Supporting Information

A Supramolecular Approach For Liver Radioembolization

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Methods

Stability of ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ in FCS

^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ was dissolved in FCS (2.5 μg/mL) and shaken in a water bath at 37 ^{o}C for 44 h. After 2, 20, and 44 h 0.1 mL samples were taken and their composition was analyzed by PD-10-SEC.

Stability of ^{99m}Tc-Maa-Ad in FCS

Lyophilized MAA (2 mg) was dissolved in 1 mL of saline (0.9% NaCl, sterile and pyrogen-free, B. Braun Medical Supplies, Inc., Oss, The Netherlands). To one portion 100 μL of a freshly eluted ^{99m}Tc-Na-pertechnetate solution (500 MBq/mL, Mallinckrodt Medical B.V.) was added and the mixture was gently stirred in a shaking water bath for 1 h at 37 ^{o}C. Thereafter, the solution was washed 2 times PBS by 2 centrifugation steps (3 min, 1,200 rpm). Next, 20 μL of Ad-TFP (10 mg/mL DMSO) was added. After allowing it to react in a shaking water bath for 1 h at 37 ^{o}C, the reaction mixture was washed 2 times with PBS by 2 centrifugation steps (3 min, 1,200 rpm) and the pellet was dissolved in 1 mL PBS. Of this solution, 0.1 mL was added to 0.9 mL of FCS and was shaken in a water bath at 37 ^{o}C up to 44 h. At 2, 20, and 44 h after incubation 0.1 mL samples were taken and their composition analysed by PD-10 SEC.

Stability of Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ and MAA-Ad complexes in FCS

Mixtures of either MAA-Ad (0.2 mg/mL) with ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ (10 μg/mL, 1 MBq) or ^{99m}Tc-MAA-Ad (0.2 mg/mL, 1 MBq) with Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ (10 μg/mL) were prepared in 0.2 mL PBS and the solutions were incubated for 1 h in a shaking water bath at 37 ^{o}C. Thereafter, the formed complexes were washed twice with PBS by centrifugation (5 min, 3,000 g) and resuspended in 0.2 mL PBS. Subsequently, 0.1 mL thereof was mixed with 1 mL FCS and shaken at 37 ^{o}C in a shaking water bath up to 44 h. At 2, 20, and 44 h after incubation 0.1 mL samples were taken and diluted in 1 mL of PBS and after spinning for 5 min at 7,000 rpm, the decay corrected radioactivity of the pellet and supernatant was measured in a dose-calibrator. Hereby a reduction, in the radioactivity of the pellet represents dissociation or instability (% of binding).

Results

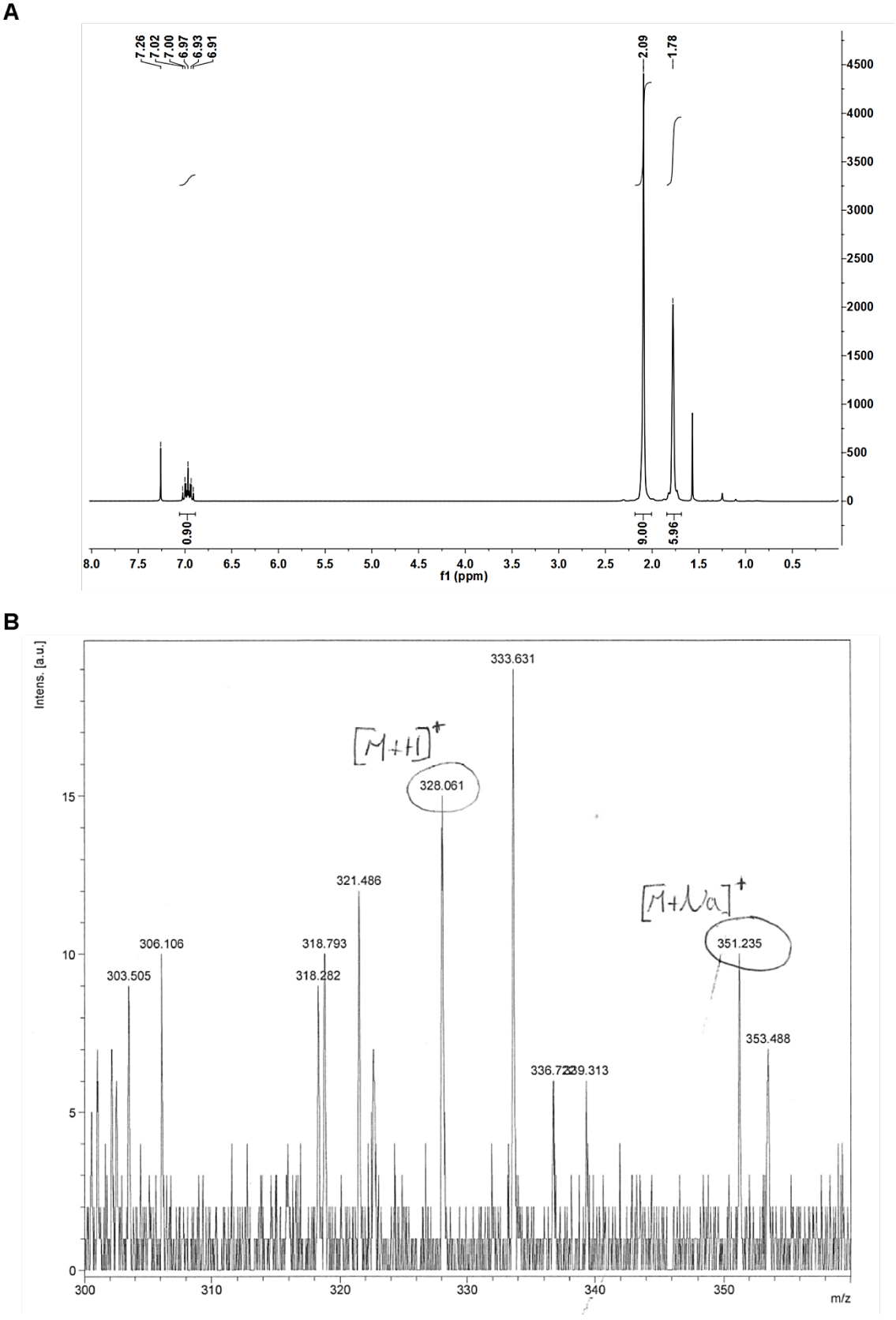


Figure S1. A) NMR of Ad-TFP measured in $CDCL_3$. B) Mass spectra of Ad-TFP, only low signals could be obtained as the compound is hard to ionize. Signals corresponding to the mass matrix are therefore clearly visible as well.

To quantify the difference of ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ accumulation after pre-administration of nothing, MAA or MAA-Ad for all the investigated organs (Table 1 and Table S1), the relative increase of ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ accumulation with regard to the ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ reference distribution (Figure 3) was calculated (Figure S2). If there was a significant increase with $p < 0.01$ this was indicated with a *. When MAA or MAA-Ad was administered i.v. the uptake in the lungs was found highest (but this difference was not significant due to large variations). While locally administered MAA or MAA-Ad resulted in significant increases in spleen, liver and kidneys; pre-administration of MAA or MAA-Ad was performed via the spleen. With the i.v. pre-administration method (Model I) the polymer accumulation increased in more organs compared to the local pre-administration method (Model II), underlining once more the fact that the system works best for the clinically more relevant model i.e. local administration. The significance data can be slightly misleading since increases from e.g. 0.1 %ID/g to 0.3 %ID/g in the brain will be displayed as significant (Table 1 and Table S1).

Table S1. The biodistribution of ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ following injection of: none (reference distribution), MAA, or MAA-Ad and the biodistribution of ^{99m}Tc-MAA-Ad administered via Models I and II. Data (expressed as the mean ± SD of the percentage of the injected dose per gram tissue (%ID/g) of 5 observations) are calculated from radioactivity counts in various tissues at 2 h post-injection of the tracer.

	Reference distribution host	**Distribution of host (^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$) following injection of indicated guest**				**Reference distribution guest**	
Tissue	**^{99m}TC-Cy5$_{0.5}$CD$_{10}$PIBMA** mean	**Model I: MAA** Mean	**Model I: MAA-Ad** Mean	**Model II: MAA** Mean	**Model II: MAA-Ad** mean	**Model I: ^{99m}Tc-MAA-Ad** mean	**Model II: ^{99m}Tc-MAA-Ad** mean
Salivary gland	3.5 ± 0.4	9.1 ± 3.3	17.3 ± 3.5	3.0 ± 0.6	3.9 ± 0.1	N.A.	8.9 ± 1.9
Stomach	4.4 ± 0.8	19.8 ± 7.6	10.8 ± 1.4	3.0 ± 0.6	2.3 ± 0.8	11.5 ± 9.1	12.7 ± 3.1
Intestines	0.8 ± 0.3	1.5 ± 0.6	1.5 ± 0.3	0.9 ± 0.0	0.7 ± 0.3	0.6 ± 0.3	1.8 ± 0.5

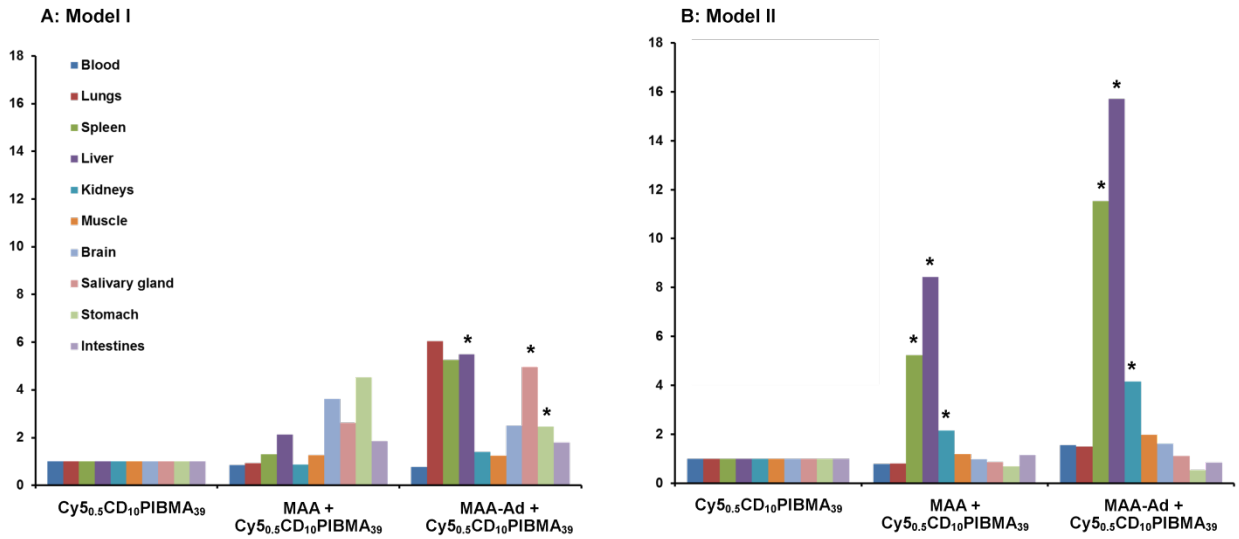
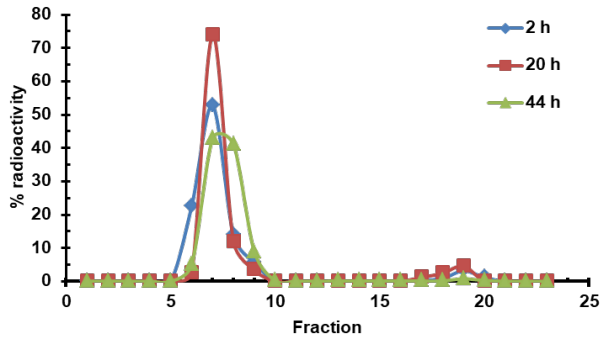


Figure S2. Relative increase of ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ after i.v. (A) or Local (B) administration of MAA or MAA-Ad with respect to ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ accumulation in the indicated organ when no particles are pre-administered. With significance of difference ($p < 0.01$) indicated by *.

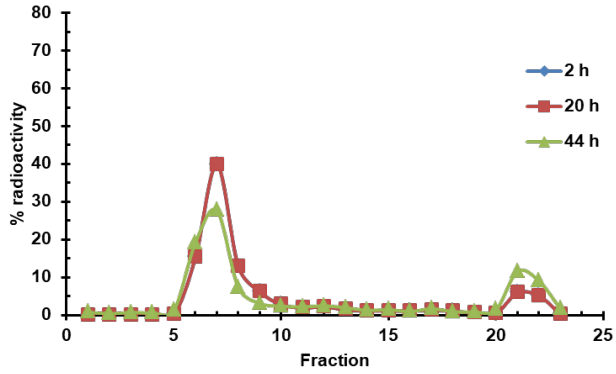
Both the individual components and the complexes formed demonstrated a high serum stability (Figure S3). No clear metabolites of the individual components could be defined. This said some dissociation of ^{99m}Tc was observed both from the individual components as from the complexes formed. Nevertheless, the complex yielded around a 80% stability at 44 h.

As the Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ polymer was not optimized for ^{99m}Tc chelation, but merely provided coordination sides by its free –COOH moieties, some dissociation of ^{99m}Tc was observed *in vivo* (Table S1, Figure S3). When this occurred, characteristic uptake in the salivary glands and stomach could be observed. As these findings did not complicate the assessment of the pre-targeting ability, no attempts were made to optimize the chelation stability.

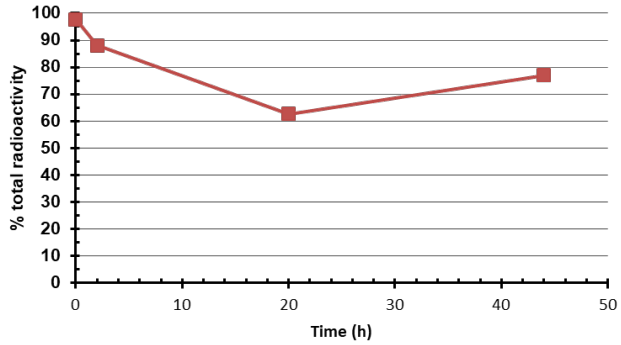
A PD-10 size-exclusion chromatography ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$



B PD-10 size-exclusion chromatography of ^{99m}Tc-MAA



C Stability of complexed ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ with MAA-Ad



D Stability of complexed Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ with ^{99m}Tc-MAA-Ad

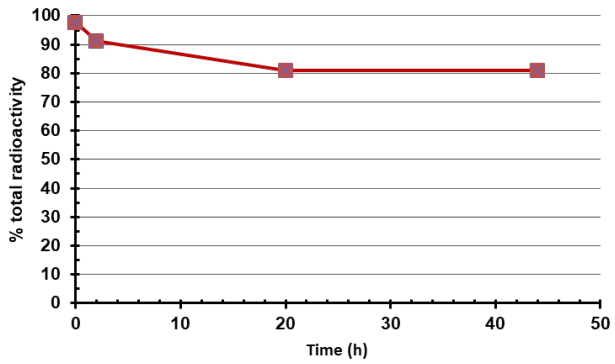


Figure S3 A) Serum stability of ^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ (peak around fraction 18 indicated smaller fragments), B) Serum stability of ^{99m}Tc-MAA (peak around fraction 22 indicated some free ^{99m}Tc). C) Serum stability of [^{99m}Tc-Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ * MAA-Ad] complexes, D) Serum stability of [Cy5$_{0.5}$CD$_{10}$PIBMA$_{39}$ * ^{99m}Tc-MAA-Ad] complexes.