

# Bottom-up construction of stimuli-responsive biomaterials from peptide and protein building blocks

Caitlin A. D'Ambrosio

Advisors: April M. Kloxin, Wilfred Chen, and Christopher J. Kloxin

Committee Members: Millicent O. Sullivan, Kevin V. Solomon, and Darrin J. Pochan

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Peptides and proteins offer a powerful and programmable foundation for the construction of stimuli-responsive materials, yet their full potential has been constrained by limitations in synthesis, purification, and functionalization. This dissertation establishes a modular biosynthetic platform using computationally designed coiled-coil peptides, termed bundlemers, as versatile building blocks for the bottom-up assembly of complex nanostructures and functional materials.

A scalable, column-free biosynthesis route to bundlemer peptides is first developed using inclusion body-intein (IBTi) fusion tags, eliminating the need for column chromatography while improving yield over conventional approaches. This platform is extended to co-express two distinct bundlemer-forming sequences as a single fusion protein, enabling hetero-tetrameric assemblies with precise control over subunit composition that is otherwise unattainable without redesigning the core peptide sequence.

Genetically encoded bioorthogonal handles, previously inaccessible through solid-phase peptide synthesis, are then integrated into the bundlemer framework. Sortase recognition motifs enable enzymatic polymerization of antiparallel bundlemers into fibrils under ambient conditions, while a dual-reactive parallel bundlemer displaying orthogonal SpyTag and sortase motifs at opposite termini enables sequential, site-specific protein layering onto the surface of E2 protein nanocages.

The modularity of this platform is further exploited to construct stimuli-responsive bottlebrushes entirely from bundlemer peptides. Site-specific incorporation of the unnatural

amino acid azidophenylalanine via amber stop codon suppression installs reactive handles into the bundlemer backbone, enabling copper-catalyzed surface grafting of a zwitterionic peptide brush. The resulting assemblies exhibit rigid-rod morphologies, pH-responsive assembly, and reversible self-healing behavior, with bristle functionalization enhancing bulk elastic stiffness. Unnatural amino acid stoichiometry is established as a handle for controlling bottlebrush dimensions.

Finally, an engineered circularly permuted light-oxygen-voltage domain (cpLOV2) with terminal reactive handles is characterized to understand how terminal peptide fusions and macromolecular scaffolds influence photocycle kinetics. Conjugation onto a bundlemer scaffold reveals that quaternary structural constraints can dramatically diverge photokinetic behavior, yielding either a reversible optogenetic switch or an intermolecularly locked, bistable ratchet, establishing design principles for light-driven protein nanomachines.

Collectively, this work demonstrates that modular biosynthesis access to functionalized peptide building blocks enables the programmable construction of stimuli-responsive materials with broad implications for synthetic biology, biomaterial design, and therapeutic delivery.