

**Improving statistical certainty of glycosylation similarity between influenza A virus
variants using data-independent acquisition mass spectrometry**

Deborah Chang¹, Joshua Klein², William Hackett², Mary Rachel Nalehua², Xiu-Feng Wan^{3,4,5,6},
Joseph Zaia^{1,2}

Supplementary File 3. Annotated tandem mass spectra for glycopeptides that were positively identified in the DDA data compared to spectra that failed to pass the scoring threshold in the DIA data.

SWZ mutant cell pp. 2-3

SWZ mutant egg pp. 4-7

SWZ wild type cell pp. 8-11

SWZ wild type egg pp. 12-21

DDA

Y P A L N V T M P N N E Q F D K

b₂ b₃ b₄ b₅ b₆ y₁₀ y₈ y₄ y₃ y₂ y₁

p. 2

+ {Hex:5; HexNAc:2}

Cosine Similarity: 0.00

Relative Intensity
100%
75%
50%
25%
0%
-25%
-50%
-75%
-100%

DDA MS2 score: 6.125312939

DIA expected MS2 score: 0

DIA

Y P A L N V T M P N N E Q F D K

200 400 600 800 1000 1200

m/z

DDA

Y P A L N V T M P N N E Q F D K

b₂ b₃

y₈ y₆ y₃ y₂ y₁

p. 3

+ {Hex5; HexNAc:3}

Cosine Similarity: 0.72

Relative Intensity
100%
75%
50%
0%
25%
50%
75%
100%

DDA MS2 score: 7.703814974

y₁
y₂
b₃
y₃
y₄
y₆
b₆
y₈

DIA

Y P A L N V T M P N N E Q F D K

b₂ b₃ b₄

y₈ y₄ y₃ y₂

250 500 750 1000 1250 1500 1750 2000

m/z

DDA

Y P A L N V T M P N N E Q F D K

b2 b3 b4 b5 b6

y10 y9 y8 y7

y5 y4 y3 y2 y1

p. 4

+ {Hex:6; HexNAc:2}

Cosine Similarity: 0.91

100%

75%

50%

25%

0%

Relative Intensity

DDA MS2 score: 11.0431677

y12

b3

y3 y4

y4 y5

y5 y6

y6 y7

y7 y8

y8

y9

y10 y10

100%

75%

50%

DIA

Y P A L N V T M P N N E Q F D K

b2 b3 b5 b6

y10 y8 y6 y5 y4 y3 y2

200

400

600

800

1000

1200

m/z

DDA

N W A G V T H N G T S S S C

y₁₂ y₁₁ y₉ y₈
b₂ b₃ b₇

p. 5

+ {Hex:5; HexNAc:2}

Relative Intensity
100%
75%
50%
25%
0%
25%
50%
75%
100%

DDA MS2 score: 8.481097693

Cosine Similarity: 0.08

y_{b2}
y_{b3}

y_{b7}
y₈
y₉

y_{8+HexNAc}
y₁₁
y₁₂

peptide

DIA

N W A G V T H N G T S S S C

200 400 600 800 1000 1200 1400

m/z

DDA

Y P A L N V T M P N N E Q F D K

b2 b3 b4 b5 b6

y8 y7 y6 y5 y4 y3 y2 y1

p. 6

+ {Hex:8; HexNAc:3}

100%

75%

50%

25%

0%

25%

50%

75%

100%

DDA MS2 score:

11.10456969

DIA expected MS2 score:

14.43348603

Cosine Similarity: 0.91

Relative Intensity

DIA

250

500

750

1000

1250

1500

1750

2000

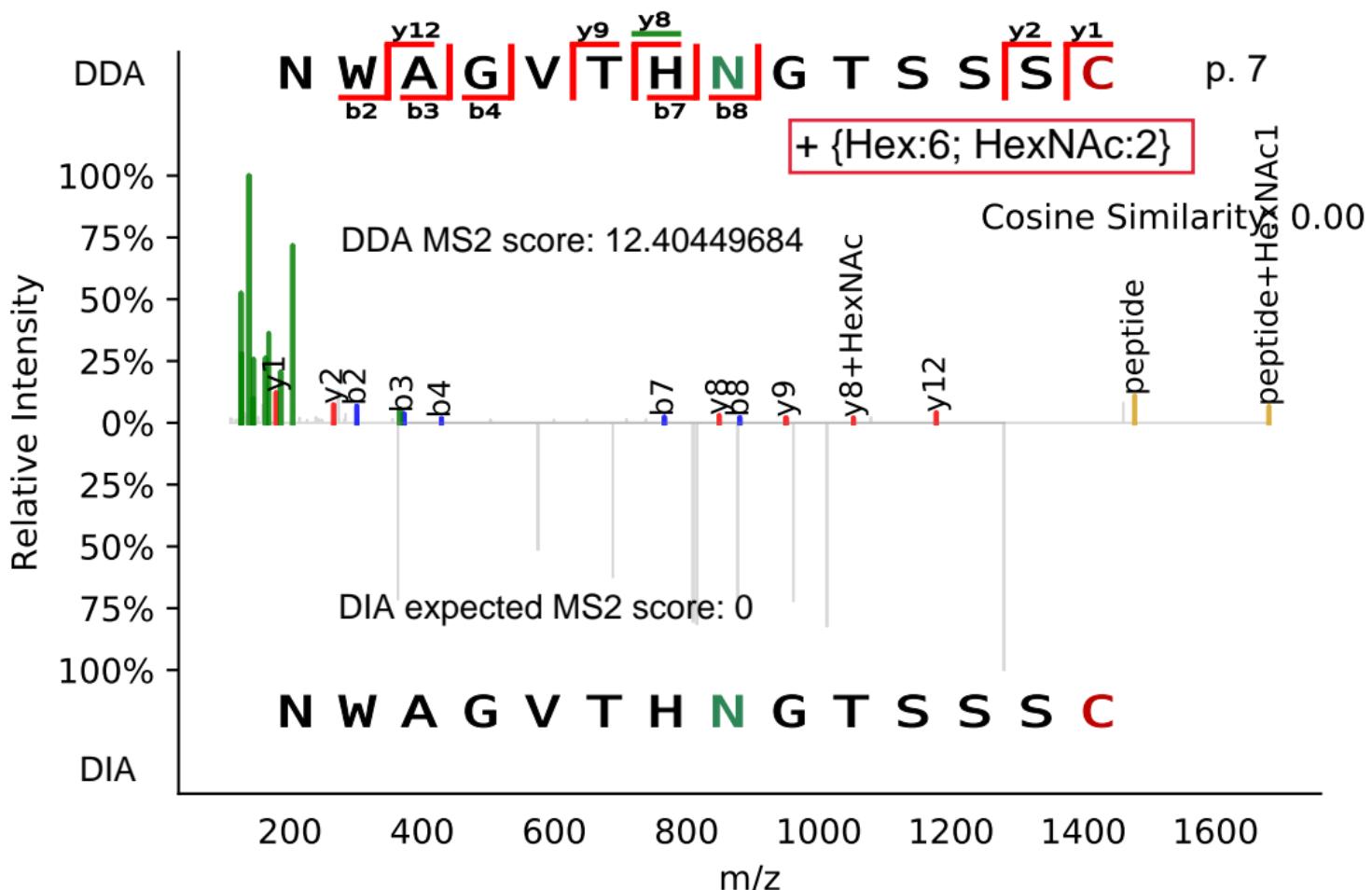
m/z

Y P A L N V T M P N N E Q F D K

b2 b3 b4 b5 b6 b7

y8 y7 y6 y5 y4 y3 y2

b10



DDA

Y P A L N V T M P N N E Q F D K

b₂ b₃ b₄ b₅ b₆

y₈ y₄ y₃ y₂ y₁

p. 8

+ {Hex6; HexNAc:3}

Cosine Similarity: 0.01

Relative Intensity
100%
75%
50%
0%
-25%
-50%
-75%
-100%

DDA MS2 score: 5.434376904

y₁
y₂
y₃
y₄
y₅
y₆
y₈

DIA

DIA expected MS2 score: 0.490990694

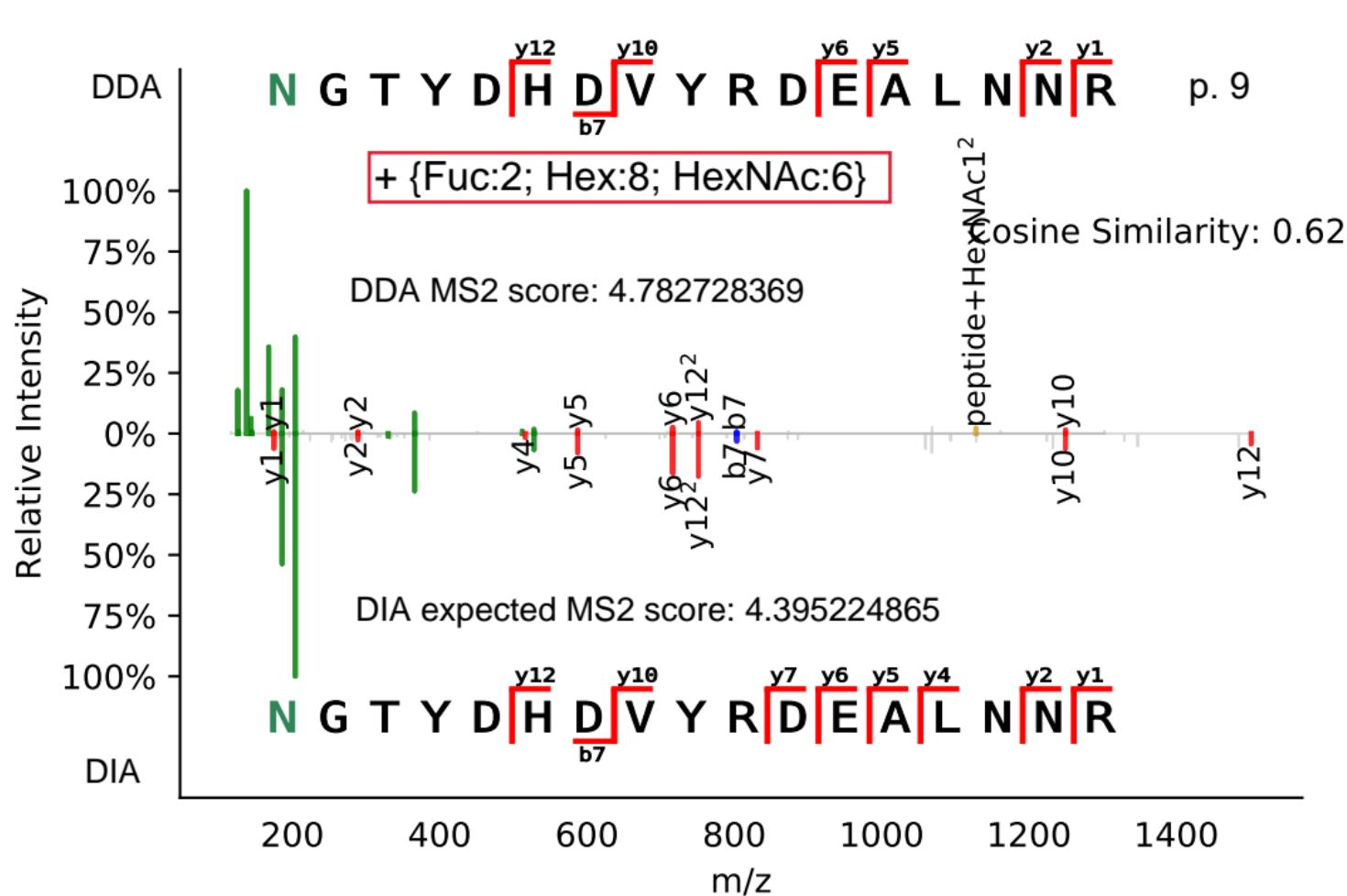
Y P A L N V T M P N N E Q F D K

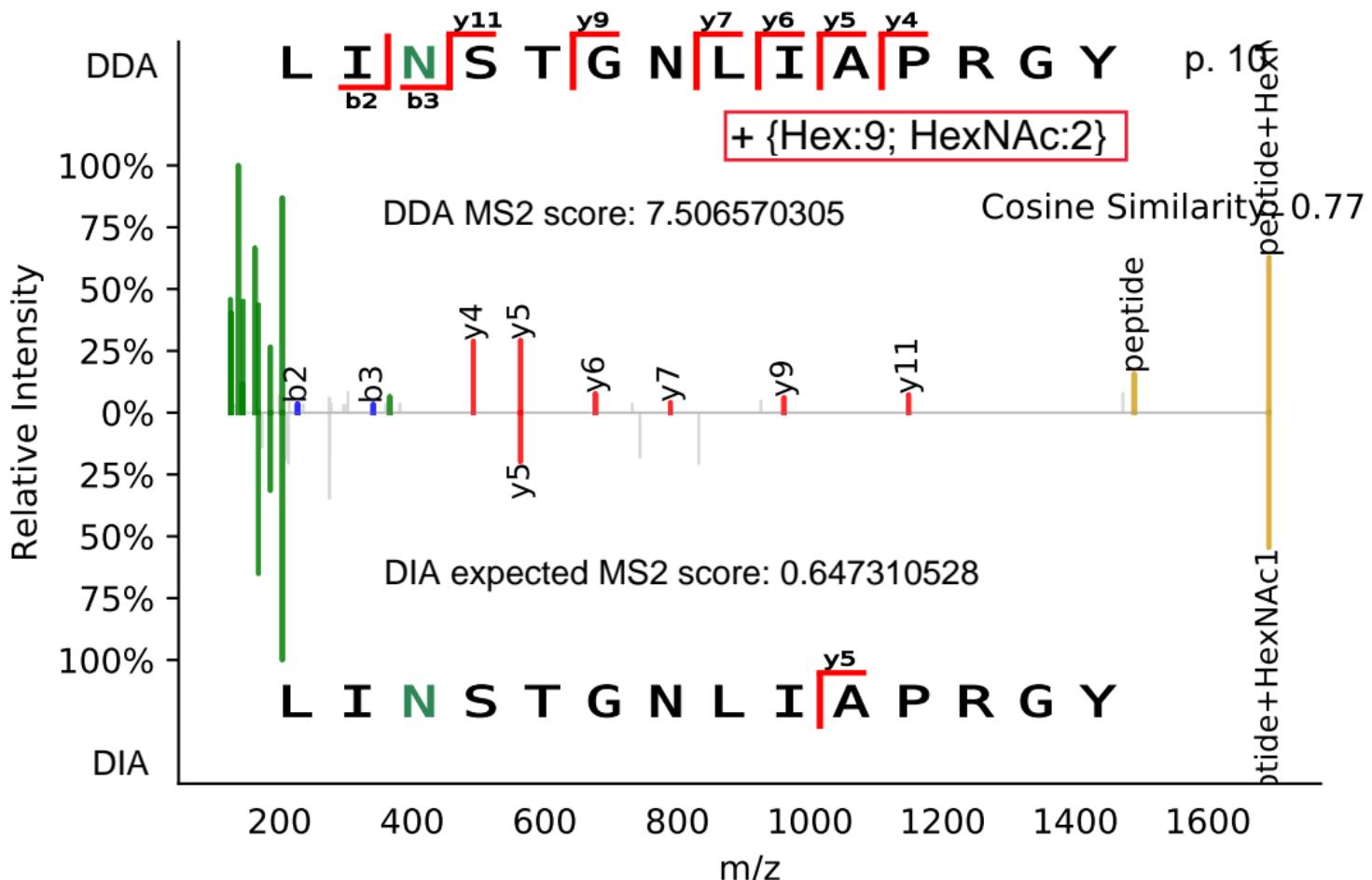
b₁

y₂ y₁

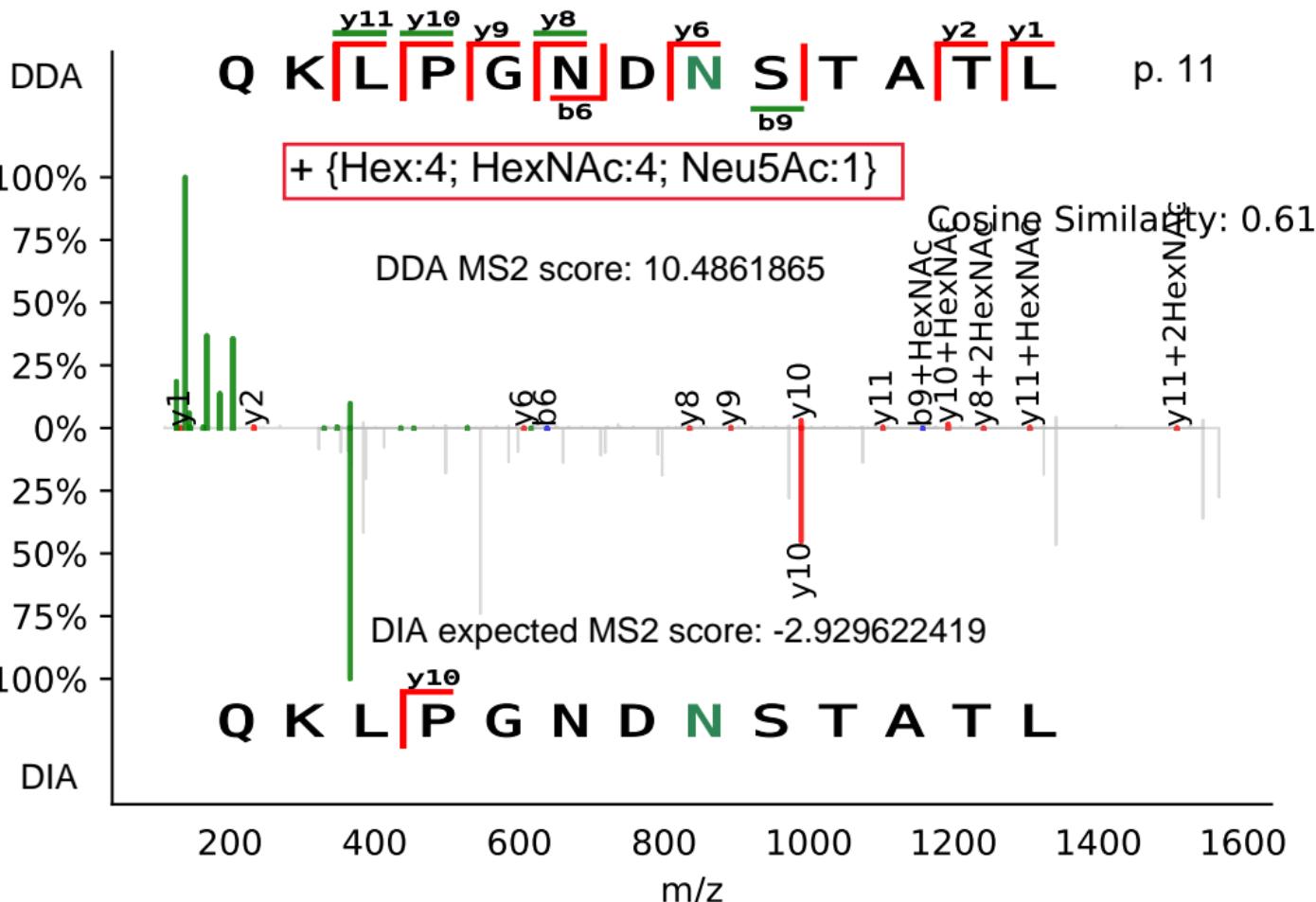
250 500 750 1000 1250 1500 1750 2000

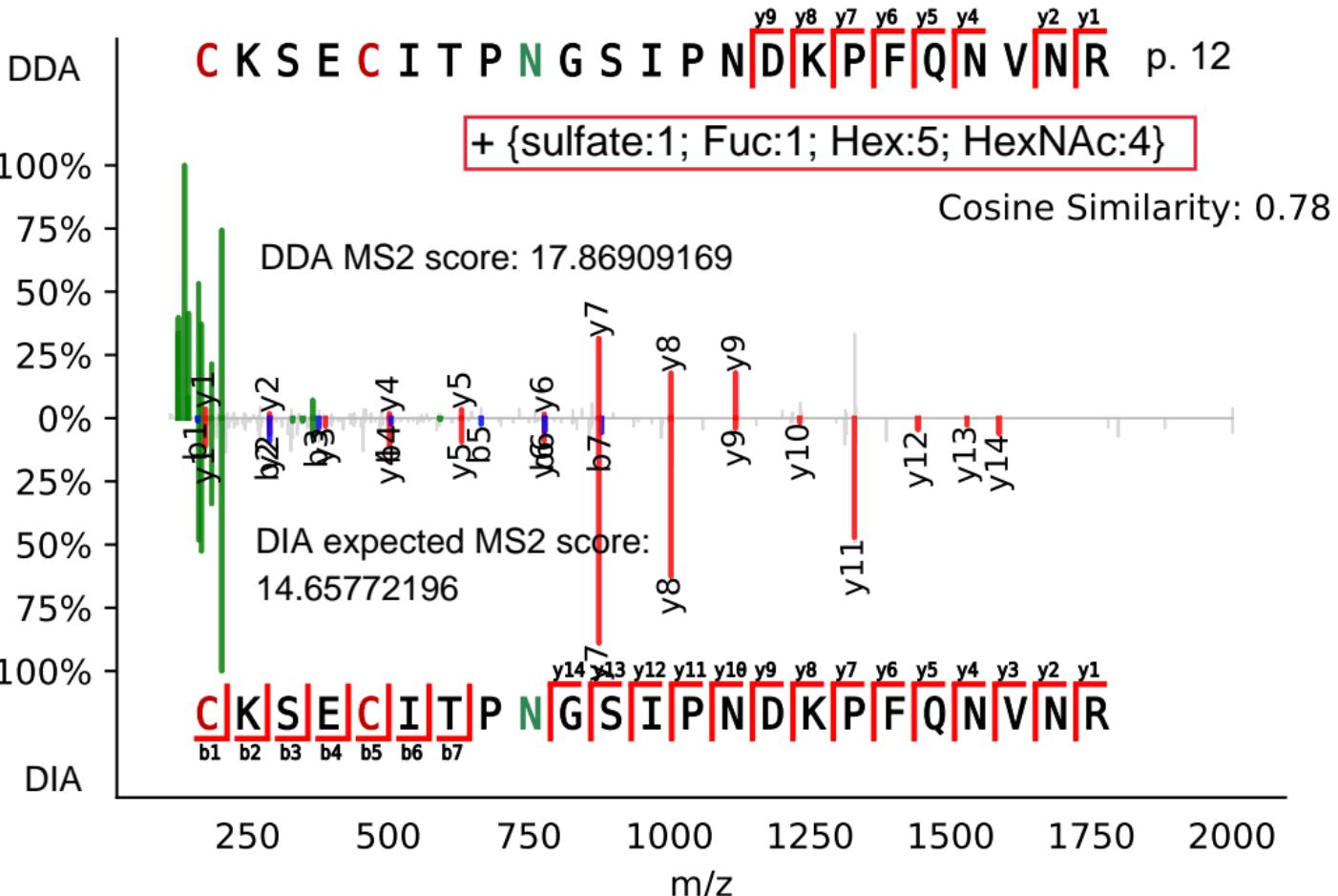
m/z

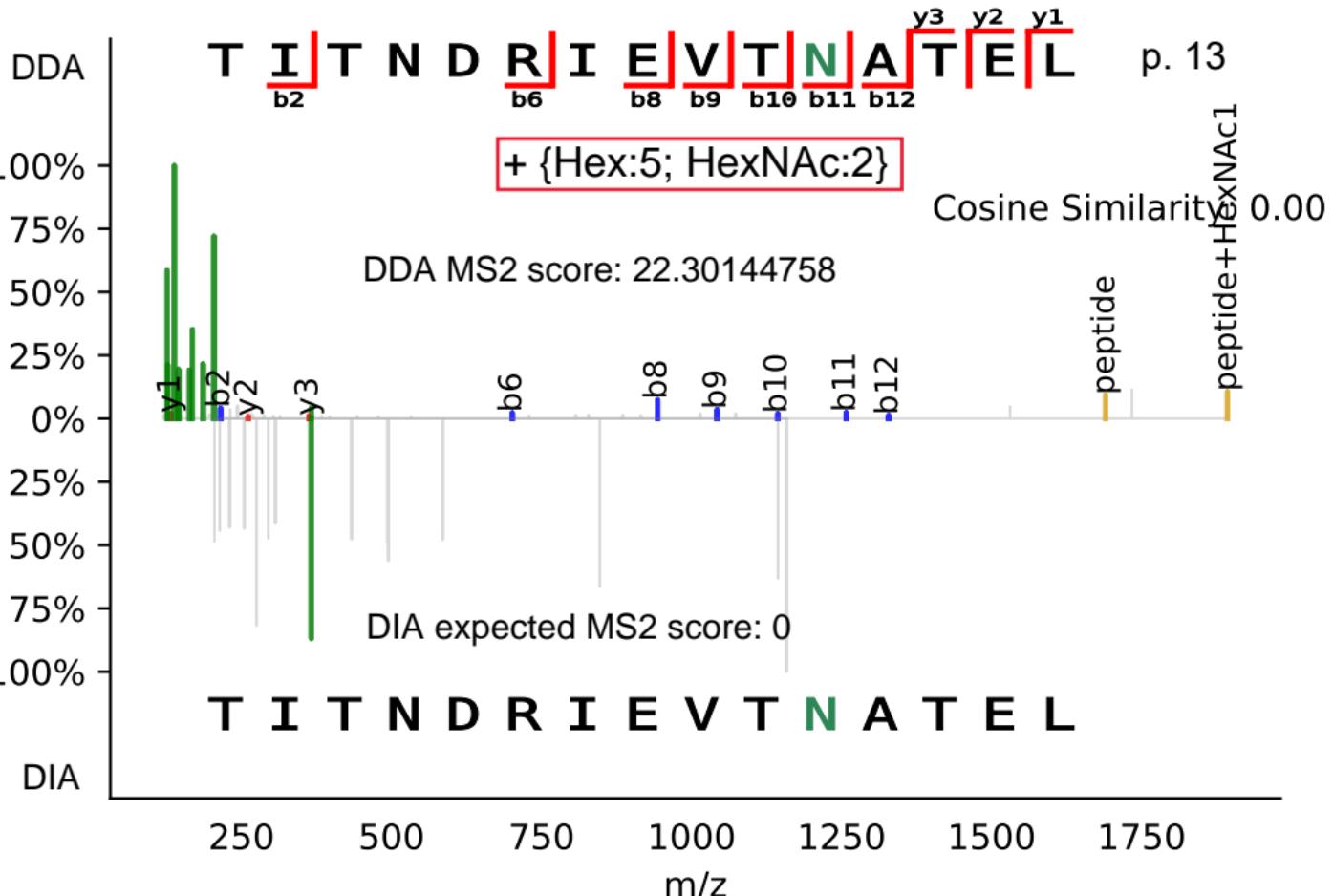




Relative Intensity







DDA

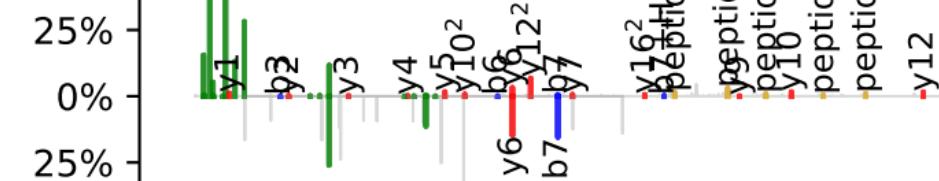
N G T Y D H D V Y R D E A L N N R p. 14

+ {Fuc:1; Hex:6; HexNAc:5}

100%
75%
50%
0%
-25%
-50%
-75%

DDA MS2 score: 46.94466977
Cosine Similarity: 0.06

Relative Intensity



DDA

N G T Y D H D V Y R D E A L N N R

500 1000 1500 2000

m/z

Relative Intensity

DDA

N G T Y D H D V Y R D E A L N N R

b3

y₁₂

y₁₀

y₇

y₆

y₅

y₄

y₃

y₂

y₁

b7

b8

+ {Fuc:1; Hex:5; HexNAc:4; Neu5Ac:1}

100%

75%

50%

25%

0%

DDA MS2 score: 21.84053733

y₁

y₂

y₃

y₄

y₅

y₆

y₁₂²

y₇

y₈

b₇ + HexNAc

peptide + HexNAc^{1,2}

y₁₀

peptide + Hex²HexNAc²

y₁₂

Cosine Similarity: 0.07

75%

50%

25%

0%

DIA expected MS2 score: -3.010299957

DIA

N G T Y D H D V Y R D E A L N N R

b7

200

400

600

800

1000

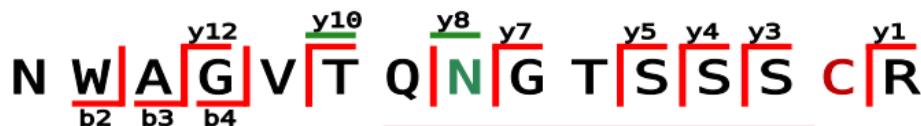
1200

1400

m/z

Relative Intensity

DDA



p. 16

+ {Hex:5; HexNAc:2}

100%

75%

50%

0%

-25%

-50%

-75%

-100%

DDA MS2 score: 28.14885584

DIA expected MS2 score: 16.75710083

DIA

m/z



peptide+HexNAc1 peptide+HexNAc1

0.80

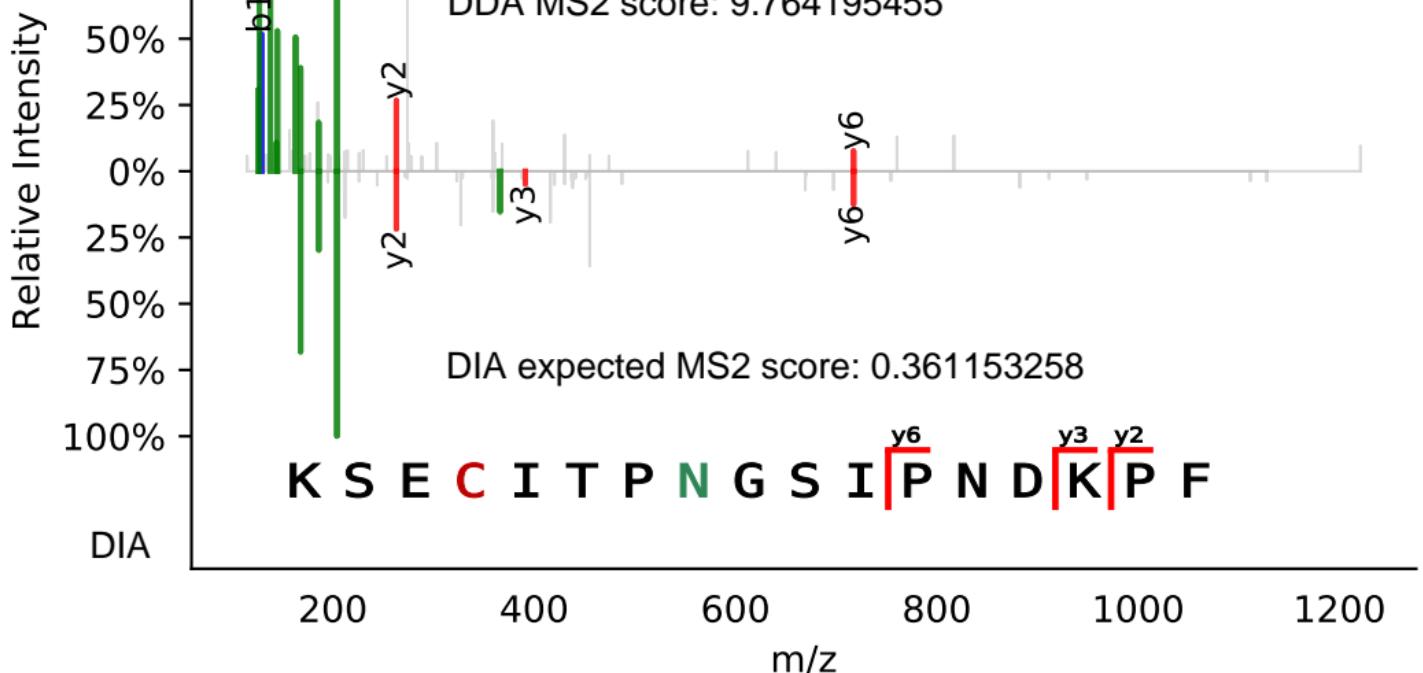
Cosine Similarity

DDA
K S E C I T P N G S I | P N D K | P F
b1

+ {Fuc:2; Hex:6; HexNAc:8}

Cosine Similarity: 0.26

DDA MS2 score: 9.764195455



1000

800

600

400

200

DDA

N G T Y D H D V Y R D E A L N N R

y₁₂
y₁₀
b₇

100%

+ {sulfate:1; Fuc:1; Hex:6; HexNAc:5}

p. 18

75%

50%

25%

0%

DDA MS2 score: 19.96899603

peptide²

peptide+HexNAc^{1,2}

peptide+Fuc^{1,2}

peptide+Hex^{1,2}

y₁₀

y₁₂

One Similarity: 0.05

Relative Intensity

75%

50%

25%

0%

DIA expected MS2 score: 0.298748646

DIA

N G T Y D H D V Y R D E A L N N R

200

400

600

800

1000

1200

1400

m/z

DDA

Y P A L N V T M P N N E Q F D K

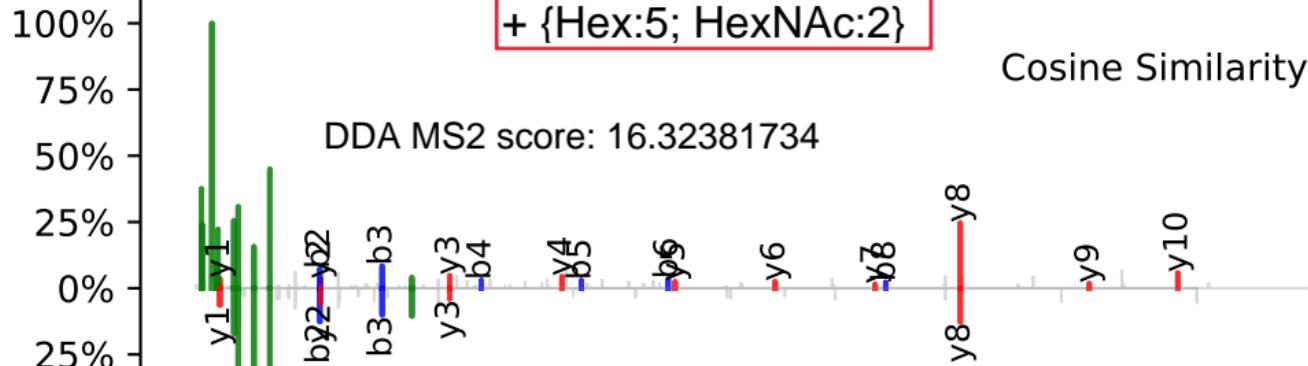
b₂ b₃ b₄ b₅ b₆ y₁₀ y₉ y₈ y₇ y₆ y₅ y₄ y₃ y₂ y₁

p. 19

+ {Hex:5; HexNAc:2}

Cosine Similarity: 0.60

Relative Intensity



DIA

Y P A L N V T M P N N E Q F D K

b₂ b₃ y₈ y₃ y₂ y₁

DIA expected MS2 score: 1.814630145

m/z

DDA

Y P A L N V T M P N N E Q F D K p. 20

y₁₄ y₁₃ y₁₂ y₁₁ y₁₀ y₉ y₈ y₇ y₆ y₅ y₄ y₃ y₂ y₁

b₂ b₃ b₄ b₅ b₆ b₇ b₈ b₁₀

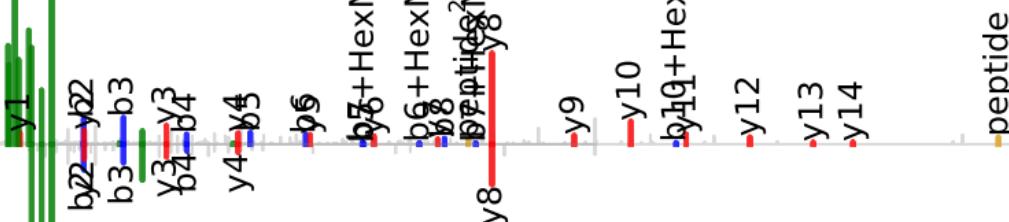
+ {Hex:7; HexNAc:2}

100%
75%
50%
25%
0%

DDA MS2 score: 50.02988162

Cosine Similarity: 0.78

Relative Intensity



75%
50%
25%
0%
75%
100%

DIA expected MS2 score: 2.39655768

100%
DIA
Y P A L N V T M P N N E Q F D K

y₈
b₂ b₃ b₄

250 500 750 1000 1250 1500 1750 2000

m/z

DDA

S E C I T P N G S I P N D K P F Q N V N R

b₂ b₃ b₄ b₅ y₁₆ y₁₄ y₁₂ y₁₁ y₁₀ y₉ y₈ y₇ y₆ y₅ y₄ y₃ y₂ y₁

p. 21

+ {Hex:5; HexNAc:2}

100%

75%

50%

0%

-25%

-50%

-75%

-100%

Relative Intensity

DDA MS2 score: 14.90839504

Cosine Similarity: 0.97

DIA

S E C I T P N G S I P N D K P F Q N V N R

b₂ b₃ b₄ b₅ y₁₆ y₁₄ y₁₁ y₉ y₈ y₇ y₅ y₄ y₃ y₂ y₁

y₁₆+HexNAc-y₁₆+HexNAc

250

500

750

1000

1250

1500

1750

2000

m/z