# *De novo* sequencing of chimeric spectra using Casanovo

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The authors declare no competing financial interest.

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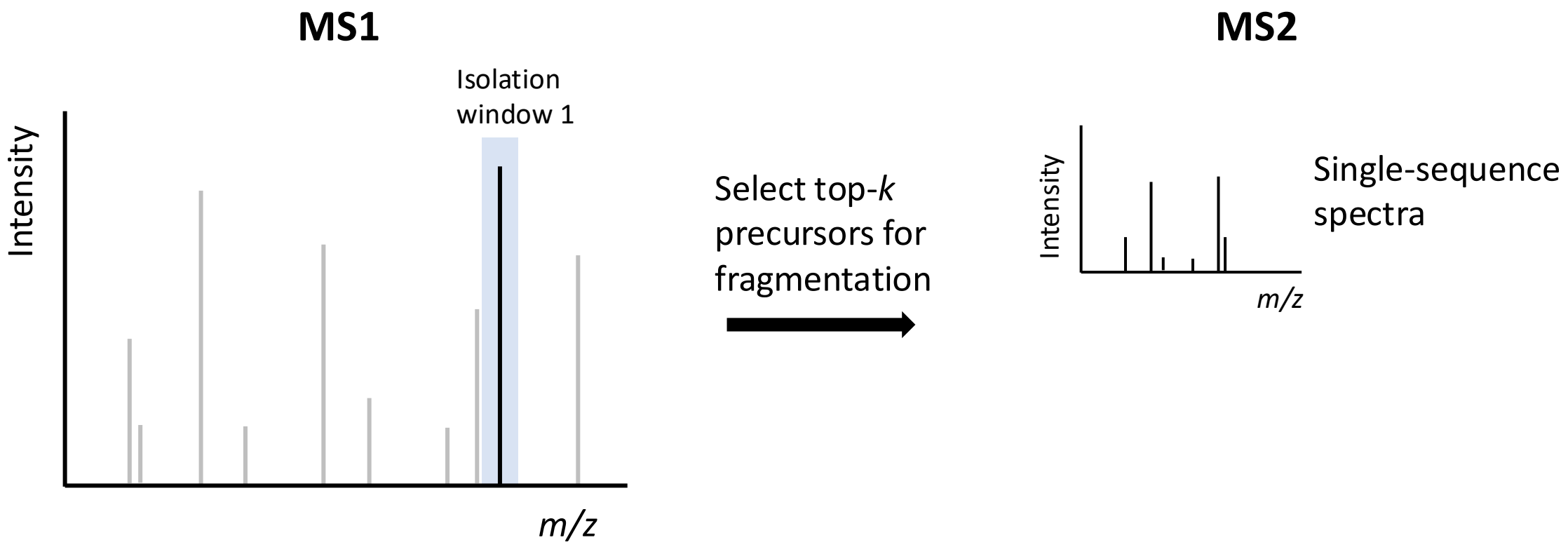
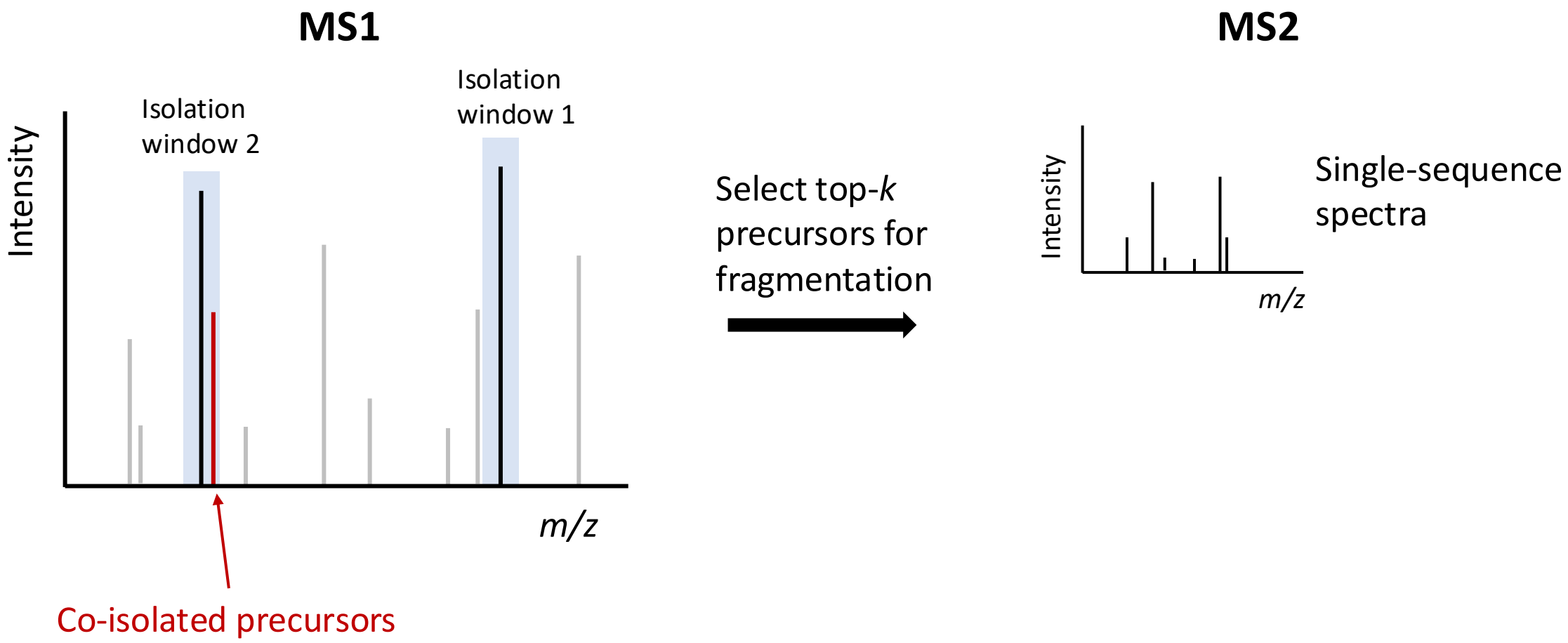
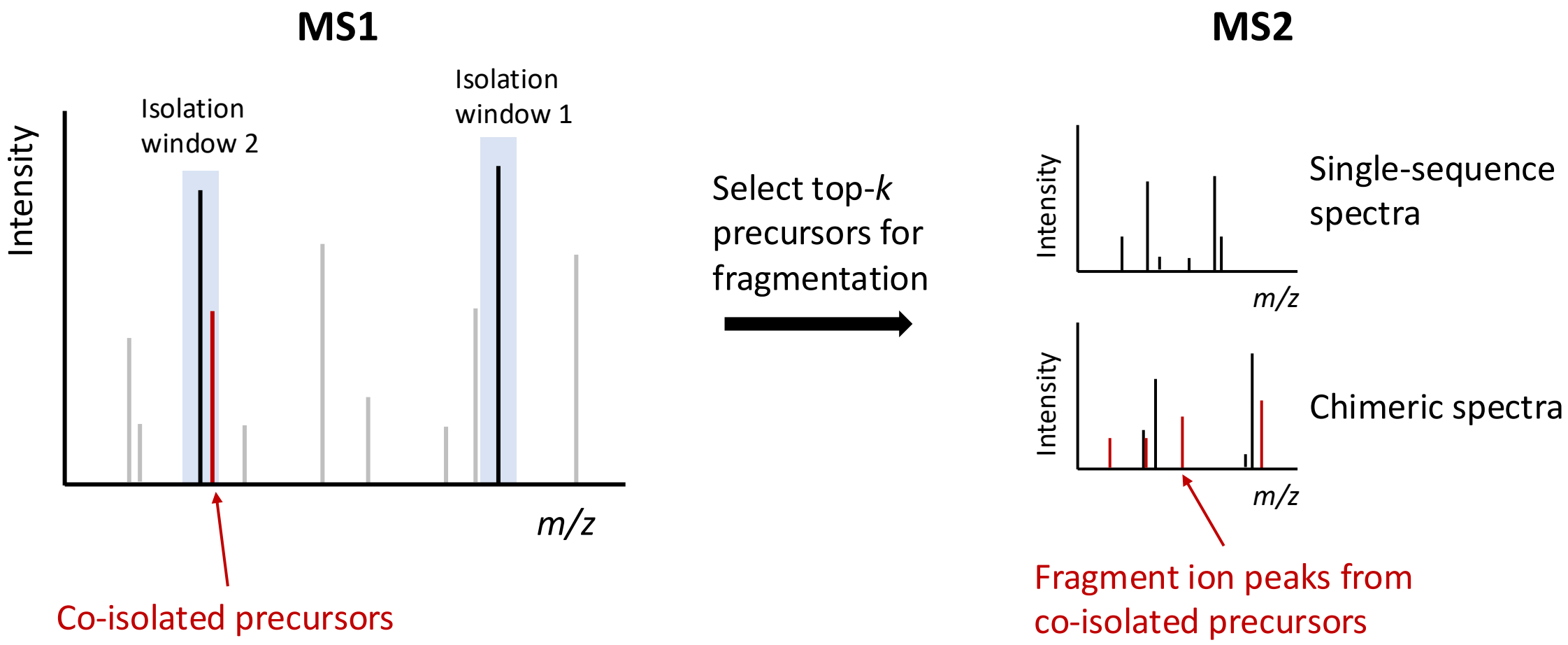
# DDA Selects and Isolates the Top-*k* Precursors for Fragmentation



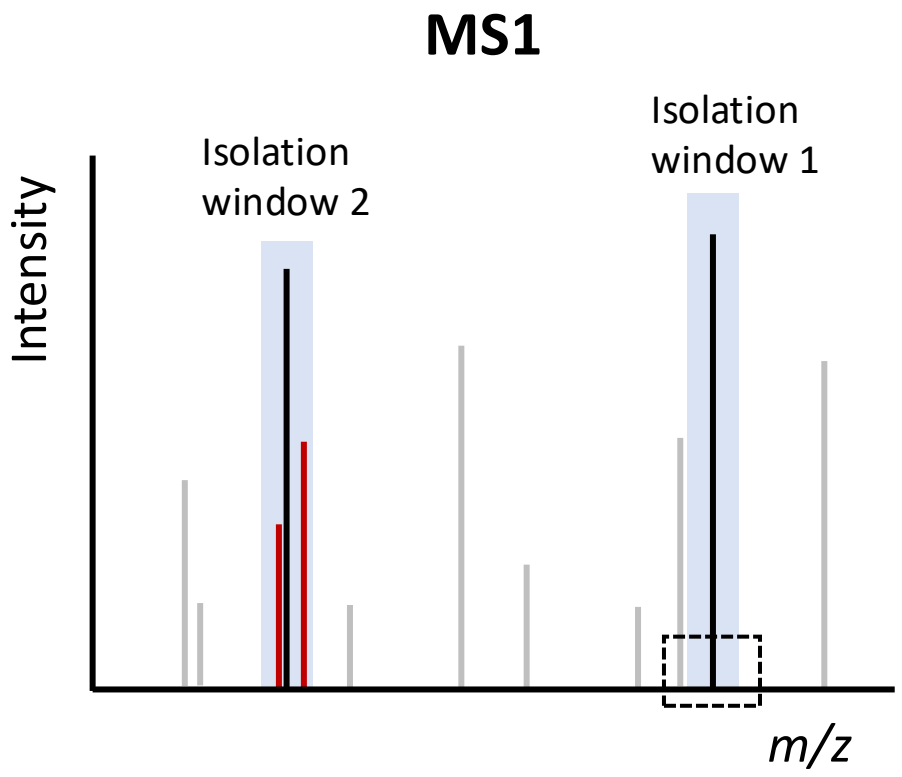
# Co-isolated Precursors in DDA Give Rise to Chimeric Spectra



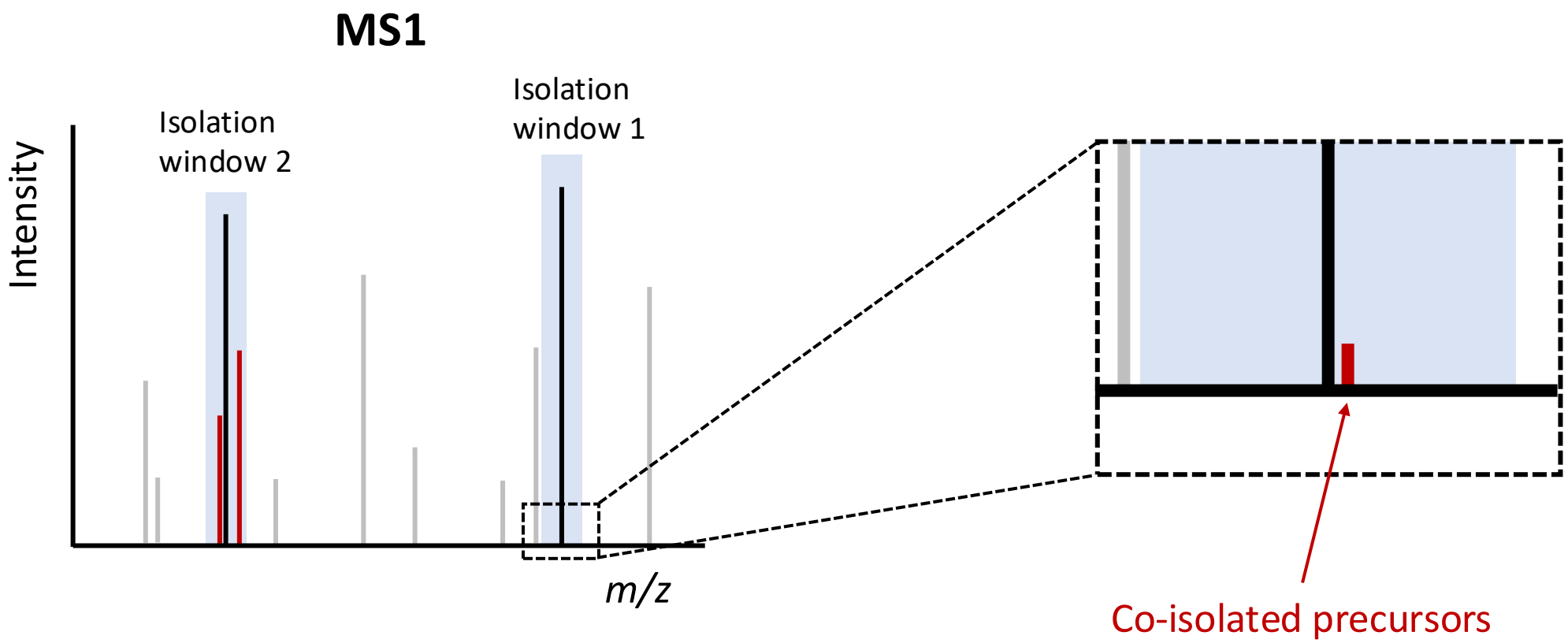
# Chimeric Spectra Contain Fragment Ions from Multiple Co-isolated Precursors



# Every Spectrum Is Potentially Chimeric



# Every Spectrum Is Potentially Chimeric — Sensitive Methods Can Reveal Hidden Co-isolated Peptides



# Existing *de novo* Methods on DDA Are Not Designed for Chimeric Spectra

| Method | Year | Model architecture | | | Chimeric spectra | Citations |
|---|---|---|---|---|---|---|
| | | CNN | Transformer | Others | | |
| DeepNovo (Tran et al. 2017) | 2017 | ✓ | | | | 564 |
| SMSNet (Karunratanakul et al. 2019) | 2019 | ✓ | | | | 97 |
| PepNet (Liu et al. 2023) | 2023 | ✓ | | | | 86 |
| Casanovo (Yilmaz et al. 2022) | 2022 | | ✓ | | | 117 |
| GraphNovo (Mao et al. 2023) | 2023 | | ✓ | | | 59 |
| $\pi$-HelixNovo (Yang et al. 2024) | 2024 | | ✓ | | | 46 |
| PointNovo (Qiao et al. 2021) | 2021 | | | ✓ | | 128 |
| pNovo 3 (Yang et al. 2019) | 2019 | | | ✓ | | 112 |

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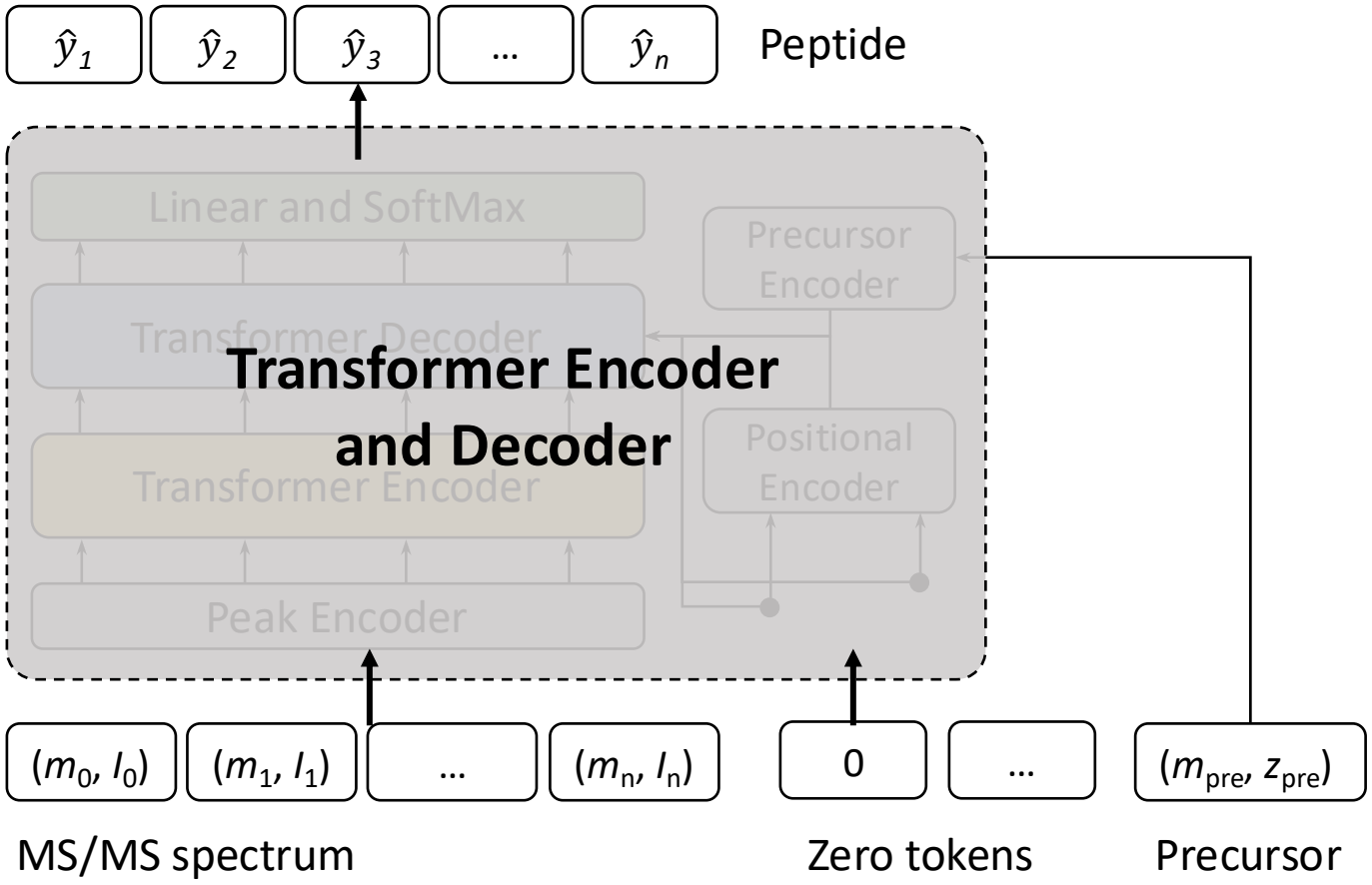
Original table is from [1], where the citations are updated by counting from Google Scholar on May 26, 2026.

[1] Bittremieux, Wout, et al. "Deep learning methods for de novo peptide sequencing." Mass Spectrometry Reviews 45.3 (2026): 507-526.

# Casanovo Is a State-of-the-Art Transformer-Based *de novo* Sequencer

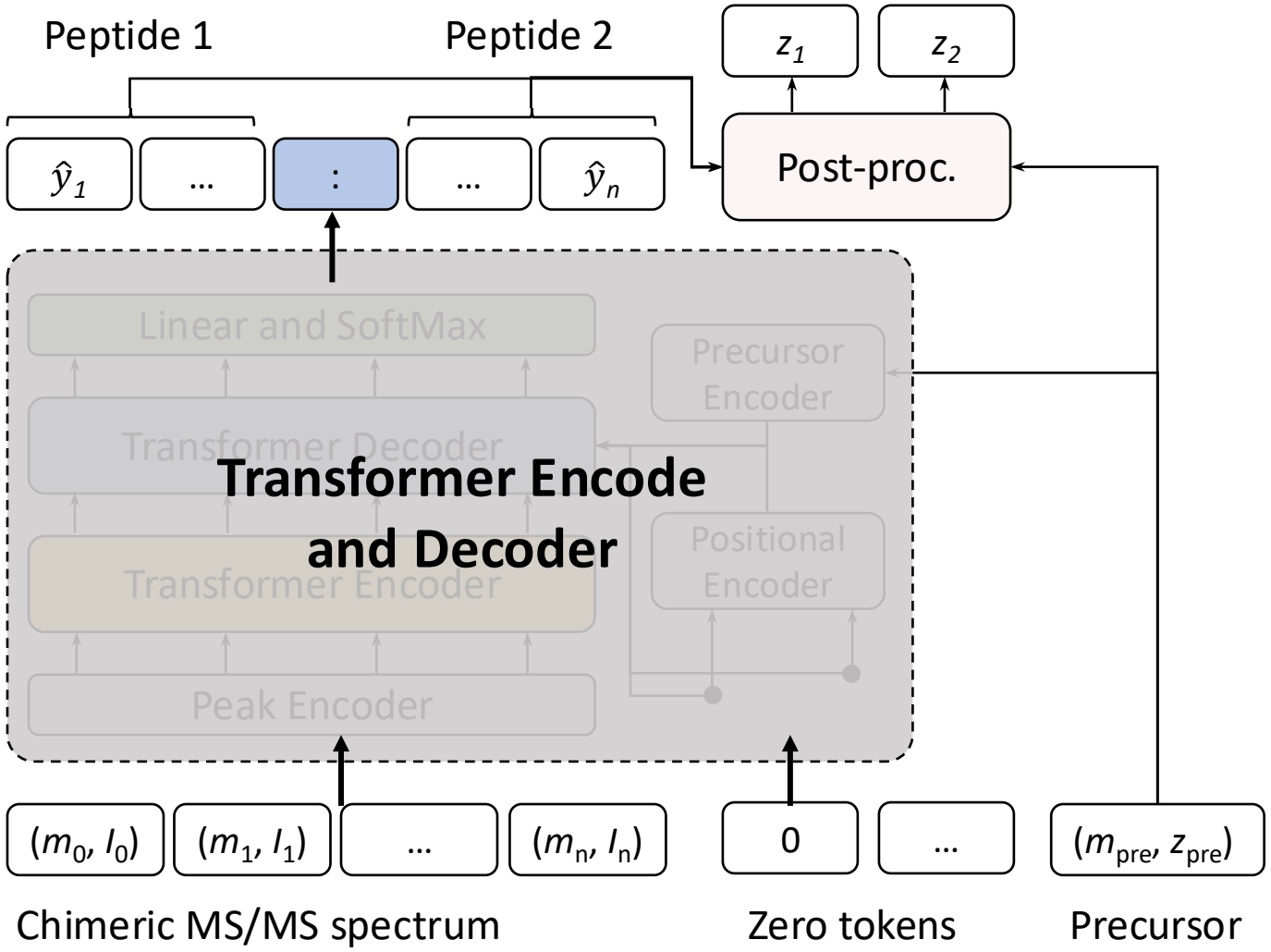



[2] Yilmaz, Melih, et al. "Sequence-to-sequence translation from mass spectra to peptides with a transformer model." Nature communications 15.1 (2024): 6427.

# Three Key Modifications Enable Casanovo to Directly Predict Chimera

**Casanovo-chim**

1. Special separator token ":" for dual-peptide output



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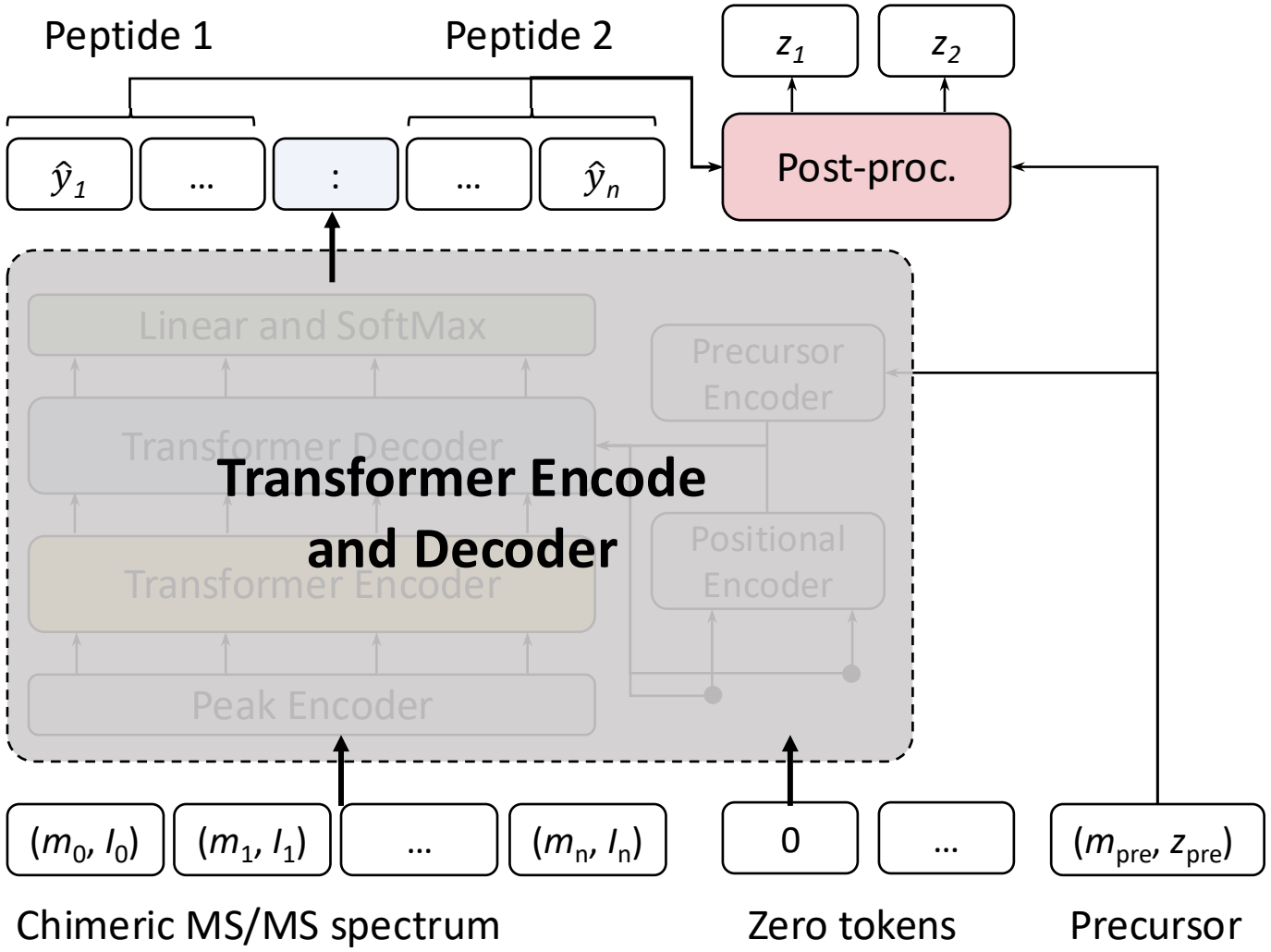
**Casanovo-chim**

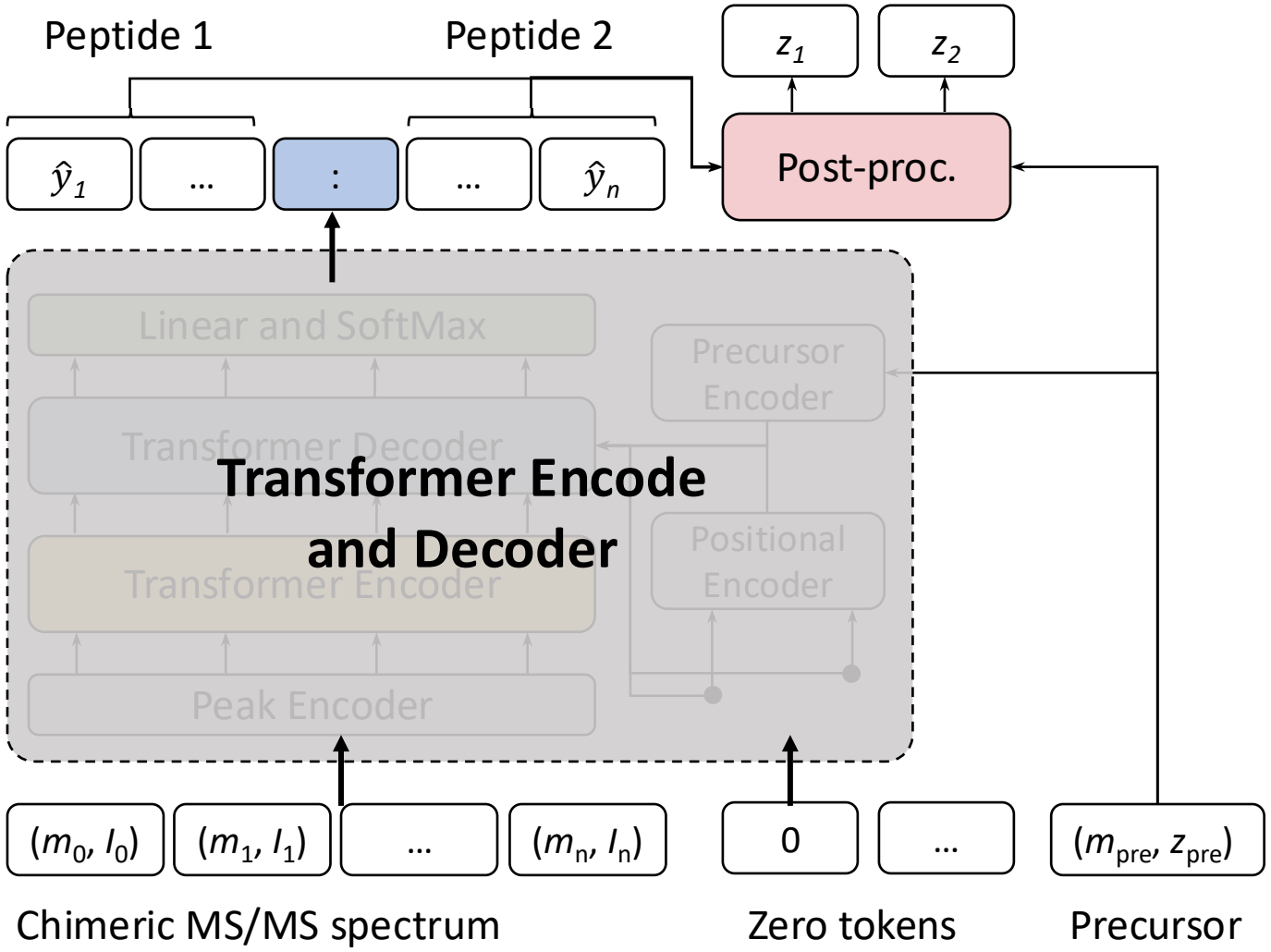
1. Special separator token ":" for dual-peptide output
2. Post-processing to correct chimeric charge states



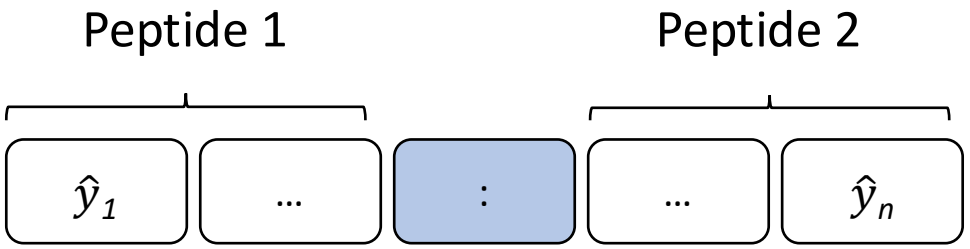
# Three Key Modifications Enable Casanovo to Directly Predict Chimera

**Casanovo-chim**

1. Special separator token ":" for dual-peptide output
2. Post-processing to correct chimeric charge states
3. Permutation-invariant loss function for chimeric output



# Permutation-Invariant Loss Function for Chimeric Output



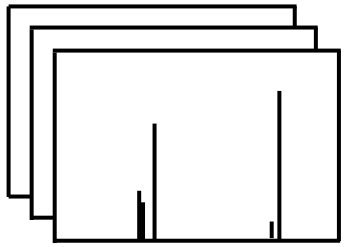

predicted peptide $\hat{y}$

$$\mathcal{L} = \min(\mathcal{H}(p_1 : p_2, \hat{y}), \mathcal{H}(p_2 : p_1, \hat{y}))$$

mean per-token
cross-entropy loss

# We Constructed a Ten-Species Chimeric and Single-Peptide Spectra Dataset Using MSFragger DDA+

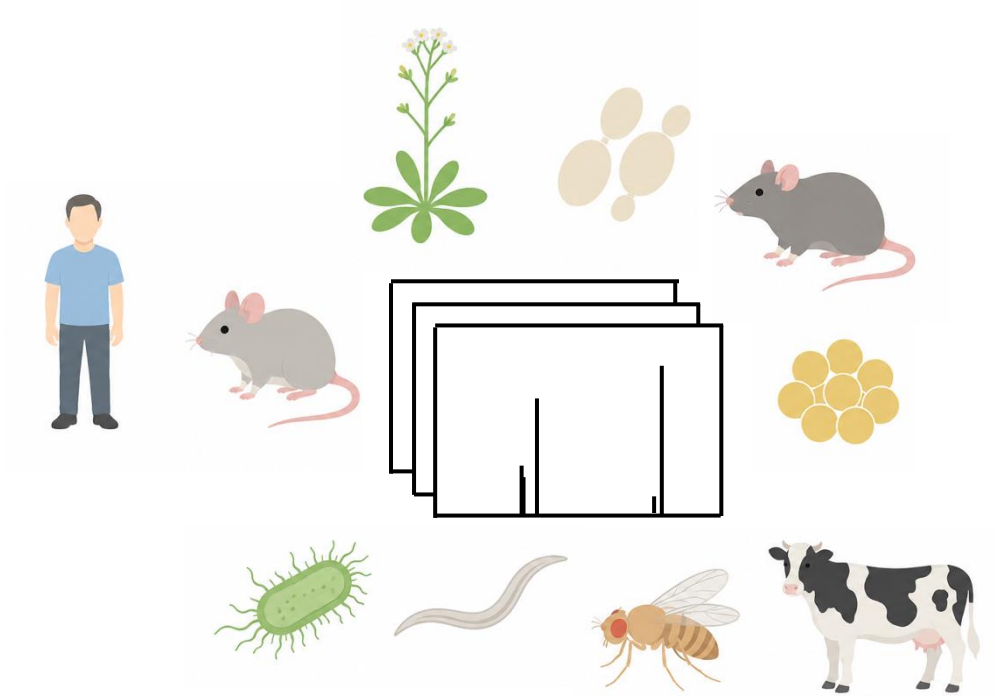
PRIDE
PRoteomics IDEntifications Database



Experimental spectra

FRAGPIPE

MSFragger DDA+



Spectra with peptide annotations across ten species

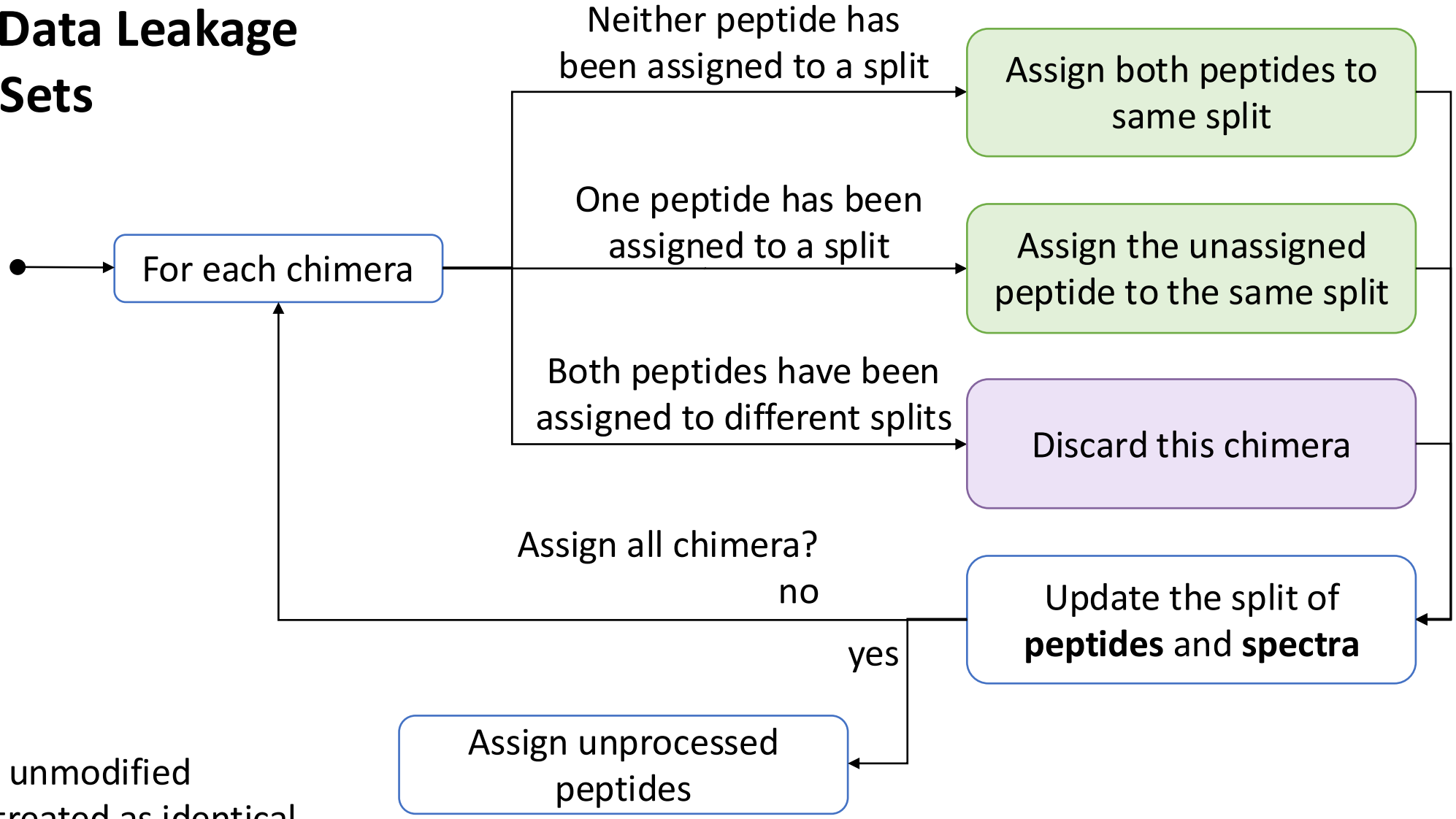
[3] Yu, Fengchao, Yamei Deng, and Alexey I. Nesvizhskii. "MSFragger-DDA+ enhances peptide identification sensitivity with full isolation window search." Nature Communications 16.1 (2025): 3329.

# Preprocessing Removes Unreliable Chimeras and Balances Peptide Representation

1. Spectra with 3+ co-isolated precursors excluded.
2. Top 2 spectra retained per unique peptide or chimeric pair (by q-value).
3. Chimeras downsampled to ≤10 per unique peptide for balanced representation.

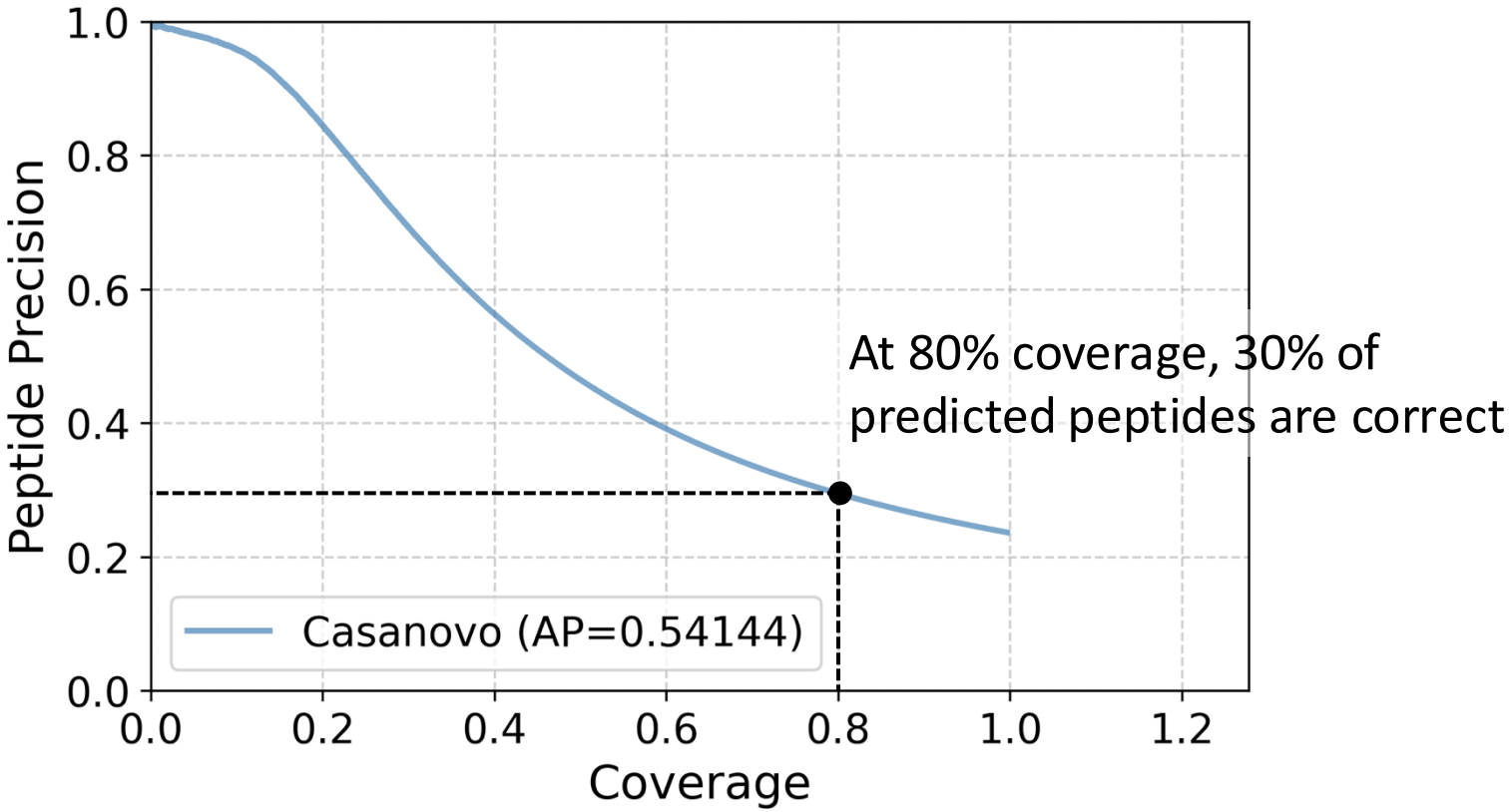
**~5M spectra from ~ 1M peptides — 53.3% chimeric**

# Peptide-Level Partitioning Prevents Data Leakage between Sets

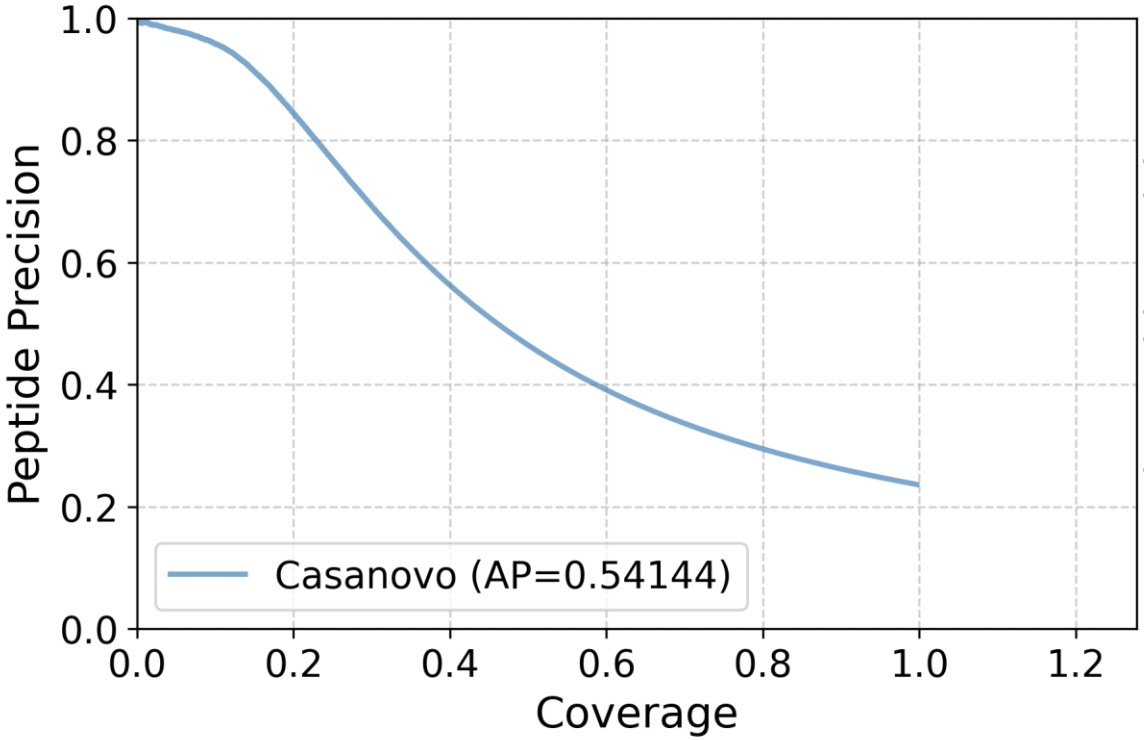


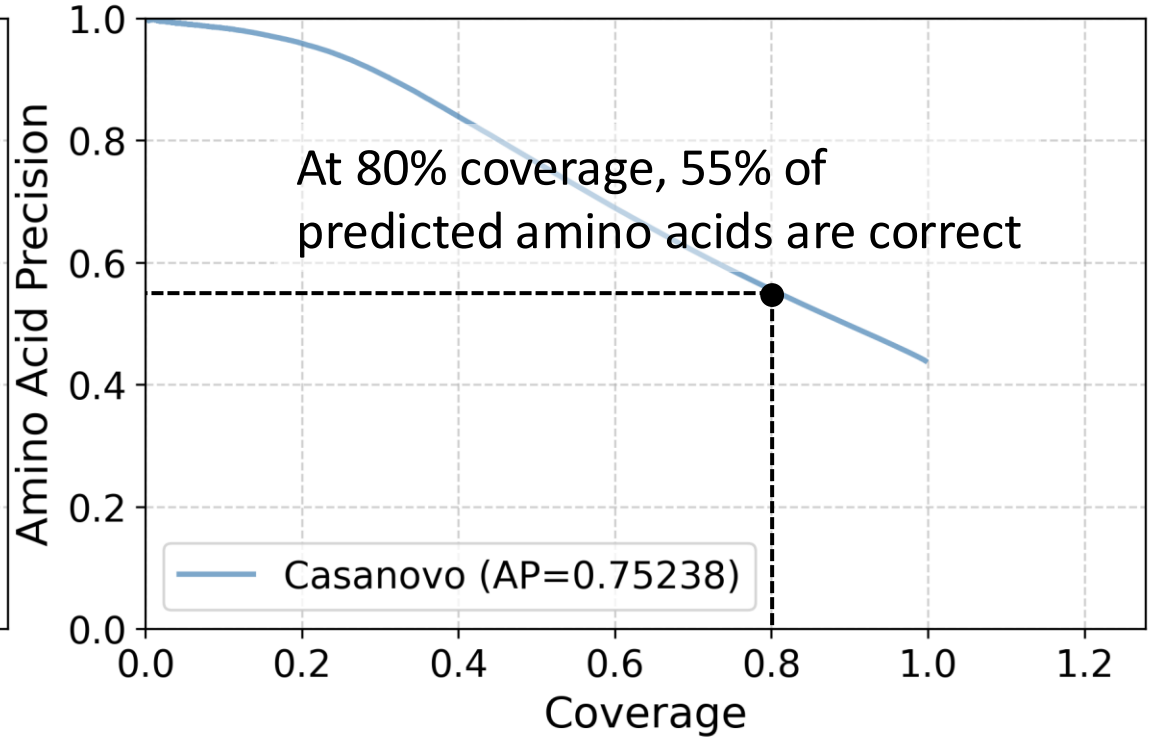

Modified and unmodified peptides are treated as identical.

# Precision-Coverage Curves Evaluate Both Accuracy and Completeness of Predictions



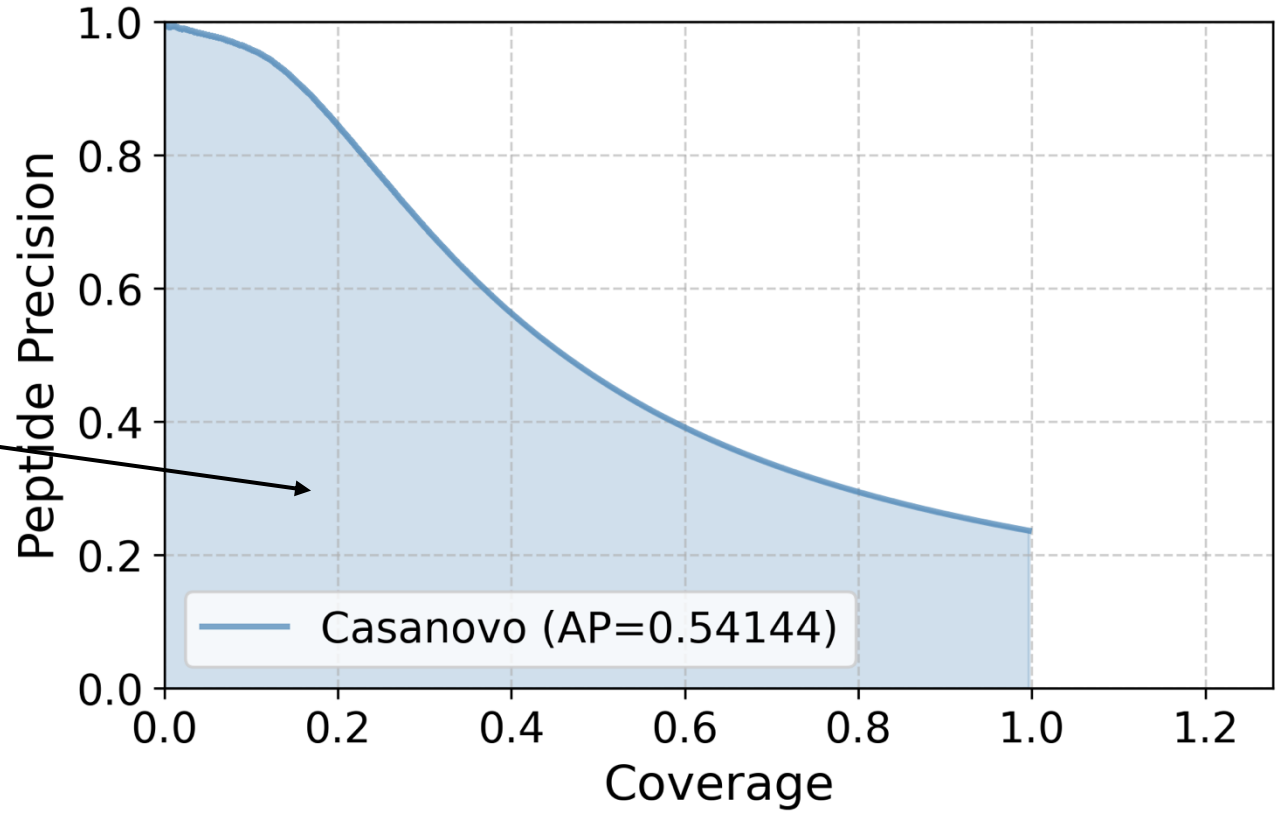
# Precision-Coverage Curve Can Also be Calculated at Amino Acid Level



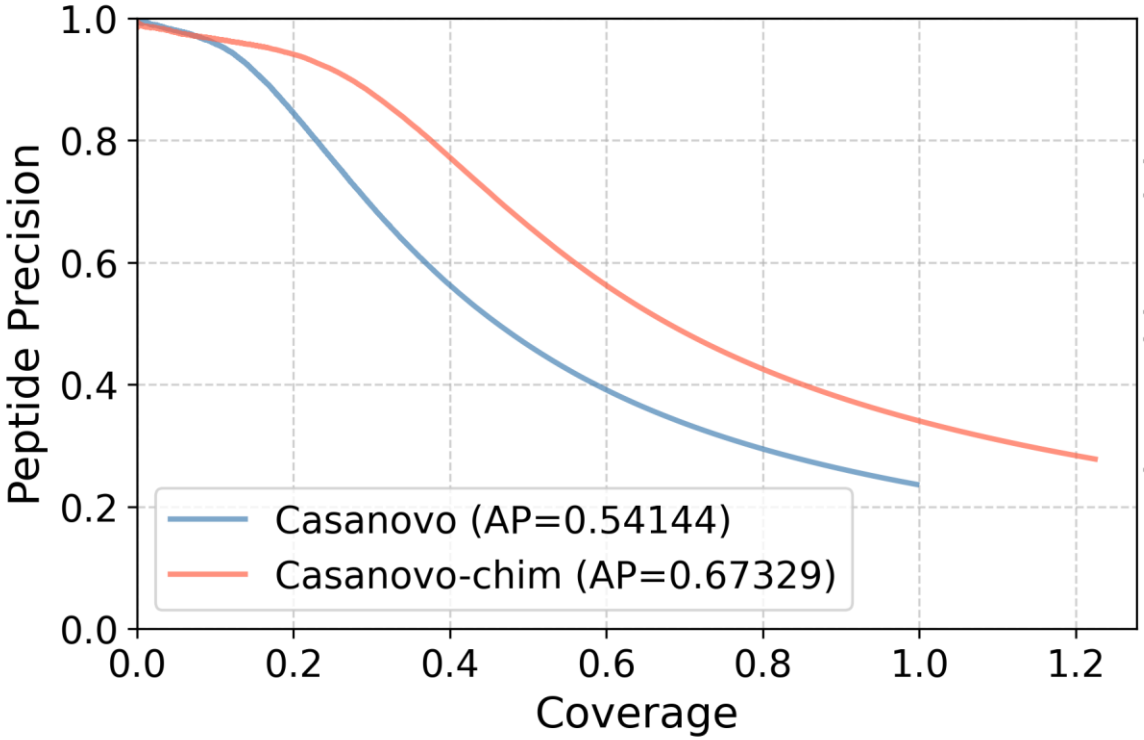



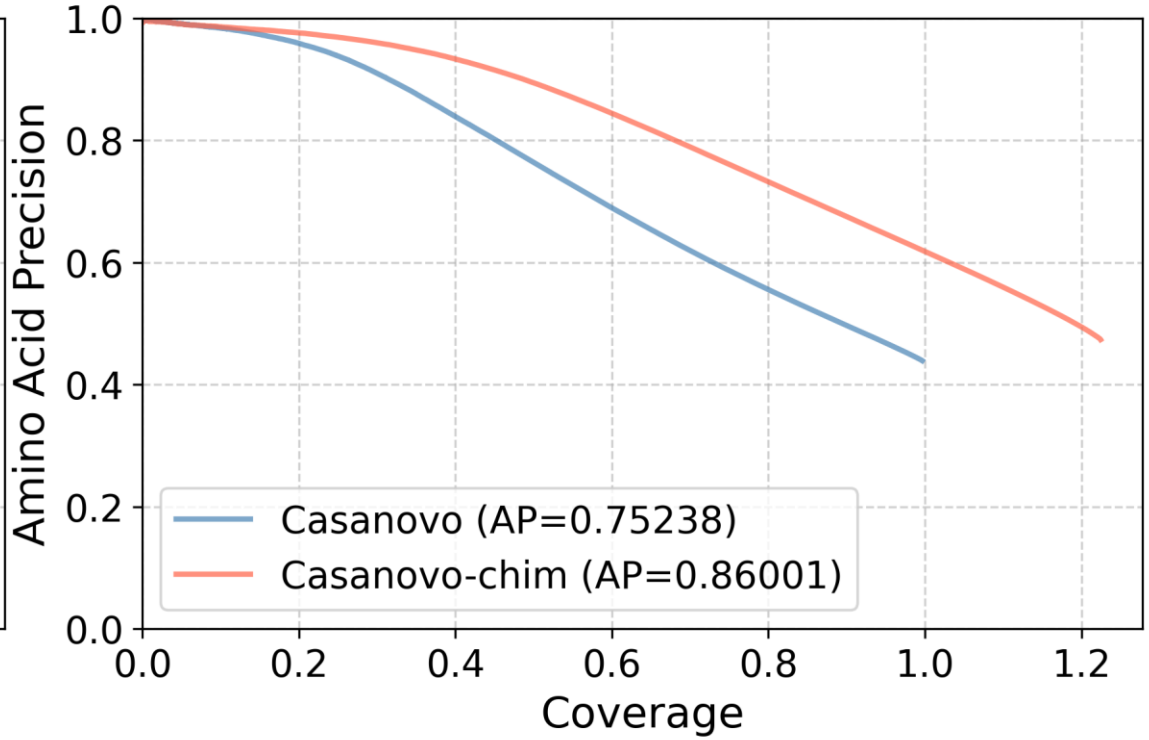
# Chimeric Predictions Can Exceed Full Coverage — Average precision (AP) Is Evaluated at Coverage ≤ 1

Area under the curve
= average precision

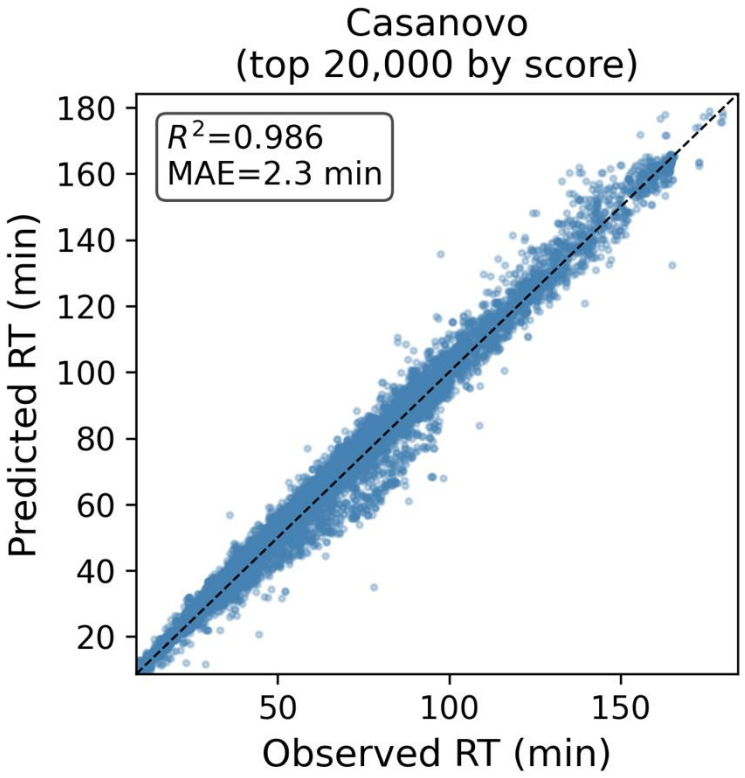


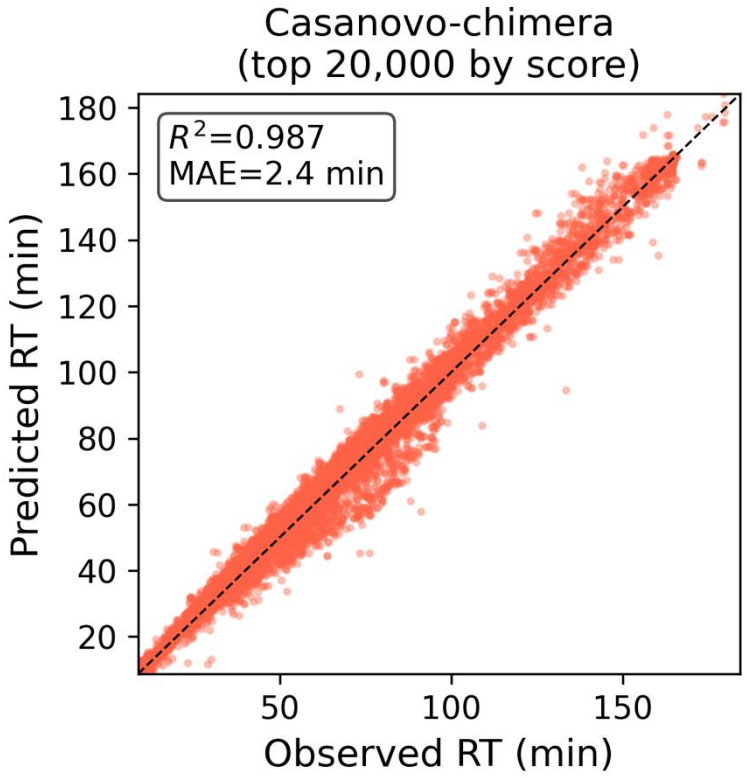
# Casanovo-chim Outperforms non-chimeric model at Both Peptide and Amino Acid Levels



# Top-Ranked Predictions Match Casanovo's Retention Time Reliability

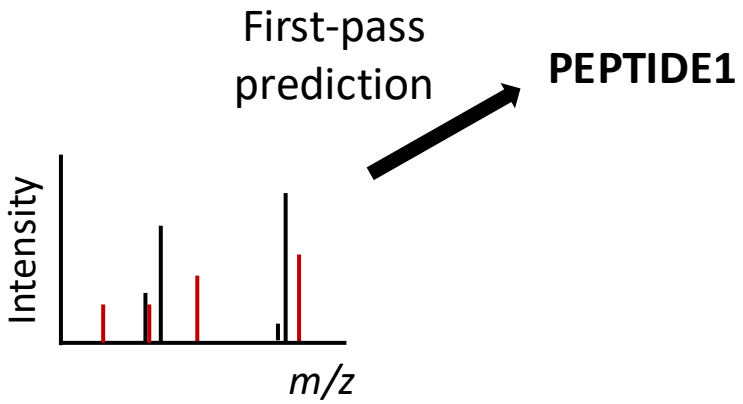



Bo Wen
Noble Lab

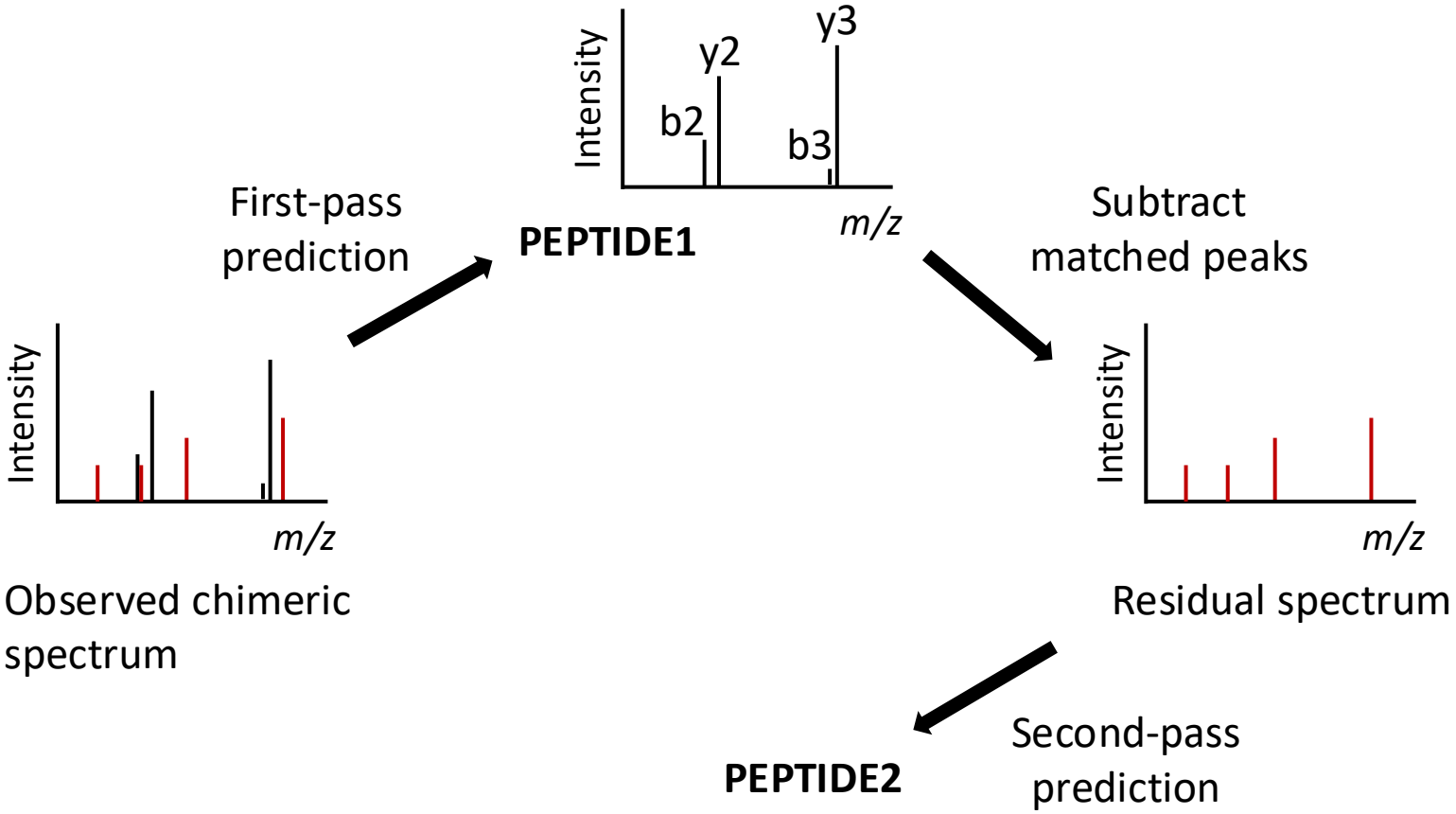
[4] Wen, Bo, et al. "Carafe enables high quality in silico spectral library generation for data-independent acquisition proteomics." Nature Communications 16.1 (2025): 9815.
[5] Wen, Bo, et al. "Carafe2 enables high quality in silico spectral library generation for timsTOF data-independent acquisition proteomics." bioRxiv (2026): 2026-03.

# An Iterative Baseline: Casanovo Performs First-Pass Prediction on Observed Spectra

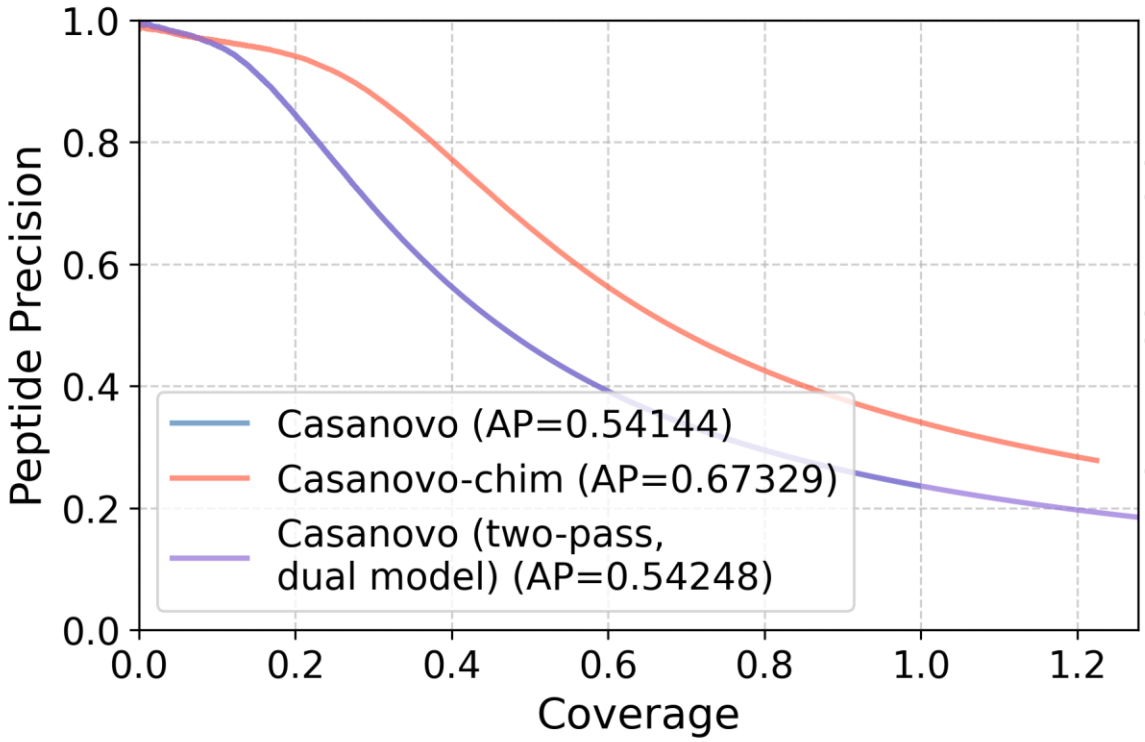


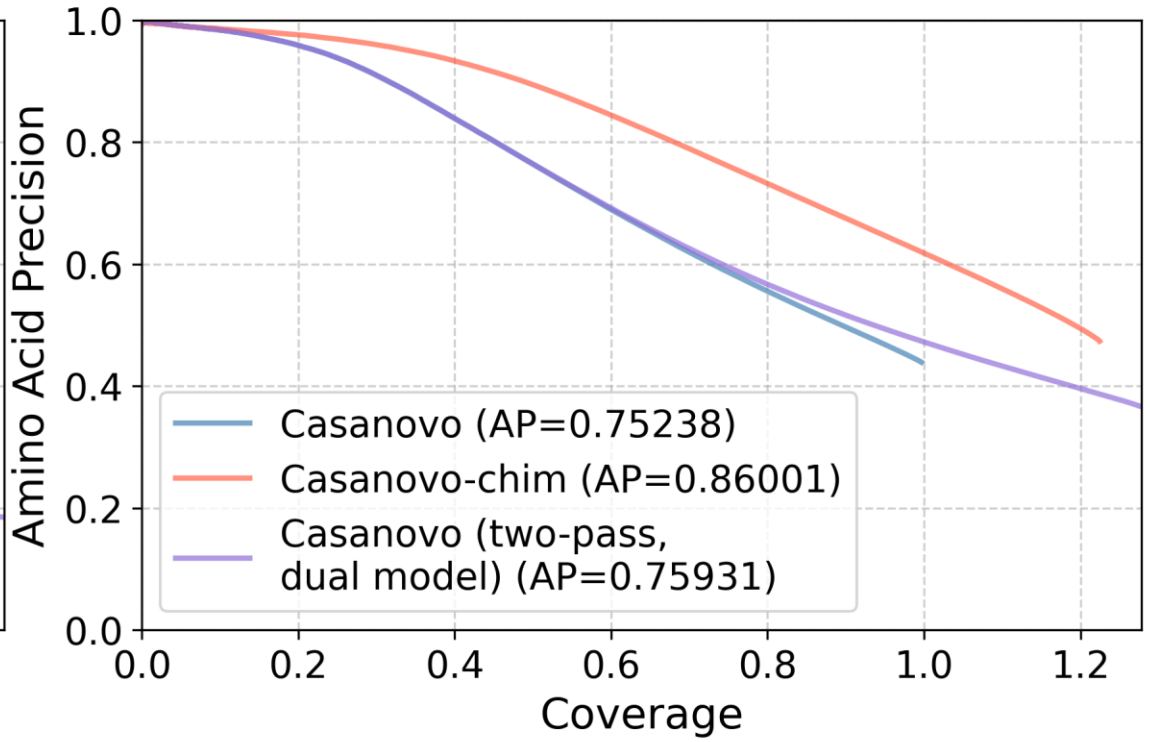

Observed chimeric spectrum

# Second-Pass Prediction Is Performed on Residual Peaks After Subtracting Annotated Peaks



# Casanovo-Chim Outperforms Two-Pass Casanovo



# Takeaway

- We extend Casanovo to directly predict peptides from chimeric spectra.
- At precision 0.8, Casanovo-Chim identifies 17,281 more unique peptides than the non-chimeric baseline — a ~37% lift.
- Casanovo-Chim predictions are validated by consistent retention time.
- Casanovo-Chim outperforms the two-pass Casanovo baseline.

# Acknowledgement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