A single amino acid substitution changes the self-assembly status of a type IV piliation secretin.

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Table S1. Strains and plasmids

Strains/plasmids	Vectors, genotypes and relevant characteristics	Reference/source
Stains		
PAP105	Δ(*lac-pro*) F′ (*lacI*^{q1} Δ*lacZM15 proAB*$^{+}$ Tn*10*))	Lab collection
BL21(DE3)	*E. coli* B F′ *ompT hsdS gal* (λDE3) *endA*	Studier *et al*. (1990)
Plasmids		
pCHAP3783	pIVEX2.3MCS-*pilQ*^{MC58} *Neisseria meningitidis* (MC58)	Guilvout *et al*. (2008)
pCHAP3731	pIVEX2.3MCS-*pulD*$^{28\text{-}660}$	Guilvout *et al*. (2008)
pCHAP3716	pIVEX2.3MCS-*pulD*$^{28\text{-}42/259\text{-}660}$	Guilvout *et al*. (2008)
pCHAP3635	pSU18 p_{lac}*pulD*	Guilvout *et al*. (1999)
pCHAP8243	pBR322 *pulS*, pelBsp-*pulAB pulC-O* Δ*pulD*	Guilvout *et al*. (1999)
pCHAP585	pUC19 p_{lac}*pulS*	Guilvout *et al*. (1999)
pDIA17	pACYC184-*lacI*^{q}	Munier *et al*. (1991)
pIVEX2.3MCS	Expression vector with T7 promoter	Roche Applied Science
pCHAP9147	pIVEX2.3MCS-pIV (f1 filamentous phage secretin)	This study
pCHAP9117	pIVEX2.3MCS-*pilQ*^{MC58} Thr$_{359}$Ser/Pro$_{613}$Leu	This study
pCHAP9119	pIVEX2.3MCS-*pilQ*^{MC58} Thr$_{359}$Ser	This study
pCHAP9120	pIVEX2.3MCS-*pilQ*^{MC58} Pro$_{613}$Leu	This study
pCHAP9121	pIVEX2.3MCS-*pulD*$^{28\text{-}660}$ Pro$_{443}$Leu	This study
pCHAP9122	pIVEX2.3MCS-*pulD*$^{28\text{-}42/259\text{-}660}$ Pro$_{443}$Leu	This study
pCHAP9124	pSU18-P_{lac}*pulD* Pro$_{443}$Leu	This study
pCHAP9129	pIVEX2.3MCS-*pilQ*^{8013} *Neisseria meningitidis* (8013)	This study
pCHAP9132	pIVEX2.3MCS-*pilQ*^{8013} *N. meningitidis* (8013) Pro$_{605}$Leu	This study
pHH41	pIVEX2.3MCS-*pilQ*^{PAK} *Pseudomonas aeruginosa* (PAK)	Hoang *et al*. (2011)
pCHAP9162	pIVEX2.3MCS-*pilQ*^{PAK} *P. aeruginosa* (PAK) Pro$_{562}$Leu	This study
pCHAP9166	pIVEX2.3MCS-*lspD Legionella pneumophila*	This study
pCHAP9167	pIVEX2.3MCS-*gspD Shewanella oneidensis*	This study
pCHAP9169	pIVEX2.3MCS-*xpsD Xanthomonas campestris*	This study
pCHAP9164	T7 promoter - *Aeromonas hydrophila* linear template	This study
pCHAP9168	T7 promoter - *Vibrio cholera* linear template	This study

Table S2. Primer sequences

Name	Primer sequence[a] (5′-3′)	Restriction site/mutation
PNN5	GGAAACATTACAGACATCAAAG	
PNN6	CCTCAATAGCGCAGGCTG	
PNN20	GATATTCTCGCCACGctgAGCATCGTTACCCTC	Pro$_{443}$Leu
PNN21	GAGGGTAACGATGCTcagCGTGGCGAGAATATC	Pro$_{443}$Leu
PNN73	CCAAAACGCTTGCCAATgcgCGCGTGCTGACCCAAAAC	Pro$_{613}$Ala
PNN74	GTTTTGGGTCAGCACGCGcgcATTGGCAAGCGTTTTGG	Pro$_{613}$Ala
PNN75	CCAAAACGCTTGCCAATggcCGCGTGCTGACCCAAAAC	Pro$_{613}$Gly
PNN76	GTTTTGGGTCAGCACGCGgccATTGGCAAGCGTTTTGG	Pro$_{613}$Gly
PNN77	CCAAAACGCTTGCCAATgtgCGCGTGCTGACCCAAAAC	Pro$_{613}$Val
PNN78	GTTTTGGGTCAGCACGCGcacATTGGCAAGCGTTTTGG	Pro$_{613}$Val
PNN79	CCAAAACGCTTGCCAATtgcCGCGTGCTGACCCAAAAC	Pro$_{613}$Cys
PNN80	GTTTTGGGTCAGCACGCGgcaATTGGCAAGCGTTTTGG	Pro$_{613}$Cys
PNN81	CCAAAACGCTTGCCAATttcCGCGTGCTGACCCAAAAC	Pro$_{613}$Phe
PNN82	GTTTTGGGTCAGCACGCGgaaATTGGCAAGCGTTTTGG	Pro$_{613}$Phe
PNN38	CGCTTGCCAATCCGagcGTGCTGACCCAAAACC	Arg$_{614}$Ser
PNN39	GGTTTTGGGTCAGCACgctCGGATTGGCAAGCG	Arg$_{614}$Ser
PNN43	CCAAAACGCTTGCCaccCCGCGCGTGCTGACCC	Asn$_{612}$Thr
PNN44	GGGTCAGCACGCGCGGggtGGCAAGCGTTTTGG	Asn$_{612}$Thr
PNN34	CGCTTGCCAATctgCGCGTGCTGACCC	Pro$_{605}$Leu
PNN35	GGGTCAGCACGCGcagATTGGCAAGCG	Pro$_{605}$Leu
PNN71	CGGCGAGATCGTCTCCCAActgAAGGTCGTGACCTCGGACAAG	Pro$_{562}$Leu
PNN72	CTTGTCCGAGGTCACGACCTTcagTTGGGAGACGATCTCGCCG	Pro$_{562}$Leu
PNN30	gaattccatatgGGAAACATTACAGACATCAAAG	NdeI
PNN31	cgcggatccggccggccTCAATAGCGCAGGCTGTTGCC	BamHI-FseI
PNN59	ggaattccatatgCAAGTAATTGAAATGAATAATTCGC	NdeI
PNN60	cgcggatccCTACAGGGCGCGTACTATGG	BamHI
Legion-For	ggaattccatatgAATGATGTCAATATTTCAACCGTAAC	NdeI
Legion-Rev	cgcgagctcTTATTTGGTCATTTTTGATGAGTATTG	SacI
Shewan-For	ggaattccatatgGAACAATATGCGGCCAACTTTAAG	NdeI
Shewan-Rev	cgcggatccTCATTGTGTTTTATCTTTGTTTTTG	BamHI
Xanth-For	ggaattccatatgGCCGATGCACCGGCGGTG	NdeI
Xanth-Rev	cgcggatccTCAGCGCGTGCGCGGCGC	BamHI
Aerom-Lin-F	ctttaagaaggagatataccatgACCGAATATTCCGCCAGCTTC	T7 promoter
Aerom-Lin-R	tgatgatgagaacccccccTTACTTGTTACCCACGAATGGC	T7 terminator
Vibrio-Lin-F	ctttaagaaggagatataccatgAACGAGTTTAGCGCCAGCTTTAAAG	T7 promoter
Vibrio-Lin-R	tgatgatgagaacccccccTCATTGCTTGGCTTCCATCTG	T7 terminator

[a] Lower case letters correspond to either restriction enzyme sequence, base substitution in mutagenic primers or additional sequence recommended for linear template production.

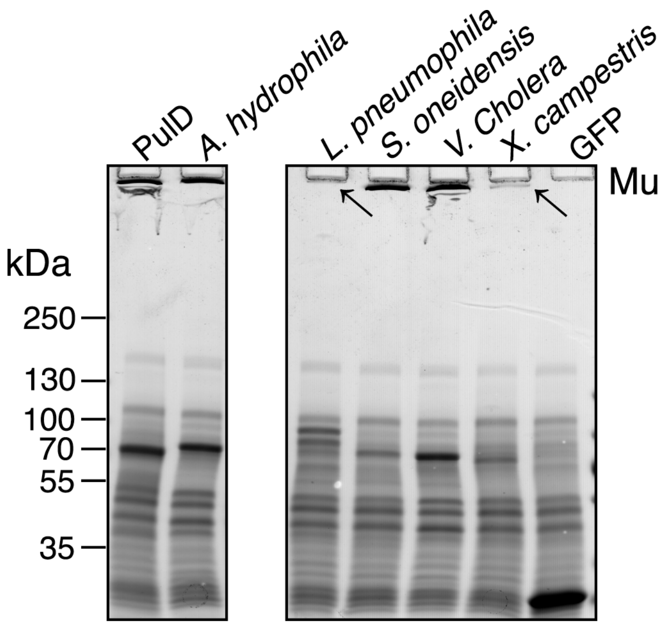

FIG S1 Multimerization of T2SS secretins. Secretins from *Klebsiella oxytoca* (PulD), *A. hydrophila* (ExeD), *L. pneumophila* (LspD), *S. oneidensis* (GspD), *V. Cholera* (EpsD), *X. campestris* (XcsD), and a green fluorescent protein (GFP) control were synthesized in the *E. coli* RTS 100 *in vitro* transcription-translation system supplemented with lecithin liposomes. Aliquots from each sample were diluted in SDS sample buffer, separated by SDS-PAGE on a 4-20% gradient gel and stained with Coomassie blue. Secretin multimers are visible at the bottom of sample wells and arrows denote trace amounts of *L. pneumophila* and *X. campestris* multimer. Mu, multimer.

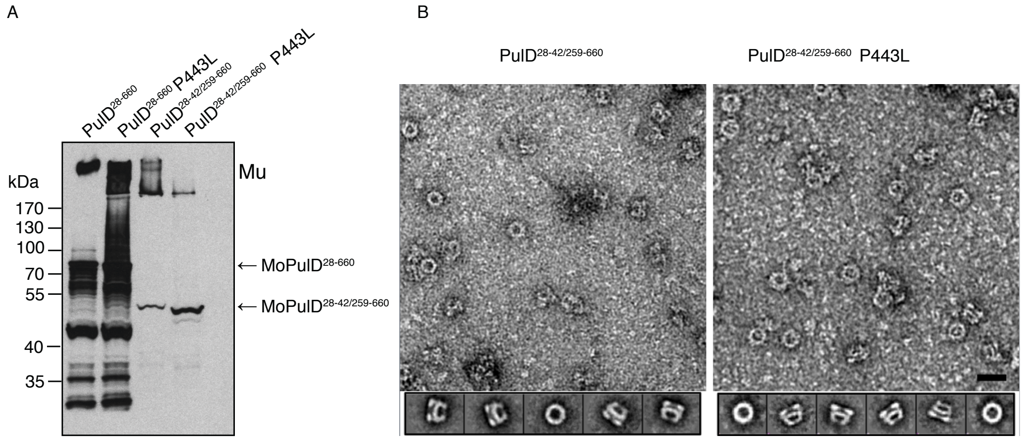

FIG S2 PulDP443L can multimerize and is structurally similar to wild type PulD. (A) Total *in vitro* synthesis reactions of full length (PulD$^{28-660}$) and truncated (PulD$^{28-42/259-660}$) PulD wild type and P443L variant analyzed by SDS-PAGE and immunoblotting with PulD-specific antisera. Mu, multimer; Mo, monomer. (B) PulD$^{28-42/259-660}$ and PulD$^{28-42/259-660}$P443L produced in the *in vitro* synthesis reaction, purified on magnetic nickel beads, negatively stained and analyzed by electron microscopy. Bottom panels show class averages of 363 (PulD$^{28-42/259-660}$) and 307 (PulD$^{28-42/259-660}$P443L) manually selected secretin particles. The scale bar represents 25 nm.