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Search for a high-mass calibrant for NALIM (Native Liquid MALDI MS): the case of mouse IgA



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Introduction

Native mass spectrometry (native MS) has the unique ability to directly observe a wide range of non-covalent biomolecular complexes formed of proteins, DNA/RNA, small molecules..., while covering a wide range of applications (dynamics, stoichiometry, affinity binding,...). So far, the go-to ionization method for native MS has been electrospray [1-3]. Recently, we developed a new native MS MALDI method using liquid spots called **Native Liquid MALDI (NALIM)**. The success of this method relies mainly on a liquid matrix that enables the gentle transfer of complexes in the gas phase while avoiding the transition through the solid state, which is the basis of classical solid-spot deposition methods. By using a soluble heterodimer, we demonstrated the proof-of-concept of this new native MALDI method [4].

One of our **research axes** is to extend applications of native liquid MALDI MS methods to the observation of membrane protein complexes. Many of these molecular assemblies have expected masses in the 100 - 300 kDa mass range. Moreover, in NALIM we mainly observe 1⁺ and 2⁺ charge states, the monocharged state being largely predominant. Consequently, this type of application requires calibrant standards at high *m/z* ratios. As no commercial calibrant exists in this mass range, we decided to produce our own calibrants, and started by investigating the usability of an immunoglobulin A as calibrant.

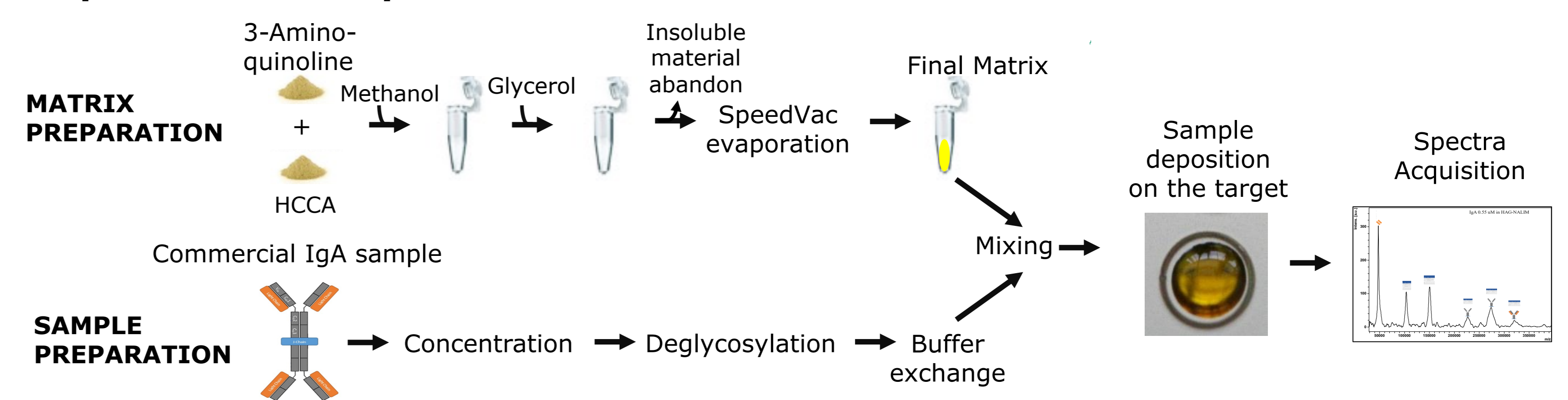
IgA: we focused our attention on this immunoglobulin as the predominant IgA forms are monomers and dimers. The monomers assemble in dimers via covalent binding with the J-Chain. The mass of this glycosylated assembly is expected to be around 360 kDa. Thus, we envisioned that this mixture, after a deglycosylation step, could be a potential calibrant covering a wide mass range in NALIM applications.

Objectives and means: find a suitable calibrant for high mass range applications in NALIM by optimizing biochemical preparation, matrix composition and extraction delay.

Materials and Methods

- The **IgA sample** is a commercial monoclonal antibody produced in mice.
- The **matrix** is a mixture of HCCA/3AQ/glycerol (1:4:6, m/m). Methanol is used to solubilize the solid chemicals and then is discarded by a vacuum concentrator system.
- Mass spectrometry instrumentation** : MALDI-TOF/TOF UltrafleXtreme (Bruker)

Experimental setup



Results

We began by optimizing the extraction delay for these high molecular mass assemblies.

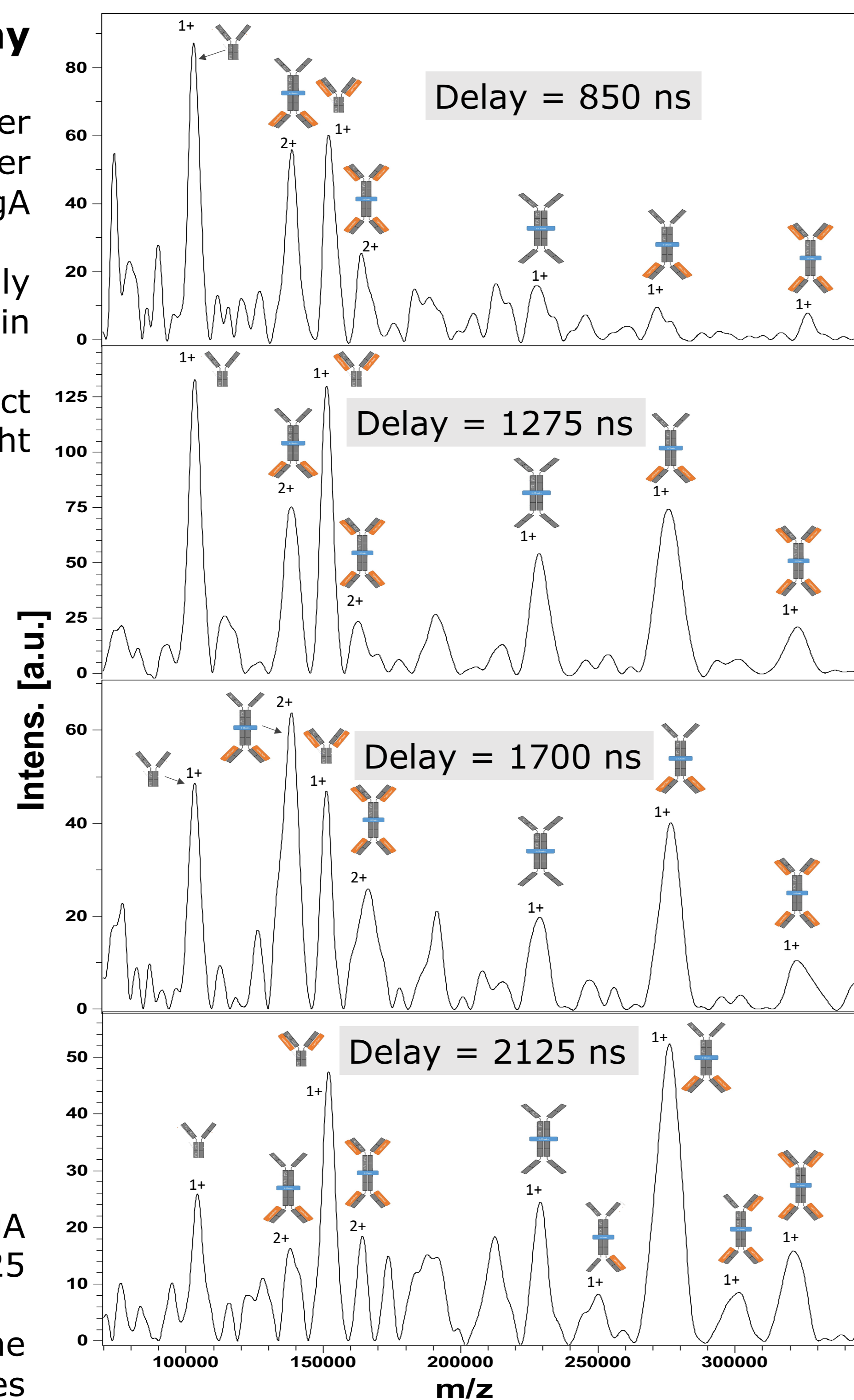
At the beginning, deglycosylation was performed using PNGase F during 24h at 37°C in soft denaturation conditions (1M urea).

Pulsed ion extraction delay (PIE delay) variation

- Spectra were recorded after deglycosylation and buffer exchange at a final IgA concentration of 0.5 μM.
- As mentioned above, we mainly observe 1⁺ and 2⁺ charge states in NALIM.
- Surprisingly, we also detect species missing 1 or more light chains (Lc).

	Monomer – 2 Lc
	Monomer
	Dimer – 4 Lc
	Dimer – 2 Lc
	Dimer

- Deglycosylation produces an IgA dimer with a mass around 325 kDa.
- By increasing the delay, the intensities of higher mass species are favored, as expected. As we are interested to have a good overview of all oligomers present in the sample, we choose an intermediate value of 1770 ns for the PIE delay for further experiments.



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The **biochemical preparation** was further optimized by adding steps of concentration and/or buffer exchange.

Optimal parameters included 2M urea concentration and 24h of deglycosylation time at 37°C.

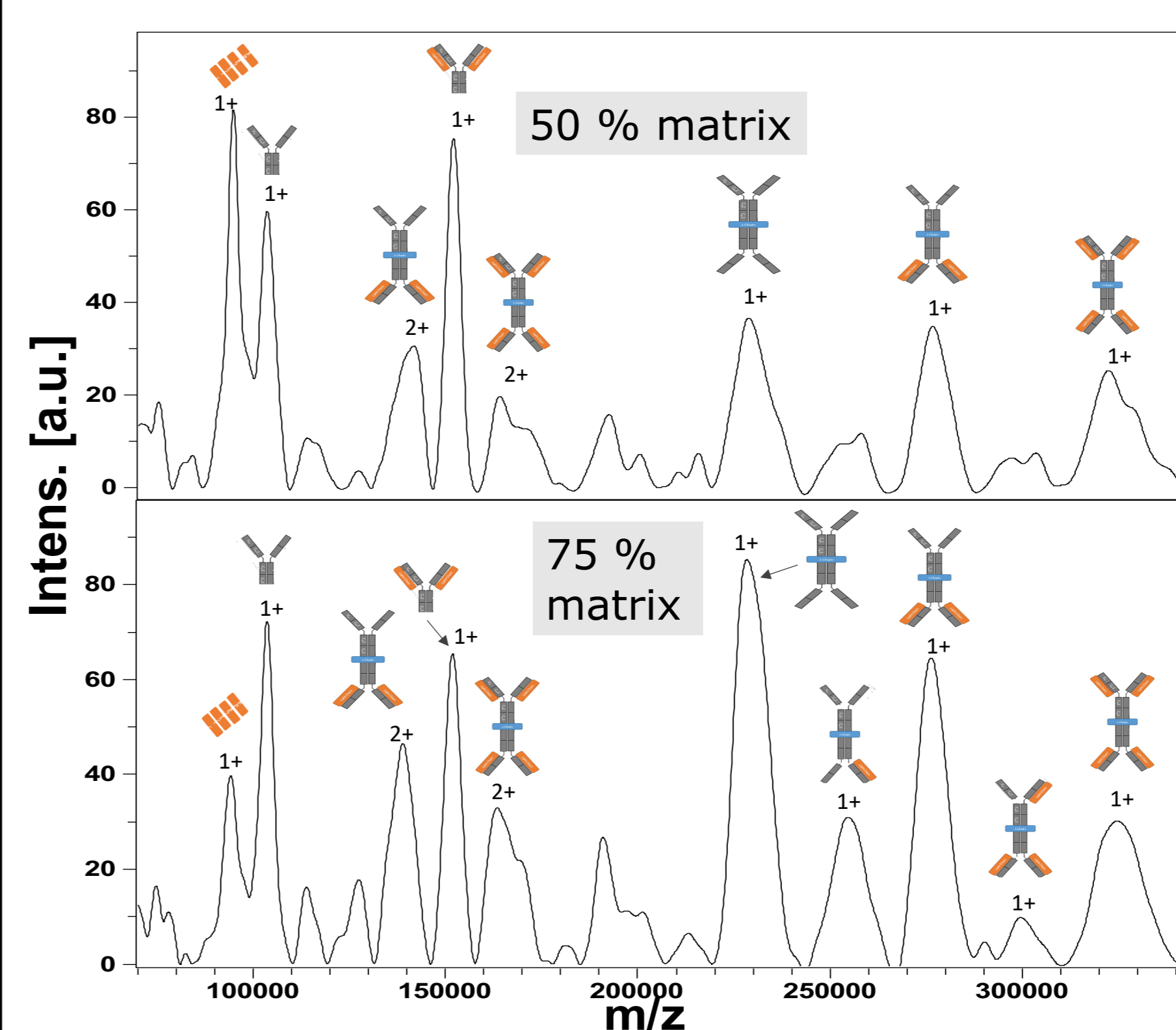
Mass determination

Here are listed the species for which masses were determined by using spots on an ultra-thin layer of HCCA [5] and then used as calibrant points.

Species (1 ⁺ charge)					
Used as calibrant point	✓	✓	✗	✓	✗

Matrix composition variation

- When mixing sample and matrix, we can set different sample factor dilutions :
 - 1/2 -> 50 % matrix final concentration
 - 1/4 -> 75 % matrix final concentration



- Spectra were recorded after deglycosylation and buffer exchange at a final IgA concentration of 0.55 μM.
- A higher concentration of matrix increases the dimer/monomer ratio, mostly by increasing the dimers intensity. This effect is less marked for species which have lost 1 to 3 Lc.
- Despite increasing urea concentration during the deglycosylation step, we still have broad peaks for the dimer species.

Discussion/Conclusion

We chose an IgA as a potential calibrant to cover the 300 kDa range thanks to Ig dimerization. NALIM analysis of a commercial mouse IgA shows that some species missing one or more Lc are present. A literature search provides the explanation: while in humans IgAs, a Lc is always bound to an Hc (heavy chain) *via* disulfide bridges, in mouse IgA Lc binds to Hc in a noncovalent manner [6]. NALIM thus clearly shows its ability to conserve noncovalent biomolecular complexes.

While this IgA preparation covers a wide mass range, the dimer species still appear as broad peaks, affecting accuracy and the quality of the calibration. The recently solved cryoEM structure of mouse IgA reveals that several N-glycosylation sites are much less accessible when the J-chain is present [7], so that dimers may be more resistant to deglycosylation than monomers.

