Lab on a Chip 2012
Electronic Supplementary Information

Fluorinated liquid enabled digital microfluidic protein handling for fully in-situ MALDI-MS analysis with surfactant aided crystallization

Andrew P. Aijian,*[a] Debalina Chatterjee[a,b] and Robin L. Garrell[b]
[a] *Biomedical Engineering Interdepartmental PhD Program, University of California, Los Angeles, CA 90095-1600, USA*
[b] *Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569, USA*
[c] *California NanoSystems Institute(CNSI), University of California, Los Angeles, CA 90095-7227, USA.*
E-mail: garrell@chem.ucla.edu; Tel: +1 310-825-4383

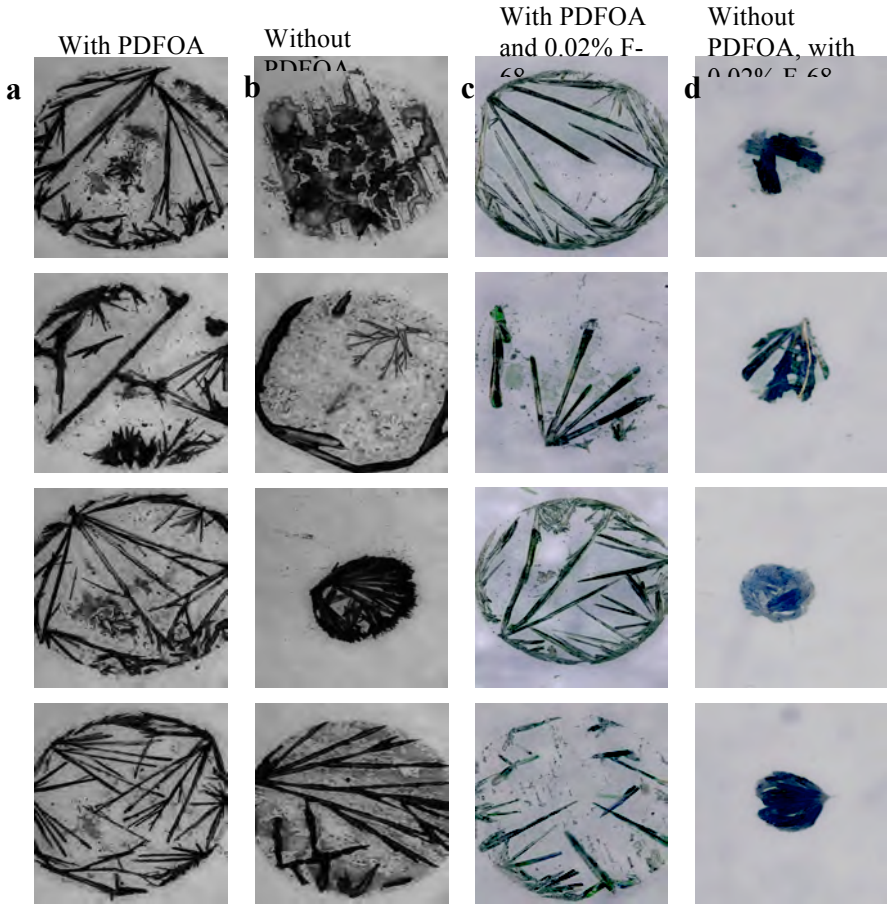


Figure S1. A comparison of typical crystal morphology for digested FITC-BSA samples crystallized on Cytop with (a) and without (b) PDFOA in the matrix solution. Samples of digested FITC-BSA containing 0.02% Pluronic® F-68 crystallized on Cytop with (c) and without (d) PDFOA in the matrix. All samples crystallized with PDFOA exhibited the typical crystal structure of long, needle-like crystals originating from the outer rim of the spot. Samples crystallized without PDFOA exhibited unpredictable crystal structure. Samples crystallized without PDFOA but with 0.02% F-68 consistently formed compact crystal clusters.

All mass spectra were acquired from a Voyager DE-STR Mass Spectrometer from Applied Biosciences. The instrument settings for all spectra acquired were as follows:

Mode of operation: Reflector
Extraction mode: Delayed
Polarity: Positive
Acquisition control: Manual

Accelerating voltage: 20000V
Grid Voltage: 66%
Mirror voltage ratio: 1.12
Guide wire 0: 0%
Extraction delay time: 170 nsec

Laser intensity: 2716 – 2866
Laser Rep Rate: 20.0 Hz
Calibration matrix: 2,5-Dihydroxybenzoic acid
Timed ion selector: Off

TIS gate width: 30
TIS flight length: 1167

Prior to analysis by MASCOT, all spectra were modified in the Data Explorer™ (Applied Biosystems) according to the following modifications:

Gaussian Smooth: 7 points
Baseline Correction: yes
Peak insertion: when necessary

All spectra were internally calibrated using either a peptide standard, trypsin autolysis peak, or both.

Below are the mass spectra used in figures 7a, 7b, and 6, respectively. The peptides identified by MASCOT are listed along with the MOWSE score and protein identification.

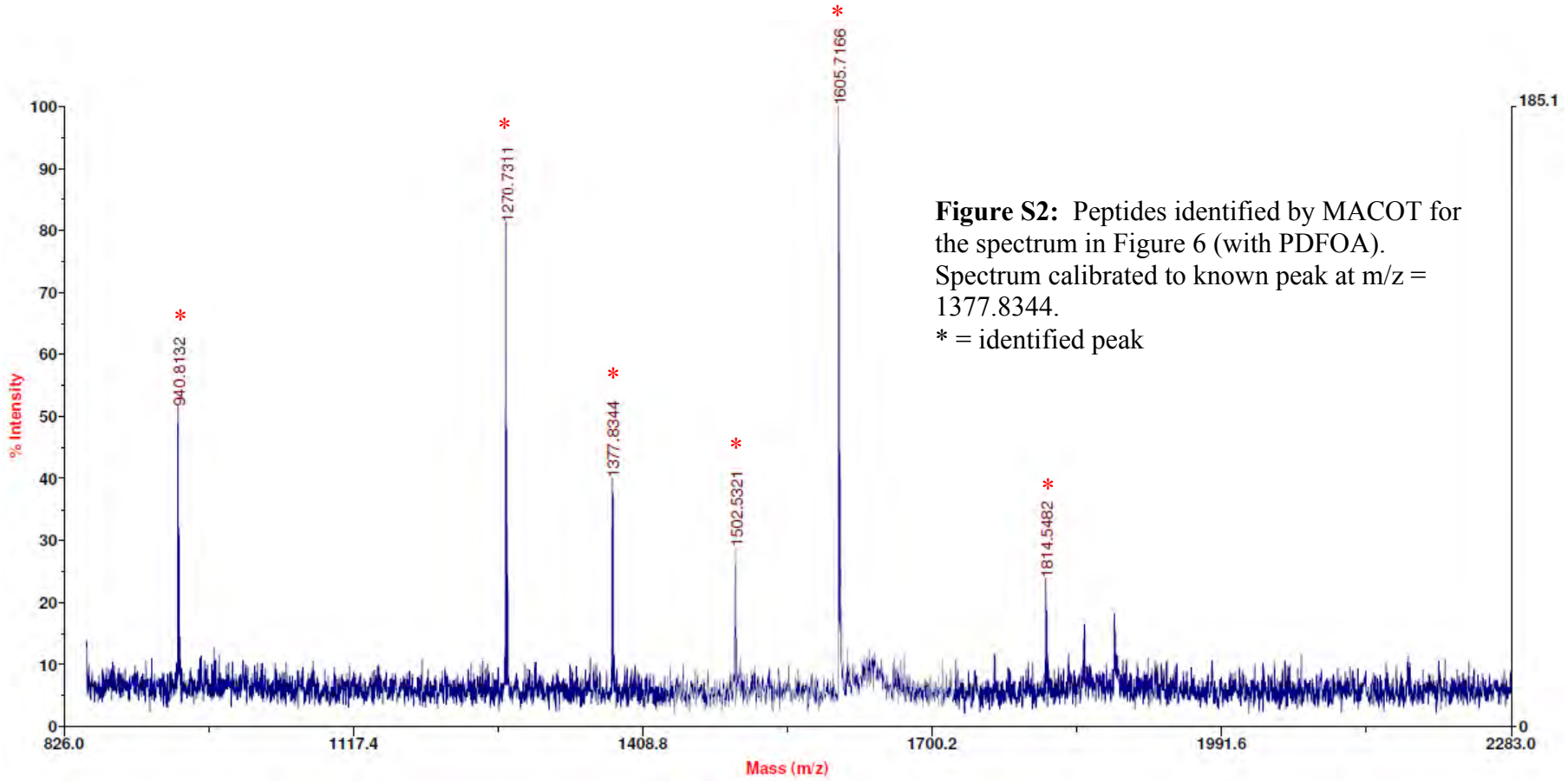


Figure S2: Peptides identified by MACOT for the spectrum in Figure 6 (with PDFOA). Spectrum calibrated to known peak at m/z = 1377.8344.
* = identified peak

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
2 - 17	1814.5482	1814.5482	1814.8952	-191	0	M.GLSDGEWQQVLNVWGK.V
18 - 32	1605.7166	1605.7166	1605.8475	-81	0	K.VEADIAGHGQEVLIR.L
33 - 43	1270.7311	1270.7311	1270.6557	59	0	R.LFTGHPETLEK.F
65 - 78	1377.8344	1377.8344	1377.8344	0	0	K.HGTVVLTALGGILK.K
120 - 134	1501.5314	1501.5314	1501.6620	-87	0	K.HPGDFGADAQGAMTK.A
147 - 154	940.8132	940.8132	940.4654	370	1	K.YKELGFQG.-

Match to: MYG_EQUBU Score: 110 Expect: 5.3e-06
Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2
Nominal mass (Mr): 17072; Calculated pI value: 7.21
NCBI BLAST search of MYG_EQUBU against nr
Unformatted sequence string for pasting into other applications
Taxonomy: Equus burchellii
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 6
Number of mass values matched: 6
Sequence Coverage: 51%

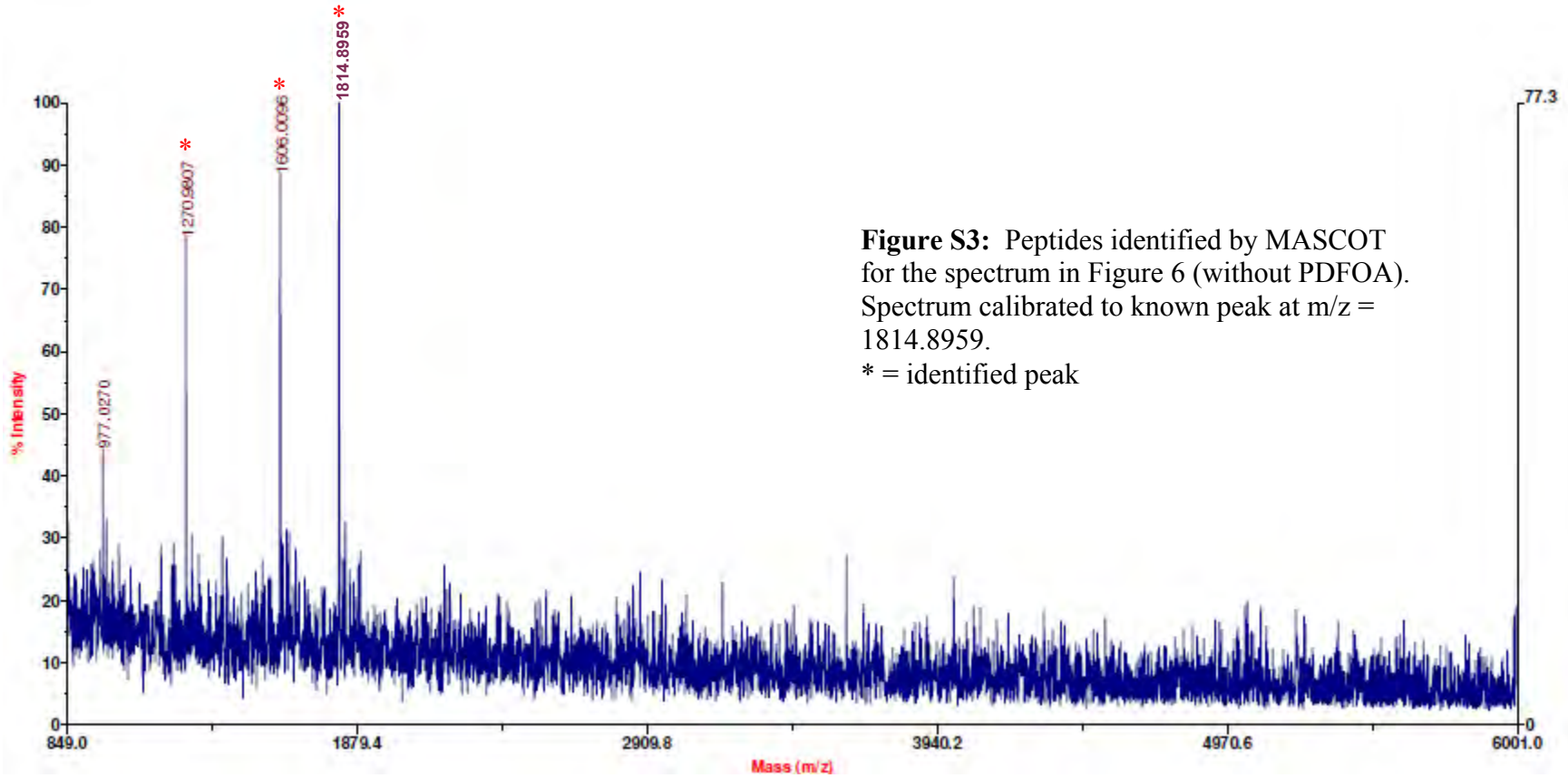


Figure S3: Peptides identified by MASCOT for the spectrum in Figure 6 (without PDFOA). Spectrum calibrated to known peak at m/z = 1814.8959.
* = identified peak

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
2 - 17	1814.8959	1814.8959	1814.8952	0	0	M.GLSDGEWQQVLNVWGK.V
18 - 32	1606.0096	1606.0096	1605.8475	101	0	K.VEADIAGHGQEVLIR.L
33 - 43	1270.9807	1270.9807	1270.6557	256	0	R.LFTGHPETLEK.F

Match to: MYG_EQUBU Score: 58 Expect: 0.83
Myoglobin OS=Equus burchelli GN=MB PE=1 SV=2
Nominal mass (Mr): 17072; Calculated pI value: 7.21
NCBI BLAST search of MYG_EQUBU against nr
Unformatted sequence string for pasting into other applications
Taxonomy: Equus burchellii
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 3
Number of mass values matched: 3
Sequence Coverage: 27%

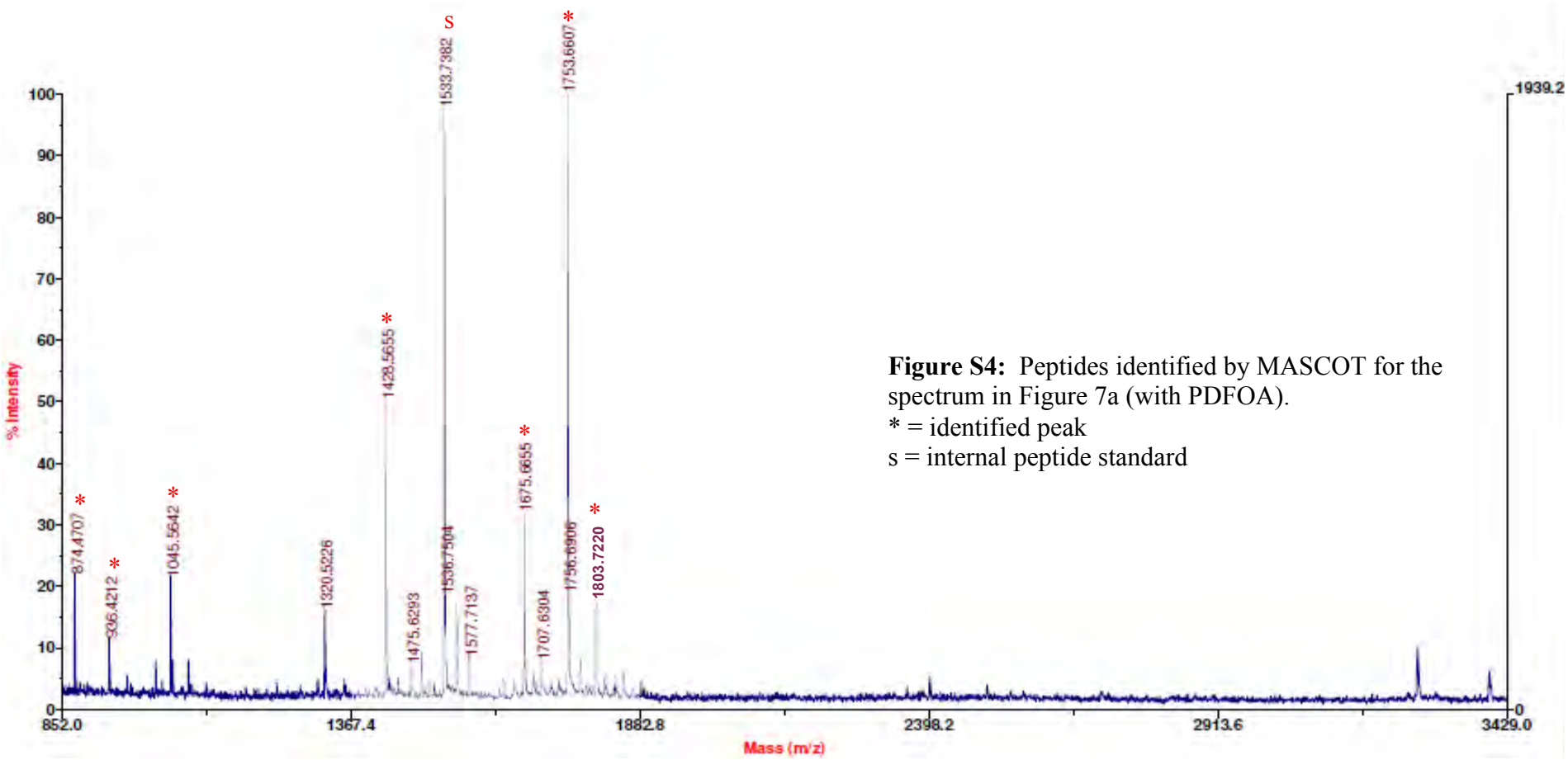


Figure S4: Peptides identified by MASCOT for the spectrum in Figure 7a (with PDFOA).
* = identified peak
s = internal peptide standard

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
33 - 39	874.4707	873.4635	873.4093	62	0	R.HGLDNYR.G
52 - 63	1428.5655	1427.5582	1427.6429	-59	0	K.FESNFNTQATNR.N
64 - 79	1753.6607	1752.6535	1752.8278	-99	0	R.NTDGSTDYGILQINSR.W
80 - 86	936.4212	935.4139	935.3708	46	0	R.WWCNDGR.T
115 - 130	1803.7220	1802.7148	1802.8886	-96	1	K.KIVSDGNGMNAWVAWR.N
116 - 130	1675.6655	1674.6582	1674.7937	-81	0	K.IVSDGNGMNAWVAWR.N
135 - 143	1045.5642	1044.5569	1044.5352	21	0	K.GTDVQAWIR.G

Match to: LYSC_CHICK Score: 155 Expect: 1.7e-10
Lysozyme C OS=Gallus gallus GN=LYZ PE=1 SV=1
Nominal mass (Mr): 16228; Calculated pI value: 9.37
NCBI BLAST search of LYSC_CHICK against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Gallus gallus
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 7
Number of mass values matched: 7
Sequence Coverage: 45%

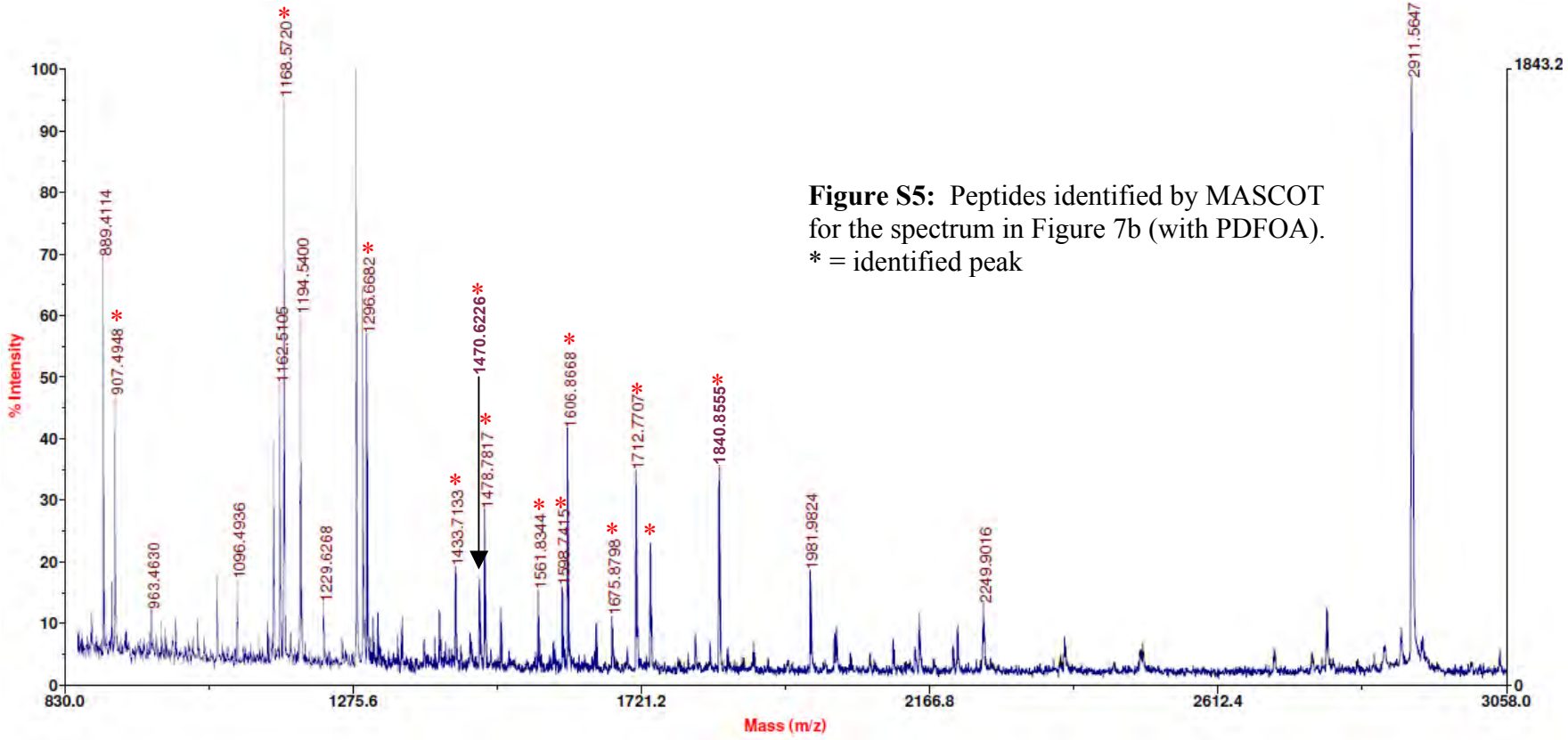


Figure S5: Peptides identified by MASCOT for the spectrum in Figure 7b (with PDFOA). * = identified peak

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
1 - 14	1606.8668	1605.8595	1605.8912	-20	3	-.MGDVEKGKKIFVQK.C
24 - 39	1675.8798	1674.8725	1674.9067	-20	2	K.GGKHKTGPNLHGLFGR.K
27 - 39	1433.7133	1432.7060	1432.7688	-44	1	K.HKTGPNLHGLFGR.K
27 - 40	1561.8344	1560.8271	1560.8637	-23	2	K.HKTGPNLHGLFGRK.T
29 - 39	1168.5720	1167.5647	1167.6149	-43	0	K.TGPNLHGLFGR.K
29 - 40	1296.6682	1295.6609	1295.7099	-38	1	K.TGPNLHGLFGRK.T
40 - 54	1598.7415	1597.7342	1597.7736	-25	1	R.KTGQAPGFTYTDANK.N
40 - 56	1840.8555	1839.8482	1839.9115	-34	2	R.KTGQAPGFTYTDANKNK.G
41 - 54	1470.6226	1469.6153	1469.6787	-43	0	K.TGQAPGFTYTDANK.N
41 - 56	1712.7707	1711.7634	1711.8166	-31	1	K.TGQAPGFTYTDANKNK.G
81 - 88	907.4948	906.4875	906.5361	-54	1	K.MIFAGIKK.K
88 - 101	1734.9427	1733.9354	1734.0039	-39	4	K.KKTEREDLIAYLKK.A
90 - 101	1478.7817	1477.7744	1477.8140	-27	2	K.TEREDLIAYLKK.A

Match to: CYC_HORSE Score: 224 Expect: 2.1e-17
Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2
Nominal mass (Mr): 11825; Calculated pI value: 9.59
NCBI BLAST search of CYC_HORSE against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Equus caballus
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 13
Number of mass values matched: 13
Sequence Coverage: 64%

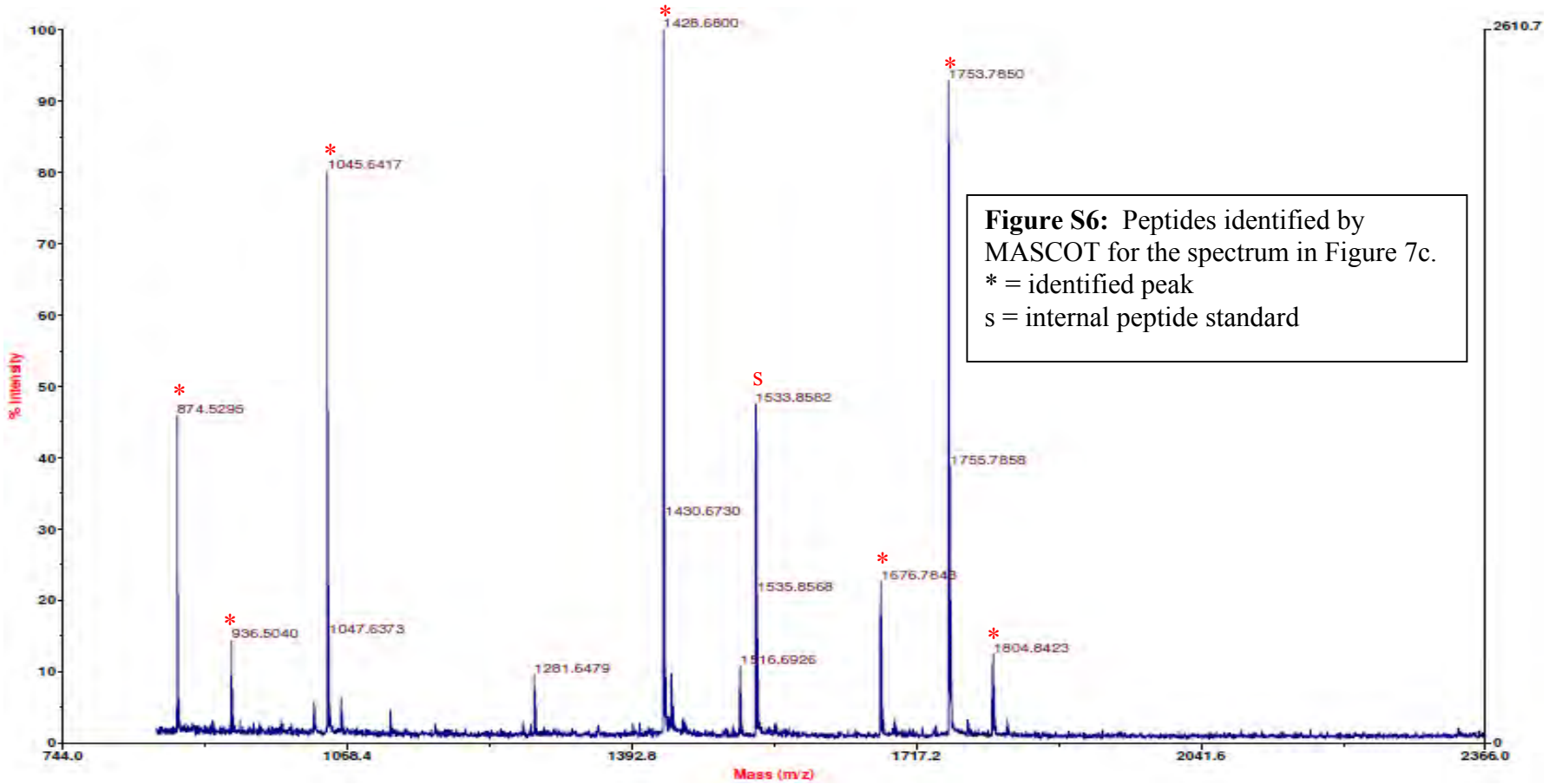


Figure S6: Peptides identified by MASCOT for the spectrum in Figure 7c.
* = identified peak
s = internal peptide standard

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
33 - 39	874.5295	873.5222	873.4093	129	0	R.HGLDNYR.G
52 - 63	1428.6800	1427.6727	1427.6429	21	0	K.FESNFNTQATNR.N
64 - 79	1753.7850	1752.7777	1752.8278	-29	0	R.NTDGSTDYGILQINSR.W
80 - 86	936.5040	935.4967	935.3708	135	0	R.WWCNDGR.T
115 - 130	1803.8526	1802.8453	1802.8886	-24	1	K.KIVSDGNGMNAWVAWR.N
116 - 130	1675.7971	1674.7898	1674.7937	-2	0	K.IVSDGNGMNAWVAWR.N
135 - 143	1045.6417	1044.6344	1044.5352	95	0	K.GTDVQAWIR.G

Match to: LYSC_CHICK Score: 151 Expect: 4.2e-10
Lysozyme C OS=Gallus gallus GN=LYZ PE=1 SV=1
Nominal mass (Mr): 16228; Calculated pI value: 9.37
NCBI BLAST search of LYSC_CHICK against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Gallus gallus
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 7
Number of mass values matched: 7
Sequence Coverage: 45%

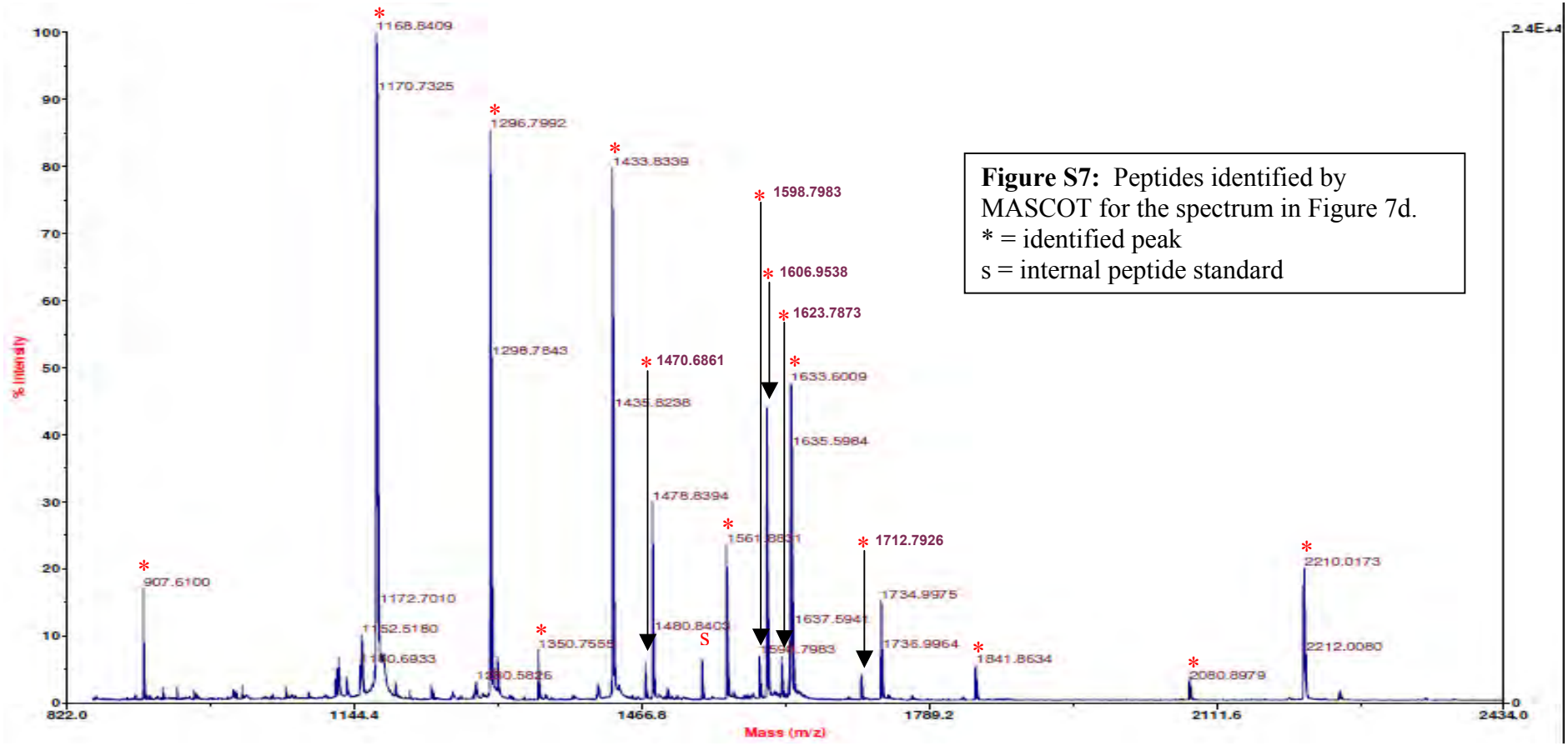
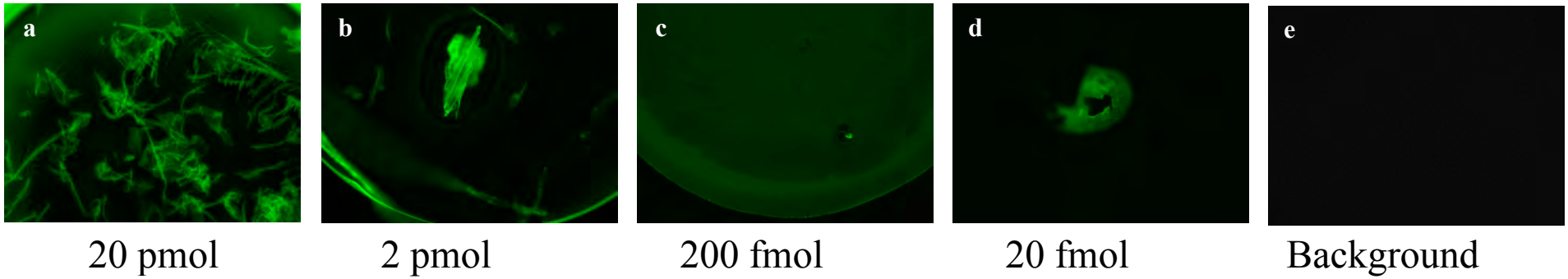


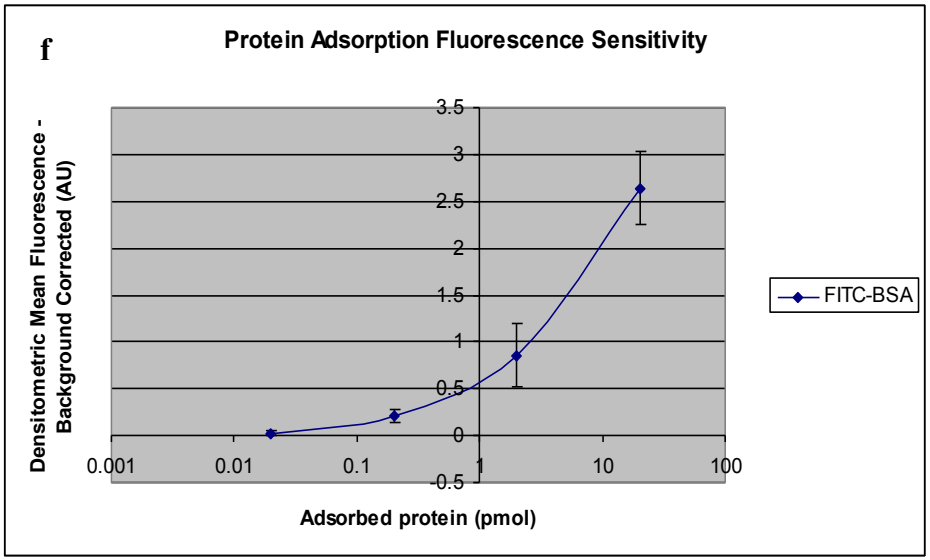
Figure S7: Peptides identified by MASCOT for the spectrum in Figure 7d.
* = identified peak
s = internal peptide standard

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
10 - 23	1633.6009	1632.5936	1632.8116	-134	1	K.IFVQKCAQCHTVEK.G
27 - 39	1433.8339	1432.8266	1432.7688	40	1	K.HKTGPNLHGLFGR.K
27 - 40	1561.8831	1560.8758	1560.8637	8	2	K.HKTGPNLHGLFGRK.T
29 - 39	1168.8409	1167.8337	1167.6149	187	0	K.TGPNLHGLFGR.K
29 - 40	1296.7992	1295.7919	1295.7099	63	1	K.TGPNLHGLFGRK.T
40 - 54	1598.7983	1597.7910	1597.7736	11	1	R.KTGQAPGFTYTDANK.N
40 - 56	1840.8652	1839.8580	1839.9115	-29	2	R.KTGQAPGFTYTDANKNK.G
41 - 54	1470.6861	1469.6789	1469.6787	0	0	K.TGQAPGFTYTDANK.N
41 - 56	1712.7926	1711.7853	1711.8166	-18	1	K.TGQAPGFTYTDANKNK.G
57 - 73	2080.8979	2079.8907	2080.0186	-62	1	K.GITWKEETLMEYLENPK.K
57 - 74	2209.0137	2208.0064	2208.1136	-49	2	K.GITWKEETLMEYLENPKK.Y
62 - 74	1623.7873	1622.7800	1622.7861	-4	1	K.EETLMEYLENPKK.Y
81 - 88	907.6100	906.6027	906.5361	74	1	K.MIFAGIKK.K
89 - 101	1606.9538	1605.9465	1605.9090	23	3	K.KTEREDLIAYLKK.A
90 - 100	1350.7555	1349.7482	1349.7190	22	1	K.TEREDLIAYLK.K

CYC_HORSE Score: 251 Expect: 4.2e-20
Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2
Nominal mass (Mr): 11825; Calculated pI value: 9.59
NCBI BLAST search of CYC_HORSE against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Equus caballus
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 15
Number of mass values matched: 15
Sequence Coverage: 79%
Matched peptides shown in Bold Red





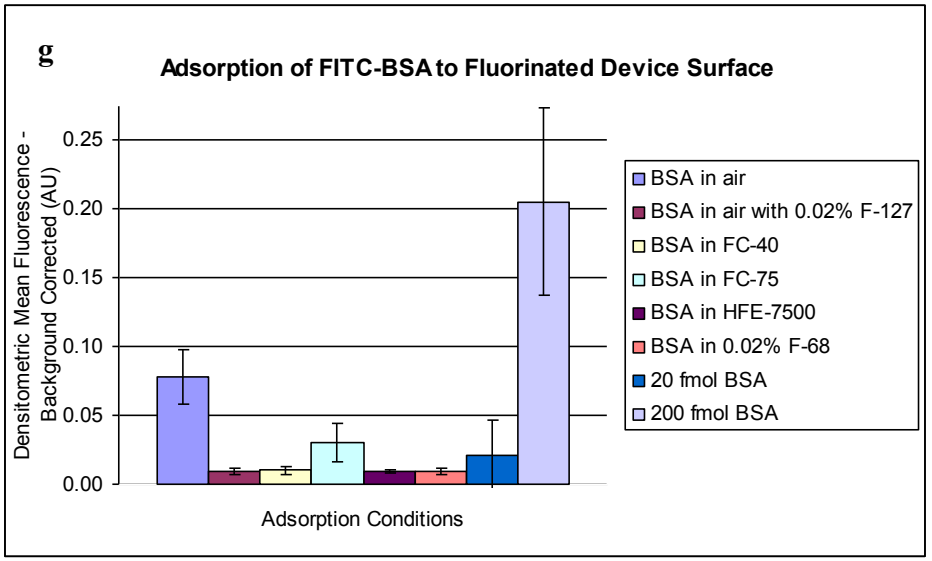


Figure S8. (a – e) Fluorescence images showing adsorption of known amounts of protein to the hydrophobic surface of the device. To determine the sensitivity of fluorescence microscopy for detecting FITC-BSA adsorbed to the device surface 2 μL drops of various, known concentrations were deposited onto a Teflon® coated surface and allowed to dry. The dried protein spots were then imaged by fluorescence microscopy. (f) The sensitivity analysis reveals that as little as 20 fmol of protein is readily detected using fluorescence microscopy. (g) Figure 4 with the addition of fluorescence intensity data for 20 and 200 fmol of adsorbed FITC-BSA (data added as the rightmost columns). The addition of Pluronics®, or engulfing the droplets in fluorinated liquids, reduces the adsorption of proteins substantially compared to a droplet without surfactant additives sitting in air (leftmost column); protein adsorption is reduced to 20 fmol or less.