Optimizing Capillary LC/MS Peptide Analysis

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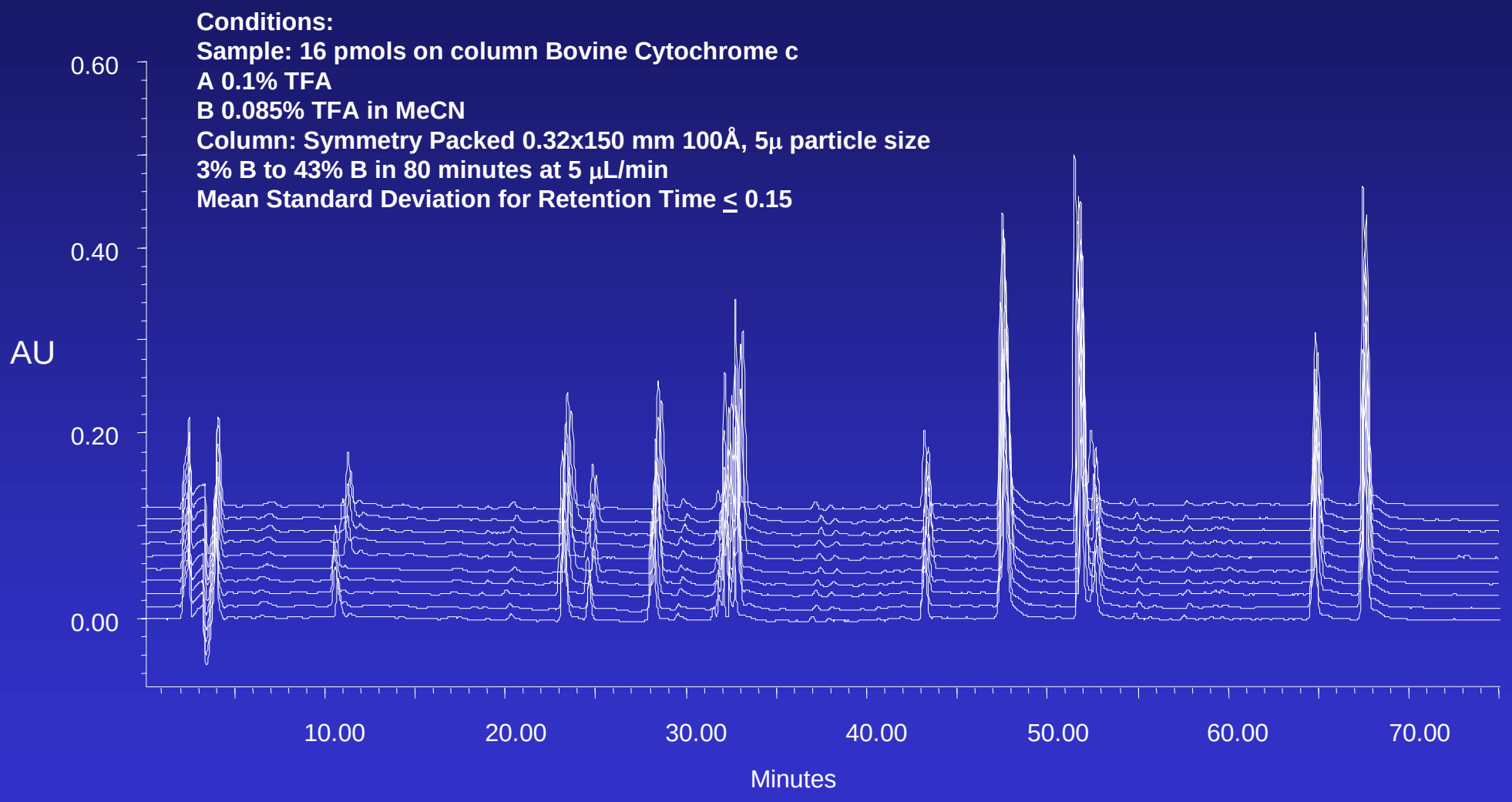
030525

Experimental Design and System

- Compare and Contrast Sensitivity and Mobile Phase effects on UV and MS Detection.
- Examine Methods for High Throughput Capillary LC/Tandem MS Methods
- System
  - **Waters CapLC, Waters LCZ 4000 or Micromass QTOF**

Tryptic Map Overlays (n = 10)



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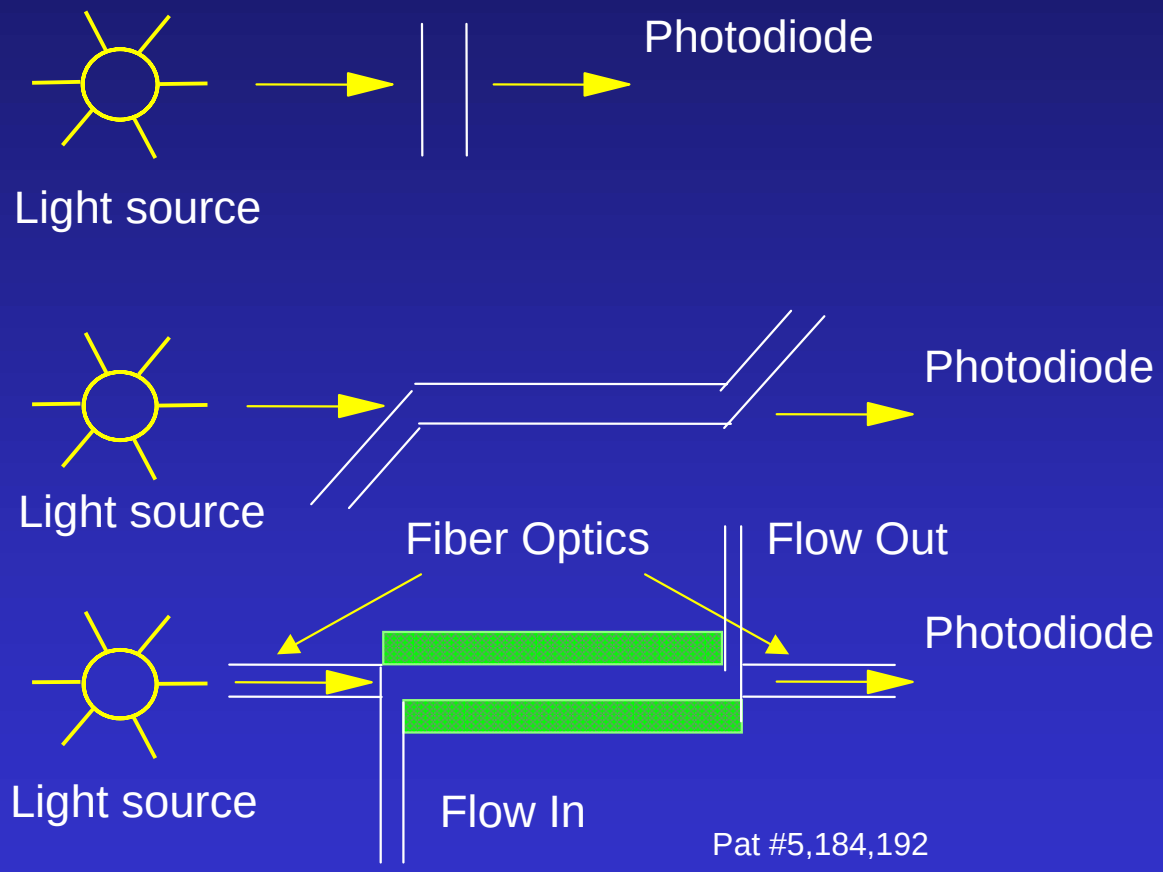
Evolution of Capillary Detector Design



Transverse Illumination
- short pathlength
- low sensitivity

Shaped Cell
- long pathlength
- better sensitivity
- stray light
- poor linearity

Light Guided
- long pathlength
- good linearity
- near quantitative throughput

How Capillary LC Increases Sensitivity

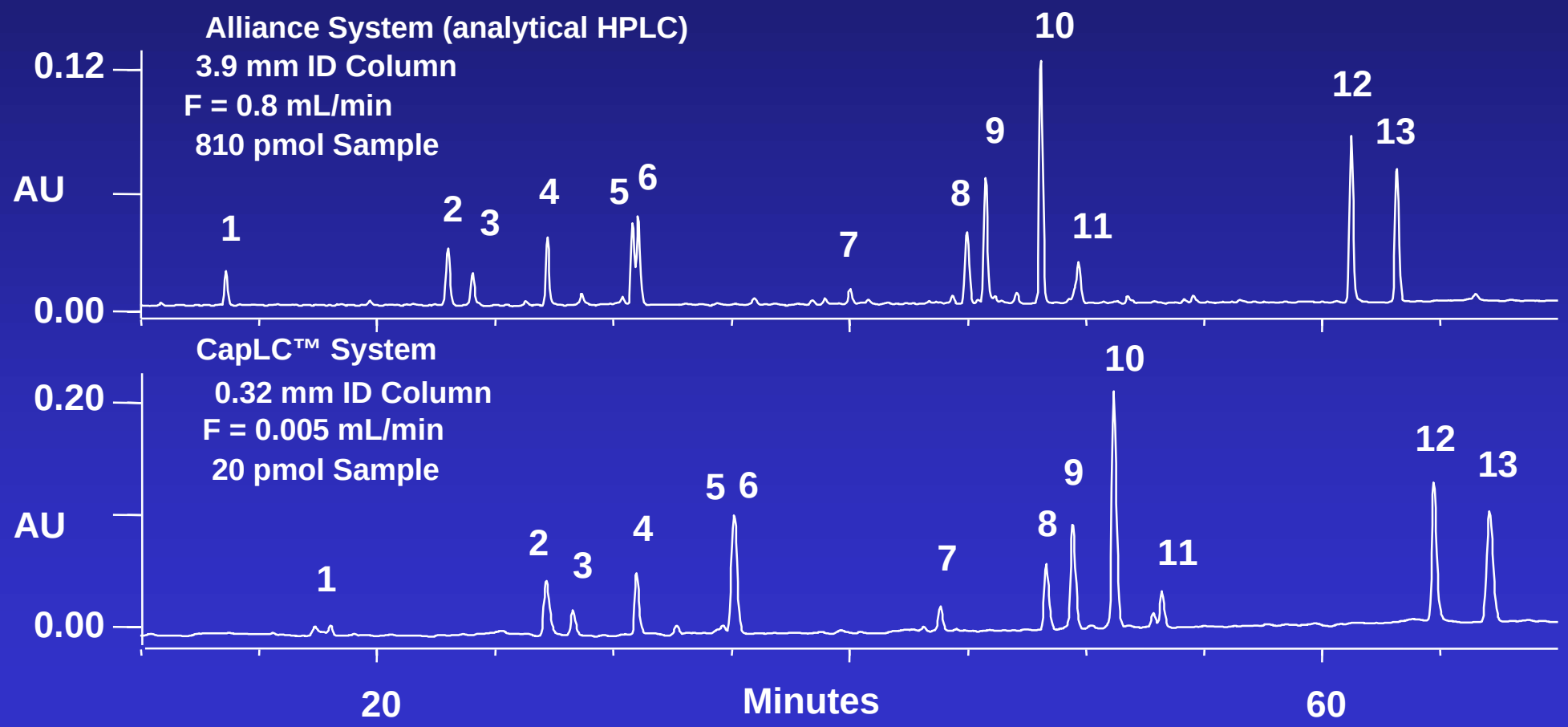
Smaller diameter = lower peak volume

Lower peak volume = higher concentration

$$\text{Increase in concentration} = \frac{(D_{larger})^2}{(D_{smaller})^2} \text{ or } \frac{(3.9)^2}{(0.32)^2} = 149 \text{ times}$$

Comparison of Capillary to Standard Chromatography

Columns: Waters Symmetry 100™ C_{18}, 100Å, 5µ particle size



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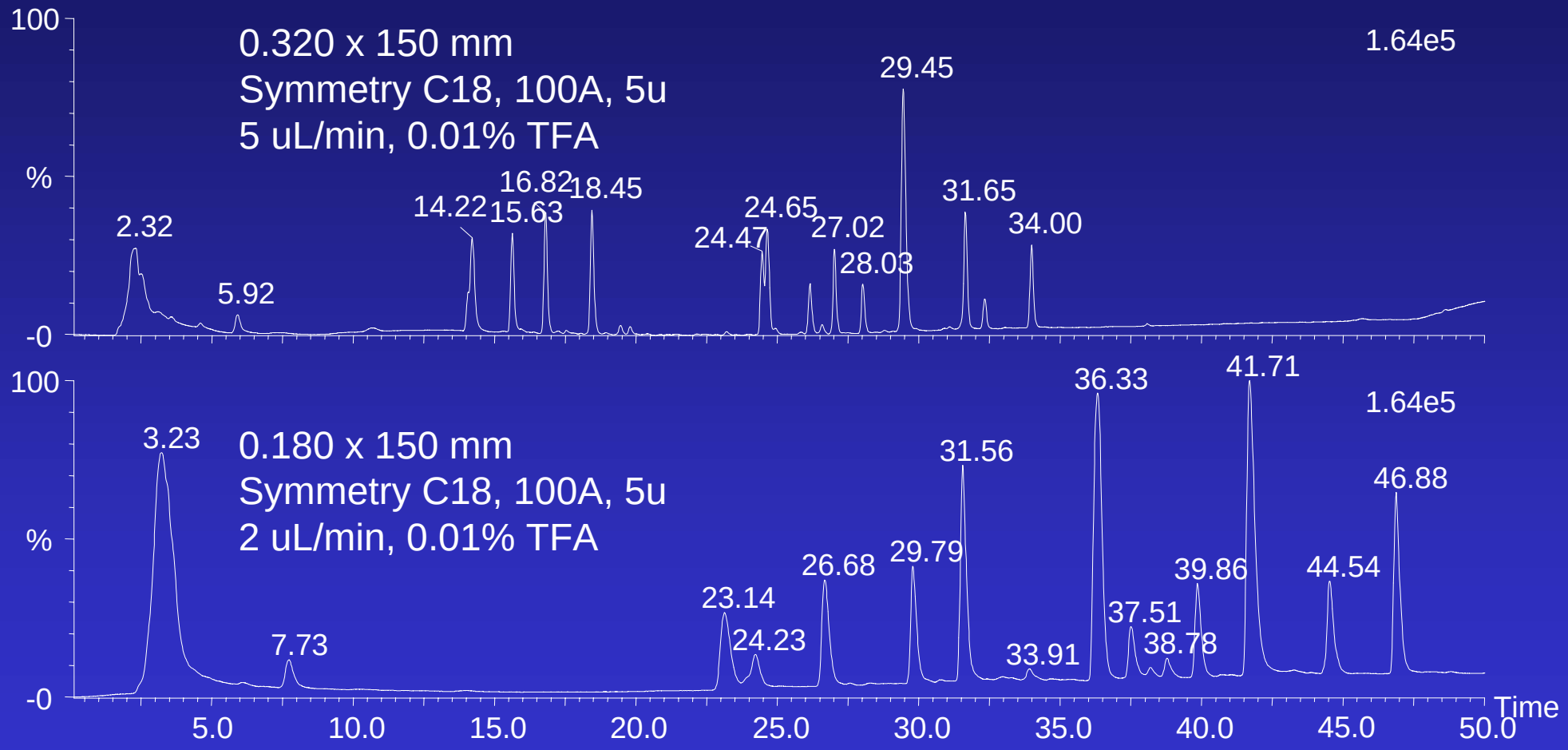
320 micron vs 180 micron
UV at 214 nm detection of 8 pmols



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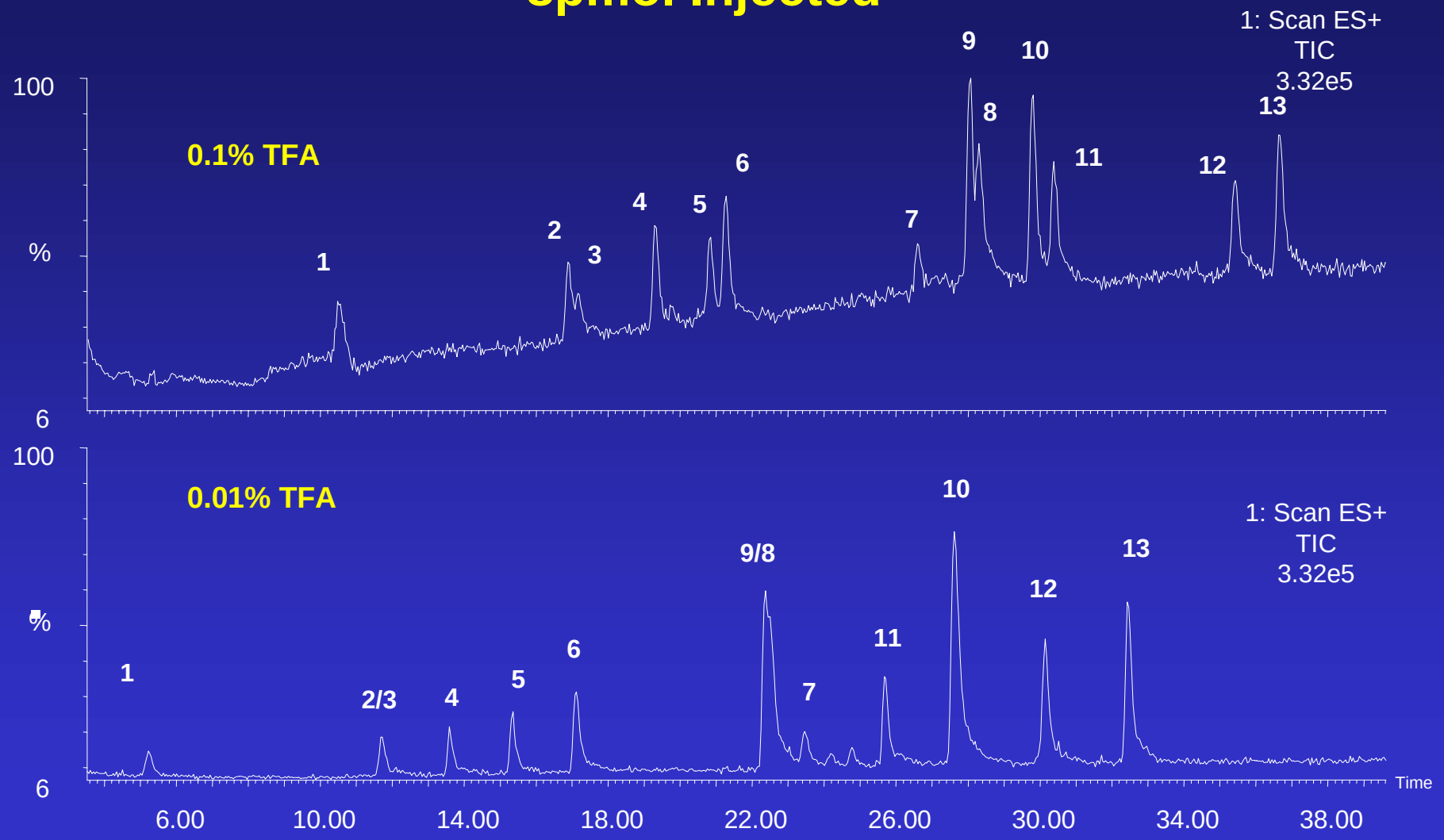
Comparison of TIC

8pmol Injected



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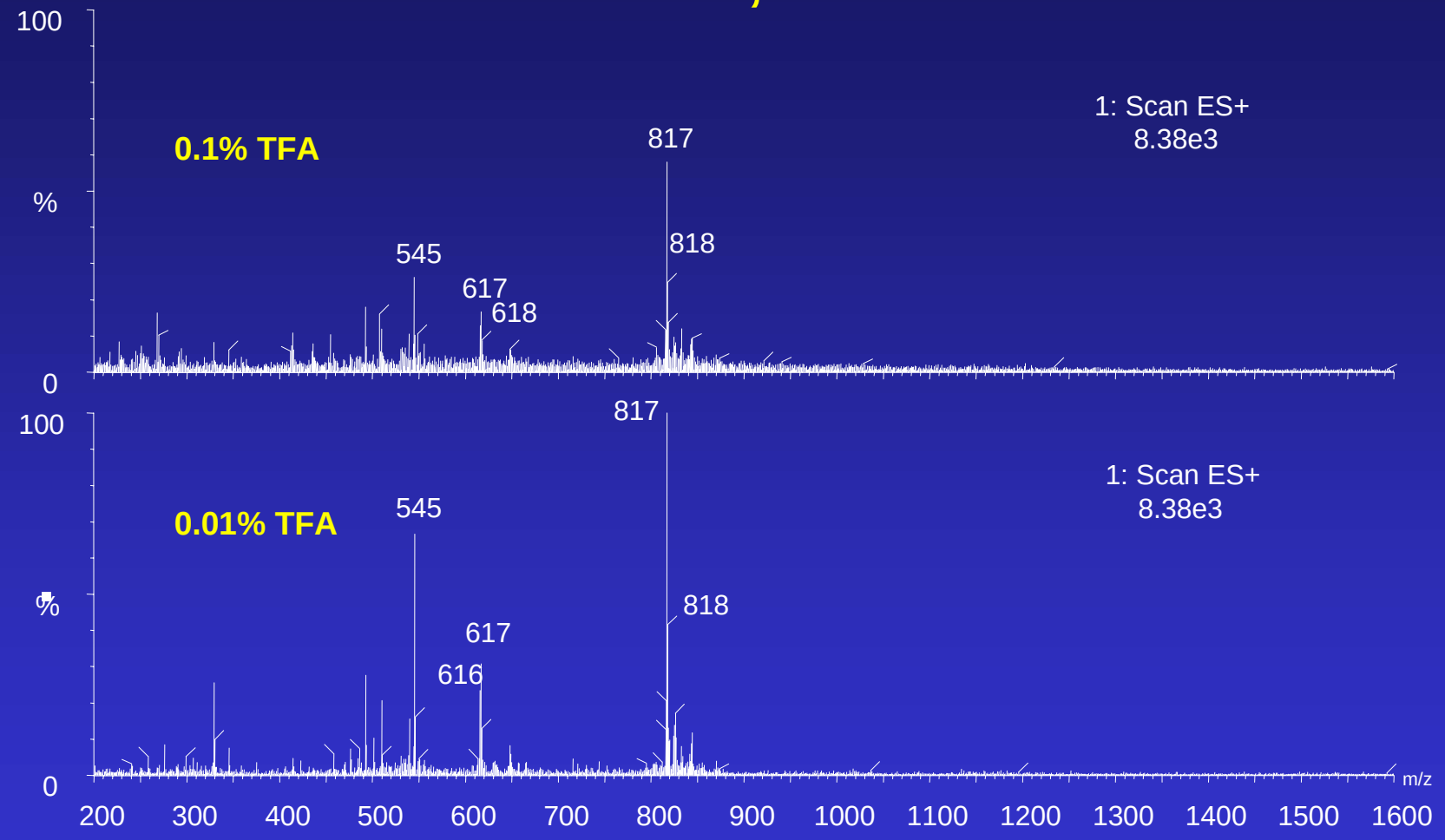
Comparison of Spectral Quality

Peak 10, 8 Pmol



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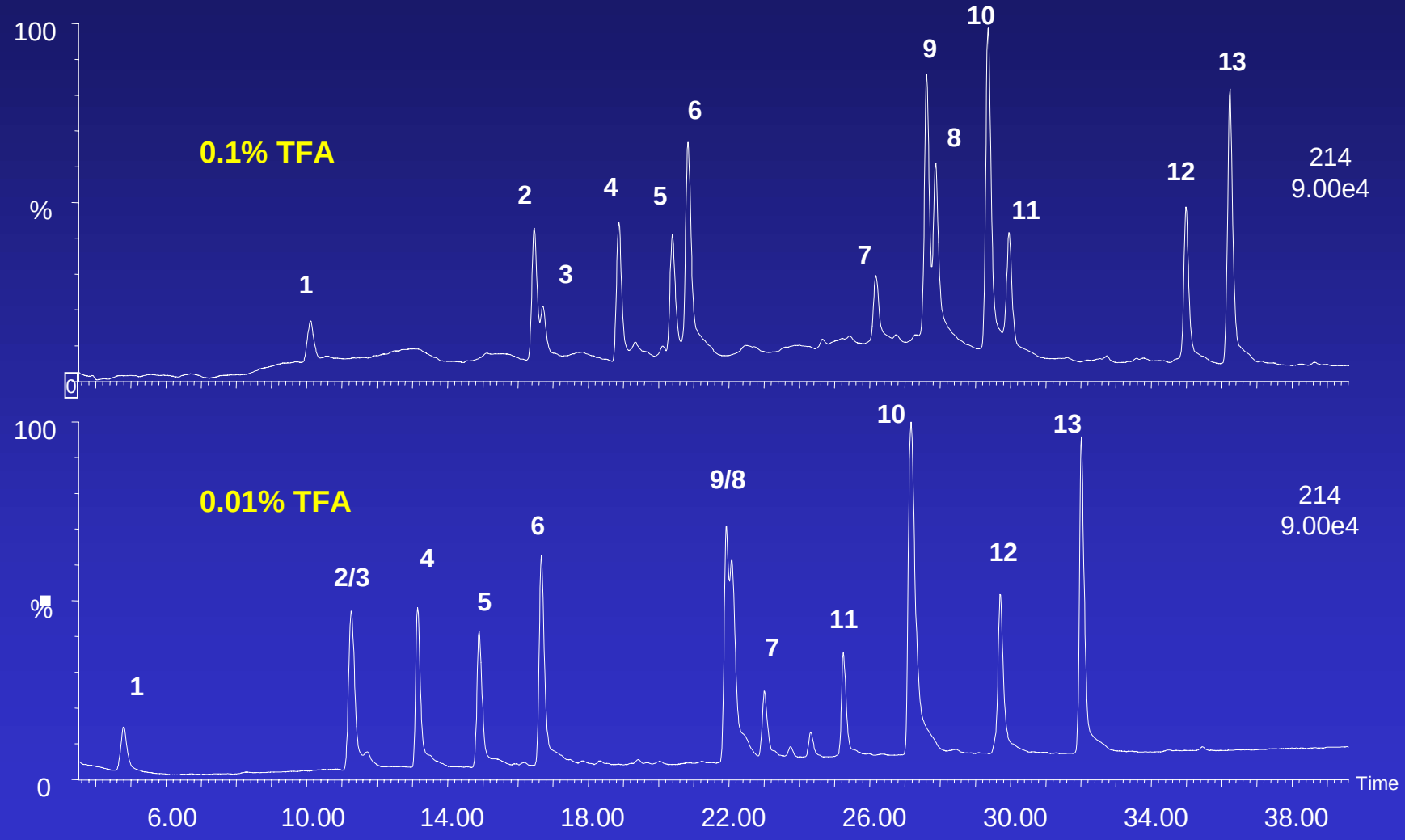
Comparison of UV Response

8 Pmol, UV at 214 nm



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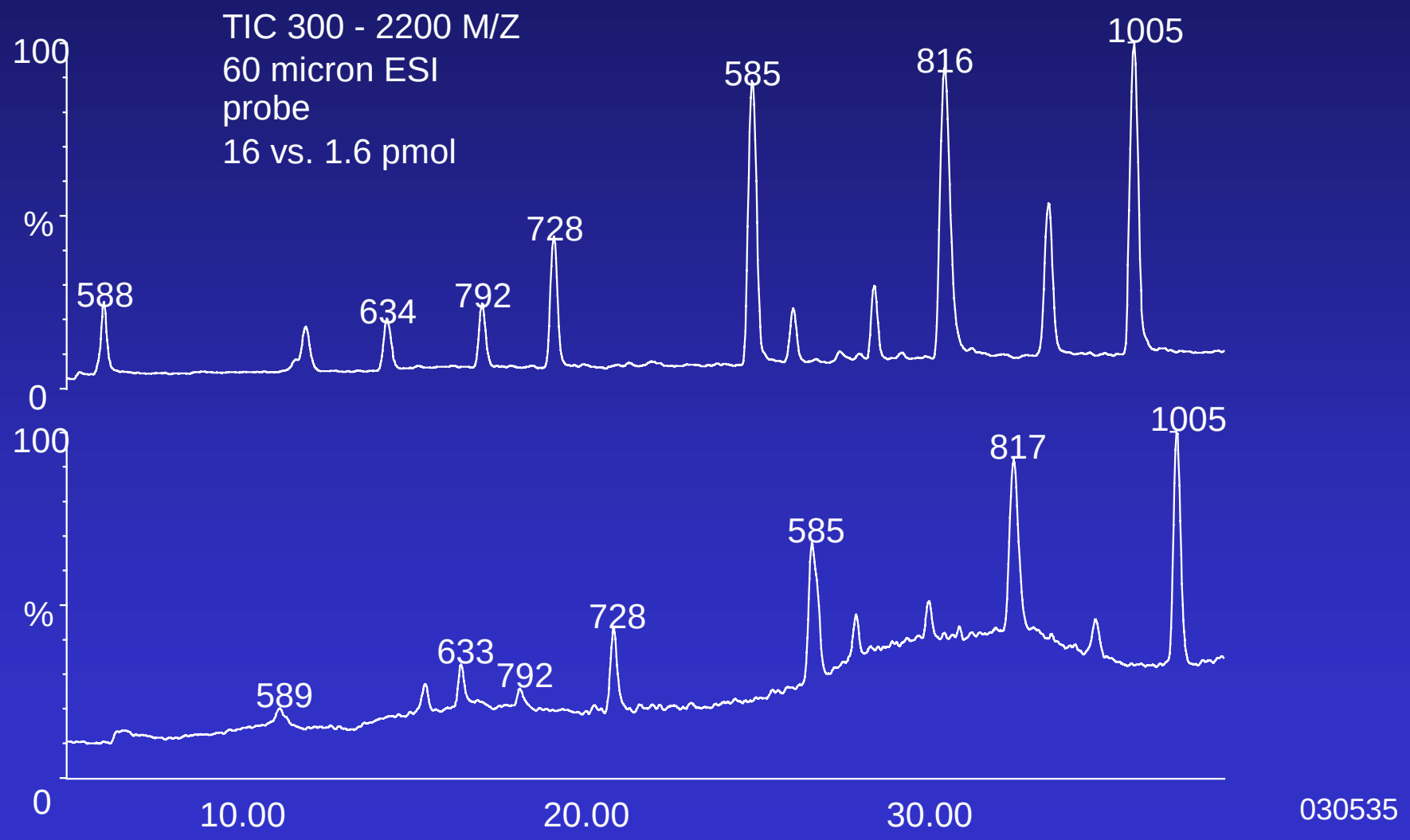
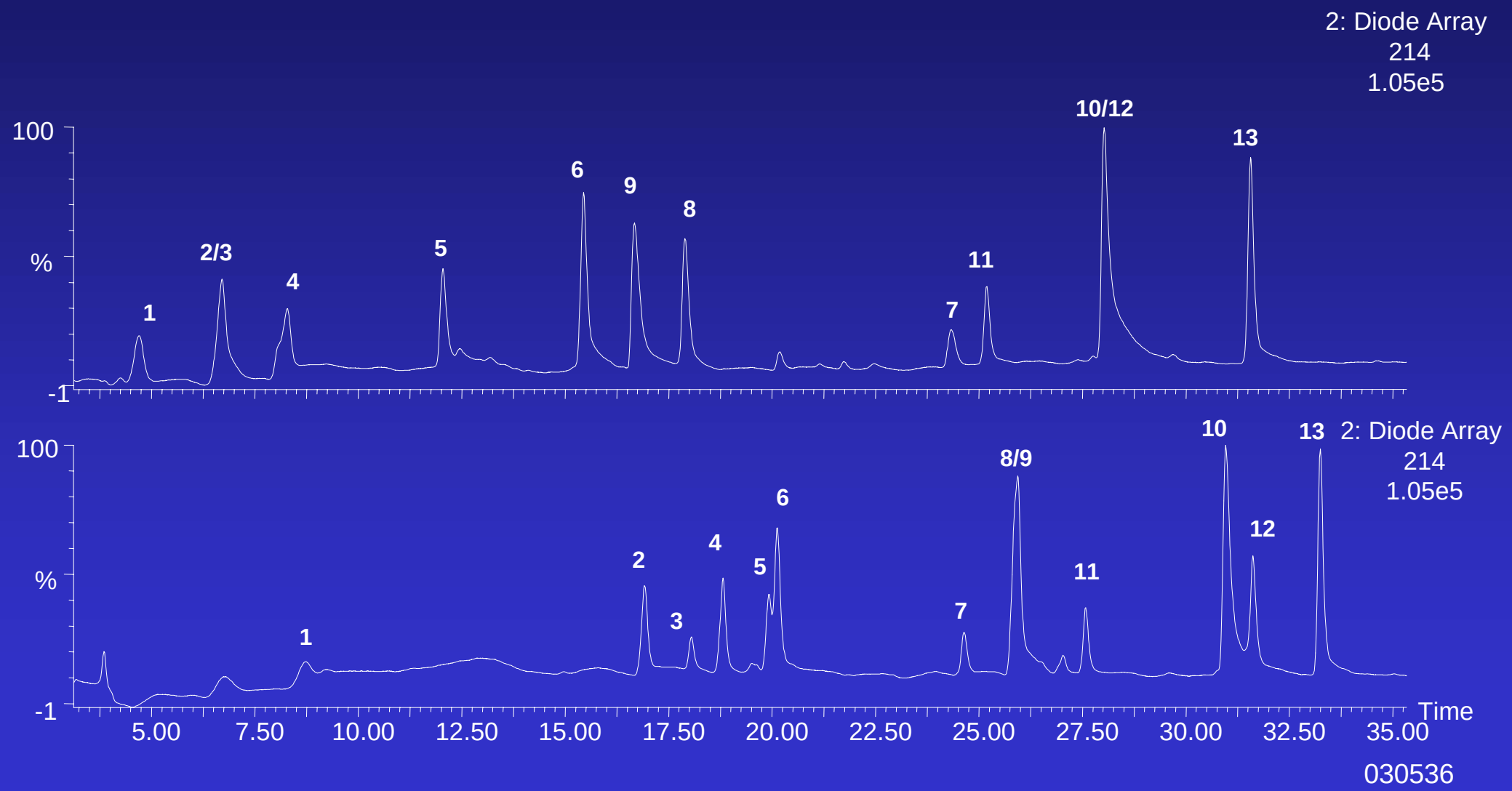
High Sensitivity MS Detection with 0.01% TFA



0.05% Acetic Acid vs. 0.05% Acetic Acid + 0.005% HFBA, UV at 214 nm



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Variable Ion Suppression with Acetic Acid and Heptafluorobutyric Acid

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LC/MS/MS Objectives

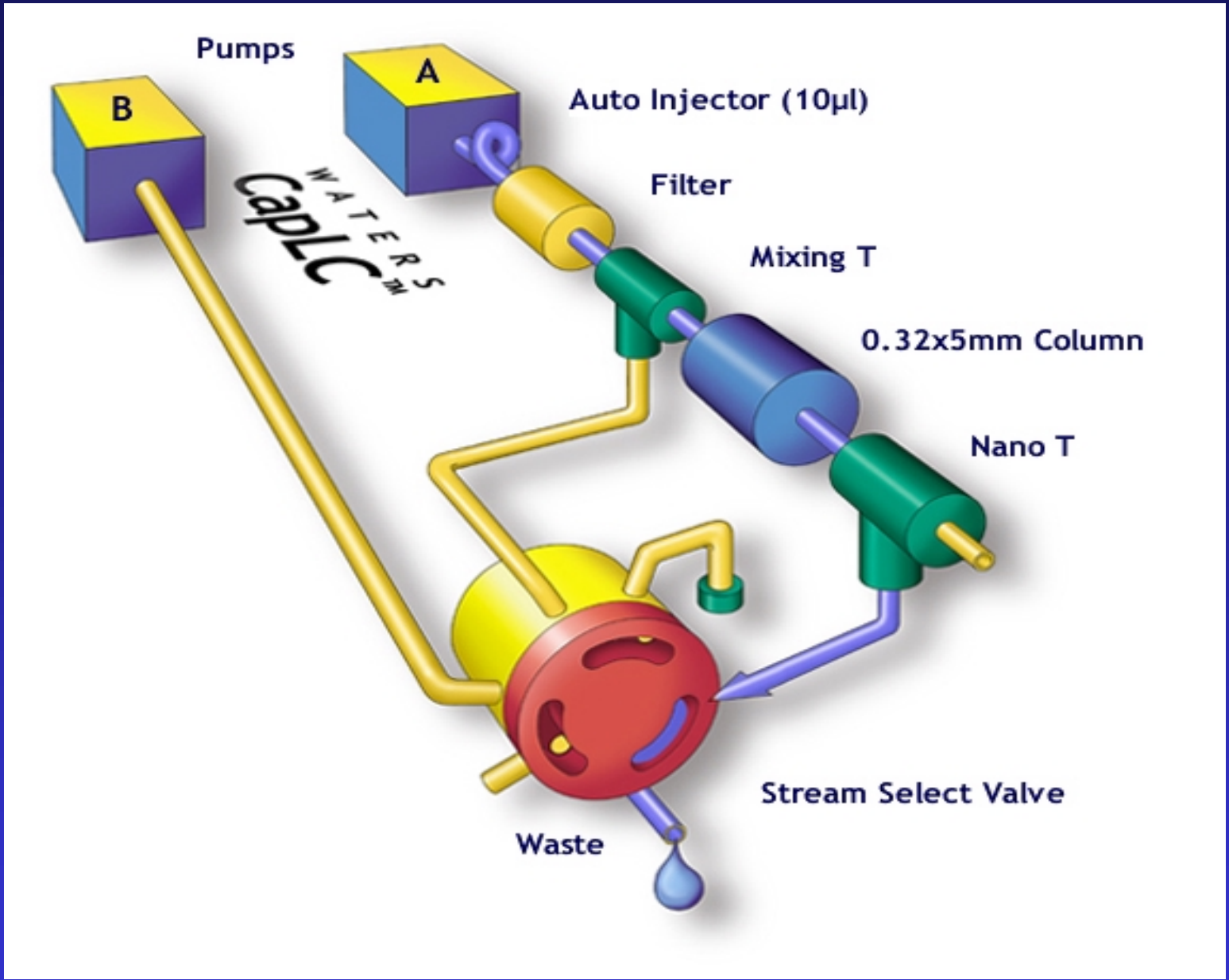
- Produce a fully integrated analytical system
  - From sample submission to protein identity
- Identify proteins in an automated fashion
- Increase Sample throughput (>6 samples /Hr)

LC/MS/MS Conditions

- Sample :ß -Galactosidase tryptic digest
- 180µmx150mm PepMap C18
- Flow rate 2µL/min
- A = 0.1% Formic Acid
- B = 0.1% Formic Acid in MeCN
- Qtof: Data dependent MS\MS mode

Gradient I (post-loading): 5 - 60%B in 35 min

Gradient II (Stream Select): High flow load at 5 % B for 3.2 minutes, 5 - 60% linear in 1 minute with a hold for 3.5 minutes and subsequent return to initial conditions


Pumps
A
B
Auto Injector (10µl)
Filter
Mixing T
0.32x5mm Column
Nano T
Stream Select Valve
Waste
WATERS
CapLC™

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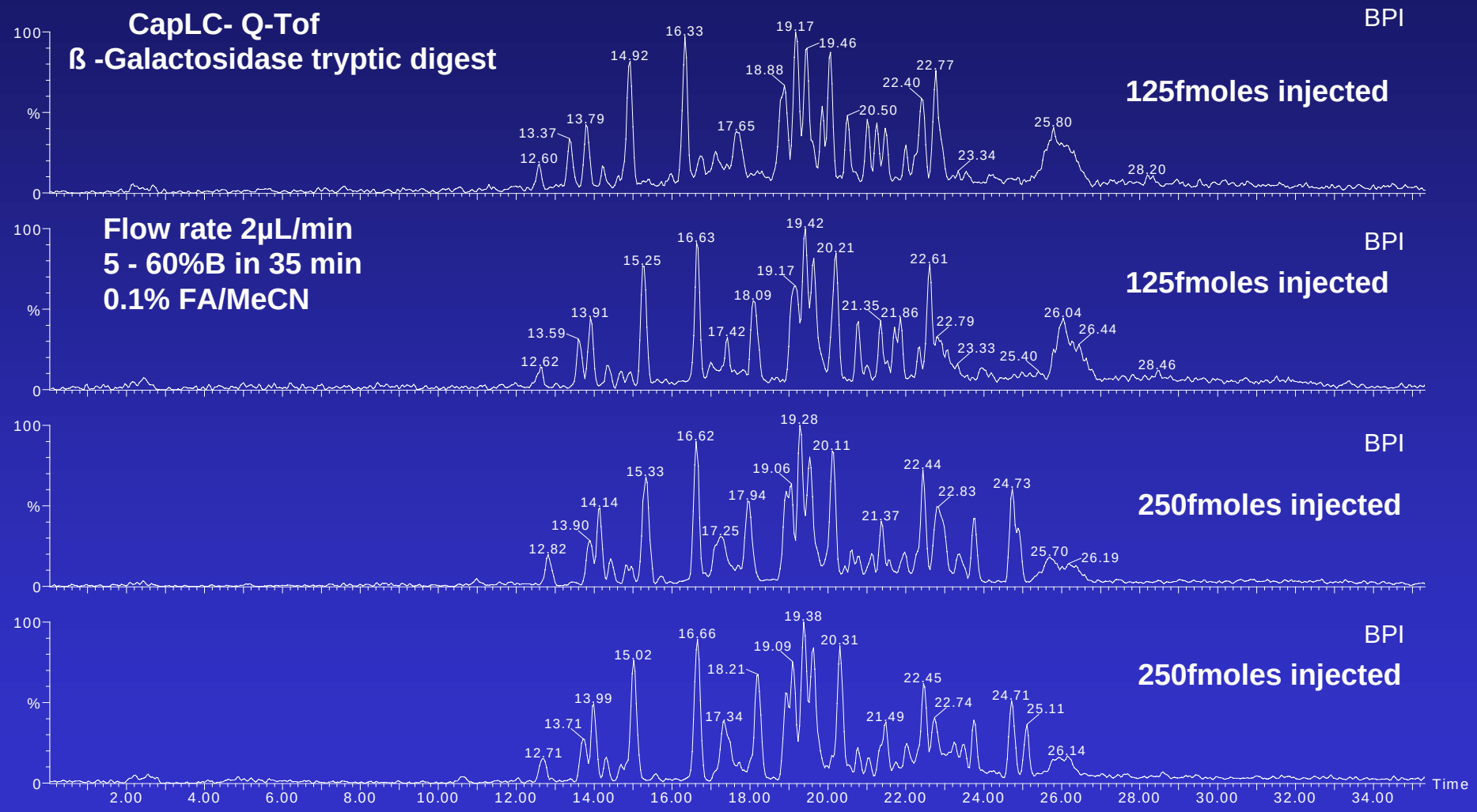
Nanospray LC/MS Peptide Map Gradient I



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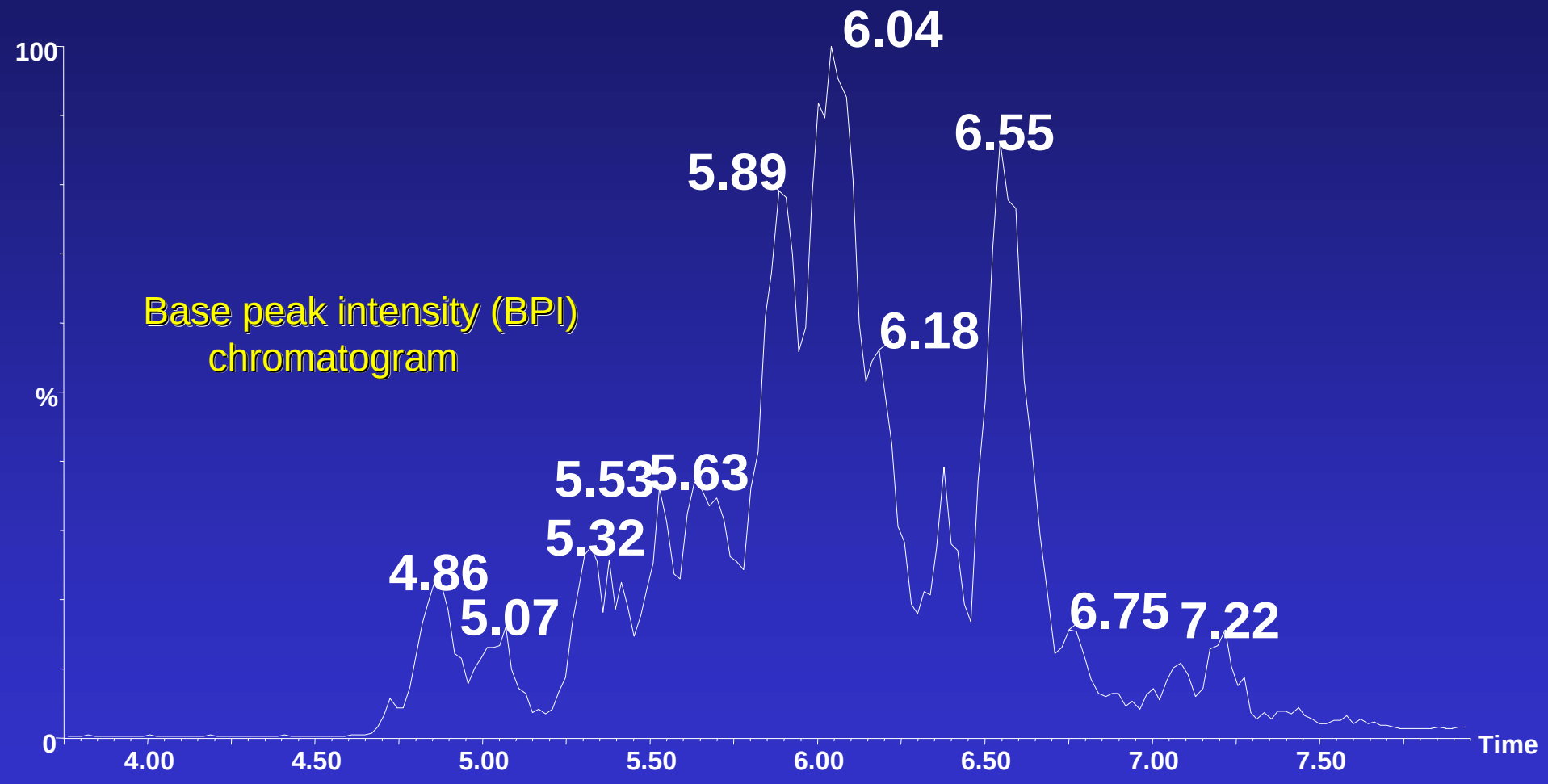
100fmole injection B-galactosidase tryptic digest Gradient II



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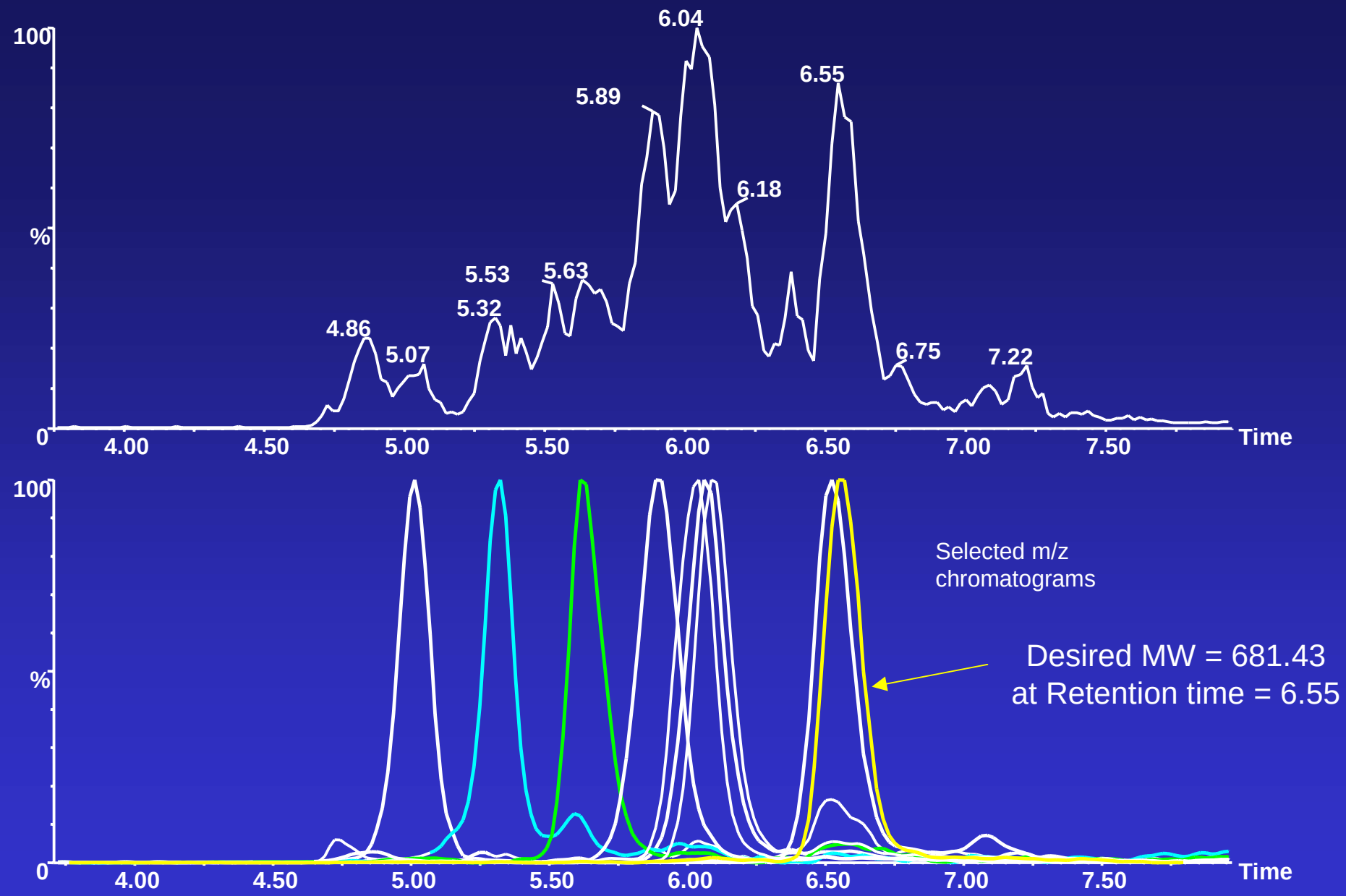
100
%
0
4.86
5.07
5.32
5.53
5.63
5.89
6.04
6.18
6.55
6.75
7.22
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
Time
Selected m/z
chromatograms
Desired MW = 681.43
at Retention time = 6.55
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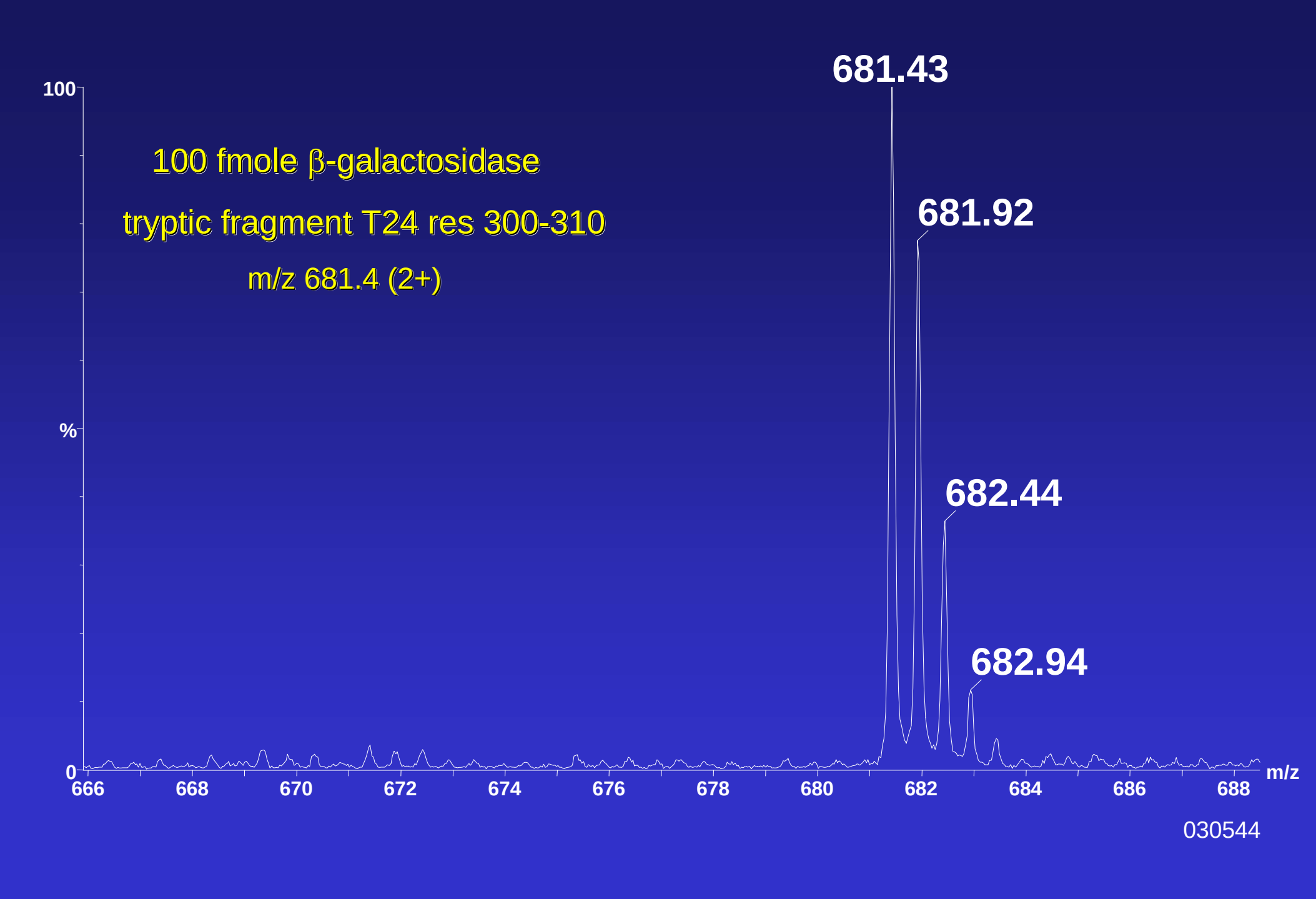
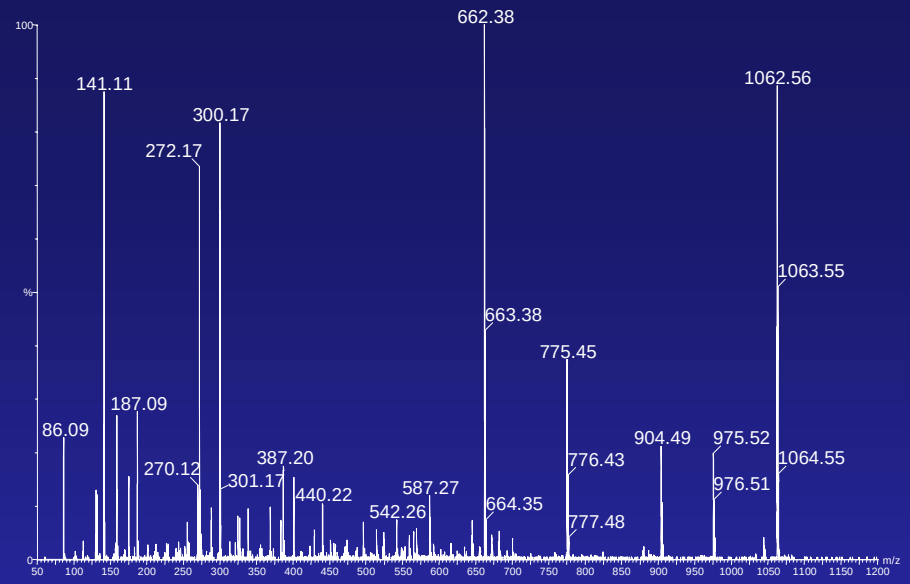
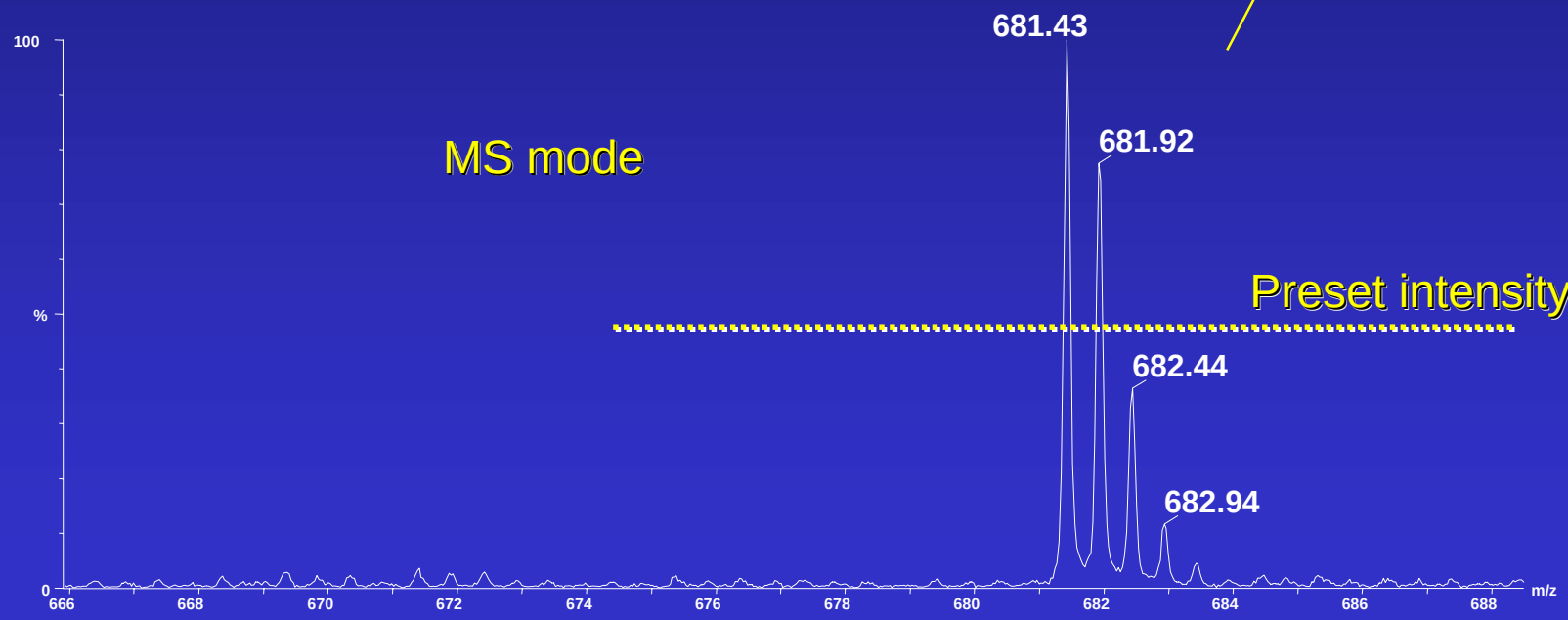

100 fmole β-galactosidase
tryptic fragment T24 res 300-310
m/z 681.4 (2+)
681.43
681.92
682.44
682.94
100
%
0
666
668
670
672
674
676
678
680
682
684
686
688
m/z
030544



Automatic MS/MS



MS mode

Preset intensity threshold

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Summary

- Capillary HPLC is becoming a routine analysis tool
- New instrument design can provide sub-picomole UV and MS detection
- Direct flow can give reproducible gradients in the low microliter per minute range
- LC/MS/MS analysis now capable of providing femtomole level sequence analysis

Thanks to Everyone

- Waters
  - Dennis DellaRovere
  - Joe Luongo
  - Frank Rubino
  - John Heden
  - Scott McLaren
- Micromass
  - Bob Bordoli
  - Chris Hughes
- Glaxo-Welcome
  - Walter Blackstock
  - Jyoti Choudhary
  - Malcolm Ward
- MaxEnt Solutions
  - John Skilling