Global Proteome Profiling of *Desulfovibrio vulgaris* Using Three-Dimensional LC-MS/MS Proteomic Platform

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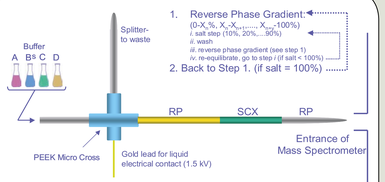
Introduction

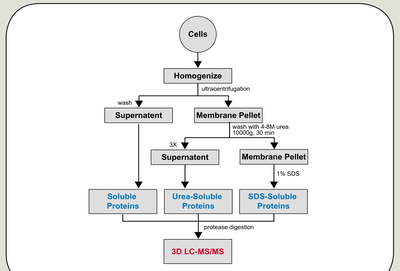
- Diversa's proteomics platform features three-dimensional liquid chromatography (3D LC) coupled with tandem mass spectrometry (MS/MS). The 3D LC system provided high peak capacity and resolving power, minimal sample loss, and accessibility to membrane proteins. The 3D separation was a highly flexible process and easily automated.
- Combining improvements in sample preparation and the development of novel software, this platform presents a comprehensive view of the *Desulfovibrio vulgaris* proteome. *D. vulgaris* proteins were isolated into three parts resulting in the collective identification of 1,386 unique proteins with an average coverage of 10 peptides per protein.
- The 3D technology provided tools for the detailed mapping of the *D. vulgaris* proteome and the differential profiling of it's stress response expression.

Assembly of 3D LC

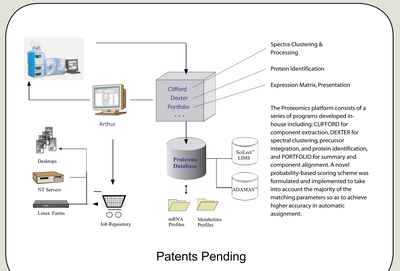


Key features:
- Minimal sample handling/loss
- SDS tolerance
- High peak capacity and better separation

Sample Preparation



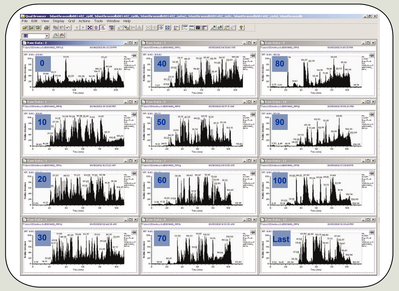
Data Analysis Platform



The Proteomics platform consists of a series of programs developed in-house including CLIFFORD for component extraction, DEXTER for spectral clustering,precursor integration, and protein identification, and PORTFOLIO for summary and component alignment. A novel probability-based scoring scheme was formulated and implemented to take into account the majority of the matching parameters so as to achieve higher accuracy in automatic assignment.

Patents Pending

12 Salt Sub-fractions in Reverse Phase 0-8 Fraction (soluble)



Protein Identifications

High Confidence and High Coverage

	Fractions	Raw Spectra	Identified Peptides	Identified Proteins
3D LC-MS/MS	Soluble	272,437	3,971	718
	Urea-solubilized	282,512	8,416	1,168
	SDS-solubilized	251,146	1,832	538
			Total	**1,386**

High Sequence Coverage

Examples

ORF03423 flavodoxin

1 MPKALIVYGS TTGNTEYTAE TIARELADAG YEVDSRDAAS VEAGGLFEGF DEVLLGCSTW GDDSIEEQDD FIPLFDSLEE 80
81 TGAQGRKVAC FGCGDSSYEY FCGAVDAIEE KLKNLGAEIV QDGLRIDGDP RARDDDIVGW AHDVRGAI

ORF05180 enolase (eno)

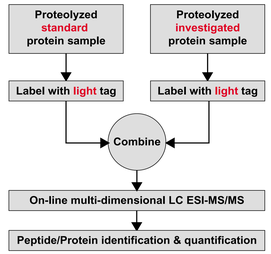
1 MSITVSVWAR EILDSRGNPT IEVEVSLESG HSGRAAVPSG ASTGTREALE LRDGDKGRVK GKGVEKAVDN VMGEIAEAVY 80
81 GLDALRQVQL DNYELLDLDGT DNKERLGANA MLGVSLATARAASSFLGLPL YQYLGGVNAK VLPVPLMNII NGGAHAPYNI 160
161 DIQEFMIMPI GAATFREALR MGAETFHTLK ALLAADGHVT SVGDEGGFAP NLKSHREALFK YITRALEESG YIPGARIALA 240
241 IDAASSEFYR DGKYHLAGEG KTFNSNEMTE WLGLFTAKYPIISIEDGLAE GDWEGWGELT YKLGIDVQGV GDDDFVNPFD 320
321 ILAQGIDEGV ANSILIKENQ IGTLTETLDT IEMAKQAAYT TVISHRSGET EDHFIADLAV GLNAGQIKTG SLCRSDRLAK 400
401 VNQLRIEED LIDAGAYFGP MIASHFEGYEG DEEFEDA

ORF03811 translation elongation factor Tu (tuf)

1 MGKEKFERKK PHVNIGTIGH IDHGKTTLTA AITKTAGLEG QGKFIAYDEI DKAPEEKURG ITIATAHVEY ETATRHYAHV 80
81 DCPGHADYIK NMIFGAGQMD GAILVVAATD GPMPQTREHI LLARQVGVPY IVVFLNKCDMVDDELLELV ELEVRELLTF 160
161 YGFPGDDVPV VRGSALKALE SDDPNSDACK PIRELLAACD SYIPEPQRDI DKPFLMPIED VFSISGHGTV VTGRVERGVI 240
241 KVGEEVEIVG IKDTTKSTCT GVEMFRKLLD QGQAGDNGALLLQGVKAEDI ERGQVLAAPG SITPHRKFKA EVYVLSKEEG 320
321 GRHTPFFSGY RPQFYFRTTD ITGNITLEEG VEMVMPGDNA TFNVELIAPI AMELGLRFAI REGGRTVGAG VVSEIVE

Blue: Identified
Black: Not Identified

Semi-Quantitation



Summary

- Our innovative 3D LC-MS/MS technology approach combines on-line chromatography separation, mass spectrometry separation and detection, as well as a novel bioinformatics software platform
- It is detergent tolerant, thus excellent for membrane proteins
- Because it is a fully automated workflow, there is minimal sample handling & loss, and ultimately requires less sample
- The 3D chromatography shows superior separation power, peak capacity, and large dynamic range
- The combination of 3D technology and novel bioinformatics platform allows for high confidence in and high coverage for protein identifications
- It is also semi-quantitative for protein expression profiling

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