Induction and Manipulation of Biomolecular Condensates Through Spatially Heterogeneous Solution Conditions

Alexander K. Buell

The study of biomolecular condensates (BMCs) is of great current interest because of the proposed roles of these types of assemblies in biological function and disease. In living cells, BMCs form in a highly heterogeneous environment and are influenced by concentration gradients of various relevant species. Furthermore, the biological functionality of the BMCs requires precise spatial control of their formation in some cases. In recent years, a number of in vitro experimental approaches have emerged that allow the generation, study, and manipulation of BMCs through the creation of well-defined spatially heterogeneous solution conditions relevant for BMC formation. In this concept article, it is presented in what way such methods can contribute to improved understanding and control of BMCs.

1. Introduction

Biomolecular condensates (BMCs)[1] are spatial regions inside living cells, ranging from nm to μm scales, that are not bounded by a biological membrane and nevertheless are highly enriched in certain compounds, notably specific proteins and nucleic acids. BMCs are increasingly recognized to play a wide range of crucial biological functions, e.g., in development and cell division (see **Figure 1** for examples), as well as in stress response[2] and transcription.[3] In particular, the transient, dynamic nature of BMCs is exploited in many of these scenarios, as the local accumulation of specific proteins and nucleic acids is only required at specific moments in the cell cycle or when a given gene needs to be transcribed. When this biological need is no longer present, BMCs can dissolve and dissociate again, and their components return to being homogeneously distributed throughout the cell or the nucleus. Many of the major protein components found in BMCs in vivo can undergo liquid−liquid phase separation (LLPS) in vitro in highly purified scenarios.[4–6] It is therefore currently widely accepted that the detailed and quantitative study of LLPS of proteins in vitro can provide valuable insight into the formation of BMCs in vivo, despite the very different physico-chemical environments. A wealth of in vitro studies, often based on systematic mutagenesis, has allowed to delineate in some cases the key interactions responsible for LLPS and BMC formation.[6–9] The main conclusion from such studies is that the formation of BMCs is often driven by aromatic amino acids and π-stacking interactions between tyrosine and arginine residues. Electrostatic interactions can be both favorable[10,11] and unfavorable[12] for BMC formation and therefore the solution pH and ionic strength are important control parameters in studies of BMCs.

The interior of eukaryotic cells is highly heterogeneous, with various membrane-bound subcompartments, such as the nucleus, endosomes, mitochondria, or chloroplasts. Furthermore, there are various sources and sinks of the key molecules that are involved in controlling BMC formation—in addition to proteins and nucleic acids mostly small metabolites, such as ATP,[13] that is locally generated from sources (ATPase) and consumed by sinks (e.g., molecular motors) as well as ions, such as, e.g., Ca^{2+} that can strongly influence LLPS,[9] that are flowing in and out of the cell through ion channels. Furthermore, individual membrane-bound compartments can differ significantly in pH from the cytosol, such as endosomes and lysozomes, and pH is known to have a strong influence on the tendency of proteins to form BMCs.[14] It is becoming increasingly clear that this heterogeneity matters for the formation of BMCs in vivo and that BMCs can even actively contribute to this heterogeneity through the creation of gradients of client molecules or pH.[15] Nonhomogeneous distribution of BMCs across the cell can be important for their functions, e.g., in development[16] and cell division[17] (Figure 1a,b). As a result, spatial control of the solution conditions in in vitro experiments represents one key additional step toward recapitulating biologically relevant BMC formation. Furthermore, in cases where BMCs are formed from multiple components in vitro, it is important in what order and in exactly what way the different components are brought into contact, as the hierarchical buildup of the resulting BMCs (see Figure 1c for a relevant example of a heterogeneous BMC in vivo) can depend on these aspects, highlighting their nonequilibrium nature in many cases.[18,19] However, the level of spatial control required to address these factors cannot routinely be reached in large bulk experimental setups. Microfluidics represents a very promising platform to achieve the required level of spatial control of the solution conditions. In this perspective, I present and discuss some recent advances in this area.

A. K. Buell
Department of Biotechnology and Biomedicine
Technical University of Denmark
Søltofts Plads, 2800 Kgs. Lyngby, Denmark
E-mail: alebu@dtu.dk

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative CommonsAttribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH

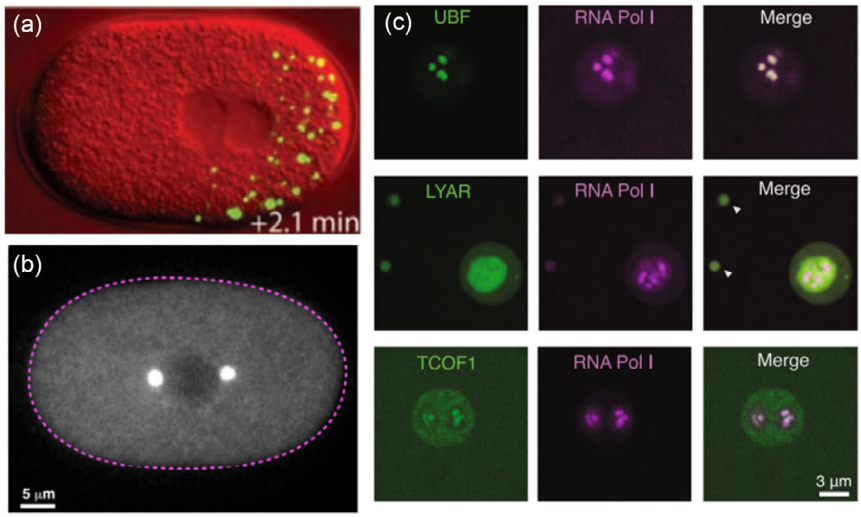

Figure 1. Examples of how BMCs are under spatial control in living cells. a) Fluorescent images of GFP::PGL-1 (green) superimposed on differential interference contrast (DIC) (red) of a *C. elegans* one cell embryo before division. The localization of the condensates to one side of the embryo will lead to an asymmetric discribution of the PGL-1 protein among the two daughter cells. Time relative to pronuclear meeting (pnm). b) *C. elegans* embryo with GFP-labeled SPD-5 assembled into spherical pericentriolar material. Cell outline in magenta. Centrosomes are nonmembrane-bound compartments that nucleate microtubule arrays, which are crucial for the separation of the genetic material during cell division. c) Representative images of *Xenopus* oocyte nucleoli expressing mRNA of GFP-tagged UBF (top row), RFP-tagged TCOF1 (middle row), and GFP-tagged LYAR (bottom row) illustrate the hierarchical and spatially nonuniform build-up of nucleoli that lies at the origin of a measurable pH gradient across this membrane-less organelle. Panel a) reproduced from,[16] panel b) reproduced from[17] and panel c) reproduced from[15] with permission.

2. The Induction of Biomolecular Condensate Formation in Bulk Solution

One major aim of in vitro studies of BMC formation is to elucidate the thermodynamics of this process,[20] often through the drawing of phase diagrams. If the latter are generated under conditions that approximate equilibrium, they allow to determine key parameters such as χ(chi), the relative interaction strength of solvent and solute.[21,22] A low or negative value of χ indicates relatively favorable solute–solvent interactions, facilitating mixing. A large positive value of χ is characteristic of a system that undergoes demixing and phase separation.[22] At a given concentration of the relevant biomolecular components, the formation of BMCs can be induced through changes in χ that can be brought about in a variety of ways, notably through a change in temperature and solution composition.[4] If temperature is used as a control variable, BMCs can be most often induced by lowering the temperature, but there are also cases in which a lower critical solution temperature leads to the formation of BMCs upon heating.[23] Depending on the size of the sample, a change in temperature can be induced homogeneously throughout the entire volume of the solution, which leads to BMC formation throughout the sample. In particular for very small sample volumes, e.g., in microcapillaries, temperatures can be very rapidly quenched on a time scale that even allows the kinetics of BMC formation to be resolved in some cases.[10] When the formation of BMCs is to be induced by a change in solution conditions, e.g., pH or salt concentration, the manual addition of a small volume of concentrated buffer or salt stock solution makes it difficult to achieve rapid homogenization of the solution.[24] Important parameters of the BMCs, such as the size distribution and in complex systems also the internal structure of BMCs,[18] can depend on the local solution conditions and the order of addition of components and therefore it can be challenging to achieve high levels of reproducibility of such experiments in bulk solution.[4] If BMC formation is to be induced rapidly through, e.g., the removal of salt,[4] and therefore dialysis or chromatographic separation are not an option due to their slow nature, sample dilution is the only manner in which this can be achieved. However, in a traditional bulk solution setup, selective dilution of only a given compound often cannot easily be achieved. Therefore, the dilution of the inhibitory component, e.g., salt, is limited to dilution factors that do not push the biomolecular components, e.g., protein and nucleic acids, outside their binodal line, i.e., outside the region where they form BMCs. Taken together, these difficulties suggest that the transition from bulk experiments to microfluidic platforms could be beneficial for the study of BMCs in a multitude of ways. While it can be challenging and requires considerable expertise to generate and operate custom-made microfluidic devices, an increasing number of turn-key microfluidic platforms (e.g., for droplet microfluidics and for Taylor dispersion (TD) analysis and diffusional sizing) are becoming available, helping to democratize microfluidics.

Alexander K. Buell did his Ph.D. and postdoctoral work in Biophysics at the University of Cambridge, UK, followed by an Assistant Professorship at the University of Düsseldorf, Germany. Since 2019, he is Full Professor of Protein Biophysics at the Technical University of Denmark, DTU. His interests are to understand and control protein assembly and aggregation in diverse areas such as biomedicine, biotechnology, as well as materials and food science.

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH

3. Spatial Control of Solution Conditions Allows Precise Control of Biomolecular Condensate Formation

The complete absence of convection, the well-defined laminar flow, and the ability to simulate the fluid and diffusive dynamics inside microfluidic devices makes such systems highly suitable for well-controlled experiments of mixing and de-mixing of molecular species.[25] Mixing is usually purely diffusive, which can be highly efficient on a micrometric length scale. To illustrate this, a typical protein with a diffusion coefficient of 10^{-10} m^2 s^{-1} diffuses about 10 μm per second. Notably, if molecules with very different diffusion coefficients are present in a mixture, efficient separation can be achieved by exposing the mixture to buffer in a co-flow geometry. This has been used to induce BMC formation by the nuclear porin FG-Nup49, where the removal of denaturant in the continuous microfluidic co-flow induced BMC formation in the centre of the main channel.[26] Even more efficient separation of differentially diffusive molecules can be achieved through TD.[27] In TD, a plug of a molecular mixture is injected into a channel or capillary and the parabolic flow profile, with high flow rates in the centre and low flow rates close to the walls leads to a differential dispersion of solutes along the flow direction, depending on the relative diffusion coefficients of the species[28] (**Figure 2**a). Highly diffusive species (e.g., small molecules or salt ions) will move efficiently between flow lines of different velocities and therefore all travel with similar average velocities and thus display small degrees of dispersion. Larger species, however, that have lower diffusion coefficients (e.g., protein molecules) will tend to diffuse less between flow lines and therefore will show a wider distribution of average velocities, leading to larger dispersion.

If, for example, salt ions and proteins are present in a mixture and are subjected to Taylor dispersion, these two types of species that differ by 1–2 orders of magnitude in diffusion coefficient, can be partly separated in a dynamical fashion. In cases where the salt acts as a suppressor of BMC formation, e.g., through screening of favorable electrostatic interactions,[4,9] this separation through TD can act as an efficient inducer of BMCs (Figure 2a). Notably, the nonequilibrium nature of this process allows the selective dilution of the suppressor, while maintaining a relatively high concentration of the protein/biomolecule (Figure 2b), something that is distinctly different from bulk measurements, as discussed above. In a recent study by our group, this feature was exploited to study BMCs at exceedingly low ionic strength. We were able to discover that even protein systems that require low ionic strength to undergo BMC formation, such as Ddx4N1 and PGL3, require a limiting minimal ionic strength in order to form BMCs, and that as the ionic strength tends to zero, the BMCs dissolve[11] (Figure 2c). This is likely caused by the fact that as the Debye length tends to values that exceed second and higher order nearest neighbour distances inside the condensate, a net repulsion due to the equal net charges of the protein molecules starts to dominate over locally attractive interactions between oppositely charged sites/patches. Taylor dispersion-induced phase separation (TDIPS) therefore allows very controlled and efficient and therefore highly reproducible removal of BMC suppressor molecules, while using minute quantities of sample (tens of nl).[11] These features are particularly suitable for large-scale screening of condensate modulating compounds. In TDIPS, BMCs form mostly in the front (center of the capillary) and back of the plug (close to the walls) because the suppressor (here: salt) concentration is minimal there (Figure 2a). The BMCs that form in the front of the plug and therefore center of the capillary are unable to interact with the capillary walls and are therefore not subject to sticking or similar issues. The exposure of the BMCs to the shear forces in the laminar flow can lead to flow focusing, as in the case of any colloidal particle

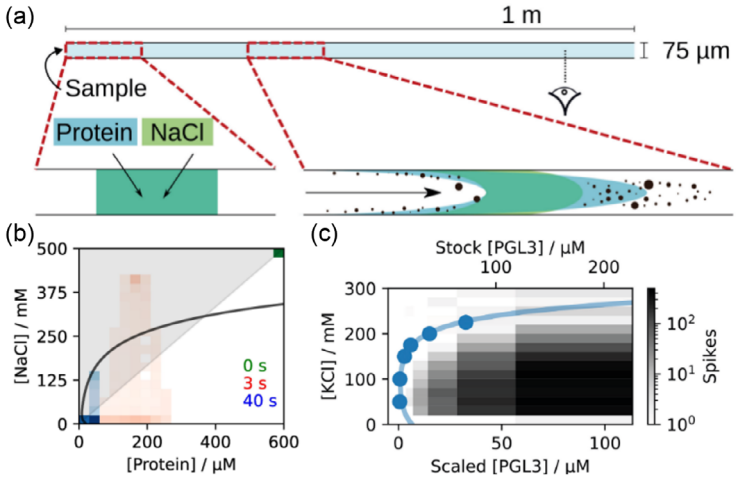

Figure 2. Illustration of Taylor dispersion-induced phase separation (TDIPS). a) The very different diffusion coefficients of salt ions and proteins lead to an efficient physical separation of proteins and salt through TD (see text for details) in laminar flow. In case the salt suppresses BMC formation (e.g., for Ddx4N1 and PGL-3), the physical separation of protein and salt induces BMC formation in the center (ahead of the plug) and at the walls (behind the plug) of the capillary. b) The nonequilibrium nature of the TD experiment allows to reproducibly generate transient extreme dilutions of the salt relative to the protein that cannot be achieved in equilibrium dilutions (the region accessible through the latter are illustrated by the area shaded in gray). Therefore, regions of the phase diagram that are normally inaccessible can be explored, such as the region of ultralow ionic strength. This time-dependent analysis of the sampled regions of the phase diagram was generated from finite element simulations with COMSOL. c) TDIPS enabled the discovery of a re-entrant transition of PGL-3 and Ddx4N1 as the solution ionic strength tends toward zero. All figure panels from[11] with permission.

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH

subjected to shear flow.[29,30] Notably, it has been shown that at sufficiently high shear rates, some condensates formed by aggregation prone proteins can undergo a transition to less reversible structures,[31] an aspect that should be kept in mind in TDIPS experiments, but that has so far not been observed in those. TDIPS is particularly powerful if a small molecule that represses condensate formation needs to be removed, but it works also in the reverse case, i.e., if a small molecular species needs to be added to induce phase separation. This is, e.g., the case of α-synuclein, where condensate formation can be induced by the addition of salt.[11,32]

In addition to the transient gradients of molecular species that can be induced through Taylor dispersion, microfluidic systems also allow the generation of relatively long-lived gradients, e.g., using dead end channels,[33] or else even permanent gradients at steady state, induced by constantly supplying and removing certain components, e.g., in H-channel geometries[34] (**Figure 3**a). In the context of such longer lived gradients, the directed motion of biomolecules and BMCs induced by such gradients can be also conveniently studied in microfluidic devices. If a molecule or particle interacts favorably with another molecular species that forms a gradient (e.g., a salt), then the molecule/particle undergoes so-called diffusiophoretic motion in the direction of increasing concentration of the gradient-forming molecule.[33]

4. Phoretic Motion of Biomolecular Condensates

It can be generally assumed that most colloidal particles, whether solid or liquid, and therefore also BMC particles/droplets, will carry a surface charge under most solution conditions. They will therefore be able to be manipulated through the application of an electric field. This has been shown in some recent studies, in which the electrophoretic mobility of individual condensate droplets was measured, either in free-flow electrophoresis[35] or

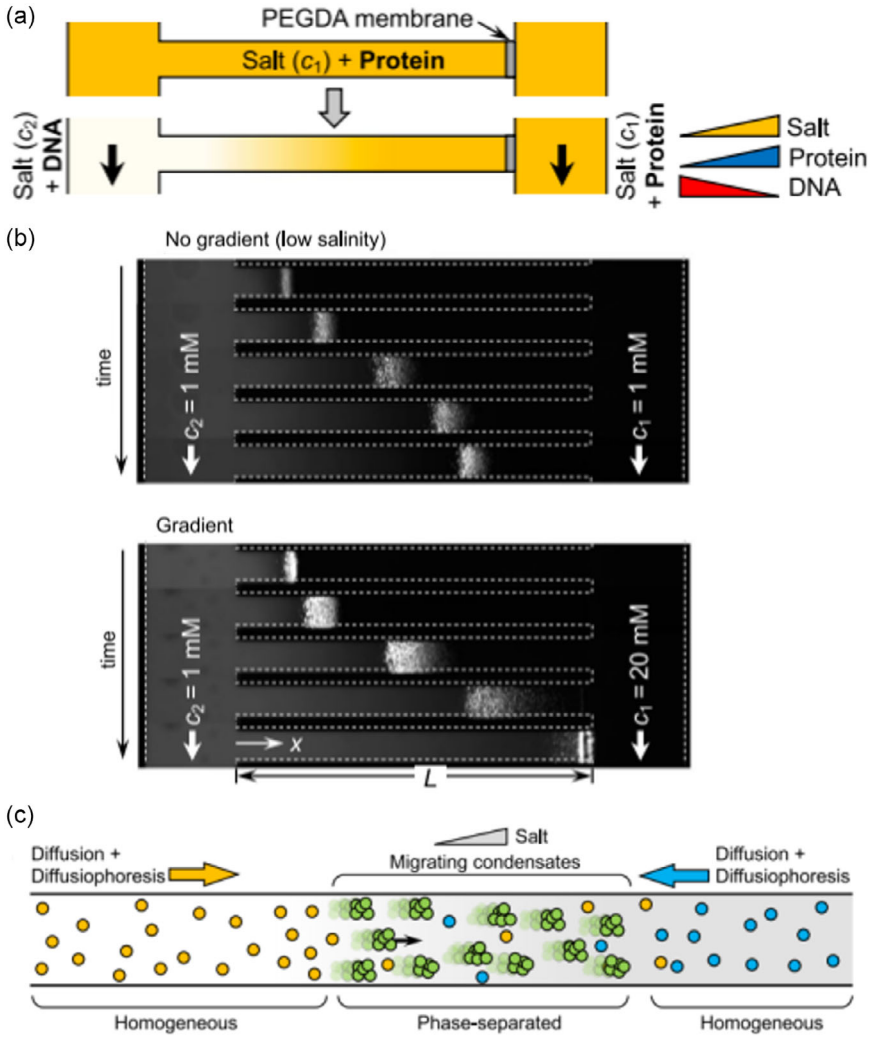

Figure 3. Diffusiophoresis of biomolecular condensates. a) An H-shaped microfluidic device is used to set up a gradient in NaCl concentration along the horizontal channel (length L = 800 μm; width = 60 μm; depth = 40 μm) and is fed with polypeptide ($[RGRGG]_5$) and ssDNAs ($[dT]_{40}$) solutions. b) Fluorescence image sequences of the [dT]40-[RGRGG]5 BMCs over a period of 60 min. The timestamps for the images are 1, 5, 20, 40, and 60 min. c) In the presence of a salt gradient, the transport of biomolecules is enhanced by diffusiophoresis, which increases their local concentration and thus promotes their phase separation. In addition, salt gradients further impart directional motility to the phase-separated condensates, thereby extending their lifetime. Yellow: ssDNA, blue: protein, green: condensates. All figure panels reproduced from[34] with permission.

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH

directly on a microscope slide.[36] It was shown that accurate determination of ζ-(zeta-)potential and surface charge, properties that are relevant for the colloidal stability of the condensates against fusion,[35] requires not only the measurement of the mobility of individual BMCs but also the application of a theoretical description appropriate for liquid assemblies, which demonstrate a much higher electrophoretic mobility for a given ζ-potential/surface-charge than expected for a solid particle.[36] Through these measurements, it was shown that the surface charge, and hence the electrophoretic mobility of condensate droplets can be modulated, or even undergo a change in sign, through changes in solution composition.[36]

It has been proposed that diffusiophoresis plays a role in large-scale pattern formation in Biology,[37] but it has also been shown that diffusiophoresis can play a significant role on the length scale and inside of individual living cells, due to the highly heterogeneous nature of cells.[38,39] Liquid droplets are known to undergo diffusiophoresis if exposed to chemical gradients, and their spherical shape is not normally affected by the diffusiophoretic movement.[40,41] It has recently been experimentally demonstrated that a salt gradient leads to diffusiophoretic motion of associative BMCs formed through coacervation of the peptide $[RGRGG]_5$ with ssDNA[34] (Figure 3). This system only forms BMCs at moderate to low ionic strengths and therefore a salt gradient provides spatial control over where BMCs of such a system can exist, in addition to being able to induce their diffusiophoretic motion. In the presence of such a salt gradient, both DNA and peptide, as well as the BMCs themselves undergo diffusiophoresis, leading overall to a clearly directed motion of the BMCs, the magnitude of which depends on the type of salt employed

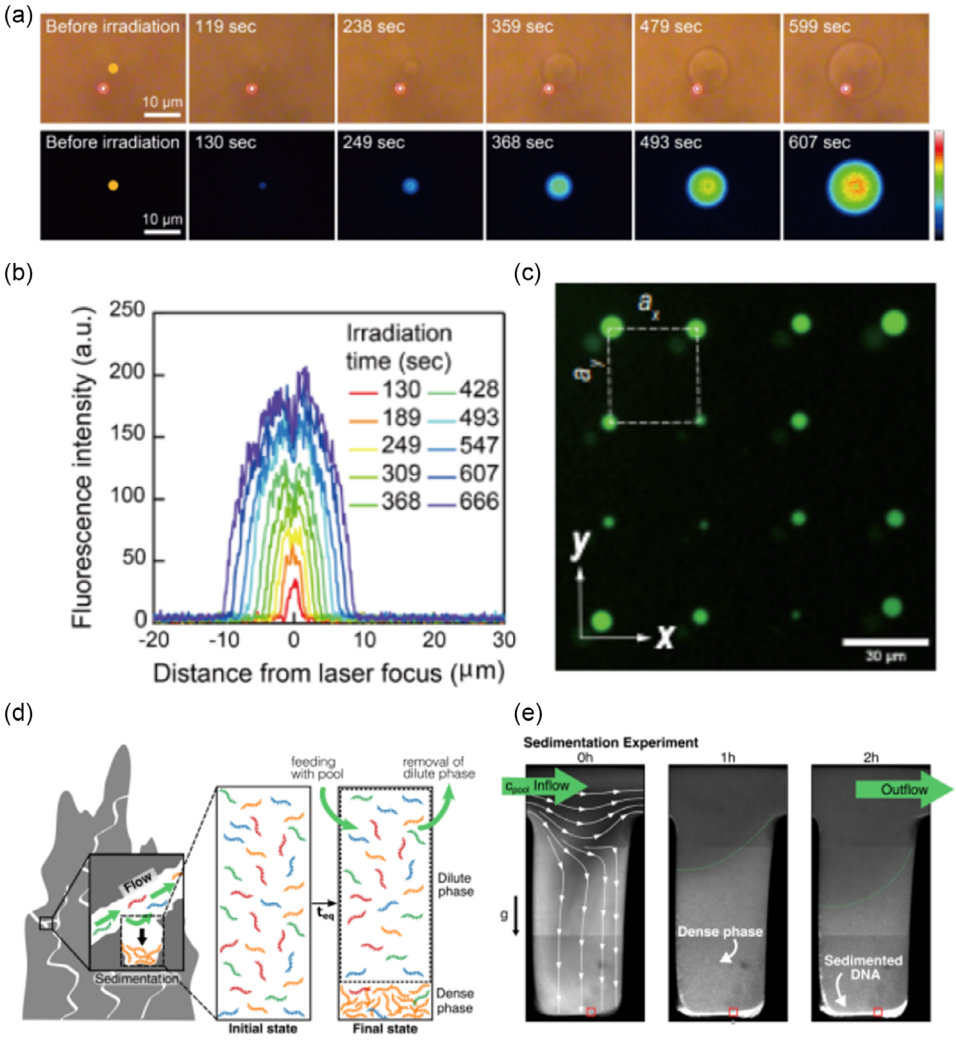

Figure 4. Spatial control of BMC formation and localization through direct action of optical, acoustic, and thermophoretic forces. a) Transmission (upper) and confocal fluorescence images (lower) of time-dependent formation of an α-synuclein BMC when a sample solution containing 200 μM α-synuclein (100 mM NaCl, 7.5% PEG) was subjected to optical trapping. The bright red spot corresponds to a focused 633 nm laser used to locate the air/solution interface. The focal position of the 1064 nm laser is indicated by a yellow spot. b) Time-dependent fluorescence intensity distribution of an α-synuclein BMC at the air/solution interface. c) Fluorescence image of poly-rA condensates aligned by the 2D acoustic field with $f_{x,y} = 49.6/49.7$ MHz. d) Left: Illustration of a time-dependent oligonucleotide flow through porous rocks on early earth. Right: At each cycle, the system phase separates into a dense and a dilute phase, the dense phase sediments and then a fraction of the dilute phase is removed and replaced with samples coming from a fixed sequence pool. e) Fluorescence time-lapse images of a microfluidic chamber with a 2 μm s^{-1} inflow of sequence pair 1. DNA that phase separates can sediment, thus is not advected out of the chamber. Panels a) + b) reproduced from,[43] panel c) from[45] and panels d) + e) from[48] with permission.

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH

(Figure 3b,c). In another recent study, it has been theoretically demonstrated that diffusiophoresis can also be expected from a system of biomolecules undergoing a cyclic, driven reaction, in which a compound oscillates between a phase-separating and a soluble state. The diffusiophoresis is in this case driven by gradients of fuel and waste molecules.[42]

5. Mechanical Manipulation of Biomolecular Condensates

In addition to studying and manipulating BMCs by applying an external electric field or by imposing a concentration gradient, direct mechanical manipulation is also possible. The ability to directly and selectively move BMCs around is a complementary strategy to achieve the same overall goal as described in the previous sections, namely control over where in a given system BMCs are present at any given time. The active and externally controlled movement of BMCs will ultimately also contribute to the establishment of spatially heterogeneous solution conditions and is therefore closely related to the more indirect ways of controlling BMC formation through spatial control of the other relevant control variables, such as concentrations of the species involved.

Given the size range of BMCs and the fact that the elevated biomolecular concentration inside BMCs leads to a distinct refractive index from that of the surrounding dilute solution in in vitro experiments, optical tweezers can be used to directly manipulate BMCs. Such mechanical manipulation is, e.g., useful to control the fusion of BMCs.[19,35] Individual biomolecules are normally too small to experience significant optical forces that would overcome their intrinsic Brownian motion, but it was recently demonstrated that an optical trap was able to induce the local formation of an individual α-synuclein BMC droplet, which was reversible upon switching off the trap[43] (**Figure 4**a,b). The authors explained this phenomenon by the transport and trapping at the air/water interface of nanoscale assemblies of α-synculein[12] by the optical trap, which are present if a crowder molecule, such as PEG, is added. Those nanometric assemblies are apparently sufficiently large to experience relevant forces that can lead to their accumulation at the irradiated spot.

A recent addition to the toolkit of direct mechanical manipulation is acoustophoresis of BMCs, using mechanical transducers to generate focused beams of condensates in microfluidic devices[44] or create a two-dimensional standing pressure field. Individual BMCs can be trapped in the potential wells created by the acoustic radiation force[45] (Figure 4c). Such acoustic trapping can be used to study the viscoelastic properties of condensates when two of them are trapped in the same potential well and the timescale of their merging is monitored, similarly to when optical tweezers are employed for the same purpose.[18]

6. Summary and Outlook

The spatial control of solution conditions, as well as the direct mechanical manipulation of BMCs, is useful for their study, as it allows well-defined experiments of the generation and further evolution of such structures, e.g., through merging. At the same time, such experiments carry the promise of allowing closer approximations of in vivo scenarios that are characterized by a high level of spatial heterogeneity. It remains, however, to be seen to what extent such simplified in vitro setups can recapitulate all the relevant processes that control BMC formation in vivo and also whether inhomogeneous spatial distributions of condensates inside living cells are controlled by chemical gradients as opposed to active transport through molecular motors.[46] In any case, the types of experiments highlighted in this Perspective represent important and courageous steps toward the aim of a better understanding of the highly complex scenarios of BMC formation in biology.

As an outlook, and as mentioned at the beginning of this Perspective article, temperature is a convenient thermodynamic variable that allows the control of LLPS and BMC formation in vitro and in vivo.[47] Thermal cycling in combination with spatial inhomogeneity caused by dense phase sedimentation has been recently demonstrated to be able to lead to sequence selection in nucleic acid phase transitions,[48] as a simulated scenario of how sequence selection might have occurred on the early earth through physical processes (Figure 4d,e). However, spatially non-uniform temperature distributions have not yet been explored in the context of BMC formation. Such scenarios are also not physically plausible to occur in living cells without external heating.[49] On the other hand, the study of BMCs in temperature gradients or thermophoretic traps[50] could be an interesting way to study the kinetics and thermodynamics of BMC formation and dissolution.

Acknowledgements

The author thanks the Novo Nordisk foundation for funding (NNFSA170028392).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: biomolecular condensate · diffusiophoresis · liquid–liquid phase separation · microfludics · Taylor dispersion

[1] S. F. Banani, H. O. Lee, A. A. Hyman, M. K. Rosen, *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 285.
[2] J. Guillén-Boixet, A. Kopach, A. S. Holehouse, S. Wittmann, M. Jahnel, R. Schlüßler, K. Kim, I. R. E. A. Trussina, J. Wang, D. Mateju, I. Poser, S. Maharana, M. Ruer-Gruß, D. Richter, X. Zhang, Y.-T. Chang, J. Guck, A. Honigmann, J. Mahamid, A. A. Hyman, R. V. Pappu, S. Alberti, T. M. Franzmann, *Cell* **2020**, *181*, 346.
[3] P. Cramer, *Nature* **2019**, *573*, 45.
[4] T. Nott, E. Petsalaki, P. Farber, D. Jervis, E. Fussner, A. Plochowietz, T. D. Craggs, D. Bazett-Jones, T. Pawson, J. Forman-Kay, A. Baldwin, *Mol. Cell* **2015**, *57*, 936.
[5] A. Patel, H. Lee, L. Jawerth, S. Maharana, M. Jahnel, M. Hein, S. Stoynov, J. Mahamid, S. Saha, T. Franzmann, A. Pozniakovski, I. Poser, N. Maghelli, L. Royer, M. Weigert, E. Myers, S. Grill, D. Drechsel, A. A. Hyman, S. Alberti, *Cell* **2015**, *162*, 1066.

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH

[6] A. Bremer, M. Farag, W. M. Borcherds, I. Peran, E. W. Martin, R. V. Pappu, T. Mittag, *Nat. Chem.* **2022**, *14*, 196.
[7] R. K. Norrild, S. von Bülow, E. Halldórsson, K. Lindorff-Larsen, J. M. Rogers, A. K. Buell, bioRxiv 2024.12.21.629870 **2024**, https://doi.org/10.1101/2024.12.21.629870.
[8] J. Wang, J.-M. Choi, A. S. Holehouse, H. O. Lee, X. Zhang, M. Jahnel, S. Maharana, R. Lemaitre, A. Pozniakovsky, D. Drechsel, I. Poser, R. V. Pappu, S. Alberti, A. A. Hyman, *Cell* **2018**, *174*, 688.
[9] M. D. Crabtree, J. Holland, A. S. Pillai, P. S. Kompella, L. Babl, N. N. Turner, J. T. Eaton, G. K. A. Hochberg, D. G. A. L. Aarts, C. Redfield, A. J. Baldwin, T. J. Nott, *Cell Rep.* **2023**, *42*, 113375.
[10] E. G. P. Stender, S. Ray, R. K. Norrild, J. A. Larsen, D. Petersen, A. Farzadfard, C. Galvagnion, H. Jensen, A. K. Buell, *Nat. Commun.* **2021**, *12*, 7289.
[11] R. K. Norrild, T. O. Mason, L. Boyens-Thiele, S. Ray, J. B. Mortensen, A. W. Fritsch, J. M. Iglesias-Artola, L. K. Klausen, E. G. P. Stender, H. Jensen, A. K. Buell, *Angew. Chem. Int. Ed.* **2024**, *63*, e202404018.
[12] S. Ray, T. O. Mason, L. Boyens-Thiele, A. Farzadfard, J. A. Larsen, R. K. Norrild, N. Jahnke, A. K. Buell, *Nat. Chem.* **2023**, *15*, 1306.
[13] A. Patel, L. Malinovska, S. Saha, J. Wang, S. Alberti, Y. Krishnan, A. A. Hyman, *Science* **2017**, *356*, 753.
[14] X. Jin, M. Zhou, S. Chen, D. Li, X. Cao, B. Liu, *Cell. Mol. Life Sci.* **2022**, *79*, 380.
[15] M. R. King, K. M. Ruff, A. Z. Lin, A. Pant, M. Farag, J. M. Lalmansingh, T. Wu, M. J. Fossat, W. Ouyang, M. D. Lew, E. Lundberg, M. D. Vahey, R. V. Pappu, *Cell* **2024**, *187*, 1889.
[16] C. P. Brangwynne, C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F. Jülicher, A. A. Hyman, *Science* **2009**, *324*, 1729.
[17] J. B. Woodruff, B. Ferreira Gomes, P. O. Widlund, J. Mahamid, A. Honigmann, A. A. Hyman, *Cell* **2017**, *169*, 1066.
[18] N. A. Erkamp, T. Sneideris, H. Ausserwöger, D. Qian, S. Qamar, J. Nixon-Abell, P. St George-Hyslop, J. D. Schmit, D. A. Weitz, T. P. J. Knowles, *Nat. Commun.* **2023**, *14*.
[19] N. A. Erkamp, I. Sanchez-Burgos, A. Zhou, T. J. Krug, S. Qamar, T. Sneideris, E. Zhang, K. Nakajima, A. Chen, R. Collepardo-Guevara, J. van Hest, P. St George-Hyslop, D. A. Weitz, J. R. Espinosa, T. P. J. Knowles, bioRxiv 2024.11.15.623768 **2024**, https://doi.org/10.1101/2024.11.15.623768.
[20] S. Ray, A. K. Buell, *J. Chem. Phys.* **2024**, *160*, 091001.
[21] D. Qian, T. C. T. Michaels, T. P. J. Knowles, *J. Phys. Chem. Lett.* **2022**, *13*, 7853.
[22] C. Brangwynne, P. Tompa, R. Pappu, *Nat. Phys.* **2015**, *11*, 899.
[23] F. G. Quiroz, A. Chilkoti, *Nat. Mater.* **2015**, *14*, 1164.
[24] J. Van Lindt, A. Bratek-Skicki, P. N. Nguyen, D. Pakravan, L. F. Durán-Armenta, A. Tantos, R. Pancsa, L. Van Den Bosch, D. Maes, P. Tompa, *Commun. Biol.* **2021**, *4*, 77.
[25] D. E. Otzen, A. K. Buell, H. Jensen, *Curr. Opin. Struct. Biol.* **2021**, *70*, 8.
[26] G. Celetti, G. Paci, J. Caria, V. Vandelinder, G. Bachand, E. A. Lemke, *J. Cell Biol.* **2020**, *219*.
[27] S. G. Taylor, *Proc. R. Soc. A* **1953**, *219*, 186.
[28] H. Cottet, J.-P. Biron, M. Martin, *Analyst* **2014**, *139*, 3552.
[29] D. D. Carlo, D. Irimia, R. G. Tompkins, M. Toner, *Appl. Phys. Sci.* **2007**, *104*, 18892.
[30] G. Segré, A. Silberberg, *Nature* **1961**, *189*, 209.
[31] Y. Shen, F. S. Ruggeri, D. Vigolo, A. Kamada, S. Qamar, A. Levin, C. Iserman, S. Alberti, P. S. George-Hyslop, T. P. J. Knowles, *Nat. Nanotechnol.* **2020**, *15*, 841.
[32] S. Ray, C. Chiodaroli, A. Farzadfard, A. Kunka, K. Helga Schott, S. Hertel, K. Mojtic, L. Kjaer Klausen, C. Galvagnion, A. K. Buell, bioRxiv 2024.11.18.624073 **2024**, https://doi.org/10.1101/2024.11.18.624073.
[33] Q. A. E. Peter, R. P. B. Jacquat, T. W. Herling, P. K. Challa, T. Kartanas, T. P. J. Knowles, *J. Phys. Chem. B* **2022**, *126*, 8913.
[34] V. S. Doan, I. Alshareedah, A. Singh, P. R. Banerjee, S. Shin, *Nat. Commun.* **2024**, *15*, 7686.
[35] T. J. Welsh, G. Krainer, J. R. Espinosa, J. A. Joseph, A. Sridhar, M. Jahnel, W. E. Arter, K. L. Saar, S. Alberti, R. Collepardo-Guevara, T. P. J. Knowles, *Nano Lett.* **2022**, *22*, 612.
[36] M. H. I. Van Haren, B. S. Visser, E. Spruijt, *Nat. Commun.* **2024**, *15*, 3564.
[37] B. M. Alessio, A. Gupta, *Sci. Adv.* **2023**, *9*, eadj2457.
[38] B. Ramm, A. Goychuk, A. Khmelinskaia, P. Blumhardt, H. Eto, K. A. Ganzinger, E. Frey, P. Schwille, *Nat. Phys.* **2021**, *17*, 850.
[39] R. P. Sear, *Phys. Rev. Lett.* **2019**, *122*, 128101.
[40] B. E. Mckenzie, H. C. W. Chu, S. Garoff, R. D. Tilton, A. S. Khair, *J. Fluid Mech.* **2022**, *949*, A17.
[41] F. Yang, S. Shin, H. A. Stone, *J. Fluid Mech.* **2018**, *852*, 37.
[42] G. Häfner, M. Müller, *ACS Nano* **2024**, *18*, 16530.
[43] K. Yuzu, C.-Y. Lin, P.-W. Yi, C.-H. Huang, H. Masuhara, E. Chatani, *Proc. Natl. Acad Sci. U S A* **2024**, *121*, e2402162121.
[44] P. Gelin, J. Van Lindt, A. Bratek-Skicki, S. Stroobants, M. Krzek, I. Ziemecka, P. Tompa, W. De Malsche, D. Maes, *Crystals* **2019**, *9*, 120.
[45] K. Nakajima, T. Sneideris, L. L. Good, N. A. Erkamp, H. Ogi, T. P. J. Knowles, bioRxiv 2024.09.16.613217 **2024**, https://doi.org/10.1101/2024.09.16.613217.
[46] A. Cochard, A. Safieddine, P. Combe, M. Benassy, D. Weil, Z. Gueroui, *EMBO J.* **2023**, *42*, e114106.
[47] A. W. Fritsch, A. F Diaz-Delgadillo, O. Adame-Arana, C. Hoege, M. Mittasch, M. Kreysing, M. Leaver, A. A Hyman, F. Jülicher, C. A Weber, *Proc. Natl. Acad Sci. U. S. A.* **2021**, *118*, e2102772118.
[48] G. Bartolucci, A. Calaça Serrão, P. Schwintek, A. Kühnlein, Y. Rana, P. Janto, D. Hofer, C. B Mast, D. Braun, C. A. Weber, *Proc. Natl. Acad Sci. U. S. A.* **2023**, *120*, e2218876120.
[49] G. Baffou, H. Rigneault, D. Marguet, L. Jullien, *Nat. Methods* **2014**, *11*, 899.
[50] M. Fränzl, T. Thalheim, J. Adler, D. Huster, J. Posseckardt, M. Mertig, F. Cichos, *Nat. Methods* **2019**, *16*, 611.

Manuscript received: January 17, 2025
Revised manuscript received: April 2, 2025
Version of record online: April 17, 2025

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH