

Research Overview

Research in my group will use organic synthesis, combinatorial methods, and analytical techniques, such as tandem liquid chromatography-mass spectrometry and nuclear magnetic resonance, to answer biologically relevant questions at the molecular level. We will also actively seek collaborations with entities such as the UT Joint Institute for Biological Sciences (JIBS) and the Oak Ridge National Laboratory (ORNL).

Specifically, undergraduates will be able to contribute to exciting projects such as the one outlined below. This project will involve undergraduates in research at the interface of chemistry and biology. Students will be able to focus on organic synthesis, biological culturing techniques, and/or analytical protocols to complement research in our laboratory. Each will be able to tailor their experience by choosing to perform a short synthesis of a molecule of interest or by choosing to analyze biological LC-MS/MS datasets.

Combining Organic Synthesis and Metabolomics to Determine *In Vivo* Molecular Recognition

Small molecule (ligand) binding interactions with proteins are key chemical regulators of cellular behavior, and determining the target for drug candidates is the first step in identifying their mode of action. As novel natural products are characterized, they are often screened for biological activity, such as cytotoxicity, and some show promise as new antibiotics. However, determining the exact mechanism of action is more difficult, and often the identity of the cellular target remains unknown, e. g. the glucosinolates (**1**) and their isothiocyanate analogues (e. g. sulforaphane [**2**], Figure 1) are known to be cytotoxic to *Helicobacter pylori* and several cancer lines,^{1, 2} but no protein-ligand interaction for these compounds has been reported.

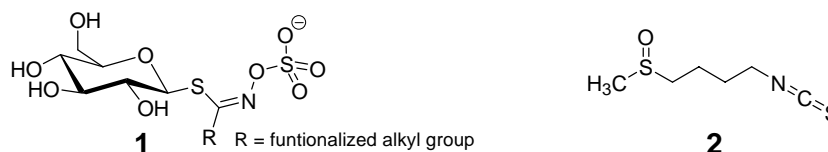


Figure 1. General structure for the glucosinolates (**1**) and the structure of sulforaphane (**2**)

Our research will rely on organic synthesis to generate biologically interesting molecules and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine protein-ligand binding partners. The strategy will initially rely on metabolomics to identify molecules that disrupt metabolism and to determine the biological pathway upon which the molecule acts by monitoring for disruption of enzymatic activity, Figure 2. Microbial cell cultures will be treated with a drug candidate, the cells will be extracted to yield the metabolome,³ and the metabolome will then be analyzed via LS-MS/MS using a triple quadrupole instrument in multiple reaction monitoring mode (MRM).⁴ This analysis method currently provides information on ~200 metabolites distributed among the core biological pathways, and has been used to determine stress responses in yeast and *Escherichia coli*.⁵ Further work will generate more potent analogues of interesting molecules, and their effects on metabolism will also be determined by LC-MS/MS.

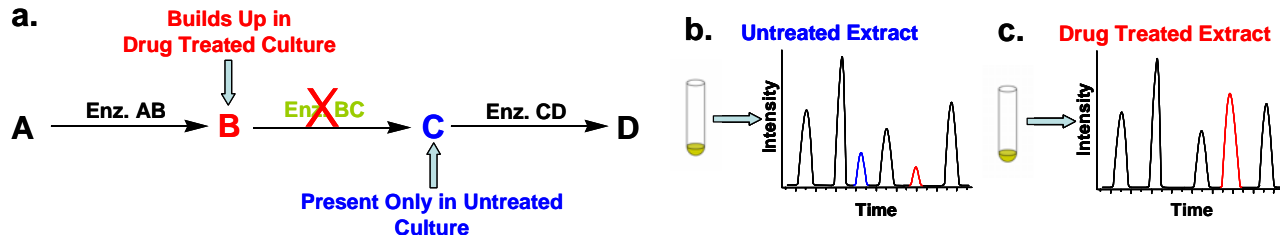


Figure 2. Metabolic disruptions will be detected by monitoring for fluctuations in metabolite concentrations. **a.)** A molecule targeting an enzyme will lead to an increase in the concentration of metabolites upstream of the inhibited enzyme and an absence in the concentration of metabolites downstream of the enzyme. **b.)** Idealized chromatogram of the metabolome from an untreated cell culture. Note the presence of compound **B** and **C** (peaks shown in red and blue, respectively). **c.)** Idealized chromatogram of the metabolome from a drug treated cell culture. Note the increase in the concentration of **B** (red peak) and the absence of **C** (lack of blue peak). Drug will be added in sub-lethal doses to avoid gross metabolic disruption due to cell death.

References

1. Fahey, J. W.; Haristoy, X.; Dolan, P. M.; Kensler, T. W.; Scholtus, I.; Stephenson, K. K.; Talalay, P.; Lozniewski, A., "Sulforaphane Inhibits Extracellular, Intracellular, and Antibiotic-Resistant Strains of *Helicobacter Pylori* and Prevents Benzo[a]Pyrene-Induced Stomach Tumors." *Proceedings of the National Academy of Sciences of the United States of America*, **2002**, 99, (11), 7610-7615.
2. Haristoy, X.; Angioi-Duprez, K.; Duprez, A.; Lozniewski, A., "Efficacy of Sulforaphane in Eradicating *Helicobacter Pylori* in Human Gastric Xenografts Implanted in Nude Mice." *Antimicrobial Agents and Chemotherapy*, **2003**, 47, (12), 3982-3984.
3. Kimball, E.; Rabinowitz, J. D., "Identifying Decomposition Products in Extracts of Cellular Metabolites." *Analytical Biochemistry*, **2006**, 358, (2), 273-280.
4. Bajad, S. U.; Lu, W. Y.; Kimball, E. H.; Yuan, J.; Peterson, C.; Rabinowitz, J. D., "Separation and Quantitation of Water Soluble Cellular Metabolites by Hydrophilic Interaction Chromatography-Tandem Mass Spectrometry." *Journal of Chromatography A*, **2006**, 1125, (1), 76-88.
5. Brauer, M. J.; Yuan, J.; Bennett, B. D.; Lu, W. Y.; Kimball, E.; Botstein, D.; Rabinowitz, J. D., "Conservation of the Metabolomic Response to Starvation across Two Divergent Microbes." *Proceedings of the National Academy of Sciences of the United States of America*, **2006**, 103, (51), 19302-19307.

Biographical Sketch

Dr. Campagna completed his B.S. in Chemistry from North Carolina State University in 2000 and conducted research with Prof. Jonathan S. Lindsey on the chemical synthesis of bacterial chromosomes. He received his Ph.D. from Princeton University in 2006, after working with Prof. Martin F. Semmelhack on a joint project with Profs. Bonnie L. Bassler and Frederick M. Hughson to characterize the chemical properties of an inter-species bacterial signaling molecule, autoinducer-2. Currently Dr. Campagna is a post-doctoral fellow with Prof. Joshua D. Rabinowitz at the Lewis-Sigler Institute for Integrative Genomics at Princeton University where he is developing mass spectrometric methods for the identification of natural products from whole cell extracts. He joined the Chemistry Department at UT Knoxville in August 2007.

Representative Publications

Semmelhack, M. F.; Campagna, S. R.; Federle, M. J.; Bassler, B. L. "An expeditious synthesis of DPD and boron binding studies," *Org. Lett.*, **2005**, 7(4), 569-572.

Miller, S. T.; Xavier, K. B.; Campagna, S. R.; Taga, M. E.; Semmelhack, M. F.; Bassler, B. L.; Hughson, F. M. "Salmonella *typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2," *Mol. Cell*, **2004**, 15(5), 677-687.

Rickard, A. H.; Palmer, R. J.; Blehert, D. S.; Campagna, S. R.; Semmelhack, M. F.; Eglund, P. G.; Bassler, B. L.; Kolenbrander, P. E. "Autoinducer-2: a concentration-dependent signal for mutualistic bacterial biofilm growth," *Mol. Micro.*, **2006**, 60(6), 1446-1456.

Campagna, S. R.; Rabinowitz, J. D. "Metabolome Quantitation Mass Spectrometry: Sensitive measurement of many core metabolites in parallel." *G.I.T. Lab. Rev.*, in press January, **2007**