Nano-Bio-Sensing Summer School
EPFL, Lausanne, Switzerland, June 29th – July 3rd, 2009

2009

LOCCANDIA

Lab-on-chip for cancer diagnosis

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DTBS/STD/09-135

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Funded by EC contract FP6-034202

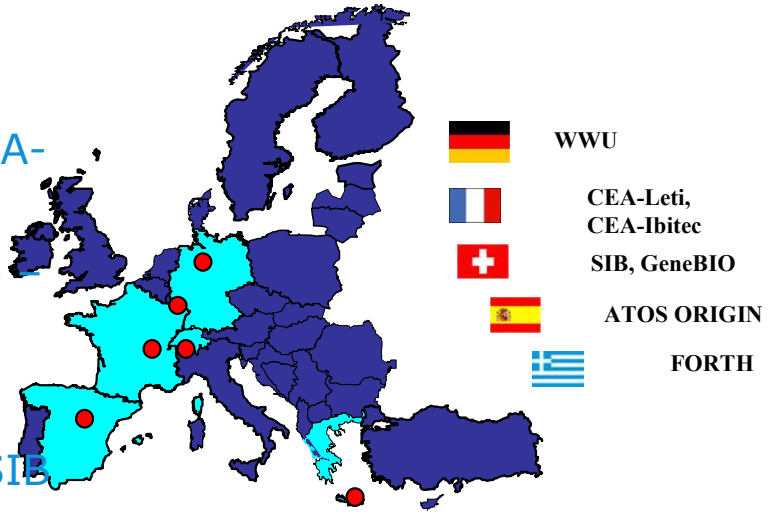
LOCCANDIA Project

FULL TITLE: Lab-On-Chip based protein profiling for CANcer DIAgnosis

Project granted by the European Commission:
Reference: FP6/2005/IST/5/034202
Call: Priority 2.5.2. Micro/nano based sub-system
Web site: http://www.loccandia.eu

List of partners:

- Atos Origin sae, Spain – ATOS
- Commissariat à l'Energie Atomique, France – CEA-LETI, CEA-IBITEC
- Foundation for Research and Technology, Greece – FORTH
- University of Münster, Germany – WWU
- Swiss Institute of Bioinformatics, Switzerland – SIB
- Geneva Bioinformatics, Switzerland - GeneBIO



Reference: Jordan B. et al. (2008), " LOCCANDIA: Lab-on-Chip Based Protein Profiling for Cancer Diagnosis ", 5th pHealth Workshop on wearable micro and nano systems for personalised health "from research into practice", Valencia, Spain.

Funded by EC contract FP6-034202

LOCCANDIA Project teams

- **ATOS:** Blanca Jordán, José F. Esteban, Manuel Perez
- **CEA-LETI:** Pierre Grangeat, Laurent Gerfault, Caroline Paulus, Grégory Strubel, Florence Ricoul, Myriam Cubizolles, Emeline Mery, Lucie Baujard Lamotte, Caroline Fontelaye, Guillaume Nonglaton, Françoise Vinet, Nicolas Sarrut, Olivier Constantin
- **CEA-LEMM:** François FENAILLE, Cédric MESMIN, Eric EZAN
- **FORTH:** Dimitris Kafetzopoulus, Manolis Tsiknakis, Sophie Kaforou, Hara Roumpaki, George Potamias, Haris Kondylakis, Manolis Kalaitz, Vangelis Kritsotakis
- **WWU:** Jürgen Schnekenburger, Verena Schick, Jasna Peter-Katalinic, Laura Bindila, Daniela Hahn, Rainer Ossig
- **SIB:** Frédérique Lisacek
- **GeneBIO:** Pierre-Alain Binz

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CAPSI Project

- Full title:
  - chaîne d'analyse protéomique multifactorielle haute sensibilité à composants intégrés
  - high sensitivity multifactorial proteomic analytical chain based on integrated components
- Project granted by the Commissariat à l'Energie Atomique (CEA)within the program "Technology for healthcare"
- CAPSI project teams:
  - CEA-LETI: Pierre Grangeat, Laurent Gerfault, Caroline Paulus, Grégory Strubel, Florence Ricoul, Myriam Cubizolles, Emeline Mery, Lucie Baujard Lamotte, Agnès Fonverne, Guillaume Nonglaton, Caroline Fontelaye, Françoise Vinet, Nicolas Sarrut, Olivier Constantin
  - CEA-iRTSV-EDyP: Jérôme Garin, Alain Dupuis, Virginie Brun, Dorothée Lebert, Mathieu Trauchessec, Annie Adrait, Christophe Bruley, Véronique Dupierris

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The Digital Patient

- The **Digital Patient** for **eHealth :**

- The combination of complementary **multimodal representations**

- The **bio-profiles** = a multiparametric description
  - morphological profiles
  - molecular profiles
  - physiological profiles

- **Imaging systems** = localisation of the information

- **Healthcare objectives** = early diagnosis, personnalized healthcare, home healthcare monitoring

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The molecular profiles

- a disease is characterized by a **molecular signature** (cancer, infectious disease, immunological disease,…)
- **multiparametric approach :** a set of genes, proteins, therapeutical molecules, functional relationships

→ Need to recognize **signatures** on **molecular profiles**

- **molecular profiles :** a fundamental information for personnalized healthcare, risk factor analysis, early detection, therapeutical planning and follow-up, drug development, system biology.

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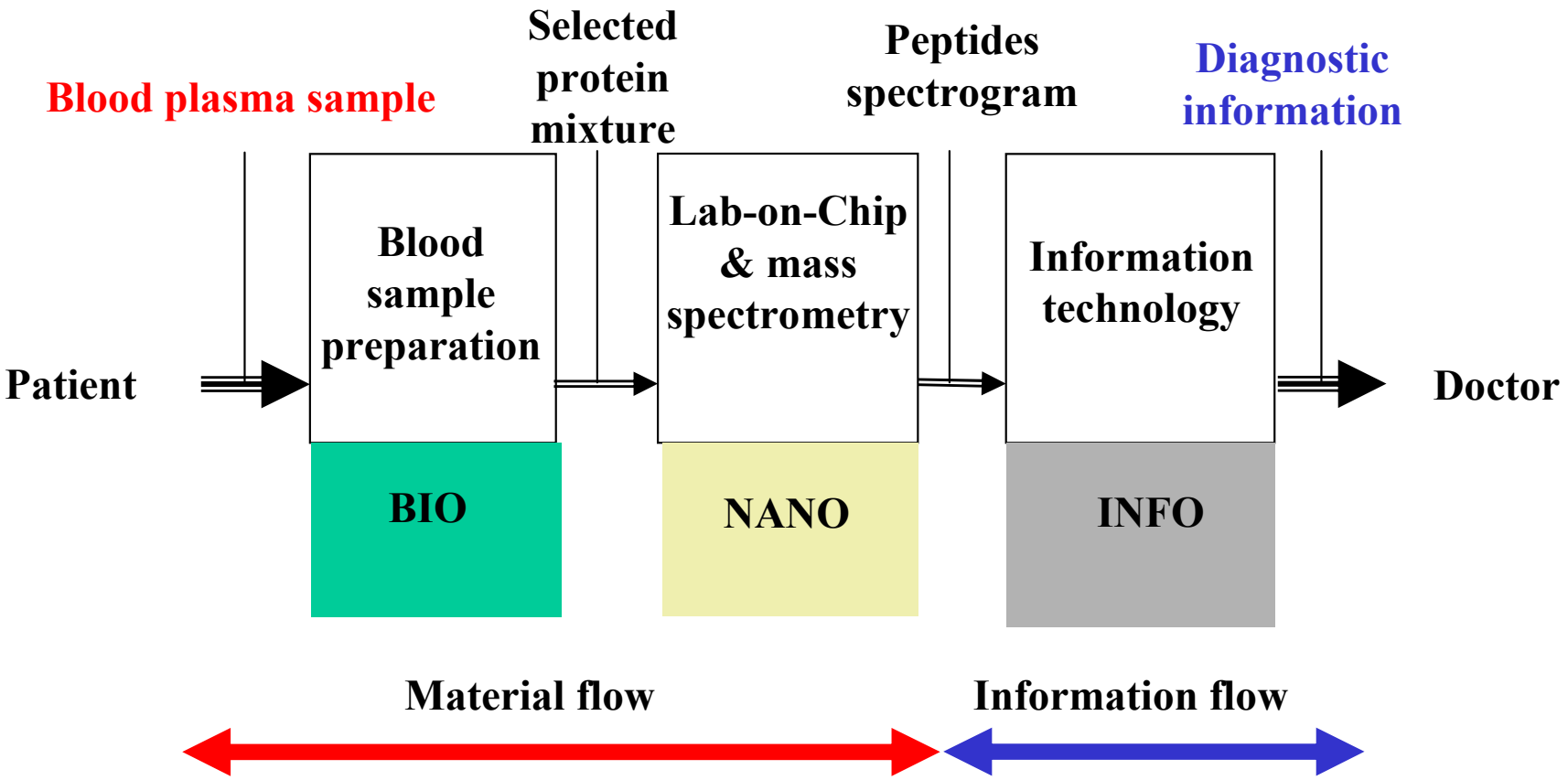
The Nano-Bio-Info-Cogno (NBIC) Convergence



The chain for high sensitivity lab-on-chip based proteomic analysis

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Lab-on-chip for in-vitro molecular diagnosis

- General trend towards micro-nano bio systems (MNBS) for in vitro diagnosis
- Microfluidics is broadly recognized as a key solution to support molecular diagnostic and point-of-care devices.
- Nanotechnologies for:
  - nanoparticles for in-vitro molecular interaction
  - surface functionalization
  - fluid handling
  - transduction
- Key requirement: to perform better, faster, cheaper and smaller analysis, but still reproducible, sensitive and reliable

Funded by EC contract FP6-034202

Conference highlight

- **Clinical proteomics**
- Micro-nano technologies for microfluidic analysis coupled with mass spectrometry
- Information processing
- Conclusion

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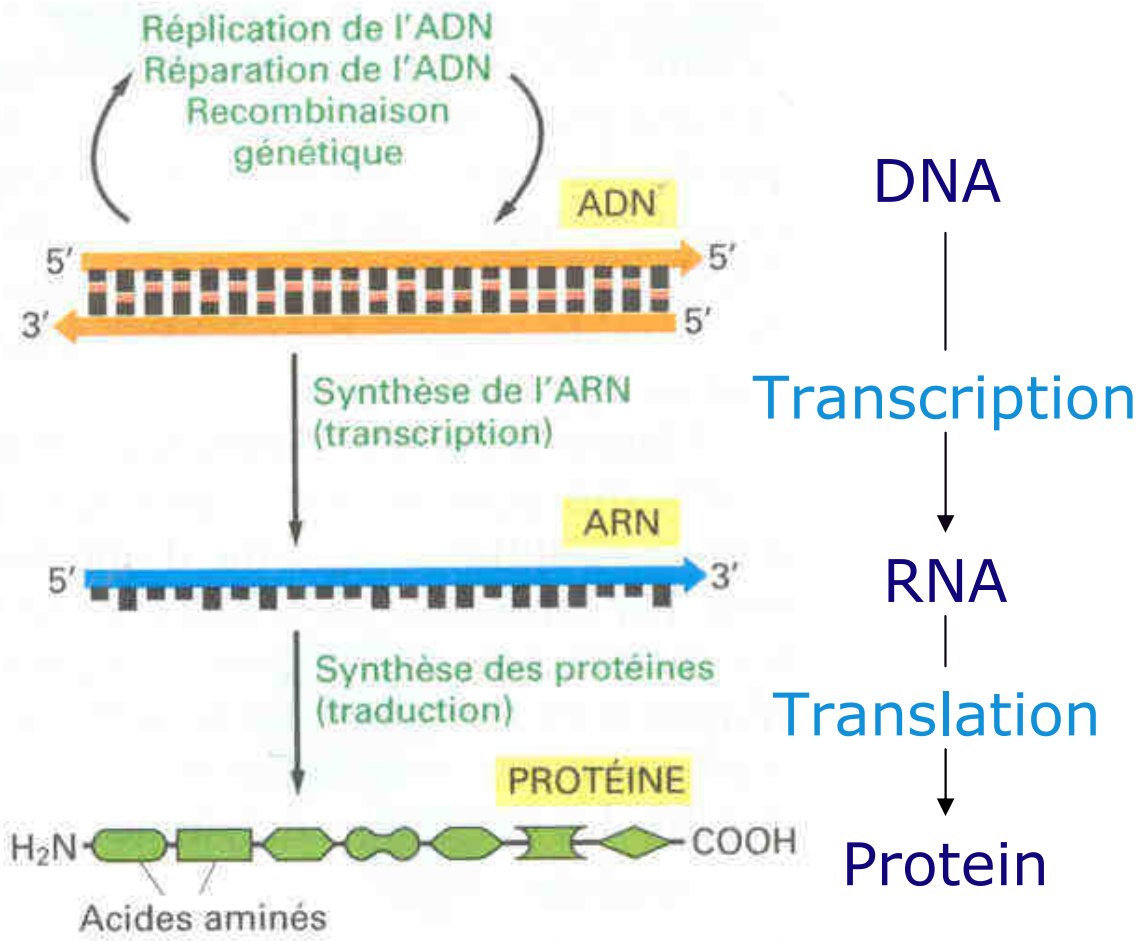
Metabolic pathway from DNA to protein



Intracellular genetic information flow from DNA to RNA (transcription) and from RNA to protein (translation)

Source: Alberts, Johnson et al.(2004), Biologie moléculaire de la cellule, Médecines-Sciences, Flammarion

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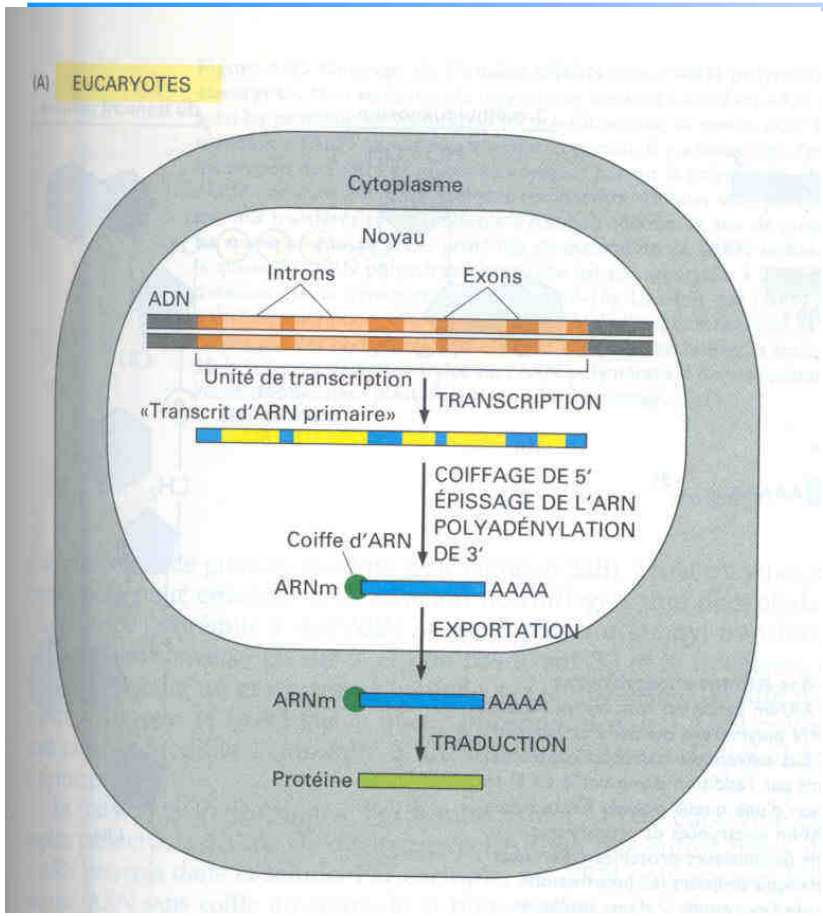
RNA splicing and isoforms



Splicing between primary RNA and messager RNA

Ref: Alberts, Johnson et al, Biologie moléculaire de la cellule, Médecines Sciences Flammarion

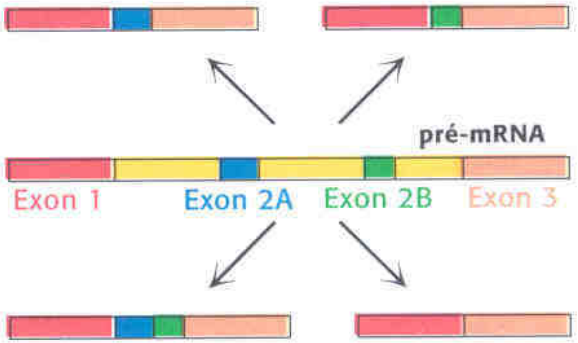


FIGURE 28.33 Schémas d'épissage alternatif. Un pré-mRNA possédant de nombreux exons est parfois épissé de façons différentes. Ici, avec deux exons alternatifs (exons 2A et 2B) présents, le mRNA peut être produit soit avec aucun de ces exons, soit avec l'un ou l'autre, soit avec les deux. Des schémas d'épissage alternatif plus complexes sont aussi possibles.

Alternative splicing produces different isoforms

Ref: Stryer, Berg, Tymoczko, Biochimie, Médecines Sciences Flammarion

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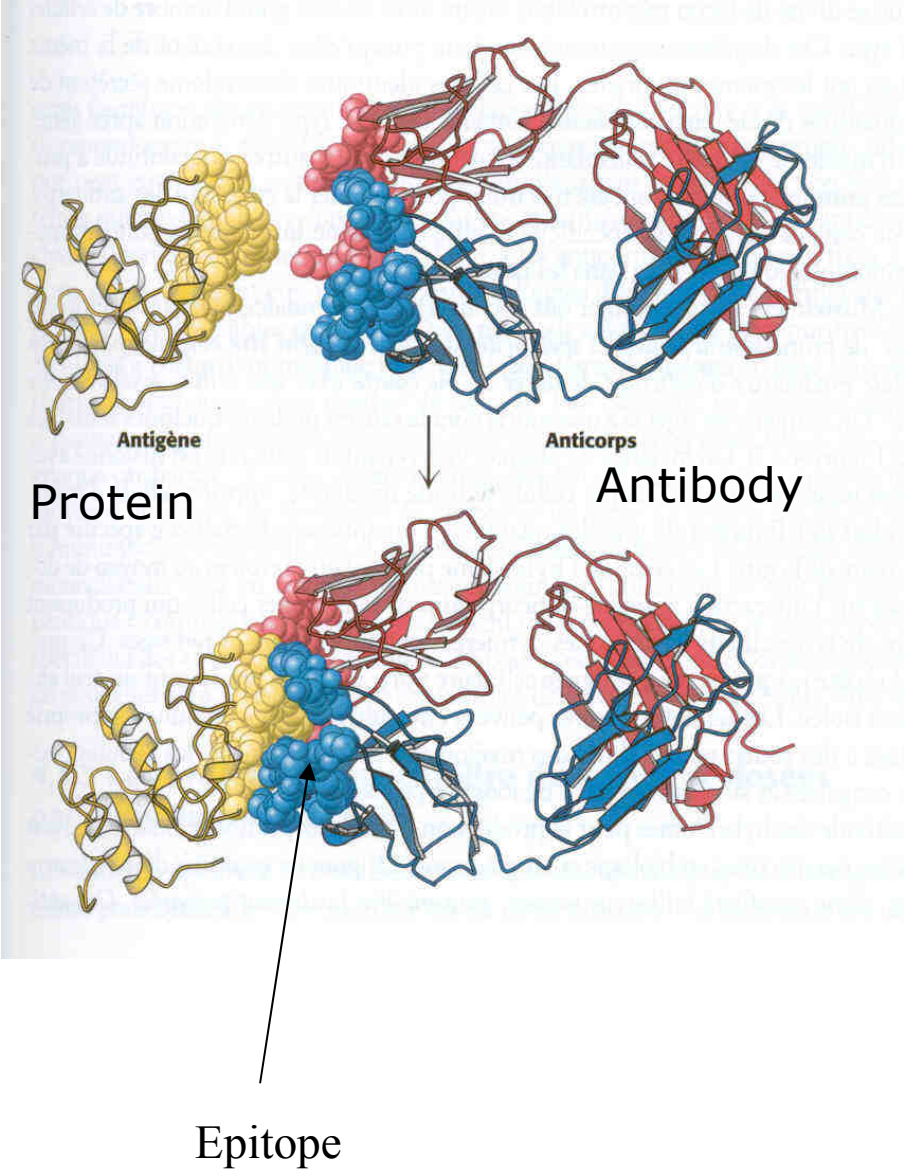
Epitope and antibody recognition



An antibody does recognize a specific group of amino-acids at the surface of the protein called **epitope**

Antibody recognition **cannot differentiate between isoforms** which include the same epitope

Ref. schéma: Stryer, Berg, Tymoczko, Biochimie, Médecines Sciences Flammarion

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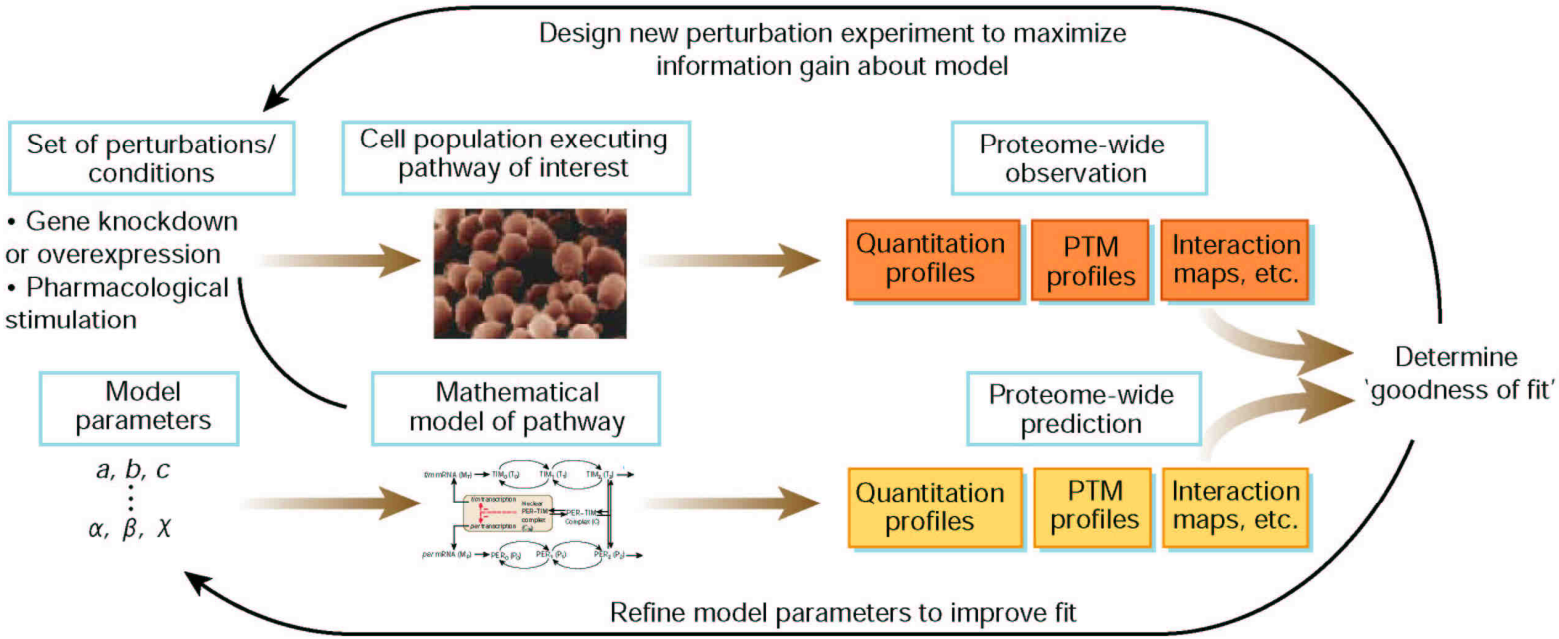
Systems biology

Proteins are key nodes in the biomolecular network studied by systems biology



Source: Aebersold R & Mann M. (2003), Nature, 422, p. 198-207

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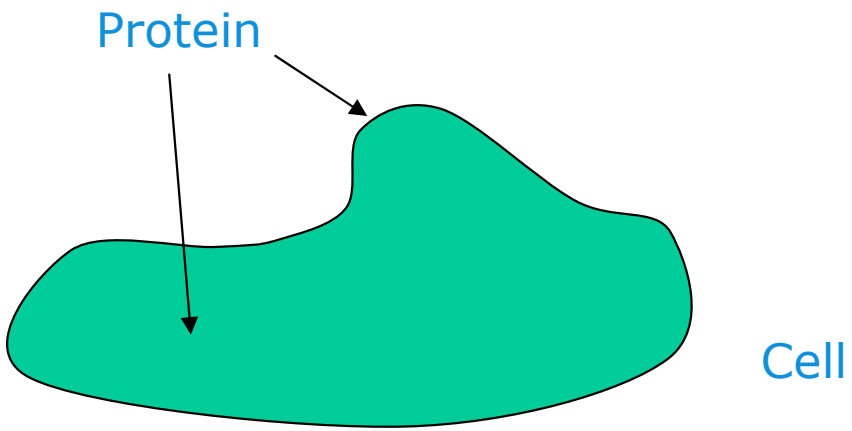
The 2 main diagnostic modes in proteomic

1. **First mode: The cell = a protein sensor**
   - The protein get attach to the membrane
   - The protein get inside the cell

- Diagnostic principle: a marker (a label) is attached to the protein and we image the cells which have captured the labelled proteins. The technique is defined by the type of marker which is used:
  - Nuclear imaging (radioactive marker)
  - Luminescent or fluorescent imaging (luminescent or fluorescent marker)
  - Nuclear Magnetic Resonance imaging (magnetic marker)



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The 2 main diagnostic modes in proteomic

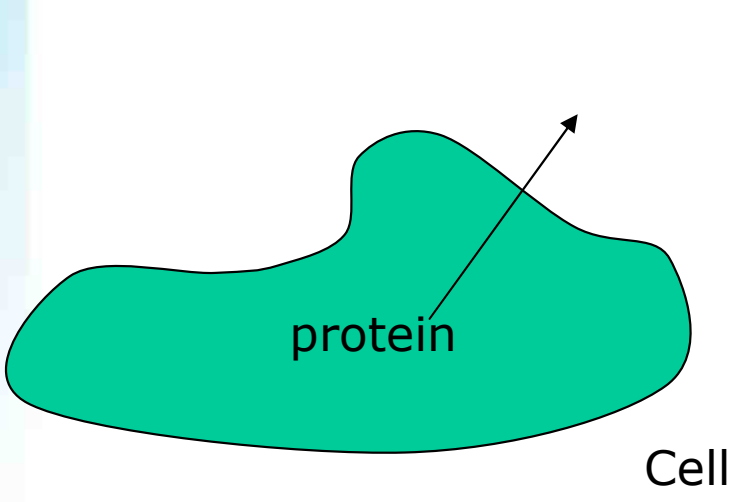
2. Second mode: The cell = a protein emitter

Diagnostic principle based on:

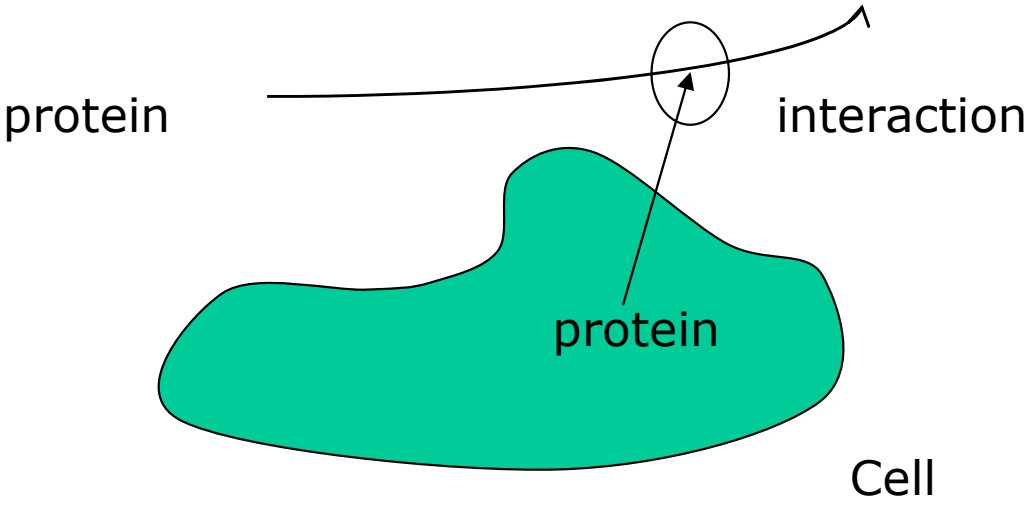
- the proteins
- The modification of protein structure
  - Translational modifications
  - Post-translational modifications

The information can be:

- direct: the target is the emitted protein
- indirect: the protein is cleaving a circulating protein that is the target



Direct measure



Indirect measure

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Proteomic, cancer, and molecular profiling

- **Proteomic:** to describe the molecular signals and the activation states in the cell and its nearby environment
- **Signal network perturbation** induces the fact that the cells do not control anymore their growth, leading to the development of tumoral lesions (unbalanced between survival proteins and apoptosis)
- Each cancer patient has his own pathologic molecular profile.
- **Multiparametric approach:** to combine several markers in order to improve the sensitivity and the specificity.
- Cancer mechanisms linked to proteins:
  - Tumor cells produce aberrant proteins
  - Tumor cells produce cleavage products which degrades proteins within the blood
  - Non-tumor cells respond to signals emitted remotely by tumoral cells

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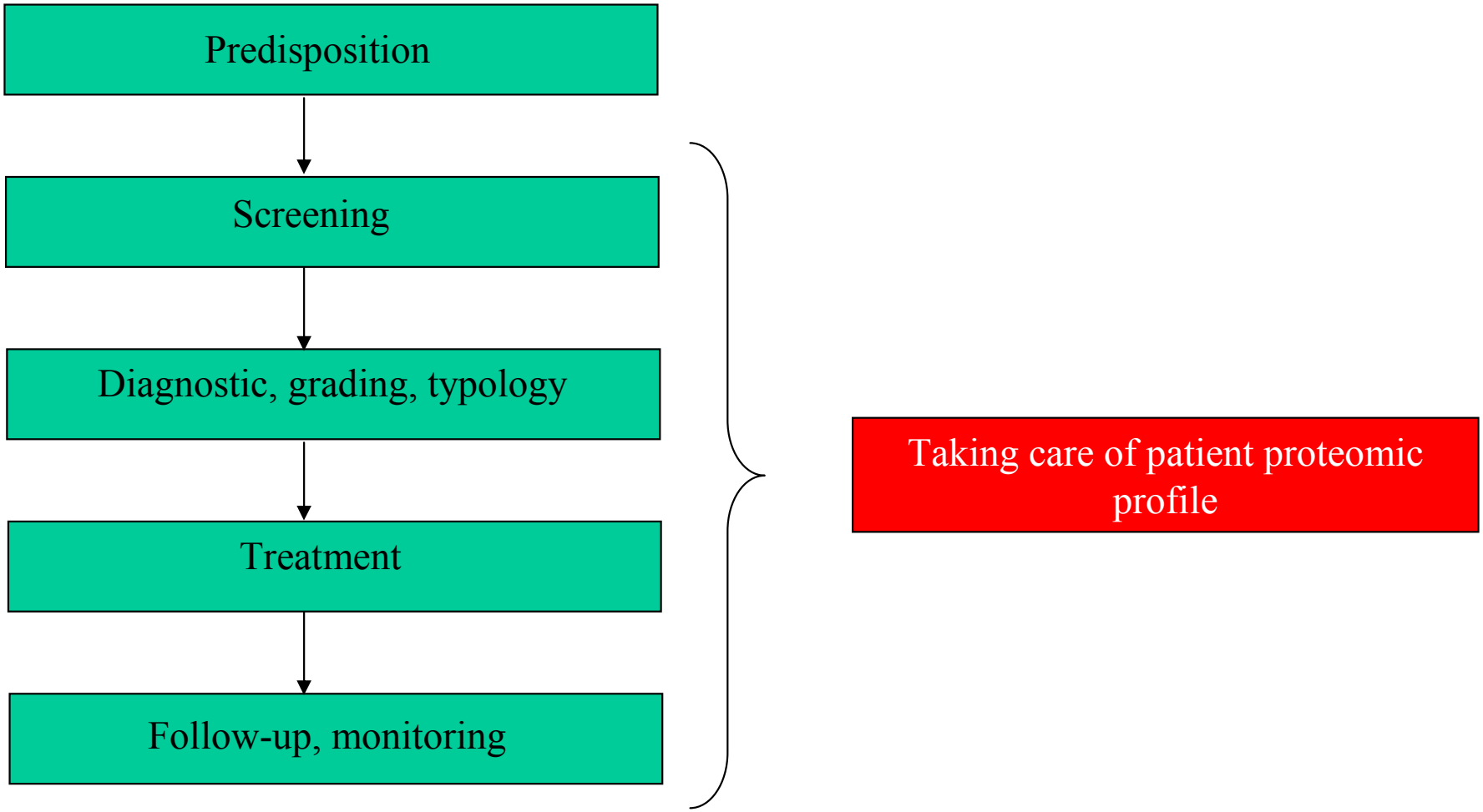
Care cycle of a disease like cancer



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The challenges of protein analysis in plasma

- High sensitivity
  - Our objective: 1 to 1000 ng/ml
- Very large dynamic ratio in protein concentration:
  - Total protein content in plasma: ~100 mg/ml
  - 1 ng/ml corresponds to a ratio of 1 to 10^8 between the targeted proteins and the total protein content
- A large protein content:
  - About 3000 proteins identified in the plasma by the HPPP

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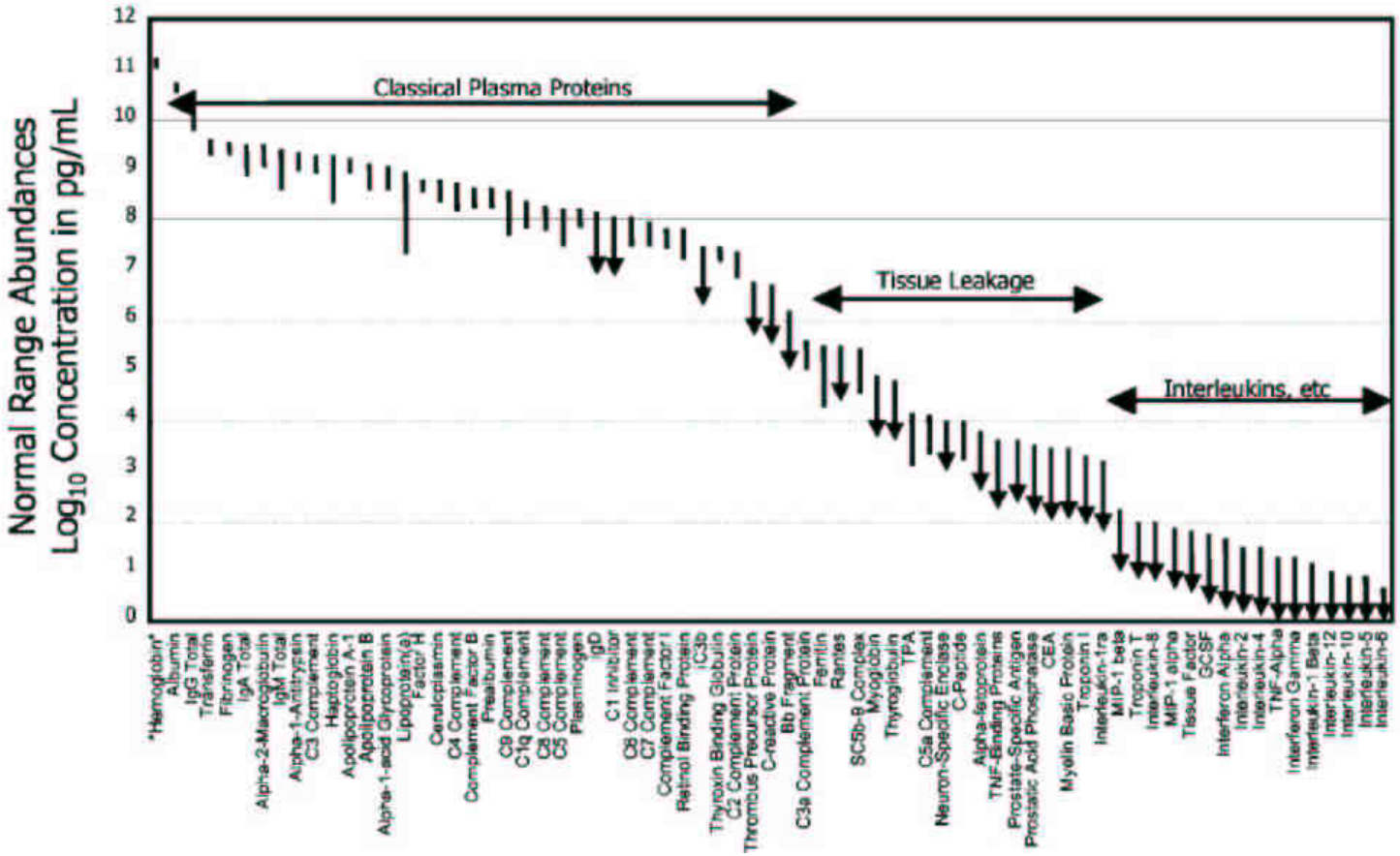
Dynamic range of protein concentration in plasma



Reference intervals for 70 protein analytes in plasma.

Abundance is plotted on a log scale spanning 12 orders of magnitude.

Source: Anderson N.L., Anderson N.G. (2003), Molecular & Cellular Proteomics, Vol 2.1, p. 50.

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The main proteomic approaches

- The immunological approaches
  - ELISA technique
  - Protein array
- The mass spectrometry approaches (MS)
  - Laser ionisation: SELDI, MALDI
  - Electrospray ionisation: ESI
- Our approach:
  - Combining:
    - immunological recognition
    - chromatography separation
    - electrospray ionisation
    - mass spectrometry separation and quantification

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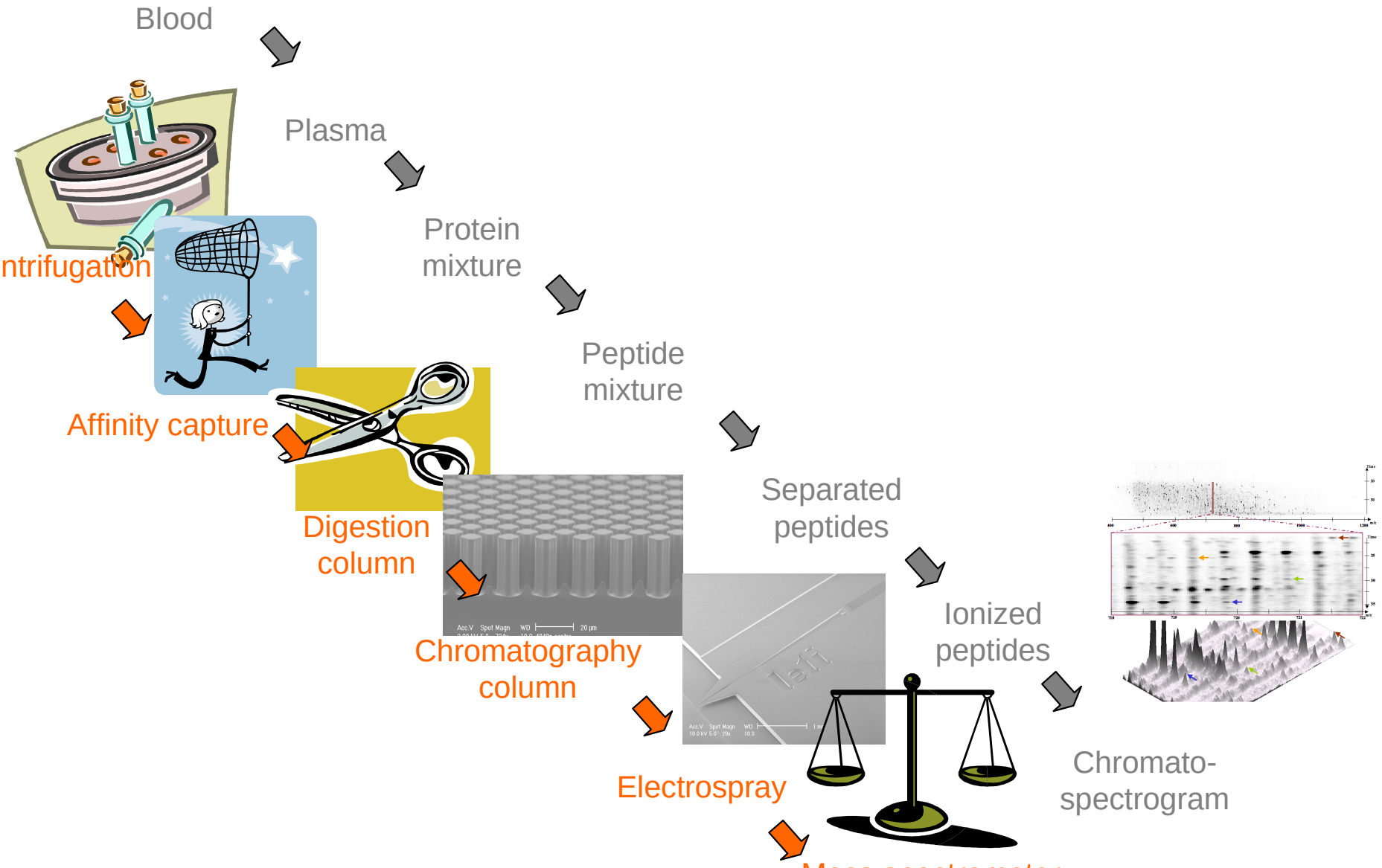
The immuno-chromato-ESI-MS analytical chain



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Conference highlight

- Clinical proteomics
- **Micro-nano technologies for microfluidic analysis coupled with mass spectrometry**
- Information processing
- Conclusion

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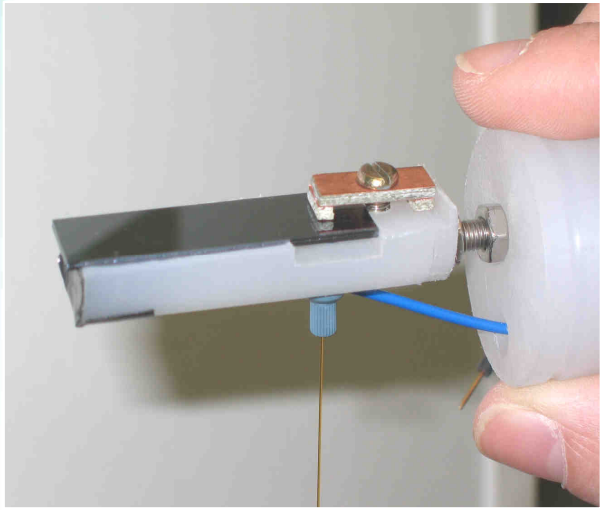
Micro-nano technology objective: lab-on-chip



- **Point of care :**

Full system from blood plasma sample to diagnostic information

- **Lab-on-Chip :**

Miniaturized integrated components to increase sensitivity (nano-LC, nano-ESI) and throughput

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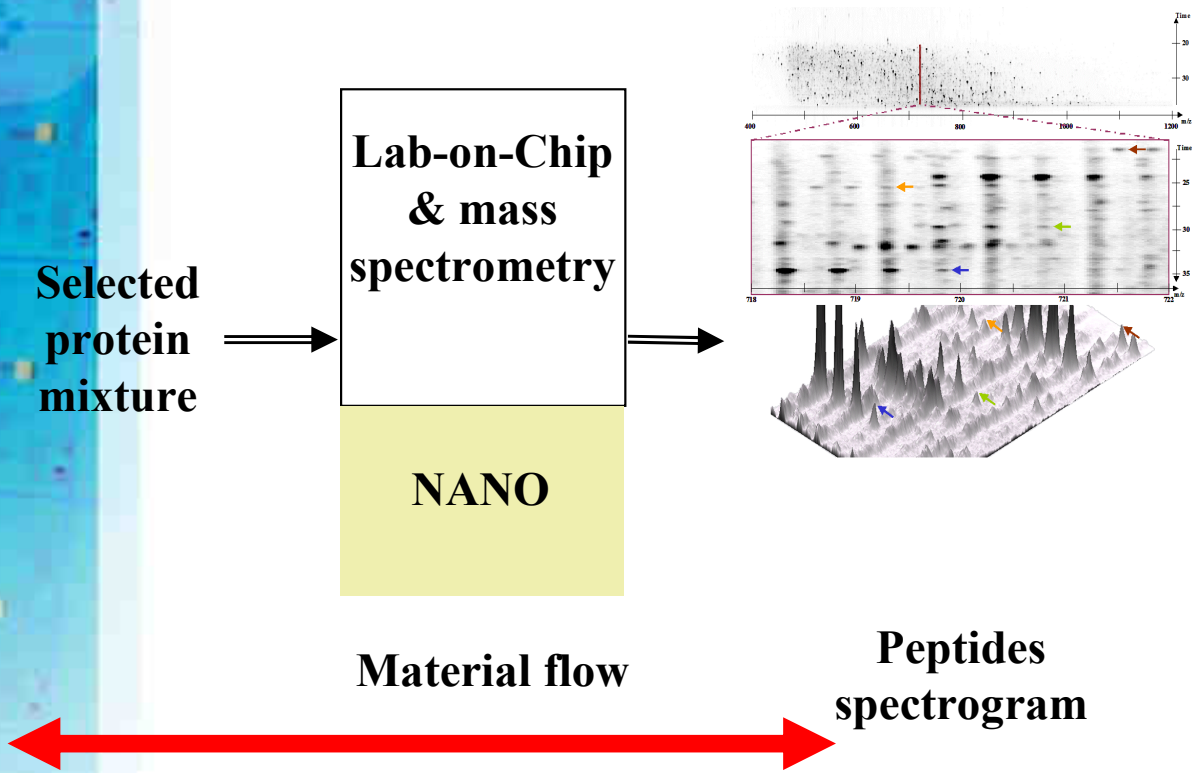
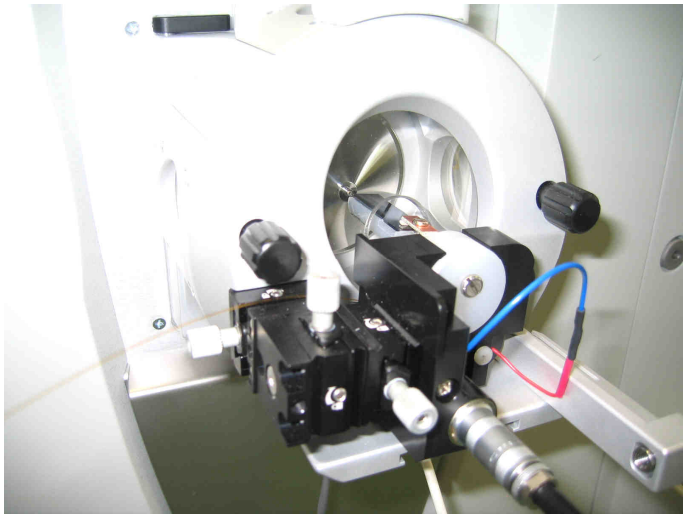
LOCCANDIA Project Goals: Lab-on-chip and mass spectrometry



- The objectives are:
  - to provide new batches of our current microsystems
  - to improve the chromatography-electrospray microsystem technology



- Two interconnected modules will be designed:
  - a protein digestion module
  - a liquid chromatography-electrospray ionisation module

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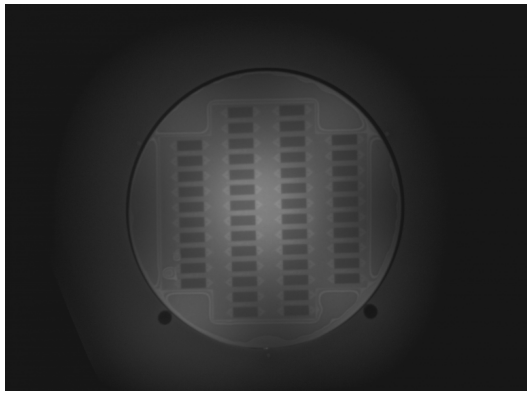
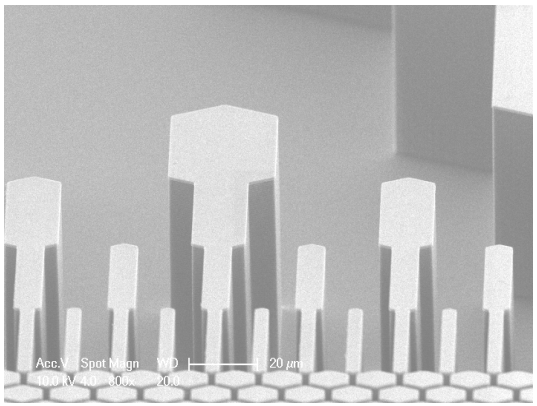
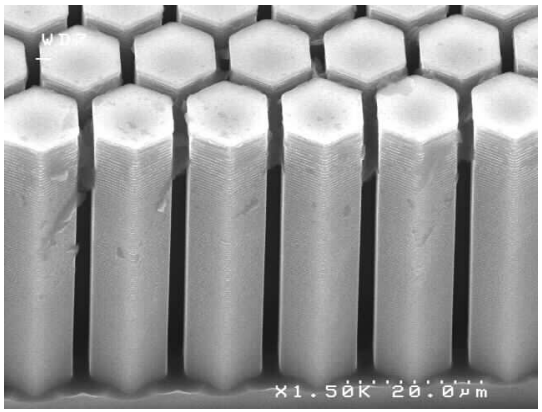
Digestion microreactor



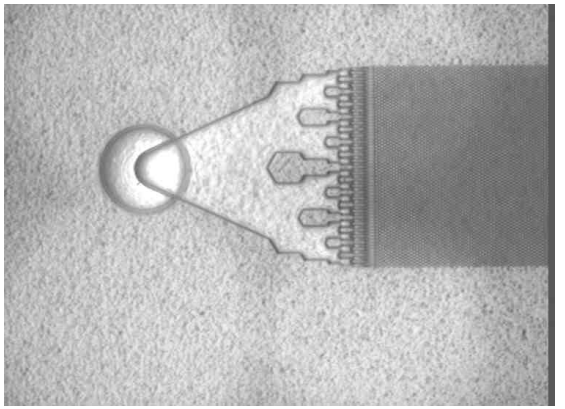
24 devices on a 200 mm silicon wafer



View of the input of the microreactor



SEM view of the hexagonal micropillars



Infrared microscope view of the microreactor

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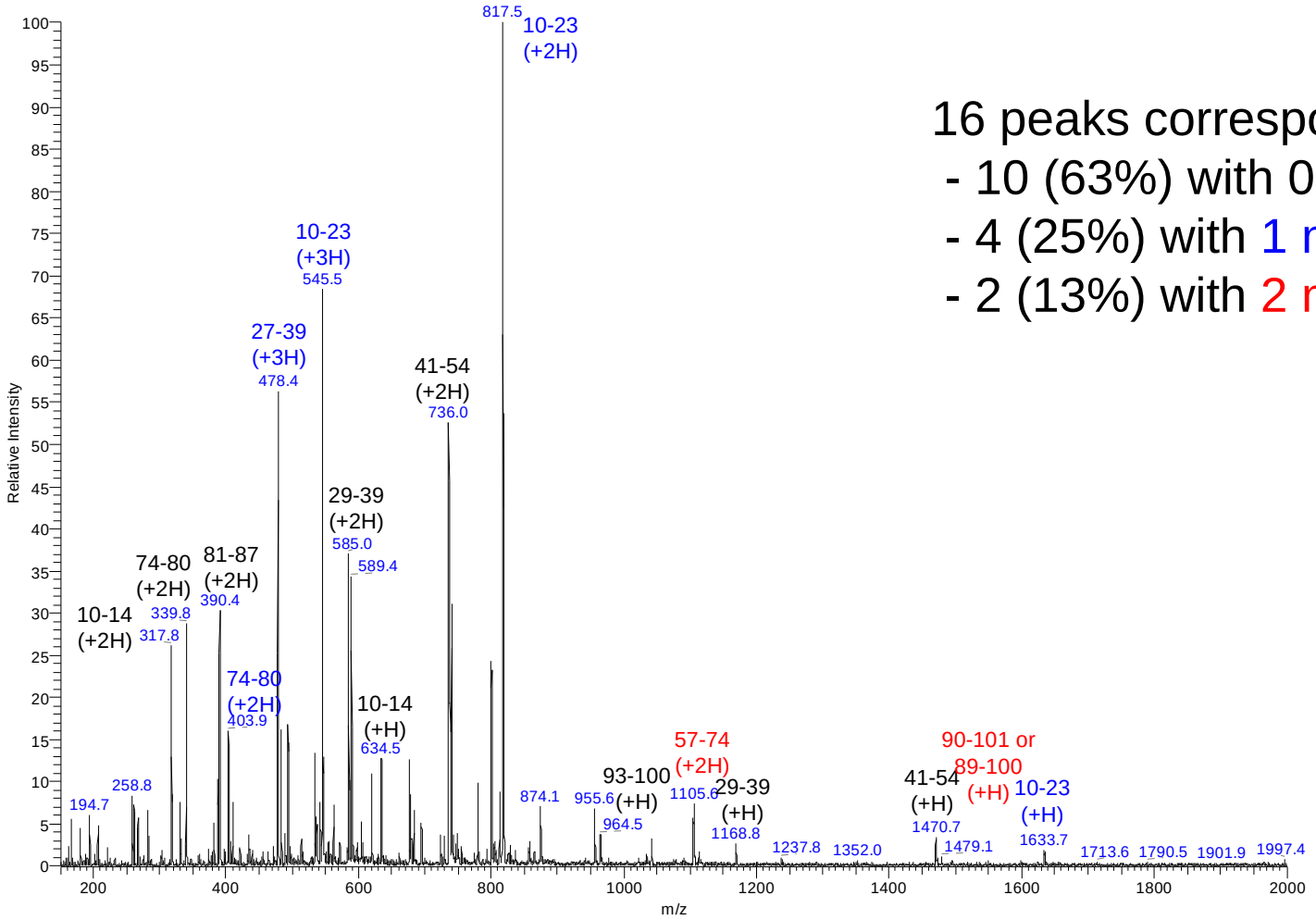
ESI-MS results: 10µl/min

Cyt C at 10pmol/µl digested at 10µl/min, pellet dissolved at 20pmol/µl in 95% H_2O, 5% ACN, 0.05% TFA – Theory: 13 peptides > 3aa



16 peaks corresponding to cyt C:
- 10 (63%) with 0 misscleavage
- 4 (25%) with 1 misscleavage
- 2 (13%) with 2 misscleavages

Cyt C on chip digestion is effective at 10µl/min

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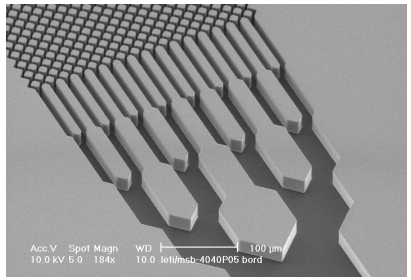
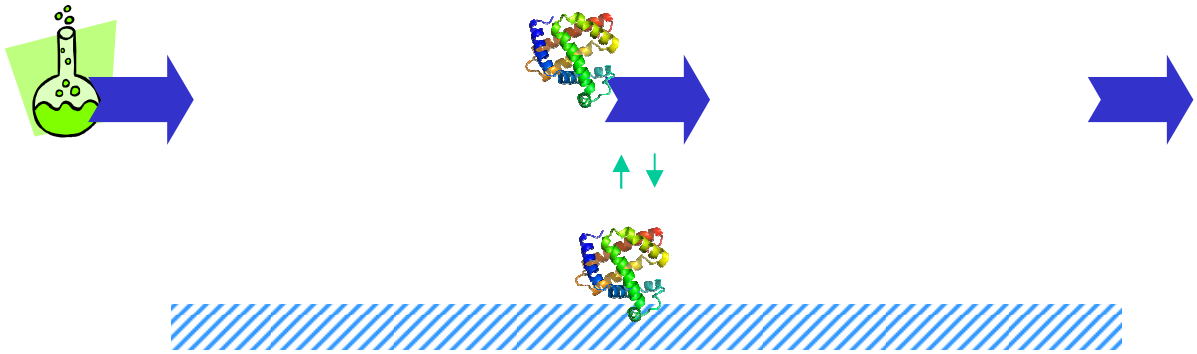
Chromatography column

Description of the chromatography column



Chromatography column



Physical effects

- Microfluidic
- Presence of 2 phases (mobile and stationary phase)
- Convection-diffusion
- Saturation effect
- adsorption
- Peptide competition
- Evolution of solvant composition

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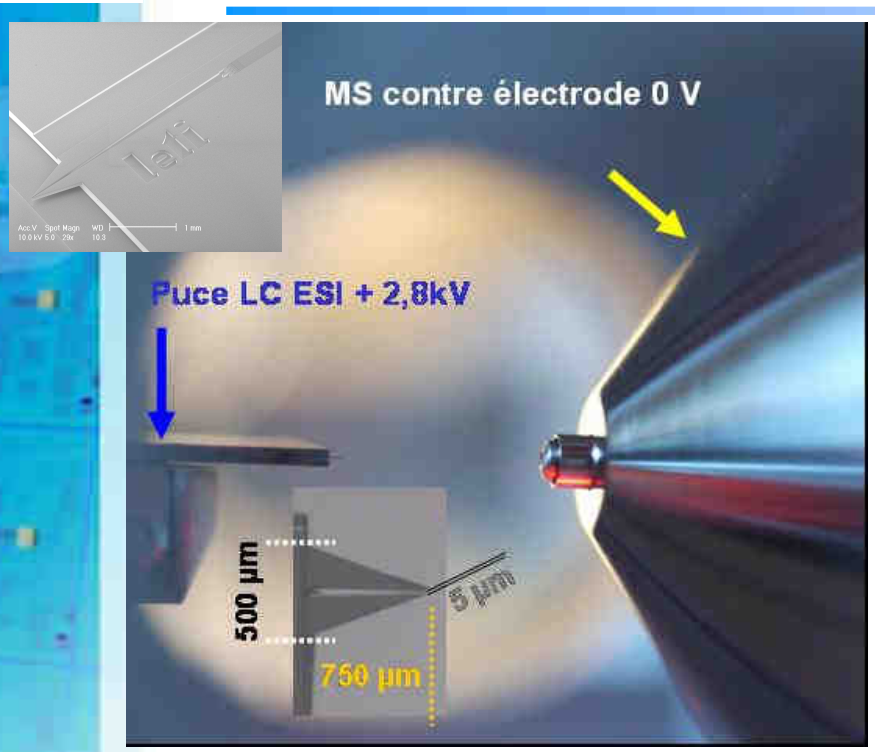
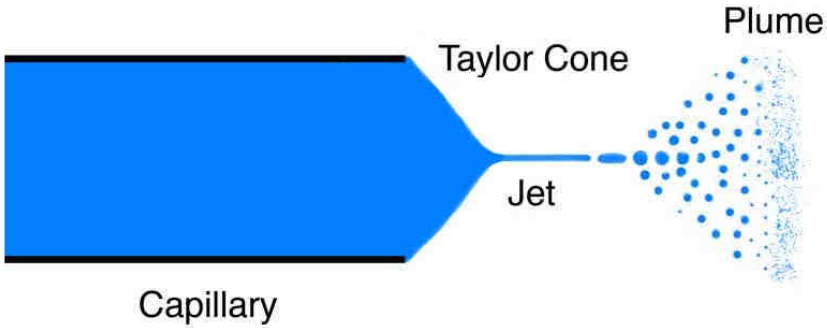
Electrospray



Electrospray in front of the mass spectrometer



Electrospray principle

Physical effects

Electro-hydro dynamic

Electro-chemistry

Peptide competition

Evolution of solvant composition

Funded by EC contract FP6-034202

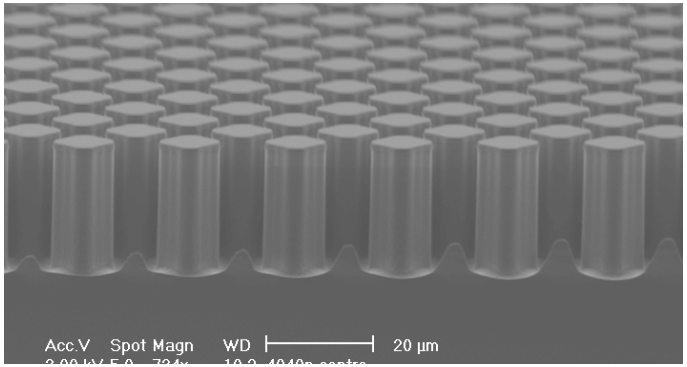
Why nano-liquid chromatography?

- In chromatography, the detection limit is proportional to the concentration of targeted molecules in elution peak
- The lower is the volume of the elution peak, the higher is the total amount detection limit in the elution peak for a concentration threshold fixed by the mass spectrometer
- Nano-chromatography refers to columns operating on nanoliter volumes
- Nano-chromatography columns allow to detect molecular quantity in the femtomolar range
- 1 femtomole of molecules (~10 pg for a molecule of 10 Kdalton) diluted in 1 mL of plasma correspond to a concentration of 1 picomole/L

Funded by EC contract FP6-034202

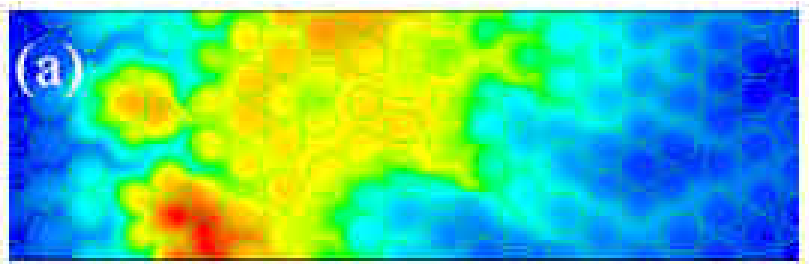
Why silicon technology ?

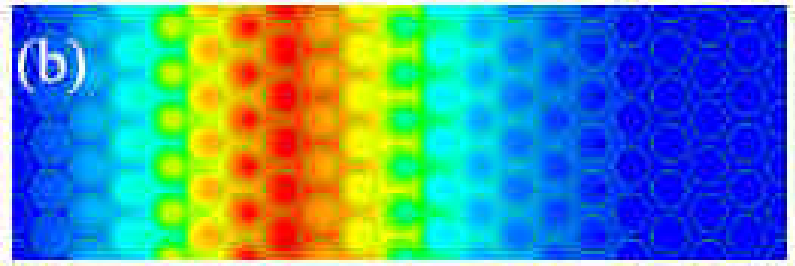
- Reproducible technology with high surface/volume ratio



Micro-pillar structure for large surface / volume ratio

- Optimized hydrodynamic flow *(Computational Fluid Dynamic study from G. Desmet et al, J. Chrom A, 2005)*





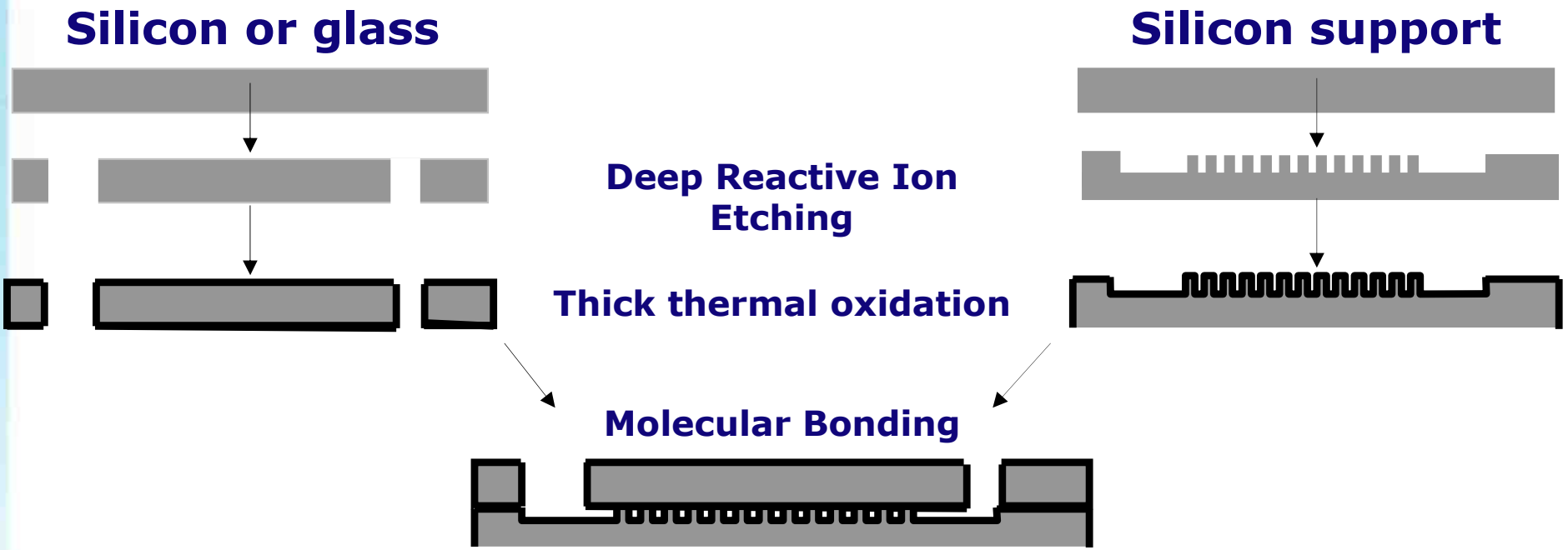
Hydrodynamic flow simulation in

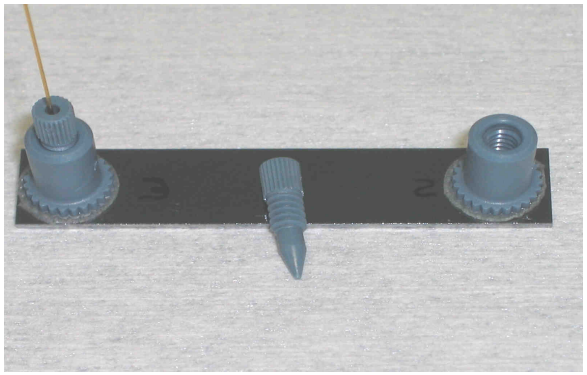
a) Capillary with bead packing

b) Perfectly ordered microstructures

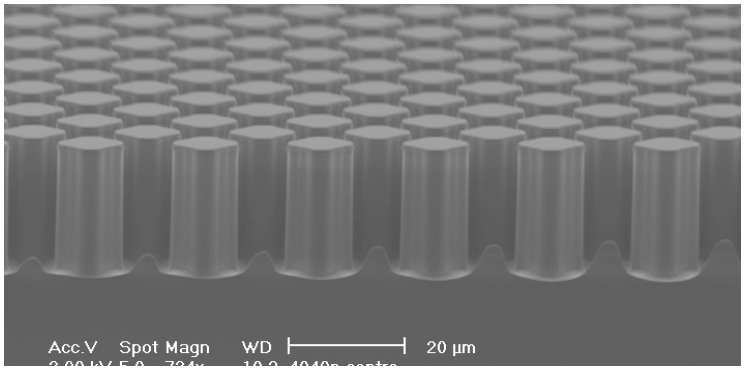
Funded by EC contract FP6-034202

Microchips fabrication: digestion chips and LC chips





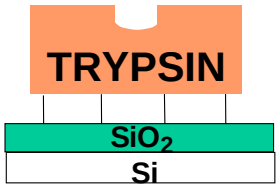
Nanoport™ assemblies for capillary connection

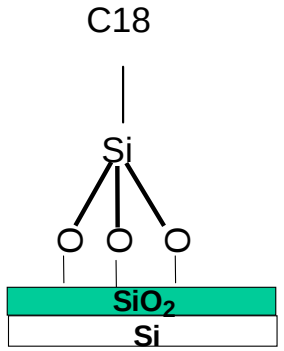


Micro-pillar structure for large surface / volume ratio

Funded by EC

Chemical treatment: grafting of trypsin and C_{18} silane





Grafting of trypsin for the digestion module

Grafting of C_{18} silane for the chromatographic module

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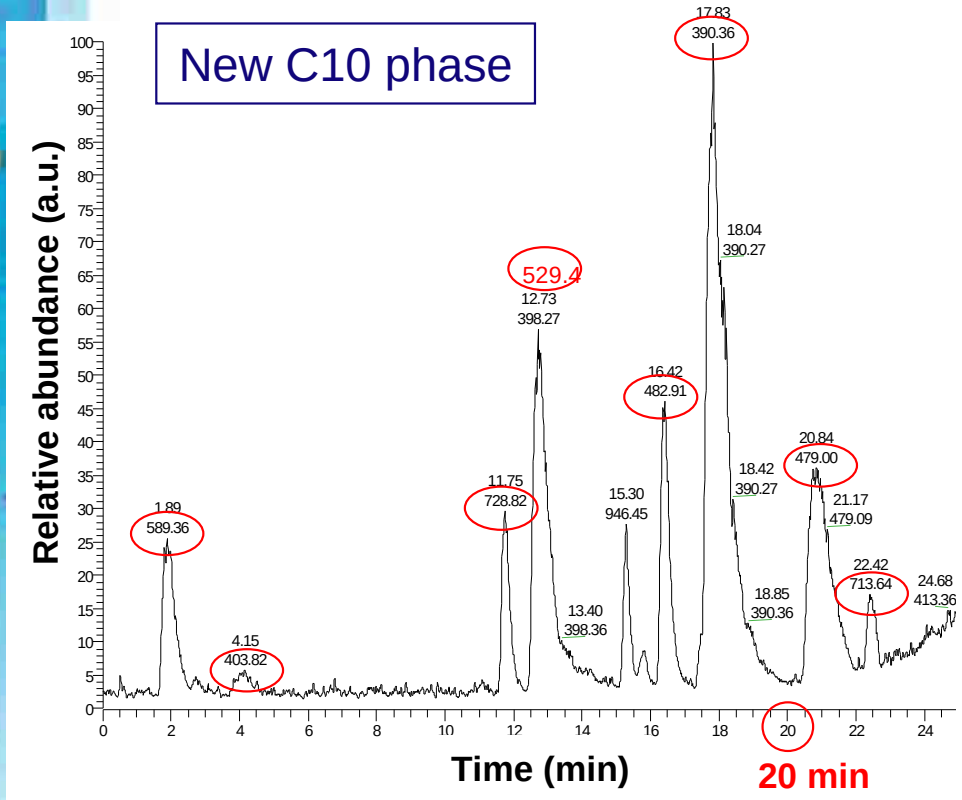
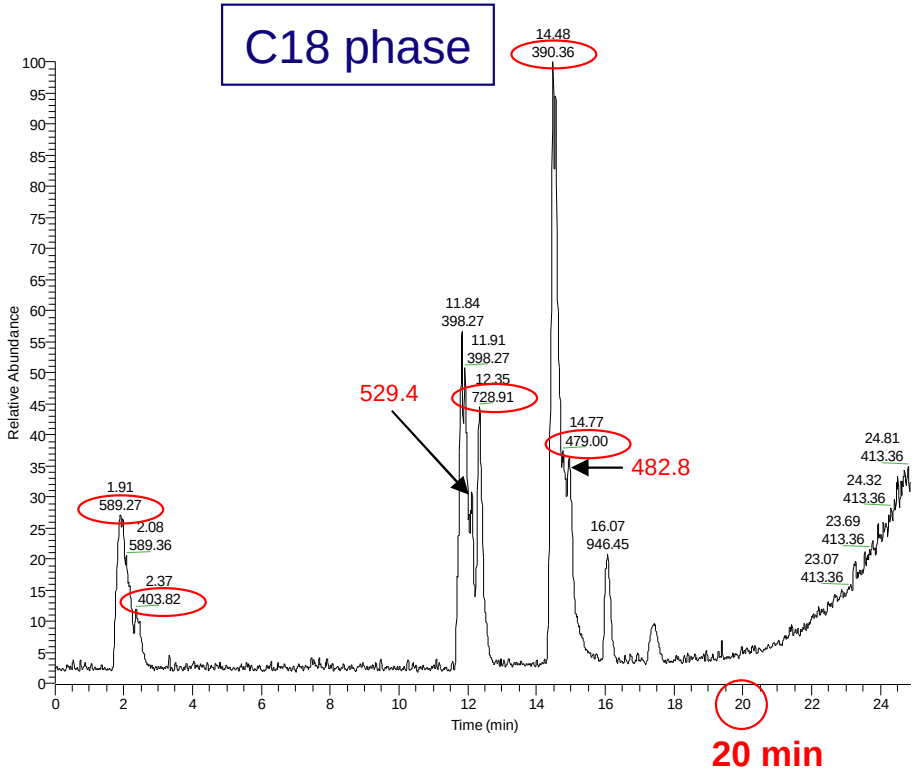
New retention phase for chromatography module





E. Mery, F. Ricoul, N. Sarrut, O. Constantin, G. Delapierre, J. Garin, F. Vinet (2008), A silicon microfluidic chip integrating an ordered micropillars array separation column and a nano-electrospray emitter for LC/MS analysis of peptides, Sensors and Actuators B, Vol. 134, Iss. 2, pp. 438-446.

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Conference highlight

- Clinical proteomics
- Micro-nano technologies for microfluidic analysis coupled with mass spectrometry
- **Information processing**
- Conclusion

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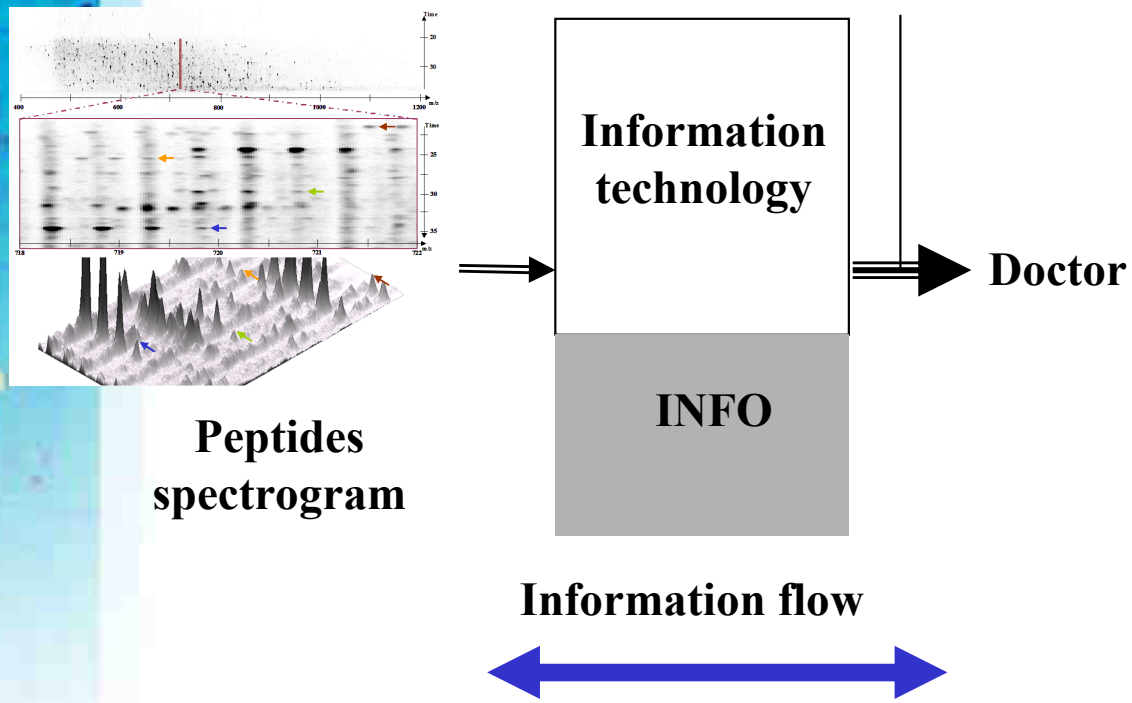
LOCCANDIA Project Goals : Information technology



To build an Integrated Clinico-Proteomics Environment (ICPE):

- a Proteomic Information Management System (PIS) for sample information management
- a Clinical Information System (CIS) for patient information management to allow clinical evaluation
- an Information and data mediation infrastructure including preprocessing, reconstruction, visualization, protein/peptide identification and data analysis modules

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The main stages in image processing

Living system to observe

Measurement system

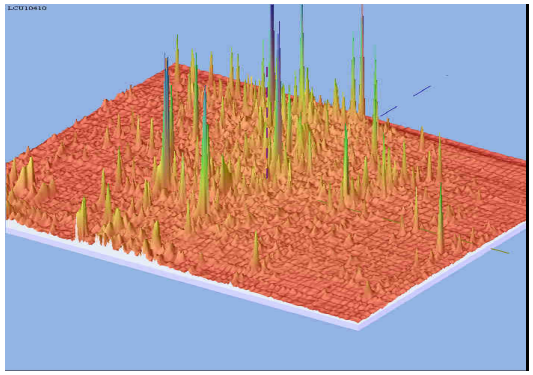
Digital electronic signal

Digital processing

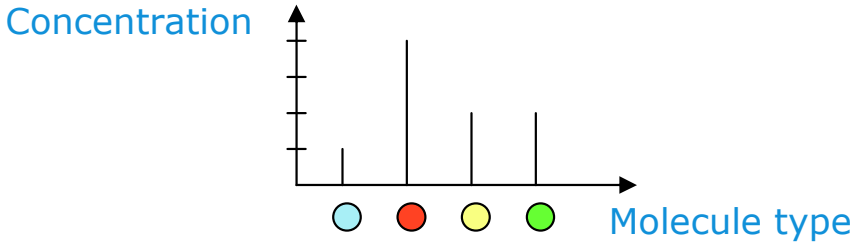
Digital representation

Data analysis

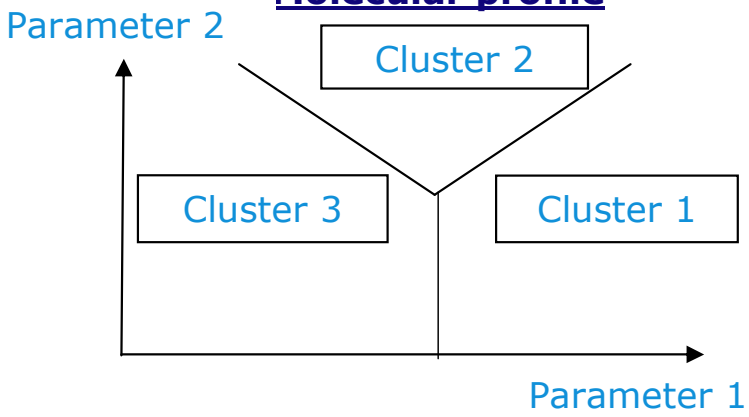
Parametric/Symbolic description



Spectrogram



Molecular profile



Sample classification

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Advanced information processing requirements

LOCCANDIA

Objectives:

- To manage the acquisition chain parameters
- To quantify the targeted proteins
- To visualize the results
- To identify contaminants
- To manage patient information
- To assist the clinician to take a decision

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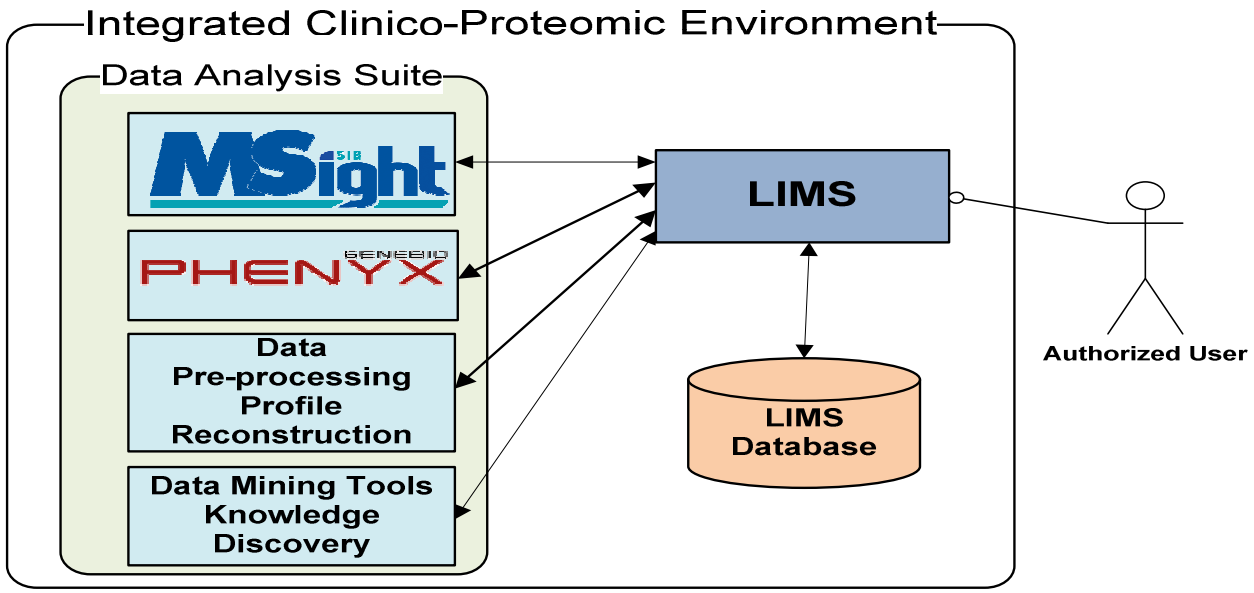
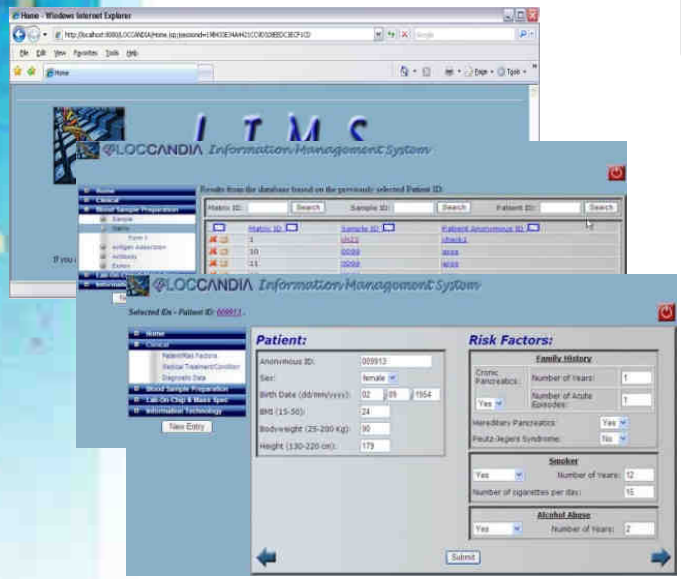
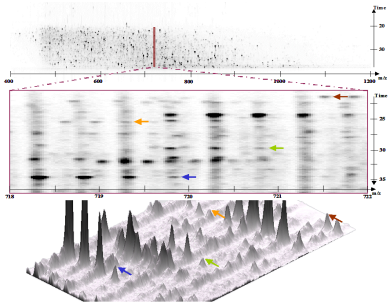
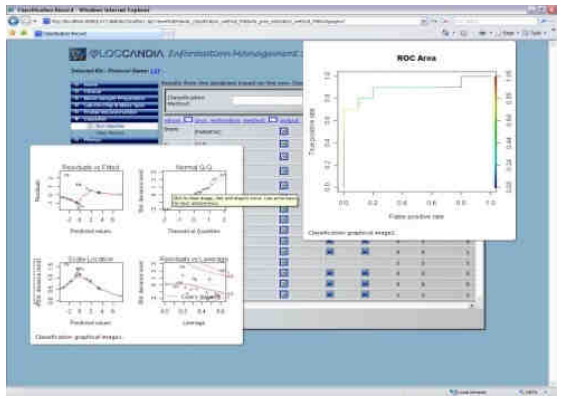
LOCCANDIA information management system (LIMS)





Identification





Parameter management: Patient, Analytical Chain → Molecular Profile Reconstruction → Visualisation → Classification, Diagnostic assistance

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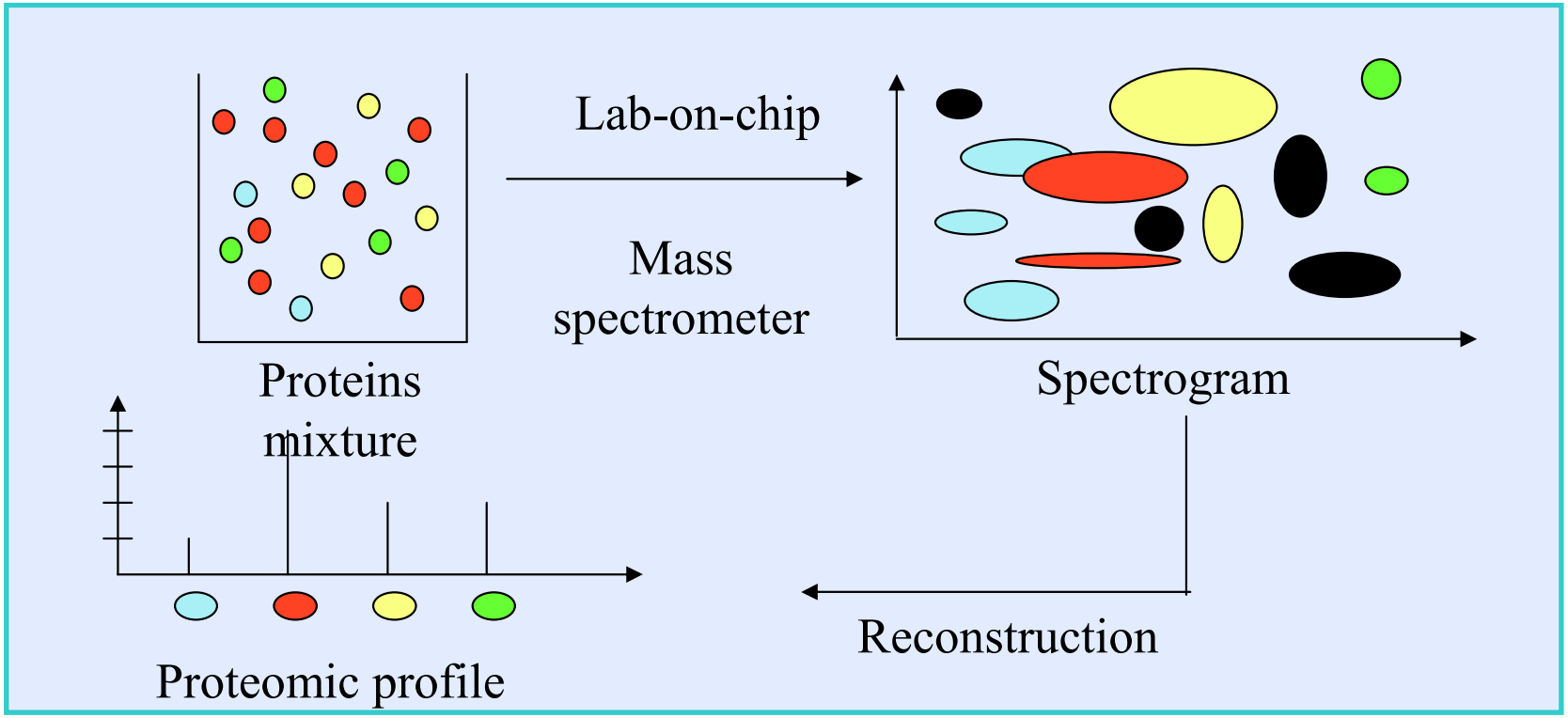
Molecular Profile reconstruction



- Three main approaches are currently investigated:
  - chemometrics approaches associated with factorial analysis
  - statistical approach associated with parametric models
  - dynamic modeling describing the physico-chemical behaviour

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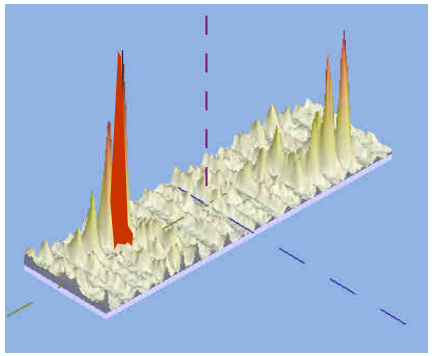
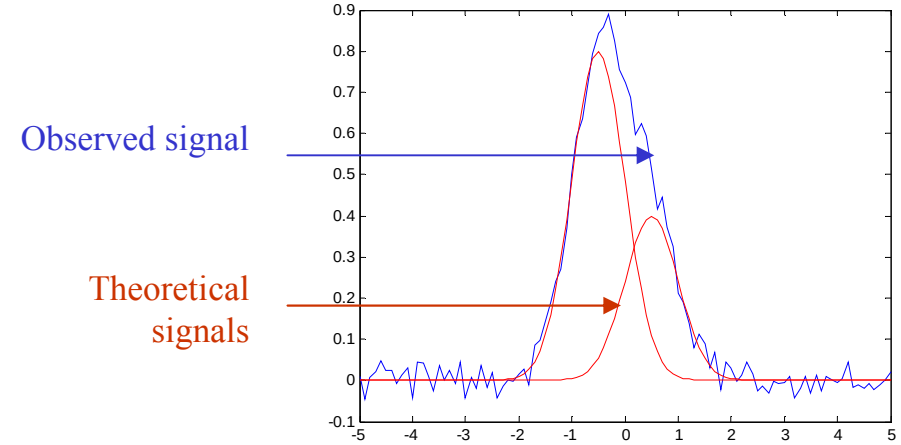
State of the art

Methods basesd on peak detection and peak parameters handling



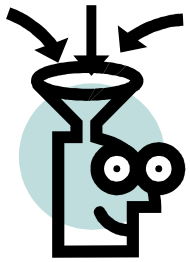
Area under the curve method

[Radulovic, D. *et al.* (2004) *Mol. Cell. Proteomics* 3, 984-997]



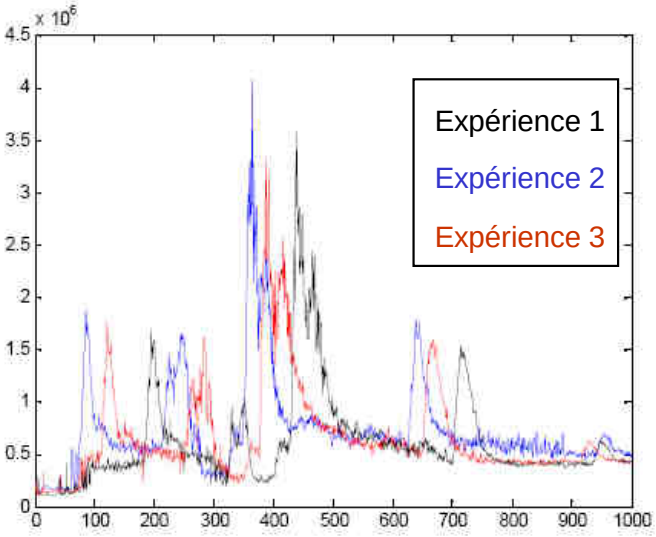
Signal mixtures

Methods based on full signal handling



Methods based on full signature signal estimation e.g.: PLS

[Idborg, H. *et al.* (2004) *Rapid Commun. Mass Spectrom.* 18, 944-954]



Shift in chromatographic retention time

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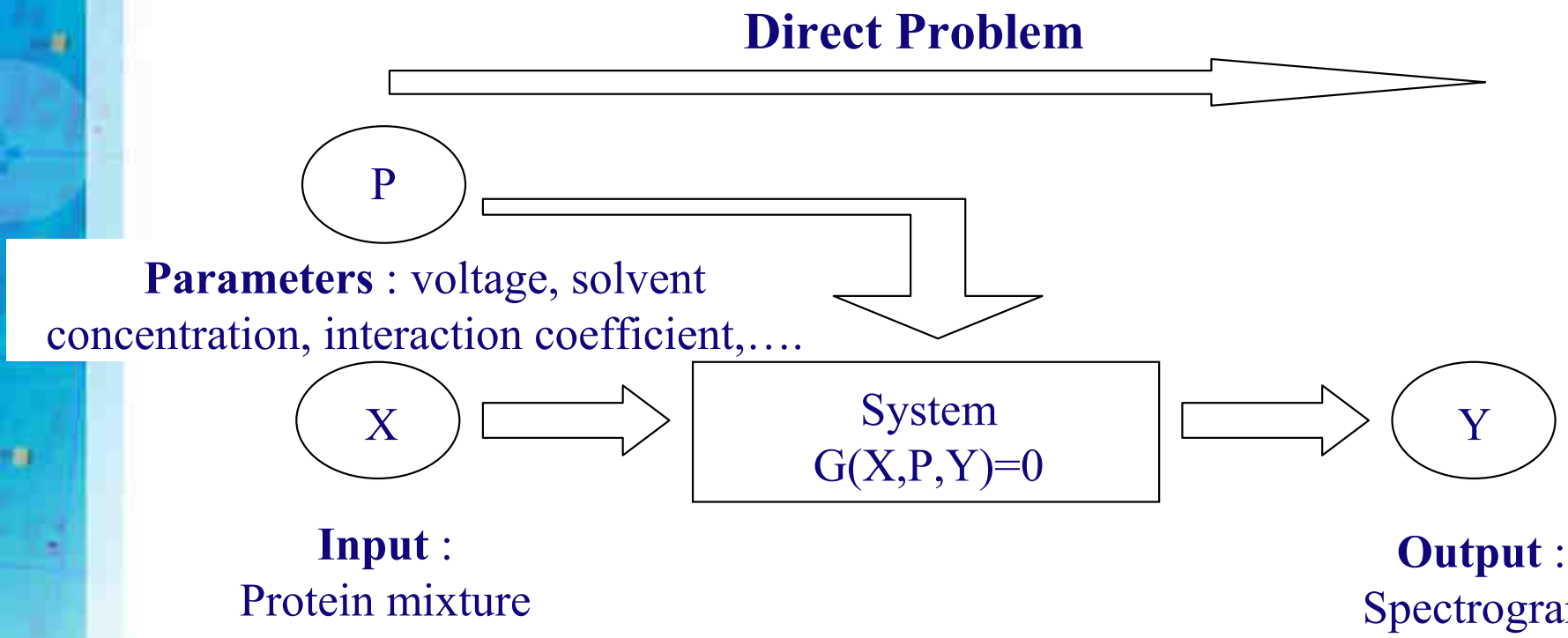
System approach



Reference:

GRANGEAT P. (2007), "Traitement de l'information", in M. LAHMANI, P. BOISSEAU, P. HOUDY, Les Nanosciences III : Nanobiotechnologies et Nanobiologie, Chapitre 13, 763-789, collection « Echelles » directed by M. LAGUES and A. LESNE, Editions Belin.

GRANGEAT P. (2009), "Data processing", in M. LAHMANI, P. BOISSEAU, P. HOUDY : Nanobiotechnology and Nanobiology, Springer. (to appear).

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Acquisition chain model for a peptide

	Modelized phenomena	Model form
Pre-analytic preparation	Dynamic processes	Gain factor
Chromatography	Buffer exchange **Convection diffusion equation**	Gaussian impulse response
Electrospray	Dynamic process with a small time scale	Gain factor
Mass spectrometry	Sampling+Integration **Mathieu equation**	Gain factor + Gaussian

The two first moments of the solution

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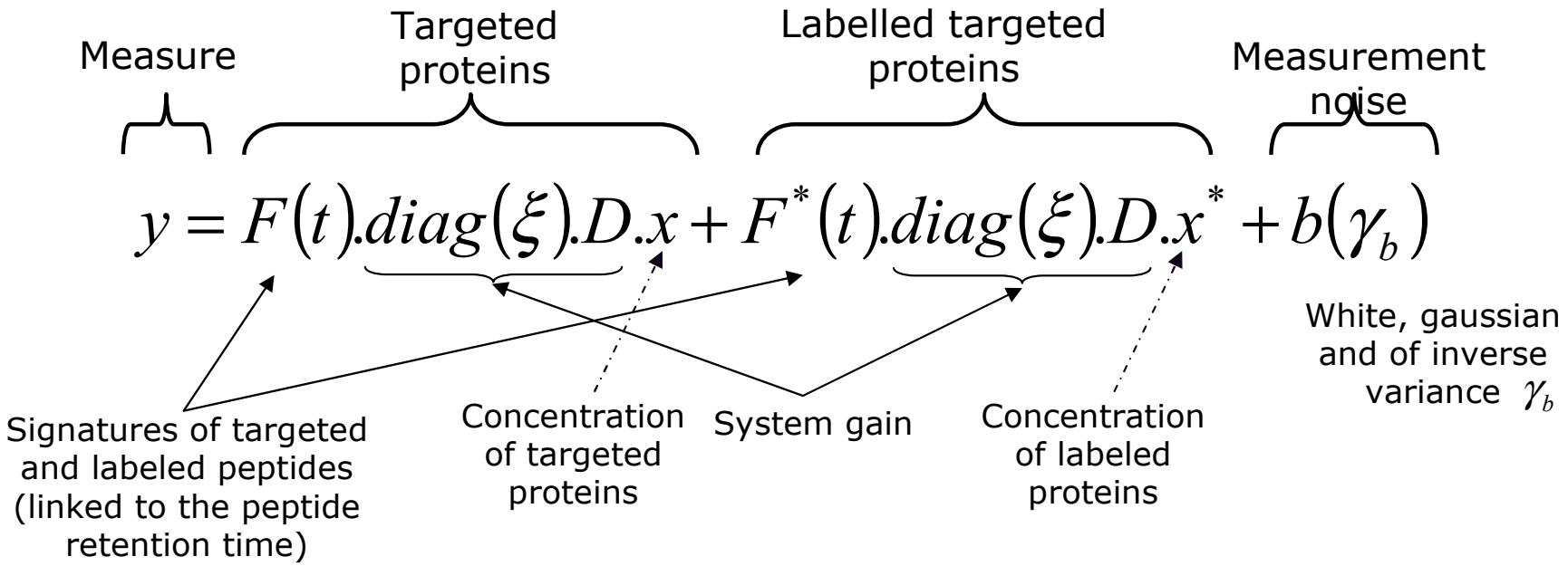
Statistical Reconstruction : direct problem

- Measurement equation :



- The unknowns:
  - the concentrations x
  - the instrument parameters (t, ξ)
  - the measurement noise parameters γ_b

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The a posteriori law:

$$p\left(\mathbf{x},\boldsymbol{\xi},\mathbf{t},\gamma_b\,|\,\mathbf{Y}\right)\propto \exp\left(-\frac{1}{2}\gamma_b\left\|\mathbf{Y}-\Theta\left(\mathbf{x},\mathbf{x}^*,\boldsymbol{\xi},\mathbf{t}\right)\right\|^2\right)$$ Noise: Gaussian law

$$\times\prod_{i=1}^{P}\exp\left(-\frac{1}{2}\gamma_x^p\left(x_p-\bar{x}_p\right)^2\right)$$ Concentration: Gaussian law

$$\times\prod_{i=1}^{I}\exp\left(-\frac{1}{2}\gamma_\xi^i\left(\xi_i-\bar{\xi}_i\right)^2\right)$$ System gain: Gaussian law

$$\times\prod_{i=1}^{I}U\left(t_i;t_i^m,t_i^M\right)$$ Retention time: uniform law

$$\times\frac{\gamma_b^{\alpha_b-1}}{\beta_b^{\alpha_b}\Gamma\left(\alpha_b\right)}\exp\left(-\frac{\gamma_b}{\beta_b}\right)$$ Noise inverse variance: gamma law

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Bayesian approach

Statistical framework

	Associated law	Notations
Direct model + noise model	Likelihood	$p\left(\boldsymbol{Y}\middle\vert \boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\right)$
Modeling the *a priori* information on the parameters	*a priori law*	$p\left(\boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\right)$
Combined model	*a posteriori law*	$p\left(\boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\middle\vert \boldsymbol{Y}\right)$

Bayes rule:

$$p\left(\boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\middle\vert \boldsymbol{Y}\right)=\frac{p\left(\boldsymbol{Y}\middle\vert \boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\right)p\left(\boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\right)}{\int p\left(\boldsymbol{Y}\middle\vert \boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\right)p\left(\boldsymbol{x},\boldsymbol{\xi},\boldsymbol{t},\gamma_b\right)\mathrm{d}\boldsymbol{x}\,\mathrm{d}\boldsymbol{\xi}\,\mathrm{d}\boldsymbol{t}\,\mathrm{d}\gamma_b}$$

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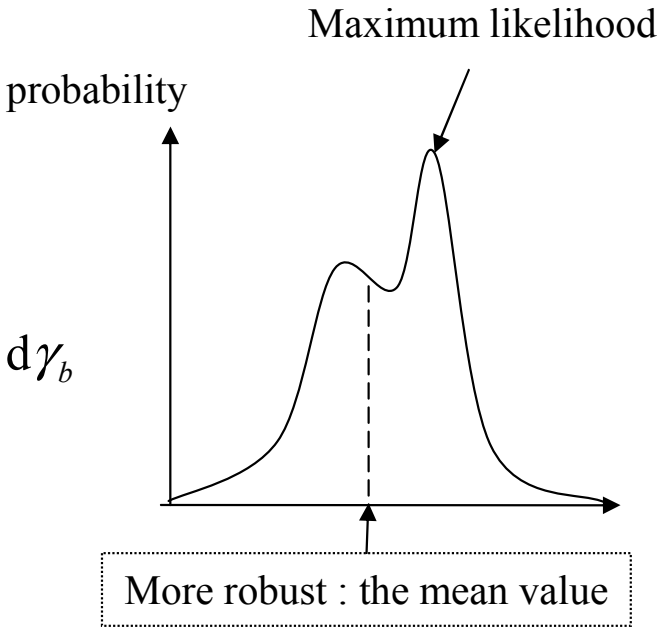
Statistical Reconstruction: the inverse problem



- Bayesian reconstruction :
  - Expectation of the A Posteriori law (EAP)

$$[\mathbf{x}, \xi, \mathbf{t}, \gamma_b] = \int [\mathbf{x}, \xi, \mathbf{t}, \gamma_b] p(\mathbf{x}, \xi, \mathbf{t}, \gamma_b | \mathbf{Y}) d\mathbf{x}\, d\xi\, d\mathbf{t}\, d\gamma_b$$

- MCMC (Monte Carlo methods based on Markov Chain) algorithm
  - random sampling of each unknown conditionally to the others
  - computation of the empirical mean of the samples

$$\left[\hat{\boldsymbol{x}} \quad \hat{\xi} \quad \hat{\boldsymbol{t}} \quad \hat{\gamma}_b\right] \approx \frac{1}{K} \sum_{k=K_0}^{K+K_0-1} \left[\boldsymbol{x}^{(k)} \quad \xi^{(k)} \quad \boldsymbol{t}^{(k)} \quad \gamma_b^{(k)}\right]$$

Reference: STRUBEL G. (2008), "Reconstruction de profils moléculaires: modélisation et inversion d'une chaîne de mesure protéomique (molecular profile reconstruction: modeling and inversion of a proteomic analytical chain)",Ph.D. thesis, INPG,Grenoble, France.

GRANGEAT P., STRUBEL G., GIOVANNELLI J.-F., BRUN V., GERFAULT L., PAULUS C., DUPUIS A., GARIN J. (2009), "Robust statistical reconstruction of protein profiles in mass spectrometry", 57th ASMS Conference on Mass Spectrometry, Philadelphia, Pennsylvania, USA, 31 Mai-4 Juin 2009.

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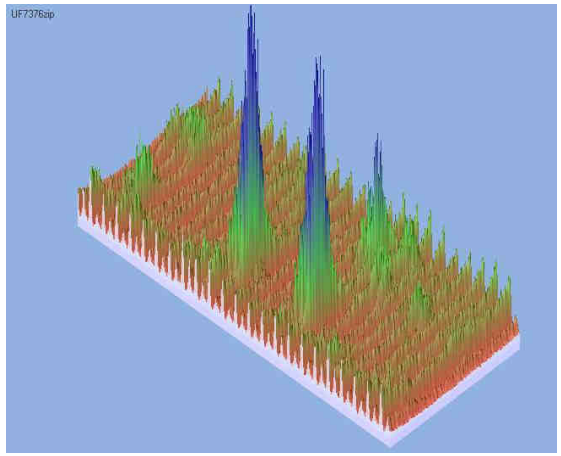
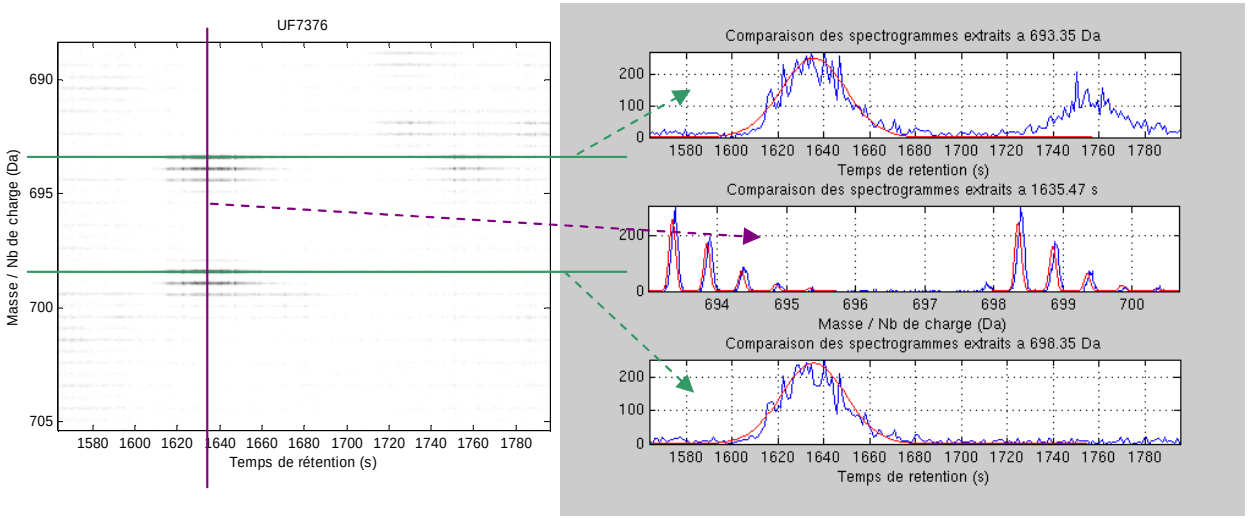
Statistical reconstruction: experimental results

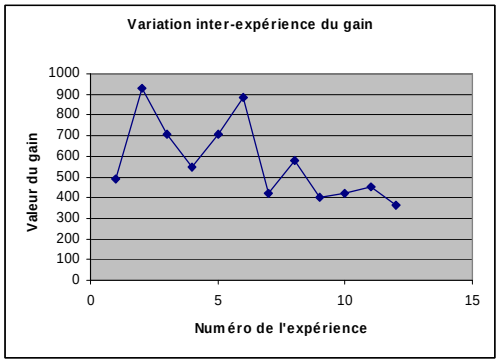


3D display of a zone within the spectrogram around one peptide peak of the targeted protein

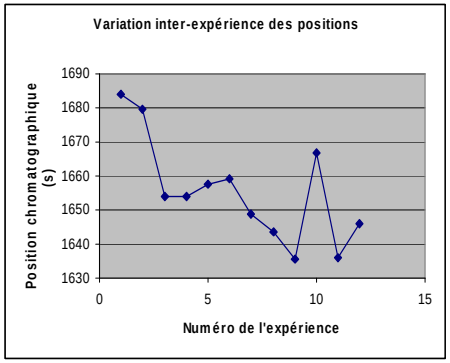


2D display of a spectrogram *extracted plot*

Comparison between adjusted model (red) and measurement (blue)



Gain variation on the set of experiences



Position variation of chromatographic peak on the set of experiences

Despite these technological variations and the noisy background, the ***average variation coefficient is 6.2 %*** *for the reconstructed protein quantity.*

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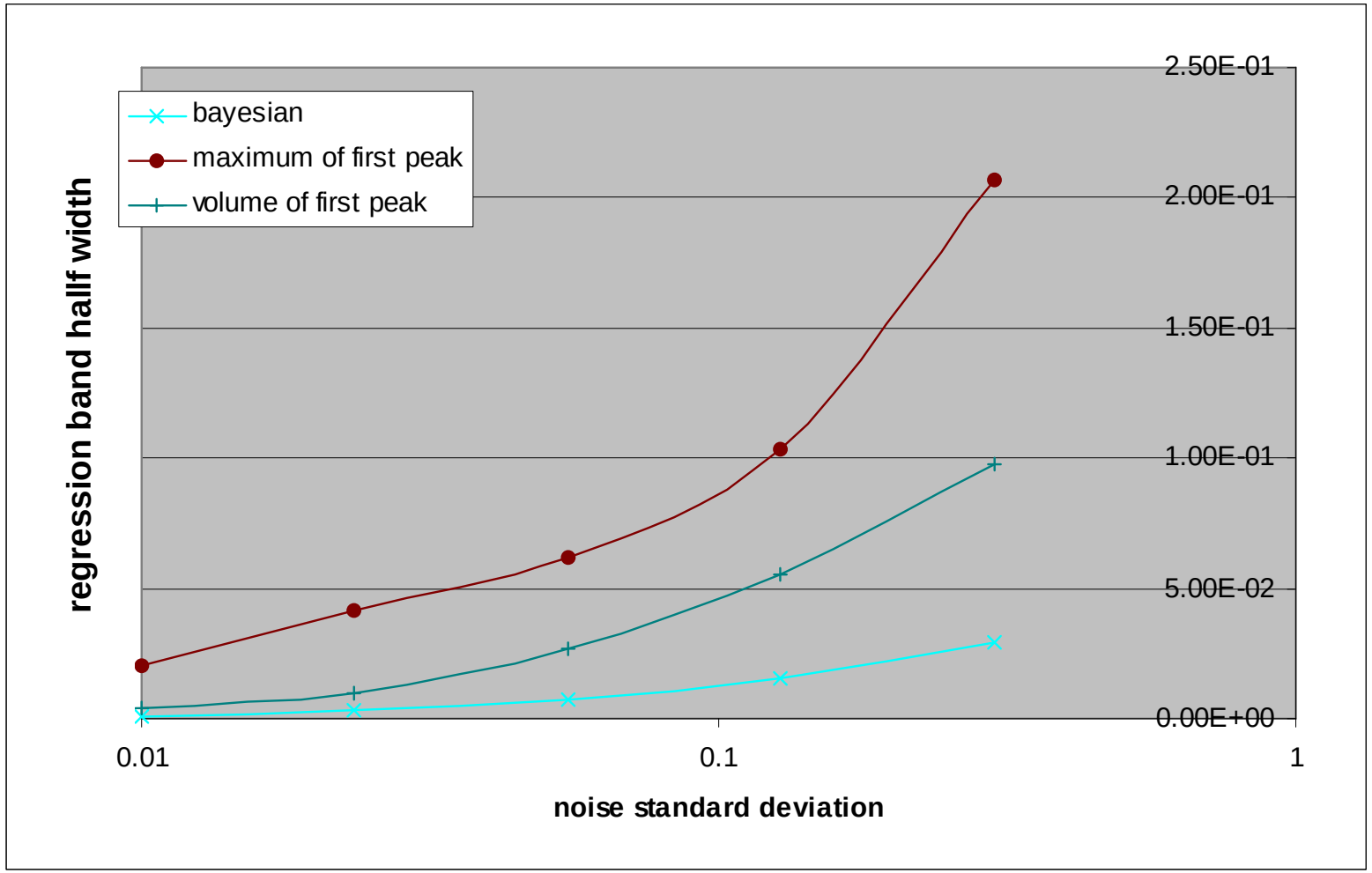
Comparison with standard methods on simulated data



Statistical reconstruction is the most robust against high noise level

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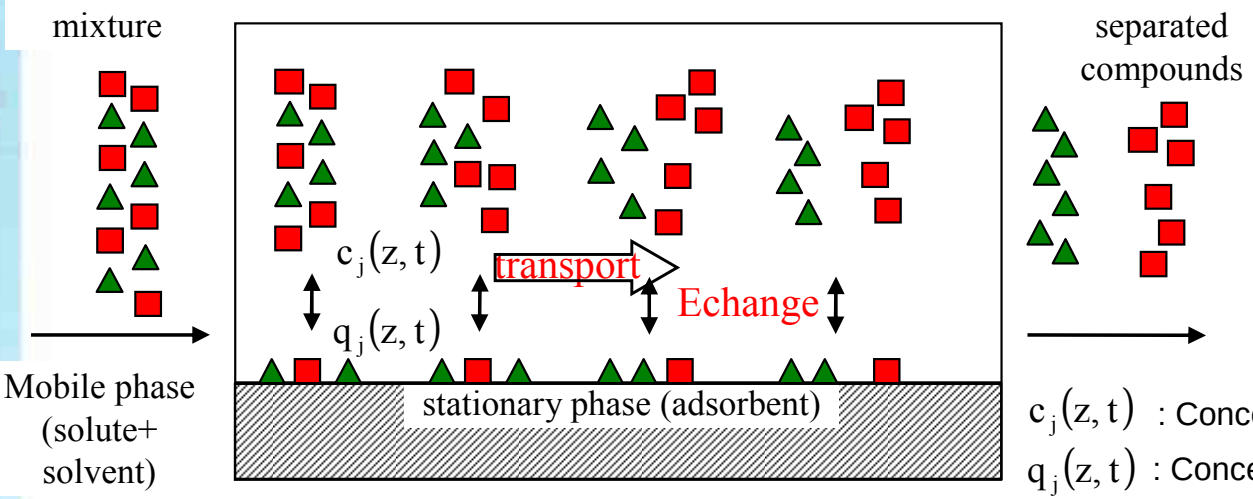
Dynamical Reconstruction: the chromatography column model

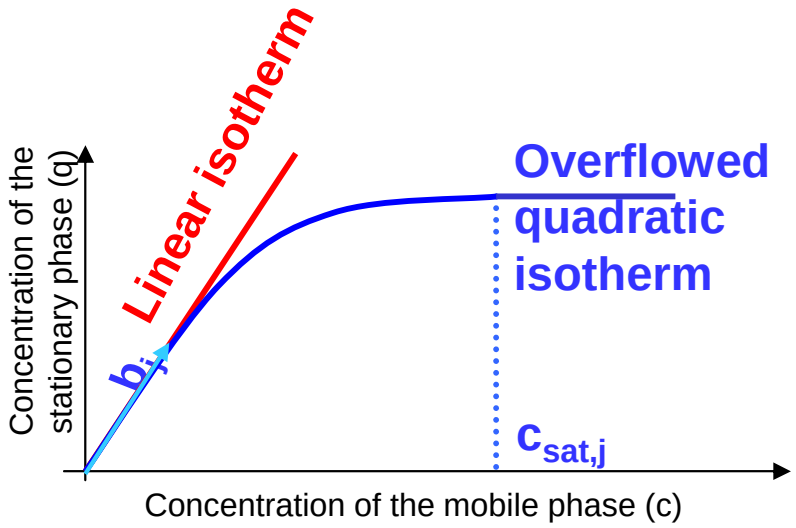


$c_j(z,t)$: Concentration of peptide j in the mobile phase

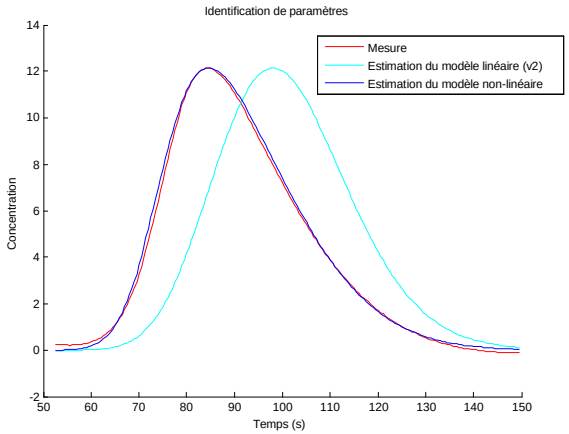
$q_j(z,t)$: Concentration of peptide j in the stationary phase

Dynamical model of the chromatography column

Rerefence: PAULUS C., GERFAULT L., BRUN V., GRANGEAT P. (2009), "Inversion of a nano-Liquid Chromatography dynamical model for quantitative proteomics", SYSID 2009: 15th Symposium on System Identification, Saint Malo, France, 6-8 juillet 2009.



Isotherm linking the concentrations in mobile and stationary phase



Comparison of measured and modelled chromatographic profiles taking into account the overflow effect

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Conference highlight

- Clinical proteomics
- Micro-nano technologies for microfluidic analysis coupled with mass spectrometry
- Information processing
- **Conclusion**

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Conclusion

- **OBJECTIVES:**
  - lab-on-chip based point of care dedicated to clinical proteomics from blood sample
  - early detection, treatment planning and follow-up of diseases with proteomic signature such as cancer
  - technological beakthrough will come from the combination of high sensitivity detection and high complexity separation
  - innovative robust molecular profile reconstruction software
- **MULTIDISCIPLINARY RESEARCHES :**
  - Micro-Nano Bio Info Cogno convergence
- **TWO CHALLENGING PROJECTS HAVE BEEN DESCRIBED :**
  - LOCCANDIA & CAPSI
  - First proof of concept are in progress
- **LONG TERM PROJECTS**

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Key information processing topics at the sensor level for micro-nano-biomedical-systems

- Inverse problem
  - profile reconstruction
  - source separation
- Statistical signal processing
- Model based signal processing
  - system identification
  - functional model
- Fluid management
- Data analysis:
  - classification
  - decision support
  - signature recognition
  - biostatistics

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