Phase separation of a microtubule plus-end tracking protein into a fluid fractal network

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Microtubule plus-end tracking proteins (+TIPs) orchestrate essential cellular processes like mitosis through dynamic condensates, yet their internal supramolecular architecture remains elusive. Our study presents a breakthrough in understanding the phase separation of Bik1, a CLIP-170 family member in budding yeast, revealing that its condensates form a fluid fractal network rather than a homogeneous liquid.

Utilizing small-angle X-ray scattering (SAXS) on preformed Bik1 droplets flowing within a microfluidic chip, we captured the nanoscale organization of the condensed phase [1]. The scattering intensity follows a distinct power-law decay, $I(q) \sim q^{-2}$, across length scales from 300 Å to 3000 Å. This behavior corresponds to a fractal dimension close to 2, providing direct experimental evidence for percolation-based models of biomolecular condensates. Real-space reconstructions derived from the SAXS data visualize a heterogeneous structure characterized by alternating protein-rich clusters and solvent-rich voids. This fractal topology explains the extraordinary material properties of Bik1 droplets: they exhibit unusually high viscosity despite maintaining a relatively low protein concentration, a feature critical for their function as the "+TIP body" in vivo.

Complementing the SAXS findings, crosslinking mass spectrometry (XL-MS) identified specific conformational rearrangements driving this transition. We observed a significant increase in self-links within the central coiled-coil domain and enhanced interactions between the N-terminal CAP-Gly domain and the C-terminal EEY/F-like motif during condensation. Truncation mutants lacking either terminal domain failed to form droplets, confirming that multivalent interactions mediated by these regions are essential for establishing the fractal network.

Our SAXS data and empirical structural model demonstrates that the fractal nature of Bik1 condensates optimizes space-filling while preserving dynamic exchange. This framework offers a template for investigating the supramolecular organization of other complex, oligomeric biomolecular condensates.

Keywords:
Phase separation, Fractal network, Biomolecular condensates, Bik1

References:

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