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

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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 burchelli
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%
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<td>1814.8959</td>
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<td>101</td>
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<td>1270.6557</td>
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**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

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 burchelli
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%
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<td>1433.7133</td>
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<td>1168.5720</td>
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**Figure S5:** Peptides identified by MASCOT for the spectrum in Figure 7b (with PDFOA). *= identified peak

**Match to: CYC_HORSE Score: 224 Expect: 2.1e-17**

Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2Nominal mass (Mr): 11825; Calculated pI value: 9.59NCBI 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%

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<td>1428.6800</td>
<td>1427.6727</td>
<td>1427.6429</td>
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<td>0</td>
<td>K.FE SNFNTQATNR.N</td>
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<td>1753.7850</td>
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**Figure S6:** Peptides identified by MASCOT for the spectrum in Figure 7c.
* = identified peak
s = internal peptide standard

**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
Number of mass values searched: 7
Number of mass values matched: 7
Sequence Coverage: 45%
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**Figure S7:** Peptides identified by MASCOT for the spectrum in Figure 7d. * = identified peak  
**s** = internal peptide standard

**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.