GOVERNO DE PORTUGAL
MINISTÉRIO DA SAÚDE

Instituto_Nacional de Saúde Doutor Ricardo Jorge

Proteomics in biomarker discovery for clinical purposes

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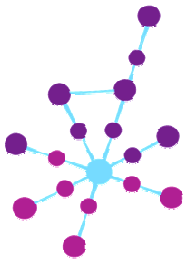

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DEPARTAMENTO DE GENÉTICA INSA I.P.

Biomarker

NIH-USA official definition:

A characteristic that is objectively measured and evaluated as indicator of normal or pathogenic biological processes or pharmacological response to a therapeutic intervention"

Biomarker still needed for

- early detection of diseases to benefit from the potential therapies.
- pharmacodynamic assessment of drug action to help guide dose and schedule
- selection of patients who will benefit from therapy (pharmacoproteomics)

impact on patient well being and financial viability of healthcare systems

Why Protein as Biomarker ?

To understand **how to control** an **environmental response** and or **treat** a particular **disease**, it is necessary to **identify the proteins** associated with these processes and **understand how they function**.

Clinical Proteomics

Dedicated to the study of the **PROTEOME PROFILE** associated with the **HEALTHY AND DISEASE STATE**, in the search for **DIAGNOSTIC / PROGNOSTIC / MONITORING BIOMARKERS** or as **TARGETS** for the development of new therapeutic approaches

Proteins are complex

- **Genes are digital in nature with a 4-letter language**, **proteins** are analog with a **20 letters language;** genes operate in a one-dimensional world and **proteins** in a **three-dimensional world**
- **Proteins is extremely complex** due to: modifications by gene mutation, RNA editing, RNA splicing, up to 400 types of covalent changes and protein processing
- **Proteins** are **dynamical**, changing their **3-dimensional structures**, **positions** in the cell, **concentrations** at different cellular sites, **sequence**s, **covalent chemistries**, and **interactions** with other proteins and molecules of many types in response to endogenous and exogenous stimuli;
- **Proteins** exhibit a 106 **dynamic range** in tissues and a 1012 dynamic range in blood, making quantification essential
- **Proteins** lack the molecular complementarity of DNA and hence **cannot be amplified** prior to measurement—thus, **higher ultrasensitive techniques** to **measure and analyze** protein molecules **is needed**

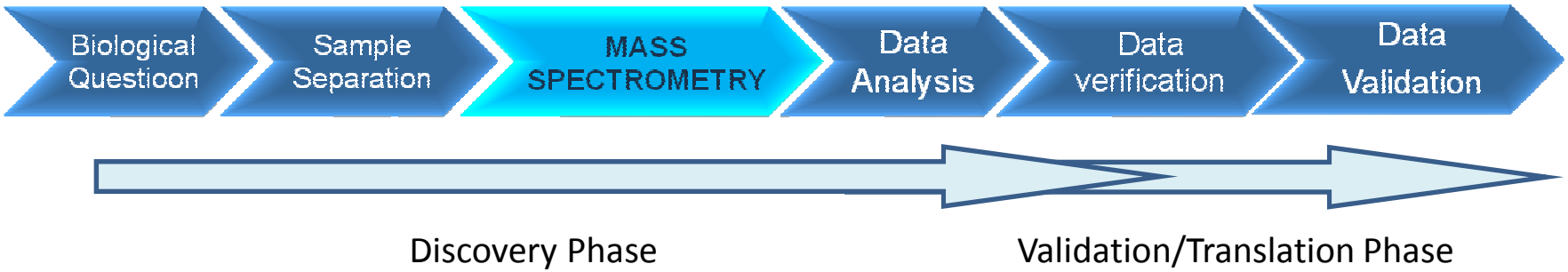
Hood et al Proteomics. 2012 Sep;12(18):2773-83

Proteomics Technology

- Discovery-based approach
- Targeted –based approach

Discovery -based approach

What proteins can be detected in this sample ?



Penque, Expert Rev Proteomics, 2007, 4:199-209

Torre et al, 2015. Book chapter in Methods in Molecular&Biology, in press

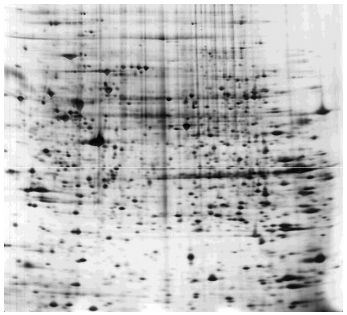
Discovery Proteomics approach

Biological Question & Sample → Sample Separation → MASS SPECTROMETRY → Data Analysis → Data Verification

Biofluid

Cell lines

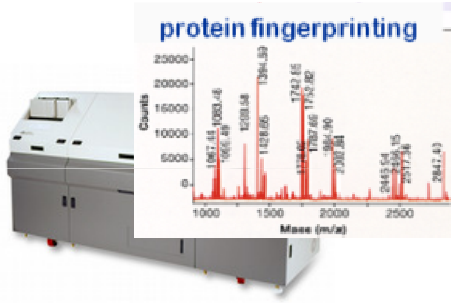
Tissue



2D-gel

HPLC

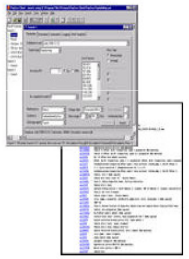
Data Aquisition



MALDITOFTOF

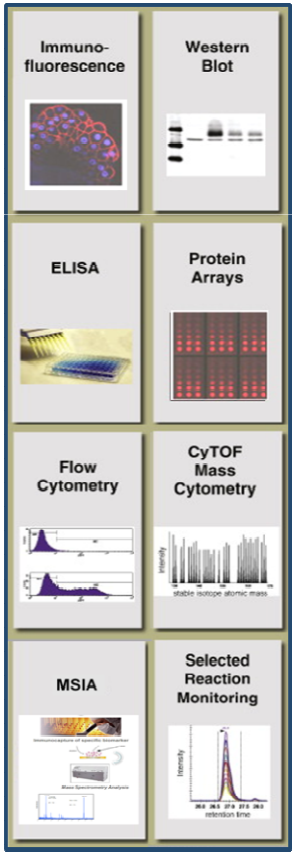
ESI-MS/MS

LC/MS/MS
Shotgun MS



Data Base Query
(GPS, Mascot, Sequest, GO, etc

Pathway/Network Analysis



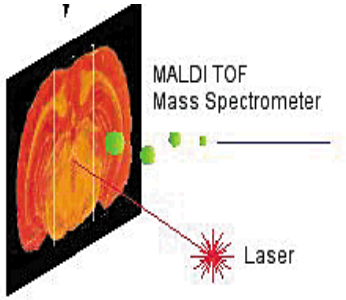
Discovery-based Proteomics approach

Biological Question & Sample → Sample Separation → MASS SPECTROMETRY → Data Analysis → Data Verification

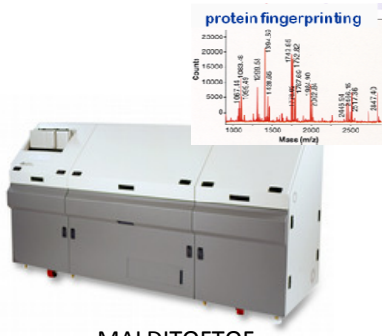
Biofluid

Cell lines

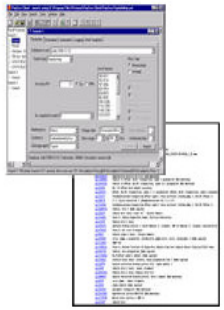
Tissue



Imaging MS



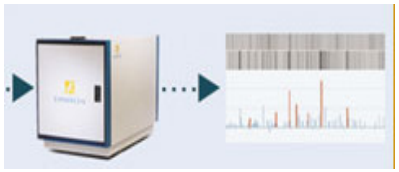
MALDITOFTOF



Data Base Query
(Mascot, Sequest, etc

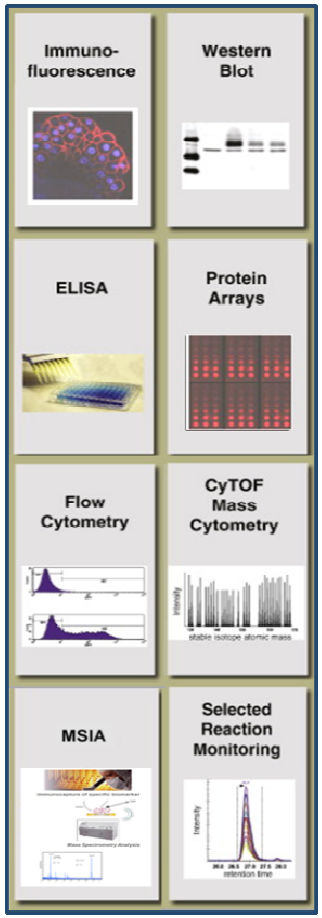
SELDI-TOF

protein CHIP



MALDI

Pathway/Network analysis



Targeted proteomics approach

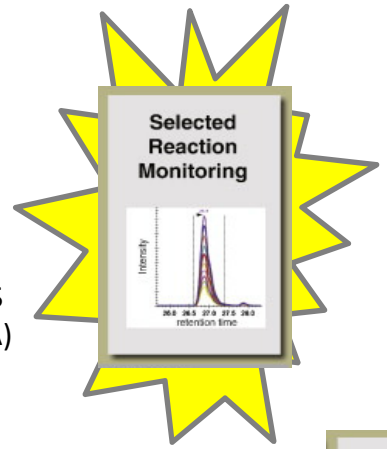
Is protein X measurable in this sample?

Shotgun proteomics

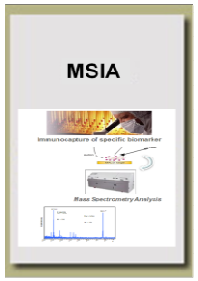
Biochemical experiments

Literature

Mass spectrometer-based



QQQ-type MS
(SRM, SISCAPA)

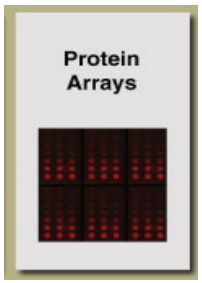


MALDI-type
(MISA, iMALDI)

SRMAtlas

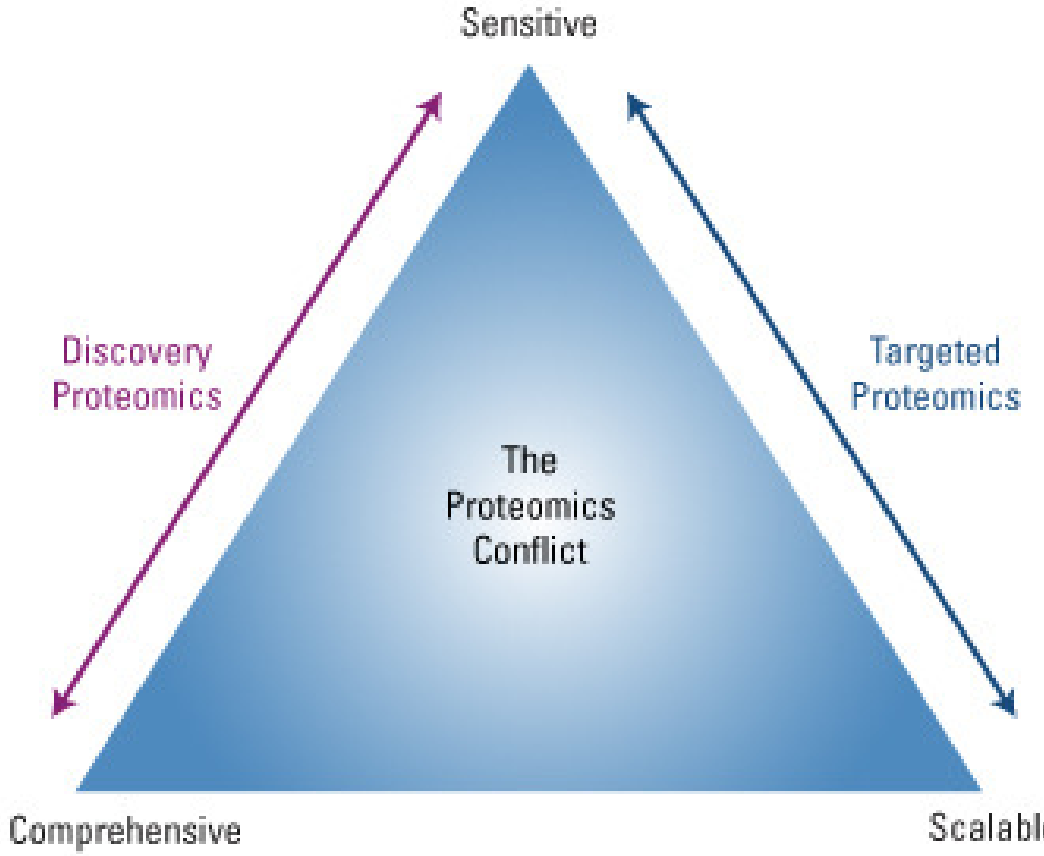
http://www.srmatlas.org/
(170,000SRM assays for human)

Antibody /Affinity - based



THE HUMAN PROTEIN ATLAS

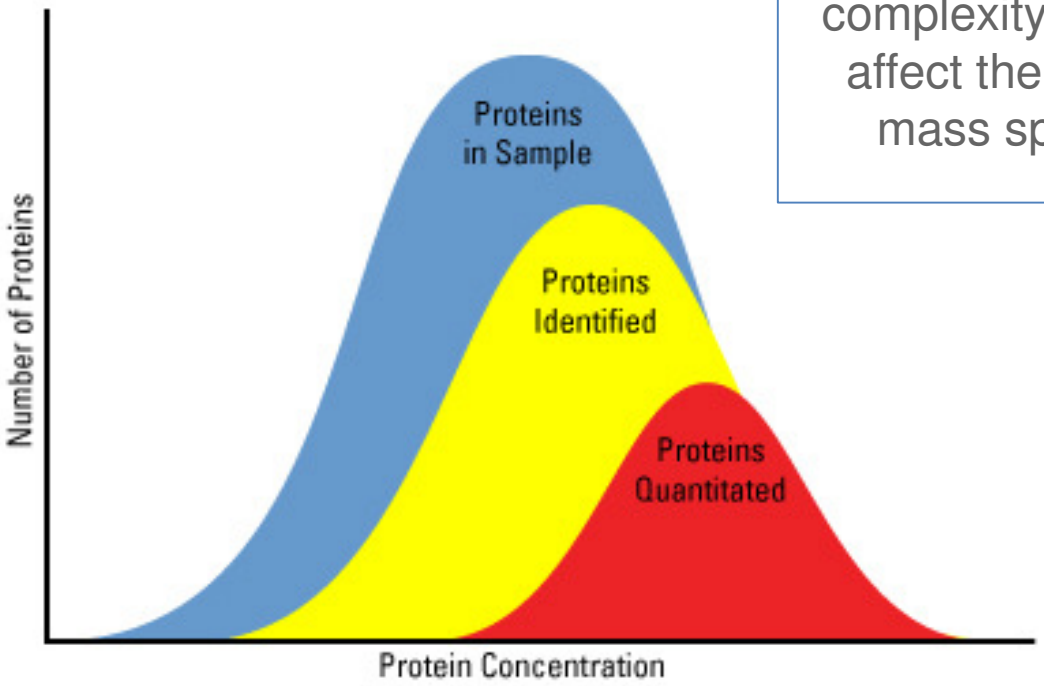
http://www.proteinatlas.org/
21,500 Abs for 11,000 genes



The balance between scope/sensitivity/scalability of discovery and targeted proteomics.

Due to the broad-scope nature and sensitivity of **discovery proteomics**, the ability to perform a **comprehensive analysis** of hundreds or **thousands of samples is limited**. Conversely, **targeted proteomic** analysis entails the **quantitation of discrete subsets of peptides**, which allows the ability to analyze these peptides across thousands of samples with the highest level of sensitivity.

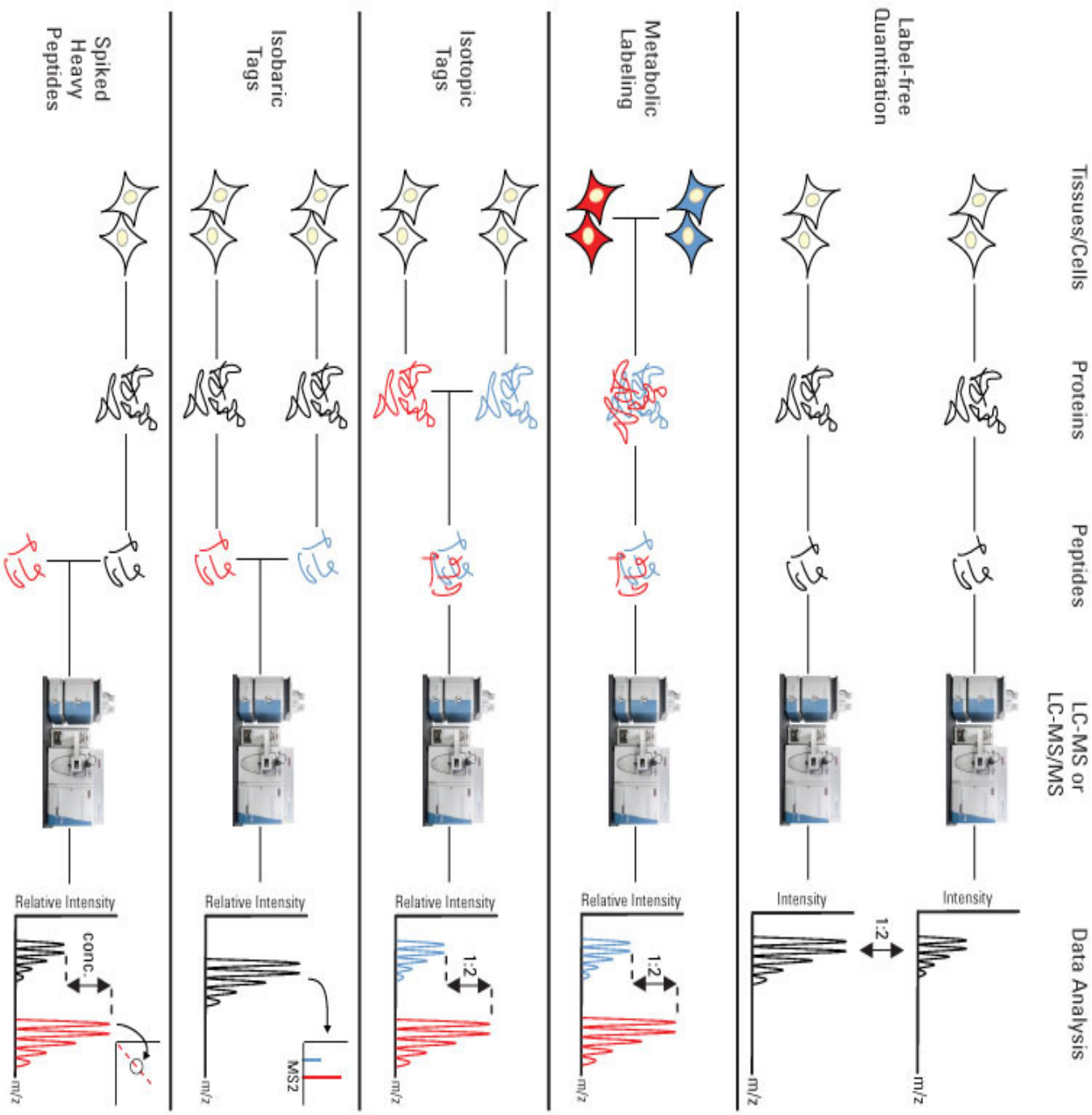
Quantitative Proteomics



Protein abundance and sample complexity are significant factors that affect the availability of proteins for mass spectrometric quantitation

Proteomics Quantitation

Absolute / Relative



Proteomics Clinical Purposes (some examples)

Instituto_Nacional de Saúde
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Missões

- Desenvolver uma plataforma I&D inovadora baseada na proteómica para validação, implementação de **biomarcadores** já existentes ou descoberta de novos biomarcadores de **diagnóstico, prognóstico e monitorização de doenças** ou como **alvos** a novos **abordagens terapêuticas**.
- Prestar **colaboração** e **serviços** de caracterização de proteínas pela proteómica
- Contribuir para o desenvolvimento da proteómica no nosso país (promoção/realização de cursos/estágios/conferências, networking) na área da proteómica

Research

Running Projects

1. **Proteomics of chronic lung diseases leading to biomarkers and therapeutic target discovery**. FCT project POCTI/SAU-MMO/56163/2004. PI: D Penque

2. ***Environmental Tobacco Smoke Exposure at Portuguese Restaurants, Bars and nightclubs: health effects and early molecular mechanisms underlying respiratory disorders***. *FCG/ACSS.* PI: T Simões & D Penque.

3. **MSIA technology development .** PI: D Penque & V Torres

4. **Obstructive sleep apnea and associated metabolic/cardiovascular disorders: understanding mechanisms towards early diagnosis and prognosis prediction**. HMSP-ICJ/0022/2011- *Junior Investigator*: A Feliciano, HPV

Chronic Lung Diseases

Cystic Fibrosis

Asthma

COPD

Biormaker Discovery of Chronic Lung Diseases

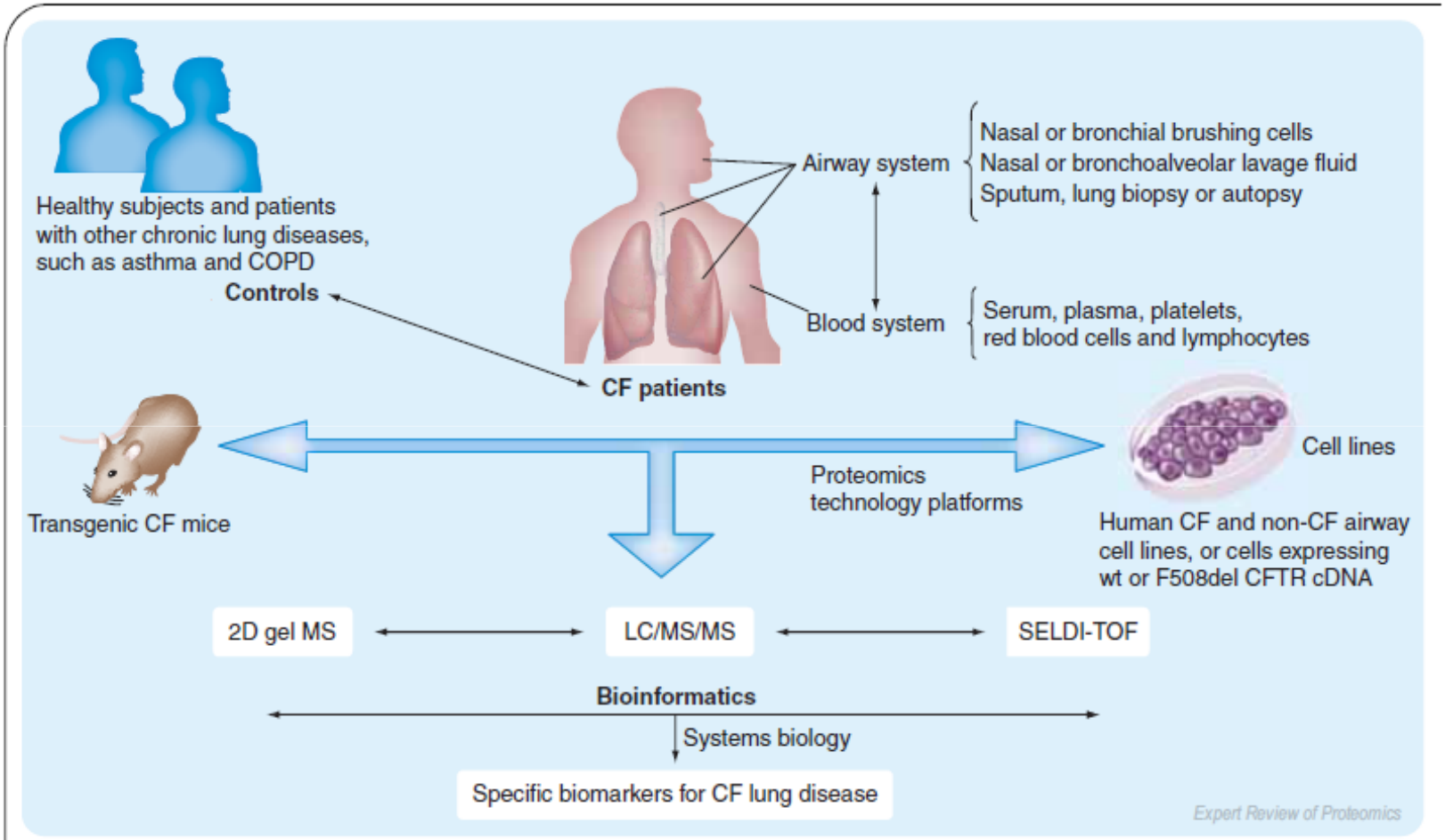
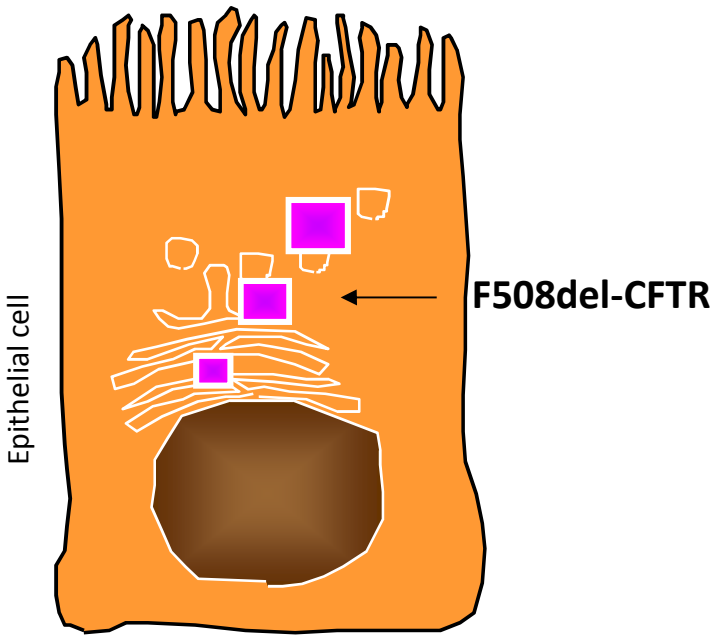


Figure 2. Schematic representation of putative proteomic strategies for the development of specific biomarkers in CF lung disease.
CF: Cystic fibrosis; CFTR: CF transmembrane conductance regulator; COPD: Chronic obstructive pulmonary disease; LC: Liquid chromatography; MS: Mass spectrometry; MS/MS: Tandem MS; SELDI: Surface-enhanced laser desorption/ionization; TOF: Time-of-flight; wt: Wild type.

D Penque Expert Rev. Proteomics 4 (2) 2007

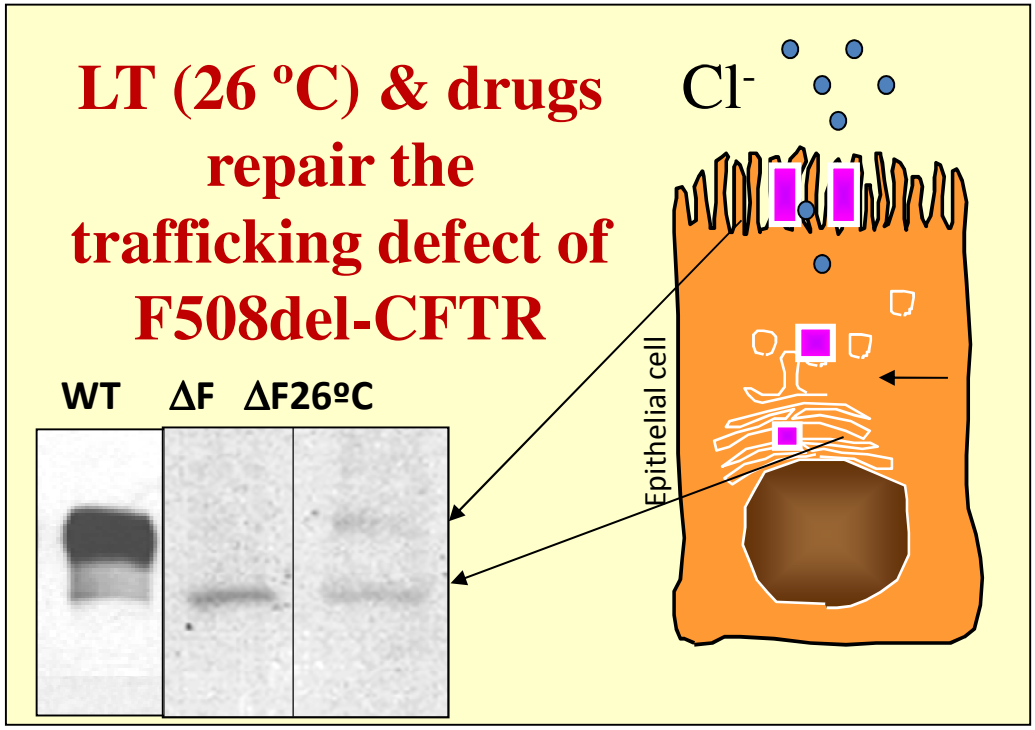
Investigating by Proteomics the trafficking defect of F508del-CFTR



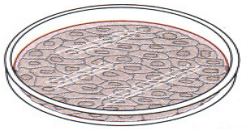
Class II

Trafficking defect

- Basic mechanism responsible for F508del-CFTR retention in ER remains to be elucidated



Low-temperature



3-10 pH

8-16% SDS-PAGE

37ºC

26ºC

24h

48h

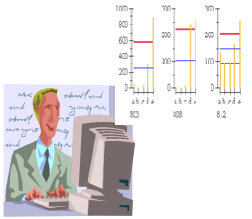
WT-CFTR

ΔF508-CFTR

BHK cells stably expressing CFTR

BHK cell line expressing WT or ΔF508del-CFTR

Progenesis PG200v2006



2D Map Analysis

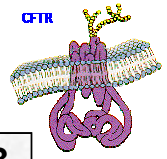
MS

MALDI-TOF-TOF 4700

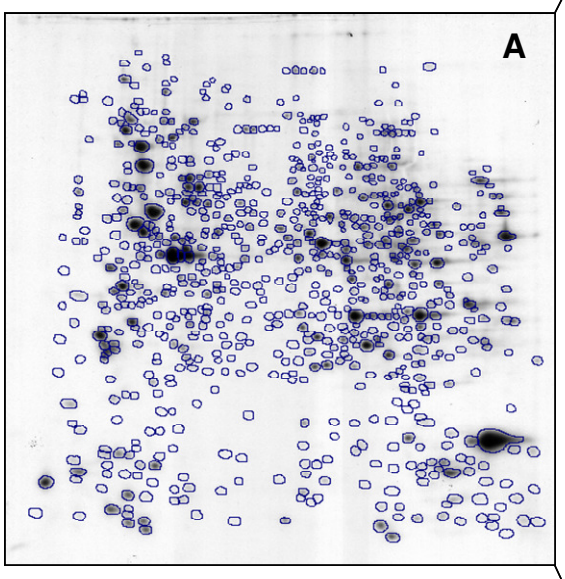
3h ^{35}S-**methionine** metabolic labelling

Gomes-Alves et al 2009

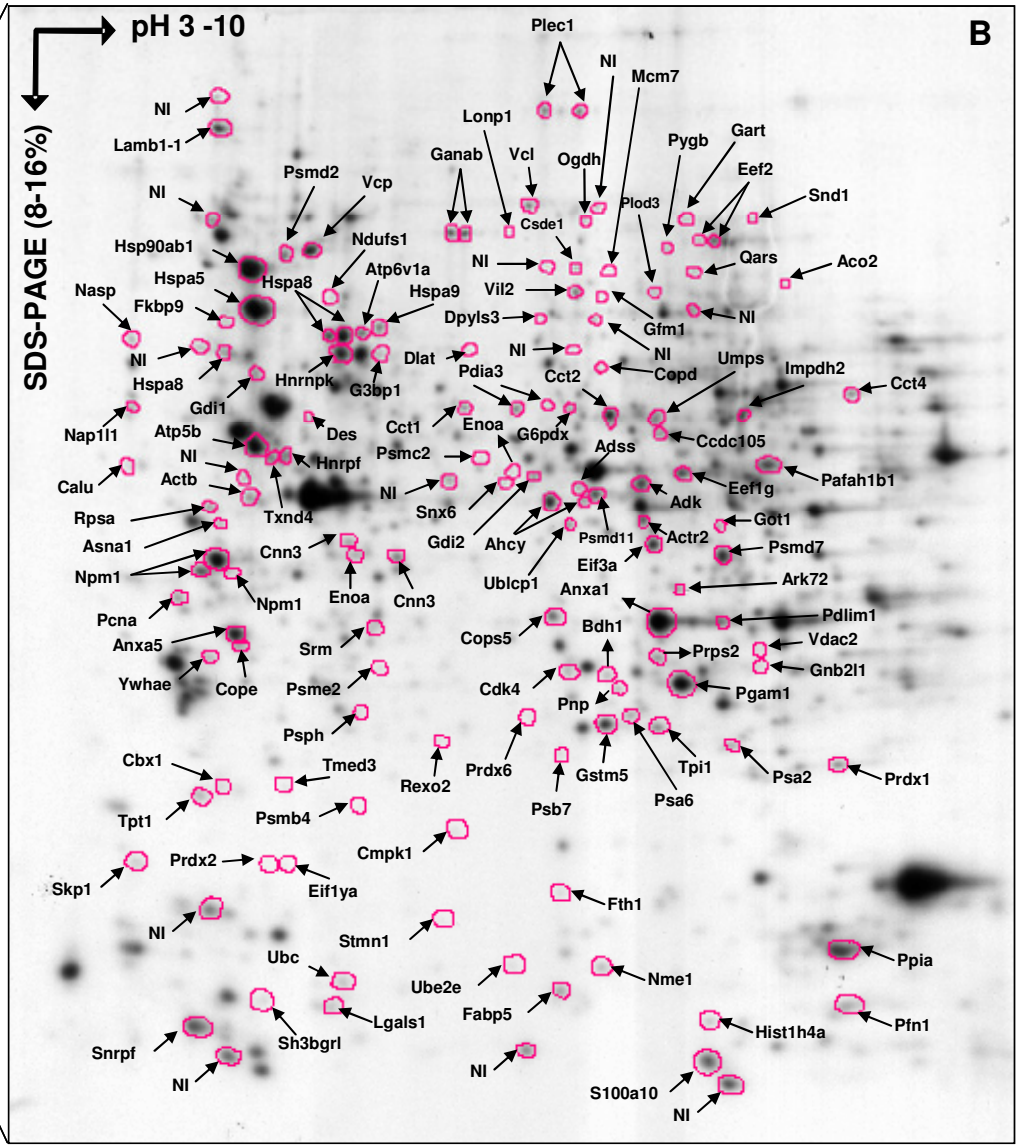
Gomes-Alves et al 2009, JOP, in press



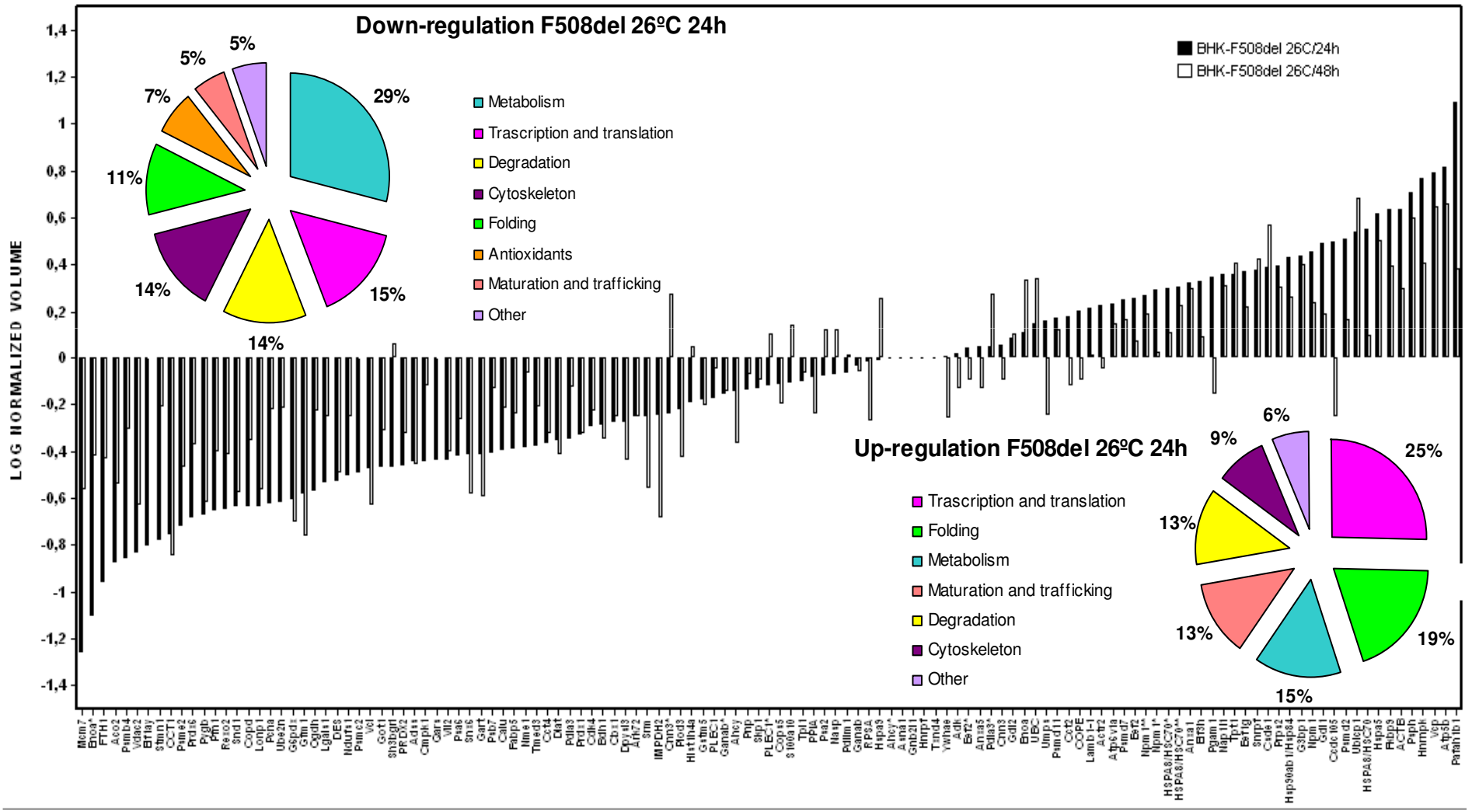
- 6 groups (cell types/conditions)
- 4-5 gels/group
- Normalized volume
- ANOVA, p < 0.05



- Differences over 1.5
- 139 protein spots differentially expressed
- **125 proteins spots identified**

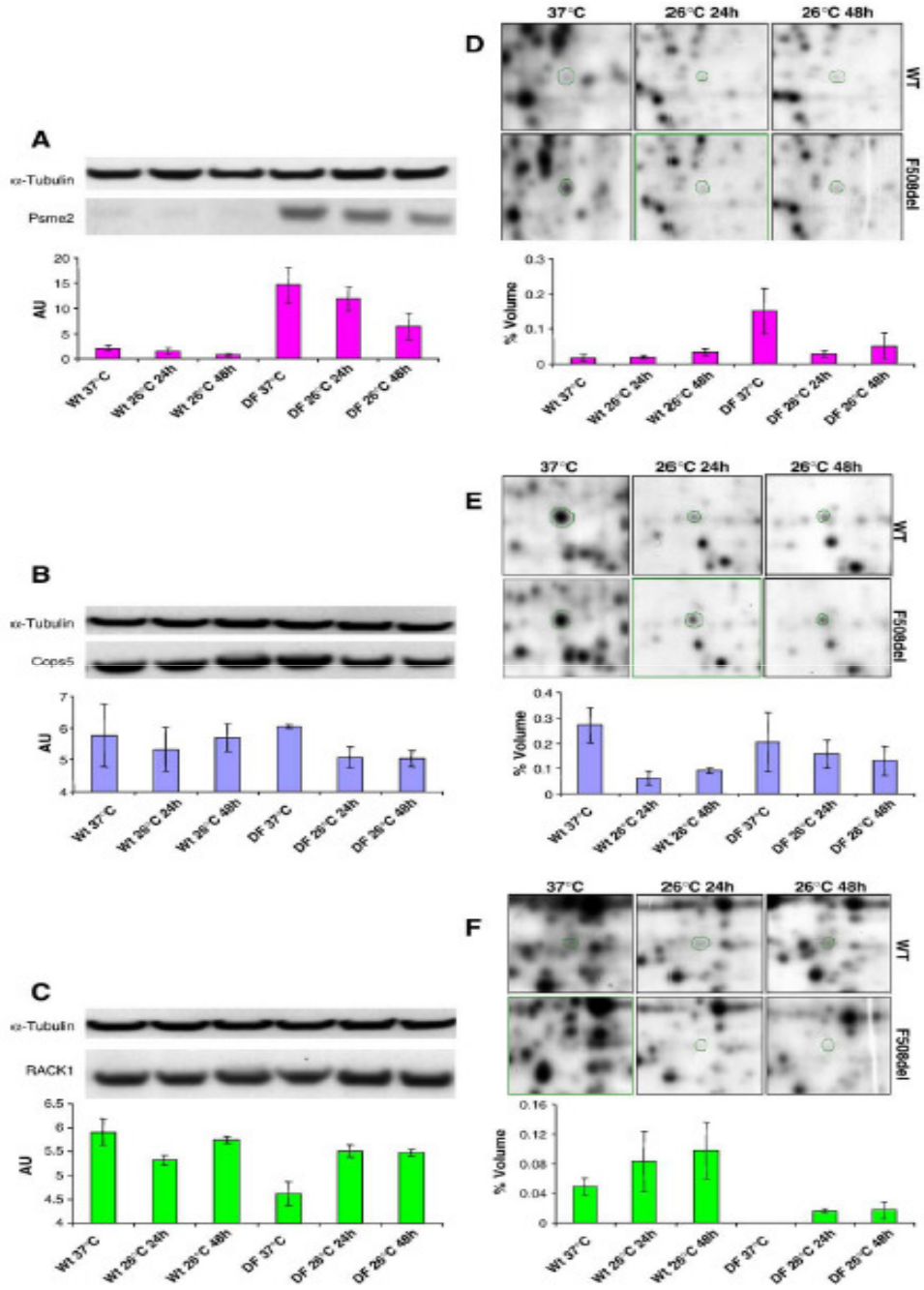


Several degradation associated proteins were down-regulated, while BiP and other UPR related proteins were found up-regulated in BHK-F508del cells under the CFTR-"rescue" treatment at low temperature.



Gomes-Alves et al 2010

Validation by western-blotting

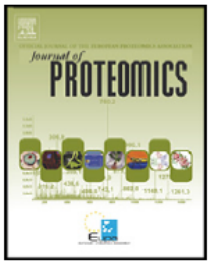


JOURNAL OF PROTEOMICS 73 (2009) 218–230

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot



Low temperature restoring effect on F508del-CFTR misprocessing: A proteomic approach

Patricia Gomes-Alves[a,b], *Sofia Neves*[a], *Ana V. Coelho*[b], *Deborah Penque*[a,*]

[a]*Laboratório de Proteómica, Departamento de Genética, Instituto Nacional de Saúde Dr Ricardo Jorge (INSA,I.P.), Lisboa, Portugal*
[b]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal*

Biochimica et Biophysica Acta 1804 (2010) 856–865

Contents lists available at ScienceDirect

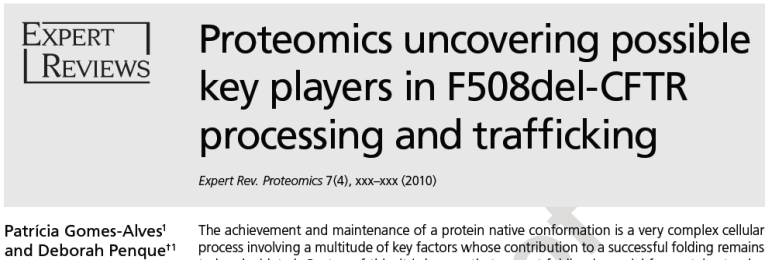
Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Rescue of F508del-CFTR by RXR motif inactivation triggers proteome modulation associated with the unfolded protein response

Patrícia Gomes-Alves [a,b], Francisco Couto [c], Cátia Pesquita [c], Ana V. Coelho [b], Deborah Penque [a,*]

[a] *Laboratório de Proteómica, Departamento de Genética, Instituto Nacional de Saúde Dr Ricardo Jorge (INSA, I.P.), Av. Padre Cruz, 1649-016 Lisboa, Portugal*
[b] *Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. Da República, 2780-157 Oeiras, Portugal*
[c] *Departamento de Informática, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal*



Low temperature

UPR

Unfolde protein response

Mutagenic Repair

(RXR) motifs

Increase folding capacity

& diminish degradation

↑ BIP, mortalin, Hsp90, Hsp70

↓ proteosome (Psme2)

Expression Reversion of some proteins involved in CFTR maturation & trafficking

e.g ↑ RACK1

Promote relocation of ΔF-508-CFTR to cell surface

research articles Journal of proteome research

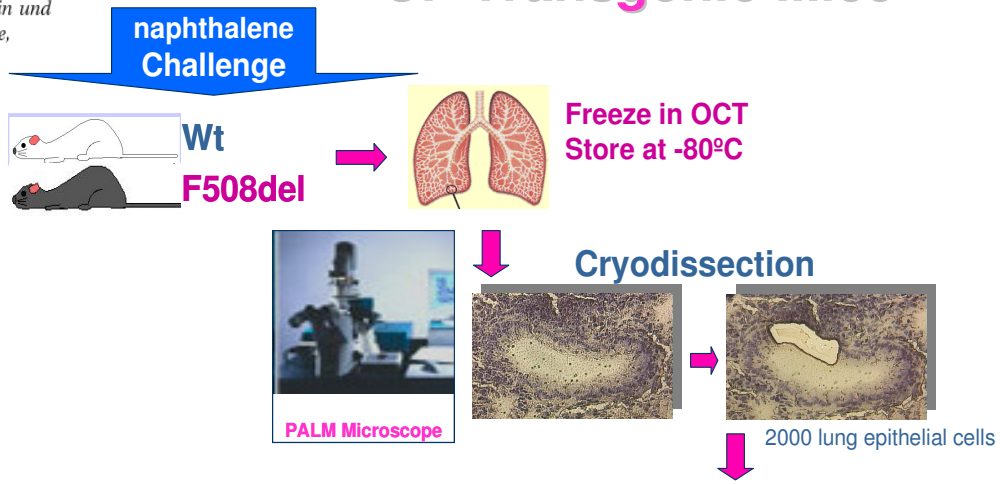
Proteomic Analysis of Naphthalene-Induced Airway Epithelial Injury and Repair in a Cystic Fibrosis Mouse Model

Isabel M. Carvalho-Oliveira,[†,‡] Nuno Charro,[‡] Jamil Aarbiou,[†] Ruvalic M. Buijs-Offerman,[†] Martina Wilke,[§] Thomas Schettgen,[∥] Thomas Kraus,[∥] Mark K. Titulaer,[⊥] Peter Burgers,[⊥] Theo M. Luider,[⊥] Deborah Penque,[*,‡,#] and Bob J. Scholte[*,†,#]

Department of Cell Biology, Erasmus Medical Centre, Rotterdam, The Netherlands, Laboratório de Proteómica, Departamento de Genética, Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa, Portugal, Department of Biochemistry, Erasmus University Medical Centre, Rotterdam, The Netherlands, Institut für Arbeitsmedizin und Sozialmedizin Universitätsklinikum Aachen, and Department of Neurology, Erasmus Medical Centre, Rotterdam, The Netherlands

Received January 9, 2009

CF Transgenic Mice



The results suggest the involvement of **prostaglandin** and **retinoic acid metabolism** in the abnormal responses of CF mutant mice to injury.

Carvalho-Oliveira et al, 2009, JPR, 8:3606-16

Carvalho-Oliveira et al, 2007, Expert Rev Mol Diag, 7:407-417

JOURNAL OF PROTEOMICS 74 (2011) 110–126

available at www.sciencedirect.com

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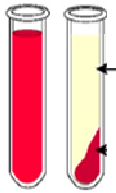
www.elsevier.com/locate/jprot

ELSEVIER

Serum proteomics signature of Cystic Fibrosis patients: A complementary 2-DE and LC–MS/MS approach

Nuno Charro[a,b], Brian L. Hood[b], Daniel Faria[c], Paula Pacheco[d], Pilar Azevedo[e], Carlos Lopes[e], António Bugalho de Almeida[e], Francisco M. Couto[c], Thomas P. Conrads[b,*,1], Deborah Penque[a,*,1]



Serum

LC/MS/MS

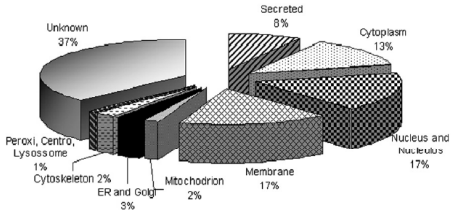
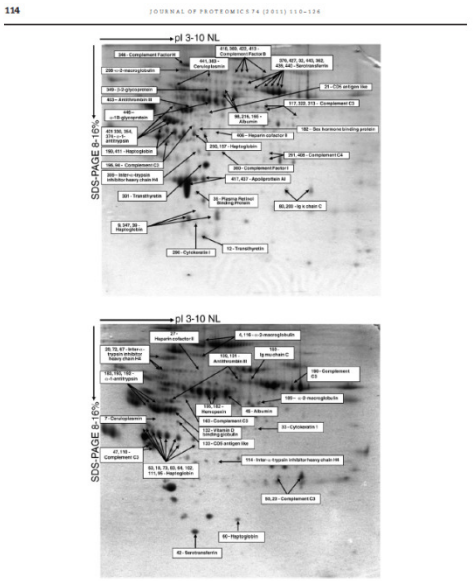


Fig. 3 – Graphical representation of the subcellular location of the identified proteins by label-free LC–MS/MS according to PIKE software.



2D-gel

Fig. 1 – (1) and (2): 2-DE reference maps of serum depleted from the six most abundant proteins from the mutational-based analysis and respiratory-based analysis, respectively, with the indication of the differentially expressed protein (ANOVA test, $p<0.05$; 4 gel replicas per group, total 20 gels). Highlighted protein spots were identified by MS (Tables 2.1 and 2.2SI).

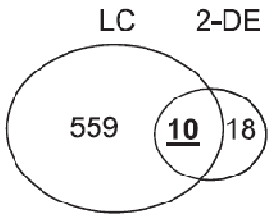


Fig. 2 – Total proteins identified in LC–MS/MS (by at least 2 peptides) and in 2-DE experiments.

Dysregulated Pathways (~70 p) :

- abnormal tissue/airway remodeling, protease/antiprotease imbalance, innate immune dysfunction,
- chronic inflammation,
- nutritional imbalance
- *P. aeruginosa* colonization.

Apolipoproteins family (VDBP, ApoA-I, and ApoB) gradually lower expression from non-CF to CF-carrier individuals and from those to CF patients,

The **enzyme NDKB** was identified only in the CF, its functions account for ion sensor in epithelial cells, pancreatic secretion, neutrophil-mediated inflammation and energy production, highlighting its physiological significance in the context of CF.

Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry xx (2009) xxx – xxx
CLINICAL BIOCHEMISTRY

SELDI-TOF biomarker signatures for cystic fibrosis, asthma and chronic obstructive pulmonary disease

Patrícia Gomes-Alves [a], Margaret Imrie [b], Robert D. Gray [b], Paulo Nogueira [c], Sergio Ciordia [d], Paula Pacheco [e], Pilar Azevedo [f], Carlos Lopes [f], António Bugalho de Almeida [f], Micaela Guardiano [g], David J. Porteous [b], Juan P. Albar [d], A. Christopher Boyd [b,1], Deborah Penque [a,*,1]

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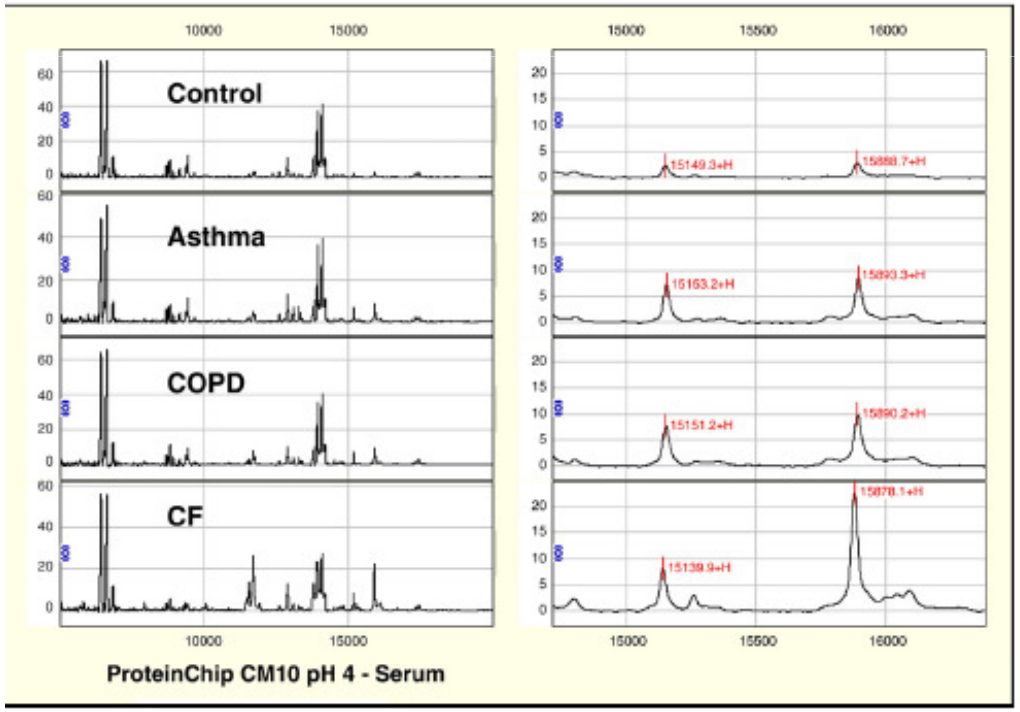

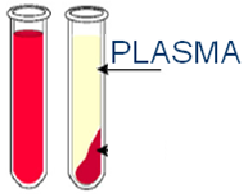

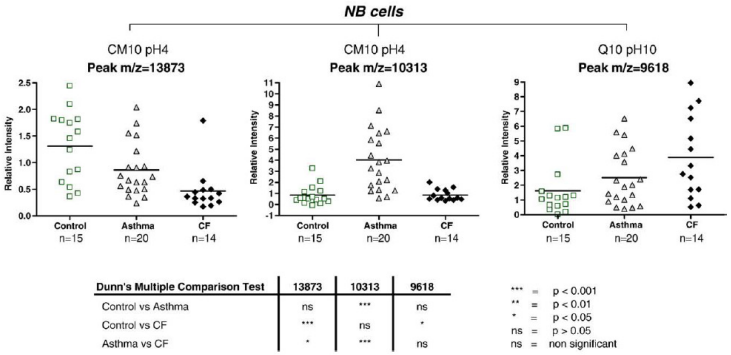

Fig. 3. Relative intensity of different protein clusters in NB cells from controls, asthma and CF patients. Data obtained on a CM10 pH 4 assay (13,873 Da and 10,313 Da) and on a Q10 pH 10 assay (9618 Da).

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Profiling the erythrocyte membrane proteome isolated from patients diagnosed with chronic obstructive pulmonary disease☆

Bruno M. Alexandre[a,b], Nuno Charro[a,b], Josip Blonder[b], Carlos Lopes[c], Pilar Azevedo[c], António Bugalho de Almeida[c], King C. Chan[b], DaRue A. Prieto[b], Haleem Issaq[b], Timothy D. Veenstra[b], Deborah Penque[a,*]

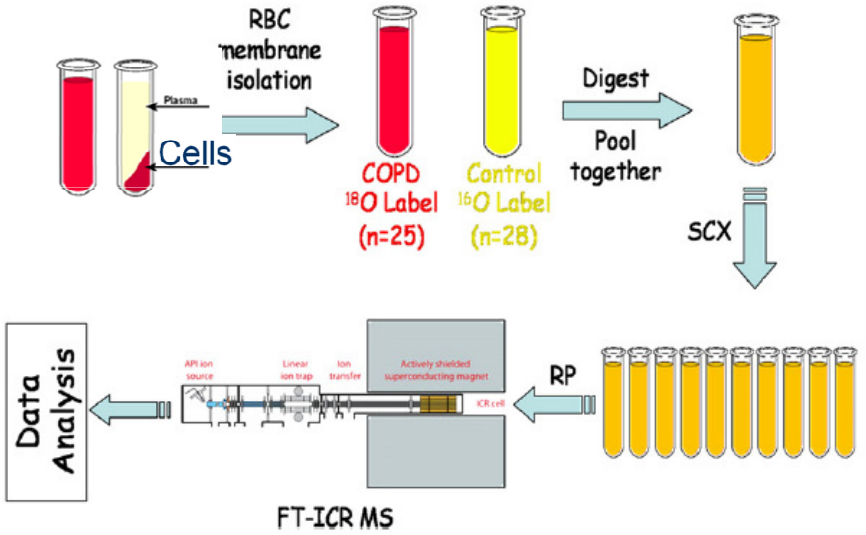


Fig. 1 – Basic scheme of methodology showing main steps of sample preparation.

219 proteins dysregulated in COPD RBCm

COPD

Most enriched Pathways :

- cell-to-cell signaling and interaction
- hematological system
- development,
- immune response,
- oxidative stress and
- cytoskeleton.

- ↓Chorein (VPS13A) > cell membrane deformation of RBC c Methemoglobin reductase

- ↓(Cytochrome CYB5R3) > COPD patients may be at higher risk for developing methemoglobinemia.

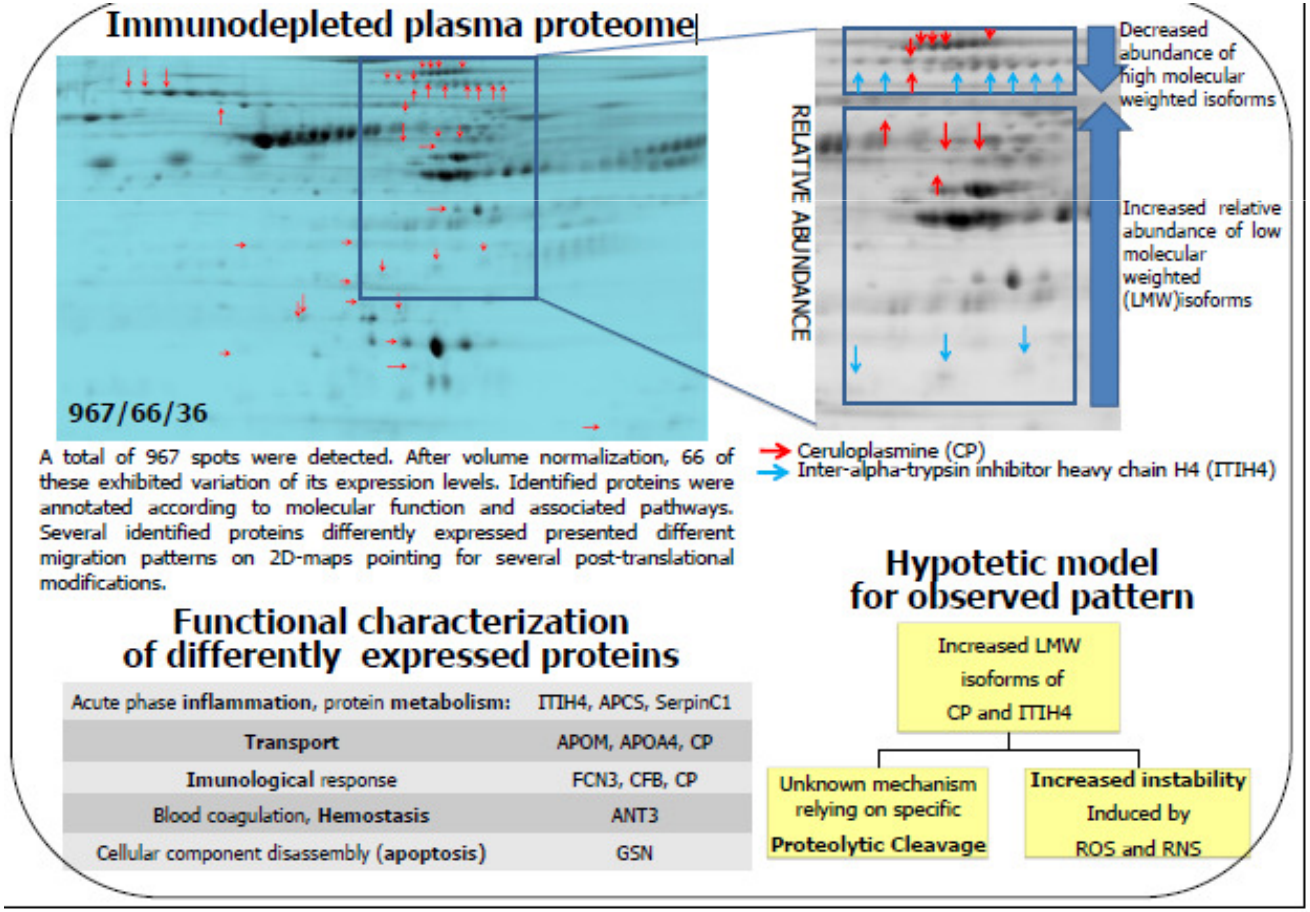
OCCUPATIONAL EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE: A STUDY IN LISBON RESTAURANTS

Solange A. Pacheco[1], Fátima Aguiar[2], Patrícia Ruivo[3], Maria Carmo Proença[2], Michael Sekera[3], Deborah Penque[1], Tânia Simões[1]

2012 Arnaldo Sampaio Award

EFFECTS OF OCCUPATIONAL EXPOSURE TO TOBACCO SMOKE: IS THERE A LINK BETWEEN ENVIRONMENTAL EXPOSURE AND DISEASE?

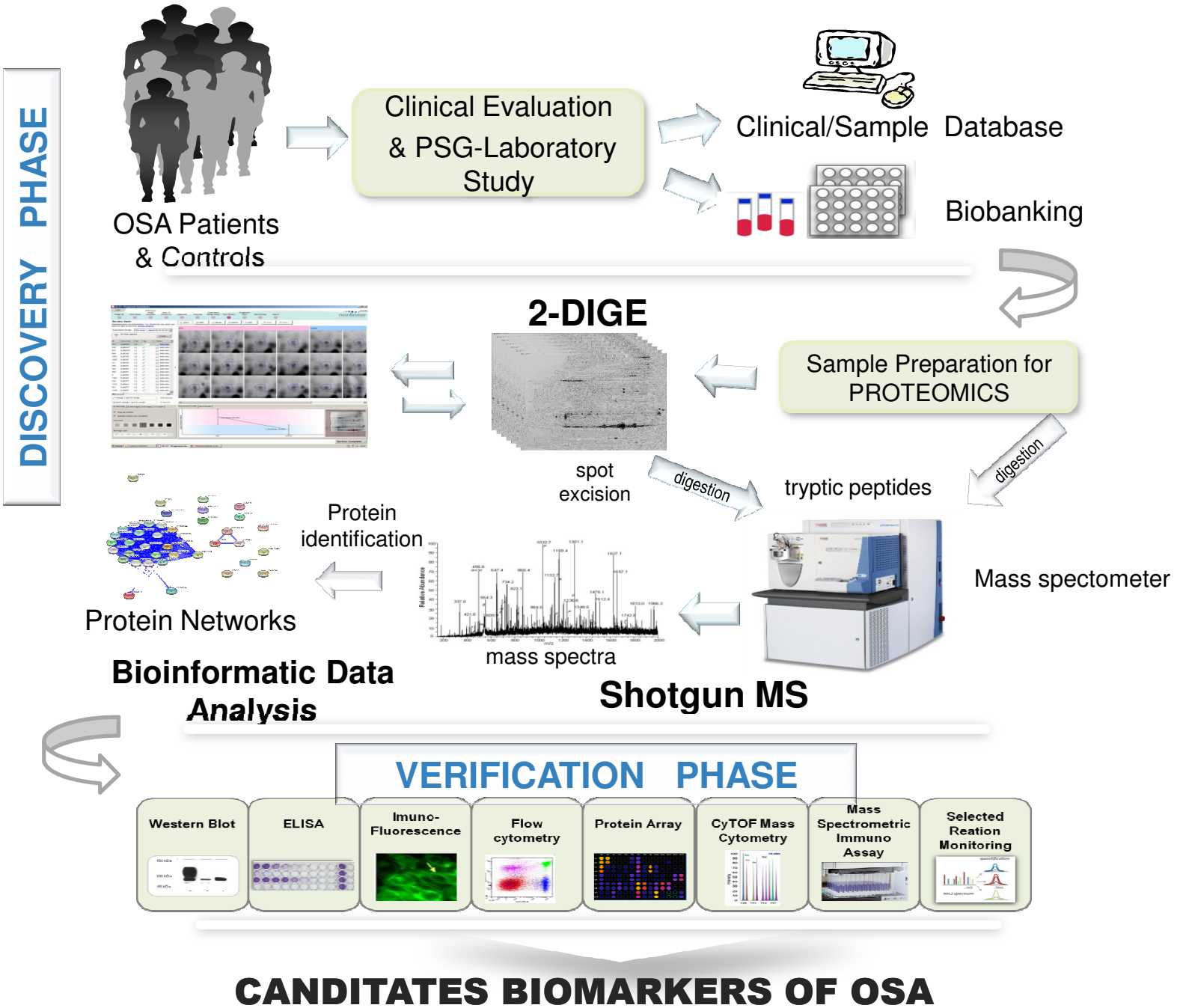
Solange A. Pacheco[1], Vukosava M. Torres[1], Henriqueta Louro[1], Filomena Gomes[1], Carlos Lopes[2], Nelson Marçal[2], Elsa Fragoso[2], Carla Martins[2], Cátia L. Oliveira[2], Manuela Hagenfeldt[1], António Bugalho-Almeida[2], Deborah Penque[1], Tânia Simões[1]



Biomarkers for Obsructive Sleep Apnea is needed

- to distinguish snoring from OSA , facilitating population screening and prevention of OSA-associated outcomes
- to provide new insights into pathophysiological aspects of OSA that underlie the increased cardiovascular and metabolic risk in general population

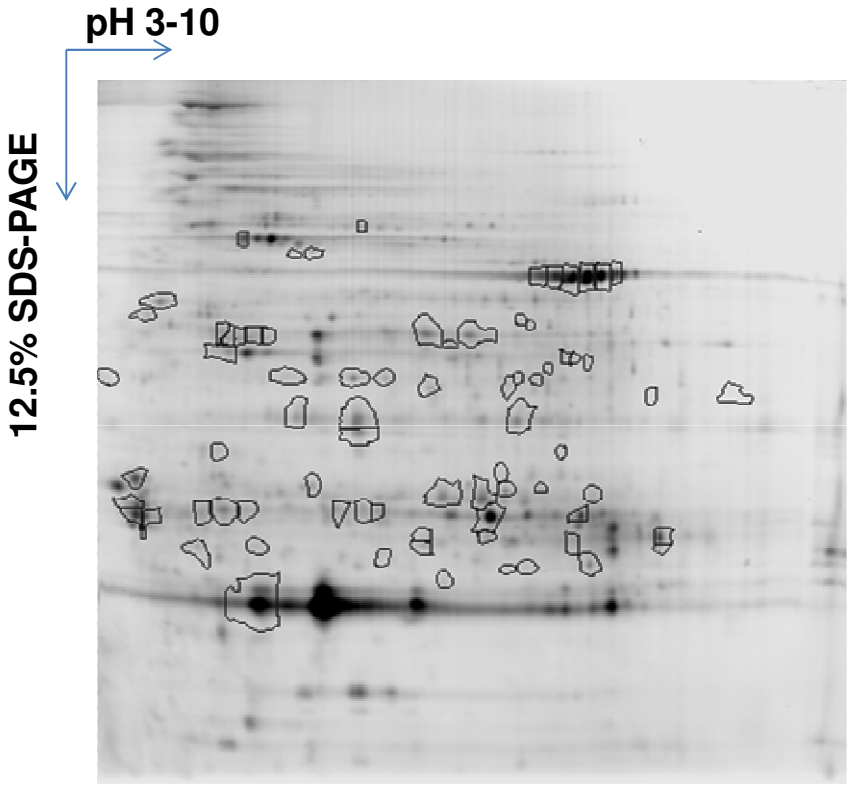
PROTEOMICS



Feliciano et al Sleep Medicine 2015

2DIGE

OSA Evening X OSA Morning X Snorers Evening X Snorers Morning



RBC Hemoglobin-depleted cytoplasmic fraction

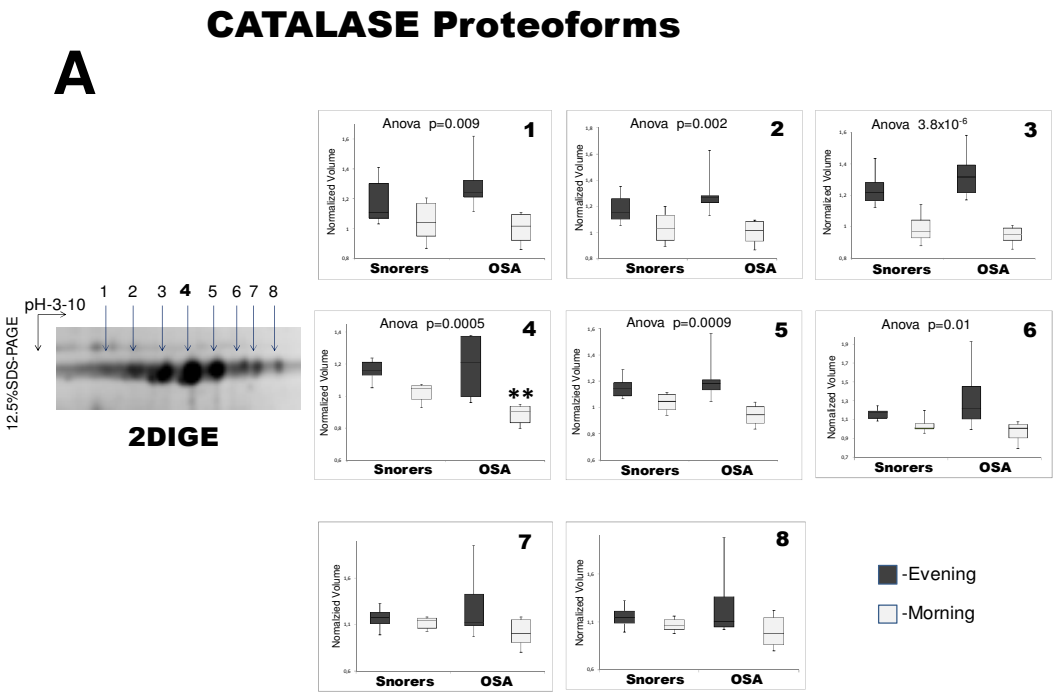
76 spots identified differentially abundant (Anova p≤ 0.05)

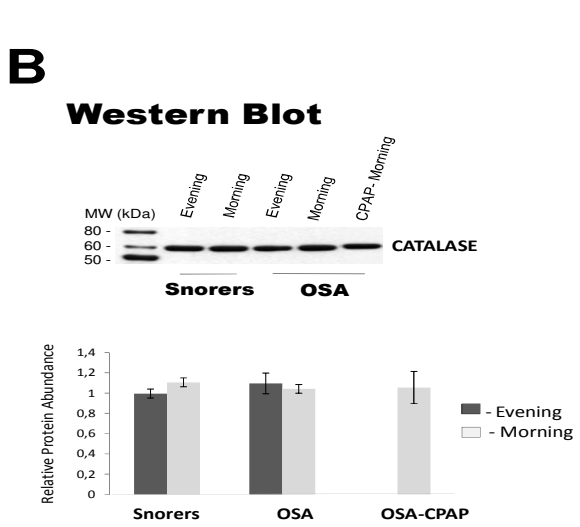
30 spots identified by MALDITOTOF, corresponding **21 different proteins**

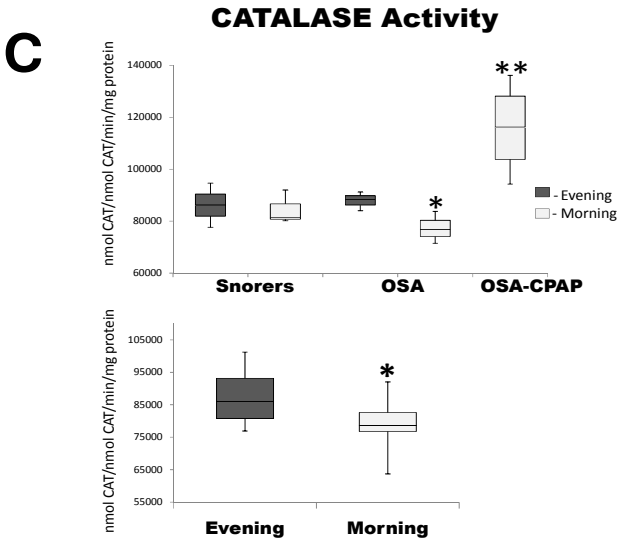
Existence of Post-translational Modifications

Discovery Phase

Validation Phase







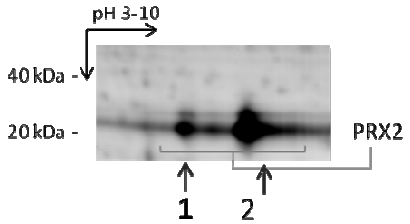
continuous positive airway pressure (**CPAP**),

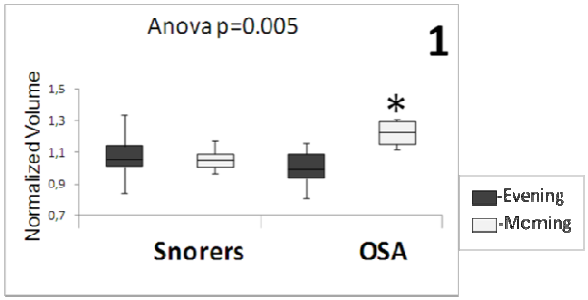
Peroxiredoxin 2 Proteoforms

Discovery Phase

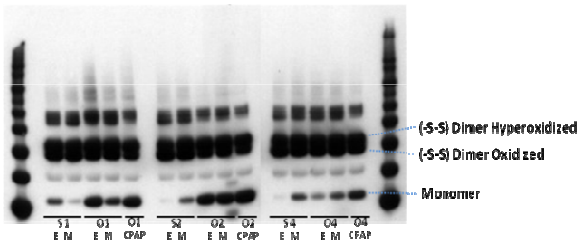
Validation Phase

2DIGE

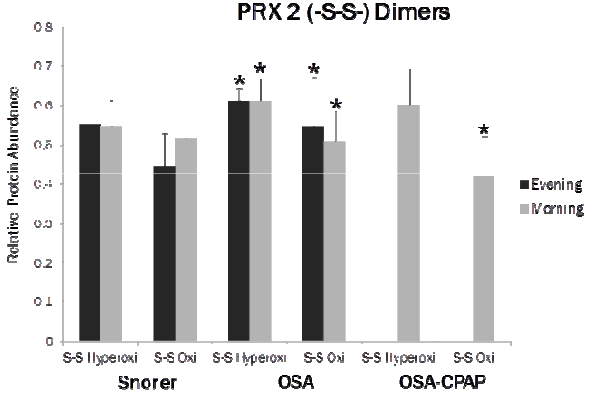




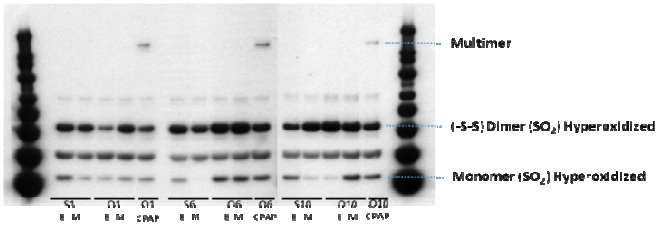
PRX2 Ab



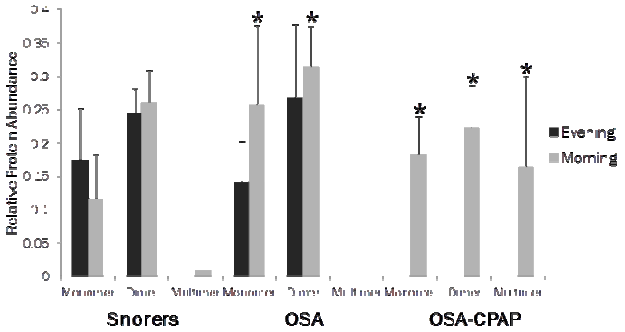
PRX 2 (-S-S-) Dimers



$PRXO_{2/3}$ Ab



$PRXSO_{2/3}$ (Hyperoxidized)



Summary

Proteomics can provide:

- New insights into the poorly-understood pathogenetic processes of diseases.
- New biomarkers for diagnosis & prognosis
- New targets for development of novel therapeutic approaches.

Acknowledgements

Team members

- INSA (Proteomics, Mol Biol, Statistic)
  - Patrícia Alves
  - Bruno Alexandre
  - Nuno Charro
  - Isabel C Oliveira*
  - Deborah Penque
  - Tânia Simões
  - M Fátima Vaz
  - Paula Pacheco
  - Paulo Nogueira
- ITQB (Mass spectrometry)
  - Ana V Coelho
- Univ Madrid (MS consultants)
  - Juan Pablo Albar
- Univ Lund
  - Peter James
- HSM/Clinic Pulmonology (patients recruitment, clinical phenotype)
  - Pilar Azevedo
  - Carlos Lopes
  - António Bugalho de Almeida
- FCUL (Bioinformtics)
  - Francisco Couto, David Santos
- Univ Edinburgh (SELDI-TOF consultants)
  - Margaret Imrie; Robert Gray
  - David Poteous; Chris Boyd
- Univ Pittisburgh (MS/consult)
  - Thomas Conrads & Brian Hood
- NCI (MS consultants)
  - Timothy Veenstra & Josip Blonder

Multidisciplinary Team IN OSA Study

Sleep Pathology Clinic (CHLN)
(Cristina Bárbara, MD, PhD;
Paula Pinto, MD, PhD)

Pathology Lab (CHLN)
(Helena Proença)

Proteomics Laboratory (INSA)
(Deborah Penque, PhD; Rune Matthiesen, PhD;
Vukosava Torres, PhD; Vesna Bozanic, PhD)

Biotech Institute (Biotempo)
(database platform design & implementation)

Division of Sleep Medicine
(BWH-Harvard Medical School)
(Atul Malhotra, PhD)

University of Arizona
R Nelson
(consultant in MSIA-Technology)

CIC bioGUNE, Spain
(Felix Elortza)

Work partially supported by FCT research grants: PCOTI/ESP/44720/2002, POCTI/MGI/40848/2001, POCTI/SAU-MMO/56163/2004, Gulbenkian Fondation-ACSS ;
Harvard Medical School-Portugal Program (HMSP-ICJ/0022/2011)

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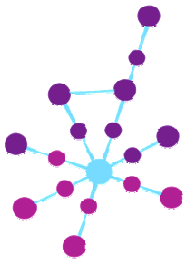

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