# Arginine-glycine-aspartic acid peptide-labeled quantum dot 705

## QD705-RGD

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| **Chemical name:** | Arginine-glycine-aspartic acid peptide-labeled quantum dot 705 | |
| **Abbreviated name:** | QD705-RGD | |
| **Synonym:** | | |
| **Agent Category:** | Peptide | |
| **Target:** | Integrin $\alpha_v\beta_3$ | |
| **Target Category:** | Receptor binding | |
| **Method of detection:** | Optical, near-infrared (NIR) fluorescence imaging | |
| **Source of signal/contrast:** | Quantum dot 705 (QD705) | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | Click on protein, nucleotide (RefSeq), and gene for more information about integrin $\alpha_v\beta_3$. |

## Background

[PubMed]

Arginine-glycine-aspartic acid peptide-labeled quantum dot 705 (QD705-RGD) is an integrin-targeted molecular imaging agent developed for near-infrared (NIR) optical imaging of tumor vasculature (1). QD705 has an emission maximum at 705 nm.

Cellular survival, invasion, and migration control embryonic development, angiogenesis, tumor metastasis, and other physiologic processes. These processes are governed at both the extracellular and intracellular levels by several factors (2, 3). Among the molecules

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that regulate angiogenesis are integrins, a superfamily of cell adhesion proteins that form heterodimeric receptors for extracellular matrix (ECM) molecules (4, 5).These transmembrane glycoproteins consist of two noncovalently associated subunits, α and β (18 α- and 8 β-subunits in mammals), which are assembled into at least 24 α/β pairs. Some integrins recognize a single ECM protein ligand, whereas some can bind several ligands. Several integrins, such as integrin $\alpha_v\beta_3$, have affinity for the arginine-glycine-aspartic acid (RGD) tripeptide motif, which is found in many ECM proteins. Integrin $\alpha_v\beta_3$ receptor expression on endothelial cells is stimulated by angiogenic factors and environments. The integrin $\alpha_v\beta_3$ receptor is generally not found in normal tissue but is strongly expressed in vessels with increased angiogenesis, such as tumor vasculature. It is significantly up-regulated in certain types of tumor cells and in almost all tumor vasculature. Increased levels of integrin $\alpha_v\beta_3$ expression are closely associated with increased cell invasion and metastasis. Molecular imaging probes carrying the RGD motif that binds to the integrin $\alpha_v\beta_3$ can be used to image tumor vasculature and evaluate angiogenic response to tumor therapy (1, 6).

Optical imaging is an imaging method that utilizes light photons emitted from bioluminescence and fluorescence probes. Fluorescence imaging in the visible light range of 395-600 nm can penetrate only to a depth of 1-2 cm and has significant background signal because of tissue autofluorescence. NIR (700-900 nm) fluorescence imaging has the advantages of relatively higher tissue penetration and lower autofluorescence from non-target tissue (6). Quantum dots (QDs) are colloidal semiconductor nanocrystals with very high levels of brightness and photostability, high molar extinction coefficients, and broad absorption with narrow and symmetric photoluminescence spectra spanning the ultraviolet to NIR (7, 8). Their fluorescent emission can be size-tuned as a function of core size. The best QD fluorophores are mostly made of cadmium selenide (CdSe) cores overcoated with a layer of ZnS (9). The potential toxicity of QDs is not well established. Not all QDs are alike, and their toxicity appears to depend on multiple physicochemical factors (10). Studies have shown a concentration-dependent *in vitro* cytotoxicity of CdSe QDs under certain conditions, and this toxicity appears to be significantly reduced by coating (11-13). The surfaces of QDs can be modified to be water soluble and biocompatible, and QDs can be attached to targeting molecules such as antibodies and peptides. Cai et al. (1) developed the first RGD peptide-labeled QDs for imaging of integrin $\alpha_v\beta_3$-positive tumor vasculature.

## Synthesis

[PubMed]

Cai et al. (1, 14) reported the synthesis of QD705-RGD using commercially available amine-modified QD705 (emission maximum, 705 nm). A detailed protocol was described by Cai et al. (14). Briefly, these QDs were built with a polyethylene glycol spacer (molecular weight 2,000) covalently attached to the QD705 surface. QD705 was first conjugated to a heterobifunctional cross-linker, 4-maleimidobutyric acid *N*-hydroxysuccinimide ester, to yield a maleimide-nanocrystal surface (maleimide-QD705).

The lysine ϵ-amino group of a potent integrin $\alpha_v\beta_3$ antagonist, c(RGDyK), was reacted with *S*-acetylthioglycolic acid *N*-hydroxysuccinimide ester. This was followed by thiol deprotection by hydroxylamine under neutral condition to yield the thiolated RGD peptide, c(RGDy(ϵ-acetylthiol)K) (RGD-SH). The maleimide-QD705 was reacted with RGD-SH for 1 h at pH 7.5. The final QD705-RGD was purified by size-exclusion chromatography. It was estimated that there were 30-50 RGD peptides per QD with a ligand coupling efficiency of 40-50%. Atomic force microscopy revealed discrete entities with smooth and uniform surface features, vertical heights of 5-7 nm, and lateral sizes of 15-20 nm. These results indicated that the structures were single QDs and that the preparation produced monodisperse single QD705-RGD particles without aggregation. These findings were confirmed by scanning electron microscopy.

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Cai et al. (1) determined the *in vitro* binding affinity of QD705-RGD in live and fixed MCF-7 (integrin $\alpha_v\beta_3$-negative human breast cancer cell line), MDA-MB-435 (human breast cancer cell line with medium integrin $\alpha_v\beta_3$-positive expression), and U87MG (human glioblastoma with high integrin $\alpha_v\beta_3$ expression) cells. These cells were blocked with 0.1% bovine serum albumin, stained, and examined under the microscope. QD705-RGD did not bind to MCF-7 (integrin-negative) cells. The integrin-positive cells (MDA-MB-435 and U87MG) were clearly visualized in fluorescence images from QD705-RGD binding. The fluorescence intensity correlated with the integrin expression level of the cell lines. This binding was blocked effectively by 2 μM c(RGDyK). Unconjugated QD705 showed no significant binding to any of the cell lines.

*Ex vivo* tumor tissue binding studies were performed by Cai et al. (1). Tumor tissues generated from MCF-7, MDA-MB-435, and U87MG cells were cryosectioned at –20 °C into 8-μm slices, which were fixed in cold acetone and then stained with either QD705 or QD705-RGD. The results were similar to those of *in vitro* cell binding. QD705 had minimal nonspecific binding to all tumor tissues. QD705-RGD showed high binding to the U87MG tumor tissue but no binding to the MCF-7 tumor tissue.

Cai et al. (1) incubated QD705 and QD705-RGD in complete mouse serum at 37 °C to evaluate the change in fluorescent signal as a function of time. Fluorescence signal intensity decreased significantly over time for both QDs. After 24 h of incubation, the intensity decreased to 73.6% and 66.4% of the original values for QD705 and QD705-RGD, respectively.

## Animal Studies

### Rodents

[PubMed]

Cai et al. (1) performed *in vivo* distribution and imaging studies of QD705-RGD in athymic nude mice bearing U87MG tumors on their shoulders (tumor size, 0.5-0.8 $cm^3$). Each mouse was administered i.v. QD705 or QD705-RGD (200 pmol of QDs, 6-10 nmol of peptide). The mice were imaged by an optical system with a custom-designed, spectrally optimized lens system. With QD705-RGD, a fluorescence signal was observed in the tumor as early as 20 min after injection. The tumor signal intensity reached a maximum at 6 h. The tumor/background ratios ($n = 3$) were 3.08 ± 1.42, 3.39 ± 1.13, 4.42 ± 1.88, and 2.09 ± 1.17 at 1, 4, 6, and 27 h after injection, respectively. With QD705, no significant fluorescent signal was observed in the tumor at all time points. Both QD705 and QD705-RGD accumulated rapidly in the liver, spleen, bone marrow, and lymph nodes. Uptake in the liver was observed as early as 10 min after injection. The liver signal from QD705-RGD was slightly lower than that of QD705. The authors suggested that the difference might be caused by the overall charge difference between QD705 and QD705-RGD. On the basis of these results, mice were euthanized at 6 h after injection, and the tumors were harvested and imaged immediately. The U87MG tumors from QD705-RGD mice showed clear signal intensity, but no signal was observed in tumors from QD705 mice. The signal from the QD705-RGD tumors was heterogeneous, and the authors suggested that the signal came mainly from the tumor vasculature. Microscope images of tumor cryosections confirmed that QD705-RGD did not extravasate and remained within the tumor vasculature.

### Other Non-Primate Mammals

[PubMed]

No publication is currently available.

### Non-Human Primates

[PubMed]

No publication is currently available.

## Human Studies

[PubMed]

No publication is currently available.

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