At the Chancellor’s Honors Banquet, which in observance of social distancing only took place in spirit this year, two GST students were recognized for their Extraordinary Professional Promise in research. Shantanu Shukla (Myles lab) was recognized for his pioneering work applying neutron- and X-ray based techniques to investigate protein structure. Connor Cooper (Parks lab) stood out for his skills in computational chemistry, which he has applied to proteins from transmembrane efflux carriers responsible for bacterial multidrug resistance to mercury metabolizing enzymes, and many more.

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Nice going and congratulations!

AWARDS AND RECOGNITIONS WON BY GST STUDENTS

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Nice going and congratulations!
GT: Hi Mircea, I hear you have started a line of work on the novel coronavirus. With your ongoing projects on the oral microbiome, a respiratory virus is perhaps not such a stretch? What drew you to the topic? Was it your previous expertise, colleagues who approached you, your creative idea, a sense of obligation to do something?

MP: I guess a little bit of each. As a postdoc I worked in an HIV lab and I made and handled infectious virus in a BSL-3 lab, so I have a bit of familiarity with nasty viruses. I also happened to have ongoing work on setting up a nanobody discovery platform in the lab. Nanobodies are single-chain synthetic proteins that are derived from the antigen-binding region of antibodies, usually the heavy chain. We use them for fishing out uncultured bacteria from the microbiome. But nanobodies can also be developed against viruses, so I decided to apply it to the emerging Covid-19 pandemic. Part of this nanobody discovery would also be developing assays for virus detection. I combined it with testing existing antibodies against the SARS-CoV-2 virus, as well as testing the antibodies that the human body produces against SARS-CoV-2 (serological assays).

GT: Why nanobodies?

MP: Nanobodies are more stable than regular antibodies. Because they are also much cheaper to produce in large quantity, they could be implemented not only for clinical detection but also for environmental testing. And different nanobodies can be specific enough to potentially discriminate between different viral strains. Nanobodies may even be developed for therapeutics, not only for detection; they may synergize with other drugs that inhibit viral attachment to cells. In addition, because they can be produced at a large scale, nanobodies may be a good approach to enrich viruses from highly diluted samples (e.g. water, surfaces) and enable testing for viral presence in the environment.

GT: How do you screen for nanobodies with biological activity?

MP: I am using a nanobody yeast display developed by a team at Harvard, that has been proven to work in discovering highly selective nanobodies against many membrane proteins. The approach can be quite rapid and lead to nanobodies against multiple viral proteins. We have identified several nanobody candidates against the SARS-CoV-2 spike protein and are in the process of purifying them and testing their characteristics. I have also tested commercial antibodies against the Spike protein and developed an ELISA (immunological assay) to detect antibodies against SARS-CoV-2 in serum samples from patients.

GT: Thank you for sharing your ongoing project with our readers. Good luck!

SELECTED RECENT PUBLICATIONS BY GST STUDENTS


Dr. Constance Bailey is an Assistant Professor in the Department of Chemistry. Her expertise is in the broad area of synthetic biology, specifically the reverse and forward engineering of biochemical pathways for the synthesis of polyketides, some of which function as antibiotics, pharmaceutical intermediates or specialty chemicals. The polyketide synthases are a fascinating class of enzymes because they have an unusual modular multidomain structure whereby a single protein can catalyze multiple sequential synthetic steps. Dr. Bailey came to UT after postdoctoral training in the lab of Professor Jay Keasling, one of the world leaders of the field of metabolic engineering. She has already worked with GST students.

Dr. Benjamin Parker is an Assistant Professor in Microbiology. He is utilizing a small insect, the pea aphid, as a model system to understand its surprisingly complex microbiome. Genetic variation in the pea aphid host opens the door to understand how various components of the microbiome, bacteria, fungi and viruses, interact. For example symbiotic bacteria can influence the aphid’s immune response against a pathogenic fungus. Meanwhile, infection with a virus can influence the aphid’s developmental decision to make wings! Dr. Parker was just selected as one of 22 Pew Biomedical Scholars by the Pew Charitable Trust, an impressive recognition of his contributions and potential for future success. Congratulations!

Dr. Andrew Steen is an Assistant Professor in Microbiology and in Earth and Planetary Science. He is an environmental microbiologist who focuses on the role of microbes in geochemical carbon and nitrogen cycling processes in aquatic and arctic environments. He does fieldwork in coastal estuaries, the open ocean, and the high arctic. He has already contributed in creative ways to advance one core mission of GST, that is to spread the expertise in computational and biostatistical data analytics. For example he has been teaching a course in the R programming language on several occasions.

Dr. Jianbin Wang joined the Department of Biochemistry & Cellular and Molecular Biology as an Assistant Professor in Spring 2020. His goal is to understand a dramatic case of genome reorganization, the programmed elimination of large tracts of DNA from the genomes of somatic cells. This occurs in a variety of biological taxa, such as certain nematodes and crustaceans. It constitutes a natural case of directed genome editing. His work on the somewhat neglected parasitic nematodes, which infest livestock, pets and humans, occupies a unique niche and represents an unusual angle for translational biomedical research on our campus. He is developing another model system for DNA elimination, the copepod Mesocyclops edax. Dr. Wang already succeeded in garnering independent NIH funding for his research as a principal investigator in his pre-faculty appointment at the University of Colorado, and just received notice that his NIH proposal at UT will be funded!

Khushboo Bafna - Conformational Sub-states and Dynamics in Human Ribonuclease Family, PhD Summer 2019 (Agarwal lab): Postdoc at Rensselaer Polytech Institute, Troy, NY, Montelione lab.

Aditya Barde - Studying the Plant-Microbe Interface of Populus Using Constructed Microbial Communities, MS Summer 2019 (Pelletier lab): Technologist, Sema4 Genetic Testing Lab, Stamford, CT.

Jennifer Childers - Investigating the functions of the plant-associated genus Variovorax in the Populus rhizosphere, MS Fall 2019 (Morrell-Falvey lab): Microbiologist, Bayer Crop Science, St. Louis, MO.

Adam Green - Computational discovery of new efflux pump inhibitors that target AcrA of the Escherichia coli AcrAB-ToLC efflux pump, PhD Fall 2019 (Smith lab): Solutions scientist at Dassault Systemes BIOVIA, Waltham, MA.

Sanjeev Dahal – Characterization of Diverse Mechanisms of Salicin Degradation in Populus Microbiome Isolates, PhD Fall 2019 (Pelletier lab): Postdoc at Queen’s U, Kingston, Ontario, Canada.

David Foutch - Network Analysis of Protein Structure Networks Upon Ligand Binding, MS Spring 2020 (Shen lab).

Manuel Ivan Villalobos Solis—Needles in a haystack of protein diversity: Interrogation of complex biological samples through specialized strategies in bottom-up proteomics uncover peptides of interest for diverse applications, PhD Summer 2020 (Hettich lab): Postdoc at ORNL.


Alfredo Blakeley-Ruiz - Extracting detailed metabolic information and connections from mammalian gut microbiomes via metaproteomics, PhD Summer 2020 (Hettich lab): Post-doc at North Carolina State University.

Connor Cooper - Computational Approaches to Understanding the Structure, Dynamics, Functions, and Mechanisms of Various Bacterial Proteins, PhD Summer 2020 (Parks lab): Continuing at ORNL.

Pawat Pattarawat - Formulation and evaluation of gemcitabine plus romidepsin + cisplatin combination for controlling tumors, PhD Summer 2020 (Hwa-Chain Wang lab): Continuing at the UT Vet School.

Best of luck to all GST alumni in jobs already begun or yet to be determined!
When Chinese scientists published the RNA sequence of the novel coronavirus, now called SARS-CoV-2, only weeks after discovering it as the source of a mysterious new type of pneumonia, the computational molecular biophysics group directed by Governor’s Chair Professor Jeremy Smith knew right away that a bundle of projects had just fallen into their lap. Any drugs that would some day inhibit the cellular entry and proliferation of the virus in human cells would surely be studied in great detail using the powerful supercomputing techniques that the team has long harnessed to understand other protein and carbohydrate assemblies. But how to invert the process and discover candidate molecules for such drugs? The team went to work right away. One attractive feature – arguably – of the coronaviruses is their large genome, the largest of all RNA viruses. A large genome translates into numerous encoded gene products. There are envelope proteins, which structure the surface of the virion and mediate cell entry, RNA polymerases and endoribonucleases that replicate and process the RNA, and phosphatases and proteases that process the original translation products made in the host cell. From our experience with the human immunodeficiency virus we know that combining a number of drugs against several disparate viral enzymes can provide a therapeutic value that none of the individual drugs can. How to find those elusive molecules?

Because of the fundamental groundwork laid by research on previous coronaviruses (some of which conducted at UT in Knoxville by the late David Brian) we know a thing or two about the workings of these viruses. The virus’s S protein is famous around the world because it forms the picturesque spikes sticking out from the spherical virus particle. It is the virus’s key to enter its host cells. Because the structure of S proteins is well known, Nicholas Smith in the group of Jeremy Smith at ORNL knew how to do the right thing: Another group had already predicted its structure using a technique known as homology modeling, based on evolutionarily conserved homologs of Spike. However, finding small molecules that might bind the S protein as tightly as the cheese to the pizza was not an easy task. This is where a second technique called ‘ensemble docking’ comes into play. The potential binding sites on a protein are highly dynamic on the scale of pico- to nanoseconds, a time scale that lends itself to computational simulation rather than experimental investigation. With ensemble docking, small molecules can be tested for their biophysically predictable affinity to the S protein, and not just the one or few conformations that were coincidentally caught in an X-ray crystal structure, but hundreds more. Because the conformations can potentially trap a drug candidate molecule leading to an inactive complex, as many as possible of these conformations should be tested out – ensemble docking instead of single docking. The Center for Molecular Biophysics was an early proponent of this approach. As Jeremy Smith explains, “we have so far searched for candidate binding partners for numerous drug target proteins relevant to other diseases. In each of the 16 cases so far, some of the small molecule ligands we predicted were later validated by experiment.”

By mid-February, within just three weeks of the original issue of the SARS-CoV-2 sequence, Smith’s team had set up the system and identified a roster of potential candidate molecules to bind. The resulting preprint publication has since been downloaded over 40,000 times from ChemRxiv. As Smith and his colleague Jerry Parks explain in a letter published in the June 4 issue of the New England Journal of Medicine, this is just a first step in the right direction.

How now to sift through millions and billions of molecules that organic chemistry makes imaginable? If the goal is to quickly find a drug that can be fast-tracked into clinical trials without having to worry about excessive side effects, then existing drug molecules are a clear winner, because their safety profile is known – at least for people not infected with Covid-19. Still, screening these thousands of molecules for their binding potential against hundreds of ensemble conformations for more than a handful of different coronavirus proteins is an enormous computational challenge. Between Jeremy Smith, and a team of experienced researchers and PhD students, who have all cut their teeth on more challenging molecular-dynamics simulations, the expertise is there to harness the leeway afforded by Summit and other goliaths of supercomputing. “Multiple drug candidates against multiple protein targets” is a challenge ideally suited to the massively parallel computing infrastructure in the Leadership Computing Facility at ORNL.

Smith and his team now work intensely with scientists at many other institutions large and small, a consortium of eight mainly regional institutions and national labs that anchors a network of collaborators at Harvard, IBM and Novartis, Google and Nvidia. Current GST students Rupesh Agarwal, Connor Cooper and Shawn Shen have turned into coronavirus researchers. Former GST faculty and students well known to those who follow GST affairs, including Sally Ellingson (University of Kentucky) and Jerome Baudry (University of Alabama Huntsville), are co-developing these techniques. And, remarkable even in the best of times, even the White House is paying attention. As described in a new preprint recently posted *, the consortium ensemble-docked libraries of thousands of small molecules against eight different SARS-CoV-2 encoded proteins, thus prioritizing their binding propensity.

Would even more computing power help? Definitely. And the computational molecular biophysics group demonstrates why and how. Already, computational advances have coaxed the Summit supercomputer to potentially dock a billion different molecules in less than a day. This advance opens the door to explore the ligand-binding potential of a universe of novel compounds, if only there were no other users competing for Summit’s attention. As new machines such as Frontier advance supercomputing from the peta- to the exa-scale, these new algorithms will become practical and accessible.

Not every compound that binds to a target will have a biochemical consequence let alone therapeutic activity; however, drugs that do

Governor’s Chair Professor Jeremy Smith directs the Center for Molecular Biophysics

Supercomputers Grease the Squeaky Wheel of Drug Discovery

* Growing Trends
There is also much room for developing machine-learning and artificial intelligence tools to make the most of the deluge of data, for example to predict the quantitative binding affinity of each potential ligand. Connor Cooper (GST PhD 2020) testifies how Covid-19 has accelerated the pace of science: "I will say one interesting thing has been learning how to deal with the rapid dissemination of new information and results in real time. For example, there were no SARS-CoV-2 X-ray or cryo-EM structures available in the Protein Data Bank in January, and now there are over 300 structures available."


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**Rupesh Agarwal** is about to graduate with a PhD from GST from the Center for Molecular Biophysics. The SARS-CoV-2 virus threw a curveball, leading him to segue into a new line of work in a hurry.

**GT: Thanks, Rupesh, for illustrating some of your experiences with ‘Growing Trends’. How did this get started?**

**RA:** After the work done by Jeremy (Smith) and Micholas (Smith) exploded in the news and social media around February, I asked Jeremy if I could join this project. This was just before the lockdown started. Soon, we started having regular video conference calls to bounce off ideas and finalize a strategy. Our goal was to come up with potential drugs that target SARS-CoV-2 viral proteins as quickly as possible, given the rate at which the pandemic was soaring, and still is. The initial methodology that we decided to use had three parts: 1) perform molecular dynamics (MD) simulations of different SARS-CoV-2 viral proteins to capture the dynamics of the proteins; 2) cluster the MD trajectories (i.e., different protein conformations generated from MD) and 3) perform virtual screening of libraries of small compounds (docking) to identify potential ligands, followed by ranking of the compounds. Simultaneously, teams at Argonne National Lab and ORNL have been using AI/ML (artificial intelligence and machine learning) to generate more robust clustering protocols to implement on MD trajectories. Omar Demerdash in our group has been leading efforts to re-score the small-molecule poses identified from docking to increase the success rate.

**GT: How did your previous expertise predispose you for this work?**

**RA:** As part of my dissertation, I have used similar methodologies to successfully identify novel inhibitors for two different therapeutically relevant protein targets. With this background, I decided to contribute to this project primarily as part of the docking team led by Jerome Baudry. With help from Micholas Smith and John Eblen, I implemented the pipeline that I created during my Ph.D. for SARS-CoV-2 and performed virtual screening on six different viral proteins. The identified compounds have been suggested for experimental validation and are currently being tested.

**GT: Any promising results to report at this point?**

**RA:** Our virtual screening predictions have been successful so far. Multiple compounds have shown expected inhibition during initial experimental screens, which is promising. Apart from these hits, we have now docked >1 billion small molecules to the main protease of SARS-CoV-2 using all of the Summit supercomputer in less than 24 hours, which is a big feat in itself and has not been achieved ever before. The results of this are currently being analyzed. This is an example how a lot of peripheral innovation is coming out of this project, which will open new areas for research in the future.

**GT: Do you want to highlight specific coworkers? Publications?**

**RA:** It is always fun to work with Micholas Smith, Jerome Baudry, and Loukas Petridis, with whom I have collaborated in the past on other projects. At the same time, it has been an awesome experience to get the opportunity to work with other scientists from ORNL like Ada Sedova, Josh Vermaas, David Rogers, and John Eblen and get the chance to learn about different aspects of supercomputing. Apart from the anchor paper, which was published recently with our protocol and results, I have also contributed to a technical paper on optimizing the docking protocol on the Summit supercomputer, which was recently accepted as a conference paper.


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**GT: Did you make new connections, find new collaborators?**

**RA:** These past few months have been a very unique experience compared to other projects because of the urgency of the work and the super-collaborative approach. I got a chance to talk and discuss my ideas with many people from different labs (national labs and universities) with varied expertise, which would not have been possible otherwise. I hope they remember me when I meet them in person! Through my involvement in Covid-19 research, I also had the opportunity to work with Life Science Tennessee, a non-profit organization, in their campaign to support biopharmaceutical research and innovation. As a part of this campaign, my letter-to-the-editor was published in the Knoxville News Sentinel newspaper.

Lastly, I would like to mention that the life of graduate students in GST (especially computational biophysicists like me) will not be the same, because our advisors now know the amount of work one can get done from home! **
The SARS-CoV-2 coronavirus has proven once again that the 21st century is the century of biology. In a practical and irrefutable display of evolution, a virus, which may well be of little significance in its original animal host, presumably bats, mutated into a formidable pathogen of humans. Managing to outfox the immune defenses of many, the virus hit the population-genetic equivalent of a jackpot, jumping into a species that not only represents a huge reservoir of potential individuals to infect but that is also networked across the globe to support exponential transmission for a long time.

AVA: Many reasons. First of all, RNA is the central molecule in my molecular biology lab, and arguably it is the central molecule in the cell. Who would have thought that our planet could ever contain too much RNA? Well, the SARS-CoV-2 coronavirus has a genome composed of RNA, not DNA.

FL: While UT’s campus was still discussing what to do, Oak Ridge National Lab had jumped in and decided to set up their own local testing lab. I was engaged in the planning of this testing capacity and contributed one of my PCR machines for this effort. The ORNL lab has now been screening ORNL personnel for several weeks to ensure a safe work environment. Also, the genetic testing technology to detect viral RNA, known as reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is being used routinely as a research tool in our labs.

AVA: Right. Well before the virus even reached Tennessee, one of my graduate students remarked “We know qPCR. Why don’t they just let us test ourselves?”

GT: Why did you think that ‘pooled testing’ would be the way to go?

AVA: In a nutshell, there was and still is no high-throughput technique that can detect the virus efficiently at negligible cost. At the same time, because the virus does not trigger strong symptoms in all those infected, one cannot simply wait for symptoms to appear before testing. In order to monitor and control the spread of the virus, it is essential to test non-symptomatic people as well. I think the hospitals who are now testing patients and health care workers before bringing them together for non-emergency procedures would attest to this. However, with current technology and resources, testing thousands of individuals of the campus population is only practical using pooling.

Finally, my research community used pooled sample screening strategies ~20 years ago to identify strains of plants that have mutations in specific genes. With careful preparation, these smart pooling strategies promised to make testing for the virus a lot cheaper and high-throughput.

GT: How did you go about it?

AVA: Back in March, I started to talk to a few colleagues on campus about the urgency to ‘do something’, use the expertise on campus to supplement the inadequate commercial testing enterprise. Tim Sparer (faculty in Microbiology) was already ahead of the curve, having started an actual project with local collaborators early on. At first, I believed that my ideas needed to resonate with funding agencies. I approached the National Science Foundation about a RAPID project (a grant program with a fast decision process), but this program had already spent nearly all its money within its first few weeks. Next, through NIM-BioS (the National Institute for Mathematical...
Gross and Heidi Goodrich-Blair threading this needle, several of us got talking and planned a project. By May we knew that our proposal was not funded – sour grapes: it would have been a modest amount of funding anyway – but those of us who were motivated got to know each other better.

FL: Meanwhile, the UT administration decided to explore the merits of a testing campaign on campus. A large committee was put together, chaired by Governor’s Chair Professor Terry Hazen. To make a long story short, over the course of many Zoom meetings, it percolated that two testing modalities have merit. One is a wastewater testing campaign that Terry Hazen is now implementing. And the second is our pooled saliva testing campaign that Albrecht and I have been working on for the past 3 months.

GT: How will those two campaigns work together?

FL: Infected people shed the virus when they use the restroom and numerous studies have shown that viral RNA biomarkers can be detected in wastewater. In other words, monitoring effluent water from residential dwellings can inform if infected persons live in the building. If the analysis of wastewater collected from a building shows a spike in viral RNA biomarkers, we will respond by asking residents to provide saliva samples. For efficiency, 5 or 10 saliva samples will be pooled together and tested as a pool. If the pool is negative, nothing will happen. If the pool is positive, we will communicate the information to the Student Health Center. Next, the individuals in positive pools will be contacted for re-testing individually with a clinically approved test, followed by isolation and contact tracing as needed. In an ideal world, we would test everybody every day but this is simply impossible. We have to apply a smart strategy to identify clusters of infection early to prevent uncontrolled spread. The integrated wastewater and pooled saliva testing campaigns can help us to efficiently identify and hopefully control the virus on campus. Of course, we must rely on the cooperation of the student population, and we all have to play by the rules and make some (minor) sacrifices for the greater good.

GT: How far along are you? When will I be asked to donate a sample?

AVA: The campaign will focus on undergraduate students living in residential housing on campus, for the time being. The testing lab is essentially ready to start processing samples. A lot needed to be put in place. Before I mention a few steps, let me underscore how important it has been that everyone involved has been pulling in the same direction. For example, our surveillance testing campaign is neither ‘research’ nor ‘clinical testing’. Instead, it falls under ‘public health surveillance’. This was negotiated at the behest of our team and with critical input from Sarah Pruett in the Office of Research, between the Director of the Student Health Center, Dr. Spencer Gregg, the Chancellor’s office and the Knox County Health Department.

AVA: Also, we have been leaning heavily on the Office of Student Life to roll out our campaign to the student population on campus. For example, Jolyon Gray and his team were key in interfacing with programmers and making sure saliva samples would be tracked reliably but in a way that maintains data privacy. Jill Zambito did an amazing job setting up a large effort to distribute the sampling kits to the UT undergraduate student population. We would need another article to acknowledge everyone who not only helped out but was instrumental in getting us to this point.

FL: Yes, the logistics behind the saliva surveillance testing campaign are complicated, and it was fantastic to experience how various people interacted and collaborated to get us across the finish line. We have the surveillance testing capacity in place and its success now depends on you! When you do receive a request to donate a saliva sample, which is super easy to do, please do your part!

Staff of the Center for Environmental Biotechnology Cynthia Swift and Tingting Xu are programming a robot to perform RNA extraction.
Matthew Keller
graduated with a BS in Chemistry from UT Chattanooga in 2018 and recently earned an MS in Analytical Chemistry from the Chemistry department at UT Knoxville. His Master’s research was done with Dr. Campagna and Dr. Hettich and focused on metabolomic investigations. His academic interests include chemical biology and mass spectrometry. Although his degrees are in chemistry, Keller has always had a significant interest in biology, and in the GST department he hopes to continue to apply chemical measurement techniques to study biology.

Emily Smith
graduated from Virginia Tech with a B.S. in biological systems engineering in 2017. She has been working at Oak Ridge National Lab as a research assistant under the supervision of Wellington Muchero investigating a set of ancient genes with shared function in plants and animals. She is excited to continue learning new computational skills to apply to biological data sets during her graduate studies. Additionally, she enjoys baking and cooking for family and friends.

Benjamin Nordick
graduated with a BS in Molecular & Cellular Biology from the University of Illinois at Urbana-Champaign. In the lab of Dr. Susan Martinis, he used bioinformatics and molecular biology techniques to study splice variants of the human leucyl-tRNA synthetase. He has experience in software engineering and looks forward to developing computational tools to accelerate the understanding of the genome.

Katherine Ostrouchov
Fueled by surviving a protracted bacterial infection, Katherine has made it her life’s goal to help characterize the human microbiome in order to better diagnose and treat disease. She plans to work alongside cutting-edge scientists in the GST program to develop her skills as a researcher as she works to unravel a critical field in medicine that is not yet understood.

Margaret Spangler
graduated with a BS in Microbiology from UTK with a minor in Hispanic studies. During her undergraduate career she researched in a biochemistry lab studying a plant-specific lactic acid transporter and its role in stress tolerance. For the next year she participated in an internship at ORNL under Jessy Labbé, focusing on computationally and experimentally discovering antimicrobial peptides in a variety of fungi. She looks forward to continuing that work in the future and expanding her expertise in fungal genetics, with the intent of a career in fungal drug discovery. In addition to research, she enjoys spending time exploring the Knoxville area with her two pups. Go vols!