Monolith Protocol MO-P-049

B. burgdorferi CspA – C9

Borrelia burgdorferi is a Gram-negative bacterium that causes Lyme disease. These spirochetes are spread by ticks and if the infection is left untreated, severe and chronic symptoms develop, including neuroborreliosis, carditis and arthritis. To be able to survive in the human host, this bacterium has evolved several sophisticated mechanisms to evade the human innate immune system. One strategy is to bind complement proteins and thus inhibit the complement cascade directly. CspA is a surface exposed protein that binds the complement protein C9 and thereby blocks the complement attack and contributes to bacterial survival in the human host.

protein – protein interaction | complement evasion

A1. Target/Fluorescent Molecule

CspA
uniprot.org/uniprot/A0A0H3BZN5

A2. Molecule Class/Organism

Bacterial surface protein/immune evasion protein
Borrelia burgdorferi

A3. Sequence/Formula

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MTKAKLNIIK LNIIAMILTL ICTSCAPFSK IDPKANANTK PKKITNPGEN TQNFEDKSGD LSASDEKIME TIASELKAIG
KELEDQKKEE NIQIAKIAKE KFDFLSTFKV GPYDLIDEDI QMKIKRTLYS SLDYKKENIE KLKEILEILK KNSKHYNIIG
RLIYHISWSI QFQIEQNLEL IQNGVENLSQ EESKSLLMQI KSNLEIKQRL KKTLNETLKV YNQNTQDNEK ILAEHFNKYY
KDFDTLKPAF Y
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A4. Purification Strategy/Source

Construction and expression of plasmids harboring the *cspA* gene encoding for CspA and purification of the proteins were previously described.[1]

A5. Stock Concentration/Stock Buffer

8.3 mg/mL | 320 µM

A6. Molecular Weight/Extinction Coefficient

25.9 kDa

[1] Kraiczy et al., J Biol Chem 279 (2004) 2421-2429

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. Add 96.9 µL of Labeling Buffer NHS to 3.1 µL of 320 µM CspA to obtain 100 µL of a 10 µM 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 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 CspA 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 6 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 CspA (~1.6 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Complement component 9 (C9)
uniprot.org/uniprot/P02748

B2. Molecule Class/Organism

Human complement protein
Homo sapiens (Human)

B3. Sequence/Formula

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MSACRSFAVA ICILEISILT AQYTTSYDPE LTESSGSASH IDCRMSPWSE WSQCDPCLRQ MFRSRSIEVF GQFNGKRCTD
AVGDRRQCVP TEPCEDAEDD CGNDFQCSTG RCIKMRLRCN GDNDCGDFSD EDDCESEPRP PCRDRVVEES ELARTAGYGI
NILGMDPLST PFDNEFYNGL CNRDRDGNTL TYYRRPWNVA SLIYETKGEK NFRTEHYEEQ IEAFKSIIQE KTSNFNAAIS
LKFTPTETNK AEQCCEETAS SISLHGKGSF RFSYSKNETY QLFLSYSSKK EKMFLHVKGE IHLGRFVMRN RDVVLTTTFV
DDIKALPTTY EKGEYFAFLE TYGTHYSSSG SLGGLYELIY VLDKASMKRK GVELKDIKRC LGYHLDVSLA FSEISVGAEF
NKDDCVKRGE GRAVNITSEN LIDDVVSLIR GGTRKYAFEL KEKLLRGTVI DVTDFVNWAS SINDAPVLIS QKLSPIYNLV
PVKMKNAHLK KQNLERAIED YINEFSVRKC HTCQNGGTVI LMDGKCLCAC PFKFEGIACE ISKQKISEGL PALEFPNEK
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B4. Purification Strategy/Source

Purified from human serum (see manufacturer website)
Complement Technology
A126

B5. Stock Concentration/Stock Buffer

1.1 mg/mL | 15 µM
10 mM sodium phosphate, pH 7.3, 145 mM NaCl

B6. Molecular Weight/Extinction Coefficient

71 kDa

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 15 µM C9 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 CspA with 196 µL of dilution buffer to obtain 200 µL of ~32 nM CspA.
4. Add 10 µL of labeled CspA (~32 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

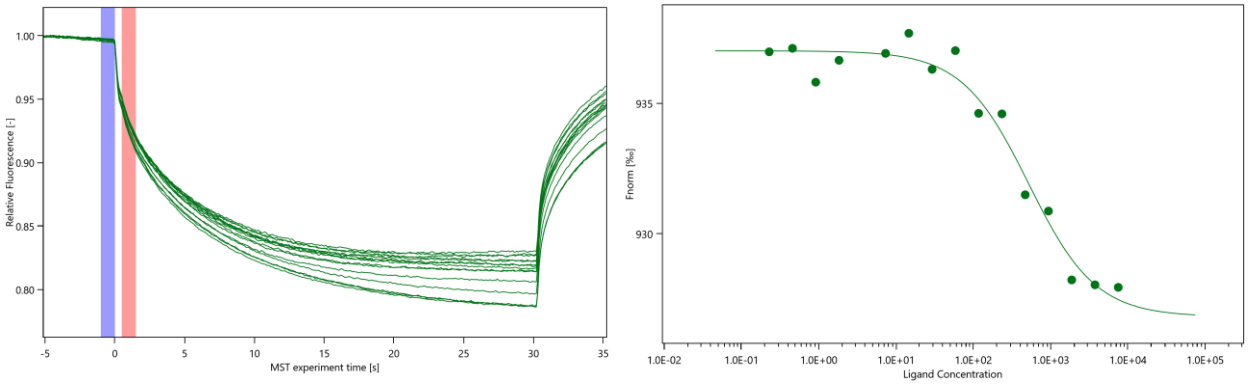
MO.Affinity Analysis v2.3 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith/#monolith-software

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
16 nM CspA | 7.5 µM – 229 pM C9 | 25°C | medium MST power | 50% excitation power

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

K_d = 559 nM



D5. Reference Results/Supporting Results

N/A

E. Contributors

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