RNA-Peptide Conjugation through an Efficient Covalent Bond Formation

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Supplemental Tables and Figures

A

	pH 4	pH 5	pH 6	pH 7
c–RNP	7331	6703	6684	5142
RNA	922	668	926	917
Sum	8253	7372	7610	6059
ratio (RNP)	0.89	0.91	0.88	0.85
trial2	0.91	0.88	0.86	0.83
trial3	0.96	0.98	0.83	0.78
Average	0.92	0.92	0.86	0.82
S.D.	0.04	0.05	0.03	0.03

B

	An16/Ac–Rev(GGS)$_3$G, 3h	scrAn16/Ac–Rev(GGS)$_3$G, 3h	scrAn16/Ac–Rev(GGS)$_3$G, 16h
c–RNP	11359.37	9794.51	7870.15
RNA	1497.92	N.D.	203.48
Sum	12857.28	9793.05	8073.62
ratio (RNP)	0.88	1.00	0.97

C

	An16/Ac–Rev(GGS)$_3$G, 3h	An16/Ac–(GGS)$_3$G, 3h	An16/Ac–(GGS)$_3$G, 14h
c–RNP	10973.12	4312.76	5801.42
RNA	748.19	5076.85	4203.42
Sum	11721.31	9389.61	10004.84
ratio (RNP)	0.94	0.46	0.58
trial2		0.33	0.57
trial3		0.34	0.56
Average		0.37	0.57
S.D.		0.07	0.01

D

pH, time	pH 5, 3h	pH 5, 3h	pH5, >14 h
c–RNP	An16/Ac–Rev(GGS)$_3$G	scrAn16/Ac–Rev(GGS)$_3$G	scrAn16/Ac–Rev(GGS)$_3$G
trial 1	0.91	0.90	0.90
trial 2	0.88	0.92	0.84
trial 3	0.94	1.00	0.97
trial 4	1.00	0.88	0.92
trial 5	0.89	0.91	0.93
Average	0.92	0.92	0.91
S.D.	0.05	0.05	0.05

Figure S1. The results of band intensity analysis of denaturing PAGE shown in (**A**) Figure 2, (**B**) 3 and (**C**) 4. Intensity of each band was analyzed by using Quantity One ver. 4.6.9 (Bio-Rad Laboratories, Inc.). N.D. means that the band has not been detected. Ratio (RNP) is shown the ratio of reacted RNA band intensity to the total RNA band intensity that equals the reaction efficiency in each condition. The averaged reaction efficiencies of covalently-linked RNP and the standard deviations were shown in (**D**). An16 and scrAn16 RNA did not show the significant difference in the reaction efficiency in the reaction with Ac-Rev-(GGS)₃G-HZ peptide.
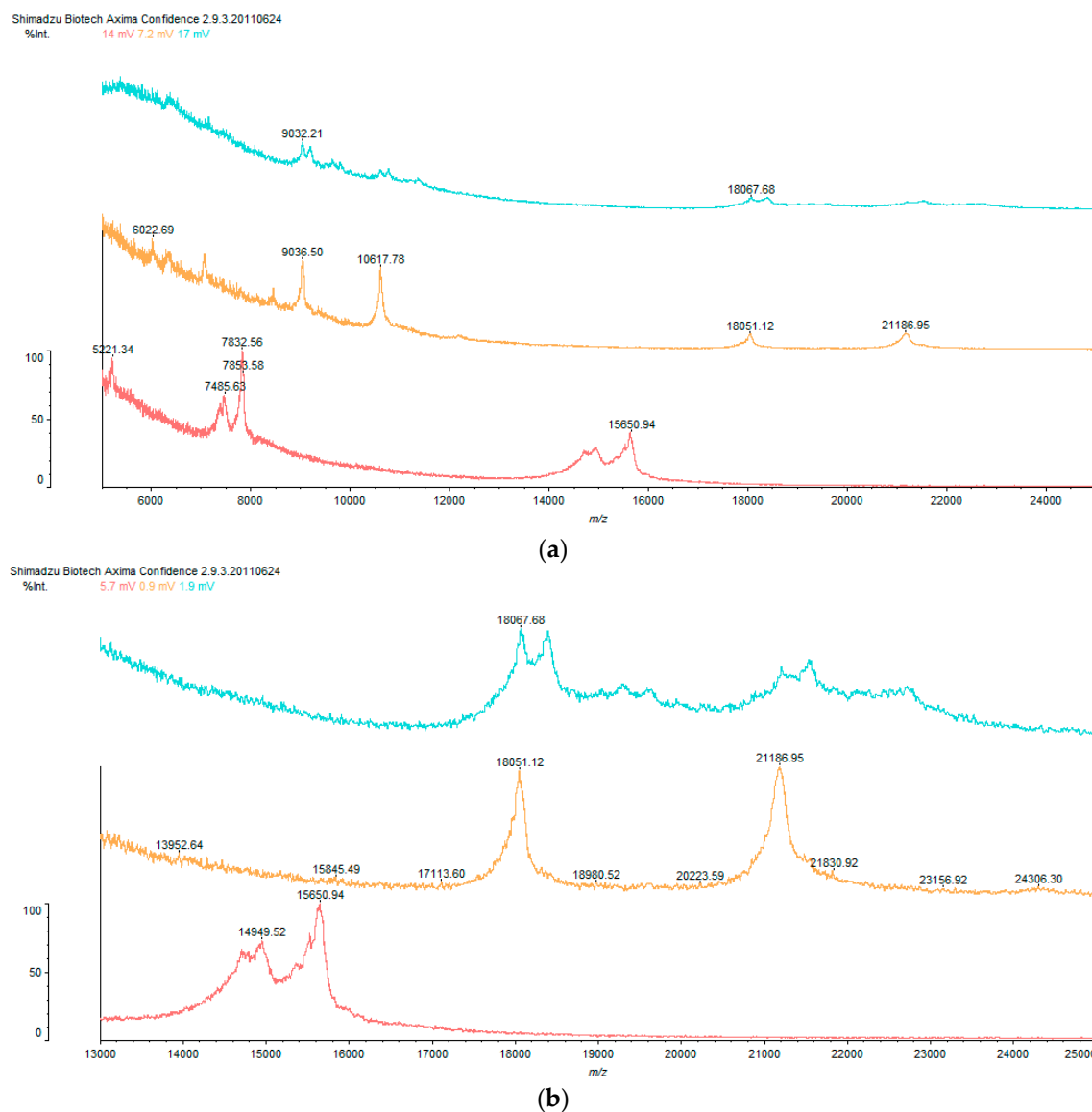
(**a**)

(**b**)

Figure S2. MALDI TOF MS analysis of the reaction solution. (**a**) MS spectra of the reaction solutions of scrAn16 and Ac-Rev-(GGS)₃G-HZ (cyan), An16 and Ac-Rev-(GGS)₃G-HZ (orange), and (**b**) An16 and Ac-(GGS)₃G (red) ranging from A) m/z 5000 to 25000 and B) m/z 13000 to 25000. The summary of results was shown in Figure S3.

Reaction solution	Found MS peaks	Calcd. MS	Difference	Difference%	Identification
An16/Ac-Rev(GGS)₃G-HZ pH5, 3h	18051.1	18017.7	33.5	0.19	c−An16/Ac−Rev(GGS)₃G
	21187.0	21171.6	15.4	0.07	c−An16/2Ac−Rev(GGS)₃G
scrAn16/Ac-Rev(GGS)₃G-HZ, 3h	18067.7	18017.7	50.0	0.28	c−scrAn16/Ac−Rev(GGS)₃G
	18388.7		*1 321.1		c−scrAn16(N+1)/Ac−Rev(GGS)₃G
	21209.9	21171.6	38.4	0.18	c−scrAn16/2Ac−Rev(GGS)₃G
	21535.6		*1 325.7		c−scrAn16(N+1)/2Ac−Rev(GGS)₃G
An16/Ac-(GGS)₃G-HZ, 14h	15650.9	15597.1	53.9	0.35	c−An16/Ac−(GGS)₃G
	14949.5	14863.8	85.8	0.58	An16 RNA (unreacted)

*1: Difference between the found peaks

Figure S3. Summary of the results of MALDI TOF MS analysis.

m/z 18051.1 for c-An16/Ac-Rev-(GGS)$_3$G, m/z 18067.7 and 18388.7 for c-scrAn16/Ac-Rev-(GGS)$_3$G (Calculated for [M+H]$^+$ was 18017.7) and 15650.9 for c-An16/Ac-(GGS)$_3$G (Calculated for [M+H]$^+$ was 15597.1) showed the successful production of covalently-linked RNP. The bigger number of peak (m/z 18388.7) in c-scrAn16/Ac-Rev-(GGS)$_3$G reaction solution should be originated from mononucleotide addition at 3'-end of RNA in transcription. In both solutions, the peaks speculated as the c-RNP that has two peptides was also observed: 21187.0 for c-An16/2Ac-Rev-(GGS)$_3$G and 21209.9 and 21535.6 for c-An16/2Ac-Rev-(GGS)$_3$G (Calculated for [M+H]$^+$ was 21171.6). The unreacted RNA (m/z 14949.5) was observed in the reaction mixture of An16 and Ac-(GGS)$_3$G because of the low reaction yield of this condition.