Biomolecular condensates

https://doi.org/10.1038/s41589-023-01488-y

In search of chemical rationales

Aseem Z. Ansari

Small molecules and drugs are not homogenously distributed across cells, and are instead enriched in distinct subcellular compartments and membraneless biomolecular condensates. A new study lays out the path to identifying chemical features or 'rationales' that confer condensate-selective partitioning of small molecules.

Like dew in the morning mist or droplets of oil dispersed in water, some biomolecules undergo phase transition to form membraneless condensates within living cells[1,2]. Akin to oil droplets in water, the internal physicochemical properties within a biomolecular condensate can differ markedly from the surrounding cellular milieu. Moreover, condensates formed by different collections of biomolecules (such as proteins, nucleic acids or metabolites) can also differ from each other. As may be expected, condensates containing distinct sets of biomolecules are implicated in distinct cellular functions. It follows that formation of aberrant condensates or loss of functional condensates would contribute to the etiology of various diseases, including neurodegeneration, cancer and viral infection. With the growing appreciation for the pervasive roles of condensates in cellular function, elucidating the physical principles that govern their formation has become an active area of scientific investigation[3]. The recent observation that several widely prescribed chemotherapeutic agents selectively partition into distinct condensates where they mediate their actions has captured the attention of scientists in academia and birthed new biotech companies[4,5]. Deciphering the 'chemical grammar' that guides the selective partitioning of small molecules into distinct biomolecular condensates is an emerging frontier that will lead to the next generation of therapies. In this issue, Kilgore et al.[6] take an important step towards identifying the chemical features — or rationales— that correlate with the ability to selectively partition into distinct condensates[6].

Using live-cell microscopy, the authors examined the subcellular distribution of a curated set of inherently fluorescent small molecules, US Food and Drug Administration-approved drugs, natural products and chemical probes[6]. These molecules exhibited non-uniform spatial distributions, consistent with selective enrichment in distinct subcellular compartments such as the nucleus, mitochondria and the nucleolus, a multilayered membraneless condensate. Although molecules that target DNA or genome-active enzymes were expected to partition to the nucleus, the unmistakable enrichment of Sunitinib — a drug that acts on multiple cell surface receptor tyrosine kinases — in the nucleolus was a surprise. This observation builds on prior reports of drugs being enriched in functional condensates that are devoid of their intended high-affinity protein targets[5]. Such discordant patterns intimate that the physicochemical properties of certain condensates may offer a more welcoming chemical environment for a particular small molecule that was designed for a target that resides elsewhere in the cell. The implications of this finding are profound for understanding unanticipated off-target effects, tissue-specific pharmacodynamics, and for harnessing chemical features to guide the selective subcellular partitioning of next-generation therapeutic agents.

In search of the chemical features that confer condensate-selective partitioning, Kilgore et al.[6] developed a library of around 1,500 molecules by appending distinct moieties (R groups) onto commonly used organic fluorophore scaffolds. The selective enrichment of different chemical probes in three distinct homotypic condensates (biochemically reconstituted using three different nucleator or driver proteins) was tested via high-throughput imaging. After separating out the contribution of the dye scaffold, hints of distinctive chemical features were discernable among the molecules that selectively partitioned into the 'transcriptional', 'nucleolar' or 'heterochromatic' condensates. A deep learning neural network trained on a subset of condensate-selective probes was then able to predict the selectivity of other tested molecules, with particular success in predicting enrichment into the heterochromatic condensates formed by HP1α (Fig. 1a). The chemical features — called rationales, based on the lexicon from the machine learning field — for all three condensates were unsurprisingly enriched in aromatic and cationic moieties, but distinct flavors superimposed on these core scaffolds appear to tilt the selective partitioning of individual molecules. This insight marks an important advance for formulating the lexicon, syntax and, eventually, the chemical grammar that governs selective partitioning of small molecules into complex subcellular condensates and compartments.

With this deep learning framework, it would be interesting to examine recently revealed molecules that partition into condensates and perturb their function by varied mechanisms. For example, using a kinetic imaging approach called DropScan, Wang et al.[7] screened 1,777 known drugs, and found that LY2835219 rapidly cleared aberrant condensates formed by oncogenic transcription factors. Although this molecule is typically deployed as CDK4/6 kinase inhibitor, it appears to moonlight as a potential lysosome-targeting LYTAC[8] to deliver 'onco-condensates'[9] to the lysosome. The first-generation deep learning approach of Kilgore et al.[6] may well yield a rationale (or two) for why LY2835219 selectively partitions into onco-condensates formed by multiple aberrant transcription factors, without seeming to perturb normal transcriptional condensates.

More saliently, that small molecule engagement can alter the fate of complex biomolecular condensates over time is a consideration that could reveal non-obvious rationales or chemical properties that effect desirable functional and therapeutic outcomes (Fig. 1b). Although dissolution of condensates by small molecules is generally deemed a desired functional or therapeutic outcome, some condensate-targeting molecules mediate their function by gelation, ripening or hardening of aberrant condensates[5]. One report applied concept of 'entropic expansion' from astrophysics to provide a mechanistic framework for the observation wherein small molecule binding to intrinsically disordered regions led to increased conformational entropy, thereby preventing the conversion of biomolecules to a pathological non-dynamic

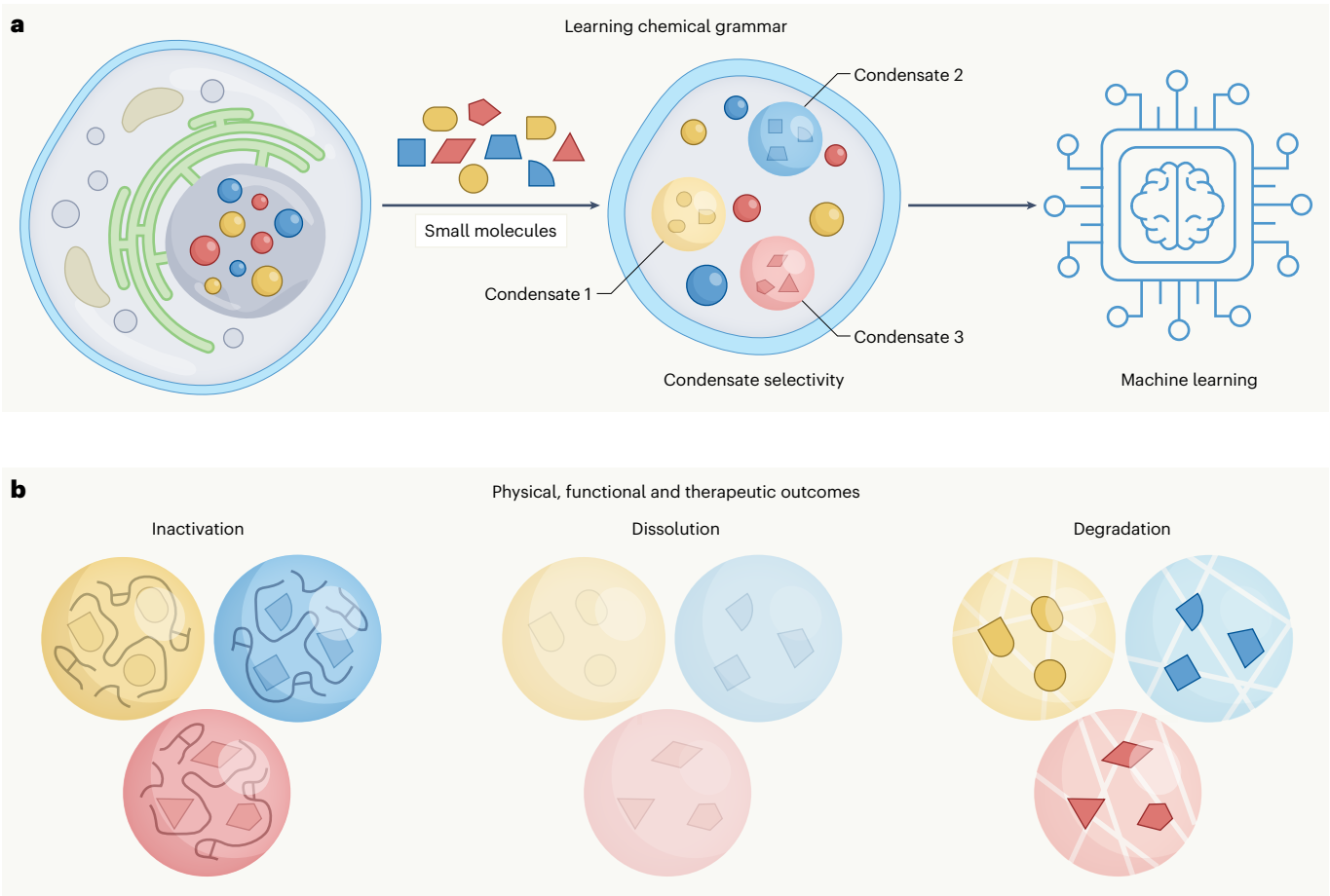

Fig. 1 | The search for chemical features that facilitate condensate-selective partitioning of small molecules. a, A cartoon of subcellular compartments with membraneless biomolecular condensates depicted as blue, red and gold spheres. The collection of small molecules with condensate-selective chemical features (rationales) is depicted in colors that are coincident with the cognate condensate. The shared rationales can be identified using deep learning algorithms. **b**, The physical, functional and therapeutic outcomes of small molecule partitioning into condensates. It is important to note that functional perturbation can occur via diverse mechanisms. Similarly, disappearance of a condensate may occur due to perturbation of condensate-forming interactions or due to degradation of the key scaffolding biomolecules.

state[10]. It remains to be explored whether such 'entropy-expanding' molecules or others that alter the material properties of condensates contain unique rationales, such as rotatable bonds or flexible chemical spacers. There is much that is nebulous and contentious in the study of biomolecular condensates[3] – however, what is unambiguous is that we are witnessing the dawn of new paradigms of drug design and exciting possibilities to effectively target thus far-undruggable drivers of disease.

Aseem Z. Ansari
Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA.
e-mail: aseem.ansari@stjude.org

Published online: 29 November 2023

References

1. *Nat. Chem. Biol.* **18**, 1289 (2022).
2. Alberti, S. & Hyman, A. A. *Nat. Rev. Mol. Cell Biol.* **22**, 196–213 (2021).
3. Mittag, T. & Pappu, R. V. *Mol. Cell* **82**, 2201–2214 (2022).
4. Klein, I. A. et al. *Science* **368**, 1386–1392 (2020).
5. Mitrea, D. M. et al. *Nat. Rev. Drug Discov.* **21**, 841–862 (2022).
6. Kilgore, H. et al. *Nat. Chem. Biol.* https://doi.org/10.1038/s41589-023-01432-0 (2023).
7. Wang, Y. et al. *Nat. Chem. Biol.* **19**, 1223–1234 (2023).
8. Banik, S. M. et al. *Nature* **584**, 291–297 (2020).
9. Mittag, T. & Ansari, A. Z. *Nat. Struct. Mol. Biol.* **28**, 543–545 (2021).
10. Heller, G. T. et al. *Sci Adv.* **6**, eabb5924 (2020).

Competing interests

The author declares no competing interests.