Quantum dot encapsulation in virus-like particles with tuneable structural properties and low toxicity

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Electronic Supplementary Information

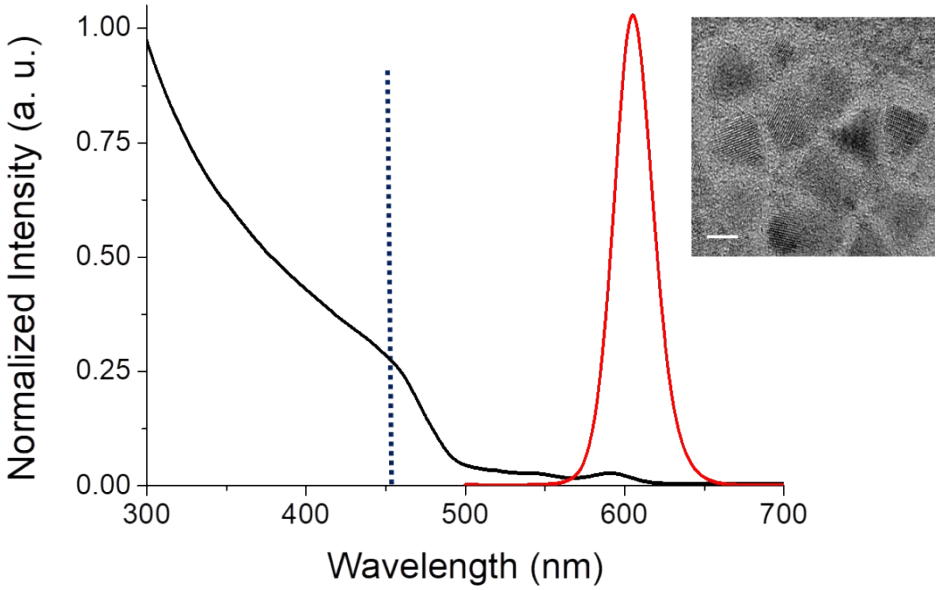

Figure ESI 1. Spectral overview and imaging parameters of QDs. Carboxylic acid-functionalized QDs have a continuous absorption in the UV-vis. region (black curve). The emission peaks around 605 nm (red curve). For *in vitro* imaging, QDs were excited at 458 nm (blue, dashed line) and emission was recorded using a long-pass filter (LP 560). Inset: TEM image of carboxylated QDs. Scale bar: 5 nm.

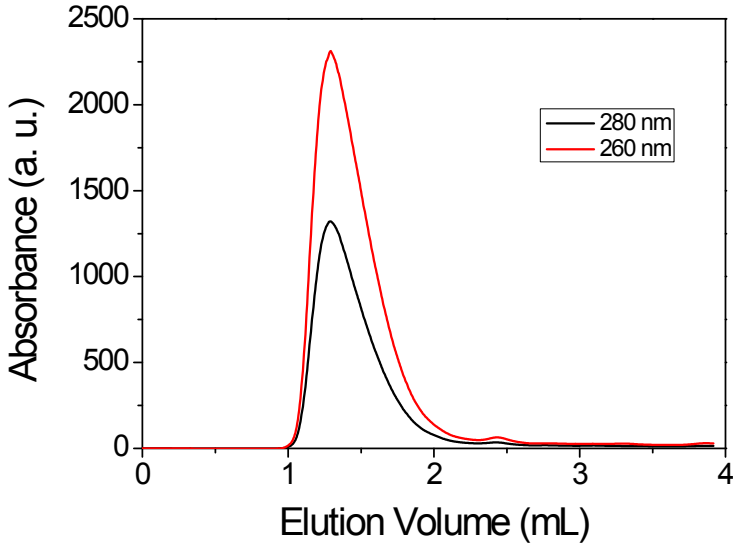

Figure ESI 2. FPLC chromatogram of wild-type CCMV capsid with T=3 symmetry. The capsid elution is followed at 280 nm (for protein, black) and at 260 nm (for RNA, red). Both curves peak at around 1.3 mL.

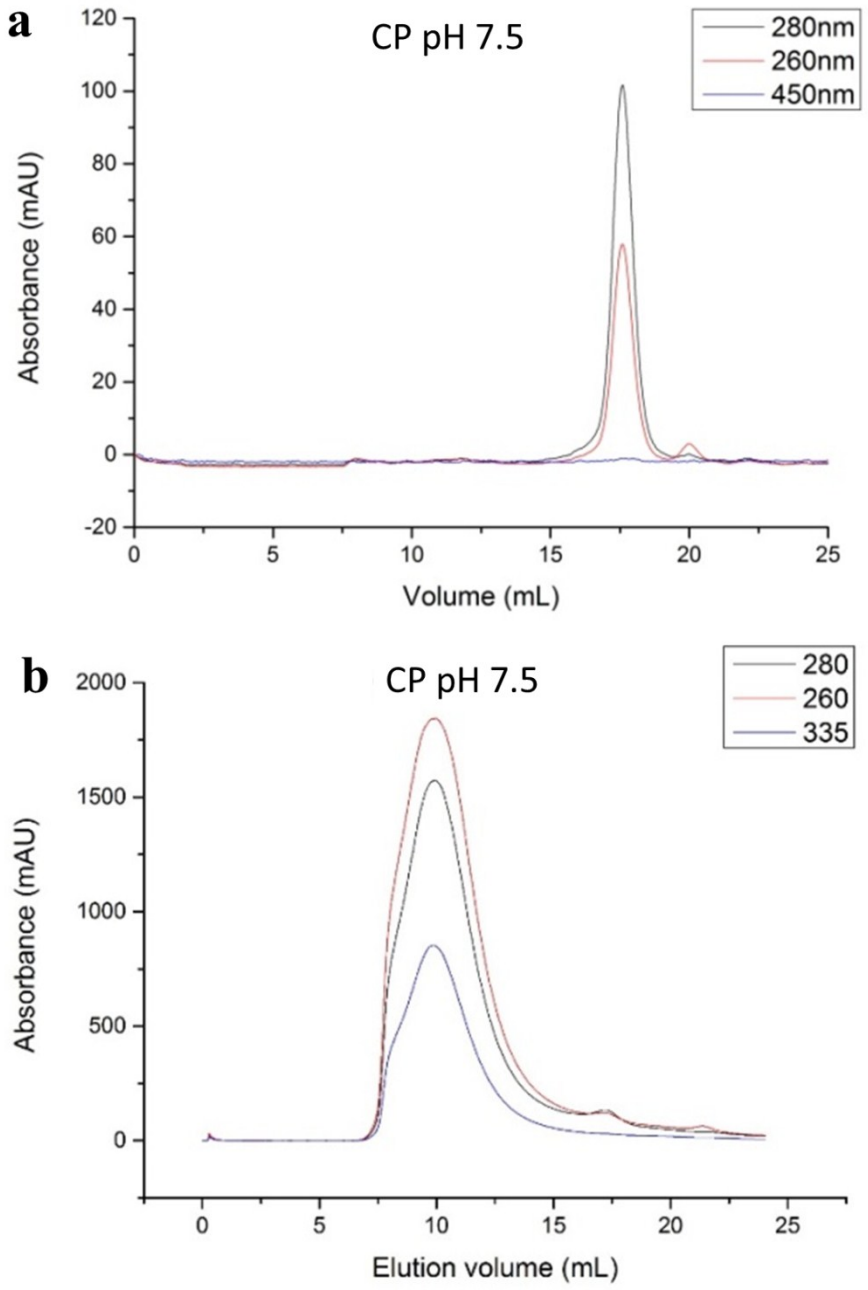

Figure ESI 3. FPLC chromatogram of CP dimers at pH 7.5 (0.3 M NaCl) in the absence **(a)** and presence **(b)** of QDs with negative surface charge.

Table ESI 1. Zeta potential values obtained in indicated buffers.

Sample	Buffer	Zeta potential (mV)
CP only (before assembly)	pH 7.5, 0.3 M NaCl	+4.1 (±2.3)
CP+QD (after assembly)	pH 7.5, 0.3 M NaCl	-14.3 (±0.8)
CP only (assembled as capsids)	pH 5.0, 1.0 M NaCl	-4.7 (±1.4)
QDs	pH 7.5, 0.3 M NaCl	-17.4 (±1.3)

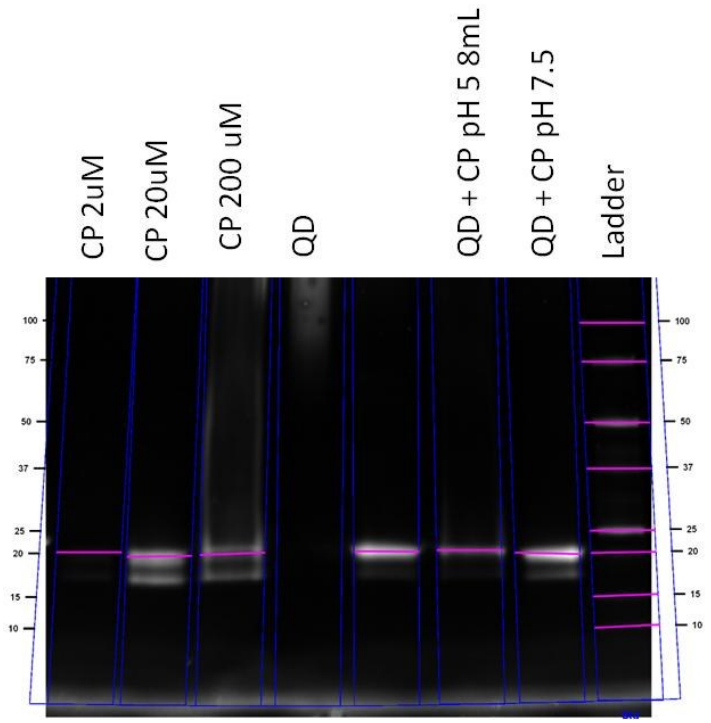

Figure ESI 4. SDS-PAGE of capsid proteins (CP) and QD/CP assemblies formed at pH 5.0 and pH 7.5.

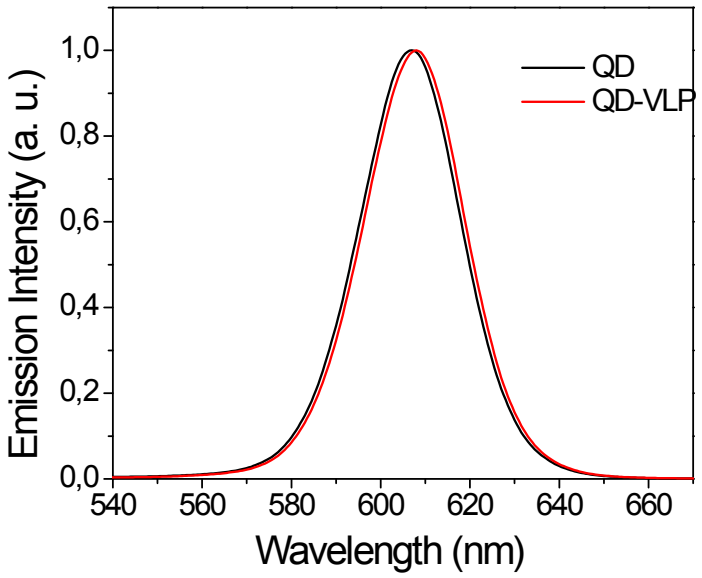

Figure ESI 5. Normalized fluorescence emission spectra of QDs recorded before (black) and after (red) the formation of QD/VLP assemblies obtained at pH 7.5. A slight red-shift is observed on the peak position after the assembly formation.

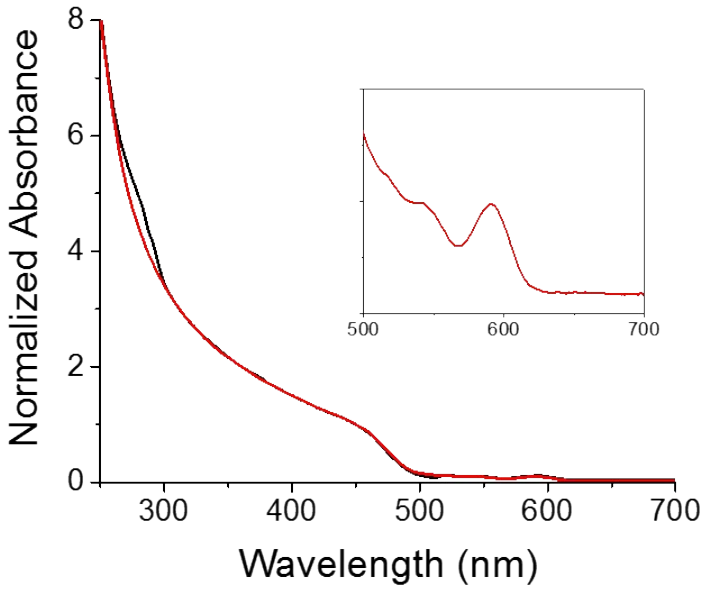

Figure ESI 6. Normalized UV vis absorption spectra of QDs (red curve) and QD/VLP assemblies obtained at pH 7.5 (black curve). The shoulder observed at 280 nm is due to protein absorption. Inset: the first excitonic peak of QDs, zoomed-in.

Calculation of the number of QD per assembly for T=1 and T=3 particles:

Particles with T=1 symmetry have 60 capsid proteins (CPs) and those with T=3 symmetry have 180 CPs. The extinction coefficient of QDs at 450 nm: 1800000 $M^{-1}cm^{-1}$
The extinction coefficient of CPs at 280 nm: 24075 $M^{-1}cm^{-1}$
Because QDs also absorb at 280 nm, we corrected CP absorption at 280 nm to exclude the contribution of QDs at this wavelength. We then calculated the QD and CP concentration using molar extinction coefficients and corrected absorbance values. The number of QDs per assembly can be obtained by dividing CP/QD ratio by 60 for T=1 assemblies and by 180 for T=3 assemblies. Accordingly, the number of QD per assembly was 3 for T=3 particles and 1.5 for T=1 particles.

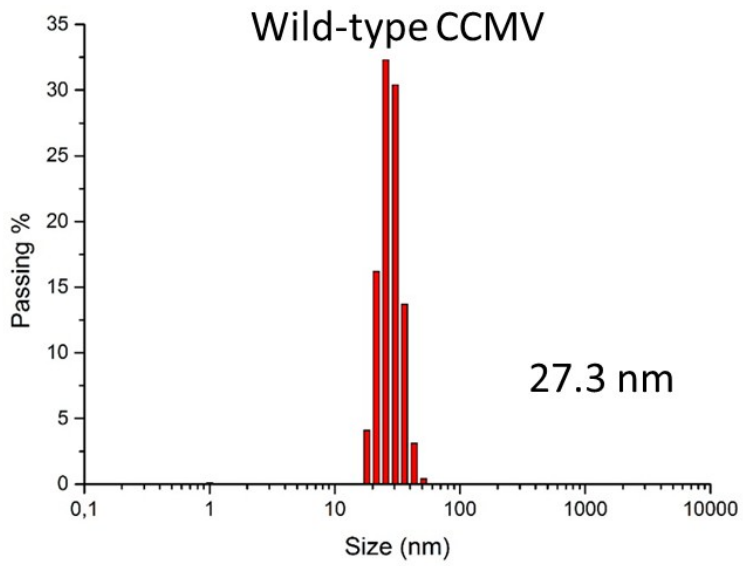

Figure ESI 7. Size distribution histogram of wild-type CCMV obtained by DLS. An average size of 27.3 nm was calculated over five separate measurements.

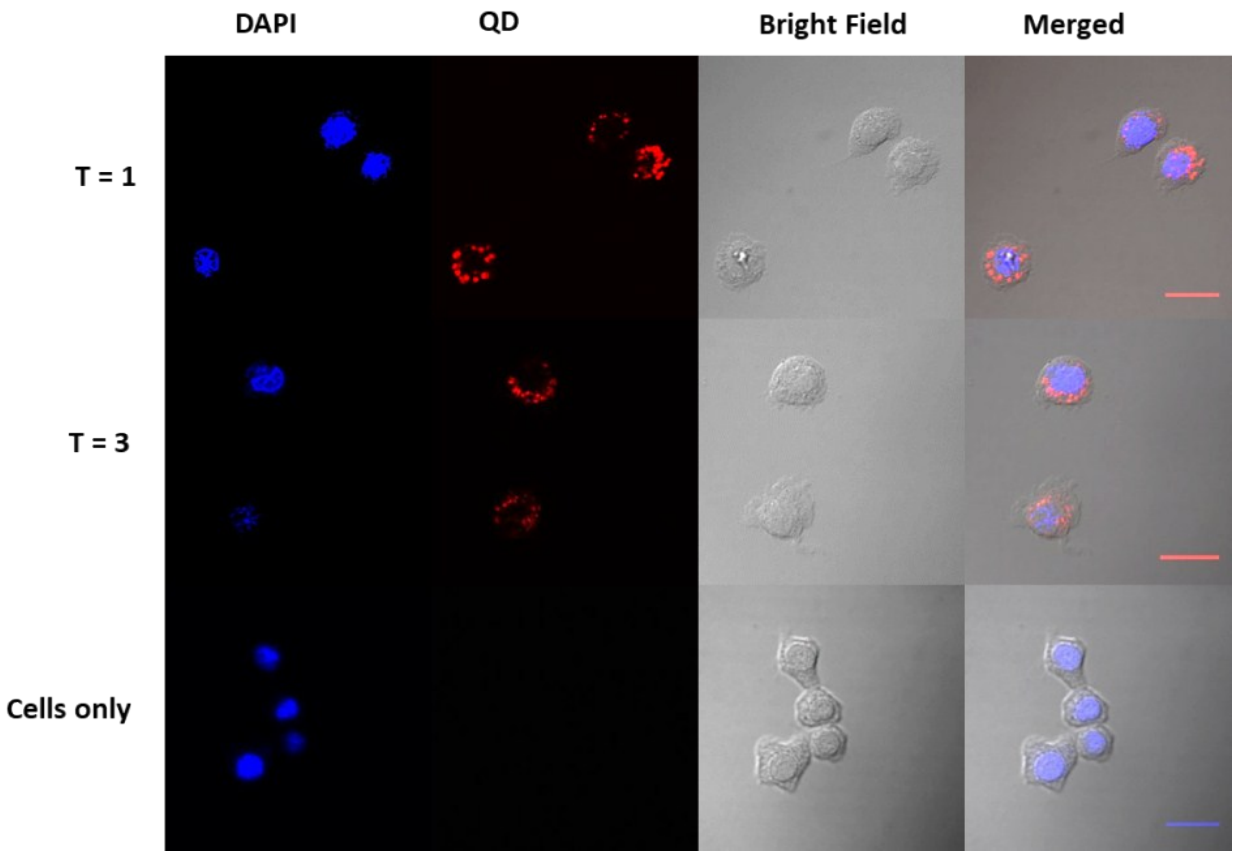

Figure ESI 8. RAW cells incubated with T=1 and T=3 particles. The nuclei of the cells are stained with DAPI (blue). Scale bar: 20 μm

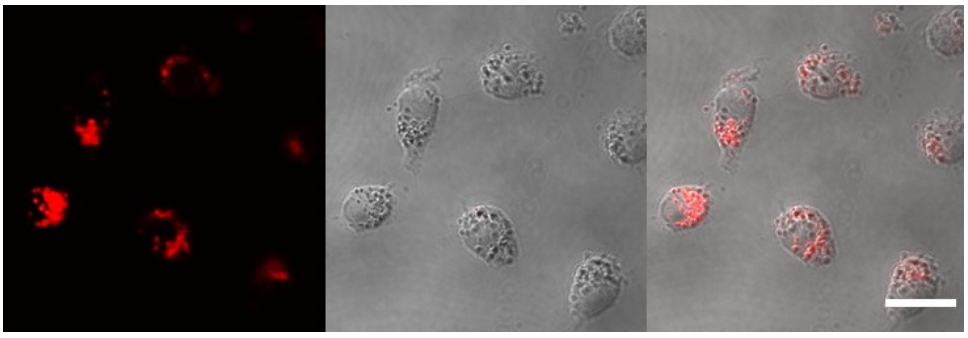

Figure ESI 9. CLSM images of RAW cells incubated with QDs. Left: bright field, middle: fluorescence, right: merged images. Scale bar: 20 μm.

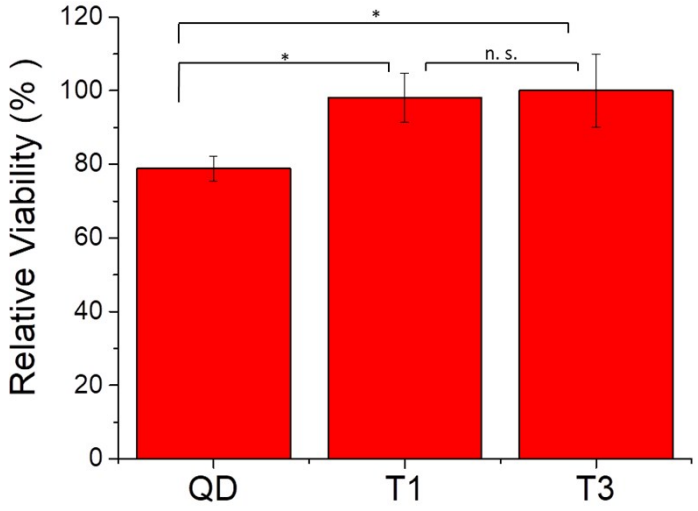

Figure ESI 10. Relative viability of RAW cells after 4 hours of incubation with QD/VLP nanoassemblies (T1 and T3) and QDs of equal QD concentration (50 nM). Unpaired t test, n.s.: non-significant ($P > 0.05$), *: significant, ($P < 0.05$).

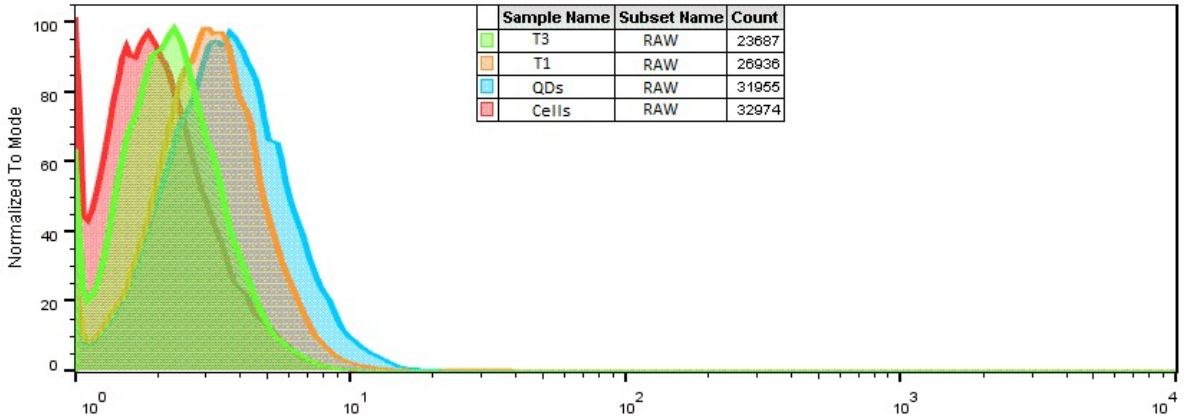

Figure ESI 11. Flow cytometry analysis of RAW cells (red, negative control) incubated with QDs (blue), T1 (orange), and T3 (green) particles for 2 hours. The histograms were plotted for populations with QD emission ($\lambda_{excitation}$: 488 nm, emission filter: 586/42). Approximately 25 000 events were monitored for each case.

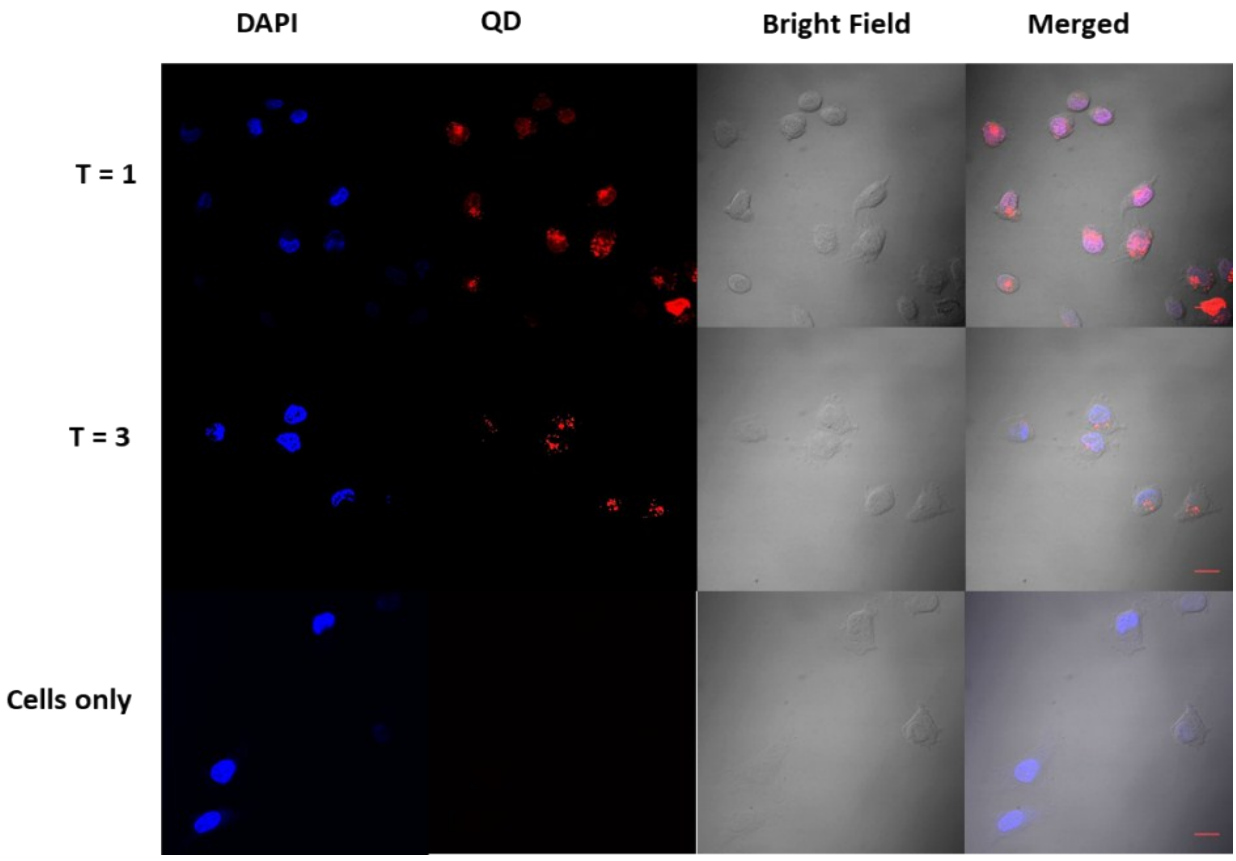

Figure ESI 12. HeLa cells incubated with T=1 and T=3 particles. The nuclei of the cells are stained with DAPI (blue). Scale bar: 20 μm