

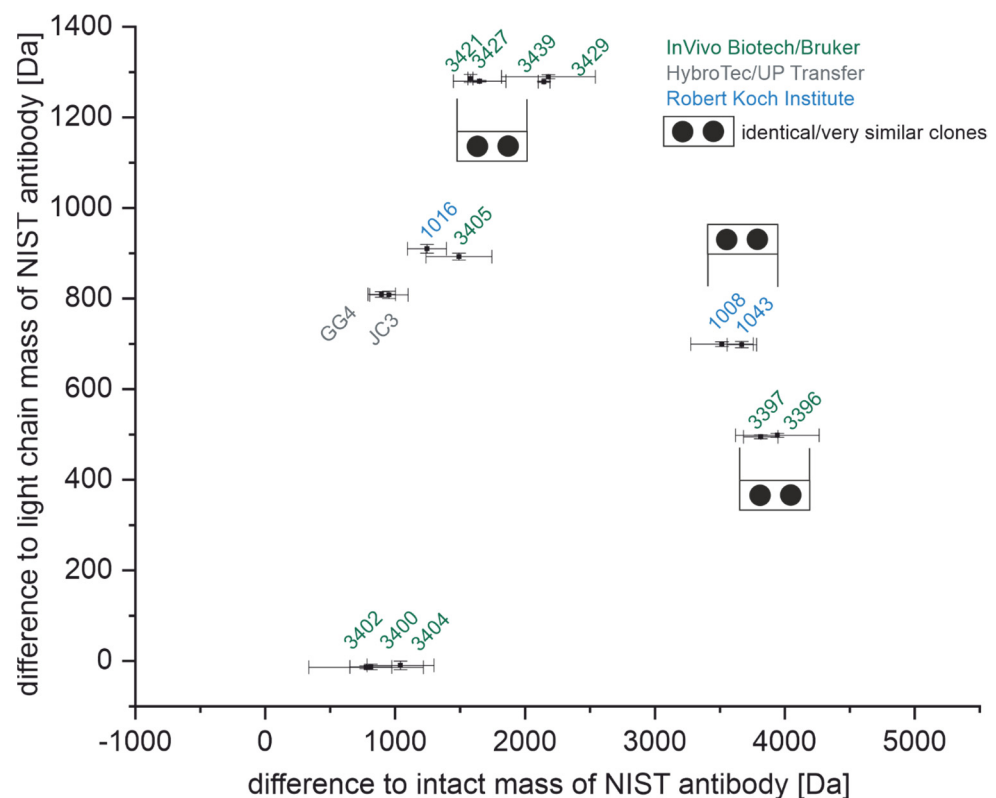
/Supplementary materials

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

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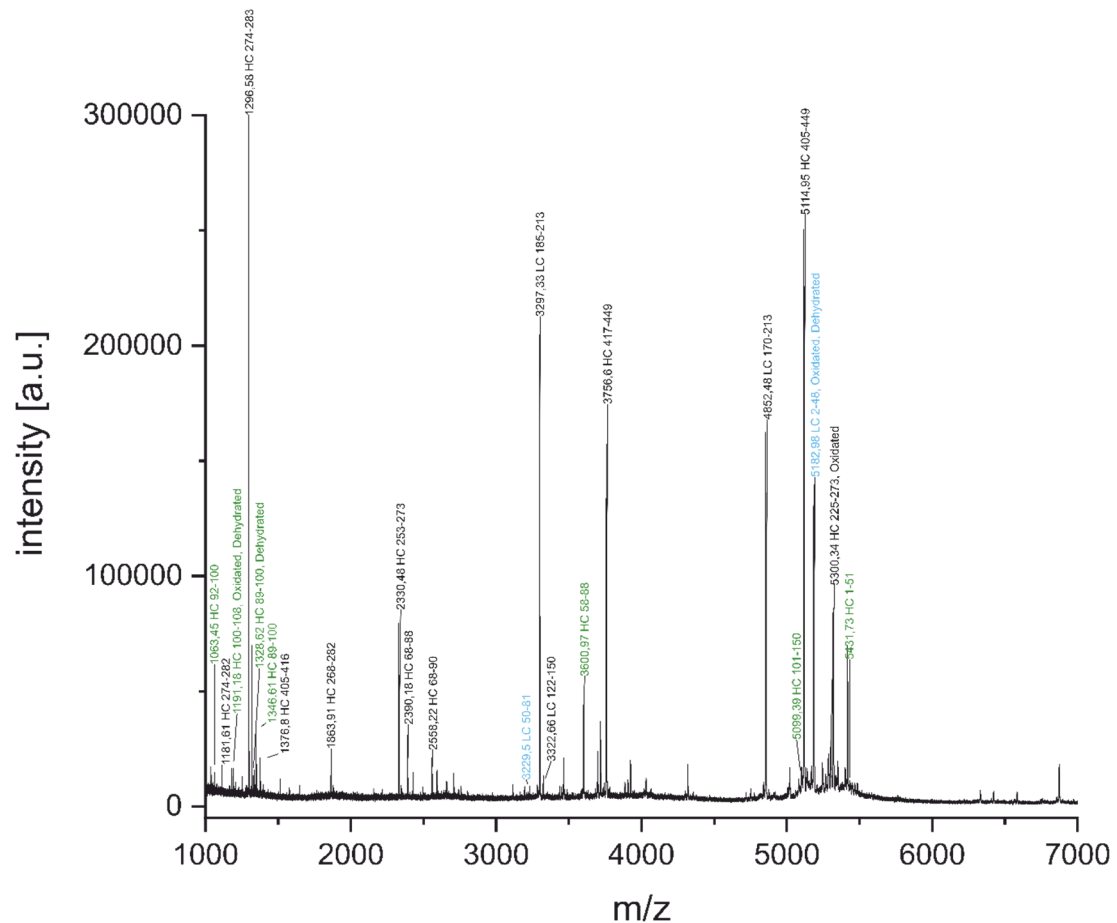
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## Determination of intact and light chain masses (IgG)



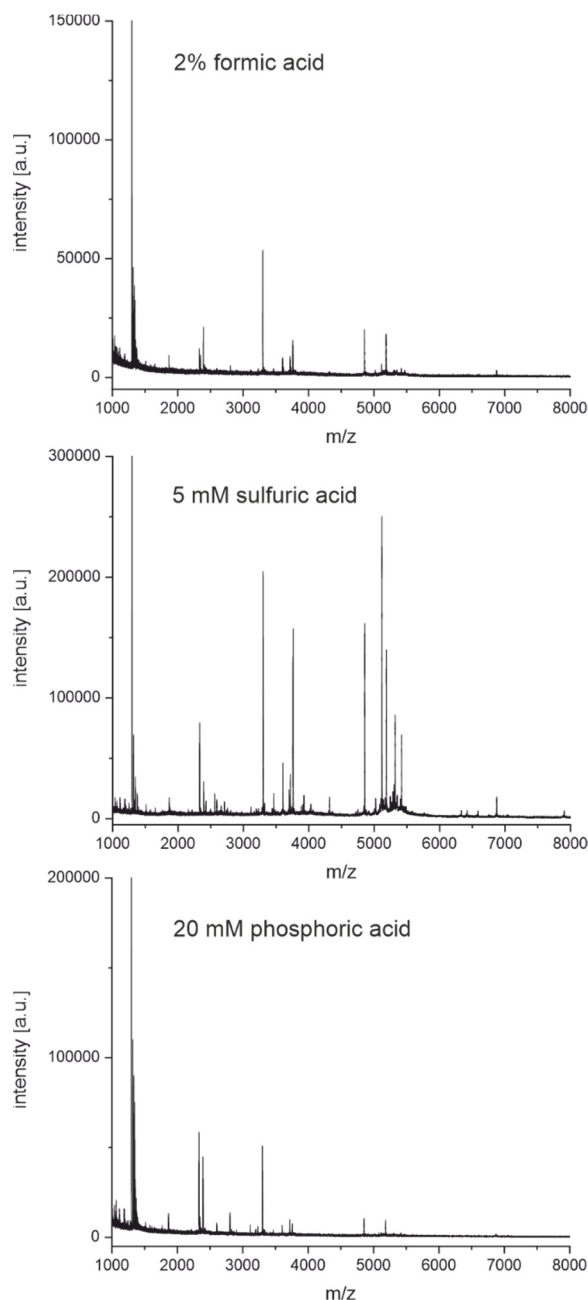
**Figure S1.** Light chain and intact masses of antibodies with overlapping mass ranges relative to the NIST antibody.

### Sequence coverage of peptide mass fingerprinting by partial acidic hydrolysis



**Figure S2.** 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%.

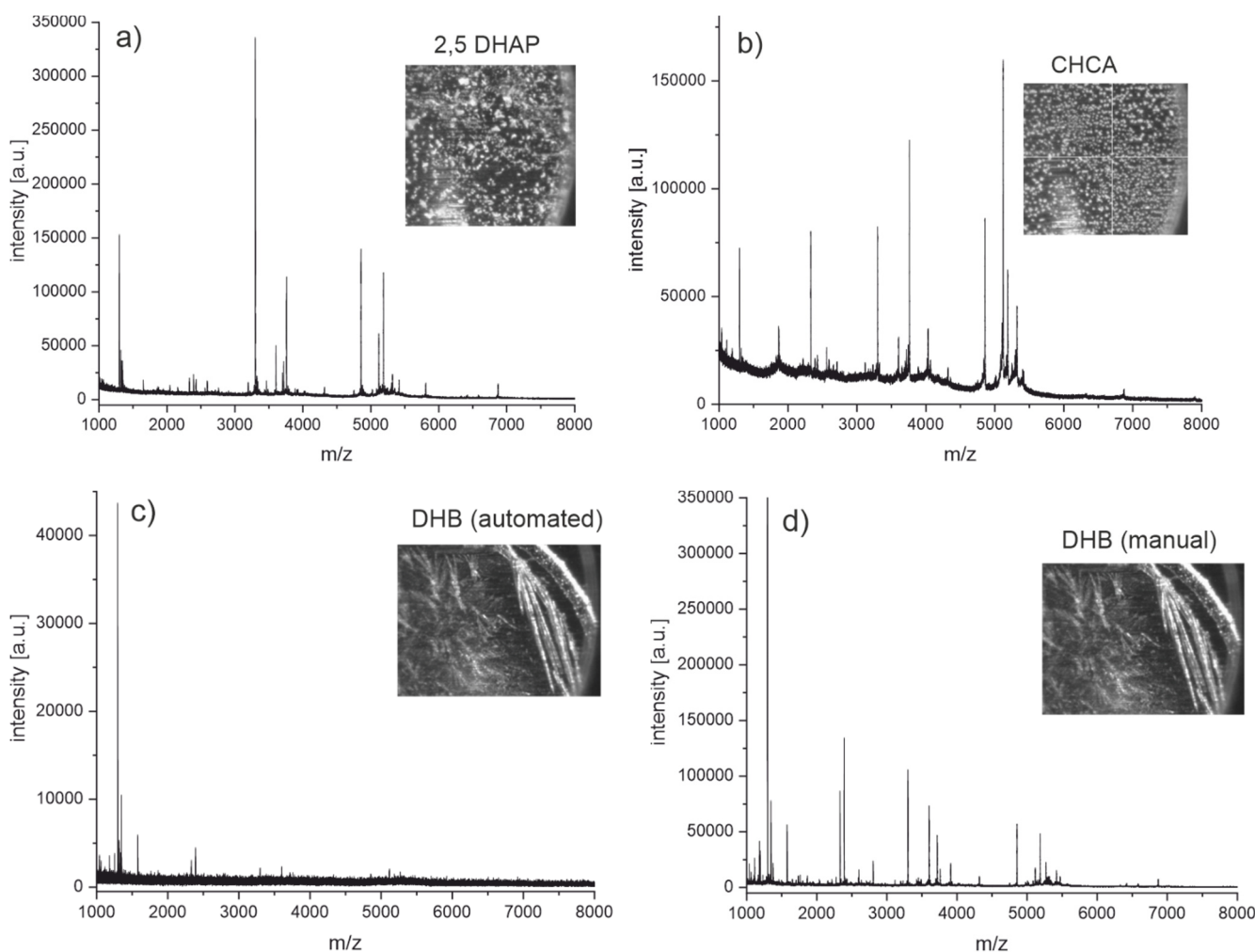
## Method optimization: Peptide mass fingerprinting with different acids as cleavage agents



**Figure S3.** Peptide mass fingerprint of NIST antibody (mAb 8671) was obtained from cleavage with 2% formic acid (S/N 16.0), 5 mM sulfuric acid (S/N 39.3), and 20 mM phosphoric acid (S/N 15.9). The signal-to-noise ratios (S/N) were calculated from the signal intensity of the monoisotopic peak at 3297.59 Da divided by the difference of maximum and minimum intensity between 3308 Da and 3316 Da.

Many diluted acids may be applied to selectively cleave at aspartic acid residues and generate fingerprints in a short time. However, as Figure S3 shows, 5 mM sulfuric acid produces the best results with a high number of peptide peaks and a good signal-to-noise ratio (S/N 39.3). 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.

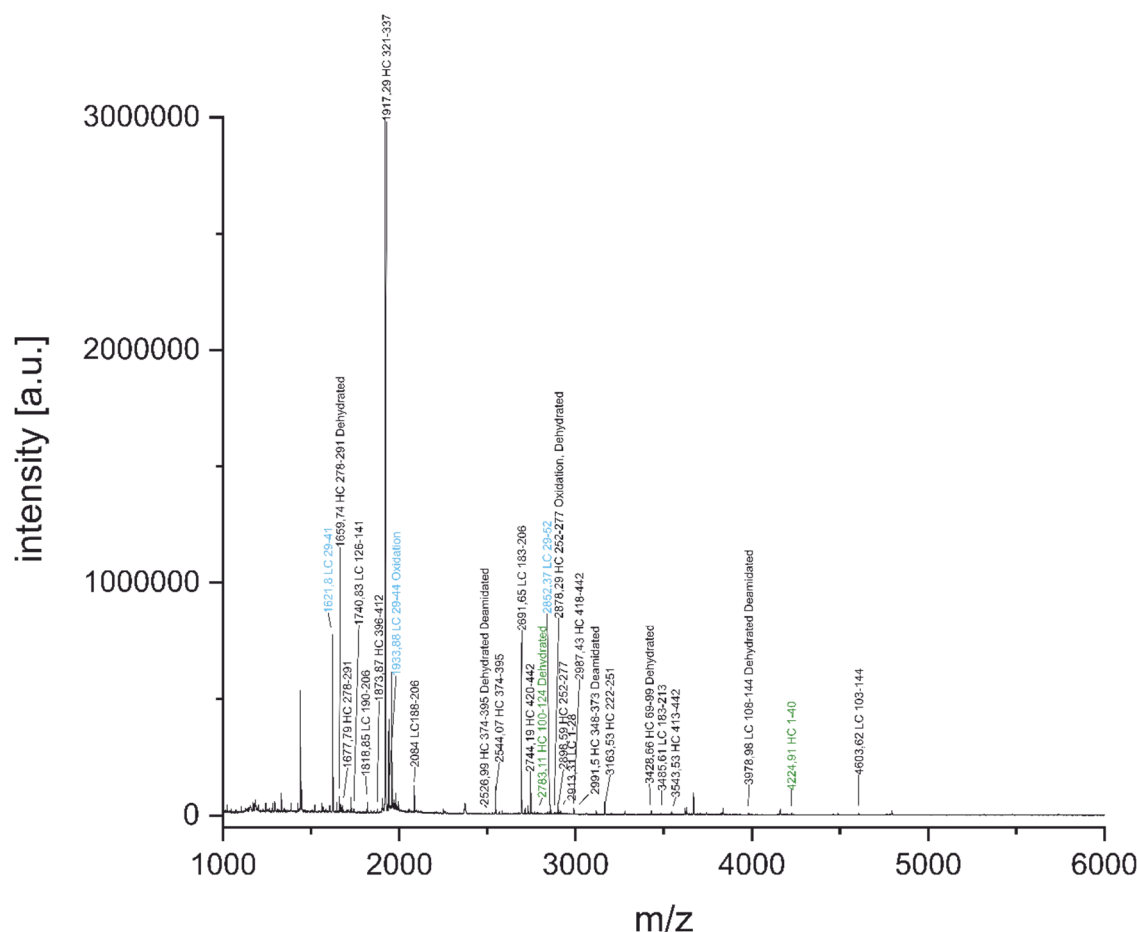
## Method optimization: MALDI matrix



**Figure S4.** 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).

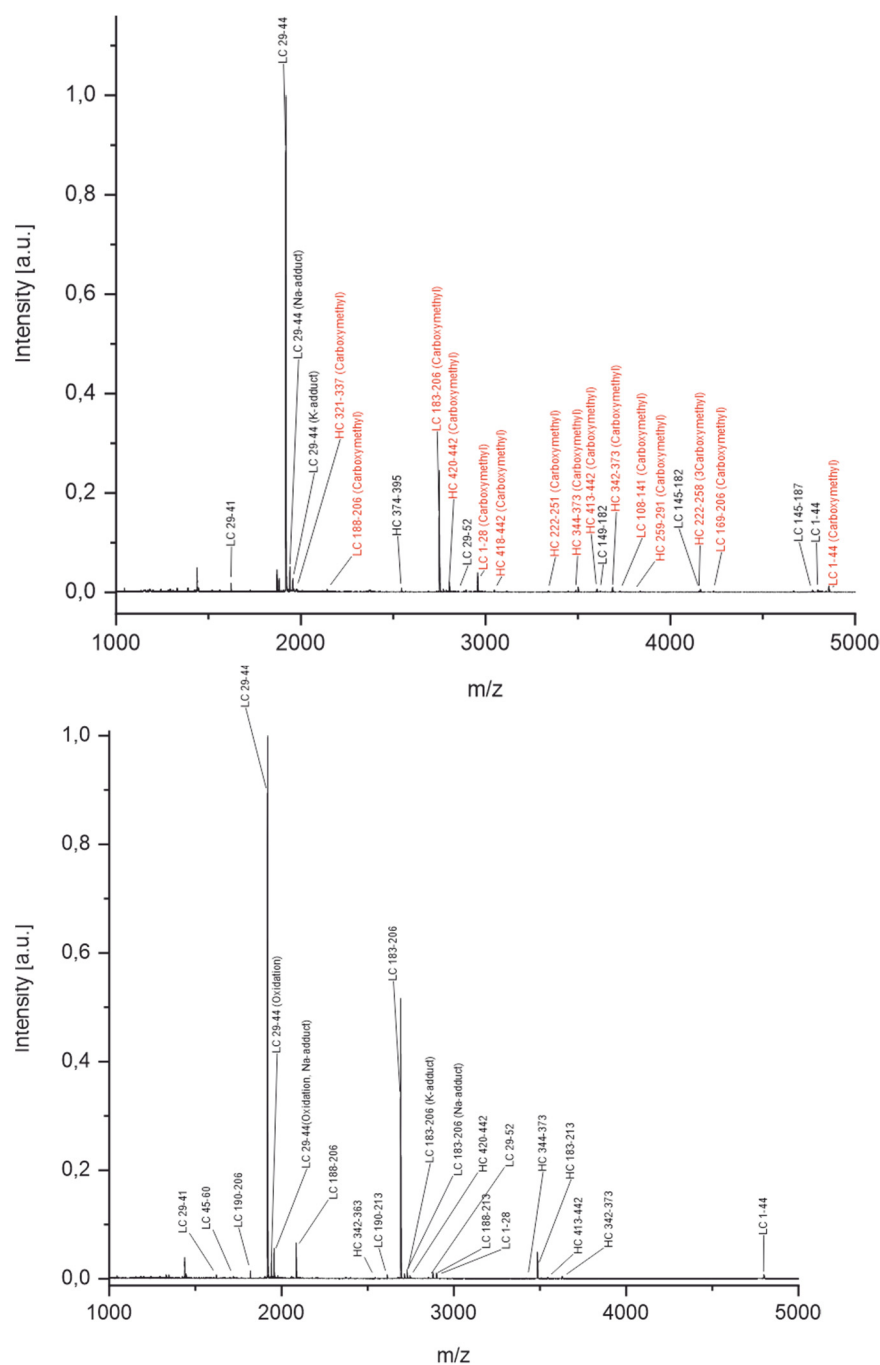
The MALDI matrix seems to play a critical role in the generation of information-rich fingerprint spectra (Figure S4). The fingerprints seem to produce the same peptides but with varying relative intensities. Furthermore, 2,5-DHAP (a) and DHB (with manual laser control, d) produce spectra with more peptides 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 the 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 by tryptic digestion



**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%.

The addition of an alkylation step did not improve the sequence coverage. In Figure S6, a comparison is shown between two fingerprints. The top fingerprint was generated by adding an alkylation step at room temperature after the denaturation of the antibody at 99 °C. The bottom fingerprint was generated by incubation of the antibody at room temperature after the denaturation at 99 °C. For both fingerprints, the sequence coverage is around 50%. This indicates that the alkylation of peptides in this context does not improve the coverage. In fact, the extra incubation time at room temperature (with or without an alkylating agent) even leads to a loss of coverage, likely caused by the refolding of the protein. Hence, alkylation should not be used in this protocol. The rapid addition of trypsin at 55 °C after the denaturing step might be crucial in order to conserve most of the denatured protein for the remainder of the 15 minutes digestion.



**Figure S6.** Tryptic digests of NIST-mab 8671 with and without the addition of an alkylation step.

Top spectrum: The antibody was incubated in 0.1 M tris-buffer (pH 7.8) with TCEP at 99 °C for 15 minutes. After the sample had cooled down to room temperature, a final concentration of iodoacetic acid (IAA) of 6 mM was added, and the sample was incubated for another 30 minutes at room temperature. Next, the temperature was raised to 55 °C, and trypsin was added. Digestion was allowed to proceed for 15 minutes. The sequence coverage was 49%. Bottom spectrum: The antibody was incubated in 0.1 M tris-buffer (pH 7.8) with TCEP at 99 °C for 15 minutes. Afterward, it was incubated for another 30 minutes at room temperature. Next, the temperature was raised to 55 °C, and trypsin was added. Digestion was allowed to proceed for 15 minutes. The sequence coverage was 50%. Peptide peaks are assigned to the NIST-mAb 8671 sequence. Peaks colored red are derived from carboxymethylated cysteine-containing peptides.

## Heavy Chain of NIST-mAb 8671

OYTLRESGPA LVKPTOTLTL TCTFSGFSLs TAGMSVgWIR QPPGKALEWL ADIWWDDKKH YNPSLKDRLT IS-  
 KDTskNOV VLKVTNMDPA DTATYYCARD MIENFYEDVW GOGTTVTVSS ASTKGPSVFP LAPSSKSTSG  
 GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS  
 NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVLEPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKENW  
 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIK KAKGQPREPO  
 VYTLPPSREE MTKNOVSLTC LVKGFYPSDI AVEWESNGOP ENNYKTTTPV LDSDGSEELY SKLTVDKSRW  
 OOGNVFSCSV MHEALHNHYT QKSLSLSPG

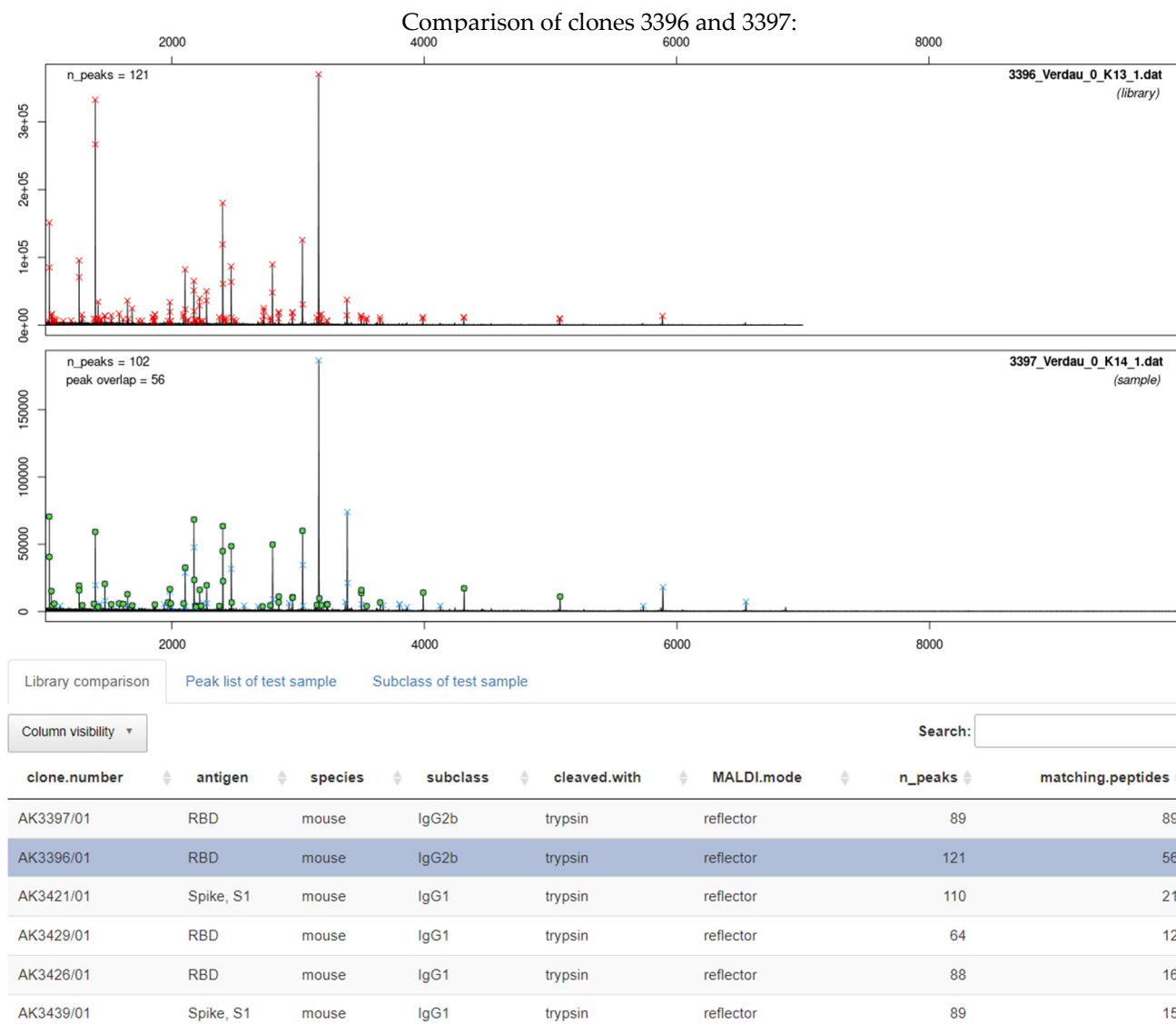
## Light Chain of NIST-mAb 8671

DIQMTQSPST LSASVGDRVT ITCSASSRVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR FSGSGSGTEF  
 LTISLQPD DFATYYCFQG SGYPFTFGGG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAK-  
 VQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

**Figure S7.** 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 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 the same clone can be detected in a library of dozens of clones (Figure S8). Both clones, 3396 and 3397, seem to be rather similar by visual examination.



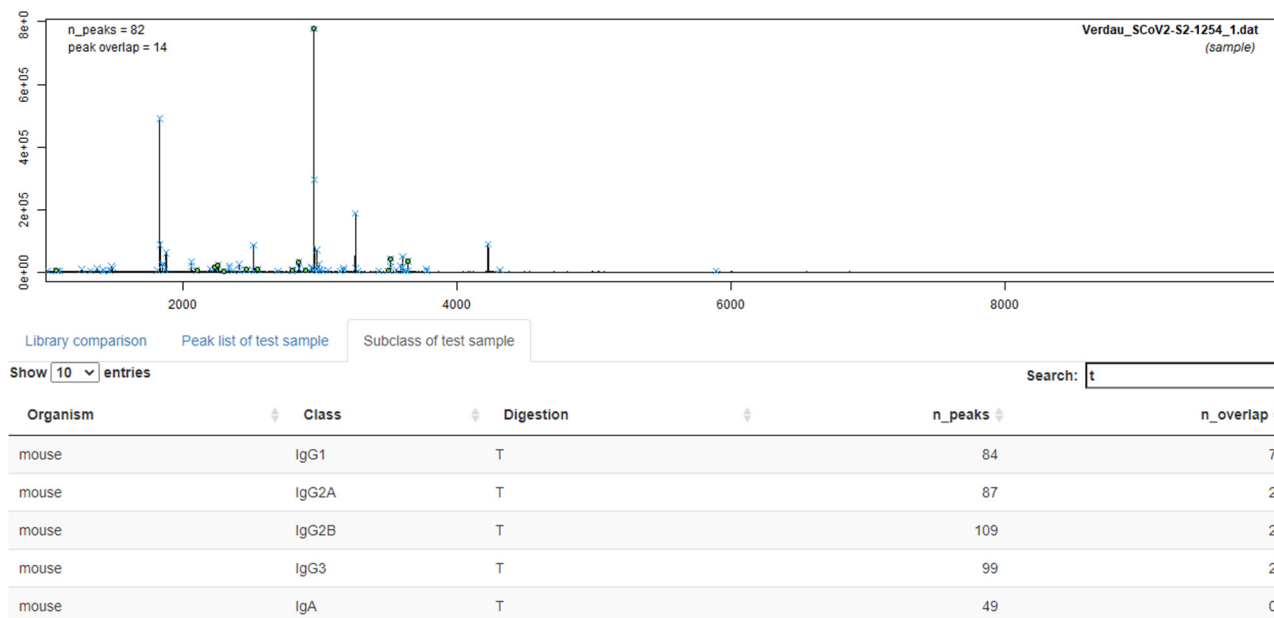
**Figure S8.** Comparison by ABID 2.0 software: The first spectrum is the library spectrum with the highest number of matching peaks with the sample spectrum (second spectrum).

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 match. 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 determination of subclasses from the antibody fingerprint spectrum (Figure S9). This works because the subclass information is inherent to the  $m/z$  values of Fc-specific peptides in the mass spectrum. ABID 2.0 was fed 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 shows the same result for clone 1254.



**Figure S9.** Screenshot of the software tool ABID 2.0. The tool 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)

Raw data are accessible on Zenodo: <https://doi.org/10.5281/zenodo.6375803>

