BIOGRAPHICAL SKETCH

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NAME: Nixon, B. Tracy

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

POSITION TITLE: Professor of Biochemistry & Molecular Biology

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 Missouri, Columbia	BA	05/1976	Secondary Education (Chemistry / German)
University of Missouri, Columbia	MS	08/1977	Genetics
Massachusetts Institute of Technology	PHD	08/1982	Cell Biology
Harvard University, Boston	Postdoc	8/82-8/83	Physiology
Massachusetts General Hospital/Harvard Genetics	Postdoc	8/83-12/86	Molecular Biology

A. Personal Statement

My long-term goal is to learn how molecular machines function in bacterial two-component signaling and transcription activation, and cellulose biosynthesis in plants. Two-component signal transduction activates ATPases that deliver vectored force to alter $E\sigma 54$ enabling it to melt promoters to regulate gene expression in bacteria. Cellulose is the main structural component of plant cell walls and is being developed as a renewable biofuel. Access to the NYSBC cryoEM facility sought in this proposal will support our studies of the assembly of cellulose synthase into trimers, further assembly of six trimers into the full cellulose synthase complex, and structural changes in trimer and full assembly that are associated with glucan chain synthesis and their coalescence into cellulose microfibrils. We anticipate that understanding this molecular machine will allow pursuit of altered cellulose that is more amenable to harvesting as a renewable biofuel and development of novel cellulose-based materials. My opportunities to work on this machine arise from our recent progress in the Center for Lignocellulose Structure and Formation, a DOE EFRC centralized at Penn State but involving coinvestigators at MIT, Cambridge University, University of Rhode Island, University of Virginia, North Carolina State University, and the Oak Ridge National Lab.

As a PhD student at MIT I conducted cell biology experiments, which I expanded to include biochemical and then structural studies to complement genetic and molecular biological approaches, administering grants funded by the NSF, DOE, USDA and NIH. Via this collection of independent and collaborative studies, I've learned that frequent communication among project members nurtures a real commitment by all parties, and constructing a realistic research plan, timeline, and budget are essential. In the latter part of my career, I have a demonstrated record of successful and productive research projects in an area of high relevance for understanding the AAA+ biological motor proteins that control the $E\sigma 54$ transcription apparatus and overcome two 30-year-old bottlenecks while developing *in vitro* cellulose synthesis from purified enzyme.

My training for research that will be supported by the requested access to the Aquilos 2 cryoFLM/cryoFIB milling device at the NYSBC emerges from three areas. First, I published groundbreaking work on the stoichiometry of CesA in the cellulose synthase complex (Nixon et al., *Science Reports,* 2016; Vandavasi et al., *Plant Physiology,* 2016) and the preparation of purified CesA from vascular and nonvascular plants (Puroshotham et al., *Proc. Natl. Acad. Sci. USA,* 2016; Cho et al., *Plant Physiol*. 2017). To extend my abilities, I learned at worshops how to perform 3D reconstructions from TEM images (random conical tilt reconstructions, Michael Radermacher; cryo-electron tomography, David Masternade and others, Vienna, Austria, May 10-18, 2019). My lab has published a negative stain 3D reconstruction of the bacterial cellulose synthase enzyme from

Gluconacetobacter hansenii, and just submitted a paper confirming our predicted stoichiometry by showing that the cytosolic domain of CESA1 from *Arabidopsis thaliana* is trimeric, mediated by a region called the PCR. Meanwhile, we combined cryo-tomography and subtomogram averaging with a novel ssNMR approach to show that *in vitro* synthesized cellulose fibrils closely resemble cellulose microfibrils in Arabidopsis cell walls (manuscript submitted). Finally, working with collaborator Andrew Neuwald, we applied the bioinformatics program DARC to identify residues essential for CESA function, developing a new module (SPARC) to examine MD simulations to raise novel mechanistic hypotheses (manuscript submitted). Finally, we have developed conditions for inducing secondary wall synthesis in protoplasts of Arabidopsis to screen mutations suggested by SPARC analysis and to provide cells for cryoFLM/cryoFIB milling for cryoET, which is the subject of this proposal.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

1982-1983 Postdoctoral research with Dr. H. Green, Harvard Medical School, Physiology.
1983-1986 Postdoctoral research with Dr. Frederick M. Ausubel, Harvard Medical School, Genetics and Massachusetts General Hospital, Molecular Biology.
1987-1993 Assistant Professor of Molecular and Cell Biology, Pennsylvania State University.
1993-2008 Associate Professor of Biochemistry and Molecular Biology, Pennsylvania State University.
2008-present Professor of Biochemistry and Molecular Biology, Pennsylvania State University.
7/02-11/02 Visiting Researcher, Dept of Plant and Microbial Biol., Univ. California at Berkeley, Berkeley, CA.
11/02-8/03 Visiting Researcher, Lawrence Berkeley Lab, University of California at Berkeley, Berkeley, CA.
7/10-3/11 Visiting Scholar, Chemistry Department, Princeton University, Princeton, NJ.
3/11-5/11 Visiting Scholar, Biochemistry Department, City University of New York, NY, NY.
2/16-present Adjunct Professor, Darst Laboratory of Molecular Biophysics, Rockefeller, NY, NY.

Honors

Undergraduate: Member Delta Phi Alpha, German Honor Society, 1973; Member Phi Kappa Phi, National Honor Society, 1975; Member Kappa Delta Pi, Education Honor Society, 1976; University Merit Scholar - tuition grant, 1972-1976; Graduated Outstanding Male Senior, College of Education, 1976;

Graduate: John M. Dalton Fellowship, 1977; Harry-Eagle Award, Tissue Culture Association, 1977; NIH-predoctoral Training Grant, 1977-1981.

Professor: Co-recipient of the DOE EFRC Ten at Ten Scientific Ideas Award for elucidating structure and function of plant cellulose synthase and cellulose synthase complex. July 29, 2019.

Invited Talks: 6th International Meeting on AAA Proteins, 2005, 2007; FASEB sponsored meeting "Positive control of transcription initiation in prokaryotes", Saxtons River, Vermont. 7/91; 7/99; 7/01; 6/03; 6/07; 6/11; EMBO-Juan March Foundation Workshop on Transcription Regulation at a Distance, Madrid, Spain, 1-15 to 1-17, 1996; John Ingraham Speaker, West Coast Bacterial Physiologist Meeting, Asilomar, CA. 12/15/02; 6/06. Prot. Soc. Annual Meeting, San Diego, CA. 7/08; SAXS Commission of the IUCr Annual Meeting, Osaka, Japan, 8/08; BNL NSLS, 03/09; 05/11; ASBMB, 04/09; Beckman Center, Urbana, IL 3/10. 9th International Conference on AAA Proteins, Kumamoto, JP, 11/11; Biophysical Society Ann. Meeting 03/11; ORNL Workshop "Future of Neutron Scattering" 04/11; Am Cryst. Assoc. 06/14; DOE EFRC review of CLSF, 1/16; XIV Cell Wall Meeting, Chania, Crete, 6/16; ACS, 04/17; CELL subgroup of the ACS annual meeting, 4/17; XV Cell Wall Meeting, Cambridge, UK, 7/19.

Journal Covers: *FASEB J* 03/01, 12/02; *Genes & Dev* 10/03, 06/06; *Structure* 04/07; *J. Mol. Biol.* 06/07, *Structure* 11/10; *Plant Physiology*, 1/2016.

C. Contributions to Science

1. **Dual Effector Theory of Growth Hormone Action.** In my PhD and first postdoctoral studies I used a tissue culture model to study differentiation of preadipocytes into fat cells. In addition to contributing to the discovery that growth hormone directly stimulated differentiation of preadipocytes, I learned that 50% of the stimulating activity in fetal calf serum was growth hormone, and most importantly, that growth hormone acted this way by *directly binding to receptors on the preadipocytes*. I also showed that muscle differentiation could likewise be stimulated by growth hormone. Observing direct action by growth hormone was so novel that it forever changed the field, forcing modification of the 80-year-old and widely accepted theory that growth hormone stimulated mesenchymal tissue growth only indirectly via somatomedins made by liver in response to the hormone. Although 36 years old, our dual effector model was cited 11 times in the last 6 years. In a current and detailed review of growth hormone and prolactin, the indirect and direct actions of growth hormone are introduced with reference to our publication (Kelley et al., 2014 in *Rec Prog Horm Res.* 48, 123-163).

a) Morikawa, M., Nixon, T. & Green, H. (1982). Growth hormone and the adipose converstion of 3T3 cells. Cell 29, 783-789.
b) Nixon, T. & Green H. (1982). Properties of growth hormone receptors in relation to the adipose conversion of 3T3 cells. *J Cell Physiol.* 15, 291-296.
c) Nixon, T. & Green, H. (1984). Contribution of growth hormone to the adipogenic activity of serum. *Endocrinology* 114, 527-532.
d) Green, H., Morikawa, M. & Nixon, T. (1985). A dual effector theory of growth-hormone action. *Differentiation* 29, 195-198. Cited 462 times.

2. **The Two-Component Signal Transduction Paradigm.** For my second and major postdoctoral studies I focused on gene regulation inside cells, asking which genes and gene products responded to external signals to direct differentiation, this time of bacteria. By applying 'bioinformatics' before the word existed, I found sequence similarity in two categories of genes that were known to work with each other in pairs to control different bacterial behaviors. I coined the phrase 'two-component regulatory systems' to contrast these with the status quo thinking in which gene regulation was mediated by single transcriptional regulators whose functional states were modified by allosteric binding of a small molecule. Departing from that picture, I described for the first time the two-component model of one protein phosphorylating another in response to environmental stimuli perceived by bacteria. The Pfam database currently lists 375,727 two-component receiver domains, arranged in 10,039 domain architectures in 8,151 species of bacteria, archea and eukaryotes. This work was a lifetime achievement, in that it gave theoretical context and a new focus to researchers studying a plethora of biological systems. My esteemed mentor Fred Ausubel cites it among his group's key accomplishments (Ausubel, 2014, *Genetics* 198, 431-434).
a) Nixon, B.T., Ronson, C.W. & Ausubel, F.M. (1986). Two component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. *Proc. Natl. Acad. USA* 83, 7850-7854.
b) Ronson, C.W., Nixon, B.T. & Ausubel, F.M. (1987). Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. *Cell* 49, 579-581.
c) Ronson, C.W., Nixon, B.T., Albright, L.M. & Ausubel, F.M. (1987). *Rhizobium meliloti ntrA* (rpoN) gene is required for diverse metabolic functions. *J. Bacteriol.* 169, 2424-2431.
d) Ronson, C.W., Astwood, P.M., Nixon, B.T. & Ausubel, F.M. (1987). Deduced products of C_4-dicarboxylate transport regulatory genes of *Rhizobium legumniosarum* are homologous to nitrogen regulatory gene products. *Nuc Acids Res.* 15, 7921-7934.

3. **Unexpected diversity in physical mechanism of two-component signal transduction.** At Penn State, my curiosity focused on how the receiver domains regulated the ATPase domains of EBPs. I learned crystallography to explore this, and first showed that regulation could be negative, with the apo receiver domain repressing the ATPase until phosphorylation triggered derepression to allow spontaneous assembly of the ATPase to its functional, oligomeric ring state. Collaborating with Nogales and De Carlo, I then discovered a dramatically different mechanism worked in a related EBP in which the phosphorylated receiver domain promotes ring assembly by stabilizing its final form. The status quo expectation that once one example of regulation in an EBP was understood all others would be too could not hold up in the light of our discovery, illustrating how unexpected diversity exists in the atomic mechanisms of two-component signal transduction even among closely related proteins. Among the papers describing those studies, three garnered cover illustrations (*FASEB J*, 2001; 2002, and *Genes & Development*, 2006). HHMI researcher Ann Stock nominated our papers for 'Faculty of 1000' status and highlighted the work in a review (Gao & Stock, 2009, *Annu Rev Microbiol.* 63, 133-154). At the time of our studies, it was also widely believed that SAXS data can only yield properties such as radius of gyration and molecular weight. We helped the scientific community depart from that status quo belief by effectively illustrating the power of *ab initio* models from SAXS data that were consistent with but also extending knowledge gained by 3D reconstructions from EM particles.
a) Jiang, J., Gu, B.H., Albright, L.M. & Nixon, B.T. (1989). Conservation between coding and regulatory elements of Rhizobium meliloti and Rhizobium leguminosarum dct genes. *J. Bacteriol.* 171, 5244-5253.
b) Lee, J.H., Scholl, D., Nixon, B.T. & Hoover, T.R. (1994). Constitutive ATP hydrolysis and transcription activation by a stable, truncated form of Rhizobium meliloti DctD, a sigma54-dependent transcriptional activator. *J Biol Chem.* 269, 20401-20409.
c) Park, S., Meyer, M.G., Jones, D.A., Yennawar, H.P., Yennawar, N.H. & Nixon, B.T. (2002). Two component signaling in the AAA+ ATPase DctD: binding Mg^{2+} and BeF_3^- selects between alternate dimeric states of the receiver domain. *FASEB J* 16, 1964-1966. Cover article.
d) De Carlo, S., Chen, B., Hoover, T.R., Kondrashkina, E., Nogales, E. & Nixon, B.T. (2006). The structural basis for regulation and function of the transcriptional activator NtrC. *Genes & Dev* 20, 1485-1495.

4. **R-finger induced rigid body role and nucleotide-driven asymmetry in the mechanism of AAA+ ATPase function.** We complemented two component regulatory domain studies with structure and function studies of the ATPase itself. In collaboration with Wemmer and Kustu, we provided the first atomic structure of a σ54-dependent AAA+ ATPase (cover article in *Genes & Dev*, 2003), and subsequently helped to advance structural understanding of σ54/DNA interactions (cover article – *J. Mol. Biol.*, 2007). Working independently, my group was among the first to use SAXS data for *ab initio* modeling of proteins, showing that loop conformations stabilized by ground and transition state analogs of ATP triggered binding of ATPase to the σ54 sigma factor of RNAP. That cover article (*Structure*, 2007) called broad attention to the use of SAXS and the exquisite sensitivity of AAA+ ATPases to different nucleotides. We then described the trigger mechanism in EBPs, discovering that an arginine sensor of one subunit drives a rigid body roll to raise loops for contacting σ54 when the R-finger sensor detects ATP bound to the neighboring subunit. That cover article (*Structure*, 2010) was described in the same issue via an invited prospective by Hoover et al. Turning our attention to the σ54-RNAP, we enticed and assisted Richard Ebright to include σ54-RNAP and NtrC1 in single molecule studies of the polymerase's clamp domain in closed and opened complexes with promoter DNA (Chakraborty et al., 2012, *Science* 337, 591-595). Most recently, we helped develop and use novel time resolved SAXS at the APS, which we combined with crystallography and 3D reconstruction from EM particles to reveal asymmetry in the chemically identical subunits of a homomeric EBP. We found that each subunit integrates its and its neighbor's experiences with ATP to form a split ring with spiral of surface loops arranged for contacting Eσ54 bound to promoter DNA (Sysoeva et al., *Genes Dev,* 2013). The APS honored the paper as a feature study, and the editor of *Cell Cycle* invited us to write a featured editorial (*Cell Cycle* 10, 2014).

a) Lee, S.Y., De La Torre, A., Yan, D., Kustu, S., Nixon, B.T. & Wemmer, D.E. (2003). Regulation of the transcriptional activator NtrC1: structural studies fo the regulatory and AAA+ ATPase domains. *Genes Dev.* 17, 2552-2563. Cover article.
b) Chen, B., Doucleff, M., Wemmer, D.E., De Carlo, S., Huang, H.H., Nogales, E., Hoover, T.R., Kondrashkina, E., Guo, L. & Nixon, B.T. (2007). ATP ground and transition states of bacterial enhancer binding AAA+ ATPases support complex formation with their target protein, sigma54. *Structure* 15, 429-440. Cover article.
c) Chen, B., Sysoeva, T.A., Chowdhury, S., Guo, L., De Carlo, S., Hanson, J.A., Yang, H. & Nixon, B.T. (2010). Engagement of arginine finger to ATP triggers large conformational changes in NtrC1 AAA+ ATPase for remodeling bacterial RNA polymerase. *Structure*, 18, 1420-1430. Cover article.
d) Sysoeva, T.A., Chowdhury, S., Guo, L. & Nixon, B.T. (2013). Nucleotide-induced asymmetry within ATPase activator ring drives σ54-RNAP interaction and ATP hydrolysis. *Genes Dev* 27, 2500-2511.

5. **Stoichiometry and function of cellulose synthase complex.** More than thirty years ago, freeze-fracture TEM images of the cellulose synthase complex revealed its 'rosette' structure, with 6 lobes organized into a circular machine of about 25 nm diameter. Until recently, it was presumed that each lobe contained 6 CesA proteins, thus a total of 36 in the full rosette, and that the resultant cellulose microfibril has 36 glucan chains. I applied modern classification tools to a collection of rosette images and molecular modeling to show that the lobes are trimeric, replacing the status quo with a new model. Collaborative work with O'Neill at ORNL showed that the catalytic subdomain of CesA forms trimers in solution, and we obtained an unpublished reconstruction of that trimer from negative stain particles (manuscript submitted). Collaborating with Zimmer, we have also learned how to purify full length CesA that when reconstituted into liposomes is functional *in vitro*. Not just making glucan chains, the membrane embedded enzyme makes microfibrils similar to those found in cell walls (manuscript submitted). On July 29, 2019 the DOE honored our elucidation of the structure and function of plant cellulose synthase and cellulose synthase complex via their Ten at Ten Scientific Ideas Award. Collaborator Zimmer's group recently capped that accomplishment by publishing the first high resolution structure of a trimeric CesA, confirming, and extending our prior work. Co-applicant Sarah Pfaff has recently developed an *in vitro* system for inducing secondary wall synthesis in plant protoplasts, showing in preliminary work that cryoFLM/cryoFIB milling are adaptable for cryo-electron tomography study of the enriched cellulose synthase complexes and microfibrils. *This work sets the foundation for our use of the Aquilos2 being sought via this proposal to the NYSBC.*

a) Nixon, B.T., Mansouri, K., Singh, A., Du, J., Davis, J.K., Lee, J.-G., Slabaugh, E., Vandavasi, V.G., O'Neill, H., Roberts, E.M., Roberts, A.W., Yingling, Y.G., Haigler, C.H. (2016). Comparative Structural and Computational Analysis Supports Eighteen Cellulose Synthases in the Plant Cellulose Synthesis Complex. *Sci. Rep.* 6:28696.
b) Vandavasi, V.G., Putnam, D.K., Zhang, Q., Petridis, L., Heller, W.T., Nixon, B.T., Haigler, C.H., Kalluri, U., Coates, L., Langan, P., Smith, J.C., Meiler, J., O'Neill, H. (2016). A Structural Study of CESA1 Catalytic Domain of Arabidopsis Cellulose Synthesis Complex: Evidence for CESA Trimers. *Plant Physiol.* **170,** 123-135. Cover article.
c) Purushotham P., Cho S.H., Díaz-Moreno S.M., Kumar M., Nixon B.T., Bulone V., Zimmer J. (2016). A single heterologously expressed plant cellulose synthase isoform is sufficient for cellulose microfibril formation in

vitro. *Proc Natl Acad Sci USA*. Sep 19. pii: 201606210. [Epub ahead of print] PubMed PMID: 27647898.

d) Cho, S.H., Purushotham, P., Fang, C., Maranas, C, Díaz-Moreno, S.M., Bulone, V., Zimmer, J., Kumar, M., Nixon, B.T. (2017). *Plant Physiol.* 175(1):146-156. Synthesis and Self-Assembly of Cellulose Microfibrils from Reconstituted Cellulose Synthase. PMID: 28768815.

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/1ZwRDklMigh57/bibliography/47347787/public/?sort=date&direction=ascending

D. Additional Information: Research Support

DOE DE-SC0001090 Cosgrove, D. (PI) 08/01/14-07/31/22
Center for Lignocellulose Structure and Formation: Structural dynamics of cellulose synthase revealed by cryoEM and DTEM.
The Center for Lignocellulose Structure and Formation is a DOE Energy Frontier Research Center lead by PI Daniel Cosgrove. The research center aims to develop a detailed nano- to meso-scale understanding of plant cell wall structure and its mechanism of assembly to provide a basis for improved methods of converting biomass into fuels. The project Nixon directs as PI is to acquire structural knowledge of the biological machine called 'rosette' that organizes multiple cellulose synthases to yield crystalline microfibers of cellulose for making plant cell walls.
Role: PI

Penn State Seed Funds Nixon (PI) 10/15/14-indefinite
Understanding Structural Asymmetry in AAA+ ATPase/σ54-RNAP/Promoter Complex
This is seed money from the Eberly College of Science and the Department of Biochemistry and Molecular Biology at Penn State to support preliminary work for the current proposal.
Role: PI

Completed Research Support (selected entries)

NIH 1S10OD026822-01 Hafenstein, S. (PI) 9/16/2019-8/20/2020
Project Title: HEI: Acquisition of a Talos Arctica G2
The Huck Institutes of Science at Penn State received funding to acquire a second TEM for cryoEM studies at Penn State University Park campus.
Role Co-investigator.

DOE DE-SC0006838 Yang (PI), Nixon (Co-PI), Tien (Co-PI) 10/1/2011-9/30/2014
Development of Quantum Dot Probes for Studies of Synergy Between Components of Wood-Degrading Fungal Enzymes
The goal was to develop a fungal system for secretion and tagging of recombinant proteins with quantum dots that can then be used in single molecule experiments to study the processivity and synergy between fungal cellulases.
Role: Nixon served as the PI of a subcontract from Princetion (DOE PRIME) to Penn State.

DOE DE-SC0001090 Cosgrove, D. (PI), Nixon (Co-PI) 8/1/2013 – 4/31/2013
DOE's Center for Lignocellulose Structure and Formation: 3D Image Reconstructions of Rosette-like Structures of Plant Cellulose Synthase
This was a seed grant for developing a cryo-EM project to determine the structure of the machine that synthesizes cellulose in higher plants, resulting in the above currently funded project.
Role: PI of the listed CLSF project.

BIOGRAPHICAL SKETCH

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NAME: SWULIUS, MATTHEW

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

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)	END DATE MM/YYYY	FIELD OF STUDY
Baylor University, Waco, Texas	BS	08/2002	Neuroscience
University of Texas Health Science Center, Houston, Texas	PHD	05/2010	Neuroscience
Caltech, Pasadena, California	Postdoctoral Fellow	09/2017	Cellular Cryotomography

A. Personal Statement

I am an expert in cryo-electron microscopy (cryo-EM), with more than 15 years of experience imaging purified protein complexes, cell extracts and whole cells (both bacterial and mammalian), and I have a history of publications using correlative cryo-fluorescence microscopy and cryo-FIB milling to investigate structures by cryo-electron tomography. I also have a history of bringing new technology to the labs I work in, and the design and execution of the newly expanded cryo-EM core facility at the Penn State College of Medicine is my most significant feat in that area. It has been running smoothly now for over two years and will serve as resource for this project. In addition to bringing this new facility to full functionality, I have been building my own lab's research program to study cytoskeletal remodeling in primary rat neuronal cultures. In my lab, we use live-cell fluorescence imaging and cryo-ET to gain insight across scales of both space and time.

B. Positions, Employment and Honors

2002 - 2004	Research Technician, Baylor College of Medicine, Department of Pathology, Houston, TX
2010 - 2017	Postdoctoral Fellow, Caltech, Division of Biology, Pasadena, CA
2017 -	Faculty Director, Penn State College of Medicine, CryoEM Core Facility, Hershey, PA
2017 -	Assistant Professor, Penn State College of Medicine, Department of Biochemistry and Molecular Biology, Hershey, PA

Other Experience and Professional Memberships

2007 -	Member, Society for Neuroscience
2011 - 2016	Member, American Society for Microbiology
2014 -	Member, Biophysical Society

Honors

2010 Dee S. and Patricia Osborne Endowed Scholarship in Neuroscience
University of Texas Health Science Center, Houston, TX

1st place in Neuroscience Research Center Poster Competition
University of Texas Health Science Center, Houston, TX

2009 2nd Place in Graduate Student Education Committee Poster Competition
University of Texas Health Science Center, Houston, TX

2005-
2007 *Training in Neuroscience* Training Grant
University of Texas Health Science Center, Houston, TX

C. Contribution to Science

1. Since starting my own lab, I have written a book chapter describing workflows for CET of cultured neurons and their automated segmentation by trained artificial intelligence. I am also co-corresponding author on a study with my Penn State colleague Federico Harte, using tomography to study changes is morphology that correspond with changes in the chemical constituency of milk micelles. I have written an invited review for a special issue of iScience on correlative light and electron microscopy, and we are currently revising our lab's first manuscript which describes interactions between actin and cofilin in neuronal growth cones during cellular outgrowth.

    a. Hylton, R. K., Heebner, J., Grillo, M., & **Swulius, M. T.** (In Revision). Cofilin Regulates Filopodial Structure and Flexibility in Neuronal Growth Cones. *Nature Communications.*

    b. Hylton RK, **Swulius M.T.** Challenges and triumphs in cryo-electron tomography. iScience. 2021 Aug 8;24(9):102959. doi: 10.1016/j.isci.2021.102959. PMID: 34466785; PMCID: PMC8383006.

    c. Hylton, R. K., Seader, V. H., & **Swulius, M. T.** (2021). Cryo-Electron Tomography and Automatic Segmentation of Cultured Hippocampal Neurons. *Methods in Molecular Biology (Clifton, N.J.)*, *2215*(Chapter 2), 25–48. http://doi.org/10.1007/978-1-0716-0966-8_2

    d. Hettiarachchi, C., **Swulius, M.T.**, and Harte, F. Determination of constituent volumes in bovine casein micelles using cryo-electron tomography*. International Dairy Journal*. 2020. PMID: 32171507

2. In my PhD, I used electron cryotomography and biochemical methods to study the structure/composition of postsynaptic densities isolated from rat brain at different developmental stages. Using quantitative analysis of the protein composition and morphology of isolated synaptic structures, my work confirmed a model of postsynaptic density assembly where early scaffolding molecules are put in place, followed by the recruitment of receptors and downstream signaling enzymes.

    a. Farley MM, **Swulius MT**, Waxham MN. Electron tomographic structure and protein composition of isolated rat cerebellar, hippocampal and cortical postsynaptic densities. *Neuroscience*. 2015 Sep 24;304:286-301. PubMed PMID: 26215919; PubMed Central PMCID: PMC4547907.

b. **Swulius MT**, Farley MM, Bryant MA, Waxham MN. Electron cryotomography of postsynaptic densities during development reveals a mechanism of assembly. *Neuroscience*. 2012 Jun 14;212:19-29. PubMed PMID: 22516021; PubMed Central PMCID: PMC3367029.

c. **Swulius MT**, Kubota Y, Forest A, Waxham MN. Structure and composition of the postsynaptic density during development. *J Comp Neurol*. 2010 Oct 15;518(20):4243-60. PubMed PMID: 20878786; PubMed Central PMCID: PMC2948241.

3. During the first half of my postdoc in Grant Jensen's lab at Caltech, my training focused on the study of the bacterial actin homologue MreB, which was thought to form an extended helical cytoskeleton under the membrane of nearly all rod-shaped bacteria (such as E. coli). I published two first author studies using ECT and correlated cryo-fluorescence microscopy showing that this is false, because there is no long helical cytoskeleton in a variety of rod-shaped bacteria, and the YFP-MreB used in pioneering studies formed artifactual helical filaments under the membrane. My first paper in Grant's lab was one of the first studies to use correlated cryo-fluorescence and cryo-electron microscopy to identify a subcellular protein structure (a bundle of GFP-tagged MreB filaments). My work helped transform the field of bacterial cell biology, which, over a decade, had grown full of models for cell shape, motility, polarity, growth and chromosome segregation that built on a helical MreB cytoskeleton.

   a. **Swulius MT**, Jensen GJ. The helical MreB cytoskeleton in Escherichia coli MC1000/pLE7 is an artifact of the N-Terminal yellow fluorescent protein tag. *J Bacteriol*. 2012 Dec;194(23):6382-6. PubMed PMID: 22904287; PubMed Central PMCID: PMC3497537.

   b. **Swulius MT**, Chen S, Jane Ding H, Li Z, Briegel A, Pilhofer M, Tocheva EI, Lybarger SR, Johnson TL, Sandkvist M, Jensen GJ. Long helical filaments are not seen encircling cells in electron cryotomograms of rod-shaped bacteria. *Biochem Biophys Res Commun*. 2011 Apr 22;407(4):650-5. PubMed PMID: 21419100; PubMed Central PMCID: PMC3093302.

4. During the second half of my postdoc I studied actomyosin-based cell division machinery in the yeast Schizosaccharomyces pombe. To accomplish this, I used a combination of cryosectioning, cryo-focused ion beam milling, and correlated cryo-fluorescence microscopy against a GFP-tagged myosin. Due to the complex dynamic nature of actomyosin machinery, we used novel structural details from ECT to guide the development of 3D coarse-grained models and explored the mechanistic implications of the ECT results. Our work pointed to a detailed model for the actomyosin ring architecture that can recapitulate both my ECT data as well as previously published fluorescence microscopy data. This interdisciplinary approach is powerful, because it uses experimental structural data to constrain and refine the modeling, and allowed us to test explicit interpretations of cryoEM images of complex and dynamic machinery.

   a. **Swulius MT**, Nguyen LT, Ladinsky MS, Ortega DR, Aich S, Mishra M, Jensen GJ. Structure of the fission yeast actomyosin ring during constriction. *Proc Natl Acad Sci* U S A. 2018 Feb 13;115(7):E1455-E1464. PubMed PMID: 29348205; PubMed Central PMCID: PMC5816141.

   b. Nguyen, L. T., **Swulius, M.T**., Mishra, M. and Jensen, G.J. (2018). Coarse-grained simulations of actomyosin rings point to a node-less model involving both unipolar and bipolar myosins. *Mol Biol Cell.* Jun 1;29(11):1318-1331.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/matthew.swulius.1/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

4100079742, TSF Strategic Research Award
SWULIUS, MATTHEW (PI)
06/01/18-06/01/2022
Structural Investigation of the NMDA receptor/CaMKII supercomplex
The goal of this study is to investigate the structure of the NMDA receptor in complex with Calcium/calmodulin-dependent kinase II using both electron cryotomography and single particle analysis
Role: PI

Completed Research Support

PSU/COM, Material Characterizations Lab seed grant
Swulius (PI)
06/01/18-12/30/20
Controlled assembly of artificial synapses for high-resolution electron imaging
The goal of this seed grant funding is to foster relations between material and life scientists between the Penn State College of Medicine and the Materials Research Institute at University Park, and it focuses on using Focused Ion Beam milling to prepare samples for high resolution imaging of synapses in a reduced-complexity environment.
Role: PI

BIOGRAPHICAL SKETCH

NAME: PFAFF, SARAH

eRA COMMONS USER NAME (credential, e.g., agency login): Not funded by NIH, so not registered.

POSITION TITLE: Ph.D. Candidate

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Rutgers University, New Brunswick, NJ	B.S.	05/2017	Biotechnology and Plant Science (double major)
Pennsylvania State University, University Park, PA	Ph.D.	expected 2022-2023	Plant Biology

A. Personal Statement

I am a highly self-motivated Ph.D. candidate who has spent the last four years of my thesis research developing and optimizing a novel system for the study of plant secondary cell wall (SCW) development. A system of this kind has not yet been published and I am currently implementing it in a variety of different studies of cell wall synthesis. As a tissue-free single-cell system, protoplasts provide many advantages for study of nascent SCW regeneration in the model species *Arabidopsis thaliana*. Being the sole developer of this new system, I am intimately aware of every critical parameter required for successful transdifferentiation of protoplasts. Years of dedicated focus has enabled me to overcome the countless obstacles presented by working in a brand new system. I have learned a variety of imaging and analysis methods from other researchers and then personally devised mechanisms by which to successfully apply them to my system. As a constant innovator, I never back away from a challenge. It is with this focus and dedication that I approach learning cryo-electron tomography (CET) to advance structural knowledge of cellulose biosynthetic machinery.

Since early in my graduate school career I have been eager to learn cryo-electron microscopy, but was not equipped to conduct research in this field. As I developed my protoplast system and demonstrated its efficacy in a variety of research areas, I attracted collaborators and forged the connections necessary to undertake such a project. Under the mentorship of Dr. Tracy Nixon and Dr. Matt Swulius, I have recently gained practical skill in the areas of vitrification by plunge freezing and focused ion beam (FIB) milling of my SCW-regenerating protoplasts. My careful and focused effort over the past months has allowed us to make great preliminary strides in this project as I have ironed out the key parameters necessary for the successful vitrification of actively-regenerating protoplasts on EM grids. I am extremely eager to continue my training in the pursuit of proficiency in CET, from sample preparation through to image acquisition and reconstruction.

My training on the Aquilos 2 at the New York Structural Biology Center will not only assist in our endeavor to solve the first near atomic resolution structure of cellulose synthase *in vivo*, but it also will support my personal goal of becoming a leader in the field of CET. As of yet, very little CET work has been done in plant systems. I seek to bridge the gap between cutting-edge techniques and the field of plant biology, where new knowledge will promote a more sustainable, food-secure future. My relentless dedication has allowed me to overcome the challenges of developing a completely novel system and then successfully applying it in new research areas in which I had no previous expertise. It is with the same dedication that I endeavor to become proficient in CET and advance our understanding of the cellular biosynthetic machinery in plants.

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B. Positions and Honors

Laboratory Assistant at Chromocell Corporation, North Brunswick, NJ (December 2013 - January 2015)

Research Assistant for Dr. Eric Lam at Rutgers University, New Brunswick, NJ (March 2014 - May 2017)

Research Assistant for Dr. Courtney Jahn with the Colorado Center for Biorefining and Bioproducts (NSF REU) at Colorado State University, Fort Collins, CO (May - July 2016)

Ph.D. Candidate with Dr. Daniel Cosgrove at Pennsylvania State University, Huck Institute of the Life Sciences (Interdepartmental Graduate Program in Plant Biology), University Park, PA (Aug 2017 - present)

2016 and Earlier

Rutgers University Academic Excellence Award (graduated top 10% of class)	
Internship Excellence Award	$200
Philip E. Maurcci Scholarship	$2,250
Best Poster Award for NSF REU Poster Session (Conference Travel Funds)	$1000

2017 to Present

American Society of Plant Biology (ASPB) Conference Travel Award	$500
NSF Graduate Research Fellowship Program (GRFP)	Honorable Mention
Eva J. Pell Distinguished Graduate Fellowship	$28,750
Huck Institute Fellowship Funds	$10,000
Robert W. Graham Endowed Fellowship	$8,000
J. Ben and Helen D. Hill Memorial Fund	$2,000
2nd Place Winner of I AM STEM speaking competition	$100
Keynote Speaker for 2021 ENVISION Conference - PSU Science-U Outreach Event	

C. Contributions to Science

Enabling the use of plant biomass as a renewable energy source has been the focus of my research goals through the various labs in which I have conducted research. In a variety of different ways, I have combined a passion for renewable energy with a focus on basic Biology research to enable use of plant material for biofuel.

1) Characterization of the aquatic plant, duckweed, for use in phytoremediation and conversion to bioenergy

As an undergraduate at Rutgers University I spent seven semesters conducting research in Dr. Eric Lam's lab, optimizing the use of the aquatic plant, duckweed, for bioremediation of wastewater and its subsequent conversion to a renewable energy source. Our lab partnered with a non-profit organization in Argentina (Njambre) to implement the first real-world application of duckweed as a bioremediation solution, to demonstrate its efficacy for countries without access to sufficient wastewater treatment options. In this collaboration I characterized the phytoremediation ability of several strains of duckweed endemic to Argentina by quantifying their growth rate, removal of nitrogen and phosphorus from wastewater, and accumulation of protein and starch for conversion to usable products. I presented my findings to our collaborators at the Rosario Institute of Biotechnology and took part in the implementation of these duckweed strains on wastewater treatment plants in Totoras and Salta, Argentina. While in the Lam Lab I also helped characterize the microbiome of duckweed and identify key symbionts that promote duckweed growth. These research projects were highlighted in my George H. Cook Honors Thesis, completed my senior year.

> Pfaff and Lam, *Differential Responses of Two Duckweed Strains to Nutrient Limitations and Media Complexities* (2017). Available upon request.

Continuing my passion for renewable energy from plant biomass, I joined the Center for Lignocellulose Structure and Formation (CLSF) at Penn State University, where I have been conducting my Ph.D. research in

Dr. Daniel Cosgrove's lab. Taking a broad, interdisciplinary approach to bioenergy-related research, the CLSF investigates the development of plant cell walls as the source of energy-rich cellulosic material used for conversion to biofuel. To effectively extract energy from plant cell walls we must understand how plants synthesize cellulose and assemble complex cell walls. My work has focused on innovating and optimizing new methods by which to conduct interdisciplinary research in the field of cell wall biology.

2) Detecting the Orientation of Newly-Deposited Crystalline Cellulose with Fluorescent CBM3

Fluorescently-tagged carbohydrate binding module 3 (CBM3-A488) binds crystalline cellulose and has been optimized for visualization of surface cellulose fibrillar patterning in onion epidermal peels (primary cell wall model system). Labeling of onion epidermal peels with CBM3-A488 was found to be pH dependent, due to the presence of negatively-charged pectins in the onion cell wall. Removal of pectin or neutralization of the charge on the CBM3 probe (at the pH of the isoelectric point) dramatically increased florescent labeling with CBM3-A488. These results hold implication for the use of protein probes to label, or enzymes to digest, cell wall samples as pH can significantly alter the penetration depth of the probe, due to electrostatic repulsion. Optimization of the labeling conditions for this new fluorescent probe revealed that CBM3-A488 can be used to selectively label the surface, or most-recently deposited, layer of cellulose in a cell wall sample for characterization of cell wall development.

> Pfaff, Wang, Wagner, Wilson, Kiemle, & Cosgrove, *Detecting the Orientation of Newly-Deposited Crystalline Cellulose with Fluorescent CBM3*. In preparation for submission in January 2022.

3) Secondary Cell Wall Regenerating Protoplasts Provide Novel Insights into the Essential Role of Xylan in Patterned Wall Formation

Deposition of xylem-type SCWs in Arabidopsis protoplasts requires xylan for proper cellulose band formation. In mutant backgrounds (*irx9* and *irx14*, xylan backbone synthesis mutants) with short-chain xylan, the synthesis of banded secondary cell walls is disrupted as visualized by cellulose and xylan labeling and confocal fluorescence microscopy. Enzymatic digestion of xylan backbones during active SCW synthesis in wild-type protoplasts displays a similar yet more distinct disruption of cellulose banding patterns in transdifferentiating protoplasts. These results indicate that xylan is required for proper cellulose bundling and that there may be a feedback mechanism between the cell wall and microtubules which guide cellulose synthases complexes as they deposit cellulose and carry vesicles containing xylan to the developing wall. Current experiments are underway to determine if microtubule patterning in protoplasts developing xylan-deficient SCWs is disrupted. This would be the first report of a feedback link between the developing cell wall and microtubule dynamics. I have conducted the entirety of this body of work under the mentorship of Dr. Daniel Cosgrove.

> Pfaff, Wagner, & Cosgrove, *Secondary Cell Wall Regenerating Protoplasts Provide Novel Insights into the Essential Role of Xylan in Patterned Wall Formation*. In preparation for submission in spring 2022.

4) Tracking Secondary Cell Wall Deposition and Lignification in Arabidopsis Inflorescence Stems

SCWs provide rigidity to the growing inflorescence stems of plants and are essential to maintaining plant stature. This study seeks to characterize the deposition of SCWs, with specific focus on lignification, in maturing Arabidopsis inflorescence stems. By analyzing the basal (base) segment of inflorescence stems of increasing height, a range of growth stages will be captured. I have stained and fluorescently imaged stem cross sections, characterizing the various stages of lignin deposition in each SCW-developing cell type. I have optimized hydroponic growth of Arabidopsis plants for C13 labeling and subsequent solid state nuclear magnetic resonance (ssNMR) studies. In collaboration with Dr. Tuo Wang (Louisiana State University), we have collected preliminary NMR results tracking the lignification of SCWs in inflorescence stems. Ongoing work seeks to capture high-resolution ssNMR data tracking the timing of deposition, interactions, hydration, and mobility of key SCW polymers. After characterization of wild-type inflorescence stem SCW development, several lignin mutants will be analyzed in collaboration with Dr. Chang-Jun Liu (Brookhaven National Lab).

Wild-type and mutant inflorescence stems will also be subjected to mechanical analysis to correlate differences in lignification with stem tensile strength. By characterizing the development of SCWs we expect to gain insights into the key components that confer mechanical rigidity to inflorescence stems to inform the production of biofuel from plants with altered lignification. This ongoing work is being conducted and coordinated by myself and Dr. Daniel Cosgrove in collaboration with the labs mentioned above.

5) Cryo-Electron Subtomogram Averaging of Cellulose Synthase Complex

Secondary cell walls (SCWs) only develop in certain cell types and provide the rigidity and structural support required for vertical growth and long-range, negative-pressure water transport. I have developed a novel system for study of plant secondary cell wall (SCW) development at the cellular level, in the model species *Arabidopsis thaliana*. By overexpression of the transcriptional factor that regulates the deposition of patterned SCWs, mesophyll protoplasts are induced to generate SCWs with thick bands of cellulose encompassing each differentiating cell. I have optimized this platform for studies of the deposition bundled cellulose and xylan, and for inducing SCW synthesis in mutant Arabidopsis backgrounds to facilitate structure/ function studies of cellulose synthase. ***For the current application***, I have shown that secondary wall formation is possible in protoplasts of an engineered mutant strain that expresses a fluorescent cellulose synthase protein 7 (YFP-CESA7). Preliminary studies show that within 18 hours of induction the fluorescent enzyme appears at the plasma membrane where cellulose is being synthesized, and that plunge-frozen, vitrified cells are suitable for cryoFIB milling to make lamellae for cryoET and subsequent subtomogram averaging. Results from this work will inform further structure/function studies of mutated CESAs of interest, based on structural data and computational analysis, which will be analyzed by super-resolution fluorescence microscopy and further CET studies. This work is being conducted by me in collaboration with Dr. Matt Swulius and Dr. Tracy Nixon (Penn State University).

D. Additional Information: Research Support and Scholastic Performance

In addition to the fellowship funds listed above in section B, my research has and will be funded by the following grant to mentors Cosgrove and Nixon:

DOE DE-SC0001090 Cosgrove, D. (PI) 08/01/14-07/31/22
Center for Lignocellulose Structure and Formation: Structural dynamics of cellulose synthase revealed by cryoEM and DTEM.

The Center for Lignocellulose Structure and Formation is a DOE Energy Frontier Research Center lead by PI Daniel Cosgrove. The research center aims to develop a detailed nano- to meso-scale understanding of plant cell wall structure and its mechanism of assembly to provide a basis for improved methods of converting biomass into fuels. The projects directed by PI Cosgrove and co-PI Nixon fund my efforts to acquire structural knowledge of the biological machine called 'rosette' that organizes multiple cellulose synthases to yield crystalline microfibers of cellulose for making plant cell walls. Role: Graduate student.

Rutgers University, New Brunswick, NJ

Double Major in Biotechnology and Plant Science, Minor in Biochemistry; Graduated Magna cum laude (GPA = 3.768)
Grading Scale: A = 4.0, B+ = 3.5, B = 3.0, C+ = 2.5, C = 2.0

Semester/Course	Level	Credits	Grade
Advanced Placement			
Biology	100	4.0	
Biology	100	4.0	
Calculus 1	100	4.0	
English Language	200	3.0	
English Literature	200	3.0	
Fall 2013			
General Chemistry and Recit	100	4.0	A
Exposition & Argument	100	3.0	B+
Calculus 2	100	4.0	A
General Psychology	100	3.0	A
The Byrne Seminars	100	1.0	Pass
Readings in Biology	100	1.0	A
Fall 2014			
Ecology People & Environ	100	3.0	A
General Physics	200	3.0	A
Plant Genetics	300	4.0	A
Organic Chemistry	300	4.0	C
Honors Research Credits	300	3.0	A
Fall 2015			
General Microbiology	300	4.0	A
General Biochemistry	400	4.0	A
Molecular Genetics	400	3.0	A
Plant Molecular Biology	500	3.0	B
Honors Research Credits	400	3.0	A
Fall 2016			
Intro to Horticulture	200	3.0	A
Topics: Wine Insights	200	1.0	A
Intro Biochemistry Lab	300	1.0	A
Sequence Analysis	300	3.0	A
Seminar in Biotech	400	1.5	A
Meth & App in Molecular Bio	400	4.0	A
G.H. Cook Honors Thesis	400	3.0	A

Semester/Course	Level	Credits	Grade
Advanced Placement			
German Language	100	3.0	
German Language	100	3.0	
World History	100	3.0	
American History	100	3.0	
Spring 2014			
General Chemistry and Recit	100	4.0	B
Intro Chem Experimentation	100	1.0	A
Animal Hand & Fit Exhibition	100	1.0	Pass
Honors Seminar I	100	3.0	A
Topics in Marine Science	200	1.5	A
Plant Science	200	3.0	A
Honors Research Credits	200	1.0	A
Basic Stats for Research	400	3.0	B+
Spring 2015			
General Physics	200	3.0	B
Honors Seminar II	200	3.0	A
Organic Chemistry	300	4.0	C+
Organic Chemistry Lab	300	2.0	B+
Honors Research Credits	300	3.0	A
Spring 2016			
General Biochemistry	400	3.0	A
Microbiology Colloquium	400	3.0	A
Molecular Genetics Lab	400	4.0	A
Plant Tissue Culture	400	3.0	A
Honors Research Credits	400	3.0	B+
Spring 2017			
Animal Hand & Fit Exhibition	100	1.0	Pass
Computational Statistics	200	3.0	A
Politics of Environ Issues	200	3.0	A
Plant Breeding	400	4.0	A
Biochemistry of Cancer	400	3.0	A
Plant Physiology	500	3.0	B+
G.H. Cook Honors Thesis	400	3.0	A

Pennsylvania State University, University Park, PA

Ph.D. Candidate in Plant Biology (Huck Institute Interdepartmental Graduate Program), Cumulative GPA = 3.960
Grading Scale: A = 4.00, A- = 3.67, B+ = 3.33, B = 3.00, B- = 2.67, C+ = 2.33, C = 2.00; R = Research

Semester/Course	Level	Credits	Grade
Fall 2017			
Plant Resour Acq & Utl	500	4.0	A
Tech in Plant Cell Biology	500	2.0	A-
Tech in Plant Molec Biology	516	2.0	A
Colloquium (Seminar Series)	500	1.0	A
Individual Studies	500	1.0	R
Fall 2018			
SNSF GRFP Fellowship Prep	500	1.0	A
Experiential Teaching	500	2.0	A
Supv Exp College Teaching	600	1.0	A
Thesis Research	600	5.0	R
Spring 2021			
PhD Dissertation Full-Time	600	-	R

Semester/Course	Level	Credits	Grade
Spring 2018			
Plant Commun & Growth	500	4.0	A
Ethics in Life Science	500	1.0	A
Colloquium (Seminar Series)	500	1.0	A
Individual Studies	500	3.0	R
Spring 2019, Fall 2019, and Spring 2020			
Thesis Research	600	9.0	R
Fall 2020			
Prof Devo for Plant Sciences	500	3.0	A
Thesis Research	600	9.0	R
Fall 2021			
PhD Dissertation Full-Time	600	-	R

PennState

The Huck Institutes of the Life Sciences
The Pennsylvania State University
201 Huck Life Sciences Building
University Park, PA 16802-5807

814-865-2625
Fax: 814-863-1357

December 21, 2021

Dear Prof. Nixon,

This letter is a statement of Penn State University's intent to purchase a FEI Aquilos 2 cyro-FIB and multimodal correlative microscopy instrument that will benefit the next phase of research in the Center for Lignocellulose Structure and Formation.

The Huck Institutes of the Life Sciences oversees the strategy, technological stance, and operation of twelve core facilities that serve life science research across the university. We solicit and listen carefully to faculty recommendations for new types of instrumentation that will move the needle on scientific capability. In so doing, we frequently partner with the Materials Research Institute and their Materials Characterization Lab (MCL), to obtain and administer instrumentation that serves the broadest portfolio of interests and disciplines.

Based on conversations with you and others, we have determined that PSU needs a FEI Aquilos 2 that allows FIB milling, SEM imaging, and fluorescent light microscopy in a single instrument, thereby providing a platform for correlative multi-modal imaging, while also allowing the production of thin electron-transparent lamellas in a cryo environment for high-resolution cryo-electron tomography in our FEI Krios microscope. PSU's has an older FIB instrument and expert MCL staff that will allow us to make rapid strides with the new instrument to reveal fundamental new things about cell structure and function.

Barring unforeseen circumstances, we will use a portion of the FY 2022 Tobacco Settlement Funds that come to PSU each year for this purpose. The PA Dept of Health is somewhat unpredictable regarding when they release those funds each year, but a ballpark estimate for delivery time is around the end of December, 2022.

We wish you well with the CLSF renewal proposal and look forward to providing a world class scientific environment to support the work of the project.

Sincerely,

James H. Marden
Professor of Biology
Assoc. Dir., Huck Institutes of the Life Sciences