

Confident Protein and Peptide Identification with Mass Spectrometry: Understanding and Exploiting Collision Energy Dependence

Ágnes Révész,¹⁾ Tibor András Rokob,¹⁾ Dániel Szabó,^{1,2)} Gitta Schlosser,²⁾ Márton Gyula Milley,¹⁾ Lilla Turiák,¹⁾ Károly Vékey,¹⁾ László Drahos¹⁾



Email: revesz.agnes@ttk.mta.hu

¹⁾ Research Centre For Natural Sciences, HAS, Budapest, Hungary

²⁾ Eötvös Loránd University, Budapest, Hungary



Paper:
J. Proteome Res.
2018, 17, 1898

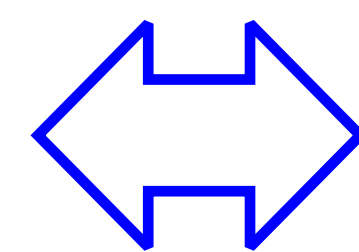
INTRODUCTION

In data-dependent analysis, peptides are identified by **database search** of **collision-induced dissociation MS/MS** spectra.

What is the optimal collision energy for peptide fragmentation?

Literature definitions

- Maximum intensity for a fragment ion type or transition
- Producing a given survival yield



Definition in this work

- **Practical approach:** peptides identified with **highest confidence**
- Confidence described as **high score:** low chance of the database search identification being random coincidence

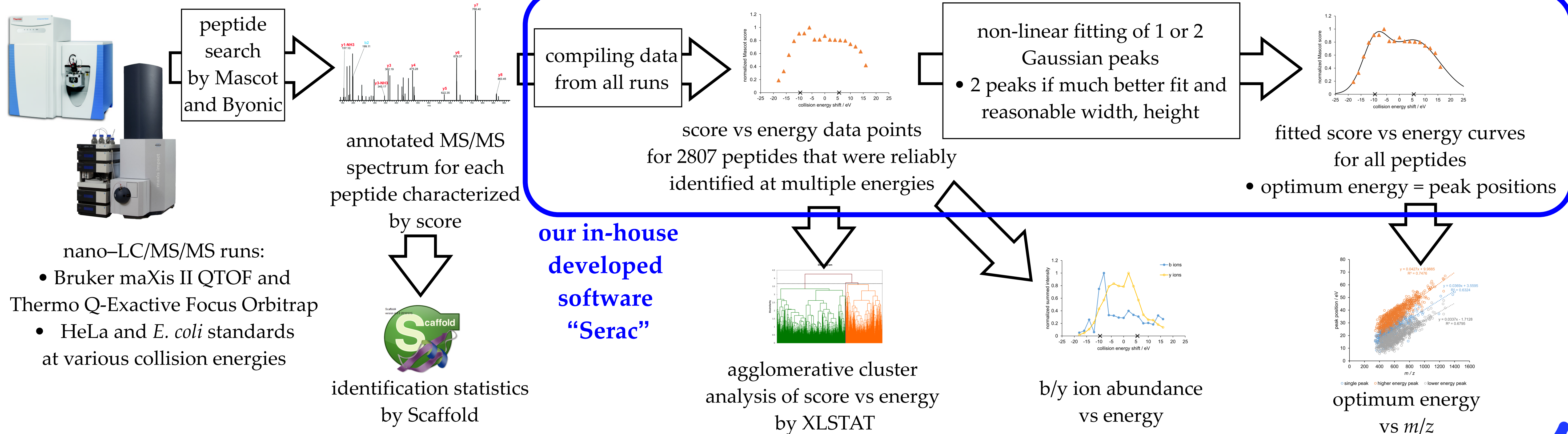
OUR AIM

- Increase
- confidence of peptide identification
 - number of identified compounds
 - sequence coverage of proteins

OUR APPROACH

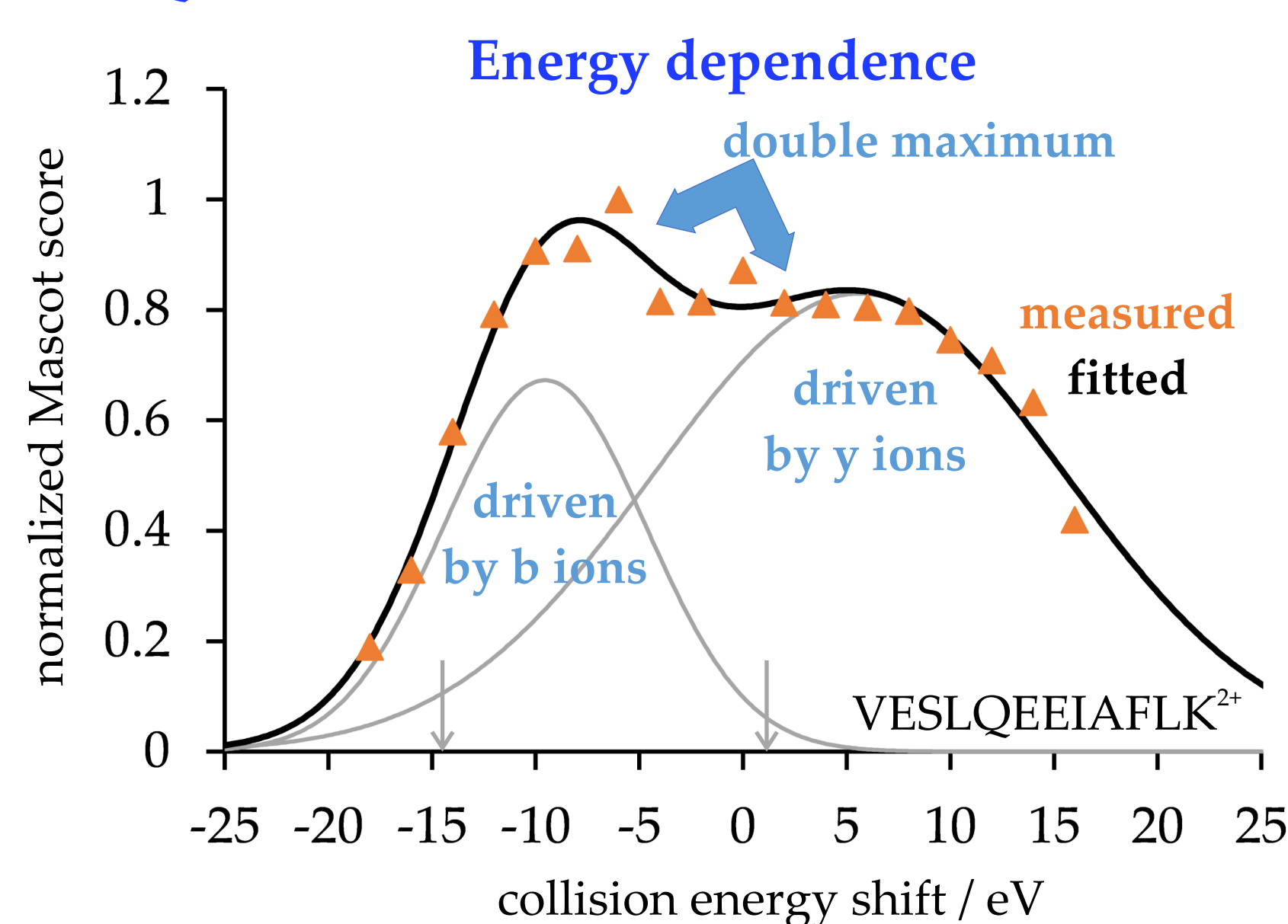
- map the score – collision energy dependence
 - find optimal settings
- using several instruments and search engines

METHODOLOGY

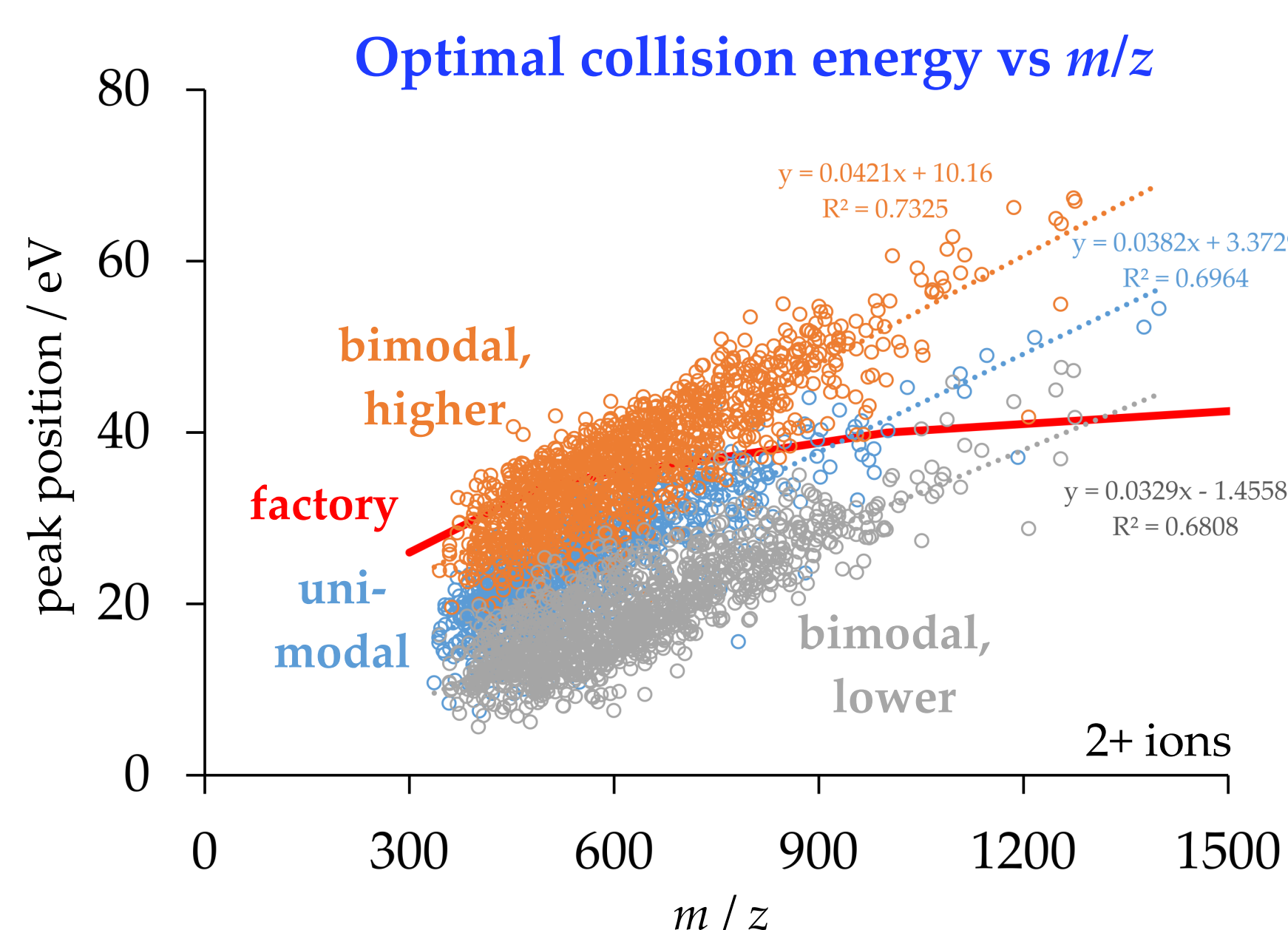


RESULTS AND CONCLUSIONS

I. QTOF / Mascot



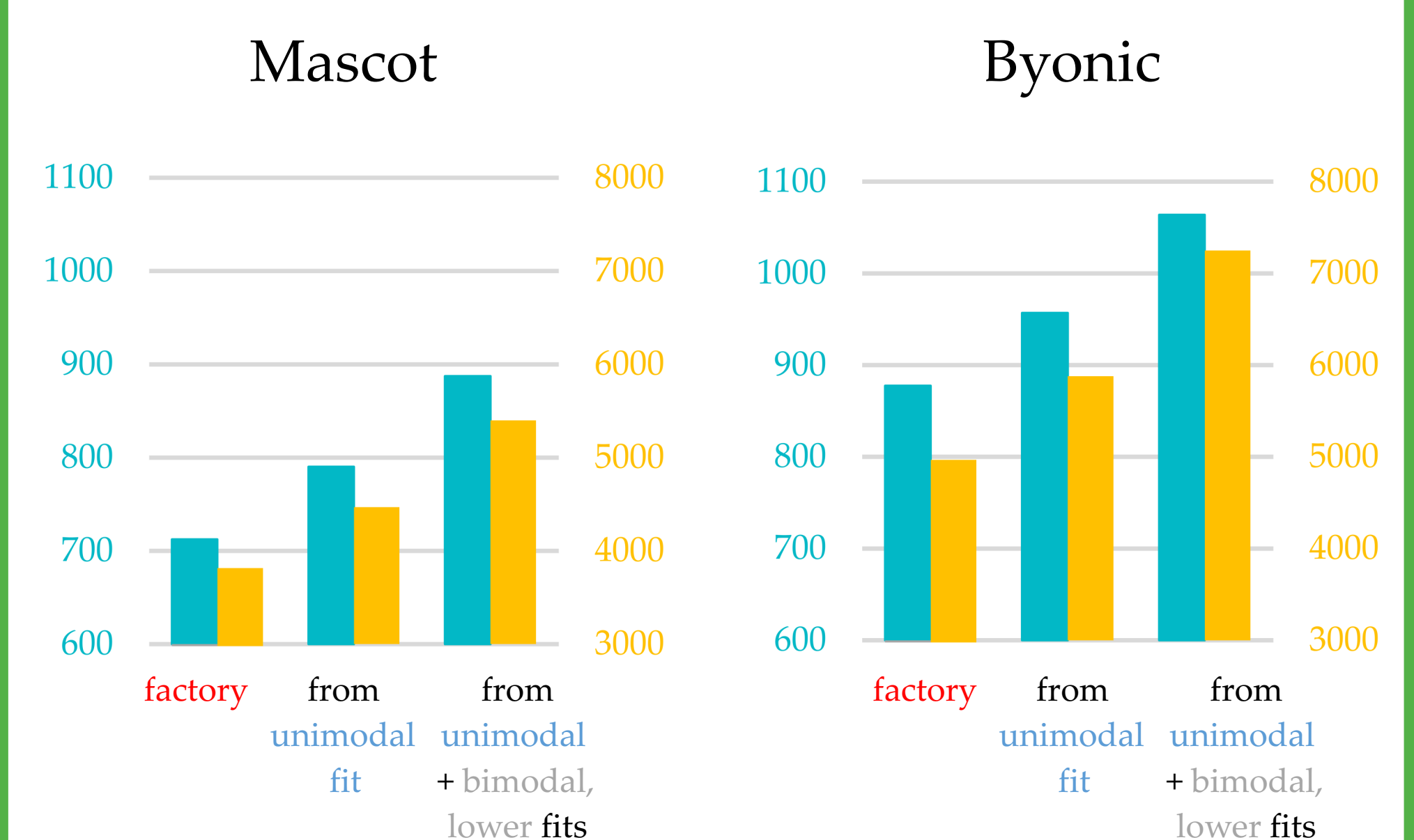
- figure shows example of a single curve
- **unprecedented bimodal behavior**
- **peaks associated with b and y ion abundance maxima**



- figure shows fitted peak positions vs. m/z
- **optimal values notably different from factory settings**

PERFORMANCE GAIN

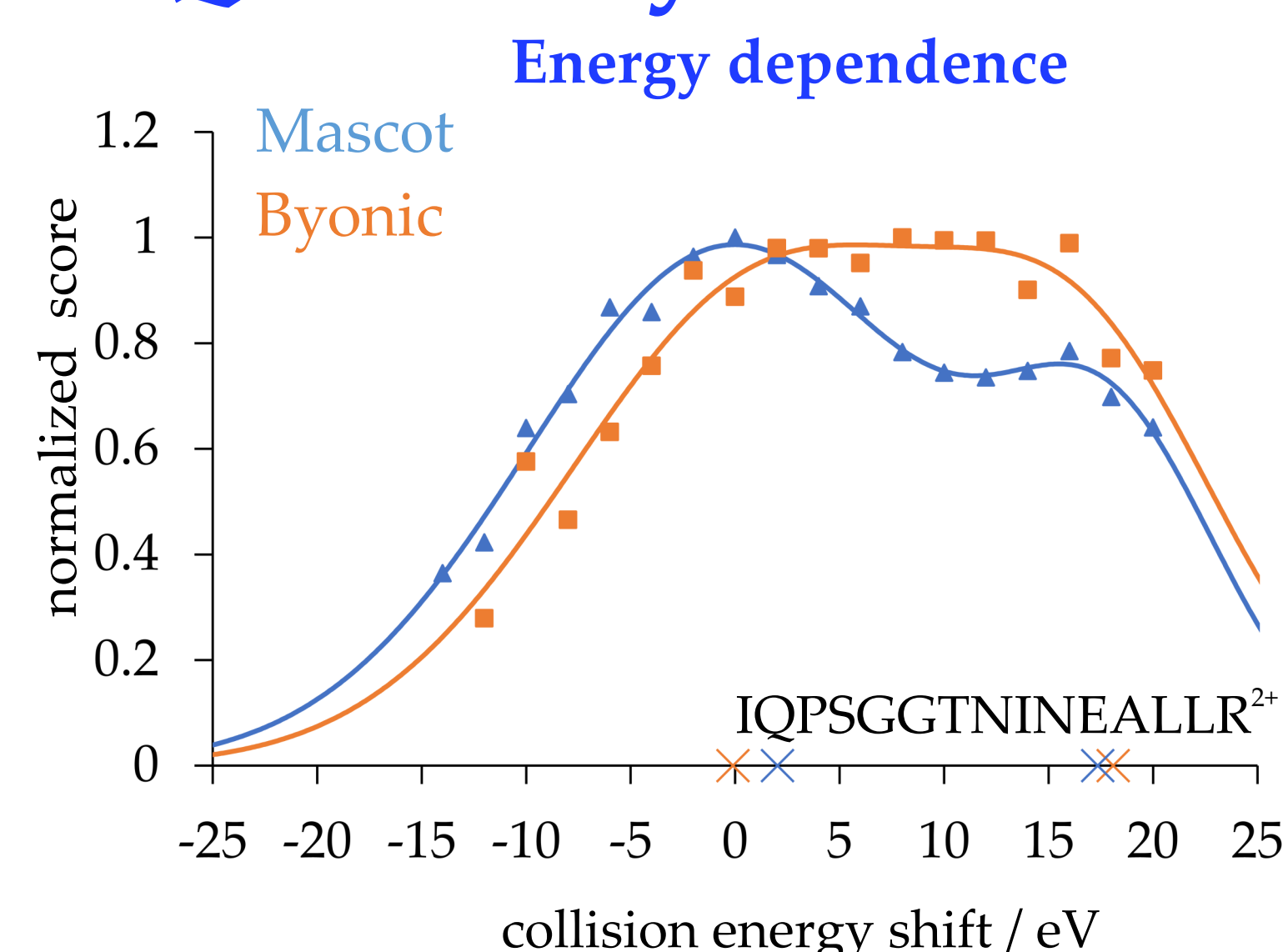
Number of identified **proteins** and **peptides** as a function of collision energy settings



Using the unimodal fit, or a combination of unimodal + bimodal lower fits, we observe increase in

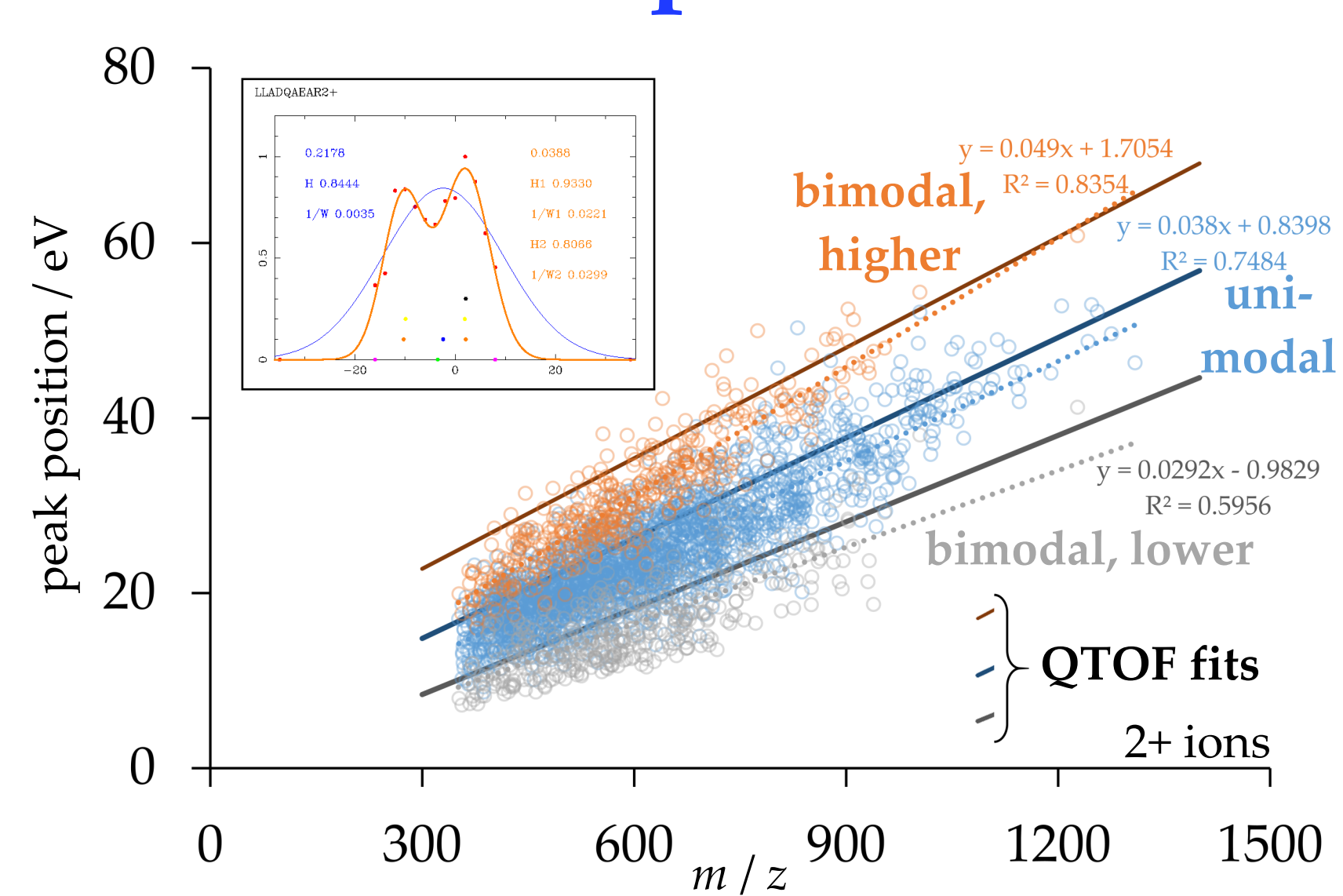
- **confidence:** score +10% / +18%
- **number:** proteins +10% / +23%
peptides +17% / +42%
- **sequence coverage:** +24% / +39%

II. QTOF / Byonic



- **comparable behavior for the other search engine**

III. Orbitrap / Mascot



- Orbitrap instrument results similar to QTOF
- **slightly lower optimal energies from fits**