

Supplement

MALDI-TOF-MS-based identification of monoclonal murine anti-SARS-CoV-2 antibodies within one hour

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Determination of intact and light chain masses (IgG)

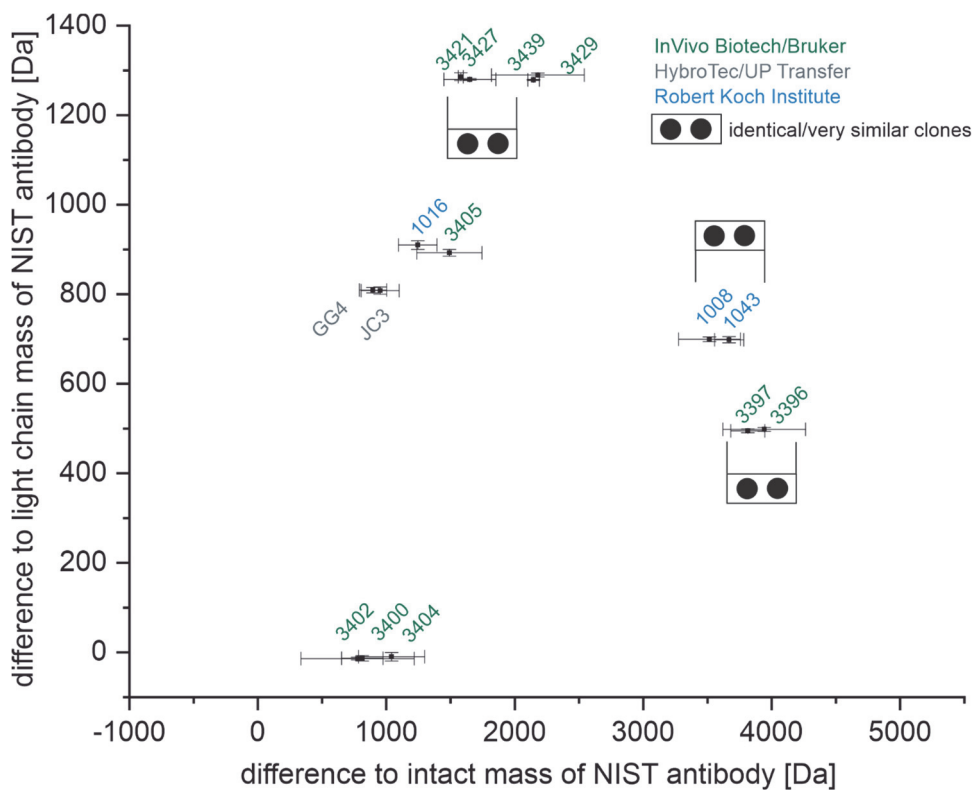


Figure S1. Light chain and intact masses of antibodies with overlapping mass ranges relative to the NIST antibody.

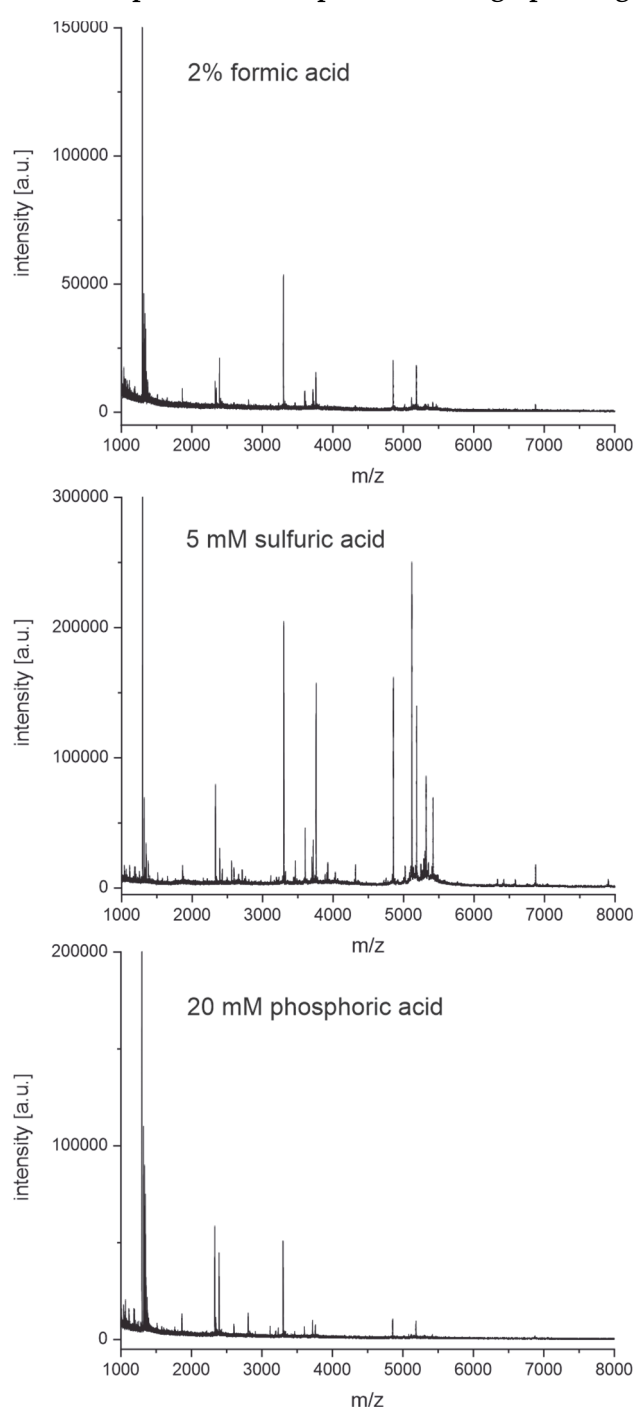
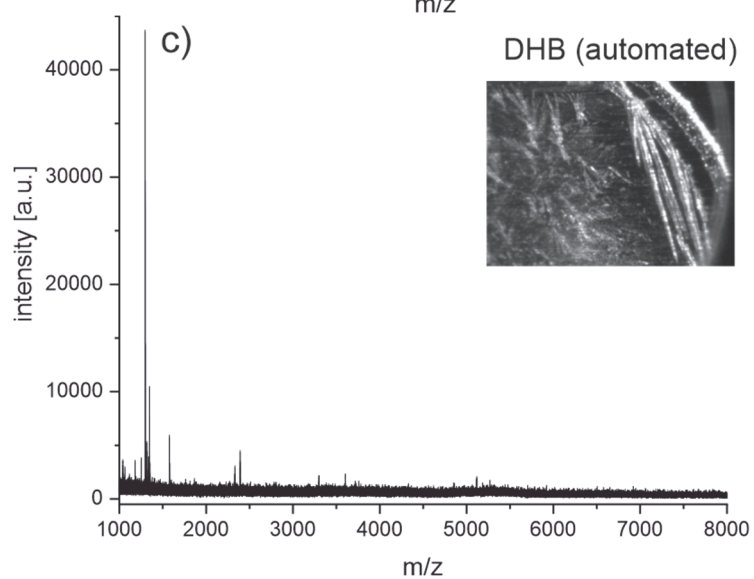
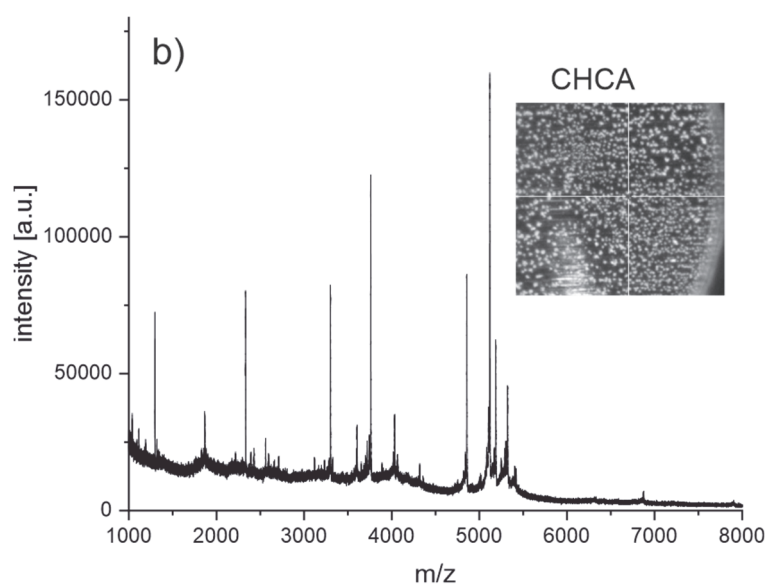
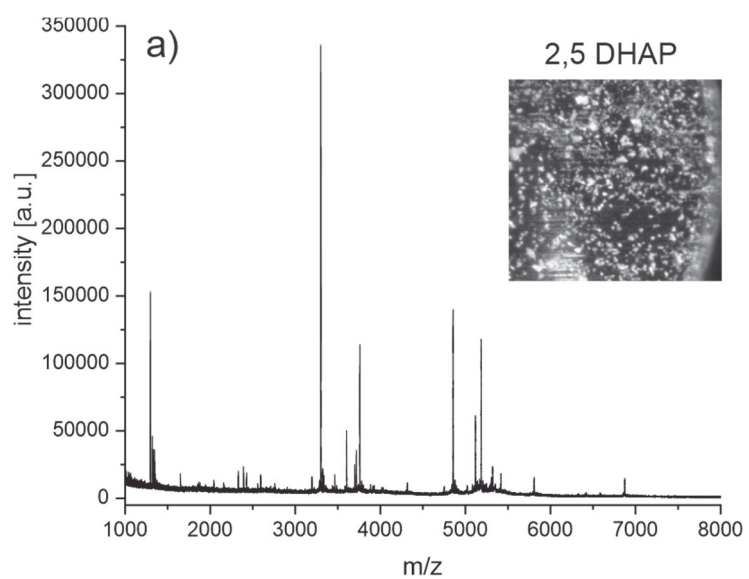
Method optimization: Peptide mass fingerprinting with different acids as the cleavage agent

Figure S2. Peptide mass fingerprint of NIST antibody (mAb 8671) was obtained from cleavage with 2% formic acid, 5 mM sulfuric acid, and 20 mM phosphoric acid.

Many diluted acids may be applied to selectively cleave at aspartic acid residues and generate fingerprints in a short time. However, as Figure S2 shows, 5 mM sulfuric acid produces the best results with a high number of peptide peaks and a good signal-to-noise ratio. Interestingly, the relative peak intensities vary greatly between the spectra, even if the peptide masses are the same. We, therefore, recommend using 5 mM sulfuric acid as a cleavage reagent to generate the fingerprints. Unwanted peptide modification, for example, formylation, is also not an issue with this cleavage reagent, compared to formic acid.

Method optimization: MALDI matrix



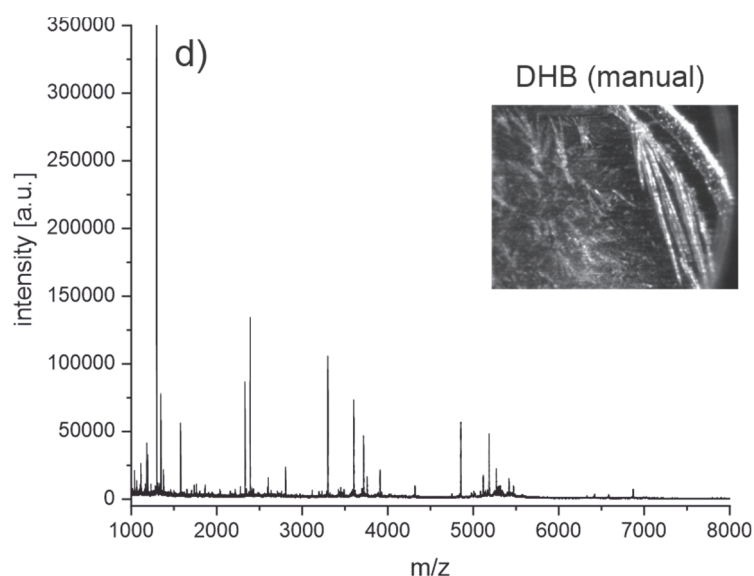


Figure S3. MALDI-TOF MS fingerprints of NIST antibody 8671 generated with different MALDI matrices: 2,5 DHAP (a), CHCA (b), DHB with an automated random pathway of the laser (c), and DHB with manual laser control (d).

The MALDI matrix seems to play a critical role in the generation of information-rich fingerprint spectra (Figure S2). The fingerprints seem to produce the same peptides but with varying relative intensities. Furthermore, 2,5-DHAP (a) and DHB (with manual laser control, d) produce spectra with more peptides and better signal-to-noise ratios than CHCA (b). However, the spectrum generated with DHB with an automated random pathway of the laser (c) is poor, even though it was acquired on the same sample spot. The sweet spot focusing can explain the differences between manual laser control and automated one. This way, the large crystals from the DHB matrix are targeted, while the automated software was not able to perform a sweet spot focusing. To facilitate automation of the workflow, we, therefore, recommend using 2,5-DHAP as the MALDI matrix. This matrix compound crystallizes homogeneously, which facilitates automation during spectra acquisition.

Sequence coverage of peptide mass fingerprinting

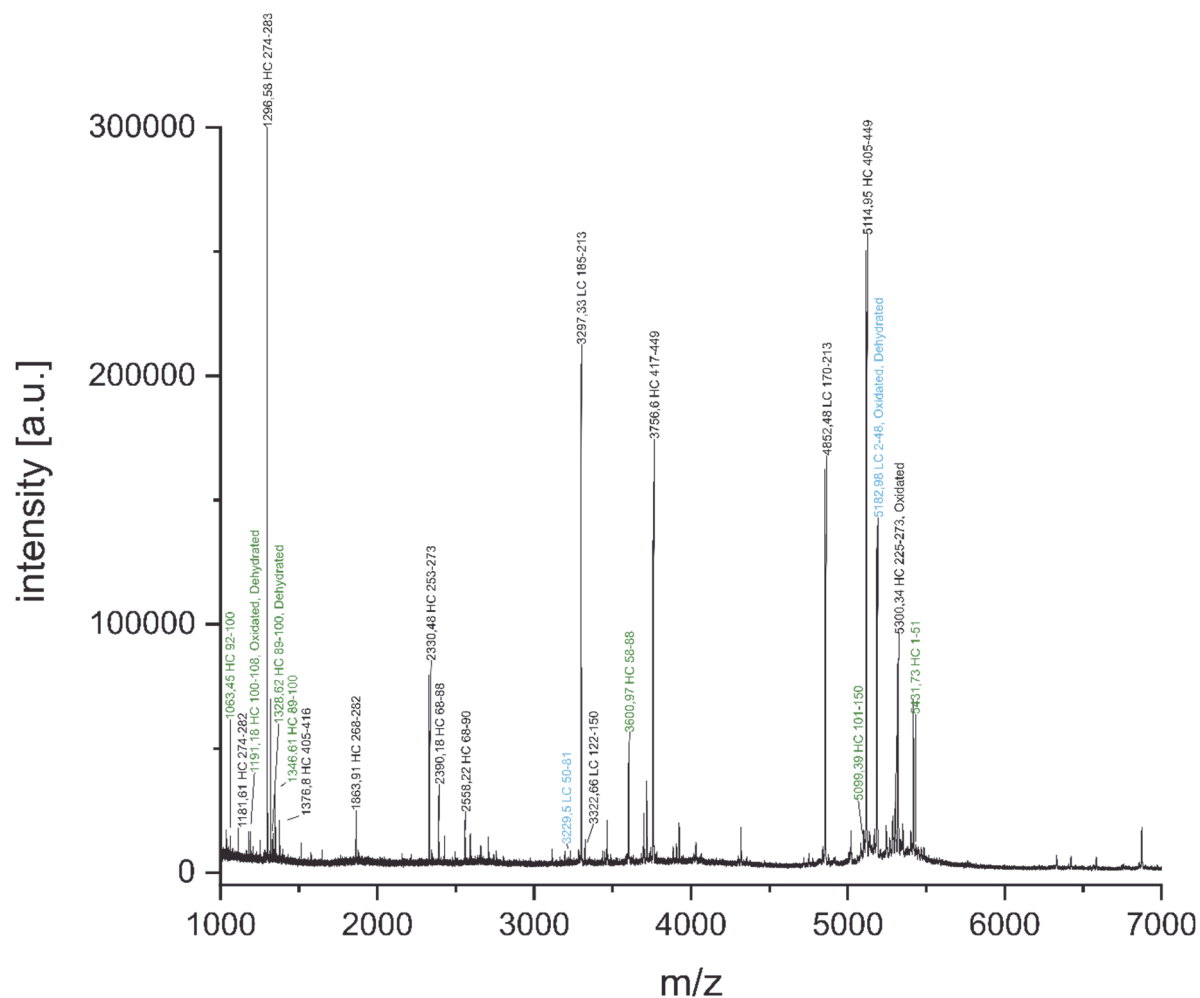


Figure S4. Acidic cleavage: MALDI-TOF fingerprint spectrum of NIST-mAb 8671 after 30 minutes incubation with diluted sulfuric acid and TCEP at 99 °C. Peptide peaks were assigned to the NIST sequence. Colored annotations mark peptides derived from CDR regions, green for the heavy chain (HC) and blue for the light chain (LC). The sequence coverage is around 61%.

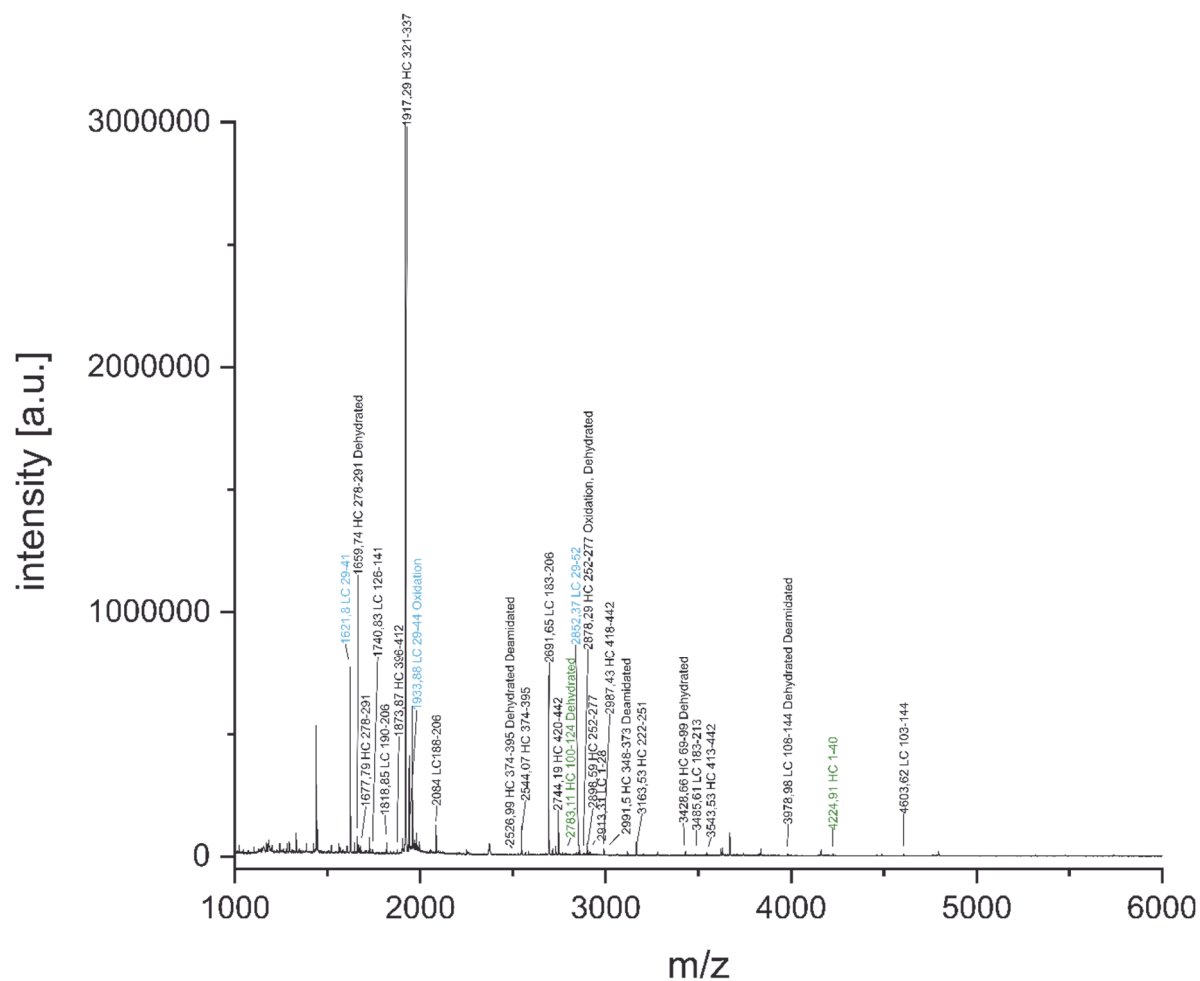


Figure S5. Tryptic digest: MALDI-TOF fingerprint spectrum of NIST-mAb 8671 after 15 minutes of incubation with TCEP at 99 °C, followed by 15 minutes of incubation with trypsin at 55 °C. Peptide peaks are assigned to the NIST sequence. Colored peaks mark peptides derived from CDR regions, green for the heavy chain (HC) and blue for the light chain (LC). The sequence coverage is around 65%.

Heavy Chain of NIST-mAb 8671

QVTLRESGPA LVKPTQTLTL TCTFSGFSL**S** **TAGMSV**GWIR QPPGKALEWL **A**DIWWDDKKH **YNPSL**KDRLT
ISKDTSKNQV VLKVTNMDPA **DTATYY**CARD **MIFNFY**FDVW GQGTTVTVSS **ASTK**GPSVFP **LAPSS**KSTSG
GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTPPAVLQSS GLYLSVSVT VPSSSLGTQT YICNVNHKPS
 NTKVDRVPEP **KSCD****K**THTCP **PC**PAPELLGG **PSV**FLPPKP KDTLMISRTP **E**VT**CV**VVDVS **HED**PEVKFNW
YVDGVEVHNA **K**TKPREEQYN STYRVVSVLT VLHQDWLNGK **EYK**CKVSNKA **LP**APIEKTIS **KAK**GQPREPQ
YTLPPSREE **M**TKNQVSLTC **LVK**GFYPSDI **A**VEWESNGQP **E**NNYK**T**TPPV **L**DS**D****G**SFFLY **S**KLTVDKSRW
QQGNVFSCSV MHEALHNHYT **Q**KSLSLSPG

Light Chain of NIST-mAb 8671

DIQMTQSPST **LS**ASVGDRVT **ITCS**ASSRVG **Y**MHWYQ**K**PG **K**APKLLI**Y****D**T **SK**LASGVPSR FSGSGSGTEF
TLTISSLQPD **D**FATYY**F**Q**G** **S**GY**P**FTFGGG TKVEIKRTVA **AP**SVF**I**PPS **DE**QLK**S**GTAS **V**VCLLN**F**Y**P**
REAKVQWKVD **N**ALQSGNSQE **S**VTEQDSKDS **T**YLSSTLTL **S**KADYE**K**HKV **Y**ACEV**T**HQGL **S**SPV**T**KS**F**NR
GEC

Figure S6. The combined sequence coverage of NIST antibody mAb 8671. Amino acids marked yellow are covered in the fingerprint spectrum generated with diluted sulfuric acid. Amino acids underlined in red are covered in the fingerprint spectrum generated with trypsin. Amino acids with bold letters are from CDR regions. The combined sequence coverage is around 82%.

Software tool ABID 2.0

In the following, a typical application of ABID 2.0 is shown, where supposed duplicates of a clone can be detected in a library of dozens of clones. Both clones, 3396 and 3397, seem to be rather similar by visual examination.

Comparison of clones 3396 and 3397:

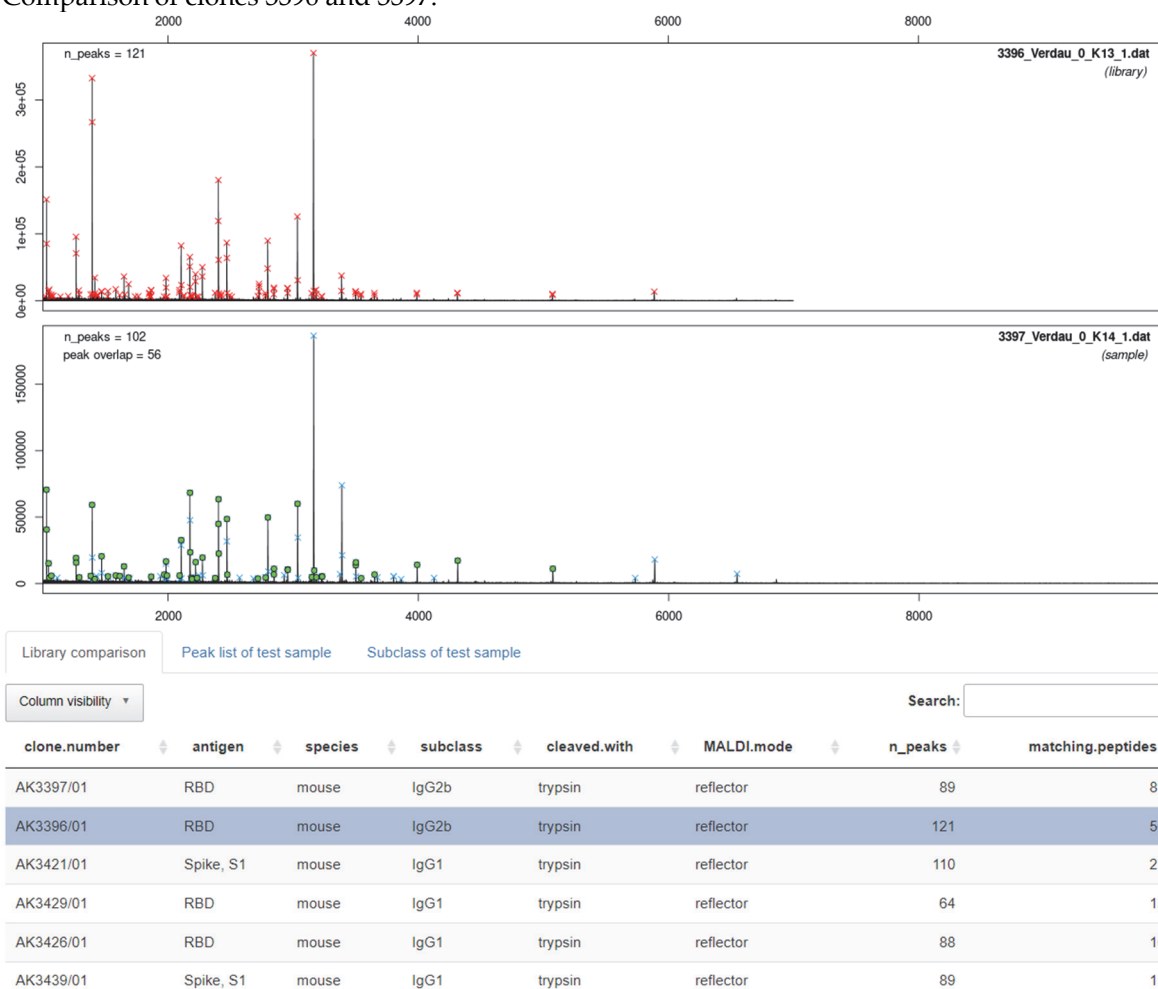


Figure S7. Comparison by ABID 2.0 software: The first spectrum is the library spectrum with the highest number of matching peaks with the sample spectrum (second spectrum). All library entries are shown in the table and ranked by the number of matching peptides (last column). The first line shows the comparison of the spectrum with itself, which leads to the result that all peaks are matching. The second line (gray bar) determines clone 3396 from the library as the highest match to the sample spectrum of clone 3397 with 56 matching peptides. The third line shows the second-best match (AK3421/01, third row) to clone 3397, which has only 21 matching peptides. The significant difference between the best hit and the second one is crucial for a decision. Based on their overlapping intact masses and light chain masses, fingerprints, subclasses, and antigens, we expect 3396 and 3397 to be very similar, if not identical.

Another feature of ABID 2.0 is the tentative determination of antibody subclasses from the fingerprint spectrum. The subclass information is inherent to the m/z values of Fc-specific peptides in the mass spectrum. ABID 2.0 was fed with peptide mass lists from *in-silico* digested Fc domains from antibodies with known sequences and subclasses. The algorithm then compares these lists with the sample spectrum to determine the best match. In Figure S11, the fingerprint spectrum of clone 1254 is loaded into the software. ABID 2.0 determines IgG1 as the subclass since the fingerprint spectrum has 7 peptide peaks that have the same masses as other IgG1 specific peptides. Conventional subclass determination by ELISA shows the same result for clone 1254.

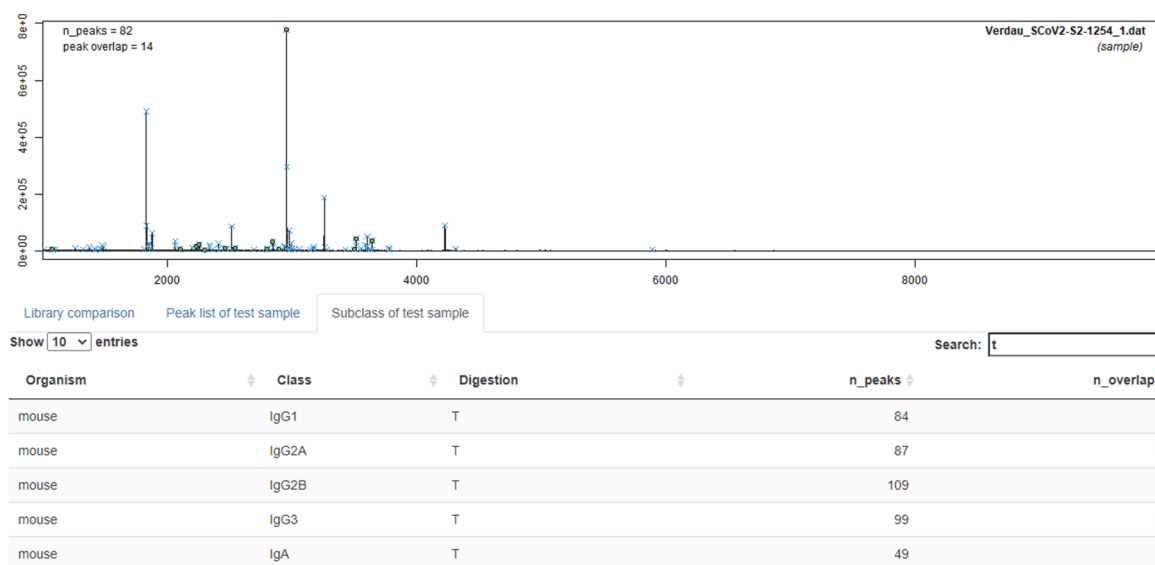
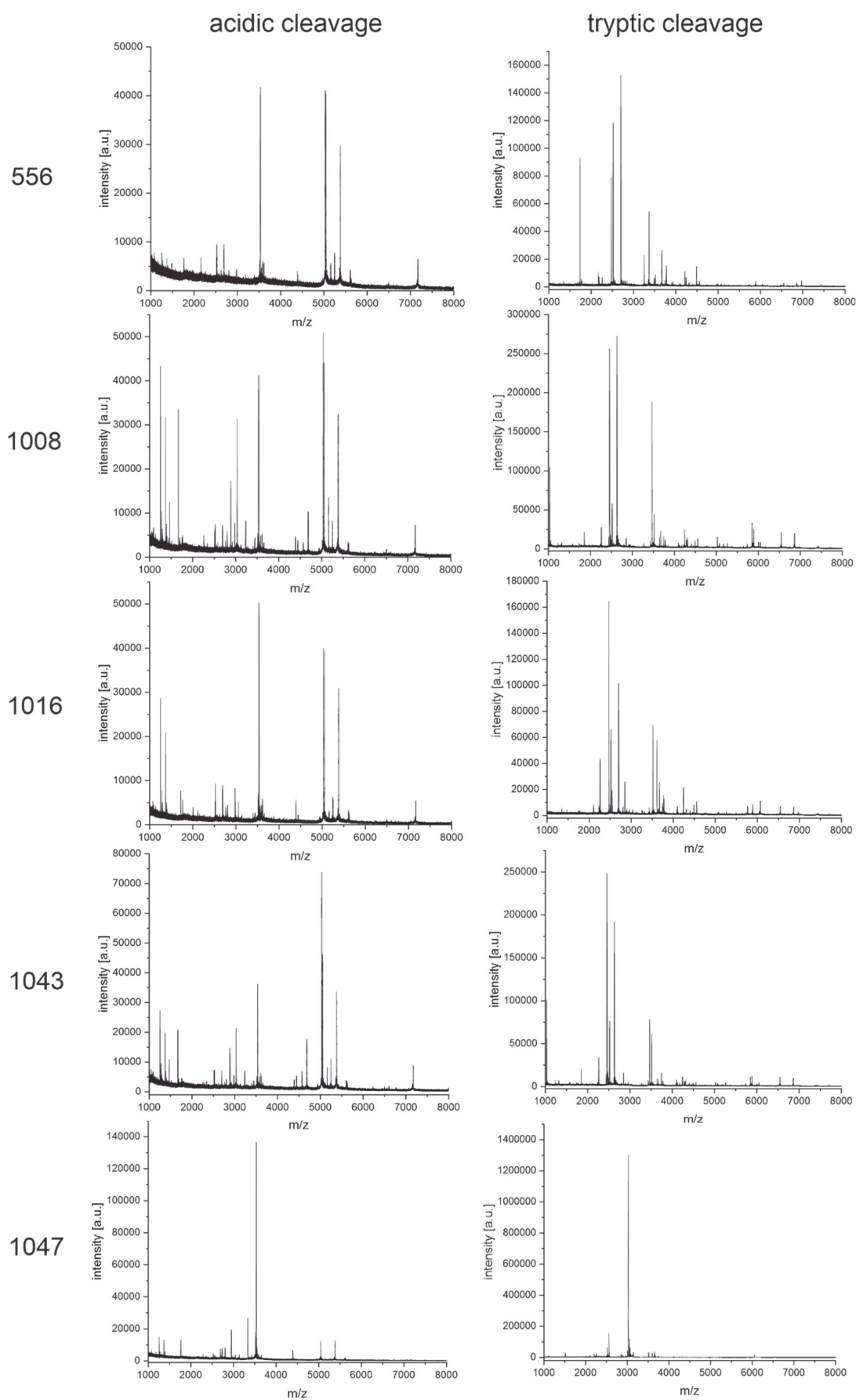
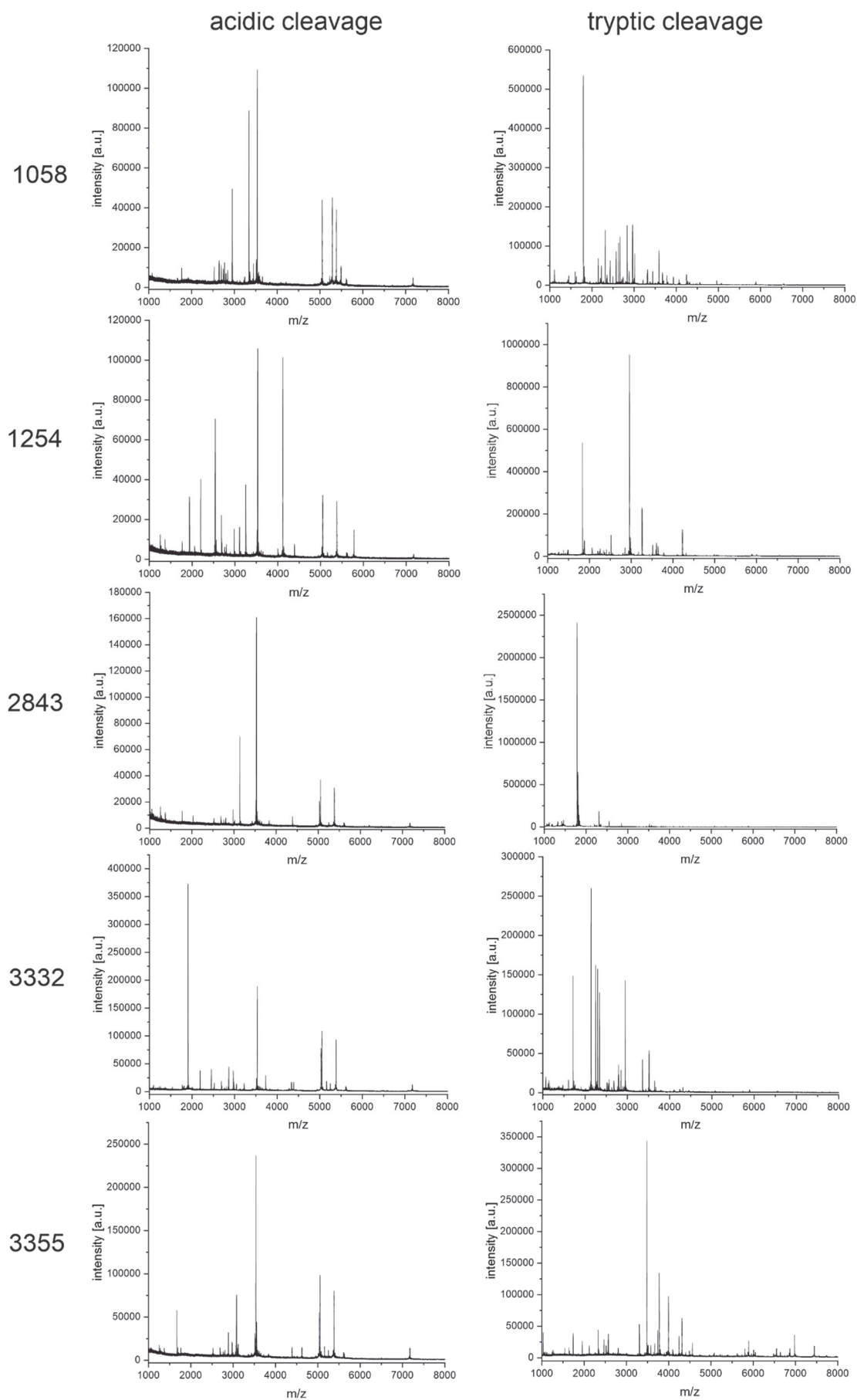


Figure S8. Antibody subclass determination: The tool ABID 2.0 compares peptide mass lists from *in-silico* digested Fc domains from antibodies with known sequences and subclasses. The fingerprint spectrum of clone 1254 contains 7 peptides that are also found in other antibodies with the subclass IgG1. Conventional subclass determination by sandwich ELISA discriminating between IgG1, IgG2a, IgG2b, and IgG3 shows the same result (data not shown).

MALDI-TOF-MS spectra of all clones (acidic and tryptic cleavage)

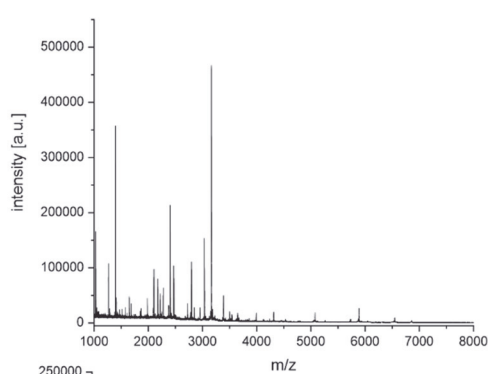
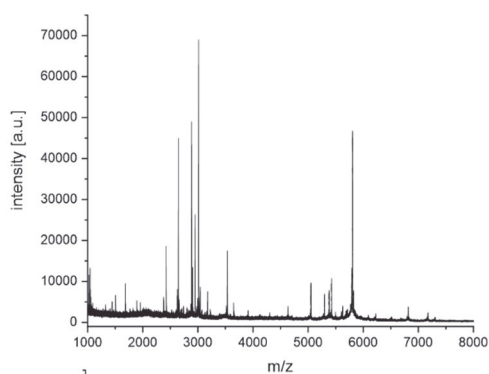




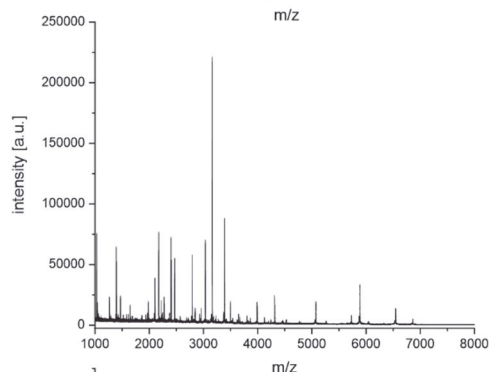
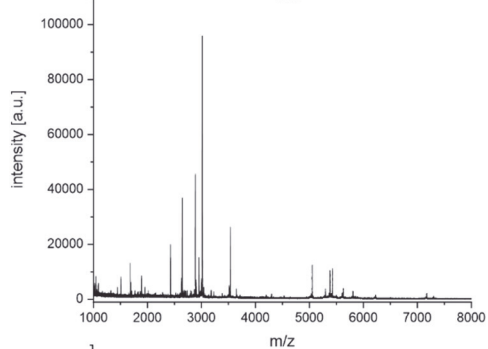
acidic cleavage

tryptic cleavage

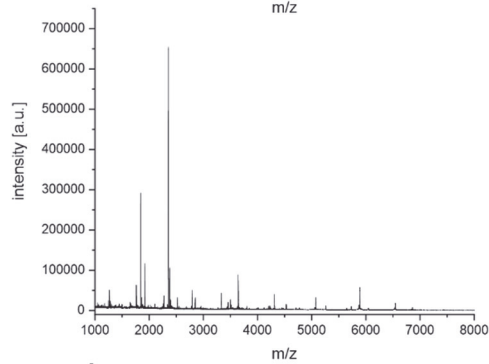
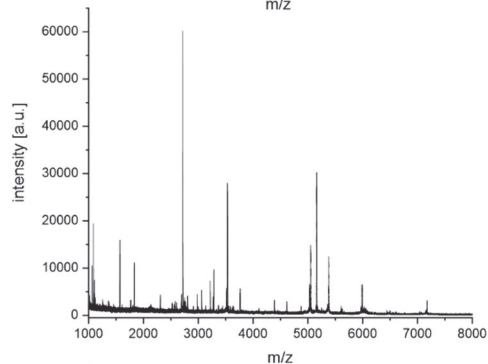
3396



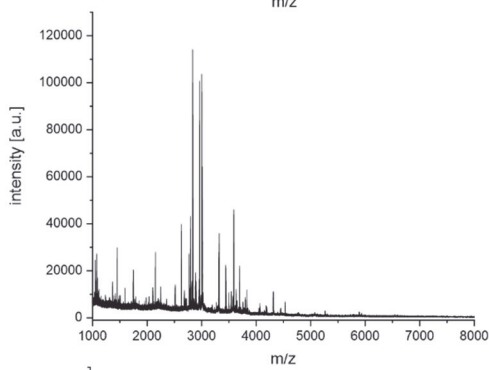
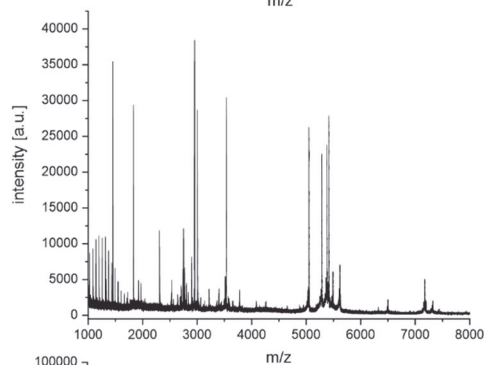
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3398



3400



3402

