Welcome to the tenth issue of Results and Discussion, a newsletter sponsored by the Office of Biomedical Research Education and Training (BRET), that is devoted to highlighting the research accomplishments and activities of our Ph.D. graduate students and postdoctoral fellows.

2020. This has truly turned into an unprecedented year as we weather COVID-19, recover from the intense early March tornado devastating parts of Nashville, and participate in the journey to end racial injustice.

As we reflect on this past year in the BRET office, we note some of the new and positive developments. Last year, the ASPIRE Program’s Data Science Essentials module won second place in the 2019 Innovations in Research and Research Education Award program sponsored by the American Association of Medical Colleges (AAMC). Launched in 2018, the module includes a didactic eight-week introduction to data science, a nine-week section to build communication and networking skills, and a series of career case sessions led by professional data scientists and hosted on site at their organization. It was developed by Ashley Brady, Ph.D., Kim Petrie, Ph.D., and Kathy Gould, Ph.D., with support from a Burroughs Wellcome Fund Career Guidance for Trainees Award.

Outcomes Research for the BRET Office continues to flourish. Director Abigail Brown, Ph.D., presented about the work of the office at the recent AAMC GREAT meeting and the ever-expanding BRET trainee database, many years in the making. Dr. Brown will also be speaking at Harvard University soon about her approaches to trainee data collection and analysis. A summary of the alumni outcomes data can be viewed on the BRET website.

Big changes came to the Quantitative and Chemical (QCB) Program – formerly the Chemical and Physical Biology Program. Dr. Tina Iverson, Professor in the Department of Pharmacology, and Dr. Vito Quaranta, Professor in the Department of Biochemistry, have been named Director and Associate Director, respectively, of the QCB. They take over from Dr. Hassane Mchaourab, who served as Director since 2015. The QCB Program was established over 15 years ago and allows graduate students the opportunity to conduct research at the interface of biology and chemistry, physics, engineering, and/or mathematics.

The career development office added two new seminar series to the list of ASPIRE programming. First, the ASPIRE Bistro for PhD Students presents professional development topics in an informal atmosphere where discussion and questions are encouraged. The Bistro series complemented the successful ASPIRE Café for Postdoctoral Fellows. All trainees were invited to the second new series – ASPIRE Job Search – which provides information and resources relevant to trainees in an active job search. The ASPIRE Bistro, Café and Job Search series will continue virtually in the fall.

Recognizing the successes of the past year in biomedical research training is all the more special with the engagement and support of our alumni, who help us by generously giving their time and support to our efforts. We are thankful for all our partners!

Sincerely,

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Worms Show Some (Dendritic) Spine

By Danielle Kopke, Ph.D.

Andrea Cuentas-Condori grew up in Lima, Peru, and obtained her B.S. under the direction of Dr. Dionicia Gamboa at the Universidad Peruana Cayetano Heredia. Her interest in molecular biology led her to the Research Experience for Peruvian Undergraduates program in which Peruvian undergraduates are paired with lab internships. Cuentas-Condori was offered an internship at Vanderbilt University.

After her internship, she decided to pursue her Ph.D. at Vanderbilt and joined the lab of David Miller, III, Ph.D., Professor of Cell and Developmental Biology, who uses the roundworm C. elegans as a model organism to study neural circuits. Neurons are composed of axons that release information and dendrites that receive information. In vertebrates, most dendrites have short, local protrusions called spines, which are important functional components of neural circuits. Spine structure and density are regulated by neural activity and are strongly correlated with learning and memory. It was generally accepted that C. elegans do not have dendritic spines.

Cuentas-Condori’s work, published recently in eLIFE, challenges previous literature and demonstrates that two C. elegans motor neurons exhibit the hallmarks of dendritic spines. The first time she observed the fluorescent protrusions that resembled spines, she couldn’t believe her eyes.

However, dendritic spines were identified in fruit flies in 2009, so she thought “maybe C. elegans do have them, but we just missed them!”

Using super-resolution microscopy, she showed that the protrusions observed in the C. elegans motor neurons were enriched in the cytoskeletal protein actin and exhibited the characteristic structures previously reported for mammalian dendritic spines. As an independent method for observing the presence of spines, Cuentas-Condori and colleagues partnered with Drs. Mei Zhen and Ben Mulcahy at the University of Toronto to use electron microscopy to make a 3D reconstruction of a motor neuron. This enabled detection of 12 dendritic spines, along with the characteristic cytoplasmic organelles. Structurally, the protrusions were very similar to mammalian dendritic spines. What about functionally?

Calcium is a key signaling molecule that mediates neuronal activity and activity-dependent synaptic plasticity (i.e. the ability to strengthen or weaken a synaptic connection in response to neuronal activity). Using sophisticated genetic techniques, Cuentas-Condori and colleagues artificially stimulated presynaptic neurons in C. elegans and observed calcium fluctuations in the corresponding dendritic spines, suggesting that the spines are functional. Furthermore, they showed that the spines respond to activity-dependent signals. When the presynaptic side was continuously stimulated during development, spine density increased compared to unstimulated control worms.

Cuentas-Condori’s work shows that C. elegans can be used as a model to study spine growth and maintenance, both of which are crucial for brain health, in normal and diseased states. She had the pleasure of presenting her findings at the 22nd International C. elegans Conference and received many kudos.

As for her next steps, she says, “I am sure that I want to do a postdoc. I think that I want to stay within the C. elegans field because the community is really collegial.”

Learn More:
This March, on the cusp of University shutdowns surrounding the Coronavirus pandemic, the BRET Office of Career Development ASPIRE Program executed the first ever Mock Interview Day. Registered graduate students and postdoctoral fellows were matched based on career interests for four rotations with alumni and Vanderbilt faculty volunteers. For 25 minutes, the interviewer asked questions and recorded their evaluations in a rubric, measuring their first impressions, the trainees’ oral responses CV/resume-based questions, how they described their research experience, communication skills, and readiness. There was a brief time of feedback before the interviewees moved to a different rotation. Overall, the event was a huge success and one that the ASPIRE Program hopes to repeat in the future.

“I thought this event was absolutely fantastic! I received helpful and constructive feedback that helped me improve my interview skills. This exercise also provided me a confidence boost that was very helpful in the real interviews I had later.”

“A few of my interviews turned into more of a networking opportunity. They suggested other people I should reach out to!”

“Getting feedback from interviewers for the particular position was invaluable. This never happens in a real interview, and they were particularly keen to do this since it was arranged that way. This was much better than a mock interview with a friend or colleague who may be an expert in something but not necessarily for the type of job you are applying for.”
Over 65,000 people develop head and neck squamous cell carcinoma (HNSCC) each year, and the 5-year survival rate is less than 50%. These statistics highlight the importance of research performed by Vanderbilt University postdoctoral fellow Shinya Sato, Ph.D., in the lab of Alissa Weaver, M.D., Ph.D., Professor of Cell and Developmental Biology. Sato’s work, published in *JCI Insight*, has looked at a signaling pathway in HNSCC that leads to blood vessel formation in the tumors.

Sato originally aspired to be a pediatrician and completed medical school in Japan, but realized research was his greater passion. During his graduate studies at Nagoya City University in Japan, Sato became interested in cancer research, in part, because of the lack of effective therapies for advanced-stage cancer patients.

“It was very sad for me,” Sato says of the lack of effective therapies. “My dream was to cure these patients completely, but I realized it is very difficult to cure cancer. Now, my hope is to improve patient lifespan.”

His own interests in cancer angiogenesis, and the recommendation of a former Weaver lab member and fellow native of Japan, led Sato to move to the US to join the Weaver lab. His work has focused on extracellular vesicles (EVs), or particles that are released from cells and help cells communicate. In order to grow, tumors need a constant supply of oxygen and nutrients which they access by stimulating new blood vessel formation in a process known as angiogenesis. Researchers hypothesized that EVs may contribute to angiogenesis, but the mechanism by which this occurred was not understood.

To address this question, Sato and his colleagues performed a proteomic analysis of EVs purified from HNSCC cells. Their analysis identified several proteins in the ephrin receptor (Eph) family that had previously been shown to regulate angiogenesis and one of the Eph proteins, EPHB2, is overexpressed in some patients. This overexpression correlated with decreased patient survival.

Additionally, Sato’s study revealed that when HNSCC cells release EVs expressing EPHB2, they bind to ephrin-B2 on endothelial cells, resulting in phosphorylation of STAT3 and promotion of angiogenesis, likely through alterations in gene expression. Sato is optimistic about the potential use of drugs that would inhibit EPHB2, especially if used in combination with pre-existing therapies, but says there are some caveats to consider.

“EPHB2 is a good target for anti-angiogenesis therapy, but we should screen patients that would benefit from such a drug, since not every patient’s tumor expresses EPHB2.”

Sato’s postdoctoral work at Vanderbilt led to his current role as Chief Physician at Kanagawa Cancer Center Research Institute in Yokohama, Japan. While he was thrilled to accept the prestigious position, Sato says returning to Japan required adjustment for his family, especially his 5-year-old son who was at first sad to leave the US.

“He’s adjusting though. One month after returning to Japan, he’s back in his routine and enjoying Japanese sushi again.”

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Learn More:
How does one obtain post-graduate career training outside of a traditional postdoctoral fellowship? Allison Eberly, Ph.D., a recent Vanderbilt University graduate, chose to pursue a Clinical Microbiology Fellowship at the Mayo Clinic in Rochester, Minnesota. Eberly began her 2-year fellowship in July of 2019, and the first thing she learned was that no two days in the clinical laboratory are ever the same. While she spends most of her mornings in laboratory rotations learning how to isolate and identify microbes, the afternoons are more variable and consist of microbiology rounds, didactic sessions, and meetings. Eberly is also on call 24/7 every third week of her fellowship, which involves carrying around an actual pager and fielding calls from in-house providers and clients regarding test results.

Eberly fell in love with microbiology when she joined the laboratory of Dr. Maria Hadjifrangiskou, Ph.D., Associate Professor of Pathology, Microbiology and Immunology, to study biofilms. However, basic research wasn’t fulfilling Eberly’s desire to help people more directly and she knew she needed to pursue a career closer to the clinic.

After participating in the Clinical Laboratory Medicine ASPIRE Module offered through the BRET Office of Career Development, Eberly learned that as a clinical microbiologist, she could combine her altruistic nature and her love for microbiology. In her current role, Eberly gets to test patient samples, determine the cause of infection, and then quickly communicate the results to the care team. If current graduate students are considering a position as a clinical microbiologist, Eberly advises them to get exposure as early in their graduate training as possible. The clinical world operates very differently than might be expected, and a lab director must wear many different hats.

What is your favorite thing about microbiology?

If I had to pick one thing, I would say biofilms. Bacterial communities, especially in complex situations where multiple bacterial species are present in an infection, are fascinating to me and we have so much to learn!

Favorite laboratory rotation so far?

While parasitology was filled with the coolest specimens, I have to say I was surprised by how much I enjoyed the mycobacteriology and mycology lab. Getting to suit up with a respirator and go into the BSL3 lab space was certainly one of the highlights, along with TB susceptibility testing and tape preps from fungal cultures. Have you ever seen Alternaria [class of fungi] under the microscope?

What is your favorite aspect about your job?

I love the variety and the unknown, which is sometimes my least favorite aspect because it makes it hard to plan the day and week.

What do you want to do after your fellowship?

Ideally, I would like to be directing a clinical laboratory in a hospital that is affiliated with an academic research center.

What time do you wake up in the morning? Go to bed at night?

In graduate school, I was a night owl, but that doesn’t work so well in the clinical world! I try to get to bed by 11pm and wake up around 6am.
The ASPIRE Scholar Fund provides support for exceptional PhD graduate students and postdoctoral fellows to pursue experiential learning opportunities that furthers their career and professional development. This fund has recently been endowed! Join others who have made this dream a reality and give generously for the next generation of scientists:

https://medschool.vanderbilt.edu/career-development/giving/
A combined desire to discover the unknown and to learn microscopy ultimately propelled Claire Strothman into the field of microtubule dynamics. Her scientific journey started with a summer research internship at Vanderbilt University in the laboratory of Qi Zhang, Ph.D., Assistant Professor of Pharmacology, during which Strothman fell in love with benchwork and live-cell imaging.

“I was lucky to get this summer job and learn how amazing microscopy is,” she said.

Strothman entered the Vanderbilt Interdisciplinary Graduate Program in 2015 and joined the lab of Marija Zanic, Ph.D., Assistant Professor of Cell & Developmental Biology, to study the properties of microtubules, the main cytoskeletal polymers within cells. Microtubules are made of α/β-tubulin dimers, which assemble in such a way that either α or β is exposed at each end. This gives each end of a microtubule different properties. One end, the “plus” end, is more dynamic, adding or losing tubulin dimers more often and faster than the other end, the “minus” end.

In her recent *Journal of Cell Biology* article, Strothman used purified proteins and total internal reflection fluorescence (TIRF) microscopy to show that key differences between minus and plus ends are dependent on the distinct tubulin dimer binding kinetics at each end. Even though it had been established that minus ends inherently grow slower and disassemble less frequently than plus ends, the mechanisms underlying these differences had not been established.

First, Strothman looked at the effects of the protective GTP (guanosine triphosphate) cap size on microtubule stability. Tubulin dimers are GTPases, which means they can bind and hydrolyze GTP to GDP (guanosine diphosphate). If GDP-bound tubulin is exposed at either end by hydrolysis of GTP, this results in destabilization of the polymer and microtubule disassembly. Therefore, the presence of a larger GTP cap could explain the increased stability of minus ends. However, she showed that both ends, despite having distinct dynamics, have comparable GTP cap sizes.

Then, Strothman showed that minus ends depolymerize slower than plus ends primarily because tubulin dimers bind more tightly to the minus end. Next, she asked whether two proteins that bind microtubules, kinesin-13 MCAK (mitotic centromere-associated kinesin) and kinesin-14 HSET, alter minus end microtubule dynamics. MCAK is a microtubule depolymerase, whereas HSET walks along microtubules to their minus ends and concentrates there. She found that HSET stabilized minus ends from MCAK-induced depolymerization, indicating that HSET modulates tubulin dynamics specifically at the minus end.

Strothman’s work has provided insight into the intrinsic and extrinsic mechanisms that govern microtubule dynamics, which have implications in the context of cancer. For instance, HSET plays an important role in tumor cell division and is overexpressed in certain types of cancer.

“This means HSET has the potential to be an effective drug target for cancer treatment,” says Strothman.

Future studies in the Zanic group aim to determine how exactly HSET regulates minus-end dynamics. As for Strothman’s future, she hopes to combine her passions for science and art in her career. Apart from her artistic side, Strothman enjoys cooking and practicing yoga, as well as playing with her cute, freckled puppy Sheila.

“Sheila’s 6-month ‘gotchaversary’ is coming up,” she says excitedly.

**Learn More:**


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**Enlightening the “Dark Side” of the Microtubule: Minus End Stability and its Regulators**

By Cayetana Arnaiz, Graduate Student

Above: Claire Strothman and her dog Sheila. Top and left side border: Images from Strothman’s research.
Faculty Spotlight:
Nancy Carrasco, M.D.

By Alexandria Oviatt, Graduate Student

Nancy Carrasco, M.D., trained as a physician at the National Autonomous University of Mexico, but ultimately pursued an academic research career. Her work on the sodium/iodide symporter (NIS) began in 1987 when she was hired by Albert Einstein College of Medicine. From there, Carrasco moved to Yale University in 2011. Carrasco thought that would be her last big move but later responded to a call from a search committee at Vanderbilt University and, in July 2019, she assumed responsibility as Chair of the Department of Molecular Physiology and Biophysics.

You received your M.D., but have ultimately pursued a career in the basic sciences. Why?

Once I was in the wards, it was not what I expected it to be. Maybe that was because I was already thinking in molecular terms, but I found it frustrating when people would ignore that aspect and say not to worry about it. I thought, “Why don’t I join a basic sciences lab to see?” I started escaping from the hospital to go to lab.

Being a woman in science can present obstacles. Has this influenced your career?

When I was hired at Albert Einstein, there were many other women on faculty – maybe 30%. Albert Einstein has an interesting history of hiring minorities, but at many other places I may have been the token woman. I have been lucky to have been surrounded by colleagues who are fair and unbiased.

How has your mentoring philosophy been shaped by your own mentors?

My postdoctoral mentor Ronald Kaback, M.D., was a very intense person. There was not a single day that was boring; there was extreme excitement or extreme depression. He was always interested in talking and thinking about science. Ron was also supportive, and I can think of one specific example: I remember being pretty new in lab, running a size exclusion column with a senior postdoc. At some point I made a mistake. I was devastated and crying at my desk when Ron came in and asked what happened. ‘I diluted the sample,’ I said. He told me, ‘It’s okay, as long as it doesn’t happen again.’ I thought that was very wise. I also learned from Ron that mentoring relationships shouldn’t end when someone leaves the lab. The support should continue indefinitely.

How have you managed your career transitions? What advice do you have for students transitioning?

Students often think they must have it all figured out, but nothing is irreversible. What’s fascinating about science is you never know the direction it will take. That’s great and challenging because you go into fields you know little about and have to educate yourself. I think that is stimulating. It is also something I love about the MPB Department – it is so scientifically diverse. At seminars some of the best questions come from people studying something completely different.

What are some of the implications of your work on the sodium/iodide symporter (NIS)?

We discovered that perchlorate binds to an allosteric site of NIS and can be transported. This has potential implications in human health, particularly in women pregnant or nursing, because NIS is expressed in the placenta. So, perchlorate can be transported to the fetus and interfere with iodine transport. As the fetus begins producing its own thyroid hormones toward the end of a pregnancy, the fetus becomes more susceptible to perchlorate uptake. This can harm development.

“Mentoring relationships shouldn’t end when someone leaves the lab. The support should continue indefinitely.”
Growing up, Kevin Manz, Ph.D., hated school – a detail that seems surprising for someone who will earn both a Ph.D. and an M.D. by 2021. Everything changed when he enrolled in a psychology course and became captivated by the biological intricacies of the brain. Manz went on to join a neuroscience lab in college and continued on to medical school at Vanderbilt University. He then made the decision to pursue his Ph.D. during his first year.

“I just felt like the types of questions that I was interested in were not being addressed in medical school. I still felt like there was a lot that I wanted to understand, and if I didn’t pursue formalized scientific training now, I probably wouldn’t be able to do it easily later in life,” he said.

Manz joined the lab of Brad Grueter, Ph.D., Assistant Professor of Anesthesiology, to study neural plasticity – the ability of neurons to change and adapt – in the brain’s reward center, the nucleus accumbens. Originally, he thought that a receptor called GABA\(_B\), which is involved in the inhibition of neural signaling, was responsible for the plasticity within a specific microcircuit in the nucleus accumbens.

“It turns out that was completely wrong, but the process of trying to figure out the mechanism of this plasticity led me to look at how the GABA\(_B\) receptor contributes to synaptic physiology in the nucleus accumbens,” he said.

To illuminate the exact role of the GABA\(_B\) receptor in the communication between neurons in the nucleus accumbens, Manz and his colleagues used whole-cell patch clamp electrophysiology and optogenetic techniques to study the neurons of genetically modified mice. They found that activation of the GABA\(_B\) receptor leads to a decrease in the strength of excitatory signaling between neurons by preventing the release of excitatory chemical messengers. Critically, they found that this process is dependent upon the interference of the GABA\(_B\) receptor in vesicular exocytosis controlled by a protein called SNAP-25.

Manz says the main significance of the work, published in the *Journal of Neuroscience*, is that it describes a mechanism of crosstalk between the inhibitory system and the excitatory system in the nucleus accumbens that has never been characterized before. The findings also have strong implications for use of the drug Baclofen to help individuals struggling with addiction. Baclofen activates the GABA\(_B\) receptor, preventing excitatory signal release by SNAP-25, and reducing the rates of drug relapse.

“This allows us to understand more clearly how the GABA\(_B\) receptor is actually shifting the reward center in such a way that makes it less likely for people to continue using drugs after they stop,” said Manz.

After completing his M.D., Manz hopes to become a critical care doctor and open his own lab devoted to studying the plasticity of microcircuits in areas of the brain involved in wakeful states. Yet, given the plasticity of his career path thus far, he may be headed for more unexpected twists that could lead to novel discoveries for many different types of patients.
DNA is constantly damaged from both internal and external factors, but cells have evolved mechanisms to recognize and repair this damage. Defects in DNA repair can lead to cancer and many other diseases. Petria Thompson and Katherine Amidon, graduate students working with David Cortez, Ph.D., Professor of Biochemistry, and Brandt Eichman, Ph.D., Chair of Biological Sciences, respectively, led the effort to elucidate the structure of a recently identified protein-DNA complex implicated in DNA repair. The study, published in *Nature Structural and Molecular Biology*, revealed that the HMCES protein creates a remarkably stable bond with damaged single-stranded DNA in order to protect the DNA from error-prone repair mechanisms.

Previously, a postdoctoral fellow in the Cortez lab characterized the SRAP domain of HMCES as important for protecting DNA from damage caused by ionizing radiation and UV exposure. As part of Thompson’s project, it was discovered that HMCES specifically recognizes abasic sites, a common form of DNA damage, and covalently crosslinks to these sites on the DNA. The Cortez lab enlisted the help of Amidon, Eichman lab member and structural biologist, to characterize the HMCES-DNA crosslink.

“It was legitimately a collaboration,” Amidon explained. “I purified the protein and handed it off to Petria. She cross-linked it to the DNA, followed by further purification, and then she gave it back to me. I put it in trays to grow crystals and did the computer analysis and structural part.”

Together, they studied the E. coli version of HMCES, YedK, which is similar in sequence and structure. Thompson and Amidon discovered that a stable covalent bond forms between a cysteine residue on the SRAP domain of YedK and an aldehyde group on damaged single-stranded DNA. This linkage, termed a thiazolidine protein-DNA crosslink, shields the damaged site from endonucleases in order to maintain genomic stability during DNA damage repair. Future studies will focus on how the crosslink is removed to proceed with abasic site repair.

Thompson, a student in the Medical Scientist Training Program, originally intended to go to Divinity School. She quickly realized, however, that she was most interested in the psychopharmacology of her Psychology class at Emory University and instead pursued research opportunities in chemistry and pharmacology labs. Before starting her M.D./Ph.D. studies at Vanderbilt, she spent two years at the NIH as a post-baccalaureate fellow. Outside of lab, she enjoys rock climbing, pottery, and works for a nonprofit called The Student National Medical Association.

Like Thompson, Amidon considered an alternative career path. She enjoys playing the trumpet and considered studying music education in college, but two of her high school courses, Chemistry and Anatomy/Physiology, piqued her curiosity in science. Ultimately, she completed a double major in Biochemistry and Microbiology at the University of Vermont and entered the Interdisciplinary Graduate Program in 2017. In her spare time, she still likes to pick up the trumpet and occasionally plays with her musical family.

Learn More:
Congratulations to our Recent Graduates!
March 2019-February 2020

Katherine Aboud
Maria Agostini
Erin Aho
Gabriela Alvarado
Erica Anderson
Jordan Anderson-Daniels
Amrita Banerjee
Theresa Barke
John Beeler
Monique Bennett
Stephanie Birnbaum
Andrew Brooks
Audra Bryan
Miles Bryan
Lauren Bryant
Iliza Butera
Stephanie Carnes
Sheridan Carrington
George Castle
Ling Chen
Jonathan Chipman
Alberto Cisneros
Bradley Clarke
Melissa Cooper
Natalie Covington
Derrick Cumberbatch
Bethany Dale
Megan Dumas
Allison Eberly
David Elion
Esther Epum
Rachel Fischer
Nora Foegeding
Oakleigh Folkes
Hubaida Fuseini
Hunter Gibbons
Elizabeth Gibson
Andrew Gordon
Kevin Graepel
Yuanjun Guo
Nolan Hartley
Scott Hinger
Angela Howard
Asante Kamkwalala
Daniel Kashima
Kevin Kelly
Tyler Kennedy
Reece Knippel
Katherine Konvinse
Danielle Kopke
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Kalen Petersen
Kristin Peterson
Meagan Postema
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Nicole Putnam
Meagan Quinlan
William Ramos-Lewis
Cristina Robinson
Gabrielle Rushing
Christi Salisbury-Ruf
Vaishali Satpute
Johanna Schafer
Marion Setliff
Robin Shafer
Erin Shockley
Cara Singer
Miranda Sowder
Michael Tackenberg
Joshua Thompson
Elijah Trefts
Jessica Tumolo
Gokhan Unlu
Garrett Warren
Melodie Yen
Jingjing Zhu
Yuantee Zhu