PNAS COMMENTARY

Percolation physics and density transition frameworks converge in biomolecular condensation

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A rapidly growing body of work in recent years has resulted in exciting advances in our understanding of the importance of biomolecular condensates (or, more generally, various forms of mesoscale to macroscale biological matter) and their transitions in biology and disease (1–3). At first glance, the physics of how liquids percolate through porous/granular materials or related concepts in network connectivity may not seem relevant to furthering our mechanistic understanding of biomolecular condensates. Interestingly, however, percolation theory has been extensively used in the related areas of polymer physics (4, 5) and phase transitions (as well as in numerous other fields). Now, in an exciting advance, Kar et al. (6) describe a combination of experimental, conceptual, and computational work that explores the connection between percolation physics and an important class of biomolecular condensation with links to neurodegenerative diseases.

Conceptual understanding in the biomolecular condensate field has been extensively guided by simple forms of nucleation and Flory–Huggins-type theories. A prediction of this type of theory is that, below a saturation concentration (c_{sat}) of a single macromolecule (e.g., protein) in a solvent, the macromolecule will exist mainly as monomers and very small clusters, because there is a size-dependent energy penalty for cluster formation. It is only above the saturation concentration that phase separation (a density transition) will occur, resulting in the formation of a dense phase (aka micrometer-sized droplets). Now, Kar et al. (6) describe a broad set of data that can provide a test of this prediction.

The proteins studied in this work are FET (FUS, EWSR1, TAF15) family proteins, with links to neurodegenerative disease, which have been extensively investigated in the field. Using a combination of imaging, dynamic light scattering (DLS), and single-particle (tracking, multiparameter fluorescence, and microfluidics-based) experiments, Kar et al. (6) show that, while phase separation is not observed in solutions below an effective c_{sat}, subsaturated solutions of these proteins contain a range of nanoscale clusters. The data indicate that clusters follow a heavy-tailed distribution, with low abundance of larger mesoscale clusters and distributions changing with total protein concentration. It is only above c_{sat} that larger micrometer-sized bodies that display coarsening appear. Fluorescence resonance energy transfer/DLS data show that cluster formation is reversible, and that protein exchanges between clusters. Together, these data draw a sharp contrast with the predictions based on nucleation theory discussed above. The authors then go on to invoke percolation theory–based ideas to offer an explanation for these observations, building on their and other previous work (4, 5, 7, 8).

In percolation theory, there exists a critical connectivity probability (p_c) at which the system undergoes a connectivity (geometric) transition, forming a system-spanning network (Fig. 1*A*). In the present work, the proteins are represented in a stickers and spacers model of associative polymers, with stickers contributing specific protein–protein interactions and spacers contributing generalized excluded volume/solvation effects. In the framework of the percolation model, this system can undergo a percolation-type connectivity transition (via specific sticker–sticker interactions) at a critical protein concentration c_{perc} (Fig. 1*B*). Thus, specific sticker–sticker interactions give rise to an additional energy scale class that contributes to system behavior. As previously described by the Pappu laboratory (8), this scenario can give rise to an interesting coupling between density and percolation transitions if the system/conditions result in c_{perc} being greater than c_{sat} but less than the dense phase concentration c_{den}. Under these conditions, a density transition results in a coupled percolation transition in the dense phase, since c_{den} is greater than c_{perc}.

How does this relate to the authors' experimental findings of molecular clusters below c_{sat} (6)? The key result from the percolation approach is that, even below the percolation threshold, smaller network clusters are still formed (Fig. 1), and the size distribution of these clusters shifts to larger sizes as the connectivity (concentration) is increased. As noted above, this is just what was observed in the authors' measurements. The authors then looked at ways to test the coupling between the two types of transitions. Indeed, using small-molecule solutes or mutations as perturbations, they find either coupled or differential effects on formation of clusters and macroscopic phase separation. These results are consistent with the existence of separate types of interactions governing generalized solubility and specific connectivity effects, and the idea that these can be perturbed selectively but can also be coupled. The above work is also complemented with simulations whose results are in keeping with the above model. Overall, the work serves to inspire a number of lines of thinking and inquiry.

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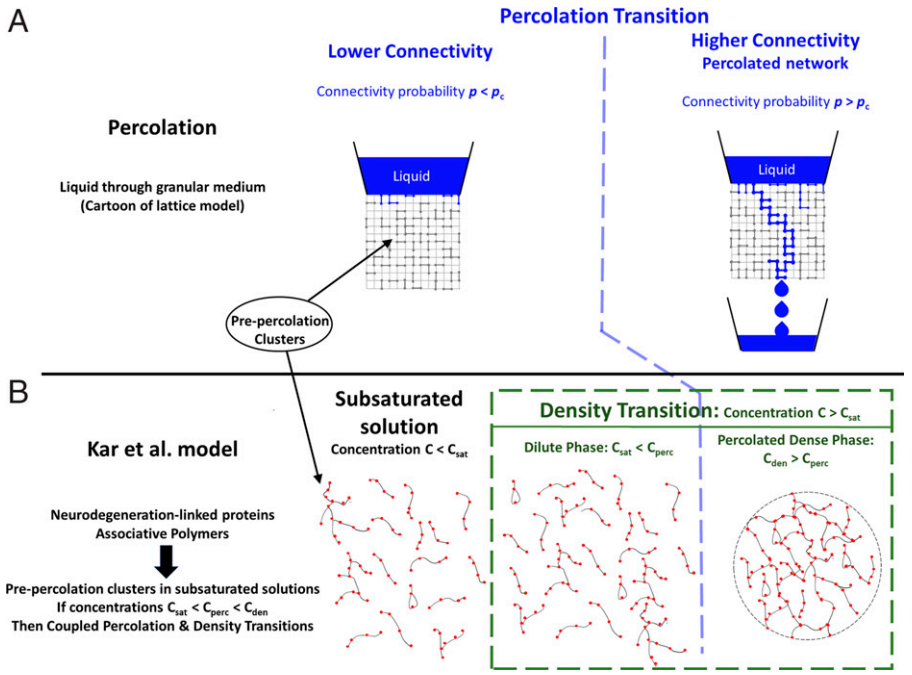

Fig. 1. Cartoon depicting (*A*) percolation of a liquid through porous/granular medium and (*B*) the model from Kar et al. (6) for prepercolation clusters in subsaturated solutions of neurodegeneration-linked proteins.

Formation of prepercolation clusters (which could be considered the equivalent of "lattice animals" in mathematics) at subsaturation concentrations raises several functional implications and interesting questions. As the authors note (6), these types of clusters form at low macromolecule concentrations that are often more in keeping with local cellular concentrations. Furthermore, cluster formation may have important consequences for the kinetics of formation of larger/macroscopic condensates in response to changing cellular conditions (including by a mechanism with analogies to "seeding" that is well known in protein aggregation processes). Several interesting questions can be raised about the structural features of the percolation clusters. While the authors' data nicely demonstrate reversibility and molecular exchange, it would be interesting to probe dynamic cluster and surface properties more directly for individual clusters, and also to understand in detail how systems transition from subsaturation cluster conditions to macroscopic condensates. In a related point, it will be important to further explore and directly test the percolation/connectivity features of the system. Extension to multicomponent systems with additional proteins, RNA, or other molecules is also of biological relevance. Indeed, multicomponent systems display more complex behavior even in simpler models of density transitions. The results are also of substantial functional interest, given the applicability of such network connectivity ideas to model complex mechanical properties and transitions of biological systems from molecular to tissue scales (9, 10).

As demonstrated by Kar et al. (6), single-particle (or single-molecule) experiments are powerful means to probe complicated systems (11). A factor in single-molecule experiments, however, is "shot noise" broadening, which results in widening of observed distributions due to inherent small number statistics. While often undesirable in single-molecule experiments, such a "shot noise broadening" effect could result in interesting consequences for small percolation clusters formed from two (or more) macromolecular species. Here, clusters containing a few to hundreds of molecules could comprise a statistically broadened composition distribution (potentially including quantization effects) that, in turn, gives rise to variations in cluster properties. Moreover, properties for even individual dynamic clusters could show temporal fluctuations, also with an analogy to fluctuations in small number experiments. Such (speculative) small number broadening effects in cluster properties may provide important functional variation and fitness advantages that would not be encoded in uniform systems such as "ensemble-averaged" macroscopic condensates or small, well-defined molecular complexes. Small clusters could also reduce the influence of mechanical forces relevant for cellular macroscopic phase separation, facilitating formation and function in small spaces, for example, in highly crowded cellular environments, or during interactions at small or tightly curved membrane structures such as lipid rafts or at synapses.

As a final discussion point, the nucleation process discussed in Kar et al. (6) can be considered in the context of a fundamental concept in chemistry and physics, that reactions or transitions with very favorable associated free-energy changes may still only occur slowly due to a substantial reaction energy barrier or other considerations. Well-known outcomes of this idea include the stability of certain highly strained molecules, locally trapped or jammed matter, and kinetic traps and the Levinthal paradox in protein folding. Thus, transitions of a system may be controlled by these factors (as well as spatial distribution and mass transfer), which can therefore also be manipulated to control the progress of a reaction (e.g., refs. 12 and 13). For example, Ranganathan and Shakhnovich (14) have explored this type of idea for biomolecular condensates and clusters using simulations, showing that a competition

between condensate coarsening and internal packing rearrangements can trap the system in smaller condensates (clusters). Such kinetic effects could also give rise to memory/hysteresis (15) and feedback (3, 16) effects, possibly with network evolution dictated by the history of the system. Future work will no doubt further explore the interplay of these and other mechanisms (17–20) in biomolecular cluster formation and physical properties.

In conclusion, exciting advances in science can occur by adapting conceptual or theoretical developments in a different field to address the problem at hand. Interesting examples related to this discussion include use of the mathematical machinery of Feynman diagrams/path integrals and renormalization group/critical phenomena (4) to address polymer physics problems, and more recent application of soft-matter physics concepts in the condensate field. The work by Kar et al. (6) represents an interesting example of such an adaptation from polymer physics and percolation theory, here used to address the problem of condensation of a class of neurodegeneration-linked proteins. The results raise important considerations pertinent to cellular conditions, regulation, and function, with relevance for fundamental and applied condensate science.

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