Structural characterization of biosimilars by HDX and top-down analysis using ECD/ETD-FTMS

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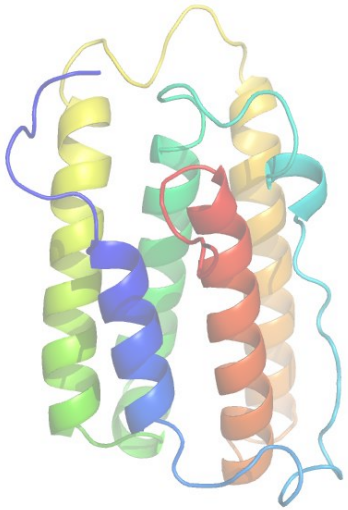


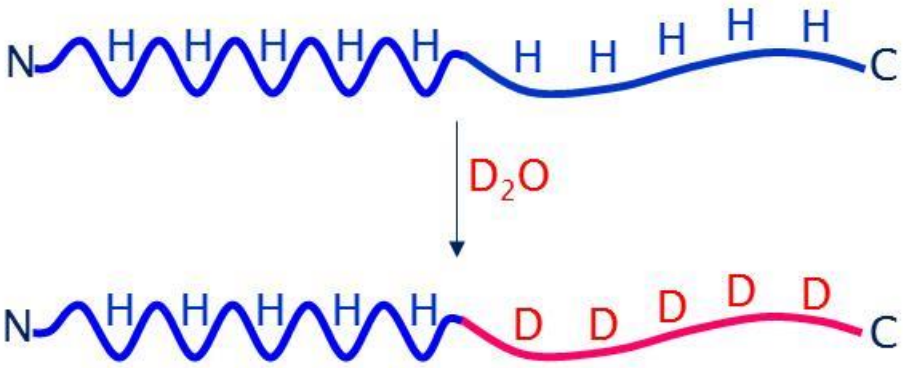
Interferon

Antibody

Talk Overview

1. Introduction of HDX-MS
2. Top-down HDX for biosimilar interferons
3. Top-down HDX for antibodies

Protein amide H/D exchange (HDX)

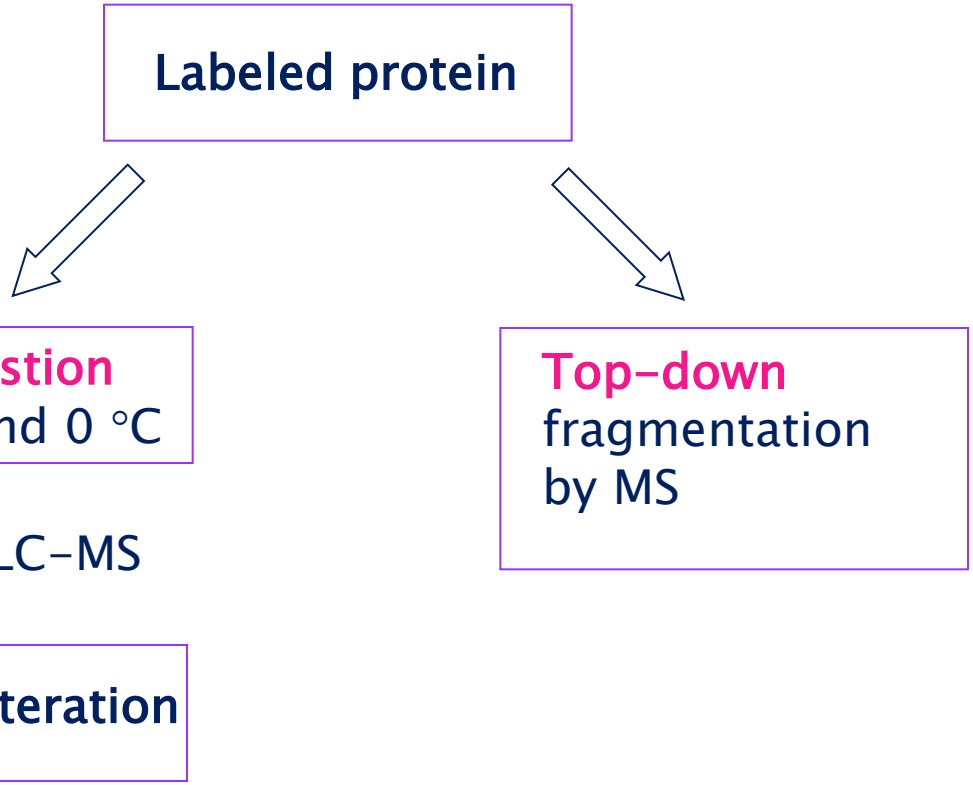


Unlabeled protein

Labeled protein

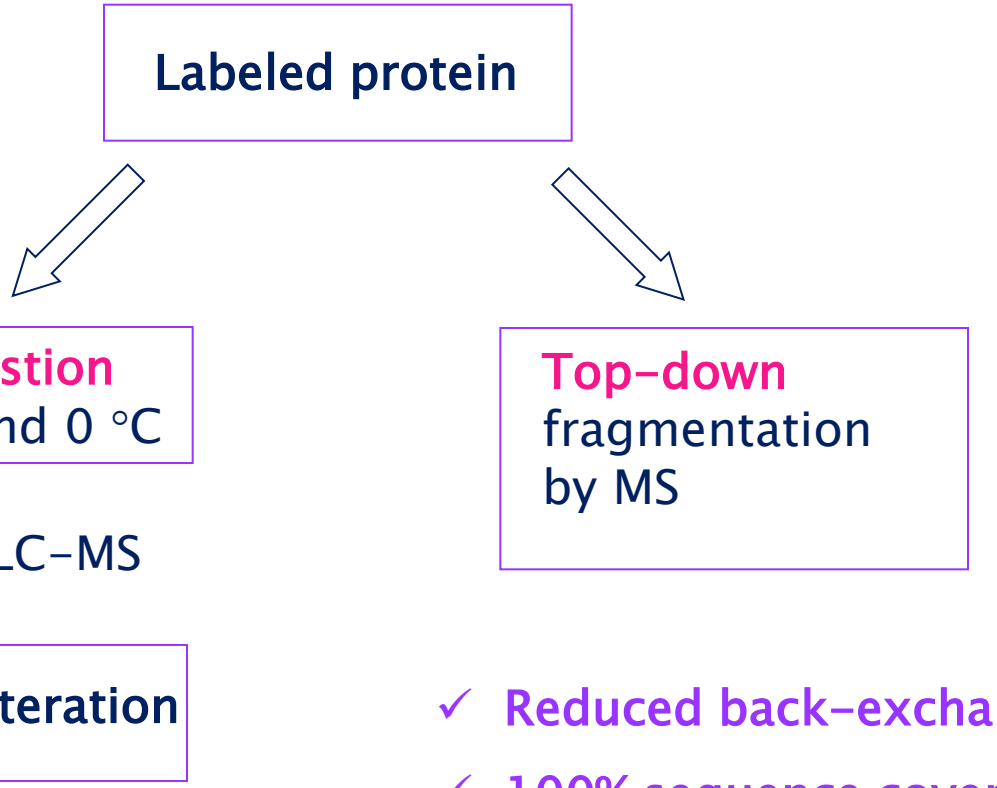
Key to HDX: locate exchanged deuterium atoms

Characterizing protein structure by HDX-MS



Peptide-level resolution
(7 – 15 residues)
High back exchange
(10% – 50%)

Characterizing protein structure by HDX-MS

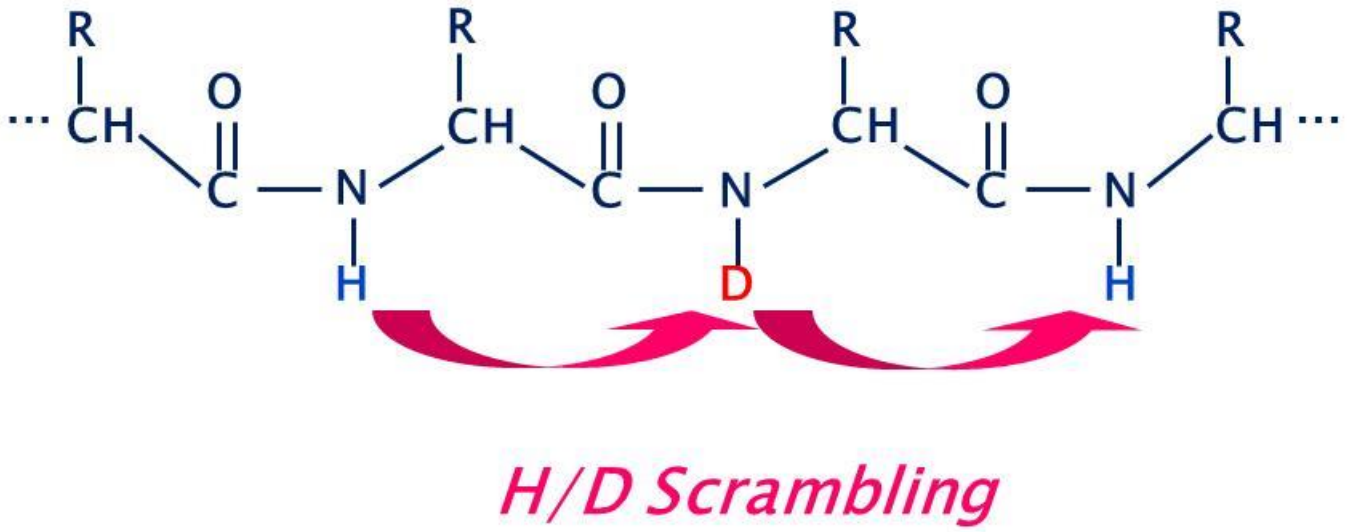


Peptide-level resolution
(7 – 15 residues)
High back exchange
(10% – 50%)

- ✓ Reduced back-exchange
- ✓ 100% sequence coverage
- ✓ Single-residue resolution
- ✓ Isoform-specific analysis

Top-down HDX

Collision-Induced Dissociation (CID)



Top-down HDX

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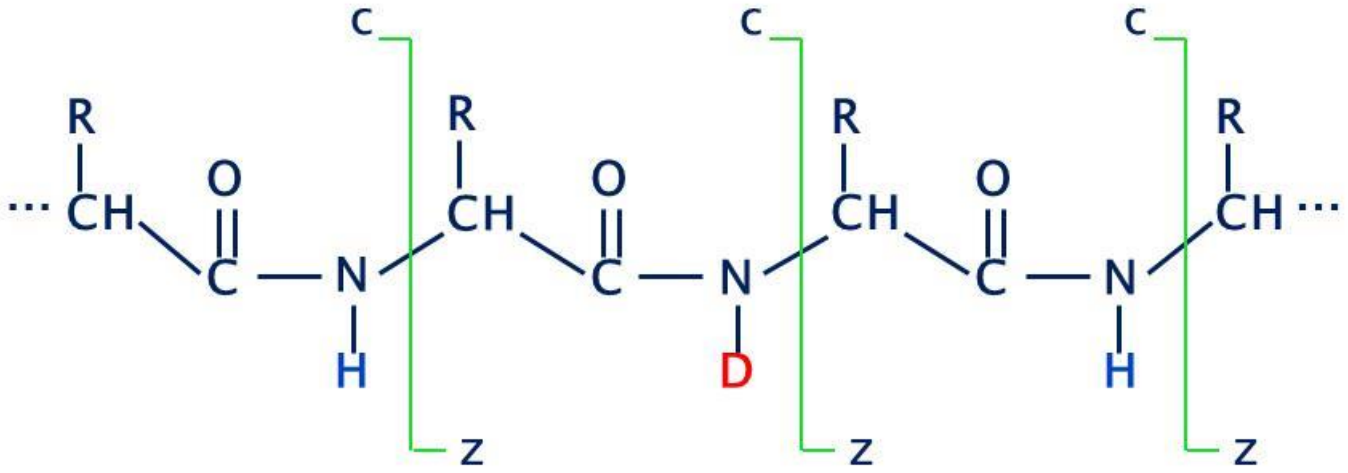
Electron Capture Dissociation (ECD) or Electron Transfer Dissociation (ETD)



Low energy, ultra-fast cleavage

H/D Scrambling test for top-down HDX/ECD

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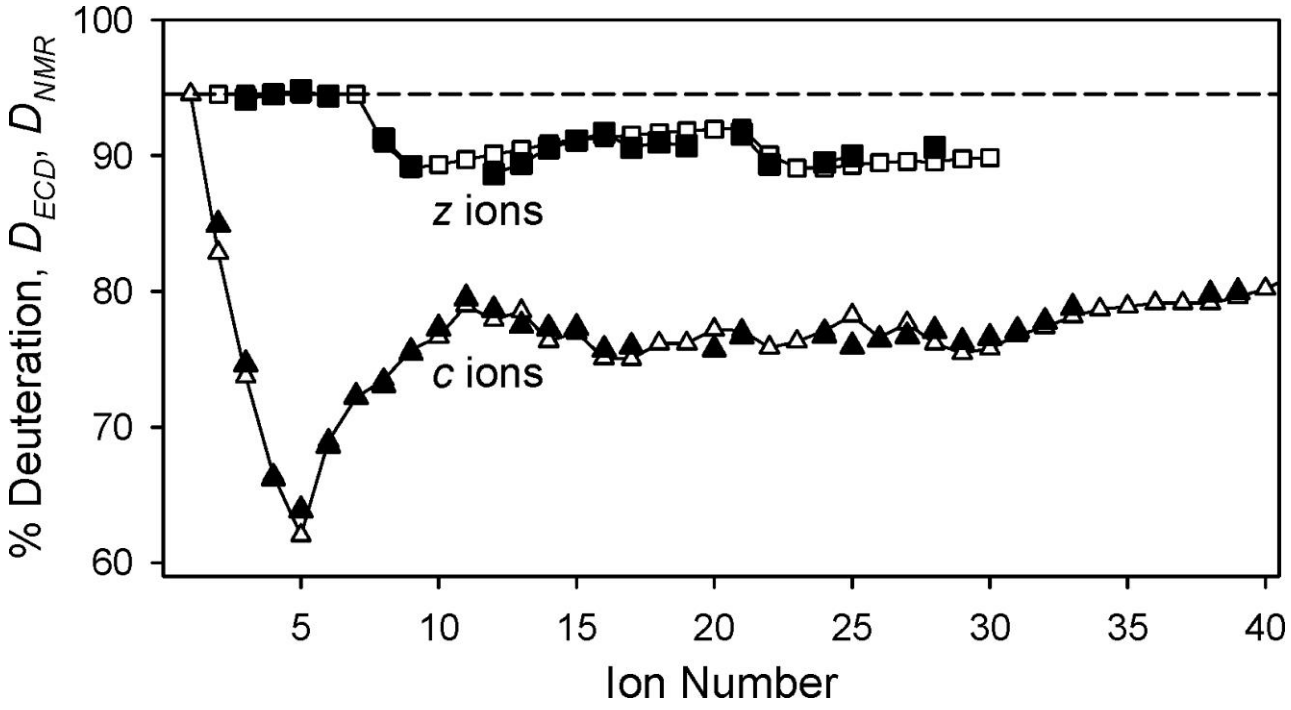
ECD = Electron Capture Dissociation



Solid symbols: D_{ECD}

Open symbols: D_{NMR}

Pan, Han, Borchers, Konermann. *JACS* 130, 11574 (2008)

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Top-down HDX for biosimilar interferons

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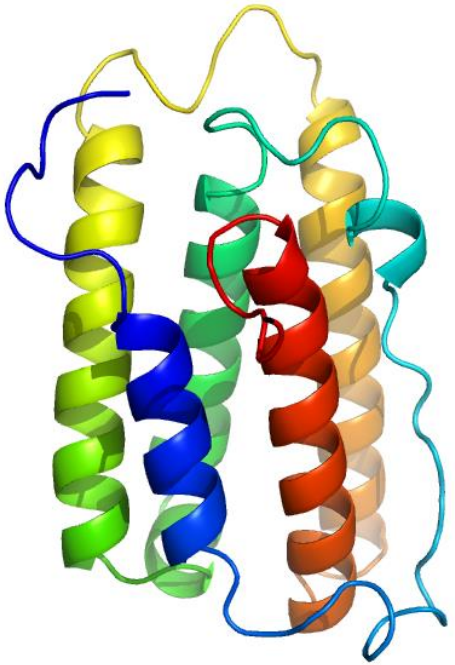
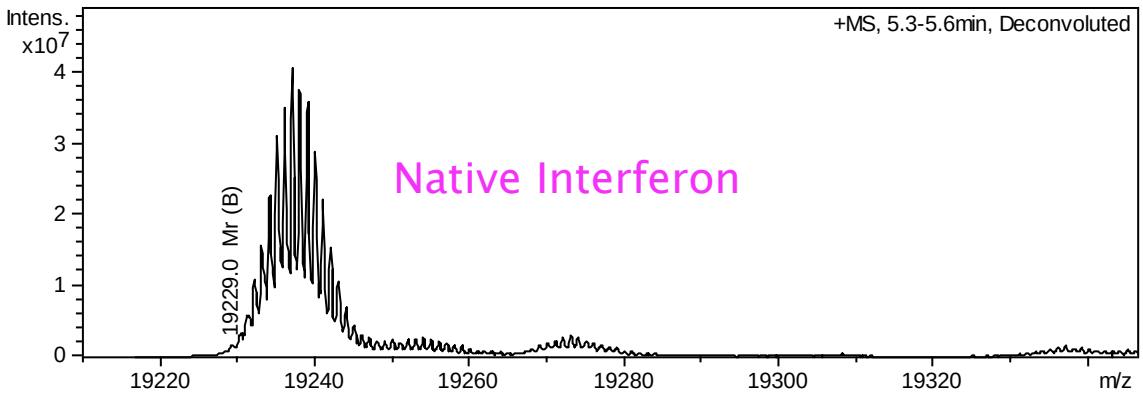
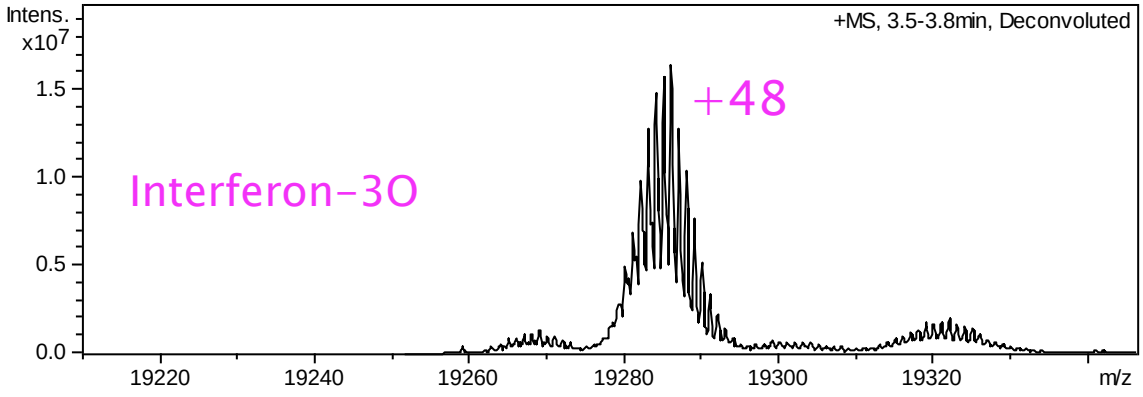
Interferon

- is used for diseases like hepatitis and leukemias
- contains two disulfide bonds
- K23R mutant has similar activity
- contains 5 Met and 2 Trp susceptible to oxidation

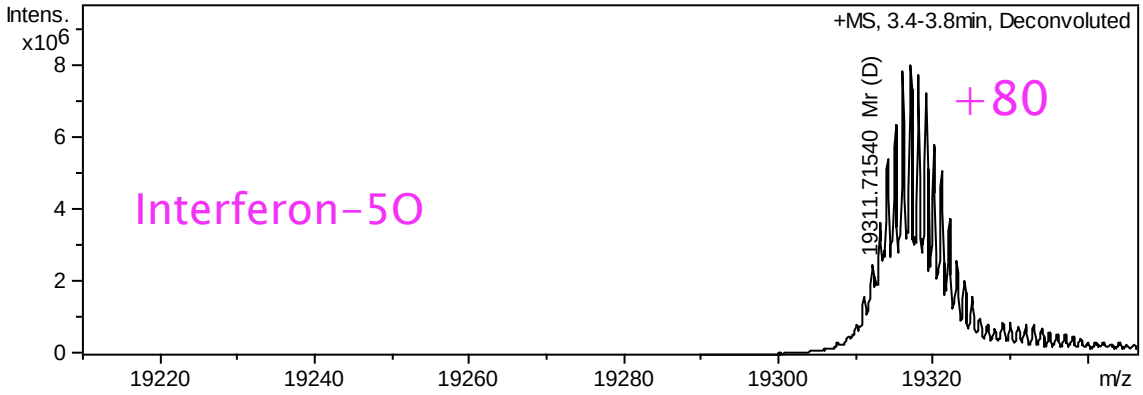


Top-down HDX for biosimilar interferons





2 h oxidation



16 h oxidation

Top-down HDX for biosimilar interferons

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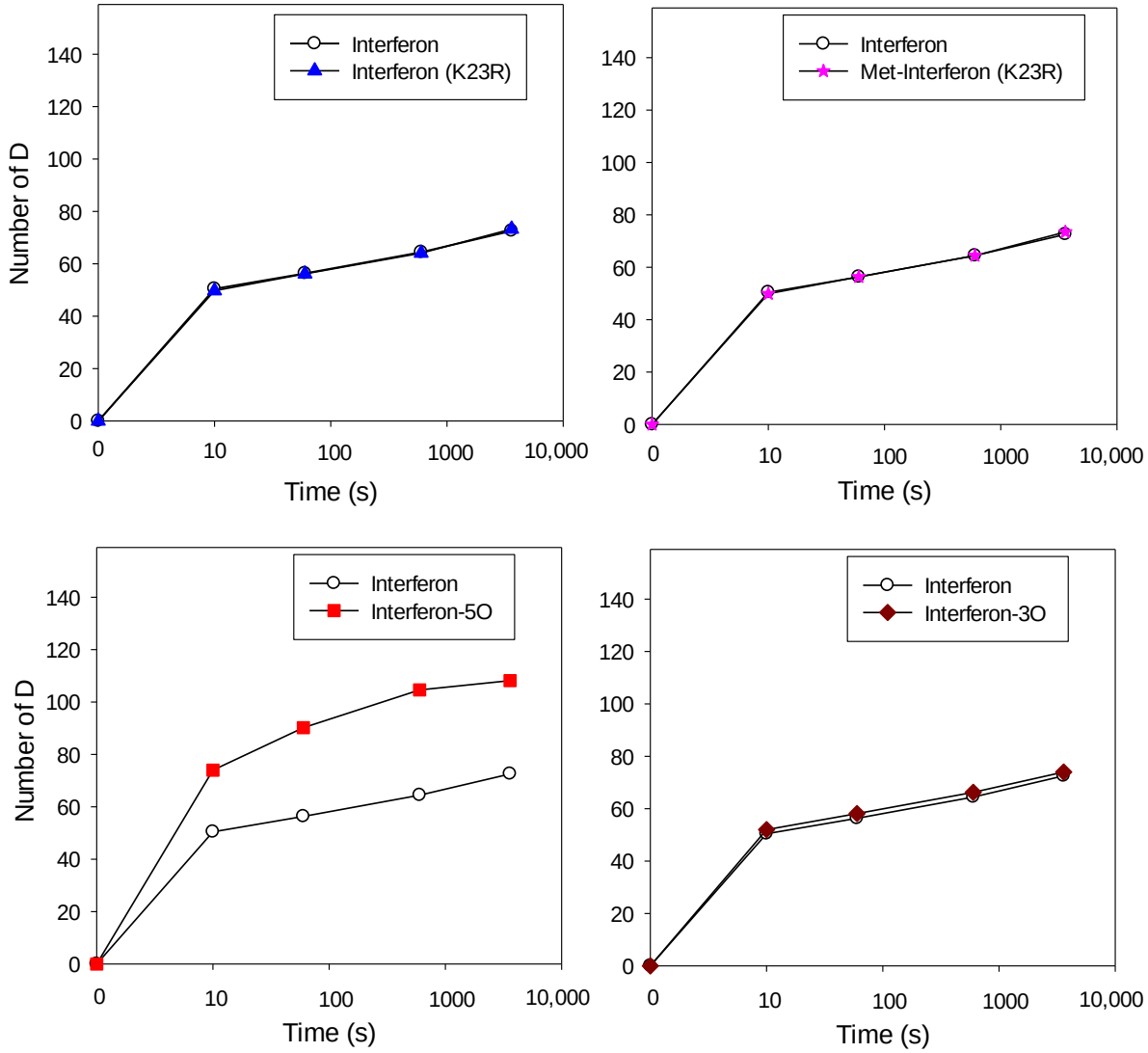
Comparison of HDX behavior between different interferons



Top-down HDX for biosimilar interferons

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Oxidation sites identified by top-down ECD

Interferon-5O:

16 21

CDLPQTHSLG SRRTLMLLAQ MRKISLFSCL KDRHDFGFPQ EEFGNQFQKA

59

ETIPVLHEMI QQIFNLFSTK DSSAAWDETL LDKFYTELYQ QLNDLEACVI

111 148

QGVGVTETPL MKEDSILAVR KYFQRITLYL KEKKYSPCAW EVVRAEIMRS

FSLSTNLQES LRSKE

Interferon-3O:

16

CDLPQTHSLG SRRTLMLLAQ MRKISLFSCL KDRHDFGFPQ EEFGNQFQKA

ETIPVLHEMI QQIFNLFSTK DSSAAWDETL LDKFYTELYQ QLNDLEACVI

111 148

QGVGVTETPL MKEDSILAVR KYFQRITLYL KEKKYSPCAW EVVRAEIMRS

FSLSTNLQES LRSKE

Top-down HDX for antibodies

To further locate the conformational changes

Disulfide reduction
at pH 2.5 and 0 °C

↓

HPLC
at pH 2.5 and 0 °C

↓

Top-down ECD

Top-down HDX for biosimilar interferons

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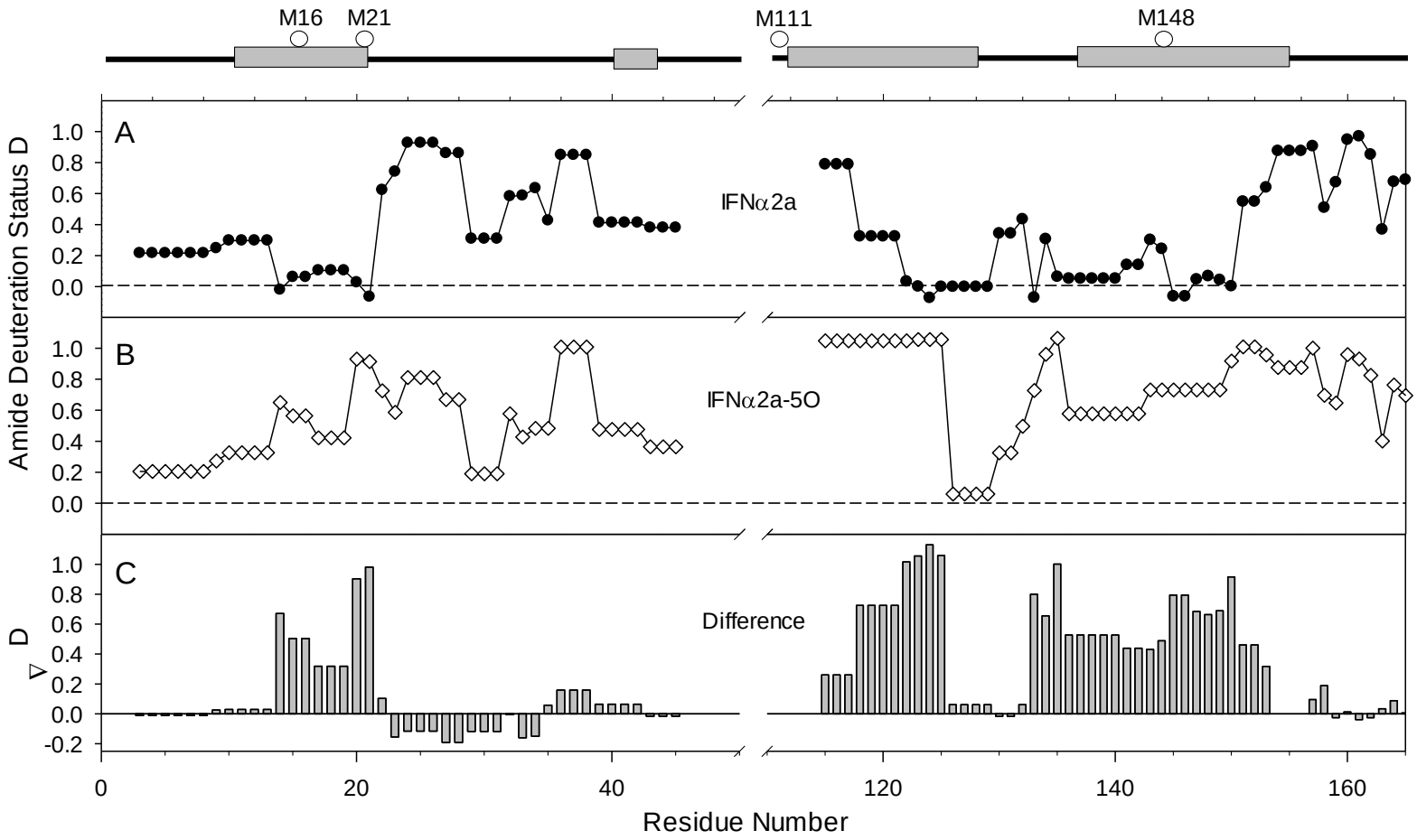
Structural difference between Interferon and Interferon-5O measured by LC-top-down



1 → complete exchange

0 → no exchnage

Top-down HDX for biosimilar interferons

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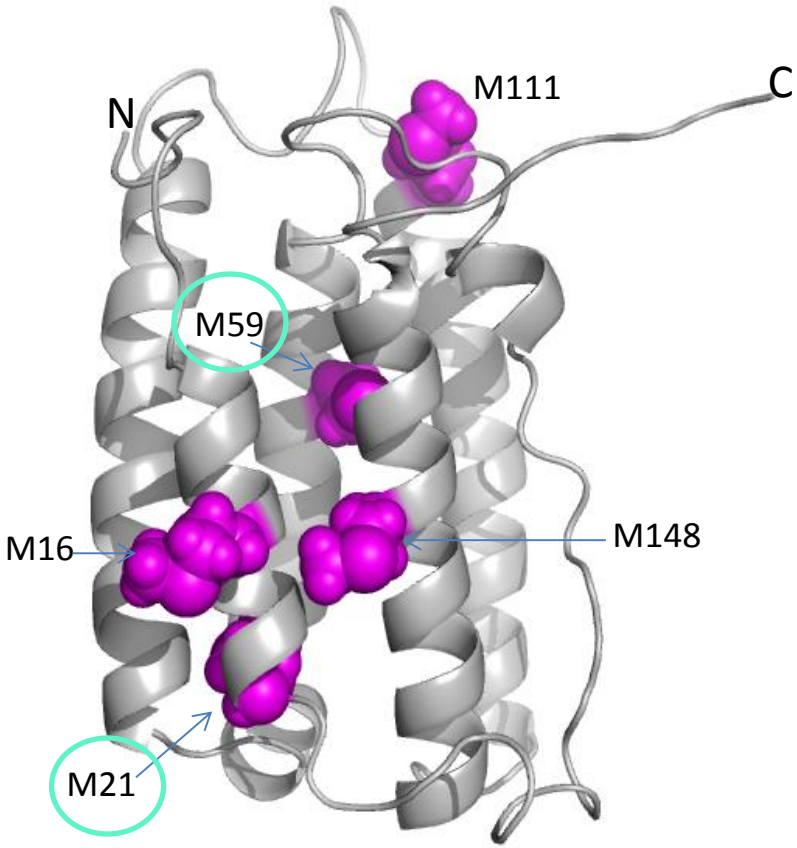
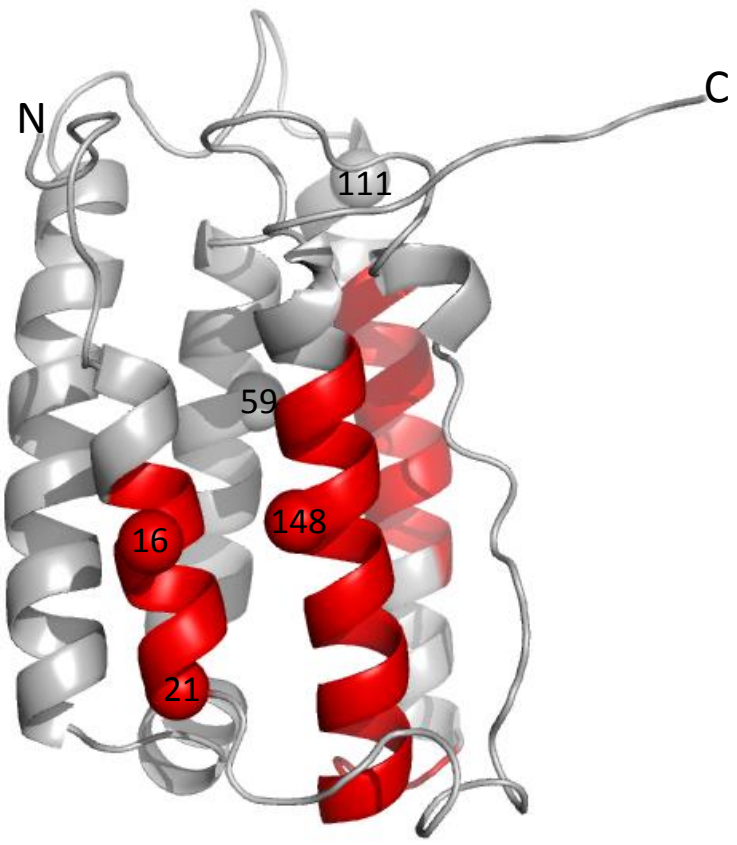
Structural difference between Interferon and Interferon-5O





Location of oxidized methionines

Pan, Borchers. *Proteomics* 2014, 14, 1249

Top-down HDX for biosimilar interferons

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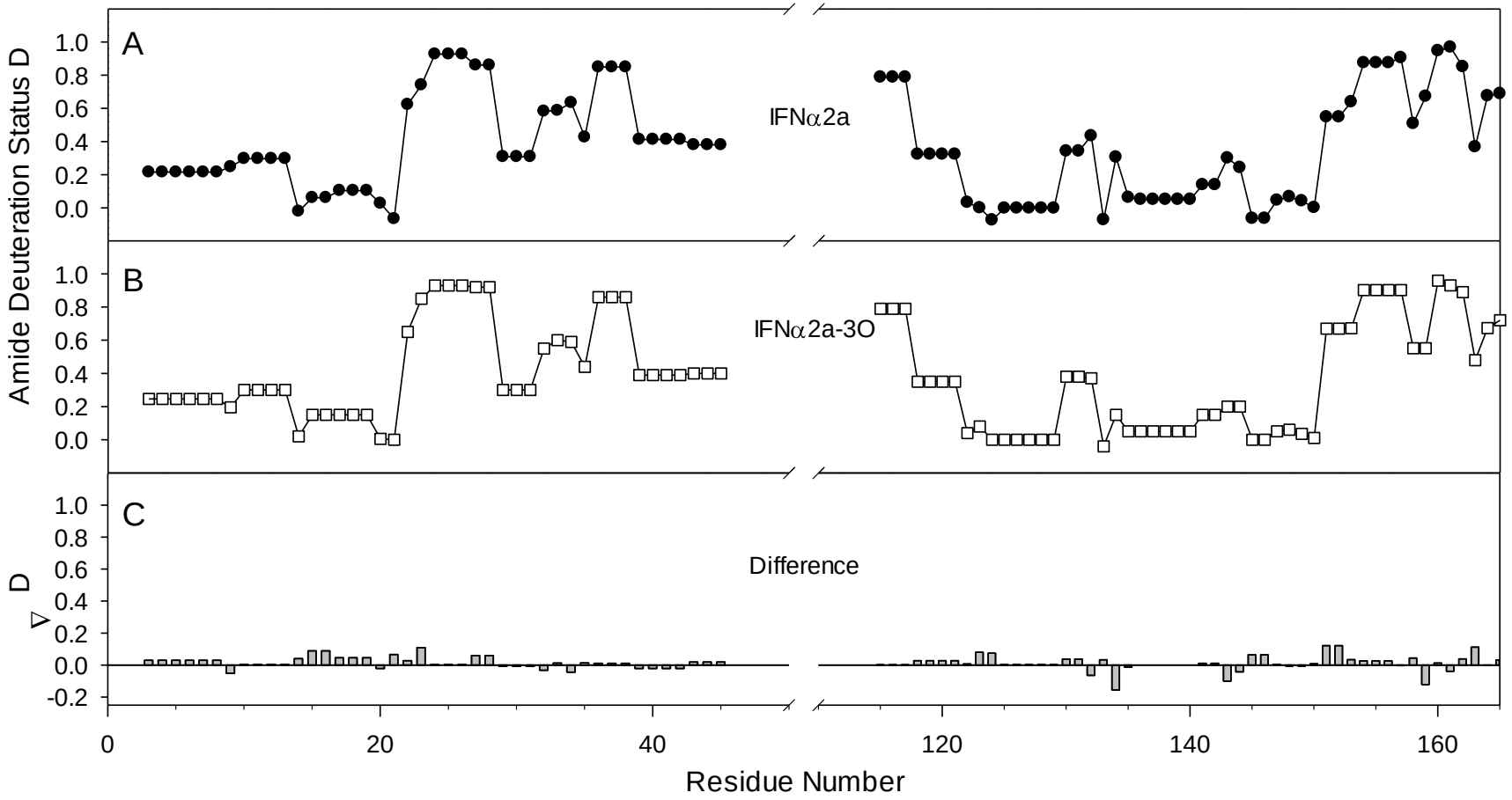
Structural comparison between Interferon and Interferon-3O



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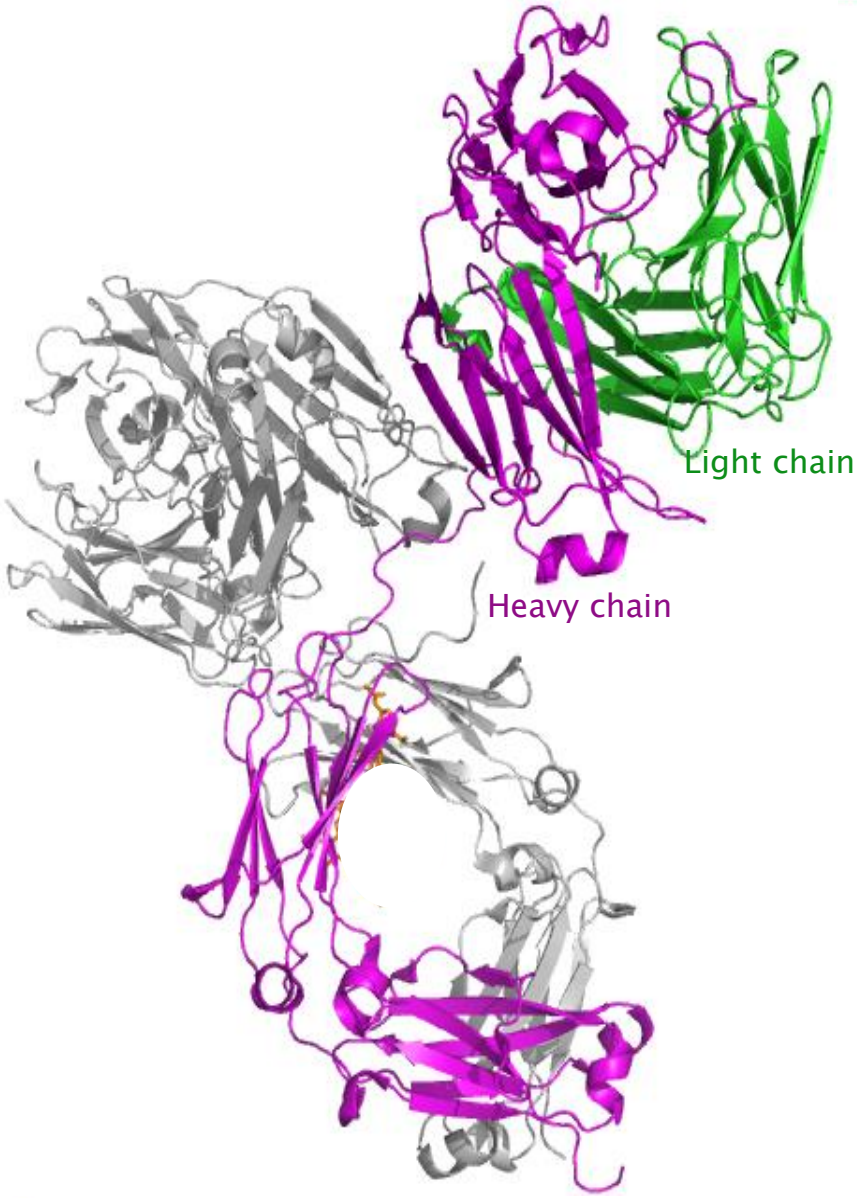
Top-down HDX for antibodies



2 light chains

2 heavy chains

> 1300 amino acids

~ 150 kDa

> 10 S-S bonds

Top-down HDX for antibodies

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To reduce back exchange,

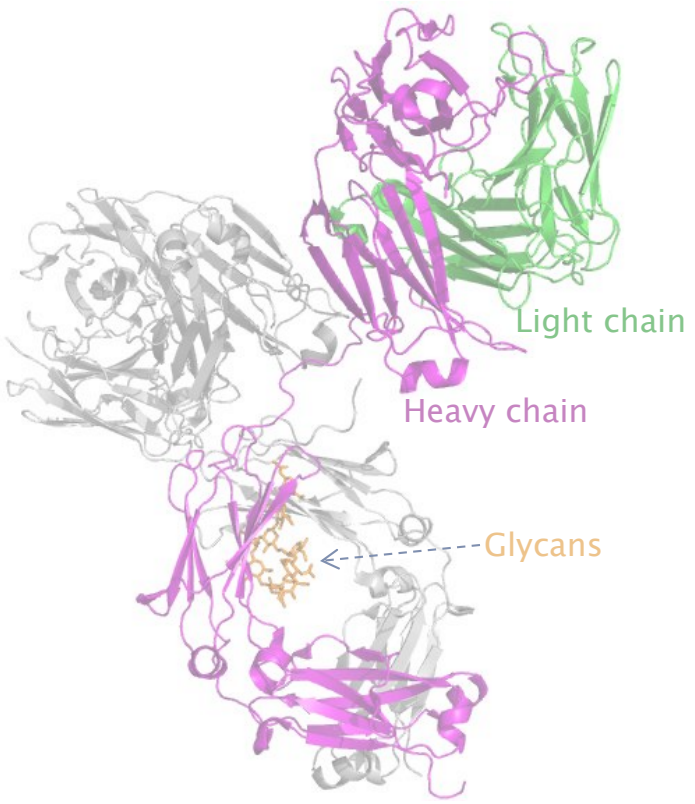
increase sequence coverage and spatial resolution

Subzero temperature LC + top-down ECD

Conclusions

- Subzero temperature HPLC significantly reduces H/D back exchange
- HDX and top-down ECD/ETD on LC time scale can pinpoint subtle conformational changes in interferon biosimilars at residue resolution
- Top-down HDX-ECD/ETD is developed for accurate higher-order structure characterization of therapeutic antibodies

Biosimilar structure analysis by FTMS



- **Higher-order structure analysis**
  by HDX-MS (top-down, bottom-up)
- **Intact mass measurement**
  at ultrahigh resolution
- **Sequencing**
  at ~ single-residue resolution
- **PTM analysis**
  including glycosylation profiling

Acknowledgements

- **UVic-Genome BC Proteomics Centre**

  Dr. Jingxi Pan

  Dr. Suping Zhang

  Albert Chou

  Darryl Hardie

- **Platform Funding**