

Figure S1. Validation of ClpP1 and ClpP2 specific polyclonal antibodies. Samples of purified ClpP1, purified ClpP2 and cells harvested from overnight cultures of WT, Δ P1, Δ P2 and Δ P1P2 strains of *P. aeruginosa* were subjected to SDS-PAGE and Western blot using primary antibodies specific for either ClpP1 or ClpP2.

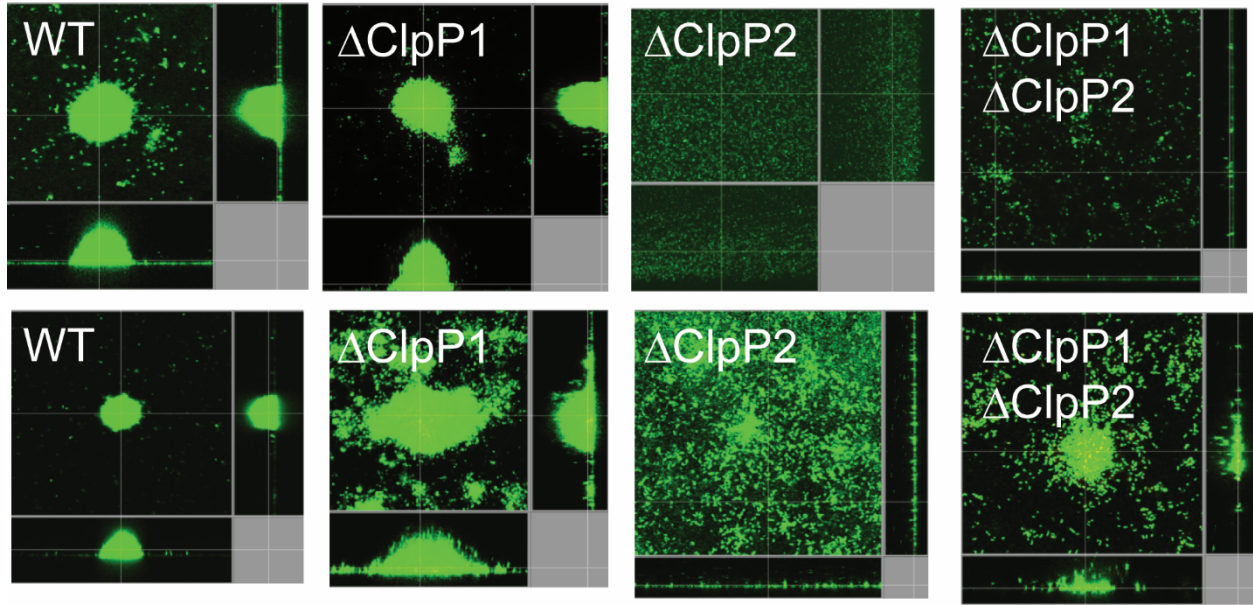
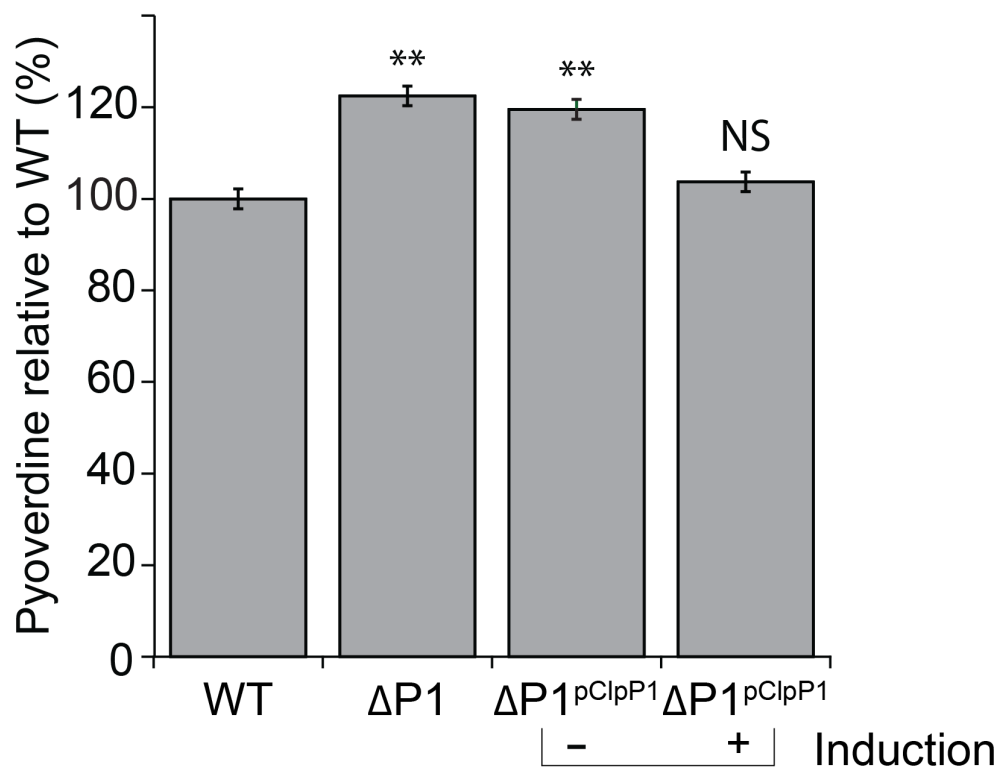


Figure S2. Microcolony assay duplicate experiments. Microcolony formation is shown for wild type *P. aeruginosa* (WT), the ClpP1 deficient strain (Δ ClpP1), the ClpP2 deficient strain (Δ ClpP2) and the strain deficient for both ClpP1 and ClpP2 (Δ ClpP1 Δ ClpP2). Experiments were carried out as described in Materials and Methods.

A.



B.

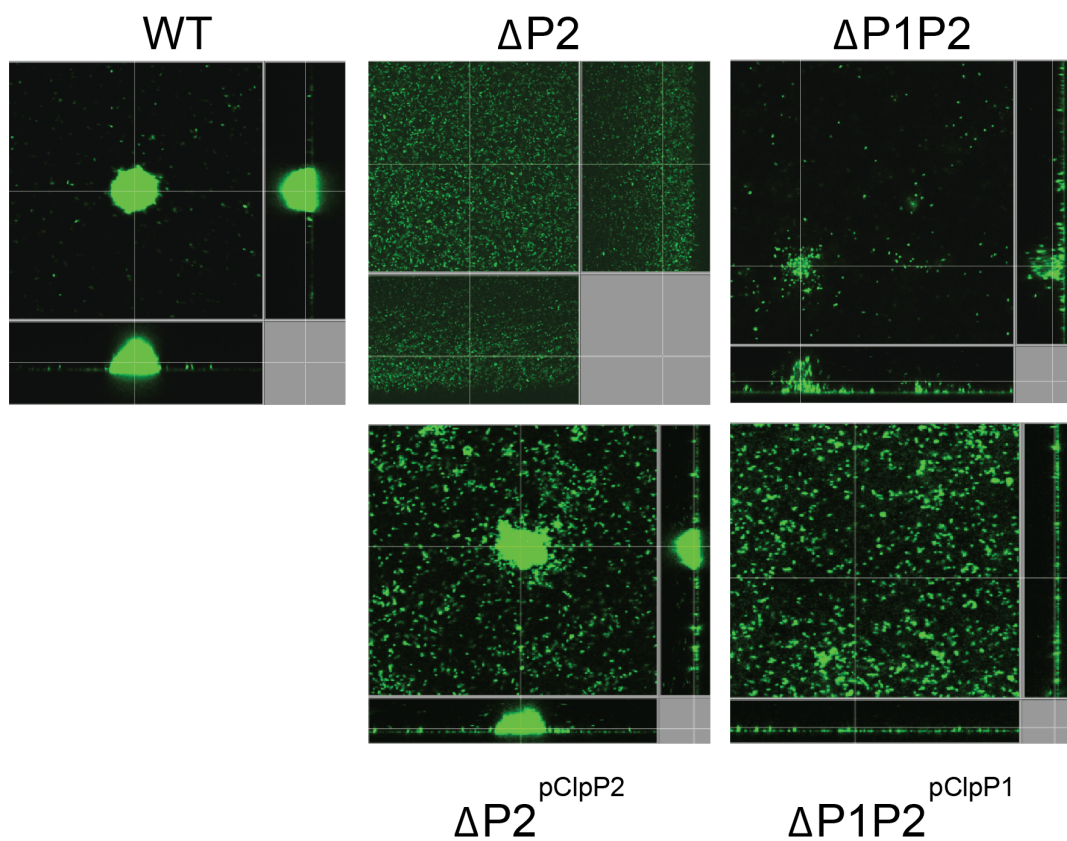


Figure S3. Rescue of pyoverdine and microcolony organization by complementation with ClpP1 or ClpP2 expression plasmids. (A) Pyoverdine levels in $\Delta P1$ are restored to wild type by the expression of ClpP1 from a plasmid (pClpP1). Values are normalized averages ($n=3$) \pm 1 SEM. Data were compared to control values (WT) by ANOVA with Dunnet's *post hoc* test (**, $P<0.005$; NS, not significant). Strains deficient for ClpP1 were transformed as previously described (1) with a pMQ71 based shuttle vector for arabinose-dependent expression of the ClpP1 gene (pClpP1). Growth conditions for cultures are the same as described for pyoverdine assays in Materials and Methods except that media was supplemented with 30 $\mu\text{g}/\text{ul}$ gentamycin for pMQ71-containing strains. 2% arabinose was used for induction conditions (+) or was omitted for controls (-). Fluorescence excitation/emission values were measured at 405/465 nm for pyoverdine quantification. (B) Microcolony formation flow cell assay by strains transformed with plasmids for the expression of ClpP1 or ClpP2. Assays carried out with strains without plasmid (WT, $\Delta P1$ and $\Delta P1P2$) are shown in the top row. The ClpP2 deficient strain ($\Delta P2$) was transformed as described in (A) and the strain deficient for both ClpP1 and ClpP2 ($\Delta P1P2$) was transformed similarly with pClpP1. Flow cell biofilm assays were carried out as described in Materials and Methods except that initial overnight cultures were grown in the presence of 30 $\mu\text{g}/\text{ml}$ gentamycin and 0.1% arabinose was present throughout the experiment.

EcClpX 1 MTDKRRK - DGSGKLLYCSFCGKSQHEVRKLIAGPSVYICDECVDL 43
PaClpX 1 MTDTRNGEDNGKLLYCSFCGKSQHEVRKLIAGPSVFICDECVDL 44

EcClpX 44 CNDIREEIKEVAPHRERSALPTPHEIRNHLDDYVIGQEQAQKV 87
PaClpX 45 CNDIREEVQEAQAESSGHKLPAPKEIRTILDQYVIGQERAKKV 88

EcClpX 88 LAVAVYNHYKRLRNGDTSNGVELGKSNILLIGPTGSGKTLLAET 131
PaClpX 89 LAVAVYNHYKRLNQRDKKDDIELGKSNILMIGPTGSGKTLLAET 132

EcClpX 132 LARLLDVPFTMADATTLTEAGYVGEDVENIQKLLQKCDYDVQK 175
PaClpX 133 LARLLNVPFTIADATTLTEAGYVGEDVENIQKLLQKCDYDVEK 176

EcClpX 176 AQRGIVYIDEIDKISRKSDNPSITRDVSGEGVQQALLKLEGTV 219
PaClpX 177 AQMGIVYIDEIDKISRKSDNPSITRDVSGEGVQQALLKLEGTV 220

EcClpX 220 AAVPPQGGRRKH PQQEFLQVDTSKILFICGGAFAGL DKVISHRVE 263
PaClpX 221 ASVPPQGGRRKH PQQEFLQVDTRNILFICGGAFAGLERV IQNRS 264

EcClpX 264 TGSIGFGATVKAKSDKASEGELLAQVEPEDLIKFGLIPEF IGR 307
PaClpX 265 RG - GIGFNAEVR SQEMGKKVGEAFKEVEPEDLVKFGLIPEFVGR 307

EcClpX 308 LPVVATLNELSEALIQILKEPKNALTQYQALFNLEGVDFLEFR 351
PaClpX 308 LPV IATLDELDEAALMQILTEPKNALTQYAKLFEMEGVDLEFR 351

EcClpX 352 DEALDAIAKKAMARKTGARGLRSIVEAALLDTMYDLPSMEDVEK 395
PaClpX 352 PDALKAVARKALERKTGARGLRSILEGILLDTMYEIPSQQDVSK 395

EcClpX 396 VVIDESVIDGQSKPLLIYGKPE - - AQQASGE 424
PaClpX 396 VVIDESVIDGSSQPLMIYENSEKPAKAAPEA 426

Figure S4. Sequence alignment of ClpX from *E.coli* (*EcClpX*) and *P. aeruginosa* (*PaClpX*). Identical residues are shown in light blue except for the Walker A motif (red), Walker B motif (orange), pore 1 loops (gray), pore 2 loops (purple), sensor 1 motif (yellow), sensor 2 motif (dark blue), arginine finger residue (green), IGF docking loops (black) and RKH motif (pink). The two proteins have 76% sequence identity. The alignment was carried out with MUSCLE (2) using default values and presented with Jalview (3).

EcClpA 1 MLNQELELSLNMAFARAREHRHEFMTVEHLLLALLSNPSAREALEACSVDLVALRQ 56
PaClpA 1 MLNRELEVTLNLAFKEARAKRHEFMTVEHLLLALLDNEAAATVLRACGANLDKLRR 56

EcClpA 57 ELEAFIEQTTPVLPASEEERDTQPTLSFQRVLQRAVFHVQSSGRNEVTGANVLVAI 112
PaClpA 57 DLQEFIDSTTPLIPQHDDERETQPTLGFQRVLQRAVFHVQSSGKREVTGANVLVAI 112

EcClpA 113 FSEQESQAAYLLRKHEVSRLDVVNFISHGTRKDEPTQSSDPGSQPNSEEQAGGEE - 167
PaClpA 113 FSEQESQAVFLLKQQS IARI DVVNYIAHGISKVPGHAHEHPQDGEQDMQDEEGGESA 168

EcClpA 168 - - - RMENFTTNLNQLARVGGIDPLIGREKELERAIQVLCRRRKNPPLLVGESGVG 219
PaClpA 169 TSNHPLDAYASNLELARQGRIDPLVGREHEVERVAQILARRRKNPPLLVGEGV 224

EcClpA 220 KTAIAEGLAWRIVQGDPVEVMADCTIYSLDIGSLLAGTKYRGDFEKRFKALLKQLE 275
PaClpA 225 KTAIAEGLAKRIVDGGQVPDLLADSVVYSLDLGALLAGTKYRGDFEKRFKALLNELR 280

EcClpA 276 QDTNSILFIDEIHTIIGAGAASGGQVDAANLIKPLLSSGKIRVIGSTTYQEFNSIF 331
PaClpA 281 KRPHAVLFIDEIHTIIGAGAASGGVMDASNLLKPVLSSEIRCIIGSTTFQEFRGIF 336

EcClpA 332 EKDRALARRFQKIDITEPSIEETVQIINGLKPKEYEAHHDVRYTAKAVRAAVELAVK 387
PaClpA 337 EKDRALARRFQKVDVTEPSVEDTYGILKGLKGRFEQHHHIEYSDEALRAAAELAAR 392

EcClpA 388 YINDRHLDPK AIDVIDEAGARARLMPVSKRKKTVNVADIESVVAR IARIPEKSVSQ 443
PaClpA 393 YINDRHMPDK AIDVIDEAGAYQRLQPEEKRVKRIEVAQVEDIVAKIARIPPKHVTT 448

EcClpA 444 SDRDTLKNLGDRLKMLVFGQDKAIEALTEAIKMARAGLGHEHKPVGSFLFAGPTGV 499
PaClpA 449 SDKELLRNLERDLKLTVFGQDDAIESLSTAIKLSRAGLKAPDKPVGSFLFAGPTGV 504

EcClpA 500 GKTEVTVQLSKALGIELLRFDMSEYMERHTVSRLIGAPPGYVGFDDQGGLLTDAVIK 555
PaClpA 505 GKTEVARQLAKALGVELVRFDMSEYMERHTVSRLIGAPPGYVGFDDQGGLLTEAITK 560

EcClpA 556 HPHAVLLLDEIEKAHPDVFNILLQVMDNGTLDNNGRKADFRNVVLMVTTNAGVRE 611
PaClpA 561 TPHCVLLLDEIEKAHPEVFNLLQVMDHGTLTDNNGRKADFRNII LIMTTNAGA EV 616

EcClpA 612 TERKSIGLIHQDNSTDAMEEIKKIFTPEFRNRLDNIWFDHLSTDVIHQVVDKFLV 667
PaClpA 617 AARASIGFNQDHTTDAMEVIKKSFTPEFRNRLDTIIQFGRLSTETIKSVVDKFLT 672

EcClpA 668 ELQVQLDQKGVSLVVSQEARNWLAEKGYDRAMGARP MARVIQDNLKKPLANELLFG 723
PaClpA 673 ELQAQL EDKRVQLVSDAARGWLAEKGYDVQMGARP MARLIQDKIKRPLAEEILFG 728

EcClpA 724 SLVDGGQVTVALDKEKNELTYGFQSAQKHKA EAAH 758
PaClpA 729 ELAEHGGV -VHVDLKGDELAFEFETA AEP A - - - 758

Figure S5. Sequence alignment of ClpA from *E. coli* (*EcClpA*) and *P. aeruginosa* (*PaClpA*). Identical residues are shown in light blue except for the Walker A motif (red), Walker B motif (orange), pore 1 loops (gray), pore 2 loops (purple), sensor 1 motif (yellow), sensor 2 motif (dark blue), arginine finger residue (green) and IGF docking loops (black). The two proteins have 66% sequence identity. The alignment was carried out with MUSCLE (2) using default values and presented with Jalview (3).

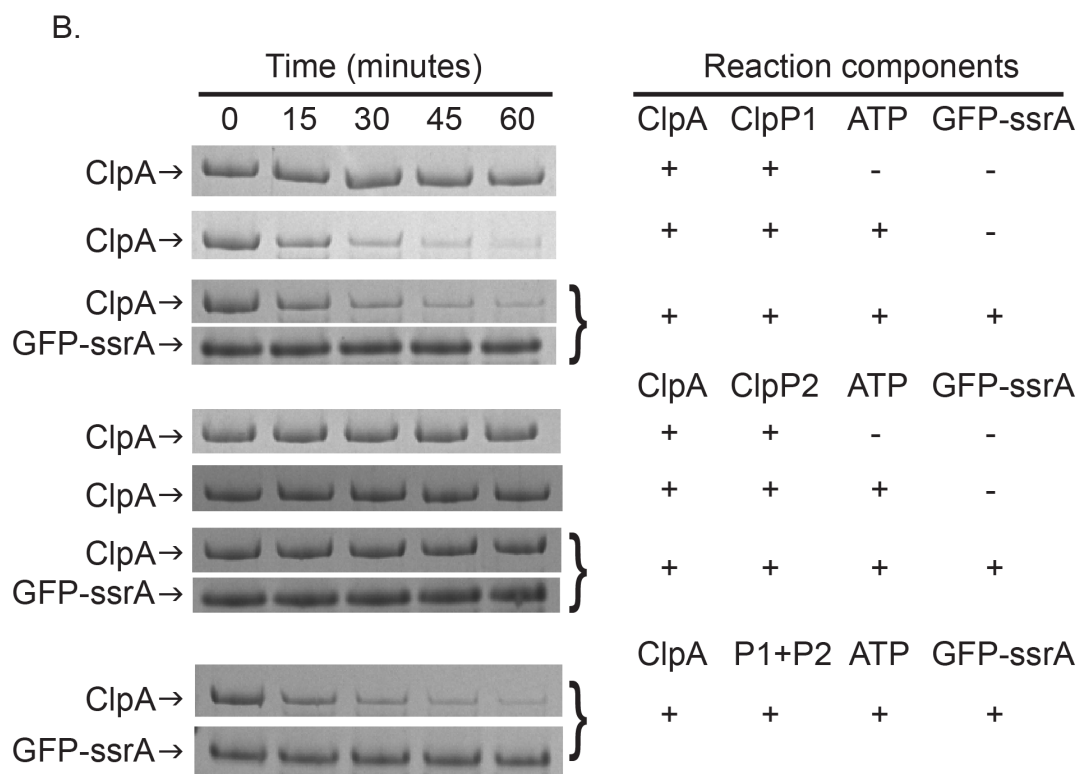
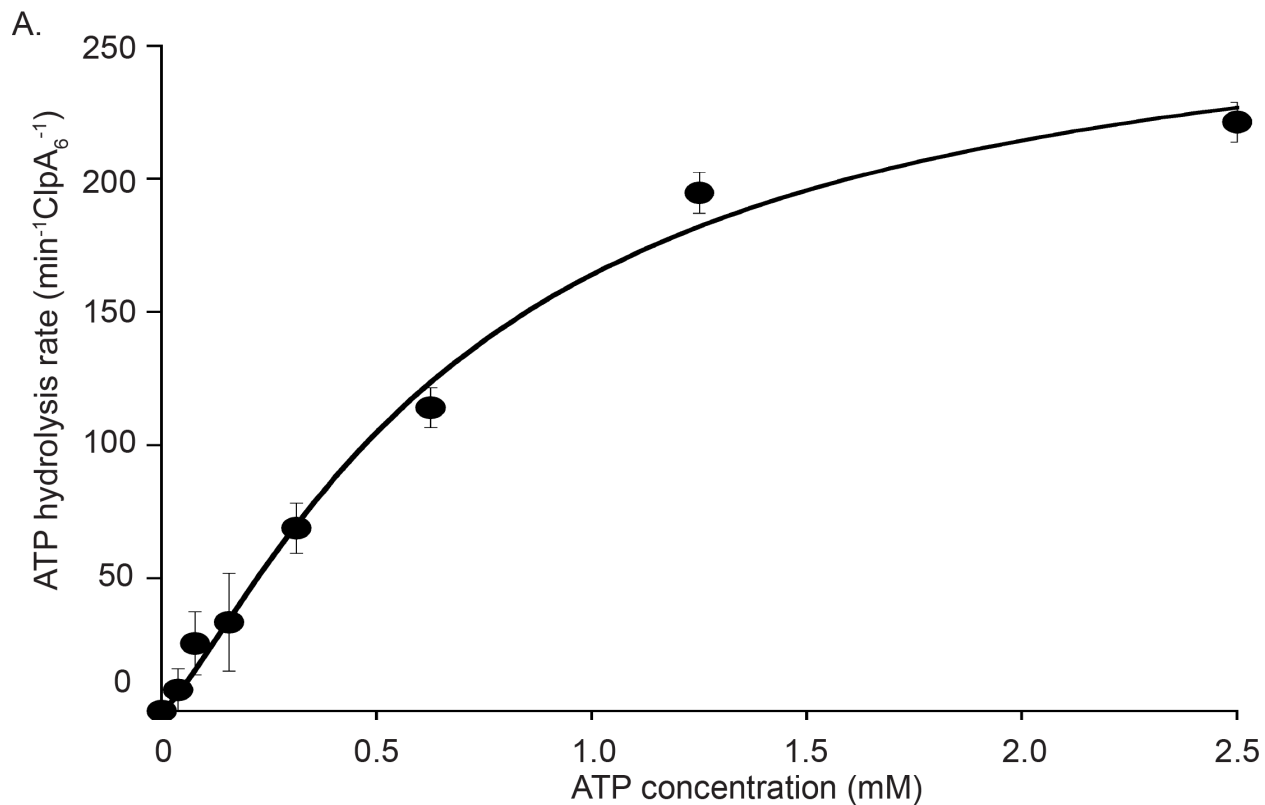


Figure S6. Enzymatic assays with purified ClpA. (A) The rate of ATP hydrolysis by *P. aeruginosa* ClpA increases as a function of ATP concentration. Data were fit to the Hill version of the Michaelis-Menten equation (solid line). Graphic analysis of the data indicated a half-maximal ATP concentration for assembly of ~0.7 mM and a Hill coefficient of 1.3. These data demonstrate cooperative assembly and active hydrolysis of ATP by purified ClpA. (B) ClpA is auto-digested by ClpP1 but not by ClpP2. Reaction mixtures were prepared in HO buffer (50 mM HEPES-KOH pH 7.6, 20 mM MgCl₂, 0.3 M NaCl, 10% glycerol, 0.5 mM DTT) using 0.1 μM ClpA₆, 0.2 μM ClpP1₁₄ or ClpP2₁₄, the ATP regeneration system (described in Material and Methods) and 2 μM GFP-ssrA. Mixtures were incubated at 30°C and aliquots were withdrawn for SDS-PAGE analysis at each indicated time point. ClpA was digested in the presence of ClpP1 and ATP regeneration system. GFP-ssrA was not digested under any conditions and did not prevent or delay digestion of ClpA, and reactions with both ClpP1 and ClpP2 behaved the same as those with ClpP1 by itself.

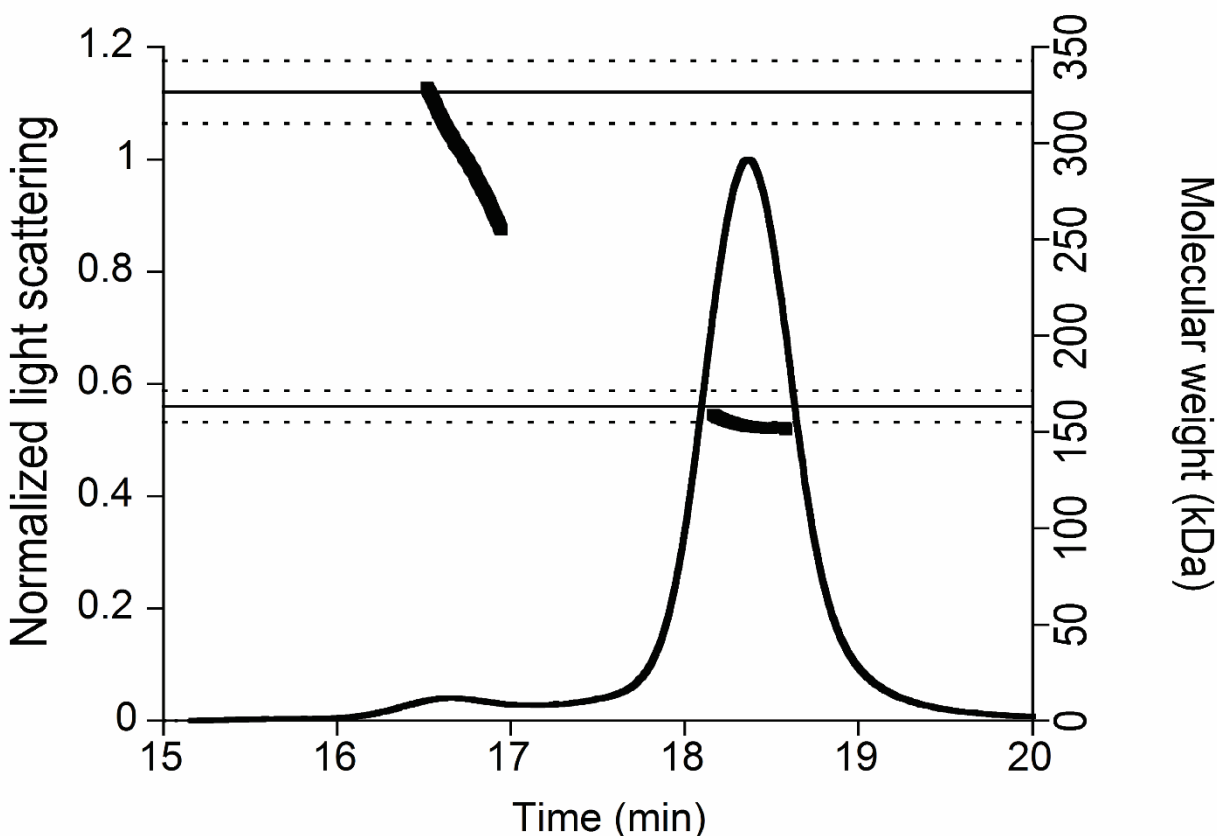


Figure S7. ClpP2 assembles as heptamers at high concentrations. SEC-MALS experiments were used to demonstrate that ClpP2 (93 μ M or 30 mg/ml loading concentration) formed heptamers (expected $M_R \sim 163$ kDa) and potentially a very small population of tetradecamers (expected $M_R \sim 327$ kDa). Dashed lines represent an error of 5% in measurement of molecular weight. Calculated molecular weights for the heptamer and tetradecamer peaks were 155 ± 0.9 kDa and 272 ± 0.2 kDa, respectively. Size exclusion chromatography was performed on a Wyatt WTC-030S5 size exclusion column using an Agilent HPLC. MALS was measured in line using a Wyatt Dawn-HELEOS instrument and concentrations were determined using a Wyatt OptilabrEX instrument. Standard Zimm-plot analysis was carried out with ASTRA software 5.3.4

(Wyatt Technology). ClpP2 samples were run in triplicate with 25 μ l load volume of 30 mg/ml protein in 0.1 M Tris pH 8, 0.15 M NaCl at room temperature.

Table S1. Primers used in this study.

Primer name	Sequence
Δ P1-pMQ 30-1	GGAATTGTGAGCGGATAACAATTTACACAGGAAACAGC TTGATGTCGAC
Δ P1-	GGAAAACCCTGGCGTTACCCGTCTTGCGATCACTCCCTAA
Δ P1-pMQ 30-3	GGGTAACGCCAGGGTTTTCCGGCTCCGCAATACTGGCGGT
Δ P1-pMQ 30-4	CCAGGCAAATTCTGTTTTATCAGACCGCTTCTGCGTTCTGA TCGAACTTGACGAGATCTTCC
Δ P2-pMQ 30-1	GGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCT CCAGGAGGGAAGCCGTCGGC
Δ P2-pMQ 30-2	GGAAAACCCTGGCGTTACCCGCAACCTCCTGGAAGTGTG
Δ P2-pMQ 30-3	GGGTAACGCCAGGGTTTTCCGTTTTCCGCAGGCCGGATAAA
Δ P2-pMQ 30-4	CCAGGCAAATTCTGTTTTATCAGACCGCTTCTGCGTTCTGATTGCATTCCG GGCCACGAC
ClpP	GAGATGAATTCGTGATTATGTCTCGCAACTCTTTTATTCCGCAC

pET2	
ClpP 1 XhoI pET2 3b	CACGCTCGAGTCAGTGATGGT GATGGTGGCCGGACCCGCTG CCGGA GACGGCCAGGTCGCGCTG
ClpP 1 NheI pMQ 71	GGGCTAGCATGTCTCGCAACTC
ClpP 1 KpnI pMQ 71	GGTACCTTAGACGGCCAGGTCGCG
ClpP 2 NdeI pET2 3b	CGTGCATCATATGAAAACCGATGACAAGGAC
ClpP 2 XhoI pET2 3b	ATGCACGCTCGAGTTATTTTTCGAACTGCGGGTGGCTCCACTGGCCAGGC AGCGTGATCTC
ClpP 2 EcoRI pMQ 71	GAATTCATGAAAACCGATGACAAGGACCG
ClpP 2 SphI pMQ 71	GCATGCTCACTGGCCAGGCAGCGT
ClpX NdeI pET2 3b	CGTGCATGCCATATGATGACTGATACCCGCAACGGCGAG

ClpX XhoI pET2 3b	GTAGCATGCTAAATGAAAACCGATGACAAGGACCGCGAAGG
ClpA AgeI pET2 3b	AGAGAACAGACCGGTGGTATGTTGAATCGAGAGCTCGAAGTCACC
ClpA HindII I pET2 3b	TCGAGTGCGGCAAGCTTTCAGGCGGGCTCCGCCGCC

Supplemental references

1. **Choi KH, Kumar A, Schweizer HP.** 2006. A 10-min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: application for DNA fragment transfer between chromosomes and plasmid transformation. J Microbiol Methods **64**:391-397.
2. **Edgar RC.** 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res **32**:1792-1797.
3. **Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ.** 2009. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics **25**:1189-1191.