Supporting Information for

DEVELOPING TARGETED HYBRID IMAGING PROBES BY CHELATOR SCAFFOLDING

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Radioiodination general information. The cyclic pentapeptide c(RGDyV) was synthesized according to Haubner et al.[1] and [Leu^{15}]-Gastrin I was purchased from Bachem (Bubendorf, Switzerland). Iodine-125 (5 mCi) was obtained from Perkin Elmer (Waltham, MA, USA) as Na^{125}I in 0.01 mM NaOH solution (pH 8-11). Iodine-125 labelling was performed at ambient temperature and labelled peptides were purified by analytical radio-RP-HPLC and fractions were collected manually. RP-HPLC analysis was carried out on an UltiMate 3000 RS UHPLC pump, Ultimate 3000 column compartment (25 °C oven temperature), UltiMate 3000 Variable Wavelength Detector (Thermo Fisher Scientific, Vienna, Austria, UV detection at 220 nm), GabiStar radio detector (Raytest; Straubenhardt, Germany) and a Jupiter 5 µm C_{18} 300 Å 150 × 4.6 mm (Phenomenex Ltd. Aschaffenburg, Germany) column with following acetonitrile (ACN)/H_2O/ 0.1 % trifluoroacetic acid (TFA) multistep gradients was used: Gradient A: flow rate of 1.0 mL/min; 0.0–2.0 min 20% ACN, 2.0–32.0 min 20–50 % ACN, 32.0–33.0 min 50–80 % ACN, 33.0–35.0 min 80 % ACN, 35.0–40.0 min 20 % ACN. Gradient B: flow rate of 1.5 mL/min; 0.0–2.0 min 0% ACN, 2.0–15.0 min 0–50 % ACN, 15.0–16.0 min 50–100 % ACN, 16.0–18.0 min 100 % ACN, 18.0–20.0 min 0 % ACN.

Radiosynthesis of [^{125}I]-[Leu^{15}]-Gastrin I via Chloramine T method. An aliquot of 10 µL of peptide stock solution (0.48 mM in H_2O) was mixed with 25 µL 0.5 M phosphate buffer, 5 µL Na^{125}I (10 MBq) solution and after adding 20 µL of freshly water-dissolved *N*-Chloro-*p*-toluenesulfonamide sodium salt in a concentration of 7.1 mM the resulting mixture was reacted for 1 min. Thereafter 25 µL 10 % (m/v) bovine serum albumin (BSA) dissolved in 0.05 M phosphate buffer was added and after 2 minutes reaction time followed by addition of 10 µL potassium iodide solution (10 % in H2O, m/v) [^{125}I]-[Leu^{15}]-Gastrin I was purified using analytical RP-HPLC (gradient A).

Radiosynthesis of [^{125}I]-c(RGDyV) via Iodogen method. Pierce™ Iodination Reagent (ThermoFisher Scientific, Vienna, Austria) was dissolved in dichloromethane to a final concentration of 2.3 mM and after transferring 150 µL to an Eppendorf tube the organic solvent was removed under argon stream. Hereafter 150 µL 0.5 M phosphate buffer (pH 7.4), 10 µL of peptide stock solution (1.7 mM in H_2O) and 5 µL Na^{125}I (10 MBq) solution was added to the 1,3,4,6-tetrachloro-3α,6α-diphenyl-glycoluril coated tube and the mixture was maintained under gentle shaking. After 10 min the reaction solution was immediately transferred to a fresh Eppendorf tube and [^{125}I]-c(RGDyV) was purified via analytical RP-HPLC (gradient B).

Supporting Reference

(1) Haubner, R., Wester, H. J., Reuning, U., Senekowitsch-Schmidtke, R., Diefenbach, B., Kessler, H., Stocklin, G., and Schwaiger, M. (1999) Radiolabeled $\alpha_v\beta_3$ integrin antagonists: A new class of tracers for tumor targeting. *J. Nucl. Med. 40*, 1061–1071.

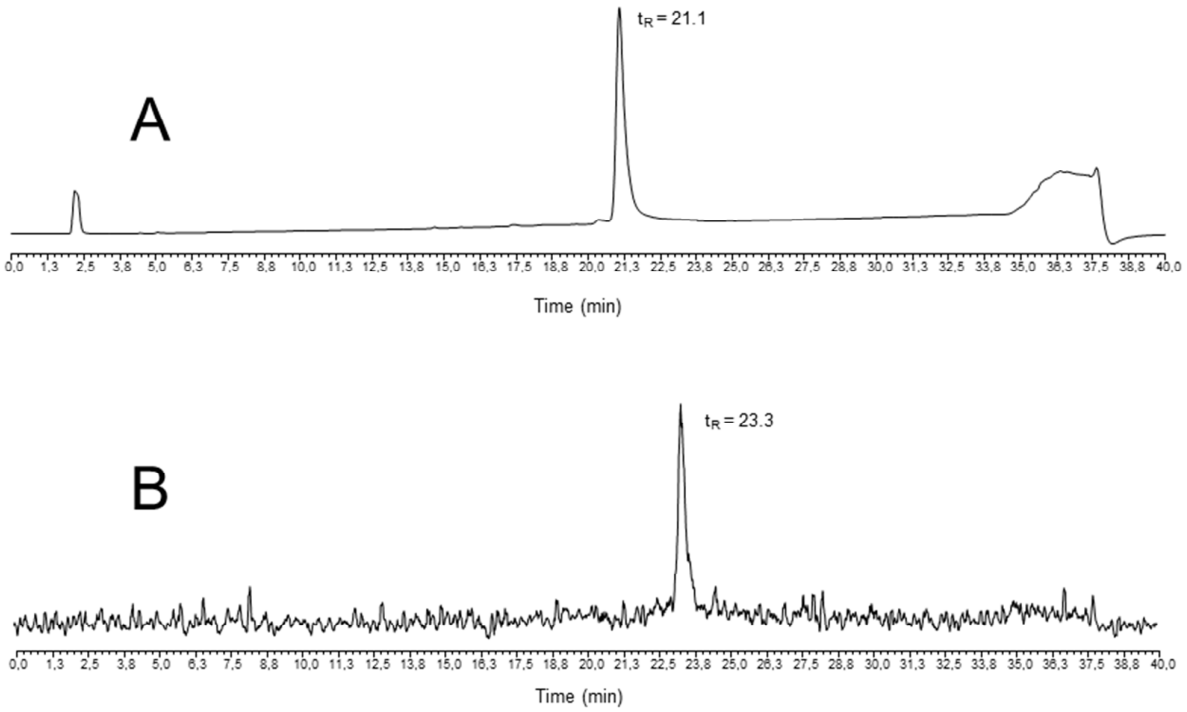

Figure S1: analytical RP-HPLC chromatogram of [Leu^{15}]-Gastrin I (**A**) and [^{125}I]-[Leu^{15}]-Gastrin I (**B**)

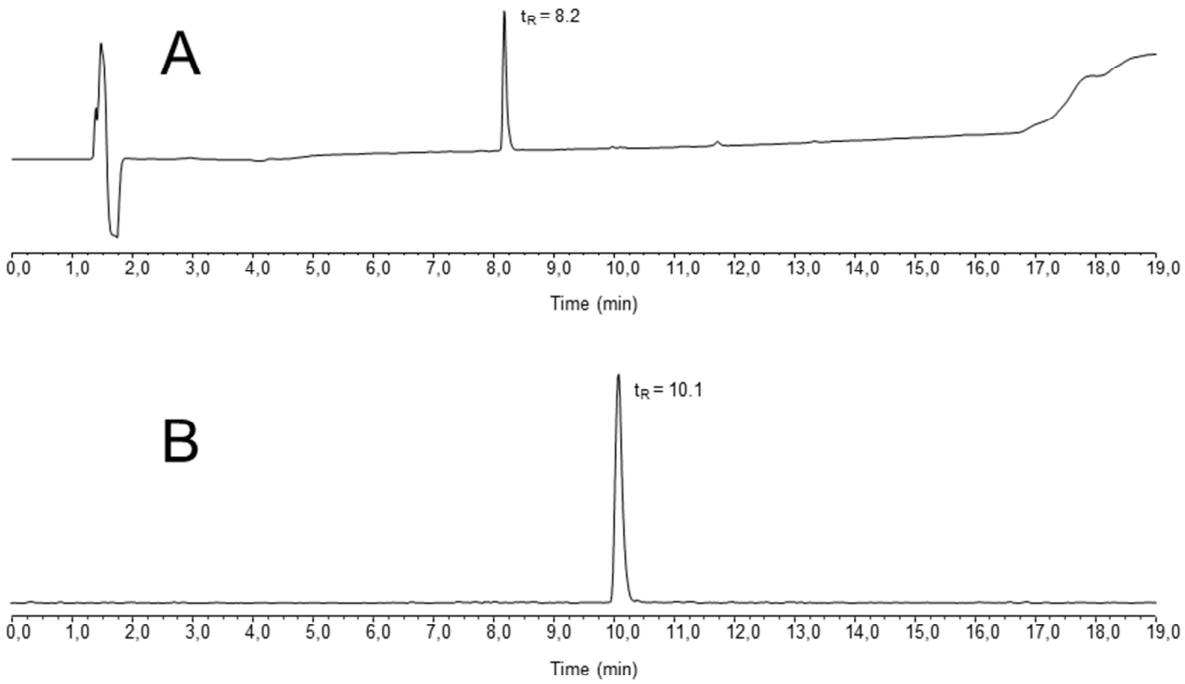

Figure S2: analytical RP-HPLC chromatogram of c(RGDyV) (**A**) and [^{125}I]-c(RGDyV) (**B**)

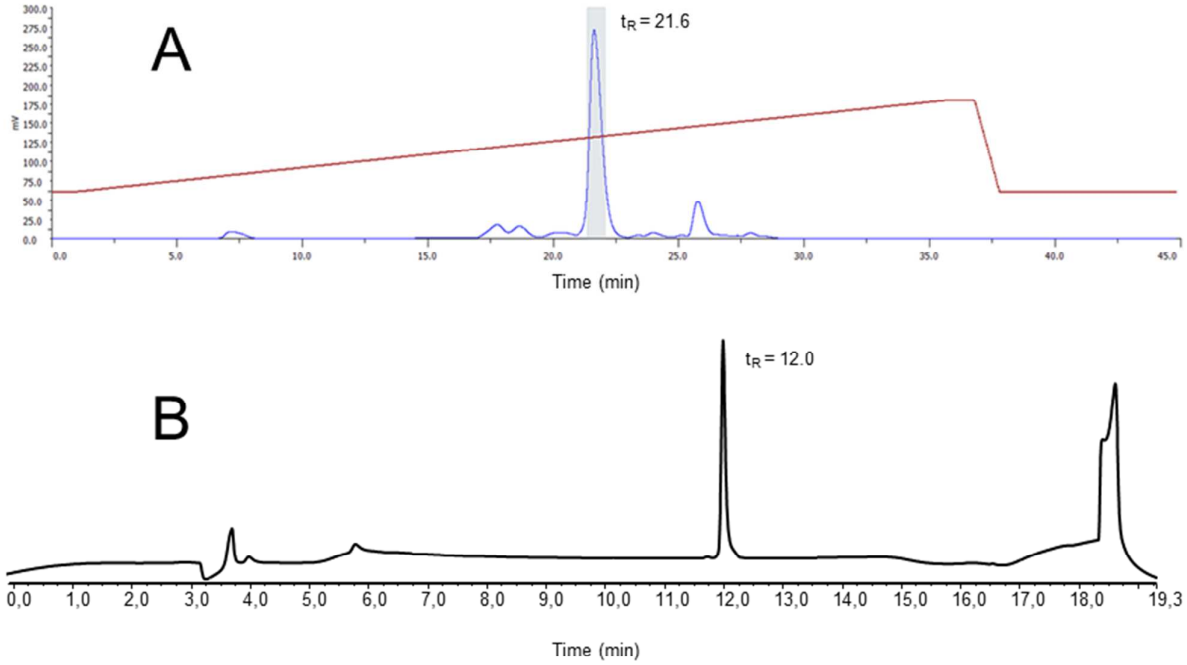

Figure S3: preparative RP-HPLC (**A**) and analytical RP-HPLC (**B**) of [3-MP^{0}-D-Glu^{1},desGlu$^{2-6}$]-minigastrin11 [**MG11-SH**]

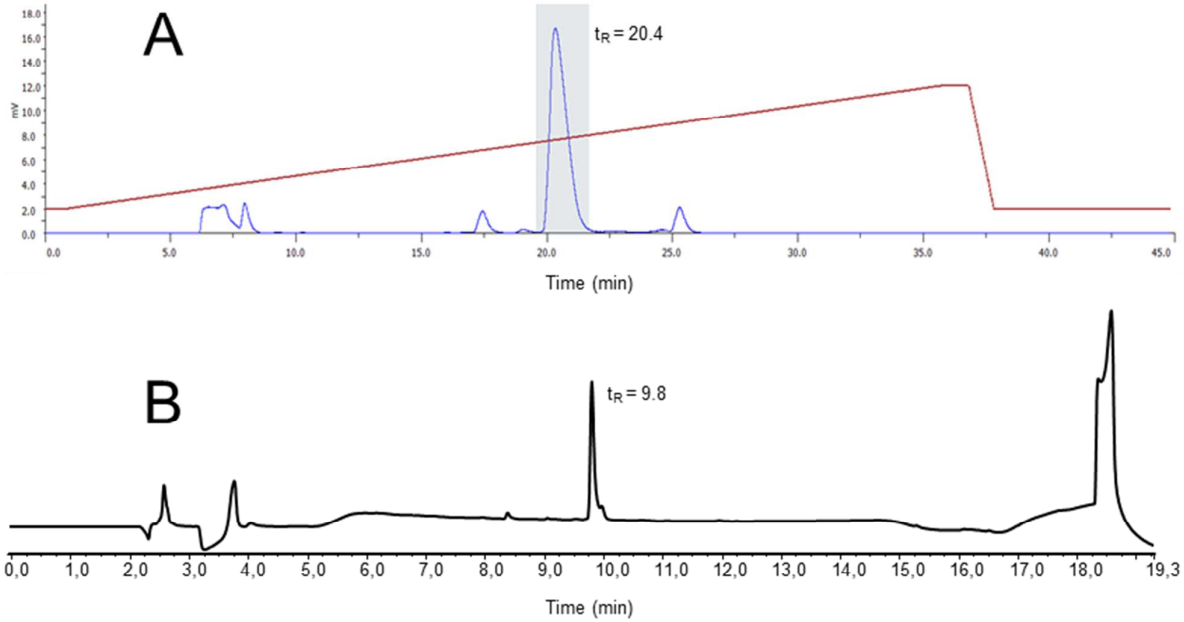

Figure S4: preparative RP-HPLC (**A**) and analytical RP-HPLC (**B**) of c(RGDfK)-$(PEG)_4$-SH [**RGD-SH**]

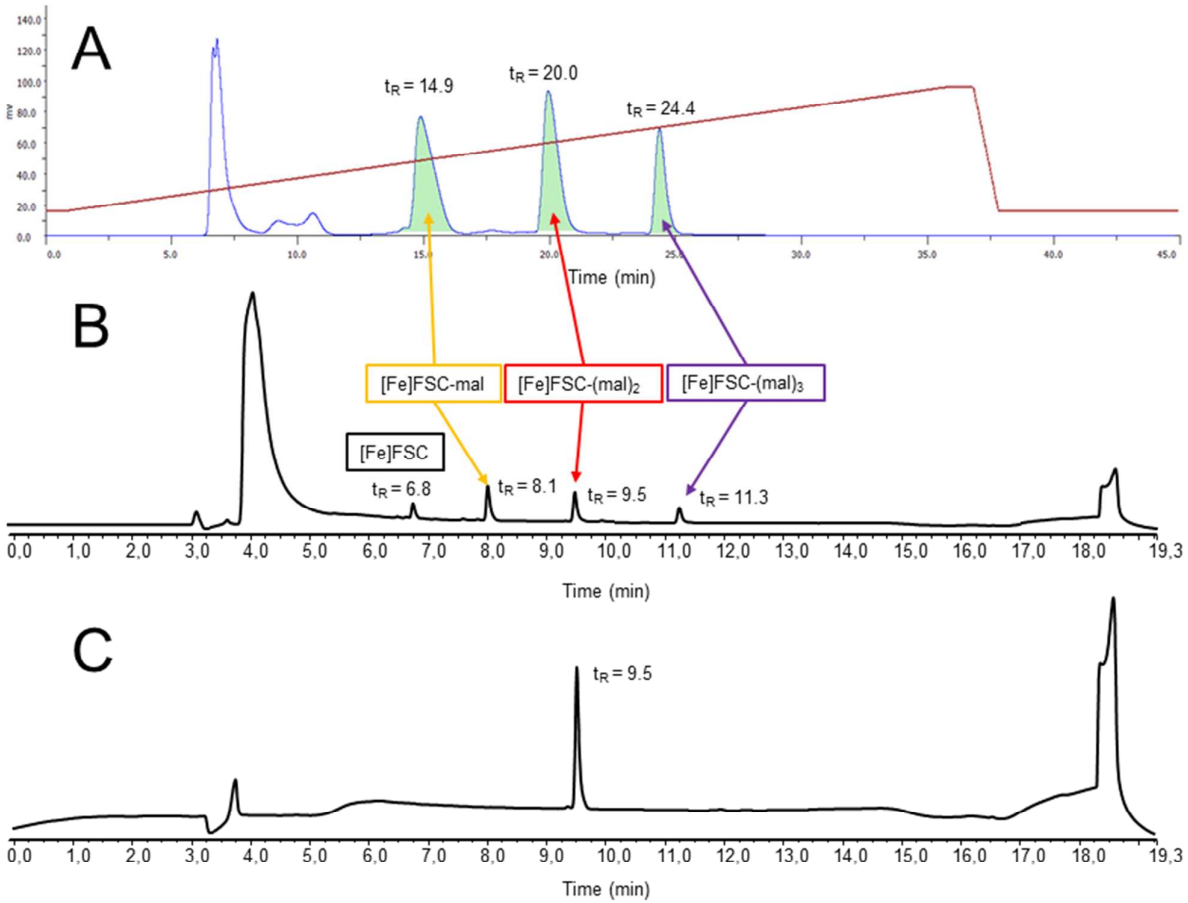

Figure S5: preparative RP-HPLC (**A**) and analytical RP-HPLC (**B**) of mono- and multiple (N-(3-maleinimidopropionyl) conjugated [Fe]FSC derivatives; analytical RP-HPLC (**C**) of [Fe]FSC-(N-(3-maleinimidopropionyl))$_2$ (**[Fe]FSC-(mal)$_2$**)

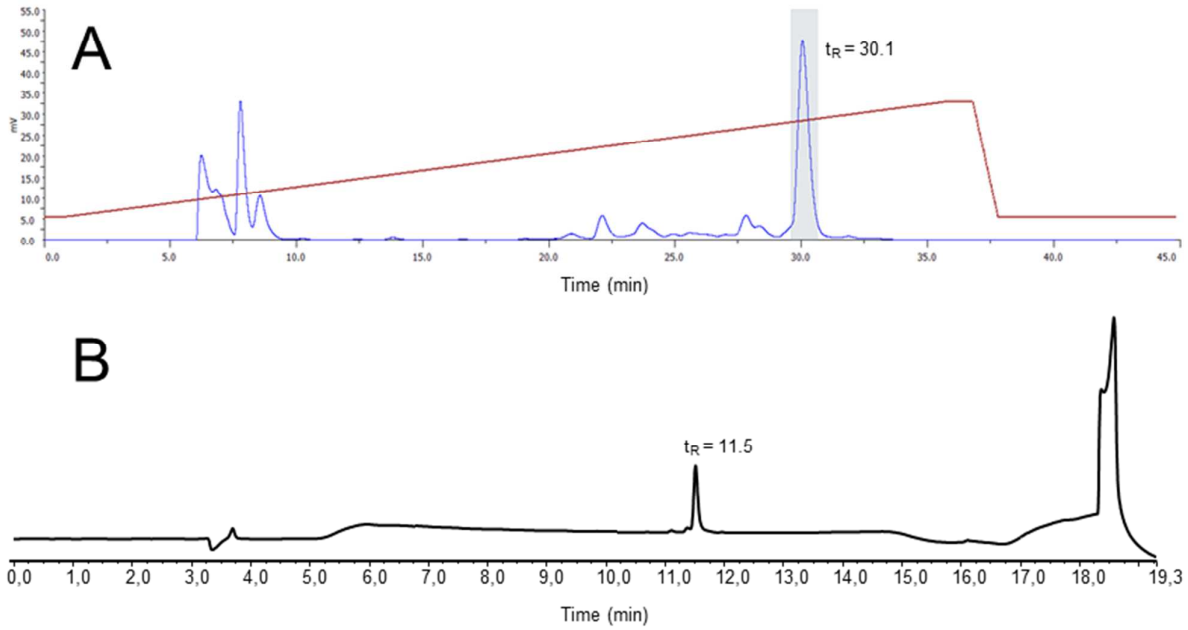

Figure S6: preparative RP-HPLC (**A**) and analytical RP-HPLC (**B**) of **Sulfo-Cyanine7-FSC-(mal)$_2$**

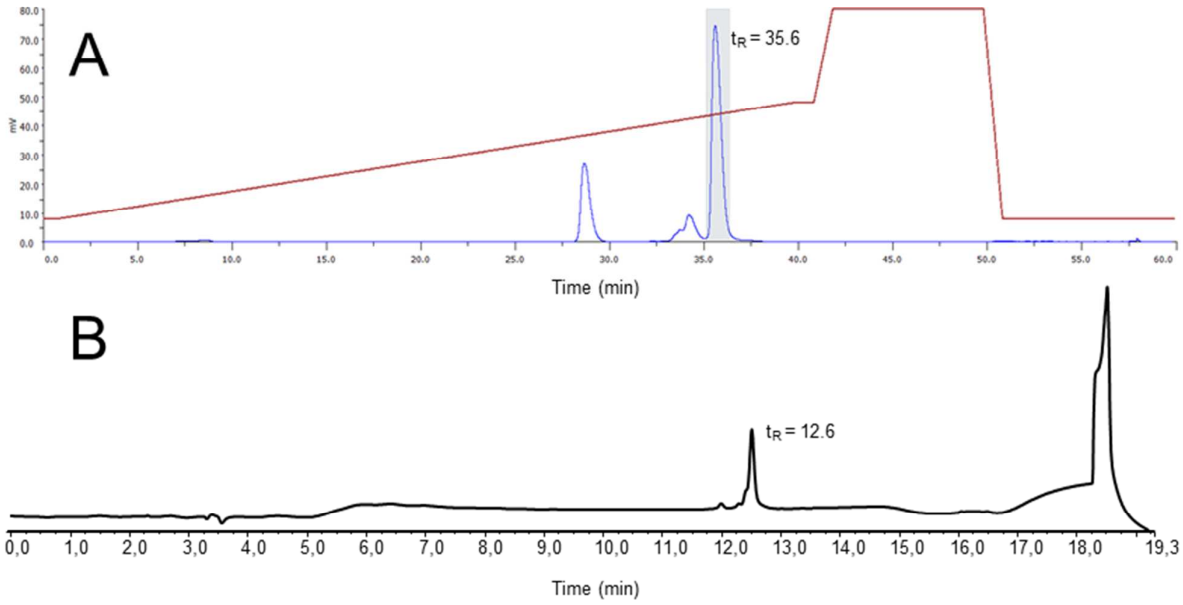

Figure S7: preparative RP-HPLC (**A**) and analytical RP-HPLC (**B**) of **Sulfo-Cy7-FSC-MG**

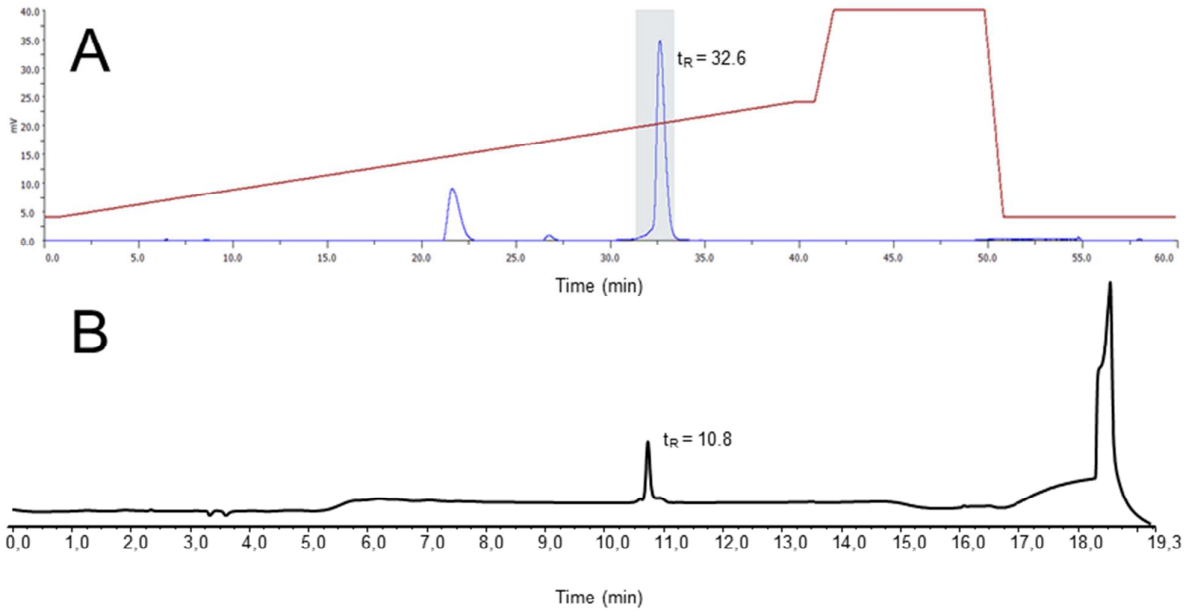

Figure S8: preparative RP-HPLC (**A**) and analytical RP-HPLC (**B**) of **Sulfo-Cy7-FSC-RGD**

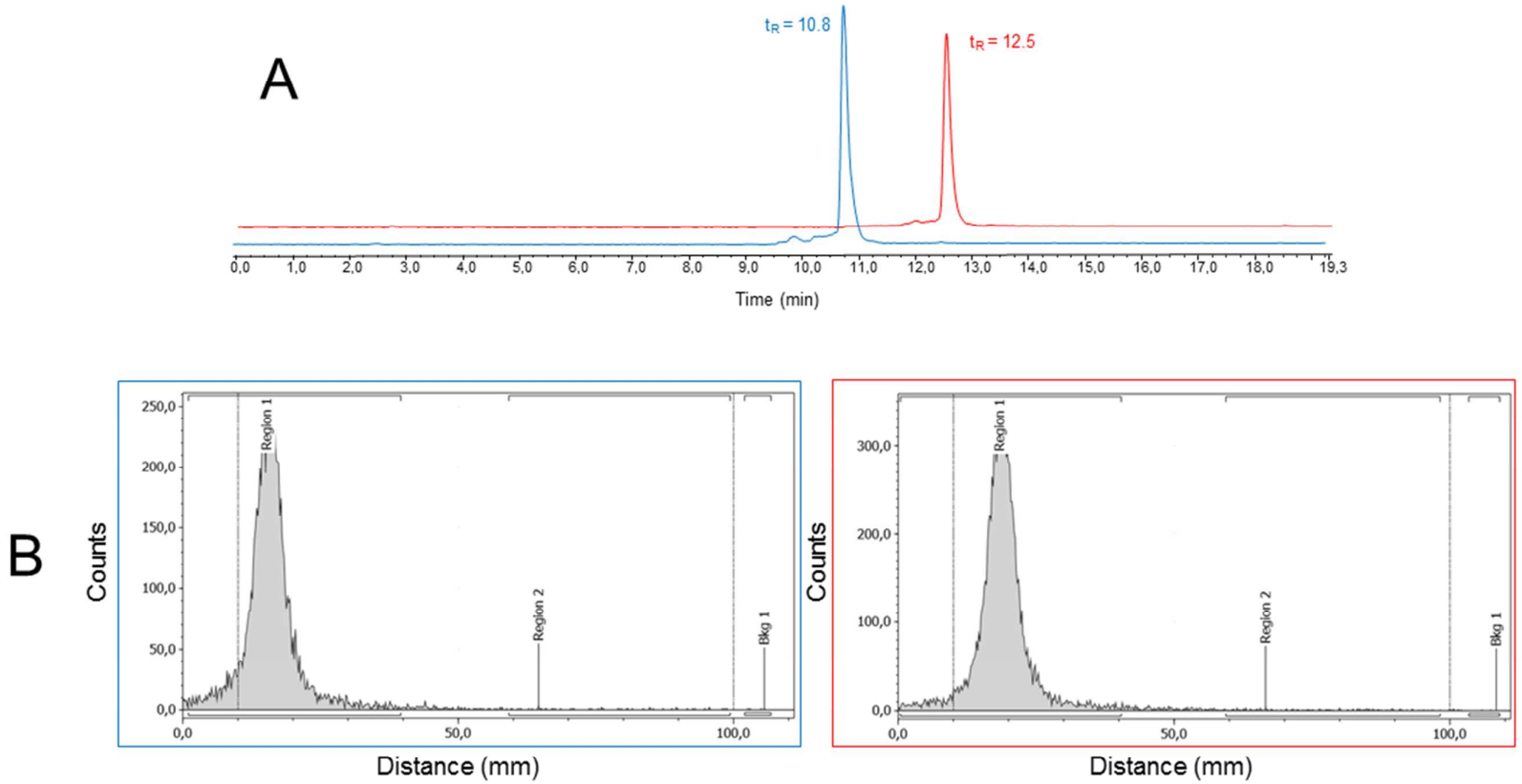

Figure S9: analytical radio-RP-HPLC (**A**) and radio-ITLC (**B**) of [^{68}Ga]Sulfo-Cy7-FSC-MG and [^{68}Ga]Sulfo-Cy7-FSC-RGD

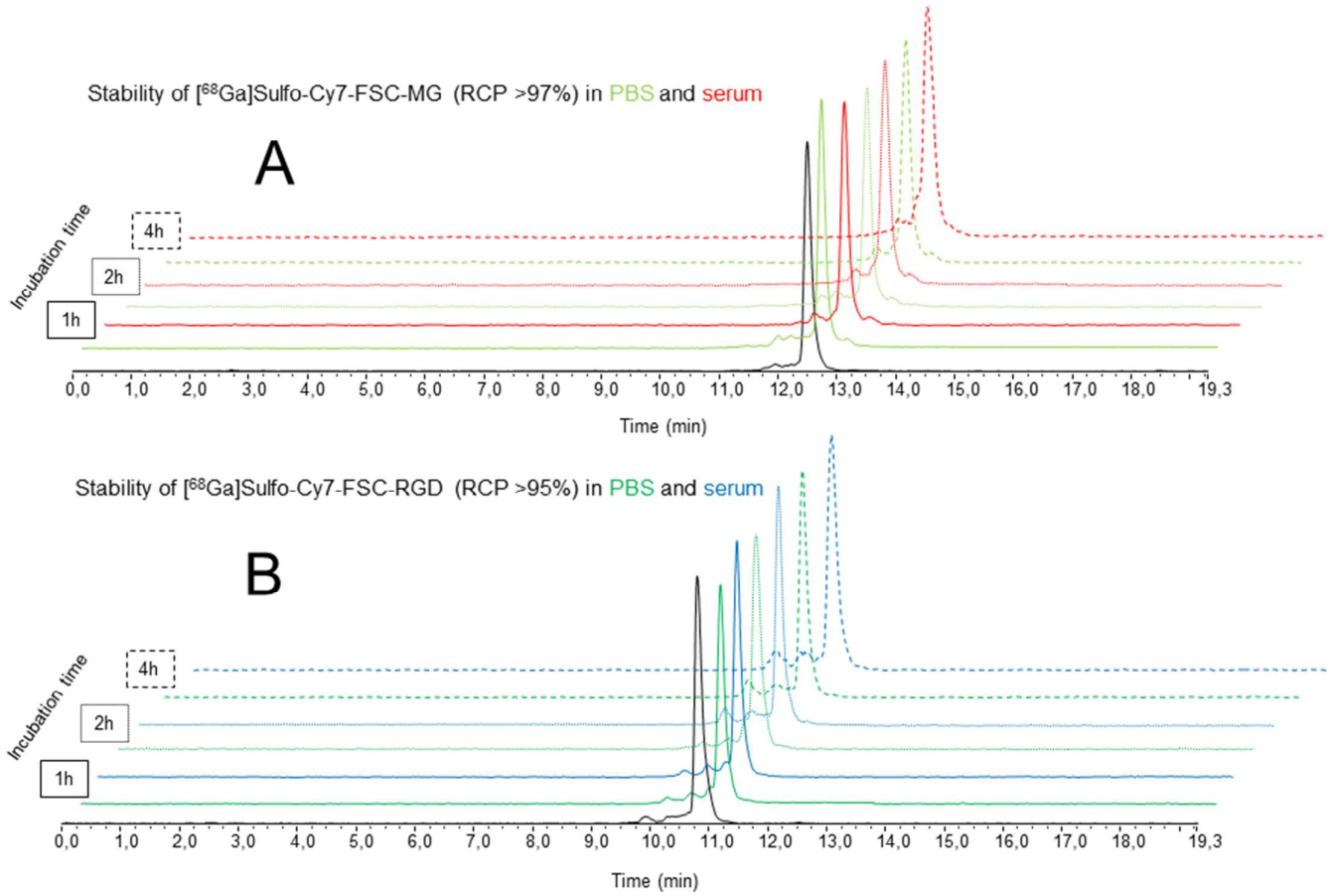

Figure S10: analytical radio-RP-HPLC to assess stability of [^{68}Ga]Sulfo-Cy7-FSC-MG (**A**) and [^{68}Ga]Sulfo-Cy7-FSC-RGD (**B**)