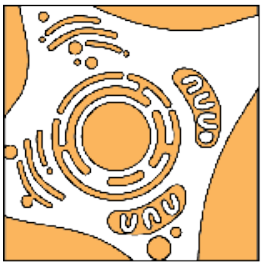
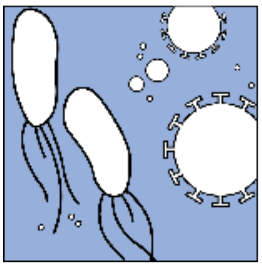




CAMB Student Newsletter

Volume 7 | Issue 4 | November 2022

In this issue

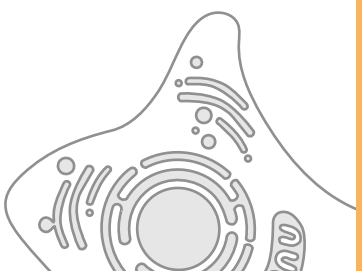
Letter from the Editors

Dear CAMB Students, Faculty, and Alumni,

Autumn has fallen upon us, and with that change of the season brings our November newsletter! We're excited to present this fun bundle of articles to read alongside a nice warm beverage of choice, preferably settled in a cozy chair. Join us in a **discussion with Alumni Dr. Emily Fabyanic** as she discusses her new and exciting opportunity at local company Spark Therapeutics. Dive into the **latest research published by CAMB G&E student Emily Lubin** as we explore her paper Histone heterogeneity: uncovering novel human disease-candidate genes. Curl up with our **Conversation with The Trainee Advocacy Alliance**, and learn about the many services they can provide for CAMB students. Yearning for some tasty autumn goodies? Check out our **Positivity Corner's Fall Recipe Roundup** and get instructions for homemade pumpkin bread and spiced caramel sauce.

For additional articles, past publications, and to learn more about the CAMB Student Newsletter team, visit our blog at cambnewsletter.wix.com/blog or follow us on Twitter at @CambNewsletter. The CAMB Newsletter team is **always in search of new writers** and **editors to join our team**! Current students interested in contributing to the CAMB Student Newsletter can reach out to us via this form to join our email list. We hope you enjoy the November 2022 issue!

Sincerely,
Kay Labella and James Gesualdi
Editors-in-Chief



Alumni Spotlight
Dr. Emily Fabyanic

Kay Labella

Peer Edited by Amber Abbott

Dr. Emily Fabyanic

Getting ready to graduate and looking to stay local? Curious what your industry options are? Had a spark with a job listing from Spark Therapeutics and want to hear more? Well, look no further, readers. In this edition of the CAMB newsletter, we welcome a special guest alum from the Pharmacology Graduate Group. Dr. Emily Fabyanic is a genomic technology scientist focusing on next-generation sequencing at Spark Therapeutics. In her present role, she is helping to found Spark's sequencing core and is working to build sequencing techniques and optimize protocols. Departments at Spark are organized by tissue system, then by disease group and therapeutic area. Dr. Fabyanic and the next-generation sequencing core work with all of these groups to help them get as much as possible out of their rare and precious samples to accelerate gene therapy development. Recently, CAMB Newsletter writers sat down with Dr. Fabayanic to discuss life after Ph.D. lab, job hunting, and how to find that perfect industry match.

What is your day-to-day like at your job?

My timetable is different depending on the day, and I have the flexibility to create my own schedule. As long as I get my work done, I can design my life around that in a pretty unique way; it's a lot like a Ph.D. in that way. I will say in industry, my volume of responsibility is a lot higher, because here, I'm working on a specific task to support people across the company instead of focusing on a narrow project. There are also systems of communication in place to manage. I like that a lot, though; I find it more fulfilling. It feels like things get done faster and make an impact on patients more quickly than the work I've done in the past.

Some days, it's a lot of meetings, talking to the therapeutic areas to gauge their needs and how to meet those needs, and design experiments with sequencing in mind. We're working closely with the other cores to design protocols that let us get impactful data. Overall, it's a lot of consulting and developing techniques and then assessing those techniques, and then evaluating what our priorities going forward need to be and how those priorities might benefit the company's progression. It's a lot like being in a new lab where things aren't quite set in stone yet. You can do a lot of creative stuff without the associated stress and academic pressure – you work on the science as a team.

Were you also looking at other small or big biopharma companies or different career trajectories?

I had decided at the point I was looking for jobs that I wasn't going to look at academic postdocs. I had researched it, but decided against it. I was looking

for local biotech or pharmaceutical companies, because we [my partner and I] had recently bought a house and I ideally wanted to stay in the area. Our other options were Boston and the Bay area. If we were to move, we wanted a house with a yard, and that seemed much more affordable here than in those locations. I was really just passively browsing at the time I applied, but I was planning to apply to every next-gen sequencing job in the area!

What are the key benefits/perks of working for your current role?

The work/life balance is so much better. At Spark, you start with 20 days off. Additionally, there's a December shutdown from around the 24th to January 1st, and we have half days every other Friday that most people take advantage of. No one goes into the lab on the weekends or is around past 5. Everyone has a much healthier relationship with work because of the structure of it. There isn't the responsibility of having to be on and thinking about the project at all times – and you're compensated well for it. As a bonus, we have all kinds of snacks; I never have to get lunch. They stock the shelves with desserts and protein bars, plus there's a fridge of Coke products and one of just LaCroix. The coffee machines are incredible, too. They use nice beans and grind them fresh, and they have so many drink options to choose from.

Even better than free food, I have a great retirement account now, and there are a lot of options for starting to save for my future. They even give us access to free financial advisors to consult with! Spark also gives me a commuter benefit of up to $350 a month. All in all, it really feels like they care and they want to keep me. With some other industrial endeavors, I think people feel like a cog and they burn out, which I felt in academia too. Spark, on the other hand, really seems like they're trying to train and retain you, and gives you access to the resources you need; they treat you very well so as not to lose you.

Another perk, and maybe my favorite, is that I'm laying the groundwork for the future. As part of the next-generation sequencing core, which is just starting up, I'm setting the foundations for precedents and protocols that will be used for years to come at Spark. Having that kind of impact is beyond great.

What have been the biggest differences between working in an academic and industry setting?

The goal-oriented work with clear expectations and timelines. Honestly, that's the best. I thrive in that environment.

How has your degree helped you in your current work?

I think a Ph.D. in general gives you time to learn how to teach yourself and to troubleshoot, and those are skills that I use everyday now. In my specific experience during my Ph.D., I developed a foundational understanding of the field, its history, and what I'm doing now from a sequencing perspective, which has helped me consult on these projects and understand what's out there and what we can modify to use for our protocols. It's a lot of knowledge that taught me how to tackle my day to day problems.

What's the best thing about your job?

I feel lucky that I get to be this creative and establish a function of the company that is so important so early in my career.

What was the job search process like for you?

It happened by accident! I wasn't ready to start applying for jobs; I was just scrolling through LinkedIn one day and I saw a job description for my current job, and it just so happened to be attuned to my Ph.D. experience. I also had very sought after expertise. It seemed like the job was built for me, so I decided to apply. The process was really quick, and I set a thesis date after. I graduated and started in January. It's

been a great fit so far! Plus, I had an internal contact, so the process went very quickly. It was a best case scenario all around!

How did your previous connections or networking opportunities with the group you ended up working for help in the interview process?

In general, if you have someone you know is working at the company you're interested in, they can facilitate informal interviews with people who might be your functional manager and speed up the interview process. It's not essential, but it really helps. If possible, they can get you the email of the person who posted the job listing and facilitate that first informal interview, and then if that goes well, they can help set up the formal interview process with human resources. I had met the person who set up my informal interview through a rotation, so it doesn't have to be someone who you have known long term, just someone who knows you a bit and can vouch for you as a scientist.

Can you tell us about the interview process at your company and how you prepared for it?

Penn Career Services helped look over my CV and resume and did mock interviews and practice negotiations with tips on what you should and shouldn't ask. The negotiation is not something you're exposed to a lot, and you're only talking to HR about it. For me, the interview itself was just panel interviews and informal sit-downs with people I might work with; since I had an internal contact I'd already talked to some of the people. Thankfully, it wasn't very stressful!

What are the key skills that companies look for during the hiring process?

Key skills they were looking for in my case was the ability to respond to questions and troubleshoot. Prove you can really do what's on your CV and are confident relaying the information you say you're versed in. If they ask you for an interview, they're interested in that information, but they also want to see that you're a good communicator and that you're interested in the company. Do some research on the company and their history. Tailoring your interview and your discussion points to that company's goals is something they want to see.

What advice do you have for current graduate students?

Stay close to the people in your program, especially those graduating before you. Be prepared to reach out if they're working somewhere you're interested in. Having internal contacts can expedite the interview process; it's not necessary but it really does help. Industry isn't as different or scary as it might seem. It's a much healthier place for me specifically, and I've really enjoyed it. I feel more fulfilled, more productive, and I've learned so much in the short period of time since joining the team at Spark, which I think speaks to how great an environment it is.

Take your time to find something that will be a good fit, but don't be afraid to keep looking if the first job you get isn't what you anticipated. You can always change trajectories, even within the same company. You're never stuck, ever. You can always adjust what you're doing to be fulfilled – it just might take time to find that. There's no pressure to get it right immediately. And advocate for yourself. Be communicative to those around you.

Though the job search can be daunting, fellow students past and present have your back! If you'd like to get in touch with this awesome alumni, you can reach out to Dr. Fabyanic on LinkedIn!

Histone heterogeneity: uncovering novel human disease-candidate genes

Sylvia Stankov
Peer Edited by Kay Labella

Histone proteins play a fundamental role in genome organization. These proteins act as spools to help condense DNA into chromatin, which is essential for fitting all our genetic material neatly into the nucleus. Histones can be chemically modified via methylation, acetylation, and other means. This impacts DNA accessibility and ultimately gene expression (1). Germline mutations in histone-encoding genes are rare and cause a fairly consistent phenotype of neurodevelopmental syndrome with craniofacial anomalies. Emily Lubin is a CAMB Genetics and Epigenetics MD/PhD candidate in Dr. Elizabeth Bhoj's lab seeking to understand the mechanisms of pathogenesis behind these mutations, with the goal of leveraging these findings towards novel treatments. Emily's recently published work is titled "Analysis of histone variant constraint and tissue expression suggest five potential novel human disease genes: *H2AFY2, H2AFZ, H2AFY, H2AFV, H1F0*" (2). This analysis is aiding the identification of causal mutations for patients with this devastating conserved phenotype.

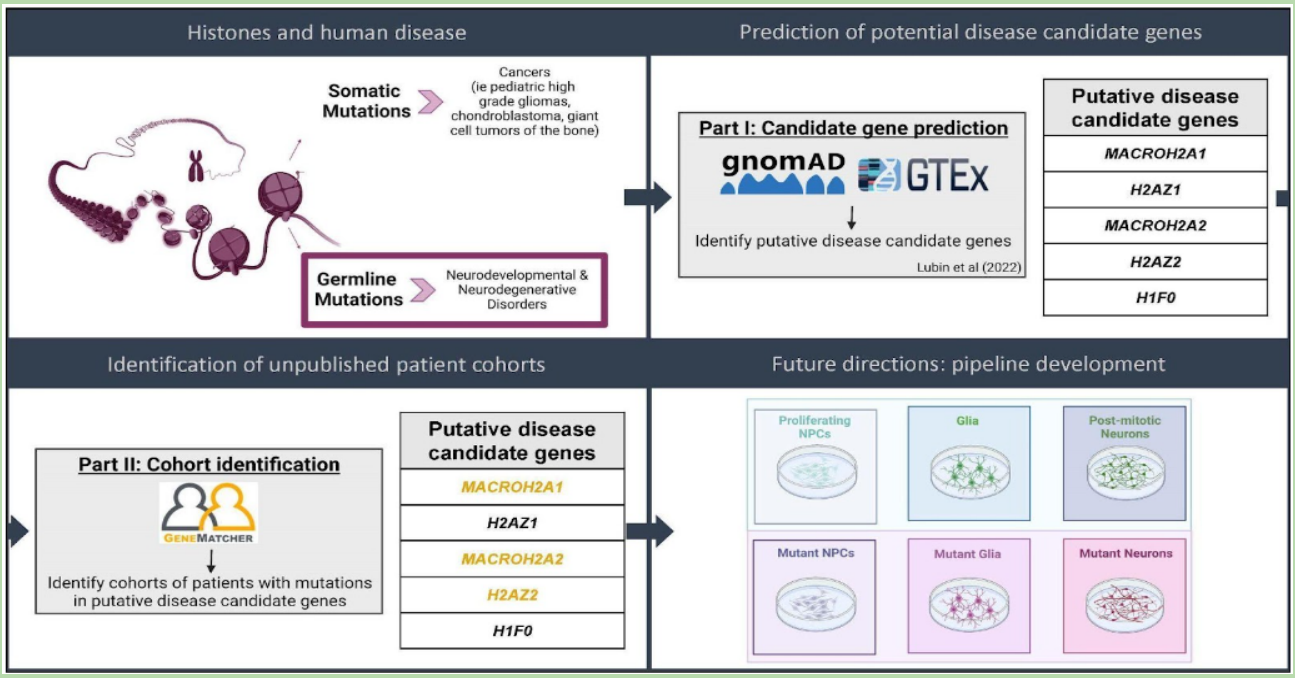
Emily Lubin, CAMB Genetics and Epigenetics MD/PhD candidate

Appreciation of histone protein-encoding genes and their roles in human disease has grown in parallel with the field itself. The replication-coupled H2A, H2B, H3 and H4 and linker H1 proteins are protected by substantial built-in redundancy, with multiple copies of each gene in the genome. The replication-independent variant histone proteins are a small subclass of histones in the nucleus with significantly less built-in redundancy. These replication-independent histones can replace replication-coupled histones and usher functional and regulatory consequences with them. In her work, Emily sought to identify which histone protein-encoding genes would most likely result in the conserved disease phenotype when mutated.

Emily began by assessing the tolerance of human histone-encoding genes to missense variation, as genes that are most crucial for the function of an organism are least able to tolerate mutations. She compiled a list of 89 histone-encoding genes and accessed their constraint metrics in gnomAD, a database composed of samples from over 140,000 donors. Seven histone-encoding genes were significantly constrained against missense mutations, and all encoded for replication-independent variant histones.

Next, Emily analyzed the tissue expression profile of histone protein-encoding genes, hypothesizing

Causal mutation – mutation responsible for a given disease



Graphical Abstract

Germline mutations in histones can cause rare pediatric neurodevelopmental and neurodegenerative disorders. To overcome limitations in clinical sequencing approaches, the team utilized constraint metrics indicating the tolerance of individual histone genes to missense or loss-of-function mutations (gnomAD), along with tissue expression analysis (GTEx) to identify putative histone-encoding disease candidate genes. Recently, three of these candidate genes were found to be mutated in unpublished cohorts of patients. Emily and the rest of Dr. Bhoj's team are working to develop a pipeline that would allow them to identify a potential therapeutic target shared among these syndromes to treat a maximum number of patients in this ultra-rare disease space.

that the most constrained genes would likely be ubiquitously expressed. She looked at 53 tissue types from over 700 donors in the GTEx database and found that all seven genes significantly constrained against missense mutations were also ubiquitously expressed. These findings suggested that mutations in these genes may indeed underpin neurodevelopmental syndrome and craniofacial anomalies. Three of these identified replication-independent histone protein-encoding genes have been associated with neurodevelopmental, neurodegeneration, and cancer phenotypes, supporting the validity of Emily's approach.

To further investigate the histone protein-encoding genes, Emily examined their tolerance to loss-of-function variation, where protein structure is predicted to be affected. She used the probability of loss-of-function intolerance (pLI) constraint score in gnomAD, which was initially developed and implemented in the ExAC database and represents just over 60,000 donors. While the smaller sample size impacted power, the analysis could still be used to screen the histone protein-encoding genes. Unexpectedly, only one gene (*H2AFY2*) met the stringent criteria of a gene predicted to be highly intolerant to loss-of-function variation. Indeed, 76% of histone protein-encoding genes overall had a score indicating tolerance to the accumulation of loss-of-function variation.

Constraint metrics – in gnomAD, metrics of pathogenicity; identifies genes subject to strong selection against various classes of mutation

GTEx – the Genotype-Tissue Expression project; public resource to study tissue-specific gene expression and regulation (https://www.gtexportal.org/home/)

gnomAD – the Genome Aggregation Database; aggregated exome and genome sequencing data with available summary data (https://gnomad.broadinstitute.org/)

Even when applying less stringent criteria, only 12% of genes were at least slightly intolerant to loss-of-function. Emily hypothesizes that some histone protein-encoding genes known to be implicated in disease did not reach significance in her screen because they encode replication-coupled histones, which may be more tolerant to mutation due to their built-in redundancy.

Subsequent analysis of tolerance to loss-of-function mutations used another computational approach. Emily assessed the loss-of-function observed/expected upper bound fraction (LOEUF) values in gnomAD, with the benefit of a larger sample size. These values fall on a continuous spectrum (unlike the initial loss-of-function analysis method above). Similar to the previous score analysis, LOEUF values for genes encoding histone proteins primarily clustered at values which predicted a tolerance to loss-of-function mutation. The eight genes likely to be highly intolerant were cross-referenced with their tissue expression in GTEx. This left six genes constrained against loss-of-function, ubiquitously expressed, and potential disease-candidates. Consistent with the missense variation analysis, all these genes encoded replication-independent, variant histone proteins. These were likely six novel disease-candidate genes; none had been previously reported to have loss-of-function mutations associated with a clinical phenotype. However, two alternative explanations of these results remained: 1) no reports exist because loss-of-function variation in these genes results in embryonic lethality or 2) missense variation is pathogenic, but in a gain-of-function or dominant negative setting, where haplo-insufficiency does not result in a neurodevelopmental phenotype. In either case, Emily's analysis supported the hypothesis that the redundancy of replication-coupled histones is protective and may compensate for mutations in an individual gene.

Emily's work revealed that few histone-encoding genes are both ubiquitously expressed and significantly constrained against missense or loss-of-function variation. The genes that were constrained unanimously encoded replication-independent, variant histone proteins. Their "vulnerability" to mutation likely stems from their comparatively reduced redundancy (versus replication-coupled histone proteins). While Emily's work assesses whole gene intolerance to mutation, a future screen to parse genes with missense or loss-of-function intolerance in specific domains may provide even more fine-tuned hits. This approach may also pick up known disease-causing mutations in genes which did not reach significance in the current screen. Greater inclusivity in databases like gnomAD and GTEx, which are primarily comprised of donors of European descent, will also aid in accurately identifying causal pathogenic variants across multiple ancestral groups.

Emily's screen additionally revealed several potential novel disease-candidate genes for the class of Mendelian disorders with neurodevelopmental syndrome and craniofacial anomalies. Since publication, Emily and Dr. Bhoj have identified previously unpublished cohorts of patients with mutations in three of the five putative disease-candidate genes. This outcome confirms that germline mutations in histone-encoding genes likely affect more children than what is currently reflected in the literature. This work is in parallel with ongoing studies dissecting the mechanism of **Bryant-Li-Bhoj Syndrome**, a neurodegenerative syndrome caused by a mutation in a replication-independent histone protein, named in recognition of the CHOP team which characterized it. Fortunately, for

The Bhoj Lab

pediatric neurodegenerative conditions, there is a therapeutic window during which treatment can help tremendously in preserving quality of life for a child over a lifetime. Emily and the rest of Dr. Bhoj's team are working to develop a pipeline where they introduce these novel disease-candidate genes into human iPSCs then differentiate those cells down lineages resulting in neural progenitor cells (NPCs), glia, and post-mitotic neurons. This approach would allow them to identify a potential therapeutic target shared among these syndromes to treat a maximum number of patients in this ultra-rare disease space.

References:

1. Martire, S. and Banaszynski, L.A. The roles of histone variants in fine-tuning chromatin organization and function. Nat Rev Mol Cell Biol (2020). https://doi.org/10.1038/s41580-020-0262-8.
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3. https://www.omim.org/entry/619720#:~:text=Bryant%2DLi%2DBhoj%20neurodevelopmental%20syndrome%2D1%20(BRYLIB1),speech%2C%20and%20delayed%20motor%20milestones.

Bryant-Li-Bhoj Syndrome:

Bryant - Li - Bhoj neurodevelopmental syndrome - 1 (BRYLIB1) is a highly variable phenotype characterized predominantly by moderate to severe global developmental delay with impaired intellectual development, poor or absent speech, and delayed motor milestones. Common features include abnormal head shape, variable dysmorphic facial features, oculomotor abnormalities, feeding problems, and nonspecific brain imaging abnormalities (3).

A Conversation with the Trainee Advocacy Alliance

James Gesauldi

The CAMB student newsletter team recently had a chance to sit down with the leadership of the **Trainee Advocacy Alliance** (**TAA**). The TAA is a collaborative group of Biomedical Graduate Studies (BGS) graduate students, Biomedical Postdoctoral Program (BPP) post-docs, and Penn faculty members from across the University who are committed to creating and maintaining a biomedical research community that embodies inclusivity, diversity, and equity.

We had an opportunity to speak with **Dan Kessler**, our CAMB graduate group Chair, along with **Jillian Eisenhauer** and **Robin Wilder**, who are CAMB colleagues currently serving as the student leadership of the TAA.

Can you tell me a little bit about the history of the Trainee Advocacy Alliance? When was the group founded and why?

What is now the Trainee Advocacy Alliance grew out of an earlier IDEAL (Inclusion, Diversity, Equity, and Learner) Research initiative started by CAMB MVP Students Prioty Sarwar and Nawar Naseer. The original name for the organization was "Minority Support Network" and it was developed in the context of the broader nationwide movement for racial equity during the summer of 2020.

Since its inception, the TAA has become a more student-driven organization that seeks to support trainees in any difficult situation through confidential discussion and counseling with trained peers. We started officially offering and promoting our counseling and mentorship services this semester.

Why did you decide to get involved in the TAA?

Robin: I had previous experience in a diversity initiative funded by the NIH, so I wanted to get involved in similar student organizations at Penn. I searched the IDEAL Research website and found the TAA, then reached out to Vanessa Martinez to join!

Jill: During undergrad I became more interested in exploring my roots as a woman of Korean descent adopted by an American family, so I ended up getting involved in Diversity, Equity, and Inclusion initiatives as well. I originally signed up to get involved as both a student mentor for CAMB GTV and a member of the TAA during the early days of the pandemic when xenophobia against Asian peoples was spiking; I wanted to help other colleagues with similar backgrounds avoid prejudiced treatment and other traumatic situations. I actually originally heard about the TAA from Robin!

When can the Trainee advocacy alliance help struggling students or postdocs?

We remain highly focused on helping students that face some level of identity-based discrimination but are of course available to help with other issues. Students can reach out to the TAA for general conflicts with their co-workers or mentors to receive counseling or advice.

We try to be available to any student facing stress, unfair treatment, bias, or any other trying situation either at Penn or in their personal life. Our goal is to have as much of an open door as possible: no problem is too small or too personal. Many of the problems brought to us fall into the traditional challenges of being a graduate student or trainee at an Ivy League institution and the associated challenges.

When a student is interested in meeting with a TAA member to discuss a problem, they can reach out via (TAA@pennmedicine.upenn.edu), and Vanessa Martinez will pair the student with a TAA member that has a relevant background or experience. Alternatively, students can browse TAA members and reach out to those members that seem appropriately qualified to help with their issue.

What sort of assistance is the TAA empowered to provide to trainees?

Our members are mainly there to listen, support, and understand the issues being brought to them by trainees, and to ask helpful follow up questions. One of our most important functions is making students aware of resources that they have access to such as Counseling and Psychological Services (CAPS) and giving them the space to decide on their own course of action. TAA members hope to help struggling students find their voice and do what they can to regain control over the difficult situation at hand.

Generally, TAA members only provide guidance to the extent that students want guidance; we are not really supposed to do anything to sway the student's course of action or provide actionable advice. Any type of outside referral to CAPS or potential intervention in a student's situation – such as having a TAA member sit in on a potentially contentious meeting between student and adviser – would only ever happen at the consent and request of the student.

How does the TAA fit into the broader goal of increasing diversity and inclusion in our research community?

Though our group is still in its early stages, having recently finished training and orienting all current TAA members, officially opening our counseling services, and advertised the TAA as a resource to this year's class of incoming PhD students, we have formed collaborative projects and partnerships with other existing inclusive organizations at Penn, such as SACNAS, E.E. Just Biomedical Society, PGWISE, and LTBGS. In fact, we are currently involved in an effort with several of these groups to raise funds and supplies for hurricane Fiona relief in Puerto Rico.

While we were initially conceived as a reactive organization oriented around helping students who are or previously were subject to stressful situations because of their identity, we are now developing a more proactive approach involving putting together resources and programming geared toward helping students through parts of the PhD process that we already know are stressful. Things like qualifying exams and thesis lab choices for example.

What is the level of commitment for students or postdocs participating in the TAA?

Each member of the TAA must complete a total of 12 hours of training on restorative communications, including one full 8 hour day of co-training both students and faculty, inclusive mentorship training, and one 3 hour training module with psychologists from CAPS. After certification, the amount of time individual members spend on TAA duties depends on the number of students that reach out to them for help, so this can vary substantially from member to member. Of course, participation in the TAA is open to all interested students and postdocs.

For any readers interested in reaching out to the TAA, members can be reached directly via their contact information available on their website. Alternatively, any students interested in becoming TAA members can reach out to Vanessa Martinez of IDEAL Research (Vanessa.MartinezPenn@pennmedicine.upenn.edu).

Navigating academic science is complicated and challenging, and asking for help when you need it is one of the most important skill sets to develop during a PhD. If you find yourself in a challenging situation, please consider reaching out to the TAA.

Fall Recipe Roundup!

Pumpkin Bread

3 ½ cups flour
2 tsp. baking soda
1 ½ tsp. salt
2 tsp. cinnamon
1 tsp. nutmeg
3 cups sugar

1 cup vegetable oil
4 eggs
⅔ cup water
2 cups cooked pumpkin

Preheat oven to 350F. Grease 2 loaf pans.

Sift dry ingredients together in a large bowl.

Mix remaining ingredients together in a small bowl. Pour into dry mixture and mix well.

Pour mixture into loaf pans. Bake at 350F for 1 hour and 15 minutes or until toothpick inserted in middle of loaf comes out clean.

Cool before removing from pans.

Spiced Caramel Sauce

120 g. (½ cup) heavy cream
1 tsp. ground cinnamon
½ tsp. ground ginger
¼ tsp. ground cardamom
⅛ tsp. ground cloves
200 g. (1 cup) granulated white sugar
85 g. (6 tbl.) butter at room temperature, cut into cubes
½ tsp. salt

Combine heavy cream and spices in a small sauce pot. Gently warm the cream over medium heat, bringing it to just above room temperature but not hot enough to steam. In a medium saucepan over low-medium heat, add all of the sugar. Make sure your pot is larger than you think it should be. With a spatula, stir the sugar as it starts to melt. The sugar will start to clump; continue to stir and push the unmelted caramel towards the center of the pot. It will become an amber color as it melts. When all sugar is dissolved, remove from heat and whisk in butter, adding a few cubes at a time. Whisk constantly until all butter is incorporated. Slowly whisk in cream and stir until caramel is smooth. Add salt to taste (but do not taste immediately). Let cool. Store in glass jars or other similar containers.

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