BIOGRAPHICAL SKETCH

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NAME: Kirchdoerfer, Robert Nicholas

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

POSITION TITLE: Assistant 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 Wisconsin, Madison	B.S	05/2006	Genetics and Biochemistry
The Scripps Research Institute	Ph.D.	05/2012	Biophysics
The Scripps Research Institute	Post-Doctoral	07/2019	Biophysics

A. Personal Statement

Research in my laboratory focuses on combining biochemical and structural biology approaches to illuminate mechanisms of virus life cycles, extending from viral RNA replication to viral entry. In particular, we study coronavirus RNA synthesis through the recombinant expression of viral protein subunits and *in vitro* reconstitution of protein-protein and protein-RNA complexes as well as assessment of viral enzyme activity. My structural biology background encompasses both X-ray crystallography and single-particle cryo-electron microscopy and is strengthened by broad skills in biophysical and biochemical characterization of protein-protein and protein-RNA interactions. My virology experience extends from negative-strand RNA viruses such as influenza and Ebola viruses to positive-strand RNA viruses such as SARS-coronavirus promoting a great breadth in perspective for generating new hypotheses and drawing parallels among viruses.

B. Positions and Honors

Positions and Employment

2006-2012 Graduate Student,
Dept. of Molecular Biology, The Scripps Research Institute, La Jolla, CA
2012-2016 Post-doctoral Fellow,
Dept. Immunology and Microbial Sciences, The Scripps Research Institute, La Jolla, CA
2016-2019 Post-doctoral Fellow,
Dept. Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA
2019- Assistant Professor
Department of Biochemistry, University of Wisconsin-Madison, Madison WI

Professional Memberships

2012-2021 Member, American Society for Virology

C. Contributions to Science

Topic, Major need or question, major findings, impact of contributions,

1. After entering host cells, viral genomes must be replicated and transcribed to produce new virions. Viral RNA genomes are dependent on virus encoded machinery for this activity. The viral RNA synthesis machineries are composed of multi-protein complexes where protein subunits interact with each other, substrates and RNA for their full activity. My studies have used both X-ray crystallography and cryo-electron microscopy to examine viral protein-protein and protein-RNA interactions. This has included studies of the influenza ribonucleoprotein complex that distinguished transcriptional and replicational states. I also examined interactions among Ebola virus proteins contributing to viral nucleocapsid assembly and function. In addition, my recent single-particle cryo-electron microscopy studies have produced the first structure of a polymerase complex from coronaviruses and illuminated interactions of the viral polymerase with viral co-factors. These studies are shaping the way these virology fields are interpreting sequence and functional data and have shifted paradigms for mechanisms of viral protein function.
    a. **Kirchdoerfer RN**, Ward AB (2019). Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat Comm. 10: 2342.
    b. Chien M, Anderson TK, Jockusch S, Tao C, Kumar S, Li X, Russo JJ, **Kirchdoerfer RN**, Ju J (2020). Nucleotide Analogues as Inhibitors of SARS-CoV-2 Polymerase. BioRxiv https://doi.org/10.1101/2020.03.18.997585
    c. **Kirchdoerfer RN**, Abelson DM, Li S, Wood MR, Saphire EO (2015). Assembly of the Ebola Virus Nucleoprotein from a Chaperoned VP35 Complex. Cell Rep. 12:140-149.
    d. Moeller A, **Kirchdoerfer RN**, Potter CS, Carragher B, Wilson IA (2012). Organization of the influenza virus replication machinery. Science. 338:1631-4.

2. To infect host cells, enveloped viruses must fuse their exterior membranes with those of the cell releasing their genomes into cells to initiate infection. For coronaviruses, the process of recognizing host receptors and carrying out the membrane fusion process is facilitated by the viral spike protein. Using cryo-electron microscopy, I determined the first structure of a human coronavirus spike. This structure demonstrated the interfolded arrangement of spike receptor-binding domains and how they cover and stabilize the spike fusion machinery. This first structure was instrumental in the design of novel mutations to stabilize the pre-fusion spike proteins of other betacoronaviruses and allowed us to determine structures of spikes of SARS- and MERS-coronavirus spikes. This also included an analysis of SARS-coronavirus spike bound to its host receptor, ACE2 which binds only a particular conformation of the spike receptor-binding domain. These studies have illuminated not only spike structures but are also producing blueprints for creation of pre-fusion spike proteins to be used as vaccine immunogens.
    a. **Kirchdoerfer RN,** Wang N, Pallesen J, Wrapp D, Turner HL, Cottrell CA, Corbett KS, Graham BS, McLellan JS, Ward AB. Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. Sci Rep. 2018 8:15701**.**
    b. Pallesen J, Wang N, Corbett KS, Wrapp D, **Kirchdoerfer RN**, Turner HL, Cottrell CA, Becker MM, Wang L, Shi W, Kong WP, Andres EL, Kettenbach AN, Denison MR, Chappell JD, Graham BS, Ward AB, McLellan JS. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. Proc Natl Acad Sci U S A. 2017 114:E7348-E7357
    c. **Kirchdoerfer RN**, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL, Corbett KS, Graham BS, McLellan JS, Ward AB. Pre-fusion structure of a human coronavirus spike protein. Nature. 2016 531:118-21.
    d. **Kirchdoerfer RN**, Bhandari M, Martini O, Sewall LM, Bangaru S, Yoon KJ, Ward AB. Structure and immune recognition of the porcine epidemic diarrhea virus spike protein. Structure. 2021 29:385-392.e5.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1jMrGuPZEIIAI/bibliography/9393999/public/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Anderson, Thomas K

eRA COMMONS USER NAME (credential, e.g., agency login): TANDERSON23

POSITION TITLE: Graduate Student Research Assistant

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin-Madison	BS	09/2015	05/2019	Biochemistry
University of Wisconsin-Madison	PhD	09/2019	05/2025	Cellular and Molecular Biology

A. Personal Statement

At the end of my freshman year at the University of Wisconsin-Madison, I joined the lab of Professor Hazel Holden (research described in **Section C**). I spent three years in Dr. Holden's lab, my first time experiencing an academic lab and learning biochemistry research techniques. This research experience initiated my pursuit of a career in science. Through further coursework and a study-abroad research experience, I established my **career goal of a tenure track faculty position at a prominent research institute.** In this academic career, I plan on **studying the replication of RNA viruses utilizing structural biology.** My extensive and diverse research experiences have prepared me for my career path.

Under the research guidance of Dr. Holden and in-lab training by senior scientist Dr. James Thoden, I became proficient at standard molecular biology techniques, recombinant protein expression, and X-ray crystallography. My research focus was to study enzymes involved in the biosynthesis of molecules (i.e., L-serine, novel carbohydrates) from pathogenic bacteria. Bacteria studied included *Mycobacterium tuberculosis, Francisella tularensis, Clostridium difficile,* and *Botulinum pertussis.* I was able to isolate distinct stages of enzymatic mechanisms by solving the structures of enzymes with cofactors and substrates or products. The overarching goal of my work was to publish high-resolution structures to be used for the development of targeted antibiotics towards the bacteria studied. My time in the Holden lab provided me with many invaluable lessons. One of the most important is that structural biology provides mechanistic details of enzymes that can be used to develop drugs targeting those enzymes.

During the spring semester of my junior year, I enrolled in an upper-level biology course, "The Biology of Viruses." Throughout the semester, I became interested in the unique and complicated mechanisms by which viruses infect and replicate within their hosts. This course was taught by two professors, Dr. Andrew Mehle and Dr. Paul Friesen. While enrolled in their class and the years that followed, I met with each professor for multiple one-on-one meetings. In these meetings, we would discuss topics in virology, their lab's research, and careers in virology (their own and in general). After this course, I wanted to find a research path that would allow me to tackle questions in virology while applying my background in structural biology.

Through my participation with the SCORE program ran by Professor Marvin Wickens at UW-Madison, I was placed in the lab of Dr. Alfredo Castello at Oxford University for two months during the summer of 2018. In the Castello lab, I studied host RNA binding proteins with dynamic activity during vaccinia virus infection. I learned several new laboratory techniques to isolate and identify these proteins of interest. Expanding my experience from recombinant enzymes, like in the Holden lab, to active viral infections was an essential step in preparing me for my graduate research project and career beyond that.

Since joining the lab of Dr. Robert Kirchdoerfer in December of 2019, I have been conducting research on coronaviruses, with cryo-electron microscopy being a primary tool used in my research. My work focuses on the

mechanisms of coronavirus RNA replication. Particularly, I focus on viral proofreading, nucleoside analog interactions, and viral replication vesicles. Proofreading is unique to coronaviruses (and immediately related virus families) among RNA viruses and permits them to have large RNA genomes. Beyond maintaining genomic identity, I am interested in how proofreading is responsible for coronaviruses ability to evade treatment by commonly used antivirals, termed nucleoside analogs, during infection. Utilizing cryo-EM and *in vivo* studies with a model coronavirus, I aim to tackle my research aims involving analyses of coronavirus proofreading, nucleoside analog efficacy, and replication vesicle structure and dynamics. Performing this work on multiple coronaviruses will allow the design of broad-acting antivirals, aiding in preventing future coronavirus pandemics.

Beginning my research career with X-ray crystallography showed me the knowledge that can be gained by structural biology. After two months of *in vivo* virus work at Oxford, I found that studying virus replication mechanics was an exciting area where I could apply my structural biology work on virus mechanics. I found a way to expand my structural background and apply it to studying viruses in the Kirchdoerfer lab. I am confident that in this setting, I can develop my structural biology expertise and virology interests by using cryo-EM to study coronavirus replication. Expanding my work to using MHV in tissue culture allows me to test hypotheses developed *in vitro*. My diverse research experiences, excellent educational background, and desire to grow as a researcher and virologist will allow me to accomplish my proposed project while providing critical details to the field of corona-virology.

B. Positions and Honors

Positions and Employment

2016 - 2019 Undergraduate Research Assistant, University of Wisconsin-Madison, Dept. of Biochemistry
2018 Undergraduate Summer Research Fellow, Oxford University, Dept. of Biochemistry
2019 - Graduate Student Research Assistant, University of Wisconsin-Madison, Dept. of Biochemistry

Other Experience

2017 - 2019 Project CRYSTAL Mentor, Dept. of Biochemistry (University of Wisconsin-Madison)
2018 Undergraduate Teaching Assistant, General Chemistry (University of Wisconsin-Madison)
2018 - 2019 Undergraduate Tutor (Chemistry, Organic Chemistry, Biochemistry)

Honors

2015 - 2018 University of Wisconsin-Madison Deans List (5/5 semesters eligible)
2017 University of Wisconsin-Madison College of Agriculture and Life Sciences Centennial Academic Merit Award
2017 University of Wisconsin-Madison Sophomore Research Fellowship (declined award)
2017 University of Wisconsin-Madison Biochemistry Undergraduate Summer Research Scholarship
2018 University of Wisconsin-Madison William F. Rank Study Abroad Scholarship
2018 University of Wisconsin-Madison Biochemistry Study Abroad Scholarship
2018 University of Wisconsin-Madison Hilldale Undergraduate Research Fellowship
2019 University of Wisconsin-Madison Graduate with Academic Distinction

C. Contributions to Science

Undergraduate Research:

During my time in the lab of Dr. Hazel Holden I studied a conserved aminotransferase involved in the biosynthesis of L-serine from three different pathogenic bacteria: *Mycobacterium tuberculosis, Clostridium difficile, Botulinum pertussis,* and *Francisella tularensis*. For this work, I cloned enzyme expression constructs, purified enzymes from *E. coli* expression systems, crystallized proteins via hanging-drop vapor diffusion, and solved enzyme structures with X-ray crystallography. For each enzyme studied, I solved two high-resolution structures (<2Å): one wildtype enzyme forming an internal aldimine with cofactor PLP and one mutant enzyme with PLP and the enzyme's product phospho-serine in the active site. This work (manuscript in preparation by Holden Lab) highlighted active site conservation across bacteria with implications on enzymatic mechanisms. For another project I took part in at the end of three years in the lab, I expressed, purified, crystallized, and solved the structure of two enzymes involved in the biosynthesis of D-*glycero*-L-*gluco*-Heptose from *Campylobacter jejuni.* This work was done as a collaboration project and resulted in two publications, for both of which I am the

second author. The Holden lab continues to use X-ray crystallography to study enzymes involved in biosynthesis from pathogenic bacteria, hopefully leading to the development of bacteria-specific antibiotics.

1) Huddleston, J. P., **Anderson, T. K.**, Girardi, N. M., Thoden, J. B., Taylor, Z., Holden, H. M., & Raushel, F. M. (2021). Biosynthesis of d-*glycero*-l-*gluco*-Heptose in the Capsular Polysaccharides of *Campylobacter jejuni*. *Biochemistry*, *60*(19), 1552–1563. https://doi.org/10.1021/acs.biochem.1c00183

2) Huddleston, J. P., **Anderson, T. K.**, Spencer, K. D., Thoden, J. B., Raushel, F. M., & Holden, H. M. (2020). Structural Analysis of Cj1427, an Essential NAD-Dependent Dehydrogenase for the Biosynthesis of the Heptose Residues in the Capsular Polysaccharides of *Campylobacter jejuni*. *Biochemistry*, *59*(13), 1314–1327. https://doi.org/10.1021/acs.biochem.0c00096

Graduate Research:

My current research position is with Dr. Robert Kirchdoerfer in the Department of Biochemistry and the Institute of Molecular Virology. Our lab studies two aspects of coronaviruses utilizing cryo-EM as an essential tool to do so: coronavirus RNA replication machinery and spike proteins. My project involves the analysis of the coronavirus proofreading machinery and viral replication vesicles. In particular, I am studying how the proofreading machinery interacts with the viral polymerase and how the polymerase functions inside these vesicles. The polymerase+proofreading complex enables coronaviruses to proofread during RNA synthesis while also evading treatment by commonly used antiviral nucleoside-analogs. This proofreading is unique to coronaviruses and its immediate virus family members among RNA viruses. I also study coronavirus replication compartments, termed DMVs, in infected cells utilizing cryo-ET. Thus far, I have helped develop expression protocols for several viral enzymes (polymerase and proofreading enzymes) from multiple coronaviruses and viral cofactors for these enzymes. I have developed *in vitro* RNA assays to confirm expected enzymatic activities and ensure that enzymes used for cryo-EM are biologically relevant. I study multiple coronaviruses, including SARS-CoV-2 the causative agent of COVID-19, in an effort to identify conserved mechanisms of replication that could be targeted by broad-acting anti-coronavirus drugs. My expertise in coronavirus recombinant protein purification led to several collaborations during the ongoing COVID-19 pandemic. As a result, I am a co-author on three recent publications (second author on one) on SARS-CoV-2 structural biology and viral polymerase and nucleoside-analog interactions.

1) Chien, M., **Anderson, T. K.**, Jockusch, S., Tao, C., Li, X., Kumar, S., Russo, J. J., Kirchdoerfer, R. N., & Ju, J. (2020). Nucleotide Analogues as Inhibitors of SARS-CoV-2 Polymerase, a Key Drug Target for COVID-19. *Journal of proteome research*, *19*(11), 4690–4697. https://doi.org/10.1021/acs.jproteome.0c00392

2) Altincekic, N., Korn, S. M., Qureshi, N. S., Dujardin, M., Ninot-Pedrosa, M., Abele, R., Abi Saad, M. J., Alfano, C., Almeida, F., Alshamleh, I., de Amorim, G. C., **Anderson, T. K.**, Anobom, C. D., … Schlundt, A. (2021). Large-Scale Recombinant Production of the SARS-CoV-2 Proteome for High-Throughput and Structural Biology Applications. *Frontiers in molecular biosciences*, *8*, 653148. https://doi.org/10.3389/fmolb.2021.653148

3) Tonelli, M., Rienstra, C., **Anderson, T. K.**, Kirchdoerfer, R., & Henzler-Wildman, K. (2021). ^{1}H, ^{13}C, and ^{15}N backbone and side chain chemical shift assignments of the SARS-CoV-2 non-structural protein 7. *Biomolecular NMR assignments*, *15*(1), 73–77. https://doi.org/10.1007/s12104-020-09985-0

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

YEAR	COURSE TITLE	GRADE

UNIVERSITY OF WISCONSIN-MADISON UNDERGRADUATE

YEAR	COURSE TITLE	GRADE
2015	Advanced General Chemistry	A
2015	Intro Communications: Inquiry & Exposition	B
2015	A Wisconsin Experience Seminar	A
2015	3rd Semester Calculus – Functions of Multivariable	AB
2016	Asian American History: Settlement and Belonging	A
2016	General Microbiology	AB
2016	Intro Organic Chemistry	A
2016	Intermediate Organic Chemistry	A
2016	First Semester Italian	DR
2016	Second Semester Introductory Biology	A
2016	Principles of Microeconomics	A
2016	Techniques in Ordinary Differential Equations	A
2017	Fundamentals of Analytical Chemistry	A
2017	Film and Media Studies: Film Comedy	AB
2017	Politics Around the World	AB
2017	Intro Organic Chemistry Lab	T
2017	General Biochemistry I	A
2017	Elementary Matrix & Linear Algebra	B
2017	General Physic II	AB
2018	General Biochemistry II	A
2018	Biology of Viruses	A
2018	History of Mathematics	B
2018	General Virology – Multiplication of Viruses	A
2018	Protein & Enzyme, Structure & Function	A
2018	Cell and Regenerative Biology Stem Cell Seminar	A
2018	Biophysical Chemistry (honors)	A
2019	Topics in Medical Biochemistry	A
2019	Biochemical Methods (Biochemistry Capstone Lab Course)	A
2019	Intro to Probability and Math Stats I	A

UNIVERSITY OF WISCONSIN-MADISON GRADUATE

2019	Responsible Conduct of Research	S
2019	Carcinogenesis and Tumor Cell Biology	A
2020	Eukaryotic Molecular Biology	A
2020	Intro to Biostatistics	A
2020	Advanced/Special Topics in Biochemistry: Biomolecular Chemistry	A

Courses at the University of Wisconsin-Madison are graded on an A – AB – B – BC – C – D – F scale. Any grade of a D or above is passing. First Semester Italian was dropped before the end of the semester resulting in a Dropped (DR) on the transcript. Intro Organic Chemistry Lab was taken at a UW system school, UW-Marathon County, over summer 2017, resulting in a T for transfer grade. Responsible Conduct of Research was graded as Satisfactory (S) or Unsatisfactory (U).