

## Supplementary Information

### **Miniaturized sample preparation on a digital microfluidics device for sensitive bottom-up microproteomics of mammalian cells using magnetic beads and mass spectrometry-compatible surfactants**

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## Supporting methods

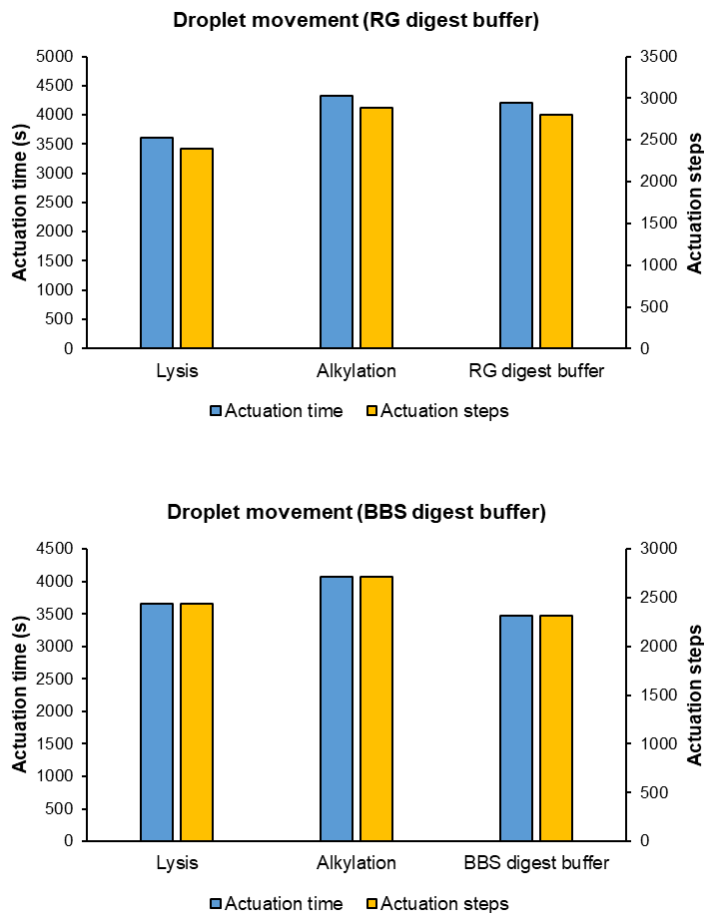
### *SP3 detergent removal assays*

For SP3 detergent removal, 2.5  $\mu\text{L}$  of BSA (2 mg/mL), 5  $\mu\text{L}$  of DTT (20 mM in 100 mM ABC buffer), and 10  $\mu\text{L}$  of aqueous detergent solution were mixed, and subsequently incubated at 56°C for 30 min. Detergents tested were Pluronic F127 (0.16% wt/vol), Pluronic F68 (0.2% wt/vol), or Tetricon 90R4 (0.2% wt/vol). After reduction, 2.5  $\mu\text{L}$  of IAA (100 mM in 25 mM ABC) were added, and samples were incubated at room temperature (RT), in the dark for 30 min. For samples to be treated by SP3, 2  $\mu\text{L}$  of a 20  $\mu\text{g}/\mu\text{L}$  SP3 magnetic bead mixture prepared in water were added to 20  $\mu\text{L}$  of final sample volume.<sup>1</sup> Protein binding was induced by addition of 52  $\mu\text{L}$  of acetonitrile (ACN), and subsequent incubation at RT for 18 min. Using a permanent magnet, beads were immobilized and the supernatant was pipetted into a new tube. Magnetically immobilized beads were further washed with 70% ethanol (EtOH), and ACN, saving both supernatants into new tubes as well. Collected SP3 supernatants were dried in a speed-vac centrifugal concentrator. For digestion, 5  $\mu\text{L}$  of trypsin (20 ng/ $\mu\text{L}$ ) were pipetted onto the pelleted beads, and samples were incubated overnight (~ 16 hrs) at 37°C in an oven. After digestion, supernatants containing peptides were transferred into new tubes, dried, and analyzed. In comparative experiments using C18-reversed phase clean-up of peptides instead of SP3 clean-up of proteins, trypsin was added to the reduced and alkylated protein. The digests were desalted using Pierce C18 Tips (10  $\mu\text{L}$  bed) according to the manufacturer protocol, but samples were eluted using either 95% ACN / 0.05% TFA, or stepwise, using 70, 80 or 90% ACN / 0.05% TFA. Eluates were dried and separately analyzed.

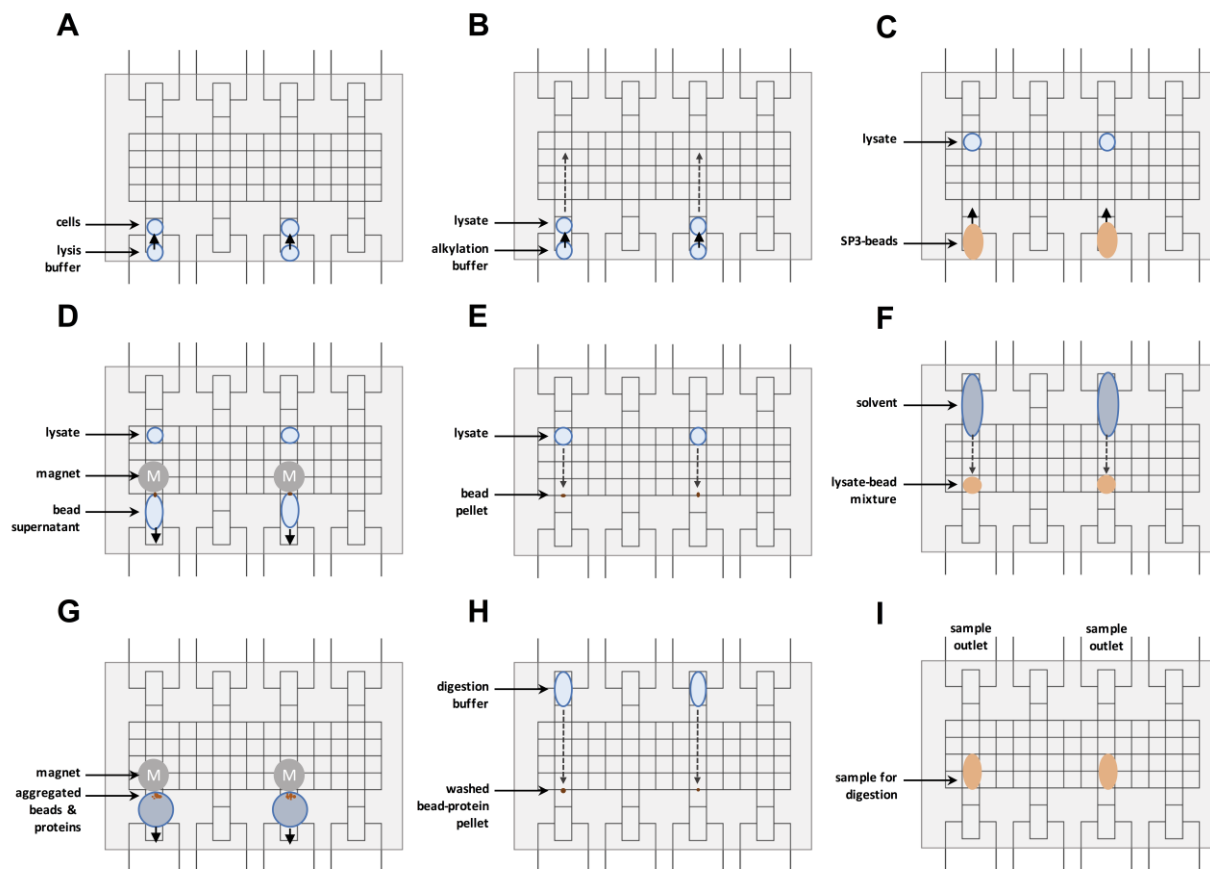
### *Linear and reflector mode MALDI-TOF MS analysis*

For analysis of polymeric detergents in SP3 supernatants and digests we modified a previously described linear mode MALDI MS method.<sup>2</sup> To reconstitute detergents to their concentration prior the SP3 procedure, dried supernatants and eluates were redissolved in 20  $\mu\text{L}$  of the ionic liquid matrix (ILM) mono-3AP-CHCA (1.5 mg/mL CHCA, 16 mM 3-aminopentane, 0.05% TFA, 70% ACN) previously described by us.<sup>3</sup> MALDI MS analysis was performed on an AB SCIEX MALDI TOF/TOF 5800 mass spectrometer (SCIEX, Darmstadt, Germany) equipped with a Nd:YAG laser and operating at a pulse rate of 400 Hz. The instrument was calibrated using a mixture of 1.5 pmol of myoglobin and 1.1 pmol of thaumatin (amount on-target, in 3 mg/mL CHCA, 0.1% TFA, 70% ACN). Spectra were acquired in positive linear mid mass mode accumulating 2,500 shots over a mass range from  $m/z$  2,000 or  $m/z$  4,000 to  $m/z$  20,000. Delayed extraction (DE1) was set to 820 ns. Peptide analysis was performed in positive reflector mode accumulating 2,000 shots over a mass range from  $m/z$  800 to  $m/z$  4,000.

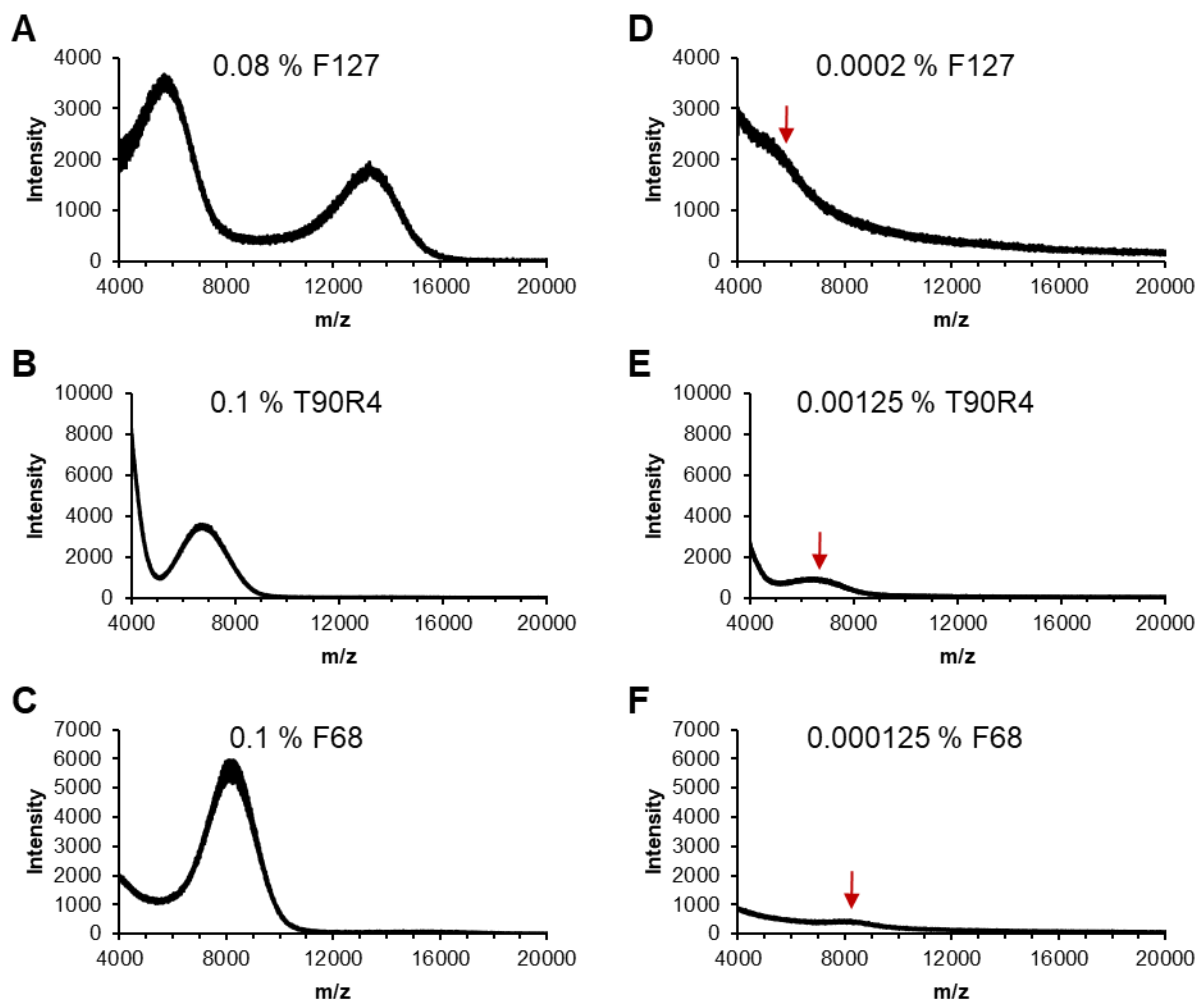
## Supporting figures



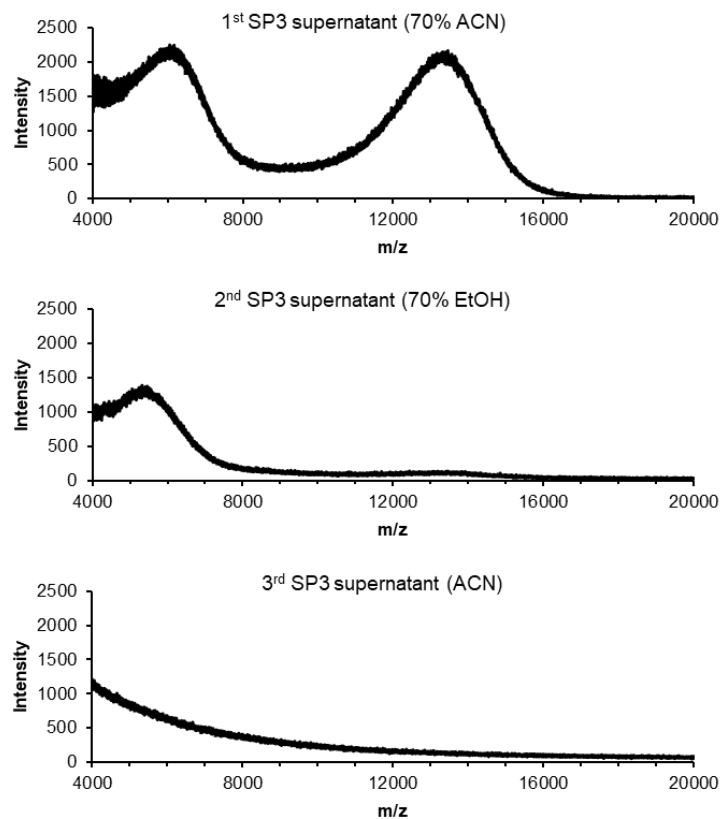
**Figure S1:** Continuous movement of reagent droplets across a series of electrodes using a programmed route. Actuation step time is defined by the transition duration (1.5 s) programmed in the MicroDrop control software. For movement experiments during cell lysis, 1.5  $\mu\text{L}$  of cell solution (approx. 500 cells/ $\mu\text{L}$  in 1x PBS and 0.08% Pluronic F68) were loaded onto the chip, and subsequently merged with 2  $\mu\text{L}$  of lysis buffer. The merged droplets were moved across a series of 8 electrodes (circular pattern). After the indicated movement time, 1.5  $\mu\text{L}$  of alkylation buffer were added to the lysates, and movement continued for the indicated time period. After performing SP3 clean-up, beads were resuspended in 2 $\mu\text{L}$  of either RG or BBS digestion buffer. The bead mixtures were moved back and forth across a series of 4 electrodes (linear pattern). Movement of BBS bead mixture stopped after 58 min, because no overlap to the next electrode was reached anymore, due to volume reduction of the droplet by evaporation.



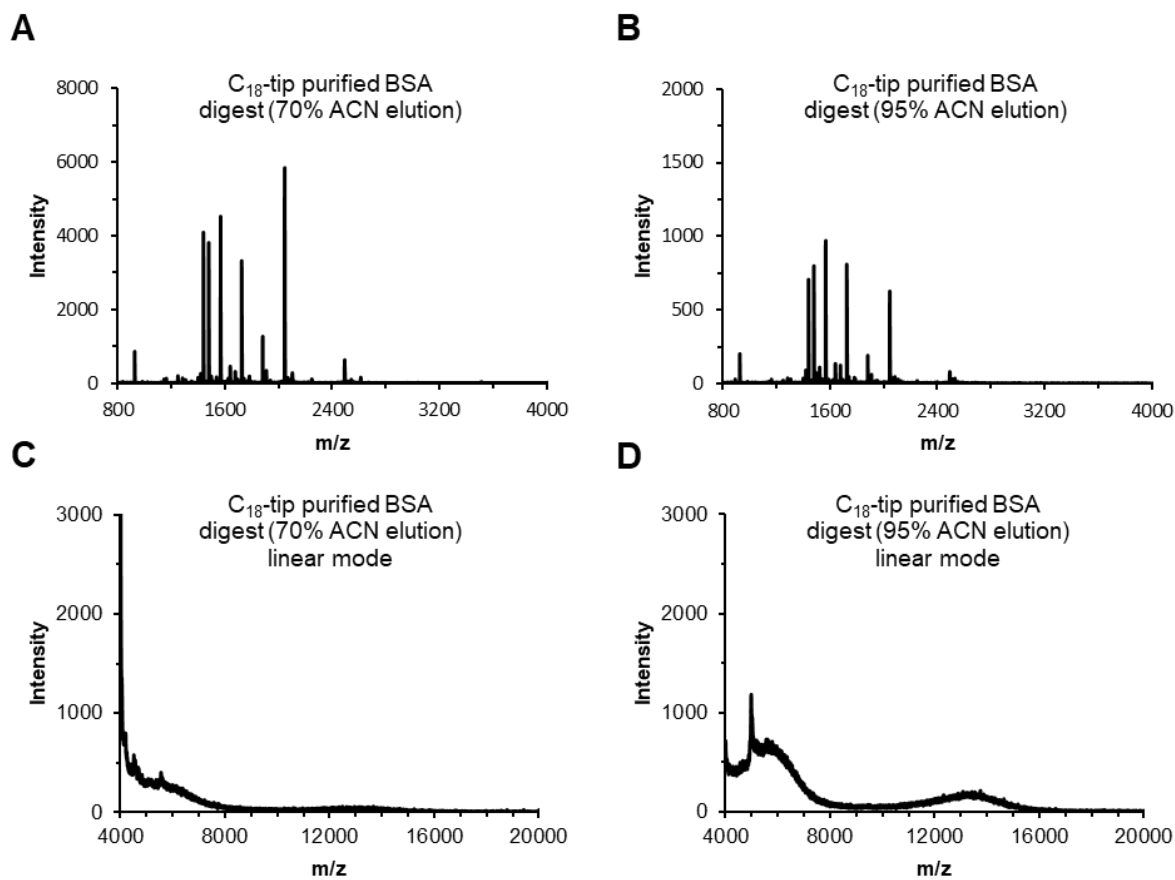
**Figure S2:** Scheme of the DMF-SP3 chip protocol. **(A)** To directly load 1  $\mu\text{L}$  of cell suspension, both the reservoir and transfer electrodes are actuated, and sample is loaded in the middle of the reservoir towards the transfer electrode. The reservoir electrode is deactivated to move the droplet onto the transfer electrode. **(B)** Alkylation buffer is added in the same manner. **(C)** SP3-bead mixture is loaded after alkylation. **(D)** Supernatant of the bead mixture is removed to pellet the beads. **(E)** The lysate is moved onto the bead pellet and subsequently mixed. **(F)** Protein-bead aggregation is induced by addition of solvent. **(G)** Aggregated beads are extracted, and supernatant is removed. Bead pellet is washed two times (not shown). **(H)** Digestion buffer is loaded and subsequently mixed with the bead pellet. **(I)** Samples for digestion are incubated in a humidified chamber for 8 hrs. Afterwards, supernatants containing peptides are separated from the beads as shown in (D), but sample is aspirated from the designated sample outlet with a GELoader tip.



**Figure S3:** Detection of polymer surfactants by linear mode MALDI MS. Spectra of DMF on-chip working concentrations of (A) Pluronic F127, (B) Tetronic 90R4, and (C) Pluronic F68, as well as spectra of the respective limit of detection (LOD) (D-F) are shown. Red arrows indicate maxima of polymer mass distributions at the LOD. Maxima of the mass distributions correspond to average masses of the polymers (5,800 Da and 13,500 Da for Pluronic F127; 7,000 Da for Tetronic 90R4; 8,000 Da for Pluronic F68). A further mass distribution of Pluronic F68 can be detected at 3,700 Da by extending the mass range down to m/z 2,000 (not shown). Matrix used was *mono*-3AP-CHCA.

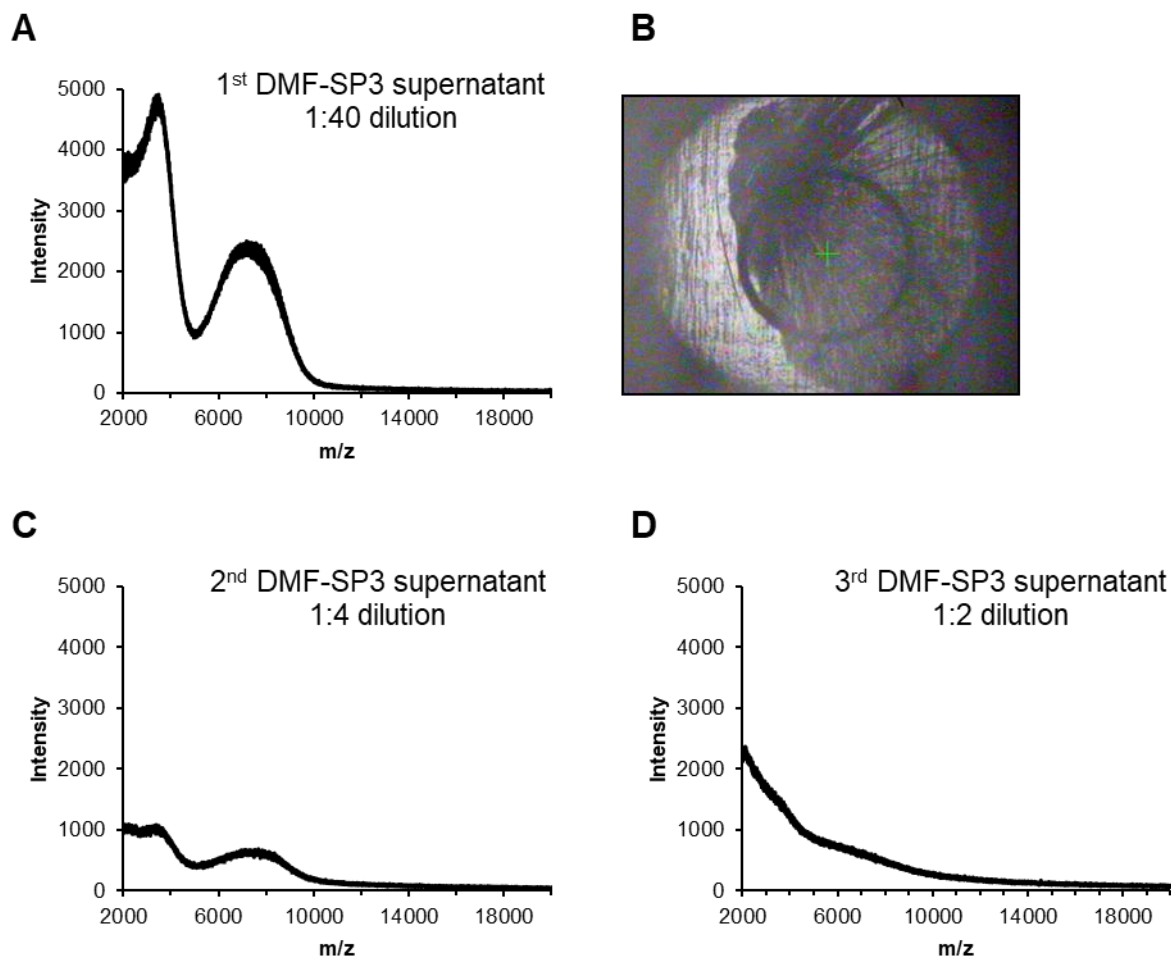


**Figure S4:** Detection of Pluronic F127 in SP3 supernatants by linear mode MALDI MS. Maxima of the polymer mass distributions were detected at approximately 5,800 Da and 13,500 Da in the 1<sup>st</sup> and 2<sup>nd</sup> SP3 supernatant, respectively, while no signal for Pluronic F127 was detected in the 3<sup>rd</sup> supernatant. Matrix used was *mono*-3AP-CHCA.

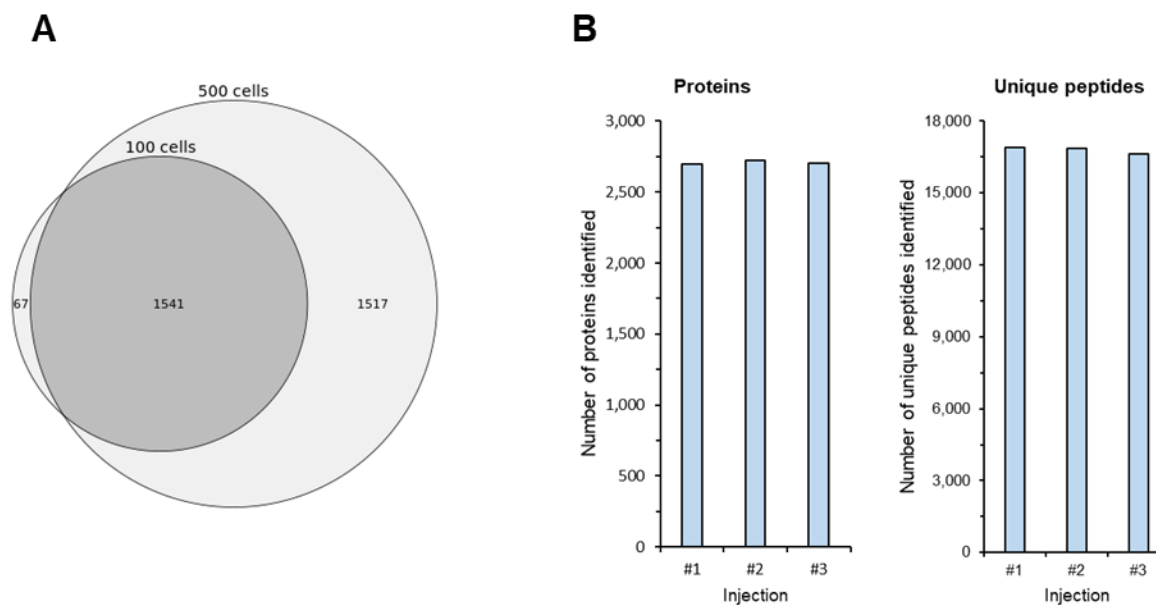


**Figure S5:** MALDI MS analysis of BSA standard digests that contained Pluronic F127 after desalting using C<sub>18</sub>-tips. **(A)** C<sub>18</sub>-tip eluate (70% ACN elution) analyzed in positive reflector mode. **(B)** C<sub>18</sub>-tip eluate (95% ACN elution) analyzed in positive reflector mode. **(C)** Linear mode analysis of C<sub>18</sub>-tip eluate (70% ACN elution). Pluronic F127 is detected with low intensity the sample. **(D)** Linear mode analysis of C<sub>18</sub>-tip eluate (95% ACN elution). Pluronic F127 mass distributions are found at 5,800 Da and 13,500 Da. Matrix was *mono*-3AP-CHCA.

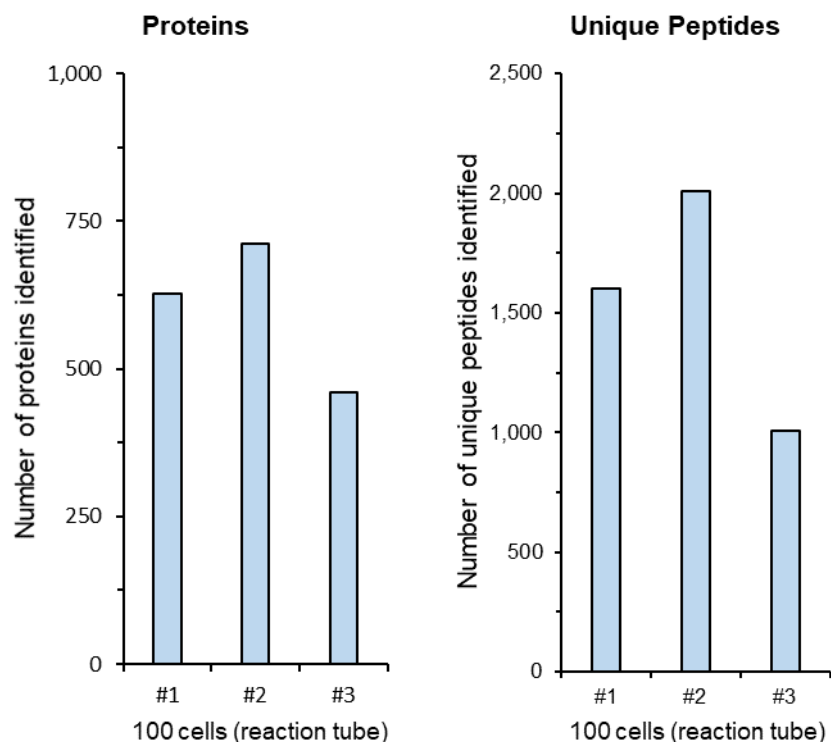




**Figure S6:** Detection of Pluronic F68 and Tetronic 90R4 in DMF-SP3 supernatants by linear positive ion mode MALDI MS. Maxima of the polymer mass distributions are found at approximately 3,700 Da (Pluronic F68) and 7,000 Da (Pluronic F68 and Tetronic 90R4) in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> SP3 supernatant. **(A)** The 1<sup>st</sup> DMF-SP3 supernatant was diluted 1:40 with ILM, due to high urea salt content impairing homogenous spot formation. **(B)** Screenshot of the instrument camera, showing an inhomogenous sample spot derived from the 1<sup>st</sup> DMF-SP3 supernatant (undiluted sample). Linear mode mass spectrum of the 2<sup>nd</sup> DMF-SP3 supernatant diluted 1:4 **(C)**, and of the 3<sup>rd</sup> DMF-SP3 supernatant diluted 1:2 **(D)**. DMF-SP3 supernatants were obtained from the urea-based lysis of 100 Jurkat T cells. Supernatants were dried and resuspended in 5 $\mu$ L of matrix. Matrix used was *mono*-3AP-CHCA.



**Figure S7:** (A) Overlap of the identified proteins from 100 Jurkat T cells cells vs. the identified proteins from 500 cells (both combined from triplicates). (B) Proteins and unique peptides identified from Jurkat T cells lysed in reagent tubes using a previously described protocol that was slightly modified.<sup>1</sup> Briefly, 14,000 cells were lysed in 8M urea, 2% CHAPS, and 5 mM DTT. Lysis was facilitated by sonication at 4°C in a Bioruptor Pico (Diagenode, Liège, Belgium) for 15 min (cycles of 30 s ‘on’, 30 s ‘off’), followed by incubation at 45°C for 30 min. Cysteines were alkylated by addition of 25 mM of IAA and 12 mM of ABC (final concentrations), and incubation in the dark for 45 min. After SP3 clean-up, beads were resuspended in 5 µL of trypsin/Lys-C mix (20 ng/µL in 50 mM TEAB). After incubation in an oven at 37°C for 16 hrs, supernatants containing peptides were dried, and stored at -20°C until further processing. For LC-MS analysis, one tenth of a sample (corresponding to material of approximately 1,400 cells) was injected.



**Figure S8:** Proteins and unique peptides identified from approximately 100 Jurkat T cells lysed in reagent tubes using a standard proteomics approach including manual sample preparation. Cells were prepared using a previously described lysis protocol that was slightly modified:<sup>1</sup> Aliquots of 5  $\mu$ L cell suspension (20 cells/ $\mu$ L) were lysed with 5  $\mu$ L of lysis buffer (final concentrations: 8M urea, 2% CHAPS, 5 mM DTT). Lysis was facilitated by sonication at 4°C in a Bioruptor Pico (Diagenode, Liège, Belgium) for 15 min (cycles of 30 s ‘on’, 30 s ‘off’), followed by incubation at 45°C for 30 min. Cysteines were alkylated by addition of 5  $\mu$ L alkylation buffer (final concentrations: 25 mM IAA, 5 mM borate buffer), and incubation in the dark for 45 min. After SP3 clean-up (2  $\mu$ L of 10 ng/ $\mu$ L bead mixture), beads were resuspended in 5  $\mu$ L of trypsin/Lys-C mix (0.8 ng/ $\mu$ L in 50 mM TEAB). After incubation in an oven at 37°C for 8 hrs, supernatants containing peptides were acidified with 1  $\mu$ L of 5% formic acid and stored at -20°C until further processing. For LC-MS analysis, the whole sample (6  $\mu$ L) was injected. The same LC-MS method as for 100 cell samples derived from DMF-SP3 was used.

## References

- 1 M. Sielaff, J. Kuharev, T. Bohn, J. Hahlbrock, T. Bopp, S. Tenzer and U. Distler, *J. Proteome Res.*, 2017, **16**, 4060–4072.
- 2 Z. Takáts, K. Vékey and L. Hegedüs, *Rapid Communications in Mass Spectrometry*, 2001, **15**, 805–810.
- 3 J. Leipert, I. Bobis, S. Schubert, H. Fickenscher, M. Leippe and A. Tholey, *Anal Bioanal Chem*, 2018, **410**, 4737–4748.