

Abstract Submitted  
for the DFD19 Meeting of  
The American Physical Society

**Self-organization of microtubules in cell-sized droplets<sup>1</sup>** YA GAI, Mechanical and Aerospace Engineering, Princeton University, SAGAR SETRU, Lewis-Sigler Institute for Integrative Genomics, Princeton University, BERNARDO GOUVEIA, HOWARD STONE, Mechanical and Aerospace Engineering, Princeton University, SABINE PETRY, Molecular Biology, Princeton University — We combine droplet microfluidics and cell-free biological systems to examine the effect of confinement and nucleation on the assembly of microtubule (MT) networks. Central to the spindle assembly is the spatial organization of MTs, a long tubular structure formed through the polymerization of tubulin dimers. Such organization is regulated by RanGTP, a GTPase associated with chromosomal activities and acting as part of a major nucleation pathway for MTs. RanGTP has been explored using *Xenopus* egg extracts, a model cell-free system for probing spindle assembly. Most extract-based assays were performed in a test tube where cell-sized confinement was missing. Therefore, we asked whether confinement can affect the MT networks. We used droplet microfluidics for encapsulating extract-based assays by generating monodisperse, extract-in-oil droplets. By varying droplet diameters and encapsulated Ran concentrations, we demonstrate that these two physical factors regulate the assembly of MT networks. Together, the two factors yield MT networks with various steady-state architectures. Our results highlight the prominent role of MT nucleation in the self-organization of MTs in cell confinement and might have direct implications in nucleation-controlled soft material processing.

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Date submitted: 26 Jul 2019

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