

SUPPLEMENTAL MATERIAL

INSIGHTS INTO THE STRUCTURAL NETWORK RESPONSIBLE FOR OLIGOMERIZATION AND ACTIVITY OF THE BACTERIAL VIRULENCE REGULATOR CLPP*

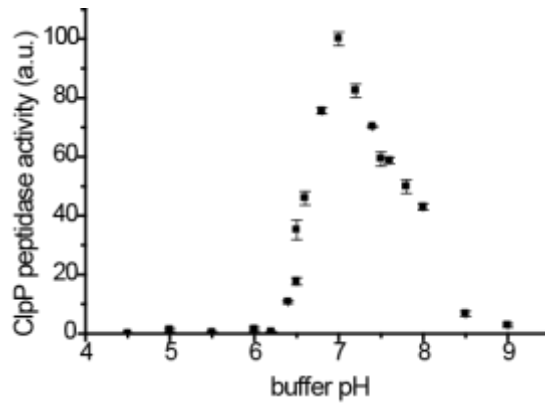
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Supplementary Figures

Supplementary Figure 1: pH-profile of ClpP peptidase activity determined by the fluorescent substrate assay.



Supplementary Tables

Supplementary Table 1: Primers used in this study

#	Name	Sequence ^a
1	SaClpP-for-rbs	GGGGACAAGTTTGTACAAAAAGCAGGCTTTGAAGGAGATAGAACCATGAATTTAATTCCTACAGTTATTGAAAC
2	SaClpP-rev-Strep	GGGGACCACITTTGTACAAGAAAAGCTGGGTGTTATTTTTCGAACTGCGGGTGGCTCCATTTTGTTCAGGTACCATCACTTC
3	Q35A-for	AGACCGTATTATTATGTTAGGTTCA GC AATTGATGACAACGTAGCAAATTCA
4	Q35A-rev	TGAATTTGCTACGTTGTCATCAATT GCT GAACTAACATAATAATACGGTCT
5	S98A-for	ATTTGTATCGGTATGGCTGCA GC AATGGGATCATTCTTATTAG
6	S98A-rev	CTAATAAGAATGATCCCATTG CT GTCAGCCATACCGATACAAAT
7	S98C-for	ACAATTTGTATCGGTATGGCTGCAT GC ATGGGATCATTCTTATTAGC
8	S98C-rev	GCTAATAAGAATGATCCCATGCAT GC AGCCATACCGATACAAATTGT
9	S98T-for	AATTTGTATCGGTATGGCTGCA ACG ATGGGATCATTCTTATTAGCAG
10	S98T-rev	CTGCTAATAAGAATGATCCCAT CGT TGCAGCCATACCGATACAAATT
11	G127AG128AG131A-for	GTAATGATTCACCAACCATTAG CTGCT GTCTCAAG CA ACAAGCAACTGAAATCGAAATTGCT
12	G127AG128AG131A-rev	AGCAATTTTCGATTTTCAGTTGCTT GTGCT TGAGCA GCAG CTAATGGTTGGTGAATCATTAC
13	Q130A-for	CACCAACCATTAGGTGGTGCT GC AGGACAAGCAACTG
14	Q130A-rev	CAGTTGCTTGTCT GC AGCACCACCTAATGGTTGGTG
15	Q132A-for	CCAACCATTAGGTGGTGCTCAAGG AGC AGCAACTGAAATCG
16	Q132A-rev	CGATTTTCAGTTGCT GC TCCTTGAGCACCACCTAATGGTTGG
17	E135A-for	GCTCAAGGACAAGCAACT GC AATCGAAATTGCTGCAAAAT
18	E135A-rev	ATTTGCAGCAATTTTCGATT GC AGTTGCTTGTCTTGGAGC
19	E135R-for	GGTGCTCAAGGACAAGCAACT AG AATCGAAATTGCTGCAAATCA
20	E135R-rev	TGATTTGCAGCAATTTTCGATT CT AGTTGCTTGTCTTGGAGCACC
21	E137A-for	CAAGGACAAGCAACTGAAATCG CA AATTGCTGCAAATCACATTTTA
22	E137A-rev	TAAAATGTGATTTGCAGCAATT GCG ATTTTCAGTTGCTTGTCTTGG
23	L144E-for	CAACTGAAATCGAAATTGCTGCAAATCACATT GAG AAAAACACGTGAAAAATTAACCCGCATTTTATC
24	L144E-rev	GATAAAATGCGGTTAATTTTTACGTGTTTT CTC AATGTGATTTGCAGCAATTTTCGATTTTCAGTTG
25	L144G-for	GAAATCGAAATTGCTGCAAATCACATT GG AAAAACACGTGAAAAATTAACCCGCAT
26	L144G-rev	ATGCGGTTAATTTTTACGTGTTTT CCA ATGTGATTTGCAGCAATTTTCGATTTTC
27	L144M-for	GAAATCGAAATTGCTGCAAATCACATT ATG AAAAACACGTGAAAAATTAACCCGCATT
28	L144M-rev	AATGCGGTTAATTTTTACGTGTTTT CAT AATGTGATTTGCAGCAATTTTCGATTTTC
29	L144R-for	GAAATCGAAATTGCTGCAAATCACATT AG AAAAACACGTGAAAAATTAACCCGCAT
30	L144R-rev	ATGCGGTTAATTTTTACGTGTTTT CT AATGTGATTTGCAGCAATTTTCGATTTTC
31	D170A-for	TGAAAAAATACAAAAAGACACAG CT CGTGATAACTTCTTAACCTGCAG
32	D170A-rev	CTGCAGTTAAGAAGTTATCACG AGCT GTGTCTTTTTGTATTTTTTCA
33	R171A-for	GTATTGAAAAAATACAAAAAGACACAGAT GC TGATAACTTCTTAACCTGCAGAAGA
34	R171A-rev	TCTTCTGCAGTTAAGAAGTTATCA GC ATCTGTGTCTTTTTGTATTTTTTCAATAC
35	R171K-for	CAAAGTATTGAAAAAATACAAAAAGACACAGAT AGG CAACTTCTTAACCTGCAGAAGAAGCTAAAGAA
36	R171K-rev	TTCTTTAGCTTCTCTGCAGTTAAGAAGTTATC CTT ATCTGTGTCTTTTTGTATTTTTTCAATACTTTG

^a attB1/attB2 sequences, Shine-Dalgarno and Kozack sequences, STREP II-tag sequence, **changed nucleotides for point mutations.**

Supplementary Table 2: Comparison of ClpP-Structure entries of the PDB

Code	Resolution	Organism	E-Helix	Barrel height	Active site	Reference
3KTG	2.4 Å	<i>Bacillus subtilis</i>	straight	9 nm	aligned	(1)
3Q7H	2.5 Å	<i>Coxiella burnetii</i>	straight	9 nm	not aligned	-
1YG6	1.9 Å	<i>Escherichia coli</i>	straight	9 nm	aligned	(2)
3P2L	2.3 Å	<i>Francisella tularensis</i>	straight	9 nm	aligned	-
2ZL0	2.6 Å	<i>Helicobacter pylori</i>	straight	9 nm	aligned	(3)
1TG6	2.1 Å	<i>Homo sapiens</i>	straight	9 nm	aligned	(4)
3STA	2.3 Å	<i>Staphylococcus aureus</i>	straight	9 nm	not aligned	(5)
3V5E	2.3 Å	<i>Staphylococcus aureus</i>	straight	9 nm	aligned	This study
3QWD	2.1 Å	<i>Staphylococcus aureus</i>	kinked (H123-K145)	8 nm	not aligned	(6)
2CBY	2.6 Å	<i>Mycobacterium tuberculosis</i>	not resolved (P125–I136)	8 nm	not aligned	(7)
2F6I	2.5 Å	<i>Plasmodium falciparum</i>	not resolved (P291–I304)	8 nm	not aligned	(8)
1Y7O	2.5 Å	<i>Streptococcus pneumonia</i> (A153P)	not resolved (P123–A139)	8 nm	not aligned	(9)
3TT6	2.6 Å	<i>Bacillus subtilis</i>	not resolved (P124–E136)	8 nm	not aligned	(10)

Supplementary Table 3: Melting Temperatures as determined by thermal shift assays

SaClpP protein	Melting temperature^a
wt	(47.3 ± 0.8 °C) 58.2 ± 0.3 °C
Q35A	58.1 ± 0.3 °C
S98A	n.d.
S98C	61.3 ± 1.0 °C
S98T	n.d.
G127AG128AG131A	60.6 ± 0.3 °C
Q130A	63.3 ± 0.3 °C
Q132A	44.6 ± 1.1 °C
E135A	62.1 ± 0.3 °C
E137A	45.1 ± 0.8 °C
L144E	(44.3 ± 0.6 °C) 58.8 ± 0.3 °C
L144G	(45.3 ± 0.8 °C) 56.6 ± 0.3 °C
L144M	(46.4 ± 1.5 °C) 56.3 ± 0.9 °C
L144R	72.3 ± 0.6 °C
D170A	(56.0 ± 0.7 °C) 60.8 ± 0.5 °C
R171A	58.5 ± 0.4 °C
R171K	58.9 ± 0.3 °C

^aMelting temperatures were determined as described in the Materials and Methods Sections. Mean values ± sd of three independent experiments are given. Minor peaks are in brackets.

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