Monolith Protocol MO-P-069

Tau – Zn^{2+} (aggregation)

Tau is a microtubule-associated protein (MAP) that is abundant in neurons, where it promotes the assembly and maintains the structure of microtubules. It is also found in the neurofibrillary tangles (NFT) in Alzheimer's disease (AD). While under normal conditions, the concentration of free metal ions in the brain is very low and does not affect neurological function, the brain tissue of AD patients has been shown to contain large amounts of transition metal ions, such as Cu^{2+}, Fe^{3+} or Zn^{2+}, and zinc concentrations in AD brains can reach as high as 10–300 µM. Low micromolar zinc concentrations have been shown to accelerate the fibrillization of human tau protein. Therefore, zinc binding to tau appears to be a substantial contributor to tauopathy and techniques to analyze and quantify the interaction between tau and zinc are essential for therapy development.

protein – ion interaction | tau | metal ions | Alzheimer's disease | aggregation

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A1. Target/Fluorescent Molecule

Tau-441 (2N4R), TauM (monomer)
uniprot.org/uniprot/P10636

A2. Molecule Class/Organism

Microtubule-associated protein
Homo sapiens (Human)

A3. Sequence/Formula

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MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG SETSDAKSTP TAEDVTAPLV
DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP
GQKGQANATR IPAKTPPAPK TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK
SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV PGGGSVQIVY KPVDLSKVTS
KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS
GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L
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A4. Purification Strategy/Source

N/A

A5. Stock Concentration/Stock Buffer

1 mg/mL | 21.8 µM

A6. Molecular Weight/Extinction Coefficient

45.9 kDa
7,575 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 27.1 µL of Labeling Buffer NHS to 22.9 µL of TauM (21.8 µM) to obtain 50 µL of a 10 µM solution.
2. Add 25 µL of DMSO to 10 µg Dye RED-NHS 2nd Generation to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 2.5 µL of the 600 µM dye solution with 47.5 µL of Labeling Buffer NHS to obtain 50 µL of a 30 µM dye solution (3x protein concentration).
4. Mix TauM and dye in a 1:1 volume ratio (100 µL final volume, 2.5% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with assay buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 100 µL of the labeling reaction from step 4 to the center of the column and let sample enter the resin bed completely.
9. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
11. Keep the labeled TauM (~1 µM) on ice in the dark.

A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

Absorbance A_{205}	1.50	Protein concentration	1.0 µM
Absorbance A_{650}	0.19	Degree-of-labeling (DOL)	0.95

B1. Ligand/Non-Fluorescent Binding Partner

Zn^{2+}

B2. Molecule Class/Organism

Divalent cation

B3. Sequence/Formula

$ZnCl_2$

B4. Purification Strategy/Source

Carl Roth GmbH
T887.1

B5. Stock Concentration/Stock Buffer

Powdered

B6. Molecular Weight/Extinction Coefficient

136.28 Da

B7. Serial Dilution Preparation

Broad dilution series

1. Dissolve 0.34 g of $ZnCl_2$ in 10 mL of ddH_2O to obtain a 250 mM $ZnCl_2$ stock solution. Add 4 drops of HCl (1.2 M) to acidify the solution and completely dissolve all $ZnCl_2$.
2. Mix 4 µL of the 250 mM $ZnCl_2$ stock with 496 µL of ddH_2O to obtain a 500 µL of a 2 mM $ZnCl_2$ solution[1].
3. Prepare a PCR-rack with 12 PCR tubes. Transfer 20 µL of the 2 mM $ZnCl_2$ solution into tube **1**. Then, add 10 µL of ddH_2O to tubes **1** to **12**.
4. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **12** to get an equal volume of 10 µL for all samples.
5. Mix 6 µL of labeled TauM (~1 µM) with 6 µL of unlabeled TauM (1 µM) and 138 µL of dilution buffer to obtain 150 µL of a ~40 nM labeled, 40 nM unlabeled TauM solution[2].
6. Centrifuge the tube at 15,000 rpm at 4°C for 15 minutes. Then, carefully transfer 130 µL of the supernatant of the tube into a new tube and mix by pipetting.
7. Add 10 µL of this solution to each tube from **12** to **1** and mix by pipetting.
8. Load capillaries immediately and run the MST measurement in 'Expert Mode'.
   (Before MST: 1 second | MST-On Time: 10 seconds | After MST: 0 seconds).
9. Remeasure capillaries after 20 minutes and 40 minutes to monitor time-dependent aggregation.
10. To demonstrate reversibility, add 0.5 µL of a buffered 100 mM EDTA solution (pH 7-8) to each tube from **12** to **1** and mix well with a 10 µL pipette[3] to obtain a final concentration of ~5 mM EDTA in each tube.

[1] Due to the low solubility of $ZnCl_2$ at non-acidic pH and higher ionic strength, as well as its tendency to form insoluble $Zn(OH)_2$ at pH > 7, the dilution series is prepared in ddH_2O (see also Krezel et al., Archives of Biochemistry and Biophysics 611 (2016) 3-19).

[2] The DOL is intentionally decreased to ~0.5 to prevent effects of dye self-quenching when tau proteins come into close proximity in dimers, oligomers or larger aggregates.

[3] Without proper mixing, insoluble aggregates can accumulate at the bottom of the tubes and the fluorescence signal of loaded capillaries will be very low.

Narrow dilution series

1. Prepare a new PCR-rack with 12 fresh PCR tubes. Mix 4.4 µL of the 2 mM $ZnCl_2$ solution with 215.6 µL of ddH_2O in tube **1** to obtain a 40 µM $ZnCl_2$ solution. Then, add 20 µL of ddH_2O to tubes **2** to **12**.
2. Prepare a **10:1** serial dilution by transferring 200 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 200 µL from tube **12** to get an equal volume of 20 µL for all samples.
3. Transfer 10 µL from each tube of the dilution series into a fresh tube to obtain a new dilution series of 12 tubes with 10 µL each. Discard the tubes from the initial dilution series.
4. Mix 6 µL of labeled TauM (~1 µM) with 6 µL of unlabeled TauM (1 µM) and 138 µL of dilution buffer to obtain 150 µL of a ~40 nM labeled, 40 nM unlabeled TauM solution.
5. Centrifuge the tube at 15,000 rpm at 4°C for 15 minutes. Then, carefully transfer 130 µL of the supernatant of the tube into a new tube and mix by pipetting.
6. Add 10 µL of this solution to each tube from **12** to **1** and mix by pipetting.
7. Incubate tubes for 30 minutes at room temperature in the dark.
8. Load capillaries and run the MST measurement in 'Expert Mode'.
   (Before MST: 1 second | MST-On Time: 10 seconds | After MST: 0 seconds).

D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

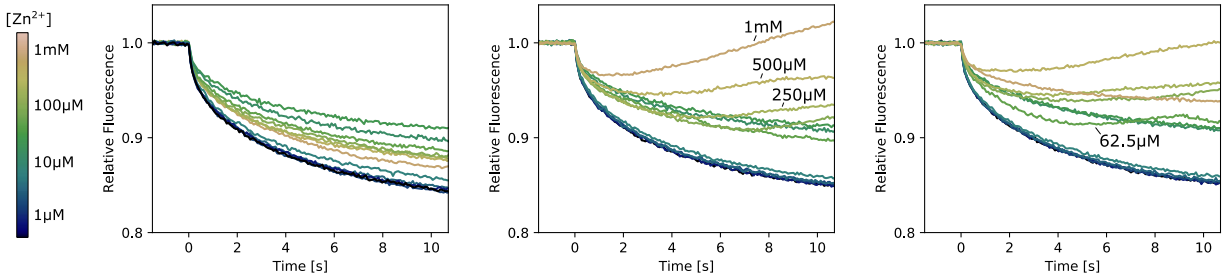
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

0.5X Phosphate-buffered saline (PBS), pH 7.4, 0.005% Pluronic® F-127
40 nM TauM | 1 mM – 488 nM (20 µM – 7 µM) Zn^{2+} | 22°C | medium MST power | 80% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

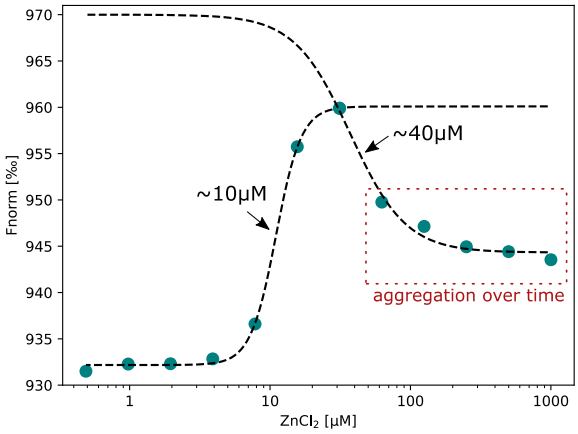
Immediately after mixing: After 20 min: After 40 min:



Zn^{2+}-induced aggregation of tau for Zn^{2+} concentrations > 50 µM after a lag time of 20–40 minutes.

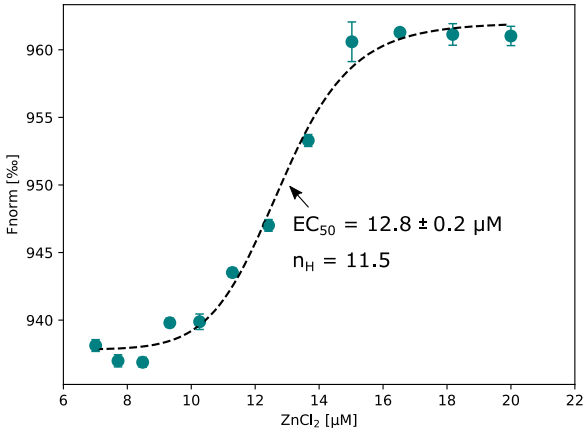
Broad dilution series

Biphasic curve (affinities of ~10 µM and ~40 µM)



Narrow dilution series

EC_{50} = 12.8 ± 0.2 µM | n_H = 11.5 (Hill coefficient)[4]



More than one type of binding sites for Zn^{2+}.

While the first binding event leads to stable tau-Zn^{2+} complexes, the second one results in aggregation over time.

D5. Reference Results/Supporting Results

K_d = 14.7 µM	Zn^{2+} – Tau-R3 peptide	Isothermal Titration Calorimetry (ITC) Li et al., Int. J. Mol. Sci. 20, 487 (2019)
K_d = 3.82 µM	Zn^{2+} – $Tau_{244–372}$	Isothermal Titration Calorimetry (ITC) Mo et al., J Biol. Chem. 284, 50, 34648–34657 (2009)

Low micromolar concentrations of Zn^{2+} dramatically accelerate fibril formation of wild-type $Tau_{244–372}$ under reducing conditions, compared with no Zn^{2+}. Higher concentrations of Zn^{2+}, however, induce wild-type $Tau_{244–372}$ to form granular aggregates in reducing conditions.

Mo et al., J Biol. Chem. 284, 50, 34648–34657 (2009)

E. Contributors

Andreas Langer[5]

[4] In many cellular processes, proteins need to respond to small changes in the concentration of their ligands with highest sensitivity, which is often achieved using 'Hill-type' cooperativity and results in very steep dose-response curves. Recently, intrinsically disordered proteins such as tau have been identified to possess a multitude of ways to function within the confines of the allosteric paradigm through mechanisms that would be extremely unfavorable or even impossible for globular proteins. (see Berlow et al., J Mol Biol. 430, 16, 2309–2320 (2018) for further reading).

[5] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com