High Throughput Screening and Characterization of Bispecifics Using Native Ion Mobility Mass Spectrometry

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Introduction

Bispecific (bsAbs) are an emerging group of biotherapeutics. While there are over 30 bsAbs in development, only two bsABs are approved for therapy.[1] Bispecific antibodies are a unique class of antibodies combining the specificities of two monoclonal antibodies (mAbs) to bind two different targets at the same time.[1,2] Generating bsAbs creates different combinations of the heavy and light chains from the original two mAbs.[1,2] The heterogeneity of the resulting bsAbs poses an analytical challenge for high throughput screening for determining the optimal antibody pairs. Here we present an LC/MS method using native and denaturing ion mobility mass spectrometry the screening and characterization of bsAbs.

Experimental

A method for generating a bsAb was adapted from Debaene et al.[3] using an in-house IgG1 (mAb A) and a commercial IgG1 standard (mAb STD). The two IgG1s were mixed with 5mM gluthathione-reduced (GSH) for 24 hours at 37°C. The resulting bsAb and both starting IgG1s were buffer exchanged into 100 mM ammonium acetate buffer using Micro Bio-spin 30 columns. The samples were introduced into the IM-QTOF using a nanoLC interface and sample introduction was performed isocratically at a flowrate of 0.4 µL/min with 200 mM ammonium acetate, pH 7. Drift times and collision cross sectional areas were determined using the IM-MS Browser. Protein deconvolution was performed using the maximum entropy algorithm found in BioConfirm for MassHunter Qualitative Analysis.

Agilent 6560 Ion Mobility Q-TOF

- Allows use of drift gases other than N_2
- Automatically controls pressure in drift tube of pure gas
- Available as an upgrade kit

Alternate Drift Gas Upgrade Kit

- Small molecule tune with fragile molecule options.
- Tunes entire system, including IM
- Capability to schedule and run in a worklist

SWARM Autotune

- IM Molecular feature extraction (iMFE) for compound extraction based on *m/z*, retention time, and drift time
- Annotations on drift scope

Ion Mobility Molecular Feature Extraction, iMFE

- Calculate collision cross section (CCS) for compounds
- Determine the CCS for compounds in a mixture identified with iMFE

Single Field Collision Cross Section, CCS

- Alternating frame acquisition with high/low collision energy
- Ramped collision energy per drift cycle
- Exportable MS/MS to Spectrum Mill and MassHunter Qualitative Analysis

Alternating High/Low Collision Energy

Native Ion Mobility Mass Spectrometry for Screening of IgGs

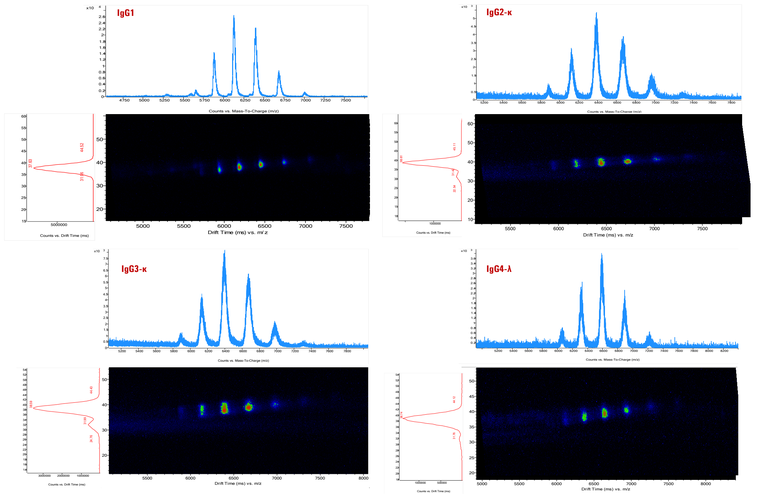

Figure 2: Native IM-MS profiling of the different subclasses of IgG1 standard **(above left),** IgG2-κ **(above right),** IgG3-κ **(bottom left),** and IgG4-λ **(bottom right)** using nanoLC with the G1992A interface with 200 mM Ammonium Acetate, pH 7.0 as the mobile phase.

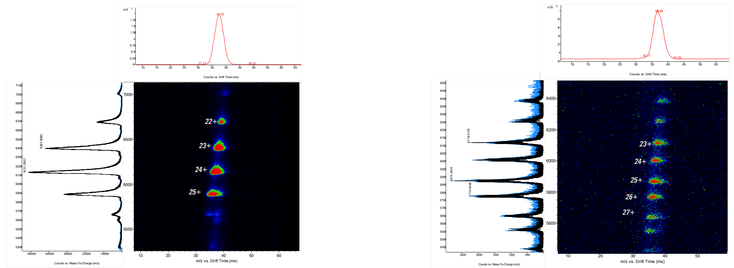

Figure 3: Native IM-MS profiling of the two IgG1s selected for generating a bispecific. The charge envelope, drift spectra and drift scope of the mAb STD **(above left)** and mAb A **(above right)** were profiled.

Preliminary Results for Bispecific

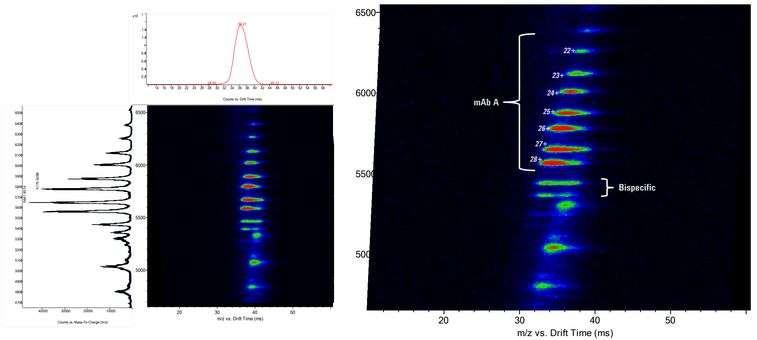

Figure 4: Native MS of the bispecific including the charge envelope and corresponding drift spectra (**above**). Zoom in of the drift scope of the charge envelope with the putative bispecific annotated and the charge envelope from mAb A (**top right**). Zoom in of the *m/z* values corresponding to the bispecific with the drift spectra, indicating the presence of two conformations.

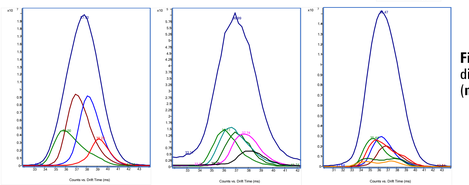

Figure 5: Overlay of the drift spectra at the different charge states of mAb STD (**left**), mAb A (**middle**), and the resulting bispecific (**right**).

Conclusion

- High throughput intact mass analysis of bispecific antibodies using native and denaturing ion mobility mass spectrometry

- [1]Spiess, Christoph, Qianting Zhai, and Paul J. Carter. "Alternative molecular formats and therapeutic applications for bispecific antibodies." *Molecular immunology* 67.2 (2015): 95-106.
- [2]Kontermann, Roland E., and Ulrich Brinkmann. "Bispecific antibodies." *Drug discovery today* 20.7 (2015): 838-847.
- [3]Debaene, François, et al. "Time resolved native ion-mobility mass spectrometry to monitor dynamics of IgG4 Fab arm exchange and "bispecific" monoclonal antibody formation." *Analytical chemistry* 85.20 (2013): 9785-9792.