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).

I. Combining Organic Synthesis and Metabolomics to Determine Drug Targets

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, 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)](image)

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, and the metabolome will then be analyzed via LC-MS/MS using a triple quadrupole instrument in multiple reaction monitoring mode (MRM). 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*. 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 blocking 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.

II. Identification of Selective Transition Metal Catalysts for the Synthesis of Complex Organic Molecules

Although chemists have had the ability to construct large libraries of ligands for transition metal catalysts, the ability to rapidly screen catalyst libraries is still lacking. Taking inspiration from nature, we will develop a strategy to identify highly robust and specific catalysts for reactions that generate complex products from simple substrates. This strategy consists of three phases: (1) design and construction of a library of ligands for transition metal catalysts, (2) screening of the library to find members capable of performing complex chemical transformations, and (3) developing a reliable assay for the visual identification of active catalysts (Figure 3).

Figure 3. A library of transition metal catalysts will be developed with the goal of identifying a set of catalysts that can perform the total synthesis of a natural product in one reaction vessel. To determine the progress of the reactions, a biological reporter strain will be used to increase the throughput of the system.
Successful catalysts will be capable of (1) selecting a metal from a pool of available candidates, (2) performing a regio- and stereospecific chemical transformation, and (3) collaborating with other catalysts to construct the desired product in a single reaction vessel. Natural products and pharmaceuticals will be targeted for synthesis as they provide a rich pool of targets from which to draw and since they often elicit responses that can be observed in vivo. The biological activity of the products will be the key to identifying active catalysts because bacterial or yeast reporters will be used as a measure of productive reaction.

Specifically, we propose that a cyclic peptide library (Figure 4) will provide catalysts capable of producing two products, benzylpenicillin and prostaglandin E$_1$ (Figure 5). The synthesis of benzylpenicillin will be accomplished in a bio-mimetic fashion utilizing a transition metal catalyzed cyclization of a peptide precursor. A successful catalyst will mediate the formation of two rings, set one stereocenter, and perform a C-H insertion (Figure 5a). The cytotoxicity of benzylpenicillin will be used as the basis for an assay that will rely on the killing of a highly colored bacterial species to indicate productive reaction.

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**Figure 4.** General scaffold for the peptide catalyst library. a) Cyclic peptide will be selected as ligands as they will provide a semi-rigid scaffold for metal binding. In this manner, they should act much like the active site of an enzyme. b) A representative member of the peptide library along with its c) predicted three dimensional structure. This ligand should have a defined structure that may provide a pocket for metal binding and selective catalysis.
Figure 5. Two target syntheses designed to test the parameters of the peptide catalyst library. a) The synthesis of benzylpenicillin will provide an initial proof of concept as it will provide a one-step synthesis of a complex molecule with a readily apparent biological activity, i.e. cytotoxicity. b) The synthesis of prostaglandin E₁ (PGE₁) will expand the utility of the methodology by both identifying a set of catalysts that can work together to synthesize a complex product in one reaction vessel and by demonstrating that the screening methodology can be expanded to biologically active molecule with more subtle activities, such as cell signaling.

The search to find catalysts for the synthesis of prostaglandin E₁ will broaden the scope of the study in three ways by (1) identifying two catalysts able to function in one pot, (2) utilizing a cyclic peptide to catalyze a reaction not found in nature, and (3) developing a biologically based assay for a non-cytotoxic molecule. Prostaglandin E₁ will be synthesized from simple olefinic starting materials in one pot via a regio- and stereospecific olefin cross metathesis reaction followed by a bio-mimetic oxidative cyclization (Figure 5). The successful cross metathesis catalyst will be able to discriminate among four possible double bonds and will form an olefin with a cis geometry. Further, a successful cyclization catalyst will form one ring, set four stereocenters, and install three oxygens. The signaling function of prostaglandin E₁ will be used to assay for productive reaction in a yeast strain containing a β-galactosidase reporter construct that is responsive to prostaglandins.

Research References: Available upon request.
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. Dr. Campagna was also a post-doctoral fellow with Prof. Joshua D. Rabinowitz at the Lewis-Sigler Institute for Integrative Genomics at Princeton University where he developed 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


