

Dynamical Component Exchange in a Model Phase Separating System: an NMR-based Approach

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ABSTRACT: Biomolecular phase separation plays a key role in spatial organization of cellular activities. Dynamic formation and rapid component exchange between phase separated cellular bodies and their environment are crucial for their function. Here, we employ a well-established phase separating model system, namely, triethylamine (TEA)-water mixture, and develop an NMR approach to detect the exchange of scaffolding TEA molecules between separate phases and determine the underlying exchange rate. We further demonstrate how the advantageous NMR properties of fluorine nuclei provide access to otherwise inaccessible exchange processes of a client molecule. The developed NMR-based approach allows quantitative monitoring of the effect of regulatory factors on component exchange and facilitates “exchange”-based screening and optimization of small molecules against druggable biomolecular targets located inside condensed phases.

Liquid-liquid phase separation has emerged as a key physicochemical principle underlying the spatial organization of biomolecules within the cell, hence reinvigorated the interest in studying this classical physical chemistry phenomenon.¹⁻⁴ Several biophysical techniques have been developed to monitor biomolecular phase separation in *in vitro* and *in vivo* conditions and determine the structural dynamics of its constituents, including proteins, nucleic acids, ions and water molecules.⁵⁻¹² A remarkable feature of phase separated cellular bodies is their rapid formation and dissolution in response to regulatory signals and dynamic component exchange with the cellular environment.¹³⁻¹⁵ The component exchange occurs not only for the so-called scaffold molecules whose phase behavior govern the formation of phase separated droplets, but also for the client molecules which are dynamically recruited to the formed droplets and exchange with the environment.¹⁶⁻¹⁸ These features are particularly advantageous in rapidly changing environments and crucial for the function of several cellular bodies.¹⁹⁻²¹

Despite its importance in physiological and potentially pathological processes, little is known on how the component exchange of membrane-less phase separated bodies is regulated by biochemical factors, e.g. mutation or chemical modifications in phase-separating biomolecules, the presence of small molecules etc. Progress in this direction requires developing experimental methods for quantitative determination of exchange rates. Here, we use a model system for phase separation, namely triethylamine (TEA)-water mixture, and develop a multi-spin NMR approach to study the exchange process between separated phases at a quantitative level. The TEA is a well-established

phase separation model system, which undergoes a reversible temperature-dependent phase separation, exhibiting lower critical solution temperature (LCST) behavior.²²⁻²⁵ As a result of phase separation, two phases are formed: a TEA-enriched condensed phase and water-enriched phase. In addition to TEA as the scaffold molecule, we investigate the exchange of a fluorine-containing client molecule between separated phases and show how the use of ¹⁹F NMR allows detection of the exchange process and quantifying its rate.

First, we examined the temperature-dependent phase separation of a mixture of TEA and dextran using microscopy. The use of dextran as crowding agent was to slow down the kinetics of the TEA phase separation process. In addition, fluorescently-labeled dextran was used in order to monitor the phase separation process. As shown in Figure 1A, at 278 K, the TEA sample exhibited a single dispersed phase, with a nearly uniform background fluorescence emission originating from the labeled dextran. We then raised the temperature to 298 K, resulting in the phase separation of the sample and the formation of a large number of variously sized spherical droplets (Figure 1A). Notably, the dextran was almost completely excluded from the formed droplets and remained in the dispersed phase.

Next, we studied the phase separation of TEA using ¹H NMR. At 278 K, where the TEA sample displays a single dispersed phase (Figure 1A), the 1D ¹H NMR spectrum showed signals at ca. 2.5 and 0.98 ppm, which belong to its methylene and methyl protons, respectively (Figure 1B). The narrow linewidth of the TEA ¹H signals reflect the large level of mobility of this small-sized molecule within the spatially homogeneous dispersed

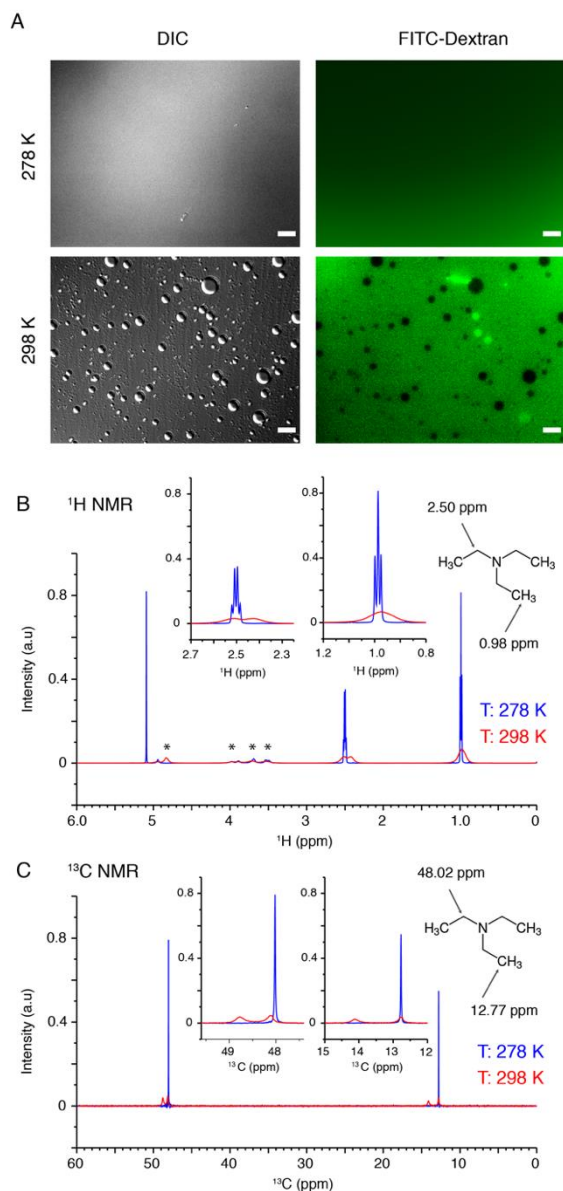


Figure 1. Liquid-liquid phase separation of triethylamine (TEA)/water/dextran mixture, probed by differential interference contrast (DIC) and fluorescence microscopy (A), and ^1H (B) and ^{13}C (C) NMR spectroscopy. ^1H signals from dextran are marked by stars in (B). The phase separation-induced broadening in TEA's methylene and methyl ^1H (B) and ^{13}C (C) signals, as well as the emergence of new ^{13}C signals can be seen. Scale bars, 10 μm .

phase. After increasing the temperature to 298 K, and the resultant phase separation, the TEA ^1H signals were strongly broadened, and consequently, the characteristic quartet and triplet splitting patterns of methylene and methyl signals were no longer observable. The phase separation-induced signal broadening was not limited to TEA signals, but also observed for the dextran signal. As dextran did not enter the formed droplets, the broadening of its signals seems to reflect the magnetic susceptibility inhomogeneity of the phase separated sample. Accordingly, the TEA signal broadening is potentially caused by three major factors: the altered mobility of TEA within the condensed interior of droplets, the chemical exchange between different

phases, and sample inhomogeneity. In addition to signal broadening, the methylene, but not methyl, signal showed the emergence of a new peak ~ 0.1 ppm upfield to the original signal.

Next, we investigated the phase separation of TEA using 1D ^{13}C NMR at natural abundance. As expected, the spectrum of the single-phase dispersed TEA sample at 278 K showed two sharp ^{13}C signals at ca. 48.02 and 12.77 ppm, belonging to methylene and methyl groups, respectively (Figure 1C). Similar to the ^1H signals, the temperature-induced phase separation led to a significant broadening of TEA's ^{13}C signals and a decrease in their intensity, likely due to the induced alteration in mobility, exchange and sample homogeneity. Unlike the ^1H spectrum, however, the ^{13}C spectrum of the phase-separated TEA sample showed two well-resolved new signals downfield of the original methylene and methyl signals. The emergence of these new ^{13}C signals (and the new ^1H signal of the methylene group, see above) pointed to the presence of an exchange process between the dispersed and condensed phases of TEA, at a rate slow on the NMR chemical shift timescale, i.e. $< 1300 \text{ s}^{-1}$ (see Figure 2A).

As a prerequisite for the quantitative investigation of dynamical exchange processes underlying the phase separation of TEA, we established the kinetics of this process through real-time 1D ^{13}C NMR experiments, where we monitored the methyl signals belonging to the dilute and condensed phase. Immediately after the temperature jump from 278 to 298 K, the signal belonging to the dilute phase was significantly broadened and partially lost its height intensity. However, further progression of the phase separation process led to the partial recovery of the linewidth and height intensity of this signal (Figure 2A). The broad signal belonging to the condensed phase followed a rather distinct trend: the gradual narrowing of the signal during the first 1-3 hours of the process was followed by a sharp decrease in peak intensity (Figures 2B). The time-dependence of peak intensities (Figures 2B) indicated the presence of three kinetic stages: an initial stage (around 60 minutes), when the condensed phase signal grew in height, a middle stage (around 60-260 minutes) during which the peak intensities remained relatively stable, and the final stage (ca. 260-600 minutes) when a sharp intensity loss was observed for the condensed phase signal. The final intensity loss of the condensed phase signal was due to the formation of large TEA droplets and their precipitation and exit from the detection zone of NMR coils. Notably, this precipitation is reverse, i.e. the TEA-enriched phase is not at the bottom but at the top of the sample, due to the lower density of TEA-enriched droplets when compared to the TEA-depleted phase. Accordingly, during kinetic stages I and III, the dominant processes are respectively droplet formation and precipitation, while the kinetic stage II represents a quasi-steady-state during which these two processes partially cancel the effect of each other on NMR signal intensities.

The study of phase separation-related exchange processes during later kinetic stages (i.e. stage III in Figure 2B) is complicated by the (reverse) precipitation-induced NMR intensity changes. To minimize the interfering effects of (reverse) precipitation, we took advantage of the reversibility of phase separation and developed an experimental scheme, where cycles of heating-cooling were employed and NMR data were collected only during the initial kinetic stage of phase separation (typically the first 15-20 minutes, depending on experiment). Using this scheme, we were able to measure a clean 2D projection plane of the ^{13}C , ^1H , ^1H HSQC-NOESY spectrum of TEA

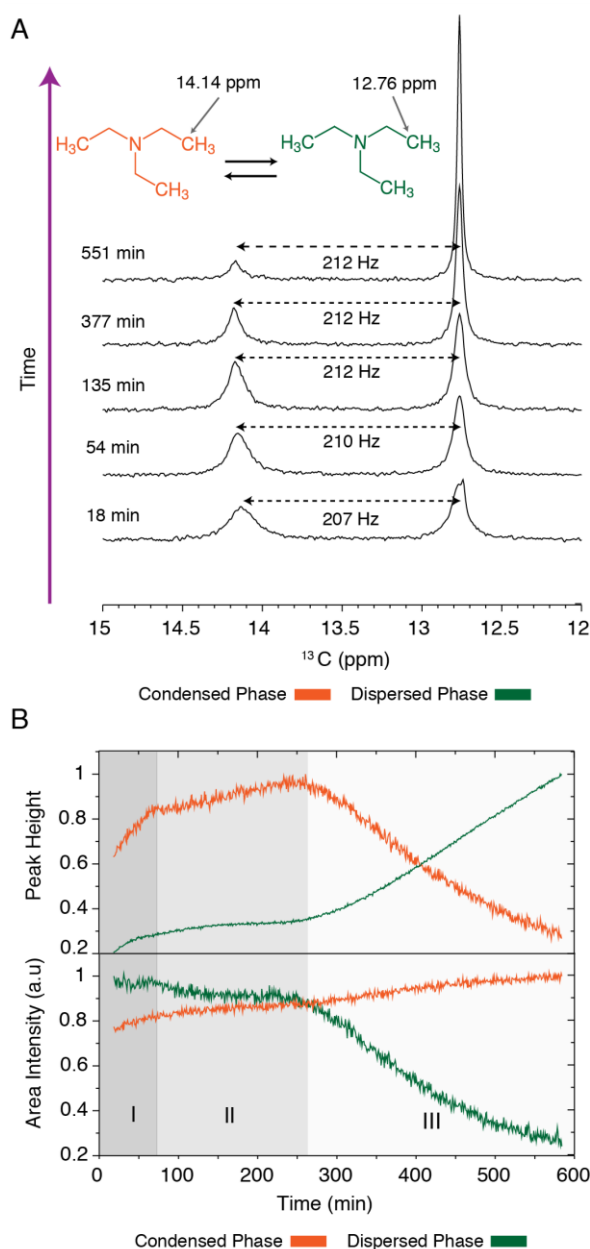


Figure 2. Kinetics of liquid-liquid phase separation in triethylamine (TEA)/water (D₂O)/dextran mixture, monitored through ¹³C methyl signals of TEA molecules. (A) Changes in the 1D ¹³C NMR spectrum of the methyl group of TEA after inducing phase separation through a temperature jump from 278 to 298K. The signals at 12.76 and 14.14 ppm originate from the dispersed and condensed phase, respectively. (B) Relative area intensity of ¹³C methyl signals of TEA from the two phases (bottom panel) and their heights (top panel) as seen in (A). Three kinetic stages of phase separation (I-III, shaded areas) can be distinguished.

TEA during phase separation. As shown in Figure 3A, ¹³C, ¹H correlation peaks were observed for TEA methylene and methyl groups from both the dispersed and condensed phases and several groups of dextran. Interestingly, the TEA ¹H signals be-

longing to the two phases showed nice correlation peaks, indicating either spatial proximity (and its consequent dipolar coupling-mediated correlation) or chemical exchange between them. The sign of cross peaks in these and complementary 2D ¹H, ¹H ROESY spectra (Figure 3B) excluded the dipolar coupling origin and confirmed the presence of chemical exchange. Notably, no correlation peaks were observed for dextran signals, consistent with the exclusion of dextran from the TEA droplets (Figure 1A).

Then, we employed the heating-cooling cycles and measured 2D ¹H, ¹H EXSY spectra of the phase separated sample at five different mixing times ranging from 15-120 ms (Figure 3C). The EXSY spectra at each mixing time were collected during the first 20 minutes after initiation of phase separation. The intensity of the exchange-induced correlation peaks displayed the characteristic dependence on mixing time. Analysis of the intensity build-up curves for the methylene and methyl groups provided an effective exchange rate, k_{ex} , of $31 \pm 5 \text{ s}^{-1}$, for the exchange of TEA molecules between two phases (Figure 3D), i.e. an exchange process that is slow on the NMR chemical shift timescale.

To further investigate the component exchange between the phase separated TEA droplets and their environment, we studied the exchange of hexafluoroisopropanol (HFIP) as a potential client molecule. The choice of HFIP was motivated by its fluorine content, as ¹⁹F nuclei are highly sensitivity to chemical environment and consequently could act as sensitive probes of exchange processes.²⁶ After establishing that HFIP (1.6% m/v) did not perturb the phase separation of TEA (Figure S1), we monitored how ¹H and ¹⁹F NMR signals of HFIP (Figure 4A) were affected by TEA phase separation. Upon temperature increase and the consequent TEA phase separation, both the methine ¹H signal and the trifluoromethyl ¹⁹F signal of HFIP displayed a small downfield shift together with significant signal broadening (Figures 4B). In addition, a new signal appeared approximately 277 Hz away from the original peak in the ¹⁹F NMR spectrum. The newly emerged peak originates from HFIP molecules inside the condensed phase, and its exchange between the two phases is expected to be slow on the NMR chemical shift timescale, i.e. slower than ca. 1700 s^{-1} . The presence of HFIP's exchange between the two TEA phases was subsequently supported by 2D ¹⁹F, ¹⁹F NOESY spectra, in which the two ¹⁹F signals showed cross-peaks of the same sign, despite the rapid rotational dynamics of trifluoromethyl groups.

To quantify the kinetics of HFIP exchange between the two phases, we employed the heating-cooling cycles described above and performed 2D ¹⁹F, ¹⁹F EXSY experiments at five different mixing times from 15-120 ms (Figures 4C,D). Analysis of the intensity build-up curves for the two exchange-related cross peaks provided a k_{ex} of $16 \pm 6 \text{ s}^{-1}$. The obtained k_{ex} value for the client HFIP is thus nearly half the value observed for the scaffold TEA molecules, suggesting that the exchange processes of the scaffold TEA and the client HFIP molecules are governed by different rate-determining steps.

In cells, the dynamic component exchange between membrane-less organelles and their environment enables their rapid formation and dissolution in response to regulatory signals.¹⁹ In addition, the rapid recruitment and dynamic exchange of client molecules with the cellular environment are of utmost

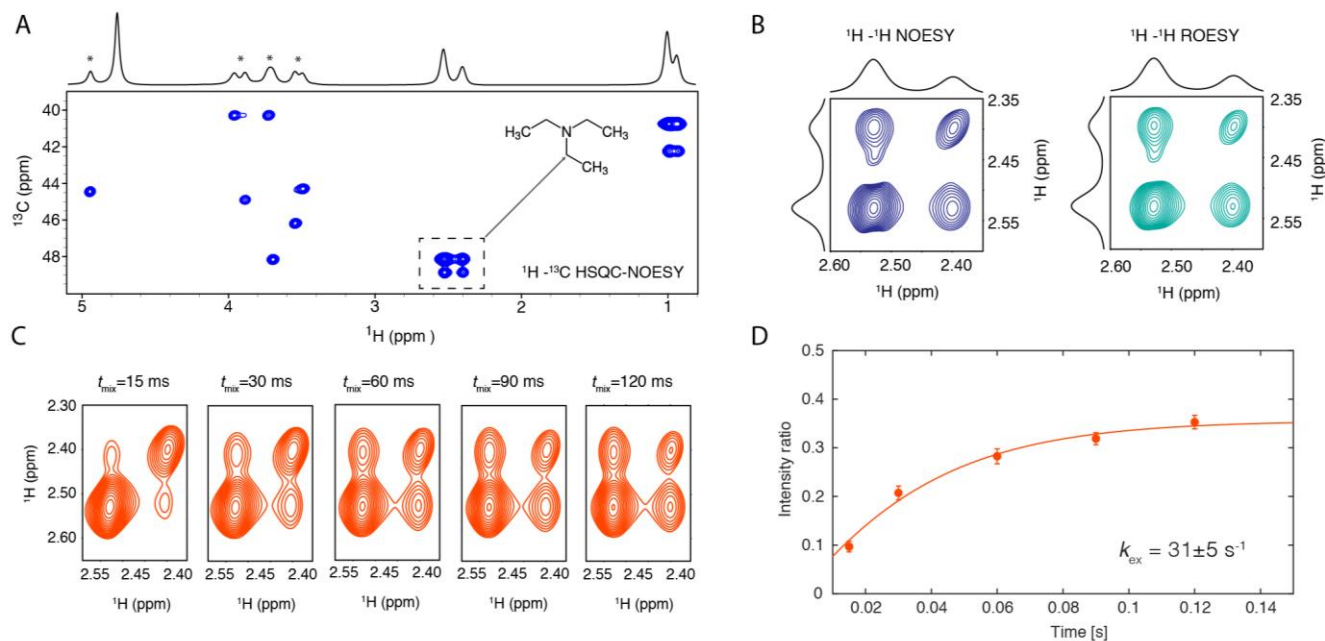


Figure 3. Detection of chemical exchange of scaffold triethylamine (TEA) molecules between dispersed and condensed phases, through NOESY-type NMR experiments. (A) 2D ^{13}C , ^1H plane of an HSQC-NOESY experiment, revealing ^1H , ^1H correlation peaks for the methylene and methyl ^1H signals of TEA molecules from the two phases. The signals belonging to the crowding agent dextran (marked by stars) did not show any correlation peak, consistent with the exclusion of dextran from the condensed phase. (B) 2D ^1H , ^1H NOESY and ROESY spectra, zoomed over the two methylene signals of TEA originating from the two phases. The same sign of correlation peaks in NOESY and ROESY spectra confirm “chemical exchange” as their source. (C) The methylene region of the ^1H , ^1H EXSY spectra of TEA, measured during the initial stage of phase separation at five different mixing times. (D) Quantitative analysis of the intensity build-up curves provides the exchange rate, k_{ex} , of TEA molecules between the two phases. Error bars are calculated by error propagation based on the spectral noise for each mixing time.

functional importance for some phase separated cellular bodies.²⁰⁻²¹ The exchange rates for the scaffold and client molecules are expected to vary over the course of non-equilibrium phase separation processes during which the size of phase-separated droplets, their number and consequently the total interface area between two phases changes (Figure 4E).⁴ Further changes in the exchange rates may be caused by droplet maturation processes, which affect the internal structure and properties of phase separated droplets.²⁷ Previous studies suggest that the phase separation of TEA obeys a nucleation-dependent kinetic regime,²⁸ resulting in alterations in the exchange rates in dependence of droplet size and numbers (Figure 4E). Here, the reversibility of temperature-dependent TEA phase separation allowed us to develop a heating-cooling cycle experimental scheme and specifically target the “initial exchange rate” for both the scaffold TEA and the client HFiP molecules. Based on our results, we argue that it is important to carefully design exchange-related experiments and measure the exchange rates at a clearly-defined kinetic stage of the phase separation process. This will enable monitoring how various regulatory factors, e.g. (bio)chemical regulatory signals, alter the component exchange processes. Thus, comparison of the exchange rates between different client molecules and droplets provides mechanistic insights into the selective recruitment and spatial distribution of biomolecules inside cells.

Our results provide an example of how a multi-spin NMR approach can be employed in detecting and quantifying exchange processes during phase separation. In particular, we showed how a shift from more widely used nuclei such as ^1H

and ^{13}C to ^{19}F may be beneficial in such studies. As shown for the client molecule HFiP (Figure 4), the large sensitivity of ^{19}F nuclei to the chemical environment leads to a larger value for the chemical shift difference between the signals originated from the two phases ($\Delta\omega$, in s^{-1}), and at a given k_{ex} , by shifting the exchange regime further towards the slow-exchange regime (by decreasing the $k_{\text{ex}}/\Delta\omega$ ratio), facilitates exchange rate determination by NMR methods. In addition, the bio-orthogonality of fluorine atoms facilitates their use for biomolecular and biological samples, especially in the context of “exchange-based” small molecule screening against biomolecular targets inside membrane-less organelles.^{26, 29-30}

The efficacy of drug candidates against biomolecular targets crucially relies on their pharmacokinetic properties ensuring their bio-availability.³¹ Similar to the case of biomolecules located inside membrane-bound organelles, drugs have to be able to efficiently enter the phase separated membrane-less compartment. Quantitative determination of exchange rates of small molecules between separated phases is thus important. For example, it has been shown that the partitioning of anti-cancer drugs into nuclear condensates is determined by the physico-chemical properties independent of their specific targets and influences their activity.³² The NMR-based approach developed in the present study enables detection and quantification of the exchange processes between different phases and thereby is an important step towards “exchange-based” screening and optimization of small molecule drug candidates.

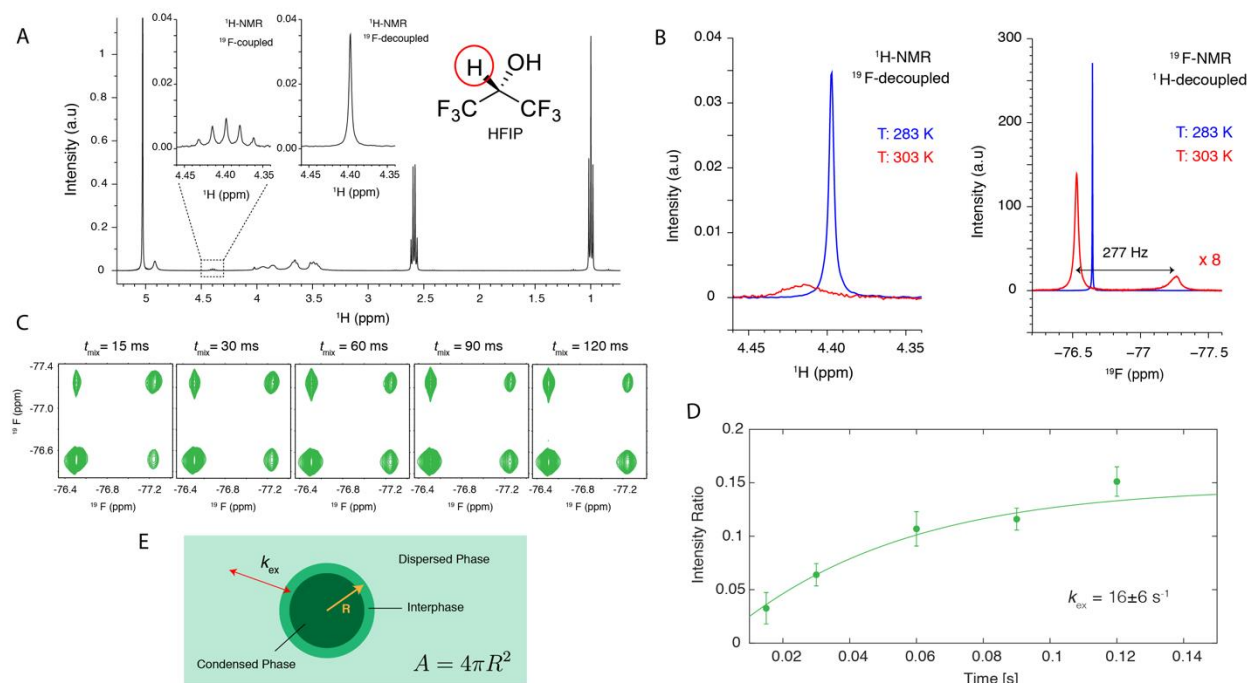


Figure 4. ^{19}F -NMR based detection of chemical exchange of a fluorine-containing client molecule, HFiP, between dispersed and condensed triethylamine (TEA) phases. (A) 1D ^1H NMR spectrum of the TEA-water sample containing HFiP (1%, v/v), before induction of phase separation. The methine proton signal of HFiP is shown in ^{19}F -coupled or decoupled spectra. (B) The effect of TEA phase separation on the methine ^1H (left panel) and trifluoromethyl ^{19}F (right panel) signals of HFiP. (C) ^{19}F , ^{19}F EXSY spectra of HFiP, measured during the initial stage of TEA phase separation at five different mixing times, displaying mixing time-dependent intensity build-up of the correlation peak. (D) Quantitative analysis of intensity build-up curves yields the exchange rate, k_{ex} , of client molecules between two phases. Error bars are calculated by error propagation based on the spectral noise for each mixing time. (E) Schematic representation of the k_{ex} dependence on the size of droplets (e.g. their radius, R), hence the surface area (A) of the interphase between the two phases.

In summary, an NMR-based approach to study component exchange in phase separating systems is presented. Using triethylamine (TEA) as a model system exhibiting reversible temperature-induced phase separation, we detect and quantify the exchange processes for the scaffold TEA, as well as a client molecule. The developed approach allows quantitative determination of the effects of regulatory factors on exchange processes and establishes the basis for exchange-based small-molecule screening against biomolecular targets located inside condensed phases.

ASSOCIATED CONTENT

Supporting Information, including Supplementary Methods (Chemicals, DIC and Fluorescence imaging, NMR experiments and data analysis) and Figure S1.

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Notes

Any additional relevant notes should be placed here.

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ABBREVIATIONS

TEA, triethylamine; HFiP, Hexafluoroisopropanol; NMR, Nuclear Magnetic Resonance.

REFERENCES

1. Banani, S. F.; Lee, H. O.; Hyman, A. A.; Rosen, M. K., Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell. Biol.* **2017**, *18* (5), 285-298.
2. Alberti, S., Phase separation in biology. *Curr. Biol.* **2017**, *27* (20), R1097-R1102.
3. Gomes, E.; Shorter, J., The molecular language of membraneless organelles. *J. Biol. Chem.* **2019**, *294* (18), 7115-7127.

4. Falahati, H.; Haji-Akbari, A., Thermodynamically driven assemblies and liquid-liquid phase separations in biology. *Soft Matter* **2019**, *15* (6), 1135-1154.
5. Elbaum-Garfinkle, S.; Kim, Y.; Szczepaniak, K.; Chen, C. C.; Eckmann, C. R.; Myong, S.; Brangwynne, C. P., The disordered P granule protein LAF-1 drives phase separation into droplets with tunable viscosity and dynamics. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (23), 7189-94.
6. Wang, J.; Choi, J. M.; Holehouse, A. S.; Lee, H. O.; Zhang, X.; Jahnel, M.; Maharana, S.; Lemaitre, R.; Pozniakovsky, A.; Drechsel, D.; Poser, I.; Pappu, R. V.; Alberti, S.; Hyman, A. A., A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. *Cell* **2018**, *174* (3), 688-699 e16.
7. Yuwen, T.; Bah, A.; Brady, J. P.; Ferrage, F.; Bouvignies, G.; Kay, L. E., Measuring Solvent Hydrogen Exchange Rates by Multifrequency Excitation (15)N CEST: Application to Protein Phase Separation. *J. Phys. Chem. B* **2018**, *122* (49), 11206-11217.
8. Ambadipudi, S.; Reddy, J. G.; Biernat, J.; Mandelkow, E.; Zweckstetter, M., Residue-specific identification of phase separation hot spots of Alzheimer's-related protein tau. *Chem. Sci.* **2019**, *10* (26), 6503-6507.
9. Emmanouilidis, L.; Esteban-Hofer, L.; Damberger, F. F.; de Vries, T.; Nguyen, C. K. X.; Ibanez, L. F.; Mergenthal, S.; Klotzsch, E.; Yulikov, M.; Jeschke, G.; Allain, F. H., NMR and EPR reveal a compaction of the RNA-binding protein FUS upon droplet formation. *Nat. Chem. Biol.* **2021**, *17* (5), 608-614.
10. Fuentes-Monteverde, J. C.; Becker, S.; Rezaei-Ghaleh, N., Biomolecular phase separation through the lens of sodium-23 NMR. *Protein Sci.* **2021**, *30* (7), 1315-1325.
11. Rezaei-Ghaleh, N.; Munari, F.; Becker, S.; Assfalg, M.; Griesinger, C., A facile oxygen-17 NMR method to determine effective viscosity in dilute, molecularly crowded and confined aqueous media. *Chem. Commun. (Camb)* **2019**, *55* (82), 12404-12407.
12. Fawzi, N. L.; Parekh, S. H.; Mittal, J., Biophysical studies of phase separation integrating experimental and computational methods. *Curr. Opin. Struct. Biol.* **2021**, *70*, 78-86.
13. Brangwynne, C. P.; Eckmann, C. R.; Courson, D. S.; Rybarska, A.; Hoege, C.; Gharakhani, J.; Julicher, F.; Hyman, A. A., Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* **2009**, *324* (5935), 1729-32.
14. Banerjee, P. R.; Milin, A. N.; Moosa, M. M.; Onuchic, P. L.; Deniz, A. A., Reentrant Phase Transition Drives Dynamic Substructure Formation in Ribonucleoprotein Droplets. *Angew. Chem. Int. Ed. Engl.* **2017**, *56* (38), 11354-11359.
15. Mediani, L.; Antoniani, F.; Galli, V.; Vinet, J.; Carra, A. D.; Bigi, I.; Tripathy, V.; Tiago, T.; Cimino, M.; Leo, G.; Amen, T.; Kaganovich, D.; Cereda, C.; Pansarasa, O.; Mandrioli, J.; Tripathi, P.; Troost, D.; Aronica, E.; Buchner, J.; Goswami, A.; Sternecker, J.; Alberti, S.; Carra, S., Hsp90-mediated regulation of DYRK3 couples stress granule disassembly and growth via mTORC1 signaling. *EMBO Rep.* **2021**, *22* (5), e51740.
16. Banani, S. F.; Rice, A. M.; Peeples, W. B.; Lin, Y.; Jain, S.; Parker, R.; Rosen, M. K., Compositional Control of Phase-Separated Cellular Bodies. *Cell* **2016**, *166* (3), 651-663.
17. Ranganathan, S.; Shakhnovich, E., Effect of RNA on Morphology and Dynamics of Membraneless Organelles. *J. Phys. Chem. B* **2021**, *125* (19), 5035-5044.
18. Ruff, K. M.; Dar, F.; Pappu, R. V., Ligand effects on phase separation of multivalent macromolecules. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118* (10).
19. Shin, Y.; Brangwynne, C. P., Liquid phase condensation in cell physiology and disease. *Science* **2017**, *357* (6357).
20. Galganski, L.; Urbanek, M. O.; Krzyzosiak, W. J., Nuclear speckles: molecular organization, biological function and role in disease. *Nucleic Acids Res.* **2017**, *45* (18), 10350-10368.
21. Sawyer, I. A.; Sturgill, D.; Sung, M. H.; Hager, G. L.; Dundr, M., Cajal body function in genome organization and transcriptome diversity. *Bioessays* **2016**, *38* (12), 1197-1208.
22. Kohler, F.; Rice, O. K., Coexistence Curve of the Triethylamine-Water System. *J. Chem. Phys.* **1957**, *26* (6), 1614-1618.
23. Hales, B. J.; Bertrand, G. L.; Hepler, L. G., Effects of 3rd Components on Critical Mixing in Water-Triethylamine System. *J. Phys. Chem.* **1966**, *70* (12), 3970-&.
24. Campbell, A. N.; Kartzmar, E., Heats of Mixing and Dielectric Constants of Some Partially Miscible Liquid Pairs. *Can. J. Chem.* **1969**, *47* (4), 619-&.
25. Kajimoto, S.; Seong, N. H.; Fukumura, H.; Dlott, D. D., Picosecond dynamics of hydrogen bond rearrangements during phase separation of a triethylamine and water mixture. *Photochem. Photobiol. Sci.* **2014**, *13* (6), 891-7.
26. Kitevski-LeBlanc, J. L.; Prosser, R. S., Current applications of 19F NMR to studies of protein structure and dynamics. *Prog. Nucl. Magn. Reson. Spectrosc.* **2012**, *62*, 1-33.
27. Feric, M.; Vaidya, N.; Harmon, T. S.; Mitrea, D. M.; Zhu, L.; Richardson, T. M.; Kriwacki, R. W.; Pappu, R. V.; Brangwynne, C. P., Coexisting Liquid Phases Underlie Nucleolar Subcompartments. *Cell* **2016**, *165* (7), 1686-1697.
28. Hobley, J.; Kajimoto, S.; Takamizawa, A.; Ohta, K.; Tran-Cong, Q.; Fukumura, H., Dynamics of liquid structure relaxation from criticality after a nanosecond laser initiated T-jump in triethylamine-water. *J. Phys. Chem. B* **2003**, *107* (41), 11411-11418.
29. Boeszoermyenyi, A.; Ogorek, B.; Jain, A.; Arthanari, H.; Wagner, G., The precious fluorine on the ring: fluorine NMR for biological systems. *J. Biomol. NMR* **2020**, *74* (8-9), 365-379.
30. Buchholz, C. R.; Pomerantz, W. C. K., (19)F NMR viewed through two different lenses: ligand-observed and protein-observed (19)F NMR applications for fragment-based drug discovery. *RSC Chem. Biol.* **2021**, *2* (5), 1312-1330.
31. Hughes, J. P.; Rees, S.; Kalindjian, S. B.; Philpott, K. L., Principles of early drug discovery. *Br. J. Pharmacol.* **2011**, *162* (6), 1239-49.
32. Klein, I. A.; Boija, A.; Afeyan, L. K.; Hawken, S. W.; Fan, M.; Dall'Agnese, A.; Oksuz, O.; Henninger, J. E.; Shrinivas, K.; Sabari, B. R.; Sagi, I.; Clark, V. E.; Platt, J. M.; Kar, M.; McCall, P. M.; Zamudio, A. V.; Manteiga, J. C.; Coffey, E. L.; Li, C. H.; Hannett, N. M.; Guo, Y. E.; Decker, T. M.; Lee, T. I.; Zhang, T.; Weng, J. K.; Taatjes, D. J.; Chakraborty, A.; Sharp, P. A.; Chang, Y. T.; Hyman, A. A.; Gray, N. S.; Young, R. A., Partitioning of cancer therapeutics in nuclear condensates. *Science* **2020**, *368* (6497), 1386-1392.

