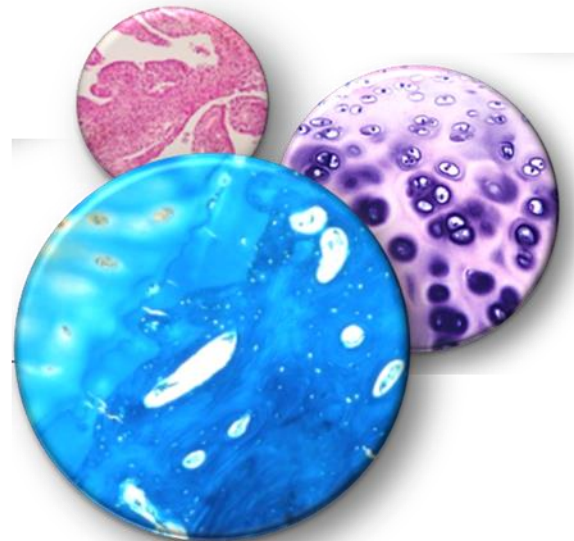


Discovery and validation of new osteoarthritis biomarkers using omics approaches across the spectrum of disease



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D-BOARD
European partnership for biomarkers discovery

www.bcru.be



Biological fluids

SERUM/PLASMA

URINE

SYNOVIAL FLUID

OMICs APPROACHS in OA

Tissues

EXTRACT

EXPLANT
SECRETOME

CARTILAGE

BONE

SYNOVIAL MEMBRANE

CELL EXTRACT

MEMBRANOME

MATRISOME

...

Cells

SECRETOME

CHONDROCYTES

OSTEOBLASTS

SYNOVIOCYTES

OSTEOCLASTS

- + Target of biomarker follow-up
- Depletion of major proteins
- Difficult to detect minor proteins

- + Joint specificity
- + Pathological pathways (inflammation,...)
- Presence in serum/urine...?

⇒ complementary approaches

GENOMIC

PROTEOMIC

TRANSCRIPTOMIC

METABOLOMIC

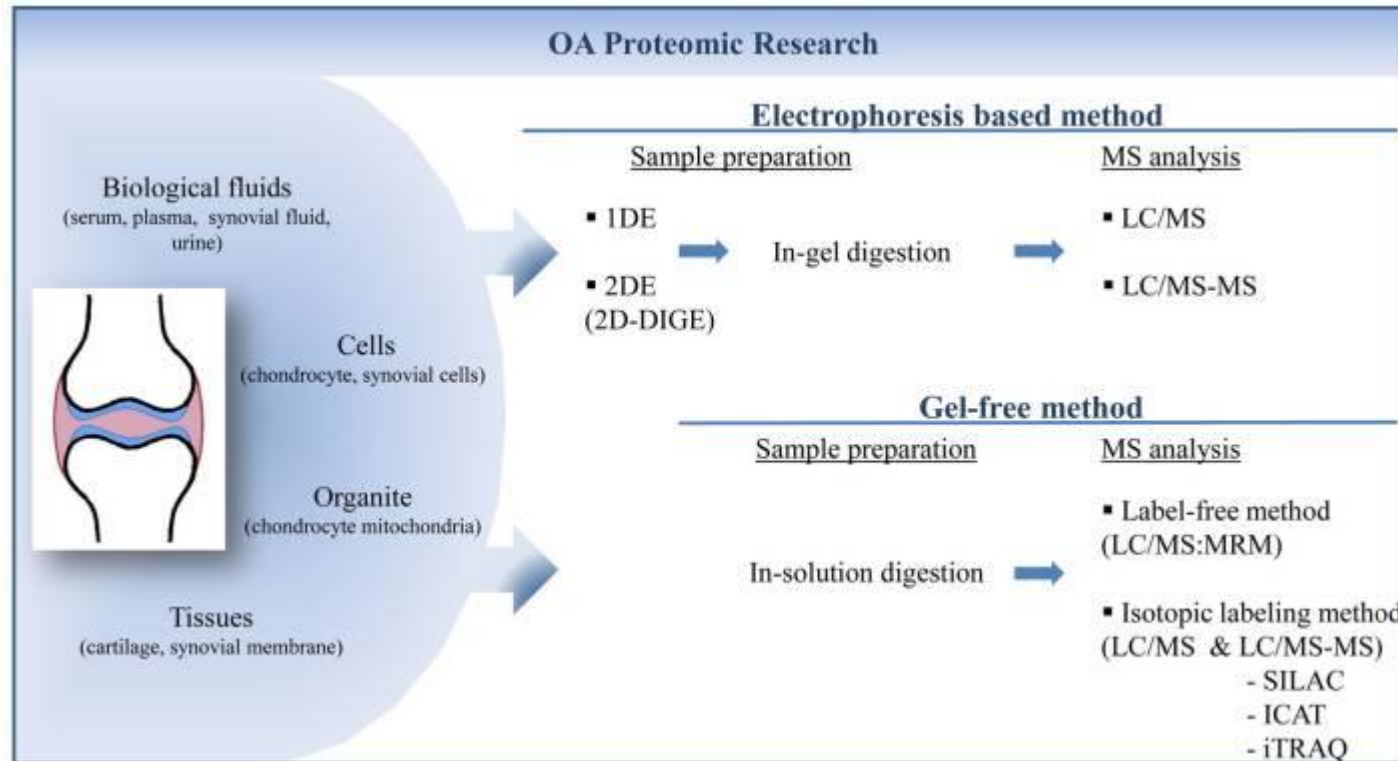
**OMICs
APPROACHS
in OA**

LIPIDOMIC



Quantitative proteomics : The state of the art

Overview of the different workflows



Gharbi M., Deberg M., Henrotin Y., Application for Proteomic Techniques in Studying Osteoarthritis: A Review, *Front Physiol.* 2011; 2: 90

Biomarker Discovery:

Differential proteomics analysis pipeline

Discovery

Verification /
Validation

High throughput
Validation

Number of sample for analysis

Number of biomarkers

- | | | |
|--|---|--|
| <ul style="list-style-type: none">• Small number of samples (3 to 10)• Looking at ± 1000 proteins at once without any <i>a priori</i>• Complex, expensive and time consuming analysis | <ul style="list-style-type: none">• Average number of samples from 10 to 100• Alternative methods• Looking at specific proteins (10-100)• Targeted quite complex and expensive methods | <ul style="list-style-type: none">• Average number of samples greater than 100• Immuno-based high throughput tests• Looking at specific proteins (<100)• Targeted and multiplex (low cost screening tests) |
|--|---|--|

Exemple of proteomic application in new OA biomarker research:
Identification of Fibulin-3 peptides as biomarkers of OA

Fibulin 3 peptides Fib3-1 and Fib3-2 are potential biomarkers of osteoarthritis

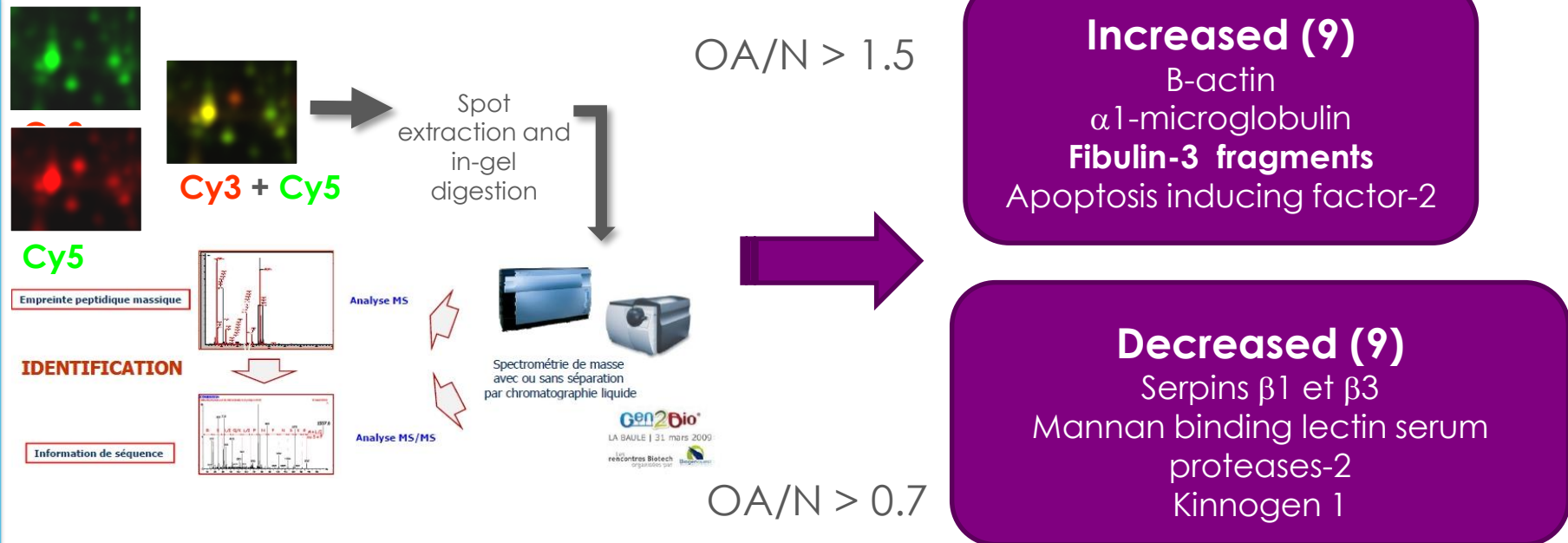
Henrotin Y et al *Arthritis Rheum* 2012, **64**(7):2260-2267

- Proteomic analysis of human urines by 2D-DIGE
- Identification of proteins or fragments of interest by mass spectrometry
- Production of specific antiserum directed against identified peptides
- Development of specific immunoassay of peptides
- Validation of peptide variation in OA population

Proteomic analysis: classical workflow of protein identification

Urinary proteome

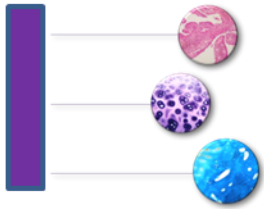
Henrotin et al. Arthritis Rheum 2012



FBLN3_HUMAN Mass: 54604 Score: 130 Queries matched: 2

EGF-containing fibulin-like extracellular matrix protein 1 precursor (Fibulin-3) (FIBL-3)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
149	685.33	1368.64	1368.54	0.09	0	58	0.00027	1	R.CVCPVSNAMCR.E
444	965.32	1928.63	1928.73	-0.10	0	72	4.2e-06	1	R.TCQDINECETTNECR.E



Fibulin-3: relation with cartilage

- Extracellular matrix protein highly expressed in cartilaginous tissue (Timpl, et al. 2003; Kobayashi, et al. 2007)
- Expressed in the mesenchyme giving rise to cartilage and bone, and plays a role in organizing the development of skeletal system (Ehlermann et al., 2003)
- Identified in OA cartilage by proteomic analysis (Vincourt et al., 2006)
- Intimately associated with (TIMP)-3, an inhibitor of metalloproteinases involved in the pathogenesis of OA (Klenotic et al., 2004 ; Kevorkian et al., 2004 ; Sahebjam et al., 2007)
- Negative regulator of chondrocytes differentiation (Wakabayashi, et al. 2010)

These bibliographic data suggest an implication of Fibulin-3 in the physiopathology of OA

Fibulin-3 fragments (Fib3-1 and Fib3-2): potential diagnostic biomarkers

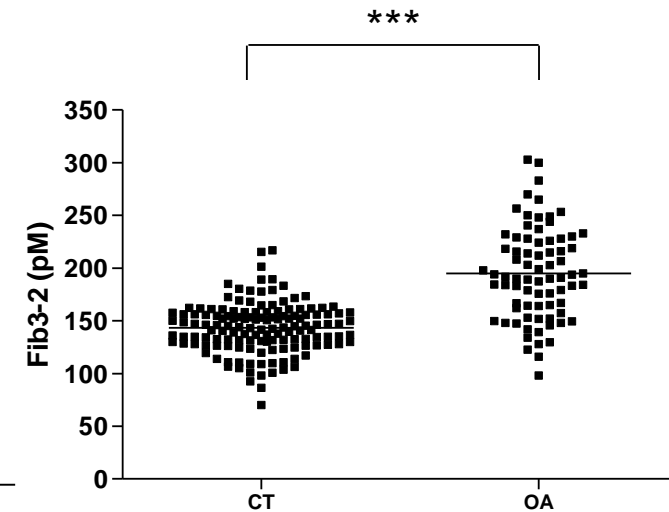
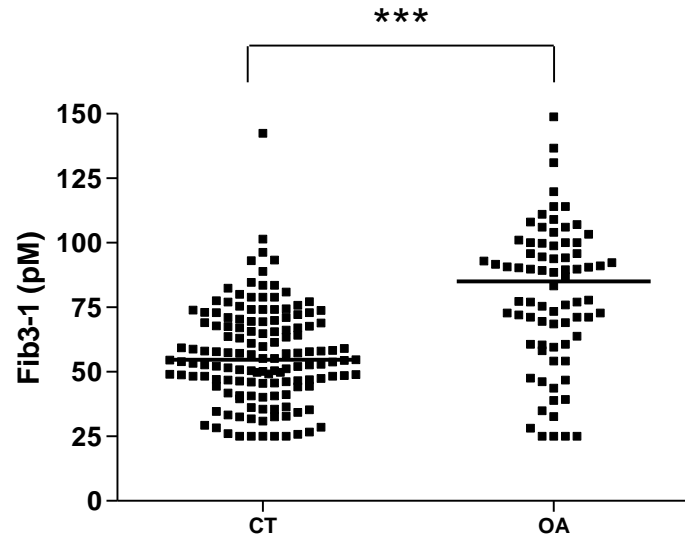
Fib3-1: **TCQDINECETTNECR**

Fib3-2: **CVCPVSNAMCR**

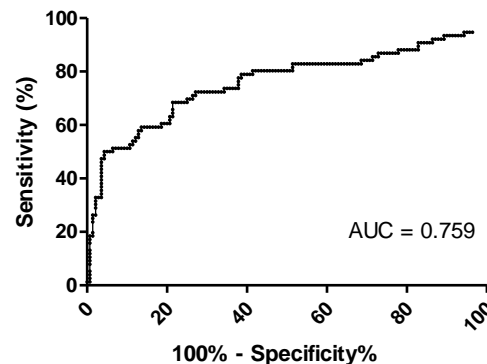
Immunization of rabbits

Antiserum production

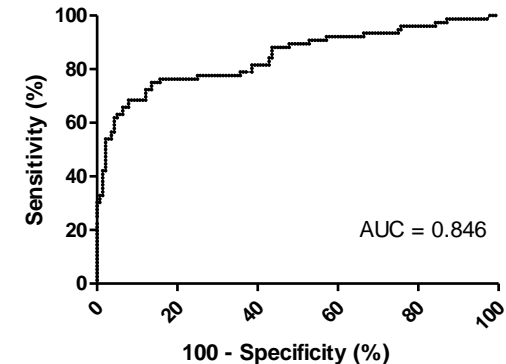
Specific immunoassays of Fib3-1 and Fib3-2 development and validation in human serum



Fib3-1: ROC curve



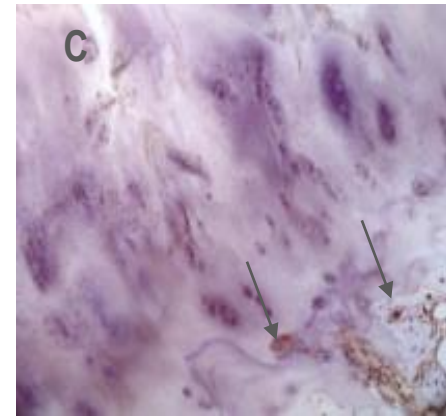
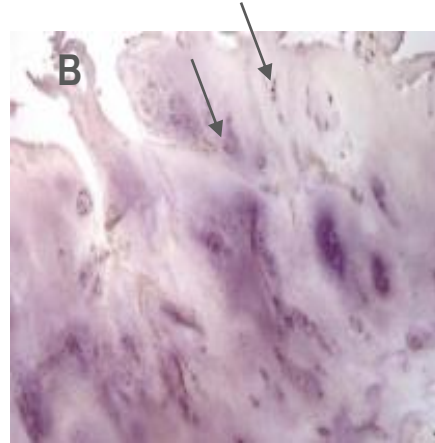
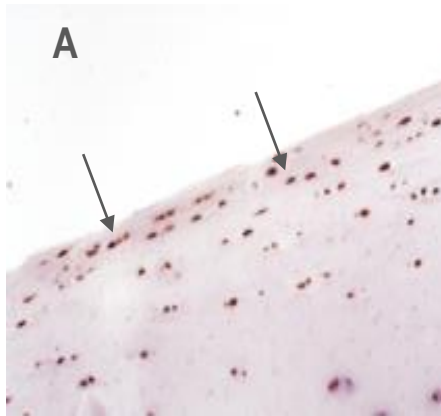
Fib 3-2: ROC curve



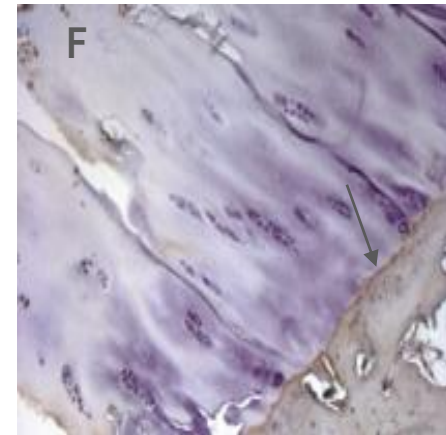
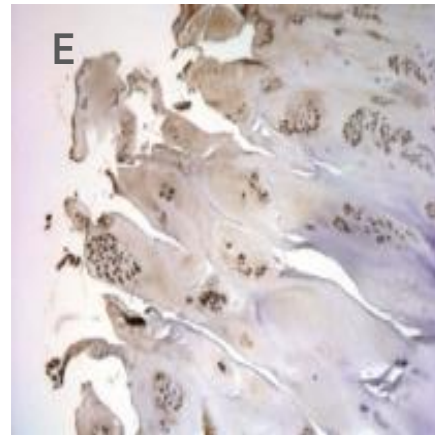
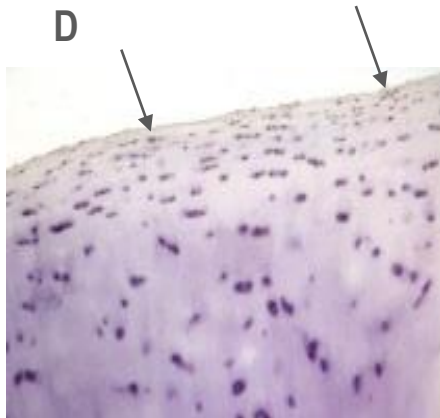
	Sensibility	Specificity
Fib3-1 (cut-off: 71.1 pM)	78.5%	68.4%
Fib3-2 (cut-off: 163.7 pM)	75.0%	86.4%

Immunolocalization of Fib3-1 and Fib3-2 (human)

Fib3-1



Fib3-2



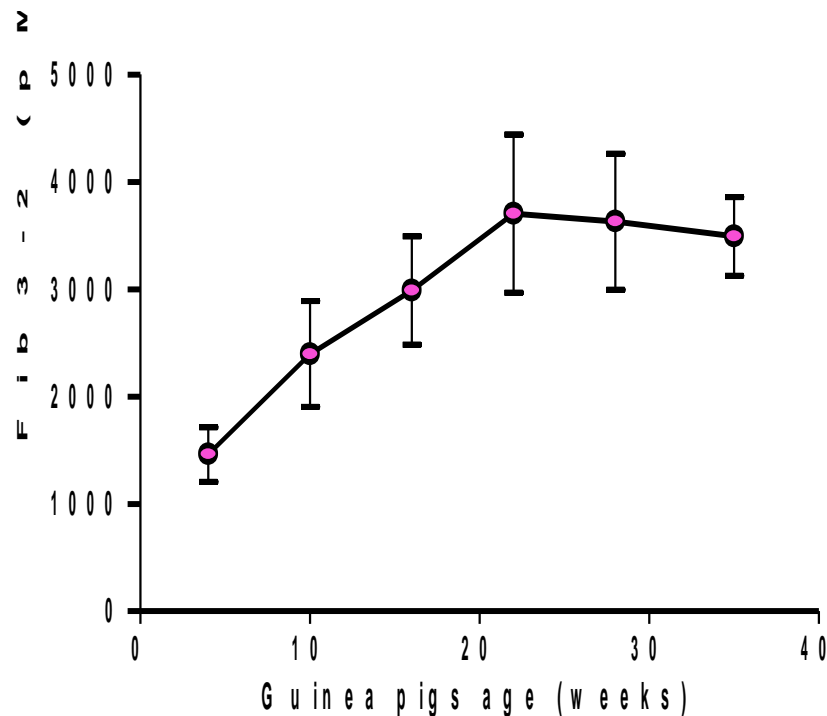
Healthy cartilage

OA cartilage
Superficial layers

OA cartilage
Bone junction

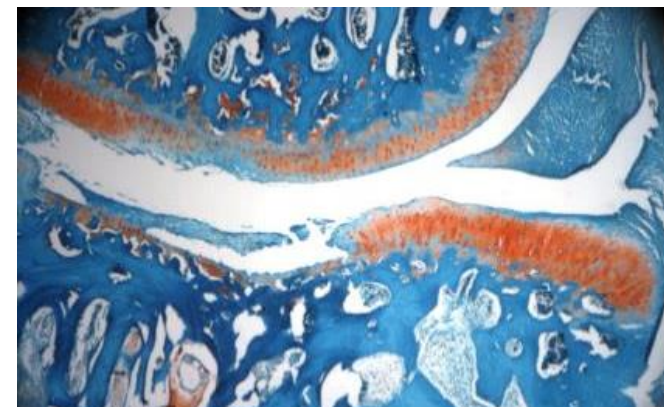
Guinea pig as spontaneous model of OA

Fib3-2 kinetic in sera and correlation with histology

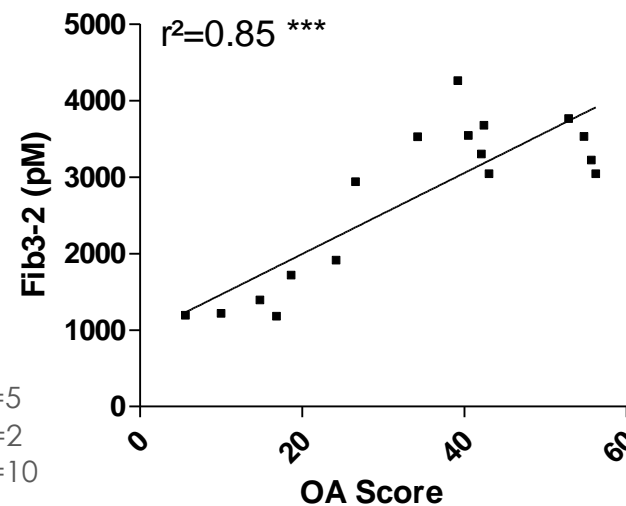


Control group
W4 n=5
W16 n=2
W35 n=10

Recommended OARSI histological OA score for guinea pig, parametric ANOVA /Dunnett's post test



35-week old guinea pig, right knee, medial



Proteomic paradox

Recovery of unknown proteins in chondrocytes - cartilage matrix

AND

Some known proteins are not found!

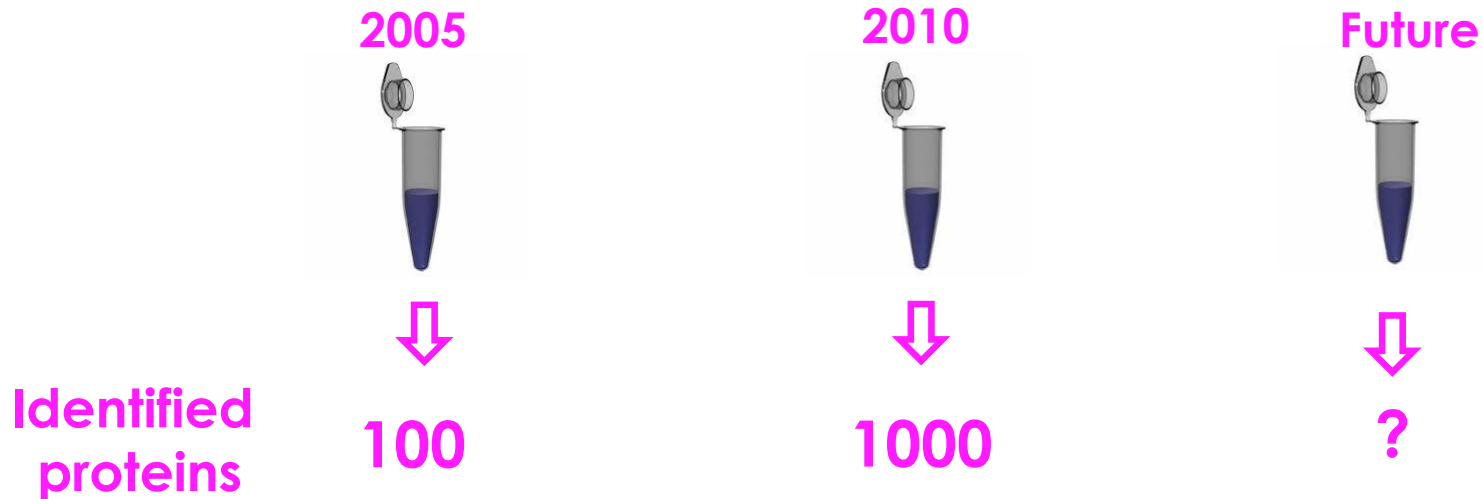
Example :

Rosenthal et al (2011) investigated protein pattern present in articular cartilage vesicles (AVCs) and didn't find TNALP or PC1, well known enzymes located in their membrane

⇒ **Due to their structure/composition/localisation,
Lot of proteins are undetectable with current technics**

Quantitative proteomics : a discipline in constant progress

- ✓ Sample preparation method / Specific depletions
- ✓ Protein labeling – label free methods
- ✓ Improvement in protein separation
- ✓ Protein fractionation method
- ✓ Sensibility of detection
- ✓ Evolution of databases



Conclusions

- New OMICs technologies **flood** us with new potential biomarkers
- **Validation** and **qualification** of one potential biomarker is a **very long and expensive process**
- It's essential to **start** OMICs studies with the **good samples** – representative – homogen...to gives us important informations - maybe on subgroups of the disease



← **bcrU** Team

Bone and Cartilage Research Unit



Thank you !

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