BIOGRAPHICAL SKETCH

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NAME: Bou-Nader, Charles

eRA COMMONS USER NAME (credential, e.g., agency login): bounaderc2

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Saint-Joseph University, Beirut	BS	2011	General Chemistry
University Lille 1, Lille	MS	2013	Chemistry, Energy and Environment
University Pierre and Marie Curie, Paris	MS	2014	Molecular Chemistry at the Interface of Biology
University Pierre and Marie Curie, Paris	PHD	09/2017	Biochemistry, Molecular and Structural Biology

A. Personal Statement

My lab's expertise is in RNA-protein biochemistry and structural biology, specifically X-ray crystallography and cryo-electron microscopy (cryo-EM). Our research focuses on mechanistic understanding of R-loops and their role in genomic integrity and gene expression. While I am a new assistant professor, my collective graduate and postdoctoral work produced a number of high-impact publications, with over 13 first author publications and 17 unique RNA-protein structures. My new lab has made significant and fast progress on several RNA-protein complexes important for immunity relying mostly on cryo-EM. Nevertheless, the limited resources at Emory and in the Georgia area for high throughput screening of cryo-EM grids and high-resolution single particle cryo-EM data acquisition is a major bottleneck. Access to the resources at NCCAT is essential for the successful completion of our funded projects and to train my current graduate student Mrs Annalise Holland who seeks to become an expert in cryo-EM.

My role on this NCCAT proposal will be to provide the necessary resources and mentorship at Emory to support Mrs Annalise Holland in her sample preparation (LysRS, DNA-RNA hybrid, tRNA), in vitro reconstitution of the complexes and cryo-EM specimen preparation for the proposed research activities at NYSBC. This is part of a funded project by NIAID (R00AI169473) defining the principles of nucleic acid immunogenicity. Moreover, I will train Mrs Holland in grid preparation and grid clipping to enhance her experience when she goes on-site at NYSBC. I am currently training Mrs Holland in data processing using Relion and CryoSPARC and I anticipate she will be in an ideal position to gain the most out of her training at NYSBC while helping the RNA field move forward by solving novel structures.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 - Assistant Professor, Emory University, Atlanta, GA
2022 - 2023 Research Fellow (postdoctoral fellow), NIDDK - National Institutes of Health, Bethesda, MD
2017 - 2022 Visiting fellow (postdoctoral fellow), NIDDK - National Institutes of Health, Bethesda, MD
2014 - 2017 Graduate student, University Pierre and Marie Curie - College de France, Paris

Honors

2023 RNA Society/Scaringe Young Scientist Award, RNA Society
2022 NIDDK Director's Award - Scientific, NIDDK
2022-2025 K99/R00 Pathway to Independence Award, NIAID
2021 Member of the Early Career Reviewer Board, eLife

2020-2022	Nancy Nossal Fellowship award, NIDDK
2020-2021	Intramural AIDS Research Fellowship IARF award, NIH
2020	NIDDK Fellow's Abstract Competition, NIDDK
2020	Member of the Early Career Reviewer Board, Journal of Biological Chemistry
2020	Fellows Award for Research Excellence, NIH
2017-2022	Postdoctoral visiting fellowship, NIDDK intramural program
2014-2017	Graduate fellowship, University Pierre and Marie Curie

C. Contribution to Science

1. **Determined the molecular basis of tRNA modification by dihydrouridine synthases:** I combined biochemistry, X-ray crystallography, small-angle X-ray scattering, and nuclear magnetic resonance to show that the human dihydrouridine synthase 2 (Dus2) uses a double-stranded RNA binding domain (dsRBD) to recognize the tertiary structure of tRNA . This dsRBD positions the tRNA in the active site of Dus2 to reduce uridine into dihydrouridine in the D-loop, a region of the tRNA named after its high content in dihydrouridine. This integrative structural biology approach uncovered the first evidence for a dsRBD that specifically recognizes tRNA, in contrast to other known dsRBDs that bind to dsRNA. This unique selectivity of Dus2 for tRNA is attributed to an N-terminal extension in the dsRBD that expands its RNA-sensing properties beyond simple dsRNAs. Taken together, this work deciphered new principles of RNA-protein interactions. Additionally, I developed a mass spectrometry-based approach to answer the 30-year-old problem of the specificity of the three conserved prokaryotic Dus enzymes that modify distinct tRNA residues. This study was significant because it revealed the mechanism dictating the selectivity and non-redundant activities of these bacterial enzymes.

    a. **Bou-Nader C**, Barraud P, Pecqueur L, Pérez J, Velours C, Shepard W, Fontecave M, Tisné C, Hamdane D. Molecular basis for transfer RNA recognition by the double-stranded RNA-binding domain of human dihydrouridine synthase 2. Nucleic Acids Res. 2019 Apr 8;47(6):3117-3126.
    b. **Bou-Nader C**, Brégeon D, Pecqueur L, Fontecave M, Hamdane D. Electrostatic Potential in the tRNA Binding Evolution of Dihydrouridine Synthases. Biochemistry. 2018 Sep 18;57(37):5407-5414.
    c. **Bou-Nader C**, Montémont H, Guérineau V, Jean-Jean O, Brégeon D, Hamdane D. Unveiling structural and functional divergences of bacterial tRNA dihydrouridine synthases: perspectives on the evolution scenario. Nucleic Acids Res. 2018 Feb 16;46(3):1386-1394.
    d. **Bou-Nader C**, Pecqueur L, Bregeon D, Kamah A, Guérineau V, Golinelli-Pimpaneau B, Guimarães BG, Fontecave M, Hamdane D. An extended dsRBD is required for post-transcriptional modification in human tRNAs. Nucleic Acids Res. 2015 Oct 30;43(19):9446-56.

2. **Established the enzymatic mechanism of flavin-dependent methylation of RNA:** TrmFO and ThyX are two methyltransferases that do not use the common methyl donor S-Adenosyl methionine (SAM), but instead rely on flavin and folate to methylate U54 in the T-loop of tRNA or dUMP into dTMP critical for DNA synthesis, respectively. The catalytic mechanism for these important enzymes was debated and revised several times mainly due to the difficulty to isolate catalytic intermediates. To circumvent this, I devised a novel synthesis in strict anaerobic conditions to produce two postulated flavin-methylene iminium species. I reconstituted both TrmFO and ThyX with these new flavin species that sustained methylation activities in vitro. This breakthrough not only demonstrated for the first time a common catalytic mechanism used by flavin-dependent methyltransferases but also paved the way for new flavin-based synthetic compounds for biotechnological applications and drug design.

    a. **Bou-Nader C**, Stull F, Pecqueur L, Simon P, Guérineau V, Royant A, Fontecave M, Lombard M, Palfey B, Hamdane D. An enzymatic activation of formaldehyde for nucleotide methylation. Nature Communications. 2021 July 27; 12(1).
    b. **Bou-Nader C**, Pecqueur L, Cornu D, Lombard M, Dezi M, Nicaise M, Velours C, Fontecave M, Hamdane D. Power of protein/tRNA functional assembly against aberrant aggregation. Phys Chem Chem Phys. 2017 Oct 25;19(41):28014-28027.

c. **Bou-Nader C**, Cornu D, Guerineau V, Fogeron T, Fontecave M, Hamdane D. Enzyme Activation with a Synthetic Catalytic Co-enzyme Intermediate: Nucleotide Methylation by Flavoenzymes. Angew Chem Int Ed Engl. 2017 Oct 2;56(41):12523-12527.

d. Hamdane D, **Bou-Nader C**, Cornu D, Hui-Bon-Hoa G, Fontecave M. Flavin-Protein Complexes: Aromatic Stacking Assisted by a Hydrogen Bond. Biochemistry. 2015 Jul 21;54(28):4354-64.

3. **Defined the mechanistic basis for tRNA control of HIV virion biogenesis:** The major structural protein of HIV-1, Gag, is targeted to the plasma membrane of the infected cell by its N-terminal matrix domain (MA). A highly basic region (HBR) on the surface of MA specifically recognizes phosphatidylinositol 4,5-bisphosphate (PIP2), a lipid uniquely present at the plasma membrane. Binding of MA to lipids is critical to allow Gag anchoring at the plasma membrane and to trigger viral particle assembly required for infectivity. Moreover, MA was shown to bind to RNA since 1997 and host tRNAs are readily crosslinked to MA in infected cells. Nevertheless, it was unknown if tRNA recognition by MA was specific or merely driven by nonspecific charge complementarity and what was the biological role of this interaction for HIV replication. To fill this gap in knowledge, I solved the first co-crystal structure of HIV-1 MA bound to a host tRNA. This question remained unanswered primarily because of the refractory behavior of MA and RNA towards crystallization, which I overcame by rational engineering of both the RNA and MA. The structure revealed that MA specifically recognizes the elbow region of the tRNA. The HBR engages mainly the phosphate backbone of both the D- and T-loop of tRNA. This shows that tRNA binding to MA occludes the lipid binding site. As a result, tRNA competes with PIP2 binding and thus inhibits Gag anchoring at the plasma membrane. Indeed, Gag mutants incompetent for tRNA binding localize prematurely at the membrane and show reduced replication efficiency. Overall, this work revealed how HIV-1 co-opts host tRNAs to delay Gag localization at the plasma membrane important for virion genesis and optimal infectivity.

   a. **Bou-Nader C**, Zhang J. Rational engineering enables co-crystallization and structural determination of the HIV-1 matrix-tRNA complex. STAR Protocols. 2022 March; 3(1):101056.

   b. **Bou-Nader C**, Muecksch F, Brown J, Gordon J, York A, Peng C, Ghirlando R, Summers M, Bieniasz P, Zhang J. HIV-1 matrix-tRNA complex structure reveals basis for host control of Gag localization. Cell Host & Microbe. 2021 September; 29(9):1421-1436.e7.

4. **Uncovered the structural basis of R-loop recognition by the S9.6 antibody:** Interested in the growing and exciting R-loop biology, I set out to address how DNA-RNA hybrids found in R-loop structures can act as antigens for the S9.6 antibody. This is important since the S9.6 antibody is widely used to map R-loops in the genome and yet it is unknown how specific S9.6 is for DNA-RNA hybrids over other nucleic acids, whether S9.6 has sequence preferences and how this antibody recognizes R-loops. To address these questions, I solved the first co-crystal structure of S9.6 free and bound to a DNA-RNA hybrid. The structure uncovered that S9.6 recognizes three consecutive 2′-hydroxyl groups on the RNA strand and interacts with six consecutive nucleotides on the DNA strand. I also determined that S9.6 has a robust preference for DNA-RNA hybrids (~160-fold) over dsRNA or dsDNA but exhibits a strong bias for GC-rich hybrids. Collectively, this study not only established a novel strategy for the specific recognition of DNA-RNA hybrid but also provided insights into the immunogenicity of duplex nucleic acids.

   a. Wei E, **Bou-Nader C**, Perry M, Fattah R, Zhang J, Leppla S, Bothra A. S9.6 Antibody–Enzyme Conjugates for the Detection of DNA–RNA Hybrids. Bioconjugate Chemistry. 2023 April 17; 34(5):834-844.

   b. **Bou-Nader C**, Bothra A, Garboczi DN, Leppla SH, Zhang J. Structural basis of R-loop recognition by the S9.6 monoclonal antibody. Nat Commun. 2022 Mar 28;13(1):1641.

5. **Established the principles of PKR inhibition by viral RNA structures:** dsRNAs are strongly immunogenic because they are recognized by pattern recognition receptors that trigger innate immune responses. For instance, PKR is an essential kinase that is activated by viral dsRNAs to shutdown global translation to curb the infection. In the constant arms race between viruses and host, some viruses have evolved non-coding RNAs that inhibit PKR's antiviral activity. Despite the critical function of PKR for innate immunity, it remains unknown how seemingly similar RNA structures can exert opposite effects on this kinase. I leveraged my knowledge of dsRBDs and the crystal structure of the adenovirus VA-I RNA, newly solved in the Zhang lab, to propose a mechanism of PKR inhibition by the adenovirus VA-I RNA. I

demonstrated that the dsRNA region formed by co-axial stacking between the 23-bp apical stem of VA-I and a strictly conserved tetrastem was both necessary and sufficient for PKR inhibition. This work identified the core effector of VA-I for PKR sequestration and most recently I solved the cryoEM structure of PKR bound to VA-I (manuscript in preparation). These insights from the VA-I RNA structure beg the question of whether the strategy used by adenovirus to inhibit PKR is shared with other viral inhibitors.

To address this question, I have focused my attention on the full-length HIV-1 TAR RNA that was suggested to inhibit PKR, although this remained ambiguous due to reports of TAR-induced activation of PKR. Using highly purified components, I have meticulously established that HIV-1 TAR inhibits PKR and that the frequently neglected TAR lower stem is critical for this function. To understand how full-length HIV-1 TAR inhibits PKR, I have solved the first crystal structure of the complete TAR RNA (manuscript in preparation). This revealed an intriguing case of convergent evolution between HIV and adenovirus that use similar RNA elements containing imperfect ~24-27 bp dsRNA segments to sequester PKR in its inactive state. I also solved most recently the structures of the TAR RNA bound to four distinct drug-like compounds. Overall, this work not only uncovered the mechanism of PKR inhibition by viral RNA structures but also shed light on novel principles of RNA-targeting by small molecules to inform the design of compounds to perturb RNA structures.

a. **Bou-Nader C**, Zhang J. Structural Insights into RNA Dimerization: Motifs, Interfaces and Functions. Molecules. 2020 Jun 23;25(12).

b. Hood IV, Gordon JM, **Bou-Nader C**, Henderson FE, Bahmanjah S, Zhang J. Crystal structure of an adenovirus virus-associated RNA. Nat Commun. 2019 Jun 28;10(1):2871. PMC6599070.

c. **Bou-Nader C**, Gordon JM, Henderson FE, Zhang J. The search for a PKR code-differential regulation of protein kinase R activity by diverse RNA and protein regulators. RNA. 2019 May;25(5):539-556.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/charles.bou-nader.1/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Holland, Kiana

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

POSITION TITLE:

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Northwest Vista College, San Antonio, TEXAS	AS	07/2020	Biology
Texas State University, San Marcos, TEXAS	BS	05/2023	Biochemistry and Microbiology

A. Personal Statement

Almost losing my mother to breast cancer changed my life and motivated me to pursue a career in STEM. Her diagnosis changed the rest of my life, and I wish it were as simple as wanting to find a cure to breast cancer, but, in reality, her diagnosis pushed me towards research, giving me a direction for my drive. As a black woman in STEM and the first of my family to go to graduate school, I quickly learned that being successful looked different from my counterparts. I have to “put on” for my whole community and represent them every minute of every lecture, at every presentation and conference. Perfection is what’s expected of me when the color of my skin naturally counts against me.

My drive landed me two of the most impactful opportunities of my early career, an NSF REU at my home institution, Texas State University, and a NIH-URISE fellowship. It was because of those two opportunities that I was introduced to the PhD track and formally began undergraduate research. Through the REU, I began my work in Dr. Xiaoyu Xue’s lab, where he focuses on DNA damage repair pathways. The project involved investigating the regulation of Mms21 SUMO E3 ligase function by the Smc5/6 components. My role in the project, over the summer, was to purify proteins of the Smc5/6 complex starting with Nse5/6. I later stayed in his lab and continued my work on purifying the Smc5/6 complex and its SIM (SUMO interacting motif) mutants, for SUMOylation in vitro assays.

The NIH-URISE fellowship allowed me to resign from the two jobs I maintained for most of my undergraduate experience and get personalized mentorship on PhD applications. I gained unique opportunities such as presenting a poster at American Biomedical Research Conference for Minoritized Scientists (ABRCMS) and giving a flash talk at the University of Texas Fall Undergraduate Research Symposium (UT- FURS) at UT Austin where I won the Best Speaker award. With my focuses set on academics, research, career development, and outreach for the American Society for Biochemistry and Molecular Biology (ASBMB) chapter at my school, I was accepted into four promising schools for my PhD: Georgia Tech University, Cornell University, Johns Hopkins University, and Emory University.

I selected Emory University because the Genetics and Molecular Biology program (GMB) emulated all the values I was looking for in a school. Here they value student advocation, recognize and respect diversity, prioritize collaborative leadership, and invest in student success regardless of the career path. In my first year at Emory I’ve received the Centennial Fellowship, I have become an Ambassador for Laney Graduate School Emory Diversifying Graduate Education (LGS-EDGE), and I have selected my mentor for my graduate studies. Going into graduate school my research interest was in cellular maintenance of genomic integrity and gene regulation with a focus on biochemical approaches which is why I chose the lab of Dr. Charles Bou-Nader. The lab's main research interests are in R-Loops that contribute to genomic instability and require strict regulation to maintain proper gene expression. Despite the importance of R-loops in biology, we have poor mechanistic understanding of how they form and are recognized by proteins to exert positive or negative impacts on genomic integrity. Motivated to fill this gap in knowledge, I aim to learn cryogenic electron microscopy (cryo-EM) to visualize how R-loops and their byproducts contribute to human health and diseases. My current research and the focus of my PhD is to define how human LysRS binds to viral DNA-RNA hybrids to catalyze

the formation of a small molecule called Ap4A. This moonlighting function of LysRS goes beyond its canonical role in translation and is an integral part of the innate immune response during mast cells activation and retroviral infections.

From this perspective, I have recently started learning cryoEM by working with my PhD advisor and the cryoEM core at Emory. I saw the process of preparing cryoEM grids and clipping them and even had the opportunity to screen them on an Arctica using EPU. While this initial training gave me promising preliminary data and solidified in my mind the different steps needed in cryoEM, I am seeking additional hands-on experience to become proficient and independent at preparing cryoEM grids and manipulating a TEM while also learning the best practices. Therefore, I am applying for a Grid Preparation and Screening (GPS/GUP3) access to go onsite at NCCAT. This unique opportunity will not only allow me to be become an expert in cryoEM grid preparation, but will also help me identify ideal conditions to visualize LysRS bound to a viral DNA-RNA hybrid. This will be a major milestone in my PhD and will advance the field forward by determining the mechanism of Ap4A synthesis by LysRS to control the innate immune response.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

Honors

2023	Outstanding Biochemistry Senior Award, Texas State University Department of Biochemistry
2023	Centennial Scholars Fellowship, Emory Laney Graduate School
2022	Recognition of High Achievement Award, Texas State University Department of Biochemistry
2022	Chemistry and Biochemistry Catalyst Award, Texas State University Department of Biochemistry

C. Contribution to Science

1. During my time as an undergraduate, I received an REU at my home institution Texas State University where I began working in Dr. Xiaoyu Xue's lab. His lab focuses on DNA damage repair pathways and my project during that summer and my final year as a senior was centered around the Smc5/6 complex. The Smc5/6 complex is found in the SUMOylation process, modifying several DNA repair proteins like Sgs1-Top-Rmi1 and Pol2. Unlike other structural maintenance of chromosome proteins in the same family, Smc5/6 contains a SUMO E3 ligase called Mms21 and it is always found in tandem with this complex. Whether other components of the 8 subunit Smc5/6 complex will affect Mms21 E3 ligase activity remains unknown. During this experience I helped develop a protocol to purify 2 out of the 8 subunits of Smc5/6, Nse5 and Nse6, along with the SIM (SUMO interacting motif) mutant. I used a tandem affinity purification strategy (Ni- and GST-affinity) coupled to a size exclusion chromatography (SEC) to purify both the wild type and mutants of Nse5 and Nse6. I confirmed the purity of my protein through SDS-PAGE. This research will contribute novel insights into DNA damage pathways that can be linked to diseases like cancer. I presented my research over this project at the symposium following my REU (see 1, below), at the American Biomedical Research Conference for Minoritized Scientists (ABRCMS) (see 2, below). I also gave a talk at the University of Texas Fall Undergraduate Research Symposium (UT- FURS) hosted by UT- Austin (see 3, below) and at the Dean's Advisory Council Meeting (see 4, below).

    1. Holland, K. A., Zhu, X., Xue, X. "Purifying Smc5/6 subunits and mutants to further investigate its E3 function". CheMIE REU Symposium, Texas State University, San Marcos, TX, August 5, 2022. (poster).
    2. Holland, K. A., Zhu, X., Xue, X. "Regulation of Mms21 SUMO E3 ligase activity by components of the Smc5/6 complex". ABRCMS, Anaheim, CA, November 2022. (poster).
    3. Holland, K. A., Zhu, X., Xue, X. "Regulation of Mms21 SUMO E3 ligase activity by components of the Smc5/6 complex". UT-FURS, UT-Austin, Austin, TX, September 24, 2022. (Talk). Awarded Best Presentation in session.
    4. Holland, K. A., Zhu, X., Xue, X. "Regulation of Mms21 SUMO E3 ligase activity by components of the Smc5/6 complex". Dean's Advisory Council Meeting, Texas State University, San Marcos, TX, December 02, 2022. (Talk).