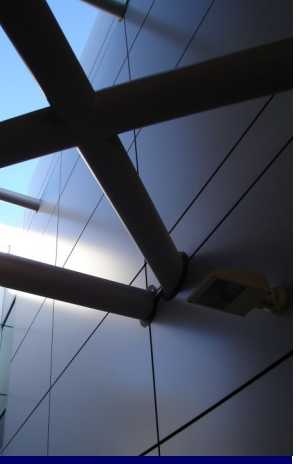

MECHANICAL ENGINEERING
DISSERTATION DEFENSE

The Department of Mechanical Engineering presents:

The Ph.D. Dissertation Defense of

Melissa Klocke

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Engineering Encapsulated Synthetic Cytoskeletal Dynamics via Nucleic Acid Nanotechnology and Genetic Circuits

Doctor of Philosophy, Graduate Program in Mechanical Engineering
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Dr. Elisa Franco, Chairperson

Programmable, synthetic cells have broad applications in sensing and drug-delivery. Current work in the development of synthetic cell components focuses on compartmentalization, and on developing the minimum required cellular machinery to carry out different processes [1, 2]. In native cells, cytoskeletal filaments are a key structure for cell division, motility, and intra-cellular transport. Harnessing these filaments for use in synthetic systems is limited by the complexity of the proteins and processes responsible for the dynamic behavior of the filaments. Synthetic tile-based DNA nanotubes, however, are comparable in length and stiffness to cytoskeletal filaments, but can be engineered to demonstrate dynamic behavior while requiring few reacting components in comparison to native systems [3, 4]. To move towards using DNA nanotubes as cytoskeletons in synthetic systems, they must demonstrate resilience to degrading enzymes found within cells, and their dynamic behavior must be automated and characterized in compartments.

Minimal cell systems execute tasks using transcription–translation (TXTL) machinery adapted from native cells. As other DNA nanotechnology has been rapidly degraded in *in vivo* environments, I first sought to assay the robustness of DNA nanotubes in an *Escherichia coli* cell-free TXTL system [5]. TXTL recapitulates physiological conditions as well as strong linear DNA degradation through the RecBCD complex. This work demonstrates that chemical modifications of the tiles making up DNA nanotubes extend their viability in TXTL for more than 24 hours. Furthermore, the addition of a Chi-site dsDNA, an inhibitor of the RecBCD complex, extends DNA nanotube lifetime significantly. These complementary approaches are a first step towards engineering resilience of DNA nanotubes for application in active minimal cell environments.

To demonstrate autonomous control of assembly and disassembly processes of nanotubes in cell-sized environments, I implemented a DNA-RNA hybrid nanotube design inside of water-in-oil droplets [6, 7]. In this design, inactive DNA tiles are activated by the presence of a trigger RNA molecule, which can be both produced and degraded by distinct enzymes [8]. A pulse of nanotube assembly-disassembly occurs when both transcribing and degrading components are present with inactive tiles in droplets. Notably, the encapsulated nanotube system required lower concentration of gene to trigger assembly than in bulk solution. Varying the concentration of gene and degrading enzymes affects both the kinetics of assembly-disassembly, and the morphology of the nanotubes. The methods developed here can be employed to develop more complex dynamics and functionalities of DNA nanotubes as synthetic cytoskeletal filaments in minimal cells.