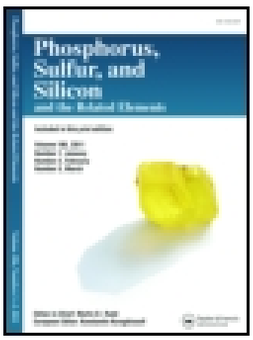

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Zwitterionic Ligands Bound to CdSe/ZnS Quantum Dots Prevent Adhesion to Mammalian Cells

Running Head: Zwitterionic Ligands Prevent Adhesion to Mammalian Cells

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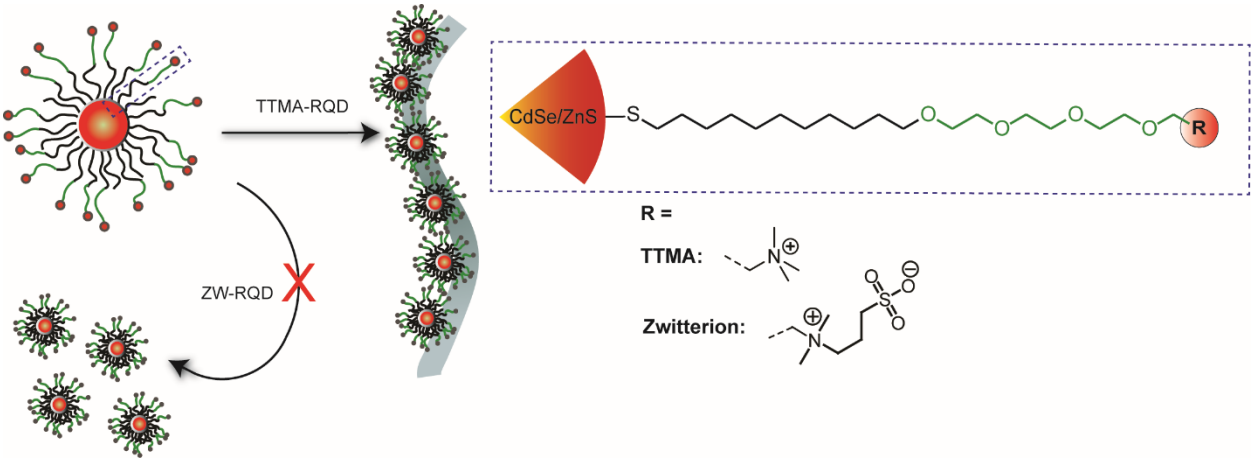
Abstract:

Zwitterionic materials are useful tools in material science and biology as they provide high water solubility while preventing non-specific interactions. Quantum dots (QDs) functionalized with zwitterionic and quaternary ammonium ligands were synthesized to investigate their interactions with the outer membrane of HeLa cells. Quaternary ammonium functionalized quantum dots adhered strongly to the cell surface while zwitterionic QDs had no cell adhesion. These results demonstrate that future non-interacting nanoparticles based on this design are possible.



Keywords: Zwitterion, Quantum Dots, Ammonium, HeLa, CHO, Adhesion

Introduction:

Zwitterions have emerged as useful tools in material science and biology due to their high water solubility while maintaining an overall neutral charge.[1] Sulfobetaines, carboxybetaines, and phosphobetaines are examples of zwitterions which do not change their charge in physiological environments such as those found in blood and plasma.[2] In addition to high water solubility, zwitterions provide a large hydration shell; water molecules surround both polar moieties providing stability.[3] This hydration shell is useful for developing materials designed for anti-fouling applications.[4] Furthermore, zwitterionic materials are useful in biology where preventing undesired adhesions to growth media and proteins are required.[5]

Zwitterions are promising materials to develop non-interacting nanoparticles for biological applications such as drug delivery. We have shown previously that zwitterionic ligands bound to gold nanoparticles provide a large hydration shell, resulting in corona free nanoparticles.[6] Herein, we have synthesized red fluorescent QDs (RQDs) functionalized with sulfobetaine zwitterionic (ZW-RQD) ligands to investigate their interactions with the outer membrane of mammalian cells. In addition, quaternary ammonium QDs (TTMA-RQD) was used as a positive control. After incubation and washing, the cationic QDs adhered strongly to the cell surface while the zwitterionic QDs had no cell adhesion. These results show that future non-interacting nanoparticles built off this design is possible.

Results and Discussion:

Scheme 1 outlines the synthesis of the TTMA[7] and zwitterion[6] ligands in addition

to the functionalization of the RQDs. RQDs were stirred with either TTMA or ZW ligands (1-RQD:7-ligand mass ratio) in a 4:1 dichloromethane:methanol solution for three days at 37 °C. The now hydrophilic RQDs were purified through dialysis to obtain pure TTMA-RQD and ZW-RQD.

Both RQDs were incubated with HeLa cells for 20 minutes and imaged using confocal microscopy to determine the degree of cell adhesion (Figure 1). Cationic RQDs show ample adhesion to the HeLa cells while ZW-RQD had no cell adhesion. In addition, both RQDs show no uptake within the cells after 20 minutes (Figure 1a, b). Longer incubation times (one hour) results in uptake of the TTMA-RQDs and was avoided to ensure cell adhesion was only observed. To investigate if similar adhesion is possible with other mammalian cells, RQDs were incubated with Chinese hamster ovary (CHO) cells and imaged using confocal microscopy (Figure S3). TTMA-RQDs show good adhesion to the surface of CHO cells while ZW-RQD had no cell adhesion.

Image analysis of the HeLa cell confocal images (Figure 2) revealed that TTMA-RQD showed high fluorescence intensity on the surface of the cell. ZW-RQD shows almost no fluorescence intensity signifying that ZW-RQDs large hydration shell prevents cell adhesion.

Experimental:

All solvents and reagents were purchased from Fisher Scientific and were used as received, unless otherwise noted. Milli-Q (MQ, 18.2 MΩ·cm at 25 °C) water was used in all steps where water was required. Detailed synthesis of TTMA[7] and zwitterionic[6]

ligands in addition to RQDs[8] can be found at their corresponding papers. Additional electron microscope images are included in the Supplemental Materials (Figures S 1 – S 2)

Functionalization of RQDs with TTMA and ZW ligands

In a 20 mL scintillation vial equipped with a stirbar was added 0.020 g of RQD. Then 4.0 mL of nitrogen purged dichloromethane (DCM) was added to dissolve the RQDs. A sample of 0.140g (1:7 mass ratio) of either TTMA or ZW ligands was dissolved in nitrogen purged methanol (MeOH, 1 mL) and directly added to the RQD solution and allowed to stir for three days at 37 °C under a nitrogen atmosphere. Afterwards, DCM and MeOH were evaporated, the vial washed three times with hexane, residual hexane evaporated and the now hydrophilic RQDs dissolved in water. Finally dialysis of TTMA-RQD and ZW-RQD in 10,000 MWCO membrane bags were performed to afford pure RQDs.

HeLa Cell Growth

HeLa cells were cultured in a humidified atmosphere (5% CO2) at 37 °C and grown in Dulbecco's modified eagle's medium (DMEM, low glucose) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/ml penicillin and 100 µg/mL streptomycin).

Incubation and Imaging of Hydrophilic RQDs with HeLa Cells

20,000 HeLa cells were plated into 1 cm^2 confocal chamber 24 h prior to RQD incubation. To perform the RQD incubation, cells were washed with PBS for 3 times followed by incubating with DMEM containing 50 nM RQDs for 20 min. After that, DMEM was discarded and cells were washed with PBS for 3 times. The confocal chamber was then placed on Zeiss LSM 510 laser scanning confocal microscope for imaging.

Image Analysis of the Confocal Images

An 8-bit grayscale format containing both fluorescent and bright field channels were obtained from least squares conformal map (LSCM). ImageJ was used to extract the fluorescent intensity channels along the cell walls.

Conclusion:

In conclusion, zwitterionic materials are useful tools as they provide high water solubility while preventing non-specific interactions. We show that non-interacting nanoparticles are possible with the functionalization of sulfobetaine zwitterionic ligands onto RQDs. The positive control, TTMA-RQD, is highly positively charged and can adhere onto the negatively charged mammalian cell membrane while ZW-RQD shows no adhesion to the surface both qualitatively and quantitatively. Nanoparticles built off this zwitterionic strategy will afford non-interacting nanoparticles for future biological applications such as drug delivery.

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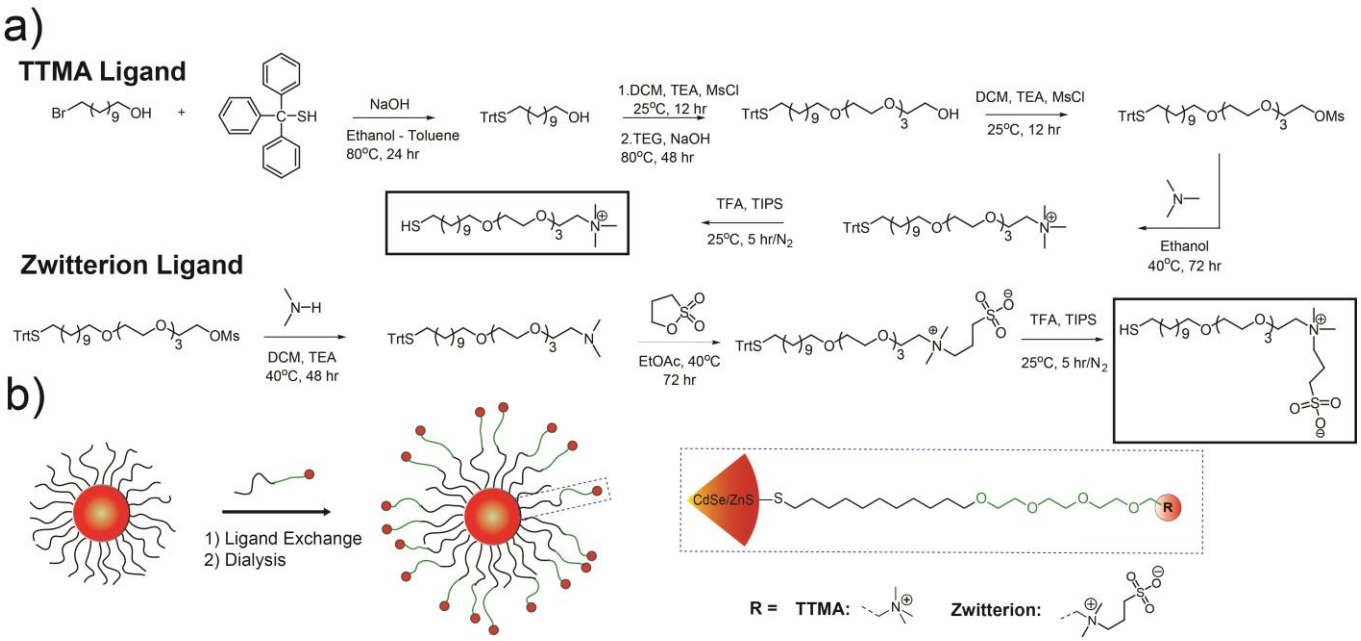

Scheme 1. a) Synthesis of TTMA and zwitterion ligands. b) Functionalization of RQDs with thiol-terminated TTMA and zwitterion ligands.

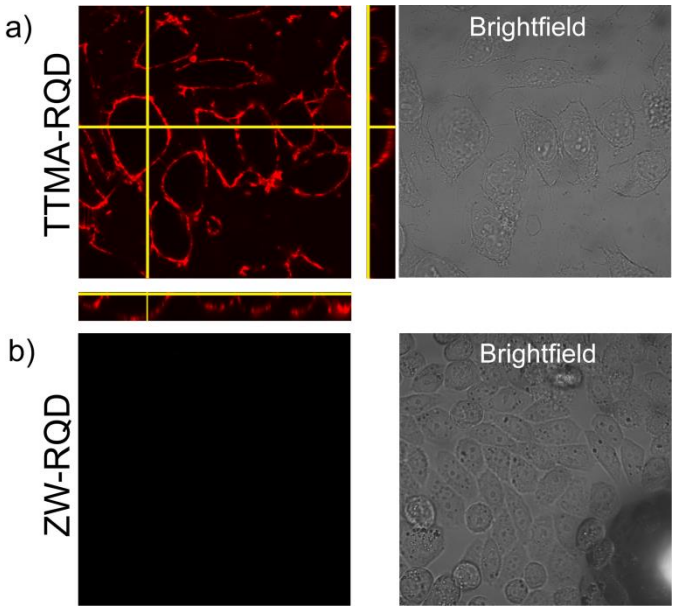

Figure 1. Confocal images of a) TTMA-RQD and b) ZW-RQD

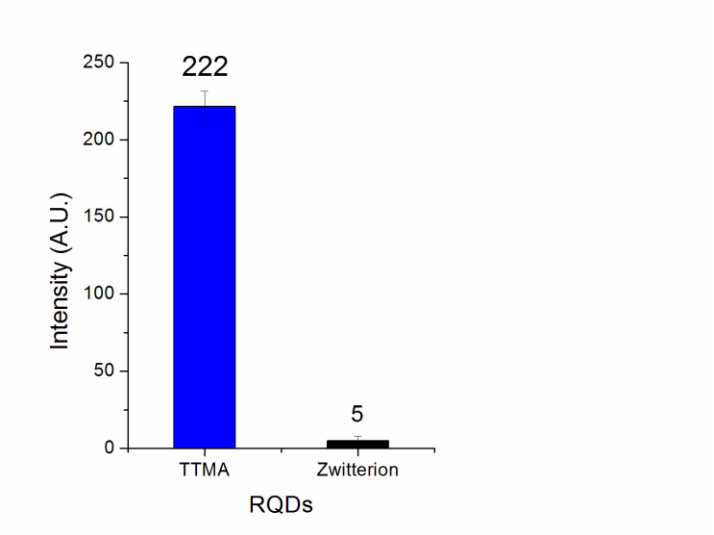

Figure 2. Fluorescence intensity of RQDs on the surface of HeLa cells. Quantification was determined by integrating over seven cells at four different points on the cell membrane.