Monolith Protocol MO-P-076

Protein X – Alpha-Synuclein

α-Synuclein is a protein that is abundant in the brain and is found mainly at the tips of neurons (presynaptic terminals). Within these structures it interacts with phospholipids and proteins. The presynaptic terminals release neurotransmitters which relays signals between neurons and is critical for normal brain function. α-Synuclein is a major component of Lewy bodies in the affected neurons in Parkinson's disease and it is also involved in the pathophysiology of Alzheimer's disease. Finding interaction partners of e.g. α-synuclein is an important step in trying to find specific biological markers of disease status or disease progression.

protein – protein interaction | alpha-synuclein | neurodegenerative diseases

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A1. Target/Fluorescent Molecule

Protein X[1]

A2. Molecule Class/Organism

Homo sapiens (Human)

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

N/A

A5. Stock Concentration/Stock Buffer

0.5 mg/mL | 15 µM

A6. Molecular Weight/Extinction Coefficient

33 kDa

A7. Dilution Buffer

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20

[1] As the data in this protocol hasn't been published yet, the target is still confidential and called 'protein X'. As soon as the data is published, the real target name will be added.

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Prepare 100 µL of a 15 µM protein X solution in ddH_2O.
2. Add 30 µL of DMSO to Dye RED-NHS (10 µg) to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 9.6 µL of the 470 µM dye solution with 90.4 µL of Labeling Buffer NHS to obtain 100 µL of a 45 µM dye solution (3x protein concentration).
4. Mix protein X and dye in a 1:1 volume ratio (200 µL final volume, 4.8% 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 dilution 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 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the resin bed completely.
9. Add 300 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 600 µL of dilution buffer and collect the eluate.
11. Keep the labeled protein X (~2.5 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

α-synuclein
uniprot.org/uniprot/P37840

B2. Molecule Class/Organism

Synuclein protein
Homo Sapiens (Human)

B3. Sequence/Formula

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MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV GSKTKEGVVH GVATVAEKTK EQVTNVGGAV VTGVTAVAQK
TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA
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B4. Purification Strategy/Source

Full-length recombinant human alpha-synuclein (GenBank Accession # NP_000336) was expressed in *E. coli* and purified from bacterial lysate using proprietary method (see manufacturer's website). Endotoxin was further removed by proprietary technique.

AnaSpec
AS-55555

B5. Stock Concentration/Stock Buffer

10 mg/mL | 690 µM

B6. Molecular Weight/Extinction Coefficient

14.5 kDa

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 10 µL of dilution buffer into tubes **1** to **16**. Then, add 10 µL of the 690 µM α-synuclein stock solution to tube **1** and mix by pipetting.
2. 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.
3. Mix 2 µL of labeled protein X (~2.5 µM) with 198 µL of dilution buffer to obtain 200 µL of ~25 nM protein X.
4. Add 10 µL of this solution to each tube from **16** to **1** and mix by pipetting.
5. Load capillaries directly.

D1. MST System/Capillaries

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

D2. MST Software

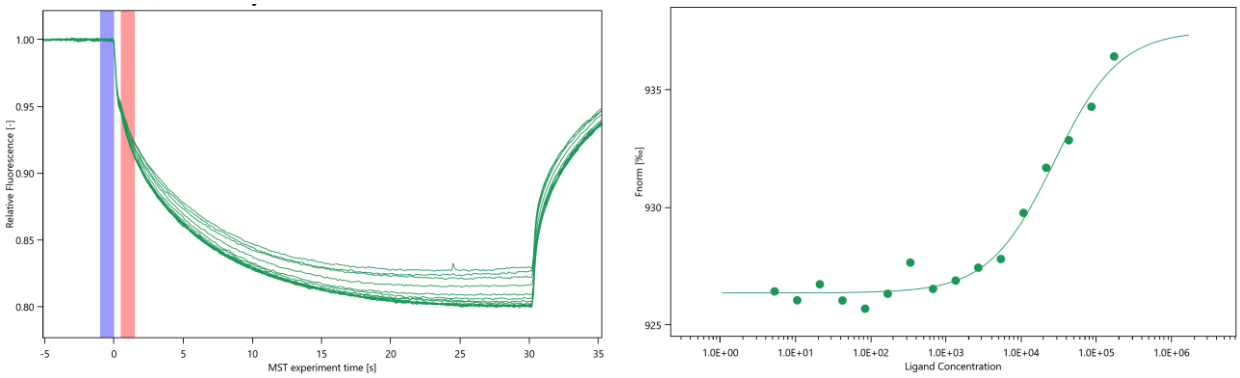
NT.Control | MO.AffinityAnalysis (NanoTemper Technologies GmbH)

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

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20
12.5 nM protein X | 172.5 µM – 5.3 nM α-synuclein | 22°C | medium MST power | 50% excitation power

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

K_d = 27 µM



D5. Reference Results/Supporting Results

N/A

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