Polymer chemistry

Liquid-like droplets of supramolecular polymers

Jennifer L. Ross

The molecules of liquid crystals and proteins can form liquid-like condensates, but such a phenomenon had not been observed for supramolecular polymers, which are held together by non-covalent bonds – until now. **See p.1011**

The separation of liquids from each other is such a common occurrence that there is an idiom about oil and water not mixing. It is more surprising when a substance that is dissolved in water spontaneously separates. If that substance forms a liquid-like state on separation, rather than aggregating into a solid, the resulting droplets can be used to confine or organize objects from the nanometre to the macroscopic scale.

In the past few years, scientists have observed that such liquid–liquid phase separation (LLPS) of proteins, RNA and DNA occurs in live cells and has crucial roles in many biological processes, including gene regulation and RNA processing and degradation[1–6]. On page 1011, Fu *et al.*[7] report the first demonstration that synthetic supramolecular polymers – macromolecules that self-assemble as a result of non-covalent interactions between the constituent monomer molecules – undergo LLPS.

The authors made the serendipitous observation that a compound known as ureidopyrimidinone glycine (UPy–Gly) polymerizes in solution to form short fibres, which then promote a condensation process that produces high-density phases containing supramolecular fibrils. Because the fibrils have a high aspect ratio (they are nanometres in diameter, and micrometres in length), these condensed assemblies of fibrils were not spherical, but instead were tactoids – that is, spindle-shaped with pointed ends (Fig. 1).

Fu and colleagues went on to rigorously characterize the shape, structure, mechanics and dynamics of the condensed phase. Using a technique called confocal laser scanning microscopy, the authors found that the tactoids were liquid-like, acting as droplets that can merge together, and that the fibrils could diffuse within the tactoids. As time advanced, the tactoids grew larger both through merging events and through continuous elongation of the fibrils. The authors also found that a minimum fibril length is needed for the condensation to occur.

Much like conventional LLPS, the condensation process observed by Fu *et al.* is driven by an increase in entropy (a measure of the number of states a system can adopt). It might seem counter-intuitive that a process in which supramolecular polymer molecules become 'more organized' than they were before leads to larger entropy, but this is the result of smaller molecules in the system being 'freed up' during tactoid formation. One indicator of the role of entropy is that the process is controlled by temperature, with higher temperatures driving faster condensation and producing smaller tactoids. Another indicator is that the tactoids continue to grow over time, increasing in length as the fibrils in them get longer.

Fu *et al.* also observed that the addition of crowding agents (inert compounds, such as polymers, the role of which is to occupy space) sped up the formation of the tactoids, increased the number that formed and reduced their size. These effects occur because crowding agents reduce the volume of solvent available for the UPy–Gly molecules, thereby increasing the effective concentration of those molecules – which then gain entropy when larger molecules in the system are pushed together. Increasing the amount of crowding agent also changed the shape of the tactoids, from bipolar (wide, spindle-shaped) condensates in which the fibrils are oriented along arcs that connect the tips of the spindle, to longer and thinner homogeneous (rod-shaped) tactoids, in which the fibrils all align with the straight axis of the tactoid. Using an X-ray scattering technique, the authors determined that the fibrils in the tactoids were hexagonally packed, and that they packed together more densely over time and at high concentrations of crowding agents.

Not only did individual fibrils in the tactoids continue to grow, but they also became less mobile, causing the tactoids to become more viscous. Similar gelation has been observed in many condensed systems that undergo LLPS, including protein systems[8–10]. Such gelation is often reversible, but irreversible protein gelation can lead to aggregation of the condensed phases, and is often observed in neurodegenerative and neuromuscular diseases[3,11,12]. Studies of how synthetic polymer systems can form gels (and of the putative

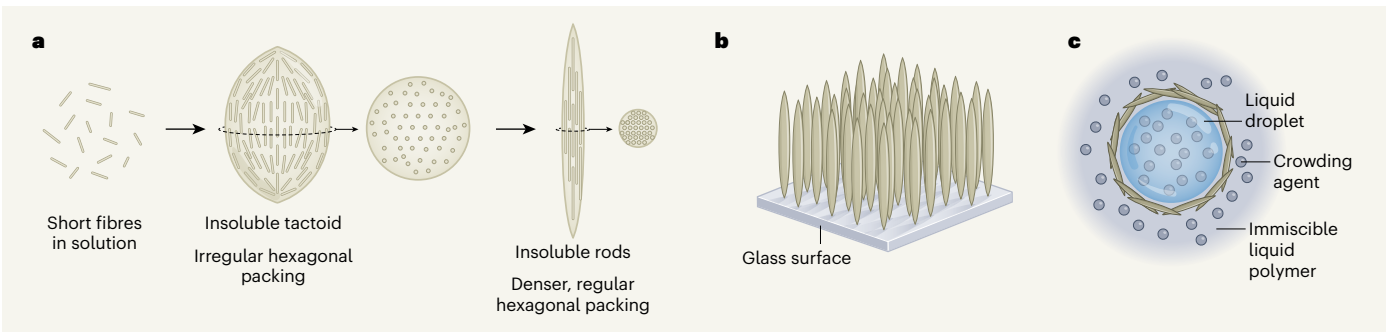

Figure 1 | Supramolecular polymers form tactoid assemblies. **a**, Supramolecular polymers self-assemble from subunits in solution and are held together by non-covalent bonds. This produces fibres that increase in length over time. Fu *et al.*[7] report that fibres of a supramolecular polymer act as micrometre-scale versions of liquid-crystal molecules. At high concentration or in the presence of crowding agents (inert compounds that take up space in solution; not shown), the fibres condense into spindle-shaped, liquid-like droplets known as tactoids, in which the fibres are packed imperfectly into a hexagonal arrangement. Over time (or with crowding agents), the fibres in the tactoids grow even longer and align, forming more densely packed rods with regular hexagonal packing. **b**, The tactoids form forest-like arrays on a glass surface. **c**, They also form crown-like arrangements at the surfaces of liquid droplets suspended in an immiscible liquid polymer.

reverse process) might shed light on how protein aggregation can be reversed, given that synthetic polymers can easily be engineered to mimic the properties of proteins.

Another intriguing attribute of Fu and colleagues' tactoids is that they can self-assemble into larger-scale structures. For example, the authors observed that tactoids on a glass coverslip aligned perpendicularly to the surface, forming an array. At first, the tactoids could diffuse laterally on the surface and coalesce. But as they aged and grew longer, they stopped coalescing and tilted, creating a forest of leaning spindles. This indicates that supramolecular polymers can undergo hierarchical assembly processes, from the nanoscale (supramolecular polymer subunits) to the millimetre scale (assemblies of tactoids), which could potentially be used to fabricate microscale bristles that, in turn, serve as scaffolds for larger structures.

By contrast, tactoids on a liquid surface formed distorted, crown-like shapes. Fu *et al.* observed this by dissolving UPy–Gly molecules in a system in which droplets of one polymer (dextran) form in another immiscible liquid polymer (polyethylene glycol; PEG). The supramolecular polymer formed from UPy–Gly at first dissolved into the PEG, but then formed condensates at the surface of the dextran droplets, eventually encapsulating the droplets. The authors found that continuous or discrete supramolecular networks could be formed at the surface of the droplets by varying the pH and concentrations of salts in the system. Similar condensation behaviour to that of UPy–Gly was observed when other supramolecular polymers were tested in the PEG–dextran system, indicating that LLPS is a general phenomenon for supramolecular polymers.

Fu and colleagues' study shows that supramolecular polymers are not immune to the laws of physics that prevail in other systems at different scales. For example, the entropic driving force that causes the condensation of the tactoids is also responsible for the condensation of liquid-crystal mesogens — nanometre-scale molecules that form liquid crystals. The authors' findings therefore open up the possibility of using supramolecular polymers as models of liquid-crystal behaviour. Although biological fibres and rods, such as virus particles, microtubules and actin filaments, have also been shown to act like liquid-crystal mesogens at the micrometre scale[13–18], they are difficult to modify. By contrast, there are many ways to modify the aspect ratio, chemical nature and chirality (handedness) of tactoids formed from synthetic supramolecular polymers, enabling them to be used as micrometre-scale models that closely relate to the molecular-scale liquid-crystal mesogens that are of interest to researchers. Future studies of the self-assembly of liquid crystals formed from supramolecular polymers might reveal the hierarchical organization of the world, from the nano- to the macroscale.

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1. McManus, J. J., Charbonneau, P., Zaccarelli, E. & Asherie, N. *Curr. Opin. Colloid Interface Sci.* **22**, 73–79 (2016).
2. Peng, P.-H., Hsu, K.-W. & Wu, K.-J. *Am. J. Cancer Res.* **11**, 3766–3776 (2021).
3. Wang, B. *et al. Sig. Transduct. Target Ther.* **6**, 290 (2021).
4. Yoshizawa, T., Nozawa, R.-S., Jia, T. Z., Saio, T. & Mori, E. *Biophys. Rev.* **12**, 519–539 (2020).
5. Hyman, A. A., Weber, C. A. & Jülicher, F. *Annu. Rev. Cell Dev. Biol.* **30**, 39–58 (2014).
6. Alberti, S., Gladfelter, A. & Mittag, T. *Cell* **176**, 419–434 (2019).
7. Fu, H. *et al. Nature* **626**, 1011–1018 (2024).
8. Boeynaems, S. *et al. Trends Cell Biol.* **28**, 420–435 (2018).
9. Sahu, S. *et al. PNAS Nexus* **2**, pgad231 (2023).
10. Xu, Y. *et al. Adv. Mater.* **33**, 2008670 (2021).
11. Zbinden, A., Pérez-Berlanga, M., De Rossi, P. & Polymenidou, M. *Dev. Cell* **55**, 45–68 (2020).
12. Ray, S. *et al. Nature Chem.* **12**, 705–716 (2020).
13. Edozie, B. *et al. Soft Matter* **15**, 4797–4807 (2019).
14. Weirich, K. L. *et al. Proc. Natl Acad. Sci. USA* **114**, 2131–2136 (2017).
15. Oakes, P. W., Viamontes, J. & Tang, J. X. *Phys. Rev. E* **75**, 061902 (2007).
16. Nyström, G., Arcari, M. & Mezzenga, R. *Nature Nanotechnol.* **13**, 330–336 (2018).
17. Maeda, H. *Langmuir* **13**, 4150–4161 (1997).
18. Dogic, Z. *Phys. Rev. Lett.* **91**, 165701 (2003).

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Evolution

Mobile DNA explains why humans don't have tails

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The lack of a tail is one thing that separates apes — including humans — from other primates. Insertion of a short DNA sequence into a gene that controls tail development could explain tail loss in the common ancestor of apes. **See p.1042**

Tails are a common feature in the animal kingdom, and all mammals have a tail at some point during embryonic development[1]. In humans, the tail disappears at the end of the embryonic phase — approximately eight weeks *in utero*[2] — although internal parts remain in the form of the tailbone. The loss of the tail is considered a distinctive characteristic of apes and might have influenced our own upright walking style. On page 1042, Xia *et al.*[3] report that the insertion of a type of mobile genetic sequence that moved around the genome during evolution, known as a transposable element, could be associated with the loss of the tail.

Most monkeys have a tail, and tails were present at the origin of the primate lineage more than 65 million years ago[4]. In fact, the absence of a tail is one way to distinguish apes from monkeys. This characteristic, or phenotype, is shared across all apes, suggesting that tail loss coincided with, or occurred shortly after, the rise of apes after they diverged from their last common ancestor with monkeys around 25 million years ago.

With this knowledge, Xia and colleagues compared prime candidate genes for tail loss across the genomes of several primate species, initially focusing on exons (the regions of DNA that code for proteins). When this approach turned out not to be fruitful, the authors extended their investigation to non-protein-coding regions that were upstream or downstream of the genes, or in the genes themselves. The latter regions are known as introns, and they commonly interrupt protein-coding sequences. Xia and colleagues found that a type of primate-specific transposable element called an Alu element[5] was inserted in an intron of the *TBXT* gene — but only in apes, and not in other primate lineages. *TBXT* is also known as *Brachyury* (meaning 'short tail') because mutations in the gene have been associated with short tails in several species, including Algerian mice (*Mus spretus*)[6] and Manx cats (*Felis catus*)[7].

But how can the insertion of a short, roughly 300-base-pair Alu element into the non-coding sequence of a gene contribute to a tailless phenotype? To answer this, Xia and colleagues further scrutinized the entire *TBXT* gene and identified another Alu element oriented in the opposite direction (inverted) in intron 5. Because inverted Alu sequences in close proximity can pair up and create double-stranded RNA structures, the authors proposed that exon 6, which resides between the two Alu sequences, might be removed from the RNA transcript straight after transcription in a process called splicing (Fig. 1).

To determine whether these two Alu sequences create alternatively spliced versions of *TBXT* RNA transcripts, Xia and colleagues took human and mouse embryonic stem cells and differentiated them so that