Supplementary Information

In vivo Demonstration of an Active Tumor Pretargeting Approach with Peptide Nucleic Acid Bioconjugates as Complementary System

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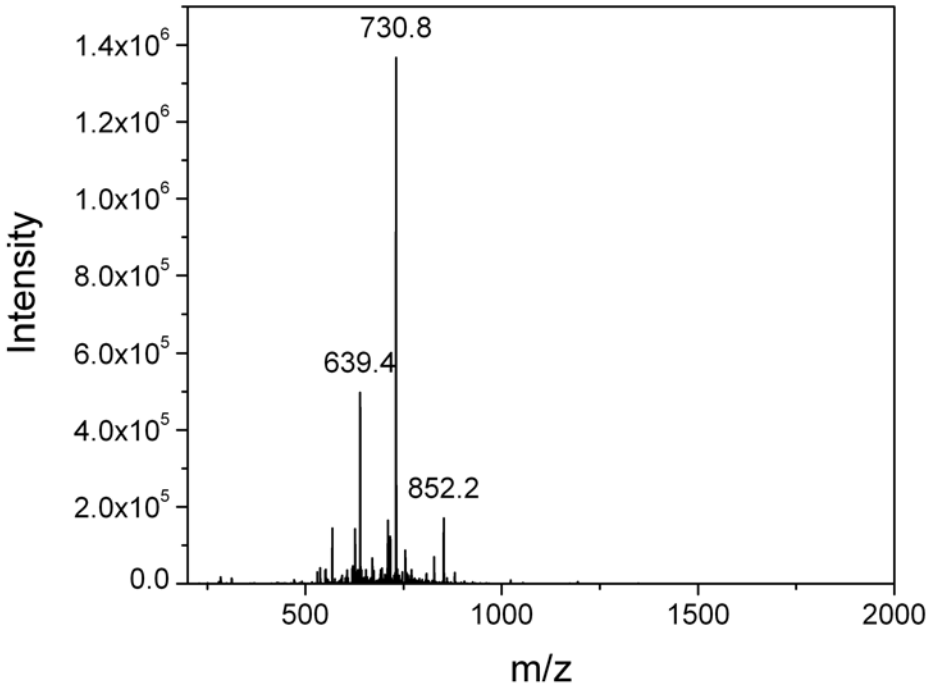

Figure S1. ESI-MS spectrum of **Cys-PNA**.

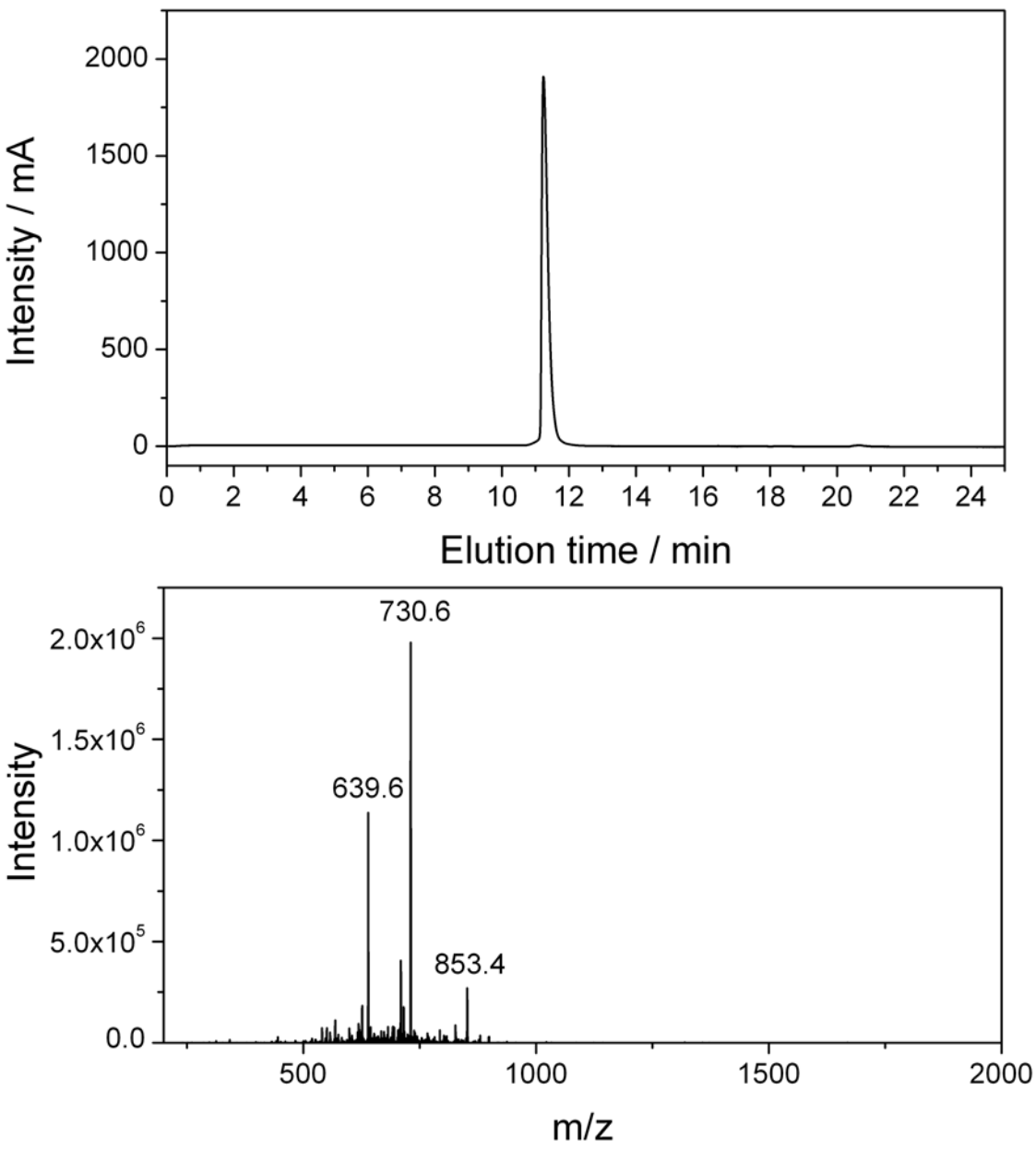

Figure S2. LC-MS spectrum of **Cys-PNA**.

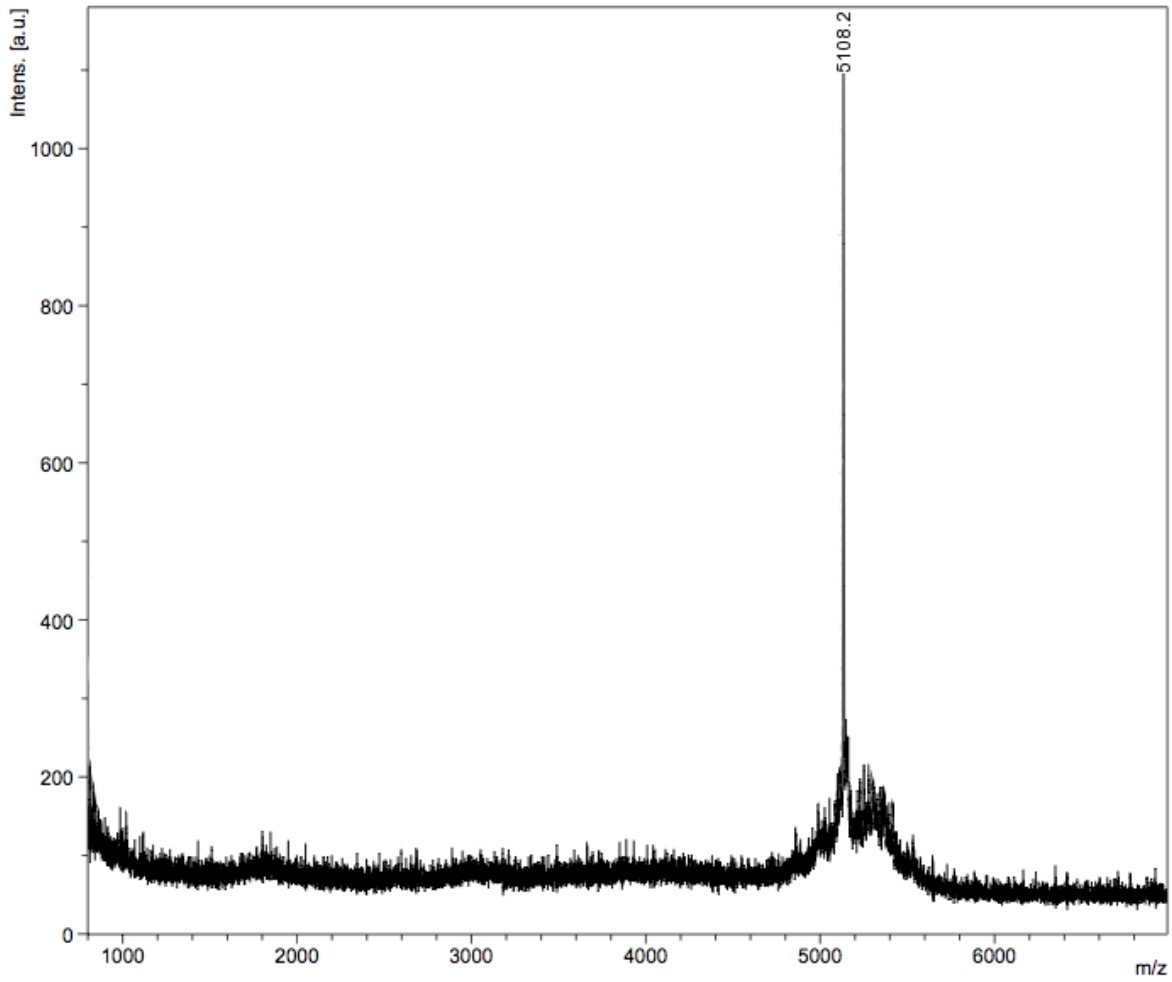

Figure S3. MALDI-TOF spectrum of **Cys-PNA**.

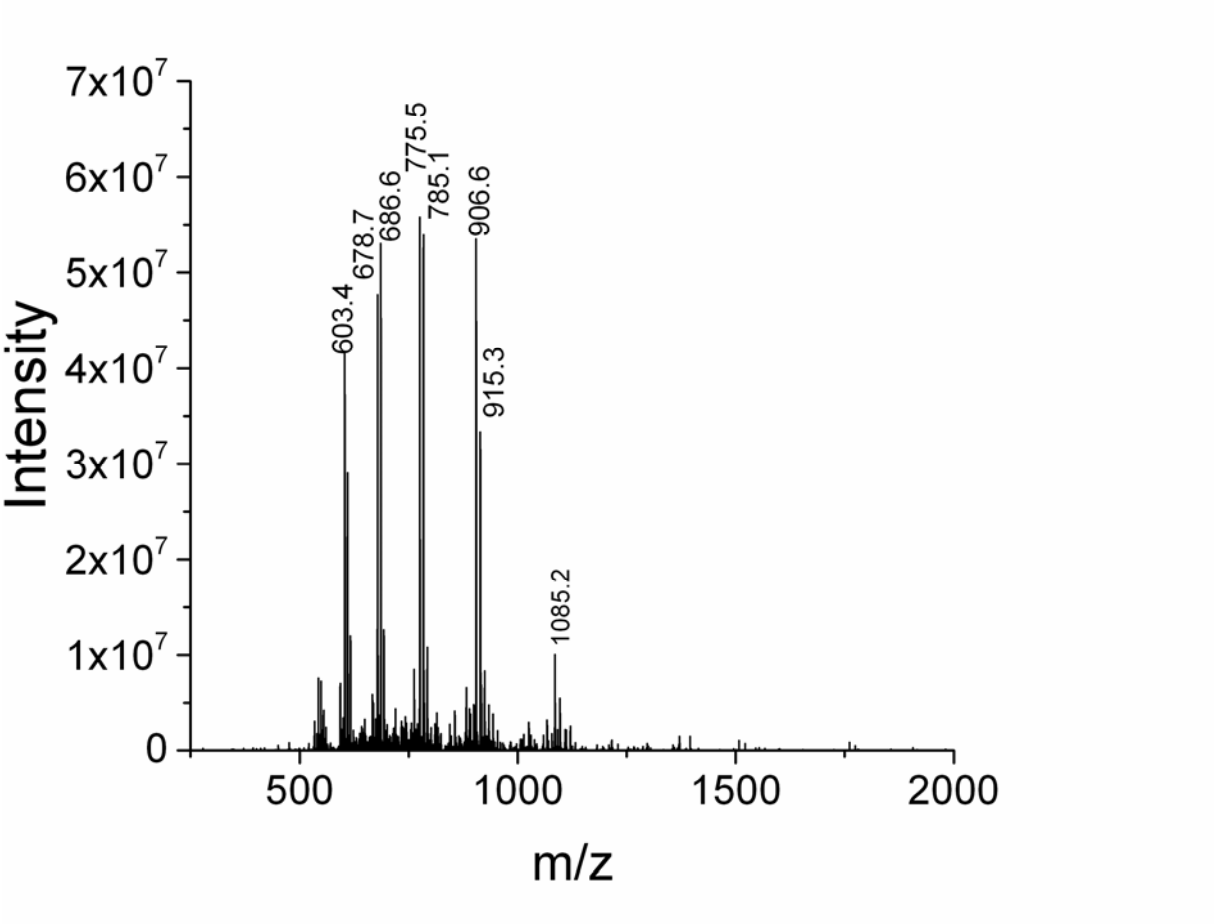

Figure S4. ESI-MS spectrum of **Dpa-PNA.**

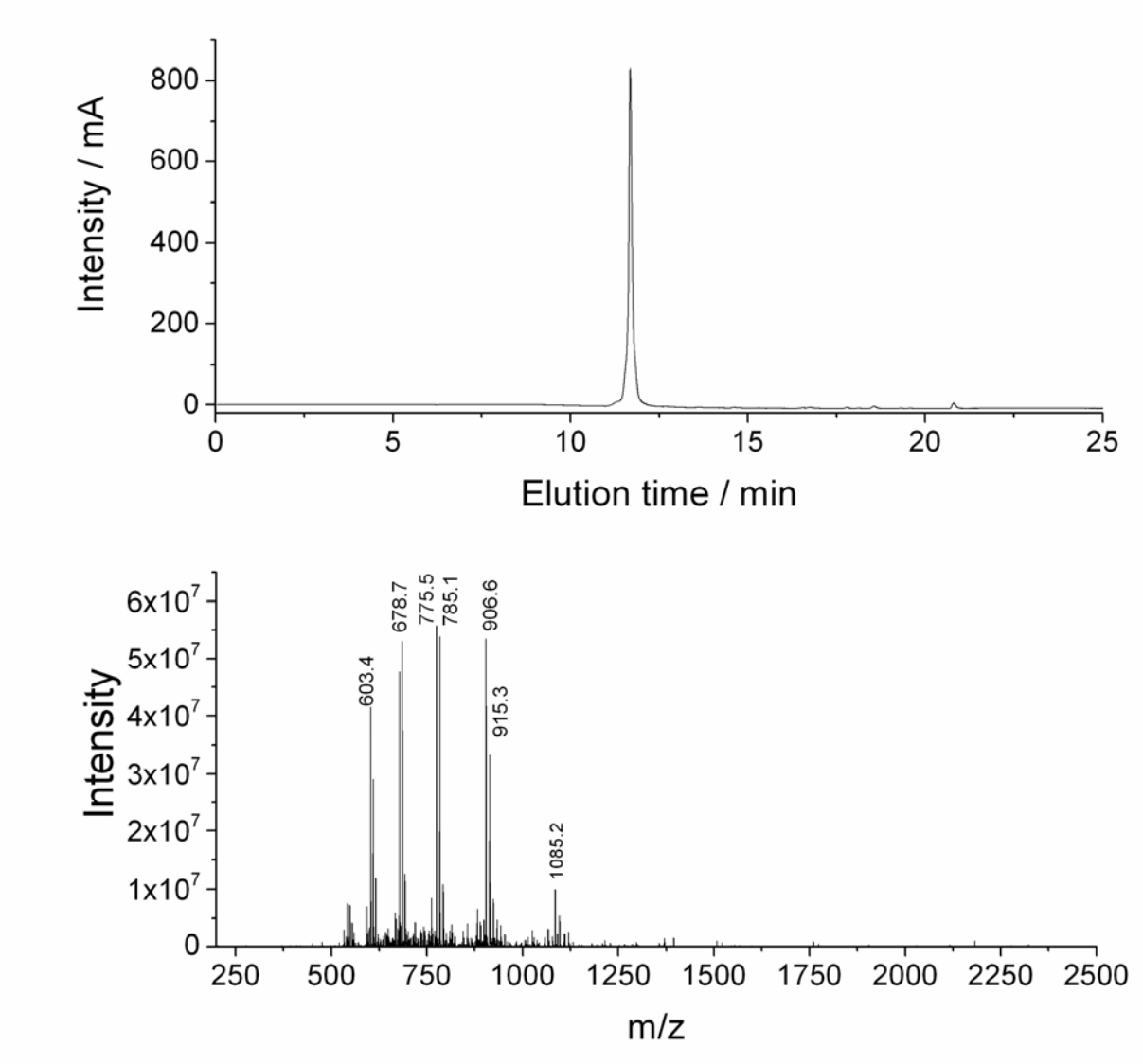

Figure S5. LC-MS spectrum of **Dpa-PNA.**

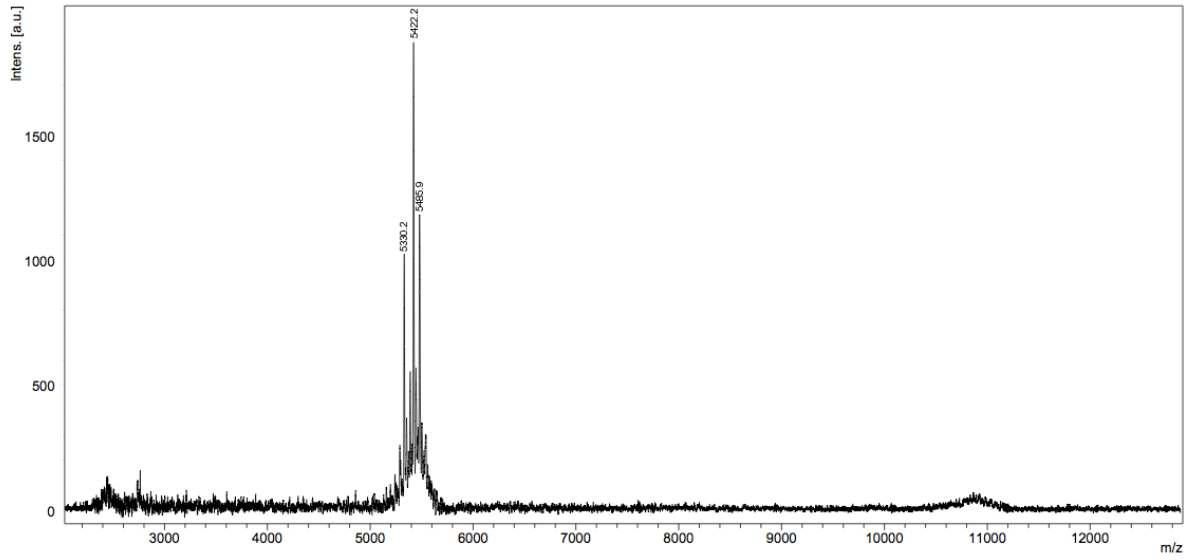

Figure S6. MALDI-TOF spectrum of **Dpa-PNA.**

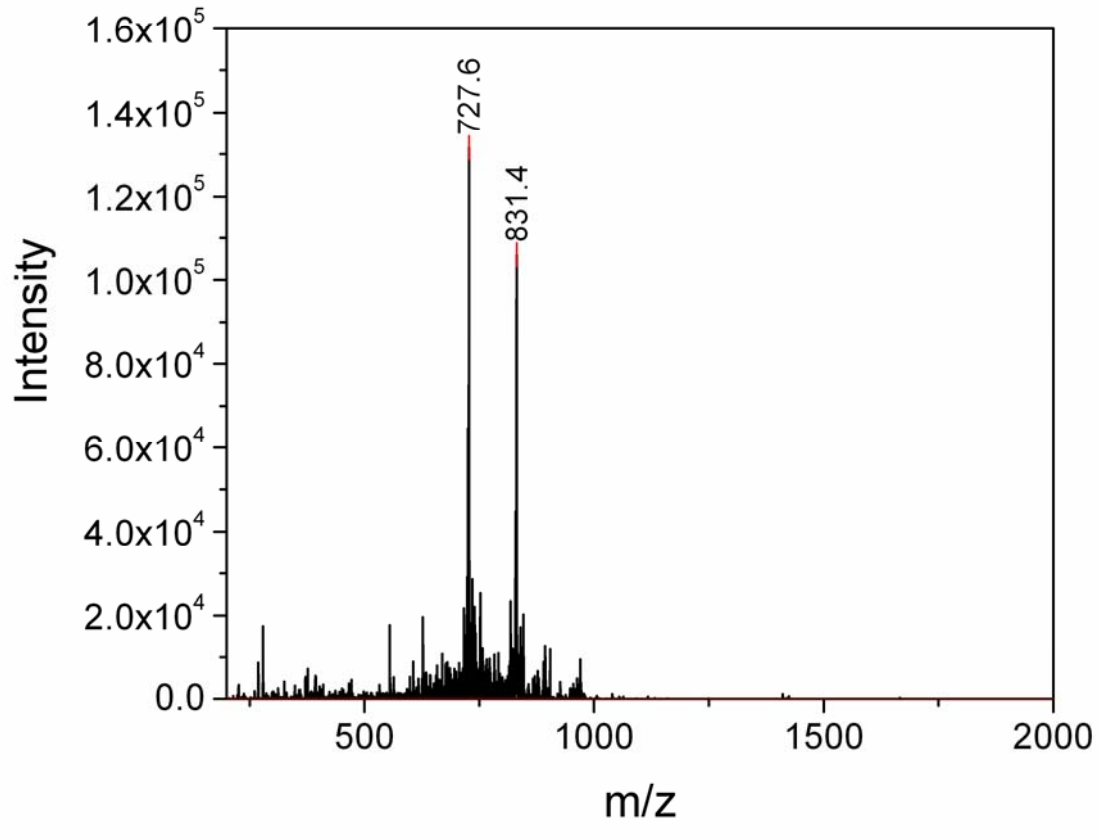

Figure S7. ESI-MS spectrum of **Dpa-Cys-PNA.**

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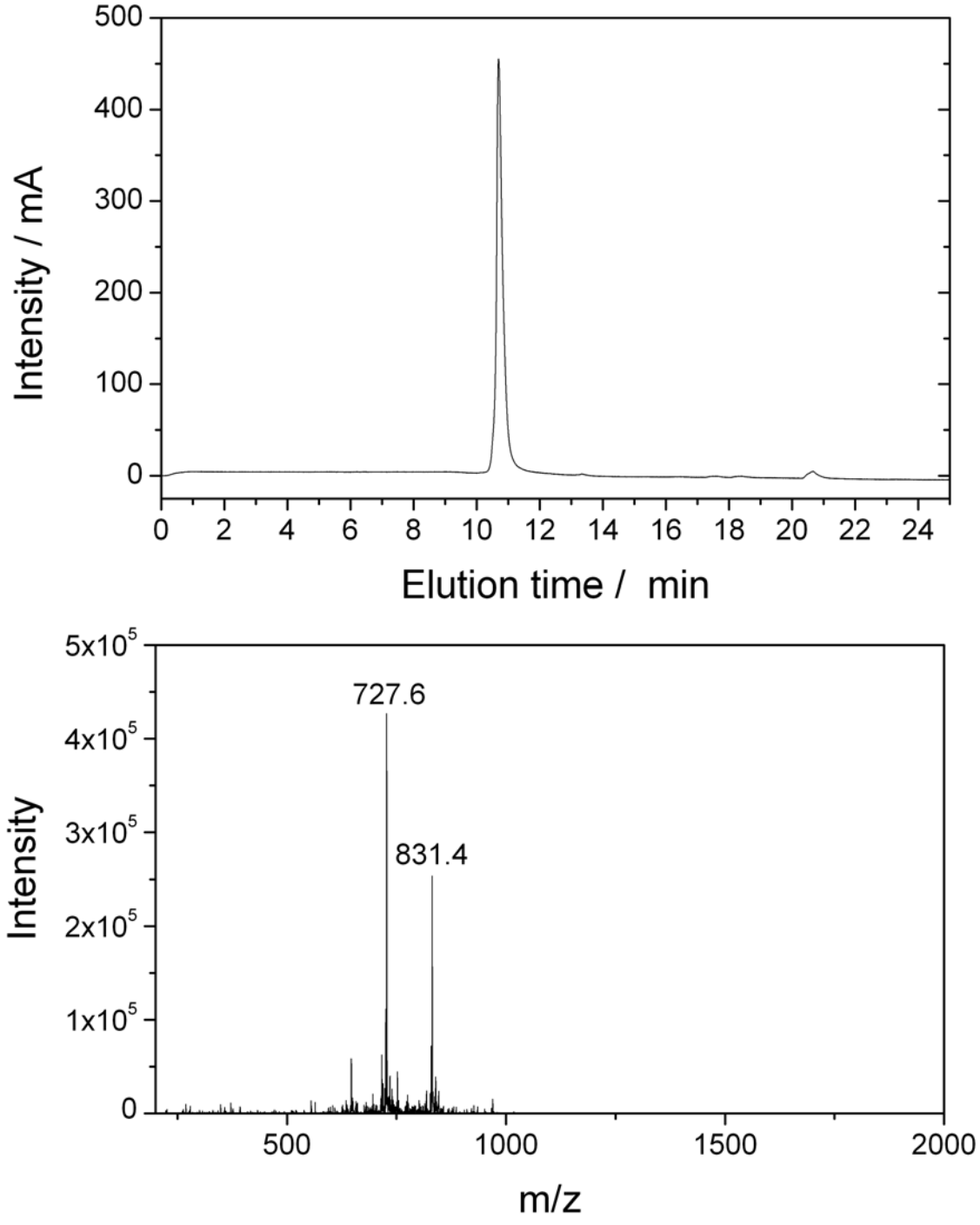

Figure S8. LC-MS spectrum of **Dpa-Cys-PNA.**

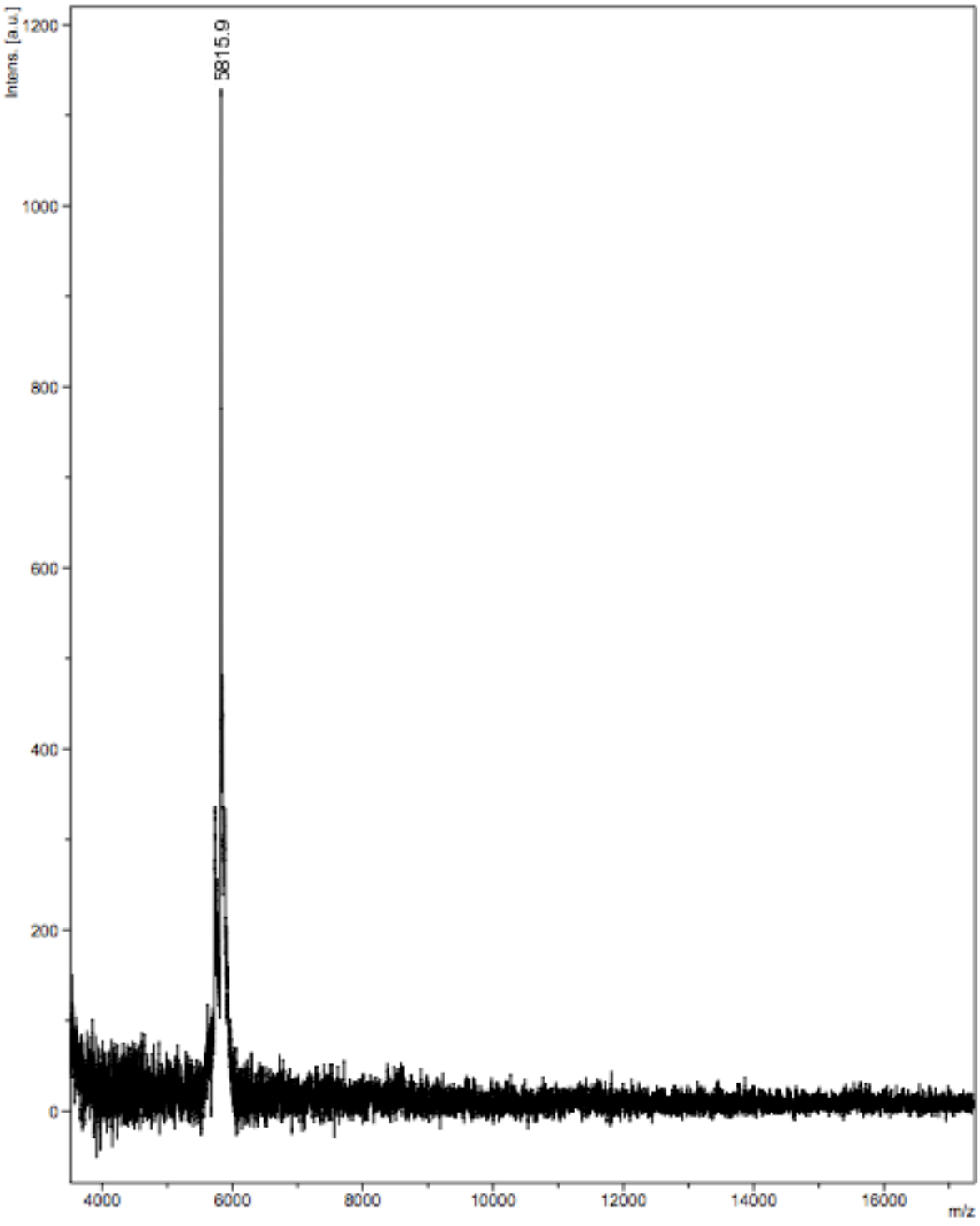

Figure S9. MALDI-TOF spectrum of **Dpa-Cys-PNA.**

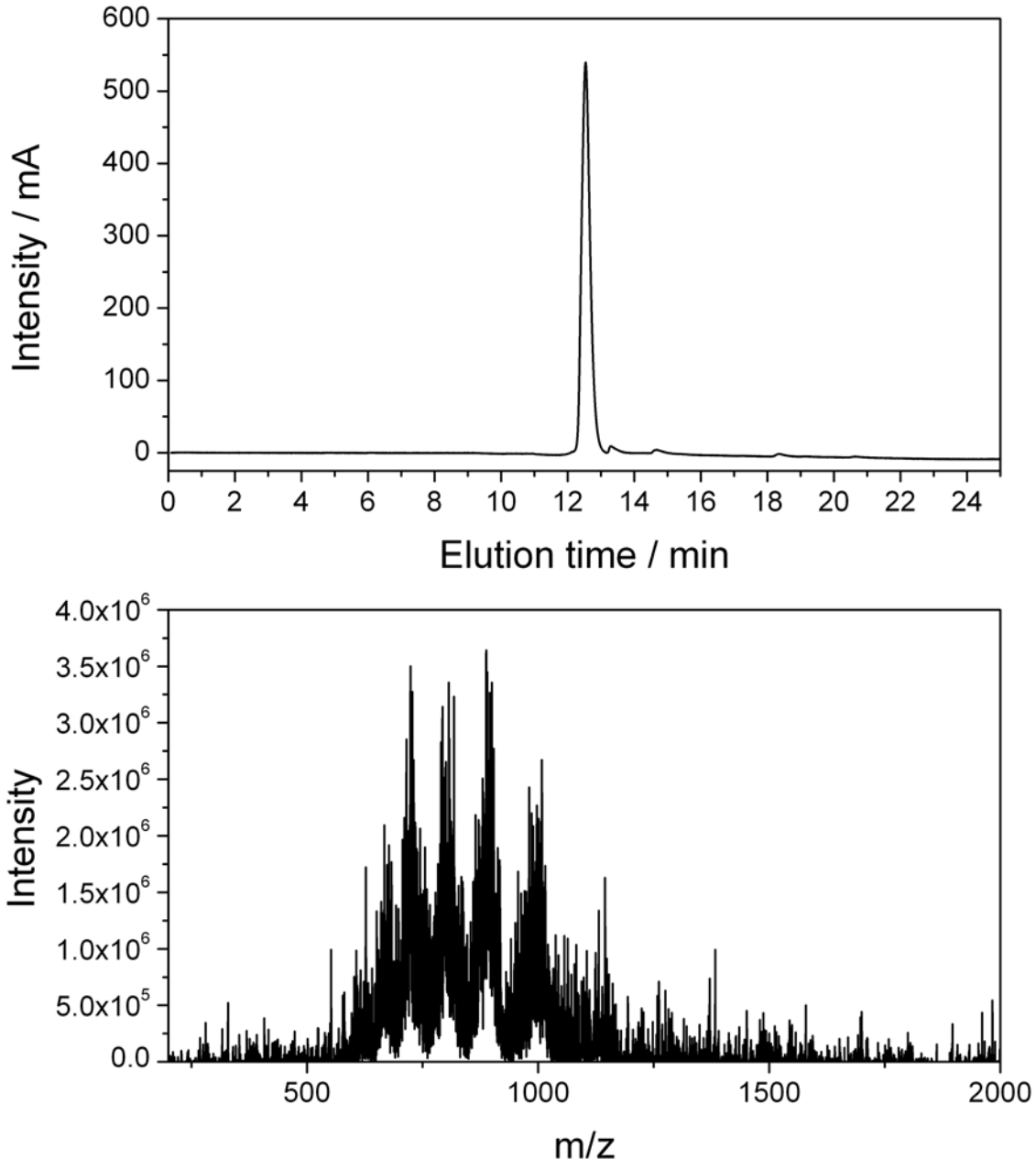

Figure S10. LC-MS spectrum of **Dpa-(Cys-PEG$_{2kDa}$)-PNA**.

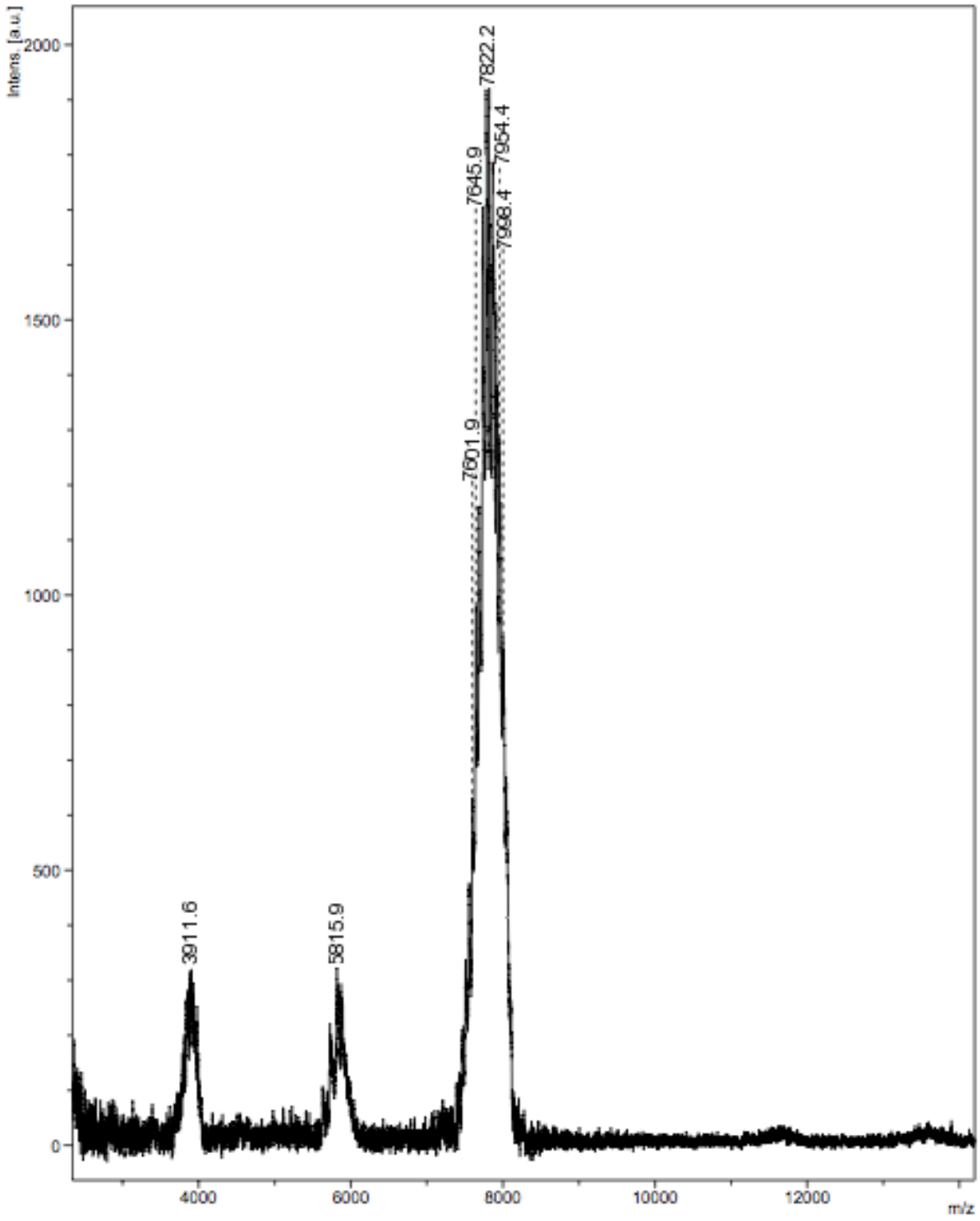

Figure S11. MALDI-TOF spectrum of **Dpa-(Cys-PEG$_{2kDa}$)-PNA**.

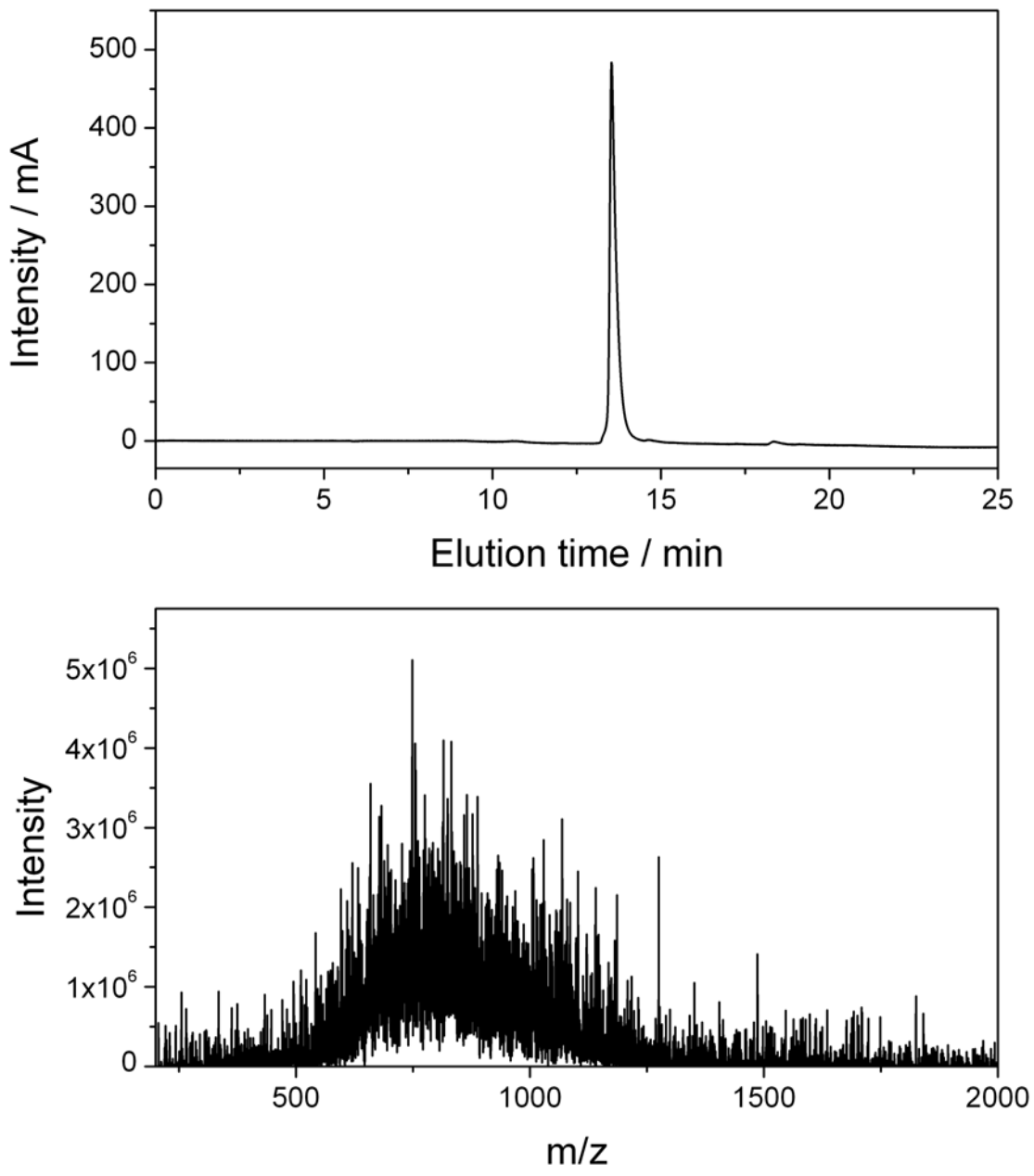

Figure S12. LC-MS spectrum of **Dpa-(Cys-PEG$_{10kDa}$)-PNA**.

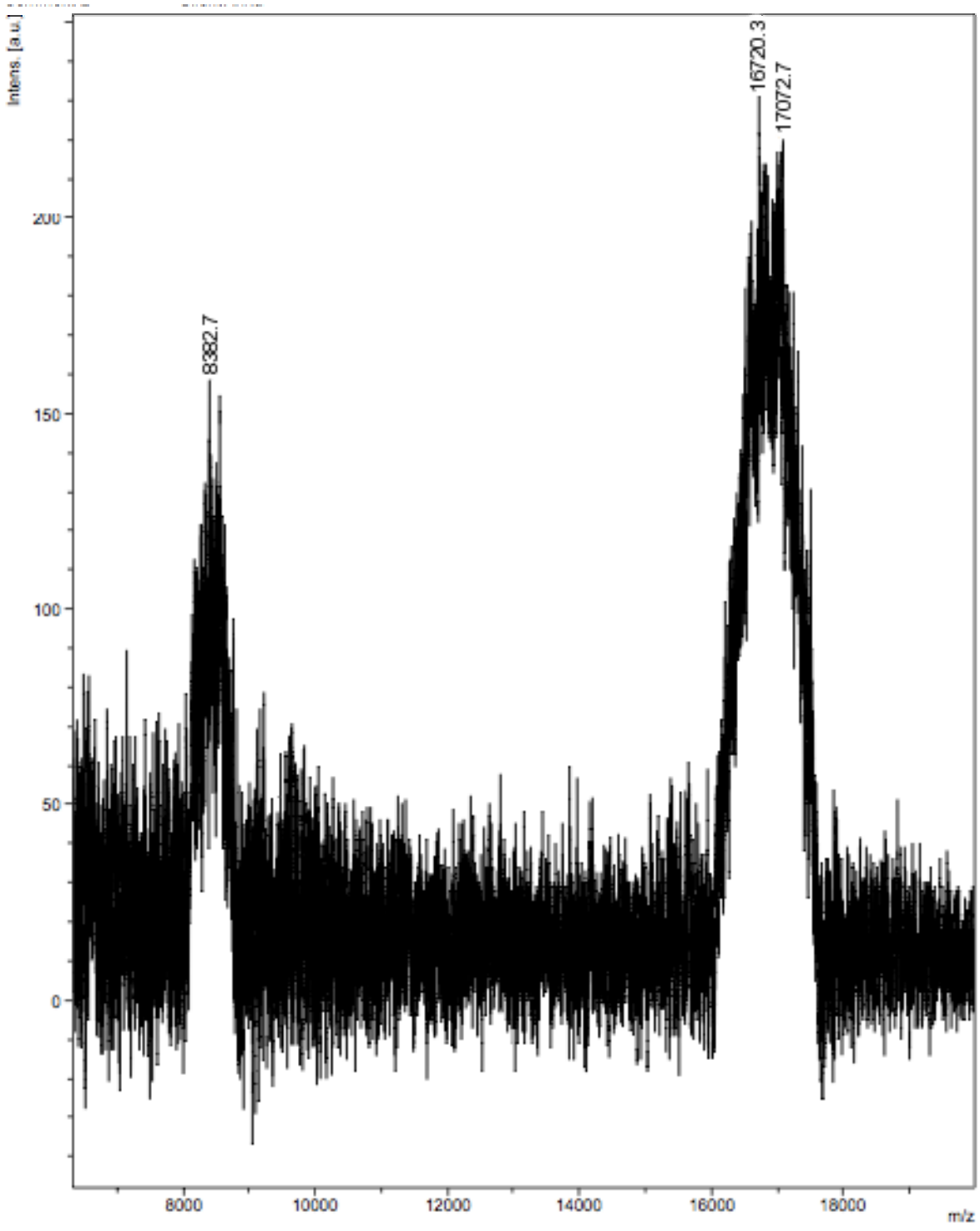

Figure S13. MALDI-TOF spectrum of **Dpa-(Cys-PEG$_{10kDa}$)-PNA**.

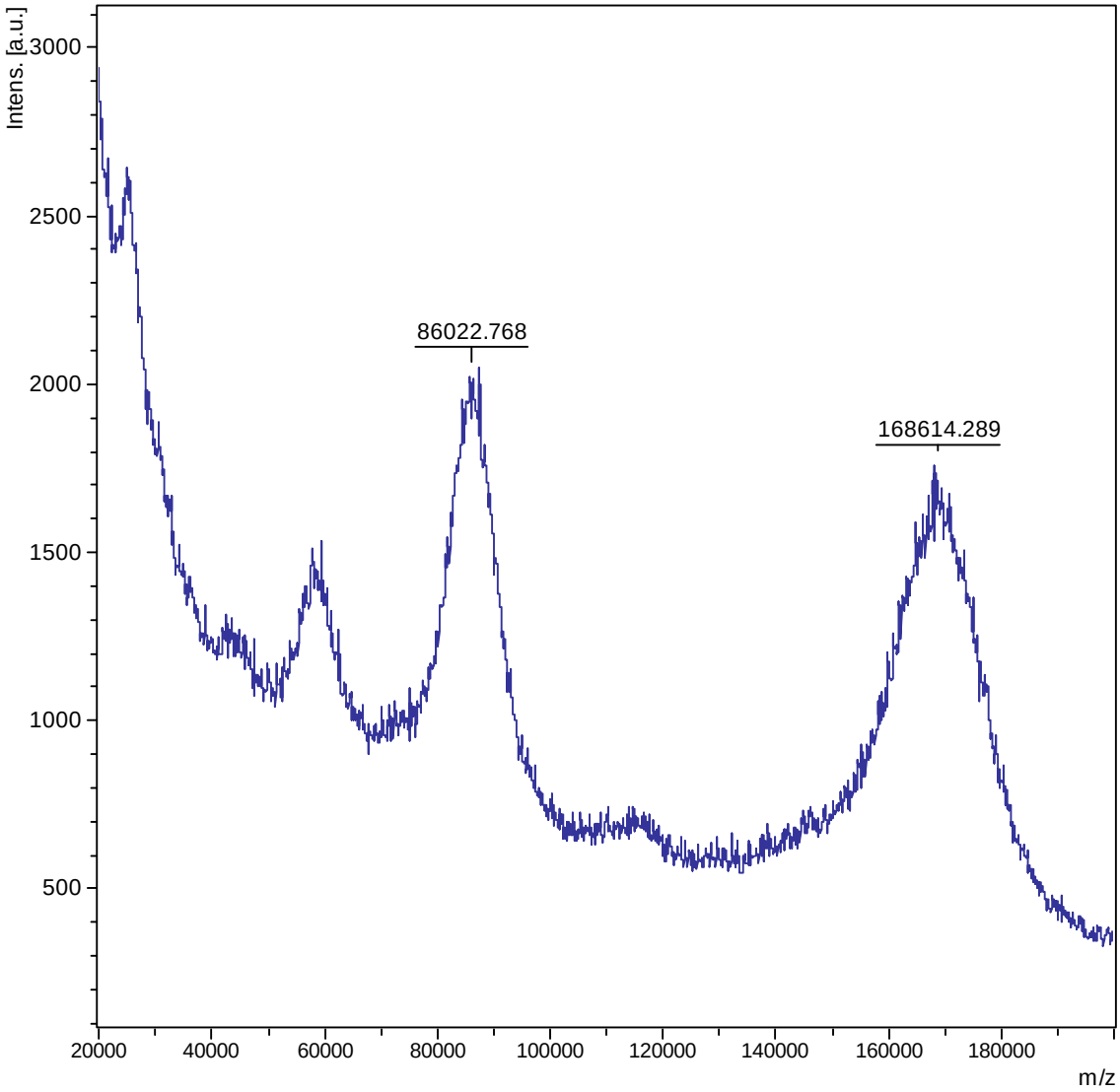

Figure S14. MALDI-TOF spectrum of **$(NOTA)_3$-C225-$(Cys\text{-}PNA)_2$.**

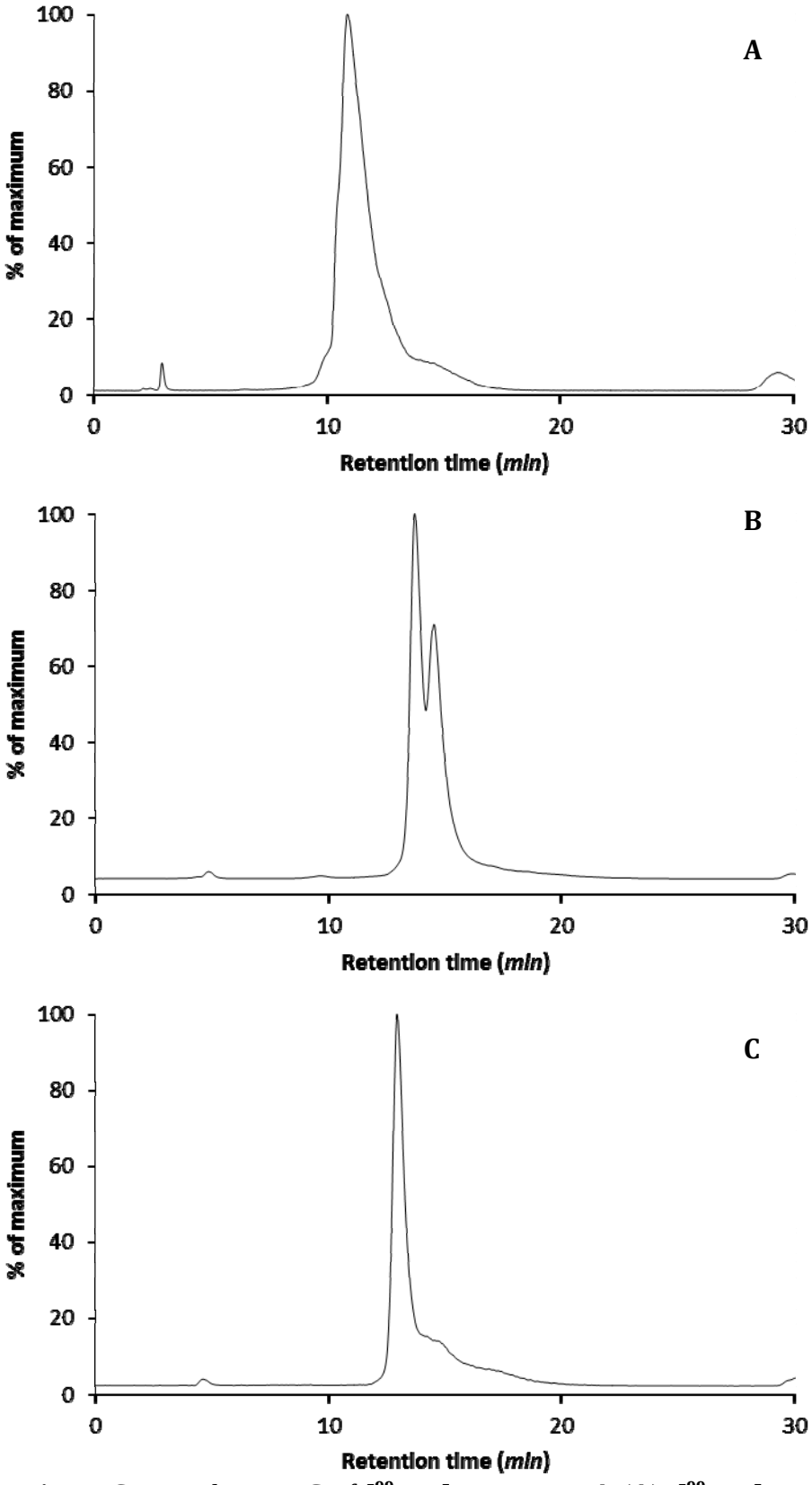

Figure S15. Radio-HPLC of **[^{99m}Tc]Tc-Dpa-PNA (A)**, **[^{99m}Tc]Tc-Dpa-(Cys-PEG$_{2kDa}$)-PNA (B)** and **[^{99m}Tc]Tc-Dpa-(Cys-PEG$_{10kDa}$)-PNA (C)**.

Table S1. Melting temperatures (T_M) for three complementary 17-mer PNA systems differing in their degree of PEGylation (0/2/10 kDa). Values were measured as duplicates.

Complementary PNA system	**T_M [°C]**
Cys-c-PNA & **Dpa-PNA**	76.5 ± 1.2
Cys-c-PNA & **Dpa-(Cys-PEG$_{2kDa}$)-PNA**	76.8 ± 1.4
Cys-c-PNA & **Dpa-(Cys-PEG$_{10kDa}$)-PNA**	76.1 ± 1.3

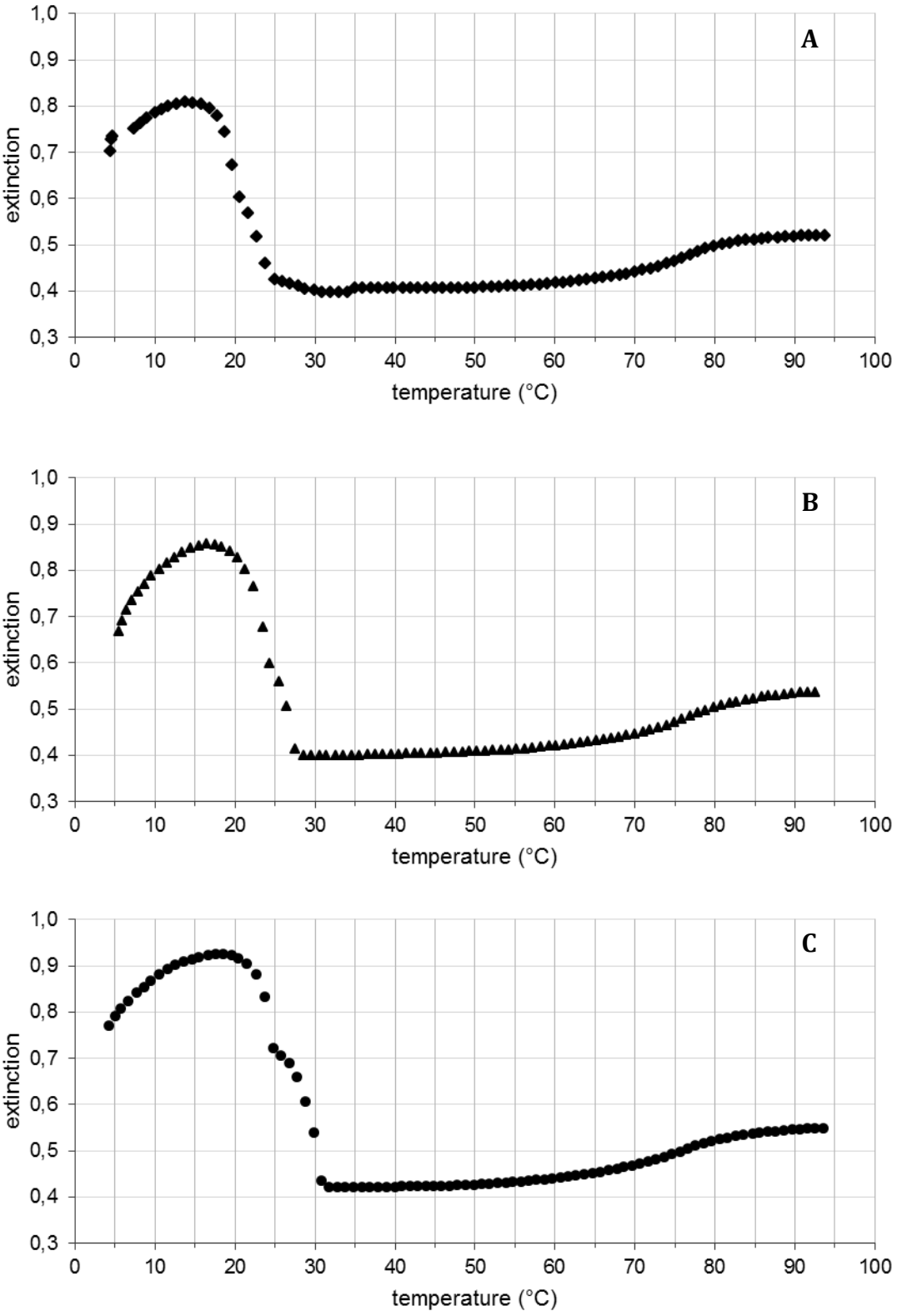

Figure S16. Melting curves of **Cys-c-PNA** & **Dpa-PNA (A)**, **Cys-c-PNA** & **Dpa-(Cys-PEG$_{2kDa}$)-PNA (B)** and **Cys-c-PNA** & **Dpa-(Cys-PEG$_{10kDa}$)-PNA (C)**.

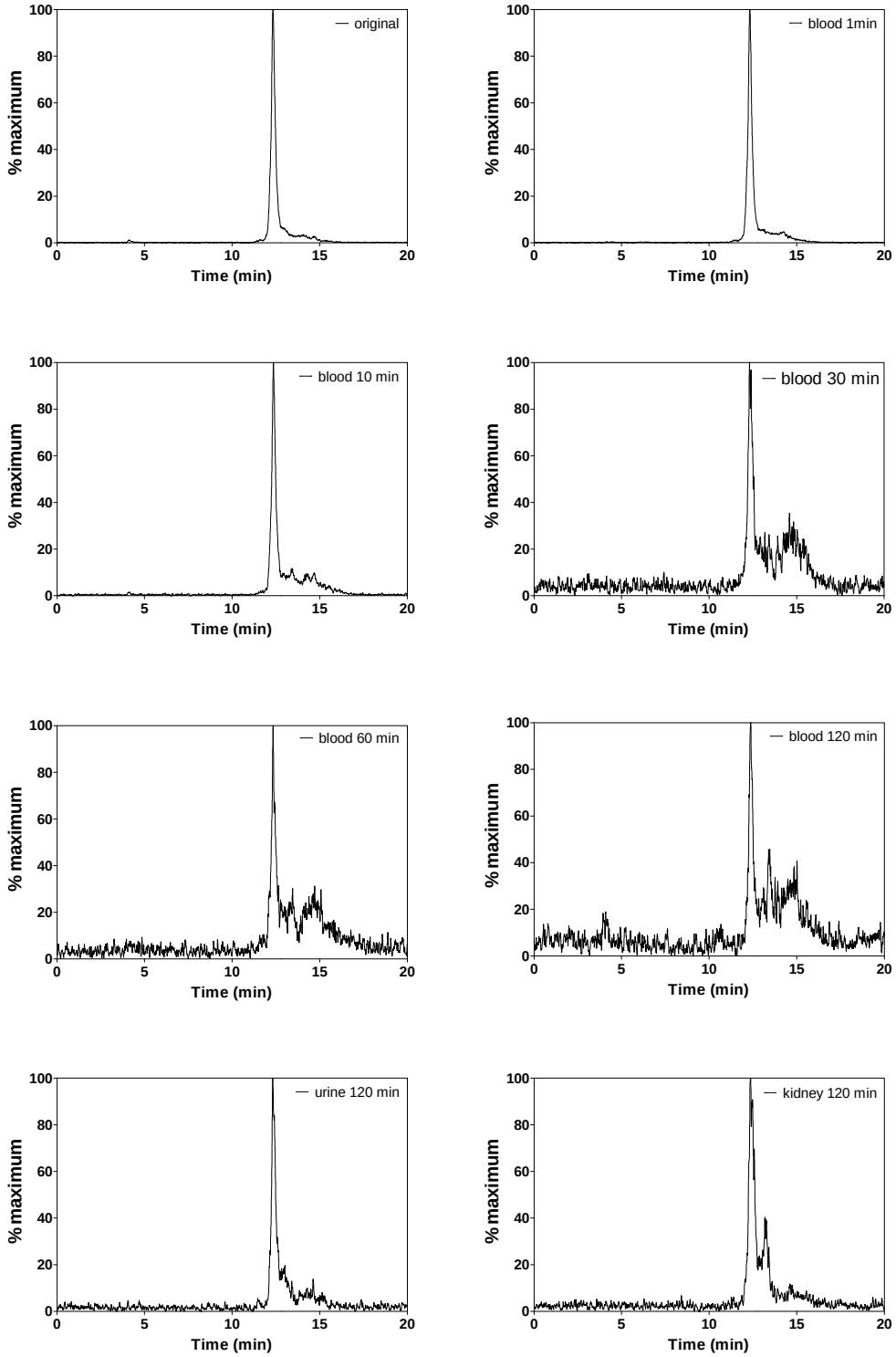

Figure S17. Radio HPLC of the **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA** (original), in rat arterial blood plasma after various times, and in extracts of kidneys and urine at 120 min after injection (the noisy signal around 16 minutes is due to low activity of the samples).

Table S2. Biodistribution of **[^{99m}Tc](Tc-Dpa)-PNA** (337±94 kBq), **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{2kDa}$)-PNA** (325±25 kBq), and **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA** (300±32 kBq) in Wistar rats after single intravenous injection at 5 min and 60 min p.i. Data are presented as %ID mean±SD (number of animals) and P values from t-test between the study groups

Compound	**[^{99m}Tc](Tc-Dpa)-PNA**		**[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{2kDa}$)-PNA**		**[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA**		0k-2k	0k-2k	0k-10k	0k-10k	2k-10k	2k-10k
Time p.i. (min)	5	60	5	60	5	60	5 min	60 min	5 min	60 min	5 min	60 min
%ID	mean	mean	mean	mean	mean	mean						
Brain	0.04 ± 0.01 (12)	0.01 ± 0 (11)	0.11 ± 0.02 (8)	0.02 ± 0.00 (8)	0.06 ± 0.01 (8)	0.02 ± 0.02 (8)	0.0000	0.0001	0.0002	0.0947	0.0001	0.7017
Pancreas	0.10 ± 0.06 (12)	0.03 ± 0.03 (11)	0.11 ± 0.01 (8)	0.15 ± 0.30 (8)	0.08 ± 0.01 (8)	0.05 ± 0.04 (8)	0.7038	0.2233	0.2885	0.1947	0.0002	0.4107
Spleen	0.14 ± 0.06 (12)	0.06 ± 0.01 (11)	0.15 ± 0.03 (8)	0.12 ± 0.08 (8)	0.22 ± 0.08 (8)	0.18 ± 0.08 (8)	0.7518	0.0239	0.0222	0.0003	0.0374	0.1886
Adrenals	0.02 ± 0.00 (12)	0.00 ± 0.00 (11)	0.03 ± 0.01 (8)	0.01 ± 0.00 (8)	0.03 ± 0.01 (8)	0.01 ± 0.01 (8)	0.0001	0.0002	0.0030	0.0301	0.2741	0.3425
Kidneys	11.2 ± 1.29 (12)	13.0 ± 1.66 (11)	12.7 ± 2.01 (8)	10.6 ± 2.18 (8)	13.0 ± 1.92 (8)	10.2 ± 1.30 (8)	0.0728	0.0216	0.0342	0.0019	0.8149	0.6971
Heart	0.22 ± 0.03 (12)	0.04 ± 0.02 (11)	0.38 ± 0.14 (8)	0.08 ± 0.03 (8)	0.29 ± 0.04 (8)	0.06 ± 0.02 (8)	0.0025	0.0037	0.0006	0.0648	0.1466	0.1336
Lung	0.70 ± 0.13 (12)	0.15 ± 0.05 (11)	1.09 ± 0.16 (8)	0.32 ± 0.13 (8)	1.84 ± 0.47 (8)	1.75 ± 1.14 (8)	0.0000	0.0017	0.0000	0.0004	0.0012	0.0050
Thymus	0.17 ± 0.05 (12)	0.03 ± 0.01 (11)	0.18 ± 0.03 (8)	0.04 ± 0.01 (8)	0.18 ± 0.04 (8)	0.04 ± 0.01 (8)	0.6824	0.1060	0.8910	0.1558	0.7711	0.5819
Thyroid	0.07 ± 0.01 (12)	0.02 ± 0.01 (11)	0.16 ± 0.22 (8)	0.04 ± 0.03 (8)	0.07 ± 0.02 (8)	0.03 ± 0.03 (8)	0.1786	0.0995	0.9229	0.3412	0.2761	0.6210
Hard. Gl.	0.08 ± 0.01 (12)	0.02 ± 0.00 (11)	0.09 ± 0.01 (8)	0.02 ± 0.01 (8)	0.08 ± 0.02 (8)	0.03 ± 0.01 (8)	0.2546	0.0038	0.4426	0.0334	0.8037	0.7293
Liver	5.45 ± 0.53 (12)	5.04 ± 0.54 (11)	5.45 ± 0.92 (8)	3.44 ± 1.21 (8)	5.64 ± 1.55 (8)	5.19 ± 1.86 (8)	0.9993	0.0018	0.7085	0.8067	0.7794	0.0547
Femur	0.30 ± 0.04 (12)	0.09 ± 0.02 (11)	0.32 ± 0.02 (8)	0.10 ± 0.02 (8)	0.28 ± 0.04 (8)	0.09 ± 0.02 (8)	0.3266	0.1690	0.4031	0.8401	0.0941	0.1956
Testicles	0.33 ± 0.13 (12)	0.12 ± 0.02 (11)	0.47 ± 0.08 (8)	0.22 ± 0.04 (8)	0.51 ± 0.22 (8)	0.19 ± 0.02 (8)	0.0210	0.0000	0.0403	0.0000	0.6060	0.1221
Intestine#	2.54 ± 0.63 (12)	3.24 ± 1.74 (11)	3.23 ± 0.26 (8)	5.17 ± 2.59 (7)	2.94 ± 0.27 (8)	6.76 ± 5.42 (8)	0.0123	0.0930	0.1244	0.0733	0.0600	0.5202
Stomach#	0.62 ± 0.12 (12)	2.51 ± 1.54 (11)	0.96 ± 0.41 (8)	2.75 ± 2.97 (7)	0.89 ± 0.14 (8)	4.31 ± 5.90 (8)	0.0198	0.8310	0.0003	0.3727	0.6961	0.5668
Carcass	54.4 ± 6.45 (12)	17.8 ± 2.9 (11)	53.5 ± 12.5 (8)	15.6 ± 2.26 (8)	64.4 ± 9.66 (8)	12.8 ± 2.24 (8)	0.8534	0.0969	0.0161	0.0012	0.0893	0.0376
Urine calc.##	22.0 ± 6.81 (12)	57.5 ± 4.26 (11)	18.5 ± 11.5 (8)	59.6 ± 5.79 (8)	8.15 ± 8.92 (8)	57.9 ± 13.21 (8)	0.4309	0.3891	0.0016	0.9211	0.0821	0.7594

with content, ## urine was calculated from the injected activity and recovery of all organs and carcass

Table S3. Biodistribution of **[^{99m}Tc](Tc-Dpa)-PNA** (337±94 kBq), **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{2kDa}$)-PNA** (325±25 kBq), and **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA** (300±32 kBq) in Wistar rats after single intravenous injection at 5 min and 60 min p.i. Data are presented as SUV mean±SD (number of animals) and P values from t-test between the study groups.

Compound	**[^{99m}Tc](Tc-Dpa)-PNA**		**[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{2kDa}$)-PNA**		**[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA**		0k-2k	0k-2k	0k-10k	0k-10k	2k-10k	2k-10k
Time p.i. (min)	5	60	5	60	5	60	5 min	5 min	60 min	60 min	5 min	60 min
SUV	mean	mean	mean	mean	mean	mean						
Blood	1.35 ± 0.21 (12)	0.24 ± 0.1 (11)	1.15 ± 0.12 (8)	0.24 ± 0.06 (8)	2.01 ± 0.35 (8)	0.34 ± 0.10 (8)	0.0001	0.0521	0.0277	0.8568	0.0000	0.0522
BAT	0.44 ± 0.04 (12)	0.08 ± 0.02 (11)	0.62 ± 0.07 (8)	0.17 ± 0.07 (8)	0.49 ± 0.07 (8)	0.15 ± 0.04 (8)	0.0405	0.0001	0.0000	0.0003	0.0046	0.3584
Hair & skin	0.80 ± 0.06 (12)	0.24 ± 0.1 (11)	0.93 ± 0.08 (8)	0.24 ± 0.02 (8)	0.86 ± 0.18 (8)	0.27 ± 0.07 (8)	0.3095	0.4714	0.0007	0.8967	0.3266	0.1888
Brain	0.04 ± 0.01 (12)	0.01 ± 0.00 (11)	0.04 ± 0.00 (8)	0.02 ± 0.02 (8)	0.07 ± 0.01 (8)	0.01 ± 0.00 (8)	0.0000	0.0064	0.9357	0.2175	0.0001	0.6622
Pancreas	0.53 ± 0.29 (12)	0.14 ± 0.15 (11)	0.35 ± 0.04 (8)	0.26 ± 0.23 (8)	0.51 ± 0.05 (8)	0.61 ± 1.16 (8)	0.9769	0.2284	0.1548	0.2189	0.0000	0.4466
Spleen	0.60 ± 0.30 (12)	0.22 ± 0.04 (11)	0.92 ± 0.27 (8)	0.70 ± 0.31 (8)	0.60 ± 0.12 (8)	0.54 ± 0.43 (8)	0.9215	0.0373	0.0304	0.0002	0.0137	0.4235
Adrenals	0.57 ± 0.11 (12)	0.14 ± 0.04 (11)	0.80 ± 0.23 (8)	0.32 ± 0.19 (8)	0.82 ± 0.15 (8)	0.25 ± 0.07 (8)	0.0006	0.0007	0.0118	0.0110	0.8535	0.3944
Kidneys	11.6 ± 1.53 (12)	13.1 ± 1.90 (11)	12.0 ± 1.33 (8)	9.29 ± 1.04 (8)	11.31 ± 1.74 (8)	9.75 ± 2.47 (8)	0.6211	0.0056	0.6869	0.0001	0.4263	0.6561
WAT	0.67 ± 0.73 (12)	0.13 ± 0.22 (11)	1.38 ± 1.46 (8)	0.17 ± 0.1 (8)	0.94 ± 0.61 (8)	0.23 ± 0.15 (8)	0.3749	0.3425	0.1698	0.6938	0.4692	0.4164
Muscle	0.39 ± 0.16 (12)	0.05 ± 0.01 (11)	0.35 ± 0.03 (8)	0.09 ± 0.04 (8)	0.30 ± 0.08 (8)	0.13 ± 0.09 (8)	0.2140	0.0212	0.5652	0.0183	0.1676	0.2966
Heart	0.57 ± 0.06 (12)	0.10 ± 0.03 (11)	0.64 ± 0.05 (8)	0.13 ± 0.03 (8)	0.81 ± 0.19 (8)	0.17 ± 0.05 (8)	0.0019	0.0047	0.0353	0.1301	0.0479	0.0950
Lung	1.06 ± 0.17 (12)	0.22 ± 0.06 (11)	2.39 ± 0.64 (8)	2.15 ± 1.27 (8)	1.41 ± 0.23 (8)	0.42 ± 0.15 (8)	0.0016	0.0016	0.0000	0.0002	0.0020	0.0030
Thymus	0.47 ± 0.09 (12)	0.10 ± 0.02 (11)	0.46 ± 0.07 (8)	0.10 ± 0.01 (8)	0.45 ± 0.05 (8)	0.12 ± 0.04 (8)	0.7261	0.0953	0.9181	0.7126	0.7956	0.1672
Hard. Gl.	0.58 ± 0.08 (12)	0.12 ± 0.03 (11)	0.58 ± 0.17 (8)	0.18 ± 0.14 (8)	0.59 ± 0.08 (8)	0.17 ± 0.04 (8)	0.6080	0.0072	0.8788	0.1759	0.8870	0.7768
Liver	0.89 ± 0.22 (12)	0.85 ± 0.25 (11)	1.12 ± 0.39 (8)	0.98 ± 0.39 (8)	1.15 ± 0.19 (8)	0.73 ± 0.28 (8)	0.0293	0.3722	0.1681	0.4097	0.8543	0.1917
Femur	0.55 ± 0.03 (12)	0.16 ± 0.02 (11)	0.45 ± 0.01 (8)	0.14 ± 0.03 (8)	0.51 ± 0.06 (8)	0.17 ± 0.04 (8)	0.0631	0.8277	0.0000	0.0541	0.0152	0.1546
Testicles	0.25 ± 0.12 (12)	0.09 ± 0.01 (11)	0.34 ± 0.13 (8)	0.13 ± 0.02 (8)	0.34 ± 0.08 (8)	0.16 ± 0.03 (8)	0.1033	0.0000	0.1403	0.0006	0.9169	0.1295

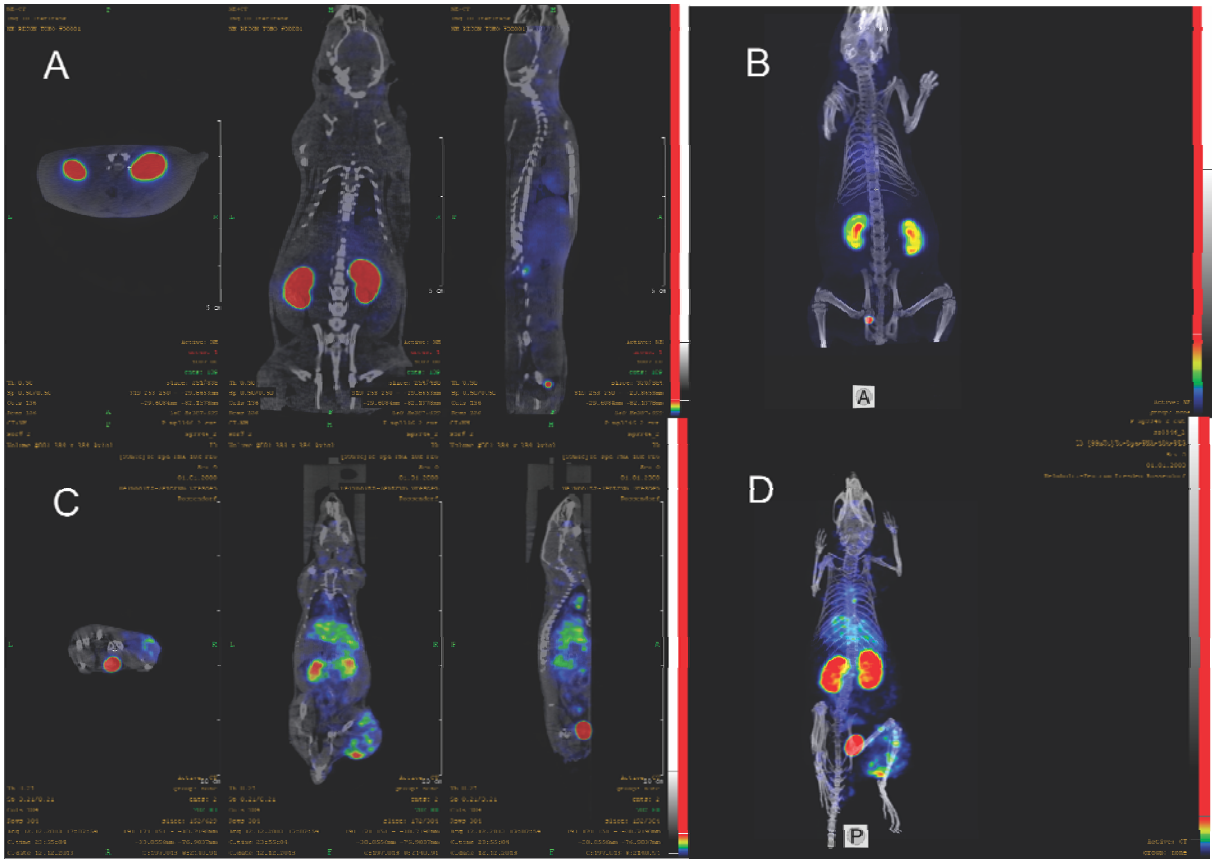

Figure S18. SPECT/CT comparison of the biodistribution of **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA** in rat and mouse. Upper images: Orthogonal (A) (transaxial, coronal, sagittal) sections and (B) maximum intensity projection of **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA** (56 MBq) in a Wistar rat (3 h p.i.). Lower images: Orthogonal (C) (transaxial, coronal, sagittal) sections and (D) maximum intensity projection of **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA** (40 MBq) in a NMRI nu/nu A431 tumor bearing mouse (3 h p.i.). The radiotracer **[^{99m}Tc](Tc-Dpa)-(Cys-PEG$_{10kDa}$)-PNA** was applied 24 h after injection of 4 nmol **(NOTA)$_3$-C225-Cys-c-PNA**.