now working to show structurally how this coupling can affect the structure of the backbone of native myosin filaments. In flight muscle, applied tension may disrupt the head-head interaction implying that the structure of the myosin heads is coupled to the structure of the myosin tails. Most recently, we have shown how interacting interfaces can be identified in a highly disordered structure using rafts of F-actin and aldolase as the test object.

a. Hu G, Taylor DW, Liu J, Taylor KA. Identification of interfaces involved in weak interactions with application to F-actin-aldolase rafts. J Struct Biol. 2018 Mar;201(3):199-209. PubMed Central PMCID: PMC5820182.

b. Hu Z, Taylor DW, Edwards RJ, Taylor KA. Coupling between myosin head conformation and the thick filament backbone structure. J Struct Biol. 2017 Dec;200(3):334-342. PubMed Central PMCID: PMC5733691.

c. Hu Z, Taylor DW, Reedy MK, Edwards RJ, Taylor KA. Structure of myosin filaments from relaxed Lethocerus flight muscle by cryo-EM at 6 Å resolution. Sci Adv. 2016 Sep;2(9):e1600058. PubMed Central PMCID: PMC5045269.

d. Wendt T, Taylor D, Trybus KM, Taylor K. Three-dimensional image reconstruction of dephosphorylated smooth muscle heavy meromyosin reveals asymmetry in the interaction between myosin heads and placement of subfragment 2. Proc Natl Acad Sci U S A. 2001 Apr 10;98(8):4361-6. PubMed Central PMCID: PMC31840.

4. In addition to my work on the crossbridge lattice of *Lethocerus* flight muscle, I have also worked on or contributed to the structure of the Z-disk as well as electron tomography of vertebrate striated muscle with Dr. Pradeep Luther. The only structure in striated muscle I have not worked on (yet) is the native thin filament. Most recently, the late John Trinick had the idea to isolate Z-disks and image them using cryoelectron tomography. His graduate student, Mara Rusu, started with procedures used by Saide and Ulrich to isolate honey bee Z-disks. When successful, they collected tilt series at the MRC Laboratory of Molecular biology and myself and my graduate student, Zhongun Hu, did the data processing to obtain a 3- D structure which we published, and is the first cryoEM structure of a Z-disk. John decided to pursue the vertebrate Z-disk and I was free to pursue the invertebrate Z-disk. My lab has also published a structure of actin filaments decorated with the myosin motor domain of smooth muscle, which showed that the actin binding cleft of a slow myosin is more open than that of a fast myosin.

a. Burgoyne T, Heumann JM, Morris EP, Knupp C, Liu J, Reedy MK, Taylor KA, Wang K, Luther PK. Three-dimensional structure of the basketweave Z-band in midshipman fish sonic muscle. Proc Natl Acad Sci U S A. 2019 Jul 30;116(31):15534-15539. PubMed Central PMCID: PMC6681754.

b. Banerjee C, Hu Z, Huang Z, Warrington JA, Taylor DW, Trybus KM, Lowey S, Taylor KA. The structure of the actin-smooth muscle myosin motor domain complex in the rigor state. J Struct Biol. 2017 Dec;200(3):325-333. PubMed Central PMCID: PMC5748330.

c. Rusu M, Hu Z, Taylor KA, Trinick J. Structure of isolated Z-disks from honeybee flight muscle. J Muscle Res Cell Motil. 2017 Apr;38(2):241-250. PubMed Central PMCID: PMC5660141.

d. Luther PK, Winkler H, Taylor K, Zoghbi ME, Craig R, Padrón R, Squire JM, Liu J. Direct visualization of myosin-binding protein C bridging myosin and actin filaments in intact muscle. Proc Natl Acad Sci U S
A. 2011 Jul 12;108(28):11423-8. PubMed Central PMCID: PMC3136262.

Complete List of Published Work in MyBibliography (172 items):

https://www.ncbi.nlm.nih.gov/myncbi/kenneth%20a..taylor.1/bibliography/public/

R35 GM139616– NIH/NIGMS. Kenneth A. Taylor (PI). cryoEM Studies of Muscle. $441,815 Annual total costs. 01/01/2021 – 12/31/2025. This grant supports studies on the atomic resolution structure of thick filaments from striated muscle. Both vertebrate and invertebrate examples are being studied.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

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NAME: Rastegarpouyani, Hosna

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

POSITION TITLE: Postdoctoral Scholar

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
University of Tehran, Tehran	BS	09/2011	Cellular & Molecular Biology
Shahid Beheshti University, Tehran	MS	09/2015	Microbiology
Florida State University, Tallahassee Florida	PHD	05/2024	Cellular & Molecular Biology
Postdoctoral Scholar	postdoc	-	Molecular Biophysics

A. Personal Statement

In 2018, I was accepted into Florida State University's Biology PhD program. After the first year at FSU, being rotated in different labs and getting to know Dr. Taylor's field of research, I became interested in Electron Microscopy sciences, an impressively powerful technology that was completely new to me. Moving on from basic biology to an interdisciplinary field of research that needs knowledge in data analysis and programming was challenging to me at first. But as I went further, I realized how eager I am to learn and explore more in this field. As of now, I have been working in the cryoEM field for more than three years and have continually learned new skills. I first began my research by trying to express and purify Ca^{+2}–insensitive Gelsolin, which is required in the filament sample preparation, using AKTA Fast Protein liquid chromatography (FPLC). Next, I started to isolate the thick filament from Rabbit psoas myofibrils and evaluate the quality of the sample for Cryo-electron microscopy by performing negatively stained Transmission Electron Microscopy (TEM). Cryo-EM has not been done on vertebrate's myosin filaments up to this point. Providing a high-resolution reconstruction of a vertebrate's myosin filament is a step in understanding the complex roles that thick filaments of all species undergo during muscle contraction. It took me almost a year to figure out how to isolate intact and full- length thick filaments from vertebrate muscle tissue. The main problem seems to arise from difficulties in preserving the apparently more fragile vertebrate thick filaments using techniques that work well for invertebrates. After optimizing the thick filament sample preparation condition for the rabbit psoas muscle, I successfully used the same method for the rabbit cardiac muscle and then the human cardiac muscle. Since a high-resolution reconstruction needs a data set from a frozen-hydrated sample, I started working on the cryo-EM sample preparation which was the most challenging part of my experiment. By fixing the sample over a grid, I managed to make a cryo-EM sample of a vertebrate thick filament in which the structure of both backbone and heads look unharmed and not disarrayed. By working on a data set of a frozen-hydrated rabbit psoas thick filament sample I built up my data processing skills in reconstructing filaments with pseudo or limited helical symmetry. In this project, I was exposed to everything from making the sample and plunge freezing it to the data collection, and the data preprocessing. However, the quality of the data was limited because of the preparation technique and the limited number of collected micrographs. Conclusive results await a newer set of data. Recently, I tried to keep the structure of filaments intact by using 2% PEG 6000 and preliminary results showed that PEGylation can also improve the cryo-EM sample quality. As opposed to crosslinkers such as glutaraldehyde, which can limit the resolution of 3D

reconstructions, PEG does not impose any such limitations. Now, I am trying to make the best quality cryo-EM sample from the human cardiac thick filament, optimize the plunge freezing condition for that, collect a large data set, and perform a 3D image reconstruction. Personally, I am very excited to work on a challenging project such as my current project, particularly since the outcome will enable people to live longer and healthier lives. Attending the Biophysical Society Annual Meetings in 2021 and 2022 and the 2022 Microscopy and Microanalysis meeting provided me with a great deal of inspiration where I had the opportunity to present my research to my peers, meet many people in different stages of their careers and enjoy exchanging ideas.

B. Positions, Scientific Appointments and Honors

Positions and Employment

Spring 2017	Teaching Assistant, University of Tehran, Department of Biology, Tehran
2017 – 2018	Researcher at Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran
2018 - 2020	Teaching Assistant, Florida State University, Tallahassee, FL
2020 - 2024	Graduate Research Assistant, Florida State University, Tallahassee, FL
2024	Postdoctoral Scholar, Florida State University, Tallahassee, FL

Other Experience and Professional Memberships

2019 -	Member, Graduate Women in Science
2020 -	Member, Biophysical Society
2021 - 2022	Event Coordinator, Graduate Women in Science
2022 -	Member, American Heart Association

Honors

2017	Outstanding academic record, Shahid Beheshti University
2018	Dean's Doctoral Scholarship for international students, Florida State University
2020	Accepted to a paid 3-month summer intern at MyoKardia Inc. which unfortunately got canceled due to the covid-19 pandemic
2021	Donna Jung Scholarship Award in fields of cryogenic studies
2022	M&M 2022 Poster Award Winner

C. Contributions to Science

1. Despite distinctly different morphology, insect flight muscle and cardiac muscle have highly conversed structural elements and utilize a similar contraction mechanism - stretch activation. Insect asynchronous flight muscle has the most highly evolved stretch activation mechanism in nature. The regular muscle activation in striated muscle involves cooperation between the tension sensor, which is the myosin tail, and the motor domain, which is myosin heads arranged in a structure called the interacting heads motif (IHM). The unbalance of length-tension relationship would disrupt the myocardial performance and lead to certain cardiomyopathies. The helical symmetry of the insect flight muscle thick filament enables us to resolve the molecular structure by using single particle cryo- electron microscopy. We have built a 3D-reconstruction of myosin filaments from the genetic model fruit fly *Drosophila melanogaster* at 4.7Å resolution and the Japanese bumble bee *Bombus ignitus* at 6Å resolution. The preliminary results were presented as posters in M&M2021 and BPS2022 and the final result has been published in the *International Journal of Molecular Sciences*. For the *Bombus ignitus* project I isolated thick filaments from the bumble bee flight muscle for mass spectrophotometry experiments. For the *Drosophila melanogaster* project, I isolated thick filaments from the fruit fly flight muscle, assessed the quality of the sample using negative staining methods, and vitrified the sample on a Quantifoil grid for the data collection. More recently, I worked out how to freeze human cardiac myofibrils for FIB milling which led to a publication in PNAS describing the different and quite unexpected, ordered arrangements of myosin heads in relaxed muscle.

a) Li, J., Rahmani, H., Yeganeh, F. A., **Rastegarpouyani, H.**, Taylor, D., Iwamoto, H., & Taylor, K. (2021). Cryo-EM structure of the flight muscle thick filament from the bumble bee, Bombus ignitus, at 6 Å Resolution. *Microscopy and Microanalysis*, *27*(S1), 1684-1686.

b) Abbasi Yeganeh F, Rastegarpouyani H, Li J, Taylor KA. Structure of the Drosophila melanogaster Flight Muscle Myosin Filament at 4.7 Å Resolution Reveals New Details of Non-Myosin Proteins. *Int J Mol Sci.* 2023;24(19):14936. Epub 20231005. doi: doi:10.3390/ijms241914936; PMCID: PMC10573858.

c) Li, J., Rahmani, H., Yeganeh, F. A., **Rastegarpouyani, H.**, Taylor, D., Iwamoto, H., & Taylor, K. (2021). Cryo-EM structure of the flight muscle thick filament from the bumble bee, *Bombus ignitus*, at 6 Å Resolution. *Int. J. Mol. Sci. 24, 377 (2023).* https://doi.org/10.3390/ijms24010377. PMID: 36613818 PMCID: PMC9820631

d) Chen L, Liu J, Rastegarpouyani H, Janssen PML, Pinto JR, Taylor KA. Structure of mavacamten-free human cardiac thick filaments within the sarcomere by cryoelectron tomography. *Proc Natl Acad Sci U S A*. 2024;121(9):e2311883121. Epub 20240222. doi: 10.1073/pnas.2311883121. PubMed PMID: 38386705; PMCID: PMC10907299.

2. Fixing Rabbit psoas thick filaments on a grid for cryo-EM purposes and improving the quality of the sample for data collection was an achievement that will be leading us to make a high-resolution 3D reconstruction of a cardiac thick filament sample for the first time. This structure would be the most useful for understanding the effects of mutations on cardiac muscle function and the pathogenesis of cardiomyopathy. I carried out the sample preparation, the sample vitrification, the data collection, and the data analysis. The result was presented as a poster in BPS2022 and M&M2022. An award was presented to me for my poster by M&M.

a) **Rastegarpouyani, H.**, Taylor, D. W., Yeganeh, F. A., Hojjatian, A., & Taylor, K. A. (2022). Isolation of Striated Muscle Thick Filaments for Cryo-EM. *Microscopy and Microanalysis*, *28*(S1), 1588-1590.

3. One of the techniques that I learned during my 3 years journey in the structural biology field is embedding methods for Transmission Electron Microscopy (TEM). I learned to fix the tissue with an aldehyde and osmium tetroxide in buffer, produce epoxy blocks, and sectioning the blocks to ultra- thin samples for applying on a grid and for TEM imaging. I sectioned and imaged some wild type and mutant mouse cardiac tissues as a part of a bigger project studying cardiomyocyte nuclear pleomorphism in a mouse model of hypertrophic cardiomyopathy. This work was recently published in the *International Journal of Molecular Sciences*.

a) Coscarella, I. L.; Landim-Vieira, M.; **Rastegarpouyani, H.**; Chase, P. B.; Irianto, J.; Pinto, J. R., Nucleus Mechanosensing in Cardiomyocytes. *Int. J. Mol. Sci. 24(17)*, 377 (2023). https://doi.org/10.3390/ijms24010377. PMID: 36613818 PMCID: PMC9820631

b) Landim-Vieira, M.; Ma, W.; Song, T.; **Rastegarpouyani, H.**; Gong, H.; Coscarella, I. L.; Bogaards, S. J. P.; Conijn, S. P.; Ottenheijm, C. A. C.; Hwang, H. S.; Papadaki, M.; Knollmann, B. C.; Sadayappan, S.; Irving, T. C.; Galkin, V. E.; Chase, P. B.; Pinto, J. R., Cardiac troponin T N-domain variant destabilizes the actin interface resulting in disturbed myofilament function. Proc Natl Acad Sci U S A 2023, 120, (23), e2221244120. PMCID: PMC10265946. DOI: 10.1073/pnas.2221244120

Ongoing research support

R35 GM139616– NIH/NIGMS. Kenneth A. Taylor (PI). cryoEM Studies of Muscle. $441,815 Annual total costs. 01/01/2021 – 12/31/2025. This grant supports studies on the atomic resolution structure of thick filaments from striated muscle. Both vertebrate and invertebrate examples are being studied.

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NAME: Jiawei Li

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

Postdoctoral Scholar

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.)*

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Florida State University, Tallahassee, FL	MS	09/2015	Microbiology
Florida State University, Tallahassee, FL	PHD	05/2024	Cellular & Molecular Biology
Florida State University, Tallahassee, FL	postdoc	-	Molecular Biophysics

A. Personal Statement

My undergraduate degree was in Physics but I changed my graduate major to Biophysics. Biology has always been the field I am most interested in and want to explore because discovery in biology contributes directly and significantly to human health, which is a big motivation in my research. In biophysics, the connection between biology and physics has spurred the development of structural biology in the past few decades. I am grateful to get admitted to the Molecular Biophysics program in Florida State University, which is an independent but comprehensive research institute, granting me all the access to the world of biophysics.
I joined in Dr. Taylor's lab in 2019, I was intrigued by the cryo-EM technique used in his lab and the biological systems they investigate – molecular structure of insect flight muscle. The cryo-EM single- particle technique has quickly developed in the last 10 years making it possible to "see" the unknown details of macromolecular in their native state. I started my work on the thick filament of *Drosophila* and *Lethocerus* flight muscle for practice, which prepared me for my project – resolving *Bombus* thick filament structure. With the newly installed detector - K3 camera and a large dataset, I successfully reconstructed this structure at 6 Å with single-particle processing. In my project, knowing about the structure of myosin heads and myosin backbone in insect flight muscle would help us to understand the stretch activation mechanism in striated muscle since they have a similar structure in terms of the axial repeat of myosin, and the highly identical/similar protein sequence also makes the insect flight muscle a suitable object to investigate the related diseases. The cryo-EM is a quickly developing field with the innovation invested in every day as it can be boosted by material science (grid preparation), algorithm development, and biochemistry (sample preparation). Therefore, attending to the conference and meeting can update me with the cutting-edge technologies and modalities.

B. Positions and

Honors Positions and

employment

2018 - 2024 Teaching Assistant and Graduate Research Assistant, Florida State University

Honors

2014 First-class scholarship, Northwest University, China
2015 First-class scholarship, Northwest University, China
2016 Second-class scholarship, Northwest University, China
2018 Dean's Doctoral Scholarship for international students, Florida State University
2024

Other experience and professional membership

2019- Member, Biophysical Society

C. Contributions to Science

1. The thick filaments in striated muscles from both vertebrates and invertebrates exhibit a high degree of similarity in the myosin tail amino acid sequence but are diverse in their protein constituents. To better understand the structure and function of asynchronous muscle thick filaments at a molecular level, those from the flight muscle of *Bombus ignitus* (order Hymenoptera) have been imaged at 6 Å resolution using cryo-EM single-particle methods. The flight muscles of *Bombus* share many general features with flight muscle thick filaments from *Drosophila* (order Diptera) and *Lethocerus* (order Hemiptera) with which they are thought to share a common ancestor. However, different structure and function of non-myosin protein densities set the three filament structures apart. Despite the absence of the annotated genome of *Bombus ignitus*, the flightin and myofilin of its thick filament were identified through the use of multiple sequence alignment, mass spectrometry, and AlphaFold model prediction, resulting in the discovery of previously unknown densities. An atomic model of flightin was also constructed de novo for the first time. I carried out the reconstruction, built the atomic model for non-myosin proteins and structure analysis.

- **Li J**, Rahmani H, Abbasi Yeganeh F, Rastegarpouyani H, Taylor DW, Wood NB, Previs MJ, Iwamoto H, Taylor KA. Structure of the Flight muscle Thick Filament from the Bumble Bee, Bombus ignitus, at 6 Å resolution. *International Journal of Molecular Sciences*. 2022 Dec 26;24(1):377.

2. Arrangement into a filament of defined length with ordered helical symmetry is influenced by contributions from non-myosin proteins, like flightin, myofilin, and paramyosin, which bind the myosin tails at various regions through different mechanisms to stabilize the assembly. With the 4.7 Å map of *Drosophila* thick filament resolved by single-particle CryoEM, we observe and can segment the myosin backbone density and non-myosin proteins from which we built an atomic model for flightin and myosin. We found three potential salt bridges and a hydrophobic interface between flightin and myosin, which showed the potential interactions between myosin and flightin within the *Drosophila* thick filament. The myosin atomic model also facilitates visualizing the charge interactions and hydrophobic interactions both within and between myosin coiled coils. My role in this project was helping to builde atomic model for flightin and myosin and data analysis.

- Abbasi Yeganeh F, Rastegarpouyani H, **Li J**, Taylor KA. Structure of the *Drosophila melanogaster* Flight Muscle Myosin Filament at 4.7 Å Resolution Reveals New Details of Non- Myosin Proteins. International Journal of Molecular Sciences. 2023 Oct

5;24(19):14936.

Ongoing research support

R35 GM139616 – NIH/NIGMS. Kenneth A. Taylor (PI). cryoEM Studies of Muscle. $441,815 Annual total costs. 01/01/2021 – 12/31/2025. This grant supports studies on the atomic resolution structure of thick filaments from striated muscle. Both vertebrate and invertebrate examples are being studied.
Role: Postdoc

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Feghhi, Maryam

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

POSITION TITLE: Teaching Assistant and Graduate Research Assistant

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)	END DATE MM/YYYY	FIELD OF STUDY
University of Tabriz, Tabriz	BS	12/2015	Solid State Physics
Urmia University of Medical Sciences	MS	05/2020	Medical Physics
Florida State University	PHD	-	Biophysics

A. Personal Statement

I am Maryam Feghhi, serving as a Research Assistant in Dr. Kenneth Taylor's lab at Florida State University since the summer of 2022. In my role, I have actively immersed myself in mastering cryo-electron microscopy (cryo-EM) techniques, focusing on sample preparation, screening, and data processing.

Looking forward, my ambitions extend to obtaining a Ph.D. degree from Florida State University and pursuing a postdoctoral fellowship in a research laboratory dedicated to the realms of cancer structural biology and drug discovery. I aspire to delve into the atomic resolution exploration of exosomes in breast cancer progression, leveraging advanced cryo-EM techniques. Ultimately, my enduring career goal is to make significant contributions to cancer research by employing cutting-edge imaging methodologies to unravel the intricate molecular mechanisms driving cancer progression and identify novel therapeutic targets.

1. Feghhi M. The distinct roles of exosomes in innate immune responses and therapeutic applications in cancer. European journal of pharmacology. 2022 October 15.
2. Feghhi M. Bystander effects induced by electron beam-irradiated MCF-7 cells: a potential mechanism of therapy resistance. Breast cancer research and treatment. 2021 May 27.
3. Feghhi M. Effect of multi-functional polyhydroxylated polyhedral oligomeric silsesquioxane (POSS) nanoparticles on the angiogenesis and exosome biogenesis in human umbilical vein endothelial cells (HUVECs). Materials & design. 2021 January 01; 197. Available from: https://www.sciencedirect.com/science/article/pii/S0264127520307620
4. Jabbari N, Akbariazar E, Feqhhi M, Rahbarghazi R, Rezaie J. Breast cancer-derived exosomes: Tumor progression and therapeutic agents. J Cell Physiol. 2020 Oct;235(10):6345-6356. PubMed PMID: 32216070.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 - Teaching Assistant and Graduate Research Assistant, Florida State University, Tallahassee, FL

C. Contribution to Science

1. My previous focus revolves around breast cancer research, specifically exploring the biomedical sophisticated radiation biology techniques, including cell culturing, treatment, high-speed fluorescence

imaging, and nano particle synthesis.

A notable achievement in my research was the groundbreaking demonstration that gallic acid effectively reduces the expression of key micro-RNAs pivotal in exosome biogenesis and cell secretion. This discovery underscores the critical role of gallic acid in combating breast cancer. Furthermore, my investigations extended to characterizing the impact of POH-POSS nanoparticles on facilitating new blood vessel growth through exosome biogenesis. Additionally, I delved into the effects of electron beam radiotherapy on both cancerous and bystander cells, contributing to novel drug discovery techniques for cancer treatment.

My current research involves the reconstruction of honey bee thick filaments from three different life stages using cryo-electron microscopy (cryo-EM) in Dr. Taylor's lab. This interdisciplinary endeavor enhances our understanding of muscle structure and function, providing valuable insights into the changes in thick filament structure during ageing.

a. Feghhi M. The distinct roles of exosomes in innate immune responses and therapeutic applications in cancer. European journal of pharmacology. 2022 October 15.
b. Feghhi M. Bystander effects induced by electron beam-irradiated MCF-7 cells: a potential mechanism of therapy resistance. Breast cancer research and treatment. 2021 May 27.
c. Feghhi M. Effect of multi-functional polyhydroxylated polyhedral oligomeric silsesquioxane (POSS) nanoparticles on the angiogenesis and exosome biogenesis in human umbilical vein endothelial cells (HUVECs). Materials & design. 2021 January 01; 197. Available from: https://www.sciencedirect.com/science/article/pii/S0264127520307620
d. Jabbari N, Akbariazar E, Feqhhi M, Rahbarghazi R, Rezaie J. Breast cancer-derived exosomes: Tumor progression and therapeutic agents. J Cell Physiol. 2020 Oct;235(10):6345-6356. PubMed PMID: 32216070.

D. Ongoing research support

R35 GM139616 – NIH/NIGMS. Kenneth A. Taylor (PI). cryoEM Studies of Muscle. $441,815 Annual total costs. 01/01/2021 – 12/31/2025. This grant supports studies on the atomic resolution structure of thick filaments from striated muscle. Both vertebrate and invertebrate examples are being studied.
Role: Graduate Research Assistant

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Pouria Gholami Tilko

eRA COMMONS USER NAME (credential, e.g., agency login): POURIA_GHOLAMI-TIKLO

POSITION TITLE: Graduate student

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)	END DATE MM/YYYY	FIELD OF STUDY
University of Kurdistan	BS	06/2014	Biotechnology
University of Tehran	MS	12/2016	Microbial Biotechnology

A. Personal Statement

In my academic journey, I pursued a Bachelor's degree in Biotechnology and later specialized in Microbial Biotechnology during my Master's program. My passion for biology and the quantitative aspects of physics and mathematics drove me to explore the intersection of these fields. This led me to the field of molecular biophysics at FSU, which aligns perfectly with my academic interests.

In 2023, I became a part of Dr. Taylor's lab, where I embarked on a fascinating learning journey in applying cryo-electron microscopy (cryoEM). I have focused on utilizing this advanced technique to investigate the structural intricacies of thick filaments in skeletal muscles, using rabbit muscle as a model for vertebrates.

Notably, the structural puzzle of thin filaments in vertebrate skeletal muscles remains unsolved. Recognizing the significance of this gap, my research aims to contribute essential data to our understanding of muscle functionality. This pursuit extends to unraveling connections between muscle-related diseases and their root causes. By gaining insights into the structural nuances, I aspire to pave the way for potential, targeted cures for these conditions. My academic journey reflects a commitment to advancing scientific knowledge and addressing critical gaps in our understanding of muscle biology.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 - Graduate student , Florida State University - Tallahassee, FL, Tallahassee, FL

Honors

2014 Ranked 6th in the 19th National Biology Olympiad for university students in Iran, National Organization for Educational Testing

2013 Outstanding Student , University of Kurdistan

C. Contribution to Science

BIOGRAPHICAL SKETCH

NAME: Nishat, Zakia

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

POSITION TITLE: Graduate Research Assistant

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Shahjalal University of Science and Technology, Sylhet	BS	10/2021	Biochemistry and Molecular Biology
Florida State University, Tallahassee, FL	PHD	12/2027 (Expected)	Molecular Biophysics

A. Personal Statement

During my junior year of my undergraduate degree, I developed a profound interest in structural biology when I learned about protein structure determination in the protein biochemistry course. The notion of solving structure and getting insight into its function, which further contributed to answering biological questions, fascinated me. However, I never directly experienced using high-end electron microscopes due to the inaccessibility to cutting-edge structural biology instruments. Instead, I aimed to get into graduate school and be trained as a structural biologist. While I had initially set my mind to pursue structure solving using electron microscopes, I redirected my plans to a related technique that also involves structure solving; i.e., molecular dynamics simulation. I have become self-taught in molecular dynamics simulation which has helped me to gain experience with computational processes that involve playing with structures and to realize the power of physics in solving structures. I taught myself intensive physics such as molecular mechanics, force field, and ligand topology generation. Finally, I was able to perform 10 nanosecond protein-ligand dynamics and submitted that as my senior year project. Since I already experienced computational structural biology, my next step is to get trained in experimental structural biology which I am currently working on. My interest in being an academic was reinforced while carrying out other research projects. I rediscovered myself as neuroplastic and I believe that I can learn anything with continued perseverance. I worked under Dr. Ajit Ghosh on identifying RNA modification in plants during stress conditions using bioinformatics analysis which resulted in my first-first author publication in Current Plant Biology. Afterward, I worked with Dr. Mostafa Kamal Masud and Dr. Yusuke Yamauchi on developing a nano-biosensor to detect autoantibodies which led to the publication in ACS Nano and Small. My first love of structural biology never faded, so I applied to biophysics Ph.D. programs and got accepted into the Molecular Biophysics Ph.D. Program at Florida State University. I chose Dr. Taylor's lab since he is seasoned in training graduate students in cutting-edge CryoEM techniques. Currently, I am working on Actin-mutant flies where I will image flight muscle without Actin protein being present using an electron microscope. Thus, I will be able to contribute to the ongoing investigation of insect flight muscle contraction mechanisms and be trained in electron microscopy.

1. Kang Y, Masud MK, Guo Y, Zhao Y, **Nishat ZS**, Zhao J, Jiang B, Sugahara Y, Pejovic T, Morgan T, Hossain MSA, Li H, Salomon C, Asahi T, Yamauchi Y. Au-Loaded Superparamagnetic Mesoporous Bimetallic CoFeB Nanovehicles for Sensitive Autoantibody Detection. ACS Nano. 2023 Feb 28;17(4):3346-3357. PubMed PMID: 36744876.
2. **Nishat ZS**, Hossain T, Islam MN, Phan HP, Wahab MA, Moni MA, Salomon C, Amin MA, Sina AA, Hossain MSA, Kaneti YV, Yamauchi Y, Masud MK. Hydrogel Nanoarchitectonics: An Evolving Paradigm for Ultrasensitive Biosensing. Small. 2022 Jul;18(26):e2107571. PubMed PMID: 35620959.
3. **Nishat ZS**, Hasan M, Islam M, Hossain T, Ghosh A. Identification of epitranscriptomic methylation marker genes in Arabidopsis and their expression profiling in response to developmental, anatomical, and environmental modulations. Current Plant Biology. 2022 June 01; 30:100247. Available from: https://www.sciencedirect.com/science/article/pii/S2214662822000135 issn: 2214-6628

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 - Graduate Research Assistant, Florida State University, Tallahassee, FL

C. Contribution to Science

1. **Undergraduate research:** For my first project, I received training in bioinformatics analysis under the supervision of Dr. Ajit Ghosh at Shahjalal University of Science and Technology. During that time, I identified 29 putative regulatory genes specific to RNA modification in the model plant Arabidopsis by using bioinformatic tools and databases. Profiling their expression in usual conditions and stressed conditions, we found significant up-regulation and down-regulation which revealed their putative contribution to stress regulation. However, the biochemical mechanism of RNA modification in stress tolerance has yet to be revealed. Our findings work as a basis for the research field of plant epitranscriptomics which would further encourage researchers to figure out the stress tolerance mechanism biochemically. Later, I became involved in the nanotechnology field under the supervision of Dr. Mostafa Kamal Masud and Dr. Yusuke Yamauchi at the Australian Institution of Bioengineering and Nanotechnology (AIBN) and the University of Queensland, Australia, while pursuing my undergraduate degree at Shahjalal University of Science and Technology. I carried out the electrochemical assay for detecting p53 autoantibody specific to ovarian cancer using the AuCoFeB biosensor. I used the Square Wave Voltammetry technique to detect the presence of p53 autoantibody and the limit of detection was 0.006 U/mL which is more sensitive than a conventional Au-based chip detection system that can detect 100ng/mL. I also drafted an extensive review on hydrogel nano-biosensors that contributed to biosensor literature.

2. **Graduate Research:** Currently, I am being trained as a structural biologist, specifically as an electron microscopist. I have begun a project to determine the structure of the flight muscle of Actin mutant flies. Once I have determined the structure, then I will compare my findings with the wild-type flies which will result in new insights about muscle contraction mechanisms.

    a. Kang Y, Masud MK, Guo Y, Zhao Y, **Nishat ZS**, Zhao J, Jiang B, Sugahara Y, Pejovic T, Morgan T, Hossain MSA, Li H, Salomon C, Asahi T, Yamauchi Y. Au-Loaded Superparamagnetic Mesoporous Bimetallic CoFeB Nanovehicles for Sensitive Autoantibody Detection. ACS Nano. 2023 Feb 28;17(4):3346-3357. PubMed PMID: 36744876.

    b. **Nishat ZS**, Hossain T, Islam MN, Phan HP, Wahab MA, Moni MA, Salomon C, Amin MA, Sina AA, Hossain MSA, Kaneti YV, Yamauchi Y, Masud MK. Hydrogel Nanoarchitectonics: An Evolving Paradigm for Ultrasensitive Biosensing. Small. 2022 Jul;18(26):e2107571. PubMed PMID: 35620959.

    c. **Nishat ZS**, Hasan M, Islam M, Hossain T, Ghosh A. Identification of epitranscriptomic methylation marker genes in Arabidopsis and their expression profiling in response to developmental, anatomical, and environmental modulations. Current Plant Biology. 2022 June 01; 30:100247. Available from: https://www.sciencedirect.com/science/article/pii/S2214662822000135 issn: 2214-6628