5.6 Gold nanoparticles conjugated with cyclic RGD and fluorescein display a highly specific imaging activity in vitro

5.6.1 Introductory remarks

Gold nanoparticles were conjugated with cyclic RGD and fluorescein and tested for their ability to stain cells in dependence upon their integrin-driven internalization. Gold nanoparticles are considered the ideal candidate for optical imaging because they are not susceptible to photobleaching and may be functionalized with several cyclic RGD peptidomimetics and fluorochromes contemporarily [9]. It is noteworthy that the structure of gold nanoparticles allows the conjugation with several biological moieties, although it is only possible an average quantification of their amount. Therefore, it is reasonable to consider that the functionalized gold nanoparticles used in this work (namely Compound DA425) were conjugated with several molecules of cyclic RGD, so that the overall signal was expected to be particularly strong. Gold nanoparticles conjugated with fluorescein alone (namely Compound DA426) were used as a control.

5.6.2 Compound DA425 positively stained prostate cancer cells

Experiments were performed on PC-3 prostate cancer cells, because of the good responsiveness of this cell line to fluorescein-conjugated RGD (**Figure 24**). MDA-MB-231 breast adenocarcinoma cells were used as a negative control for the evidences previously underlined (see **paragraph 5.5.2**).

PC-3 and MDA-MB-231 cells were exposed to a 4 hours treatment with Compounds DA425 and DA 426. It was not possible to exactly quantify the amounts of cyclic RGD on gold nanoparticles. However, it was just possible to determine an average molecular formula. On this basis, the ratio between fluorescein and cyclic RGD on each nanoparticle should be 1 : 4 circa, and every gold nanoparticle should contain from 10 to 15 cyclic RGD molecules. Given that Compound DA425 showed a 50 times increased affinity in solid-phase receptor-binding assays (data not shown) in comparison with Compound 13 or 15, Compound DA425 and Compound DA426 were administered at a concentration of 1 µM (ten-fold less than Compound 13 or 15). Compound DA425 was demonstrated to positively stain prostate cancer cells, in a very specific way. In fact, the confocal analysis of cells stained with Compound DA425 revealed that the signal given by fluorescein and the reflection spectrum given by gold were perfectly merged and localized near the cell adhesive contacts. Therefore, this ultimately suggested that the internalization took place in the membrane zones where integrin αvβ3 is mainly expressed, which are focal contacts.

Notably, breast adenocarcinoma cells subjected to the treatment previously described did not show any sign of fluorescence or reflection. In addition, the control Compound DA426 gave no signal at all on both cell lines, suggesting that the effectiveness and the specificity of Compound DA425 were due to the presence of the cyclic RGD peptidomimetic (**Figure 25**).

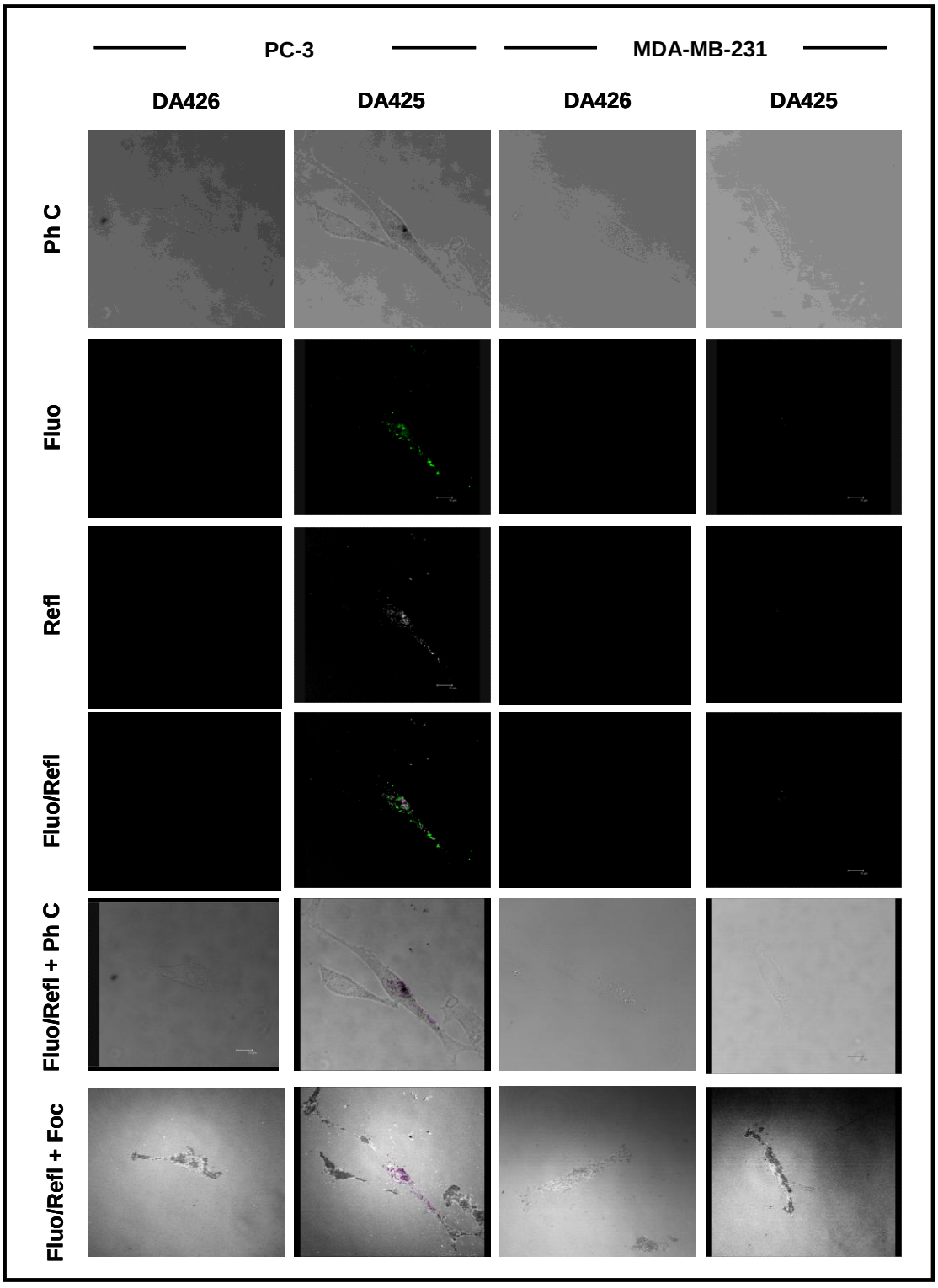

Figure 25. *Biological activity of functionalized gold nanoparticles on PC-3 and MDA-MB-231 cells. PC-3 and MDA-MB-231 cells were incubated 4 hours with 1 µM Compound DA426 or DA425. The signal provided by Compound DA425 was detectable in PC-3, but not in MDA-MB-231 cells. In particular, fluorescein signal and reflection spectrum perfectly merged and were localized near the cell adhesive contacts. Ph C: Phase Contrast; Fluo: Fluorescein; Refl: Reflection; Foc: Focal adhesions. Grey: gold; Green: fluorescein; Purple: merge. Original magnification: 63x.*