# Preparation and Biological Investigation of Luminescent Water Soluble CdTe Nanocrystals

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## 1. INTRODUCTION

Semiconductor nanocrystals, often referred to as Quantum dots (QDs) represent a material of condensed matter with the number of atoms present on the surface being inversely proportional to the diameter. Thus, QDs less than 2nm have more than 50% of their total atoms residing on the surface. Due to their quantum confinement controlled properties where the diameter of the crystallite is smaller than the equilibrium separation distance of the exciton (electron–hole pair); QDs possess unique characteristics which can make them potentially useful in a wide range of applications. QDs with high fluorescent Quantum Yields (QYs) or Quantum Efficiencies (QEs) have been utilised in areas such as optoelectronics, [(1)] fluorescent biological imaging [(2), (3), (4)] and biofunctionalisation [(5), (6)].

Nanocrystal stability is of paramount importance for their use in such instances and consequently, surface structure and reconstruction must play a crucial role. To enhance the QY of the nanocrystals, epitaxial-type shells of a semiconducting material with a larger band gap, such as ZnS, CdS [(7), (8)] have been grown on the surface to effectively cap the surface defects. The transfer of QDs possessing large QYs which are stable in organic media to an aqueous phase via ligand exchange [(9)] and photooxidation of the surface via irradiation [(10), (11)] have also been used to produce stable, highly luminescent water soluble QDs. This increased luminescence makes the QDs particularly interesting for live cell imaging and diagnostics. [(12)] QDs possess considerable advantages over conventional organic dyes due to their higher degree of photostability, tuneable emission spectra and broadband excitation over a wide wavelength range. Much work in this area has centred on the conjugation of highly luminescent QDs to proteins, peptides and other biological molecules and their subsequent addition to live cell cultures to elucidate the mechanistic pathways of their movement [(13), (14), (15)]

To our knowledge limited work has been undertaken with regard to unmodified thiol stabilised QDs for use as biological imaging reagents. Here we report the use of commercially available thiols for CdTe QDs stabilisation, their potential for luminescence enhancement and investigations into their use as live cell imaging probes.

## 2. EXPERIMENTAL

### 2.1 General procedures

Aluminium Telluride ($Al_2Te_3$) was obtained from Cerac Inc. All other chemicals and reagents were obtained from Sigma-Alrich. All manipulations in the preparation and addition of precursors were carried out under vacuum or argon by Schlenk techniques.

UV-Vis absorption spectra were recorded using a Cary 50 Conc. UV-Vis spectrophotometer. Photoluminescence (PL) measurements were performed on a Cary Eclipse fluorescence spectrometer. A Heittich Universal 32 machine at 3500 rpm was used for centrifugation.

### 2.2 Preparation of thiol capped CdTe QDs

Water soluble CdTe QDs have been prepared according to published procedure. [(10)] 100 ml of millipore water (18MΩ.cm) was degassed by bubbling argon for approximately 1 hour. $Cd(ClO_4)_2 \cdot 6H_2O$ (0.737 g, 1.76 mmol) and thioglycolic acid (TGA) stabiliser (0.324g, 3.51 mmol) (Cd:thiol ratio – 1:2) were added and the pH

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was adjusted to 11.2 – 11.3 by the addition of a 2M NaOH solution. $H_2Te$ gas, generated from $Al_2Te_3$ (0.128 g, 0.29 mmol) via dropwise addition of a 0.5M $H_2SO_4$ solution was bubbled under a slow argon flow for approximately 10 minutes (Cd:Te ratio – 2:1). The resultant, non luminescent solution was then refluxed until the desired emission wavelength and subsequently size, determined via photoluminescence (PL) spectroscopy was reached. The solution was then reduced down by rotary evaporation to ~1/5 the original volume and size selectively precipitated via the addition of isopropanol. Upon isopropanol addition, the solution became turbid, was stirred and then precipitated using centrifugation. The resultant fractions were re-dissolved in millipore water and stored at 4°C for further characterisation and use.

A variety of commercially available thiols, summarised in table 1, were used as QD stabilisers in an effort to improve the luminescent properties and resultant QY for an aqueous synthetic preparation. The fluorescence QY of the CdTe QDs was measured against a standard of Rhodamine 6G in absolute ethanol which has a 95% quantum efficiency in the region 260-280nm.

### 2.3 Photoetching

50µl of purified thiol stabilised CdTe nanocrystals were diluted with 3 $cm^3$ millipore water. These solutions were irradiated with a water filtered, high pressure Mercury (Hg) lamp (in air). Field of irradiation was >350nm, but was also altered to >450nm by use of an optical filter. The solutions were stirred throughout. PL and absorption spectra were taken to monitor any optical effects.

### 2.4 Ageing

Freshly prepared thiol stabilised CdTe nanocrystal solutions before purification were diluted with millipore water and stored in darkness at room temperature and open to air. At particular daily intervals, 50µl of these solutions were diluted with 3 $cm^3$ of millipore water. UV-vis and PL spectra were taken to monitor any optical effects due to the ageing process.

### 2.5 Biolabelling and live intracellular visualisation using confocal fluorescence microscopy

The human THP-1 monocyte cell line was obtained from the European Collection of Animal Cell Cultures (ECACC, Salisbury, UK). Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM l-glutamine/L, 100 µg penicillin/ml and 100 mg streptomycin/ml, and incubated at 37°C in 5% CO2. To induce monocyte to macrophage differentiation, THP-1 cells were cultured in the presence of 100 ng/ml PMA for 72 hours. Cells were then washed three times with HBSS before use. THP-1 cells were incubated with a 1 in 100 dilution with the QDs. Live cell imaging was performed in Lab-Tek chambered coverglass slides (Nunc). Images were acquired by fluorescence microscopy (Nikon Eclipse TE 300) and on the UtraView Live Cell Imager confocal microscopy workstation (Perkin-Elmer Life Sciences, Warrington, UK) (Nikon Eclipse TE 2000-U). Processing was performed using Ultra View LCI.

## 3. RESULTS AND DISCUSSION

### 3.1 Preparation of thiol stabilised CdTe QDs

CdTe nanocrystals have been prepared from $Cd(ClO_4)_2$ and $H_2Te$ gas in water in the presence of the stabiliser and at the pH of 11.2 – 11.3 according to published procedure. [10] This water based synthesis of CdTe QDs involved the use of several different thiol stabilisers. The QYs were calculated using Rhodamine 6G as standard. The results are summarised in Table 1.

### 3.2 Photoetching

In terms of quantum efficiencies, non aqueous synthesis of QDs produce nanocrystals with superior luminescent properties than that of their water based counterparts. [16], [17] Photoetching of the QDs can however lead to an enhancement of the luminescence properties and somewhat bridge the gap between the aqueous and non-aqueous preparations.

Bao *et al.* [11] report an increase up to 85% QY at room temperature upon illumination over a long period of time. However, their luminescence spectra experience a red shift with illumination, indicating some particle growth with time. Here we show that for TGA capped CdTe quantum dots, illumination under certain conditions not only brings about an increase in luminescence but can also show a blue shift indicating a reduction in nanocrystal size similar to the results of Gaponik *et al.* [10] Increasing the luminescence via photoactivation suggests an annealing and reconstruction of the nanocrystal surface indicating a removal of surface defect states which could reduce the emission via nonradiative pathways. These defect states are broader, less pronounced and reside at higher wavelengths than the intrinsic band-gap emission. [18] At room temperature they can appear to cumulatively add to the intrinsic emission leading to an unsymmetrical spectrum. However, at low temperature this band can be further emphasised and shown, no longer as part of the intrinsic emission, but as an independent feature. [19] Upon illumination, this can be removed or reduced and a greater symmetrical nature can be restored to the intrinsic spectrum.

**Table 1:** Stabilisers used and correspondent quantum yields. Stable refers to QD solutions kept at 4°C for longer than 3months, moderately stable – less than 3months and unstable – less than 1month.

| Stabiliser name | Stability of solution | Quantum Yield |
|---|---|---|
| Thioglycolic acid (TGA) | stable | 16% |
| 3-Mercapto-1,2-propanediol | stable | 12% |
| L-cysteine | stable | 15% |
| N-(2-Mercaptopropionyl)-Glycine | moderately stable | 6% |
| Glutathione | unstable | 25% |

Figures 1 and 2 show the resultant UV-vis and luminescence spectra of TGA capped CdTe QDs irradiated for independent periods of time, with a filter and without a filter respectively. While various other stabilisers were tested, luminescence increases were not as substantial and quenching, followed by coagulation of the colloid was far quicker. This indicates a loss of stability with irradiation possibly due to the decomposition of the thiol at the nanocrystal surface. The results for TGA stabilised QDs proved most pronounced. In figure 1 the absorption spectra initially red shifts but it then remains constant until the sample decomposition point is reached. The luminescence intensity increases rapidly (over three fold increase) with irradiation time until the decomposition point is also reached, which is followed by a reduction in intensity, an increase in the peak width half maximum value (PWHM) and a red shift to higher wavelengths. This was also accompanied by a distinctive colour change within the solution. Decomposition occurred after 70 minutes of irradiation; however the same photochemical degradation is not seen in figure 2, where only a single fold increase is observed.

In figure 2, the luminescence spectrum is seen to increase in intensity with irradiation time while no marked changes are noticed in the absorption spectrum. While no discernable PL wavelength shift is noted in figure 1, a slight blue shift to lower wavelength is evident in figure 2, thus indicating a slight reduction in overall crystallite size. No filter is present which indicates that the irradiation between 350 – 450nm causes a decrease in crystallite size, indicating an increase in band-gap energy but also results in an intensity enhancement of a lesser extent.

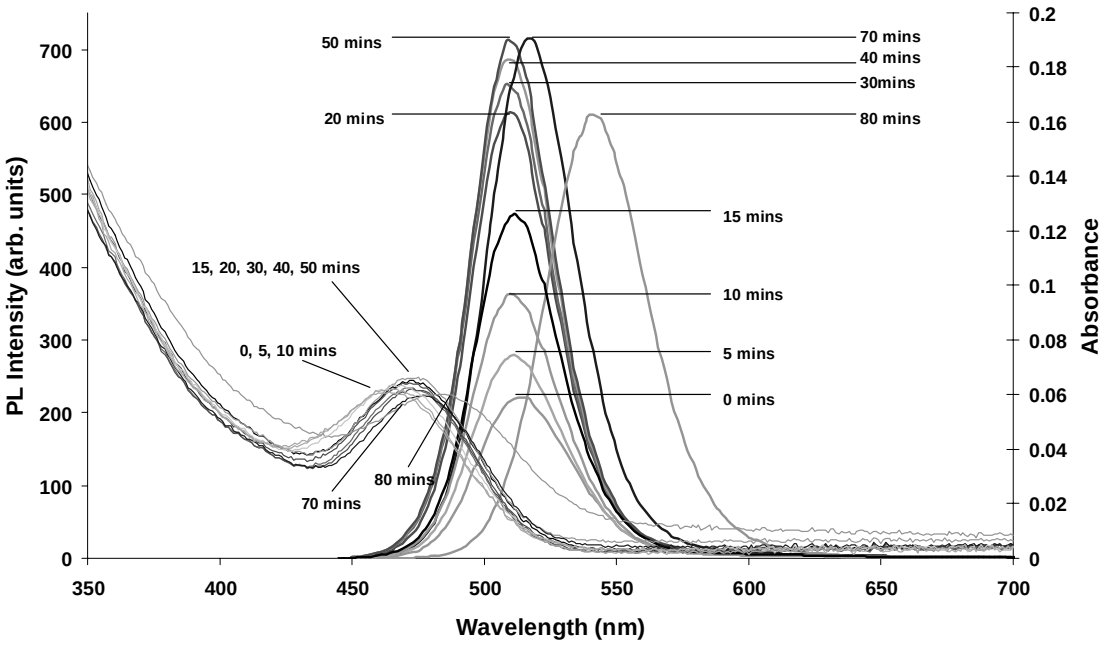


**Figure 1:** UV-vis and normalised room temperature luminescence spectra for optical filter mediated photoetching of TGA capped CdTe QDs.

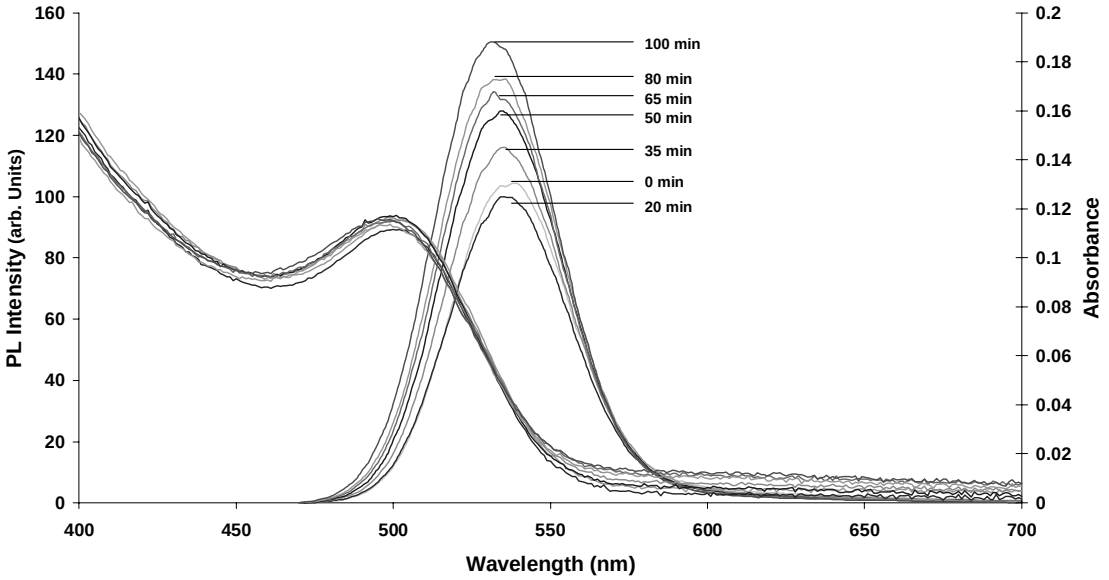


**Figure 2:** UV-vis and normalised room temperature luminescence spectra for photoetched TGA capped CdTe QDs.

Bol *et. al.* [20] postulate that oxygen is photochemically absorbed upon irradiation and efficiently reduces nonradiative recombination. This oxidation or hydrolysis of the available thiol groups can result in the formation of holes within the nanocrystal monolayer and accelerate the photooxidation process. Also, due to the large surface to volume ratio of the QDs, uncapped surface atoms can subsequently lead to the presence of

surface dangling bonds. These bonds which act as exciton traps may become photoactively oxidised, therefore removing a nonradiative pathway for decay resulting in increased luminescence intensity. [21] Another possibility is the photoactivation of so called "dark dots", whose fluorescence adds to the overall luminescence of the system. [22] Most commonly, an increase in luminescence is achieved by capping of the surface with a semiconducting material possessing a larger band-gap such as CdS or ZnS. This is generally however accompanied by an increase in size. This layer effectively caps the defects present on the surface thus reducing the number of nonradiative recombination channels and increasing the overall quantum efficiency. A photochemically catalysed reaction involving incorporation of the thiol into an epitaxial-like layer is possible, but for this to be occurring here it must also be accompanied by a restructuring of the nanocrystal surface as no increase in size is observed. A decrease is actually seen under certain conditions indicating a photocorrosive process as a result of irradiation.

### 3.3 Ageing

To monitor the effect of time and the presence of excess reagents on the optical properties of thiol capped CdTe QDs, samples possessing various different stabilisers were kept in darkness but open to air at room temperature. While all samples were analysed, TGA stabilised QDs once again gave the most substantial results.

Figure 3 shows the well established absorption and emission profiles of thiol stabilised CdTe quantum dots. As can be seen, the luminescence intensity increased quite substantially (two fold) over the period of ageing. Unlike the photoetched samples where the quantum dots were purified by size selective precipitation and then re-dissolved in millipore water, the samples left for ageing were not purified. They contained all excess reagents used in the reaction including unreacted stabiliser molecules and cadmium ions.

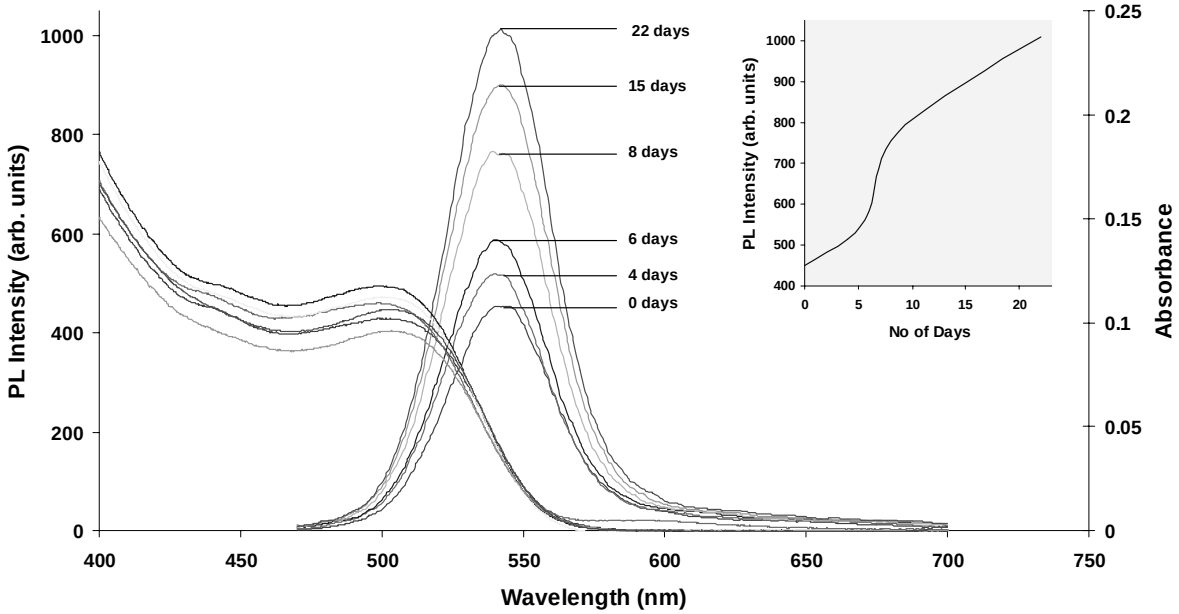


**Figure 3:** UV-vis and normalised room temperature luminescence spectra of TGA capped CdTe QDs aged for a total of 22 days.

No change in the absorption or emission wavelengths suggests that the intensity increase is from structural reorganisation of both the surface and the lattice framework itself. Thus as more ions and molecules are present it can be concluded that with time we see and increase in surface passivation due to further reaction of the excess reagents. Zhang *et.al.* [23] have also shown that the carboxylic groups of the bound and excess stabiliser molecules present play a crucial role in PL enhancement. These carboxylic groups can interact with the excess $Cd^{+}$ ions on the surface via a secondary coordination resulting in an increased stabilisation and

subsequent PL enhancement. This has also been shown for carboxylic rich polymers and poly (ethylene glycol) capped CdS nanoclusters. [24]

As ageing increases PL intensity most substantially for TGA, (sterically least demanding molecule) it can be concluded that reagent sizes and steric effects play a major role in enhancement of the surface passivation. The gaps in surface coverage resulting in dangling bonds and increased trap states must be most efficiently capped by additional incorporation of TGA. This is allowed by the size and steric compatibility of the TGA molecules to effectively diffuse to the $Cd^{2+}$ rich nanocrystal surface and bind. The other thiols used, possess extra or different functional groups than the TGA molecule. While these functional groups could play a role in secondary interactions with the surface, they may subsequently prevent other unreacted thiol molecules from successfully binding to the surface, thus resulting in no increased luminescence intensity with time.

### 3.4 Investigation of CdTe QDs in live cell imaging

QDs stabilised with the various thiols outlined in table 1, were added to a culture of THP-1 cells. THP-1 cells were differentiated to macrophages as described in materials and methods and quantum dots added at 1 in 100 dilution followed by incubation for the indicated times at 37°C. Vigorous washing followed the incubation period to detach the loosely bound quantum dots.

Figure 4 shows the phase contrast (A) and immunofluorescent images (B (green) C (red)) of L-cysteine stabilised QDs binding to the outer cell membrane of THP-1 cells. This experiment shows that L-cysteine stabilised QDs are biocompatible with the macrophage like-THP-1 cells, as no obvious toxicity was observed. Enhanced particle clustering was observed in particular around the outer membrane of the cells. Additional experimentation revealed that after prolonged exposure i.e. 6 hours, these particles did not accumulate inside the macrophages (data not shown).

In Figure 5 we investigated the ability of 3-mercapto-1, 2-propanediol stabilised QDs to label macrophages. Figure 5 shows only minimal particle binding to the cell surface following 30 minutes of coincubation at 37°C. Next we investigated glutathione stabilised CdTe QDs (Figure 6). Interestingly, the glutathione stabilised QDs did not penetrate the cell membrane nor did they adhere to the cells surface, they only displayed intracellular localisation in dead / compromised cells.

We assessed the ability of TGA stabilised QDs to act as suitable labels for biological imaging. Figure 7 shows intense particle binding of highly luminescent QDs to the cell surface following 5 minutes of a coincubation time. Further experimentation, revealed in figure 8, panel A, that TGA stabilised QDs are internalised by macrophages and sequestered to the cytoplasm within 10 minutes. Next we evaluated the effects of photoetched TGA capped QDs. Panel B of Figure 8 shows the intensely fluorescent photoetched TGA QDs with a cytoplasmic location in THP-1 cells. These QDs possess far greater luminescent properties than their non etched counterparts (Panel A, figure 8 and panel B, figure 7) and show a greater optical enhancement of the cell region.

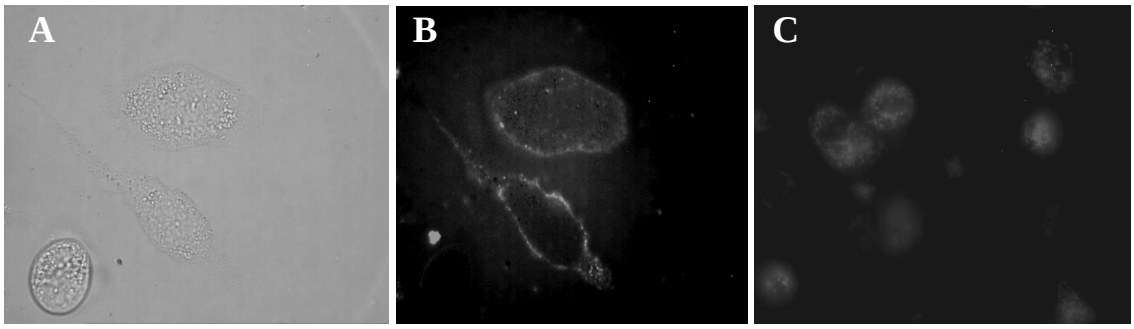


**Figure 4:** Phase contrast (A) and immunofluorescent images of green L-Cysteine (B) and red L-Cysteine (C) stabilised QDs adhering to the outer membrane the macrophages.

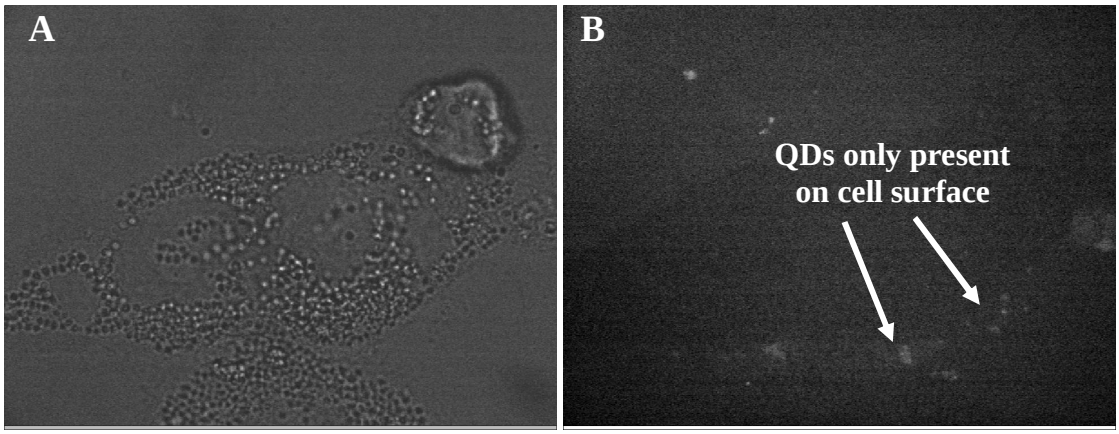


**Figure 5:** Phase contrast (A) and immunofluorescent image (B) of 3-mercapto-1,2-propanediol stabilised QDs attached to the outer membrane the macrophages.

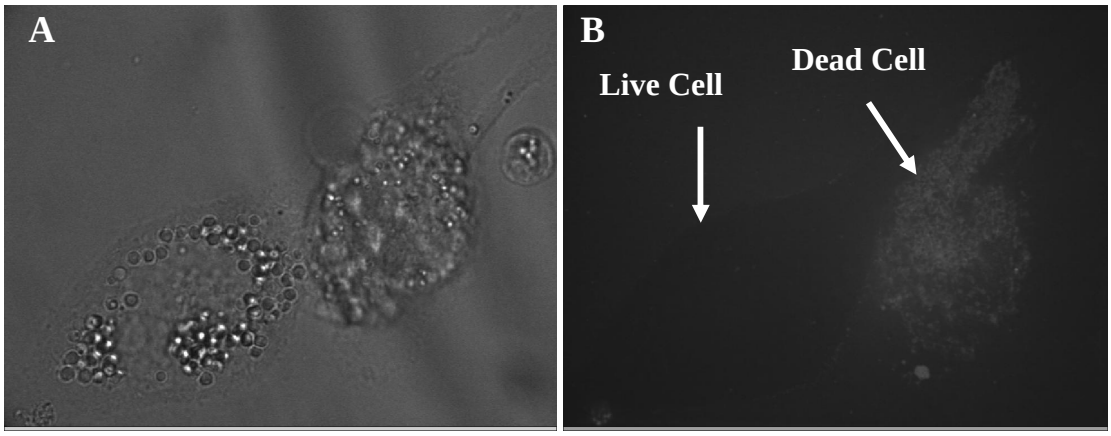


**Figure 6:** Phase contrast (A) and immunofluorescent image (B) of glutathione stabilised QDs excluded from live cells and present only inside a dead cell.

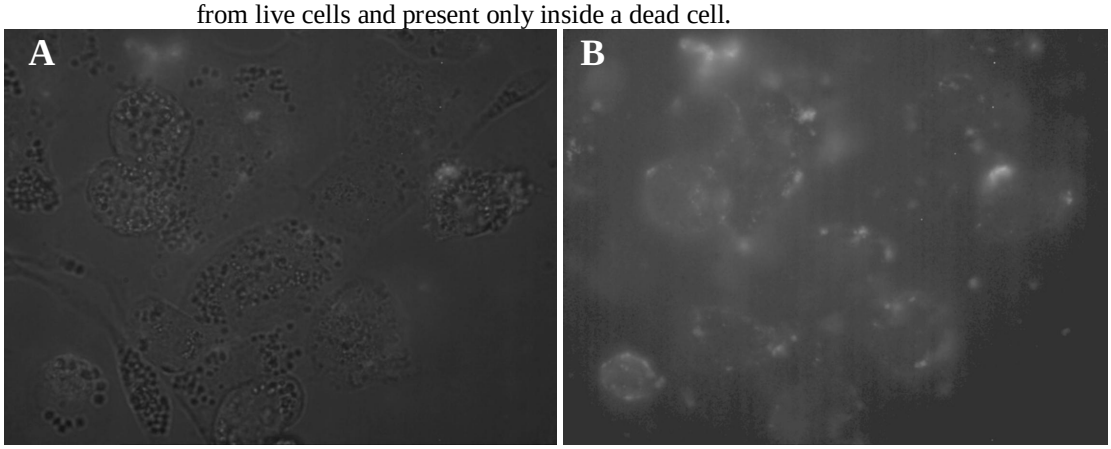


**Figure 7**: Phase contrast (A) and immunofluorescent image (B) of TGA stabilised QDs adhering to the macrophage cell surface following short coincubation treatment.

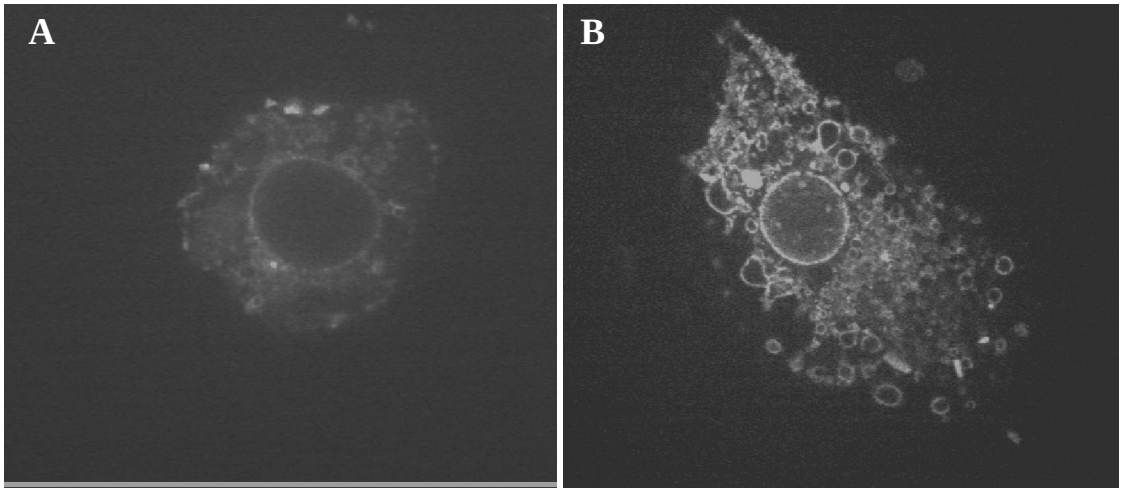


**Figure 8:** Immunofluorescent images of non photoetched TGA (A) and photoetched TGA (B) stabilised QDs displaying an intracellular location in macrophages.

## 4. CONCLUSIONS

We have shown that for TGA stabilised QDs the luminescence quantum yield can be increased significantly, up to three fold, under certain irradiation conditions. Similar increases in luminescence can also be achieved by ageing the QDs solutions to allow excess unreacted precursors to adhere and bind to the crystallite surface.

We have also tested the thiol stabilised QDs as potential biolabels. These preliminary studies have investigated the effects of differential stabilisers on CdTe QD activity. Here we show L-cysteine stabilised QDs localising to the outer cell membrane in living cells. We demonstrate the potential role of TGA stabilised CdTe QDs as excellent live cell imaging tools as they exhibit strong luminescence and excellent photostability. In addition, the ability of TGA stabilised CdTe QDs to traverse the cell membrane of macrophages is a formidable quality that may potentially be harnessed for imaging and therapeutics. Modulating the delivery of QDs to subcellular locations in living cells opens a myriad of potential applications ranging from drug delivery to examination of intracellular processes.

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