Electronic Supplementary Material (ESI) for Analytical Methods.

Cy5/BHQ dye–quencher pairs in fluorogenic qPCR probes: effects of charge and hydrophobicity

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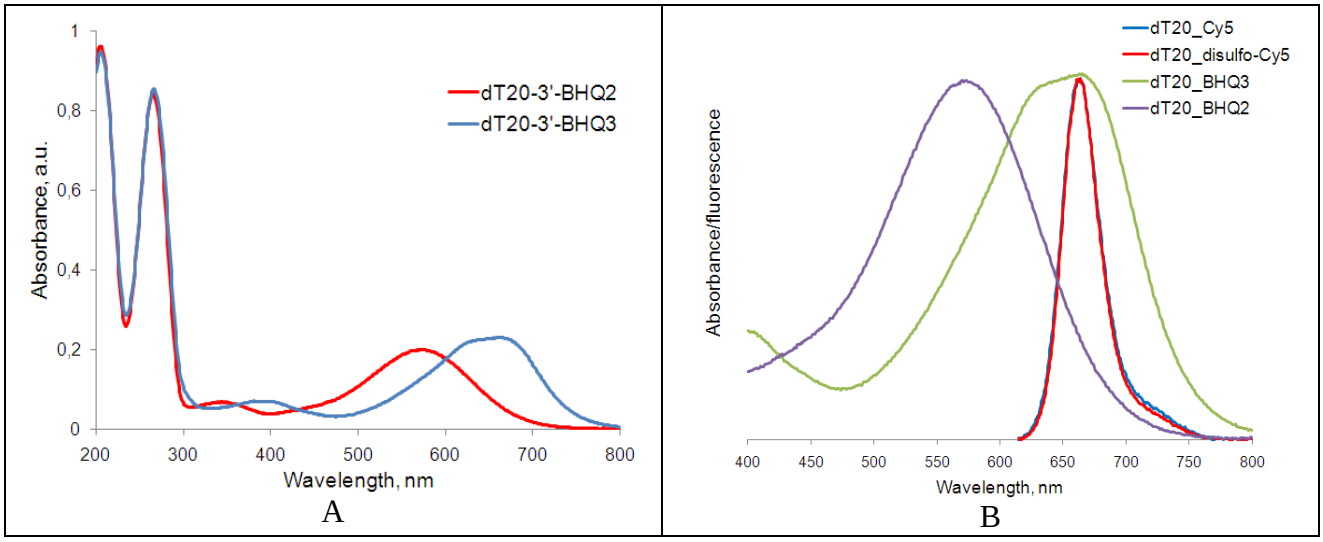


Fig. S1. A) UV/Vis spectra of model T_{20} oligonucleotides modified with BHQ2 and BHQ3 quenchers; B) normalized BHQ2 and BHQ3 absorbance vs Cy5 and disulfo-Cy5 fluorescence on model T_{20} oligonucleotides.

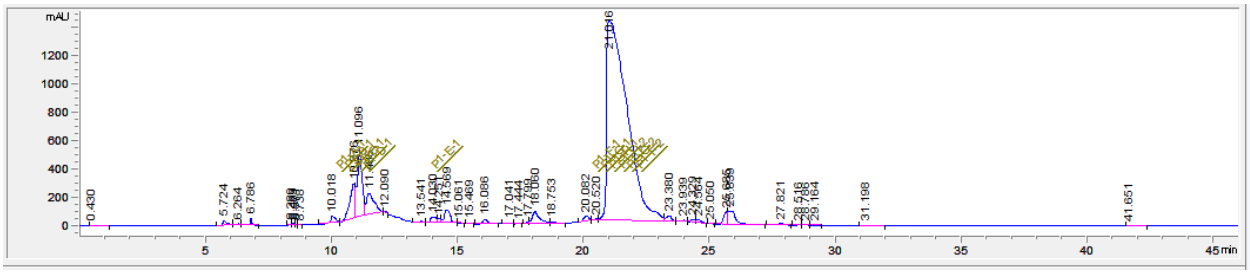


Fig. S2-1. RP-HPLC traces of reaction mixture - sCy5-azide conjugation with **<u>unpurified</u>** G3z-BHQ2 oligonucleotide

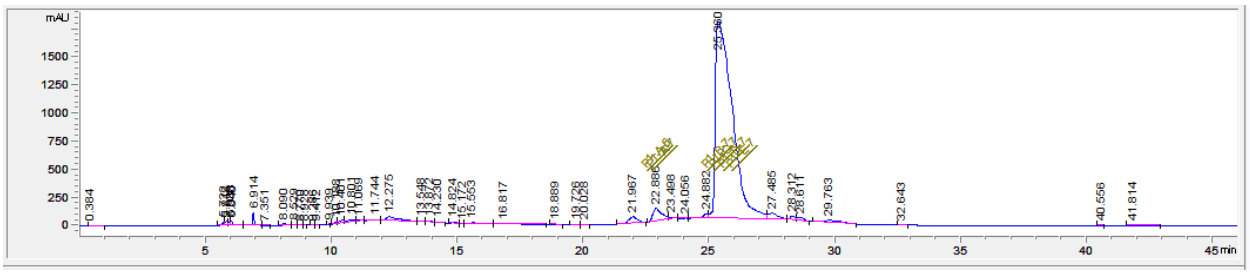
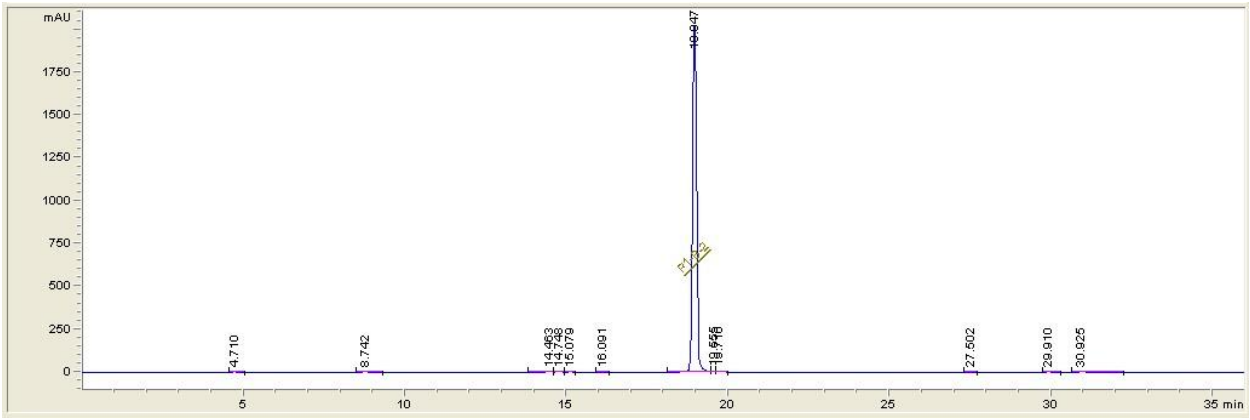


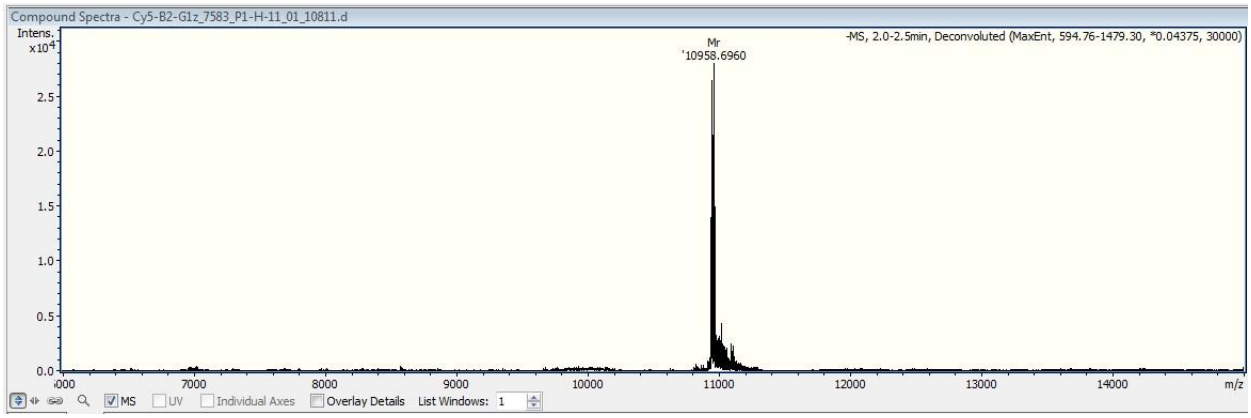
Fig. S2-2. RP-HPLC traces of reaction mixture - Cy5-azide conjugation with **<u>purified</u>** G3z-BHQ3 oligonucleotide

Table S1. Taqman probes and their ESI-MS data.

#	Name	Sequence, 5′→3′	Calcula-ted mass	Detected mass
1	Cy5-B2-G1z	**Cy5**-CTGAGCTTTAGTYAAGGCAAATAATGCTCAG-**BHQ2**	10936.2 10951.3	10943.7 10958.7
2	Cy5-B2-G2z	**Cy5**-CAGCGTCTAGTGATCCCGTTATTGGC-**BHQ2**	9346.2	9354.4
3	Cy5-B2-G3z	**Cy5**-TACCCAACTGAAGCAGCAACAGGGTA-**BHQ2**	9375.3	9383.5
4	Cy5-B2-G4z	**Cy5**-CTGAACYTGTCGGCCATCCTTTGGTT-**BHQ2**	9297.2 9312.2	9305.4 9320.4
5	Cy5-B2-P4z	**Cy5**-CACTCTGACTACTACCTTTAAACAGAGCG-**BHQ2**	10198.8	10206.5
6	Cy5-B2-P8z	**Cy5**-GGACTGCAGTCRTTGCTGTTGAAC-**BHQ2**	8776.8 8792.8	8785.3 8801.3
7	sCy5-B2-G1z	**sCy5**-CTGAGCTTTAGTYAAGGCAAATAATGCTCAG-**BHQ2**	11094.1 11109.2	11089.6 11104.6
8	sCy5-B2-G2z	**sCy5**-CAGCGTCTAGTGATCCCGTTATTGGC-**BHQ2**	9504.1	9500.3
9	sCy5-B2-G3z	**sCy5**-TACCCAACTGAAGCAGCAACAGGGTA-**BHQ2**	9533.2	9529.3
10	sCy5-B2-G4z	**sCy5**-CTGAACYTGTCGGCCATCCTTTGGTT-**BHQ2**	9455.1 9470.1	9451.3 9466.3
11	sCy5-B2-P4z	**sCy5**-CACTCTGACTACTACCTTTAAACAGAGCG-**BHQ2**	10356.7	10352.5
12	sCy5-B2-P8z	**sCy5**-GGACTGCAGTCRTTGCTGTTGAAC-**BHQ2**	8934.7 8950.7	8931.2 8947.2
13	Cy5-B3-G1z	**Cy5**-CTGAGCTTTAGTYAAGGCAAATAATGCTCAG-**BHQ3**	11196.7 11211.7	11203.9 11218.9
14	Cy5-B3-G2z	**Cy5**-CAGCGTCTAGTGATCCCGTTATTGGC-**BHQ3**	9606.6	9614.7
15	Cy5-B3-G3z	**Cy5**-TACCCAACTGAAGCAGCAACAGGGTA-**BHQ3**	9635.7	9643.6
16	Cy5-B3-G4z	**Cy5**-CTGAACYTGTCGGCCATCCTTTGGTT-**BHQ3**	9557.6 9572.6	9565.5 9580.5
17	Cy5-B3-P4z	**Cy5**-CACTCTGACTACTACCTTTAAACAGAGCG-**BHQ3**	10459.2	10466.8
18	Cy5-B3-P8z	**Cy5**-GGACTGCAGTCRTTGCTGTTGAAC-**BHQ3**	9037.3 9053.3	9045.5 9061.5
19	sCy5-B3-G1z	**sCy5**-CTGAGCTTTAGTYAAGGCAAATAATGCTCAG-**BHQ3**	11354.6 11369.6	11349.9 11364.9
20	sCy5-B3-G2z	**sCy5**-CAGCGTCTAGTGATCCCGTTATTGGC-**BHQ3**	9764.5	9760.4
21	sCy5-B3-G3z	**sCy5**-TACCCAACTGAAGCAGCAACAGGGTA-**BHQ3**	9793.6	9789.5
22	sCy5-B3-G4z	**sCy5**-CTGAACYTGTCGGCCATCCTTTGGTT-**BHQ3**	9715.5 9730.5	9711.4 9726.4
23	sCy5-B3-P4z	**sCy5**-CACTCTGACTACTACCTTTAAACAGAGCG-**BHQ3**	10617.1	10612.7
24	sCy5-B3-P8z	**sCy5**-GGACTGCAGTCRTTGCTGTTGAAC-**BHQ3**	9195.2 9211.2	9191.4 9207.4

Fig. S3-1. HPLC profile and ESI MS of **Cy5-B2-G1z** probe.





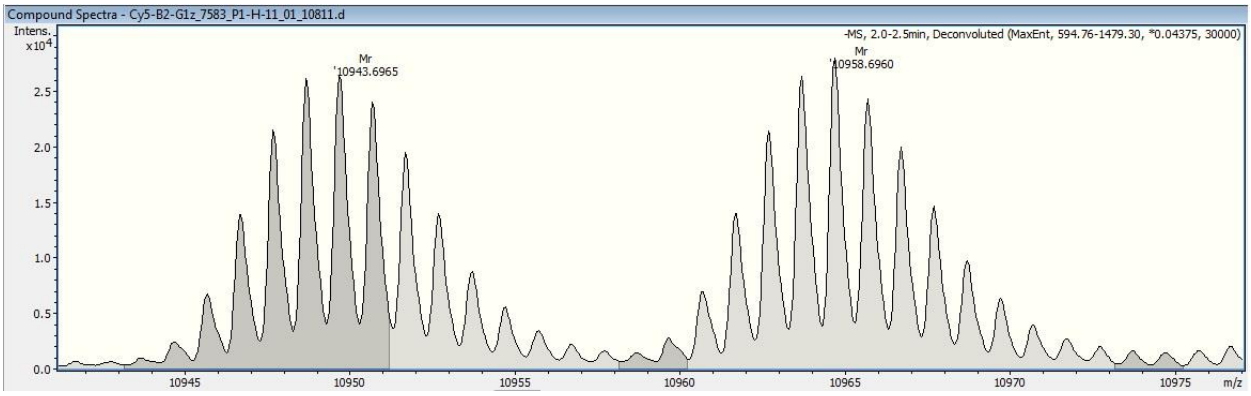
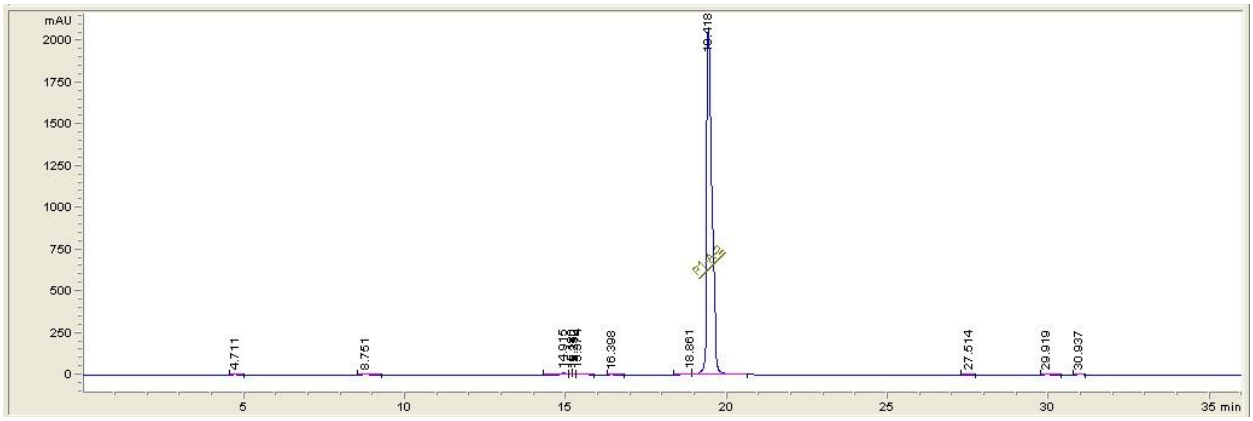


Fig. S3-2. HPLC profile and ESI MS of **Cy5-B2-G2z** probe.



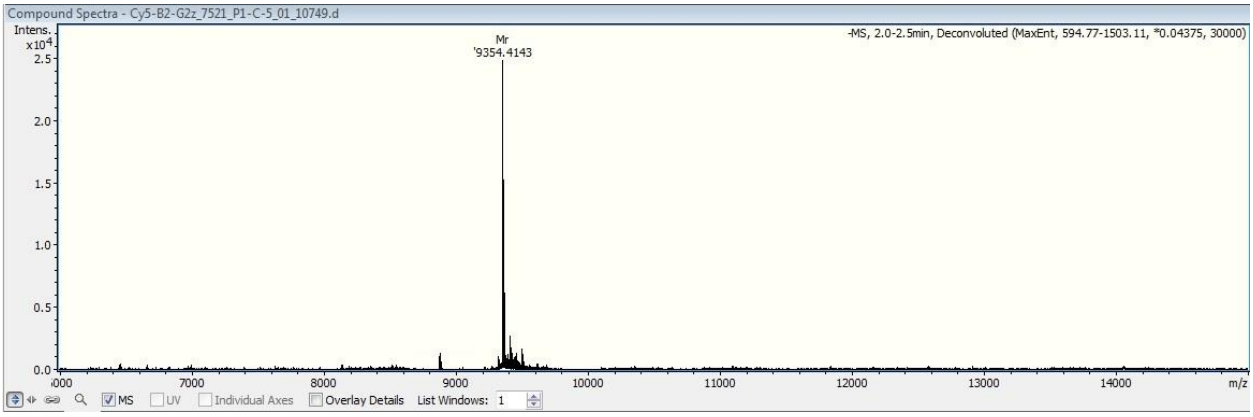
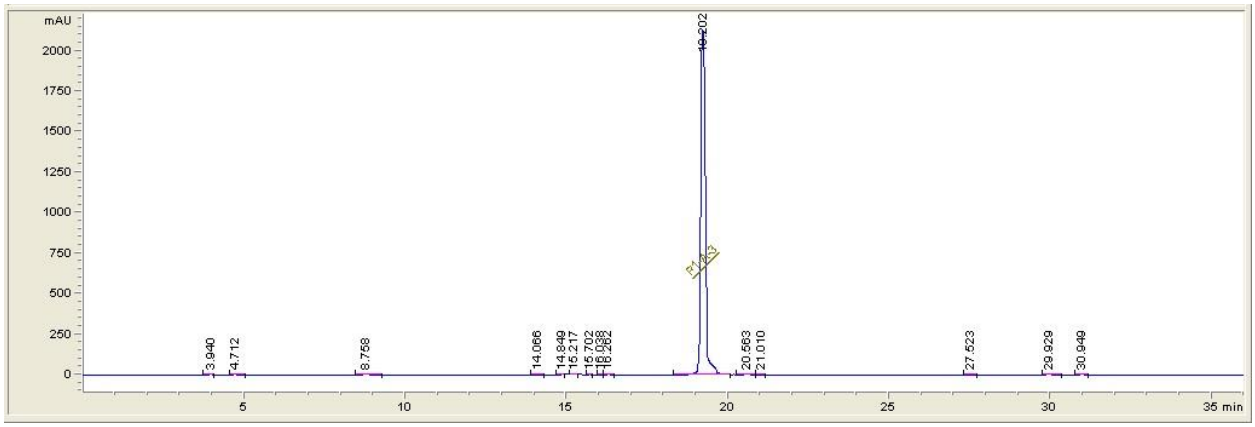


Fig. S3-3. HPLC profile and ESI MS of **Cy5-B2-G3z** probe.



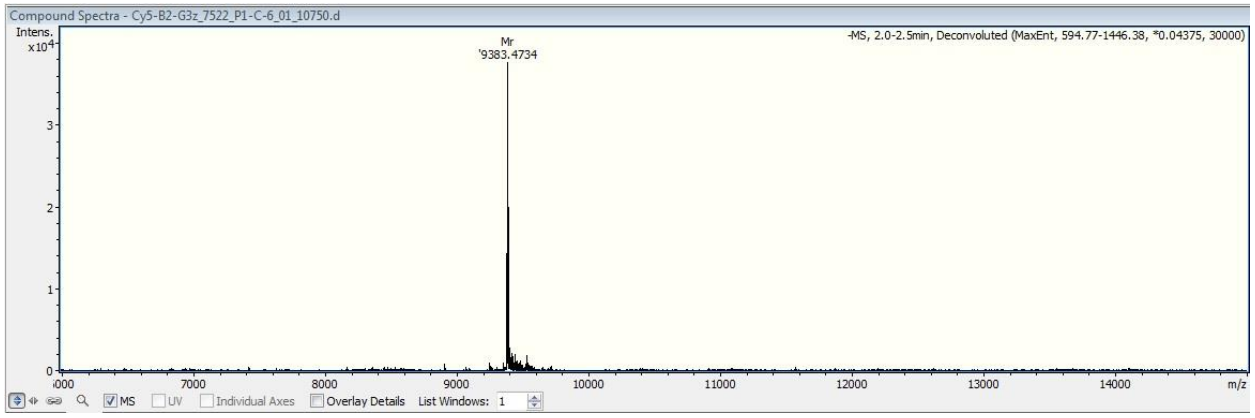
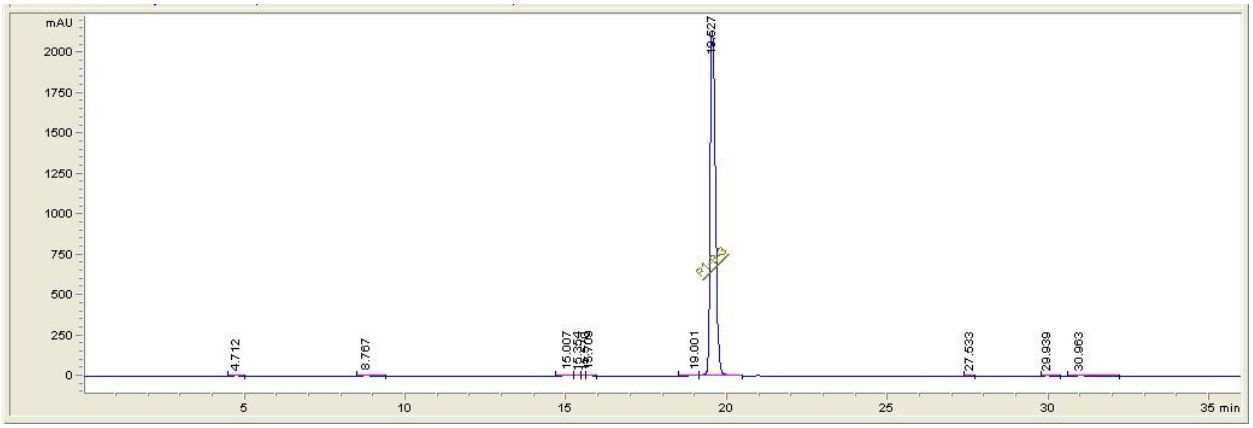
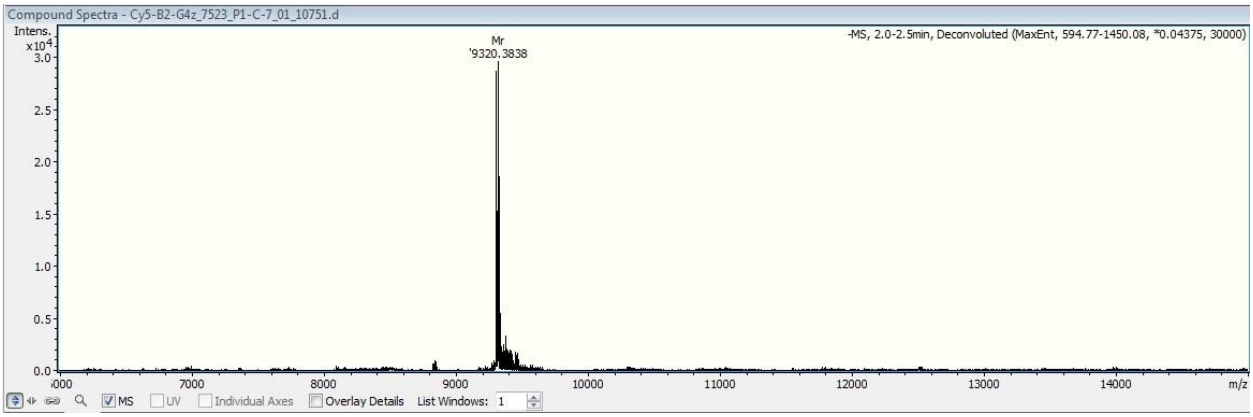


Fig. S3-4. HPLC profile and ESI MS of **Cy5-B2-G4z** probe.





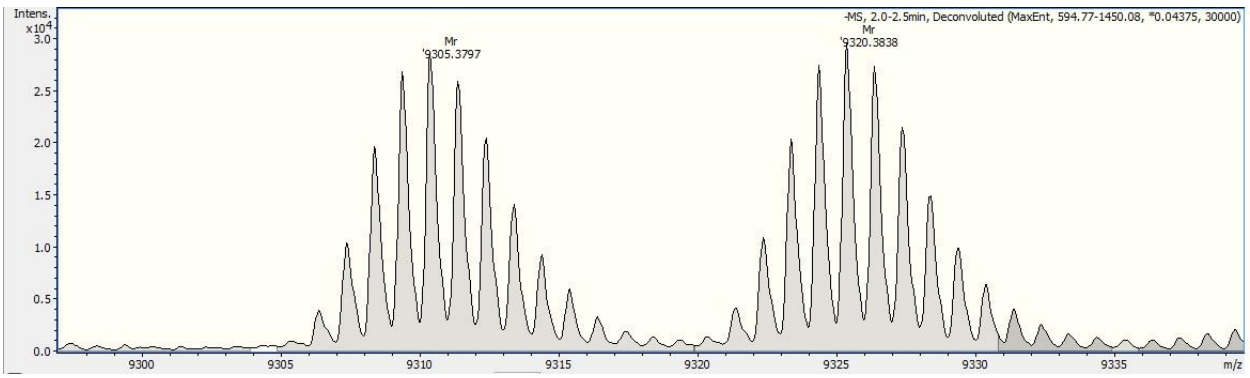
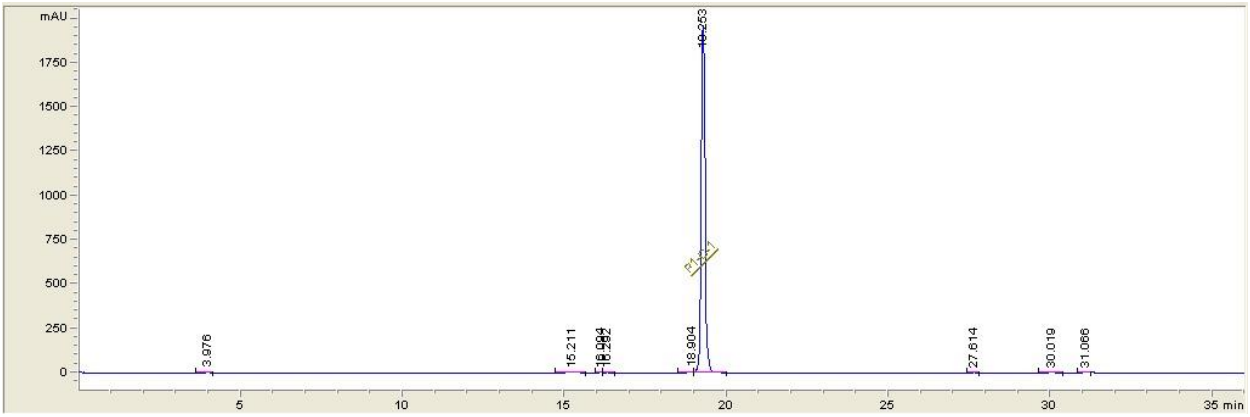


Fig. S3-5. HPLC profile and ESI MS of **Cy5-B2-P4z** probe.



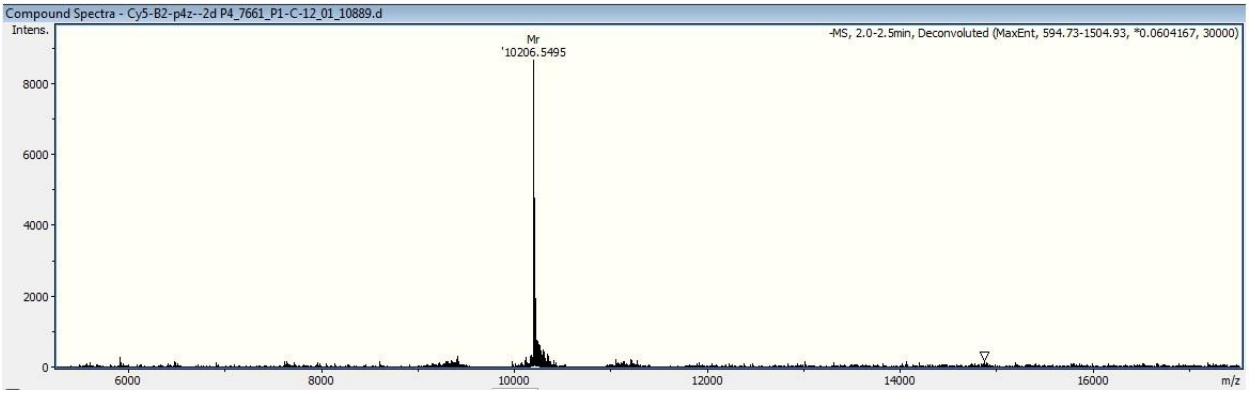
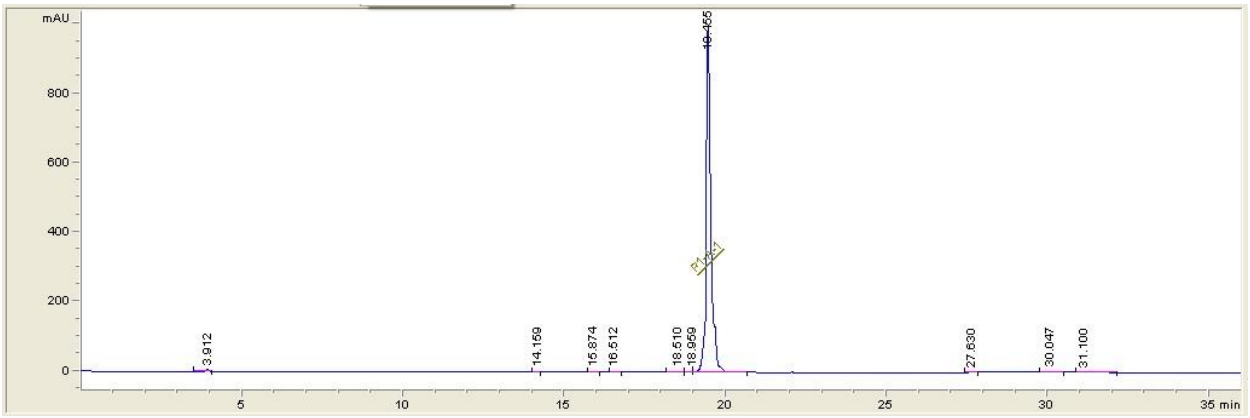
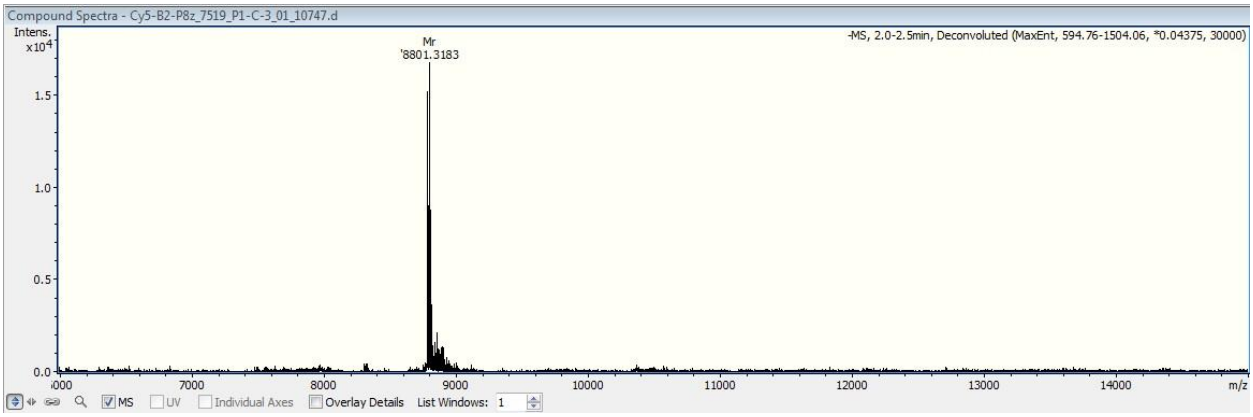


Fig. S3-6. HPLC profile and ESI MS of **Cy5-B2-P8z** probe.





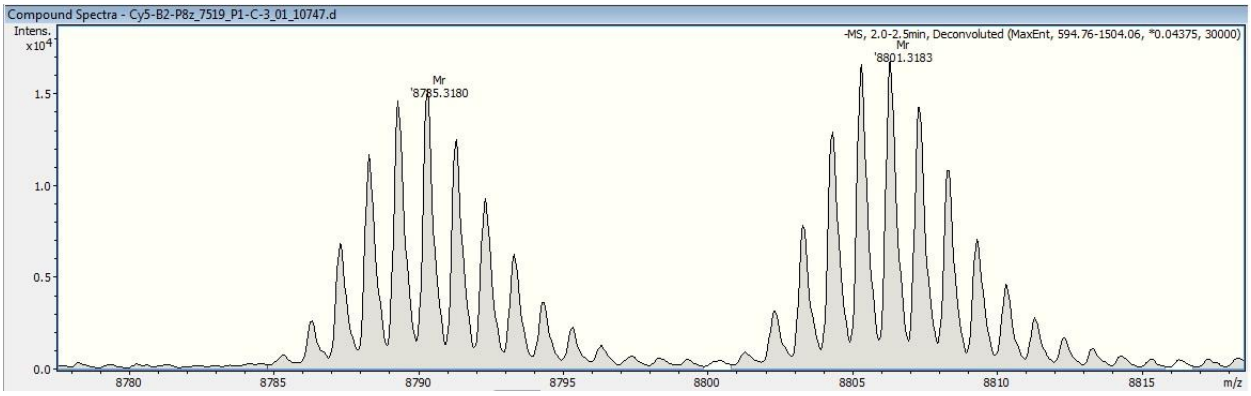


Fig. S3-7. HPLC profile and ESI MS of **sCy5-B2-G1z** probe.

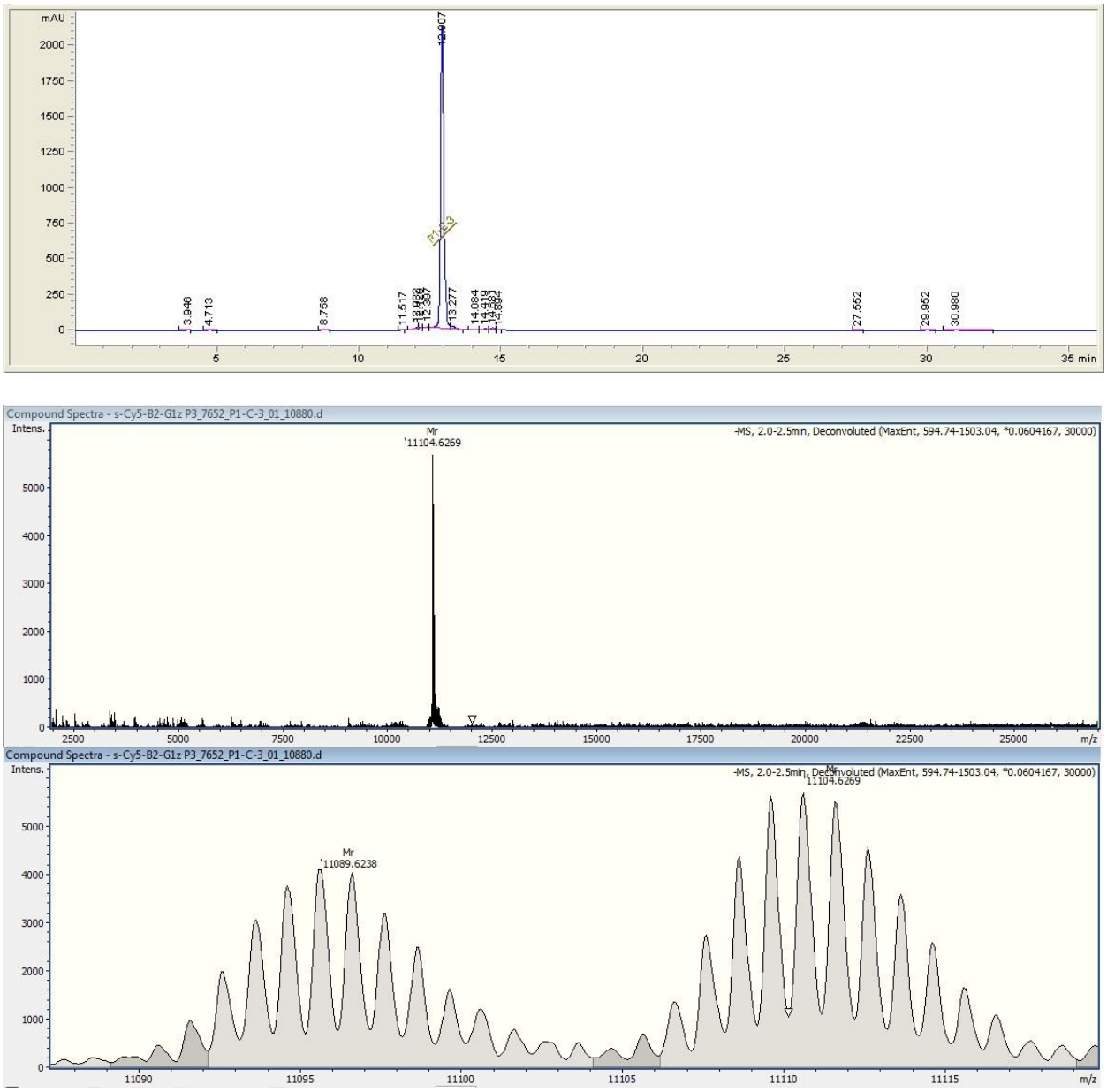
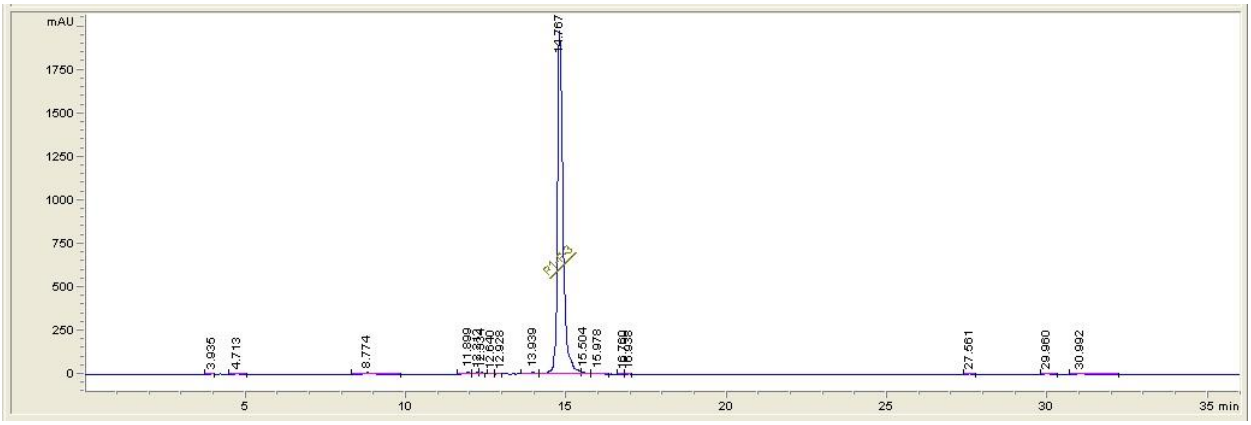


Fig. S3-8. HPLC profile and ESI MS of **sCy5-B2-G2z** probe.



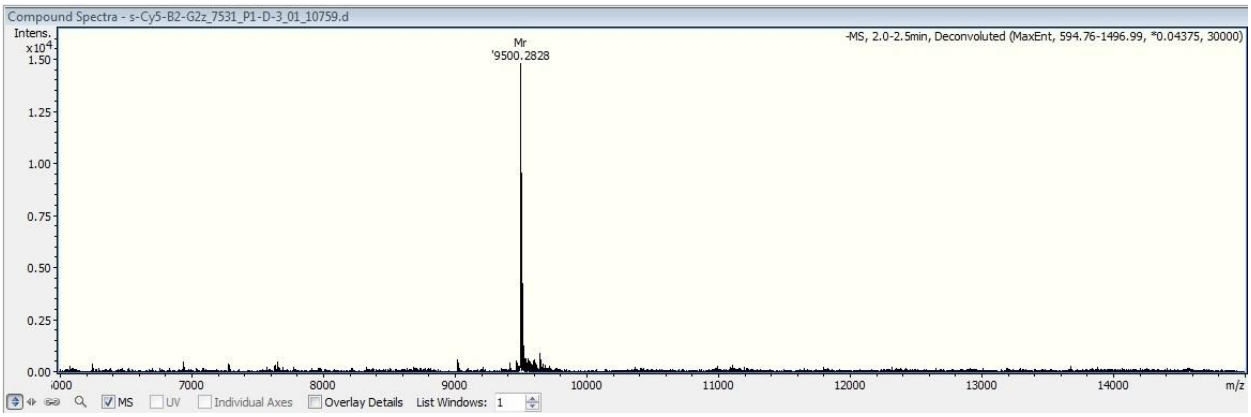
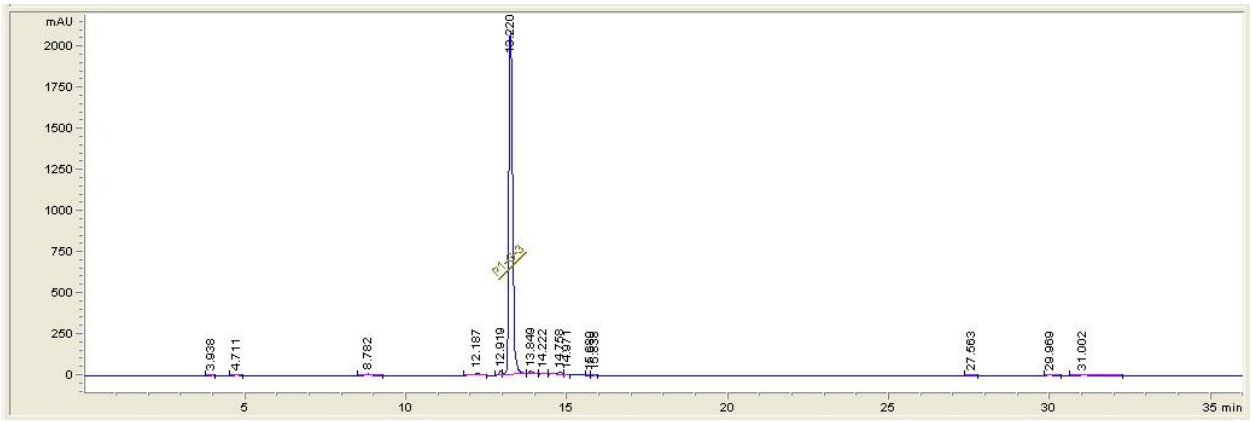


Fig. S3-9. HPLC profile and ESI MS of **sCy5-B2-G3z** probe.



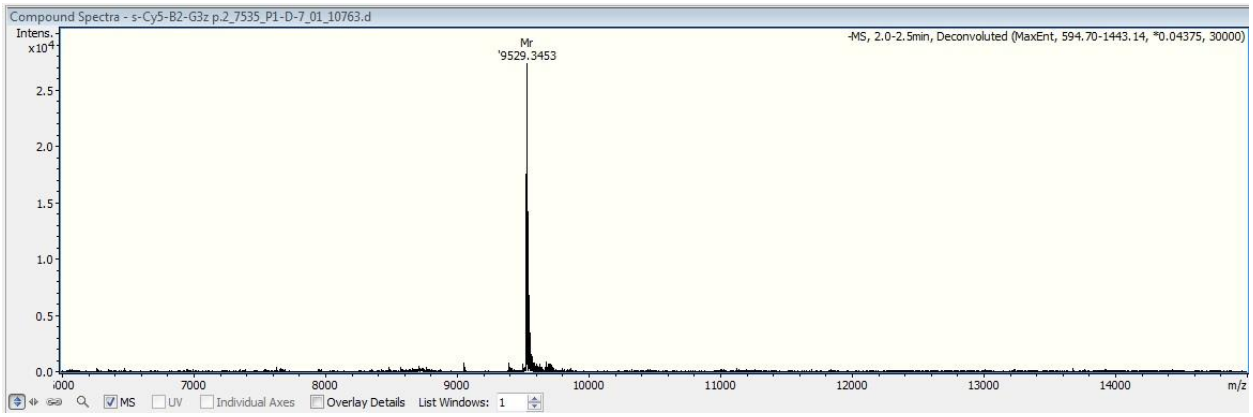
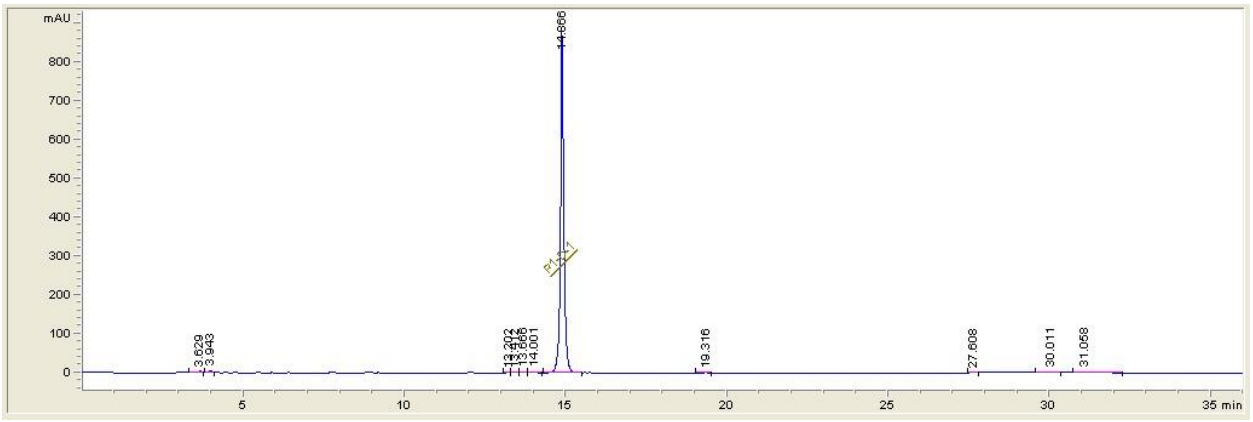
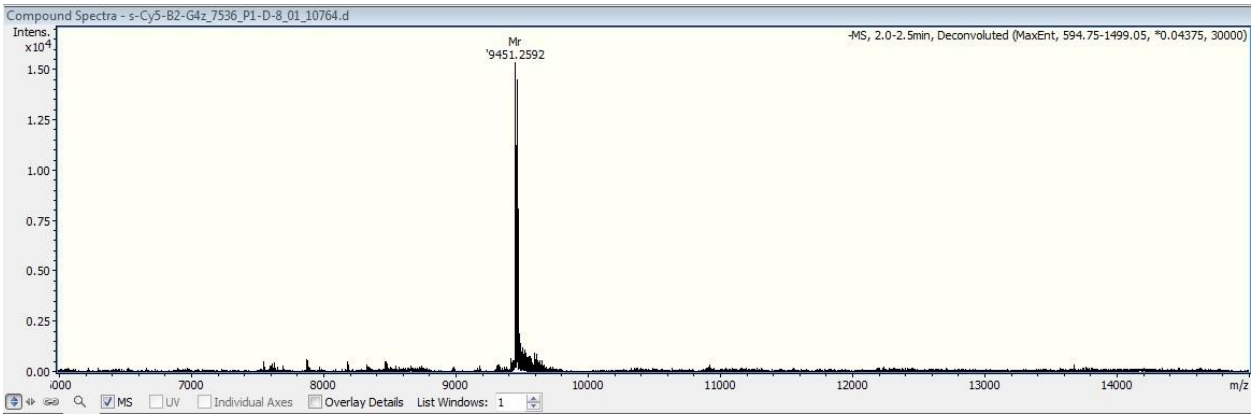


Fig. S3-10. HPLC profile and ESI MS of **sCy5-B2-G4z** probe.





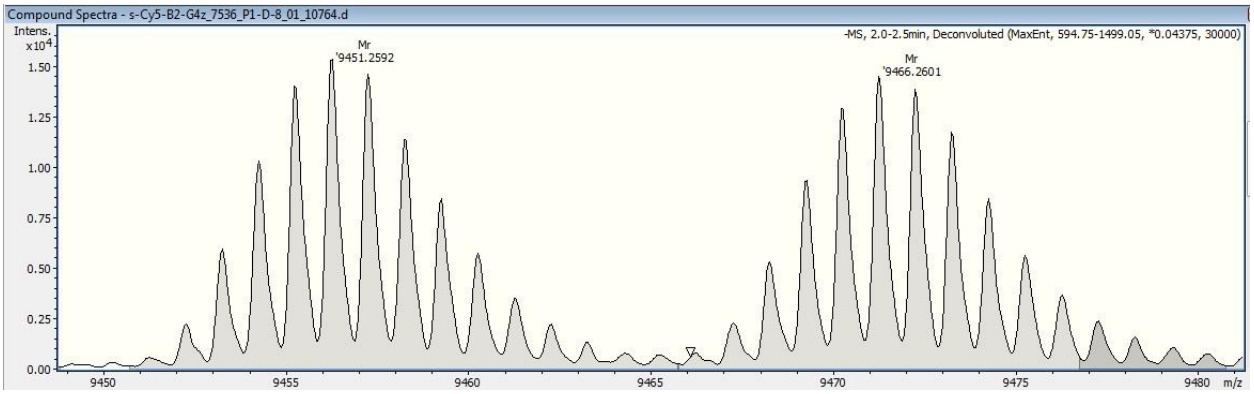
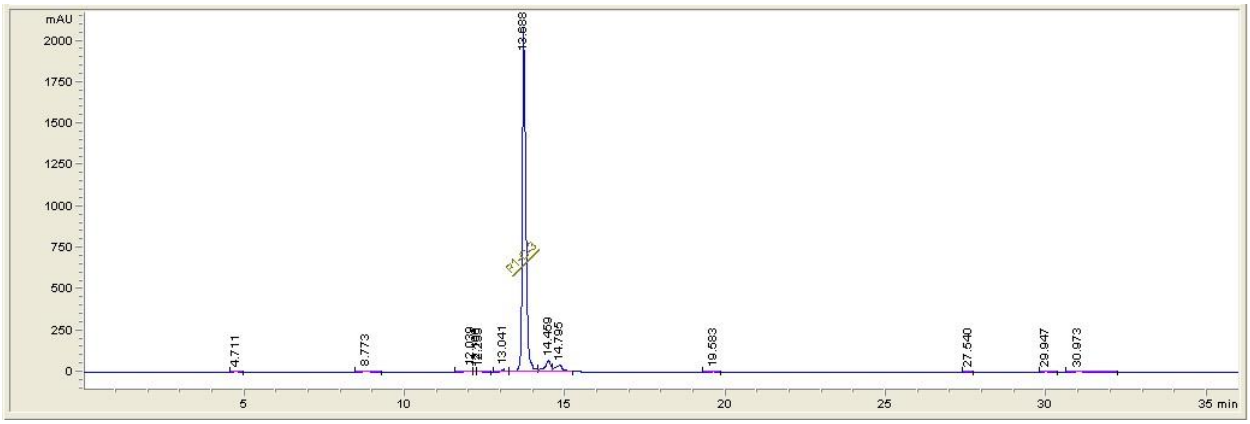


Fig. S3-11. HPLC profile and ESI MS of **sCy5-B2-P4z** probe.



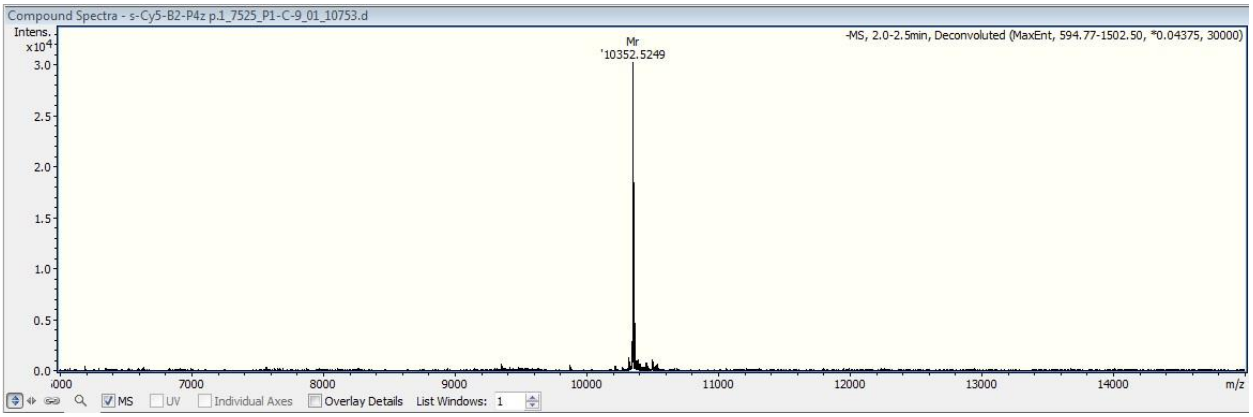
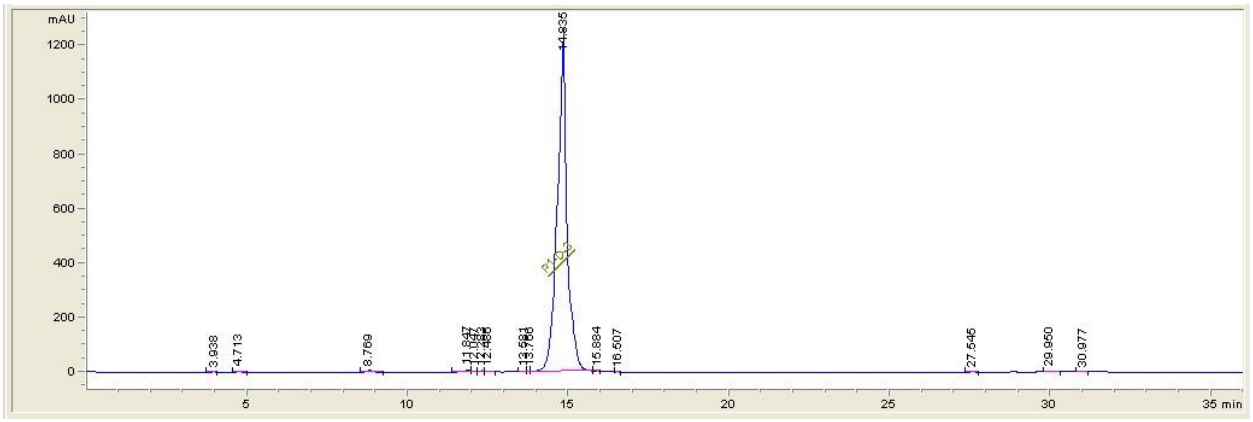
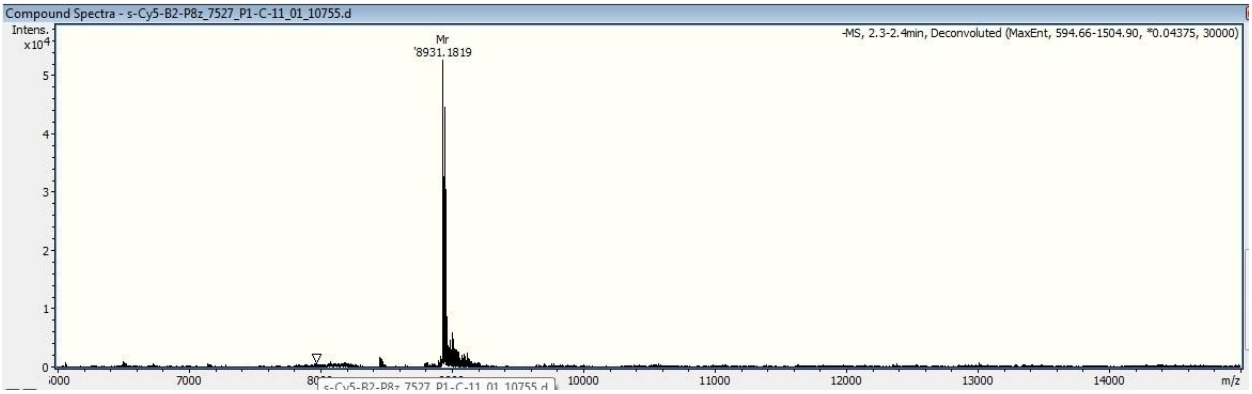


Fig. S3-12. HPLC profile and ESI MS of **sCy5-B2-P8z** probe.





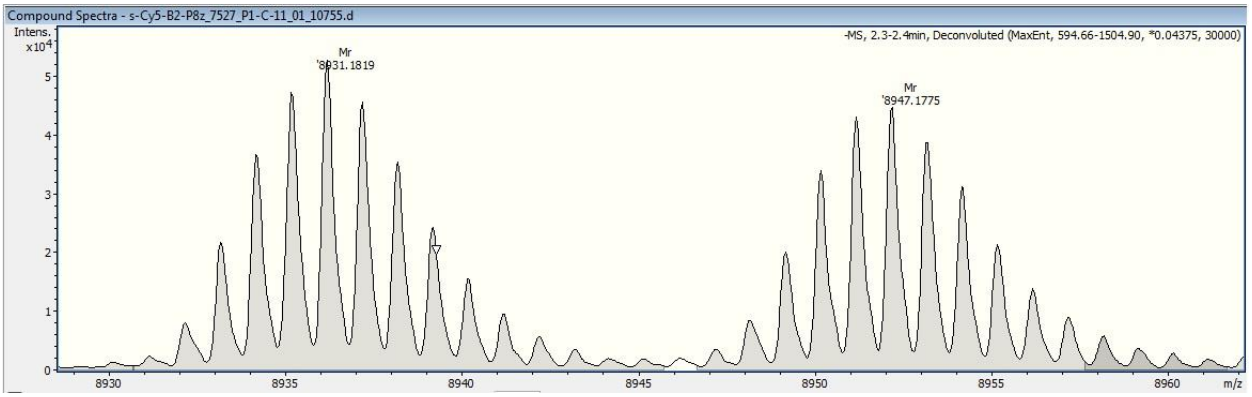
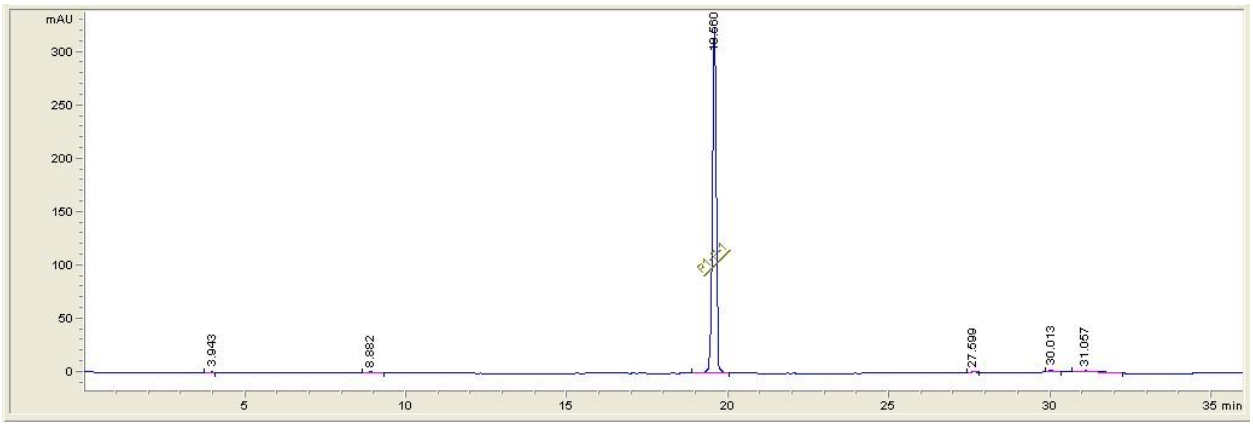
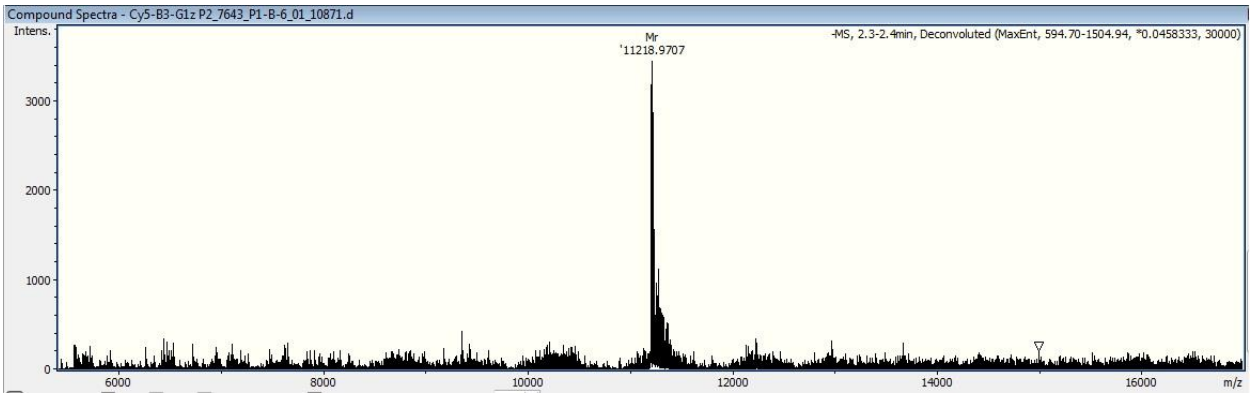


Fig. S3-13. HPLC profile and ESI MS of **Cy5-B3-G1z** probe.





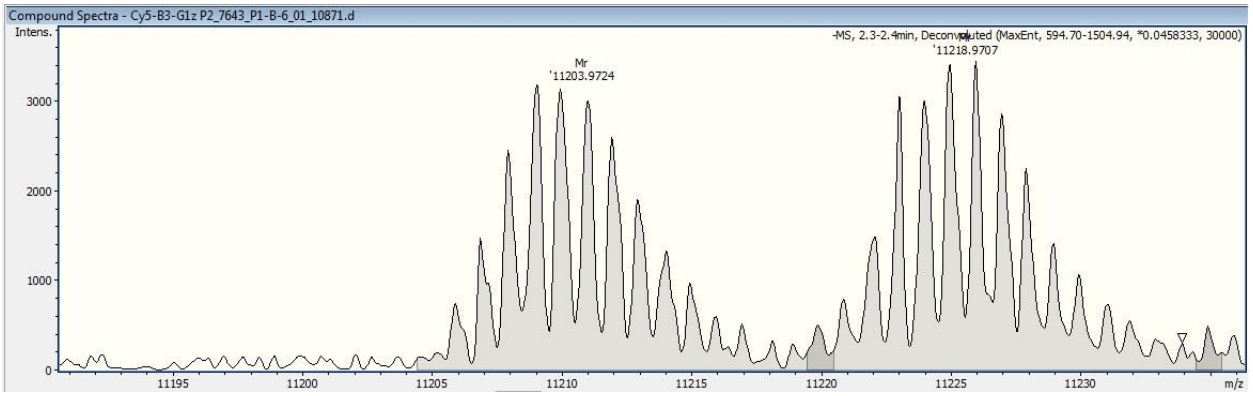
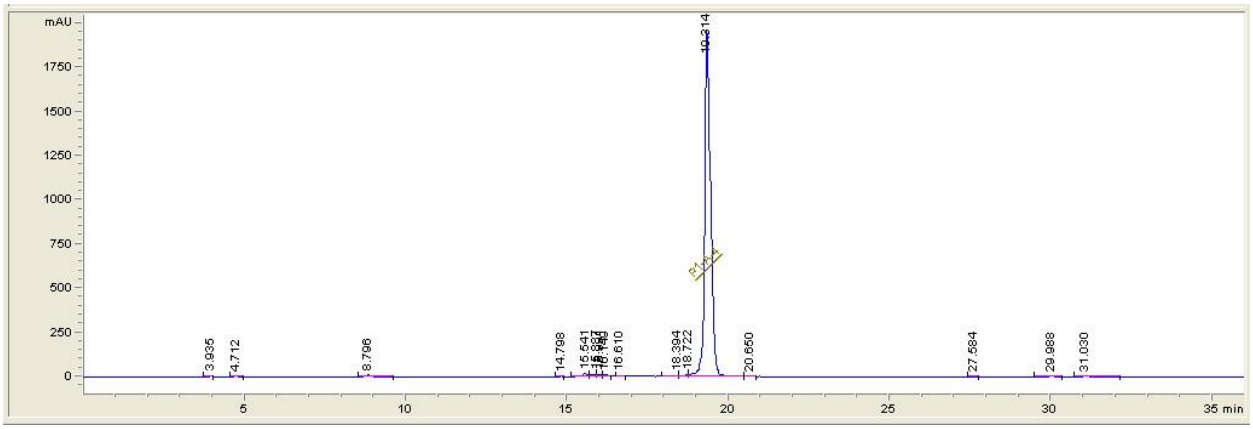


Fig. S3-14. HPLC profile and ESI MS of **Cy5-B3-G2z** probe.



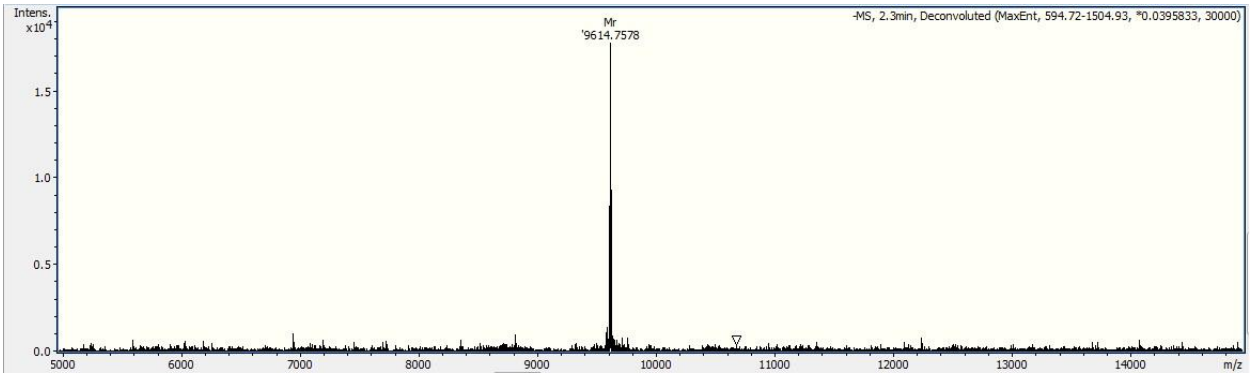
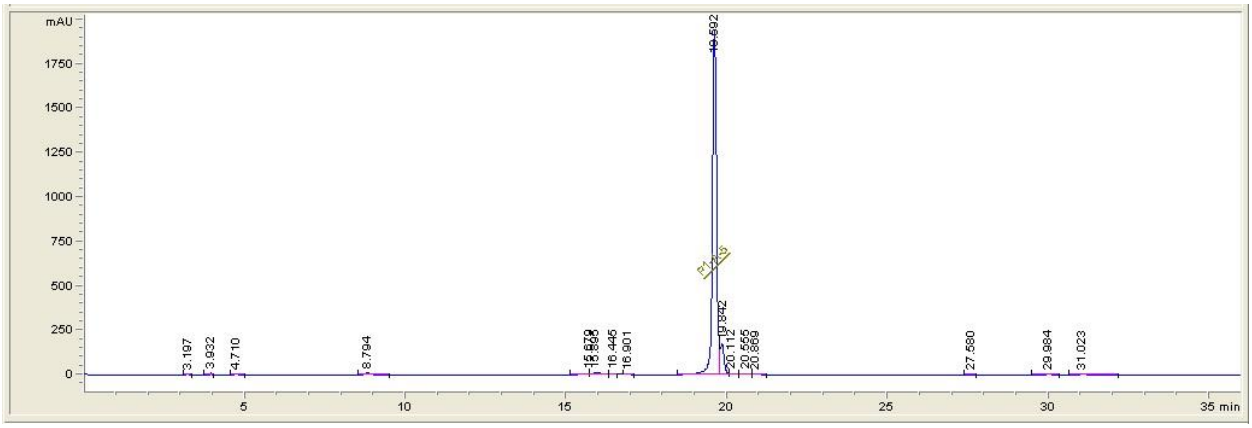


Fig. S3-15. HPLC profile and ESI MS of **Cy5-B3-G3z** probe.



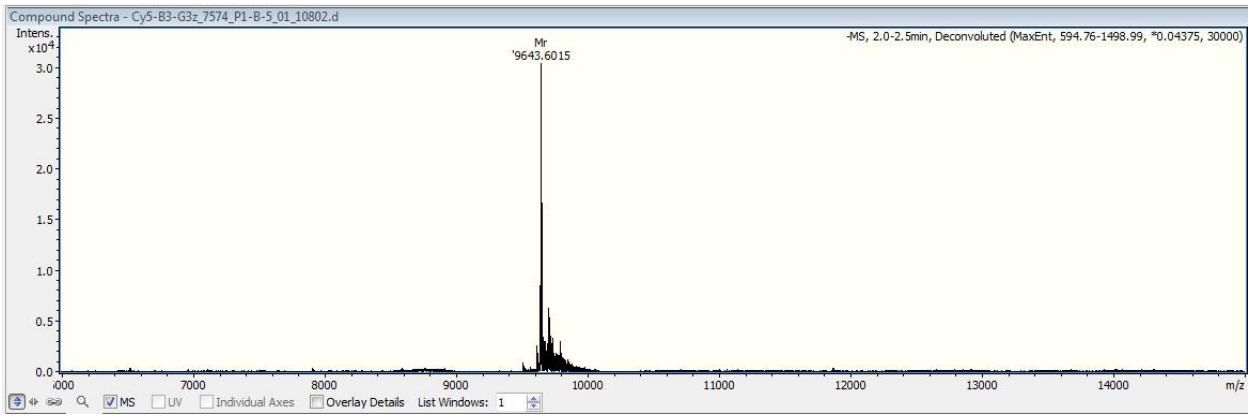
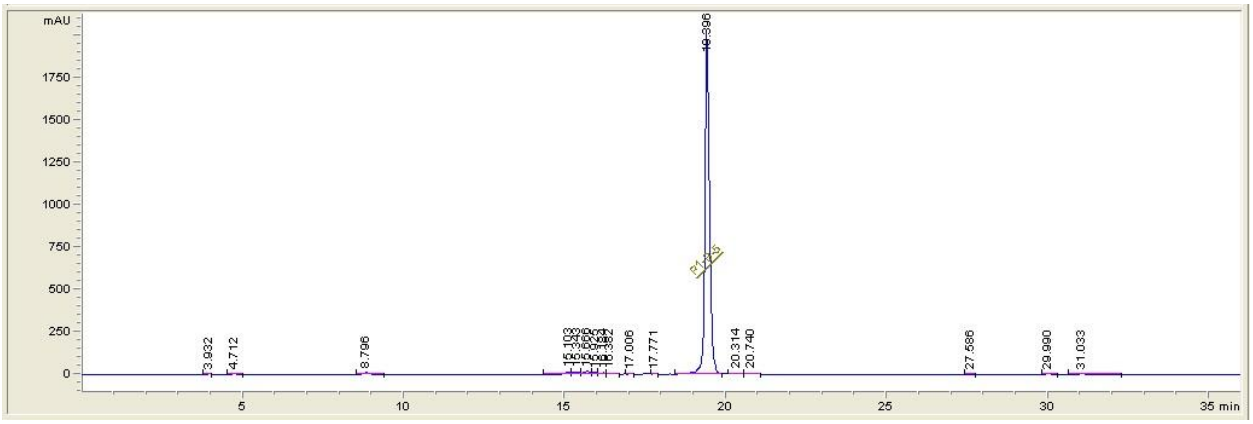


Fig. S3-16. HPLC profile and ESI MS of **Cy5-B3-G4z** probe.



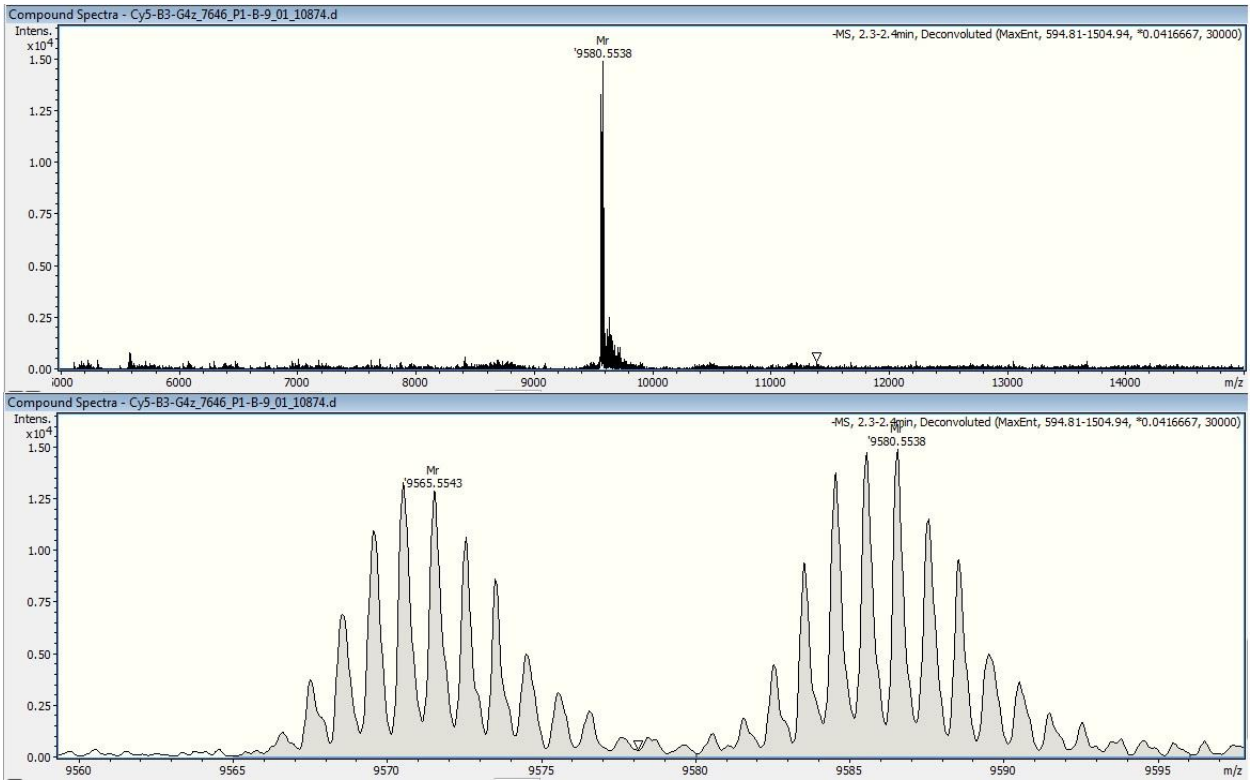
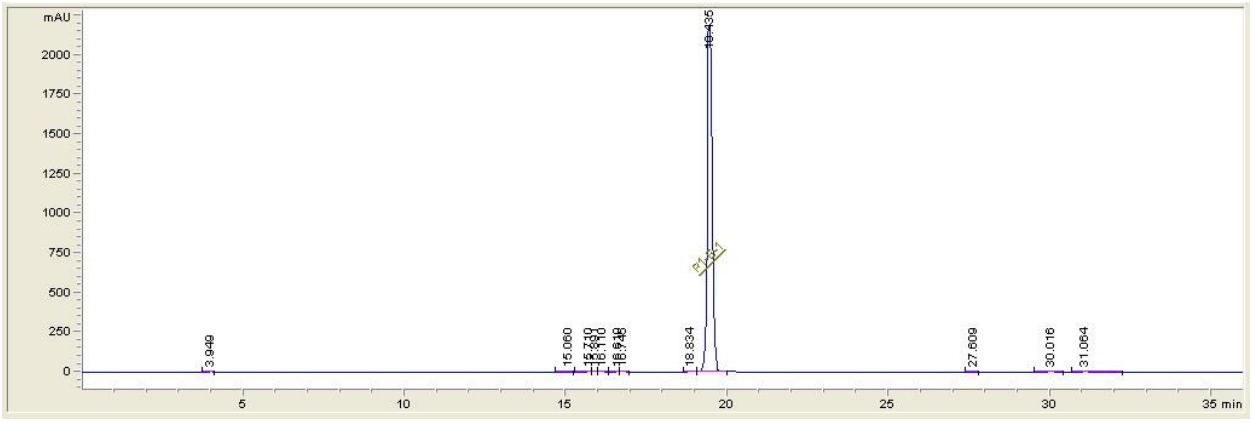


Fig. S3-17. HPLC profile and ESI MS of **Cy5-B3-P4z** probe.



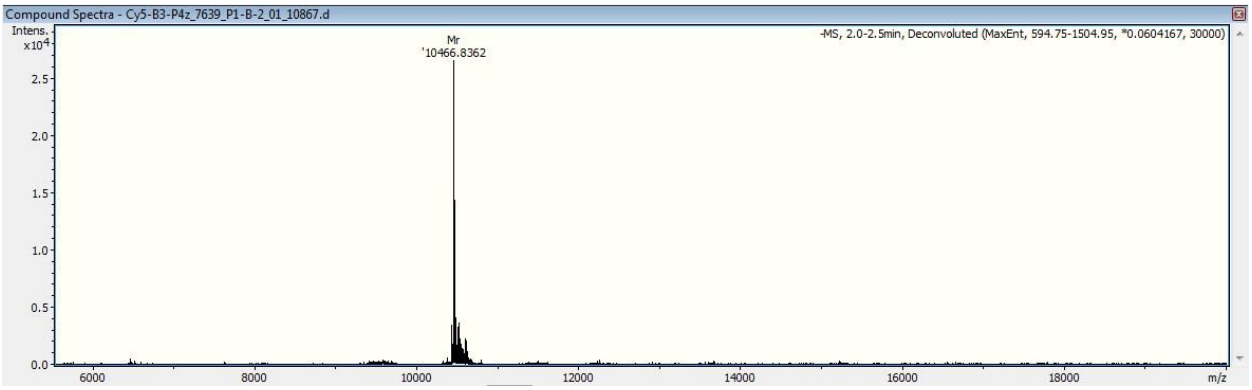
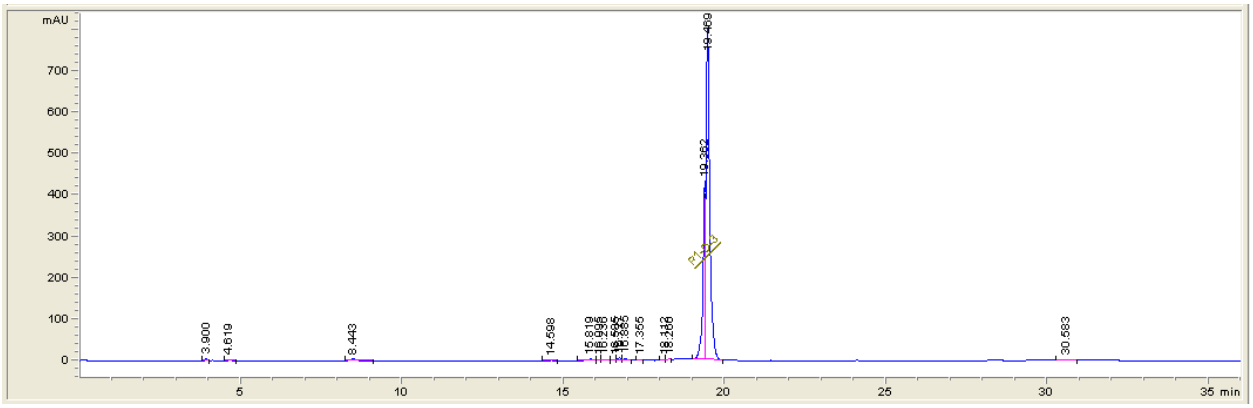
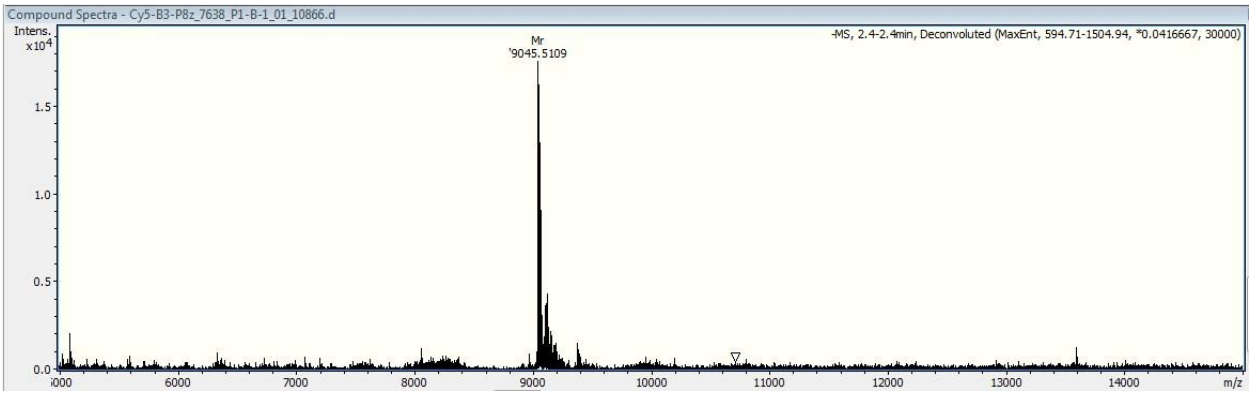


Fig. S3-18. HPLC profile and ESI MS of **Cy5-B3-P8z** probe.





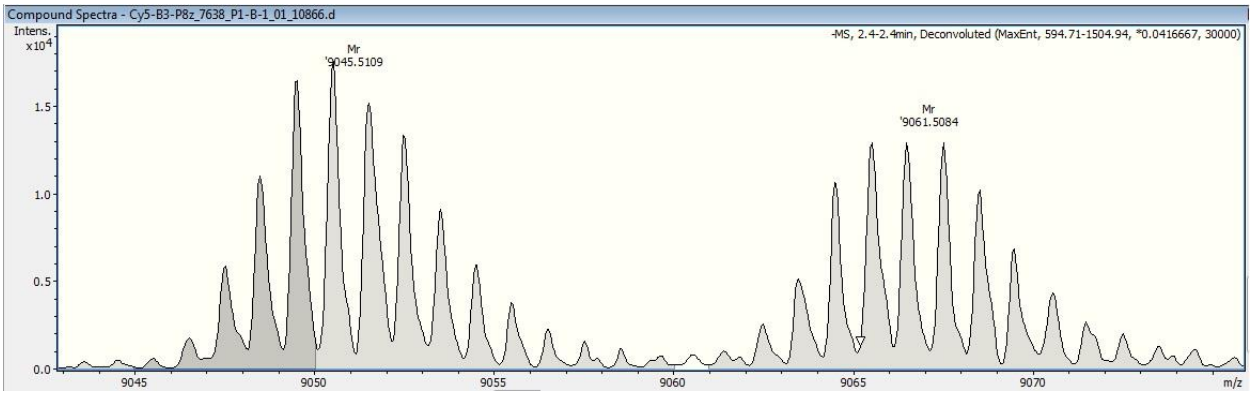
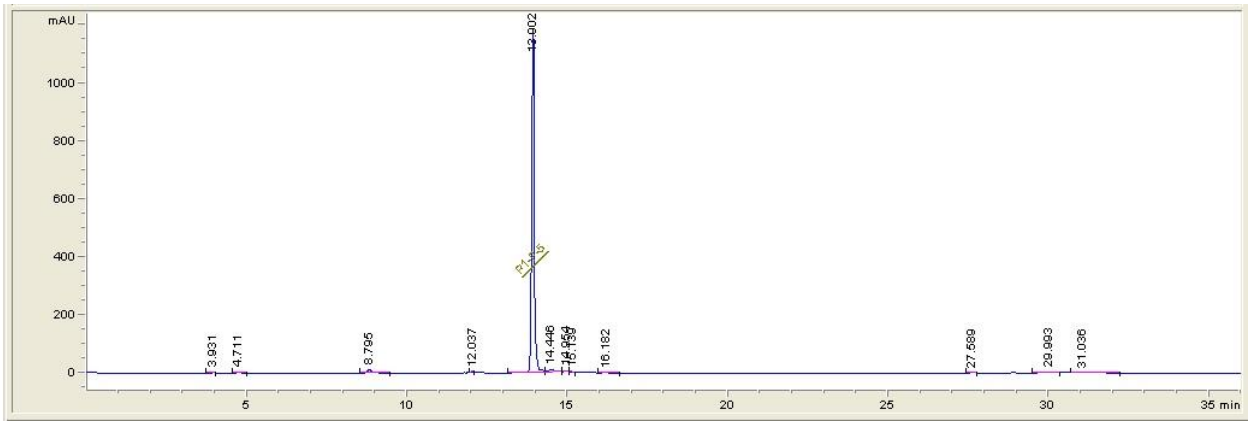
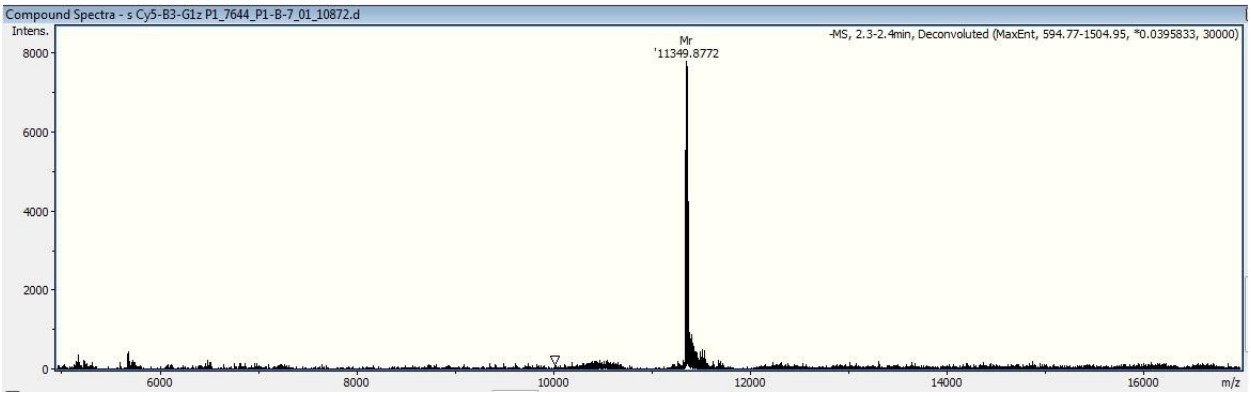


Fig. S3-19. HPLC profile and ESI MS of **sCy5-B3-G1z** probe.





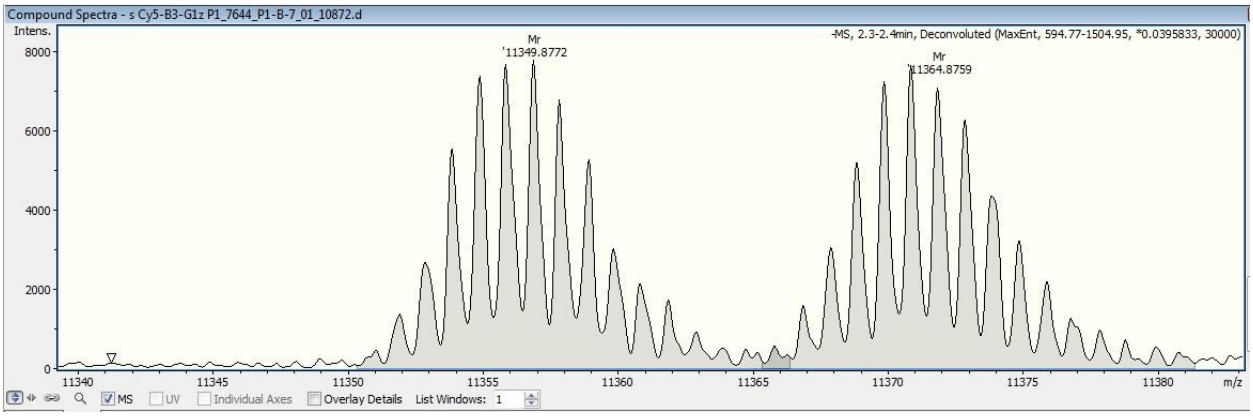
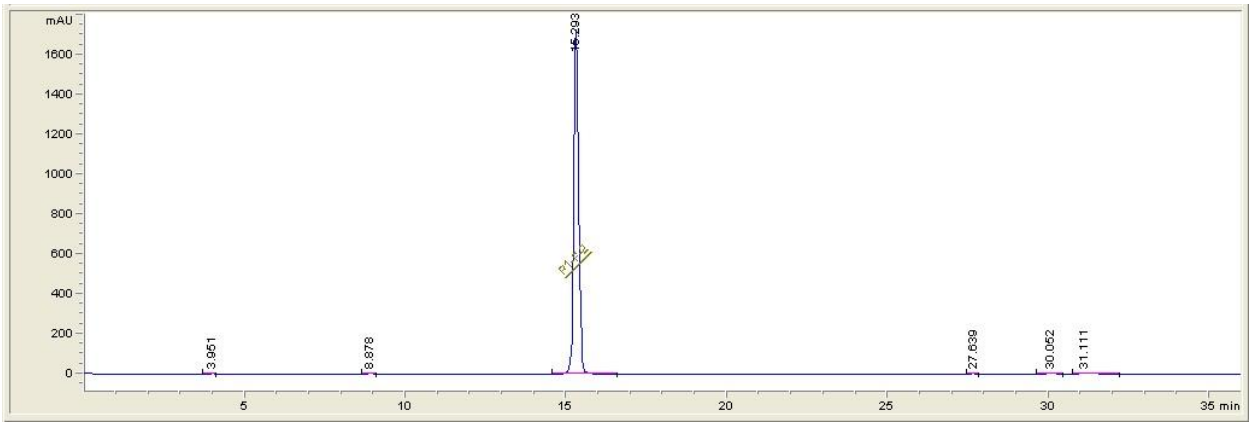


Fig. S3-20. HPLC profile and ESI MS of **sCy5-B3-G2z** probe.



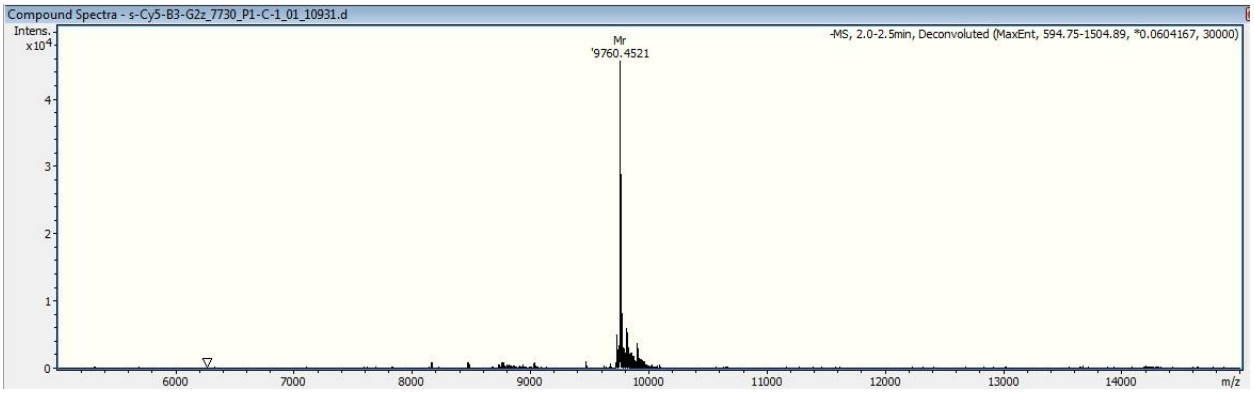
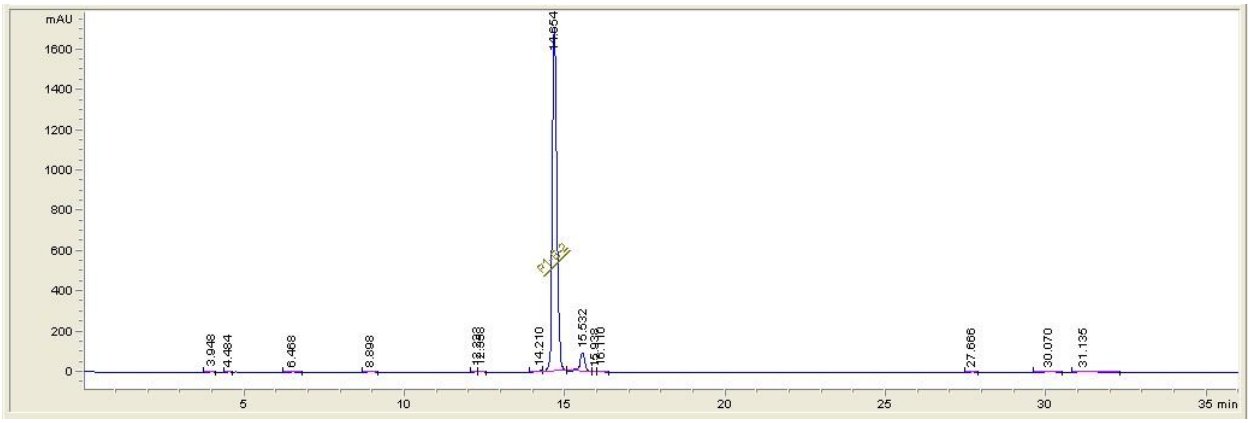


Fig. S3-21. HPLC profile and ESI MS of **sCy5-B3-G3z** probe.



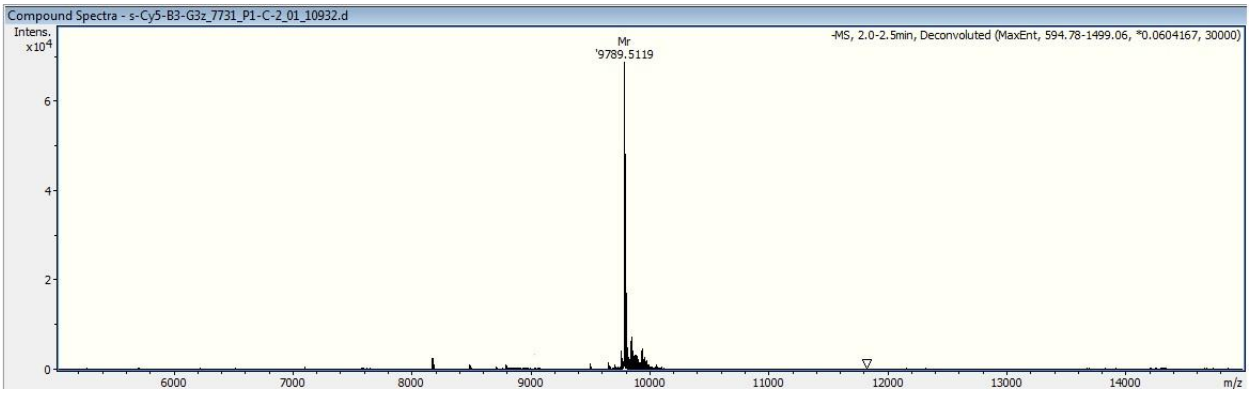
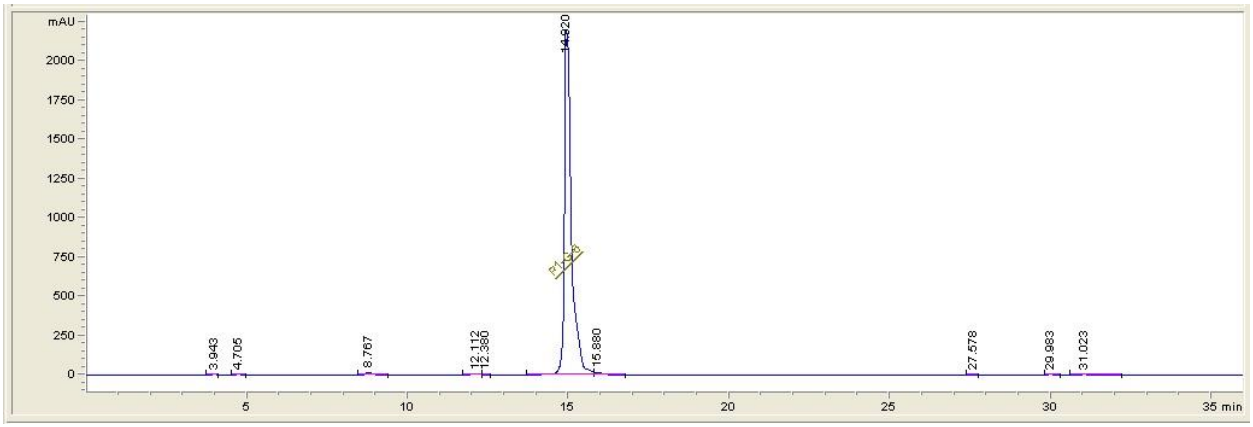


Fig. S3-22. HPLC profile and ESI MS of **sCy5-B3-G4z** probe.



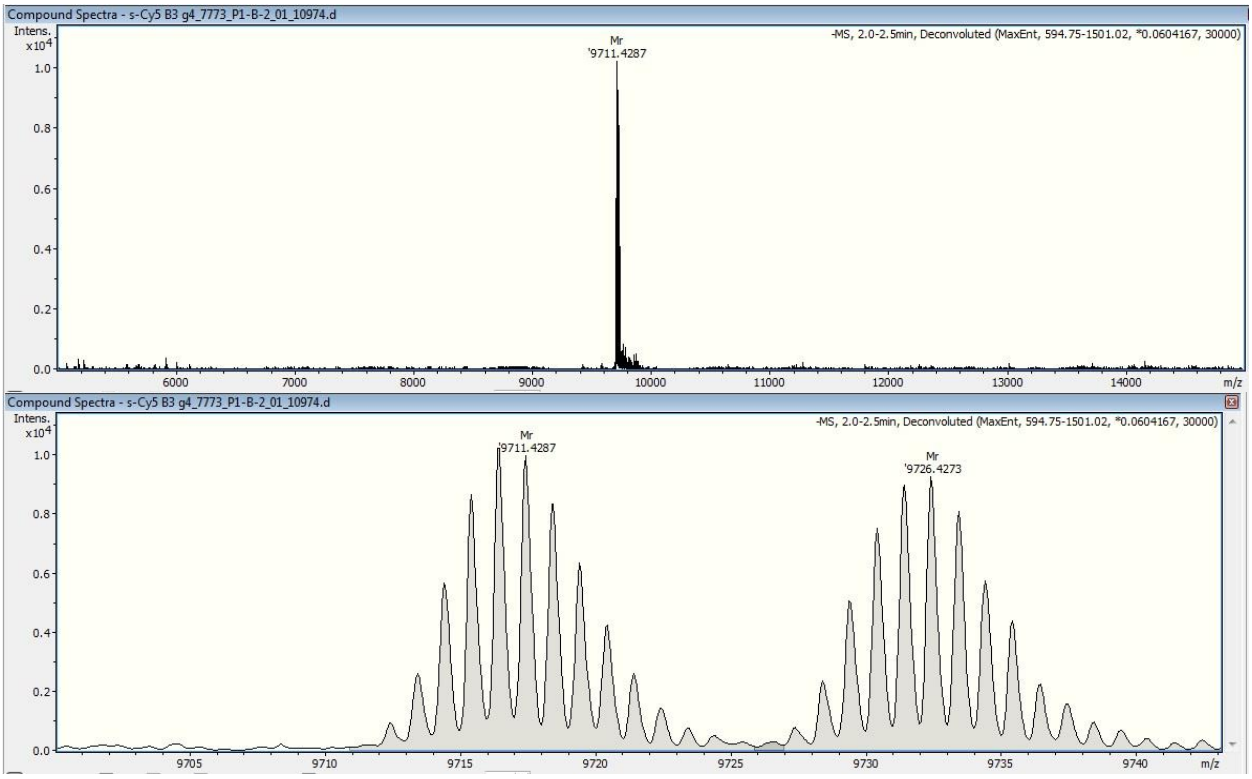
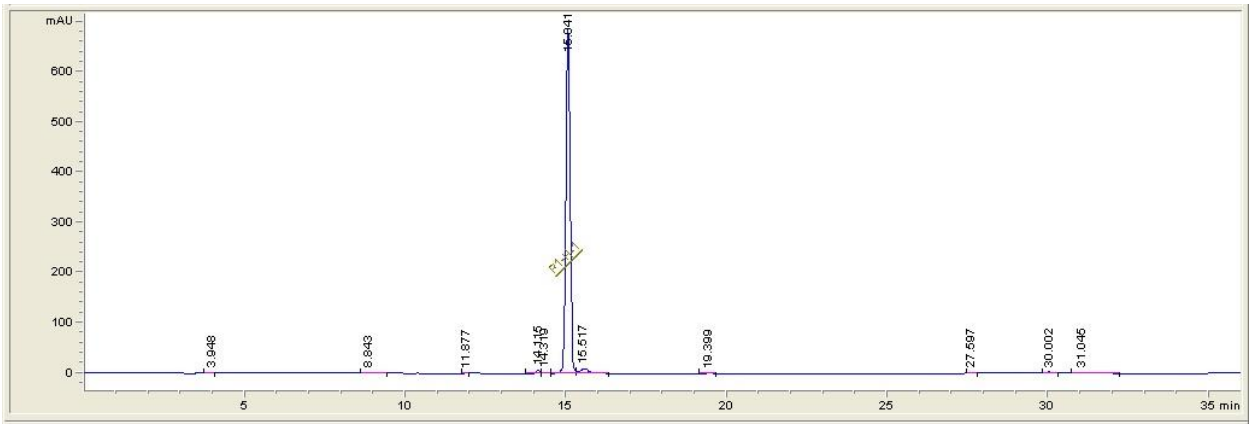


Fig. S3-23. HPLC profile and ESI MS of **sCy5-B3-P4z** probe.



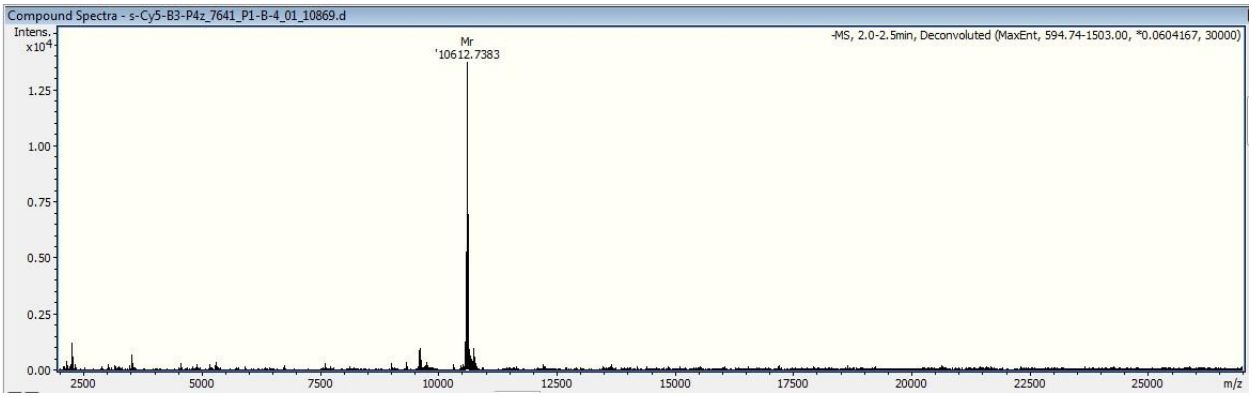
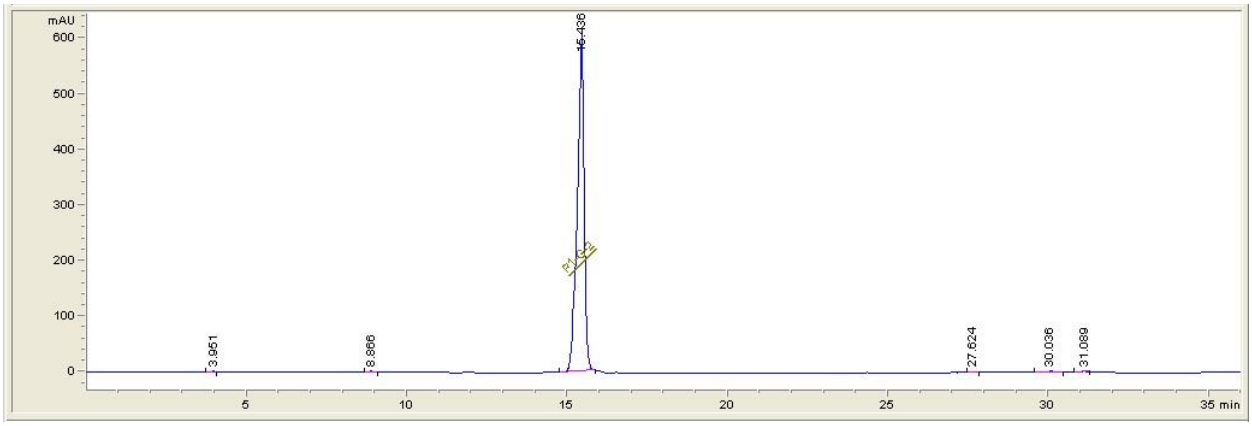
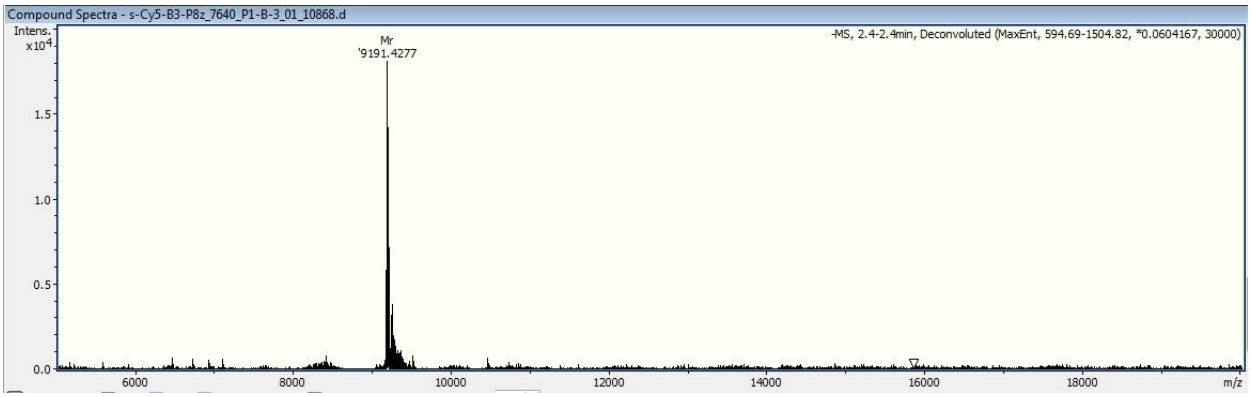


Fig. S3-24. HPLC profile and ESI MS of **sCy5-B3-P8z** probe.





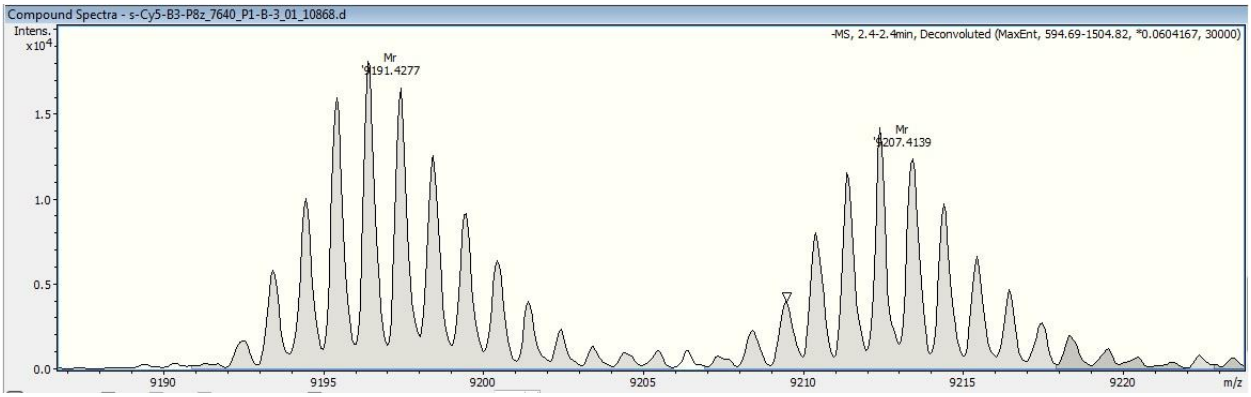


Table S2. Melting of Cy5/sCy5–BHQ2/BHQ3 labeled probes with fluorescence detection.

Probe	Dye/quencher	Tm, °C
G1	Cy5/BHQ2	64.3
	sulfo Cy5/BHQ2	68.9
	Cy5/BHQ3	62.3
	sulfo Cy5/BHQ3	67.4
G2	Cy5/BHQ2	50.2
	sulfo Cy5/BHQ2	46.2
	Cy5/BHQ3	51.7
	sulfo Cy5/BHQ3	48.2
G3	Cy5/BHQ2	58.8
	sulfo Cy5/BHQ2	62.8
	Cy5/BHQ3	54.2
	sulfo Cy5/BHQ3	58.8
G4	Cy5/BHQ2	50.7
	sulfo Cy5/BHQ2	46.2
	Cy5/BHQ3	50.2
	sulfo Cy5/BHQ3	44.1
P4	Cy5/BHQ2	48.7
	sulfo Cy5/BHQ2	52.7
	Cy5/BHQ3	48.7
	sulfo Cy5/BHQ3	51.2
P8	Cy5/BHQ2	58.3
	sulfo Cy5/BHQ2	55.3
	Cy5/BHQ3	62.3
	sulfo Cy5/BHQ3	59.8

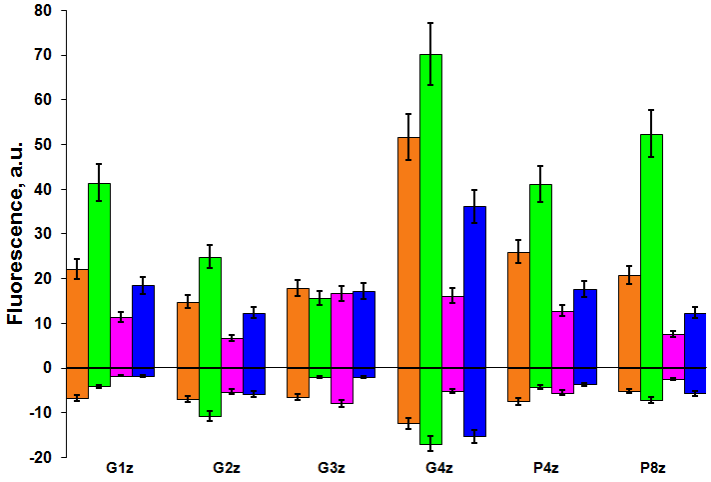
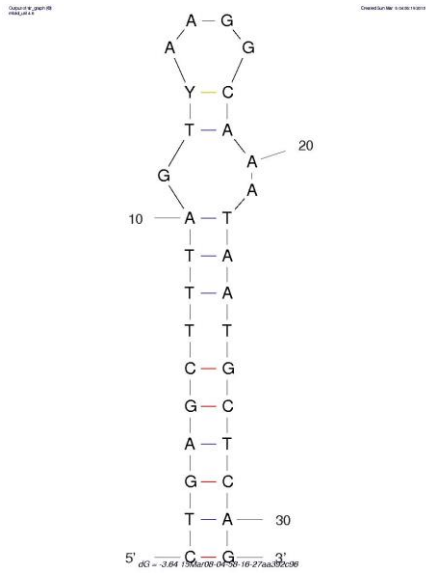
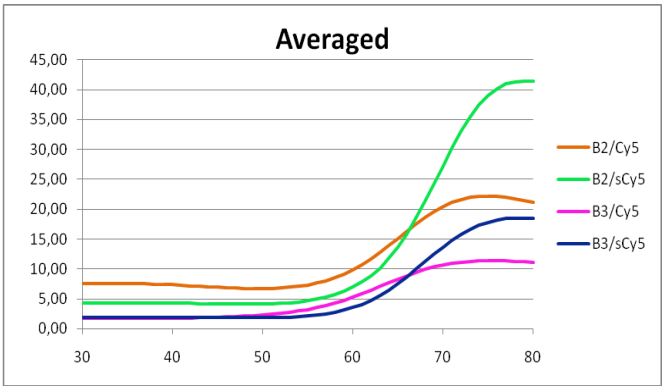


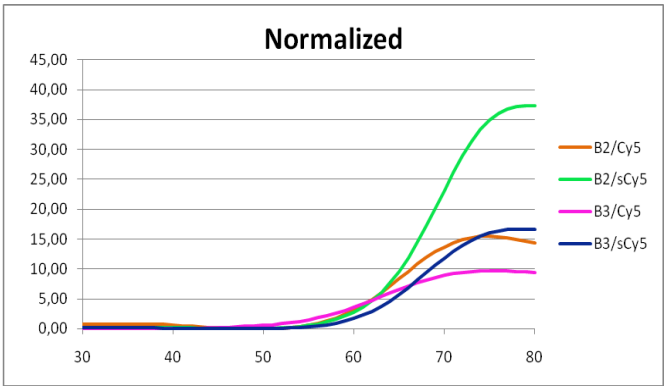
Fig S4. Fluorescence background (negative) and light-up (positive) of structured and molten probes; Cy5-B2, sCy5-B2, Cy5-B3, sCy5-B3.

Fig. S5-1. Mfold secondary structure, raw and processed melting and qPCR data for **G1z** probes.

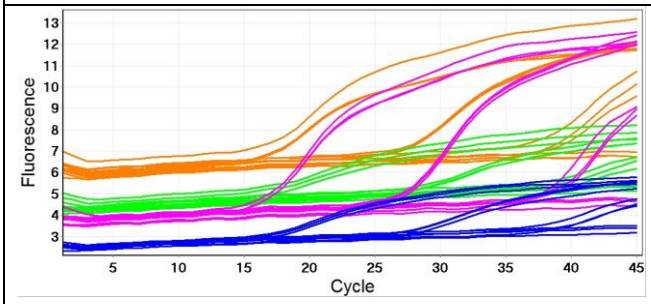


Melting





qPCR - raw data



qPCR - normalized data

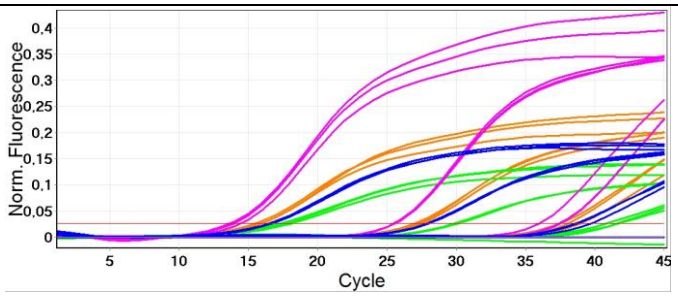
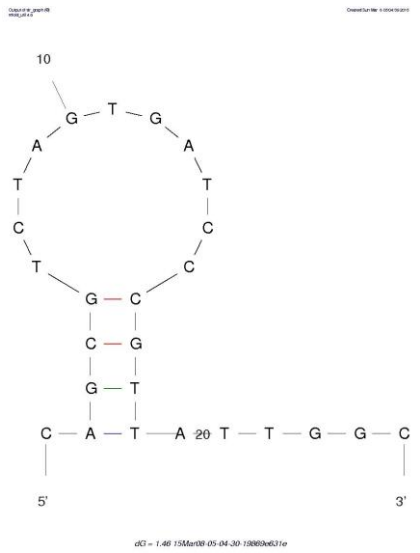
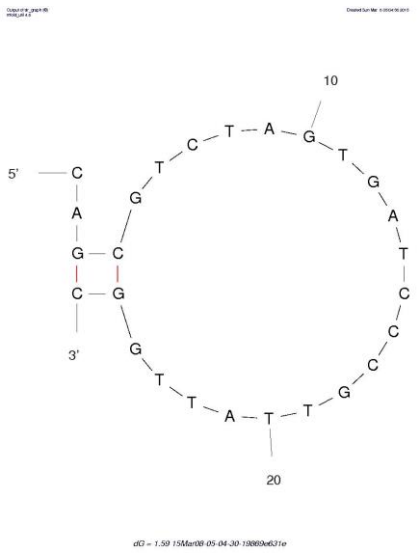
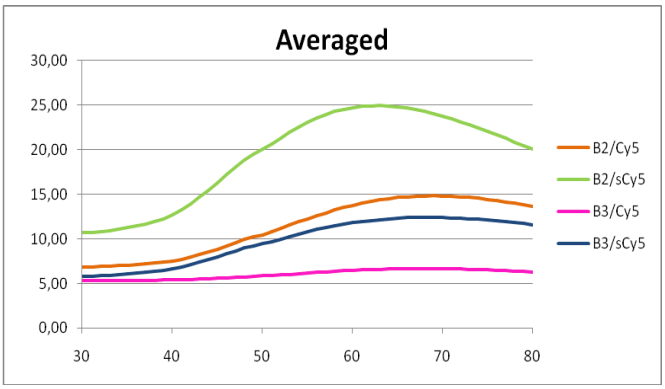


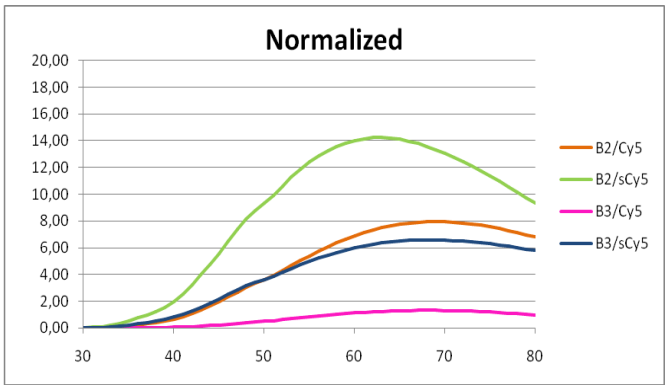
Fig. S5-2. Mfold secondary structure, raw and processed melting and qPCR data for **G2z** probes.



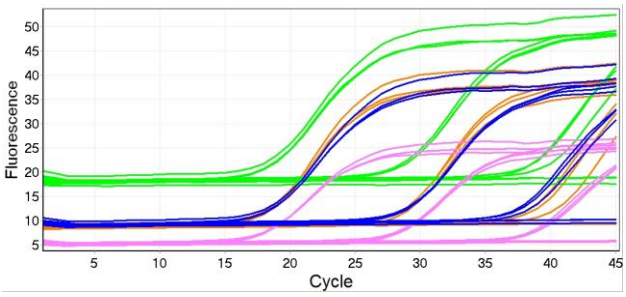


Melting





qPCR - raw data



qPCR - normalized data

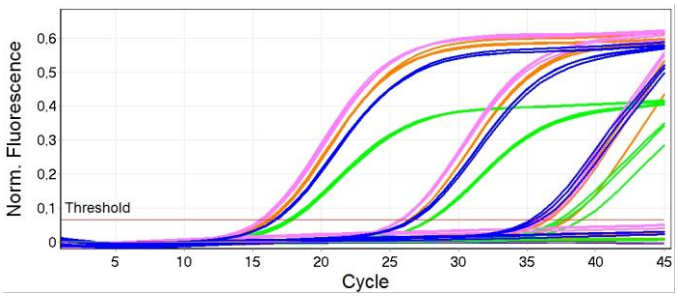
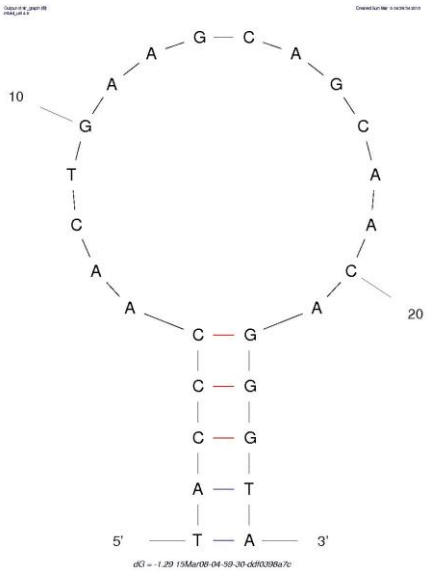
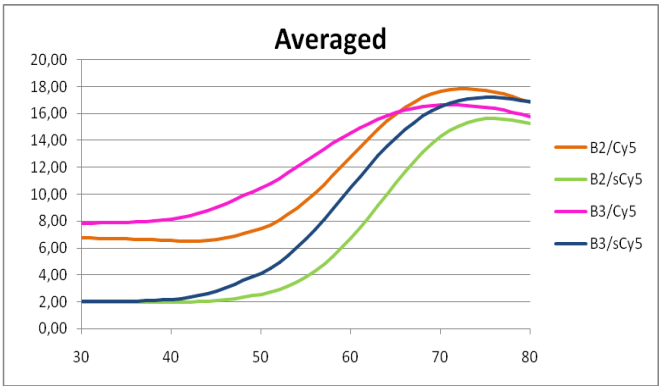
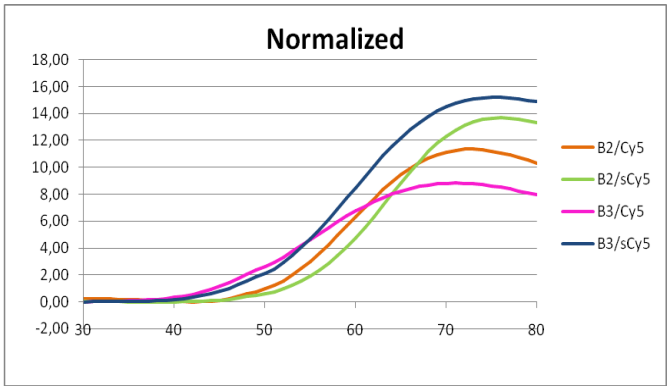


Fig. S5-3. Mfold secondary structure, raw and processed melting and qPCR data for **G3z** probes.

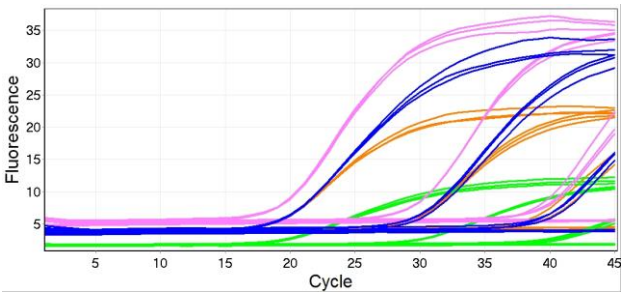


Melting





qPCR - raw data



qPCR - normalized data

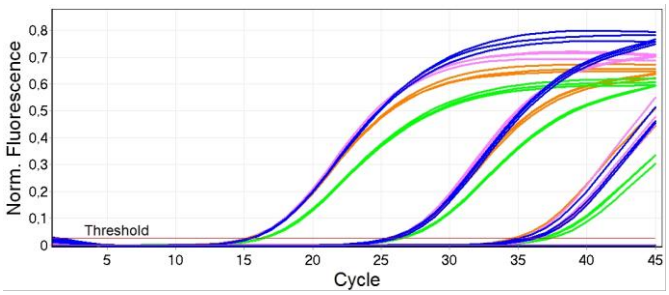
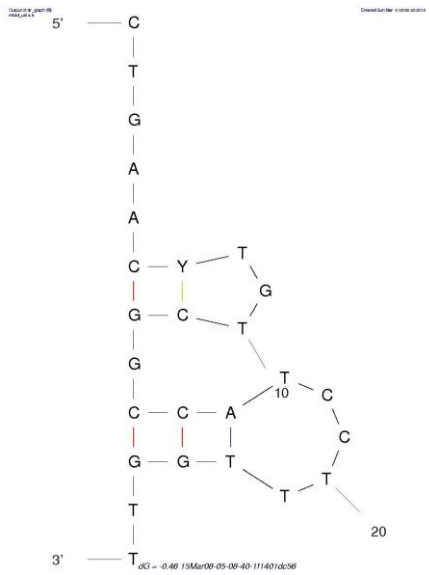
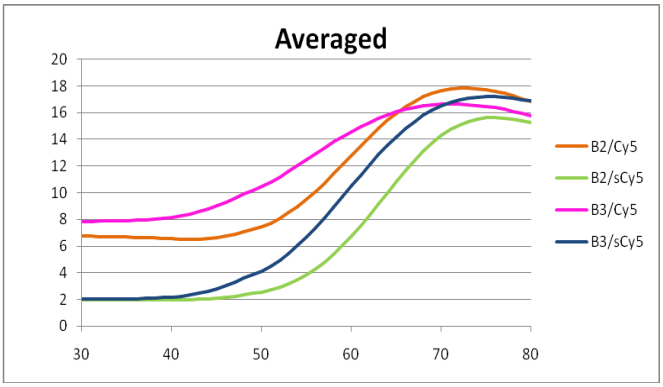
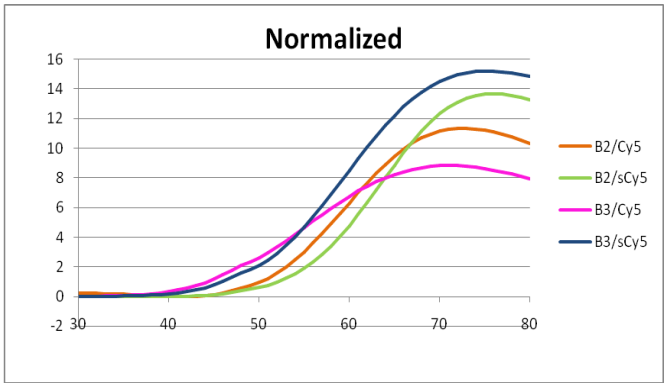


Fig. S5-4. Mfold secondary structure, raw and processed melting and qPCR data for **G4z** probes.

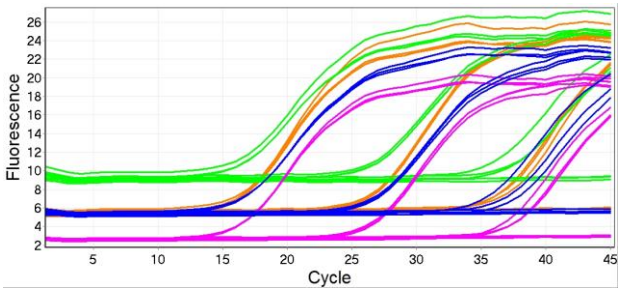


Melting





qPCR - raw data



qPCR - normalized data

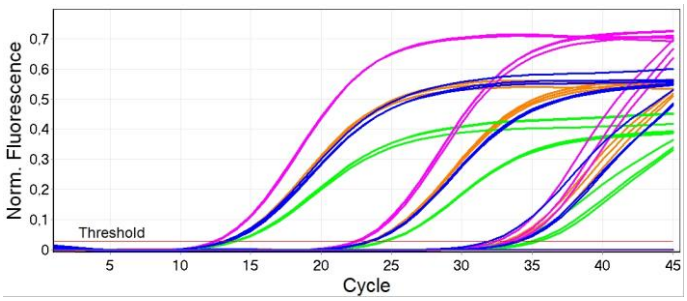
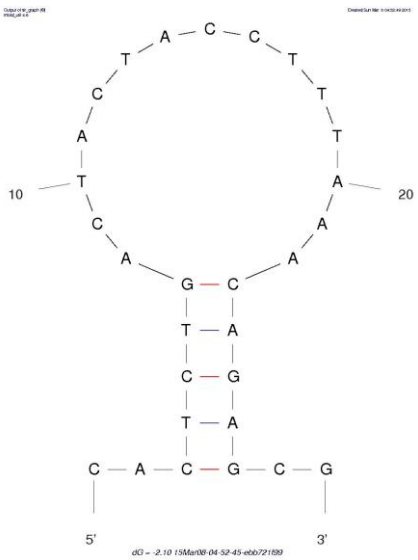
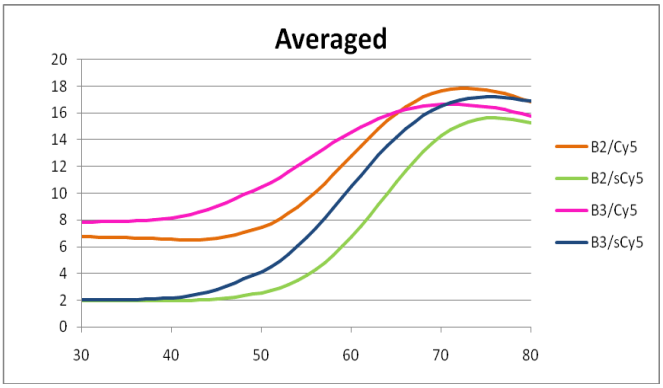
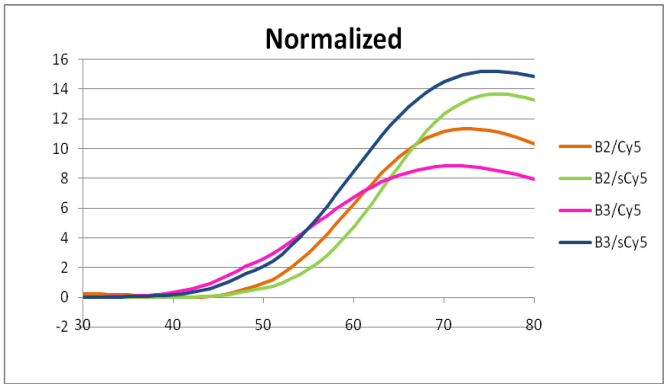


Fig. S5-5. Mfold secondary structure, raw and processed melting and qPCR data for **P4z** probes.



Melting





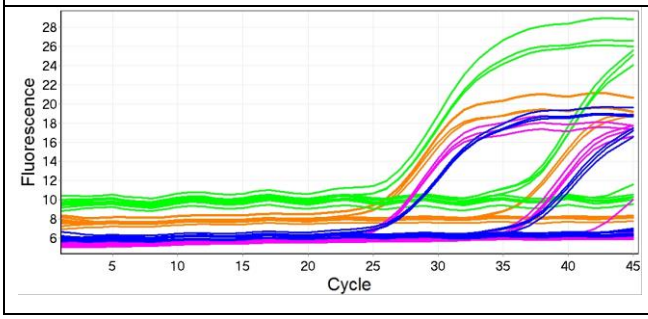
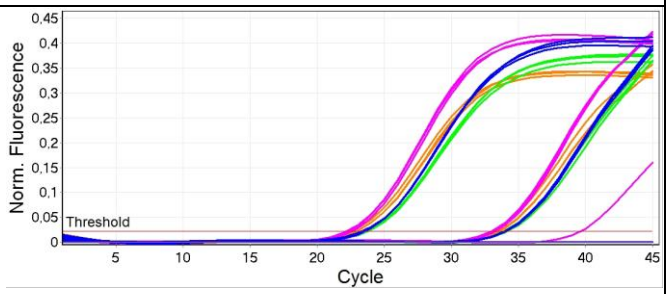
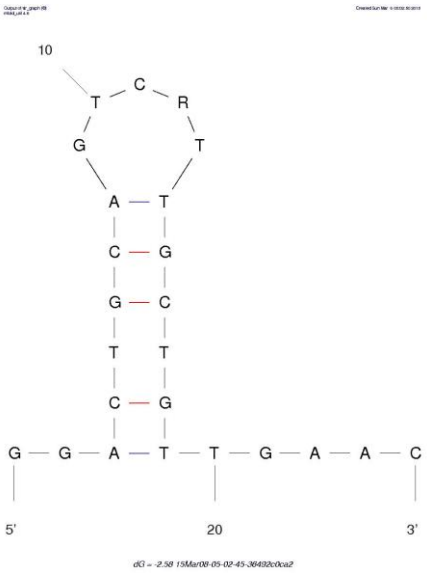
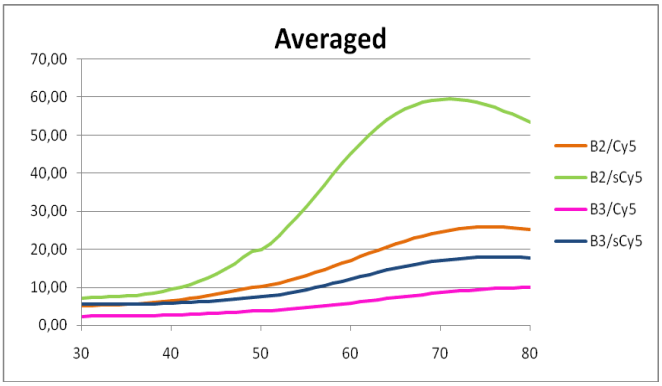
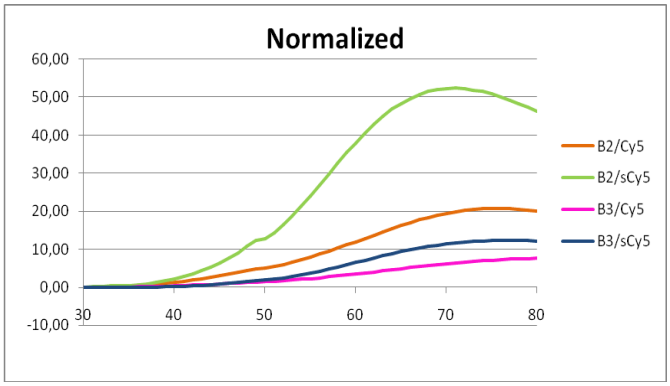
qPCR - raw data	qPCR - normalized data
	

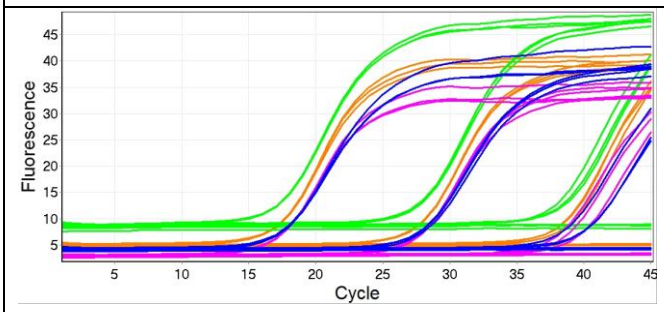
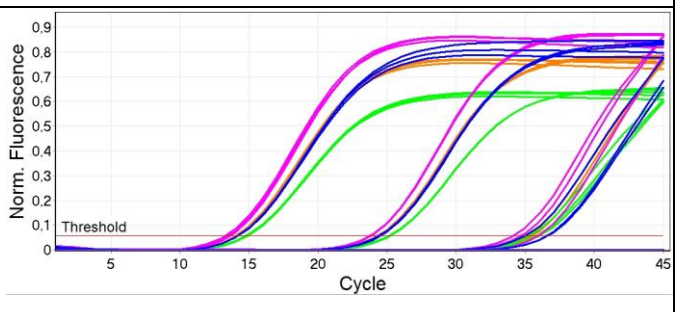
Fig. S5-6. Mfold secondary structure, raw and processed melting and qPCR data for **P8z** probes.



Melting





qPCR - raw data	qPCR - normalized data
	

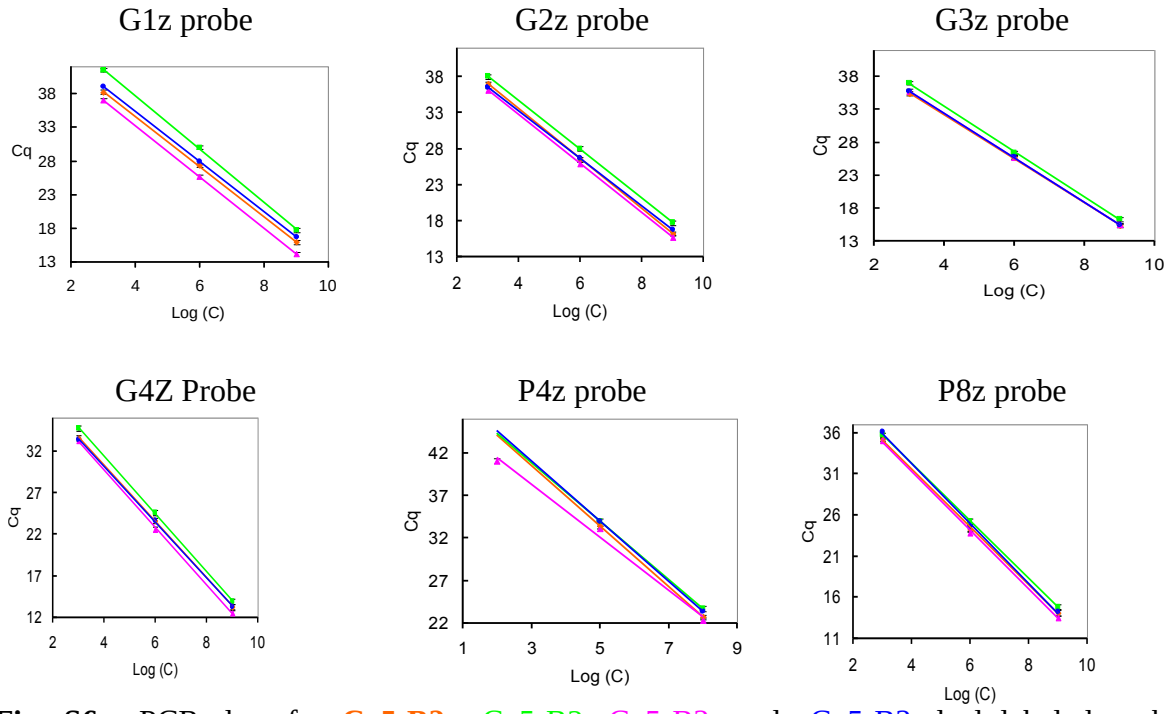


Fig. S6. qPCR data for Cy5-B2, sCy5-B2, Cy5-B3, and sCy5-B3 dual labeled probes; plot: dependence of quantification cycle number on the logarithm of DNA target concentration. For numerical values see Table S3†.

Table S3. qPCR data for Cy5-B2, sCy5-B2, Cy5-B3, and sCy5-B3 dual labeled probes.

Probe	Probe sequence, 5′-3′	qPCR data				
		Target DNA, copies/ reaction	Cq, cycles			
			Cy5-B2	sCy5-B2	Cy5-B3	sCy5-B3
G1z	**Dye**-CTG AGC TTT AGT YAA GGC AAA TAA TGC TCA G-**Quencher**	$1.0\cdot10^9$ $1.0\cdot10^6$ $1.0\cdot10^3$	15.9±0.2 27.3±0.3 38.2±0.4	17.7±0.3 28.0±0.3 38.0±0.4	14.1±0.2 25.6±0.3 37.0±0.3	16.7±0.1 28.0±0.2 39.0±0.4
G2z	**Dye**-CAG CGT CTA GTG ATC CCG TTA TTG GC-**Quencher**	$1.0\cdot10^9$ $1.0\cdot10^6$ $1.0\cdot10^3$	16.2±0.2 26.5±0.2 37.0±0.3	17.7±0.3 28.0±0.2 38.0±0.3	15.7±0.3 25.9±0.2 36.1±0.3	16.7±0.2 26.7±0.4 36.5±0.3
G3z	**Dye**-TAC CCA ACT GAA GCA GCA ACA GGG TA-**Quencher**	$1.0\cdot10^9$ $1.0\cdot10^6$ $1.0\cdot10^3$	15.3±0.2 25.7±0.3 35.4±0.3	16.3±0.3 26.4±0.3 37.0±0.3	15.4±0.3 25.6±0.3 35.7±0.3	15.4±0.3 25.8±0.3 35.7±0.3
G4z	**Dye**-CTG AAC YTG TCG GCC ATC CTT TGG TT-**Quencher**	$1.0\cdot10^9$ $1.0\cdot10^6$ $1.0\cdot10^3$	13.3±0.1 23.6±0.2 33.6±0.3	13.9±0.3 24.6±0.3 34.7±0.3	12.5±0.3 22.6±0.3 33.2±0.3	13.3±0.3 23.6±0.3 33.4±0.3
P4z	**Dye**-CAC TCT GAC TAC TAC CTT TAA ACA GAG CG-**Quencher**	$1.0\cdot10^8$ $1.0\cdot10^5$ $1.0\cdot10^2$	22.7±0.1 33.4±0.3 -	23.7±0.1 34.0±0.2 -	22.3±0.2 33.1±0.2 41.0±0.5	23.4±0.2 34.0±0.2 -
P8z	**Dye**-GGA CTG CAG TCR TTG CTG TTG AAC-**Quencher**	$1.0\cdot10^9$ $1.0\cdot10^6$ $1.0\cdot10^3$	14.1±0.2 24.3±0.2 35.2±0.3	14.8±0.3 25.2±0.2 35.7±0.3	13.5±0.3 23.8±0.3 35.0±0.3	14.2±0.4 24.5±0.2 36.1±0.3

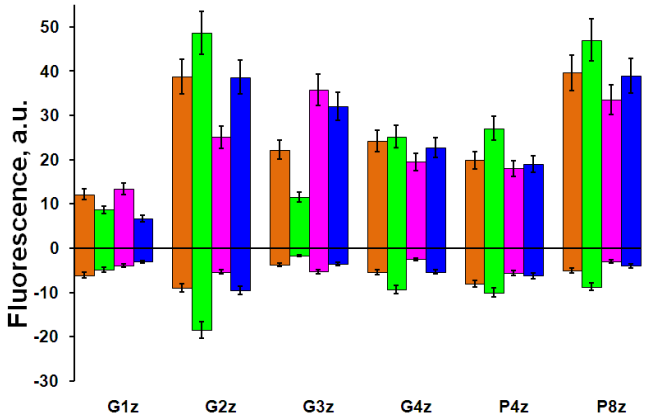


Fig. S7. Fluorescence light-up (positive) and background (negative) in qPCR; – Cy5-B2, – sCy5-B2, – Cy5-B3, – sCy5-B3.