

RESEARCH IN THE WANDLESS GROUP

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Overview – We employ an interdisciplinary approach to studies of biological systems, combining a bit of synthetic chemistry with biochemistry, cell biology, and structural biology. More specifically, the lab concentrates on the invention of molecules and techniques that enable better studies of biological processes. In short, we invent tools for biology and we are motivated by approaches that enable new experiments with unprecedented control. These new techniques may also provide a window into mechanisms involved in maintaining cellular homeostasis. Protein quality control is a particular interest at present.

New Approaches for Conditional Control of Protein Function – We recently developed a new experimental system in which the stability of a specific protein depends on the presence or absence of a cell-permeable molecule. We started with a well-studied protein-ligand pair: the FKBP12 protein and a high-affinity, synthetic ligand called Shield-1. FKBP can typically be fused to any other protein without affecting the stability of the resulting fusion protein, so we screened a library of FKBP sequences to identify mutants that are unstable in the absence of Shield-1. Additional screening enriched for fusion proteins that are stabilized by Shield-1, and further characterization of these mutants revealed that the most destabilizing mutants caused a 50-fold to 100-fold reduction in the expression levels of the proteins to which they were fused.

The instability that is conferred by the FKBP-derived destabilizing domains (DDs) is reasonably general for a wide variety of proteins, both large and small, nuclear and cytoplasmic, even membrane-bound proteins. The system works in cultured mammalian cells and in living mice. Publication 34 (in the list of publications on this website) describes this system in detail. This new technique allows rapid and reversible elimination of a specific protein either in cell culture or in mice. Building on these early successes, we have expanded this technology in several useful ways. First, we have engineered second and third DD systems using orthogonal protein-ligand combinations. We can now tunably regulate the expression levels of two (or three) different proteins or small collections of proteins using different cell-permeable small molecules. Second, we have engineered a DD system that functions in the opposite sense. The fusion protein is stable in the absence of the ligand, and administration of the ligand causes the fusion protein to be rapidly degraded. Third, we have engineered an experimental system that works in the yeast, *Saccharomyces cerevisiae*.

Given the general utility of the DD technology, we expect it to be widely useful for the biological community. We are committed to making these reagents available to any qualified investigators. Between Sept of 2006 and the end of 2009 we have distributed over 400 "kits" that include DNA plasmids encoding the DDs as well as aliquots of the stabilizing ligands.

Protein Quality Control in Mammalian Cells – The DDs can be thought of as model substrates that have the potential to bridge our understanding of the physical basis of protein stability in vitro with intracellular stability. The ability to conditionally regulate the structures of these domains using high-affinity ligands allows us to quantitatively correlate specific biophysical properties with biological stability. There are two main questions that we would like to understand in this area. First, we focus on the DD proteins themselves. What property or properties of these DDs leads them to be recognized and degraded by the cellular quality control machinery? Second, we would like to have a more complete picture of the proteins that are involved in these quality control surveillance pathways. Efforts to address both of these questions are currently underway.

One might ask whether or not we will learn anything about "normal" protein degradation in cells by studying an engineered, artificial protein such as one of our engineered DDs. Why not study the degradation machinery responsible for turning over GAPDH or ribonucleotide reductase? First, cells expressing DD-fusions under permissive conditions are not required to adapt to unusual conditions. They need to make no dramatic changes to their degradative machinery to accommodate this engineered protein. So, when we switch to the non-permissive state by withdrawing Shield-1 we will be looking at native-like intracellular conditions with high temporal control. Second, the use of our DDs to switch-on degradation is very specific, and we can make this switch without perturbing cellular homeostasis (contrast with temperature-sensitive mutants). Finally, cells may utilize a relatively small number of quality control surveillance proteins to monitor the general "folding health" of their contents. Once we identify members of this QC machinery that recognize our DDs, it will be easy to test if these proteins are also involved in regulating the lifetimes of any number of endogenous cellular proteins using a variety of techniques. We believe that our engineered destabilizing domains comprise a unique set of reagents that will allow us to probe cellular protein degradation in new ways.

Protein-Protein Interactions & Bifunctional Molecules – One of the interests of the Wandless lab has been the study 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 ternary 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. See reference 16 in the publications list for more information.

We have pursued several additional uses for bifunctional molecules as probes of cellular signaling pathways. With Takanari Inoue and Tobias Meyer, we used new rapamycin derivatives to rapidly localize signaling proteins to the inner leaflet of the plasma membrane in order to study the roles of these various domains in different signaling networks. Many cellular signaling processes are initiated at this location, and we use this rapid perturbation method to probe the functional roles of different proteins (small G-proteins and kinases) in specific signaling pathways. 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 in living cells with very fast temporal resolution. See publications 30 and 35 for more information.