Enhanced Cancer Theranostics with Self-Assembled, Multi-labeled siRNAs

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† Jenny Zilberberg and David Sabatino contributed equally to this work.

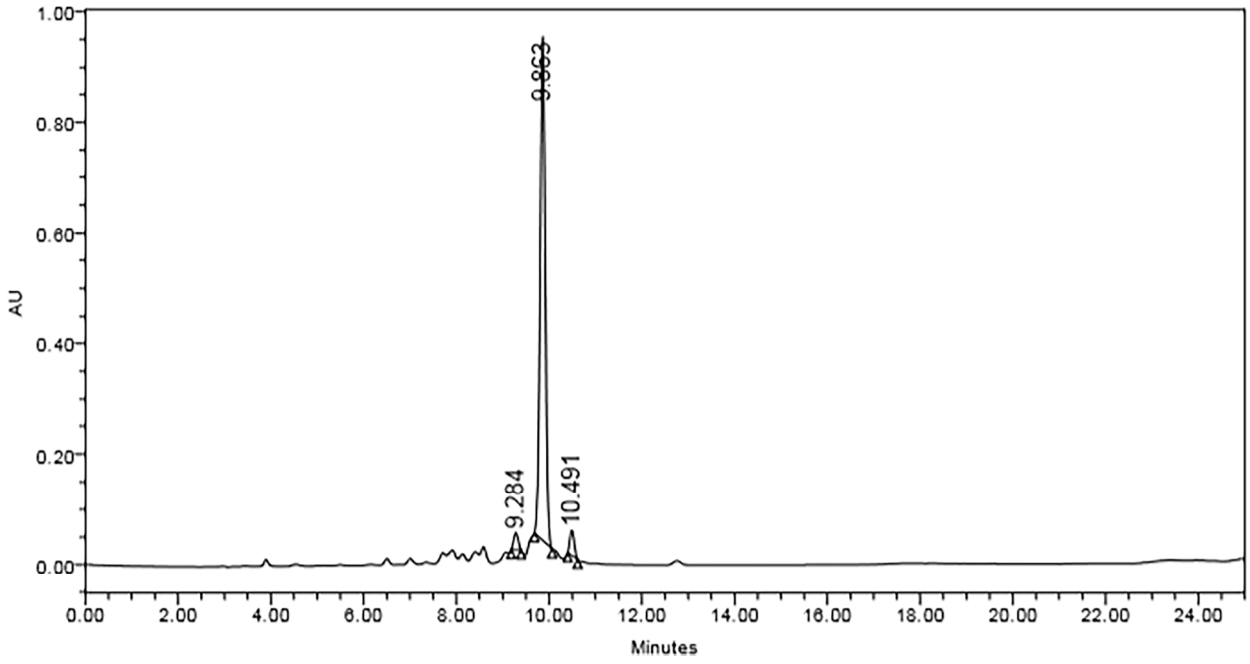
Supplementary Materials

Within this supporting information:

Name	Sequence[a]	Percent Yield (%)[b]	Percent Purity (%)[c]	Mass[d]
GRP78A1	5'-AUC AGA AUC UUC CAA CAC U-3'	91	>99%	5949.6 (5949.9)
GRP78S1	5'-AGU GUU GGA AGA UUC UGA U-3'	92	>99%	6103.7 (6103.9)
GRP94S1	5'-GAA GAA GCA UCU GAU UAC C-3'	90	>99%	6068.8 (6069.0)
GRP75S1	5'-ACU GAC UCG GAG AAU ACA A-3'	91	>99%	6091.8 (6092.2)
V-78A194A1	2'-3'-CUU CUU CGU AGA CUA CUG G-5' rU 3'-5'-AUC AGA AUC UUC CAA CAC U-3'	83	>99%	12929.7 (12929.9)
V-78A178A2	2'-3'-CCU CGC GUA AUC AUG AUC U-5' rU 3'-5'-AUC AGA AUC UUC CAA CAC U-3'	81	>98%	12275.4 (12276.6)
Y-78A194A175A1	2'-3'-CUU CUU CGU AGA CUA CUG G-5' 5'-UUG UA UCU CCG AGU CAG U-3'-5'-rU 3'-5'-AUC AGA AUC UUC CAA CAC U-3'	62	>98%	18335.9 (18357.2)
FITC-78A1	FITC-NH-$(CH_2)_6$-5'-AUC AGA AUC UUC CAA CAC U-3'	52	>97%	6516.7 (6518.5)
FITC-78S1	FITC-NH-$(CH_2)_6$-5'-AGU GUU GGA AGA UUC UGA U-3'	48	>98%	6670.8 (6672.5)
FITC-94S1	FITC-NH-$(CH_2)_6$-5'-GAA GAA GCA UCU GAU UAC C-3'	51	>97%	6635.9 (6636.8)
FITC-75S1*	FITC-NH-$(CH_2)_6$-5'-ACU GAC UCG GAG AAU ACA A-3'	N/A	>99%	6629.3 (6628.8)
FITC-V-78A194A1	2'-3'-CUU CUU CGU AGA CUA CUG G-5'-$(CH_2)_6$-NH-FITC rU 3'-5'-AUC AGA AUC UUC CAA CAC U-3'	22	>95%	13013.1 (12884.6)
FITC-V-78A178A2	2'-3'-CCU CGC GUA AUC AUG AUC U-5'-$(CH_2)_6$-NH-FITC rU 3'-5'-AUC AGA AUC UUC CAA CAC U-3'	20	>95%	12969.1 (12843.9)

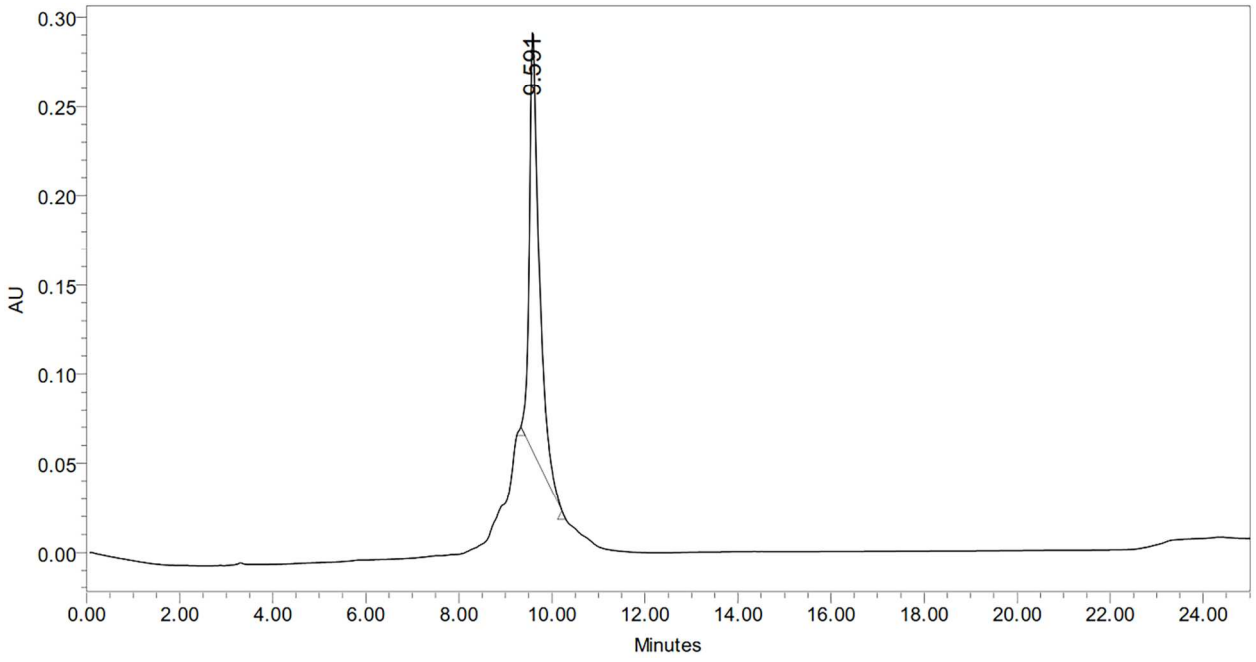
Table S1. [a]Linear, V- and Y-shaped RNA sequences designed to target GRP-78, 94 and 75. [b] Determined by UV-Vis Spectroscopy. [c] Obtained by RP-IP-HPLC using 0.1 mM TEAA in 0-45% MeCN, pH: 7.2 over 20 min. [d] Calculated mass (observed mass) by ESI-MS in negative mode (Novatia LLC, Newton, PA). * Sample purchased from ChemGenes (Wilmington, MA)

Figure S1 RP IP HPLC ANALYSIS OF LINEAR GRP78A1



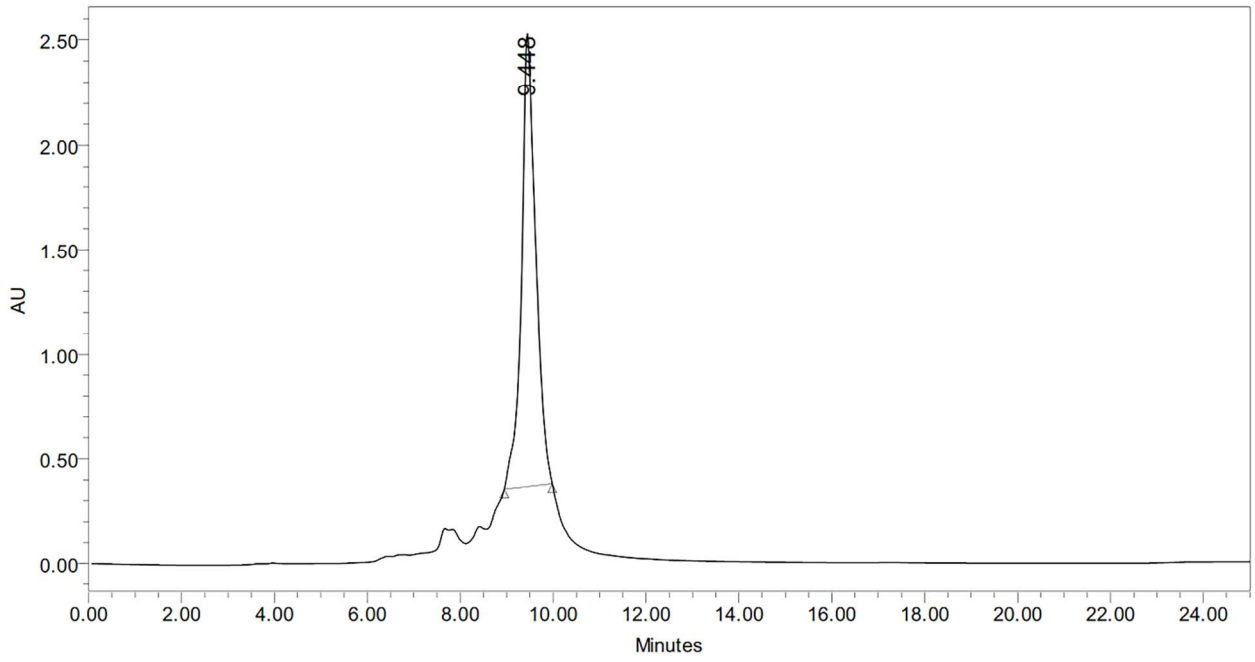
	RT	% Area	Area (μV*sec)
1	9.28	2.78	211473
2	9.86	93.29	7107854
3	10.49	3.93	299732

Figure S2 RP IP HPLC ANALYSIS OF LINEAR GRP78S1



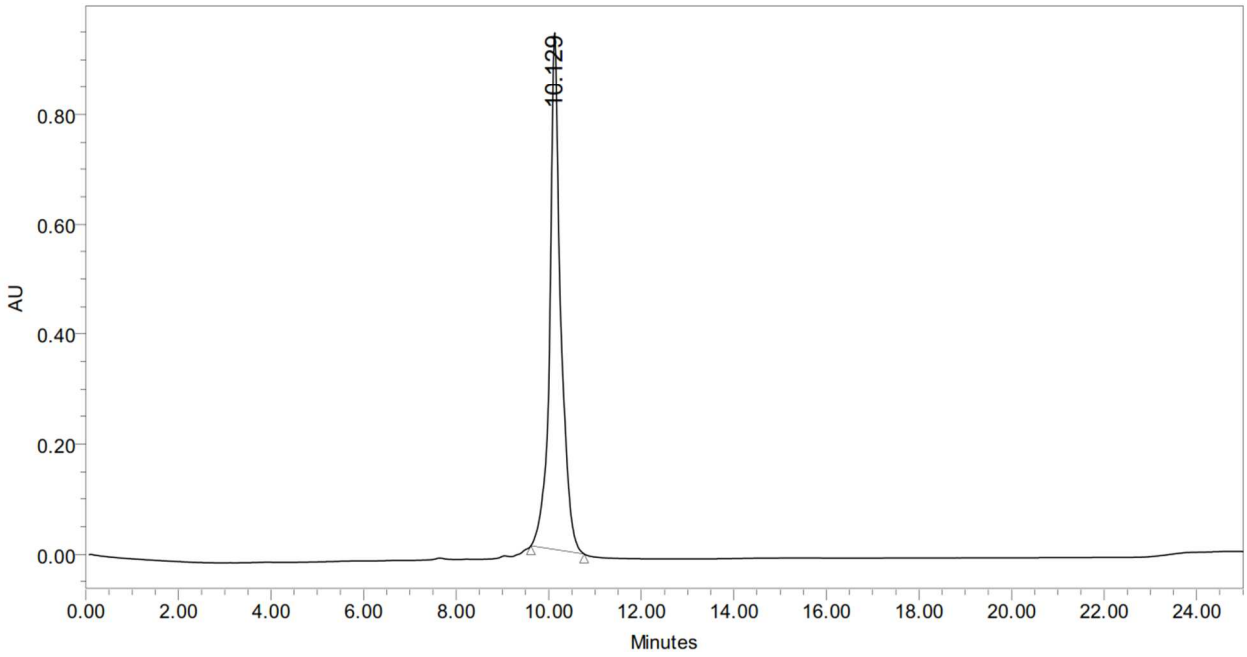
	RT	% Area	Area (µV*sec)
1	9.59	100.00	3449874

Figure S3 RP IP HPLC ANALYSIS OF LINEAR GRP94S1



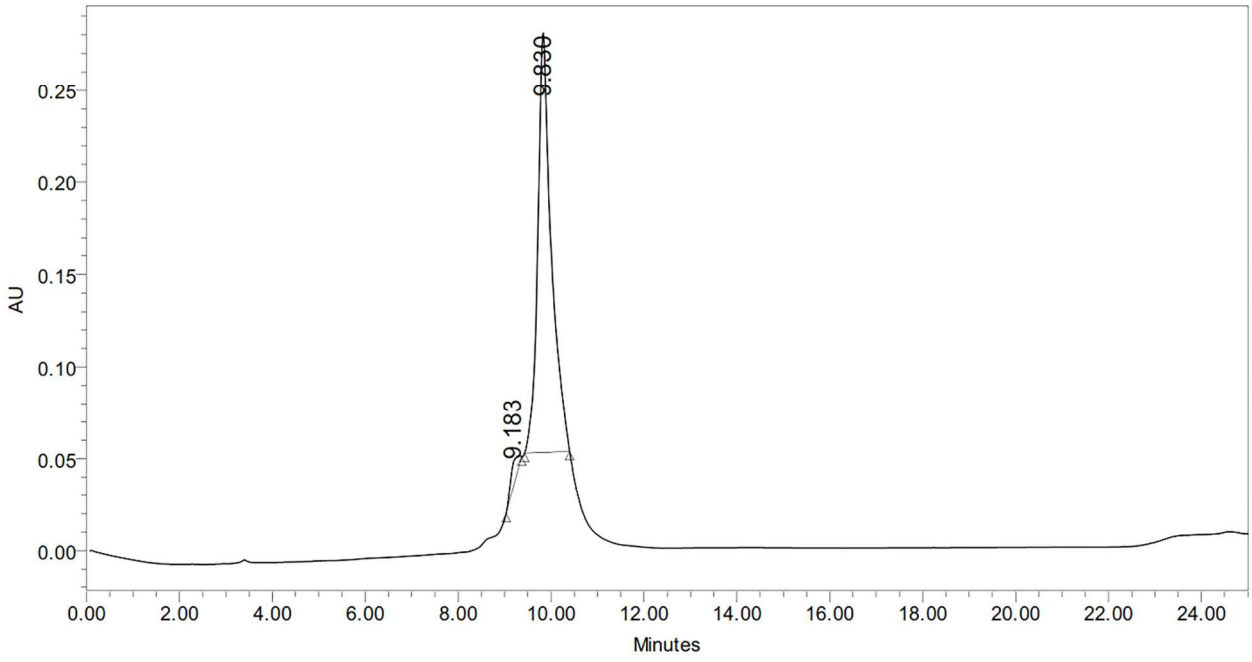
	RT	% Area	Area (μV*sec)
1	9.45	100.00	45867536

Figure S4 RP IP HPLC ANALYSIS OF V-GRP78A194A1



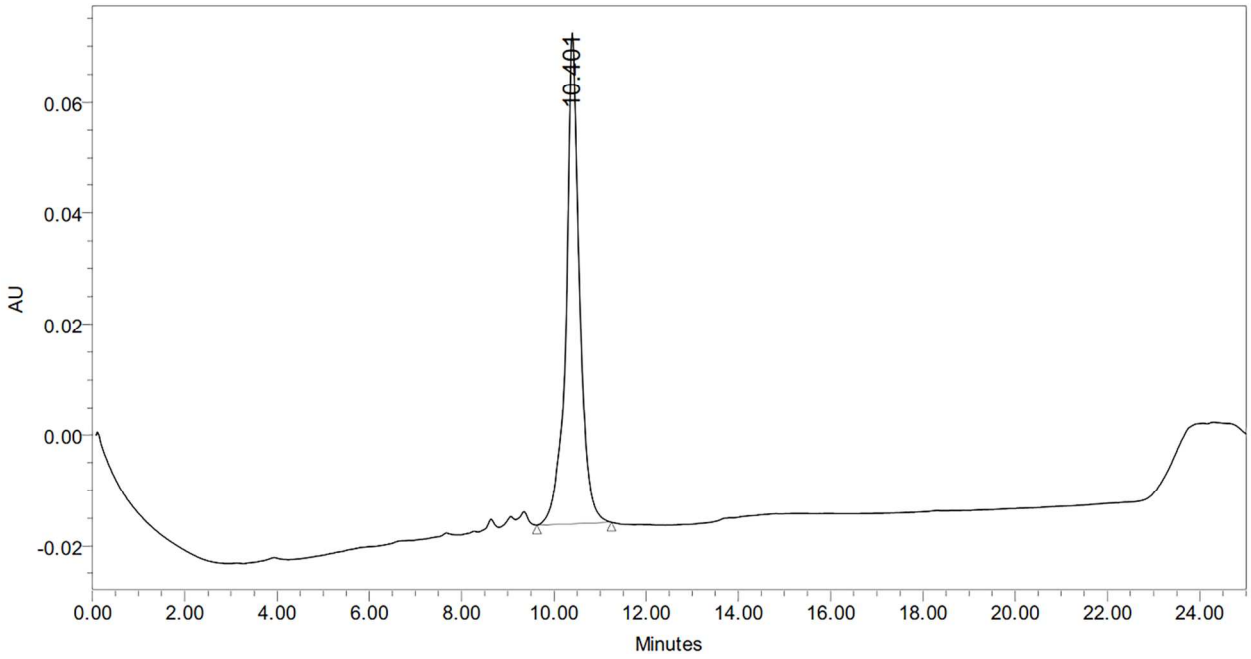
	RT	% Area	Area (μV*sec)
1	10.13	100.00	15461777

Figure S5 RP IP HPLC ANALYSIS OF V-GRP78A178A2



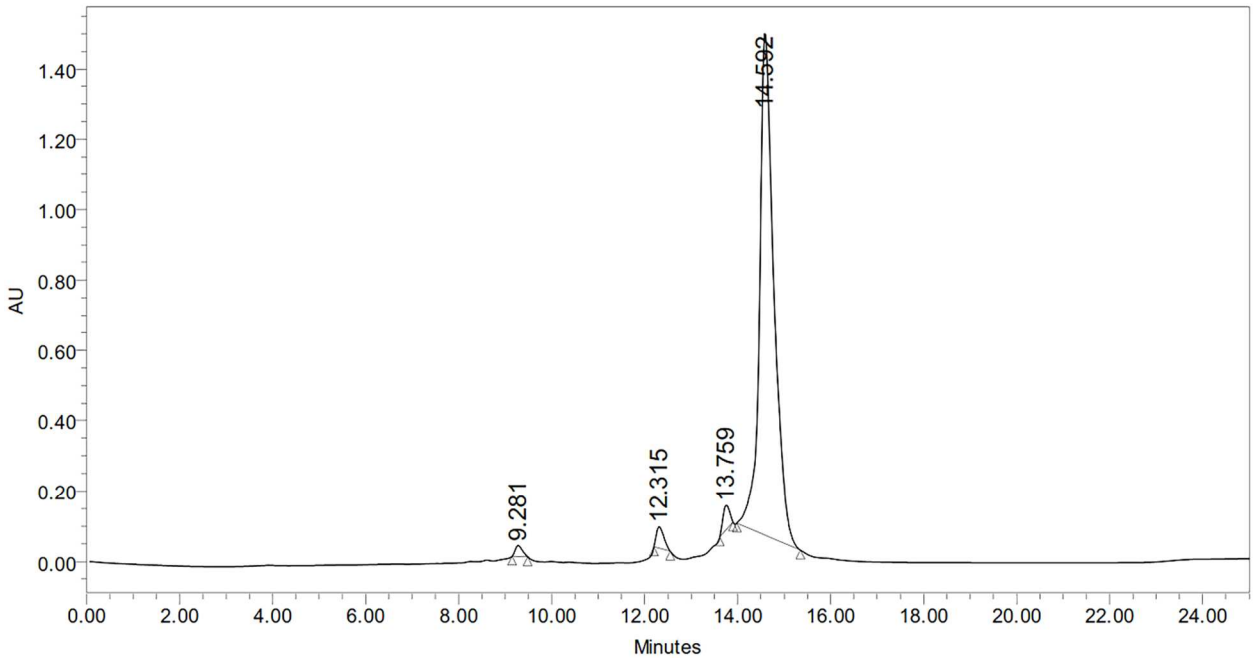
	RT	% Area	Area (μV*sec)
1	9.18	3.07	146735
2	9.83	96.93	4626292

Figure S6 RP IP HPLC ANALYSIS OF Y-GRP78A194A175A1



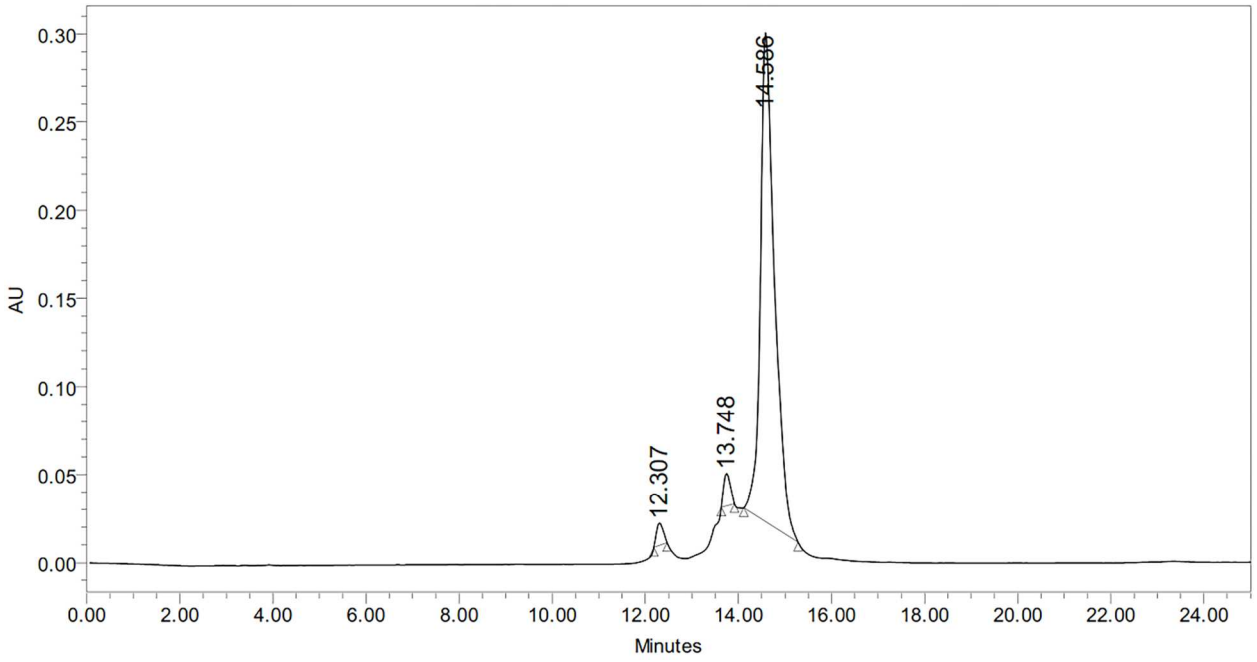
	RT	% Area	Area (μV*sec)
1	10.40	100.00	1797312

Figure S7 RP IP HPLC ANALYSIS OF FL-GRP78A1 (260nm)



	RT	% Area	Area (µV*sec)
1	9.28	1.00	324879
2	12.32	2.10	678728
3	13.76	2.10	680619
4	14.59	94.80	30706756

Figure S8 RP IP HPLC ANALYSIS OF FL-GRP78A1 (488nm)



	RT	% Area	Area (μV*sec)
1	12.31	2.00	123746
2	13.75	2.95	181980
3	14.59	95.05	5866825

Figure S9 ESI-MS ANALYSIS OF FL-GRP78A1

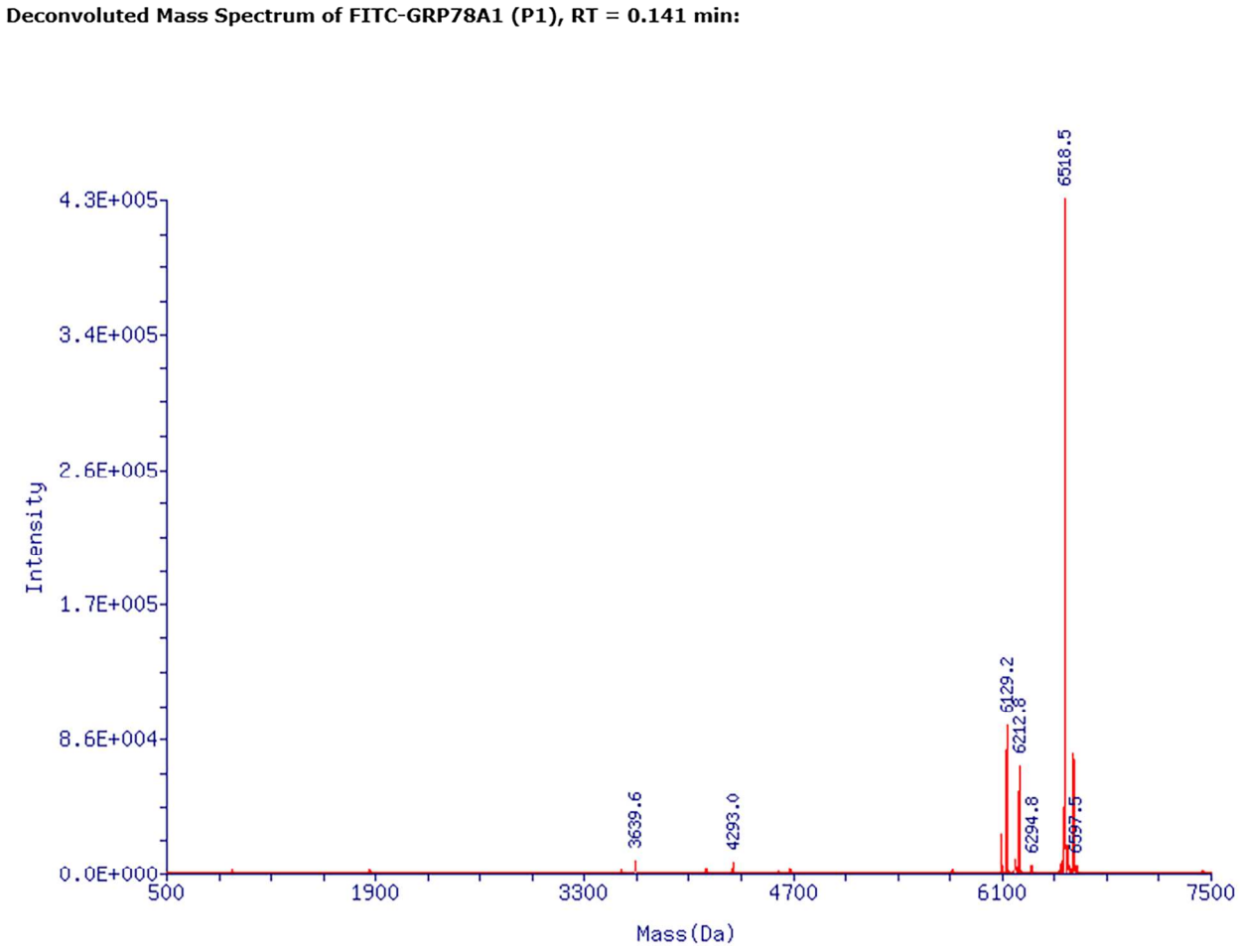
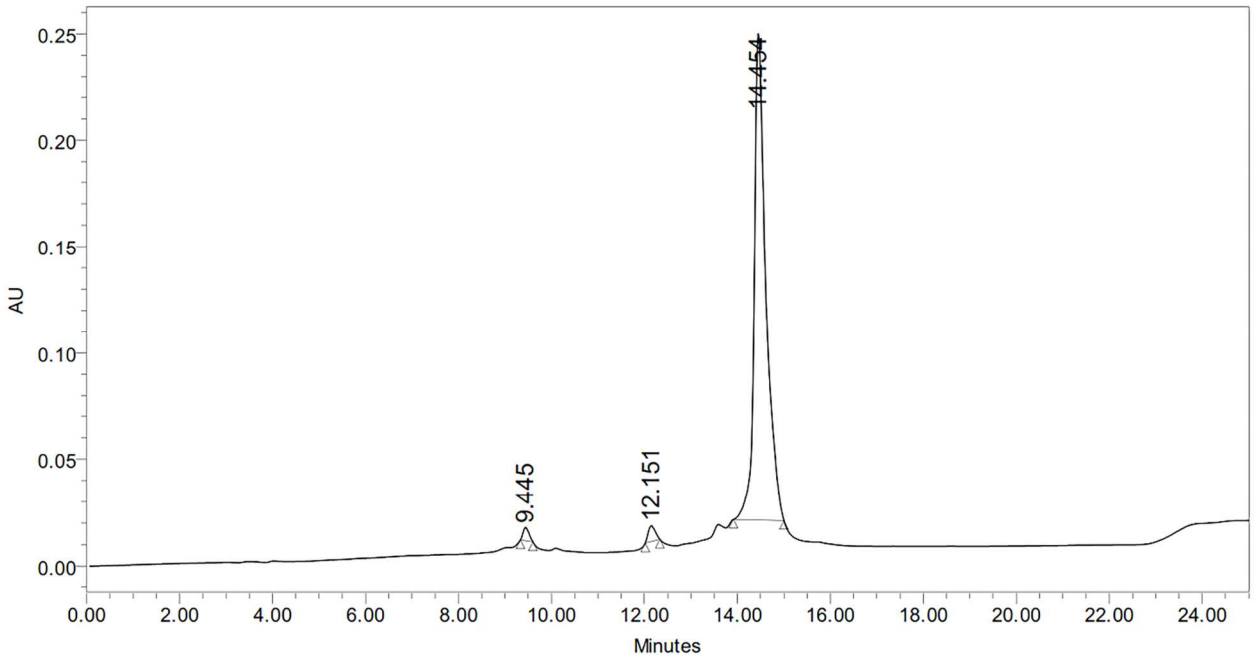
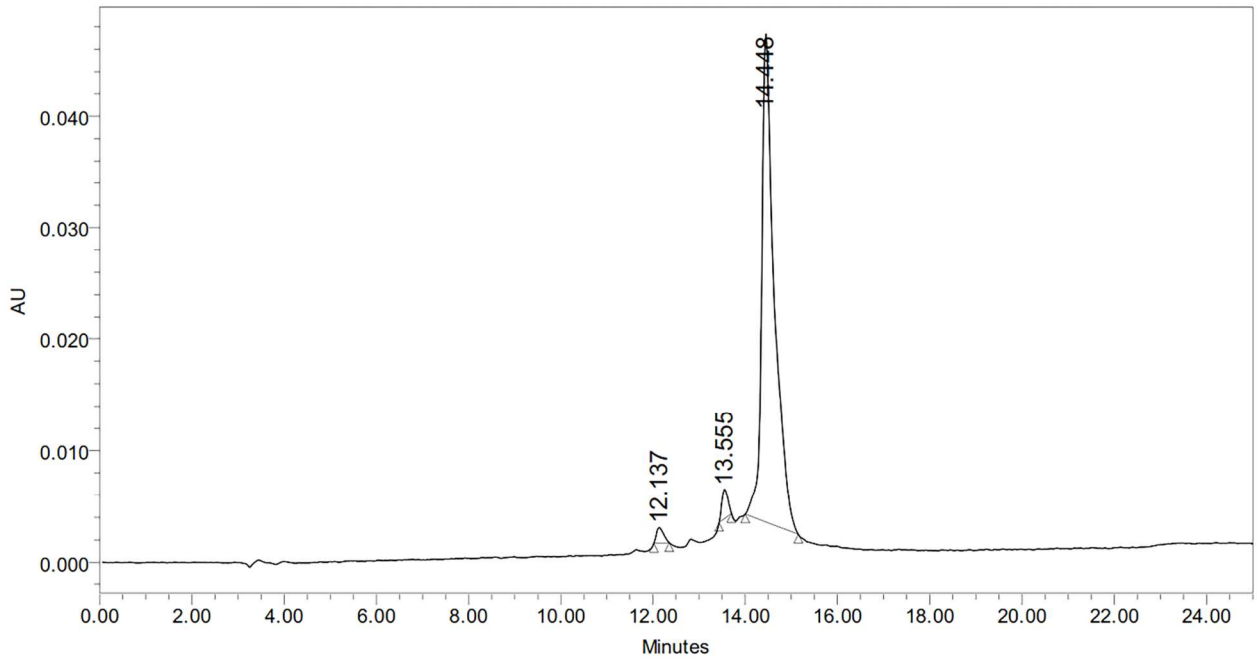


Figure S10 RP IP HPLC ANALYSIS OF FL-GRP78S1 (260nm)



	RT	% Area	Area (μV*sec)
1	9.45	1.29	53177
2	12.15	1.86	76557
3	14.45	96.84	3981950

Figure S11 RP IP HPLC ANALYSIS OF FL-GRP78S1 (488nm)



	RT	% Area	Area (μV*sec)
1	12.14	1.69	14785
2	13.56	2.70	23639
3	14.45	95.61	836278

Figure S12 ESI-MS ANALYSIS OF FL-GRP78S1

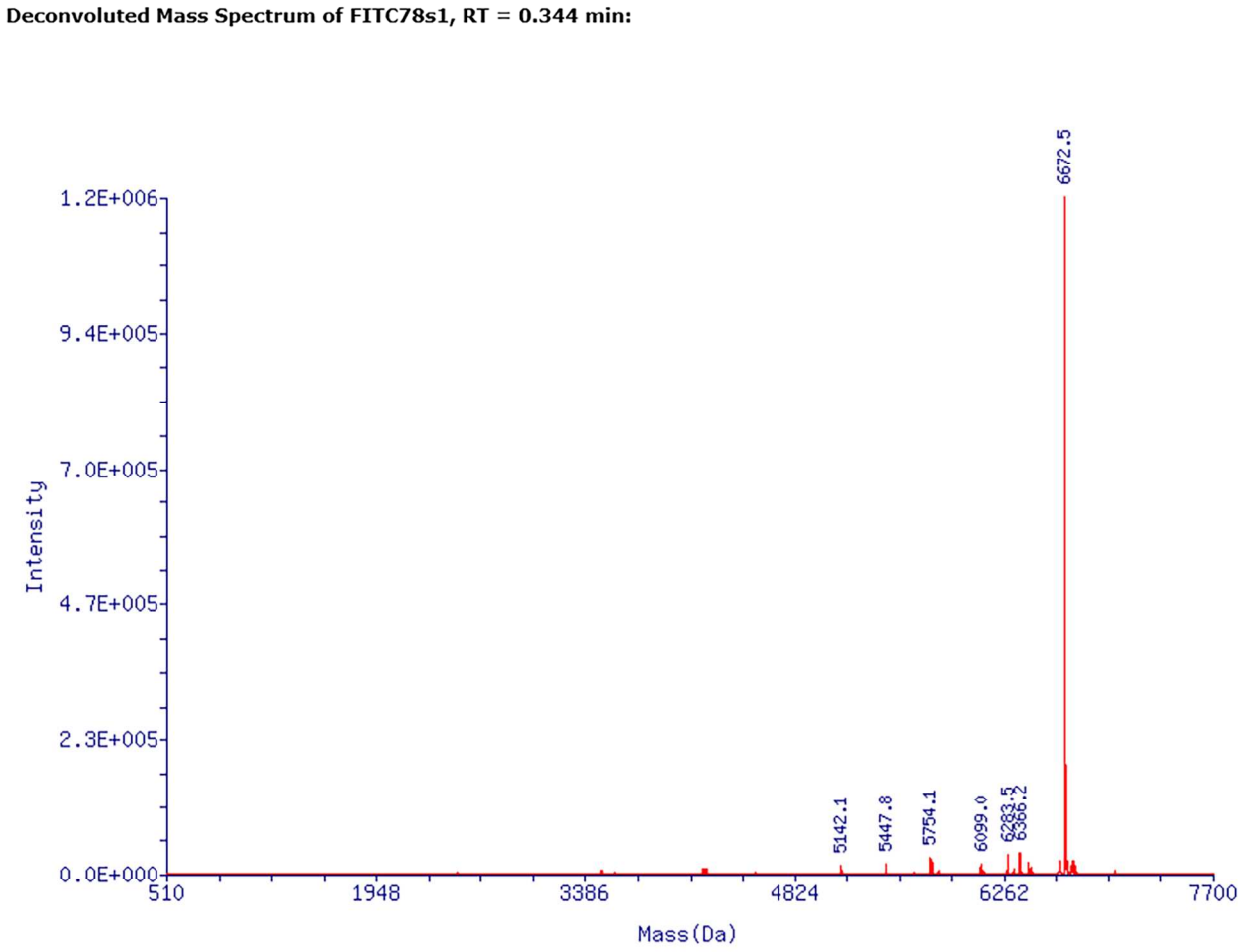
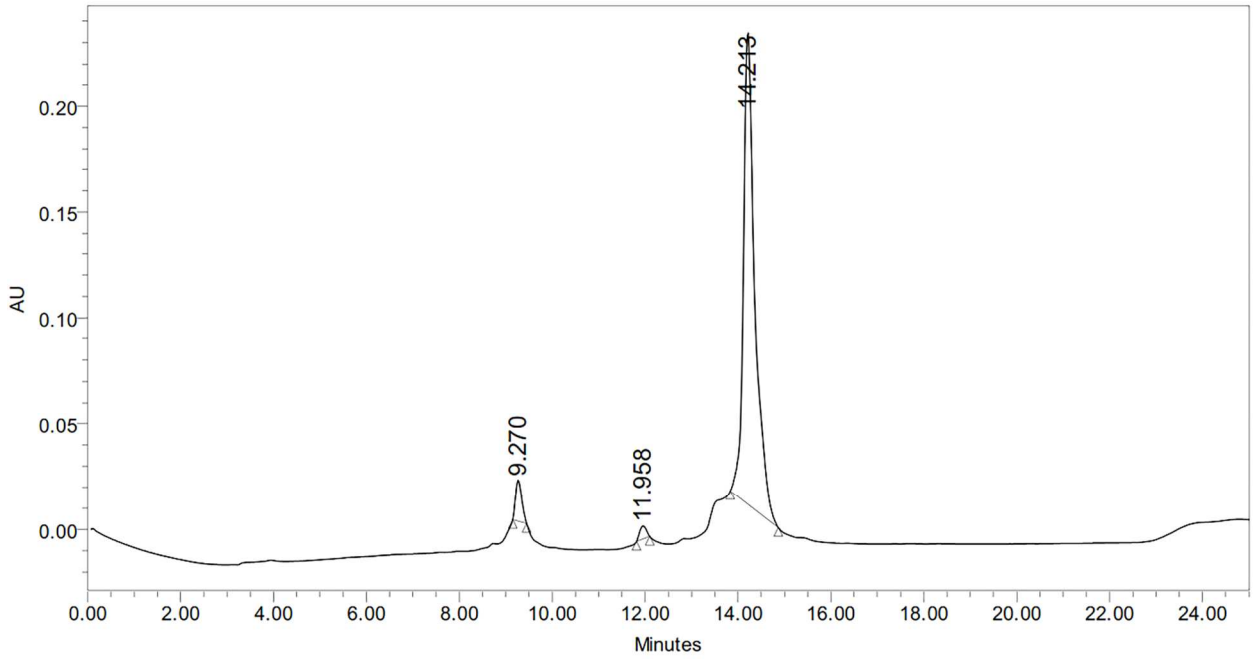


Figure S13 RP IP HPLC ANALYSIS OF FL-GRP94S1 (260nm)



	RT	% Area	Area (μV*sec)
1	9.27	4.44	180347
2	11.96	1.47	59631
3	14.21	94.09	3818389

Figure S14 ESI-MS ANALYSIS OF FL-GRP94S1

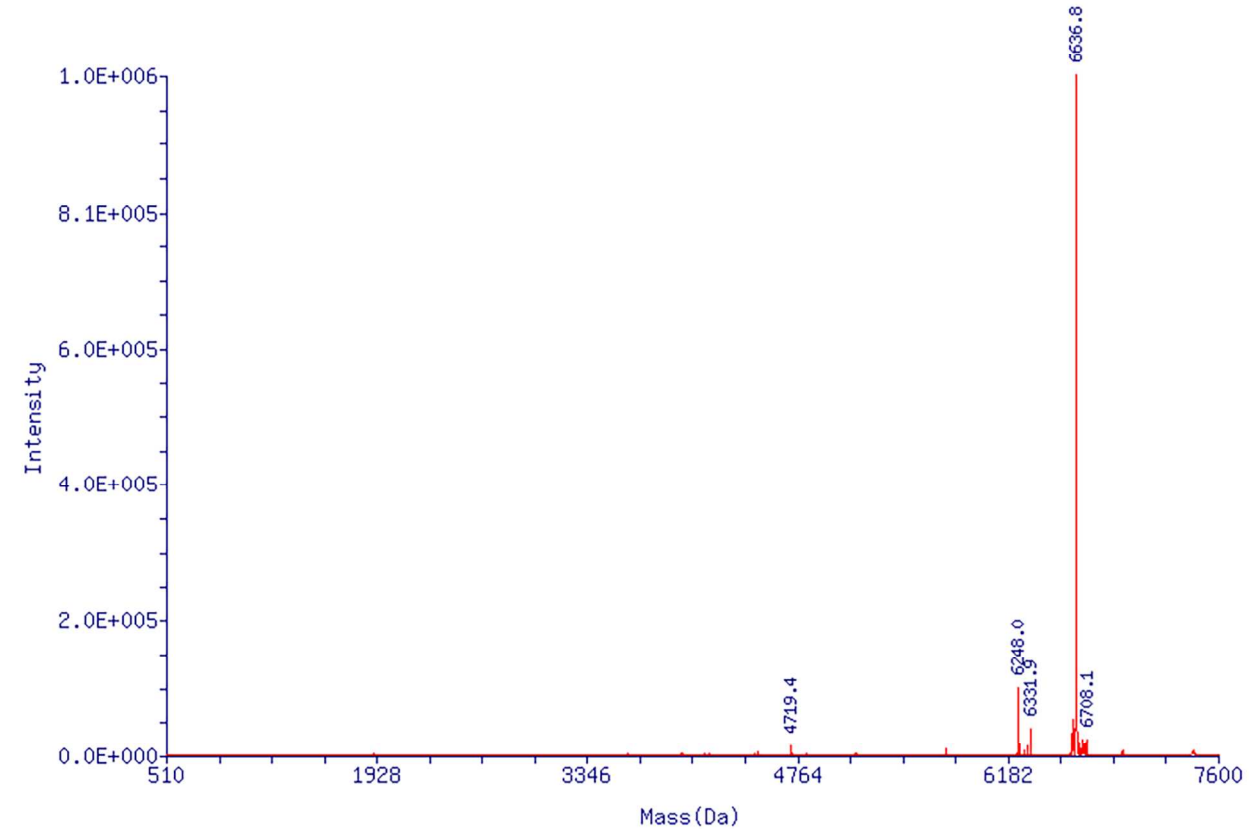
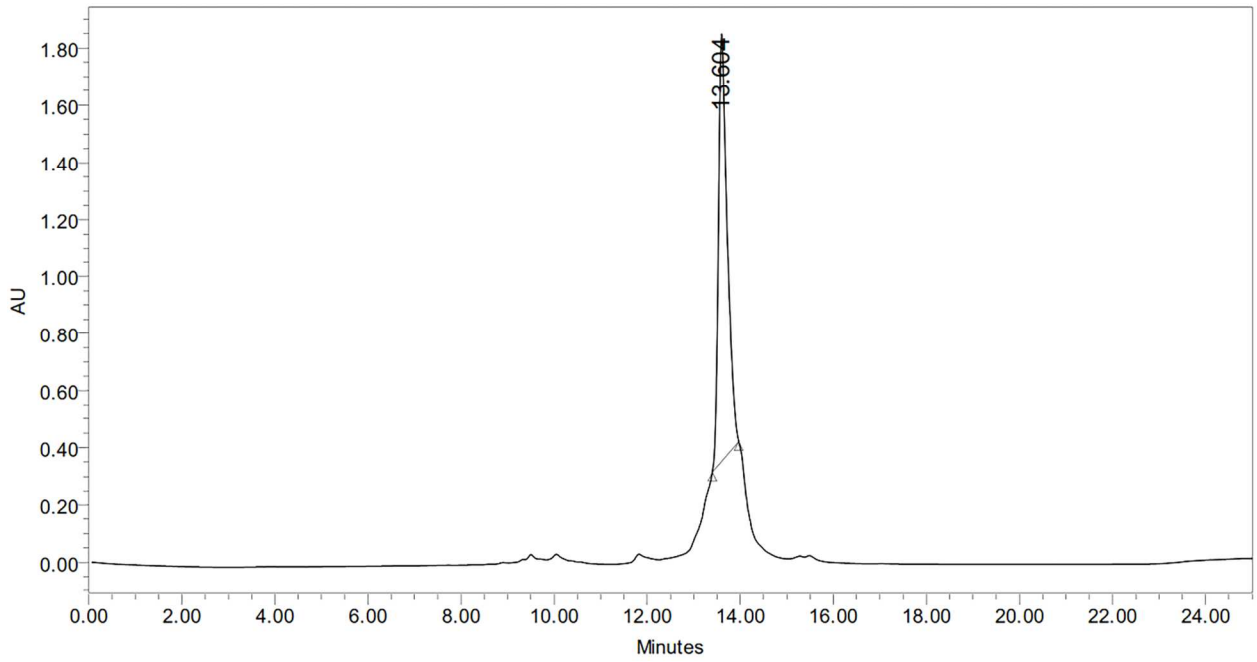
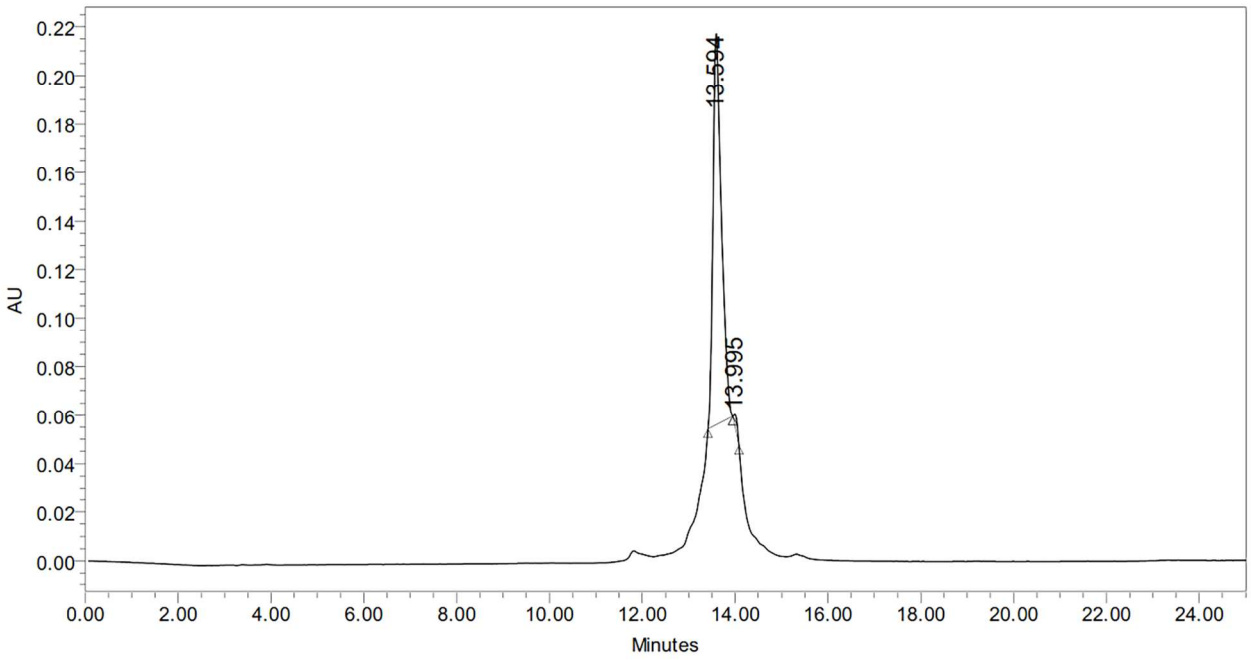


Figure S15 RP IP HPLC ANALYSIS OF FL-V-GRP78A194A1 (260nm)



	RT	% Area	Area (μV*sec)
1	13.60	100.00	19780457

Figure S16 RP IP HPLC ANALYSIS OF FL-V-GRP78A194A1 (488nm)



	RT	% Area	Area (μV*sec)
1	13.59	98.58	2031207
2	14.00	1.42	29335

Figure S17 ESI-MS ANALYSIS OF FL-V-GRP78A194A1

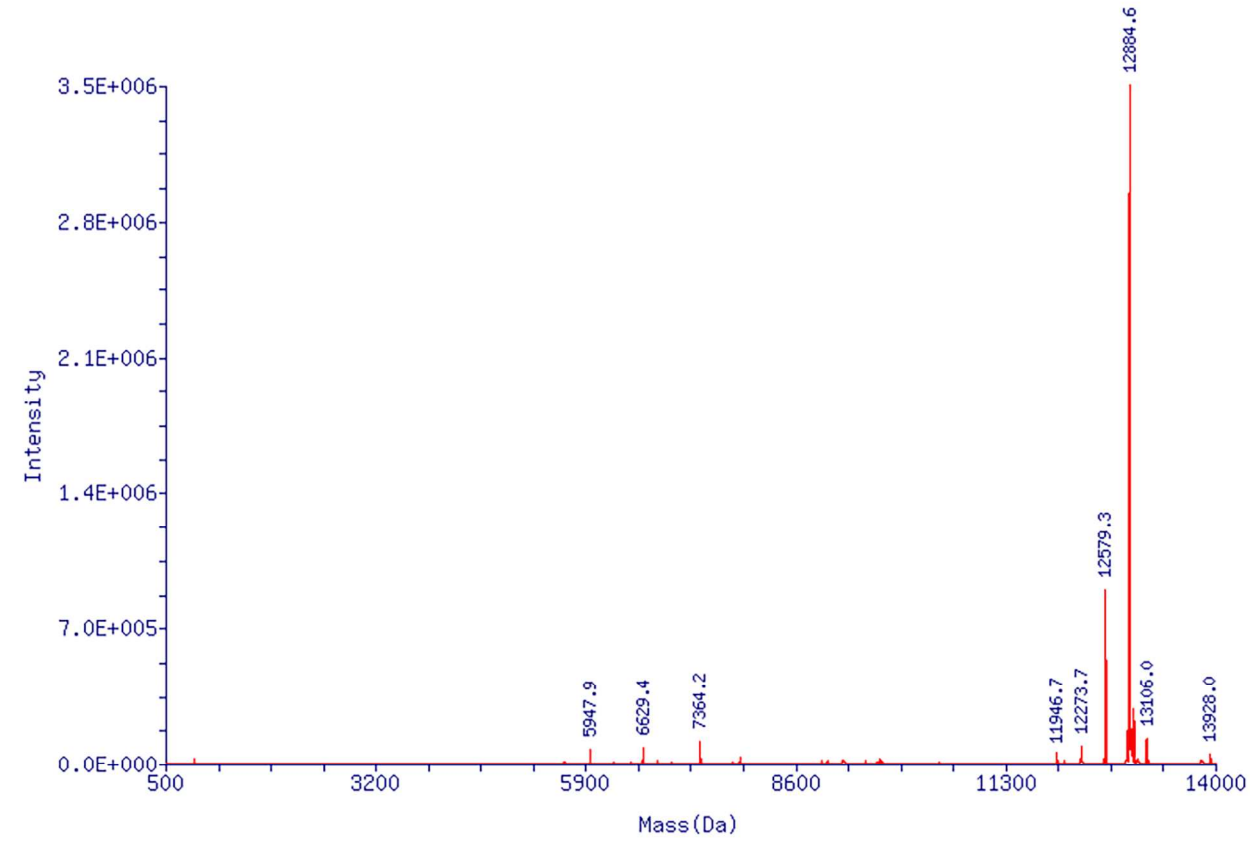
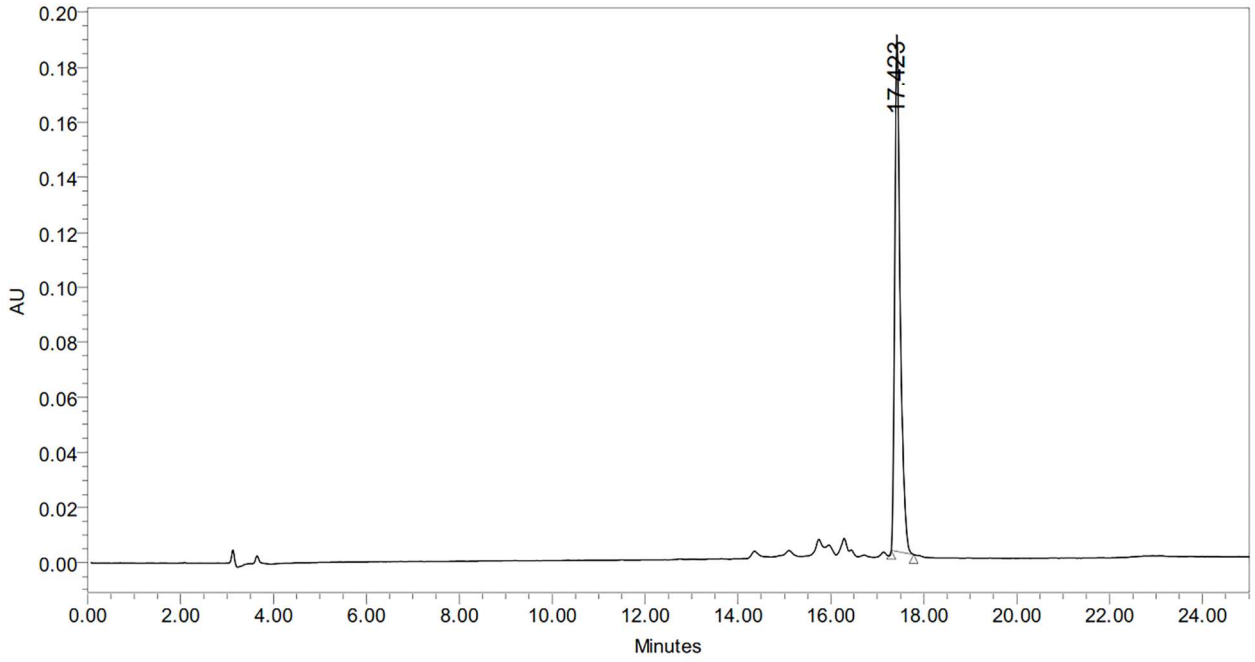
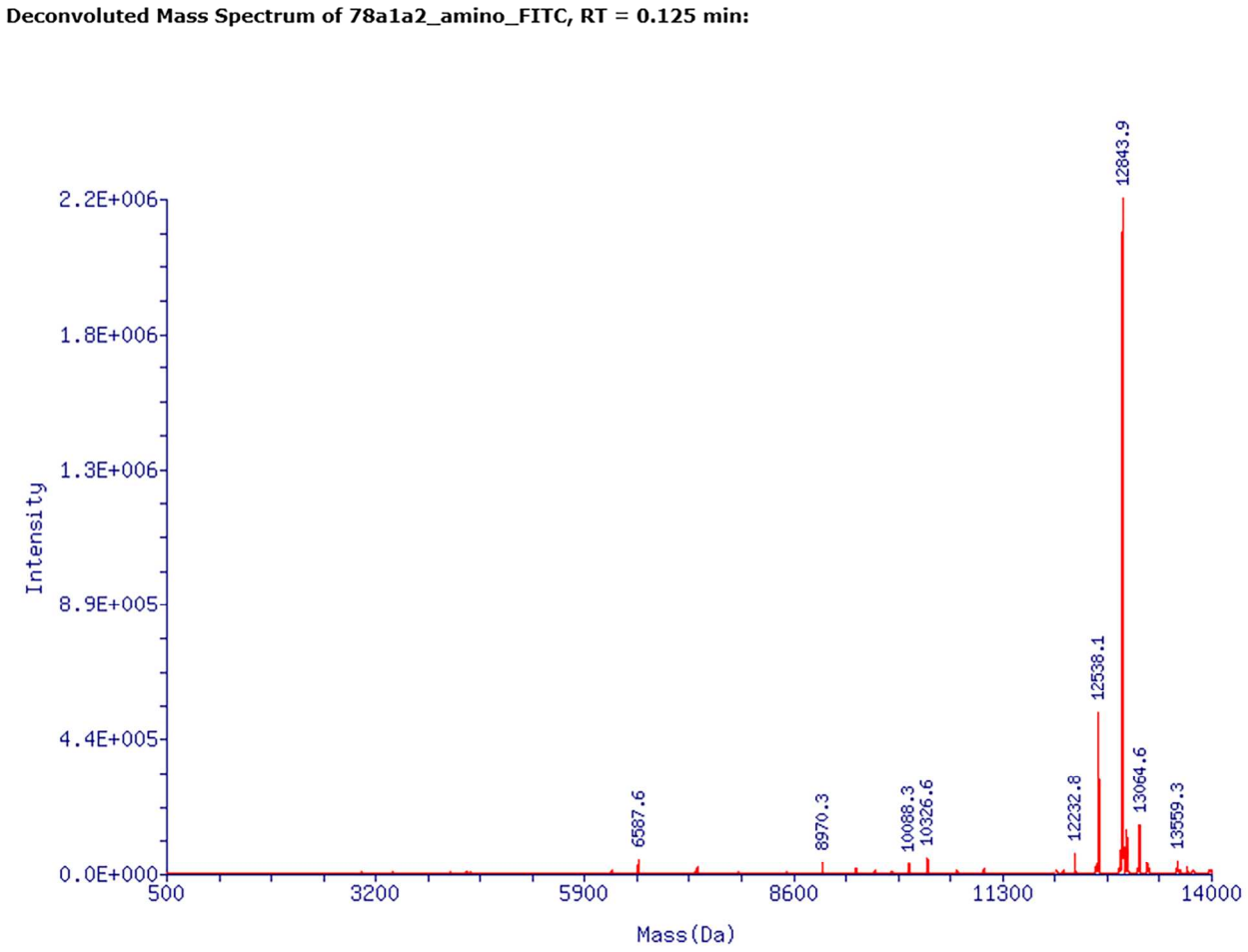


Figure S18 RP IP HPLC ANALYSIS OF FL-V-GRP78A178A2 (488nm)



	RT	% Area	Area (μV*sec)
1	17.42	100.00	1533861

Figure S19 ESI-MS ANALYSIS OF FL-V-GRP78A178A2



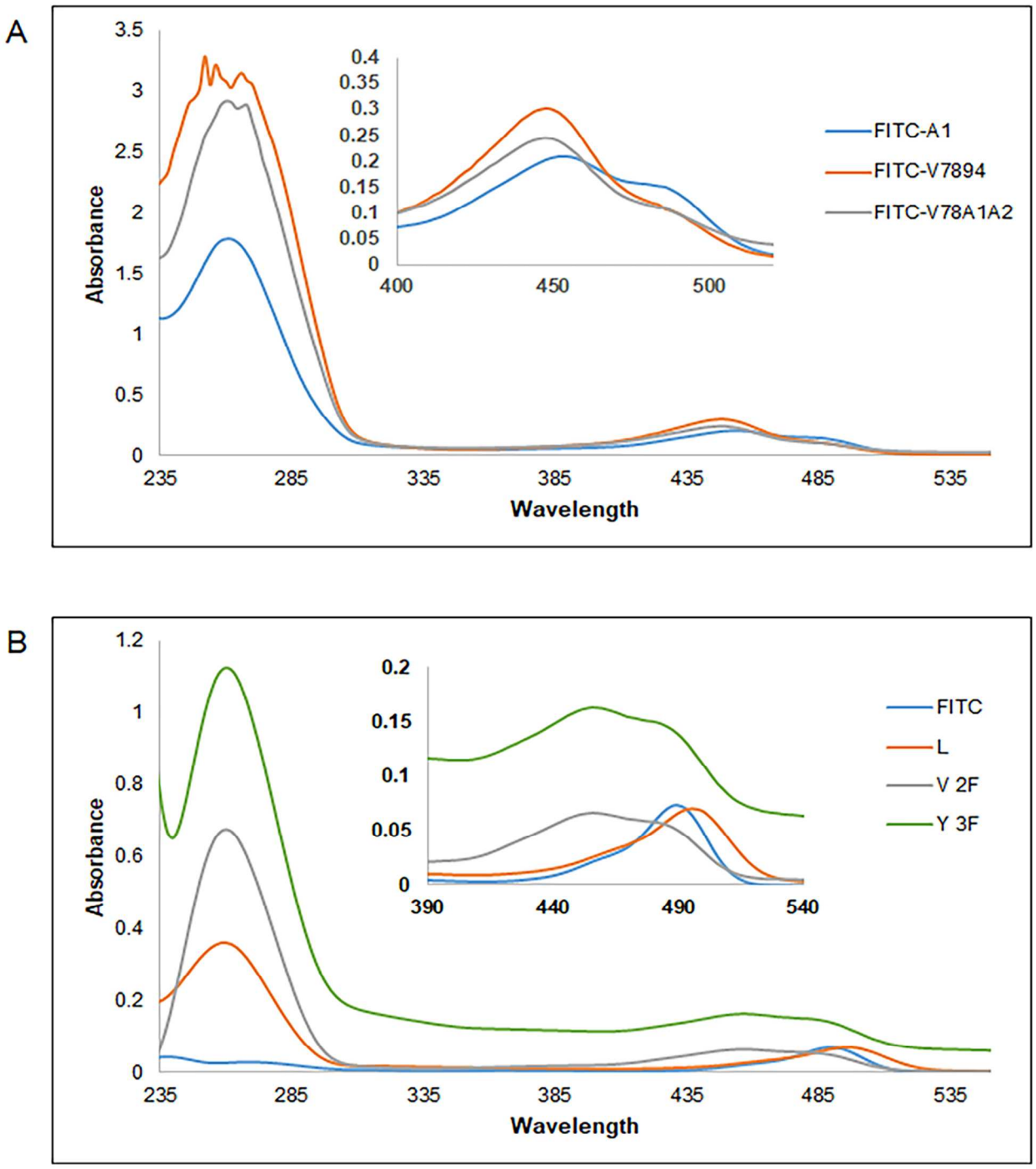


Figure S20 UV absorption spectra of FITC-labeled template siRNAs (**A**) and multi-FITC siRNAs (**B**). All siRNA hybrids were prepared in annealing buffer (1.25 μM, 10 mM Tris, 50 mM NaCl, 1 mM EDTA, pH 7.5 – 8.0.

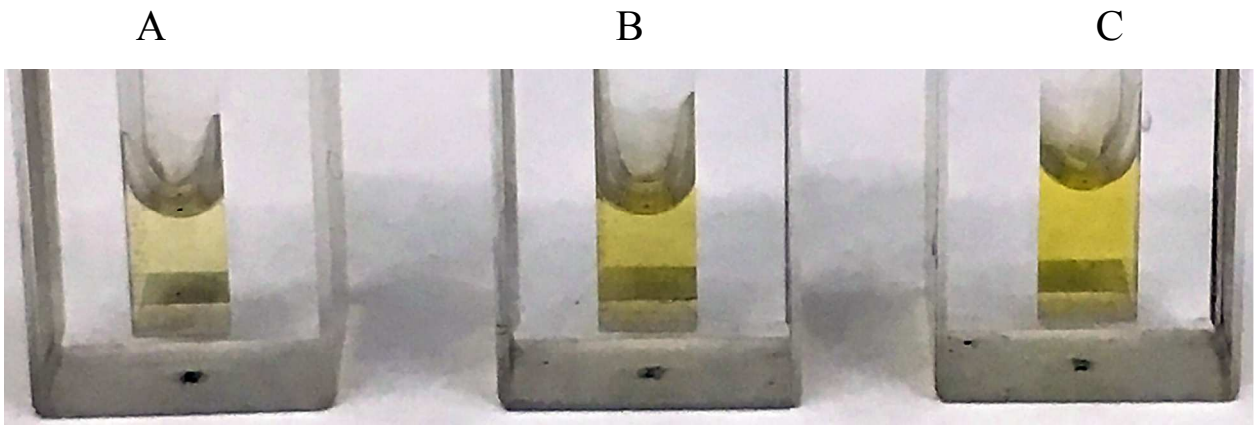


Figure S21 Multi-FITC siRNA samples pre-dilution for fluorescence spectroscopy analysis. Linear 78A1:FL-78S1 (A), V-78A194A1:FL-78S1FL-94S1 (B) and Y78A194A175A1:FL-78S1FL-94S1FL-75S1 (C). siRNA hybrids were prepared in annealing buffer (1.25 μM, 10 mM Tris, 50 mM NaCl, 1 mM EDTA, pH 7.5 – 8.0).

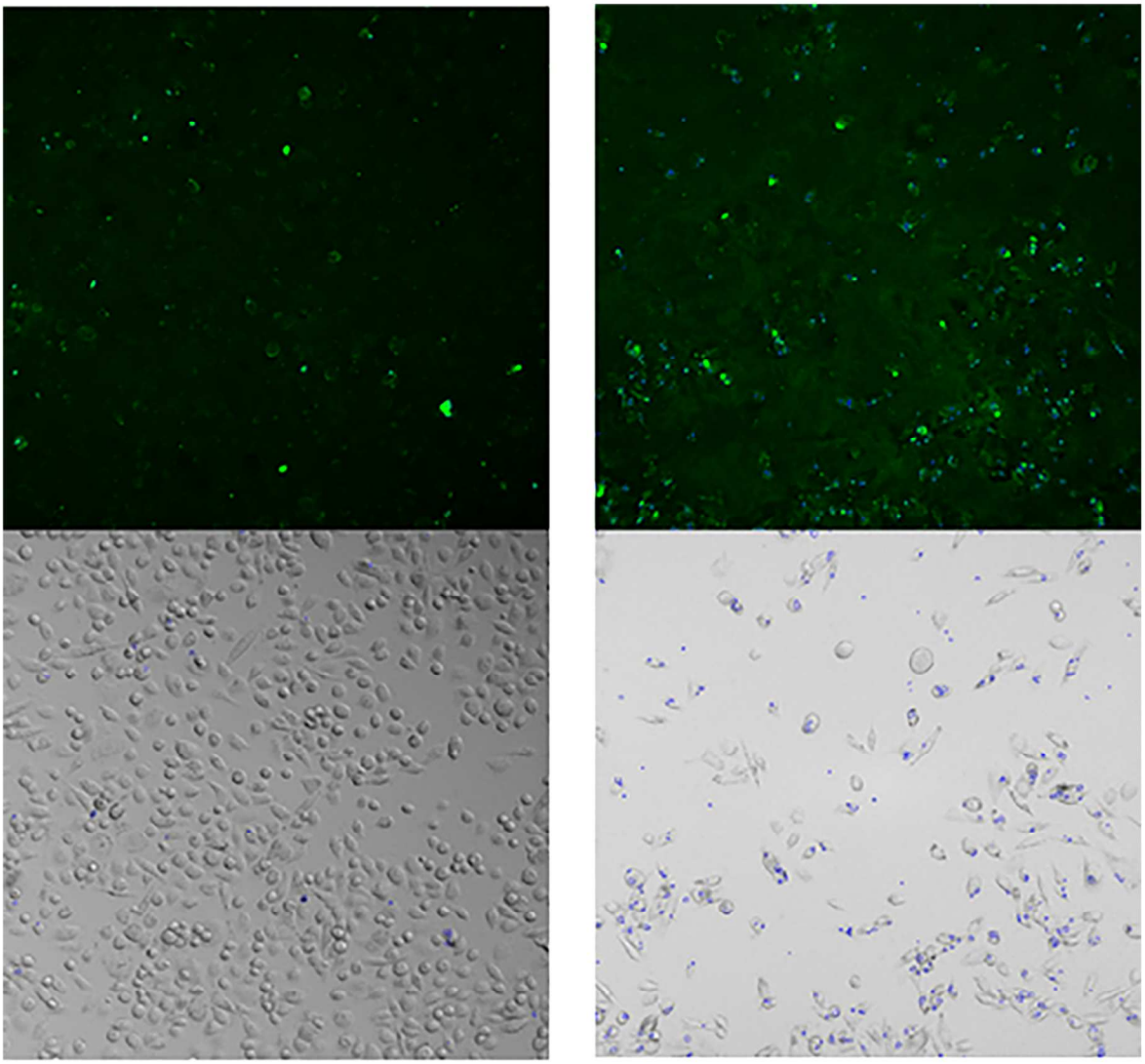


Figure S22 Fluorescent microscope images of PC-3 cells treated with V-shape FITC-siRNA containing 2 FITC probes 4 and 24 hours post transfection.

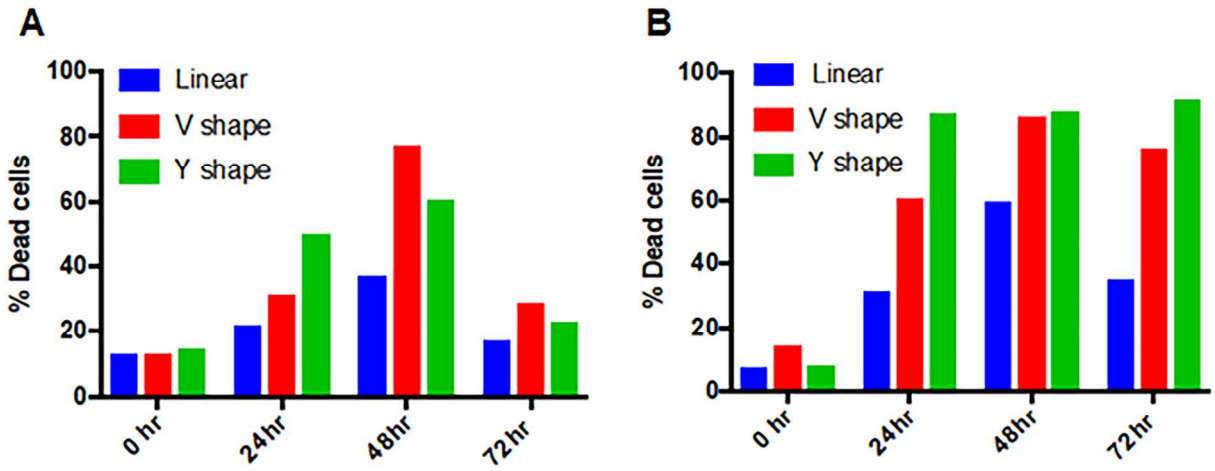


Figure S23 Cell viability determined using PI on flow cytometry, adhered cells (**A**) and supernatant cells (**B**). After 24, 48 and 72 h, the supernatant was collected, and the remaining cells were removed using trypsin. Cell samples were pelleted and re-suspended in 1% BSA in PBS (1 mL) and stained using PI according the manufacturer's recommendation.