Supplementary Information for Hierarchical Mechanical Modeling of Cytoskeletal Filament Networks

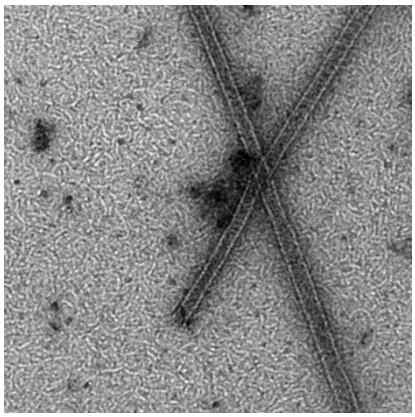

Figure 1: Preliminary negative-stain EM images of the Microtubule and K401-streptavidin (KSA) motor cluster system. Visible aggregates of K401 may be responsible for large-scale dynamics, even though the traditional microscopic picture expects KSA dimers. Insight and quantitative analysis of these details will guide the best approach for mechanical models.

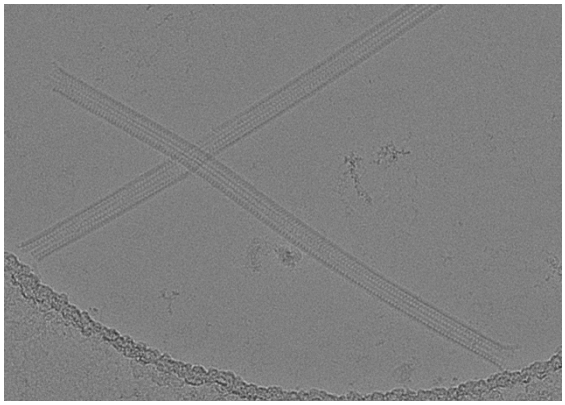

Figure 2: Preliminary cryo-EM images of Microtubules without KSA. Extracting detailed information of the microtubule lattice is important for extracting population statistics of motors relative to a given microtubule binding site.

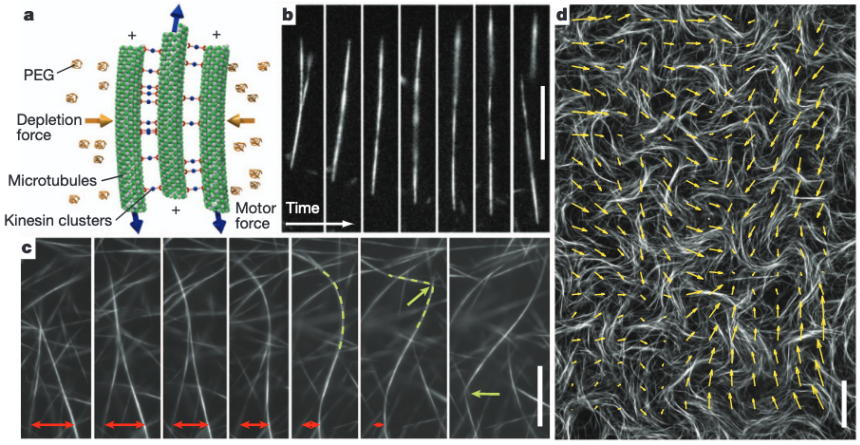

Figure 3: Reprinted from [1]. (a) Supposed microscopic picture of the microtubule system. Depletion forces facilitate bundle formation by inducing an effective attraction between filaments, while Kinesin clusters crosslink microtubules and generate sliding motion. (b) Individual microtubule bundles merge, extend, and fall apart. (c) Bundles in a percolated network exhibit the dynamics from (b) on short time-scales to produce robust dynamic steady states at long-times and large-scales. (d) Large-scale dynamics of interacting microtubule bundles are considered “active gels”, viscoelastic networks characterized by a bundle velocity field (yellow arrows). Their study has gained enormous popularity with the hopes to engineer self-healing materials and discover principles of biological organization.

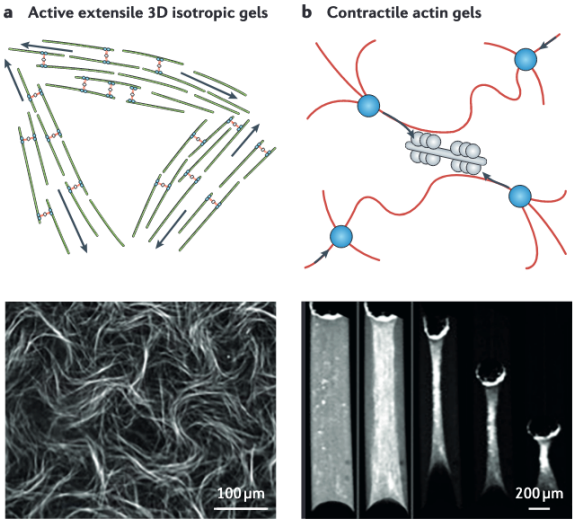

Figure 4: Reprinted from [2]. Biological active gels—assemblies of cytoskeletal filaments whose dynamics is generated by local energy consumption by molecular motors—exhibit a wide range of macroscopic behavior. (a) The microtubule-based system we study is usually characterized by chaotic, extensile sliding motion of microtubule bundles, which generates autonomous fluid flow at large-scales. (b) Active gels can also generate large-scale contractions. In an actomyosin system, myosin motors organize into mini-filaments that crosslink and entangle actin. The resulting macroscopic network contracts to a fraction of its initial size. Cryo-EM can reveal the microscopic mechanisms responsible for the fantastic hierarchical self-assembly of active gels across experimental conditions. They provide key insight into the spontaneous self-assembly of in-vivo structures instrumental in cellular function like the spindle[3,4].

[1] Sanchez, Tim, et al. "Spontaneous motion in hierarchically assembled active matter." *Nature* 491.7424 (2012): 431-434.
[2] Needleman, D., Dogic, Z. Active matter at the interface between materials science and cell biology. *Nat Rev Mater* **2**, 17048 (2017).
[3] Fürthauer, Sebastian, and Michael J. Shelley. "How cross-link numbers shape the large-scale physics of cytoskeletal materials." *Annual Review of Condensed Matter Physics* 13 (2022): 365-384.
[4] Cossio, Pilar, and Gerhard Hummer. "Bayesian analysis of individual electron microscopy images: Towards structures of dynamic and heterogeneous biomolecular assemblies." *Journal of structural biology* 184.3 (2013): 427-437.