**BIOGRAPHICAL SKETCH**

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Santiago-Frangos, Andrew

eRA COMMONS USER NAME (credential, e.g., agency login): asantia3

POSITION TITLE: Postdoctoral Researcher

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 |
|---|---|---|---|
| University of Leicester, Leicester, Leicestershire | BS | 04/2012 | Biochemistry |
| Johns Hopkins University, Baltimore, MD | PHD | 05/2018 | Biology |
| Montana State University, Bozeman, MT | Postdoctoral | NA | Molecular Biology / Biophysics |

**A. Personal Statement**

After graduating from the University of Leicester with highest honors, I pursued my Ph.D. at Johns Hopkins University in Dr. Sarah Woodson's laboratory. As a graduate student I developed several biophysical assays designed to understand the role of Hfq (host factor for Q-beta phage replication) as an RNA chaperone. As a doctoral student I established or maintained diverse collaborations with experts in bacterial regulatory RNAs, protein structure prediction and structural biology, namely with Dr. Susan Gottesman, Dr. Jeffrey Gray and Dr. Ben Luisi. Each of these scientists has contributed to my professional development and their input has helped enrich my understanding of the field. In the course of this work, I showed that the disordered C-terminus of Hfq acts as a nucleic acid mimic to regulate the RNA binding and RNA annealing activities of the structured core. Collectively, this work resulted in seven papers (including five first-author publications), an award for best thesis and invitations to present my work at two international conferences. I also honed my teaching and mentoring skills as a teaching assistant for Biochemistry classes and labs, for which I was given the Victor Corces Teaching award. Additionally, I volunteered to help supervise many undergraduate, masters and Ph.D. rotation students. I plan to continue mentoring students during my postdoctoral career and I admire Dr. Wiedenheft's commitment to mentoring. Dr. Wiedenheft is the founder and director of the "Montana Wild Virus Hunt", an infectious disease workshop designed to engage rural high school students, with a focus on Native American communities. I helped teach the workshop this year and I look forward to similar opportunities in the future. In addition to developing my skills as a mentor and instructor, my primary motivation for coming to the Wiedenheft lab is to expand on my expertise in protein-nucleic acid interactions by studying the impact of DNA modifications on the process of new spacer acquisition in the Type I-F CRISPR-Cas system. The proposed project will strengthen my training in microbiology, protein biochemistry, and provide me with new expertise in structural biology and single-molecule techniques. To enhance my training and to expand my professional network, Dr. Wiedenheft and I have developed a team of collaborators and co-mentors. Dr. David Rueda from Imperial College London in the UK, has agreed to help me execute the single-molecule experiments outlined in this proposal. I am confident that I have assembled a team of mentors that will help me achieve my long-term plan to establish an independent career and use a combination of single-molecule and traditional biochemistry approaches to study protein-nucleic acid interactions.

**B. Positions and Honors**

**Positions and Employment**

2007 - 2007 Lab Intern, La Jolla Bioengineering Institute, San Diego, CA
2009 - 2009 Lab Intern, Cyprus Institute of Neurology and Genetics, Nicosia, CY
2010 - 2011 Industrial Placement, GlaxoSmithKline, Stevenage, UK
2011 - 2012 Senior Research Thesis, University of Leicester, Leicester, UK
2012 - 2018 Graduate Researcher, Johns Hopkins University, Baltimore, MD
2018 - Postdoctoral Researcher, Montana State University, Bozeman, MT

**Other Experience and Professional Memberships**

2015 - 2018 Member, RNA Society
2016 - *Ad hoc* reviewer for *EMBO J, Mol Cell, NAR, PLOS One*

**Honors**

2012 BSc awarded with First Honors, University of Leicester
2014 Victor Corces Teaching Award for Biochemistry, Johns Hopkins University
2015 Poster Award for Outstanding RNA Research, RNA Society
2016 Travel Award, RNA Society
2018 Saul Roseman Award for Outstanding Research in Biochemistry, Johns Hopkins University
2018 Nominated - Harold M. Weintraub Graduate Student Award, Johns Hopkins University
2019 Postdoctoral Fellow of the Life Sciences Research Foundation, Simons Foundation

**C. Contribution to Science**

**1. A disordered protein domain destabilizes binding of RNA to a structured domain.**

Many bacteria transcribe small regulatory RNAs (sRNAs) to regulate the expression of stress response genes and virulence factors. Bacterial sRNAs act by base-pairing with a complementary sequence in the target mRNA, either sequestering the ribosome binding site or rendering it more accessible for translation. In *E. coli* and many other bacteria, the RNA chaperone Hfq accelerates and stabilizes sRNA-mRNA base pairing. Hfq assembles into a homo-hexameric ring with three RNA binding surfaces: the rim, the top, and the bottom of the ring. Basic rim residues anneal RNAs. The structured hexameric-ring of Hfq is surrounded by disordered C-terminal domains (CTDs). Disordered domains are widespread in RNA and DNA–binding proteins, but their functions are difficult to study and are typically ignored. This is certainly true for Hfq and function of the CTDs remained unclear for over two decades. My work showed that the CTDs of Hfq inhibit non-specific RNA interactions with the basic rim of the core and promote the release of annealed dsRNA product. Therefore, the CTDs accelerate Hfq turnover, allowing a given Hfq ring to quickly bind to and anneal the excess of sRNA–mRNA pairs present in the cell. Additionally, I showed that the CTDs are necessary for kinetic competition between sRNAs for Hfq, enabling Hfq to rapidly search through RNA substrates in the cell. In collaboration with Dr. Kavita Kumari and Dr. Daniel Schu in Susan Gottesman's lab, we showed that the CTDs establish a hierarchy of sRNAs *in vivo*, such that some sRNAs accumulate more than others. Hfq has a central role in RNA metabolism, therefore understanding the role of the disordered CTDs in Hfq–RNA interactions are important for understanding bacterial pathogenesis and advancing synthetic biology.

a) **Santiago-Frangos A**, Kavita K, Schu D, Gottesman S, Woodson S. C-terminal domain of the RNA chaperone Hfq drives sRNA competition and release of target RNA. *Proceedings of the National Academy of Sciences*. 2016 October 11; 113(41):E6089-E6096.

b) Panja S, Małecka EM, **Santiago-Frangos A**, Woodson SA. Quantitative analysis of RNA chaperone activity by native gel electrophoresis and fluorescence spectroscopy. *Methods Mol Bio*. In Press

**2. Acidic disordered protein domains can mimic nucleic acids.**

The above study revealed the function of *E. coli* Hfq's CTD in RNA-protein interactions. However, the mechanism by which the CTD inhibited non-specific RNA binding and promoted dsRNA product release were unknown. Similarly, it was unclear whether the rapidly evolving CTDs of other bacterial Hfqs performed a similar function. Therefore, I set out to determine the mechanism by which the CTD modulates RNA–protein interactions, which enabled us to predict whether autoregulation occurs in other bacterial Hfqs. Sequence analysis highlighted an enrichment of acidic residues in CTD tips of Hfqs active in RNA annealing. Additionally, I used fluorescence anisotropy to demonstrate that the acidic CTD tips interact with the basic rim of Hfq. Crucially, the rim residues most important for CTD binding were also most important for RNA annealing, as determined by stopped-flow spectroscopy. Furthermore, RNAs competed against CTDs for the basic rim. My results supported a model in which acidic CTD tips mimic nucleic acids and compete for RNA binding sites on the Hfq ring. Furthermore, in a recent collaborative effort I have shown that the auto-regulatory role of the CTD is conserved from gammaproteobacterial Hfqs (*E. coli*) through to alphaproteobacterial Hfqs (*Caulobacter crescentus*). This observation suggests that the large sequence diversity of Hfq CTDs serves to tweak its auto-inhibitory strength. These

CTD sequence adjustments may occur to accommodate the acquisition of new sRNAs and the subsequent establishment of a new interconnected regulatory network. Evolution of a generalized, flexible, substrate mimic, like Hfq's disordered CTD, may be common in proteins that interact with diverse substrates and must recycle on a fast timescale.

a) **Santiago-Frangos A** †, Fröhlich KS †, Jeliazkov JR, Małecka EM, Marino G, Gray JJ, Luisi BF, Woodson SA, Hardwick SW. Caulobacter Crescentus Hfq Structure Reveals a Conserved Mechanism of RNA Annealing Regulation. *Proceedings of the National Academy of Sciences*. 2016 October 11; 113(41):E6089-E6096.

b) Woodson SA, Panja S, **Santiago-Frangos A**. 2018. Proteins that chaperone RNA regulation. *Microbiol Spectrum* 6(4):RWR0026-2018. doi:10.1128/microbiolspec.RWR-0026-2018.

c) **Santiago-Frangos A**, Woodson S. Hfq chaperone brings speed dating to bacterial sRNA. *Wiley Interdisciplinary Reviews: RNA*. 2018 July; 9(4):e1475-.

d) **Santiago-Frangos A**, Jeliazkov J, Gray J, Woodson S. Acidic C-terminal domains autoregulate the RNA chaperone Hfq. *eLife*. 2017 August 09; 6:-.

† Indicates co-1st authors.

### 3. Acidic residues increase the selectivity of a basic RNA binding site.

In my first publication, I examined the role of highly conserved acidic residues that flank the basic rim active site of Hfq, in RNA-protein interactions. At first glance it seemed perplexing that negatively charged residues would be so highly conserved in a protein that must interact with a highly negatively charged substrate. I used a combination of Electrophoretic Mobility Shift Assays (EMSAs), fluorescence anisotropy, tryptophan quenching, and stopped flow spectroscopy to show that acidic residues flanking the arginine-rich active sites limit binding of off-target RNAs to Hfq, increasing the selectivity of Hfq's chaperone function.

a) Panja S†, **Santiago-Frangos A**†, Schu D, Gottesman S, Woodson S. Acidic Residues in the Hfq Chaperone Increase the Selectivity of sRNA Binding and Annealing. *Journal of Molecular Biology*. 2015 November; 427(22):3491-3500.

### 4. High-throughput techniques may improve the biophysical characteristics of biopharmaceuticals.

As an undergraduate at the GlaxoSmithKline Biopharm R&D department, I applied my growing knowledge of molecular biology and protein biochemistry to improve the stability of therapeutic antibodies. Many potentially therapeutic proteins are discarded late in the development pipeline due to poor stability. We sought to increase the number of successful candidate biopharmaceuticals by instituting mechanisms in early development stages that would pre-select for protein stability, along with high target affinity. In collaboration with another scientist, Susannah Ford, I designed phage display techniques to select therapeutic antibodies with improved stabilities and developed a protocol to measure their stabilities in a high-throughput and small-scale manner. Antibodies which arose from my selection procedure tended to be more stable than those generated from default protocols.

a. **Santiago-Frangos A**, Ford S. Therapeutic antibodies with improved stabilities can be enriched during early product development. *GSK Biopharm R&D National Meeting*; 2011; Stevenage, United Kingdom.

b. **Santiago-Frangos A**. Enrichment of thermodynamically stable therapeutic antibodies during early product development. *Year in Industry Dissertation*. 2011.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1jqzaknVtsaYNO/bibliography/55270912/public/

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

**Scholastic Performance**

| YEAR | COURSE TITLE | GRADE |
|---|---|---|
| | UNIVERSITY OF LEICESTER | |
| 2008 | Molecular Biochemistry | 68 |
| 2008 | Macromolecules in Action | 69 |
| 2008 | Cell and Developmental Biology | 71 |
| 2008 | Genes | 65 |
| 2009 | An Introduction to Physiology | 81 |
| 2009 | Environmental and Evolutionary Biology | 63 |
| 2009 | Microbiology | 85 |
| 2009 | I.T. and Numeracy Skills for Biologists A | 65 |
| 2009 | I.T. and Numeracy Skills for Biologists B | 72 |
| 2009 | Animal and Plant Diversity | 69 |
| 2009 | Study and Communication Skills | P |
| 2009 | Animal and Plant Physiology | 70 |
| 2009 | Chemistry for Biologists | 67 |
| 2009 | Microbiology I | 62 |
| 2009 | Microbiology II | 68 |
| 2009 | Gene Expression & Regulation | 60 |
| 2009 | Metabolic Regulation | 66 |
| 2010 | Molecular Machines | 61 |
| 2010 | Molecular Cell Biology | 65 |
| 2010 | Research Skills | 74 |
| 2010 | Bioinformatics of Genes | 72 |
| 2010 | Year in Industry | P |
| 2011 | Year in Industry | P |
| 2011 | Cancer Cell and Molecular Biology | 68 |
| 2011 | Virology | 79 |
| 2012 | Microbial Biotechnology | 80 |
| 2012 | Protein Complexes: From Cells to Molecules | 76 |
| | JOHNS HOPKINS UNIVERSITY | |
| 2012 | Responsible Conduct in Research | P |
| 2012 | Advanced Molecular Biology | A |
| 2012 | Advanced Cell Biology | A |
| 2012 | Graduate Biophysical Chemistry | A+ |
| 2013 | Genomes & Development | B+ |
| 2012 | Proteins & Nucleic Acids | B+ |
| 2013 | Introduction to Computing in Biology | A |
| 2014 | Topics in Biochemistry | A |
| 2016 | RNA | A- |

Approximate conversions of University of Leicester undergraduate module scores to US GPAs, according the Fulbright commission, are as follows: 70-100 % = 4.0, 65-69 % = 3.7, 60-64 % = 3.3. The year in industry and scientific ethics courses are graded as P (pass) or F (fail).

**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: Hunt, Clay

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

POSITION TITLE: Postdoctoral Researcher

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 |
|---|---|---|---|
| Montana State University, Bozeman, MT | BS | 05/2006 | Mechanical Engineering |
| Montana State University, Bozeman, MT | PHD | 11/2017 | Materials Science |
| Montana State University, Bozeman, MT | Postdoctoral | 02/2021 | Cryo-EM |

**A. Personal Statement**

After graduating from Montana State University, I went to Tucson, AZ to pursue a career. During that time, I realized I wanted to further my education, and returned to Montana for that purpose. As a graduate student in the lab of Dr. Stephen Sofie, I contributed to the understanding of sintering in ceramics and solid oxide fuel cells. As a doctoral student, I established a collaboration with Dr. John Neumeier, an expert in the field of materials science; Dr. Lisa Davis, an expert in mathematical modeling, Dr. Sarah Codd, a leading scientist in nuclear magnetic resonance imaging (NMRI), and Dr. Chris Organ, a renowned contributor in the field of evolution. Each of these individuals have shown me a different facet of science, and academics. The influence of each of these amazing individuals has impacted me greatly. In the course of this work, I developed the discovery of synergistic sintering aids for cubic zirconia, developed a method to quantify as a function of time the degradation rates of solid oxide fuel cells, wrote an explicit function that explains the density dependence of the activation energy of a sintering ceramic, and derived a thermodynamic model explaining why small particles of metal in an electro-catalyst do not degrade when their surface is contaminated with small amounts of a metal oxide. Collectively, this work resulted in six publications (including three first-author publications). I also had the opportunity to teach and mentor as a teaching assistant for materials science classes and labs, and a MATLAB programming lab. Additionally, I worked to mentor several undergraduate students. Finally, I volunteered as a taekwondo instructor for the university-offered taekwondo class, as well as the taekwondo club. During my time with the Wiedenheft lab, I have had the opportunity to develop a mentoring relationship with one of Prof. Wiedenheft's graduate students. I have also taken the opportunity to continue my involvement with the university taekwondo program. As the instructor of both the university-offered taekwondo class and the taekwondo club, I have the opportunity to encourage critical, evidence-based thinking in the practice of punching and kicking. With the Wiedenheft lab, I have found myself in two distinct roles. In one role, I am developing cryo-EM image-processing capabilities, and am sharing my limited understanding of mathematics, and physics to help my biology colleagues understand the form and function of electron microscopy and image processing. In the other role, I am very much a student of the biologists with whom I work. The learning I am experiencing in this role is unprecedented. I have stepped outside of my background field, and into the realm of biology. I have found new ways to communicate any understanding I have, and am constantly learning both new ways to think about what I thought I understood, and the ways of a fundamental science. My primary motivation for coming to the Wiedenheft lab is to expand on my understanding of electron microscopy, and to assist in the establishment of a cryo-EM facility in Bozeman, MT. The proposed project will strengthen my training in cryo-EM data acquisition, and image processing.

**B. Positions and Honors**

**Positions and Employment**

2010 - 2017 Graduate Researcher, Montana State University, Bozeman, MT
2017 - 2018 Materials Engineer, Glacigen Materials LLC, Bozeman, MT
2018 - 2019 Adjunct Professor, Montana State University, Bozeman, MT
2019 - Postdoctoral Researcher, Montana State University, Bozeman, MT

**Other Experience and Professional Memberships**

2016 - *Ad hoc* reviewer for *MRX*

**Honors**

2010 Awarded Benjamin Fellowship, Montana State University

**C. Contribution to Science**

**1. Rate of Degradation Quantification for Solid Oxide Fuel Cells.**

A solid-oxide fuel cell (SOFC) is a device made from metal oxides that harvest electrical energy from the bonding of hydrogen to oxygen in the formation of water. This reaction gives off two electrons per formed water molecule that can be used to do electrical work. The reaction ionizing the $H_2$ molecule on the anode side of the SOFC is catalyzed by nickel metal. Because the nickel metal is close to its melting point at SOFC operating temperatures, the finely dispersed nickel particles used to catalyze $H_2$ ionization tend to agglomerate. As this agglomeration occurs, SOFC performance decreases. Many studies have been undertaken to decrease both total degradation, and the degradation rate of the SOFC. However, no method of quantifying the instantaneous degradation rate of an SOFC existed. I developed an algorithm that fit SOFC data to a sum of differentiable functions. The time derivative of the fit of the SOFC data was then directly evaluated, and quantified.

c) **C. D. Hunt**, M. S. Zachariasen, D.R. Driscoll, S.W. Sofie, "Degradation rate quantification of solid oxide fuel cell performance with and without $Al_2TiO_5$ addition" International Journal of Hydrogen Energy 2018

d) Welander, Martha. Zachariason, Marley. Sofie, **Hunt, Clay**. Stephen. Walker, Robert. "Operando Studies of Redox Resilience in ALT Enhanced NiO-YSZ SOFC Anodes" Journal of the Electrochemical Society. 2018

**2. Thermodynamic model explaining the inhibition of nickel.**

The above study revealed a dependence of the SOFC degradation rate on the quantity of aluminum oxide added to the anode of the SOFC. SOFC anodes with aluminum oxide were studied with scanning electron microscopy, atom probe tomography, and electron force microscopy. A typical anode of a SOFC is made of a mixture of nickel metal that has been finely dispersed among yttria-stabilized zirconia (YSZ) particles. The regions of the SOFC anode where nickel metal particles are touching the YSZ particles, and are also touching the anode atmosphere are the only region of the cell where the SOFC reaction that forms water and electricity can take place. Thus, the agglomeration of nickel during SOFC operation degrades the functionality of the SOFC. During SOFC operation, the nickel is hot enough that it flows almost like water on wax paper: small beads of water or nickel metal coalesce into large beads of water or nickel metal to form a structure with less surface area than the finely dispersed water or nickel metal. These studies revealed that small nickel particles with even smaller aluminum oxide particles on the surface of the small nickel particles did not migrate to form large structures. Rather, dimples formed on the surface of the small particles decorated with even smaller aluminum oxide particles. I developed a thermodynamic model based on the idea that a dimpled sphere has a greater surface area than a smooth sphere of the same size. The energy of a surface is directly proportional to the surface area. Thus, increasing the surface area of a small nickel particle is thermodynamically forbidden. This forms the basis for the thermodynamic model that explains the arrest of catalyst particle migration when the metal particles are decorated with even smaller metal-oxide particles.

e) D. Driscoll, **C.D. Hunt**, D. Perea, S.W. Sofie, "Diffusion Caging: Thermodynamic Arrest of Ostwald Ripening," -Under Review

**3. Explanation of why activation energy of a sintering ceramic appears to change with density.**

Sintering is a diffusion process by which the molecules, atoms, or ions composing the powder particles of a powder compact move around to bind the powder particles together. This phenomenon occurs exclusively below the melting temperature of the sintering material, and most commonly occurs in snow. Sintering is often employed to process technical ceramics such as yttria-stabilized zirconia, and aluminum oxide because the melting temperature of these materials is prohibitively high. During sintering in a crystalline structure, an atom (ion or molecule) moves from one equilibrium in a crystal lattice to another equilibrium position in a crystal lattice. Statistically, this motion is driven by the minimization of energy. The new equilibrium position of the atom that has diffused during sintering is lower in energy than the equilibrium position the atom previously occupied. During sintering, a powder compact densifies. This densification is a result of the centers of the powder particles moving together. In other words, densification takes place on the scale of the powder particles; not on the atomic scale. During densification, little change occurs to the atomic structure of the material. However, the field of sintering in materials science talks at great lengths about how the apparent activation energy changes with density. The work I did on this subject resulted in an explicit relationship between the activation energy of volume diffusion (the energy barrier an atom must overcome to diffuse from one equilibrium position to another equilibrium position in a crystal lattice), the activation energy of grain-boundary diffusion (the energy barrier an atom must overcome to diffuse from one equilibrium position to another equilibrium position in a grain boundary region) to sintered density, and sintering temperature.

b) **C. D. Hunt**, K. Newhouse, D.R. Driscoll, S.W. Sofie, "Explicit definition of apparent activation energy Part I: Separation of sintering mechanisms" -**Under review**

c) **C. Hunt**, L Davis, A. Parker, K. Newhouse, S. Sofie, "Explicit definition of apparent activation energy Part II: Data simulation and predictive model " **Under review**.

All Mentioned Work Available Upon Request: klayhunt@gmail.com

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

**Scholastic Performance**

| YEAR | COURSE TITLE | GRADE |
|---|---|---|
| | MONTANA STATE UNIVERSITY | |
| 2010 | Modern Optics | A |
| 2010 | Partial Differential Equations II | A |
| 2010 | Continuum Mechanics | A |
| 2010 | Advanced Engineering Analysis I | A |
| 2010 | Theory of Magnetic Resonance Imaging I | A |
| 2011 | Advanced Engineering Analysis II | A |
| 2011 | Transport Phenomena | A |
| 2011 | Advanced Ceramics | A |
| 2011 | Quantum Mechanics II | A- |
| 2011 | Mathematical Physics I | A |
| 2012 | Novel Materials for Physics and Engineering | B+ |
| 2012 | Statistical Mechanics | B |
| 2012 | Thermodynamics | A |
| 2012 | Condensed Matter Physics I | A |
| 2013 | Classical and Statistical Thermodynamics | A |
| 2014 | Research and Methods in Engineering | A |
| 2015 | Advanced Materials Science I | A |
| 2017 | Elastic and Inelastic Analysis I | A |

Grades reported on standard A – F grade scale