CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Ultrasensitive Discovery of Circulating Nausea-Inducing Factors

Name, degree, title, institution, and contact information including the email address of each PI

Hojoon Lee, PhD
Assistant Professor
Department of Neurobiology
Northwestern University
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Raymond Moellering, PhD
Associate Professor
Department of Chemistry
University of Chicago
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Do you have any current or pending grant applications that potentially overlap with this application? Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

(ENTER THE OVERLAP HERE)

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☒	**NO** ☐
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

Ultrasensitive Discovery of Circulating Nausea-Inducing Factors

Lay Summary

Nausea is an unpleasant sensation, which is believed to have evolved as a defense mechanism against physiological distress[1]. A frequent cause of nausea is food poisoning; the ensuing vomiting helps expel the causative toxins. A variety of other conditions can cause nausea, including gastrointestinal ailments, anxiety, pregnancy, and motion sickness[2]. Nausea is also an adverse side-effect of many medications and a major deterrent for cancer or diabetes therapy. Nearly half of patients undergoing chemotherapy experience nausea, seriously impacting their quality-of-life. The cost of nausea on the U.S. economy is estimated at ~$10 billion annually[3]. However, very little is known about the molecular basis of nausea. In this proposal, our multidisciplinary team will leverage critical expertise and ultra-sensitive proteomic technologies to identify the molecules ('alarm signals' produced by the body) that contribute to causing nausea, which will set the stage for future studies to potentially treat this ailment.

High-risk/High-reward Statement

There is intense interest to understand how the brain senses the state of the body (e.g., NIH's Notice of Special Interest: Promoting Research on Interoception and Its Impact on Health and Disease). Nausea has been challenging to tackle, because the symptoms are variable and subjective. In particular, mice cannot vomit, making nausea assessments harder. Nevertheless, mice treated with emetic agents (e.g, apomorphine) display other signs of 'nausea', including expressions of distress and nausea-specific behaviors (pica – consumption of nonedible items – and conditioned taste aversion[4,5]). We recently established conditions that evoke nausea-like symptoms in mice with motion sickness (MS), enabling us to take advantage of the powerful resources available for mouse studies. Leveraging these exciting new developments, we propose to generate a novel molecular profile of the altered proteome and metabolome landscape in cerebral spinal fluid (CSF) surrounding the nerves that 'sense' and transmit nausea signals. Creation of this hypothesis-generating map requires an animal model that recapitulates this complex phenomenon and ultrasensitive -omics methods to detect and quantify candidate proteins and metabolites from very small CSF samples. This dataset and the candidate signaling molecules it will identify can form the basis of additional funding from conventional sources and transform research on nausea.

Application Statement

Pioneering work by Borison et al. (1951)[6] identified a 'chemoreceptor trigger zone' in the caudal brainstem that mediates nausea and vomiting. This brain region, encompassing the area postrema (AP), nucleus of the tractus solitarius (NTS), and the dorsal motor nucleus of the vagus (DMV), is exposed to the fourth ventricle and lacks a proper blood-brain barrier (Fig. 1A). Artificially stimulating neurons in this area caused nausea-like responses in mice[4]. Therefore, it was hypothesized that the chemoreceptor trigger zone would detect circulating toxins, pathogens and other alarm signals produced in the body to initiate nausea. The presence of such factors was supported by experiments in cats where blockage of CSF flow in ventricles prevented emesis from motion sickness[7]. However, previous efforts to identify nausea-inducing signals have been largely unsuccessful, hampered by lack of sensitive diagnostic tools.

More recently, GDF15, a TGF-beta family protein, was identified as a circulating signal that causes anorexia. GDF15 is produced by malignant tumors, many diseases, and even strenuous exercise, therefore it seems to function as an alarm signal to inform the brain of physiological stress[8,9]. The genetic testing company 23andMe also identified GDF15 as the culprit for severe morning sickness (hyperemesis gravidarum) in pregnant women[10]. GDF15 acts on neurons in the AP via the cognate receptor GFRAL to cause anorexia and vomiting. Notably, in our preliminary studies, MS activated a discrete population of brainstem neurons that do not express GFRAL (see Fig. 1A). This suggests the presence of diverse pathways for nausea induction, but the molecular substrate(s) responsible for this process remain to be uncovered.

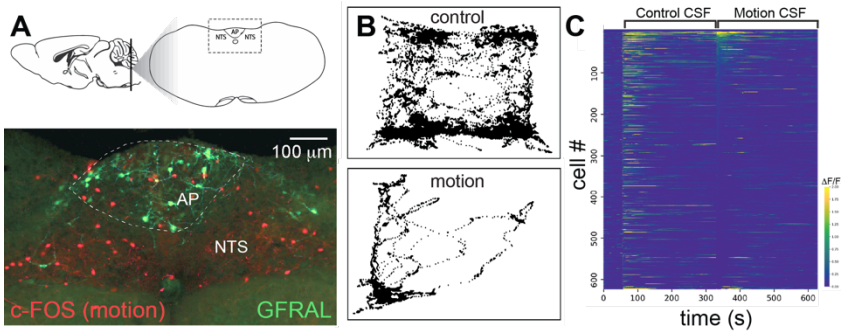

Figure 1. Induction of motion sickness (MS) in mice. A*, MS activates neurons in the area postrema (AP) and the nucleus tractus solitarius (NTS). Brain sections from a MS mouse were stained with antibodies against c-FOS and GFRAL.* ***B****, Mice with MS show reduced locomotion. Traces tracking locomotor activity were recorded for 30 mins following control vs motion-stimulation.* ***C****, Calcium responses in brainstem neurons in vitro after treatment of CSF collected from control and MS animals.*

Our hypothesis is that motion stimuli produce alarm signals alerting circumventricular brainstem nausea neurons. Accumulation of alarm signals in the CSF may explain the persistent, lingering nausea symptoms even after conclusion of the motion stimulus[11]. **Our goal for this project is to identify novel alarm signals produced by MS using a proteomics approach**. To do this, we propose to use state-of-the art proteomic/metabolomic analyses of CSF (Aim 1) and develop an in vitro assay to assess CSF-dependent activity in brainstem neurons (Aim 2).

To accomplish our goals in the proposed period and in preparation for this application, we (H.L. and R.M.) have already engaged in foundational work for this project. The two PIs leading this project leverage strong expertise and successful track records in their respective fields. The Lee group has extensive experience in sensory neurobiology in mouse models[12]. The Moellering group has critical expertise in advanced proteomic and metabolomic approaches[13,14]. The two groups will collaborate seamlessly with regular 'super-group' meetings and frequent communication via email and Slack.

Preliminary results: Based on published information[15] and our extensive pilot work, we determined a reliable condition for inducing motion sickness in mice with a 40° vertical tilt nutating shaker. After 30 minutes of stimulation at 60 rpm, mice exhibited behavioral and neurological signs of nausea. Motion sick animals were in a hunched, stationary posture (Fig. 1B) and displayed piloerection, fecal incontinence, orbital tightening (squinting) for ~30 mins after conclusion of rotation. We also performed immunostains for c-FOS, a marker for neuronal activity, on sectioned brain tissues and detected significant activity in the chemoreceptor trigger zone after rotational shaking (Fig. 1A).

Aim 1. Identify nausea signals in CSF using advanced proteomic approaches

To identify candidate alarm signal(s), we propose to perform proteomic analysis of CSF collected from motion-stimulated vs. control animals. We will induce MS in wild-type mice using the rotating shaker, and harvest CSF through the obex using a glass capillary needle. In a pilot study, we collected CSF from MS animals (n=3) and performed mass spectrometry. We detected ~200 unique proteins, including cytokines and metabolic proteins, in the CSF sample. This preliminary work established the feasibility of collecting CSF samples from MS animals. Now, we propose to apply a micro-scale proteomic processing workflow combined with isotopic quantitative barcoding and detection of CSF proteins on a state-of-the-art Thermo Eclipse Tribrid mass spectrometer (see letter of support from UChicago Proteomics Core). We will compare the CSF proteomes of control (no MS treatment) and MS-induced mice (n=8 per cohort) by collecting CSF fluid from each, precipitating protein using cold acetone and performing standard tryptic digests. To permit more sensitive and multiplexed comparison of samples, we will isotopically label the ensuing tryptic peptides from each sample using 10-plex TMT barcodes, which enables determination of not only proteins present in these samples, but highly precise quantitation of their relative abundance between wild-type and MS mice[13,16,17]. TMT-labeled proteomes from control and MS-treated mice will be pooled together and analyzed on a nano-LC-coupled Eclipse Tribrid mass spectrometer (Fig. 2). This instrument is extremely sensitive – even permitting single-cell proteomic applications – and will be run using optimized settings for detection of TMT-labeled peptides and between-run detection and quantitation using a workflow employed by us to boost detection and quantitation of proteins from very small

clinical samples[14]. Standard data processing using this approach will enable robust detection of proteins in the CSF across biological replicates within each cohort (e.g., each mouse) and will be combined to identify proteins whose abundance are statistically altered in the MS-induced mice for further study in Aim 2. In parallel, this workflow could be repeated to specifically detect posttranslationally modified proteins, for example phosphorylated and glycosylated species, as the altered PTM states could be important signals as well. After completing these analyses, we will build a short-list of candidate proteins enriched in the motion sickness condition. We expect alarm signal(s) to be secreted factors and we will cross-reference Allen Brain Atlas and other gene expression databases to eliminate broadly expressed protein candidates.

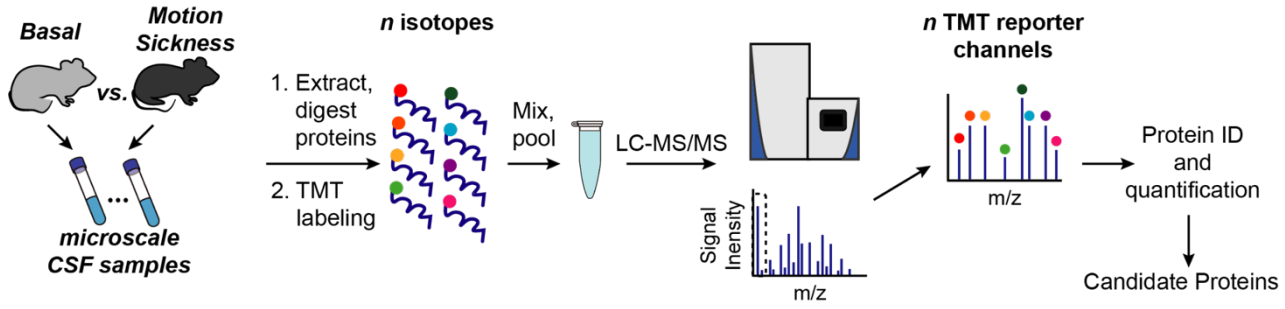

Figure 2. Quantitative proteomic profiling of MS-related proteins. *CSF extraction from basal and MS-induced mice will be collected and processed for microscale TMT barcoding and analysis by LC-MS/MS. Instrument targeting of TMT-labeled peptides using in-line FAIMS device as well as between-run ion detection will provide deep and quantitative profile of the proteins present in each sample and the relative abundance between control and MS mice.*

Aim 2. Establish a bioassay using dissociated brainstem neurons to detect CSF- dependent responses

The goal of this aim is to develop a calcium-reporter assay to rapidly detect nausea-inducing factors. To do this, we will use mice expressing a GFP-based calcium indicator, GCaMP6s[12,18], in neurons (this can be achieved using mouse strains, such as Thy1-GCaMP, or stereotaxic injection of viral vectors, such as AAV-Synapsin1-GCaMP, into the brainstem[12]). We will extract brainstem tissue containing the AP/NTS regions from these mice, dissociate the tissue with papain and plate the isolated cells on a laminin coated coverslip. Calcium imaging will be performed under an inverted Zeiss confocal microscope. Changes in GCaMP fluorescence will be used to detect neurons responding to the applied stimulus. Using this platform, we will test: 1) *CSF from MS mice vs CSF from control mice*; 2) *CSF collected from different timepoints* during motion stimulation to determine when the nausea factor(s) is most active; 3) *Candidate nausea factors* (recombinant proteins from commercial sources) short-listed from proteomic/metabolomic analyses in Aim 1. Our preliminary results show great promise for the proposed approach – we observed a handful of brainstem neurons responding to MS-CSF (Fig. 1C). However, we also plan to test alternative fluorescent sensors for other intracellular signaling pathways that may be involved in nausea detection, such cADDIS (Montana Molecular) for cAMP[4] signaling.

We anticipate that the work proposed here will lead to an enduring synergistic collaboration between the two PIs resulting in NIH R01 grants, high-impact publications, etc. Our long-term goal is, ultimately, to alleviate the debilitating symptoms of nausea. Despite the prevalence of clinical cases of nausea and current treatment options are inadequate (e.g. broadly acting anticholinergics or antihistamines[15]) Our proposed studies are expected to provide better understanding of the pathophysiology of nausea and help develop therapeutic strategies.

References

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Program Director/Principal Investigator (Last, First, Middle): Lee, Hojoon

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 06/01/2023 THROUGH 05/31/2025

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Hojoon Lee	PD/PI		0.18		114,047	2,281	636	2,917
TBD - Research Tech	Technician	9			39,672	30,498	8,509	39,007
SUBTOTALS →						32,779	9,145	41,924

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	
Consumables	10,576
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
Animal Care & Purchases	10,000

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 62,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 62,500

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only*.	41,924	43,181			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	10,576	9,319			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	10,000	10,000			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	62,500	62,500			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	62,500	62,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 125,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See attached page 8.

Budget Justification – Chicago Biomedical Consortium Catalyst Award (06/01/23 – 5/31/25)

PI: Hojoon Lee
The Lee lab will be performing the animal experiments and leading studies proposed in Aim 2.

PERSONNEL

Senior Personnel
Dr. Lee, Principal Investigator, will commit 2% academic effort (0.18 academic months) to the project. He will carry out a subset of the experiments described in the proposal and supervise design and implementation of all proposed experiments, data presentation at meetings and writing manuscripts.

Other Personnel
Research Technologist (TBD, 9 calendar months each year)
Support is requested for a research technologist who will conduct the experiments, as well as participate in study design, data analysis, presentation at meetings and manuscript preparation.

MATERIALS AND SUPPLIES

Animals Purchase & Care (Y1: $10,000, Y2: $10,000)
Support is requested for the purchase of wild type and transgenic mice for our experiments. Purchase of mouse strains will be necessary in the earlier more exploratory stages of the project. Some of these mice will be bred and maintained.

Supplies (Y1: $10,576, Y2: $9,319)
Support is requested for the purchase of molecular biology consumables.

Salaries have been increased by 3% annually each September.

Employee benefits have been calculated based on the following rates:
- 09/01/22 – 08/31/23…. 27.9% (provisional)
- 09/01/23 – 08/31/24 and thereafter…27.9% (provisional)

No Indirect Costs per Sponsor's application guidelines.

*** Receipt of this grant will not result in duplication of funding or reduction of support.**

	Year 1	Year 2	Total
Faculty Salary	$2,281	$2,349	$4,630
Faculty Fringe	$636	$655	$1,291
Research Tech Salary	$30,498	$31,413	$61,911
Research Tech Fringe	$8,509	$8,764	$17,273
Materials and Supplies/Animals	$20,576	$19,319	$39,895
Total	$62,500	$62,500	$125,000

Program Director/Principal Investigator (Last, First, Middle): Raymond Moellering

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH
	06/01/2023	05/31/2025

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Raymond Moellering	PD/PI			0.15	50,251	2,513	636	3,149
Pratyasha Chakraborty	GRA	3			37,000	9,250	0	9,250
SUBTOTALS →						11,763	636	12,399

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	
Reagents and supplies for -omics sample processing and mass spectrometry analysis (e.g., tandem mass tag [TMT] chemical labels, desalting columns, instrument consumables and laboratory consumables (tubes, buffers, etc.).	48,480
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
Graduate Student Fees	1,621

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 62,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 62,500

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only*.	12,399	12,770			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	48,480	48,109			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	1,621	1,621			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	62,500	62,500			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	62,500	62,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 125,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Raymond Moellering, Ph.D. (P.I., 0.15SM per year): Dr. Moellering is an Associate Professor of Chemistry, a Core Fellow of the Institute for Genomics and Systems Biology, and Director of the Integrated Proteomics Platform the University of Chicago. Dr. Moellering has extensive expertise in chemical biology, biorthogonal chemistry, synthetic biologics. In collaboration with Dr. Lee, Dr. Moellering will direct and supervise the proteomics- and metabolomics-related work in Aim 1 of this proposal.

Pratyasha Chakraborty, Graduate Student (3 CM per year): Ms. Chakraborty is a Chemistry graduate student in Dr. Moellering's lab. She will participate in all aspects of the proteomics and metabolomics study planning and design, quantitative data analysis, participation in discussions with Drs. Lee and Moellering and their groups, presentation of research in progress in scientific meetings, and preparation of scientific publications.

Funds ($48,480 for Y1 and $48,109 for Y2) are requested for consumable materials, reagents, and supplies to perform the proposed micro-scale metabolomics and proteomics studies.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Hojoon Lee**

eRA COMMONS USER NAME (credential, e.g., agency login): hl2547

POSITION TITLE: **Assistant Professor, Northwestern University**

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	Completion Date	FIELD OF STUDY
Seoul National University, South Korea	B.S.	02/1997	Molecular Biology
Seoul National University, South Korea	M.S.	02/1999	Molecular Biology
University of California Los Angeles	Ph.D.	03/2007	Biological Chemistry
University of California, San Diego and Columbia University, New York / HHMI	Postdoctoral training	12/2018	Neuroscience

A. Personal Statement

I am an assistant professor in the Department of Neurobiology at Northwestern University (start date: January 1st, 2019). My interest lies in understanding the assembly and function of sensory circuits. As a graduate student with Dr. Edward De Robertis at UCLA, I studied how the nervous system is patterned during gastrulation [Cell (2006), Developmental Cell (2008), Genes & Development (2009)]. During post-doctoral training with Dr. Charles Zuker at Columbia University, I focused on the assembly of the peripheral taste system. This is a particularly attractive model system for developmental neuroscience, since the rapid, lifelong turnover of the taste receptor cells on the tongue allows observation of wiring events in adults. We identified connectivity molecules which confer wiring specificity to sweet- vs bitter-taste receptor cells [Nature 548:330-333 (2017)].

While studying taste circuits, I was fascinated to observe that strongly bitter tastants often induce retching or gagging reflexes in mice and reasoned that this could be an entry point to identify the neural circuitry underlying nausea and/or vomiting. Since establishing my independent research group at Northwestern University, we have accumulated a considerable amount of preliminary data, which form the foundation for my proposal, “Ultrasensitive Discovery of Circulating Nausea-Inducing Factors”.

My published body of work demonstrates that I have tackled fundamentally important questions in biology throughout my career. Dr. Zuker encouraged his post-doctoral trainees to develop projects with an independent research focus; this was both exhilarating and challenging (especially considering that all Zuker lab projects targeted only the highest-impact scientific journals). However, I have developed a profound appreciation for the transformative nature of high-risk, high-reward projects. I am also well-versed in diverse methodologies, including molecular biology, mouse genetics, biochemistry, and in vivo functional imaging.

The Covid-19 pandemic has been particularly challenging for a new PI. We had just moved into my newly renovated lab space in February 2020, when we were shutdown. At the very least, this translated into 6 months of salary and animal maintenance costs with no returns in scientific productivity. Although my institution provided me with generous start-up funds, this unforeseen global hardship has brought on financial strain and significant delay in research productivity for my lab.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

1997 – 1999 Graduate Student, Seoul National University, Advisor: Kyungjin Kim
1999 – 2002 Republic of Korea Air Force (mandatory military service)
2002 – 2007 Graduate Student, UCLA, Advisor: Edward M. De Robertis
2007 – 2008 Postdoctoral Researcher, UCLA/HHMI, Advisor: Edward M. De Robertis
2008 – 2014 Postdoctoral Fellow, UCSD/HHMI & Columbia University, Advisor: Charles Zuker
2014 – 2018 Associate Research Scientist, Columbia University, Advisor: Charles Zuker
2019 – present Assistant Professor, Northwestern University

Honors and Awards

1998 Best Poster Award, International Symposium of Asia and Oceania Society for Comparative Endocrinology, South Korea

2005 David Sigman Best Poster Award, Dept. of Biological Chemistry, University of California Los Angeles

2008 Finalist, Helen Hay Whitney and Jane Coffin Childs Foundation Fellowships

2019 Whitehall Foundation Research Grant Award

2022 National Academy of Sciences Kavli Fellow

C. Contributions to Science

1. Extracellular network of interacting proteins that patterns the embryonic dorsal-ventral axis

The classical experiment of making a two-headed newt by transplanting a small piece of embryonic tissue (the 'Organizer') onto another embryo showed that a whole new brain can be created by signals from a mere handful of cells. Ensuing molecular work determined that the main output of this donor tissue are secreted antagonists of growth factors, rather than instructive signals. For example, secreted BMP antagonists Chordin, Noggin and Follistatin, are secreted from the Organizer, locally restricting BMP activity to promote neural fate. As a graduate student, my first interest was in uncovering the function of *sizzled*. This gene is strongly expressed on the ventral (belly) side of the *Xenopus laevis* gastrula embryo, but curiously affected dorsal (brain) development. Notably, Sizzled required the presence of Chordin for activity. What is the function of Sizzled? To understand this, I performed biochemical studies to show that Sizzled promotes Chordin activity by protecting it from degradation from Tolloid proteases such that:

Sizzled ----| Tolloid ----| Chordin ----| BMP

In this way, the extracellular BMP signaling is regulated by multiple layers of interacting proteins, to ensure robust embryonic development. This study also identified a new and unexpected function for the Frizzled cysteine-rich protein domain (CRD) in inhibiting metalloproteinases. This protein domain was previously known as Wnt-binding domains, thus identifying a potential extracellular site for crosstalk between BMP and Wnt signaling pathways.

a. **Lee, H. X.**, Ambrosio, A. L., Reversade, B., and De Robertis, E. M. (2006). Embryonic dorsal-ventral signaling: secreted frizzled-related proteins as inhibitors of tolloid proteinases. **Cell**, 124:147-159. PMCID: PMC2486255
Highlighted in:
Mullins, M (2006) Tolloid gets Sizzled competing with Chordin. ***Developmental Cell*** *10:154-156.*
Kimelman, D. and Szeto, D.P. (2006). Chordin cleavage is sizzling. ***Nature Cell Biology*** *8:305-307.*

I leveraged these findings to establish transformative concepts and new tools. Using biochemical assays developed in the first study, I screened secreted proteins produced during gastrulation for previously uncharacterized protein-protein interactions, to find that 1) a ventrally-expressed chordin-like protein Crossveinless2 antagonizes BMP signaling[b], 2) Crescent, a dorsally-expressed secreted Frizzled related protein promotes neural tissue development by promoting Chordin stability[c], and 3) BMP4 directly inhibits Tolloids to provide negative feedback regulation[d].

b. Ambrosio*, A. L., Taelman*, V. F., **Lee*, H. X.**, Metzinger, C., Coffinier, C., and De Robertis, E. M. (2008). Crossveinless-2 is a BMP feedback inhibitor that binds Chordin/BMP to regulate Xenopus embryonic patterning. **Developmental Cell**, 15:248-260. PMCID: PMC2581521
** Co-first author*

c. Ploper, D., **Lee, H.X.,** and De Robertis, E.M. (2011). Dorsal-ventral patterning: Crescent is a dorsally secreted Frizzled-related protein that competitively inhibits Tolloid proteases. **Developmental Biology**, 352:317-328. PMCID: PMC3088358

d. **Lee, H. X.**, Mendes, F. A., Plouhinec, J.L., and De Robertis, E. M., (2009). Enzymatic regulation of pattern: BMP4 binds CUB domains of Tolloids and inhibits proteinase activity. **Genes & Development**, 23:2551-2562. PMCID: PMC2779747

Overall, my work illuminated the intricate network of extracellular protein-protein interactions, which establish and fine-tune the BMP gradient (high ventrally, but low dorsally) to specify the dorsal-ventral body plan of the embryo, and laid the foundation for ensuing studies, which recreated gastrulation by computational modeling.

2. Re-wiring the taste system

Taste receptor cells (TRCs) dispatch taste information afferent fibers from taste neurons, such that sweet TRCs signal to sweet ganglion neurons, bitter TRCs to bitter neurons, sour to sour, etc. However, TRCs have short lifespans of only about two weeks and are continuously replaced. *How is the fidelity of signal transmission preserved in the face of rapid TRC turnover?* We reasoned that TRCs must express dedicated connectivity molecules to guide wiring with correct partner neurons and compared RNA-seq data from bitter TRCs vs sweet TRCs (these should never cross-wire, in order to prevent bitter toxins from tasting sweet and appetitive). We combined single-cell functional imaging and mouse genetics to demonstrate that sweet and bitter TRCs use distinct Semaphorin connectivity molecules in a deterministic fashion to guide wiring of the peripheral taste system. Indeed, we engineered animals whereby bitter neurons now respond to sweet tastants, sweet neurons respond to bitter, or with sweet neurons responding to sour stimuli[a]. Together, these results uncovered the basic logic of the wiring of the taste system at the periphery[b], and illustrated how a sensory circuit preserves signaling integrity despite rapid and stochastic turnover of receptor cells.

a. **Lee, H.**, Macpherson, L.J., Parada, C.A., Zuker, C.S., and Ryba, N.J.P. (2017) Rewiring the taste system. **Nature** 548, 330–333. PMCID: PMC5805144
Highlighted in:
Li, J. and Luo, L. (2017) Neurobiology: A bitter-sweet symphony. Nature, 548:285-287
Graham, D. (2017) Solving an identity crisis in the taste system. Lab Animal, 46:357
Wired (2017) What a mouse's mixed-up taste buds say about the brain.
Popular Science (2017) We finally know how your tongue tells your brain what you're tasting.

b. Fowler, B., Ye, J., Humayun, S., **Lee, H.**, and Macpherson, L.J. (2022) Revisiting the taste map: regional specialization of the tongue revealed by gustatory ganglion imaging. **iScience** *in press*

Complete list of published work in NCBI/MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/hojoon.lee.1/bibliography/public/

D. Additional Information: Research Support

Ongoing Research Support

Research Grant Award, Whitehall Foundation 04/01/2020 – 03/31/2023
Neural Circuits Underlying Taste Behavior
The goal of this project is to identify molecular markers for taste-responsive cells in the mammalian brainstem.

Completed Research Support

Pilot Grant, NSF-Simons Center for Quantitative Biology 07/01/2021 – 06/30/2022
Mapping the Transcriptional Path of Taste Cell Specification
The goal of this project is to understand taste receptor cell differentiation.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Moellering, Raymond E.

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

POSITION TITLE: Associate Professor of Chemistry

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 Arizona; Tucson, AZ	B. S.	05/2005	Chemistry; Biochemistry and Molecular Biophysics
Harvard University; Cambridge, MA	A.M.	05/2009	Chemistry & Chemical Biology
Harvard University; Cambridge, MA	Ph.D.	05/2010	Chemistry & Chemical Biology
The Scripps Research Institute; La Jolla, CA	Postdoctoral	12/2014	Chemical Biology

A. Personal Statement

I am an Associate Professor in the Department of Chemistry as well as a Core Fellow in the Institute for Genomics and Systems Biology and the Faculty Director for the Proteomics Platform core facility at The University of Chicago. My research interests involve the application of chemical biology, synthetic chemistry, and proteomics to solve challenging problems in biology and human health. We posit that in order to understand molecular information flow under basal or diseased states we must be able to probe biomolecular function and organization in native environments across scales of space – ranging from subcellular complexes, single cells, tissues to live animals – and dynamic kinetic regimes. We have endeavored to accomplish these goals through interconnected programs in chemical probe design, synthesis and optimization; integrated “top-down” and “bottom-up” proteomic profiling technology development; application of these probes and technologies for deep interrogation of signaling mechanisms – with a specific emphasis on metabolism – that are known or hypothesized to be dysregulated in diseases like cancer, aging, chronic inflammation and metabolic disorders. We subsequently apply this information to the development of new chemical strategies for the synthesis of potent and selective inhibitors of causal proteins in disease, with a particular emphasis on those that have historically been difficult to target by conventional small molecule drugs. Ultimately, my research program aims to shift the current paradigm of how we study interactions and activity within the proteome, and in parallel change the definition of tractable proteomic space for the development of diagnostics and therapeutics.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2020 - Associate Professor, Department of Chemistry, University of Chicago, Chicago, IL.
2015 - Member, University of Chicago Comprehensive Cancer Center, Chicago, IL.
2015- Core Fellow, Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL.
2015- Assistant Professor, Department of Chemistry, University of Chicago, Chicago, IL.
2011-2014 Postdoctoral fellow, Department of Chemical Physiology, TSRI, La Jolla, CA.
2005-2010 Doctoral student, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA.

Other Experience and Professional Memberships

2017- Founder and Consultant, Anastasis Biotec Ltd.
2014- 2016 Member, Scientific Advisory Board, Trojantec Ltd.
2011- Member, American Chemical Society
2008- Member, American Association for Cancer Research

Honors (selected)

2020 Sloan Research Fellowship
2019 NSF CAREER Award
2017 NIH Director's New Innovator Award
2017 Research Scholar Award, American Cancer Society
2016 Mary Kay Foundation Cancer Research Award
2015 V Scholar Award, V Foundation for Cancer Research
2014 K99/R00 Pathway to Independence Award, National Cancer Institute/NIH
2014 Dale F. Frey Award for Breakthrough Scientists, Damon Runyon Cancer Research Foundation
2011 HHMI-Damon Runyon Postdoctoral Fellowship
2009 Lilly Singapore Centre for Drug Discovery Keystone Symposia Scholarship
2008 American Association for Cancer Research Centennial Predoctoral Fellowship
2008 Vertex Pharmaceuticals Excellent Graduate Research Award
2008 Gerald B. Grindey Memorial Scholar-in-training Award, AACR
2007 ACS/Gesellschaft Deutscher Chemiker Invited Scholar Exchange

C. Contributions to Science

1. The field of proteomics aims to provide a comprehensive accounting of the compliment of proteins in a biological sample of interest. In the decade since the development of orbitrap mass spectrometers and analysis algorithms, the field of proteomics has found mainstream applications in basic chemical, biological and clinical research. Despite the maturity of these technologies, standard proteomic platforms are typically limited to providing two pieces of information: whether a specific protein is present in a sample and the relative abundance of a protein of interest within a sample. While powerful, these approaches omit information on cellular heterogeneity, spatial localization, they are often not directly compatible with limited abundance primary tissues, and importantly, they do not provide quantitative information on protein activity. My laboratory is creating chemical proteomic technologies that move away from signal-averaged, abundance-based proteomic profiling approaches that are primarily applied in cell culture. We are focused on establishing two new paradigms in the application of chemical proteomic technologies to provide novel insights into the role of metabolic enzymes to tumor development and metastasis – simultaneously moving away from bulk cell culture and cell lysate-based experiments. We have developed a modular platform enabling the direct interrogation of enzyme *activity* in single cells with sub-cellular and intercellular spatial resolution. This technology, named ADPL, does not require any genetic manipulation of biological samples (i.e. compatible with patient samples) and should be portable to essentially any active protein or enzyme of interest in a wide array of biological contexts. On the other end of the spectrum, we are developing highly selective small molecule probes to directly image metabolic enzyme activities in live animals. More generally, further development of these unique proteomic technologies will enable the widespread interrogation of metabolic activity in cancer and other diseases at scale and resolution currently out of reach with standard proteomic methods.

   a. Chang JW, Bhuiyan M, Tsai HM, Zhang HJ, Li G, Fathi S, McCutcheon DC, Leoni L, Freifelder R, Chen CT & **Moellering RE*.** "In vivo imaging of the tumor-associated enzyme NCEH1 with a covalent PET probe." *Angew. Chem. Int. Ed.,* **2020**; 59:15161-15165.
   b. Li G, Eckert MA, Chang JW, Montgomery JE, Chryplewicz A, Lengyel E & **Moellering RE***. "Ultrasensitive, multiplexed chemoproteomic profiling with soluble activity-dependent proximity ligation. *Proc. Natl. Acad. Sci. U.S.A.,* **2019***,* 116(43): 21493-500.
   c. Li G & **Moellering RE*.** "A concise, modular antibody-oligonucleotide conjugation strategy based on disuccinimidyl ester activation chemistry." *ChemBioChem*, **2019**; 20*:*1599-1605.
   d. Li G, Montgomery JE, Eckert MA, Chang JW, Tienda SM, Lengyel E & **Moellering RE***. "An activity-dependent proximity ligation platform for spatially resolved quantification of active enzymes in single cells." *Nat*. *Commun.,* **2017**; *8*(1): 1775.

2. To complement the targeted (*i.e.* bottom-up) ultrasensitive activity-based profiling methods above, my group is also developing and applying global (*i.e* top-down) proteomic platforms to understand functional organization of and crosstalk within biomolecules and the proteome. This includes new proteome-wide interaction profiling methods to interrogate the functional consequences of protein posttranslational modifications, dynamic protein-metabolite and protein-protein interaction networks, and how these events are orchestrated within and between cells.
    a. Huang JX, Coukos JE & **Moellering RE***. "Interaction profiling methods to map protein and pathway targets of bioactive ligands." *Curr. Opin. Chem. Biol.*, **2020**; 54: 76-84.
    b. McCutcheon DC, Lee G, Carlos A, Montgomery JE & **Moellering RE*.** "Photoproximity profiling of protein-protein interactions in cells." *J. Am. Chem. Soc.*, **2019**; 142(1): 146-53.
    c. Huang JX, Lee G, Cavanaugh K, Chang JW, Gardel M & **Moellering RE***. "High throughput discovery of functional protein modifications with hotspot thermal profiling**.** *Nat. Methods,* **2019;** 16(9): 894-901.
    d. Eckert MA, Coscia F, Chryplewicz A, Chang JW, Hernandez KM, Pan S, Tienda SM, Nahotko DA, Li G, Blazenovic I, Lastra RR, Curtis M, Yamada SD, Perets R, McGregor SM, Andrade J, Fiehn O, **Moellering RE**, Mann M & Lengyel E. "Proteomics reveals NNMT as a master metabolic regulator of cancer-associated fibroblasts." *Nature,* **2019**; 569(7758): 723-8.

3. My group is working on harnessing selective chemical transformations on peptides and proteins to develop new classes of peptidomimetics targeting protein-protein and protein-DNA interactions. I have developed synthetically stabilized peptides and miniproteins targeting critical protein-protein interfaces in several oncogenic complexes, including Notch1, β-catenin and RAB25. Several of these probe molecules are now commercially available and have been used in dozens of published studies into oncogenic signaling in cancer and other biological contexts. These studies represent seminal proof-of-concept cases for the development of direct acting inhibitors of multi-protein transcription factor complexes, either with stabilized peptidomimetics or more traditional small molecules, and there are several compounds in this class currently in pre-clinical and clinical testing. Current areas of research in our lab are focused on developing novel chemistries to modify and augment peptides and proteins, with a focus on bioorthogonal reactions that can operate in aqueous conditions. Building on these chemistries we are focused on developing new classes of larger, tertiary domain mimics that fall in between traditional small molecules and protein therapeutics – so-called synthetic biologics – to target more difficult protein-protein and protein-DNA interactions.
    a. Speltz TE, Qiao Z, Swenson CS, Shangguan X, Coukos JS, Lee CW, Thomas, DM, Santana J, Fanniing, SW, Greene GL, **Moellering RE***. "Targeting MYC with modular synthetic transcriptional repressors derived from bHLH DNA-binding domains." *Nat. Biotechnol.* **2022**. doi: 10.1038/s41587-022-01504-x.
    b. Montgomery JE, Donnelly JA, Fanning SW, Speltz TE, Coukos JS, Greene GL & **Moellering RE***. "Versatile peptide macrocyclization with Diels-Alder cycloadditions." *J. Am. Chem. Soc,* **2019**, 141(41): 16374-81.
    c. Mitra S, Montgomery JE, Kolar MJ, Li Gang, Jeong KJ, Peng B, Verdine GL, Mills GB & **Moellering RE***. "Stapled peptide inhibitors of RAB25 target context-specific phenotypes in cancer." *Nat. Commun*., **2017**; *8*(1): 660.
    d. **Moellering RE**, Cornejo M, Davis TN, Del Bianco C, Aster JC, Blacklow SC, Kung AL, Gilliland DG, Verdine GL & Bradner JE. "Direct inhibition of the NOTCH transcription factor complex." *Nature.* **2009**; *462*:182-188.

4. A fundamental question in biochemistry is how metabolic status is communicated throughout cells and tissues. Integrated with our work on protein modifications my group has pursued the question of whether reactive metabolites, which arise though the oxidative chemical manipulations performed by metabolic enzymes, chemically modify proteins or other biomolecules in native environments. Beyond this initial question are functional extensions, including whether such modifications are selective, occur at high or low stoichiometry, impart structural and functional consequences, if they represent inherent regulatory mechanisms conserved through evolution, and ultimately, whether these modifications may serve as a way to integrate signals from metabolism to other cell signaling pathways. My group has discovered several previously unknown protein post-translational modifications that are formed by the non-enzymatic

reaction between reactive primary metabolites and proteins in diverse organisms. These findings have provided the first evidence of a direct connection between glycolytic flux and alterations in the structure and function of key proteins, including glycolytic enzymes themselves, pointing to the existence of an ancient and likely biomedically important intrinsic feedback pathway in mammalian cells. More directly, this work implicates reactive metabolite-induced modifications as a mechanism to integrate core metabolism with numerous other signaling pathways. We are continuing to explore the capacity for these reactive metabolites and the modifications they form to serve as diffusible signals in both normal and diseased biological processes. Additionally, we have developed several small molecules that regulate these pathways and the disease phenotypes that result from them.

a. Coukos JS, **Moellering RE*.** "Methylglyoxal forms diverse mercaptomethylimidazole crosslinks with thiol and guanidine pairs in endogenous metabolites and proteins." *ACS Chem. Biol.,* **2021**; 16(11):2453-2461.
b. Bollong MJ, Lee G, Coukos JS, Yun H, Zambaldo C, Chang JW, Chin EN, Ahmad I, Chatterjee AK, Lairson L*, Schultz PG* & **Moellering RE***. "A metabolite-derived protein modification integrates glycolysis with KEAP1-NRF2 signaling." *Nature,* **2018**; *562*(7728): 600-4.
c. Chang JW, Montgomery JE, Lee G **& Moellering RE***. "Chemoproteomic profiling of phosphoaspartate modifications in prokaryotes." *Angew. Chem. Int. Ed.,* **2018**; *57*(48): 15712-6.
d. **Moellering RE*** & Cravatt BF*. "Functional lysine modification by an intrinsically reactive primary glycolytic metabolite." *Science*, **2013**; *341*(6145): 549-53. * - Co-corresponding authors

5. Tumor progression is marked by altered cellular metabolism, which is followed by microenvironmental changes in oxygen content and extracellular pH. The direct cause and consequences of these alterations are unknown, however their correlation to tumor aggressiveness and clinical endpoints is firmly established. In line with my general interests in deregulating metabolic signaling in cancer, I sought to further our understanding of acidosis in selecting for more malignant phenotypes in aggressive cancers. By developing several novel in vitro assays of cell migration and invasion coupled with methods to acclimate cells to grow under low pH conditions, I was able to show that melanoma cells selected by environmental acidosis display increased motility, in vitro invasion and metastasis in SCID mice. Transcriptional profiling also highlighted several pathways involved in these phenotypes for further study. Importantly, this study reinforced the hypothesis that extracellular acidosis results in selection of stable, resistant populations, rather inductive changes to the existing tumor population.

a. **Moellering RE**, Black K, Krishnamurthy C, Baggett BK, Stafford P, Rain M, Gatenby RA & Gillies RJ. "Acid treatment of melanoma cells selects for invasive phenotypes." *Clin Exp Metastasis.* **2008**; *25*:411-25.

PHS OTHER SUPPORT
For All Application Types – DO NOT SUBMIT UNLESS REQUESTED

There is no "form page" for reporting Other Support. Information on Other Support should be provided in the format shown below.

*Name of Individual: Raymond Moellering
Commons ID: rmoeller

Other Support – Project/Proposal

Title: “CAREER: Reactivity-Driven Metabolic Signaling: A Feature not a Flaw in Metabolic Regulation”

*Major Goals: The goal is to Develop an integrated suite of chemical probes, proteomic methods and cellular model systems to discover and quantify the role of a prototypical intrinsic reactive metabolite, methylglyoxal, in regulating cellular homeostasis and complex phenotypes in cells and whole organisms.
*Status of Support: Active

*Project Number: CHE1945442

*Name of PD/PI: Moellering, R.

*Source of Support: NSF

*Primary Place of Performance: University of Chicago

*Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2019-11/2024

* Total Award Amount (including Indirect Costs): $730,000

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)	Amount Per Year
1. 2021	.8 Academic	$ 146,000
2. 2022	.8 Academic	$ 146,000
3. 2023	.8 Academic	$ 146,000

Title: “Chemical Proteomic Platform Development "

*Major Goals: The goal is to develop novel chemical probes to target causal proteins in disease.

*Status of Support: Active

*Project Number: FG-2020-12839

*Name of PD/PI: Moellering, R.

*Source of Support: Alfred Sloan Foundation

*Primary Place of Performance: University of Chicago

*Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2020-09/14/2022

* Total Award Amount (including Indirect Costs): $75,000

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)	Amount Per Year
2. 2021	.02 Academic	$ 37,500

Title: "Reading and Writing Metastatic Protein Signatures in Breast Cancer"

*Major Goals: Design, synthesis and proteomic characterization of activity probes targeting protein families implicated in breast cancer malignancy

*Status of Support: Active

*Project Number: CCR21663985

*Name of PD/PI: Moellering, R.

*Source of Support: Susan G. Komen Research Programs

*Primary Place of Performance: University of Chicago

*Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2021-08/2024

* Total Award Amount (including Indirect Costs): $450,000

* Person Months (Calendar/Academic/Summer) per budget period

Year (YYYY)	Person Months (##.##)	Amount Per Year
1. 2021	.50 Academic	$ 150,000
2. 2022	.50 Academic	$ 150,000
3. 2023	.50 Academic	$ 150,000

Title: "Single-Cell Protein Activity Profiling in Breast Cancer Cells and Tissues"

*Major Goals: The goal is to create modular, high-throughput proteomic platforms for multiplexed activity profiling in primary patient tissues and cells.

*Status of Support: Active

*Project Number: 1R33CA269094-01

*Name of PD/PI: Moellering, R PI; Tay, S. Co-I

*Source of Support: NIH

*Primary Place of Performance: University of Chicago

*Project/Proposal Start and End Date: (MM/YYYY) (if available): 06/2022-04/2025

* Total Award Amount (including Indirect Costs): $1,148,001

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	1 Academic
2. 2023	1 Academic
3. 2024	1 Academic

Title: "Mapping Protein Social Network Dynamics with Photoproximity Profiling Platforms"

*Major Goals: The goal is to develop and validate several complementary, light-dependent proximity profiling platforms capable of detecting protein interaction dynamics in live cells with high spatial and temporal resolution, as well as minimal perturbation to the cellular environment.

*Status of Support: Active

*Project Number: GM145852-01

*Name of PD/PI: Moellering, R.

*Source of Support: NIH

*Primary Place of Performance: University of Chicago

*Project/Proposal Start and End Date: (MM/YYYY) (if available):09/2022-08/2026

* Total Award Amount (including Indirect Costs): $1,304,172

* Person Months (Calendar/Academic/Summer) per budget period

Year (YYYY)	Person Months (##.##)
1. 2022	1 Academic
2. 2023	1 Academic
3. 2024	1 Academic
4. 2025	1 Academic
5. 2026	1 Academic

Title: "In vivo detection of TNBC aggressiveness using activity based probes"

*Major Goals: The goal is to leverage our knowledge of chemical probe design and synthesis, in vivo TNBC modeling, and live animal imaging to assess if clinically-relevant correlations between active NCEH1 and tumor aggressiveness exist.

*Status of Support: Pending

*Project Number: GRANT13490514

*Name of PD/PI: Moellering, R.

*Source of Support: NIH

*Primary Place of Performance: University of Chicago

*Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2022-03/2024

* Total Award Amount (including Indirect Costs): $451,000

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	.50 Academic
2. 2023	.50 Academic

Title: “Targeting the Hypoxic Response in TNBC with Synthetic Transcriptional Repressors”

*Major Goals: The goal is to develop a novel class of direct transcription factor inhibitors to simultaneously block XBP1 and HIF1a, which are two master regulators of the hypoxic stress response in triple negative breast cancer (TNBC) that is critical for tumor growth, survival and metastasis.

*Status of Support: Pending

Project Number:

Name of PD/PI: (PI: Moellering, R; PI:Rosner, M; PI: Oakes, S.)

*Source of Support: NIH

*Primary Place of Performance: University of Chicago

Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2023-03/2028

* Total Award Amount (including Indirect Costs): $4,015,620

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	1.5 Summer
2. 2023	1.5 Summer
3. 2024	1.5 Summer
4. 2025	1.5 Summer
5. 2026	1.5 Summer

***Overlap** (summarized for each individual):

Funding in grant CCR21663985 is focused on developing and validating small molecule chemical probes and microfluidic cell processing protocols to enrich circulating tumor cells from patient blood samples prior to ADPL profiling. These Aims are all distinct from those proposed in the 1R33CA269094-01 proposal.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature:_____

Date: _____

THE UNIVERSITY OF CHICAGO

November 16, 2022

Raymond E. Moellering, PhD
Associate Professor
Department of Chemistry
University of Chicago

Hojoon Lee, PhD
Assistant Professor
Department of Neurobiology
Northwestern University

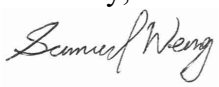
Dear Raymond and Hojoon,

I am happy to express my support for your CBC-Catalyst proposal entitled "Ultrasensitive Discovery of Circulating Nausea-Inducing Factors."

As you know, the Proteomics Platform at the University of Chicago is a mass spectrometry-based proteomics research facility located in the Knapp Center for Biomedical Discovery (KCBD). Dr. Raymond Moellering is the faculty director of the Platform. I am the technical director and manage its day-to-day operations. The mission of the facility is to work in collaboration with groups from UChicago and external organizations or companies to develop and utilize top-notch proteomics techniques to answer both basic and translational scientific questions. The platform has state-of-the-art mass spectrometry instruments including Thermo Scientific Orbitrap Eclipse Tribrid Mass Spectrometer and Thermo Scientific Orbitrap Exploris 480 Quadrupole Mass Spectrometer coupled with UltiMate 3000 RSLCnano liquid chromatography system and FAIMS Pro Interface. The Proteomics Platform has the capabilities for various proteomics analysis such as global, targeted and quantitative proteomics, deep proteome analysis, post-translational modification analysis, thermal profiling analysis, immunopeptidomics, and clinical proteomics. The Platform also supports scientific consultation tailored to each project, proteomics sample preparation, and education to help researchers design, perform, and interpret complex proteomics data.

We are looking forward to assisting you and your team perform micro-scale quantitative proteomic detection efforts using the Eclipse instrument and TMT labeling to identify and measure factors present in the cerebral spinal fluid of mice experiencing motion sickness-induced nausea.

Sincerely,



Shao Huan Samuel Weng
Proteomics Platform Director
University of Chicago

AT THE FOREFRONT
UChicagoMedicine
Comprehensive Cancer Center

November 17, 2022

Raymond E. Moellering, PhD
Associate Professor
Department of Chemistry
University of Chicago

Hojoon Lee, PhD
Assistant Professor
Department of Neurobiology
Northwestern University

Dear Raymond and Hojoon,

As the Technical Director of The Metabolomics Platform at the University of Chicago, I am happy to express my support for your CBC-Catalyst proposal entitled "**Ultrasensitive Discovery of Circulating Nausea-Inducing Factors**."

Our platform can provide you with expert metabolomics and lipidomics assays. These will include separation, characterization, identification, and quantification of a diverse group of polar metabolites and non-polar lipid species .The facility is equipped with state-of-the-art high resolution Thermo Scientific Orbitrap IQ-X Tribrid mass spectrometer (+UVPD) coupled to a Vanquish Horizon UHPLC system to provide steady-state measurements and is skilled in performing stable isotope labeling using 13C, 15N, and deuterated labeled metabolites. We have experience in quantifying polar metabolites and non-polar lipid species in a wide variety of matrices such as cells, culture medium, bodily fluid and flash-frozen tissue samples.We support hypothesis-generating untargeted and hypothesis-driven targeted metabolomics studies from the experimental design stage through data acquisition, data analysis, and interpretation.

We are looking forward to assisting you and your team perform micro-scale quantitative metabolomics to identify and measure polar and lipid-based metabolites present in the cerebral spinal fluid of mice experiencing motion sickness-induced nausea.

Sincerely,

Hardik Shah
Technical Director
Metabolomics Platform
University of Chicago Medicine Comprehensive Cancer Center

900 E 57th street, KCBD-4142, CHICAGO, IL 60637-1470
PHONE: 1-773-834-8830