Chemistry & Biology, Vol. 12, November, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.chembiol.2005.11.002

New Light on Quantum Dot Cytotoxicity

As quantum dots are beginning to be used for in vivo imaging, the question of their long-term effect on cell viability is becoming critical. In this issue of *Chemistry & Biology*, Lovrić and colleagues examine the likely role of reactive oxygen species in quantum dot cytotoxicity [1].

Quantum dots or qdots are a new breed of nonorganic nanocrystalline fluorescent probes, which have recently caught the interest of many biologists and generated much hope, some hype, but also skepticism (for recent reviews, see for instance ref. [2, 3, 4]). A consensus has emerged concerning their superior photophysical properties, but mixed reports on their stability or compatibility with long-term live imaging might be attributed to the fact that there are currently almost as many quantum dots as there are reports about them. This diversity has multiple origins. First, qdots can be synthesized using different materials and protocols, leading to various final products. Second, qdots need to be solubilized in aqueous buffers using additional chemical steps. At this stage too, a vast number of solutions have been proposed and tested. Finally, biological functionalization adds a third level of diversity.

Whereas initial reports have described successes in areas including single-molecule detection [5], single-cell tracking [6], and whole embryo [7] or animal in vivo imaging [8], the need for a serious assessment of qdot's potential long-term cytotoxicity has garnered much needed attention in recent publications [9, 10, 11]. Although limited to cadmium (Cd) chalcogenide materials (CdS, CdSe, or CdTe cores, with or without a ZnS shell), these studies have globally shown (1) that qdots become toxic to cells when present at micromolar concentrations in the growth medium, and (2) that toxicity is directly related to the accessibility of the core surface cadmium atoms to the surrounding medium. Kirchner et al. have also shown that at high concentrations, any type of nanoparticle will reduce cell viability [11]. Therefore, different mechanisms of cytotoxicity may be at play (Figure 1).

Two questions need to be addressed in order to better fight qdot cytotoxicity at the concentrations (<<1 μM) used in actual imaging applications. First, what can prevent Cd atoms from being accessible to or potentially released into the surrounding medium? And second, what are the biochemical mechanisms resulting in cytotoxicity?

Past studies have answered the first question relatively satisfactorily (Figure 1). Core Cd atom accessibility is linked to the permeability to oxygen [12] and protons [13] of the different extra layers of materials that are added to the core (shell and ligands). Diffusion of oxygen to the surface of the shell or core can result in oxidation (or photo-oxidation upon UV excitation) of these crystalline materials, and enable subsequent release of Cd^{2+} ions [9]. Protons (in low pH environments) on the other hand can lead to protonation of the coordination groups of the ligands and their subsequent detachment from the qdot surface [13]. In other words, a shell will reduce qdot toxicity by delaying the oxidation of the core, as will a solubilization layer comprised of long, crosslinked chains rather than short (usually not crosslinked) monomers [9, 14, 15].

The second question, the biochemical mechanisms of cytotoxicity of qdots, is slowly beginning to be answered. One early study by Derfus et al. documented the effect of qdots on cultures of primary hepatocytes, the liver being a prime target of Cd toxicity [9]. Measurement of Cd^{2+} concentrations in solutions of qdots exposed to different treatments (exposure to air or UV excitation) showed a direct correlation between Cd^{2+} release and the extent of cytotoxicity. However, it was only assumed that the mechanism of cytotoxicity was the inactivation of essential mitochondrial proteins through Cd-sulfhydryl group interactions, as reported in the literature [16].

One possible alternative mechanism involving the creation of reactive oxygen species (ROS) such as free radicals (hydroxyl radical: •OH and superoxide: $\bullet O_2^-$) and singlet oxygen ($^1O_2$) has been suggested in recent papers [17, 18, 19, 20], including the current article by Lovrić et al. [1]. Samia et al. have shown that singlet oxygen can be generated in toluene by bare CdSe qdots, and in water when conjugated with a photosensitizer [20]. Singlet oxygen generation via a photosensitizer involves the photoexcitation of the sensitizer, subsequent intersystem crossing from its singlet state to its triplet state, and finally energy transfer from the sensitizer triplet state to an oxygen molecule (Figure 2). This energy transfer converts triplet O_2 ($^3O_2$) to singlet O_2, a species known to cause irreversible damage to nucleic acids, enzymes, and cellular components such as mitochondria and both plasma and nuclear membranes. Other ROS (free radicals such as •OH and $\bullet O_2^-$) have been detected by electron paramagnetic resonance (EPR) in solutions containing qdots. In a brief report, Green and Howman showed DNA nicking and free radical generation from both CdSe and CdSe/ZnS qdots [18]. Ipe et al. have, on the other hand, pointed out that the conduction band potential of CdSe does not allow reduction of oxygen to superoxide after photoexcitation, but could produce hydroxyl radicals [19]. In core/shell CdSe/ZnS qdots, the ZnS shell creates even higher energy barriers between oxidizing molecules and the CdSe core, preventing the production of either •OH or $\bullet O_2^-$. Accordingly, Ipe et al. discovered traces of •OH radicals in solutions of irradiated core qdots, but found no evidence of free radicals generated from irradiated core/shell qdots [19]. This suggests that Green and Howman core/shell results could be due to the degradation of the ZnS shell upon irradiation, illustrating once more the importance of a well-protected core.

Using a combination of morphological characterizations and biochemical assays, Lovrić et al. now provide convincing evidence for the production of ROS in live

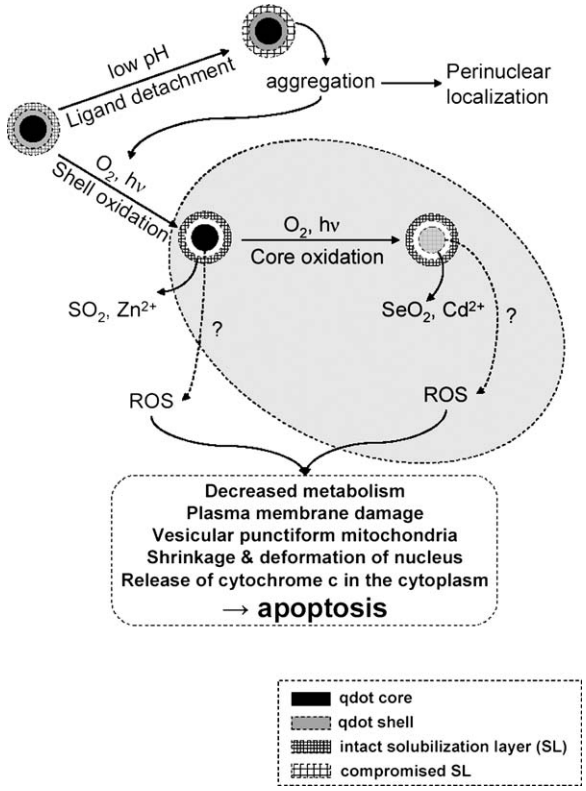

Figure 1. Schematics of the Different Mechanisms Resulting in Quantum Dot Cytotoxicity

Core/shell qdots can undergo several degradation processes in the cell: their solubilization layer (hatched) can lose its integrity, resulting in precipitation and aggregation; the shell (black) can be oxidized; and finally, the core (gray) can be oxidized. By mechanisms that remain poorly understood, reactive oxygen species (ROS) can be generated, triggering a chain of apoptotic events. The experiments described by Lovrić et al., who used core-only qdots, would correspond to the grayed area.

cells incubated with CdTe qdots solubilized with mercaptopropionic ligands [1]. Although the qdots used in this study are expected to be relatively unstable due to their short ligands and the absence of a shell, and therefore rather toxic (see above), they are in this respect ideally suited to the characterization of qdot cytotoxicity.

The authors first describe extensive cellular damage of the plasma membrane, mitochondria and nucleus, after 12–24 hr of qdot exposure, accompanied by a reduced metabolism in a dose- and time-dependent manner, all characteristic of a stressful cellular context. At the highest qdot concentrations (~1 μM), cell death is observed and occurs via apoptosis, as shown by the release of cytochrome c into the cytoplasm. However, the absence of apparent caspase involvement indicates a nonclassical mechanism of apoptosis. The authors finally demonstrate the involvement of ROS using a fluorescence assay producing negative results in the presence of antioxidants. A current picture summarizing the previously available data on cytotoxicity and these new results is presented on Figure 1, illustrated with hypothetical core/shell qdots.

Understanding and addressing the mechanisms of qdot cytotoxicity is necessary to harness their potential for live cell or animal imaging, and to exploit them for photodynamic therapy applications [20, 21] (Figure 2). As illustrated by the work of Lovrić et al., combining both capabilities may be a challenge as they require opposite characteristics. But as such qdots could enable physicians not only to image tumors, but also to selectively target them for destruction with a single probe, this goal remains worth pursuing.

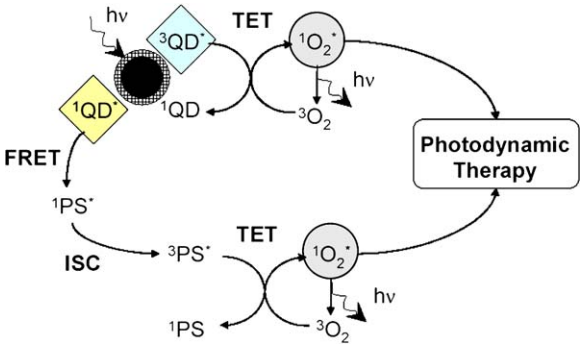

Figure 2. Reactions Involved in the Production of Reactive Oxygen Species via Photoexcitation of a Qdot

Two possible mechanisms can be invoked, one involving the singlet excited state of the qdot ($^1QD^*$, yellow path), and the other its dark triplet state ($^3QD^*$, blue path). In the first case, a photosentisizer (PS) is needed to generate the ROS (singlet oxygen $^1O_2^*$, gray circle) via a cascade of fluorescence energy transfer (FRET), intersystem crossing (ISC), and triplet energy transfer (TET); in the second, singlet oxygen is directly obtained by TET from the qdot to triplet oxygen. The singlet oxygen species can return to its triplet state by phosphorescence in the near-infrared within a timescale of several microseconds, or be involved in further reactions such as those involved in photodynamic therapy. Adapted in part from ref. [20].

James M. Tsay and Xavier Michalet
Department of Chemistry & Biochemistry
University of California, Los Angeles
607 Charles E. Young Drive East
Los Angeles, California 90095

Selected Reading

1. Lovric, J., Cho, S.J., Winnik, F.M., and Maysinger, D. (2005). Chem. Biol. *12*, this issue, 1227–1234.
2. Alivisatos, A.P. (2004). Nat. Biotechnol. *22*, 47–52.
3. Michalet, X., Pinaud, F.F., Bentolila, L.A., Tsay, J.M., Doose, S., Li, J.J., Sundaresan, G., Wu, A.M., Gambhir, S.S., and Weiss, S. (2005). Science *307*, 538–544.
4. Medintz, I.L., Uyeda, H.T., Goldman, E.R., and Mattoussi, H. (2005). Nat. Mater. *4*, 435–446.
5. Dahan, M., Levi, S., Luccardini, C., Rostaing, P., Riveau, B., and Triller, A. (2003). Science *302*, 442–445.
6. Jaiswal, J.K., Mattoussi, H., Mauro, J.M., and Simon, S.M. (2003). Nat. Biotechnol. *21*, 47–51.
7. Dubertret, B., Skourides, P., Norris, D.J., Noireaux, V., Brivanlou, A.H., and Libchaber, A. (2002). Science *298*, 1759–1762.
8. Gao, X., Cui, Y., Levenson, R.M., Chung, L.W.K., and Nie, S. (2004). Nat. Biotechnol. *22*, 969.
9. Derfus, A.M., Chan, W.C.W., and Bhatia, S.N. (2004). Nano Lett. *4*, 11–18.
10. Shioara, A., Hoshino, A., Hanaki, K.-I., Suzuki, K., and Yamamoto, K. (2004). Microbiol. Immunol. *48*, 669–675.
11. Kirchner, C., Liedl, T., Kudera, S., Pellegrino, T., Munoz Javier, A., Gaub, H.E., Stolzle, S., Fertig, N., and Parak, W.J. (2005). Nano. Lett. *5*, 331–338.
12. Aldana, J., Wang, Y.A., and Peng, X. (2001). J. Am. Chem. Soc. *123*, 8844–8850.

13. Aldana, J., Lavelle, N., Wang, Y., and Peng, X. (2005). J. Am. Chem. Soc. *127*, 2496–2504.
14. Kim, S., and Bawendi, M.G. (2003). J. Am. Chem. Soc. *125*, 14652–14653.
15. Guo, W., Li, J.J., Wang, Y.A., and Peng, X.G. (2003). Chem. Mater. *15*, 3125–3133.
16. Rikans, L.E., and Yamano, T. (2000). J. Biochem. Mol. Toxicol. *14*, 110–117.
17. Bakalova, R., Ohba, H., Zhelev, Z., Nagase, T., Jose, R., Ishikawa, M., and Baba, Y. (2004). Nano Lett. *4*, 1567–1573.
18. Green, M., and Howman, E. (2005). Chem. Commun. 121–123.
19. Ipe, B.I., Lehnig, M., and Niemeyer, C.M. (2005). Small *1*, 706–709.
20. Samia, A.C., Chen, X., and Burda, C. (2003). J. Am. Chem. Soc. *125*, 15736–15737.
21. Balakova, R., Ohba, H., Zhelev, Z., Ishikawa, M., and Baba, Y. (2004). Nat. Biotechnol. *22*, 1360–1361.