

## Supporting Information

**Impact of Cellulose Nanocrystals on the Aggregation and Initial Adhesion of**

***Pseudomonas fluorescens* Bacteria**

**Xiaohui Sun<sup>1</sup>, Qingye Lu<sup>1</sup>, Yaman Boluk<sup>1,2</sup>, Yang Liu<sup>1,\*</sup>**

<sup>1</sup>Department of Civil and Environmental Engineering, Faculty of Engineering, University of Alberta, Edmonton, AB, T6G 2W2, Canada

<sup>2</sup>National Institute for Nanotechnology, National Research Council of Canada, Edmonton AB T6G 2M9, Canada

\* To whom correspondence should be addressed: Email: [yang.liu@ualberta.ca](mailto:yang.liu@ualberta.ca),

Phone: 780-492-5115 Fax: 780-492-0249

Number of pages: 7 (including cover sheet)

Number of figures: 4

Number of tables: 1

Text: CNC volume fraction calculations; Microscopy glass slides/Glass cover slips cleaning protocol; Zeta potential measurements of glass slides; Depletion potential calculations; Dissipation change in QCM-D experiments. Figure. S1, Fluorescent microscopy images of bacterial aggregation; Figure. S2 Zeta potential distribution; Figure. S3, Theoretical DLVO interaction energies between bacteria and glass surface with and without CNC; Figure. S4, QCM-D study on bacterial adsorption onto CNC coated silica surfaces; Table S1, Input parameters in the DLVO calculations; References.

### **CNC volume fraction calculations:**

Density of CNC is  $1.5\text{g}\cdot\text{cm}^{-3}$  (Sun et al. 2012).

$$1\% \text{ (Wt) CNC volume fraction} = \frac{1\%}{\text{Density of CNC}} = \frac{0.01\text{g}\cdot\text{g}^{-1}\text{H}_2\text{O}}{1.5\text{g}\cdot\text{cm}^{-3}} = 6.6\times 10^{-3}\text{cm}^3\cdot\text{g}^{-1}\text{H}_2\text{O} = 6.6\times 10^{-3}\text{ml}\cdot\text{ml}^{-1}\text{H}_2\text{O}$$

In the current study, 1 mL 1.0% (wt) CNC suspension was added to 1 mL of the bacterial suspension, thus CNC weight concentration was 0.5%.

$$0.5\% \text{ (Wt) CNC volume fraction} = \frac{6.6\times 10^{-3}\text{ml}\cdot\text{ml}^{-1}}{2} = 3.3\times 10^{-3}\text{ml}\cdot\text{ml}^{-1}$$

### **Microscopy glass slides/Glass cover slips cleaning protocol:**

Prior to each experiment, the slide pieces ( $1\text{cm} \times 1\text{cm}$ ) were thoroughly rinsed with deionized (DI) water to remove visible impurities (large particles). Subsequently, the slides were immersed in 1N HCl and sonicated for 10 minutes to remove grease. After sonication, the slides were rinsed with sterilized ultrapure water, 70% ethanol, and sterilized ultrapure water successively. Finally, the drying process was achieved in a biosafety cabinet (CLASS II Type A2, Microzone Cor., Canada). The clean slides were reserved as bare slides used in the bacterial aggregation and adhesion experiments (Hwang et al. 2012).

### **Zeta potential measurements of glass slides:**

To determine the surface charge of bare glass slide surfaces, silicon dioxide particles (approx. 99%; particle size 0.5-10  $\mu\text{m}$  with 80 % being between 1-5  $\mu\text{m}$ . Sigma - Aldrich) were used. Zeta potential of silicon dioxide particles was measured in 10 mM NaCl solution with no pH adjustment (pH = 6.0-6.2) using a Malvern Zetasizer Nano-ZS (Model: ZEN3600, Malvern Instruments Ltd, Worcestershire, UK) at 25 °C. Zeta potential is reported to be  $-36.3 \pm 0.15$  mV in this study.

### **Depletion potential calculations:**

The depletion potential  $W$  for the same colloidal spheres with the radius  $R$  in the presence of rod-like particles is given as:

$$W(h) = -\frac{2}{3}k_B T \varphi_r \frac{L}{D} \frac{R}{D} \left(1 - \frac{h}{L}\right)^3 \quad (3)$$

where  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature,  $L$  is the length,  $D$  is the diameter,  $\varphi_r$  is the volume fraction of rod-like particles (CNC particles here),  $R$  is the diameter and  $h$  is the surface-to-surface distance of bigger spherical colloidal particles (bacterial cells

here). One can estimate the needed depletant concentration if the attraction energy of  $-3 k_B T$  is assumed sufficient to induce the phase separation of large particles (Lekkerkerker and Tuinier 2011).

Parameters used in the current study:

$$L = 100 \text{ nm}$$

$$D = 10 \text{ nm}$$

$R = 440$  and  $410$  nm (Equivalent Radii) for *P. flu* CHA0 and *P. flu* CHA19-WS cells respectively

$$\varphi_r = 3.3 \times 10^{-3} \text{ ml} \cdot \text{ml}^{-1}$$

According to equation 3, the depletion potential  $W(h)$  was about  $-1 k_B T$ , which is less than  $-3 k_B T$ . Therefore, the depletion aggregation was unfavorable in the current study.

#### **Dissipation change in QCM-D experiments:**

$\Delta D$  is defined by the following equation:

$$\Delta D = \frac{E_{lost}}{2\pi E_{stored}} \quad (4)$$

where  $E_{lost}$  is the energy lost during one oscillation cycle,  $E_{stored}$  is the total energy stored in the oscillator.  $\Delta D$  represents the sum of all processes that induce energy losses in the oscillating system (Rodahl et al. 1995).

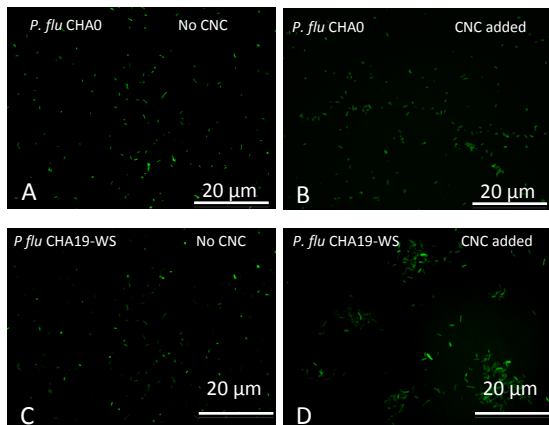


Figure. S1 Fluorescent microscopy images of bacterial aggregation. *P. flu* CHA0 without (A) and with CNC (B); *P. flu* CHA19-WS without (C) and with CNC (D).

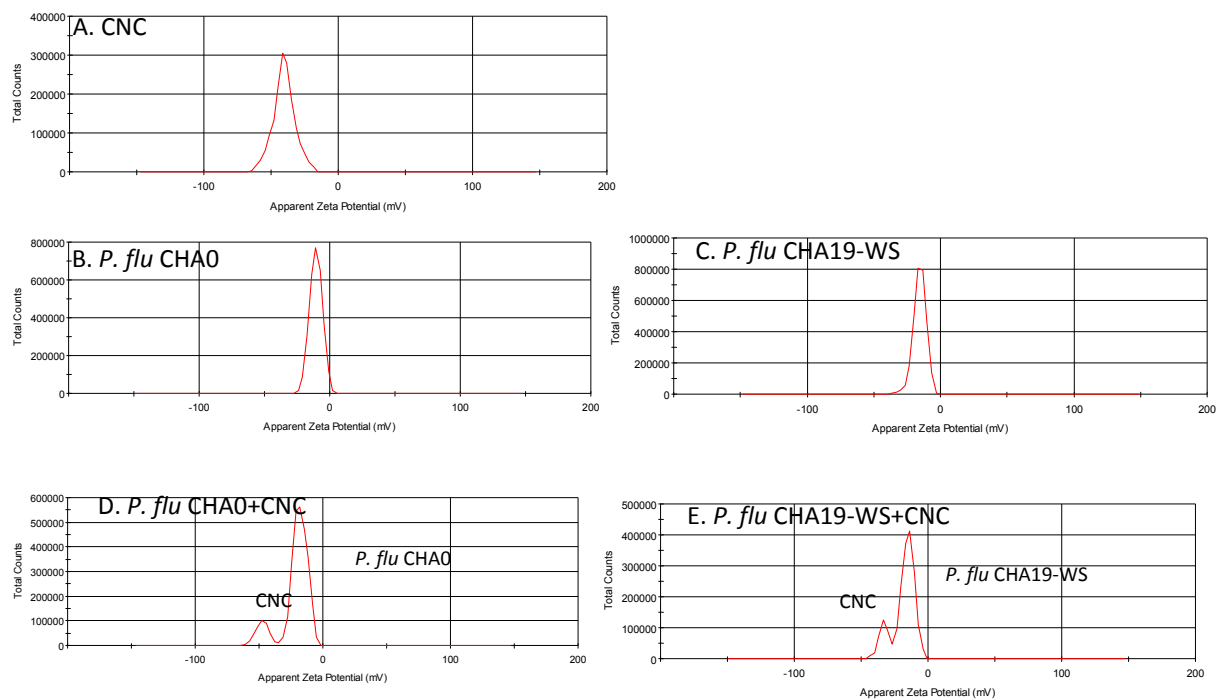


Figure. S2 Representative zeta potential distribution (in 10 mM NaCl solution with no pH adjustment). (A) CNC; (B) *P. flu* CHA0; (C) *P. flu* CHA19-WS; (D) *P. flu* CHA0 and CNC mixture; (E) *P. flu* CHA19-WS and CNC mixture.

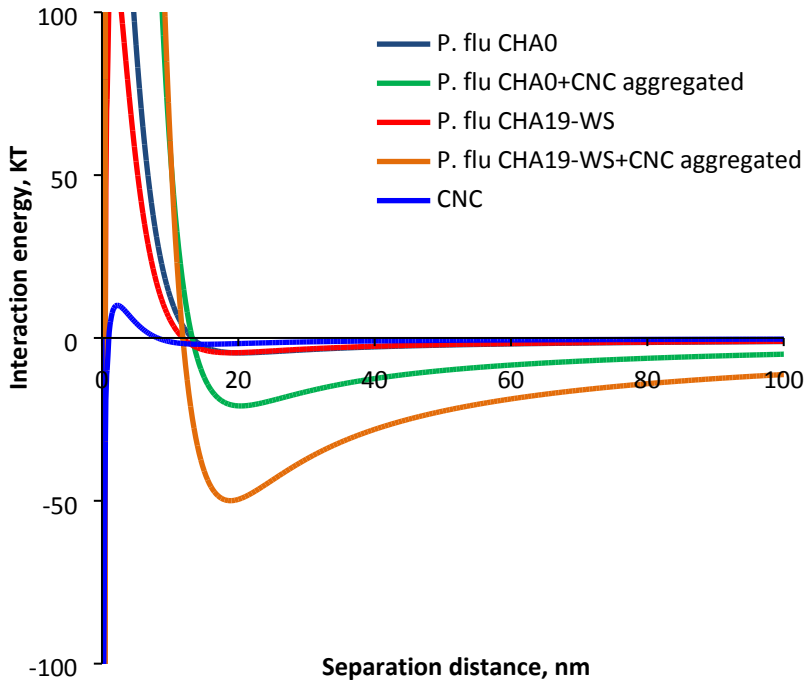


Figure. S3 Theoretical DLVO interaction energies between bacteria and the silica surface with and without CNC, and DLVO interaction energies between CNC particles and the silica surface.

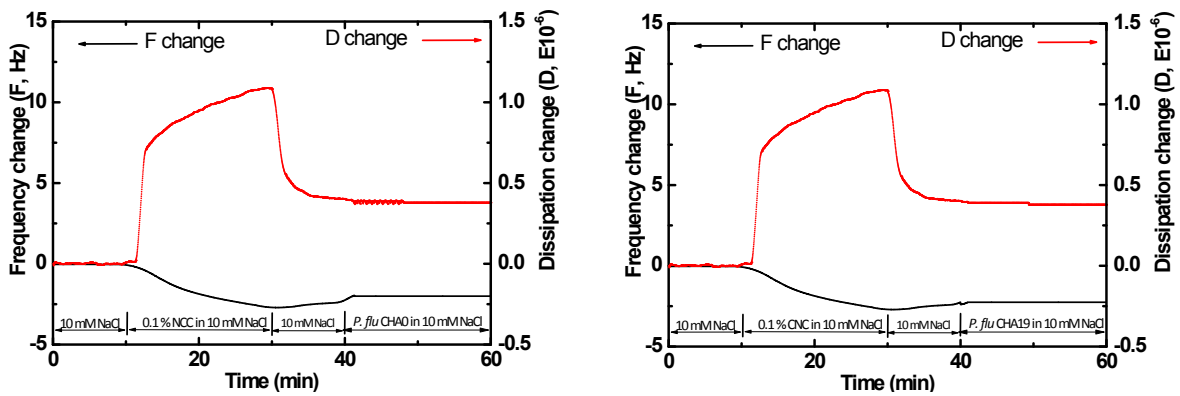


Figure. S4 QCM-D study on bacterial adsorption onto CNC coated silica surfaces. (A) *P. flu* CHA0; (B) *P. flu* CHA19-WS

Table S1 Input parameters in the DLVO calculations

Symbol	Value used
$a_1$ , equivalent radius of <i>P. flu</i> CHA0 cells (m)	$4.4 \times 10^{-7}$
$a_2$ , equivalent radius of <i>P. flu</i> CHA19-WS cells (m)	$4.1 \times 10^{-7}$
$a_3$ , equivalent radius of CNC particles (m)	$2.04 \times 10^{-8}$
$\zeta_1$ , zeta potential of <i>P. flu</i> CHA0 (mv)	-18.78
$\zeta_2$ , zeta potential of <i>P. flu</i> CHA19-WS (mv)	-16.22
$\zeta_3$ , zeta potential of CNC (mv)	-42.30
$\zeta_4$ , zeta potential of glass surface/silica crystal surface (mv)	-36.30
$A_{123}$ , bacterium-water-silica Hamaker constant (J)	$6.16 \times 10^{-21}$ (Rijnaarts et al. 1995a and b)
$A_{123}$ , CNC-water-silica Hamaker constant (J)	$4.4 \times 10^{-20}$
relative permittivity	80.1
vacuum permittivity	$8.854 \times 10^{-12}$
$\kappa^{-1}$ , Debye length (m)	$3.3 \times 10^8$

Note: 1. In NaCl solution, the electric double layer Debye length  $\kappa^{-1}$  is given by  $\kappa^{-1} = \frac{0.304}{\sqrt{[NaCl]}} nm$ , where [NaCl] in the unit of M(Lu et al. 2011).

2. The Hamaker constant for CNC-water-silica is calculated based on the equation  $A_{132} = (\sqrt{A_{11}} - \sqrt{A_{33}})(\sqrt{A_{22}} - \sqrt{A_{33}})$ , where  $A_{11}$  is Hamaker's constant for cellulose particles,  $A_{22}$  is Hamaker's constant for silica, and  $A_{33}$  is Hamaker's constant for water. Hamaker's constants for cellulose, silica, and water used in this study were  $1.1 \times 10^{-20}$  J (Boluk et al. 2012),  $2.0 \times 10^{-20}$  J (Lomboy et al. 2011), and  $3.7 \times 10^{-20}$  J (Lerner et al. 2012), respectively.

## References

- Boluk Y, Zhao LY, Incani V. 2012. Dispersions of Nanocrystalline Cellulose in Aqueous Polymer Solutions: Structure Formation of Colloidal Rods. *Langmuir* 28(14): 6114-6123.
- Hwang G, Kang S, El-Din M, Liu Y. 2012. Impact of an extracellular polymeric substance (EPS) precoating on the initial adhesion of *Burkholderia cepacia* and *Pseudomonas aeruginosa*. *Biofouling* 28:525-38.
- Lekkerkerker HNW and Tuinier R. 2011. *Colloids and depletion*. Springer 100.
- Lerner RN, Lu QY, Zeng HB, Liu Y. 2012. The effects of biofilm on the transport of stabilized zerovalent iron nanoparticles in saturated porous media. *Water Research* 46(4): 975-985.
- Lomboy G, Sundararajan S, Wang KJ, Subramaniam S. 2011. A test method for determining adhesion forces and Hamaker constants of cementitious materials using atomic force microscopy. *Cement and Concrete Research* 41(11): 1157-1166.
- Lu QY, Wang J, Faghihnejad A, Zeng HB, Liu Y. 2011. Understanding the molecular interactions of lipopolysaccharides during *E. coli* initial adhesion with a surface forces apparatus. *Soft Matter* 7:9366-79.
- Rijnaarts HH, Norde W, Bouwer EJ, Lyklema J, Zehnder AJ. 1995a. Reversibility and mechanism of bacterial adhesion. *Colloids and Surfaces B: Biointerfaces* 4(1):5-22.
- Rijnaarts HHM, Norde W, Lyklema J, Zehnder AJB. 1995b. The isoelectric point of bacteria as an indicator for the presence of cell-surface polymers that inhibit adhesion. *Colloids and Surfaces B-Biointerfaces* 4:191-7.
- Rodahl M, Hook F, Krozer A, Brzezinski P, Kasemo B. 1995. quartz-crystal microbalance setup for frequency and q-factor measurements in gaseous and liquid environments. *Review of Scientific Instruments* 66(7): 3924-3930.
- Sun, X.H., Danumah, C., Liu, Y. and Boluk, Y. (2012) Flocculation of bacteria by depletion interactions due to rod-shaped cellulose nanocrystals. *Chemical Engineering Journal* 198, 476-481.