Electronic Supplementary Material

In Vivo Biodistribution of Radiolabeled Acoustic Protein Nanostructures

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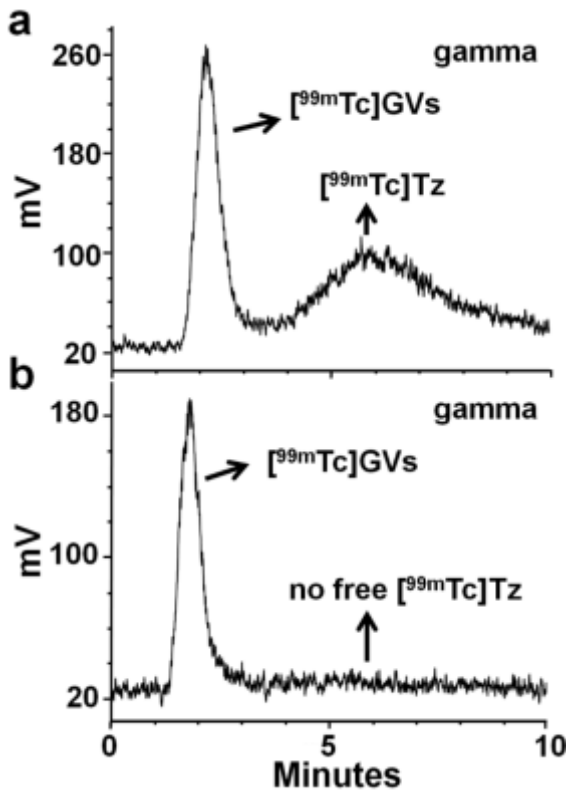

Fig. S1 Size exclusion HPLC chromatograms (gamma detection). **a** Chromatogram of the reaction mixture containing both [^{99m}Tc]GVs and free [^{99m}Tc]Tz (3). **b** Purified [^{99m}Tc]GVs showing no residual [^{99m}Tc]Tz.

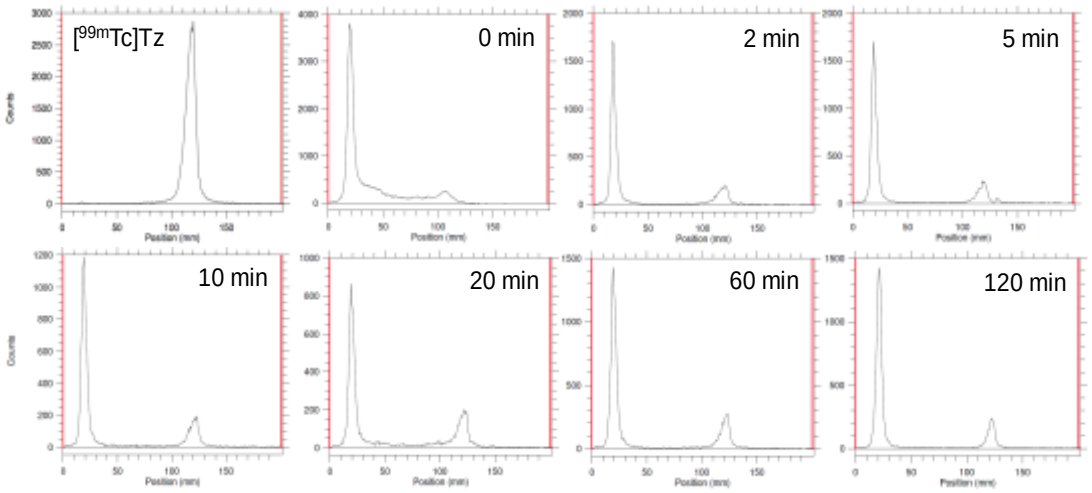

Figure S2. Radio-TLC analysis of [^{99m}Tc]Tz (Top-left). Radio-TLC analysis of [^{99m}Tc]GVs incubated at 37 ^{o}C in plasma at the indicated time points. Radio-TLC was eluted with 75% methanol and 25% water.

Table S1. Stability in plasma. Table reports the stability of [^{99m}Tc]GVs in plasma at 37 ^{o}C for up to 120 minutes.

Sample	% [^{99m}Tc]GVs	% Compound 3
[^{99m}Tc]Tz	0	100
Plasma[^{99m}Tc]GVs 0 min	87	13
Plasma[^{99m}Tc]GVs 2 min	80	20
Plasma[^{99m}Tc]GVs 5 min	78	22
Plasma[^{99m}Tc]GVs 10 min	78	22
Plasma[^{99m}Tc]GVs 20 min	71	29
Plasma[^{99m}Tc]GVs 60 min	76	24
Plasma[^{99m}Tc]GVs 120 min	81	19

Table S2. Activity in plasma layers. Table reports the % Activity in the GV and plasma layers after centrifugal flotation at 60 and 120 minutes.

Time	% Activity in GV layer	% Activity in plasma layer
60 min	31	69
120 min	44	56

Table S3. 3D Segmentation quantification. Table reports the mean and SEM of the volume of segmented organs.

Organs	Liver	Spleen	Lungs	Gall Bladder	Duodenum	Bladder
Volume (cm^3)	1.964	0.166	0.609	0.028	0.427	0.183
SEM (n=6)	0.176	0.017	0.032	0.005	0.082	0.020

Table S4. Dynamic Uptake quantification. Table reports the mean and SEM of the uptake rate constants. Rate constants were calculated using a linear regression for all organs using the 0-10 min range except for the duodenum for which the 30-60 min range was used.

Organs	Liver	Spleen	Lungs	Gall Bladder	Duodenum	Bladder
Uptake Rate Constants (%ID/cc/min)	2.760	0.584	1.433	5.254	0.935	0.422
SEM (n=6)	0.270	0.181	0.467	1.762	0.266	0.174

Video S1. Spatio-temporal biodistribution of [^{99m}Tc]GVs in mouse. *In vivo* dynamic SPECT co-registered with microCT images showing the spatio-temporal biodistribution of [^{99m}Tc]GVs in mouse organs in the axial, coronal and sagittal planes. **a** Movies show SPECT data, 68 s between each frame, duration of observation was 102 min between 18 and 120 min following [^{99m}Tc]GVs injection. **b** Movies show SPECT data for the first 17 minutes following [^{99m}Tc]GVs injection, 78 s between each frame including the first frame acquired before injection. SPECT data are filtered with an isotropic 1.6 mm Gaussian filter. The threshold used for the MIP is 0.5.