

A Triantennary Dendritic Galactoside-Capped Nanohybrid with a ZnS/CdSe Nanoparticle Core as a Hydrophilic, Fluorescent, Multivalent Probe for Metastatic Lung Cancer Cells

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Semiconductor nanocrystals have generated pronounced fundamental and technical interests in recent years. The functionalization with specific surface sites on quantum dots (QDs) for selective molecular attachment is a promising approach for their application as bioprobes, especially in cellular imaging, drug delivery, and nanosensors.¹ QDs have narrow emission bandwidth, symmetrical and tunable profiles according to their size and materials composition, excellent photostability, and broad absorption spectra, making them as the best choice for fluorescent probes. The most common method to keep QDs stable and water soluble for biocompatible purpose is to chemically attach a hydrophilic organic surfactant onto the surface of nanocrystals. However, the corresponding QD-based nanohybrids have never been explored.

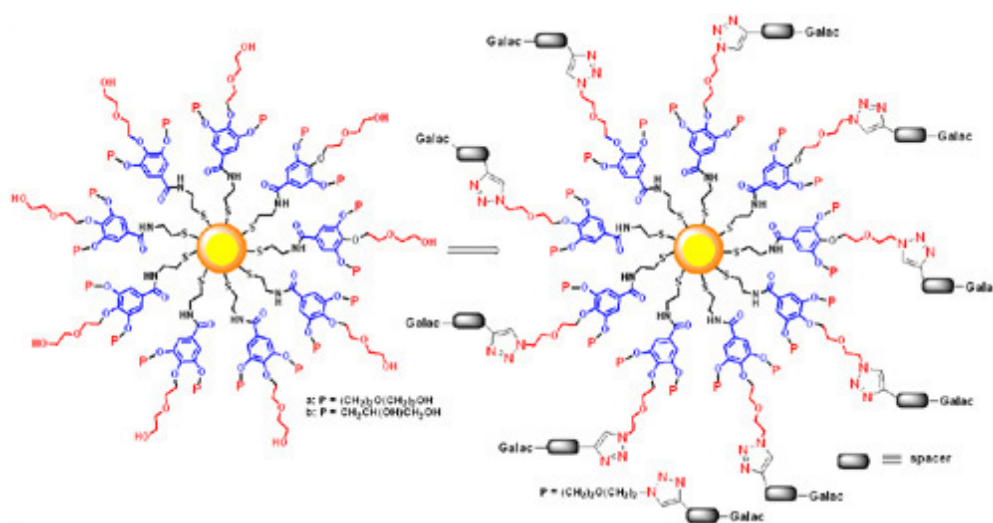


Figure 1

We have recently documented the first successful synthesis of water-soluble nanohybrids between gallamide-based, tri-/penta-podal dendrons and CdSe/ZnS core/shell NPs as fluorescent probes (**Fig. 1**).² In addition, the nanoparticle size distribution for one of the nanohybrids, nanohybrid-12, ranges from 4.5-5.5 nm, as determined by

high resolution transmission electron microscopy (HR-TEM) measurements. The TEM images clearly indicate the uniform crystallinity of the nanocrystals in the nanohybrid (**Fig. 2**).

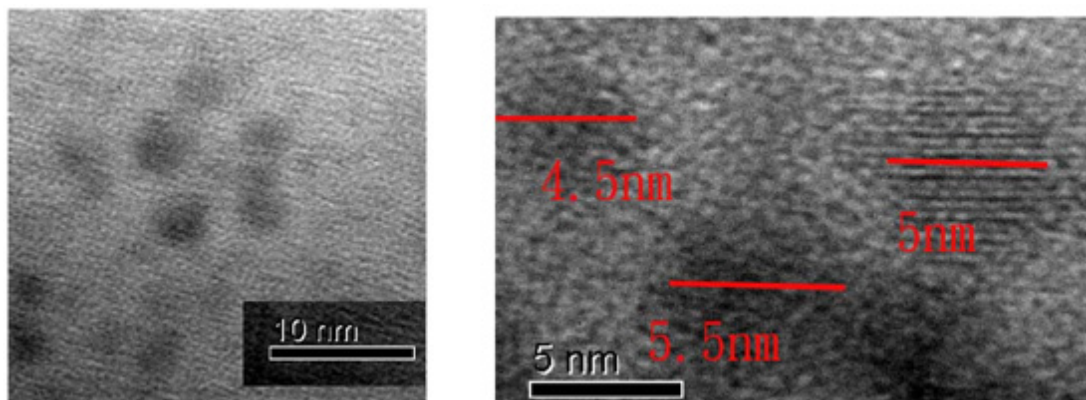


Figure 2. HR-TEM images of nanohybrid-12

To understand the origin of the facile endocytosis profiles by cancer cells and utilize the specific galactose recognition by cancer cells, we sought to prepare dendritic nanohybrids with galactoside-encapsulated CdSe/ZnS combination and examined their trans-membrane, photo-dynamic profiles through lung cancer cells in view of the enhanced cellular recognition by favorable multiple binding.

To examine whether the tripodal galactosidic nanohybrid-12 can be delivered inside cancer cells upon endocytosis uptake, cancer cell incubation experiments in the presence of these nanohybrids were carried out. Highly metastatic A549 lung cancer cells were treated with 10 μM of nanohybrid 12.³ The mixture was incubated in serum-free medium at 37 ° C for 3-12 hours. Confocal microscope images were taken with Z-clipping. It was found that lung cancer cells can efficiently take up nanohybrid-12 in 2-3 hours (**Fig. 3**). In particular, the endocytosis process was more pronounced for lung cancer cells that were undergoing mitosis. The result suggests that the membrane composition for the cancer cells undergoing mitosis tend to exert stronger interaction with polyhydroxyl-ended nanohybrids, thus facilitating their faster delivery inside cancer cells. The non-cytotoxic profile for nanohybrid-12 incubated for 2-5 days in serum-free media suggests that nanohybrid-12 with galactoside peripheral units could be useful for long-term cellular fluorescent imaging (data not shown).

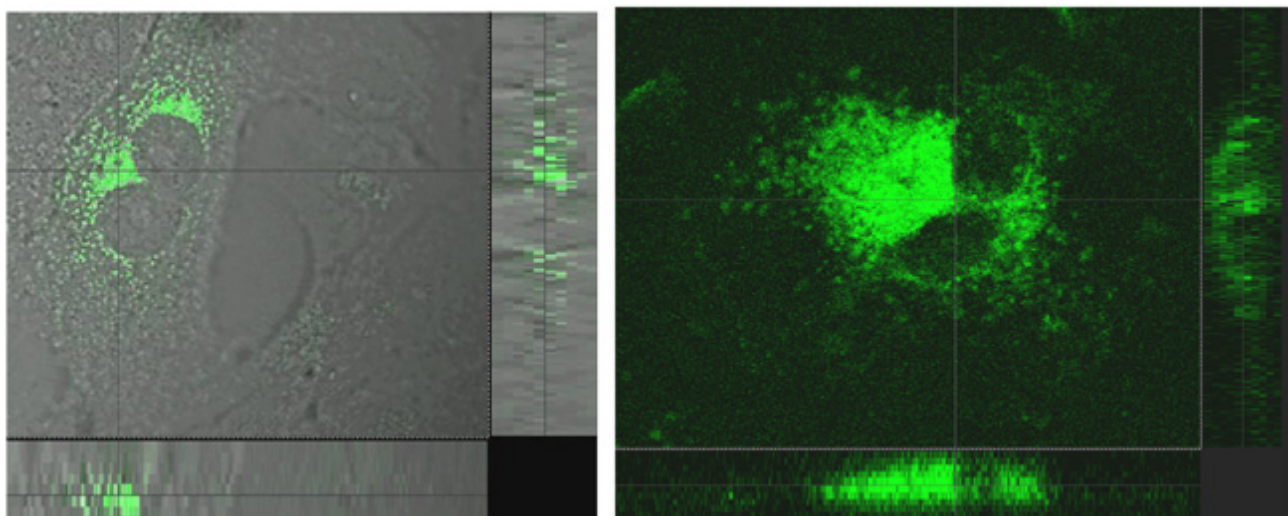


Figure 3. Two representative images taken from incubation experiments of A549 lung cancer cells with 10 μM of nanohybrid-12 for 3 hours. A stacked bright-field and confocal microscopic image with Z-clipping along x and y

axes are shown.

To examine if the galactoside-capped, gallamide-based nanohybrid-**12** exerts any cancer cell differentiation during uptake event, we treated nanohybrid-**12** with low metastasis CL1-1 lung cancer cells. In marked contrast, it was found that essentially all the nanohybrids remained outside or on the cellular surface under the same incubation conditions (**Fig. 4**). The results further support that the translocation of nanohybrid-**12** inside A549 lung cancer cells was indeed through receptor-mediated endocytosis. Therefore, nanohybrid-**12** may serve as a useful drug carrier with cancer cell differentiation, allowing for photodynamic tracing studies.

In conclusion, we have demonstrated a successful example of using biphasic extraction system for efficient dative/covalent ligand exchange on the surface of CdSe/ZnS nanocrystals. The resultant nanohybrid becomes highly water soluble with intact morphology of the nanocrystal core. The nanohybrid-**12** can be smoothly delivered inside metastatic lung cancer cells via receptor-mediated endocytosis as evidenced by confocal microscopic analysis. A low metastasis CL1-1 lung cancer cell blocks the translocation of nanohybrid-**12** into cell endosomes. Furthermore, lung cancer cells that are undergoing active mitosis tend to uptake the nanohybrids more efficiently and remain sustainable in serum-containing media for several days, which shed light on its potential application as a photodynamic drug carrier for apoptosis studies. The tri-peripheral site multivalent nanoprobe is potentially useful for studying the multimeric carbohydrate interactions, understanding endocytic process as well cell adhesion and recognition.

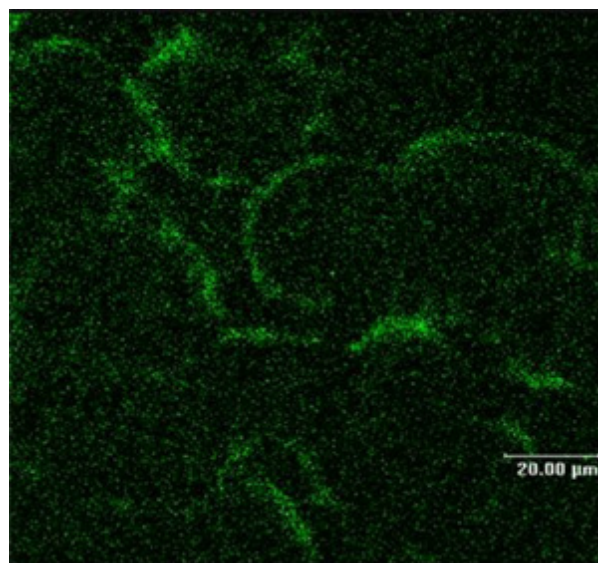


Figure 4. Images taken from incubation experiments of CL1-1 lung cancer cells with 2 μ M of tripodal, galactosidic nanohybrid-**12** for 2-3 hours.

References

1. Alivisatos, A. P. *Nature* **1994**, 370, 354.
2. Chen, C.-T.; Pawar, V. D.; Munot, Y. S.; Chen, C.-C.; Hsu, C.-J. *Chem. Commun.* **2005**, 2483.
3. Experimental procedures, spectral data, ^1H and ^{13}C NMR spectra, (HR)-TEM images and incubation studies for nanohybrid-**12** are available free of charge via the Internet at <http://www.acs.org> .