Experimental report

22/08/2022

Proposal: 8-04-901 **Council:** 10/2020

Title: Influence of aluminum ions and nanoparticles on the protein tau dynamics and aggregation

Research area: Biology

This proposal is a new proposal

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Samples: Protein - CHNOS

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	4	2	15/02/2021	17/02/2021
IN15 Standard	2	3	23/02/2021	26/02/2021
D11	1	0		
D22	1	0		

Abstract:

The protein tau is an intrinsically disordered protein of 441 amino acids in its largest form. It is involved in Alzheimer's disease and can form amyloid fibers, which consist of a stack of beta-sheets forming an elongated fiber. In addition, the amyloid deposits containing tau were also found to contain substantial amounts of aluminum. The aluminum can induce tau aggregation and can promote neurotoxicity in-vivo. While Al3+ was shown to accelerate tau aggregation in-vivo, the molecular insights, center-of-mass diffusion, internal dynamics, beta-sheets formation, are still to be studied to better understand the effect of aluminum on tau aggregation.

In a previous experiment at the ILL (proposal 8-04-863), we observed a significant effect of the aluminum sample holder on protein diffusion and internal dynamics. We hereby propose to investigate the effect of Al3+ and nanoparticles of oxidized aluminum on tau aggregation using Teflon-coated sample holder. The unique energy transfer range accessible with the combination of IN15 and BATS option of IN16B will be used to accurately characterize center-of-mass diffusion and internal dynamics during tau aggregation.

Experimental report, proposal 8-04-901

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August 22, 2022

Scientific background. Protein amyloid aggregation is associated with neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. In particular, the protein tau is involved in Alzheimer's disease, the hallmark of which is proteinaceous aggregates known as amyloids (*1*). Multiple evidences point to small oligomeric species as transient on-pathway aggregates that induce the cytotoxicity (*2*). Hence, the first goal of this experiment was to monitor the dynamics (using quasi-elastic neutron scattering - QENS - and neutron spin-echo - NSE) of the protein tau during the aggregation process to obtain information on the transient oligomers. Furthermore, we suspect the aluminum to influence the aggregation kinetics and pathway. We thus used nickel-coated neutron sample holder and aluminum nano-particles to test the hypothesis as a second goal. However, this measurement was not conclusive and only the first goal is reported here.
Experimental procedure - QENS. The protein was dissolved in the aggregation buffer (30 mM HEPES, 0.15 M NaCl, 5 mM DTT at pH 7.4 in pure D2O), and the obtained protein solution was used to dissolve the amount of heparin powder that corresponds to a molar ratio 4:1 tau:heparin. The measurements performed on IN16B were done in BATS mode (*3*). The data were recorded using scans of 15 minutes each for a total time of 20 hours. To analyze the time series, a sliding average was performed by taking a window of 8 scans, such that each data set represents a measurement of 2 hours. Subsequently, the signal of the empty sample container was subtracted from the data prior to the analysis. The resolution function was modeled using a pseudo-Voigt profile. The signal of the fibrillation buffer was modeled using the following equation,

$$S_{D_2O}(q,\omega) = a_1 \mathcal{L}_{\gamma_1}(q,\omega) + a_2 \mathcal{L}_{\gamma_2}(q,\omega) \tag{1}$$

Therein, a_1 and a_2 are scalars and L_{γ_1,γ_2} are normalized Lorentzian line shapes, inspired by the standard model for water (*4*). The data on tau fibrillation were analyzed using the following model (after ruling out other models),

$$S(q,\omega) = R(q,\omega) \otimes A_q \mathcal{L}_\Gamma(q,\omega) + \beta_{D_2O} S_{D_2O}(q,\omega) \tag{2}$$

where $R(q,\omega)$ is the resolution function, $A_q = A_q(q)$ a scalar that is free to take any value for each momentum transfer q and β_{D_2O} a scaling factor for the D_2O signal which accounts for the volume fraction excluded by the proteins (here $\beta_{D_2O} = 0.94$). All components of the buffer are included in the D_2O signal. All BATS data analysis were performed using the nPDyn software v3.0 (*5*).
Experimental procedure - NSE. All neutron spin-echo measurements were performed in IN15 at the ILL (*6*). The protein tau at a concentration of 60 mg/mL was measured for the initial state before the addition of heparin in the fibrillation buffer. The measurement of the initial state was done at two wavelengths, 10 and 12 Å and 6 momentum transfer q values, 0.025, 0.034, 0.042, 0.056, 0.067 and 0.078 $Å^{-1}$. Along with the samples, the buffer was measured for subtraction to the signal as well as the transmission signal at the low q limit for normalization. Subsequently, the heparin was added to the sample in a molar ratio 4:1 tau:heparin and a series of 2 hours scans at Å was recorded for 20 hours in total during the fibrillation. Eventually, the final, aggregated, state was measured again at 10 and 12 Å. The data were analyzed using the stretched exponential function:

$$I(q,t) = exp^{-\left(D_c q^2 t\right)^\beta} \tag{3}$$

where D_c is the apparent collective diffusion coefficient, and β is associated with the homogeneity of the dynamics of the system. Other models were used and compared using the Bayesian information criterion. The data were fitted for all q-values at once.

Main results. The collective motions on a time scale of 100 ns are investigated using NSE. For an intrisically disordered protein (IDP) such as tau, large scale motions of the disordered regions and center-of-mass diffusion of the proteins will contribute to the rate of decrease of the signal with time. The collective diffusion coefficient D_c decreases quickly during the aggregation process from 5.9 Å^2/ns initially, to 4.5 Å^2/ns at 5 hours and more slowly after 5 hours to reach 4.2 Å^2/ns at 20 hours (Figure 1). The evolution of D_c can be fitted using a double exponential (see dotted orange line on Figure 1), where the faster decay rate coincides with the evolution of the Thioflavin T (an amyloid-specific fluorescent probe) fluorescence signal for the first 10 hours. The parameter β is lower than 1 for the whole aggregation reaction, indicating a significant heterogeneity of the dynamics. The aggregation process is expected to diminish the contribution of the center-of-mass diffusion in the first hours of aggregation as we observe the formation of numerous and large aggregates with electron microscopy and atomic force microscopy. Therefore, after 5 hours, we attribute the QENS signal to the internal dynamics alone. It appears that tau has an initial apparent diffusion coefficient D_i of 14 Å^2/ns, which decreases to a first minimum of 8 Å^2/ns at 5 h, subsequently increases to almost 13 Å^2/ns at 7.5 h, passes to a second minimum of 8 Å^2/ns at 12 h before coming back close to its initial value at the end of the measurement. We attribute the first minimum to the formation of the so-called amyloid cross-β structure, which consists of a stack of β-sheets formed within individual monomers. The maximum occurs when the structure stabilizes. The second minimum is attributed to the formation of larger aggregates, formed by bundles of amyloid fibers.

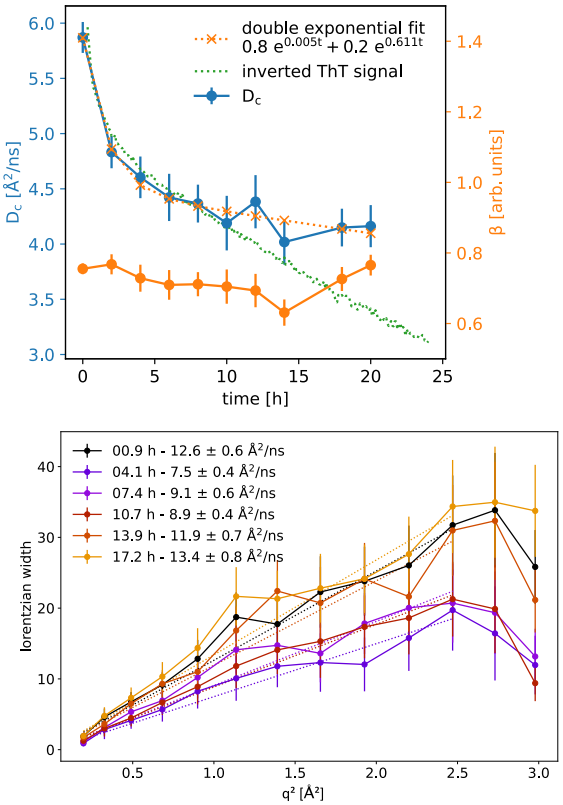

Figure 1: **Top.** Fitted parameters of eq. 3 as a function of time for the NSE data. The blue line with error bars corresponds to D_c, the orange line with error bar to β and the green dotted line to a kinetic measurement using a amyloid-specific fluorescent probe, the Thioflavin T (ThT) and the orange dotted line to a fit of $D_c(t)$ with a double exponential model. **Bottom.** Fitted width of the Lorentzian as the function momentum transfer q^2 for different kinetic times ranging from 0 h (black) to 17 h (yellow). The fitted value for the apparent diffusion coefficient is given in legend.

References

1. F. Chiti, C. M. Dobson, *Annual Review of Biochemistry* **86**, 27–68 (2017).
2. B. Winner *et al.*, *Proceedings of the National Academy of Sciences* **108**, 4194–4199, ISSN: 0027-8424, 1091-6490 (Mar. 2011).
3. M. Appel, B. Frick, A. Magerl, *Scientific Reports* **8**, 13580, ISSN: 2045-2322 (Sept. 2018).
4. J. Qvist, H. Schober, B. Halle, *The Journal of Chemical Physics* **134**, 144508, ISSN: 0021-9606 (Apr. 2011).
5. K. Pounot, *Kpounot/nPDyn: V3.0.0*, Zenodo, Nov. 2021.
6. P. Schleger *et al.*, *Physica B: Condensed Matter*, Proceedings of the International Conference on Neutron Scattering **241–243**, 164–165, ISSN: 0921-4526 (Dec. 1997).