Liquid-liquid phase separation, biomolecular condensates, puncta, non-stoichiometric supramolecular assemblies, membraneless organelles, and bacterial chemotaxis are best understood as emergent phenomena with switch-like behaviour.

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Liquid-liquid phase separation (LLPS) is currently of great interest in cell biology. LLPS is an example of what is called an emergent phenomenon – an idea that comes from condensed-matter physics. Emergent phenomena have the characteristic feature of having a switch-like response. I show that the Hill equation of biochemistry can be used as a simple model of strongly cooperative, switch-like, behaviour. One result is that a switch-like response requires relatively few molecules, even ten gives a strongly switch-like response. Thus if a biological function enabled by LLPS relies on LLPS to provide a switch-like response to a stimulus, then condensates large enough to be visible in optical microscopy are not needed.

I. INTRODUCTION

In cell biology, there has been an explosion of interest in what is variously called “liquid/liquid phase separation” (LLPS), “biomolecular condensates”, “puncta”, “non-stoichiometric supramolecular assemblies” or “membraneless organelles”. Of the five terms, four have been recently invented by biologists, while LLPS is a well-established term in thermodynamics. LLPS is illustrated in Figure 1, where liquid olive oil (yellow), and liquid vinegar (black), have phase separated. There are a number of good recent reviews, see for example.

FIG. 1. Liquid/liquid phase separation of balsamic vinegar (black) and olive oil (yellow). From Wikimedia, author Drazmovde, made available under a Creative Commons Attribution 4.0 International license.

Here, I want to point out to biologists that LLPS is just one example of a broad class of phenomena, called emergent phenomena below, but in brief, they are phenomena that can occur (emerge) only when many molecules (or other objects) interact. Other examples of emergent behaviour in cell biology are known, as are other examples in biology. For example the collective behaviour of flocks of birds and swarms of insects are emergent phenomena. The idea of emergent phenomena is arguably the most interesting and productive idea to arise in physics in the second half of the twentieth century. About one third of the Nobel Prizes in Physics awarded from 1970 to 2000, were for aspects of emergent phenomena. Now in the early twenty-first century, we find that there are many examples of what looks like an emergent phenomenon in cell biology. It is the perfect time to apply what we know about emergent phenomena in non-living systems, to cell biology.

II. DEFINING LIQUID-LIQUID PHASE SEPARATION (LLPS)

I start by defining what a liquid is. The definition of a liquid is simple. It is a phenomenological not a molecular definition. Liquids are distinguished from solids by the fact that when a force is applied to a liquid it flows, whereas solids do not. Thus parts of the cytoplasm or nucleoplasm that exhibit flow, are liquids.

Now that we know what a liquid is, then LLPS is just two distinct coexisting liquids. An example is shown in Figure 1. Both the balsamic vinegar and the olive oil satisfy the definition of a liquid, and they coexist. The influential work of Brangwynne and coworkers found that P granules in the cytoplasm of Caenorhabditis elegans behaved much like droplets of balsamic vinegar in olive oil, the granules flowed and coalesced.

As the definition of liquid is phenomenological not based on molecular behaviour, it goes against how a liquid is defined, to use molecular behaviour to decide if something is a liquid or not. So an experimental technique such as Fluorescence Recovery After Photobleaching (FRAP) that studies dynamics of molecules, cannot determine if a region in a cell is liquid or not. If a region
of a cell with a high concentration of a particular protein is characterised via a technique such as FRAP that studies molecular properties, it is more sensible to refer to it as a biomolecular condensate or non-stoichiometric assembly etc, rather than LLPS.

III. LLPS IS AN EXAMPLE OF AN EMERGENT PHENOMENON

LLPS is an example of an emergent phenomenon. By definition, emergent phenomena are phenomena that only appear when many molecules interact and act as one. One or two molecules cannot show emergent phenomena. Emergent behaviour is also sometimes referred to as behaviour where “more is different”, following an iconic (amongst physicists) article by arguably the most distinguished condensed matter physicist of the postwar period, Philip Anderson.

In the case of liquids and LLPS, the most obvious emergent (particularly if microscopy is the technique) phenomenon is the ability to flow. A single molecule, or a dimer, cannot flow but a condensate of hundreds or more molecules can. This flow can then be described by an equation, the Stokes equation, which applies to liquids but not to single molecules.

Within the Stokes equation, the liquid is described solely by its viscosity, which describes how fast or how slowly a liquid flows when pushed. A characteristic and fundamental feature of emergent properties (here viscosity), is that although they are determined by the molecular properties, they give very little information on these molecular properties. For example, the viscosity of droplets of the Caenorhabditis elegans protein LAF-1, has been estimated to be $34 \pm 5 \text{ Pa s}$. This viscosity is determined by the molecule LAF-1, however, the number of different molecular interactions that can give rise to a viscosity of $34 \text{ Pa s}$ is infinite, and so measuring the viscosity tells you almost nothing about LAF-1.

The ability to flow is just one of the emergent phenomena of LLPS, there are others. For example, a cell relies on an emergent phenomenon then the function is performed by tens of molecules, not thousands. I will try and show this, by following Sourjik and Berg, and using the Hill equation.

IV. LLPS IS NOT THE ONLY EXAMPLE OF AN EMERGENT PHENOMENON IN CELLS

The role of emergent phenomena in E. coli chemotaxis has been extensively studied. I think those studying LLPS can learn from this work. So I will briefly review it, highlighting the features in common with LLPS.

A. E. coli chemotaxis

Bacteria such as E. coli can sense gradients in the concentration of food molecules such as aspartate, with impressive accuracy. This enables them to swim to regions of higher food concentration, which is called chemotaxis. The role of the emergent phenomenon here is to provide the cooperativity needed to increase the sensitivity with which the bacteria sense their environment.

The receptors in E. coli chemotaxis form long-lived assemblies in which they interact cooperatively. The receptors do not assemble cooperatively, or flow. However, the emergent behaviour that drives signal transduction is known to be analogous to that in LLPS. Duke and Bray modelled E. coli chemotaxis using a condensed-matter physics model (called the Ising model) also used to model LLPS. Thus, if the biological function of LLPS is to provide cooperativity that it is very closely analogous to the behaviour seen in E. coli chemotaxis, despite the absence of liquid behaviour in the chemotaxis receptors.

V. TEN MOLECULES IS ENOUGH FOR STRONGLY EMERGENT BEHAVIOUR

Emergent behaviour is an extreme example of cooperative behaviour. This is true in the sense that emergence is defined to mean that there is no limit to the strength of the cooperativity, because there is no limit to the number of molecules that can interact and cooperate. However, in practice even relatively small numbers of molecules, tens or fewer, can often result in strong cooperativity. So even if a biological function relies on a set of biomolecules capable of emergent behaviour, it may be that in practice the function is performed by tens of molecules, not thousands. I will try and show this, by following Sourjik and Berg, and using the Hill equation.
A. Understanding emergent biological functions using the Hill equation of biochemistry

The Hill equation was originally developed to study the cooperative oxygen-binding behaviour of the four sub-units of haemoglobin.\textsuperscript{25,26} Note that as only a maximum of four subunits can cooperate, this is not an emergent phenomenon.

\[ T + nC \rightleftharpoons TC_n \]

For \( n = 4 \), this is a standard model for oxygen binding to haemoglobin, but if we allow \( n \) to take any value, it can model emergence.

If the function we are interested in is sequestration of the target \( T \), then the figure of merit for the function is

\[ \text{fraction of target } T \text{ in condensates} = \frac{1}{1 + K_d/[C]^n} \]

which is the Hill equation.\textsuperscript{24,25} \( K_d \) is a dissociation constant. The concentration of sequestering molecules needed to sequester the target only depends on the value of the dissociation constant \( K_d \) — it is a characteristic of emergent phenomena that molecular details only matter in so far as they contribute to the value of one or a few parameters.

The fraction of a target recruited by a condensate of ten molecules is plotted in Figure 2. Note that the onset of sequestration is relatively sharp even for \( n = 10 \) molecules, and is very switch-like for \( n = 50 \). It is characteristic feature of emergent phenomena that behaviour can be switch-like.\textsuperscript{5,7,14,15}

In the language of the Hill equation, the formation of even a small condensate has a very large value of the Hill coefficient \( n \), and that the Hill coefficient increases with size. As LLPS is a true emergent phenomenon, the Hill coefficient can become arbitrarily large.

VI. CONCLUSION

There has been an explosion of interest in LLPS/“biomolecular condensates”/“non-stoichiometric supramolecular assemblies”\textsuperscript{1–3,5,6,10–13} The ability of these condensates/assemblies to grow very large (eg when a protein is overexpressed) suggests that they are an example of an emergent phenomenon. This is an exciting possibility; emergent behaviour has already been shown to be a powerful way of understanding \textit{E. coli} chemotaxis\textsuperscript{16} and it may be equally useful in understanding the many systems now known to have biomolecular condensates.

Focusing on the cooperative nature of emergent behaviour will, I think, be more useful than focusing on the liquid-like nature of the condensates. Other than in work of\textsuperscript{27} the liquid-like nature of the condensates has not been shown to be directly relevant to function. If cooperativity is directly functional, it is worth noting that, as we showed above using the Hill equation, strong cooperativity is achieved with only ten or so molecules. Thus, condensates/assemblies of ten molecules may be perfectly functional, although hard to detect using conventional microscopy.
VII. ACKNOWLEDGEMENTS

It is a pleasure to acknowledge M. Bienz, X. Darzacq and D. Frenkel for helpful discussions. Figure 1 is from Wikimedia, author Drazmoyde.