

# **SPECT/NIRF Dual Modality Imaging for Detection of Intraperitoneal Colon Tumor with an Avidin/Biotin Pretargeting System**

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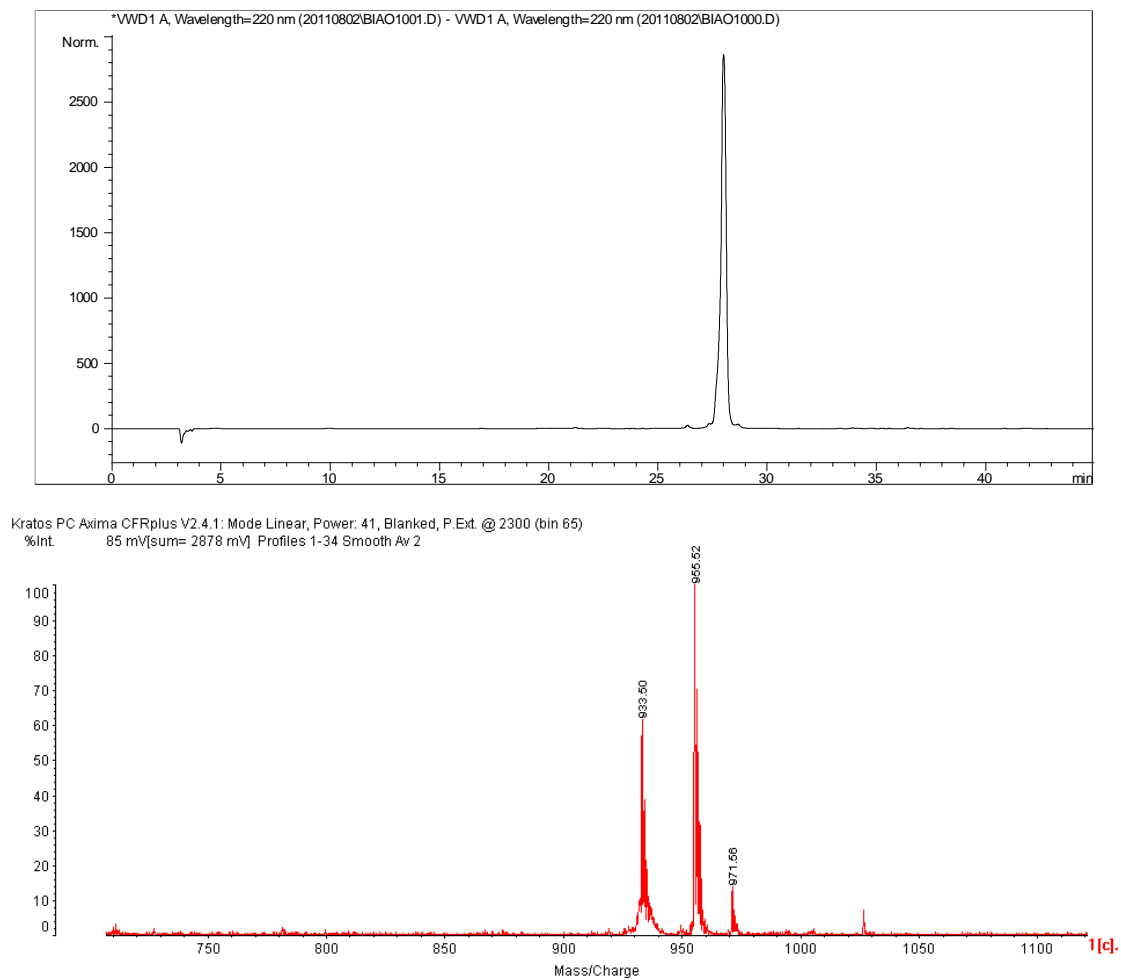
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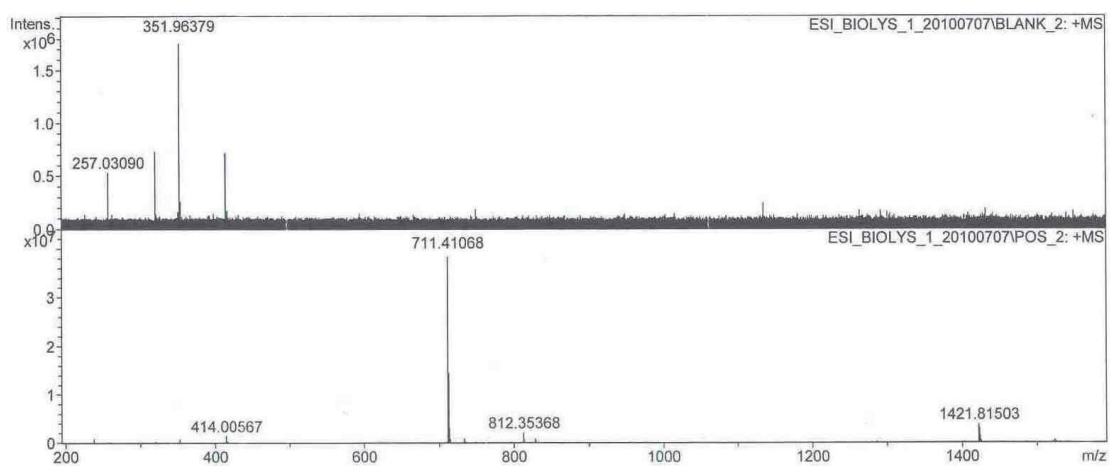
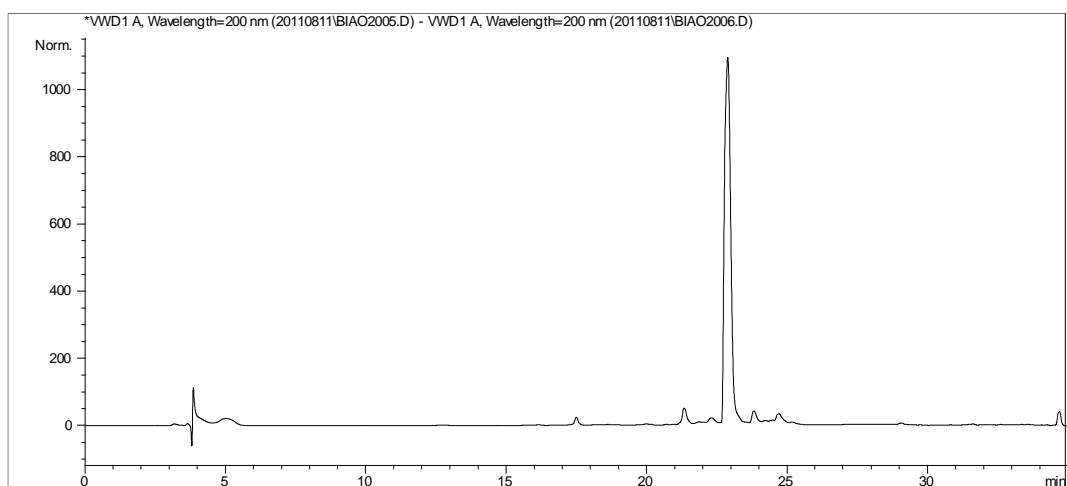
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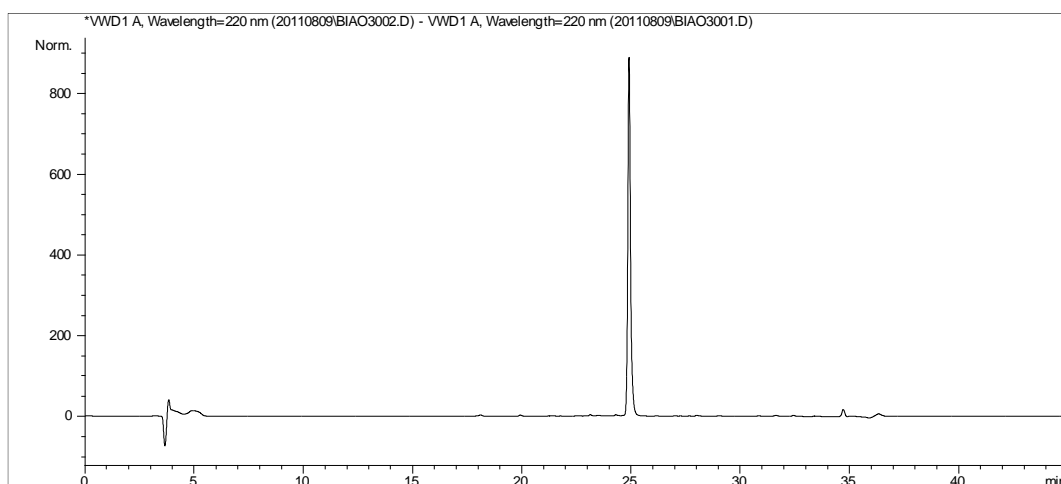
## Supplementary information



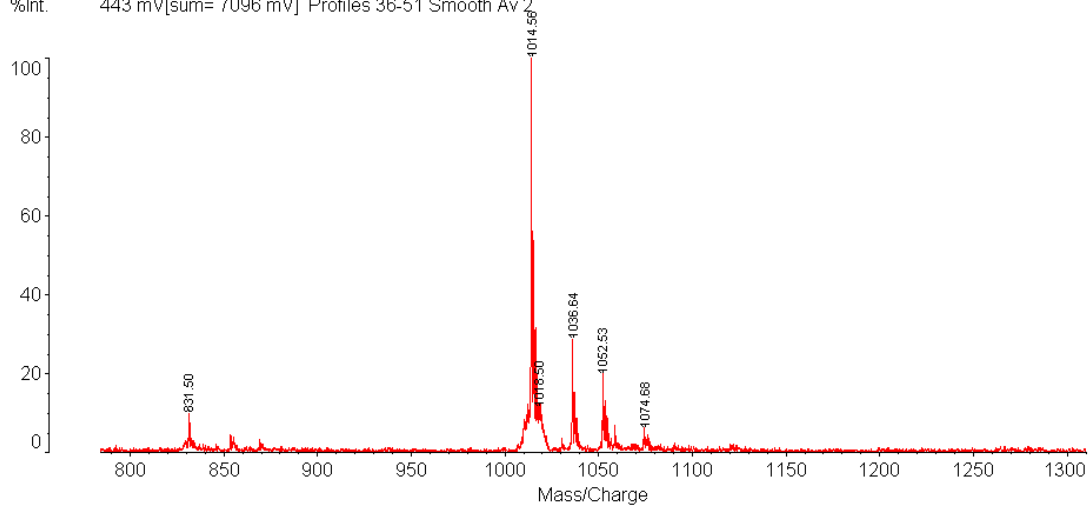
**SFigure.1 HPLC and MS analysis of Biotin-PEG4-lysine(Dde)-Fmoc. HPLC analysis (C18 column):  $t_R = 28.2$  min, purity > 95%. MALDI-TOF-MS:  $[M+H]^+$  m/z 933.5 (calc 933.5);  $[M+Na]^+$  m/z 955.5 (calc 955.5);  $[M+K]^+$  m/z 971.6 (calc 971.5); MW : 932.5 Yield: 52.1 %.**



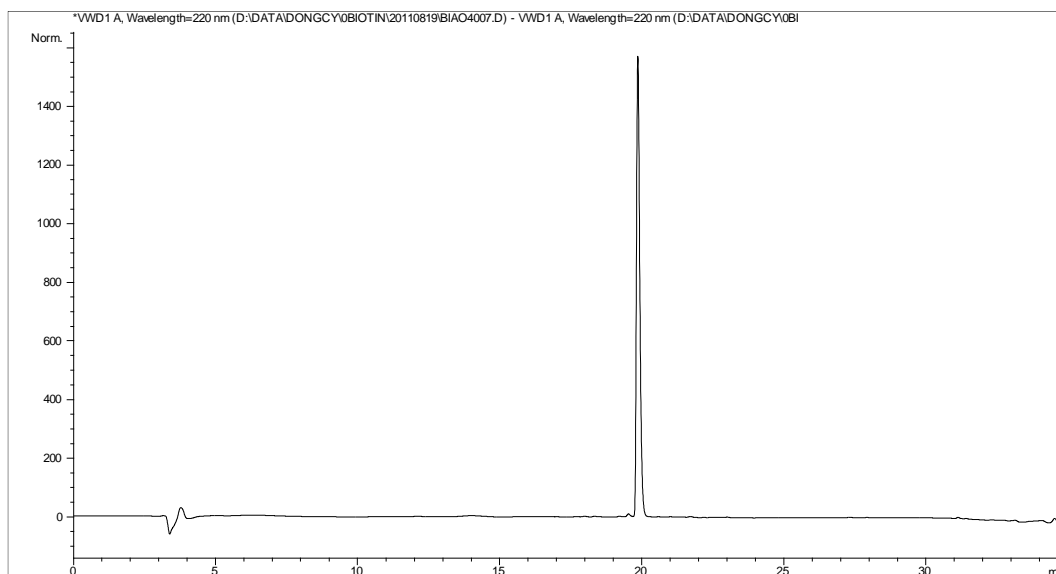
**Figure.2 HPLC and MS analysis of Biotin-PEG4-lysine(Dde)-NH<sub>2</sub>. HPLC analysis (C18 column): *t*R = 23.0 min, purity > 95%. ESI-MS: [M+H]<sup>+</sup> m/z 711.4 (calc 711.4); MW : 711.4; Yield: 82.9 %.**



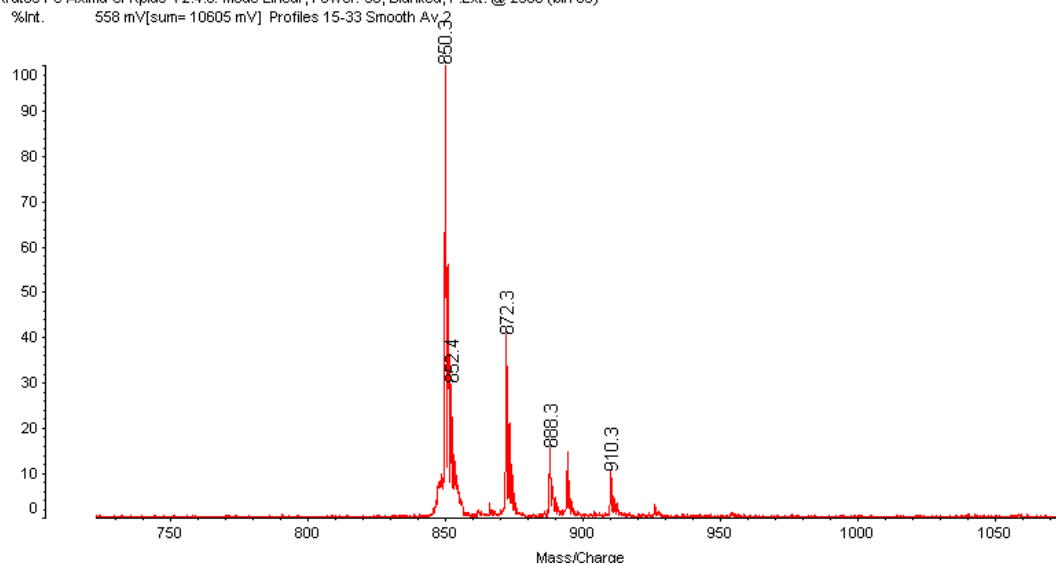
Kratos PC Axima CFRplus V2.4.1: Mode Linear, Power: 38, Blanked, P.Ext. @ 2300 (bin 65)  
 %Int. 443 mV[sum= 7096 mV] Profiles 36-51 Smooth Av 2



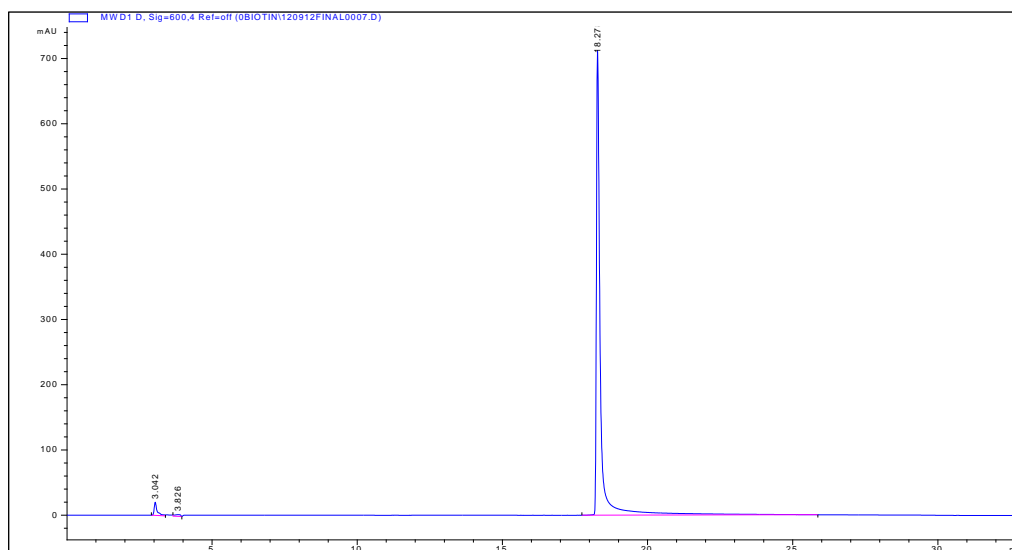
**SFigure.3 HPLC and MS analysis of Biotin-PEG4-lysine(Dde)-HYNIC. HPLC analysis (C18 column):  $t_R = 24.9$  min, purity > 95%. MALDI-TOF-MS:  $[M+H]^+$  m/z 1014.6 (calc 1014.4);  $[M+Na]^+$  m/z 1036.6 (calc 1036.4);  $[M+K]^+$  m/z 1052.5 (calc 1052.4); MW : 1013.4 Yield: 58.2 %.**



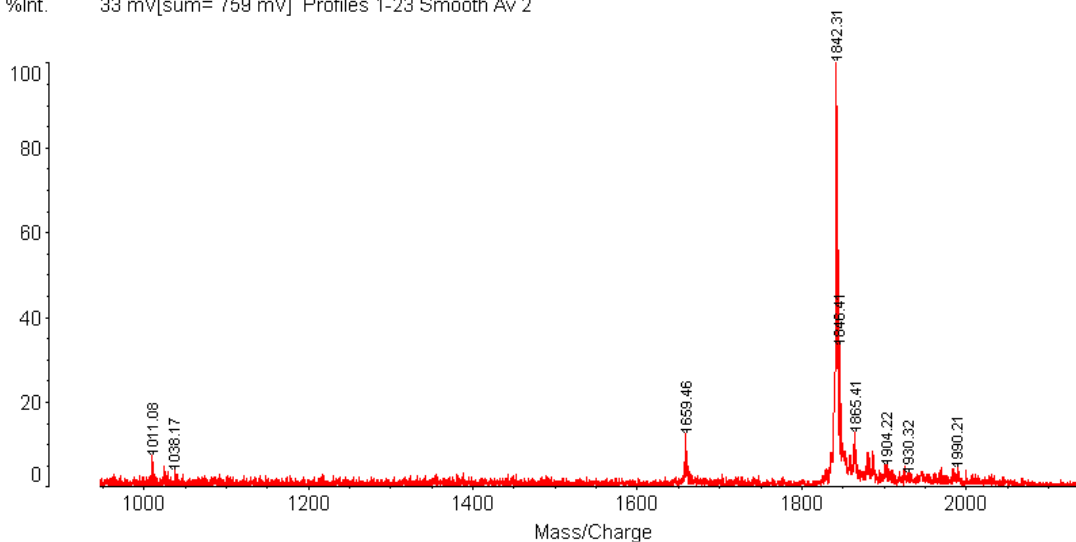
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 38, Blanked, P.Ext. @ 2300 (bin 65)  
 %Int. 558 mV[sum= 10605 mV] Profiles 15-33 Smooth Av. 2



**Figure.4 HPLC and MS analysis of Biotin-PEG4-lysine(NH<sub>2</sub>)-HYNIC. HPLC analysis (C18 column): *t*R = 20.1 min, purity > 95%. MALDI-TOF-MS: [M+H]<sup>+</sup> m/z 850.3 (calc 850.3); [M+Na]<sup>+</sup> m/z 872.3 (calc 872.3); [M+K]<sup>+</sup> m/z 888.3 (calc 888.3); MW : 849.3; Yield: 87.0 %.**



Kratos PC Axima CFRplus V2.4.1: Mode Linear, Power: 46, Blanked, P.Ext. @ 1500 (bin 53)  
 %Int. 33 mV[sur= 759 mV] Profiles 1-23 Smooth Av 2



**Figure.5 HPLC and MS analysis of Biotin-PEG4-lysine(Cy5.5)-HYNIC. HPLC analysis (C18 column): *t*R = 18.6 min, purity > 95%. MALDI-TOF-MS: [M+H]<sup>+</sup> m/z 1842.3 (calc 1838.0); MW : 1838.0; Yield: 52.1 %.**

	1 h	2 h	4 h
Tumor/Blood	8.58 ± 1.93	19.28 ± 3.63	21.46 ± 5.78
Tumor/Heart	9.35 ± 1.06	16.62 ± 2.35	11.43 ± 4.59
Tumor/Lung	2.79 ± 0.39	4.88 ± 0.41	4.60 ± 1.93
Tumor/Kidney	0.12 ± 0.03	0.10 ± 0.02	0.07 ± 0.04
Tumor/Liver	2.62 ± 1.11	4.49 ± 1.02	2.37 ± 1.38
Tumor/Spleen	4.06 ± 1.11	7.26 ± 1.53	6.86 ± 2.75
Tumor/Intestine	2.22 ± 0.42	5.71 ± 1.62	4.46 ± 0.97
Tumor/Stomach	2.47 ± 1.40	3.97 ± 0.51	2.78 ± 1.17
Tumor/Bone	5.71 ± 1.45	10.46 ± 0.61	7.35 ± 2.05
Tumor/Muscle	7.20 ± 1.57	9.05 ± 3.18	9.20 ± 2.48

**STable.1 Tumor-to-normal organ ratios of <sup>99m</sup>Tc-HYNIC-lys(Cy5.5)-PEG<sub>4</sub>-biotin at 1, 2, and 4 hours after pretargeted injection of avidin into nude mice bearing LS180 colon tumors.**