



Biological Profiling

• Microarrays

- cDNA arrays
- oligonucleotide arrays
- whole genome arrays
- Proteomics
 - yeast two hybrid
 - PAGE techniques



Proteomics - PAGE techniques

- Proteins can be run through a poly acrylamide gel (similar to that used to seqparate DNA molecules).
- Can be separated based on charge or mass.
- 2D Page separates a protein extract in two dimensions.











Identifying a protein 'blob'

- Take the small pieces and run through a mass spectrometer. This gives an accurate measurement of the weight of each.
- The total weight and mass of trypsin digested fragments is often enough to identify a protein.
- The mass spec is known as a MALDI-TOFF







How to build a protein network

- What is there
- High throughput 2D PAGE
- Automatic analysis of 2D Page
- How is it connected
- Yeast two hybrid screening
- Building and analysing the network
- An example



Data obtained

- Depending on sample, you get a profile of potential protein-protein interactions that can be used to predict functional protein complexes.
- False positives are frequent.
- Can be confirmed by affinity purification etc.







Protein Networks











Predictive value of networks

Jeong et al., (2001) Lethality and Centrality in protein networks. Nature 411 p41

- In the yeast genome, the essential vs. unessential genes are known.
- Rank the most connected genes
- Compare known lethal genes with rank order

k	fraction	%lethal
<6	93%	21%
>15	0.7%	62%





















Synapse proteo	mes		
#PSD	proteins	000000	
Collins et al Yoshimura et al Jordan et al Peng et al Li et al 151 Satoh et al Walikonis et al	620 441 401 328 46 29	Postsynaptic Density 0.25 µm Total PSD proteins	1124
	110		1469
Consensus PSD	466 (2 or	more studies)	1168
Armstrong, 2007	,		





The synaptic proteome is enriched for proteins			
containing signa	alling relate	ed domains	
5 5	U		
		0/ 14	
	% MASC	% Mouse	ratio
Protein kinase	11.8	3.75	3.16
Ser/Thr protein kinase	10.2	1.69	6.05
SH3	8.06	1.51	5.33
Pleckstrin-like	5.91	1.25	4.72
PDZ/DHR/GLGF	5.91	0.74	8.04
Small GTP-binding domain	5.38	1.49	3.62
Pleckstrin homology	4.84	1.08	4.49
Calcium-binding EF-hand	4.84	1.65	2.93
C2	4.30	0.82	5.26
IQ calmodulin-binding region	3.76	0.31	12.0



Annotation of	MASC prote	eins expect (random)
Schizophrenia	33	3
Bipolar disorder	12	
Depression	14	
Mental retardation	n 23	
LTP	44	3
Rodent spatial lear	rning 32	2
Rodent fear condit	tioning 25	
Armstrong, 2007	(186)	·















Synapse proteome predictions

• Biology:

- LTP change in neuron response after experience (electrophysiological)
- Mouse KOs
- Network Analysis
 - vertex degree (number of protein interactions)
 - network diameter (average shortest path after simulated protein deletion)



















core network properties

architecture relates to function small world nature gives robustness underlying modular substructure modules have specific functionality

what about dynamics?

- regulation within network
- evolution from simple nervous systems
- expression patterns across brain regions





















Name	motif (ENSP00000279593)	Start
PXSP motif	PRSP	1114
not named ?	CxxCxxxxNLYDIxED	1242
fyn site	Y	1246
PXSP motif	PQSP	1282
CanKII binding	RQHSYD	1300
РКС	S	1303
РКС	S	1323
p85 P13K binding	YxxM	1336
fyn site	Y	1336
AP-2 binding	YEKL	1474
fyn site	Υ	1474
CK2 site	S	1481
PDZ binding	ESDV	1481

Vertebrate NR2 cytoplasmic C-terminal motifs absent in invertebrates



































