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Strategies to detect phase separation in cells

Despite the tremendous progress in studying biomolecular condensates, it remains a challenge to reliably detect and quantify condensates produced by liquid-liquid phase separation (LLPS) in living cells. In particular, liquid droplets produced by LLPS and stretches of chromosomes decorated with nuclear proteins that do not undergo LLPS can share several properties, like an appearance as 'foci'and dynamic turnover of molecules. To address this issue, we are currently developing experimental and computational strategies to distinguish both cases. On the one hand, we use half-FRAP experiments, in which half of the condensate is bleached and the intensity in both halves is followed. We find that such experiments can distinguish between a liquid droplet formed by LLPS and a scaffold that is decorated with transiently binding proteins. We show that an anti-correlation between the intensities in both halves is a robust signature of LLPS that is related to the preferential internal mixing of condensates, and we establish a quantitative relationship between this signature and the interfacial energy at the boundary of the condensate. On the other hand, we work on a computational workflow to retrieve key information about cellular condensates from published large-scale datasets, including the type and number of molecules in each condensate. We use this information to infer the biophysical nature of the condensates and to compare it to the expectations for LLPS. Both approaches are complementary to each other and will hopefully improve our understanding of biomolecular condensates in cells.

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