Supplementary Material

Type III secretion filaments as scaffolds for inorganic nanostructures

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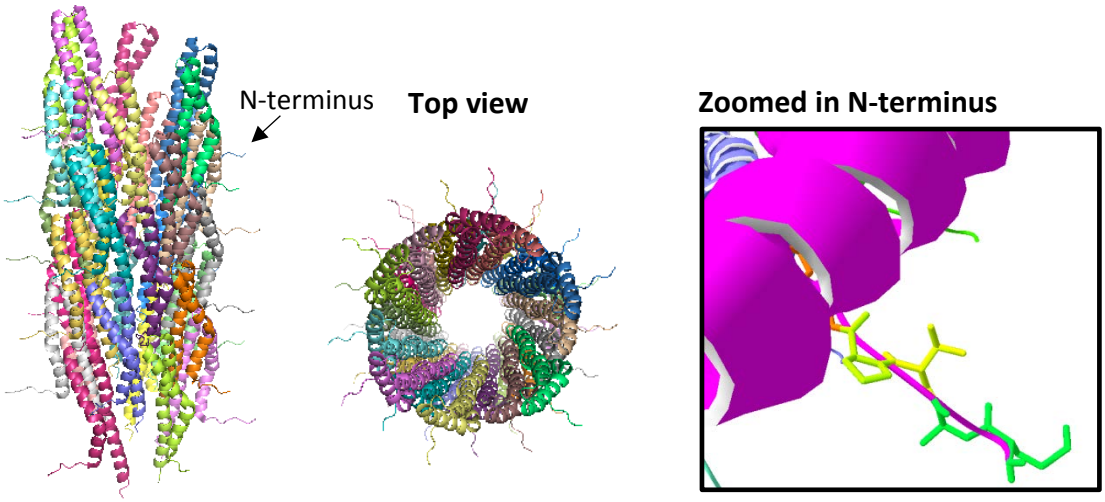

Figure S1. Atomic model of oligomerized PrgI needle from Loquet *et al.* (PDB code 2LPZ) [1]

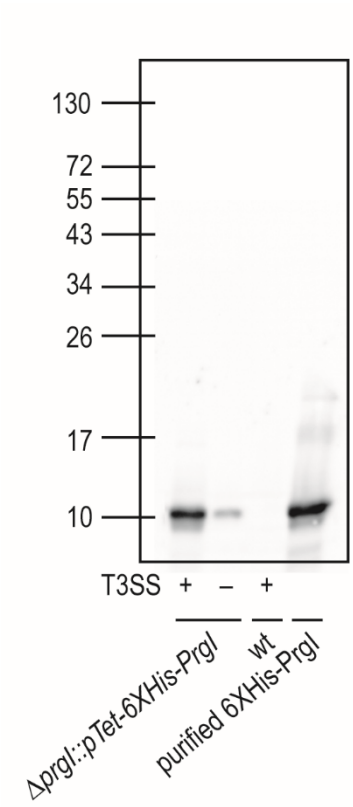

Figure S2. Anti-polyhistidine western blot showing presence of 6XHis-PrgI in samples containing needles sheared from the Δ*prgI* strain with plasmid-borne 6XHis-PrgI, grown with and without T3SS-inducing conditions. These are compared with non-modified needles sheared from wild type cells and recombinant, purified 6XH-PrgI. Cells expressing 6XH-PrgI from the *pTet* promoter were induced with 42 ng/ml aTc.

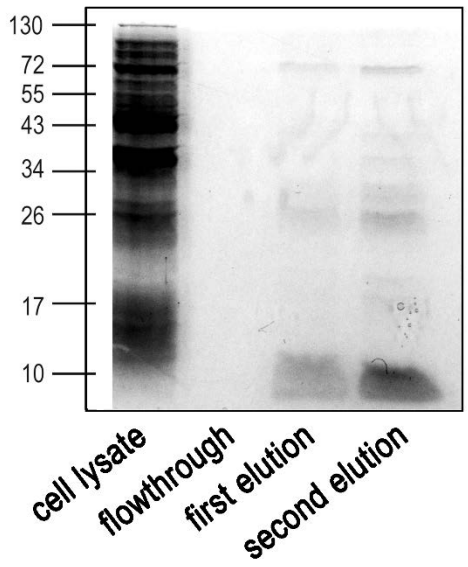

Figure S3. SDS-PAGE showing stages in 6XHis-PrgI purification from soluble BL21 cell lysate using Ni^{2+} affinity column chromatography.

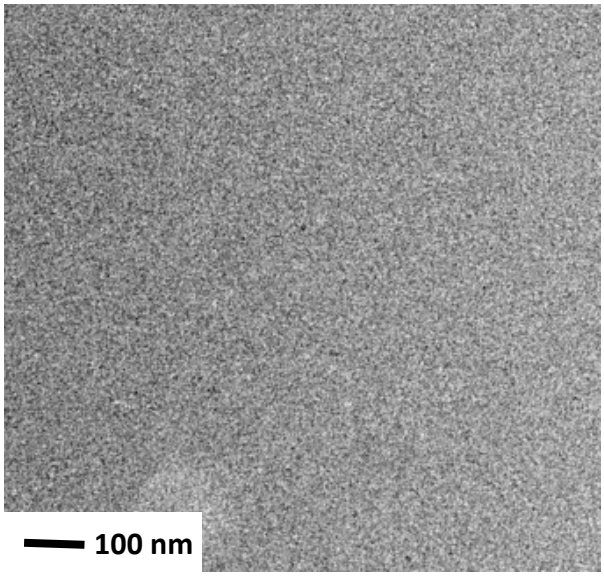

Figure S4. Recombinant 6XHis-PrgI$^{Q26A/K50A}$, when expressed and purified, does not assemble into filaments. Image from 17 days after purification.

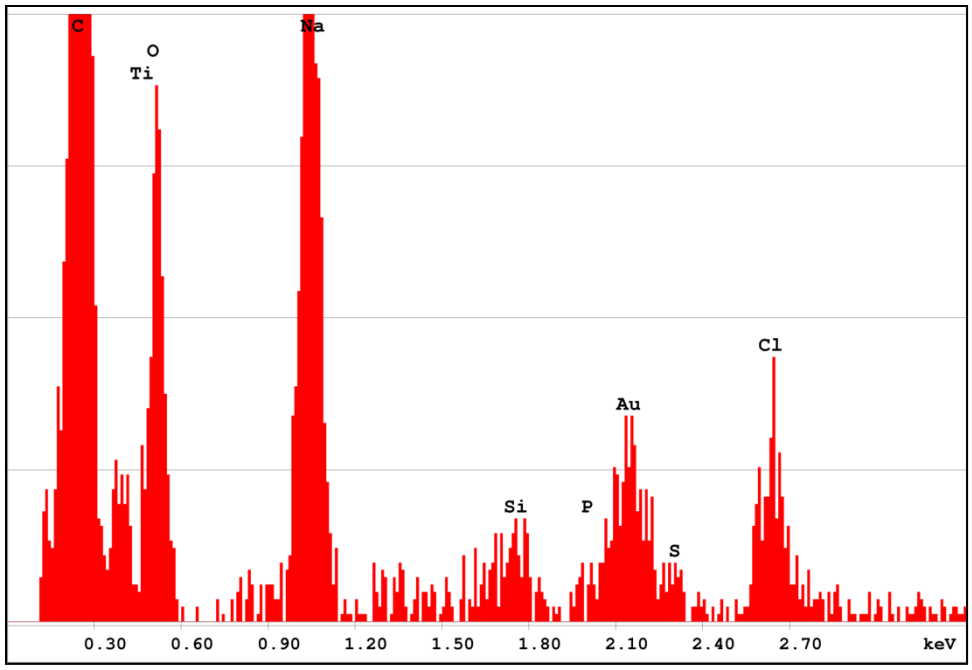

Figure S5. Energy dispersive X-ray spectroscopy confirmed that the contiguous structures observed with TEM contained Au after several washes in 20 mM HEPES buffer. Other peaks include Si/Ti/O from the substrate which the structures were deposited on, Na/Cl from salts in the buffer, and organic elements from the proteins.

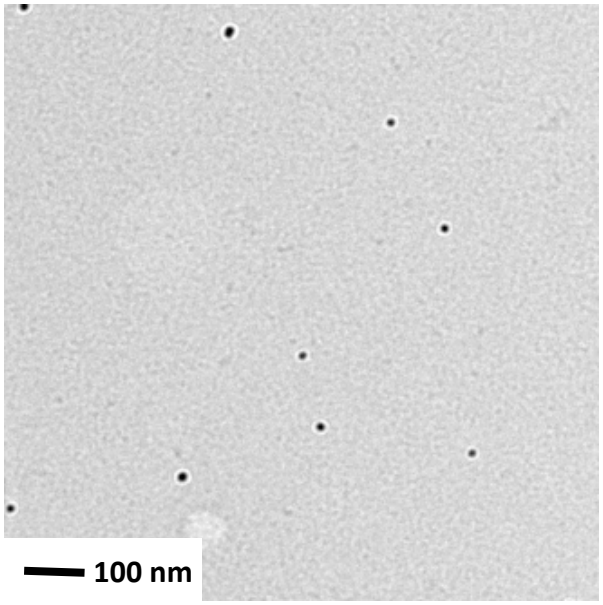

Figure S6. Non-stained TEM image showing growth of Au particles, but lack of contiguous, filament-based networks, using wild type recombinant PrgI filaments in Au reduction conditions.

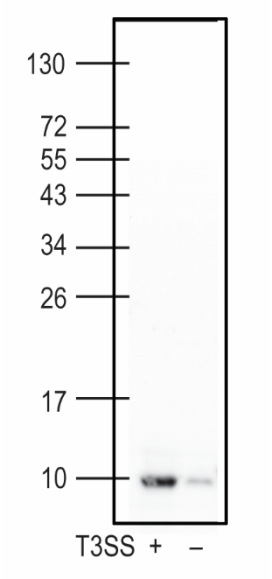

Figure S7. Anti-polyhistidine western blot showing presence of 6XHis-PrgI in samples containing needles sheared from the genomically modified 6XHis-PrgI strain, in T3SS inducing and non-inducing conditions.

Table SI. Plasmid constructs used in this study. Strains are wild type (wt) *S. enterica* and variants of the same unless otherwise indicated.

	Strain	**Plasmid**	**Description**
pAA17	wt	SptP-6XH-DH-FL	*SptP*-DH-FLAG tag/CmR/ColE1
pAA18	Δ*flhCD*	SptP-6XH-DH-FL	same
pAA19	Δ*prgI*Δ*flhCD*	SptP-6XH-DH-FL	same
pAA20	*6XH-prgI* Δ*flhCD*	SptP-6XH-DH-FL	same
pAA21	Δ*flhCD*	pTet-6XHis-prgI	aTc-inducible 6XH-PrgI/KanR/p15a
pAA22	Δ*flhCD*	pTet-prgI	aTc-inducible PrgI/KanR/p15a
pAA23	BL21 *E. coli*	pET28b -6XHis-prgI$^{Q26A/K50A}$	Recombinant 6XHis-prgI$^{Q26A/K50A}$/KanR/p15a
pAA24	BL21 *E. coli*	pET28b-6XH-prgI	Recombinant 6XH-PrgI /KanR/ColE1

Table SII. Primers used in this study.

Primers used for creating Δ*flhCD* strain using recombineering [2]

Insertion of *cat-sacB* cassette in genome, first round of recombineering
Forward primer: GTGCGGCTACGTCGCACAAAAATAAAGTTGGTTATTCTGGTGTGACGGAAGATCACTTCG Reverse primer: TGACTTACCGCTGCTGGAGTGTTTGTCCACACCGTTTCGGATCAAAGGGAAAACTGTCCATAT
Deletion of *cat-sacB* cassette in second round of recombineering to make scarless knock-in:
Forward primer: GTGCGGCTACGTCGCACAAAAATAAAGTTGGTTATTCTGGCCGAAACGGTGTGGACAAAC Reverse primer: TGACTTACCGCTGCTGGAGTGTTTGTCCACACCGTTTCGG
Sequencing primers used to confirm clean deletion:
Forward primer (upstream of *flhC*): GAGGCTGCGTTATACGTCACAATG Reverse primer (downstream of *flhD*): CAACAGCGGAAGGATGATGTCGT

Primers used for creating Δ*prgI* strain and integration of *6XHis-prgI* in the *S. enterica* genome [2]

Insertion of *cat-sacB* cassette in genome, first round of recombineering:
Forward primer: AGGCCATTGGTATTTCCCAAGCCCACTTTAATTTAACGTAAATAAGGAAGTCATTATCAAAGGGAAA ACTGTCCATAT Reverse primer: TAACGGCATTCTCAGGGACAATAGTTGCAATCGACATAATCCACCTTATAACTGATGTGACGGAAG ATCACTTCG
Deletion of *cat-sacB* cassette in second round of recombineering to make clean deletion:
Forward primer: AAAAGATGACTGGCTCAAGGGGCGCTCATTTCAGTACGGGGCGGAAGGTTATATCAAAATGAGCCC AGGCCATTGGTATTTCCCAAGCCCACTTTAATCAGTTATAAGGT Reverse primer: CAATGTCCGTTTCCATAGACCTGATATTGACCGCCTGCCCTATAACGGCATTCTCAGGGACAATAGT TGCAATCGACATAATCCACCTTATAACTGATTAAAGTGGGCTT
Sequencing primers used to confirm clean deletion:
Forward primer (homology to *prgH*): CGGAAGGTTATATCAAAATGAGCCC Reverse primer (homology to *prgJ*): TTTCCATAGACCTGATATTGACCGC
Insertion of *6XHis-prgI* in *prgI* locus:
Forward primer: CCCAAGCCCACTTTAATTTAACGTAAATAAGGAAGTCATTATGCACCACCACCACCACCACGCAACA CCTTGGTCAGG Reverse primer: GGACAATAGTTGCAATCGACATAATCCACCTTATAACTGATTAACGGAAGTTCTGAATAATGGC

Primers for cloning PrgI from *S. enterica* genome

Forward primer: AGTATCGAATTCATGAGATCTATGGCAACACCTTGGTCAG
Reverse primer: AGTATCCTCGAGTTAGGATCCTTAACGGAAGTTCTGAATAATGG

Primer for appending 6XHis-tag N-terminally to PrgI

Forward primer:
AGTATCGAATTCATGAGATCTATGGCACATCATCACCATCACCACACACCTTGGTCAG
Reverse primer: AGTATCCTCGAGTTAGGATCCTTAACGGAAGTTCTGAATAATGG

Primers for making V65A and V67A solubility-enhancing mutations in PrgI via Quikchange

Forward primer: GCAATCGAACACGGCAAAAGCCTTTAAGGATATTGATG
Reverse primer: CATCAATATCCTTAAAGGCTTTTGCCGTGTTCGATTGC

Primer for cloning PrgI into pET28b(+) vector

Forward primer: AGTATCCATATGGCAACACCTTGGTCAGGCTATCTG
Reverse primer: AGTATCGGTCTCACTTA ACGGAAGTTCTGAATAATGGC

References

1. Loquet A, Sgourakis NG, Gupta R, Giller K, Riedel D, Goosmann C, Griesinger C, Kolbe M, Baker D, Becker S, *et al.* 2012 Atomic model of the type III secretion system needle. *Nature* **486**, 276-279. (doi:10.1038/nature11079)
2. Thomason L, Court DL, Bubunenko M, Costantino N, Wilson H, Datta S, Oppenheim A. 2007 Recombineering: genetic engineering in bacteria using homologous recombination. *Curr. Protoc. Mol. Biol.* **78**, 1.16.1–1.16.24. (doi:10.1002/0471142727.mb0116s78)