Supplementary material

MALDI imaging in human skin tissue sections: focus on various matrices and enzymes

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Figure S1

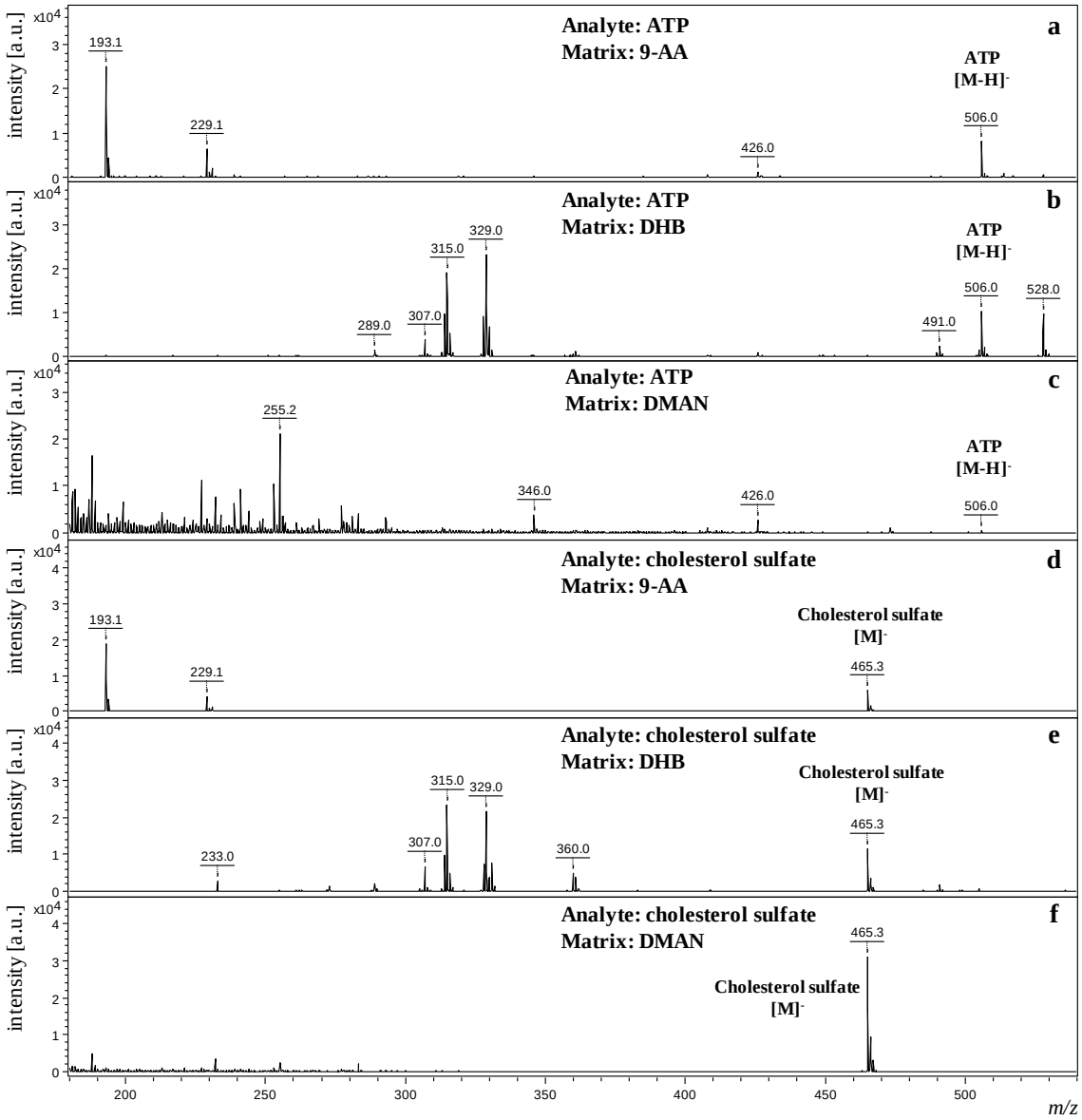

Fig. S1 Standard on-target preparation (ground steel target, Bruker Daltonics) of pure adenosine triphosphate (ATP) and cholesterol sulfate with various matrices: **a** ATP and 9-aminoacridine (9-AA); **b** ATP and 2,5-dihydroxybenzoic acid (DHB); **c** ATP and 1,8-bis(dimethylamino)naphthalene (DMAN); **d** cholesterol sulfate and 9-AA; **e** cholesterol sulfate and DHB; **f** cholesterol sulfate and DMAN

Preparation details:
Matrix solutions:
9-AA, 20 $mgmL^{-1}$ in methanol–deionized water (50:50, v/v)
DHB, 20 $mgmL^{-1}$ in acetonitrile–deionized water (30:70, v/v)
DMAN, 20 $mgmL^{-1}$ in ethanol
Sample solutions:
ATP, 1 $mgmL^{-1}$ in deionized water
Cholesterol sulfate, 1 $mgmL^{-1}$ in 2-propanol–deionized water (50:50, v/v)
Preparation protocol:
Equal volumes (2 µL each) of sample solution and matrix solution were pre-mixed. Subsequently, an aliquot (0.5 µL) was applied onto a ground steel target.

Figure S2

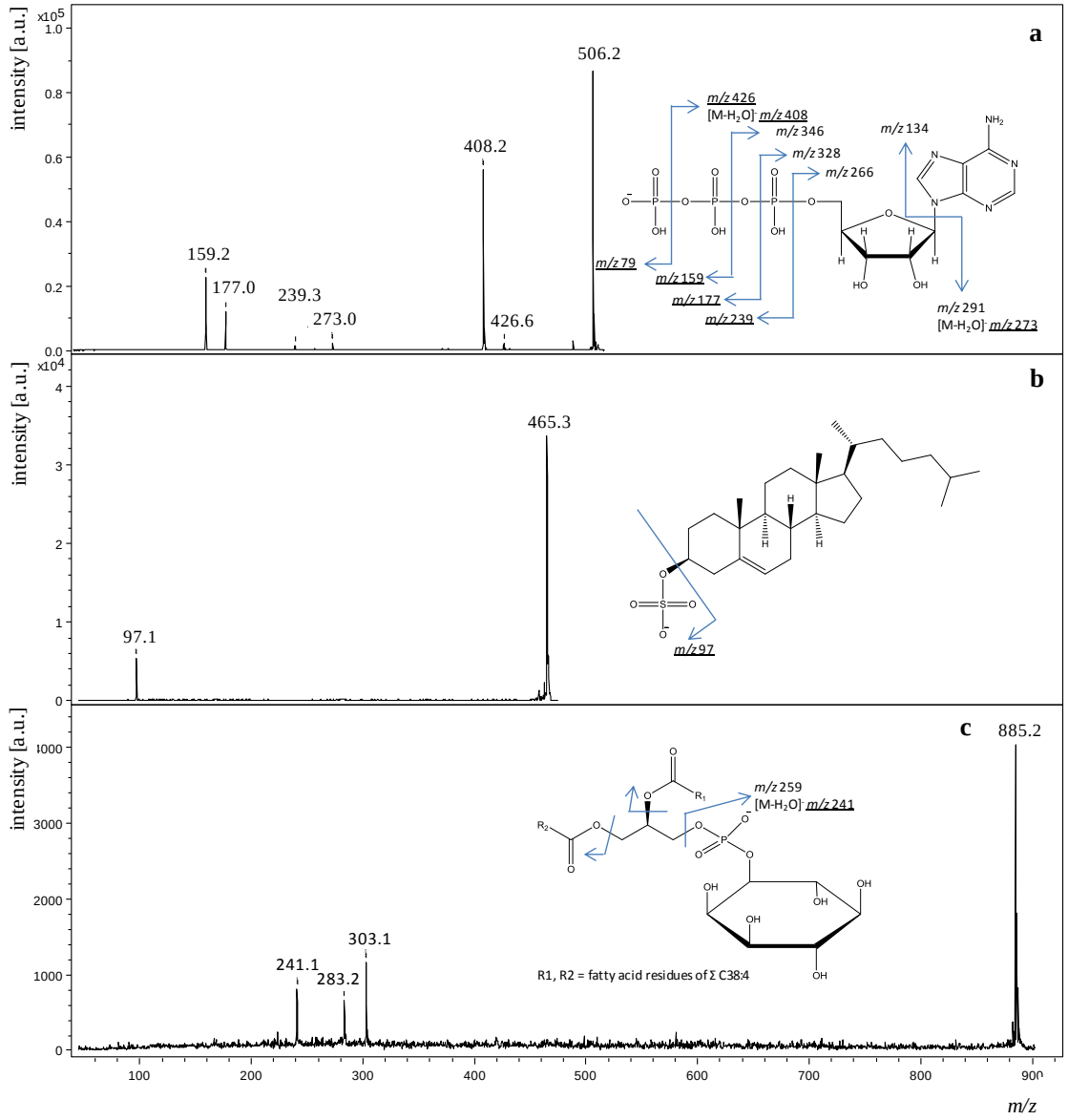

Fig. S2 PSD spectra recorded directly in a human skin tissue section using 9-AA as the matrix and operating in the negative reflector mode: **a** ATP $[M-H]^-$ *m/z* 506; **b** Cholesterol sulfate $[M]^-$ *m/z* 465; **c** phosphatidylinositol C38:4 $[M-H]^-$ *m/z* 885. Structural information and potential cleavage sites are presented within the figure

Figure S3

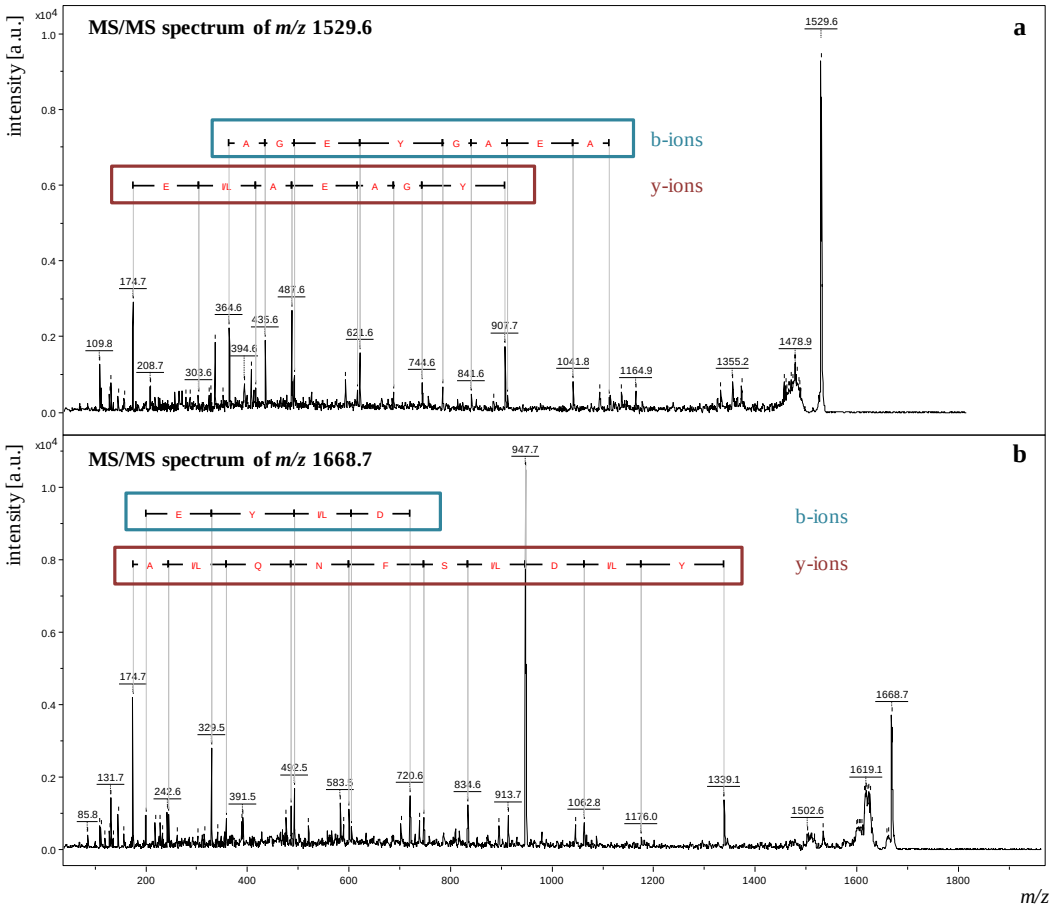

Fig. S3 MS/MS spectra of *m/z* 1529.6 (**a**) and *m/z* 1668.7 (**b**) recorded in a skin tissue section eluate after mixing with α-cyano-4-hydroxycinnamic acid matrix. Series of b-ions and y-ions are annotated in the spectra, respectively. Data were exported to an in-house MASCOT database search platform (Matrix Science). Results were as follows: **a** VGAHAGEYGAEALER, protein: HBA_HUMAN, score: 69; **b** SLEYLDLSFNQIAR, protein: LUM_HUMAN, score: 74

Figure S4

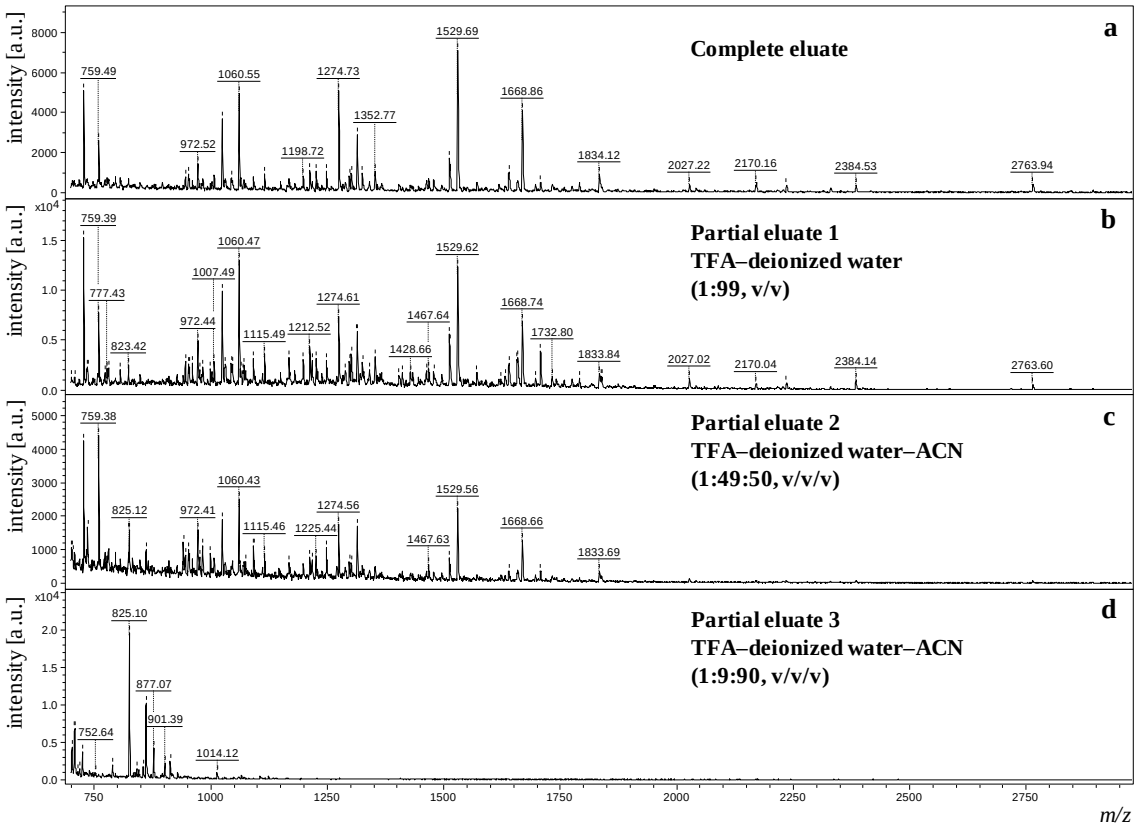

Fig. S4 MS spectra of the different eluates derived from skin tissue sections: **a** complete eluate with TFA–deionized water (1:99, v/v), TFA–deionized water–ACN (1:49:50, v/v/v) and TFA–deionized water–ACN (1:9:90, v/v/v); **b** partial elution with TFA–deionized water (1:99, v/v); **c** partial elution with TFA–deionized water–ACN (1:49:50, v/v/v); **d** partial elution with TFA–deionized water–ACN (1:9:90, v/v/v)
TFA trifluoroacetic acid, *ACN* acetonitrile

Figure S5

Elastin, Homo sapiens

P15502, **ELN_HUMAN**

(UniProtKB/Swiss-Prot)

Molecule processing:

[1-26] Signal peptide; [27-786] Elastin

Theoretical cleavage sites of:

Trypsin T

Cleavage sites*: R, K

No. of potential cleavages: 39

Pepsin P

Cleavage sites*: F, T, W, L

No. of potential cleavages: 97

Identified peptides from elastin:

Elastase E

Cleavage sites**: A, V, S, G, L/I

No. of potential cleavages: >100

Identified peptide by MALDI-MSI:

< > RFPGVGVLPGVPT

Elastase E

In-solution digestion of human elastin

Identified peptides by MALDI-TOF/TOF-MS (selection):

< >

* The cleavage specificities (cleavage rules and exceptions) of trypsin and pepsin can be looked up in the open ExPASy Bioinformatics Resource Portal http://web.expasy.org/peptide_cutter/peptidecutter_enzymes.html

The cleavage occurs at the right side (C-terminal direction) of the marked amino acid.

** Manufacturer´s information sheet (Promega)

Fig. S5 Sequence of elastin (ELN_HUMAN): T theoretical cleavage sites of **trypsin**; P theoretical cleavage sites of **pepsin**; E identified peptide from elastin subsequent to on-tissue digestion using **elastase**; E identified peptides from elastin subsequent to in-solution digestion of purified human elastin by use of **elastase**