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Supplementary information:

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

Christian F. Pantoja [a], Markus Zweckstetter [a, b *], Nasrollah Rezaei-Ghaleh [b, c *]

[a] Translational Structural Biology Group, German Center for Neurodegenerative Diseases (DZNE), Von-Siebold-Strasse 3a, D-37075 Göttingen, Germany.

[b] Department of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Faßberg 11, D-37077 Göttingen, Germany.

[c] Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany.

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1. Chemicals and materials:

Triethylamine (TEA) **Sigma-Aldrich (≥ 99.5%),** fluorescein isothiocyanate dextran (FITC-dextran) **Sigma-Aldrich** average molecular mass Mw 500.000, DextranT500 **Pharmacosmos** average molecular mass Mw 450.000-550.000, Deuterium oxide (D_2O) **Sigma-Aldrich** 99.9 atom % D, 1,1,1,3,3,3-Hexafluor-2-propanol HFIP **Sigma-Aldrich (≥ 99%)**. Deionized water was used for the preparation of aqueous solutions.

2. NMR sample preparation

2.1. Triethylamine/D_2O/Dextran

A volume of 180 μL was prepared with the following composition (12% m/v) Triethylamine and (15% m/v) DextranT500 dissolved in D_2O 99.9%**.** After the complete dissolution at a lower temperature, the complete volume was transferred to a 3.0 mm Wildman NMR tube.

2.2. Triethylamine/D_2O/Dextran/ HFIP

Similar to the protocol described before 180 μL of solution (1.6% m/v) 1,1,1,3,3,3-Hexafluor-2-propanol, (12% m/v) Triethylamine and (15% m/v) DextranT500 dissolved in D_2O 99.9% was prepared. Following the complete dissolution at a lower temperature, the volume was transferred to a 3.0 mm Wildman NMR tube.

3. Methods and instrumentation

3.1. DIC and Fluorescence images

A Leica DM600B microscope with 63x objective (water immersion) was employed to collect the DIC and fluorescence images. The sample was prepared using (50 mM) Triethylamine (TEA) dissolved in (15%) fluorescein isothiocyanate dextran (FITC-dextran) aqueous solution. A temperature regulation system was employed to record the imaging at 278K and 298K. The obtained images were processed using FIJI (NIH) software [1].

3.2. NMR spectroscopy

3.2.1. ^{1}H-NMR & ^{13}C-NMR

^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker 600 MHz Spectrometer equipped with a triple resonance cryogenic probe. Standard Bruker pulse sequence 1D ^{1}H and INEPT ^{13}C were chosen for data recording. ^{1}H NMR spectra were collected with a spectral width of SW: 10.0 ppm and INEPT ^{13}C NMR spectra were recorded using a spectral width of 60.0 ppm.

3.2.2. Real time ^{13}C-NMR

Kinetic data were acquired using the INEPT ^{13}C experiment (Bruker pulse sequence) and Topspin subroutines in a Bruker 600 MHz spectrometer equipped with a triple resonance cryogenic probe. The identical parameter set described before was used in this case. The 1D ^{13}C-NMR kinetic data were processed using Topspin 4.0 (Bruker) and analyzed employing a homemade Scilab [2] routines.

3.2.3. ^{1}H-^{13}C HSQC-NOESY

2D ^{1}H-^{13}C HSQC-NOESY [3] experiment was collected in a Bruker 700 MHz spectrometer with a triple resonance cryogenic probe. Standard Bruker pulse sequence was employed. The direct spectral window was set up to SW2: 10.0 ppm and the indirect spectral window SW1: 28.0 ppm. The narrow SW1 allowed us to fold all the peaks into the actual windows and record all the NMR signals in the carbon dimension but also reducing considerably the experimental time needed. The complex point recorded in the indirect dimension was t_1 = 128 using 4 scans by increment.

3.2.4. ^{1}H-^{1}H NOESY & ROESY

2D ^{1}H-^{1}H NOESY [3] and 2D ^{1}H-^{1}H ROESY [3] experiments were collected in a Bruker 700 MHz spectrometer with a triple resonance cryogenic probe. Standard Bruker pulse sequences were used in both cases. The spectral window for direct dimension and indirect dimension was set up to 2.5 ppm. A total of t_1=80 complex points was chosen for the indirect dimension. 2D ^{1}H-^{1}H NOESY was recorded with a mixing time of 200 ms and 2D ^{1}H-^{1}H ROESY with spinlock 25 KHz. Each experiment was set up to 4 scans by increment in the indirect dimension. 2D NMR data were processed using Topspin 4.0 (Bruker) and analyzed by NMRFAM-SPARKY[4].

3.2.5. ^{1}H-^{1}H NOESY/EXSY using heating and cooling cycles

The initial temperature was set up to 278 K where the system remained a uniform phase. 1D ^{1}H-NMR spectra of the system were recorded for this initial condition. Thereafter, a temperature rise triggered the phase separation process with a lag time of a few minutes until the temperature equilibrium was reached by the sample. Then a 2D ^{1}H-^{1}H NOESY/EXSY [3] was recorded using a single mixing time. The parameter set was optimized to reduce the experimental time of this experiment below 15 min. Finally, once the experiment was completed the temperature was returned to 278 K to reestablish the uniform phase condition. 1D ^{1}H-NMR spectra were recorded to corroborate that the sample was recovered completely. After verifying that the sample was fully recovered the next mixing times were measured in a similar way.

3.2.6. Chemical Exchange Rate by ^{1}H-^{1}H NOESY/EXSY

After recording the complete set of mixing times using 2D ^{1}H-^{1}H NOESY/EXSY experiments, the intensity ratio between the cross peak and diagonal peak was fitted to the exchange model of two states[5]:

Equation S1.

$$\frac{I_{AB}}{I_{AA}} = \frac{P_A P_B[1 - \exp(-k_{ex}\tau_{mix})]}{P_A[P_A + P_B \exp(-k_{ex}\tau_{mix})]}$$

assuming that $R_{1,A} = R_{1,B} = R_1$. The analysis of the 2D NOESY/EXSY spectra were carried out using Sparky and fitting to the current model was performed by in-house Matlab[6] routines.

3.2.7. ^{1}H NMR & ^{19}F NMR (Triethylamine/D2O/Dextran/HFIP)

^{1}H-NMR and ^{19}F-NMR experiments were recorded using a Bruker 400 MHz Spectrometer equipped with a room-temperature TBO probe. Standard Bruker pulse sequences were used to collect the ^{1}H-NMR (19F decoupled) spectrum and ^{19}F-NMR (^{1}H decoupled) spectra. The spectral window was set up to SW: 10.0 ppm in the case of 1H-NMR and SW: 30 ppm in the case of ^{19}F-NMR.

3.2.8. Kinetics of LLPS monitored by ^{19}F NMR

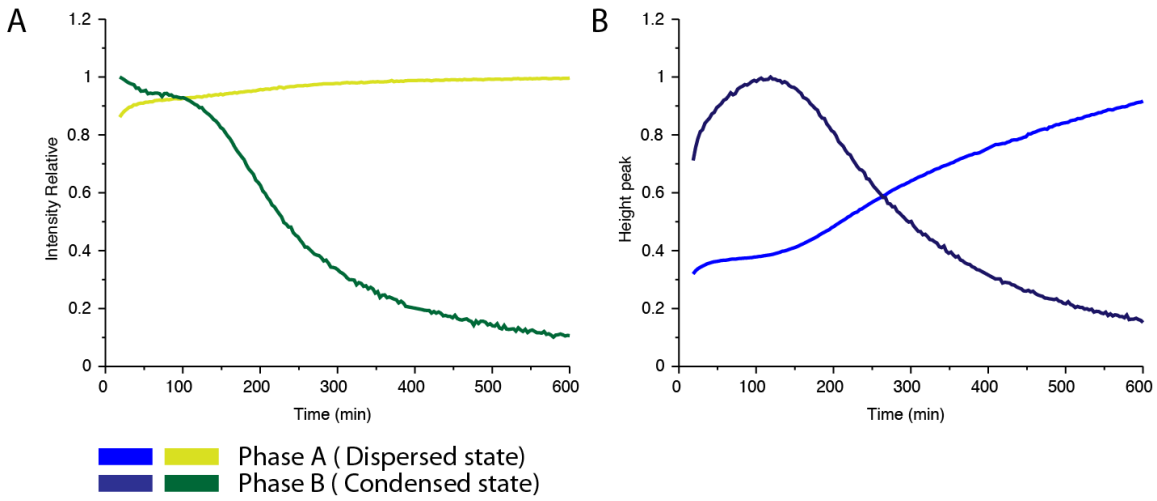

Figure S1. Kinetic evolution of liquid-liquid phase separation of TEA/D_2O/Dextran/HFiP mixture followed by ^{19}F-NMR. Relative intensity (A) and peak height (B) are reported for the fluorine signals of HFiP originated from the dispersed and condensed states. Note the similarity between the kinetic stages observed for the client HFiP molecule and the scaffold Triethylamine molecule shown in **Figure 2**.

3.2.9. ^{19}F-^{19}F NOESY/EXSY

^{19}F-^{19}F NOESY experiment was recorded using a Bruker 400 MHz Spectrometer equipped a room-temperature TBO probe. The probe allows ^{19}F excitation on a broadband inner coil and ^{1}H decoupling on an outer coil. Modification to the standard 2D NOESY experiment was introduced to include 1H decoupling during acquisition but also refocusing of the heteronuclear coupling ^{1}H-^{19}F before the mixing period. The strategy of Heating and cooling cycles (see above, section 3.2.5) was used to collect the complete set of mixing times (15 ms,

30 ms, 60 ms, 90 ms and 120 ms). Spectral window in both dimension was setup to 4.0 ppm and t_1=40 complex points were chosen to reconstruct the indirect dimension.

The complete data set of 2D ^{19}F-^{19}F NOESY/EXSY was processed using Topspin 4.0 and analyzed using Sparky. The corresponding intensity ratio between the cross peak and diagonal peak was fitted to the exchange model of two states described before in equation S1 using in-house Matlab[5] routines.

4. List of acronyms and symbols

TEA	Triethylamine
HFIP	Hexafluoroisopropanol
LLPS	Liquid-liquid Phase Separation
LCST	Lower critical solution temperature
DIC microscopy	Differential Interference Contrast microscopy
NMR	Nuclear Magnetic Resonance
HSQC	Heteronuclear Single Quantum Coherence
NOESY	Nuclear Overhauser Enhancement Spectroscopy
ROESY	Rotating frame Overhauser Enhancement Spectroscopy
EXSY	Exchange Spectroscopy
k_{ex}	Chemical exchange constant
$\Delta\omega$	Chemical shift difference

5. References

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