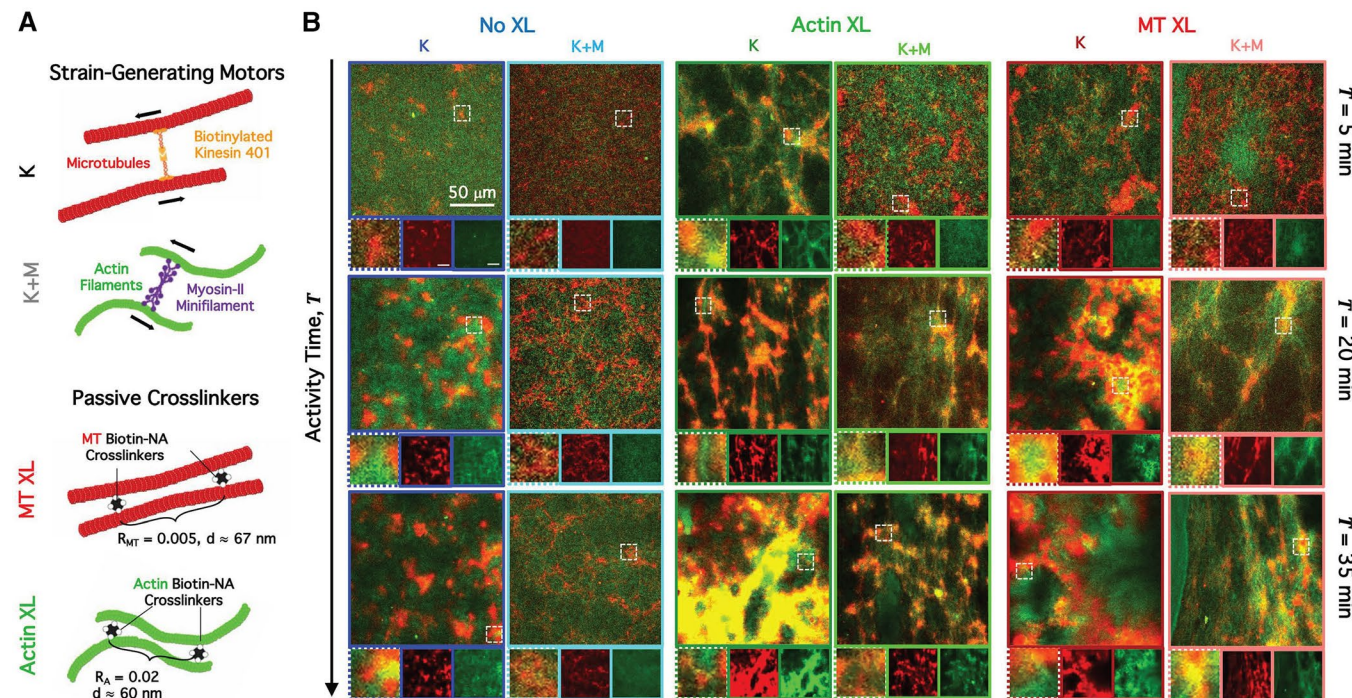


The cytoskeleton is a non-equilibrium multifunctional composite of diverse protein filaments, motors, and crosslinkers that cooperate and compete to enable diverse cellular structures and processes. The composite nature of the cytoskeleton, which confers its signature versatility and programmability, is one of its hallmarks. Yet, current active matter platforms, inspired by the cytoskeleton, are limited to single force-generating components and/or substrates. Here, we engineer composites of microtubules and actin driven by kinesin and myosin motors—**breaking new ground in active matter design** by incorporating multiple independently tunable force-generating components and active substrates.

We discover that motor competition delays the onset of active dynamics and suppresses de-mixing, while crosslinking hastens this onset by enhancing network connectivity. Importantly, the emergent dynamics and non-equilibrium properties we reveal can be programmed by very subtle changes in substrate connectivity and activity.

Active double-motor composites and actin and microtubules display a rich array of structures and dynamics



RJ McGorty, CJ Currie, J Michel, M Sasanpour, C Gunter, KA Lindsay, MJ Rust, P Katira, M Das, JL Ross, RM Robertson-Anderson. Kinesin and myosin motors compete to drive rich multiphase dynamics in programmable cytoskeletal composites. PNAS nexus 2, pgad245 (2023). DOI: 10.1093/pnasnexus/pgad245

In this work, our collaboration developed protocols for engineering and characterizing tunable three-dimensional composite networks of co-entangled actin filaments and microtubules.

Such composites undergo active restructuring and ballistic motion, driven by myosin II and kinesin motors, and are tuned by the relative concentrations of actin, microtubules, motor proteins, and passive crosslinkers.

By sharing these protocols and results in a video-based journal format, we lower the barriers for researchers to study active cytoskeletal mechanics and empower and train the next-generation workforce in biomaterial science.

M. Sasanpour, D.H. Achiriloaie, G. Lee, G. Leech, M. Hendija, K.A. Lindsay, J.L. Ross, R.J. McGorty, and R.M. Robertson-Anderson, 2022. *Reconstituting and characterizing actin-microtubule composites with tunable motor-driven dynamics and mechanics*. *JoVE* (Journal of Visualized Experiments), **186**, e64228 (2022). DOI: 10.3791/64228.



Still frame capture from the video-based protocol, which is available from JoVE (Journal of Visualized Experiments)

Our collaboration developed and hosted a 2-day hackathon at UC Santa Barbara that brought together 11 trainees (ranging from high school students to postdoctoral scholars) and 5 faculty to identify needs and develop software solutions for high-throughput analyses of complex active matter systems.

Before the hackathon, only 23% were somewhat or very comfortable with concepts of data screening, a number that increased to 100% after the event, based on post-event surveys.

91% of respondents felt that the software developed at the hackathon would be useful to researchers, both in their own groups and to other groups, within the upcoming year.

We are now integrating these codes into research frameworks and will publish our results and all codes in the upcoming reporting period.



DMREF team members working collaboratively to develop software codes for high-throughput analyses

Our collaboration engaged two high school students in data science and database design projects through a summer internship program at University of San Diego.

The students expanded their existing programming skills and worked collaboratively with the PIs to determine the optimal platform, programming language and server to host the database.

They regularly presented their work to the full DMREF collaboration. One of the high schools students continues to work on the project along with one of her high school teachers.

We anticipate continued involvement of high school students on this project through our annual summer high school internship program, providing excellent training and workforce development opportunities in data science and integrated computational and experimental approaches.



High school students conducting research and presenting their work on database design at Univ of San Diego

Variable Name	Type	Description
material_name	String (ASCII) (short)	Name of the material (from front page)
Detailed composition (components, concentrations, etc.)	String (ASCII) (long)	Not a whole protocol, but an overview on how the network was created (which materials + concentration of each)
media	Img	Tiff image
Acquisition details (e.g. frame rate, image size, number of frames, filters used)	String (ASCII) (long)	All important details
Metadata (if available)	If available	Text or File upload
process_notes	String (ASCII) (long)	Likely will be a block of text where a user can input relevant information about the creation of their material.

Home Page

Anderson McGorty Database of Materials [FORMAT]

Material Name	Composition	Method of Acquisition	Date
Material A	actin/myosin/MT/etc	confocal/olympus/etc	7/18/23
Material B	actin/myosin/MT/etc	confocal/olympus/etc	7/19/23
Material C	actin/myosin/MT/etc	confocal/olympus/etc	7/20/23
Material D	actin/myosin/MT/etc	confocal/olympus/etc	7/21/23
Material E	actin/myosin/MT/etc	confocal/olympus/etc	7/22/23
Material F	actin/myosin/MT/etc	confocal/olympus/etc	7/23/23
Material G	actin/myosin/MT/etc	confocal/olympus/etc	7/24/23
Material H	actin/myosin/MT/etc	confocal/olympus/etc	7/25/23
Material I	actin/myosin/MT/etc	confocal/olympus/etc	7/26/23
Material J	actin/myosin/MT/etc	confocal/olympus/etc	7/27/23

