Maldi-MS (Autoflex Speed):

(+)-Maldi-MS (or (–)-Maldi-MS) analyses were performed on an *Autoflex Speed* time-of-flight mass spectrometer (*Bruker Daltonics*, Bremen, Germany) equipped with a *Bruker* smartbeam^{TM}-II laser (355 nm wavelength) in the reflectron (or in the linear) mode. Around 1000 spectra were summed at a scan rate of 2 kHz in the mass range between (*m/z* 800 and 4'000).

Mass calibration (examples):

Mass calibration (peptides, mass range ca. 350 – 4'000 Da): Monoisotopic masses were calibrated with an accuracy ≤ 5 ppm using signals from the HCCA matrix at *m/z* 379.09246 and a mixture of peptides composed of Bradykinin 1-7 ($[M+H]^+_{mono}$ = *m/z* 757.39916), angiotensin II (1'046.54180), angiotensin I (1'296.68480), Subtance P (1347.73540), Bombesin (1619.82230), renin substrate (1'758.93261), ACTH clip 1-17 (2093.08620), ACTH clip 18-39 (2'465.19830) and Somatostatin 28 (3'147.47100) all obtained from the *Bruker* peptide calibration standard II mixture.

Mass calibration (proteins, mass range ca. 4'000 – 20'000 Da): Average masses were calibrated using signals from a protein mixture composes of Insulin ($[M+H]^+_{avg}$ = *m/z* 5'734.52), Cytochrome C (6'181.05), Myoglobin (8476.66), Ubiquitin (8'565.76), Cytochrom C (12'360.97), and Myoglobin (16'952.31) all obtained from the *Bruker* Protein calibration standard I mixture.

Mass calibration (proteins, mass range ca. 10'000 – 70'000 Da): Average masses were calibrated using signals from a protein mixture composes of Cytochrome C ($[M+H]^+_{avg}$ = *m/z* 12'360), Protein A (44'613), Albumin (66'431) all obtained from the *Bruker* Protein calibration standard II mixture.

Target used:

- Ground steel target (Bruker, various matrices possible) or
- AnchorChip target with 0.6 µm Anchors (Bruker, various matrices possible).

Preparation of Matrix: Ask the service department if necessary.

Sample preparation (e.g.): Samples were dissolved in a solution of 50 µl H_2O + 0.1% trifluoroacetic acid. A volume of 1 µl was spotted on a ground steel target prepared with α-cyano-4-hydroxycinnamic acid matrix (HCCA, Bruker). After 1 min, the spot was rinsed with 7 µl of an aqueous solution containing 10 mM ADHP buffer.

(TLC-) Maldi of lipids (Lipid A as example and has to be adapted accordingly)

MALDI-TOF-MS experiments were performed on a *Autoflex Speed* time-of-flight mass spectrometer (*Bruker Daltonics*, Bremen, Germany), equipped with a smartbeam^{TM} II laser (354 nm wavelength, *Bruker*), in the reflectron (or in the linear) mode. Monoisotopic masses were calibrated with an accuracy ≤ 5 ppm using signals from the HCCA matrix at *m/z* 379.09246 and a mixture of peptides composed of Bradykinin 1-7 ($[M+H]^+_{mono}$ = *m/z* 757.39916), angiotensin II (1'046.54180), angiotensin I (1'296.68480), Subtance P (1347.73540), Bombesin (1619.82230), renin substrate (1'758.93261), ACTH clip 1-17 (2093.08620), ACTH clip 18-39 (2'465.19830) and Somatostatin 28 (3'147.47100) all obtained from the *Bruker* peptide calibration standard II mixture. The MALDI matrix was prepared by dissolving 15 mg of 5-chloro-2-mercapto-benzothiazole (CMBT) in 1 ml of MeOH/$CHCl_3$ (3:2, *v/v*). Samples were dissolved in 20 µl of MeOH/$CHCl_3$ (3:2, *v/v*) and mixed to the matrix solution in a ratio of 1:1 (*v/v*). A volume of 0.5 µl was spotted on a ground steel target. Finally, MALDI spectra (2'000 shots) were recorded between m/z 800 and 3'600 in the negative ionization mode.

HPTLC-Maldi-TOF-MS: Prior to MS measurements, the lipid A extract was washed once with 50 µl acidified EtOH (20 ml 95% (*v/v*) EtOH mixed with 100 µl 4 N HCl), supplemented with 5 µl H_2O, and further washed twice with 50 µl 95% (v/v) EtOH. Then, samples were dissolved in 20 µl $CHCl_3$/MeOH (4:6, v/v); 15 µl of the mixture were further diluted with 45 µl of the same solvent and supplemented with piperidine (1 µl/ml). The TLC-Maldi were performed according to *Fuchs et al.* **(1)**. HPTLC Kieselgel 60 F254 aluminium backed sheets (50x75 mm; *Merck* Millipore) were pre-run in $CHCl_3$, and dried at 120 °C for 20 min. Lipid A extracts (~ 300 µg), dissolved in $CHCl_3$/MeOH (4:1, *v/v*), were applied to the plates, which were developed in $CHCl_3$/MeOH/H_2O/NH_4OH (35:25:4:2, *v/v/v/v*), and visualized with a solution of primuline, according to *White et al.* **(2)**. TLC plates were coated with 2x 100 µl of MALDI-matrix solution (20 mg/ml 5-chloro-2-mercaptobenzothiazole (CMBT) in MeOH/$CHCl_3$ (3:2, *v/v*), sonicated, and filtered through 0.2 µm pore PTFE filters) using an electrospray nebulizer (4 bars Ar, 0 V), and plugged into a dedicated TLC-MALDI adapter target (*Bruker*). The MS was calibrated with a 1:1 mixture of Peptide II standards (*Bruker*, see above) in solution and ATT matrix (10 eq ATT_{sat} in MeOH/H_2O (1:1, *v/v*) and 1 eq 100 mg/ml ammonium citrate). MALDI spectra were recorded between m/z 800 and 3'600 for each TLC lane at 0.5 mm length resolution, with 11 spots measurements over 5 mm width and 10 shots/measurements.

1. Fuchs, B., Schiller, J., Süß, R., Zscharnack, M., Bader, A., Müller, P., Schürenberg, M., Becker, M., and Suckau, D. (2008) Analysis of stem cell lipids by offline HPTLC-MALDI-TOF MS. *Anal. Bioanal. Chem.* **392**, 849-860
2. White, T., Bursten, S., Federighi, D., Lewis, R. A., and Nudelman, E. (1998) High-resolution separation and quantification of neutral lipid and phospholipid species in mammalian cells and sera by multi-one-dimensional thin-layer chromatography. *Anal. Biochem.* **258**, 109-117

Maldi-MS (Autoflex I, until end of 2017)

(+)-Maldi-MS (or (–)-Maldi-MS) analyses were performed on an *Autoflex* time-of-flight mass spectrometer (*Bruker Daltonics*, Bremen, Germany) equipped with a nitrogen laser (337 nm wavelength) in the reflectron (or in the linear) mode. Around 500 spectra were summed at a scan rate of 10 Hz in the mass range between (*m/z* 800 and 4'000).

Mass calibration (examples):

Mass calibration (peptides, mass range ca. 350 – 4'000 Da): Monoisotopic masses were calibrated using signals from the HCCA matrix at *m/z* 379.09 and a mixture of peptides composed of Bradykinin 1-7 ($[M+H]^+_{mono}$ = *m/z* 757.40), angiotensin II (1'046.54), angiotensin I (1'296.68), Subtance P (1347.74), Bombesin (1619.82), renin substrate (1'758.93), ACTH clip 1-17 (2093.09), ACTH clip 18-39 (2'465.20) and Somatostatin 28 (3'147.47) all obtained from the *Bruker* peptide calibration standard II mixture.

Mass calibration (proteins, mass range ca. 4'000 – 20'000 Da): Average masses were calibrated using signals from a protein mixture composes of Insulin ($[M+H]^+_{avg}$ = *m/z* 5'734.52), Cytochrome C (6'181.05), Myoglobin (8476.66), Ubiquitin (8'565.76), Cytochrom C (12'360.97), and Myoglobin (16'952.31) all obtained from the *Bruker* Protein calibration standard I mixture.

Mass calibration (proteins, mass range ca. 10'000 – 70'000 Da): Average masses were calibrated using signals from a protein mixture composes of Cytochrome C ($[M+H]^+_{avg}$ = *m/z* 12'360), Protein A (44'613), Albumin (66'431) all obtained from the *Bruker* Protein calibration standard

Target used:

- Prespotted AnchorChip target prepared with a-cyano-4-hydroxycinnamic acid as matrix (HCCA, Bruker) or
- Ground steel target (Bruker, various matrices possible) or
- AnchorChip target with 0.6 µm Anchors (Bruker, various matrices possible).

Matrix preparation: Ask the service department if necessary.

Sample preparation (e.g.): Samples were dissolved in a solution of 50 µl H_2O + 0.1% trifluoroacetic acid. A volume of 1 µl was spotted on prespotted anchor chip target prepared with α-cyano-4-hydroxycinnamic acid matrix (HCCA, Bruker). After 1 min, the spot was rinsed with 7 *µ*l of an aqueous solution containing 10 mM ADHP buffer.