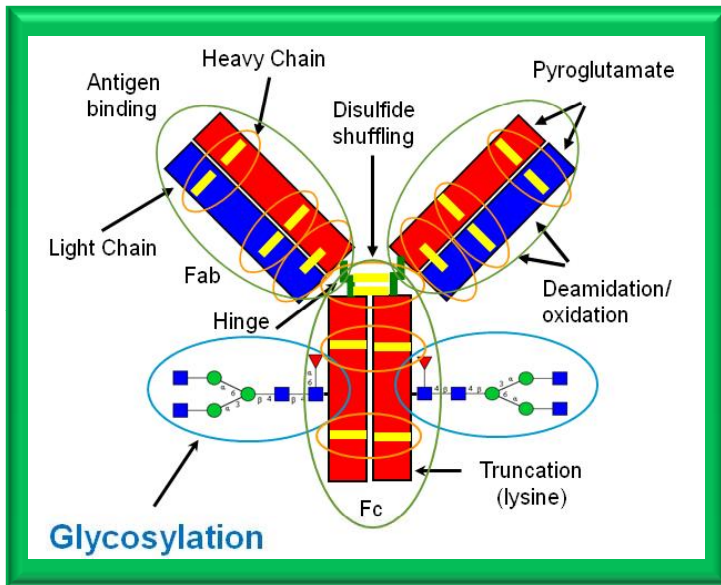




mAb-Glyco Chip Kit

Background

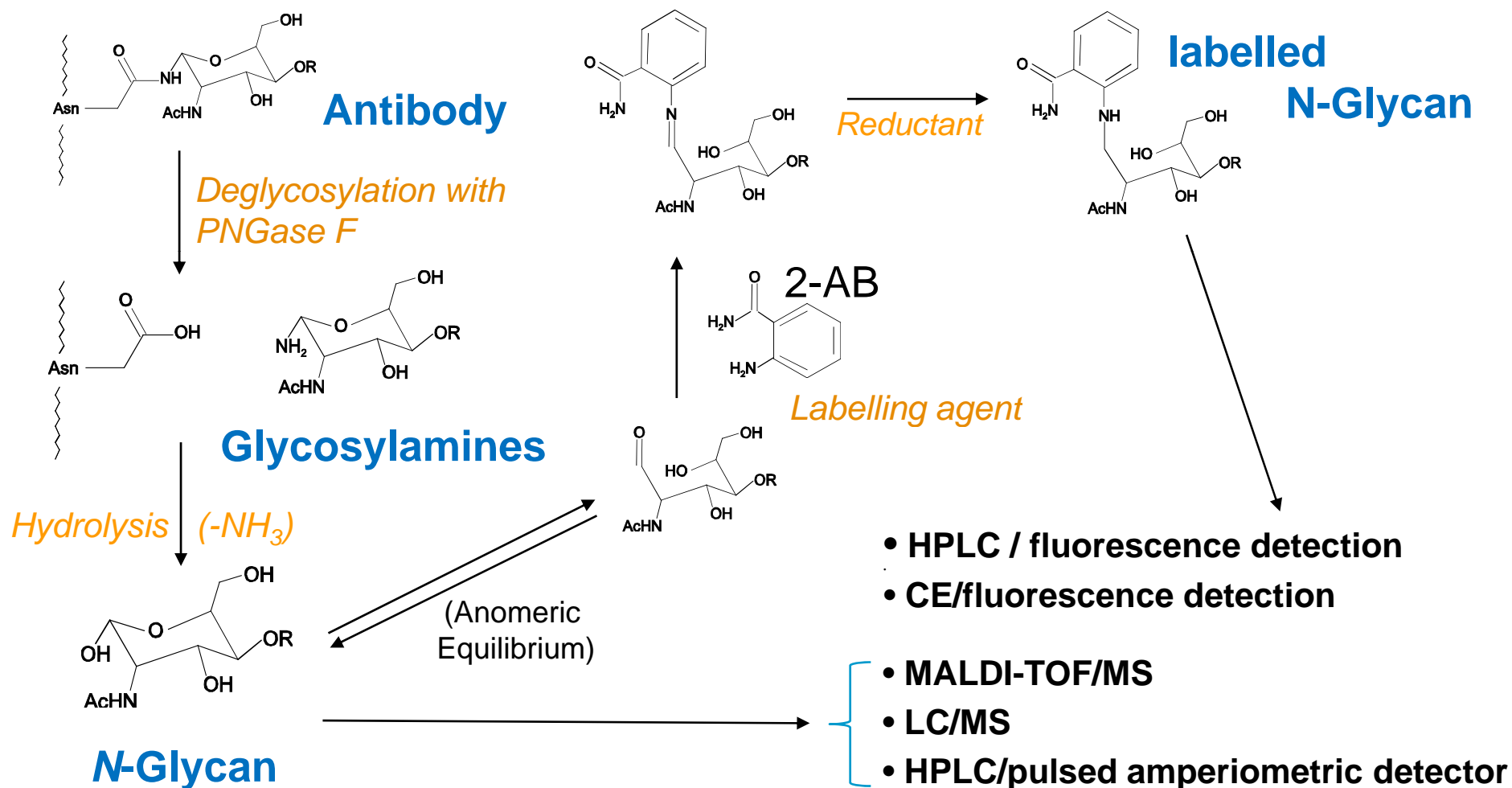
- Importance of monoclonal antibody characterization



- Monoclonal antibodies (mAbs) are glycoproteins bearing complex oligosaccharide moieties
- Presence/absence and glycosylation profile impacts therapeutic efficacy, pharmacokinetics, immunogenicity, stability, etc.
- Glycosylation is influenced by many factors e.g. cell line in which the mAb is produced or other production conditions (pH T media)
- Hence, characterization of glycan profiles is of vital importance throughout the various phases of development of these therapeutic mAbs

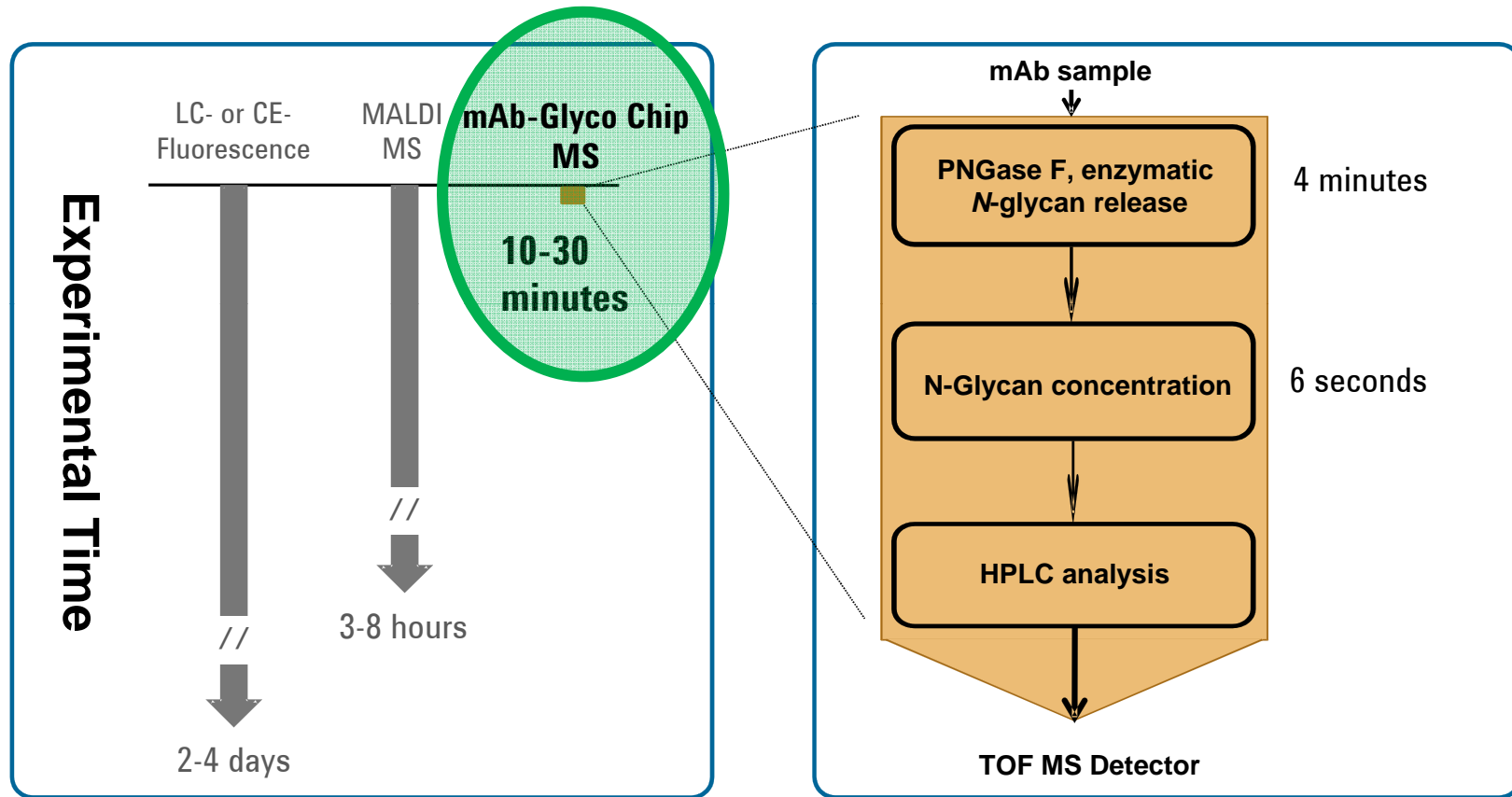
Glycan Characterization

- Typical workflows



On-Chip Glycan Characterization

- *Revolution in N-glycan analysis*



mAb-Glyco-Chip Kit

– A complete turnkey solution for N-glycan characterization

Reagent Pack



mAb-Glyco Chip



Reporting Templates

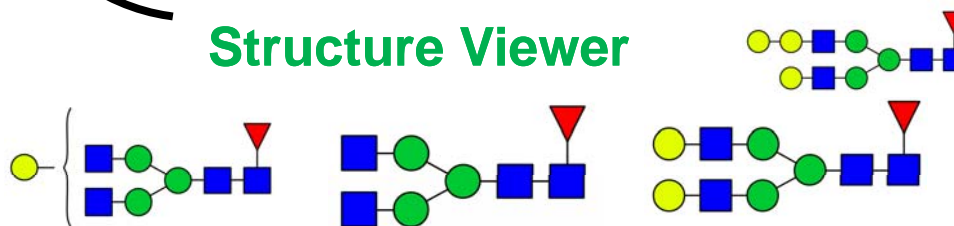
Compound Label	RT	Mass	Name	Formula	Tgt Mass	Diff (ppm)	Isomer(s) Present	Total Volume	Total Vol%
Cpd 1: Man5 N	9.068	1233.4475	Man5 N	C46H79N3O35	1233.4494	-1.52	1233.4494	134626	5.2
Cpd 3: Man4 N	9.082	1071.3956	Man4 N	C40H69N3O30	1071.3966	-0.9	1071.3966	6937	0.3
Cpd 4: 1000 OA OG N	9.103	1112.4235	1000 OA OG N	C42H72N4O30	1112.4231	0.31		34631	1.3
Cpd 5: 2000 OA OG N	9.133	1315.5029	2000 OA OG N	C50H89N5O35	1315.5025	0.3		59267	2.3
Cpd 9: Man4 N	9.17	1071.3928	Man4 N	C40H69N3O30	1071.3966	-3.55	1071.3966	6937	0.3
Cpd 13: 1100 OA OG N	9.23	1258.4825	1100 OA OG N	C48H82N4O34	1258.481	1.15	1258.481	45257	1.7

Total workflow solution

Accurate Mass Glycan Database

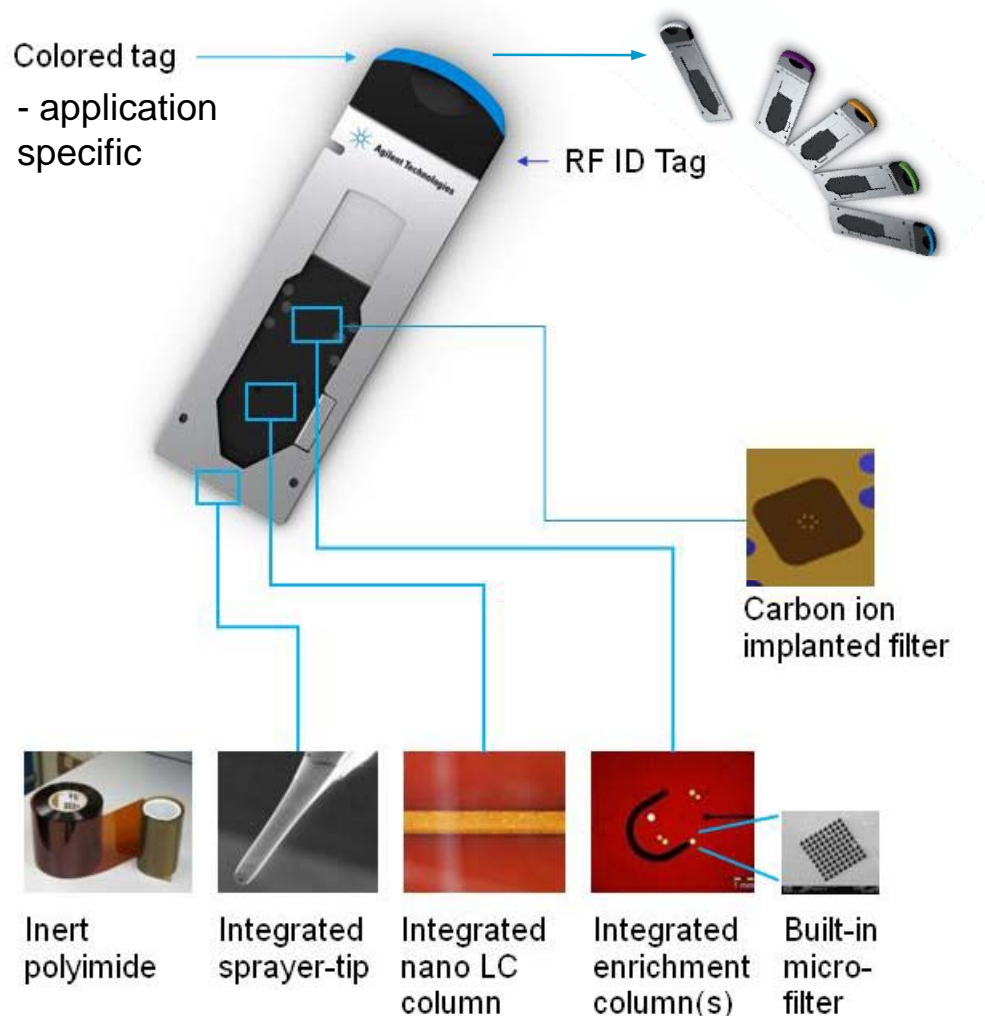
Compound Name	Formula	Mass	RT (min)	CAS	ChemSpider	IUPAC Name
Man4	C40H69N3O30	1,072.3000				Man4:3Man4:4Man4:3Man4:4Man4:4Man4:4Man4
1000 OA OG N	C42H72N4O30	1,112.4274				
1000 OA OG	C42H72N4O30	1,113.4075				
Man5	C46H79N3O35	1,233.4941				Man5:3Man5:4Man5:3Man5:4Man5:4Man5:4Man5
Man5	C46H79N3O35	1,234.4540				Man5:3Man5:4Man5:3Man5:4Man5:4Man5:4Man5
1100 OA OG N	C48H82N4O34	1,258.4036				
1100 OA OG	C48H82N4O34	1,259.4056				
1010 OA OG N	C48H82N4O34	1,274.4796				
1010 OA OG	C48H82N4O34	1,275.4590				
2000 OA OG N	C50H89N5O35	1,315.5021				GAMan3:2Man4:3Man4:3Man4:4Man4:4Man4:4Man4:4Man4
2000 OA OG	C50H89N5O35	1,316.4853				GAMan3:2Man4:3Man4:3Man4:4Man4:4Man4:4Man4:4Man4
Man4	C40H69N3O30	1,399.5023				Man4:3Man4:4Man4:3Man4:4Man4:4Man4:4Man4
Man4	C40H69N3O30	1,398.4825				Man4:3Man4:4Man4:3Man4:4Man4:4Man4:4Man4
1110 OA OG N	C48H82N4O34	1,420.5397				
1110 OA OG	C48H82N4O34	1,421.5190				
2100 OA OG N	C50H89N5O35	1,403.5042				GAMan3:2Man4:3Man4:3Man4:4Man4:4Man4:4Man4:4Man4
2100 OA OG	C50H89N5O35	1,402.5443				GAMan3:2Man4:3Man4:3Man4:4Man4:4Man4:4Man4:4Man4
2010 OA OG N	C50H89N5O35	1,477.5533				
2010 OA OG	C50H89N5O35	1,478.5335				

Structure Viewer



Agilent HPLC-Chip II

– General key features



Fully integrated design

- Patented laser-ablated channels
- Nano-analytical column
- Enrichment column(s)
- Micro-valve connections
- Nano-electrospray tip
- Micro-filters
- Carbon ion implanted filter

Enhanced performance

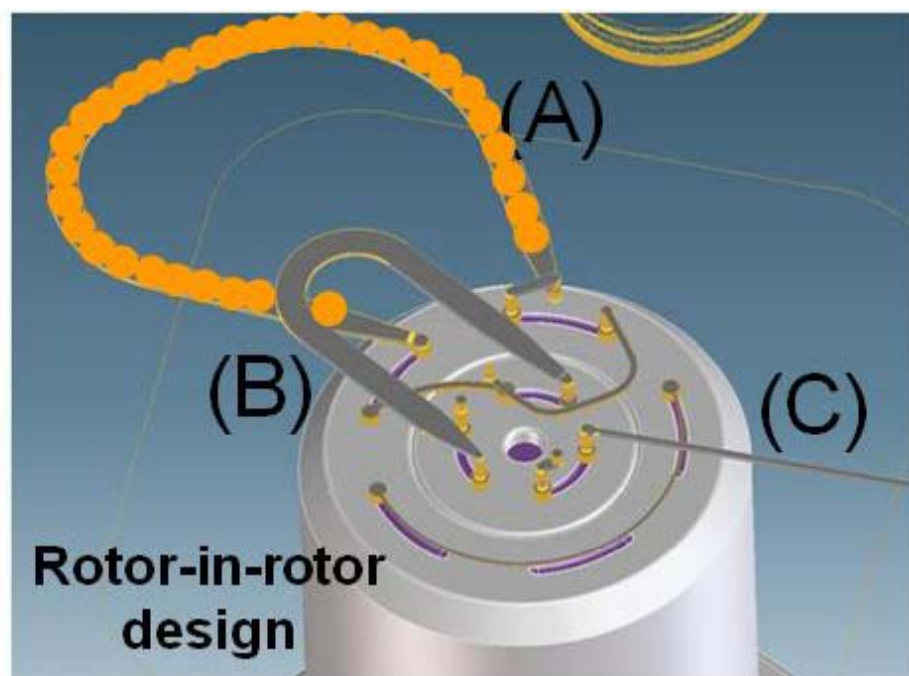
- Zero dead volume for better chromatographic performance
- LC/MS sensitivity
- Improved run-to-run reproducibility and chip life-time

Robustness & ease-of-use

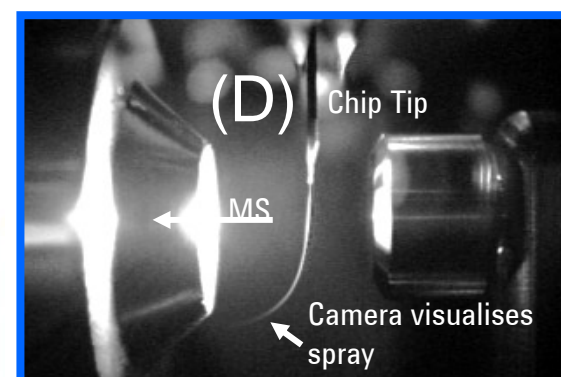
- No clogging of spray needle
- Plug-&-Play replacement
- Improved surface characteristics for optimal contact and sealing

mAb-Glyco-Chip

– *Integrated design*

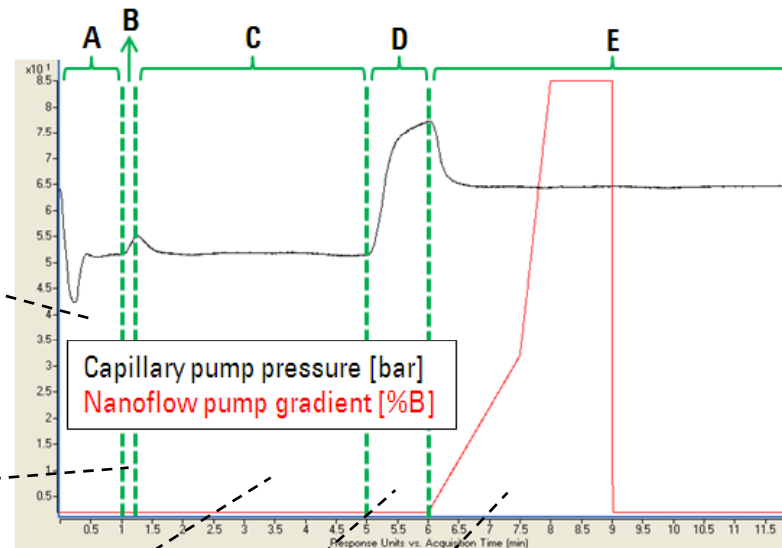
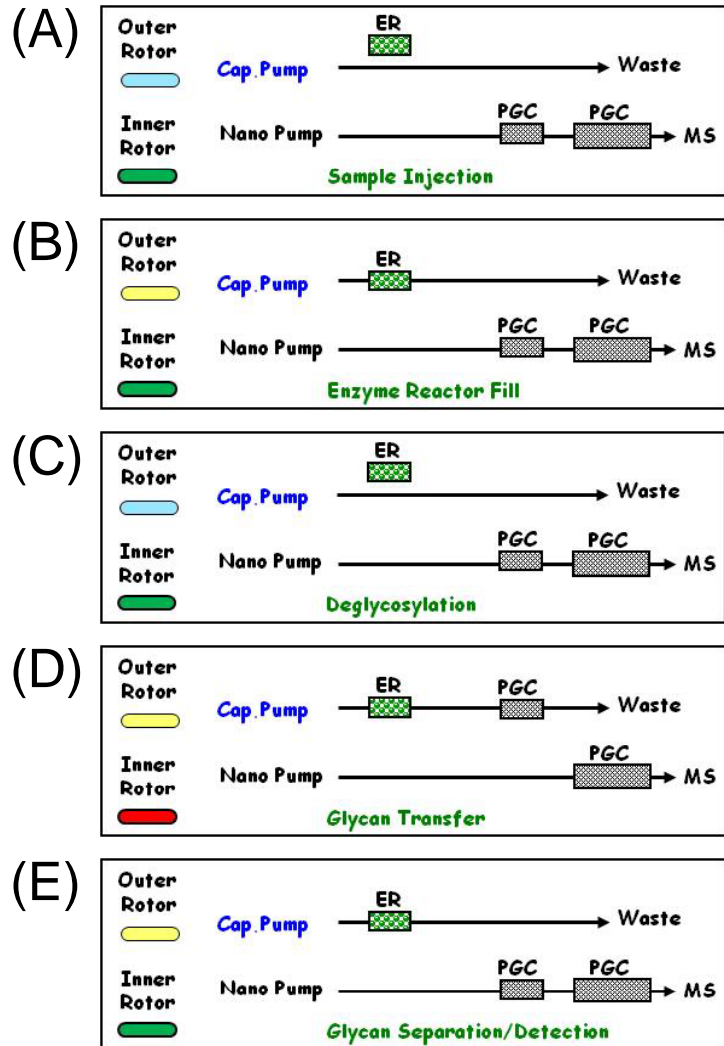


- (A) Enzyme reactor packed with immobilized **PNGase F** beads for on-chip deglycosylation of mAbs
- (B) Enrichment and (C) analytical columns for trapping and the separation of cleaved *N*-glycans
- (D) Electro spray tip for direct transfer to (Q)TOF detection



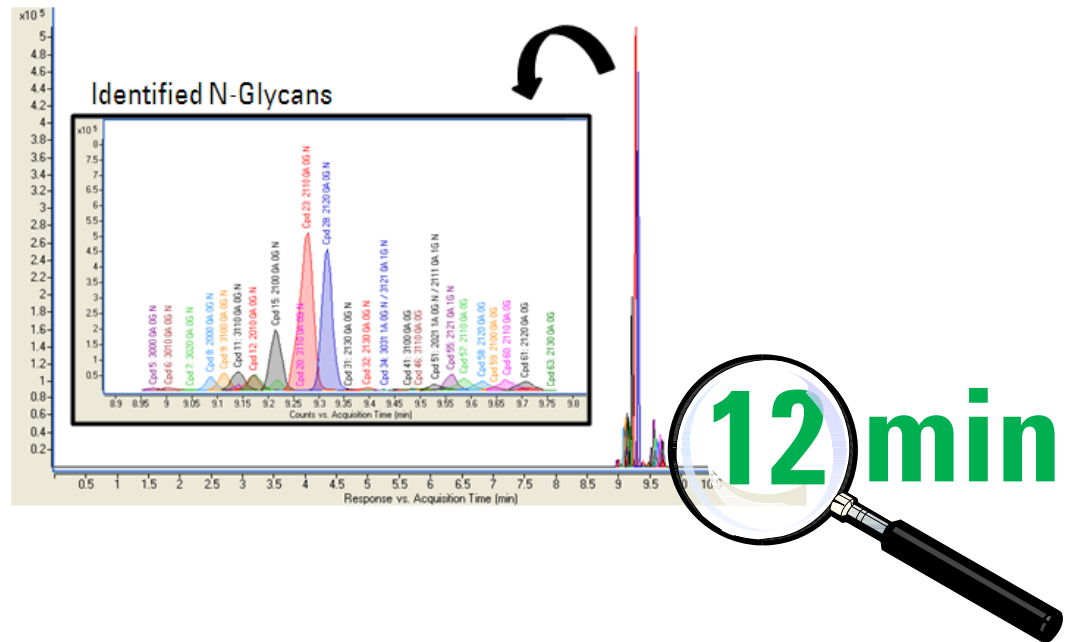
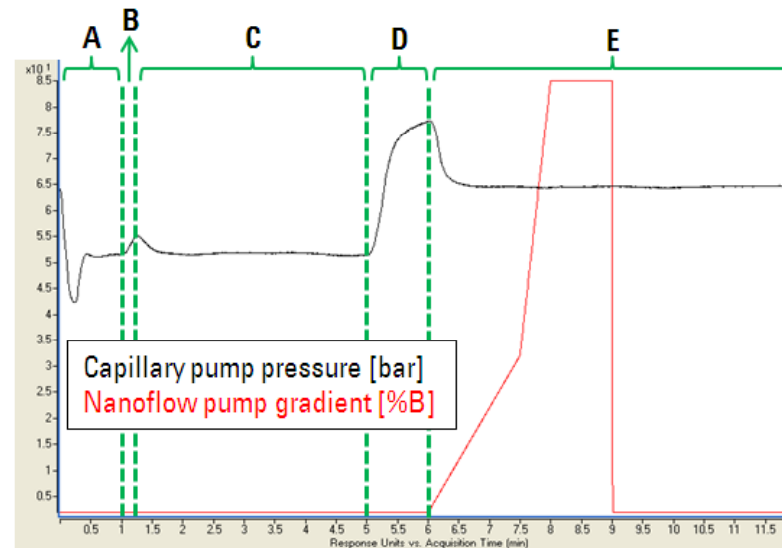
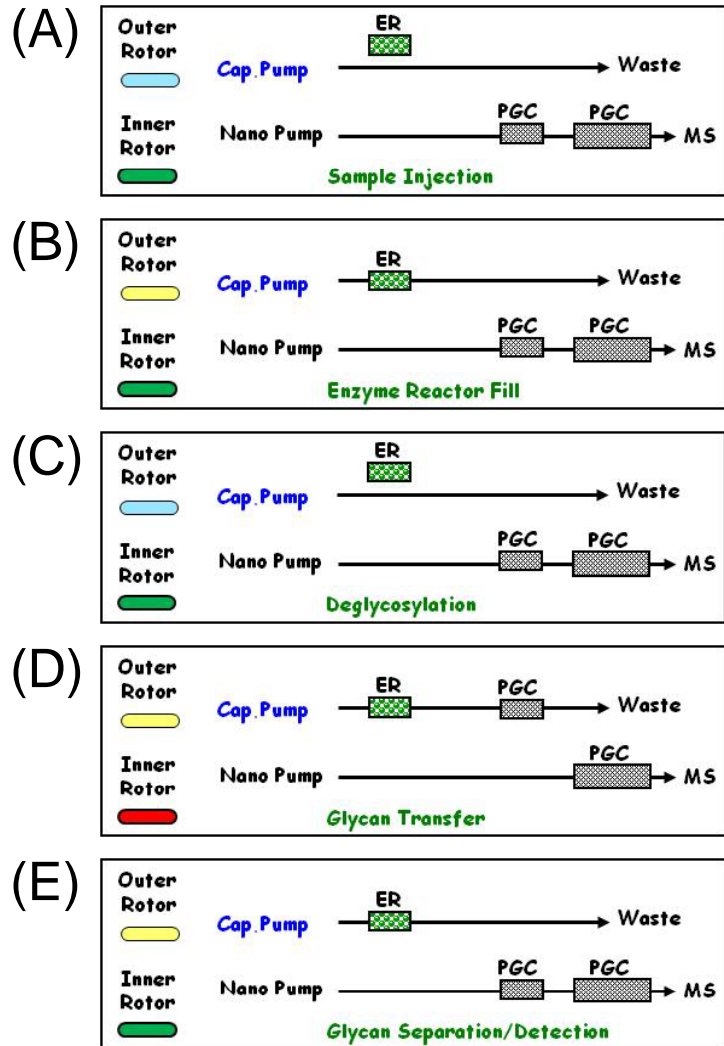
mAb-Glyco-Chip

– On-Chip workflow



mAb-Glyco-Chip

– On-Chip workflow

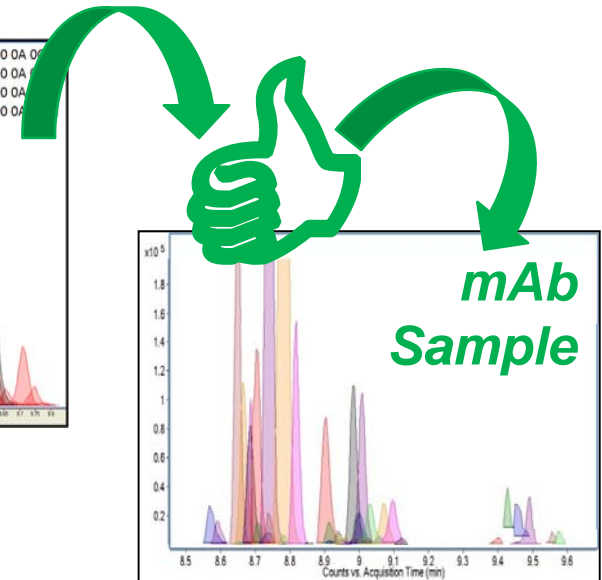
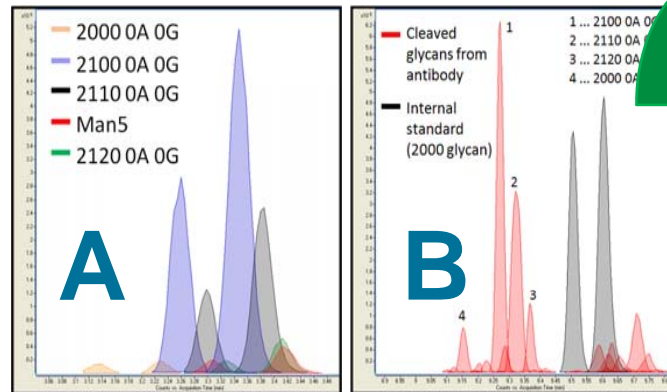


mAb-Glyco Chip Reagent Pack

– Includes all chemicals and reagents needed

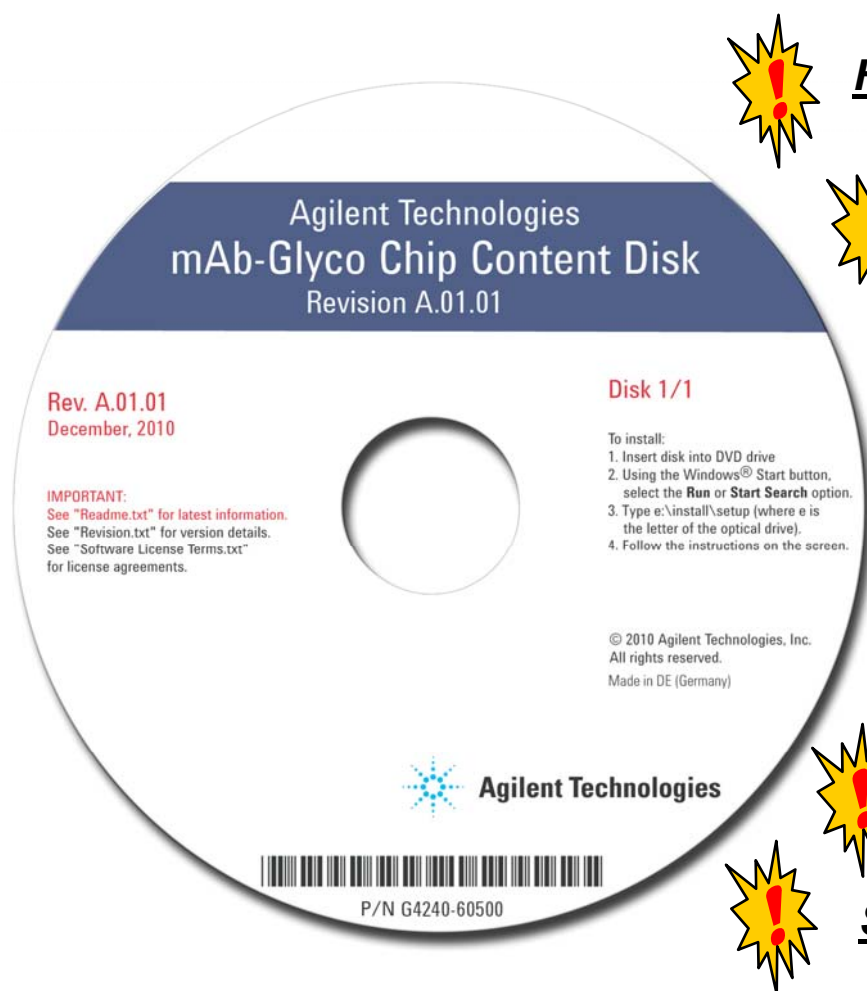


- System Conditioning Reagent
- Deglycosylation buffers = loading mobile phase
- **A**: Glycan-std's to verify chromatographic performance
- **B**: mAb-std to verify function of enzyme reactor



mAb-Glyco-Chip Content Disk

– *Completeness*



HTML interface for easy installation of content

Chip.ini and firmware updater

mAb-Glyco Chip user documents

Acquisition and data analysis methods

**Acc. Mass-Glycan structure database
144 entries of typical mAb glycans***

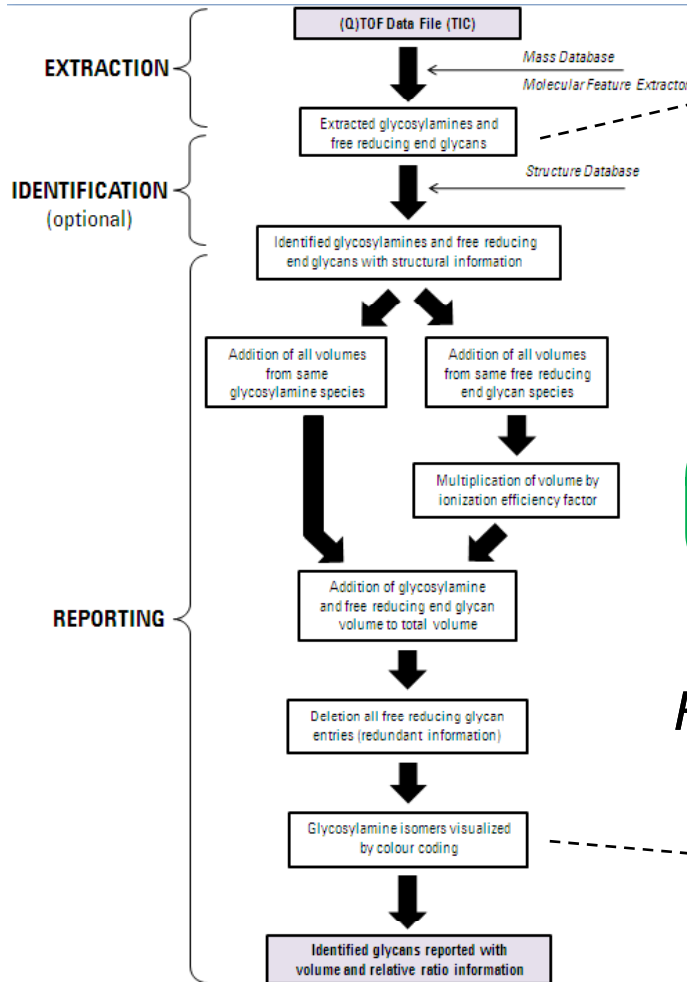
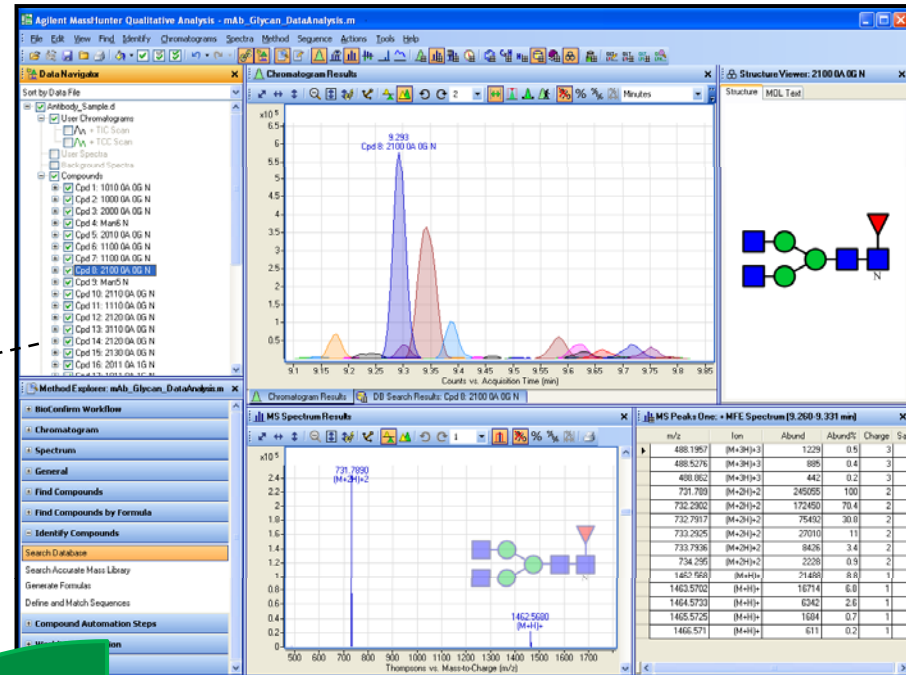
Example files and reporting templates

Supporting documents

* can be modified

Data Processing

– Answers in minutes



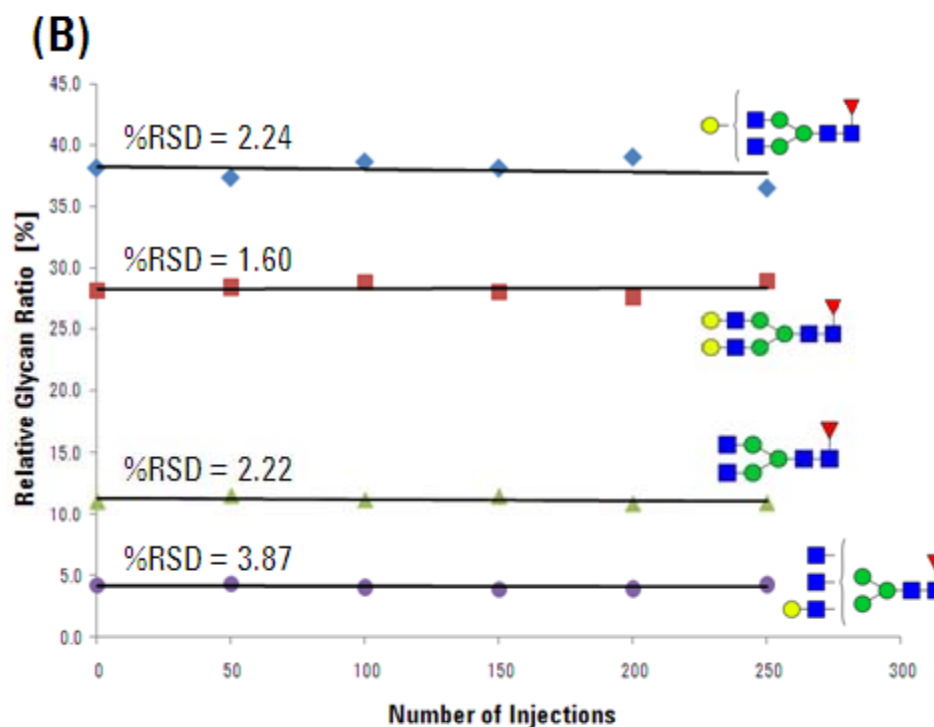
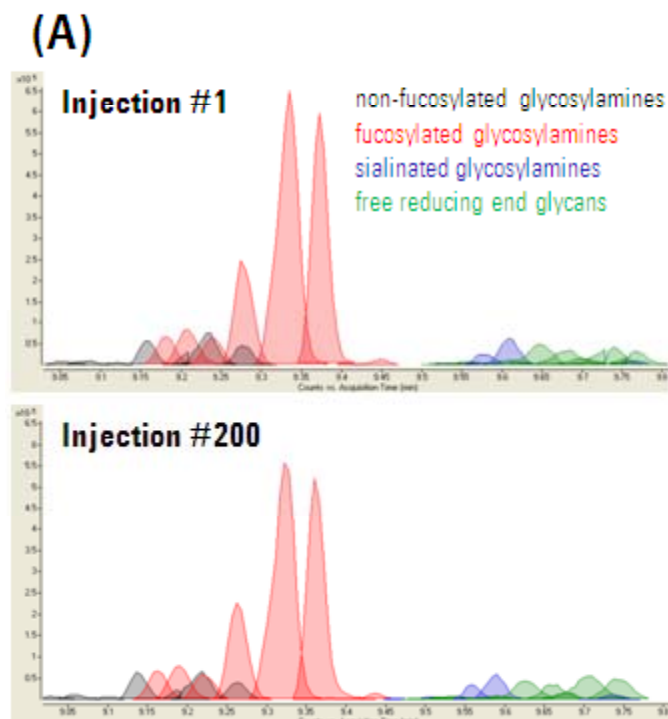
Glycan results of an extraction and identification procedure with MassHunter Qualitative Analysis Software. Sample: Antibody Standard from the mAb-Glyco Chip reagent pack

Name	Formula	Tgt Mass	Diff (ppm)	Isomer(s) Present	Total Volume	Total Vol%
2121 0A 1G N	C79H132N6O58	2092.7564	0.93		8906	0.3
1100 0A 0G N	C48H82N4O34	1258.481	-0.58	1258.481	40030	1.5
2120 0A 0G N	C68H115N5O49	1785.6661	1.83		11972	0.5
3100 0A 0G N	C64H108N6O44	1664.6398	0.46		10792	0.4
2110 0A 0G N	C62H105N5O44	1623.6132	1.39		284929	10.8
Man5 N	C46H79N3O35	1233.4494	-1.36	1233.4494	134626	5.1
2100 0A 0G N	C56H95N5O39	1461.5604	1.66		2044974	77.4
1100 0A 0G N	C48H82N4O34	1258.481	1.15	1258.481	40030	1.5
2010 0A 0G N	C56H95N5O40	1477.5553	-0.5		5076	0.2
1110 0A 0G N	C54H92N4O39	1420.5339	0.03		864	0.0
Man4 N	C40H69N3O30	1071.3966	-3.55	1071.3966	6937	0.3
2000 0A 0G N	C50H85N5O35	1315.5025	0.3		59267	2.2
1000 0A 0G N	C42H72N4O30	1112.4231	0.31		34631	1.3
Man4 N	C40H69N3O30	1071.3966	-0.9	1071.3966	6937	0.3
Man5 N	C46H79N3O35	1233.4494	-1.52	1233.4494	134626	5.1

* Reporting templates automatically process glycan hits by clustering and merging isomeric structures.

mAb-Glyco-Chip Kit

– Chip life-time test with 10 Chips → 200-300 injections (IgG)



Long-term stability and robustness of the mAb-Glyco Chip: (A) Extracted glycan pattern of the analysed antibody at injection number 1 and 200; (B) Relative glycan ratio as function of number of injections performed (4 most intense N-glycans). Sample: IgG from bovine serum (Sigma), 75 ng on-column.

Conclusion

– *mAb-Glyco Chip Kit*

- Up to 100 x faster than existing methods for the characterization of *N*-glycans on monoclonal antibodies (mAbs)
- An automated turnkey solution for on-chip deglycosylation, *N*-glycan separation and high sensitivity TOF/QTOF detection
- Integrated comprehensive data processing for automated glycan identification, quantification and reporting
- Provides a robust workflow solution that aids in removing a major bottleneck during the development phase of mAb-based biotech drugs enabling the analyst to provide answers fast

