Monolith Protocol MO-P-047

Vitronectin – Hic2

Streptococcus pneumoniae is a respiratory tract pathogen that can cause mucosal and respiratory diseases such as sinusitis, otitis media and pneumonia, but also life-threatening diseases such as sepsis and meningitis. This Gram-positive bacterium has evolved several sophisticated mechanisms to evade the human innate immune system. One strategy is to bind human complement regulators to inhibit the complement system directly on the surface of the pathogen. The surface exposed protein Hic2 binds the complement inhibitor vitronectin and thereby uses its function to block the complement attack.

protein – protein interaction | complement evasion

A1. Target/Fluorescent Molecule

Vitronectin
uniprot.org/uniprot/D9ZGG2

A2. Molecule Class/Organism

Human complement regulators
Homo sapiens (Human)

A3. Sequence/Formula

```
MAPLRPLLIL ALLAWVALAD QESCKGRCTE GFNVDKKCQC DELCSYYQSC CTDYTAECKP QVTRGDVFTM PEDEYTVYDD
GEEKNNATVH EQVGGPSLTS DLQAQSKGNP EQTPVLKPEE EAPAPEVGAS KPEGIDSRPE TLHPGRPQPP AEEELCSGKP
FDAFTDLKNG SLFAFRGQYC YELDEKAVRP GYPKLIRDVW GIEGPIDAAF TRINCQGKTY LFKGSQYWRF EDGVLDPDYP
RNISDGFDGI PDNVDAALAL PAHSYSGRER VYFFKGKQYW EYQFQHQPSQ EECEGSSLSA VFEHFAMMQR DSWEDIFELL
FWGRTSAGTR QPQFISRDWH GVPGQVDAAM AGRIYISGMA PRPSLAKKQR FRHRNRKGYR SQRGHSRGRN QNSRRPSRAT
WLSLFSSEES NLGANNYDDY RMDWLVPATC EPIQSVFFFS GDKYYRVNLR TRRVDTVDPP YPRSIAQYWL GCPAPGHL
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A4. Purification Strategy/Source

Purified from human plasma (see manufacturer's website)
Sigma-Aldrich GmbH
V8379

A5. Stock Concentration/Stock Buffer

Lyophilized powder

A6. Molecular Weight/Extinction Coefficient

75 kDa

A7. Dilution Buffer

50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20

A8. Labeling Strategy

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

A9. Labeling Procedure

1. Resuspend 100 µg of vitronectin in 10 µL of ddH_2O to obtain a 13.3 µM vitronectin solution.
2. 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.
3. Mix 8.5 µL of the 470 µM dye solution with 91.5 µL of Labeling Buffer NHS to obtain 100 µL of a 40 µM dye solution (3x protein concentration).
4. Mix vitronectin and dye in a 1:1 volume ratio (200 µL final volume, 4.25% 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 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 vitronectin (~2.2 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Hic2 (N-terminal fragment of *S. pneumoniae* Factor H-binding inhibitor of complement surface protein PspC (PspC11.4) without the signal sequence)
uniprot.org/uniprot/Q9F888

B2. Molecule Class/Organism

Bacterial outer membrane protein/adhesin
Streptococcus pneumoniae

B3. Sequence/Formula

```
TEKEVTTQVA TSSNKANKSQ TEHMKAAKQV DEYIEKMLSE IQLDRRKHTQ NVGLLTKLGA IKTEYLRGLS VSKEKSTAEL
PSEIKEKLTA AFEQFKKDTL KSGKKVAEAQ KKAKDQKEAK QEIEALIVKH KGREIDLDRK KAKAAVTEHL KKLLNDIEKN
LKKEQHTHTV ELIKNLKDIE KTYLHKLDES TQKAQLQKLI AESQSKLDEA FSKFKNGLSS SSNSGSSTK
```

B4. Purification Strategy/Source

The S. pneumoniae A66 DNA sequence coding for Hic2 was PCR amplified using specific primers and chromosomal DNA as a template. The PCR product was cloned after *Nhe*I/*Hind*III (NEB) digestion into the similar digested pTP1 vector resulting in the pHic2 plasmid. For protein production the plasmid was transformed into *E. coli* BL21(DE3) and expression of the recombinant N-terminally His_6-tagged protein was induced with 1 mM IPTG. The His_6-tagged Hic2 was purified by Ni^{2+} affinity chromatography with the Protino Ni-TED prepacked column kit according to manufacturer's instructions (Machery-Nagel, Dueren, Germany). The protein was dialyzed against appropriate buffer before the MST experiments. The purity of the expressed protein was controlled on SDS-PAGE with silver staining.

B5. Stock Concentration/Stock Buffer

2.1 mg/mL | 80 µM
50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20

B6. Molecular Weight/Extinction Coefficient

26 kDa

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Add 20 µL of a 80 µM Hic2 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 2 µL of labeled vitronectin (~2.2 µM) with 198 µL of dilution buffer to obtain 200 µL of a ~22 nM vitronectin solution.
4. Add 10 µL of labeled vitronectin (~22 nM) 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 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

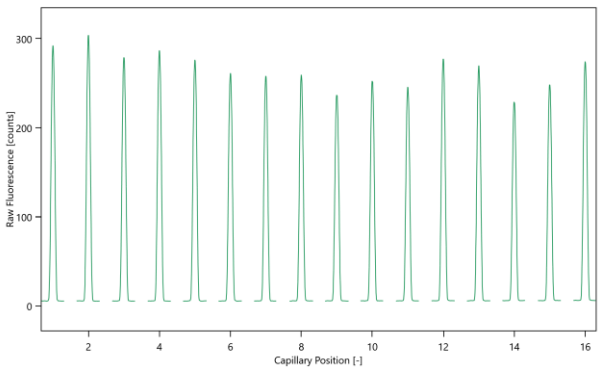
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

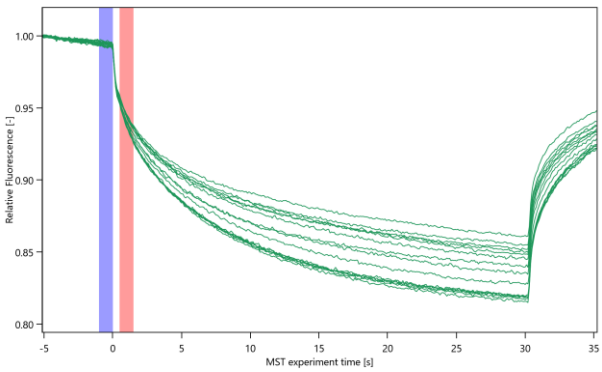
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

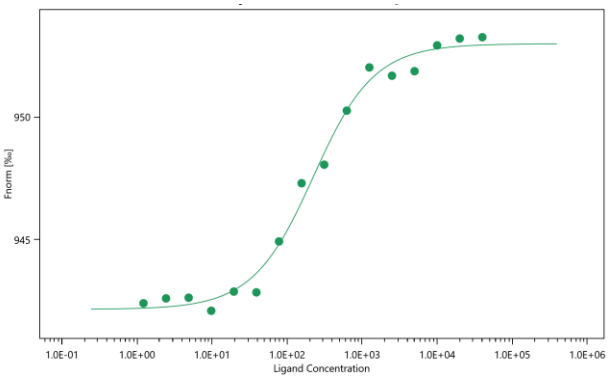
50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20
11 nM vitronectin | 40 µM – 1.2 nM Hic2 | 22°C | medium MST power | 60% excitation power

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

K_d = 220 nM Kohler et al., Thromb. Haem. 113(1), 125-142 (2015)







D5. Reference Results/Supporting Results

K_d = 1.51 µM Surface Plasmon Resonance (SPR)
Kohler et al., Thromb. Haem. 113(1), 125-142 (2015)

E. Contributors

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