

# Serum degradation analysis by MALDI/ToF: a new method and tool

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# Introduction

One of the potential risks in a biomarker discovery study is that samples with different preservation time and conditions are compared. For example, pathological serum samples collected over several years and cryo-preserved in biobanks could be compared with control samples recently obtained from healthy donors. The statistical analysis aimed at the identification of differences between the two groups could identify as biomarkers of a pathological condition signals whose difference could only depend on the degradation process of the pathological samples. Pre-analytical variables, like patient conditions, venipuncture details, etc..., may also alter the analysis of blood derived samples. Here, we propose a simple method, mass spectrometry based, able to assess the integrity of serum samples by evaluating their absolute and relative fibrinopeptide contents.

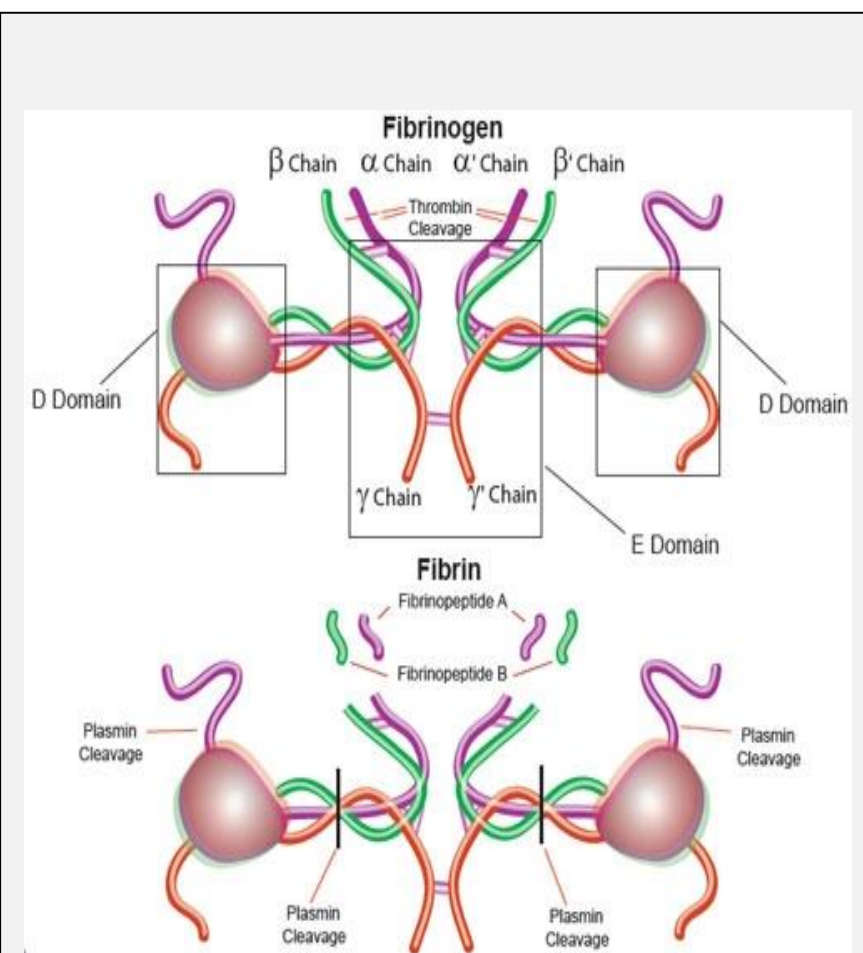


Figure 1: mechanism of  
fibrinogen activation.  
(From Sigma-Aldrich Co. LLC.)

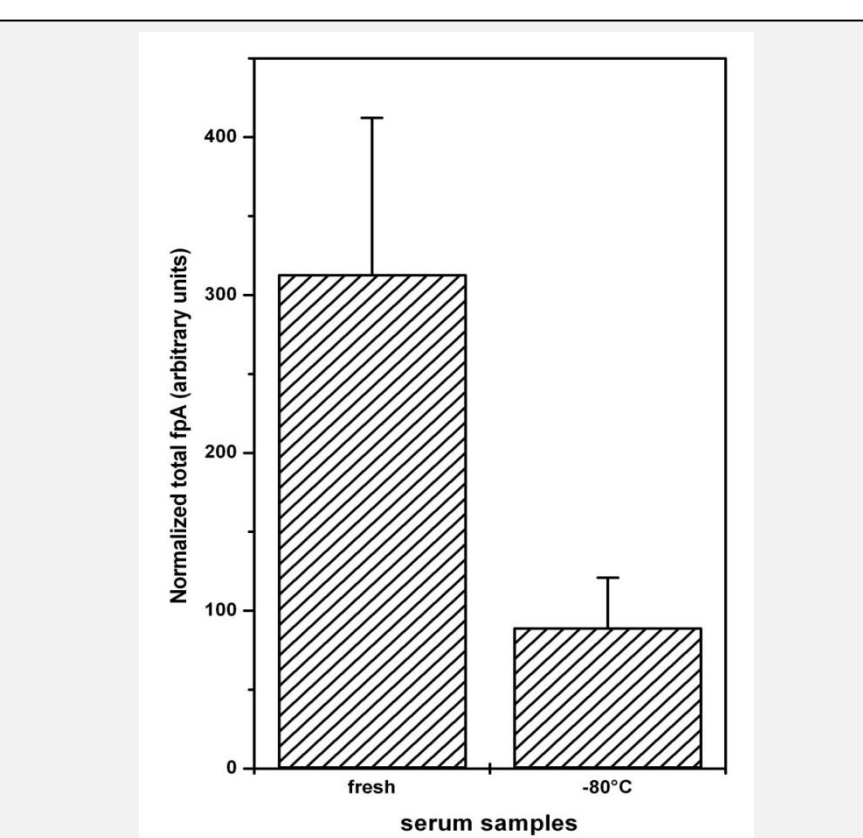


Figure 3: comparison of total fpA abundance in fresh and cryopreserved samples

## FpA degradation

The peptidomic profile of a fresh serum sample is mainly characterized by signals due to fibrinopeptides, generated during the coagulation process (fig. 1), mainly fibrinopeptide A (fpA) and its degradation products (table 1).

A recent study showed a gradual degradation of the fpA molecule, as a result of the preservation process [1]. In the sera stored for 18 months at -80°C, a significant percentage decrease of the most conserved molecules of fpA was observed along with the percentage increase of the most degraded forms. Figure 2 shows the MALDI/ToF spectra of a fresh (panel A) and a poorly preserved (panel B) serum sample. Moreover, also the total amount of fpA, in all its forms, in the cryo-preserved samples is clearly lower than the one calculated for fresh sera (fig. 3). These data are summarized in figure 4 where the percentage of total fpA is plotted against the percentage of high MW fpA for each sample, and in figure 5 which shows the differences, in terms of percentage, between fresh and cryopreserved samples for each fpA form.

The high susceptibility of fpA to degradation suggests a possible use of this molecule as quality indicator of the cryo-preserved serum samples.

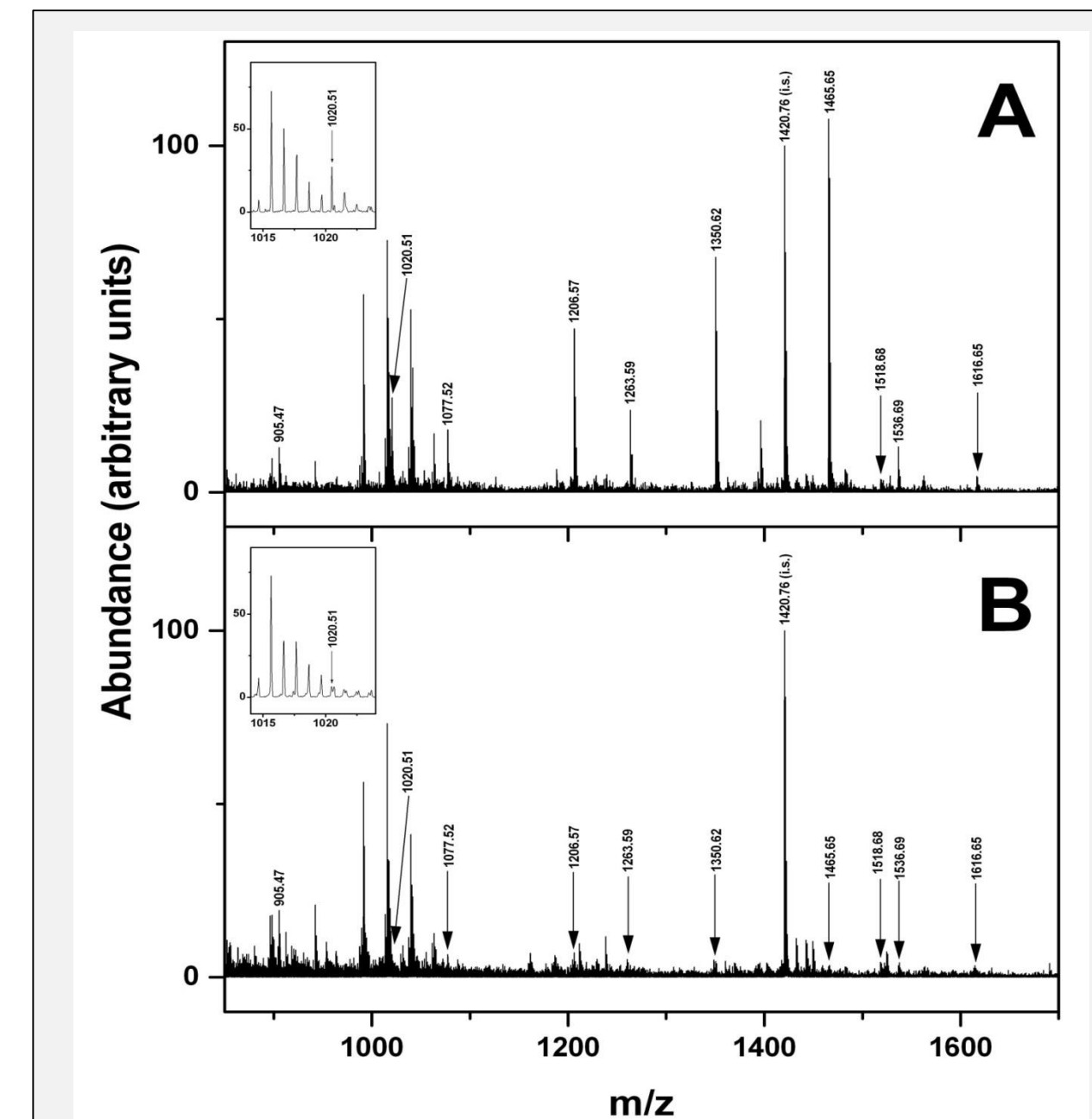


Figure 2: comparison of MALDI/ToF spectra from fresh and cryopreserved samples

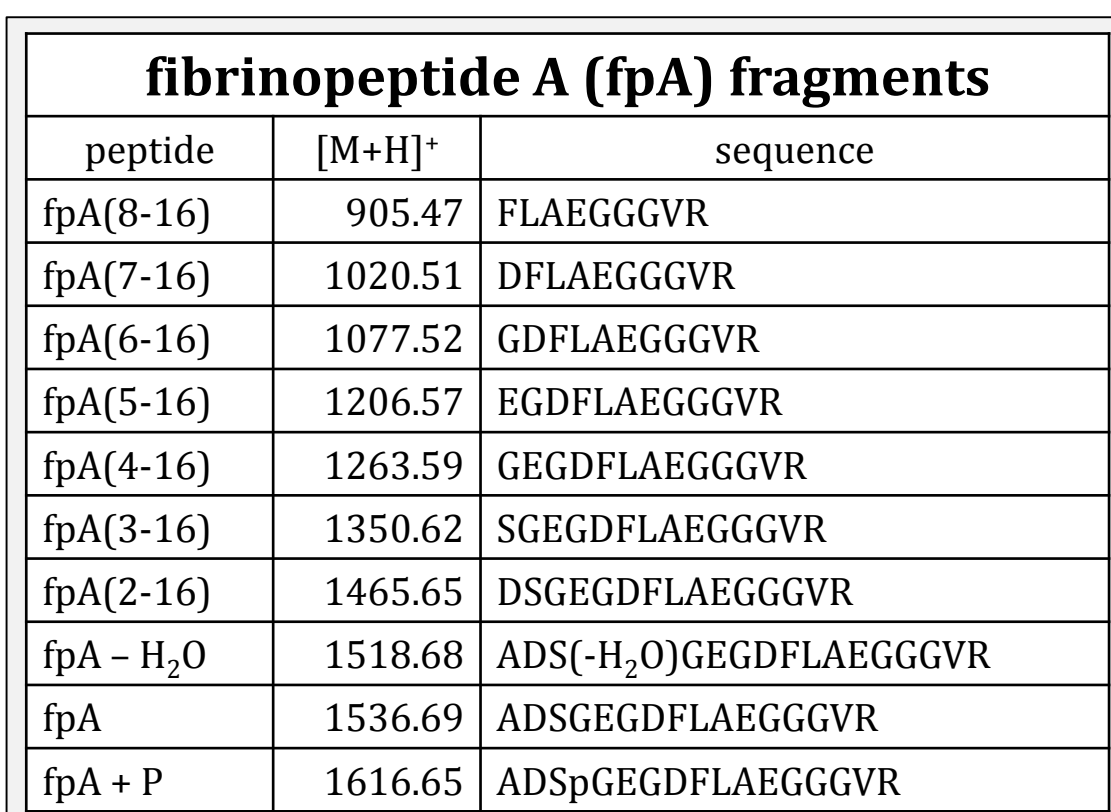


Table 1: fpA peptides

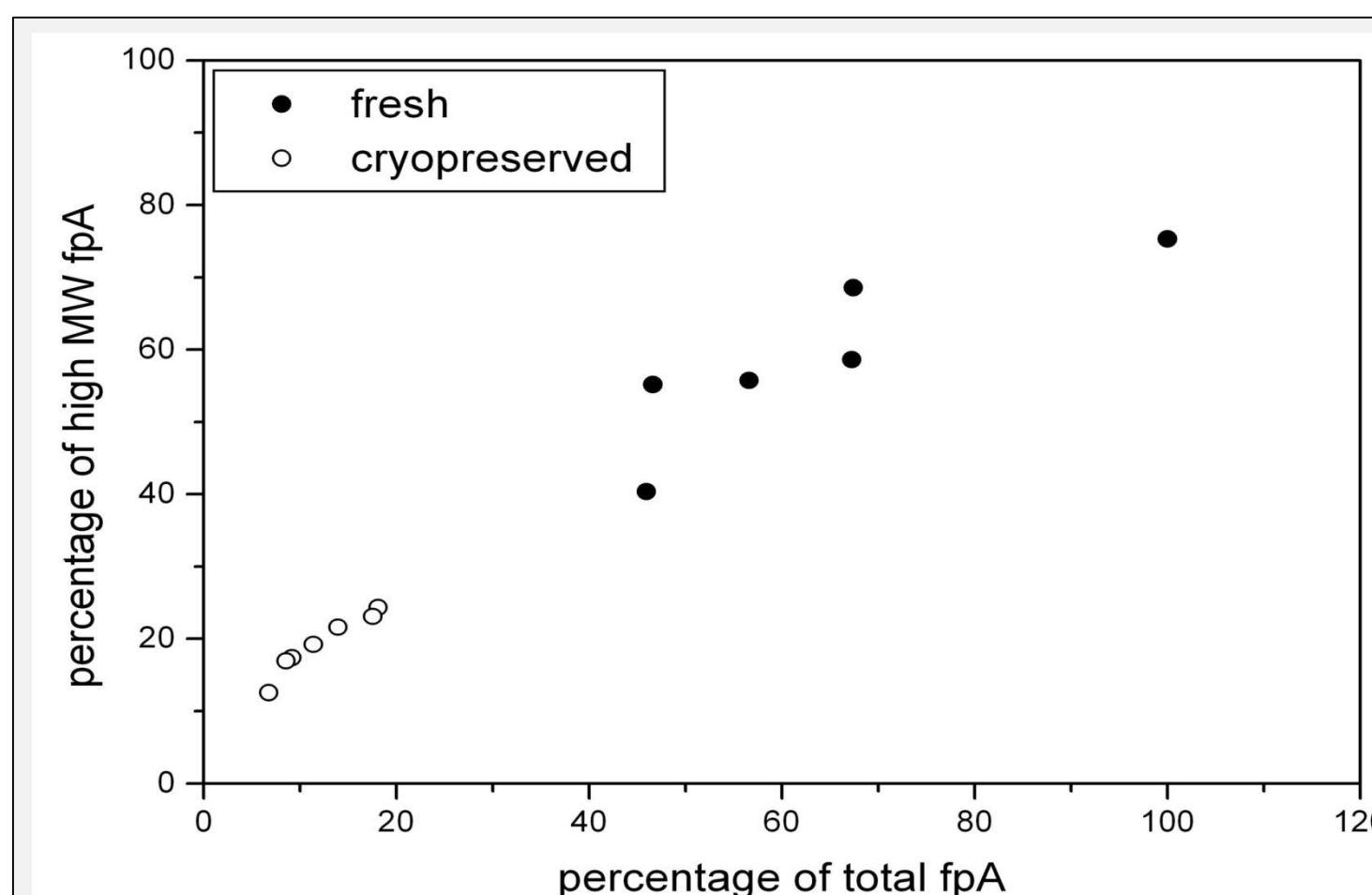


Figure 4: the percentage of total fpA plotted against the percentage of high MW fpA for each sample

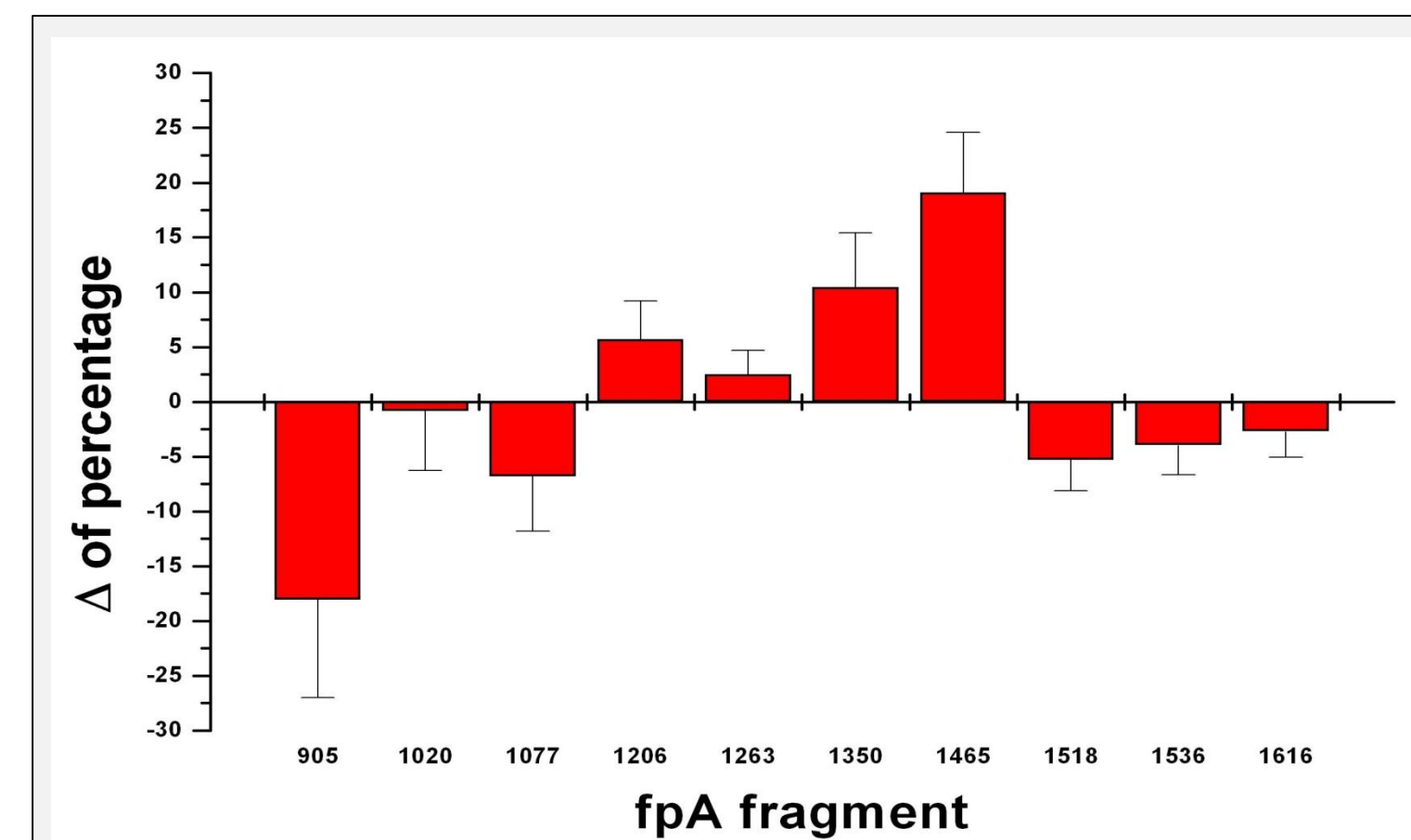


Figure 5: differences of percentage between fresh and cryopreserved samples for each fpA form

## Web based degradation analysis

We developed a web tool to assess the integrity of serum samples by evaluating their fibrinopeptide contents. The tool was built in a LAMP (Linux, Apache, MySQL, PHP) environment and is available on-line to all interested researchers.

The tool processes input spectra to extract peak lists which are then elaborated for noise reduction. FpA peptides are identified and compared in order to compute both their total abundance and the percent contribution of each peptide. If a reference spectrum from a sample of good quality is provided, total abundances are expressed as percent of its total abundance. A qualitative score is assigned to spectra by taking into account both their total abundance and the ratio between abundances of more and less degraded forms.

The screenshot displays the SeraDeg web application interface. At the top, there is a navigation bar with the text "SeraDeg Sera Degradation" and a logo. Below the navigation bar, there is a header section with the text "SeraDeg Sera Degradation Analysis Build April 1, 2017" and a message: "This system is under active development, please forgive us for possible errors and send us your comments, criticisms and congratulations, if any." Below the header, there is a main content area with the title "SeraDeg at once: Upload file and perform analysis". The main content area contains a paragraph: "For repeated analysis with different parameters on the same data, we suggest you to upload the spectra file separately. It will be available for six months. Go to the upload page first and then to the analysis interface later." Below this paragraph, there is a note: "NB! Move your mouse over the icon for a contextual help. Should you have any problem, see the help page." The main content area is divided into two sections: "Job information" and "Input data". The "Job information" section contains a form with the following fields: "Job name:" (with the value "seradeg41983"), "Your email:" (with a placeholder "your email"), and "Country:" (with a placeholder "country"). The "Input data" section contains a form with the following fields: "Archive file:" (with a "Browse..." button and a note "No file selected. (see example file)"), "Reference spectrum name:" (with a placeholder "reference spectrum name"), "Median file parameters:" (with a placeholder "median file parameters"), "Normalization peak:" (with radio buttons for "No" and "Yes" and a value "1420.76 m/z"), and "Half window size: 30" and "Signal / noise ratio: 2.5". At the bottom of the form, there is a "SUBMIT" button.

Figure 6: input form

**SarvaDeg**  
Sarva Degradation Analysis  
Build: Apr 1, 2017

**Input data**  
Job name: saradeg31459  
Run on: Monday 20th of June 2017 19:26:22 AM

**Spectrum under analysis**  
Spectrum label: C15\_C\_35  
FPA peptides overall abundance: 952.93  
Low high mass proportion: 29.99% / 70.01%

**Score**  
Bar chart showing scores for different peptide classes: Low mass peptides (29.99%), High mass peptides (70.01%).

**FFA peptide mass pattern**  
Bar chart showing peptide masses and their relative abundances. The x-axis represents peptide mass (m/z) and the y-axis represents relative abundance (0% to 40%).

**Distribution of peptide abundances**  
Bar chart showing peptide masses and their relative abundances. The x-axis represents peptide mass (m/z) and the y-axis represents relative abundance (0% to 40%).

**MS/MS spectra**  
12 individual MS/MS spectra for different peptide masses: 905.44, 1020.49, 1073.23, 1206.50, 1263.58, 1295.64, 1445.63, 1516.68, 1536.76, 1516.99, 1536.99, 1616.99.

**Peptide**  
Table with 5 columns: Peptide, Group, Reference m/z, Absolute abundance, Percent abundance.

Peptide	Group	Reference m/z	Absolute abundance	Percent abundance
FFA (6-16)	low	905.48	61.75	6.48%
FFA (1-16)	low	1020.49	90.75	9.53%
FFA (1-16)	low	1073.23	57.15	5.99%
FFA (5-16)	none	1206.55	162.23	17.02%
FFA (1-16)	none	1263.58	57.64	6.05%
FFA (1-16)	high	1350.62	202.19	21.22%
FFA (2-16)	high	1445.63	237.27	24.71%
FFA (1-20)	none	1516.68	24.24	2.54%
FFA (1-16)	none	1536.76	9.72	1.02%
FFA - P	none	1616.98	0.03	0.00%

MS/MS values of peptides have been adjusted with reference to the m/z value of the marker, when found.  
MS/MS Abundance of peptides have been normalized on reference to the abundance of the marker, when found.

Figure 7: detailed graphical output

## References

1. Mangerini R, Romano P et al. Anal Biochem 2011, 417:174-81

Color codes (Abundance vs Reference)			Star codes (High mass vs Low mass peptide)			Color codes for values										
★	Abundance > 66.6%		★★★★	High mass > 80%		Value	Low mass peptides abundance values									
★	Abundance > 33.3% and <= 66.6%		★★★★	High mass > 60% and <= 80%		Value	Low mass peptides abundance values									
★	Abundance < 33.3%		★★★★	High mass > 40% and <= 60%		Value	High mass peptides abundance values									
			★★★★	High mass < 40%		Value	Corrected abundance values									
NBI For low abundances, one red star is assigned, independently from the high mass percentage						Value	Whole spectrum related values									
Label	Score	High mass peptides (%)	Overall abundance	Abundance vs reference	Detection	165-46 m/z	162-49 m/z	167-53 m/z	120-52 m/z	120-53 m/z	120-54 m/z	140-53 m/z	155-68 m/z	158-76 m/z	169-83 m/z	
C66_C_SP [Graphs]	★★★★	73.61%	862.39	337.45%	5.40	68.46	68.46	51.1	168.2	51.6	262.2	297.3	24.2	9.7	0.0	
C65_C_SP [Graphs]	★★★★	73.78%	891.29	367.00%	4.84	59.6	73.4	60.3	168.6	61.2	188.1	20.1	8.0	0.0	0.0	
C65_D_SP [Graphs]	★★★★	74.12%	890.49	352.72%	4.15	49.9	71.6	47.0	147.3	58.5	194.3	288.0	23.8	10.2	0.0	
C65_B_SP [Graphs]	★★★★	73.72%	851.88	337.42%	4.25	44.5	69.4	50.6	142.2	62.2	163.5	276.1	24.9	9.0	0.0	
C473_C_SP [Graphs]	★★★★	51.16%	814.49	322.61%	20.89	78.2	133.0	67.7	166.6	50.9	150.0	134.5	26.2	5.3	0.0	
C372_D_SP [Graphs]	★★★★	69.02%	781.20	309.43%	16.61	13.0	30.0	23.6	50.6	51.8	206.2	332.2	33.7	32.6	0.0	
C216_C_SP [Graphs]	★★★★	68.48%	772.76	306.09%	7.85	48.0	97.0	39.5	102.5	45.4	141.2	263.0	24.2	10.4	0.0	
C213_D_SP [Graphs]	★★★★	62.72%	760.21	301.12%	12.02	51.5	100.4	45.9	109.9	52.9	140.5	192.9	19.7	5.2	0.0	
C312_C_SP [Graphs]	★★★★	70.72%	753.00	298.26%	4.73	42.6	81.2	40.4	109.7	51.7	173.5	223.4	20.1	10.4	0.0	
C300_A_SP [Graphs]	★★★	50.29%	174.37	69.07%	17.82	17.5	28.5	15.3	24.4	13.9	26.7	35.3	7.8	5.1	0.0	
C171_C_SP [Graphs]	★★★	61.52%	173.44	68.70%	13.02	9.8	26.4	10.6	33.0	11.0	29.9	44.8	5.3	2.7	0.0	
C214_D_SP [Graphs]	★★★	44.73%	173.00	68.62%	21.98	25.7	32.5	13.2	21.6	10.7	26.5	31.3	7.9	3.6	0.0	
C133_A_SP [Graphs]	★★★	56.79%	170.42	67.50%	13.29	17.3	25.3	11.8	24.1	11.6	31.4	40.2	8.6	0.0	0.0	
C190_B_SP [Graphs]	★★★	76.66%	168.09	66.58%	7.77	7.8	12.9	7.0	18.8	9.9	37.6	64.4	6.7	3.0	0.0	
C204_B_SP [Graphs]	★★★	68.49%	166.79	66.06%	9.07	11.0	20.7	7.8	19.7	9.8	29.1	56.6	9.1	2.9	0.0	
C151_B_SP [Graphs]	★★★	38.00%	166.47	66.94%	26.89	27.2	30.9	14.2	29.9	11.5	22.7	21.6	6.4	2.1	0.0	
C308_C_SP [Graphs]	★★★	52.42%	163.90	64.92%	18.59	13.2	30.7	10.4	30.2	9.6	24.8	35.0	6.6	3.2	0.0	
C143_B_SP [Graphs]	★★★	46.21%	87.19	34.54%	24.69	9.5	23.5	3.9	10.4	3.8	10.3	21.4	3.0	1.4	0.0	
S123237_A_SP [Graphs]	★★★	37.58%	86.98	34.46%	26.37	15.3	14.6	8.4	14.5	4.0	9.5	13.6	4.4	1.6	0.0	
S122033_A_SP [Graphs]	★★★	42.38%	85.22	33.76%	22.99	11.0	16.4	8.0	12.5	4.9	10.8	15.2	3.9	2.6	0.0	
12504621_C_SP [Graphs]	★★★	37.39%	84.90	33.63%	26.54	8.9	15.9	12.5	10.5	10.4	10.0	12.2	0.0	0.0	0.0	
C138_C_SP [Graphs]	★★★	47.63%	83.84	33.21%	23.34	7.1	20.6	5.0	11.6	3.5	8.1	11.5	4.3	2.1	0.0	
S120219_D_SP [Graphs]	★★★	24.26%	82.96	32.86%	36.65	21.1	19.0	8.5	10.6	5.3	6.7	8.8	2.8	0.0	0.0	
S118804_C_SP [Graphs]	★★★	49.65%	82.57	32.70%	23.62	4.2	10.0	10.1	12.6	7.5	8.8	14.3	9.6	5.4	0.0	
S117726_C_SP [Graphs]	★★★	49.68%	81.62	32.33%	21.62	4.2	13.3	9.6	11.1	9.2	8.3	17.2	9.8	4.5	0.0	

Figure 7: list of spectra, ordered by decreasing total abundance, the assigned score and their features