

Liquid extraction surface analysis mass spectrometry for protein analysis directly from clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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MASS SPECTROMETRY

Background

- Rise of antibiotic resistance—new methods of characterisation for bacteria required
- Mass spectrometry widely applied for both characterisation and identification of microorganisms
- Ideal arrangement—study of **live cells directly on substrate**

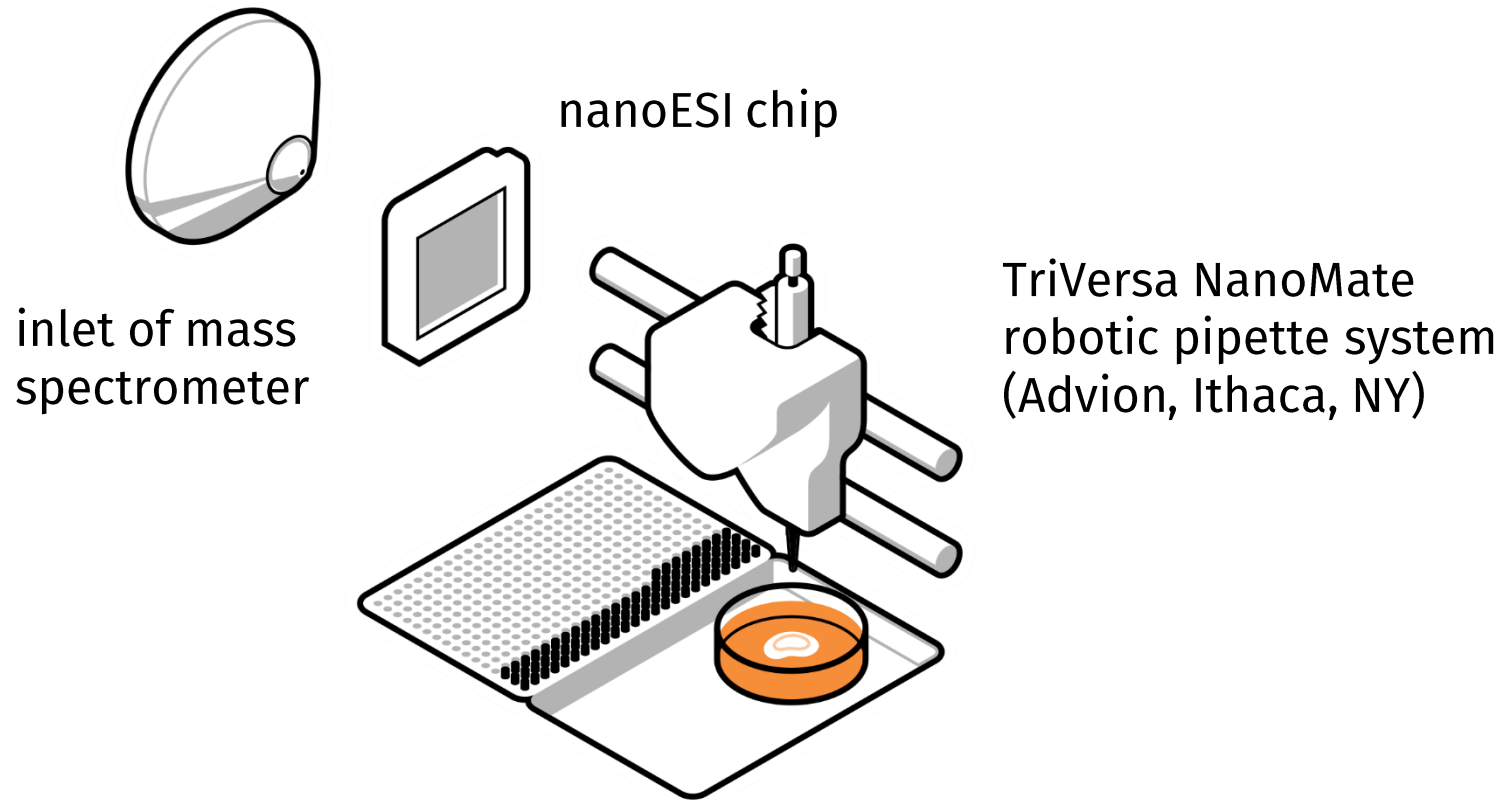


Liquid extraction surface analysis (LESA)

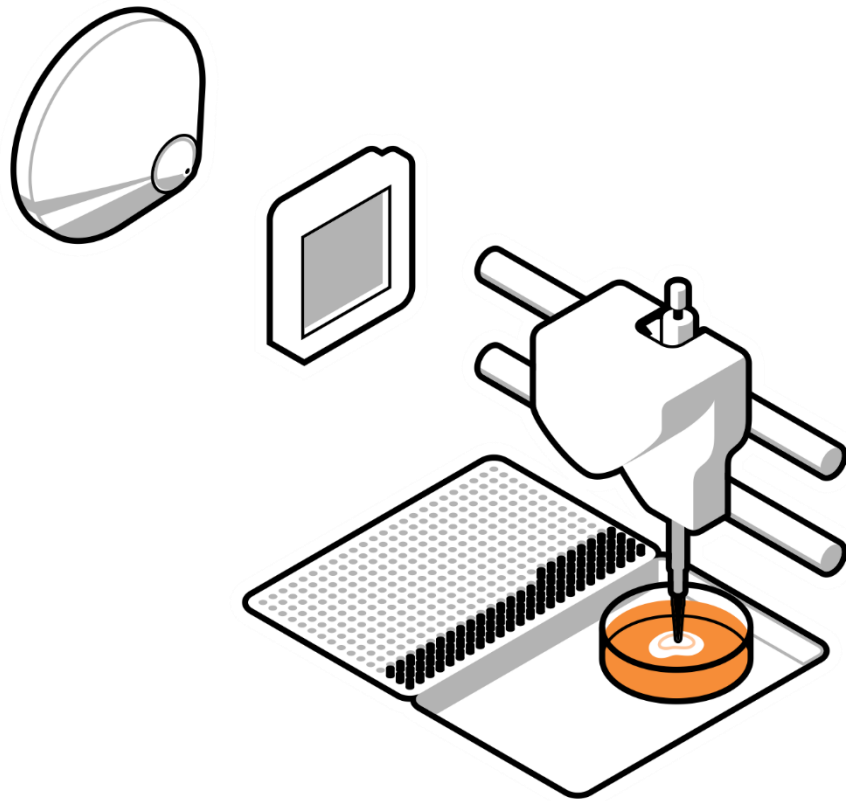
- Microjunction-based extraction followed by nanoelectrospray ionisation
- **No sample preparation**
- All classes of analytes accessed—up to and including **proteins**



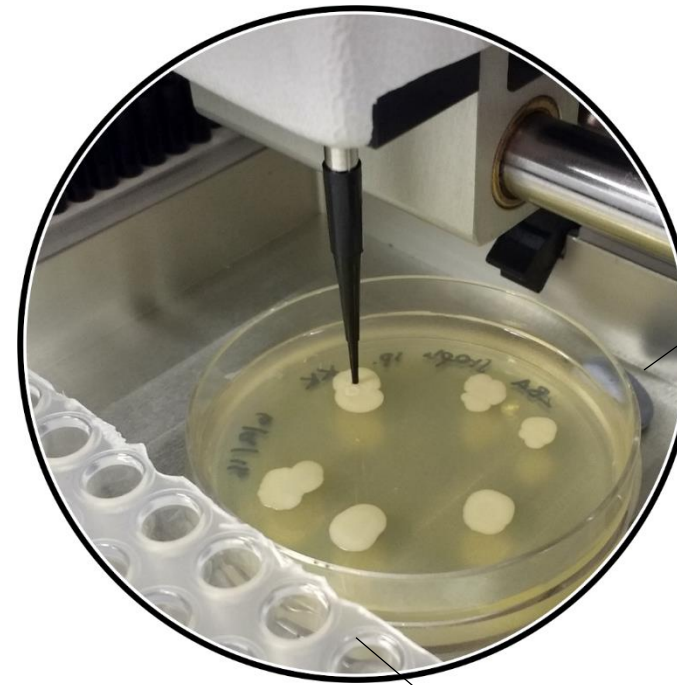
LESA-MS—how it works



LESA-MS—how it works



Sampling

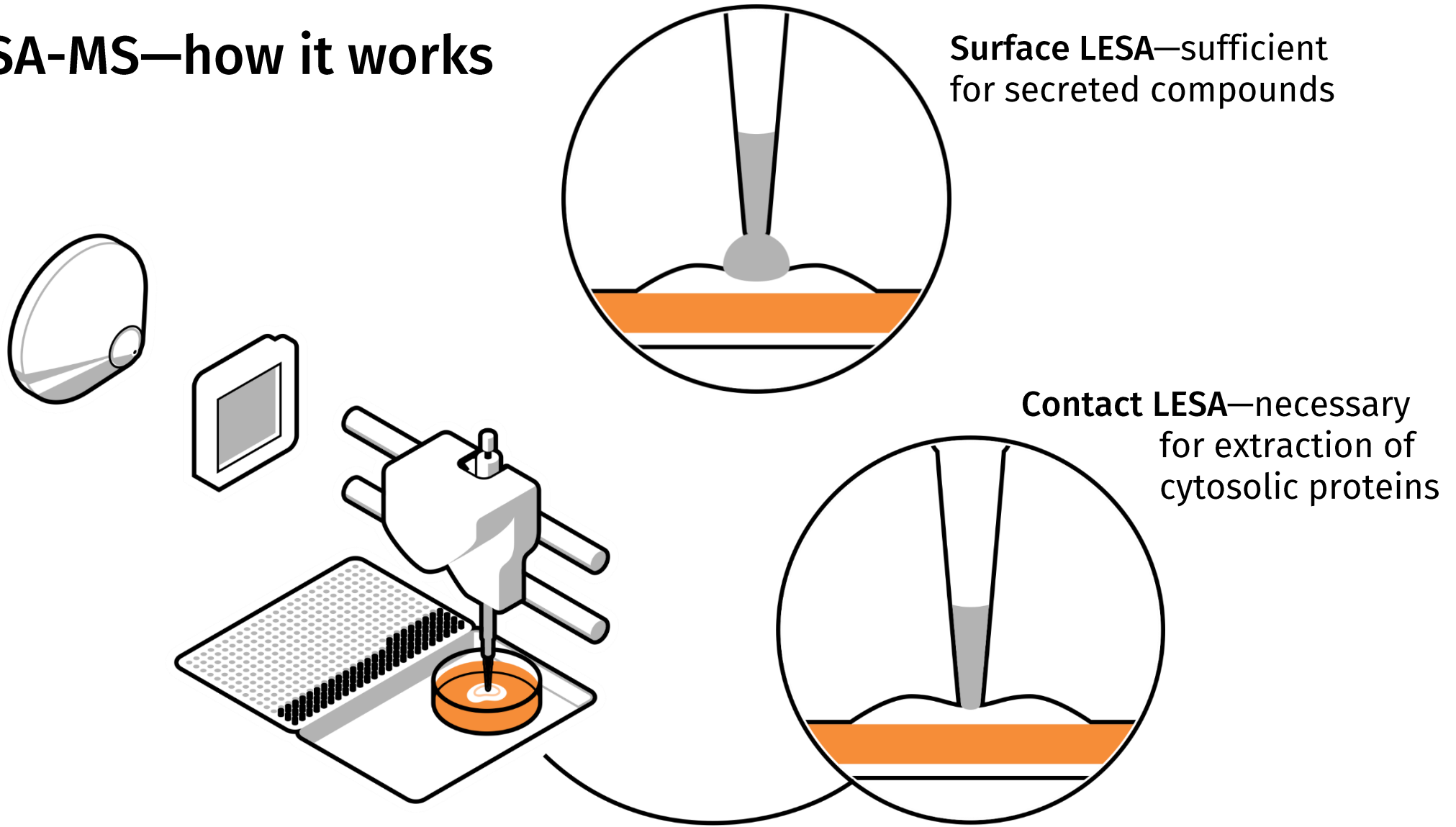


adhesive
putty

half a 96-well plate



LESA-MS—how it works

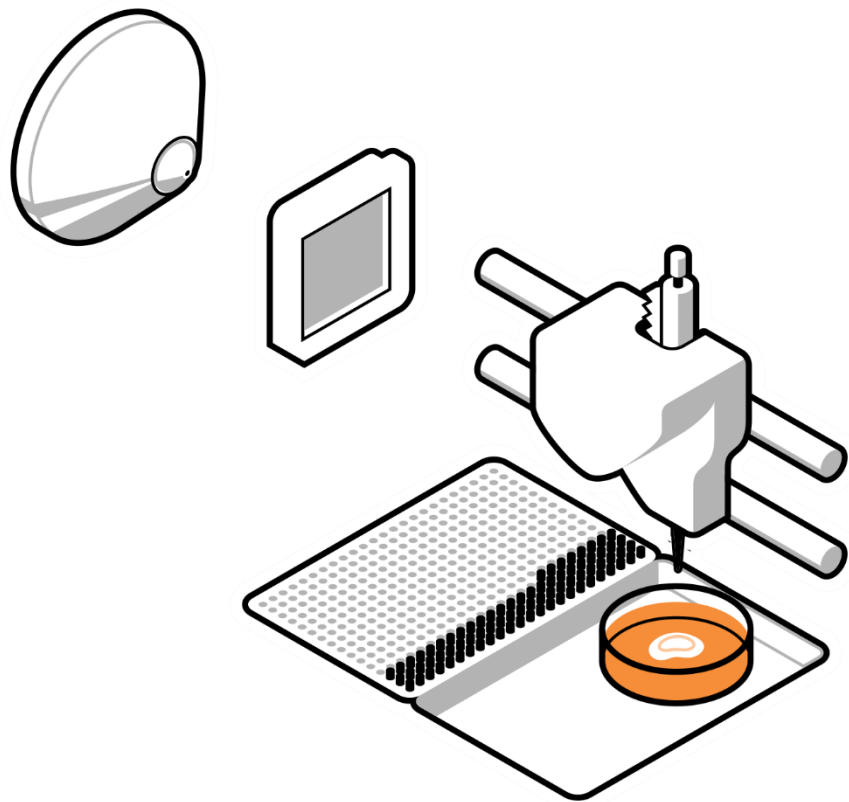


Surface LESA—sufficient for secreted compounds

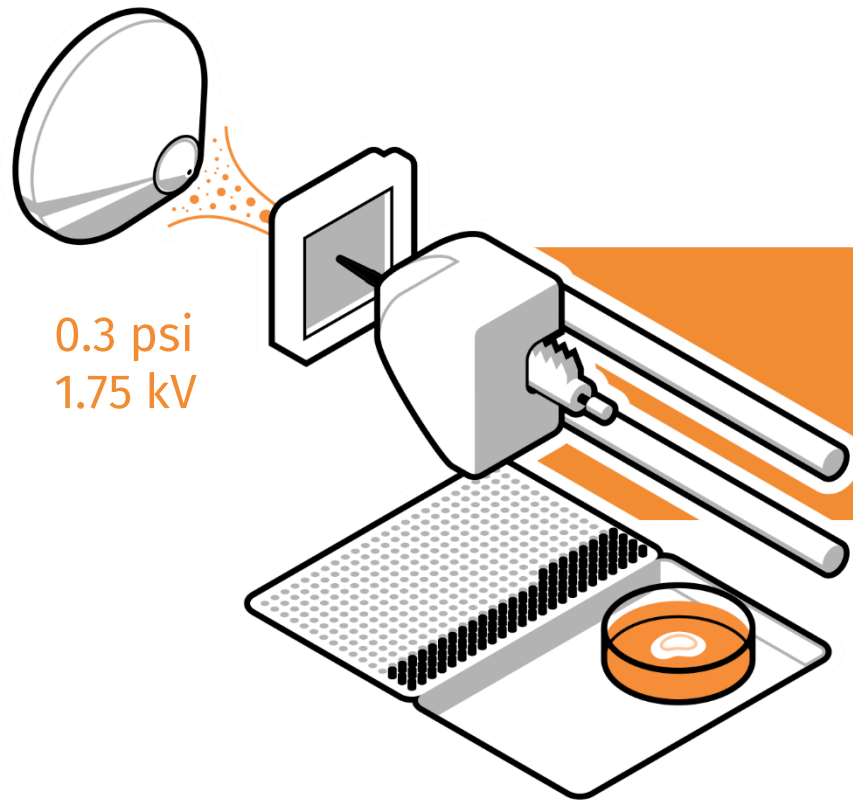
Contact LESA—necessary for extraction of cytosolic proteins



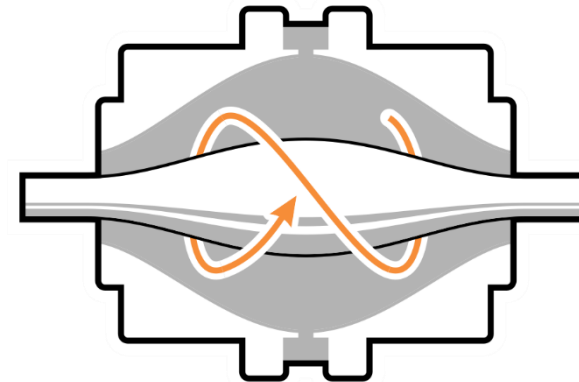
LESA-MS—how it works



LESA-MS—how it works



Analysis:
ThermoFisher Orbitrap Elite
resolution 120000
at m/z 400

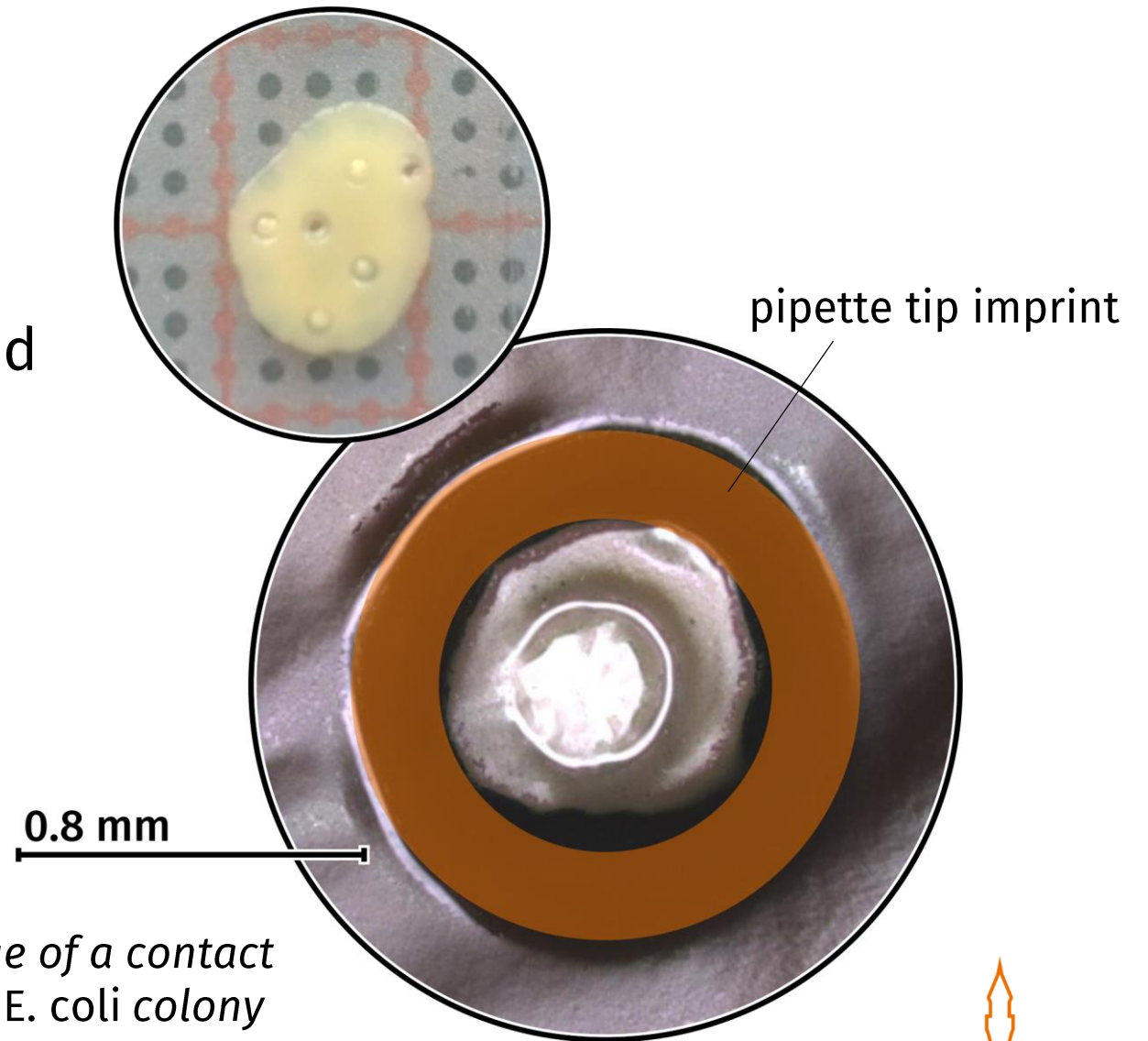


5 minutes acquisition
10 μ scans/scan



Sampling

- **40:60:1** or **50:45:5**
acetonitrile, water and formic acid
- dwell 3-15 s



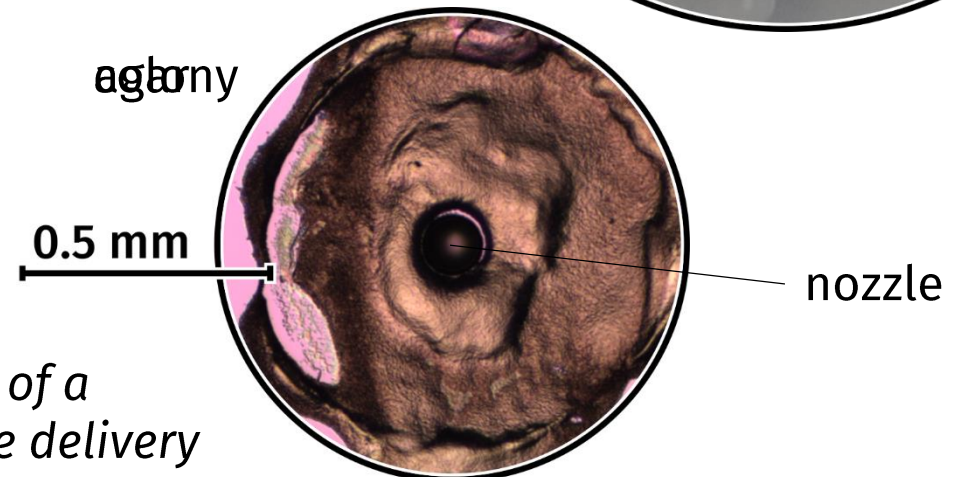
*Confocal microscope image of a contact
LEESA sampling spot on an E. coli colony*



Sampling challenges

- Correct sampling height **crucial**
 - Too high—no contact established, no cytosolic protein
 - Too low—risk of lifting colony material and blocking the nozzle

nanoESI chip
400 nozzles



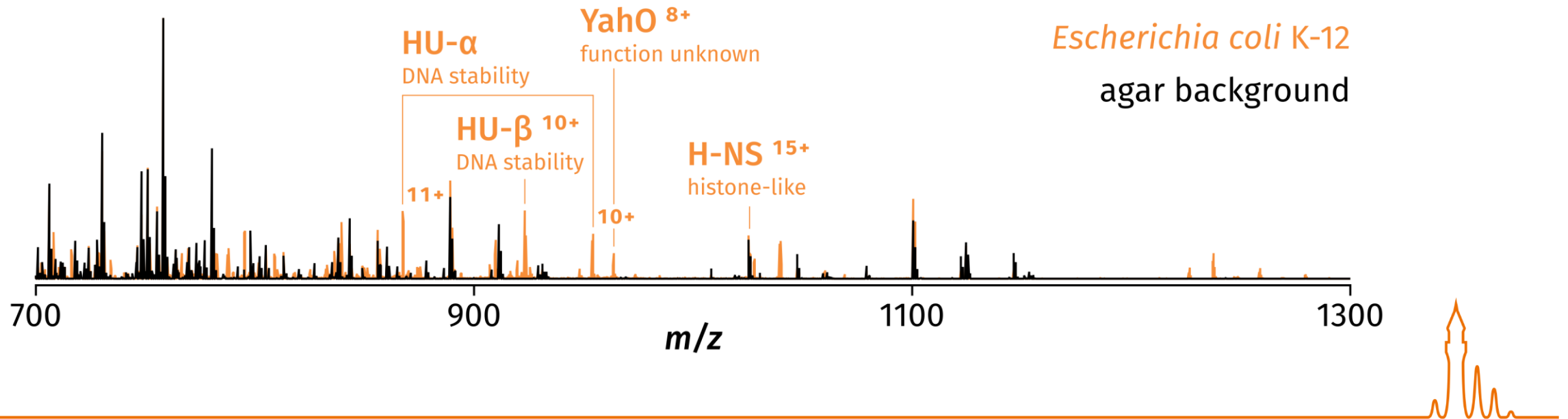
Optical microscope photograph of a nanoESI nozzle following sample delivery



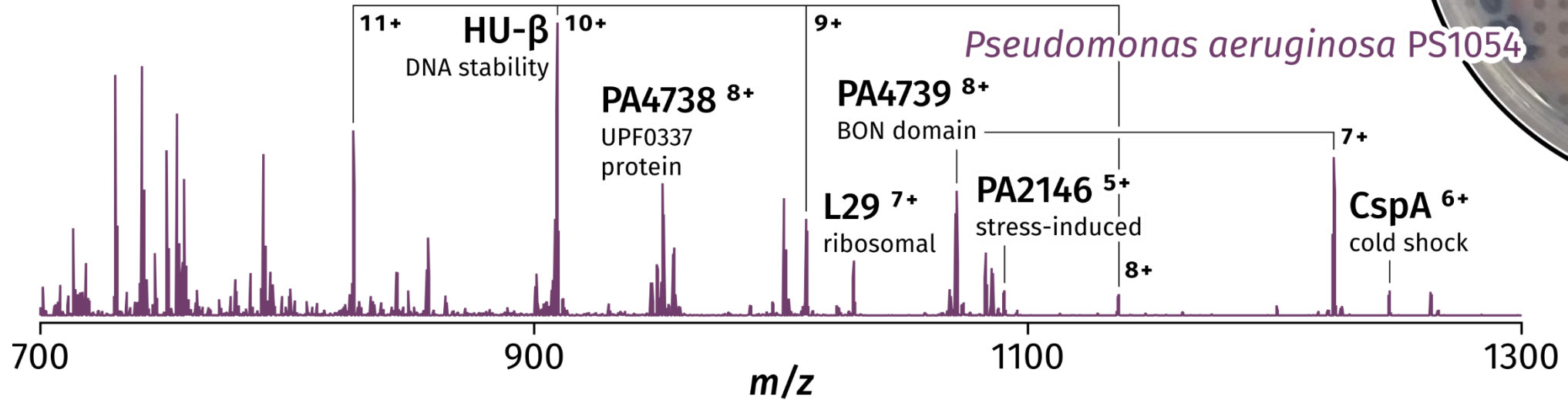
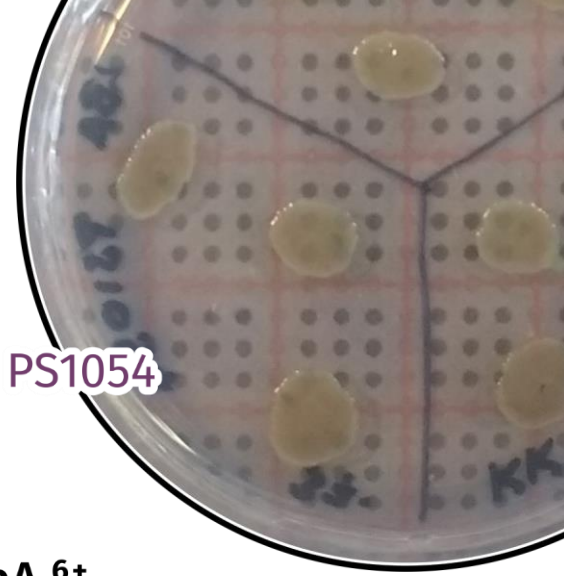
Initial work—*E. coli* K-12 model

- Development of the contact protocol
- Proteins and lipids observed

All mass spectra acquired following incubation at 37°C for 24 hours

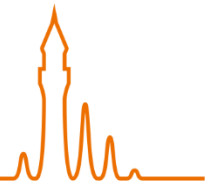


The clinical isolates—*Pseudomonas*

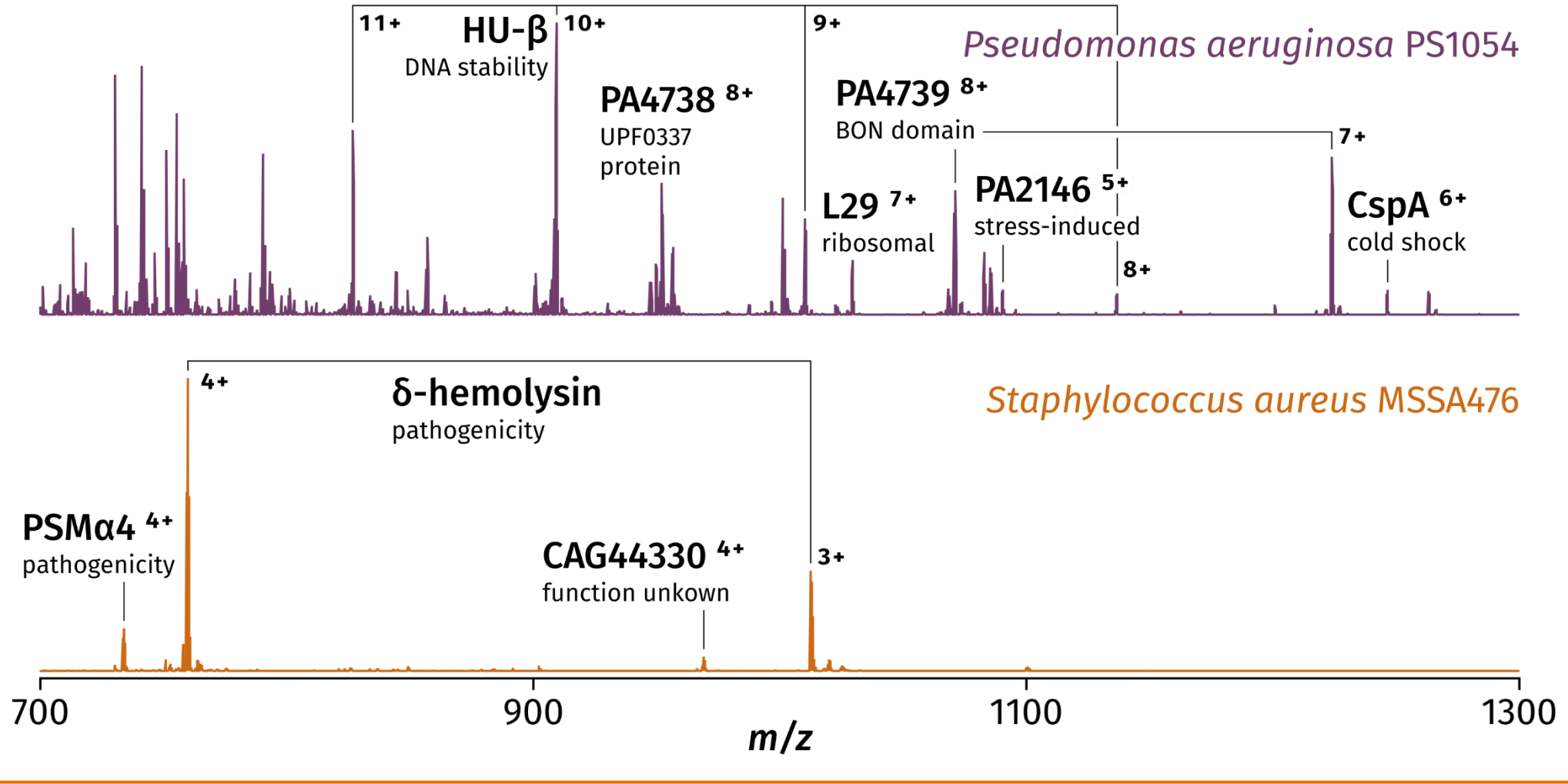


Full mass spectrum acquired following incubation at 37°C for 24 hours

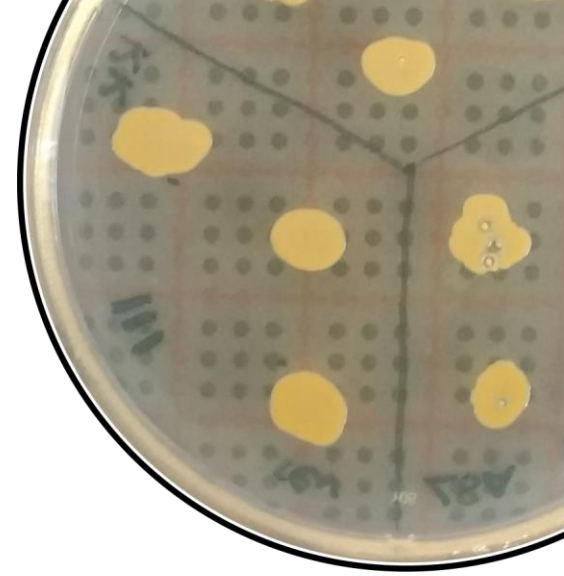
- Gram-negative
- Protein-rich mass spectra (20+ unique species)



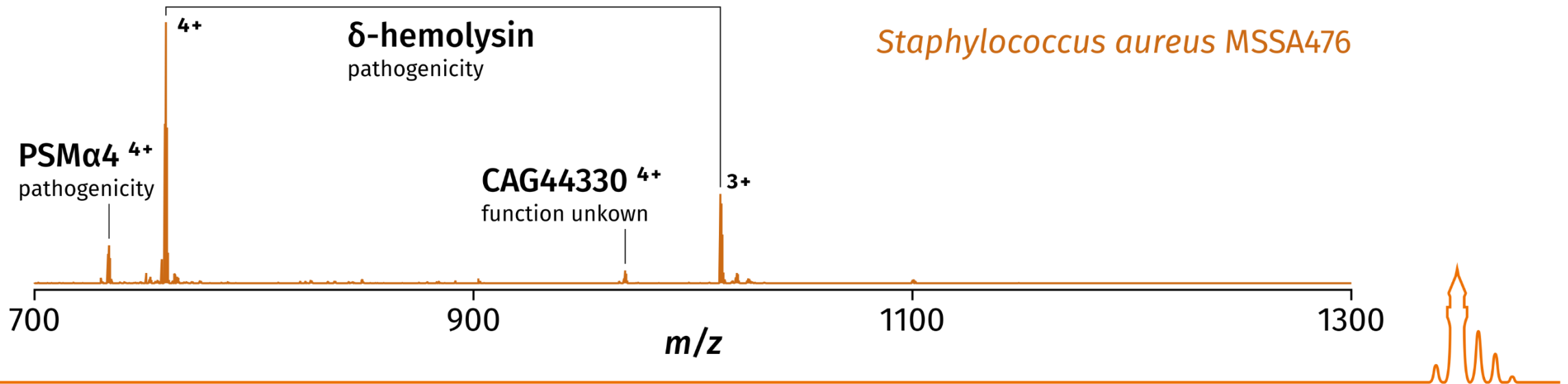
The clinical isolates



The clinical isolates—*Staphylococcus*



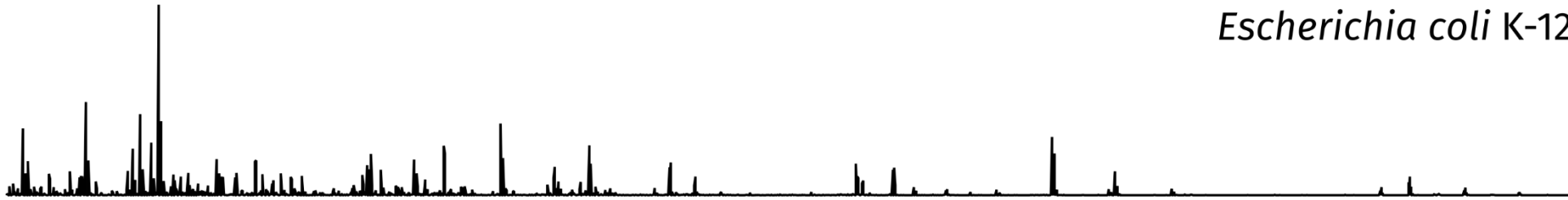
- Gram-positive
- Mass spectra dominated by **secreted peptides**



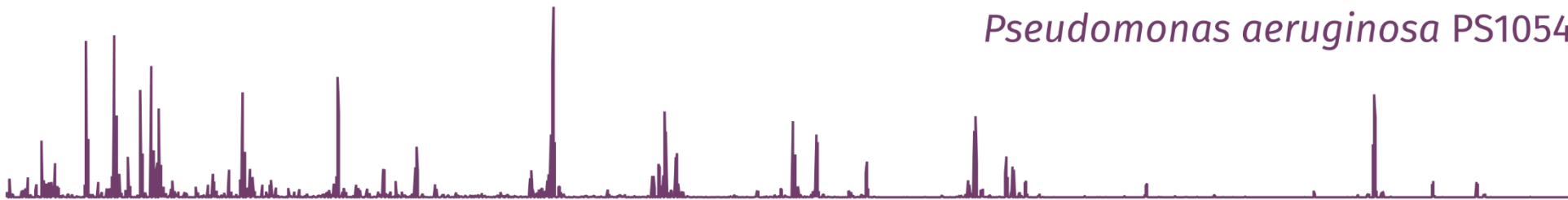
Direct comparison

All mass spectra acquired following incubation at 37°C for 24 hours

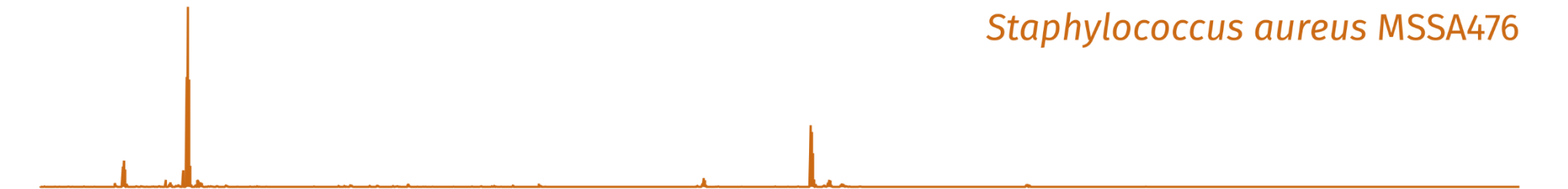
Escherichia coli K-12



Pseudomonas aeruginosa PS1054



Staphylococcus aureus MSSA476



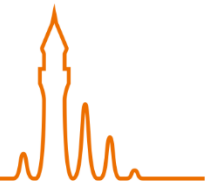
700

900

m/z

1100

1300



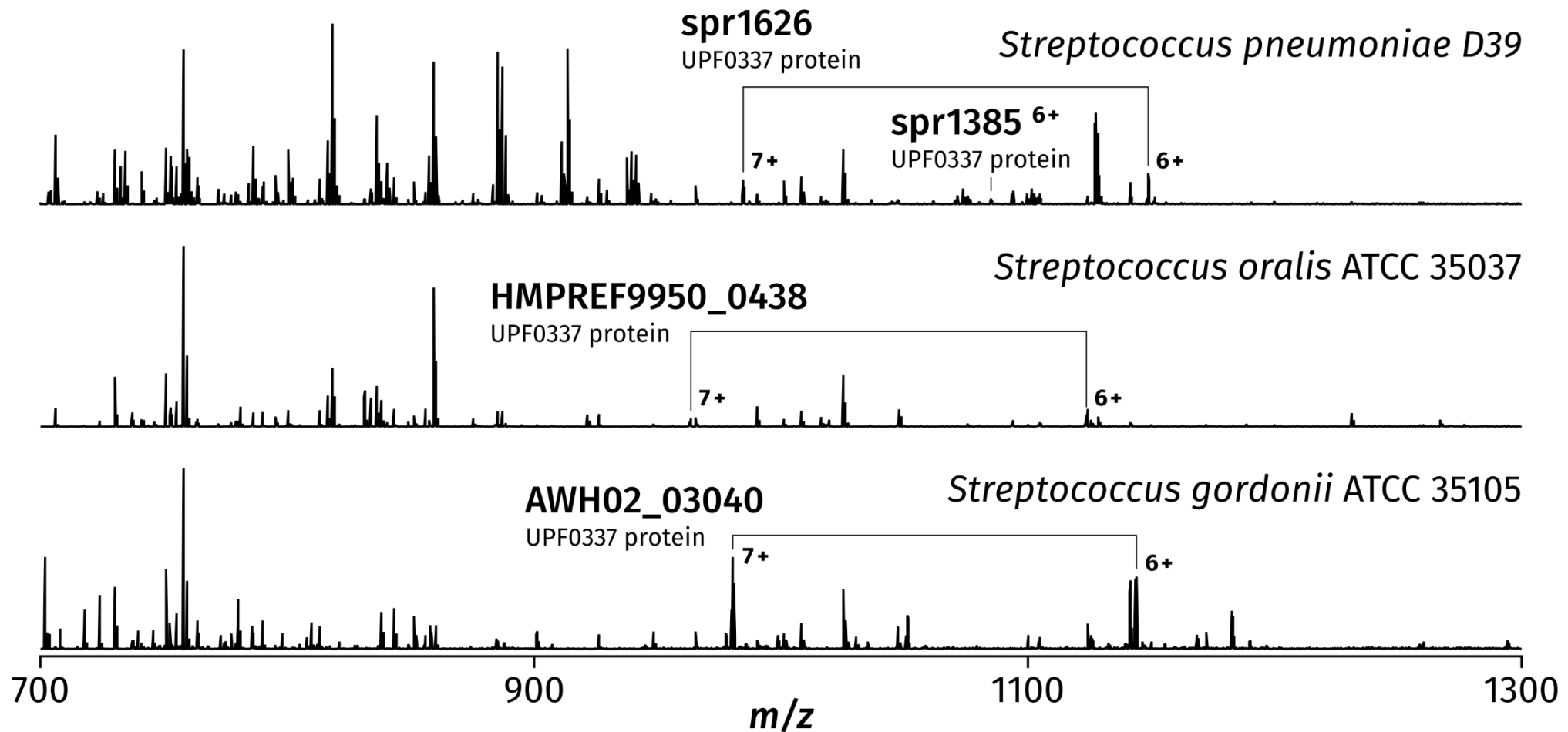
Streptococci

- Gram-positive, encapsulated
- Viridans group streptococci—notoriously difficult to distinguish by MALDI TOF MS



Streptococci

All mass spectra acquired following semi-anaerobic incubation at 37°C for 24 hours



The UPF0337 (CsbD-like) family

- Seen in **every species** probed so far
- Stress response; exact function unknown

Species	Protein	Observed mass
<i>E. coli</i>	YjbJ	8320.08
<i>P. aeruginosa</i>	PA4738	7606.81
<i>S. aureus</i>	SAOUHSC_00845	6883.45
<i>S. pneumoniae</i>	spr1385	6503.43
	spr1626	6884.53
<i>S. oralis</i>	HMPREF9950_0438	6734.46
<i>S. gordonii</i>	AWH02_03040	6854.65



MS/MS protein identification

- Mass spectra acquired over a range of bacterial growth conditions
- Fragmentation by **CID**
- Identification by **ProSightPC 3.0**
- Databases constructed using proteomes available through UniProt



MS/MS protein identification

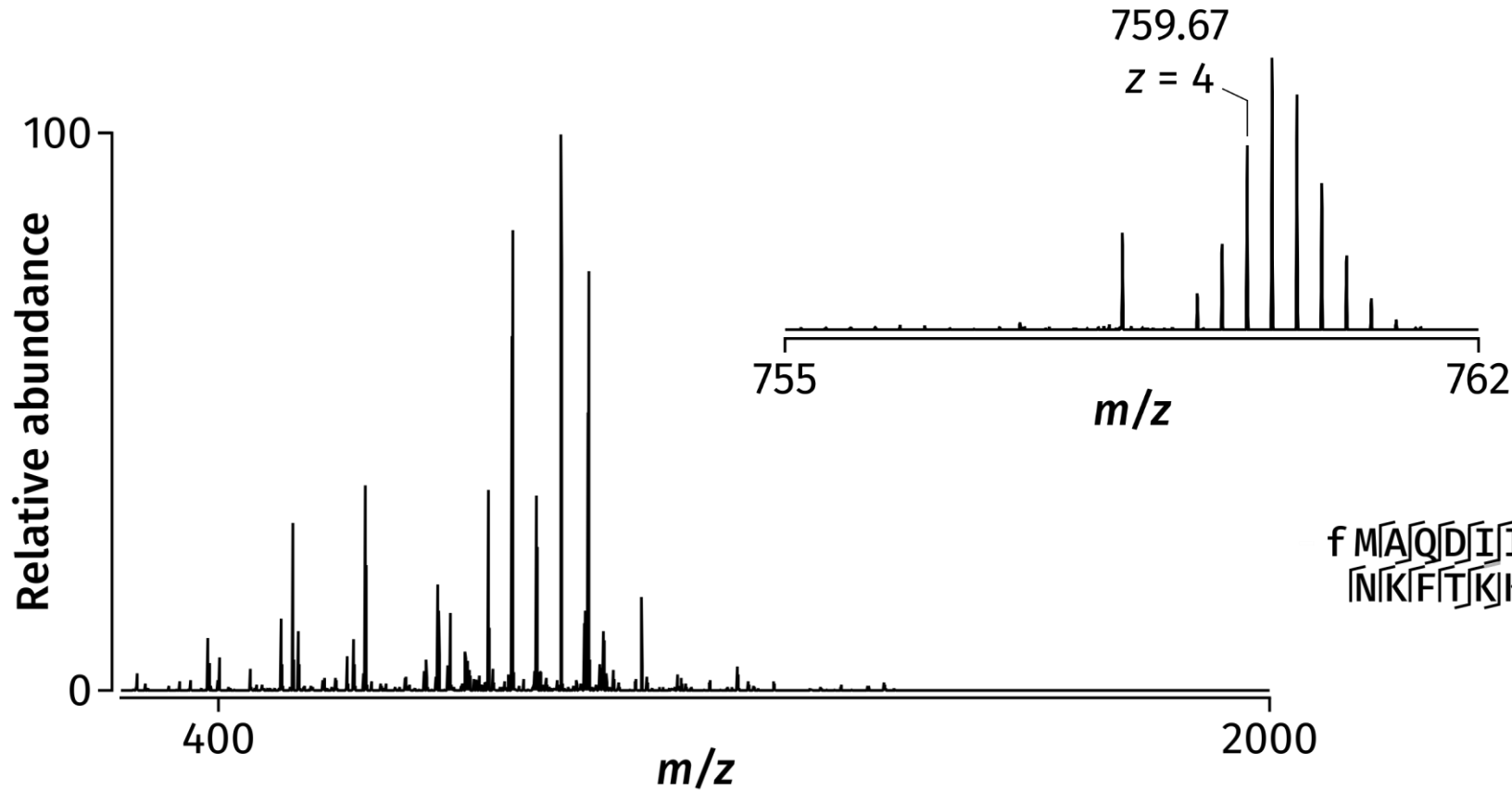
Species	Strain	Proteins identified	Sequence coverages (%)
<i>Escherichia coli</i>	K-12	15	11-91
	BL21 mCherry		
<i>Pseudomonas aeruginosa</i>	PS1054	15	14-67
<i>Staphylococcus aureus</i>	MSSA476	6	89-100
<i>Streptococcus pneumoniae</i>	D39	2	76-85
<i>Streptococcus oralis</i>	ATCC 35037	1	82
<i>Streptococcus gordonii</i>	ATCC 35105	1	71
Total		40	



MS/MS protein identification

***S. aureus* δ -hemolysin**
well-characterised toxin

M_{obs} 3034.64 Da
0.8 ppm



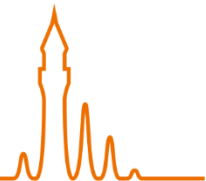
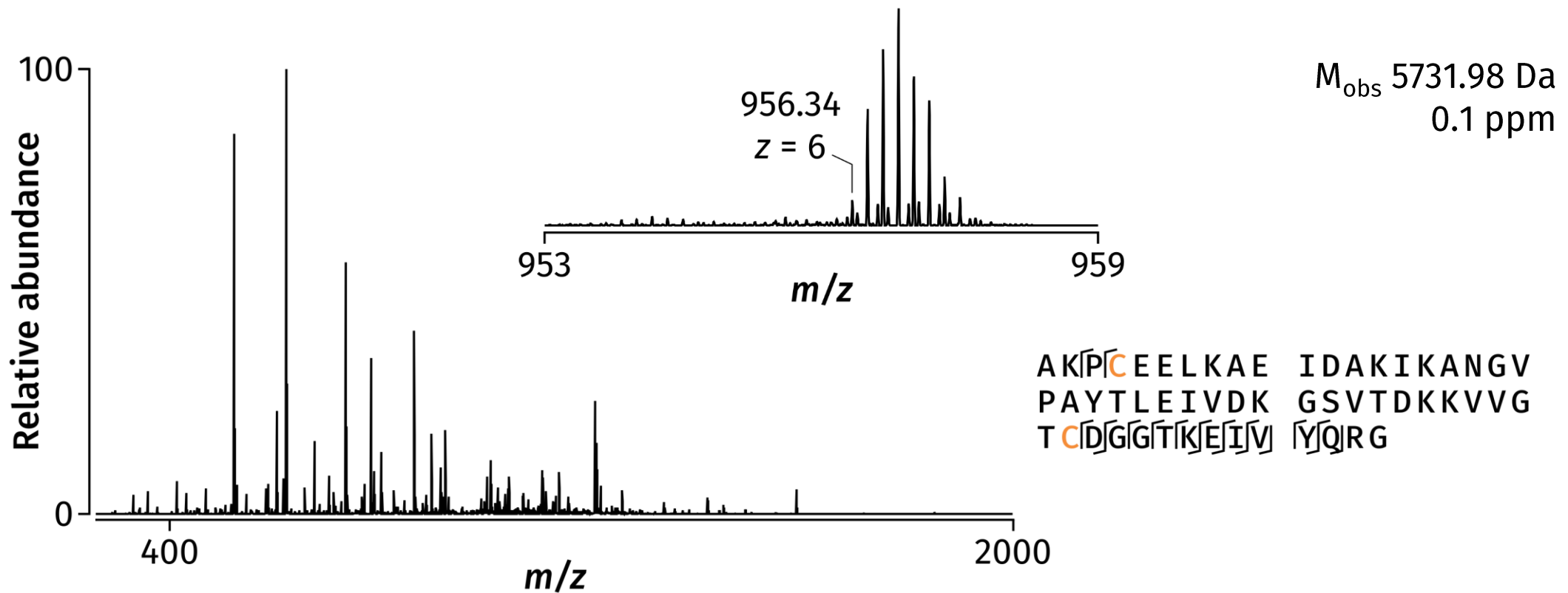
f M A Q D I I S T I S D L V K W I I D T V
N K F T K K



MS/MS protein identification

***P. aeruginosa* PA0039**

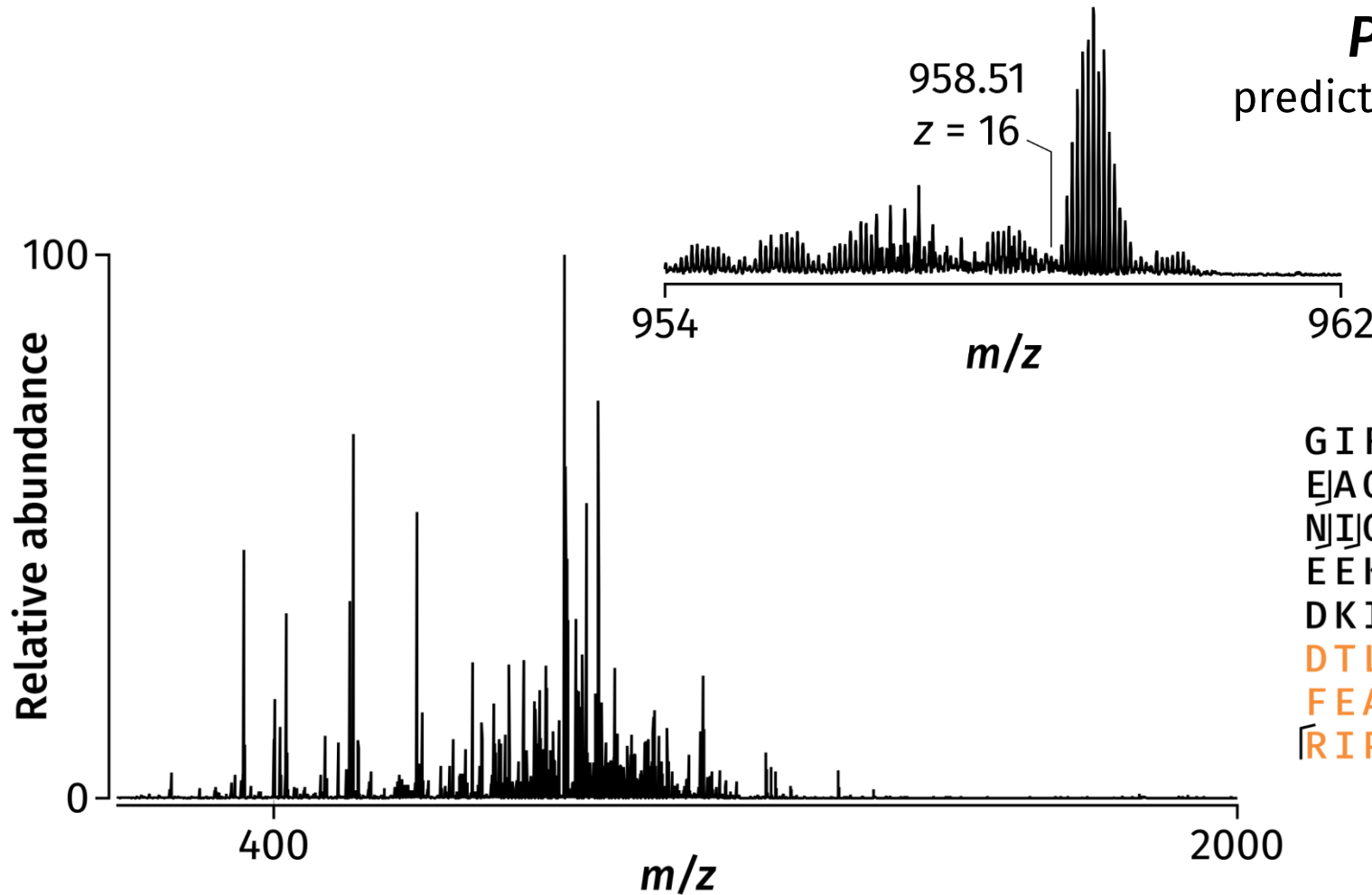
predicted DUF1161 protein,
function unknown



MS/MS protein identification

***P. aeruginosa* PA5178**
predicted stress response protein

M_{obs} 15320.09 Da
3.5 ppm

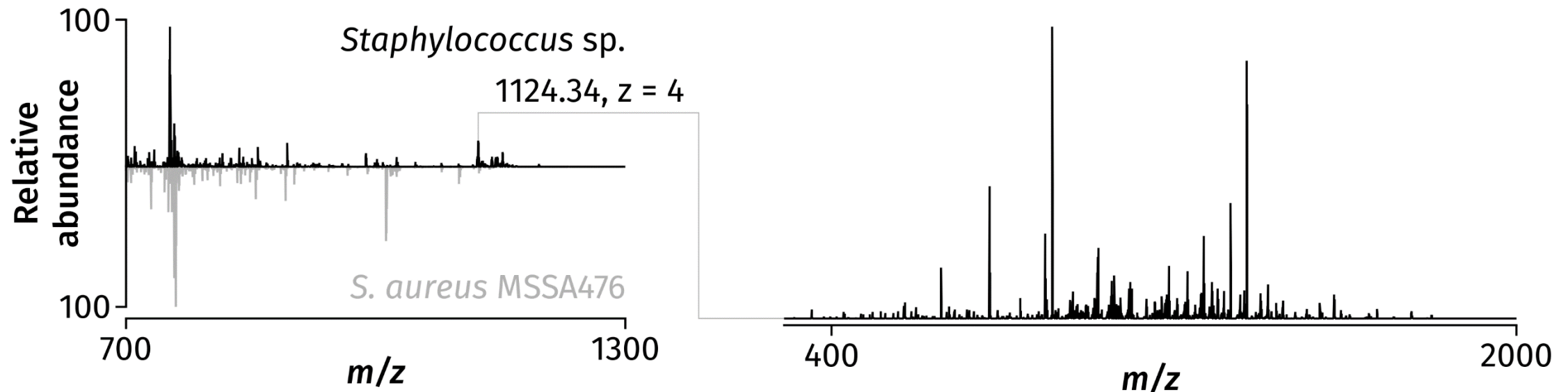


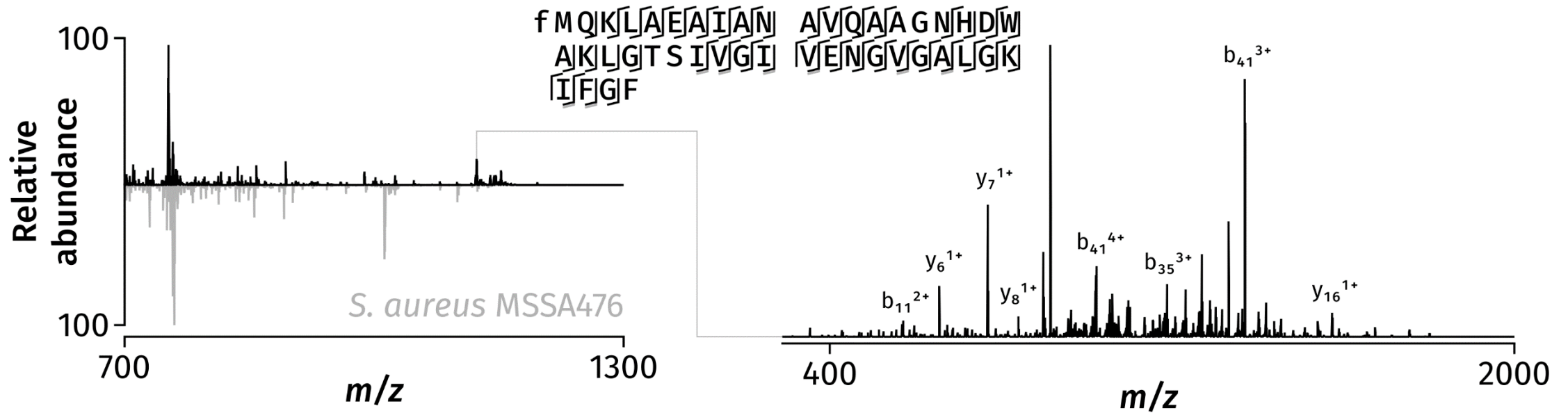
G I F A J F V J K E J A J G	E J K L W D J T L T G H
E J A Q A A E S L K E	H V A K V G L G N J P
N J I Q V S V E G D K	V I A S G E J V A S Q
E E K E K I L L A L	G N J V A G V S S V E
D K I T V A Q A A P	E A R F V T V K K G
D T L S A I A K A E	Y G N A N A Y M K I
F E A N K P M L S H	P D K I I Y P G Q V L
R I P E	



Staphylococcus sp.

- In-house strain originally thought to be *S. epidermidis*





<i>Staphylococcus</i> sp.	MQKLA EAIAN	AVQA AAGNHDW	AKLG T SIVGI	VENG VGALGK	IFGF
	M KLAEAIAN	V+AA + DW	KL G T SIV I	VE+GV LGK	IFGF
<i>S. epidermidis</i>	MSKLA EAIAN	TVKAA QDQDW	TKLG T SIVDI	VESG VSVL GK	IFGF

75% sequence homology



Conclusions

- LESA MS suitable for top-down identification and characterisation of proteins in bacteria
- Detected proteins not previously observed by any other technique
- Potential for differentiation of species



Acknowledgements

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