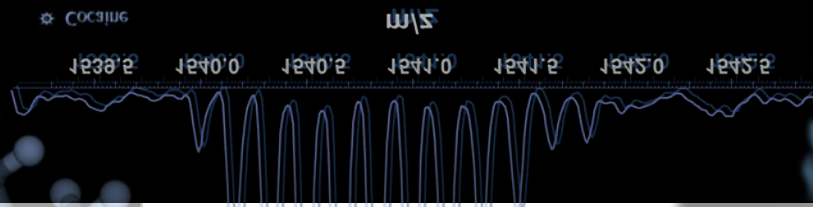
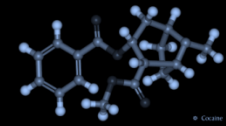


Yale/NIDA Neuroproteomics Center



Protein Identification & Protein Profiling in the
Neuroproteome
Kathy Stone





Outline

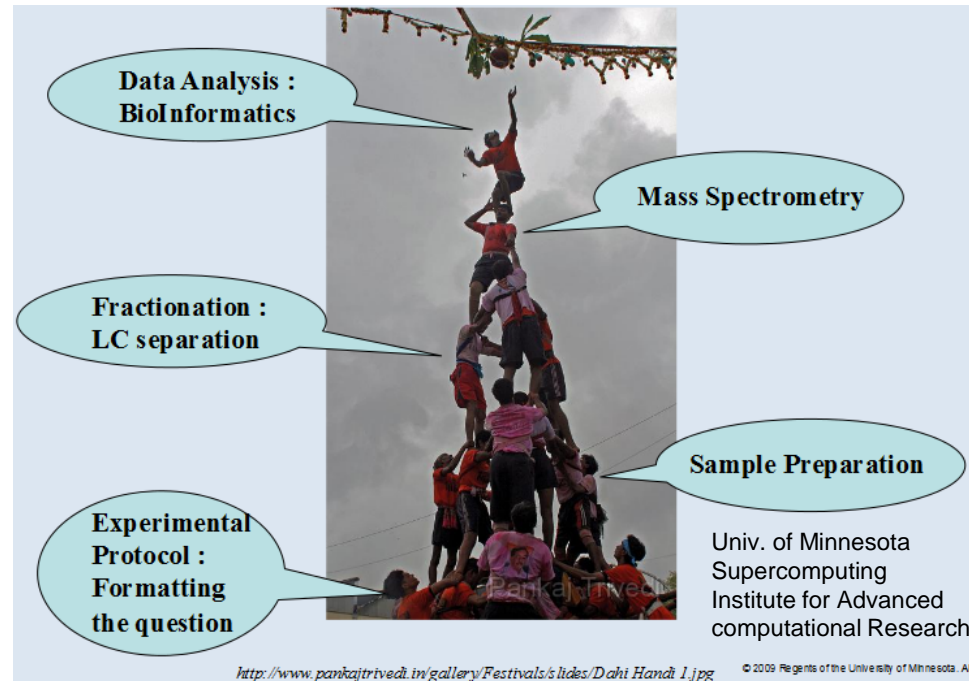


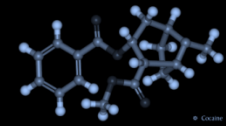
➤ Overview of the Neuroproteomics Cores Synergy

- Protein Profiling and Identification, Biophysics, and Phosphoinositide Analysis Core
- Protein Post-Translational Modification Identification & Profiling Core (PTM-core)
- Targeted Proteomics Core
- Bioinformatics and Biostatistics Core (BBC)
- The synergy propels the Center forward in going from Discovery to verification/validation of biomarkers of drug addiction.

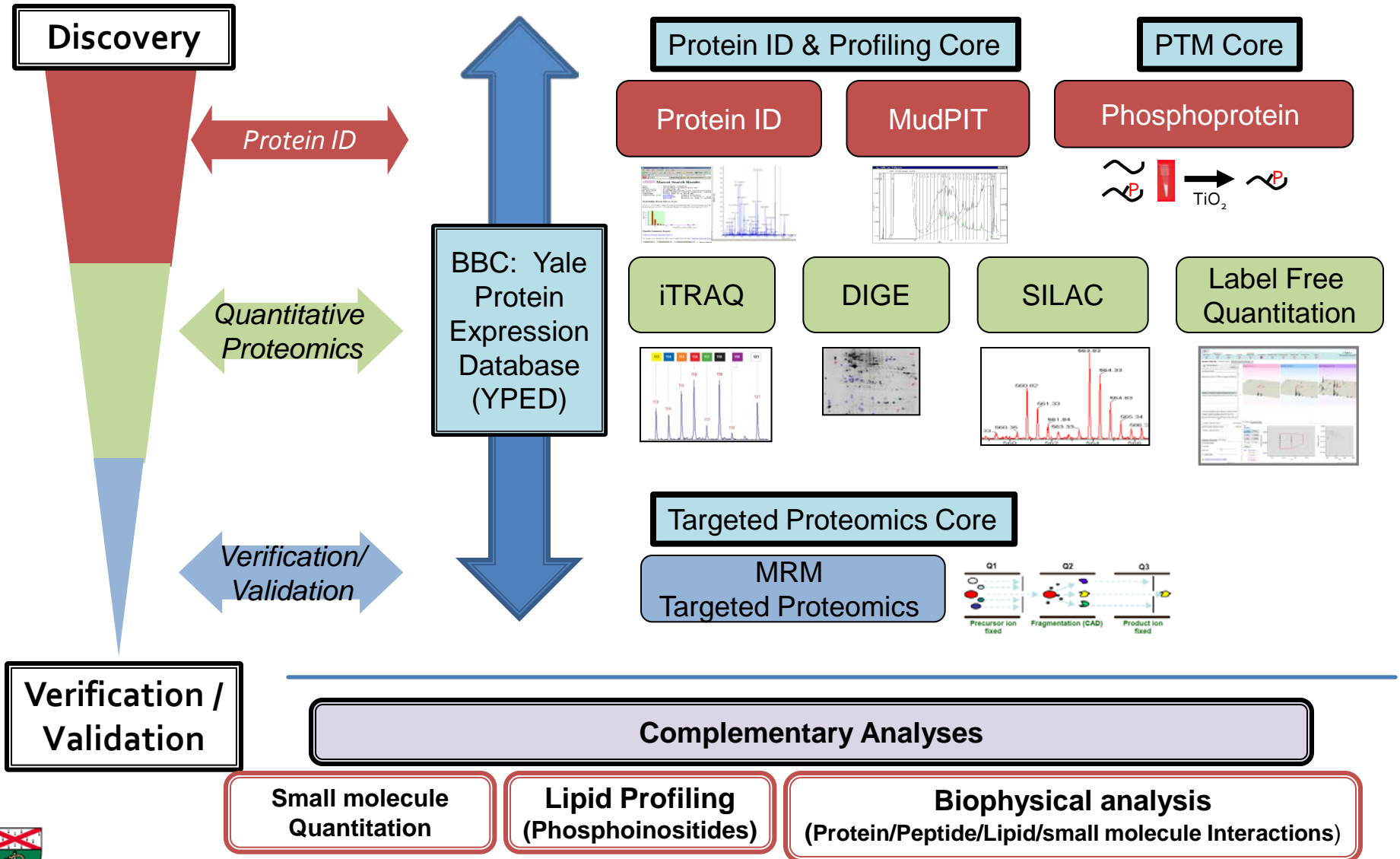
➤ Projects overview

- complex proteome analyses
 - ❖ Human cerebellum
 - ❖ Choroid plexus
- Protein Profiling
 - ❖ iTRAQ
 - ❖ DIGE
 - ❖ Label Free Quantitation (LFQ)
 - ❖ SILAC/SILAM

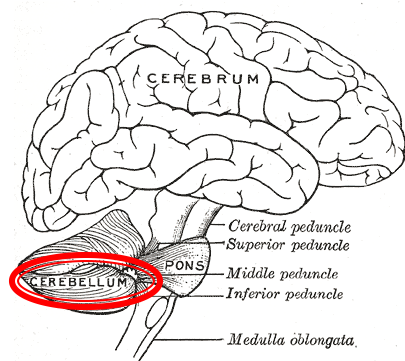




Proteomic Discovery to Verification / Validation



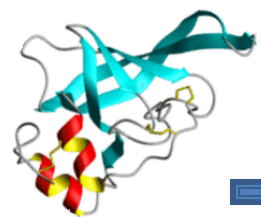
Protein Identification: Mapping the human cerebellum proteome



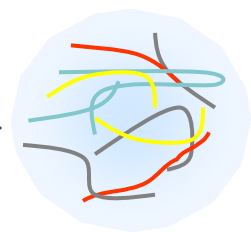
RIPA buffer
0.1%SDS, 0.1%
Triton X100,
0.01%deoxycholate



Probe sonication;
Chloroform/MEOH
ppt



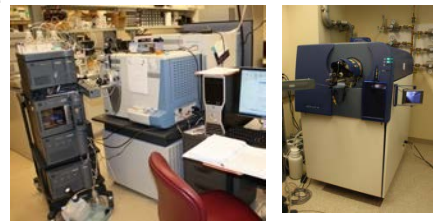
Complex
protein
mixture



Lys C/tryptic
digestion in 1%
RapiGest™ SF
Surfactant



20 SCX fractions
collected offline



Each cation fraction is
analyzed by LC MS/MS
analysis on the LTQ
Orbitrap Elite and AB
5600 TripleTOF®



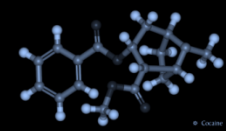
	# Distinct Proteins Identified
Total # ID with Sig. Mascot Score	4,403
Total #ID with ≥2 Sig. peptides*	3,374

Swissprotein database, homo sapiens taxonomy
Protein Score threshold 56, *1% FDR

Pilot Project: Rob Kitchen

Toward Improved Label-free Quantification of MS/MS Spectra Using Predicted Proteomes Based on Second-generation RNA Sequencing

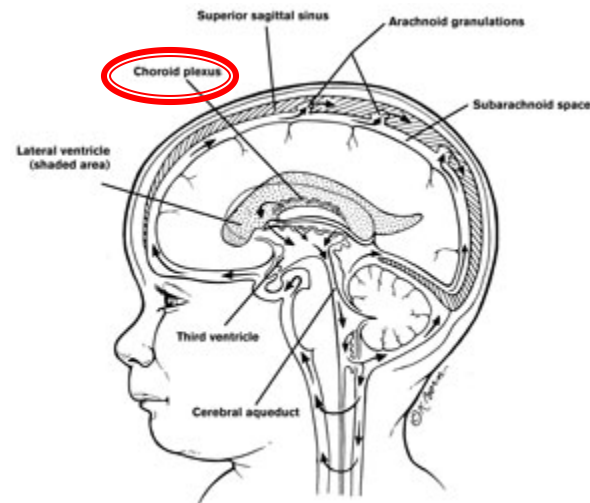




Mapping of the Rat Choroid Plexus (CP) Proteome

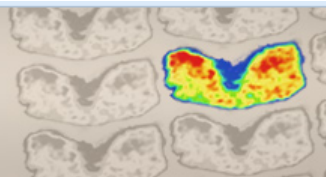


- CP has been implicated as a target site for brain drug delivery*
- By mapping the Rat CP proteome, we hoped to gain insight on the CP function
- over 1,400 proteins were identified with ≥ 2 Significantly identified peptides (Mascot protein score = 32)



The molecular functions associated with various proteins of the CP proteome indicate that it is a blood–cerebrospinal fluid (CSF) barrier that exhibits high levels of metabolic activity.

Translational Psychiatry



Citation: *Translational Psychiatry* (2012) 2, e139; doi:10.1038/tp.2012.64
Published online 10 July 2012

A molecular characterization of the choroid plexus and stress-induced gene regulation

[Open](#)

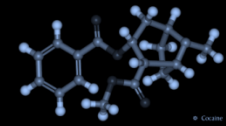
M Sathyanesan¹, M J Girenti¹, M Banas¹, K Stone², C Bruce², E Guilchick², K Wilczak-Havill², A Nairn^{1,2}, K Williams², S Sass³, J G Duman³ and S S Newton¹

Pilot Project: Sam Sathyanesan

Development of Cerebrospinal Fluid Markers for Neuropsychiatric Disorders

*Duncan et. al, *Pharm Res* 2005;22:1011-1037





Protein Profiling



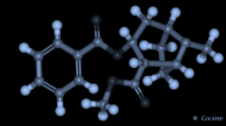
Commonly utilized Protein Profiling Techniques

	DIGE	iTRAQ	SILAC	Label Free Quantitation
labeling	chemical	chemical	metabolic	None
Quantitation	protein	peptide	peptide	Peptide
# of samples compared	2 with 1 internal standard	8	2.....	Unlimited #
Sample amount	50-100µg	25-50µg	?	1µg
Sample type	Any (cells, tissue, biological fluids...)	Any (cells, tissue, biological fluids...)	Cells only	Any (cells, tissue, biological fluids...)
PTM analysis	Yes	difficult	yes	Yes with fractionation
challenges	Very acidic, basic, large or small MW proteins may be lost	Labeling efficiency and buffer composition	Only suitable for cells	Data analysis

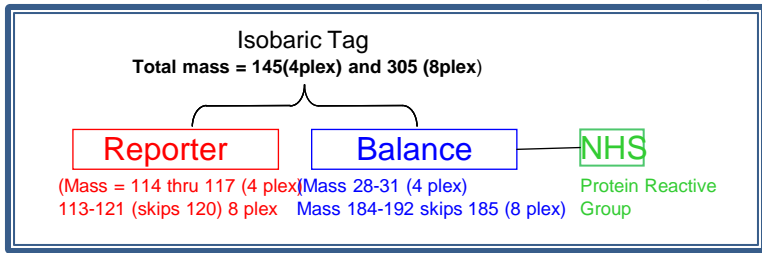
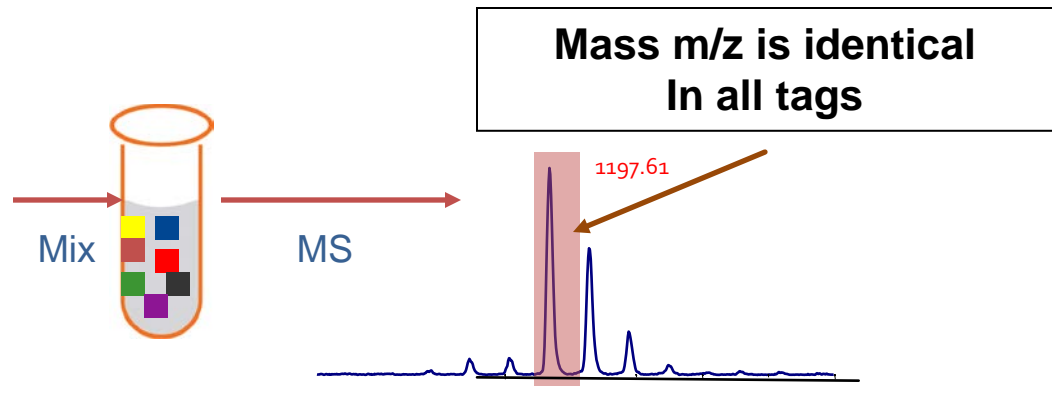
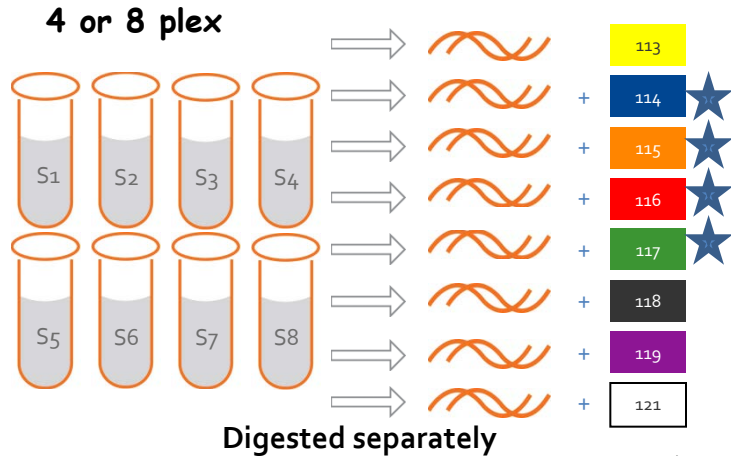
DIGE=2D differential gel electrophoresis
 iTRAQ=isobaric tags for relative quantitation

SILAC=stable isotopic labeling by amino acids in cell culture

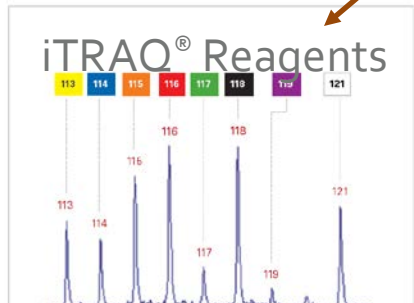
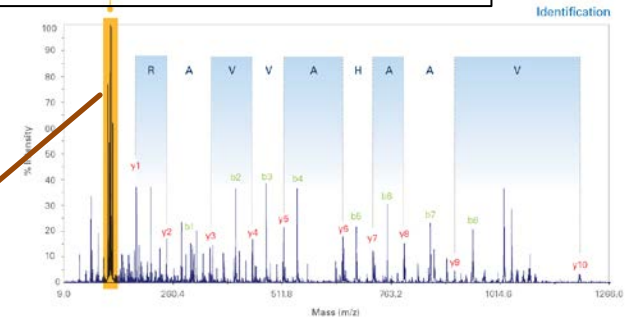




Multiplex protein Quantitation using iTRAQ[®] 4 and 8plex



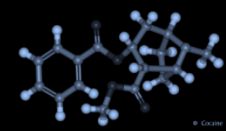
MS/MS reveals the reporter ions



AB SCIEX 5600 TripleTof[®]

ProteinPilot (v4.5) Quantitation and Loading into YPED





iTRAQ Analysis: WT vs neuronal nicotinic acetylcholine receptor Knockout

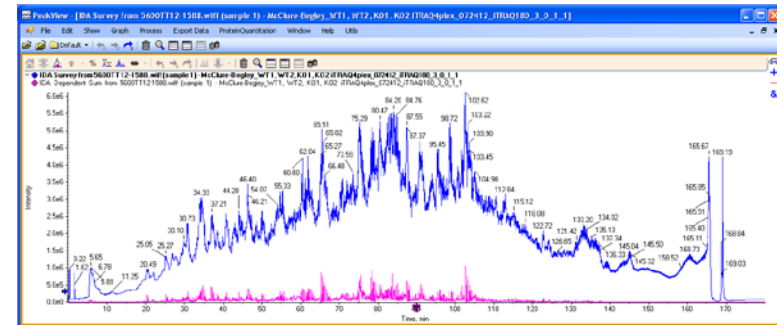


Background

- Neuronal Nicotinic acetylcholine receptors containing $\alpha 4$ and $\beta 2$ subunits are the principal receptors in the mammalian central nervous system that bind nicotine with high affinity
- These receptors are involved in nicotine dependence, mood disorders, neurodegeneration and neuroprotection
- GOAL: use iTRAQ to understand their protein:protein interactions

Experiment

- Mouse alpha4 WT and KO plus (1) or minus (2) amine-reactive crosslinker prior to an IP
- 4 plex
 WT1=114 KO1=116
 WT2=115 KO2=117



YPED

iTRAQ4plex Results for Sample: A4 4-plex WT/KO ProGroup UniSwiss

Execution Date	Program Version	Database	Search Engine
27 Jul 2012 10:39	ProteinPilot 4.0	UniSwiss	ProGroup
Summary Statistics Protein Score >=2			A4 4-plex WT/KO ProGroup
#of proteins with 1 peptide identified			82
#of proteins with >= 99% confidence (protein score 2.0)			151
#of proteins with >= 95% confidence (protein score 1.3)			158
#of proteins with >= 90% confidence (protein score 1.0)			161
Range of 115:114 ratios			0.0103 - 23.7684
Range of 116:114 ratios			0.2168 - 14.9968
Range of 117:114 ratios			0.0108 - 69.1831
Ratio	115:114	116:114	117:114
Observed Bias Correction	1	1	1

[View iTRAQ Sample Information](#) Panther Summary Cutoff 1.5

[View False Positive Analysis](#) -Select-

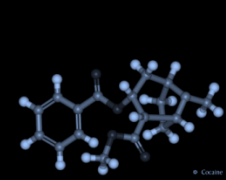
151 proteins found, displaying 1 to 20.
 [First/Prev] 1, 2, 3, 4, 5, 6, 7, 8 [Next/Last]

Protein Score	Protein ID	Protein Name	Percent Coverage	# Distinct Peptides in Ratios	115:114 ratio	115:114 P value	116:114 ratio	116:114 P value	117:114 ratio	117:114 P value
36.23	NFM_MOUSE	Neurofilament medium polypeptide OS=Mus musculus GN=Nefm PE=1 SV=4	34.91	8	0.4571	0.4847	3.8371	0.0086	0.0457	0.0051



Pilot Project: Tristan McClure-Begley

Defining the nAChR Interactome



iTRAQ Results: 1 peptide from the identified Neurofilament medium polypeptide protein

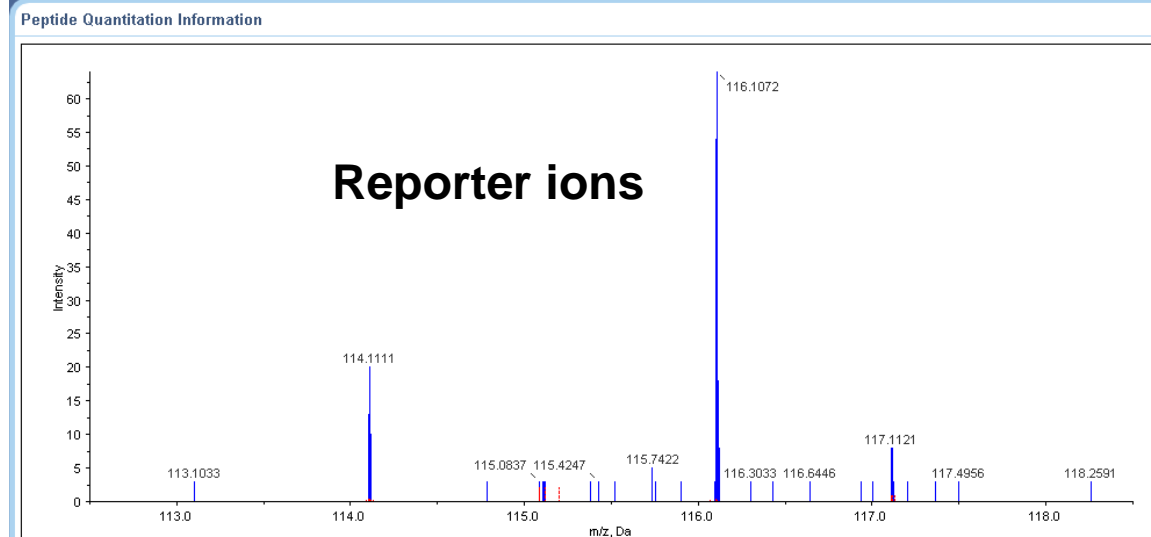


Protein Quant				Protein ID				Spectra				
Proteins Detected												
N	Unused	Total	% Cov	Accessio...	Name	Species	Peptides(95%)	115:114	116:114	117:114	Biological Processes	Molecular Functions
122	3.66	7.70	20.8	trjF7A3A...	Neurofilament heavy polypeptide OS=Mus musc...	MOUSE	5	0.0109	3.0200	0.0175		
32	19.99	25.33	40.0	spjP0855...	Neurofilament light polypeptide OS=Mus muscu...	MOUSE	22	3.4041	4.7424	1.4191		
14	36.23	36.28	34.9	spjP0855...	Neurofilament medium polypeptide OS=Mus mu...	MOUSE	24	0.4571	3.8371	0.0457		
33	19.90	20.03	21.9	trjB1AGX...	P140 gene OS=Mus musculus GN=Scrtin PE=4...	MOUSE	11	0.0112	2.7290	0.0108		

ProteinPilot™ Report

Peptide Quantitation												
Used	Annotation	Conf	Sequence	Modifications	Cleavages	ΔMass	Theor m/z	Theor z	Spectrum	115:114	116:114	117:114
<input checked="" type="checkbox"/>	auto	85	FEEEAR	iTRAQ4plex@N-term		-0.0036	462.7308	2	1.1.1.1813.4	0.0000	0.0000	0.0000
<input checked="" type="checkbox"/>	auto	99	LRDDTEAAIR	iTRAQ4plex@N-term	missed R-D...	-0.0029	435.2411	3	1.1.1.2115.5	0.0000	0.0000	0.0000
<input checked="" type="checkbox"/>	auto	90	LTEAARQNH	iTRAQ4plex@N-term		0.0318	431.2414	3	1.1.1.1968.4	0.0000	0.0000	0.0000
<input checked="" type="checkbox"/>	auto	93	SPVEEVKPKPEAK	iTRAQ4plex@N-term iTRAQ4plex(KO@7 iTRAQ4plex(KO@9 iTRAQ4plex(KO@13		0.0026	504.3062	4	1.1.1.2116.5	0.0000	3.3434	0.1275
<input checked="" type="checkbox"/>	auto	99	VQSLQDEVAFLR	iTRAQ4plex@N-term		0.0043	774.9288	2	1.1.1.3301.7	0.5256	1.4432	
<input type="checkbox"/>	auto - discor...	99	AQVQLDSHLEEDIHR	iTRAQ4plex@N-term		0.0052	513.0084	4	1.1.1.2594.7			

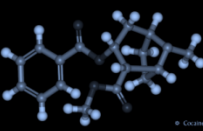
Identified peptide



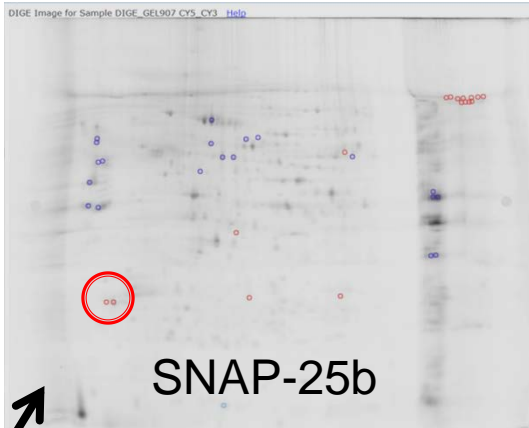
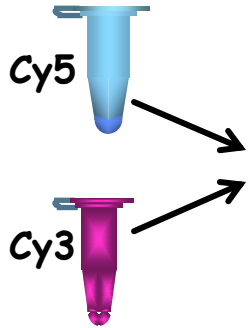
Only the WT 114 and 116 Have reporter ions – the KO (115 and 117) was not visible

❖ the ratios of proteins associated with Neuronal Nicotinic acetylcholine receptors are decreased in KO

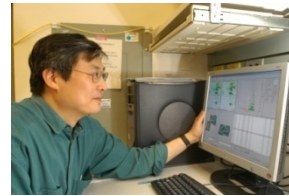




DIGE Protein Profiling and Protein Identification Workflow



Typhoon 9410 Image



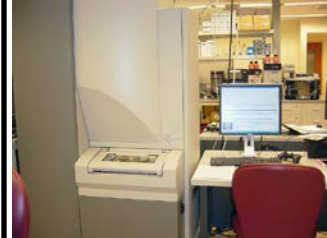
Decyder Software Analysis



Protein spots differentially regulated are robotically picked and digested with trypsin



Protein identification is based on a Mascot search using a combined peptide mass fingerprint and MS/MS database search (AB GPS Explorer)



AB 4800 MALDI-Tof/Tof



YPED
DIGE Results for Sample: DIGE_GEL907 MASCOT IPI_mouse Gly-Gly

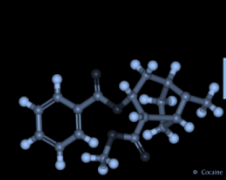
Execution Date	DeCyder Version	Database	Search Engine
2008-12-03 22:41:46.0	5.00	IPI_mouse Gly-Gly	MASCOT
#spots picked			38
#spots analysed			38
#spots with proteins Id'd			37
#proteins Id'd with 1 peptide			0
Median Cy5/Cy3 (all spots)			1.3399999999999999
Range Cy5/Cy3 (all spots)			-3.26 -- 2.4899
#spots with C5/C3 >= 2 fold difference (all spots)			14
Median Cy5/Cy3 (proteins Id'd)			1.3107
Range Cy5/Cy3 (proteins Id'd)			-3.26 -- 2.4899
#spots with C5/C3 >= 2 fold difference (proteins Id'd)			14
DB Search Score Cutoff (scores greater than cutoff are significant p<0.05)			60

View DIGE Image DIGE Sample Information Panther Summary Cutoff 1.5

37 proteins found, displaying 1 to 20.
[First|Prev|1, 2|Next|Last]

Spot Number	Protein ID	Protein Name	DB Search Score	Total Ion Score	Percent Coverage	Peptide #	CY5:CY3 Ratio
1946	IPI00125635	Tax_Id=10090 Gene_Symbol=Snap25 Isoform SNAP-25b of Synaptosomal-associated protein 25 Mascot detail	341	286	118	25	-3.26
1948	IPI00125635	Tax_Id=10090 Gene_Symbol=Snap25 Isoform SNAP-25b of Synaptosomal-associated protein 25 Mascot detail	331	259	134	25	-2.4841
584	IPI00845595	Tax_Id=10090 Gene_Symbol=Dnm1 Isoform 2 of Dynamin-1 Mascot detail	187	127	42	33	-2.4356
619	IPI01347110	Tax_Id=10090 Gene_Symbol=Kif73 Karatel-1 Mascot detail	81	38	46	23	-2.4193





Proteomic Analysis for identification of Cysteine string protein α (CSP α) protein clients



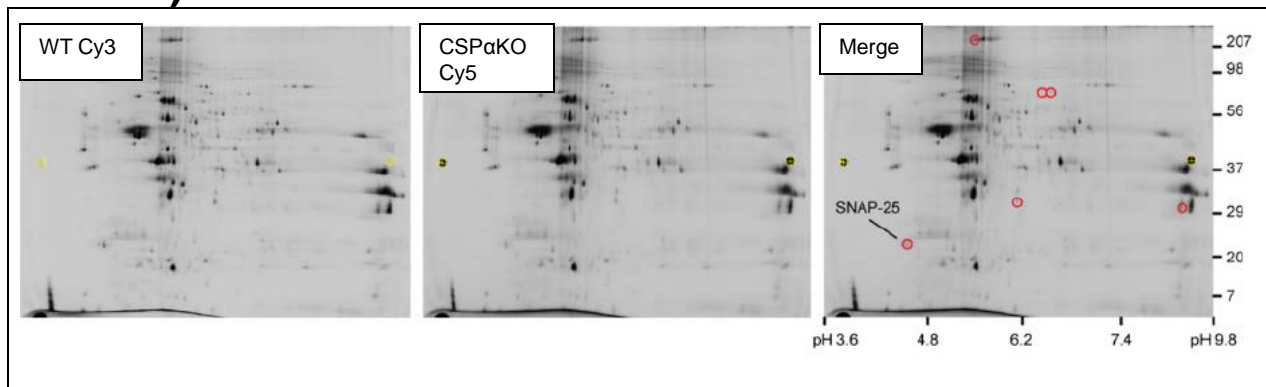
- Utilized DIGE & iTRAQ protein profiling techniques
- located 22 proteins that are selectively decreased in CSP α knockout synapses
- includes two that bind to the CSP α chaperone, SNAP-25 and GTPase dynamin 1.
- DIGE proved to be effective in determining the proteins that were changing expression between the WT and KO (13 gels used)

Cell PRESS **Neuron Article**

Identification of CSP α Clients Reveals a Role in Dynamin 1 Regulation

Yong-Quan Zhang,^{1,6} Michael X. Henderson,^{1,6} Christopher M. Colangelo,³ Stephen D. Ginsberg,⁵ Can Bruce,^{3,4} Terence Wu,³ and Sreeranga S. Chandra^{1,2,*}

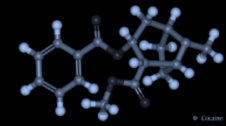
¹Program in Cellular Neuroscience, Neurodegeneration and Repair, Department of Neurology
²Department of Molecular Cell and Developmental Biology
Yale University, New Haven, CT 06536, USA
³W. M. Keck Foundation Biotechnology Resource Laboratory, Mass Spectrometry Resources
⁴Department of Molecular Biophysics and Biochemistry
Yale University, New Haven, CT 06510, USA
⁵Center for Dementia Research, Nathan Kline Institute and Department of Psychiatry, New York University Langone Medical Center, New York, NY 10962, USA
⁶These authors contributed equally to this work
*Correspondence: sreeranga.chandra@yale.edu
DOI 10.1016/j.neuron.2012.01.029



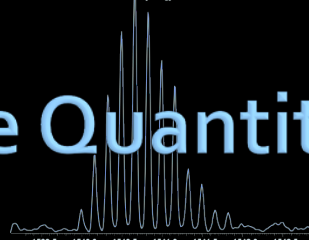
Pilot Project: Sreeranga Chandra

Phosphoinositides, Synaptic Signaling, Drug Addiction, and Neurodegeneration



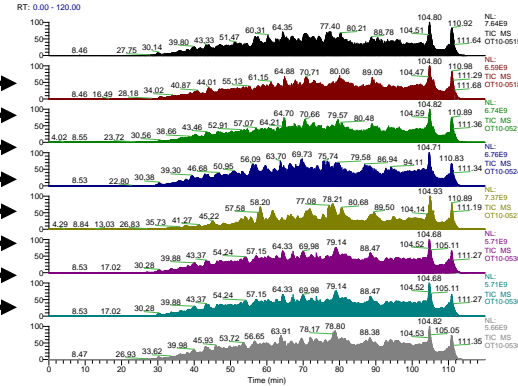
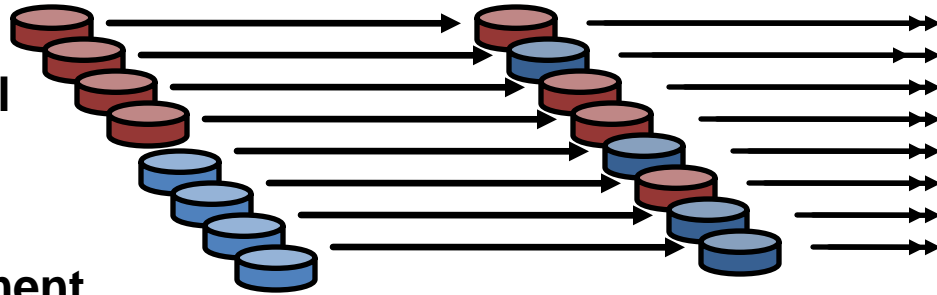


Label Free Quantitation (LFQ)



Control

Experiment



Cells,
Tissue,
Biological
Fluids

Sample
Processing
Ab,
Depletion,
Protein
Forest,
OFFGEL
etc.

Enzymatic
Digestion

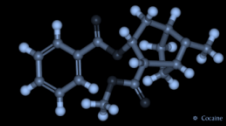
Samples are
Block
Randomized
& run with 3
technical
replicates

LC-MS



- Performed using Non Linear Dynamics Progenesis LC-MS software
- The runs are aligned
- Normalized
- Comparisons are based on the relative intensities of extracted ion chromatograms of complex samples





LFQ Analysis on Sample Set LF12-033



Experiment Design

Condition	control S1, S2, S3	Extinction S4, S5, S6	Reconsolidation S7, S8, S9
Replicates	8	7	8

Experiment: compared control vs extinction or reconsolidation of memories associated with cocaine self-administration in rats.

Proteins

Protein building options

Protein grouping Group similar proteins

Protein quantitation Using only features with no protein conflicts

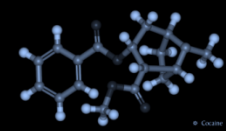
ATP Synthase subunit beta
Shows up regulation
In the reconsolidation

Accession	Peptides	Score	Anova (p) *	Fold	Tags	Description	Average Normalised Abundances		
							control S1, S2, S3	Extinction S4, S5, S6	Reconsolidation S7, S8, S9
ATPA_RAT	10	496.18	0.04	7.31		ATP synthase subunit alpha, mitochondrial OS=Rattus norvegicus GN=Atpa1 PE=1 SV=2	1.56e+005	1.57e+005	1.14e+006
ATPB_RAT	10	485.54	8.46e-003	47.59		ATP synthase subunit beta, mitochondrial OS=Rattus norvegicus GN=Atpb1 PE=1 SV=2	2.16e+004	2.50e+004	1.03e+006
HBA_RAT	6	470.83	0.25	5.47		Hemoglobin subunit alpha-1/2 OS=Rattus norvegicus GN=Hba1 PE=1 SV=3	8.23e+005	8.65e+005	4.50e+006
SPTN1_RAT	15	464.27	0.23	1.22		Spectrin alpha chain, non-erythrocytic 1 OS=Rattus norvegicus GN=Sptan1 PE=1 SV=2	1.26e+006	1.33e+006	1.09e+006
RTN1_RAT	8	436.97	0.04	1.27		Reticulon-1 OS=Rattus norvegicus GN=Rtn1 PE=2 SV=1	9.62e+006	1.22e+007	9.84e+006
NCAM1_RAT	6	423.96	0.16	1.17		Neural cell adhesion molecule 1 OS=Rattus norvegicus GN=Ncam1 PE=1 SV=1	9.23e+006	1.08e+007	9.25e+006
ODPA_RAT	14	411.81	0.16	1.22		Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial OS=Rattus norvegicus GN=Pdha1 PE=1 SV=2	6.83e+006	8.32e+006	7.91e+006
STX1A_RAT	5	410.34	1.39e-003	1.89		Syntaxin-1A OS=Rattus norvegicus GN=Stx1a PE=1 SV=1	4.35e+006	5.29e+006	2.80e+006
CXAI_RAT	21	408.07	0.14	1.19		Gap junction alpha-1 protein OS=Rattus norvegicus GN=Gja1 PE=1 SV=2	1.14e+007	9.96e+006	1.19e+007
SCG2_RAT	10	408.00	0.61	1.13		Secretogranin-2 OS=Rattus norvegicus GN=Scg2 PE=2 SV=1	4.50e+006	4.07e+006	4.59e+006

Pilot Project: Mary Torregrossa

Identification of Proteins that are Differentially Activated by Drug Cue Memory Extinction and Reconsolidation in the Amygdala and Nucleus Accumbens using Phosphoproteomics

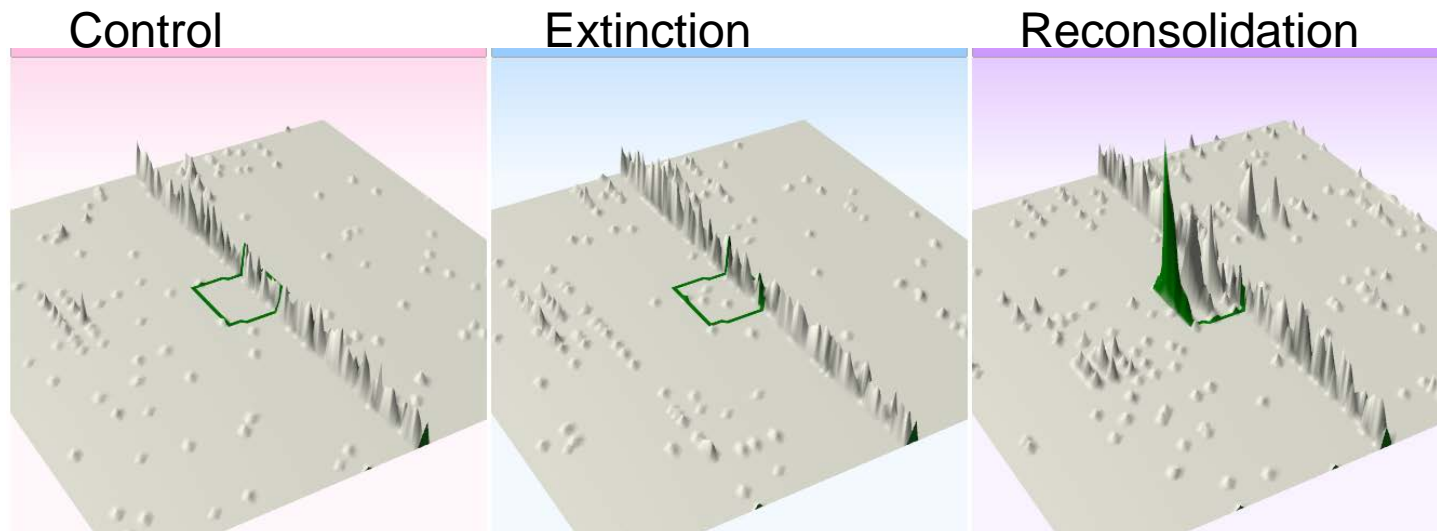


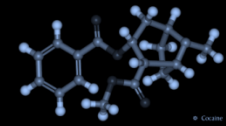


3D Montage View of an ATP synthase subunit beta peptide



- The Montage view can be used to verify a peptide change
- This shows one peptide:
m/z=594.3253, triply charged, mass MH+= 1780.954
Sequence identified : IMNVIGEPIDERGPIK
Peptide score:45.6
Fold change = 1.03e+4



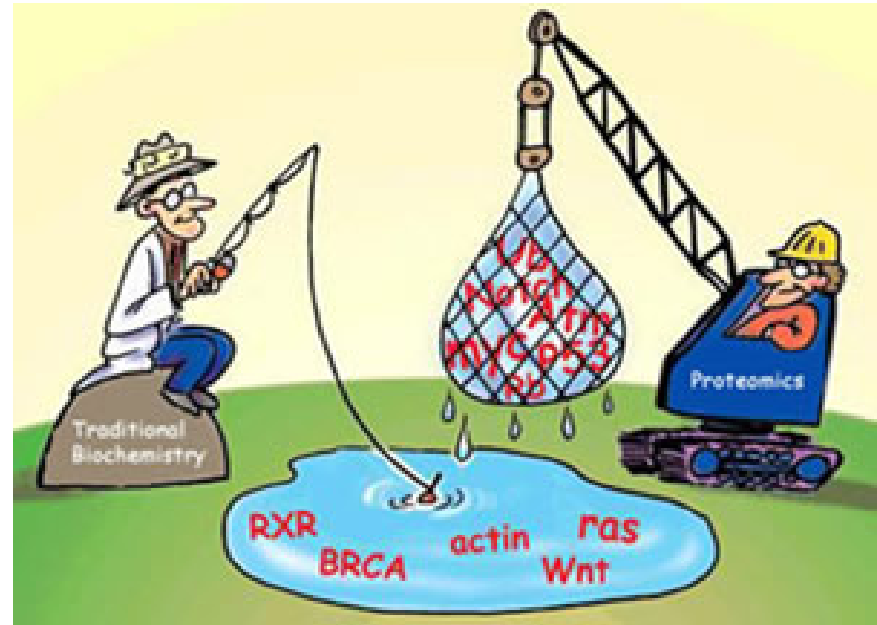


Summary



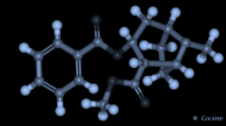
protein profiling: Allows one to find differences in sample spectra very quickly using a small amount of material. When those differences are noted, one may proceed to identify and purify larger amounts of material using other types of array. This material can then be used to characterize the protein and assays can be developed for research or diagnostic purposes.

(http://www.genomicglossaries.com/content/ex_bio.asp)

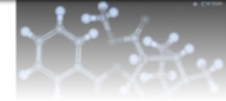
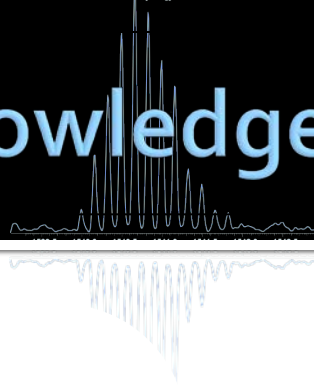


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