Reductive Amination Combining Dimethylation for Quantitative Analysis of Early Stage Glycated Proteins

Qing-He Tong†‖, Tian-Yang Yan‡‖, Tao Tao†, Lei Zhang†, Li-Qi Xie*† and Hao-jie Lu*†‡§

† Shanghai Cancer Centre and Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, P. R. China.

‡ Department of Chemistry, Fudan University, Shanghai 200433, P. R. China

§ Key Laboratory of Glycoconjuates Research Ministry of Public Health, Fudan University, Shanghai 200032, P. R. China

Table of contents

Table S-1 Number of exogenous glycation sites of myoglobin generated under different reaction time and reductant concentration.

Table S-2 The ratio of endogenous glycated peptides detected in the serum of diabetic patients without complicated retinal detachment over that in the serum of diabetic patients with complicated retinal detachment.

Table S-3 Comparison of protein glycation in the serum of diabetic patients with or without complicated retinal detachment.

Figure S-1 MALDI-TOF mass spectra of glycated/non-glycated and dimethylated peptides.

Figure S-2 MALDI-TOF mass spectra in quantifying 1:1 mixed RAD labelled glycated peptide.

Figure S-3 MALDI-TOF mass spectra of glycated myoglobin peptides before and after boric acid enrichment using different material to peptides ratios.

Figure S-4 MALDI-TOF mass spectrum of 1:1 mixed, light and heavy labelled and enriched peptide, in which heavy labelled peptide was mixed with digested ASF.

Figure S-5 MALDI-TOF mass spectra of glycated peptide reduced with $NaBD_3CN$ and then reduced with $NaBD_3CN$ and CD_2O.

Figure S-6 MALDI-TOF mass spectra of equivalently mixed light and heavy labelled peptide of different concentrations under optimized reduction condition.

Figure S-7 Mass spectrum of a false identified glycated peptide.

Table S-1. Number of exogenous glycation sites of myoglobin generated under different reaction time and reductant concentration.

$NaBH_3CN$（mM） / Time（hr）	300	75	18.75	4.69
4	1	0	0	0
12	4	2	1	1
24	6	4	1	1
32	9	4	1	1
48	11	4	2	1
60	15	5	2	1
108	19	6	3	1

Table S-2. The ratio of endogenous glycated peptides detected in the serum of diabetic patients without complicated retinal detachment over that in the serum of diabetic patients with complicated retinal detachment.

Protein	Glycated peptide (the red represents glycation site)	$R_{(L/H)}$*
A2MG	GEAFTLKgATVLNYLPKgCIR	0
A2MG	GPTQEFKgK	0
A2MG	MVSGFIPLKPTVKgMLER	0.772
A2MG	MVSGFIPLKgPTVK	0.566
A2MG	GEAFTLKgATVLNYLPK	0.548
A2MG	ATVLNYLPKgCIR	0.427
A2MG	DLKgPAIVK	0.510
ALBU	DVCKNYAEAKg	0
ALBU	KgVPQVSTPTLVEVSR	0.951
ALBU	YICENQDSISSKgLK	0.779
ALBU	CASLQKgFGER	0.605
ALBU	ADLAKgYICENQDSISSKgLK	0.232
ALBU	VTKgCCTESLVNR	0.027
ALBU	KgQTALVELVK	0.348
ALBU	ADLAKgYICENQDSISSK	0
ANT3	FDTISEKgTSDQIHFFFAKgLNCR	0
ANT3	SKgFSPENTR	0.842
APOH	VSFFCKgNK	1.526
APOH	TDASDVKgPC	0.039
B3KXH0	TPVIVTLKgENER	0
CO3	KgGYTQQLAFR	0.921

CO3	ACEPGVDYVYKgTR	0.758
CO3	GVFVLNKgK	0.708
CO3	LDKgACEPGVDYVYK	0.467
CO4A	GTLKgVLR	0.314
COMP	AVAEPGIQLKgAVK	0
FA5	DIASGLIGLLLICKgSR	infinity
FETA	VAKgGYQELLEK	0.672
FETA	YIQESQALAKgR	0.490
FETA	SCGLFQKgLGEYYLQNAFLVAYTK	0.430
FETUA	CNLLAEKgQYGFCK	1.647
GELS	EVQGFESATFLGYFKgSGLK	0
GELS	LKgATQVSK	0.534
GSYCP1	VLGEKgETLLYENK	0
HBA	MFLSFPTTKgTYFPHFDLSHGSAQVK	0.587
HBA	AAWGKgVGAHAGEYGAEALER	infinity
ITIH1	KgAAISGENAGLVR	0
ITIH1	VTAWKgQYR	0
ITIH1	LWAYLTIQELLAKgR	0.537
ITIH2	AKgGKgTAGLVR	0
ITIH2	GKgTAGLVR	0.351
ITIH2	MKgQTVEAMK	0.250
ITIH2	KgFYNQVSTPLLR	0.236
ITIH2	KgLGSYEHR	0.212
ITIH2	LSKgIQKgNVK	0.113

ITIH3	VQPKgQLVK	0
ITIH3	TAGLVKgASGR	1.141
ITIH3	AVSQGKgTAGLVK	0.899
ITIH3	GISMLNKgAR	0.098
ITIH4	FKgPTLSQQQK	0.560
KMT2D	KgDGDLDTDELLK	0
POSTN	IITGPEIKgYTR	0
PZP	ATVLNYLPKgCIR	infinity
PZP	MVSGFIPLKgPTVK	infinity
RET4	MKgYWGVASFLQK	0
TCRG1	EIKgEEPK	infinity
THRB	KgSPQELLCGASLISDR	0.768
TRFE	KgSCHTAVGR	0.056
TRFE	CLVEKgGDVAFVK	infinity
TRFL	KgSCHTAVDR	0.572
TSP1	AEKgGFLLLASLR	0.473
TSP4	AVAEPGIQLKgAVK	0
TSP4	SKgTGPGEHLR	0
UBF1	KgDYEVELLR	0
VTDB	DYEKgNKgVCK	0
VTDB	LCDNLSTKgNSK	0.250
VTDB	KgLCMAALK	0.248
VTDB	GQELCADYSENTFTEYKgKgK	0.087

* $R_{(L/H)}$=(S1/S2)/(S1'/S2'), in which $R_{(L/H)}$ was the ratio of light and heavy labelled glycated peptides from certain protein. S1 and S2 represented the signal strength of light and heavy labelled glycated peptides, respectively. S1' and S2' was the median of

the signal strength of all light and heavy labelled non-glycated peptides that belong to the protein, respectively. Infinity indicates that the peptide was only identified in the serum of diabetic patients without complicated retinal detachment, while 0 indicates that the peptide was only identified in the serum of diabetic patients with complicated retinal detachment.

Table S-3. Comparison of protein glycation in the serum of diabetic patients with or without complicated retinal detachment.

Protein	**$R_{(L/H)}$***
A2MG	0.548
ALBU	0.477
ANT3	0.421
APOH	0.783
CO3	0.733
CO4A	0.314
FETA	0.490
FETUA	1.647
GELS	0.267
HBA	0.587
ITIH1	0.537
ITIH2	0.236
ITIH3	0.899
ITIH4	0.560
THRB	0.768
TRFE	0.056
TRFL	0.572
TSP1	0.473
VTDB	0.248

$R_{(L/H)}$: The ratio of glycation in the serum of diabetic patients without complicated retinal detachment over that in the serum of diabetic patients with complicated retinal detachment.

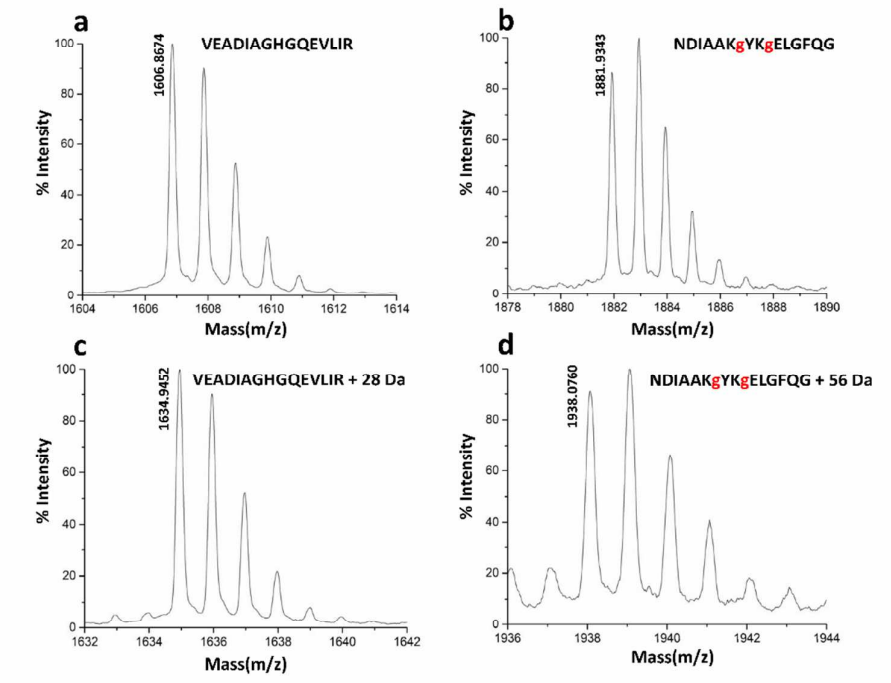

Figure S-1. MALDI-TOF mass spectra of (a) non-glycated peptide of myoglobin VEADIAGHGQEVLIR; (b) glycated peptide of myoglobin NDIAAK**g**YK**g**ELGFQG; (c) dimethylated peptide VEADIAGHGQEVLIR; (d) dimethylated peptide NDIAAK**g**YK**g**ELGFQG.

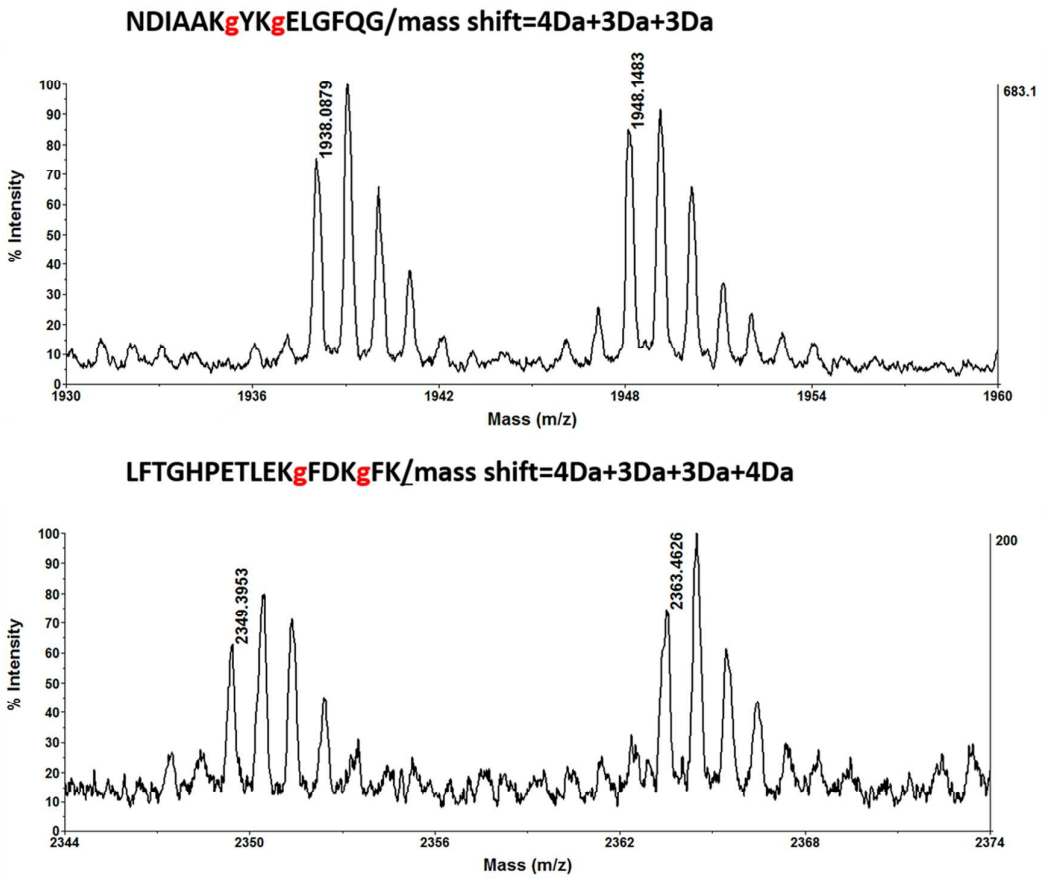

Figure S-2. MALDI-TOF mass spectra in quantifying 1:1 mixed RAD labelled glycated peptide. The mass shift of heavy and light RAD labelled myoglobin peptides accords with 4 m + 3 n (m represents the number of N-termini and Lys residues, n represents the number of glycated sites).

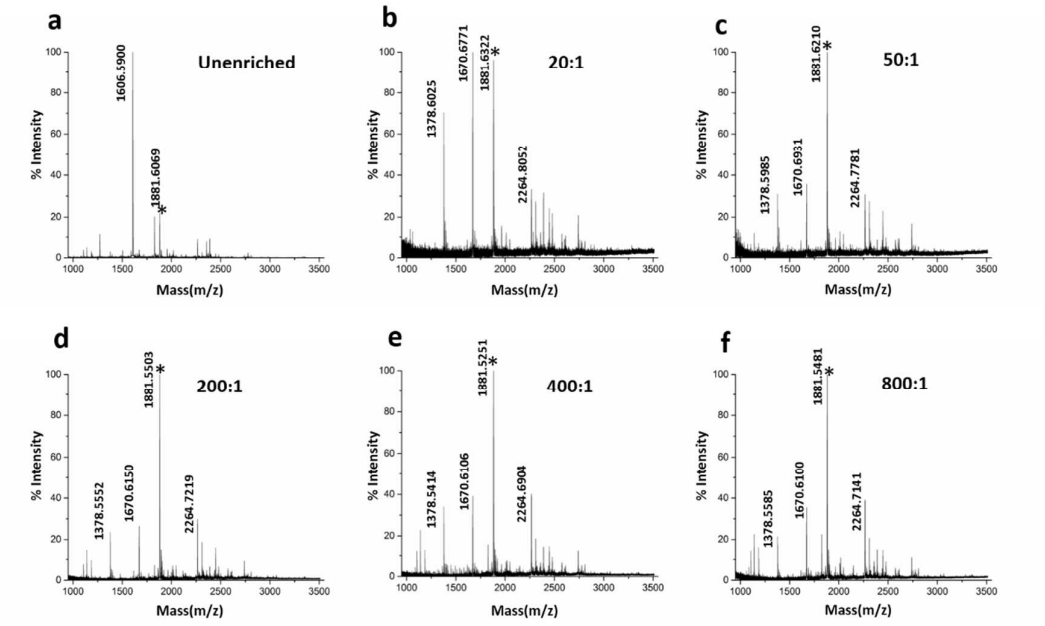

Figure S-3. MALDI-TOF mass spectra of glycated myoglobin peptides (a) before and after boric acid enrichment using material to peptides ratios at (b) 20:1, (c) 50:1, (d) 200:1, (e) 400:1 and (f) 800:1. * represents RAD labelled glycated peptide of ALELFRNDIAAK**g**YK.

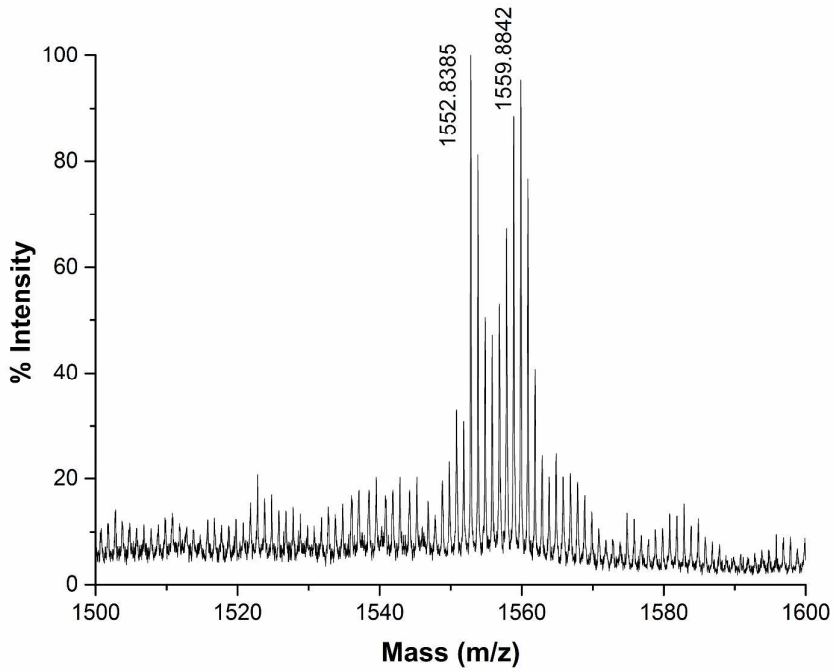

Figure S-4. MALDI-TOF mass spectrum of 1:1 mixed, light and heavy labelled and enriched peptide YIGIVK**g**QAGLER, in which heavy labelled peptide was mixed with digested ASF during enrichment before mixed with enriched light labelled peptide.

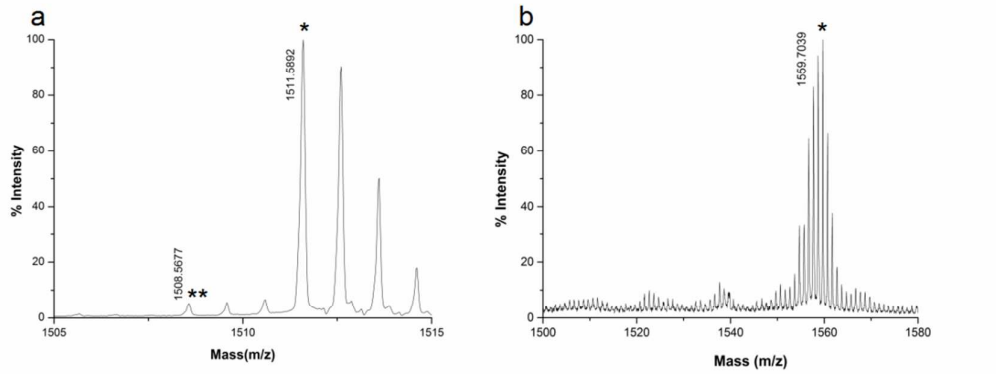

Figure S-5. MALDI-TOF mass spectra of glycated peptide YIGIVKgQAGLER (a) reduced with $NaBD_3CN$ and then (b) reduced with $NaBD_3CN$ and CD_2O. * represents reductive product and ** represents reactant.

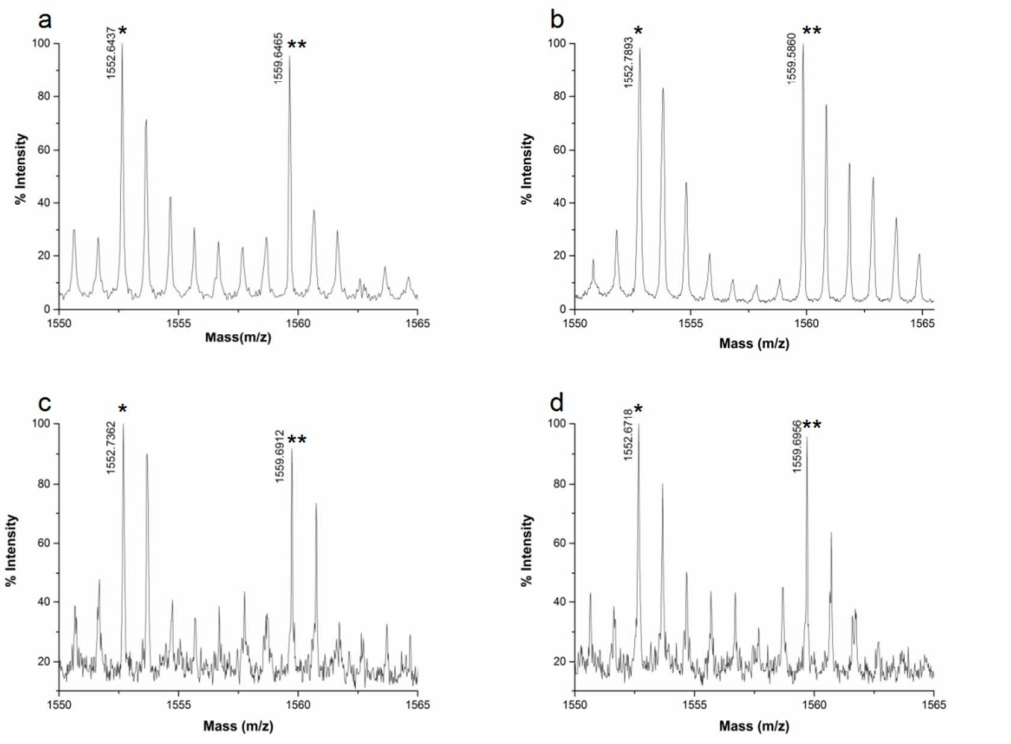

Figure S-6. MALDI-TOF mass spectra of isovolumetrically mixed light and heavy labelled peptide YIGIVK**g**QAGLER with the same concentration of (a) 1 mg/mL, (b) 0.1 mg/mL, (c) 0.01 mg/mL (d) 0.001 mg/mL, which were reduced with the optimized condition. * represents light labelled peptide reduced in water and ** represents heavy labelled peptide reduced in 5mM glucose.

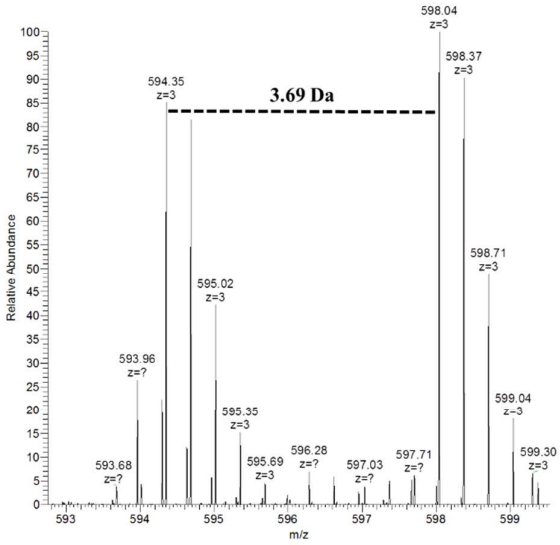

Figure S-7. Mass spectrum of a false identified glycated peptide, the mass shift is inconsistent with 4 m + 3 n (m represents the number of N-termini and Lys residues, n represents the number of glycated sites).