Database of proteinogenic amino acid reference spectra for Bismuth-cluster ToF-SIMS. I. Negative polarity

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The number of time-of-flight secondary ion mass spectrometry studies on biological tissues and cells strongly increased since the development of primary ion sources that allow not only elemental but also molecular analysis. Still, substantial fragmentation during ionic bombardment results in a large amount of peaks, rendering data analysis complex. Complete and trustable sets of reference spectra for the main biological building blocks such as proteins, sugars and lipids are required. Here, the authors provide a database of reference spectra for the 21 proteinogenic amino acids + glycine for bismuth instruments. Paper I of this two-part article series contains the negative polarity spectra and Paper II the positive polarity spectra. These reference spectra were obtained with the primary ion Bi_3^+ and spectra for the primary ions Bi_1^+ and Bi_3^{++} are contained in the supplementary material as ASCII files. Published by the AVS. <https://doi.org/10.1116/1.5024987>

Keywords: ToF-SIMS, amino acid, mass spectrometry, fragmentation

Accession #: 01426, 01427, 01428, 01429, 01430, 01431, 01432, 01433, 01434, 01435, 01436, 01437, 01438, 01439, 01440, 01441, 01442, 01443, 01444, 01445, 01446, 01447

Technique: SIMS

Host Material: Silicon (100) wafer

Instrument: IONTOF TOF-SIMS.5

Major Species in Spectra: C, H, N, O, S

Minor Species in Spectra: Si, Cl

Published Spectra: 22

Spectra in Electronic Record: 66

Published Figures: 44

Spectral Category: Reference

INTRODUCTION

Partial amino acid databases for time-of-flight secondary ion mass spectrometry (ToF-SIMS) reference mass spectra have been already conducted based on the now largely replaced monoatomic argon (Ref. 1) and caesium (Ref. 2) primary ion sources. The characteristic molecular fragmentation in ToF-SIMS measurements, however, depends on the primary ion impacting the surface. The current standard primary ion source is the bismuth/manganese liquid metal ion source. For bismuth and bismuth cluster primary ions, only limited lists attributing prominent peaks to specific amino acids can be found in the literature, e.g., Ref. 3. Here, we provide a database of original reference spectra for the 21 proteinogenic amino acids + glycine as well as the complete corresponding peak lists determining the fragmentation products. Fragments which uniquely represent specific amino acids are highlighted in Table 24. This Paper I of the database contains the negative polarity spectra. Positive polarity spectra are contained in Paper II (Ref. 4).

The amino acids were purchased through Sigma Aldrich (Article number: 09416, purity > 99.0%). Each amino acid was dissolved in H_2O (Sigma Aldrich, Article number: 270733) and 0.1 M solutions were so prepared. Host silicon wafers of $1 \times 1 \text{ cm}$ size were first washed by submersion in subsequent ultrasound baths of $2 \times 15 \text{ min}$ in acetone and $1 \times 15 \text{ min}$ in ethanol, and then plasma-treated for a duration of 10 min to render the surface hydrophilic. The 0.1 M amino acid solutions were subsequently drop-deposited inside a laminar flow chamber on separate silicon wafers. It was made sure that each deposited droplet is spread over the entire wafer surface before leaving the wafers in the laminar flow chamber for drying.

The spectra were obtained on a ToF-SIMS.5 instrument (IONTOF) equipped with a 25 keV bismuth-manganese liquid metal

ion gun. The instrument was operated in high current bunched mode. The primary ion dose was kept below the static limit ($10^{12} \text{ ions/cm}^2$) for each spectrum and a low-energy electron flood gun was used to compensate charging effects. The raster size was $200 \times 200 \mu\text{m}^2$ with a raster resolution of 128×128 , a cycle time of 100 μs , and primary ion currents of 1.54 pA for Bi_1^+ , 0.37–0.4 pA for Bi_3^+ , and 0.44 pA for Bi_3^{++} . Fifty scans were acquired per single mass spectrum. All presented spectra are normalized to their respective total ion count. Note that variations in mass resolution among the reference spectra (see Fig. 23) are due to crystallization geometry differences between the amino acids.

Figures and tables presented contain only attributions for the most prominent peaks. Complete peak-lists attributing fragmentation products to 50–100 main peaks for each amino acid are included in the attachment. In the figures, the full molecule is referred to with “M.” Polymerization products are marked with “p.p.” peaks originating from the substrate with “s.” and contaminants with “c.” Major peaks where a unique attribution was not possible, e.g., due to an overlap beyond mass resolution of two potential fragmentation products, are marked with “n.a.” standing for *not (uniquely) attributed*. In the label of the supplementary material files, the *Spectrum ID#* uniquely identifies each amino acid. Mass spectra acquired using Bi_3^+ , Bi_1^+ , and Bi_3^{++} primary ions are further identified by the file-suffix “01,” “02,” and “03,” respectively, and peak lists in ASCII format by the file-suffix “04.”

SPECIMEN DESCRIPTION (ACCESSION # 01426, 01427, 01428, 01429, 01430, 01431, 01432, 01433, 01434, 01435, 01436, 01437, 01438, 01439, 01440, 01441, 01442, 01443, 01444, 01445, 01446, 01447)

Host Material: Silicon (100) wafer

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CAS Registry #: 7440–21-3

Host Material Characteristics: Homogeneous; solid; single crystal; semiconductor

Chemical Name: L-alanine (01426), L-arginine monohydrochloride (01427), L-asparagine (01428), L-aspartic acid (01429), L-cysteine (01430), L-cystine (01431), L-glutamic acid (01432), L-glutamine (01433), glycine (01434), L-histidine monohydrochloride (01435), L-4-hydroxyproline (01436), L-isoleucine (01437), L-leucine (01438), L-lysine monohydrochloride (01439), L-methionine (01440), L-phenylalanine (01441), L-proline (01442), L-serine (01443), L-threonine (01444), L-tryptophan (01445), L-tyrosine (01446), L-valine (01447)

Source: Sigma Aldrich

Host Composition: Si and SiO₂

Form: Crystalline powder dissolved in DI H₂O and subsequently drop-deposited on the host wafer

Lot Number: L-alanine: BCBS2461V, L-arginine monohydrochloride: 1361811V, L-asparagine: 1371738V, L-aspartic acid: BCBR6815V, L-cysteine: BCBR4500V, L-cystine: BCBS1903V, L-glutamic acid: 1423805V, L-glutamine: BCBR7569V, glycine: 1119375V, L-histidine monohydrochloride: BCBS1876V, L-4-hydroxyproline: BCBL2666V, L-isoleucine: 1423806V, L-leucine: BCBQ9986V, L-lysine monohydrochloride: BCBN9886V, L-methionine: 1423807V, L-phenylalanine: BCBQ1493V, L-proline: BCBP4505V, L-serine: 1336081V, L-threonine: BCBQ4901V, L-tryptophan: BCBR0168V, L-tyrosine: BCBR7417V, L-valine: BCBM0163V

Structure: L-alanine: C₃H₇NO₂, L-arginine monohydrochloride: C₆H₁₄N₄O₂·HCl, L-asparagine: C₄H₈N₂O₃, L-aspartic acid: C₄H₇NO₄, L-cysteine: C₃H₇NO₂S, L-cystine: C₆H₁₂N₂O₄S₂, L-glutamic acid: C₅H₉NO₄, L-glutamine: C₅H₁₀N₂O₃, glycine: C₂H₅NO₂, L-histidine monohydrochloride: C₆H₉N₃O₂·HCl, L-4-hydroxyproline: C₅H₉NO₃, L-isoleucine: C₆H₁₃NO₂, L-leucine: C₆H₁₃NO₂, L-lysine monohydrochloride: C₆H₁₄N₂O₂·HCl, L-methionine: C₅H₁₁NO₂S, L-phenylalanine: C₉H₁₁NO₂, L-proline: C₅H₉NO₂, L-serine: C₃H₇NO₃, L-threonine: C₄H₉NO₃, L-tryptophan: C₁₁H₁₂N₂O₂, L-tyrosine: C₉H₁₁NO₃, L-valine: C₅H₁₁NO₂

History and Significance: The proteinogenic amino acids are the building blocks of all proteins in the known life forms. Complete spectra databases of organic molecules facilitate the interpretation of ToF-SIMS data from biological specimen. However, it should be kept in mind that in biological specimens amino acids are incorporated into larger molecules (proteins, peptidoglycans, etc.), which affects the fragmentation patterns.

As Received Condition: Samples received in powder form

Analyzed Region: Regions of 200 × 200 μm² on maximally homogeneous specimen sites

Ex Situ Preparation/Mounting: 0.1 M amino acid solutions were prepared in H₂O and drop-deposited on separate 1 × 1 cm large plasma-treated Si-wafers in a laminar flow chamber.

In Situ Preparation: None

Charge Control: Low energy electrons

Temp. During Analysis: 300 K

Pressure During Analysis: 5.6 × 10⁻⁸ Pa

Preanalysis Beam Exposure: None

INSTRUMENT CONFIGURATION

Manufacturer and Model: IONTOF TOF-SIMS.5

Analyzer Type: Time-of-flight

Detector Type: Microchannel plate

Experiment Type: Mass spectra

Oxygen Flood Source: None

Other Flood Source: Low energy electrons

Energy Acceptance Window: 20 eV

Postacceleration Voltage: 10 000 eV

Sample Bias: 0 eV

Specimen Normal-to-analyzer (Θ_e): 0°

Ion Sources

Ion Source 1 of 1

Purpose of this Ion Source: Analysis beam

Ion Source Manufacturer: IONTOF (Münster, Germany)

Ion Source Model: 25 keV Bi/Mn cluster source

Beam Mass Filter: Electrodynamic mass filter

Beam Species and Charge State: Bi₃⁺

Beam Gating Used: None

Beam Voltage: 25 000 eV

Net Beam Voltage (impact voltage): 25 000 eV

Ion Pulse Width: 29.3 ns

Ion Pulse Rate: 10 kHz

Pulsed Beam Current: 0.00037–0.0004 nA

Current Measurement Method: Faraday cup

Beam Raster Size: 200 × 200 μm²

Beam Incident Angle: 45°

Source-to-Analyzer Angle: 45°

ACKNOWLEDGMENTS

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REFERENCES

1. Preinstalled database on IONTOF GmbH instruments.
2. N. T. Samuel, M. S. Wagner, K. D. Dornfeld, and D. G. Castner, *Surf. Sci. Spectra* **8**, 163 (2001).
3. M. Urbini, V. Piteto, F. de Notaristefani, F. Scaldaferrì, A. Gasbarrini, and L. Tortora, *Anal. Bioanal. Chem.* **409**, 6097 (2017).
4. M. Kawecki and L. Bernard, *Surf. Sci. Spectra* **25**, 015002 (2018).

ALANINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01426	71.0139	$C_3H_3O_2^-$	Alanine
...	72.0091	$C_2H_2NO_2^-$	...
...	86.0248	$C_3H_4NO_2^-$	...
...	88.0404	$C_3H_6NO_2^-$	...
...	89.0482	$C_3H_7NO_2^-$	...

Accession #	01426-01, 01426-02, 01426-03, 01426-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_3H_4NO_2^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

ARGININE (HYDROCHLORIDE)			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01427	41.0145	CHN_2^-	Arginine
...	58.0411	CH_4N_3^-	...
...	131.0826	$\text{C}_5\text{H}_{11}\text{N}_2\text{O}_2^-$	...
...	156.0779	$\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2^-$	...
...	158.0935	$\text{C}_6\text{H}_{12}\text{N}_3\text{O}_2^-$	...
...	173.1044	$\text{C}_6\text{H}_{13}\text{N}_4\text{O}_2^-$	...

Accession #	01427-01, 01427-02, 01427-03, 01427-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2^-$
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

ASPARAGINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01428	41.9985	CNO ⁻	Asparagine
...	72.0091	C ₂ H ₂ NO ₂ ⁻	...
...	113.0357	C ₄ H ₅ N ₂ O ₂ ⁻	...
...	114.0197	C ₄ H ₄ NO ₃ ⁻	...
...	131.0462	C ₄ H ₇ N ₂ O ₃ ⁻	...

Accession #	01428-01, 01428-02, 01428-03, 01428-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH ⁻ , OH ⁻ , C ₂ H ⁻ , CH ₂ N ⁻ , C ₄ H ₅ N ₂ O ₃ ⁻
Primary Ion Dose	4.73 × 10 ¹¹ cm ⁻²
Primary Species	Bi ₃ ⁺
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	200 × 200 μm ²

ASPARATIC ACID			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01429	98.0248	$C_4H_4NO_2^-$	Asparatic acid
...	88.0404	$C_3H_6NO_2^-$	...
...	115.0037	$C_4H_3O_4^-$	...
...	117.0193	$C_4H_5O_4^-$	...
...	132.0302	$C_4H_6NO_4^-$	...

Accession #	01429-01, 01429-02, 01429-03, 01429-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_4H_7NO_4^-$
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

CYSTEINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01430	31.9726	S^-	Cysteine
...	32.9804	HS^-	...
...	56.9804	C_2HS^-	...
...	57.9757	CNS^-	...
...	72.0091	$C_2H_2NO^-$	...
...	74.0070	$C_2H_4NS^-$	...
...	86.0248	$C_3H_4NO_2^-$	...
...	102.9859	$C_3H_3O_2S^-$	...
...	120.0125	$C_3H_6NO_2S^-$	...

Accession #	01430-01, 01430-02, 01430-03, 01430-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_3H_7SNO_2^-$
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

CYSTINE

Spectrum ID #	Mass (Da)	Species	Peak Assignment
01431	31.9726	S^-	Cystine
...	32.9804	HS^-	...
...	63.9447	S_2^-	...
...	64.9525	HS_2^-	...
...	74.0070	$C_2H_4NS^-$	...
...	104.0176	$C_3H_6NOS^-$	...
...	120.0125	$C_3H_6NO_2S^-$	...
...	151.9845	$C_3H_6NO_2S_2^-$	...
...	239.0166	$C_6H_{11}N_2O_4S_2^-$	...

Accession #	01431-01, 01431-02, 01431-03, 01431-04
Host Material	Silicon wafer
Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_6H_{11}S_2N_2O_4^-$
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$
Comment	Region of interest (ROI), defined by a minimum threshold of the HS_2^- signal, was applied to eliminate the substrate signal.

GLUTAMIC ACID			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01432	59.0139	$C_2H_3O_2^-$	Glutamic acid
...	71.0139	$C_3H_3O_2^-$	...
...	72.0091	$C_2H_2NO_2^-$	...
...	74.0248	$C_2H_4NO_2^-$	...
...	85.0295	$C_4H_5O_2^-$	...
...	100.0404	$C_4H_6NO_2^-$	...
...	102.0560	$C_4H_8NO_2^-$	...
...	128.0353	$C_5H_6NO_3^-$	...
...	131.0350	$C_5H_7O_4^-$	...
...	146.0459	$C_5H_8NO_4^-$	...

Accession #	01432-01, 01432-02, 01432-03, 01432-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_5H_9NO_4^-$
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

GLUTAMINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01433	41.9985	CNO ⁻	Glutamine
...	58.0298	C ₂ H ₄ NO ⁻	...
...	72.0091	C ₂ H ₂ NO ₂ ⁻	...
...	74.0248	C ₂ H ₄ NO ₂ ⁻	...
...	82.0298	C ₄ H ₄ NO ⁻	...
...	99.0564	C ₄ H ₇ N ₂ O ⁻	...
...	128.0353	C ₅ H ₆ NO ₃ ⁻	...
...	145.0619	C ₅ H ₉ N ₂ O ₃ ⁻	...

Accession #	01433-01, 01433-02, 01433-03, 01433-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH ⁻ , OH ⁻ , C ₂ H ⁻ , CH ₂ N ⁻ , C ₅ H ₇ N ₂ O ₃ ⁻
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi ₃ ⁺
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	200 × 200 μm ²

GLYCINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01434	58.0060	$C_2H_2O_2^-$	Glycine
...	72.0091	$C_2H_2NO_2^-$	...
...	74.0248	$C_2H_4NO_2^-$	...

Accession #	01434-01, 01434-02, 01434-03, 01434-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH ⁻ , OH ⁻ , C ₂ H ⁻ , CH ₂ N ⁻ , C ₂ H ₅ NO ₂ ⁻
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi ₃ ⁺
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	200 × 200 μm ²

HISTIDINE (HYDROCHLORIDE)			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01435	50.0036	C_3N^-	Histidine
...	65.0145	$C_3HN_2^-$	...
...	80.0380	$C_4H_4N_2^-$	...
...	81.0458	$C_4H_5N_2^-$	...
...	93.0458	$C_5H_5N_2^-$	...
...	104.0254	$C_5H_2N_3^-$	...
...	108.0567	$C_5H_6N_3^-$	...
...	137.0357	$C_6H_5N_2O_2^-$	...
...	154.0622	$C_6H_8N_3O_2^-$	...

Accession #	01435-01, 01435-02, 01435-03, 01435-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_6H_9N_3O_2^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

HYDROXYPROLINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01436	65.9985	C_3NO^-	Hydroxyproline
...	66.0349	$C_4H_4N^-$	...
...	71.0139	$C_3H_3O_2^-$	...
...	72.0091	$C_2H_2NO_2^-$	...
...	82.0298	$C_4H_4NO^-$	...
...	86.0248	$C_3H_4NO_2^-$	...
...	99.0088	$C_4H_3O_3^-$	...
...	110.0248	$C_5H_4NO_2^-$	...
...	128.0353	$C_5H_6NO_3^-$	...
...	130.0510	$C_5H_8NO_3^-$	...

Accession #	01436-01, 01436-02, 01436-03, 01436-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , O^- , C_2H^- , CH_2N^- , $C_5H_9NO_3^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

ISOLEUCINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01437	72.0091	$C_2H_2NO_2^-$	Isoleucine
...	85.0169	$C_3H_3NO_2^-$	...
...	100.0404	$C_4H_6NO_2^-$	...
...	114.0560	$C_5H_8NO_2^-$	...
...	128.0717	$C_6H_{10}NO_2^-$	...
...	130.0874	$C_6H_{12}NO_2^-$	...

Accession #	01437-01, 01437-02, 01437-03, 01437-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , O^- , C_2H^- , CH_2N^- , $C_6H_{13}NO_2^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

LEUCINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01438	72.0091	$C_2H_2NO_2^-$	Leucine
...	86.0248	$C_3H_4NO_2^-$	...
...	114.0560	$C_5H_8NO_2^-$	...
...	128.0717	$C_6H_{10}NO_2^-$	...
...	130.0874	$C_6H_{12}NO_2^-$	...

Accession #	01438-01, 01438-02, 01438-03, 01438-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_6H_{10}NO_2^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

LYSINE (HYDROCHLORIDE)			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01439	71.0139	$C_3H_3O_2^-$	Lysine
...	72.0091	$C_2H_2NO_2^-$	...
...	86.0248	$C_3H_4NO_2^-$	...
...	128.0717	$C_6H_{10}NO_2^-$	...
...	130.0874	$C_6H_{12}NO_2^-$	...
...	145.0983	$C_6H_{13}N_2O_2^-$	...

Accession #	01439-01, 01439-02, 01439-03, 01439-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_6H_{14}N_2O_2^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

METHIONINE

Spectrum ID #	Mass (Da)	Species	Peak Assignment
01440	31.9726	S^-	Methionine
...	32.9804	HS^-	...
...	46.9961	CH_3S^-	...
...	57.9757	CNS^-	...
...	62.9910	CH_3OS^-	...
...	72.0091	$C_2H_2NO_2^-$	...
...	73.0169	$C_2H_3NO_2^-$	...
...	98.0248	$C_4H_4NO_2^-$	...
...	100.0404	$C_4H_6NO_2^-$	...
...	114.9859	$C_4H_3O_2S^-$	...
...	134.0281	$C_4H_8NO_2S^-$	...
...	148.0438	$C_5H_{10}NO_2S^-$	...
...	164.0387	$C_5H_{10}NO_3S^-$	...

Accession #	01440-01, 01440-02, 01440-03, 01440-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , C_2H^- , CH_2N^- , $C_5H_8NO_2S^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

PHENYLALANINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01441	49.0084	C_4H^-	Phenylalanine
...	72.0091	$C_2H_2NO_2^-$	...
...	73.0084	C_6H^-	...
...	91.0553	$C_7H_7^-$	...
...	97.0084	C_8H^-	...
...	103.0553	$C_8H_7^-$	...
...	118.0662	$C_8H_8N^-$	...
...	147.0452	$C_9H_7O_2^-$	...
...	164.0717	$C_9H_{10}NO_2^-$	...

Accession #	01441-01, 01441-02, 01441-03, 01441-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_9H_{11}NO_2^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

PROLINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01442	66.0349	$C_4H_4N^-$	Proline
...	71.0139	$C_3H_3O_2^-$	...
...	72.0091	$C_2H_2NO_2^-$	...
...	112.0404	$C_5H_6NO_2^-$	...
...	114.0560	$C_5H_8NO_2^-$	...

Accession #	01442-01, 01442-02, 01442-03, 01442-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , C_2^- , CH_2N^- , $C_5H_6NO_2^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

SERINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01443	59.0139	$C_2H_3O_2^-$	Serine
...	71.0139	$C_3H_3O_2^-$	...
...	74.0248	$C_2H_4NO_2^-$	...
...	87.0088	$C_3H_3O_3^-$	...
...	104.0353	$C_3H_6NO_3^-$	...

Accession #	01443-01, 01443-02, 01443-03, 01443-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , O^- , C_2H^- , CH_2N^- , $C_3H_7NO_3^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

THREONINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01444	72.0091	$C_2H_2NO_2^-$	Threonine
...	74.0248	$C_2H_4NO_2^-$	...
...	118.0510	$C_4H_8NO_3^-$	...

Accession #	01444-01, 01444-02, 01444-03, 01444-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , O^- , C_2H^- , CH_2N^- , $C_4H_9NO_3^-$
Primary Ion Dose	$5.11 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.0004 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

TRYPTOPHAN			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01445	49.0084	C_4H^-	Tryptophan
...	108.0455	$C_6H_6NO^-$	...
...	116.0506	$C_8H_6N^-$	...
...	136.0530	$C_8H_8O_2^-$	...
...	142.0662	$C_{10}H_8N^-$	...
...	157.0771	$C_{10}H_9N_2^-$	...
...	162.0686	$C_{10}H_{10}O_2^-$	...
...	203.0826	$C_{11}H_{11}N_2O_2^-$	...

Accession #	01445-01, 01445-02, 01445-03, 01445-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_{11}H_{12}N_2O_2^-$
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

TYROSINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01446	41.9985	CNO ⁻	Tyrosine
...	93.0346	C ₆ H ₅ O ⁻	...
...	119.0502	C ₈ H ₇ O ⁻	...
...	134.0611	C ₈ H ₈ NO ⁻	...
...	163.0401	C ₉ H ₇ O ₃ ⁻	...
...	180.0666	C ₉ H ₁₀ NO ₃ ⁻	...

Accession #	01446-01, 01446-02, 01446-03, 01446-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH ⁻ , OH ⁻ , C ₂ H ⁻ , CH ₂ N ⁻ , C ₉ H ₁₀ NO ₃ ⁻
Primary Ion Dose	4.73 × 10 ¹¹ cm ⁻²
Primary Species	Bi ₃ ⁺
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	200 × 200 μm ²
Comment	ROI, defined by a minimum threshold of the C ₉ H ₁₀ NO ₃ ⁻ signal, was applied to reduce the substrate signal.

VALINE			
Spectrum ID #	Mass (Da)	Species	Peak Assignment
01447	72.0091	$C_2H_2NO_2^-$	Valine
...	85.0295	$C_4H_5O_2^-$	...
...	100.0404	$C_4H_6NO_2^-$	...
...	116.0717	$C_5H_{10}NO_2^-$	...

Accession #	01447-01, 01447-02, 01447-03, 01447-04
■ Host Material	Silicon wafer
■ Technique	SIMS
Secondary Source Polarity	Negative
Mass Range Minimum	0 Da
Mass Range Maximum	250 Da
Species Used for Mass Calibration	CH^- , OH^- , C_2H^- , CH_2N^- , $C_5H_{11}NO_2^-$
Primary Ion Dose	$4.73 \times 10^{11} \text{ cm}^{-2}$
Primary Species	Bi_3^+
Primary Ion Pulse Width	29.3 ns
Pulsed Beam Current	0.00037 nA
Beam Raster Size	$200 \times 200 \mu\text{m}^2$

EXPANDED $[M-H]^-$ PEAKS FOR EACH AMINO ACID SPECTRUM

Accession #

01426-01, 01427-01, 01428-01, 01429-01, 01430-01,
 01431-01, 01432-01, 01433-01, 01434-01, 01435-01,
 01436-01, 01437-01, 01438-01, 01439-01, 01440-01, 01441-01,
 01442-01, 01443-01, 01444-01, 01445-01, 01446-01, 01447-01

SPECTRAL FEATURES TABLE

Mass (Da)	Peak assignment	Peak present in following amino acids	Peak most prominent in
26.0036	CN ⁻	all amino acids	—
31.9726	S ⁻	cysteine, cystine, methionine	cysteine, cystine, methionine
32.9804	HS ⁻	cysteine, cystine, methionine	cysteine, cystine, methionine
41.0145	CHN ₂ ⁻	arginine, Histidine	arginine
41.9985	CNO ⁻	all amino acids	—
42.0111	C ₂ H ₂ O ⁻	all amino acids	—
42.0349	C ₂ H ₄ N ⁻	all amino acids	—
44.9982	CHO ₂ ⁻	all amino acids	—
46.9961	CH ₃ S ⁻	cysteine, cystine, methionine	methionine
49.0084	C ₄ H ⁻	most amino acids	—
50.0036	C ₃ N ⁻	all amino acids but glycine	—
56.9804	C ₂ HS ⁻	cysteine, cystine, methionine	cysteine, cystine, methionine
57.9757	CNS ⁻	cysteine, cystine, methionine	cysteine, cystine, methionine
58.0060	C ₂ H ₂ O ₂ ⁻	all amino acids	—
58.0298	C ₂ H ₄ NO ⁻	all amino acids	—
58.0411	CH ₄ N ₃ ⁻	arginine	arginine
59.0139	C ₂ H ₃ O ₂ ⁻	all amino acids	—
62.9910	CH ₃ OS ⁻	theoretically none	methionine
63.9447	S ₂ ⁻	cystine	cystine
64.9525	HS ₂ ⁻	cystine	cystine
65.0145	C ₃ HN ₂ ⁻	multiple amino acids	histidine
65.9985	C ₃ NO ⁻	all amino acids	—
66.0349	C ₄ H ₄ N ⁻	multiple amino acids	hydroxyproline, proline
71.0139	C ₃ H ₃ O ₂ ⁻	all amino acids but glycine	—
72.0091	C ₂ H ₂ NO ₂ ⁻	all amino acids	—
73.0084	C ₆ H ⁻	some heavier aminoacids	—
73.0169	C ₂ H ₃ NO ₂ ⁻	all amino acids	—
74.0070	C ₂ H ₄ NS ⁻	cysteine, cystine, methionine	cysteine, cystine
74.0248	C ₂ H ₄ NO ₂ ⁻	all amino acids	—
80.0380	C ₄ H ₄ N ₂ ⁻	histidine (and negligibly in some others)	histidine
81.0458	C ₄ H ₅ N ₂ ⁻	asparagine, glutamine, histidine	histidine
82.0298	C ₄ H ₄ NO ⁻	multiple amino acids	—
85.0169	C ₃ H ₃ NO ₂ ⁻	multiple amino acids	—
85.0295	C ₄ H ₅ O ₂ ⁻	multiple amino acids	—
86.0248	C ₃ H ₄ NO ₂ ⁻	multiple amino acids	—
87.0088	C ₃ H ₃ O ₃ ⁻	hydroxyproline, serine, threonine	serine
88.0404	C ₃ H ₆ NO ₂ ⁻	multiple amino acids	—
89.0482	C ₃ H ₇ NO ₂ ⁻	multiple amino acids	—
91.0553	C ₇ H ₇ ⁻	phenylalanine, tryptophan, tyrosine	phenylalanine
93.0346	C ₆ H ₅ O ⁻	multiple amino acids	—
93.0458	C ₅ H ₅ N ₂ ⁻	multiple amino acids	histidine
97.0084	C ₈ H ⁻	phenylalanine, tryptophan, tyrosine	phenylalanine, tryptophan
98.0248	C ₄ H ₄ NO ₂ ⁻	multiple amino acids	—
99.0088	C ₄ H ₃ O ₃ ⁻	asparagine, asparatic acid, hydroxyproline, threonine	—

Mass (Da)	Peak assignment	Peak present in following amino acids	Peak most prominent in
99.0564	C ₄ H ₇ N ₂ O ⁻	multiple amino acids	—
100.0404	C ₄ H ₆ NO ₂ ⁻	multiple amino acids	—
102.0560	C ₄ H ₈ NO ₂ ⁻	multiple amino acids	—
102.9859	C ₃ H ₃ O ₂ S ⁻	cysteine, cystine	cysteine
103.0553	C ₈ H ₇ ⁻	phenylalanine, tryptophan, tyrosine	phenylalanine
104.0176	C ₃ H ₆ NOS ⁻	cysteine, cystine	cystine
104.0254	C ₅ H ₂ N ₃ ⁻	histidine (and negligibly in some others)	histidine
104.0353	C ₃ H ₆ NO ₃ ⁻	hydroxyproline, serine, threonine	serine
108.0455	C ₆ H ₆ NO ⁻	multiple amino acids	—
108.0567	C ₅ H ₆ N ₃ ⁻	histidine	histidine
110.0248	C ₅ H ₄ NO ₂ ⁻	multiple amino acids	—
112.0404	C ₅ H ₆ NO ₂ ⁻	multiple amino acids	—
113.0357	C ₄ H ₅ N ₂ O ₂ ⁻	glutamic acid, glutamine, asparagine, histidine	asparagine, histidine
114.0197	C ₄ H ₄ NO ₃ ⁻	asparagine, aspartic acid, hydroxyproline, threonine	asparagine
114.0560	C ₅ H ₈ NO ₂ ⁻	multiple amino acids	isoleucine, leucine, proline
114.9859	C ₄ H ₃ O ₂ S ⁻	methionine	methionine
115.0037	C ₄ H ₃ O ₄ ⁻	aspartic acid	aspartic acid
116.0506	C ₈ H ₆ N ⁻	phenylalanine, tryptophan	tryptophan
116.0717	C ₅ H ₁₀ NO ₂ ⁻	valine	valine
117.0193	C ₄ H ₅ O ₄ ⁻	aspartic acid	aspartic acid
118.0510	C ₄ H ₈ NO ₃ ⁻	asparagine, aspartic acid, hydroxyproline, threonine	threonine
118.0662	C ₈ H ₈ N ⁻	phenylalanine	phenylalanine
119.0502	C ₈ H ₇ O ⁻	tyrosine	tyrosine
120.0125	C ₃ H ₆ NO ₂ S ⁻	cysteine, cystine	cysteine, cystine
128.0353	C ₅ H ₆ NO ₃ ⁻	glutamine, glutamic acid, hydroxyprolin	glutamine, glutamic acid, hydroxyprolin
128.0717	C ₆ H ₁₀ NO ₂ ⁻	isoleucine, leucine, lysine	isoleucine, leucine, lysine
130.0510	C ₅ H ₈ NO ₃ ⁻	glutamic acid, glutamine, hydroxyproline, proline	hydroxyproline
130.0874	C ₆ H ₁₂ NO ₂ ⁻	isoleucine, leucine, lysine	isoleucine, leucine, lysine
131.0350	C ₅ H ₇ O ₄ ⁻	glutamic acid	glutamic acid
131.0462	C ₄ H ₇ N ₂ O ₃ ⁻	asparagine	asparagine
131.0826	C ₅ H ₁₁ N ₂ O ₂ ⁻	arginine	arginine
132.0302	C ₄ H ₆ NO ₄ ⁻	aspartic acid	aspartic acid
134.0281	C ₄ H ₈ NO ₂ S ⁻	methionine	methionine
134.0611	C ₈ H ₈ NO ⁻	tyrosine	tyrosine
136.0530	C ₈ H ₈ O ₂ ⁻	tryptophan	tryptophan
137.0357	C ₆ H ₅ N ₂ O ₂ ⁻	histidine	histidine
142.0662	C ₁₀ H ₈ N ⁻	tryptophan	tryptophan
145.0619	C ₅ H ₉ N ₂ O ₃ ⁻	glutamine	glutamine
145.0983	C ₆ H ₁₃ N ₂ O ₂ ⁻	lysine	lysine
146.0459	C ₅ H ₈ NO ₄ ⁻	glutamic acid	glutamic acid
147.0452	C ₉ H ₇ O ₂ ⁻	phenylalanine	phenylalanine
148.0438	C ₅ H ₁₀ NO ₂ S ⁻	methionine	methionine
151.9845	C ₃ H ₆ NO ₂ S ₂ ⁻	cystine	cystine
154.0622	C ₆ H ₈ N ₃ O ₂ ⁻	histidine	histidine
156.0779	C ₆ H ₁₀ N ₃ O ₂ ⁻	arginine	arginine
157.0771	C ₁₀ H ₉ N ₂ ⁻	tryptophan	tryptophan

Mass (Da)	Peak assignment	Peak present in following amino acids	Peak most prominent in
158.0935	$\text{C}_6\text{H}_{12}\text{N}_3\text{O}_2^-$	arginine	arginine
162.0686	$\text{C}_{10}\text{H}_{10}\text{O}_2^-$	tryptophan	tryptophan
163.0401	$\text{C}_9\text{H}_7\text{O}_3^-$	tyrosine	tyrosine
164.0387	$\text{C}_5\text{H}_{10}\text{NO}_3\text{S}^-$	methionine	methionine
164.0717	$\text{C}_9\text{H}_{10}\text{NO}_2^-$	phenylalanine	phenylalanine
173.1044	$\text{C}_6\text{H}_{13}\text{N}_4\text{O}_2^-$	arginine	arginine
180.0666	$\text{C}_9\text{H}_{10}\text{NO}_3^-$	tyrosine	tyrosine
203.0826	$\text{C}_{11}\text{H}_{11}\text{N}_2\text{O}_2^-$	tryptophan	tryptophan
239.0166	$\text{C}_6\text{H}_{11}\text{N}_2\text{O}_4\text{S}_2^-$	cystine	cystine