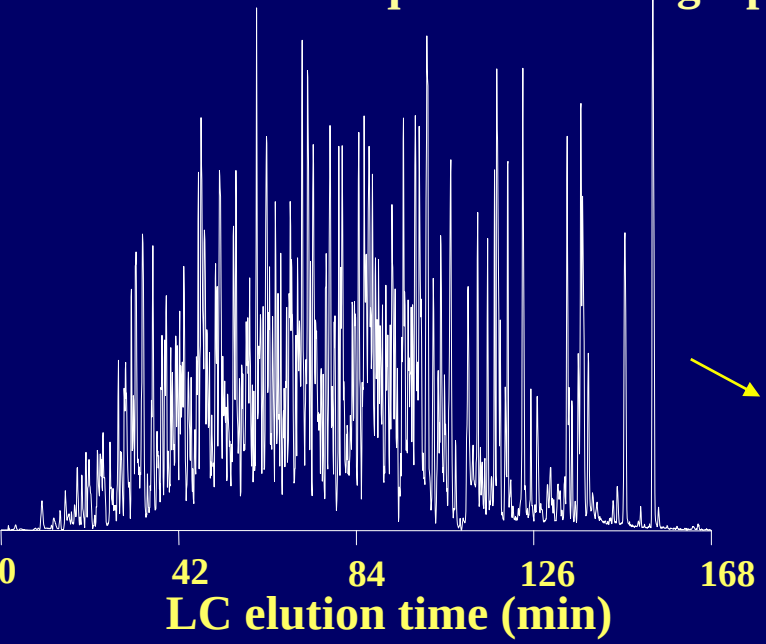
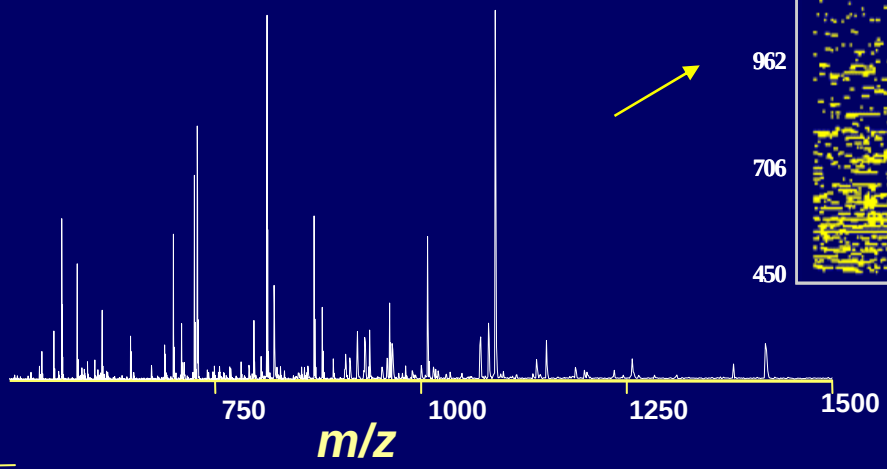
Approach for high throughput microbial proteomics

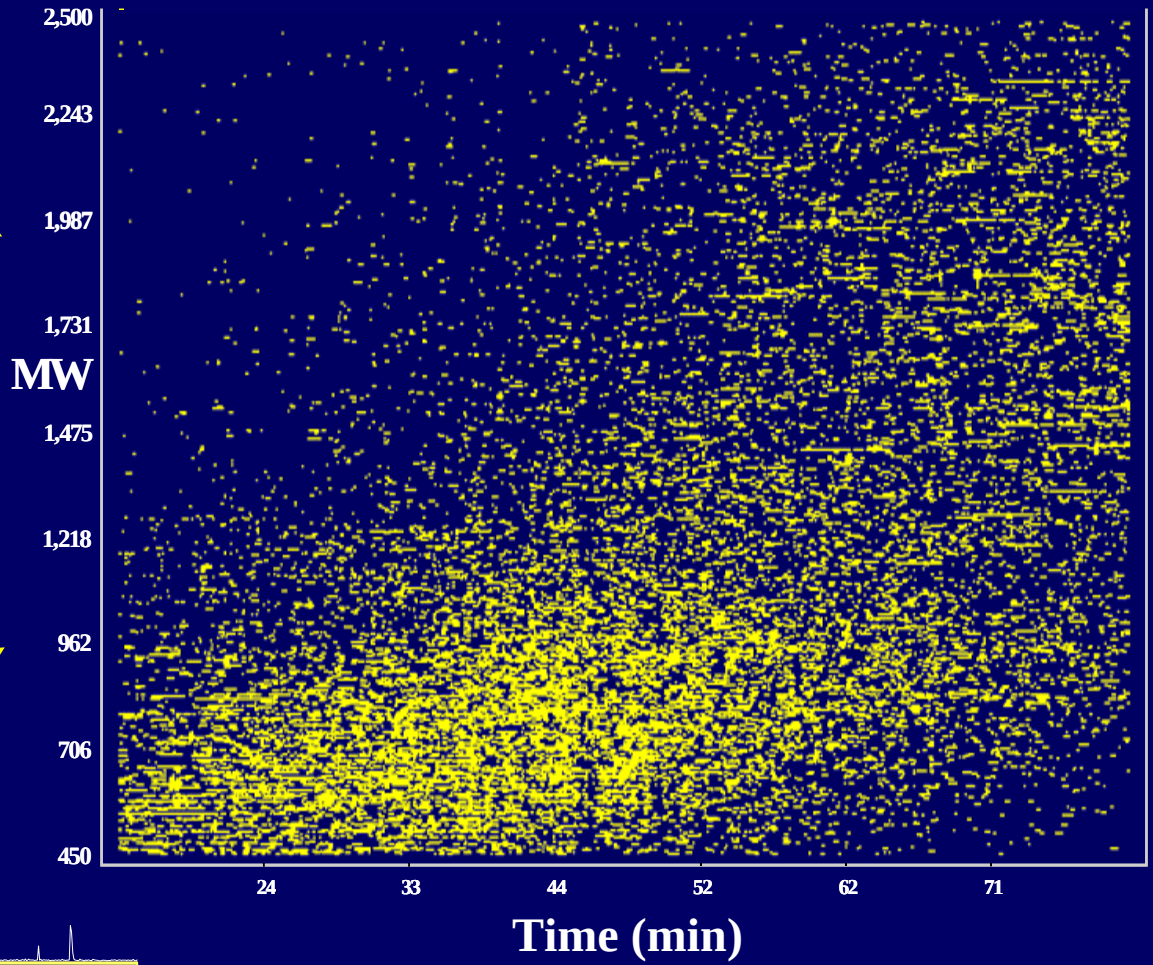
Dimension one: liqiud chromatography



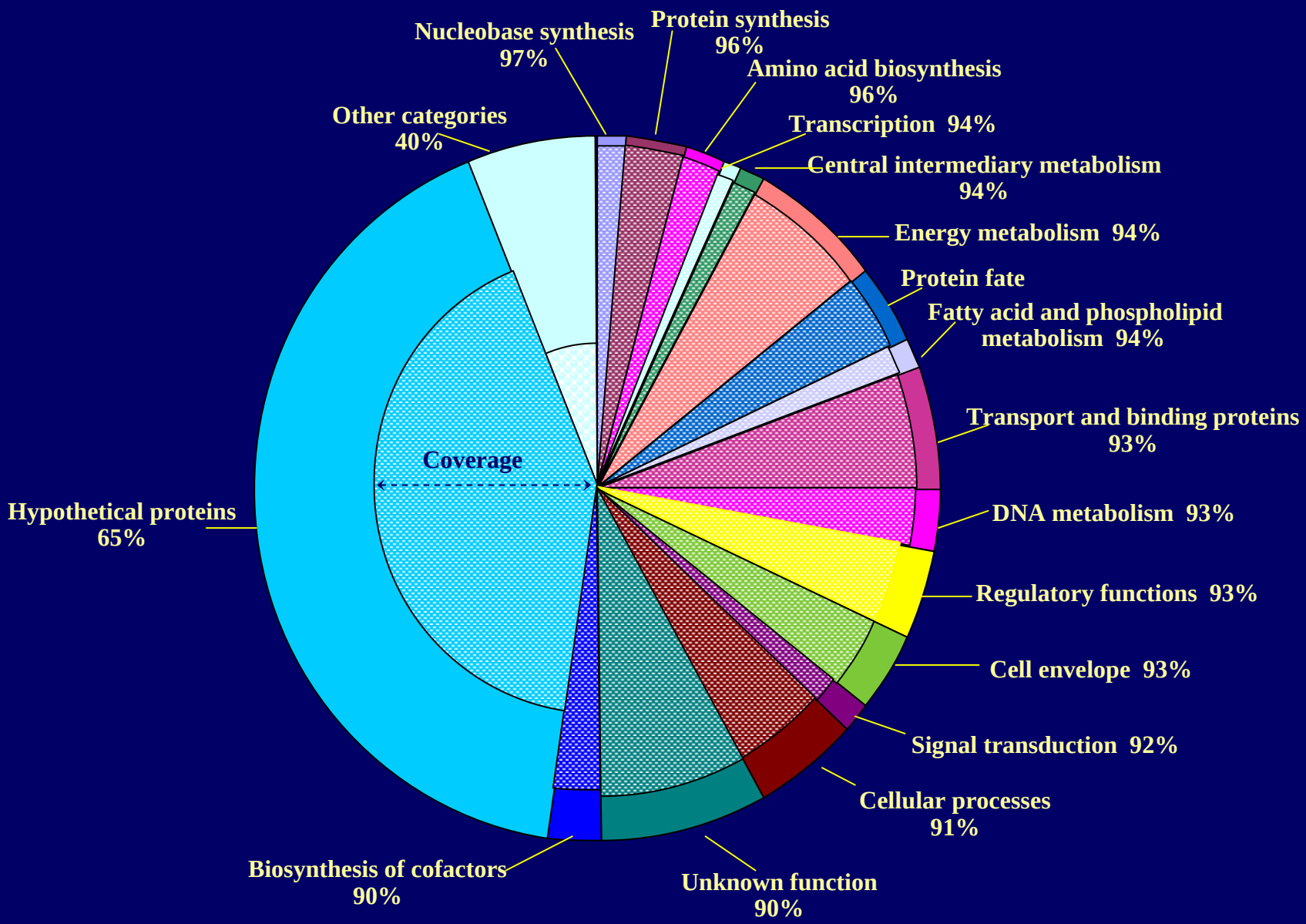
Dimension two: mass spectrometry



2-D display of detected peptides



Shewanella oneidensis MR-1



3862 of 4931 predicted ORFs (78%) covered by AMT tags

Shewanella; ordered by relative MS abundance (aerobic CR 19.1)

Reference	Description	MW (kDa)	Abundance	Peptides observed
SO0217	**translation elongation factor Tu**	**43**	**10000**	**62**
SO3311	**histidyl-tRNA synthetase**	**47**	**10000**	**7**
SO2178	**cytochrome c551 peroxidase [2 hemes]**	**36**	**6600**	**13**
SO4509	**formate dehydrogenase, alpha subunit**	**106**	**5600**	**17**
SO1825	**MotA/TolQ/ExbB proton channel family protein**	**49**	**4800**	**28**
SO0608	**ubiquinol-cytochrome c reductase, iron-sulfur subunit**	**21**	**3200**	**6**
SO0244	**ribosomal protein S14**	**11**	**3100**	**6**
SO0848	**periplasmic nitrate reductase precursor**	**92**	**3000**	**11**
SO1824	**conserved hypothetical protein**	**25**	**2700**	**18**
SO1931	**2-oxoglutarate dehydrogenase, E2 comp., dihydrolipoamide succinyltransferase**	**43**	**2700**	**11**
SO3532	**isoleucyl-tRNA synthetase**	**106**	**2500**	**12**
SO3146	**DNA-binding protein, H-NS family**	**15**	**2500**	**8**
SO0704	**chaperonin GroEL**	**57**	**2200**	**48**
SO2086	**phenylalanyl-tRNA synthetase, beta subunit**	**87**	**2100**	**10**
SO2839	**hypothetical protein**	**16**	**2100**	**3**
SO3310	**conserved hypothetical protein**	**22**	**2100**	**8**
SO0426	**pyruvate dehydrogenase complex, E3 component, lipoamide dehydrogenase**	**51**	**2000**	**22**
SO3942	**serine protease, HtrA/DegQ/DegS family**	**47**	**2000**	**18**
SO4320	**agglutination protein [TAT?]**	**52**	**2000**	**13**

[Many others]

SO0612	stringent starvation protein b	**16**	**2.4**	**2**
SO4250	deoxyuridine 5-triphosphate nucleotidohydrolase	**16**	**2.4**	**2**
SO2569	hypothetical protein	**12**	**2.2**	**1**
SO1339	conserved hypothetical protein	**10**	**2.1**	**1**
SO0568	conserved hypothetical protein	**15**	**2.1**	**3**
SO4311	cyay protein	**12**	**1.9**	**2**
SO0527	conserved hypothetical protein	**12**	**1.5**	**1**
SO0109	conserved hypothetical protein	**9**	**1.4**	**1**
SO4329	conserved hypothetical protein	**10**	**1.4**	**1**

Shewanella oneidensis MR-1; CR 19.1 vs. CR19.2

Sub-oxic only

SO4235	3-isopropylmalate dehydrogenase
SO4053	methyl-accepting chemotaxis protein
SOA0153	heavy metal efflux pump CzcA family
SO3101	conserved hypothetical protein
SO4598	heavy metal efflux pump, CzcA family
SO0581	hypothetical protein
SO1020	NADH dehydrogenase I, B subunit
SO4520	oxygen-independent coproporphyrinogen III oxidase, putative

Aerobic only

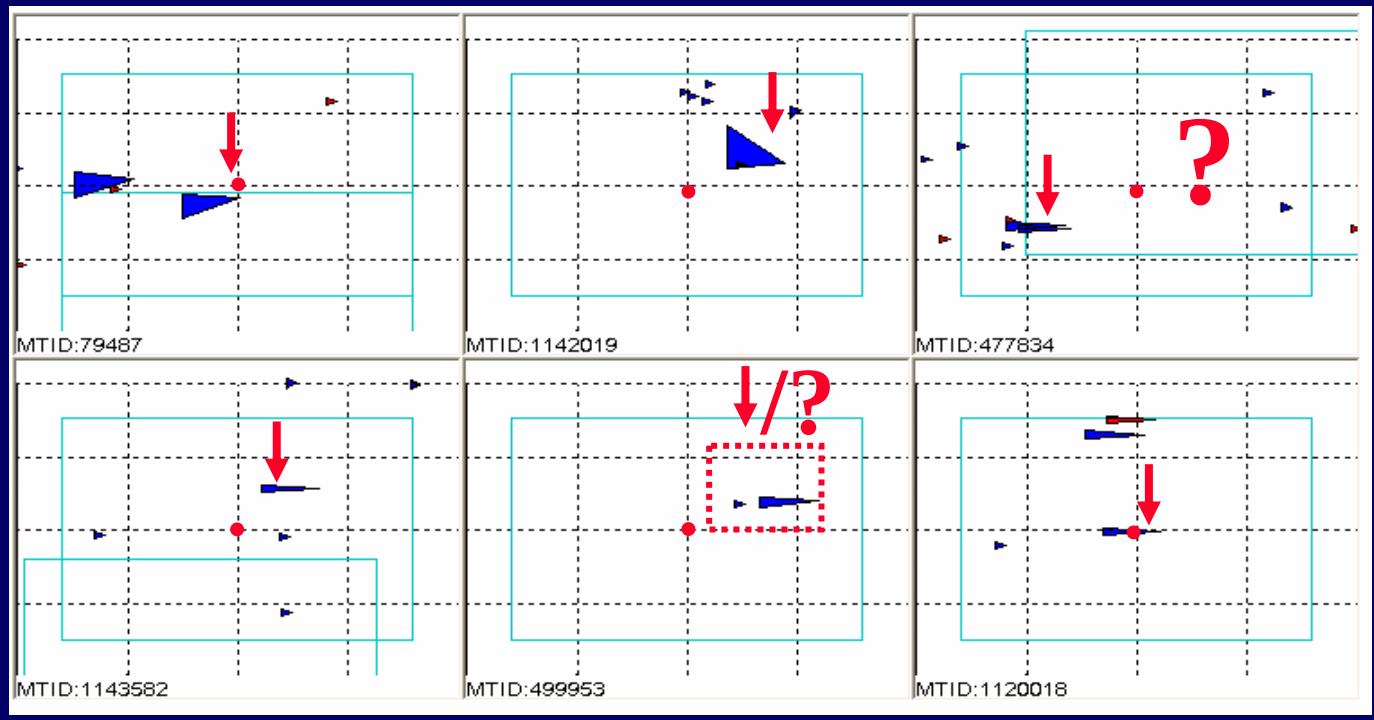
SO0417	pilin, putative
SO4511	formate dehydrogenase, C subunit, putative
SO1700	hypothetical protein
SO2565	intracellular proteinase inhibitor domain protein
SO0674	prophage MuSo1, protein Gp32, putative
SO3157	lipoprotein, putative
SO2720	conserved hypothetical protein
SOA0061	parA protein putative
SO0518	outer membrane efflux family protein, putative

ORF View of the six AMT tags detected for SO1673

Outer membrane protein OmpW, putative (23 kDa)

Analysis 1

Time



Mass

Reference	Mass Tag	Mono MW	Aerobic Analysis 1	Aerobic Analysis 2	CR 19.1 Analysis 3	Sub-oxic Analysis 1	Sub-oxic Analysis 2	CR 19.2 Analysis 3
SOA0154	1258837	3116.4						
SOA0154	1287719	3146.6						
SOA0154	1288202	1982.9						
SOA0154	1560718	2098.0						
SO1673	79487	1007.6						
SO1673	477834	1082.6						
SO1673	499953	2785.4						
SO1673	1120018	1787.9						
SO1673	1142019	2802.4						
SO1673	1143582	1901.0						
SO1673	1314538	2369.2						

PNNL prototype high throughput proteomics lab

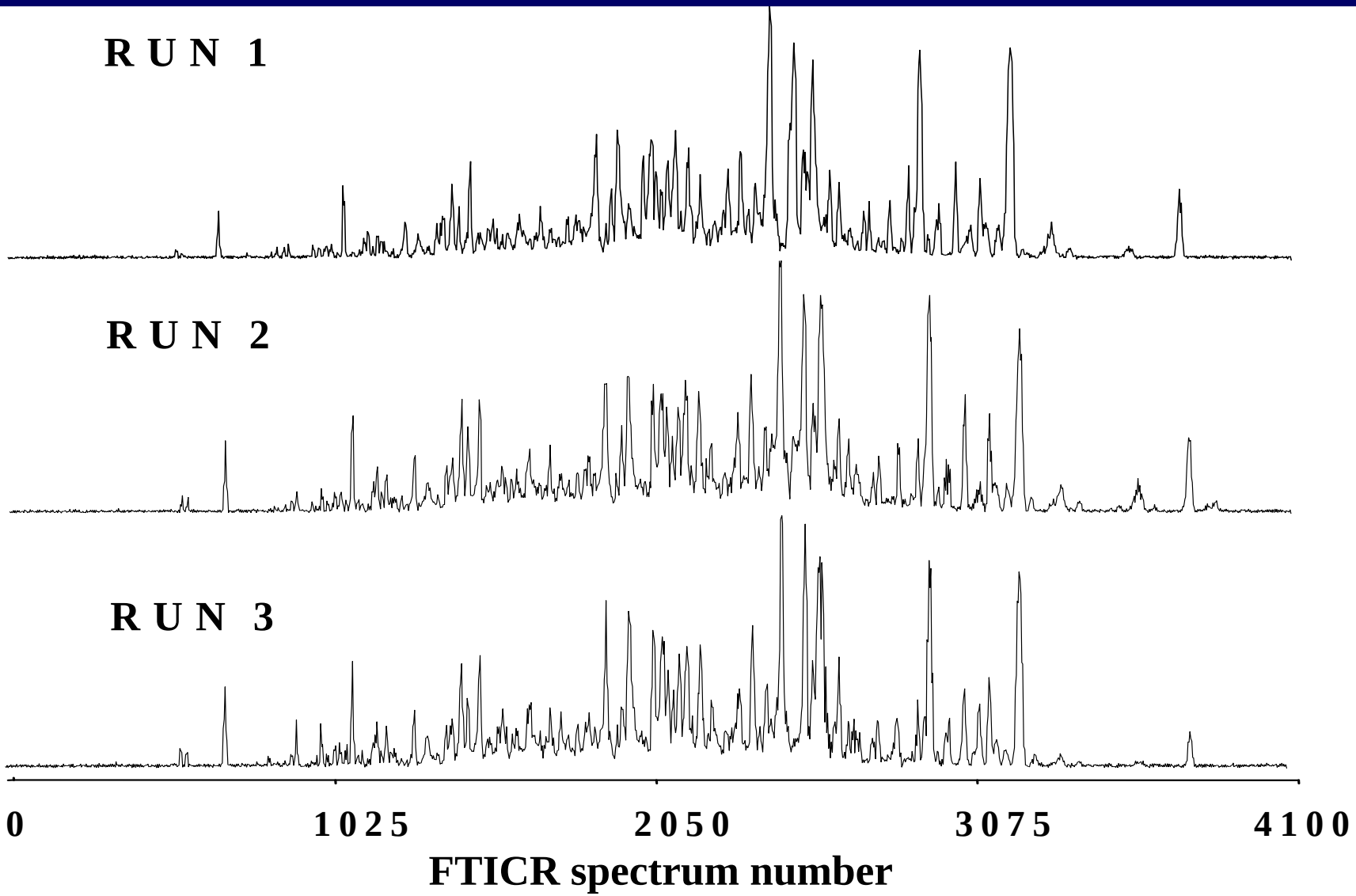
- Separate lab dedicated to production
- Test bed for automation of technology and QA/QC procedures
- Need for careful separation from technology development
  - Different staffing and mind set
  - Data production oriented

Prototype high throughput capillary LC-FTICR

Mass spectrometer cluster for PMT/AMT tag generation using tandem mass spectrometry

Automation essential for high throughput and data quality

Three overnight ‘back-to-back” analyses



Summary

• ~35% of *S. oneidensis* proteins identified under both aerobic and oxygen-limited conditions (1667 aerobic, 1759 sub-oxic).

• A limited set of proteins identified exclusively under aerobic or oxygen-limited conditions; a much greater set of proteins also observed at significantly different levels.

• Chemostat runs using ^{15}N enriched -media, scheduled shortly, will enable more precise determinations of relative abundance changes.

• Much proteomic data is not yet effectively used (e.g. from modified proteins).

• Replicate analyses essential; important to develop statistically sound levels of quality for all measurements.