Gellman Group Foldamer Reunion and Alumni Research Symposium

August 9 - August 11, 2019
Madison, Wisconsin

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Gellman Group Foldamer Reunion and Research Symposium

Friday, August 9, 2019: Opening Reception

5:00 pm - 8:00 pm Grainger Hall, Room 1530 Capital Cafe

Saturday, August 10, 2019: Alumni Research Symposium

7:45 am - 8:30 am Gathering and refreshments

8:30 am - 8:45 am Opening remarks, Will Pomerantz and Jon Lai

Session 1: From Foldamers to Immunology and Biology
8:45 am - 10:15 am 12 minute talks/3 minute Q&A

10:15 am - 10:45 am Coffee break

Session 2: From Foldamers to Therapeutics
10:45 am - 12:30 pm 12 minute talks/3 minute Q&A

12:30 pm - 2:00 pm Lunch

Session 3: Frontiers in Peptide and Foldamer Chemistry
2:00 pm - 3:30 pm 12 minute talks/3 minute Q&A

3:30 pm - 4:00 pm Coffee break

Session 4: Beyond Foldamers
4:00 pm - 5:30 pm 12 minute talks/3 minute Q&A

5:30 pm Closing remarks, Julie Plotkin and Sam Gellman

Saturday, August 10, 2019: Symposium Dinner and Reception

6:30 pm - 9:30 pm Fluno Center

Sunday, August 11, 2019: Informal Breakfast

9:00 am - 12:00 pm Memorial Union, Tripp Commons

Map of reunion event locations is on page 13.

Alumni Research Symposium Detailed Schedule and Speaker Abstracts

Session 1: From Foldamers to Immunology and Biology

Session Chairs: Alumni: Terra Potocky, Regeneron Pharmaceuticals; Current Gellmanite: Shi Liu

8:45 am – 10:15 am 15 minute time slots; 12 minute talks/3 minute Q&A

Paul Savage, Brigham Young University

"*Converting bacterial antigens into virus-like vaccines to generate high affinity antibodies to capsular glycans*"

Vaccines based on bacterial capsular glycans typically do not generate memory responses and stimulate production of only low-affinity antibodies. Obstacles for generation of optimal responses include the polyvalent nature of the glycans and lack of T cell help allowing class switching from IgM to IgG. To overcome these obstacles, we are synthesizing the repeating units of bacterial glycans, conjugating them on virus-like particles and adding a potent adjuvant for natural killer T cells. The resulting vaccines elicit cognate T cell help for B cells, class switching and affinity maturation leading to production of nanomolar affinity antibodies and memory responses.

Christian Hackenberger, FMP Berlin

"*Better structured? Protein conjugates for multivalent targeting*"

Multivalent ligand–receptor binding mediates several essential biological interactions. A particularly prominent example is the cellular uptake of the influenza virus, as viral infection occurs by binding of sialic acid terminated cell-surface glycans to the trimeric viral membrane protein hemagglutinin (HA).

For the design of an influenza inhibitor, we proposed to take advantage of the structural parameters of the HA-trimer. This concept allowed us to engineer a monodisperse multivalent protein-scaffold with tunable valency, spatial arrangement and ligand identity. The obtained conjugates were tested in *in vivo* infection inhibition assays revealing a highly potent inhibitor against human and avian viral strains with impressive binding and inhibition parameters.

Jon Lai, Albert Einstein College of Medicine

"*Protective human monoclonal antibodies against Chikungunya virus isolated from convalescent patients in the Bronx*, NY"

Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus that causes persistent arthritis in a subset of human patients. We report the isolation and functional characterization of monoclonal antibodies (mAbs) from two patients infected with CHIKV in the Dominican Republic. Single B cell sorting yielded a panel of 46 human mAbs of diverse germline lineages that targeted epitopes within the E1 or E2 glycoproteins. MAbs that recognized either E1 or E2 proteins exhibited neutralizing activity. Viral escape mutations localized the binding epitopes for two E1 mAbs to sites within domain I or the linker between domains I and III; and for two E2 mAbs between the β-connector region and the B-domain. Two of the E2-specific mAbs conferred *in vivo* protection in a stringent lethal challenge mouse model of CHIKV infection, whereas the E1 mAbs did not. These results provide insight into human antibody response to CHIKV and identify candidate mAbs for therapeutic intervention.

Meg Schmitt, University of Colorado Denver

"*Multifunctional M13 phage particles as the components of molecular diagnostics*"

The nanoscale size, simple life cycles, and speed and ease with which they can be prepared, manipulated, and characterized render phages attractive and versatile systems for myriad biotechnological applications. M13 phage are most the commonly used system for phage display, which involves the creation and screening of large libraries of peptides or proteins displayed on the surface of the phage particle as a fusion to one of the coat proteins. We have described the first phage display libraries of an alternative scaffold protein, Sso7d from the hyperthermophilic archeon *Sulfolobus solfataricus* and demonstrated proof-of-concept evolution of binding molecules for a variety of sizes and shapes of target proteins. Generating highly decorated phage is challenging because the small size and close packing of phage coat proteins as well as the phage assembly process all place constraints on the display of fusion proteins. One approach to overcome the bias against the display of large proteins on phage is to develop functional proteins of reduced size. We have recently utilized the Sso7d library to identify nanomolar binding proteins against a Mycobacterium tuberculosis protein which is secreted in urine during active tuberculosis infections. From a diagnosis standpoint, targeting biomarkers that are not blood-borne is of particular import in locations where medical infrastructure and personnel are lacking. We are converting the evolved Sso7d variant along with monoclonal antibodies into non-invasive diagnostic tests for active tuberculosis infection. From an application standpoint, phage particles are attractive as molecular recognition systems because they feature improved physical stability and cost of production relative to traditional antibody reagents. Because all of the components of the sensors are created and assembled as part of the phage life cycle process, these sensors will be incredibly inexpensive to manufacture.

Runhui Liu, East China University of Science and Technology

"*New synthetic strategies to construct peptide polymers with biological functions*"

Peptide polymers have been extensively studied in a broad field including biological applications. Although the synthesis of peptide polymers is considered as a common and routine chemistry, it still has unsolved shortcomings. The current imperfect synthesis also hindered the development of this field. To address some of the long-lasting challenges in peptide polymer synthesis, we developed news synthetic strategies and explored the biological functions of some resulting polypeptides.

Michael Giuliano, College of Charleston

"*Sequential and environmental determinants of neuropeptide conformation*"

The neuropeptides are a class of signaling peptides that encompass a broad array of often interrelated functions. These include analgesia, nociception, and the regulation of appetite, circadian rhythms, and mood. Despite their importance, many neuropeptides also lack representation in the protein data bank, and few existing structures are determined under conditions that accurately mimic their signaling environment - the biological membrane. This talk provides some highlights of our efforts to biophysically characterize and determine NMR structures of select neuropeptides and their fragments in both aqueous buffer and in the presence of lipid bicelles.

10:15 am – 10:45 am Coffee Break

Session 2: From Foldamers to Therapeutics

Session Chairs: Alumni: Erik Hadley, Stemcell Technologies; Current Gellmanite: Naomi Biok

10:45 am – 12:30 pm 15 minute time slots; 12 minute talks/3 minute Q&A

Gui-Bai Liang, Independent consutlant

"*Novel GPR40 agonists for the treatment of the type 2 diabetes*"

Detailed SAR analysis led to the design of a novel class of highly pre-organized GPR40 agonists that are very potent and selective *in vitro* and are efficacious in preclinical animal models.

Matt Woll, PTC Therapeutics

"*To splice or not to splice: Can small molecules answer the question?*"

Orally bioavailable small molecules that target RNA and RNA related processes have a delivery and distribution advantage over oligonucleotides. However, targeting specific RNA sequences or structures with small molecules remains a significant challenge. We have recently reported success in shifting SMN2 pre-mRNA splicing to include exon 7, thus increasing levels of full length SMN protein in SMA patient derived cells. Chemical optimization of these molecules has yielded compounds that increase SMN protein and ameliorate disease phenotype in mouse models of SMA. Two optimized compounds have advanced to human clinical trials and have demonstrated a dose-dependent effect on SMN2 splicing and SMN protein increase in humans.

Justin Murray, Amgen

"*Discovery of tarantula venom-derived* $Na_V1.7$ *inhibitory peptides*"

Drug discovery research on new pain targets with human genetic validation, including the voltage-gated sodium channel $Na_V1.7$, is being pursued to address the unmet medical need for chronic pain and the rising opioid epidemic. As part of early research efforts on this front, we have previously developed $Na_V1.7$ inhibitory peptide-antibody conjugates with tarantula venom-derived GpTx-1 toxin peptides with extended half-life in rodents but only moderate *in vitro* activity and without *in vivo* activity. We identified the more potent peptide JzTx-V from our natural peptide collection and improved its selectivity against other sodium channel isoforms through positional analoging. Here we report utilization of the JzTx-V scaffold in a peptide-antibody conjugate and architectural variations in linker, peptide loading, peptide charge, and antibody attachment site that resulted in a compound with improved target exposure and moderate activity in a $Na_V1.7$-dependent pharmacodynamic model but requires further optimization to identify a conjugate that can fully engage $Na_V1.7$ *in vivo*.

Emily English, Gemstone Biotherapeutics

"*Gemstone Biotherapeutics*"

Gemstone Biotherapeutics is a clinical stage regenerative medicine company that was spun out of Johns Hopkins University. CEO Emily English will share lessons learned over the course of her last two years' work, since joining the company in 2017.

Zvi Hayouka, The Hebrew University

"*Development of novel antimicrobial peptides*"

Pathogenic infections present a relentless threat to human health, while the overuse of antibiotics in medicine and agriculture has contributed to the rise of dangerous antibiotic-resistant pathogens. Another major concern is the phenomenon of bacterial persistence that manage to evade the effect of antibiotics by entering a state of dormancy. In my lab we aim to cope with bacterial resistance and persistence by developing peptide-based, antimicrobial agents. We are exploring three unique types of compounds: random antimicrobial peptide mixtures, bacterial toxin-antitoxin inhibitors, and quorum-sensing quenchers to disrupt bacterial cell-to-cell communication. These are novel approaches with immense potential for the development of treatments against problematic pathogens to improve human health.

Ross Cheloha, Boston Children's Hospital/Harvard Medical School

"*Exploration of cell surface protein operation using non-natural protein-peptide conjugates*"

Cell surface proteins are essential for intercellular communication and the maintenance of homeostasis. Characterizing the precise roles that cell surface proteins fulfill and devising methods to selectively activate them are challenging. We have produced conjugates comprised of peptides and camelid single domain antibodies (nanobodies) that serve to report on and modify cell surface protein movement and function. These conjugates have proven valuable for monitoring protein internalization, modulating peptide immunogenicity and selectively activating specific G-protein coupled receptors.

12:30 pm – 2:00 pm Lunch

Session 3: Frontiers in Peptide and Foldamer Chemistry

Session Chairs: Alumni: Tim Peelen, Lebanon Valley College; Current Gellmanite: Kate Kurgan

2:00 pm – 3:30 pm 15 minute time slots; 12 minute talks/3 minute Q&A

Seth Horne, University of Pittsburgh

"*Foldamer mimics of protein tertiary structure*"

The structural diversity of proteins is vast and the foundation for their functional versatility in nature. Impressive strides have been made in developing artificial backbones that fold in defined ways; however, leveraging this precedent to reproduce complex tertiary folding patterns remains a significant challenge. This talk will describe recent work toward a general method for creating foldamer mimics of protein tertiary structure through the systematic alteration of backbone covalent structure in natural sequences. The impact of backbone modification on structure, folding thermodynamics, and biological properties will be surveyed in several prototype systems.

James Checco, University of Illinois

"*Exploring the signaling of D-amino acid-containing neuropeptides*"

Neuropeptides are cell-to-cell signaling molecules used to facilitate communication among neurons within the central nervous system. Although peptides translated by the ribosome contain exclusively L-amino acid residues, neuropeptides in several animals undergo the enzyme-catalyzed isomerization of an amino acid residue from the L-stereoisomer to the D-stereoisomer. However, because L- to D-residue isomerization is challenging to detect by many peptide characterization methods, D-amino acid-containing peptides are difficult to identify and little is known about the full consequences of L- to D-residue isomerization. Our research aims to advance our understanding of the role of L- to D-residue isomerization in neuropeptide signaling.

David Mortenson, Scripps Research Institute

"*(Quasi)racemic crystallization for the study of native and non-native protein structure*"

Proteins are chiral entities, and as a result, are able to crystallize in only 65 of 230 possible three-dimensional space groups. Previous studies have indicated that cocrystallization of L and D forms of a protein of interest (prepared via chemical synthesis) can facilitate crystal growth from otherwise recalcitrant proteins, due to the accessibility of additional space groups. This talk will focus on applications of racemic and "nearly-racemic" crystallization methods to the study of heterochiral protein-protein interactions (i.e., occurring between L- and D-polypeptides), and assessment of how non-natural amino acids fit into native protein structures.

(David E. Mortenson, Jay D. Steinkruger, Dale F. Kreitler are all involved in this Gellman lab work.)

Soo Hyuk Choi, Yonsei University

"*Unconventional peptide helices with switchable handedness*"

Mixed helices, such as the β-peptide 12/10-helix and the 1:1 α/β-peptide 11/9-helix arise from two types of intramolecular hydrogen bonds with alternating directionality. β- or α/β-peptides that contain cis-2-aminocycloalkanecarboxylic acid could display both right- and left-handed mixed-helical conformations in solution. These unconventional peptide helices with switchable handedness are potential scaffolds for stimuli-responsive functional oligomers. In that regard, we have explored diverse internal and external factors that may control the helical screw sense with a variety of examples: incorporation of a central residue with a specific constraint, modification of terminal capping, length of oligomers, solvent conditions.

Josh Price, Brigham Young University

"*How much is that salt bridge worth? The context-dependence of non-covalent interaction strength in peptides and proteins*"

Non-covalent interactions play a critical role in protein folding and conformational stability. Understanding these interactions is crucial to developing methods for predicting protein secondary, tertiary and quaternary structure, especially for proteins with limited sequence homology to well-characterized proteins. Efforts to assess the strength of a non-covalent interaction between two amino acid residues typically rely on double mutant cycle analysis, which depends on the assumption that the two residues of interest interact exclusively with each other and not with any other nearby amino acids. This assumption is useful to a first approximation but seems unlikely to be generally true given the structural complexity of most proteins. Instead, one might expect the strength of some binary non-covalent interactions to be substantially influenced by one or more additional nearby amino acid side chains. Computational predictions, bioinformatic studies, and triple mutant cycle analyses suggest that the synergistic coupling of three groups (i.e., a ternary interaction; sometimes called cooperativity) is feasible within the complicated architecture of proteins. Here we show that placing a non-polar or aromatic side chain between two oppositely charged residues along the solvent-exposed face of an α-helix facilitates a synergistic three-way interaction that would not otherwise be possible. We also discuss recent progress toward understanding how site-specific protein PEGylation influences the strength of a nearby salt bridge.

Younghee Shin, Seoul National University

"*Discovery of neuro-protective small molecule regulating proteostasis by regulating PERK signaling in stress responsive manner*"

A number of neurodegenerative diseases including tauopathies are caused by an abnormal proteostasis and accumulation of aggregation-prone proteins in neurons. In order to find small molecules suppressing Tau protein aggregation in cells, we performed a phenotype-based screening using HEK293 Tau BiFC (Bimolecular Fluorescence Complementation)-Venus cells. SB1617 was selected as a leading compound after the structure activity relationship study with its high potency in reducing tau protein oligomerization and low cytotoxicity. We obtained a possible target protein list of SB1617 by applying FITGE (Fluorescence difference in two-dimensional gel electrophoresis) and TS-FITGE (thermal shift FITGE) methods. Further gene knockdown experiments for those proteins, biophysical tests and in vitro bio-functional tests revealed that SB1617 activates PERK signaling under the cell stressed conditions. Stimulated PERK signaling by SB1617 caused sustained EIF2alpha phosphorylation, which suppressed global protein synthesis and relieved ER workload. The downstream of PERK signaling, ATF4 level was transiently enhanced by SB1617 that is a transcription factor regulating autophagic genes. When Traumatic Brain Injury model mice were treated with SB1617, hippocampal and cortical region showed enhanced autophagy and increased level of Brain-derived neurotrophic factor (BDNF) compared to the control group. SB1617 was effective in ameliorating motor neuron behavior in TBI mice. Further elucidation of mechanism regarding conditional PERK activation and proteostasis regulation will accelerate developing therapeutics for neurodegenerative diseases.

3:30 pm - 4:00 pm Coffee Break

Session 4: Beyond Foldamers

Session Chairs: Alumni: Marlies Hager, University of Illinois at Urbana-Champaign; Current Gellmanite: Victor Outlaw

4:00 pm – 5:30 pm 15 minute time slots; 12 minute talks/3 minute Q&A

Nick Fisk, University of Colorado Denver

"*Dissecting the factors that determine the fidelity of the genetic code using sense codon reassignment*"

I will describe our work on orthogonal pair-directed partial genetic code reassignments as a step toward exploring the space of possible genetic codes. We have developed a simple fluorescence-based screen to quantitatively evaluate the extent to which the meaning of sense codons can be reassigned in *E. coli* by introduced orthogonal translational machinery. Comparisons of the efficiency of reassignment at different codons and between orthogonal pair systems enable the dissection of factors contributing to the fidelity of translation.

Engineering the genetic code requires a detailed understanding of the molecular and systems level interactions governing the entire process of translation. The extent to which the degeneracy of the genetic code can be broken is not obvious *a priori*, and quantitative measurements of directed sense codon reassignments provide a unique data set that contributes to unraveling the *in vivo* importance of factors such as tRNA abundance, aminoacylation level, elongation factor binding efficiency, tRNA modifications, and codon-anticodon interaction energy in determining translational fidelity. The method of using orthogonal pairs to precisely direct the incorporation of amino acids in response to sense codons additionally allows a more focused examination of the physiological effects of amino acid substitutions.

We show that to some extent each of the more than 30 sense codons we evaluated can be reassigned to another amino acid in *E. coli*. We quantify the efficiency and specificity of sense codon reassignment of these codons and use this data set to begin to tease apart the relative quantitative importance of the factors that contribute to the efficiency of translation. The fluorescence-based screen is readily applied in a high throughput mode to select tRNA/aminoacyl tRNA synthetase pair variants from large libraries. The main finding of these studies are that the *E. coli* genetic code is readily reassignable and genome-wide amino acid substitutions are well tolerated.

Matt Windsor, American Chemical Society

"*Directing eyeballs to your science*"

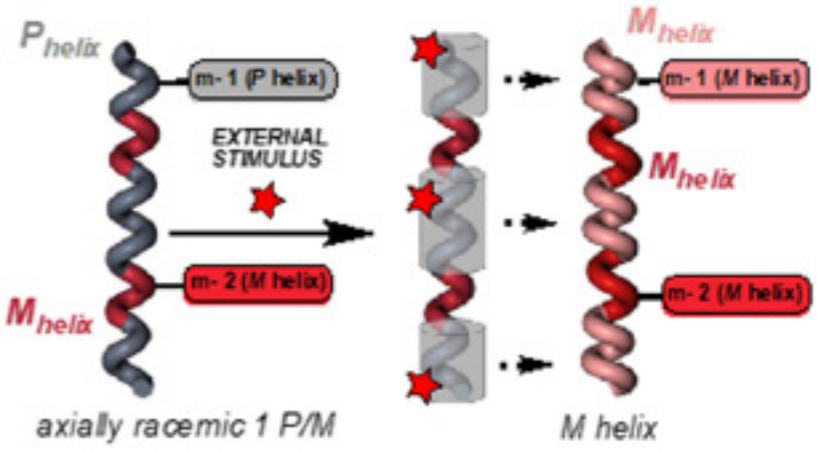
What's the value in doing good science if no one knows it exists? With an ever-increasing number of journals, scientific sub-specialties and conferences, scientists need to take a more active role in helping their audience find them. As a science communication professional at a scientific society (e.g., the American Chemical Society), I'll share actions you can take to ensure your work gets the attention it deserves.

Félix Freire, Univeristy of Santiago de Compostela

"*Stimuli-responsive helical polymers*"

The helical sense control of dynamic helical polymers via external stimuli[1-2] has been widely explored during the last decade due to the potential of these materials to act as sensors, chiroptical switches, chiral stationary phases[3] or chiral catalysts, among other applications.

In helical polymers, the helical sense is determined by the chirality of the pendant.[1] Thus, P or M helical senses can be selectively obtained depending on the absolute configuration of the chiral pendant group (R/S). Nolte[4] and Yashima[2] research groups have demonstrated that when the pendant bears more than one chiral center, the group closer to the backbone controls the helical sense. As for the distance between the chiral group and the polymeric backbone, Veciana et al.[5] have shown that the capability for helical induction decreases when the chiral center is shifted away along the pendant chain. Herein, I will show through different examples, that to control the helical sense of a dynamic helical polymer it is necessary not only to control the chirality of the pendant group but also its conformational composition. [6-9]



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Tom Stein, Hagerstown Community College

"*Bioprinting and the art of career synthesis*"

This presentation highlights the establishment of bioprinting at Hagerstown Community College in Maryland. For context, I will include personal career choices since working in the Gelman lab that made bioprinting appear the obvious next step.

Will Pomerantz, University of Minnesota

"*Confessions of a fluorophile: development of protein-observed fluorine NMR (PrOF NMR) to study molecular recognition*"

Protein-protein interaction inhibitor discovery has proven difficult due to the large surface area and dynamic interfaces of proteins and thus offers an important challenge in molecular recognition. This short talk will describe one methodology developed in my lab inspired by my early research in the Gellman group using 2H NMR and 19F NMR spectroscopy. I will first describe how protein-observed 19F NMR or (PrOF NMR) can be implemented for detecting protein-ligand interactions by screening low complexity molecules (fragments), drug-like molecules, and peptidomimetics. New developments using this method, including multi-protein screening, will be illustrated in our efforts towards developing isoform selective inhibitors of bromodomain-histone interactions to study epigenetic gene regulation. These studies have led us to formulate design rules for the development of selective inhibitors through engagement of structured-water networks, the discovery of new halogen bonding interactions in a previously inaccessible binding site, and through the use of 3D-enriched fragment libraries leading to novel heterocycle leads. The speed, ease of interpretation, and low concentration of protein needed for binding experiments affords a new method to discover and characterize both native and new ligands for bromodomains and may find utility in the study of additional epigenetic "reader" domains.

Dan Appella, NIH/NIDDK

"*Peptide nucleic acid foldamers: from design to application*"

Using chemical features that are important for foldamers, backbone modifications of Peptide Nucleic Acids (PNAs) are used to alter the binding properties to complementary nucleic acid sequences with the goal of improving diagnostic devices.

5:30 *pm* ***Closing remarks, Julie Plotkin and Sam Gellman***

Foldamer Reunion Geography

Friday, August 9, 2019: Opening Reception

5:00 pm - 8:00 pm Grainger Hall, Room 1530 Capital Cafe (location 1)

Saturday, August 10, 2019: Gellman Alumni Research Symposium

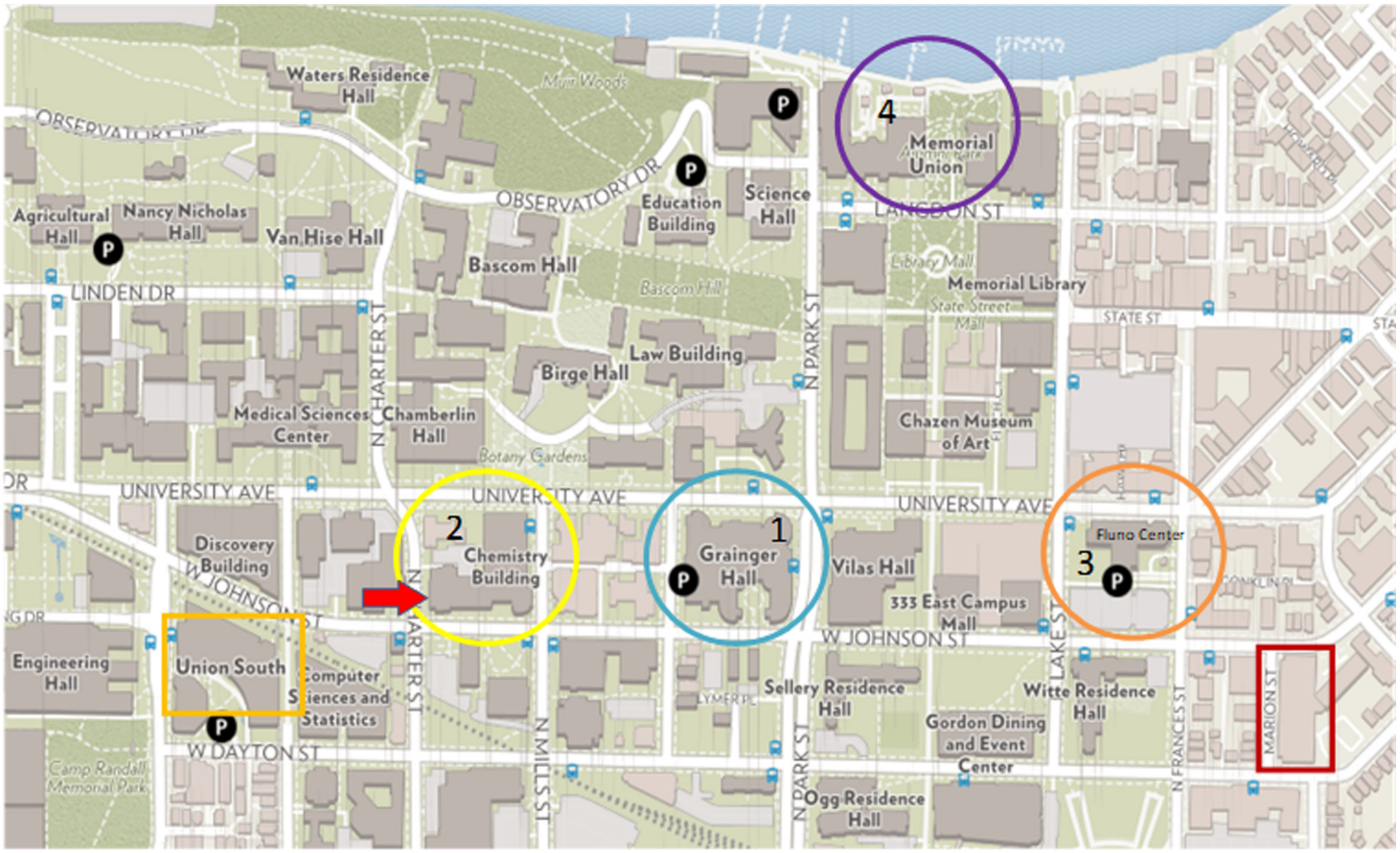
7:45 am - 6:00 pm Chemistry building, 1101 University Ave (location 2)

Saturday, August 10, 2019: Symposium Dinner and Reception

6:30 pm - 9:30 pm Fluno Center, Executive dining room, 601 University Ave (location 3)

Sunday, August 11, 2019: Informal Breakfast

9:00 am - 12:00 pm Memorial Union, Tripp Commons, 800 Langdon St (location 4)



Symposium hotels:

Union South Hotel: 1308 W. Dayton Street

DoubleTree Hotel: 525 W. Johnson Street

We are so glad that each of you were able to join us this weekend to celebrate Sam and his mentorship to us over the years. As organizers, we had a lot of fun conversations throughout the last 18 months, planning the event and reminiscing about our days in the Gellman group. We hope that each of you have an opportunity this weekend to visit with old friends, connect across the Gellman generations to make new friends, and share stories of great science. After the event, we will be emailing an electronic version of this document to all the alumni, and we'll be including some more information (e.g. alumni contact information) for everyone.

This event would not have been possible without an incredible amount of "on location" work from Sam, Julie Plotkin, and Karen Stephens.

Sam, we thank you for being a great reason for all of us to gather together this weekend. We appreciate all the hard work and time you dedicated to making this "reunion" a reality.

Julie, we thank you for all your work identifying and coordinating with venues for our meals and social events. We are grateful for the time you spent making this weekend such a success.

Karen, this event would not have been possible without you. Thank you so much for your organization in Madison from making sure the numbers of attendees matched the numbers of meals planned to handling venues that could accommodate us as the numbers grew to helping us identify resouces in Madison when we bounced ideas around. Given that none of us are living in Madison right now, your presence during the planning was invaluable in bring the vision of the reunion to reality. We are deeply grateful.

Thank you to all of our speakers. Seeing the myriad ways in which our time working with Sam have shaped what we are each doing today was an inspriring experience.

Thank you to each of our session chairs, both alumni and current group members. We are grateful to you for helping keep us on time and stimulating lively discussion.

Gellman alums, please keep in touch, even if it is just a brief update to Sam that gets included in a future group letter.

We wish you safe travels back to your homes.

Jon Lai
Will Pomerantz
Meg Schmitt