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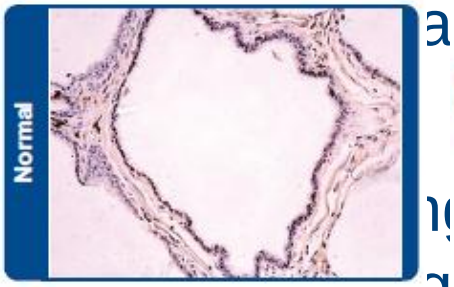
Novel biomarkers of chemical-induced asthma: a murine model

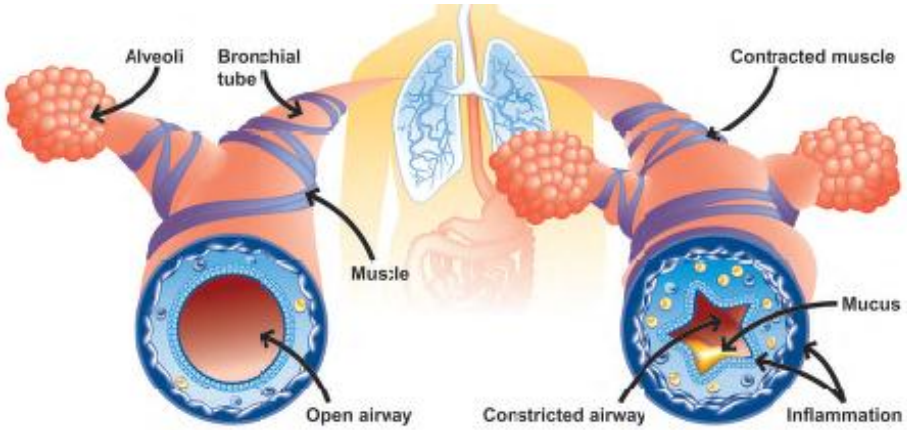
Jeroen Vanoirbeek

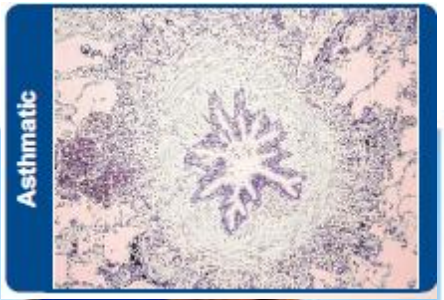
Asthma

1. Chronic airway disease:
   prevalence: 5-10%, 300.000 people affected world-wide

2. Reversible airflow limitation, airway hyperreactivity &
   a
   ı(
   coughing







Asthma

1. Rapid increase of allergy and asthma in past 40 years

2. Changes in gene pool unlikely

3. Changes in “environment”?
   - Hygiene, lifestyle, diet, ...
   - Air pollution (indoor, outdoor)
   - Specific chemicals?

4. Mechanisms?

Occupational asthma

1. Definition:
   - occupational asthma is a disease characterized by variable airflow limitation and/or airway hyperresponsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace

2. 9-15% of all adult asthma is due to exposures on the work floor

3. The most common cause of work-related lung diseases

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Bernstein et al., 1999; Mapp et al., 2005

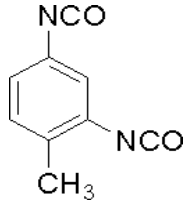
Occupational agents

High molecular weight (HMW) compounds (≥ 5 kDa)

- Animal proteins
- Plant proteins
- Enzymes

Low molecular weight (LMW) compounds (< 5 kDa)

- Chemicals
- Metals
- Wood dust
- Pharmaca



Immune sensitization

IgE mediated or non-IgE mediated

LMW sensitizers

Isocyanates, persulphates,…

No immune sensitization

Non-IgE mediated

LMW irritants

Chlorine, Ammonia,…

IgE mediated

HMW allergens

Flour, Lab animals,…

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Occupational agents

- Diisocyanates
  - Highly reactive, low molecular weight compounds
  - Most common cause of chemical-induced occupational asthma
  - Used for the production of polyurethanes, foams, paints, etc

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Skin - lung

- Development of chemical-induced asthma
  - Primary route of exposure and initiation of immune response = respiratory tract
  - Regulation and prevention of OA almost exclusively focusses on airborne exposures
  - Despite reduction in workplace respiratory exposure, diisocyanate asthma continues to occur

Focus on skin exposure

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Redlich C and Herrick C, Curr Opin Allergy Clin Immunol 2008

Occupational asthma

Mechanisms ?

- Role of chemical properties ?
  - chemical reactivity
  - irritant properties
- Pathways of sensitization ?
  - via dermal route ?
- Immunological mechanisms ?
  - usually no specific IgE antibodies
  - cellular mechanisms

Implications

- Hazard identification
  - Prediction of asthmogens
- Prevention
  - Avoid skin contact
- Surveillance & diagnosis
  - Identification of sensitized subjects
  - Identification of sensitizing agent in affected subjects

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Aim

To identify (early) biomarkers of chemical-induced asthma and sensitization to chemicals using proteomics techniques

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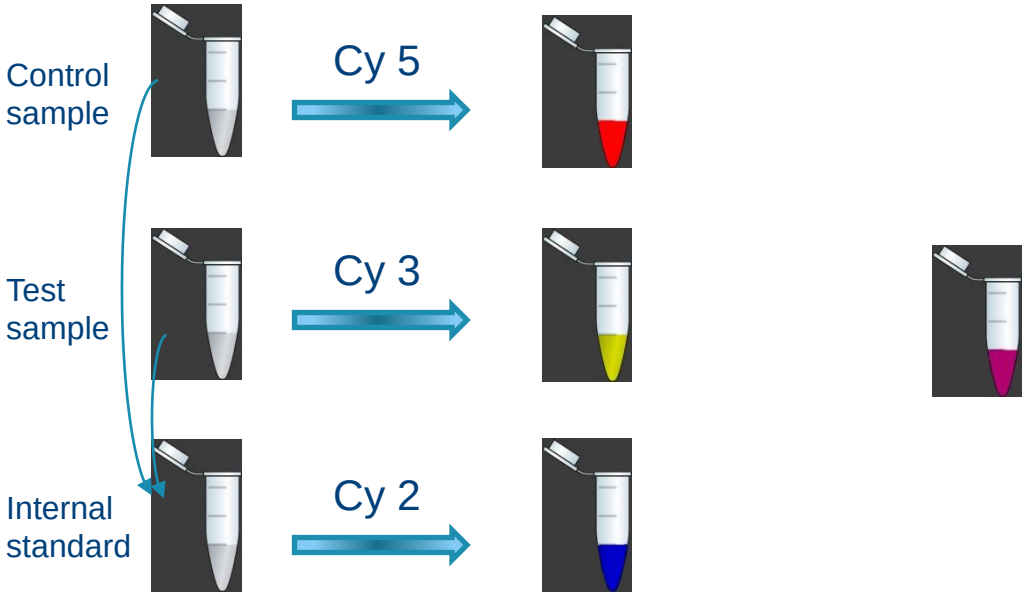
Proteomics

- Proteomics is a global strategy in which all proteins (the proteome) derived from a cell, tissue, body liquid or whole organism, are simultaneously visualized and identified

Two-dimensional difference gel electrophoresis: 2D-DIGE

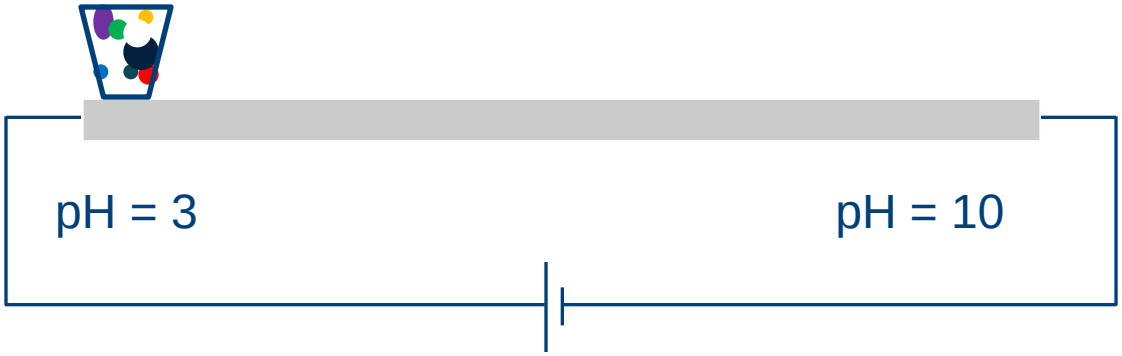


Two-dimensional difference gel electrophoresis: 2D-DIGE

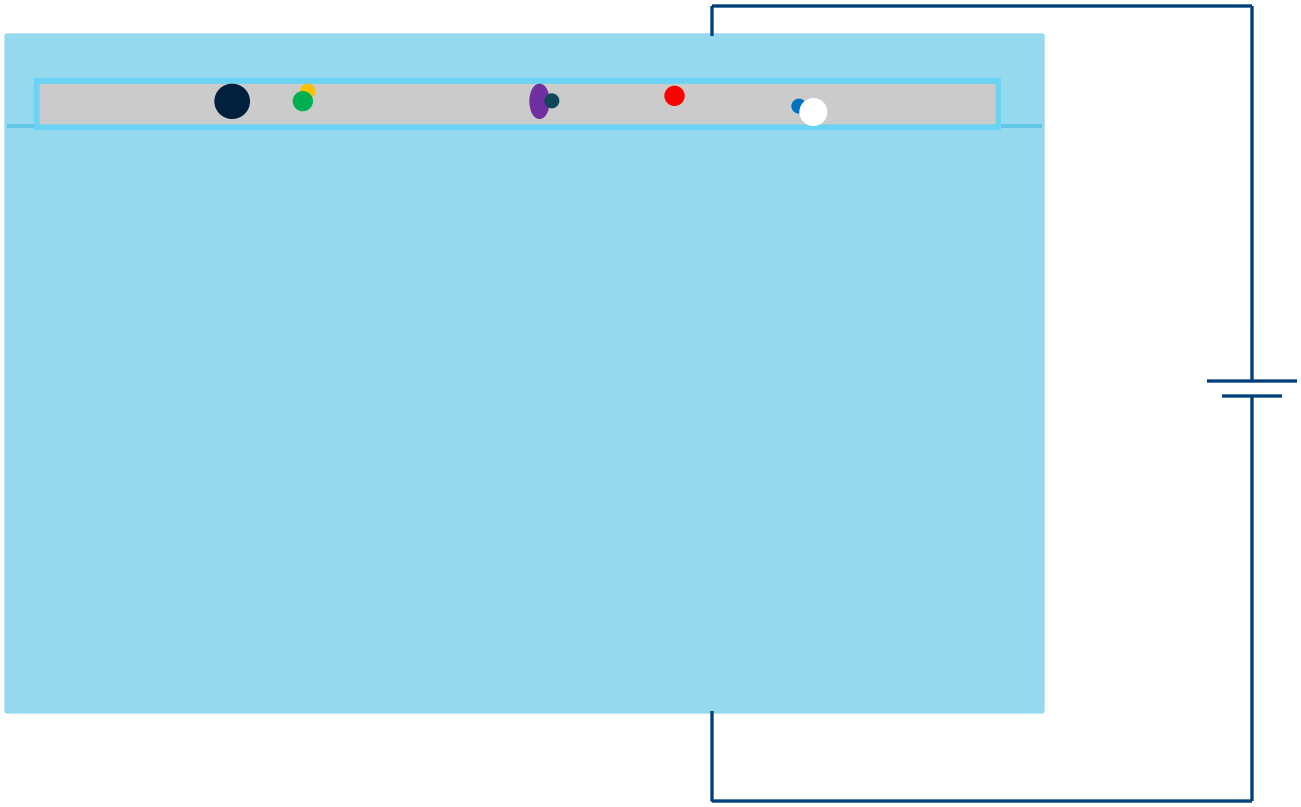
pH = 3

pH = 10

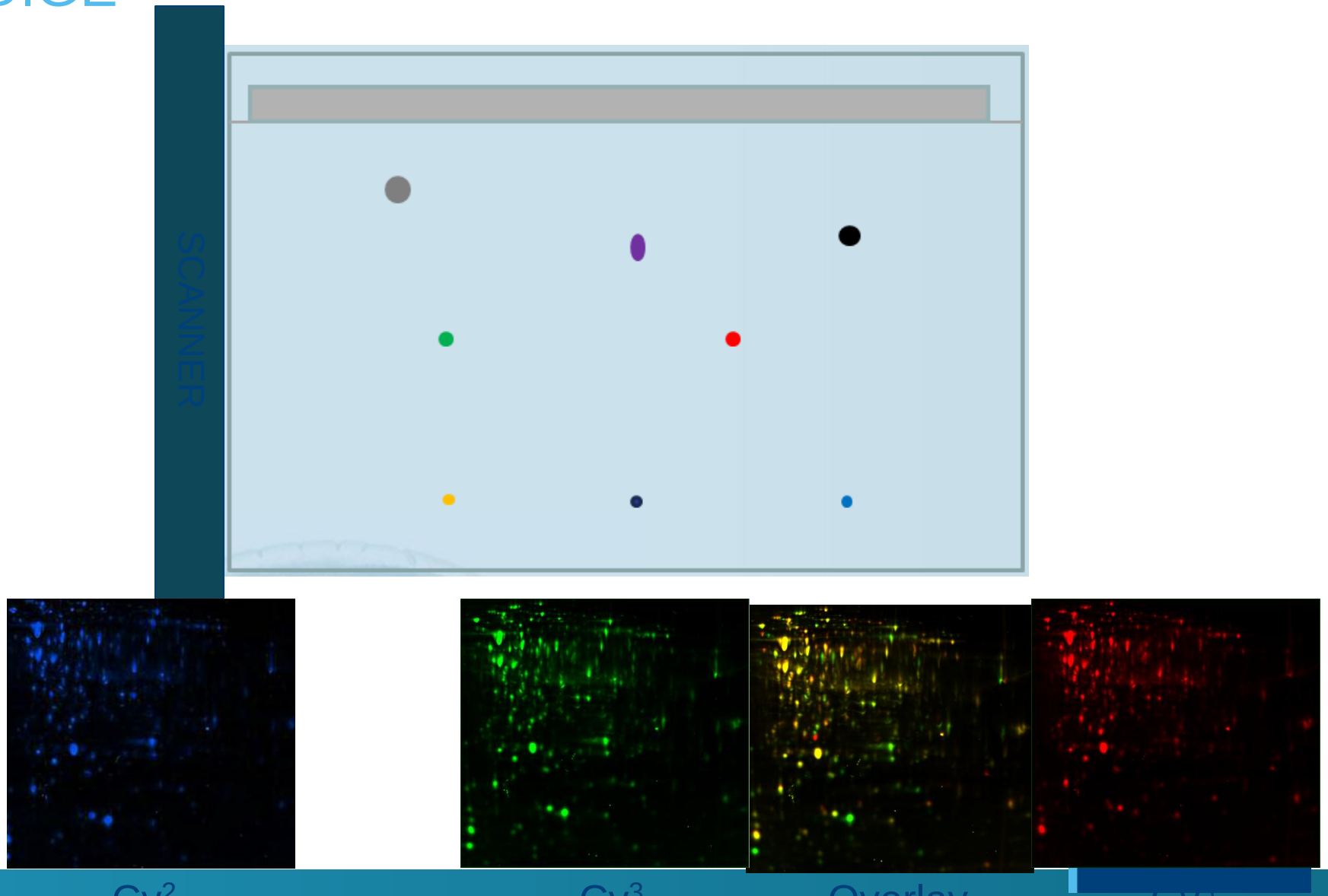
Two-dimensional difference gel electrophoresis: 2D-DIGE



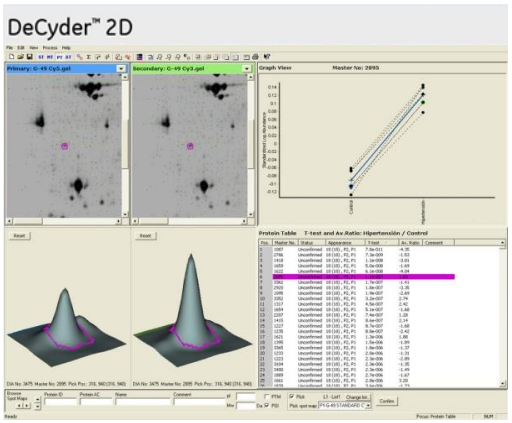
Two-dimensional difference gel electrophoresis: 2D-DIGE

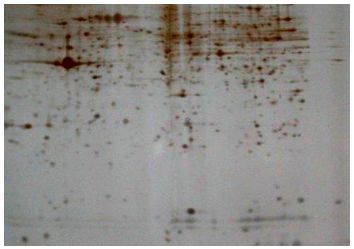


Two-dimensional difference gel electrophoresis: 2D-DIGE

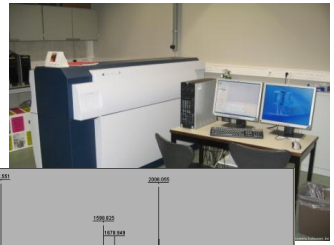


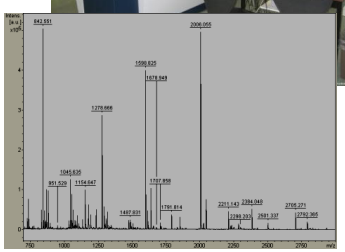
Two-dimensional difference gel electrophoresis: 2D-DIGE



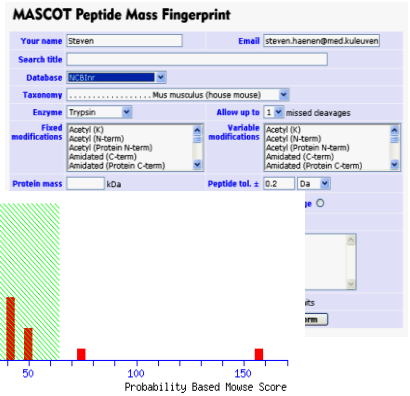


Trypsin Digest





MALDI-TOF MS



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Proteomics in chemical-induced asthma

- Classical research: focus on immune related cells and cytokines, resulting in many insights, but exact mechanisms of OA still unclear

- Development of new & sensitive methods → new approaches

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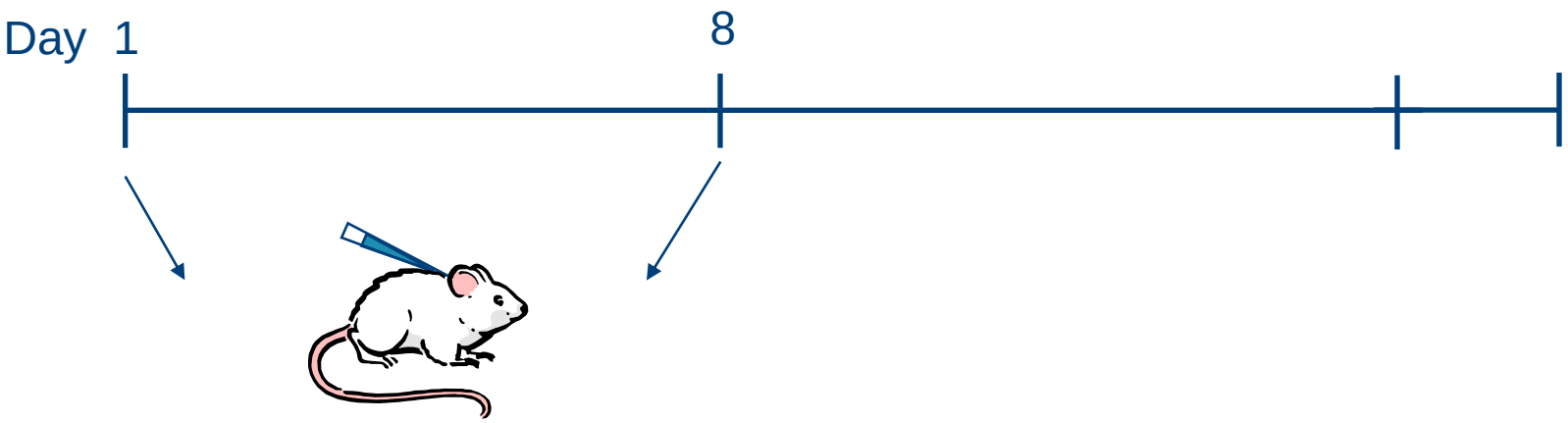
Proteomics in chemical-induced asthma



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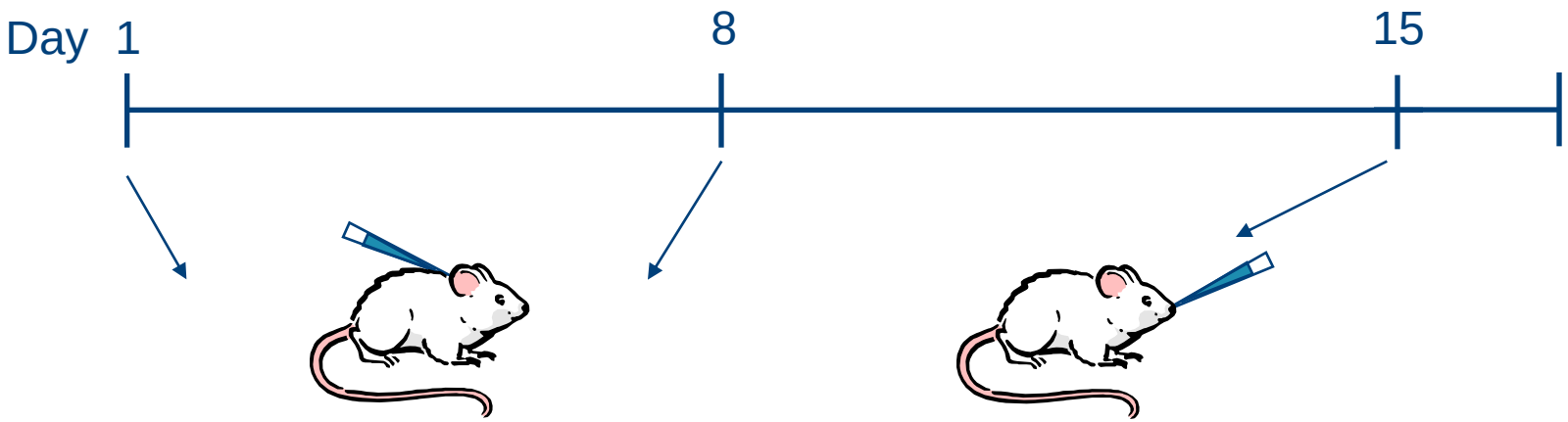
Vanoirbeek et al., 2003, 2004, 2008; De Vooght et al., 2010

Proteomics in chemical-induced asthma



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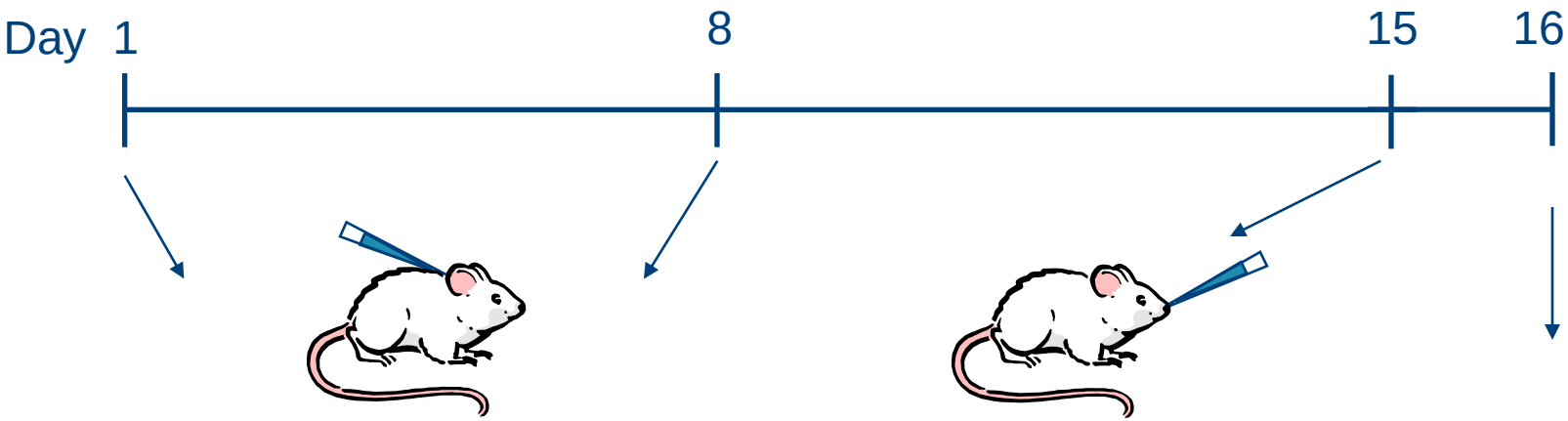
Proteomics in chemical-induced asthma



Dermal treatment on both ears (20 µl) with 0.3%TDI or AOO (vehicle)

Airway challenge: 0.01% TDI or AOO (vehicle)

AHR (methacholine)

sensitization

challenge

Sample collection:
- auricular lymph nodes
- serum
- BAL

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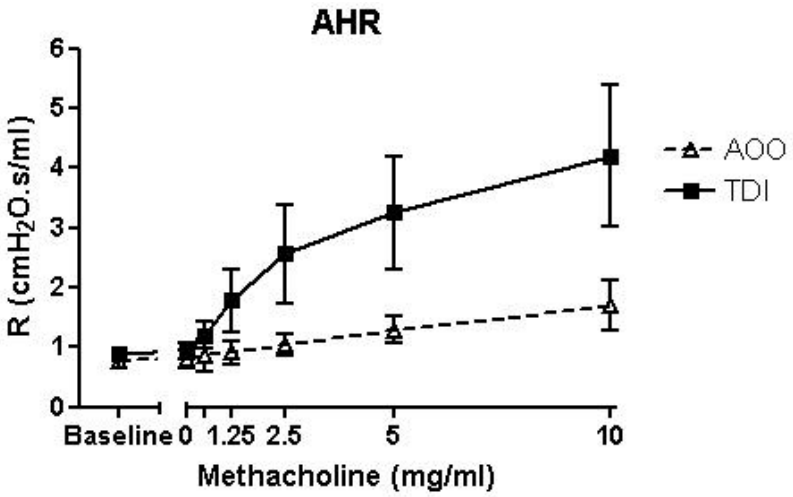
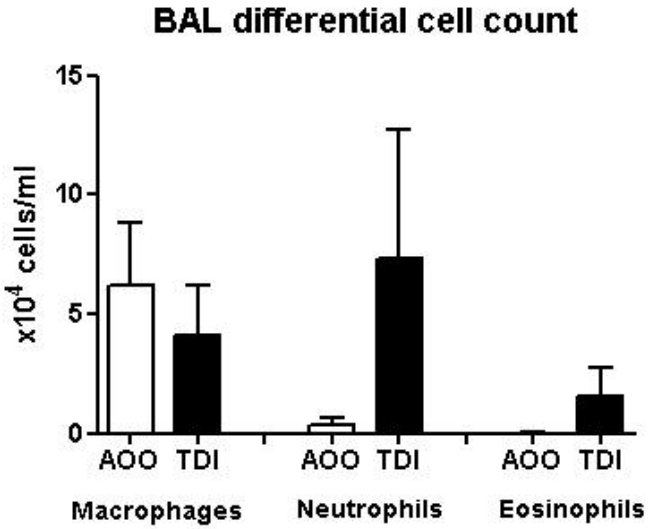
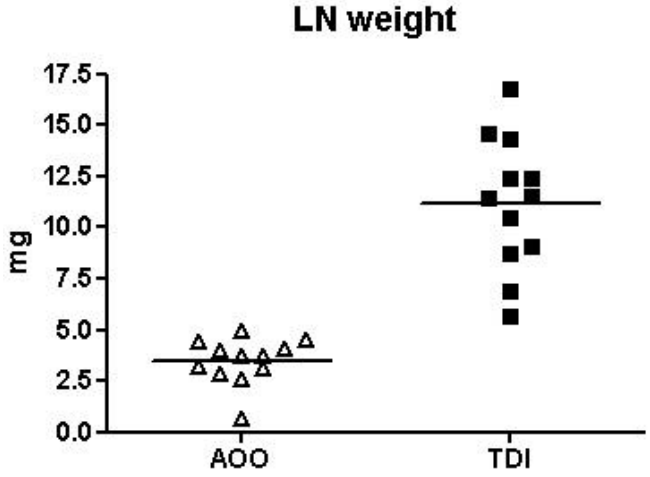
Vanoirbeek et al., 2003, 2004, 2008; De Vooght et al., 2010

Proteomics in chemical-induced asthma







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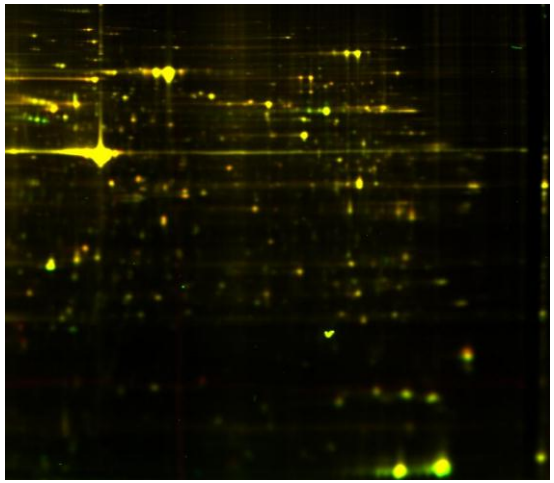
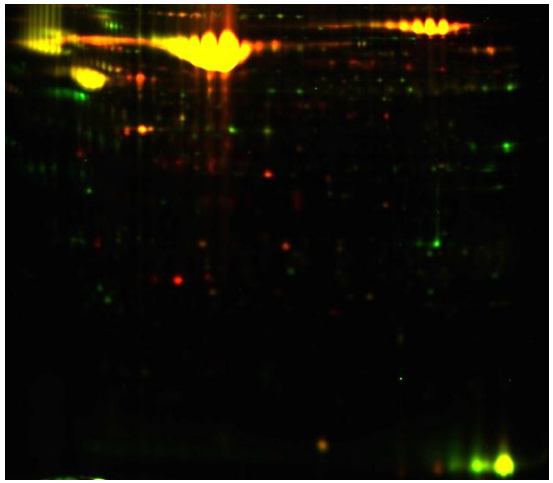
Proteomics in chemical-induced asthma

Auricular lymph nodes



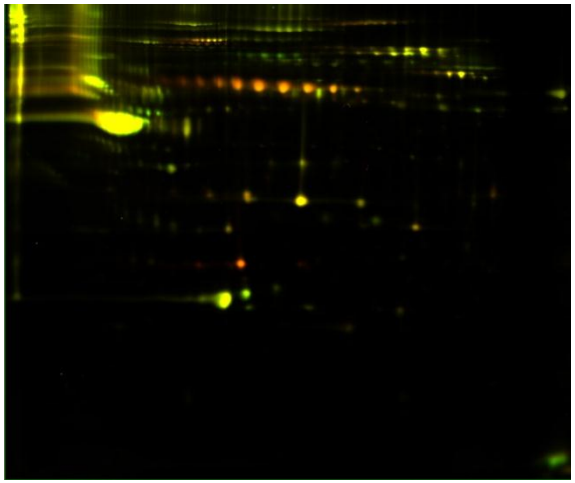
p < 0.01	
Diff. Spots	53
Identified	27
Up	14
Down	13

BAL



p < 0.01	
Diff. Spots	210
Identified	72
Up	55
Down	17

Serum



p < 0.01	
Diff. Spots	40
Identified	18
Up	9
Down	9

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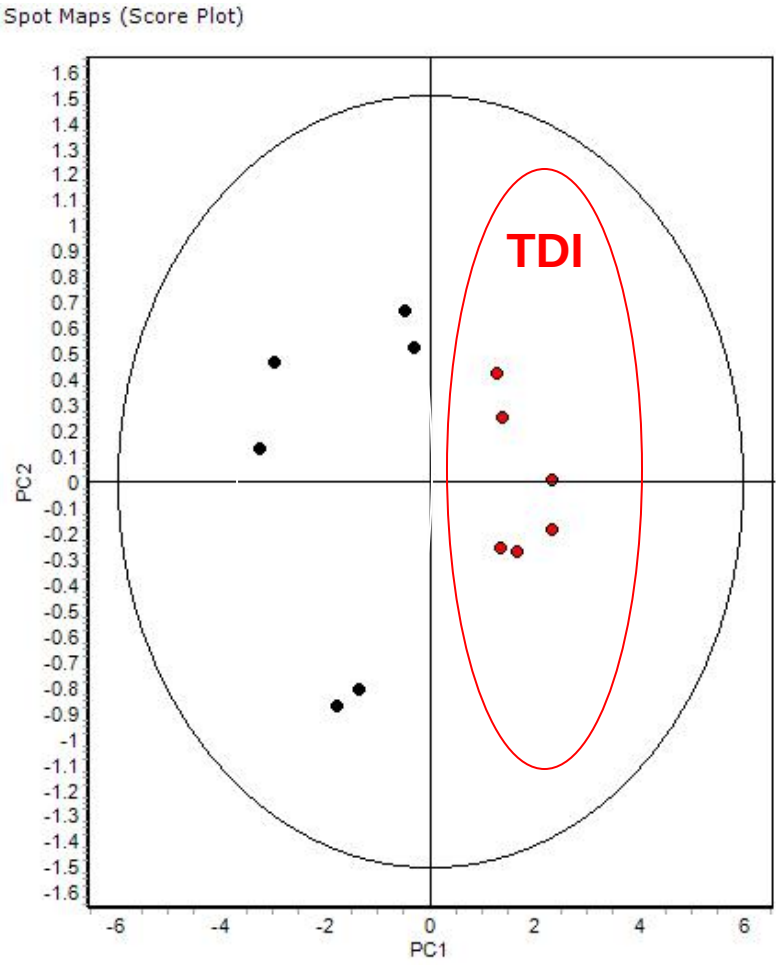
Proteomics in chemical-induced asthma



Auricular lymph nodes

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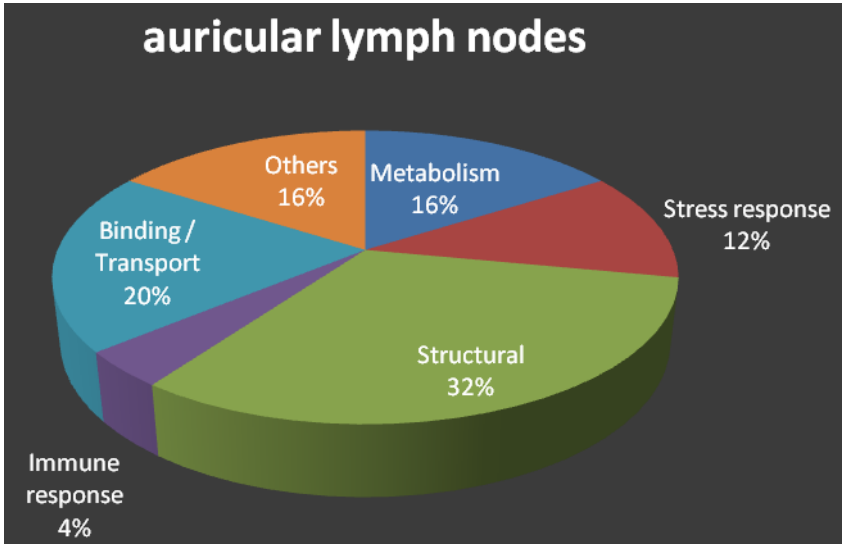
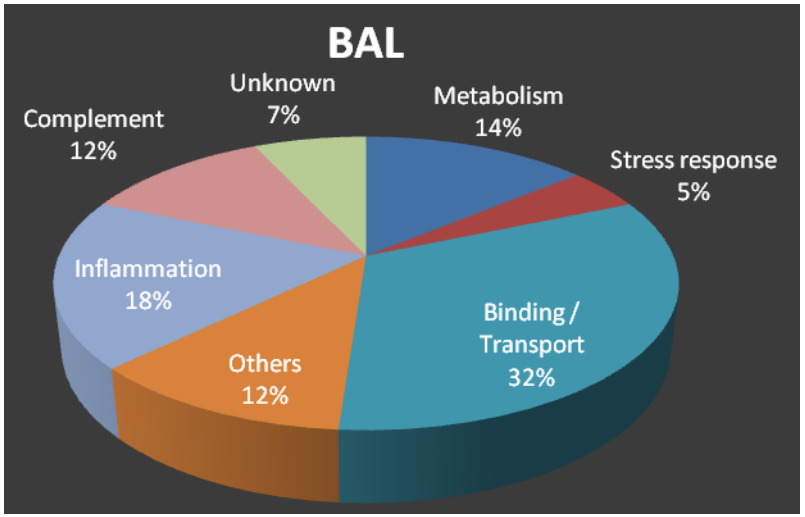
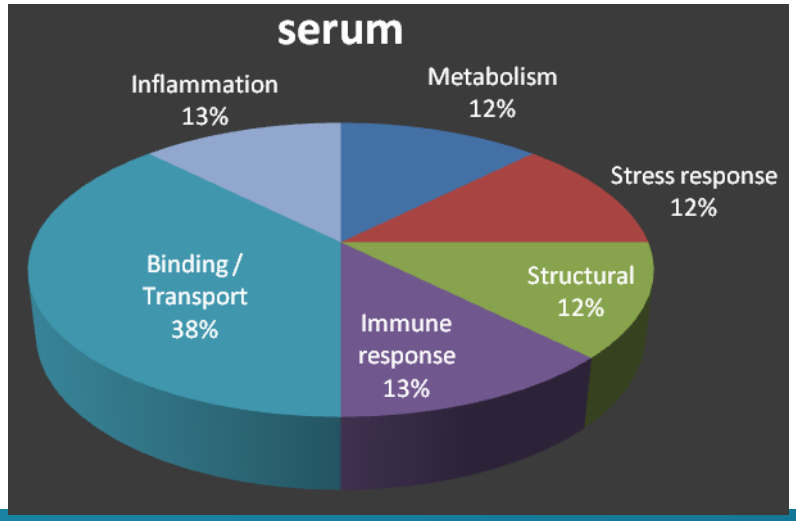
Proteomics in chemical-induced asthma







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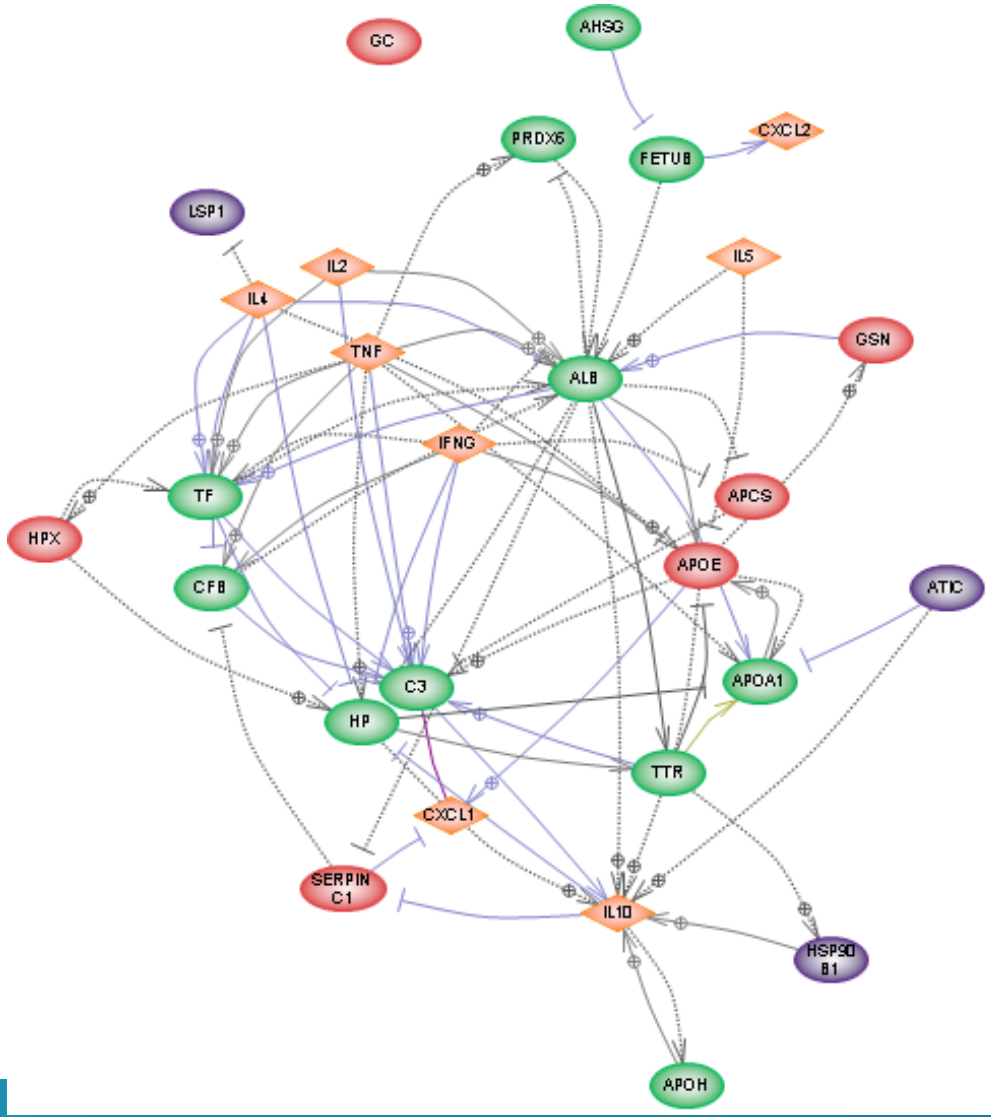
Proteomics in chemical-induced asthma



auricular lymph nodes

bronchoalveolar lavage

serum

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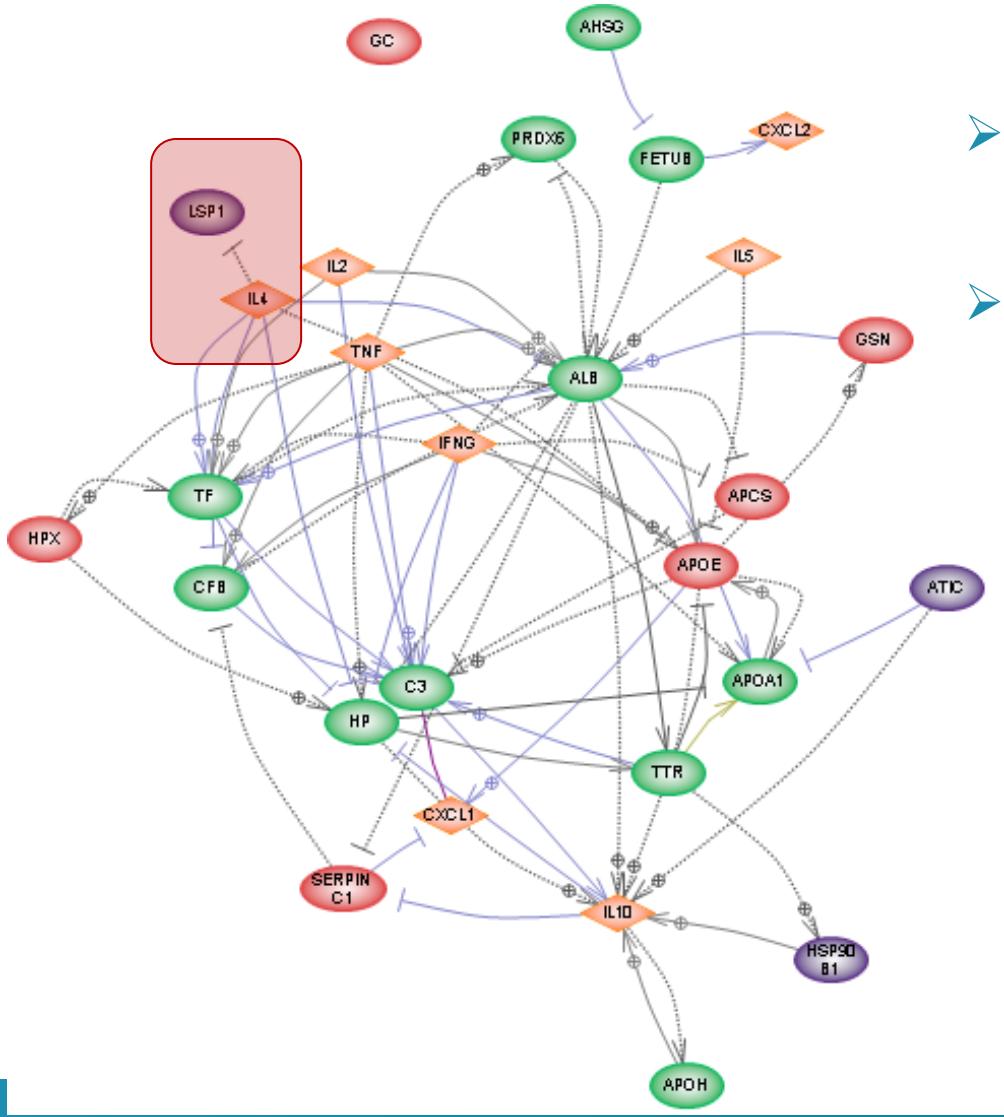
Proteomics in chemical-induced asthma



- Lymphocyte specific protein-1 ↓
- Literature:
  - Expression:
    - lymphocytes
    - neutrophils
    - macrophages
  - Chemotaxis & activation neutro
  - Overexpression → dysfunction
  - Regulated by IL-4

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Vanoirbeek et al., 2008, De Vooght et al., 2009

Conclusions

1. First systematic & systemic proteomics approach in a model of occupational asthma

2. Dermal sensitization and a single airway challenge leads to profound proteome changes in multiple compartments

3. Physiological and immunological changes are reflected by changes in the proteome

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Proteome changes in auricular lymph nodes and serum after dermal sensitization to toluene diisocyanate in mice

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Proteome changes after dermal sensitization

1. So far, no study has focused on early time points

2. Human (early) biomarker research is complicated: only when disease has established

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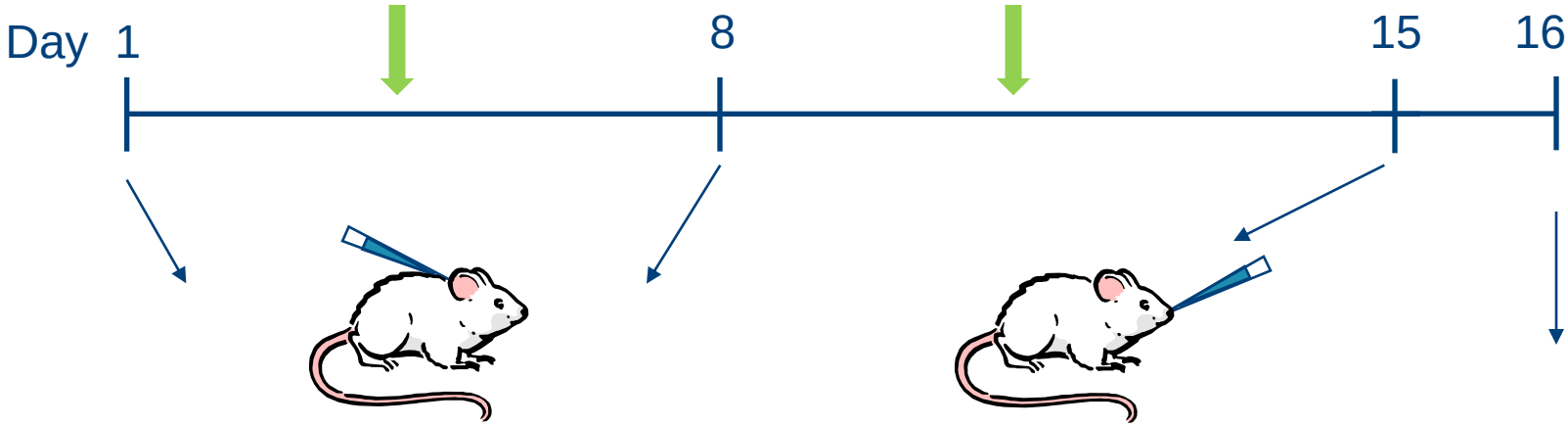
Proteome changes after dermal sensitization

1. So far, no study has focused on early time points

2. Human (early) biomarker research is complicated: only when disease has established

3. Rationale: investigate changes in the proteome during sensitization to identify (early) markers of sensitization

Proteome changes after dermal sensitization



Dermal treatment
on both ears (20 µl) with
0.3%TDI or AOO (vehicle)

Oropharyngeal
aspiration: 0.01% TDI
or AOO (vehicle)

Sample collection:
- serum
- auricular lymph nodes

AHR
(methacholine)

sample collection:
- auricular lymph nodes
- serum

sensitization

challenge

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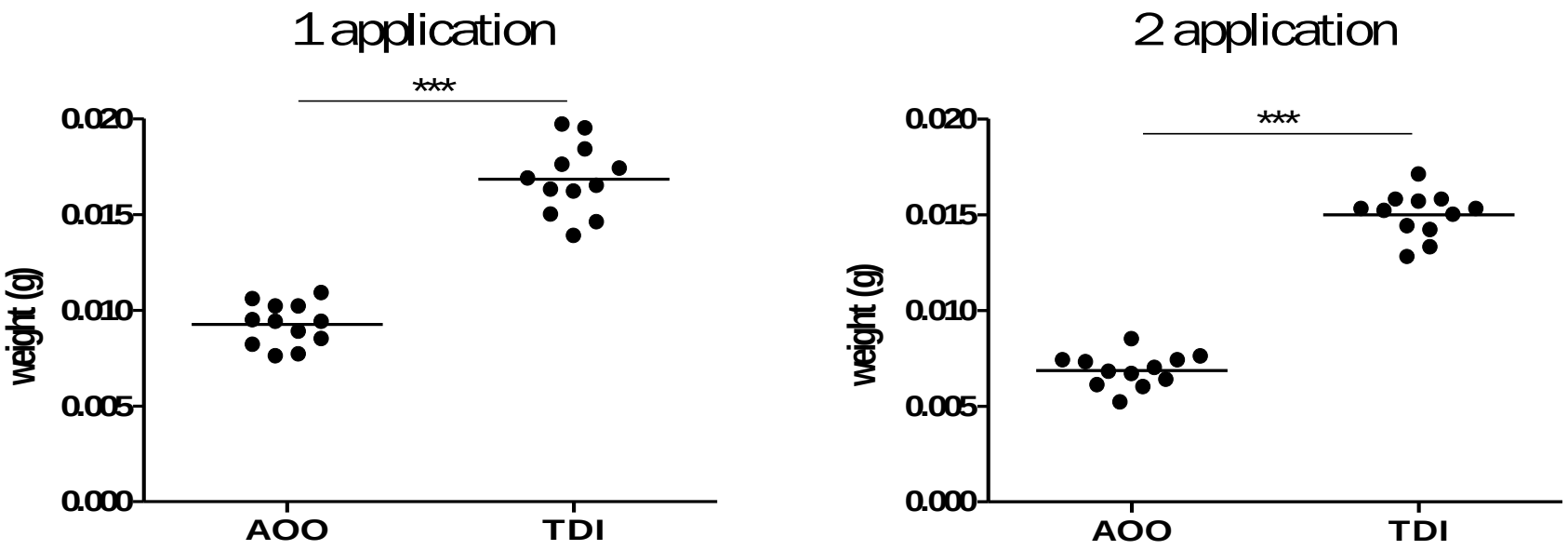
Proteome changes after dermal sensitization

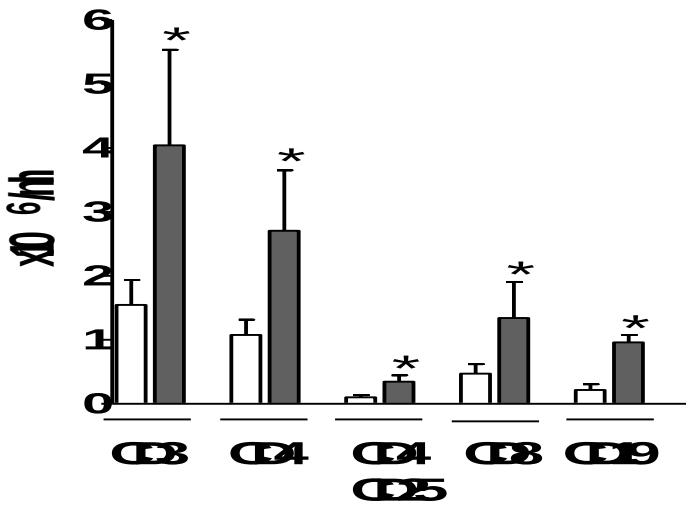
Auricular lymph node weight



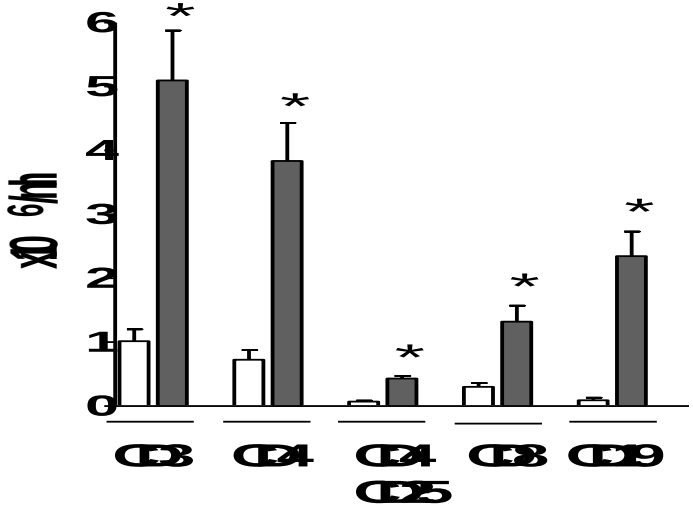
Proteome changes after dermal sensitization

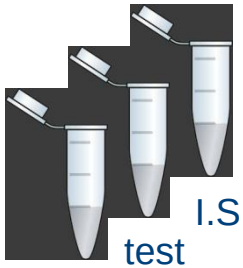
Lymphocyte subpopulations

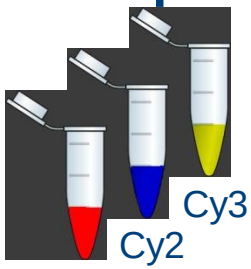
1 application



2 applications

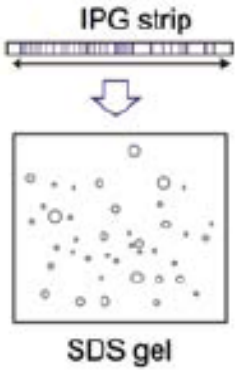




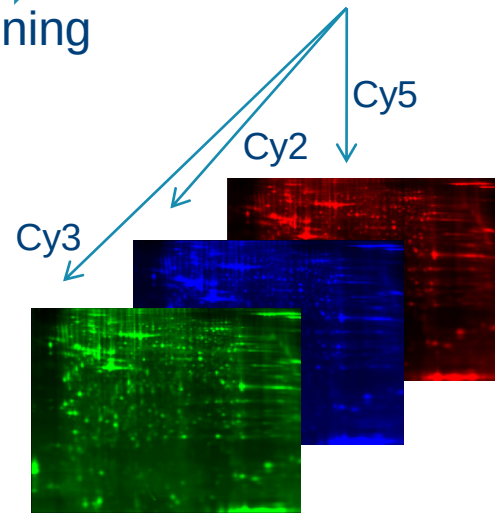


1 labeling

2 separation

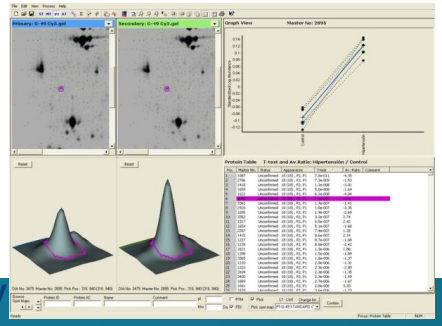


3 scanning

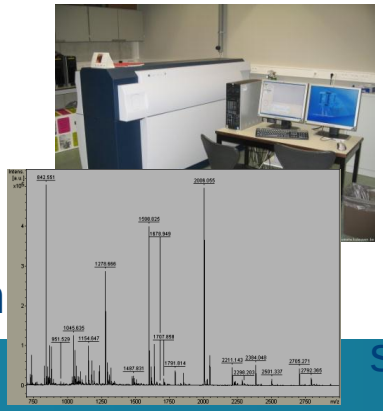


2D-DIGE workflow

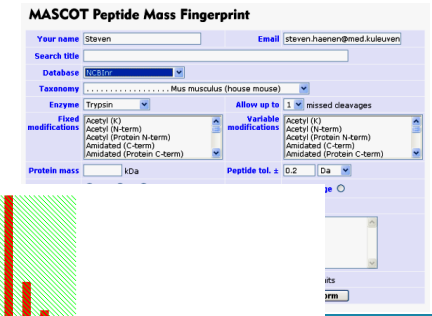
Decyder analysis 4

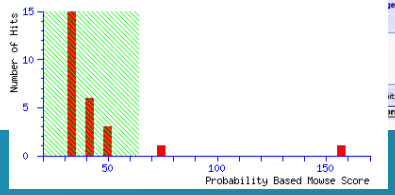


5 mass spectrometry



6 identification

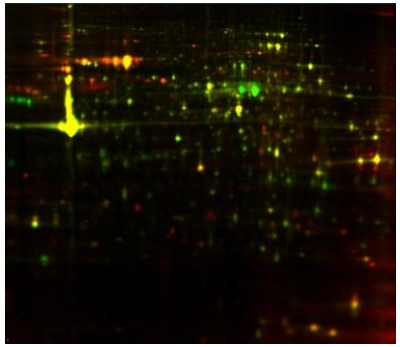




Proteome changes after dermal sensitization

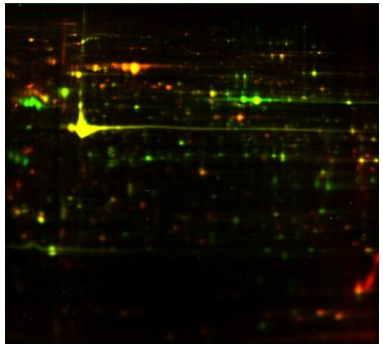
auricular lymph nodes

1 application



p < 0.01	
Diff. spots	38
Identified	26
Up	15
Down	11

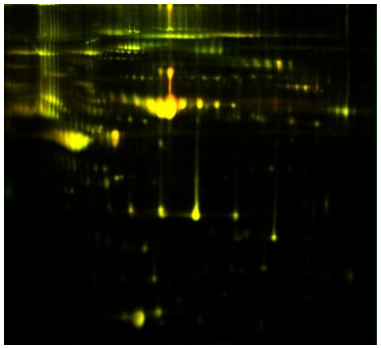
2 applications



p < 0.01	
Diff. spots	58
Identified	35
Up	19
Down	16

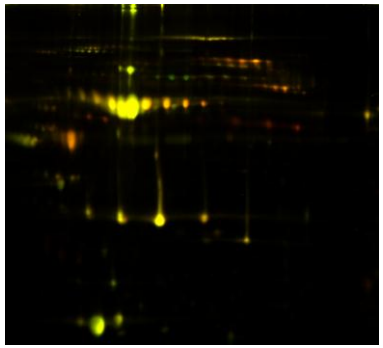
serum

1 application



p < 0.01	
Diff. spots	7
Identified	3
Up	3
Down	0

2 applications



p < 0.01	
Diff. spots	16
Identified	10
Up	4
Down	6

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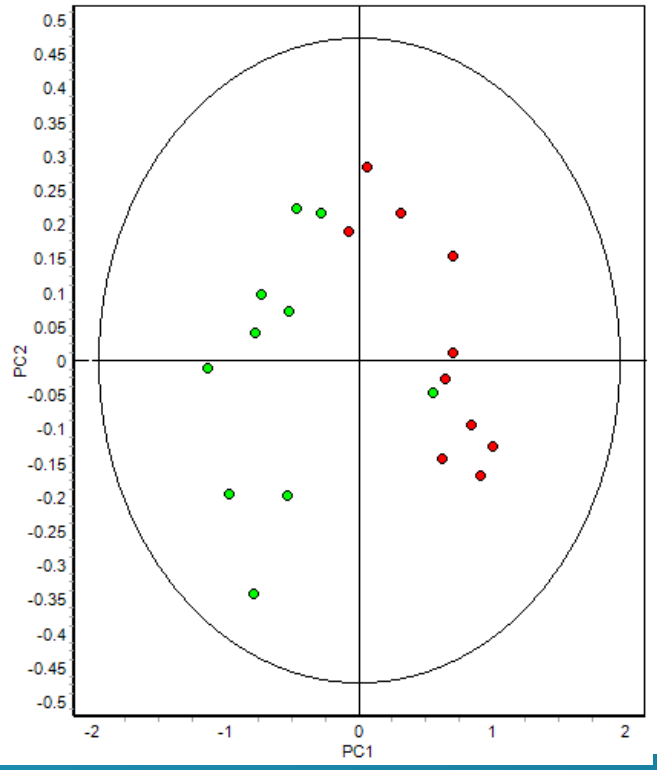
Proteome changes after dermal sensitization

auricular lymph nodes

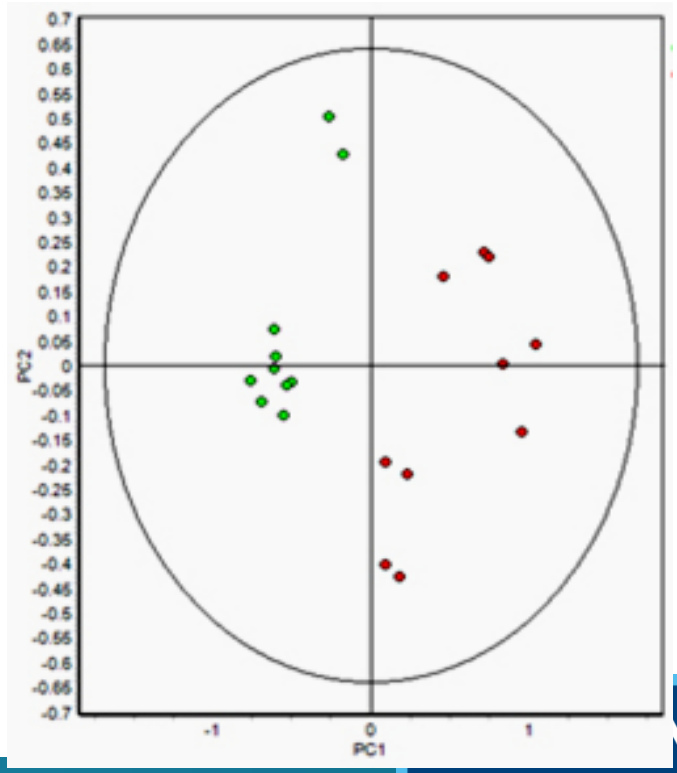
1 application



AOO

TDI

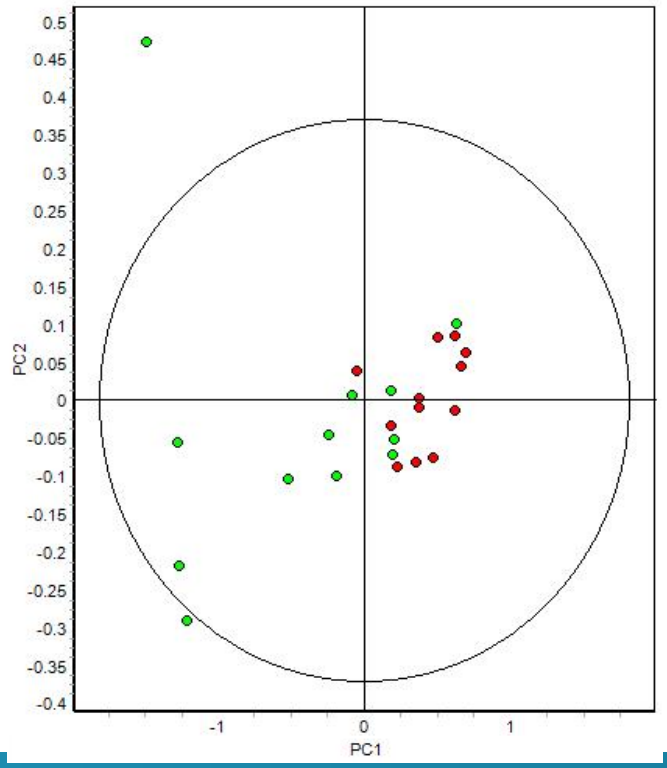
2 applications



Proteome changes after dermal sensitization

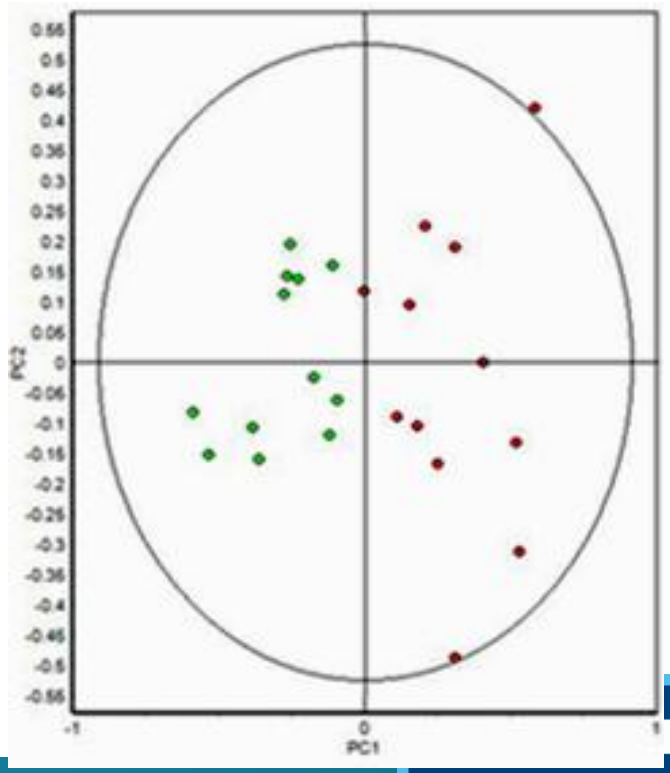
serum

1 application



AOO
TDI

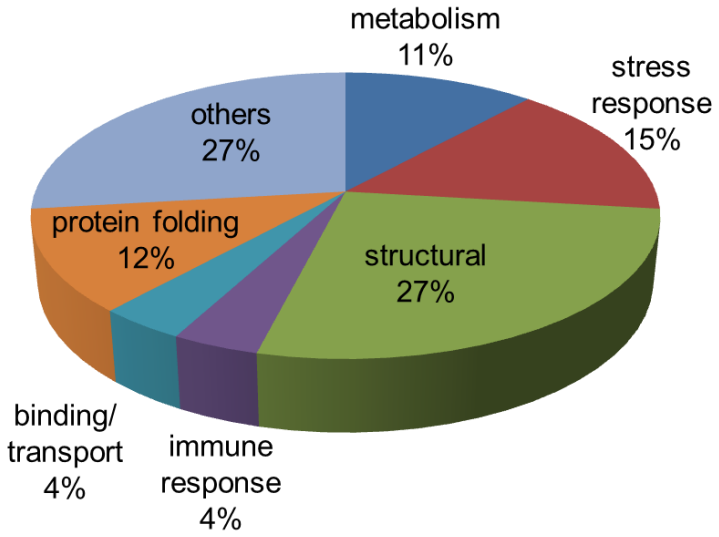
2 applications



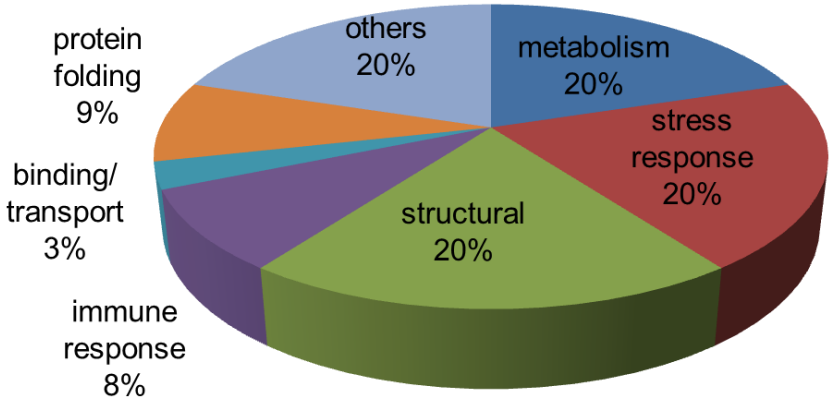
Proteome changes after dermal sensitization

auricular lymph nodes

1 application



2 applications



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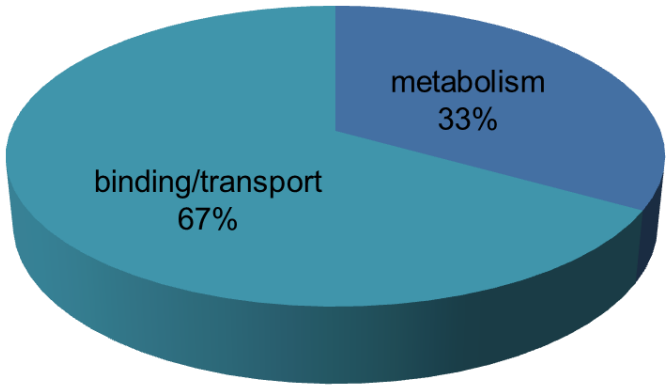
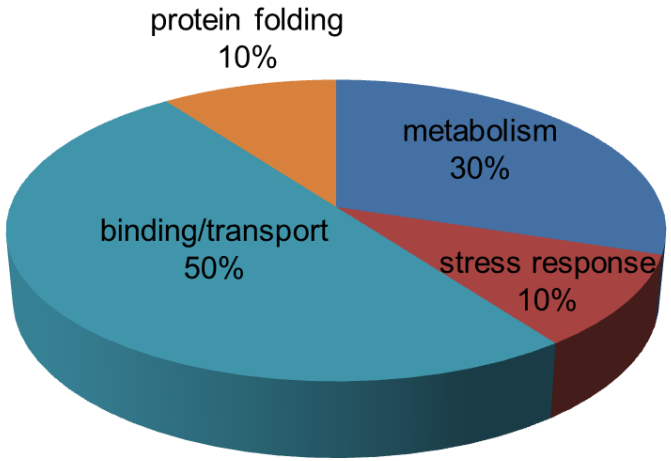
Proteome changes after dermal sensitization

serum

1 application



2 applications



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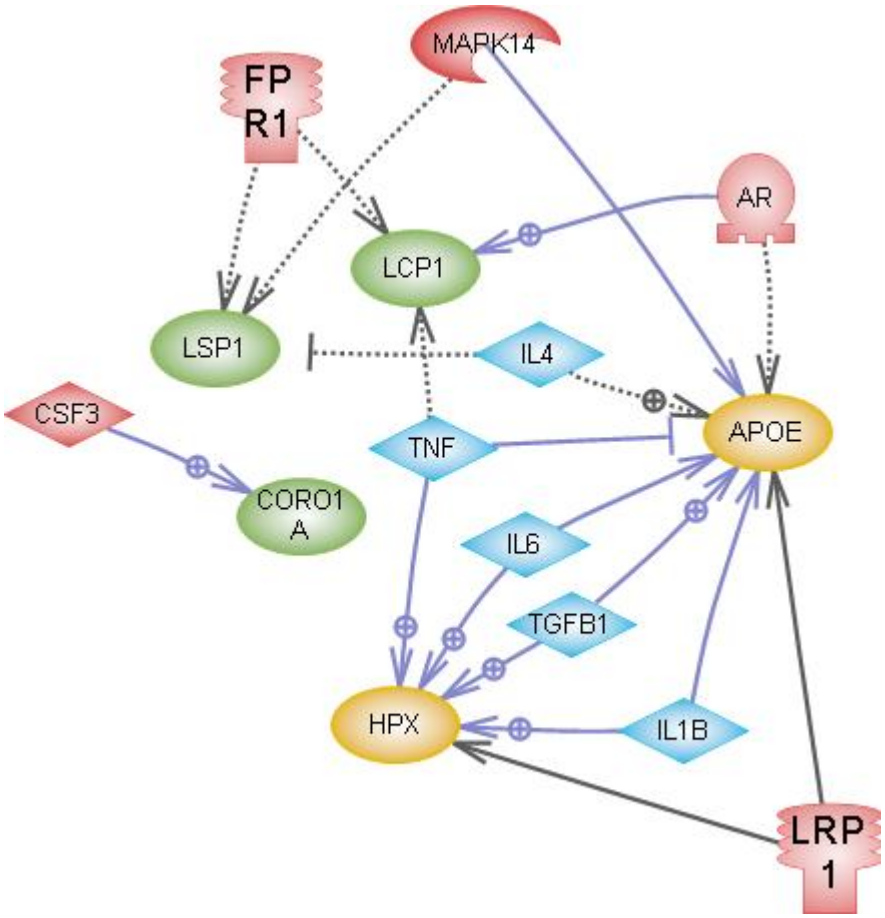
Proteome changes after dermal sensitization



auricular lymph nodes

serum

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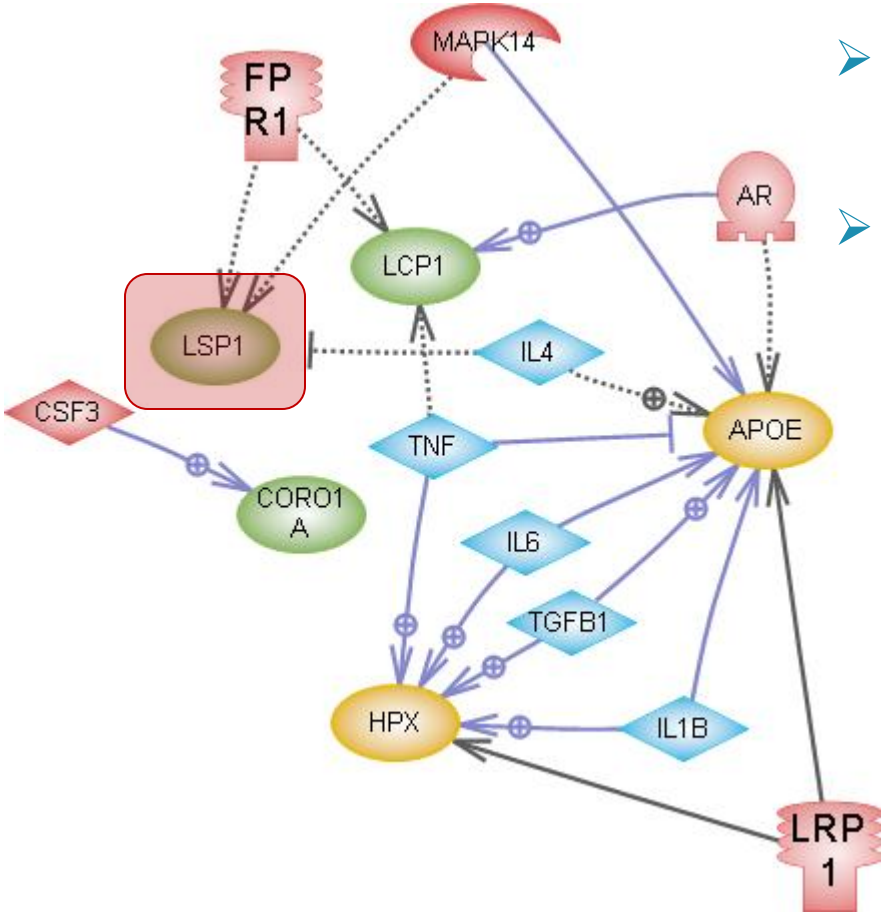
Proteome changes after dermal sensitization



- Lymphocyte specific protein-1
- Literature:
  - Expression:
    - lymphocytes
    - neutrophils
    - macrophages
  - Chemotaxis & activation neutro
  - Overexpression → dysfunction
  - Regulated by IL-4
  - Down regulated in complete model *(Haenen et al., 2010)*

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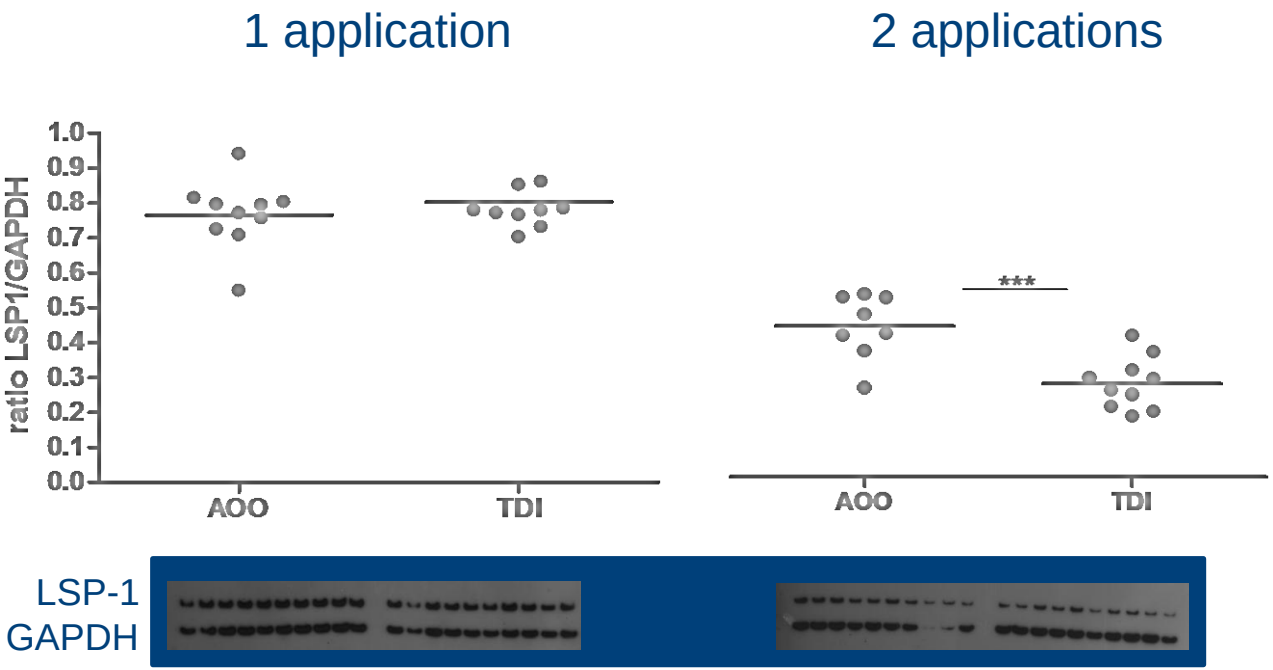
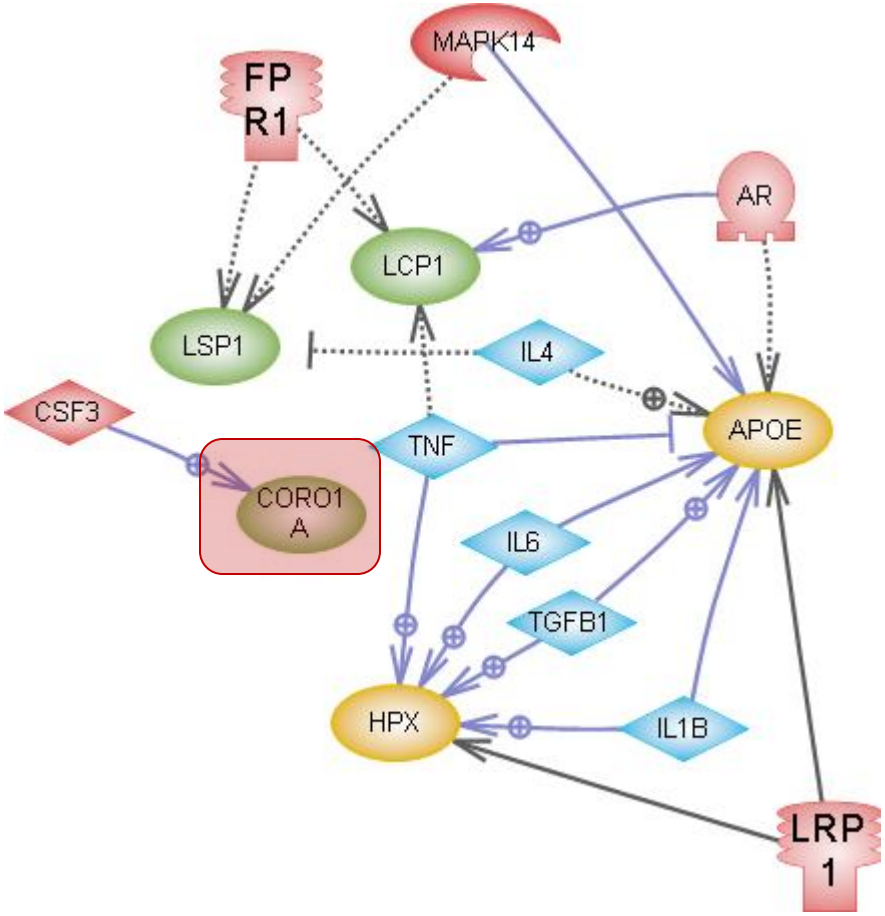
Proteome changes after dermal sensitization

LSP-1



Proteome changes after dermal sensitization



- Coronin 1a
- Literature:
  - Member of well-conserved family of coronin proteins
  - Mediates Ca^{2+} release
  - Involvement T cell activation and proliferation

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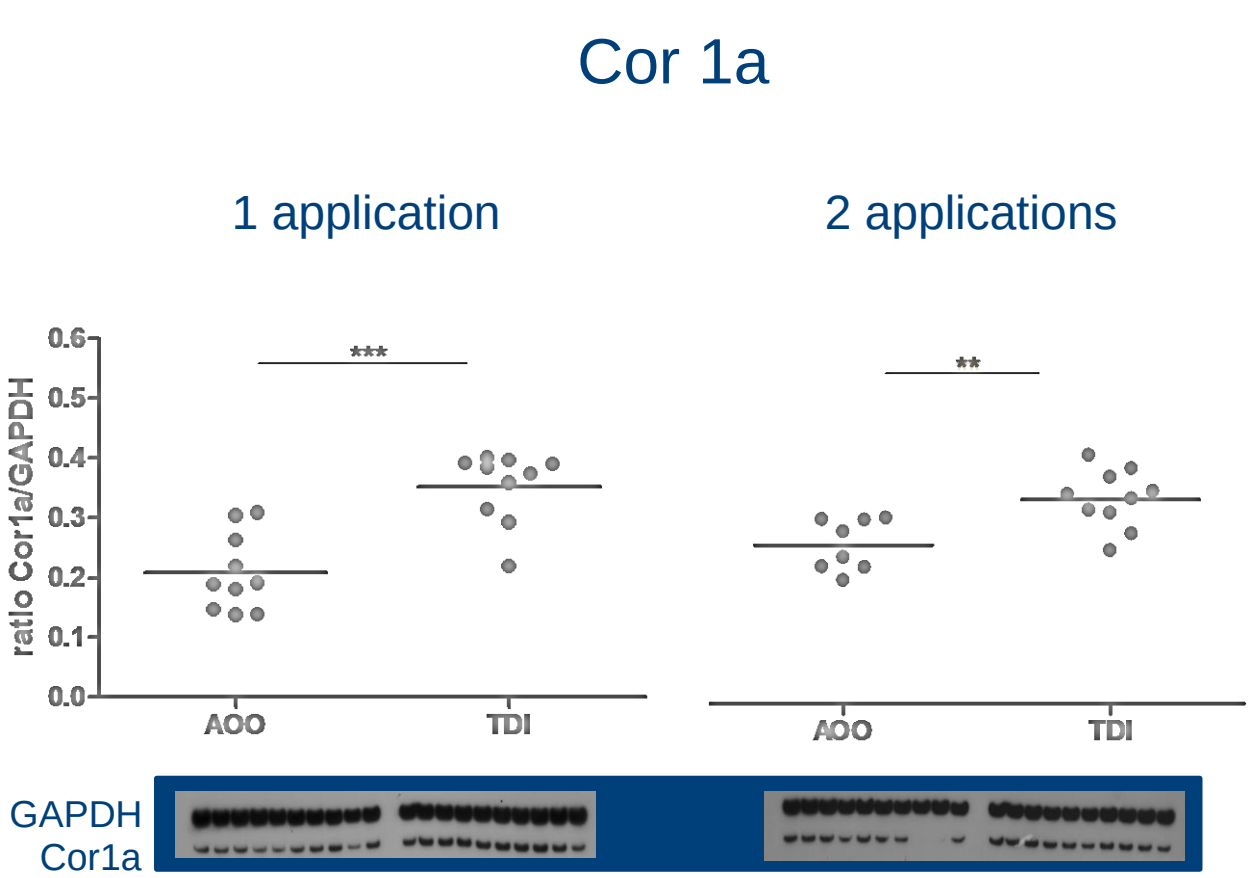
Proteome changes after dermal sensitization

Cor 1a



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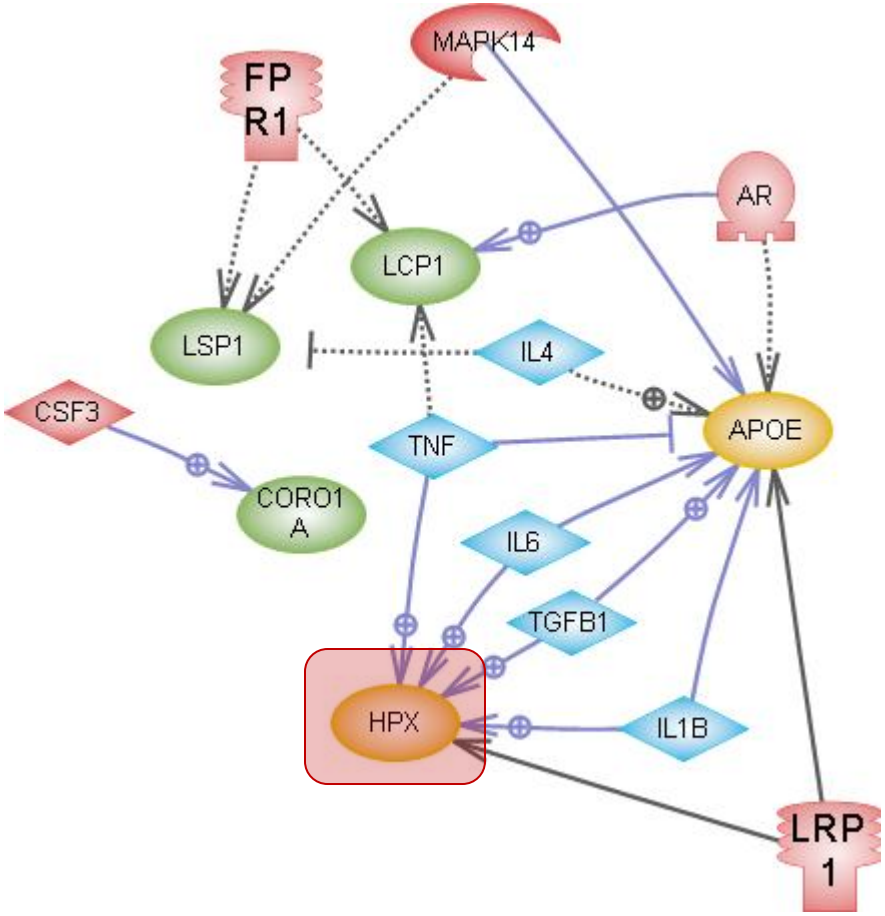
Proteome changes after dermal sensitization



- Hemopexin
- acute phase protein
- Literature:
  - Involved in innate body defense
  - Upregulated during inflammation
  - Also in complete model (BAL and serum)

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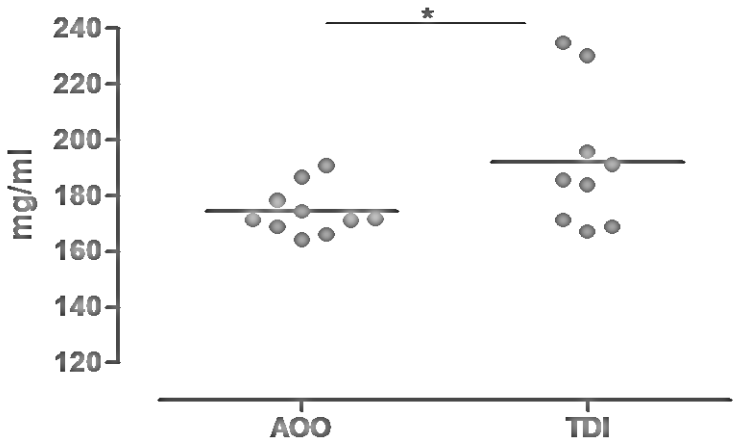
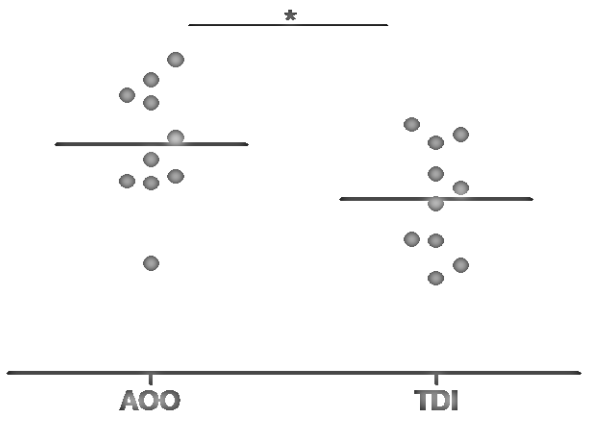
Proteome changes after dermal sensitization

Hemopexin

1 application



2 applications



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Conclusions

1. Sensitization causes profound differences in the proteome of TDI-sensitized mice compared to control mice

2. A subset of proteins were confirmed in an independent set of mice

3. Validation needed in human exposed workers

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