# Kynon Jade Benjamin

## K99 POSTDOCTORAL FELLOW · COMPUTATIONAL BIOLOGIST

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Research Interests: Neurological disorders, health disparities, bioinformatics, computational biology, machine learning

## Summary\_

**COMPUTATIONAL GENETICIST** with expertise in bioinformatics, machine learning, and molecular biology. Authored and/or contributed to 8 publications, 24 presented posters and presentations national, regional, and local meetings, receiving 6 awards including 3 first place. Experience in computational pipeline developed and implementation.

## **Education & Training**

## Lieber Institute for Brain Development; Johns Hopkins University, Departments of Neurology and Psychiatry

Baltimore, MD

POSTDOCTORAL FELLOW: "GENETIC AND ENVIRONMENTAL REGULATION OF GENE EXPRESSION IN NEUROPSYCHIATRIC DISORDERS"

Oct 2017 - Present

- Computational analysis in four postmortem brain regions for genetic and epigenetic ancestry (**Python, R, Machine learning**)
  - Identified and characterized ancestry-related expression differences in postmortem brain from AA and EA individuals
  - Designed, developed, and implemented GPU-based eQTL and fine-mapping analysis pipeline
  - Designed, developed, and implemented mash modeling pipelines for differential expression and eQTL analyses
  - Designed, developed, and implemented pipeline for determining genetic bases of ancestry-related expression differences
- · Computational analysis in postmortem brain for schizophrenia (Python, R, Linux, Machine learning)
  - Identified new insights into the role of the caudate in schizophrenia and potential new lines of treatment
  - Designed, developed, and implemented genotype imputation pipeline for eQTL analysis
  - Designed, developed, and implemented limma-voom differential expression pipeline for post-mortem samples
  - Designed, optimized, and implemented machine learning pipelines with feature reduction and ranking
  - Prioritized genes and genetic variants associated with schizophrenia risk via functional genomics (TWAS, Colocalization, SMR)
  - Examined dopamine system and the affect of antipsychotics within the caudate nucleus
  - Designed, developed, and implemented co-expression network analysis (Autoencoders, WGCNA)
- Human cell lines and organoids for neurodevelopment (Python, R, Linux)
  - Collaborated with bench biologist bulk and single-cell RNA-sequencing and genotyping
  - Designed, developed, and implemented single-cell RNA-sequencing pipeline
  - Designed and preformed genetic verification of postmortem dura-derived induced pluriponent stems cells
- NIH T32 Training Program in Psychiatry
- MOSAIC Postdoctoral Career Transition Award to Promote Diversity (K99/R00): MD0169640

## Texas A&M University, Department of Veterinary Pathobiology

College Station, TX

 $Ph.D.\ in\ Genetics;\ Dissertation:\ ``Understanding\ the\ imprinting\ mechanism\ of\ \textit{UBE3A}\ for\ the rapeutic\ intervention''$ 

Aug 2012 - Aug 2017

- Computational and molecular characterization of the expression patterns of long non-coding RNA of *Ube3a/UBE3A* antisense and novel *Ube3a* isoform 4 in the imprinting of *Ube3a* 
  - Analyzed more than **5 TB** of RNA-seq, stranded and unstranded, paired- and single-end datasets for mouse and human over a range of tissues and cell types for spatiotemporal regulation (**R**, **Linux**)
  - My pipeline reduced overall processing time by 90%, decreased storage by 60% by removing unnecessary intermediate conversion files, increased quality control and improved novel isoform detection
  - ANOVA, student's T-test, post-hoc Tukey's HSD statistical analysis conducted in R significantly reducing computational time from programs like PRISM and Excel
  - Utilized quantitative real-time PCR, Sanger sequencing, electrophoresis, and cloning for long non-coding RNA analysis and alternative splicing verification
- Developed interactive **R** script to analyze **1.2 TB** of fluorescent image based high-throughput screening data assay to reactivate paternal *Ube3a* allele in ES cell derived neurons for therapeutic intervention (**Stem cells, ImageJ, R**)
  - Developed **Shiny** web application for automatic high-throughput screen analysis and logistic regression algorithm for hit analysis (**Shiny**, **Octave**, **R**)
  - Supervised a team of undergraduates in molecular data acquisition and basic laboratory work
  - Utilized immunofluorescence microscopy and mouse embryonic stem cell technology
- Findings:
  - 1. Neuron-specific spatiotemporally regulated *Ube3a* antisense is an extensively processed transcript with 5' capping, 3' polyadenylation and alternative splicing
  - 2. Proposed new model for imprinting mechanism of *Ube3a* involving a temporally regulated novel paternal specific isoform, denoted isoform 4
  - 3. Developed high-throughput screening assay for drug discovery in ES cell-derived neurons

Kynon JM Benjamin · CV 1

B.S. IN BIOMEDICAL ENGINEERING; UNDERGRADUATE RESEARCH/SUMMER UNDERGRADUATE RESEARCH PROGRAM

Nov 2010 - Jun 2012

- Investigated increasing drug loading into nanofibers to determine the mechanical integrity of fibers as a function of percent drug
- Prepared, evaluated, and analyzed electrospun poly( $\epsilon$ -caprolactone) and poly(vinyl alcohol) nanofibers via SEM, fluorescence assays, pH change, and mechanical tester
- Developed semi-automatic MATLAB script for mechanical tester analysis reducing processing time by  $\sim 70\%$
- Developed liquid chromatography mass spectrometry (LC-MS) method for the analysis of naproxen drug release from polymeric scaffolds in media and PBS
- Supervised and trained undergraduate and graduate students on nanofiber technique

#### Indiana University, Purdue University, Indianapolis

Indianapolis, IN

Aug 2009 - Jul 2010

LIFE-HEALTH SCIENCE INTERNSHIP INTERN

- Investigated cement degradation of dicalcium phosphate dihydrate for cranial implants
- Prepared, evaluated, and analyzed monocalcium phosphate monohydrate(MCPM)/ $\beta$ -tricalcium phosphate cements and MCPM/nanocrystalline hydroxyapatite cements independently
- Developed skills in degradation analysis using X-ray diffraction, percent mass loss, pH change, and in vitro live/dead cell assay

## **Publications**

#### Published

- 1. **Benjamin, KJM**, Chen, Q, Jaffe, AE, Stolz, JM, Collado-Torres, L, Huuki, LA, Burke, EE, Arora, R, Feltrin, AS, Barbosa, AR, Radulescu, E, Pergola, G, Shin, JH, Ulrich, WS, Deep-Soboslay, A, Tao, R, the BrainSeq Consortium, Hyde, TM, Kleinman, JE, Erwin, JA, Weinberger, DR, and Apuã CM Paquola. "Analysis of the caudate nucleus transcriptome in individuals with schizophrenia highlights effects of antipsychotics and novel risk genes". *Nature Neuroscience*. 2022. PMID: 36319771.
- 2. D'Ignazio, L, Jacomini, RS, Qamar, B, **Benjamin, KJM**, Arora, R, Sawada, T, Diffenderfer, KE, Pankonin, AR, Hendriks, WT, Bragg, DC, Paquola, ACM, and Jennifer A Erwin. "Variation in TAF1 expression in female carrier induced pluripotent stem cells and human brain ontogeny has implications for adult neostriatum vulnerability in X-linked Dystonia Parkinsonism". *eNeuro*. 2022. PMID: 35868859.
- 3. Sawada, T, **Benjamin, KJM**, Brandtjen, AC, Tietze, E, Allen, SJ, Paquola, ACM, Kleinman, JE, Hyde, TM, and Jennifer A Erwin. "Generation of four postmortem dura-derived iPS cell lines from four control individuals with genotypic and brain-region-specific transcrptomic data available through the BrainSEQ consortium". *Stem Cell Research*. 2020. PMID: 32446240.
- 4. Sawada, T, Chater, TE, Sasagawa, Y, Yoshimura, M, Fujimori, N, Tanaka, K, **Benjamin, KJ**, Paquola, ACM, Erwin, JA, Goda, Y, Nikaido, I, and Tadafumi Kato. "Developmental Excitation-Inhibition Imbalance Underlying Psychoses Revealed by Single-Cell Analyses of Discordant Twins-Derived Cerebral Organoids". *Molecular Psychiatry*. 2020. PMID: 32764691.

#### Submitted

- 1. **Benjamin, KJM**<sup>+</sup>, Arora, R<sup>+</sup>, D'Ignazio, L, Hyde, TM, Kleinman, JE, Weinberger, DR, Paquola, ACM, and Jennifer A Erwin. "Effects of sex on transcriptional associations with schizophrenia across dorsolateral prefrontal cortex, hippocampus, and caudate nucleus". [pre-print]. 2022. DOI: https://doi.org/10.1101/2022.09.30.22280452.
- 2. **Benjamin, KJM**, Katipalli, T, and Apuã CM Paquola. "dRFEtools: Dynamic recursive feature elimination for 'omics". [pre-print]. 2022. DOI: https://doi.org/10.1101/2022.07.27.501227.
- 3. Sawada, T, Barbosa, A, Araujo, B, McCord, AE, D'Ignazio, L, **Benjamin, KJM**, Feltrin, A, Arora, R, Brandtjen, A, Kleinman, JE, Hyde, TM, Weinberger, DR, Paquola, ACM, and Jennifer A Erwin. "Ventral forebrain organoids derived from individuals with schizophrenia recapitulate perturbed striatal gene expression dynamics of the donors' brains". [pre-print]. 2022. DOI: https://doi.org/10.1101/2022.05.26.493589.
- 4. Tietze, E, Barbosa, AR, Euclydes, V, Cho, HJ, Lee, YK, Feltrin, A, van de Leemput, J, Di Carlo, P, Sawada, T, **Benjamin, KJ**, Brentani, H, Kleinman, JE, Hyde, TM, Weinberger, DR, Ursini, G, McKay, R, Paquola, ACM, Shin, JH, and Jennifer A Erwin. "Single-cell analysis of human trophoblast stem cell specification reveals activation of fetal cytotrophoblast expression programs including coronavirus associated host factors and human endogenous retroviruses". [pre-print]. 2020. DOI: https://doi.org/10.1101/2020.08.29.273425.

## **Scholarship**

Extramural Funding

NIMHD K99MD0169640: MOSAIC Baltimore, MD

COMPREHENSIVE COMPUTATIONAL ANALYSIS OF GENETIC AND REGULATORY DIFFERENCES BETWEEN INDIVIDUALS WITH AFRICAN AND EUROPEAN ANCESTRIES ACROSS FOUR BRAIN REGIONS: \$947000

Dec 2021 - Nov 2023

NIMH T32MH015330: Fellowship Baltimore, MD

ANALYSIS OF THE CAUDATE NUCLEUS TRANSCRIPTOME IN INDIVIDUALS WITH SCHIZOPHRENIA HIGHLIGHTS EFFECTS OF

Jun 2019 - May 2021

ANTIPSYCHOTICS AND NOVEL RISK GENES; SUPPORTS NIH LEVEL SALARY AND PROFESSIONAL DEVELOPMENT

College Station, TX

**Great Lakes STEM Scholarship** 

July 2014

Intramural Funding

\$2500 AWARD

**CVM Advanced Developmental Training Travel Award** College Station, TX

UPTO \$2500 FOR TRAVEL EXPENSIVES

May 2015

CVM Graduate Student Research Trainee Grant

College Station, TX

\$5000 SEED MONEY RESEARCH AND SUPPLIES TO GENERATE PRELIMINARY DATA

May 2014

Texas A&M Institute for Genome Sciences and Society (WSGI) Graduate Traineeship

College Station, TX

COMPUTATIONAL ANALYSIS OF 50 IDIOPATHIC ANGELMAN SYNDROME PATIENTS; SUPPORTS HALF OF SALARY

Sep 2013 - Aug 2014

## **Presentations**

#### **Invited Talk/Oral Presentations**

**International Conference on Intelligent Biology and Medicine** Philadelphia, PA

ORAL: "DREFTOOLS: DYNAMIC RECURSIVE FEATURE FLIMINATION FOR OMICS"

August 2022 New York, NY

**MERIT Emerging Leaders Symposium** 

July 2022

INVITED TALK: "LARGE-SCALE COMPUTATIONAL GENOMICS: NEW, REUSE, AND DEVELOP"

Baltimore, MD

**Johns Hopkins Postdoctoral Conference** 

April 2019

ORAL: "COMPUTATIONAL ANALYSIS OF GENETIC AND TRANSCRIPTIONAL LANDSCAPES OF THE CAUDATE NUCLEUS IN SCHIZOPHRENIA" **Defense Threat Reduction Agency** 

Fort Belvoir, VA

Invited talk: "Understanding the imprinting mechanism of *Ube3a* for therapeutic intervention"

July 2017

**Center for Computational Biology & Bioinformatics (UCSD)** 

June 2017

INVITED TALK: "UNDERSTANDING THE IMPRINTING MECHANISM OF Ube3a FOR THERAPEUTIC INTERVENTION"

Falls Church, VA

San Diego, CA

INVITED TALK: "THE Ube3a ANTISENSE TRANSCRIPT UNDERGOES EXTENSIVE PROCESSING AND IS SPATIOTEMPORALLY REGULATED IN

June 2017

Laboratory of Molecular Virology & Pathogenesis (MHRP)

Inova Translational Medicine Institute

Silver Spring, MD

INVITED TALK: "THE Ube3a ANTISENSE TRANSCRIPT UNDERGOES EXTENSIVE PROCESSING AND IS SPATIOTEMPORALLY REGULATED IN THE BRAIN"

June 2017

**Texas A&M Student Research Week** 

College Station, TX March 2016

ORAL: "INVESTIGATING NOVEL Ube3a ISOFORM 4 IN THE IMPRINTING OF Ube3a" **Genetics and Genomics (G2) Seminar Series** 

College Station, TX

Invited talk: "Investigating novel *Ube3a* isoform 4 in the imprinting of *Ube3a*"

February 2016

**College of Veterinary Medicine (CVM) Neuroscience** 

College Station, TX

INVITED TALK: "NEURODEVELOPMENTAL DISORDERS: INSTABILITY OF CHROMOSOME 15Q11-Q13" **Texas A&M Student Research Week** 

April 2015 College Station, TX

ORAL: "DEVELOPMENT OF EMBRYONIC STEM CELL-DERIVED NEURONAL CULTURES FOR HIGH-THROUGHPUT DRUG SCREENING"

February 2014

## **Poster Presentations**

**American Society of Human Genetics** Los Angeles, CA

POSTER: "GENETIC AND ENVIRONMENTAL REGULATION OF CAUDATE NUCLEUS TRANSCRIPTOME IN SCHIZOPHRENIA"

October 2022

**Society of Neuroscience** San Diego, CA POSTER: "COMPUTATIONAL ANALYSIS OF GENETIC AND TRANSCRIPTIONAL LANDSCAPES OF THE CAUDATE NUCLEUS IN November 2018 SCHIZOPHRENIA" **Texas A&M Imaging Sciences Spotlight Series** College Station, TX POSTER: "HIGH-THROUGHPUT DRUG SCREENING OF MOUSE EMBRYONIC STEM CELL-DERIVED NEURONS" January 2016 College of Veterinary Medicine (CVM) Symposium College Station, TX POSTER: "HIGH-THROUGHPUT DRUG SCREENING OF MOUSE EMBRYONIC STEM CELL-DERIVED NEURONS" January 2016 Texas A&M Health Science Center Symposium College Station, TX POSTER: "DEVELOPMENT OF EMBRYONIC STEM CELL-DERIVED NEURONAL CULTURES FOR HIGH-THROUGHPUT DRUG SCREENING" April 2014 **Texas A&M Genetics Graduate Student Recruiting Symposium** College Station, TX POSTER: "DEVELOPMENT OF EMBRYONIC STEM CELL-DERIVED NEURONAL CULTURES FOR HIGH-THROUGHPUT DRUG SCREENING" February 2014 **National Society of Black Engineers Fall Regional Conference** Rochester, NY POSTER: "DRUG LOADING OF NAPROXEN SODIUM ON THE DEGRADATION CHARACTERISTICS OF POLYCAPROLACTONE" October 2011 **Biomedical Engineering Society Annual Meeting** Hartford, CT POSTER: "DRUG LOADING OF NAPROXEN SODIUM ON THE DEGRADATION CHARACTERISTICS OF POLYCAPROLACTONE" October 2011 **NSF LSAMP/RPI SURP** Troy, NY POSTER: "DRUG LOADING OF NAPROXEN SODIUM ON THE DEGRADATION CHARACTERISTICS OF POLYCAPROLACTONE" August 2011 Walter Lincoln Hawkins '32 Graduate Research Conference Troy, NY POSTER: "COMPARISON OF MCPM-TCP AND MCPM-HA CEMENT DEGRADATION" April 2011 **Purdue Biomaterials Research Symposium** West Lafayette, IN POSTER: "COMPARISON OF MCPM-TCP AND MCPM-HA CEMENT DEGRADATION" April 2010 **Life-Health Sciences Internship Poster Session** Indianapolis, IN POSTER: "COMPARISON OF MCPM-TCP AND MCPM-HA CEMENT DEGRADATION" April 2010 Annual Glenn Research Center Summer Poster Session Cleveland, OH POSTER: "BIOFUELS AS AN ALTERNATIVE FUEL SOURCE FOR AVIATION" August 2009 **Panels Hopkins URM Accepted Applicants Virtual Visit** Virtual March 2021 PANEL: "HOPKINS GRADUATE ACCEPTED APPLICANTS VISIT FACULTY/FELLOWS PANEL" Johns Hopkins, School of Medicine; Second Look Visit Baltimore, MD PANEL: "JOHNS HOPKINS GRADUATE BIOMEDICAL EDUCATION PROGRAMS ACCEPTED APPLICANTS, FACULTY AND POSTDOCTORAL March 2019 FELLOWS PANEL" Johns Hopkins, School of Medicine; Biomedical Scholars Association Event Baltimore, MD PANEL: "NAVIGATING AND PREPARING FOR A CONFERENCE" November 2018 **Teaching LIBD rstats Club** Baltimore, MD JOURNAL CLUB SESSION LEADER Apr 2020 - Currently • Suggested R programming related topics for discussion • Developed lesson plan for R programming topics in bioinformatics Lecture history of session: https://bit.ly/30gpcZk Summer Undergraduate Research Virtual Exchange (SURVE) Baltimore, MD **LECTURER** Jul 2020 - Aug 2020 Development learning objectives and activities for SURVE

Baltimore, MD

4

Jun 2020 - Jul 2020

KYNON JM BENJAMIN · CV

· Primary instructor for virtual journal club, where I helped development 8-week journal club curriculum

• Development activities for article critique, and demonstrated and evaluated presentations.

Basic Science Institute (BSI)- Summer Internship Program (SIP)

Collaborative virtual lecturer

JOURNAL CLUB GROUP LEADER

## Texas A&M University, Department of Biochemistry

College Station, TX

GRADUATE TEACHING ASSISTANT Aug 2012 - Jun 2013

- · Laboratory instructor for Genetics course
- · Primary instructor for one class and assisted with second class each semester
- · Gave introductory lecture, followed by hands-on supervising of student experiements
- Evaluated student performance and assigned grades

#### **Graduate Teaching Association/Academy for Future Facility**

College Station, TX

STEERING COMMITTEE MEMBER

Jan 2014 - Jul 2016

- Worked collaboratively in the committee, where I helped run workshops and seminars for professional development, including but not limited to:
  - Philosophy of Teaching Statement
  - Teaching in Large Classes
  - Teaching with Technology
  - Developing Assessments
- Maintained and troubleshoot Blackboard, including but not limited to:
  - Generating assessments
  - Transferring 15 GB of data between website platforms
  - Troubleshooting any computer technological issues experienced by other committee members

## **Honors & Distinctions**

- 2022 **Recipient**, International Conference on Intelligent Biology and Medicine: Travel Award
- 2021 MOSAIC (K99/R00) Fellow, NIMHD, Lieber Institute for Brain Development
- 2019 T32 Postdoctoral Fellow, Johns Hopkins School of Medicine, Department of Psychiatry
- 2019 **1**<sup>st</sup> **Place**, Johns Hopkins Postdoctoral Conference: Oral Presentation
- 2017 Scholar Finalist, Data Incubator
- 2016 **Nomination**, Excellence in Research at Texas A&M Student Research Week
- 2016 Honorable Mention, Texas A&M Imaging Sciences Spotlight Series
- 2015 **Recipient**, CVM Advanced Developmental Training Travel Award
- 2015 **Recipient**, Cold Spring Harbor *Drosophila* Neurobiology Course
- 2014 **Recipient**, Great Lakes STEM Scholarship
- 2014 4<sup>th</sup> Place, Texas A&M Genetics Graduate Student Recruiting Symposium Poster Presentation
- 2014 Recipient, CVM Graduate Student Research Trainee Grant
- 2013 **Recipient**, Texas A&M Institute for Genome Sciences and Society (WSGI) Graduate Traineeship
- 2011 Recipient, NSF funded LSAMP Research Experience for Undergraduates at RPI
- 2011 **1**st **Place**, Walter Lincoln Hawkins '32 Graduate Research Conference: Poster Presentation
- 2011 **1**st **Place**, National Society of Black Engineers Fall Regional Conference: Poster Presentation
- 2011 **Recipient**, Rensselaer's Professional Leadership Program
- 2009 **Recipient**, NASA Science Technology Institute Summer Scholars Program
- 2009 **Recipient**, Life Health Science Internship

## Service

#### **Professional Activities**

### Lieber Institute for Brain Development Postdoctoral Association (LIBD-PDA)

Baltimore, MD

PRESIDENT

Apr 2020 - Current

- · Established LIBD-PDA
- Obtained universal transportation benefit for all LIBD employees
- Obtained pipeline for joint appointments for all LIBD postdoctoral fellows
- Managed, planned, and organized first LIBD-PDA Virtual Retreat

### **Johns Hopkins Postdoctoral Association**

Baltimore, MD

CO-PRESIDENT

Aug 2019 - Jul 2020

- Advocated for postdoc interests during COVID19 pandemic
- Secured funding for Annual Postdoctoral Conference
- Advocated for inclusion of postdocs in Faculty Learner Misconduct Policy
- Secured improvements for LGBTQ+ health insurance
- Advocated and advised JHU university wide postdoctoral database

JHPDA Po	licy and Advocacy Committee	Baltimore, MD Aug 2018 - Jul 2019
<ul><li>Organize</li><li>Organize</li></ul>	d for minority mental health support d annual postdoctoral survey d seminars on domestic and international financial wellness d and secured improvements for postdoc childcare options	
Genetics (	iraduate Student Association	College Station, TX
TREASURER		Jul 2013 - Jul 2015
	d meetings and annual symposium and maintained budget	
CVM Grad	uate Student Association	College Station, TX
TREASURER		Jul 2014 - Jul 2015
	d meetings and yearly outreach event and maintained budget	
National S	ociety of Black Engineers	Troy, NY
Treasurer		Jun 2011 - Jun 2012
<ul><li>Develope</li><li>Wrote the</li></ul>	managed the transition from on-campus to off-campus banking system d system for reimbursements and invoicing inaugural handbook for the new treasurer position and maintained \$70000 annual budget	
Rensselaer's Professional Leadership Program		Troy, NY
FELLOW		Aug 2011 - May 2012
Selective le	adership program designed to bridge the gap between school and the workplace.	
Schizonhr	Journal Reviewer enia Bulletin	
ONE REVIEW		2021
Profess	sional Activities	
2020-	<b>President</b> , Lieber Institute for Brain Development Postdoctoral Association	Baltimore, MD
2019-2020	Co-President, Johns Hopkins Postdoctoral Association	Baltimore, MD
2019-2020	Postdoctoral Representative, Faculty Senate; Johns Hopkins, School of Medicine	Baltimore, MD
2019-2020	Postdoctoral Representative, Postdoctoral Affairs Advisory Board	Baltimore, MD
2019-2020	Postdoctoral Representative, Institute for Excellence in Education Board of Directors	Baltimore, MD
2018-	<b>Member</b> , JHPDA Diversity Postdoctoral Alliance Committee	Baltimore, MD
2018-2019	Member/Co-Chair, JHPDA Policy and Advocacy Committee	Baltimore, MD
2018-2019	Postdoc Member, JHU University Health Services Committee	Baltimore, MD
2013–2016	<b>Member</b> , Graduate Teaching Association/Academy for Future Faculty Steering Committee	College Station, TX
2015–2016	<b>Student Member</b> , Genetics Graduate Student Association Academics Committee	College Station, TX
2014–2015	<b>Student Member</b> , Genetics Graduate Student Association Awards Committee	College Station, TX
<b>Profes</b> :	sional Memberships	
Internatio	nal Conference on Intelligent Biology and Medicine	
MEMBER		2022-
American	Society of Human Genetics	
MEMBER		2022-
Society fo	r Neuroscience	
MEMBER		2017-
National S	ociety of Black Engineers	
M /C	PTER TREASURER	2010-2016