Genedata
solutions in silico

Genmab

> ***Compared to the CEX-based process, the time required for data acquisition and processing was reduced by 95% and the number of person hours dedicated to data processing was reduced by 70%.***
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**INDUSTRY**
Biotechnology

**CUSTOMER SINCE**
2015

**ABOUT GENMAB**
Genmab is an international biotechnology company specializing in the creation and development of differentiated antibody therapeutics for the treatment of cancer.

**GENEDATA SOLUTION**
EXPRESSIONIST

# Developing a Generic HT Assay for DuoBody® Characterization

## Background

Genmab is an international biotechnology company that specializes in creating and developing antibody biotherapeutics for treating cancer. The innovative DuoBody technology platform facilitates generation of human IgG1 bispecific antibodies (bsAb) using a fast, scalable process. Predicting the behavior of bsAbs based on the activity of their parent antibodies is difficult, and therefore Genmab has developed a specific method for screening bsAb libraries (Figure 1 overleaf). Briefly, two IgG1 antibody libraries (X and Y) are produced separately (Step 1), purified (Step 2), and recombined to generate a bsAb library containing the complete set of parent antibody permutations (Step 3). After recombination, the bsAb library is screened for desired functionalities (Step 4).

Targeted mutations in the heavy chain third constant (CH3) domain allow heterodimerization under mild reducing conditions, with the process being essentially unidirectional with typical yields of >95%[1–3]. The homogeneity of the final bsAb product is determined to monitor process efficiency and product quality. To process multiple bsAb screening libraries, such a characterization would ideally be highly generic, provide unbiased and relevant molecular information, and operate efficiently in a high-throughput manner..

## Main Challenges

### Replacing inefficient existing LC-based assay technologies

A commonly used conventional method for characterizing antibody products is cation-exchange (CEX) chromatography. However, due to substantial charge heterogeneity, this method frequently requires retesting. The alternative orthogonal method hydrophobic-interaction (HIC) chromatography also has limitations and peak resolution is usually low. These methods are laborious and require lengthy eluent profiles and buffer exchanges. In addition, they also require internal control runs using homodimers to assign peaks, further increasing the time required for analysis.

### Speeding and simplifying data processing and analysis

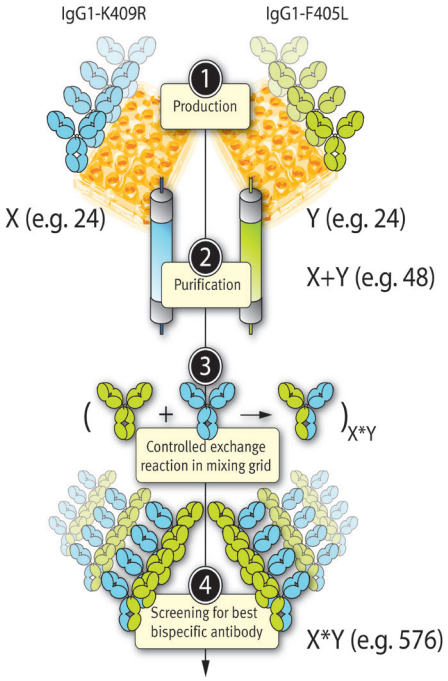
In CEX profile analysis, charge heterogeneity can lead to overlapping peak profiles that frequently require manual peak integration. In HIC profiles, overlapping peaks caused by low resolution are often impossible to interpret.

### Increasing throughput to accommodate numbers of samples

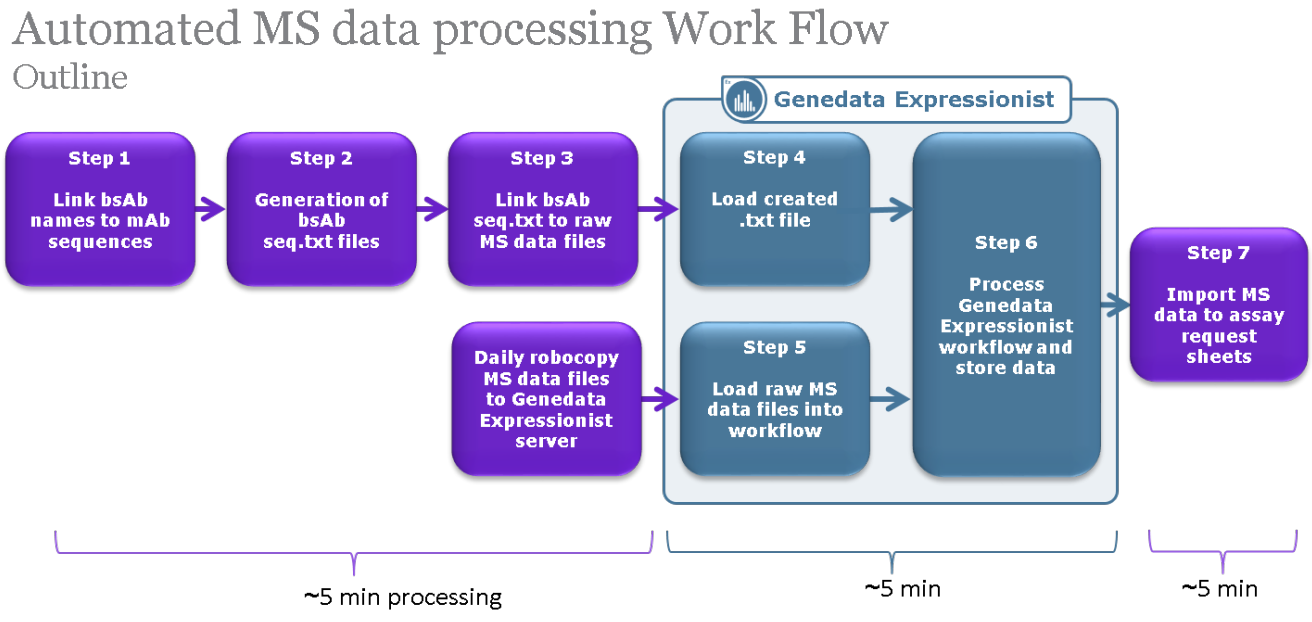
The large number of bsAb variants generated in a screening library means that ideally, the analysis method should be as fast as possible. The laborious nature of CEX-based methods means that such approaches would represent a significant bottleneck in screening.

### Achieving standardization and reproducibility

The unpredictable behavior of antibody variants during CEX chromatographic separation and the frequent requirement for an additional orthogonal separation means that analyses using CEX-based methods are not standardized.




❶ *The DuoBody bsAb library screening process*




❷ *The Genmab DuoBody bsAb data analysis procedure (slide presented by Dr Ewald van den Bremer at PEGS Summit 2017 and PepTalk 2018)*

## Solution

### Switching to MS-based analysis methods

Compared to CEX-based methods, MS-based analysis provides significantly more information on the physical composition of samples. In addition to the relative ratios of expected molecular species, the presence and relative ratios of additional species (for example, sequence variants or glycosylated forms) can be determined.

### Efficiently processing large volumes of MS data

The additional levels of information provided by MS analysis lead to larger volumes of data. As an enterprise level platform designed to facilitate and accelerate MS data processing in biopharmaceutical characterization, Genedata Expressionist was an enabling component in developing an MS-based assay for automated analysis of bsAb variants.

## Benefits

### Accelerated processing and analysis using generic LC-MS–based methods

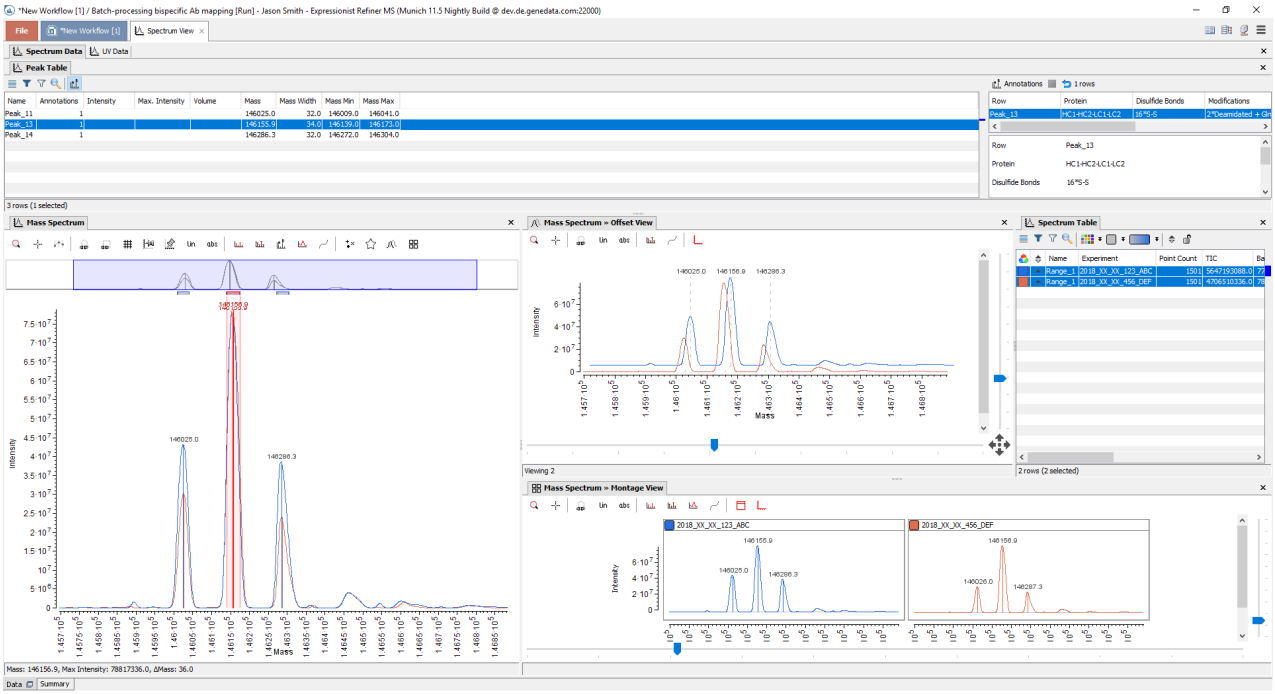
The LC method used in the LC-MS analysis of bsAbs is a generic, fast-gradient method suitable for analysis of any antibody. This means that no time-consuming buffer exchange, chromatographic separation optimization, or additional orthogonal methods are required.

### Fast and efficient data processing and analysis through automation of the workflow

The Genmab DuoBody bsAb data analysis procedure can be divided into three stages (Figure 2). First, FASTA-format files containing the sequences of the respective parent antibody heavy and light chains are created (Steps 1–3).
Second, these files are automatically sent together with the MS raw data to the Genedata Expressionist server where they are loaded into a fully automated, custom-built data analysis workflow (Steps 4–6). Within this workflow, individual activities use global and sample-specific parameters to process each raw data file. For example, an Intact Protein activity deconvolutes mass spectra and a Protein Mapping activity rapidly analyzes multiple disulfide variants and screens for glycosylated species.
Finally, the analysis results are reported in a format compatible with automated import into downstream data processing (Step 7).

### Standardization providing globally comparable, high-quality results

Always using the same method for analysis and subsequent data processing enables meaningful comparisons of results across and between entire bsAb libraries. This standardized method greatly facilitates benchmarking and process monitoring in the production of bsAb therapeutics for research applications.



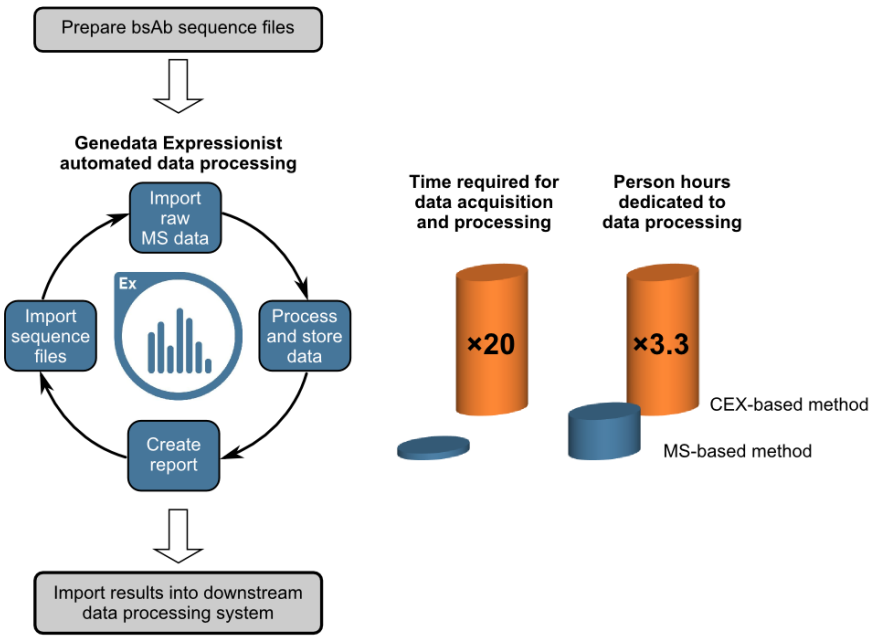

❸ *Processing high-content MS information using Genedata Expressionist delivers deeper insights into samples. The displayed samples contain a DuoBody and spiked homodimers.*

### Detailed visualization and reporting delivering deeper insights into sample makeup

Using MS-based methods, mass calculations provide an additional level of confidence in the identification of each molecular species. These species can be manually investigated using the software's Spectrum View (Figure 3). The Genedata Expressionist workflow creates a report containing detailed results for each sample, ready for import into existing data infrastructures

## Summary

Using Genedata Expressionist, scientists at Genmab established an automated MS-based high-throughput assay that provides qualitative and quantitative information on a bsAb preparation in a fraction of the time and effort required by conventional CEX-based methods. Compared to the CEX-based process, the time required for data acquisition and processing was reduced by 95% and the number of person hours dedicated to data processing was reduced by 70% (Figure 4).




❹ *The Genmab DuoBody bsAb data analysis procedure and time savings obtained by the adoption of the automated MS-based data analysis workflow.*

### References

1. Labrijn, A.F., Meesters, J.I. et al. Controlled Fab-arm exchange for the generation of stable bispecific IgG1. Nat Protoc. 2014 Oct;9(10):2450-63. doi: 10.1038/nprot.2014.
2. Labrijn, A.F., Meesters, J.I. et al. Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange. Proc. Natl. Acad. Sci. USA. 2013 Mar 26;110(13):5145-50. doi: 10.1073/pnas.1220145110.
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