Characterization of the Ruler Protein Interaction Interface on the Substrate Specificity Switch Protein in the *Yersinia* Type III Secretion System

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Running title: YscP binding interface on YscU

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KEYWORDS:
Yersinia pseudotuberculosis; Type III secretion system (T3SS); nuclear magnetic resonance (NMR); secretion; gram-negative bacteria; protein conformation; YscU; YscP; YscI

SUPPORTING INFORMATION

Supplemental Figure 1: Similarity of YscP and its homologs. **(A)** Sequence alignment of the YscP homologues (FliK and PscP) Sequence alignment of YscP homologues**.** MAFFT sequence alignment (1) of YscP homologues from different T3SS-containing bacteria. The alignment contains YscP from *Yersinia pseudotuberculosis*, YscP* from *Yersinia enterocolitica*, FliK from *Shigella flexneri*, FliK* from *Salmonella typhimurium* and PscP from *Pseudomonas aeruginosa*. The amino acids are numbered according to their positions in YscP from *Yersinia pseudotuberculosis*. Conserved residues are highlighted in red, and similar residues are shown in yellow. (B) The YscP homologues FliK (blue) and PscP (green) have a conserved "ball-and-chain" architecture with a flexible N-terminal segment and a folded C-terminal domain. The structural alignment was created using the DALI server (2) based on the NMR structure of FliK from *Salmonella typhimurium* (PDB code: 2RRL) (3) and the crystal structure of PscP (green) from *Pseudomonas aeruginosa* (PDB code: 5CUK) (4).

A

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                 ....|....10....|....20....|....30....|....40....|....50....|....60....|....70....|....80....|....90....|....100...|....110
YscP     MNKITT--RSPL--EPEYQPLGKPHHALQACVDPEQALLHNNKGNCHPKEESLKPVRPHDL----GKKEGQKGDGLRAHAPLAATSQPGRKEVGLKPQHNHQNNHDFNLSPLAEGATNRA
YscP*    MNKITT--RSPL--EPEYQPLGKLHHDLQARADPEQALLHNNKGNRHPKEEPRRPVRPHDL----GKKEGQKGDGLRAHAPLEATFQPGRKEVGLKPQHNHQNNHDLNLSPLAEGVTNRA
PscP     MLKLNAVDTAPLVSSDTLAPLPPLRAQQIA---PEQAL---------PAHRPPAPRPPFDK----GDETTEAA--ATADAPTST-------------------------PLAD------
FliK     MIRLAPLITADV---DTTT-LPGGKASDAA-QDPLTLLSEALAGE--TTTDKAAPQLL---------VATDKP--TTKGEPLVS-------------------------DILAD------
FliK*    MITLPQLITTDT---DMTAGLTSGKTTGSA-EDPLALLAGALGAD--GAQGKDARITLADLQAAGGKLSKELL--TQHGEPGQA-------------------------VKLAD------

                 ....|....120...|....130...|....140...|....150...|....160...|....170...|....180...|....190...|....200...|....210...|....220...|....
YscP     HLYQQDS---RFDDRVESIINALMPLAPFLEGVTCEIGTSSESPCEPSGHDELFVQQSPIDSAQPVQLNTKPTVQPLNPAADGAEVIVWSVGRETPASIAKNQRDSRQKRLAEEPLALH-
YscP*    HLYQQDS---RFDDRVESIINALMPLAPFLKGVTCEIGTSSESPCEPSGHDELFVQQSPIDSVQPVQLNTKPTVQPLNPAADGAEVIVWSVGRETPASIAKNQRDSRQKRLAEEPLPLH-
PscP     ---QPAA---PAADRPPTIRQPPMPVAADATPTPTPTPTPT---------------PTPTPTPTPT-----PTVSP-----SG------SVARQAPAVSARVAAST----QAREPASV--
FliK     --AQQADLLIPVDETLPVINDEQ---STSTPLTTAQEMTLAAVADKNTTKDE----KADDLNEDVT-----ASLSALFAMLPGFDNT--PKVTDAES--------------TVLPA----
FliK*    LLAQKAN---ATDETLTDLTQAQHLLSTLTP--SLKTSALAALS-KTAQHDE----KTPALSDEDL-----ASLSALFAMLPGQPVAT-PVAGETPAEN-----------HIALESLLRG

                 .230....|....240....|....250....|....260....|....270....|....280....|
YscP     -QKALPEICPPAVSATPDDHLVARWCATPVTEVAEKSARFPYKATVQSEQLDMTELADRSQH-------------------------------------------------------------
YscP*    -QEALPEVCPPAVSATPNDHLVARWCATPVAEVAEKSARFLHKAIVQSEQLDMTELADRSQHLTDGVDSSKDTIELPRPEEPLPLHQEALPEVAEKSARFQHKATVQSEQLDMTELAARS
PscP     --SAPPVDEPPLVPVSSHPQIAGR-----THERPQPGPGFPAKT--------AAEVAPTAQA----------SVQA-SPPAP-----------------------------------------
FliK     --------EKPTLFT-----KLTS--AQLTTAQPDDAPGTPAQP--------LTPLVAEAQS----------KAEVISTPSP-----------------------------------------
FliK*    DMPSAPQEETHTLSFSEHEKGKTE--ASLARASDDRATGPALTP--------LVVAAAATSA----------KVEVDSPPAP-----------------------------------------

                   290....|....300....|....310....|....320....|....330....|....340....|....350....|....360....|....370....|....380....|....390....|....
YscP     --LTDGVDSSKDTIEPPRPEELLLPREETLPEMYSLSFTAEVV-TP-GDHLLATM------RATRLASVSEQLIQLAQRLAVELELRGGSSQVTQLHLNLPELGAIMVRIAEIPGKLHVE
YscP*    QYLTDGVDSSKDTIEPPRPEELLLPREETLLEMYSLSFTAEVV-TP-GDHLLATM------RATRLTSVSEQLIQLAQRLAVELELRGGSSQVTQLHLNLPELGAIMVRIAEIPGKLHVE
PscP     --TAGGEGRGEERRQPGETDPSALEPDDQA------PVPLEAMQTP-GDRLLARLLASSGSRPLPLADLARLLDAVQGRIQVA---SAAESHAARLQVRLPQLGAVEVQVLHGHGQLQIE
FliK     --VT---ADASPLITPHQTQP--LP-----------TVAAEVLSAELGSH--------------------EWQQSLSQHISLF---TRQGQQSAELRLHPQDLGEVQISLKVDDNQAQIQ
FliK*    --VTH--GAAMPTLSSATAQP--LP-----------VASAEVLSAELGSH--------------------EWQQTFSQQVMLF---TRQGQQSAQLRLHPEELGQVHISLKLDDNQAQLQ

                 400....|....410....|....420..  .....  430....|....440....|....450...
YscP     LIASREALRILAQGSYDLLERLQRI---EPTQL--------DTQASDDSE--QESRQKRHVYEEWEAEE----------------------
YscP*    LIASQEALRILAQGSYDLLERLQRI---EPTQL--------DTQASGDSE--QESRQKRHVYEEWEAEE----------------------
PscP     ISASPGSLALLQQARGELLERLQRLHPEQPVQL--------TTNQQQDSG--QRSRQHRYLHEEWQAE----------------------
FliK     MISPHQHVRAALEAALPVL-ETQLA--ESGIQLGQSNISGESFSGQQQAASQQQQSQHTANHEPLAGEDDDTLPVPVSLQGRVTGNSGVDIFA
FliK*    MVSPHSHVRAALEAALPML-ETQLA--ESGIQLGQSSISSESFAGQQQSSS-QQQSSRAQHTDAFGAEDDIALAAPASLQAAARGNGAVDIFA
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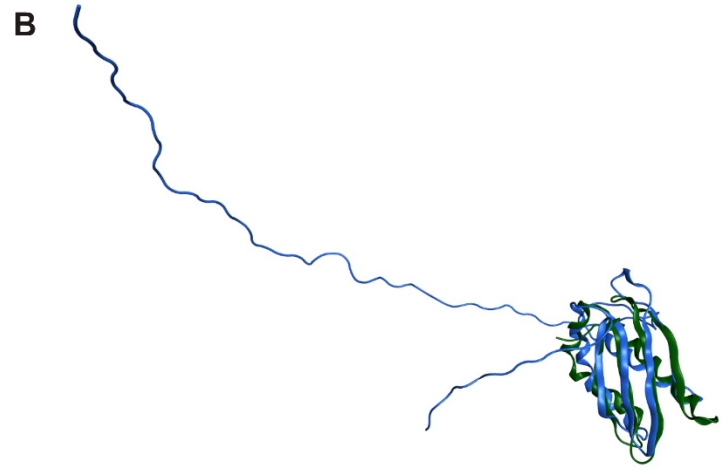
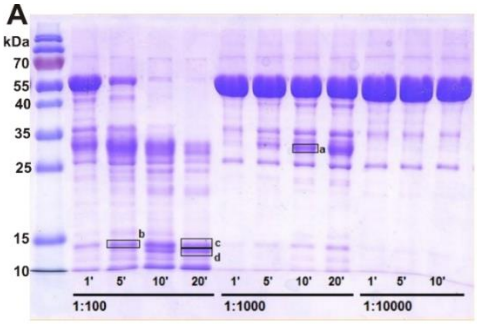
B



Supplemental Figure 2: Limited trypsin proteolysis of YscP. (A) SDS-PAGE of YscP fragments produced by trypsin proteolysis. A 22µM solution of YscP was mixed with trypsin at molar ratios of 1:100, 1:1000 and 1:10000 at 4 °C in 25mM Tris (pH 7.4) containing 150mM NaCl and 2mM DTT. Aliquots were taken at 1, 5, 10 or 20 minutes, and proteolysis was terminated by adding SDS-PAGE loading buffer and heating the samples to 90 °C. The gel fragments cut out for mass spectroscopy are labeled a, b, c and d. (B) LC-MS/MS results for the selected SDS-PAGE bands. The matched peptides are shown in red. (C) Summary of sequence coverage from different bands.

A



C

Sample	Reference spectrum	Mascot Score	Protein precursor mass (Da)	Peptides matched	Sequence coverage (%)
a	Yop proteins translocation protein P OS=*Yersinia pestis*	1091	50388	15	37.1
b	Yop proteins translocation protein P OS=*Yersinia pestis*	587	50388	9	22.6
c	Yop proteins translocation protein P OS=*Yersinia pestis*	624	50388	11	27.3
d	Yop proteins translocation protein P OS=*Yersinia pestis*	644	50388	13	33.8

B

Band a

MNKITTRSPL EPEYQPLGKP HHALQACVDF EQALLHNNKG NCHPKEESLK
PVRPHDLGKK EGQKGDGLRA HAPLAATSQP GRKEVGLKPQ HNHQNNHDFN
LSPLAEGATN RAHLYQQDSR FDDRVESIIN ALMPLAPFLE GVTCETGTSS
ESPCEPSGHD ELFVQQSPID SAQPVQLNSK PTVQPLNPAA DGAEVIVWSV
GRETPASIAK NQRDSRQKRL AEEPLALHQK ALPEICPPAV SATPDDHLVA
RWCATPVTEV AEKSARFPYK ATVQSEQLDM TELADRSQHL TDGVDSSKDT
IEPPRPEKLL LPREETLPEM YSLSFTAPVV TPGDHLLATM RATRLASVSE
QLIQLAQRLA VELELRGGSS QVTQLHLNLP ELGAIMVRIA EIPGKLHVEL
IASREALRIL AQGSYDLLER LQRIEPTQLD FQASDDSEQE SRQKRHVYEE
WEAEE

Band b

MNKITTRSPL EPEYQPLGKP HHALQACVDF EQALLHNNKG NCHPKEESLK
PVRPHDLGKK EGQKGDGLRA HAPLAATSQP GRKEVGLKPQ HNHQNNHDFN
LSPLAEGATN RAHLYQQDSR FDDRVESIIN ALMPLAPFLE GVTCETGTSS
ESPCEPSGHD ELFVQQSPID SAQPVQLNSK PTVQPLNPAA DGAEVIVWSV
GRETPASIAK NQRDSRQKRL AEEPLALHQK ALPEICPPAV SATPDDHLVA
RWCATPVTEV AEKSARFPYK ATVQSEQLDM TELADRSQHL TDGVDSSKDT
IEPPRPEKLL LPREETLPEM YSLSFTAPVV TPGDHLLATM RATRLASVSE
QLIQLAQRLA VELELRGGSS QVTQLHLNLP ELGAIMVRIA EIPGKLHVEL
IASREALRIL AQGSYDLLER LQRIEPTQLD FQASDDSEQE SRQKRHVYEE
WEAEE

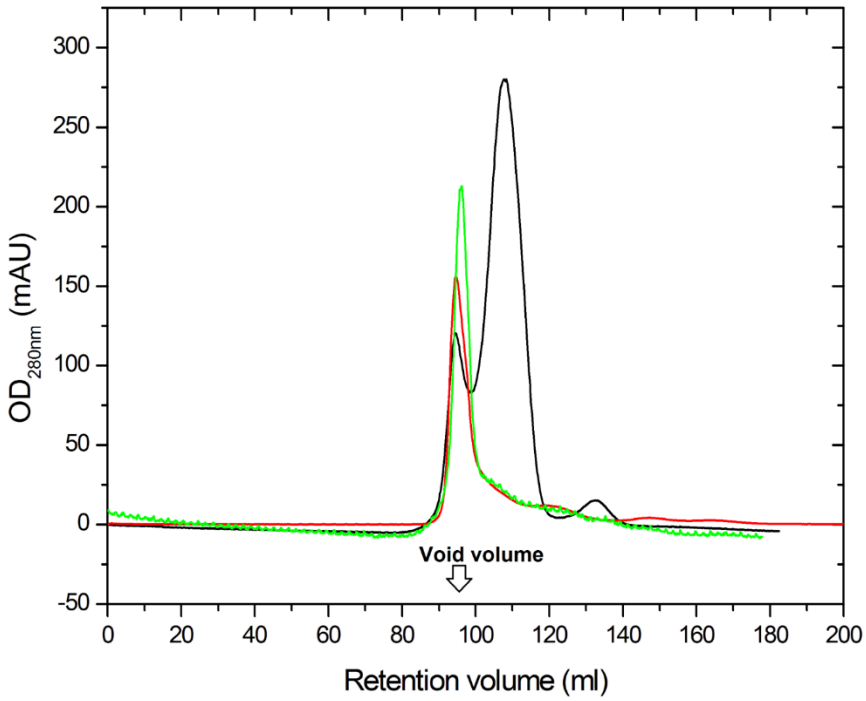
Band c

MNKITTRSPL EPEYQPLGKP HHALQACVDF EQALLHNNKG NCHPKEESLK
PVRPHDLGKK EGQKGDGLRA HAPLAATSQP GRKEVGLKPQ HNHQNNHDFN
LSPLAEGATN RAHLYQQDSR FDDRVESIIN ALMPLAPFLE GVTCETGTSS
ESPCEPSGHD ELFVQQSPID SAQPVQLNSK PTVQPLNPAA DGAEVIVWSV
GRETPASIAK NQRDSRQKRL AEEPLALHQK ALPEICPPAV SATPDDHLVA
RWCATPVTEV AEKSARFPYK ATVQSEQLDM TELADRSQHL TDGVDSSKDT
IEPPRPEKLL LPREETLPEM YSLSFTAPVV TPGDHLLATM RATRLASVSE
QLIQLAQRLA VELELRGGSS QVTQLHLNLP ELGAIMVRIA EIPGKLHVEL
IASREALRIL AQGSYDLLER LQRIEPTQLD FQASDDSEQE SRQKRHVYEE
WEAEE

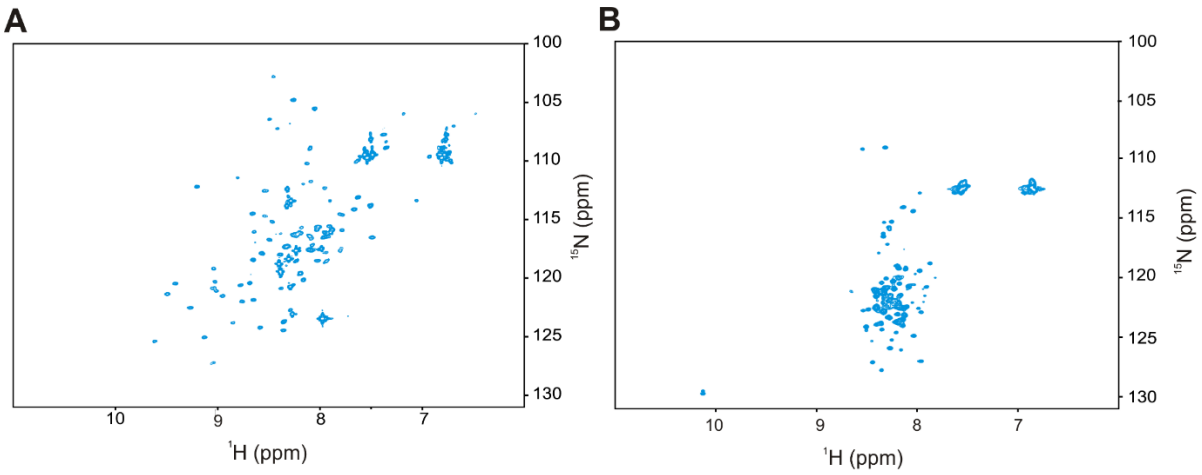
Band d

MNKITTRSPL EPEYQPLGKP HHALQACVDF EQALLHNNKG NCHPKEESLK
PVRPHDLGKK EGQKGDGLRA HAPLAATSQP GRKEVGLKPQ HNHQNNHDFN
LSPLAEGATN RAHLYQQDSR FDDRVESIIN ALMPLAPFLE GVTCETGTSS
ESPCEPSGHD ELFVQQSPID SAQPVQLNSK PTVQPLNPAA DGAEVIVWSV
GRETPASIAK NQRDSRQKRL AEEPLALHQK ALPEICPPAV SATPDDHLVA
RWCATPVTEV AEKSARFPYK ATVQSEQLDM TELADRSQHL TDGVDSSKDT
IEPPRPEKLL LPREETLPEM YSLSFTAPVV TPGDHLLATM RATRLASVSE
QLIQLAQRLA VELELRGGSS QVTQLHLNLP ELGAIMVRIA EIPGKLHVEL
IASREALRIL AQGSYDLLER LQRIEPTQLD FQASDDSEQE SRQKRHVYEE
WEAEE

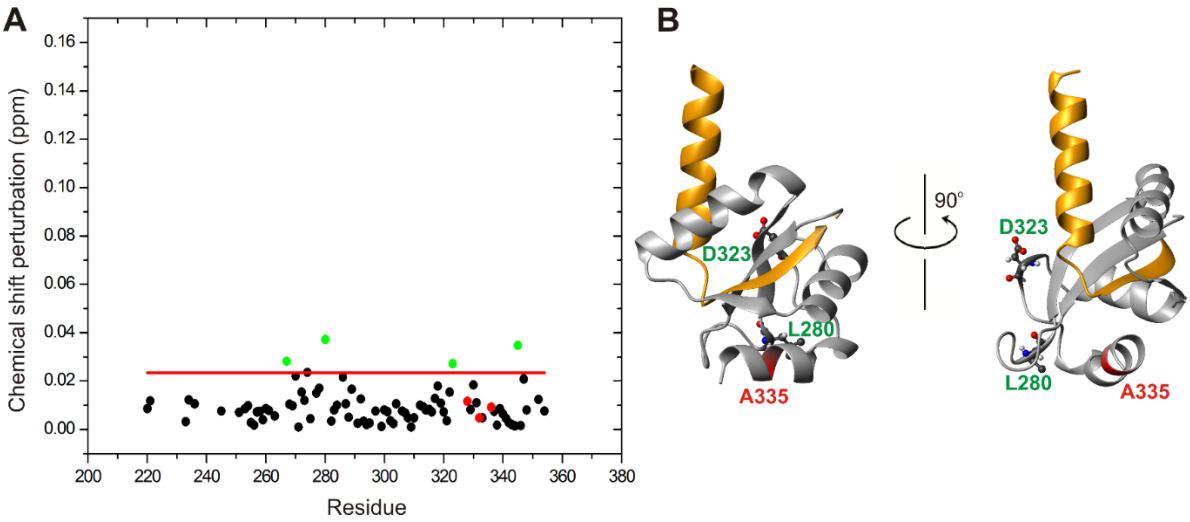
Supplemental Figure 3: Molecular weight of YscP constructs estimated from size exclusion chromatography. Size exclusion chromatogram of YscP (black), YscP$^{342-442}$ (red) and YscP$^{203-442}$ (green) performed on a 26/60 Sepharyl S-100HR column. YscP was eluted as a monomer (elution volume of 110 ml). The other peaks corresponded to protein contaminants from the purification process. YscP$^{342-442}$ and YscP$^{203-442}$ were eluted in the column void volume (95 ml), suggesting that they formed high molecular weight multimers or aggregates.



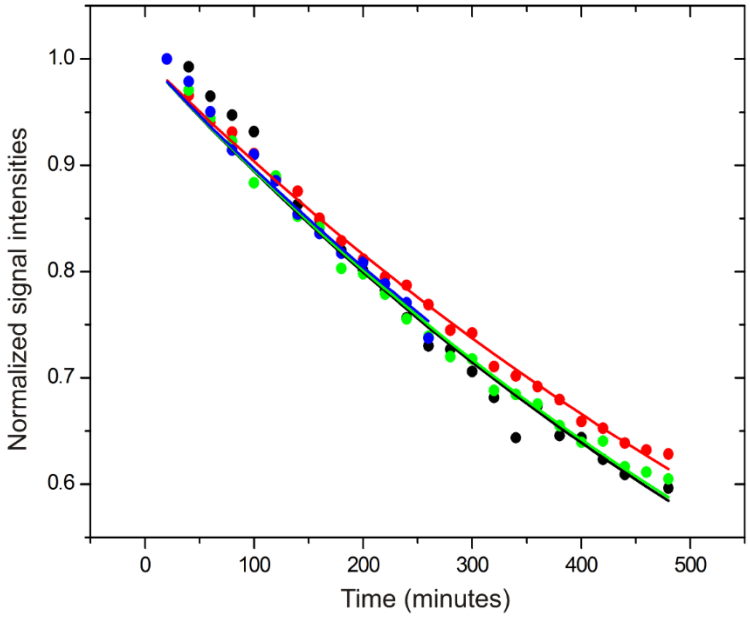
Supplemental Figure 4: NMR spectra of YscP$^{342-442}$ and YscP$^{203-445}$. (**A**) ^{1}H-^{15}N HSQC spectrum of ^{15}N labeled YscP$^{342-442}$. (**B**) ^{1}H-^{15}N HSQC spectrum of ^{15}N labeled YscP$^{203-445}$. The NMR spectra were acquired at a protein concentration of 50 µM in PBS at 25 °C.



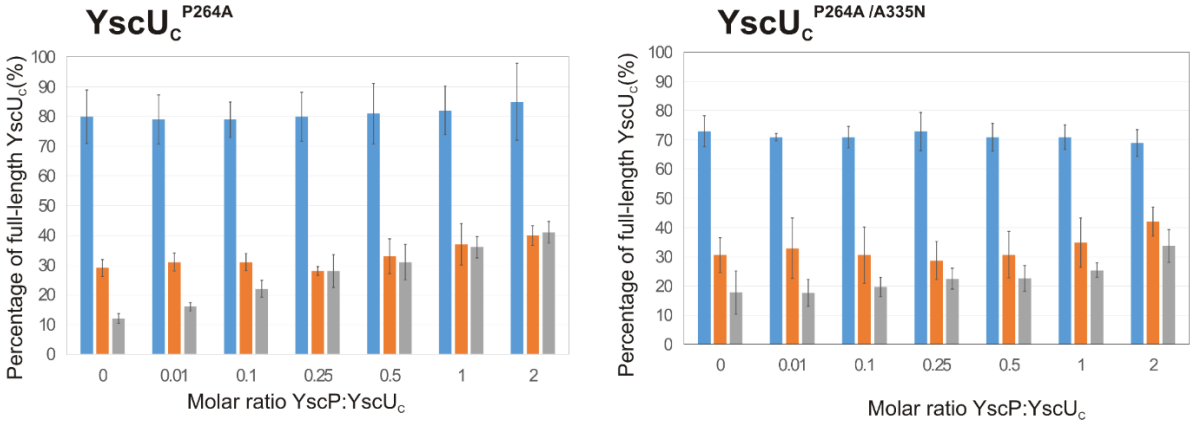
Supplemental Figure 5: Mutation of alanine 335 to asparagine in YscU abolishes YscP binding. (A) Chemical shift perturbations (ppm) between free ^{15}N-labeled $YscU_C^{A335N}$ and ^{15}N-labeled $YscU_C^{A335N}$ in complex with YscP were calculated with equation [1] and are plotted against the $YscU_C$ residue number. The threshold used to define significant chemical shift changes is indicated by a red line. The residues with the largest chemical shift perturbations is green while YscP binding residues in wild-type $YscU_C$ are marked as red. (B) The residues with the largest chemical shift perturbations from panel A (I267, L280, D323 and N345) are shown in green on the $YscU_C$ structure. The mutated position, 335, is shown in red.



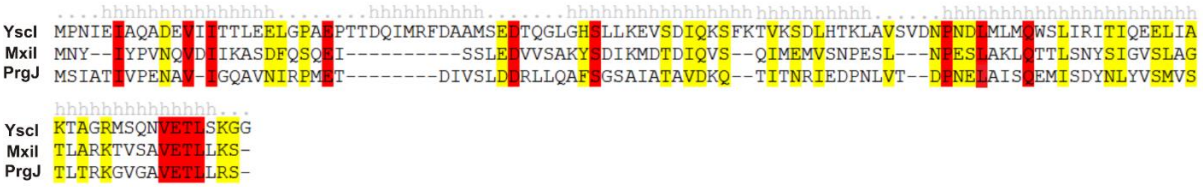
Supplemental Figure 6: Dissociation kinetics of $YscU_C$ in the absence and presence of YscP. Dissociation kinetics of $YscU_C$ in the absence of YscP (black) and with $YscU_C$:YscP molar ratios of 1:0.5 (red), 1:1 (green), or 1:2 (blue) at pH 7.4 monitored by 1D ^{1}H NMR-spectroscopy at 37 °C in PBS with 1 mM TCEP. The solid lines represent fits to single exponential decay functions. Raw data were obtained by integration of the methyl resonances of $YscU_{CC}$.



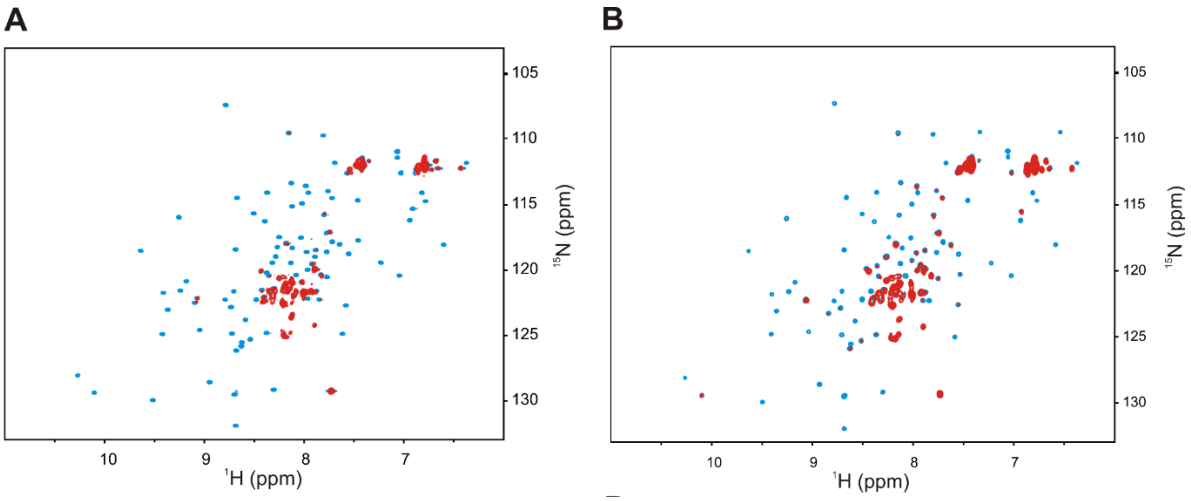
Supplemental Figure 7: YscP binding interferes with auto-cleavage of $YscU_C^{P264A}$ showing by immunoblot analysis of the proportion of full-length $YscU_C^{P264A}$ and $YscU_C^{P264A/A335N}$ in the samples after 0 (blue), 1 (orange), 2 (grey) days' incubation at 37 °C. Each sample generates 2 bands in the western blot (see Fig 6) SDS-PAGE, with the top band corresponding to full-length $YscU_C$ and the bottom band to the fragment $YscU_{CC}$. The relative abundance of the full-length protein ($YscU_C$ variants) was calculated by dividing the full-length protein integration in a given image by that for the total protein. Values shown in the graphs are means ± the standard deviation of the mean (the experiment was repeated 4 times).



Supplemental Figure 8: Sequential alignment of YscI homologs. MAFFT sequence alignment (1) of YscI (*Yersinia pseudotuberculosis*) and the homologues MxiI (*Shigella flexneri*) and PrgJ (*Salmonella typhimurium*). The amino acids are numbered according to their positions in YscI of *Yersinia pseudotuberculosis*. Conserved residues are highlighted in red and similar residues are shown in yellow. The secondary structure of YscI was predicted using DSSP and PSIPRED (5,6) and is indicated by the letter "h".



Supplemental Figure 9: YscP and YscI do not bind to the same binding site on $YscU_C$. (A) Overlay of $^{1}H–^{15}N$ HSQC spectra of ^{15}N-labeled $YscU_C^{wt}$ in the absence (blue) and presence (red) of YscI at a protein concentration of 50 µM in PBS, TCEP 1 mM at 37 °C. (**B**) Overlay of $^{1}H–^{15}N$ HSQC spectra of ^{15}N-labeled $YscU_C^{A335N}$ in the absence (blue) and presence (red) of YscI at a protein concentration of 50 µM in PBS, TCEP 1 mM at 37 °C.



SUPPLEMENTAL REFERENCES:

1. Katoh, K., Misawa, K., Kuma, K. i., and Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**, 3059-3066
2. Holm, L., and Rosenström, P. (2010) Dali server: conservation mapping in 3D. *Nucleic Acids Research* **38**, W545-W549
3. Mizuno, S., Amida, H., Kobayashi, N., Aizawa, S.-I., and Tate, S.-i. (2011) The NMR Structure of FliK, the Trigger for the Switch of Substrate Specificity in the Flagellar Type III Secretion Apparatus. *Journal of Molecular Biology* **409**, 558-573
4. Bergeron, J. R., Fernandez, L., Wasney, G. A., Vuckovic, M., Reffuveille, F., Hancock, R. E., and Strynadka, N. C. (2016) The Structure of a Type 3 Secretion System (T3SS) Ruler Protein Suggests a Molecular Mechanism for Needle Length Sensing. *The Journal of biological chemistry* **291**, 1676-1691
5. Kabsch, W., and Sander, C. (1983) Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* **22**, 2577-2637
6. Jones, D. T. (1999) Protein secondary structure prediction based on position-specific scoring matrices1. *Journal of Molecular Biology* **292**, 195-202

SUPPLEMENTAL TABLE

Supplemental Table S1: Bacterial strains and plasmids used in this study

Strains, plasmids, or constructs	Description[a]	Reference
E. coli strains		
BL21	IPTG-inducible T7 RNA polymerase	Studier, Moffat
Top 10	Commercial one-shot competent cells	Invitrogen
BL21 (DE3) pLysS	IPTG-inducible T7 RNA polymerase Cm^r	Studier, Moffat
Y. pseudotuberculosis strains		
YPIII(pIB102)	wild-type, parental strain, Km^r	
YPIII(pIB75)	*ysc*U null strain, Km^r	Levander et al., 2002
YPIII(pIB69)	*ysc*P null strain, Km^r	Levander et al., 2002
YPIII *ΔyscP, ΔyscU*	Double *ΔyscP, ΔyscU* strain	Edquist *et al*., 2003
Plasmids		
pGEX-6P3	Commercial vector with N-term. GST-fusion Cb^r	GE Healthcare
pBADmycHis A	Commercial vector for L-ara induced expression Cb^r	Invitrogen
pET20b(+)	T7-promoter commercial. C-terminal His6 tag Cb^r	Qiagen
pETMBP-1a	Commercial vector with N-terminal His tag	GE Healthcare
pNIC28-Bsa4	Commercial vector with N-terminal His tag	Opher Gileadi
Constructs		
His_6-MBP-$YscP^{342-442}$	*yscP*$^{342-442}$ in pETMBP-1a	This study
His_6-$YscP^{203-445}$	*yscP*$^{203-445}$ in pNIC28-Bsa4	This study
YscI-His_6	*yscI* full length in pET20b(+)	This study
GST-YscP	*yscP* full length in pGEX-6P3	This study
YscU	*yscU* full length in pBADmycHis A	Frost *et al*., 2012
$YscU^{L280N}$	*yscU*^{L280N} in pBADmycHis A	This study
$YscU^{E332A}$	*yscU*^{E332A} in pBADmycHis A	This study
$YscU^{A335N}$	*yscU*^{A335N} in pBADmycHis A	This study
$YscU^{E336A}$	*yscU*^{E336A} in pBADmycHis A	This study
$YscU^{A268F}$	*yscU*^{A268F} in pBADmycHis A	This study
$YscU^{V292T}$	*yscU*^{V292T} in pBADmycHis A	This study
$YscU^{A268F, A335N}$	*yscU*$^{A268F, A335N}$ in pBADmycHis A	This study
$YscU^{V292T, A335N}$	*yscU*$^{V292T, A335N}$ in pBADmycHis A	This study
GST-$YscU_C$	*yscU*$_C$ in pGEX-6p-3	Frost et al., 2012
GST-$YscU_C^{A335N}$	*yscU*$_C^{A335N}$ in pGEX-6p-3	This study

[a] Km^r: kanamycin resistance; Cb^r: carbenicillin resistance; Cm^r: chloramphenicol

Supplemental Table S2: Primers used in this study

Primer name	Primer sequence (5'- 3')	Restriction sites
Primers for sub-cloning		
fw_pGEX_yscU$_C$	cgcggatcctactatcaatatattaaggaactta	*Bam*HI
rv_pGEX_yscU$_C$	tcccccggggttaattagctaccaccactgatgag	*Sma*I
fw_pGEX_yscP$^{203-455}$	tacttccaatccatggaaactccggccagtatag	*Bsa*I
rv_pGEX_ yscP$^{203-455}$	tatccacctttactgtca ttcttcagcctcccactcc	*Bsa*I
fw_pGEX_yscP$^{342-442}$	gcttccatgggcgcgaccaggctggc	*Nco*I
rw_pGEX_yscP$^{342-442}$	atgcggccgctcactgacgtgactcctgttcac	*Not*I
fw_pET_yscI	ggaattccatatgccgaacatagaaatagctcagg	*Nde*I
fw_pET_yscI	ccgctcgagccccccttcgacaaggtt	*Xho*I
Primers for site-directed mutagenesis		
fw_pBAD_yscU$_{FL}$	catgccatggtgagcggagaaaagacagag	*Nco*I
rv_yscU$_{FL}$_L280N	gaatgttaccaacggatttggtgtttcccctcgcttg	
fw_yscU$_{FL}$_L280N	caagcgaggggaaacaccaaatccgttggtaacattc	
rv_pBAD_yscU$_{FL}$	cggaattcttataacatttcggaatgttgtttc	*Eco*RI
rv_yscU$_{FL}$_E332A	gcacttcagctgtggccgctatttgctcagccgg	
fw_yscU$_{FL}$_E332A	ccggctgagcaaatagcggccacagctgaagtgc	
rv_yscU$_{FL}$_A335N	gccatcgtagcacttcatttgtggcctctatttgc	
fw_yscU$_{FL}$_A335N	gcaaatagaggccacaaatgaagtgctacgatggc	
rv_yscU$_{FL}$_E336A	ctagccatcgtagcactgcagctgtggcctctatttg	
fw_yscU$_{FL}$_E336A	caaatagaggccacagctgcagtgctacgatggctag	
rv_yscU$_{FL}$_A268F	gcttgtaaagaataccaatgaaaatatgggtcggattag	
fw_yscU$_{FL}$_A268F	ctaatccgacccatattttcattggtattctttacaagc	
rv_yscU$_{FL}$_V292T	gcgcacagtctgcgtttgggcatcggtatatttgaatg	
fw_yscU$_{FL}$_V292T	cattcaaatataccgatgcccaaacgcagactgtgcgc	