Monolith Protocol MO-P-039

FYN – Staurosporine

FYN is a non-receptor tyrosine-protein kinase that plays a role in many biological processes including regulation of cell growth and survival, cell adhesion, integrin-mediated signaling, cytoskeletal remodeling, cell motility, immune response and axon guidance. Staurosporine is a relatively non-selective protein kinase inhibitor, which blocks many kinases to different degrees. Staurosporine is often used as a general method for inducing apoptosis.

protein – small molecule interaction | kinase | inhibitor

A1. Target/Fluorescent Molecule

Tyrosine-protein kinase FYN
uniprot.org/uniprot/P06241

A2. Molecule Class/Organism

Non-receptor tyrosine-protein kinase
Homo sapiens (Human)

A3. Sequence/Formula

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MKDVWEIPRE SLQLIKRLGN GQFGEVWMGT WNGNTKVAIK TLKPGTMSPE SFLEEAQIMK KLKHDKLVQL YAVVSEEPIY
IVTEYMNKGS LLDFLKDGEG RALKLPNLVD MAAQVAAGMA YIERMNYIHR DLRSANILVG NGLICKIADF GLARLIEDNE
YTARQGAKFP IKWTAPEAAL YGRFTIKSDV WSFGILLTEL VTKGRVPYPG MNNREVLEQV ERGYRMPCPQ DCPISLHELM
IHCWKKDPEE RPTFEYLQSF LEDYGHHHHH H
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A4. Purification Strategy/Source

Crelux GmbH

A5. Stock Concentration/Stock Buffer

10 mg/mL | 321 µM
20 mM Tris-HCl, pH 8.5, 156 mM NaCl, 5 mM DTT

A6. Molecular Weight/Extinction Coefficient

31 kDa
49,640 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.005% TWEEN® 20

A8. Labeling Strategy

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

A9. Labeling Procedure

1. Add 98.4 µL of Labeling Buffer NHS to 1.6 µL of 321 µM FYN to obtain 100 µL of a 5 µM solution.
2. Use the A-Column to perform a buffer exchange into Labeling Buffer NHS.
   a. Invert A-Column to suspend slurry and twist off bottom (twist slightly in both directions).
   b. Loosen the cap of the column and place it in a 1.5 mL microcentrifuge collection tube.
   c. Centrifuge at **1500 × g** for **1 min** to remove excess liquid.
   d. Add 300 µL of Labeling Buffer NHS and centrifuge at **1500 × g** for **1 min** (3x).
   e. Place 100 µL of the 10 µM FYN solution in the center of the resin.
   f. Place the sample in a **new** microcentrifuge collection tube and centrifuge at **1500 × g** for **2 min**.

   The collected flow-through should yield around 100 µL of ~3 µM FYN (~60 % recovery).
3. Add 30 µL of DMSO to 10 µg RED-NHS dye to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
4. Mix 3.2 µL of the 470 µM dye solution with 96.8 µL of Labeling Buffer NHS to obtain 100 µL of a 15 µM dye solution (~5x protein concentration).
5. Mix FYN and dye in a 1:1 volume ratio (200 µL final volume, 1.6% final DMSO concentration).
6. Incubate for 30 minutes at room temperature in the dark.
7. 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.
8. 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.
9. Add 200 µL of the labeling reaction from step 5 to the center of the column and let sample enter the bed completely.
10. Add 400 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
12. Centrifuge for 5 min at 14,000 rpm and 8°C.
13. Keep the labeled FYN (~0.6 µM) on ice in the dark.

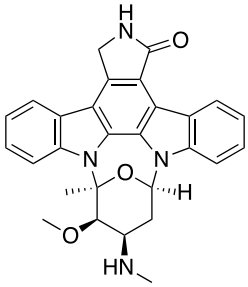
A10. Labeling Efficiency

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

Absorbance A_{280}	0.04	Protein concentration	0.6 µM
Absorbance A_{650}	0.41	Degree-of-labeling (DOL)	2.7

B1. Ligand/Non-Fluorescent Binding Partner

Staurosporine



B2. Molecule Class/Organism

Protein kinase inhibitor
Streptomyces staurosporeus

B3. Sequence/Formula

$C_{28}H_{26}N_4O_3$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
S5921

B5. Stock Concentration/Stock Buffer

23.3 mg/mL | 50 mM
DMSO

B6. Molecular Weight/Extinction Coefficient

466.53 Da

B7. Serial Dilution Preparation

1. Transfer 1 µL of 50 mM Staurosporine into 99 µl of DMSO to obtain 100 µL of a 500 µM Staurosporine solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 500 µM Staurosporine solution into tube **1**. Then, transfer 10 µL of DMSO into tubes **2** to **16**.
3. 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.
4. Add 27 µL of dilution buffer to each tube and mix well by pipetting.
5. Mix 12.8 µL of labeled FYN with 787 µL of dilution buffer to obtain 800 µL of ~16 nM FYN.
6. Add 10 µL of 16 nM labeled Fyn-kinase to 16 fresh tubes
7. Add 0.8 µl of Staurosporine dilution to each tube from **16** to **1** and mix by pipetting.
8. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

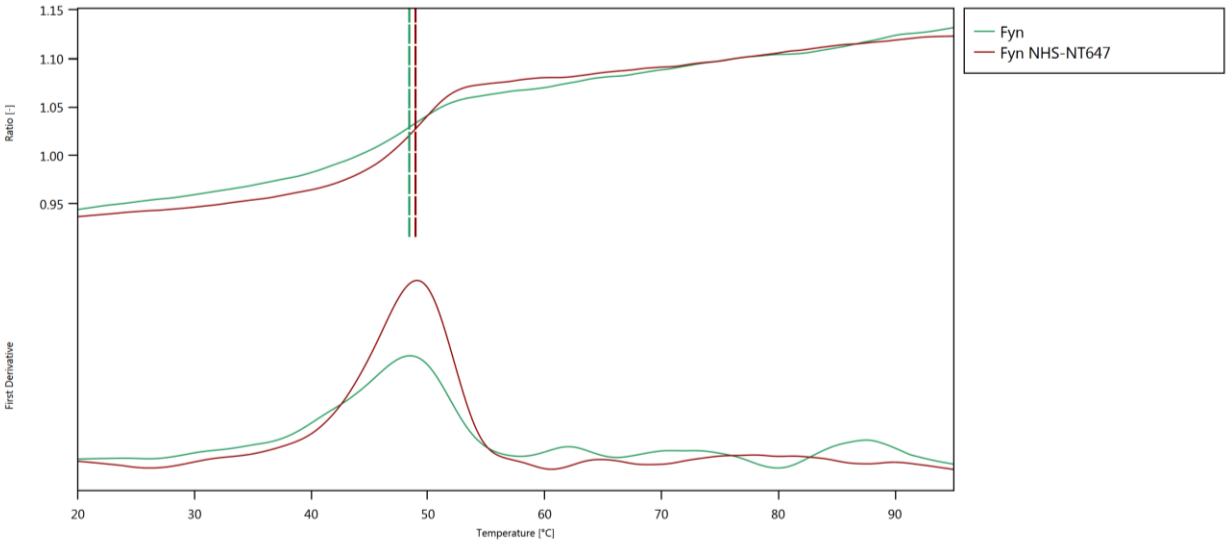
B8. SD-Test

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.

C. Applied Quality Checks

Validation of structural integrity of labeled FYN using Prometheus NT.48:
nanotempertech.com/prometheus

Fyn	10 µL of 1 µM FYN in dilution buffer	T_m = 48.3°C
Fyn NHS-NT647	10 µL of B-Column eluate in dilution buffer (~1 µM)	T_m = 49.0°C



D1. MST System/Capillaries

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

D2. MST Software

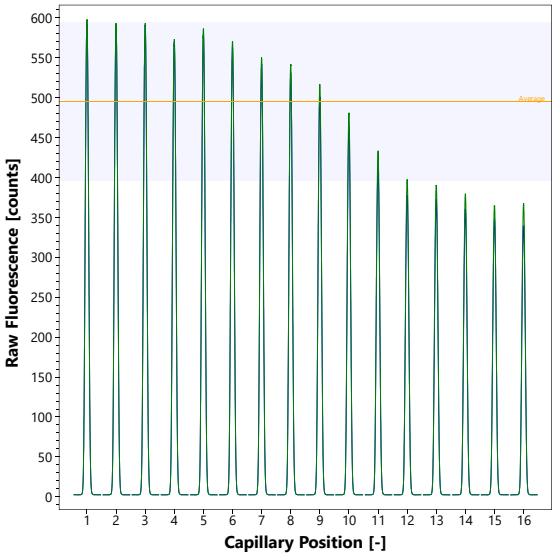
MO.Control v1.6 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

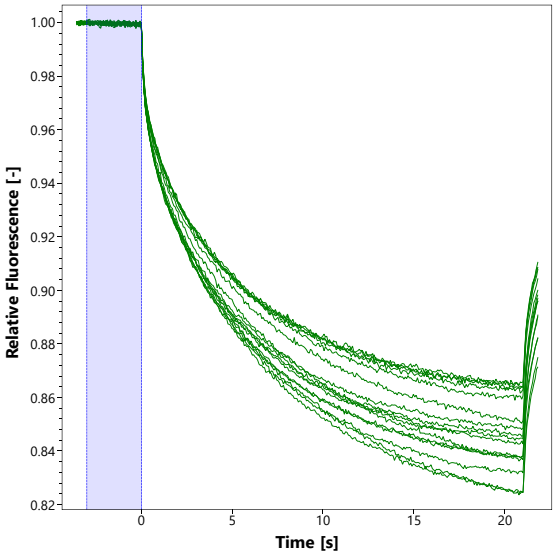
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

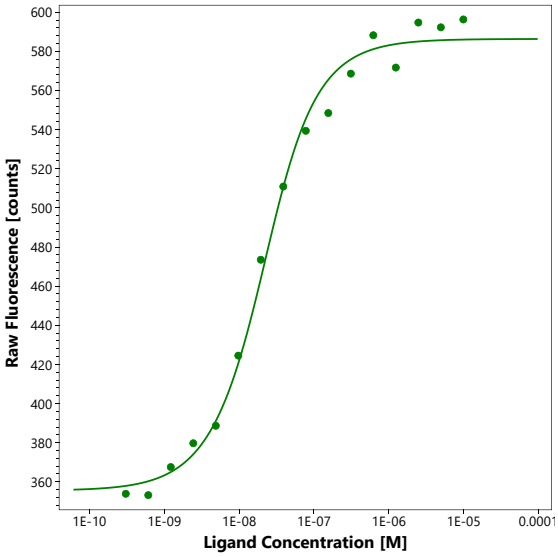
10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2% DMSO, 0.005% TWEEN® 20
15 nM FYN | 10 µM – 0.31 nM Staurosporine | 25°C | medium MST power | 20% excitation power

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

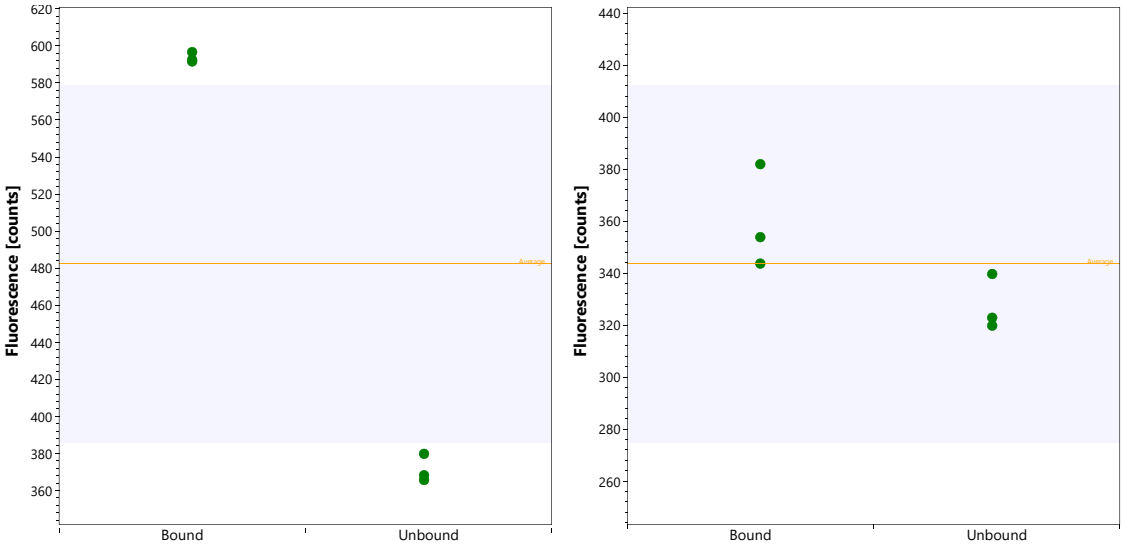
K_d = 14.1 nM







SD-Test:



D5. Reference Results/Supporting Results

N/A

E. Contributors

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