

# Ion Funnel Transmission Increases Number of Protein Identifications in Complex Proteomics Samples

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## Introduction

Ion traps are widely used as workhorses in life science research due to their high spectral rate and unmatched MS/MS sensitivity; however there is a high demand to further push their limits. An improved ion transfer using funnel-ion guide allows for a substantial increase of ion flux. With a new ion trap MS (Bruker amaZon) the expected increase in sensitivity is examined for proteomics applications.

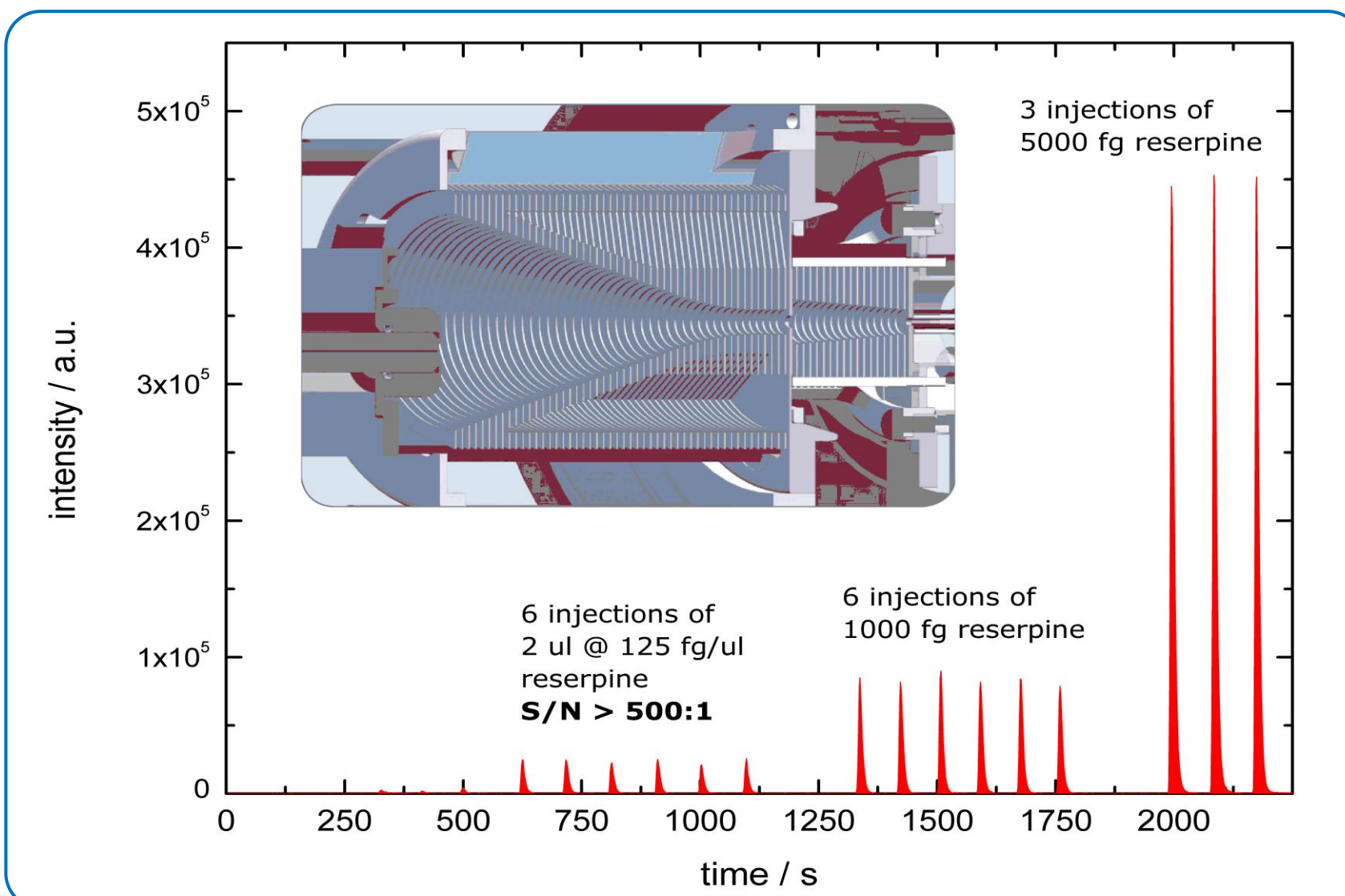


Fig. 1 Subsequent injections of different amounts of reserpine into the amaZon. 250fg reserpine on column (flow rate: 200 µl/min) result in a S/N > 500:1.

## Methods

Unlike any other ion guide a funnel system focuses ions from a large initial volume into the exit orifice. Ions exit the capillary with considerable kinetic energy and a strong beam divergence, due to the strong gas expansion at this stage. The funnel accepts ions within a wide beam angle and thermalizes their kinetic energy by collisional damping. An axial potential gradient drives the ions further down the funnel, where the geometric confinement focusses the ions and delivers them through the small exit aperture to the next pumping stage.

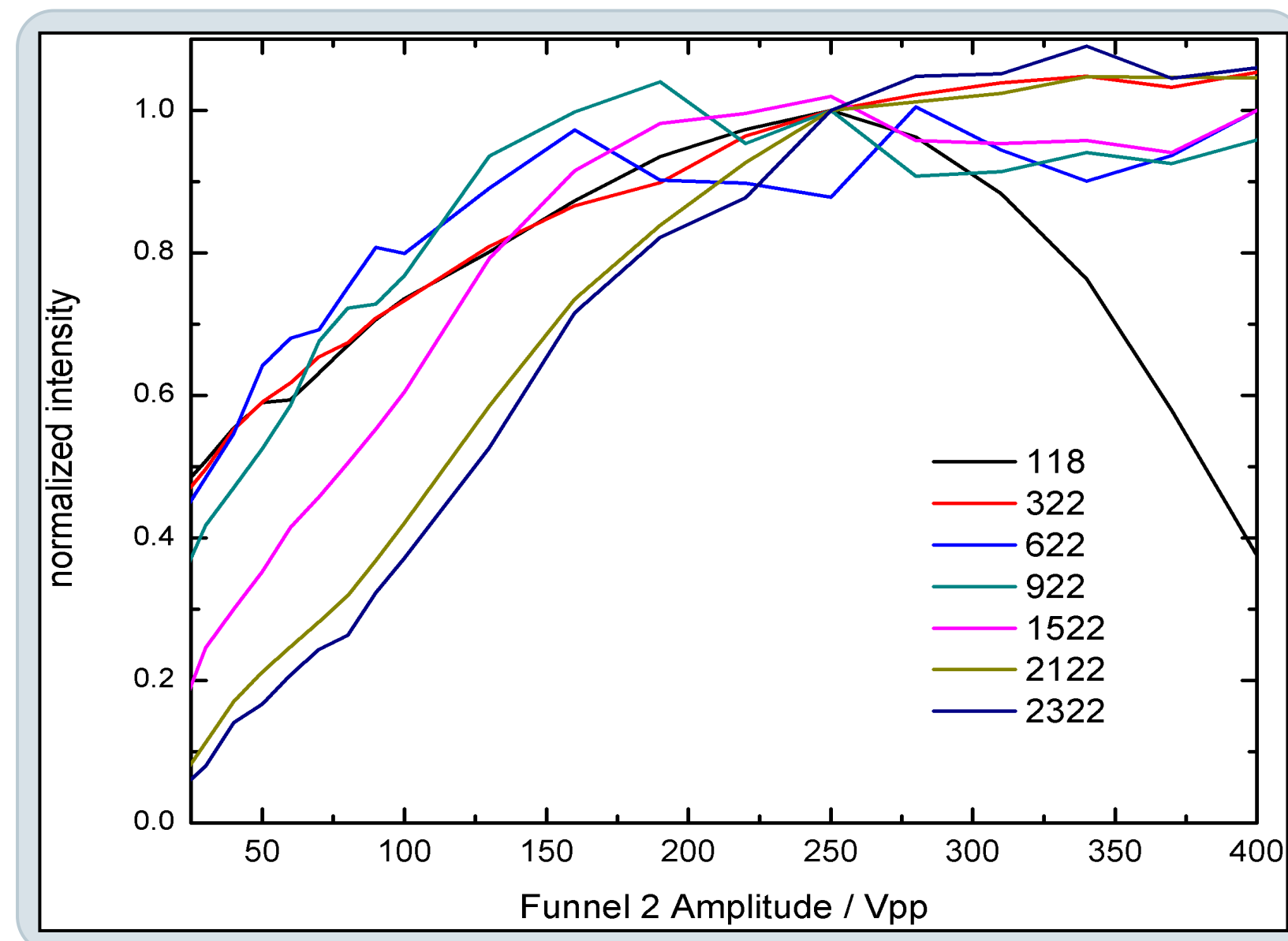


Fig. 2 Mass dependency of ion funnel transmission. Normalized ion intensities for a broad range of ion masses as function of the radio frequency amplitude applied to the funnel device.

## Results

### Mass (in-)dependency

Ion funnel transmission shows little mass selectivity For m/z values relevant for proteomics (>300 Th) transmission shows a broad RF optimum nearly identical for all masses between 322 and 2322 Th, superseding any compromises like RF stepping of other ion guides as used in other trap devices (Fig. 2).

### Transmission efficiency

Compared to skimmer ion optics MSMS sensitivity for reserpine injections could be increased 10fold resulting in a signal to noise > 500 for 250fg on column (Fig 1).

### Protein Identification

Besides the increase in MSMS sensitivity an increased ion flux into a trap is expected to result in a significantly faster duty cycle during data dependent autoMSMS experiments. Thus more precursor ions can be fragmented in the same amount of time. Replicate injections of 5 ng of an *E.coli* digest were separated using 90 min nanoLC gradients at 300 nl/min. Protein identification results (Mascot 2.2) from the funnel instrument (amaZon) were compared to the previous instrument generation (HCT ultra).

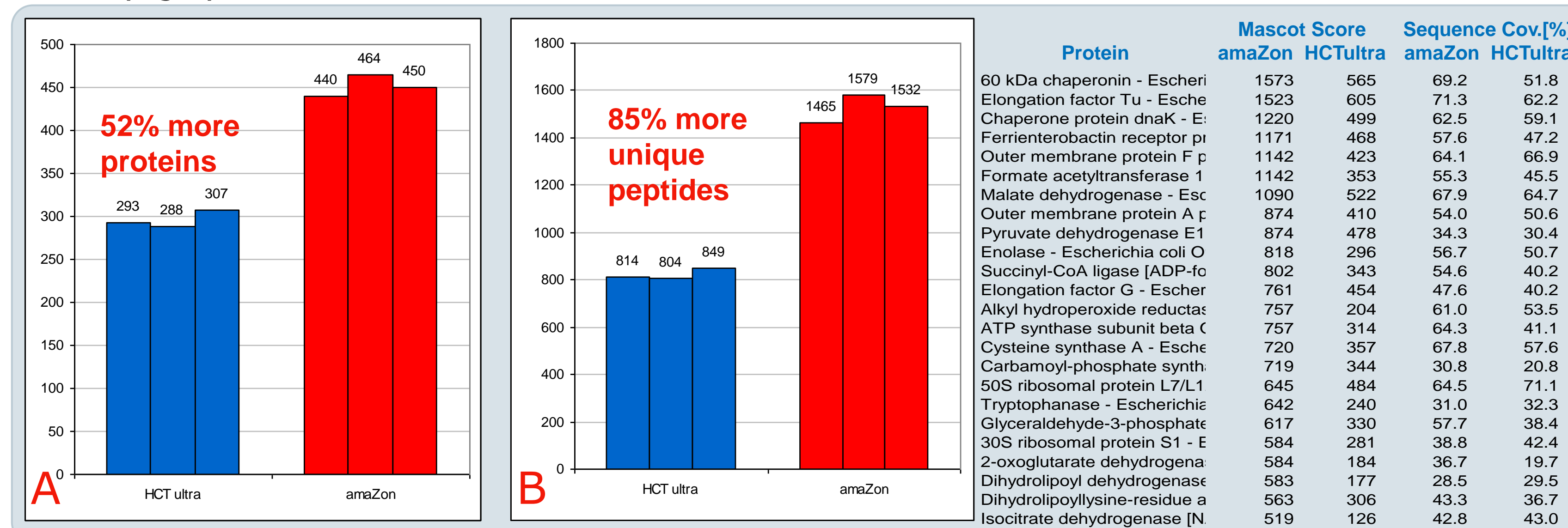


Fig. 3 Protein identification results from 5 ng *E.coli* digest separated using a 90 min nano LC gradient (300 nl/min). A: Number of identified proteins (Mascot, FDR 1.5%); B: Number of unique peptide sequences (ions score >30). C: List of top 25 protein hits with Mascot protein score and sequence coverage.

Number of proteins increased 52%, the number of identified unique peptides 85% (Fig. 3).

Even for the top matching hits data quality could be increased significantly, leading to higher Mascot scores and sequence coverage for individual proteins.

The average ions score for all peptide hits above identity threshold increased from 42.9 to 49.8, showing the general benefit for spectra quality.

## Conclusions

Ion funnel transmission for an ion trap MS results in:

- 10x increase in ion flux
- 10x higher MSMS sensitivity
- 50% more identified proteins
- more confidence and higher sequence coverage even for high abundant proteins