

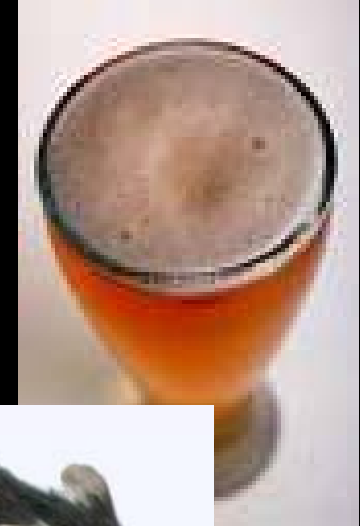
# Sample Preparation and Fractionation for Proteomics

Ben Herbert

# Two types of Proteomes.

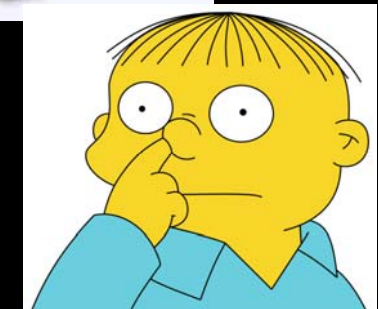
## 1. Model proteomes.

- Completely sequenced genome.
- Free of contaminating proteomes.



## 2. Clinical proteomes.

- Partial genome sequence.
- Contaminating proteomes.



## Hypothesis-driven or not ?

- Non-hypothesis driven

- Larger numbers of IDs
- may be more suited to 'shotgun' techniques
- LC-MS
- 1-D gel and LC-MS

- Hypothesis driven

- Fewer IDs required
- more suited to 2-D gel techniques
- MALDI or LC-MS for IDs
- 1-D gel and LC-MS

Many projects employ a mix of techniques to obtain broader coverage of the proteome

## What outcomes are required ?

- Protein ID lists – catalogue of the proteome
  - may be better to use LC-MS and 1-D gels
  - one sample
- Comparison of two or more samples
  - 2-D gels
  - labeling technologies (DIGE) and sensitive stains
  - 1-D gels and LC-MS or iTRAQ etc

# How much sample do you have ?

- Micrograms of protein
  - LC-MS
  - 1-D gels and LC-MS
  - Many IDs possible with low sample amount
- 2-D gels with nanogram detection stains or DIGE
- MS analysis can be difficult – requires high sensitivity and good technique

# How much sample do you have ?

- Milligrams of protein
  - preparative 2-D gels – fluorescent stain and coomassie
  - fractionation
    - isoelectric or molecular weight fractionation
    - membrane preparations

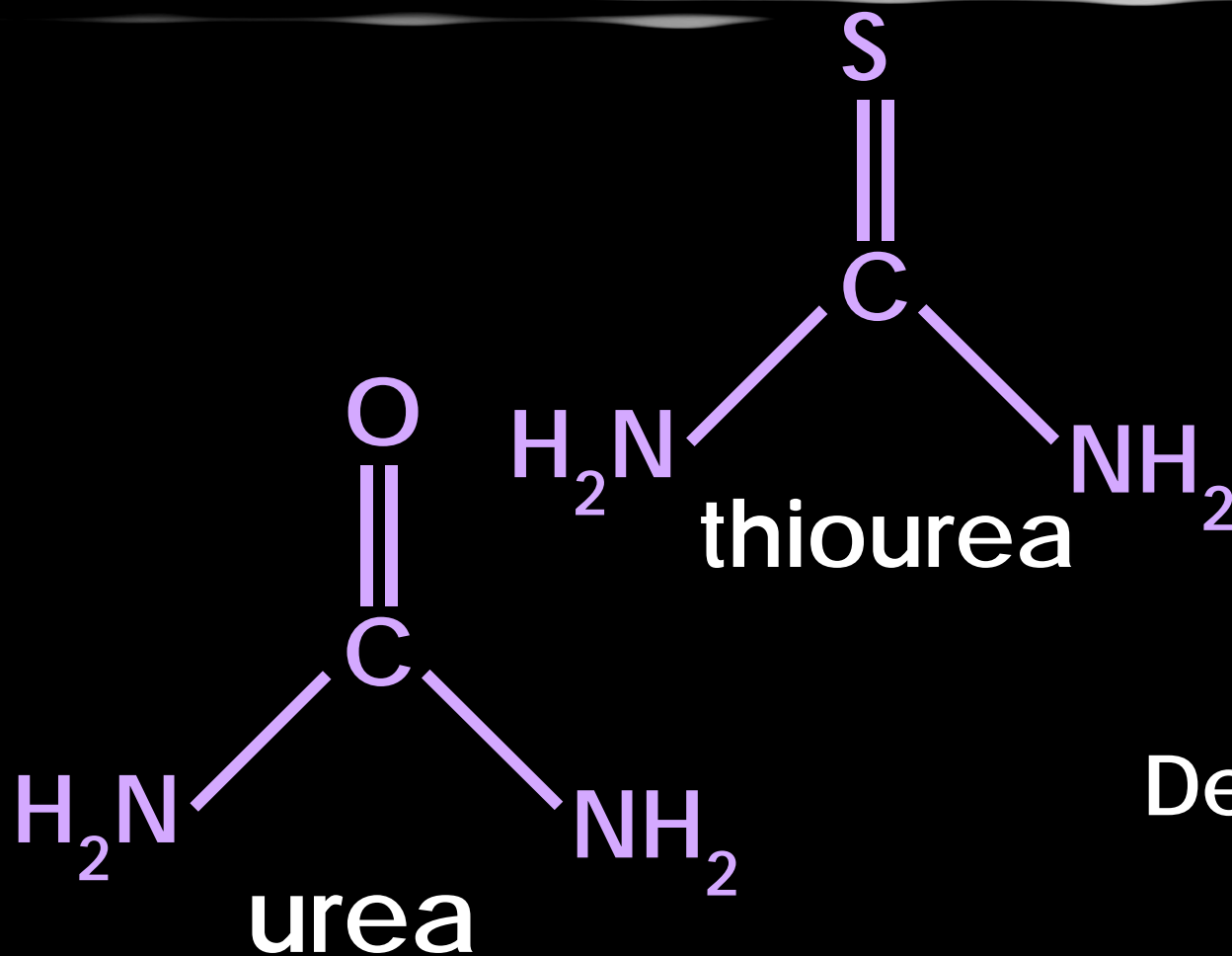
**if you can't solubilise  
- you can't analyse**

**Step 1: extract/solubilise the protein from the biological sample**

**Step 2: remove any substances which may interfere with the analysis**

**Step 3: maintain protein solubility throughout separation**

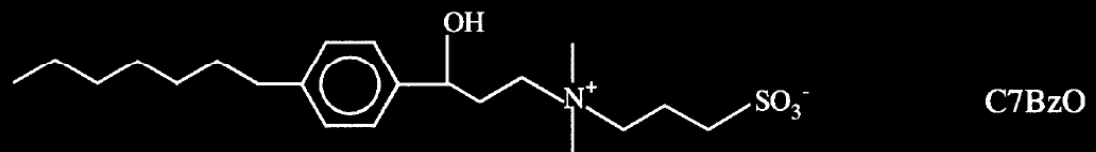
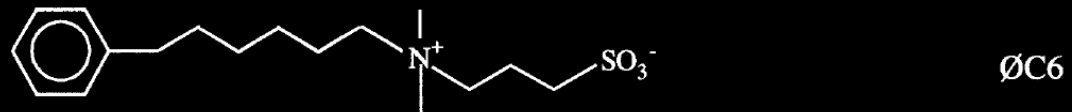
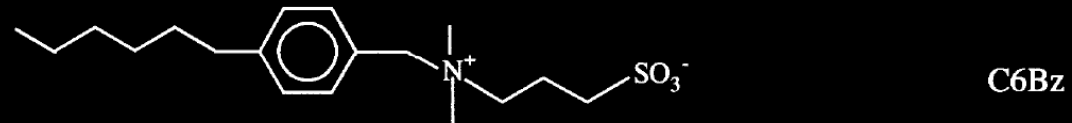
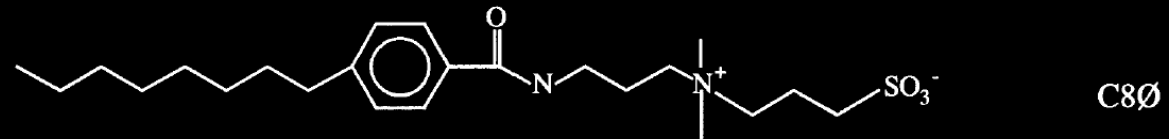
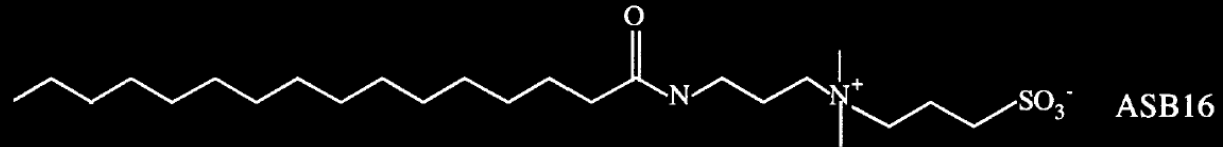
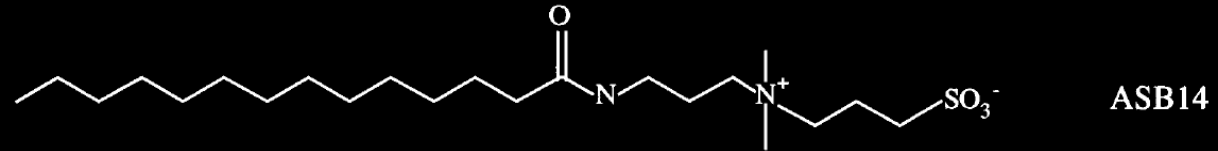
sample preparation  
- chaotropes and detergent



Detergent

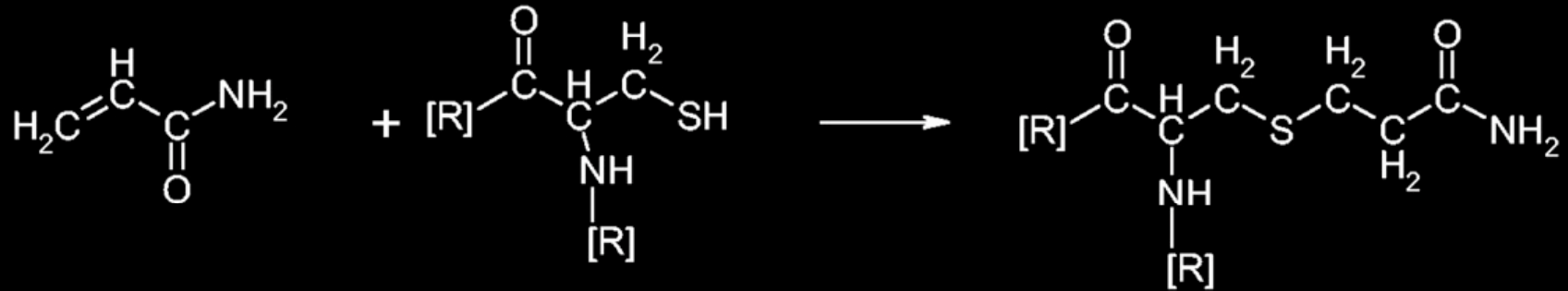
# sample preparation

- agents tensio-actifs inventés par le Thierry Rabilloud



# Reduce and alkylate your proteins before electrophoresis

ACRYLAMIDE



ACR

Protein cysteine residue

S-(2-carbamoylethyl)-cysteine adduct

**Simple (1step) alkylation chemistry – reduce with TBP**

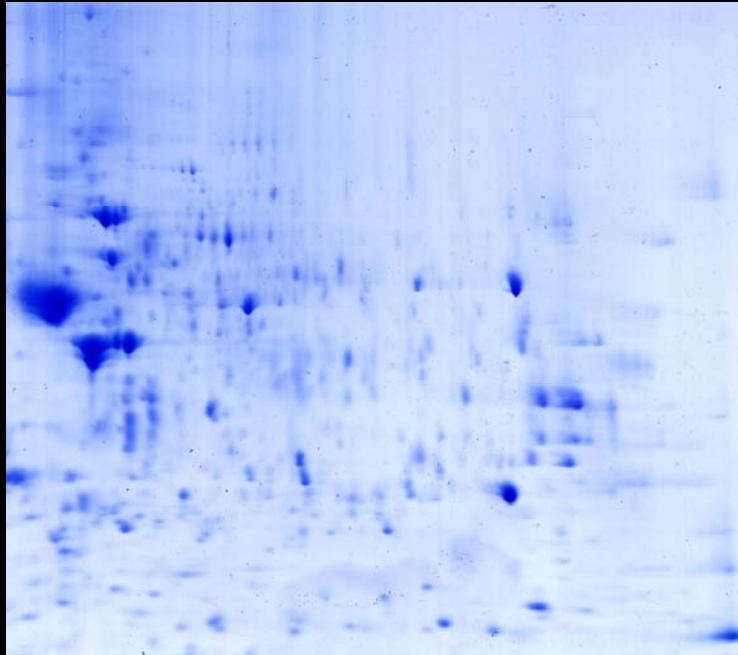
**acrylamide does not react with phosphines**

**neutral cys-adducts**

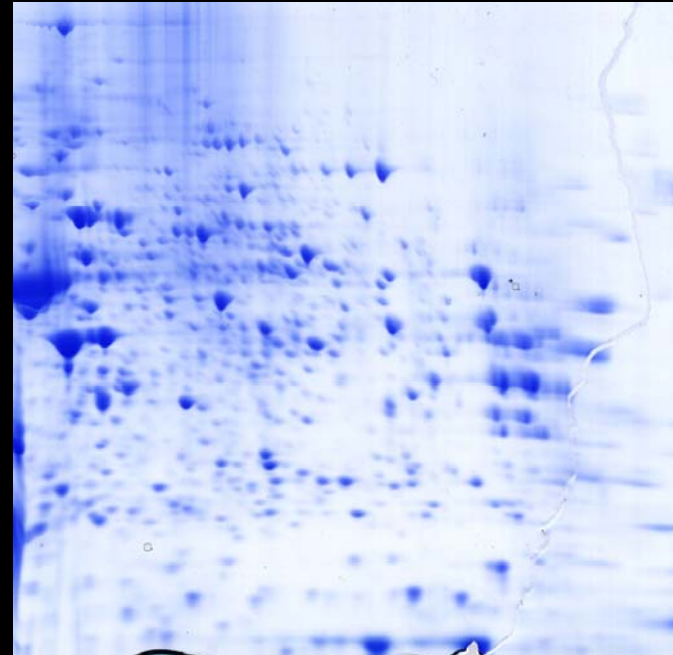
**suitable for isoelectric focusing**

# reduction and alkylation

- ratio of protein to alkylating agent



TBP reduction



Acrylamide alkylated

# sample cleanup - salts

*salts and buffers are the number one cause of problems with 2-D gel separations*

## Technical Information

	<b>BIO-SPIN P-6</b>	<b>MICRO BIO-SPIN P-6</b>	<b>BIO-SPIN P-30</b>	<b>MICRO BIO-SPIN P-30</b>
Packed support	Special Grade Bio-Gel P-6 gel	Special Grade Bio-Gel P-6 gel	Special Grade Bio-Gel P-30 gel	Special Grade Bio-Gel P-30 gel

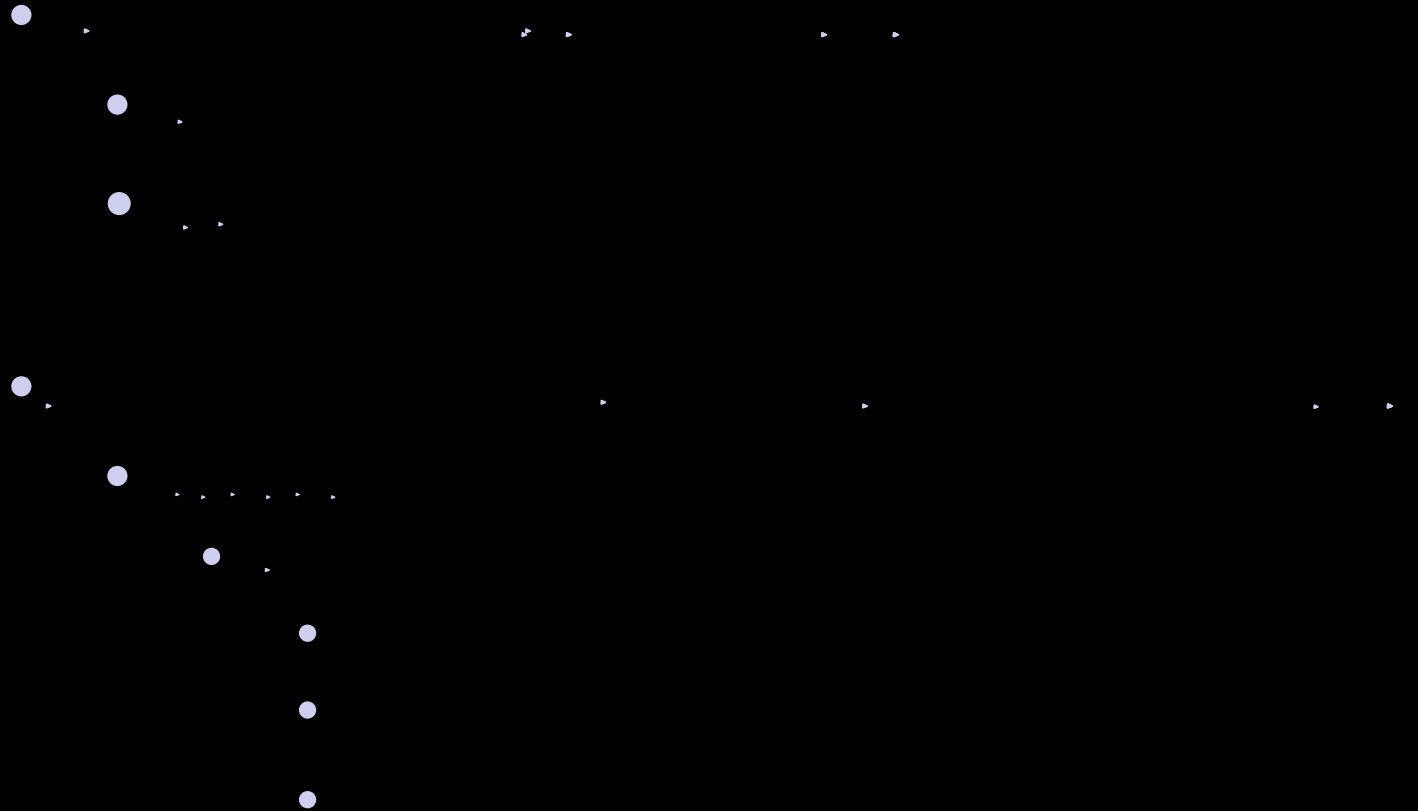
## equilibrate with urea, thiourea, detergent

Application	Desalting and buffer exchange	Desalting and buffer exchange	Desalting, and nucleotide and small molecule removal	DNA sequencing reaction mixtures (Tris), and small molecule removal
Bed volume	1.1 ml	0.7 ml	1.1 ml	0.7 ml
Retention and recovery	90% recovery of 20 bases/bp, 99% retention of salts	90% recovery of 20 bases/bp, 99% retention of salts	95% recovery of 22 bases/bp, 98% retention of dNTPs	95% recovery of 22 bases/bp, 98% retention of dNTPs
Exclusion limit, globular proteins	6,000 daltons	6,000 daltons	40,000 daltons	40,000 daltons
Sample volume	50–100 µl	10–75 µl	50–100 µl	10–75 µl
Centrifuge type	Swinging bucket	Microcentrifuge	Swinging bucket	Microcentrifuge
Autoclavable	Yes	Yes	Yes	Yes

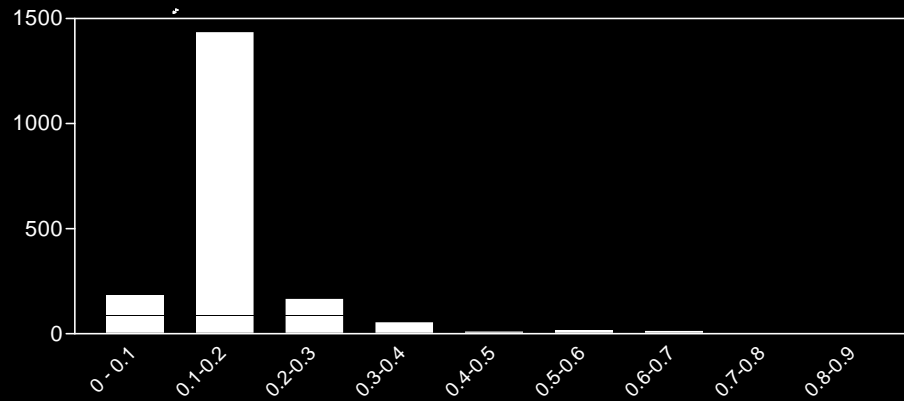
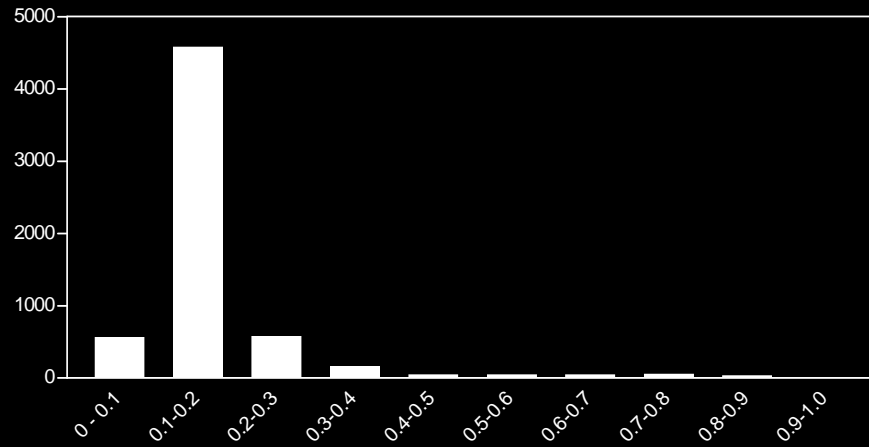
# Membrane Proteomics

methods for fractionation  
solubilisation and separation

# Proteome analysis - *2-D or not 2-D*



# How big is the problem?



# Why can't we see more proteins on gels?

.

•

•

•

•

✓

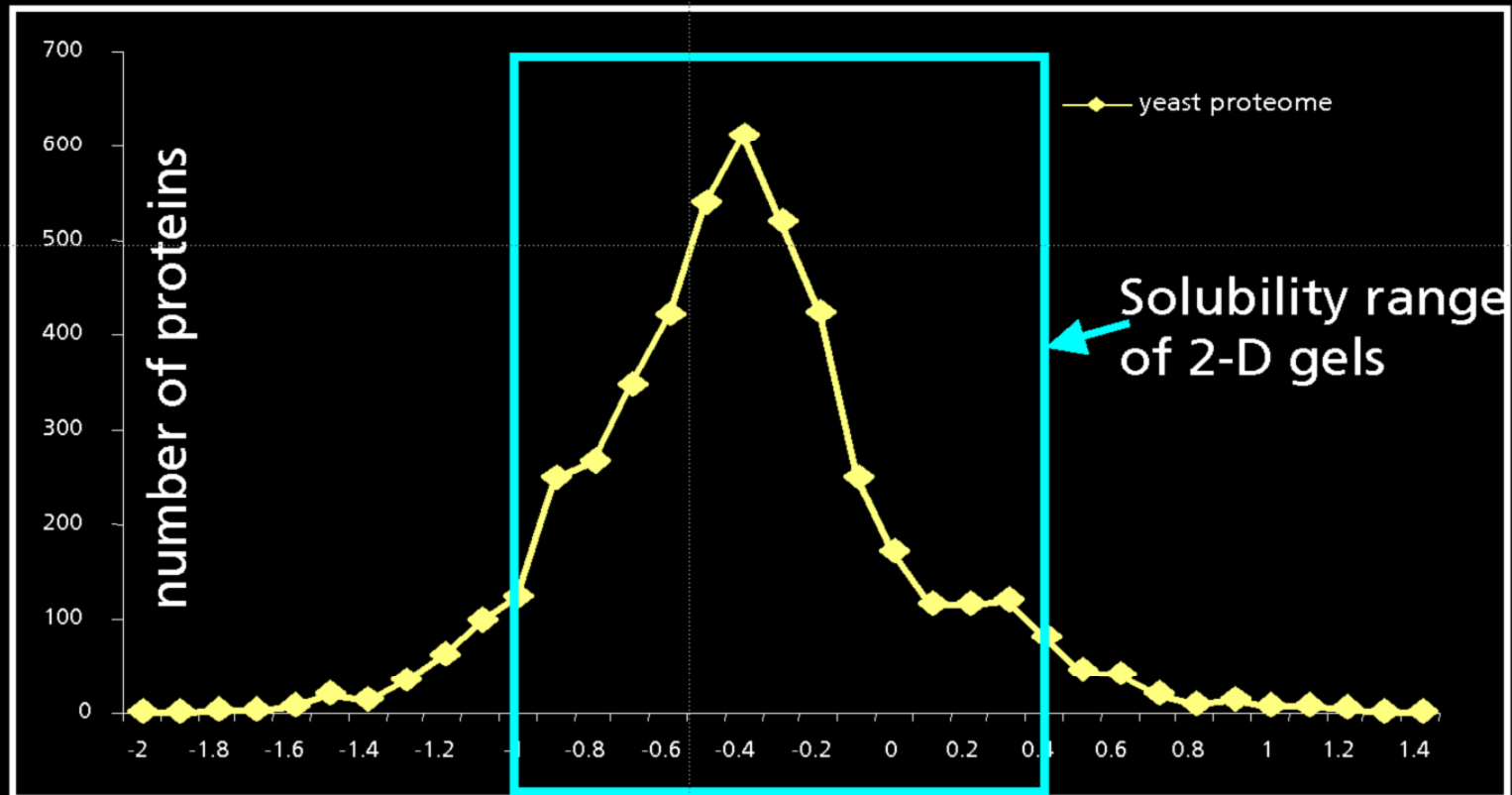
•

•

•

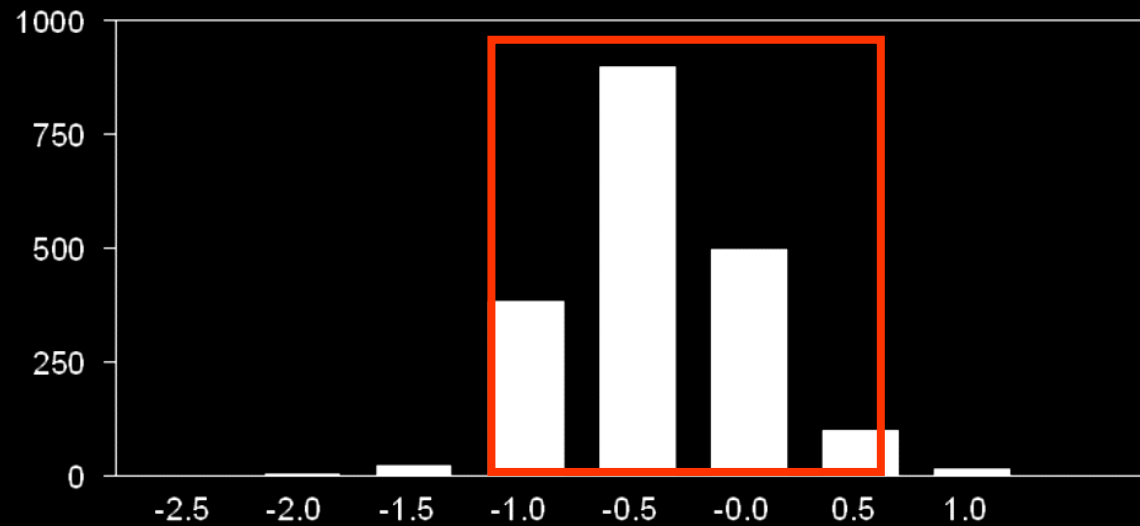
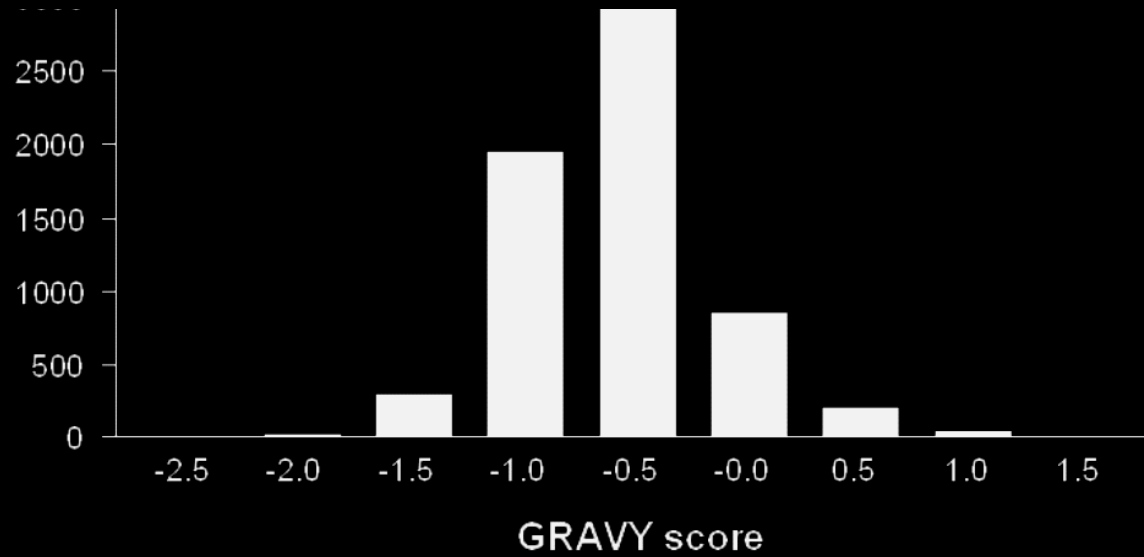
solubility is not the major problem

## *S. cerevisiae*

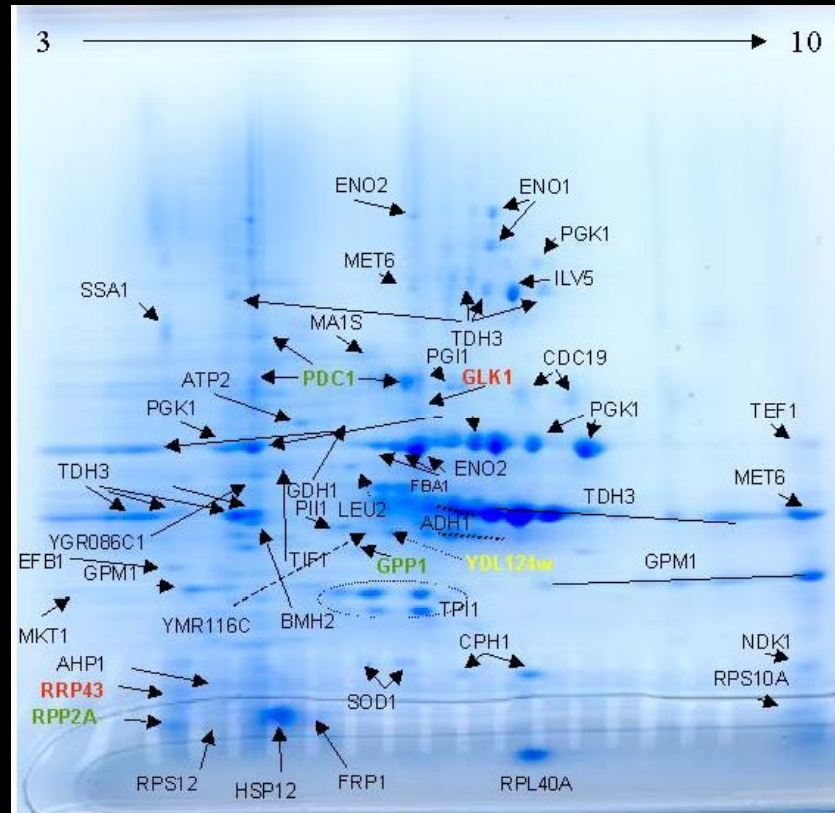


Hydrophobicity by GRAVY score

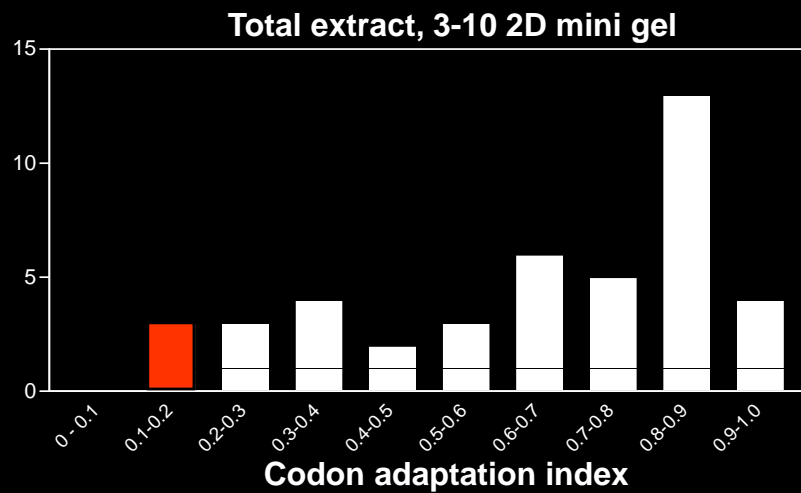
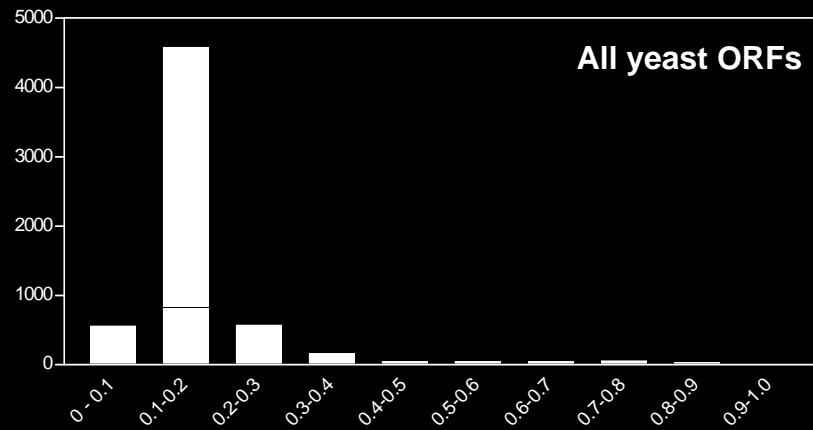
# Membrane proteins – *most should be soluble*



# Yeast total extract - *no membrane proteins*



# Yeast total extract - *data summary*



# Yeast membrane prep – *sodium carbonate*

Eur J Biochem

1296

EJB EB1293

aw 31/3/0 17:17

ALDEN

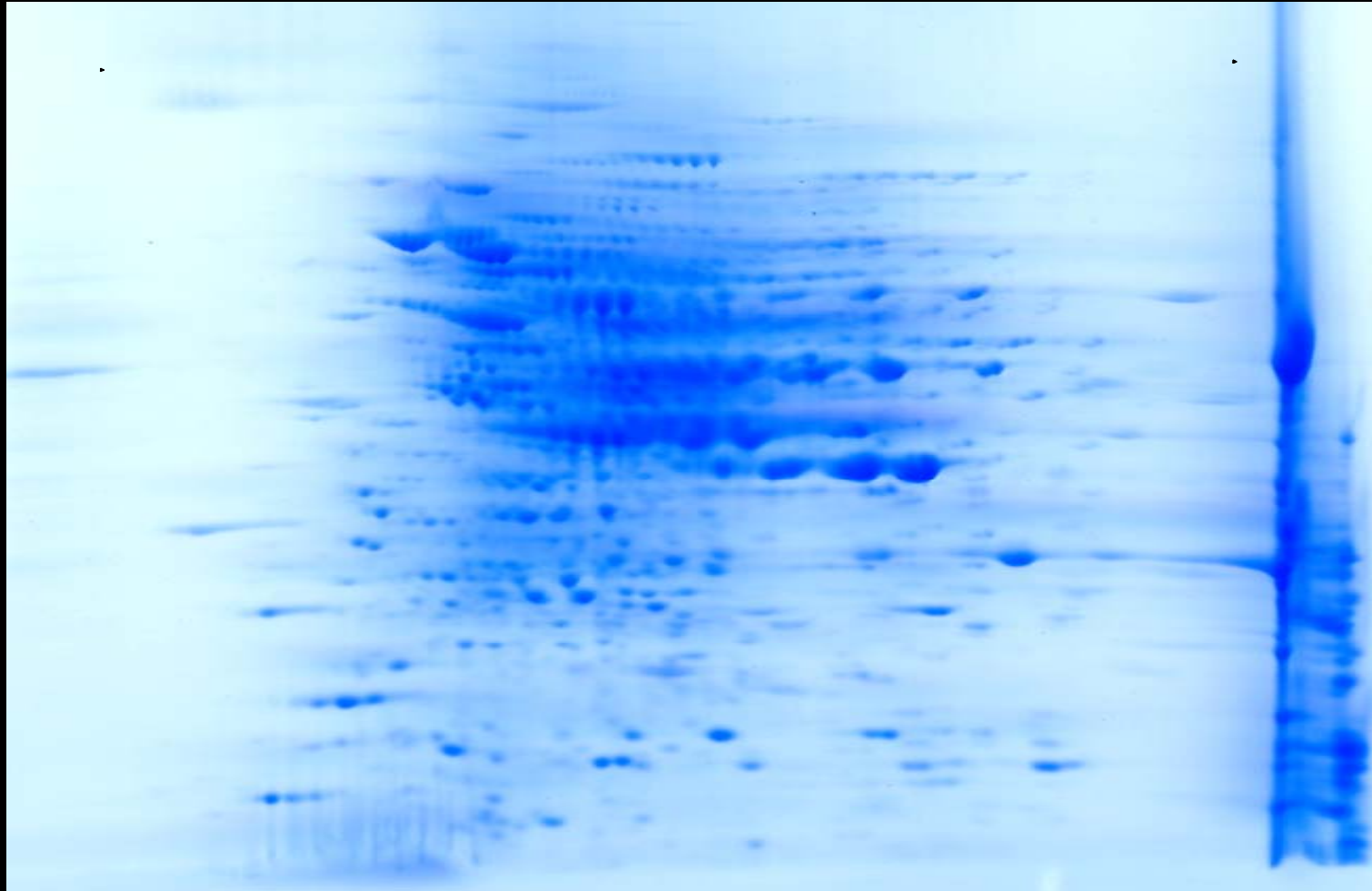
*Eur. J. Biochem.* 267, 1–12 (2000) © FEBS 2000

## Proteomic analysis of the *Escherichia coli* outer membrane

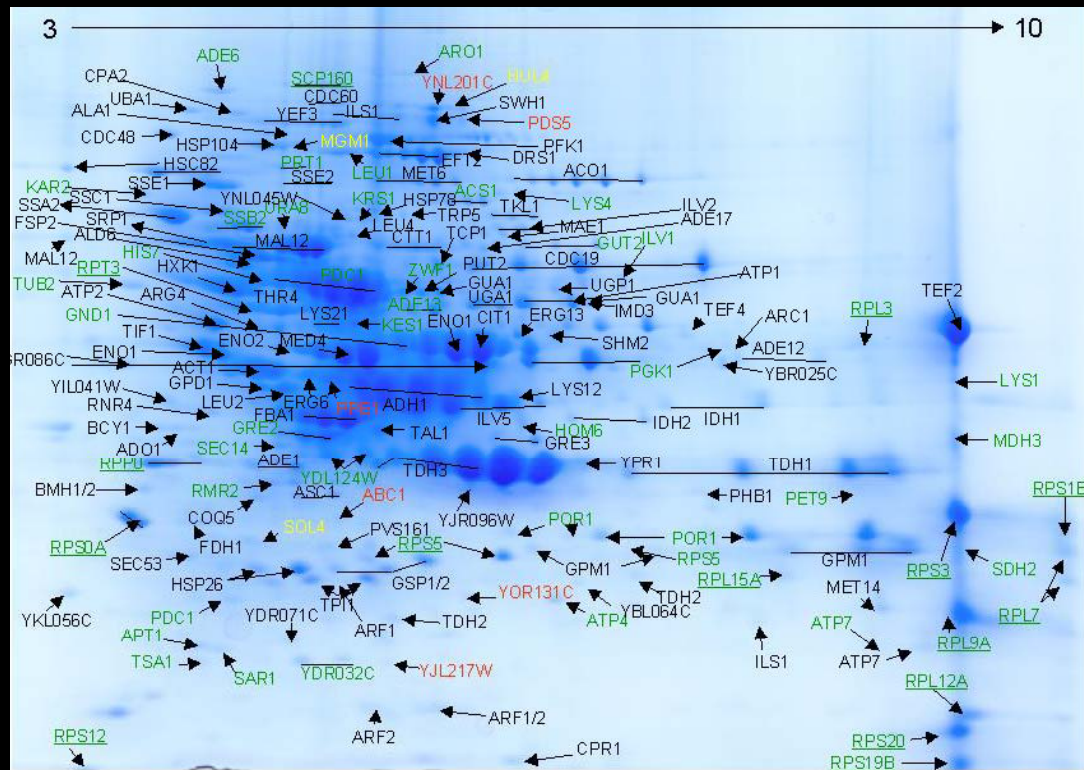
**Mark P. Molloy<sup>1</sup>, Ben R. Herbert<sup>2</sup>, Martin B. Slade<sup>1</sup>, Thierry Rabilloud<sup>3</sup>, Amanda S. Nouwens<sup>1</sup>, Keith L. Williams<sup>2</sup> and Andrew A. Gooley<sup>2</sup>**

<sup>1</sup>Australian Proteome Analysis Facility, Department of Biological Sciences, Macquarie University, Sydney, Australia; <sup>2</sup>Proteome Systems Limited, Sydney, Australia; <sup>3</sup>DBMS/BECP, CEA-Grenoble, France

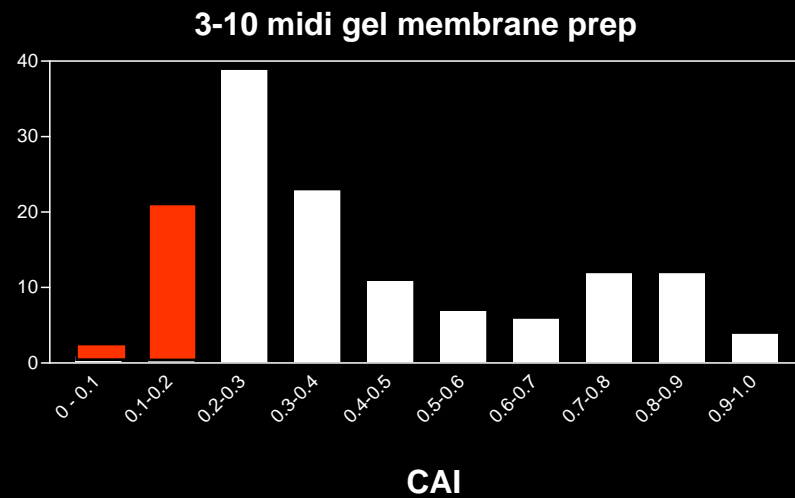
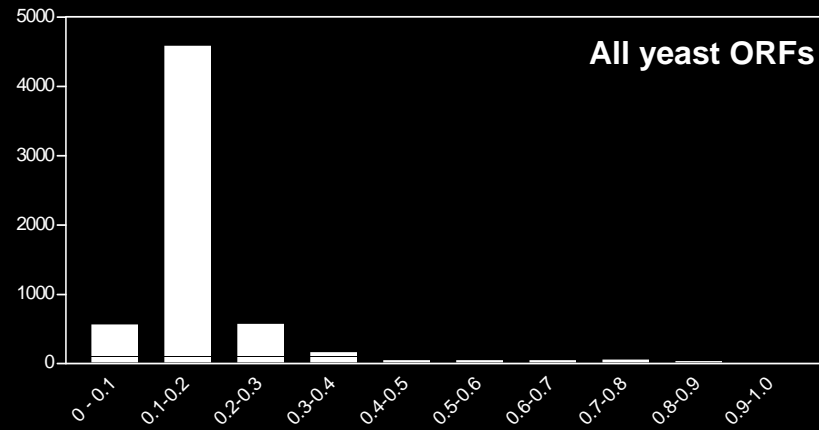
Yeast membrane 2-D gel - *urea/thiourea/ASB14*



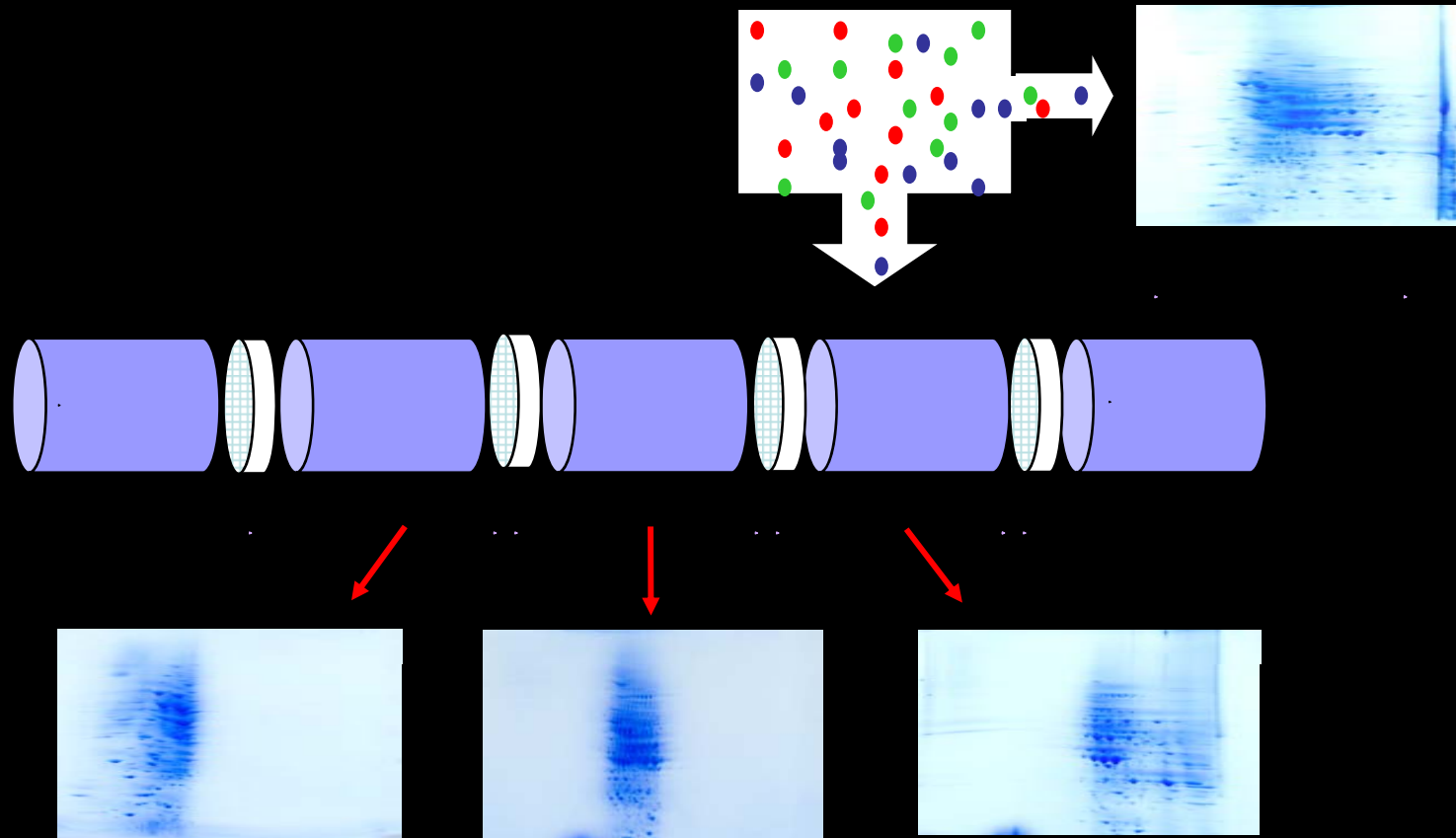
# Yeast membrane prep - *20% integral membrane*

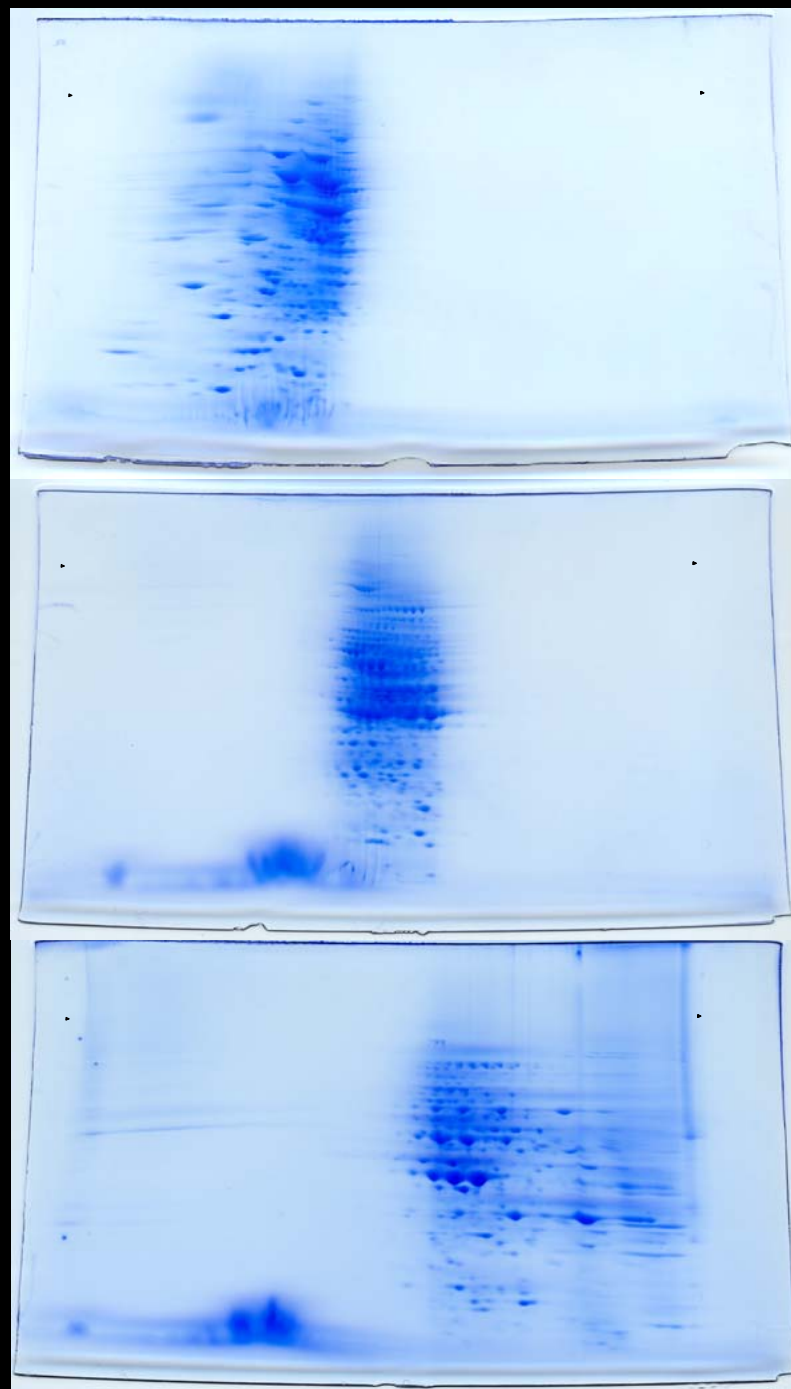
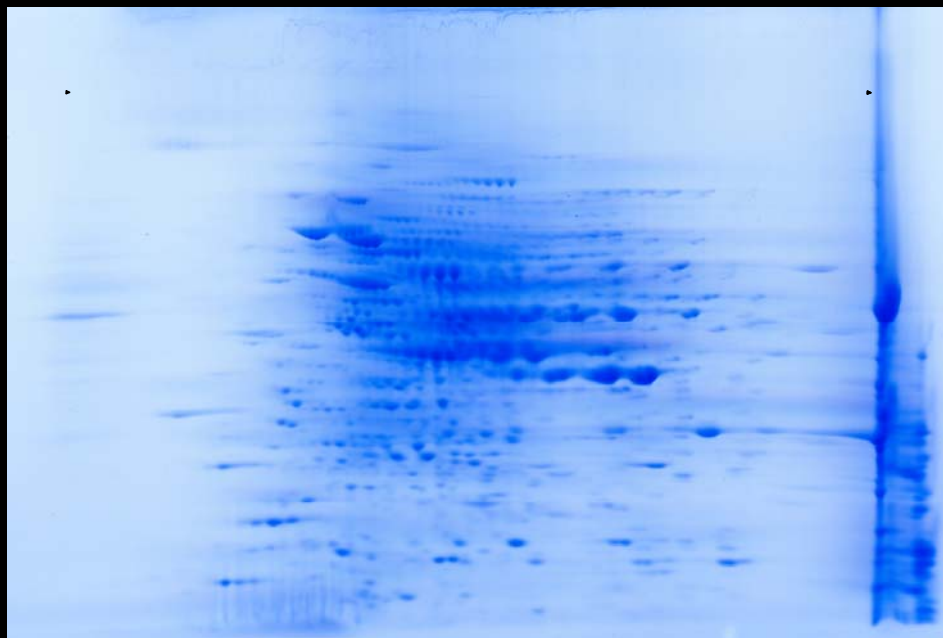


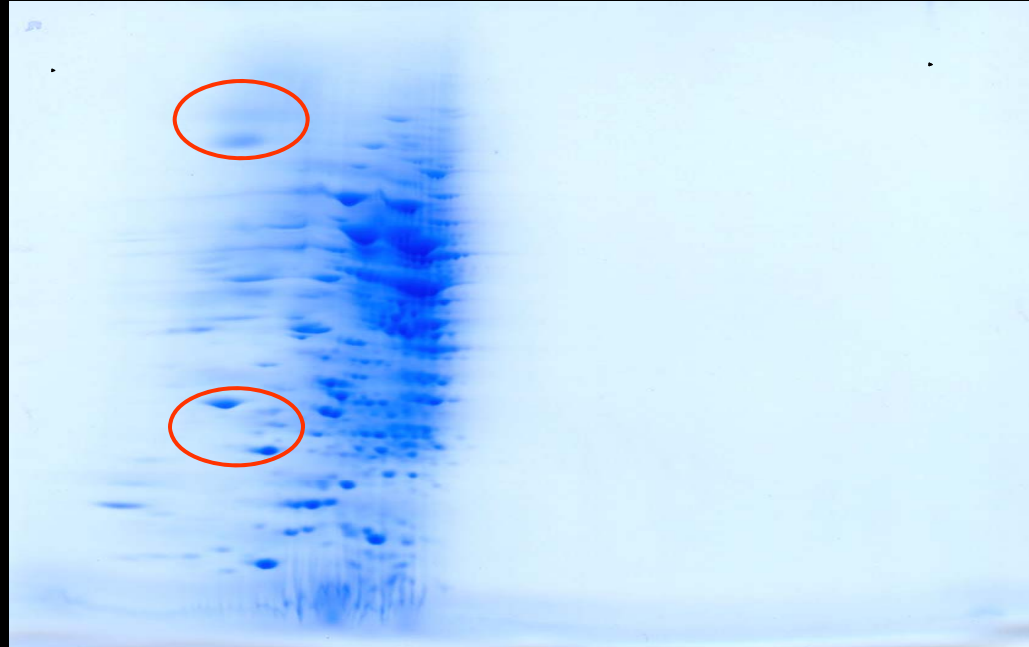
# Yeast membrane extract – *an improvement*



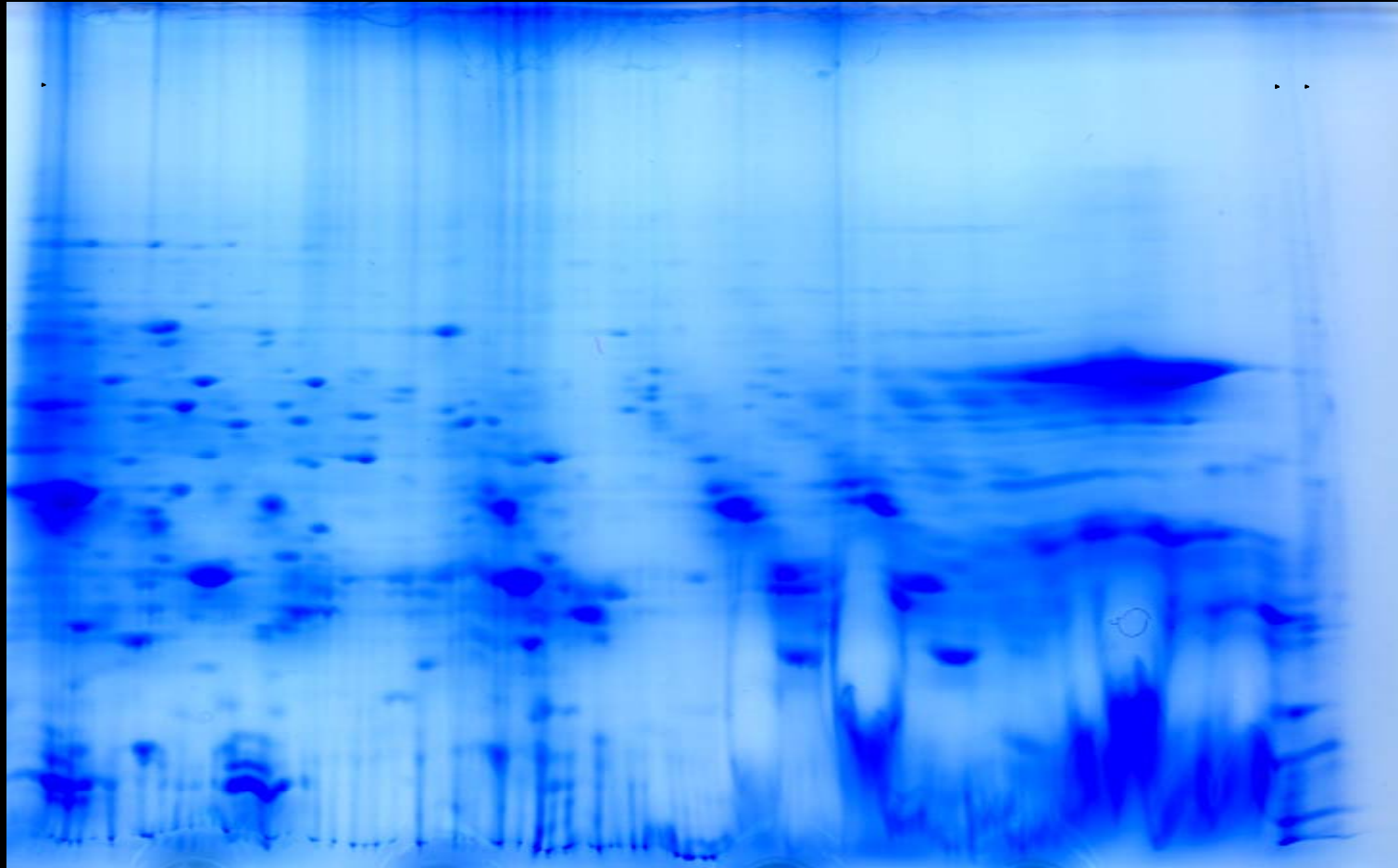
# Yeast membrane - *isoelectric fractionation*



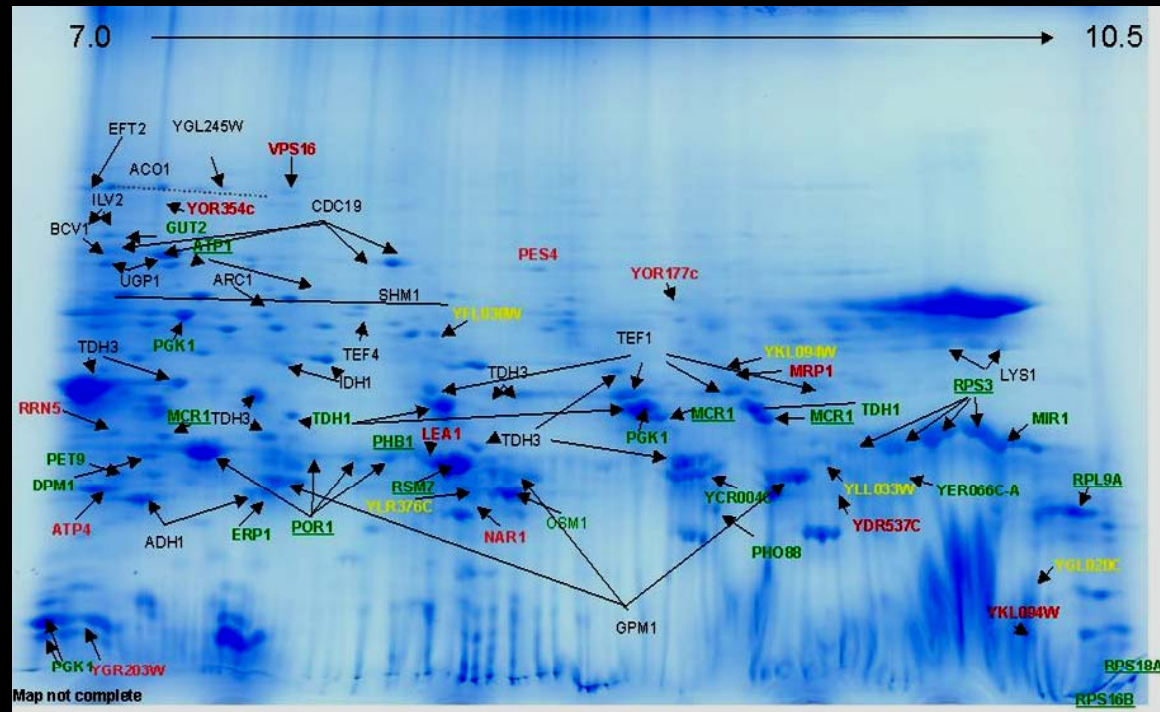




# Yeast membrane alkaline fraction

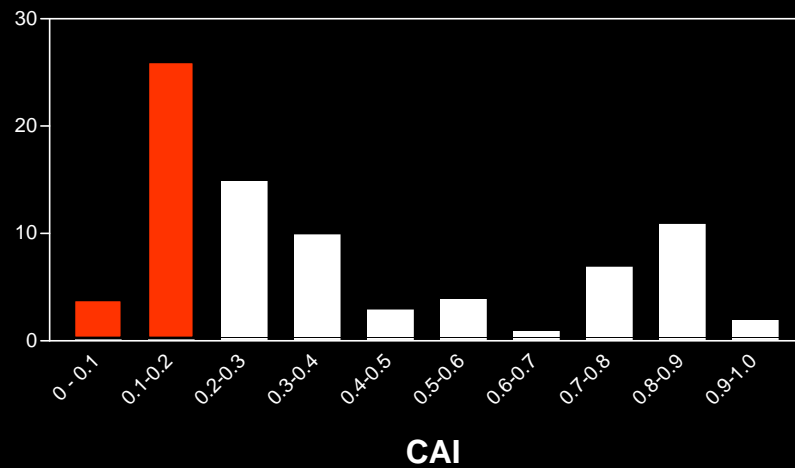
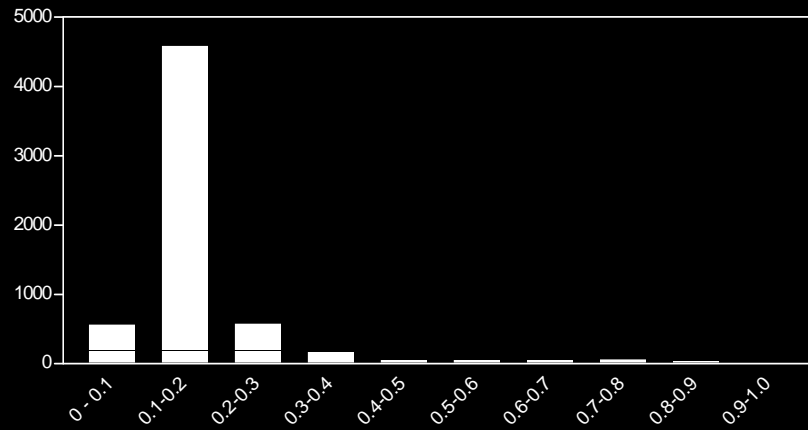


# Alkaline yeast membrane – *data summary*

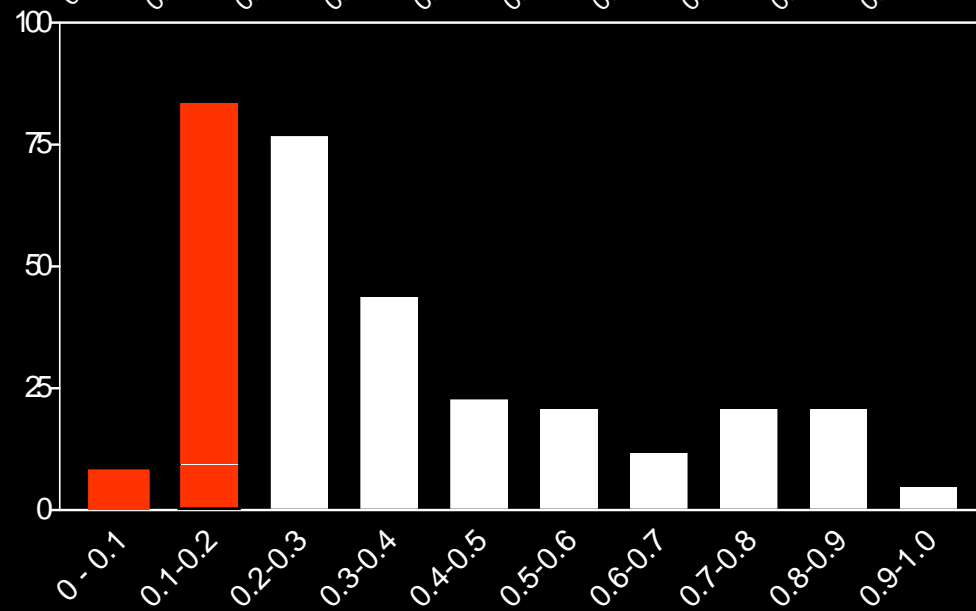
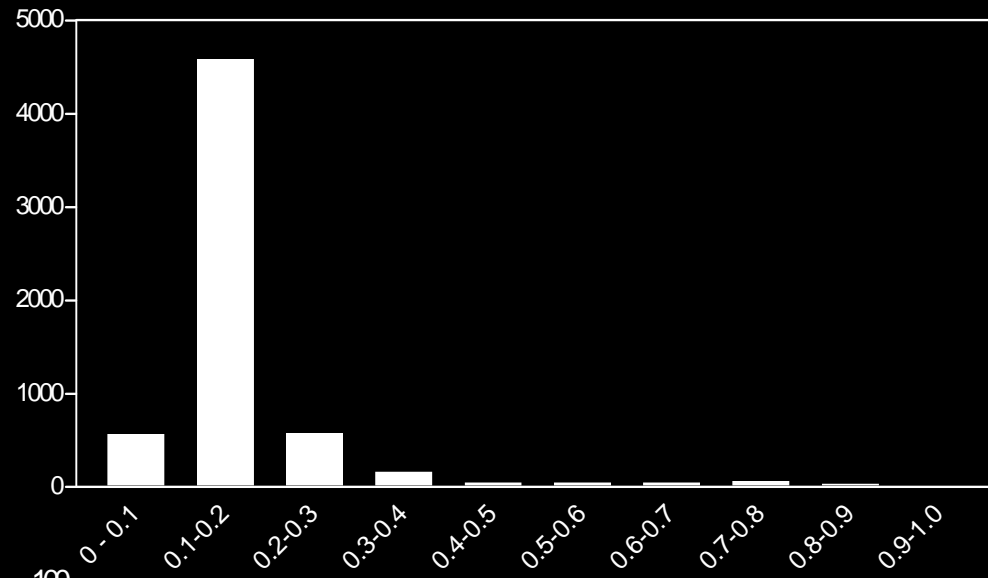


# Alkaline yeast membrane - *data summary*

Codon adaptation index, all yeast ORFs



# Yeast membrane – *overall summary so far*



# Yeast membrane – *overall summary so far*



## **Unseen Proteome: Mining below the Tip of the Iceberg to Find Low Abundance and Membrane Proteins**

Susanne K. Pedersen,<sup>†</sup> Jenny L. Harry,<sup>†</sup> Lucille Sebastian,<sup>†</sup> Jasmine Baker,<sup>†</sup> Mathew D. Traini,<sup>†</sup>  
John T. McCarthy,<sup>†</sup> Abi Manoharan,<sup>†</sup> Marc R. Wilkins,<sup>†</sup> Andrew A. Gooley,<sup>†</sup>  
Pier Giorgio Righetti,<sup>‡</sup> Nicolle H. Packer,<sup>†</sup> Keith L. Williams,<sup>†</sup> and Ben R. Herbert<sup>\*,†</sup>

*Proteome Systems, 35 Waterloo Rd, North Ryde, Sydney, NSW 2113, Australia, and the Department of Agricultural and Industrial Biotechnologies, University of Verona, 37134 Verona, Italy*

**Received December 5, 2002**

Abundant and hydrophilic nonmembrane proteins with isoelectric points below pH 8 are the predominant proteins identified in most proteomics projects. In yeast, however, low-abundance proteins make up 80% of the predicted proteome, approximately 50% have  $pI$ 's above pH 8 and 30% of the yeast ORFs are predicted to encode membrane proteins with at least 1 trans-membrane span. By applying highly solubilizing reagents and isoelectric fractionation to a membrane fraction of yeast we have purified and identified 780 protein isoforms, representing 323 gene products, including 28% low abundance proteins and 49% membrane or membrane associated proteins. More importantly, considering the frequency and importance of co- and post-translational modifications, the separation of protein isoforms is essential and two-dimensional electrophoresis remains the only technique which offers sufficient resolution to address this at a proteomic level.

# Membrane proteins on 2-D – *reality*

•

•

—

•

•