**In vivo optical imaging of integrin α\_V-β\_3 in mice using multivalent or monovalent cRGD targeting vectors**

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**Abstract**

**Background:** The cRGD peptide is a promising probe for early non-invasive detection of tumors. This study aimed to demonstrate how RAFT-c(-RGDfK-)\(_4\), a molecule allowing a tetrameric presentation of cRGD, improved cRGD-targeting potential using in vivo models of α\_V-β\_3-positive or negative tumors.

**Results:** We chose the human embryonic kidney cells HEK293(β\(_3\)) (high levels of α\_V-β\_3) or HEK293(β\(_1\)) (α\_V-β\_3-negative but expressing α\_V and β\(_1\)) engrafted subcutaneously (s.c.) in mice. Non-invasive in vivo optical imaging demonstrated that as compared to its monomeric cRGD analogue, Cy5-RAFT-c(-RGDfK-)\(_4\) injected intravenously had higher uptake, prolonged retention and markedly enhanced contrast in HEK293(β\(_3\)) than in the HEK293(β\(_1\)) tumors. Blocking studies further demonstrated the targeting specificity and competitive binding ability of the tetramer.

**Conclusion:** In conclusion, we demonstrated that Cy5-RAFT-c(-RGDfK-)\(_4\) was indeed binding to the α\_V-β\_3 receptor and with an improved activity as compared to its monomeric analog, confirming the interest of using multivalent ligands. Intravenous injection of Cy5-RAFT-c(-RGDfK-)\(_4\) in this novel pair of HEK293(β\(_3\)) and HEK293(β\(_1\)) tumors, provided tumor/skin ratio above 15. Such an important contrast plus the opportunity to use the HEK293(β\(_3\)) negative control cell line are major assets for the community of researchers working on the design and amelioration of RGD-targeted vectors or on RGD-antagonists.

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**Background**

The tripeptide sequence Arg-Gly-Asp (RGD) \([1,2]\) is a well known motif recognizing and interacting with integrin, a family of transmembrane heterodimeric glycoproteins composed of one α and one β subunits \([3,4]\). The structure of a cyclic pentapeptide containing RGD was optimized in order to provide a high affinity and selectivity for the α\_V-β\_3 integrin \([5]\), an integrin overexpressed at the sur-
face of activated endothelial cells during angiogenesis [6,7] and in various types of tumor cells [8-11]. Radiolabeled cRGD peptides in combination with nuclear imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been extensively studied for imaging of αvβ3 expression in experimental tumors [12]. More recently, the development of in vivo optical imaging techniques and of various fluorescent-cRGD conjugates were also described for imaging cancer in mice [12-18]. In addition, it was shown that presenting multiple copies of the cRGD motif was usually associated with improved properties of the probes [16,19]. In this aim, our group has developed a novel tetrameric molecule by grafting four copies of cRGD onto a cyclic decapeptide platform called RAFT (Regioselectively Addressable Functionalized Template) [17,18,20]. When injected intravenously in nude mice bearing s.c. human carcinoma IGROV1 (PET) and single photon emission computed tomography (SPECT) have been extensively studied for imaging of αvβ3 expression in experimental tumors [12]. More recently, the development of in vivo optical imaging techniques and of various fluorescent-cRGD conjugates were also described for imaging cancer in mice [12-18]. In addition, it was shown that presenting multiple copies of the cRGD motif was usually associated with improved properties of the probes [16,19]. In this aim, our group has developed a novel tetrameric molecule by grafting four copies of cRGD onto a cyclic decapeptide platform called RAFT (Regioselectively Addressable Functionalized Template) [17,18,20]. When injected intravenously in nude mice bearing s.c. human carcinoma IGROV1 tumors, expressing a low level of αvβ3, cyanine 5-labeled RAFT-c(-RGDFK-)4 showed a better tumor contrast than its monomeric analog [18].

In the present study, we took advantage of a particular tumor model for addressing RGD-mediated targeting specificity in vivo. This model derived from the naturally αv-positive and β3-negative HEK293 cell line was initially transfected by a plasmid encoding the human β3 chain, forming a strongly αvβ3-positive HEK293(β3) stable clone. In addition, HEK293(β3), an αvβ3-negative control overexpressing the β3 chain instead of the β3, had been also established. As their parent cell line HEK293, we show that the 2 β3 or β1 subclones are forming tumors when injected subcutaneously into athymic nude mice. Using these tumor models, our different RGD-based molecules and competition experiments, we demonstrate the extremely good specificity and improved tumor accumulation and retention of the Cy5-labeled RAFT-c(-RGDFK-)4 probe as compared to its monomeric analog. Since RGD-based antiangiogenic therapies are currently under investigation, and that cRGD can also serve as a ligand in human nuclear medicine, optimization of its specificity and drug delivery properties is of major importance for clinical applications.

**Results**

**In vitro binding studies**

HEK293(β3) and HEK293(β1) cells are stable transfectants of human β3 and β1 subunit, respectively, from the human embryonic kidney cell line. Western blot analysis showed that αv was strongly expressed in both cell lines, and confirmed the successful transfection of β3 or β1 subunits (Fig 1A). This phenotype was also confirmed by FACS analysis performed with the anti-human αvβ3 antibody [18]. These 2 cell lines, were then observed using confocal laser scanning microscopy (CLSM) after incubation with Cy5-labeled RAFT-c(-RGDFK-)4, cRGD, or RAFT-c(-RβADfK-)4. As shown in Fig. 1B, none of these peptides bound to the HEK293(β3) cells. As expected also, the RAFT-c(-RβADfK-)4 control peptide did not bind to the αvβ3-positive HEK293(β3) cells. In contrast, cRGD and RAFT-c(-RGDFK-)4 were reacting with HEK293(β3) cells moderately and very strongly, respectively.

**Establishment of paired αvβ3-positive and αvβ3-negative tumor models**

A s.c. inoculation of HEK293(β3) or HEK293(β1) cells in nude mice lead to tumor formation. This suggested that overexpression of β3 or β1 did not modify the known tumorigenicity of the parental HEK293 cell line (see ATCC number CRL-1573). Histological examination with hematoxylin and eosin (H.E.) staining shows that either HEK293(β3) or HEK293(β1) xenografts are composed of nodular cell masses and stroma (Fig 2). Immunohistochemical labeling of tumor sections shows positive αvαv staining in HEK293(β3) cells but not in HEK293(β3) and a similar low to moderate vascularization as indicated by the CD 31-labeling of both tumors. Thus expression of the β3 chain was not lost during tumor growth and was not affecting the tumor vasculature.

**Whole body optical imaging**

Nude mice bearing s.c. tumor xenografts of HEK293(β3) or HEK293(β1) cell line received an i.v. injection of 10 nmol Cy5-labeled RAFT-c(-RGDFK-)4, cRGD, or RAFT-c(-RβADfK-)4 and were imaged at different time points during 2 days. As shown in Fig. 3a, four hours after injection a stronger tumor uptake was observed for RAFT-c(-RGDFK-)4 than for cRGD, while the control probe RAFT-c(-RβADfK-)4 was not retained in the tumor. Interestingly, the αvβ3-negative HEK293(β3) tumors did not take-up the RAFT-c(-RGDFK-)4 peptide, demonstrating the specificity of RAFT-c(-RGDFK-)4 for the αvβ3 integrin. The quantitative analysis also showed that the tetramer and the monomer reached similar maximal tumor uptake 5 to 30 min postinjection (p.i.) (Fig. 3b). Between 30 min an 4 hr p.i., the tetramer's signal remained very elevated in the tumor (65 472 ± 90 to 61 875 ± 3434 photons/pixel) while a marked decrease (from 63 744 ± 3 031 to 28 349 ± 9 727 photons/pixel) was measured with the cRGD. At later time points RAFT-c(-RGDFK-)4 always showed a better tumor accumulation than the monomer. The negative control probe RAFT-c(-RβADfK-)4 was rapidly washed-out from the tumors. In normal skin, all 3 probes exhibited similar kinetic curves, except at early time points (5 min to 1 hr) where cRGD showed a somewhat stronger non-specific diffusion (Fig 3b). Finally, the tumor contrast (T/S ratio) was markedly enhanced with RAFT-c(-RGDFK-)4. Four hr p.i. the T/S ratio reached the value of 15.9 ± 3.6 with RAFT-c(-RGDFK-)4. This was significantly higher than that of the monomeric cRGD (5.9 ± 2.0), or the 1.4 ± 0.1
ratio obtained for the control probe. Importantly, RAFT-c(-RGDFK-)₄ did not accumulate in the αᵥβ₃-negative HEK293(β₁) xenografts. Indeed, the measured signal obtained with RAFT-c(-RGDFK-)₄ in HEK293(β₁) tumors was similar to that of RAFT-c(-RβADfK-)₄ in HEK293(β₃) tumors.

Confocal microscopic observation of RGD-Cy5 conjugate distribution
Tumors of mice treated as mentioned above were excised 3 or 24 hr p.i. and analyzed by CLSM imaging (Fig. 4). Cy5-RAFT-c(-RGDFK-)₄ was massively internalized by tumor cells as shown at a higher magnification in the insert (Fig. 4B). While virtually each tumor cell was strongly labeled at 3 hr, it was still easily detectable in a large proportion of tumor cells after 24 hr (data not shown). A similar pattern was obtained with the monomeric cRGD although the intensity of the signal was lower (Fig. 4C). No specific fluorescence was found with the control peptide Cy5-RAFT-c(-RβADfK-)₄ (Fig 4D).

Blocking study
In order to further establish the in vivo specificity of Cy5-RGD conjugates, 10 nmol Cy5-RAFT-c(-RGDFK-)₄ or Cy5-cRGD were coinjected with 300 nmol unlabeled tetrameric RGD or 1200 nmol unlabeled monomeric cRGD. The differences in the injected doses of unlabeled molecules were calculated in order to maintain equal concentrations of the competing RGD motifs. As shown in Fig. 5A, the tumor uptake of Cy5-RAFT-c(-RGDFK-)₄ was significantly reduced in the presence of "cold" (unlabeled) monomer and this effect was more obvious when the "cold" tetramer was used. As an example, at 3 hr p.i. the signal intensities were significantly decreasing (p < 0.0001) from 65 472 ± 80 without competitor to 34 339 ± 6 402 in the presence of "cold" cRGD (reduction of 50%) and down to 12 894 ± 2 504 when the "cold" tetramer was in excess (reduction of 80%). This blocking effect was obvious on the corresponding images (Fig. 5A, left panel). In addition, it is important to note that the strong decrease of the signal in the tumors was observed...
while the kidneys were showing identical intensities. This indicated that, as expected, the non-specific renal uptake of the tetrameric RGD was not affected by the presence of the different competitors. Similarly, a reduction of at least 50 to 60% was obtained when Cy5-cRGD was used for labeling (Fig. 5B). The blocking effect of both competitors was very strong even if RAFT-c(-RGDfK-)4 was slightly more efficient.

Discussion

RGD-based peptides are certainly the most frequently used molecules for tumor targeting and are currently in use for selective drug delivery and tumor imaging in preclinical models or in clinical trials. In this study we present evidences that presenting four copies of the cRGD motif on our RAFT carrier greatly improves cRGD-mediated tumor targeting in vivo of \( \alpha_\beta_3 \)-positive tumors.

In vitro and in vivo, the \( \alpha_\beta_3 \)-positive HEK293(\( \beta_3 \)) cells and tumors were very strongly recognized by Cy5-RAFT-c(-RGDFK-)4 but not by the negative control RAFT-c(-RGADfK-)4. In addition, the \( \alpha_\beta_3 \)-negative HEK293(\( \beta_1 \)) samples remained negative after staining with the RGD or RGD-based peptides. Furthermore, fluorescence images of both cultured cells and excised tumors clearly demon-

strate the stronger labeling of HEK293(\( \beta_3 \)) cells by Cy5-RAFT-c(-RGDFK-)4 as compared to its monomeric analogue, confirming the enhanced receptor binding achieved when multiple RGD motifs are presented by a single template. The tetrameric RGD exhibited also stronger signal intensity in tumors, longer retention and much better contrast as compared to its monomeric analogue. Such effects could be explained by its augmented receptor-binding affinity due to the polyvalency effect [17,19,20] and increased molecular size which certainly delays the circulation and tumor retention time of the Cy5-RAFT-c(-RGDFK-)4. Finally, the active internalization of the Cy5-RAFT-c(-RGDFK-)4 probe may also contribute to its improved accumulation in the tumor cells. As shown on the tumor sections, the internalization was very strong since most of the signal was coming from the cytoplasm of the target cells. This suggests that such vector could be highly efficient to deliver drugs intracellularly. Multivalent presentation of ligands is improving significantly the targeting of tumors and several highly efficient targeting molecules allowing a multivalent presentation of RGD have been described [21-27]. Nonetheless, for some of these molecules the chemical formulation is poorly characterized and thus the number of ligand motifs being added on a polymer is random and cannot be controlled. In addition, the conformation of these molecules is not constrained. It is thus impossible to separate spatially the different biological functions presented by a single molecule nor it is possible to know its exact structure. These problems are avoided using RAFT-c(-RGDFK-)4 because the chemistry we use is regio- and chemo-selective. Thus the synthesis and purification of the final molecules are perfectly controlled even at gram scale. In addition, the RAFT architecture allows a spatial separation between the targeting and "drug-delivery" domains. Finally, RAFT is also interesting because its geometry allows a presentation of four RGD motifs at a very high density on its small surface.

To further confirm the receptor binding specificity of the Cy5-labeled RGD tetramer, blocking experiments were performed in vivo. In agreement with other reports using the monomeric cRGD [13,14,16], we observed an almost complete inhibition of cRGD accumulation in the presence of an excess of "cold" cRGD or RAFT-c(-RGDFK-)4. More interestingly the opposite experiment showed that while cRGD was able to block roughly 50% of Cy5-RAFT-c(-RGDFK-)4 accumulation, the presence of an excess of unlabeled tetramer was reducing by more than 80% the Cy5-RAFT-c(-RGDFK-)4 signal in the tumor. Finally Cy5-RAFT-c(-RGDFK-)4 shows higher renal uptake than Cy5-cRGD. This was observed in either tumor-bearing or normal mice. This renal retention is likely to be non-specific since it was not modified by the presence of an excess of unlabeled tetramer or monomer.

Figure 2
Hematoxylin-eosin (H.E.) staining and immunohistochemical staining with anti-CD31 and anti-\( \alpha_\beta_3 \) in HEK293(\( \beta_3 \)) or HEK293(\( \beta_1 \)) s.c. xenografts. Original magnification: × 20 objective lenses.
HEK293(β3) and HEK293(β1) might be an interesting duo of tumors forming from cell lines which differ only by their integrin β3 status. One is αVβ3-positive and the other is αVβ3-negative. Another model, M21 was also described. The αVβ3-positive M21 and its αVβ3-negative variant M21-L human melanoma cell lines were the first reported paired models for in vivo evaluation of αVβ3 receptor binding specificity of RGD peptides [13,28,29]. M21-L cells were selected and maintained as a stable variant of M21 unable to synthesize the α chain but with normal levels of the β chain [30]. Here, we present another duo of αVβ3-positive and negative s.c. tumor xenografts. HEK293(β3) and HEK293(β1) cell lines are stable transfectants of the human embryonic kidney cell line HEK293, overexpressing the human integrin β3 and β1 subunits respectively. While the original HEK293 cells express high levels of αV but negligible levels of α5, β3 and β1, HEK293(β3) expresses impressive amounts of αVβ3, and HEK293(β1) cells mainly form the αVβ1 receptor (another known receptor of fibronectin and vitronectin). This model is of great interest for the in vivo study of RGD-based targeting vectors since tumor/skin ratio of more than 15 can be obtained. Such a large dynamic is allowing precise measurements of the impact of treatments or chemical modifications possibly affecting the RGD-mediated targeting. In addition, the negative control cell line is a major asset to confirm the specificity of these RGD-delivery systems.
Conclusion

Using such paired tumor models, we demonstrated that RAFT-c(-RGDfK-)₄ is specific for the \( \alpha_v \beta_3 \) receptor and internalized. In addition, due to its multifunctional backbone, it can carry multiple biological functions on a single, spatially and chemically defined molecule. Finally, the production of large quantities of perfectly controllable batches makes of RAFT-c(-RGDfK-)₄ a powerful and versatile synthetic vector for clinical applications like targeted-drug delivery or molecular imaging of cancer. Ultimately, our goal will be to combine these two applications and to use RAFT-c(-RGDfK-)₄ for imaging and quantification of its targeted-drug delivery efficiency.

Methods

**RGD-Peptides Synthesis and Fluorescent Labeling**

The detailed protocol for synthesis of RGD peptides was reported previously [20]. Here, a brief description was given below for the strategy of RGD multimerization and fluorescence labeling. RAFT is a cyclic decapeptide (c [-Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-]) with up to six lysine residues. Protection of the lysine in position 1, 3, 6, or 8 and of the two in positions 2 and 7 results in RAFT molecules having two orthogonally addressable domains pointing on either side of the cyclopeptide backbone. On the upper face, four copies of the c [-RGDfK-] peptide were grafted.
via an oxime bond (R1-O-N=C-R2) for recognition of the integrin. On the opposite side of RAFT, Cy5 mono NHS (N-hydroxysuccinimide) ester (Amersham Biosciences, Uppsala, Sweden) was added on the lysine chain (c [-KKPKGAKKPG-]) [17]. As a negative control probe, Cy5-labeled RAFT-c(-RGDfK-)4 was also synthesized in a similar way. Changing the G amino-acid by a β-Ala abolishes RGD-mediated affinity for the integrins. All the probes were dissolved in phosphate-buffered saline (PBS) for the \textit{in vitro} and \textit{in vivo} application.

\textbf{Cell Lines and Culture Conditions}

HEK293(β3) and HEK293(β1) cells, stable transfectants of human β3 and β1 subunit, respectively, from the human embryonic kidney cell line (kindly provided by J-F. Gourvest, Aventis, France) were cultured in DMEM enriched with 4.5 g.L$^{-1}$ glucose and supplemented with 1% glutamine, 10% fetal bovine serum (FBS), 50 units/ml penicillin, 50 μg/ml streptomycin and 700 μg/ml Geneticin (G418 sulfate, Gibco, Paisley, UK). The 2 cell lines were cultured at 37°C in a humidified 95% air:5% CO$_2$ atmosphere.

\textbf{Western blot analysis of integrin subunit expression}

Cells were lysed in RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40, 1 mM NaF, 1 mM Na$_3$VO$_4$, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg/ml for each of leupeptin, aprotinin, and pepstatin) for 30 min on ice, and then the lysates were centrifuged at 17000 × g for 15 minutes at 4°C. The protein concentration of the supernatant was quantified using a protein assay kit (Bio Rad Labs., Richmond, CA). Aliquots of protein (40 μg) were subjected to electrophoresis on 7–10% polyacrylamide
gels containing 0.1% SDS, followed by electrophoretic transfer onto PVDF-membranes, Hybond™-P (Amersham Biosciences UK Limited, Little Chalfont, Buckinghamshire, UK). The membranes were then incubated with primary antibody: rabbit anti-human integrin α5 polyclonal antibody (1:5000; Chemicon International, Inc., Temecula, CA), rabbit anti-integrin β3 tail serum (1:1500; kindly provided by Dr C. Albíges-Rizo, Grenoble, France) or mouse anti-human β3 monoclonal antibody (clone VI-PL2, 1:100; BD Biosciences PharMingen, San Diego, CA). To monitor equal protein loading, membranes were also probed for actin using rabbit anti-actin polyclonal antibody (1:1000; Sigma) or for HSP70 using mouse anti-HSP70 monoclonal antibody (1:5000; Affinity BioReagents Inc.). For visualization, horseradish peroxidase (HRP)-conjugated secondary antibodies, followed by ECL™ immunodetection (Amersham Biosciences UK Limited) were used.

**Animal, Tumor Models and Histochemistry**
Animal procedures were in agreement with the EEC guidelines. Female athymic Swiss nude mice, purchased from Janvier (Le Genest Saint Isle, France) at 6–8 weeks of age were used and maintained under specific pathogen-free conditions. Subcutaneous (s.c.) injection of 20 × 10⁶ HEK293(β3) or HEK293(β1) cells suspended in 200 μl of PBS into the right flank of mice resulted in formation of 6–8 mm-diameter tumors after 4–6 weeks. Immunostaining with mouse anti-human integrin α5β3 monoclonal antibody, clone LM609 (1:100; Chemicon) was performed on acetone-fixed cryosections using M.O.M. immunodetection (peroxidase) Kit (Vector laboratories, Inc., Burlingame, CA). Rat anti-mouse CD31 monoclonal antibody, clone MECA32 (1:3000; BD Biosciences PharMingen) staining was performed on methanol-fixed cryosections using Strept-ABComplex/HRP immunodetection Kit (DakoCytomation). The nuclei were counterstained with hematoxylin.

**In Vitro Studies**
Cells were seeded on sterilized 18-mm-diameter glass coverslips in 12-well plates (3 × 10⁵ cells per well), and incubated overnight at 37°C. Afterwards, the cells were washed with PBS and incubated at 37°C in the presence of Cy5-labeled peptides RAFT-c(-RGDFK-)₄, cRGD or RAFT-c(-RβADfK-)₄ at final concentration of 0.1 μM for 30 min. They were then washed with PBS, fixed with 2% paraformaldehyde at room temperature for 10 min. The nuclei were stained with 5 μM Hoechst 33342, and the coverslips were inverted onto glass slides using Mowiol (Calbiochem, San Diego, CA) mounting medium. The slides were observed with a confocal laser scanning microscopy (CLSM) (LSM510, Zeiss, France).

**In Vivo Optical Imaging of Tumor-bearing Mice**
The mice bearing s.c. HEK293(β3) or HEK293(β1) tumors at diameter of 6–8 mm were used for imaging experiments. They received intravenous (i.v.) injection of Cy5-labeled peptides RAFT-c(-RGDFK-)₄, cRGD or RAFT-c(-RβADfK-)₄ at 10 nmol for each mouse (n = 3–4 for each probe). For the blocking experiments, s.c. HEK293(β1) tumor-bearing mice (n = 2–4 for each group) received coinjection of Cy5 labeled RAFT-c(-RGDFK-)₄ or cRGD (10 nmol/mouse) together with unlabeled RAFT-c(-RGDFK-)₄ (300 nmol/mouse) or cRGD (1200 nmol/mouse). Four times higher molar concentration of cRGD was used than that of tetramer to have same number of cRGD motifs.

Fluorescence reflectance imaging was performed using a Hamamatsu optical imaging system described previously [17,18]. In brief, imaging was carried out in a dark box, and anesthetized animal was illuminated with a monochromatic 633 nm light (50 μW.cm⁻²). The re-emitted fluorescence was filtered using a colored glass filter RG 665 (optical density > 5 at the excitation wavelength 633 nm) and collected with a cooled (-70°C) digital charge-coupled device (CCD) camera (Hamamatsu digital camera C4742-98-26LWGS, Hamamatsu Photonics K.K., Japan). All fluorescence images were acquired using 100 ms of exposure time, with other related parameters kept constant throughout the experiment. Images were acquired as 16-bit TIFF files which can provide a dynamic of up to 65535 grey levels. Image processing used in this study, including setting LUT (look-up-table) range and measurement of the fluorescence intensity for each region of interest (ROI), were performed using the Wasabi software (Hamamatsu). It is also important to note that all the images are presented without background subtraction. For quantifying tumor contrast, the mean fluorescence intensities of the tumor area (T) and that of the distant skin area (S) were calculated; dividing T by S produced the ratio between tumor tissues and background level.

**Histological Distribution of RGD-peptides in Tumors**
At 3 and/or 24 hr after i.v. injection of 10 nmol of Cy5labeled RAFT-c(-RGDFK-)₄, cRGD or RAFT-c(-RβADfK-)₄, the mice were euthanized and tumors were excised, frozen in liquid nitrogen and stored at -80°C. Sections of 20–30 μm thickness were fixed with 2% paraformaldehyde at room temperature for 10 min. The nuclei were stained with 5 μM Hoechst 33342, and the coverslips were mounted using Mowiol and kept at 4°C in the dark until observation using CLSM.

**Statistical Analysis**
All the data are given as mean ± standard (SD) of n independent measurements. Statistical analysis was performed...
using two-tailed nonparametric Mann-Whitney t-test. Statistical significance was assigned for values of $p < 0.05$.

Authors’ contributions
ZJ and ILC were in charge of the experiments. VI ran the in vivo imaging. SF, DB and PD were in charge of the synthesis of the molecules, MCF and ILC designed the study. All authors read and approved the final manuscript.

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