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National Institute for General Medical Sciences
RISE
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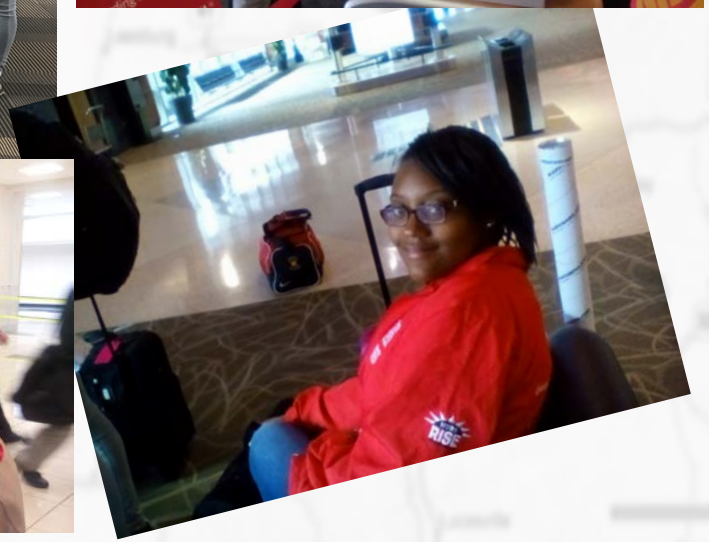

Follow the ‘Red Coats’


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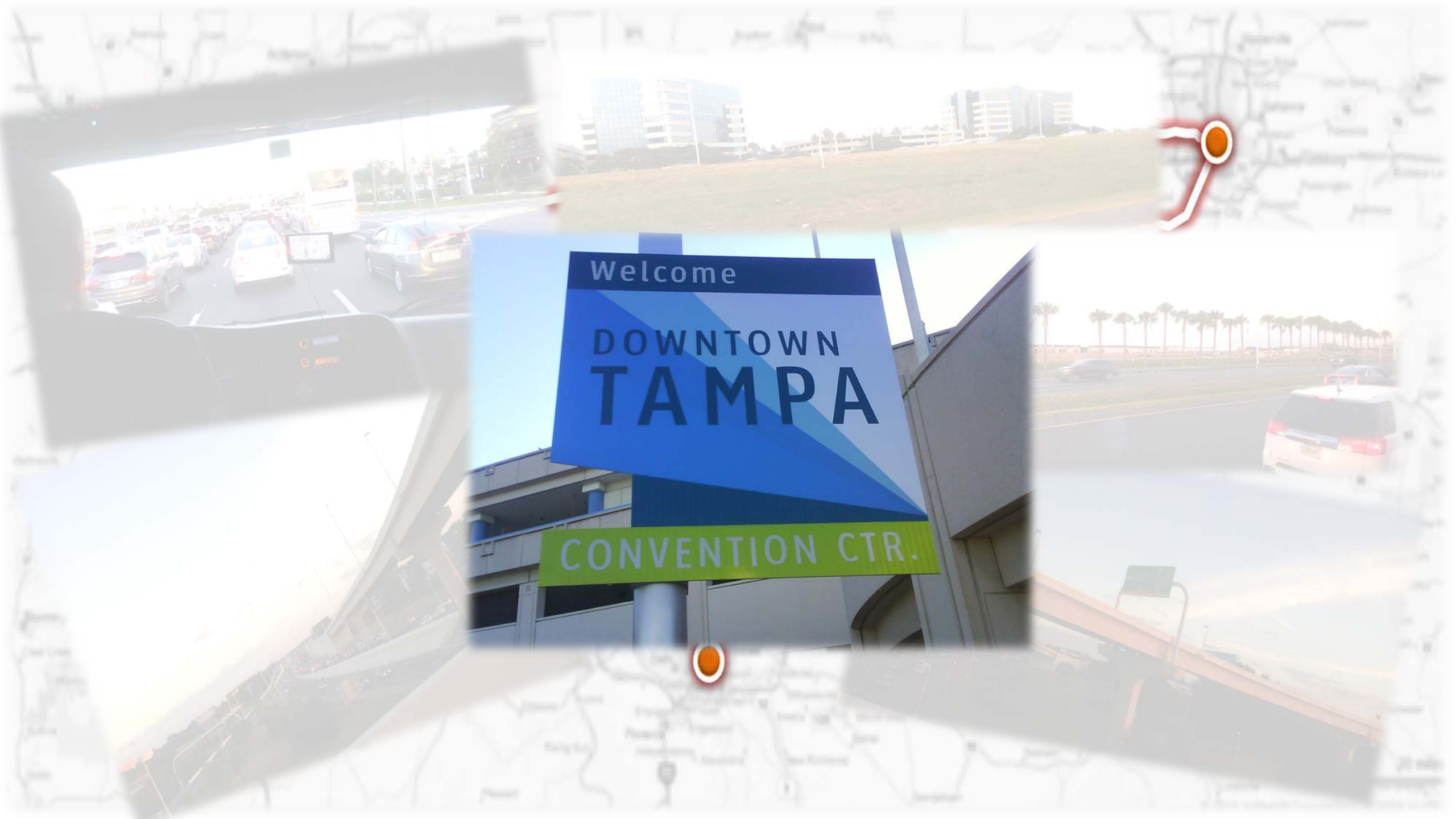
Welcome
DOWNTOWN
TAMPA
CONVENTION CTR.

Rams are in the 'House'

Oral and Poster Presentation Winners

- Lanazha Belfield – Senior Biology Major
- Beverly Dosso – Senior Chemistry Major
- Victoria Sedwick – Senior Chemistry Major

WINSTON SALEM STATE UNIVERSITY

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Undergraduate Student Training in Academic Research

Thermodynamic Regulation of Cardiovascular Stability in Type 2 Diabetes

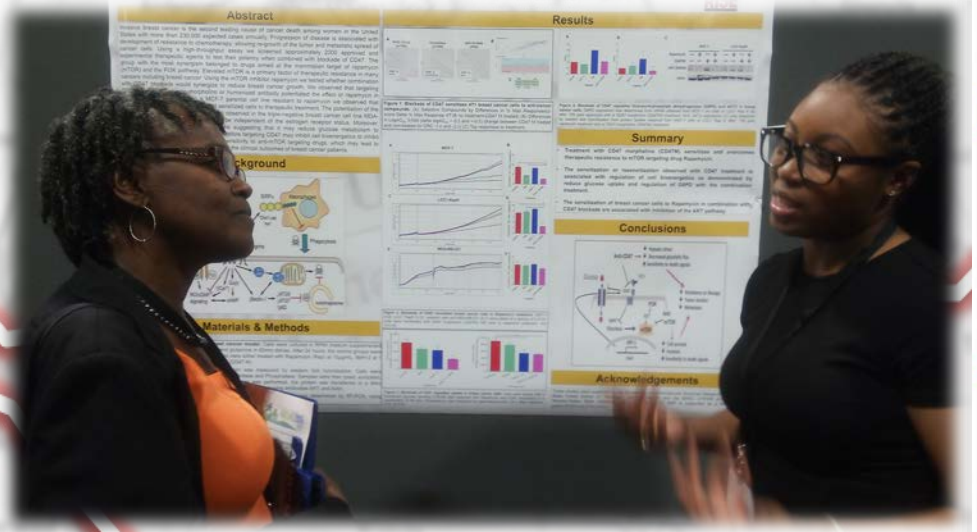
Lanazha Belfield

Mentor: Dr. Tennille D. Presley

Biomedical Research Infrastructure Center

November, 2016

Physiology - Best Oral Presentation



Cancer Biology

Best Poster Presentation

WINSTON-SALEM STATE UNIVERSITY
Biomedical Research Infrastructure Center

Blockade of CD47 Synergizes with Rapamycin to Overcome Therapeutic Resistant Breast Cancer

Beverly Dosso[1], Yismeilin R. Feliz Mosquea[2], Katherine L. Cook[2], David R. Soto-Pantoja[2]
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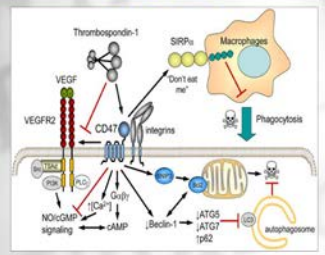
Abstract

Invasive breast cancer is the second leading cause of cancer death among women in the United States with more than 230,000 expected cases annually. Progression of disease is associated with development of resistance to chemotherapy, allowing re-growth of the tumor and metastatic spread of cancer cells. Using a high-throughput assay we screened approximately 2000 approved and experimental therapeutic agents to test their potency when combined with blockade of CD47. The group with the most synergism belonged to drugs aimed at the mammalian target of rapamycin (mTOR) and the PI3K pathway. Elevated mTOR is a primary factor of therapeutic resistance in many cancers including breast cancer. Using the mTOR inhibitor rapamycin we tested whether combination with CD47 blockade would synergize to reduce breast cancer growth. We observed that targeting CD47 using an antisense morpholino or humanized antibody potentiated the effect or rapamycin in MCF-7 cells. Moreover using a MCF-7 parental cell line resistant to rapamycin we observed that combination with anti-CD47 treatment sensitized cells to therapeutic treatment. The potentiation of the antigrowth effect of rapamycin was also observed in the triple-negative breast cancer cell line MDA-MB-231 indicating that this effect may be independent of the estrogen receptor status. Moreover, targeting CD47 reduced glucose uptake suggesting that it may reduce glucose metabolism to sensitize cells to rapamycin therapy. Therefore targeting CD47 may inhibit cell bioenergetics to inhibit pro-tumorigenic pathways and restore sensitivity to anti-mTOR targeting drugs, which may lead to novel therapeutic combinations to improve the clinical outcomes of breast cancer patients.

Background



Materials & Methods

In vitro LCC1 Rap R and MCF7 breast cancer model: Cells were cultured in RPMI medium supplemented with 10% FBS, penicillin/streptomycin, and glutamine in 60mm dishes. After 24 hours, the control groups were left untreated and the experimental groups were either treated with Rapamycin (Rap) at 10μg/mL, B6H12 at 1 μg/mL, and or antisense CD47 morpholino (CD47 M).

Western Blot Analysis: Protein expression was measured by western blot hybridization. Cells were harvested with 1mL of RIPA buffer containing Protase and Phosphatase. Samples were then lysed, sonicated, and a BSA was conducted. After Electrophoresis was performed, the protein was transferred to a Nitro cellulose membrane. The membrane was probed with the following antibodies AKT, and Actin.

PCR Analysis: Cells were harvested in Trizol and gene expression was determined by RT-PCR, using specific primers to G6PD AND HPRT.

Results

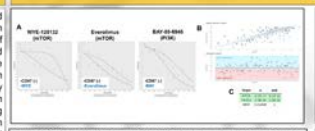


Figure 1. Blockade of CD47 sensitizes 4T1 breast cancer cells to anti-cancer compounds. (A) Selective Compounds by Differences in % Max Responses(z-score Delta % Max Response 4T1B no treatment-CD47 M treated) (B) Differences in LogAC$_{50}$ 3-fold (delta logAC$_{50}$ > 0.5 and <-0.5) change between CD47 M treated and non-treated for CRC -1.n and -2.n) (C) Top responses to treatment.

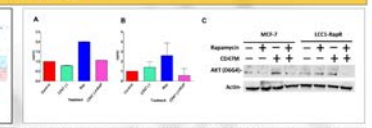


Figure 4. Blockade of CD47 regulates Glucose-6-phosphate dehydrogenase (G6PD) and AKT2 in breast cancer cells. G6PD expression was determined from mRNA obtained from MCF-7 (A) cells or LCC1 Rap R (B) after 72h post rapamycin and or CD47 morpholino (CD47M) treatment. N=2. AKT2 expression (C) was measured by western blot hybridization from protein lysates obtained from MCF-7 cells or LCC1 Rap R after 72h post rapamycin treatment and or CD47 morpholino (CD47M) treatment.

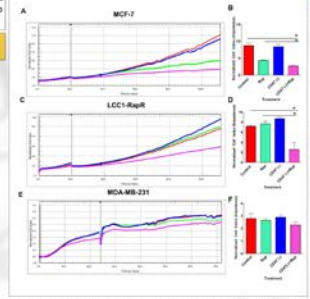


Figure 2. Blockade of CD47 sensitizes breast cancer cells to Rapamycin treatment. MCF-7 (A,B), LCC1 RapR (C,D) resistant cells and MDA-MB-231 (E,F) were plated at a density of 2 X10^5. Cells were transfected with CD47 morpholino (CD47M) 48h prior to rapamycin treatment. n=3 *p<0.05

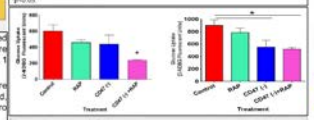
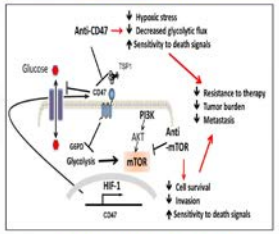


Figure 3. Blockade of CD47 regulates uptake in breast cancer cells. Cells were treated with a fluorescent glucose homolog 2-NDGB after treatment with Rapamycin and CD47 morpholino or a combination of the two. Fluorescence was measured by spectrophotometry 24 h after treatment. n=3. *p<0.05

Summary

- Treatment with CD47 morpholino (CD47M) sensitizes and overcomes therapeutic resistance to mTOR targeting drug Rapamycin.
- The sensitization or resensitization observed with CD47 treatment is associated with regulation of cell bioenergetics as demonstrated by reduce glucose uptake and regulation of G6PD with the combination treatment.
- The sensitization of breast cancer cells to Rapamycin in combination with CD47 blockade are associated with inhibition of the AKT pathway.

Conclusions



Acknowledgements

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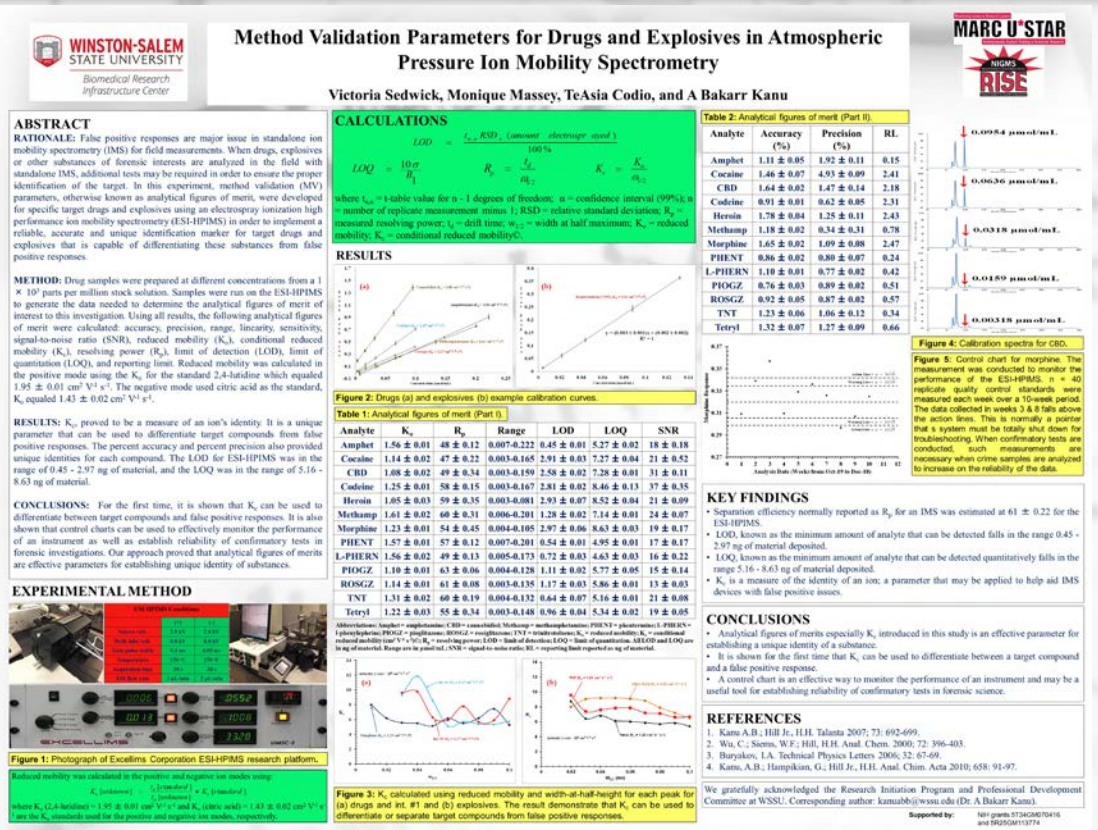

Method Validation Parameters for Drugs and Explosives in Atmospheric Pressure Ion Mobility Spectrometry

Victoria Sedwick, Monique Massey, TeAsia Codio, and A Bakarr Kanu

WINSTON-SALEM STATE UNIVERSITY
Biomedical Research Infrastructure Center

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ABSTRACT

RATIONALE: False positive responses are major issue in standalone ion mobility spectrometry (IMS) for field measurements. When drugs, explosives or other substances of forensic interests are analyzed in the field with standalone IMS, additional tests may be required in order to ensure the proper identification of the target. In this experiment, method validation (MV) parameters, otherwise known as analytical figures of merit, were developed for specific target drugs and explosives using an electrospray ionization high performance ion mobility spectrometry (ESI-HPIMS) in order to implement a reliable, accurate and unique identification marker for target drugs and explosives that is capable of differentiating these substances from false positive responses.

METHOD: Drug samples were prepared at different concentrations from a 1 × 10^3 parts per million stock solution. Samples were run on the ESI-HPIMS to generate the data needed to determine the analytical figures of merit of interest to this investigation. Using all results, the following analytical figures of merit were calculated: accuracy, precision, range, linearity, sensitivity, signal-to-noise ratio (SNR), reduced mobility (K_o), conditional reduced mobility (K_c), resolving power (R_p), limit of detection (LOD), limit of quantitation (LOQ), and reporting limit. Reduced mobility was calculated in the positive mode using the K_o for the standard 2,4-lutidine which equaled 1.95 ± 0.01 $cm^2 V^{-1} s^{-1}$. The negative mode used citric acid as the standard, K_o equaled 1.43 ± 0.02 $cm^2 V^{-1} s^{-1}$.

RESULTS: K_o proved to be a measure of an ion's identity. It is a unique parameter that can be used to differentiate target compounds from false positive responses. The percent accuracy and percent precision also provided unique identities for each compound. The LOD for ESI-HPIMS was in the range of 0.45 - 2.97 ng of material, and the LOQ was in the range of 5.16 - 8.63 ng of material.

CONCLUSIONS: For the first time, it is shown that K_o can be used to differentiate between target compounds and false positive responses. It is also shown that control charts can be used to effectively monitor the performance of an instrument as well as establish reliability of confirmatory tests in forensic investigations. Our approach proved that analytical figures of merits are effective parameters for establishing unique identity of substances.

EXPERIMENTAL METHOD

Figure 1: Photograph of Excellims Corporation ESI-HPIMS research platform.

Reduced mobility was calculated in the positive and negative ion modes using:

$$K_o\,(unknown) = \frac{t_d\,(standard)}{t_d\,(unknown)} * K_o\,(standard)$$

where K_o (2,4-lutidine) = 1.95 ± 0.01 $cm^2 V^{-1} s^{-1}$ and K_o (citric acid) = 1.43 ± 0.02 $cm^2 V^{-1} s^{-1}$ are the K_o standards used for the positive and negative ion modes, respectively.

CALCULATIONS

$$LOD = \frac{t_{\alpha,n} \cdot RSD_y \cdot (amount \cdot electrospr \cdot used)}{100\,\%}$$

$$LOQ = \frac{10\sigma}{B_1} \qquad R_p \approx \frac{t_d}{\omega_{\frac{1}{2}}} \qquad K_o = \frac{K_c}{\omega_{\frac{1}{2}}}$$

where $t_{\alpha,n}$ = t-table value for n - 1 degrees of freedom; α = confidence interval (99%); n = number of replicate measurement minus 1; RSD = relative standard deviation; R_p = measured resolving power; t_d = drift time; $w_{\frac{1}{2}}$ = width at half maximum; K_o = reduced mobility; K_c = conditional reduced mobility©.

RESULTS

Figure 2: Drugs (a) and explosives (b) example calibration curves.

Table 1: Analytical figures of merit (Part I).

Analyte	K_o	R_p	Range	LOD	LOQ	SNR
Amphet	1.56 ± 0.01	48 ± 0.12	0.007-0.222	0.45 ± 0.01	5.27 ± 0.02	18 ± 0.18
Cocaine	1.14 ± 0.02	47 ± 0.22	0.003-0.165	2.91 ± 0.03	7.27 ± 0.04	21 ± 0.52
CBD	1.08 ± 0.02	49 ± 0.34	0.003-0.159	2.58 ± 0.02	7.28 ± 0.01	31 ± 0.11
Codeine	1.25 ± 0.01	58 ± 0.15	0.003-0.167	2.81 ± 0.02	8.46 ± 0.13	37 ± 0.35
Heroin	1.05 ± 0.03	59 ± 0.35	0.003-0.081	2.93 ± 0.07	8.52 ± 0.04	21 ± 0.09
Methamp	1.61 ± 0.02	60 ± 0.31	0.006-0.201	1.28 ± 0.02	7.14 ± 0.01	24 ± 0.07
Morphine	1.23 ± 0.01	54 ± 0.45	0.004-0.105	2.97 ± 0.06	8.63 ± 0.03	19 ± 0.17
PHENT	1.57 ± 0.01	57 ± 0.12	0.007-0.201	0.54 ± 0.01	4.95 ± 0.01	17 ± 0.17
L-PHERN	1.56 ± 0.02	49 ± 0.13	0.005-0.173	0.72 ± 0.03	4.63 ± 0.03	16 ± 0.22
PIOGZ	1.10 ± 0.01	63 ± 0.06	0.004-0.128	1.11 ± 0.02	5.77 ± 0.05	15 ± 0.14
ROSGZ	1.14 ± 0.01	61 ± 0.08	0.003-0.135	1.17 ± 0.03	5.86 ± 0.01	13 ± 0.03
TNT	1.31 ± 0.02	60 ± 0.19	0.004-0.132	0.64 ± 0.07	5.16 ± 0.01	21 ± 0.08
Tetryl	1.22 ± 0.03	55 ± 0.34	0.003-0.148	0.96 ± 0.04	5.34 ± 0.02	19 ± 0.05

Abbreviations: Amphet = amphetamine; CBD = cannabidiol; Methamp = methamphetamine; PHENT = phentermine; L-PHERN = l-phenylephrine; PIOGZ = pioglitazone; ROSGZ = rosiglitazone; TNT = trinitrotoluene; K_o = reduced mobility; K_c = conditional reduced mobility (cm² V⁻¹ s⁻¹); R_p = resolving power; LOD = limit of detection; LOQ = limit of quantitation. All LOD and LOQ are in ng of material. Range are in panel red.; SNR = signal-to-noise ratio; RL = reporting limit reported as ng of material.

Figure 3: K_o calculated using reduced mobility and width-at-half-height for each peak for (a) drugs and int. #1 and (b) explosives. The result demonstrate that K_o can be used to differentiate or separate target compounds from false positive responses.

Table 2: Analytical figures of merit (Part II).

Analyte	Accuracy (%)	Precision (%)	RL
Amphet	1.11 ± 0.05	1.92 ± 0.11	0.15
Cocaine	1.46 ± 0.07	4.93 ± 0.09	2.41
CBD	1.64 ± 0.02	1.47 ± 0.14	2.18
Codeine	0.91 ± 0.01	0.62 ± 0.05	2.31
Heroin	1.78 ± 0.04	1.25 ± 0.11	2.43
Methamp	1.18 ± 0.02	0.34 ± 0.31	0.78
Morphine	1.65 ± 0.02	1.09 ± 0.08	2.47
PHENT	0.86 ± 0.02	0.80 ± 0.07	0.24
L-PHERN	1.10 ± 0.01	0.77 ± 0.02	0.42
PIOGZ	0.76 ± 0.03	0.89 ± 0.02	0.51
ROSGZ	0.92 ± 0.05	0.87 ± 0.02	0.57
TNT	1.23 ± 0.06	1.06 ± 0.12	0.34
Tetryl	1.32 ± 0.07	1.27 ± 0.09	0.66

Figure 4: Calibration spectra for CBD.

Figure 5: Control chart for morphine. The measurement was conducted to monitor the performance of the ESI-HPIMS. n = 40 replicate quality control standards were measured each week over a 10-week period. The data collected in weeks 3 & 8 falls above the action lines. This is normally a pointer that s system must be totally shut down for troubleshooting. When confirmatory tests are conducted, such measurements are necessary when crime samples are analyzed to increase on the reliability of the data.

KEY FINDINGS

- Separation efficiency normally reported as R_p for an IMS was estimated at 61 ± 0.22 for the ESI-HPIMS.
- LOD, known as the minimum amount of analyte that can be detected falls in the range 0.45 - 2.97 ng of material deposited.
- LOQ, known as the minimum amount of analyte that can be detected quantitatively falls in the range 5.16 - 8.63 ng of material deposited.
- K_o is a measure of the identity of an ion; a parameter that may be applied to help aid IMS devices with false positive issues.

CONCLUSIONS

- Analytical figures of merits especially K_o introduced in this study is an effective parameter for establishing a unique identity of a substance.
- It is shown for the first time that K_o can be used to differentiate between a target compound and a false positive response.
- A control chart is an effective way to monitor the performance of an instrument and may be a useful tool for establishing reliability of confirmatory tests in forensic science.

REFERENCES

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4. Kanu, A.B.; Hampikian, G.; Hill Jr., H.H. Anal. Chim. Acta 2010; 658: 91-97.

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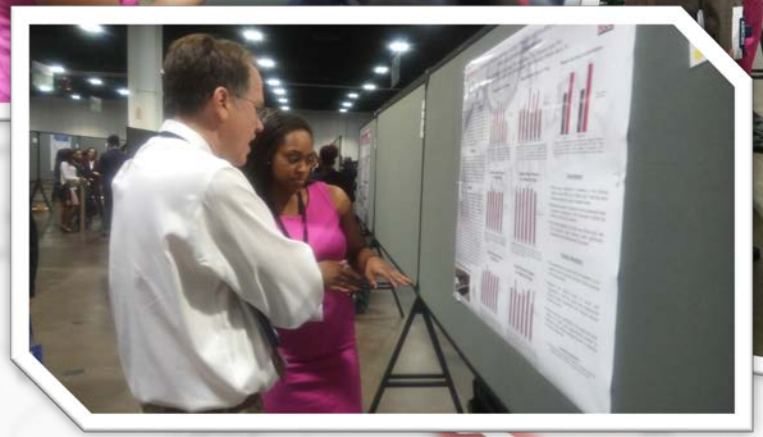
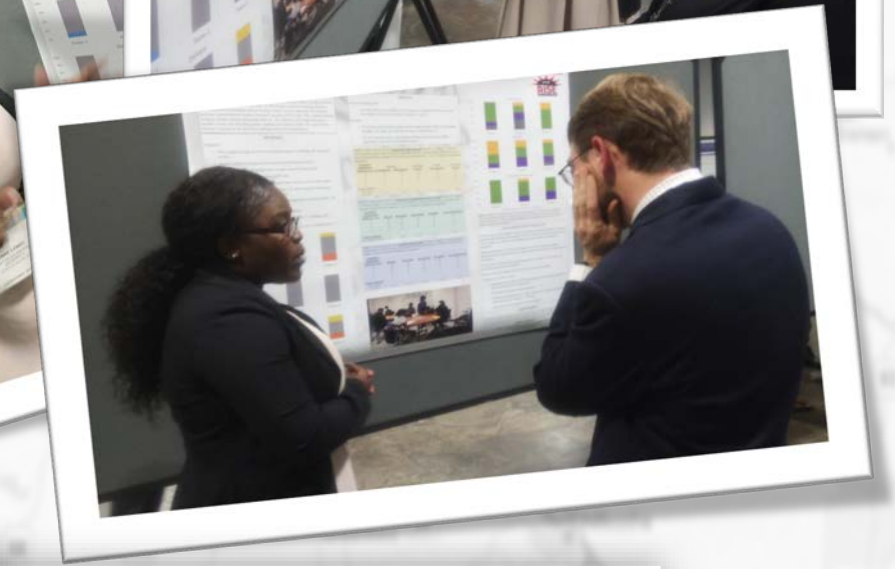
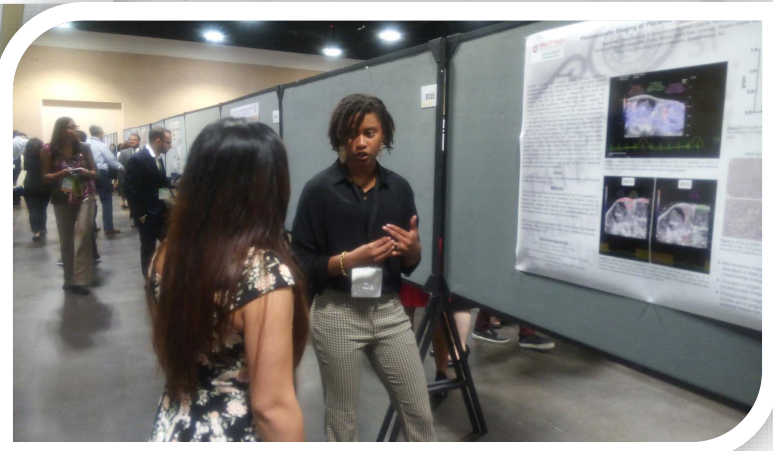
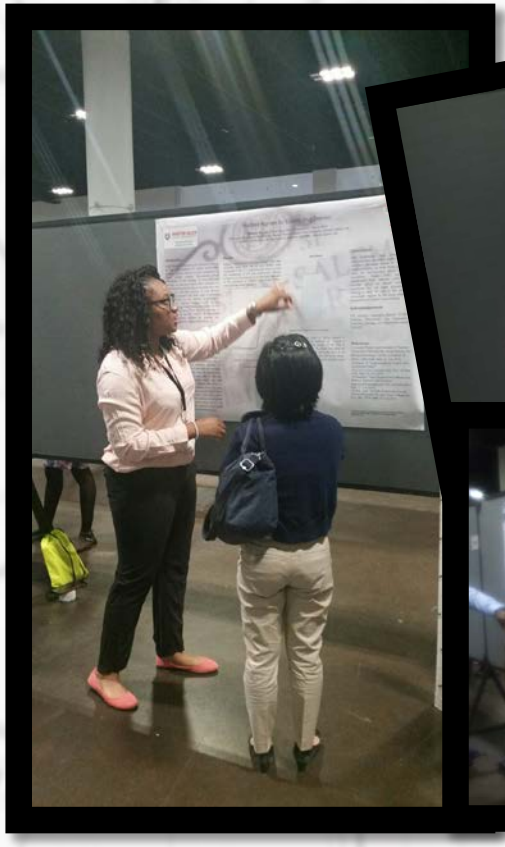
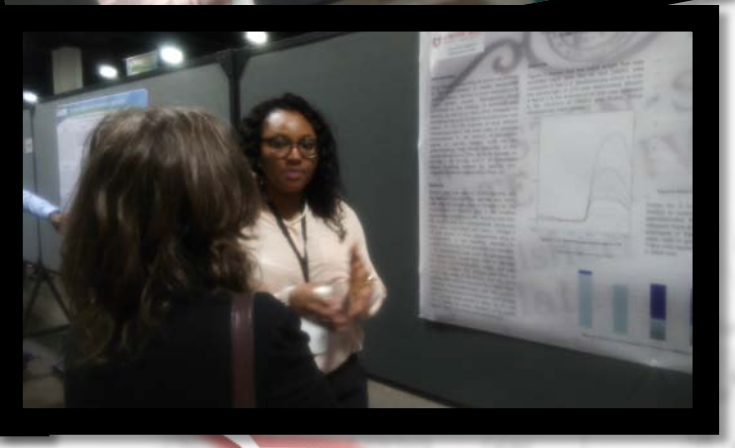
Chemistry - Best Poster Presentation

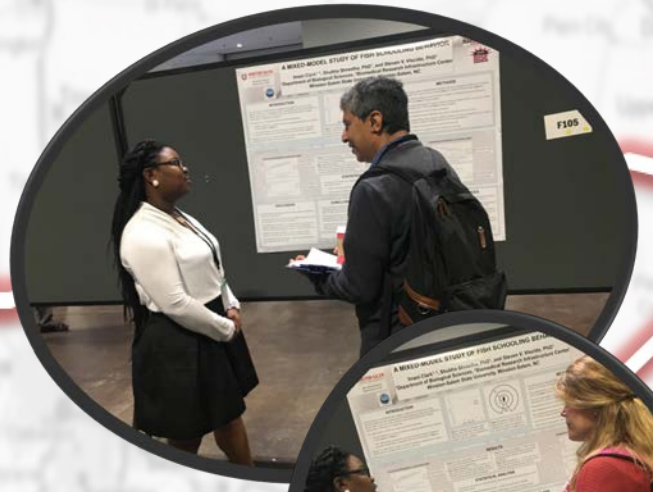








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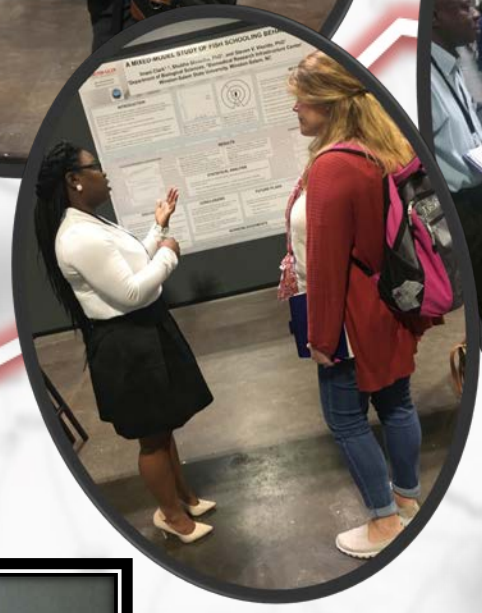

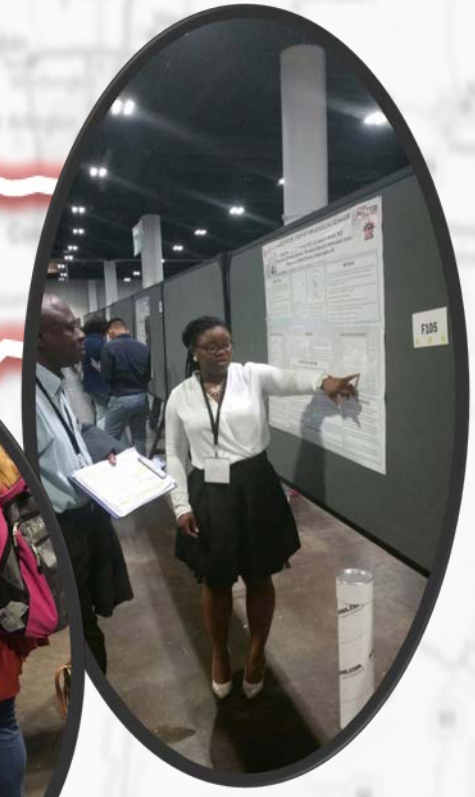
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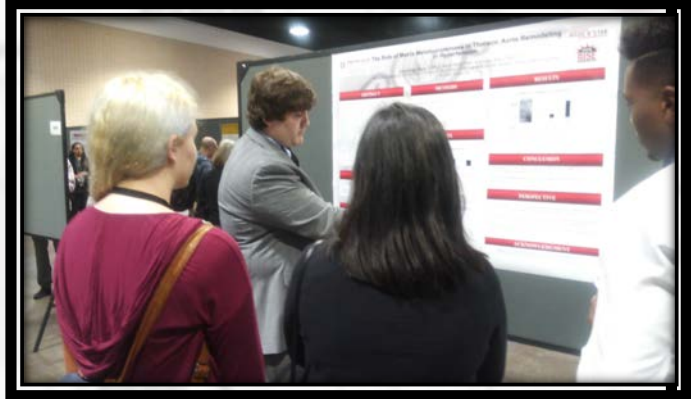

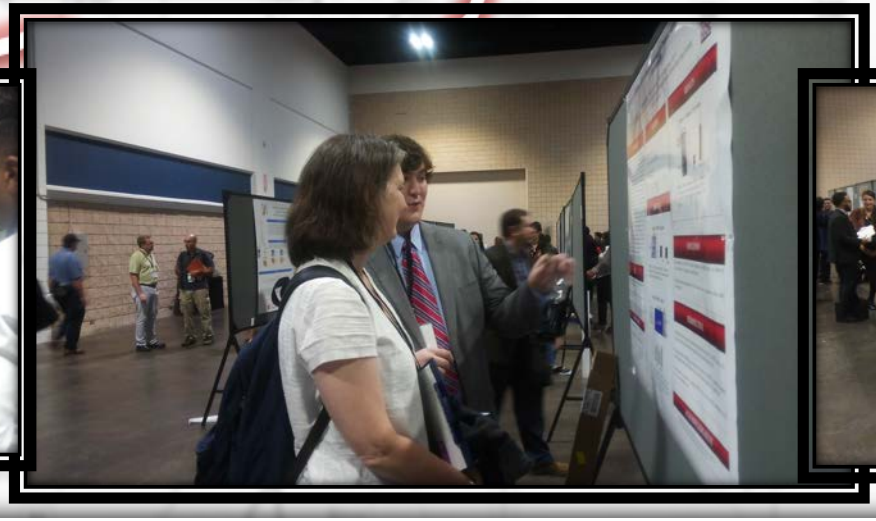

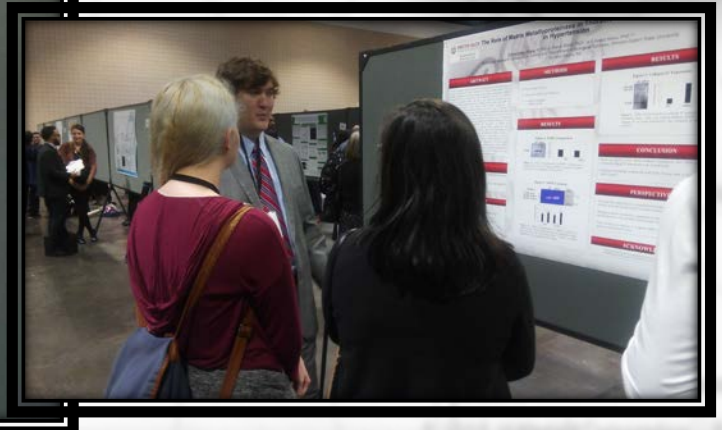


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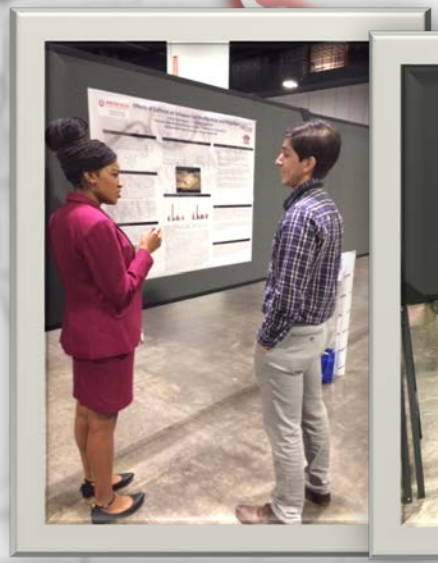

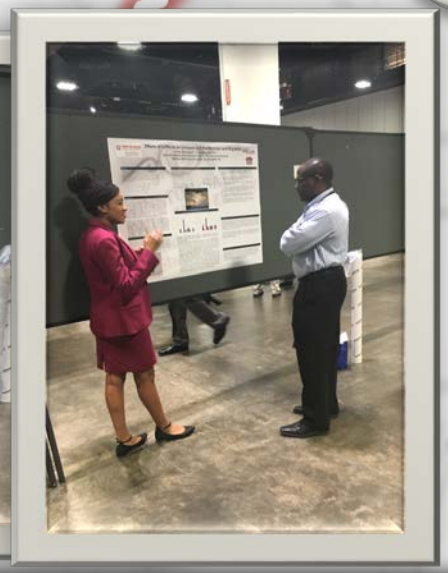




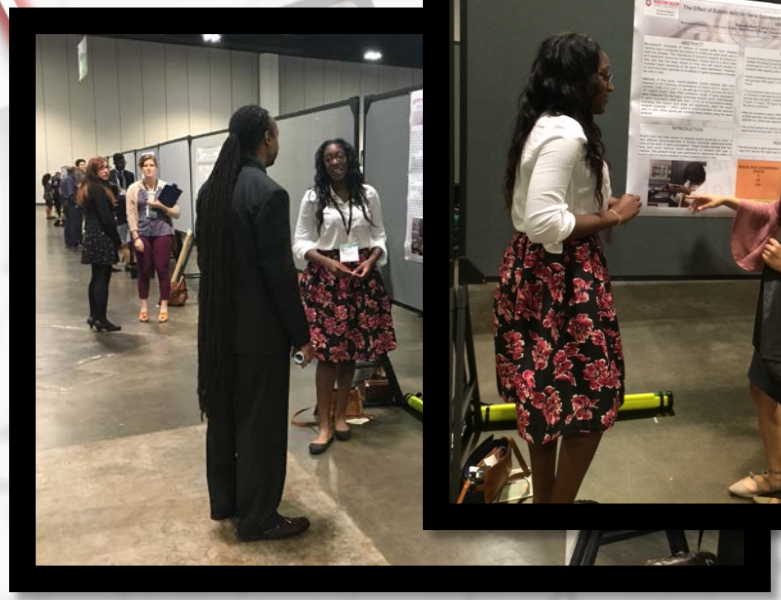

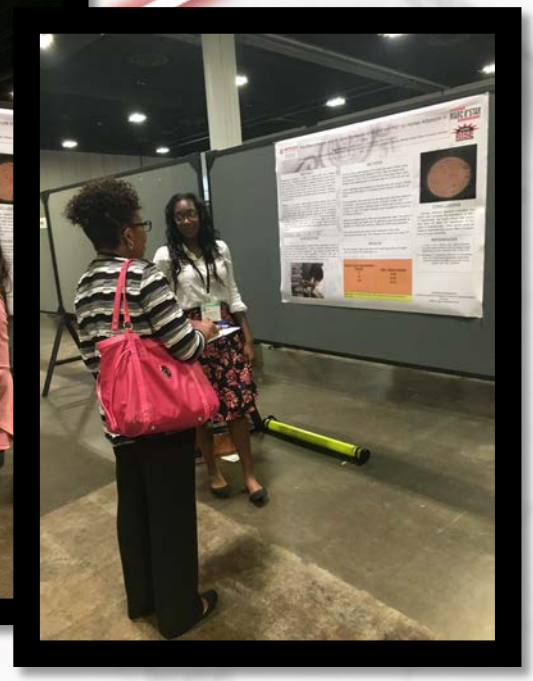

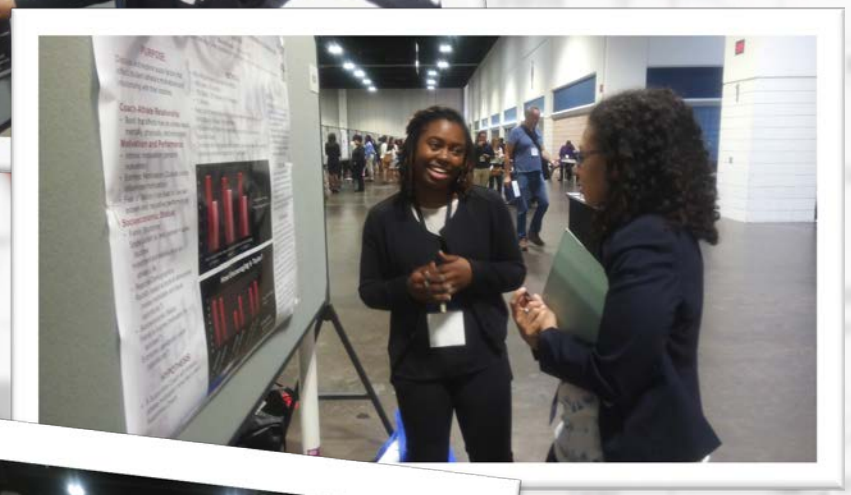

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Lanazha Belfield	Biology	Senior	MARC Scholar
Beverly Dosso	Chemistry	Senior	MARC Scholar
Anne Lenzo	Biology	Junior	MARC Scholar
Adriance Edington	Biology	Junior	MARC Scholar
Eric Pridgen	Math	Senior	MARC Affiliate
Kiana Rushdan	Biology	Senior	MARC Affiliate
Christopher Shew	Biology	Senior	MARC Affiliate
Zipporah Foster	Psychology	Senior	MARC Affiliate
Victoria Sedwick	Chemistry	Senior	MARC Affiliate
Ziaqueria Short	Biology	Sophomore	NIGMS RISE
Joshua Waller	Exercise Physiology	Sophomore	NIGMS RISE
Lakhia Fuller	Biology	Sophomore	NIGMS RISE
Guy Blackmon	Biology	Junior	NIGMS RISE
Zakiyah Henry	Biology	Sophomore	NIGMS RISE
Thomas Fair	Exercise Physiology	Junior	NIGMS RISE
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