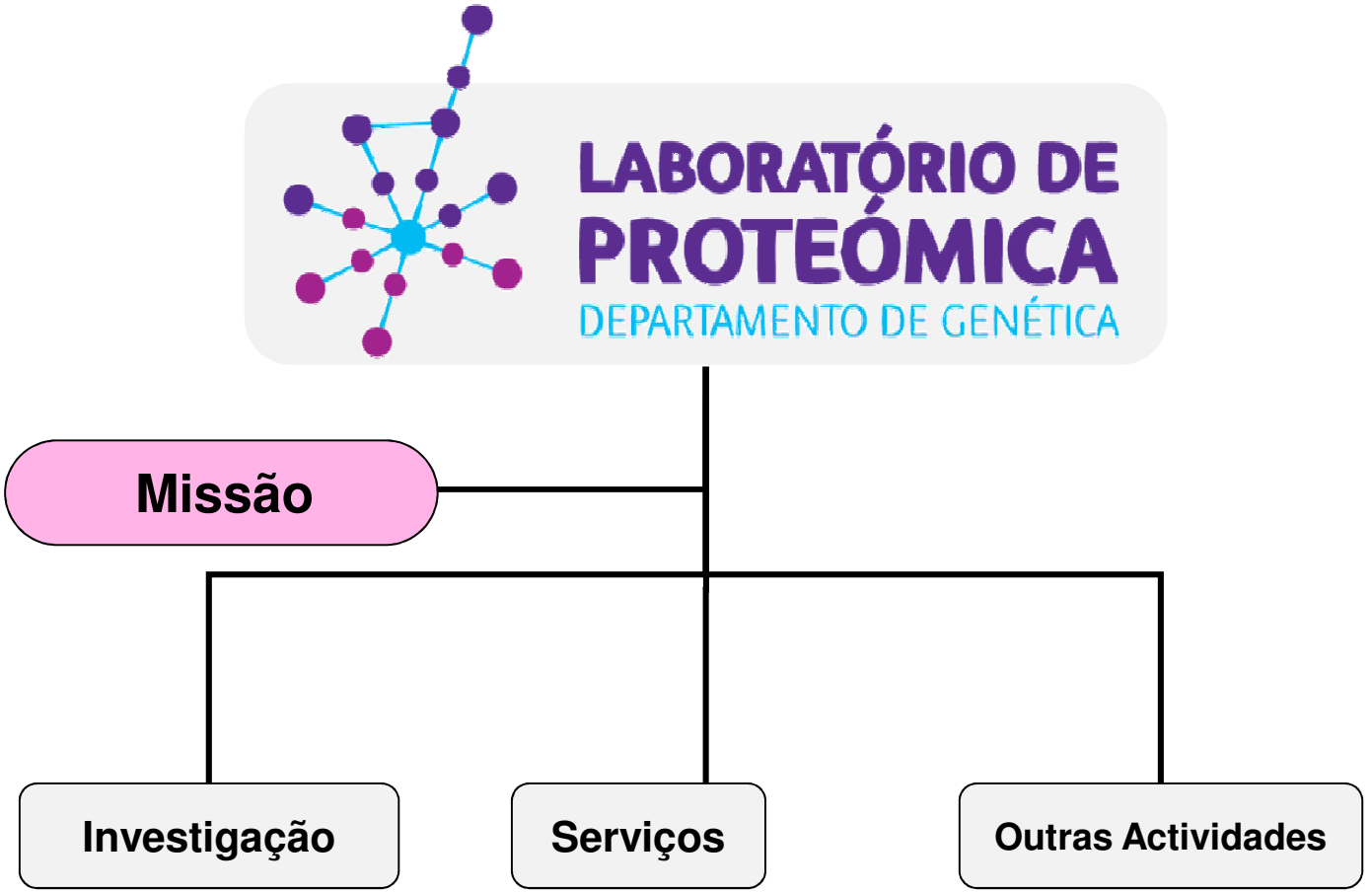
Instituto_Nacional de Saúde Doutor Ricardo Jorge



Missão

- Desenvolver uma plataforma I&D e Inovação (IDI) de proteómica para **validação e implementação** de **biomarcadores já existentes** e/ou **descoberta de novos** biomarcadores de diagnóstico, prognóstico e monitorização de doenças, ou ainda como alvos de novas abordagens terapêuticas.
- **Prestar serviços** de identificação/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

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**.

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

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**

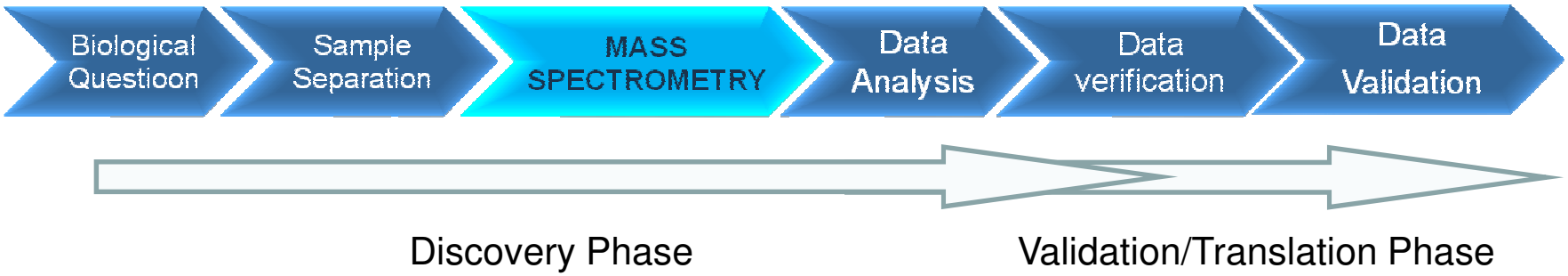
Hood et al Proteomics. 2012 Sep;12(18):2773-83

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

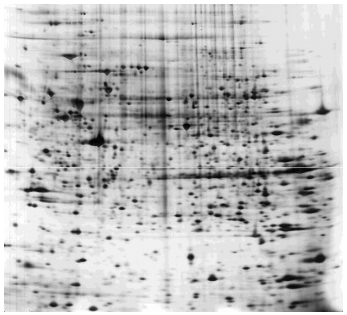
Discovery Proteomics approach

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

Biofluid

Cell lines

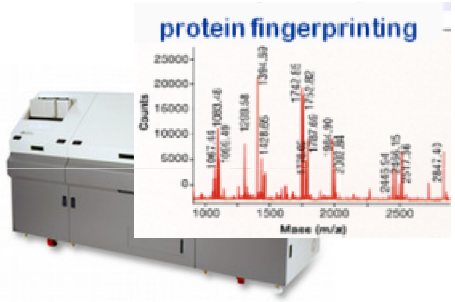
Tissue



2D-gel

HPLC

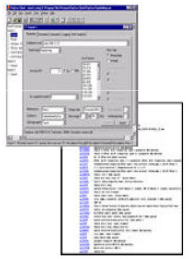
Data Aquisition



MALDITOFTOF

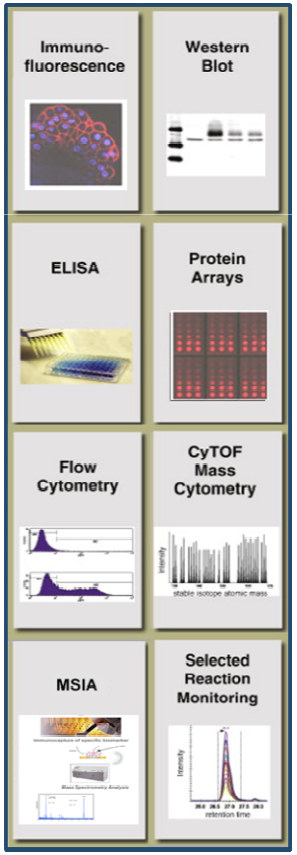
ESI-MS/MS

LC/MS/MS
Shotgun MS



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

Pathway/Network Analysis



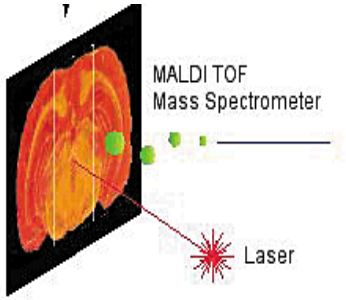
Discovery-based Proteomics approach

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

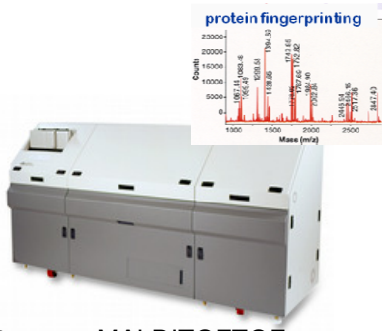
Biofluid

Cell lines

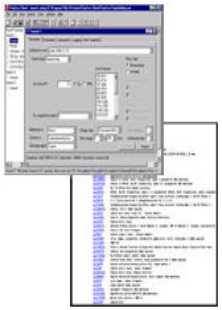
Tissue



Imaging MS



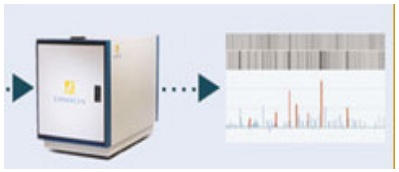
MALDITOFTOF



Data Base Query
(Mascot, Sequest, etc

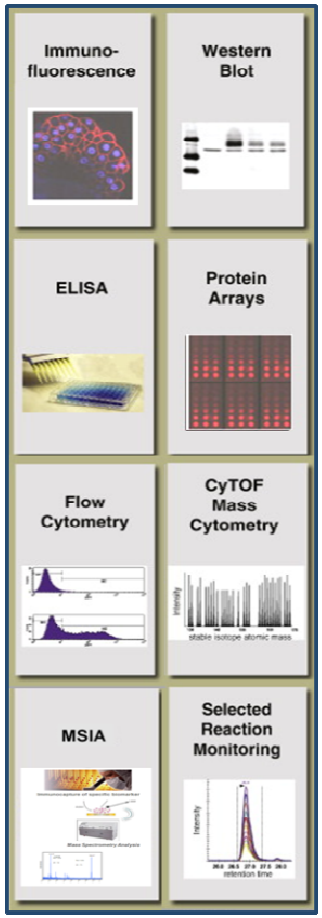
SELDI-TOF

protein CHIP



MALDI

Pathway/Network analysis



Targeted proteomics approach

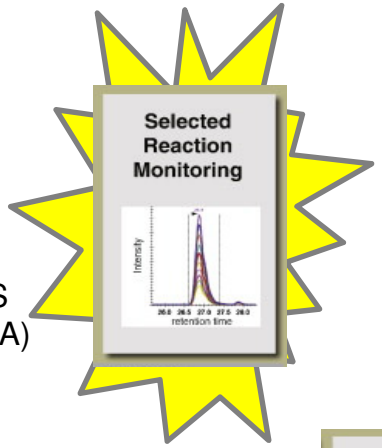
Is protein X measurable in this sample?

Shotgun proteomics

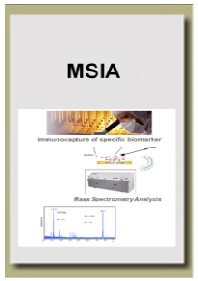
Biochemical experiments

Literature

Mass spectrometer-based



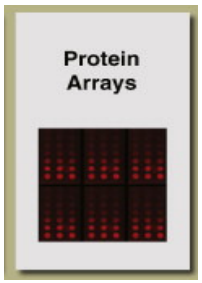
QQQ-type MS
(SRM, SISCAPA)



MALDI-type
(MISA, iMALDI)

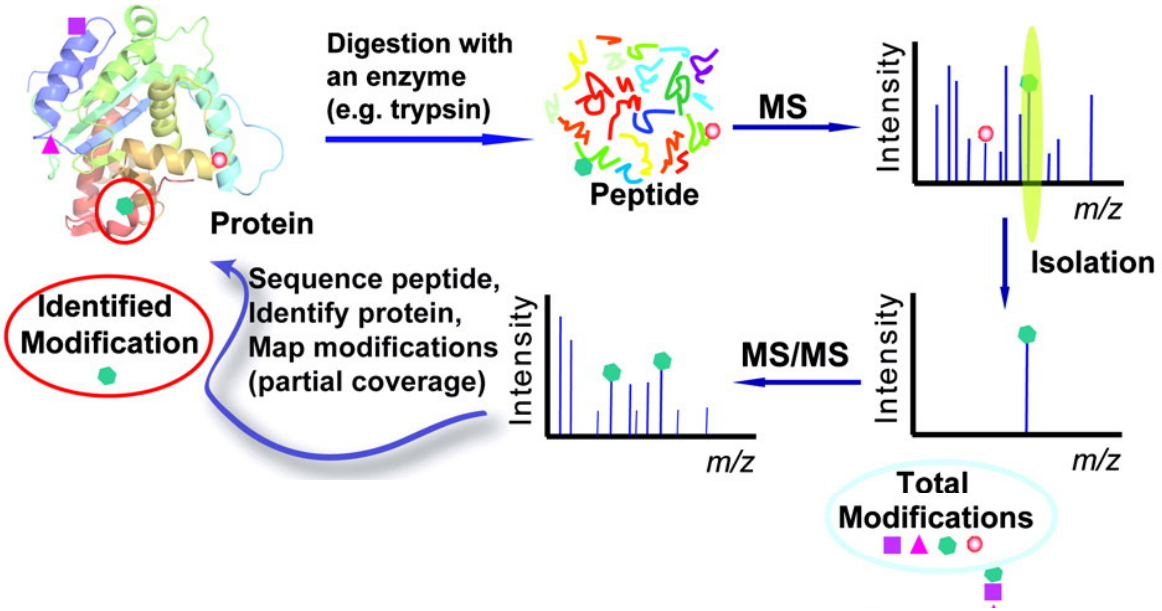
SRMAtlas
http://www.srmatlas.org/
(170,000SRM assays for human)

Antibody /Affinity - based

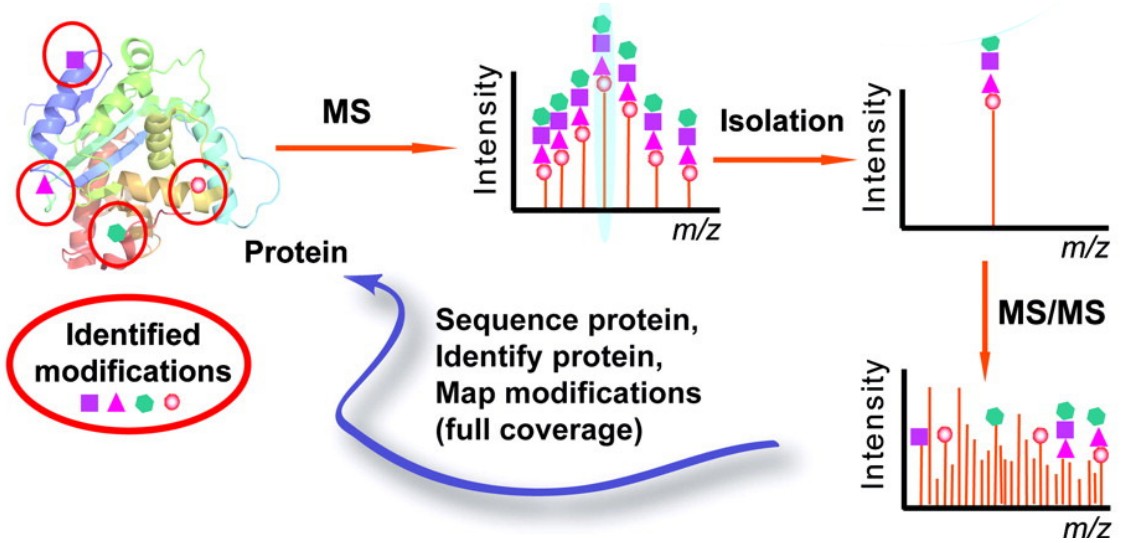


THE HUMAN PROTEIN ATLAS
http://www.proteinatlas.org/
21,500 Abs for 11,000 genes

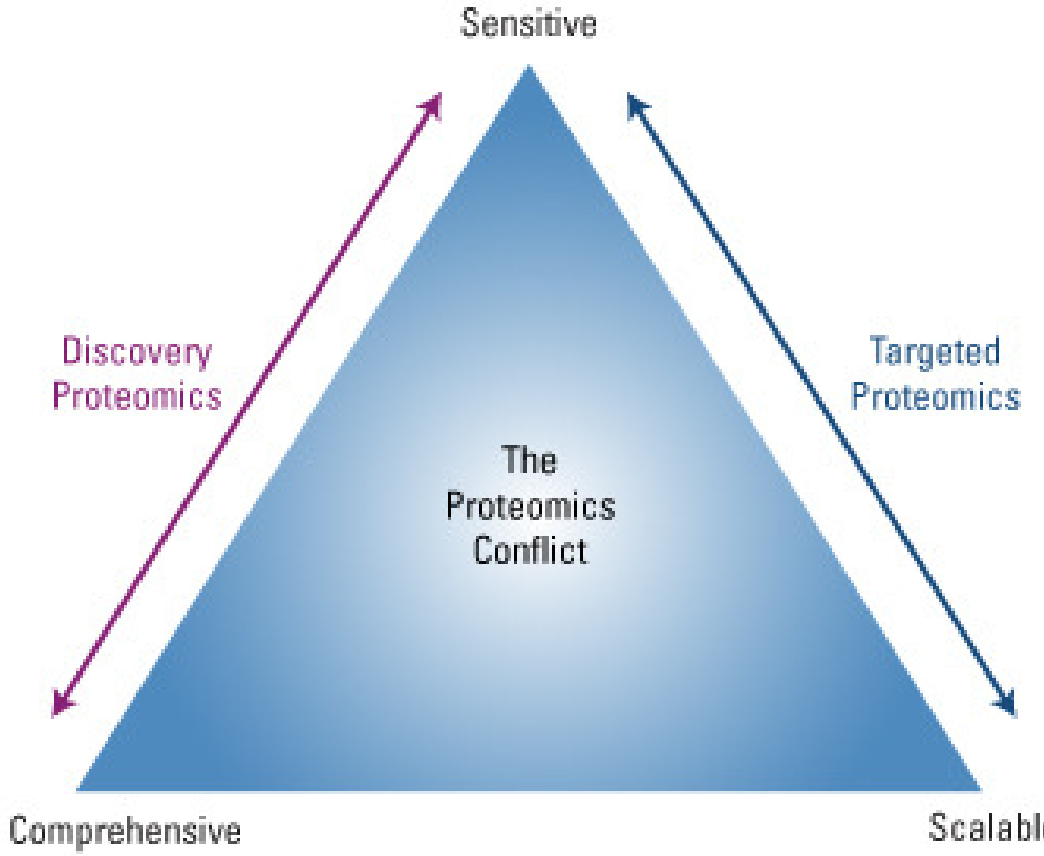
Bottom-up MS approach



Top-down MS approach



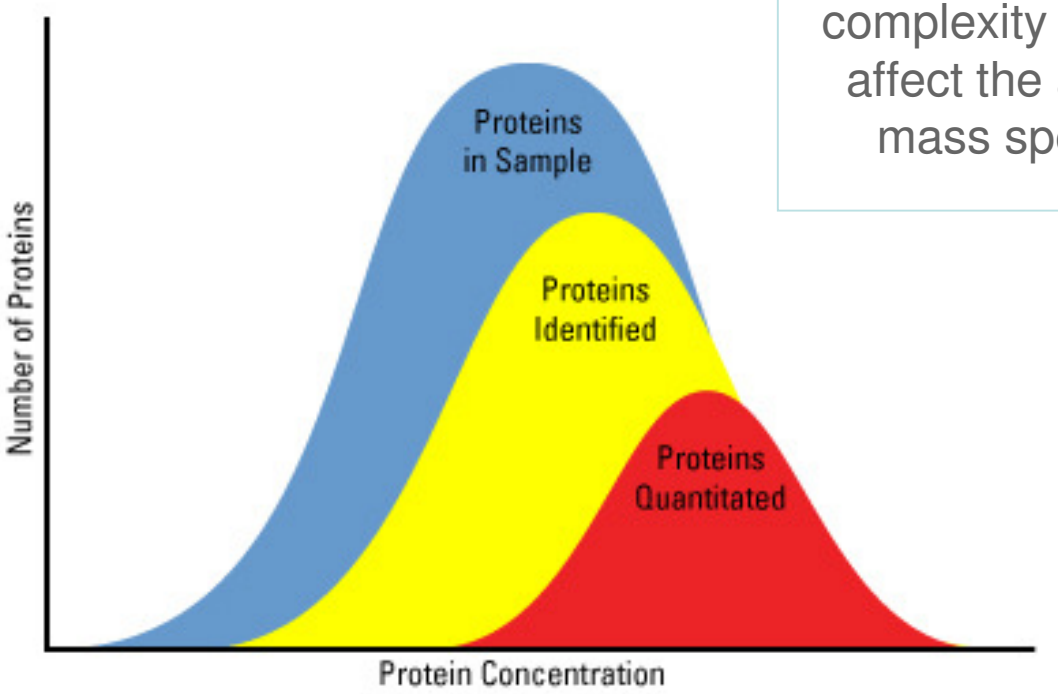
More info about **Proteoforms**



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.

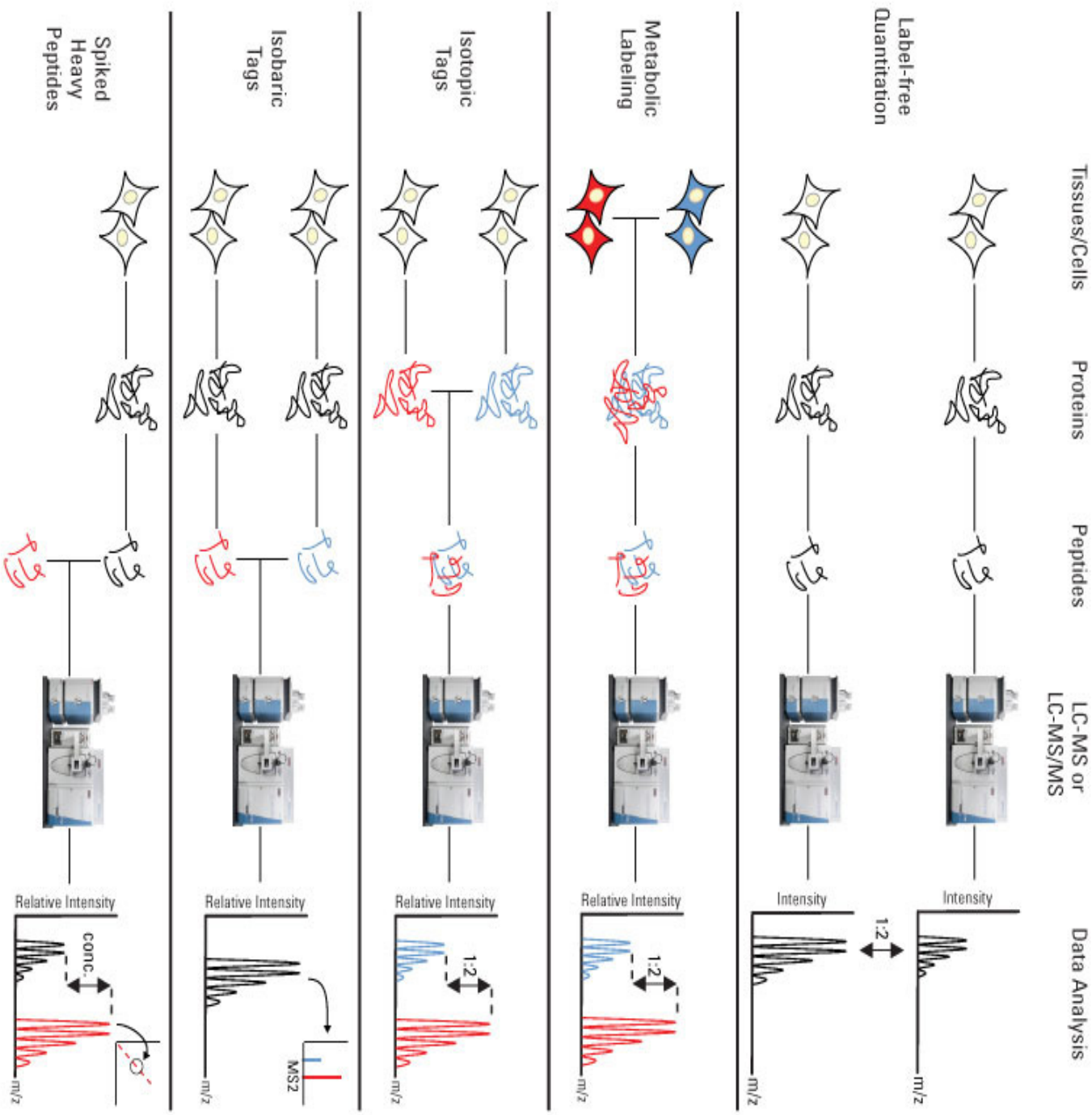
Quantitative Proteomics



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

Proteomics Quantitation

Absolute / Relative



I&D:
CLINICAL & Translational Proteomics

- Chronic Lung Diseases (e.g., CF, asthma, COPD)
- Environmental Exposition (e.g., Tobacco smoke, secondhand smoke)
- Obstructive Sleep Apnea & OSA-Associated Diseases (cardiometabolics)
- Mass Spectrometric Immune Assay (MSIA) to clinical application

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*

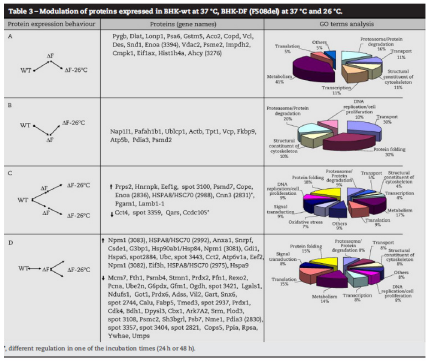


Table 3 – Modulation of proteins expressed in RHK-wt at 37 °C, RHK-DF (F508del) at 37 °C and 26 °C.

Protein expression behaviour	Proteins (gene names)	GO terms analysis
A	Pygb, Dlat, Longl, Psa6, Gstm5, Acn2, Copd, Vcl, Des, Snd1, Enoa (3394), Vdac2, Psme2, Impdh2, Crapk1, Eif5ax, Histlh4a, Ahcy (3276)	
B	Nap1l1, Pafah1b1, Ublcp1, Acth, Tpt1, Vcp, Fkbp9, Atp5h, Pdia3, Psmd2	
C	↑ Prpo2, Hnrnpk, Eef1g, spot 3100, Psmd7, Cope, Enoa (2836), HSPA8/HSC70 (2988), Cnn3 (2831)*, Pgam1, Lamb1-1 ↓ Crt4, spot 3359, Qprs, Cdc105*	
D	↑ Npm1 (3083), HSPA8/HSC70 (2992), Anxa1, Snrp1, Cadr1, G3bp1, Hnrpkobl/Hnrpk, Npm1 (3083), Cd11, Hspa1, spot2884, Ubc, spot 3443, Crt2, Atp6v1a, Eef2, Npm1 (3083), Eif3h, HSPA8/HSC70 (2975), Hspa5 ↓ Mcm7, Pfn1, Psmb4, Stmn1, Prdx2, Pfn1, Reno2, Pcna, Ube1h, Gfpt1, Cgi1h, spot 3421, Lgals1, Ndufs1, Got1, Prdx6, Adas, Vil2, Gart, Sms6, spot 2746, Calu, Fabp5, Tmed1, spot 2917, Prdx1, Cd44, Ddh1, Dpysl3, Cbr1, Ark7A2, Srm, Plod3, spot 3108, Psmc2, Sh3bgrl, Prb7, Nme1, Pdia3 (2830), spot 3357, spot 3404, spot 2821, Copa5, Ppia, Rpsa, Ywhae, Umps	

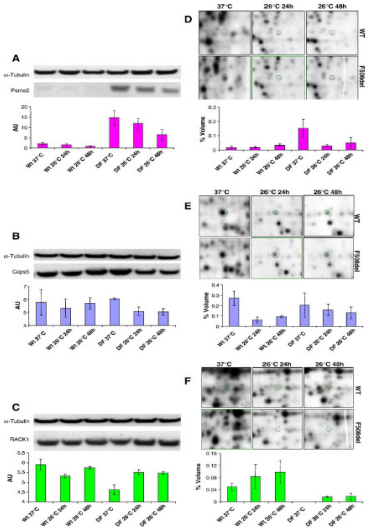
* different regulation in one of the incubation times (24 h or 48 h).

Biochimica et Biophysica Acta 1804 (2010) 856–865

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

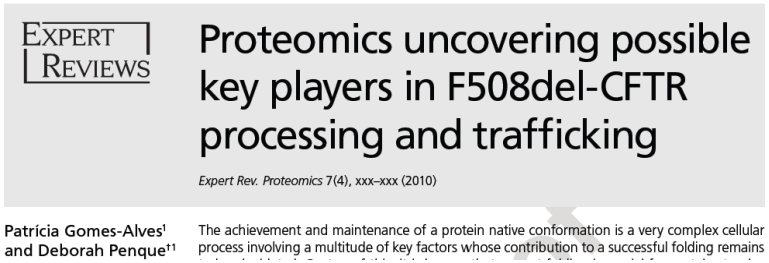
journal homepage: www.elsevier.com/locate/bbapap



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

Unfolded protein response

Mutagenic Repair
(RXR) motifs

↑ **BIP, mortalin, Hsp90, Hsp70**

Increase folding capacity & diminish degradation

↓ **proteosome (Psme2)**

Expression Reversion of some proteins involved in CFTR maturation & trafficking

e.g ↑ RACK1

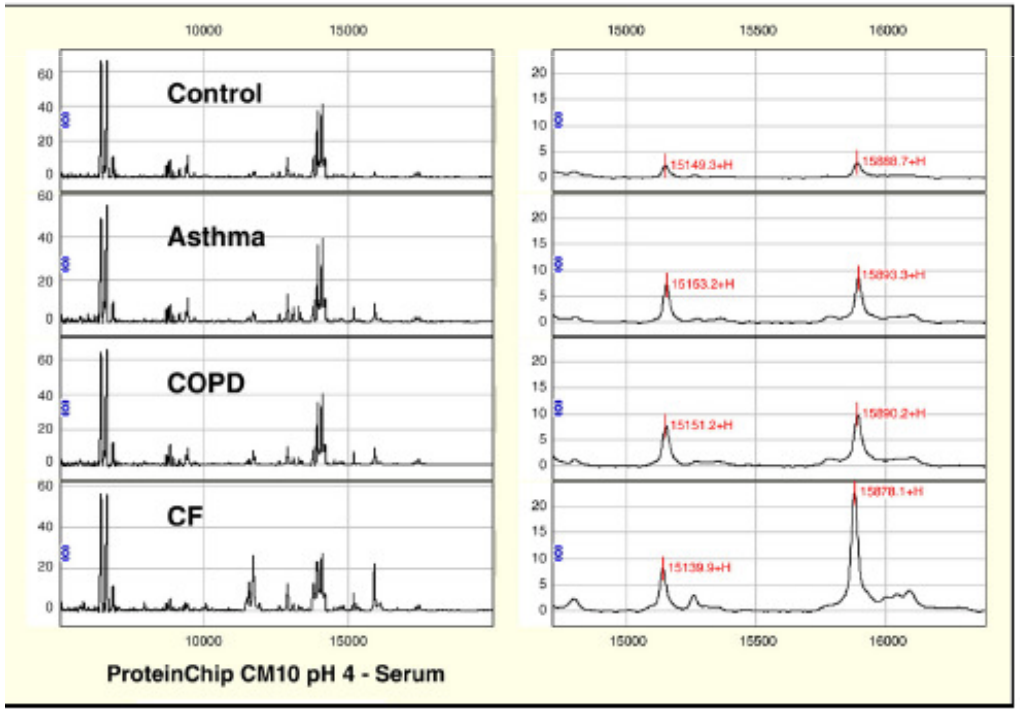
Promote relocation of ΔF-508-CFTR to cell surface

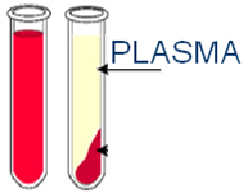
Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry xx (2009) xxx – xxx
CLINICAL BIOCHEMISTRY

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]

[a] Laboratório de Proteómica, Departamento de Genética, INSA-IP, Av. Padre Cruz, 1649-016 Lisboa, Portugal
[b] Medical Sciences (Medical Genetics), University of Edinburgh, Molecular Medicine Centre, Western General Hospital, Edinburgh, UK
[c] Departamento de Epidemiologia, INSA-IP, Lisboa, Portugal
[d] Laboratory of Proteomics, CNB-CSIC, Universidad Autónoma de Madrid, Madrid, Spain
[e] Unidade de Biologia Molecular, Departamento de Genética, INSA-IP, Lisboa, Portugal
[f] Clínica Universitária de Pneumologia, Hospital Santa Maria, Lisboa, Portugal
[g] Hospital São João, Universidade do Porto, Porto, Portugal





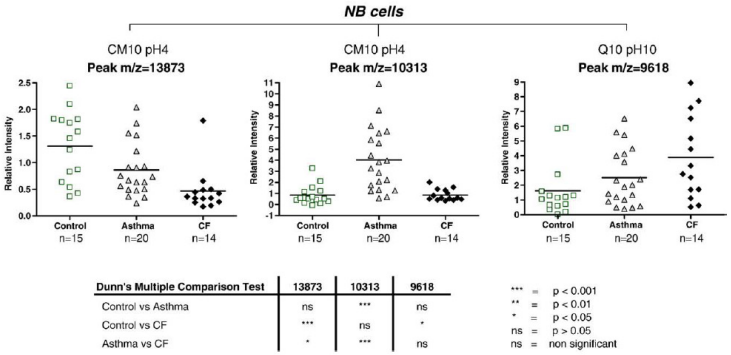


Fig. 3. Relative intensity of different protein clusters in NB cells from controls, asthma and CF patients. Data obtained on a CM10 pH 4 assay (13,873 Da and 10,313 Da) and on a Q10 pH 10 assay (9618 Da).

research articles Journal of proteome research

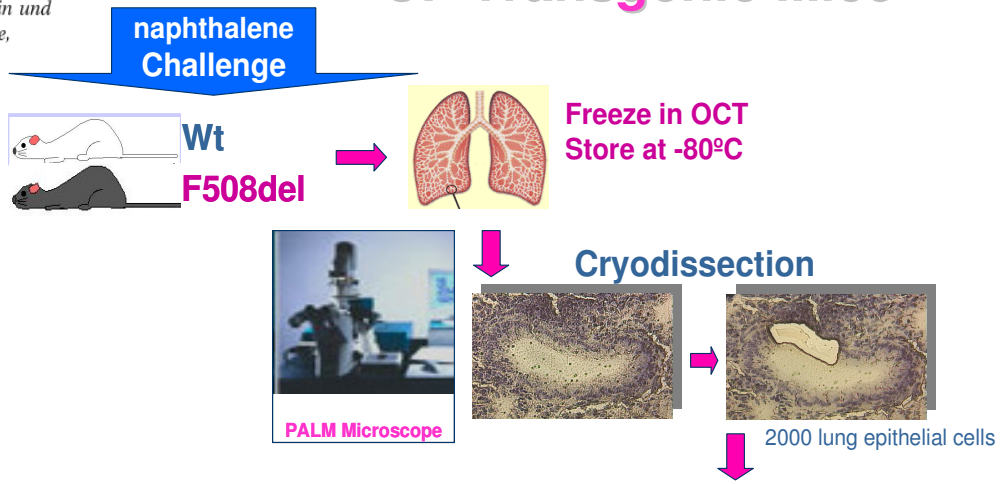
Proteomic Analysis of Naphthalene-Induced Airway Epithelial Injury and Repair in a Cystic Fibrosis Mouse Model

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

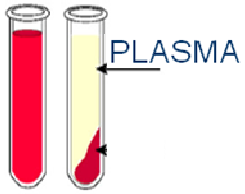
ScienceDirect

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]



LC/MS/MS

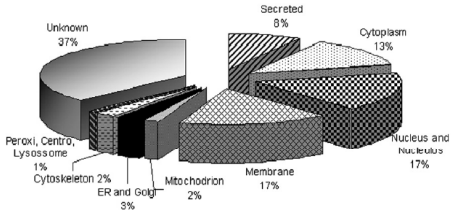
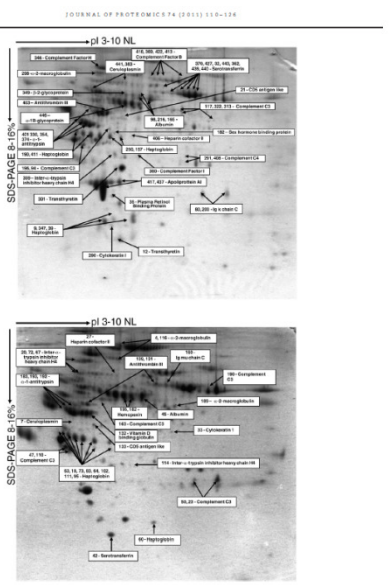


Fig. 3 – Graphical representation of the subcellular location of the identified proteins by label-free LC–MS/MS according to PIKE software.



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).

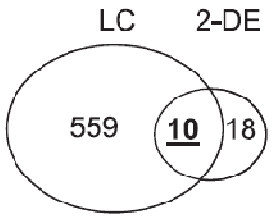


Fig. 2 – Total proteins identified in LC–MS/MS (by at least 2 peptides) and in 2-DE experiments.

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.

JOURNAL OF PROTEOMICS XX (2012) XXX–XXX

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

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,*]

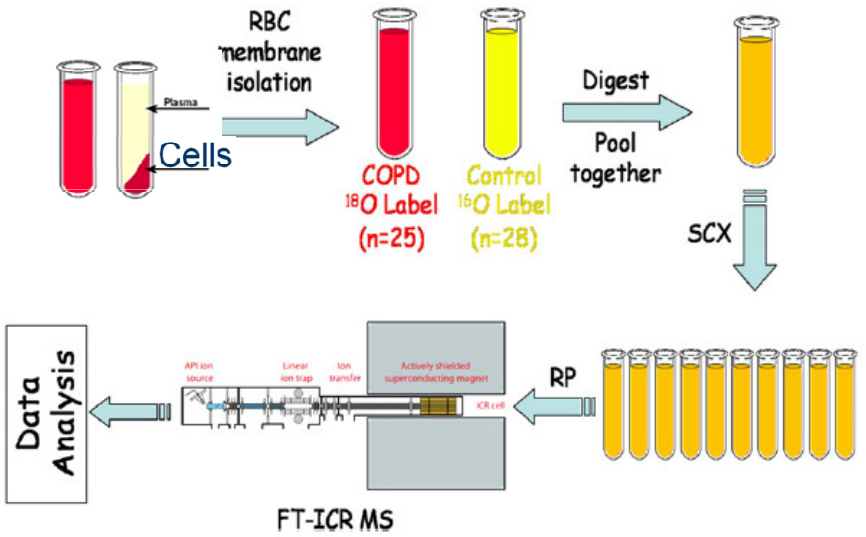


Fig. 1 – Basic scheme of methodology showing main steps of sample preparation.

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.

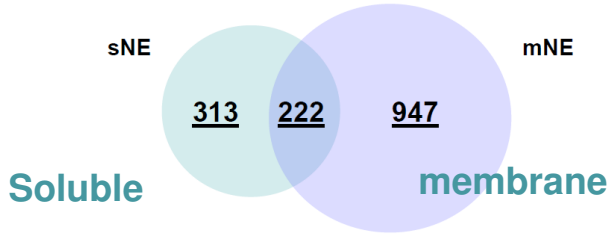
- ↓ Chorein (VPS13A) > cell membrane deformation of RBC c Methemoglobin reductase

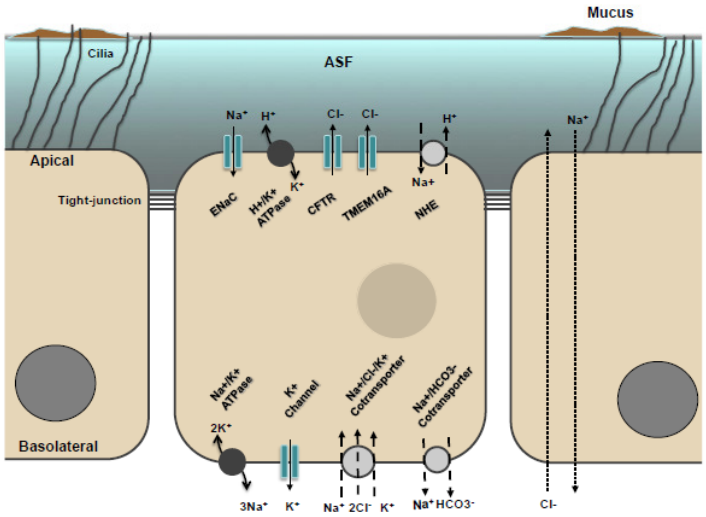
- ↓ (CYB5R3) > COPD patients may be at higher risk for developing methemoglobinemia.

Cellular Pre-fraccionation

⇩

LC/MS/MS





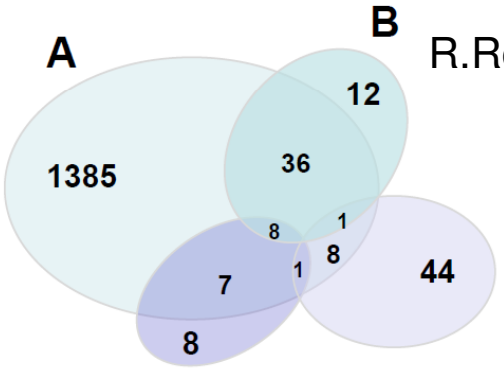
Main NE Proteome Function:

- fluid volume/ionic regulation
- physical barrier maintenance
- detoxification & immunological defence

available at www.sciencedirect.com
ScienceDirect
www.elsevier.com/locate/jprot
ELSEVIER
PROTEOMICS

Molecular profiling of the human nasal epithelium: A proteomics approach

Tânia Simões[a,1], Nuno Charro[a,1], Josip Blonder[b], Daniel Faria[c], Francisco M. Couto[c], King C. Chan[b], Timothy Waybright[b], Haleem J. Isaaq[b], Timothy D. Veenstra[b], Deborah Penque[a,]*



R.Rosa *et al*.2006

C Lee *et al.*

D Min-Man *et al.*

Proteome similarities between NE & LE support the applicability of NE to assess lung diseases

LABORATÓRIO DE PROTEÓMICA
DEPARTAMENTO DE GENÉTICA

2015
Projects running as Collaborator (n=3)

1. ***Development and validation of Vaso-occlusion early in a Mendelian Model of Vascular Disease*** (PIC/IC/83084/2007) **PI: J Lavinha** (INSA).
   Participantes LabP: D Penque, F Vaz.

2. **Investigação em doenças neurodegenerativas. Tiago Outeiro, (PI) e Hugo Miranda .** Instituto de Medicina Molecular, da Unidade de Neurociências. **Alfa-sinucleina como biomarcador sanguíneo para a doença de Parkinson** (FCT exploratory project), **Hugo Miranda** do IMM, PI .

Participantes LabP: D Penque

3. **Proteómica de exsudados em feridas crónicas e em pé diabético: a procura de biomarcadores diferencias.** Belarmino Barata. FCUL. (PREMIADO)

Participantes LabP: D Penque , V Torres & F Vaz.

4. **ProbeCOPD. Protease activitybased probes for Chronic Obstructive Pulmonary Disease diagnostics** PI. Susana Lucas e Rui Moreira , FARMID, (FCT APROVADO)

Associação da Faculdade de Farmácia para a Investigação e Desenvolvimento (FARMID)

Participantes LabP: D Penque , V Torres & F Vaz.

LABORATÓRIO DE PROTEÓMICA
DEPARTAMENTO DE GENÉTICA

Submitted projects (Calls 2015)

Under Evaluation

DIABETES ASSOCIADA A SÍNDROME DA APNEIA OBSTRUTIVA DO SONO: efeito terapêutico e molecular da ventilação não invasiva versus exercício físico (INFARMED 2015).

PI: D Penque

Publications

- Since 2007- **42 publications** in **international** peer-reviewed journals and 30 Posters/**8** Oral selected presentations in International Congress.

PROTEOMICS EDUCATIONAL ACTIVITIES

- Since 2007, the Lab is the coordinator of the '**Clinical Proteomics' and 'Protein Investigation' Courses/Modules** as part of the **PHD PROG**RAM in Medical and Life Science, Faculdade Ciências Médicas, Universidade Nova de Lisboa.

- 4 PhDs and 3 MSc theses in Proteomics field were concluded so far.
- At this moment, 4 PhD students (1 pharmacist, 1 veterinarian and 2 medical doctors) and 2 MSc students (biologists) are concluding their thesis program.

LAB PARTICIPATION IN INTERNATIONAL PROTEOMICS ACTIVITIES (most relevants)

- (2011-2013) (2014-2016) **- European Proteomics Association (EuPA)-**Communication Conference Committee
- (2005-2007) (2007-2009) - **European Proteomics Association (EuPA)** – Education Committee.
- 2014- Jury member of the **Young Investigator Award** at 2014-HUPO&EUPA Meeting, Madrid, Spain.
- 2012- Jury member of the **Best Young Investigator Award** at the EUPA & BBSPR Meeting, Glasgow, UK.
- 2012- Local organizer support of the inaugural meeting of the Top-Down Proteomics Consortium, Cascais, Portugal.
- (2010, 2012, 2014)- Organizer of the **Proteomics Photo&Graphic Art Contest.** EuPA-CC Dissemination Initiative.
- 2010- Organizer of the **Annual European Association Conference & Rede-ProCura** , Estoril, Portugal
- 2010-Jury Member of the Young Investigator Award at EuPA& ProCura Meeting hold in Estoril, Portugal
- 2007- Jury member of the Best Poster/Communication Awards at SSP& EuPA Meeting, Valencia, Spain.
- Since 2011 - The PI of the Lab is member of the **Associated Editorial Board-Europe of the Journal of Integrated OMICS: a methodological journal (JIOMICS)** (http://www.jiomics.com/index.php/jio/index)
- Since 2008- The PI of the Lab is member of the **Editorial Board of the Journal of Proteomics –Elsevier** (http://www.journals.elsevier.com/journal-of-proteomics).

RELEVANT COLLABORATIONS

- **Peter James**, PhD, Prof /group leader at **University of Lund, Sweden** (pioneer in protein sequencing by MS) .
- **Randall Nelson**, PhD, Prof/group leader **State University of Arizona, USA** (pioneer in MSIA technology)
- **Atul Malhotra**, MD, PhD, **San Diego Health System's Division of Pulmonary and Critical Care, California.**
- **Cristina Bárbara**, MD, PhD, **Diretor/Clinica de Pneumologia, CHLN**; coordinator of Programa Prioritário em Doenças Respiratórias, SNS.
- **Hugo Vicente Miranda**, PhD, group leader at **IMM Lisboa**
- **Rui Moreira**, PhD, Director/group leader, iMed.ULisboa, **Faculdade de Farmácia, UL**.
- **RNEM-** Rede Nacional de Espectrometria de Massa (coordinator: Helena Florencia, PhD).
- **Rede-ProCura-** Portuguese Proteomics Association (presidente: D Penque, PhD)
- **ToxOmics**- Research Center for Toxicogenomics & Human Health, UNL, (coordinator: José Rueff, PhD)