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Enhancement of photobactericidal activity of chlorin-e6-cellulose nanocrystals by covalent attachment of Polymyxin B.

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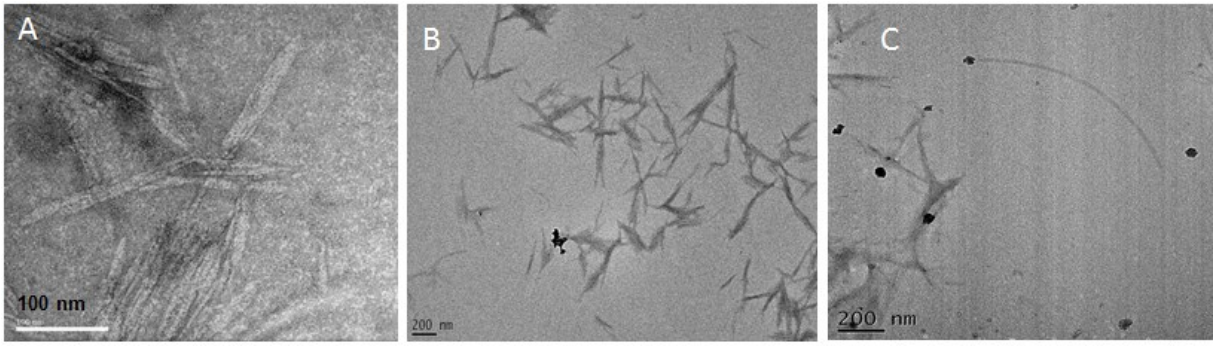

Fig S1. TEM images of samples dispersed in water: unmodified CNCs (A), $CNCs_{ox}$ **1** (B) and $CNCs_{C6\text{-}PMB}$ **3** (C). All samples were stained with uranyl acetate for better contrast.

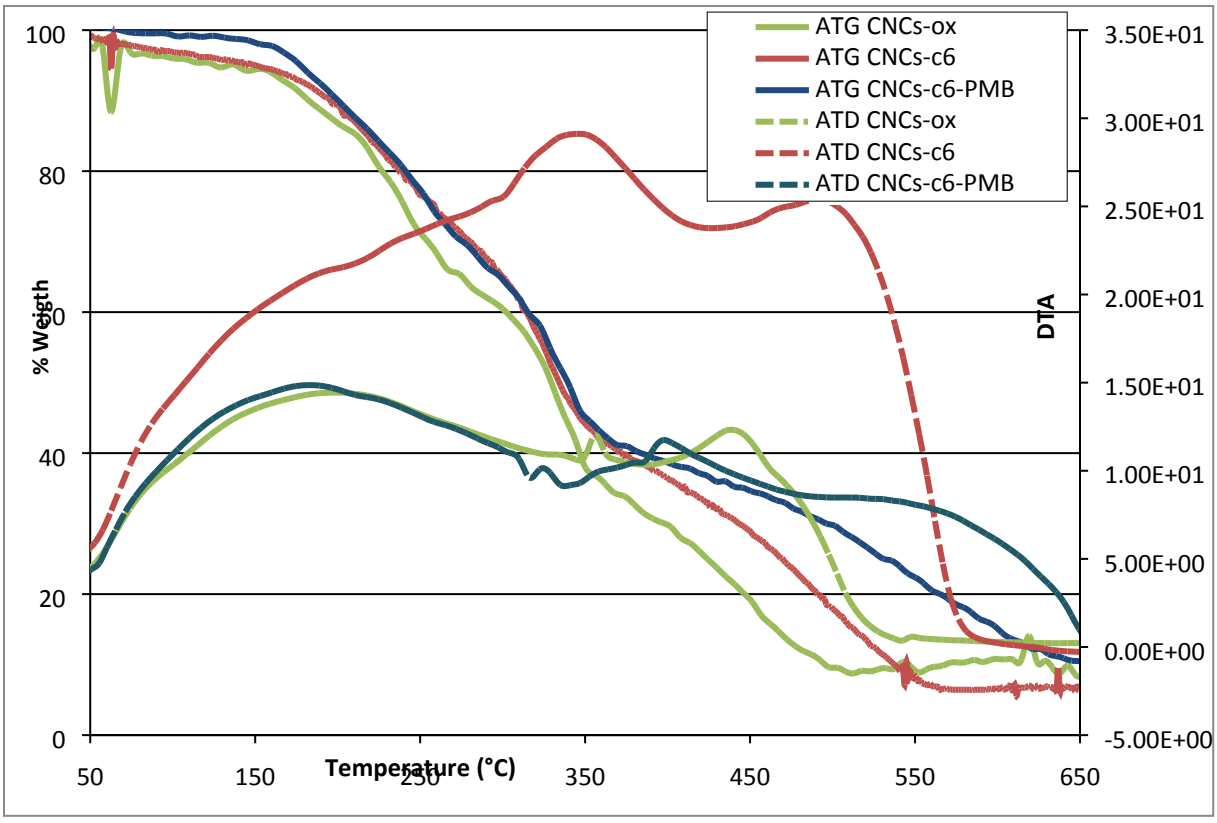

Fig S2. Thermogravimetric analyses (TGA) and differential thermal analyses (DTA) of dried samples : $CNCs_{ox}$ **1**, $CNCs_{C6}$ **2** and $CNCs_{C6\text{-}PMB}$ **3**.

Singlet oxygen production :

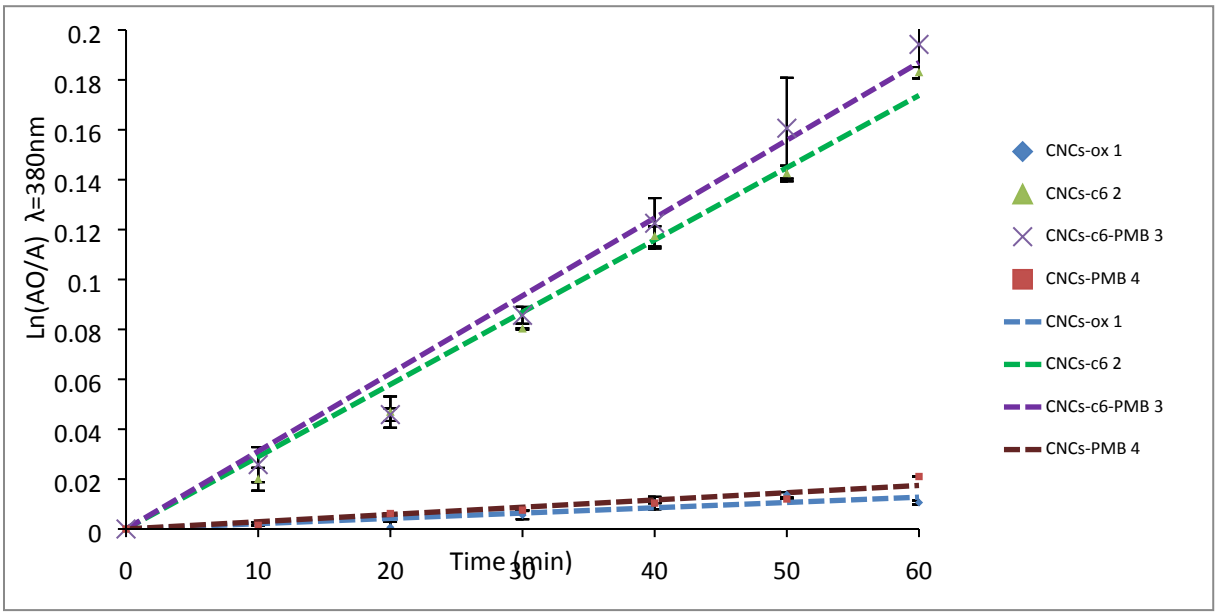

Fig S3. Photooxidation of 9,10-dimethylanthracene (DMA, 95µM) under light irradiation (850 LUX) in presence of $CNCs_{ox}$ **1** (0.164 mg/mL), $CNCs_{C6}$ **2** (0.129 mg/mL), $CNCs_{C6\text{-}PMB}$ **3** (0.191 mg/mL), or $CNCs_{PMB}$ **4** (0.164 mg/mL) in *N-N*-dimethylformamide. Values represent means ± standard deviation of three separate experiments.

Photooxidation of 9,10-dimethylanthracene (DMA)

Nanocrystals were suspended in a solution of spectroscopic grade of DMF (3 mL) (**$CNCs_{ox}$ (1)**: 0.164 mg/mL **; $CNCs_{c6}$ (2)**: 0.129 mg/mL **; $CNCs_{c6\text{-}PMB}$ (3):** 0.191 mg/mL **; $CNCs_{PMB}$ (4)**: 0.164 mg/mL). 60 µL of a fresh solution of DMA in DMF (4.85 mM) were added to the suspension in a quartz cuvette. The photooxidation was studied by following the decrease of the absorbance (A) at 380 nm, under light irradiation (850 LUX, λ= 400 – 690 nm) and vigorous agitation. The rate of photooxidation was illustrated by a linear least-squares fit of the semilogarithmic plot of Ln (A_O/A) versus time.

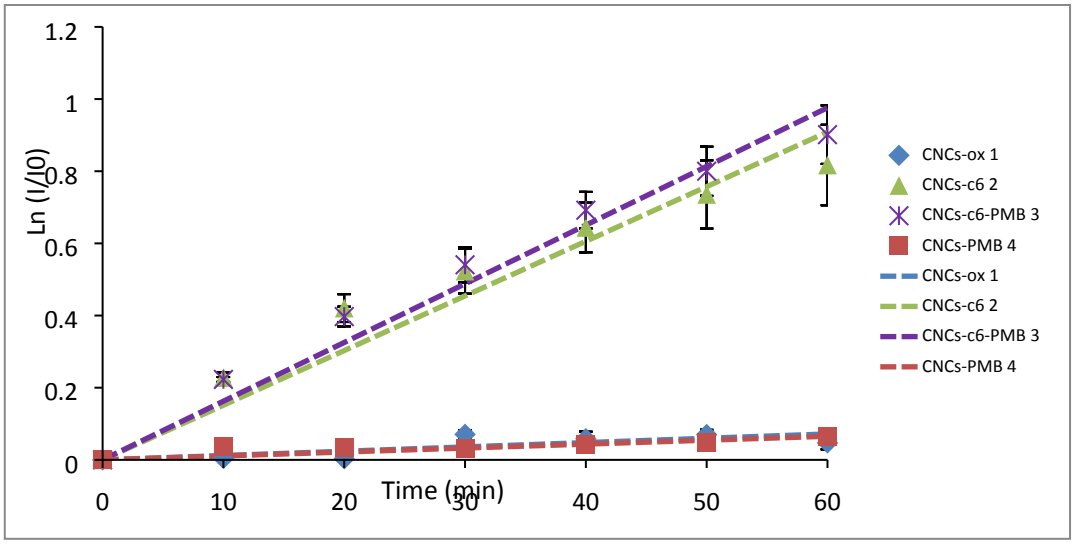

Fig S4. Photooxidation of SOSG (10 µM) under red light irradiation (400 LUX) in presence of CNCsox 1 (0.164 mg/mL), CNCsC6 2 (0.129 mg/mL), CNCsC6-PMB 3 (0.191 mg/mL), or CNCsPMB 4 (0.164 mg/mL) in DPBS (0.1X) . Values represent means ± standard deviation of three separate experiments.

Photooxydation of Singlet Oxygen Sensor Green Reagent (SOSG)

Nanocrystals were suspended in a solution of DPBS (Dulbecco's phosphate-buffered saline, pH 7.0-7.3) (0.1X, 1 mL) : **$CNCs_{ox}$ (1)**: 0.164 mg/mL ; **$CNCs_{c6}$ (2)**: 0.129 mg/mL ; **$CNCs_{c6\text{-}PMB}$ (3):** 0.191 mg/mL ; **$CNCs_{PMB}$ (4)**: 0.164 mg/mL. 2 µL of a fresh solution of SOSG in methanol (~5mM) was added to the suspensions. The photooxidation was studied by following the fluorescence emissions of the solution at 535 nm (I) (λ_{exc} = 485 nm), under a red light irradiation (red LED, 400 LUX). The rate of photooxidation was illustrated by a linear least-squares fit of the semilogarithmic plot of Ln (I/I_0) versus time. As expected, an identical result was obtained in physiological conditions.

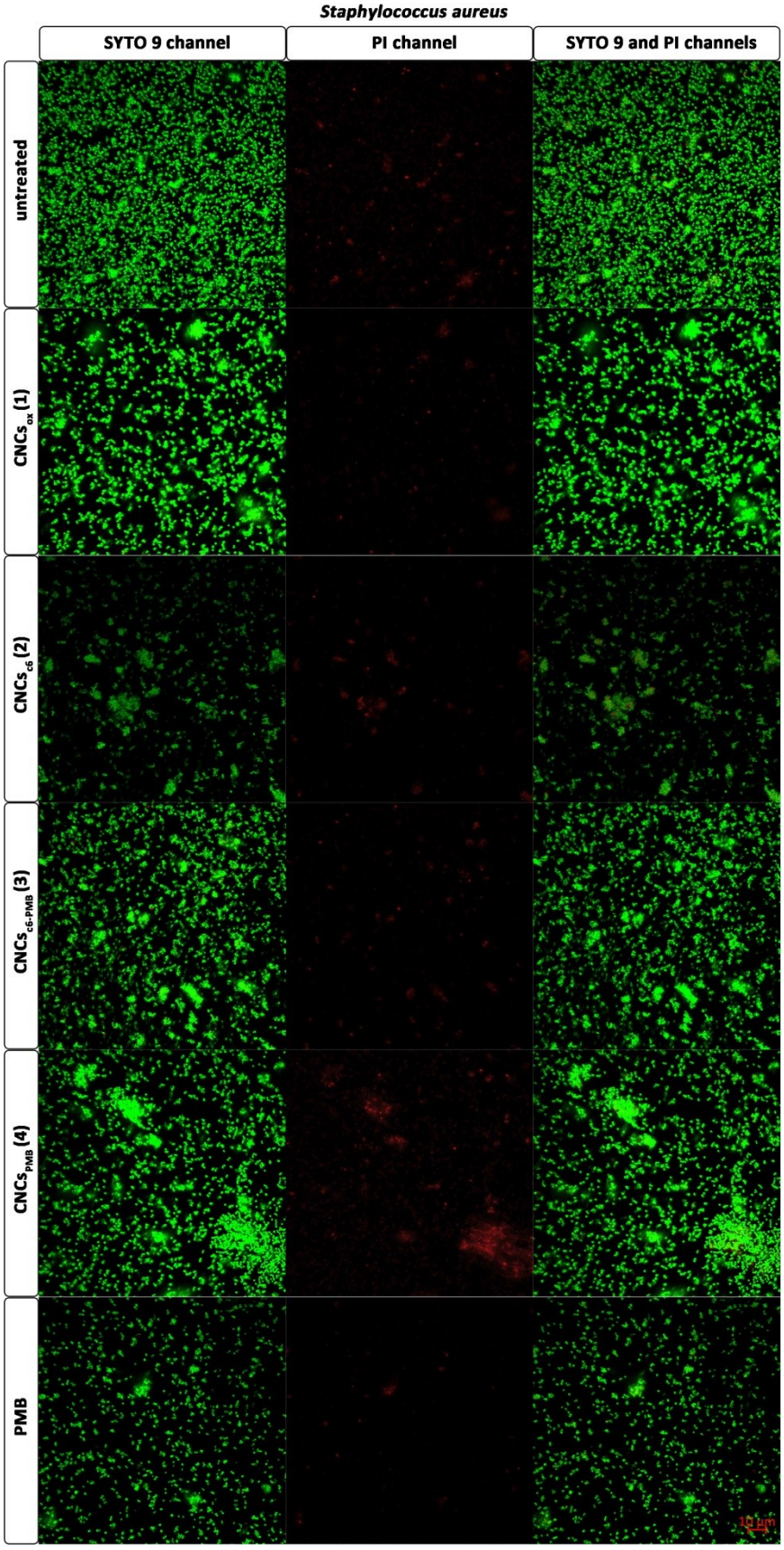

Fig S5. Confocal laser scanning microscopy imaging of *S. aureus* in different conditions: untreated, in contact with the different CNCs or free PMB. LIVE/DEAD® BacLight™ was used to observe the viability of bacteria. Bacteria with intact cell membranes stain fluorescent green (SYTO9), whereas bacteria with damaged membranes stain fluorescent red (PI). Images were obtained after 30 min of contact at room temperature.

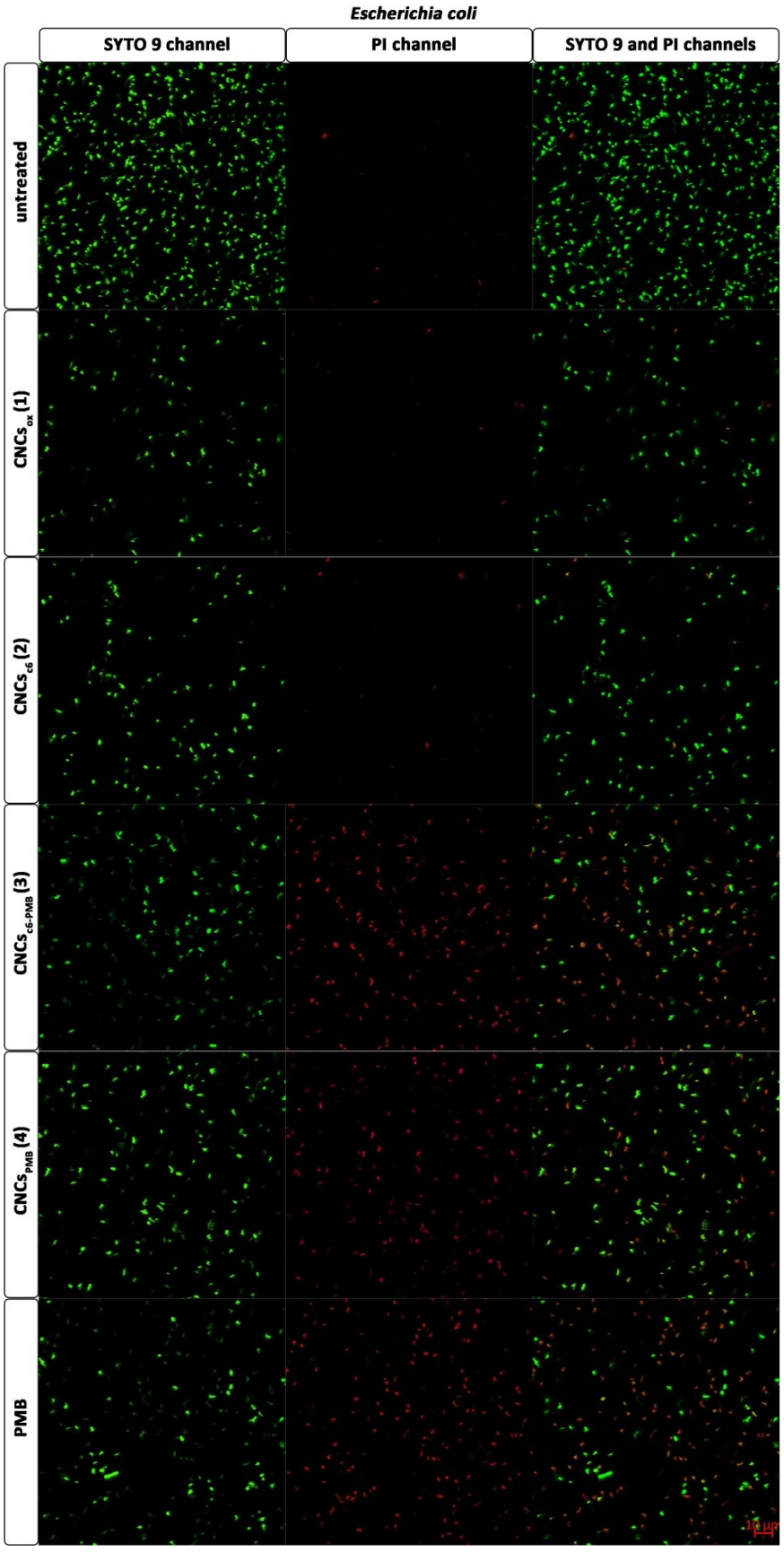

Fig S6. Confocal laser scanning microscopy imaging of *E. coli* in different conditions: untreated, in contact with the different CNCs or free PMB. LIVE/DEAD® BacLight™ was used to observe the viability of bacteria. Bacteria with intact cell membranes stain fluorescent green (SYTO9), whereas bacteria with damaged membranes stain fluorescent red (PI). Images were obtained after 30 min of contact at room temperature.

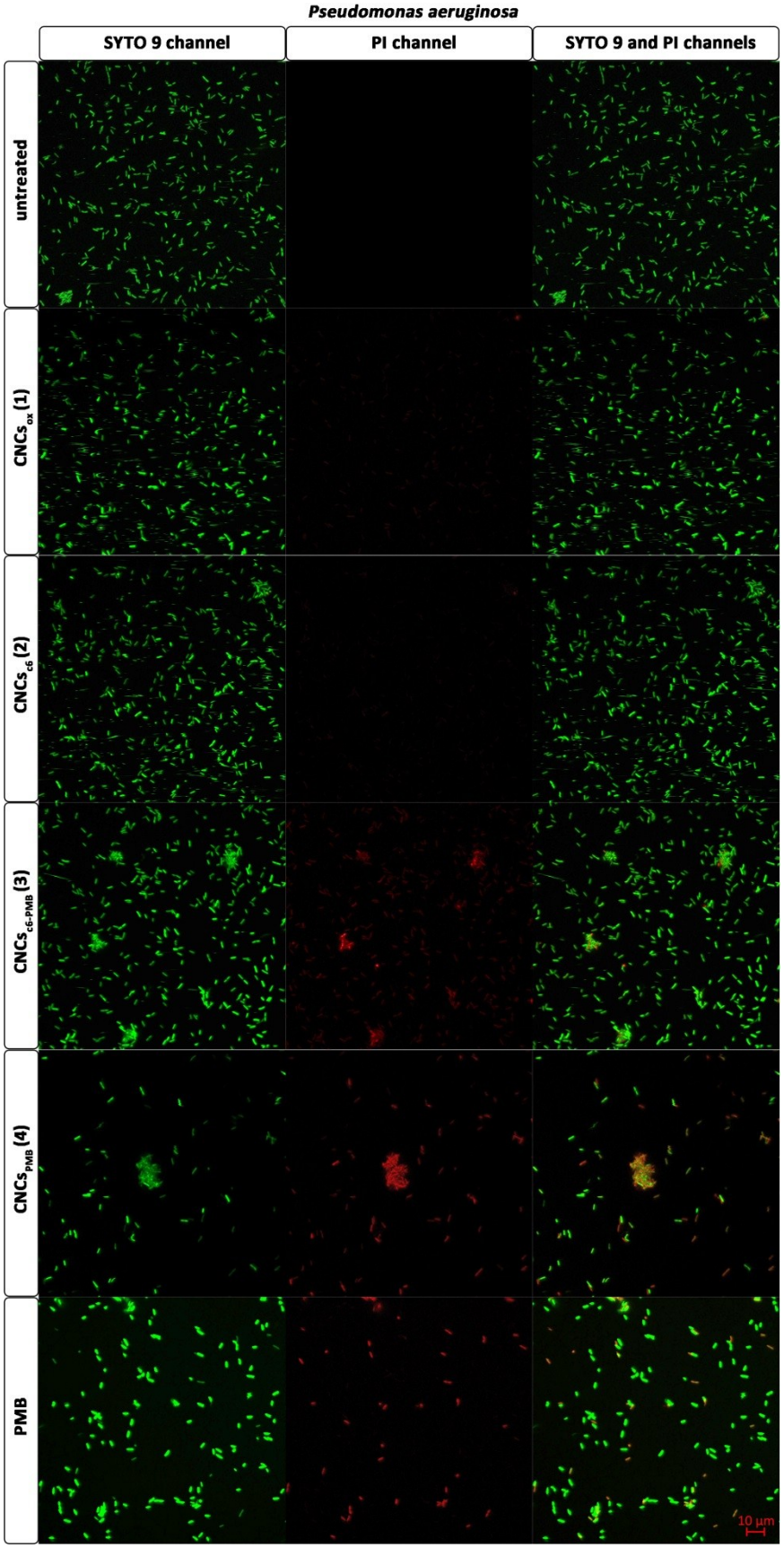

Fig S7. Confocal laser scanning microscopy imaging of *P. aeruginosa* in different conditions: untreated, in contact with the different CNCs or free PMB. LIVE/DEAD® BacLight™ was used to observe the viability of bacteria. Bacteria with intact cell membranes stain fluorescent green (SYTO9), whereas bacteria with damaged membranes stain fluorescent red (PI). Images were obtained after 30 min of contact at room temperature

Degree of substitution DS :

Based on the maximum amount of units possible (Table S1) and their theoretical elementary analysis, a theoretical result for each nanocrystal was calculated (Table S2)

Table S1. Maximum Theorical DS for each CNCs. Each unit has been represented by abbreviations: "COOH" for carboxyl units, "OH" for hydroxyl units, "c6" for units bearing a chlorin-e6-spermine, "PMB" for polymyxin units and "*N*-acyl" for *N*-acylisourea by-products units.

	COOH	OH	c6	PMB	*N*-acyl	Σ
$CNCs_{ox}$ (1)	0.41	0.59	0	0	0	1
$CNCs_{c6}$ (2)	0.15	0.59	0.21	0	0.05	1
$CNCs_{c6\text{-}PMB}$ (3)	0	0.59	0.21	0.15	0.05	1
$CNCs_{PMB}$ (4)	0.15	0.59	0	0.21	0.05	1

Table S2. Theoretical elementary analysis of the different CNCs (calculated with ChemBioDraw®)

	C	H	N	O
$CNCs_{ox}$ (1)	43.0	5.5	0.000	51.4
$CNCs_{c6}$ (2)	48.4	6.2	3.306	42.1
$CNCs_{c6\text{-}PMB}$ (3)	50.3	6.7	5.610	37.4
$CNCs_{PMB}$ (4)	46.5	6.4	4.217	42.9

Then, for each CNCs, the formula (Equation) has been applied to afford the estimation of DS (Table S3).

$$DS = maximun\ DS\ \ X\ \frac{increase\ of\ nitrogen\ content\ (experimental)}{increase\ of\ nitrogen\ content\ (theoretical)}$$

Equation. Calculation of the DS

Table S3. Estimated DS

	c6	PMB
$CNCs_{c6}$ (2)	0.19	0
$CNCs_{c6\text{-}PMB}$ (3)	0.19	0.12
$CNCs_{PMB}$ (4)	0	0.20

The yield of COOH on CNCs ox is 92 %. The combination of this yield with the oxidation degree then allowed the calculation of chlorin-e6 density on nanocrystals: 0.92x21% = 19% where 21% correspond to DS maximum if only one half of the carboxylic substituted glucose units was used to graft the chlorin-e6.