Supplementary Data

for

Hyper-truncated Asn355- and Asn391-glycans modulate the activity of neutrophil granule myeloperoxidase

Harry C. Tjondro\textsuperscript{a,b,1}, Julian Ugonotti \textsuperscript{a,b,1}, Rebeca Kawahara \textsuperscript{a,b,1}, Sayantani Chatterjee \textsuperscript{a,b,1}, Ian Loke\textsuperscript{c}, Siyun Chen\textsuperscript{d}, Fabian Soltermann\textsuperscript{d}, Hannes Hinneburg\textsuperscript{a,b,}, Benjamin L. Parker\textsuperscript{e}, Vignesh Venkatakrishnan\textsuperscript{f}, Regis Dieckmann\textsuperscript{f}, Oliver C. Grant\textsuperscript{e}, Johan Bylund\textsuperscript{h}, Alison Rodger\textsuperscript{a,b}, Robert J. Woods\textsuperscript{g}, Anna Karlsson-Bengtsson\textsuperscript{f}, Weston B. Struwe\textsuperscript{d}, Morten Thaysen-Andersen\textsuperscript{a,b,*}

\textsuperscript{a}Department of Molecular Sciences, Macquarie University, Sydney, NSW, Australia
\textsuperscript{b}Biomolecular Discovery Research Centre, Macquarie University, Sydney, NSW, Australia
\textsuperscript{c}Cordlife Group Limited, Singapore, Singapore
\textsuperscript{d}Department of Chemistry, University of Oxford, Oxford, United Kingdom
\textsuperscript{e}Department of Physiology, University of Melbourne, Melbourne, VIC, Australia
\textsuperscript{f}Department of Rheumatology & Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
\textsuperscript{g}Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia, United States
\textsuperscript{h}Department of Oral Microbiology and Immunology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
\textsuperscript{i}Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

\textsuperscript{1}Contributed equally

*Corresponding author:
Dr Morten Thaysen-Andersen, PhD
Department of Molecular Sciences - Macquarie University, NSW-2109, Sydney, Australia
Ph: +61 2 9850 7487 - Email: morten.andersen@mq.edu.au
Annotation and Fragmentation Key

- Mannose (162.0528 Da)
- Galactose (162.0528 Da)
- N-Acetylglucosamine (203.0794 Da)
- N-Acetylgalactosamine (203.0794 Da)
- N-Acetylneuraminic acid (291.0954 Da)
- Fucose (146.0579 Da)
- Cross-ring fragment (unspecified)
- Acetyl group (42.0106 Da)

- Indicates mostly Y ions (includes oxygen of glycosidic linkage)
- Indicates mostly Z ions (excludes oxygen of glycosidic linkage)

- Reduced reducing end
- Phosphate (79.9700 Da)

Supplementary Data S1
Manually annotated PGC-LC-ESI-CID-MS/MS (-) spectra of reduced N-glycans (alditols) released from nMPO
Glycan #1

Observed m/z 571.24 (1-), RT: ~17.8 min

[M-H]⁻ 571.23 Da

*Ambiguity of the cross-ring fragments
Glycan #2

Observed m/z 587.30 (1-), RT: ~16.9 min
[M-H]⁻: 587.23 Da
Glycan #3

Observed m/z 733.36 (1-), RT: ~21.1 min

[M-H]⁻ 733.28 Da

Precursor ion - Acetyl

Precursor ion - Acetyl-H₂O

* Ambiguity of the cross-ring fragments
Glycan #4

Observed m/z 749.36 (1-), RT: ~18.4 min
[M-H]⁻ 749.28 Da

Note: Only one isomer was observed for M₂, which is biosynthetically predicted to be α₁,6-linkage isomer based on experience with neutrophil glycosylation. The α₁,3-isomer, typically of lower abundance in human neutrophils, was not detected.
Glycan #5a

Observed m/z 895.46 (1-), RT: ~24.3 min

[M-H]· 895.34 Da

Note: Identified as the α1,6-Man isomer based on PGC LC elution time, its high abundance and experience with neutrophil N-glycosylation.

Precursor ion -Acetyl

Precursor ion -Acetyl-H₂O

* Ambiguity of the cross-ring fragments
Glycan #5b

Observed m/z 895.46 (1-), RT: ~26.4 min
[M-H] 895.34 Da

Note: This low abundance glycan was annotated as the α1,3-Man isomer based on PGC LC elution time and experience with neutrophil N-glycosylation.

* Ambiguity of the cross-ring fragments
Glycan #6

Observed m/z 911.44 (1-), RT: ~22.9 min

[M-H] 911.33 Da

* Ambiguity of the cross-ring fragments
Glycan #7

Observed m/z 1057.54 (1-), RT: ~31.1 min
[M-H]^- 1057.39 Da

* Ambiguity of the cross-ring fragments
Glycan #8a

Note: The outer mannose residue has been placed on the α1,6-Man arm based on biosynthetic rules and may be predicted to be α1,6-linked based on PGC LC elution pattern (relative to the M4b isomer). However the mannosyl linkage of this residue was left unassigned since no MS/MS evidence is present to support this.

Observed m/z 1073.40 (1-), RT: ~18.3 min
[M-H]^- 1073.38 Da
Glycan #8b

Observed m/z 1073.40 (1-), RT: ~21.3 min

[M-H]⁻ 1073.38 Da

Note: The outer mannose residue has been placed on the α1,6-Man arm based on biosynthetic rules and may be predicted to be α1,3-linked based on PGC LC elution pattern (relative to the M4a isomer). However, the mannosyl linkage of this residue was left unassigned since no MS/MS evidence is present to support this.

Precursor ion -Acetyl

Precursor ion -Acetyl-H₂O

* Ambiguity of the cross-ring fragments
Note: The outer mannose residue has been placed on the α1,6-Man arm based on biosynthetic rules. Unknown if this residue is found in a α1,3- or α1,6-linkage since no MS/MS evidence to support either so left unassigned.

Observed \( m/z \) 1219.44 (1-), RT: ~28.5 min

\([M-H]^-\) 1219.44 Da
Glycan #10

Observed m/z 617.22 (2-), RT: ~18.1 min  
[M-H-] 1235.44 Da

Ambiguity of the cross-ring fragments
Glycan #11a

Observed m/z 1381.62 (1-), RT: ~30.4 min

[M-H]⁻ 1381.49 Da

Note: Structure predicted from known biosynthetic rules, but no MS/MS support for exact topology of outer mannose residues.
Glycan #11b

Observed m/z 1381.62 (1-), RT: ~33.2 min
[M-H]- 1381.49 Da

Note: Structure predicted from known biosynthetic rules, but no MS/MS support for exact topology of outer mannose residues.

* Ambiguity of the cross-ring fragments
Glycan #12

Observed m/z 698.36 (2-), RT: ~20.7 min
[M-H]⁻ 1397.49 Da

*Ambiguity of the cross-ring fragments
Glycan #13

Observed m/z 779.38 (2-), RT: ~20.9 min
[M-H]⁻ 1559.54 Da

-2 Hex

-2

-2

-2

-4 Hex

-3 Hex

-2 Hex

* Ambiguity of the cross-ring fragments
Glycan #14a

Observed m/z 738.23 (2-), RT: ~17.6 min
[M-H] - 1477.46 Da

Note: Based on knowledge of the M6P pathway, the phosphate moiety appears on outer mannose residue, but no direct MS/MS evidence to support the exact position of the phosphate.
Glycan #14b

Observed m/z 738.23 (2-), RT: ~18.5 min

[M-H]⁻ 1477.46 Da

Note: Based on knowledge of the M6P pathway, the phosphate moiety appears on outer mannose residue, but no MS/MS evidence to support the exact position.

* Ambiguity of the cross-ring fragments
Glycan #15

Observed m/z 819.32 (2-), RT: ~17.8 min  
[M-H]⁻ 1639.51 Da

Note: Based on knowledge of the M6P pathway, the phosphate moiety appears on outer mannose residue, but no MS/MS evidence to support the exact position of the phosphate.
**Glycan #16**

Observed m/z 1260.60 (1-), RT: ~33.7 min

$[\text{M-H}]^- 1260.47$ Da

---

**Note:** From biosynthetic pathway, the single β1,2-GlcNAc residue is predicted to occupy the α-1,3 arm but no MS/MS evidence to support this, so here left unassigned.
Glycan #17

Observed m/z 731.28 (2-), RT: ~26.1 min

[M-H]⁻ 1463.55 Da

*Ambiguity of the cross-ring fragments
Glycan #18

Observed m/z 783.28 (2-), RT: ~22.3 min

[M-H]: 1567.56 Da

Note: This glycan is annotated as the α2,6-sialyl linkage isomer based on the early PGC-LC elution and experience with neutrophil N-glycan profiling. The elongated antenna is known to frequently occupy the α1,3-arm rather than the α1,6-arm of neutrophil N-glycans and have thus been assigned as such.
Glycan #19a

Observed m/z 856.31 (2-), RT: ~27.9 min

\[ \text{[M-H]} \times 1713.62 \text{ Da} \]

Note: This glycan is annotated as the α2,6-sialyl linkage isomer based on the early PGC-LC elution and experience with neutrophil N-glycan profiling. The elongated antenna is known to frequently occupy the α1,3-arm rather than the α1,6-arm of neutrophil N-glycans and have thus been assigned as such.

* Ambiguity of the cross-ring fragments
Glycan #19b

Note: This glycan is annotated as the α2,3-sialyl linkage isomer based on the late PGC-LC elution and experience with neutrophil N-glycan profiling. The elongated antenna is known to frequently occupy the α1,3-arm rather than the α1,6-arm of neutrophil N-glycans and is thus assigned as such.

Observed m/z 856.31 (2-), RT: ~35.6 min
[M-H]- 1713.62 Da

FA1G1S1b

* Ambiguity of the cross-ring fragments
Note: This low abundance glycan is possibly the isomer where the Sia-Gal-GlcNAc antenna occupy the α1,6-arm rather than the α1,3-arm common for neutrophil N-glycans but no direct MS/MS evidence to support this.

Glycan #19c

Observed $m/z$ 856.31 (2-), RT: ~43.8 min

$[\text{M-H}]^-$ 1713.62 Da

*Ambiguity of the cross-ring fragments*
Glycan #20a

Observed m/z 957.85 (2⁻), RT: ~29.3 min

[M-H⁻]: 1916.70 Da

Note: This glycan is annotated as the α2,6-sialyl linkage isomer based on the early PGC-LC elution and higher abundance (relative to Glycan #20b) and experience with neutrophil N-glycan profiling. The elongated antenna is known to frequently occupy the α1,3-arm rather than the α1,6-arm of neutrophil N-glycans and is thus represented as such.

FA2G1S1a

* Ambiguity of the cross-ring fragments
Glycan #20b

Note: This glycan is annotated as the α2,3-sialyl linkage isomer based on the later PGC-LC elution and lower abundance (relative to Glycan #20a) and experience with neutrophil N-glycan profiling. The elongated antenna is known to frequently occupy the α1,3-arm rather than the α1,6-arm of neutrophil N-glycans and have thus been assigned as such.

Observed m/z 957.85 (2−), RT: ~37.3 min

[M-H]− 1916.70 Da

FA2G1S1b

* Ambiguity in the cross-ring fragments
Glycan #21

Observed m/z 1038.88 (2-), RT: ~31.3 min

[M-H]⁻ 2078.75 Da

Note: This glycan is annotated as the α2,6-sialyl linkage isomer based on the early PGC-LC elution and experience with neutrophil N-glycan profiling. The arm position of the sialic acid could not be deduced.
Note: This glycan has been annotated as the α2,6-/α2,6-sialyl isomer based on the early PGC-LC elution time.
Note: This glycan has been annotated as the α2,6-/α2,3-sialyl isomer based on the late PGC-LC elution time (relative to Glycan #22a) and experience with neutrophil N-glycosylation. Not possible to position the α2,6- and α2,3-sialic acid to specific arms.

Observed m/z 1184.52 (2-), RT: ~39.4 min

[M-H] - 2369.84 Da

FA2G2S2b

m/z 1916.96

* Ambiguity of the cross-ring fragments
Glycan #23

Observed $m/z$ 1111.91 (2-), RT: ~29.9 min

$[\text{M-H}]^-$ 2224.82 Da

Note: No MS/MS evidence to support fucosyl-linkage type or the position of this outer arm residue, hence these features relating to the fucosyl moiety have been left unassigned. The sialyl-linkage can be predicted to be $\alpha$2,6-sialyl from other characterised structures, but the arm position of this residue is unknown.
Supplementary Data S2A
Examples of Byonic-annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of peptides identified with non-glyco PTMs (i.e. Met and Trp mono- and di-oxidation and Tyr mono-chlorination) from the analysis of unenriched peptide mixtures of nMPO
Human myeloperoxidase (P05164)

**Mono-oxidised methionine-containing peptide**

629\textcolor{red}{KLM}EQYGTPNNIDIWMGGVSEPLKR\textcolor{black}{641} (identification confidence level, PEP 2D: 2.4\times10^{-20})
Human myeloperoxidase (P05164)

*Mono-oxidised tryptophan-containing peptide*

$^{198}\text{WLPAEYEDGFSLPYG}^\text{WTPGVKR}^{219}$ (identification confidence level, PEP 2D: $1.9 \times 10^{-15}$)
Human myeloperoxidase (P05164)

Di-oxidised tryptophan-containing peptide

$^{629}$KLMEQYGTPNNIDI$^W$MGGVSEPLKR$^{641}$ (identification confidence level, PEP 2D: $1.9 \times 10^{-18}$)

R. KLM$^{[+16]}$EYGTPNNIDIW$^{[+32]}$MGGVSEPLKR. K z=4, scan#=48200, scan time=73.5488
Supplementary Data S2B
Byonic-annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of N- and C-terminal truncation variants of the MPO α- and β-chain identified from the analysis of unenriched peptide mixtures of nMPO
Human myeloperoxidase (P05164)
α-chain N-terminal (truncation variant)

$^{164}\text{GVTCPEQDKYR}$ (identification confidence level, PEP 2D: $4.0 \times 10^{-11}$)
Human myeloperoxidase (P05164)
α-chain N-terminal (truncation variant)
$^{165}$VTCEQDKYR (identification confidence level, PEP 2D: $5.7 \times 10^{-7}$)
Human myeloperoxidase (P05164)
α-chain N-terminal (truncation variant)

$^{166}_{\text{T}}$CPEQDKYR (identification confidence level, PEP 2D: $3.3 \times 10^{-8}$)
Human myeloperoxidase (P05164)  
α-chain N-terminal (truncation variant)  
SLMFMQWGQLLDHDLDFTEPAAR^{272} (identification confidence level, PEP 2D: 1.9x10^{-23})
Human myeloperoxidase (P05164) 
β-chain N-terminal (truncation variant) 

\[ \text{277}^{TVGCETSCVQQPPCFPLKIPPNDPR} \] (identification confidence level, PEP 2D: \(8.7\times10^{-14}\))
Human myeloperoxidase (P05164)

**β-chain N-termini** (truncation variant)

\[ ^{278} \text{GVNCETSCVQQPPCFPLKIPPNDPR} \] (identification confidence level, PEP 2D: \(1.1 \times 10^{-15}\))
Human myeloperoxidase (P05164)

**β-chain N-terminal** (truncation variant)

$^{279}VNCETSCVQQPPCFPLKIPPNDPR$ (identification confidence level, PEP 2D: $1.5 \times 10^{-17}$)

G.VNC[+57]ETSC[+57]VQQPPC[+57]FPLKIPPNDPR. I z=2, scan# = 44243, scan time = 69.6922
Human myeloperoxidase (P05164)

β-chain C-terminal

DFVNCSTLPALNASWREAS$^{745}$ (identification confidence level, PEP 2D: $1.4 \times 10^{-8}$)

R. DFVN$^{[+876]}$C$^{[+57]}$STLPALNASWREAS. - z=3, scan # = 62968, scan time = 87.0878
Supplementary Data S3A

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn323 identified from the analysis of the unenriched peptide mixtures of nMPO.
Human myeloperoxidase (P05164)
SCPACPGS$N$ITIR (Asn323 peptide)

Obs. $m/z$ 716.83 (2+)
Obs. $[\text{M+H}]^+$ = 1432.66 Da
Calc. $[\text{M+H}]^+$ = 1432.66 Da
RT: 42 min

Non-glycosylated peptide
Human myeloperoxidase (P05164)
SCPACPGS\textit{NITIR} (Asn323 peptide)

Obs. \(m/\zeta\) 818.32 (2+)
Glycan: 203.07 Da (GlcNAc\textsubscript{1})
Obs. \([\text{M+H}]^+\) = 1635.64 Da
Calc. \([\text{M+H}]^+\) = 1635.74 Da
RT: 42 min

Glycoform #1

SCPACPGS\textit{NITIR}

\begin{align*}
\text{b}_2^{1+} & = 248.07 \\
\text{b}_3^{1+} & = 301.13 \\
\text{b}_4^{1+} & = 329.13 \\
\text{y}_7^{1+} & = 345.12 \\
\text{y}_8^{1+} & = 416.16 \\
\text{y}_9^{1+} & = 530.79 \\
\text{y}_10^{1+} & = 610.80 \\
\text{y}_11^{1+} & = 694.85 \\
\text{y}_12^{1+} & = 840.46 \\
\text{y}_13^{1+} & = 906.49 \\
\text{y}_14^{1+} & = 1017.51 \\
\text{y}_15^{1+} & = 1100.55 \\
\text{y}_16^{1+} & = 1185.61 \\
\text{y}_17^{1+} & = 1220.60 \\
\text{y}_18^{1+} & = 1388.66 \\
\end{align*}
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. m/z 891.36 (2+)
Glycan: 349.14 Da (GlcNAc1Fuc1)
Obs. [M+H]+ = 1781.72 Da
Calc. [M+H]+ = 1781.80 Da
RT: 41 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. $m/z$ 919.91 (2+)
Glycan: 406.15 Da (GlcNAc$_2$)
Obs. $[M+H]^+$ = 1838.84 Da
Calc. $[M+H]^+$ = 1838.83 Da
RT: 42 min
Human myeloperoxidase (P05164) SCPACPGS\textbf{NITIR} (Asn323 peptide)

Obs. m/z 992.95 (2+)

Glycan: 552.22 Da (GlcNAc$_2$Fuc$_1$)

Obs. [M+H]$^+$ = 1984.90 Da
Calc. [M+H]$^+$ = 1984.88 Da

RT: 42 min

Glycoform #4

\begin{align*}
\text{SCPACPGS} & \text{NITIR} \\
& \text{y}_8^{1+} + \text{GlcNAc} \\
& \text{y}_{11}^{1+} + \text{GlcNAc} \\
& \text{y}_{11}^{1+} + \text{GlcNAc}_2\text{Fuc}_1
\end{align*}
Human myeloperoxidase (P05164)
SCPACPGS\textsubscript{NITIR} (Asn323 peptide)
Obs. \textit{m/z} 1000.93 (2+)
Glycan: 568.21 Da (GlcNAc\textsubscript{2}Hex\textsubscript{1})
Obs. [M+H]\textsuperscript{+} = 2000.86 Da
Calc. [M+H]\textsuperscript{+} = 2000.88 Da
RT: 42 min
Human myeloperoxidase (P05164)
SCPACPGS\text{NITIR} (Asn323 peptide)
Obs. \(m/z\) 1073.97 (2+)
Glycan: 714.27 Da (GlcNAc\text{2}Hex\text{1}Fuc\text{1})
Obs. [M+H]\(^+\) = 2146.94 Da
Calc. [M+H]\(^+\) = 2146.94 Da
RT: 42 min
Human myeloperoxidase (P05164)
SCPACPGS\textit{NITIR} (Asn323 peptide)

Obs. \(m/z\) 1081.98 (2+)
Glycan: 730.26 Da (GlcNAc\textsubscript{2}Hex\textsubscript{2})
Obs. \([M+H]^+\) = 2162.96 Da
Calc. \([M+H]^+\) = 2162.93 Da

RT: 40 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. m/z 1155.49 (2+)
Glycan: 876.32 Da (GlcNAc₂Hex₂Fuc₁)
Obs. [M+H]⁺ = 2308.98 Da
Calc. [M+H]⁺ = 2308.99 Da
RT: 42 min

Glycoform #8
SCPACPGSNITIR

Obs. m/z 1155.49 (2+)
Glycan: 876.32 Da (GlcNAc₂Hex₂Fuc₁)
Obs. [M+H]⁺ = 2308.98 Da
Calc. [M+H]⁺ = 2308.99 Da
RT: 42 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. \( m/z \) 775.64 (3+)
Glycan: 892.3172 Da (\( \text{GlcNAc}_2\text{Hex}_3 \))
Obs. \([\text{M+H}]^+ = 2324.92 \) Da
Calc. \([\text{M+H}]^+ = 2324.98 \) Da
RT: 39 min
Human myeloperoxidase (P05164)  
SCPACPGS\text{N}ITIR (Asn323 peptide)  
Obs. \( m/z \) 1236.03 (2+)  
Glycan: 1038.38 Da (GlcN_{2}Hex_{3}Fuc_{1})  
Obs. [M+H]\(^{+}\) = 2471.06 Da  
Calc. [M+H]\(^{+}\) = 2471.04 Da  
RT: 42 min
Human myeloperoxidase (P05164)
SCPACPGS\textbf{N}ITIR (Asn323 peptide)

Obs. \( m/z \) 1244.02 (2+)
Glycan: 1054.37 Da (GlcNAc\textsubscript{2}Hex\textsubscript{4})

Obs. \([M+H]^+ = 2487.04 \text{ Da}\)
Calc. \([M+H]^+ = 2487.04 \text{ Da}\)

RT: 42 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. $m/z$ 884.03 (3+)
Glycan: 1216.4 Da (GlcNAc$_2$Hex$_5$)
Obs. [M+H]$^+$ = 2650.09 Da
Calc. [M+H]$^+$ = 2649.09 Da
Off-by-X (Byonic) : 1
RT: 40 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. m/z 937.37 (3+)
Glycan: 1378.48 Da (GlcNAc$_2$Hex$_6$)
Obs. [M+H]$^+$ = 2810.11 Da
Calc. [M+H]$^+$ = 2811.14 Da
Off-by-X (manual) : -1
RT: 40 min
Human myeloperoxidase (P05164)
SCPACPGS\textit{NITIR} (Asn323 peptide)
Obs. $m/z$ 1487.60 (2+)
Glycan: 1540.53 Da (GlcNAc$_2$Hex$_7$)
Obs. [M+H]$^+$ = 2974.20 Da
Calc. [M+H]$^+$ = 2973.20 Da
Off-by-X (manual) : 1
RT: 40 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. m/z 1527.08 (2+)
Glycan: 1700.528 Da \((\text{GlcNAc}_2\text{Hex}_7\text{Phos}_1)\)
Obs. \([\text{M+H}]^+\) = 3053.16 Da
Calc. \([\text{M+H}]^+\) = 3053.20 Da
RT: 41 min
Human myeloperoxidase (P05164)
SCPACPGSN\textbf{N}ITIR (Asn323 peptide)
Obs. $m/z$ 1567.57 (2+)
Glycan: 1700.53 Da (GlcNAc$_2$Hex$_7$Phos$_2$)
Obs. $[M+H]^+ = 3134.14$ Da
Calc. $[M+H]^+ = 3133.20$ Da
Off-by-X (manual) : 1
RT: 44 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. m/z 1568.07 (2+)
Glycan: 1702.58 Da (GlcNAc\textsubscript{2}Hex\textsubscript{8})
Obs. [M+H]\textsuperscript{+} = 3135.14 Da
Calc. [M+H]\textsuperscript{+} = 3135.25 Da
RT: 40 min
Glycoform #18

Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. \( m/z \) 1000.06 (3+)
Glycan: 1565.56 Da (GlcNAc\(_3\)Hex\(_5\)Fuc\(_1\))
Obs. \([M+H]^+\) = 2998.18 Da
Calc. \([M+H]^+\) = 2998.23 Da
RT: 40 min
Human myeloperoxidase (P05164)
SCPACPGS\textbf{N}ITIR (Asn323 peptide)
Obs. \( m/z \) 994.39 (3+)
Glycan: 1548.55 Da (\( \text{GlcNAc}_3\text{Hex}_4\text{NeuAc}_1 \))
Obs. \( [M+H]^+ = 2981.17 \) Da
Calc. \( [M+H]^+ = 2981.21 \) Da
RT: 43 min

Glycoform #19
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. m/z 1563.60 (2+)
Glycan: 1694.6 Da (\(\text{GlcNAc}_3\text{Hex}_4\text{NeuAc}_1\text{Fuc}_1\))
Obs. [M+H]+ = 3126.20 Da
Calc. [M+H]+ = 3127.27 Da
Off-by-X (manual): 1
RT: 44 min
Human myeloperoxidase (P05164)
SCPACPGSNITIR (Asn323 peptide)
Obs. m/z 1110.81 (2+)
Glycan: 1897.68 Da
(GlcNAc$_4$Hex$_4$NeuAc$_1$Fuc$_1$)
Obs. [M+H]$^+$ = 3330.43 Da
Calc. [M+H]$^+$ = 3330.35 Da
RT: 42 min

Glycoform #21

SCPACPGSNITIR
Human myeloperoxidase (P05164)
SCPACPGS\textbf{N}ITIR (Asn323 peptide)
Obs. $m/z$ 1261.84 (3+)
Glycan: 2350.83 Da ($\text{GlcNAc}_4\text{Hex}_5\text{NeuAc}_2\text{Fuc}_1$)
Obs. $[\text{M+H}]^+ = 3783.52$ Da
Calc. $[\text{M+H}]^+ = 3783.50$ Da
RT: 44 min

Glycoform #22
Supplementary Data S3B

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn355 identified from the analysis of the unenriched peptide mixtures of nMPO.
Human myeloperoxidase (P05164)
NLR\textbf{N}MSNQLGLLLAVNQR (Asn355 peptide)
Obs. $m/z$ 653.33 (3+)
Obs. $[\text{M+H}]^+ = 1957.99$ Da
Calc. $[\text{M+H}]^+ = 1957.04$ Da
RT: 59 min

Non-glycosylated peptide

y_1^{1+} y_2^{1+} y_3^{1+} y_4^{1+} y_5^{1+} y_6^{1+} y_8^{1+} y_9^{1+} b_1^{1+} b_{10}^{1+}
Human myeloperoxidase (P05164)
NLRNMSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 1080.57 (2+)
Glycan: 203.08 Da (GlcNAc₁)
Obs. [M+H]⁺ = 2160.14 Da
Calc. [M+H]⁺ = 2160.11 Da
RT: 54 min

Glycoform #1

N L R N M S N Q L
y8
G
y7
L L
y5
A
y4
V N
y2
Q
y1
R

y8
175.12
303.18
400.19
516.29
587.39
642.32
813.49
988.54

y7
1+

y5
1+

y4
1+

y3
1+

y2
1+

y1
2+
Glycoform #2

Human myeloperoxidase (P05164)
NLRNMSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 1182.10 (2+)
Glycan: 406.16 Da (GlcNAc$_2$)
Obs. [M+H]$^+$ = 2363.20 Da
Calc. [M+H]$^+$ = 2363.20 Da
RT: 56 min
Human myeloperoxidase (P05164)
NLRNMSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 842.76 (3+)
Glycan: 568.21 Da (GlcNAc$_2$Hex$_1$)
Calc. [M+H]$^+$ = 2525.25 Da
Off-by-X (Manual) : 1
RT: 53 min
Human myeloperoxidase (P05164)
NLRNMSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 896.44 (3+)
Glycan: 730.26 Da (GlcNAc$_2$Hex$_2$)
Obs. [M+H]$^+$ = 2687.32 Da
Calc. [M+H]$^+$ = 2687.30 Da
RT: 52 min
Human myeloperoxidase (P05164) NLRNMSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 950.79 (3+)
Glycan: 892.3172 Da (GlcNAc₂Hex₃)
Obs. [M+H]⁺ = 2850.37 Da
Calc. [M+H]⁺ = 2849.36 Da
Off-by-X (manual) : 1
RT: 53 min

Glycoform #5
Human myeloperoxidase (P05164)
NLR\textsubscript{N}M\textsubscript{S}N\textsubscript{Q}L\textsubscript{G}L\textsubscript{L}A\textsubscript{V}N\textsubscript{Q}R (Asn355 peptide)
Obs. \(m/z\) 753.86 (4+)
Glycan: 1054.37 Da (GlcNAc\textsubscript{2}Hex\textsubscript{4})
Obs. \([\text{M+H}]^+ = 3012.44\) Da
Calc. \([\text{M+H}]^+ = 3011.41\) Da
Off-by-X (manual) : 1
RT: 55 min

Glycoform #6

\text{NLRNMSNQLGLLAVNQR}\ (\text{Asn355 peptide})
\text{Obs. \(m/z\) 753.86 (4+)}
\text{Glycan: 1054.37 Da (GlcNAc\textsubscript{2}Hex\textsubscript{4})}
\text{Obs. \([\text{M+H}]^+ = 3012.44\) Da}
\text{Calc. \([\text{M+H}]^+ = 3011.41\) Da}
\text{Off-by-X (manual) : 1}
\text{RT: 55 min}
Human myeloperoxidase (P05164)
NLR
MSNQLGLLAVNQR (Asn355 peptide)

Obs. \( m/z \) 794.37 (4+)
Glycan: 1216.4 Da (GlcNAc\(_2\)Hex\(_5\))
Obs. \([M+H]^+\) = 3174.48 Da
Calc. \([M+H]^+\) = 3173.46 Da
Off-by-X (manual) : 1
RT: 53 min

Glycoform #7

\( Y_1^{2+} \)
\( Y_2^{2+} \)
\( Y_3^{1+} \)
\( Y_4^{1+} \)
\( Y_5^{1+} \)
\( Y_6^{1+} \)
\( Y_7^{1+} \)
\( Y_8^{1+} \)

\( Y_1^{2+} \) + GlcNAc
\( Y_2^{2+} \) + GlcNAc
\( Y_3^{1+} \) + Hex
\( Y_4^{1+} \) + GlcNAc
\( Y_5^{1+} \) + GlcNAc
\( Y_6^{1+} \) + GlcNAc
\( Y_7^{1+} \) + GlcNAc
\( Y_8^{1+} \) + GlcNAc

RT: 53 min
Human myeloperoxidase (P05164)
NLRNMSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 834.89 (4+)
Glycan: 1378.48 Da (GlcNAc$_2$Hex$_6$)
Obs. [M+H]$^+$ = 3336.56 Da
Calc. [M+H]$^+$ = 3335.51 Da
Off-by-X (manual) : 1
RT: 53 min

Glycoform #8

\[
\text{NLRNMSNQLGLLAVNQR}
\]

\[
y_8 \quad y_7 \quad y_6 \quad y_5 \quad y_4 \quad y_3
\]

1080.56
204.09
138.05
145.05
587.33
870.52
1048.56
1080.56
145.05
587.33
870.52
1048.56

RT: 53 min
Human myeloperoxidase (P05164)
NLRNMSNQLGLLAVNQR (Asn355 peptide)

Obs. \( m/z \) 875.65 (+4)
Glycan: 1540.53 Da (GlcNAc\(_7\)Hex\(_7\))
Obs. \([M+H]^+\) = 3499.6 Da
Calc. \([M+H]^+\) = 3497.57 Da
Off-by-X (manual) : 2
RT: 53 min

Glycoform #9

Y\(_1^{2+}\) 1081.06
Y\(_2^{1+}\) 204.09
Y\(_3^{1+}\) 303.16
Y\(_4^{1+}\) 366.14
Y\(_5^{1+}\) 587.33
Y\(_6^{1+}\) 700.41
Y\(_7^{1+}\) 913.50
Y\(_8^{1+}\) 1049.06

Y\(_1^{2+}\) + GlcNAc
Hex
Y\(_2^{1+}\) + GlcNAc
Hex\(_2\)
Y\(_3^{1+}\) + GlcNAc
Hex\(_3\)

RT: 53 min
Glycoform #10

NLR\text{\textsuperscript{\text{N}}}M\text{\textsuperscript{\text{S}}}N\text{\textsuperscript{\text{Q}}}L\text{\textsuperscript{\text{G}}}L\text{\textsuperscript{\text{L}}}A\text{\textsuperscript{\text{V}}}N\text{\textsuperscript{\text{Q}}}R

RT: 53 min

Human myeloperoxidase (P05164)
NLR\text{\textsuperscript{\text{N}}}M\text{\textsuperscript{\text{S}}}N\text{\textsuperscript{\text{Q}}}L\text{\textsuperscript{\text{G}}}L\text{\textsuperscript{\text{L}}}A\text{\textsuperscript{\text{V}}}N\text{\textsuperscript{\text{Q}}}R (Asn355 peptide)

Obs. \textit{m/z} 1221.21 (3+)
Glycan: 1702.58 Da (GlcNAc\textsubscript{2}Hex\textsubscript{8})
Obs. [M+H]\textsuperscript{+} = 3661.63 Da
Calc. [M+H]\textsuperscript{+} = 3659.62 Da
Off-by-X (manual) : 2
RT: 53 min
Supplementary Data S3C

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn391 identified from the analysis of unenriched peptide mixtures of nMPO.
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. $m/z$ 708.69 (+3)
Obs. $[M+H]^+$ = 2124.07 Da
Calc. $[M+H]^+$ = 2124.05 Da
RT: 81 min

Non-glycosylated peptide

ALLPFDNLHDDPCLLTNR

Obs. $m/z$ 708.69 (+3)
Obs. $[M+H]^+$ = 2124.07 Da
Calc. $[M+H]^+$ = 2124.05 Da
RT: 81 min
Human myeloperoxidase (P05164)  
ALLPFDNLHDDPCLLTN\(\text{N}\)R (Asn391 peptide)  
Obs. \(m/z\) 1164.03 (2+)  
Glycan: 203.08 Da (GlcNAc\(_1\))  
Obs. [M+H]\(^+\) = 2327.06 Da  
Calc. [M+H]\(^+\) = 2327.13 Da  
RT: 75 min  

Glycoform #1
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. m/z 844.03 (3+)
Glycan: 406.16 Da (GlcNAc$_2$)
Obs. [M+H]$^+$ = 2530.09 Da
Calc. [M+H]$^+$ = 2530.21 Da
RT: 80 min
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. m/z 898.09 (3+)
Glycan: 568.21 Da (GlcNAc₂Hex₁)
Obs. [M+H]+ = 2692.18 Da
Calc. [M+H]+ = 2692.26 Da
RT: 77 min
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLT\textsubscript{N}R (Asn391 peptide)
Obs. $m/z$ 952.45 (3+)
Glycan: 730.26 Da (GlcNAc$_2$Hex$_2$)
Obs. [M+H]$^+$ = 2855.35 Da
Calc. [M+H]$^+$ = 2854.31 Da
Off-by-X (manual) : 1
RT: 78 min

Glycoform #4
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. $m/z$ 1000.79 (3+)
Glycan: 876.32 Da (GlcNAc$_2$Hex$_2$Fuc$_1$)
Obs. [$M+H]^+ = 3000.37$ Da
Calc. [$M+H]^+ = 3000.37$ Da
RT: 77 min

Glycoform #5

A L L P F D N L | H D D | P C L L T N R

$y_7^{1+}$ +
GlcNAc

$y_7^{1+}$

$Y_1^{2+}$
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLT\textsuperscript{NR} (Asn391 peptide)
Obs. $m/z$ 1006.46 (3+)
Glycan: 892.3172 Da (GlcNAc$\textsubscript{2}$Hex$\textsubscript{3}$)
Obs. [M+H]$^+$ = 3017.38 Da
Calc. [M+H]$^+$ = 3016.37 Da
Off-by-X (manual) : 1
RT: 76 min

Glycoform #6

\[
\begin{align*}
\text{ALLPFDNLHDDPCLLT}&\text{NR} \\
y_3^{1+} + \text{GlcNAc} &
y_4^{1+} + \text{GlcNAc} &
y_7^{1+} + \text{GlcNAc} \\
&
\end{align*}
\]
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. \( m/z \) 1054.87 (3+)
Glycan: 1038.38 Da (GlcNAc\(_2\)Hex\(_3\)Fuc\(_1\))
Obs. \([M+H]^+\) = 3162.61 Da
Calc. \([M+H]^+\) = 3162.43 Da
RT: 79 min

Glycoform #7

\[
\text{ALLPFDNLHDDPCLLTNR}
\]

\( y_{10}^{+1} + \text{GlcNAc}\)
\( y_{7}^{+1} + \text{GlcNAc}\)
\( y_{4}^{+1} + \text{GlcNAc}\)
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. m/z 1589.72 (2+)
Glycan: 1054.37 Da (GlcNAc$_2$Hex$_4$)
Obs. [M+H]$^+$ = 3178.44 Da
Calc. [M+H]$^+$ = 3178.42 Da
RT: 77 min

Glycoform #8

\[ \gamma_7^{1+} + \text{GlcNAc} \]

\[ \gamma_{10}^{1+} + \text{GlcNAc} \]

\[ \gamma_{11}^{1+} + \text{GlcNAc} \]

\[ \gamma_{12}^{1+} + \text{GlcNAc} \]

\[ \gamma_{15}^{1+} + \text{GlcNAc} \]

\[ \gamma_{1}^{1+} \]
Human myeloperoxidase (P05164)

ALLPFDNLHDDPCLLTN (Asn391 peptide)

Obs. $m/z$ 1108.49 (3+)

Glycan: 1221.46 Da (GlcNAc$_2$Hex$_4$Fuc$_1$)

Obs. $\left[M+H\right]^+ = 3323.47$ Da

Calc. $\left[M+H\right]^+ = 3324.48$ Da

Off-by-X (manual) : 1

RT: 80 min

Glycoform #9

$\gamma_{y10}^{1+}$ + GlcNAc

$\gamma_{y7}^{1+}$ + GlcNAc

$\gamma_{y4}^{1+}$ + GlcNAc

$\gamma_{y3}^{1+}$ + GlcNAc

$\gamma_{y10}^{1+}$ + GlcNAc
**Human myeloperoxidase (P05164)**

ALLPFDNLHDDPCLLTNR (Asn391 peptide)

Obs. \( m/z \) 1671.23 (2+)

Glycan: 1216.42 Da (GlcNAc\(_2\)Hex\(_5\))

Obs. \([M+H]^+\) = 3341.46 Da

Calc. \([M+H]^+\) = 3340.47 Da

Off-by-X (manual) : 1

RT: 76 min
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLT\textbf{N}R (Asn391 peptide)
Obs. \textit{m/z} 1162.84 (3+)
Glycan: 1362.48 Da (Glc\textit{N}Ac$_2$\textit{Hex}_5\textit{Fuc}_1$
Obs. [M+H]$^+$ = 3486.52 Da
Calc. [M+H]$^+$ = 3486.53 Da
RT: 80 min
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. m/z 1168.85 (3+)
Glycan: 1378.48 Da (GlcNAc$_2$Hex$_6$)
Obs. [M+H]$^+$ = 3504.55 Da
Calc. [M+H]$^+$ = 3502.53 Da
Off-by-X (manual) : 2
RT: 77 min

Glycoform #12
ALLPFDNLHDDPCLLTNR

RT: 76.90 AV: 1 NL: 8.76E6
T: FTMS + p NSI d Full ms2 1168.8485@hcd30.00 [110.0000-3595.0000]
Human myeloperoxidase (P05164) ALLPFDNLHDDPCLLTNR (Asn391 peptide) Obs. m/z 1222.87 (3+)
Glycan: 1540.53 Da (GlcNAc$_2$Hex$_7$) Obs. [M+H]$^+$ = 3666.61 Da Calc. [M+H]$^+$ = 3664.59 Da Off-by-X (manual) : 2 RT: 74 min
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. \( m/z \) 1276.89 (3+)
Glycan: 1702.58 Da (GlcNAc\(_2\)Hex\(_8\))
Obs. \([M+H]^+ = 3828.67 \text{ Da}
Calc. \([M+H]^+ = 3826.63 \text{ Da}
Off-by-X (manual) : 2
RT: 74 \text{ min}

Glycoform #14

\[\text{ALL}[PFDNLHDDPCLLTNR}\]

\(Y_7^{1+} + \text{GlcNAc}\)
\(1164.57\)
\(Y_{10}^{1+} + \text{GlcNAc}\)
\(1251.60\)
\(Y_{12}^{1+} + \text{GlcNAc}\)
\(1443.65\)
\(Y_{15}^{1+}\)
\(2030.90\)
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. m/z 1225.21 (3+)
Glycan: 1548.55 Da (GlcNAc₃Hex₄NeuAc₁)
Obs. [M+H]⁺ = 3673.63 Da
Calc. [M+H]⁺ = 3672.59 Da
Off-by-x (manual) : 1
RT: 79 min

Glycoform #15

\[ \text{ALLPFDNLHDDPCLLTNR} \]
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. m/z 1279.56 (3+)
Glycan: 1710.60 Da (GlcNAc$_3$Hex$_5$NeuAc$_1$)
Obs. [M+H]$^+$ = 3836.68 Da
Calc. [M+H]$^+$ = 3834.65 Da
Off-by-X (manual) : 2
RT: 78 min

Glycoform #16

A L L
y$_{15}$
P F D N L H D D P C L L T N R

y$_1^{1+}$ + GlcNAc

y$_1^{2+}$ + GlcNAc
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. \( m/z \) 1333.58 (3+)
Glycan: 1872.65 Da (GlcNAc\(_3\)Hex\(_6\)NeuAc\(_1\))
Obs. \([M+H]^+ = 3998.74\) Da
Calc. \([M+H]^+ = 3996.70\) Da
Off-by-X (Manual) : 2
RT: 78 min
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. $m/z$ 1298.91 (3+)
Glycan: 1768.64 Da (GlcNAc$_4$Hex$_5$Fuc$_1$)
Obs. [M+H]$^+ = 3894.73$ Da
Calc. [M+H]$^+ = 3892.69$ Da
Off-by-X (manual) : 2
RT: 74 min
Supplementary Data S3D

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn483 identified from the analysis of the unenriched peptide mixtures of nMPO.
Human myeloperoxidase (P05164)
SYNDSVDPR (Asn483 peptide)
Obs. m/z 964.89 (2+)
Glycan: 897.35 Da (GlcNAc₂Hex₂Fuc₁)
Obs. [M+H]⁺ = 1928.79 Da
Calc. [M+H]⁺ = 1928.79 Da
RT: 28 min
Human myeloperoxidase (P05164)
SYNDSVDPR (Asn483 peptide)
Obs. \( m/z \) 972.89 (2+)
Glycan: 913.35 Da (GlcNAc\(_2\)Hex\(_3\))
Obs. \([M+H]^+\) = 1944.78 Da
Calc. \([M+H]^+\) = 1944.78 Da
RT: 27 min
Human myeloperoxidase (P05164)
SYN\(NdSVDPR\) (Asn483 peptide)
Obs. \(m/z\) 1045.92 (2+)
Glycan: 1059.40 Da (GlcNAc\(_2\)Hex\(_3\)Fuc\(_1\))
Obs. \([M+H]^+\) = 2090.84 Da
Calc. \([M+H]^+\) = 2090.83 Da
RT: 28 min
Human myeloperoxidase (P05164) 
SYNDSDVPR (Asn483 peptide) 
Obs. m/z 1126.89 (2+) 
Glycan: 1221.46 Da (GlcNAc$_2$Hex$_4$Fuc$_1$) 
Obs. [M+H]$^+$ = 2252.78 Da 
Calc. [M+H]$^+$ = 2252.89 Da 
RT: 27 min
Human myeloperoxidase (P05164)

SYNSVDPR (Asn483 peptide)

Obs. m/z 1135.45 (2+)

Glycan: 1216.423 Da (GlcNAc$_2$Hex$_5$)

Obs. [M+H]$^+$ = 2269.90 Da

Calc. [M+H]$^+$ = 2268.89 Da

Off-by-X (manual) : 1

RT: 25 min
Human myeloperoxidase (P0516)

SY
N
D
S
V
D
P
R
(Asn483 peptide)

Glycoform #6

Obs. m/z 1208.48 (2+)

Glycan: 1362.481 Da (GlcNAc₂Hex₅Fuc₁)

Obs. [M+H]^+ = 2415.96 Da

Calc. [M+H]^+ = 2414.95 Da

Off-by-X (manual) : 1

RT: 27 min
Human myeloperoxidase (P05164)
SYNDSVDPR (Asn483 peptide)
Obs. \( m/z \) 1147.46 (2+)
Glycan: 1241.46 Da (GlcNAc\(_3\)Hex\(_3\)Fuc\(_1\))
Obs. \([M+H]^+\) = 2293.92 Da
Calc. \([M+H]^+\) = 2293.92 Da
RT: 27 min
Human myeloperoxidase (P05164)
SYNDSVDPR (Asn483 peptide)
Obs. m/z 1374.04 (2+)
Glycan: 1694.6 Da
(GlcNAc$_3$Hex$_4$Fuc$_1$NeuAc$_1$)
Obs. [M+H]$^+$ = 2747.08 Da
Calc. [M+H]$^+$ = 2747.06 Da
RT: 29 min
Human myeloperoxidase (P05164) SYNSVDSPR (Asn483 peptide)
Obs. m/z 1024.39 (3+)
Glycan: 2039.74 Da (GlcNAc$_3$Hex$_6$Fuc$_1$NeuAc$_1$)
Obs. [M+H]$^+$ = 3071.17 Da
Calc. [M+H]$^+$ = 3071.17 Da
RT: 28 min
Human myeloperoxidase (P05164)
SYNDSVDPR (Asn483 peptide)
Obs. m/z 1249.50 (2+)
Glycan: 1444.53 Da
(GlcNAc$_4$Hex$_3$Fuc$_1$)
Obs. [M+H]$^+$ = 2498.00 Da
Calc. [M+H]$^+$ = 2497.00 Da
Off-by-X (manual): 1
RT: 27 min
Human myeloperoxidase (P05164)
SYNDSVDPR (Asn483 peptide)
Obs. m/z 1038.41 (+3)
Glycan: 2059.74 Da
(GlcNAc$_4$Hex$_5$Fuc$_1$NeuAc$_1$)
Obs. [M+H]$^+$ = 3113.23 Da
Calc. [M+H]$^+$ = 3112.20 Da
Off-by-X (manual): 1
RT: 28
Human myeloperoxidase (P05164)  
SYNDSVDPR (Asn483 peptide)  
Obs. m/z 984.39 (+3)  
Glycan: 1897.62 Da  
(GlcNAc$_4$Hex$_4$Fuc$_1$NeuAc$_1$)  
Obs. [M+H]$^+$ = 2951.17 Da  
Calc. [M+H]$^+$ = 2950.15 Da  
Off-by-X (manual) : 1  
RT: 28 min
Supplementary Data S3E

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn729 identified from the analysis of unenriched peptide mixtures of nMPO.
Human myeloperoxidase (P05164)
DFVNCSTLPALNLASWR (Asn729 peptide)
Obs. m/z 982.99 (2+)
Obs. [M+H]^+ = 1964.98 Da
Calc. [M+H]^+ = 1963.95 Da
Off-by-X (manual) : 1
RT: 93 min

Non-glycosylated peptide
Human myeloperoxidase (P05164)
DFV\textbf{N}CSTLPALNLASWR (Asn729 peptide)
Obs. \( m/z \) 1084.53 (2+)
Glycan: 203.08 Da (GlcNAc\(_1\))
Obs. \([M+H]^+\) = 2168.06 Da
Calc. \([M+H]^+\) = 2167.03 Da
Off-by-X (manual) : 1
RT: 91 min

Glycoform #1

\begin{align*}
\text{Obs.} \quad \text{m/z} & \quad 1084.03 \\
\text{Calc.} \quad \text{m/z} & \quad 1085.03 \\
\text{Off} & \quad \text{by} \\
\text{X} (\text{manual}) & \quad 1
\end{align*}
Human myeloperoxidase (P05164)
DFVNCSTLPALNLASWR (Asn729 peptide)
Obs. m/z 1157.56 (2+)
Glycan: 349.14 Da (GlcNAc1Fuc1)
Obs. [M+H]+ = 2314.12 Da
Calc. [M+H]+ = 2313.09 Da
Off-by-X (manual) : 1
RT: 91 min
Human myeloperoxidase (P05164)
DFVNCSTLPALNLASWR (Asn729 peptide)
Obs. \( m/z \) 1185.57 (2+)
Glycan: 406.15 Da (GlcNAc\(_2\))
Obs. \([\text{M}+\text{H}]^+\) = 2370.14 Da
Calc. \([\text{M}+\text{H}]^+\) = 2370.11 Da
RT: 90 min

Glycoform #3

\[
\begin{align*}
\text{DFV} & \quad \text{N} \\
\text{C} & \quad \text{S} \\
\text{T} & \quad \text{L} \\
\text{P} & \quad \text{A} \\
\text{L} & \quad \text{N} \\
\text{L} & \quad \text{A} \\
\text{S} & \quad \text{W} \\
\text{R} & \quad \\
\end{align*}
\]
Human myeloperoxidase (P05164)
DFVNCSTLPALNLASWR (Asn729 peptide)
Obs. m/z 1259.10 (2+)
Glycan: 552.22 Da (GlcNAc$_2$Fuc$_1$)
Obs. [M+H]$^+$ = 2517.20 Da
Calc. [M+H]$^+$ = 2516.17 Da
Off-by-X (manual) : 1
RT: 89 min

Glycoform #4
Human myeloperoxidase (P05164)
DFV\text{N}CSTLPALNLASWR (Asn729 peptide)
Obs. \textit{m/z} 1267.1 (2+)
Glycan: 568.21 Da (GlcNAc$_2$Hex$_1$)
Obs. [M+H]$^+$ = 2533.20 Da
Calc. [M+H]$^+$ = 2532.16 Da
Off-by-X (Manual) : 1
RT: 90 min

Glycoform #5

\text{DFV\text{N}CSTLPALNLASWR}
Human myeloperoxidase (P05164)

DFVN CSTLPALNLASWR (Asn729 peptide)

Obs. m/z 893.75 (3+)

Glycan: 714.27 Da (GlcNAc₂Man₁Fuc₁)

Obs. [M+H]⁺ = 2679.25 Da

Calc. [M+H]⁺ = 2678.22 Da

Off-by-X (manual) : 1

RT: 89 min

Glycoform #6
Human myeloperoxidase (P05164)

DFVNCSTLPALNLASWR (Asn729 peptide)

Obs. $m/z$ 899.09 (3+)

Glycan: 730.26 Da ($\text{GlcNAc}_2\text{Hex}_2$)

Obs. $[\text{M+H}]^+ = 2695.27$ Da

Calc. $[\text{M+H}]^+ = 2694.21$ Da

Off-by-X (manual): 1

RT: 88 min
Human myeloperoxidase (P05164)
DFVN\text{CSTLPALNLASWR} (Asn729 peptide)
Obs. \textit{m/z} 947.77 (3+)
Glycan: 876.32 Da (GlcNAc$_2$Hex$_2$Fuc$_1$)
Obs. [M+H]$^+$ = 2841.31 Da
Calc. [M+H]$^+$ = 2840.27 Da
Off-by-X (manual): 1
RT: 89 min

Glycoform #8
Human myeloperoxidase (P05164)

DFV\textsuperscript{N}CSTLPALNLASWR (Asn729 peptide)

Obs. \textit{m/z} 953.44 (3+)

Glycan: 892.3172 Da (GlcNA\textsubscript{c2}Hex\textsubscript{3})

Obs. [M+H]\textsuperscript{+} = 2858.32 Da

Calc. [M+H]\textsuperscript{+} = 2856.27 Da

Off-by-X (manual) : 1

RT: 88 min
Human myeloperoxidase (P05164)

**DFVNCSTLPALNLASWR (Asn729 peptide)**

Obs. $m/z$ 1001.79 (3+)

Glycan: 1038.38 Da (GlcNAc$_2$Hex$_3$Fuc$_1$)

Obs. [M+H]$^+$ = 3003.37 Da

Calc. [M+H]$^+$ = 3002.33 Da

Off-by-X (manual) : 1

RT: 87 min
Human myeloperoxidase (P05164)  
DFVN{N}CSTLPALNLASWR (Asn729 peptide)  
Obs. m/z 1510.67 (2+)  
Glycan: 1054.37 Da (GlcNAc\textsubscript{2}Hex\textsubscript{4})  
Obs. [M+H]\textsuperscript{+} = 3020.34 Da  
Calc. [M+H]\textsuperscript{+} = 3018.32 Da  
Off-by-X (manual) : 2  
RT: 87 min
Human myeloperoxidase (P05164)
DFVNCSTLPALNLASWR (Asn729 peptide)
Obs. m/z 1055.81 (3+)
Glycan: 1221.46 Da (GlcNAc₂Hex₄Fuc₁)
Obs. [M+H]⁺ = 3165.43 Da
Calc. [M+H]⁺ = 3164.38 Da
Off-by-X (manual) : 1
RT: 87 min

Glycoform #12

D F
b₂
V
y₁₄
N
y₁₃
C
y₁₂
b₅
S
y₁₁
T
y₁₀
L
y₉
P
y₈
A
y₇
L
y₆
N L
y₄
A
y₃
S W R
Human myeloperoxidase (P05164)
DFVNCSTLPALNLASWR (Asn729 peptide)
Obs. \( m/z \) 1061.48 (3+)
Glycan: 1216.4 Da (GlcNAc\(_2\)Hex\(_5\))
Obs. [M+H]\(^+\) = 3182.44 Da
Calc. [M+H]\(^+\) = 3180.37 Da
Off-by-X (manual) : 2
RT: 87 min

Glycoform #13

\[
\text{DFVNCSTLPALNLASWR}
\]
Human myeloperoxidase (P05164)
DFVNCSTLPALNLASWR (Asn729 peptide)
Obs. m/z 1664.73 (2+)
Glycan: 1362.481 Da (GlcNAc$_2$Hex$_5$Fuc$_1$)
Obs. [M+H]$^+$ = 3328.46 Da
Calc. [M+H]$^+$ = 3326.43 Da
Off-by-X (manual) : 2
RT: 87 min

Glycoform #14

D F V N
b$_2$
V N
y$_{13}$
C
y$_{12}$
b$_5$
S T
y$_{10}$
L
y$_9$
P A
y$_7$
L
y$_6$
N L A
y$_3$
S W R
Human myeloperoxidase (P05164)
DFV\text{N}CSTLPALNLASWR (Asn729 peptide)
Obs. \textit{m/z} 1115.50 (3+)
Glycan: 1378.48 Da (GlcNAc\textsubscript{2}Hex\textsubscript{6})
Obs. [M+H]\textsuperscript{+} = 3344.50 Da
Calc. [M+H]\textsuperscript{+} = 3342.43 Da
Off-by-X (manual) : 2
RT: 87 min

Glycoform #15
Human myeloperoxidase (P51064)
DFVN\text{CSTLPALNLASWR} (Asn729 peptide)
Obs. \( m/z \) 1220.53 (3+)
Glycan: 1694.6 Da (GlcNAc\textsubscript{3}Hex\textsubscript{4}NeuAc\textsubscript{1}Fuc\textsubscript{1})
Obs. [M+H]\(^+\) = 3659.59 Da
Calc. [M+H]\(^+\) = 3658.55 Da
Off-by-X (manual): 1
RT: 88 min