Electronic Supplementary Material (ESI) for Dalton Transactions

Supporting Information

Copper-64 labelling of triazacyclononane-triphosphinate chelators

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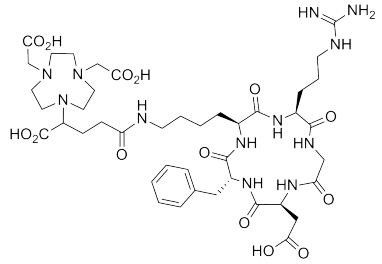
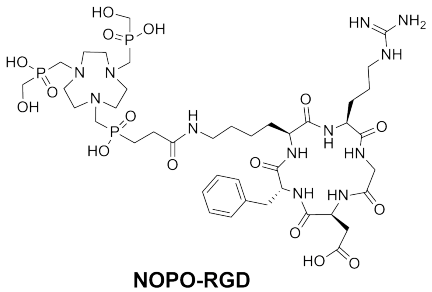
1. Materials and reagents

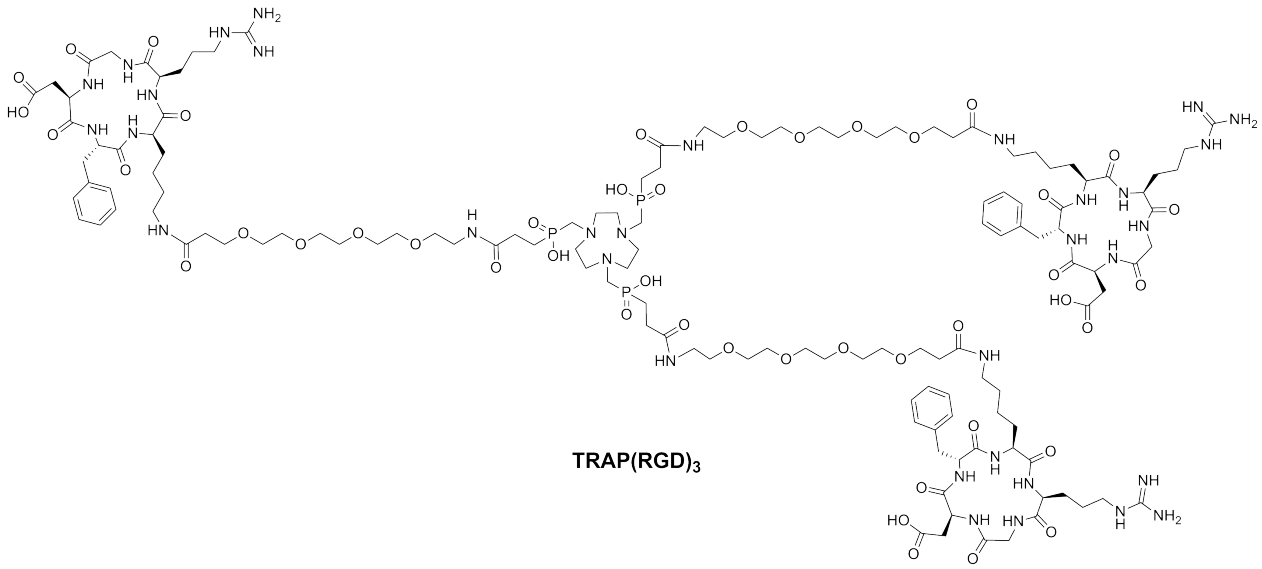
^{64}Cu was obtained from ACOM (Montecosaro Scalo, Italy) in 0.1 M HCl. Content of non-^{64}Cu metals according to the manufacturer: Pb ≤ 0.129 µg/mL, Ni ≤ 0.394 µg/ml, Cu ≤ 0.165 µg/mL, Zn ≤ 0.338 µg/mL, Fe ≤ 0.465 µg/mL. NODAGA-RGD was purchased from ABX (Radeberg, Germany), NOTA[1], DOTA[2], TRAP-H[3], TRAP-Pr[4], NOPO[5], TRAP(RGD)$_3$[6] and NOPO-RGD[5] were prepared as described in the literature. A sample of DEDPA was kindly provided by Carlos Platas Iglesias, Universidade da Coruña, Spain. Buffers (HEPES, sodium acetate) and water (Ultrapur) were purchased from Merck (Darmstadt, Germany).



NODAGA-RGD



NOPO-RGD



TRAP(RGD)$_3$

Figure S1: Structures of RGD peptide conjugates.

2. ^{64}Cu-labelling

Non-buffered solutions: For experiments at pH 3, 100 µL of ^{64}Cu in 0.1 M HCl was mixed with 9.9 mL of water. 90 µL of that solution was mixed with 10 µL of ligand solution, resulting in ligand concentrations of 0.1, 0.3, 1, 3 and 10 µM. Activity of added ^{64}Cu was in the range of 1.9–2.5 MBq (0.2–0.3 pmol). Labelling was done for 5 min at 25 °C, whereafter ^{64}Cu incorporation was determined by radio-TLC (see below).

Labelling in NaOAc buffer: Mixing 10 µL of 100 µM solution (1 nmol of ligand in total) of TRAP(RGD)$_3$, NOPO-RGD and NODAGA-RGD with 5 µL of 1 M aq. NaOAc and additon of 7 µL of ^{64}Cu in 0.1 M HCl (~ 3 MBq) resulted in pH 5.6. The mixtures was left standing for 5 min at 25 °C and then evaluated by radio-TLC (see below).

Labelling in HEPES buffer: TRAP(RGD)$_3$, NOPO-RGD and NODAGA-RGD (10 µL of 100 µM solution, 1 nmol) were mixed with 80 µL of aq. HEPES solution (7.2 g of HEPES + 6 mL water) and 10 µL of ^{64}Cu (~ 4 MBq) in 0.1 M HCl was added, which resulted in pH 5.7. The mixtures were incubated for 5 min at 37 °C and evaluated by radio-TLC (see below).

Preparation of the tracer for in vivo injection: TRAP(RGD)$_3$ (5 nmol) in water (50 µL) was mixed with 5 µL of aq. HEPES solution and 0.1 M NaOH (40 µL). ^{64}Cu in 0.1 M HCl (50 µL, 120 MBq, 13.2 pmol) was added (final pH ~ 4.4) and the solution was heated for 30 min to 95 °C. ^{64}Cu-labelled tracer was purified by solid phase extraction, using a C8 light cartridge (Waters), preconditioned with 10 mL of ethanol and 10 mL of water. The reaction mixture was passed over the cartridge, purged with 1 mL of water in order to remove free ^{64}Cu, and the product eluted with 1 mL of ethanol. After addition of water (1 mL) and PBS (1 mL), the ethanol was evaporated in vacuo and the solution simultaneously concentrated to 1 mL. Before injection, the formulation was filtered over a 0.22 µM sterile filter.

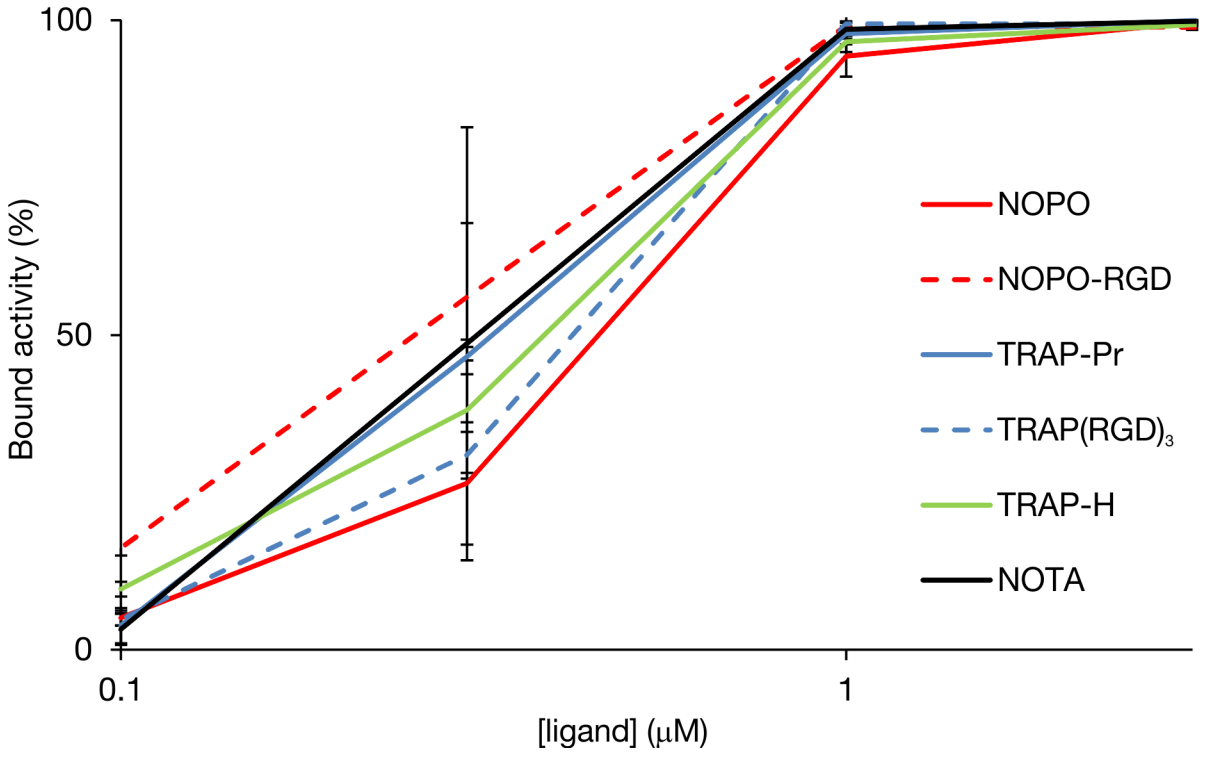


Figure S2: ^{64}Cu incorporation as function of chelator concentration.

2.1. Comment on metal-to-ligand ratio and incorporation efficiency

Even though the labelling with low activity of ^{64}Cu showed to be highly efficient using low chelator amount, e.g. 0.1–1 nmol (Figure 1), the labelling efficiency showed to be highly dependent on the metal-to-ligand (M:L) ratio. Labelling with higher activity of ^{64}Cu and similar ligand concentration did not lead to comparable activity incorporation. Therefore, for preparation of higher doses, e.g. for injection purposes, the strategy similar to that for routine labelling with ^{177}Lu is of choice, i.e., calculation of a well-defined, known optimal excess of chelator. On the contrary, when labelling of e.g. 1 nmol of TRAP chelators with ^{68}Ga, quantitative activity incorporation independent on the absolute activity is observed.

For example, labelling of 0.1 nmol (c = 1 μM) of precursors with ~ 2.2 MBq of ^{64}Cu (~ 0.24 pmol, M:L ratio 1:417) resulted in quantitative ^{64}Cu incorporation at r.t. Contrary, labelling of a ten times higher concentration of TRAP(RGD)$_3$ (c = 10 μM, 1 nmol) with ^{64}Cu (120 MBq, 13.2 pmol, pH 3.1 adjusted with aq. HEPES) resulted in a M:L ratio of 1:75, which was too low to yield labelled product, even at 95 °C. However, a radiochemical yield of > 95 % was reached by labelling 5 nmol of the precursor (M:L ratio is then 1:378). In further experiments, adjusting the pH to 4.4 helped to reduce the reaction time to 20 min at 95 °C. Specific activities were typically ranging around 20 GBq/μmol.

3. Analysis

^{64}Cu incorporation by chelators was evaluated by TLC (silica 60 coated alumina sheets, Merck), using 0.1 M aq. EDTA as mobile phase. Labelled chelators/conjugates stay at the origin (R_f = 0), whereas unbound ^{64}Cu is complexed by EDTA and moves with the front (R_f = 0.9–1.0).

4. EDTA challenge and stability in human plasma

The chelators (1–2 nmol of each) were labelled with ~ 12 MBq of ^{64}Cu, using a mixture of ^{64}Cu in 0.1 M HCl (7 μL) and 1 M NaOAc (5 μL), pH 5.7, 5 min reaction at 95 °C. Full labelling was confirmed by TLC. Then, each sample was diluted with PBS to reach a volume of 50 μL. 20 μL of the solutions were added to 100 μL of 0.1 M EDTA and left standing at room temperature. Samples for TLC analysis were withdrawn after 1, 2 and 12 h.

The stability of ^{64}Cu-labelled NOPO-RGD, TRAP(RGD)$_3$ and NODAGA-RGD was tested also in human plasma, for which purpose 20 μL of the solutions containing labelled conjugate (prepared as described before) were transferred to 100 μL of plasma, and incubated at room temperature. Samples for TLC analysis were taken after 1, 2 and 12 h of incubation.

5. log*P* determination

50 μL (~ 0.5 MBq) of PBS solution of a purified tracer was mixed with 450 μL of PBS and 500 μL of n-octanol. After 1 min of vigorous shaking, the phases were separated by centrifugation and the activity contained in 100 μL aliquots of both water and n-octanol phases were measured in a γ-counter (1480 WIZARD^{TM}, PerkinElmer Wallac). Experiments were repeated 8 times.

6. Small animal PET imaging

The animal model for $\alpha_v\beta_3$ integrin expression was described before in detail.[6] Briefly, CD-1 athymic nude mice were used, bearing tumor xenografts (M21 human melanoma with high $\alpha_v\beta_3$ integrin expression) on the right shoulder.

MicroPET imaging was performed using small animal PET/CT scanner (Siemens Inveon). ^{68}Ga-TRAP(RGD)$_3$ (12 MBq, prepared as described[6]) or ^{64}Cu- TRAP(RGD)$_3$ in PBS (30 MBq) was injected to the tail vain of a mouse anaesthetised with isoflurane. PET data was recorded for 15 min, 75 min p.i. (^{68}Ga- and ^{64}Cu-TRAP(RGD)$_3$) and 18 h p.i. (^{64}Cu-TRAP(RGD)$_3$ only). Images were reconstructed using Inveon Research Workplace software; 3D ordered-subsets expectation maximum (OSEM3D) algorithm without scanner and attenuation correction. All animal experiments were performed in accordance with general animal welfare regulations in Germany.

References

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