

## **Synthetic Biology-Driven Approach to Repurpose Polyamides (STORM)**

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**Background/Objectives.** STORM aims to discover and design novel methods and enzymatic pathways for bioconversion and reuse of synthetic polymers. Nylon-6 (PA6) is a synthetic polymer highly recalcitrant to degradation, whose environmental and economic footprint could be reduced through polymer degradation and upcycling technologies. Currently, little understanding on PA6 biological degradation exists and no direct enzymatic degradation pathway has been identified. With an increase advancement in systems biology approaches, computational modeling, sequence-based homologue searching, and molecular docking allows us to elucidate PA6 biological basis of degradation using several target enzymes classes. Here, we investigate a direct relationship between the PA6 material morphology and topology and efficiency of enzymatic degradation using several modeling approaches as well as experimental testing. The intercept between polymer morphology and enzyme kinetics will allow us to propose model pathway for degradation of PA6 previously unattainable by existing biological systems.

**Approach/Activities.** STORM contains both polymer degradation and polymer morphology modeling approaches. For degradation, the research concept involves integration of computational modeling, elucidating of the biological catalyst for degradation via homologue searches and evolutionary analyses, experimental laboratory testing of enzyme efficacy, and finally, optimization of enzyme expression in model organisms. Enzymes of interest are fed into enzyme-substrate models to down-select potentially effective enzymes. Homology searches and alignments are then used to identify target enzymes in currently available organisms. Laboratory testing is performed on the down-selected enzymes to determine degradation efficacy and parameters. These enzymes will then be engineered into a model organism for overexpression capable of degrading PA6 at higher rates.

**Results/Lessons Learned.** This work provides the first known technology for degradation and upcycling of recalcitrant PA6 polymers. Polymer morphology experimentation has shown significant PA6 degradation into its oligomer components of dimers, trimers, and tetramers after incubation with a down-selected targeted enzyme. PA6 films have been developed using crystalline and amorphous chains to illustrate the specific degradation approaches needed for the differing morphologies of PA6. Current research has highlighted the challenges in enzymatically degrading high crystalline structures of PA6, illustrating that two separate degradation strategies may be necessary. Key risks include low yields of enzymes, cost of genetic engineering of organisms, and effective connection of the degradation portion of work. These identified risks are mitigated through proper enzyme down-selection prior to testing, experimental testing prior to overexpression, and the use of teaming partners at Debut Biotechnology and the U.S. Department of Energy's (DOE's) User Facilities, which provide technical expertise in these areas.