

Supplementary Materials

for 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. 100 randomized monoclonal antibody samples were purchased from InVivo BioTech GmbH (Hennigsdorf, Germany). Bovine Serum Albumin (BSA, 23209) reference sample, Coomassie Bradford reagent (23200), trifluoroacetic acid > 99,5 % (TFA, 85183) and Pierce C₁₈ Tips (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-MS calibration

For MALDI-MS calibration, a mixture of two poly(methyl methacrylate) polymers with average molar weights of 1960 g/mol and 5050 g/mol was 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-propenyliden]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
PMMA 10mer + K ⁺	1042.27
PMMA 15mer + K ⁺	1542.85
PMMA 20mer + K ⁺	2043.43
PMMA 25mer + K ⁺	2544.01
PMMA 30mer + K ⁺	3044.59
PMMA 35mer + K ⁺	3545.17
PMMA 40mer + K ⁺	4045.75
PMMA 45mer + K ⁺	4546.33
PMMA 50mer + K ⁺	5046.91
PMMA 55mer + K ⁺	5547.48
PMMA 60mer + K ⁺	6048.06
PMMA 65mer + K ⁺	6548.64
PMMA 70mer + K ⁺	7049.22
PMMA 75mer + K ⁺	7549.80

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 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 ($\lambda=595\text{ nm}$). The concentrations were automatically calculated using the software *Gen5*. **Figure S1** shows the antibody mass of each sample prior to protein cleavage.

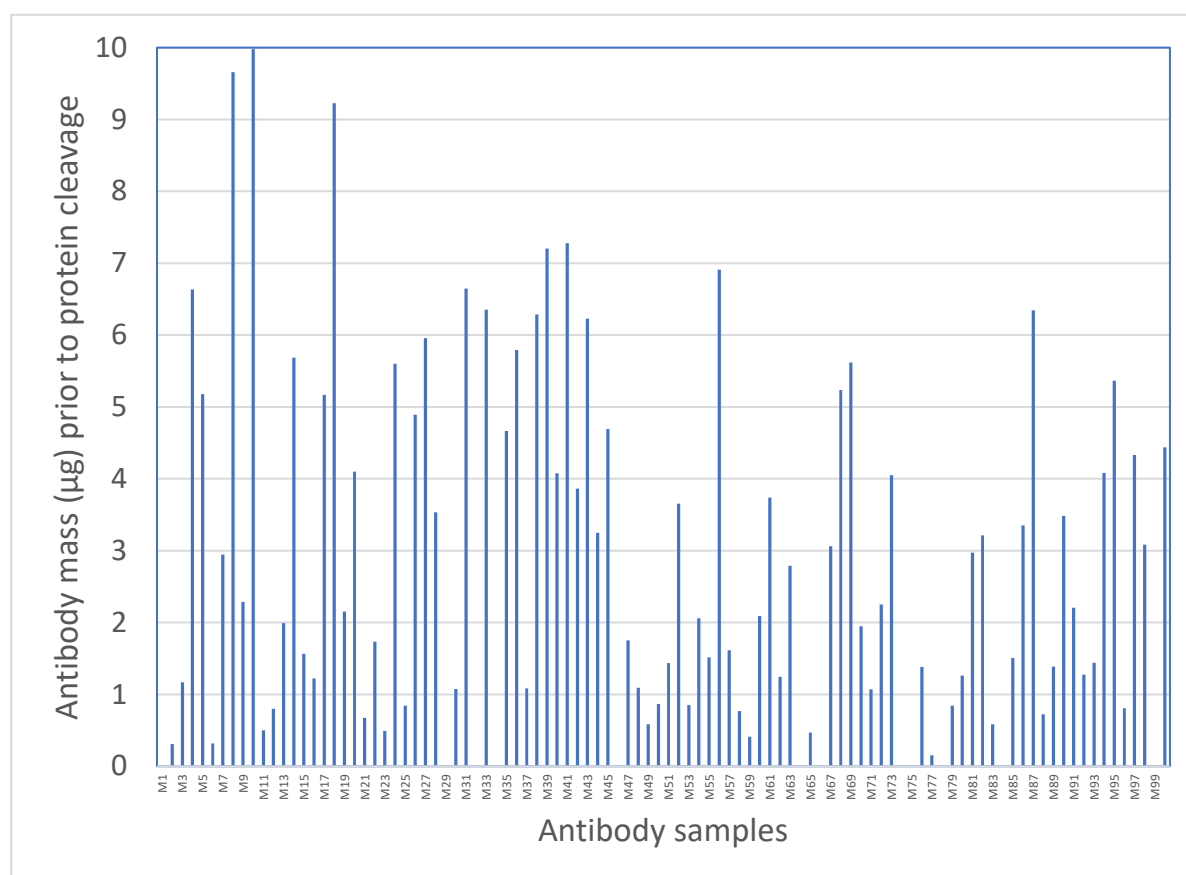


Figure S1. Antibody concentration of each sample prior to protein cleavage determined via Bradford-Assay.

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

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 S2** 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.

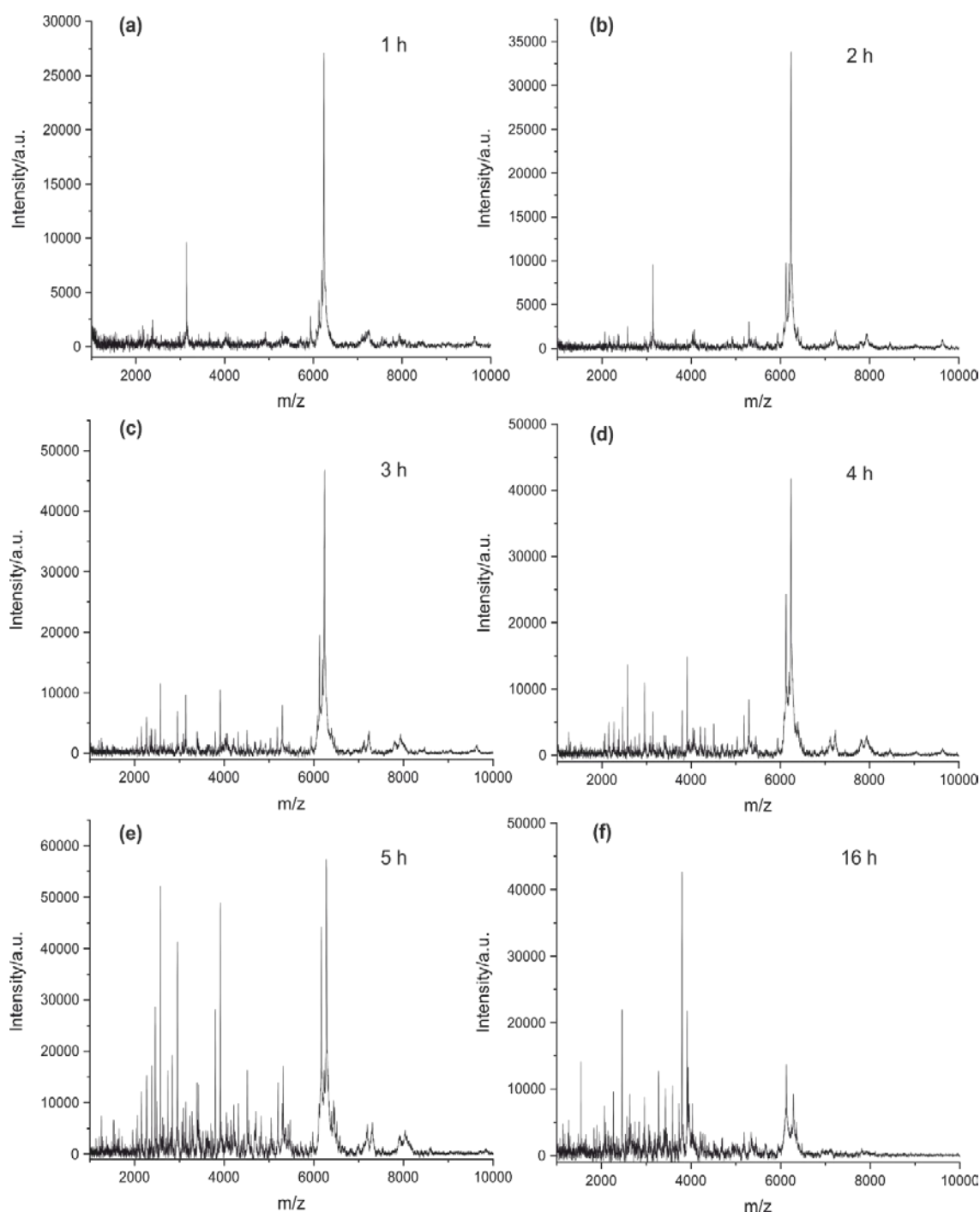


Figure S2. Optimization of protein cleavage time. MALDI-TOF-MS spectra of mouse IgG1 cleaved at 90 °C for one (a), two (b), three (c), four (d), five (e), and sixteen hours (f).

Blinded random sampling

(a)

Compare to Library

Best Match: M001 **Score: 31** **certainty: likely**

Antibody Library

M001 (Mouse; IgG2ak) Matching Peaks: 73
 M094 (Mouse; IgG2ak) Matching Peaks: 42
 M060 (Mouse; IgG) Matching Peaks: 30
 M033 (Mouse; n.a.) Matching Peaks: 29
 M040 (Mouse; IgG2bk) Matching Peaks: 29

(b)

Compare to Library

Best Match: M010 **Score: 35** **certainty: likely**

Antibody Library

M010 (Mouse; IgG2bk) Matching Peaks: 97
 M040 (Mouse; IgG2bk) Matching Peaks: 62
 M013 (Mouse; IgG2b) Matching Peaks: 55
 M041 (Mouse; IgG3k) Matching Peaks: 53
 M031 (Mouse; n.a.) Matching Peaks: 49

(c)

Compare to Library

Best Match: M036 **Score: 41** **certainty: most likely**

Antibody Library

M036 (Mouse; IgG1) Matching Peaks: 107
 M027 (Mouse; IgG1k) Matching Peaks: 66
 M026 (Mouse; IgG1k) Matching Peaks: 63
 M028 (Mouse; IgG1k) Matching Peaks: 61
 M056 (Mouse; IgG1k) Matching Peaks: 59

(d)

Compare to Library

Best Match: M079 **Score: 28** **certainty: likely**

Antibody Library

M079 (Mouse; IgG1) Matching Peaks: 90
 M063 (Mouse; IgG1k) Matching Peaks: 62
 M052 (Mouse; IgG1k) Matching Peaks: 61
 M062 (Mouse; n.a.) Matching Peaks: 60
 M026 (Mouse; IgG1k) Matching Peaks: 59

(e)

Compare to Library

Best Match: M082 **Score: 15** **certainty: likely**

Antibody Library

M082 (Mouse; IgG1k) Matching Peaks: 72
 M009 (Mouse; IgG1) Matching Peaks: 57
 M052 (Mouse; IgG1k) Matching Peaks: 55
 M019 (Mouse; IgG1) Matching Peaks: 52
 M027 (Mouse; IgG1k) Matching Peaks: 52

Figure S3. Software-based results of the random survey sampling. Randomly selected samples were (a) M1, (b) M10, (c) M36, (d) M79 and (e) M82. Samples were successfully recognized as shown in the highest number of matching peaks.

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.

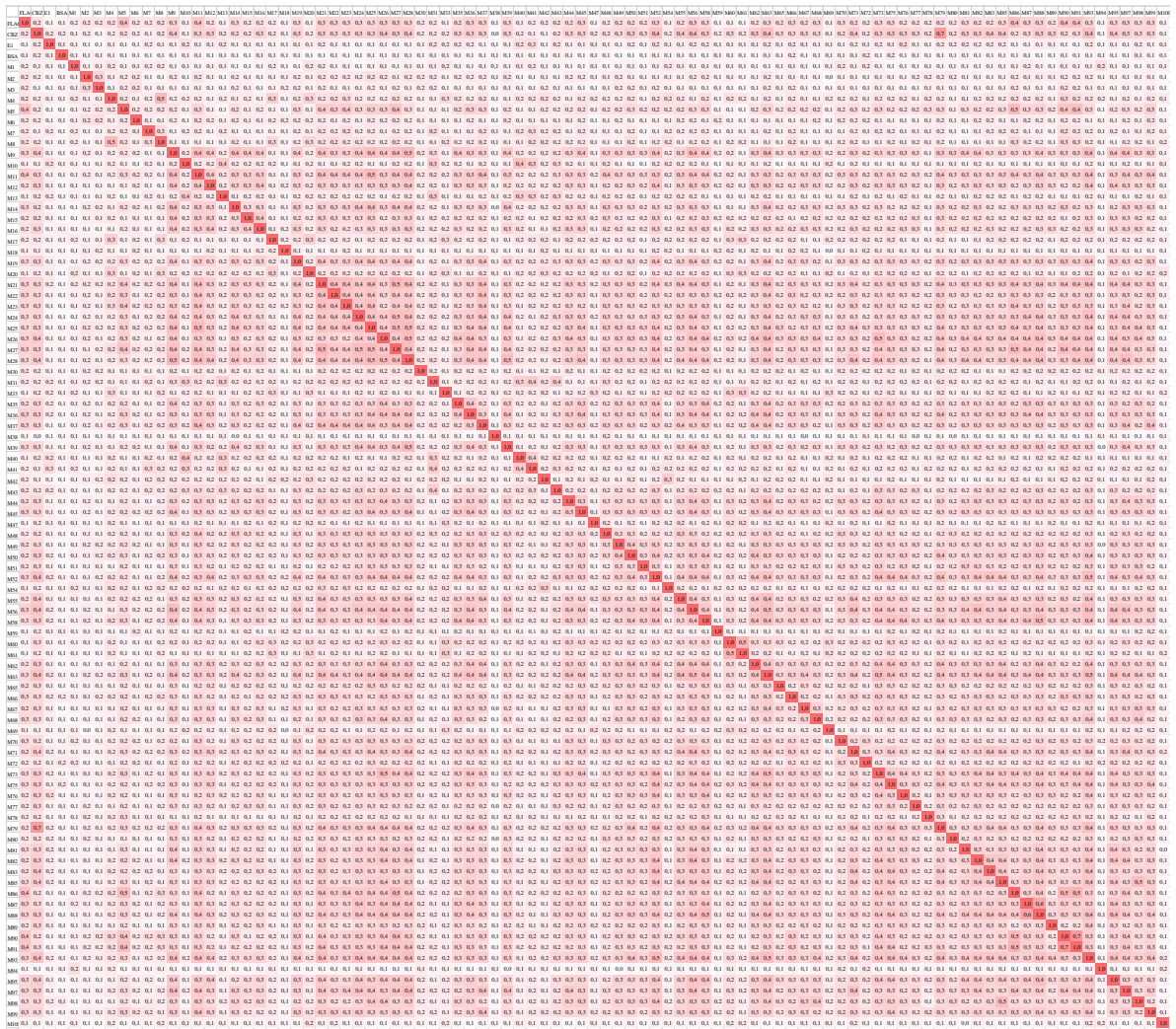


Figure S4. Correlation matrix of all 89 antibodies. The color intensity correlates with an increasing number of matching peaks.

ABID Software

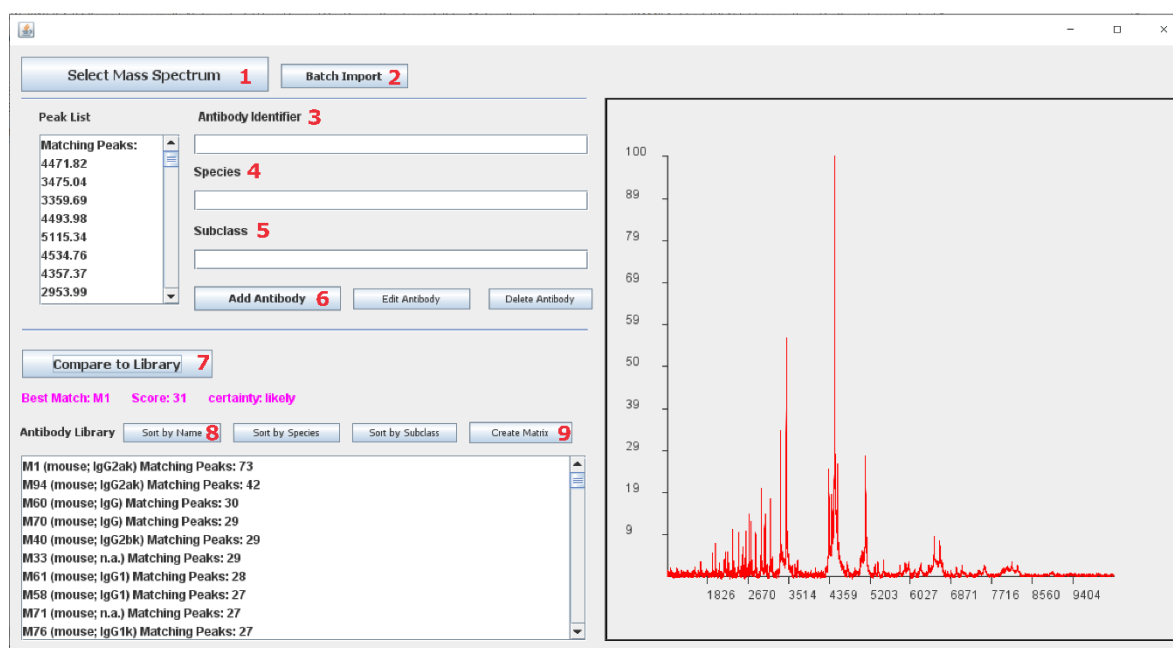


Figure S5. Screenshot of the Software Tool ABID.

The Software Tool ABID is a standalone Java application runnable 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 in 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 by others and used for further studies.

Fingerprint spectra of all antibody samples