# The Beta Roll Peptide as a Reversible, Calcium Sensitive, and Modular Scaffold for the Engineering of Biomolecular Recognition

Mark A. Blenner[1], Geza R. Szilvay[1], Oren Shur[1], Donald Cropek[2], Scott A. Banta[1]

Beta roll peptide motifs are composed of repetitive calcium binding nonamers. These motifs are found in several proteases, lipases and haemolysins, and are defined by the consensus: GGXGXDXUX, where U is an aliphatic amino acid and X is any amino acid. Repeating peptide scaffolds have been increasingly utilized as a stable and modular interface for biomolecular recognition of target molecules. These repeat peptides can be concatenated to increase the area available to design biomolecular interaction, making possible the design of proteins that can bind targets of varying size. Calcium binding triggers the folding of the beta roll repeats such that two variable amino acids of every other nonamer form an interface on one side of the beta roll. Thus, the binding of calcium controls the assembly of the interface designed to bind its target, and in the absence of calcium, the target can be released from the beta roll.

Here we present the engineering and characterization of beta roll motifs from a Serralysin and adenylate cyclase. The functional folding of the beta roll peptide was determined using circular dichroism (CD) spectroscopy, dynamic light scattering (DLS) and fluorescence resonance energy transfer (FRET). CD spectroscopy indicated that unstructured peptide formed calcium induced beta sheet secondary structure for N- and C- terminally capped beta roll constructs. Similarly, DLS experiments indicated a decrease in the hydrodynamic diameter of beta roll peptides upon calcium binding. FRET experiments similarly showed a calcium dependent decrease in fluorophore separation. In an effort to understand the roles of regions adjacent to the beta-roll, both the N- and C- terminal capping groups were truncated in several logical positions, based on secondary structure prediction models. We found that either N-terminal or C-terminal capping was sufficient for calcium induced beta roll folding. This is consistent with studies on leucine-rich repeat proteins, where capping is necessary to stabilize the hydrophobic core.