Dynamics and instabilities of contractile actin networks in artificial cells

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Contractile actin network have an essential role in many cellular processes including cell division, intracellular transport and cell motility. While the molecular components involved are largely known, we still do not understand what controls the large scale properties of these networks. Synthetic, cell-free systems offer a valuable platform to explore cytoskeletal dynamics over a broad range of parameters, detached from the complexity of the living cell. We generate bulk actin networks in cell-sized compartments by introducing cytoplasmic *Xenopus* egg extracts, which contain all the components of the actin machinery, into water-in-oil droplets. Importantly, the presence of actin turnover in our system allows these networks to attain a dynamic steady state characterized by contractile actin flows which persist for hours. The properties of the reconstituted actin networks can be modulated by introducing different nucleators, as well as auxiliary proteins such as crosslinkers and disassembly factors. We utilize this system to explore in a systematic manner how the interplay between actin assembly and disassembly processes, network connectivity and myosin motor activity, shapes the large-scale structure, turnover and flows of contractile actin networks. We find that under a broad range of conditions, the network undergoes homogenous contraction despite large spatial variations in network density, and that this contraction rate is inversely proportional to the actin disassembly rate. Depending on the conditions and the size of the droplets, we observe either a symmetric state in which the network contracts towards the center of the droplets and exhibits a spherically symmetric density and flow pattern, or a polar state in which the contraction center is localized near the droplet's boundary. In the symmetric state, the contraction center is actively maintained near the middle of the droplet, reminiscent of actin-based centering mechanisms found in living cells. During symmetry breaking, the system transitions from this symmetric state to a polar state, mimicking cellular symmetry breaking as seen for example during motility initiation or spindle migration in mammalian oocytes.