Amine- and carboxyl-quantum dots affect membrane integrity of bacterium *Cupriavidus metallidurans* CH34

Supporting information

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Experimental methods

Flow Field Flow Fractionation measurements. In addition to the fluorescence correlation spectroscopy measurements, the size distribution characterization of the QDs dots was carried out with an asymmetrical flow field flow fractionation (aFlFFF) system (AF2000 Focus, Postnova Analytics) coupled on-line with UV/VIS spectrophotometer. The software AF2000 Control (Postnova Analytics) was used to control the FFF system and detector. Measured channel dimensions are: tip-to-tip length, 27.5 mm; inlet triangle breadth and length, 20 and 34 mm, respectively; outlet triangle breadth and length, 5 and 10 mm. Regenerated cellulose membrane were employed with cut-off of 1 was employed as accumulation wall. 350-µm spacer was employed. The 10^{-2} M MOPS (pH = 7.0) used as carrier solution was filtered on a 0.1 µm-pore size teflon filter (Postnova Analytics). The optimized focusing and elution conditions were: inlet flow rate V_{in} = 0.2 mL min^{-1}; focus flow rate, V_{foc} = 1.8 mL min^{-1}; crossflow rate, V_c = 1.00 mL min^{-1} and outlet flow rate V_{out} = 1 mL min^{-1} . The respective elution conditions were: V_c = 1 mL min^{-1} and V_{out} = 1 mL min^{-1}

The hydrodynamic radius of QDs was obtained by calibration of the UV detector, injecting 50 µL of mixture containing latex particles (Postnova Analytics) in 10^{-2} M MOPS with diameter of 22, 58 and 97 nm. The following relationship between hydrodynamic radius, R_H and the retention time, t_r was obtained: $\log R_H = (\log t_r + 0.176)$.

Ultrafiltration and ICP-MS measurements of Zn and Cd concentrations. To evaluate the amount of Cd and Zn released from QDs in bacterial exposure medium microcon centrifugal filter devices (MCFD, 3kD cut-off, Millipore) were used to isolate the dissolved from the particulate fractions. As recommended by the manufacturer, MCFD were pre-rinsed with 0.1 M NaOH and 3 times with Milli-Q water to remove organic surfactants from the membrane. MCFD were conditioned with experimental medium by performing at least 3 consecutive runs

with 250 µL of the experimental medium. Blanc samples containing only experimental medium, or medium and algae in the absence of QDs were run in parallel. Controls containing mixtures of 10^{-7} M Zn and Cd as nitrates allowing to evaluate potential losses of Zn and Cd to the filter and walls of the microcon device. The amount of Cd and Zn in the retentate and filtrate was measured by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, Elan DRC II) following acidification with HNO_3 (0.2% v/v, Baker instra grade). Typically, 250 µL of experimental medium was centrifuged for 60 min at 13000 × g. Total Cd and Zn recoveries were about 85%. About 40% of Cd and 15% of Zn losses to the filter and walls of the MCFD were found in the control experiments with 2×10^{-7} M Zn and Cd. Experiments were performed in triplicate.

Determination of Cd , Zn and Se content in bacteria

Cd, Zn and Se content in bacterium was measured by ICP-MS, following the isolation of bacteria from the QD containing medium by gently centrifugation, rinse with 10^{-2} M MOPS and digestion with concentrated HNO_3 (suprapur, Baker) at 100°C over 1 hour. Experiments were performed in triplicate.

Effect of QDs on bacterial growth determination

The effect of QDs on bacterial growth was followed by the measurement of the optical density at 600 nm (OD_{600}) by microplate reader (MRX, Dynatech Laboratory). For this purpose, bacteria pre-exposed to 200 nM of QDs for one hour in 10^{-2} M MOPS, pH = 7.0, were isolated by gentle centrifugation, washed with 10^{-2} M MOPS and re-suspended in the liquid growth medium 284 to an initial OD_{600} =0.05. Experiments were performed in triplicate.

Results

Average values and distribution of QDs hydrodynamic radius determined by asFlFFF-UV.

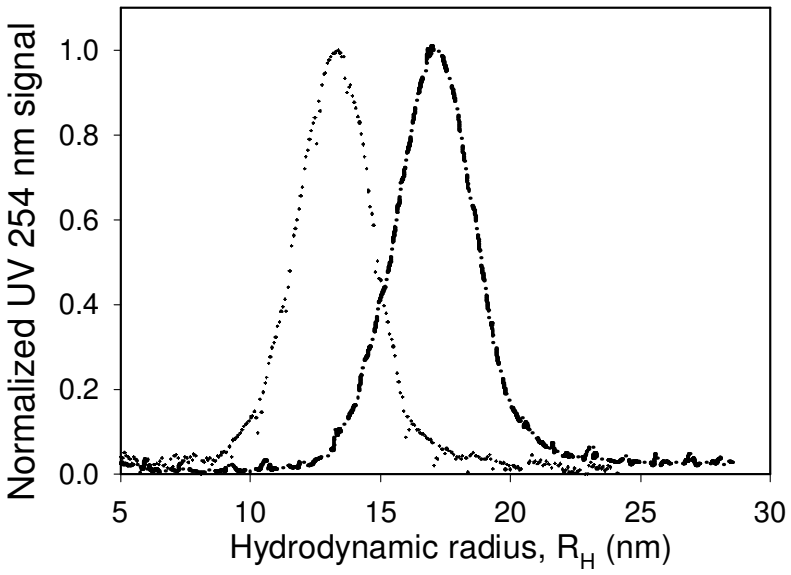

Figure S1. Hydrodynamic radius distribution obtained by asFlFFF-UV for 1μM amine- (dashed line) and carboxyl- (dotted line) PEG-QDs in 10^{-2}M MOPS, pH= 7.0, immediately after preparation of the QDs dispersion, injection volume 50 μL.

The weight average hydrodynamic radius of the QDs determined in 10^{-2}M MOPS, pH = 7.0, were 13.5 nm and 17.4 nm for carboxyl and amine-PEG-QDs. The polydispersity index in both cases was between 1.02 and 1.04, showing the homogeneity in hydrodynamic radius of the studied QDs.

Effect of bacteria on the QDs fluorescence and number of particles in the medium

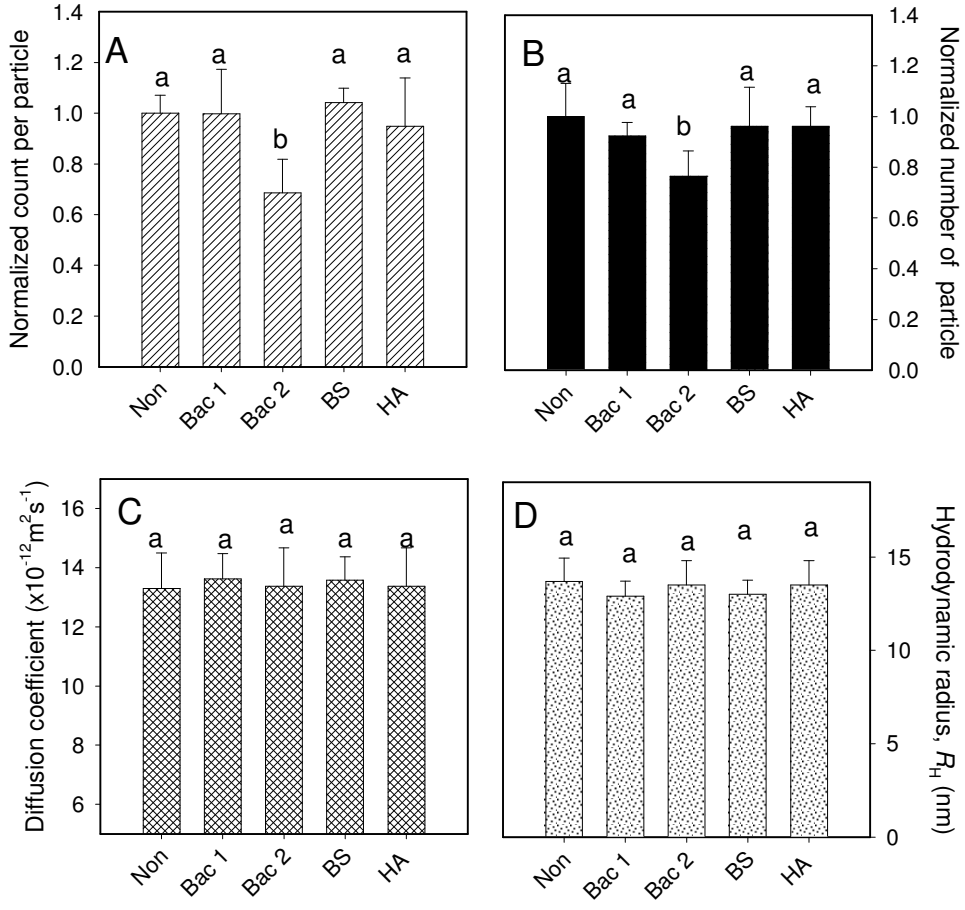

Figure S2. Influence of different medium components on (**A**) fluorescence properties, (**B**) number, (**C**) diffusion coefficient and (**D**) average hydrodynamic radius of freely diffusing particles in dispersions containing 20 nM carboxyl-PEG-QDs, 10^{-2} M MOPS, pH = 7.0 and time of contact of 60 min in the absence (*Non*) and presence of 30 mg L^{-1} HA (*HA*); 10^4 bacterial cells ml^{-1} (*Bac 1*); 10^5 bacterial cells mL^{-1} (*Bac 2*) and bacterial supernatant (*BS*). Number of particles in the confocal volume of the FCS microscope and florescence of individual particles (count per particle, CPP) were normalized to the initial values determined before addition of different organic material. Each point represents a mean value of 9 measurements. Different letters indicate significant differences between means ($p < 0.05$, Student-Neuman-Keuls test), N = 9.

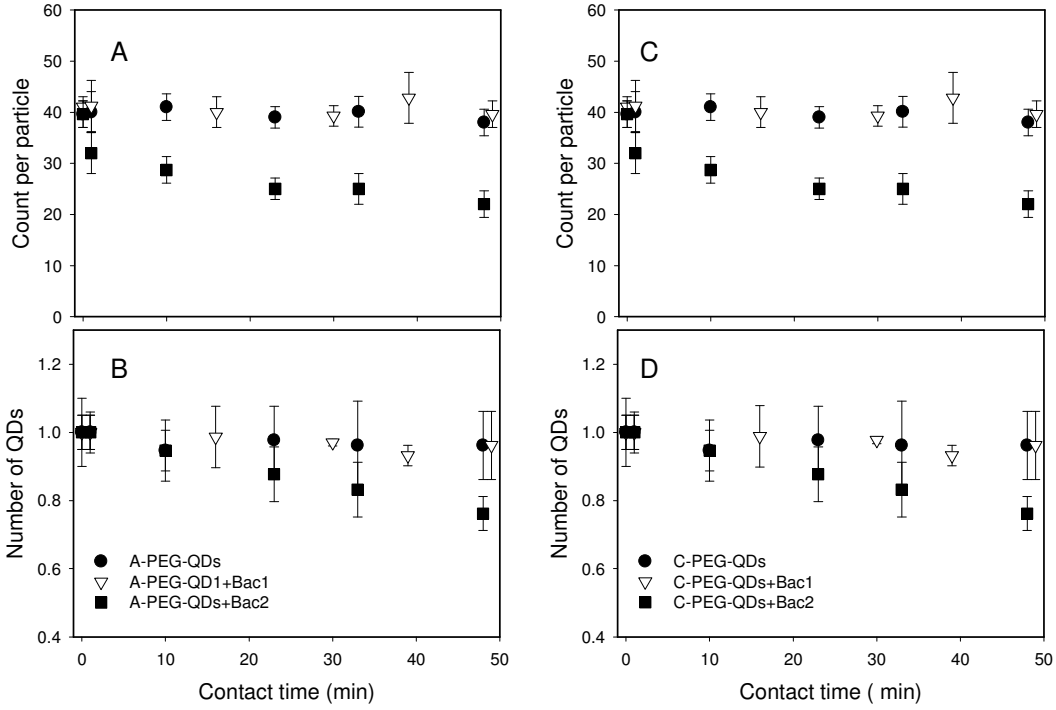

Figure S3. Evolution of single particle fluorescent intensity (**A**, **C**) and number of freely diffusing particles (**B**, **D**) in the dispersion containing 20 nM amine- (**A**, **B**) or carboxyl-PEG-QDs (**C**, **D**), in the absence and presence of 10^4 cells mL^{-1} (*Bac 1*) or 10^5 cells mL^{-1} (*Bac 2*) of *C. metallidurans*; 10^{-2} M MOPS, pH = 7.0. Number of particles in the confocal volume of the FCS microscope in the presence of bacteria was normalized to the initial values determined before bacterial addition. Each point represents a mean value of 9 measurements.

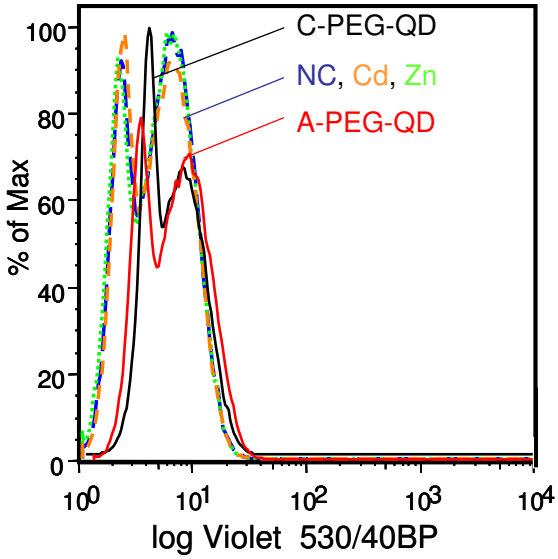

Figure S4. Fluorescence as a percentage of maximum of *C. metallidurans* alone (blue dashed line), C. *metallidurans* exposed 60 min to 200 nM amine- (red line) or carboxyl-PEG-QDs (black line), Zn and Cd nitrates (green and orange dashed lines) detected by Flow Cytometry; excitation wavelength = 488 nm; emission wavelength = 530 nm .

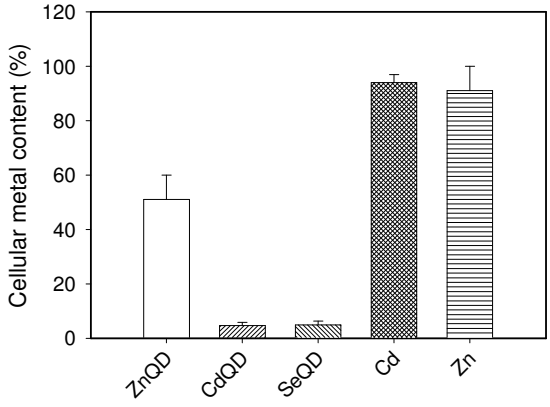

Figure S5. Cellular metal content (adsorbed plus internalized) of *C. metallidurans* as a percentage of the initial metal amount in the experimental medium before bacterial addition in the presence of 20 nM of amine-PEG-QDs (ZnQD, CdQD and SeQD). Zn and Cd denote cellular metal in the presence of 1 μM of Zn^{2+} as $Zn(NO_3)_2$ and Cd^{2+} as $Cd(NO_3)_2$.

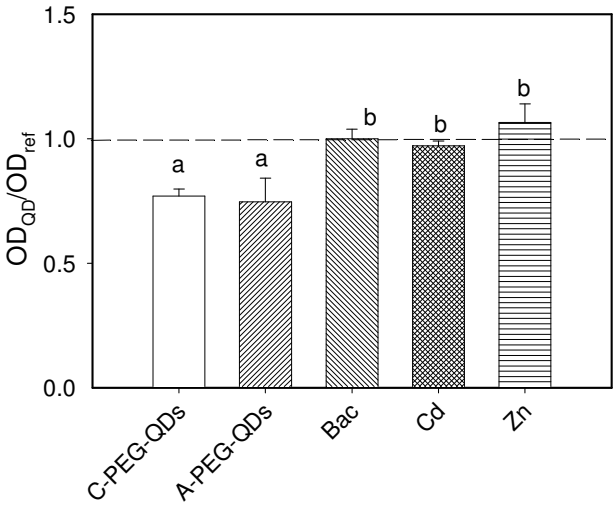

Figure S6. Optical density (OD) of the bacterial suspension following pre-exposed for 60 min to 200 nM of amine- or carboxyl- PEG-QDs or 200 nM Cd^{2+} or Zn^{2+} as added as nitrate salts in 10^{-2} M MOPS, pH=7.0. Optical density was measured at 600 nm for the cultures at 48h growth time and divided by the OD obtained for bacteria in the absence of QDs. Each point represents a mean value of 3 measurements. Different letters indicate significant differences between means ($p < 0.05$, Student-Neuman-Keuls test, N = 3).