Toward Multiscale Intracellular Rheology: A Probe-Aware, TFM-Compatible Measurement Framework

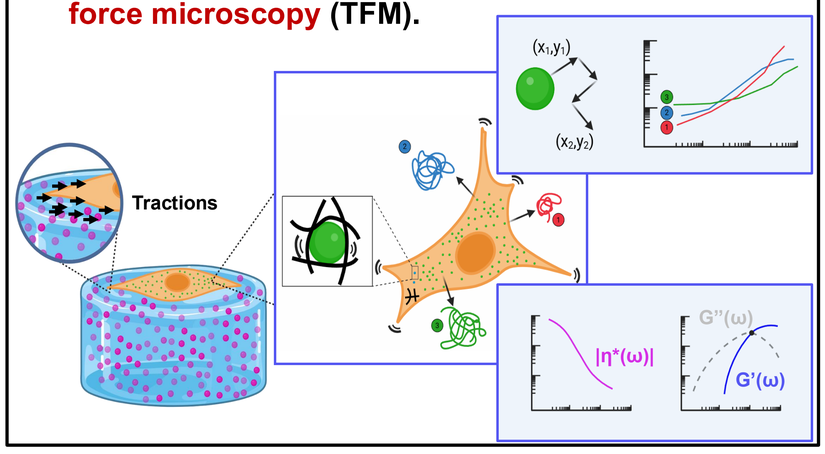
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Problem:

- Understanding the mechanical state of the cytoplasm, viscosity and fluidity is rarely considered in collective cell migration studies.
- Intracellular microrheology is conditioned by both probe choice and acquisition settings.
- Intracellular rheology captures only one dimension of cellular mechanics: the internal material state and its consequences for intracellular transport and remodeling.

Idea: To combine intracellular micro- and nanorheology using genetically encoded multimeric nanoparticles (GEMs) and fluorescent lipid droplets (LDs) with traction force microscopy (TFM).

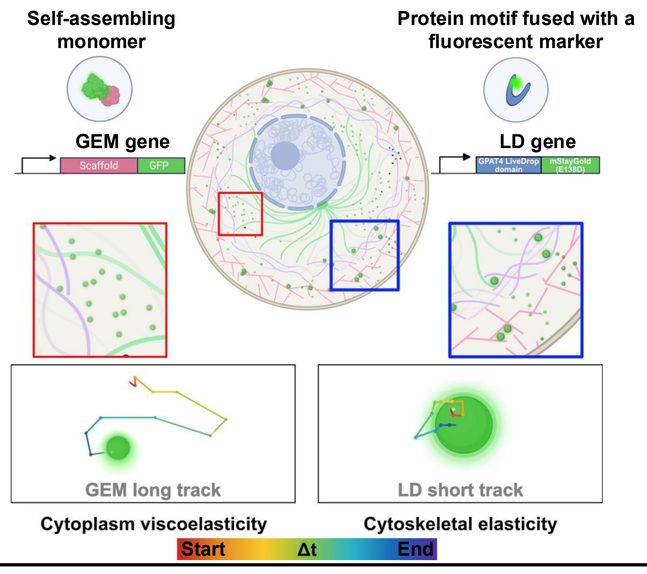


Plan:

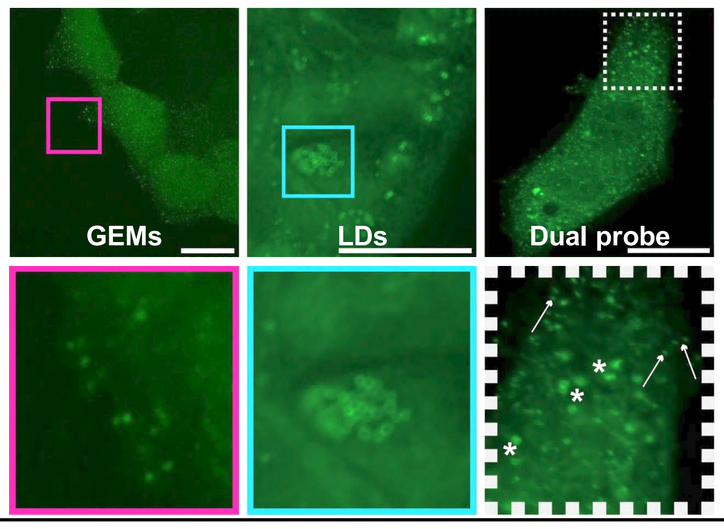
- Establishing a dual-tracer intracellular tool for multiscale rheology.
- MSD-derived intracellular rheology descriptors are acquisition-conditioned, with probe-specific stability windows.
- Separation of GEM and LD spots in dual-probe cells using control-derived FACS-like classification.
- Co-registered GEM nanorheology and TFM maps provide a TFM-compatible force-context benchmark.

Probe Engineering

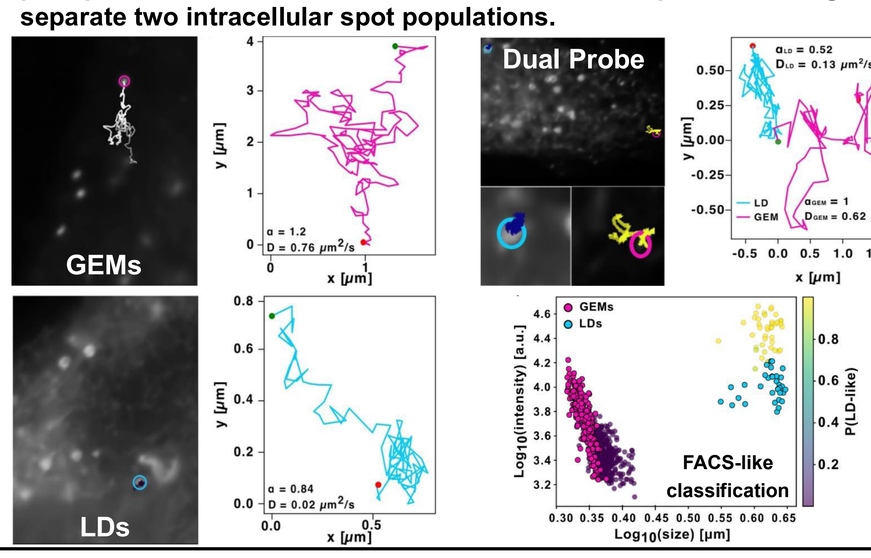
GEMs and fluorescent LDs schematic



We designed both probes to be detected in the same spectral channel to minimize acquisition complexity and keep imaging overhead low.



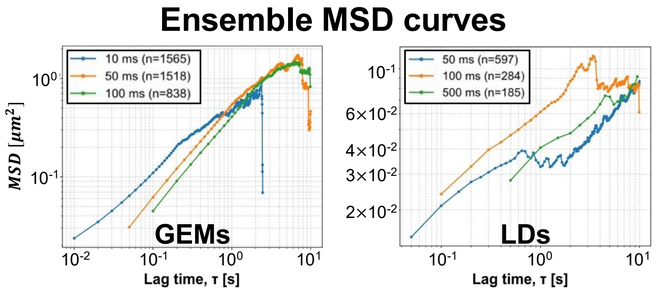
We performed single particle tracking analysis on both probes. In parallel, we implemented a FACS-like analytical framework based on per-spot feature vectors extracted from time-lapse recordings to separate two intracellular spot populations.



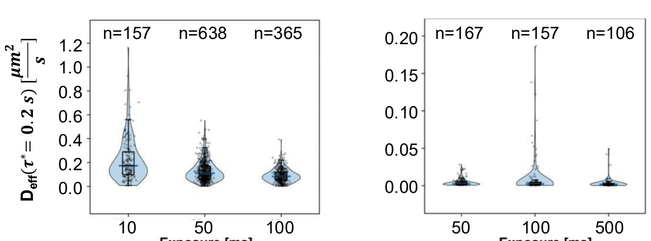
All scale bars: 25 μm

Probe Validation

Ensemble MSD curves



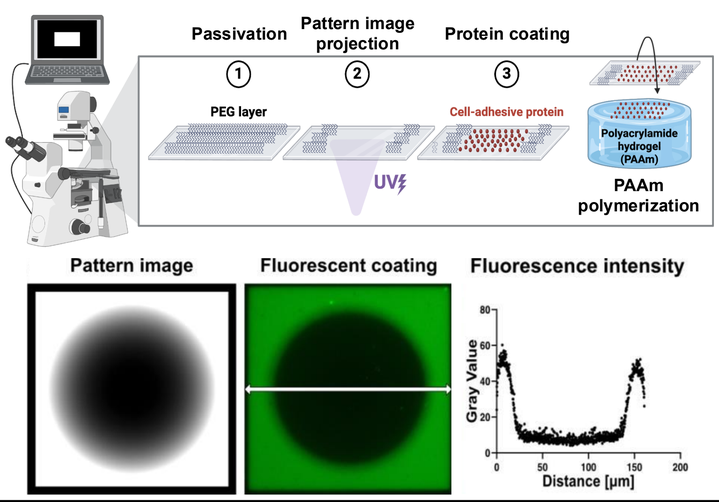
MSD-derived diffusion coefficient after trajectory-level QC filtering

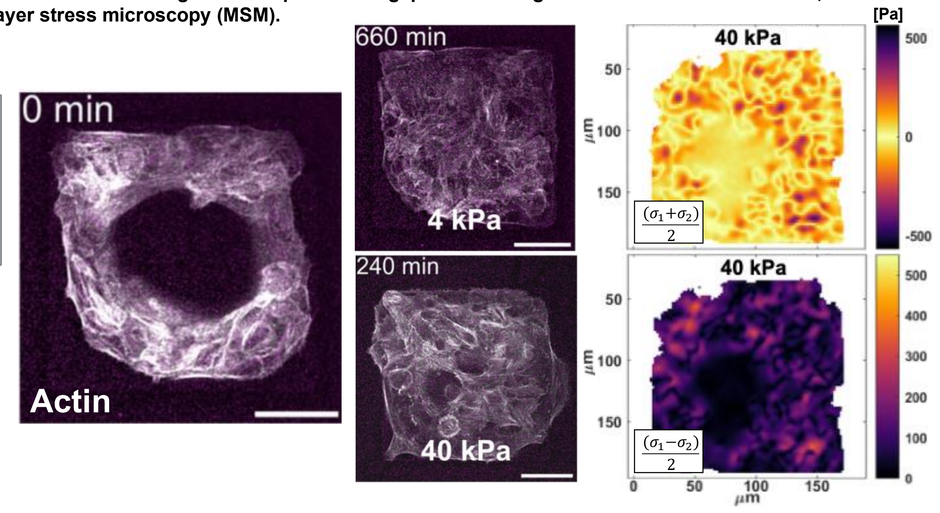


TFM validation

Photopatterned adhesive islands on gels for stiffness-controlled migration assays and stress mapping

Light-Induced Molecular Adsorption of Proteins (LIMAP) and the PRIMO system (Alvéole, France) enable the fabrication of micropatterned substrates in which ECM proteins are selectively adsorbed onto defined regions. For example, gradient patterns can be used to generate reproducible gaps for cell migration studies. In this context, traction force fields can be converted into collective-scale descriptors using monolayer stress microscopy (MSM).

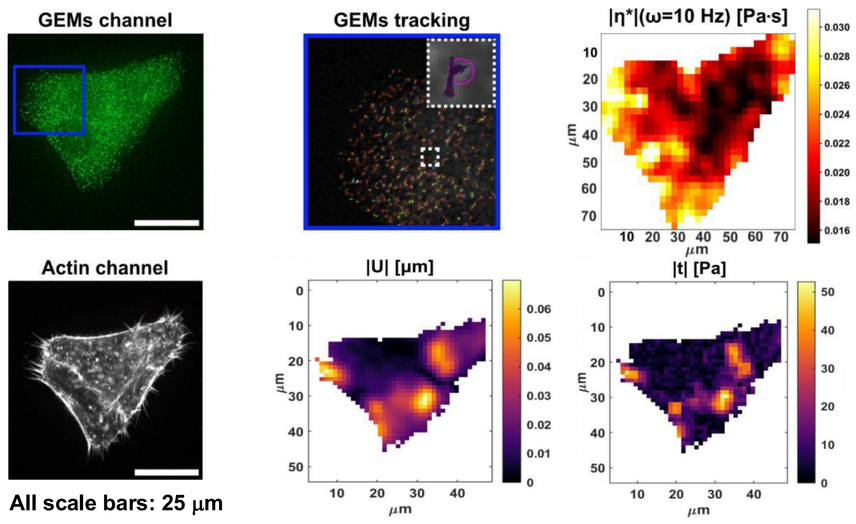




All scale bars: 50 μm

Proof-of-concept TFM + GEMs

We pair GEM-based intracellular nanorheology with TFM in a two-channel imaging workflow. This combined readout provides a way to interpret intracellular material state together with force generation within the same experimental framework.



All scale bars: 25 μm

Conclusions & Future Directions

- **Comparability is a design choice:** for each probe and analysis pipeline, stable acquisition settings should be empirically defined and clearly reported so that observed differences reflect biology.
- **Intracellular rheology, stratified using FACS-like gates,** could be interpreted alongside tissue-level force transmission within the same experiment, strengthening the link between intracellular material state, interfacial force generation, and long-range mechanical coordination.
- **Same-cell co-registration** of intracellular rheology across scales with traction output remains an aspirational goal. The dual-probe approach represents a practical step in that direction by providing multiscale intracellular readouts in a channel-efficient format that could be paired with TFM in future studies.
- **Together, these advances** suggest a tractable path from probe- and acquisition-aware intracellular readouts to geometry-controlled experiments that link the crowded intracellular environment to inside-out force output quantified by traction measurements.

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