Electronic Supplementary Material (ESI) for Analytical Methods.

Quantification of prospective type 2 diabetes mellitus biomarkers by stable isotope dilution with bi-labeled standard glycated peptides

Alena Soboleva,[1,2†] Maciej Modzel,[3†] Anna Didio,[1,2] Halina Płóciennik,[3] Monika Kijewska,[3] Tatiana Grischina,[1] Tatiana Karonova,[4,5] Tatiana Bilova,[6] Vasily Stefanov,[1] Piotr Stefanowicz,[3] Andrej Frolov[2*]

Supplementary Information

[1]Department of Biochemistry, St. Petersburg State University; [2]Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry; [3]Faculty of Chemistry, University of Wroclaw; [4]Federal Almazov North-West Medical Research Centre; [5]The First Pavlov St. Petersburg State Medical University; [6]Department of Plant Physiology and Biochemistry, St. Petersburg State University

[†]These authors contributed equally to the manuscript

*Corresponding author:

Dr. Andrej Frolov

Leibniz Institute of Plant Biochemistry

Department of Bioorganic Chemistry

Weinberg 3, 06120,

Halle/Saale, Germany

Tel. +49 (0) 345 55821350

Fax. +49 (0) 345 55821309

Email: afrolov@ipb-halle.de

Directory

Tables

Table S1 Physical and biochemical parameters of the T2DM patients, as well as corresponding diabetes-related and non-related therapy

Proband ID	Height (cm)	Body weight (kg)	BMI	Age	Therapy		HbA_{1C} (%)	Albumin (µg/µl serum)	Complications
					Diabetes-specific	Diabetes-unspecific			
1	165	97	35.6	50	metformin, vildagliptin	valsartan, verapamil, rosuvastatin, fenofibrate	9.5	35.2	neuropathy, nephropathy, retinopathy
2	162	89.5	34.1	64	metformin, vildagliptin	atorvastatin, perindopril, indapamide	8.0	44.0	neuropathy
3	175	98.5	32.2	56	metformin, gliclazide	losartan, levothyroxine, acetylsalicylic acid, magnesium hydroxide	8.6	48.0	neuropathy
4	157	70	28.4	65	metformin	-	7.6	41.4	neuropathy, retinopathy
5	158	105.0	42.0	72	metformin, glibenclamide	metoprolol, digoxin, atorvastatin, famotidine, dabigatran, torasemide, azilsartan	8.1	47.0	neuropathy

BMI, body mass index; HbA_{1C}, glycated hemoglobin

Table S2 Physical and biochemical parameters of the non-diabetic individuals, as well as corresponding therapy, not related to diabetes

Proband ID	Height (cm)	Body weight (kg)	BMI	Age	Therapy		HbA_{1C} (%)	Albumin (µg/µl serum)	Complications
					Diabetes-specific	Diabetes-unspecific			
6	162	78	29.7	54	-	enalapril	5.8	47.3	-
7	156	82	33.7	67	-	-	6.2	46.0	-
8	171	77	26.6	57	-	acetylsalicylic acid	5.4	49.2	-
9	158	75	30.0	65	-	losartan, indapamide, metoprolol, acetylsalicylic acid, atorvastatin	6.0	46.4	-
10	158	64.1	25.7	64	-	-	4.9	45.9	-

BMI, body mass index; HbA_{1C}, glycated hemoglobin

Table S3 Source and mass analyzer settings applied for QqTOF-MS experiments

Parameter	**Settings**
MS Conditions	
Ionization mode	Positive
Ion spray voltage	3.5 kV
Nebulizer gas temperature	300 °C
Nebulizer gas flow	5 L/min
Front mirror voltage	7000
Mid mirror voltage	1669.2
Back mirror voltage	1250
Mass to charge ratio (*m/z*) range	400 – 2000
Resolution	36,000 – 39,000
Scan rate/frequency	3 Hz

Table S4 Instrument settings applied for Orbitrap-LIT-MS experiments

Parameter	Setting
MS conditions	
Ionization mode	Positive
Mass analyzer	LIT-Orbitrap (FT-scan)
Ion spray voltage (IS)	4.0 kV
Nebulizer gas	35 psig
Auxillary gas	10 psig
Capillary temperature	285 °C
Mass to charge ratio (*m/z*) range	400 – 2000
Resolution	30000
MS/MS conditions	
Ionization mode	Positive
Mass analyzer	LIT-Orbitrap (FT-scan)
Ion spray voltage (IS)	4.0 kV
Fragmentation	Collision activated dissociation
Isolation width	2 Da
Charge state rejected	1+
Normalized collision energy	35%
Activation frequency	0.25
Activation time	30 ms

Table S5 Protein recoveries and normalized total UV (595 nm) densities calculated for individual samples separated by SDS-PAGE

Sample	Concentration (mg/mL)	Intensity (AU)
1	58.6	18600
2	56.0	19200
3	64.5	17400
4	52.2	20200
5	62.4	19801
6	82.4	17999
7	82.3	18392
8	45.3	19966
9	36.2	20350
10	58.0	20254

Table S6 Sensitivity and linearity parameters obtained for the mixture of stable isotope-labeled peptide standards

Label	*m/z*	*z*	t_R (min)	LOD (mol)	LOQ (mol)	LDR	Slope	Intercept	R^2
4	757.6958	3	38.8	2.5E-13	1.0E-12	2.5E+02	7.76E+03	-7.46E+04	0.99
5	701.9948	3	36.1	1.0E-13	2.5E-13	0.4E+03	9.70E+04	-1.44E+05	0.99
6	736.3859	3	45.8	1.0E-13	1.0E-12	1.0E+02	1.00E+06	-6.00E+06	0.99

All parameters were determined in three independent dilution series

Table S7 Elemental composition of glycated peptides detected in plasma protein digests and corresponding synthetic stable isotope-labeled internal standards

Label	Sequence	m/z_{calc}	$m/_{observed}$	z	Elemental composition	Error (ppm)
1	DSTYSLSSTLTLSK$_{Am}$ADYE-$^{13}C_6$ $^{15}N_2$KK(Dab)K	2821.3459	2821.3357	3	C_{118} $^{13}C_6$ H$_{196}$ N$_{26}$ $^{15}N_2$ O$_{44}$ S	-3.62
2	ADLAK$_{Am}$YICENQDSISS-$^{13}C_6$ $^{15}N_2$KK(Dab)K	2597.2233	2597.2099	3	C_{106} $^{13}C_6$ H$_{180}$ N$_{26}$ $^{15}N_2$ O$_{38}$ S$_2$	-5.16
3	VFDEFK$_{Am}$PLVEEPQNLI-$^{13}C_6$ $^{15}N_2$KK(Dab)K	2757.4179	2757.4079	3	C_{124} $^{13}C_6$ H$_{204}$ N$_{26}$ $^{15}N_2$ O$_{35}$ S	-3.62
4	DSTYSLSSTLTLSK$_{Am}$ADYE-$^{13}C_6$ $^{15}N_2$K	2278.1049	2278.1055	3	C_{91} $^{13}C_6$ H$_{153}$ N$_{19}$ $^{15}N_2$ O$_{41}$	0.21
5	ADLAK$_{Am}$YICENQDSISS-$^{13}C_6$ $^{15}N_2$K	2111.0061	2111.0037	3	C_{81} $^{13}C_6$ H$_{145}$ N$_{20}$ $^{15}N_2$ O$_{36}$ S$_2$	-0.05
6	VFDEFK$_{Am}$PLVEEPQNLI-$^{13}C_6$ $^{15}N_2$K	2214.1770	2214.1788	3	C_{96} $^{13}C_6$ H$_{162}$ N$_{19}$ $^{15}N_2$ O$_{33}$	0.78
7	DSTYSLSSTLTLSK$_{Am}$ADYEK	2270.0952	2270.0907	3	$C_{97}H_{158}N_{21}O_{41}$	1.92
8	ADLAK$_{Am}$YICENQDSISSK	2102.9895	2102.9922	3	$C_{87}H_{145}N_{22}O_{36}S$	1.29
9	VFDEFK$_{Am}$PLVEEPQNLIK	2206.1628	2206.1646	3	$C_{102}H_{163}N_{21}O_{33}$	0.88

Table S8 Label-free quantification of individual glycation in plasma samples obtained from T2DM patients and non-diabetic individuals (controls)

Label	Protein name	Peptide sequence	t_R	*m/z*	*z*	Site[a]	S (counts)		Ratio T2DM/Control
							T2DM	Controls	
7	Kappa chain C region	DSTYSLSSTLTLSK$_{Am}$ADYEK				K_{75}	8.06E+04 ± 2.83+04	4.43E+04 ± 1.47E+04	1.82
8	HSA	ADLAK$_{Am}$YICENQDSISSK				K_{287}	2.88E+06 ± 1.26E+06	1.26E+06 ± 3.76E+05	2.28
9	HSA	VFDEFK$_{Am}$PLVEEPQNLIK				K_{402}	2.10E+06 ± 9.73E+05	1.20E+06 ± 4.13E+05	1.76

The sites of plasma protein glycation were quantified by the label-free approach using RP-HPLC-ESI-QqTOF-MS

Figures

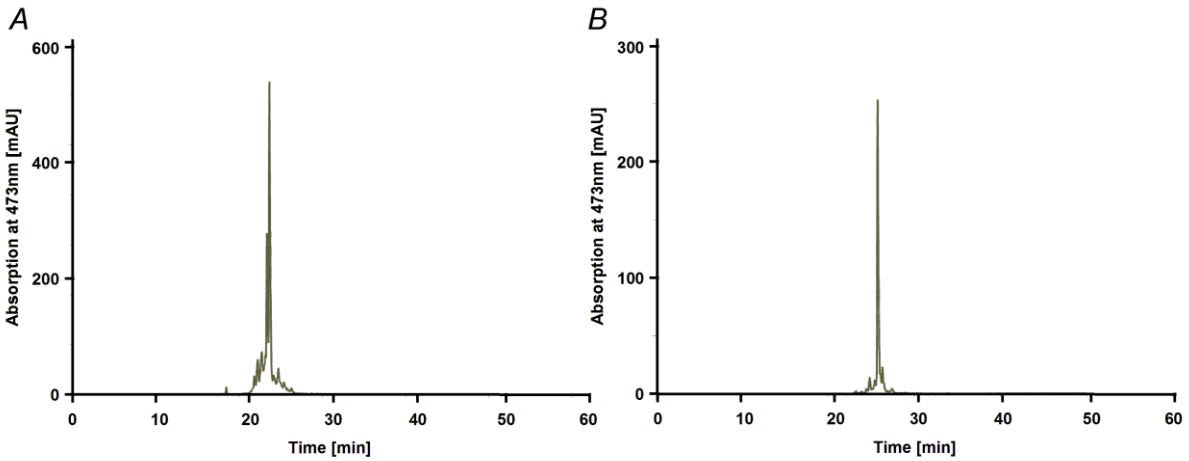

Figure S1 Reversed phase chromatograms of the peptide ADLAK$_{Am}$YICENQDSISS-$^{13}C_6{}^{15}N_2$KK(Dab)K (**2**) and peptide VFDEFK$_{Am}$PLVEEPQNLI-$^{13}C_6{}^{15}N_2$KK(Dab)K (**3**), obtained by IP-RP-HPLC using water and acetonitrile as eluents A and B, respectively, both containing 0.1% (v/v) TFA as ion pair reagent.

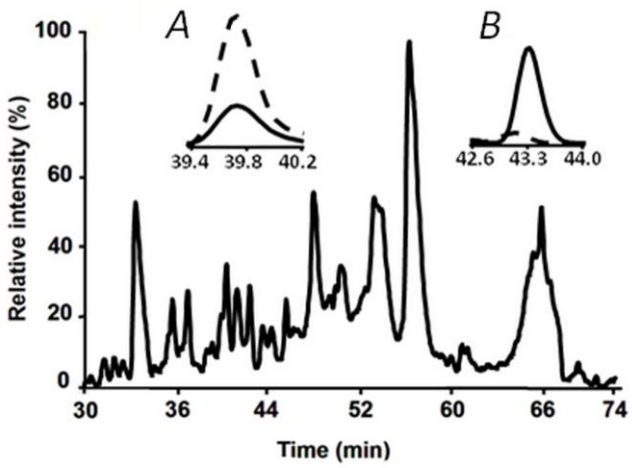

Figure S2 LC-MS analysis of T2DM pooled plasma spiked prior to the tryptic digestion with the bi-labeled peptides DSTYSLSSTLTLSKAmADYE-13C615N2KK(Dab)K and VFDEFKAmPLVEEPQNLI-13C615N2KK(Dab)K. Total ion chromatograms acquired for a pooled plasma sample, spiked with bi-labeled standard peptides, hydrolyzed with trypsin, and the extracted ion chromatograms (XICs, segments) of *m/*z 757.70 ± 0.02 (solid) and 760.37 ± 0.02 (dashed) corresponding to $[M+3H]^{3+}$ ions of the peptides DSTYSLSSTLTLSK$_{Am}$ADYEK and DSTYSLSSTLTLSK$_{Am}$ADYE[$^{13}C_6{}^{15}N_2$-K], respectively (A) and XICs of *m/*z 736.37 ± 0.02 and 739.04 ± 0.02 corresponding to $[M+3H]^{3+}$ ions of the peptides VFDEFK$_{Am}$PLVEEPQNLI[$^{13}C_6{}^{15}N_2$-K], respectively (B).

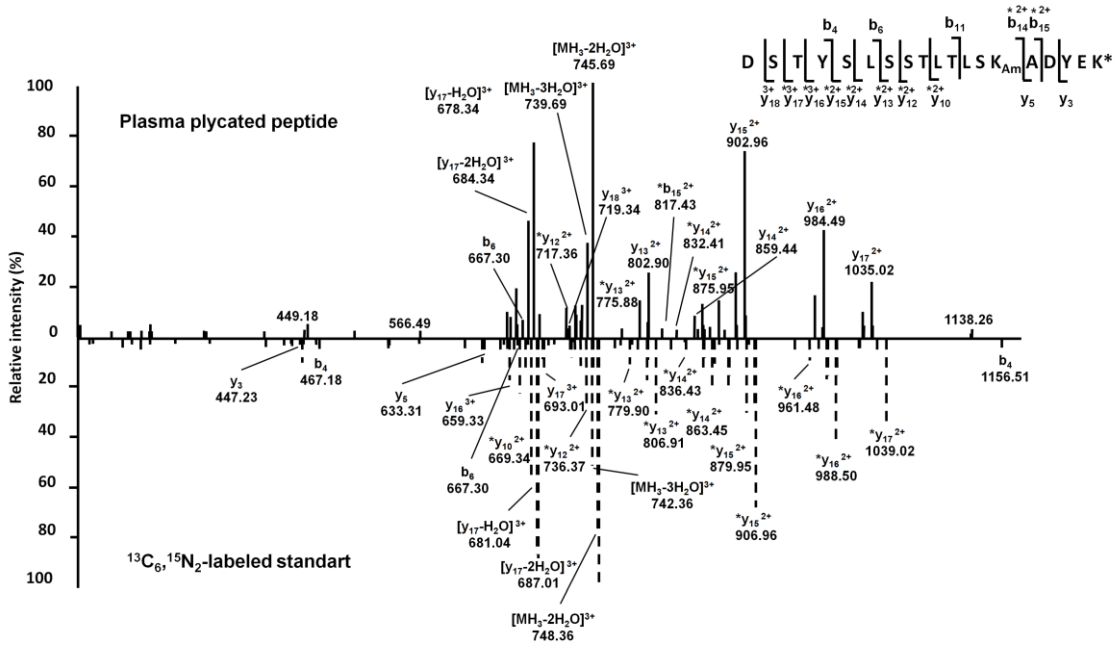

Figure S3 MS/MS spectra acquired for *m/*z 757.7 (solid) and 760.4 (dashed), corresponding to $[M+3H]^{3+}$ quasi-molecular ions of the peptide DSTYSLSSTLTLSK$_{Am}$ADYEK (solid) and DSTYSLSSTLTLSK$_{Am}$ADYE[$^{13}C_6{}^{15}N_2$-K] (dashed).

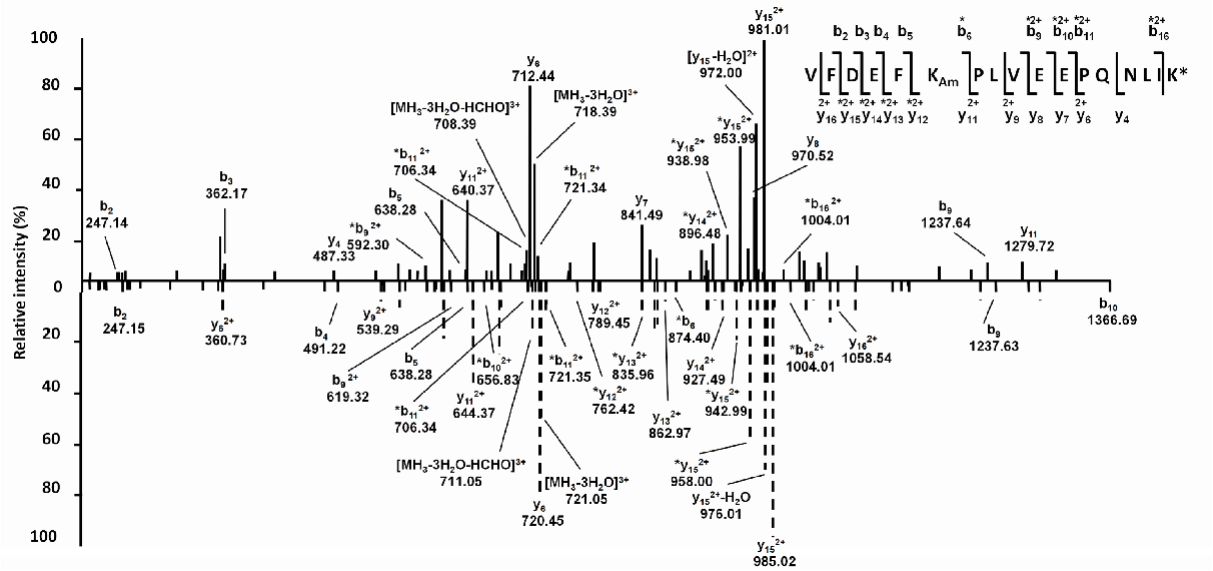

Figure S4 MS/MS spectra acquired for *m/*z 736.4 (solid) and 739.0 (dashed), corresponding to $[M+3H]^{3+}$ quasi-molecular ions of the peptide VFDEFK$_{Am}$PLVEEPQNLIK (solid) and VFDEFK$_{Am}$PLVEEPQNLI[$^{13}C_6{}^{15}N_2$-K] (dashed), respectively.