Rapid Profiling mAbs with Recombinant Prokaryotic Lectins using Ion Mobility Spectrometry

Using Singly-Charged Electrospray Ions Preserves Molecular Conformation and Allows Protein-protein Complexes to be Studied without Charge Distortion

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Motivation

A need exists to screen the glycosylation patterns of biotherapeutic mAbs more rapidly. Lectin binding assays have been used historically in a variety of testing formats. The impurity of plant-derived lectins can lead to data interpretation problems, as encountered earlier with an ion mobility detection scheme (Engel). Recently, recombinant prokaryotic lectins (RPL) have become commercially available. They are purer and provide greater specificity than plant-based lectins. We chose to further evaluate the use of ion mobility spectrometry as the detection scheme for RPL-mAb binding because this technique is rapid (3min), automatable and potentially useful for identifying antibody variants.

Approach

Charge-Reduced Electrospray

Highly-charged electrospray droplets containing analyte molecules were passed through a cloud of bipolar air ions. The air ions effectively reduced the charge on electrospray droplets to one charge. This well-established charge-reduction method (Kaufman; Hogan; Maisser) leads to coulombically-undistorted analyte ions, thus preserving molecular conformation.

Biotherapeutic antibodies and RPLs

Cetuximab ('Cetux', Erbitux) and Tocilizumab ('Tocil', Actemra) were analyzed before and after deglycosylation with PNGase-F. Native and deglycosylated mAbs were reacted with a 2x excess of RPLs and cations (Ca++, Mg+ and Mn++), then buffer exchanged into 25 mM NH_4Ac. Reaction mixtures were diluted to [mAb] = 25 µg/mL before analysis. The naming convention of the RPLs corresponds to the targeted glycans; aGal: α-galactose, Gal1: β-galactose and LacNAc, Fuc1: α-fucose, Man2: α-mannose and Sia3: α-Neu5Ac. RPLs were provided by Glycoselect, Ltd.

Ion Mobility Spectrometry

IMgenius, a simple bench-top ion mobility spectrometer from IonDX, Inc. (top panels) was used to determine the mobility constant K, or 1/ K of singly-charged electrospray ions of mAbs, RPLs and mAb-RPL complexes.

References

Engel, NY, et al., JASMS, 28, 77-86, 2017
Kaufman, SL., et al., Anal. Chem., 68, 1895-1904, 1996;
Kaufman, SL, J. Aerosol Sci., 29,537-552, 1998.

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Fig. 1

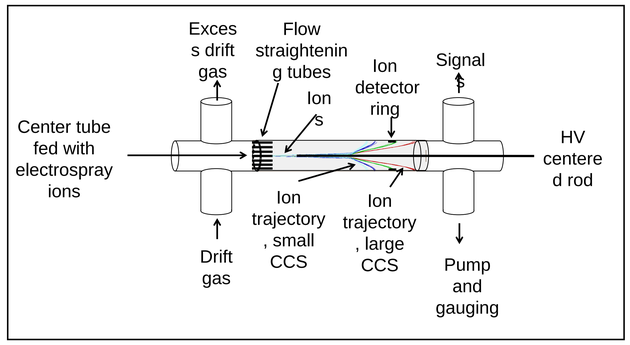


Fig. 1 Instrument description: Enlarged cross-sectional view shows ion trajectories (colored lines) fanning out and terminating on a ring electrode (short black lines) positioned of the inside of the cylindrical body of the spectrometer. A ramped voltage applied to the center rod deflects ions onto the ring electrode.

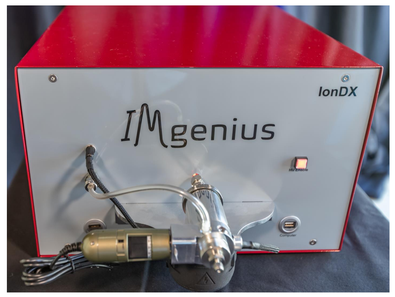
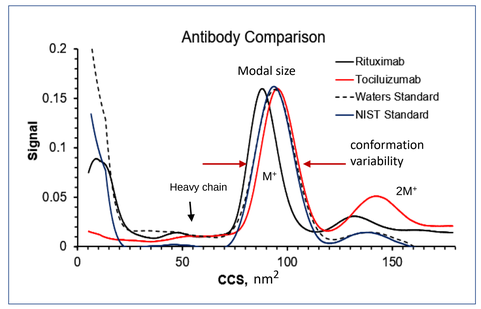


Fig. 2



Results

Fig. 2. Data interpretation. Raw data is in the form of ion current vs center rod voltage, which is transformed to plots of ion current vs collision cross section (CCS). Spectra are acquired in 3 min. Ion mobility spectra of antibodies are overlaid for comparison. Each antibody has a slightly different modal size. The width of the peaks correspond to the variation in conformation.

Fig. 3. Ion mobility spectra of Tocil M+ ions before and after deglycosylation. The CCS values for the deglycosylated molecules is slightly smaller than the native molecules, revealing a smaller conformation due to the loss of glycans. Reaction with aGal shifts the native Tocil to a larger size and shifts deglycosylated Tocil insignificantly, as expected for an experimental control.

Fig. 4. Ion mobility spectra of Cetux M+ before and after deglycosylation. The CCS values for the deglycosylated molecules is slightly smaller than the native molecules, revealing a smaller conformation due to the loss of glycans. Reaction with aGal shifts the native Cetux to a slightly larger size (compared to Tocil in Fig. 3) and shifts degly Cetux insignificantly, as expected for an experimental control.

Figs. 5 - 8. Tocil was reacted with Gal1, Fuc1, Man2 and Sia3. Ion mobility spectra before and after reaction show various levels of shift to larger molecular size following reaction with the RPLs. Gal1 and Sia3 show a small shift while the shift for Fuc1 and Man2 is much larger. Peaks in the spectra at ~ 140 nm^2 correspond to the dimer of Tocil. The Tocil dimer also shifts to larger size following reaction with Fuc1, but not with Man2. In Fig. 6 and 7, the entire peak shifts and becomes broader, while in Fig 5 and 8 the largest change is peak broadening. The latter implies that only the largest constituents in Tocil react with these two RPLs since the left side of the peak does not shift, while the right side of the peaks became wider.

Fig. 3:

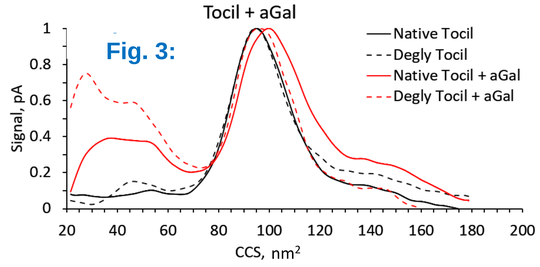
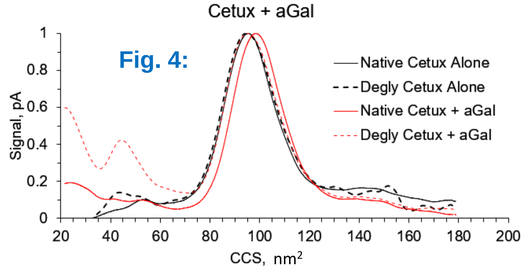


Fig. 4:



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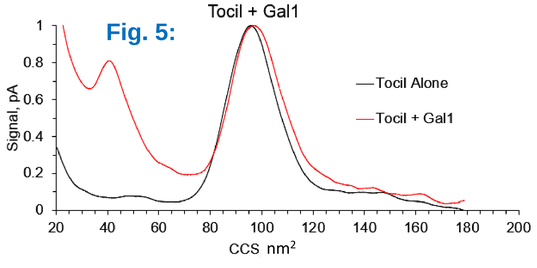

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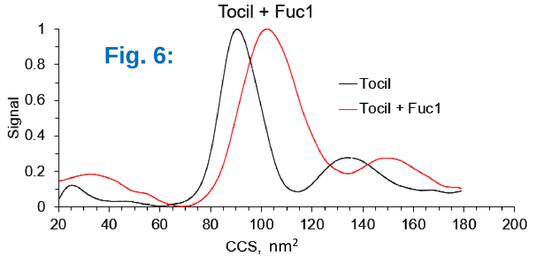

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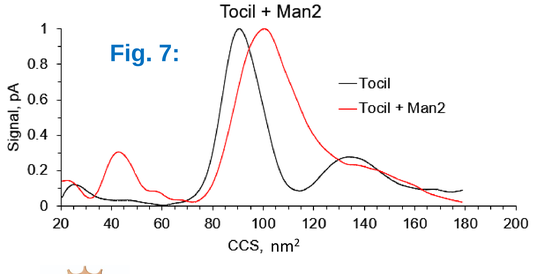
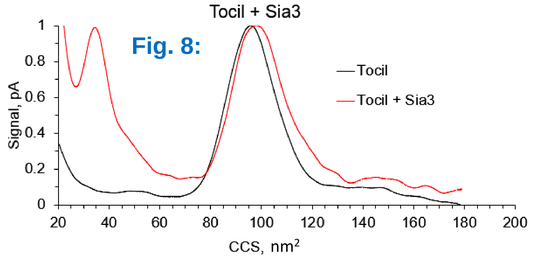


Fig. 8:



Summary

1) We demonstrated the use of RPL lectins as a technique for rapidly screening (3 min) biotherapeutic antibodies for glycosylation.
2) Simple comparison of the mobility of M+ ions of antibodies before and after reaction with RPLs allows determination of different glycoforms on the antibodies.

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