SmartEnzymes™

Enzymatic Strategies for Characterization of Biopharmaceuticals

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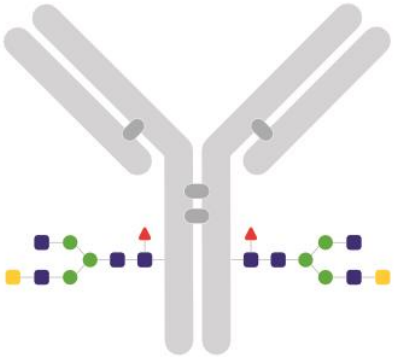
SmartEnzymes™

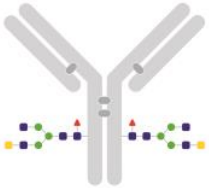
GENOVIS

Antibody Digestion	Proteomics	Glycan Profiling
FabALACTICA®	GingisREX®	FucosEXO™
FabDELLO™ NEW!	Antibody Deglycosylation	GalactEXO™
FabRICATOR®	GlycINATOR®	GalNAcEXO™
FabRICATOR®Z	IgGZERO®	OglyZOR®
FabULOUS™	Antibody Conjugation	OmniGLYZOR™ NEW!
GingisKHAN®	GlyCLICK®	OpeRATOR®
GlySERIAS™ NEW!	TransGLYCIT™ NEW!	PNGase F NEW!
	Affinity Purification	SialEXO®
	GlycOCATCH®	

Middle-level LC-MS Analysis

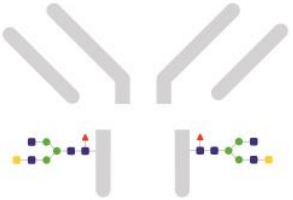


Top-down



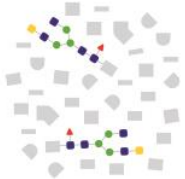
- Intact mass
- High-throughput
- Low resolution

Middle level



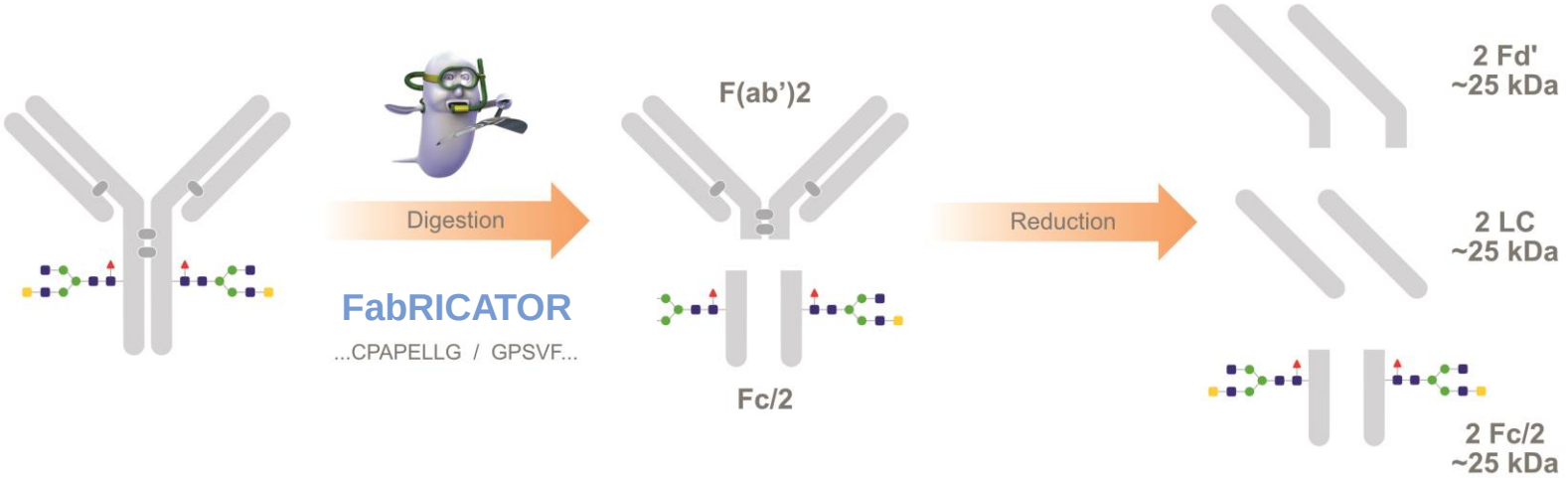
- High resolution
- Limited sample prep
- Fast analysis time

Bottom-up



- High resolution
- Extensive sample prep
- Long analysis time
- Site specific modifications

FabRICATOR®

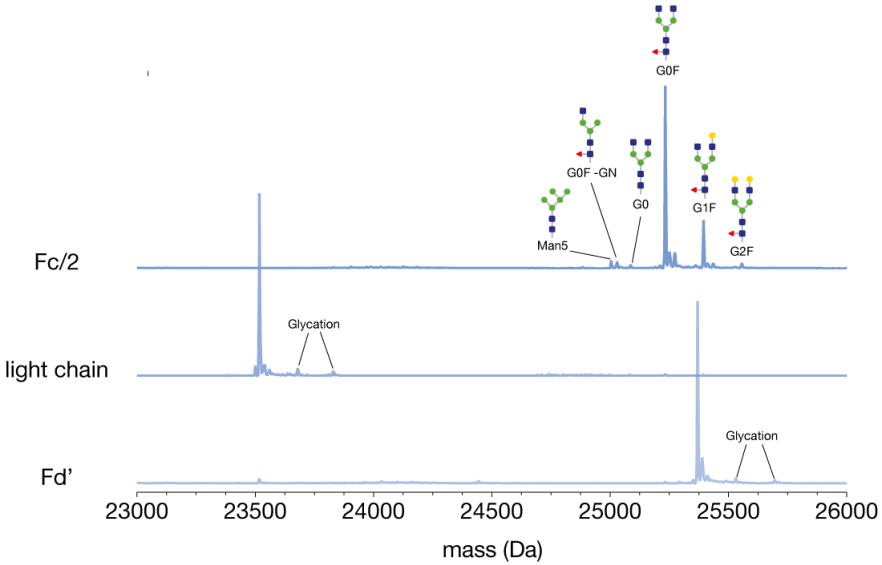


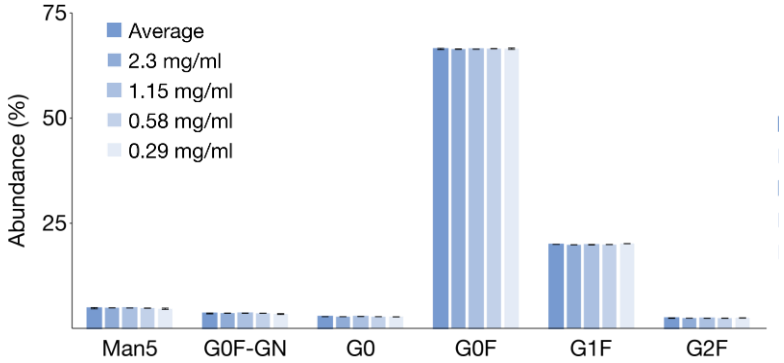
- One specific digestion site
- Digests human IgG_{1-4}
- No over-digestion
- No optimization needed

- 30 min at 37°C
- pH 5.5 – 8.0
- Physiological buffers
- Platform method

30 MINUTES

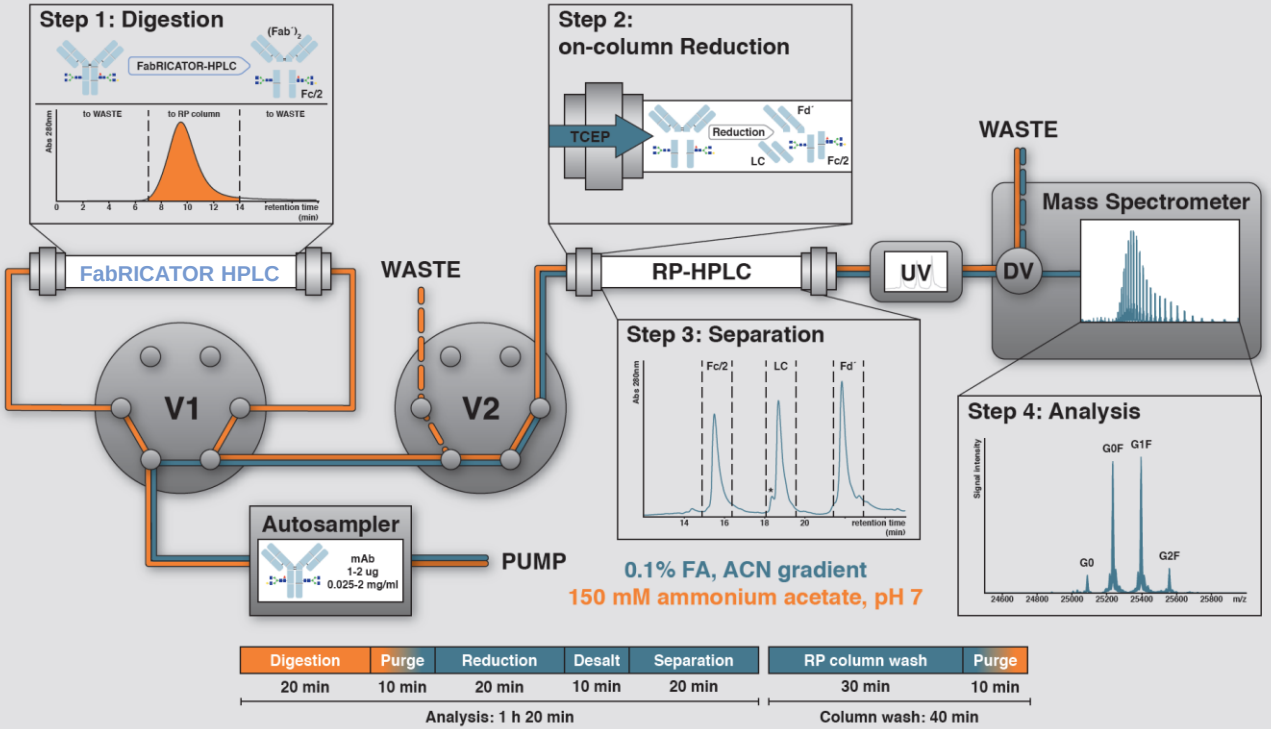
Analysis of Many CQAs by Middle-level LC-MS





- Analysis of several different CQAs in one analysis
- A robust assay without the need for optimization

FabRICATOR-HPLC Workflow



FabALACTICA®

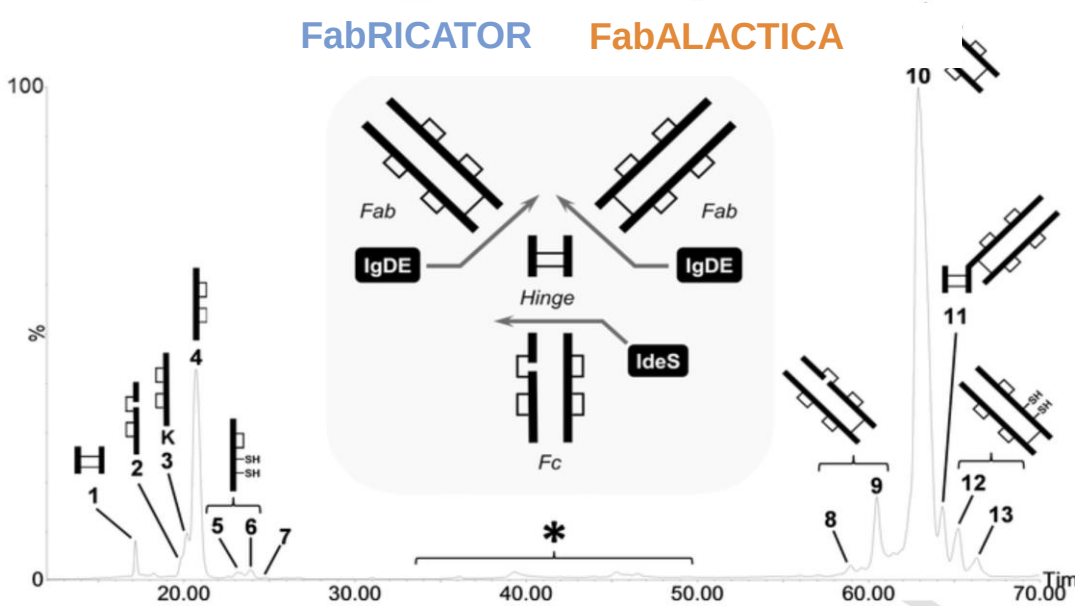
- Human IgG1 specific cysteine protease
- Digests the upper hinge
- Generating intact Fc and Fab fragments

- Robust protocol
- No over-digestion
- No reducing agents

O/N
REACTION

Middle-up Analysis of mAb Disulfide Bridges

FabRICATOR FabALACTICA



- Combined digestion FabALACTICA (IgdE) & FabRICATOR (IdeS)
- Separation on di-phenyl column
- Monitor free sulfhydryls without labeling
- Applicable to any IgG1

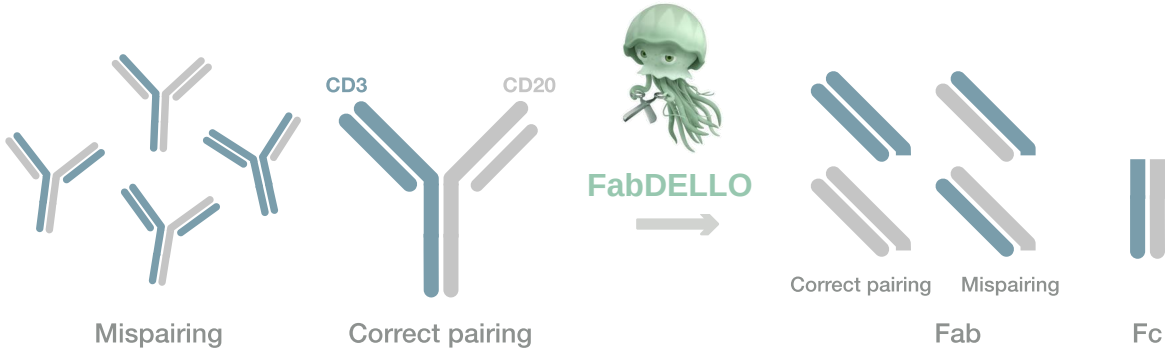
Faid, V. *et al.*, 2017. Journal of Pharmaceutical and Biomedical Analysis, 149, pp.541–546.

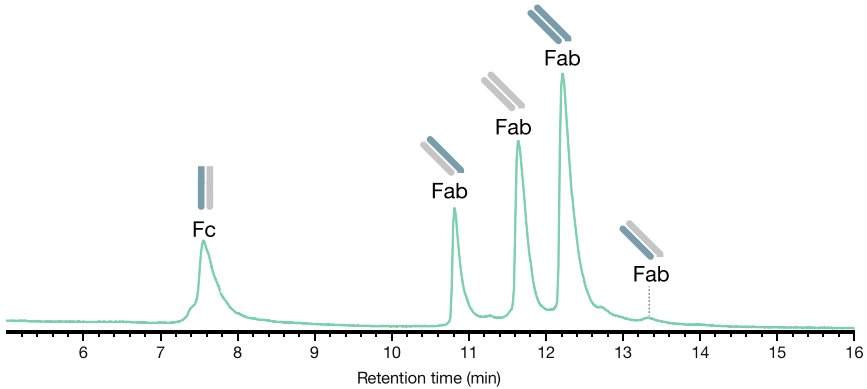
FabDELLO™

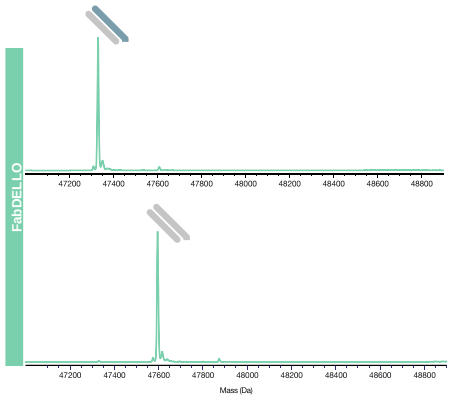
- Protease with digestion of human IgG1 at a single site above the hinge
- No need for reducing conditions
- 2 h incubation at 37°C
- Active on mAbs with mutated hinge regions, such as LALA antibodies
- Generates intact Fab and Fc fragments

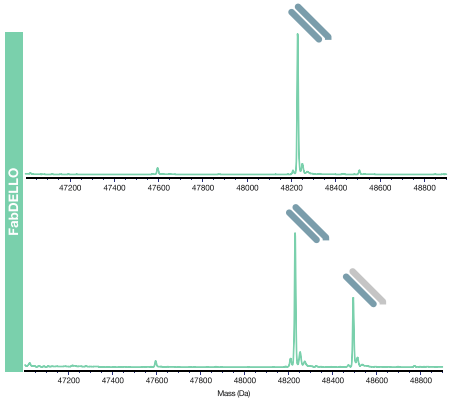
2 HOURS

Characterization of Bispecific Antibodies

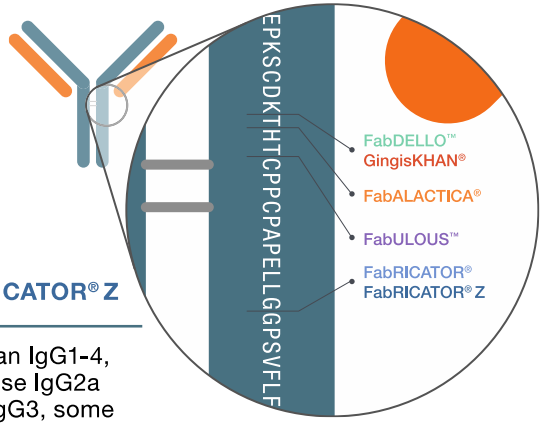






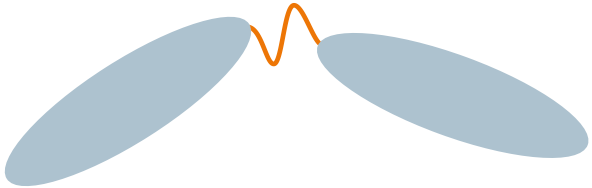


IgG Proteases Comparison

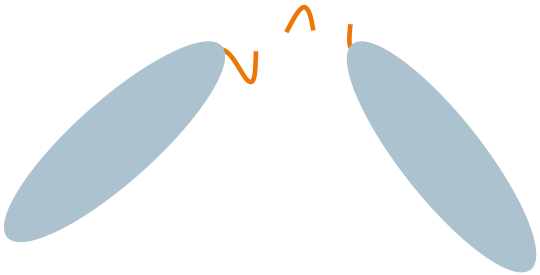


Enzyme	FabRICATOR®	FabALACTICA®	FabDELLO™	GingisKHAN®	FabULOUS™	FabRICATOR® Z
IgG species and subclasses	Human IgG1-4, mouse IgG2a and IgG3, some classes of rat, monkey, rabbit and sheep	Human IgG1	Human IgG1	Human IgG1	Human IgG, mouse, rat, goat, sheep and rabbit	Human IgG1-4, Mouse IgG2a and IgG3, some classes of monkey, rabbit and sheep
Digestion site (human IgG1)	LLG / GPS	DKT / HTC	CDK / THT	CDK / THT	THT / CPP	LLG / GPS
Above / below hinge (human IgG1)	Below	Above	Above	Above	Above	Below
Reaction requirements	Physiological buffers	Physiological buffers	10 mM $CaCl_2$. Do not use phosphate buffers!	2 mM cysteine	Reducing conditions	Physiological buffers
Reaction time	30 min	O/N	2 h	1 h	1 h	2 h
pH	5.5 - 8	6 - 8	7 - 8.5	8	6.5 - 8	5.5 - 8

GlySERIAS™

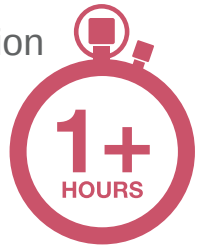


GlySERIAS

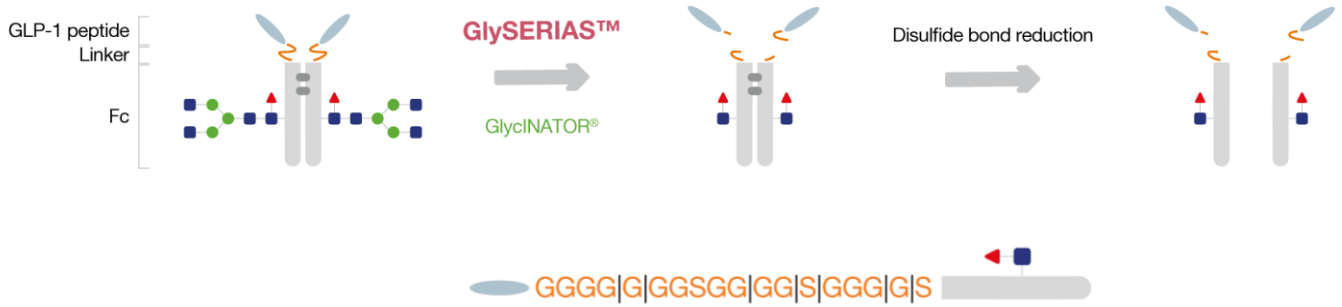


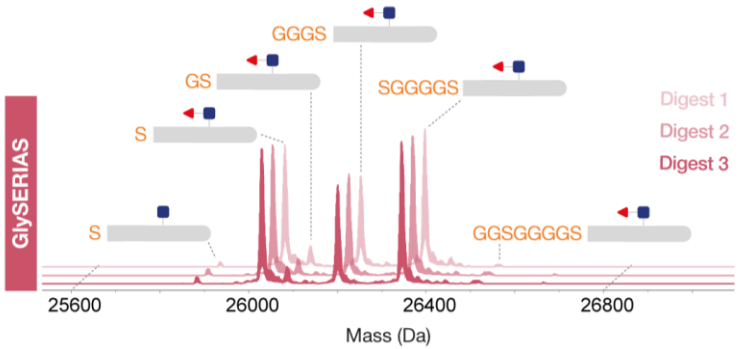
- Unique enzyme digesting flexible linkers in fusion proteins.
- Active on GS linkers such as G_4S and G_xS_y linkers, and polyglycine (G) linkers.
- 1 hour reaction (optimization for individual proteins may be required.

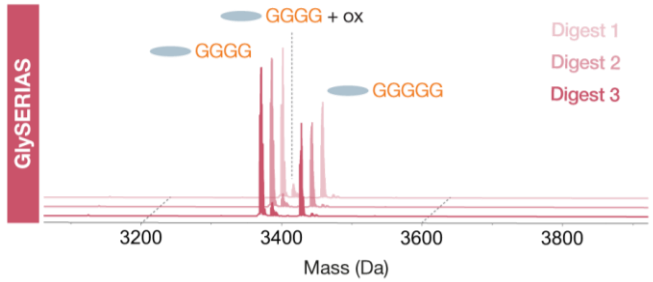
- No reducing agent or co-factor needed.
- Improves resolution and reduces complexity of fusion protein analysis.
- A middle-level approach for characterization of fusion protein components.



Digestion of a GS-linked Fusion Protein



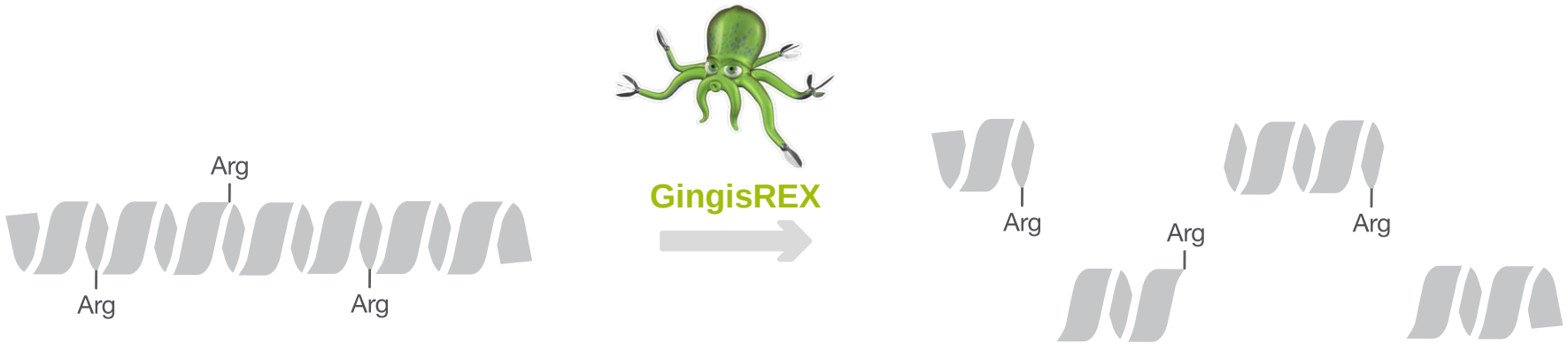




GingisREX®

GENOVIS

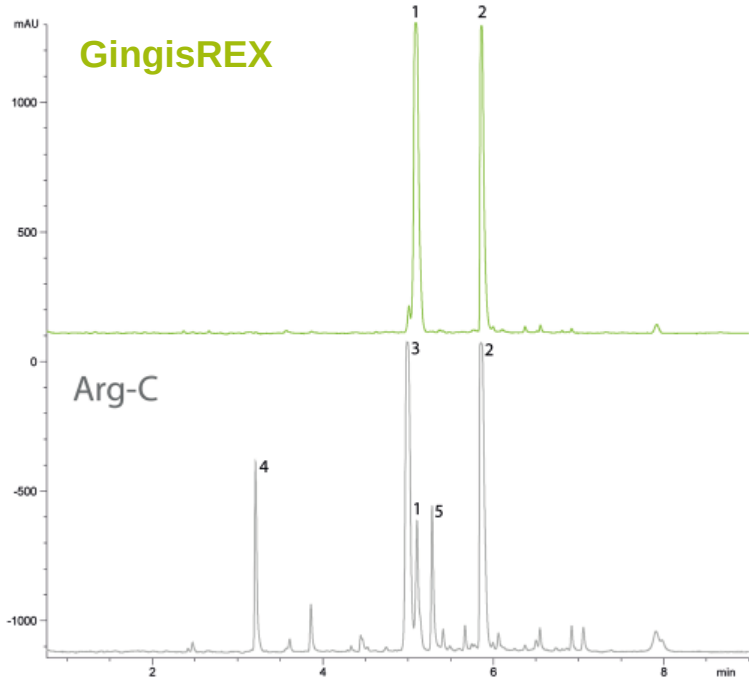
Arginine Specific Protease



- Cysteine protease
- Specific activity on Arg-X residues
- Active on Arg-Pro linkages

- Active in 6M urea
- pH 5.0-9.0
- Inhibited by guanidine-HCl

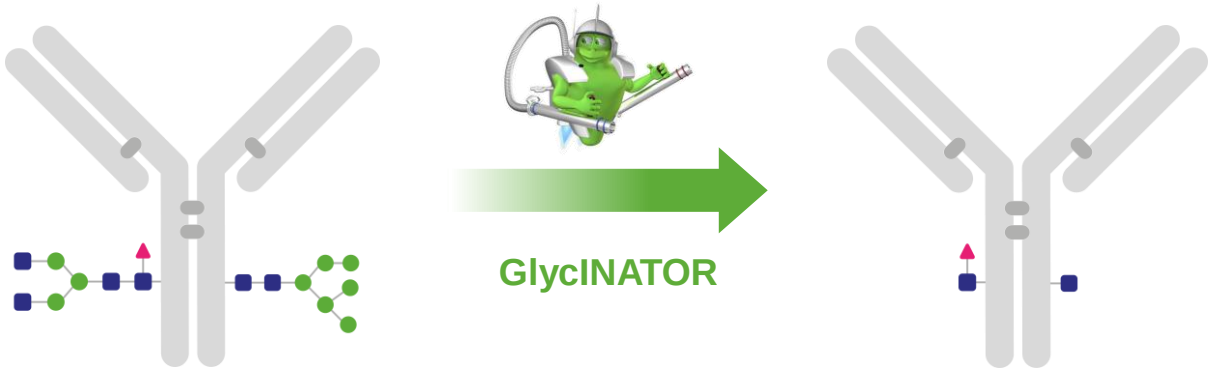
Peptide Mapping of Oxidized Insulin β-chain



PEPTIDE NO.	AMINO ACID SEQUENCE
Intact protein	FVNQHLCGSHLVEALYLVCGERGFFYTPKA
1	GFFYTPKA
2	FVNQHLCGSHLVEALYLVCGER
3	GFFYTPK
4	FVNQHLCGSH
5	LVEALYLVCGER + Na

Sequences of oxidized insulin β-chain digested by GingisREX or Arg-C.
Green indicates arginine residues and red indicates lysine residues.

GlycINATOR®

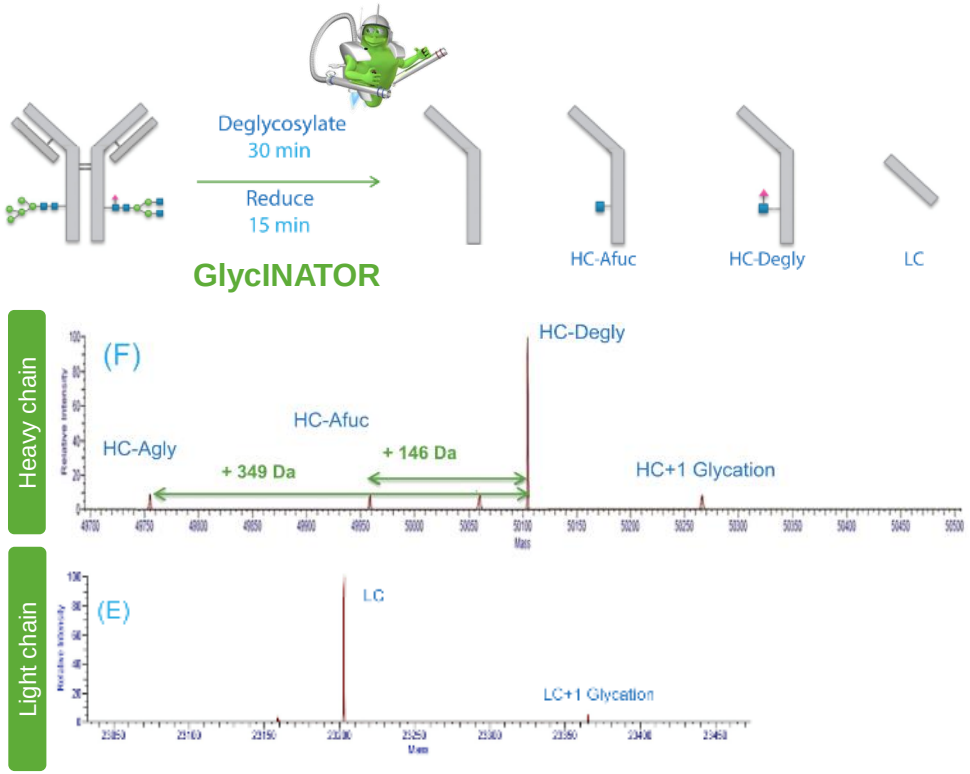


- GlycINATOR is an IgG specific endoglycosidase
- Removes all glycoforms of IgG
- Specific for the Fc-glycans
- 30 min incubation at 37°C

- Glycan removal abolishes ADCC
- Enables rapid analysis of afucosylation
- Native reduction of sample complexity
- Enhanced sensitivity in oxidation or glycation workflows

30 MINUTES

Afucosylation Assay using GlycINATOR



Afucosylation Assay

- Core afucosylation
- N-glyco occupancy
- High-throughput analysis
- Cell line screening
- Process development

Liu, S. & Zang, L., 2016. Analytical Biochemistry, pp.1–10.

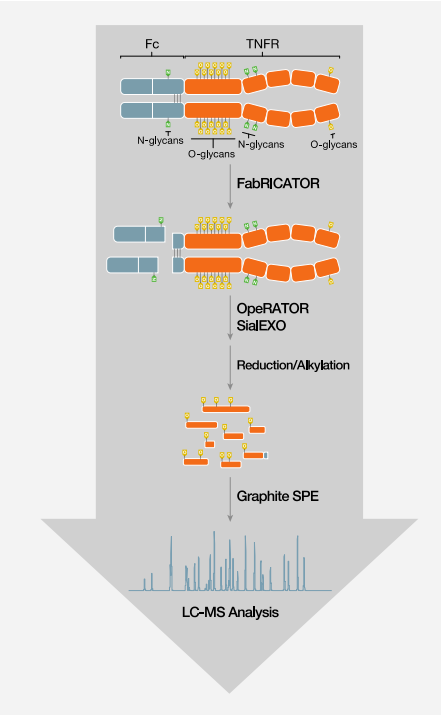
OpeRATOR®

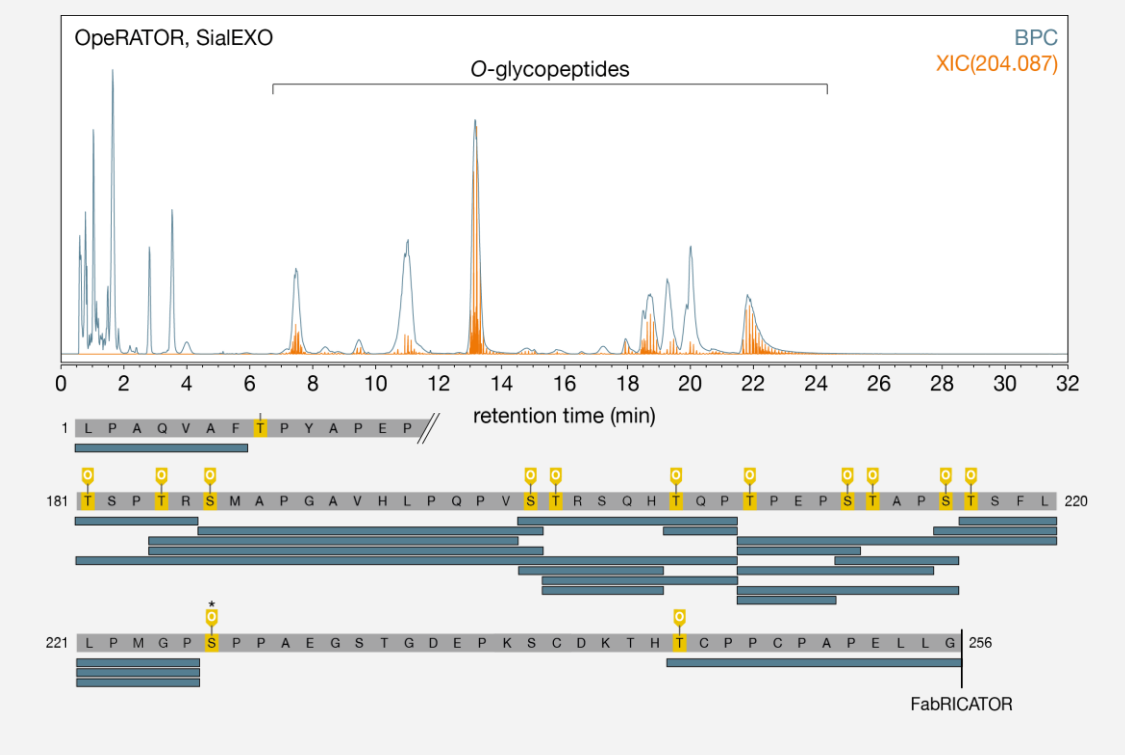
O-glycoprotease from *A. muciniphila*

- *O*-glycoprotein specific protease
- Hydrolyzes glycoproteins N-terminally to the *O*-glycosylated serine or threonine residues
- No activity on *N*-glycosylation sites
- Significantly reduced activity on sialylated *O*-glycosylation sites
- A sialidase (SialEXO) is included with OpeRATOR

2+ HOURS

Mapping *O*-glycosylation sites in etanercept





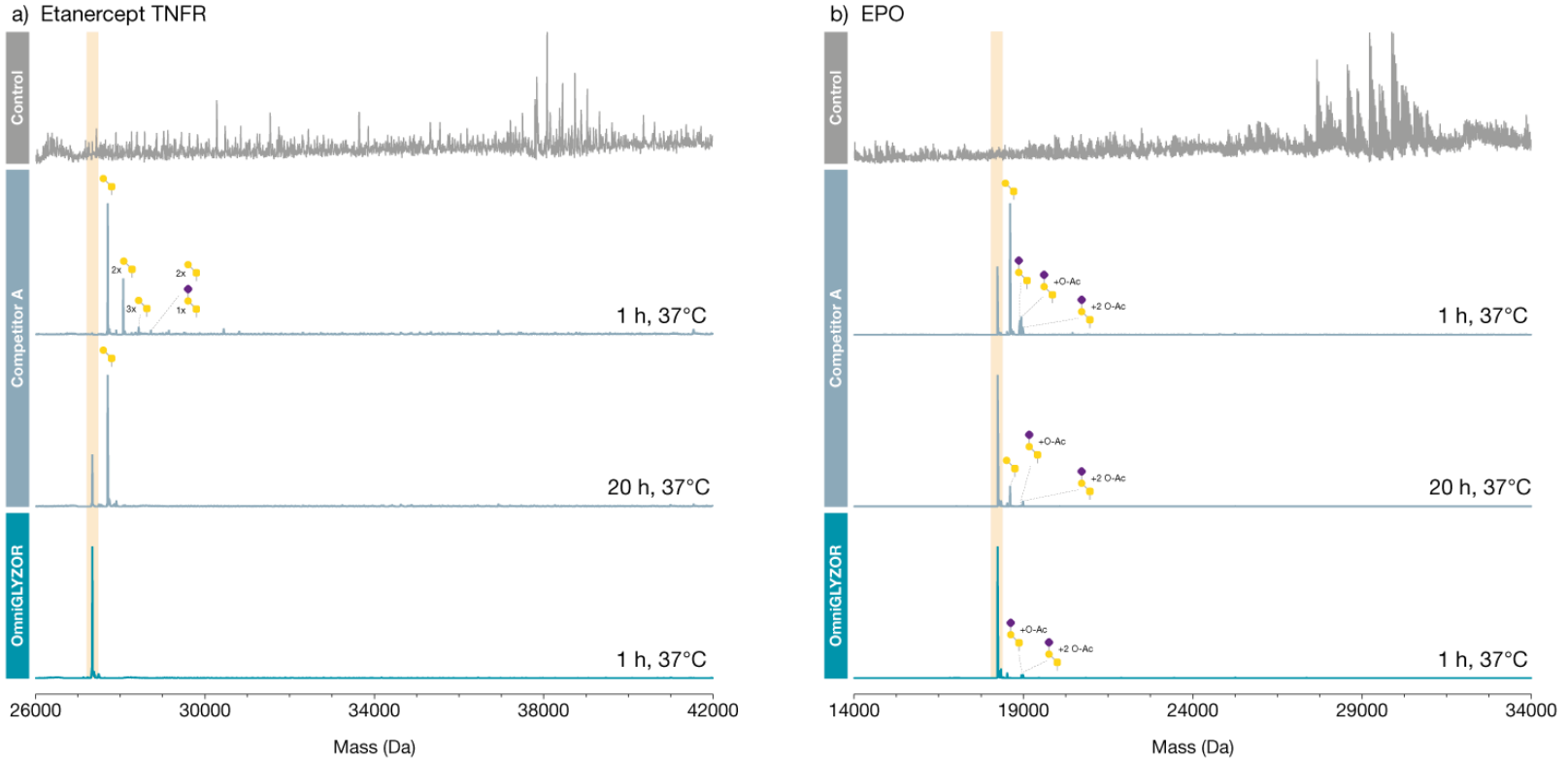
- All *O*-glycosylation sites of etanercept could be identified
- No enrichment needed
- Site-specific mapping without ETD fragmentation

OmniGLYZOR™

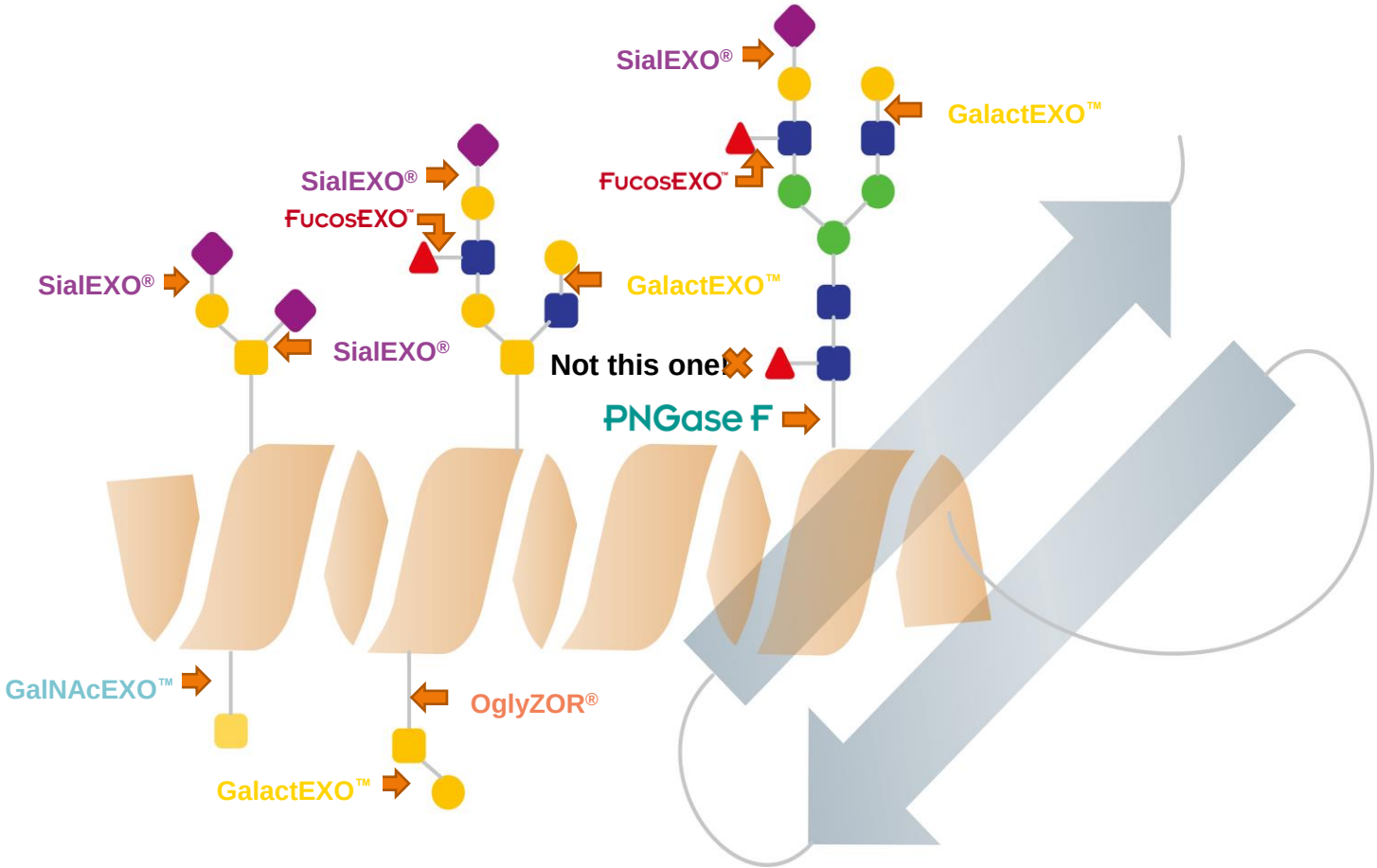
- Contains a mix of immobilized enzymes for fast and efficient removal of N- and simple mucin-type O-glycans (mono- and disialyl core 1 and Tn antigen).
- Activities included: PNGase F, O-glycosidase, sialidase, α-GalNAcase.
- 1-4 h workflow
- Ready-to-use spin column format – no enzyme interfering in the analysis.
- Compatible with LC-MS.
- *Rapi*Gest™* included

* RapiGest™ SF Surfactant from Waters Corporation is included in OmniGLYZOR. RapiGest™ is a trademark of Waters Corporation.

Efficient Removal of Glycans from Etanercept and EPO

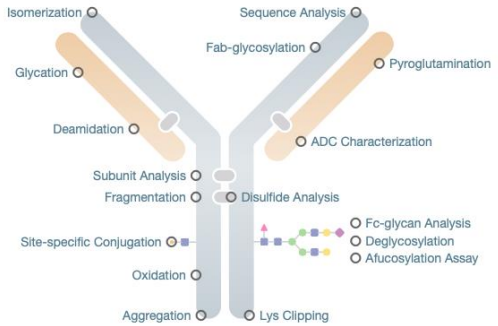


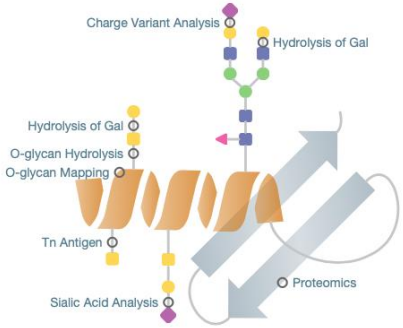
Tools for Characterization of Complex Glycoproteins



Resources – www.genovis.com

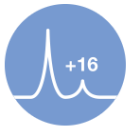
SmartEnzymes Applications





Mass Spectrometric Analysis

Subunit Analysis



Oxidation

Fc-glycan Analysis

Sialic Acid Linkage Analysis

Unmasking Charge Variants

Tn Antigen

SmartStories

Weiming Yang
Johns Hopkins University

OpeRATOR changes the game in the field of O-linked glycoproteomics

Hanieh Khalili
University of East London

Antibody mimetics generation using GingisKHAN

Bastiaan Duivelshof
University of Geneva

Generating site-specific ADCs using the GlyCLICK technology

Min Kyung So
KBIO Osong Medical Innovation Foundation

FabRICATOR for Intact Analysis of Biologics

Dan Bach Kristensen
Symphogen A/S

FabRICATOR MagIC produce F(ab)2 subunits in less than 30 minutes

Valegh Faid
LFB Biotechnologies

FabALACTICA in non-reducing study of antibody disulphides

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