Supplementary Material

Equations

Equation 1. $A_{280c} = A_{280} - A_{309} \times CF$

Measured absorbance of albumin: A_{280}

Measured absorbance of ADIBO: A_{309}

Corrected absorbance of albumin: A_{280c}

Correction factor (CF) = 0.8658

Equation 2. Absorbance = ε ($M^{-1}cm^{-1}$) × concentration (mol/L) × length (cm)

Equation 3. DOF = $[(A_{309} / \varepsilon_{309, ADIBO})/(A_{280c} / \varepsilon_{280, Albumin})]$

$\varepsilon_{309, ADIBO}$ ($M^{-1}cm^{-1}$) = 12000

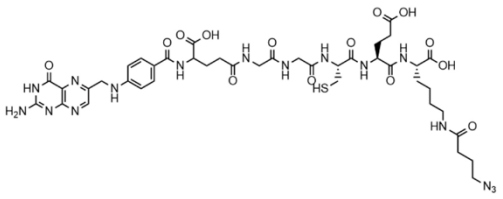
$\varepsilon_{280, Albumin}$ ($M^{-1}cm^{-1}$) = 35295.5

Concentration of albumin (mol/L) = A_{280c} / ($\varepsilon_{280, Albumin}$ x length)

Concentration of ADIBO (mol/L) = A_{309}/ ($\varepsilon_{309, ADIBO}$ x length)

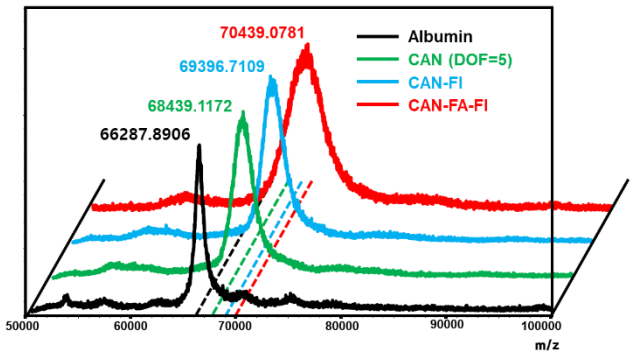
DOF: Degree of functionalization (= Number of ADIBO per albumin)

A



N2-(4-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzamido)-4-carboxybutanoyl)glycylglycyl-L-cysteinyl-L-glutamyl-N6-(4-azidobutanoyl)-L-lysine (N_3-folate), Exact Mass: 1026.37 g/mol

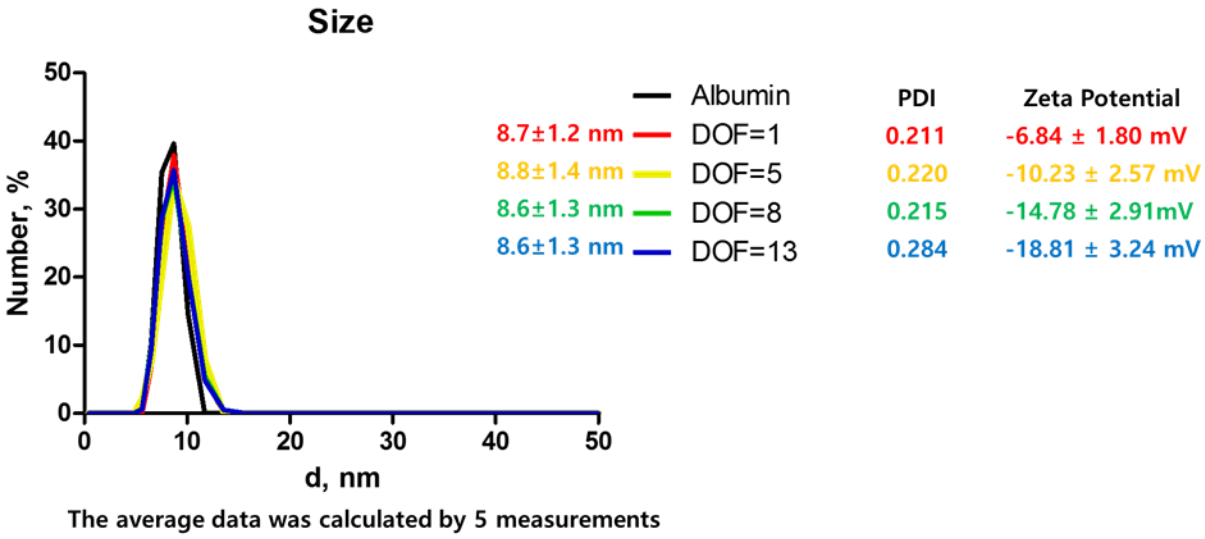
B



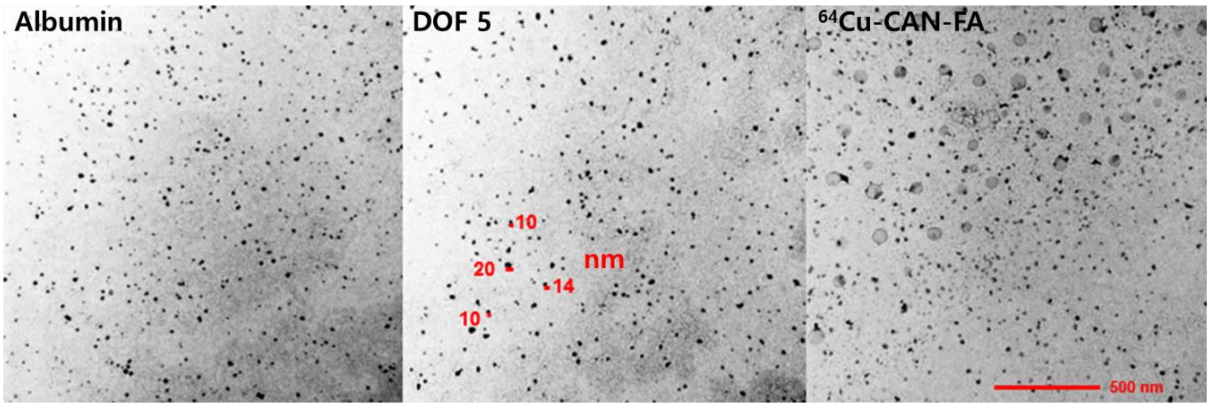
C

Type	MW (mean)	Conjugation No. of per Albumin	Remarks
Albumin	66475.09	0.00	Control
CAN	68439.11	5.48	DOF
CAN-FI	69396.71	FI/CAN = 1.24	Exact mass: 766.97 g/mol
CAN-FA-FI	70439.07	FI/CAN-FA = 1.01	Exact Mass: 1026.37 g/mol

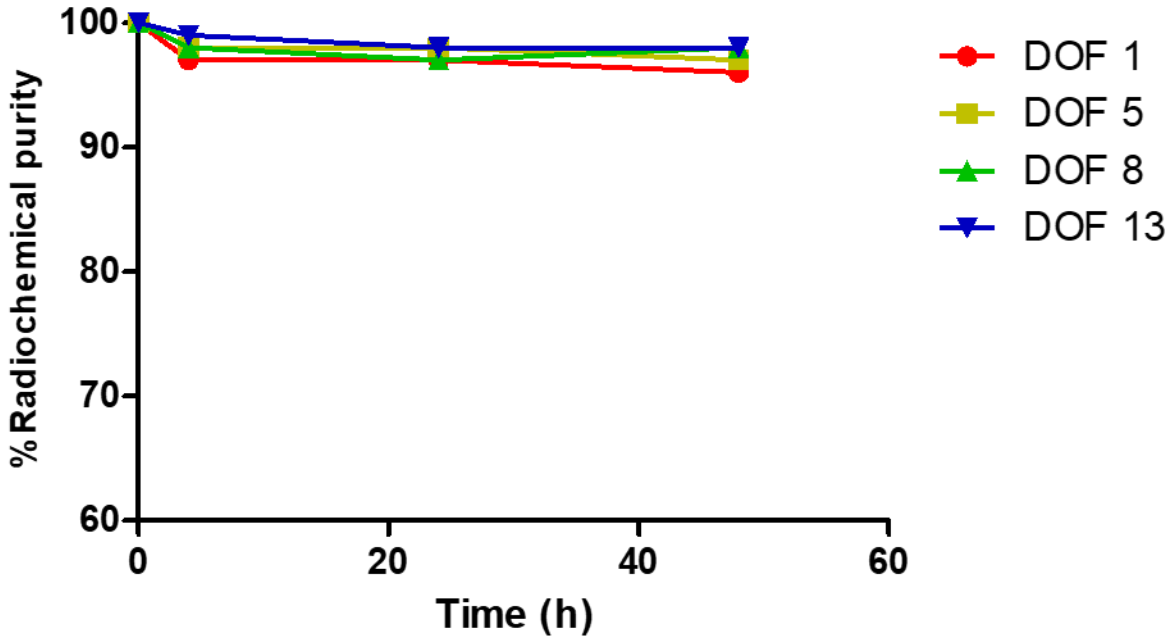
Supplementary Figure S1. Preparation of folic acid (FA) and fluorescence (FI) conjugated albumin. (A) Chemical structure of N_3-folate. (B) MALDI-TOF showed increased mass according to reaction. (C) The increased molecular weight according to reaction and conjugation number of attached ADIBO, FI, and FA was calculated



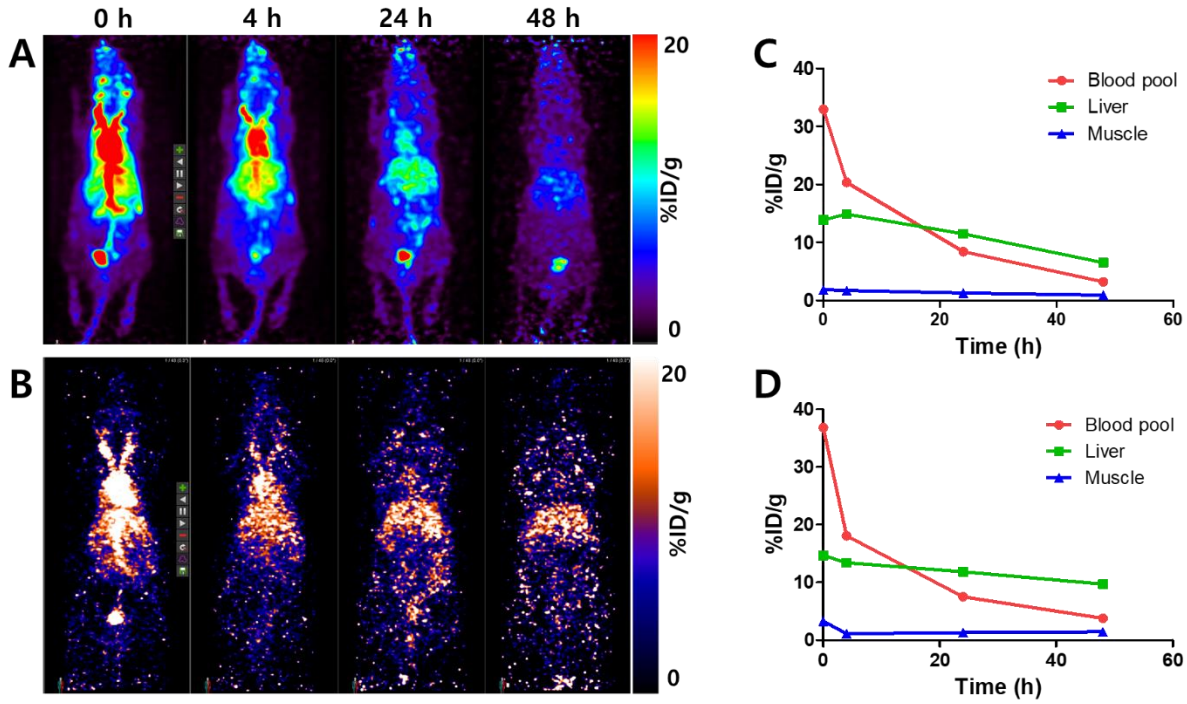
Supplementary Figure S2. Size and zeta potential of CAN. Average hydrodynamic diameters and zeta potentials of albumin and CANs in PBS using the DLS system



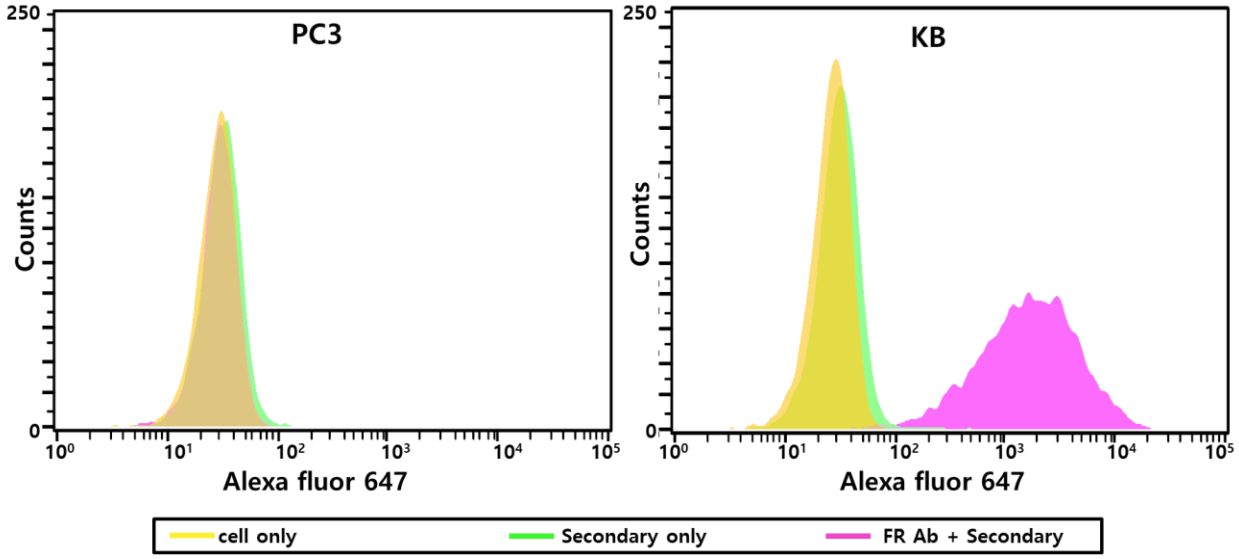
Supplementary Figure S3. TEM images of albumin and CANs. The morphology of albumin, CAN with DOF5, and ^{64}Cu-CAN-FA was examined by TEM



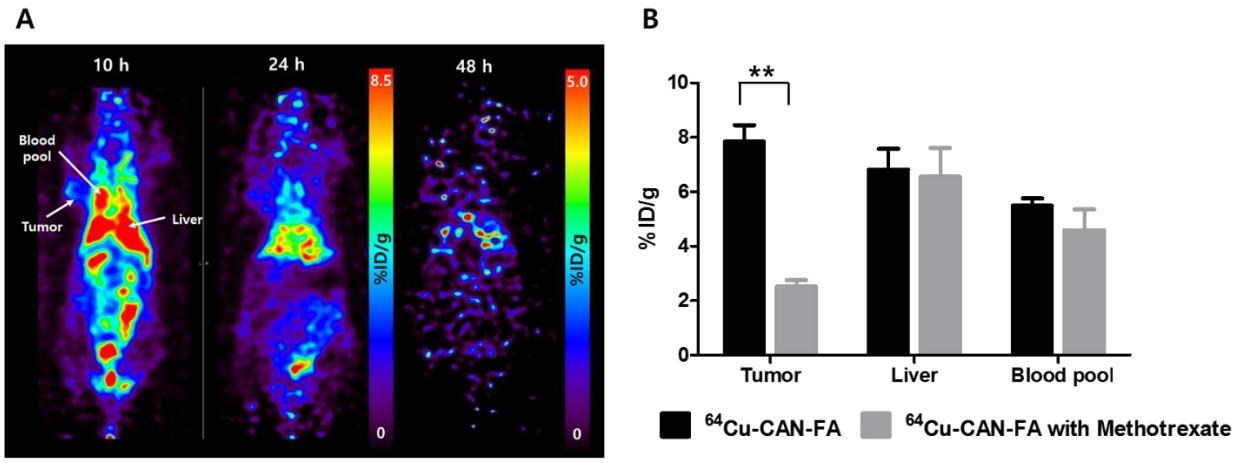
Supplementary Figure S4. Labeling stability of ^{64}Cu-CAN. Labeling stability test of each CAN after click reaction with ^{64}Cu-NOTA-N_3. The radiochemical purity was checked by radio TLC chromatogram and a percentage of value at Rf = 0.0–0.1



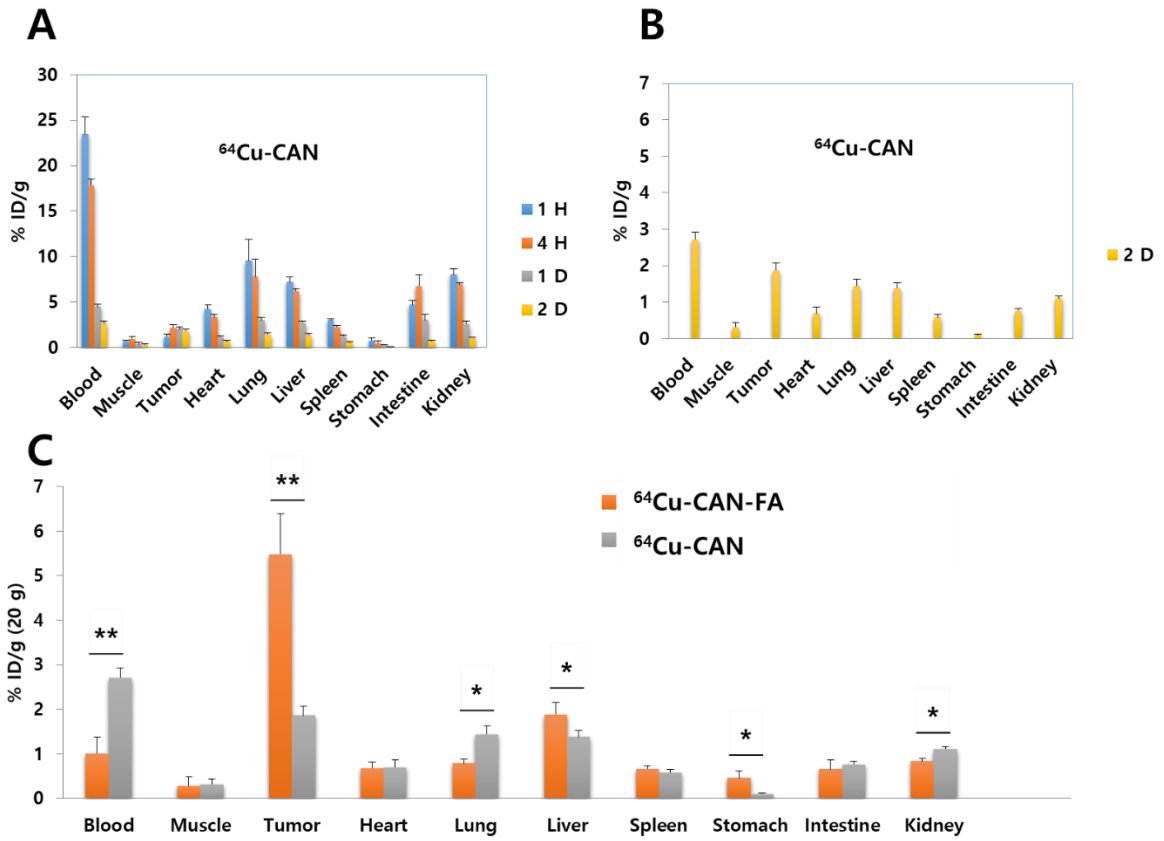
Supplementary Figure S5. Comparison of biodistribution of ^{64}Cu-CAN and ^{177}Lu-CAN. The image-based comparison of two different radioisotope-labeled CANs. (A) ^{64}Cu-labeled CAN, representative small animal PET image at 0, 4, 24, and 48 h post-injection. (B) ^{177}Lu-labeled CAN, representative nano SPECT/CT image at 0, 4, 24, and 48 h post-injection. (C and D) The graph shows similar biodistribution values between the two different radioisotope-labeled CANs. Both used the DOF of 5 (DOF5)



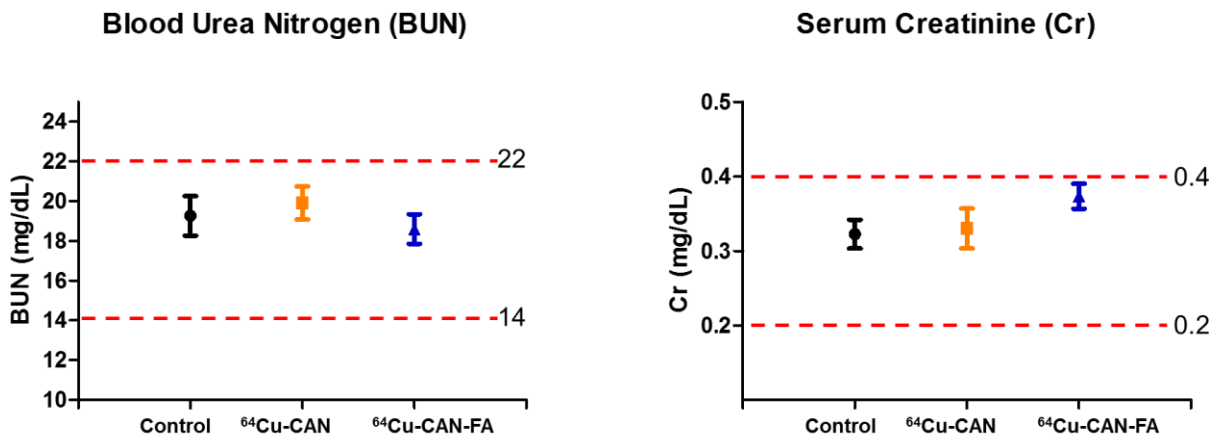
Supplementary Figure S6. Folate receptor antibody showed specific binding to KB but not PC3 cells



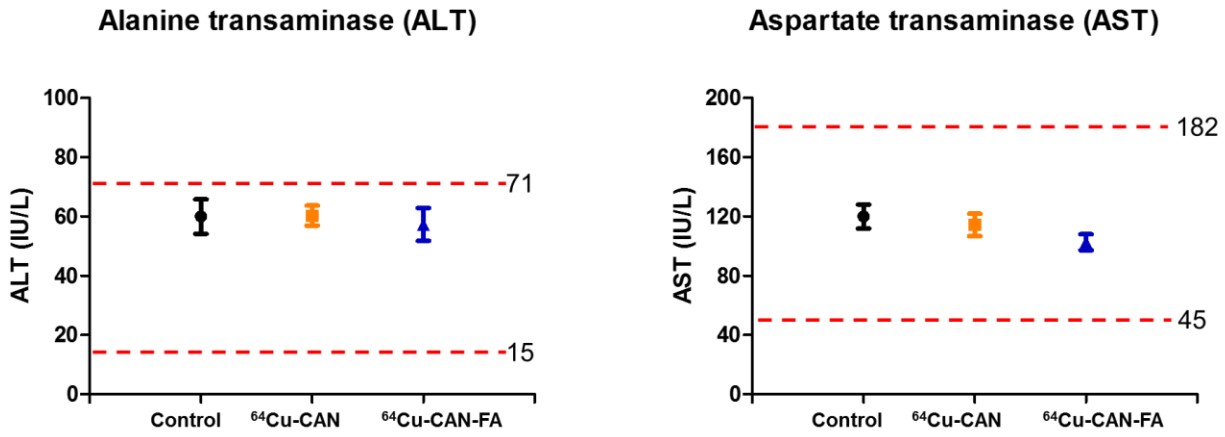
Supplementary Figure S7. Methotrexate injection study. (A) *In vivo* PET scans were obtained 10, 24, and 48 h post-injection of 64Cu-CAN-FA and methotrexate (n = 3). (B) The quantified uptakes were compared at 24 h post-injection with the ^{64}Cu-CAN-FA and ^{64}Cu-CAN-FA with methotrexate groups. The tumor uptake was significantly reduced in the ^{64}Cu-CAN-FA with methotrexate group than in the ^{64}Cu-CAN-FA group ($P < 0.01$). There was no significant difference in the liver and blood pool



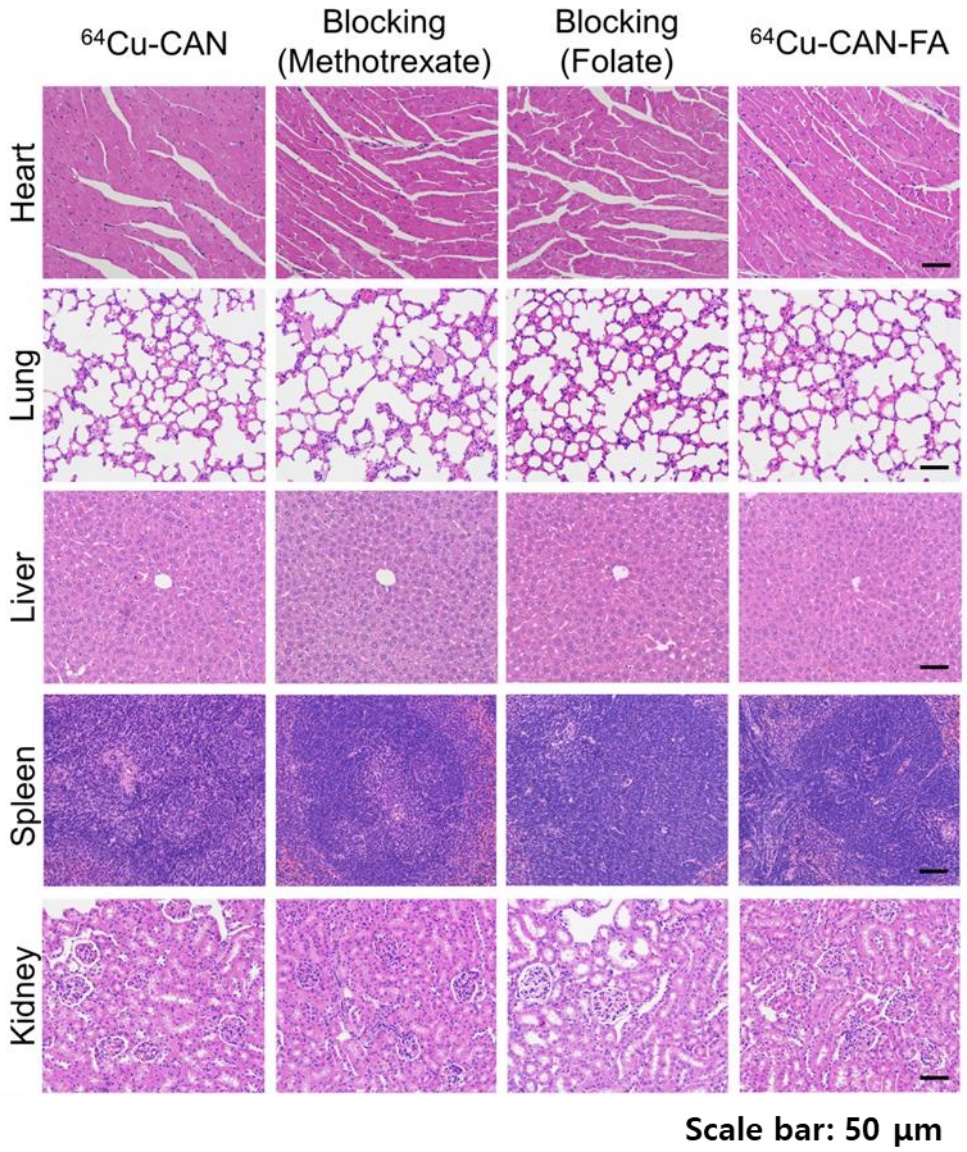
Supplementary Figure S8. (A, B) *Ex vivo* biodistribution results using ^{64}Cu-CAN in the KB model at 1, 4, 24, and 48 h after the injection. (C) Comparison between ^{64}Cu-CAN-FA (orange) and ^{64}Cu-CAN (gray) in KB model at 48 h after injection. *: $P < 0.05$, **: $P < 0.01$



Supplementary Figure S9. Blood urea nitrogen (BUN) and creatinine (Cr) in normal mice, 48 h after injection of saline as a control, ^{64}Cu-CAN, and ^{64}Cu-CAN-FA (n = 4). Dashed lines denote the normal ranges of parameters for normal mice



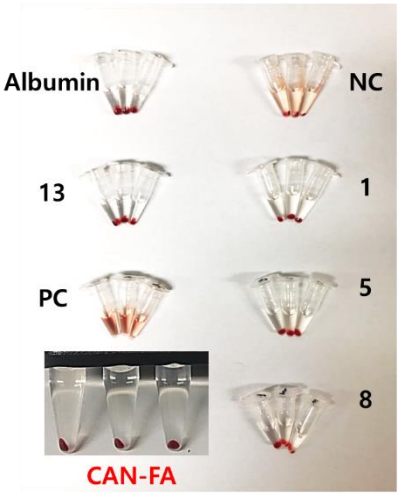
Supplementary Figure S10. Alanine transaminase (ALT) and aspartate transaminase (AST) in normal mice, 48 h after injection of saline as a control, ^{64}Cu-CAN, and ^{64}Cu-CAN-FA (n = 4). Dashed lines denote the normal ranges of parameters for normal mice



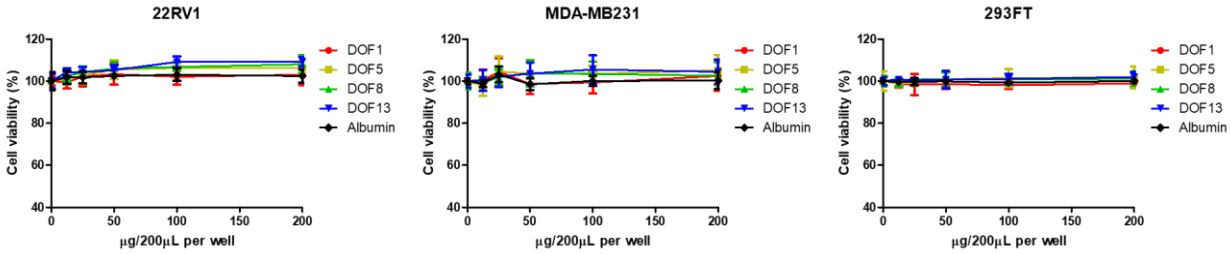
Supplementary Figure S11. Hematoxylin and eosin (H&E) staining of main organs and tumor sections. H&E showed no damage in normal tissues, including the heart, lungs, liver, spleen, or kidneys

Treatment Group		Optical density (O.D)	Hemolytic Potential (%)*
Negative control (Saline, NC)		0.160 ± 0.006	
Positive control (Triton-x 100, PC)		0.280 ± 0.002	
Albumin		0.132 ± 0.009	None
CAN	DOF1	0.138 ± 0.005	
	DOF5	0.156 ± 0.006	
	DOF8	0.158 ± 0.005	
	DOF13	0.162 ± 0.004	1.389
	CAN-FA	0.159 ± 0.005	None

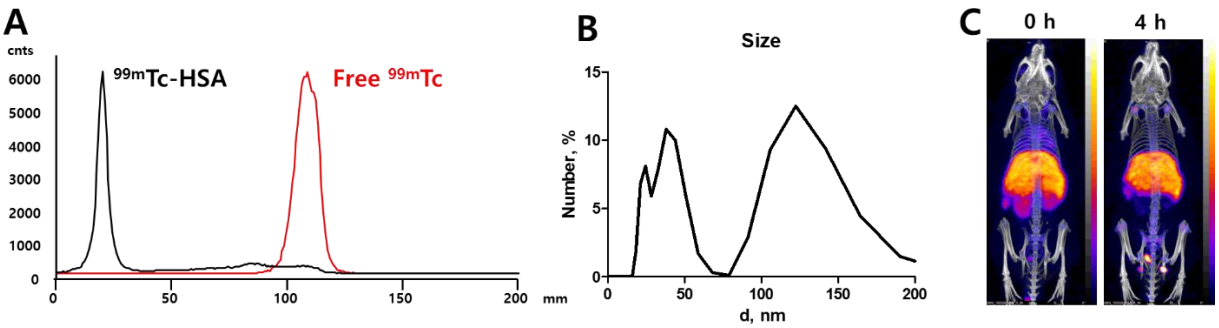
100(Treatment Group-NC) / (PC-NC)



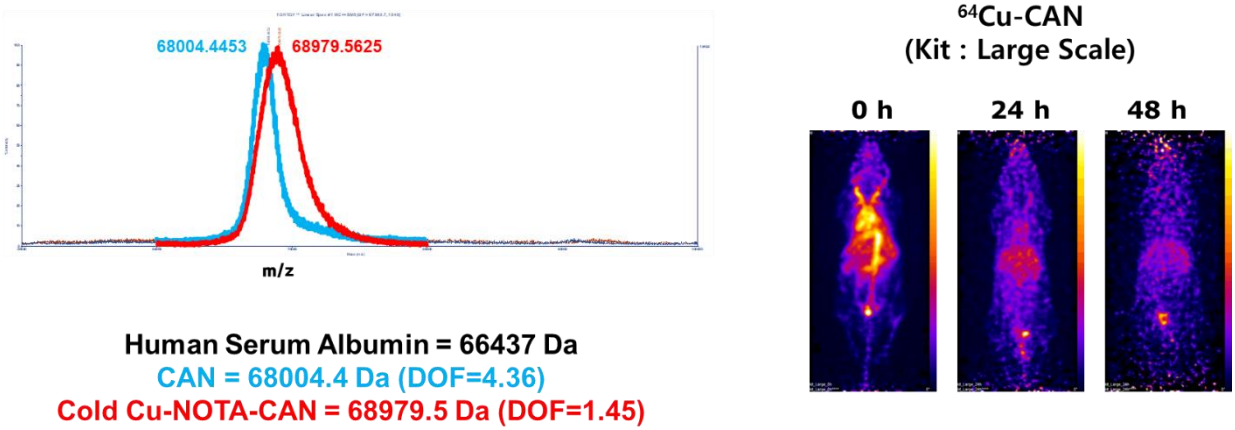
Supplementary Figure S12. Blood compatibility test. A value of over 5% hemolytic potential indicates that blood hemolysis occurred. No hemolysis was observed in any of the experimental groups



Supplementary Figure S13. Cell viability assay. No cytotoxic effects were observed in all 3 cell lines and >95% of the cells were viable



Supplementary Figure S14. Radiolabeling, size, and *in vivo* imaging of ^{99m}Tc-HSA. (A) Radio TLC chromatograms of conventionally prepared ^{99m}Tc-HSA. (B) Average hydrodynamic diameters. (C) Representative nano SPECT/CT image at 0 and 4 h after injection. As shown in the graph, the size was not uniform. The image shows the liver immediately after the injection



Supplementary Figure S15. MALDI-TOF result and *in vivo* Imaging of ^{64}Cu-CAN from large scale synthesis. The representative MALDI-TOF analysis results. Blue denotes CAN and red denotes cold Cu-labeled CAN. PET images were obtained by attaching isotopes to the CAN and are shown on the right

Supplementary Table S1. Table of UV-vis measurements for estimation of DOF

A

Reaction ratio	Peak intensity at 280 nm (A_{280})					Peak intensity at 309 nm (A_{309})				
	Set 1	Set 2	Set 3	Mean	SD	Set 1	Set 2	Set 3	Mean	SD
Rxn R1	0.092	0.165	0.169	0.142	0.043	0.035	0.056	0.059	0.05	0.013
Rxn R3	0.225	0.224	0.219	0.223	0.003	0.124	0.125	0.119	0.123	0.003
Rxn R6	0.282	0.281	0.292	0.285	0.006	0.194	0.193	0.2	0.196	0.004
Rxn R8	0.319	0.33	0.329	0.326	0.006	0.243	0.25	0.251	0.248	0.004
Rxn R11	0.385	0.372	0.402	0.386	0.015	0.317	0.302	0.335	0.318	0.017
Rxn R17	0.588	0.628	0.601	0.606	0.02	0.557	0.598	0.568	0.574	0.021
Rxn R23	0.728	0.729	0.71	0.722	0.011	0.714	0.717	0.696	0.709	0.011

B

Reaction ratio	A_{280}	Corrected A_{280} (A_{280c})	A_{309}	UV-based DOF (B)	MALDI TOF-based DOF (A)	B / A
Rxn R1	0.142	0.099	0.050	1.5	1.0	1.46
Rxn R3	0.223	0.116	0.123	3.1	3.4	0.92
Rxn R6	0.285	0.115	0.196	5.0	5.3	0.95
Rxn R8	0.326	0.111	0.248	6.6	6.8	0.97
Rxn R11	0.386	0.110	0.318	8.5	8.4	1.01
Rxn R17	0.606	0.107	0.574	15.8	13.9	1.13
Rxn R23	0.722	0.107	0.709	19.6	17.3	1.13

Supplementary Table S2. Quantified organ uptakes of CANs with different DOFs measured from PET imaging (n = 4 for each group)

DOF1	Blood pool (%ID/g)		Liver (%ID/g)		Muscle (%ID/g)	
	Average	SD	Average	SD	Average	SD
0 h	29.26	0.67	16.91	0.76	2.79	0.90
4 h	18.13	0.56	12.85	0.90	2.35	0.16
24 h	8.52	0.04	8.59	1.28	1.80	0.05
48 h	3.61	0.07	4.78	1.21	1.27	0.22

DOF5	Blood pool (%ID/g)		Liver (%ID/g)		Muscle (%ID/g)	
	Average	SD	Average	SD	Average	SD
0 h	31.27	2.97	14.16	0.54	1.83	0.04
4 h	19.62	1.50	14.82	0.61	2.14	0.32
24 h	8.81	0.80	11.19	0.16	1.81	0.53
48 h	3.41	0.21	5.53	0.48	0.83	0.09

DOF8	Blood pool (%ID/g)		Liver (%ID/g)		Muscle (%ID/g)	
	Average	SD	Average	SD	Average	SD
0 h	31.25	1.04	17.25	1.50	2.19	0.38
4 h	18.52	0.05	17.89	1.81	1.96	0.10
24 h	7.55	0.05	18.96	0.00	1.71	0.25
48 h	2.46	0.15	8.59	1.30	0.58	0.04

DOF13	Blood pool (%ID/g)		Liver (%ID/g)		Muscle (%ID/g)	
	Average	SD	Average	SD	Average	SD
0 h	29.27	0.85	18.27	0.91	1.89	0.18
4 h	13.48	0.31	23.00	0.86	2.34	0.15
24 h	4.40	0.27	28.13	0.72	0.98	0.03
48 h	1.15	0.10	9.33	0.15	0.63	0.19

Supplementary Table S3. Table of pharmacokinetics of CAN with different DOFs

	$T_{1/2\alpha}$ (h)		$T_{1/2\beta}$ (h)		AUC (%ID/g x h)	
	Average	**SD**	**Average**	**SD**	**Average**	**SD**
DOF1	1.26	0.19	20.64	0.52	493.30	3.50
DOF5	0.82	0.24	18.28	1.68	519.57	41.59
DOF8	0.83	0.09	16.42	1.03	468.00	3.10
DOF13	1.32	0.08	16.10	0.34	319.70	1.80