Rapid Identity Assays for mAb Development, Production Control and Release

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Anja Resemann[1], Waltraud Evers[1], Yue Ju[2], Guillaume Tremintin[3], Detlev Suckau[1]

[1] Bruker Corporation, Bremen, Germany
[2] Bruker Corporation, Billerica, MA, USA
[3] Bruker Corporation, San Jose, CA, USA

Overview

BioPharma Compass 3.0 software supports the routine analysis of biopharmaceuticals, both with **LC-ESI** and **LC-free MALDI** workflows.

Here we discuss applications of MALDI to high throughput **clone selection** and to the rapid identification of mAb samples, e.g., for **Fill & Finish Operations**, **within 20 min** from intact mAb sample to identification report.

Introduction

During biopharmaceutical development (e.g., clone selection) and production (rapid release identity testing) there is a requirement for fast analysis return times to accelerate decision making and reduce costs. We utilized rapid protein digest methods and integrated MALDI-TOF sample analysis with a software workflow to compare measurements against a reference attribute profile. This comparison was used in clone selection workflows to screen glycan profiles in intact Fc-domains and to provide antibody identities rapidly, based on differentiating abundant peptides in peptide mass fingerprints.

Protocols were developed to achieve analysis return times from intact antibody samples to automatic identity confirmation based on trypsin/Lys-C digests of 15 min and for Fc-glycoprofiling within 1/2 hour.

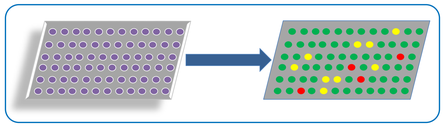

Fig. 1 Pass/fail results of the analysis are mapped directly to the position of samples on the MALDI plate

Methods

Several antibodies were used in this study either as medicinal formulations (NIST mAb, Adalimumab, Trastuzumab. Panitumumab, Cetuximab and Natalizumab).

For **clone selection** they were digested using IdeS (Genovis), diluted into DHAP or sinapinic acid MALDI matrix and the 2^+ charge state was used for Fc-glycan profiling in linear mode MALDI-MS analysis.

For **rapid release identity testing** they were incubated in 50% trifluoroethanol/ 50mM DTT (5min, 50°C) digested using trypsin/Lys-C (Promega), 5min after dilution with digestion buffer (***Fig. 5***).

Samples were analyzed by MALDI on an autoflex maX (Bruker) in reflector mode using CCA matrix. Automatically acquired spectra were processed in BioPharma Compass 3.0 (Bruker). Antibody identity was confirmed based on the peptide profile similarity while rapid glycan profiling was based on Fc-linked glycan profiling.

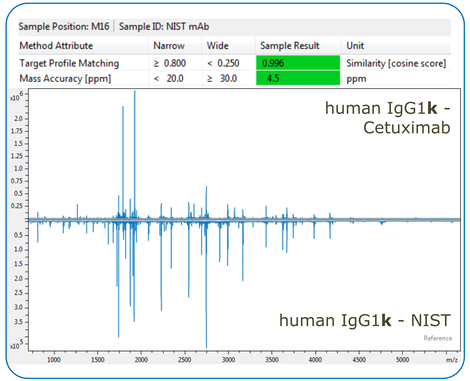

Fig. 2 Samples can be compared to reference data:
- **automatically** (top: cosine similarity score of NIST vs. NIST glycan profiles – see **Fig. 3**)
- **visually** (bottom: butterfly plot of cetuximab vs. NISTmAb tryptic digest – see **Fig. 4**)

Clone Selection

Digest and sample preparation time of IdeS digestion and MALDI sample preparation was 1/2 hr. Major glycans such as G0F, G1F, G2F and G3F were assayed by direct profiling of the Fc-domain of monoclonal antibodies. Spectra acquisition and processing were completed in less than 10 sec/sample. Different attributes such as the match of the glycan profile with a QTOF reference profile (***Fig. 3***) with a certain score (***Fig. 2, top***) or the test for G0F as being the base peak glycan were reported in the software providing multiple data points rapidly to decide which clones to select for further rounds of screening.

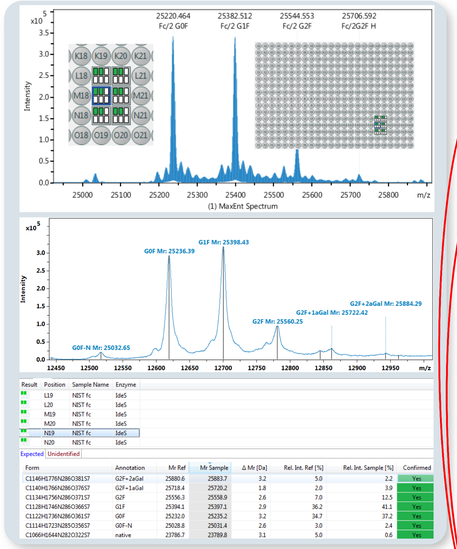

Fig. 3 NISTmAb Fc glycoprofiles from maXis ESI-UHR-QTOF (**top**, deconvoluted, Mr mono) and from autoflex maX MALDI-TOF (**middle**, 2^+, Mr ave). Inserts show the sample plate view with the traffic light reporting fields as explained in **Fig. 2, top**. Detailed match report **bottom**.

Rapid Identity Testing

The quality of the MALDI peptide mass fingerprints from all tested antibody digests was high (average: 70% sequence coverage for LCs and 40% for HCs) providing for an identity assay largely based on the differentiating peptides, i.e., peptides derived from the CDR of ~120 residues of the antibodies; 4-13 peptides were used in these profiles in addition to 6 abundant common peptides. Profiles of these antibodies allowed for their automated distinction based on cosine similarity scoring (CSS) with CSS>0.9 as acceptance criterion, non-matching identities yielded CCS values of 0.2-0.6. In addition, butterfly plots allow the visual confirmation of the ID provided by the software (***Fig. 2 bottom***).

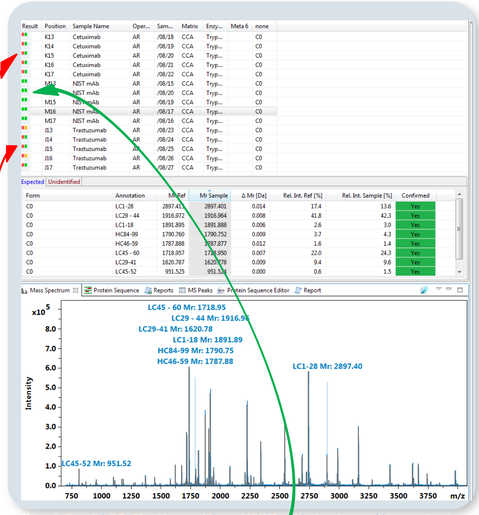

Fig. 4 Rapid ID Testing with a NISTmAb test profile against another NISTmAb rapid digest successfully confirmed NISTmAb. Matches against cetuximab and trastuzumab failed according to cosine similarity scores.
■■: ID not confirmed / Mass accuracy within MS Tol.

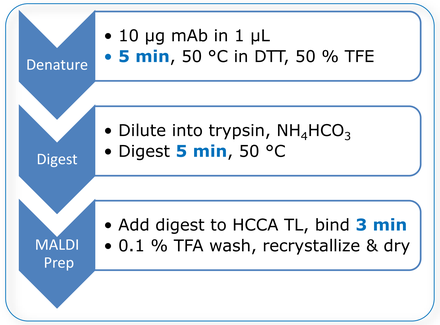

Fig.5 Fifteen min Rapid Identity Testing Chemistry

The rapid ID testing result for NISTmAb (Pos. M16) is presented in the form of a pass/fail list (***Fig.4***) that displays the Rel. Int. of the peptides present in the selected dataset and a reference peptide profile.

Conclusions

- BioPharma Compass allows to take advantage of MALDI MS spectra through comparison of target/reference profiles with test samples
- Typical applications supported:
  - screening in early development, such as **clone selection**
  - **Rapid Release ID Testing** during Fill & Finish operations in approx. 15 min
  - QC of incoming goods such as Tween20 vs Tween 80 differentiation. (not shown)
  - 2-AB glycan profiling (LC-free, not shown)

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