Hint1 Regulated Hydrogelation of Nucleoside Phosphoramidate Functionalized Self-Assembling Peptides

The non-covalent association of molecules is a ubiquitous feature of life. Protein scaffolds, components of the extracellular matrix, and even duplexed nucleic acids all undergo supramolecular assembly. To generate synthetic self-assembled nanostructures, a wide variety of small molecule motifs have been developed that utilize hydrogen bonding, electrostatics, and hydrophobic interactions to contribute to the assembly process. Growing interest in the regulation of supramolecular assembly has produced a variety of responsiveness motifs. The governing effect of these motifs is exerted through physical and chemical cues such as ultrasound, temperature, pH, reduction and oxidation, and enzymatic responsiveness. Enzymes are of interest for the regulation of supramolecular interactions as they allow the mimicry of biological systems. They are catalytic in nature, able to be regulated by various biomolecules and ligands, and are biocompatible.

To create a responsive and tunable self-assembly system, we have developed Histidine Triad Nucleotide Binding Protein 1 (HINT1) responsive nucleoside phosphoramidate pro-gelators (PPGs). HINT1 has been well characterized as a nucleoside phosphoramidase and acyl-adenylate hydrolase. In this system, HINT1 releases self-assembling peptides from the blocking effect of nucleoside phosphoramidate moieties, allowing the peptides to assemble into supramolecular nanofibers. In aqueous solution, these nanofibers have been observed to form hydrogels. Characterization of these materials with oscillatory rheometry and transmission electron microscopy has yielded data demonstrating PPGs with different nucleosides in the parent structure giving rise to morphologically different nanofiber structures after HINT1 activation. We are utilizing chemical biological tools such as small molecule inhibitors, catalytically dead HINT1 mutants, and slow substrates to investigate the role of HINT1 active site and catalytic activity on the regulation of PPG assembly. Inhibitors have been shown to block HINT1 activity on PPG substrates, and Hint1 H112N catalytically dead mutant has been shown to be unable to activate self-assembly. Nucleoside thiophosphoramidate substrates have been demonstrated to stabilize the covalent adenylated active site intermediate generated during the HINT1 catalytic cycle. We are currently synthesizing slow PPG substrates bearing a thiophosphoramidate group to further tune the kinetic activation of these substrates for self-assembly. Our goal is to develop an adaptable system for the construction of biologically responsive materials that may be assembled *in situ* in response to HINT1 activity.