Missão

Investigação  Serviços  Outras Actividades
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**.

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, sequences, 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.

Proteomics Technology

• Discovery-based approach

• Targeted –based approach
Discovery -based approach

What proteins can be detected in this sample?

Discovery Phase

Validation/Translation Phase

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

Data Acquisition
- 2D-gel
- MALDI-TOF-TOF
- HPLC
- ESI-MS/MS
- LC/MS/MS

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

Pathway/Network Analysis

Methods:
- Immuno-fluorescence
- Western Blot
- ELISA
- Protein Array
- Flow Cytometry
- CyTOF Mass Cytometry
- MSIA
- Selected Reaction Monitoring
Discovery-based Proteomics approach

- **Biological Question & Sample**
- **Sample Separation**
- **Mass Spectrometry**
- **Data Analysis**
- **Data Verification**

**Methods:**
- MALDITOF/TOF
- Imaging MS
- Protein CHIP
- SELDI-TOF

**Data Base Query:**
(Mascot, Sequest, etc)

**Techniques:**
- Immuno-fluorescence
- Western Blot
- ELISA
- Protein Arrays
- Flow Cytometry
- CyTOF Mass Cytometry
- MSIA
- Selected Reaction Monitoring

**Pathway/Network analysis**
Targeted proteomics approach

Is protein X measurable in this sample?

Shotgun proteomics  
Biochemical experiments

Hypothesis  
Prediction Candidate proteins

Hypothesis Test  
Measurement of candidates

Results  
Translation to clinic

Mass spectrometer-based

QQQ-type MS (SRM, SISCAPA)

MALDI-type (MISA, iMALDI)

Antibody/Affinity-based

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

SRMA Atlas
http://www.srmatlas.org/
(170,000SRM assays for human)
Bottom-up MS approach

- Digestion with an enzyme (e.g. trypsin)
- Sequence peptide, identify protein, map modifications (partial coverage)

Top-down MS approach

- Sequence protein, identify protein, map modifications (full coverage)

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.
Protein abundance and sample complexity are significant factors that affect the availability of proteins for mass spectrometric quantitation.
Proteomics
Quantitation
Absolute / Relative

Spike
Heavy
Peptides

Isobaric
Tags

Isotopic
Tags

Metabolic
Labeling

Label-free
Quantification

Tissues/Cells

Proteins

Peptides

LC-MS or
LC-MS/MS

Data Analysis

Relative Intensity
Relative Intensity
Relative Intensity
Relative Intensity

Intensity

m/z
m/z
m/z
m/z

concentration

m/z
m/z
m/z
m/z

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

Patricia Gomes-Alves\textsuperscript{a,\textdagger}, Sofia Neves\textsuperscript{a}, Ana V. Coelho\textsuperscript{b}, Deborah Penque\textsuperscript{a,\textdagger}

\textsuperscript{a}Laboratório de Proteómica, Departamento de Genética, Instituto Nacional de Saúde Dr. Ricardo Jorge (INSAJ), Lisboa, Portugal
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Rescue of F508del-CFTR by RXR motif inactivation triggers proteome modulation associated with the unfolded protein response

Patricia Gomes-Alves\textsuperscript{a,\textdagger}, Francisco Couto\textsuperscript{c}, Cátia Pesquita\textsuperscript{c}, Ana V. Coelho\textsuperscript{b}, Deborah Penque\textsuperscript{a,\textdagger}

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Low temperature

**UPR**

Unfolded protein response

- Increase folding capacity
- & diminish degradation

**Mutagenic Repair**

(RXR) motifs

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

Patricia 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 Guaridano g, David J. Porteous b, Juan P. Albar a, A. Christopher Boyd b, I. Deborah Penke a, h, i

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Fig. 3. Relative intensity of different protein clusters in NB cells from controls, asthma and COPD patients. Data obtained on a CM10 pH 4 assay (15,973 Da and 11,831 Da) and on a Q10 pH 10 assay (19,165 Da).
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
Serum proteomics signature of Cystic Fibrosis patients: A complementary 2-DE and LC-MS/MS approach

Nuno Charro\textsuperscript{a,b}, Brian L. Hood\textsuperscript{b}, Daniel Faria\textsuperscript{c}, Paula Pacheco\textsuperscript{d}, Pilar Azevedo\textsuperscript{e}, Carlos Lopes\textsuperscript{e}, António Bugalho de Almeida\textsuperscript{f}, Francisco M. Couto\textsuperscript{g}, Thomas P. Conrads\textsuperscript{b,n}, Deborah Penque\textsuperscript{a,n,1}

Dysregulated Pathways (~70 p):
- abnormal tissue/airway remodeling,
- protease/antiprotease imbalance,
- innate immune dysfunction,
- chronic inflammation,
- nutritional imbalance
- \textit{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.
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.
Molecular profiling of the human nasal epithelium: A proteomics approach

Tanía Simões\textsuperscript{a,1}, Nuno Charro\textsuperscript{a,1}, Josip Blonder\textsuperscript{b}, Daniel Faria\textsuperscript{c}, Francisco M. Couto\textsuperscript{c}, King C. Chan\textsuperscript{b}, Timothy Waybright\textsuperscript{b}, Haleem J. Isaaq\textsuperscript{b}, Timothy D. Veenstra\textsuperscript{b}, Deborah Penque\textsuperscript{a,\textsuperscript{b}}

Main NE Proteome Function:
- fluid volume/ionic regulation
- physical barrier maintenance
- detoxification & immunological defence

Proteome similarities between NE & LE support the applicability of NE to assess lung diseases
   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-sinucleína como biomarcador sanguíneo para a doença de Parkinson (FCT exploratory project), Hugo Miranda do IMM, PI.
   Participantes LabP: D Penque.

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

4. ProbeCOPD. Protease activity based 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.
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 PROGRAM 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 relevant)

- (2011-2013) (2014-2016) - European Proteomics Association (EuPA)-Communication Conference Committee
- 2014- Jury member of the Young Investigator Award at 2014-HUPO&EUPO 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- 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.
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)
• **ToxOmic**s- Research Center for Toxigenomics & Human Health, UNL, (coordinator: José Rueff, PhD)