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Supporting Information

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Supporting Information

for

Photocrosslinking of RNA and photoMet Containing Amphiphilic α-Helical Peptides

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Experimental details of the following:

1. General
2. Syntheses of peptides
3. Photo cross-linking
4. Fluorescence anisotropic assay
5. RNA footprinting

1. General: Fmoc-protected α-amino acids and Rink Amide MBHA resin were purchased from Novabiochem (San Diego, CA). Dimethylformamide (DMF), *N,N*-diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), 1,2-dichloromethane (DCM), and piperidine were purchased from Sigma-Aldrich and used as supplied.

For all RNA-related applications, water was autoclaved and treated with 0.1% diethylpyrocarbonate (DEPC).

2. Syntheses of peptides: The peptides were synthesized manually using Fmoc solid-phase peptide chemistry on Rink amide MBHA resin with loading levels of 0.4-0.6 mmol/g resin. All peptides were synthesized in 0.38 μmol scale using a protocol for the solid phase synthesis. The detail of the syntheses of peptides has been described elsewhere[1]. PhotoMet, the photo-active unnatural amino acid, was synthesized as described previously. All peptides were acetylated at the N terminus. All peptides were confirmed by an Auto Flex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany) equipped with a 337 nm nitrogen laser and 1.2 m flight tube. HPLC traces are shown in Figure S1. All peptides were run on an Agilent 1100

instrument.

Table S1. Mass spectrometry data for the peptide described in this study.[a]

Peptide	Calculated mass[b]	Found mass
ARRP	2029.375	2029.264
L1Mp	2027.365	2027.372
R2Mp	1984.348	1984.328
R3Mp	1984.348	1984.023
L4Mp	2027.365	2027.039
L5Mp	2027.365	2027.274
R6Mp	1984.348	1984.118
L7Mp	2027.365	2027.340
L8Mp	2027.365	2027.295
R9Mp	1984.348	1984.307
R10Mp	1984.348	1984.259
L11Mp	2027.365	2027.249
L12Mp	2027.365	2027.258
R13Mp	1984.348	1984.339
L14Mp	2027.365	2027.055
A15Mp	2069.412	2069.235
G16Mp	2083.428	2083.337

[a] Each number represents photoMet mutant of ARRP at the designated position. The sequence of ARRP is Ac-LRRLLRLLRRLLRLAG-NH_2. [b]All mass data of Mp containing peptides were detected as N_2 was deleted, ($M-N_2+H^+$). In contrast, ARRP was detected as ($M+H^+$).

3. Photocrosslinking: Photocrosslinking was performed in 10 ~ 30 μL scale. Cross-linking buffer contains 50 mM Tris, 1 mM $MgCl_2$, 150 mM NaCl, 1 mM DTT at pH 7.5. Briefly, mixtures of a peptide and preannealed ^{32}P labeled RRE RNA in described concentrations were incubated for 10 min on ice using 150 μL PCR tubes. The reaction mixtures were irradiated at 302 nm by a UV transilluminator (SL-20 High Performance DNA Image Visualizer™, SeouLin Bioscience. Co., Ltd, Korea) for 10 min and incubated for 10 min on ice again. Cross-linking with a various concentration of R10MP is shown in Figure S2. Reaction under nonspecific harsh condition with a series of Mp mutant is shown in Figure S3.To scale up cross-link adduct for footprinting experimernts, 50 nM radiolabeled RRE were irradiated in the presence of 50 μM R10Mp in 300 μL scale.

4. Fluorescence anisotropy: Fluorescence anisotropy (FA) measurements were performed on a PerkinElmer LS55 equipped with a thermo-controlled water circulator as described.[1]

5. RNA footprinting

Labeling of RRE RNA. RRE probe (~ 80 pmoles) was radioactively labeled at the 5'-end by using 500 μCi of [γ-^{32} P] ATP (New England Biolabs) and 50 units of polynucleotide kinase (New England Biolabs), after the hydrolysis of 5'-phosphate by 20 units of alkaline phosphatase (CIP, New England biolabs). The same amount of RRE RNA was 3'-end-labeled using cytidine 3",5"-bis(phosphate) (PerkinElmer) catalyzed by T4 RNA ligase 1 (New England Biolabs) following manufacture's protocol. Labeled probe was separated from small nucleotides by passage through G-25 Sephadex beads (Sigma).

RNA footprinting Assay. A labeled solution of ~10 nM RRE RNA was heated to 65 ℃ for 5 min and slowly cooled to room temperatu re over in a buffer containing 20 mM 4-(2-hydroxyethyl) piperazine-1-ethansulfonic acid (HEPES), 1 mM $MgCl_2$, 5 mM KCl, and 140 mM NaCl at pH 7.4. To the resulting solution, 0.2 units or 0.1 unit (for 5'-end-labeled or 3'-end-labed RRE, respectively) of RNase T1 (Ambion) was added and incubated for 15 min at room temperature. Alkaline hydrolysis was performed at 95^{o}C for 15 min in the presence of 4 μg of the yeast RNA using alkaline hydrolysis buffer (Ambion). Then, the fragmented RNA was obtained by ethanol precipitation and dried out. Resulting RNA was electrophoresed using 20% polyacrylamide-7 M urea gel at 1000 V for 8 h. The gel was dried over 40 min at 80ºC using an aspirator. The gel was exposed to a phosphorimager screen and individual bands were quantified on a FLA-3000 and analyzed with Multi Gauge Ver. 3.0 software (Fuji Photo).

References

[1] S. Hyun, H. J. Kim, N. J. Lee, K. H. Lee, Y. Lee, D. R. Ahn, K. Kim, S. Jeong, J. Yu, *J. Am. Chem. Soc.* **2007**, *129*, 4514-4515..

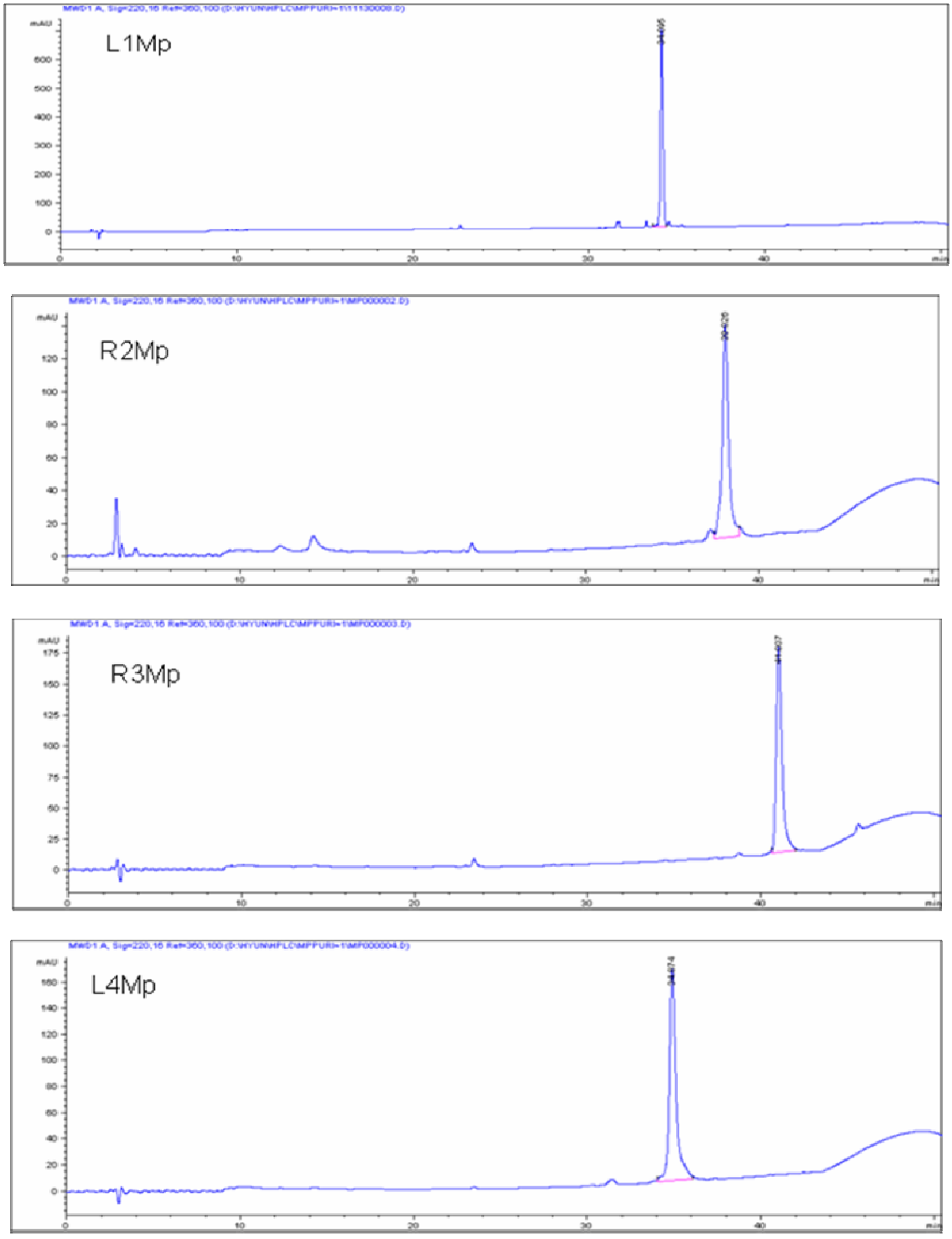

Figure S1. Chromatograms of ARRP Mp mutants. A Phenomenex C_{18} (3 µm, 4.6 x 150 mm) column was used as the stationary phase. For the mobile phase, buffer A (water with 0.1% v/v TFA) and buffer B (acetonitrile with 0.1% *v/v* TFA) were used as a gradient. The gradient conditions are as follows: 5 min, 5% B followed by linear gradient 5-60% B over 35 min. Each peptide was labeled on a respective chromatogram.

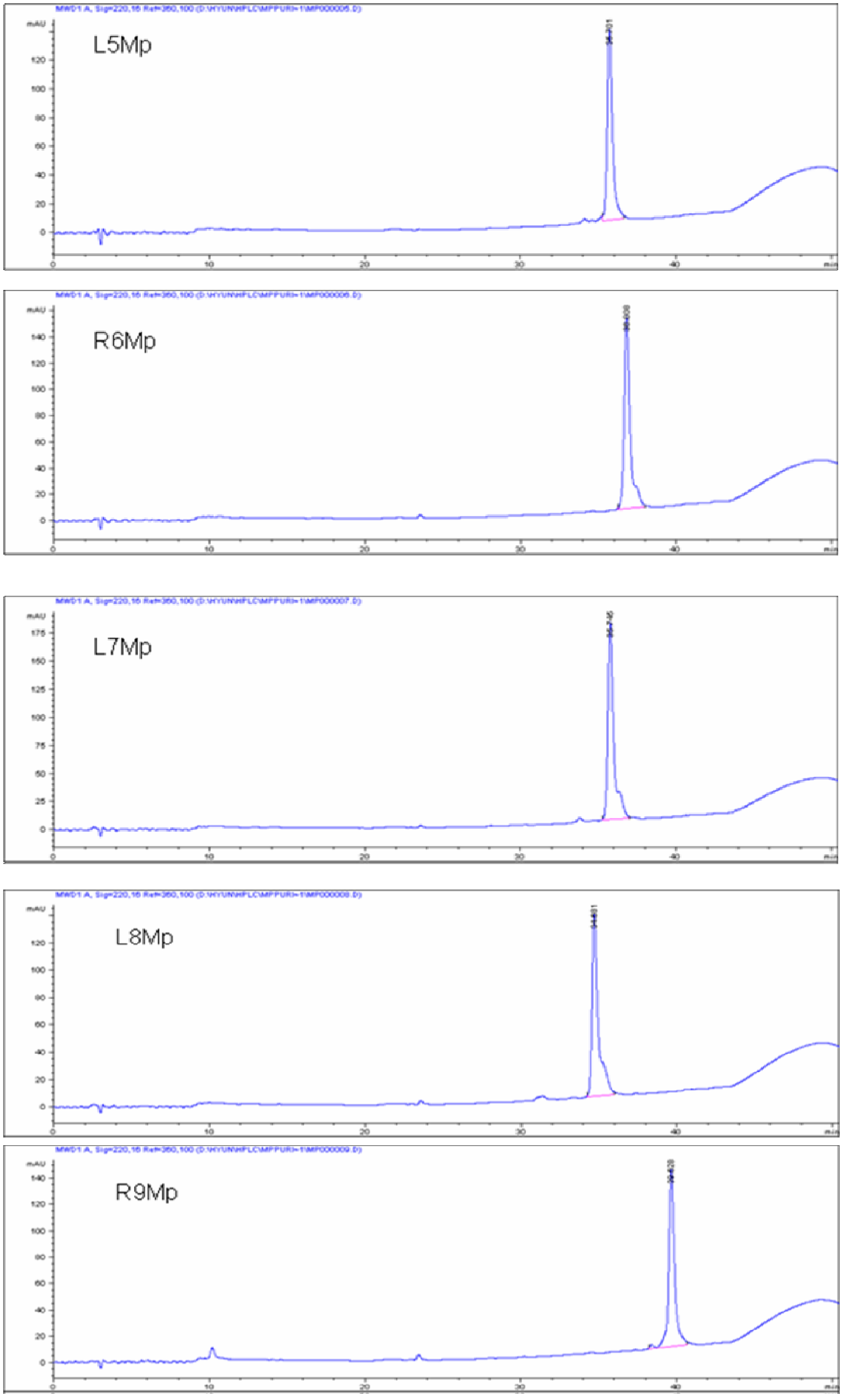

Figure S1. Cont.

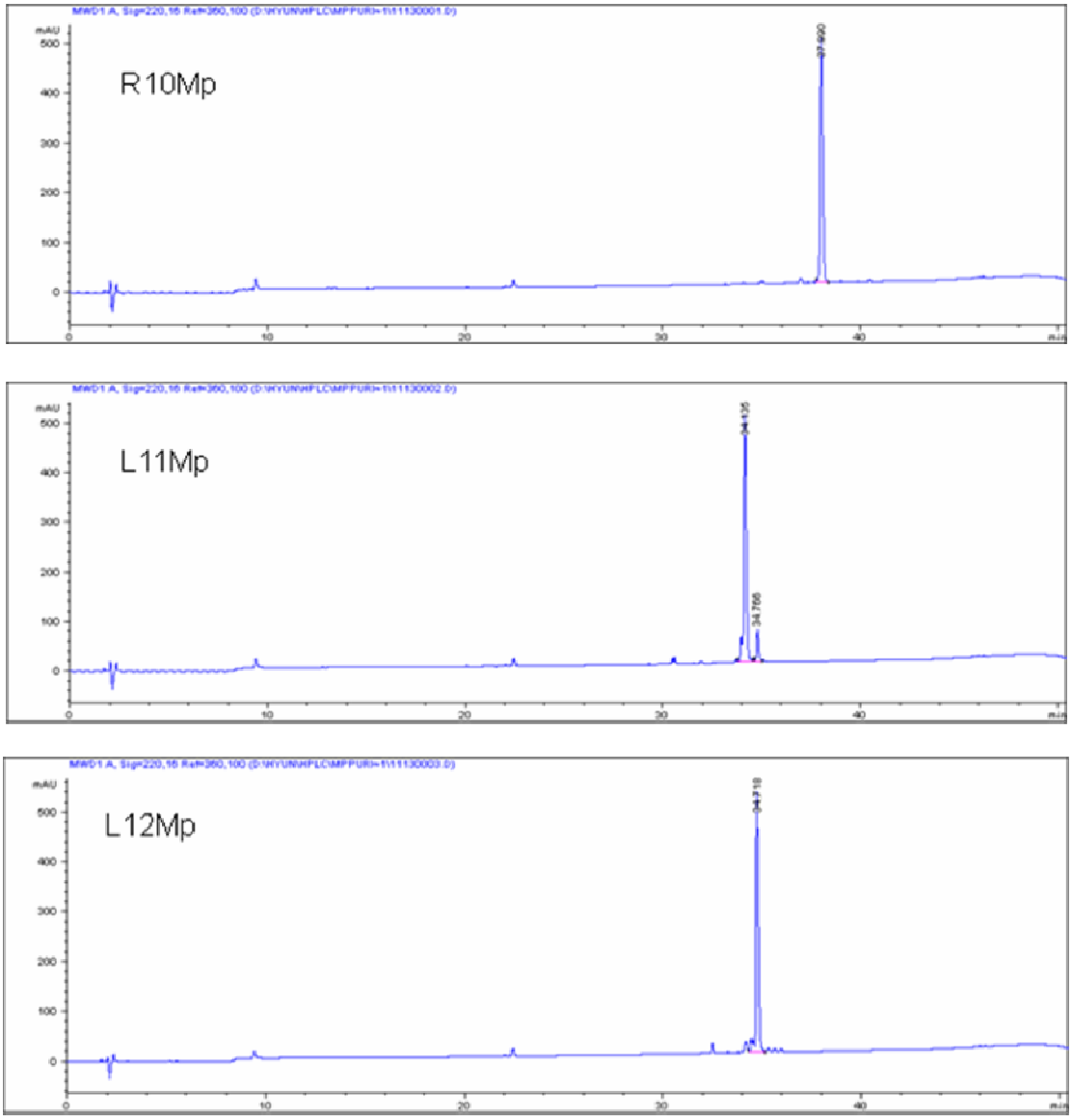

Figure S1. Cont.

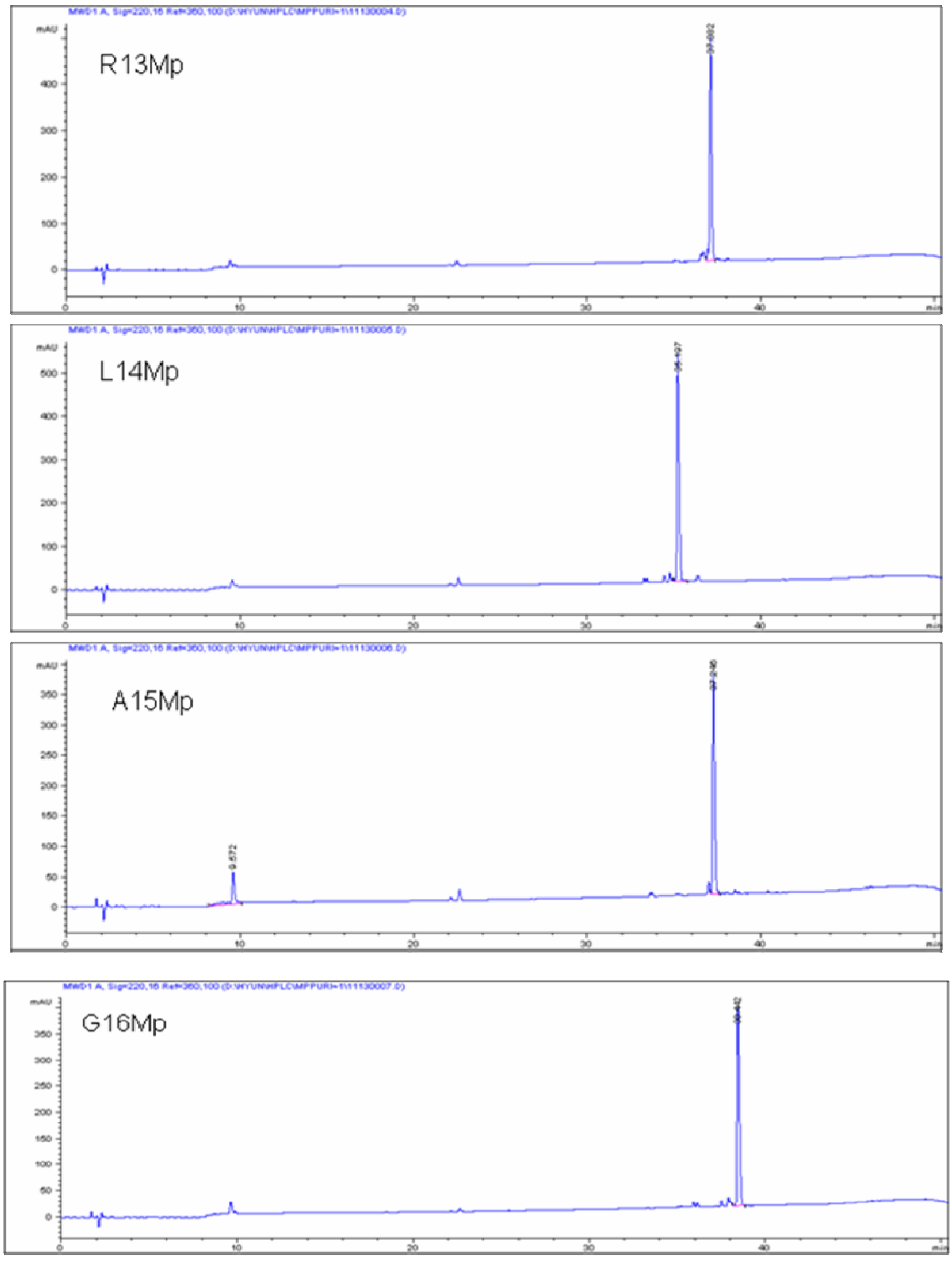

Figure S1. Cont.