Monolith Protocol MO-P-058

Beta-Amylase – Serpin-Z4

Both, amylases and serpins, are highly abundant enzymes in all kingdoms of life. Beta-Amylase is a glycohydrolase that is crucial for seed germination by breaking down starch into smaller carbohydrates that serve as an energy source. Serpins are known to inhibit various serine proteases, but have recently been shown to possess non-inhibitory functions as well.

protein – protein interaction | amylase | serpin | plant proteins | germination

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A1. Target/Fluorescent Molecule

Beta-Amylase
uniprot.org/uniprot/P82993

A2. Molecule Class/Organism

Amylases
Barley (*Hordeum vulgare cv. Harrington*)

A3. Sequence/Formula

```
MEVNVKGNYV QVYVMLPLDA VSVNNRFEKG DELRAQLRKL VEAGVDGVMV DVWWGLVEGK GPKAYDWSAY KQLFELVQKA
GLKLQAIMSF HQCGGNVGDA VNIPIPQWVR DVGTRDPDIF YTDGHGTRNI EYLTLGVDNQ PLFHGRSAVQ MYADYMTSFR
ENMKEFLDAG VIVDIEVGLG PAGEMRYPSY PQSHGWSFPG IGEFICYDKY LQADFKAAAA AVGHPEWEFP NDAGQYNDTP
ERTQFFRDNG TYLTEKGRFF LAWYSNNLIK HGDRILDEAN KVFLGYKVQL AIKISGIHWW YKVPSHAAEL TAGYYNLHDR
DGYRTIARML KRHRASINFT CAEMRDSEQS SQAMSAPEEL VQQVLSAGWR EGLNVACENA LPRYDPTAYN TILRNARPHG
INQSGPPEHK LFGFTYLRLS NQLVEGQNYV NFKTFVDRMH ANLPRDPYVD PMAPLPRSGP EISIEMILQA AKPKLQPFPF
QEHTDLPVGP TGGMGGQAEG PTCGMGGQVK GPTGGMGGQA EDPTSGMGGE LPATM
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A4. Purification Strategy/Source

Expressed as C-terminal His-tag fusion in *Escherichia coli* SHuffle cells
Purification via Ni-NTA affinity chromatography and gel filtration

A5. Stock Concentration/Stock Buffer

2.95 mg/mL | 32 µM
Phosphate-buffered saline (PBS, pH 7.4), 2 mM DTT, 5% glycerol

A6. Molecular Weight/Extinction Coefficient

59.6 kDa
92,250 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate-buffered saline (PBS, pH 7.4), 0.1% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* NT-647-NHS (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 69 µL of Labeling Buffer NHS to 31 µL of 32 µM Beta-Amylase to obtain 100 µL of a 10 µM solution.
2. Add 30 µL of DMSO to 10 µg NT-647-NHS to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 6.4 µL of the 470 µM dye solution with 93.6 µL of Labeling Buffer NHS to obtain 100 µL of a 30 µM dye solution (3x protein concentration).
4. Mix Beta-Amylase and dye in a 1:1 volume ratio (200 µL final volume, 3.2% 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 400 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 Beta-Amylase (~2 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Serpin-Z4
uniprot.org/uniprot/P06293

B2. Molecule Class/Organism

Serpins
Barley (*Hordeum vulgare cv. Harrington*)

B3. Sequence/Formula

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MATTLATDVR LSIAHQTRFA LRLRSAISSN PERAAGNVAF SPLSLHVALS LITAGAAATR DQLVAILGDG GAGDAKELNA
LAEQVVQFVL ANESSTGGPR IAFANGIFVD ASLSLKPSFE ELAVCQYKAK TQSVDFQHKT LEAVGQVNSW VEQVTTGLIK
QILPPGSVDN TTKLILGNAL YFKGAWDQKF DESNTKCDSF HLLDGSSIQT QFMSSTKKQY ISSSDNLKVL KLPYAKGHDK
RQFSMYILLP GAQDGLWSLA KRLSTEPEFI ENHIPKQTVE VGRFQLPKFK ISYQFEASSL LRALGLQLPF SEEADLSEMV
DSSQGLEISH VFHKSFVEVN EEGTEAGAAT VAMGVAMSMP LKVDLVDFVA NHPFLFLIRE DIAGVVVFVG HVTNPLISA
```

B4. Purification Strategy/Source

Expressed as C-terminal His-tag fusion in *Escherichia coli* SHuffle cells
Purification via Ni-NTA affinity chromatography and gel filtration

B5. Stock Concentration/Stock Buffer

2.06 mg/mL | 80 µM
Phosphate-buffered saline (PBS, pH 7.4), 2 mM DTT, 5 % glycerol

B6. Molecular Weight/Extinction Coefficient

42.3 kDa
25,440 $M^{-1}cm^{-1}$ (ε_{280})

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 80 µM Serpin-Z4 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 7 µL of labeled Beta-Amylase with 193 µL of dilution buffer to obtain 200 µL of ~70 nM Beta-Amylase.
4. Add 10 µL of 70 nM labeled Beta-Amylase to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

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

D2. MST Software

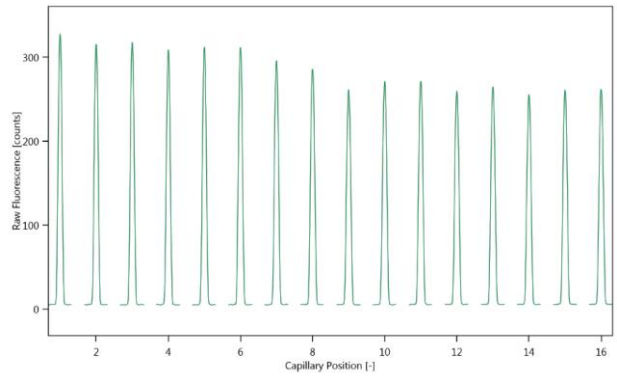
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

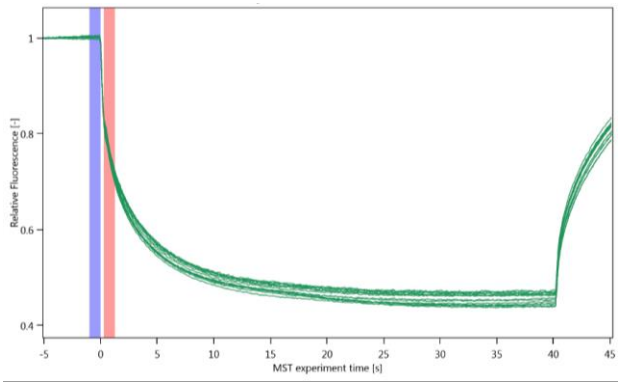
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

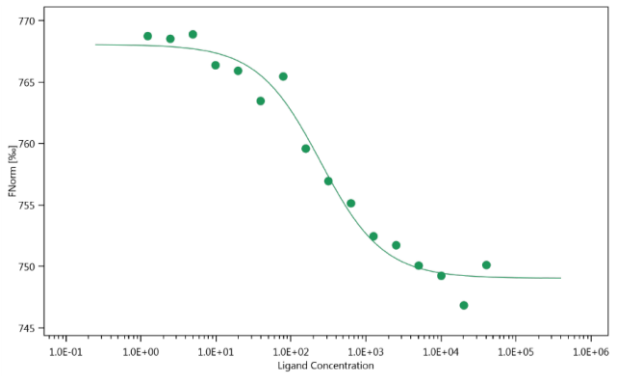
Phosphate-buffered saline (PBS, pH 7.4), 0.1% TWEEN® 20
35 nM Beta-Amylase | 40.5 µM – 1.24 nM Serpin-Z4 | 25°C | low MST Power | 50% excitation power

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

K_d = 215 ± 14 nM







D5. Reference Results/Supporting Results

K_d = 105 nM	MicroScale Thermophoresis (MST) – inverted assay
K_d = 316 nM	Surface Plasmon Resonance (SPR)
K_d = 142 nM	Surface Plasmon Resonance (SPR) – inverted assay
	Cohen and Fluhr, Plant Direct (2018) 1–12

E. Contributors

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