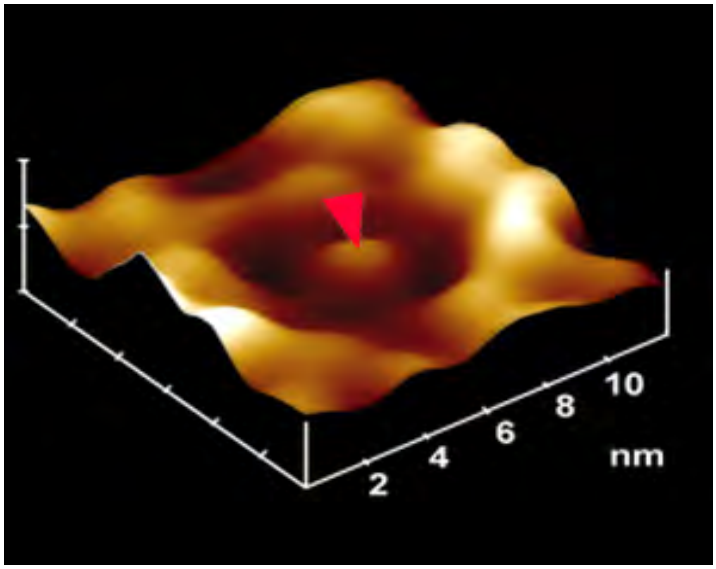
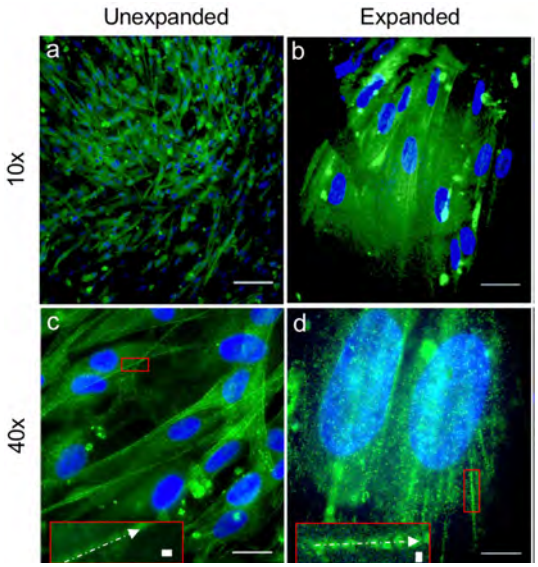
NBSI PROGRESS REPORT 2020



Atomic Force Micrograph of the Neuronal Porosome Complex

Porosome: The secretory portal involved in neurotransmission



Expansion Microscopy on Human Skeletal Muscle Cells

A new modality in nanoscale imaging using an ordinary light microscope

NanoBioScience Institute (NBSI)

http://www2.med.wayne.edu/physiology/nanobioscience/nanobioscience.htm

School of Medicine

Wayne State University

Progress Report
Prof. Bhanu P. Jena
Director, NBSI
George E. Palade University Professor & Distinguished Professor
March 5, 2020

NBSI was established in 2000 at the School of Medicine immediately after my arrival from Yale University School of Medicine and is the first and only existing '**NanoBioScience Institute**' on our campus in this cutting-edge technology. The primary objective of the institute was to establish a strong interdisciplinary program in the Nano Sciences & Nano Medicine at the Medical School and the University. In summary, with no seed funding from the University, NBSI continues to contribute to the academic and research progress in the field, and to bring recognition to the School of Medicine and to Wayne State University. Some of the past and present contributions of NBSI are highlighted below:

NBSI Highlights (Past & Present)

1. **NBSI continues to bring together a large group of cross-campus interdisciplinary faculty and student groups to study Nano Science, Nano Medicine, & Nano Technology.**
2. **NBSI has facilitated joint grant applications and funding from the NSF, NIH, DOD, and other private sources.**
3. **NBSI Director (Jena) has developed a team-taught, campus-wide multidisciplinary NanoBioScience Course (PSL7215) in the Dept. of Physiology at the Medical School, which is in its 14th year of offering. The class size continues to grow, and in the current class, we had 30 students from across campus. Students from other schools including from the University of Michigan are attracted to this course, and many have opted to continue their higher education such as Ph.D. and MD at Wayne State University.**
4. **NBSI has been selected as one of the top four Nano Institutes in the US. PROVIDED AS AN ATTACHMENT TO THIS REPORT (http://www2.med.wayne.edu/physiology/nanobioscience/pdfs/Nanotechnology%20Standing.pdf).**
5. **The NBSI Director (Jena) together with the late Prof. Ahmed H. Zewail (1999 Nobel Laureate in Chemistry) has helped establish the $150 million Asian NanoScience Institute in South Korea and served as its Co-Director (2002-2006).**
6. **The NBSI Director (Jena) was organizer and Chair of the *International Nano Science Symposium* in 2002 at Wayne State University.**
7. **The NBSI Director (Jena) was Co-Organizer and Co-Chair of the *International Nano Science Symposium* in 2002 at Pusan National University in South Korea (Supported by Samsung and Govt. of Korea).**
8. **The NBSI Director (Jena) was organizer and Chair of an *International Conference on Nano Science in the Understanding of Nature* in 2005 in Aiche, Japan, at the World Expo to show case the Medical School and Wayne State University. WSU President and Michigan Governor attended the conference. (Supported by a $250,000 grant from Toyota Corp.)**

9. **The NBSI Director (Jena) was selected by the National Science Foundation (2003-2009) to serve as one of the 4-member Site Visit Team to assist in the establishment of the Nano Biotechnology Center at Cornell University in Ithaca.**

10. **The NBSI Director and NBSI Co-Director and member Prof. Charles Manke, Associate Dean of Engineering, WSU, were both invited in 2015 by the President of Târgu Mureș University in Romania, to help establish a Nano Science Institute in that university and to establish strong collaborative research partnership with NBSI, which is ongoing.**

11. **The NBSI Director (Jena) together with Prof. Roger Kornberg (2006 Nobel Laureate in Chemistry) serves in the Advisory Board and is helping in the establishment of a $3 Billion STEM University in India, funded by the Anil Agarwal Foundation: (https://www.med.wayne.edu/news/2016/07/13/bhanu-jena-invited-to-contribute-to-indias-3-billion-vedanta-university-project/). This has helped initiate the establishment of both research and clinical ties between NBSI, Wayne State University and the proposed Vedanta University.**

12. **NBSI has fostered the establishment of several national and international scientific collaborations, grant applications, and meetings.**

13. **The NBSI Director (Jena) was invited in 2017 by the Rustaveli National Science Foundation of the Republic of Georgia, together with the NBSI international collaborator Prof. Mzia G. Zhvania, Head of Chemical Neuroscience, Ilia State University, Tbilisi, Georgia, to establish a nano neuroscience Ph.D. program, where Ph.D. students funded by the foundation will visit NBSI to work in various member laboratories. This proposal was funded, resulting in annual international scientific meetings and student exchange and research collaborations:**
    https://cellularneurosciencephd.iliauni.edu.ge/?lang=en;

    http://bnsma2019.iliauni.edu.ge

14. **NBSI Director (Jena) has published six books (one *in-press:* *https://www.springer.com/gp/book/9783030444952#aboutAuthors*) on Nano Science and over 170 scientific papers in the field, some which are listed below.**

15. **The NBSI Director (Jena) received the Honorary Scientist Award from the 130-year old "*Victor Babes National Institute of Pathology*" [https://www.ivb.ro/v3/] of Romania and invited to serve on its Advisory Board. Research collaborations between NBSI and the Victor Babes National Institute initiated.**

    https://today.wayne.edu/medicine/news/2017/11/29/romanian-institute-of-research-and-development-in-pathology-honors-dr-jena-30000

16. **NBSI establishes research collaborations with Delhi University, where the NBSI Director (Jena) serves as an Honorary Professor for life.**

    https://today.wayne.edu/medicine/news/2017/04/10/delhi-university-names-bhanu-jena-lifetime-honorary-professor-29786

17. **The NBSI Director (Jena) has been a Keynote Speaker at both in 2019 and 2020 Boston Nano Conference:** (https://www.nanoworldconference.com/nwc-2019/featured-speakers); (https://nanoworldconference.com/featured-speakers)

18. **The NBSI Director (Jena) together with Physiology Chair J.-P. Jin has actively participated with colleagues across campus, in putting together a Ph.D. Concentration in Biophysics, involving the Departments of Physiology, Physics, Bioengineering and Chemical Engineering & Materials Science at all the three colleges (Medicine, Engineering & Liberal Arts and Sciences) within WSU.**

19. **Personally, the NBSI Director (Jena) has actively participated in joint grant writing and submission with NBSI Members, resulting in three successful extramural awards: 2 from the NSF (2007-2010; 2011-2016: EB00303, CBET1066661) and a one from the NIH (2019-2020: NIH 2R56 NS079429-04A1) which is currently active.**

20. **Recognizing the surge in metabolic disease (Hypertension, Heart Disease, Diabetes, Cancer, Mental Illness, etc) in urban localities around the world including Detroit, the NBSI Director (Jena) has recently collaborated with NBSI colleagues to put together a proposal for a new "Center for Metabolism & Movement" to be established within the NanoBioScience Institute. A summary of the 'Center Proposal' is provided at the end of this progress report.**

21. **NBSI Member Robert J. Wessells publication "Sestrins are evolutionarily conserved mediators of exercise benefits. (2020) *Nature Communications* 11, 190" is in 99% of readership for all journal articles.**

22. **NBSI Member K. Zhang in collaborating with H. Nguyen at Wayne State University Chemistry Department, developed a new technology using synthesized "sugar" (glycopolymer) as heparanase inhibitors to treat diabetes and diabetic nephropathy. As the modified "sugar" is specific and non-toxic, this new technology is groundbreaking both conceptionally and technically (US patent W063-0067USP1, 2019).**
23. **NBSI Member K. Zhang has commercialized technology entitled "Generation of an affinity-purified rabbit anti-CREBH polyclonal antibody" (WSU licensed to Kerafast In.) attracts many national and international orders for the critical CREBH reagent each year. The commercialized technology has financially benefit the University (out of the university portion of the sale income, 10% goes to the school general fund and 15% to the department).**

24. **Some examples of recent extramural funding by WSU NBSI Members:**

-NIH NS079429 **A. Dombkowski** (PI), **B. P. Jena** (Co-I) 08/15/19-07/31/20 [$539,000]
TITLE: The role of non-coding RNAs in epilepsy of tuberous sclerosis complex and focal cortical dysplasia type 2B.

-NIH **R. J. Wessells** (PI) 09/30/18-05/31/21 [$1,690,000]
TITLE: Octopamine controls adaptation to endurance exercise in drosophila.

-NIH SPORE CEP **S. Arslanturk** (PI) 11/01/19-10/31/21 [$539,000]
TITLE: Early detection of aggressive prostate cancer through molecular biomarker identification using deep learning.

-1R01DK110314 **X. Chen** (PI), **K. Zhang** (Co-I) 09/01/16-08/31/21 [$1,155,125]
TITLE: Proinsulin ER export and beta cell ER homeostasis in health and diabetes.

-NIH DK090313 **K. Zhang** (PI) 01/01/16-06/30/21 [$2,060,293]
TITLE: Regulation of Circadian Metabolism by the Hepatic Transcription Factor CREBH.

-NIH AR066634 **D. Fang** (PI), **K. Zhang** (PI) 07/01/14-06/30/19 [$1,250,000]
TITLE: Simultaneous Targeting of IRE1a in B Cells and Macrophages for Lupus Therapy.

-NIH DK109036 **K. Zhang** (Co-I) 03/01/16-02/29/21 [$1,891,650]
TITLE: Role of Inositol requiring enzyme 1 in regulating angiogenesis for diabetic wound repair.

Collaborative Studies at the NBSI

International NBSI Collaborations

NBSI-Ilia State University & I. Beritashvili Center, Tbilisi, Georgia

Jena-Zhvania Collaboration (2003-present): Prof. Mazia G. Zhvania, Professor of Neuroscience, Ilia State University & Head of Laboratory of Brain Ultrastructure and Nanoarchotecture, I. Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia, and Prof. Jena's group have been collaborating for over a decade on the structure-function of the neuronal porosome complex. Prof. Zhvania is a leading investigator in the cutting-edge technology and field of Nano Science and Nano Medicine, focused on neurobiology and neuronal diseases, critical in the development of new neurological treatment modalities, drug development, and therapy. Prof. Mzia G. Zhvania is also a leading expert on the 'porosome', a supramolecular structure involved in neurotransmission, and is one of the first to establish the field of Nano Science in

Georgia. The expertise and instrumentation available in the Zhvania group and her Georgian colleagues have provided high-resolution electron microscopy (EM), and EM tomography capabilities for the study of the brain, individual neurons, and supramolecular structures at the nerve terminal such as the porosome, the secretory portal for neurotransmission. The Jena laboratory has helped train students from the Zhvania group in atomic force microscopy and associated biophysical approaches, and in the recent past, Dr. Nato Kotaria and Ms. Vera Okuneva from the Zhvania group received fellowship from Georgia to come and spend 6 and 3 months respectively, to training in the Jena laboratory at WSU. This collaboration between the Zhvania and the Jena group has resulted in the publication of two scientific papers listed below:

1. Cho, W.J., Lee, J-S., Ren, G., Zhang, L., Shin, L., Manke, C.W., Potoff, J., Kotaria, N., Zhvania, M.G., Jena, B.P. (2011) Membrane-directed molecular assembly of the neuronal SNARE complex. *J. Cell. Mol. Med.* 15:31-37.

2. Cho, W-J., Jeremic, A., Rognlien, K. T., Zhvania, M.G., Lazrishvili, I., Tamar, B., Jena, B.P. (2004). Structure, isolation, composition and reconstitution of the neuronal fusion pore. *Cell Biol. Int.* 28:699-708.

In the past decade, Prof. Zhvania has received grant funding to host five international meetings, including one in 2019 on nanomedicine involving neurons and neurological disorders, which were attended and participated by Jena and colleagues. The meeting funded the attendance and research presentation of Prof. Jena and one of his year 1 MD-Ph.D. Students Sebastian Pernal to the meeting. The meeting also supported the attendance and presentation by Prof. Daniel A. Walz, collaborator and advisor of NBSI. Prof. Jena and Zhvania have applied for an NIH-Fogarty Foundation grant. In May 2017, a new Nano neuroscience Ph.D. Program was launched in Ilia State University in Tbilisi, Georgia through funding from the Georgian Government's Rustaveli Science Foundation. Graduate student are expected to come and work in NBSI Lab's funded by the Georgian Govt. In 2019 Prof. Zhvania and a colleague visited Wayne State University and met several NBSI members to establish new research collaborations.

NBSI-Karolinska Institute, Stockholm, Sweden

Jena-Larsson Collaboration (2012-present): Prof. Lars Larsson, Professor of Physiology, Karolinska Institute, Stockholm, Sweden and the Jena group have collaborated in several aspects of muscle physiology, recently submitted a joint NIH Grant, and published the following studies:

1. Li M, Deguchi T, Näreoja T, Jena BP, Hänninen P, Larsson L. (2015) Nanometric features of myosin filaments extracted from a single muscle fiber to uncover the mechanisms underlying organized motility. *Arch Biochem Biophys*. 2015 Oct 1;583:1-8. doi: 10.1016/j.abb.2015.06.010.PMID: 26116379

2. Kuhn ER, Naik AR, Lewis BE, Kokotovich KM, Li M, Stemmler TM, Larsson L, Jena BP. (2018) Nanothermometry Reveals Calcium-Induced Remodeling of Myosin. *ACS Nano Letters* October 22, 2018, DOI:10.1021/acs.nanolett.8b02989

3. Cacciani N, Salah H, Li M, Akkad H, Backeus A, Hedström Y, Jena BP, Larsson L. (2019) Chaperone co-inducer BGP-15 mitigates contractile dysfunction of the soleus muscle in a rat ICU model. *Acta Physiologica.* DOI:

10.1111/apha.13425

Funded by the Karolinska Institute, Post-Doctoral Student Dr. Mishan Lee has visited the Jena lab. to conduct research on muscle efficiency. Results from the study (March-April 2017) and earlier studies, was part of the manuscript #2 (above), and was used to put together a proposal to NIH for joint funding.

NBSI-University of Windsor, Windsor, Ontario, Canada

Jena-Trant Collaboration (2017-present): Prof. John F. Trant and the Jena lab. have been collaborating on determining optimal carbohydrate formulations as cell and tissue cryoprotectants the molecular level mechanisms involved in such protections. This is an ongoing collaboration.

NBSI-University of Melbourne, Melbourne, Australia

Stemmer-Reid Collaboration (2009-present): Development and testing of new chemical labeling strategies for mass spectrometry-based analysis of biomolecules. Over the last few years I have had the good fortune to collaborate with Dr. Gavin Reid from The University of Melbourne in Melbourne Australia. Gavin and I have complementary research interests and backgrounds. His group has developed novel chemical labeling strategies and has examined the behavior of novel chemical entities in the gas phase during MS analysis. I have contributed by evaluating the new reagents in my lab with a focus on novel approaches to analysis of phosphopeptides. The field of signaling and signal transduction research has benefited from this work and it has laid the groundwork for additional advances in targeted proteomic analysis.

1. Smith S.A., Kalcic C.L., Safran K.A., Stemmer P.M., Dantus M., Reid G.E.: Enhanced characterization of singly protonated phosphopeptide ions by femtosecond laser-induced ionization/dissociation tandem mass spectrometry (fs-LID-MS/MS). J Am Soc Mass Spectrom. 21:2031-40, 2010. PMID: 20888783
2. Palumbo, A.M., Smith, S.A., Kalcic, C.L., Dantus, M., Stemmer, P.M. and Reid, G.E.: Tandem Mass Spectrometry Strategies for Phosphoproteome Analysis. Rev. Mass Spectrom Rev. 2011, 30(4):600-25. PMID: 21294150
3. Lu, Y., Zhou, X., Stemmer, PM., Reid, GE.: Sulfonium Ion Derivitization, Isobaric Stable Isotope Labeling and Data Dependent CID- and ETD-MS/MS for Enhanced Phosphopeptide Quantitation, Identification and Phosphoprylation Site Characterization. J Am Soc Mass Spectrom. 2012, 23(4):577-93. PMID: 21952753; PMCID: PMC4228788
4. Zhou X, Mester C, Stemmer PM, Reid GE. Oxidation-induced conformational changes in calcineurin determined by covalent labeling and tandem mass spectrometry. Biochemistry. 2014 Nov 4;53(43):6754-65. doi: 10.1021/bi5009744. Epub 2014 Oct 20. PMID: 25286016

NBSI-University of Windsor, Windsor, Canada

Stemmer-Rueda Collaborations (2014-present): Bioinformatics for interpretation of omic data sets. Dr. Luise Rueda from the University of Windsor in Windsor CA has been a valued collaborator for the past three years. Our work is focused

on interpretation of proteomic data sets that have hundreds to thousands of quantified elements. Our first project together was presented at the 2016 Great Lakes Bioinformatics and the Canadian Computational Biology Conference in Toronto.

1. Mrinalini Pandit, Mina Maleki, Nicholas J Carruthers, Paul M. Stemmer, Luis Rueda. Prediction of Calmodulin-binding Proteins.

NCBS, Bangalore, India

Wessells-Brockmann Collaboration (2017-present): The Wessells group has collaborated with Axel Brockmann's group in NCBS Bangalore, India, and published one paper.

Sujkowski, A., Ramesh, D., Brockmann, A., Wessells, R. (2017). Octopamine drives endurance exercise adaptations in *Drosophila*. *Cell Rep*. 21(7):1809-1823. PMID:29141215. PMCID: PMC5693351.

The Director (Jena) together with NBSI colleagues continue to participate in international institution building, gaining international recognition and visibility to Wayne State University, and expand research and academic collaborations on various fronts in nanobioscience and nanomedicine:

1. Established the Asian NanoScience Institute in South Korea and served as its Co-Director (2002-2006).
2. Development of the Institute of NanoMedicine in University of Delhi, India (ongoing).
3. Development of the NanoBioScience Institute in Tbilisi, Georgia (ongoing).
4. Invited to help establish a $3 billion Vedanta University, Odisha, India (ongoing).
5. Development of the "Center for Metabolism and Movement", focused on the 'Human Skeletal Muscle Cell Atlas', Wayne State University, (ongoing).

Selected National NBSI Collaborations

NBSI-Lawrence Berkeley Laboratory, CA

Jena-Ren Collaboration (2004-present): Dr. Gary Ren, Lawrence Berkeley Laboratory, CA, have been collaborating on t-/v-SNARE and porosome structures using cryo-EM. This ongoing collaboration has resulted in the funding of one DOE User Proposal and the following 5 research publications. Three additional manuscripts are in preparation.

1. Cho, W.J., Lee, J-S., Ren, G., Zhang, L., Shin, L., Manke, C.W., Potoff, J., Kotaria, N., Zhvania, M.G., Jena, B. P. (2011). Membrane-directed molecular assembly of the neuronal SNARE complex. *J. Cell. Mol. Med.* 15:31-37.
2. Cho, W-J., Shin, L., Ren, G., Jena, B. P. (2009). Structure of membrane-associated neuronal SNARE complex: Implication in neurotransmitter release. *J. Cell Mol. Med*. 13:4161-4165.
3. Cho, W-J., Ren, G., Lee, J-S., Jeftinija, K., Jeftinija, S., Jena, B.P. (2009). Nanoscale three-dimensional contour map of protein assembly within the astrocyte porosome complex. *Cell Biol. Int.* 33:224-229.
4. Cho, W-J, Ren, G., Jena, B.P. (2008). EM 3D contour maps provide protein assembly at the nanoscale within the neuronal porosome complex. *J. Microscopy* 232:106-111.

5. Cho, W-J, Jeremic, A., Jin, H., Ren, G., Jena, B. P. (2007). Neuronal fusion pore assembly requires membrane cholesterol. *Cell Biol. Int.* 31:1301-1308.

NBSI-University of Vermont, Burlington, VT

Jena-Taatjes Collaboration (2000-present): Prof. Douglas J. Taatjes, Department of Pathology, University of Vermont College of Medicine, VT, have been collaborating on t-/v-SNARE and porosome structures using various imaging modalities. This ongoing collaboration has resulted in the funding of one NIH grant and the following 11 research publications.

1. Cho, S.-J., Quinn, A.S., Stromer, M.H., Dash, S., Cho, J., Taatjes, D.J., Jena, B.P. (2002). Structure and dynamics of the fusion pore in live cells. *Cell Biol. Int.* 26:35-42.
2. Jeremic, A., Quinn, A.S., Cho, W-J., Taatjes, D.J., Jena, B. P. (2006). Energy-dependent disassembly of self-assembled SNARE complex: observation at nanometer resolution using atomic force microscopy. *J. Am. Chem. Soc.* 128:26-27.
3. Wang, S., Lee, J-S., Bishop, N., Jeremic, A., Cho, WJ., Chen, X., Mao, G., Taatjes, D.J., Jena, B.P. (2012). 3D organization and function of the cell: Golgi budding and vesicle biogenesis to docking at the porosome complex. *Histochem. Cell Biol.* 137:703-718.
4. Lee, J-S., Hou, X., Bishop, N., Wang, S., Flack, A., Cho, WJ., Chen, X., Mao, G., Taatjes, D.J., Sun, F., Zhang, K., Jena, B.P. (2013). Aquaporin-assisted and ER-mediated mitochondrial fission: A hypothesis. *Micron* 47:50-58.
5. Taatjes, D.J., Rand, J.H., Jena, B.P. (2013). Atomic force microscopy: high resolution dynamic imaging of cellular and molecular structure in health and disease. *J. Cell Physiol.* 228:1949-1955.
6. Hou, X., Lewis, K.T., Wu, Q., Wang, S., Chen, X., Flack, A., Mao, G., Taatjes, D.J., Sun, F., Jena, B.P. (2014). Proteome of the porosome complex in human airways epithelia: Interaction with the cystic fibrosis transmembrane conductance regulator (CFTR). *Journal of Proteomics* 96:82-91.
7. Kovari, L.C., Brunzelle, J.S., Lewis, K.T., Cho, W.J., Lee, J-S., Taatjes, D.J., Jena, B.P. (2014). X-ray solution structure of the native neuronal porosome-synaptic vesicle complex: Implication in neurotransmitter release. *Micron* 56:37-43.
8. Lewis, K.T., Maddipati, K.R., Taatjes, D.J., Jena, B.P. (2014). Neuronal porosome lipidome. *J. Cell. Mol. Med.* 18:1927-1937.
9. Jena, B. P., Taatjes, D.J. (2014). NanoCellBiology: Multimodal Imaging in Biology & Medicine *Pan Sanford Publishing Pte. Ltd.* p1-400, ISBN: 9789814411790.
10. Rajagopal, A., Kulkarni, S., Lewis, K.T., Chen, X., Maarouf, A., Kelly, C.V., Taatjes, D. J., Jena, B.P. (2015). Proteome of the insulin-secreting Min6 porosome complex: Involvement of Hsp90 in its assembly and function. *Journal of Proteomics* 114:83-92.
11. Naik, A.R., Kulkarni, S.P., Lewis, K.T., Taatjes, D.J., Jena, B.P. (2016) Functional reconstitution of the porosome complex in live cells. *Endocrinology* 157:54-60.
12. Jena, B. P., Gatti, D. L., Arslanturk, S., Pernal, S., Taatjes, D. J. (2019) Human Skeletal Muscle Cell Atlas: Unraveling Cellular Secrets Utilizing 'Muscle-on-a-Chip', Differential Expansion Microscopy, Mass Spectrometry, Nanothermometry and Machine Learning. *Micron* 177: 55-59.
13. Pernal, S., Liyanaarachchi, A., Gatti, D. L., Arslanturk, S., Formosa, B., Pulvender, R., Kuhn, E.R., Ramos, R., Naik, A.R.,George, K., Arslanturk, S., Taatjes, D. J., Jena, B. P. (2019). Differential Expansion Microscopy. *bioRxiv* 699579; doi: https://doi.org/10.1101/699579
14. Naik, A.R., Kuhn, E.R., Lewis, K.T., Kokotovich, K.M., Maddipati, K.R., Chen, X., Hörber, J.H.K., Taatjes, D.J., Potoff, J.J., Jena, B.P. (2019) Self-assembly and biogenesis of the cellular membrane are dictated by membrane stretch and composition. *J. Phys. Chem. B.* 123: 6997-7005.
15. Pernal, S., Liyanaarachchi, A., Gatti, D. L., Arslanturk, S., Formosa, B., Pulvender, R., Kuhn, E.R., Ramos, R., Naik, A.R.,George, K., Arslanturk, S., Taatjes, D. J., Jena, B. P. (2020). Nanoscale imaging using differential expansion microscopy. *Histochem. Cell Biol. (in-press).*

NBSI-Northwestern University & Synchrotron Research Center, Chicago, IL

Jena-Brunzelle Collaboration (2014-present): Prof. Joseph S Brunzelle, and the Jena lab. have been collaborating on determining the molecular structure of the native neuronal porosome complex using solution X-ray and neutron scattering studies. This ongoing collaboration has resulted in the following research publication.

1. Kovari, L.C., Brunzelle, J.S., Lewis, K.T., Cho, W.J., Lee, J-S., Taatjes, D.J., Jena, B.P. (2014). X-ray solution structure of the native neuronal porosome-synaptic vesicle complex: Implication in neurotransmitter release. *Micron* 56:37-43.

NBSI-UPenn, Philadelphia, PA

Stemmler-Dancis Collaboration (2002-present): Prof. Andrew Dancis, Division of Hematology, Department of Medicine at the University of Penn, Philadelphia has been collaborating with Dr. Timothy Stemmler, Department of Pharmaceutical Sciences, WSU to elucidate the molecular and atomic basis for cellular metal regulation using a variety of Biophysical techniques. This work has been NIH funded for the past 11 years and resulting in 8 publications.

1. Dzul, S.*; Rocha, A.; Rabat, S.; Kandegedara, A.*; Kusowski, A.*; Pain, J.; Murari, A.; Pain, D.; Dancis, A.; Stemmler, T.L.; "In vitro characterization of a novel Isu homologue from *Drosophila melanogaster* for de novo FeS-cluster formation." *Metallomics*, 2017,*9*, 48-60.
2. Rodrigues, A.V.*; Kandegedara, A.*; Rotondo, J.A.*; Dancis, A.; Stemmler, T.L. "Iron Loading Site on the Fe-S Cluster Assembly Scaffold Protein is Distinct from the Active Site" *BioMetals,* 2015, *28,* 567-76.
3. Pandey, A.; Gordon, D.M.; Pain, J.; Stemmler, T.L.; Dancis, A.; Pain, D. "Frataxin directly stimulates mitochondrial cysteine desulfurase by exposing substrate-binding sites and a mutant Fe-S cluster scaffold protein with frataxin-bypassing ability acts similarly." *J. Biol. Chem.*, 2013, *288*, 36773-86.
4. Cook, J.D.*; Kondapalli, K.C.*; Rawat, S.*; Childs, W.C.*; Murugesan, Y.*; Dancis, A.; Stemmler, T.L. "Molecular details of the yeast frataxin-Isu1 interaction during mitochondrial Fe-S cluster assembly" *Biochem.,* 2010, *49* (3), 8756-65.
5. Stemmler, T.L.; Lesuisse, E.; Pain, D.; Dancis, A. "Frataxin and mitochondrial Fe-S cluster biogenesis" *J. Biol. Chem.,* 2010, *285* (35), 26737-43.
6. Kondapalli, K.C.*; Dancis, A.; Stemmler, T.L. "Molecular interaction between Frataxin and Ferrochelatase during Heme Assembly" in Bioinorganic Chemistry; Cellular Systems and Synthetic Models, ACS Symposium Series 1012, Eric C. Long and Michael J. Baldwin, Eds., American Chemical Society, 2009, 17-30.
7. Kondapalli, K.C.*; Kok, N.M.*; Dancis, A.; Stemmler, T.L. "Drosophila Frataxin: an iron chaperone during cellular [2Fe-2S] cluster bioassembly" *Biochem.*, 2008, *47,* 6917-27.
8. He, Y.*; Alam, S.L.; Proteasa, S.V.*; Zhang, Y.*; Lesuisse, E.; Dancis, A.; Stemmler, T.L. "Yeast Frataxin Solution Structure, Iron Binding and Ferrochelatase Interaction," *Biochem.*, 2004, *43,* 16254-62.

NBSI-Northwestern University, Chicago, IL

Stemmler-Rosenzweig Collaboration (2003-present): Prof. Amy Rosenzweig, Department of Chemistry at Northwestern University has collaborated with Dr. Stemmler to characterize the molecular and atomic characteristics of particulate methane monooxygenase, a protein isolated from several methantropic bacteria that converts methane to methanol. In the process of removing the greenhouse gas methane, it converts the gas to a viable liquid energy source, methane. This work, as well as additional collaborations, has resulted in 15 publications.

1. Fisher, O.S.; Kenney, G.E.; Ross, M.O.; Ro, S.Y.; Lemma, B.E.; Batelu, S.*; Thomas, P.M.; Sosnowski, V.C.; DeHart, C.J.; Kelleher, N.L.; Stemmler, T.L.; Hoffman, B.M.; Rosenzweig, A.C. "Characterization of a long over looked copper protein from methane- and ammonia-oxidizing bacteria", *Nature Comm.,* 2018, *9,* 4276-88.
2. Ro, S.Y.; Ross, M.O.; Deng, Y.W.; Batelu, S.*; Lawton, T.J.; Hurley, J.D.; Stemmler, T.L.; Hoffman, B.M.; Rosenzweig, A.C.; "From micelles to bicelles: effect of the membrane on particulate methane monooxygenase activity." *J. Biol. Chem*., 2018, *293*, 10457-10465. PMID: 29739854.
3. Purohit, R.; Ross, M.O.; Batelu, S.*; Kusowski, A.*; Stemmler, T.L.; Hoffman, B.M.; Rosenzweig, A.C. "A Cu^{+}-specific CopB transporter: Revising P_{1B}-type ATPase classification." *Proc. Natl. Acad. Sci, U.S.A.*, 2018, *115*. 2108-13.
4. Smith, A.T.; Barupala, D.*; Stemmler, T.L.; Rosenzweig, A.C. "Discovery and characterization of a novel metal binding domain involved in cadium, cobalt, and zinc transport" *Nature Chemical Biology,* 2015, *11,* 678-84.
5. Sirajuddin, S.; Barupala, D.*; Helling, S.; Marcus, K.; Stemmler, T.L.; Rosenzweig, A.C. "Effects of Zinc on Particulate Methane Monooxygenase Activity and Structure" *J Biol Chem,* 2014, *289,* 21782-94.
6. Zielazinski, E.L.: González-Guerrero, M.; Subramanian, P.*; Stemmler, T.L.; Argüello, J.M.; Rosenzweig, A.C. "Sinorhizobium meliloti Nia is a P(1B-5)-ATPase expressed in the nodule during plant symbiosis and is involved in Ni and Fe transport." *Metallomics*, 2013, *12*, 1614-23.
7. Zielazinski, E.L.; Cutsail III, G.E.; Hoffman, B.M.; Stemmler, T.L.; Rosenzweig, A.C. "Characterization of a Cobalt-Specific P_{1B}-ATPase" *Biochem.,* 2012, *51* (40), 7891-900.
8. Smith, S.M.; Rawat, S.*; Telser, J.; Hoffman, B.M.; Stemmler, T.L.; Rosenzweig, A.C. "Crystal structure and characterization of particulate methane monooxygenase from *Methylocystis* species strain M" *Biochem.*, 2011, *59* (1), 10231-40.
9. Traverso, M.E.; Subramanian, P.*; Davydov, R.; Hoffman, B.M.; Stemmler, T.L.; Rosenzweig, A.C. "Identification of a hemerythrin-like domain in a P1B-type transport ATPase" *Biochem.,* 2010, *49* (33), 7060-8.
10. Balasubramanian, R.; Smith, S.M.; Rawat, S.*; Yatsunyk, L.A.; Stemmler, T.L.; Rosenzweig, A.C. "Oxidation of methane by a biological dicopper center" *Nature,* 2010, *465,* 115-9.
11. Hakemian, A.S.; Kondapalli, K.C.*; Telser, J; Hoffman, B.M.; Stemmler, T.L.; Rosenzweig, A.C. "The metal centers of particulate methane monooxygenase from *Methylosinus trichosporium* OB3b" *Biochem.,* 2008, *47,* 6793-801.
12. Sazinsky, M.H.; LeMoine, B,; Orofino, M.; Davydov, R.; Bencze, K.Z.*; Stemmler, T.L.; Hoffman, B.M.; Argüello, J.M.; Rosenzweig, A.C. "Characterization and Structure of a Novel Zn^{2+} and [2Fe-2S]-Containing Copper Chaperone from *Archaeoglobus fulgidus,*" *J. Biol. Chem.*, 2007, *282,* 25950-9.
13. Lieberman, R.L.; Kondapalli, K.C.*; Shrestha, D.B.; Hakemian, A.S.; Smith, S.M.; Telser, J.; Kuzelka, J.; Gupta, R.; Borovik, A.S.; Lippard, S.J.; Hoffman, B.M.; Rosenzweig, A.C.; Stemmler, T.L. "Characterization of the particulate methane monooxygenase metal centers in multiple redox states by X-ray absorption spectroscopy," *Inorg. Chem.*, 2006, *45,* 8372-81.
14. Hakemian, A.S.; Tinberg, C.E.; Kondapalli, K.C.*; Tesler, J.; Hoffman, B.M.; Stemmler, T.L.; Rosenzweig, A.C. "The copper chelator methanobactin from *Methy-losinus trichosporium* OB3b binds copper(I)," *J. Am. Chem. Soc.*, 2005, *127*, 17142-3.
15. Lieberman, R.L.; Shrestha, D.B.; Doan, P.E.; Hoffman, B.M.; Stemmler, T.L.; Rosenzweig, A.C. "Purified particulate methane monooxygenase from *Methylococcus capsulatus* (Bath) is a dimer with both mononuclear copper and a copper-containing cluster," *Proc. Natl. Acad. Sci, U.S.A.*, 2003, *100,* 3820-3825.

NBSI-Florida International University, FL

Stemmler-Rosen Collaboration (2001-present): Prof. Barry Rosen, Department of Cellular Biology and Pharmacology, Florida International University, collaborates with Dr. Stemmler to better understand the molecular and atomic details of how As binding proteins regulate metalloid homeostasis and drive methylation of the metalloid. There work together has developed into 8 publications.

1. Pawitwar, S.; Nadar, V.; Kandegedara, A.*; Stemmler, T.L.; Rosen, B.; Yoshinaga, M. "Biochemical characterization of ArsI: a novel C-As lyase for degradation of environmental organoarsenicals." *Environmental Science and Technology,* 2017, *51,* 11115-25

2. Kumar, N.V.; Yang, J.; Pillai, J.K.; Rawat, S.*; Solano, C.; Kumar, A.; Grotli, M.; Stemmler, T.L.; Rosen, B.P.; Tamas, M.K. "Arsenic directly binds to and activates the yeast AP-1-like transcription factor Yap8" *Molecular and Cellular Biology,* 2016, *36,* 913-922.
3. Ye, J.; He, Y.*; Skalicky, J.; Rosen, B.P.; Stemmler, T.L. "Resonance assignments and secondary structure predictions of the As(III) metallochaperone ArsD in solution" *BioMol. NMR Assn.,* 2011, *5* (1), 109-112.
4. Yang, J.; Rawat, S.*; Stemmler, T.L.; Rosen, B.P. "Arsenic binding and transfer by the ArsD As(III) metallochaperone" *Biochem.,* 2010, *49* (17), 3658-66.
5. Kandegedara, A.; Thiyagarajan, S.; Kondapalli, K.C.*; Stemmler, T.L.; Rosen, B.P. "Role of bound Zn(II) in the CadC Cd(II)/Pb(II)/Zn(II)-responsive repressor," *J. Biol. Chem.,* 2009, *284,* 14958-65.
6. Ordóñez, E.; Thiyagarajan, S.; Cook, J.D.*; Stemmler, T.L.; Gil, J.A.; Mateos, L.M.; Rosen, B.P. "Evolution of metalloid binding sites in transcriptional regulators" *J. Biol. Chem.*, 2008, *283,* 25706-14.
7. Qin, J.; Fu, H.-L.; Ye, J.; Bencze, K.Z.*; Stemmler, T.L.; Rawlings, D. E.; Rosen, B. P. "Convergent Evolution of a New Arsenic Binding Site in the ArsR/SmtB Family of Metalloregulators", *J. Biol. Chem.*, 2007, *282,* 34346-55.
8. Ramírez-Solís, A.; Mukopadhyay, R.; Rosen, B.P.; Stemmler, T.L. "Experimental and Theoretical Characterization of Arsenite in Water: Insights into the Coordination Environment of As-O," *Inorg. Chem.,* 2004, *43*, 2954-9.

University of Georgia, Athens, GA

Stemmler-Harrop Collaboration (2013-present): Prof. Todd Harrop, Department of Chemistry, University of Georgia, Athens, GA and Dr. Stemmler have been collaborating on the characterization a series of small molecule metallocomplexes that show catalytic reactivity and structural similarities to human enzyme active sites.

1. Rhine, M.A.; Rodrigues, A.V.*; Urbauer, R.J.B.; Stemmler, T.L.; Harrop, T.C. "Proton-Induced Reactivity of NO^- from a {CoBI}8 Complex." *J. Am. Chem. Soc.,* 2014, *136,*12560-3.
2. Walter, M.R.; Dzul, S.P.*; Rodrigues, A.V.*; Stemmler, T.L.; Tesler, J.; Conradie, J.; Ghosh, A.; Harrop, T.C. "Synthesis of Co(II)-NO(-) Complexes and their reactivity as a Source of Nitroxyl." *J. Am. Chem. Soc.*, 2016, *138,* 12459-71.
3. Steiner, R.A.; Dzul, S.P.*; Stemmler, T.L.; Harrop, T.C. "Synthesis and Speciation-Dependent Properties of a Multimetallic Model Complex of NiSOD That Exhibits Unique Hydrogen-Bonding." *Inorganic Chemistry,* 2017, *56,* 2849-62.
4. Truong, P.T.; Gale, E.M.; Dzul, S.P.*; Stemmler, T.L.; Harrop, T.C. "Steric Enforcement about One Thiolate Donor Leads to New Oxidation Chemistry in a NiSOD Model Compound." *Inorganic Chemistry,* 2017*, 56,* 7761-80.
5. Truong, P.T.; Broering, E.P.; Dzul, S.P.*; Chkraborty, I.; Stemmler, T.L.; Harrop, T.C.; "Simultaneous nitrosylation and N-nitrosation of a Ni-thiolate model complex of Ni-containing SOD." *Chem. Science,* 2018, *9*, 8567-74.

University of Maryland, Baltimore, MD

Stemmler-Michel Collaboration (2015-present): Prof. Sarah Michel, Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD and Dr. Stemmler have been collaborating on the characterization of Au based antibiotic drugs and on human gene regulation proteins.

1. Shimberg, G.D.; Michalek, J.L.; Oluyadi, A.D.; Rodrigues, A.V.*; Beth E. Zucconi, B.E.; Neu, H.; Ghosh, S.; Sureschandra, K.; Wilson, G.M.; Stemmler, T.L.**;** Michel, S.L.J. "Cleavage and Polyadenylation Specificity Factor 30: An RNA binding Zinc Finger Protein with an unexpected 2Fe-2S cluster." *Proc. Natl. Acad. Sci, U.S.A.*, **2016**, *113,* 4700-5.
2. Ok, K.; Li, W.; Neu, H.M.; Batelu, S.*; Stemmler, T.L.; Kane, M.A.; Michel, S.L.J.; "The role of gold in inflammation and tristetraprolin activity." *Chemistry,* 2020**,** *26,* 1535-47.
3. Pritts, J.D.; Hursey, M.S.; Michalek, J.L.; Batelu, S.*; Stemmler, T.L.; Michel, S.L.J.; "Unraveling the RNA binding properties of the Iron-Sulfur Zinc Finger Protein CPSF30." *Biochemistry,* 2020,*59,* 970-82.

NBSI- University of Missouri Kansas City

Stemmer-Persechini Collaboration (2000-present): Dr. Anthony Persechini from the University of Missourri Kansas City has been a valued collaborator for over 20 years. Our work is focused on the biochemical mechanisms of calmodulin-dependent signaling. Calmodulin is a key regulator of almost every process in mammalian cells. The work I have done to detail the mechanisms by which calmodulin binds with positive cooperativity in a Ca2+-dependent manner to the calmodulin-binding domains of target proteins and to delineate the role of the different Ca2+ binding sites in calmodulin are shown in the following papers. Our current work is focused on profiling the full calmodulin binding proteome using crosslinking and LC-MS/MS.

1. Persechini A, Yano K, Stemmer PM.: Ca(2+) binding and energy coupling in the calmodulin-myosin light chain kinase complex. J Biol Chem. 2000 Feb 11;275(6):4199-204. PMID: 10660583
2. Persechini A, Stemmer PM.: Calmodulin is a limiting factor in the cell. Trends Cardiovasc Med. 2002 Jan;12(1):32-7. PMID: 11796242
3. Ohashi I, Pohoreki R, Morita K, Stemmer PM.: Alcohols increase calmodulin affinity for Ca2+ and decrease target affinity for calmodulin. Biochim Biophys Acta. 2004 May 3;1691(2-3):161-7. PMID: 15110996

NBSI-University of Michigan

Wessells-Lee Collaboration (2017-present): University of Michigan. Wessells group has long-term collaboration with Jun-Hee Lee's lab which has resulted in one paper (below) and two submitted NIH grants.

1. *Kim, M.J., *Sujkowski, A., Namkoong, S., Gu, B., Cobb, T., Kim, B., Ho, A., Cho, C.S., Semple, I, Ro, S.H, Davis, C., Brooks, S.V., Karin, M., *Wessells, R.J., *Lee, J.H. (2020). Sestrins are evolutionarily conserved mediators of exercise benefits. *Nature Communications* 11, 190.

NBSI-University of Virginia

Wessells-Yan Collaboration (2017-present): Wessells group has collaborated multiple times with Zhen Yan's group, which has resulted in two publications (below) and three submitted NIH grants.

1. Xu, P., Damschroder, D., Zhang, M., Ryall, K.A., Adler, P.N., Saucerman, J.J., Wessells, R.J., Yan, Z. (2019). Atg2, Atg9 and Atg18 in mitochondrial integrity, cardiac function and healthspan in *Drosophila*. *J Mol Cell Cardiol*. 127:116-124. PMID:30571977.
2. Laker RC, Xu P, Ryall KA, Sujkowski A, Kenwood BM, Chain KH, Zhang M, Royal MA, Hoehn KL, Dirscoll M, Adler PN, Wessells RJ, Saucerman JJ, Yan Z. (2014) A novel MitoTimer reporter gene for mitochondrial

content, structure, stress and damage in vivo. *J Biol Chem*. Apr 25;289(17):12005-15. PMID: 24644293. PMCID: PMC4002107.

NBSI-Brown University

Wessells-Rand Collaboration (2017-present): Wessells group has collaborated with David Rand's group, resulting in one publication (below).

1. Sujkowski, A., Spierer, A.N., Rajagopalan, T., Bazzell, B., Safdar, M., Imsirovic, D., Arking, R., Rand, D., Wessells, R. (2018). Mito-nuclear Interactions Modify *Drosophila* Exercise Performance. *Mitochondrion* S1567-7249(18):30061-30068. PMID:30408593.

NBSI-Northwestern University

Zhang-Deyu Fang (2008-present): Hosmer Allen Johnson Professor, Department of Pathology, Feinberg School of Medicine
Northwestern University. This ongoing collaboration has resulted in multiple NIH grant and at least 15 research publications.

1. Wei, J., Yuan, Y., Wang, Y., Zhang, Y., Xu, Y., Yang, Z.*, Li, F., Gao, B., Jin, C., Melo-Cardenas, J., Chen, L., Pan, H., Wang, J., He, F., Zhang, K.#, Fang, D.# 2018 Hrd1-ERAD controls the hepatokine FGF21 production through K27-linked polyubiquitination of CREBH. *EMBO J*. 37(22). pii: e98942.
   [# *Corresponding authors*] [Impact factor: 10.6]
2. Wang, J., Qiu, Y.*, Yang, Z.*, Kim, H., Qian, Q., Sun, Q., Zhang, C., Yin, L., Fang, D., Back, S., Kaufman, R.J., Yang, L., and Zhang, K. 2018. Inositol Requiring Enzyme 1 Prevents Hepatic Steatosis through Processing microRNAs. *Science Signal.* *(Science)* 11(530). pii: eaao4617. [Impact factor 7, one of the most authentic *Science* sister journals].
3. Yang, Y., Kong, S., Zhang, Y., Melo-Cardenas, J., Gao, B., Zhang, Y., Zhang, D. D., Zhang, B., Song, J., Thorp, E., Zhang, K., Zhang, J., Fang, D. 2018. The endoplasmic reticulum--resident E3 ubiquitin ligase Hrd1 controls a critical checkpoint in B- cell development in mice. *J Biol Chem.* 293(33):12934-12944. [Impact factor: 4.258].
4. Wei, J., Yuan, Y., Xu, Y., Zhang, Y., Wang, Y., Peek, C.B., Yang, Y., Gao, B., Jin, C., Melo-Cardenas, J., Zhang, K., Wang, J., He, F., Fang, D. 2018. ER-associated ubiquitin ligase HRD1 programs liver metabolism by targeting multiple metabolic enzymes. *Nature Commun.* 9(1):3659. [Impact factor 12.353].

NBSI-University of Chicago

Zhang-Chuan He (2019-present): John T. Wilson Distinguished University Professor and HHMI investigator at the University of Chicago.

NBSI-Ohio State University

Zhang-Qinghua Sun (2008-present): Professor and Assistant Dean, College of Public Health of Ohio State University.

1. Wang Y, Li R, Chen R, Gu W, Zhang L, Gu J, Wang Z, Liu Y, Sun Q, Zhang K, Liu C. 2020. Ambient fine particulate matter exposure perturbed circadian rhythm and oscillations of lipid metabolism in adipose tissues. *Chemosphere.* 251:126392.
2. Wang, J., Qiu, Y.*, Yang, Z.*, Kim, H., Qian, Q., Sun, Q., Zhang, C., Yin, L., Fang, D., Back, S., Kaufman, R.J., Yang, L., and Zhang, K. 2018. Inositol Requiring Enzyme 1 Prevents Hepatic Steatosis through Processing microRNAs. *Science Signal.* *(Science)* 11(530). pii: eaao4617. [Impact factor 7, one of the most authentic *Science* sister journals].

3. Bhattacharya, A., Sun, S., Wang, H., Liu, M., Long, Q., Yin, L., Kersten, S., Zhang, K., Qi, L. 2018. Hepatic Sel1L-Hrd1 ERAD manages FGF21 levels and systemic metabolism via CREBH. *EMBO J*. (2018) 37(22). pii: e99277 [Impact factor: 10.6].

NBSI-Case Western Reserve University

Zhang-Sanjay Rajagopalan (2010-present): Herman K. Hellerstein Professor and Division Chief, Department of Internal Medicine and Radiology, Case Western Reserve University

NBSI-New York University

Zhang-Lung-Chi Chen (2010-present): Professor, Department of Environmental Medicine, NYU School of Medicine

NBSI-University of Michigan

Zhang-Jiandie Lin (2009-present): Bradley M Patten Collegiate Professor in the Life Sciences, University of Michigan Medical School

Lei Yin (2011-present): Associate Professor in Integrated Molecular Physiology, University of Michigan Medical School

Ling Qi (2017-present): Professor in Integrated Molecular Physiology, University of Michigan Medical School

1. Wang, J., Qiu, Y.*, Yang, Z.*, Kim, H., Qian, Q., Sun, Q., Zhang, C., Yin, L., Fang, D., Back, S., Kaufman, R.J., Yang, L., and Zhang, K. 2018. Inositol Requiring Enzyme 1 Prevents Hepatic Steatosis through Processing microRNAs. *Science Signal.* ***(Science)*** 11(530). pii: eaao4617. [Impact factor 7, one of the most authentic *Science* sister journals].
2. Zhang, P., Kuang, H., He, Y., Idiga, S., Li, S., Chen, Z., Yang, Z.*, Cai, X., Zhang, K., Potthoff, M., Xu, Y., Lin, J. 2018. NRG1-Fc improves metabolic health via dual hepatic and central action. *JCI Insight* 2018, 3(5). pii: 98522. doi: 10.1172/jci.insight.98522.

NBSI-Louisiana State University

Zhang-Thomas Gettys (2016-present): John H. Hernandez Professor of Health Promotion, Pennington Biomedical Research Center

NBSI Collaborations Within Wayne State University

Jena-Potoff-Manke (2007-2011): NSF Supported Project (2007-2011)– Prof. Jeffrey Potoff (PI)/Jena (Co-PI)/Manke (Co-PI); NSF-CBET 0730768 – Bioengineering and Molecular Simulation Studies to Understand Membrane Fusion. Results from experiments and simulations funded by this work are described detail in the preliminary data. This grant provided partial funding for two graduate students Mrs. Zeena Issa (Chemical Engineering) and Ms. Leah Shin (Physiology), and undergraduate student, Rebecca Lindsey (Chemical Engineering). To date, this ongoing project has produced 6 peer-reviewed manuscripts [1-6], one of which was featured on the cover of the Journal of Physical Chemistry B [2]. Four scientific presentations were made at national and international meetings.

1. Cho, W.J., Lee, J-S., Ren, G., Zhang, L., Shin, L., Manke, C.W., Potoff, J., Kotaria, N., Zhvania, M.G., Jena, B. P. (2011). Membrane-directed molecular assembly of the neuronal SNARE complex. *J. Cell. Mol. Med.* 15:31-37.
2. Issa, Z., Manke, C.W., Jena, B. P., Potoff, J.J. (2010). Ca^{2+} bridging of apposed phospholipid bilayer *J. Phys. Chem.* 114:13249-13254.
3. Potoff, J.J., Issa, Z., Manke Jr, C.W., Jena, B. P. (2008). Ca^{2+}-Dimethylphosphate complex formation: providing insight into Ca^{2+} mediated local dehydration and membrane fusion in cells. *Cell Biol. Int.* 32:361-366.
4. Jena, B. P. (2009). Porosome: the secretory portal in cells. *Biochemistry*. 49:4009-4018.
5. Jena, B.P. (2010) Secretory vesicles transiently dock and fuse at the porosome to discharge contents during cell secretion. *Cell Biol. Int.* 34:3-12.
6. Jena, B.P. (2009) Functional organization of the porosome complex and associated structures facilitating cellular secretion. *Physiology* 24:367-376.

Jena-Potoff-Manke (2011-2016): NSF Supported Project (2011-2016)– Potoff (PI)/Jena (Co-PI)/Manke (Co-PI); NSF-CBET 1066661 – Elucidation of Membrane Fusion Mechanisms Using a Combined Simulation and Experimental Approach. The Jena component of this grant provided partial funding for two graduate students Ms. Amanda Flack (Physiology, who graduated with a Ph.D. in June 2014) and Mr. Kenneth T. Lewis (Physiology, current doctoral candidate). With support from the Dept. of Physics & Astronomy, two graduate students Ms. Maheshika Perera (Physics) and Mr. Suvra S. Laha (Physics). Laha successfully completed his doctoral program in the laboratory and is doing post-doctoral studies. Undergraduate students, Ms. Sanjana Kulkarni (Biology); Ms. Amulya Rajagopal (Physics); Mr. Brandon Laethem (Biology); and Mr. Malek Ghandour (Biology), continue to work on various associated projects in the laboratory. A manuscript submitted with Amulya Rajagopal and Sanjana Kulkarni as lead authors, has been published [6], and a second manuscript with Sanjana Kulkarni as co-lead author with Graduate Student Akshata Naik (Physiology) is published [8] in the journal *Endocrinology*. Studies by Rajgopal and Kulkarni were twice selected and funded for their participation at the ***2014 NCUR Kentucky, and the 2015 NCUR Washington Conference***. Additionally, Ms. Rajagopal received the prestigious 2014 ***"George B. & Eveline R. Beard Endowed Student Prize"*** for her work. Ms. S. Kulkarni is completing her Senior Thesis in the lab. In the past three years, besides the three graduate and four undergraduate students, four high school students (Rohin Patel, Alina Shafikova, Naveen Karthik, and Cara Skrzycki) have actively participated in summer research in the laboratory, with Naveen Karthik making the Semifinalist in the ***2014 Siemens Math & Science Competition***. Ms. Alina Shafikova now a freshman, continues to progress her work in the laboratory, and proposes to work toward her Senior Thesis dissertation in the lab. To date, this ongoing project has produced 8 peer-reviewed manuscripts [1-8], one [2] of which was featured on the cover of the Journal of Histochemistry and Cell Biology, and another published in the Journal of Proteomics [4] was selected as F1000 prime. Additional manuscripts with Brandon Laethem as lead author in one, and Kenneth T. Lewis as lead author in three manuscripts, are in preparation. Eight scientific presentations were made at national and international meetings during this funding period.

1. Lee, J-S., Jeremic, A., Shin, L., Cho, WJ., Chen, X., Jena, B.P. (2012). Neuronal porosome proteome: Molecular dynamics and architecture. *J. Proteomics* 75:3952-3962.
2. Wang, S., Lee, J-S., Bishop, N., Jeremic, A., Cho, WJ., Chen, X., Mao, G., Taatjes, D.J., Jena, B.P. (2012). 3D organization and function of the cell: Golgi budding and vesicle biogenesis to docking at the porosome complex. *Histochem. Cell Biol.* 137:703-718.
3. Kovari, L.C., Brunzelle, J.S., Lewis, K.T., Cho, W.J., Lee, J-S., Taatjes, D.J., Jena, B.P. (2014). X-ray solution structure of the native neuronal porosome-synaptic vesicle complex: Implication in neurotransmitter release. *Micron* 56:37-43.
4. Hou, X., Lewis, K.T., Wu, Q., Wang, S., Chen, X., Flack, A., Mao, G., Taatjes, D.J., Sun, F., Jena, B.P. (2014). Proteome of the porosome complex in human airways epithelia: Interaction with the cystic fibrosis transmembrane conductance regulator (CFTR). *Journal of Proteomics* 96:82-91.
5. Jena, B. P. (2015) Porosome Discovered Nearly 20 Years Ago Provide Molecular Insights into the Kiss-and-Run Mechanism of Cell Secretion. *J Cell Mol. Med.* J. 2015 May 28. PMID: 26033351.
6. Rajagopal, A., Kulkarni, S., Lewis, K.T., Chen, X., Maarouf, A., Kelly, C.V., Taatjes, D. J., Jena, B.P. (2015). Proteome of the insulin-secreting Min6 porosome complex: Involvement of Hsp90 in its assembly and function. *Journal of Proteomics* 114:83-92.
7. Naik AR, Lewis KT, Jena BP. (2015) The Neuronal Porosome Complex in Health and Disease. *Exp. Biol. Med.* (Maywood) OnlineFirst, published on August 11, 2015 as doi:10.1177/1535370215598400.
8. Naik, A.R., Kulkarni, S.P., Lewis, K.T., Taatjes, D.J., Jena, B.P. (2016) Functional reconstitution of the porosome complex in live cells. *Endocrinology* 157:54-60.
9. Laha SS, Naik AR, Kuhn ER, Alvarez M, Sujkowsky A, Wessells RJ, Jena BP. (2017) Nano thermometry measure of muscle efficiency. *Nano Letters* 2017 Jan 23. DOI: 10.1021/acs.nanolett.6b05092
10. Lewis KT, Maddipati KR, Naik AR, Jena BP. (2017) Unique lipid chemistry of synaptic vesicle and synaptosome membrane revealed using mass spectrometry. *Chemical Neuroscience*. 2017 DOI: 10.1021/acschemneuro.7b00030. PMID: 28244738.
11. Arachchige MP, Laha SS, Naik AR, Lewis KT, Naik R, Jena BP. (2017) Functionalized nanoparticles enable tracking the rapid entry and release of doxorubicin in human pancreatic cancer cells. Micron. 2017 Jan;92:25-31. doi: 10.1016/j.micron.2016.10.005.PMID: 27846432.
12. Jena BP, Stemmer PM, Wang S, Guangzhao M, Lewis KT, Walz DA. (2017) Human platelet vesicles exhibit distinct size and proteome. J. Proteom Res. DOI: 10.1021/acsjproteom. 7b00309.

Jena-Potoff (2018-present)

1. Naik, A.R., Kuhn, E.R., Lewis, K.T., Kokotovich, K.M., Maddipati, K.R., Chen, X., Hörber, J.H.K., Taatjes, D.J., Potoff, J.J., Jena, B.P. (2019) Self-assembly and biogenesis of the cellular membrane are dictated by membrane stretch and composition. *J. Phys. Chem. B.* 123: 6997-7005.

Jena-Stemmler Collaboration (2008-present): Prof. Timothy L. Stemmler, Department of Pharmaceutical Sciences, have been collaborating on t-/v-SNARE structure-function in neurons using CD spectroscopy. This ongoing collaboration has resulted in the following research publications.

1. Shin, L., Cho, W-J., Cook, J., Stemmler, T., Jena, B. P. (2010). Membrane lipids influence protein complex assembly-disassembly. *J. Am. Chem. Soc.* 132:5596-5597.
2. Cook, J.D., Cho, W.J., Stemmler, T.L., Jena, B. P. (2008). Circular dichroism (CD) spectroscopy of the assembly and disassembly of SNAREs: the proteins involved in membrane fusion in cells. *Chem. Phys. Lett.* 462:6-9.
3. Kuhn, E. R., Naik, A. R., Lewis, B. E., Kokotovich, K. M., Li, M., Stemmler, T. M., Larsson, L., Jena, B. P. (2018) Nanothermometry reveals calcium-induced remodeling of myosin. *ACS Nano Letters* October 22, 2018, DOI: 10.1021/acs.nanolett.8b02989.

Jena-Chen Collaboration (2012-present): Prof. Xuequn Chen, Department of Physiology, have been collaborating on composition of the porosome complex using mass spectrometry. This ongoing collaboration has resulted in the following research publications. X. Chen, Associate Professor of Physiology, Wayne State University School of Medicine Received first NIH R01 Grant in 2016.

1. Lee, J-S., Jeremic, A., Shin, L., Cho, WJ., Chen, X., Jena, B.P. (2012). Neuronal porosome proteome: Molecular dynamics and architecture. *J. Proteomics* 75:3952-3962.
2. Wang, S., Lee, J-S., Bishop, N., Jeremic, A., Cho, WJ., Chen, X., Mao, G., Taatjes, D.J., Jena, B.P. (2012). 3D organization and function of the cell: Golgi budding and vesicle biogenesis to docking at the porosome complex. *Histochem. Cell Biol.* 137:703-718.
3. Hou, X., Lewis, K.T., Wu, Q., Wang, S., Chen, X., Flack, A., Mao, G., Taatjes, D.J., Sun, F., Jena, B.P. (2014). Proteome of the porosome complex in human airways epithelia: Interaction with the cystic fibrosis transmembrane conductance regulator (CFTR). *Journal of Proteomics* 96:82-91.
4. Rajagopal, A., Kulkarni, S., Lewis, K.T., Chen, X., Maarouf, A., Kelly, C.V., Taatjes, D. J., Jena, B.P. (2015). Proteome of the insulin-secreting Min6 porosome complex: Involvement of Hsp90 in its assembly and function. *Journal of Proteomics* 114:83-92.
5. Fang J, Liu M, Zhang X, Sakamoto T, Taatjes DJ, Jena BP, Sun F, Woods J, Bryson T, Kowluru A, Zhang K, Chen X. (2015) COPII Dependent ER Export: a Critical Component of Insulin Biogenesis and Beta Cell ER Homeostasis. *Mol Endocrinol.* 29: 1156-1169.
6. Lee JS, Caruso JA, Hubbs G, Schnepp P, Woods J, Fang J, Li C, Zhang K, Stemmer PM, Jena BP, Chen X. Molecular architecture of mouse and human pancreatic zymogen granules: protein components and their copy numbers. *Biophys Rep.* 2018;4(2):94-103. doi: 10.1007/s41048-018-0055-1. Epub 2018 Apr 26. PMID: 29756009
7. Naik, A.R., Kuhn, E.R., Lewis, K.T., Kokotovich, K.M., Maddipati, K.R., Chen, X., Hörber, J.H.K., Taatjes, D.J., Potoff, J.J., Jena, B.P. (2019) Self-assembly and biogenesis of the cellular membrane are dictated by membrane stretch and composition. *J. Phys. Chem. B.* 123: 6997-7005.

Jena-Sun Collaboration (2014-present): Prof. Fei Sun, Department of Physiology, have been collaborating on composition of the porosome complex in human airways epethelia. This ongoing collaboration has resulted in the following research publication.

1. Hou, X., Lewis, K.T., Wu, Q., Wang, S., Chen, X., Flack, A., Mao, G., Taatjes, D.J., Sun, F., Jena, B.P. (2014).

Proteome of the porosome complex in human airways epithelia: Interaction with the cystic fibrosis transmembrane conductance regulator (CFTR). *Journal of Proteomics* 96:82-91.

Jena-Maddipati Collaboration (2014-present): Prof. Krishna R. Maddipati, Director, **Wayne State University Lipidomics Facility**, have been collaborating on determining the lipid composition of the porosome complex and in membrane biogenesis. This ongoing collaboration has resulted in the following research publication. An NIH grant proposal is in progress.

1. Lewis, K.T., Maddipati, K.R., Taatjes, D.J., Jena, B.P. (2014). Neuronal porosome lipidome. *J. Cell. Mol. Med.* 18:1927-1937.
2. Lewis KT, Maddipati KR, Naik AR, Jena BP. (2017) Unique lipid chemistry of synaptic vesicle and synaptosome membrane revealed using mass spectrometry. *Chemical Neuroscience*. 2017 DOI: 10.1021/acschemneuro.7b00030. PMID: 28244738.
3. Naik, A.R., Kuhn, E.R., Lewis, K.T., Kokotovich, K.M., Maddipati, K.R., Chen, X., Hörber, J.H.K., Taatjes, D.J., Potoff, J.J., Jena, B.P. (2019) Self-assembly and biogenesis of the cellular membrane are dictated by membrane stretch and composition. *J. Phys. Chem. B.* 123: 6997-7005.

Jena-Stemmer Collaboration (2016-present): Prof. Paul M. Stemmer, Director, **Wayne State University Proteomics Facility**, have been collaborating on determining the proteome of the porosome complex and secretory vesicle chemistry. This ongoing collaboration has resulted in the following research publication. An NIH grant proposal is in progress.

1. Jena BP, Stemmer PM, Wang S, Guangzhao M, Lewis KT, Walz DA. (2017) Human platelet vesicles exhibit distinct size and proteome. *J. Proteom Res.* DOI: 10.1021/acsjproteom. 7b00309.
2. Naik, A. R., Pernal, S., Lewis, K. T., Wu, Y., Wu, H., Carruthers, N. J., Stemmer, P. M., Jena, B. P. (2019) Human skeletal muscle cells on engineered 3D platform express key growth and developmental proteins. *ACS Biomaterials Science & Engineering* DOI: 10.1021/acsbiomaterials.8b01338.
3. Kuhn, E.R.; Naik, A.R.; Lewis, B.E.*; Kokotovich, K.M.; Li. M.; Stemmler, T.L.; Larsson, L.; Jena, B.P.; "Nanothermometry reveals calcium-induced remodelling of Myosin." *Nano. Letters*, 2018, *18*, 7021-9.
4.

Jena-Kovari Collaboration (2014-present): Prof. Ladislau Kovari, and the Jena lab. have been collaborating on determining the molecular structure of the native neuronal porosome complex using solution X-ray and neutron scattering studies. This ongoing collaboration has resulted in the following research publication.

1. Kovari, L.C., Brunzelle, J.S., Lewis, K.T., Cho, W.J., Lee, J-S., Taatjes, D.J., Jena, B.P. (2014). X-ray solution structure of the native neuronal porosome-synaptic vesicle complex: Implication in neurotransmitter release. *Micron* 56:37-43.

Jena-Kim Collaboration (2015-present): Prof. Hyeong-Reh Kim, Department of Pathology, have been collaborating on the use of pH- and temperature-sensitive nanoparticles in cancer detection and therapy. This ongoing collaboration has resulted in the following research publication.

1. Najy A, Dyson G, Jena BP, Lin C-Y, Kim H-R. (2016) Matriptase Activation and Shedding through PDGF-D mediated Extracellular Acidosis. *Am J. Physiol Cell Physiol* 310: C293-C304.

Jena-Kelly Collaboration (2014-present): Prof. Chris Kelly, Department of Physics & Astronomy, have been collaborating on t-/v-SNARE and porosome structure-function in beta cells of the endocrine pancreas using super resolution microscopy. Jointly, Prof's. Kelly and Jena revealed the effects of HSP90 on the supramolecular structure of the porosome secretory portal. In brief, inhibition of HSP90 resulted in deformations in porosome assembly and function. They achieved this through complimentary methods in proteomics, optical imaging, and electron microscopy. Ongoing collaborative studies are exploring the effects of membrane bending on the organization of the porosome on the plasma membrane. Through engineering nanoscale membrane curvature and Polarized Localization Microscopy, preliminary data suggests a passive sorting and aggregation method of porosomes at membrane buds. Additionally, Profs. Kelly and Jena have jointly advised graduate and undergraduate students. In particular, Dr. Suvra S. Laha earned his Ph.D. in physics by studying the magnetic properties and biomedical applications of nanomaterials. Dr. Laha discovered mechanisms for regulating the relaxation rates and temperatures for supraparamagnetic nanoparticles by balancing the Brownian and Neel relaxation rates via diverse nanoparticle syntheses. This ongoing collaboration has resulted in the following research publication. Christopher V. Kelly, Associate Professor of Physics & Astronomy, Wayne State University Received NSF Career Award in 2016

1. Rajagopal, A., Kulkarni, S., Lewis, K.T., Chen, X., Maarouf, A., Kelly, C.V., Taatjes, D. J., Jena, B.P. (2015). Proteome of the insulin-secreting Min6 porosome complex: Involvement of Hsp90 in its assembly and function. *Journal of Proteomics* 114:83-92.

Jena-Mao Collaboration (2012-2019): Prof. Guangzhao Mao, Chair, Department of Chemical Engineering & Material Science, and the Jena group have been collaborating on porosome structure-function using AFM. This ongoing collaboration has resulted in the following research publications.

1. Wang, S., Lee, J-S., Bishop, N., Jeremic, A., Cho, WJ., Chen, X., Mao, G., Taatjes, D.J., Jena, B.P. (2012). 3D organization and function of the cell: Golgi budding and vesicle biogenesis to docking at the porosome complex. *Histochem. Cell Biol.* 137:703-718.
2. Hou, X., Lewis, K.T., Wu, Q., Wang, S., Chen, X., Flack, A., Mao, G., Taatjes, D.J., Sun, F., Jena, B.P. (2014). Proteome of the porosome complex in human airways epithelia: Interaction with the cystic fibrosis transmembrane conductance regulator (CFTR). *Journal of Proteomics* 96:82-91.
3. Jena BP, Stemmer PM, Wang S, Guangzhao M, Lewis KT, Walz DA. (2017) Human platelet vesicles exhibit distinct size and proteome. *J. Proteom Res.* DOI: 10.1021/acsjproteom. 7b00309.

Jena-Walz Collaboration (2015-present): Prof. Daniel A. Walz and the Jena lab. have been collaborating on understanding the proteome of the human platelet and the different vesicles within.

1. Lee JS, Agrawal S, von Turkovich M, Taatjes DJ, Walz DA, Jena BP. (2012) Water channels in platelet volume regulation. *J Cell Mol Med.* 2012 Apr;16(4):945-9. doi: 10.1111/j.1582-4934.2011.01362.x. PMID: 21692982
2. Jena BP, Stemmer PM, Wang S, Guangzhao M, Lewis KT, Walz DA. (2017) Human platelet vesicles exhibit distinct size and proteome. *J. Proteom Res.* DOI: 10.1021/acsjproteom. 7b00309.

Jena-Gatti Collaboration (2018-present) Domenico L. Gatti, Associate Professor of Biochemistry, Microbiology and Immunology, and the Jena lab. have been collaborating to establish the Human Skeletal Muscle Cell Atlas. In addition to the following manuscript, they have jointly submitted the Chan-Zuckerberg grant in 2018, the Human Science Frontier Award with colleagues in Italy in 2019, and two NIH R01's in 2019 and one revised R01 in 2020.

1. Jena BP, Gatti DL, Arslanturk S, Pernal S, Taatjes DJ., "Human skeletal muscle cell atlas: Unraveling cellular secrets utilizing 'muscle-on-a-chip', differential expansion microscopy, mass spectrometry, nanothermometry and machine learning.", *Micron.* 117, 55-59, 2019.
2. Pernal, S., Liyanaarachchi, A., Gatti, D. L., Arslanturk, S., Formosa, B., Pulvender, R., Kuhn, E.R., Ramos, R.,

Naik, A.R.,George, K., Arslanturk, S., Taatjes, D. J., Jena, B. P. (2019). Differential Expansion Microscopy. *bioRxiv* 699579; doi: https://doi.org/10.1101/699579
3. Gatti, D. L., Arslanturk, S., Lal, S., Jena, B. P. (2019). Deep learning strategies for differential expansion microscopy. *bioRxiv* 743682; doi: https://doi.org/10.1101/74368
4. Pernal, S., Liyanaarachchi, A., Gatti, D. L., Arslanturk, S., Formosa, B., Pulvender, R., Kuhn, E.R., Ramos, R., Naik, A.R.,George, K., Arslanturk, S., Taatjes, D. J., Jena, B. P. (2020). Nanoscale imaging using differential expansion microscopy. *Histochem. Cell Biol. (in-press).*

Jena-Arslanturk Collaboration (2018-present) Suzan Arslanturk, Assistant Professor of Computer Science, and the Jena lab. have been collaborating to establish the Human Skeletal Muscle Cell Atlas. In addition to the following manuscript, they have jointly submitted Chan-Zuckerberg grant in 2018, two NIH R01's in 2019 and one revised R01 application in 2020.

1. Jena BP, Gatti DL, Arslanturk S, Pernal S, Taatjes DJ., "Human skeletal muscle cell atlas: Unraveling cellular secrets utilizing 'muscle-on-a-chip', differential expansion microscopy, mass spectrometry, nanothermometry and machine learning.", *Micron.* 117, 55-59, 2019.
2. Pernal, S., Liyanaarachchi, A., Gatti, D. L., Arslanturk, S., Formosa, B., Pulvender, R., Kuhn, E.R., Ramos, R., Naik, A.R.,George, K., Arslanturk, S., Taatjes, D. J., Jena, B. P. (2019). Differential Expansion Microscopy. *bioRxiv* 699579; doi: https://doi.org/10.1101/699579
3. Gatti, D. L., Arslanturk, S., Lal, S., Jena, B. P. (2019). Deep learning strategies for differential expansion microscopy. *bioRxiv* 743682; doi: https://doi.org/10.1101/74368
4. Pernal, S., Liyanaarachchi, A., Gatti, D. L., Arslanturk, S., Formosa, B., Pulvender, R., Kuhn, E.R., Ramos, R., Naik, A.R.,George, K., Arslanturk, S., Taatjes, D. J., Jena, B. P. (2020). Nanoscale imaging using differential expansion microscopy. *Histochem. Cell Biol. (in-press).*

Jena-Dombkowski Collaboration (2017-present) Alan Dombkowski, Associate Professor of Pediatrics and the Jena lab. are collaborating on the role of non-coding RNAs in epilepsy of tuberous sclerosis complex and focal cortical dysplasia type 2B in children. Jena serves as a Co-I on a recently funded (2019) NIH grant on the subject where Dombkowski serves as PI.

Jena-Lisak-Benjamins Collaboration (2017-present) Professor Joyce A. Benjamins and Professor Robert P. Lisak of Neurology and the Jena lab. are collaborating on B Cell Secretory Factors and Neuronal and Oligodendroglia Toxicity. They have jointly submitted a proposal to NMSS. Results of this collaboration have recently been published.

1. Benjamins JA, Nedelkoska L, Touil H, Stemmer PM, Carruthers NJ, Jena BP, Naik AR, Bar-Or A, Lisak RP. Exosome-enriched fractions from MS B cells induce oligodendrocyte death. *Neurol Neuroimmunol Neuroinflamm.* 2019;6:e550. doi:10.1212/NXI.0000000000000550

Jena-Matthew Collaboration (2018-present) Howard Matthew, Vice-Chair and Professor of Chemical Engineering & Materials Science and the Jena lab. are collaborating on a muscle-bone organoid.

Jena-Pellett collaborations: Prof. Pellett has been studying how herpesvirus virions acquire their envelope and how newly enveloped virions are transported to, and then released from the cell surface. An ongoing project in the laboratory has included collaboration between the Pellett and Jena laboratories.

Jena-Grossman Collaboration (2018-present) Lawrence I. Grossman, Director and Professor at the Center for Molecular Medicine and Genetics and the Jena lab's are collaborating on elucidating the interaction between players of the mitochondrial electron transport chain. A joint manuscript and an NIH R01 proposal is in progress.

Jena-Kezhong Collaboration (2019-present) Kezhong Zhang, Professor at the Center for Molecular Medicine and Genetics and the Jena lab's are collaborating on elucidating the molecular mechanism involved in the biogenesis, distribution and secretion of lipid droplets in the liver and skeletal muscles.

1. Lee JS, Caruso JA, Hubbs G, Schnepp P, Woods J, Fang J, Li C, Zhang K, Stemmer PM, Jena BP, Chen X. Molecular architecture of mouse and human pancreatic zymogen granules: protein components and their copy numbers. *Biophys Rep.* 2018;4(2):94-103. doi: 10.1007/s41048-018-0055-1. Epub 2018 Apr 26. PMID: 29756009

Kelly-Potoff Collaboration (2015-present): Profs. Kelly and Potoff have an emerging collaboration to reveal the nanoscopic mechanisms of membrane bending. Prof. Potoff brings his expertise in computational simulations and revealing the molecular details of membrane organization. Prof. Kelly brings his expertise in super-resolution optical methods and nanoengineering. Jointly, they are exploring the mechanism by which lipids of varying shape and phase may contribute to spontaneous membrane bending and initiate endocytosis.

Kelly-Granneman Collaboration (2015-present): Professor James Granneman, Department of Molecular Medicine and Genetics, Wayne State University School of Medicine, specializes in adipose tissue cell and molecular biology, target identification and high throughput screening for novel obesity and diabetes therapeutics. This growing collaboration combines Prof. Granneman's expertise in endocrinology and metabolism with Prof. Kelly's expertise in nanoscale biological processes. Jointly, they aim to understand the supramolecular structures created on the phospholipid surface of lipid droplets within fat cells. Through revealing the cooperative protein behavior, they hope to further advance therapeutic approaches for regulating lipolysis.

Rosenspire-Caruso-Stemmer (2005-present): Analysis of Toxicant and disease mechanisms. Using mass spectrometry we have profiled phosphoproteomes and signaling molecules. The findings have been the first to demonstrate that the Lyn kinase is a key node in mercury toxicity. In this ongoing project we have performed discovery analysis by profiling mass spectrometry focused on the phophoproteome and targeted quantitation using Multiple Reaction Monitoring (MRM) of phosphopeptides in Lyn kinase. We are expanding this project to examine the contribution of genetic background to mercury toxicity mediated by phosphorylation changes in Lyn kinase and Syk kinase. The following publications are papers from my research group in collaboration with Drs. Al. Rosenspire and Joe caruso.

1. Caruso JA, Stemmer PM.: Proteomic profiling of lipid rafts in a human breast cancer model of tumorigenic progression. Clinical and Experimental Metastasis, 2011, 28(6):529-540. PMID: 21533873; PMCID: PMC3827680
2. Caruthers, N.J., Stemmer, P.M., Shin, N., Dombkowski, A., Caruso, J.A., Gill, R., Rosenspire, A.: Mercury Alters B-Cell Protein Phosphorylation Profiles. J Proteome Res. 2014, 13(2):496-505. PMID: 24224561; PMCID: PMC4167842
3. Caruso, J.A., Stemmer, P.M., Dombkowski, A., Caruthers, N.J., Gill, R., and Rosenspire, A.J.: A systems toxicology approach identifies Lyn as a key signaling phosphoprotein modulated by mercury in a B lymphocyte cell model. Toxicology and Applied Pharmacology, 2014;276(1):47-54. PMID: 24440445; PMCID: PMC4005802

Dombkowski-Stemmer Collaboration (2008-present): Dr. Alan Dombkowski and I have collaborated on various projects in which advanced analysis of proteomic data sets is required. Our shared publications are represented in the previous sections and also include the following.

1. Dombkowski AA, Batista CE, Cukovic D, Carruthers NJ, Ranganathan R, Shukla U, Stemmer PM, Chugani HT, Chugani DC. Cortical Tubers: Windows into Dysregulation of Epilepsy Risk and Synaptic Signaling Genes by MicroRNAs. Cereb Cortex. 2014 Dec 1. PubMed PMID: 25452577.

Jena-Pellett Collaborations: Viruses are nanomachines that can be targeted for destruction by nanomachines designed for that purpose, as was done in a study performed in collaboration with Dr. Lawrence Lum and other investigators from the Karmanos Cancer Institute, Henry Ford Hospital, and the Wayne State Division of Infectious Diseases.

1. Lum, L.G., M. Ramesh, A. Thakur, S. Mitra, A. Deol, J.P. Uberti, and P.E. Pellett. 2012. Targeting cytomegalovirus infected targets with T-cells armed with anti-CD3 x anti- CMV bispecific antibody. Biology of Blood and Marrow Transplantation 18:1012-1022.

Pellett-Kovari collaborations: The Pellett laboratory is collaborating with the laboratory of Prof. Ladislau Kovari to study the structural aspects of the function of a conserved herpesvirus protein that is involved in virion envelopment and egress.

Wessells-Jin Collaboration:

1. Cao, T., Sujkowski, A., Cobb, T., Wessells, R.J., Jin, JP. (2020) The glutamic acid-rich long C-terminal extension of troponin T has a critical role in insect muscle functions. *J Biol Chem* doi: 10.1074/jbc.RA119.012014. PMID:32024695.

Wessells-Christian Reynolds. Collaboration has resulted in one publication.
Damschroder, D., Reynolds, C., Wessells, R. (2018). *Drosophila tafazzin* mutants have impaired capacity. *Physiol. Rep.* 6(3) doi: 10.14814/phys2.13604. PMID: 29405656. PMCID: open access.

Wessells-Jena. Collaboration has resulted in one publication and one submitted NIH grant.

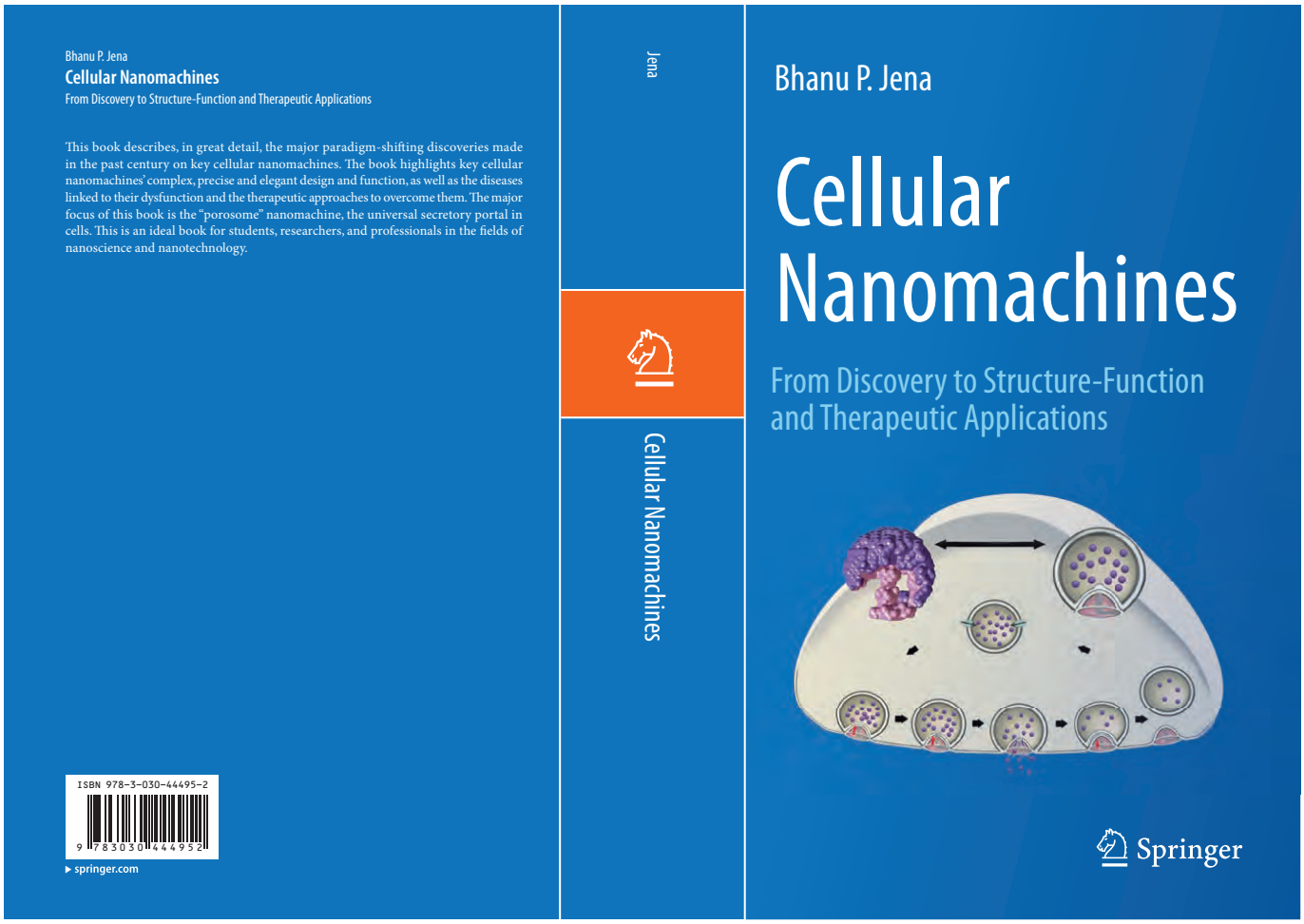
1. Laha, S., Naik, A., Kuhn, E., Alvarez, M., Sujkowski, A., Wessells, R., Jena, B. (2017) Nanothermometry measure of muscle efficiency. *Nano Lett* 8:17(2), 1262-1268. PMID:28112520. PMCID: open access.

Wessells-Robert Arking. Collaboration has resulted in three publications.

1. Sujkowski, A., Bazzell, B., Carpenter, K., Arking, R., Wessells, R.J. (2015) Endurance exercise and selective breeding for longevity extend *Drosophila* healthspan by overlapping mechanisms. *Aging* 7(8):535-552. PMID:26298685. PMCID:PMC4586100.
2. Piazza, N., Gosangi, B., Devilla, S., Arking, R., Wessells, R.J. (2009). Exercise-training in young *Drosophila melanogaster* reduces age-related declines in mobility and cardiac function. *PLoS One*. 4(6):e5886. PMID:19517023
3. Sujkowski, A., Spierer, A.N., Rajagopalan, T., Bazzell, B., Safdar, M., Imsirovic, D., Arking, R., Rand, D., Wessells, R. (2018). Mito-nuclear Interactions Modify *Drosophila* Exercise Performance. *Mitochondrion* S1567-7249(18):30061-30068. PMID:30408593.

Wessells-Sokol Todi. Collaboration has resulted in one submitted paper and two submitted NIH grants.

NBSI BOOK IN-PRESS:



PATENT

1. **Inventor: Jena, B.P.** Nano thermometry measure of muscle efficiency. U.S. Provisional Patent:US 62/498,015. Establishes a novel approach to determine muscle efficiency, with promise for early diagnosis and treatment of various metabolic disorders including cancer.

2. **Inventors:** Nguyen, H., **Zhang, K.,** Loka, R. Title: Sulfated Glycopolymers as Potent and Specific Heparanase Inhibitors for Diabetic Therapy. U.S. Provisional Patent No.: W063-0067USP1 | WSU 20-1581 1; Date: October 21, 2019

3. **Inventors:** Kaufman, R., **Zhang, K.** Title: Compositions and Methods for Modulating the Acute Phase Response. U.S. US patent pub. No.: US2007/0111258A1; Pub. Date: May 17, 2007
International pub. No.: WO/2007/002493; Pub. Date: April 1, 2007

CURRENT NBSI FOCUS

1. Among the current focus of NBSI is establishment of the **'Human Skeletal Muscle Cell Atlas'** and utilize the combined power of differential expansion microscopy (DiExM) and machine learning and AI approaches to study cellular structure-function at the nanoscale using an ordinary diffraction limited microscope. Application of DiExM in diagnostic pathology is also being explored.

2. **Recognizing the surge in metabolic disease (Hypertension, Heart Disease, Diabetes, Cancer, Mental Illness, etc) in urban localities around the world including Detroit, the NBSI Director (Jena) has recently collaborated with NBSI members and national and international experts, to put together a proposal for a new "Center for Metabolism & Movement" to be established within the NanoBioScience Institute at Wayne State University. A summary of the proposed Center is provided below:**

Center for Metabolism & Movement

OVERVIEW: The Center for Metabolism and Movement (CMM) will be the genesis of a new field of study, the 'Science and Engineering of Metabolism and Movement', that will provide substantial new insights into the two critical interdependent life processes and to harness this knowledge to benefit society. How metabolism generates energy and how energy is used in generating movement is well known; insights into the emergent properties and nuances of these two highly coordinated processes in a living organism remains to be understood. Skeletal muscle, used for locomotion, is the largest metabolic organ, whereas the brain drives both locomotion and metabolism. The Center's outreaching goal is to unravel the poorly understood coordinated workings of the brain and skeletal muscle in experimental cross-talk using engineered tools and approaches while utilizing machine learning to establish neural networks that will provide a deep systems level understanding of the two processes. This objective is aligned with NSF's Big Idea of "understanding the rules of life" via "convergence research" by merging approaches, tools and technologies from a diverse field of science and engineering. The Center will adopt integrative strategies that "harnesses the data revolution" utilizing 'machine learning', to obtain a systems level understanding of metabolism and movement. Furthermore, the transforming education and career paths to be developed in the Center will contribute to bridge the knowledge gaps in our understanding of brain-skeletal muscle cross-talk. Results from the study will provide a breakthrough in our understanding of the most critical determinants in the survival and success of an organism and open a window to elucidate a wide range of life processes such as the brain neural network and cognition, the skeletal muscle cell atlas, and provide new and novel ideas in the development of tools and approaches for a broad spectrum of applications in the sciences. CMM will be characterized as a highly interdisciplinary and multi-institutional NSF Science and Technology Center, featuring close collaborations among neurobiologists, muscle physiologists, physicists, engineers and computer scientists. The scope and duration of the Center will enable a breadth and depth of activities not possible by other funding mechanisms. The funding scale, the collective contribution of investigators with diverse expertise and Center management, will allow research, education, training and knowledge transfer activities to be supported well beyond what is possible via an uncoordinated individual investigator-directed project.

INTELLECTUAL MERIT: CMM will focus on understanding the fundamental mechanism of how brain and skeletal muscle communicate to regulate metabolism and movement in an organism. How do brain and skeletal muscles adapt to inputs from each other? How does exercise impact the neural network of the brain? and How can machine learning be used to capture key elements in brain-muscle cross-talk in understanding metabolism and movement? Will be addressed. This new knowledge will provide a window to our understanding of a wide range of life processes and in the development of tools and approaches for a broad spectrum of applications in the sciences. The Center will additionally contribute to the technological advancements in tool-building and in new and novel approaches required for the proposed study and their application in chemistry, physics, engineering, and the biological sciences.

BROADER IMPACT: In addition to contributing to the knowledge and technology base on metabolism and movement, and their application, the Center will have a transforming impact on society by educating the public on the two most critical determinants of life and utilization of the new and leading technologies. The science and technology base to be provided by the Center will serve as a resource to build on programmatic strengths for the development of new technologies and their use and commercialization, for the transfer of knowledge to the broader scientific community and to public and private sectors, in the development of new courses and education programs for graduate and postdoctoral fellows, and for the recruitment of new faculty. The advanced tools and technologies created in the Center will provide an unprecedented understanding of life processes and will be optimized and delivered for broader application in the investigation of a wide range of biological processes. The Center will serve as a pipeline of trained scientists in the field who from the very beginning, are trained to engage the public. This highly interdisciplinary Center with a futuristic vision will serve the rapidly growing application of science and technology in service to humanity.

PROJECT DESCRIPTION

A. Center Rationale: Metabolism and movement, the two most critical determinants in the survival and success of an organism, are tightly regulated by concerted signaling between the brain and skeletal muscle. Sensory perception in the organism is converted to synaptic transmissions through motor neurons, which then feed downstream to effector cells. Crucial to carrying out the resulting biomechanical processes are two important cellular players: mitochondria and myosin. Although the skeletal muscle, mitochondria, myosin, and the neurons that control them, have been intensively investigated on their own, their coordinated involvement in metabolism and movement remains poorly understood. The **grand challenge** to be addressed by the Center for Metabolism and Movement (CMM) will be to obtain a fundamental understanding of the cross-talk between the brain and skeletal muscle and mitochondria and myosin. This objective is aligned with NSF's Big Idea of "understanding the rules of life" via "convergence research" by merging approaches, tools and technologies from a diverse field of science and engineering. The Center will adopt integrative strategies that "harnesses the data revolution" utilizing 'machine learning', to obtain a systems level understanding of metabolism and movement. Furthermore, the transforming education and career paths to be developed in the Center will contribute to bridge the knowledge gaps in our understanding of brain-skeletal muscle cross-talk in the regulation of metabolism and movement in organisms. Results from the study will provide a **breakthrough** in our understanding of the most critical determinants in the survival and success of an organism and open a window to elucidate a wide range of life processes such as the brain neural network and cognition, the skeletal muscle cell atlas, the role of mitochondria in driving metabolism and mechanical function of skeletal muscles, and provide new and novel ideas in the development of tools and approaches for a broad spectrum of applications in the sciences.

A bottom-up multiscale approach, from single molecule 3D imaging using differential expansion microscopy developed by members of the Center team, combined with team-expertise in cryo-electron microscopy, TIRF and STED, solution X-ray and neutron scattering, to engineered brain and skeletal muscle 'Tissue-on-a-Chip' developed by team members, makes the proposed research possible. Small animal models like the *Drosophila* where center members have developed unique genetic variants and exercise regiems, allow biochemical and biomechanical manipulations to determine the role of exercise on the remodeling of mitochondria and myosin in neurons and skeletal muscle. Similarly, team expertise in the noninvasive imaging of brain and muscle tissues in larger animals and humans utilizing Magnetoencephalography, MEG, Positron Emission Tomography, PET, and functional Magnetic Resonance Imaging, fMRI, and in the computational tools of image analysis, allows for the first time and provide a platform to determine the brain-muscle and mitochondria-myosin cross-talks required for understanding metabolism and movement. Information gained from experimental studies will be integrated with computational generative tools ranging from molecular dynamics simulations of protein ensembles to steady-state and time-course simulations of cell metabolism and protein expression, and analytical tools of machine learning, to obtain a systems-level understanding.

Our vision is to create a new field – the 'Science and Engineering of Metabolism and Movement' via a multi-disciplinary and multi-institutional approach, by bringing together muscle physiologists, neuroscientists, computer engineers and machine learning experts. This new Center is dedicated to establishing a knowledge and technology base on

metabolism and movement, the two most critical determinants for the survival and success of an organism. **The mission** of CMM is to understand foundational mechanisms of metabolism and movement at the interface of physiology and biophysics. The discoveries made by CMM will be critical to obtain a fundamental understanding of the cross-talk between the brain and skeletal muscle and mitochondria and myosin, regulating metabolism and movement. Three goals are at the heart of CMM's mission: 1: Reveal how brain and skeletal muscles communicate to regulate metabolism. 2: Reveal how skeletal muscles adapt to brain inputs and vice versa. 3: Cross-cutting technology development and integration. Develop advanced computational and instrumentation platforms to answer and analyze key research questions proposed in goals 1 & 2. Integrate strategies for knowledge dissemination. Develop graduate-level courses in the field, train students and disseminate new knowledge and application with the greater scientific community and the public. The approaches will be characterized by a highly interdisciplinary and multi-institutional NSF Science and Technology Center, featuring close collaborations among neurobiologists, muscle physiologists, physicists, engineers and computer scientists. CMM will serve as a platform for the design and development of new tools and technologies and will act as a template for educating a new generation of researchers with in-depth understanding of a broad spectrum of disciplines, capable of performing research across disciplinary boundaries, and who are trained to engage the public. The interdisciplinary challenges of a systems level understanding of metabolism and movement being addressed at CMM is beyond the scope and duration of individual research groups or single institutions. The funding scale, the collective contribution of investigators with diverse expertise applicable to the projects, and the experience-based management of the Center, will allow research, education, training and knowledge transfer activities to be supported well beyond what is possible by any of the existing NSF STC's. The science and technology base to be provided by the Center will serve as a resource to build on programmatic strengths, for the development of new technologies and their use and commercialization, for the transfer of knowledge to the broader scientific community and to public and private sectors, in the development of new courses and education programs for graduate and postdoctoral fellows, and for the recruitment of new faculty. This highly interdisciplinary Center with a futuristic vision will serve the rapidly growing application of science and technology in service of humanity.

B. Center Plan: While studies report the tight correlation between metabolism and brain function **(1)**, there exists a brain-muscle cross-talk regulating metabolism and movement homeostasis **(2,3)**, which is little understood. At the cellular level, mitochondria are tasked with the synthesis of ATP, the molecule that fuels life processes including the molecular motor protein myosin for movement. There is significant evidence supporting a close relationship between mitochondrial function and the morphology of skeletal muscles **(4,5)**, and conversely the contribution of myosin on mitochondrial DNA maintenance **(6)**. Clearly, there is a void in our knowledge on these cross-talks and therefore, the Centers outreaching goal is to unravel the coordinated workings of the brain and skeletal muscle and mitochondria and myosin in the regulation of metabolism and movement to gain a systems-level molecular understanding. To accomplish this objective, *Drosophila*, and human primary neuron and muscle-on-a-Chip, will serve as the primary model systems. Noninvasive studies on small animals and humans will be conducted utilizing Magnetoencephalography (MEG), Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI) to determine brain and muscle activity and metabolism. In the first five years, CMM will have the following three major thrusts. **Thrust 1: Brain-skeletal muscles cross-talk to regulate metabolism. (1a)** How different afferent and efferent inputs from the brain impacts skeletal muscle efficiency, motility and

metabolism. **(1b)** How exercise-induced exosomes released from skeletal muscles impact brain metabolism. **Thrust 2: Adaptation of skeletal muscle and brain inputs. (2a)** How visual cues related to exercise, a psychobiological model of endurance performance, impact skeletal muscle metabolism and function. **(2b)** How exercise and sedentary states impact mitochondria and myosin remodeling in both the skeletal muscle and the brain. **Thrust 3: Cross-cutting technology development and integration.** Development and integration of computational methods and cross-cutting tools and instrumentation platforms that will help in elucidating the questions being asked and in the dissemination of knowledge. (3a) Deep learning platform to capture key elements in brain-muscle cross-talk, function and regulation of metabolism and movement. Thrusts 1 and 2 are enabled and connected by a cross-cutting toolset program that will help in the advancement of instrumentation platforms and computational methods required to elucidate the questions being asked. Computational approaches will be used to capture key elements in brain-muscle cross-talk, function and regulation of metabolism and movement. Generative computational tools, ranging from molecular dynamics simulations of protein ensembles to steady-state and time-course simulations of cell metabolism and protein expression; and analytical tools of machine learning, will be utilized to obtain a systems-level understanding of the cross-talks pertaining to metabolism and movement. Information from PET, MEG and fMRI on the areas of the brain that are activated during movement and their consumption of oxygen and substrates will be used to constrain the *in silico* reconstruction of a time and space resolved map of the cellular metabolism in these areas. The center will also study the features of brain hierarchical processing of images by measuring visual cortical activity with fMRI and MEG and mapping its decoding (translation) into the hierarchical features of pre-trained deep neural network (DNN) processing the images. Likewise, the brain response to sensory stimuli from movement will be mapped into the corresponding response of recurrent neural networks (RNN) to the same time series of stimuli. Part of this focus will be the continuing development of additional technologies like (a) Differential Expansion Microscopy (DiExM) (b) Direct Adhesion Nanothermometry (DANT) in determining muscle efficiency. (c) 'Engineered Tissue-on-a-Chip'.

Thrust 1: Brain-skeletal muscles cross-talk to regulate metabolism. This thrust encompasses two primary research themes: (1a) How different afferent and efferent inputs from the brain impacts skeletal muscles metabolism; (1b) How exercise-induced exosomes released from skeletal muscles impact brain metabolism. Both themes will continually be connected to themes in thrust 2 and to computational methods required to elucidate the questions being asked, and to the cross-cutting toolset program that will help in the advancement of the instrumentation platforms.

(1a) How different afferent and efferent inputs from the brain impacts skeletal muscles metabolism is poorly understood. Enabling this understanding will fill gaps in our broader understanding of brain-skeletal muscle communication in the regulation of metabolism. Our implementation strategy involves the participation of CMM Center investigator **Donal O'Leary**, Director of the Cardiovascular Research Program, who is an internationally recognized expert in the area of afferent and efferent inputs on skeletal muscle function. To understand the effect of efferent and afferent inputs on brain and muscle metabolism, the coordinated involvement of CMM Center investigators **Otto Muzik, Jessica S. Damoiseaux** and **Susan M. Bowyer**, experts on the use of noninvasive MEG, PET and fMRI on brain function and tissue metabolism, with state-of-the-art equipment and facilities at their disposal, will be able to make major advancement. During exercise afferents from skeletal muscle become active due to insufficient O_2 delivery. These afferents travel to the brain and elicit

activation of autonomic nervous system afferents. Insufficient cardiac output results in these afferents to become hyper active and are responsible for excessive increases in sympathetic output causing profound peripheral vasoconstriction. This vasoconstriction includes the heart and even the under-perfused skeletal muscle which thereby elicits a positive-feedback, a vicious cycle scenario acting as an effective amplifier of sympathetic activity. Such positive feedback scenarios may also arise from cardiac afferents as coronary vasculature becomes vasoconstricted. The O'Leary laboratory, using animal models to study this phenomenon, have developed approaches to dampen both the extent of skeletal muscle afferent activation and the effects of sympathetic activity, to be able to dissect out at the physiological level, afferent and efferent inputs to skeletal muscles. Noninvasive MEG, PET and fMRI in the laboratories of CMM Center investigators **Otto Muzik, Jessica S. Damoiseaux** and **Susan M. Bowyer**, in combination with *in silico* reconstruction of both steady state and dynamic cellular metabolic fluxes, will be used to determine brain and skeletal muscle activity and metabolism in animals. Skeletal muscle biopsies from animals from these studies will serve in determining at the cellular and molecular level, the remodeling of mitochondria and myosin, at the morphological, compositional and functional level in the laboratories of **Douglas J. Taatjes** (Electron Microscopy, STED, STORM), **Bhanu P. Jena** (DiExM, Atomic Force Microscopy), **Christopher V. Kelly** (TIRF Microscopy), **Lawrence I. Grossman** (Electron transport chain proteins, mitochondrial biochemistry)**, Lars Larsson** (gene expression and myosin-actin motility assays), **Paul Stemmer** (Mass Spectrometry: Proteomics) and **Krishna Maddapati** (Mass Spectrometry: Lipidomics).

(1b) How exercise-induced exosomes released from skeletal muscles impact brain metabolism. Exosomes are 40-120 nm membrane-bound vesicles released from cells, have been recognized as a major natural carrier of miRNA, non-coding RNA and proteins, contributing to cellular communication **(7)**. Blood samples from sedentary and exercised animals used in studies outlined in **1a** will be used to isolate and determine circulating exosomes for characterization in the laboratories of CMM investigators **Alan Dombkowski, Bhanu P. Jena (8) [Fig 1]**, **Paul Stemmer and Krishna Maddapati**. The Dombkowski lab. routinely isolates exosomes both from surgically resected human brain tissue, muscles and blood, which is then characterized by the Jena lab. using the Zeta Sizer, AFM and immunoblot analysis **[Fig 1]**. These studies will be further complemented using engineered muscle and brain 'Tissue-on-a-Chip' microphysiological stretchable platform developed in the **Jena lab. [Fig 2]** to obtained exosomes released from skeletal muscle cells in culture following various regimes of stretched (exercised) and sedentary states and study their impact on the metabolism of brain neurons in culture and the remodeling of mitochondria. The Jena laboratory has optimized a stretchable micropatterned 3D microphysiological platform for use in studies that recapitulates organized and parallel growth of muscle fibers **(9) [Fig 2]** expressing key myogenic and mitochondrial proteins **(9)**. The proteome (**Paul Stemmer**), lipidome (**Krishna Maddapati**) and miRNA (**Alan Dombkowski**) in the isolated exosomes, and the compositional remodeling of neurons as a result of exposure to skeletal muscle-derived exosomes from exercised and sedentary states, will be assessed. Structural and functional remodeling of the mitochondria in cultured brain neurons and whole brain subjected to the exosomes, will be assessed using 3D Differential Immuno Expansion Microscopy (DiExM) combined with machine learning in the Jena lab. **[Fig 3] (10, and manuscript under review)**. Time-course metabolomics and RNA-seq will be carried out on neurons from different brain regions exposed to skeletal muscle-derived exosomes, to be able to determine ongoing cellular activities and gene expression profiles that define the type of cellular process in progress. Neuronal activity will also be assessed using calcium imaging

using fluorophore combined electrophysiological recordings in the laboratories of **Inna Slutsky**. To understand the effect of the exosomes on the neuronal circuits of the brain, genetically engineered indicators combined with two-photon microscopy and epifluorescence fiberscopes applied to different brain regions of mice will be used [Fig 4] **(Slutsky lab., 11-14)**. Complementing these studies, the effects of exosomes derived from blood plasma of sedentary and exercised animals on brain metabolism will be assessed using MEG, PET and fMRI and metabolic simulations in the laboratories of **Otto Muzik, Jessica S. Damoiseaux** and **Susan M. Bowyer**.

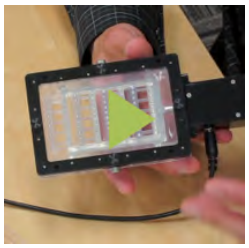


Figure 1. Atomic force micrograph of isolated exosomes from resected human brain tissue demonstrates an average size of 60 nm and a distribution of 30-115 nm. ***(Jena, Dombkowski Lab)***.

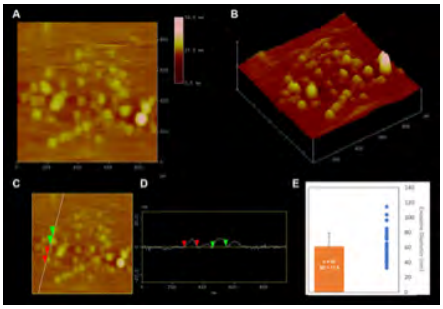


Figure 2. Multimodal tension device with stretch capacity mimicking both in tension and frequency, exercising native human skeletal muscles on a micropatterned 3D stretchable platform. **(7, *Jena Lab*)**.

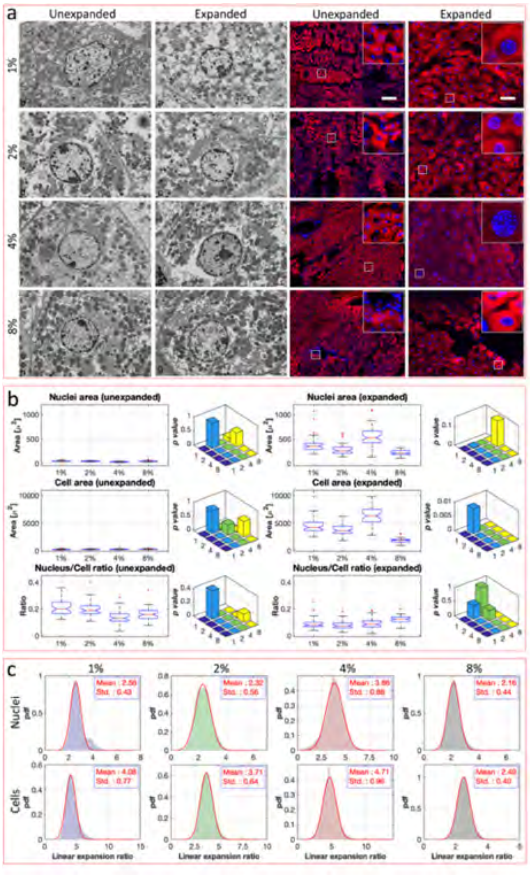


Figure 3. Modified approach using sodium polyacrylate-induced up to 8-fold linear expansion of rat hepatocytes prefixed in 4% para formaldehyde (PFA), corresponding to a 512-fold increase in cell volume. **a**. (LEFT TWO PANELS) Representative transmission electron micrographs of the unexpanded and expanded rat liver tissue demonstrating no visible change between the 2%, 4% and 8% PFA fixed tissue, while the 1% expanded cell reflects some loss of cytosolic material. (RIGHT TWO PANELS) Light fluorescent micrographs of rat liver sections pre-fixed in different concentrations of PFA and stained using lysotracker red R18 dye for the cell and DAPI blue for the nucleus, demonstrates maximum expansion (up to 8-fold in linear expansion) in the 4% PFA-fixed hepatocytes. **b**. Significant changes in the cell and nucleus area and the ratios between them in unexpanded and expanded cells, is demonstrated. **c**. Distribution of the linear fold expansion in the hepatocyte nucleus and cell body demonstrates from 2- to 8-fold expansion, with the 4% PFA-fixed cell demonstrating maximal expansion. ***(Jena, Gatti, Arslanturk, -Manuscript under review)***.

In 2015, as opposed to the invention of an imaging tool, the substrate was enlarged to enable nanoscale imaging using an ordinary diffraction limited light microscope **(15)**. In this simple yet novel approach termed expansion microscopy (ExM) **(15)**, a hydration-competent polymer (sodium polyacrylate) is used to physically expand biological specimens to be imaged at nm scale, using a light microscope **(15-17)**. ExM holds great promise for a fundamental understanding of the cell at the nanometer scale using a simple diffraction limited light microscope. While the protocol for expansion and the retention of intracellular antigen has progressed in the past 3 years since invention of ExM, the 4-fold linear expansion limit, while retaining the immune-fluorescently labeled integrity and correction for the anisotropic expansion of intracellular organelles, remains a major hurdle. Using a modified protocol, the

Jena Lab. has overcome the 4-fold linear expansion limit, while retaining the immune-fluorescently labeled integrity, achieving an 8-fold linear expansion of tissues to achieve a volumetric expansion of 512-fold [Fig 3] **(manuscript under review)**. To establish a correction factor that could be applied to the DiExM images, the **Jena lab.** in collaboration with proposed CMM Center investigators **Domenico L. Gatti** and **Suzan Arslanturk**, have developed a Matlab® based interactive machine learning application for the analysis of both expanded and unexpanded images, capable of recognizing fine cellular changes affecting the size, shape and distribution of organelles and/or macromolecules. This machine learning approach will also be further developed in the Center to predict expanded status of cells and of any remodeling at the nanometer scale in the skeletal muscle and brain tissue **(10, and manuscript under review)**.

Time-course metabolomics and RNA-seq will be carried out on neurons from different brain regions exposed to skeletal muscle-derived exosomes, to be able to determine ongoing cellular activities and gene expression profiles that define the type of cellular process in progress. Neuronal activity will also be assessed using calcium imaging using fluorophore combined electrophysiological recordings in the laboratories of **Inna Slutsky**. To understand the effect of the exosomes on the neuronal circuits of the brain, genetically engineered indicators combined with two-photon microscopy and epifluorescence fiberscopes applied to different brain regions of mice will be used [Fig 4] **(Slutsky lab., 11-14)**. Complementing these studies, the effects of exosomes derived from blood plasma of sedentary and exercised animals on brain metabolism will be assessed using PET and fMRI and metabolic simulations in the laboratories of **Otto Muzik, Jessica S. Damoiseaux**.

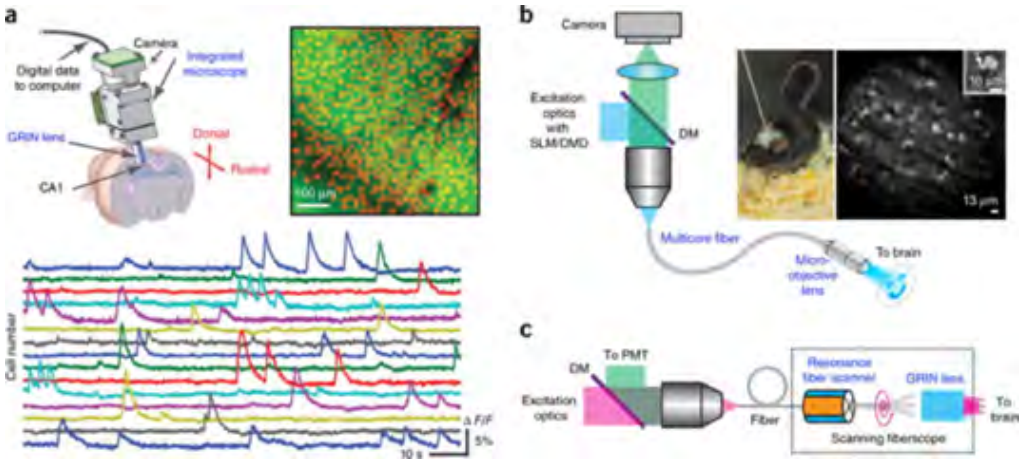


Figure 4. Neural activity in conscious freely behaving mouse. a: Epifluorescence miniaturized head-mounted microscope in a GCaMP3-transfected mouse and signals from 15 different neuronal cells. **b**: Epifluorescence calcium imaging showing a portion of a single interneuron (inset) and **c**: Schematics of a two-photon scanning fibroscope **(11)**.

Thrust 2: Adaptation of skeletal muscle and brain inputs.

(2a) How visual cues related to exercise, a psychobiological model of endurance performance, impact skeletal muscle metabolism and function. CMM investigator **Robert J. Wessels** has identified a subset of neurons whose activity is necessary and sufficient to drive exercise adaptations even in sedentary animals. Octopamine (the invertebrate analog of norepinephrine) secretion mediates the effect of these neurons on exercise adaptation and transient stimulation of neurons

that secrete octopamine is completely sufficient to provide metabolic and performance benefits of exercise to sedentary animals. The Wessells lab. has begun to extend these observations into human subjects, using virtual reality headsets to simulate the experience of exercise in sedentary people [Fig 5].

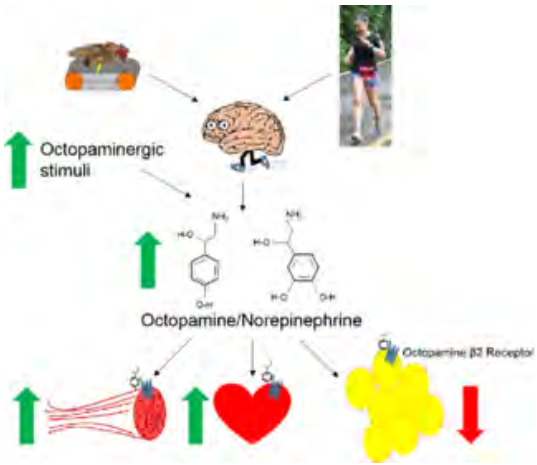


Figure 5. Schematic drawing illustrating the role of octopamine secretion during exercise and visual cues mediating brain neuroplasticity (**Wessells** lab.).

In pilot experiments with healthy young humans, >62% of subjects without prior exposure to exercise or to virtual reality, experienced increased norepinephrine release and heart rate that was similar to the increases observed when the same subjects exercised on a bike.

The energy demand of the brain is nearly 20% of all energy consumption, and higher cognitive functions are associated with increased brain metabolism and the expression of metabolic related genes. Understanding the relationship between visual cues and their impact on skeletal muscle and brain metabolism will be a unique and novel contribution by CMM investigators. **Jessica S. Damoiseaux**, will assess exercise-induced remodeling of brain networks using fMRI and tractography. **Susan M. Bowyer** using MEG, will add a dimension of high temporal resolution to this exploration of how human brain processes language, hearing, vision, and sensory inputs during exercise. PET studies by **Otto Muzik** will assess noninvasively key metabolic fluxes (i.e., O_2, glucose consumption) in both skeletal muscles and brain, thus providing the experimental basis for simulations/reconstructions by computational means of the brain and muscle metabolisms, as they reciprocally adapt to changing energy requirements **(18-22)** [Fig 6].

Figure 6. Schematic representation of multiscale integration of cellular events, metabolic indices, and bold fMRI signal acquisitions during synaptic activity in the brain. Figure represents EPSP generation in postsynaptic neurons (1, N) and glutamate-transporter current in astrocytes (1, A) (green circles). Early energy requirements of neurons are met by lactate oxidation in neuronal mitochondria, resulting in decreased extracellular lactate levels (2) and oxygen concentration (2) (red circles). As illustrated by combined PET measurements of glucose utilization, blood flow, and oxygen consumption, an uncoupling between CMRGlu, CBF,and $CMRO_2$ occurs, resulting in a decreased oxygen extraction fraction (OEF) and increased lactate production (3); the ANLS model provides a metabolic context for these observations with an increased glucose uptake into astrocytes (3) that is processed through aerobic glycolysis, resulting in the replenishment of the extra-cellular lactate pool (3) (blue circles). A direct glucose uptake by neurons under basal conditions and a potential lactate overflow into the circulation during activation may occur (dotted arrows). The delayed transient aerobic glycolysis occurring in astrocytes, which does not require an increase in oxygen consumption, results in excess oxyhemoglobin in the activated area, producing the positive bold signal (4) (gold circle). The initial dip, corresponds to oxygen utilization, resulting in increased deoxyhemoglobin, which occurs during the early phase

of synaptic activity (4). CBF: cerebral blood flow; CMRGlu: cerebral metabolic rate of glucose; $CMRO_2$: cerebral metabolic rate of oxygen; EPSP: excitatory postsynaptic potential **(18)**.

(2b) Impact of exercise and sedentary states on lipid droplet biogenesis and on mitochondria and myosin remodeling. Space flight and inactivity has been reported to induce a programmed shift from slow to fast type II fibers in human skeletal muscles **(23-26)**. However, the molecular mechanism of this process remains unclear. Since physical activity in humans and animal models is known to upregulate the expression of the transcriptional coactivator PGC-1α involved in mitochondrial biogenesis and found to rescue animal models of myopathy **(27)**, the impact of exercise and on the increased and decreased expression of PGC-1 on myosin and mitochondria remodeling in both the skeletal muscle and brain, will be studied using the fruit fly *Drosophila* and skeletal muscle and brain neuron cultures on the 3D microphysiological platform **(9)** **[Fig 2]**.

Similar to the disuse-induced muscle wasting in experimental rat model and in humans established in the **Lars Larsson** lab. **(28-31)**, the Wessells lab. has designed a novel exercise **(32,33)** and disuse paradigm for *Drosophila melanogaster*. The movement of flies is restricted, limiting availability of space for their movement using a foam plug. The disuse assay system is suitable for both large-scale fly genetics and for longitudinal studies to follow individual changes in physiology of the fly under various conditions and during aging. Similarly, an earlier study **(34)** shows that the *Drosophila* homolog of the vertebrate exercise response gene PGC-1α *spargel* (srl), is sufficient to induce exercise-dependent phenotypes. Reduction of srl expression levels acutely compromises negative geotaxis ability and reduces exercise-induced improvement in both negative geotaxis and time to exhaustion. To test the hypothesis that overexpression of *spargel* would potentiate efficiency of the fly muscle in the use of ATP, in a recent study from the Jena lab. **(27)** utilizing cadmium telluride quantum dots (CdTe QDs) as nano thermometers in direct association with isolated skeletal muscles obtained from wild type *Drosophila melanogaster* outcrossed genetic background control flies (y^1w^1*UAS-srl)* and flies with muscle-specific *spargel* overexpression (*mef2>UAS-srl)*, demonstrate *spargel* overexpression indeed potentiates muscle efficiency in the fly. Remodeling of the neural network in the fly brain, and of myosin and mitochondria in the fly brain and flight muscle, and lipid droplet biogenesis in them, will be assessed using: (i) DiExM developed in the **Jena lab.**, (ii) solution x-ray and neutron scattering studies in the **Joseph S. Brunzelle** and **Jena labs. (35)**, (iii) cryo-electron microscopy in the **Gang Ren** laboratory and (iv) ER-mediated lipid droplet biogenesis in the laboratory of **Kezhong Zhang**. The fly brain is composed of nearly 100,000 neurons **(36)**. Large dimension, high-resolution imaging is important for neural circuit visualization as neurons have both long- and short-range patterns, from axons and dendrites to synapses at nerve terminals. Although EM is the conventional approach for nanoscale imaging, tomograms are time consuming, tedious and expensive, and the retrieval of structural information segmented from high-density images within large volume datasets continues to remain challenging. Fluorescent probes will therefore be used to localize synapse, mitochondria and various mitochondria-associated proteins and myosin isoforms, to determine their distribution and arrangement at nanoscale in 3D in the fly primarily using DiExM and complemented with cryo-EM and X-ray and neutron scattering studies. Biochemical remodeling of the brain and skeletal muscle myosin and mitochondria will be assessed using mass spectrometry.

To assess **functional changes to the neural circuits in the fly brain**, genetically targeted optical electrophysiology will be performed in the laboratory of **Inna Slutsky**. In the same way that genetically encoded fluorescent sensors revolutionized

the study of intracellular calcium signals, **ArcLight technology (37)** now enables optical measurements in intact neural circuits of membrane potential that underlies neuronal information processing and will be used to assess functional remodeling of neural circuits in the fly brain in exercise and sedentary states. Using this approach, multiple fluorescent voltage indicators will be genetically targeted and expressed in neurons of the fly brain, enabling reliable recording of individual electrical events simultaneously in multiple neurons according to published procedures **(37)**.

Thrust 3: Cross-cutting technology development and integration. Development and integration of computational methods and cross-cutting tools and instrumentation platforms that will help in elucidating the questions being asked and in the dissemination of knowledge **(Section D)**.

3a. Computational: Develop a machine learning platform to capture key elements in brain-muscle cross-talk, function, and regulation of metabolism and movement. The Center will focus on studies aimed at understanding how the brain and skeletal muscles metabolism are interconnected, and how machine learning can be used to capture key elements in brain-muscle cross-talk to understand metabolism and movement. These studies will be performed in the laboratories of **Domenico L. Gatti, Suzan Arslanturk and Bhanu P. Jena (10).**

The skeletal muscle component: Skeletal muscles account for more than a third of our body weight, and myocytes metabolism has a major impact on whole-body homeostasis. For instance, myocytes are responsible for ~75% of the insulin-stimulated clearance of glucose from the blood after a meal **(38).** Using human myocytes differentiated into myotubes it is possible to accurately assess: (i) mitochondrial respiratory activity by high resolution oximetry and fluxes of major reporter metabolites by **SeaHorse technology**; (ii) mitochondrial morpho-functional properties by confocal microscopy imaging using specific fluorescent probes to detect morphology, mitochondrial membrane potential (mt$\Delta\Psi$), reactive oxygen/nitrogen species, mtCa^{2+}; (iii) expression of transcription factors and down-stream targets involved in mitochondrial biogenesis, mitophagy, dynamics, antioxidant response, and mitochondrial Unfolded Protein Response (mtUPR). Furthermore, as cellular molecular motors (myosin) consume energy in the form of ATP and convert it into mechanical work to generate force and movement, the heat loss by such a motor while performing work provides a way of quantifying its efficiency. When energy used by a molecular motor results in greater heat loss and less mechanical work performed, it reflects lower efficiency. Based on this principle, the nanothermometry (NT) approaches developed by the Jena lab. can be used to make precise measurements of mitochondria and myosin efficiency **(27)**. Using a small number of experimentally derived parameters as constraints, the complexity of human cells metabolism can be explored computationally through state-of-the-art genome-scale metabolic models (GEMs) **(39-40)**. Starting from the generic metabolic network of a human cell, reconstruction of a context-specific GEM captures the active subset of metabolism present in a particular tissues and cell types **(41,42)** **[Fig 7]**.

A comprehensive myocyte GEM, iMyocyte2419, was recently reconstructed (**43*)*** and can be downloaded from the repository of curated models. It consists of 5,590 reactions in eight different compartments, 4,448 metabolites (2,396 unique), and 2,419 genes. iMyocyte2419 GEM will be adopted in the Center as the basic GEM for muscle metabolism, with updates/corrections based on locally acquired proteomic and transcriptome data. The metabolic pattern of human muscle cells in culture is expected to vary considerably depending on the growth conditions and phase. For example:

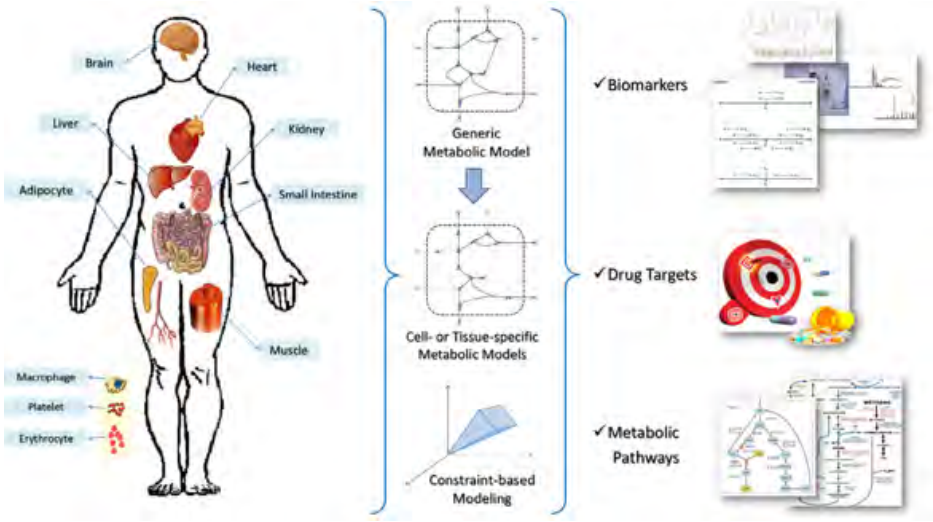


Figure. 7. Constraint-based analysis of human genome-scale metabolic network models. The path from generic to tissue specific models is shown, and their application for predicting biomarkers, drug targets, and analyzing metabolic pathways **(42)**.

1. During log-phase on 2D/3D substrate cells will maximize the biomass synthetic reaction.

2. During stationary phase on either 2D or 3D substrates cells will minimize the energy demand and therefore ATP synthesis.

3. During stretching exercise on a 3D platform cells will maximize the synthesis of ATP to support contraction.

4. After prolonged exercise myosin synthesis and mitochondrial biogenesis will be induced.

The reorganization of metabolism in these states will be reconstructed using the iMyocyte2419 model within the theoretical frame of Flux Balance Analysis (FBA). FBA can thus be defined as the use of linear programming to identify the best cost function:

$$\min_{v}/\max\ \boldsymbol{c^T v} \quad s.t. \begin{cases} \boldsymbol{Sv} = \dfrac{d\boldsymbol{x}}{dt} = \boldsymbol{0} \\ \boldsymbol{v_{i,min} \le v_i \le v_{i,max}} \end{cases}$$

Where $\boldsymbol{S}$ is the *stoichiometric matrix*, $\boldsymbol{v}$ is a vector of *fluxes*, with $\boldsymbol{v_{i,min} \le v_i \le v_{i,max}}$ upper and lower limits determined experimentally, and $\boldsymbol{c}$ is a vector of weights indicating how much each reaction (such as the biomass reaction when simulating maximum growth) contributes to the objective function. The output of FBA is a particular flux distribution, $\boldsymbol{v}$, which maximizes or minimizes the objective function. Thus, the most important step in the application of FBA is the choice of the *objective function* that allows the identification of a particular functional state in the space of possible solutions. Some examples of objective function, with direct application to the functional states of brain and muscle cells are:

Minimize or maximize ATP production: find conditions of optimal energy efficiency.

Minimize (or maximize) nutrient uptake: find conditions to carry out a particular function with minimal or maximal consumption of certain nutrients, i.e., when testing the performance of myocytes contraction using glucose, fatty acids, or ketone bodies as substrates (or in neurons participating in highly active neural networks).

Maximize metabolite production: find the maximal production rate of a chosen metabolite, i.e., the sets of amino acids involved in the biosynthesis of myosin and/or mitochondrial proteins (or a specific neuromediator).

Maximize biomass formation: determine the maximal growth rate of a cell in a given environment. For example, this metabolic pattern would reflect the replication of muscle stem cells after an injury leading to muscle loss.

Maximize biomass and metabolite production: find the best compromise between cell growth and metabolite production. For example, myocytes propagated in a new culture after extensive exercise on a 3D stretching platform will both reenter log-phase (biomass maximization) and show evidence of exercise adaptation (increased myosin synthesis).

Based on proteomic and transcriptome data, gene expression effects (including those produced by specific knock-outs) on metabolism will be investigated by constraining the associated reaction or reactions to specific values. *Dynamic* ME simulations, which add into the basic framework of FBA additional constraints on protein concentration dynamics that alter the metabolic response to environmental fluctuations, will be used to predict the substrate utilization hierarchy in the presence of multiple substrates, and thus provide an understanding of proteome allocation and metabolism under the complex and transient environments of brain and muscle cells, whose substrate preferential utilization is rapidly changing in response to their reciprocal regulation.

The brain component: The brain is composed of several cell types, including neurons and astrocytes. Highly optimized GEM's are available for the cerebral cortex, hippocampus, and lateral ventricles neurons and glia cells, and for cerebellum granular and molecular layer cells and for Purkinje cells. These GEM's allow highly refined simulations of metabolic fluxes in these individual cell types. However, as a result of the intimate connections between these cells, brain metabolism will be modeled by multi-cell-type metabolic networks. Flux constraints suitable for modeling neurons and glia metabolism will be derived (*in vitro*) from cell cultures biochemical studies (e.g., by SeaHorse technology, see above) and (*in vivo*) from dynamic quantitative PET imaging (**Muzik laboratory**). PET scans with ^{15}O-O_2, ^{15}O-H_2O, ^{18}F-FDG, co-registered to T1-weighted magnetic resonance (MR) images, will typically be used to measure blood volume, blood flow and Cerebral metabolic rate of oxygen (CMR_{O2}) and glucose (CMR_{glu}) consumption, which are sufficient to constrain the FBA models. This methodology is well suited to measure absolute regional values of glucose and oxidative metabolism in both the brain and muscle tissue during rest and exercise condition. Currently there are no experiments that have directly monitored such interactions in vivo, and the fate of enhanced glucose uptake in tissues is still unclear. The Center approach will be unique in applying PET validated methodology to study the interaction between the brain (control system) and muscle tissue (actuator) with respect to overall energy consumption and will set clear boundary conditions for metabolic reconstructions using genome-scale metabolic models (GEMs). The time resolution of PET imaging will be overcome by combining functional brain imaging with magnetoencephalography (MEG) *in the* **Boyer lab**. Special generative adversarial forms of neural networks (GANs) will also be employed to achieve denoising and both spatial and time hyperresolution of brain and muscle images. Ultimately, the combination of PET, fMRI and MEG imaging with FBA/dynamic ME metabolic reconstructions and simulations will lead to the development of a **metabolic map** of brain and skeletal muscles with millisecond and voxel scale resolution, and thus reveal the reciprocal influence and regulation of these two organs.

3b. Experimental:

1. DiExM (**Jena, Gatti** and **Arslanturk** lab.).

2. Direct Adhesion Nanothermometry (DANT) in determining muscle efficiency (**Jena** lab.).

3.'Human Muscle-on-a-Chip' microphysiological 3D stretchable platform (**Jena** and **Matthews** Lab.).

As an example, components 1-3, will focus on **optimizing expansion while retaining integrity of the tissue.** Immunofluorescent imaging and detection of biological samples rely on identifying epitopes via primary antibodies followed by fluorescent-tagged secondary antibodies, therefore the optimal resolution could be at the size of the probe, which is nearly 30 nm. In contrast, if primary peptide antibodies tagged with a fluorophore are used for labeling, the probe size is reduced by half to about 10-15 nm. With a 1000-fold expansion in 3D of a fluorescently labeled tissue, one could reach the scale of single molecular imaging using a diffraction limited fluorescent microscope. Therefore, optimization of the expansion protocol (while retaining tissue integrity, will be an important early achievement of CMM investigators.

The **Jena, Gatti,** and **Arslanturk** laboratories**.** have made the first iteration of this objective by making major adjustments to the original ExM protocol to achieve >500-fold expansion in 3D, while retaining the immuno-fluorescently labeled integrity of the tissue **[Fig 3]**. Additionally, the group has demonstrated expansion to be anisotropic i.e., differential expansion between tissues, between cells in the same tissue, between intracellular organelles and within an organelle.

Component 4 will bring brain and muscle together **at the intersection of imaging, FBA, and Deep Learning.** PET/MEG/FBA metabolic reconstruction of myocytes and neurons/glia metabolism will be only a preliminary step in the development of the **Center's ambitious goal and completely novel approach** to understand the complex relationship between *form* and *function* in these cells. A widely accepted paradigm of cell biology is that cell morphology and function are not only 'interconnected', but mutually 'deterministic'. In no other cells is this connection more evident than in muscle cells, where the spatial localization of energy producing mitochondria near myosin fibers and axonal ending is critical for contraction and communication. On this basis, *Deep Learning* tools will be developed to reveal the correlation between the metabolic activity of brain and muscle cells and their images. In fact, while it is *intuitively* obvious that cell morphology (i.e., the image of a muscle cell) and functions (i.e., its energy demands and contraction performance) are intimately connected, it is only an artificial neural network that, when exposed to a set of images and corresponding functions (the training set), can quantitate this connection and use it for prediction of function from morphology (image function), or morphology from function (function image), for samples that were never previously seen by the network.

The biochemical *markers* of metabolic fluxes collected in resting and/or active muscle and brain cells both *in vitro* and *in vivo*, and the corresponding metabolic networks calculated by FBA will be matched to the cells morphology as determined by the advanced form of fluorescence microscopy developed at the center (i.e., DiExM). 'Deep Learning' *neural networks* will be trained to associate a particular metabolic vector (the label of each image) to a specific DExM image **[Fig 8]**. We anticipate that such trained network will then be capable of inferring the metabolic state and performance of myocytes and neurons simply from their images, without the need for all the biochemical analyses that were necessary to initially train the network. In the future, this approach could be used to infer the metabolic state and performance of muscle and brain cells from normal and diseased individuals simply from imaged biopsy material, without the need for laborious, expensive or invasive biochemical tests. Likewise, a Deep Learning platform will be developed to associate particular patterns of

activations in the brain (as for example imaged by MEG/MRI) to the brain and muscle metabolic states calculated by FBA and used to train the network.

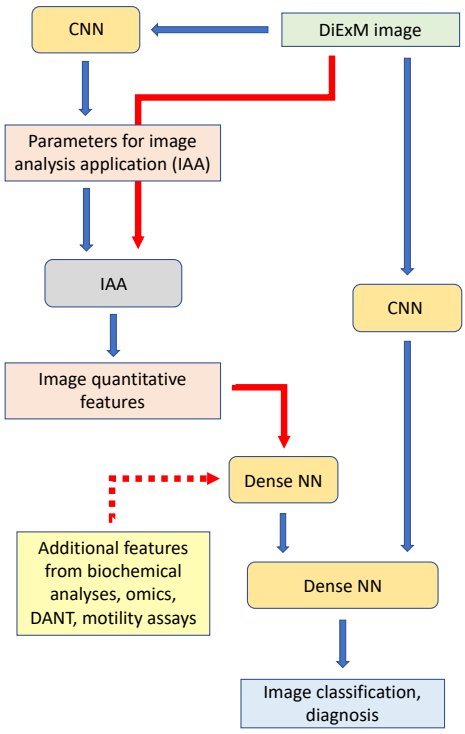


Figure 8. End-to-end pipeline for diagnostic assignment of DiExM images. DiExM images are first passed through a CNN to assign parameters required by an image analysis application (IAA) that produces a vector of quantitative features. These features (as well as additional features from other potential sources) are then channeled through a dense network and finally merged with a separate CNN that detects morphological characteristics. The output of the pipeline is a diagnostic class assignment.

C. Team Description: CMM brings together eight core partner institutions (Wayne State University, Case Western Reserve University, University of Vermont, Argonne National Laboratory, Lawrence Berkeley National Laboratory, Henry Ford Hospital, Tel Aviv University and the Karolinska Institute) with key facilities in conjunction with expert researchers. As outlined in the application, the expertise of the participating faculty and senior personnel afford a convergent research team to address the grand challenges outlined in our center plans, by integrating core strengths in the biology of brain, muscle and metabolism – expert area 1 (Wayne State University, Karolinska Institute, Tel Aviv University, Henry Ford Hospital, Case Western Reserve, University of Vermont), machine learning – expert area 2 (Wayne State University, Tel Aviv University), cross-cutting tools and technology development – expert area 3 (Wayne State University, University of Vermont, Argonne National Laboratory, Lawrence Berkeley National Laboratory). Several key scientific contributions by CMM's PI's (briefly outlined in Section 4b) set the stage for the proposed effort and have been critical to delineating the CMM thrusts. Given space limitations, just a few examples are **Lawrence I. Grossman**, a leading expert on cell metabolism and mitochondria biology, working on the electron transport chain proteins of the organelle. Similarly, **Walter F. Boron** is an expert on cell pH regulation and gas (O_2 and CO_2) transport, critical for cellular metabolism and survival. **Bhanu P. Jena** is a cell biologist with extensive experience on studies involving secretion, skeletal muscle cell biology, cell structure-function and tool development (DiExM, Nanothermometry, Muscle-on-a-Chip). All three of the above CMM members currently serve as founding directors of research institutes and large research programs, with decades of experience in research and education program development and administration, at both the national and international level.

D. Integration of Strategies: Develop graduate-level courses in the field, train students and disseminate new knowledge and application developed at the Center to the greater scientific community and the public. The primary objective is to develop, integrate, and disseminate knowledge and skills that are derived from the generated data and their analysis for a systems-level understanding. Existing and new University graduate courses will be designed to train the next generation of scientists to harness the capability and capacity of computers, bioinformatics and data analytics for a comprehensive understanding of systems biology. A working group of faculty and graduate students in College/School of Engineering, Liberal Arts and Sciences, Education, and Medicine, in conjunction with the University's Provost "Big Data Initiative" are

invested in this undertaking. New courses and methodologies will be placed in the public domain through Coursera and/or similar web-based platforms. All research programs in the center and in each of its participating partner institutions will be integrated into one or more graduate-level course(s), to be offered in various disciplines such as Physiology, Physics, Chemical Engineering and Computer Science. All our participating groups will participate in training the next generation of graduates and postdoctoral fellows in this new field. We will partner with the **Detroit Science Center** to bring new and novel discoveries, inventions and their application to the public.

The PI (**Jena**) is the Founding Director of the NanoBioScience Institute, established in the year 2000, and oversees all the multi-disciplinary studies in the institute involving the College of Arts and Sciences, the College of Engineering, and the School of Medicine at Wayne State University, with the participation of over 50 faculty and 100 students at any given time. The PI, with appointment in all three colleges, continues to mentor with colleagues, undergraduate, graduate and post-doctoral students in Chemical Engineering, Computer Science, Physics and Physiology, and is involved in the summer research program for high school students. As Director of the NanoBioScience Institute and its multi-disciplinary program, the PI has developed innovative curriculum to enhance retention rates. With colleagues, some who are participating in the proposed CMM, the PI has developed a nanoscience course (PSL 7215), which he directs and instructs with colleagues from the Dept. of Physics, Chemical Engineering, Computer Science and Engineering, Biochemistry, and Pharmacy. The course is used as a vehicle to discuss recent advances in the field. The PI has developed a new multi-disciplinary cross-campus concentration: "Biophysics & Biomaterials", between the Dept. of Physiology, Physics, and Chemical Engineering. This concentration covers a broad range of topics from various imaging modalities (AFM, EM, TIRF, STED, etc) to the development of simulation codes and applications using tools such as LAMMPS and NAMD. In the proposed CMM Center, this course will be further developed using the OpenCourseWare model. New knowledge and technology developed in the proposed Center will similarly be integrated into a number of courses in Physiology, Physics, Computer Science and Engineering. The further development and integration of a broad range of topics, from introductory statistical mechanics to the development of simulation codes and applications using tools such as LAMMPS and NAMD will be implemented. This approach provides the opportunity to a large body of students from high school through undergraduate, graduate and post-doctoral level and help enhance the participation of underrepresented groups, thereby providing a broad impact. For example, the Center will hold workshops and seminars every 3 months on DiExM technology combined with machine learning to bring the technology to the greater scientific community to learn and to use in their own research. The **Jena, Gatti** and **Arslanturk** lab. have developed a Matlab® based interactive application for the analysis of control and DiExM images, which has already been made public and is available for download at the investigator's website together with a video tutorial. The application first identifies each organelle (segmentation) and then extracts quantitative information about various features of nuclei, cytoplasm, and other imaged molecules or organelles of importance via feature engineering. In light of the different levels of expansion undergone by different cellular compartments, this quantitative information is extremely valuable to make precise interpretation of DiExM images of cells in various states that affect cellular and sub-cellular dimensions. However, at this time the application requires intervention by the user for fine-tuning the segmentation of organelles and adjustments of parameters which are used in image analysis, due to changes from image to image depending on various factors (i.e., resolution, brightness, type of staining, number of channels, cell density, etc.). The

application is robust enough that the ranges of parameter values that assure optimal performance are quite large. A goal of the Center is to further improve the application by completely automating the feature engineering process. For this purpose, a separate Convolutional Neural Network (CNN) will be trained with a very large set of DiExM images that have been hand-labeled with the correct set of parameters for optimal feature extraction. In this case, the CNN will not work as a *classifier*, but will instead actuate a form of *regression*, providing as the output of its final layer the required set of parameters for input to the image analysis application. Ultimately, this application will be combined with the machine learning application to construct an end-to-end pipeline for automated DiExM image analysis, classification **[Fig 9]**.

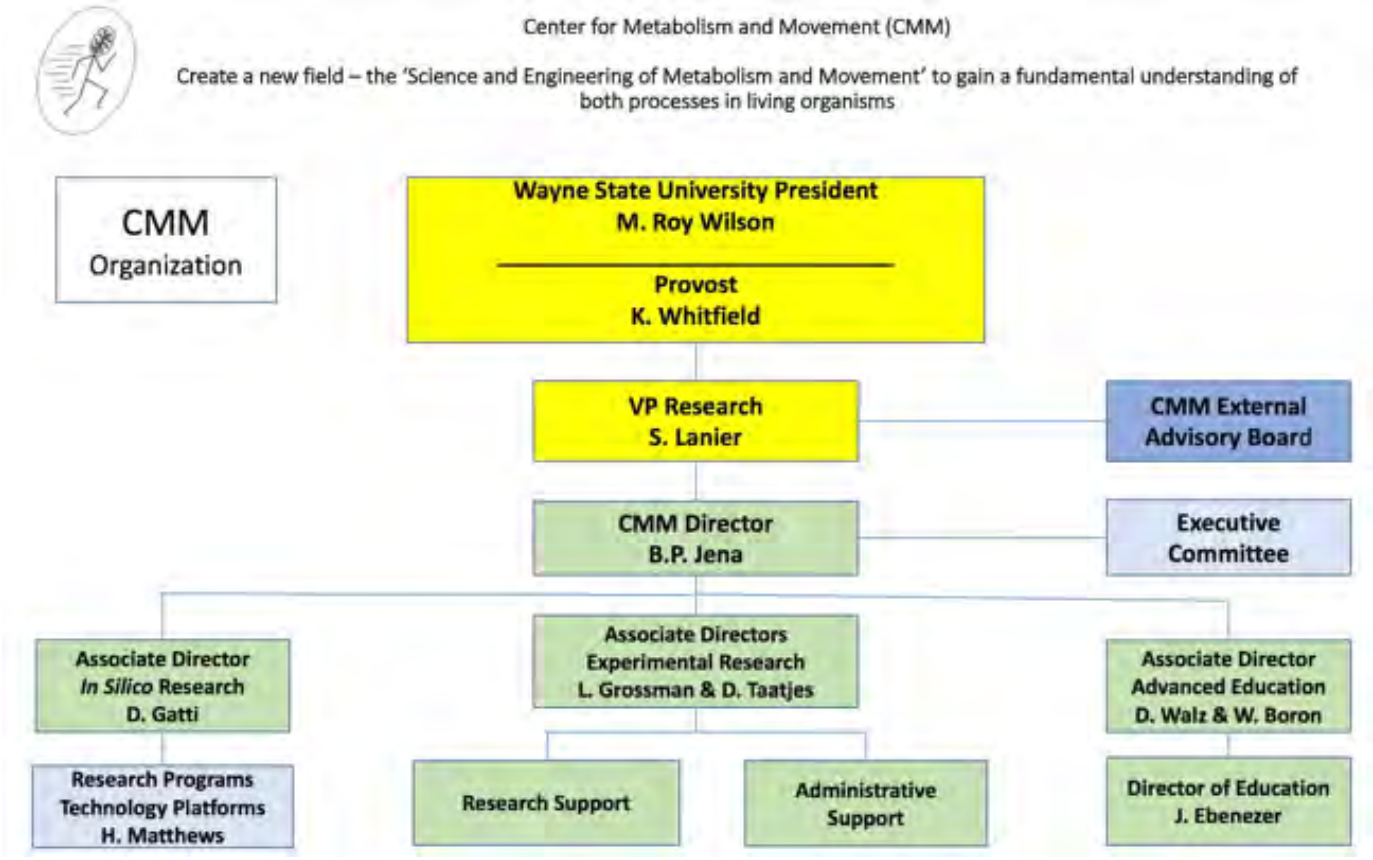


Figure 9. Center for Metabolism and Movement (CMM) Organization Chart.

The organization of CMM is structured to promote excellence in integration strategies to achieving the science and educational objectives of the Center. Starting with WSU **President Dr. M. Roy Wilson**, a scientist and educationist, and a member of the US National Academy, **Prof. Dr. Keith E. Whitfield**, Provost, and an accomplished scholar in neuroscience, and **Prof. Dr. Stephen M. Lanier**, Vice President for Research, and an accomplished scientist in pharmacology and educator, are all invested in the promotion of research and academic excellence at Wayne State University. The excellent facilities and laboratory space of the new iBio building where the proposed Center will be located, and the collegial and collaborative atmosphere there, fostered and nurtured by Prof. Lanier, is a testament of WSU's commitment for CMM. The experience and track record of the **PI and Director Prof. Dr. Bhanu P. Jena** in the establishment of such scientific and academic centers of excellence, in the integration of research and education activities and strategies to patent and commercialize new products, tools and approaches developed at CMM, is exemplified by the establishment of the

NanoBioScience Institute at WSU, the Korea Nano Science Institute and a company 'QPathology' in Boston, MA. Members of the Advisory Board of QPathology involve experts in machine learning and artificial intelligence like Prof. Dr. John D. Halamka, Dean of Technology at Harvard and molecular and gene editing expert and scientist Prof. George M. Church of Harvard and MIT, will also be consulted. Similarly, **Prof. Dr. Lawrence I. Grossman**, Director of the Center for Molecular Medicine & Genetics, and **Prof. Dr. Daniel A. Walz**, Associate Director of Advanced Education at CMM, with 35 years of research and administrative experience, including as former Vice President of Research and Dean of the Graduate College at Wayne State University, will help in integrating and implementing the research and education activities at CMM.

This highly experienced management team structure **[Fig 9]**, will help foster research, education, training and knowledge transfer activities by funding mechanisms and making available existing resources within the participating groups and their organizations. The science and technology base to be provided by the Center will serve as a resource to build on programmatic strengths, for the development of new technologies and their use and commercialization, for the transfer of knowledge to the broader scientific community and to public and private sectors, in the development of new courses and education programs for graduate and postdoctoral fellows, and for the recruitment of new faculty. The Center will serve as a pipeline of trained scientists in the field who from the very beginning, are trained to engage the public. This highly interdisciplinary Center with a futuristic vision, will serve the rapidly growing application of science and technology in service to humanity.

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Massachusetts Institute of Technology, Cambridge	Biotech Process Engineering Center	http://web.mit.edu/bpec/
Massachusetts Institute of Technology, Cambridge	Whitehead Center	www.wi.mit.edu
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State Univ. of N.Y. Stony Brook	Center for Biotech	www.biotech.sunysb.edu
Tuskegee Univ., Ala.	Center for Plant Biotechnology Research	www.tuskegee.edu
Univ. of Cincinnati, Ohio	Genome Research Institute	http://medcenter.uc.edu
Univ. of Colorado, Denver	Center for Pharmaceutical Biotech	www.uchsc.edu/sop/biotech/overview.html
Univ. of Florida, Gainesville	Center for Regenerative Health Biotech	www.biotech.ufl.edu
Univ. of Idaho, Moscow	Environmental Biotech Institute	http://image.fs.uidaho.edu
Univ. of Illinois, Urbana-Champaign	Biotech Center	www.biotec.uiuc.edu
Univ. of Maryland, College Park	Biotechnology Institute	www.umbi.umd.edu
Univ. of Maryland, Rockville	Center for Advanced Research in Biotechnology	http://carb.umbi.umd.edu
Univ. of Maryland, Shady Grove	Center for Advanced Research in Biotech	http://shadygrove.umbi.umd.edu
Univ. of Minnesota, Minneapolis	Academic Health Center	www.ahc.umn.edu
Univ. of Southern California, Los Angeles	Center for Computational and Experimental Genomics	www-hto.usc.edu
Univ. of Tennessee, et al	Tennessee Biotech Association	www.tnbio.org
Univ. of Texas, College Station	Crop Biotech Center	http://cbc.tamu.edu
Univ. of Texas, Houston	Health Science Center	http://biotech.uth.tmc.edu
Univ. of Wisconsin, Madison	Biotechnology Center	www.biotech.wisc.edu
Utah State Univ., Logan	Center for Integrated Biosystems	www.biosystems.usu.edu
Virginia Polytechnic Institute and State Univ., Blacksburg	Fralin Biotech Center	www.biotech.vt.edu
Washington State Univ., Pullman	Center for Integrated Biotechnology	www.biotechnology.wsu.edu
Yale Univ., New Haven, Conn.	Keck Biotechnology Lab	http://keck.med.yale.edu
Nanobiotechnology		
Arizona State Univ., Tempe	BioDesign Institute	www.biodesign.asu.edu
California Institute of Technology, Pasadena	Nanosystems Biology Cancer Center	www.caltechcancer.org
Cornell Univ., Ithaca, N.Y.	Nanobiotechnology Center	www.nbtc.cornell.edu
Wayne State Univ., Detroit, Mich.	NanoBioScience Institute	www.med.wayne.edu/nanobioscience/
Web sites of interest		
Academic Microarray Core Facilities	www.nslij-genetics.org/microarray/core.html	
Biotechnology Educational Resources	www.nal.usda.gov/bic/Education_res	
Biotechnology on the Web	www.cato.com/biotech	
Biotech-Related WWW Sites and Documents	www.nal.usda.gov/bic/www.html	
GradSchools.com	www.gradschools.com/listings/menus/biotechnology_menu.html	