Glu-C – an orthogonal and alternative enzyme for protein quantitation by LC-MS/MS

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Science
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EBF, 17th November 2016

LC-MS/MS for protein quantification

- Pros
  - Highly selective (metabolites, degradation products)
  - Improved accuracy
  - Multiplexing (simultaneous analysis of multiple proteins)

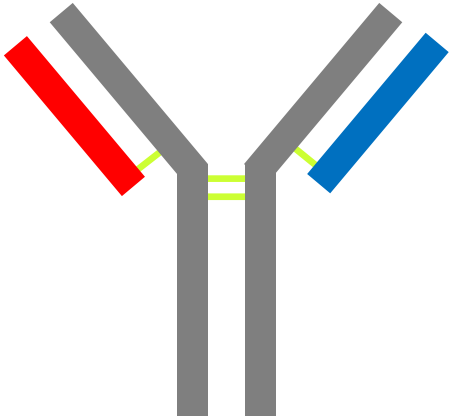
- Technique
  - Targeted “bottom-up” → sensitivity and specificity
  - Proteins are digested → analysis at peptide level (signature peptide approach)
  - Trypsin – most commonly used

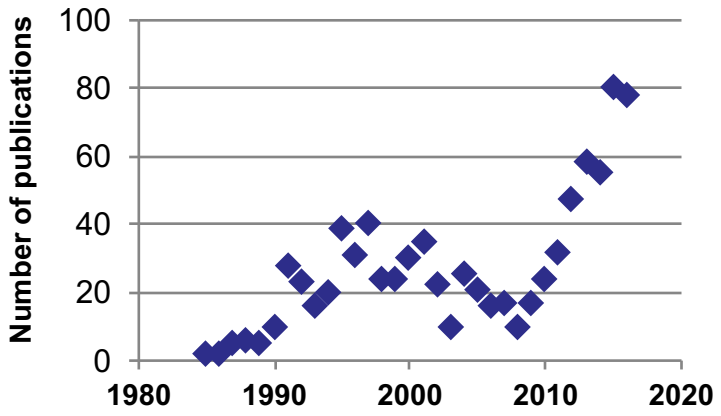
Glu-C

- Glutamyl endopeptidase
- Bacterial Ser-protease (*Staphylococcus aureus*)
- Cleaves after Glu and Asp
- Favors Glu at pH8 in ammonium buffer
- Product are peptides with acidic C-terminal → orthogonal to trypsin
- Price comparable to trypsin
- Patented generic tryptic signature peptides (2014 March: US, 2016 February: EU)

Model analyte – bispecific IgG1

- Bispecific mAbs
  - Different Fab regions → different specificity → complex activity
  - Highly specific and complex functions and mechanism of action
  - Less adverse side effects
  - Complex structure
  - Increasing interest on bispecific mAbs





Searched for "bispecific antibodies" on ScienceDirect (abstract/title)

Method Development Workflow

- *In silico* digestion

↓ Selection of the appropriate digesti… enzyme
↓ Initial BLAST screen

- Peptide mapping (HRAM-MS)

- Su…

- Sa… and LC-MS/MS optimization

Generic LC-MS/MS quantitation method

Goal

- To set up a generic mAb quantitation method in pre-clinical matrices using Glu-C digestion

- Requirements:
  - Simple sample preparation
  - LLOQ of 1 µg/mL using 10 µL sample

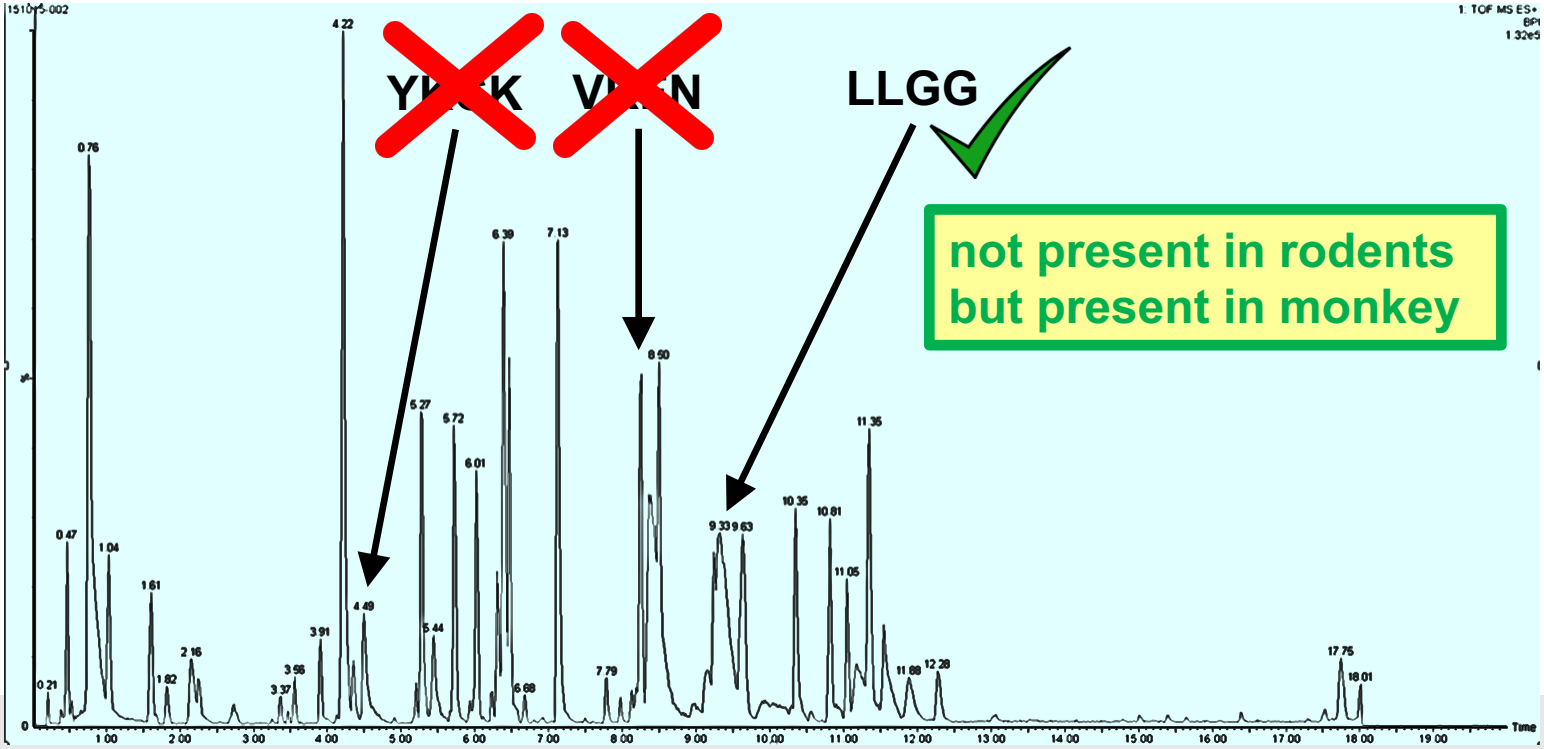
Instrumentation

- Peptide mapping
  - Waters Synapt G2 Q-TOF
  - MS^E acquisition

- Quantitation
  - Waters Xevo TQ-S
  - Waters Acquity UPLC
  - LC separation: Acquity HSS T3 column (100 x 2.1 mm, 1.7 µm particles)

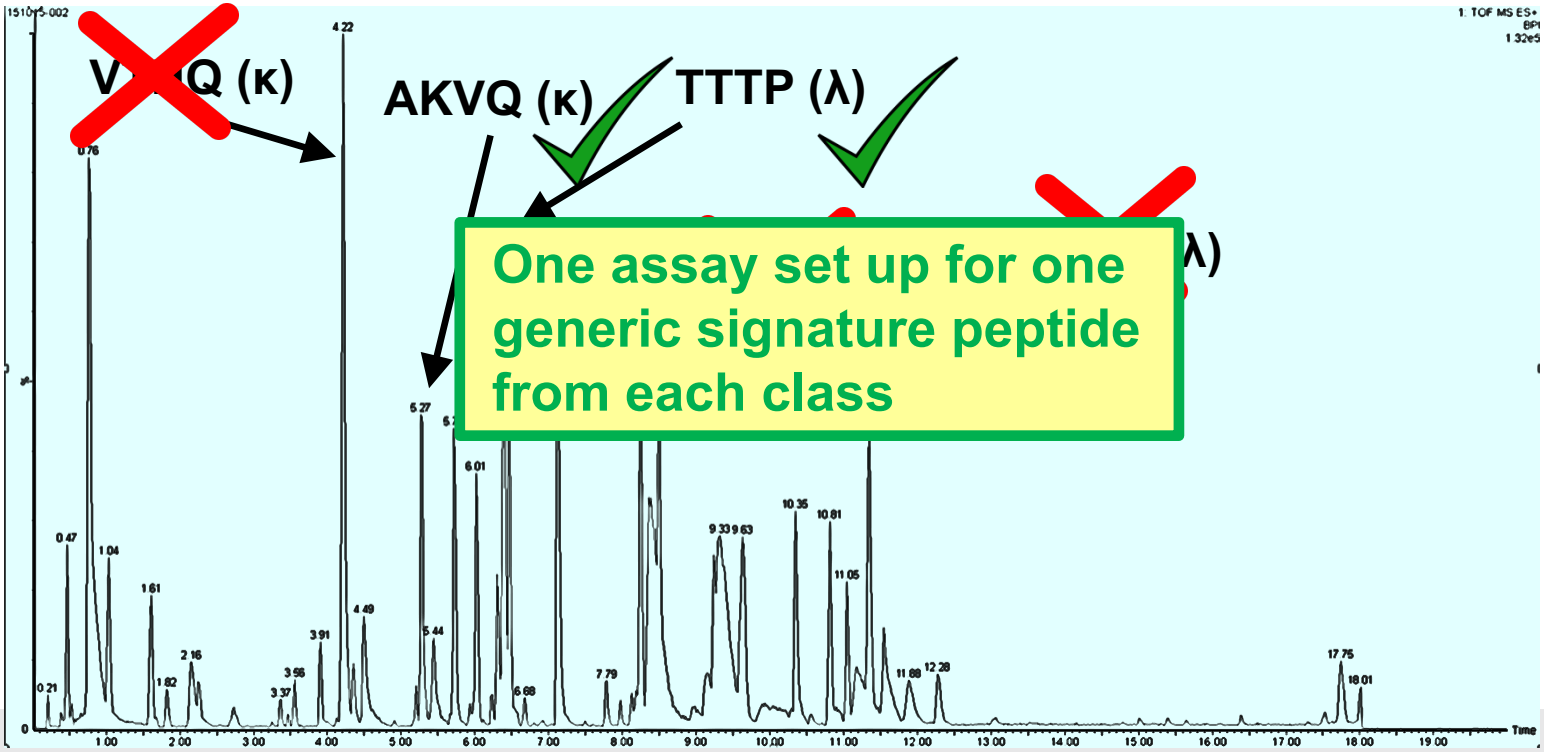
Peptide mapping

- Generic signature peptides
  - Peptides from the heavy chain constant domain
  - Few are unique to human
  - Those that are – are not highly sensitive



Peptide mapping

- Generic signature peptides
  - What about monkey? → Light chains come in handy
  - Two light chain classes: **λ** and **κ** → different constant region

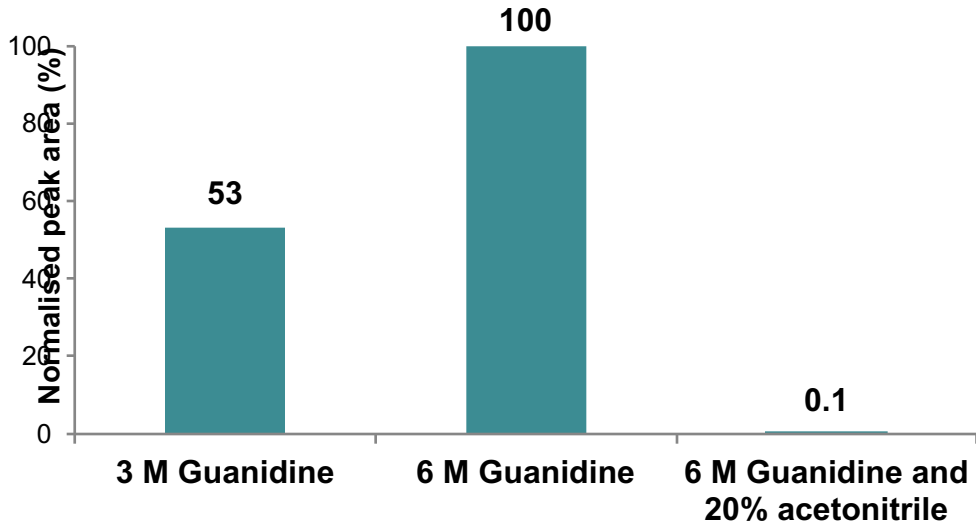


Digestion optimization

- Sample preparation
  - pellet digestion*
- Digestion conditions

LLGG normalised peak area		
Enzyme conc. (µg/mL)	Digestion time	
	2 h	overnight
0.3	0.50	31
3.0	17	93
30	34	100

- Denaturing reagent



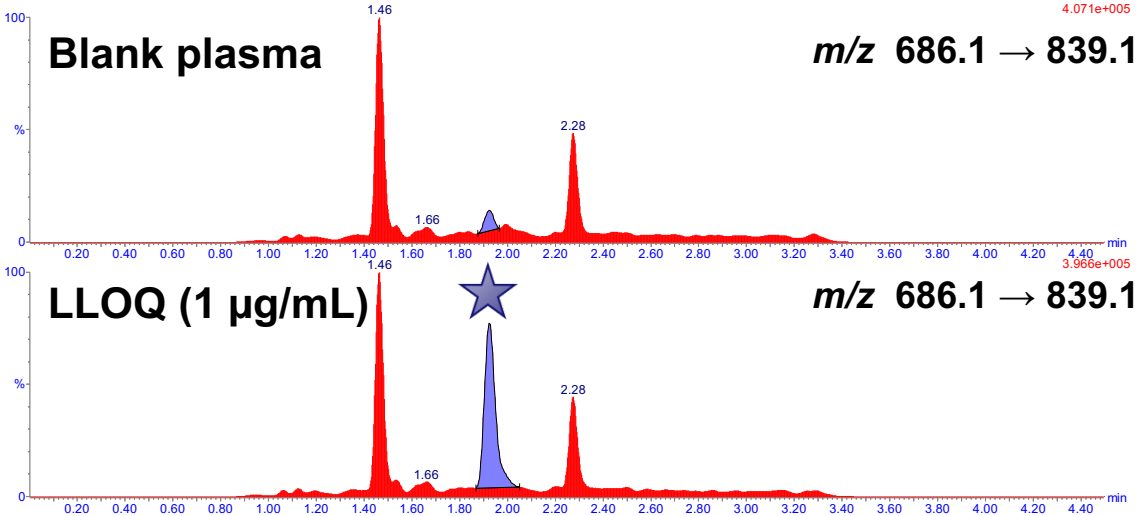
- Optimal conditions
  - overnight digestion with 3.0 µg/mL Glu-C
  - 6 M guanidine

*Richard Kay (2015, November). Developing LC-MS/MS methods for quantifying mAbs: Transitioning from pre-clinical to clinical matrices. Presented at EBF[8th] Open Meeting, Barcelona

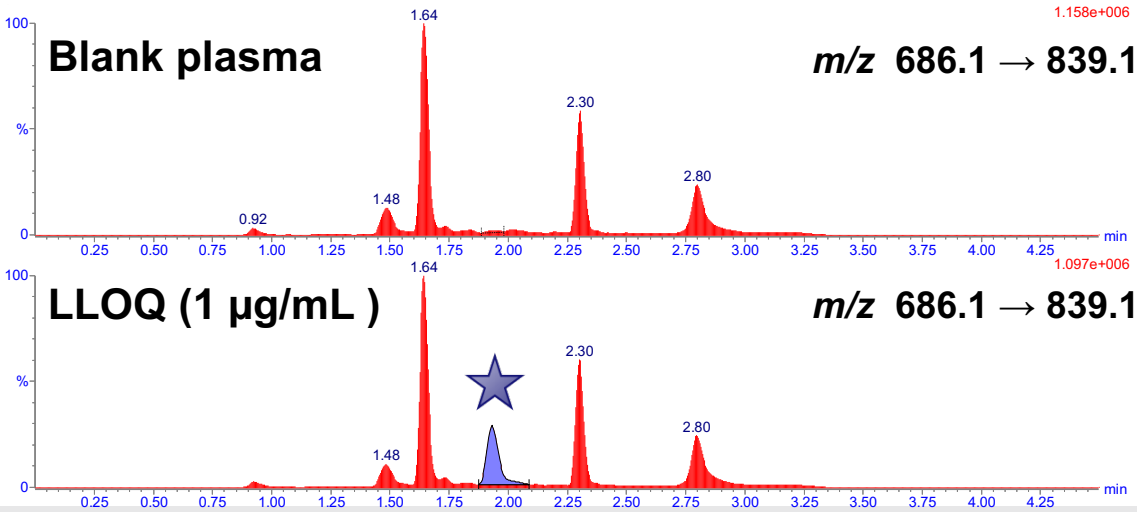
IgG1 heavy chain LC-MS/MS

Generic signature peptide: LLGGPSVFLFPPKPKDTLMISRTPE

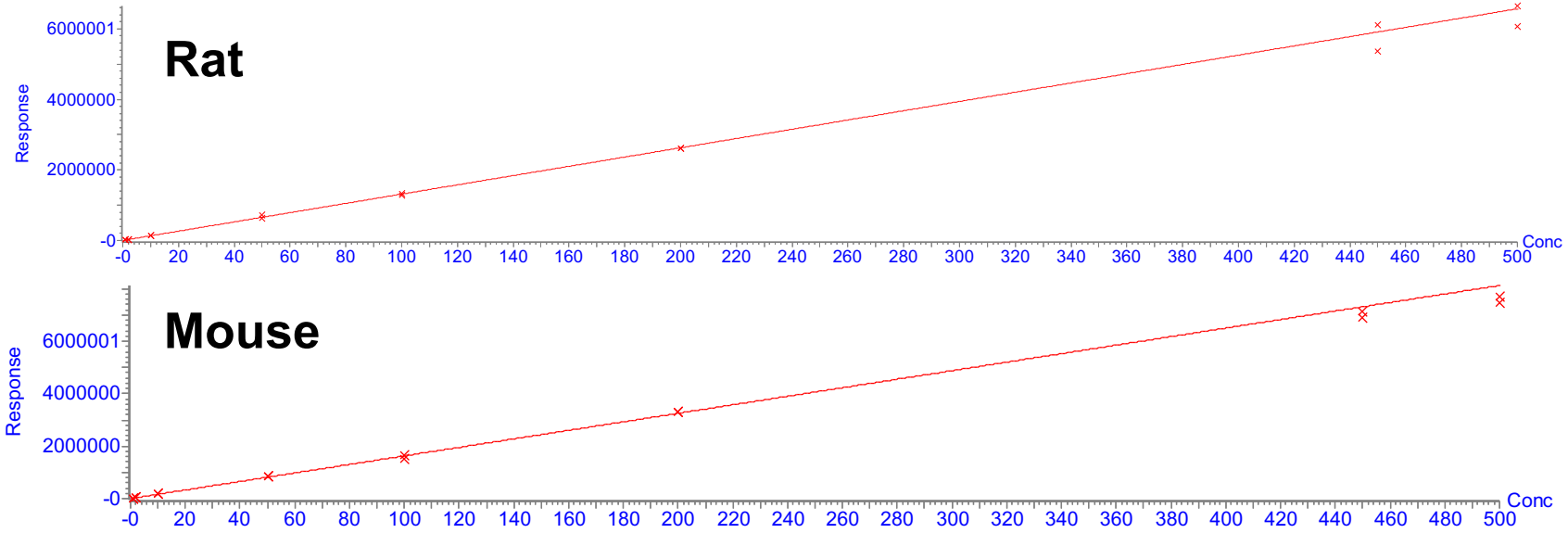
Rat



Mouse



IgG1 heavy chain LC-MS/MS precision and accuracy



IgG1 heavy chain peptide in rat

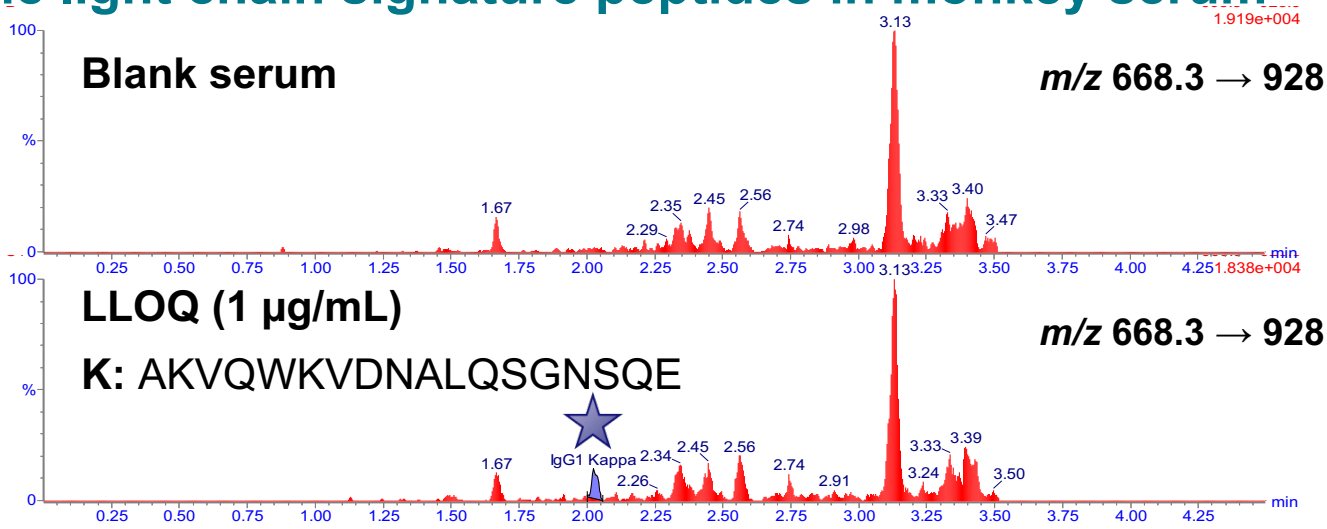
QC level	Conc. (µg/mL)	Mean (µg/mL)	%CV	%RE
LLOQ	1	1.0	4.2	-2.1
Low	3	2.9	3.4	-5.1
Medium	40	38.8	7.0	-3.0
High	400	364.6	6.7	-8.9

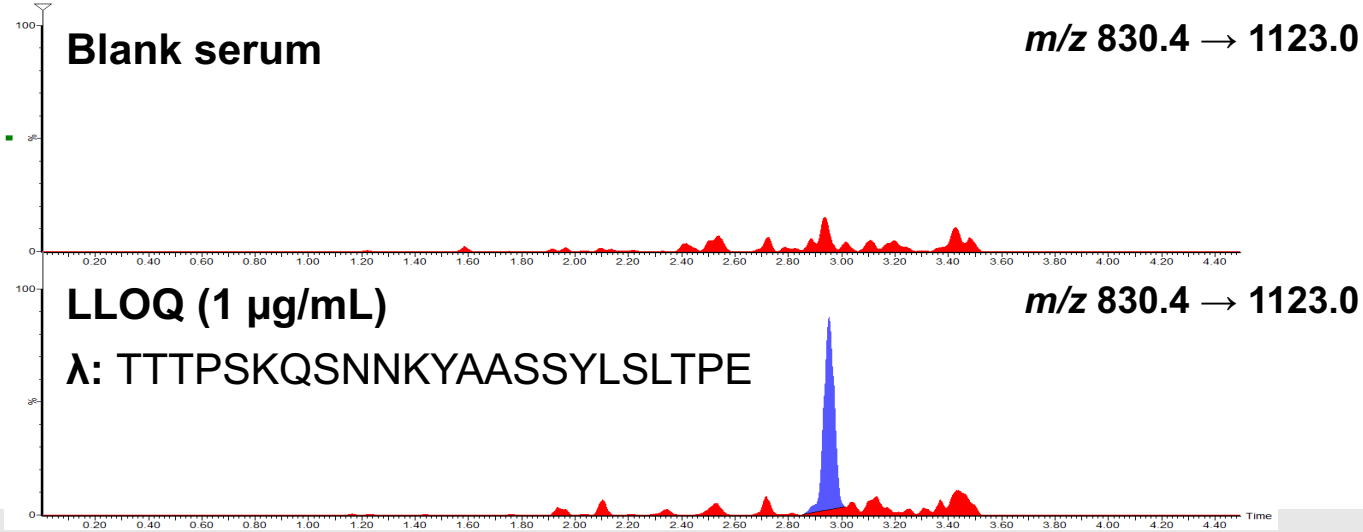
IgG1 heavy chain peptide in mouse

QC level	Conc. (µg/mL)	Mean (µg/mL)	%CV	%RE
LLOQ	1	0.9	3.2	-4.9
Low	3	2.7	6.7	-8.2
Medium	40	40.5	3.3	1.2
High	400	398.0	3.2	-0.5

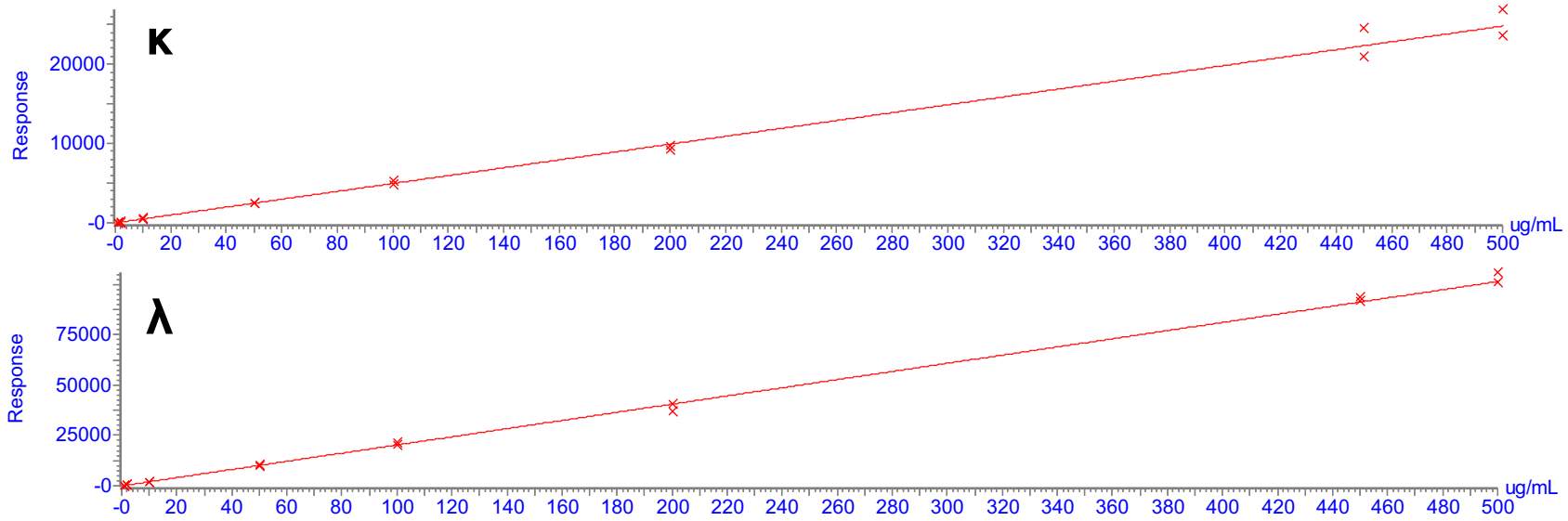
IgG1 light chain LC-MS/MS

Generic light chain signature peptides in monkey serum





IgG1 light chain LC-MS/MS precision and accuracy



IgG1 light chain κ peptide in monkey

QC level	Conc. (µg/mL)	Mean (µg/mL)	%CV	%RE
LLOQ	1	0.93	14.6	-6.7
Low	3	3.18	17.1	6.1
Medium	40	40.43	4.5	1.1
High	400	408.75	3.6	2.2

IgG1 light chain λ peptide in monkey

QC level	Conc. (µg/mL)	Mean (µg/mL)	%CV	%RE
LLOQ	1	1.00	10.9	0.0
Low	3	2.78	4.8	-7.2
Medium	40	37.63	1.7	-5.9
High	400	385.93	3.9	-3.5

Conclusions

- Glu-C digestion provides an efficient alternative means for quantification of biopharmaceuticals in biological samples
  - Two assays have been developed
    1. Heavy chain generic peptide → rodents
    2. Two light chain generic peptides (κ and λ) → monkey samples
  - Satisfactory assay performance, even with no IS
  - Sensitivity (LLOQ) is comparable to a trypsin digestion approach
  - Work around current patent

- in the pipeline
  - Cross validation against ligand binding assay data

Acknowledgements

Colleagues at LGC

- Kjetil Hansen*
- Mark Hows
- Richard Kay@

Novimmune

- Emilie Escoffier
- Robert Nelson

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Thank you

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