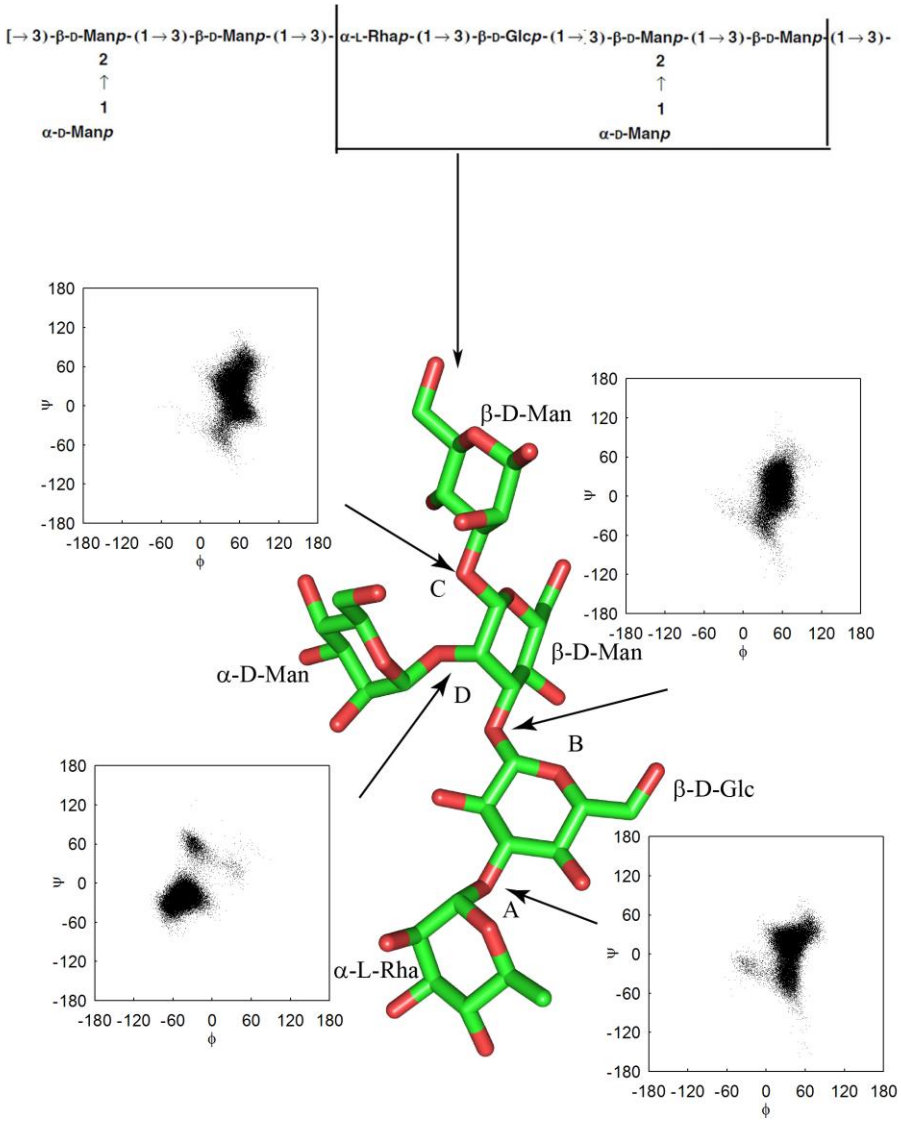
Supplemental information

Methods and Results

Generation of Psl spatial structure. An energy minimized spatial structure model of Psl polysaccharide repeat unit (α-L-Rhap-(1→3)-β-D-Glcp-(1→3)-[α-D-Manp-(1→2)]-β-D-Manp-(1→3)-β-D-Manp-(1-1)-OH) was generated by the Glycam Biomolecule Builder (Woods Group. (2005-2013) GLYCAM Web. Complex Carbohydrate Research Center, University of Georgia, Athens, GA. http://www.glycam.com). Oligosaccharides are usually highly flexible under physiological conditions, but the model generated by Glycam is a single static structure. Therefore, we employed a molecular dynamics (MD) simulation in aqueous solution (TIP3P water model) {William L. Jorgensen, 1983 #2061} to study the dynamic properties of Psl exopolysaccharide. The MD simulation was performed for 100 ns under NPT canonical ensemble using the NAMD program {James C. Phillips, 2005 #2063} with a 2-fs integration step and the CHARMM36 all-atom carbohydrate force field. The trajectories were monitored and analyzed using the program VMD {Humphrey W, 1996 #2062}. Glycosidic linkage conformation was represented by dihedral angles ϕ (H1-C1-Ox-Cx) and ψ (C1-Ox-Cx-Hx), defined by the hydrogen atoms at the linkages. The final structural model of Psl repeat unit is shown in following image. Atoms are indicated by different colors: carbon, green; oxygen, red; nitrogen, blue. Insets present the predicted dihedral angle distributions of the glycosidic linkage. The average glycosidic linkage dihedral angles for Psl repeat unit are compiled in panel B.

A



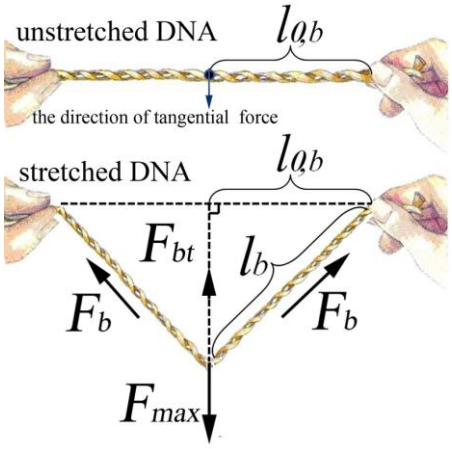
B

Average glycosidic linkage dihedral angles for Psl polysaccharide repeat unit

glycosidic linkage	ϕ(°)	ψ(°)
α-L-Rhap-(1-3)-β-D-Glcp	36±15	8±25
β-D-Glcp-(1-3)-β-D-Manp	50±13	13±25
β-D-Manp-(1-3)-β-D-Manp	53±14	22±28
α-D-Manp-(1-2)-β-D-Manp	-44±26	-18±22

The possibility of a DNA strand to promote the T4P-mediated bacterial migration.

The bacterial T4P works by extension and retraction of the pili to pull the bacterium forward {Skerker, 2001 #1931}; {Bertrand, 2010 #1930}. If bacteria use eDNA as the surface to drive bacterial migration within biofilm, then one would question whether a DNA strand could handle the force due to the retraction of T4P. Bacteria attached to a surface can generate forces ≈ 100 pN via retraction of T4P (F_{T4P}) {Opitz, 2009 #2037}. The tensile strength of DNA ($F_{TS\ of\ DNA}$) is ≈ 500 pN {Opitz, 2009 #2037}. If a bacterium moves along the DNA strand by T4P, the strength of DNA would be large enough to sustain the retracting of T4P since the tensile strength of DNA (500 pN) is much larger than the force generated by T4P (F_{T4P} = 100 pN). If the release of Psl occurred while a bacterium was crawling on an eDNA strand, it would also lead to the forming of an eDNA-Psl fiber. If T4P is bound vertically to a dsDNA strand, can the DNA bear the force from the retraction of T4P? A DNA strand is able to bear a maximum tensile strength F_b = 476 ± 84 pN {Opitz, 2009 #2037}. At the breaking point, the stretched (l_b) and unstretched ($l_{0,b}$) length of DNA conforms to the equation: $l_b / l_{0,b}$ = 2.14 ± 0.20 {Opitz, 2009 #2037}. As a result, when pulled by a maximum tangential force (F_{max}), the DNA strand will be bent and generate an equal and contrary force (F_{bt}), which is equal to



$2\times\sqrt{1-(\frac{l_{0,b}}{l_b})^2}\times F_b = 842$ pN according to Pythagorean Theorem. The maximum tangential force (F_{max}) is much more than the force of T4P (F_{T4P} = 100 pN), thus making it possible for a DNA strand to promote the retraction of T4P at vertical direction.

Legends for supplemental figures

Figure S1 The location of eDNA-Psl fibers web in a pellicle of PAO1. Serial horizon optical section images of a 5 μm-thick pellicle were shown. Psl was stained by HHA-TRITC in red. eDNA and bacterial chromosomal DNA were stained by SYTO9 in green. The chromosomal DNA in bacteria were concentrated dots, yet eDNA appeared diffused and had fiber-look.

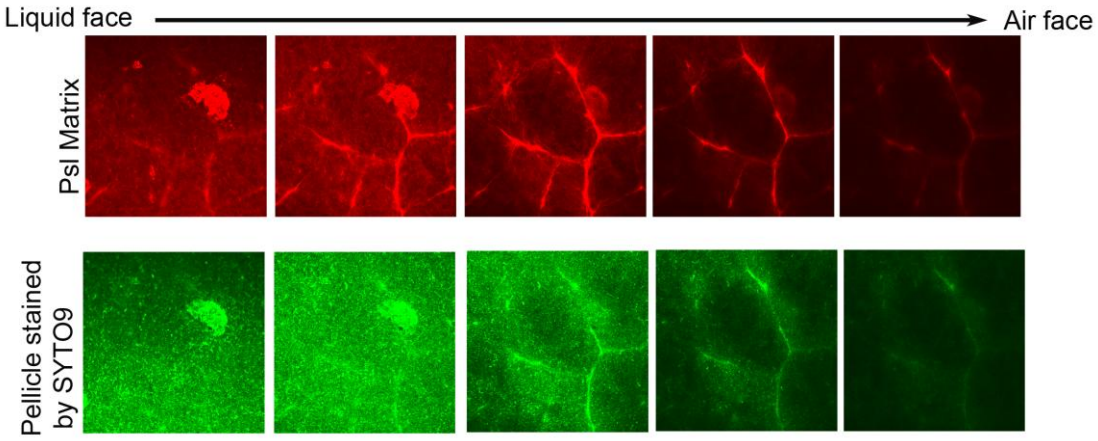


Figure S2 The radial pattern eDNA-fiber webs observed at biofilm microcolony initiation stage. (A) eDNA-fiber webs in pellicles of the wild type *P. aeruginosa* strain PAO1 at biofilm microcolony initiation stage. The big arrow indicated a radial pattern eDNA-fiber web and the small white arrows indicated the clearly visible eDNA fibers in the area with less bacteria. **(**B) The radial pattern Psl-fiber web at biofilm microcolony initiation stage. Psl was stained by HHA-TRITC (red). (C) The radial pattern eDNA fibers (indicated by white arrows) found in the pellicle of *algC* mutant, an exopolysaccharide-synthesis-deficient strain. eDNA were stained in red by PI. (D) The radial pattern eDNA-fibers in the flow-cell biofilm of *algC* mutant. eDNA were stained in green by SYTOX Green. Scale bar: 5 μm.

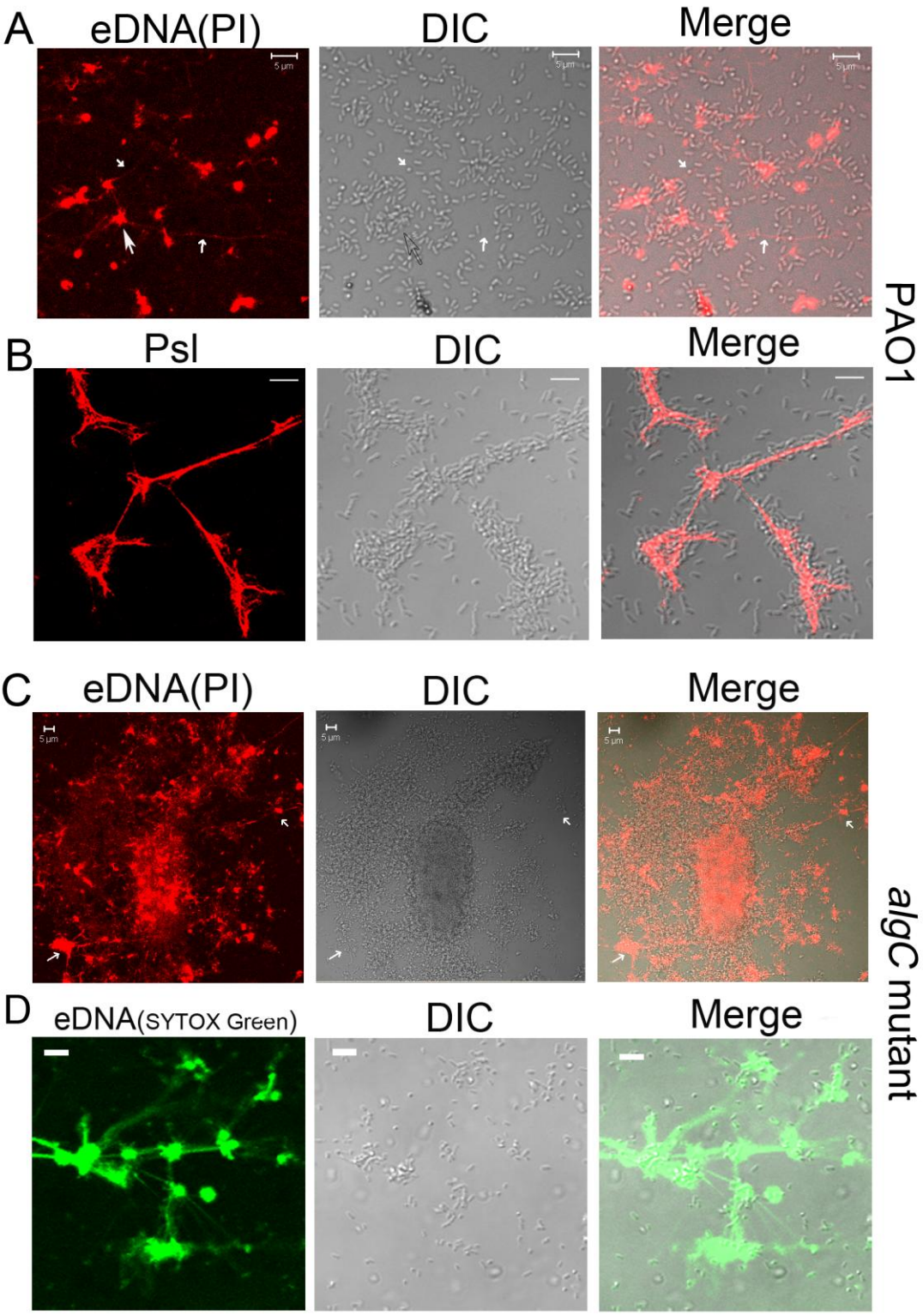

A
eDNA(PI)
DIC
Merge
B
PsI
DIC
Merge
PAO1
C
eDNA(PI)
DIC
Merge
D
eDNA(SYTOX Green)
DIC
Merge
algC mutant

Figure S3 Secretion of eDNA matches the timing of Psl release. (A) The Psl release from bacteria of *P. aeruginosa* PAO1 in a shaking culture. (B) eDNA secretion was increased during the period of Psl release from bacteria in a PBS buffer at standing condition. Psl was detected by immuno-dot blotting with anti-Psl serum.

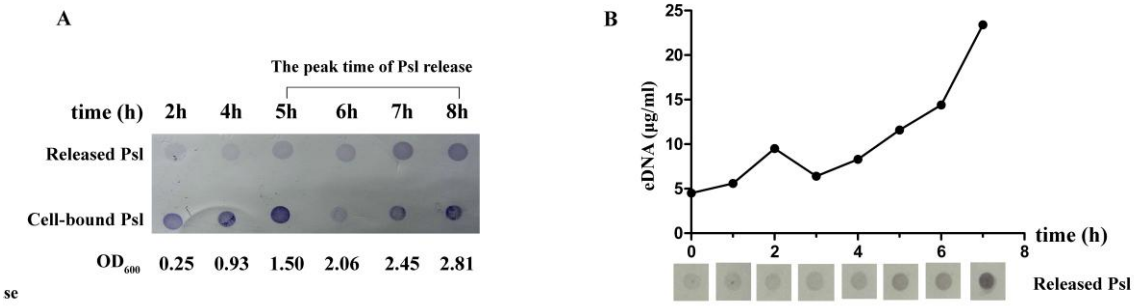


Figure S4 The effect of DNase I on the Psl and eDNA matrix in pellicles of wild type strain PAO1. Shown were the selected images of Psl/eDNA matrix in the middle layer of pellicles after 46 hours growth with DNase I. Psl was stained in red by HHA-TRITC and DNA was stained in green by SYTO9. The corresponding DIC images and the photograph of pellicles in the chambers were also shown. Scale bar: 10 μm.

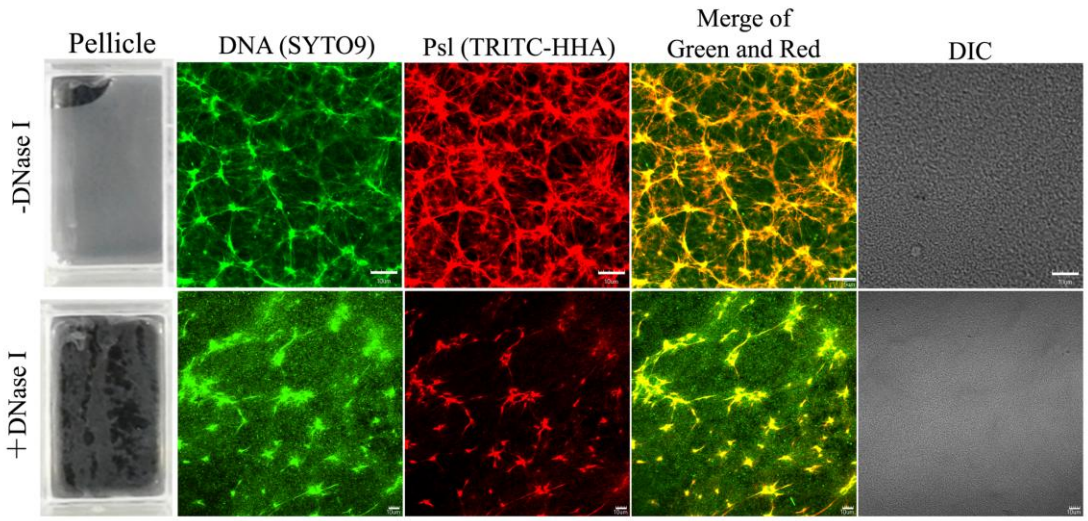


Figure S5 The eDNA fibers (indicated by the arrow) were found in the pellicle of PAO1 strain after 46 hours treatment with manosidase (Psl-digested enzyme). Psl was stained in green by HHA-FITC and eDNA was stained in red by PI. The corresponding DIC image was shown. Scale bar: 10 μm.

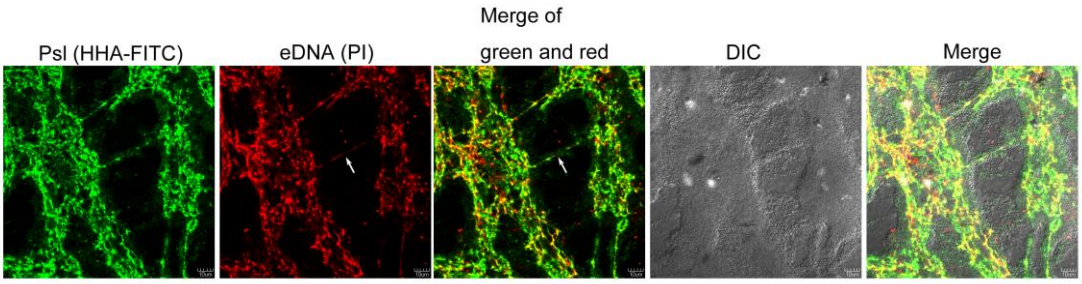


Figure S6 The comparison of DNase I treatment on the biofilm biomass of Psl-negative strain WFPA800 and wild type strains PAO1. Shown are microtiter dish biofilm assay of PAO1 and WFPA800 with/without DNase I treatment. DNase I treatment caused 50% reduction of biofilm biomass in Psl-negative strain compared with the non-treatment control. The same treatment only resulted in a 10% reduction of biofilm biomass in wild type strain.