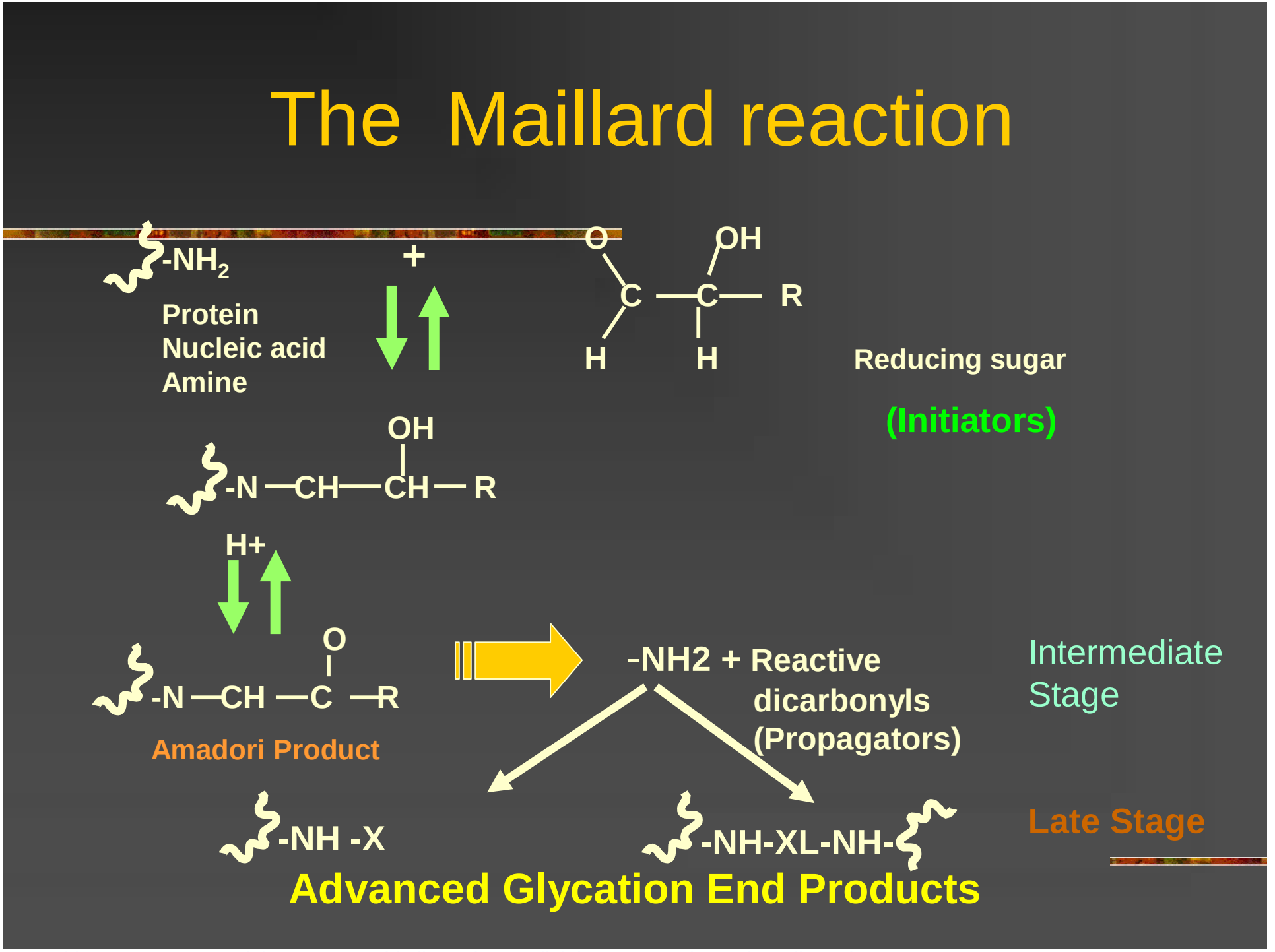
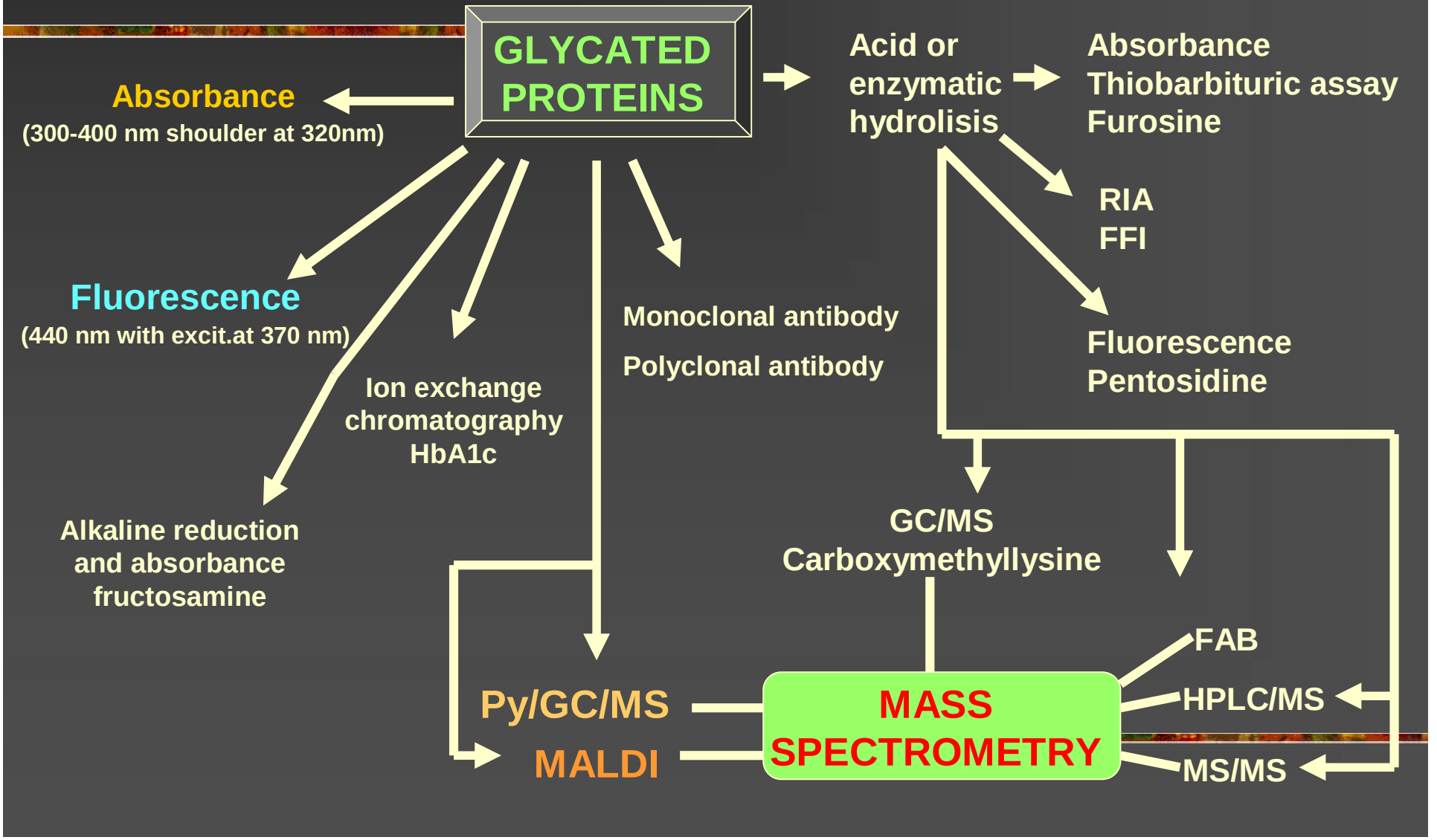
MASS SPECTROMETRY IN NON-ENZYMATIC PROTEIN GLYCATION STUDIES

R.Seraglia, A.Lapolla and P.Traldi

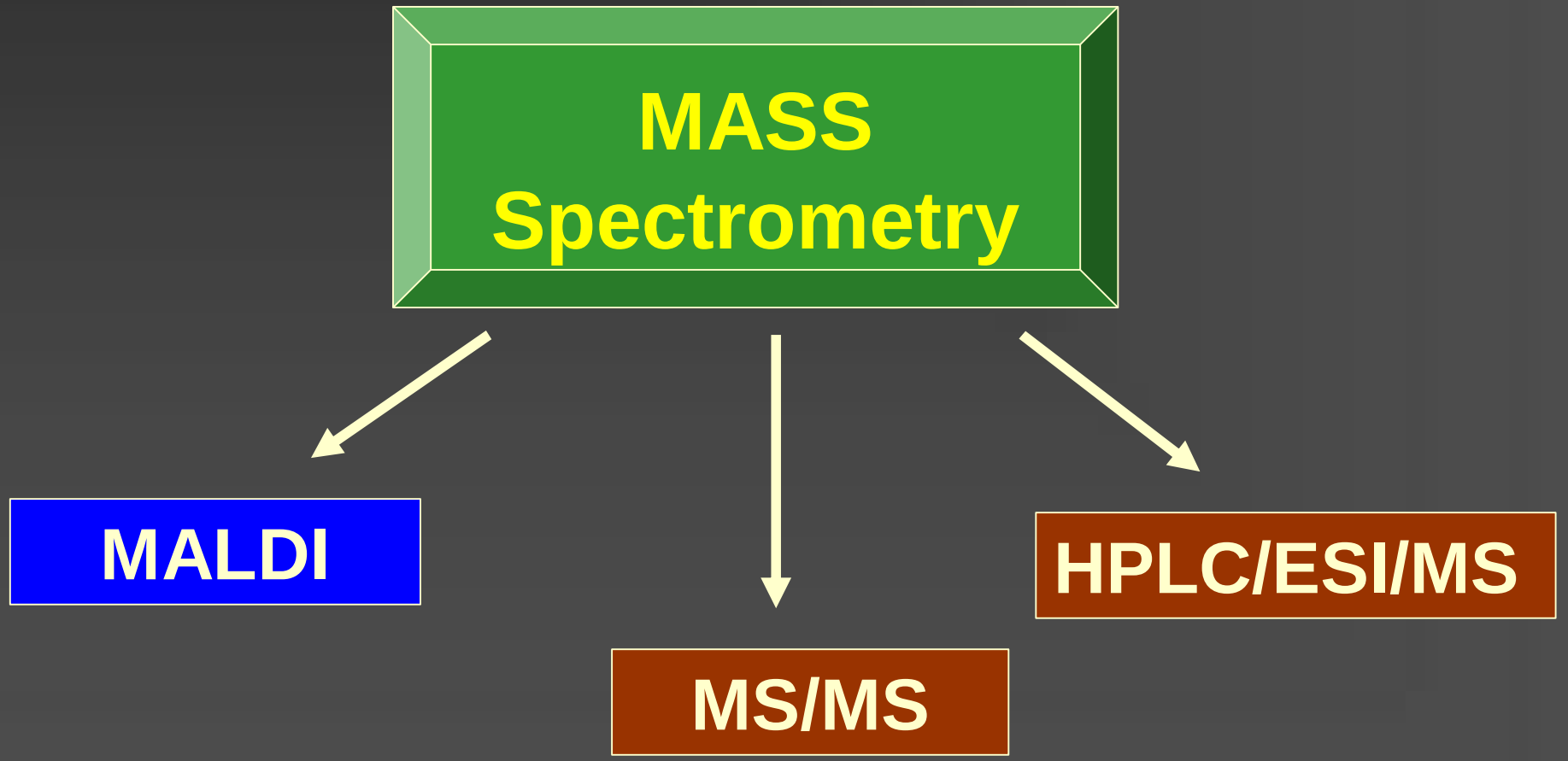
The Maillard reaction



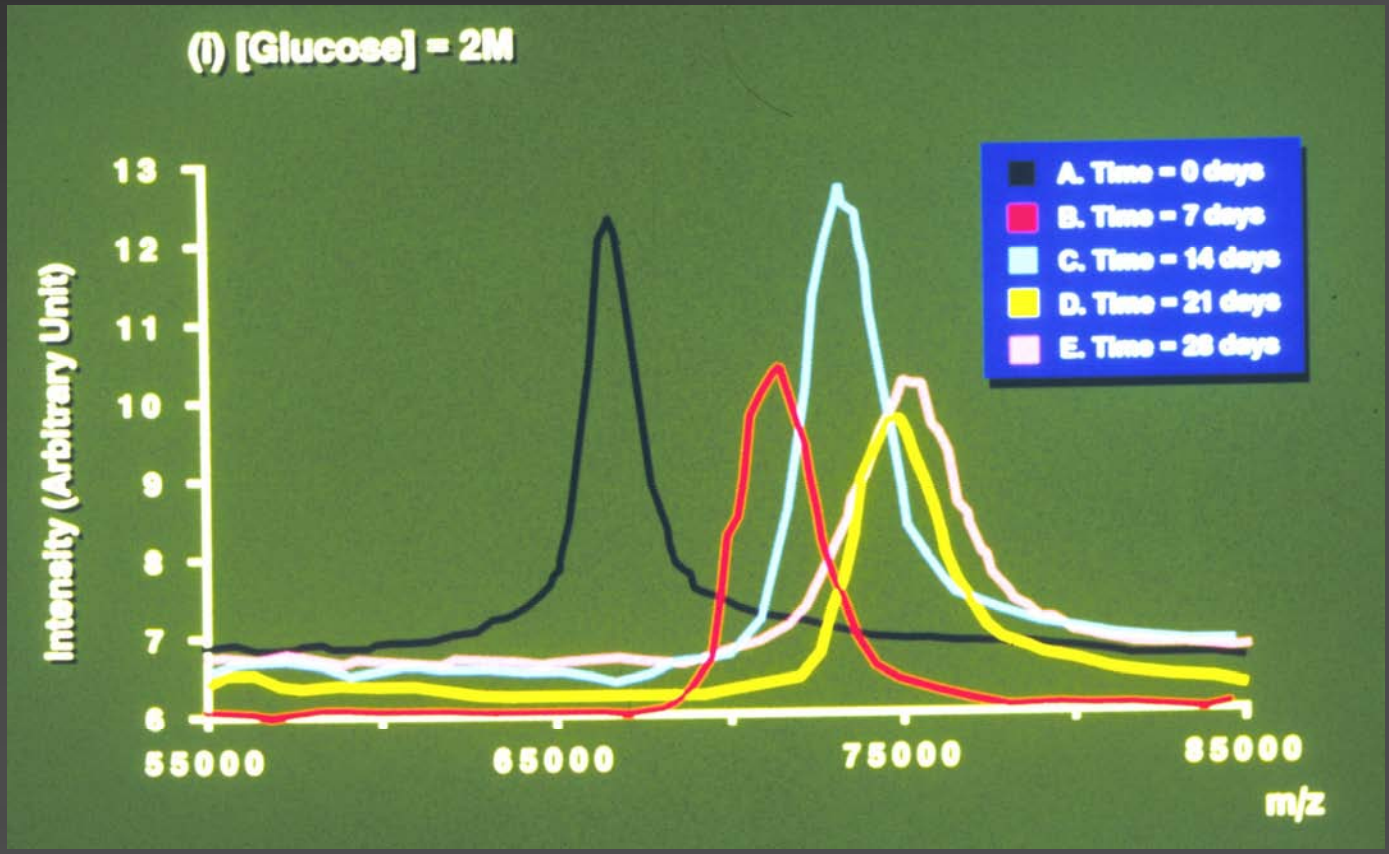
Strategies for glycated proteins analysis



Strategies for Glycated Protein Analysis:
Mass Spectrometric Approaches

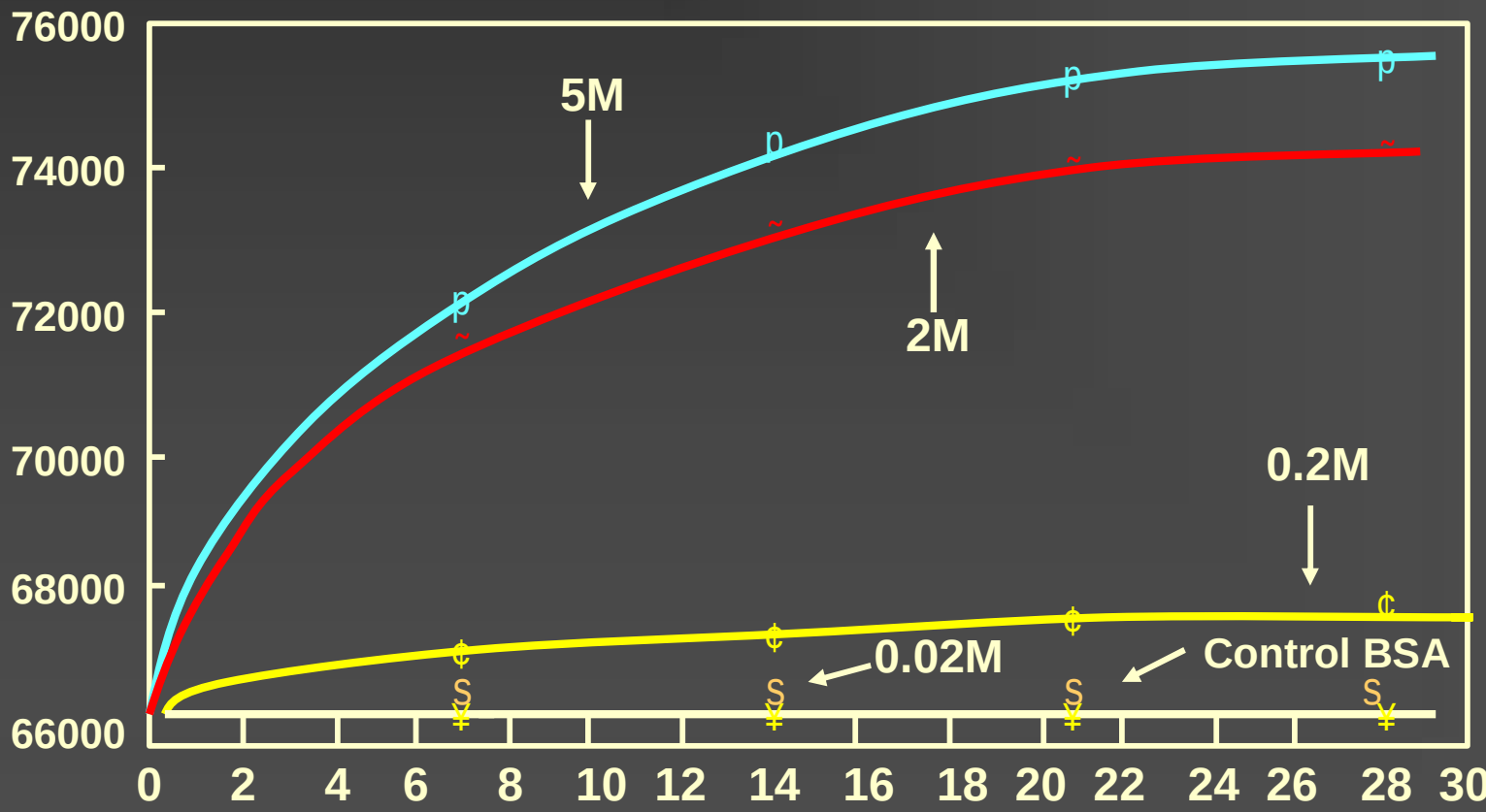


MALDI spectra of BSA at different incubation times



A.Lapolla,BBA 1994

Molecular Mass vs incubation time for BSA incubated wth glucose at different concentrations



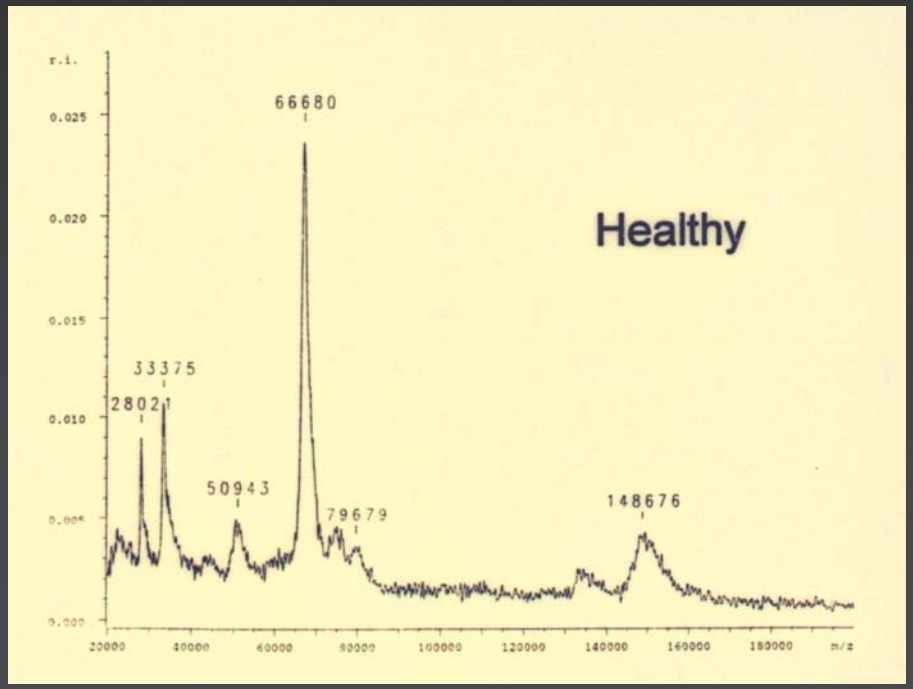
A.Lapolla,BBA 1994

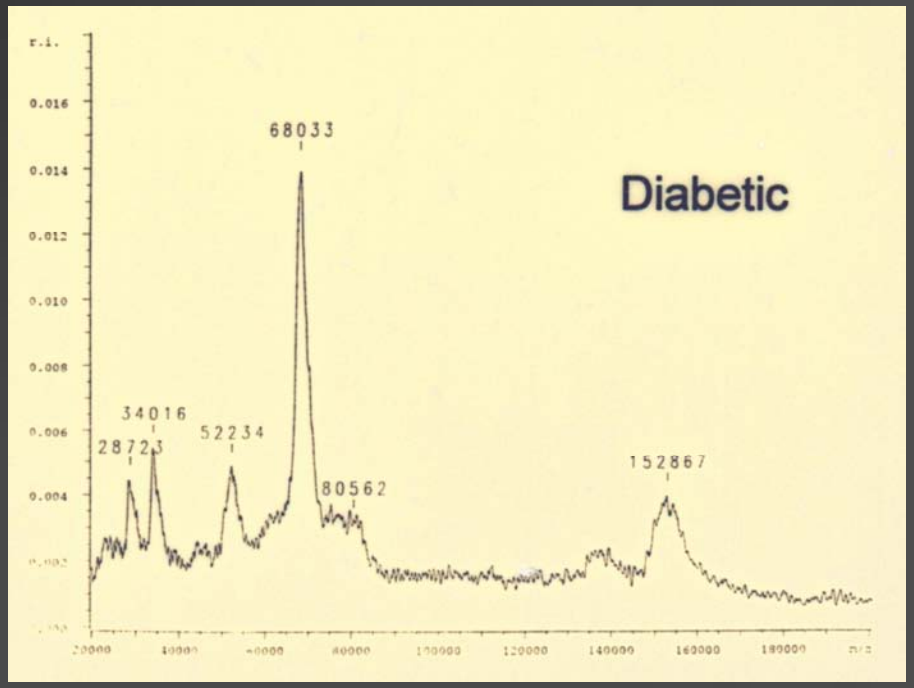
MALDI in the Study of Glycated Plasma Proteins

A.Lapolla,D.Fedele,R.Seraglia, S.Catinella, L.Baldo, R.Aronica, P.Traldi

Diabetologia 1995

MALDI spectra of plasma protein fractions from a healthy and a diabetic subject

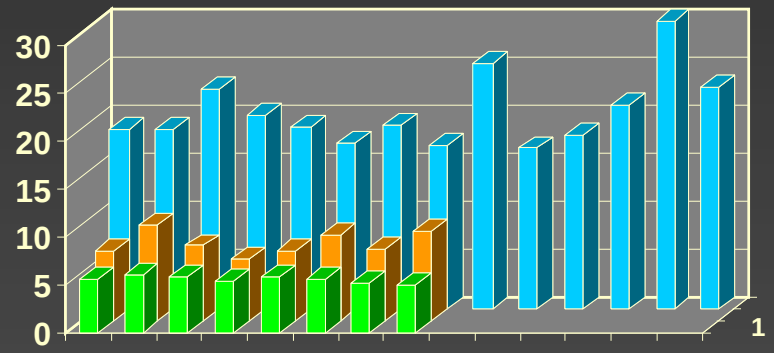




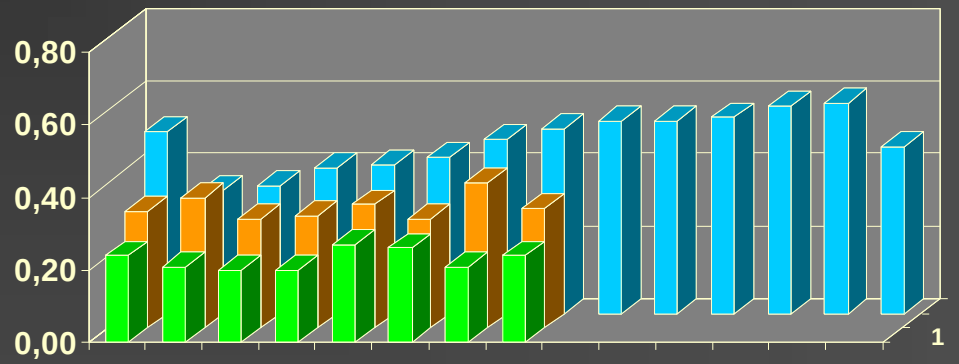
Glycation parameters

¢ NGT ¢ well controlled DM ¢ poorly controlled DM

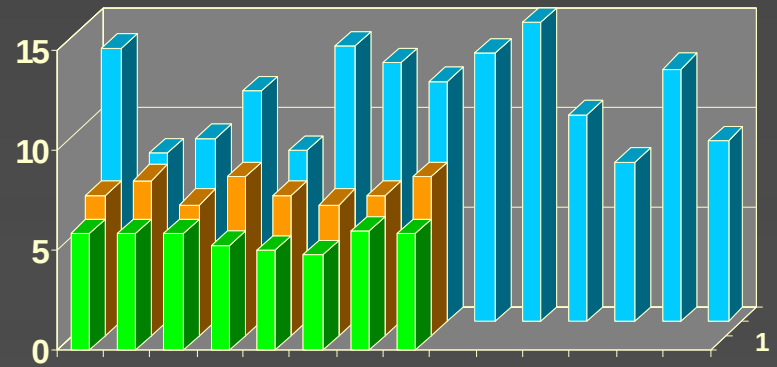
FPG (mmol/l)



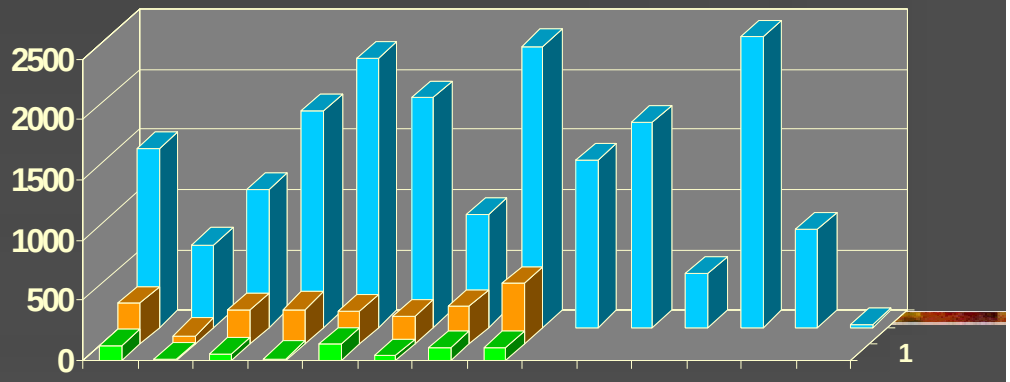
Furosine (μgFur/mg prot)



HbA1c (%)



ΔM HSA

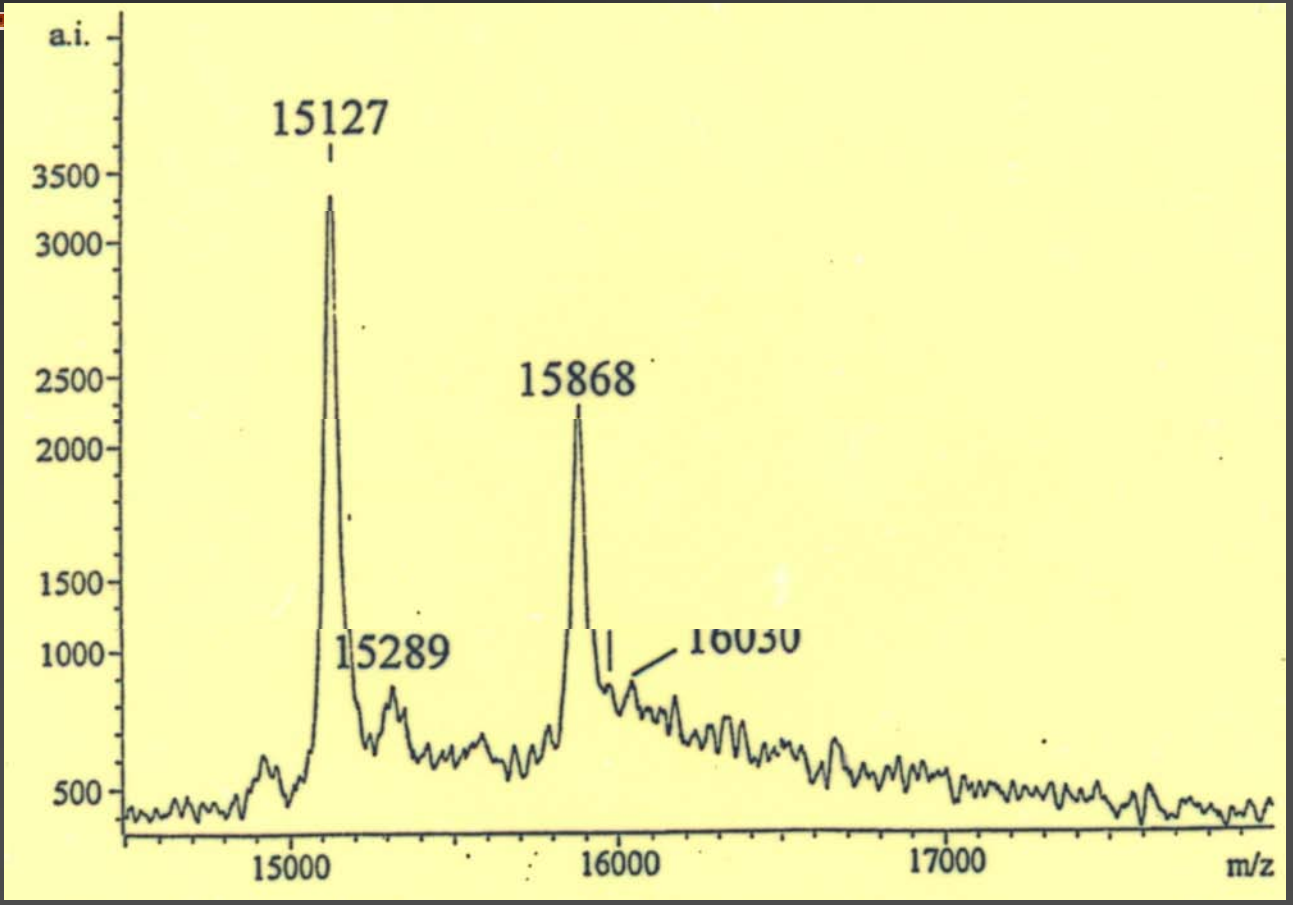


MALDI in the Study of Glycated Globins

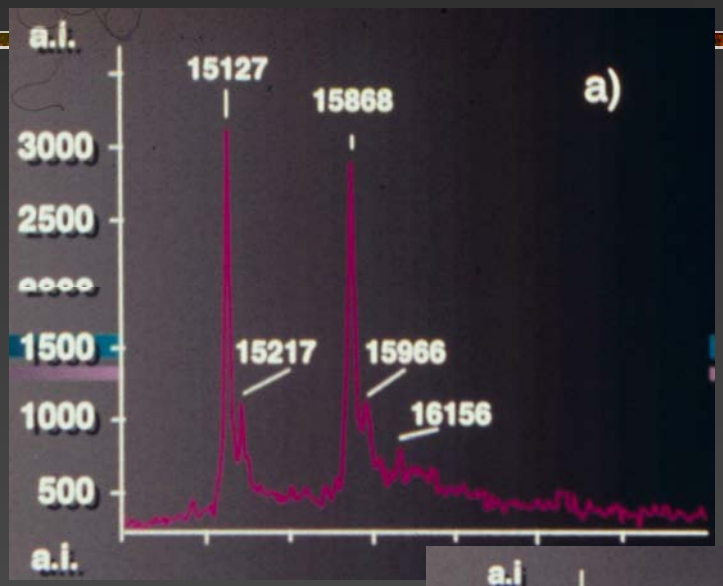
A.Lapolla, P.Traldi, et all.

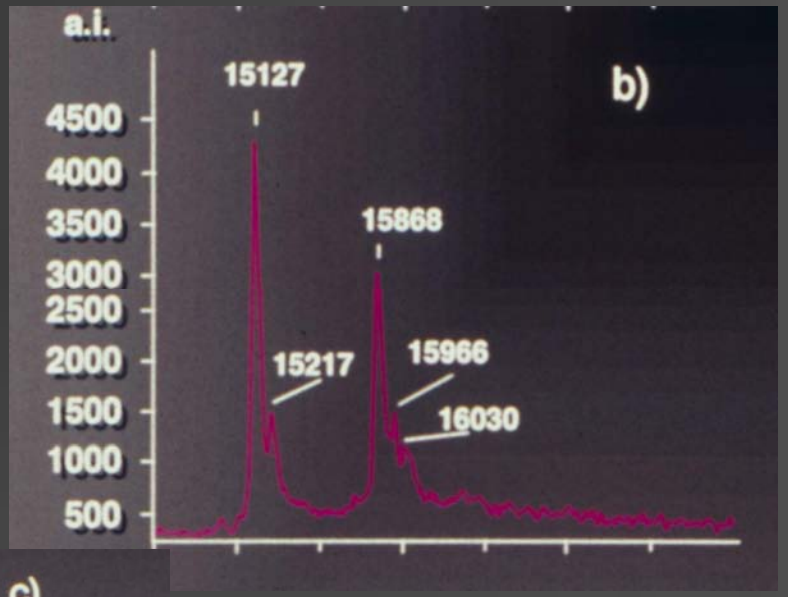
Rapid Commun Mass Spectrom 1996
Rapid Commun Mass Spectrom 1998
Clin Chem 1999

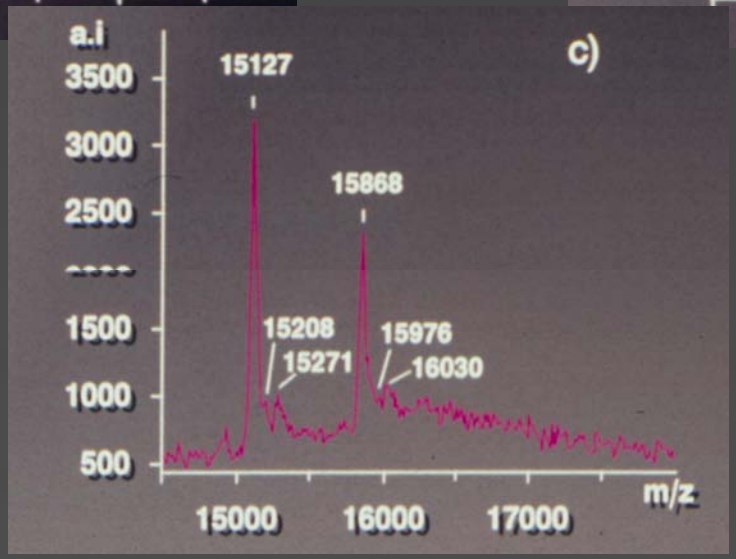
MALDI spectra of health subject erythrocyte globin



MALDI spectra of diabetic patients erythrocyte globin

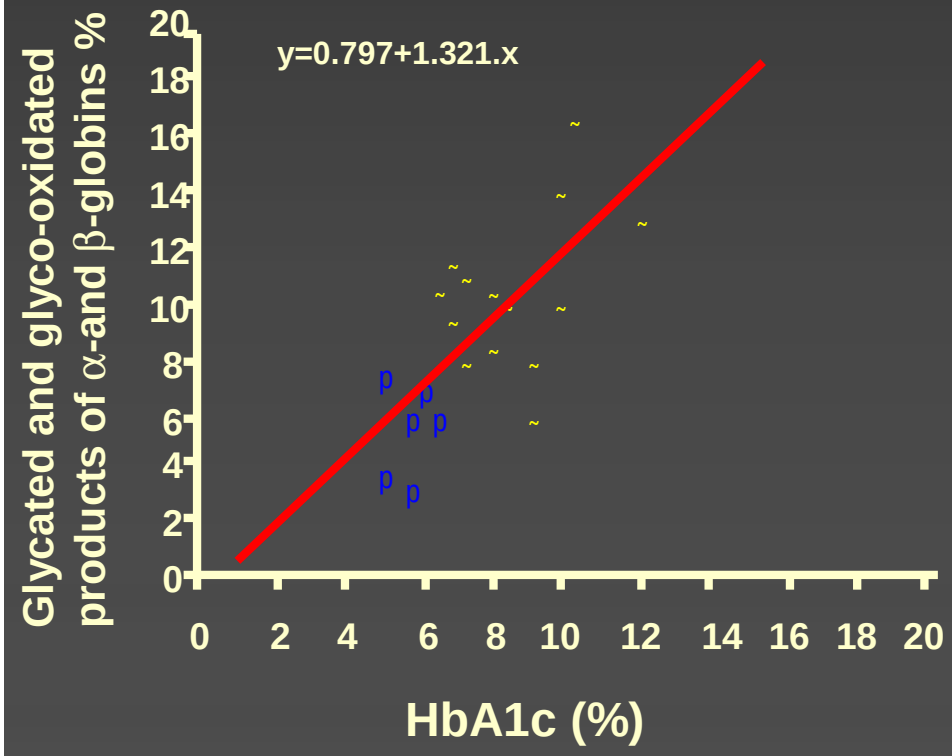


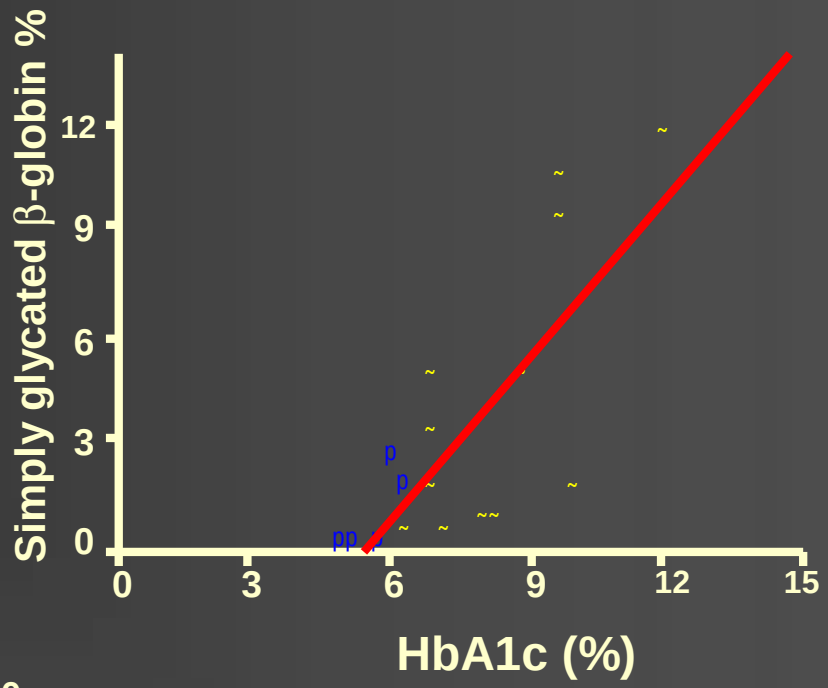


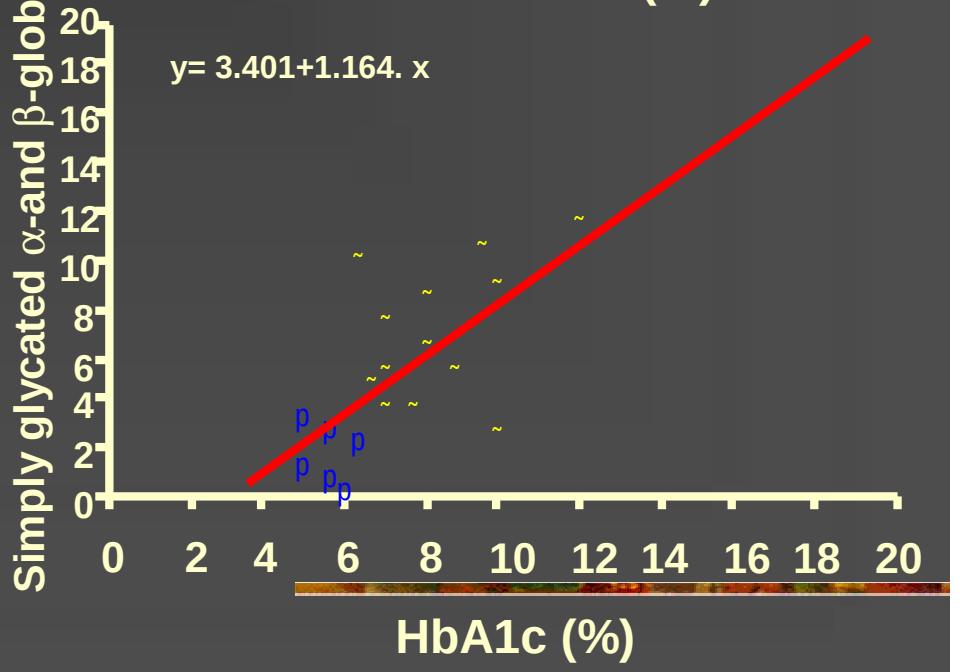


Plot of % of globins obtained by MALDI vs HbA1c

p healthy ˜ diabetic







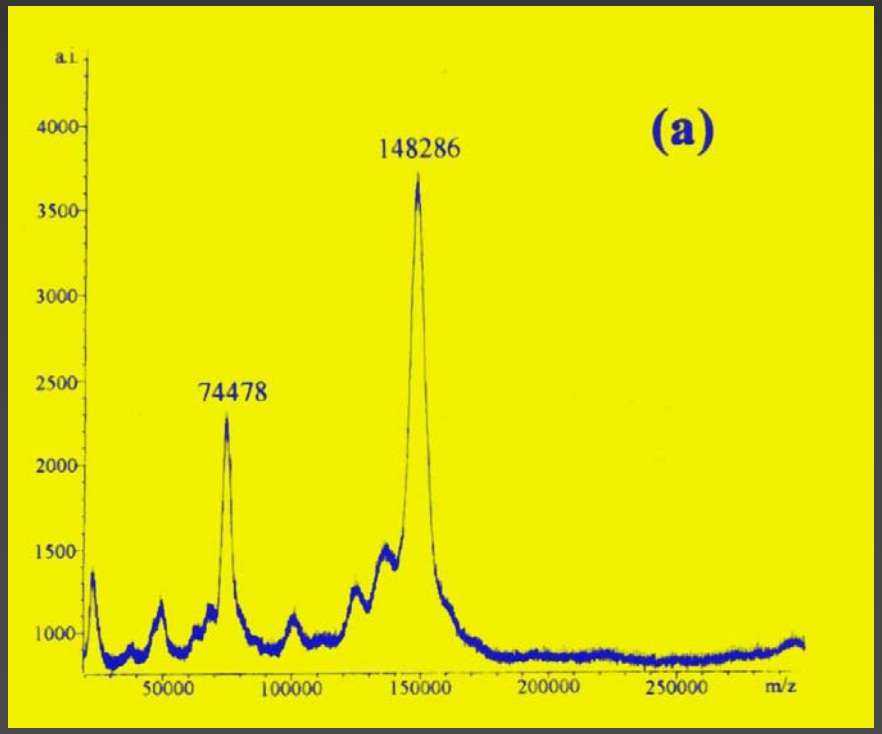
MALDI n the Study of Glycated γ-Globulins

A.Lapolla, P.Traldi et al.

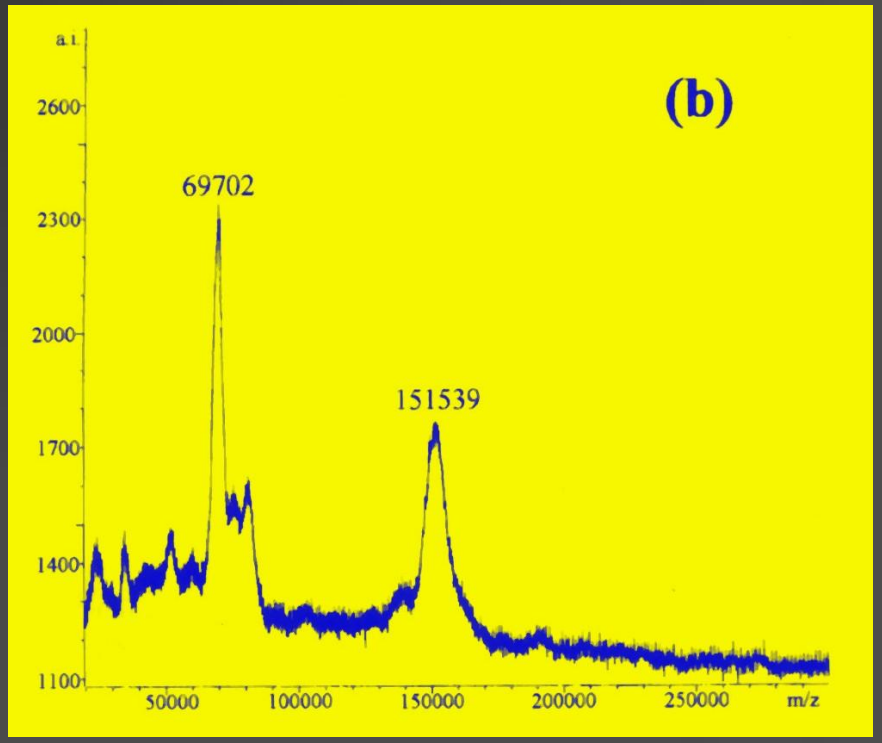
Rapid Commun Mass Spectrom 1997

J Am Soc Mass Spectrom 2000

MALDI mass spectra of IgG

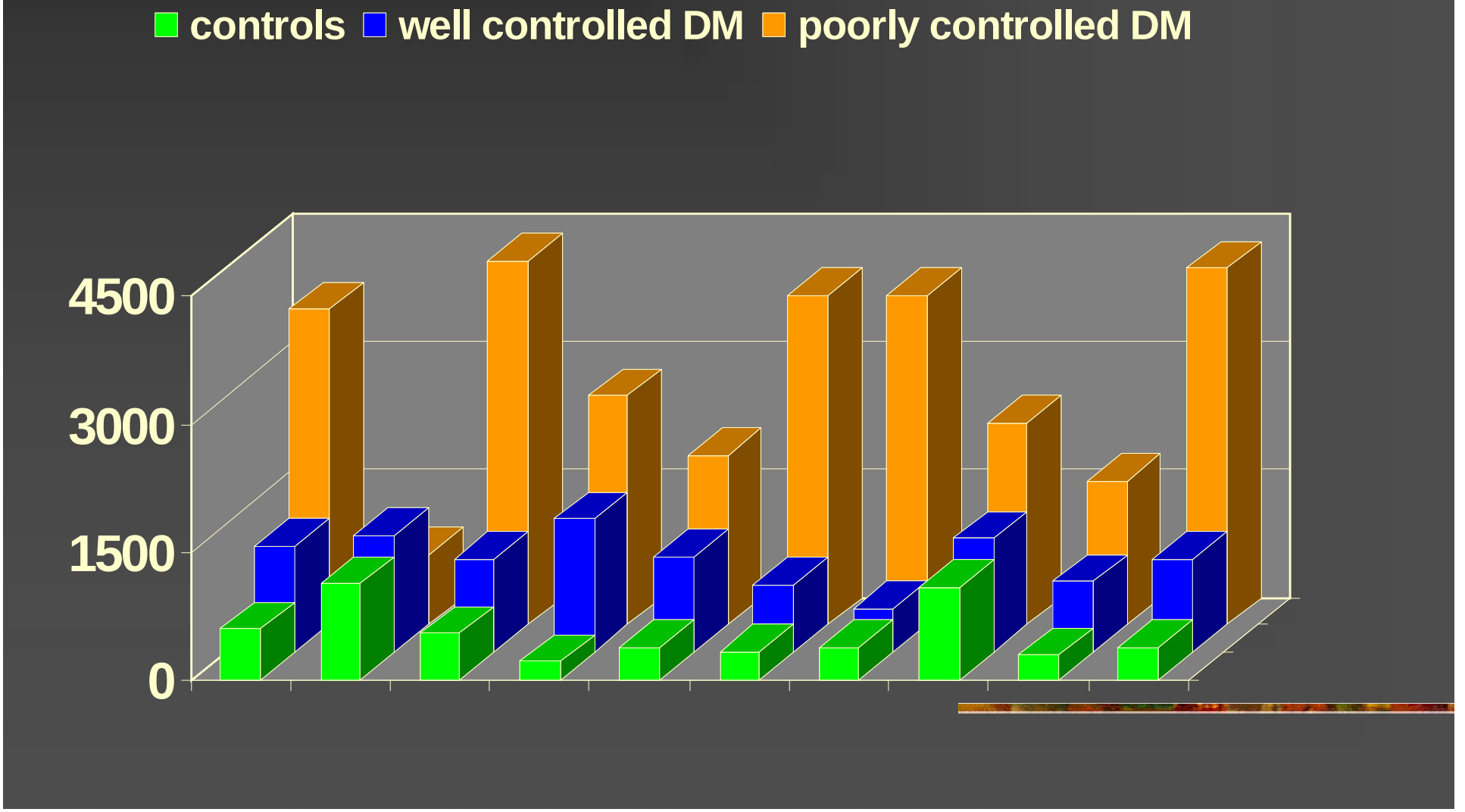


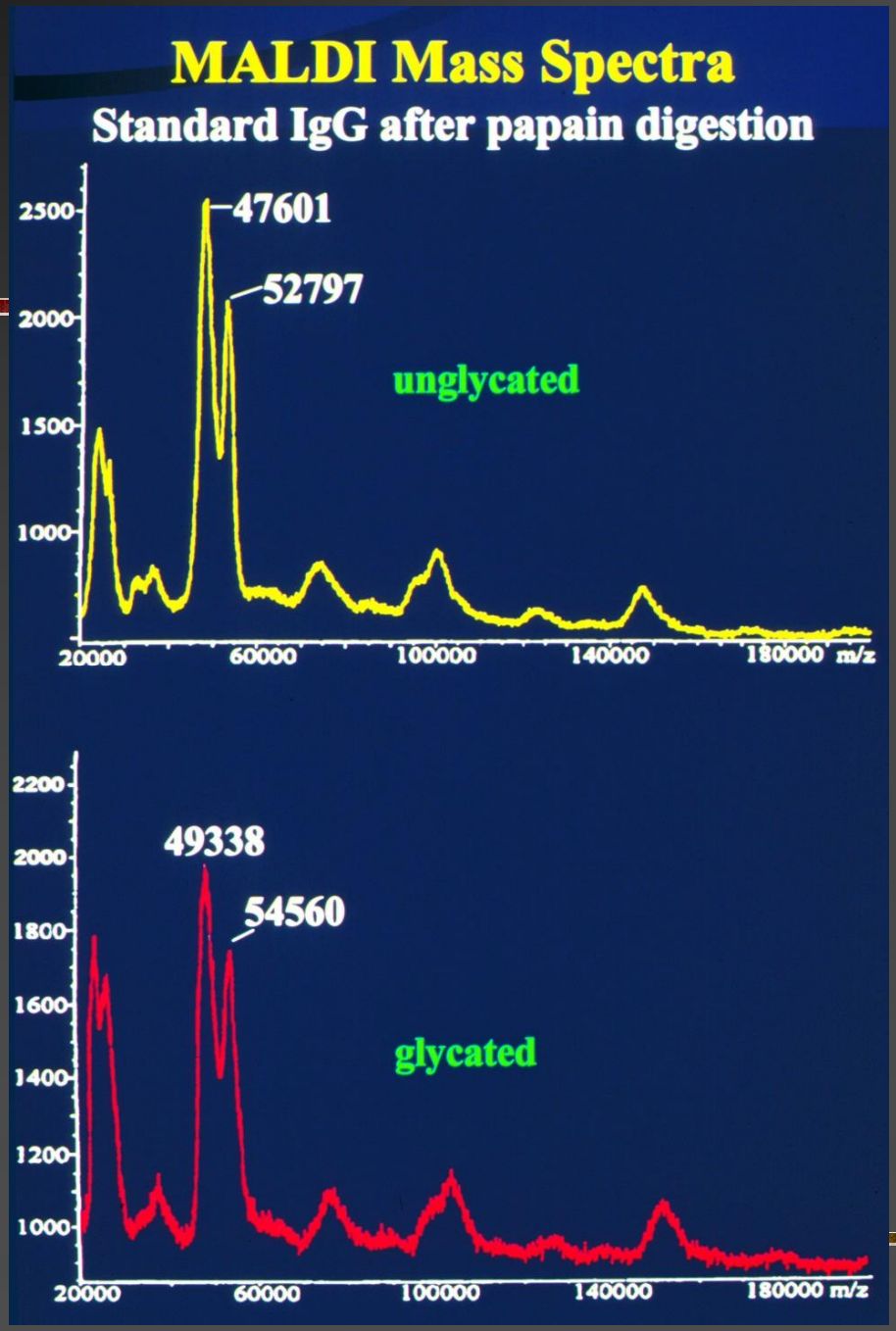
Standard IgG

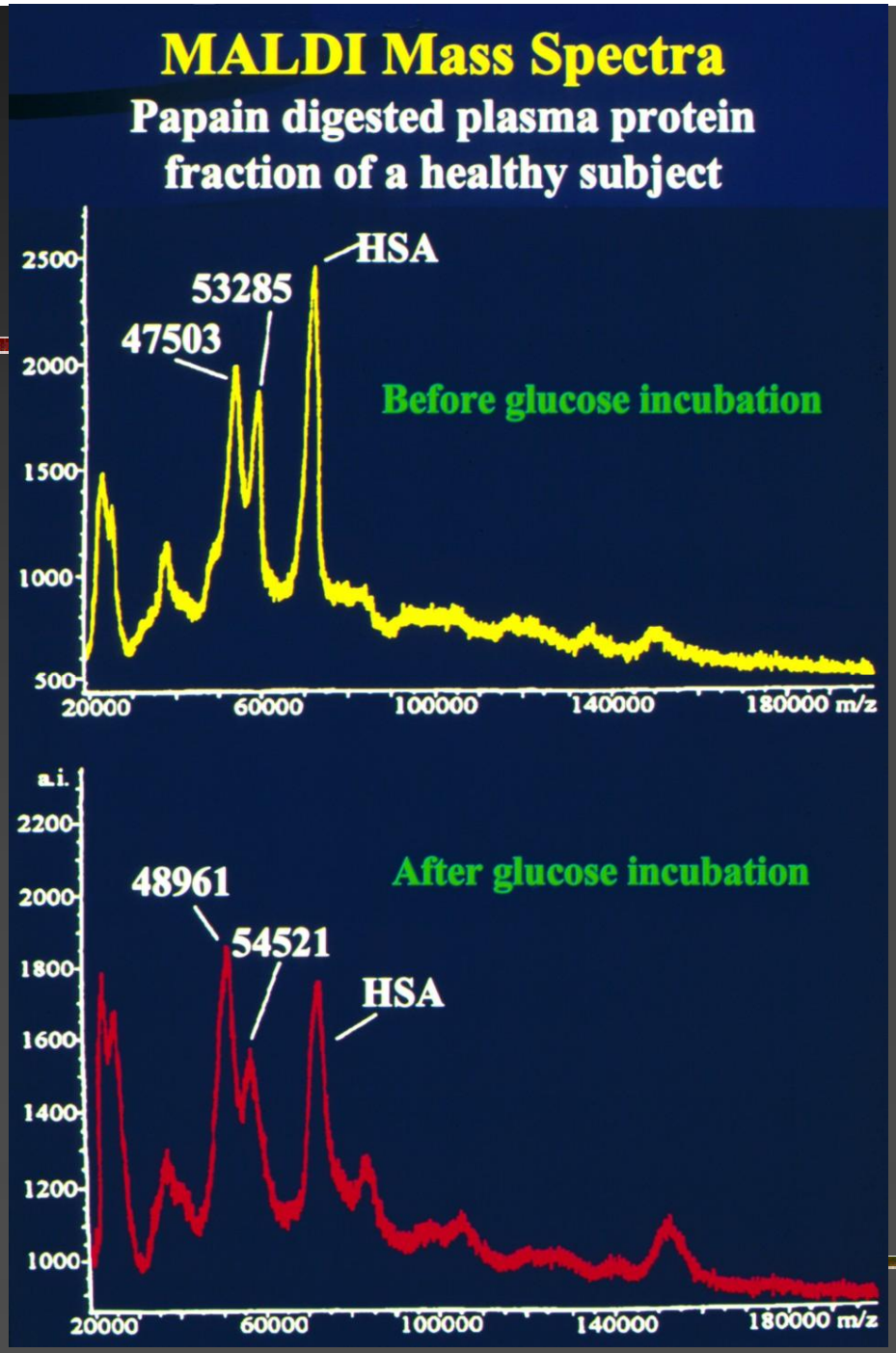


IgG protein fraction of a poorly controlled diabetic subject

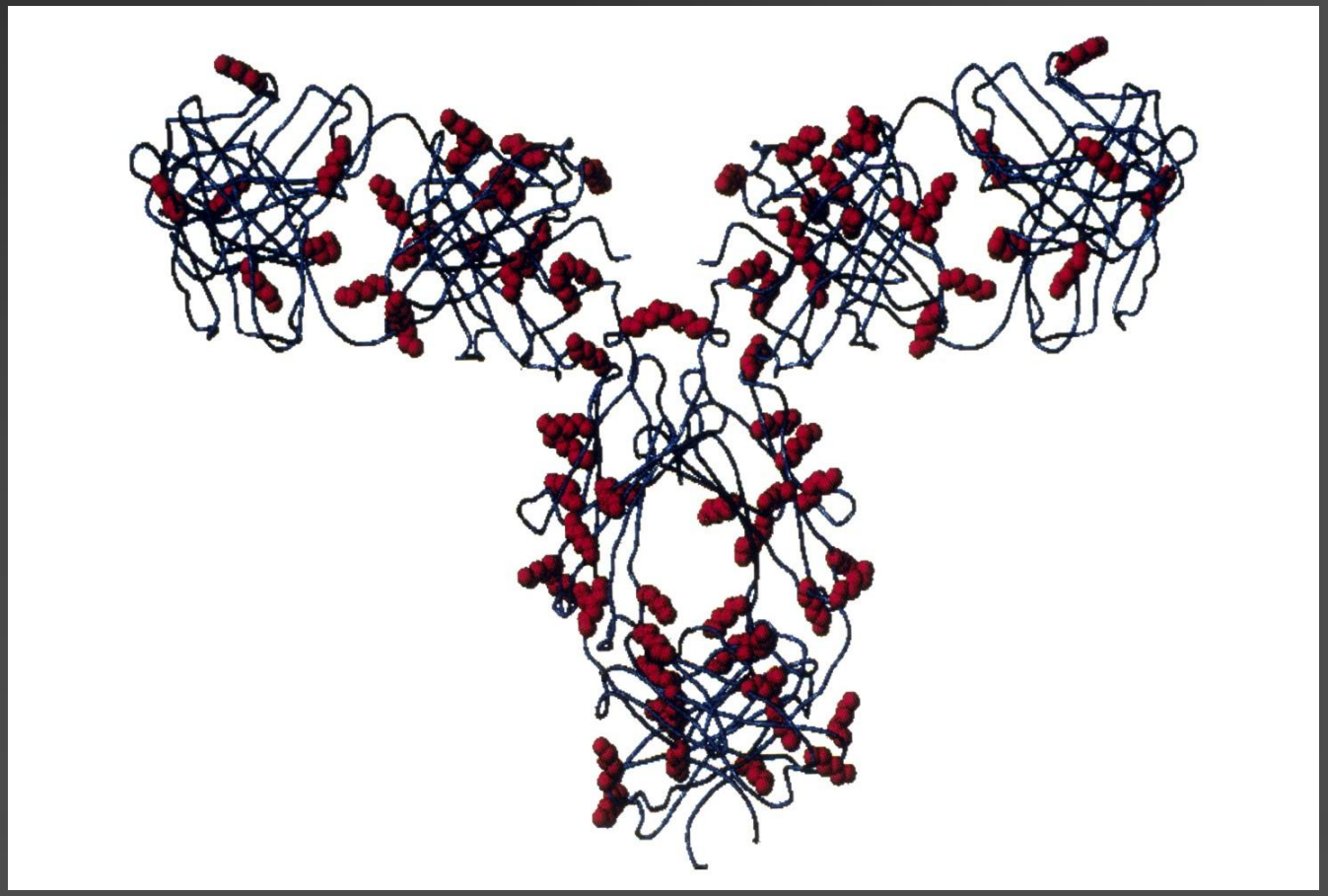
ΔM values of IgG



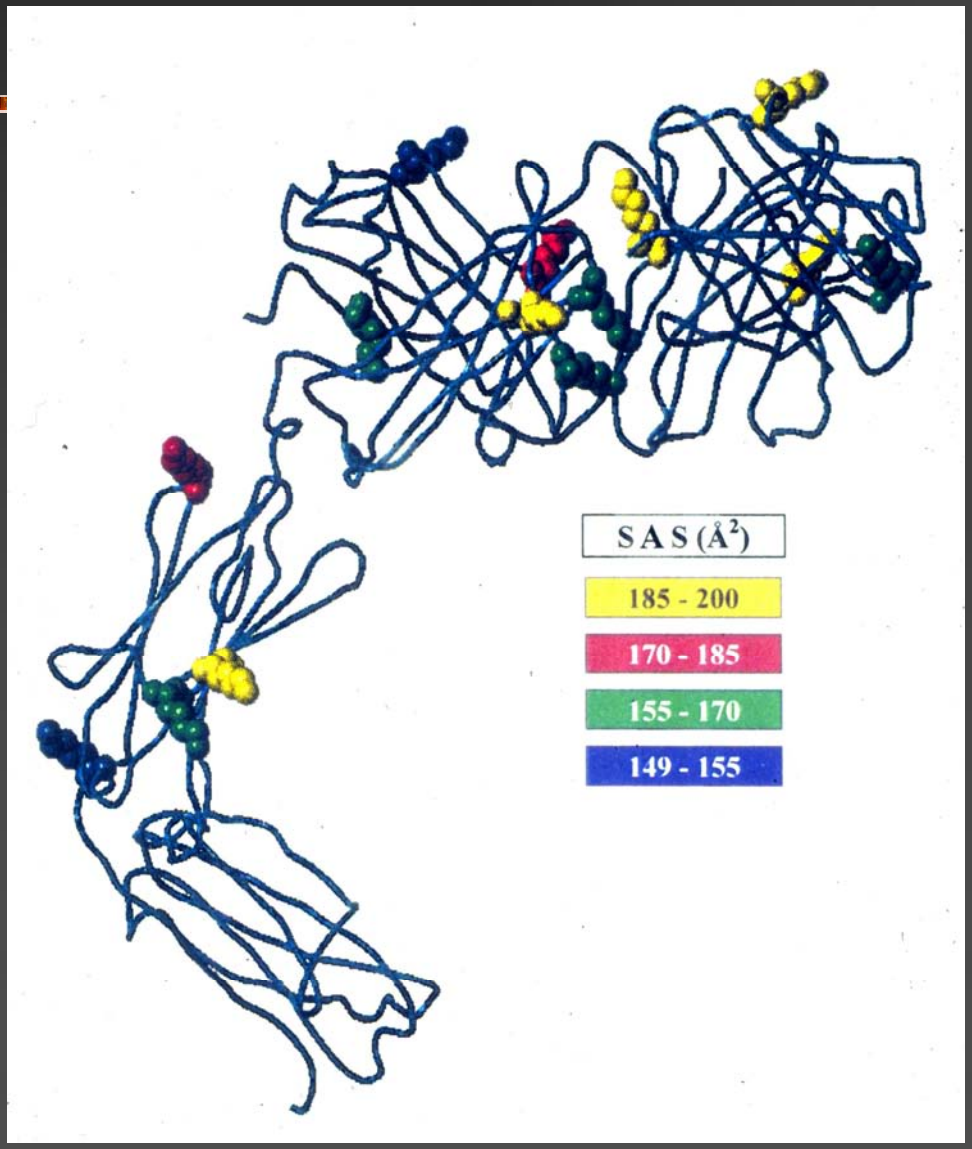




αcarbon trace of IgG



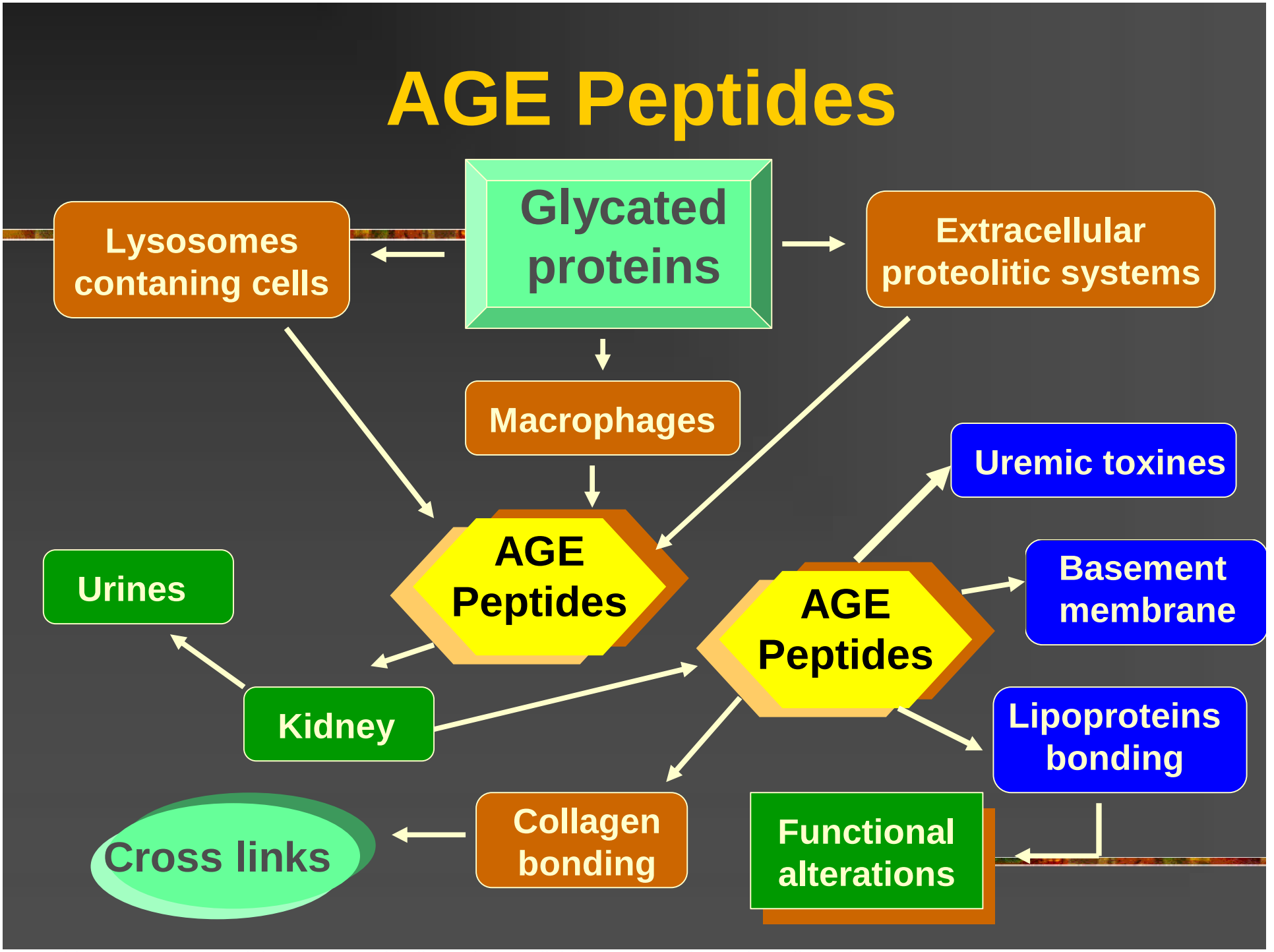
SAS of lysine residues of IgG



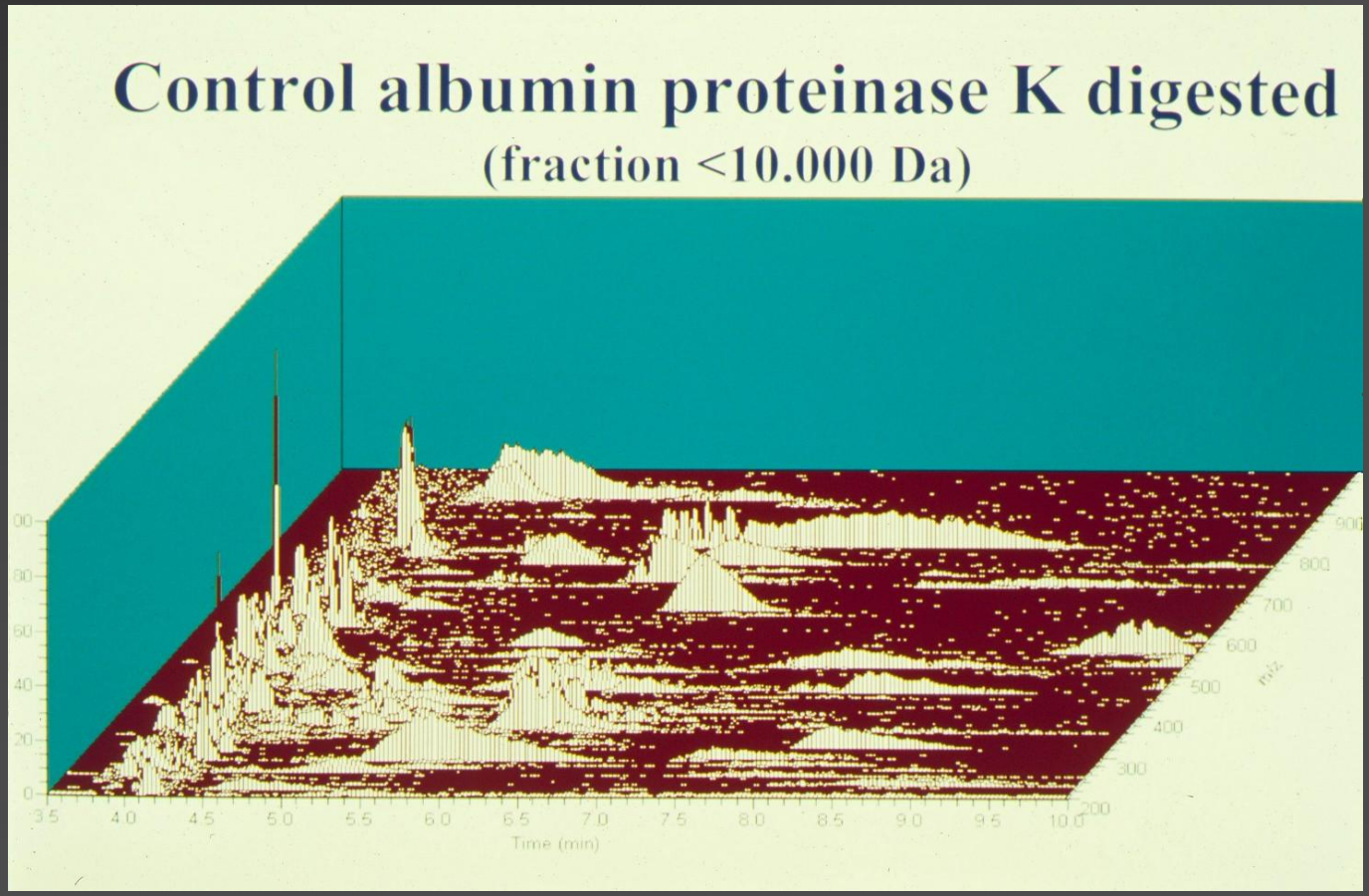
Mass Spectrometric Approaches in the Study of AGE Peptides

A.Lapolla, D.Fedele, L.Martano, CN Aricò, M.Garbeglio, R.Seraglia, D.Favretto, P.Tradi

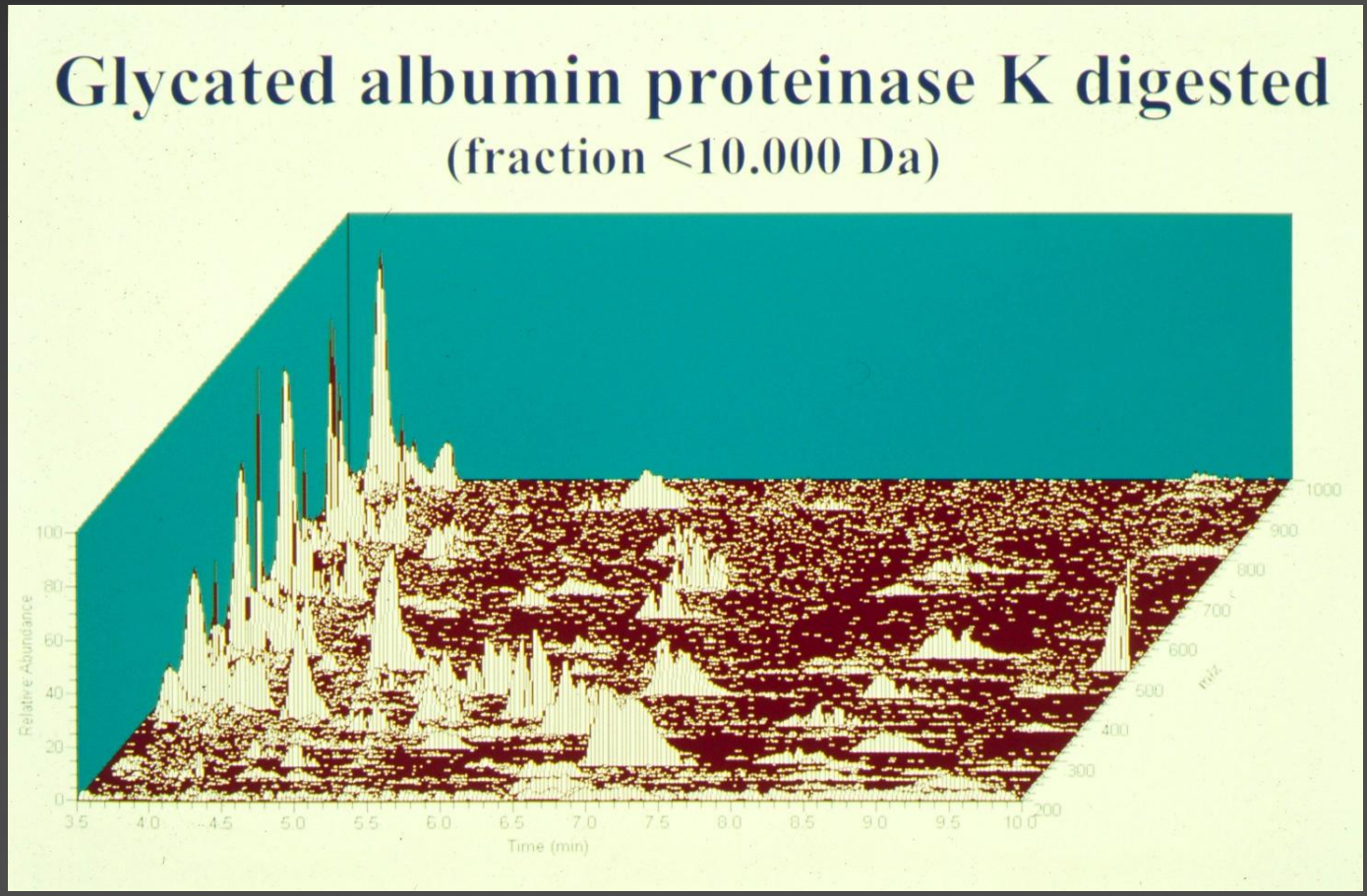
J Mass Spectrom 2001



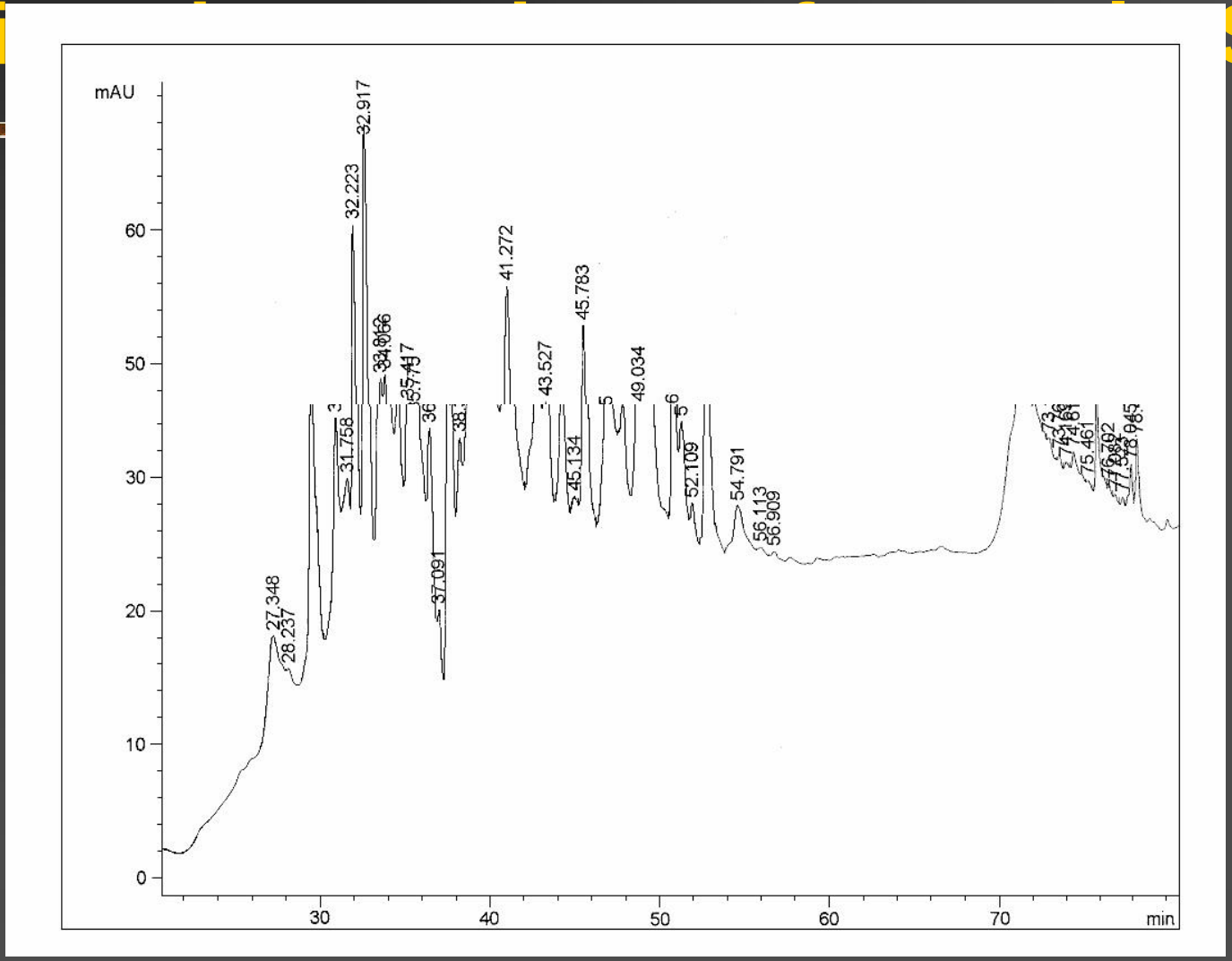
Ionic species vs retention time reconstructed from HPLC/ESI/MS runs of control Albumin



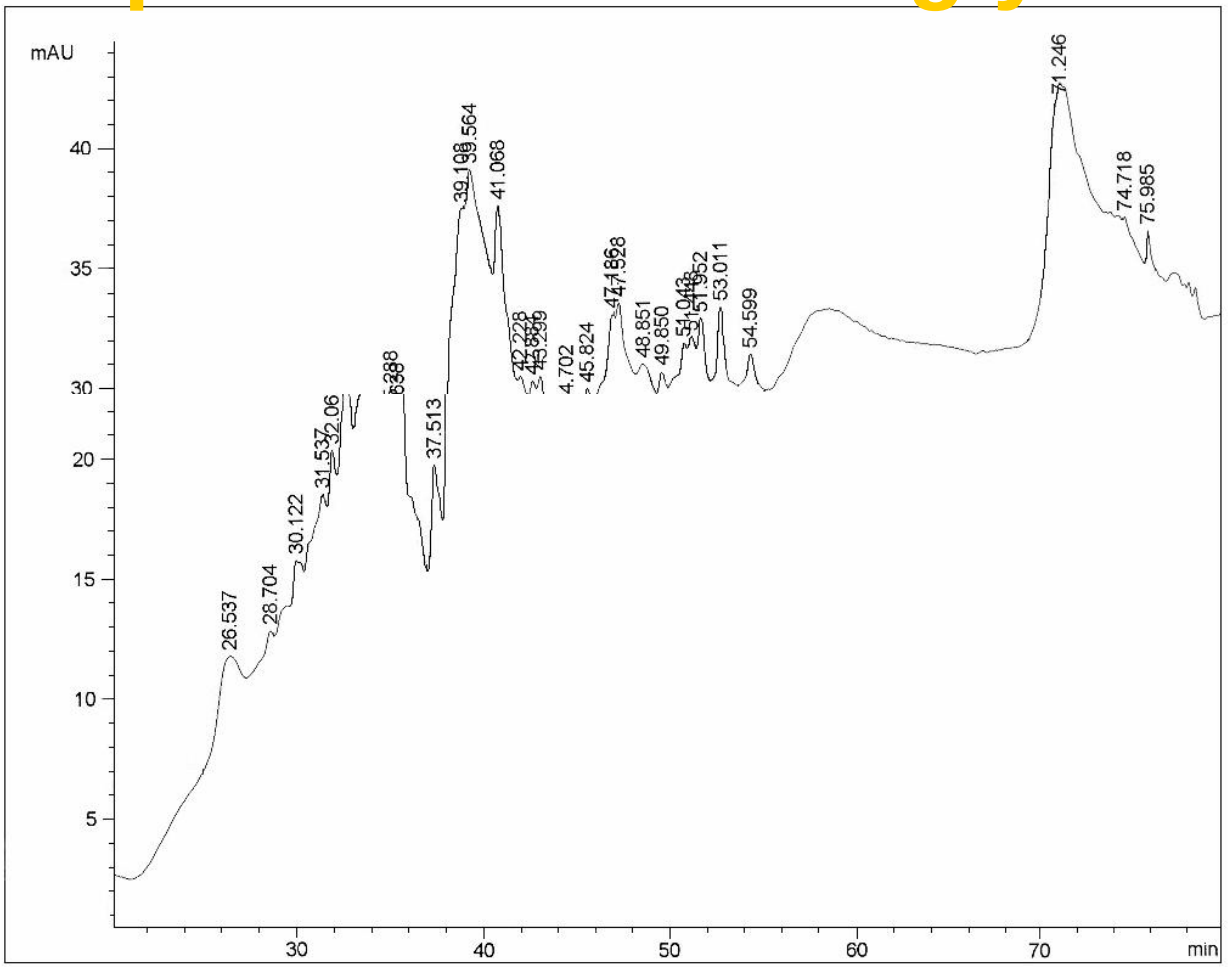
Ionic species vs retention time reconstructed from HPLC/ESI/MS runs of glycated Albumin



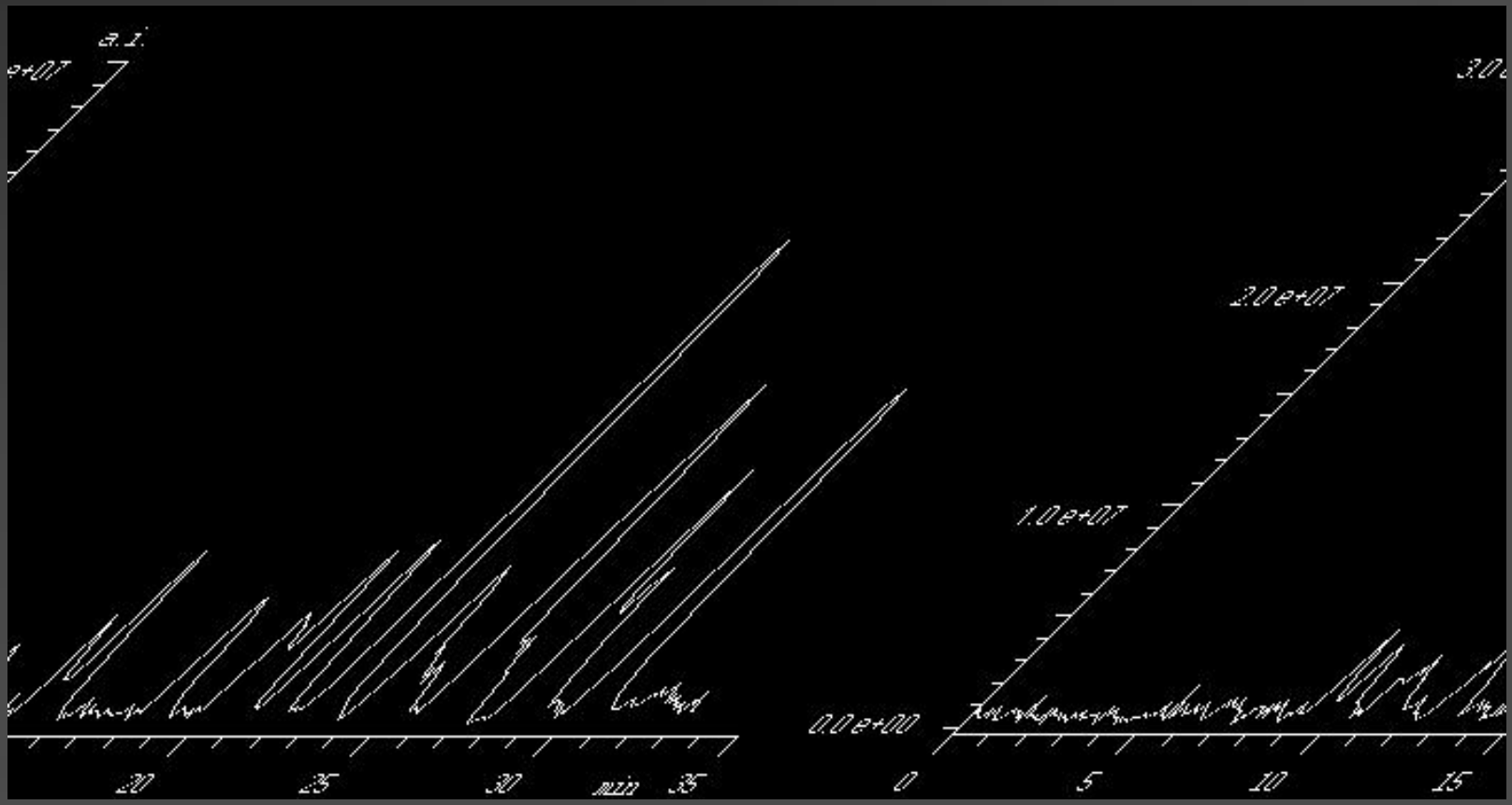
UV(214nm) Chromatogram of trypsin di SA



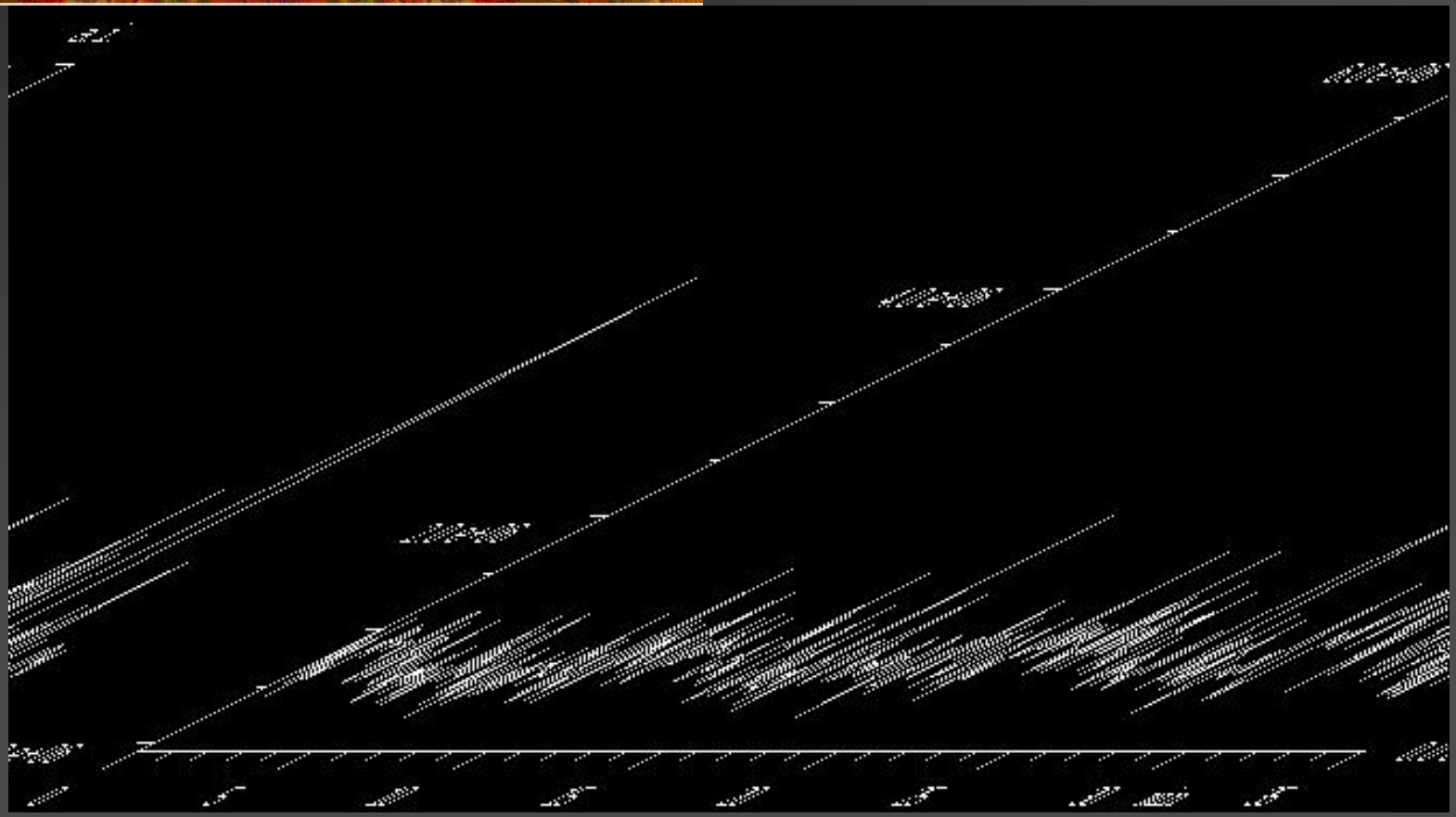
UV(214nm) Chromatogram of trypsin digestion products *in vitro* glycated HSA



Total ion current chromatogram of trypsin digestion products of control HSA



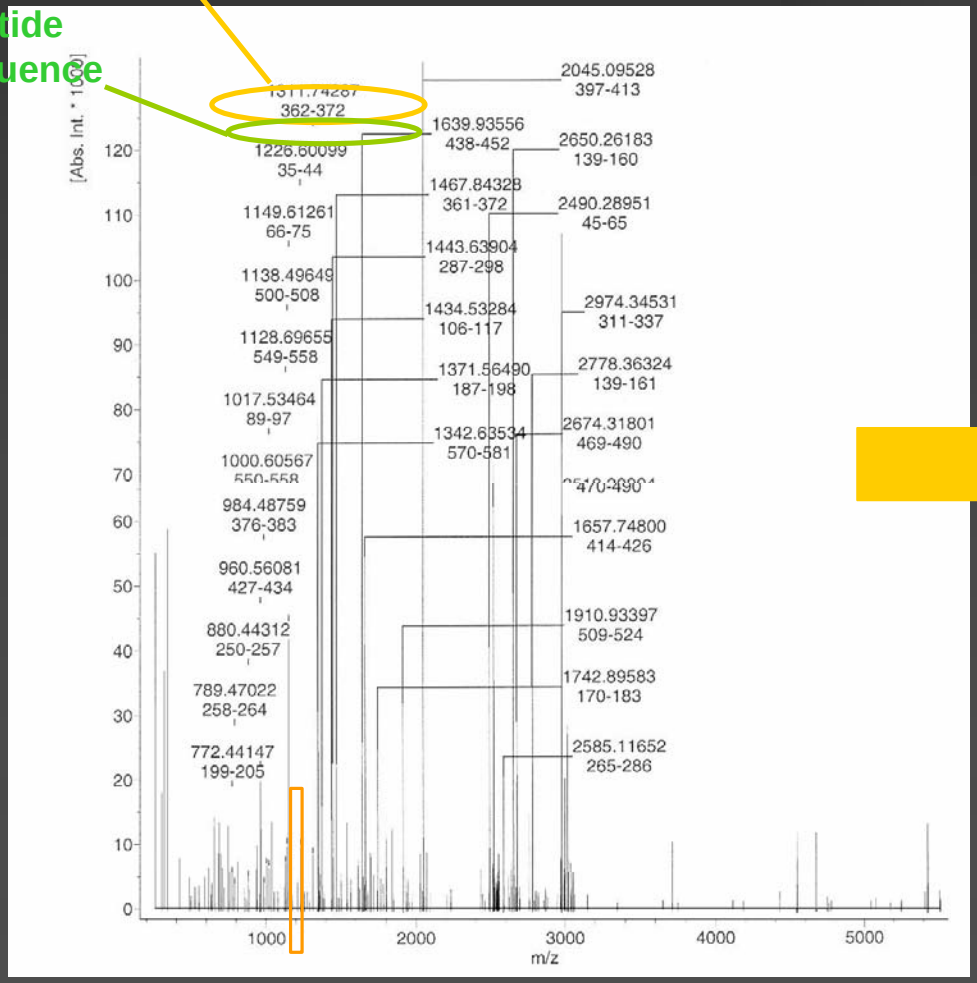
Total ion current chromatogram of trypsin digestion products *in vitro* glycated HSA

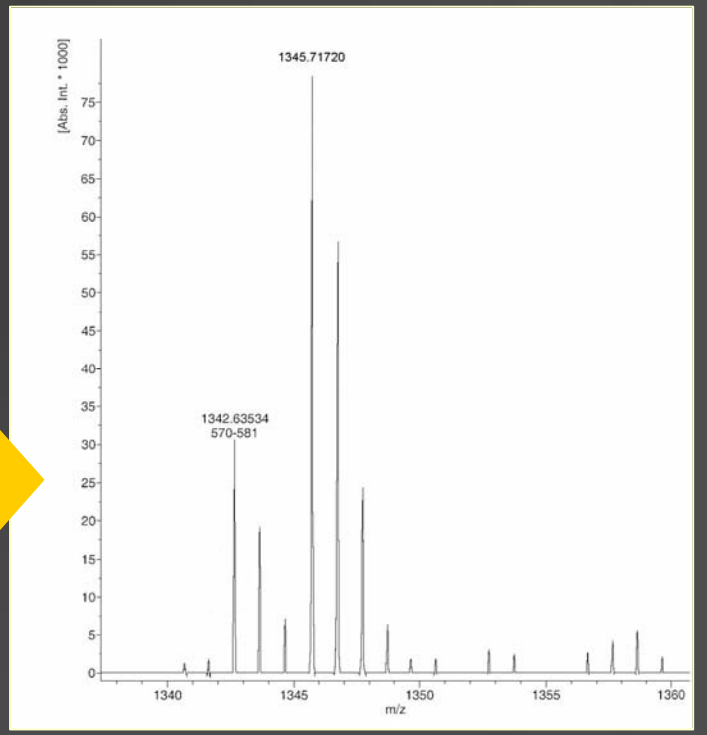


ESI-MS spectrum TIC chromatogram run of of trypsin digestion products of control HSA

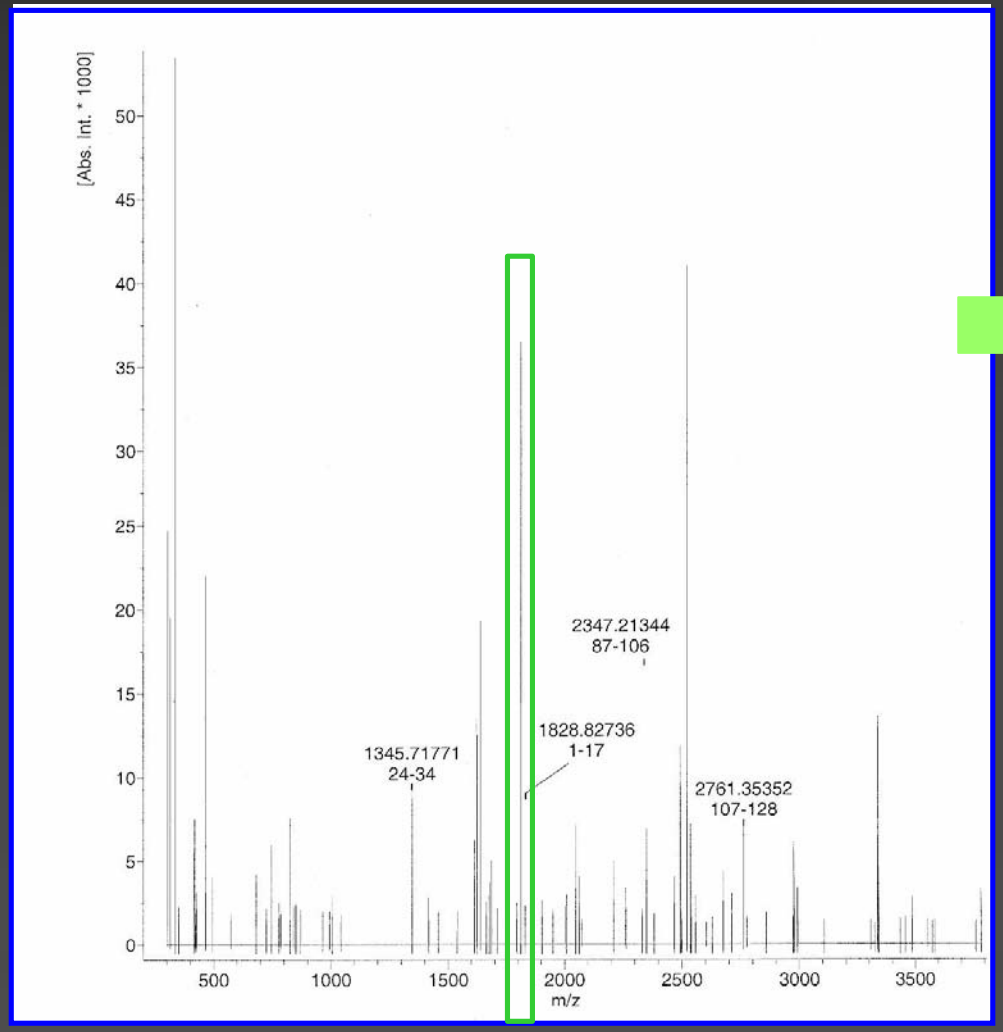
accurate mass

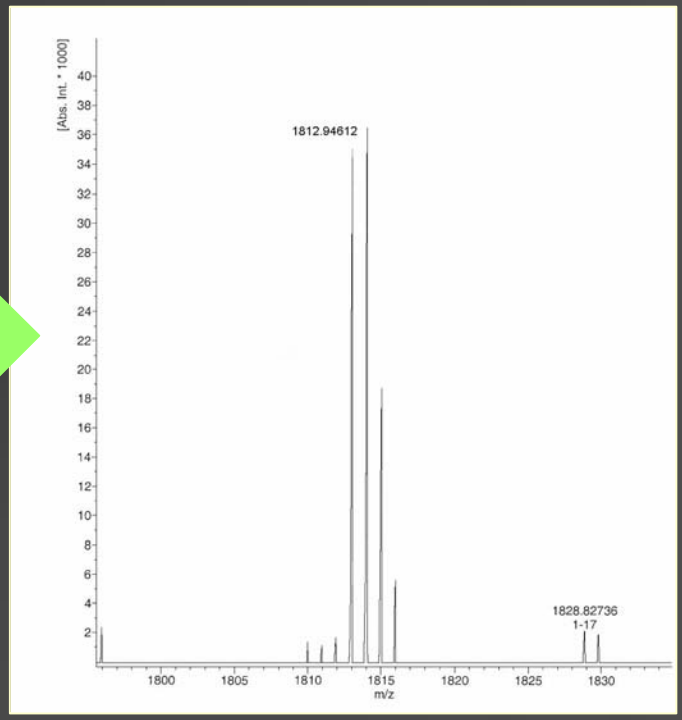
peptide sequence





ESI-MS spectrum TIC chromatogram run of trypsin digestion products *in vitro* glycated HSA





Petides identified by accurate mass measurements based on HSA sequence

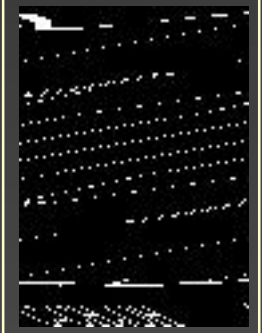
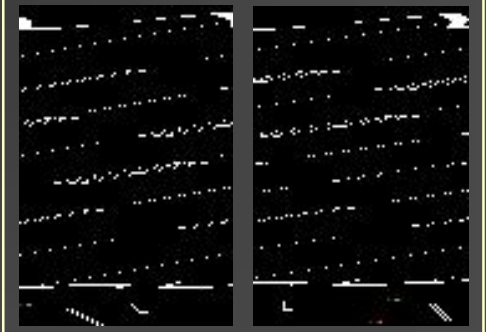
1 DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
51 KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKQEPERNE
101 CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
201 ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
251 LECADDRADL AKYICENQDS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
301 DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
351 KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
401 YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
451 DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPCFSA LEVDETYVPK
501 EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
551 FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL

Colour code:
DAHK: sequences revealed only in control HSA
DAHK: sequences revealed only in glycated HSA
DAHK: sequences revealed both in control HSA and glycated HSA
DAHK: sequences glycated (+162u)
DAHK: sequences probably involved in cross-linking

Possible modifications of peptides due to glycation processes

Possible modifications of single peptides		Mass increment
$N=CH\text{-}(CHOH)_4\text{-}CH_2OH$	Peptide+ $C_6H_{10}O_5$	+162.052824
$N=CHCH_2C(=O)\text{-}(CHOH)_2\text{-}CH_2OH$	Peptide+ $C_6H_{10}O_5 - H_2O$	+144.042259
$N=CHCH_2C(=O)C(=O)CH_2CH_2OH$	Peptide+ $C_6H_{10}O_5 - 2H_2O$	+126.031694
$N=CHCH_2C(=O)C(=O)CH=CH_2$	Peptide+$C_6H_{10}O_5 - 3H_2O$	+108.021129
$N=CH\text{-}(CHOH)_4\text{-}CH_2OPO_3H_2$	Peptide+ $C_6H_{10}O_5 + HPO_3$	+242.019156
$N=CHCH_2C(=O)\text{-}(CHOH)_2\text{-}CH_2OPO_3H_2$	Peptide+ $C_6H_{10}O_5 + HPO_3 - H_2O$	+224.008591
$N=CHCH_2C(=O)C(=O)CH_2CH_2OPO_3H_2$	Peptide+ $C_6H_{10}O_5 + HPO_3 - 2H_2O$	+205.998026
*(PEPTIDES WITH MORE THAN 1 LYSINE)	Peptide+$2C_6H_{10}O_5$	+324.105648
*	Peptide+$3C_6H_{10}O_5$	+486.158472
*	Peptide+$4C_6H_{10}O_5$	+648.211296
$N=CHCH_2OH$ $NHCH_2C(=O)H$	Peptide+C_2H_2O	+42.010565
$NHCHC(=O)H$	Peptide+C_2O	+39.994915
$NHCH_2C(=O)OH$	Peptide+C_2H_2O	+58.005479

Possible cross-links

	Possible cross-links			
Structure				
	Peptide 1+ Peptide 2+ $C_6H_6O_3$	Peptide 1+ Peptide 2+ 4C	Peptide 1+ Peptide 2+ 2C –2H	Peptide 1+ Peptide 2+ 2C
Mass increment	+126.031694	+48.0	+21984350	+24.0

Ionic species detected in ESI spectra of glycation products of samples and their possible origin and sequence

Control HSA Observed mass	Glycated HSA Observed mass	Position and modification	Sequence	Theoretical mass
1667.76435				
	1675.8402	160-162+437-445+24u	RYK+CCKHPEAKR+24u	1675.8399
	1677.80277	429-432+565-574+48u	NLGK+ETCFAEEGKK+48u	1677.8032
	1686.3338			
1695.69501				
	1708.9774			
1742.89583		146-159	HPYFYAPELLFFAK	1742.846
1762.81877				
1798.95955				
	1812.94612	226-240+162u	AEFAEVSKLVTDLTKK+162u	1812.94823
	1828.82736	163-174+349-351+126u	AAFTECCQAADK+AK+126u	1828.833495
		187-190/561-564+546-557+22u	DEGK/ADDK+AVMDDFAAFVEK(1Met-ox)+22u	1828.81895
1848.81111				
	1903.047747	206-212+526-534+48u	FGERAFK+QTALVELVK+48u	1903.058
1909.12529				
1910.93397		485-500	RPCFSALEVDETYPK	1910.9322

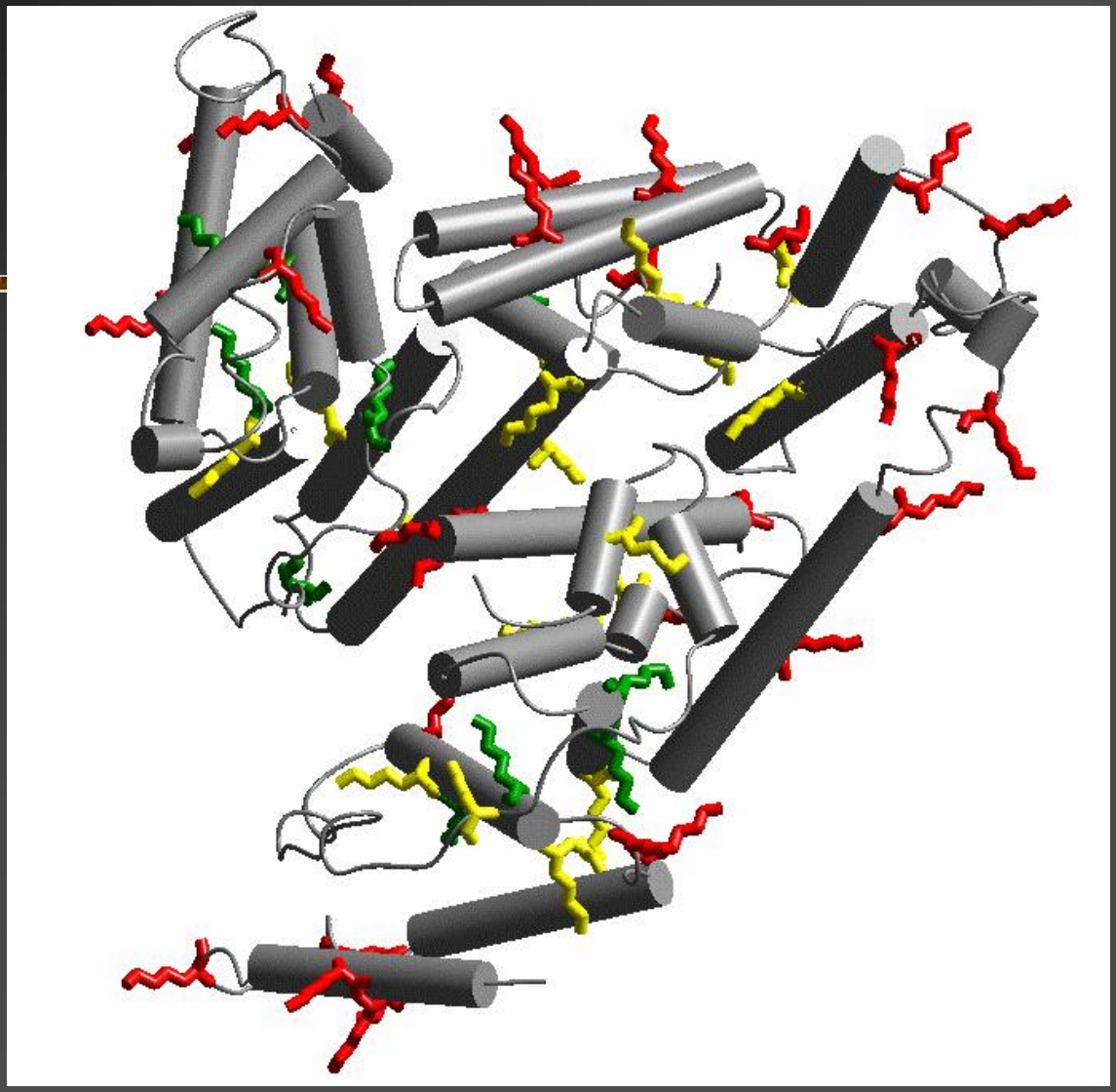


Figure 9. The most solvent -exposed lysine residues color coded according to their range of fractional solvent accessible surfaces (red: 0.5 – 1.0, more exposed; yellow: 0.3 – 0.5, less exposed; green: 0.1 – 0.3, buried).

Conclusions

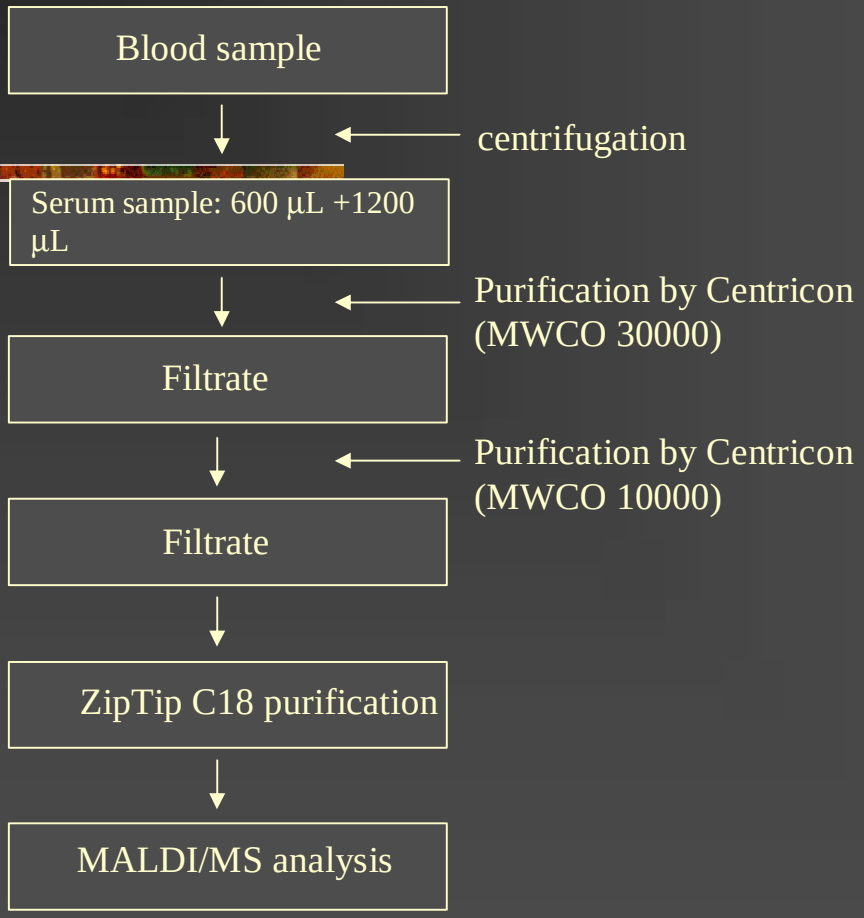
The data obtained from *in vitro* glycation of HSA pointed out these crucial points:

- the enzymatic digestion of glycated protein is more difficult than that of genuine protein;
- glycation can modify the site of enzyme cleavage;
- the presence of a large number of species originating from glycation-induced cross-linking processes;
- the MS/MS spectra of doubly charged glycated peptides are not particularly useful for sequence investigation but allow an unequivocal identification of their chemical nature.

An in vivo investigation on AGE-peptides:

preliminary results

MALDI sample preparation



MALDI conditions:

Ion positive reflectron mode
Grid voltage: 72 %
Extraction delay time: 150 nsec
Acceleration Voltage: 20 kV
Guide wire: 0.05%
Mirror voltage ratio: 1.12

Matrix: α-cyano-4-hydroxycinnamic acid