

Supporting Information for

Exploring Glycated Sites in Human Serum Albumin: Impact of Sample Processing Techniques on Detection and Analysis

Namita Kumari,^{*a} Madhumati S Vaishnav^{a,b} Sathyanarayana Srikanta^b P. R. Krishnaswamy^a and Navakanta Bhat^a

^a Centre for Nano Science and Engineering, Indian Institute of Science, Bengaluru 560012, India

^b Samatvam Endocrinology Diabetes Center, Jnana Sanjeevini Diabetes Hospital and Medical Center, Bengaluru, India

Table S1 The serum sample details (HbA1c, serum albumin concentration and % GA).

Sample	% HbA1c	% GA	Albumin (g/dL)
1	5.7	19.7	4.4
2	7	20.9	4.8
3	5.8	24.1	4.1
4	7.5	26.9	4.4
5	9.7	27.9	4.4
6	11.2	31.1	4.2
7	9.9	33.9	3.8
8	10.4	34.4	4.2
9	8.1	35.8	3.9
10	12.1	39.5	4.4
11	14	41.8	4.2
12	16.3	50.3	4.3
13	18.1	51.4	4.1
14	15.9	53	3.7
15	17.7	53.8	4.2
16	15.6	62.3	3.2

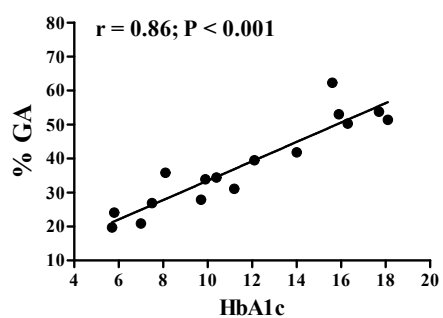


Fig. S1 Scatter plot and regression analysis representing the correlation between % GA with HbA1c (N = 16).

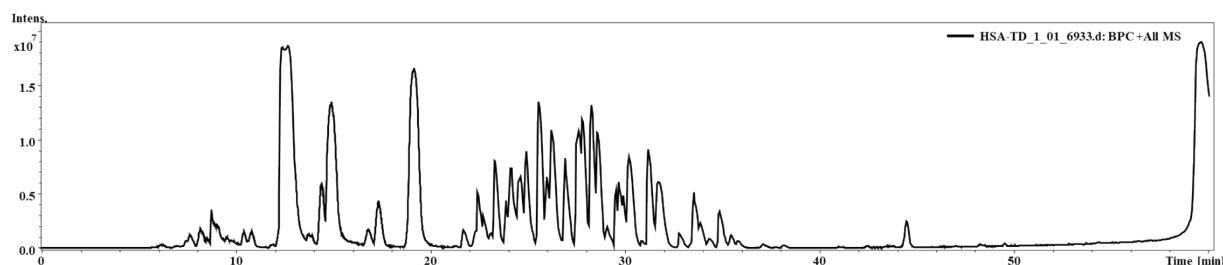


Fig. S2 The LC-MS chromatogram of trypsin digested HSA (Sigma).

Table S2. The list of tryptic peptides detected from the HSA (Sigma) indicating their sequence, retention time and observed MS and MS/MS charge states.

No	PEPTIDE (Sequence)	Observed MS (Charge)	MS/MS (Charge)	Rt (min)
1	DAHK (1-4)	470.2 (1+)		6.8 - 7.3
2	SEVAHR (5-10)	698.3 (1+)		9.3 - 9.7
3	DLGEENFK (13 - 20)	476.21(2+)	476.21(2+)	26.3
4	ALVLIIFAQYLQQCPFEDHVK (21 - 41)	830.74 (3+)	830.74 (3+)	33.8
5	LVNEVTEFAK (42 - 51)	575.29 (2+)	575.29 (2+)	27.4
6	TCVADESAENCDK (52 - 64)	749.75 (2+)	749.75 (2+)	22.3
7	SLHTLFGDK (65-73)	509.25 (2+)	509.25 (2+)	27.5
8	LCTVATLR (74 - 81)	467.27 (2+)	467.27 (2+)	26.4
9	ETYGEMADCCAK (82 - 93)	717.78 (2+)	717.78 (2+)	25
10	QEPERNECFLQHK (94 - 106)	572.28 (3+)	572.28 (3+)	24.1
11	DDNPNLPR (107 - 114)	470.74 (2+)	470.74 (2+)	24.1
12	LVRPEVDVMCTAFHDNEETFLK (115 - 136)	663.58 (4+)	663.58 (4+)	29.4
13	YLIEIAR (138-144)	464.26 (2+)	464.26 (2+)	27.8
14	HPYFYAPELFFAK(146 - 159)	581.98 (3+)	581.98 (3+)	32.8
15	YK (161 - 162)	310.1 (1+)	310.1 (1+)	8 - 9.3
16	AAFTECCQAADK (163 - 174)	686.29 (2+)	686.29 (2+)	24.2
17	LDELRDEGK (182 - 190)	537.78 (2+)	537.78 (2+)	23.3
18	ASSAK (191-195)	463.2 (1+)	464.75 (1+)	7.4
19	QR (196 - 197)	303.1 (1+)		9.8-11.1
20	LK (198-199)	260.2 (1+)		8 - 9.6
21	FGER (206 - 209)	508.2 (1+)		21.4 - 22.3
22	AFK (210-212)	365.2 (1+)		12.9-14.2
23	AWAVAR (213 - 218)	673.37 (1+); 337.2 (2+)		25.4-26.2
24	LSQR (219 - 222)	503.3 (1+); 252.1 (2+)	503.3 (1+)	10.5
25	AEFAEVSK (226 - 233)	440.71 (2+)	440.71 (2+)	24.8
26	LVTDLTK (234-240)	395.22 (2+)	395.22 (2+)	26.1
27	VHTECCHGDLLECADDR (241 - 257)	696.62 (3+)	696.62 (3+)	25.2
28	ADLAK (258 - 262)	259.16(2+); 517.31 (1+)	517.31 (1+)	13.9
29	YICENQDSISSK (263 - 274)	722.33 (2+)	722.33 (2+)	24.2

30	LKECCEKPLEK (275 - 286)	773.91 (2+); 516.28 (3+); 387.46 (4+)	773.91 (2+); 516.28 (3+)	23.8
31	SHCIAEVENDEMPADLPSLAADFVESK (287-307)	992.43 (3+)	992.43 (3+)	31.6
32	DVCK (308 - 311)	521.25 (1+)	521.25 (1+)	10.5
33	NYEAK (318 - 323)	695.35 (1+); 348.18 (2+)	695.35 (1+)	17
34	DVFLGMFLYEYAR (324 - 336)	812.41 (2+)	812.41 (2+)	34.9
35	RHPDYSVVLLL (337 - 348)	489.96 (3+)	489.96 (3+)	29.2
36	TYETTLEK (352 - 359)	492.76 (2+)	492.76 (2+)	24.6
37	CCAAADPHECYAK (360 - 372)	519.54 (3+)	519.54 (3+)	22.6
38	VFDEFKPLVEEQNLK (373 - 389)	682.71 (3+)	682.71 (3+)	30.1
39	QNCLEFQLGEYK (390 - 402)	553.26 (3+)	553.26 (3+)	29.6
40	FQNALLVR (403 - 410)	960.58 (1+); 480.80 (2+)	480.80 (2+)	28.3
41	YTK (411 - 413)	411.24 (1+)	411.24 (1+)	9.4
42	KVPQVSTPTLVEVSR (414-428)	820.48 (2+)	820.48 (2+)	27
43	NLGK (429 - 432)	431.27 (1+)	431.27 (1+)	10.9
44	VGSK (433 - 436)	390.23 (1+)		7.1 - 8.3
45	CCK (437 - 439)	467.18 (1+)	467.18 (1+)	7.6
46	HPEAK (440 - 444)	581.31 (1+)	581.31 (1+)	7.6
47	MPCAEDYLSVVLNQLCVLHEK (446-466)	840.08 (3+); 630.31 (4+)	840.08 (3+); 630.31 (4+)	33.5
48	TPVSDR (467 to 472)	673.39 (1+); 337.2 (2+)		25.9 - 26.4
49	VTK (473-475)	347.22 (1+)		7.1 - 7.8
50	CCTESLVNR (476-484)	569.76 (2+)	569.76 (2+)	24.6
51	RPCFSALEVDETYVVK (485-500)	637.65 (3+)	637.65 (3+)	28.1
52	EFNAETFTFHADICTLSEK (501-519)	754.36 (3+)	754.36 (3+)	29.9
53	QIK (522 - 524)	388.27 (1+)		9.8 - 10.8
54	QTALVELVK (526-534)	500.82 (2+)	500.82 (2+)	28.6
55	ATK (539 - 541)	319.21 (1+)		7.1 - 7.3
56	EQLK (542 - 545)	517.31 (1+)		12.2 - 12.6
57	AVMDDFAAFVEK (546-557)	671.83 (2+)	671.83 (2+)	31.1
58	CCK (558 - 560)	467.18 (1+)	467.18 (1+)	7.6
59	ADDK (561 - 564)	448.21 (1+)	448.21 (1+)	7.4
60	KLVAASQAALGL (574-585)	571.36 (2+)	571.36 (2+)	28.7

1 DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
51 KICVADESAE NCDKSLHTLF GDKLCTVAIL RETYGEMADC CAKQEPERNE
101 CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKLYLY EIARRHPYFY
151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
201 ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
251 LECADDRADL AKYICENQDS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
301 DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLR LA
351 KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
401 YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
451 DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPCFSA LEVDETYVVK
501 EFNAETFTFH ADICTLSEK E RQIKKQ TALV ELVKHKPKAT KEQLKAVMDD
551 FAAFVEKCCK ADDKETCFAE EGK KLVAASQ AALGL

Fig. S3 The peptide sequence detected in the tryptic digest of HSA (Sigma); peptides detected with MS/MS confirmation is marked yellow and green, the peptides with only MS confirmation are indicated blue color fonts (two different shades indicate two different peptides), whereas the red colored peptide sequence could not be detected.

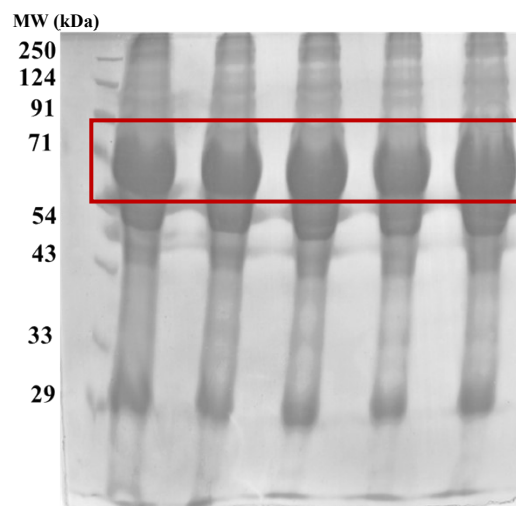


Fig. S4 SDS-PAGE gel electrophoresis of five serum samples (% GA = 62.3, 53, 50, 41.8, 34.4) for isolating albumin (circle indicating the albumin band) from other serum proteins.

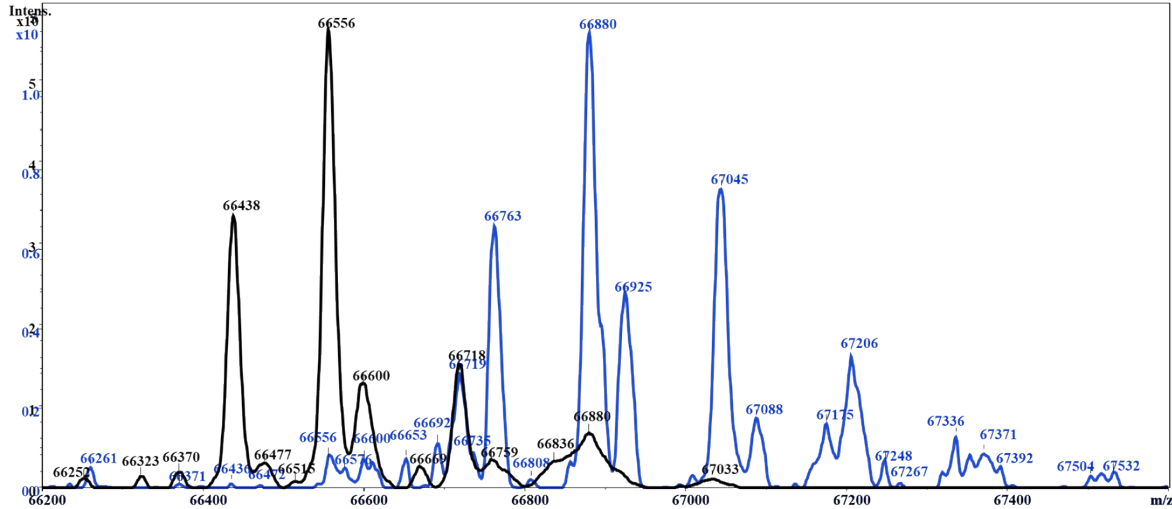


Fig. S5 Overlay of mass spectrum of LGA (less glycated, black) and MGA (more glycated, blue) of the serum sample (% GA = 41.8). Deconvolution has been done using the maximum entropy algorithm.

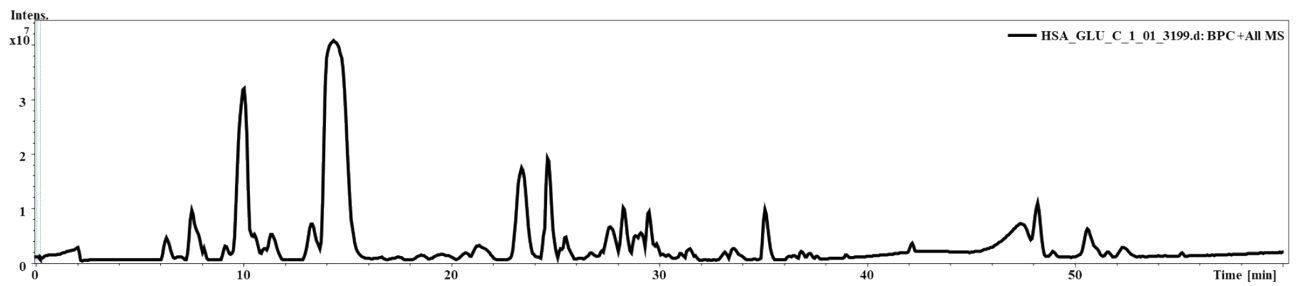


Fig. S6 The LC-MS chromatogram of Glu-C digested HSA (Sigma).

1 DAHKSEVAHR FKDLGEE¹NFK ALVLIAFAQY LQQCPFEDHV KLVNE²VTEFA
51 KTCVADE³SAE NCDKSLHTLF GDKLCTVATL RETYGE⁴MADC CAKQEPERNE
101 CFLQHKDDNP NLPRLVRPEV⁵ DVMCTAFHDN EETFLKKYLY EIARRHPYFY
151 APELLFFAKR YKAAFT⁶ECCQ AADKAACLLP KLDEL⁷RDEGK ASSAKQRLKC
201 ASLQKFGERA FKAWAVARLS QRFPAE⁸FAE VSKLVTDLTK VHTE⁹CCHGDL
251 LECADDRADL AKYICEN¹⁰QDS ISSKLKE¹¹CCE KPLLEKSHCI AEVENDEMPA
301 DLPSLAADFV ESKDVCKNYA EAKDVFLGMF¹² LYEYARRHPD YSVVLLRLA
351 KTYE¹³TTLKCAAAADPHECY AKVFDE¹⁴FKPL VEEPQNLIKQ NCELFEQLGE
401 YKFQNALLRV YTKKVPQVST PTLVE¹⁵VSRNL GKVGSKCKKH PEAKRMPCAE
451 DYLSVVLNQL CVLHE¹⁶KTPVS DRVTKCCTES LVNRRPCFSA LEVDE¹⁷TYVPK
501 EFNAE¹⁸TFTFH ADICTLSEKE RQIKKQ¹⁹TALV ELVKHKPKAT KEQLKAVMDD
551 FAAFVE²⁰KCKC ADDKETCFAE EGK²¹KLVAASQ AALGL

Fig. S7 The peptide sequence detected in the Glu-C digest of HSA (Sigma); peptides detected with MS/MS confirmation is marked yellow and green, the peptides with only MS confirmation are indicated blue color fonts, whereas the red colored peptide sequence could not be detected.

Table S3. The list of peptides detected from the Glu-C digest of HSA (Sigma) indicating their sequence, retention time and observed MS and MS/MS charge states.

	PEPTIDE (Sequence)	MS (Charge)	MS/MS (Charge)	Rt (min)
1	DAHKSE (1-6)	686.31(1+); 343.65 (2+)	686.31(1+); 343.65 (2+)	6.6
2	VAHRFKDLGEE (7 – 17)	650.83 (2+); 434.22 (3+)	650.83 (2+); 434.22 (3+)	24.5
3	NFKALVLIFAQYLQQPFEDHVKLVNE (18 – 45)	1112.25(2+); 834.69(3+); 667.95(4+)	1112.25(2+); 834.69(3+); 667.95(4+)	36.4
4	VTE (46 - 48)	348.17 (1+)		8.8 – 10.2
5	FAKTCVADE (49 – 57)	1040.47 (1+); 520.74 (2+)	520.74 (2+)	21.8
6	SAE (58 – 60)	306.12 (1+)	306.12 (1+)	8.1
7	NCDKSLHTLFGDKLCTVATLRE (61 – 82)	1289.64(2+); 860.09(3+); 645.32 (4+)	645.57 (4+)	30.5
8	TYGE (83 – 86)	469.19 (1+)	469.19 (1+)	10.5
9	MADCCAKQEPERNE (87 – 100)	869.35 (2+); 579.90 (3+)	869.35 (2+)	10.2
10	CFLQHKDDNPPLRVRPE (101 – 119)	1174.58 (2+); 783.40 (3+); 587.8 (4+); 470.44 (5+)	587.8 (4+); 470.44 (5+)	28.4
11	VDVMCTAFHDNEE (120 – 132)	783.80 (2+); 522.87 (3+)	783.80 (2+)	29.9
12	TFLKKYLYE (133 – 141)	602.83 (2+); 402.32 (3+)	602.83 (2+); 402.32 (3+)	29.8
13	IARRHPYFYAPE (142 – 153)	760.39 (2+); 507.26 (3+); 380.70 (4+)	760.39 (2+); 507.26 (3+)	27.7
14	LLFFAKRYKAAAFTE (154 – 167)	852.97 (2+); 568.98 (3+); 426.99 (4+)	568.98 (3+); 426.99 (4+)	31.3
15	CCQAADKAAACLLPKLDE (168 – 184)	654.97 (3+)		30.2- 30.4
16	LRDE (185 - 188)	532.27(1+)	532.27(1+)	8.8
17	RAFKAWAVARLSQRFPKAE (209 – 227)	558.81 (4+); 447.25 (5+)		28.6- 28.9
18	FAE (228 - 230)	366.16 (1+)	366.16 (1+)	17
19	VSKLVTDLTKVHTE (231 – 244)	785.44 (2+); 523.96(3+); 393.22 (4+)	785.44 (2+); 523.96(3+); 393.22 (4+)	28.1
20	CCHGDLLE (245 – 252)	1003.39 (1+); 502.20 (2+)	502.20 (2+)	28
21	CADDRADLAKYICE (253 – 266)	567.25 (3+)	567.25 (3+)	29.7
22	NQDSISSKLKE (267 – 277)	624.82 (2+); 416.88 (3+)	624.82 (2+); 416.88 (3+)	24.6
23	SKDVCKNYAE (312 – 321)	607.28 (2+); 405.18 (3+)		10.1 - 10.4
24	AKDVFLGMFLYE (322 – 333)	716.86 (2+)	716.86 (2+)	36.7
25	YARRHPDYVLLLLRLAKTYE (334 – 354)	641.60 (4+); 513.48 (5+)	641.60 (4+); 513.48 (5+)	32.5
26	TTLE (355 – 358)	463.23 (1+)	463.23 (1+)	15
27	KCCAAADPHECYAKVFDE (359 – 376)	724.30 (3+); 543.47 (4+)		28 - 28.4
28	FKPLVEEPQNLIKQNCE (377 – 393)	1043.53 (2+); 696.02 (3+); 522.27 (4+)	696.02 (3+)	30

29	LFEQLGE (394 – 400)	835.42 (1+); 418.21 (2+)	835.42 (1+); 418.21 (2+)	31.6
30	YKFQNALLVRYTKKVPQVSTPTLVE (401 – 425)	731.41 (4+); 585.33 (5+)	585.33 (5+)	30.2
31	AKRMPCAE (443 – 450)	481.73 (2+)	481.73 (2+)	10.1

	Glycated Lysine residue	PEPTIDE (Sequence)	MS (Charge)	MS/MS (Charge)	Rt (min)
1	K-12	VAHRFKDLGEE	1300.6 (1+); 650.8 (2+); 434.2(3+)	650.8 (3+); 434.2(3+)	26.1
2	K-64	NCDKSLHTLFGDKLCTVATLRE	914.11(3+); 685.84 (4+); 548.87 (5+)	548.87 (5+)	31
3	K-136/K-137	TFLKKLYE	1366.71 (1+); 683.86 (2+); 456.24 (3+)	683.86 (2+); 456.24(3+)	30.4
4	K-159/K-162	LLFFAKRYKAAFTE	934.0 (2+); 623.00 (3+); 467.5 (4+)	467.5 (4+)	31.4
5	K-233	VSKLVTDLTKVHTE	866.47 (2+); 577.98 (3+); 433.74 (4+)	577.98 (3+)	29
6	K-276	NQDSISSKLKE	705.85 (2+); 470.9 (3+)	705.85 (2+); 470.9 (3+)	26.4
7	K-313	SKDVCKNYAE	688.3 (2+); 459.2 (3+)		10.1 - 10.6
8	K-323	AKDVFLGMFLYE	797.89 (2+)		36.1 -36.5
9	K-351	YARRHPDYSVLLLLRLAKTYE	909 (3+); 682.12 (4+); 545.9 (5+)	682.12 (4+); 545.9 (5+)	32.9
10	K-359	KCCAAADPHE	660.76 (2+)		9.5 - 10.0
11	K-378/K-389	FKPLVEEPQNLKQNC	1124.56 (2+); 750.04 (3+); 567.78 (4+)		30.5 - 30.8
12	K-475	KTPVSDRVTKCCTE	921.93 (2+); 614.95 (3+)		10.1 - 10.9
13	K-525	RQIKKQTALVE	783.43 (2+); 492.62 (3+)	783.43 (2+); 492.62 (3+)	12.7
14	K-545	QLKAVMDDFAAFVE	873.42 (2+); 582.61 (3+)	582.61 (3+)	35.1
15	K-564	KCKKADDKE	658.28 (2+); 439.18 (3+)	439.18 (3+)	5.8
16	K-574	GKKLVAASQAALGL	774.9 (2+); 496.96 (3+)	774.9 (2+); 496.96 (3+)	29.9
32	DYLSVVLNQLCVLHE (451 – 465)		901.46 (2+); 601.30 (3+)	901.46 (2+); 601.30 (3+)	37
33	KTPVSDRVTKCCTE (466 – 479)		840.9 (2+); 560.94 (3+); 420.95 (4+)		10 - 10.6
34	SLVNRPCFSALE (480 – 492)		774.90 (2+); 516.93 (3+)	516.93 (3+)	29.6
35	VDE (493 – 495)		362.16 (1+)	362.16 (1+)	9.4
36	TYVPKEFNAE (496 – 505)		599.29 (2+)	599.29 (2+)	28.3
37	TFTFHADICTLSE (506 – 518)		771.35 (2+)	771.35 (2+)	32
38	TFTFHADICTLSEKE (506 – 520)		899.9 (2+); 600.28 (3+)	600.28 (3+)	30.7
39	RQIKKQTALVE (521 – 531)		657.39 (2+); 438.60 (3+)	438.60 (3+)	10.3
40	LVKHKPKATKE (532 – 542)		639.89 (2+); 426.93 (3+)	639.89 (2+)	6.4
41	QLKAVMDDFAAFVE (543 – 556)		792.39 (2+); 528.59 (3+)	792.39 (2+); 528.59 (3+)	35.1
42	KCKKADDKETCF AEE (557 – 571)		945.35 (2+); 630.57 (3+)		10 - 10.6
43	TCFAEE (567 – 571)		756.28 (1+)	756.28 (1+)	26.4
44	GKKLVAASQAALGL (572 – 585)		663.91 (2+); 442.94 (3+)	663.91 (2+); 442.94 (3+)	29.4

Table S4. The list of peptides detected from the Glu-C digest of glycated HSA (with 100 mM glucose for 28 days) indicating their sequence, retention time and observed MS and MS/MS charge states.

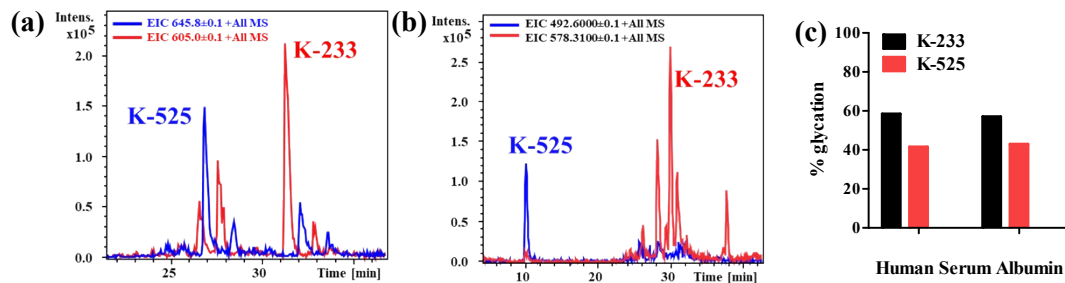


Fig. S8. (a) Overlaid extracted ion chromatogram of glycosylated lysine residue [K-525, K*QTALVELVK, MH⁺ = 1290.69; m/z = (645.8)²⁺ and K-233, AEFAEVSK*LVTDLTK, MH⁺ = 1812.89; m/z = (605.0)³⁺] from the tryptic digest of HSA (b) Overlaid extracted ion chromatogram of glycosylated lysine residue [K-525, RQIKK*QTALVE, MH⁺ = 1475.79; m/z = (492.60)³⁺ and K-233, VSKLVTDLTK*VHTE, MH⁺ = 1731.88; m/z = (578.0)³⁺] from the Glu C digest of HSA (c) The relative extent of glycation at two lysine residues (K-525 and K-233) in human serum albumin after the in-solution proteolytic digestion using trypsin and Glu-C enzymes.