

Supplementary Materials

of the paper

Fast Confirmation of Antibody Identity by MALDI-TOF-MS Fingerprints

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Materials

BAM-mab 01 (Anti-CBZ) and BAM-mab 02 (Anti-E1) monoclonal antibodies were obtained from BAM Division 1.8. One hundred randomized monoclonal antibody samples were purchased from InVivo BioTech GmbH (Hennigsdorf, Germany). Bovine serum albumin (BSA, 23209), Coomassie Bradford reagent (23200), trifluoroacetic acid > 99,5 % (TFA, 85183) and Pierce C₁₈ Tips, 10 µL bed (87782) were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). α-Cyano-4-hydroxycinnamic acid > 99 % (CHCA, 70990), trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene] malononitrile > 99 % (DCTB, 87884) and dithiothreitol > 99 % (DTT, D0632) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Protein G Mag Sepharose Xtra (11545664) was purchased from GE Healthcare (Chicago, Illinois, USA). Formic acid 98 % (FA, 94318) was obtained from Honeywell Fluka. Tris(2-carboxyethyl)phosphine hydrochloride 98 % (TCEP, 580560) was purchased from Calbiochem (San Diego, USA). Acetonitrile 99.95 % (ACN, 2697) and tetrahydrofuran 99.9 % (THF, 775) were purchased from Th. Geyer GmbH & Co. KG (Renningen, Germany). Lab water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) with a resistivity of > 18.2 Ω and TOC value of < 5 ppb. Poly(methyl methacrylate) (PMMA) was obtained from PSS Polymer Standard Service GmbH.

MALDI TOF MS calibration

For MALDI MS calibration, a mixture of two poly(methyl methacrylate) polymers (PMMA) with average molar masses of 1960 g/mol and 5050 g/mol were used. In order to enhance ionization and facilitate peak recognition, a few crystals of potassium trifluoroacetate were added to the trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) MALDI matrix before mixing it with the polymer. A reference list containing the masses of the potassium adducts of the n-mers of the polymer mixture was then used to calibrate the instrument. **Table S1** contains this information.

Table S1. Reference mass list for the potassium adducts of the PMMA mixture.

PMMA n-mer + K ⁺	m/z, average mass
10-mer	1042.27
15-mer	1542.85
20-mer	2043.43
25-mer	2544.01
30-mer	3044.59
35-mer	3545.17
40-mer	4045.75
45-mer	4546.33
50-mer	5046.91
55-mer	5547.48
60-mer	6048.06
65-mer	6548.64
70-mer	7049.22
75-mer	7549.80

Optimization of protein cleavage time

Optimization of protein cleavage time was performed by applying different reaction times to the same antibody sample during protein cleavage. **Figure S1** shows the fingerprint obtained from antibody cleavage at one hour (a), two hours (b), three hours (c), four hours (d), five hours (e), and sixteen hours (f). The most information-rich spectrum was obtained after 5 h of protein cleavage.

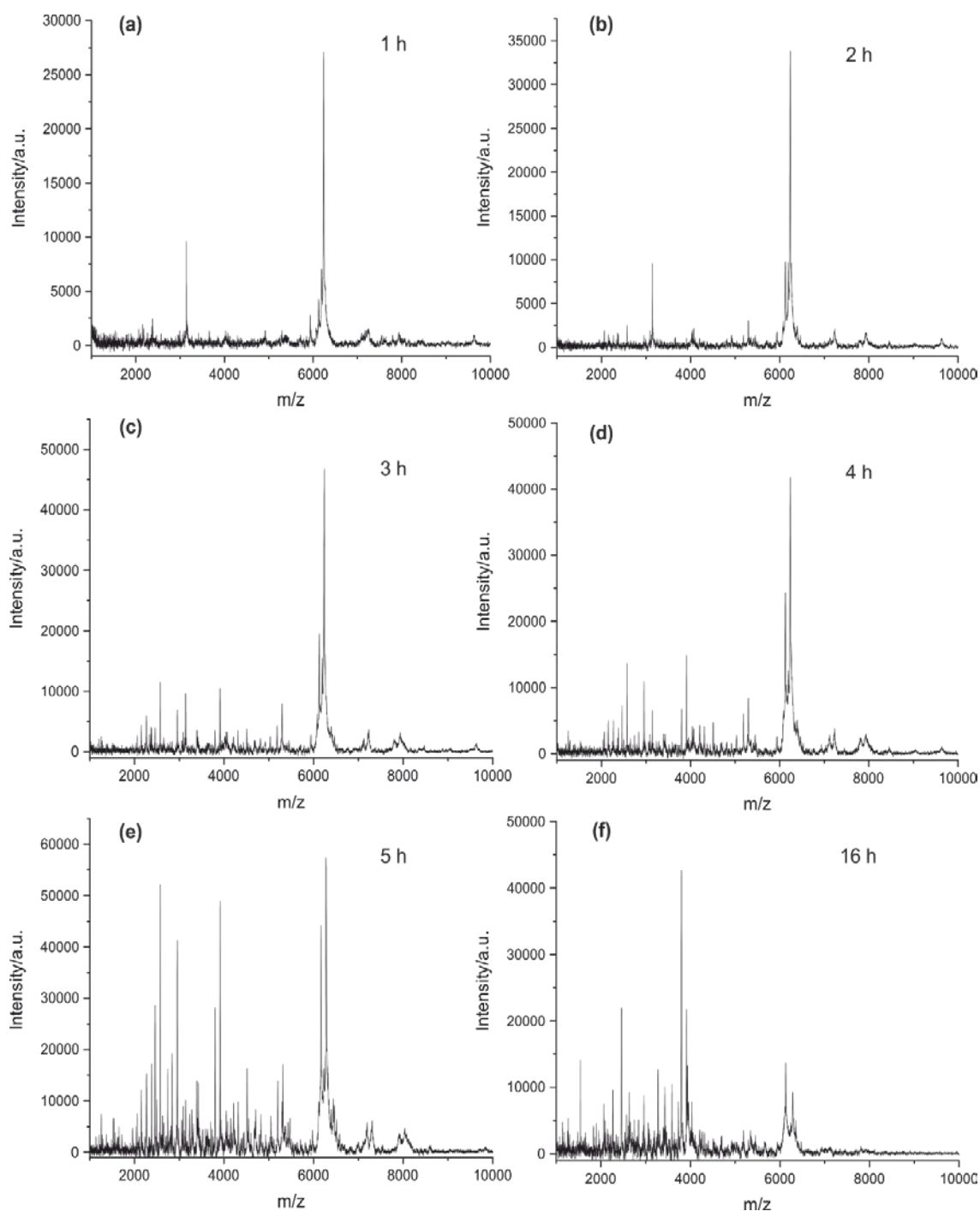


Figure S1. Optimization of protein cleavage time. MALDI-TOF-MS spectra of mouse IgG₁ cleaved at 90 °C for one (a), two (b), three (c), four (d), five (e), and sixteen hours (f).

Bradford assay

Prior to protein cleavage, the concentration of the antibodies was determined via Bradford Assay. For each sample, three aliquots of 10 μL were mixed with 200 μL of Bradford reagent on a microtiter plate. The calibration of the assay was performed with the human therapeutic antibody Adalimumab. The mixture of sample aliquots and Bradford reagent was put in the dark for 10 min before measuring the absorption of each cavity with a microplate reader (595 nm) The concentrations were automatically calculated using the software *Gen5*. **Figure S2** shows the antibody amount of each sample prior to protein cleavage.

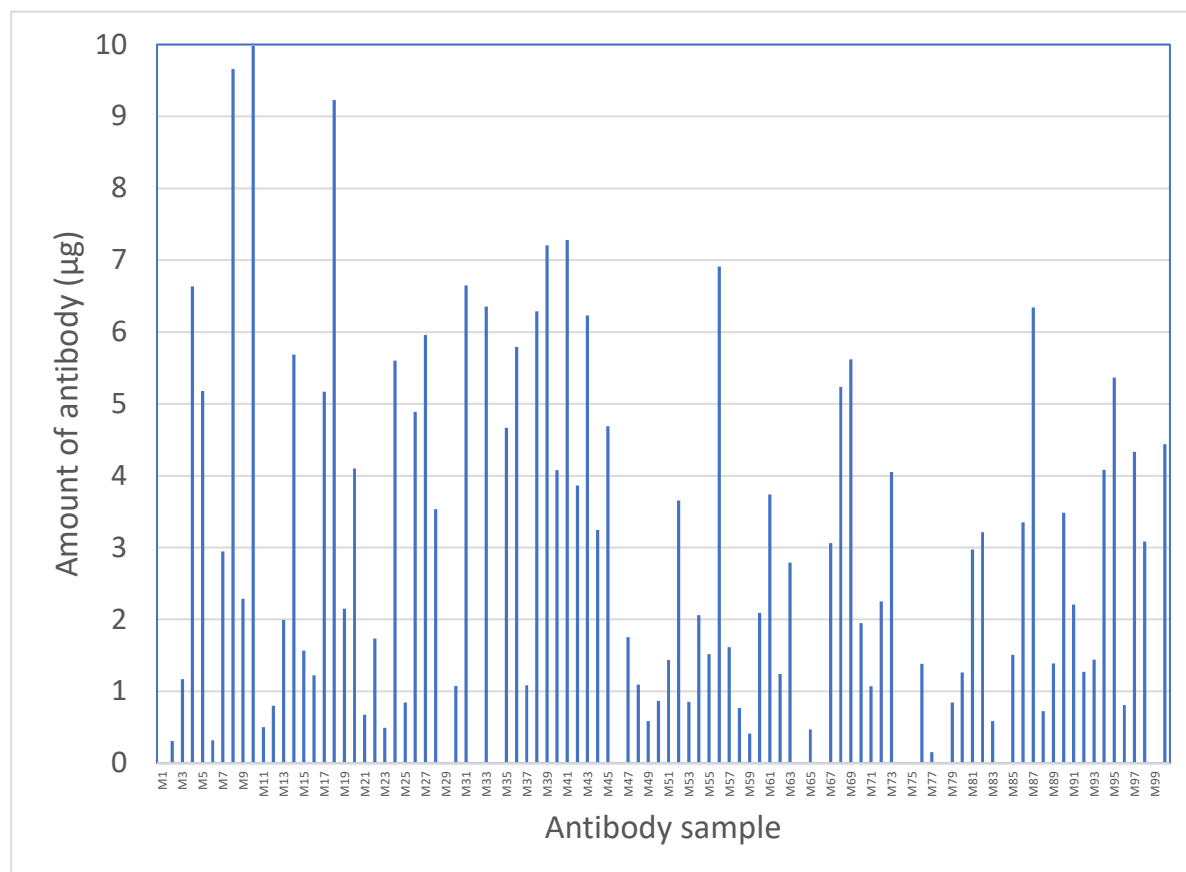


Figure S2. Antibody amount of each sample prior to protein cleavage determined via Bradford assay. For several samples, the concentration of the antibody was below the limit of detection of the Bradford-Assay ($\sim 1 \mu\text{g}$). In some of these cases, it was not possible to obtain an information-rich fingerprint spectrum. However, the majority of the samples (89 in total), even if below the quantification limit of the Bradford assay, still gave decent fingerprint spectra. Samples with antibody concentrations above $1 \mu\text{g}$ always resulted in information-rich spectra.

Description of ABID software

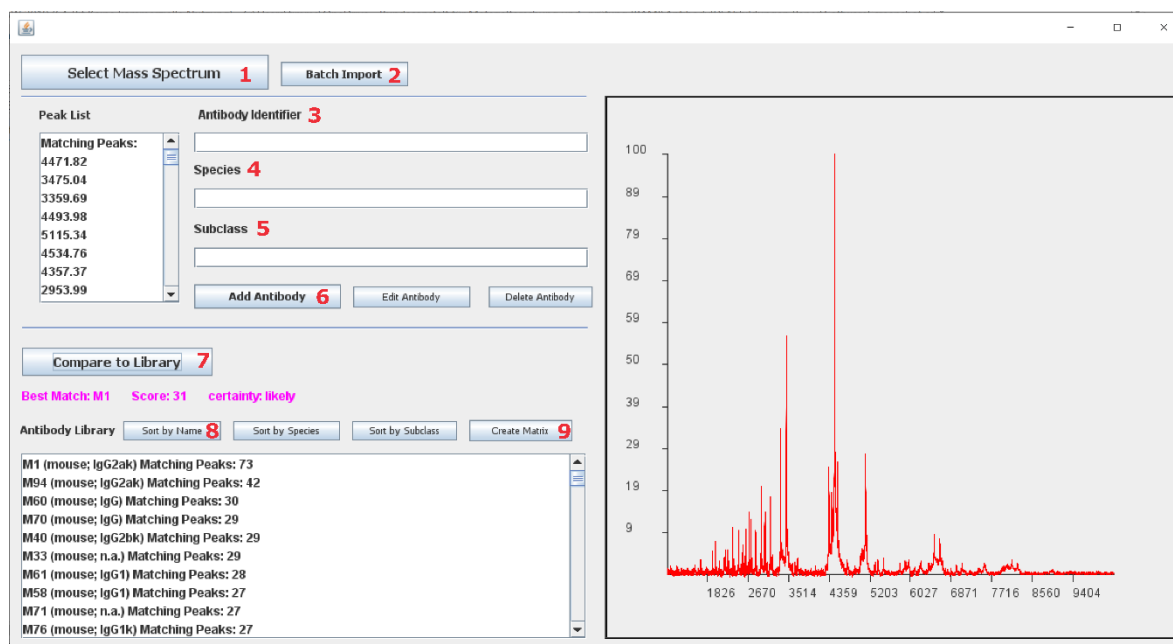


Figure S3. Screenshot of the software tool ABID.

The Software Tool ABID is a standalone Java application running on any device with Java version 8 or higher. Click on ABID.jar to open a new interface showing several buttons and options. Select your ASCII file (e.g., *.txt, *.dat) fingerprint spectrum file (“Select Mass Spectrum”) and load it into the software (1). For an uploaded spectrum, the name of the antibody must be defined (3). If known, the species of origin (4) and subclass (5) of the antibody can be added. Click on “Add Antibody” (6) to add the fingerprint to the library. To compare an uploaded spectrum to a created library, click on “Compare to Library” (7). The library is automatically sorted by the number of matching peaks. The user can also sort the library by names of the antibodies, subclass, and species (8). To create a correlation matrix, click on “Create Matrix” (9). An ASCII file is generated that can easily be imported into Excel.

The attached .zip files to this publication contain the Java software tool ABID and all the 89 antibody fingerprints generated during this study as ASCII files. Using the button “Batch Import” (2), the user can import multiple spectra at once by selecting the directory of the spectra. This way, the same library that the authors used for this work can be easily created and used for further studies.

Specificity of antibody fingerprints (correlation matrix)

Figure S4 shows the correlation matrix for all 89 antibodies used in this study. The number of matching peaks for every antibody is given in a normalized value. The intensity in color correlates with a rising number of matching peaks.

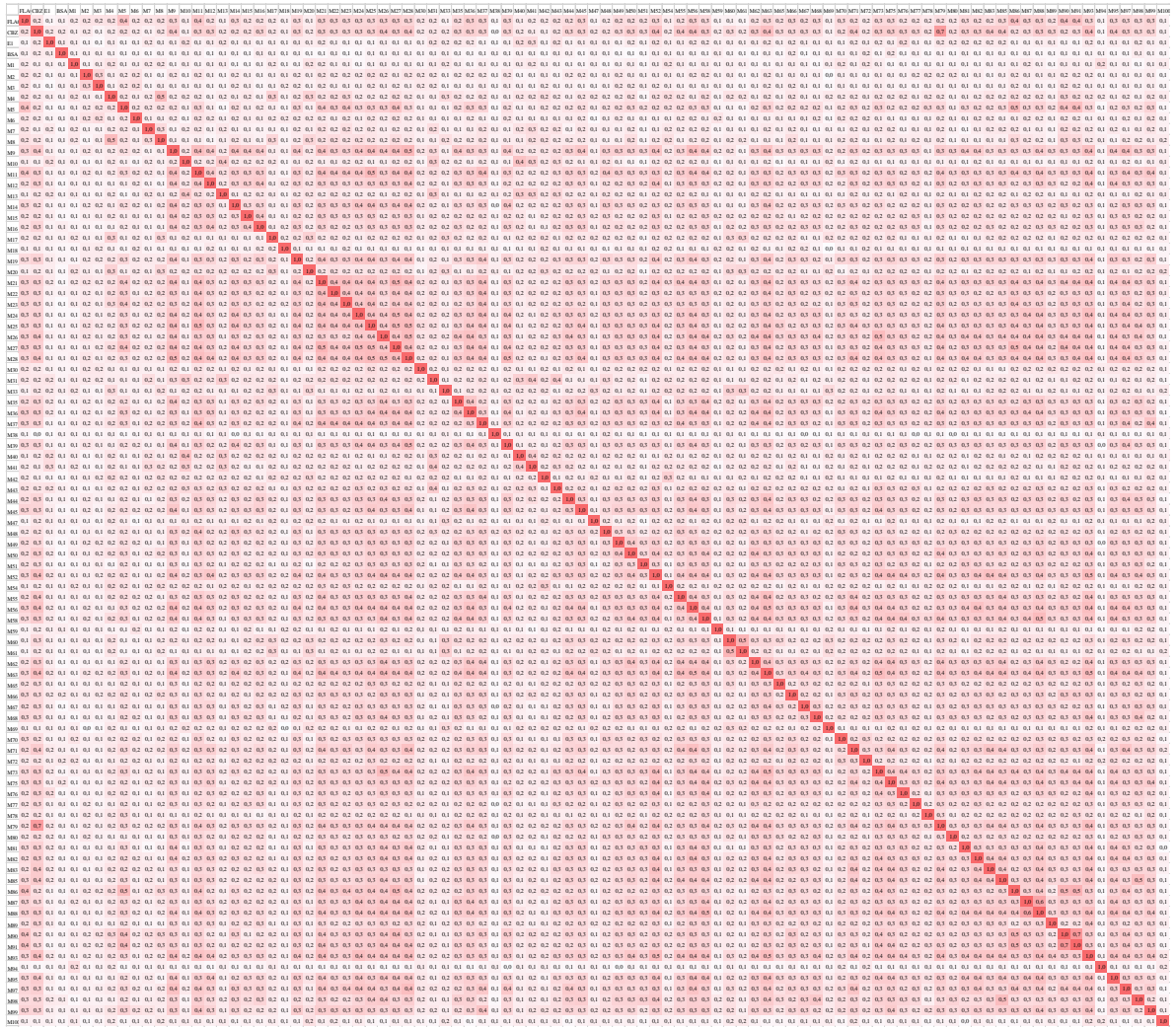


Figure S4. Correlation matrix of all 89 antibodies. The color intensity correlates with an increasing number of matching peaks.

Blinded random sampling

(a)

Compare to Library

Best Match: M1 Score: 31 certainty: likely

Antibody Library Sort by Name Sort by Species

M1 (mouse; IgG2ak) Matching Peaks: 73
 M94 (mouse; IgG2ak) Matching Peaks: 42
 M60 (mouse; IgG) Matching Peaks: 30
 M40 (mouse; IgG2bk) Matching Peaks: 29
 M33 (mouse; n.a.) Matching Peaks: 29

(b)

Compare to Library

Best Match: M10 Score: 35 certainty: likely

Antibody Library Sort by Name Sort by Species

M10 (mouse; IgG2bk) Matching Peaks: 97
 M40 (mouse; IgG2bk) Matching Peaks: 62
 M13 (mouse; IgG2b) Matching Peaks: 55
 M41 (mouse; IgG3k) Matching Peaks: 53
 M31 (mouse; n.a.) Matching Peaks: 49

(c)

Compare to Library

Best Match: M36 Score: 41 certainty: most likely

Antibody Library Sort by Name Sort by Species

M36 (mouse; IgG1) Matching Peaks: 107
 M27 (mouse; IgG1k) Matching Peaks: 66
 M26 (mouse; IgG1k) Matching Peaks: 63
 M28 (mouse; IgG1k) Matching Peaks: 61
 M56 (mouse; IgG1k) Matching Peaks: 59

(d)

Compare to Library

Best Match: M79 Score: 27 certainty: likely

Antibody Library Sort by Name Sort by Species

M79 (mouse; IgG1) Matching Peaks: 89
 M63 (mouse; IgG1k) Matching Peaks: 62
 M52 (mouse; IgG1k) Matching Peaks: 61
 M62 (mouse; n.a.) Matching Peaks: 60
 M26 (mouse; IgG1k) Matching Peaks: 59

(e)

Compare to Library

Best Match: M82 Score: 15 certainty: likely

Antibody Library Sort by Name Sort by Species

M82 (mouse; IgG1k) Matching Peaks: 72
 M9 (mouse; IgG1) Matching Peaks: 57
 M52 (mouse; IgG1k) Matching Peaks: 55
 M19 (mouse; IgG1) Matching Peaks: 52
 M27 (mouse; IgG1k) Matching Peaks: 52

Figure S5. Software-based results of the blinded samples. Randomly selected samples were (a) M1, (b) M10, (c) M36, (d) M79 and (e) M82. Samples were successfully recognized based on the highest number of matching peaks.

Fingerprint spectra of all antibodies

