Monolith Protocol MO-P-063

Factor H – Hic (surface of intact bacteria)

Streptococcus pneumoniae is a Gram-positive bacterium that 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 Hic binds the human complement inhibitor Factor H and thereby uses its function to block the complement attack. Here, we use Hic heterologously expressed on the surface of *Lactococcus lactis* (intact bacteria) to analyze the binding of the human complement regulator Factor H. The plasmid-encoded Hic protein is anchored to the peptidoglycan via Sortase A on the surface of the bacterial strain and this system is used as a tool to analyze the function of the protein of interest on an avirulent bacterial strain lacking all other *S. pneumoniae proteins*. *L. lactis* containing an empty vector (not expressing Hic on the surface) is used as a negative control.

protein – protein interaction | whole bacteria

A1. Target/Fluorescent Molecule

Factor H
uniprot.org/uniprot/P08603

A2. Molecule Class/Organism

Complement protein
Homo sapiens (Human)

A3. Sequence/Formula

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MRLLAKIICL MLWAICVAED CNELPPRRNT EILTGSWSDQ TYPEGTQAIY KCRPGYRSLG NVIMVCRKGE WVALNPLRKC
QKRPCGHPGD TPFGTFTLTG GNVFEYGVKA VYTCNEGYQL LGEINYRECD TDGWTNDIPI CEVVKCLPVT APENGKIVSS
AMEPDREYHF GQAVRFVCNS GYKIEGDEEM HCSDDGFWSK EKPKCVEISC KSPDVINGSP ISQKIIYKEN ERFQYKCNMG
YEYSERGDAV CTESGWRPLP SCEEKSCDNP YIPNGDYSPL RIKHRTGDEI TYQCRNGFYP ATRGNTAKCT STGWIPAPRC
TLKPCDYPDI KHGGLYHENM RRPYFPVAVG KYYSYYCDEH FETPSGSYWD HIHCTQDGWS PAVPCLRKCY FPYLENGYNQ
NYGRKFVQGK SIDVACHPGY ALPKAQTTVT CMENGWSPTP RCIRVKTCSK SSIDIENGFI SESQYTYALK EKAKYQCKLG
YVTADGETSG SITCGKDGWS AQPTCIKSCD IPVFMNARTK NDFTWFKLND TLDYECHDGY ESNTGSTTGS IVCGYNGWSD
LPICYERECE LPKIDVHLVP DRKKDQYKVG EVLKFSCKPG FTIVGPNSVQ CYHFGLSPDL PICKEQVQSC GPPPELLNGN
VKEKTKEEYG HSEVVEYYCN PRFLMKGPNK IQCVDGEWTT LPVCIVEEST CGDIPELEHG WAQLSSPPYY YGDSVEFNCS
ESFTMIGHRS ITCIHGVWTQ LPQCVAIDKL KKCKSSNLII LEEHLKNKKE FDHNSNIRYR CRGKEGWIHT VCINGRWDPE
VNCSMAQIQL CPPPPQIPNS HNMTTTLNYR DGEKVSVLCQ ENYLIQEGEE ITCKDGRWQS IPLCVEKIPC SQPPQIEHGT
INSSRSSQES YAHGTKLSYT CEGGFRISEE NETTCYMGKW SSPPQCEGLP CKSPPEISHG VVAHMSDSYQ YGEEVTYKCF
EGFGIDGPAI AKCLGEKWSH PPSCIKTDCL SLPSFENAIP MGEKKDVYKA GEQVTYTCAT YYKMDGASNV TCINSRWTGR
PTCRDTSCVN PPTVQNAYIV SRQMSKYPSG ERVRYQCRSP YEMFGDEEVM CLNGNWTEPP QCKDSTGKCG PPPPIDNGDI
TSFPLSVYAP ASSVEYQCQN LYQLEGNKRI TCRNGQWSEP PKCLHPCVIS REIMENYNIA LRWTAKQKLY SRTGESVEFV
CKRGYRLSSR SHTLRTTCWD GKLEYPTCAK R
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A4. Purification Strategy/Source

Purified from human serum (see manufacturer's website)
Merck
341274

A5. Stock Concentration/Stock Buffer

1.03 mg/mL | 6.6 µM
PBS, pH 7.2

A6. Molecular Weight/Extinction Coefficient

155 kDa

A7. Dilution Buffer

Phosphate-buffered saline (PBS, pH 7.4), 0.005% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 25 µL of DMSO to Dye RED-NHS 2nd Generation (10 µg) to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
2. Mix 3.5 µL of the 600 µM dye solution with 96.5 µL of Labeling Buffer NHS to obtain 100 µL of a 21 µM dye solution (~3x protein concentration).
3. Mix 100 µl Factor H (6.6 µM)and dye in a 1:1 volume ratio (200 µL final volume, 1.8% final DMSO concentration).
4. Incubate for 30 minutes at room temperature in the dark.
5. 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.
6. 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.
7. Add 200 µL of the labeling reaction from step 3 to the center of the column and let sample enter the resin bed completely.
8. Add 300 µL of dilution buffer after the sample has entered and discard the flow through.
9. Place column in a new collection tube, add 600 µL of dilution buffer and collect the eluate.
10. Keep the labeled Factor H (~1 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Hic (expressed on the surface of intact *Lactococcus lactis*)
uniprot.org/uniprot/Q9F888

B2. Molecule Class/Organism

Factor H binding surface protein (pspC11.4)
Streptococcus pneumoniae

Expression strain: *Lactococcus lactis*

B3. Sequence/Formula

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MFASKNERKV HYSIRKFSIG VASVAVASLF MGSVVHATEK EVTTQVATSS NKANKSQTEH MKAAKQVDEY IEKMLSEIQL
DRRKHTQNVG LLTKLGAIKT EYLRGLSVSK EKSTAELPSE IKEKLTAAFE QFKKDTLKSG KKVAEAQKKA KDQKEAKQEI
EALIVKHKGR EIDLDRKKAK AAVTEHLKKL LNDIEKNLKK EQHTHTVELI KNLKDIEKTY LHKLDESTQK AQLQKLIAES
QSKLDEAFSK FKNGLSSSSN SGSSTKPETP QPETPKPEVK PELETPKPEV KPEPETPKPE VKPEPETPKP EVKPELETPK
PEVKPEPETP KPEVKPEPET PKPEVKPEPE TPKPEVKPEL ETPKPEVKPE LETPKPEVKP EPETPKPEVK PELETPKPEV
KPEPETPKPE VKPELETPKP EVKPEPETPK PEVKPELETP KPEVKPEPET PKPEVKPEPE TPKPEVKPEP ETPKPEVKPE
LETPKPEVKP ELETPKPEVK PEPETPKPEV KPELETPKPE VKPELEIPKP EVKPDNSKPQ ADDKKPSTPN NLSKDKQSSN
QASTNENKKQ GPATNKPKKS LPSTGSISNL ALEIAGLLTL AGATILAKKR MK
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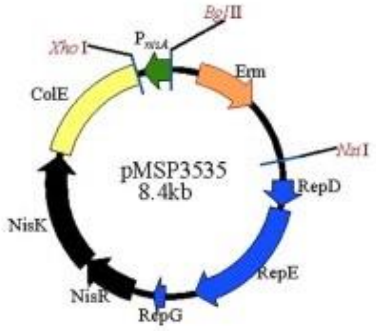
Vector to express Hic on the surface of *Lactococcus lactis*:



MCS pMSP3535:
- BglII - BamHI - SpeI - SmaI - PstI - SphI - XbaI - XhoI -

MCS pMSP3545:
- BglII - NcoI - PstI - SphI - KpnI - SpeI - XbaI - XhoI -

B4. Purification Strategy/Source

Two strains were used in this experiment, LL16 - *Lactococcus lactis* pMSP3545 (negative control containing the empty vector) and LL17 - *Lactococcus lactis* pMSP3545-*hic* (expressing Hic on the surface). The construct was prepared as previously described[1]. The two *L. lactis* strains were grown statically on GM17 agar plates (37,5 g/L M17 Medium (Oxoid), 0.5% Glucose, 5 µg/mL Erythromycin and 1.2% agar) overnight at 30°C. Thereafter, the bacteria were grown statically in 10 mL GM17 broth (37,5 g/L M17 Medium, 0.5% Glucose, 5 µg/mL Erythromycin) overnight at 30°C. Bacteria were added to new tubes with fresh GM17 medium and started growing at an OD_{600} of 0.1. To induce expression of Hic on the surface, the bacteria were grown to mid-log phase (OD_{600} of 0.5), followed by 1 hr induction with 1 µg/mL Nisin. After induction, the bacteria were centrifuged (3750 × g, 6 min). The pellets were then resuspended in PBS and set to an OD_{600} of 20. The surface expression of Hic was confirmed by flow cytometry using an anti-His pAb.

B5. Stock Concentration/Stock Buffer

5×10^9 bacteria/mL
PBS

B6. Molecular Weight/Extinction Coefficient

68 kDa

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the bacterial suspension[2] 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 Factor H (~1 µM) with 198 µL of dilution buffer to obtain 200 µL of ~10 nM labeled Factor H.
4. Add 10 µL of ~10 nM labeled Factor H 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

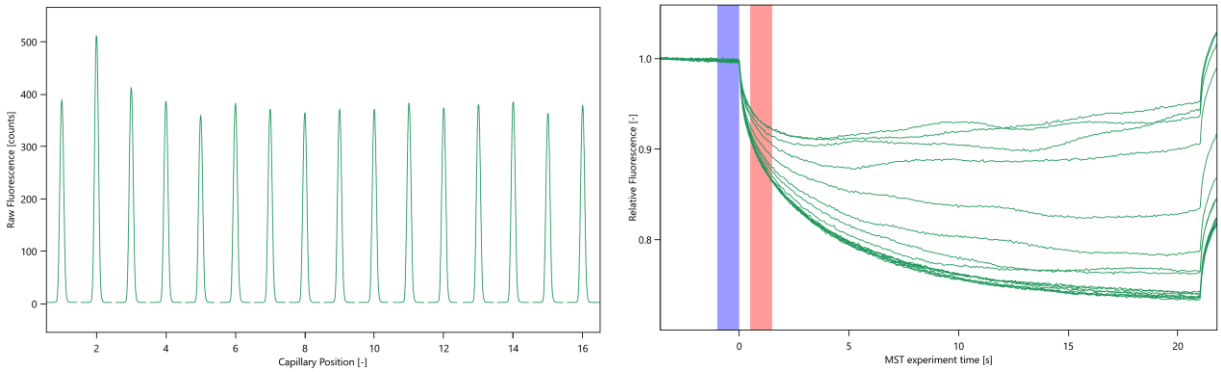
[1] Kohler et al., Thromb Haemost 113(1): 125-142 (2015)

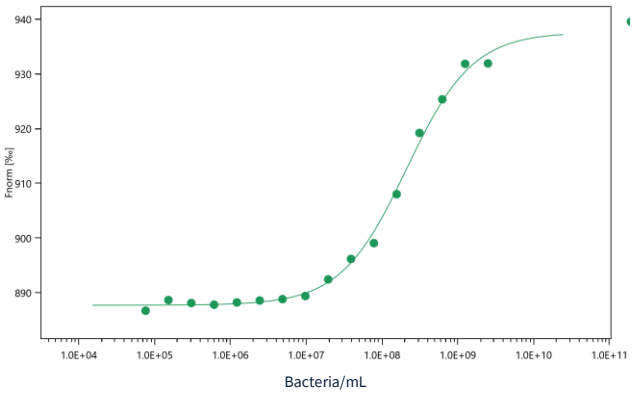
[2] There was no difference in the binding curve using 0.5×10^9/ml compared to 2.5×10^9/ml bacteria as a start concentration.

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

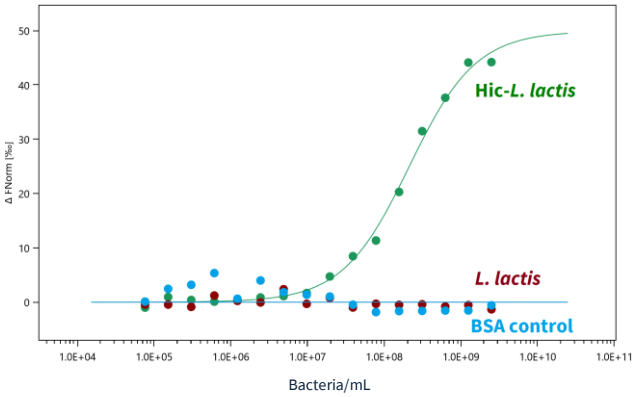
Phosphate-buffered saline (PBS, pH 7.4), 0.005% TWEEN® 20
10 nM Factor H | 2.5 x 10^9 -7.63 x 10^4 bacteria/mL | 22°C | high MST Power | 40% excitation power

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





Negative controls: *L. lactis* lacking Hic on the surface (red curve) and RED-NHS labeled BSA interacting with Hic-*L. lactis* (blue curve)



D5. Reference Results/Supporting Results

K_d = 23 nM Surface Plasmon Resonance (SPR)
Janulczyk et al., J Biol Chem 275(47): 37257-63 (2000)

E. Contributors

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