

## Monitoring and Imaging Hypoxic Cells using Perfluorinated Near-Infrared Fluorescent Micelles

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### Introduction:

In the poorly formed blood vessel networks of solid tumors, hypoxic regions, or areas of low oxygen concentration, develop due to the ineffective delivery of oxygen by the cells. These hypoxic tumors are often resistant to conventional treatment methods [1]. Therefore, the development of a complementary strategy to detect and deliver oxygen to hypoxic tumors would improve treatment response. This could be achieved with multifunctional nanoparticles that are designed to report hypoxia and deliver oxygen to the target tissue. Specifically, quantum dots (Qdots), nanometer semiconductor particles, are used in optical imaging because of their near infrared fluorescence properties, brightness, photostability and potential for multivalent functionalization. In this study, we functionalized Qdots with perfluorocarbons to form novel micelles. We chose perfluorocarbons because of their oxygen carrying abilities [2]. To solubilize the nanoparticle in aqueous solution, an amphiphilic molecule was also prepared and used to formulate the micelle construct [3]. Thus, we developed new perfluorocarbon-coated quantum dots that are capable of monitoring and imaging hypoxic cells. In addition, the materials can effectively deliver oxygen to hypoxic tissues, which will improve the treatment of these difficult tumors.

### Materials:

PbS Quantum Evidot was acquired from Evident Technology (Troy, NY). Perfluorodecane-thiol, perfluorocetyl bromide, toluene, and hexane were purchased from Sigma Aldrich (St. Louis, MO). Oxygen UHP was obtained from Airgas (St. Louis, MO) and deionized water was obtained from MillQ System (Billerica, MA).

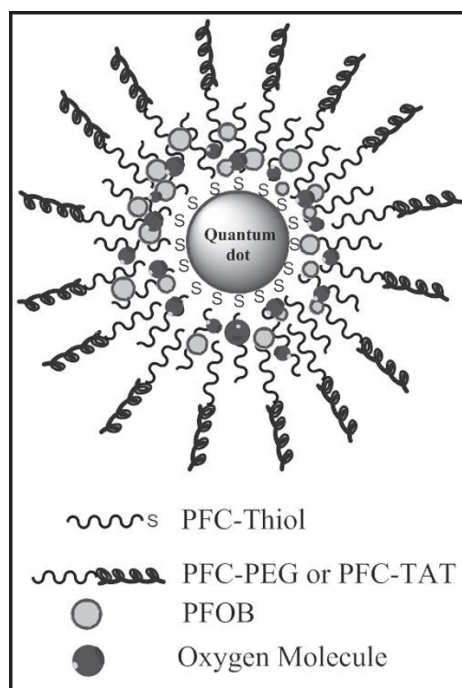


Figure 1: Micelle construct.

### Synthesis of the Micelle:

Lead sulfide quantum dots (Qdots, 10 mg/mL) were functionalized with multiple perfluorocarbon surfactants. First, perfluorodecane (PFC) thiol was added to Qdots based on a previous research patent, resulting in a 0.05 volume ratio of Qdots in PFC-thiol [4]. The trioctylphosphine oxide (TOPO) ligands originally coating the Qdots were replaced by the PFC-thiol surfactant. Second, polyethylene glycol-perfluorocarbon (PFC-PEG, 16 mg) was synthesized and added to 20  $\mu$ l of the 0.05 concentrated sample PFC-thiol Qdot solution, based on concentration calculations ensuring that each individual quantum dot would be completely coated with PFC-PEG instead of forming large aggregates.

After centrifugation, the suspension of the micelle in deionized water (200  $\mu$ L) illustrated that the PFC-PEG

efficiently made the Qdots hydrophilic. To a new solution, with a similar approach, 20  $\mu$ l of PFOB saturated the solution according to surface area calculations. The final Qdot construct formed is shown in Figure 1.

The novel micelle construct was then saturated in an oxygenated environment for two hours. Fluorescent quenching was observed, due to PFOB's ligand structure, which effectively holds oxygen molecules. An attempt at restoring the fluorescence was completed by displacing the oxygen molecules with nitrogen, but to prove the PFOB containing the oxygen was the cause of the quenching, the Qdots and samples functionalized with each surfactant addition were also submitted to the oxygenated environment for comparison of fluorescent emission.

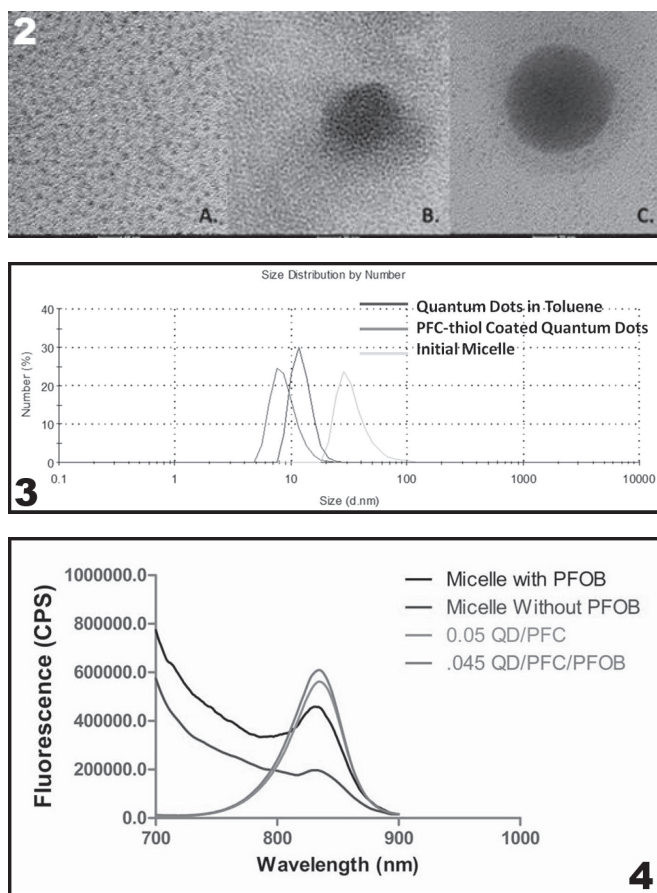


Figure 2, top: TEM images; A) Qdot in toluene, B) Micelle without PFOB, and C) Micelle with PFOB.

Figure 3, middle: DLS for each surfactant addition.

Figure 4, bottom: Fluorescence of micelles compared to surfactant additions.

### Characterization:

The size of the initial PbS Qdots and Qdots with each surfactant coating addition was confirmed by transmission electron microscopy (TEM) images taken on the FEI Tecnai Spirit 120KV, and the hydrodynamic diameter measurements from the dynamic light scattering (DLS) information obtained from the Malvern Zetasizer Nano ZS. The change in size after each surfactant layer addition and of the final micelle (with and without PFOB) was compared. Fluorescence emission data was recorded from the Fluorolog-3 spectrophotometer (Horiba Jobin Yvon). An optimized concentration was determined via the emission efficiency comparison. The quenching effect was also observed via the emission measurement of the micelle construct.

### Results and Conclusions:

The TEM images (Figure 2-A) for the PbS Qdots in toluene collaborated with the DLS (Figure 3) measurement of the

hydrodynamic diameter including the TOPO ligands, proving the 2 nm size. As hypothesized, the replacement of the Qdot TOPO ligands in the addition of the relatively smaller PFC-thiol ligand was shown by the slight decrease in the DLS size. The production of varying concentrations of PFC-thiol and Qdots showed that low amounts of PFC-thiol or high amounts of Qdots can cause the unsuccessful coating and Qdot precipitation. A temporal evolution of the fluorescence of the remaining concentrations of 0.05 and 0.2 volumes of Qdots in PFC-thiol proved that emission was not concentration dependent after 10  $\mu$ l of Qdot. The 0.05 and 0.1 concentrated samples were observed over five days and the 0.05 sample had a fairly high fluorescence, for a less concentrated sample, making it the feasible choice.

With the next surfactant addition, DLS data (Figure 3) showed an increase in size indicating the PFC-PEG surfactant successfully coated the Qdots. After centrifugation, the brown pellets formed were suspended in water demonstrating that the successful formation of hydrophilic micelle. TEM images of the micelle are shown in Figure 2-B. Micelles containing PFOB were successfully created via similar procedure and DLS (Figure 3) and TEM (Figure 2-C) information obtained showed no size increase compared to the micelle without PFOB. The fluorescence of the micelle containing PFOB showed improved emission efficiency due to the better coating of Qdots in this construct.

Oxygenation of the micelle construct was successful and quenched fluorescence was observed. De-oxygenation with nitrogen and restoring the fluorescence of this construct need to be further investigated.

### Conclusions:

Qdots were successfully functionalized with perfluorocarbons compounds and solubilized in an aqueous solution for the monitoring and imaging of hypoxic cells. The micelle construct was also proven capable of containing oxygen to be delivered to hypoxic cell regions.

### Acknowledgements:

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### References:

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