"Smart" multivalent fluorescent nanoparticles for cell imaging and drug delivery

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We have synthesized geldanamycin (GA)-polyacrylate coated CdSe/ZnS nanocrystal (NC) conjugates with well-controlled NC/polymer/GA ratios and studied their uptake by HCT116 colon cancer cells. NC-GA conjugates were readily transported into living HCT116 cells with a strong correlation to the GA/NC ratio, while other examined NCs were not, indicating the crucial role of the geldanamycin in the uptake event. This is the first example of dose-responsive drug-mediated uptake of NCs by living cells.

Semiconductor nanocrystals, commonly called quantum dots (QDs), are very promising fluorescent tools for live cell and tissue imaging because of their exceptional brightness and slow photobleaching, compared to the organic fluorescent dyes.[1, 2] Recent advances in synthesis of core-shell quantum dots[3, 4] as well as in the design of functionalized polymer coatings[5, 6] has greatly enhanced quantum yield and improved stability of quantums dots in colloid solutions. In addition, functionalization of polymer-coated QD's allows chemical derivatizations and biocompatibility.

Since quantum dots are considerably larger than organic molecules, their transport into live cells is a considerable challenge. Earlier drastic microinjection[7] and electroporation[8-10] techniques suffer from narrow scope and undesired cell stress respectively, are now being replaced with lipofection[9-11] and polypeptide based delivery methods.[6, 9] Specific targeting is normally provided through conjugation of various antibodies to the polyfunctional quantum dot.[6, 8, 12, 13] Unfortunately the latter approach greatly increases the size of fluorescent probes and does not cover all possible intracellular targets. Therefore conjugation of small target-specific drug molecules to fluorescent nanocrystals will enable fluorescent tagging of numerous intracellular targets and greatly expand the scope of fluorescent imaging in live cells. Geldanamycin is suitable for this purpose due to its known rapid uptake rates by cancer cells[14] as well as high affinity for the HSP90 protein complex.[15] Here we report our studies on the synthesis of quantum dot-geldanamycin fluorescent probes and their uptake by live HCT116 colon cancer cells.

First we have synthesized 2.2 nm CdSe nanocrystals with absorbance and emission maxima at 488 and 505 nm respectively,[4] capped them with ZnS[3] and coated the resulting ~3 nm CdSe/ZnS nanoparticles by 130 kDa octylamine-grafted *poly*(*t*-butylacrylate-*co*-ethyl acrylate-*co*-methacrylic acid).[6]

Then using the optimized EDC coupling protocol, specifically optimized for our nanoparticles, synthesized polymer-coated nanoparticles were coupled to the amino-functionalized geldanamycin in various ratios to yield nanocrystal-geldanamycin conjugates **4a-**

d (NC-GA) in 1:285, 1:81, 1:29 and 1:17 ratios respectively (**Fig. 1**). We have also synthesized quantum dots conjugated to the amino-functionalized PEG-extended geldanamycin in 1:10 ratio **5** (NC-PEG-GA) and to the large excess of N-*t*-BOC protected hexyldiamine **6** (NC-NH-BOC) for control purposes. Synthesized conjugates were quantified based on absorbance at 488 and 550 nm and extinction coefficients of fluorescent nanocrystals and geldanamycin moieties respectively (**Fig. 2**).

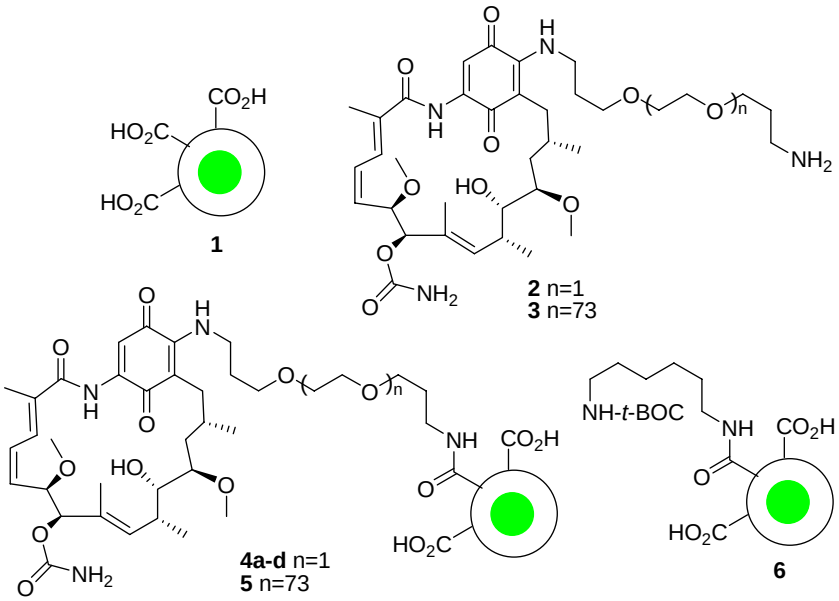

Figure 1. Synthesized nanoparticle conjugates.

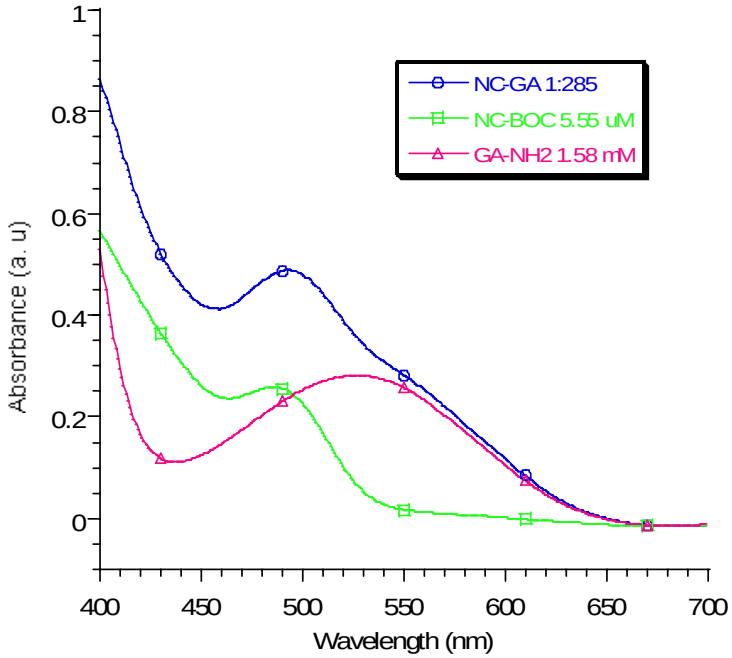

Figure 2. UV-visible spectra of normalized NC conjugates **4a**, **6** and aminogeldanamycin **3** in ethanol.

Fluorescence spectra of conjugates **4a-d** indicated an effective quenching of nanocrystal fluorescence, which correlates with the loading of the covalently attached geldanamycin. In case of NC-GA conjugate **4a** with 1:285 NC/GA covalent loadings, fluorescence is quenched completely due to FRET. Exposure of the conjugate **4a** to daylight for 7 days or 25W UV-lamp at 254 nm for 5h respectively results in a complete recovery of the fluorescence, presumably due to photodecomposition of the geldanamycin chromophore. Remarkably, the physical mixture of NC-COOH **1** and GA-NH_2 displays a fluorescence level nearly identical to the NC-BOC control **6**. This indicates that chemical conjugation is required to bring significant population of quencher GA molecules in close proximity to polymer-coated nanocrystals.

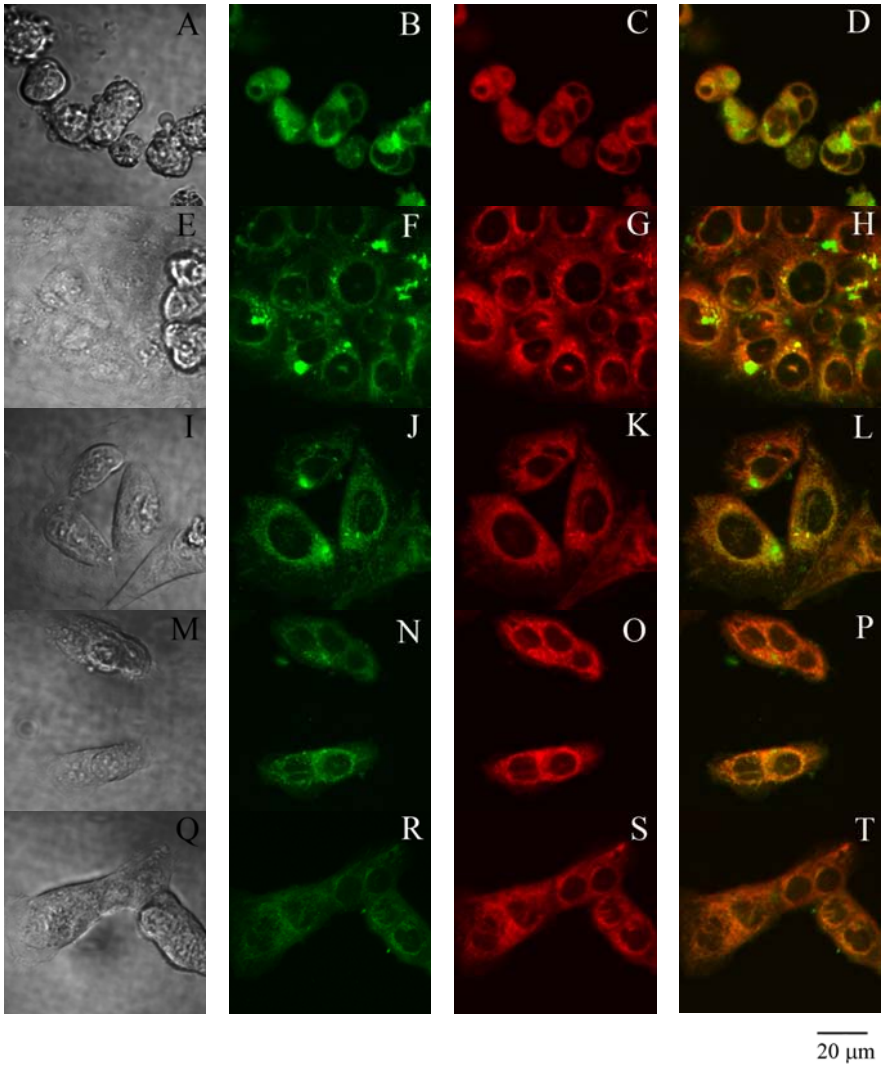

Figure 3. Uptake of NC-GA nanoparticles **4a** (A-D), **4b** (E-H), **4c** (I-L), **4d** (M-P) and **6** (Q-T) by HCT116 cells. (A, E, I, M, and Q) transmission image; (B, F, J, N and R) UV-excited green emission corresponding to quantum dot fluorescence as well as cellular autofluorescence; (C, G, K, O, and S) nile blue staining cellular membranes; (D, H, L, P, and T) the merged image of quantum dot and nile blue fluorescence.

We have tested the uptake of synthesized NC conjugates by live HCT116 cells using confocal microscopy (**Fig. 3**). Overnight incubation of the NC-GA conjugates **4a-c**, with geldanamycin loadings 1:285 (A-D), 1:81 (E-H) and 1:29 (I-L), resulted in virtually complete labeling of all cells in the culture and decreasing efficiency of uptake in the following order: **4a**>**4b**>**4c**. During confocal imaging, the UV-excited fluorescence signal, at first hardly visible, remarkably enhances within few seconds and then stabilizes. This is consistent with rapid degradation of unconjugated geldanamycin that normally occurs with light exposure, and anticipated disruption of GA quenching of NC signal. Uptake of the conjugate **4d** (M-P) with only 1:10 geldanamycin loading as well as NC-COOH **1** (Q-T) were negligible. Later series of experiments rule out competitive PEG-mediated, hydrophobicity-driven or spontaneous geldanamycin co-assisted theories of the NC uptake. Specifically, PEG conjugate **5** with 1:10 loading of geldanamycin, and a mixture of NC-COOH **2** and GA-NH_2 were not transported into HCT116 cells. Therefore, we have concluded that covalently linked geldanamycin acts as an effective carrier for transport of NC-GA conjugates into live HCT116 cells. Granular structure of cells in case of uptake of NC-GA conjugates **4a** and **4b** (Fig. 3 A, E) indicates some toxic effects at higher nanoparticle concentrations. Remarkably, the uptake of NC-GA conjugate **4c** has no evident effect on cell morphology, as compared to the control untreated cells. The vast majority of NC-labeled cells have passed membrane integrity tests with propidium iodide, indicating that cells are still viable.

We have demonstrated a doze-responsive uptake of multivalent nanoparticles into live cells based on the covalent attachment of geldanamycin molecules to the exterior of the nanoparticles. Further research on the mechanism of uptake and the generalizability of NC-GA as well as other NC-drug conjugates in terms of uptake by cancer cells is underway.

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