Experimental report

16/09/2024

Proposal: 8-04-938 **Council:** 10/2022

Title: Dynamics of the tau protein in liquid-liquid phase separation

Research area: Biology

This proposal is a resubmission of 8-04-925

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Samples: tau LLPS

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	3	3	05/04/2024	08/04/2024

Abstract:

Liquid-liquid phase separation (LLPS) of proteins is currently one of the hottest topics in cell biology. LLPS generates intracellular membrane-less compartments in biological cells, within which distinct biochemical processes occur. Most relevant in the context of human health, LLPS has been suggested recently to play a role in neurodegeneration. Indeed, amyloid aggregation of the intrinsically disordered protein tau, one of the pathological hallmarks of Alzheimer¿s, has been suggested to nucleate through LLPS. Our consortium has been using neutron spectroscopy to study the dynamics of tau in various forms, including pathological fibers. We now embark on a research program to study tau in LLPS, with a particular focus on changes in protein and hydration-water dynamics that occur during and after amyloid formation. We will study internal protein dynamics and center-of-mass (global) diffusion of tau with and without LLPS and during aging within LLPS. We will determine if internal and external protein dynamics of tau are altered upon LLPS and during aging. This revised resubmission of #86725 (Feb 21) now provides the missing sample preparation details requested by the review panel.

Dynamics of the tau protein in liquid-liquid phase separation

During the experiment on IN16B in Doppler mode, full QENS spectra ($30\,\mu eV \leq \hbar\omega \leq 30\,\mu eV$, $0.19\,Å \leq q \leq 1.89\,Å$) were recorded for homogeneous and phase-separating tau solutions.

Lyophilised tau powder was rehydrated in D_2O-buffer (20 mM HEPES, pD 7). The solution contained deuterated polyethylene glycol (PEG) at 8 % for phase-separating samples. On overview of the measured samples is given in table 1 Deuterated PEG was synthesised by Dr. Jürgen Allgaier at JCNS (molar mass from NMR end group analysis: $M_n = 8950\,g\,mol^{-1}$, polymerization degree from GPC: M_w/M_n=1.02, deuteration degree of the PEG chain without starter C_4H_9: 99.3 %)

Each sample at each temperature was fitted over all q values. We fitted one or two Lorentzians for the buffer depending on the presence of PEG, and two Lorentzians for the protein contribution. We fitted two models. One imposed Fickian diffusion for the centre-of-mass (COM) diffusion and jump diffusion for the internal dynamics. The second Fickian for both processes.

In Fig. 2, the dependency of the Lorentzian linewidth with q^2 is shown as calculated from the fit results (diffusion coefficients and residence time). The models agree on the COM diffusion D. It very similar between the samples (2σ) which is unexpected since the presence of PEG increases the crowding which should reduce D. We speculate that a change in the effective hydrodynamic size of tau compensates for the change in crowding.

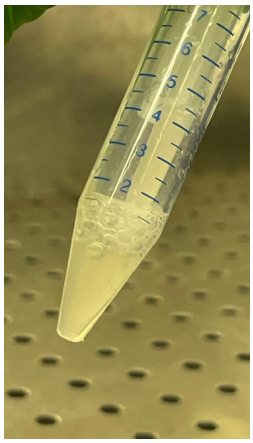


Fig. 1: The solution of rehydrated tau ($75.6\,mg\,L^{-1}$ tau and 8 % (w/V) PEG) was turbid before being placed into the IN16B sample holder.

As to internal dynamics (Fig. 2 right, bottom), there is a large difference between the corresponding diffusion coefficients. For well-folded proteins, this would be an unusual result of crowding [1].

Also, the linewidths increase steeply and exceed the instrument's maximal energy transfer in the Doppler mode (30 µeV). The smallest q values dominate the fit in this case. This effect is even more pronounced at higher temperatures. For the LPPS sample at 320 K, the Lorentzian broadens to a nearly flat line (Fig. 2 e) Under these conditions, it becomes difficult to discern if the diffusive processes are Fickian of jump-like.

Table 1: Measured sample conditions. c(tau): protein concentration calculated from powder weight and tau's specific volume, φ: molecular volume fraction, c(dPEG): concentration of PEG, LLPS :the presence of phase separation, T: temperature. Full QENS spectra were recorded with 6 h counting time per sample and temperature.

c(tau) $[mg\,mL^{-1}]$	φ_{tau}	c(dPEG) [%(w/V)]	LLPS	T [K]
75.6	0.0548	0	no	280, 300, 320
75.6	0.0548	8	yes	280, 300, 320
38.9	0.0274	0	no	280, 320
3.9	0.0027	8	yes	320

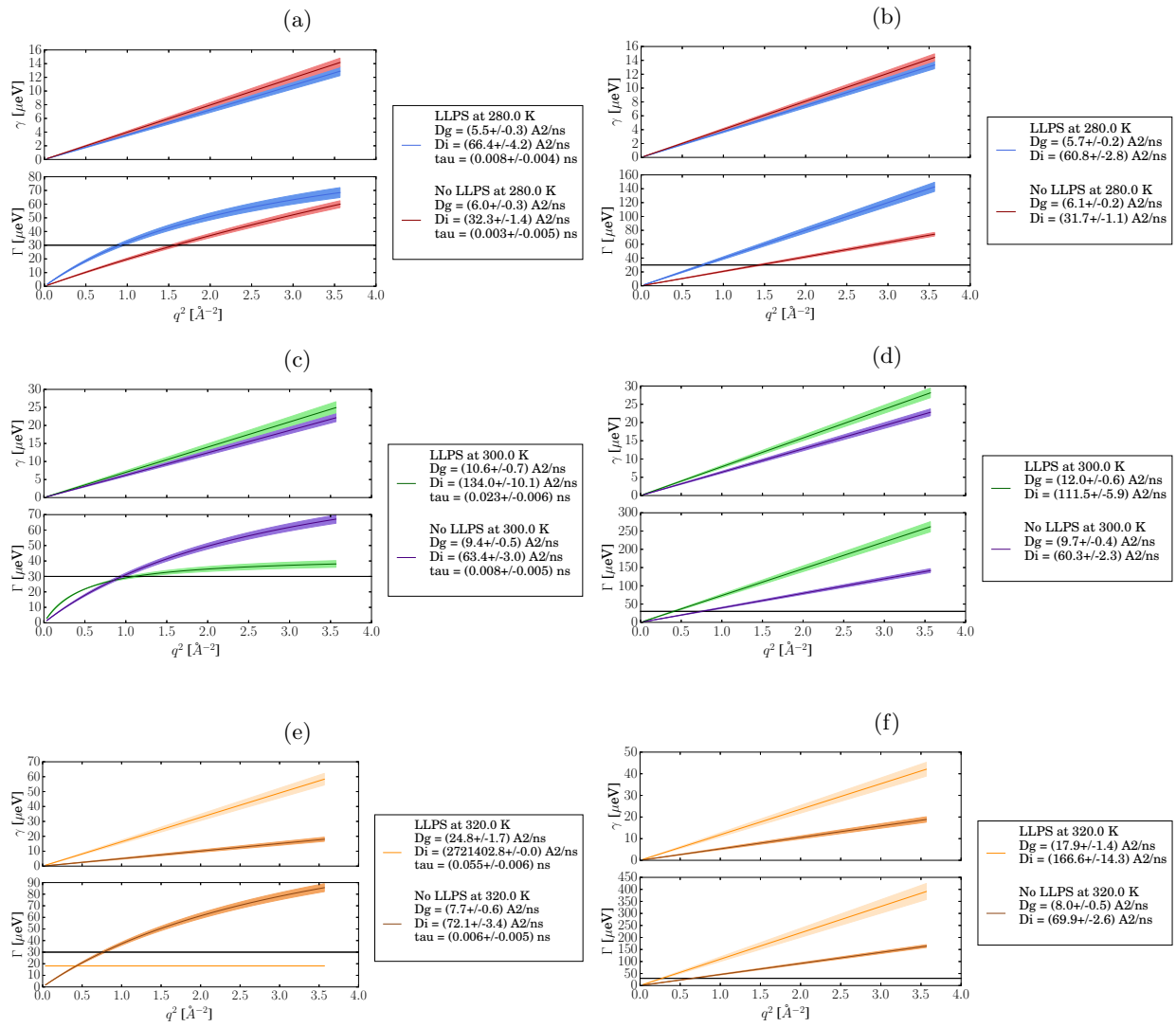


Fig. 2: The dependency of the linewidth versus q^2 for center-of-mass (γ) and internal (Γ) dynamics was calculated from the fit results. The results come from two fit which imposed either global Fickian and internal jump-diffusion (a, c, e), or global and internal Fickian diffusion (b, d,f).

References: [1] M. Grimaldo et al. en. *Quarterly Reviews of Biophysics* 52 (2019).