Research in the Wandless Group

The Wandless lab employs an interdisciplinary approach to studies of biological processes, combining synthetic chemistry with biochemistry, cell biology, and structural biology. However our core science is synthetic chemistry – the ability to create new molecules. Rather than focusing on the development of new synthetic methods or the synthesis of molecules isolated from nature, my laboratory concentrates on molecules that possess novel functions. Specifically, we synthesize molecules that are designed to:

- provide insights into cellular processes by elucidating signaling pathways.
- utilize endogenous cellular proteins for entirely new purposes.
- probe the molecular interactions between small molecules and macromolecules.

Chemists bring a different perspective and techniques to biological problems, and while chemistry will not provide answers to all biological questions, there are certain problems that stand to benefit from a very molecular approach.

Some of the richest and most exciting research opportunities can be found at the interfaces of traditional disciplines, and our particular focus lies at the interface of chemistry and biology. The challenges are numerous for a group whose core competency is synthetic chemistry. To address biological problems we must either learn and incorporate many new biological techniques or we must forge collaborations to provide these capabilities. In order to maximize the intellectual ownership of our research, my laboratory strives to incorporate as many of the relevant techniques as is practical into the lab.

Advantages of this approach include the ability to exercise control over the timing and operational details of the relevant experiments. The wide-ranging experimental requirements also provide a broad and locally deep training experience for the students performing the research. Students in my lab often perform synthetic chemistry, cloning and protein expression, enzymology, and mammalian cell culture in order to characterize the compounds that they design. As a result, the students are challenged with multiple learning curves, which provides solid interdisciplinary training in order to complete a particular study. On balance, I believe that incorporating these techniques and investing in the scientific infrastructure of the lab is the more rewarding strategy. However, we cannot be experts at everything, so we collaborate with other groups in order to perform certain experiments. For example, it would be cost prohibitive to build our own facility for high-resolution studies of living mammalian cells, so we work with Tobias Meyer (Molecular Pharmacology) to perform these experiments. Similarly, we collaborated with Kasturi Haldar (Northwestern Medical School) to study malaria parasites. Our most longstanding collaboration is with Jerry Crabtree's lab, and this relationship has been extremely fruitful.

Protein-Protein Interactions & Bifunctional Molecules – One of the primary goals of my laboratory has been the design, synthesis and evaluation of synthetic molecules that are capable of binding to two different proteins. These molecules are typically comprised of two ligands for their respective proteins, and the ligands are linked by a covalent tether. In cases where the tether between the ligands is relatively long, both proteins can simultaneously bind to the bifunctional molecule to form a trimeric complex. The formation of a trimeric complex creates an environment wherein interactions between the two proteins are possible. These nascent protein-protein interactions may contribute either favorably or unfavorably to the overall stability of the trimeric complex, and we have shown that these protein-protein interactions may endow
the bifunctional molecules with biophysical and biological properties that are significantly different from the monomeric ligands that comprise them. In one of our first studies, we demonstrated that a trimeric complex is more stable than one might expect based on the stabilities of the two constituent dimeric complexes.

Bifunctional molecules that possess prohibitively short tethers create a very different environment. In this case, unfavorable protein-protein interactions significantly destabilize the trimeric complex relative to the two dimeric complexes, and the bifunctional molecule will partition between its two protein receptors as a function of the respective dissociation constants as well as the concentrations of all three species. We have capitalized on this behavior to design a bifunctional molecule containing one ligand that is capable of inhibiting an essential cellular enzyme and displaying cytotoxicity. However, the other ligand binds tightly to a very abundant cellular protein called a presenter protein, and this binding event does not elicit any measurable cellular response. Thus, in the presence of both proteins, the bifunctional molecule is effectively "detoxified" by the presenter protein.

However, if the presenter protein is either absent or binds weakly to its ligand, the bifunctional molecule is free to inhibit the essential enzyme and cause cytotoxicity. Bifunctional molecules such as these display context-dependent biological activity. Their ability to cause a biological response depends on the presence or absence of a biologically silent presenter protein rather than the target protein. As a result, we have decoupled a molecule's ability to elicit biological activity from the presence or absence of the target protein. Two different cells that possess identical amounts of the same target protein (but different presenter proteins) will respond differently to a bifunctional molecule. With a knowledge of a cellular environment, which is increasingly available using genomic and proteomic techniques, we can design and synthesize bifunctional molecules that display predictable selectivity for different proteins or cell types. Using a bifunctional molecule capable of inhibiting dihydrofolate reductase, we have demonstrated selective cytotoxicity toward malaria parasites but not against the host mammalian cells that possess the protective protein as well as the target protein. We have demonstrated this detoxification strategy in cell-free assays using purified proteins, cell-based assays against the *Plasmodium falciparum* malaria parasite, and using *Plasmodium*-infected mice.

From the outset of these studies, we have been working to understand the molecular details that give rise to the unique properties of these bifunctional molecules. One line of investigation has focused on the nature of the putative protein-protein interactions. We have primarily used multidimensional NMR spectroscopy and electrospray mass spectrometry to investigate these interactions, and although we have published only one manuscript in this area to date, these efforts continue in our laboratory. Another valuable mechanistic aspect of these bifunctional molecules is the predictive power that is provided if information about the constituent protein-ligand interactions is available. We have formulated quantitative models that incorporate multiple equilibria to account for all possible molecular species (monomeric as well as dimeric and trimeric complexes) and potential dissociation constants. These models can be used qualitatively to discern whether or not a trimeric complex contributes to a particular binding equilibrium. Furthermore, fitting of experimental data to the model provides quantitative information about the stability of participating trimeric complexes. Our ability to model equilibria involving the trimeric complexes has been very valuable both for designing experimental systems as well as for providing quantitative data following experiments.

We are pursuing several additional uses for bifunctional molecules as probes of
cellular signaling pathways. In one case, we have covalently linked a high affinity protein ligand to several different membrane-associated molecules. These bifunctional molecules are designed to insert into cell membranes and recruit diffuse cytosolic proteins to the inner leaflet of the plasma membrane in living cells. Many cellular signaling processes are initiated at this location, and we use this rapid perturbation method to probe the functional roles of different proteins in specific signaling pathways. We have prepared fusion constructs between the ligand binding domain and different signaling proteins (e.g., small G-proteins and kinases), and we will transfect these constructs into cells and observe their behavior in living cells upon treatment with the membrane-anchored FKBP ligand. The use of these cell-permeable small molecules to perturb cellular signaling events enjoys several advantages over traditional genetic approaches, the most significant of which is the ability to measure real-time cellular responses on the millisecond timescale. The ability to observe living mammalian cells has been enhanced by a collaboration with Tobias Meyer's lab at Stanford Medical School.

**Synthetic Probes of Mammalian Checkpoints** – Another significant focus of our research has been the elucidation of cell cycle checkpoints that monitor microtubule integrity. Dozens of different small molecules, both natural products and synthetic compounds, bind to microtubules and interfere with their normal functions. Examples include taxol, vincristine, and epothilone, and many of these compounds are used as anticancer drugs. When normal microtubule functions are disrupted by these drugs, cells typically undergo cell cycle arrest at the metaphase-anaphase transition of mitosis. Early pharmacologic models postulated that these drugs caused significant damage to the cellular cytoskeleton, thus causing cytotoxicity in rapidly dividing cells such as cancer cells. It was later realized that these drugs display biological activity at concentrations that are significantly lower than the concentrations that are required to either depolymerize or polymerize microtubules. The second generation of pharmacologic models postulate that these drugs inhibit a microtubule-specific process called dynamic instability. However, this model only begs the question of how these drugs inhibit microtubule dynamics, and more significantly, how cells monitor microtubule dynamics and sense that microtubule function is impaired.

We set out to test this model using synthetic chemistry to prepare phomopsin A. Phomopsin is one of the most potent anti-tubulin molecules yet discovered, and through the synthesis of the natural product as well as a variety of closely related nonnatural products, we are testing these models of antimitotic drug action. We are correlating *in vitro* measurements of microtubule dynamic instability with the cellular effects of these molecules. A high degree of correlation provides evidence in favor of the dynamic instability model, and a lack of agreement point to an alternative model. We plan to use phomopsin and its variants to gain insights into the molecular mechanisms underlying this checkpoint. The most challenging aspect of these studies is the synthesis of phomopsin and its variants, and we are presently close to completing the total synthesis of phomopsin and have recently completed the synthesis of a related natural product called ustiloxin. Even though we never set out to develop new synthetic methodology, the challenges associated with phomopsin's synthesis dictated that we devise new solutions for unsolved problems. We have developed a new method to prepare α,β-dehydroamino acids. Our approach provides a stereospecific method to prepare these compounds from β-hydroxyamino acids, which are readily available. We have also demonstrated the utility of nucleophilic aromatic substitution to prepare hindered tertiary alkyl-aryl ethers such as that found in phomopsin.

Our most recent efforts in the checkpoint field are aimed at understanding the early events that lead to activation of the DNA damage checkpoint. Mammalian cells
can detect distinct types of DNA damage caused by a variety of different events (e.g.,
UV or ionizing radiation, alkylation). Several of the key downstream checkpoint signaling
proteins have been identified, and one of the central players is a protein kinase called
ATR, which is essential for the DNA damage checkpoint. ATR's kinase activity is
covalementy inhibited by a natural product called wortmannin, and we have synthesized
several variants of wortmannin in order to isolate ATR and identify additional checkpoint
proteins that associate with ATR. ATR is the earliest known player in the DNA damage
checkpoint and it responds to a variety of different types of DNA damage. However, we
do not yet know the molecular details underlying this recognition event. To probe these
early events, we have also synthesized a variety of DNA molecules that possess specific
types of damage. We have prepared phosphoramidites of the cis-syn thymidine dimers
that arise from UV damage to DNA, and these molecules have been specifically
incorporated into both oligonucleotides and large, circular plasmids. Through a
collaboration with Karlene Cimprich, we can add our synthetic molecules to cell-free
Xenopus extracts and monitor checkpoint responses. We are currently analyzing
different types of DNA damage to pinpoint the specific lesions that activate the
checkpoint. These studies may allow us to identify not only the exact molecular
structures of the DNA lesions leading to checkpoint activation, but also the proteins that
act as cellular sensors of DNA integrity.