

# Enhanced Peptide Identification with an Ultra High Resolution Mass Spectrometer

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Analysis of biological samples frequently involves the identification of peptides from low amounts of complex samples. Confident identification of these peptides requires rapid generation of high quality, high sensitivity MS and MS-MS data. The maXis TOF mass spectrometer incorporates novel technical innovations, which together produce an unprecedented level of data quality, resulting in a significant increase in the number of peptides identified from challenging samples.

## Introduction

For complex samples, resolution is required from both the LC and the mass spectrometer. Modern day LC systems exploit rapid LC gradients that offer increased speed of analysis. Very narrow peak widths are produced, commonly only 1 sec wide compared to 30 sec with a standard LC. This effectively increases the peptide concentration reaching the mass spec and, therefore, results in increased sensitivity. It is however, essential that the mass spec duty cycle is fast enough to detect these peaks as they rapidly emerge from the LC system and that enough data points are acquired from each peak profile, particularly if quantification is desired.

## Experimental

**Complex sample:** 100 ng of *E. coli* cells. Low abundance sample: 500 amol Bovine serum albumin (BSA). Both samples were digested with trypsin.

**LC systems and gradients:** Ultimate 3000 (Dionex) and EASY-nLC (Bruker). Gradient: from 5 to 40% ACN, 0.1% formic acid, in 90 min (*E. coli*) or 10 min (BSA), flow rate 300 nL/min. Analytical column: 75  $\mu\text{m}$   $\times$  150 mm, trapping column, 100  $\mu\text{m}$   $\times$  10 mm; Acclaim PepMap100 C18 (Dionex).

**MS system:** Bruker Daltonics maxis ultra high resolution TOF.

## Results

**Complex sample:** From 100 ng of digested *E. coli* cells, 690 proteins were identified with a stringent false positive detection rate of 0.57% (Figure 1). 8507 spectra were acquired and searched by Mascot 2.2. The Swissprot 56.1 database was used, with taxonomy restricted to *E. coli*. The modifications included were carbamidomethyl Cys (fixed) and oxidized Met (variable).

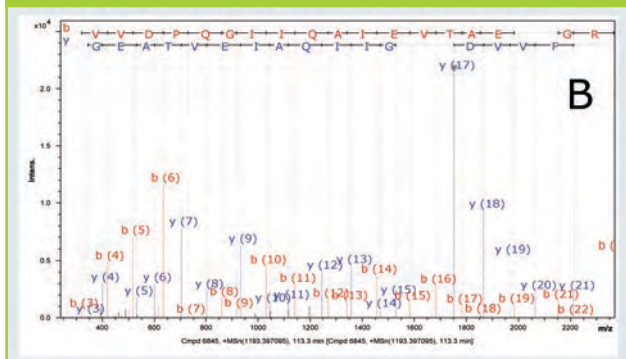
Figure 1: ProteinScope screenshot of protein list (start and end). 690 proteins were identified from 100 ng digested *E. coli* cells.

Info	OK	Accession	Protein	MW [kDa]	pI	Scores	Peptides	SC
	<input type="checkbox"/>	EFTU_ECOLI	Elongation factor Tu - Escherichia coli (strain K12)	43.3	5.2	4999...	26	74.4
	<input type="checkbox"/>	CH60_ECO24	60 kDa chaperonin - Escherichia coli O139:H28 (strain E24377A / ETEC)	57.3	4.7	4182...	29	72.1
	<input type="checkbox"/>	PFLB_ECOLI	Formate acetyltransferase 1 - Escherichia coli (strain K12)	85.3	5.6	3279...	36	59.5
	<input type="checkbox"/>	FEPA_ECOLI	Ferrienterobactin receptor precursor - Escherichia coli (strain K12)	82.1	5.3	3140...	25	54.2
	<input type="checkbox"/>	MDH_ECOH5	Malate dehydrogenase - Escherichia coli O9:H4 (strain H5)	32.3	5.5	2824...	17	76.0
	<input type="checkbox"/>	DNAK_ECOH5	Chaperone protein dnaK - Escherichia coli O9:H4 (strain H5)	69.1	4.7	2562...	34	58.8
	<input type="checkbox"/>	ODP1_ECO57	Pyruvate dehydrogenase E1 component - Escherichia coli O157:H7	99.6	5.4	2403...	36	50.5
	<input type="checkbox"/>	OMPF_ECOLI	Outer membrane protein F precursor - Escherichia coli (strain K12)	39.3	4.6	2358...	16	56.6
	<input type="checkbox"/>	PGK_ECOH5	Phosphoglycerate kinase - Escherichia coli O9:H4 (strain H5)	41.1	4.9	2321...	16	64.3
	<input type="checkbox"/>	ATPB_ECOLI	ATP synthase subunit beta O5=Escherichia coli (strain ATCC 8739 / ...)	50.3	4.8	1995...	20	63.3

**690 identified proteins (0.57% false discovery rate, separate random decoy database)**

	<input type="checkbox"/>	COABC_EC...	Coenzyme A biosynthesis bifunctional protein coaBC O5=Escherichia...	43.4	7.8	25.8 ...	1	8.9	679
	<input type="checkbox"/>	PTGCB_ECOLI	PTS system glucose-specific EIICB component O5=Escherichia coli (s...	50.6	9.3	25.2 ...	2	6.5	680
	<input type="checkbox"/>	YGIW_ECOLI	Protein ygiW O5=Escherichia coli (strain K12) GN=ygiW PE=1 SV=1	14.0	4.9	25.0 ...	1	6.9	681
	<input type="checkbox"/>	YDCL_ECOLI	Uncharacterized lipoprotein ydCL O5=Escherichia coli (strain K12) GN...	24.4	9.2	24.5 ...	1	11.3	682
	<input type="checkbox"/>	YJDC_ECOLI	HTH-type transcriptional regulator yjDC - Escherichia coli (strain K12)	21.9	4.8	24.4 ...	1	12.6	683
	<input type="checkbox"/>	YCAR_ECOH5	UPF0434 protein ycaR - Escherichia coli O9:H4 (strain H5)	6.9	4.8	24.1 ...	1	15.0	684
	<input type="checkbox"/>	HSCA_ECOH5	Chaperone protein hscA O5=Escherichia coli O9:H4 (strain H5) GN=...	65.6	4.8	24.0 ...	1	4.5	685
	<input type="checkbox"/>	FENR_ECOLI	Ferredoxin--NADP reductase O5=Escherichia coli (strain K12) GN=fp...	27.7	6.2	23.9 ...	1	3.6	686
	<input type="checkbox"/>	GSHR_ECOLI	Glutathione reductase - Escherichia coli (strain K12)	48.7	5.6	23.5 ...	1	5.1	687
	<input type="checkbox"/>	NUOB_ECOLI	NADH-quinone oxidoreductase subunit B O5=Escherichia coli (strain ...)	25.0	5.5	22.8 ...	1	4.1	688
	<input type="checkbox"/>	AROB_ECOLI	3-dehydroquinate synthase O5=Escherichia coli (strain K12) GN=aro...	38.9	5.7	22.5 ...	1	2.2	689
	<input type="checkbox"/>	LTAE_ECOLI	Low specificity L-threonine aldolase - Escherichia coli (strain K12)	36.5	5.8	21.6 ...	1	4.2	690

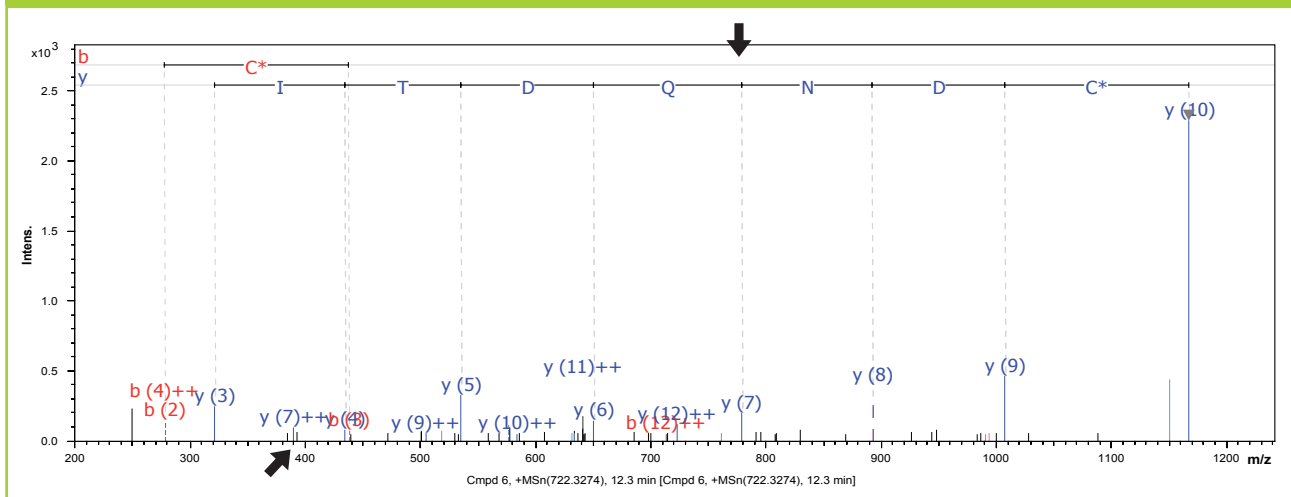
**Figure 2:** Example fragment spectrum delivering high sequence coverage.



cell into orthogonal acceleration. In addition, techniques have been employed to reduce energy distribution in the direction of flight and to ensure a precise starting position for orthogonal deflection.

In combination, these technical developments result in high quality, information rich spectra at high resolution (40000) with incredible mass accuracy (low to sub ppm) at the MS and MS-MS level along with fast acquisition speeds. A substantial increase in the number of peptides identified from both complex and low abundance samples with an increase in significance and certainty for the peptides identified has been demonstrated.

**Figure 3:** Fragment spectrum from a 500 amol BSA digest.



4917 peptides matched the identity threshold with an average Mascot score of 58.8. Thus, over 58% of MS-MS spectra were identified as significant — demonstrating the high quality of the MS-MS data produced. See Figure 2 as an example.

**Low abundance samples:** From 500 amol BSA, 13 unique peptides were identified, representing a sequence coverage of 23% and an overall Mascot scoring of 412 (Figure 3). Here, taxonomy was restricted to mammals. Again, peptides were confidently identified based on high quality MS-MS data.

Hence, these results prove maXis' capability to provide high MS resolving power to separate potentially overlapping signals in a complex sample and, therefore, to allow correct mass assignments. Ultimately, these features directly translate into high identification rates with excellent Mascot scores — even on a time scale that keeps pace with modern ultrafast LC systems.<sup>1</sup>

**Conclusion**

The key proteomics goals are identifying more peptides with more certainty. The maXis ultra high resolution TOF mass spectrometer brings together novel design technologies to reach these goals. The technological advances include a hexapole collision cell together with an ion cooler which provide efficient fragmentation of ions from a broad mass range and efficient transfer of fragment ions from the collision

**Reference**

1. Bruker Daltonics Application Note ET-16.



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