Enhancing the Scope for Screening Protein-Ligand Interactions by Non-Covalent Mass Spectrometry

Dr Rebecca J. Burnley

UCB Celltech
Slough, UK

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Atsumi, living with epilepsy

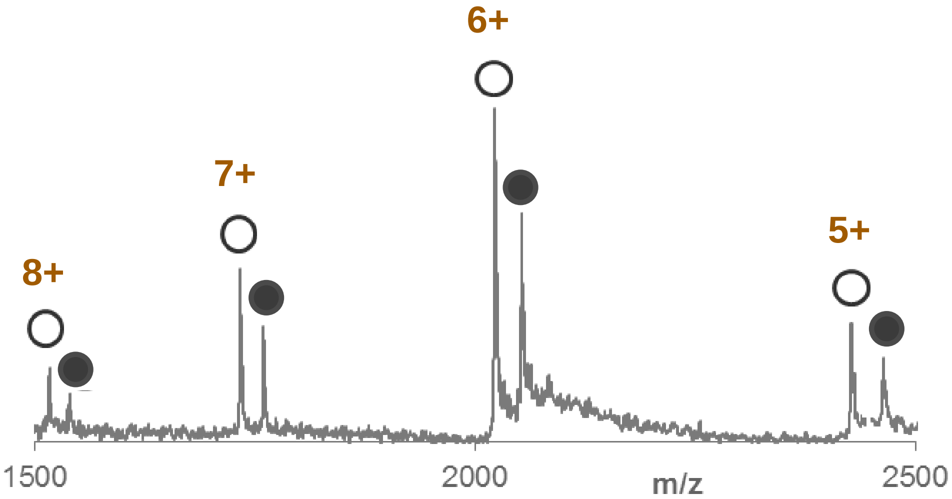
Inspired by patients.
Driven by science.

Non-Covalent Mass Spectrometry in NCE Drug Discovery

NCE = new chemical entity

Orthogonal Screen for Hit Identification

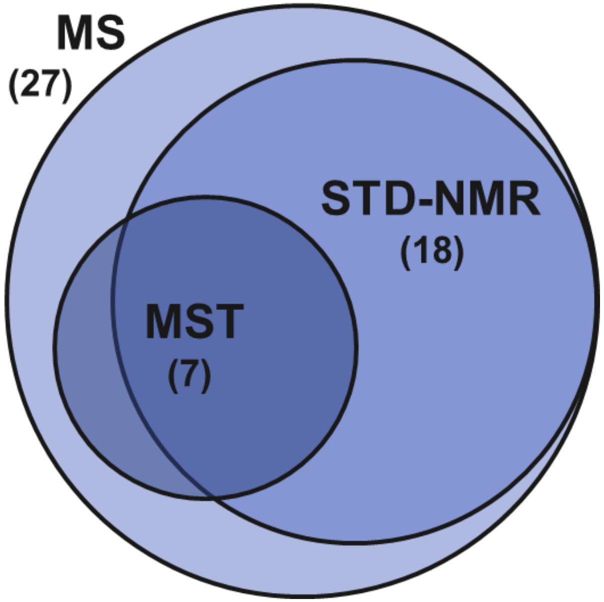
- Library of 10s-1000s of fragments (~200 Da) or compounds (~500 Da)
- Binding to purified protein target (10 kDa - 100 kDa): native-like state
- Automated native MS protocol; Tof or Q-Tof MS

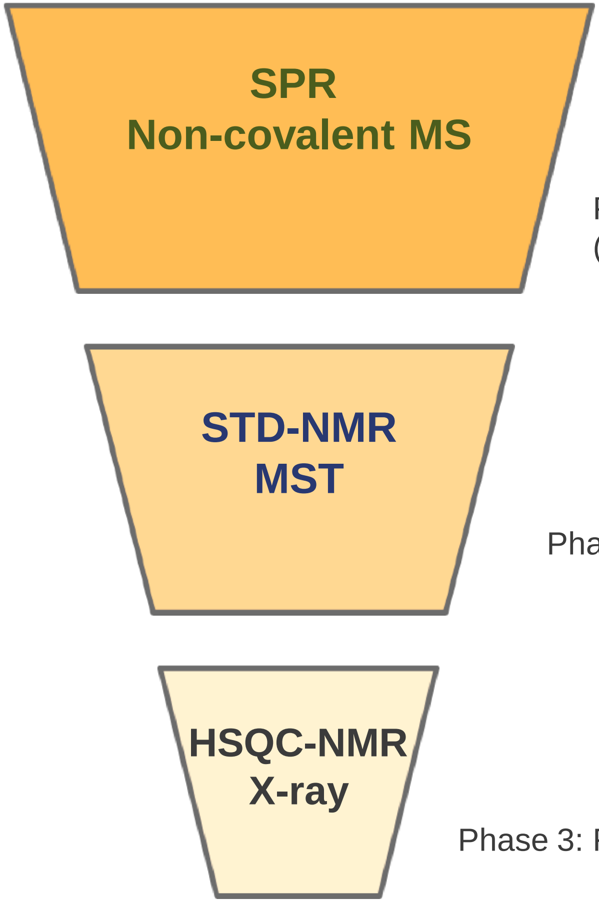


Integrating Non-Covalent MS into In-House Screening Approach

19 hits, 2% hit-rate

27 hits, 2.5% hit-rate





SPR
Non-covalent MS

Phase 1: screen entire library (>1000 compounds)

STD-NMR
MST

Phase 2: confirm hits from phase 1

HSQC-NMR
X-ray

Phase 3: Further analysis of confirmed hits

STD-NMR = Saturation Transfer Difference NMR
MST = Microscale Thermophoresis

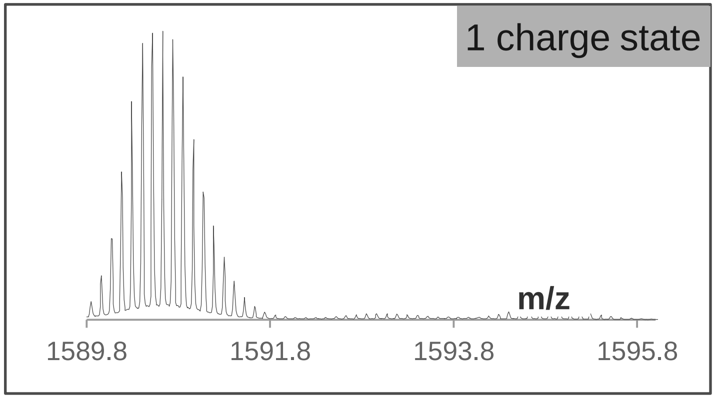
SPR = surface plasmon resonance

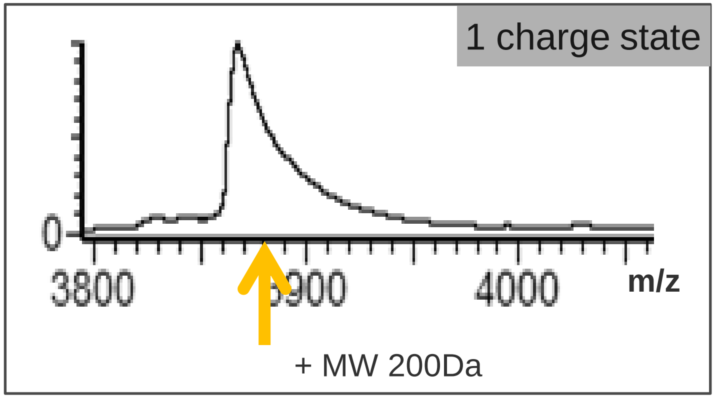
Hannah Maple

Limitations of Screening by MS

- Measurement of binding limited by ‘spectral resolution’ - adducts, peak tailing, desalting
  [source conditions/ accelerating voltages must be kept to a minimum to retain compound binding]

- Speed/ Sensitivity:
  Medium throughput,
  Non-specific binding (ESI artefact, concentration)



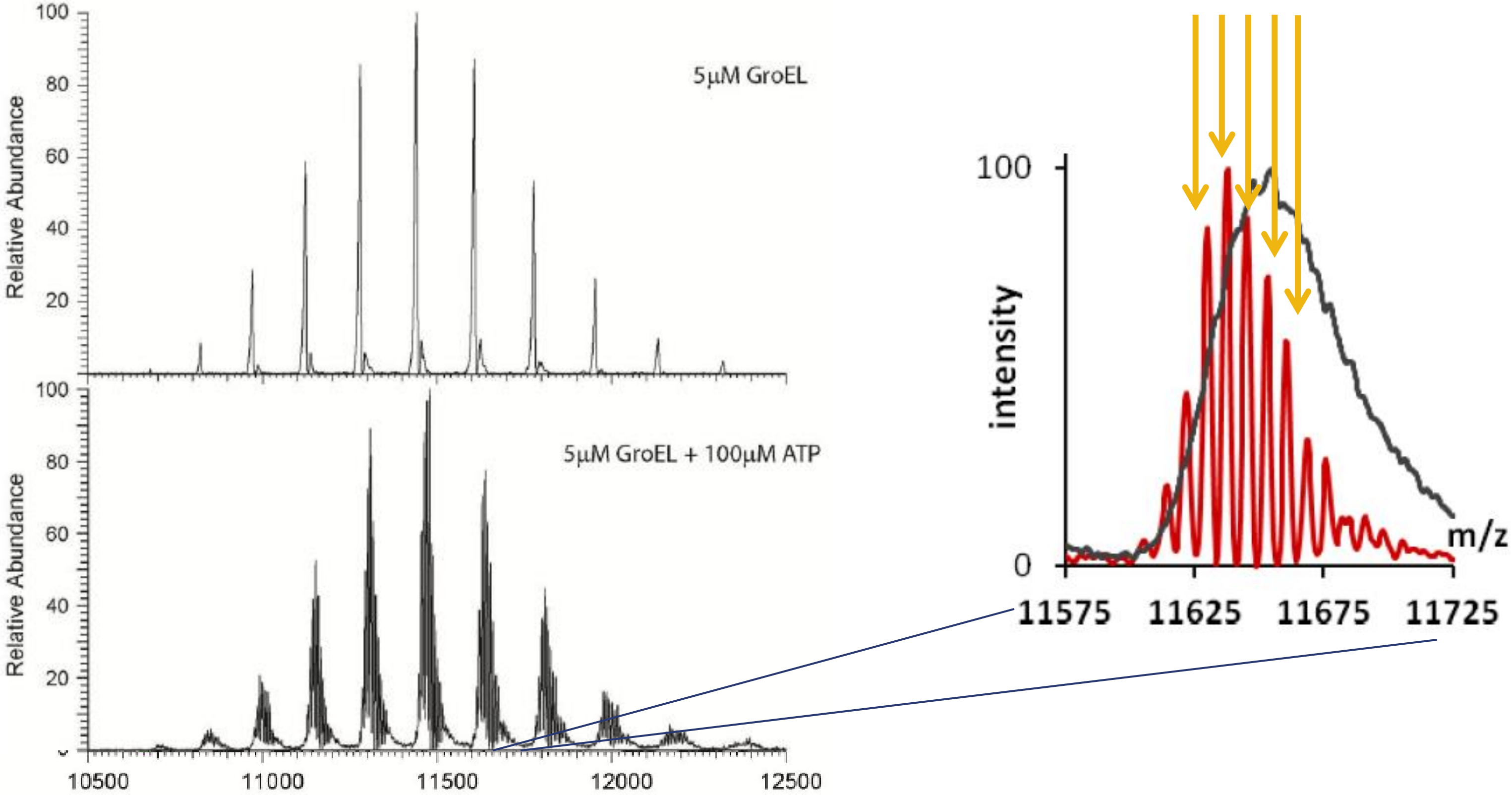


+ MW 200Da

Orbitrap MS Analysis of Large Protein Complexes

800 kDa GroEL + 507 Da ATP

Individual ATP molecules binding to GroEL

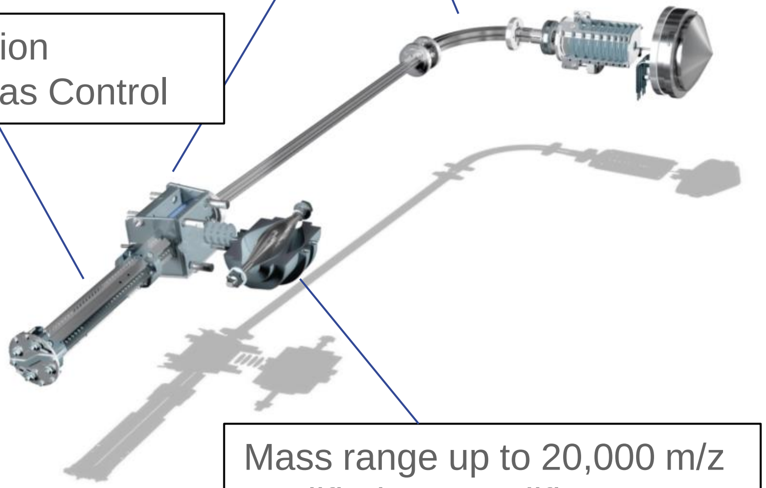


TEST for protein-ligand analysis (smaller proteins, weak binding)
COMPARE to existing technology

Rose RJ et al, 2012, Nature Methods

Native Mass Spectrometry – Instrumentation

Thermo Scientific™ Exactive™ Plus EMR Orbitrap mass spectrometer



High Mass Transmission in
Bent flatapole, Octapole & C-Trap

In-source
dissociation
energy

HCD Cell Desolvation
Automated HCD Gas Control

Mass range up to 20,000 m/z
Modified Preamplifier
32 ms transient available

Native Mass Spectrometry – Sample Introduction

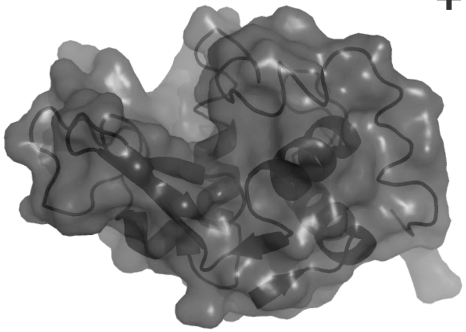
Thermo Scientific™ Exactive™ Plus EMR Orbitrap mass spectrometer

Advion Triversa Nanomate

Automated chip-based nanoESI from 96- or 384- well plate

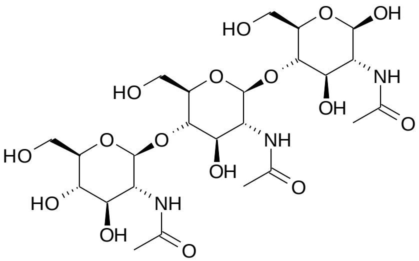
Protein-Ligand Binding on Exactive Plus EMR MS

MODEL SYSTEM

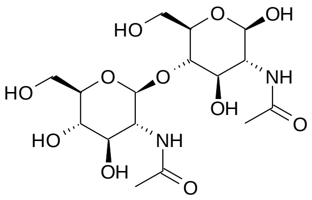


10 µM lysozyme

+



+



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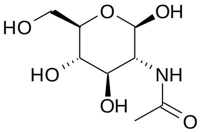
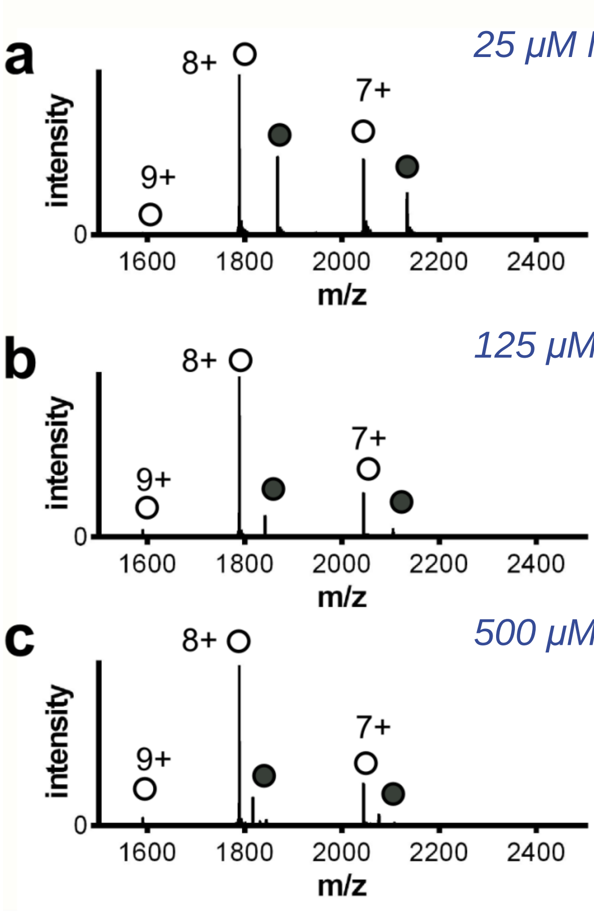


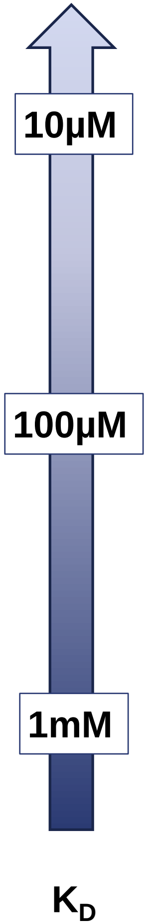


25 µM NAG3

125 µM NAG2

500 µM NAG

1 scan (< 1 sec)



10µM

100µM

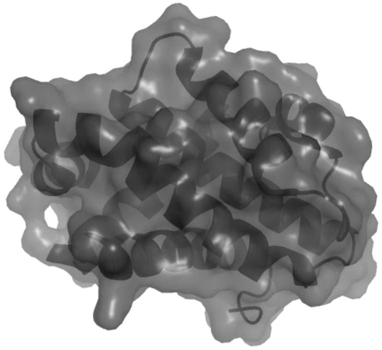
1mM

K_D

Maple HJ et al, 2014, Rapid Comm Mass Spectrom

Protein-Ligand Binding on Exactive Plus EMR MS

DRUG DISCOVERY TARGET



10 µM Bcl-xL

+

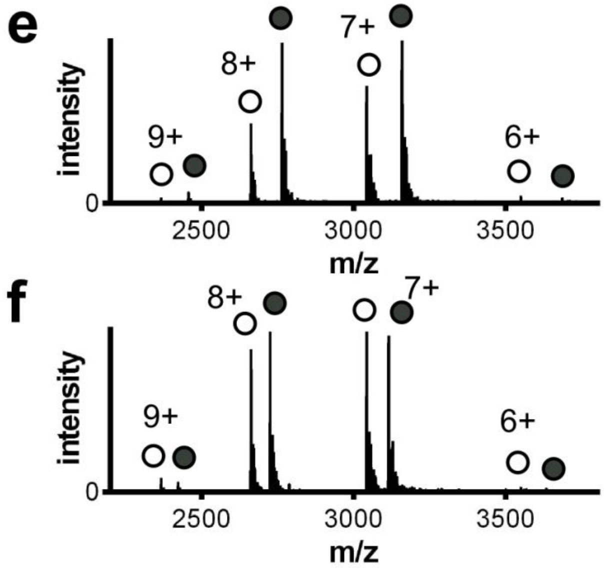
ABT737

Mw 812 Da

+

'cpd 1'

Mw 502 Da

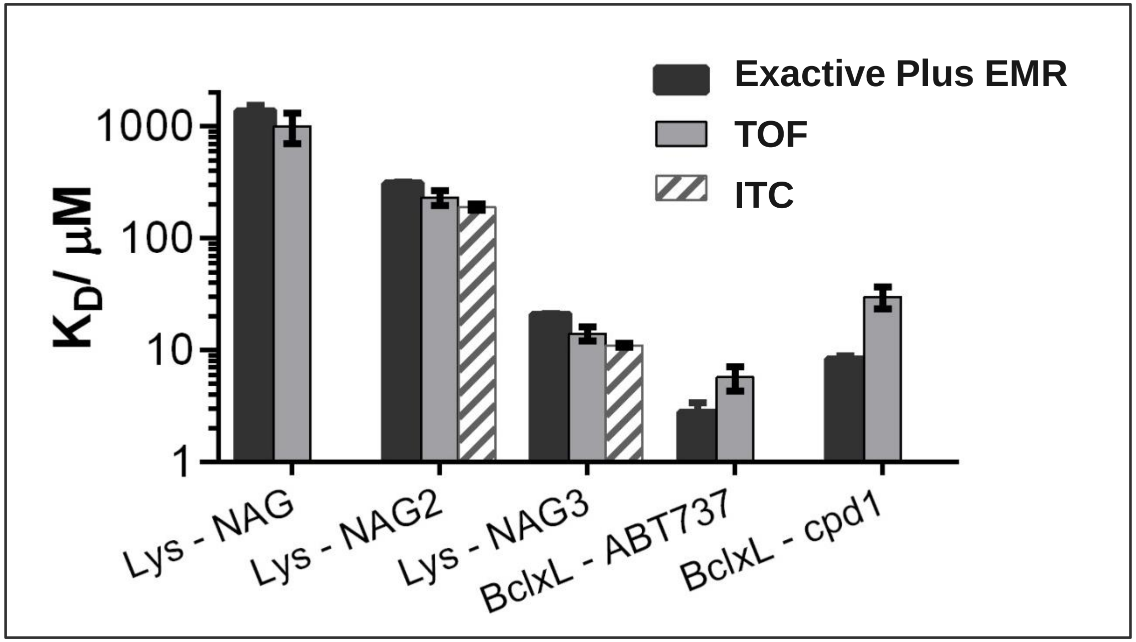


1 scan (< 1 sec)

Maple HJ et al, 2014, Rapid Comm Mass Spectrom

Protein-Ligand Binding on Exactive Plus EMR MS

Estimation of binding strength, comparison with existing technology

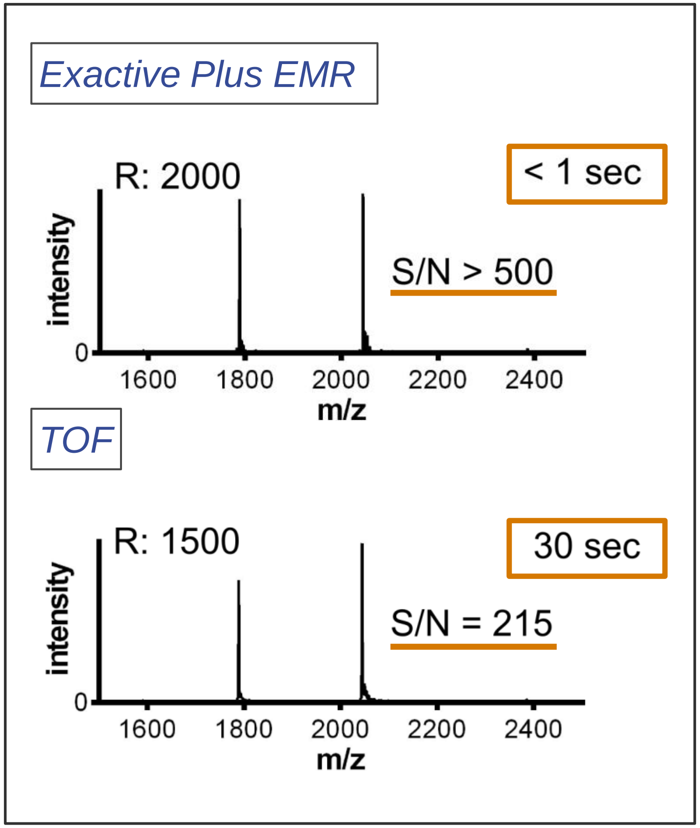


K_D/ µM	E+ EMR	TOF	ITC
Lys-NAG	1375 ± 159	1000 ± 300	
Lys-NAG2	311 ± 7	230 ± 35	189 ± 11
Lys-NAG3	21 ± 0.4	14 ± 2	11 ± 0.4
BclxL- ABT737	2.8 ± 0.6	5.7 ± 1.4	
BclxL-cpd1	8.3 ± 0.5	30 ± 6.6	

Maple HJ et al, 2014, Rapid Comm Mass Spectrom
Maple HJ et al, 2011, J Med Chem

Sensitivity and Spectral Resolution

625 nM lysozyme



Exactive Plus EMR

< 1 sec

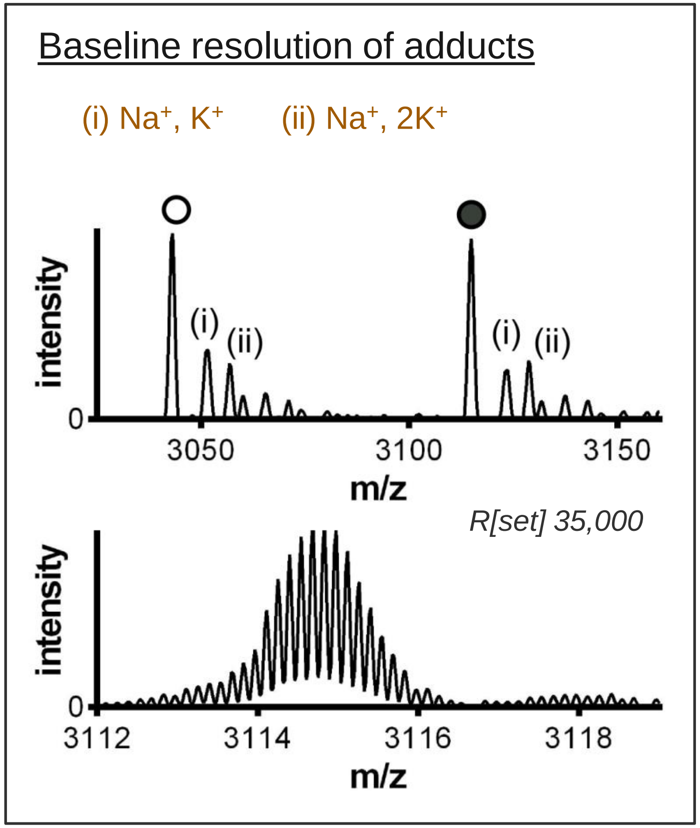
S/N > 500

TOF

30 sec

S/N = 215

BclxL



Baseline resolution of adducts

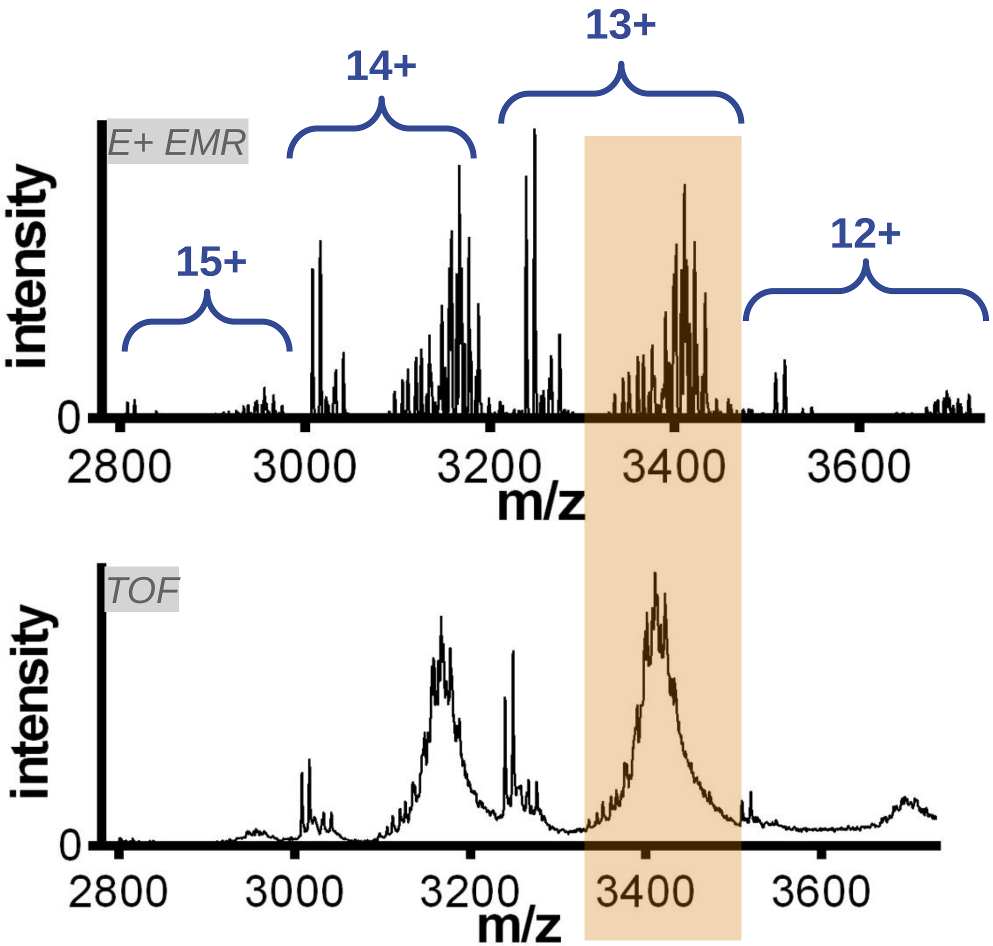
(i) Na^+, K^+ (ii) Na^+, $2K^+$

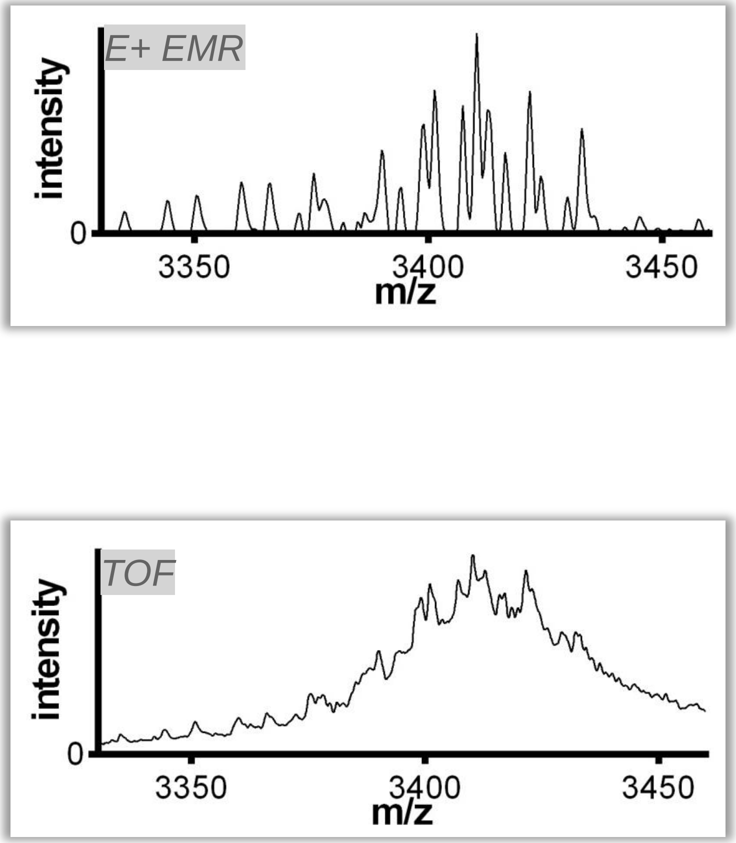
R[set] 35,000

Maple HJ et al, 2014, Rapid Comm Mass Spectrom

Heterogeneous Proteins: Glycoproteins

40 kDa highly glycosylated protein:

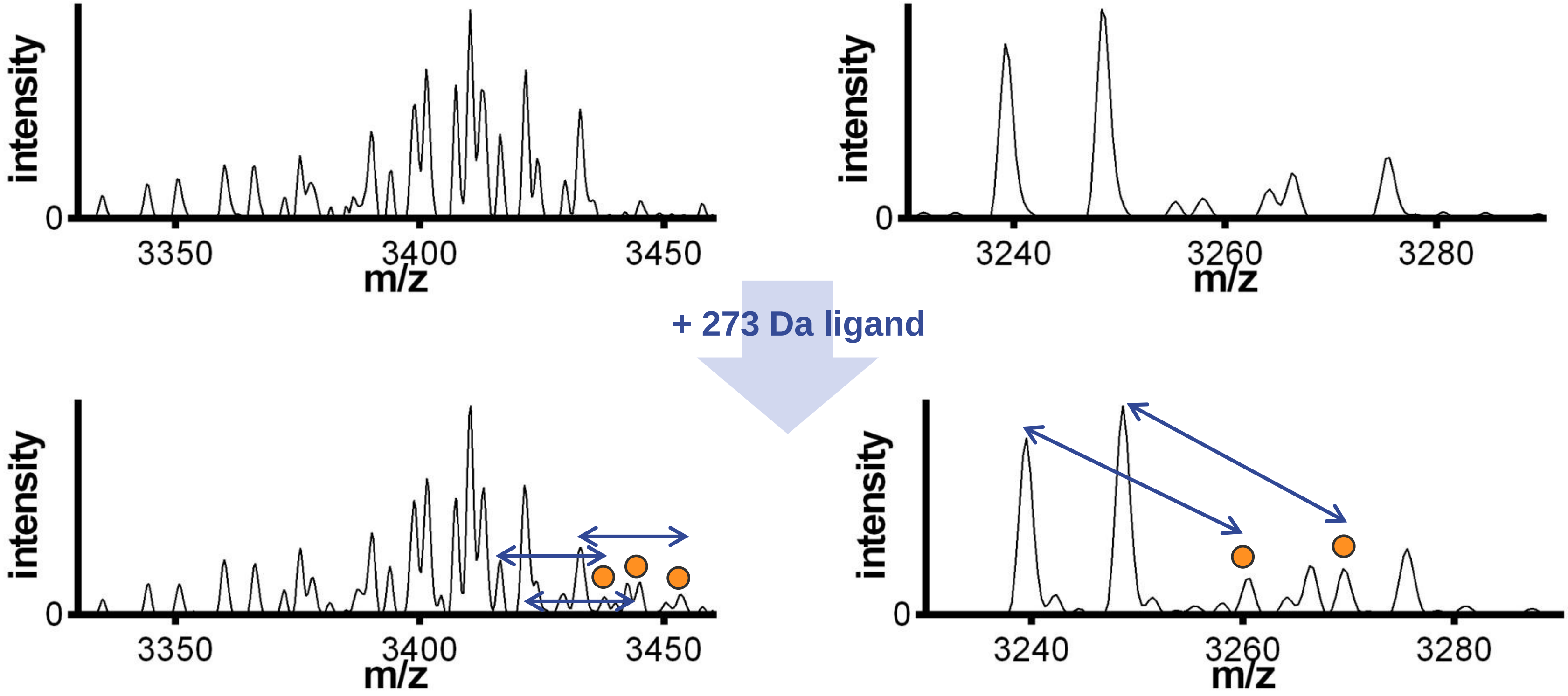




Maple HJ et al, 2014, Rapid Comm Mass Spectrom

Measuring Fragments Binding to Glycosylated Proteins

Typical approach: enzymatic deglycosylation. May affect protein structure/ stability/ function.



Ligand binding observed directly with glycosylated protein

Maple HJ et al, 2014, Rapid Comm Mass Spectrom

Conclusions

Non-Covalent MS can be used to screening small compounds and fragments for hit identification in drug discovery

Evaluation of Exactive Plus EMR for this application (model systems):

Maintains non-covalent protein-ligand interactions during analysis (K_D to mM)

Spectra obtained within seconds - sensitive

Good spectral resolution, good peak shape

⇒ **More reliable measurement of binding of SMALL or WEAKLY-BINDING compounds**

⇒ **Faster analysis of interactions**

⇒ **Lower concentrations: reduced sample consumption, reduced non-specific artefacts**

Acknowledgements

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Mark Baumert
Mark Allen

Thank You!


Atsumi, living with epilepsy

Inspired by patients.
Driven by science.