Monolith Protocol MO-P-074

Tau – EGCG

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). Epigallocatechin-3-gallate (EGCG) is an active phytochemical of green tea that has shown its potency against various diseases including aggregation inhibition of tau.

protein – small molecule interaction | tau | Alzheimer's disease

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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.

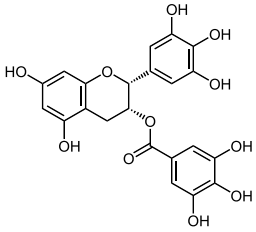
A10. Labeling Efficiency

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

Epigallocatechin-3-gallate (EGCG)



B2. Molecule Class/Organism

Catechin

B3. Sequence/Formula

$C_{22}H_{18}O_{11}$

B4. Purification Strategy/Source

Fisher Scientific GmbH
15214248

B5. Stock Concentration/Stock Buffer

10 mg

B6. Molecular Weight/Extinction Coefficient

458.37 Da

B7. Serial Dilution Preparation

1. Dissolve 10 mg of EGCG in 436 µL of DMSO to obtain a 50 mM EGCG stock solution.
2. Mix 20 µL of the 50 mM EGCG stock with 80 µL of DMSO to obtain a 10 mM EGCG solution.
3. Mix 4 µL of the 10 mM EGCG solution with 196 µL of dilution buffer to obtain 200 µL of 200 µM EGCG solution.
4. Mix 10 µL of the 200 µM EGCG solution with 190 µL of dilution buffer to obtain 200 µL of a 10 µM EGCG solution.
5. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 10 µM EGCG solution into tube **1**. Then, add 10 µL of dilution buffers to tubes **2** to **16**.
6. 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 **16** to get an equal volume of 10 µL for all samples.
7. Mix 8 µL of labeled TauM (~1 µM) with and 192 µL of dilution buffer to obtain 200 µL of a ~40 nM labeled TauM solution.
8. Add 10 µL of this solution to each tube from **16** to **1** and mix by pipetting.
9. Incubate for 10 minutes at room temperature in the dark before loading capillaries.

B8. SD-Test

1. Prepare the SD-mix: Dilute 400 µL of 10% SDS and 40 µL of 1 M DTT in 560 µL water to obtain a solution containing 4% SDS and 40 mM DTT.
2. Transfer 7 µL of the SD-mix to six PCR tubes.
3. Add 7 µL from the remainder of tubes **1** to **3** and **14** to **16** to the tubes containing 7 µL SD-mix. Mix well by pipetting.
4. Place samples on a heat block set to 95°C for 5 minutes to denature the protein, then allow to cool at 25°C for 10 minutes before loading into capillaries.

D1. MST System/Capillaries

Monolith Pico – RED (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

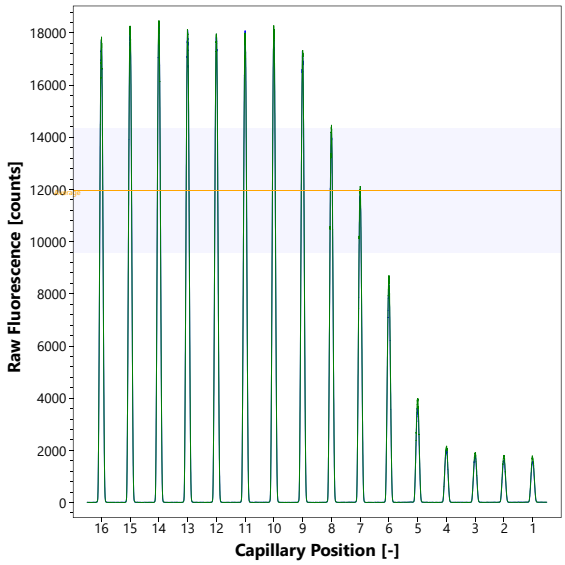
MO.Control v2.0.1 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

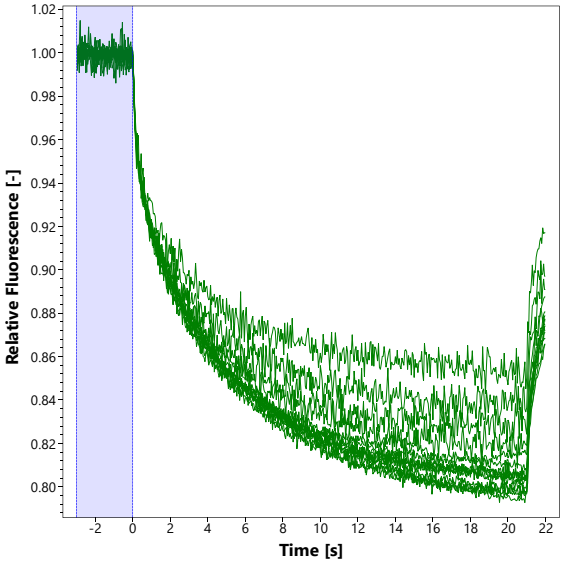
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

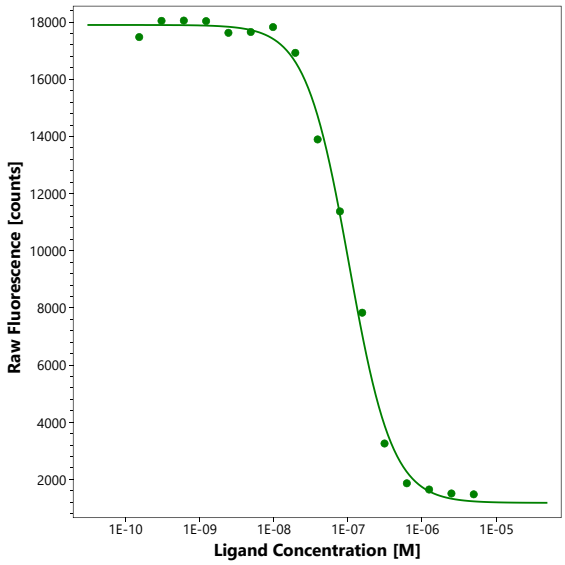
Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127
20 nM TauM | 5 µM – 153 pM EGCG | 25°C | medium MST power | 5% excitation power

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

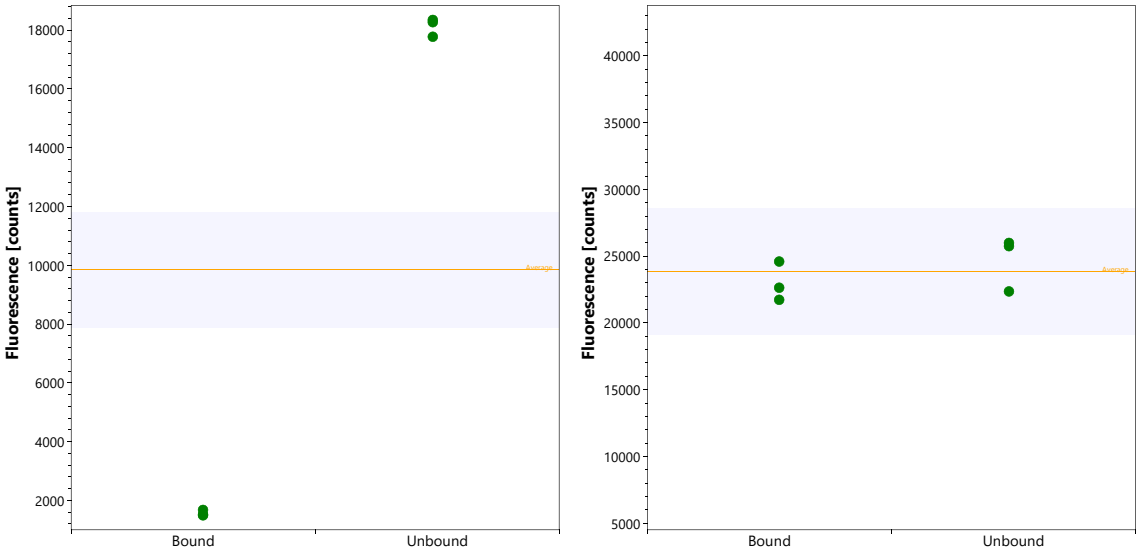
EC_{50} = 104 nM







SD-Test[1]:



D5. Reference Results/Supporting Results

K_d = 74 nM Isothermal Titration Calorimetry (ITC)
Sonawane et al., Scientific Reports (2020) 10:12579

E. Contributors

Andreas Langer[2]

[1] Due to the ligand-dependent fluorescence changes, an SD-test was performed to confirm binding-dependent change. As the changes in initial fluorescence were concluded to be binding-specific, the initial fluorescence data was used for binding curve fit and K_d determination. Note that after longer incubation (several hours), the SD-test starts to become negative since EGCG can covalently crosslink to tau, see also Zhao et al., Inflammation & Allergy - Drug Targets, 2013, 12, 308-314.

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