Identification of effect of N-glycan modification and its sialylation on proteolytic stability and glucose-stabilizing activity of glucagon-like peptide 1 by site-directed enzymatic glycosylation

Huan Liu[#a], Zengwei Liang[#a], Yu Wang[#a], Yingze Li[a], Ya Wang[a], Xin Guo[c,d], Wanyi Guan[*b], Wei Zou[*a], Zhigang Wu[*a]

[a] College of Food and Biology, Hebei University of Science and Technology, Shijiazhuang, Hebei 050018, China
[b] Ministry of Education Key Laboratory of Molecular and Cellular Biology, Hebei Key Laboratory of Molecular and Cellular Biology, College of Life Sciences, Hebei Normal University, Shijiazhuang, Hebei 050024, China
[c] Research Center, Hebei Province hospital of Chinese Medicine, Affiliated Hospital of Hebei University of Traditional Chinese Medicine, Shijiazhuang, Hebei 050011, China
[d] Department of Pathology and Laboratory Medicine, Department of Pathology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan
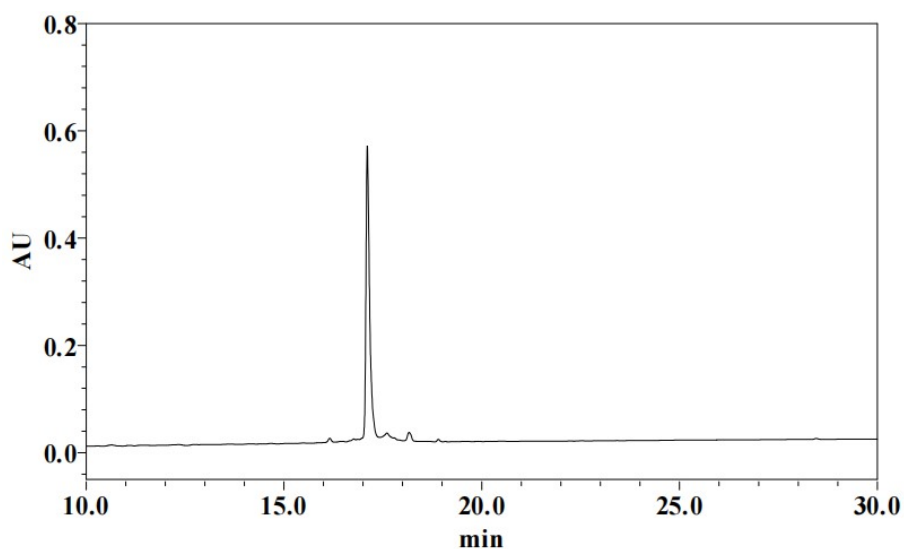
Supporting Information

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Fig. S1 HPLC chromatographs and ESI-MS spectra of glycosylated GLP-1 analogues

Glc-GLP-1-1

H—H-A-E-G-T-F-T-S-N-V——S-S-Y-L-E-G-Q-A-A-K——E-F—I-A-W-L-V-K-G-R——G—OH
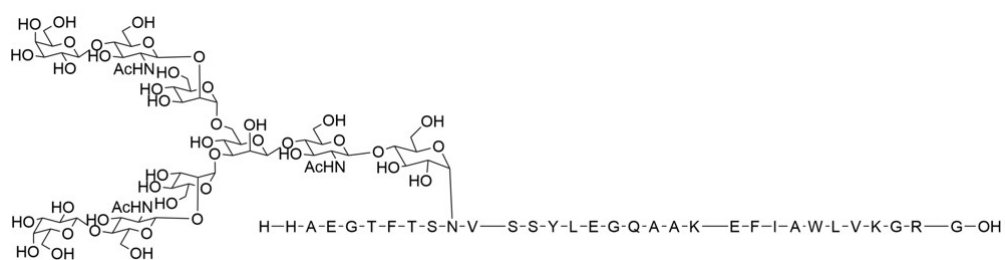
HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 17.107 min.

MS: calculated 3514.7369; found 3515.4704.
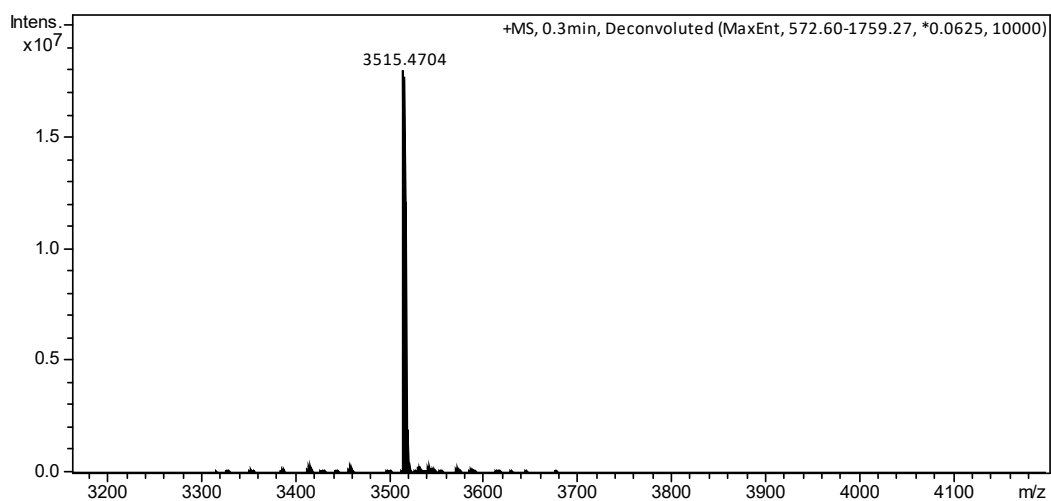
Glycan-GLP-1-1(G2)

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. $T_R = 16.087$ min.
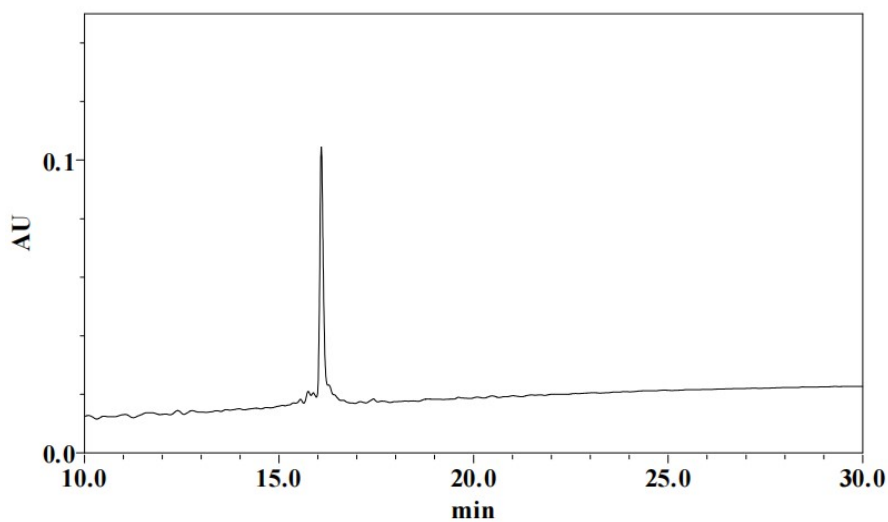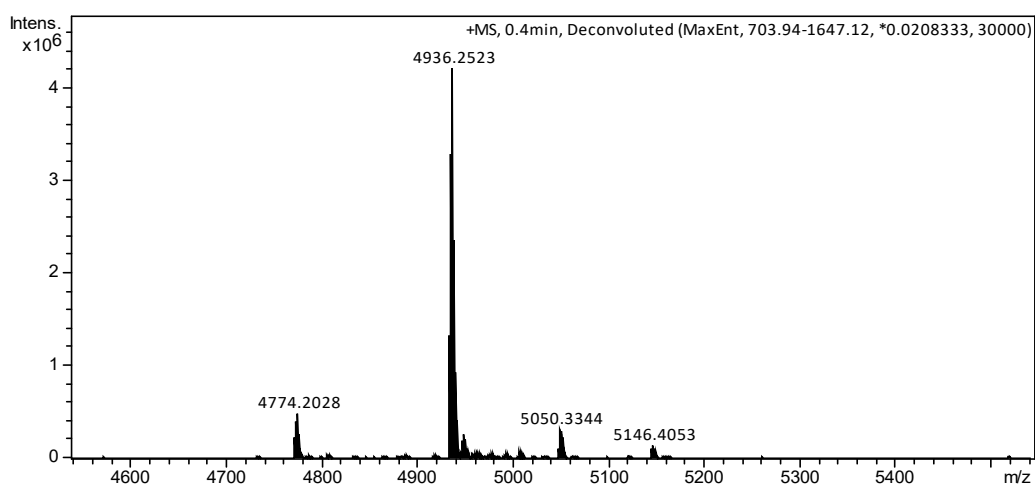

MS: calculated 4935.2391; found 4936.2523.
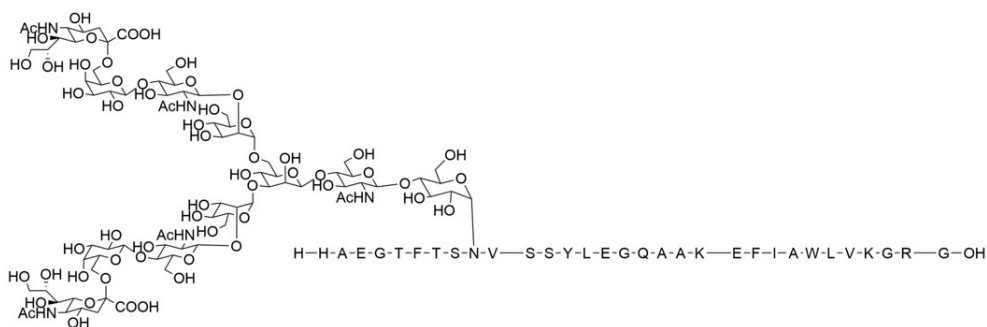
Glycan-GLP-1-1(G2S2)

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 15.599 min.
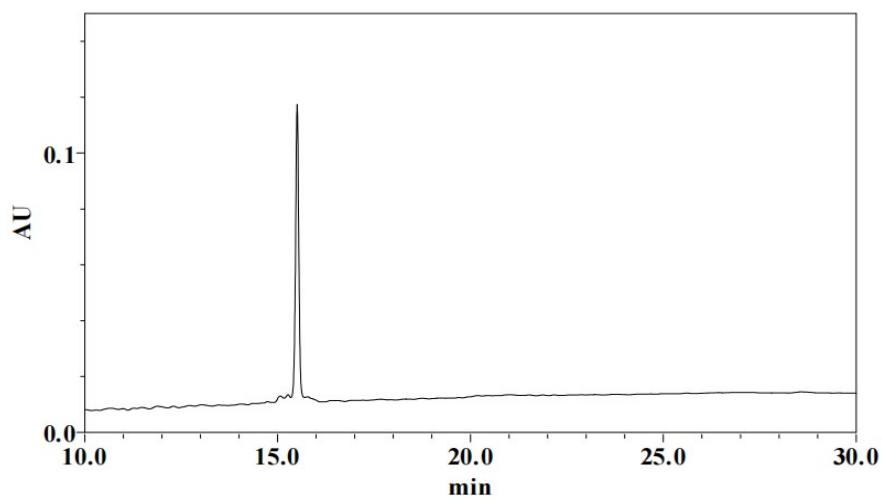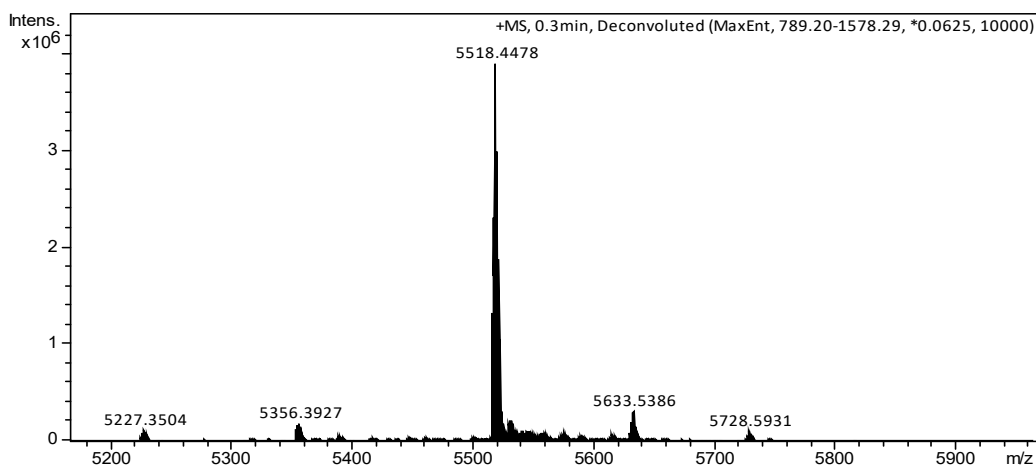

MS: calculated 5517.4299; found 5518.4478.

Glc-GLP-1-3

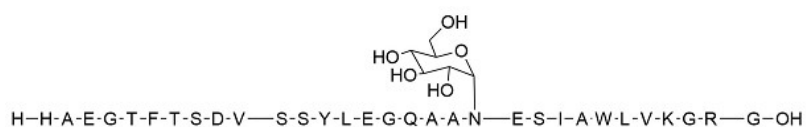H–H-A-E-G-T-F-T-S-D-V—S-S-Y-L-E-G-Q-A-A-N—E-S-I-A-W-L-V-K-G-R—G–OH

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 13.345 min.
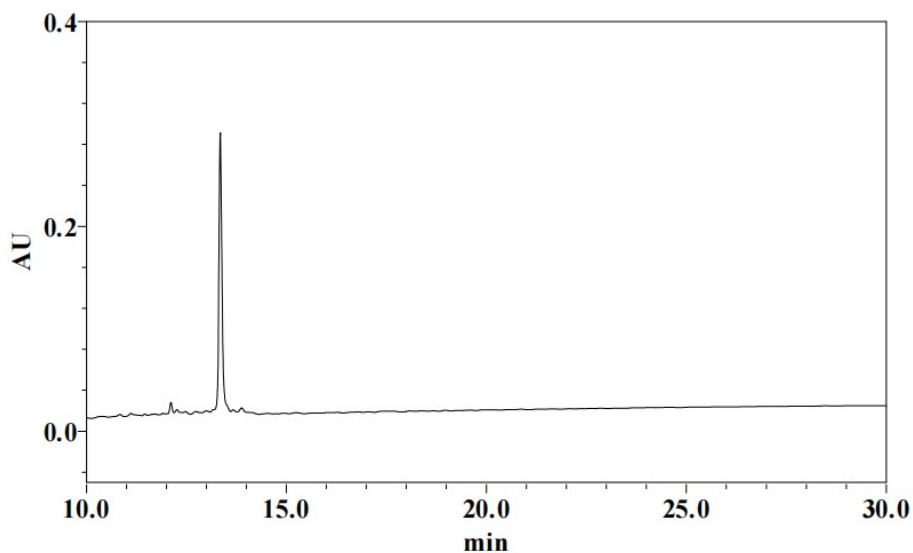

MS: calculated 3441.6325; found 3442.6382.

Glycan-GLP-1-3(G2)

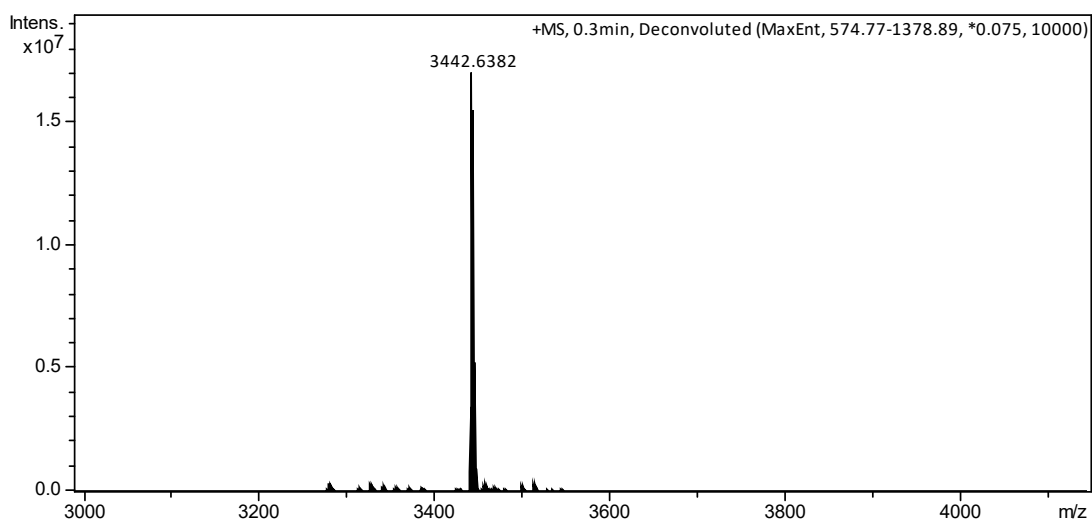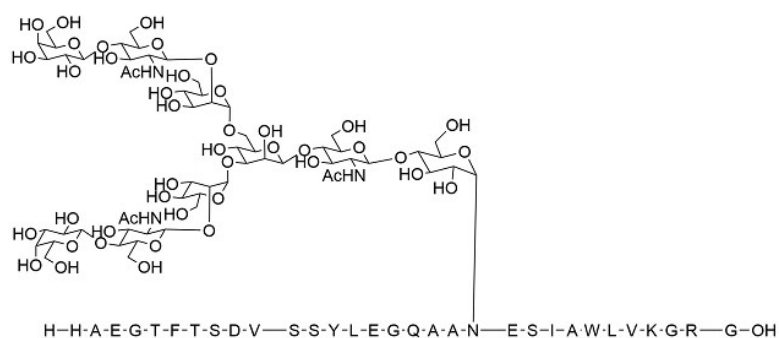H–H·A·E·G·T·F·T·S·D·V——S·S·Y·L·E·G·Q·A·A·N——E·S·I·A·W·L·V·K·G·R——G–OH

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 12.764 min.

MS: calculated 4862.1347; found 4863.1389.

Glycan-GLP-1-3(G2S2)

H–H-A-E-G-T-F-T-S-D-V—S-S-Y-L-E-G-Q-A-A-N—E-S-I-A-W-L-V-K-G-R—G–OH

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 12.394 min.
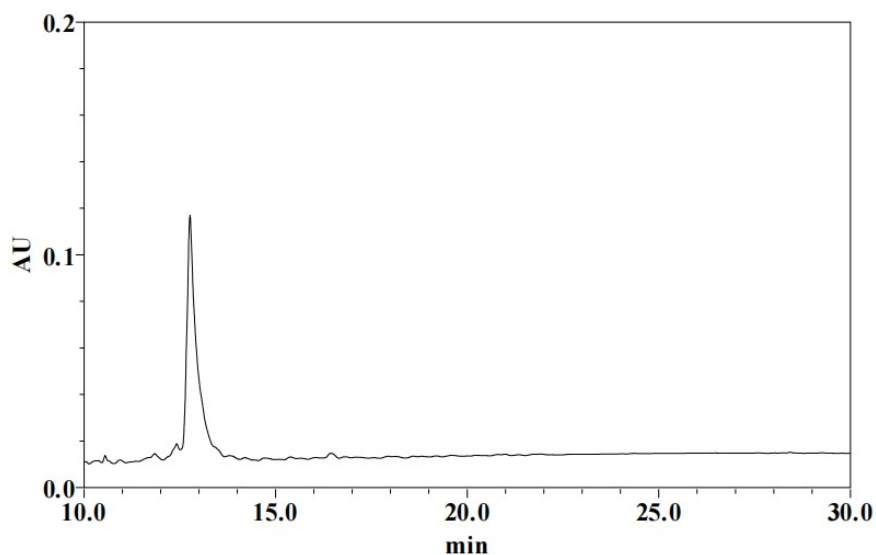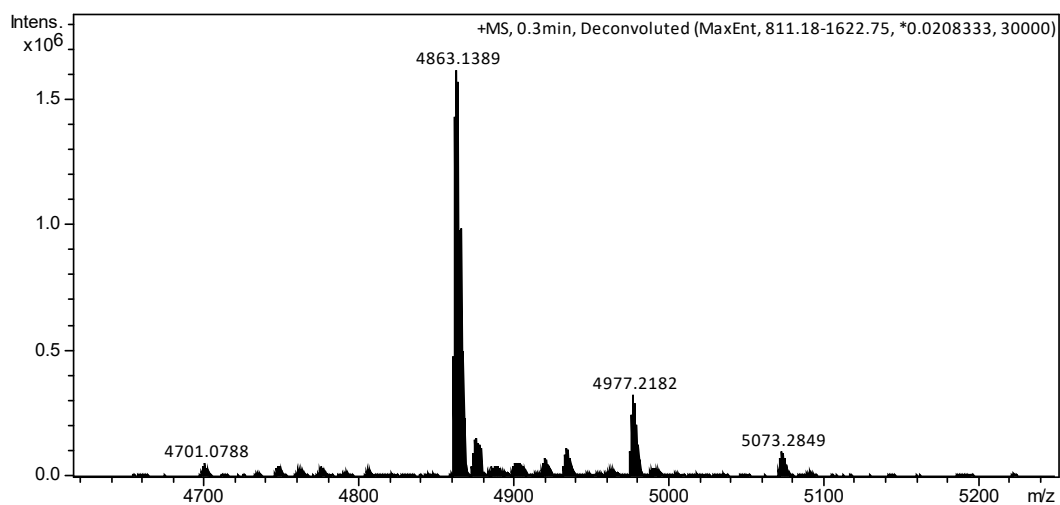

MS: calculated 5444.3255; found 5445.3584.

Glc-GLP-1-5

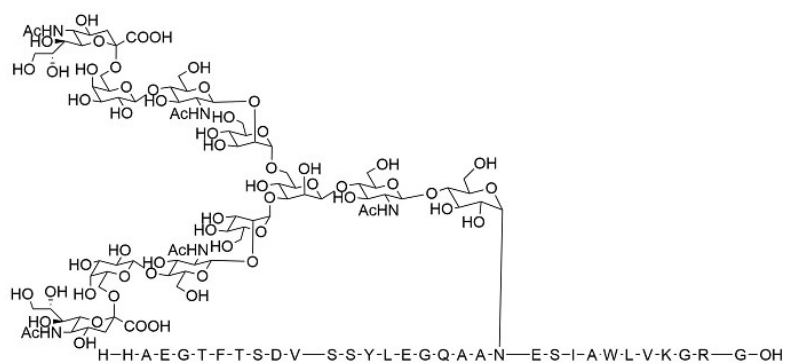H—H-A-E-G-T-F-T-S-D-V—S-S-Y-L-E-G-Q-A-A-K—E-F-I-A-W-L-V-N-G-T—G—OH

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 19.384 min.
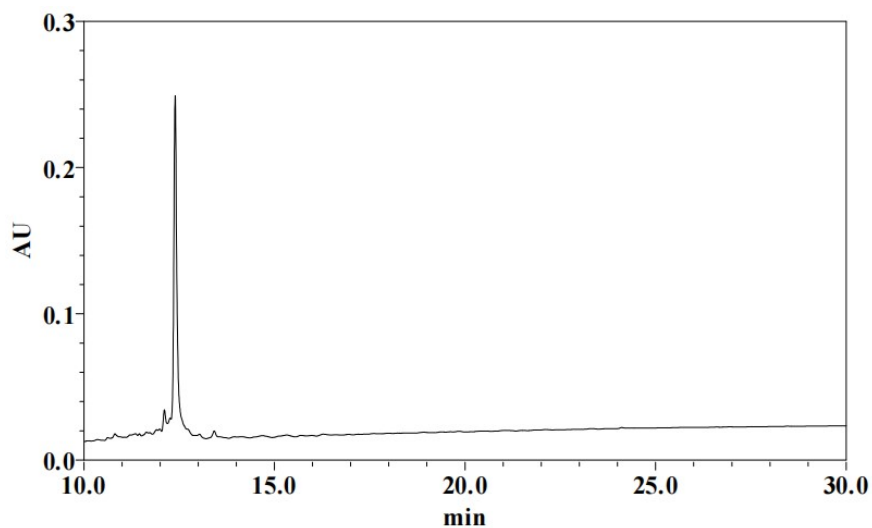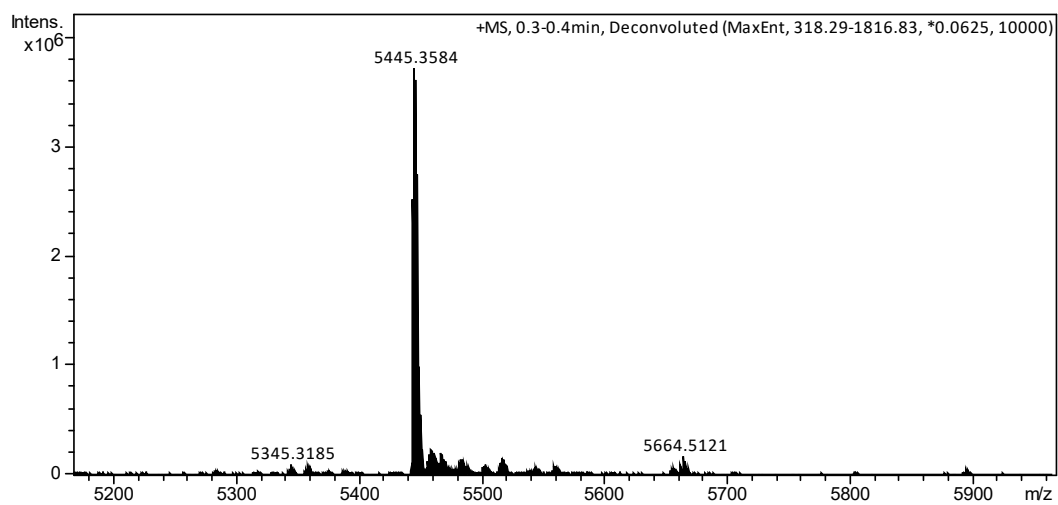

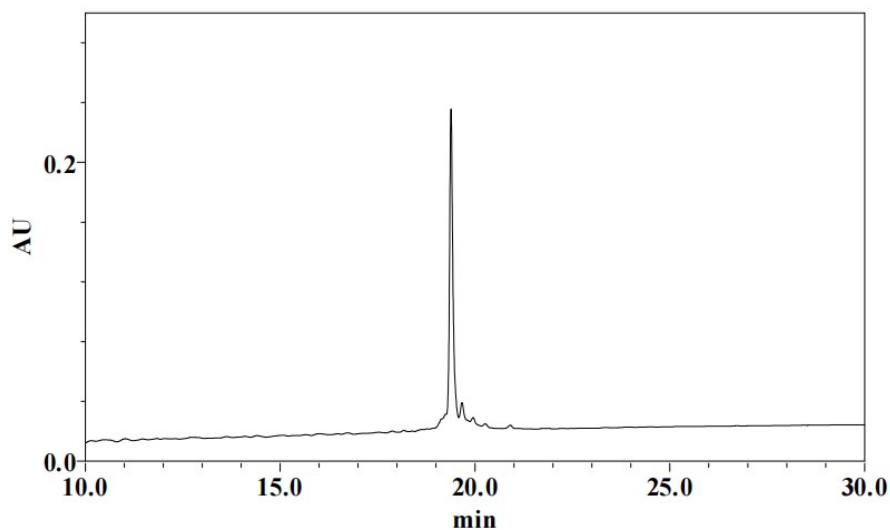**MS:** calculated 3446.6154; found 3447.6287.

Glycan-GLP-1-5(G2)

H—H-A-E-G-T-F-T-S-D-V—S-S-Y-L-E-G-Q-A-A-K—E-F-I-A-W-L-V-N-G-T—G—OH

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 18.252 min.

MS: calculated 4867.1176; found 4868.1329.
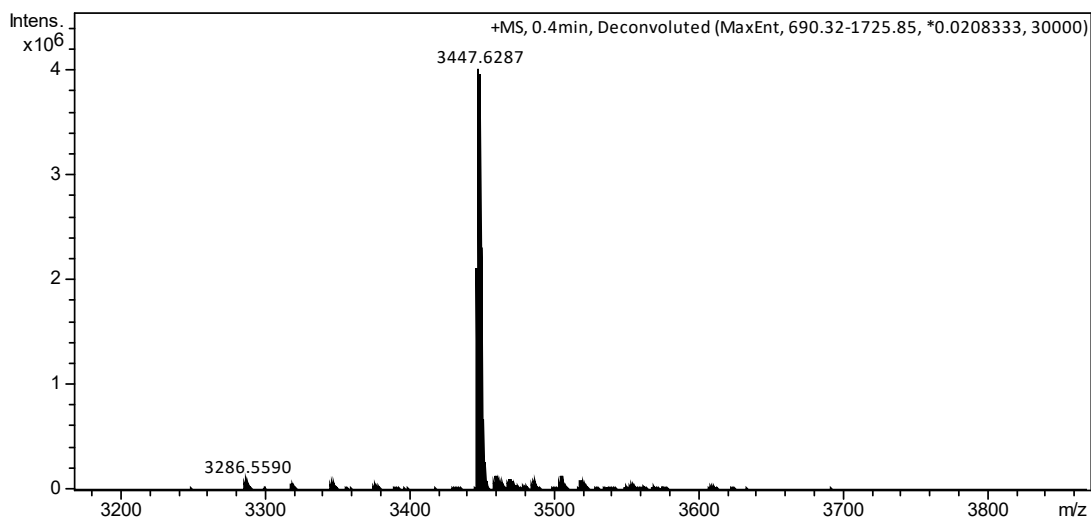
Glycan-GLP-1-5(G2S2)

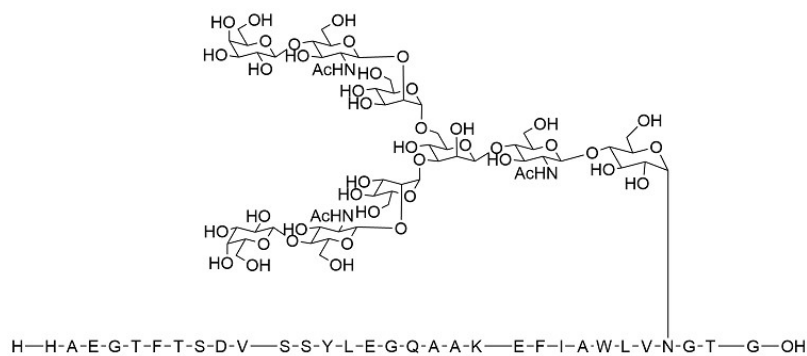H—H-A-E-G-T-F-T-S-D-V—S-S-Y-L-E-G-Q-A-A-K—E-F-I-A-W-L-V-N-G-T—G—OH

HPLC-C18-UV: The column (4.6 mm × 250 mm) was eluted with a linear gradient of 20-60% acetonitrile (ACN) containing 0.1% TFA within 35 min at a flow rate of 1 mL/min. T_R = 18.160 min.
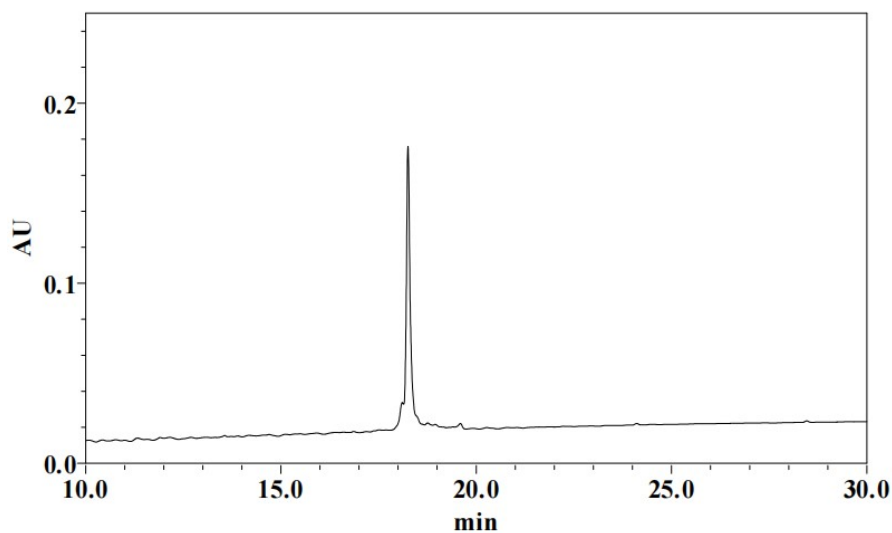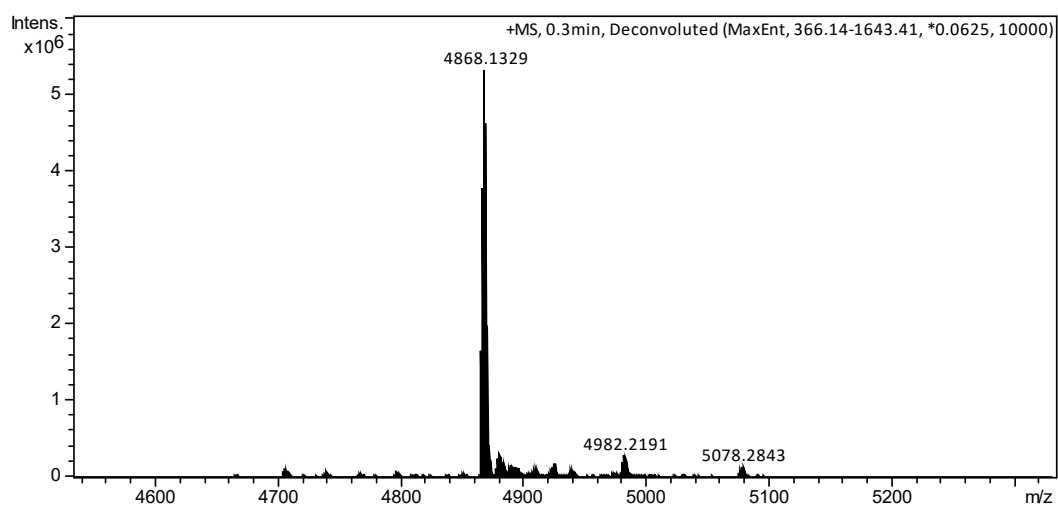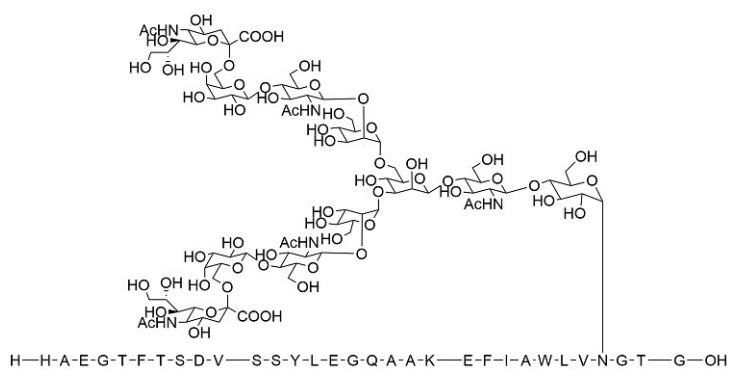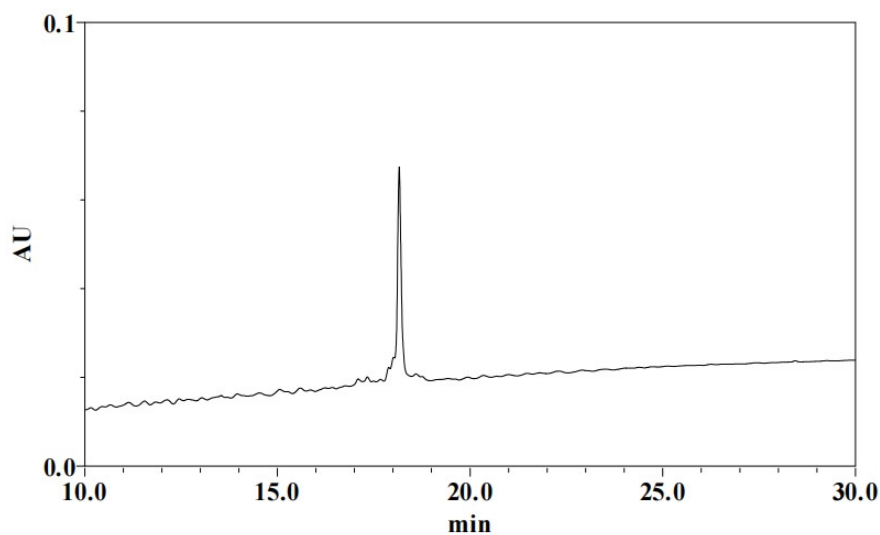

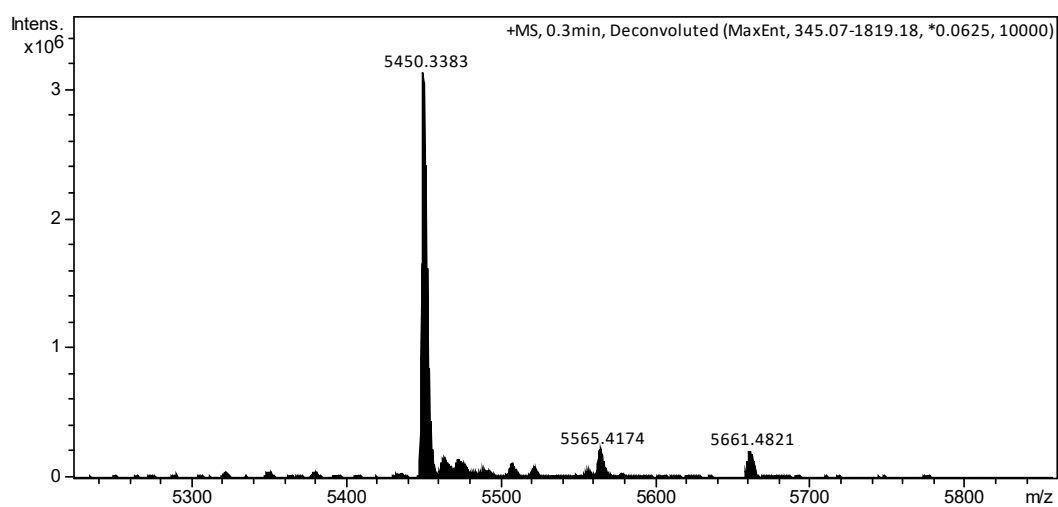**MS:** calculated 5449.3085; found 5450.3383.

Table S1 Retention times, theoretical and observed molecular weights of glycosylated GLP-1 analogues

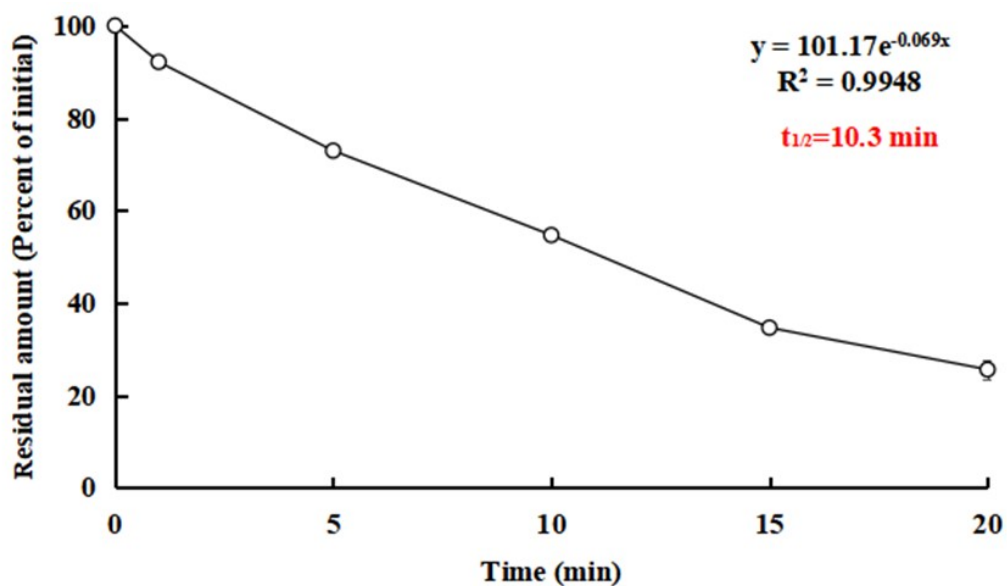| Peptide ID	Glycosylation site	Glycoform	Retention time (min)	ESI-MS analysis	
				Calcd mass[b]	Observed[c]
1	15N	Glc	17.107	3514.74	3515.47
		G2	16.087	4935.24	4936.25
		G2S2	15.599	5517.43	5518.45
2	16N	Glc	N.A.[a]	3531.74	N.A.
3	26N	Glc	13.345	3441.63	3442.64
		G2	12.764	4862.13	4863.14
		G2S2	12.394	5444.33	5445.36
4	34N	Glc	N.A.	3433.59	N.A.
5	34N	Glc	19.384	3446.62	3447.63
		G2	18.252	4867.12	4868.13
		G2S2	18.160	5449.31	5450.34

[a] N.A.: Not applicable. [b] Molecular weight of free base. [c] $[M+H]^+$.

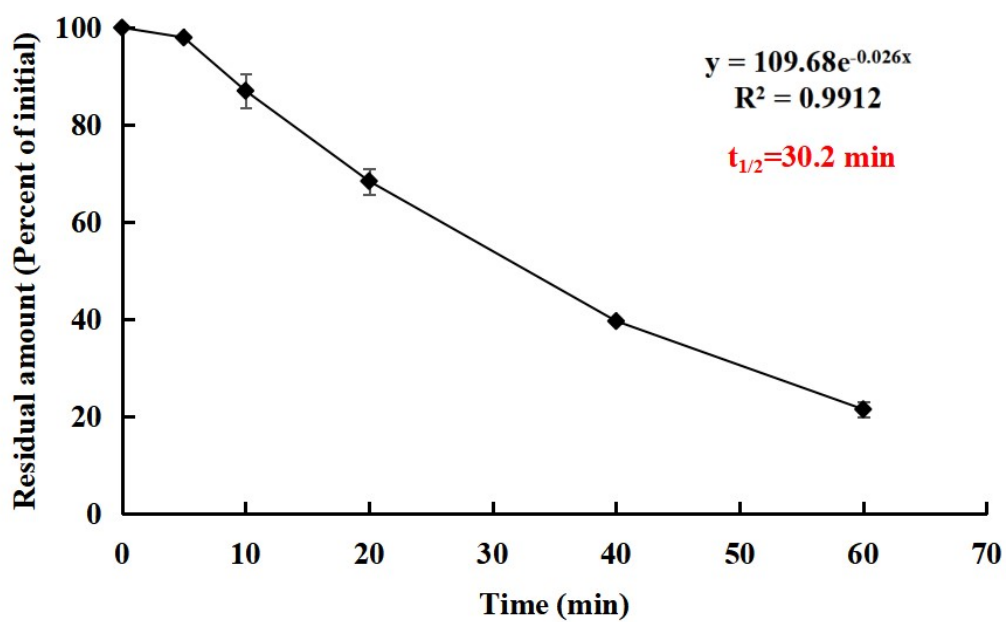**Table S2** The calculated ratio of secondary structure elements for native GLP-1 and glycan-GLP-1 analogues

Variants	α-helix	Antiparallel	Parallel	β-turn	Radom coil
GLP-1	13.80%	43.00%	2.30%	20.70%	27.40%
Glycan-GLP-1-1(G2)	13.70%	45.10%	2.30%	20.00%	26.90%
Glycan-GLP-1-1(G2S2)	13.80%	45.20%	2.20%	20.00%	26.80%
Glycan-GLP-1-3(G2)	13.40%	45.80%	2.30%	19.90%	26.70%
Glycan-GLP-1-3(G2S2)	13.30%	46.20%	2.30%	19.70%	26.70%
Glycan-GLP-1-5(G2)	13.50%	46.00%	2.30%	19.60%	26.80%
Glycan-GLP-1-5(G2S2)	13.40%	45.10%	2.20%	20.10%	26.90%

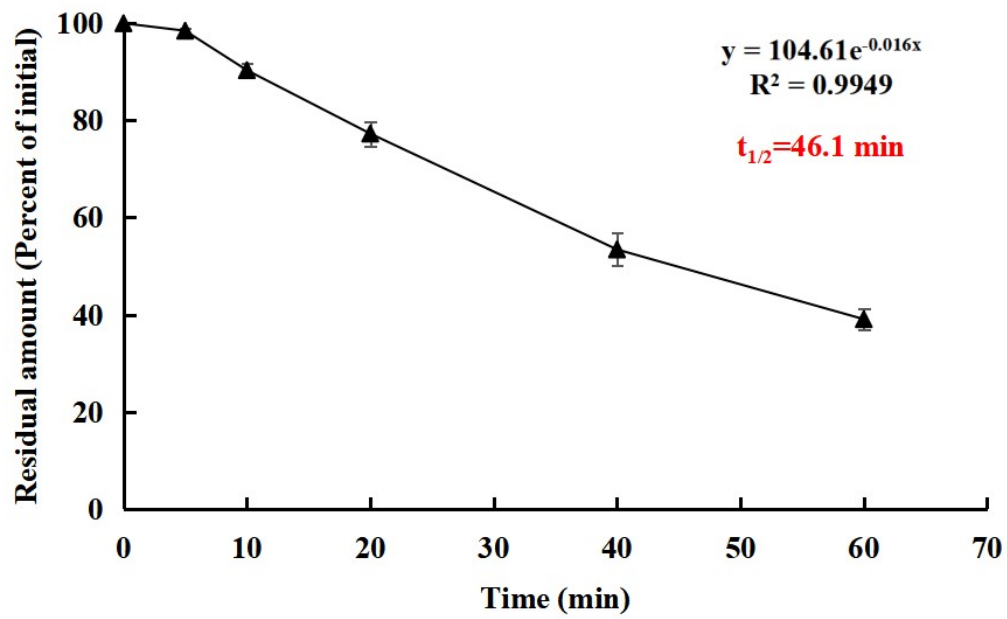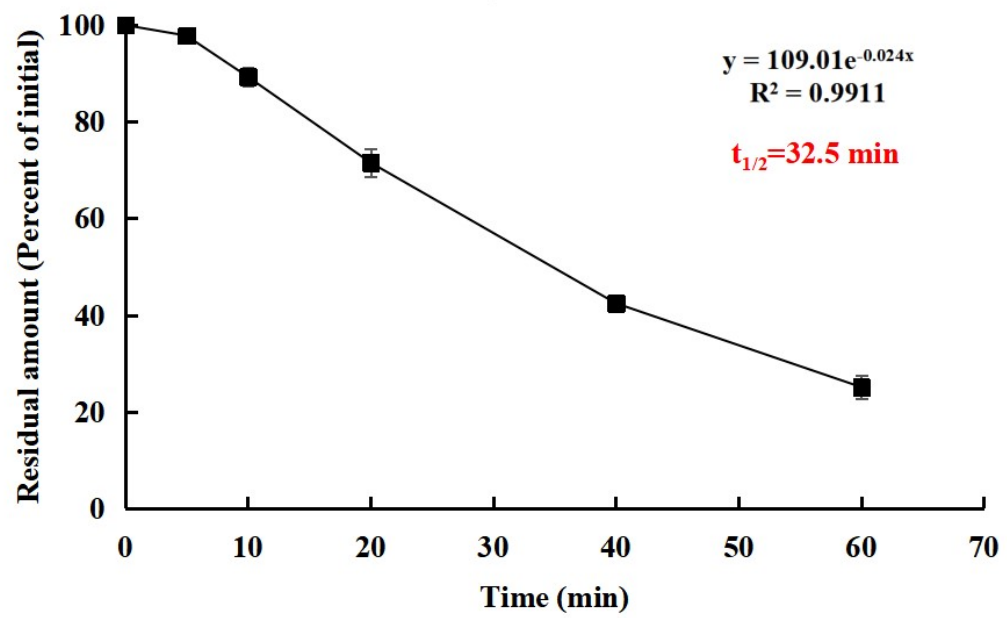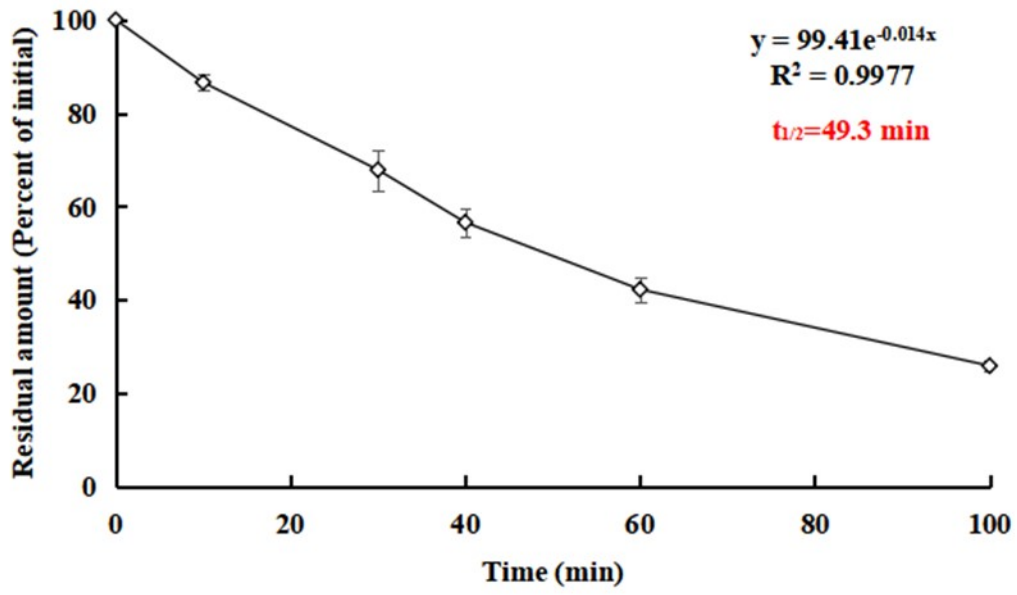**Fig. S2** Degradation profiles of GLP-1 isoforms incubated with DPP-IV

GLP-1

$$y = 101.17e^{-0.069x}$$
$$R^2 = 0.9948$$
$$t_{1/2}=10.3 \text{ min}$$

Glc-GLP-1-1

$$y = 109.68e^{-0.026x}$$
$$R^2 = 0.9912$$
$$t_{1/2}=30.2 \text{ min}$$

Glc-GLP-1-3

$y = 104.61e^{-0.016x}$
$R^2 = 0.9949$

$t_{1/2}$=46.1 min

Glc-GLP-1-5

$y = 109.01e^{-0.024x}$
$R^2 = 0.9911$

$t_{1/2}$=32.5 min

Glycan-GLP-1-1(G2)

$y = 99.41e^{-0.014x}$
$R^2 = 0.9977$

$t_{1/2}=49.3$ min

Glycan-GLP-1-1(G2S2)

$y = 103.6e^{-0.005x}$
$R^2 = 0.9931$

$t_{1/2}=146$ min

Glycan-GLP-1-3(G2)

$y = 104.92e^{-0.004x}$
$R^2 = 0.9933$

$t_{1/2} = 182.5$ min

Glycan-GLP-1-3(G2S2)

$y = 105.78e^{-0.002x}$
$R^2 = 0.9926$

$t_{1/2} = 380$ min

Glycan-GLP-1-5(G2)

$y = 103.55e^{-0.004x}$

$R^2 = 0.9895$

$t_{1/2} = 182.5$ min

Glycan-GLP-1-5(G2S2)

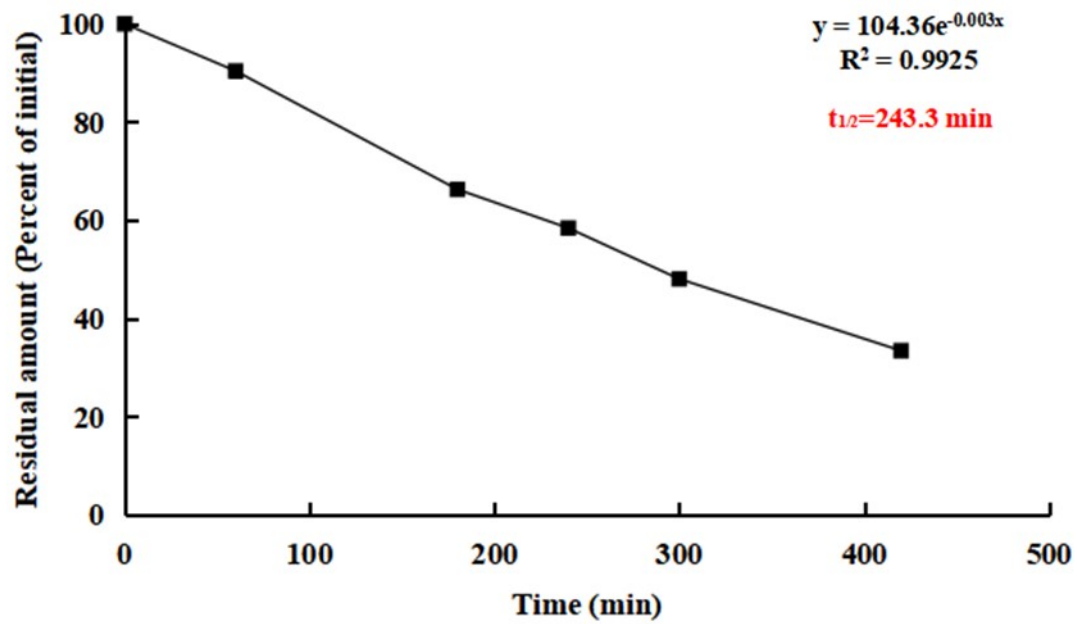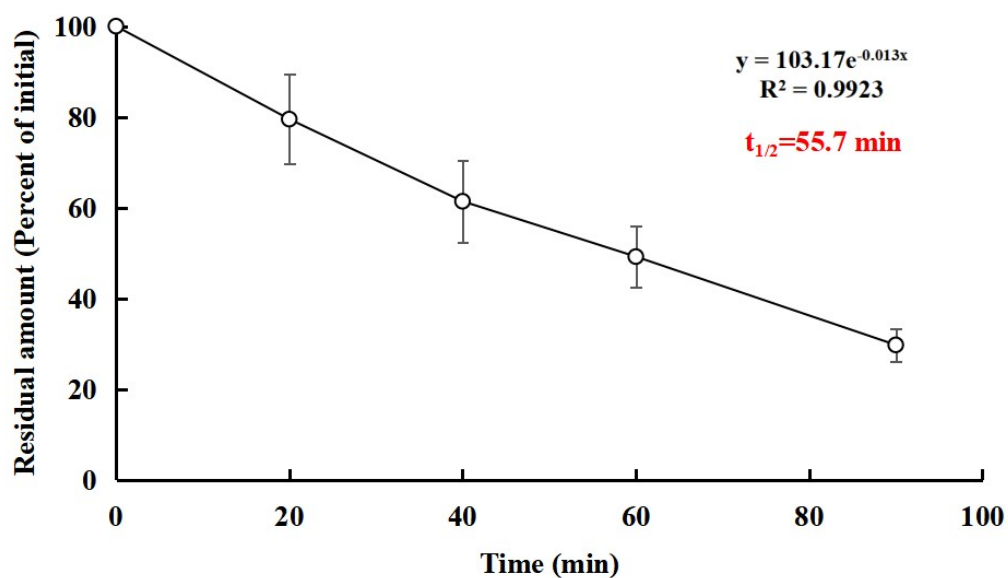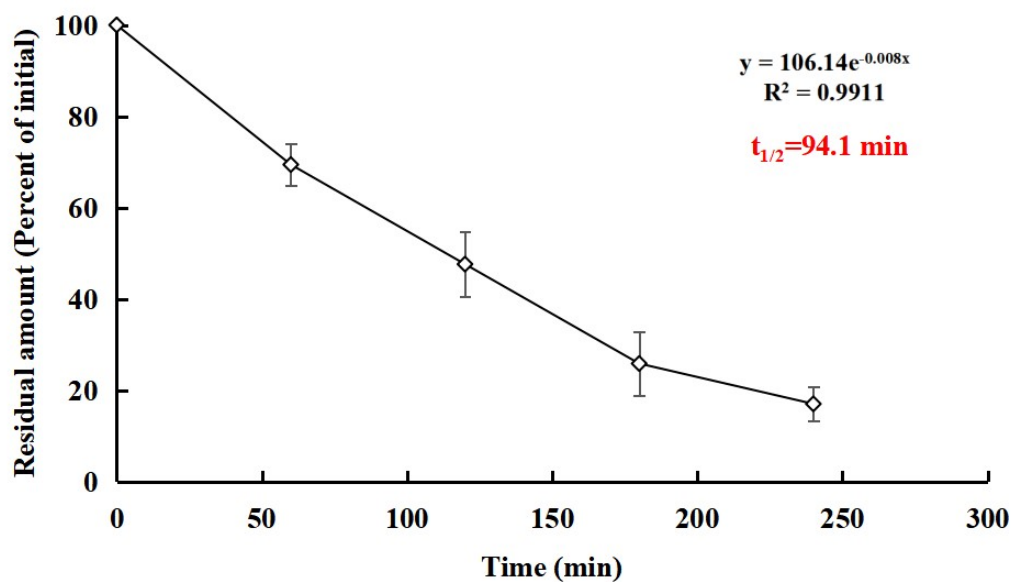$y = 104.36e^{-0.003x}$

$R^2 = 0.9925$

$t_{1/2} = 243.3$ min
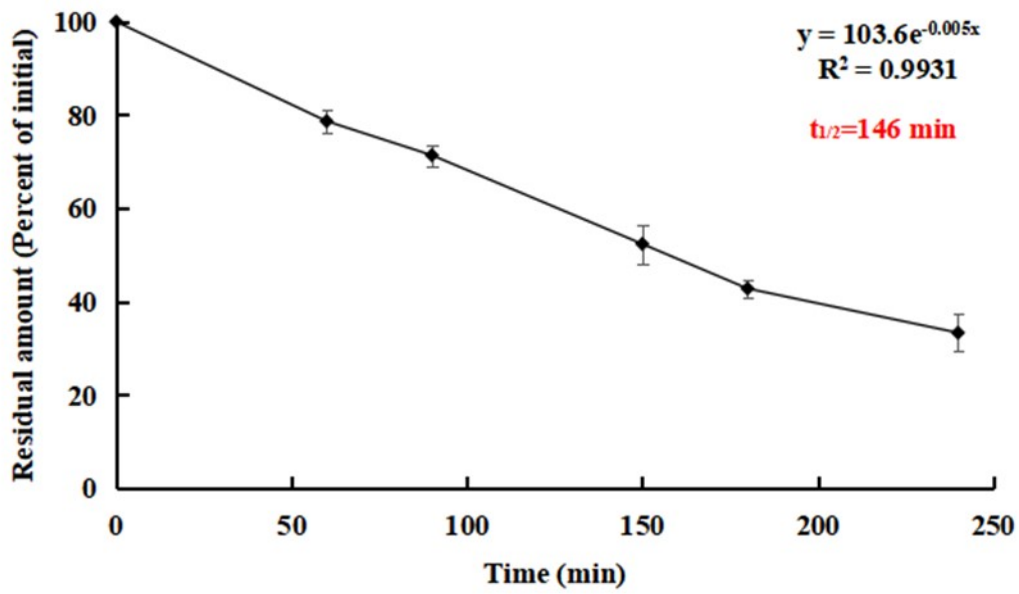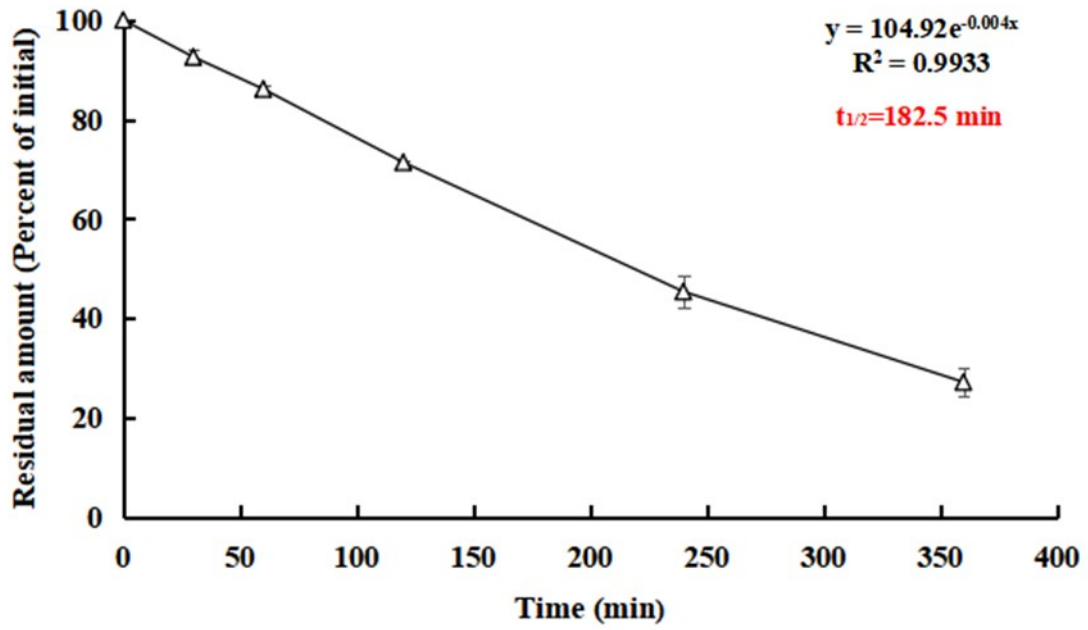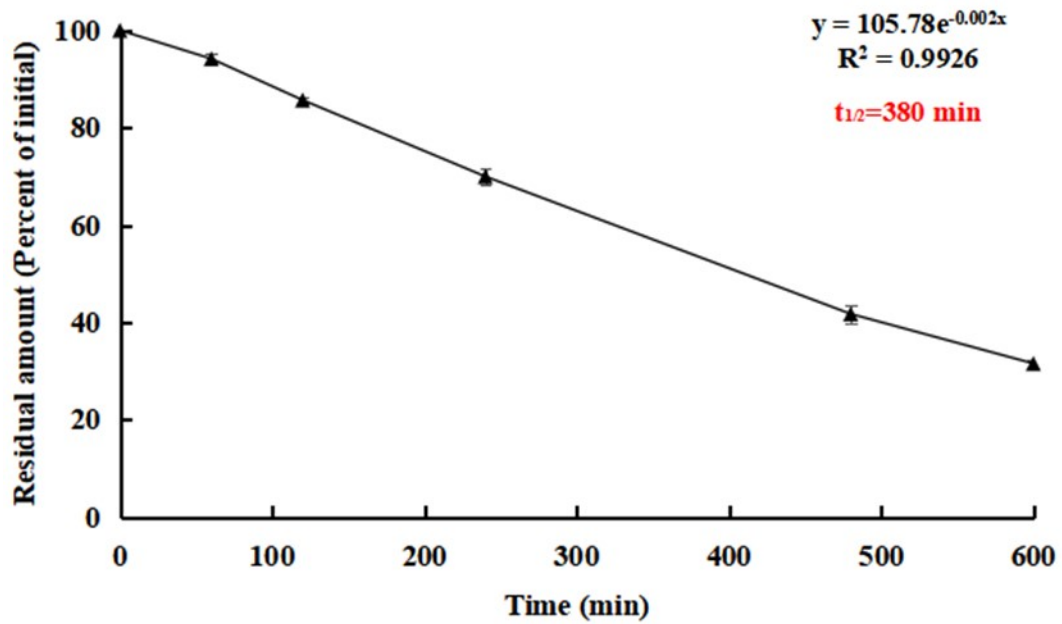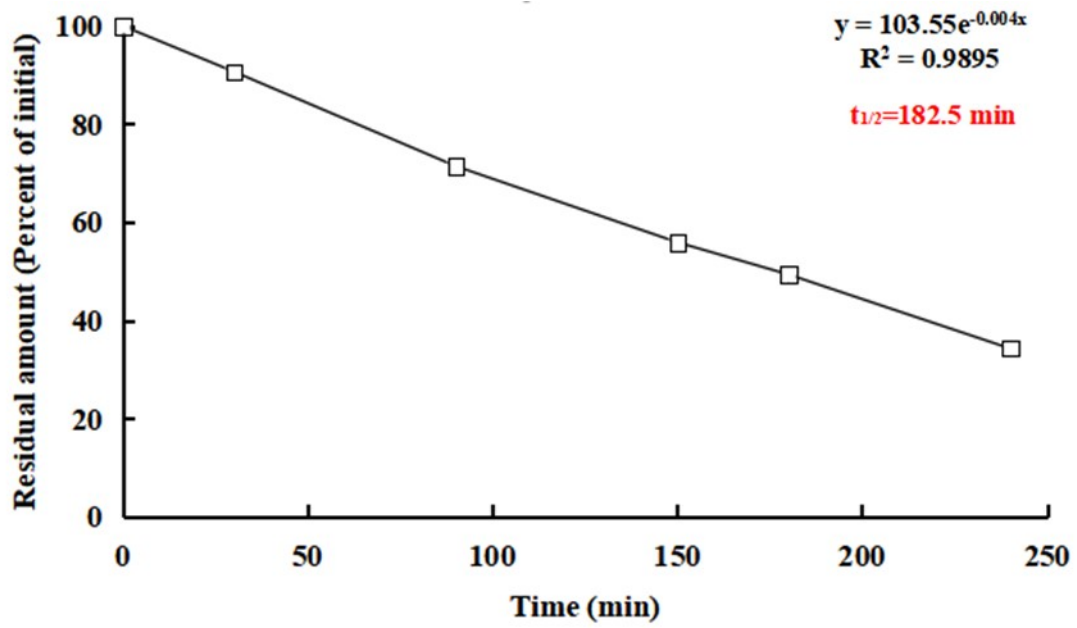
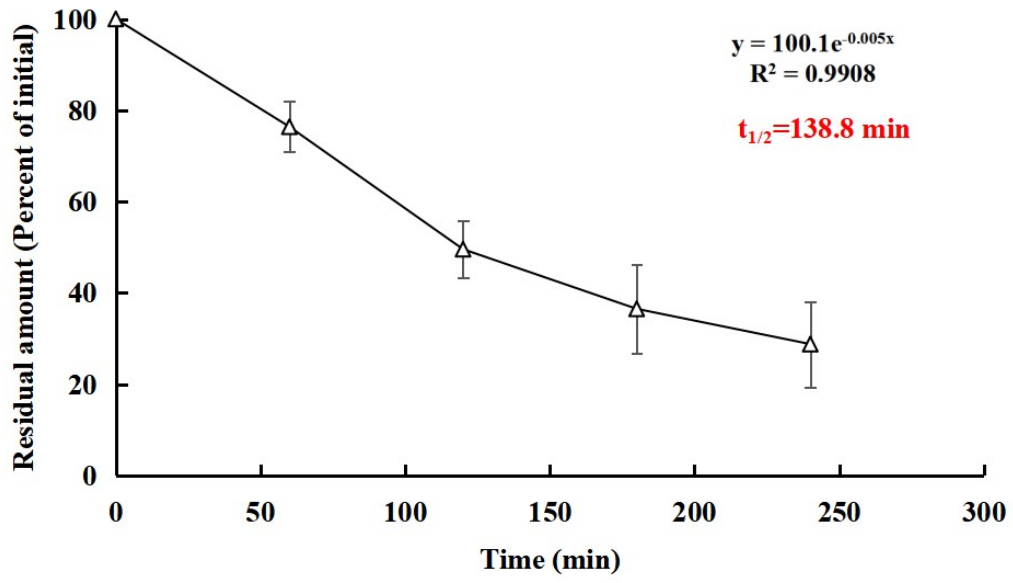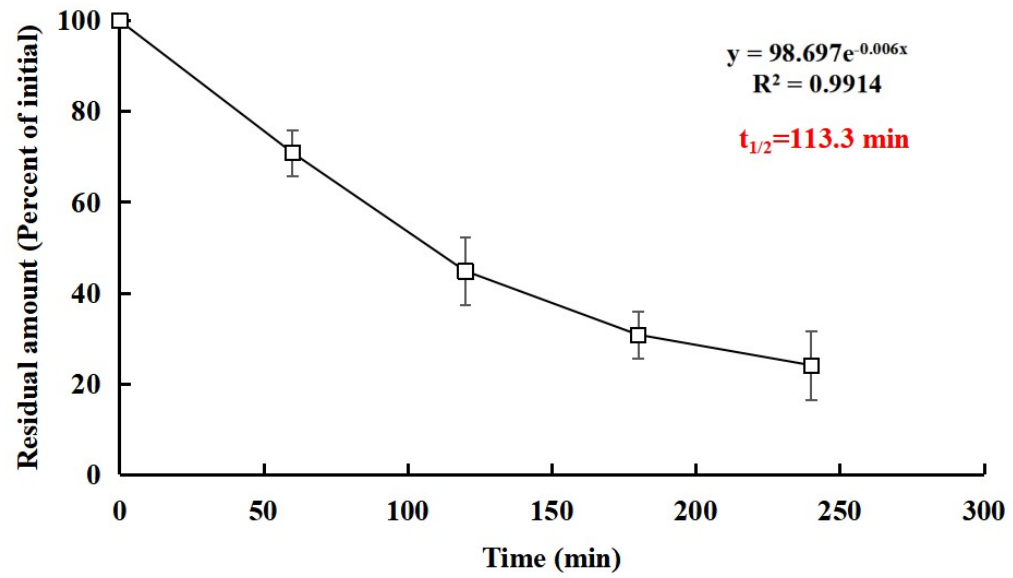**Fig. S3** Degradation profiles of GLP-1 isoforms incubated with mouse serum

GLP-1

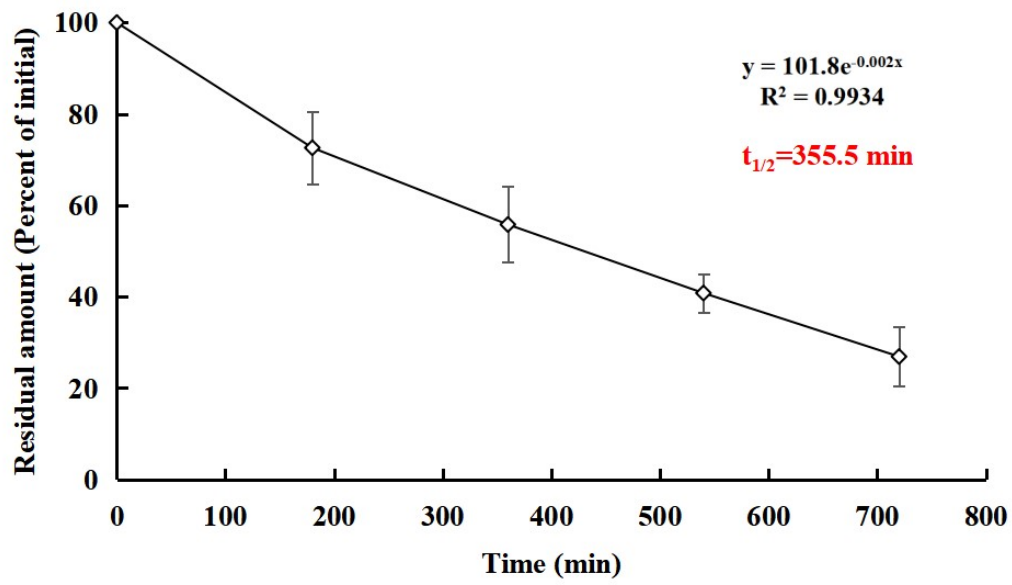$y = 103.17e^{-0.013x}$
$R^2 = 0.9923$

$t_{1/2} = 55.7$ min

Glc-GLP-1-1

$y = 106.14e^{-0.008x}$
$R^2 = 0.9911$

$t_{1/2} = 94.1$ min

Glc-GLP-1-3

$y = 100.1e^{-0.005x}$
$R^2 = 0.9908$

$t_{1/2}$=138.8 min

Glc-GLP-1-5

$y = 98.697e^{-0.006x}$
$R^2 = 0.9914$

$t_{1/2}$=113.3 min

Glycan-GLP-1-1(G2)

$y = 101.8e^{-0.002x}$
$R^2 = 0.9934$

$t_{1/2}$=355.5 min

Glycan-GLP-1-1(G2S2)

$y = 104.44e^{-0.001x}$
$R^2 = 0.9947$

$t_{1/2}$=736.6 min

Glycan-GLP-1-3(G2)

$$y = 104.65e^{-0.001x}$$
$$R^2 = 0.9923$$

$t_{1/2}$=738.6 min

Residual amount (Percent of initial) vs Time (min)

Glycan-GLP-1-3(G2S2)

$$y = 100.34e^{-0.0005x}$$
$$R^2 = 0.9915$$

$t_{1/2}$=1393.1 min

Residual amount (Percent of initial) vs Time (min)

Glycan-GLP-1-5(G2)

$y = 109.15e^{-0.002x}$
$R^2 = 0.9939$

$t_{1/2}=390.4$ min

Glycan-GLP-1-5(G2S2)

$y = 108.75e^{-0.001x}$
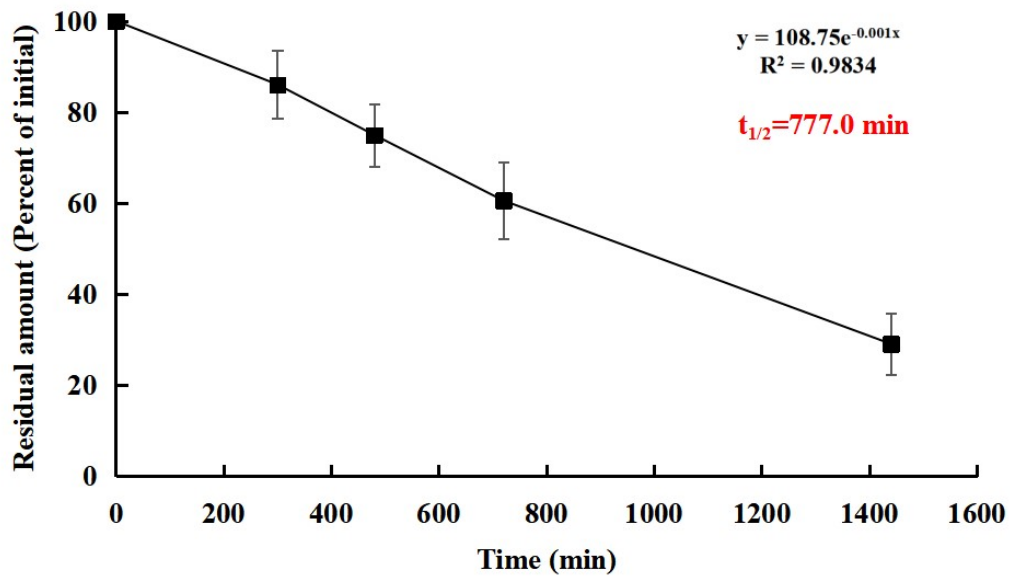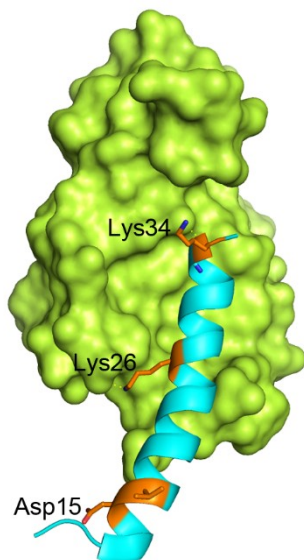$R^2 = 0.9834$

$t_{1/2}=777.0$ min
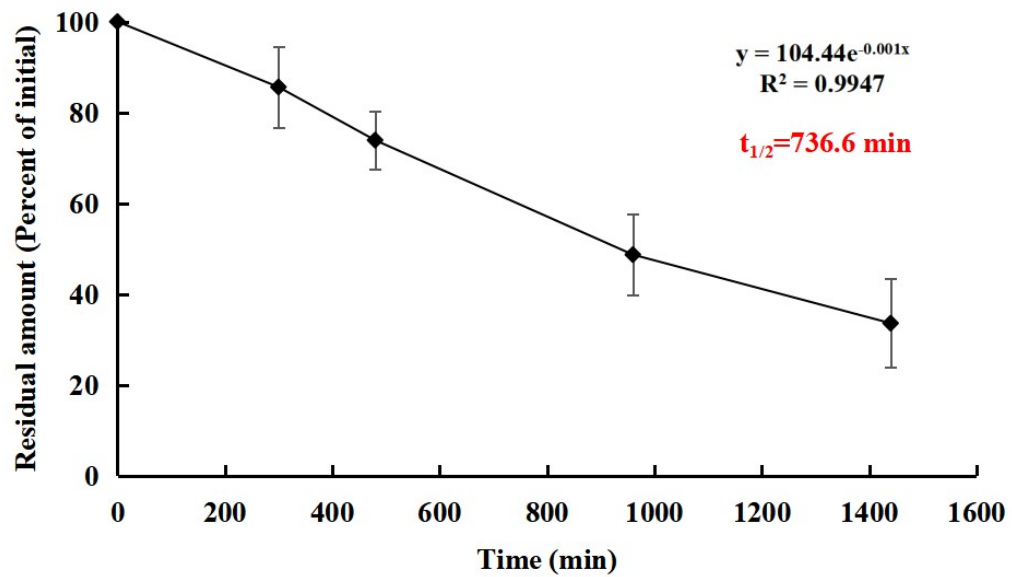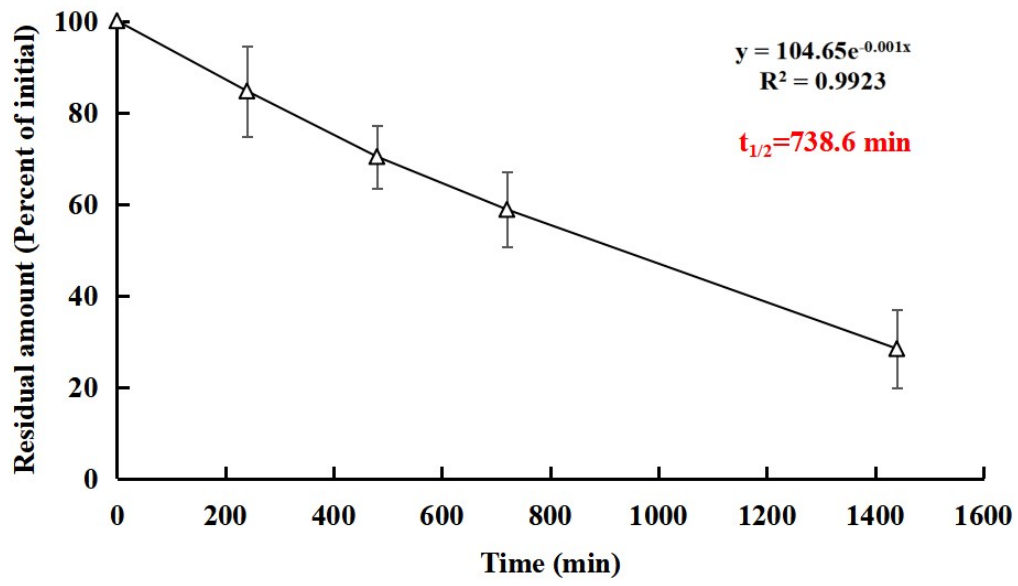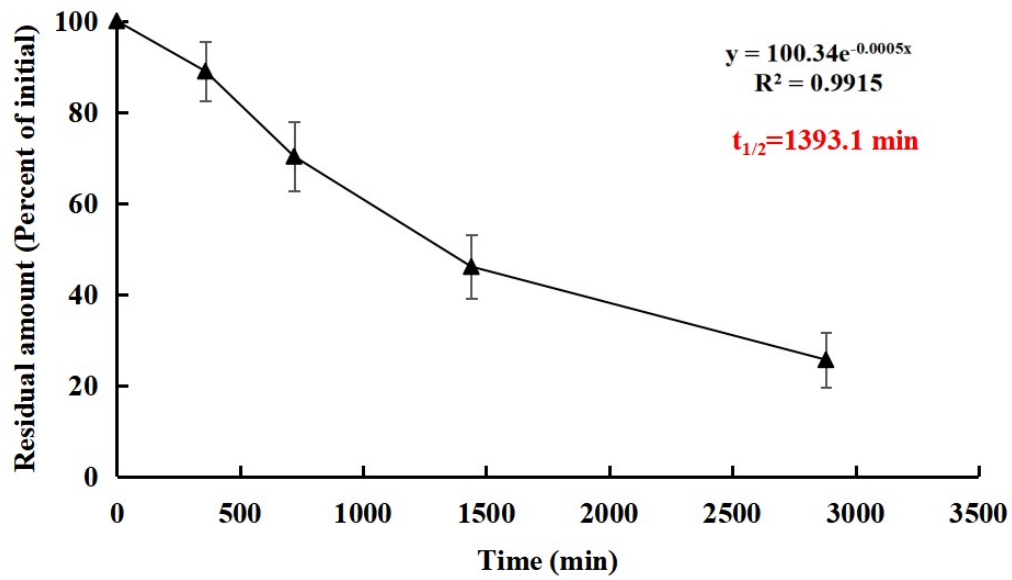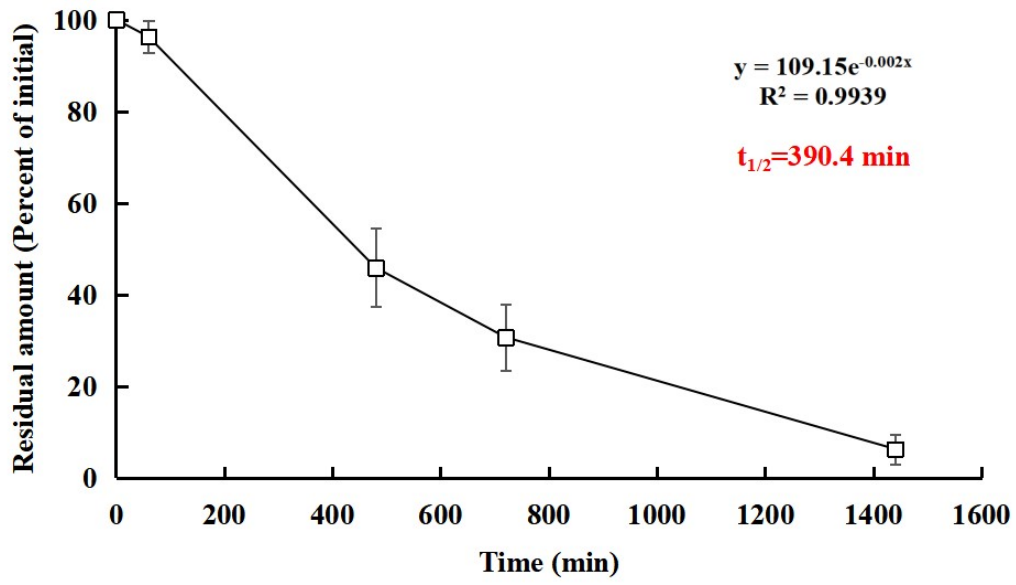
Fig. S4 GLP-1-bound extracellular domain (ECD) of the GLP-1 receptor (GLP-1R) (derived from crystal structure of PDB ID: 3IOL)[1]. GLP-1R is shown in green while GLP-1 is in cyan. The amino acids at positions 15, 26 and 34 of GLP-1, which were glycosylated in this study, are highlighted in orange and shown as sticks.

Reference

1. C. R. Underwood, P. Garibay, L. B. Knudsen, S. Hastrup, G. H. Peters, R. Rudolph and S. Reedtz-Runge, *J Biol Chem*, 2010, **285**, 723-730.