COHA Translational Fellowship Opportunity
for Residency-Trained Veterinary Specialists

Atopic dermatitis and Staphylococci in dogs: host-microbe interaction and treatment discovery

Area of Research: This team is focused on the interaction between cutaneous immune system and microbes. The main research of this group involves the interaction between Staphylococci and keratinocytes in canine and human skin disorders.

University/Department:
University of Florida, College of Veterinary Medicine, Dept. of Small Animal Clinical Sciences

Primary Mentor:
Domenico Santoro, DVM, MS, DrSc, PhD
Diplomate, ACVD, ECVD, ACVM (Bacteriology/Mycology & Immunology)
Associate Professor
Department of Small Animal Clinical Sciences
College of Veterinary Medicine
University of Florida
E-Mail: dsantoro@ufl.edu

Mentor Team:
1. Luis R. Martinez, MS, MBA, PhD
   Associate Professor
   Department of Oral Biology
   College of Dentistry
   University of Florida
2. Robert W. Huigens III, PhD
   Associate Professor
   Dept. of Medicinal Chemistry
   College of Pharmacy
   University of Florida,

Description of Potential Research Project(s):
The focus of this research training will be the evaluation of natural antimicrobial molecules against cutaneous infections in dogs with atopic dermatitis. The skin possesses a vast and important local immune system. This is characterized by several immune cells and soluble substances (e.g. host defense peptides and free fatty acids) that compose the skin associated immune response in mammals. Such system when altered, like in inflammatory skin disease (e.g. atopic dermatitis), may be responsible for the development of recurrent skin infections. In fact, atopic patients (dogs and people), in which deficiencies in different compartments of the local immune response are present, frequently require multiple rounds of antibiotics increasing the selection for resistant microorganisms.

The goal of this research effort is to investigate the pathomechanisms associated with skin infection in allergic dogs and potentially identify new treatment options to increase the natural immune defenses against bacterial pathogens.

Additional Training Opportunities:
During this fellowship, the fellow will be amply exposed to translational and comparative research. Multiple opportunities are available on campus at the University of Florida. University of Florida is one of the few academic institutions to have a dedicated CTSI unit on campus. In addition, the fellow will be encouraged to participate to human and veterinary dermatology grand rounds and journal clubs. The fellow will also be actively encouraged to participate in NIH- and CTSI-based seminars (e.g. grant writing). Finally, comparative and translational medicine workshops and seminars are constantly offered at the University of Florida. Drs. Santoro, Martinex and Huigens have active collaborations with researchers in other colleges offering additional learning venues and collaborative opportunities.

Fellowships are for 2 years and provide stipend and employee benefits at the NIH post-doctoral pay scale. Fellows may supplement their stipend with up to

25% effort towards clinical work, if such work is in alignment with the research and career development plan.

All fellowships will have a start date of fall 2022.

Biosketches of primary mentor and mentor team

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: **Domenico Santoro**

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

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Naples "Federico II" – Italy	D.V.M. (*magna cum laude*)	07/2001	Veterinary Medicine
University of Naples "Federico II" – Italy	Dr.Sc.	01/2006	Veterinary Internal Medicine
University of Illinois at Urbana-Champaign	Diplomate, ACVD & ECVD	05/2010	Veterinary Dermatology
University of Illinois at Urbana-Champaign	M.S.	05/2010	Veterinary Immuno-dermatology
University of Illinois at Urbana-Champaign	Ph.D.	11/2013	Veterinary Immuno-dermatology
University of Florida	Diplomate, ACVM	12/2017	Veterinary Bacteriology/Mycology and Immunology

A. Personal Statement

The goal of this fellowship, *"engage veterinary clinical specialists in inter-disciplinary research teams"*, perfectly fits with my role of educator and scientist. The possibility to shape new clinical-researchers is extremely appealing to me. I am a promoter of the "*clinician-scientist*" professional figure in which a specialized clinician is exposed to bench work research. This figure, being heavily involved in both a clinical and a laboratory setting, is instrumental for translational and comparative medicine functioning as bridge between the laboratory and the patients. In addition, I am strongly interested and heavily involved in comparative dermatology, as demonstrated by my recent publications.

My current research field involves the study of the pathomechanisms involved in atopic dermatitis (AD), keratinocytes alterations, and innate immune defenses against microbes with particular focus on host defense peptides and staphylococcal infections in AD. I am a trained immunologist/microbiologist and clinical dermatologist with amount 20 years of experience in veterinary medicine and almost 10 years of expertise in veterinary dermatology and immunology/microbiology. During the past 10 years, I have worked on the structural and immunological alterations present in the skin of atopic dogs and the host immune-defenses against microbes. Part of such work has been done using a canine animal model for AD here at the University of Florida. In addition, since 2010, I am part of and founder of the International Committee on Atopic Diseases of Animals (ICADA), and since 2015, I am the chair of the skin task force for the Veterinary Clinical Immunology Society. During my PhD, I have also been involved in clinical trials and study design of many projects involving cell cultures and preventative treatments for AD mastering several immunological methodologies. Finally, in the past 10 years I have been involved in study design and data analysis using various statistical tests and software and worked with many

collaborators nationally and internationally on the innate immune defenses in canine skin. For these reasons, I am exceptionally well qualified to serve as primary mentor for this fellowship.

B. Positions and Honors

Positions and Employment

2021-present	Associate Professor in Dermatology, at University of Florida, College of Veterinary Medicine, Florida, USA.
2013-present	Courtesy Assistant Professor in Dermatology, at University of Florida, College of Medicine, Florida, USA.
2015- 2021	Assistant Professor in Dermatology, at University of Florida, College of Veterinary Medicine, Florida, USA.
2013-2015	Post-Doctoral Associate in Dermatology, at University of Florida, College of Veterinary Medicine, Florida, USA
2010-2013	Visiting Clinical Instructor/PhD student in Immune-dermatology, at University of Illinois at Urbana-Champaign, College of Veterinary Medicine, Illinois, USA
2007-2010	ACVD & ECVD Resident/Master student in Dermatology, at University of Illinois at Urbana-Champaign, College of Veterinary Medicine, Illinois, USA
2006-2007	Intern in Dermatology at NC State University Veterinary Teaching Hospital, College of Veterinary Medicine, NC State University, USA
2002-2006	Post-graduate training in Veterinary Internal Medicine and Clinical Research (Research Doctorate – DrSc) at Faculty of Veterinary Medicine, University of Naples, Italy

Other Experience and Professional Memberships

American Association of Veterinary Immunologists (2019-)
American College of Veterinary Microbiologists (ACVM) (2017-)
Veterinary Clinical Immunology Society (VCIS) – chair for the skin task force (2015-)
Veterinary Clinical Immunology Society (VCIS) – infectious diseases task force (2015-)
Veterinary Clinical Immunology Society (VCIS) – diagnostic immunology task force (2015-)
American College of Veterinary Dermatology (ACVD) (2010-)
European College of Veterinary Dermatology (ECVD) (2010-)
American Academy of Veterinary Dermatology (AAVD) (2006-)
European Society of Veterinary Dermatology (ESVD) (2004-)

Honors

Elective Small Animal Climnician of the year (May 2021)
Brasley Fellowship Award – University of Illinois (August 2010 – June 2013)
Best clinical research presentation at the Phi Zeta Research day (April 2010)
Second best short communication at the 23rd ESVD/ECVD meeting (September 2009)
Second best clinical research presentation at the Phi Zeta Research day (April 2009)
Golden Oar Award (hospital service award) (April 2008)

C. Contribution to Science (*trainee)

Canine atopic dermatitis: For more than 10 years, my research has focused on gathering a better understanding of canine atopic dermatitis. Specifically, I have been conducted and involved in many basic science projects investigative the expression of structural proteins in atopic dermatitis (e.g., filaggrin and host defense peptides - HDPs). With a major interest in innate immunology and HDPs, I have focused my attention to the host-microbe interaction occurring at the skin level. This expertise allowed me to lead a subgroup of the ICADA in writing the most recent guidelines for the diagnosis of canine atopic dermatitis as well as the role of skin barrier and host-microbe interaction in the pathogenesis and treatment of canine atopic dermatitis. Finally, recently, I have been involved in a project looking in the expression of microRNA in the skin of atopic dogs.

1. Shiumitzu S*, Gillen J*, Hernandez-Bures A*, Frasca S, **Santoro D**. Evaluation of IL-17, IL-22, IL-31 and their receptors in the skin of atopic dogs: an immunofluorescence and in situ hybridization study. *Research in Veterinary Science* 2021; 136: 74-80.
2. **Santoro D**, Di Loria A, Mirante T, Oliveira DM, Laudanna C, Malanga D, Dattilo V, Iaccino E, Marsella R, Ciaramalla P. Identification of differentially expressed microRNAs in the skin of experimentally-sensitized naturally-affected atopic beagles by Next Generation Sequencing. *Immunogenetics* 2020; 72: 241-250.
3. Hensel P, **Santoro D**, Favrot C, Hill P, Griffin C, and the ICADA. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Veterinary Research* 2015; 11: 196.
4. **Santoro D**, Marsella R, Pucheu-Haston CM, Eisenschenk MNC, Nuttall T, Bizikova P, and the ICADA. Review: Pathogenesis of canine atopic dermatitis: skin barrier and host-microorganism interaction. *Veterinary Dermatology* 2015; 26: 84-e25.
5. **Santoro D**, Marsella R, Ahrens K, Graves TK, Bunick D. Altered mRNA and protein expression of filaggrin in the skin of a canine animal model for atopic dermatitis. *Veterinary Dermatology* 2013; 24: 329-e73.

Host defense peptides: In the past 10 years, my main research focus has been the investigation on the involvement of host defense peptides (HDPs) in the pathogenesis of atopic dermatitis in dogs. Below I inserted the most representative studies on this topic. Over the past 10 years, I was able to demonstrate the presence of HDPs in canine skin and how their expression varies in atopic versus healthy dogs. In addition, I was able to demonstrate that atopic skin has a different skin distribution and secreted amount of HDPs compared to healthy canine skin. Finally, I have been involved in studies evaluating the effects of plat extracts as alternative method to increase the expression of HDPs in canine atopic dermatitis.

1. **Santoro D**, Archer L, Kelley K. A defective release of host defense peptides is present in canine atopic skin. *Journal of Comparative Immunology, Microbiology & Infectious Diseases* 2019; 65: 65-69.
2. **Santoro D**, Ahrens K, Vesny R, Navarro C, Gatto H, Marsella R. Evaluation of the in vitro effect of Boldo and Meadowsweet plant extracts on the expression of antimicrobial peptides and inflammatory markers in canine keratinocytes. Research in Veterinary Sciences 2017; 115: 255-262.
3. **Santoro D.**, Ahrens K., Marsella R., Segre M. Evaluation of antimicrobial peptides and cytokine production in primary keratinocyte cell culture from healthy and atopic beagles. *Experimental Dermatology* 2015; 24: 317-319.
4. **Santoro D.**, Bunick D., Graves T.K., Segre M. Evaluation of canine antimicrobial peptides in infected and noninfected chronic atopic skin. Veterinary Dermatology 2013; 24: 39-47.e10.
5. **Santoro D**, Marsella R, Bunick D, Graves TK, Campbell KL. Expression and distribution of canine antimicrobial peptides in the skin of healthy and atopic beagles. *Veterinary Immunology and Immunopathology* 2011; **144**: 382–388.

Feline atopic syndrome: In the past few years, as part of the ICADA, I have been involved in the determining the guidelines for the diagnosis of feline atopic syndrome (FAS). Such guidelines have as ultimate goal to make clinician more aware of how to reach a correct diagnosis of allergies in cats. My involvement in feline allergies was based on a recently completed project on the use of bacterins for the treatment of FAS. Such study was based on the need for more affordable, effective and safe treatments for FAS. We were able to show that the intradermal administration of actinomycetales bacterins is an effective, cheap and safe treatment option for FAS.

1. **Santoro D,** Archer L, Fagman L. Intradermal immunotherapy with actinomycetales preparations as treatment for feline atopic syndrome: a randomized, placebo controlled, double-blinded study. *Veterinary Dermatology* [Epub 2021 April 23].
2. **Santoro D**, Pucheu-Haston CM, Prost C, Mueller R, Jackson H. Clinical signs and diagnosis of feline atopic syndrome: detailed guidelines for a correct diagnosis. *Veterinary Dermatology* 2021; 32: 26-e6.
3. Halliwell R, Pucheu-Haston C, Olivry T, Prost C, Jackson H, Banovic F, Nuttall T, **Santoro D**, Bizikova P, Mueller R. Feline Allergic Diseases: Introduction and proposed nomenclature. *Veterinary Dermatology* 2021; 32: 8-e2.

Microbiology: Working on HDPs and cutaneous innate immunity, I felt as necessary need to explore the microbiological aspect of the interaction between host and microbes. This curiosity led me to investigate the antimicrobial effects of HDPs against multidrug resistant bacteria as well as the antimicrobial effects of skin washes and plant extracts and their interaction with regularly used disinfectants. The series of studies listed below are only a representative section of the microbiological studies I have performed over the years. One of the last manuscripts in this section was performed by my resident at the time (Dr. Boyd) on the use of antimicrobials, at the ontological concentrations available, against multidrug resistant bacteria. This study has showed how bacterial culture of external ear infection is not reliable giving concrete guidelines to clinicians on how to choose the appropriate ear mediation in course of bacterial otitis.

1. **Santoro D**, Kher LD, Chala V, Navarro C. *In vitro* antimicrobial activity of host defense peptides and chlorhexidine against resistant *Staphylococcus pseudintermedius* isolates from dogs. Accepted by *Veterinary Dermatology*
2. Boyd M, **Santoro D**, Gram D. *In vitro* antimicrobial activity of topical otological antimicrobials and Tris-EDTA against resistant *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* isolates from dogs. Veterinary *Dermatology* 2019; 30: 139-e40
3. **Santoro D**, Bohannon M, Ahrens K, Navarro C, Gatto H, Marsella R. Evaluation on the effects of 0.1% Peumus boldus leaf and Spiraea ulmaria plant extract combination on bacterial colonization in canine atopic dermatitis: A preliminary randomized, placebo controlled, double-blinded study. Research in Veterinary Sciences 2018; 118: 164-170.
4. **Santoro D.** Evaluation of the secretion of antimicrobial peptides and antimicrobial effect of skin wash in atopic and healthy dogs: a preliminary study. Veterinary Dermatology 2018; 29: 402-e132. PMID: 29963726
5. **Santoro D**, Maddox CW. Canine antimicrobial peptides are effective against resistant bacteria and yeasts. Veterinary Dermatology 2014; 25: 35-e12. PMID: 24215268

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1pqvrLdmlbjw8t/bibliography/public/

D. Additional Information: Research Support (Past 5 years) (*trainee)

Ongoing Research Support
Santoro D (PI), Walton S (Co-PI), Shmalberg J (Co-PI), White A (Co-I), Pucheu-Haston C. (Co-I) Intradermal immunotherapy with a heat-killed actinomycetales bacterin preparation as adjunctive treatment for canine pythiosis: a multicentric, randomized, controlled, clinical trial. ***ACVD research grant*** **$39,918**

Santoro D (PI). Evaluation of anti-inflammatory, anti-oxidant and cytotoxicity effects of C60 on canine keratinocytes. ***Fuller Research LLC.*** **$54,991.25**
Santoro D (PI) Investigation on the *in vitro* effects of resveratrol on peripheral blood mononuclear cells harvested from healthy and atopic dogs. ***Westie Foundation of America research grant*** **$35,056**

Santoro D (PI), Kher L (Co-I)* Effect(s) of atopic dermatitis-associated cytokines on *Staphylococcus pseudintermedius*: an *in vitro* and *ex vivo* study. **A*KC – ACORN research grant* $14,999**

Santoro D. (PI) Investigation on the molecular crosstalk between canine atopic skin and microbes: unraveling potential pathomechanisms for chronic skin infections. ***AKC – OAK research grant*** **$79,369**

Santoro D (PI), Kelley K (Co-I) Ultrastructural evaluation on the alterations of host defense peptide secretion present in the canine atopic skin: a correlative light and electron microscopy study. ***ESVD major research grant $16,250***

Santoro D. **(PI)**, Kher L. (Co-I)* Prevalence of *mecA*, *mecC* and *Panton-Valentine-Leukocidin* genes in clinical isolates of *Staphylococcus pseudintermedius* from dermatological canine patients. ***SACS Departmental research grant $3,630***

di Loria A. (PI), **Santoro D. (Co-PI)** Evaluation of the cutaneous immunological milieu and leptin expression in dogs naturally affected by *Leishmania infantum/chagasi* before and after meglumine antimoniate treatment. **ESVD major research grant €19,976 ($22,403)**

Completed Research Support

Santoro D. (PI). Effects of the new Epiotic® on bacterial load and antimicrobial peptides secretion in the ears of atopic dogs: a double blinded, randomized, placebo-controlled study. **Virbac Corp. $84,007.20**

Santoro D. (PI), Chong E. (Co-PI)* Evaluation of the serum and cutaneous levels of chemokines (CCL17, CCL22, CCL27, and CCL28) in atopic dogs and their correlation with severity of canine atopic dermatitis. **A*KC – ACORN research grant* $14,967**

Santoro D. (PI) Investigation on the effects of Linkskin® spray on the cutaneous microbiome in canine atopic dermatitis: a double blinded, randomized, placebo-controlled study. ***DRN, Inc.* $71,851**

Maglione R (PI), Ferrara M (Co-PI), Ciccarelli D (Co-I), di Loria A (Co-I), **Santoro D (Co-PI)** Prevalence of *Dirofilaria repens* and coinfections with *D. immitis*, *Leishmania infantum*, and selected tick-borne diseases in privately-owned healthy dogs in the Naples area, Italy. ***ESVD practitioner research grant $5,591.04***

Santoro D. (PI). MIC and MBC of Ketochlor and AMPs against *S. pseudintermedius*, *P. aeruginosa* and *M. pachydermatis*: an *in vitro* study. **Virbac Corp. $44,625.84**

Santoro D. (PI) Anti-inflammatory and cytotoxicity study of nanoparticles. ***Earth Science Laboratories, Inc*. $32,482.50**

Cobiella D. (PI)*, **Santoro D. (Co-PI)**, Marsella R. (Co-I), Gram D. (Co-I). Evaluation of vascular endothelial growth factor (VEGF)-A protein levels in the stratum corneum and serum of healthy, atopic, and demodectic dogs. **University of Florida Foundation *research grant* $2,237.20**

Santoro D. (PI), Kher L.D. (Co-PI)*, Schultz G. (Co-I) In vitro efficacy of nano-sulfur against planktonic and biofilm state of resistant bacteria. **A*KC – ACORN research grant* $14,958**

Santoro D. (PI), Kher L.D. (Co-PI)*, Schultz G. (Co-I) In vitro efficacy of nano-sulfur against planktonic and biofilm state of resistant bacteria. **University of Florida Foundation *research grant* $10,000**

Santoro D. (PI), Marsella R. (Co-I) Is defective secretion of antimicrobial peptides associated with reduced killing effects in atopic keratinocytes?: Part I. **American Kennel Club – ACORN research grant $12,958**

BIOGRAPHICAL SKETCH

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

NAME: Luis R. Martinez

eRA COMMONS USER NAME (credential, e.g., agency login): MICRO_1

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Puerto Rico, Mayaguez, PR	BS	06/1998	Industrial Microbiology
Long Island University, Brooklyn, NY	MS	05/2001	Microbiology
Albert Einstein College of Medicine, Bronx, NY	PhD	05/2006	Microbiol. & Immunol.
Marine Biological Laboratory, Woods Hole, MA	Other training	08/2006	Molecular Mycology
Albert Einstein College of Medicine, Bronx, NY	Post-Doc	06/2011	Infectious Diseases
Cold Spring Harbor Laboratory, CSH, NY	Other training	04/2012	Prot. Purific. & Charact.
Pace University, New York, NY	MBA	05/2014	Marketing Management

A. Personal Statement

I have a broad background in infectious diseases, with specific training and expertise in microbial pathogenesis, drug development, and immunology. My primary research goals are directed toward understanding the complex interactions of infectious microorganisms with the immune system, as the balance in this interplay impacts whether host damage occurs. My science has provided several opportunities for mentoring students at every level and, to date, I have had the chance to mentor 26 undergraduate, 22 graduate (20 masters and 2 PhD), and 26 medical (1 MD/PhD) students. I am currently mentoring 3 post-doctoral trainees. My approach to mentoring these trainees is by incorporating the wonder and enthusiasm I feel about science to engage my trainees and excite them. During weekly lab meetings, I make it a point to discuss with and advise the students on aspects of their work and professional development. Moreover, I have graduate students and post-doctoral associates as the first line of "near peer" training of undergraduate or visiting trainees.

As PI on several NIH grants, my awards have provided research opportunities for my trainees. I have previously investigated the therapeutic potential of nitric oxide delivered in sustained release by the nanoparticles for the treatment of diverse infectious diseases including methicillin-resistant *Staphylococcus aureus* (MR*S*A)-associated cutaneous infections. I also showed that nitric oxide releasing nanoparticles enhance wound healing. *This work led to our licensing of the nanoparticle system to a biotechnology firm.* Perhaps, most importantly, I am excited for the opportunity these grants have provide me to train and prepare capable students (≥97% of my students have pursued a biomedical career in diverse sectors including government, healthcare, and pharmaceutical & biotechnology), from all backgrounds including under-represented minorities (<5% of academic positions) as myself and women (<30% of NIH funded investigators) who can successfully pursue careers in the biomedical sciences and medicine. Notably, the outcomes are great and several of my trainees have received institutional, regional, and national awards. *These publications in therapeutic development against bacterial skin pathogens are associated to the work proposed in this application:*

a. **Martinez, L. R.**, G. Han, M. Chacko, M. R. Mihu, M. Jacobson, P. Gialanella, A. J. Friedman, J. D. Nosanchuk, and J. M. Friedman. 2009. Antimicrobial and healing efficacy of sustained release nitric oxide nanoparticles against *Staphylococcus aureus* skin infection. **J Invest Dermatol. 129:**2463-9. PMID: 19387479 http://www.nature.com/jid/journal/v129/n10/full/jid200995a.html {Highlighted in commentary in J Invest Dermatol. **129:**2335-7}
b. Han, G., **L. R. Martinez**, M. R. Mihu, A. J. Friedman, J. M. Friedman, and J. D. Nosanchuk. 2009. Nitric oxide

releasing nanoparticles are therapeutic for *Staphylococcus aureus* abscesses. **PLoS One. 4(11):**e7804. PMID: 19915659 https://pubmed.ncbi.nlm.nih.gov/19915659/

c. Mihu, M. R., U. Sandkovsky, G. Han, J. M. Friedman, J. D. Nosanchuk, and **L. R. Martinez**. 2010. The use of nitric oxide releasing nanoparticles as a treatment against *Acinetobacter baumannii* in wound infections. **Virulence. 1:**62-7. PMID: 21178416 https://pubmed.ncbi.nlm.nih.gov/21178416/ {Highlighted commentary on article in Virulence. 1:6-7; https://pubmed.ncbi.nlm.nih.gov/21178407/}

d. Mihu, M. R., V. Cabral, R. Pattabhi, M. T. Tar, K. P. Davies, A. J. Friedman, **L. R. Martinez**, and J. D. Nosanchuk. 2016. Sustained nitric oxide releasing nanoparticles interfere with methicillin-resistant *Staphylococcus aureus* adhesion and biofilm formation in a rodent central venous catheter model. **Antimicrob Agents Chemother. 61:** e02020-16. PMID: 27821454 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5192117/

B. Positions and Honors:

Positions

9/1999-5/2000 Teaching Assistant, Department of Biology, Long Island University, Brooklyn, NY
9/2001-5/2006 Doctoral Research, Department of Microbiology and Immunology, Einstein, Bronx, NY 7/2006-5/2011 Post-Doctoral Fellow, Infectious Diseases, Department of Medicine, Einstein, Bronx, NY
8/2009-12/2010 Adjunct Assistant Professor, Department of Biology, Bronx Community College, Bronx, NY 9/2010-5/2014 Assistant Professor, Department of Biomedical Sciences, LIU-Post, Brookville, NY (*Promoted to Associate Professor with Tenure in 2014*)
11/2013-5/2014 Adjunct Assistant Professor, Department of Health Professions, York College, Queens, NY 6/2011-8/2017 Adjunct Clinical Faculty, Department of Medicine, Einstein, Bronx, NY
6/2014-8/2017 Associate Professor, Department of Biomedical Sciences, New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY
8/2017-12/2019 Associate Professor, Department of Biological Sciences, The Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX
1/2020-present Associate Professor, Department of Oral Biology, University of Florida College of Dentistry, Gainesville, FL

Professional Memberships: American Association for the Advancement of Science (AAAS; 2015), American Society for Microbiology (ASM; 2001), International Society for Human and Animal Mycology (2014), Medical Mycological Society of the Americas (MMSA; 2012)

Honors: UTEP Edge Fellow (2019); UTEP Outstanding Research Performance Award (2018-2019); NYIT Faculty Scholar (distinguished speaker; 2017); AAAS Leshner Public Engagement Fellow in Infectious Diseases (2017-2018); ABRCMS Judges' Travel Subsidy Program (2015 and 2017); Thomas J. Walsh Junior Investigator Award of the MMSA (2014); Long Island Business News, Health Care Hero Award (2011); Long Island University Research Faculty Committee Award (2011-2014); Benjamin Cummings/MACUB Student Research Award (2011-2013); Einstein, Dennis Shield Travel Award for Outstanding Post-doctoral Research (2010); NIH, Loan Repayment Program (2010-2014); NIH, Molecular Pathogenesis Training Fellow (2006-2010); ASM General Meeting Minority Travel Award (2007); Scholar, Woods Hole Marine Biological Laboratory (2006); FASEB-MARC Travel Award to Experimental Biology Annual Meeting (2001)

Other Experience

Ad hoc reviewer for scientific journals (selected): Antimicrobial Agents and Chemotherapy, Journal of Antimicrobial Chemotherapy, Journal of Applied Microbiology, BMC Microbiology, Brazilian Journal of Microbiology, Cellular Microbiology, Frontiers Cellular and Infection Microbiology, Frontiers Microbiology, Fungal Genetics and Biology, Infection and Immunity, Journal of Infectious Diseases, Interdisciplinary Perspectives in Infectious Diseases, Journal of Invasive Fungal Infections, Journal of Medical Microbiology, mBio, Medical Mycology, Microbes and Infection, Microbial Ecology, Microbial Pathogenesis, Microbiology, Nature Communications, Nature Medicine, Journal of Neuroinflammation, Neurotoxicity Research, PLoS Neglected Tropical Diseases, PLoS One, Scientific Reports, Virulence.

Editorial experience: Associate Editor for Frontiers in Microbiology (2011-2018); Editorial Board of Infection and Immunity (2017-present) and mBio (2021-present); Mycology Section Editor (2018-present) for Current Tropical Medicine Reports; Special Issue for Pathogens (2020); Topic Editor for Frontiers Cellular and Infection Microbiology (2020)

Member of Peer-review panels: NIH Member Conflict: AIDS and Related Research (ZRG1 AARR-P; 2020), NIH Pathogenic Eukaryotes Study Section (PTHE; 2020), Antimicrobial Resistance (2018-2019) and Arthritis- Post-traumatic Osteoarthritis peer review panels of the Peer-reviewed Medical Research Program for the Department of Defense Congressionally Directed Medical Research Programs (2018), Military Infectious Diseases Basic Research Award of the Defense Medical Research and Development Program of the Department of Defense of the United States (2012, 2016, 2017), Louisiana's NSF Experimental Program to Stimulate Competitive Research (2012), Puerto Rico Science, Technology and Research Trust (2015-2016), British Society for Antimicrobial Chemotherapy Grant Programme (2015), ASM Robert D. Watkins Graduate Research Fellowship program (2015-2016)

Leadership Activities: ASM's Committee on Microbiological Issues Impacting Minorities (2012-present), ASM's Communication Committee's Member Empowerment Taskforce (2015-2019), AAAS Leshner Public Engagement Fellow in Infectious Diseases (2017-2018), AAAS Early Career Science Communication Award Committee (2018-present), ASM's Host-Microbe Biology Strategic Planning Group (2018), ASM's Microbe Program Committee (2019-2021) and Track Leader of the Profession of Microbiology (2020-present).

C. Contributions to Science: (* Undergraduate, † graduate, and ‡ medical student; x URM)

1. **Application of nitric oxide-releasing nanotechnology for treatment of cutaneous infections:** My lab is interested in exploring the therapeutic potential of nitric oxide delivered in sustained release by the nanoparticles for the treatment of cutaneous infections. I have shown that nitric oxide releasing nanoparticles enhance wound healing. In fact, the simplicity and the stability of this nanotechnology makes it a very attractive treatment modality in many conditions, including combat or disaster situations, especially since they have proven high efficacy against multi-drug bacteria that are challenging the success of antibiotic use.
   a. Friedman, A. J., K. Blecher, D. Schairer, C. Tuckman-Vernon, P. Nacharaju, D. Sanchez, P. Gialanella, **L. R. Martinez**, J. M. Friedman, and J. D. Nosanchuk. 2011. Improved antimicrobial efficacy with nitric oxide releasing nanoparticle generated S-nitrosoglutathione. **Nitric Oxide. 25**:381-386. PMID: 21946032 https://www.sciencedirect.com/science/article/pii/S108986031100468X?via%3Dihub
   b. Han, G., L. N. Nguyen, C. Macherla†, Y. Chi, J. M. Friedman, J. D. Nosanchuk, and **L. R. Martinez**. 2012. Nitric oxide releasing nanoparticles accelerate wound healing by promoting fibroblast migration and collagen deposition. **Am J Pathol. 180:**1465-73. PMID: 22306734 http://ajp.amjpathol.org/article/S0002-9440(12)00015-6/fulltext
   c. Macherla†, C., D. A. Sanchez*x, M. Ahmadi*, E. M. Vellozzi, A. J. Friedman, J. D. Nosanchuk, and **L. R. Martinez**. 2012. Nitric oxide releasing nanoparticles for treatment of *Candida albicans* burn infections. **Front Microbiol. 3:**193. PMID: 22306734 http://journal.frontiersin.org/article/10.3389/fmicb.2012.00193/full
   d. Ahmadi*, M., H. H. Lee†, D. A. Sanchez*x, A. J. Friedman, M. T. Tar, K. P. Davies, J. D. Nosanchuk, and **L. R. Martinez**. 2016. Sustained nitric oxide releasing nanoparticles induce cell death in *Candida albicans* yeast and hyphal cells preventing biofilm formation *in vitro* and in a rodent central venous catheter model. **Antimicrob Agents Chemother. 60:**2185-2194. {Article featured in the website Nanowerk. http://www.nanowerk.com/nanotechnology-news/newsid=42433.php} PMID: 26810653 http://aac.asm.org/content/60/4/2185.long

2. **Pathogenesis of Multi-drug resistant *Acinetobacter baumannii*:** *A. baumannii* is a frequent cause of hospital-acquired pneumonia. Clinical approaches are limited since *A. baumannii* strains isolated from patients are resistant to current antimicrobials. *A. baumannii* can survive desiccation and during outbreaks has been recovered from various sites in the patients' environment. I developed and used stainless steel washers as abiotic surface to investigate *A. baumannii* biofilm formation and desiccation, thus mimicking the surfaces found in the hospital setting. I have extensively studied *A. baumannii*-phagocytes interactions. In addition, my laboratory has shown that alcohol exacerbates *A. baumannii* pneumonia altering the effector functions of macrophages and neutrophils.
   a. Asplund†, M. B., C. Coelho, R. J. Cordero, and **L. R. Martinez**. 2013. Alcohol impairs J774.16 macrophage-like cell antimicrobial functions *in Acinetobacter baumannii* infection. **Virulence. 4:**467-472 PMID: 23863607 http://www.tandfonline.com/doi/full/10.4161/viru.25641#.VOPshfnF_l4 {Highlighted commentary on article in Virulence. 4:435-436}
   b. Orsinger-Jacobsen*, S. J., S. S. Patel†, E. M. Vellozzi, P. Gialanella, L. Nimrichter, K. Miranda, and **L. R. Martinez**. 2013. Use of a stainless-steel washer platform to study *Acinetobacter baumannii* adhesion and biofilm formation on abiotic surfaces. **Microbiology. 159:** 2594-2604. PMID: 24025603

http://mic.sgmjournals.org/content/159/Pt_12/2594.long {Cover of the issue}

c. Grguric-Smith*, L. M., H. H. Lee†, J. A. Gandhi†, M. B. Brennan†, C. M. DeLeon-Rodriguez, C. Coelho, G. Han, and **L. R. Martinez**. 2015. Neutropenia exacerbates infection by *Acinetobacter baumannii* clinical isolates in a murine wound model. **Frontier Microb. 6:**1134. PMID: 26528277 http://journal.frontiersin.org/article/10.3389/fmicb.2015.01134/abstract

d. Krishnamoorthy†, S., B. P. Shah†, H. H. Lee†, and **L. R. Martinez**. 2015. Microbicides alter the expression and function of RND-type efflux pump AdeABC in biofilm-associated cells of *Acinetobacter baumannii* clinical isolates. **Antimicrob Agents Chemother. 60:**57-63. PMID: 26459900 http://aac.asm.org/content/60/1/57.long

3. **Impact of methamphetamine (METH) on infection and immunity:** I demonstrated that METH-in addition to its neuropsychiatric effects- alters host immune behavior. I developed a mouse model of METH use and infection to show that the microbial disease is significantly exacerbated by the drug. METH alters the phagocytosis and killing of microbes by macrophages, reduces effective T cell responses, modifies cytokine activation, and changes the antibody profiles *in vivo*. My laboratory showed that METH disrupts the blood brain barrier integrity *in vivo* via the modulation of the expression of tight junction and adhesion proteins resulting in extensive brain alterations including an increase in the susceptibility of the CNS to microbial infections. *Our article in mBio reached mainstream audience after being featured in The LA Times. http://www.latimes.com/news/science/sciencenow/la-sci-sn-meth-lung-infection-20130730,0,2202905.story*. Notably, I use *C. neoformans* and *Staphylococcus aureus* as microbial models to study host-pathogen interactions in the setting of METH due to their involvement in brain and skin infections, respectively.

a. Eugenin, E. A., J. M. Greco*, S. Frases, J. D. Nosanchuk, and **L. R. Martinez**. 2013. Methamphetamine alters blood brain barrier protein expression facilitating central nervous system infection by neurotropic *Cryptococcus neoformans*. **J Infect Dis. 208:** 699-704. PMID: 2532099 http://jid.oxfordjournals.org/content/208/4/699.long {Cover of the issue}

b. Patel†, D., G. M. Desai†, S. Frases, R. J. Cordero, C. M. DeLeon-Rodriguez, E. A. Eugenin, J. D. Nosanchuk, and **L. R. Martinez**. 2013. Methamphetamine enhances *Cryptococcus neoformans* pulmonary infection and dissemination to the brain. **mBio. 4:**e00400-13. PMID:23900172 http://mbio.asm.org/content/4/4/e00400-13.full {Editor's pick and article featured in The Los Angeles Times. http://www.latimes.com/news/science/sciencenow/la-sci-sn-meth-lung-infection- 20130730,0,2202905.story}

c. Radu, M. R., J. Roman-Sosa, A. K. Varshney, E. A. Eugenin, B. P. Shah†, H. H. Lee†, L. N. Nguyen, A. J. Guimaraes, B. C. Fries, J. D. Nosanchuk, and **L. R. Martinez**. 2015. Methamphetamine alters the antimicrobial efficacy of phagocytic cells during methicillin-resistant *Staphylococcus aureus* skin infections. **mBio. 6:**e01622-15. PMID: 26507236 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4626859/

d. Aslanyan‡, L., H. H. Lee, V. V. Ekhar†, R. L. Ramos, and **L. R. Martinez**. 2019. Methamphetamine impairs IgG1-mediated phagocytosis and killing of *Cryptococcus neoformans* by J774.16 macrophage-and NR-9640-microglia-like cells. **Infect Immun. 87 (2):** pii: e00113-18. PMID: 30510106. https://iai.asm.org/content/early/2018/11/28/IAI.00113-18.long

4. **Characterization of *Cryptococcus neoformans* biofilms:** I developed the tools necessary and was responsible for characterizing biofilm formation in the AIDS-related pathogenic encapsulated fungus *C. neoformans*. I demonstrated that one could distinguish protective from non-protective antibodies by their effects on polysaccharide shedding by *C. neoformans*. I established that protective antibodies prevented biofilm formation while non-protective antibodies had no effect on this phenomenon and went on to show that biofilm formation interfered with drug action against *C. neoformans*. *I wrote a chapter on C. neoformans biofilms in the new edition of ASM's prestigious book, Microbial Biofilms, published in 2015 (http://www.asmscience.org/content/book/10.1128/9781555817466).*

a. **Martinez, L. R.,** and A. Casadevall. 2005. Specific antibody can prevent fungal biofilm formation and this effect correlates with protective efficacy. **Infect Immun. 73:**6350-62. PMID: 16177306 http://iai.asm.org/content/73/10/6350.long

b. **Martinez, L. R.,** E. Christaki, and A. Casadevall. 2006. Specific antibody to *Cryptococcus neoformans* glucurunoxylomannan antagonizes antifungal action against cryptococcal biofilms *in vitro*. **J Infect Dis. 194:**261-66. PMID: 16779734 http://jid.oxfordjournals.org/content/194/2/261.long

c. **Martinez, L. R.**, M. R. Mihu, G. Han, S. Frases, R. J. Cordero, A. Casadevall, A. J. Friedman, J. M. Friedman, and J. D. Nosanchuk. 2010. The use of chitosan to damage *Cryptococcus neoformans* biofilms. **Biomaterials. 31**:669-79. PMID: 19819009 http://www.sciencedirect.com/science/article/pii/S0142961209010552

d. Abdulkareem†, A. F., H. H. Lee†, M. Ahmadi*, and **L. R. Martinez**. 2015. Fungal serotype-specific differences in bacterial-yeast interactions. **Virulence.** 6:654-9. PMID: 26132337 http://www.tandfonline.com/doi/full/10.1080/21505594.2015.1066962 {Highlighted commentary on article in Virulence. 6:677-678; https://www.ncbi.nlm.nih.gov/pubmed/26364987}

5. **Social Aspects of Science: Education, Public Engagement, and Diversity Issues:** I am an advocate for scientific citizenship and communication with the public. I wrote an Op-Ed in a local newspaper advocating against funding cuts for basic science and medical research in the US (http://www.newsday.com/opinion/commentary/a-cut-in-research-would-wound-u-s-1.13701060). Recently, I assessed the diversity of graduates from bachelor's, master's, and doctoral degree neuroscience programs. In addition, I demonstrated that limited pre-medical microbiology-related knowledge among medical students might be associated with increased in perceived stress when learning this content or during their clinical rotations. Furthermore, I first authored a comprehensive study that analyzes the factors that contribute to success in >300 under-represented minority life scientists.
   a. Shah‡, H., and **L. R. Martinez**. 2016. Current approaches to implement citizen science in the classroom. **J Microbiol Biol Educ. 17:**17-22. PMID: 27047583 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4798802/
   b. Ramos, R. L., K. Alviña, and **L. R. Martinez**. 2017. Diversity of graduates from bachelor's, master's, and doctoral degree neuroscience programs in the United States. **J Undergrad Neurosci Educ. 16:**A6-A13. PMID: 29371835 http://www.funjournal.org/wp-content/uploads/2017/11/june-16-6.pdf
   c. Ramos, R. L., E. Guercio, and **L. R. Martinez**. 2017. Pre-medical preparation in microbiology among applicants and matriculants in osteopathic medical school in the United States. **J Microbiol Biol Educ. 18:**1-6. http://www.asmscience.org/docserver/fulltext/jmbe/18/3/jmbe-18-61.pdf?expires=1513106170&id=id&accname=guest&checksum=463E8E2003299D8577F6A3F5DDD7 D6E0
   d. **Martinez, L. R.**, D. W. Boucaud, A. Casadevall, and A. August. 2018. Factors contributing to the success of NIH-designated underrepresented minorities in academic and non-academic research positions. **CBE Life Sci Educ. 17:**ar32. PMID: 29799320 https://www.lifescied.org/doi/abs/10.1187/cbe.16-09-0287?url_ver=Z39.88- 2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

Complete List (*85 publications*) of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/10c9nOi9rn8An/bibliography/48062664/public/?sort=date&direction=descending

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

Current Research Support

1 R01 AI145559, **Martinez (PI), 6/2019-5/2024**

Title: Unraveling *Cryptococcus neoformans* mechanisms of brain invasion and colonization

The major goal of this project: (1) Determine the role of *C. neoformans* GXM on blood brain barrier physical integrity *in vivo*; (2) Establish the cell signaling mechanisms by which *C. neoformans* GXM induces human brain microvascular endothelial cell barrier permeability; and (3) Study the impact of GXM-induced inhibition of microglial cell migration and control of *C. neoformans* brain invasion *in vivo*.

Completed Training Support

1 R15 GM117501-01, **Martinez, 8/2016-7/2019** (**Former PI**; Project Collaborator; since UTEP was ineligible for the R15 Academic Research Enhancement Award (AREA) mechanism, I transferred this award to Dr. Raddy Ramos, a colleague at NYIT COM)

Title: Impact of methamphetamine induced IL-6 production on wound healing and inflammation

The major goal of this project: (1) Investigate the impact of methamphetamine on wound polymorphonuclear leukocytes recruitment and repair *in vivo* and (2) Assess the role of methamphetamine on IL-6 production in the setting of wound healing and inflammation *in vivo*.

BIOGRAPHICAL SKETCH

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

NAME: Huigens III, Robert William

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

POSITION TITLE: Associate Professor of Medicinal Chemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of North Carolina at Greensboro	B.A.	08/03	Biology (Chem. Minor)
University of South Florida	Transfer	07/04	Organic Chemistry
North Carolina State University	Ph.D.	07/09	Organic Chemistry
University of Illinois at Urbana-Champaign	Postdoctoral	03/13	Organic Chemistry

A. Personal Statement

The overarching goal of my laboratory is to tackle intractable biomedical problems through an interdisciplinary research program utilizing synthetic organic chemistry, medicinal chemistry and chemical biology. Our group is primarily involved in natural product-inspired discovery approaches that aim to: (1) utilize available alkaloids as starting point to rapidly generate complex and diverse compound libraries for drug discovery, (2) advance hit compounds from our diverse alkaloid-derived small molecule library for specific disease areas (e.g., drug addiction), and (3) discover new antibacterial agents that eradicate biofilms and kill tuberculosis. In addition to our primary efforts, we collaborate with other groups to design and synthesize novel small molecules for therapeutic development related to cancer and myotonic dystrophy. In our first eight years at the University of Florida, our lab has published 40 papers on these topics, and we look forward to continuing efforts related to the development of novel halogenated phenazine antibacterial agents that eradicate surface-attached biofilms.

Publications relevant to this application:

1. Garrison, A. T.; Abouelhassan, Y.; Kallifidas, D.; Tan, H.; Kim, Y. S.; Jin, S.; Luesch, H.; **Huigens III, R.W.** "An Efficient Buchwald-Hartwig/Reductive Cyclization for the Scaffold Diversification of Halogenated Phenazines: Potent Antibacterial Targeting, Biofilm Eradication and Prodrug Exploration." *J. Med. Chem.* **2018**, *61*, 3962-3983.
2. Yang, H.; Kundra, S.; Chojnacki, M.; Liu, K.; Fuse, M.A.; Abouelhassan, Y.; Kallifidas, D.; Zhang, P.; Huang, G.; Jin, S.; Ding, Y.; Luesch, H.; Rohde, K.H.; Dunman, P.M.; Lemos, J.A.; **Huigens III, R.W.** "A Modular Synthetic Route Involving *N*-Aryl-2-Nitrosoaniline Intermediates Leads to a New Series of 3-Substituted Halogenated Phenazine Antibacterial Agents." *J. Med. Chem.* **2021**, *64*, 7275-7295. (Featured Article; this work was selected to be on Front Cover of *J. Med. Chem.*)

B. Positions and Honors

Positions and Employment

2020-present Associate Professor of Medicinal Chemistry, University of Florida
2014-2017 Affiliate Assistant Professor of Chemistry, University of Florida
2013-2020 Assistant Professor of Medicinal Chemistry, University of Florida
2009-2013 American Cancer Society Postdoctoral Fellow, UIUC (Advisor: P. Hergenrother)
2004-2009 Graduate Research & Teaching Assistant, North Carolina State University (Advisor: C. Melander)
2003-2004 Graduate Research & Teaching Assistant, University of South Florida (transfer)

2001-2003 Undergraduate Research & Teaching Assistant, University of North Carolina at Greensboro

Other Experience and Professional Memberships

2005-present Member, American Chemical Society

Honors

2020 Teaching Service Excellence Incentive Award (UF College of Pharmacy)
2020 NIH Reviewer; Combating Antibiotic-Resistant Bacteria Interdisciplinary Research Units (CARBIRU)
2019 Young Investigator Award, American Chemical Society (Division of Organic Chemistry)
2018 Teaching Service Excellence Incentive Award, University of Florida, College of Pharmacy
2018-present Maximizing Investigators' Research Award; National Institute of General Medical Sciences
2018 Year 2 Outstanding Teaching Team Award, College of Pharmacy, University of Florida
2018-present American Cancer Society Research Scholar
2017 Teaching Service Excellence Incentive Award, University of Florida, College of Pharmacy
2017 Year 2 Outstanding Teaching Team Award, College of Pharmacy, University of Florida
2017 Ad Hoc Reviewer; Partnerships for Countermeasures Against Select Pathogens: Therapeutics, Immunotherapeutics, and Vaccines Review Panel
2017 Teacher of the Year, College of Pharmacy, University of Florida
2016 NIH Early Career Reviewer; Anti-Infective Drug Discovery and Mechanism of Resistance (DDR)
2015 Young Investigator Award, Center for Biofilm Engineering, Montana State University
2015 Young Investigator Award, American Chemical Society, Division of Medicinal Chemistry
2010-2013 American Cancer Society Postdoctoral Fellow, University of Illinois at Urbana-Champaign
2008-2009 Jimmy V Predoctoral Scholar, North Carolina State University
2007 Teaching Excellence Award in Organic Chemistry, North Carolina State University
2006-2007 Phi Lambda Upsilon Treasurer, North Carolina State University
2005 Phi Lambda Upsilon, North Carolina State University
2004 Phi Beta Kappa, Univ. of North Carolina at Greensboro
2003 American Chemical Society Organic Chemistry Award, Univ. North Carolina at Greensboro
2003 Student Excellence Award (Honors College), Univ. of North Carolina at Greensboro
2003 Eberhart Award (Biology Department), Univ. of North Carolina at Greensboro
2003 Graduated *Summa Cum Laude*, Univ. of North Carolina at Greensboro
2001-2002 Beta Beta Beta President, University of North Carolina at Greensboro
2001 Beta Beta Beta, University of North Carolina at Greensboro

C. Contributions to Science

In eight years since beginning my independent career at the University of Florida, our lab has published 40 papers in the areas of (1) complex molecule synthesis through ring distortion, from indole alkaloids, for drug discovery, (2) the identification, development and biological studies related to novel biofilm-eradicating agents, and (3) the development of small molecules for the treatment of myotonic dystrophy. We have developed a ring distortion approach that enables the rapid generation of complex and diverse small molecules from indole alkaloids and are now expanding to other alkaloids. Our innovative tryptoline ring distortion strategy has enabled the rapid synthesis of diverse and complex small molecules from commercially available indole alkaloids (e.g., yohimbine, vincamine). These efforts aim to address the lack of complex structures used in screening libraries used for drug discovery and identify biologically active compounds for pre-clinical assessment. Currently, we have synthesized ~350 complex and diverse small molecules from various alkaloids, which are being screened for an array of biological activities important for human health. We recently identified a series of diverse and novel scaffolds from yohimbine and vincamine that demonstrate anticancer and antiplasmodial (antimalarial) activities in whole cell assays. In addition, an array of GPCR drug target antagonists have been identified with potential therapeutic applications in opioid addiction, cancer and diabetes. In addition, our lab has discovered multiple series of small molecules that eradicate bacterial biofilms following our initial investigations into phenazine antibiotics (halogenated phenazines, halogenated quinolines/nitroxoline, *N*-arylated NH125 analogues). We are now carrying out mechanistic studies with RNA-seq technology to study biofilm viability with our potent halogenated phenazine biofilm killers, and developing prodrug strategies to translate our halogenated phenazines to target biofilm infections. In the proposed studies, we aim to utilize our alkaloid ring distortion efforts to discover and develop novel compounds to treat drug addiction.

Currently, our work is supported by a MIRA grant (1R35GM128621-01: "Indole Alkaloids and Phenazine Antibiotics: New Platforms for Drug Discovery", which supports our lab's programs related to the development of novel biofilm-eradicating halogenated phenazine small molecules, RNA-seq studies related to biofilm survival and complex molecule synthesis from indole alkaloids for drug discovery and chemical biology; role: PI), a Research Scholar Grant from the American Cancer Society (131947-RSG-18-013-01-CDD: "Alkaloid Ring Distortion: A Platform for New Cancer Therapies", which aims to establish a cancer discovery platform by utilizing alkaloids to enable the discovery and development of novel anticancer agents using *in vitro* and *in vivo* models; role: PI) and an R-21 (1R21AI159191: "Optimization and Mechanistic Studies of Halogenated Phenazines and Quinolines as Anti-Tuberculosis Therapeutics", which supports the biological study of halogenated phenazine and quinoline analogues against tuberculosis with an emphasis on advancing lead compounds to infection models in animals; role: MPI (Kyle Rohde is the primary contact for this grant).

1. **Development of a tryptoline ring distortion strategy of complex indole alkaloids (PI at UF).** Our lab is developing ring-distortion strategies that utilizes available complex alkaloids (most are indole alkaloids). Using this approach, we have been able to access novel ring-cleaved, ring-rearranged and ring-expanded indole/spirooxindole small molecules. To date, we have rapidly synthesized ~350 novel complex and diverse small molecules from these available alkaloids. Through biological screening efforts, we have identified several hit compounds with interesting biological activities, including: modulation of the antioxidant response element (ARE) in cancer cells, HIF-dependent antiproliferative activities in colorectal cancer cells, anti-inflammatory activities in macrophages. One of our exciting hit compounds is a yohimbine-derived compound we found to demonstrate potent anti-malarial activity against *Plasmodia falciparum* (EC_{50} = 333 nM against multidrug-resistant Dd2 cells; >60-fold more potent than the parent indole alkaloid, which was inactive when evaluated at the highest test concentration). Recently, we have identified several new ring distortion compounds to demonstrate highly differential antagonistic profiles against a panel of diverse GPCR drug targets. From this screen, we discovered vincamine-derived compound **V2a** to possess selective antagonistic activities against hypocretin receptor 2 (HCRTR2), a GPCR that has relevance to heroin addiction in mice. We advanced **V2a** to animal studies in mice and found this novel small molecule to demonstrate *in vivo* efficacy in multiple conditioned place preference models of morphine addiction. These collective findings demonstrate the potential for the ring distortion of indole alkaloids to lead to important biological discoveries in unrelated disease areas, such as the current opioid crisis in United States.

    a. Paciaroni, N. G.; Ratnayake, R.; Matthews, J. H.; Norwood IV, V. M.; Arnold, A. C.; Dang, L. H.; Luesch, H.; **Huigens III, R.W.** "A Tryptoline Ring Distortion Strategy Leads to Complex and Diverse Biologically Active Molecules from the Indole Alkaloid Yohimbine." *Chem. Eur. J.* **2017**, *23*, 4327-4335. *(Cover Art Selection)*
    b. Paciaroni, N.G.; Perry, D.; Norwood IV, V.M.; Murillo-Solano, C.; Collins, J.; Tenneti, S.; Chakrabarti, D.; **Huigens III, R.W.** "Re-engineering of Yohimbine's Biological Activity through Ring Distortion: Identification and Structure-Activity Relationships of a New Class of Antiplasmodial Agents." *ACS Infect. Dis.* **2020**, *6*, 159-167. *(Selected as a "Featured Article"; Front Cover Art Selection)*
    c. Norwood IV, V.M.; Brice-Tutt, A.; Eans, S.O.; Stacy, H.; Shi, G.; Ratnayake, R.; Rocca, J. R.; Abbou, K. A.; Li, C.; Luesch, H.; McLaughlin, J. P.; **Huigens III, R.W.** "Preventing Morphine Seeking Behavior through the Re-engineering of Vincamine's Biological Activity." *J. Med. Chem.* **2020**, *63*, 5119-5138. *(Front Cover Art Selection)*
    d. Paciaroni, N.G.; Norwood IV, V.M.; Ratnayake, R.; Luesch, H.; **Huigens III, R.W.** "Yohimbine as a Starting Point to Access Diverse Natural Product-Like Agents with Re-programmed Activities against Cancer-Relevant GPCR Targets." *Bioorg. Med. Chem.* **2020**, *28*, 115546. (*Invited to be part of the "Chem/bio performance diversity" special issue in BMC*)
    e.
2. **Halogenated Phenazine (HP) antibacterial and biofilm-eradicating agents (PI at UF).** A major aim of my lab is to discover new small molecules that address problems associated with antibiotic resistance and tolerance (persistent biofilms). From our combined interest in phenazine antibiotic natural products, synthetic medicinal chemistry and bacterial biofilms, we have discovered a series of halogenated phenazines that are capable of potently eradicating staphylococcal (MRSA, MRSE) and vancomycin-resistant *Enterococcus faecium* biofilms. We have also discovered that these compounds slowly kill MRSA persister cells in non-biofilm (stationary) culture, demonstrate potent anti-tuberculosis (anti-MtB) activity while showing negligible cytotoxicity against HeLa cells and red blood cells (no membrane lysing activity). In addition, our lab demonstrated that HPs induce rapid iron starvation in MRSA biofilms using RNA-seq technology and has ongoing translational efforts related to HPs through the development of novel prodrug strategies.

a. Garrison, A. T.; Abouelhassan, Y.; Kallifidas, D.; Bai, F.; Ukhanova, M.; Mai, V.; Jin, S.; Luesch, H.; **Huigens III, R.W.** "Halogenated Phenazines that Potently Eradicate Biofilms, MRSA Persister Cells in Non-Biofilm Cultures and *Mycobacterium tuberculosis*." *Angew. Chem. Int. Ed.*, **2015**, *54*, 14819-14823.
b. Garrison, A. T.; Abouelhassan, Y.; Kallifidas, D.; Tan, H.; Kim, Y. S.; Jin, S.; Luesch, H.; **Huigens III, R.W.** "An Efficient Buchwald-Hartwig/Reductive Cyclization for the Scaffold Diversification of Halogenated Phenazines: Potent Antibacterial Targeting, Biofilm Eradication and Prodrug Exploration." *J. Med. Chem.* **2018**, *61*, 3962-3983.
c. Abouelhassan, Y.; Zhang, Y.; Jin, S.; **Huigens III, R.W.** "Transcript Profiling of MRSA Biofilms Treated with a Halogenated Phenazine Eradicating Agent: A Platform for Defining Cellular Targets and Pathways Critical to Biofilm Survival." *Angew. Chem. Int. Ed.* **2018**, *57*, 15523-15528.
d. Yang, H.; Kundra, S.; Chojnacki, M.; Liu, K.; Fuse, M.A.; Abouelhassan, Y.; Kallifidas, D.; Zhang, P.; Huang, G.; Jin, S.; Ding, Y.; Luesch, H.; Rohde, K.H.; Dunman, P.M.; Lemos, J.A.; **Huigens III, R.W.** "A Modular Synthetic Route Involving *N*-Aryl-2-Nitrosoaniline Intermediates Leads to a New Series of 3-Substituted Halogenated Phenazine Antibacterial Agents." *J. Med. Chem.* **2021**, *64*, 7275-7295. (Featured Article; this work was selected to be on Front Cover of *J. Med. Chem.*)

3. **Scaffold hopping discovery of novel 8-hydroxyquinoline antibacterial and biofilm-killing agents (PI at UF).** Following our initial discovery that 1-hydroxy-2-bromophenazine demonstrated potent antibacterial activity, we used a scaffold hopping strategy to related 8-hydroxyquinolines that demonstrate an array of antibacterial and antibiofilm activities. The 8-hydroxyquinoline scaffold is more amiable to diversification via synthesis and we have discovered new active sub-classes that have been generated from distinct synthesis pathways. Collectively, our 8-hydroxyquinoline analogues (including nitroxoline) have demonstrated broad spectrum antibacterial and biofilm eradication activities. We have also discovered that gallic acid, a phytochemical, dramatically potentiates the antibacterial activities of a several halogenated quinolines. It is impressive to note that gallic acid potentiated one of our potent halogenated quinolines (MIC < 1 μM) >10,000-fold in MIC potentiation assays against *S. aureus*, which is the most dramatic antibacterial potentiation we could find in the literature. Gallic acid potentiates select halogenated quinolines against a broad spectrum of bacterial pathogens (*S. aureus, S. epidermidis, A. baumannii, K. pneumoniae*), including multidrug-resistant clinical isolates.

   a. Basak, A.; Abouelhassan, Y.; Norwood IV, V. M.; Bai, F.; Nguyen, M.; Jin, S.; **Huigens III, R.W.** "Synthetically Tuning the 2-Position of Halogenated Quinolines: Optimizing Antibacterial and Biofilm Eradication Activities via Alkylation and Reductive Amination Pathways." *Chem. Eur. J.* **2016**, *22*, 9181-9189. *(Hot Article; Cover Art Selection)*
   b. Abouelhassan, Y.; Yang, Q.; Nguyen, M. T.; Rolfe, M.; Yousaf, H. Schultz, G. S.; **Huigens III, R.W.** "Nitroxoline: A Broad-Spectrum Persister Cell- and Biofilm-Eradicating Agent Against Pathogenic Bacteria." *Int. J. Antimicrob. Agents* **2017**, *49*, 247-251.
   c. Garrison, A. T.; Abouelhassan, Y.; Yang, H.; Yousaf, H. H.; Nguyen, T.; **Huigens III, R.W.** "Microwave-Enhanced Friedländer Synthesis for the Rapid Assembly of Halogenated Quinolines with Antibacterial and Biofilm Eradication Activities against Drug Resistant and Tolerant Bacteria." *Med. Chem. Commun.* **2017**, *8*, 720-724. *(Hot Article; Invited for Themed Issue: "New Talent: Americas")*
   d. Basak, A.; Abouelhassan, Y.; Kim, Y. S.; Norwood IV, V. M.; Jin, S.; **Huigens III, R.W.** "Halogenated Quinolines Bearing Polar Functionality at the 2-Position: Identification of New Antimicrobial Agents with Enhanced Activity against *Staphylococcus epidermidis.*" *Eur. J. Med. Chem.* **2018**, *155*, 705-713.

4. **Identification of *N*-arylated NH-125 analogues as rapid killers of microbial persister cells (PI at UF).** NH-125 is a membrane-active antibacterial agent that has an interesting reported biological profile, demonstrating *in vivo* efficacy in cancer and neurological studies in mice. Although, membrane-lysing agents are typically not pursued for biomedical applications due to toxicity concerns, we felt that with the known efficacy of this compound *in vivo*, NH-125 could potentially be a starting point for the development of rapid-killing antimicrobials. We have designed and synthesized novel *N*-arylated analogues of NH-125 that proved to rapidly eradicate persister cells and biofilms in bacteria and fungi. In future studies, we aim to use these compounds in RNA-seq experiments with MRSA biofilms to gain a better understanding of biofilm viability.

   a. Basak, A.; Abouelhassan, Y.; Zuo, R.; Yousaf, H.; Ding, Y.; **Huigens III, R.W.** "Antimicrobial Peptide-Inspired NH125 Analogues: Bacterial and Fungal Biofilm-Eradicating Agents and Rapid Killers of MRSA Persisters." *Org. Biomol. Chem.* **2017**, *15*, 5503-5512. *(Cover Art Selection)*

b. Abouelhassan, Y.; Basak, A.; Hussain, Y.; **Huigens III, R.W.** "Identification of *N*-Arylated NH125 Analogues as Rapid Eradicating Agents against MRSA Persister Cells and Potent Biofilm Killers of Gram-Positive Pathogens." *ChemBioChem* **2017**, *18*, 352-357. *(Cover Art Selection)*
c. Abouelhassan, Y.; Zhang, P.; Ding, Y.; **Huigens III, R.W.** "Rapid Kill Assessment of an *N*-arylated NH125 Analogue against Drug-Resistant Microorganisms." *Med. Chem. Commun.* **2019**, *10*, 712-716. *(Invited article for themed collection, "Antimicrobial Resistance, 2019.")*

5. **"Complexity-to-Diversity" of natural products (adrenosterone) to address the lack of chemical diversity in screening libraries (postdoctoral at UIUC).** A significant amount of my postdoc was spent developing a new strategy to construct complex and diverse compounds from adrenosterone (a steroid) for high throughput screening efforts and drug discovery. This strategy utilized reactions that dramatically altered the complex ring system (i.e., ring cleavage, ring expansion) of available natural products with complex, fused ring systems to rapidly generate small molecules with a high degree of skeletal diversity and complexity. Adrenosterone contains three ketone/enone functional groups positioned in the A, C and D rings of the steroidal skeleton enabling efficient ring distortion to generate unique complex compounds.

    a. **Huigens III, R.W.**; Morrison, K.C.; Hicklin, R.W.; Flood Jr., T.A.; Richter, M.F.; Hergenrother, P.J. "A ring-distortion strategy to construct stereochemically complex and structurally diverse compounds from natural products." *Nature Chem.* **2013**, *5*, 195-202.

Complete List of Published Work in MyBibliography:
www.ncbi.nlm.nih.gov/pubmed/?term=Huigens+RW