Comparative Analysis of Fibronectin Using *In Situ* ToF-SIMS, SPI-MS, and dropDESI-MS in a Microfluidic Reactor

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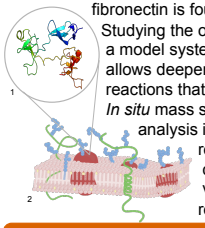
Introduction

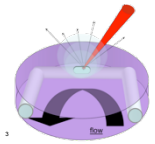
Fibronectin is a protein within the extracellular matrix of animal cells. It is an important biomolecule due to its role in cell differentiation, growth, kinesis and adhesion. Biological responses as such occur within aqueous environments and are mediated through membrane recognition and signaling; where fibronectin is found to play a role.

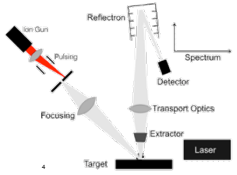


Studying the outer molecular surface of fibronectin, a model system for proteins, in aqueous solution allows deeper insight into the micro-biochemical reactions that occur during these processes. *In situ* mass spectrometry (MS) comparative analysis in aqueous solution accurately represents fibronectin's chemical components, made possible by the vacuum compatible microfluidic reactor.

Experimental Design

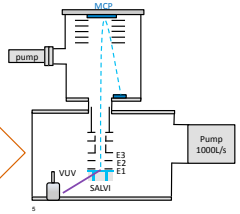
System for Analysis at the Liquid Vacuum Interface (SALVI). PDMS microfluidic block placed in vacuum chamber enabling *in situ* liquid MS.
- Microfluidic channel
- Aperture for direct liquid probing



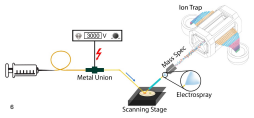


Time-of-flight secondary ion mass spectrometer (ToF-SIMS)
- Interface surface technique
- Bismuth liquid metal ion beam
- Monitors positive and negative emitted ions

Advanced light source single photon ionization mass spectrometer (ALS SPI-MS)
- Interface surface technique
- Synchroton vacuum ultraviolet (VUV) photon beam
- Determines appearance energy (AE)



Drop desorption electrospray ionization mass spectrometer (dropDESI-MS)
- Ambient conditions
- Electrode spray ion source
- Capillary generates charged micro droplets



Results

ToF-SIMS

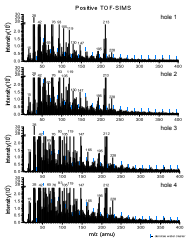


Table 1. Peak identification from the ToF-SIMS positive spectra m/z (mass-to-charge ratio) values.

m/z	amino acid fragment	amino acid
30	CH_4N^+	glycine
43	$CH_3N_2^+$	arginine
44	$C_2H_6N^+$	alanine
45	CHO_2^-	cysteine
56	$C_3H_6N^+$	lysine
59	$CH_3N_2^+$	arginine
60	$C_2H_6NO^-$	serine
61	$C_2H_5S^-$	methionine
68	$C_4H_6N^+$	proline
69	$C_4H_5O^-$	threonine
70	$C_4H_8N^+$	proline
71	$C_3H_3O_2^-$	serine
72	$C_4H_{10}N^+$	valine
73	$C_2H_5N_2^-$	arginine
74	$C_3H_8NO^+$	threonine
76	$C_2H_6NS^-$	cysteine
80	$C_5H_6N^+$	proline
81	$C_4H_5N_2^+$	histidine
82	$C_4H_8N_2^+$	histidine
83	$C_5H_7O^+$	valine
84	$C_5H_{10}N^+$	lysine

m/z	amino acid fragment	amino acid
86	$C_5H_{12}N^+$	leucine
87	$C_3H_3N_2O^+$	asparagine
88	$C_3H_6NO_2^-$	aspartic acid
98	$C_4H_4NO_2^-$	asparagine
100	$C_5H_{14}N_2^+$	arginine
101	$C_5H_{13}N_2^+$	arginine
102	$C_4H_8NO_2^-$	glutamic acid
104	$C_3H_{10}NS^+$	methionine
107	$C_7H_7O^+$	tyrosine
110	$C_6H_8N_2^+$	histidine
112	$C_5H_{14}N_2^+$	arginine
117	$C_5H_9O^+$	methionine
120	$C_9H_{10}N^+$	phenylalanine
130	$C_{11}H_8N^+$	tryptophan
131	$C_9H_7O^+$	phenylalanine
132	$C_8H_6O^+$	phenylalanine
136	$C_8H_{10}NO^+$	tyrosine
147	$C_9H_7O_2^+$	tyrosine
159	$C_{11}H_{11}N_2^+$	tryptophan
170	$C_{10}H_8NO^+$	tryptophan

SPI-MS

The m/z spectral plot for photoionization efficiencies (PIE) from 8.0 to 11.0 eV with a step size of 0.1 eV.

Table 2. Possible peak identification from the SPI-MS positive spectra.

m/z	amino acid fragment	amino acid [1,2,3,4]
14	NH^+	ammonium
41	$C_2H_3N^+$	alanine fragment
56	$C_2H_6NO^+$	amino acid fragment
80	$C_4H_6N_2^+$	histidine fragment
101	$C_4H_8NO_2^+$	glutamic acid
148	$C_5H_{14}NO^+$	methionine-ammonium
172	$C_{11}H_{14}N_2^+$	tryptophan fragment
218	$C_9H_{11}N_2O_3^+$	Gln-Ala
278	$C_{10}H_{23}N_2SO_3^+$	Gln-Met
292	$C_{15}H_{18}NO_3^+$	Tyr-Leu
336	$C_{15}H_{18}N_3O_3^+$	Tyr-Arg
353	$C_{14}H_{17}N_3SO_4^+$	Cys-Tyr-Asp immonium
368	$C_9H_{21}Si_3O_3^+$	PDMS interference (R)
429	$C_{18}H_{29}N_4O_6^+$	Lys-Ile-Gly-Asp
433	$C_{19}H_{29}N_2S_2O_6^+$	Cys-Val-Cys-Leu
505	$C_{21}H_{29}N_6SO_5^+$	Asn-Arg-Cys-Asn
580	$C_{23}H_{34}N_5SO_{7}^+$	Cys-Val-Thr-Asp-Ser-Gly
595	$C_{26}H_{34}N_4O_8^+$	Ser-Tyr-Arg-Ile-Gly
656	$C_{27}H_{38}N_7O_{10}^+$	Glu-Ser-Lys-Pro-Glu-Ala
732	$C_{34}H_{46}N_{10}O_{11}^+$	Tyr-Asp-Asn-Gly-Lys-His
808	$C_{34}H_{53}N_8O_{12}^+$	Gly-Thr-Ser-Tyr-Val-Val-Gly-Glu
884	$C_{37}H_{57}N_9S_2O_{13}^+$	Trp-Met-Met-Val-Asp-Cys-Thr

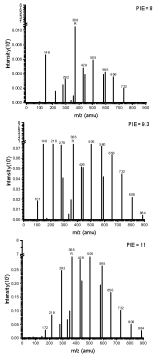
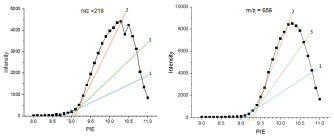


Table 3. Summary of Estimated AE Values

m/z	AE (eV)
56	9.7+/-0.1
101	8.5+/-0.1
172	9.8+/-0.1
218	8.9+/-0.1
278	8.7+/-0.1
292	9.2+/-0.1
353	9.2+/-0.1
429	9.2+/-0.1
433	9.3+/-0.1
505	9.2+/-0.1
580	9.2+/-0.1
595	9.2+/-0.1
656	9.2+/-0.1
732	9.1+/-0.1
808	9.1+/-0.1
884	9.1+/-0.1

Estimated appearance energies (AE) for selected m/z values computed. Linear regression analysis was also used. The three colored lines are 1) the onset AE value limit; 2) the extreme PIE value limit; and (3) the average between lines 1 and 2 which estimate the average AE.



dropDESI-MS

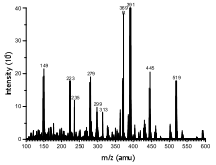


Table 4. Possible peak identification from the drop DESI-MS positive spectra.

m/z	amino acid fragment	amino acid [1,2,3,4]
149	$C_5H_{13}N_2O^+$	methionine-ammonium
223	$C_9H_{11}N_2O_3^+$	Tyr-Leu
235	$C_{10}H_{11}N_2O_3$	Asn-Tyr
279	$C_{10}H_{23}N_2SO_3^+$	Gln-Met
299	$C_{13}H_{19}N_2O_4^+$	Lys-Pro-Gly
313	$C_{10}H_{25}N_2SO_4^+$	Tyr-Met
369	$C_9H_{21}Si_4O_3^+$	PDMS interference (R)
391	$C_{18}H_{19}N_2O_5^+$	Thr-Tyr-Glu
445	$C_{18}H_{29}N_4O_6^+$	Gly-Arg-Ile-Thr
519	$C_{18}H_{29}N_6O_8^+$	Gln-Asp-Thr-Arg

Discussion

Compounds and constituents of the fibronectin samples show complementary results based on the identified amino acid fragments. Possible m/z identifications for ALS SPI-MS and dropDESI-MS were calculated based on fibronectin's amino acid sequence, while values for ToF-SIMS were comparable to previously conducted experiments. The microfluidic reactor successfully enabled different MS techniques in aqueous solution. Only fibronectin in aqueous solution has been studied so far in MS. Our results suggest the need for further research of large biomolecules to understand their surface compositions in aqueous solution, accurately representing their natural environment.

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References

Literature
[1] N. Xia, C. J. May, S. L. McArthur and D. G. Castner, *Langmuir*, 2002, **18**, 4090-4097.
[2] M. S. Wagner, T. A. Horbett and D. G. Castner, *Langmuir*, 2003, **19**,1708-1715.
[3] J. Wald, C. Muller, M. Wahl, W. Hoth-Hannig, M. Hannig, M. Kopnarski and C. Ziegler, *Phys. Status Solidi A*, 2010, **207**, 831-836.
[4] C. Bruning, S. Hellweg, S. Dambach, D. Lipinsky and H. F. Arlinghaus, *Surf. Interface Anal.*, 2006, **38**, 191-193.
[5] O. D. Sanni, M. S. Wagner, D. Briggs, D. G. Castner and J. C. Vickerman, *Surf. Interface Anal.*, 2002, **33**, 715-728.
[6] M. S. Wagner and D. G. Castner, *Langmuir*, 2001, **17**, 4649-4660.
[7] S. Muramoto, D. J. Graham, M. S. Wagner, T. G. Lee, D. W. Moon and D. G. Castner, *J. Phys. Chem. C*, 2011, **115**, 24247-24255.
[8] C. Santamaria, E. Chatelain, Y. Jackson, Q. Miao, B. Ward, F. Chappuis and M. Ndao, *BMC Infect. Disease*, 2014, **14**:302.
[9] L. Yang, X. Yu, Z. Zhu, T. Thevuthasan, and J. Cowin, *J. Vac. Sci. Technol.*, 2011, **29**, 061101-1-10

Cartoons
[1] https://www.rcsb.org
[2] http://scientificrussia.ru/articles/konferencija-po-biomembranam-v-mfti
[3] L. Yang, X. Yu, Z. Zhu, T. Thevuthasan, and J. Cowin, *J. Vac. Sci. Technol.*, 2011, **29**, 061101-1-10
[4] http://www.iontof.com/
[5] R. Kaiser, P. Maksyutenko, C. Ennis, F. Zhang, Z. Gu, S. Krishtal, A. Mebel, O. Kostko, and M. Ahmed, *Faraday Discus.*, 2010, **147**, 429-478
[6] Courtesy of LBNL

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