Published online: May 22, 2020

ADVANCED SCIENCE Open Access

www.advancedscience.com

# Virus Disinfection from Environmental Water Sources Using Living Engineered Biofilm Materials

*Jiahua Pu, Yi Liu, Jicong Zhang, Bolin An, Yingfeng Li, Xinyu Wang, Kang Din, Chong Qin, Ke Li, Mengkui Cui, Suying Liu, Yuanyuan Huang, Yanyi Wang, Yanan Lv, Jiaofang Huang, Zongqiang Cui, Suwen Zhao, and Chao Zhong**

Dr. J. Pu, Y. Liu, J. Zhang, B. An, Dr. Y. Li, Dr. X. Wang, K. Li, M. Cui, S. Liu, Dr. Y. Huang, Y. Wang, Dr. J. Huang, Prof. C. Zhong[+],[++]
Materials and Physical Biology Division
School of Physical Science and Technology
ShanghaiTech University
Shanghai 201210, China
E-mail: zhongchao@shanghaitech.edu.cn

K. Din, Prof. S. Zhao
iHuman Institute
ShanghaiTech University
Shanghai 201210, China

K. Din, Y. Lv, Prof. S. Zhao
School of Life Science and Technology
ShanghaiTech University
Shanghai 201210, China

C. Qin, Prof. Z. Cui
State Key Laboratory of Virology
Wuhan Institute of Virology
Chinese Academy of Sciences
Wuhan 430071, China

C. Qin
Wuxi Biologics Co., Ltd.
Suzhou 215100, China

S. Liu, Y. Lv
Shanghai Institute of Applied Physics
Chinese Academy of Sciences
Shanghai 201800, China

ACS Macro Letters

Letter

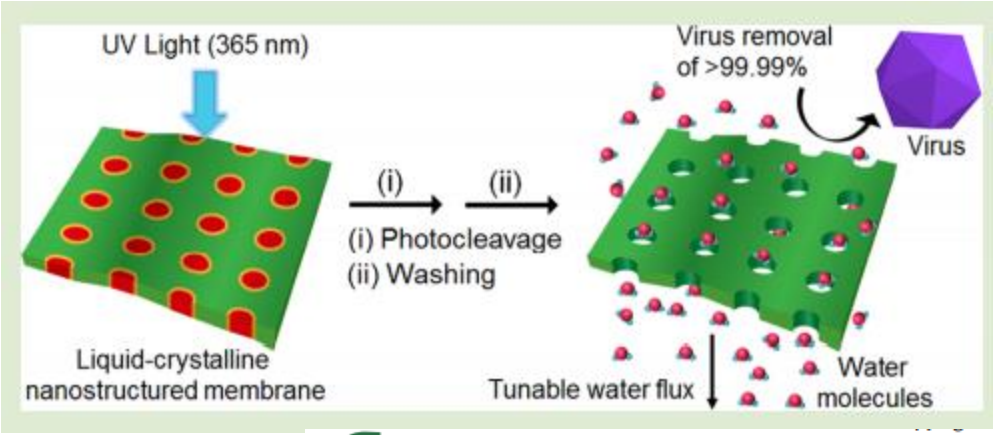
Cite This: ACS Macro Lett. 2019, 8, 1303−1308

pubs.acs.org/macroletters

# Polymerizable Photocleavable Columnar Liquid Crystals for Nanoporous Water Treatment Membranes

Monika Gupta,[†] Yuto Suzuki,[†] Takeshi Sakamoto,[†] Masafumi Yoshio,[†,§] Shotaro Torii,[‡] Hiroyuki Katayama,[*,‡] and Takashi Kato[*,†]

[†]Department of Chemistry and Biotechnology, School of Engineering, and [‡]Department of Urban Engineering, School of Engineering, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan




- **Disinfection byproducts**
- **Resistant viruses**
- **Sizes smaller than membrane pores**

ACS AUTHORCHOICE

Environmental Science & Technology

Environ. Sci. Technol. 2020, 54, 2068−2077

pubs.acs.org/est

Critical Review

# Required Chlorination Doses to Fulfill the Credit Value for Disinfection of Enteric Viruses in Water: A Critical Review

Andri Taruna Rachmadi, Masaaki Kitajima, Tsuyoshi Kato, Hiroyuki Kato, Satoshi Okabe, and Daisuke Sano*

• The **influenza virus (H1N1)** was used as a model viral contaminant.

• **CsgA :**

CsgA protein monomers are secreted from bacterial cells and can self assemble into amyloid nanofibers, and are a major component of *E. coli* biofilms.

• **C5 peptides:**

Influenza-virus-binding peptide. C5 (amino acid sequence: ARLPR) has been shown to bind to **hemagglutinin** (HA), a membrane glycoprotein of influenza virus.

• ***E. Coli*** was transformed to produce engineered fusion monomers that combined CsgA with C5.

• **Polypropylene filler** materials colonized by the CsgA-C5 biofilms were utilized to disinfect river water samples with influenza titers as high as $1 \times 10^7$ PFU $L^{-1}$.

• A suicide gene circuit is designed and applied in the engineered strain that strictly limits the growth of bacteria.

• Highlights the unique biology-only properties of living substances for material applications.

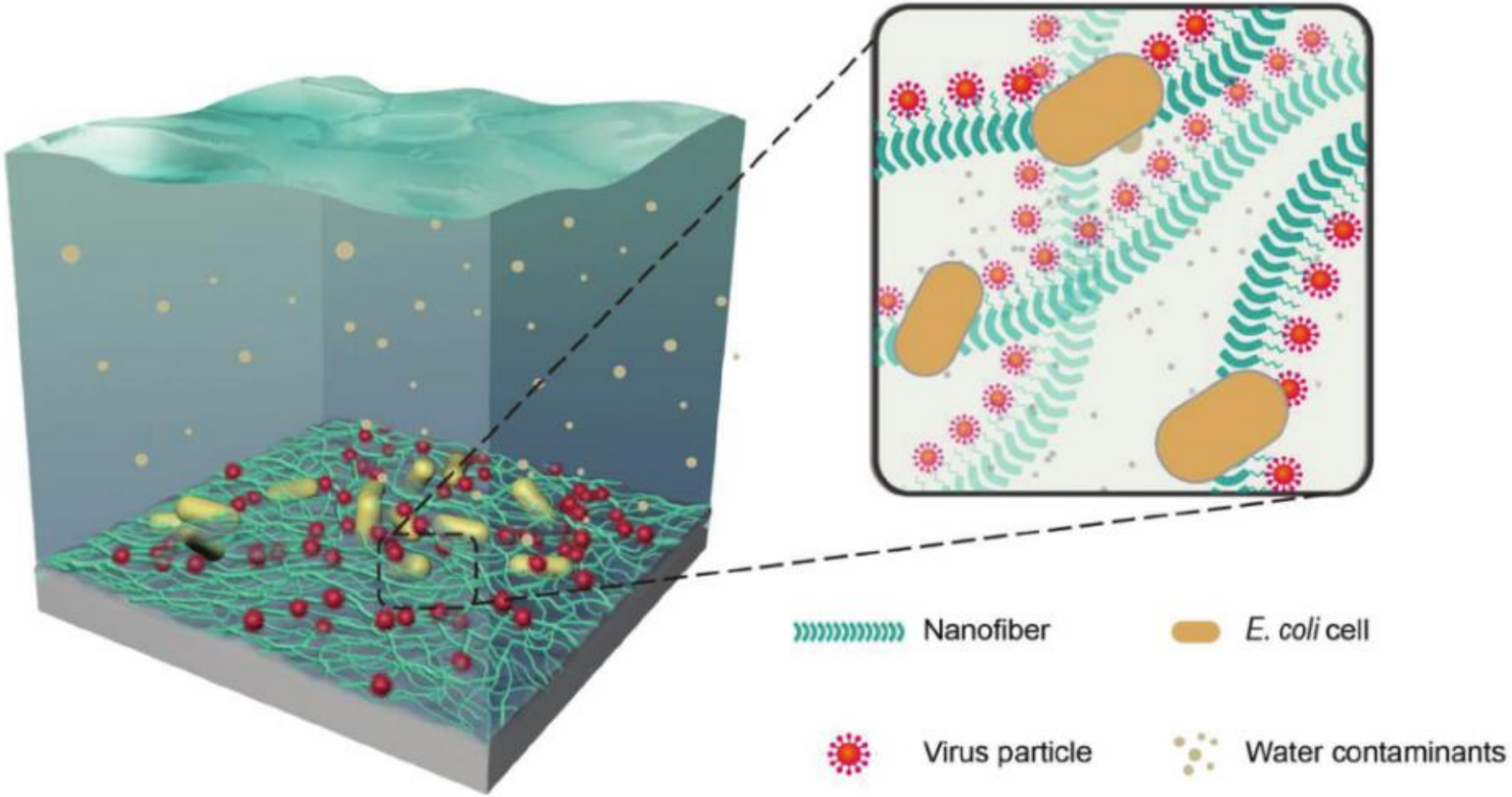



**Figure 1. Schematic diagram of the engineered biofilms for disinfecting viruses from water. CsgA-C5 proteins can be secreted out of the bacteria cells and self-assemble into the amyloid fibers comprising the extracellular matrix of engineered biofilms.**

# C5-CsgA interaction with hemagglutinin

- **C5** influenza-virus-binding peptide has a **high affinity to hemagglutinin**.
- **MODELLER** to build the homology models of **monomer** and **pentamer** fibrillar CsgA-C5.
- **Glide** to get the complex of the monomer CsgA-C5 and hemagglutinin through induced fit docking (IFD).
- Molecular dynamics simulations of the interaction between a CsgA-C5 fusion monomer and hemagglutinin by **GROMACS.** ( $\Delta G_{bind}$ value was about $-62 \pm 22$ kcal $mol^{-1}$).

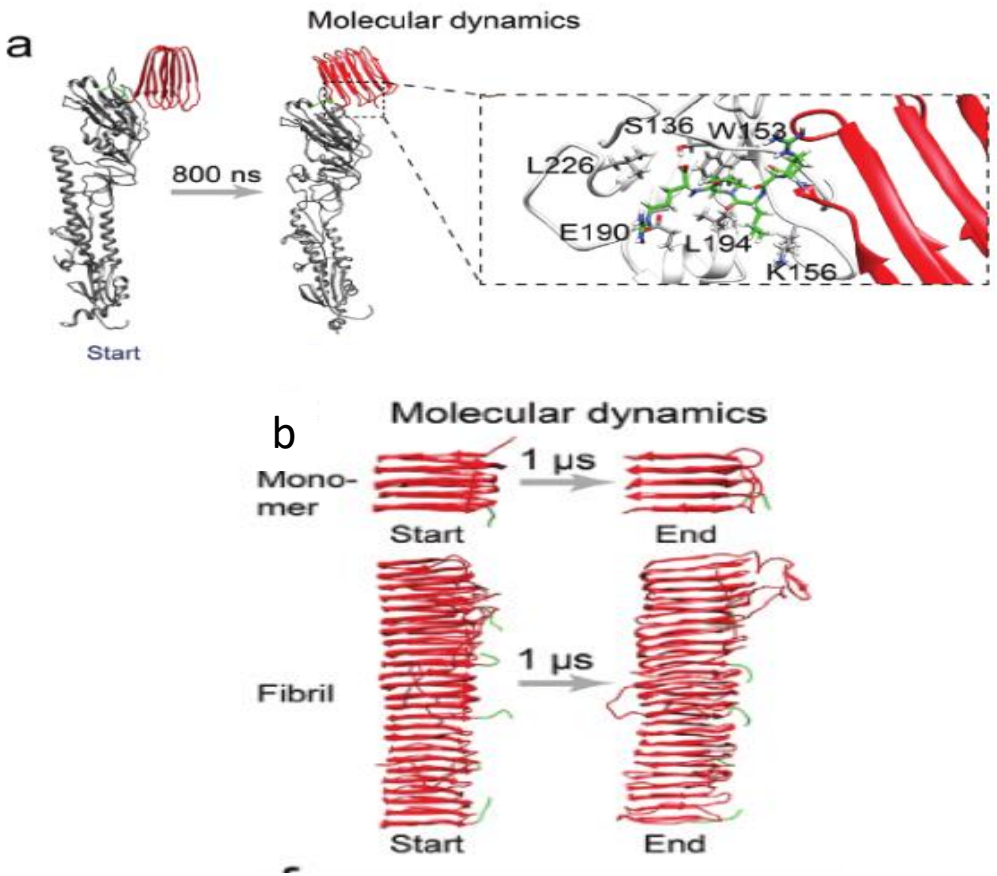



**Figure 2.**

**a) Start and end simulated structures representative of CsgA-C5 monomer interacting with hemagglutinin revealed by molecular dynamics simulations. Simulation time was 800 ns. The interactions among the key residues is expanded.**

**b) The start and end simulated structures for the CsgA-C5 monomer (top) and fibril (bottom) revealed by molecular dynamics simulations. Simulation time was 1 μs.**

# Recombinant *E. coli* cells:

- A recombinant gene combining CsgA and its biological secretion signal (ss) sequence with C-terminal C5 peptide tags was obtained by polymerase chain reaction (PCR).
- The CsgA-C5 fragment was then inserted into **peT-22b** expression vector via T4 ligase.
- Similarly, **CsgA** and polyhistidine tags containing fragment was inserted into peT-22b expression vector via T4 ligase as a control plasmid.
- To obtain the CsgA-C5 or CsgA protein over-expressing cells, pET-22b-CsgA-C5 or pET-22b-CsgA was transformed into commercially available ***E. coli* BL21 (DE3)** cells.
- **Ni-NTA resin** column was used to separate the His-tagged protein from the lysed cell suspension.
- CsgA monomers and CsgA-C5 monomers, migrated as single bands at **14.1 and 14.6 kDa**, respectively, under **SDS polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting.**

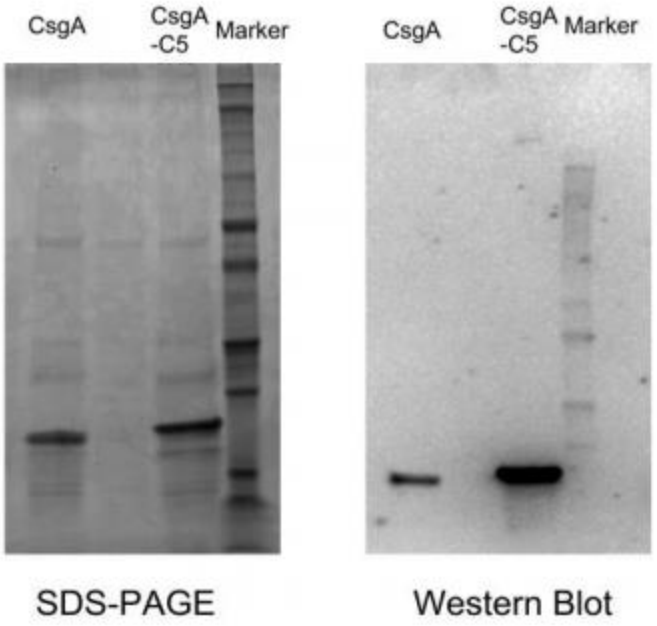
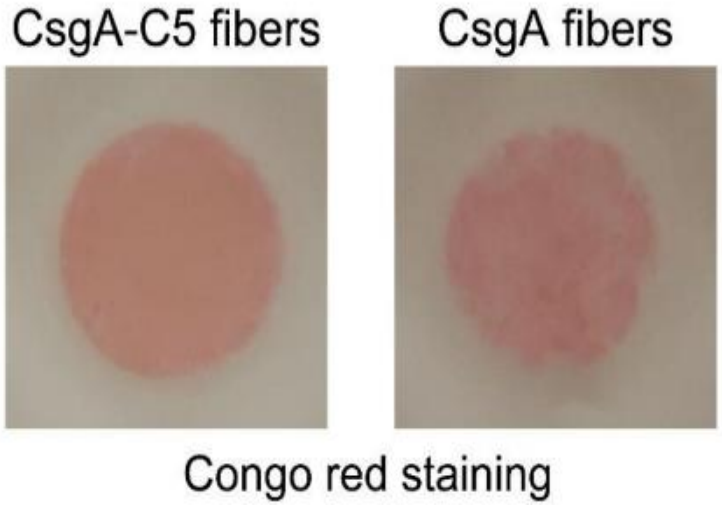
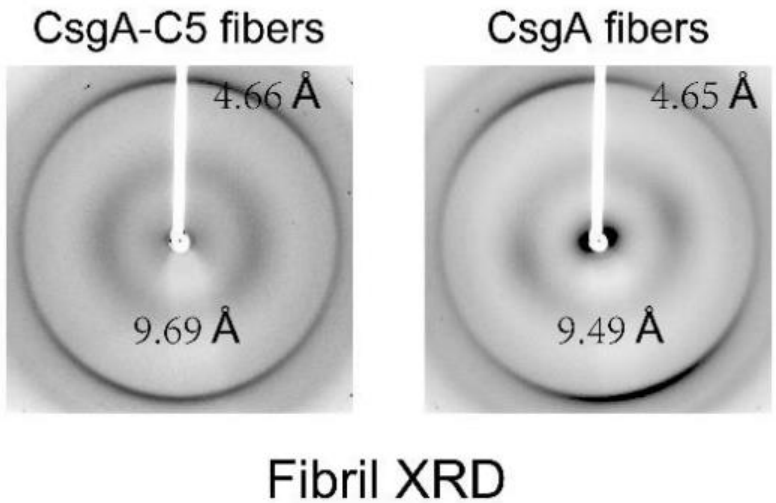


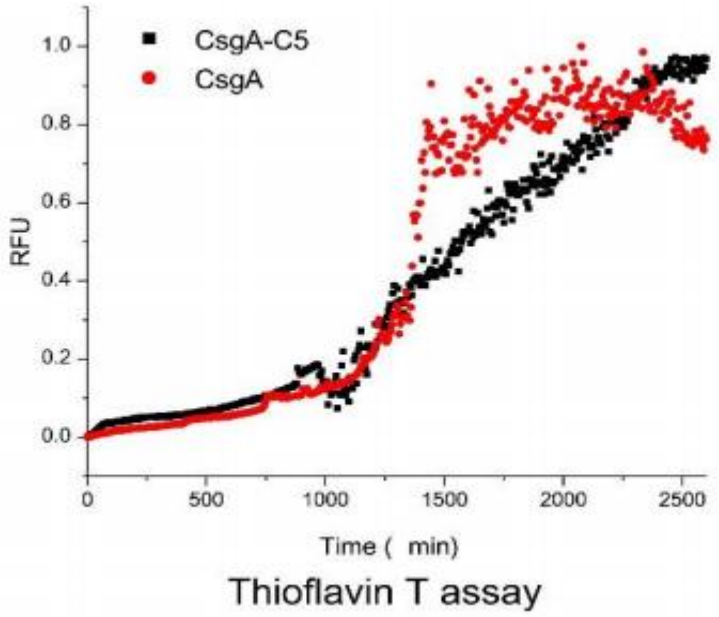

**Figure 3. Coomassie light blue stained SDS-PAGE gels and western blots with anti-His antibodies confirm purification of the expressed proteins by cobalt-resin columns.**

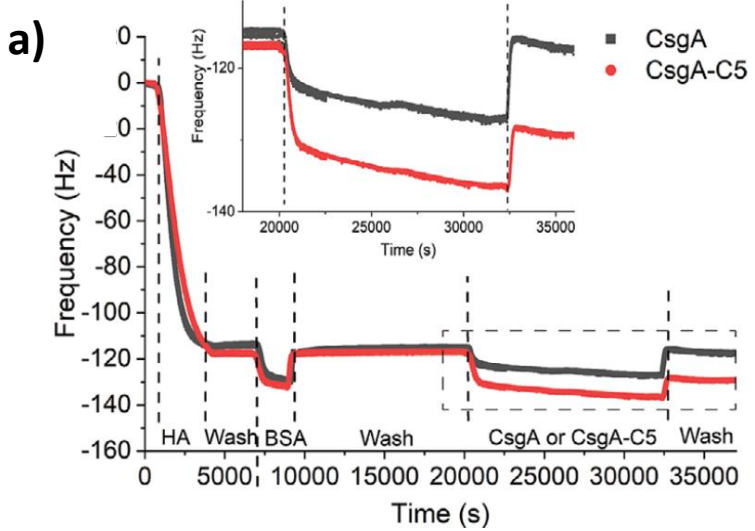
- **Congo red staining:** CsgA-C5 or CsgA protein solutions tested for amyloid.
- **Thioflavin T assay:** 438 nm excitation and 495 nm emission.
- QCM (**Quartz Crystal Microbalance):** Changes in resonance frequency (ΔF) of a quartz crystal are recorded to measure the amount of the proteins deposited on the substrates. Silicon substrates coated with HA were used.
- **X-ray fiber diffraction: T**he protein samples display typical cross-beta diffraction patterns, characteristic of amyloid fibrils. The meridional reflection at ~4.7 Å corresponds to the inter-β strand spacing and the equatorial reflection at ~6–11 Å corresponds to the distance between stacked β sheets.

**Figure 4**:

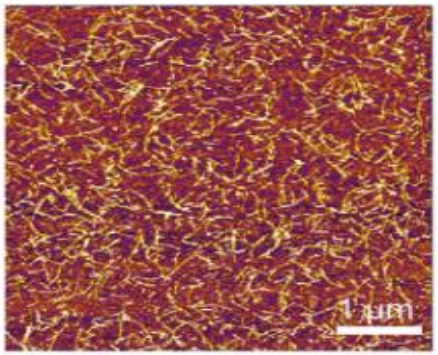





b)




c)

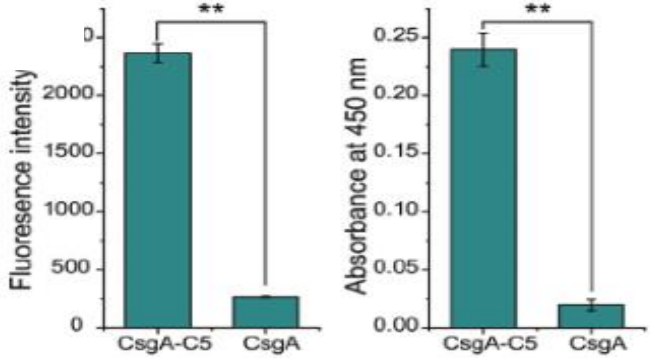



**Figure 5. a) QCM analysis of the affinity strength between CsgA-C5 monomers and hemagglutinin. The inset image shows the zoomed-in curves in the range of 18 000–36 000 s. b) AFM height image showing the morphology of self-assembled CsgA-C5 fibers. ec Confocal immunofluorescence intensity (left) and ELISA (right) analysis to quantitatively assess the binding of CsgA-C5 and CsgA nanofibers to hemagglutinin. Results are means ± s.e.m. of three independent samples (n = 3). **P < 0.01, Student's t-test.**

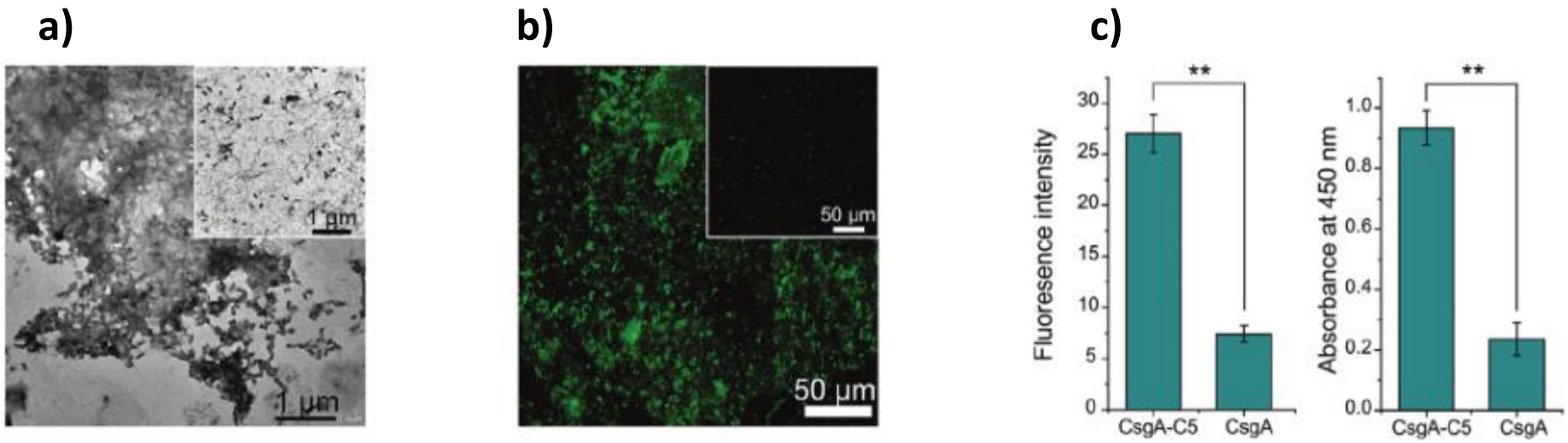



**Figure 6. a) TEM and b) immunofluorescence images of the CsgA-C5 and CsgA (inset) fibers binding with whole viruses. c) Immunofluorescence intensity (left) and ELISA (right) analysis to assess the binding of CsgA-C5 (and CsgA) nanofibers with whole virus particles. Results are means ± s.e.m. of three independent samples (n = 3). **P < 0.01, Student's t-test.**

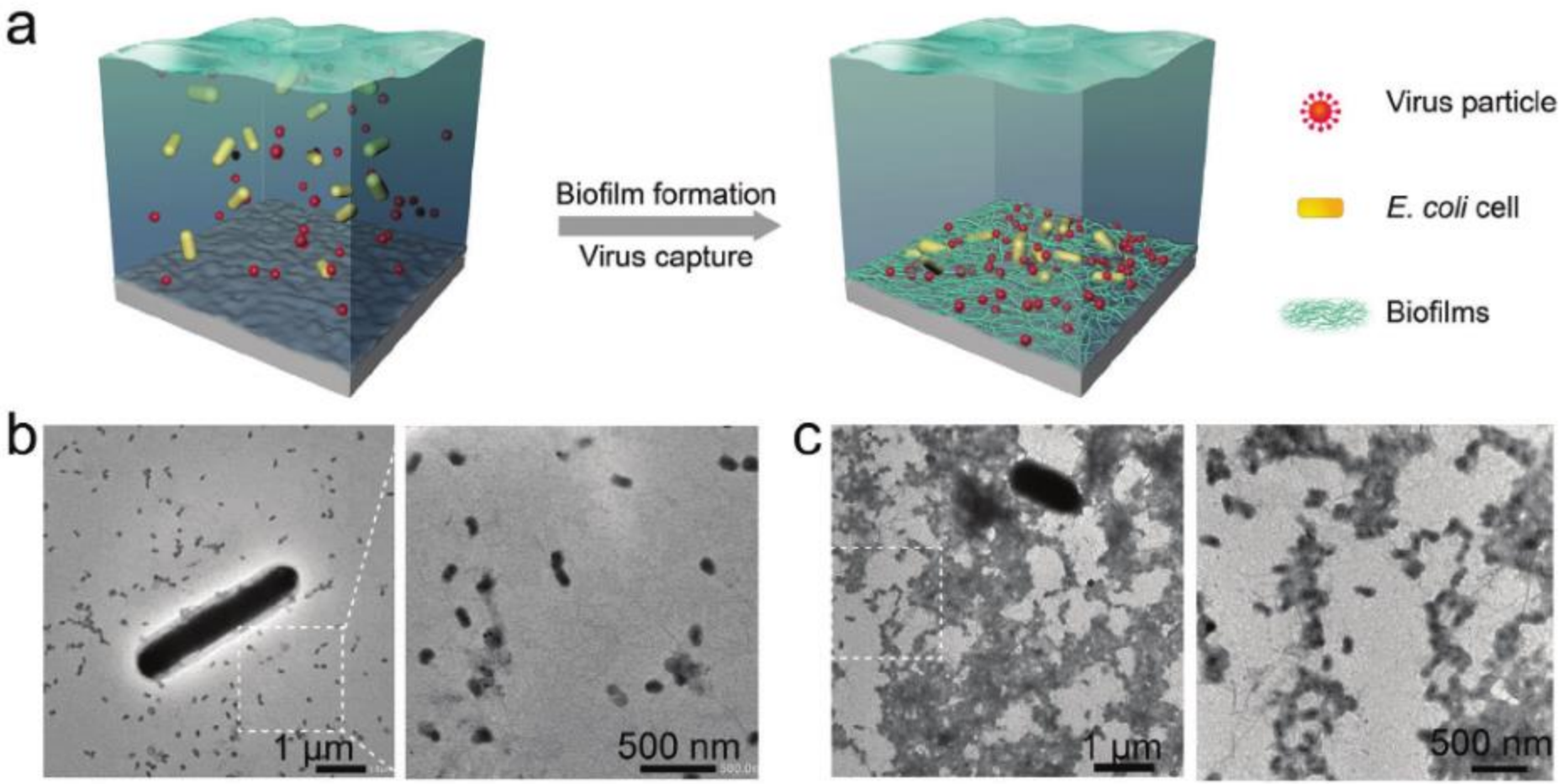



**Figure 7. Virus disinfection using engineered functional biofilms. a) Schematic of engineered E. coli cells that self-assemble into biofilms upon expression and extracellular secretion of CsgA-C5 proteins and capture viruses present in water with extracellular nanofibers. b) TEM images of the unbound status of bacterial cells and viruses (before biofilm formation) (an image of the zoomed-in area is shown at the right). c) TEM images of the CsgA-C5 biofilms binding with virus particles (an image of the zoomed-in area is shown at the right).**

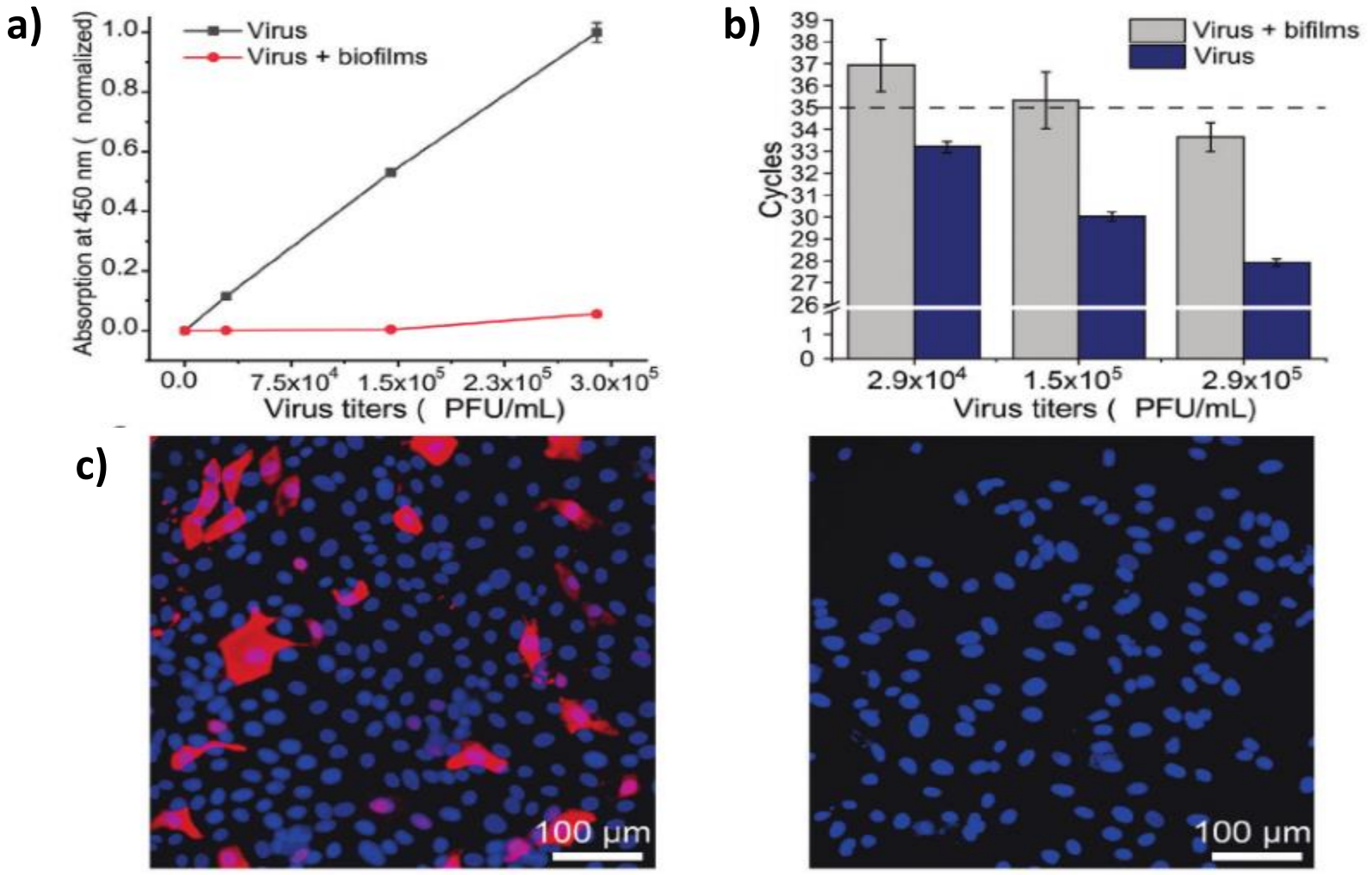



**Figure 8. a) ELISA and b) qPCR analysis of supernatants from a gradient series of virus titers samples that were incubated with biofilms. Results show means ± s.e.m. of three independent samples (n = 3). c) Biofilms were exposed to influenza virus samples (7 × 104 PFU mL−1) in PBS, and the sample supernatant was then used to inoculate cells from the influenza-susceptible MDCK (Madin–Darby canine kidney) cell line. Inoculated cells were then analyzed using a mouse monoclonal antibody against the influenza virus nucleoprotein to detect virus particles that had successfully infected cells.**

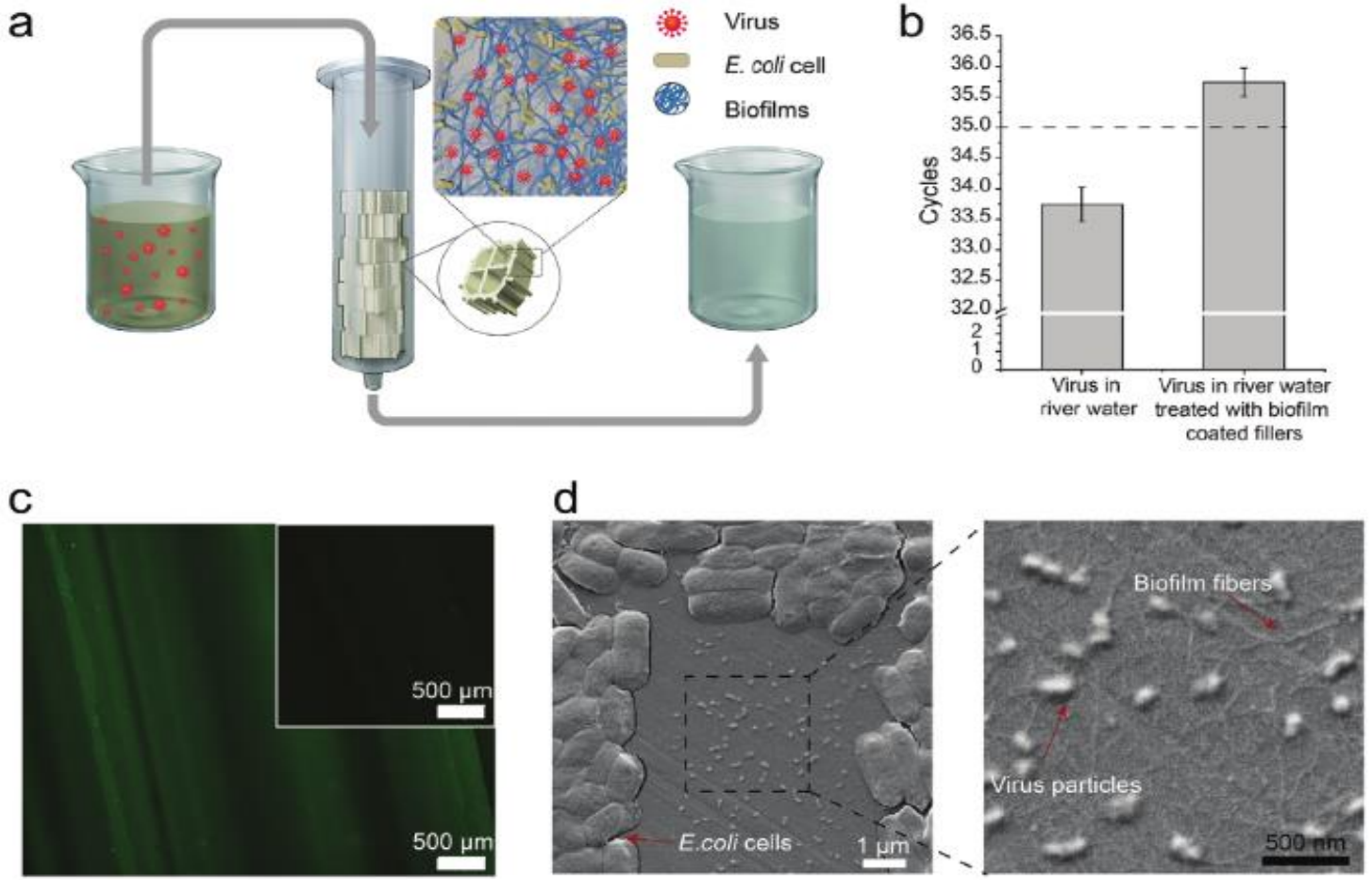



**Figure 9. Integrating engineered functional biofilms with industrial filler materials for virus elimination from river water. a) Schematic for polypropylene industrial filler material colonized by our engineered CsgA-C5 biofilms and used to eliminate viruses from river water. b) qPCR analysis of field samples after virus-spiked river water samples were passed over the immobilized biofilms. Results show means ± s.e.m. of three independent samples (n = 3). c) Immunofluorescence images of the biofilm-coated polypropylene industrial filler material after passage of the field water samples, stained against hemagglutinin. The inset image refers to the bare filler materials as a control test sample. d) SEM images of the virus particles bound to the CsgA-C5 biofilms (zoomed-in images are shown at the right). E. coli cells, amyloid fibers, and virus particles are indicated with arrows.**

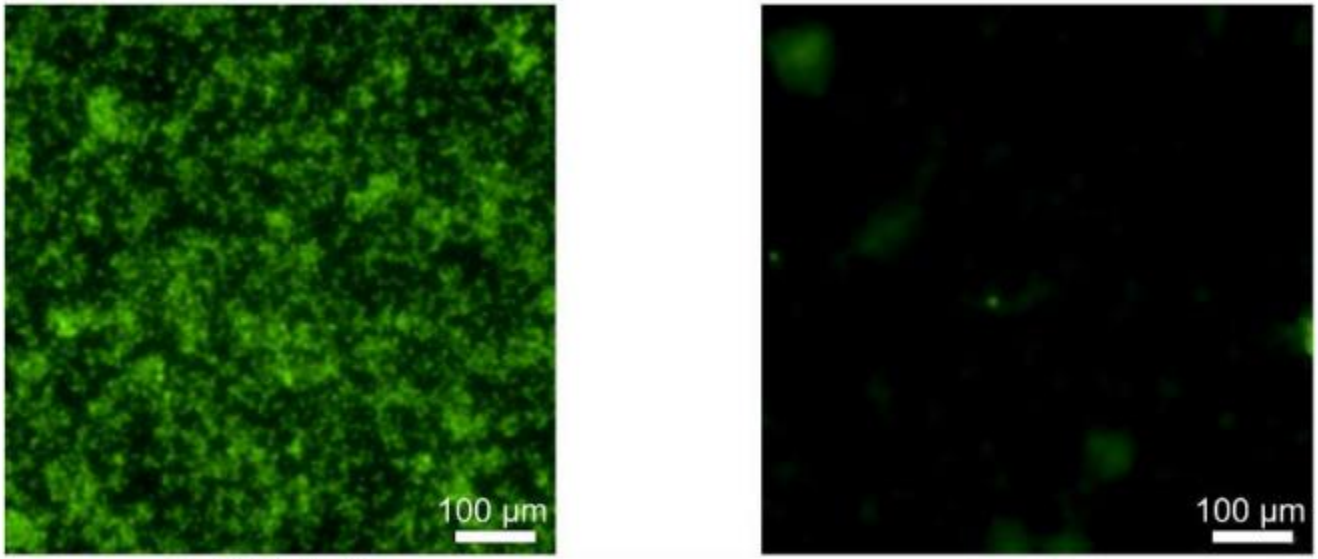
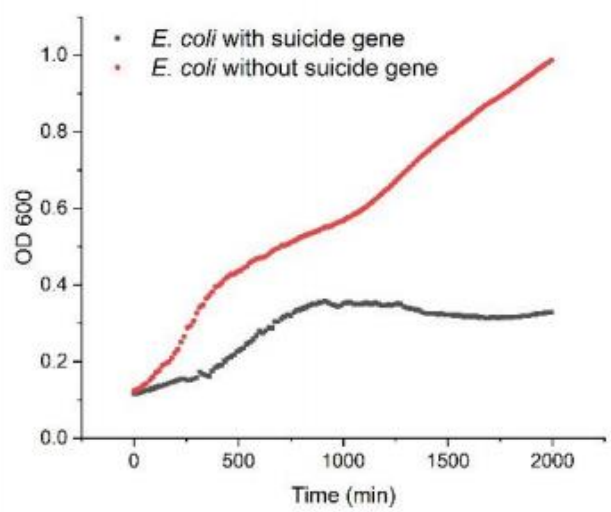
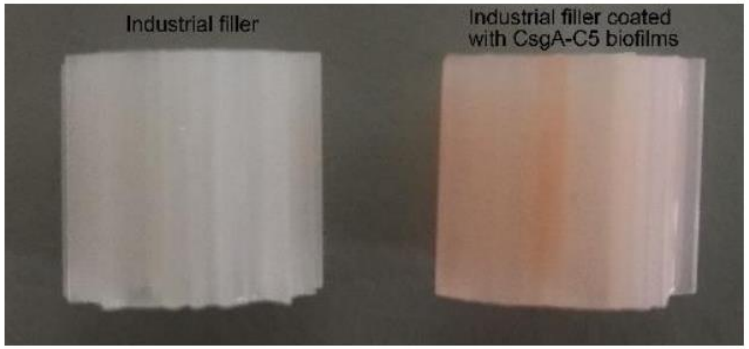



**Figure 10**. Immunofluorescence images showing the different binding behaviors of influenza virus particles with CsgA-C5 and CsgA biofilms.

- A quorum sensing-enabled **suicide gene circuit** into engineered bacteria.
- The suicide gene circuit consisting of a lysis protein ($\phi$x174E), activated by the expression of LuxI-LuxR, was introduced into engineered *E. coli*. The luxI gene producing an acylhomoserine lactone (a quorum sensing signal), can activate the suicide gene expression regulated by LuxR repressor.
- In this way, when the cell density reaches a critical threshold, expression of the toxin protein ($\phi$x174E) will be initiated, thus resulting in bacterial death.

**Figure 11.**



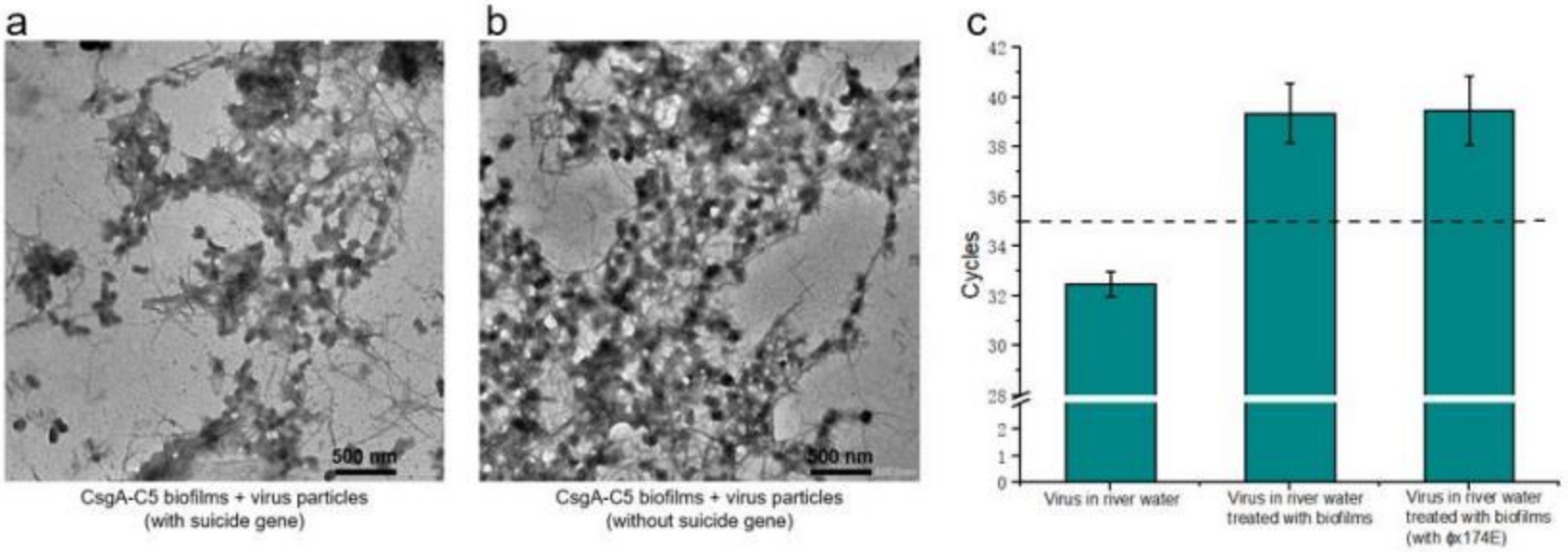



**Figure 12. Virus disinfection from river water using engineered bacteria harboring a suicide gene circuit. TEM images showing binding of virus particles using the CsgA-C5 biofilms expressed by bacteria harboring a suicide gene circuit (a) or without a suicide gene circuit (b); c, qPCR analysis of the supernatants from virus samples (3.5x107 PFU/L) that were incubated with biofilms produced by bacteria with/without a suicide gene. Results show means ± s.e.m. of three independent samples (n = 3).**

# THANK YOU