



Collision Cross-section Measurements of Precursor and Selected Fragmentation Products in

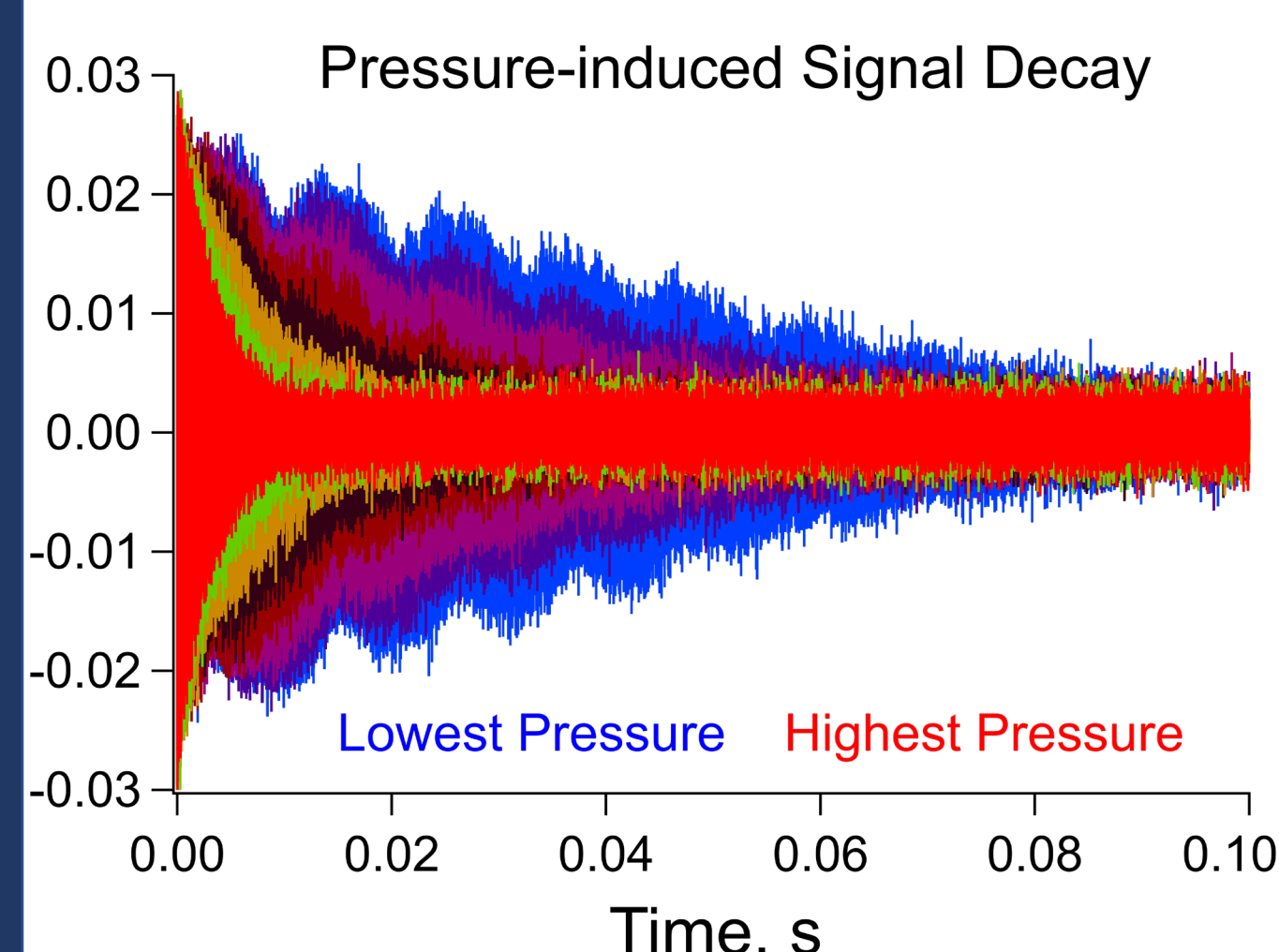
Single Experiments by SORI – CRAFTI

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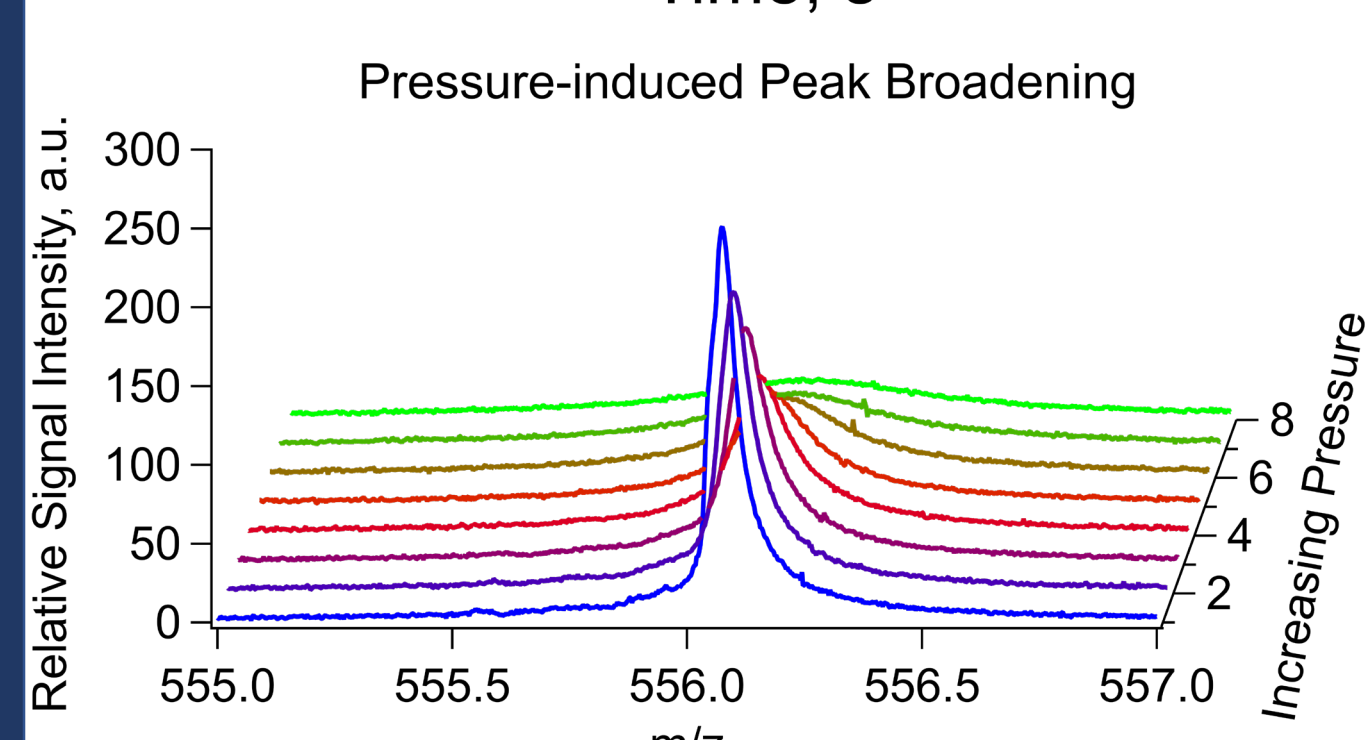
Overview

- FTICR – MS is highly amenable to pre-measurement ion activation
- CRAFTI: FT – MS method to measure ion – neutral collision cross-sectional areas (σ)
- SORI: ion activation strategy which uses off-resonance excitation to dissociate ions through multiple low energy collisions
- SORI – CRAFTI: ion activation then cross-section measurement of selected ions (precursor or products)
- Leucine – enkephalin (LE): a mass spectrometry standard often used for testing new analytical strategies
- LE (and products) wrap around small metal cations in compact conformations



Background

- Cross sectional Areas by Fourier Transform Ion cyclotron resonance mass spectrometry (CRAFTI) uses FTICR-MS and neutral collision gas to measure ion-neutral collision cross-sectional areas (σ) [1]
- Neutral collision gas leaked into trapping cell before ion excitation



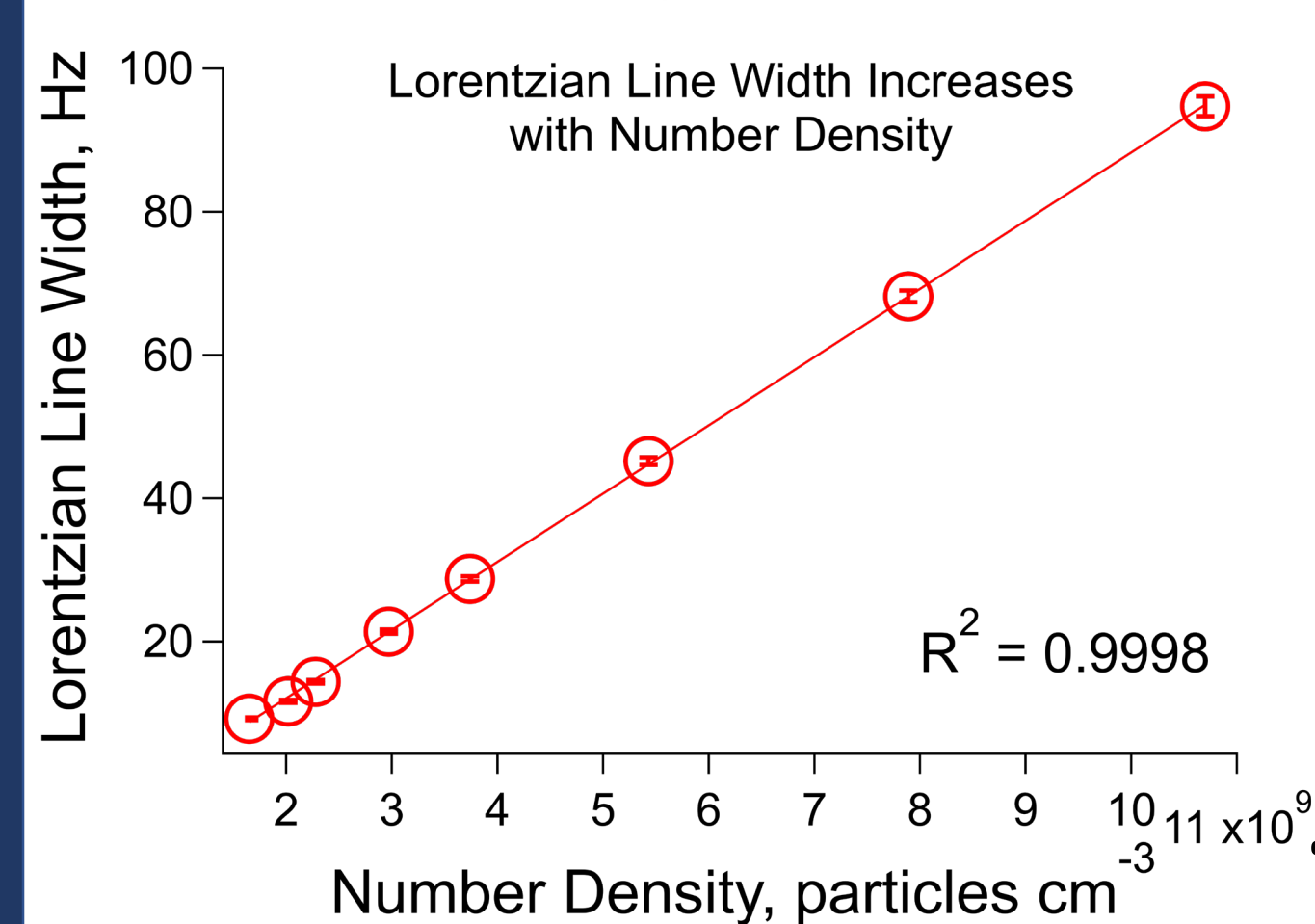
- Ion – neutral collisions dephase the coherent ion packet, shortening the time domain signal as a function of gas pressure (top left)

- Frequency domain signal peak broadens as a function of gas pressure (middle left)

- The frequency domain peak full width at half max (FWHM) as a function of neutral number density (bottom left) is used to calculate the collision cross-section using the CRAFTI equation [2]:

$$\sigma = \frac{FWHM}{n_{neutral}} \frac{m_{ion}}{q} \frac{\pi d}{\beta V_{pp} t_{exc}}$$

- MultiCRAFTI measures cross-sections of two ions simultaneously with results conveyed as ratio of the two cross-sections

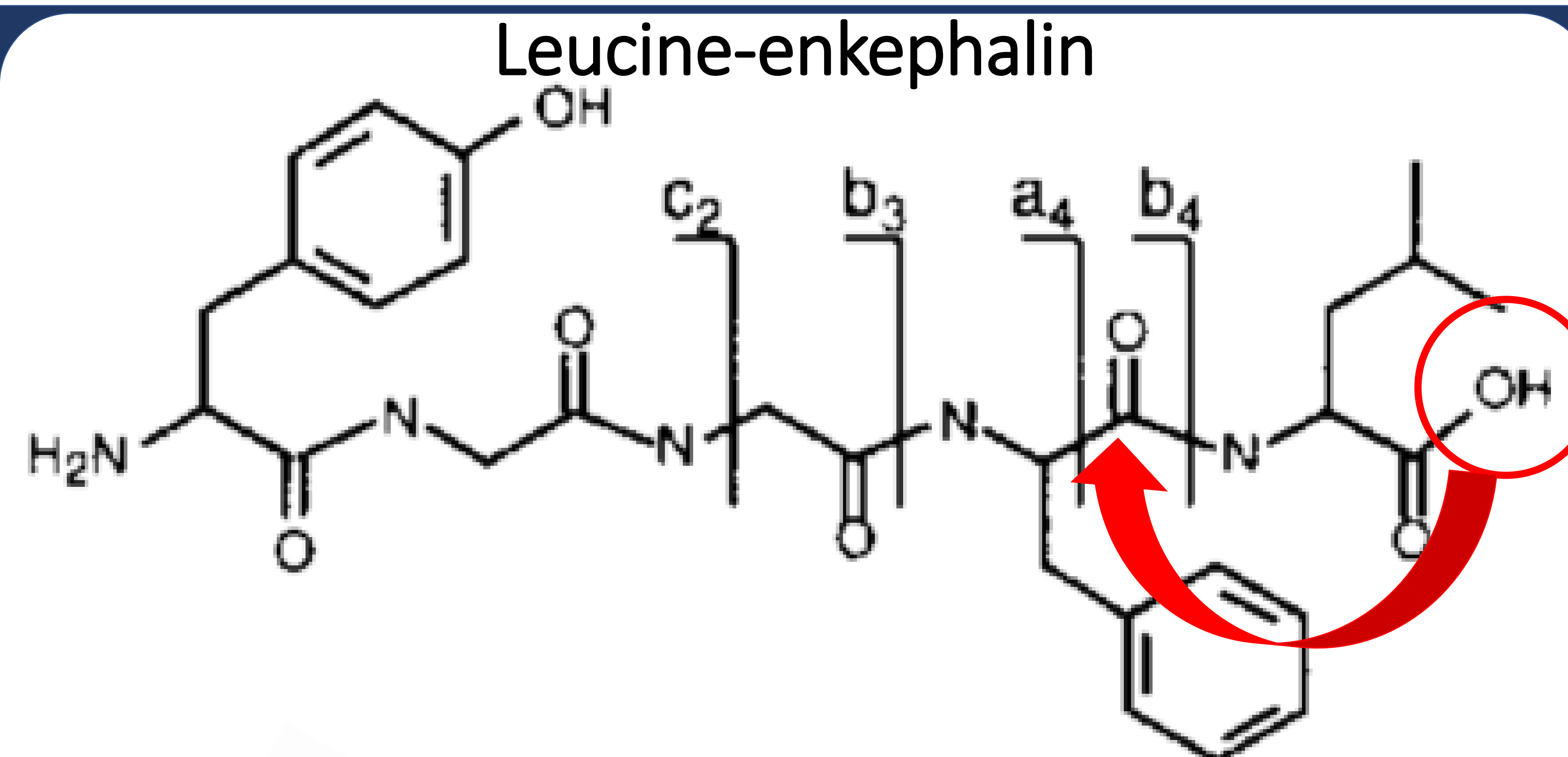


The Problem

- Can ion activation and cross-section measurements be coupled together?
- How does leucine-enkephalin fold when coordinated to alkali metal cations?
- How do alkali metal cations alter leucine-enkephalin's fragmentation pattern?

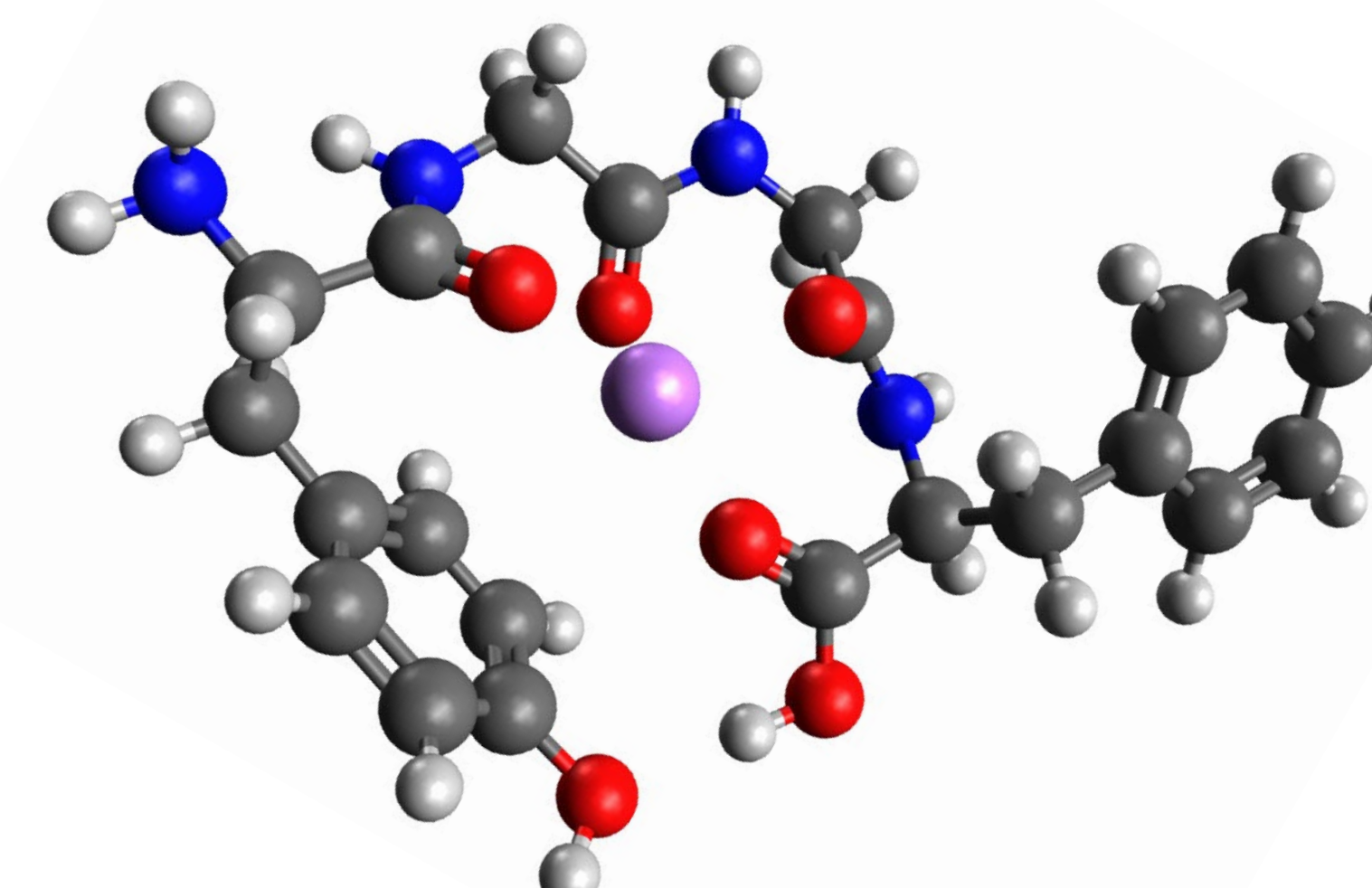
Methods

- Bruker APEX 47e FTICR-MS with ESI source; controlled by Predator Data System
- **SORI event used to activate ions prior to CRAFTI measurements**
- Conformational search using Spartan '18 MMFF force field
- Spartan '18 & NWChem to determine low energy structures (B3LYP/6-31+G*)
- Theoretical collision cross-sections using IMoS

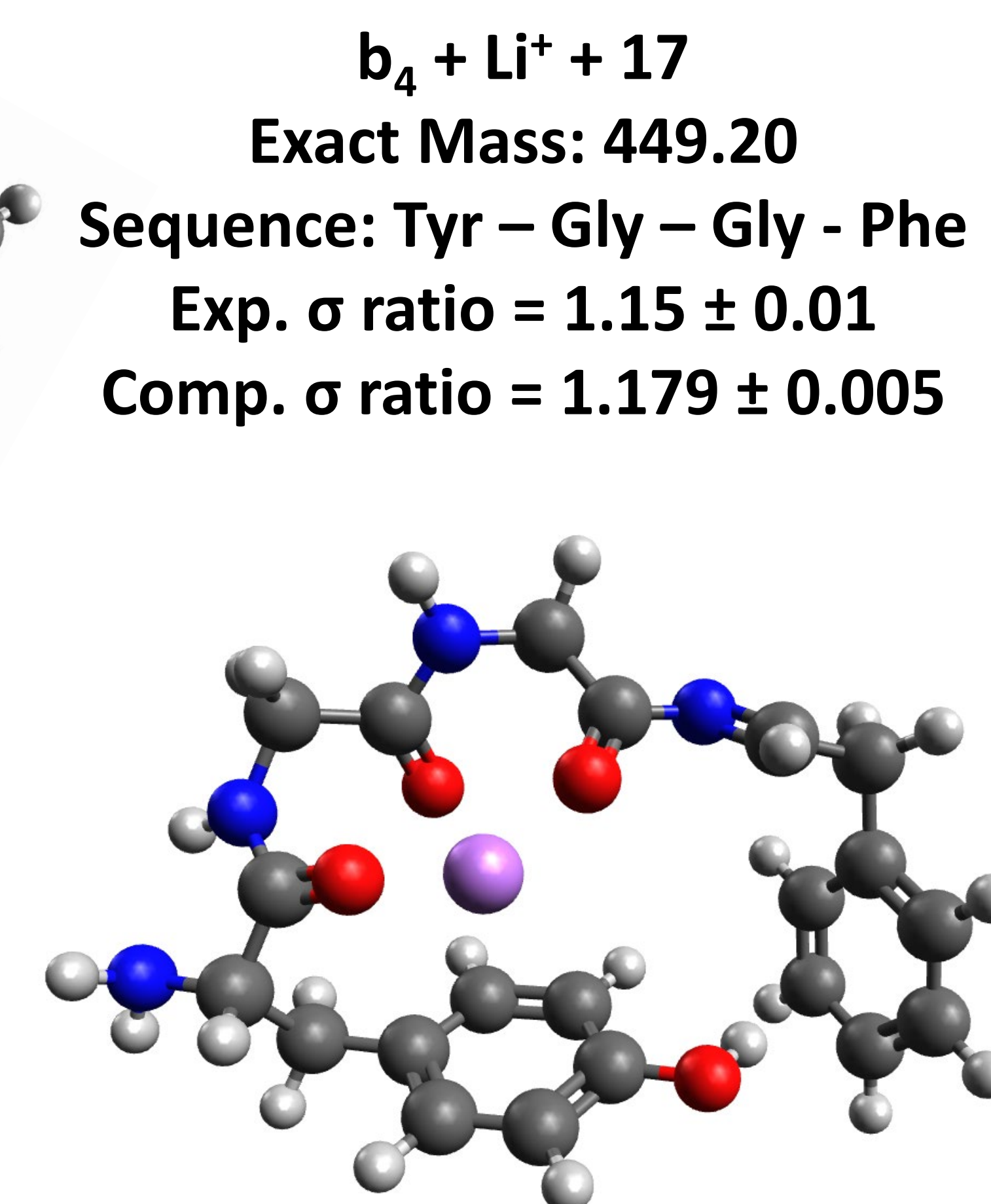


Results: SORI – CRAFTI Analysis of [LE + M]⁺

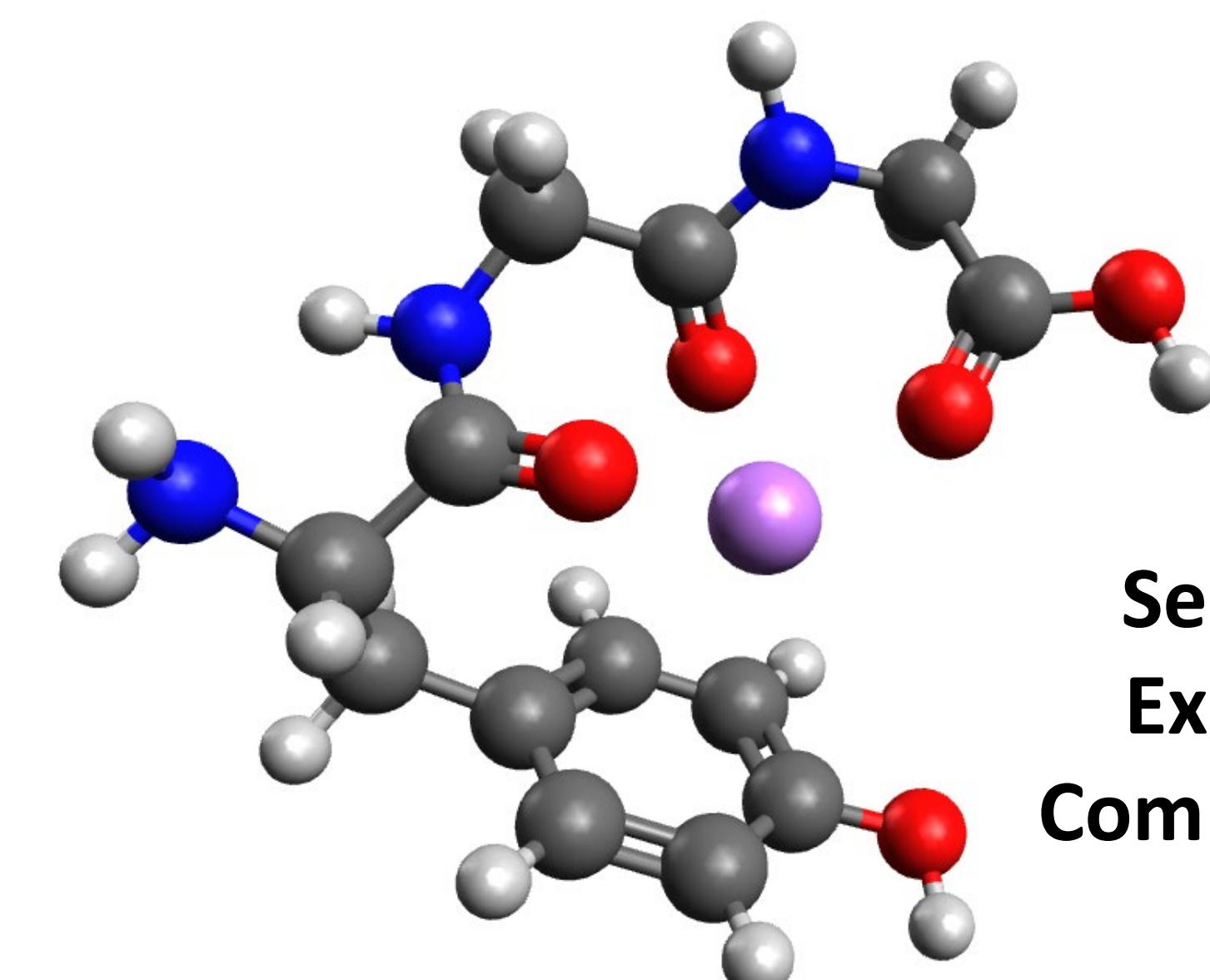
[LE + Li]⁺ / [Fragment + Li]⁺ Cross-section Ratio



a₄ + Li⁺ - 1
Exact Mass: 403.20
Sequence: Tyr – Gly – Gly - Phe
Exp. σ ratio = 1.23 ± 0.01
Comp. σ ratio = 1.26 ± 0.01



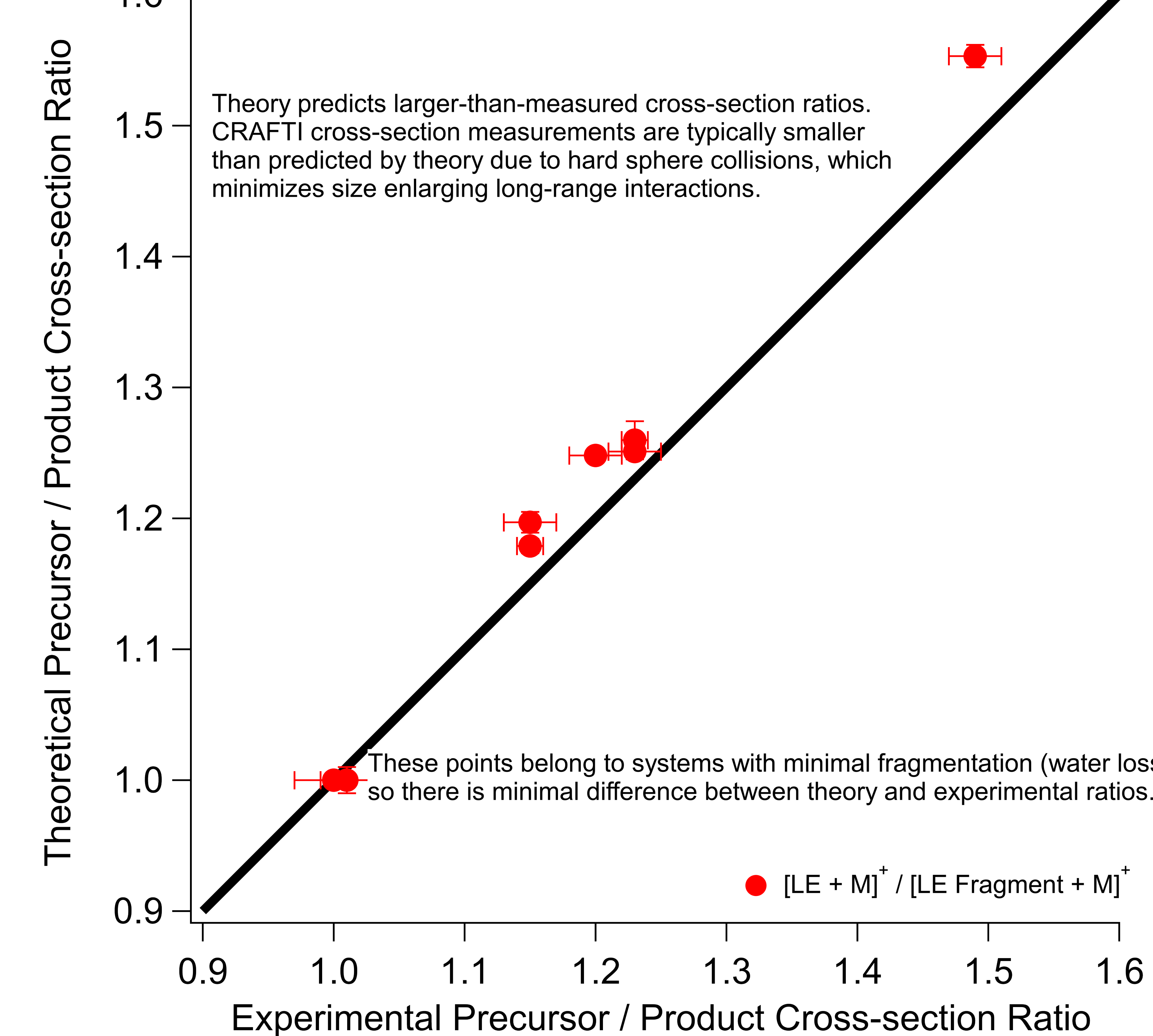
b₄ + Li⁺ + 17
Exact Mass: 449.20
Sequence: Tyr – Gly – Gly - Phe
Exp. σ ratio = 1.15 ± 0.01
Comp. σ ratio = 1.179 ± 0.005



b₃ + Li⁺ + 17
Exact Mass: 302.13
Sequence: Tyr – Gly - Gly
Exp. σ ratio = 1.49 ± 0.02
Comp. σ ratio = 1.553 ± 0.009

+ 17 is a mobile hydroxy group from original c-terminus [3] (red arrow above)
 [LE + H]⁺ doesn't dissociate in this manner [4]

SORI – CRAFTI Ratio Accuracy (vs Theory)



Conclusions

- SORI – CRAFTI: cross-section analysis of precursors and product ions
 - Likely amenable to other Ion Activation – CRAFTI strategies
- LE + Li⁺/Na⁺/K⁺ dissociates to form b₄ + M⁺ + 17
 - 17 = -OH from c-terminus
- Theory and experiment agree well enough to characterize precursor and product gas phase structures
- Future work: -OH transfer mechanism, MSⁿ – CRAFTI on smaller b_n + M⁺ + 17 fragments, unfolding – CRAFTI experiments on larger peptides

References

- [1] Yang, F., Voelkel, J., Dearden, D.V.: Anal. Chem. **84**, 4851-4857 (2012)
- [2] Anupriya, Gustafson, E., Mortensen, D.N., Dearden, D.V.: J. Am. Soc. Mass Spectrom. **29**, 251-259 (2018)
- [3] Tang, X., Ens, W., Standing, K.G., Westmore, J.B.: Anal. Chem. **60**, 1791-1799 (1988)
- [4] Schnier, P.D., Price, W.D., Strittmatter, E.F., Williams, E.R.: J. Am. Soc. Mass Spectrom. **8**, 771-780 (1997).

LE image in second column from this paper.

Acknowledgements

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