Monolith Protocol MO-P-045

ATP Binding Cassette Transporter – Phosphite

The periplasmic binding proteins from bacterial phosphite ATP-binding cassette transporters are involved in the uptake of phosphorus compounds, which are essential for cell structure, metabolism and signaling. In many natural habitats the availability of inorganic phosphate is low enough to limit microbial growth, and under these phosphate-depleted conditions some bacteria utilize phosphite as an alternative source of phosphorus.

protein – ion interaction | ABC transporters | phosphite | His_6-tag

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A1. Target/Fluorescent Molecule

ABC transporter periplasmic phosphonate-binding protein (Te_PtxB)
uniprot.org/uniprot/Q11919

A2. Molecule Class/Organism

ATP binding cassette transporter
Trichodesmium erythraeum (strain IMS101)

A3. Sequence/Formula

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MLGLILKKNL FTVLFLACLS LVSCSNSNIQ KSENKANPQK LVVALLPDES AATVIQNNKG LEMYLENKLN
KDIELFVSTD YSSMIEVASK GRLDLAYFGP LSYVLAKTKS NIEPFAALEK DGKNTYQALV IGNAEAGINS
YEKIEGKIMA YGDQASTSSH LIPKSMLKQK QLKAGENYEE VFVGAHDAVA IAVANGKAQA GGLSKPIFTA
LIERGTIDKN KVIIIAESKP FPQYPWTMRS DLDSELKTQI QQAFLELEDK AILKPFKADA FTLVTDQDYD
VVRNLGEVLE LNFEQLNK
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A4. Purification Strategy/Source

Expressed in *E. coli* BL21 (DE3) with N-terminal His_6-tag, purified by immobilized Ni-affinity chromatography and gel filtration chromatography
Produced at the University of Sheffield[1]

A5. Stock Concentration/Stock Buffer

250 µM
25 mM Tris-HCl, pH 7.4, 200 mM NaCl

A6. Molecular Weight/Extinction Coefficient

33 kDa
20,400 $M^{-1}cm^{-1}$ (ε_{280})

[1] See Bisson et al., Nature Communications 2017, 8(1), 1–12 for full details.

A7. Dilution Buffer

50 mM HEPES, pH 7.4, 250 mM NaCl, 0.05% TWEEN® 20

A8. Labeling Strategy

Monolith His-Tag Labeling Kit RED-tris-NTA (MO-L008, NanoTemper Technologies GmbH)
1* 125 pmol RED-tris-NTA Dye

A9. Labeling Procedure

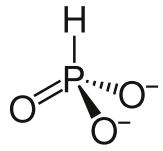
1. Suspend 125 pmol RED-tris-NTA Dye in 25 µL of dilution buffer to obtain a 5 µM dye solution.
2. Prepare a 100 nM dye solution by mixing 2 µL of dye (5 µM) and 98 µL of dilution buffer.
3. Adjust the concentration of Te_PtxB to 200 nM in dilution buffer in a volume of 100 µL.
4. Mix 100 µL of Te_PtxB protein (200 nM) with 100 µL of dye (100 nM).
5. Incubate for 30 minutes at room temperature in the dark.
6. Centrifuge the labeled Te_PtxB for 10 min at 4°C and 15,000 × g.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Phosphite (as sodium phosphite dibasic pentahydrate)



B2. Molecule Class/Organism

Inorganic compound

B3. Sequence/Formula

$Na_2HPO_3 \cdot 5H_2O$

B4. Purification Strategy/Source

Sigma-Aldrich, UK
04283

B5. Stock Concentration/Stock Buffer

Powdered

B6. Molecular Weight/Extinction Coefficient

216.04 Da

B7. Serial Dilution Preparation

1. Dissolve 5.4 g of sodium phosphite dibasic pentahydrate in 20 mL of ddH_2O, titrate the phosphite to pH 7.4 and make up to 25 mL in a volumetric flask to obtain a 1 M sodium phosphite solution.
2. Prepare 30 µL of an 80 µM solution of phosphite in dilution buffer.
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 80 µM phosphite solution into tube **1**. Then, transfer 10 µL of dilution buffer tubes **2** to **16**.
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 **16** to get an equal volume of 10 µL for all samples.
5. Add 10 µL of 100 nM labeled Te_PtxB to each tube from **16** to **1** and mix by pipetting.
6. Incubate for 15 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

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

D2. MST Software

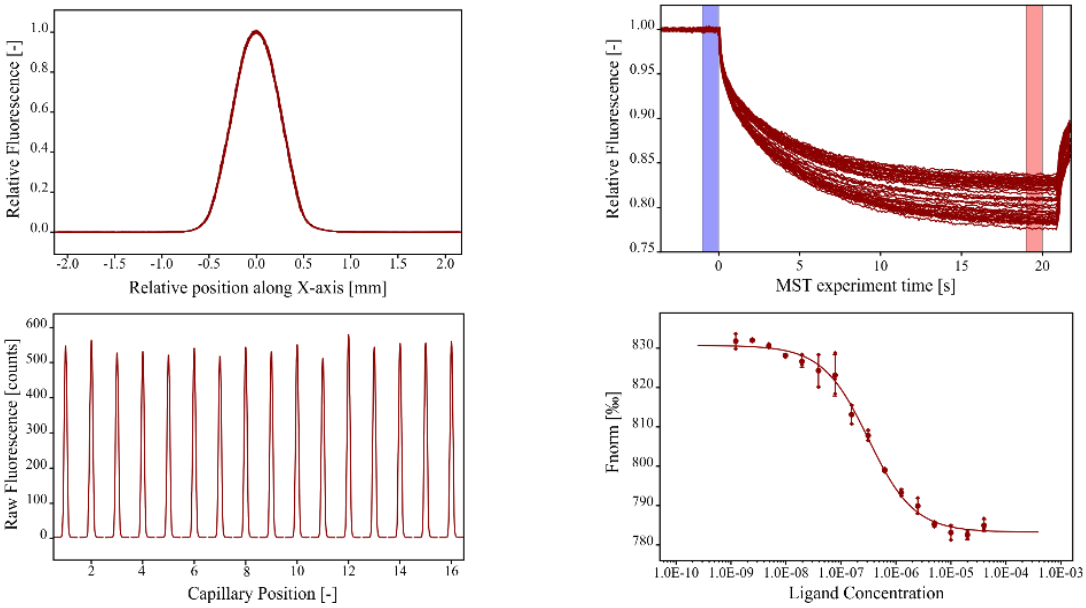
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

50 mM HEPES, pH 7.4, 250 mM NaCl, 0.05% TWEEN® 20
50 nM Te_PtxB | 40 µM – 1.2 nM phosphite | 22°C | medium MST power | 20% excitation power

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

K_d = 170 ± 70 nM Bisson et al., Nature Communications 2017, 8(1), 1–12



D5. Reference Results/Supporting Results

K_d = 289 ± 64 nM Isothermal Titration Calorimetry (ITC)
Bisson et al., Nature Communications 2017, 8(1), 1–12

E. Contributors

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