GEMs: Genetically Encoded Multimeric Nanoparticles for Biophysical Analysis of Cells

Bright, stable, and easy to use nanoparticles for high-throughput nanorheology at the mesoscale range, eliminating the need for highly sensitive microscopes.

Technology

The Holt Lab has developed 50nm diameter genetically encoded multimeric (GEM) nanoparticles, comprised of the encapsulin domain from *Quasibacillus thermotolerans* (QtE) and a GFP fluorescent tag fused at the C-terminus, for probing the biophysical properties of the cell at the mesoscale length scale (10nm-100nm) with fluorescent microscopy. Inspired by previous findings that encapsulin self-assembles into a 240-subunit icosahedral compartment with a 42-nm diameter, the research team added a GFP cloud to the encapsulin core scaffold to yield a 50nm-GEM with 240 fluorophores, improving upon the team's previously published 40-nm GEM designs (*Carlini et al., Dev Cell 2020*). In the constructed 50nm-GEM plasmid (Figure 1), the open reading frame (ORF) for *Quasibacillus thermotolerans* encapsulin was optimized for yeast expression and synthesized as a gene block for cloning the DNA fragment in yeast and mammalian vectors. Quantitative measurements of GEM nanoparticle dynamics in single *S. cerevisiae* cells determined the 50-nm GEMs have a significantly lower mean diffusivity across all cells, when compared to the previously reported 40nm-GEMs. For 50nm-GEMs, a wide trajectory spread is noted, reflecting cell heterogeneity. Conversely, 40nm-GEMs show reduced heterogeneity and a more confined data spread, suggesting that the cytoplasmic environment is more heterogeneous at the 50 nm scale compared to the 40 nm scale. Importantly, there is no evidence of barriers obstructing GEM movement, as indicated by the absence of sharp declines in Gaussian step size distributions. Furthermore, because the GEM nanoparticles are derived from a thermostable organism in a different kingdom than mammalian, there is a reduced likelihood of protein-specific interactions between the GEM and native proteins. In summary, the 50nm-GEMs effectively enable high-throughput nanorheology with routine lab equipment, allowing a broader range of researchers to study the biophysical properties of cells and various disease pathologies.

Development Status

We have been using the technology to study how the physical properties of the cytoplasm and nucleus change during cancer progression, aging, and in response to mechanical stress.

Background

Alterations in the biophysical properties of cells have been linked to the progression of numerous diseases, including neurodegeneration, cancer, and fibrosis. Aberrant phase separation in densely packed cellular environments has been associated with harmful effects, such as genomic instability, altered gene expression, DNA damage, disrupted cell signaling,

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cellular senescence, and compromised protein quality control. A significant challenge in studying cellular crowding is the lack of tools to analyze macromolecular complexes of varying sizes, particularly at the mesoscale range. Nanorheology, which infers biophysical properties from the motion of tracer particles, has been one of the most effective methods for characterizing the cell interior. However, introducing probes for nanorheology into cells has glaring drawbacks. Methods such as microinjection can damage cells and dilute the cytoplasm, complicating the determination of whether observed changes in crowding are due to intrinsic cellular alterations or artifacts introduced by the method itself. Additionally, microinjection is laborious, low-throughput, and requires highly sensitive microscopes to achieve sufficient temporal resolution. Given the current shortage of techniques to study the physical properties of cells and the physiological impacts of environmental perturbations, there is a pressing need for innovative technologies that can elucidate how the intracellular environment affects mesoscale macromolecules.

Applications

- Nano-rheology research tool for mammalian cells.
  - Applications to aging-related diseases, neurodegeneration, cancers, and Amyotrophic Lateral Sclerosis (ALS).
- Nano-rheology research tool for yeast and plant cells.
  - GEMs enable biophysical analysis of yeast and plant cytoplasm.
- Nano-rheology research for all other systems tested including *C. elegans, Drosophila, and E. coli.*

Advantages

- **Bright, stable, and easy to use:** GEM fluorescence enables single particle tracking at a very high frame rate, without the need for highly sensitive microscopes.
- **Improved data reliability:** GEMs reduce artificial perturbations on the cell, with no dilution or membrane disruption.
- **Reduced risk of protein-specific interactions:** GEM nanoparticles are biorthogonal and derived from an organism in a different kingdom than native proteins.
- **Precise single particle tracking:** Defined icosahedral geometry and size enables physical analysis of GEM motion.

Intellectual Property

NYU has filed a U.S. provisional patent application covering: 1) the compositions of the polynucleotides and vectors that form GEMs; 2) the method of producing GEMs; and 3) the method for probing the cell using GEMS or vectors.

References

1. Liam J Holt, et. al , https://pubmed.ncbi.nlm.nih.gov/39005449/