

**D-/L-isothymidine incorporation in core sequence of aptamer BC15
enhanced its binding affinity to hnRNP A1 protein**

Electronic Supplementary Information

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Content

Figure S1. HPLC separation of BC15-31 and the variants (BC15-31 as example, the trace shown here is representative for the purification of all variants of the parent oligonucleotide, regardless of the number and location of the isoT residues in each case). Dionex UltiMate 3000 HPLC, XBridge™ OST C18 column (2.5 μm, 10 mm × 50 mm), gradient program: 15-35% eluent A in 35 min (A: CH₃CN; B: 0.1 M Et₃N-CH₃COOH in water, pH = 7.7), column temperature 40 °C, flow rate 1.5 mL/min.

Figure S2. Purity identification of BC15-31 and the variants by analytical HPLC (Since all oligos had similar traces upon their initial purification (Figure S1) and the analogous oligo peak were collected in all cases, the post-purification assay data are expected to be very similar for all oligos, parent BC15-31 oligo and 4 randomly selected variants were assayed to representatively verify the purity. Trace a: parent BC15-31, trace b: BC15-31-3_L, trace c: BC15-31-26_D, trace d: BC15-31-3_L30_D, trace e: BC15-31-3_D15_D). Agilent 1200 HPLC, XBridge™ OST C18 column (2.5 μm, 10 mm × 50 mm), gradient program: 0-25% eluent A in 35 min (A: CH₃CN; B: 0.1 M Et₃N-CH₃COOH in water, pH = 7.7), column temperature 25 °C, flow rate 1.5 mL/min.

Figure S3. MALDI-TOF-MS of BC15-31 and its variants.

Figure S4. Surface plasmon resonance of BC15 (A) and BC15-31 (B) and BC15-27 (C) and NC (D) with hnRNP A1. DNA sequences are given in the section of ‘Materials and Methods’. The concentrations of protein were 5, 10, 50, 100, 200, 500, 1000nM, respectively. The data were fitted in a Langmuir Binding (1:1) model.

Figure S5. ELISA results of mismatched BC15-31 variants and hnRNP A1.

Figure S6. Characterization of Bis-D-/L-isoT-modified BC15-31 combine with hepatocarcinoma slides by fluorescent inverted microscope imaging (10×). BC15-31 and blank as control. Hepatocarcinoma slides were incubated with FAM-labeled different aptamers. Basal brightness of three groups is different because of the various hepatocarcinoma slides thickness. Bar = 100 μm.

Table S1. Mass spectrometry analysis results of the potential protein targets of BC15-31 (A) and BC15 lane (B)

Table S2. Sequences of mismatched BC15-31 variants.

Table S3. BC15-31 and D/L-isoT modified variants.

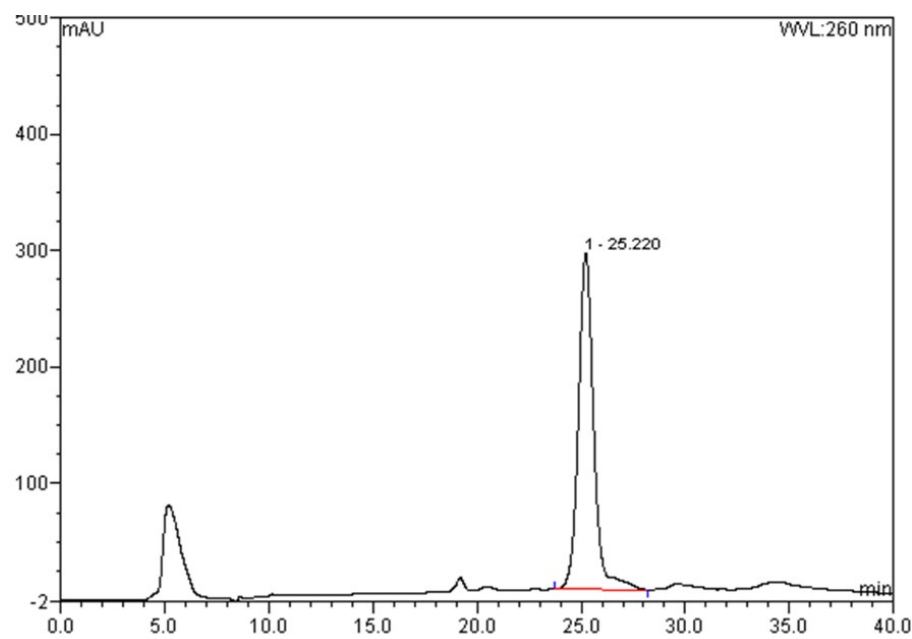


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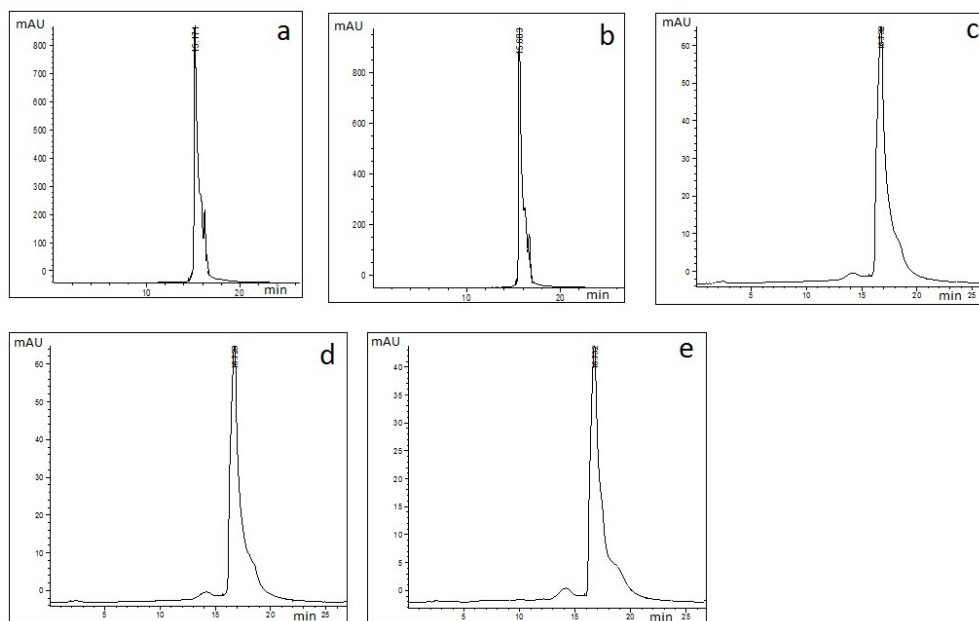


Figure S2. Purity identification of BC15-31 and the variants by analytical HPLC (Since all oligos had similar traces upon their initial purification (Figure S1) and the analogous oligo peak were collected in all cases, the post-purification assay data are expected to be very similar for all oligos, parent BC15-31 oligo and 4 randomly selected variants were assayed to representatively verify the purity. Trace a: parent BC15-31, trace b: BC15-31-3_L, trace c: BC15-31-26_D, trace d: BC15-31-3_L30_D, trace e: BC15-31-3_D15_D). Agilent 1200 HPLC, XBridge™ OST C18 column (2.5 μ m, 10 mm \times 50 mm), gradient program: 0-25% eluent A in 35 min (A: CH₃CN; B: 0.1 M Et₃N-CH₃COOH in water, pH = 7.7), column temperature 25 °C, flow rate 1.5 mL/min.

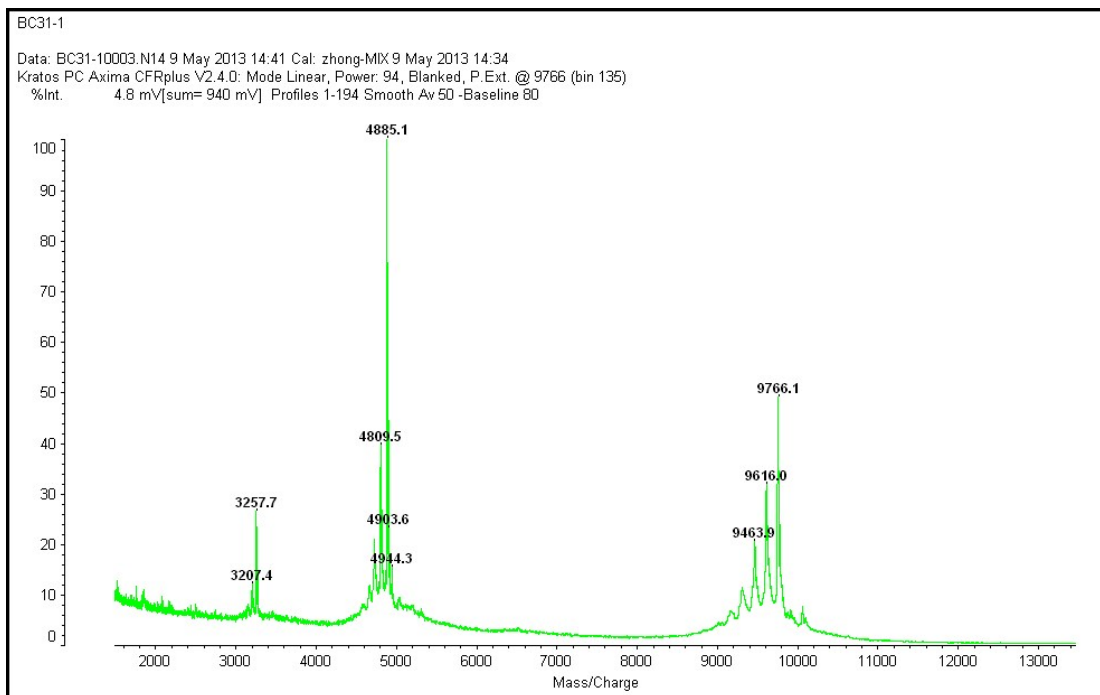
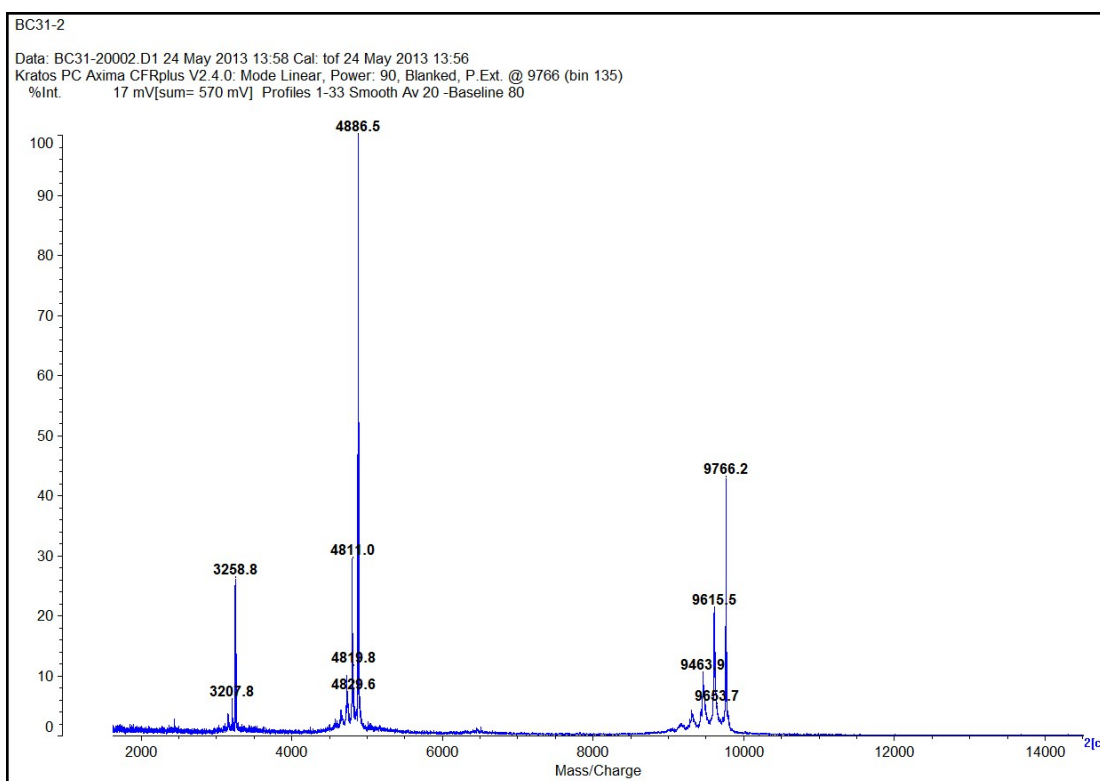
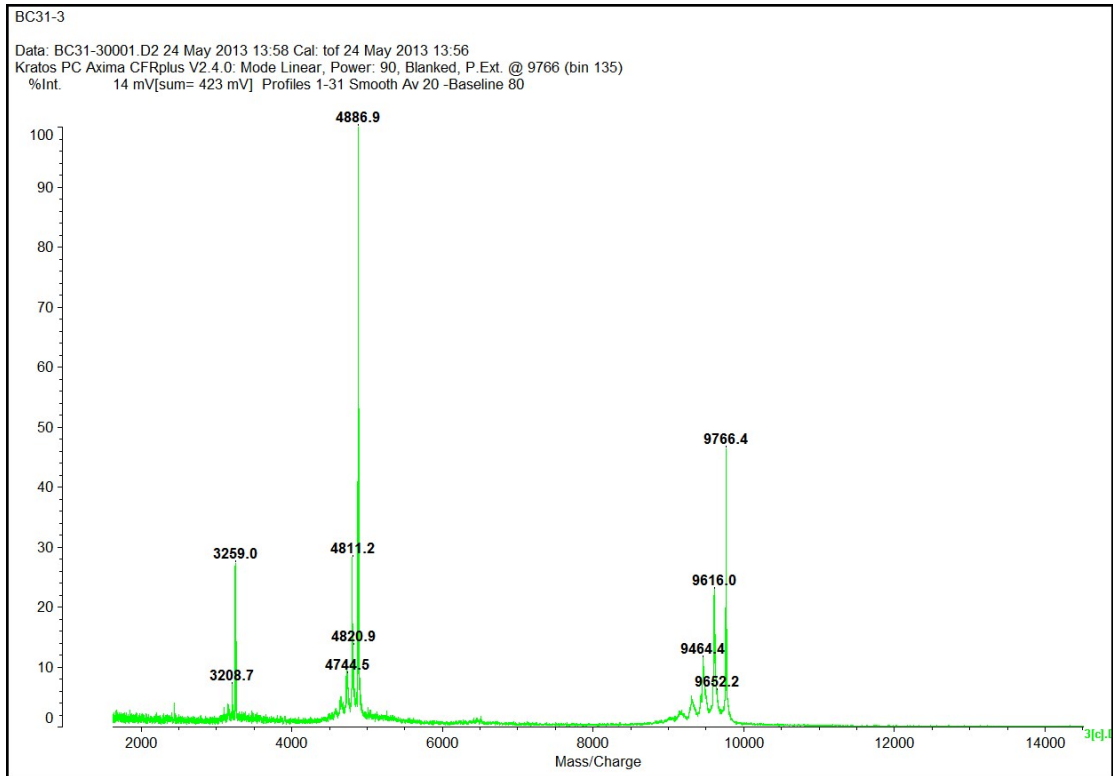


Figure S3. MALDI-TOF-MS of BC15-31 and its variants.

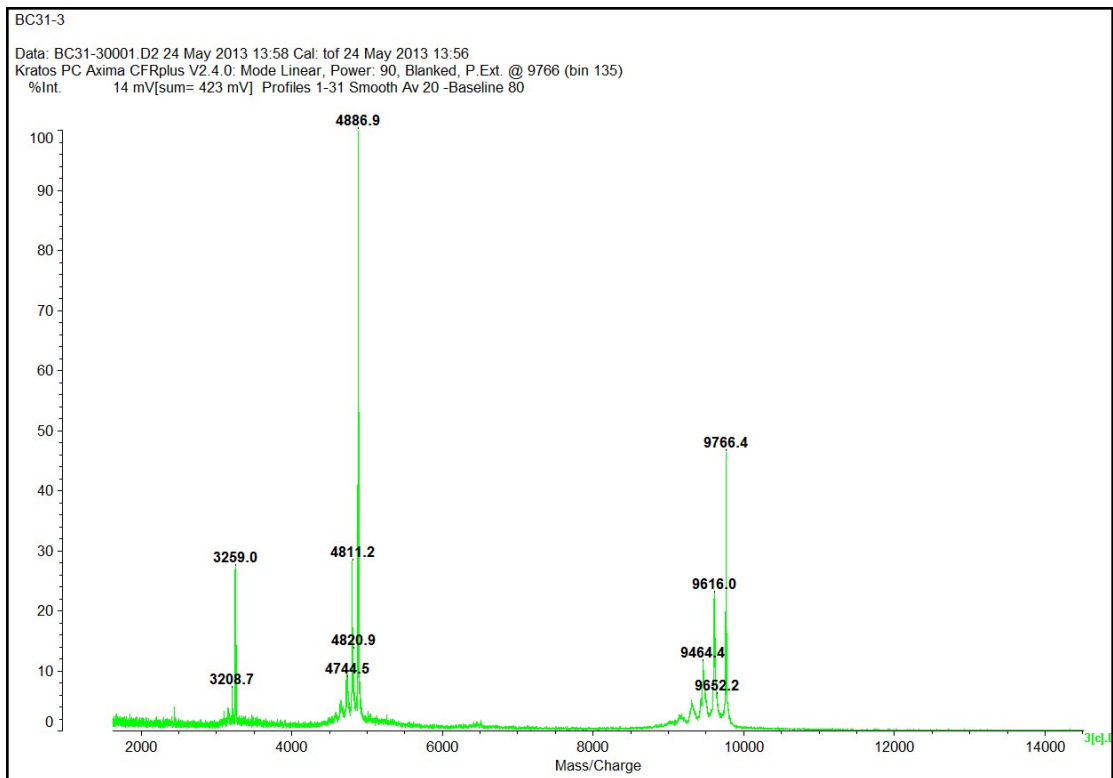
BC15-31, Found: 9766; Calcd: 9765.



BC15-31-3L, Found: 9766; Calcd: 9765.



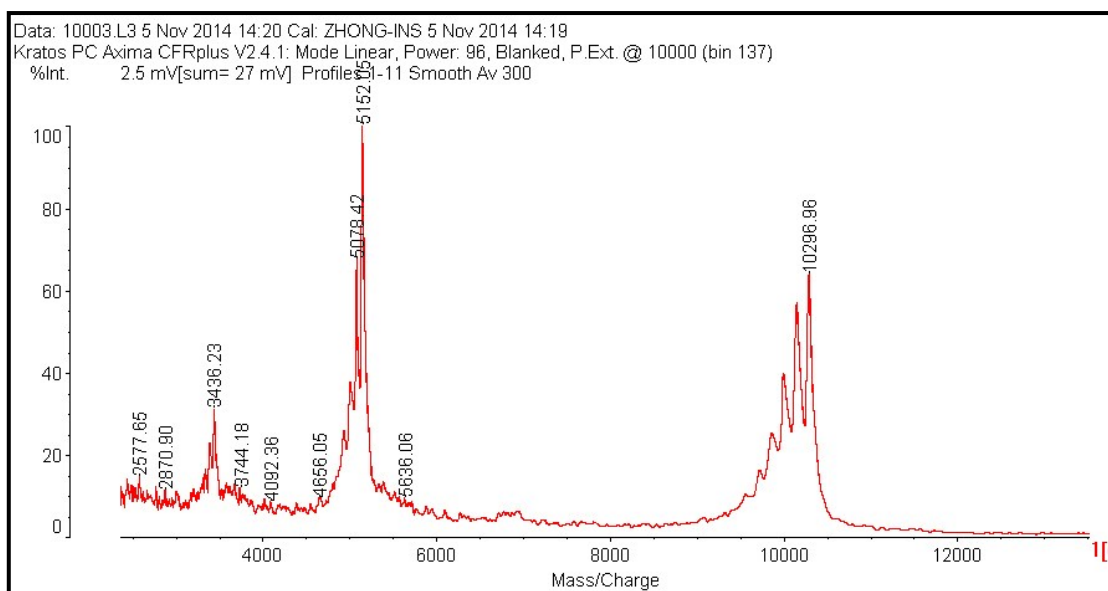
BC15-31-3D, Found: 9766; Calcd: 9765.



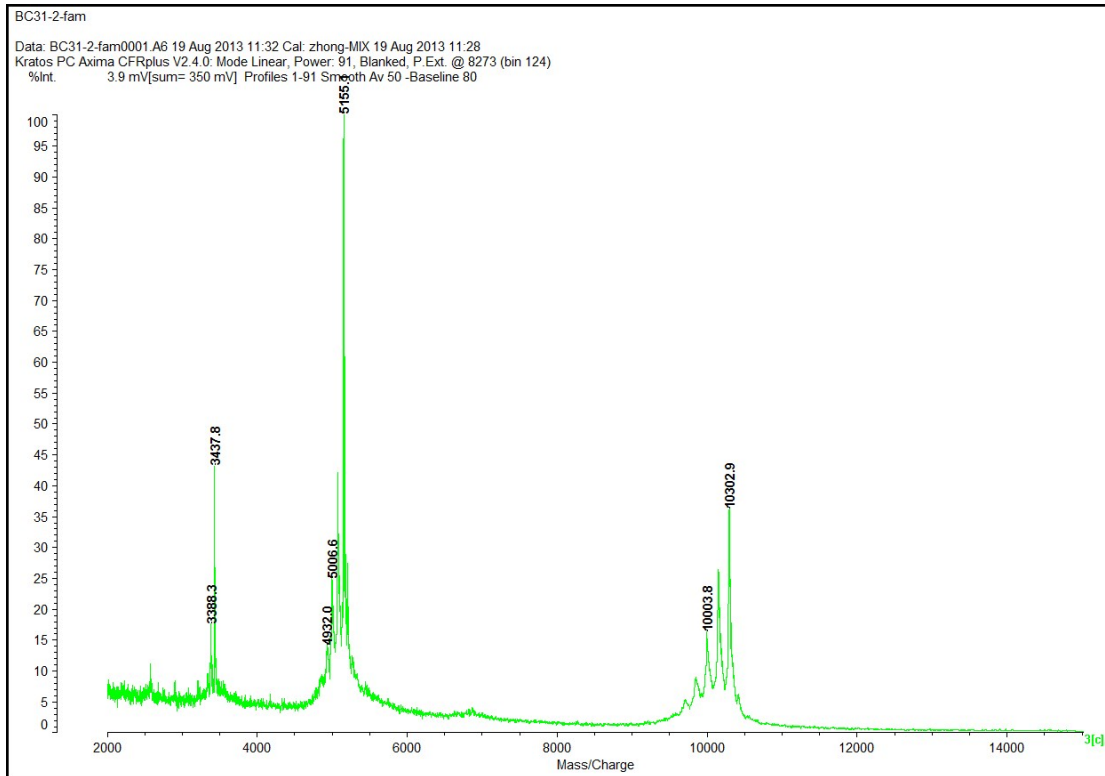
BC15-31-3L/30L, Found: 9766; Calcd: 9765.



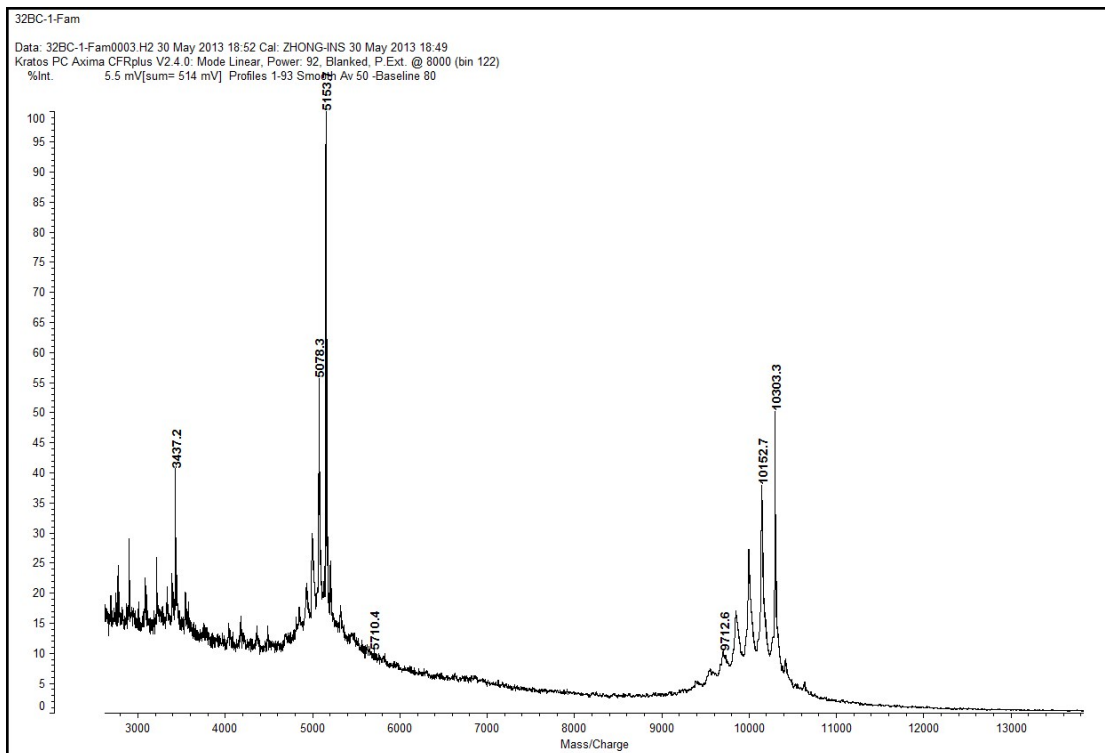
BC15-31-1L/30L, Found: 9766; Calcd: 9765.



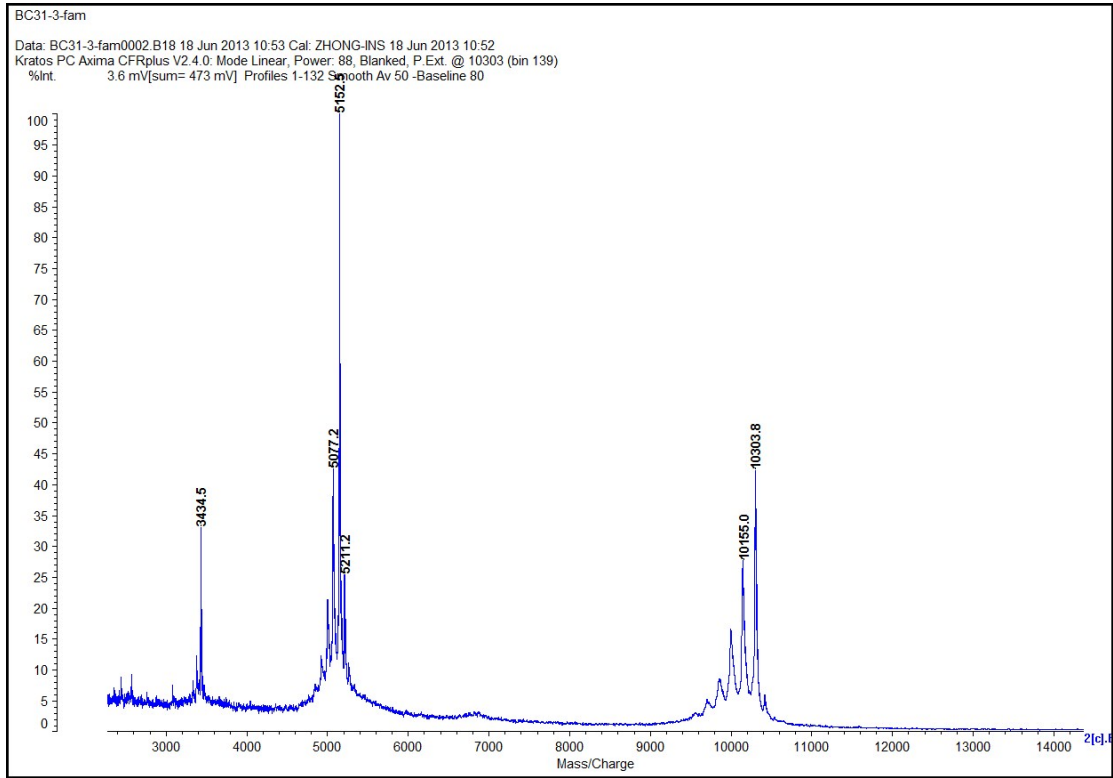
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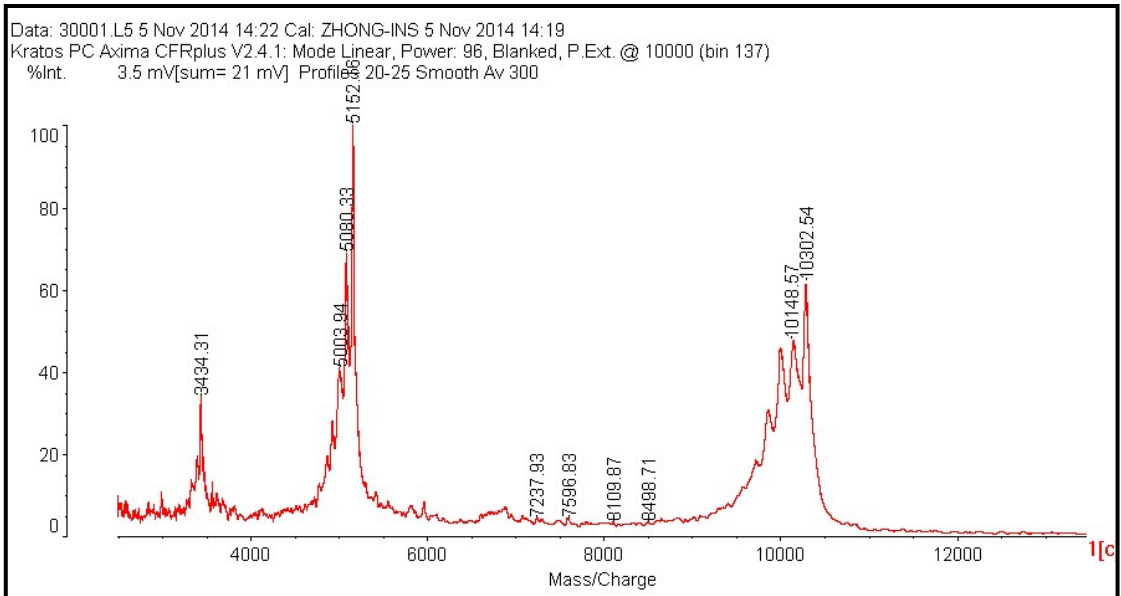
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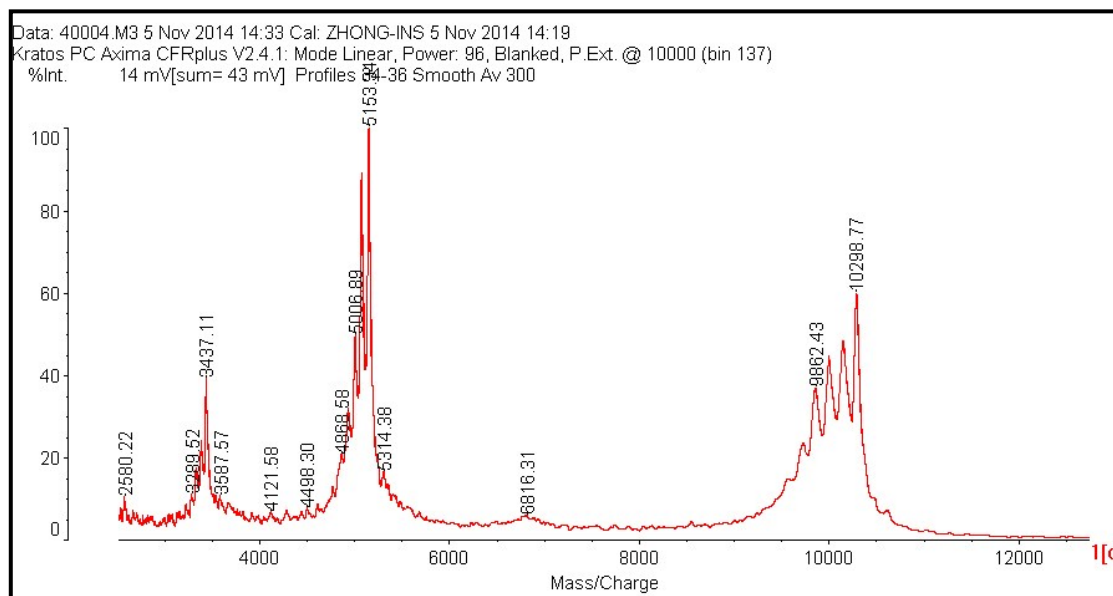
Fam-BC15-31-1L/3L, Found: 10303; Calcd: 10302.



Fam-BC15-31-3L/30L, Found: 10303; Calcd: 10302.



Fam-BC15-31-3D/30D, Found: 10302; Calcd: 10302.



Fam-BC15-31-3L/30D, Found: 10299; Calcd: 10302.

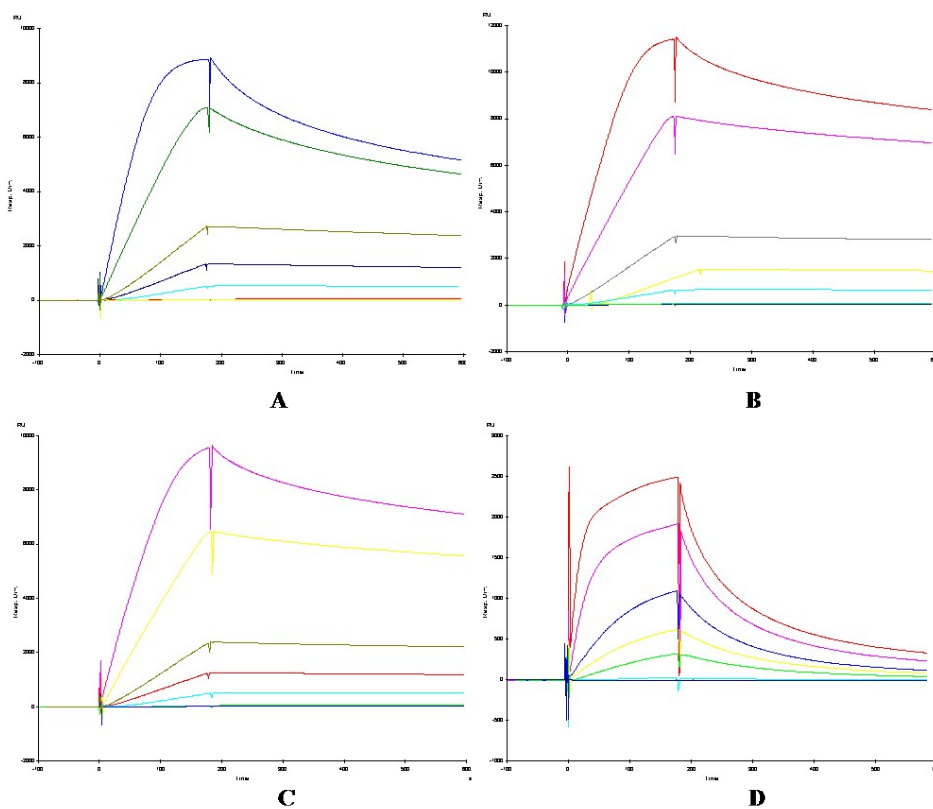


Figure S4. Surface plasmon resonance of BC15 (A) and BC15-31(B) and BC15-27(C) and NC(D) with hnRNP A1.

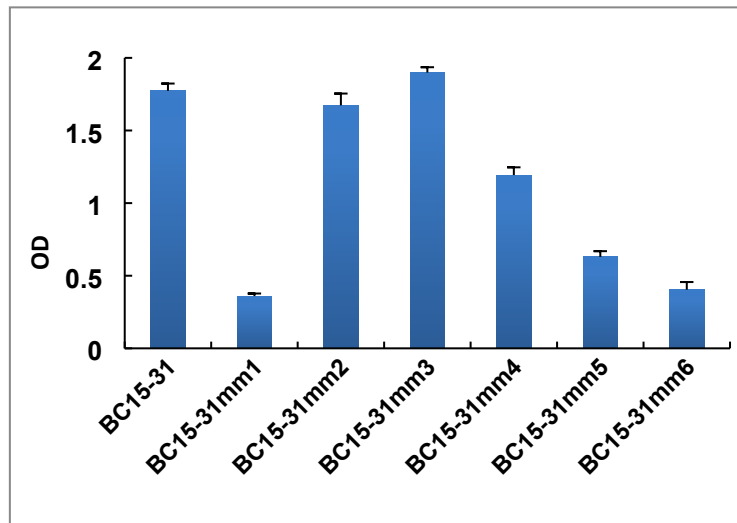


Figure S5. ELISA results of mismatched BC15-31 variants and hnRNP A1.

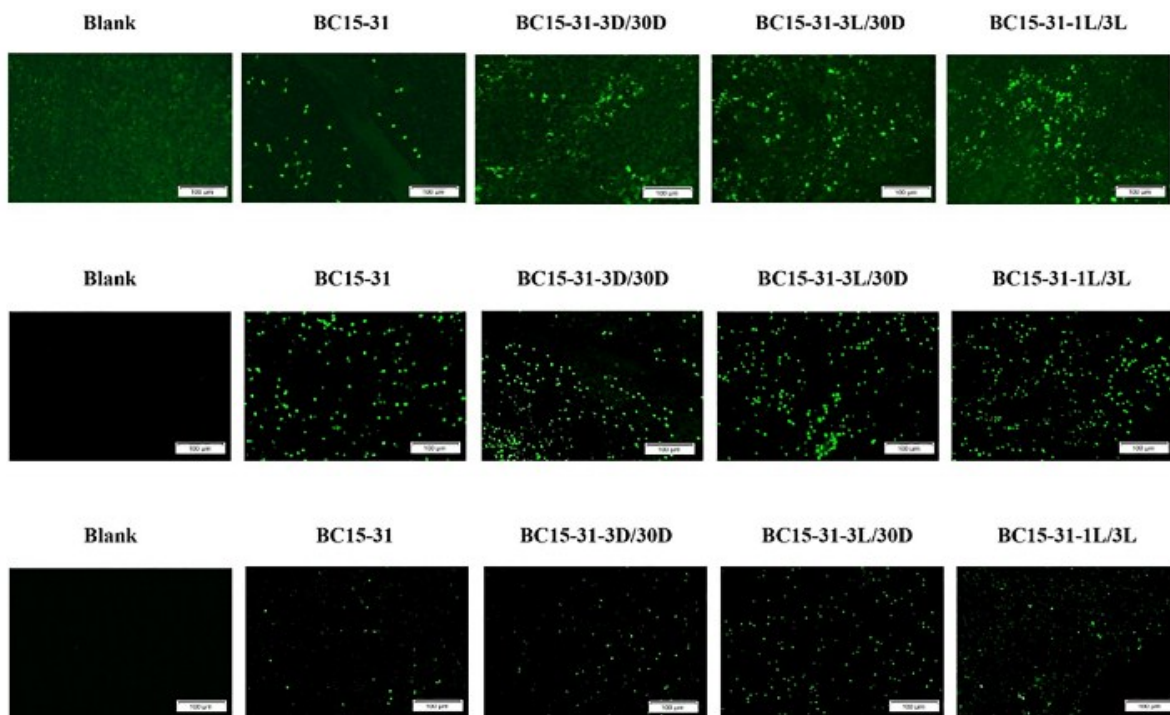


Figure S6. Characterization of Bis-D-/L-IsoT-modified BC15-31 combine with hepatocarcinoma slides by fluorescent inverted microscope imaging (10×). BC15-31 and blank as control.

Table S1. Mass spectrometry analysis results of the potential protein targets of BC15-31 (A) and BC15 lane (B)

A

Accession	Description	Score
F8W617	Heterogeneous nuclear ribonucleoprotein A1 OS=Homo sapiens GN=HNRNPA1 PE=2 SV=2 - [F8W617_HUMAN]	1053
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 - [ROA2_HUMAN]	833
Q15717	ELAV-like protein 1 OS=Homo sapiens GN=ELAVL1 PE=1 SV=2 - [ELAV1_HUMAN]	146
Q13151	Heterogeneous nuclear ribonucleoprotein A0 OS=Homo sapiens GN=HNRNPA0 PE=1 SV=1 - [ROA0_HUMAN]	123
Q02878	60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=1 SV=3 - [RL6_HUMAN]	107

B

Accession	Description	Score
F8W617	Heterogeneous nuclear ribonucleoprotein A1 OS=Homo sapiens GN=HNRNPA1 PE=2 SV=2 - [F8W617_HUMAN]	961
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 - [ROA2_HUMAN]	920
Q15717	ELAV-like protein 1 OS=Homo sapiens GN=ELAVL1 PE=1 SV=2 - [ELAV1_HUMAN]	137
Q02878	60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=1 SV=3 - [RL6_HUMAN]	124
Q13151	Heterogeneous nuclear ribonucleoprotein A0 OS=Homo sapiens GN=HNRNPA0 PE=1 SV=1 - [ROA0_HUMAN]	121
P04264	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 - [K2C1_HUMAN]	106

Table S2. Sequences of mismatched BC15-31 variants

Name	Sequence
BC15-31mm1	5'-TCT CCC CACCTA CCT CCC CTC TCT CTC TAT C-3'
BC15-31mm2	5'-TGT GGC GAG GTA GGT CCC CTG TGT GTG TATC-3'
BC15-31mm3	5'-TGT CCC GAG GTA GGT GGG GTG TGT GTG TATC-3'
BC15-31mm4	5'-TGT GGC GAG GTA GGT GGG GTC TCT CTC TATC-3'
BC15-31mm5	5'-TGT GGC GAG GTA GGT GGG GAG AGA GAG TATC-3'
BC15-31mm6	5'-TGT GGC GAG GTA GGT GGG GAC ACA CAC TATC-3'

Table S3. BC15-31 and D/L-isoT modified variants

Name	Sequence(5'-3')	MALDI-TOF-MS	
		Calcd.	Found
BC15-31	5'-TGTGGCGAGGTAGGTGGGGTGTGTGTGTATC-3'	9765	9766
BC15-31-3L	5'-TGT _L GGCGAGGTAGGTGGGGTGTGTGTGTATC-3'	9765	9766
BC15-31-3D	5'-TGT _D GGCGAGGTAGGTGGGGTGTGTGTGTATC-3'	9765	9766
BC15-31-15L	5'-TGTGGCGAGGTAGGT _L GGGGTGTGTGTGTATC-3'	9765	9766
BC15-31-15D	5'-TGTGGCGAGGTAGGT _D GGGGTGTGTGTGTATC-3'	9765	9766
BC15-31-22L	5'-TGTGGCGAGGTAGGTGGGGTGT _L GTGTGTATC-3'	9765	9766
BC15-31-22D	5'-TGTGGCGAGGTAGGTGGGGTGT _D GTGTGTATC-3'	9765	9766
BC15-31-24L	5'-TGTGGCGAGGTAGGTGGGGTGTGT _L GTGTATC-3'	9765	9766
BC15-31-24D	5'-TGTGGCGAGGTAGGTGGGGTGTGT _D GTGTATC-3'	9765	9766
BC15-31-26L	5'-TGTGGCGAGGTAGGTGGGGTGTGTGT _L GTATC-3'	9765	9766
BC15-31-26D	5'-TGTGGCGAGGTAGGTGGGGTGTGTGT _D GTATC-3'	9765	9766
BC15-31-28L	5'-TGTGGCGAGGTAGGTGGGGTGTGTGTGT _L ATC-3'	9765	9766
BC15-31-30L	5'-TGTGGCGAGGTAGGTGGGGTGTGTGTGTAT _L C-3'	9765	9766
BC15-31-30D	5'-TGTGGCGAGGTAGGTGGGGTGTGTGTGTAT _D C-3'	9765	9766
BC15-31-3L/30L	5'-TGT _L GGC GAG GTA GGT GGG GTG TGT GTG TAT _L C-3'	9765	9766
BC15-31-1L/30L	5'- _L GT GGC GAG GTA GGT GGG GTG TGT GTG TAT _L C-3'	9765	9766
BC15-31-1L/3L	5'- _L GT _L GGC GAG GTA GGT GGG GTG TGT GTG TATC-3'	9765	9766
BC15-31-3L/30D	5'-TGT _L GGC GAG GTA GGT GGG GTG TGT GTG TAT _D C-3'	9765	9766
BC15-31-3D/30D	5'-TGT _D GGC GAG GTA GGT GGG GTG TGT GTG TAT _D C-3'	9765	9766
BC15-31-15D/30D	5'-TGTGGC GAG GTA GGT _D GGG GTG TGT GTG TAT _D C-3'	9765	9766
BC15-31-3L/15D	5'-TGT _L GGC GAG GTA GGT _D GGG GTG TGT GTGTATC-3'	9765	9766
BC15-31-3D/15D	5'-TGT _D GGC GAG GTA GGT _D GGG GTG TGT GTG TATC-3'	9765	9766