Monolith Protocol MO-P-070

WSC3 – Laminarin

The ability for plant-root associated fungi to colonize their hosts depends on e.g. their capacity to remodel the cell surface to resist stress and to limit plant immune recognition. The fungal cell wall is the first cellular structure that is exposed to the plant host. WSC3 is a lectin-like protein involved in β-glucan remodeling at the fungal cell wall in the root endophyte *Serendipita indica*. It is an integral fungal cell wall component that binds long-chain β1-3 glucan. In this study the binding between the WSC3 protein and laminarin (β1-3-glucan) was analyzed.

protein – glycan | plants | fungal lectins

A1. Target/Fluorescent Molecule

WSC3
uniprot.org/uniprot/G4TKP7

A2. Molecule Class/Organism

Lectin / plant immune suppressor
Serendipita indica (strain DSM 11827) (Root endophyte fungus)

A3. Sequence/Formula

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MLSLNLLAVA LVGAASSVLA TPALVNRAVT PVNKPSIPAG PGKTYFYRGC YDELKGINHT GGKNALNHDI TSSLPSVTLE
SCVAGCAAKN YKLAGVMNGK TCACDNAISS KAPKLDDKEC SLPCTGNAGE ICGGVWHYST YYTNKSTPLP VPTSPATVGK
GPDSYVHLGC FVDRVQYRTL GGASFSSANM TPTACTKFCR DQKYRLAGVE HGNECWCGVH LVSPELASPV LSTSSDCAQA
CSGDSTSVCG DDDRINVYGA VDDPNVLDPA ILVGTNLLNQ YLSVGCYSDS YEKRLLDGYS FEADDMTATK CAVNCFSKDY
KYAGLELGKQ CFCGNTVDDS QEVDGSKCDT PCAGDRAHSC GGGLRVDLYK SLLLSIF
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A4. Purification Strategy/Source

*Si*WSC3 was recombinantly expressed and purified as previously described[1]. Briefly, the *SiWSC3* gene was cloned into the vector pPIC9 for expression in the yeast *Pichia pastoris* (Invitrogen, Karlsruhe, Germany). For selection of transformed *P. pastoris* cells the vector contained the *HIS4* gene enabling growth on a histidine-deficient medium. For protein expression the *Si*WSC3-His-expressing transformant was grown for 3 days at 28°C, pelleted by centrifugation, resuspended in new medium and grown for 24 h at 28°C with 220 rpm of shaking. The cell free supernatant was pre-purified and then purified on a column with Ni-NTA sepharose, eluted with 300 mM imidazole, followed by dialysis against water over night.

A5. Stock Concentration/Stock Buffer

N/A

[1]See Wawra et al., New Phytol. 222(3): 1493-1506 (2019) for more information.

A6. Molecular Weight/Extinction Coefficient

39 kDa
37,820 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

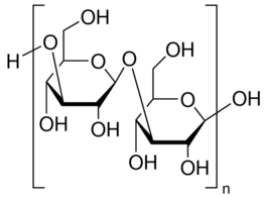
37 mM MES, pH 5, 75 mM NaCl, 0.05% TWEEN® 20

A8. Labeling Strategy

Fluorescent labeling of WSC3 using the Biotium CF594 succinimidyl ester protein labeling kit according to the manufacturer's protocol (Biotium Inc.).
#92216

B1. Ligand/Non-Fluorescent Binding Partner

Laminarin (soluble β1-3-glucan with β1-6-linkages consisting of ~30 glucose units)



B2. Molecule Class/Organism

β1-3-glucan
Laminaria digitata

B3. Sequence/Formula

N/A

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
L9634

B5. Stock Concentration/Stock Buffer

25 mg/mL | 5 mM

B6. Molecular Weight/Extinction Coefficient

N/A

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 5 mM laminarin solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
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 4 µL of labeled p38-α (~2 µM) with 196 µL of dilution buffer to obtain 200 µL of ~40 nM p38-α.
4. Add 10 µL of 1 µM labeled WSC3 to each tube from **16** to **1** and mix by pipetting.
5. Load capillaries immediately.

D1. MST System/Capillaries

Monolith NT.115 Green (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

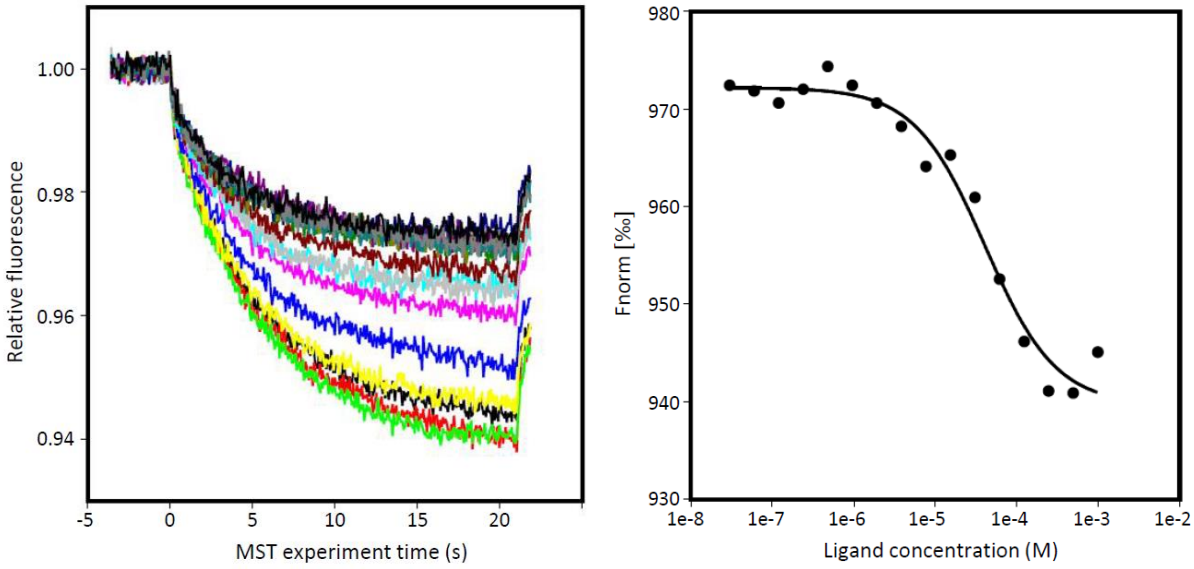
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

37 mM MES, pH 5, 75 mM NaCl, 0.05% TWEEN® 20
500 nM WSC3 | 2.5 mM – 38 nM laminarin | 22°C | high MST power | 60% excitation power

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

K_d = 20.2 ± 10.3 µM Wawra et al., New Phytol. 222(3): 1493-1506 (2019)



D5. Reference Results/Supporting Results

K_d = 12.5 ± 8.8 µM Isothermal Titration Calorimetry (ITC)
Wawra et al., New Phytol. 222(3): 1493-1506 (2019)

E. Contributors

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