Development of organ on chip technology for predictive toxicology and applied pharmacology

Eric Leclerc

CNRS

The University of Tokyo

Universite de Technologie de Compiegne

cnrs advancing the frontiers

LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820

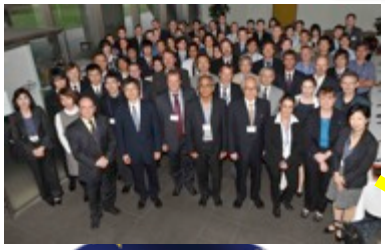
INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

東京大学 THE UNIVERSITY OF TOKYO

LIMMS – 23 years of history

IIS in old Roppongi Campus

1995



2001

Komaba II Campus

International Unit UMI 2820

2004

2008

2011: The Great Earthquake

2011

EUJO LiMMS

2012

Mirror structure in Hospital

2014

2016

iLite RHU program

New research axis

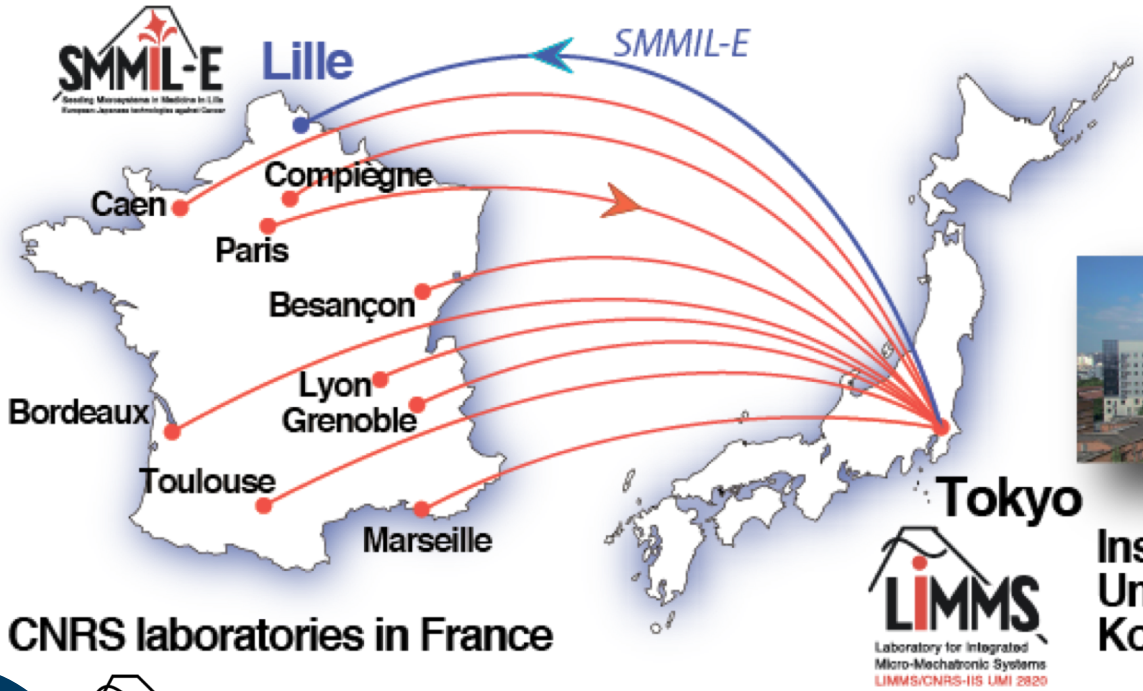
2016

iLite

Today

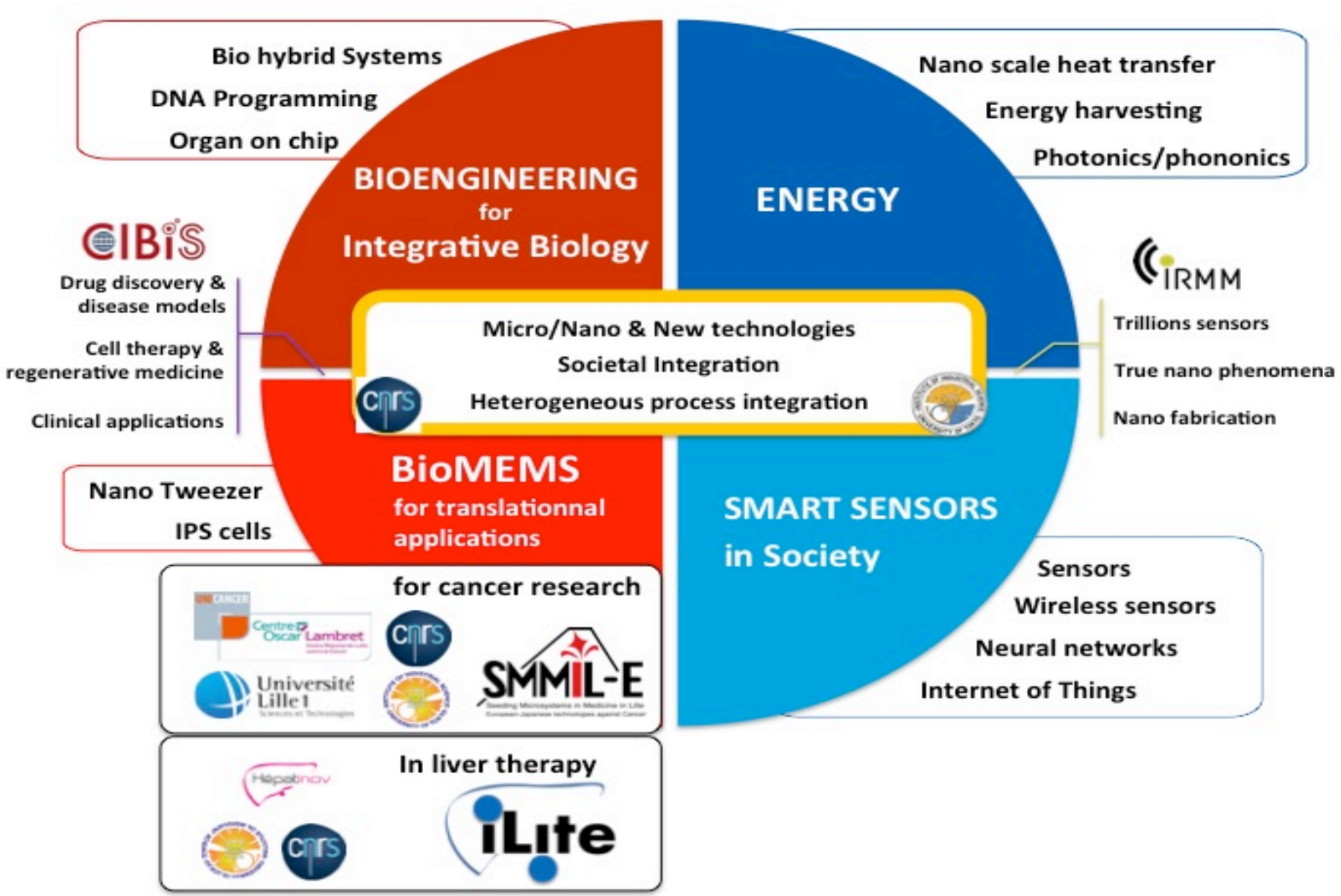
LIMMS Operation

- Established in **1995**, UMI since 2004, **16 host Japanese laboratories**
- **219** (**176** French, **9** Japanese, **26** other nationalities) persons welcomed at LIMMS
  - CNRS senior researchers **(37)**
  - **JSPS Post-Doc fellows (71)** + contract Postdocs (**20**) + PhD students (**13**) + Trainees (**36**)
  - EUJO-LIMMS members **(21)**
  - research engineers **(5)**, administrative staffs **(15)**
  - Industries **(2)**



Institute of Industrial Science
University of Tokyo
Komaba Campus

Scientific LIMMS activities



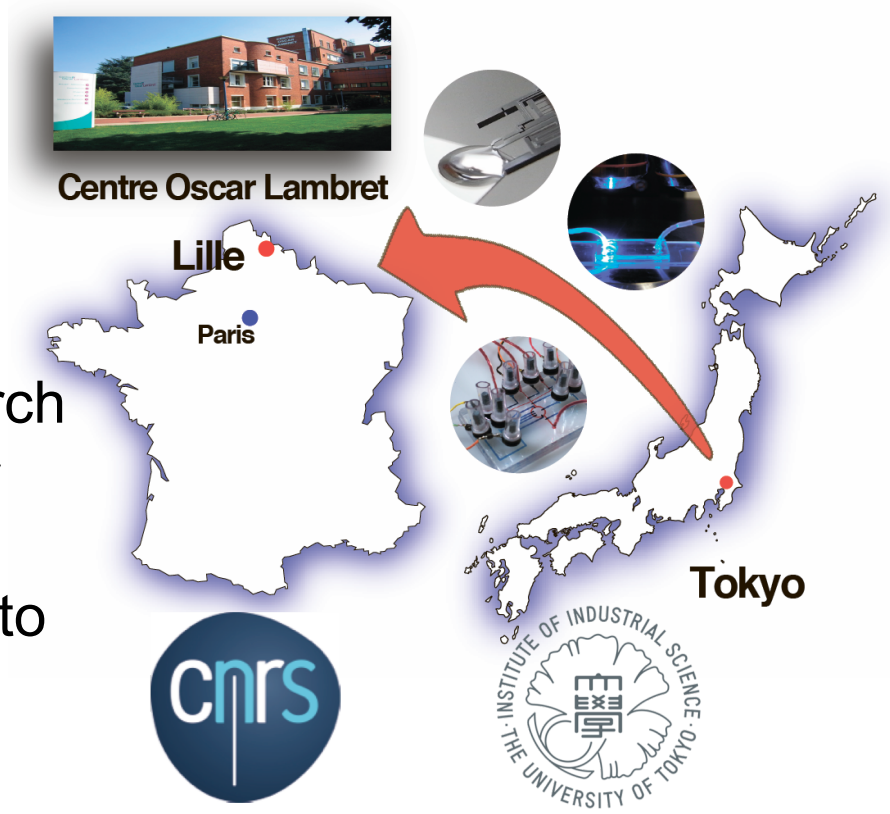
Translational research - SMMiLE

Seeding Microsystems in Medecine in Lille –
European Japanese Technologies against Cancer

SMMiL-E:

Mirror structure of LIMMS

↳IIS research site abroad



- France-Japan collaboration research to implement BioMEMS for cancer therapy
- Located at Centre Oscar Lambret to develop BioMEMS in a hospital
- ONCO-Lille being a major cancer research center in France
- Top ranked hospital in cancer therapy/treatment

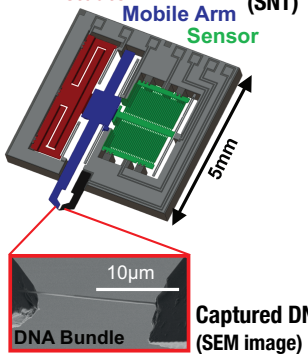
Translational research - SMMiLE research outputs

SMMiL-E
Seeding Microsystems in Medicine in Lille
European-Japanese technologies against Cancer

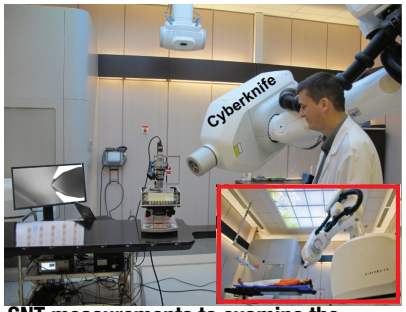
WP1: Resistance in biomolecular mechanism

- DNA degradation under X-ray (radiotherapy)

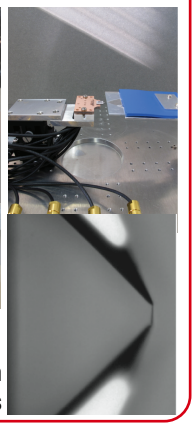
Application: Therapy and combined therapies



Captured DNA (SEM image)



SNT measurements to examine the effect of irradiation

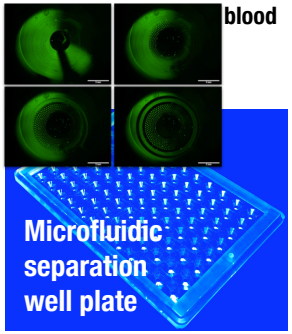


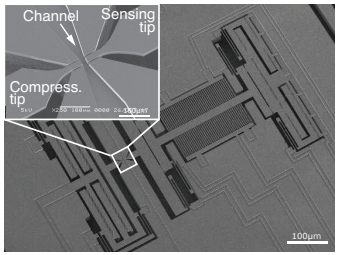
Setup and SNT with DNA between tips

WP2: Cellular evaluation and diagnosis

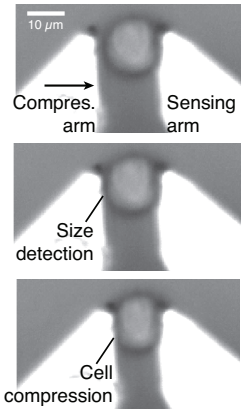
- Circulating tumor cell sorting
- Mechanical characterization of CTCs

Application: Diagnosis, therapy monitoring





Cell characterization device with built-in microfluidics



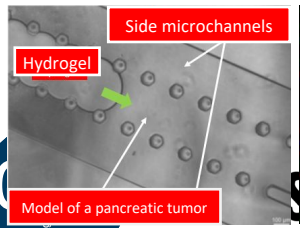
Biophysical characterization of single cells

WP3: Tumors and therapeutic targets

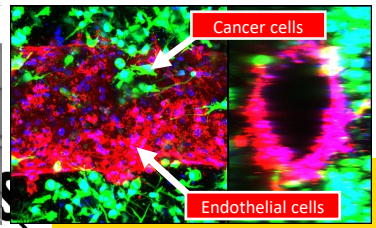
- Developing tumor angiogenesis
- Cellular motility and metastatic processes

Application: Mechanism of action, drug evaluation

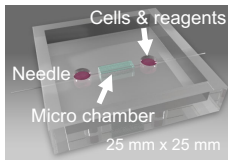
Organ-on-a-chip (pancreatic cancer)



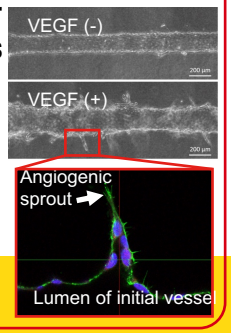
***In vitro* metastasis model**



Vasculature formation



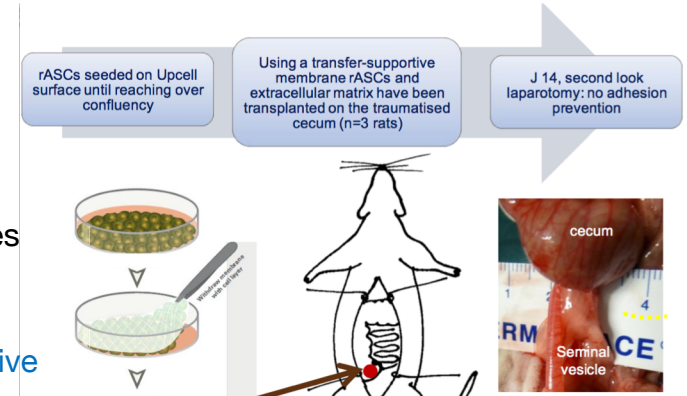
Tumor angiogenesis



WP4: Biological adhesives and neotissues

- Biological glues
- Cellular fibers
- Patch and new-tissues

Application: Reconstructive surgery



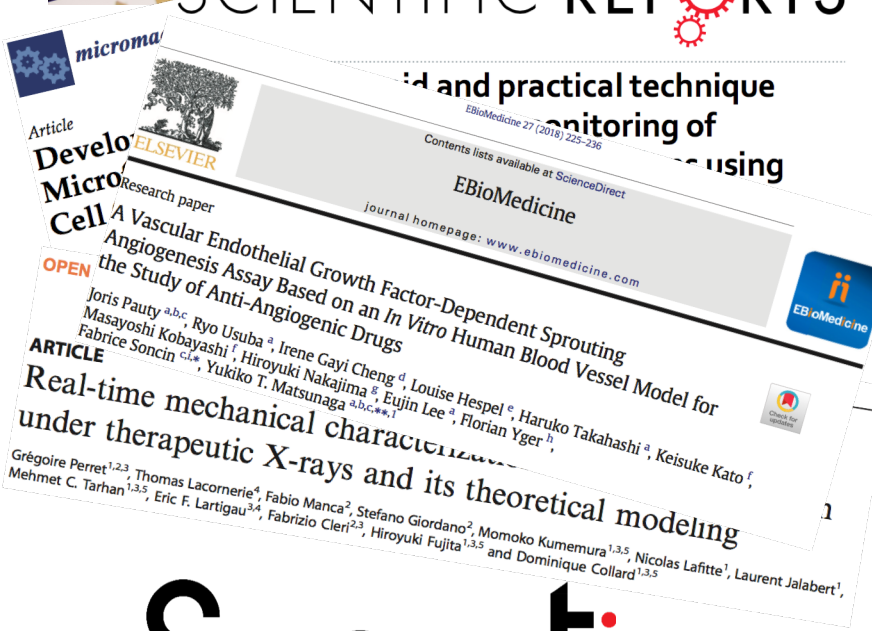
Laboratory for Integrated Micro-Mechatronic Systems
LIMMS/CNRS-IIS UMI 2820
THE UNIVERSITY OF TOKYO

Translational research - SMMiLE main scientific outputs

SMMiL-E
Seeding Microsystems in Medicine in Lille
European-Japanese technologies against Cancer

Scientific production in numbers:

Research projects (labeled/total):	7/12
Researchers in Lille (current/exp. by oct):	8/14
Research grants (granted/under evaluation):	2/7
Journal articles on related topics:	13
Conf. proceedings (Smmil-e projects):	8
Patents:	2+1
Industrial contact:	
- Start-up company (incubation):	1
- Labcom:	1
Other funding:	
- CPER budget:	6M€
- Additional CPER budget: (for Recruitment of a young PI)	0.6M€



CNRS advancing the frontiers

LIMMS
Laboratory for Integrated Micro-Mechatronic Systems
LIMMS/CNRS-IIS UMI 2820

INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

東京大学
THE UNIVERSITY OF TOKYO

Translational research - iLite

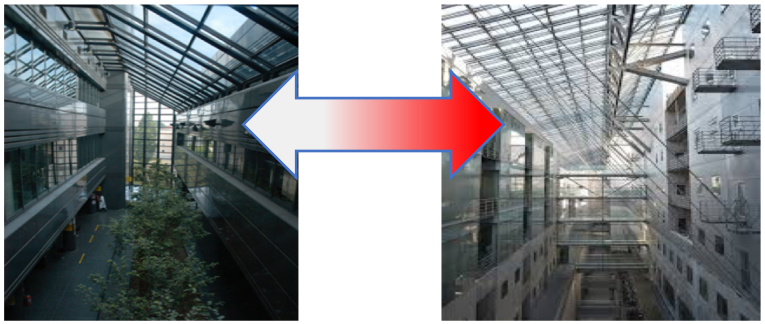
iLite
Innovation in Liver Tissue Engineering

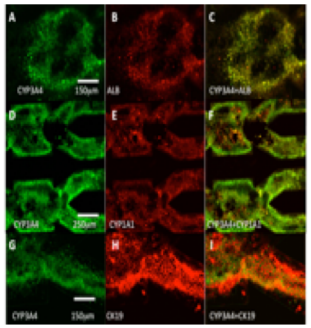
- iLite = **I**nnovation in Liver Tissue Engineering technologies for **CLINICAL demand** on liver therapy

French and Japanese joint

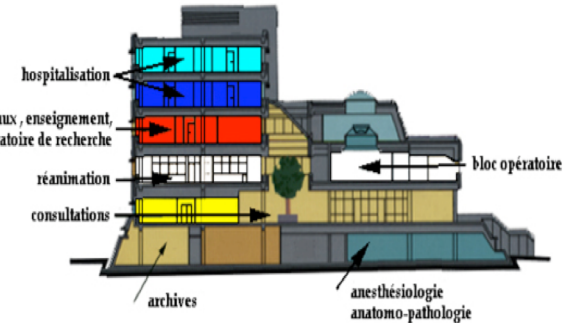
- Biologists, clinicians, engineers
- Public/private consortium (4SMEs)
- 8.5 M euros, 0.4M at LIMMS Tokyo

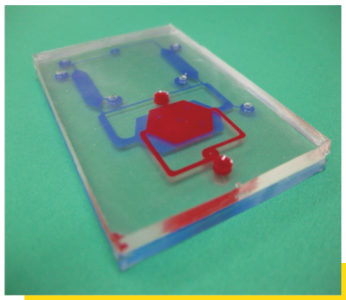
French hospital **IIS engineering**





Organ on a chip, Liver iPS & Innovative disease models





Clinical Research Facilities

Device Technologies

cnrs

INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

Hépatinov

AGENCE NATIONALE DE LA RECHERCHE ANR-16-RHUS-0005

INVESTISSEMENTS D'AVENIR

東京大学 THE UNIVERSITY OF TOKYO

Translational research - iLite main scientific outputs

Innovation in Liver Tissue Engineering

- MoU signed : 24th of October 2016/ Consortium agreement: October 2017
- 2 LIMMS-IIS publications accepted, 4 submitted, 2 in preparations
- Visit of Pr Legallais, Pr Duclos Vallee at IIS in Dec 2016 /June 2017
- Visit of Pr Sakai, Pr Okitsu at Hepatinov in Feb/Nov 2017
- 3 joint workshops (2 Japan and one in France, 9 Japanese Professors)
- 2 Joint PhD (S Matsumoto: Fujii/Leclerc, A Essaouiba Sakai/Leclerc/Legallais), 3 Post doc fellows, Students exchanges (2 PhD)
- New joint research proposal (RISE, C2C, ANSES, ANR, Kakenhi, ERC)
- Extended collaboration to Univ of Tokyo (Pr Okitsu, Pr Miyajima, Pr Takeuchi)

Academic and industrial contexts

How to evaluate the metabolism and effects of xenobiotics

Complex, numerous parameters, species specificity, cost

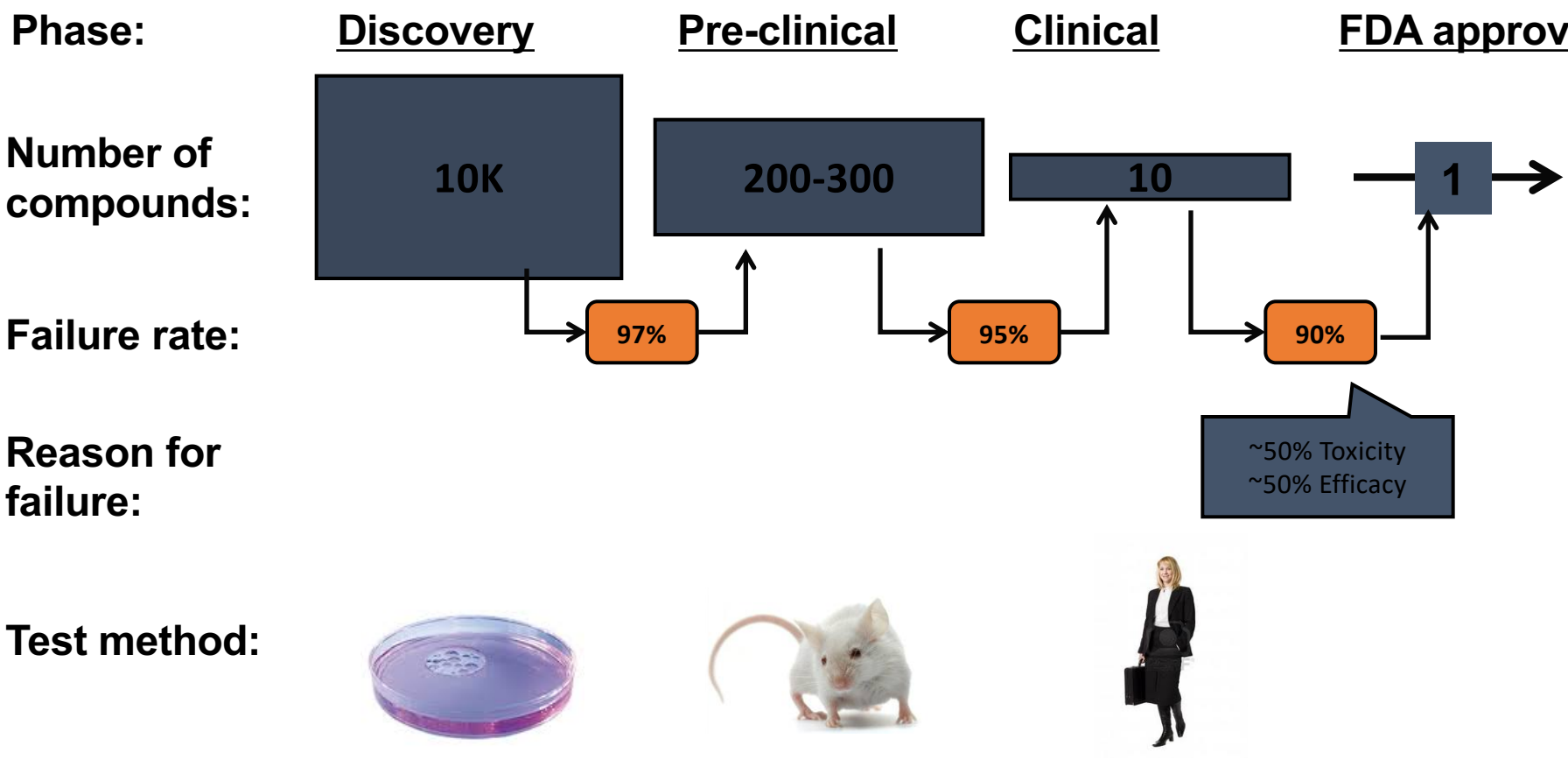
Loss of cellular functions

Industries: cosmetic, pharmaceutical, chemical

EU Directive REACH

THE PROBLEM

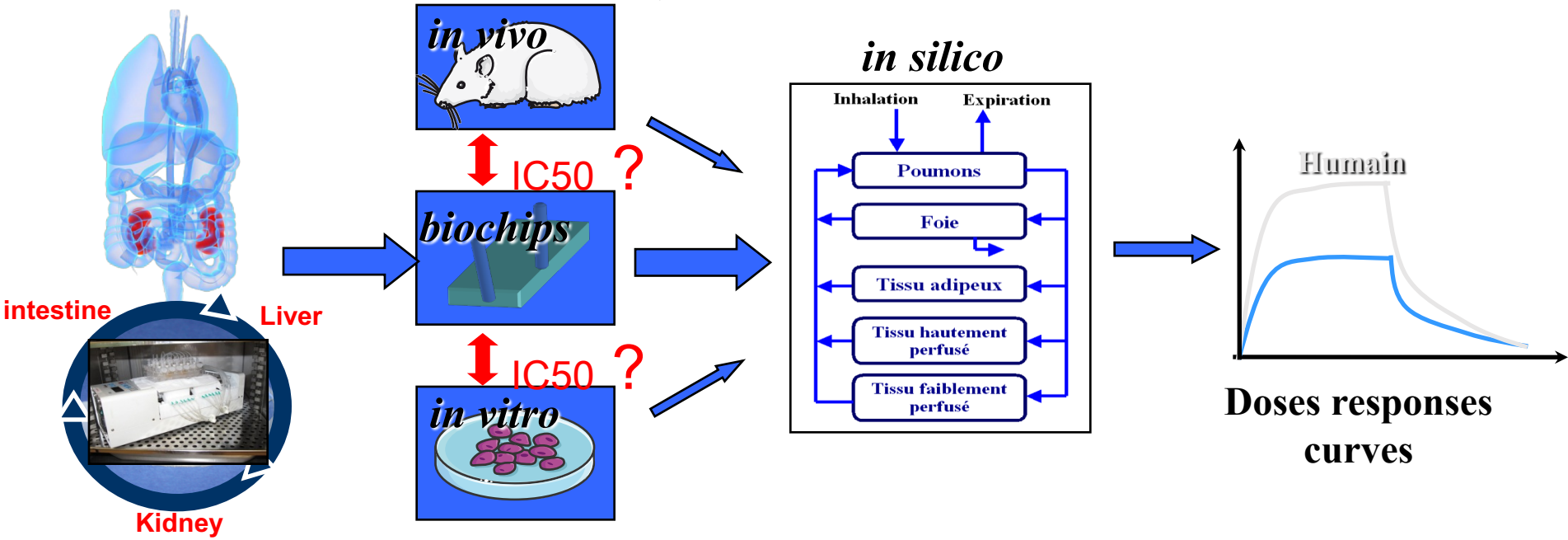
Time: 10-12 Years
Cost: $1B



Human on chip concept

Objectives: Better predictivity of toxicity and pharmacokinetics

Problems : Pertinence of actual models, costs



Solutions:

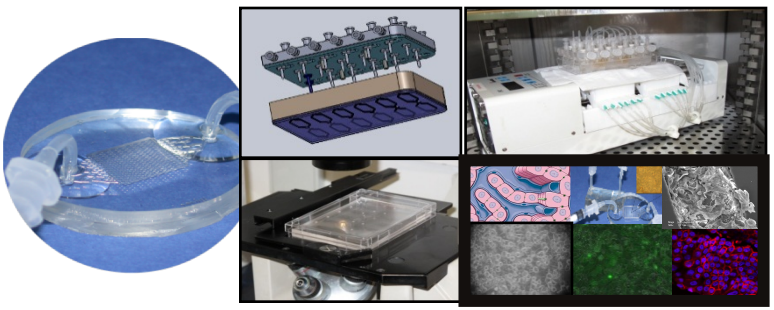
Organ on chip new experimental models; In silico integration

Comparison *in vivo/ in vitro* on reference compounds

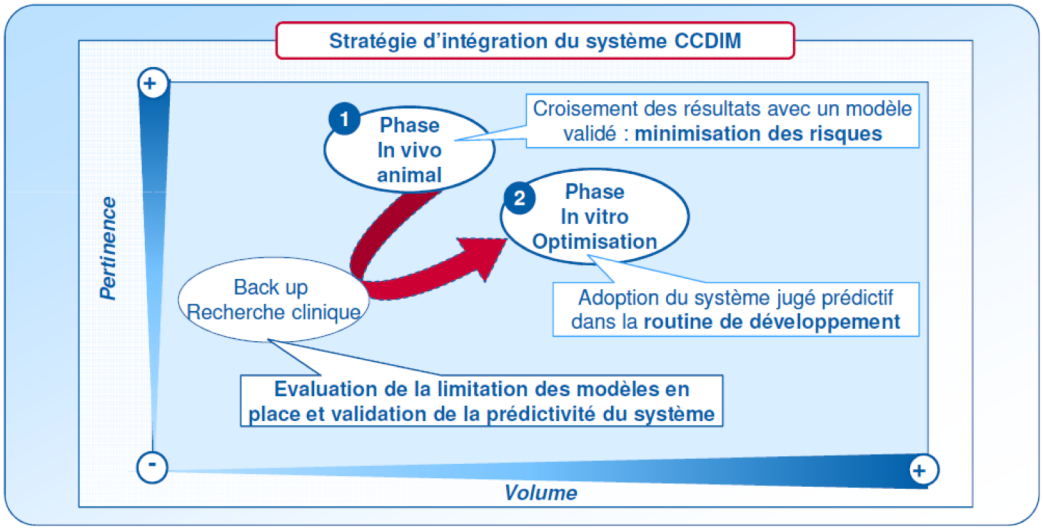
Toward human screening

Advantages of the microfluidic human like biochips

Solution : Miniaturisation of the human physiology: human on chip



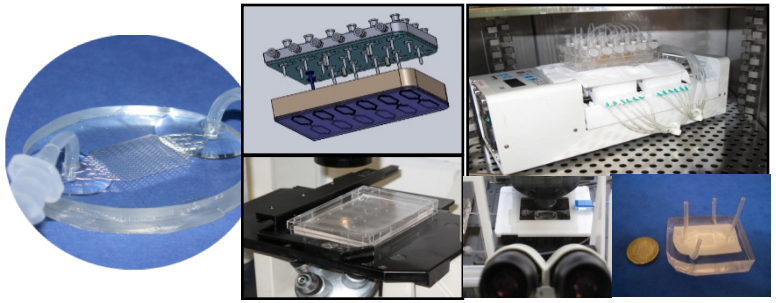
miniaturisation = *parallelisation, cost reduction*
design = *middle througput (100 molecules after HTP)*
integration = *robotisation, real time analysis*
physiology = *functional cell/tissue*
functional= ***Human predictive toxicology***



in vitro **optimisation**
=
pre clinical back up with marketing values
=
Costs reduction
False positive reduction
Animal studies reduction

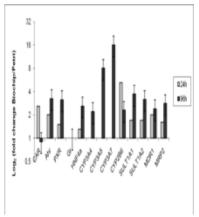
Overall strategy

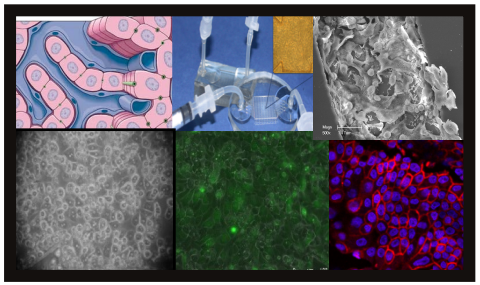
Conception and fabrication of the biochip:



* material, design, packaging, models
  - patent technology (fluidic platform)

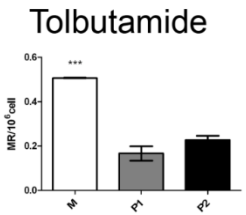
Bio ingeenering: bioartificial organs:

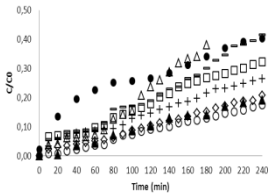




* liver, kidneys, intestine, etc…
* cultures protocols (flow rates, cells, etc…)
* compatible with biological tools (PCR, etc…)
  - patent protocol (stem cells)

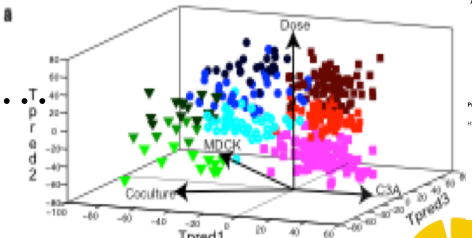
Functional tissues and biochips

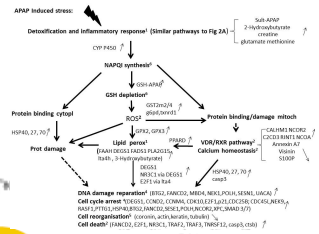




* Comparison of biochips, animal and petri data

Predictive toxicology:

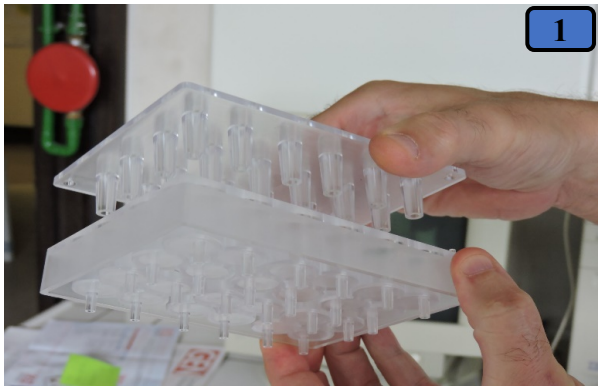


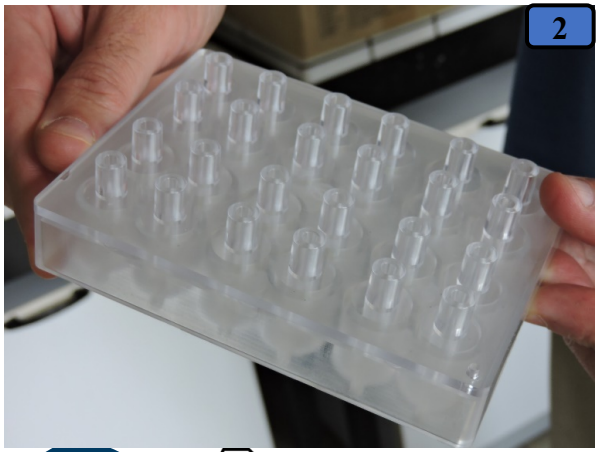


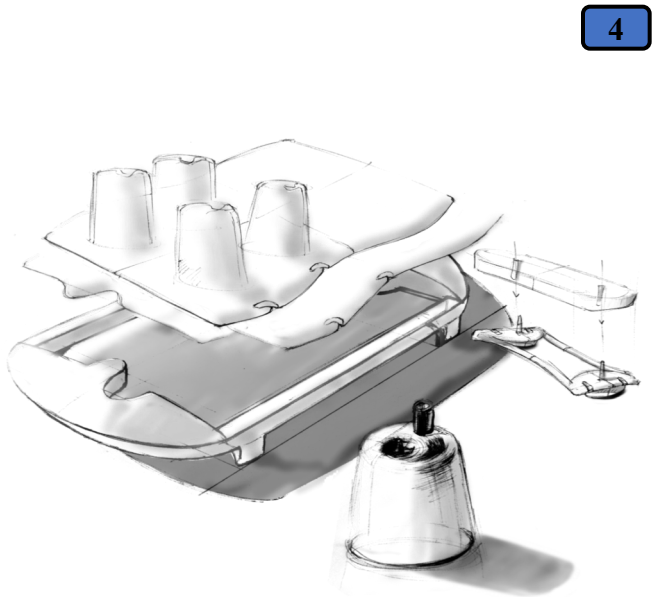
* models, omics, biomarkers, gene networks, …
  - patent oriented application (screenings)
  - know how transfert

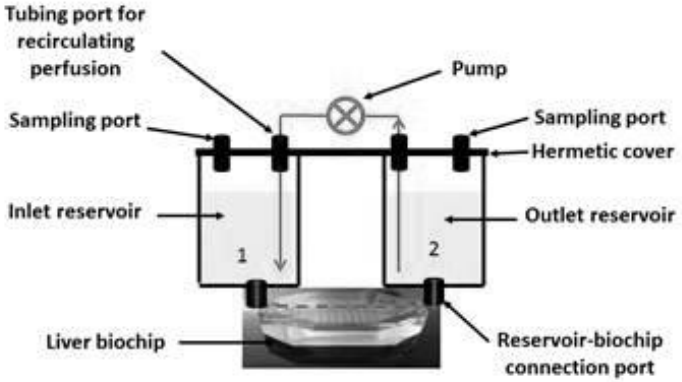
NEW MOLDED IDCCM CASE AND ITS BIOCHIPS

Assembling steps of the IDCCM case and the biochips

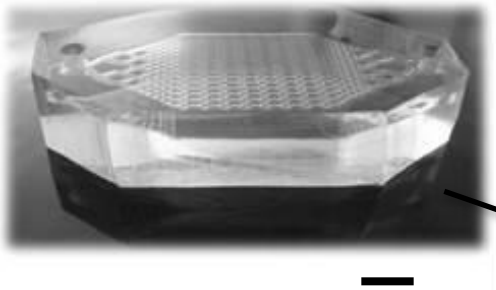






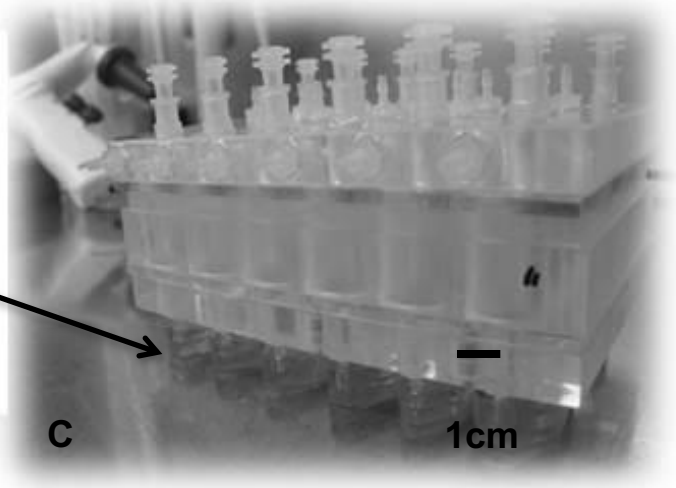


A



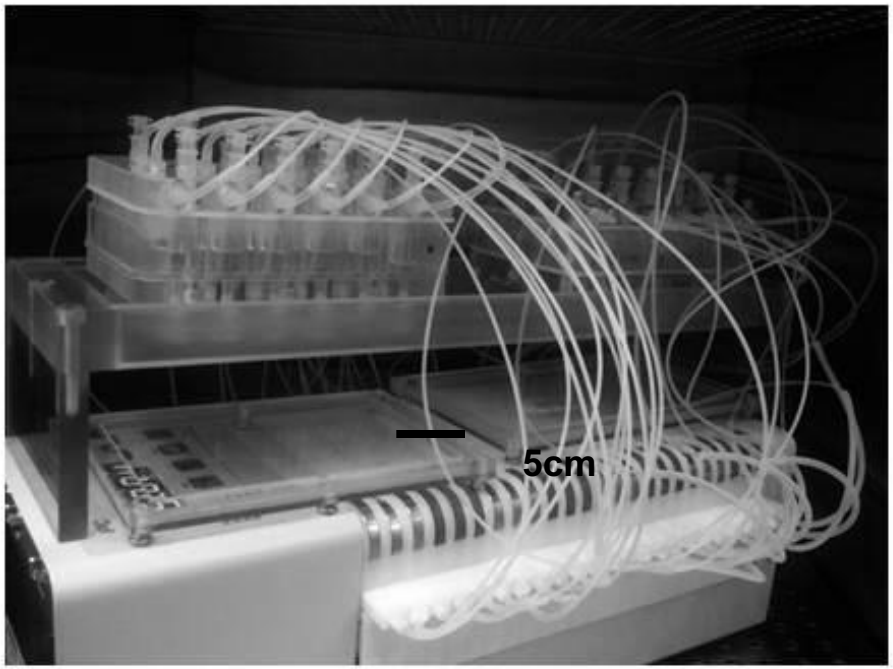
B

5mm



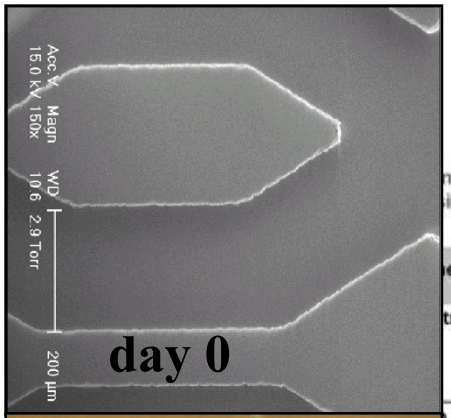
C

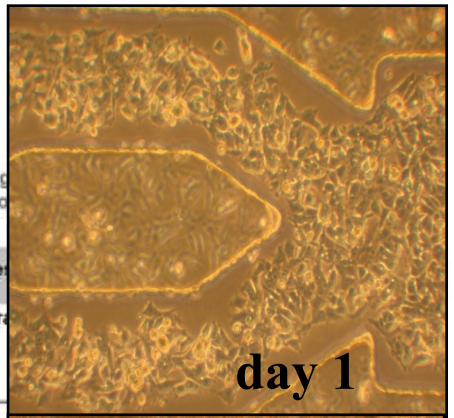
1cm

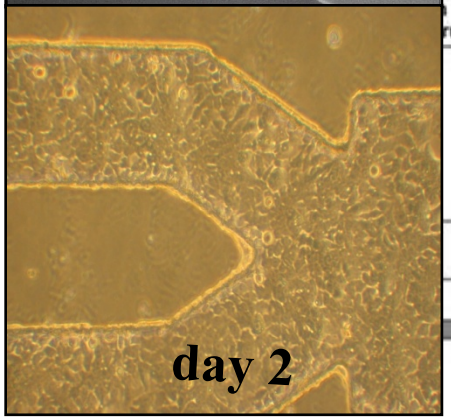


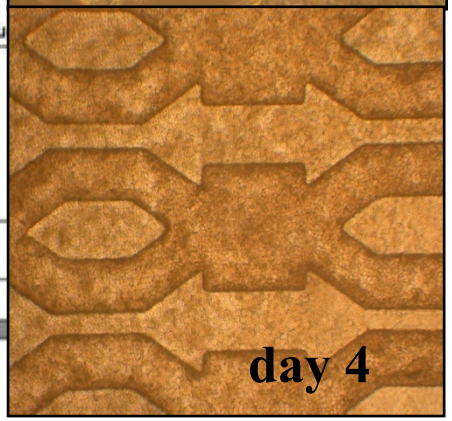
5cm

D



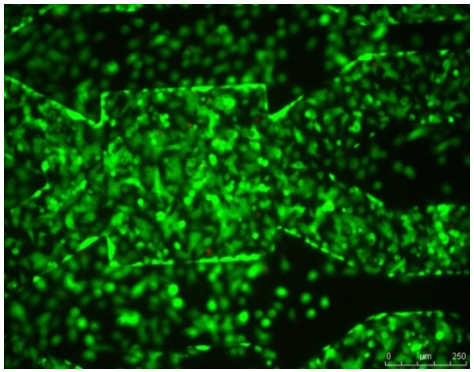




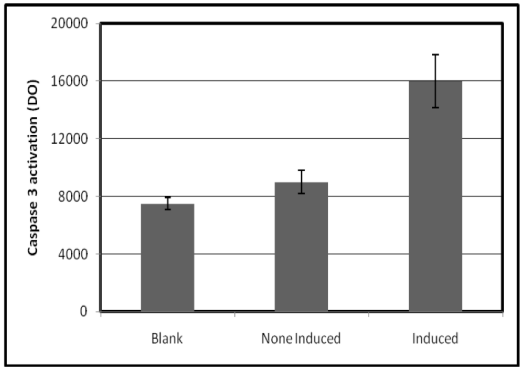


Baudoin et al., JBE 2011; sensor actuator, 2012, biotech prog 2014

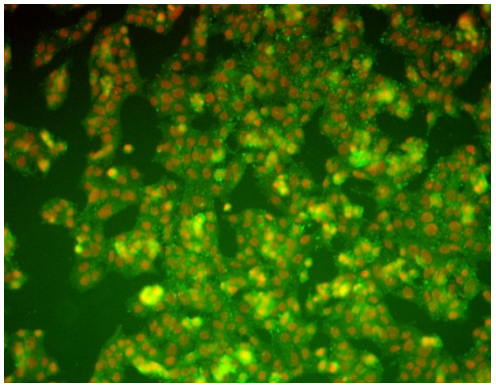
Viability, basal apoptotic situation in biochips of liver cells



Calcein AM/IP: viable cells



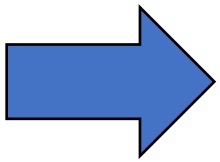
No caspase 3 activation

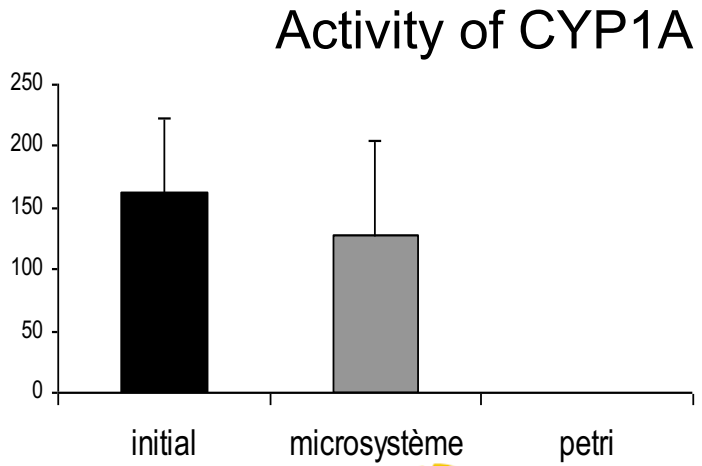


IP/Annexin V ; ++control (actinomycin D)

RTqPCR (U991)

	Day 4	
	Petri	Microfluidic Biochip
CYP3A4	100	1078
CYP1A2	100	18011
CYP1A1	100	16903
CYP2B6	100	182
CYP3A7	100	1660
CYP3A5	100	765
SULT1A1	100	353
SULT1A2	100	379
UGT1A	100	791
GR	100	106
CAR	100	71
PXR	100	286
AhR	100	258
HNF4α	100	261
MDR1	100	249
MRP2	100	393





Prot et al, Biotech Bioeng, 2011,Cheng et al., BMC genomics, 2012

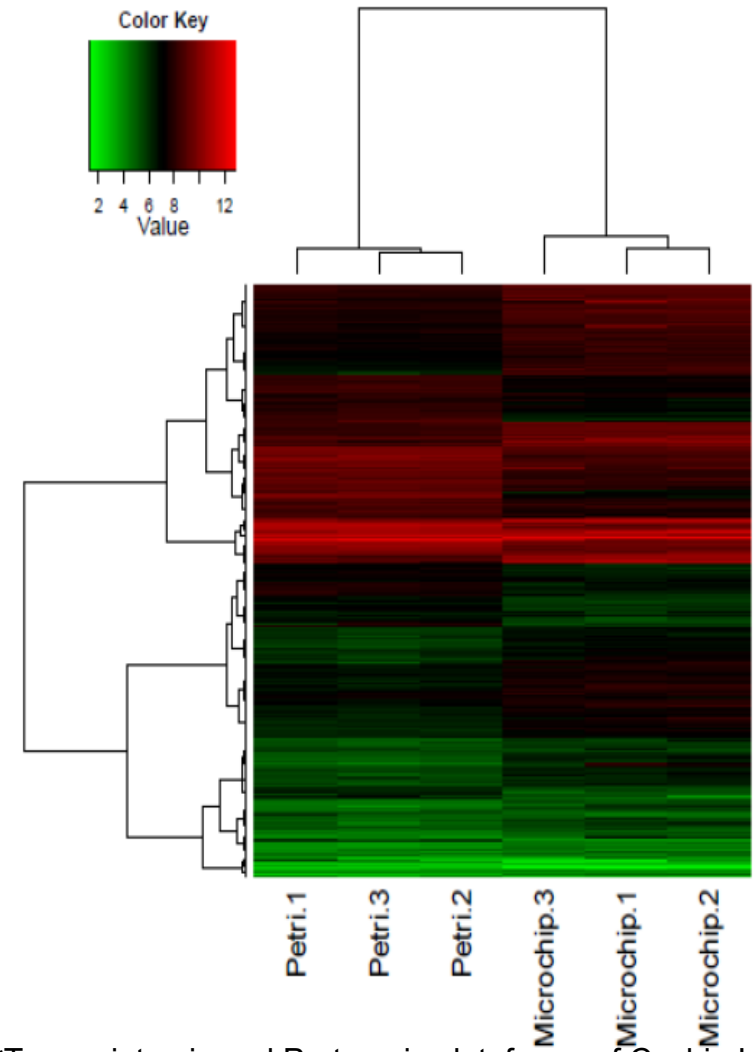
cnrs advancing the frontiers

LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820

INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

東京大学 THE UNIVERSITY OF TOKYO

Omics comparison in HepG2/C3a biochips and Petri



At the gene level:

4012 genes affected by the microfluidic cultures

At the protein level:

111 identified proteins affected by the microfluidic cultures

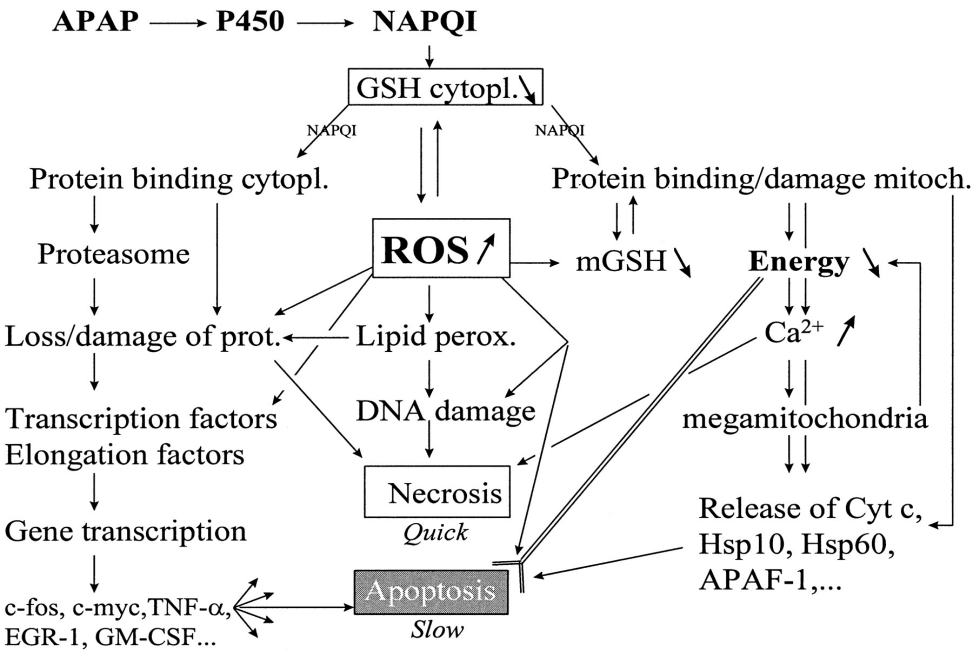
At the metabolome level:

40 identified metabolites affected by the microfluidic cultures

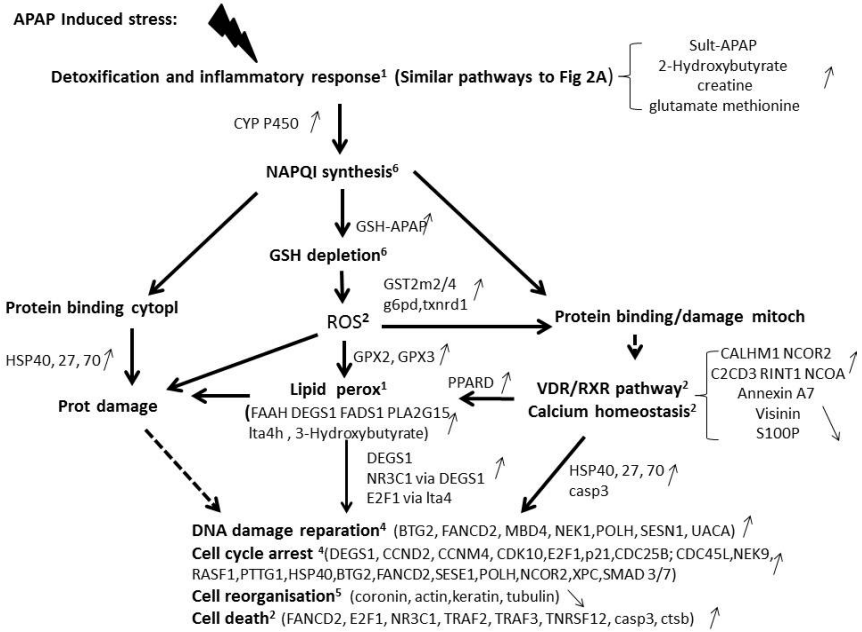
*Transcriptomic and Proteomic plateforms of Cochin Institute

Pathway and toxic network reconstruction, APAP application

Context: identification of the mechanism of toxicity



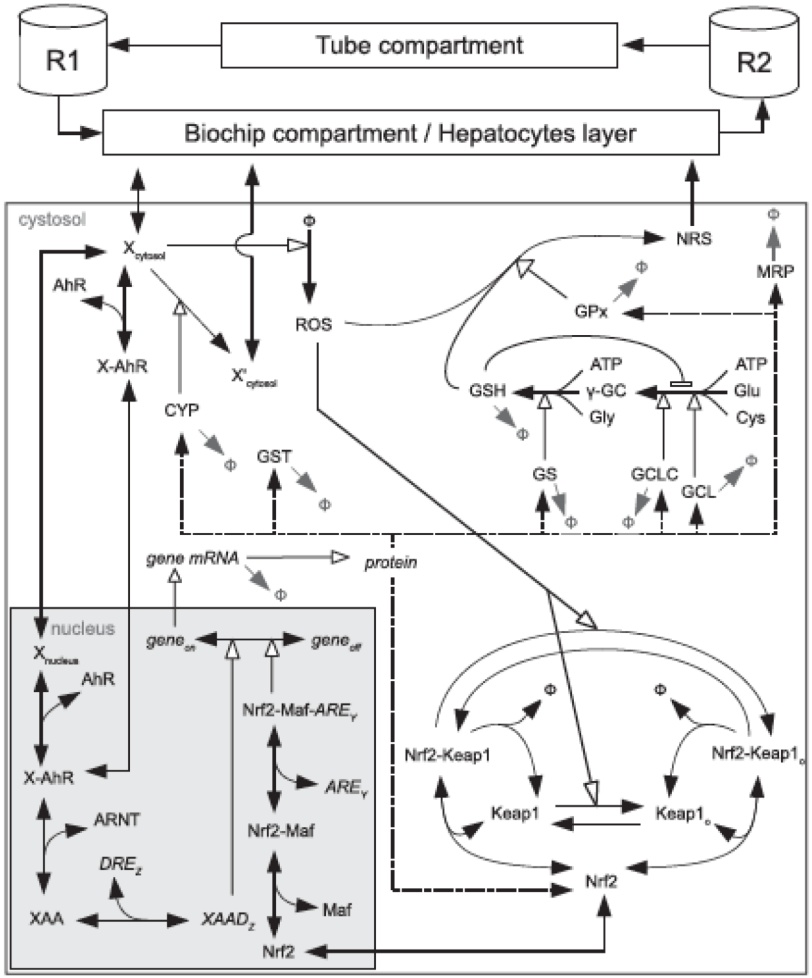
APAP *in vivo* data

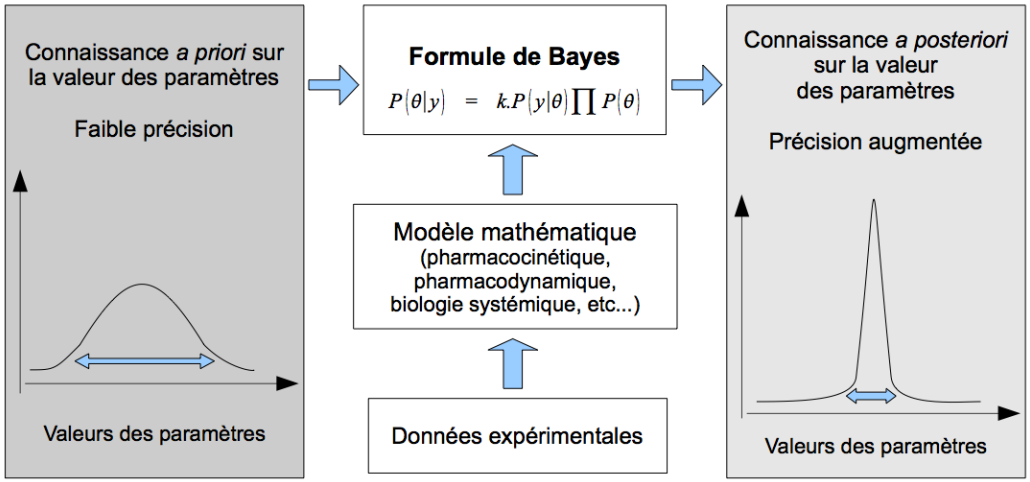


APAP biochip reconstruction

Prot et al, PlosOne 2012; Prot et al., TAAP 2012

Modeling strategy based on Bayesian statistics of a PK-Nrf2-GSH model





Leclerc et al; tox in vitro , 2014
Leclerc et al. Cell bio tox, 2015
Leclerc et al., J applied tox, in press

cnrs advancing the frontiers

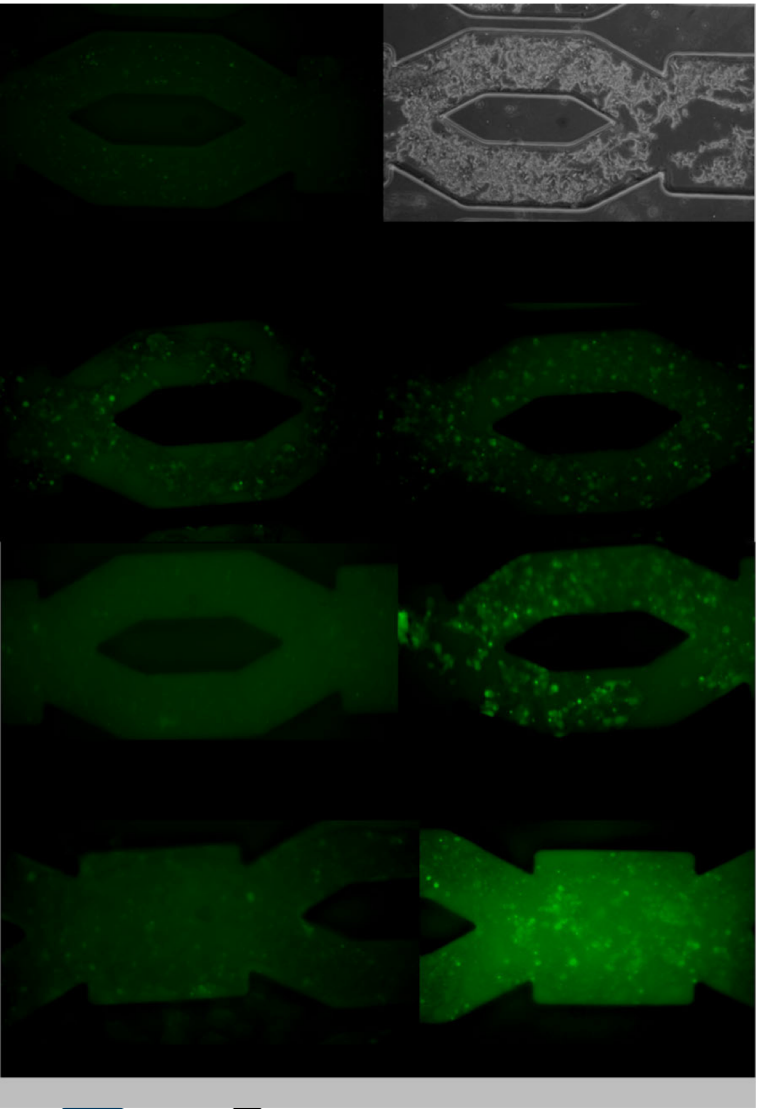
LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820

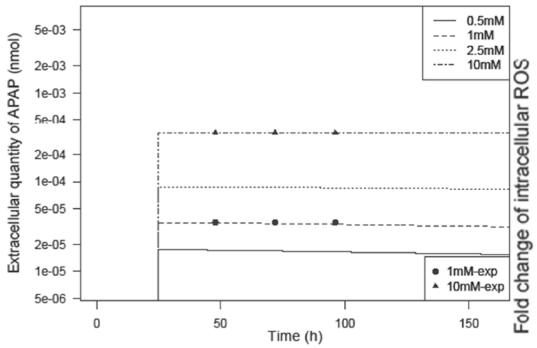
Collab chaire Tox Pred. UTC; INERIS

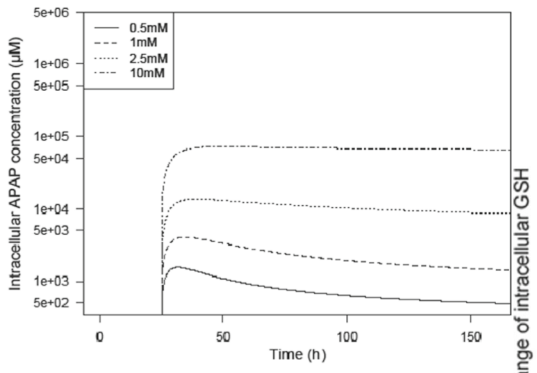
INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

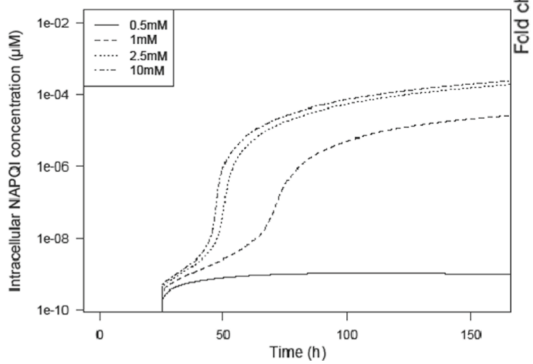
東京大学 THE UNIVERSITY OF TOKYO

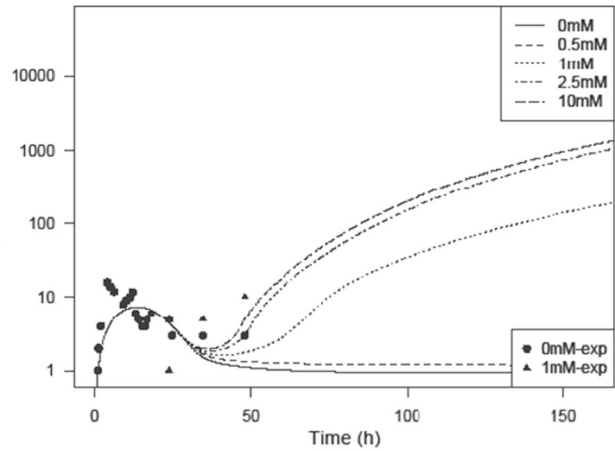
APAP-NAPQI toxicity via in silico PK-Nrf2-GSH model in HepG2/C3a

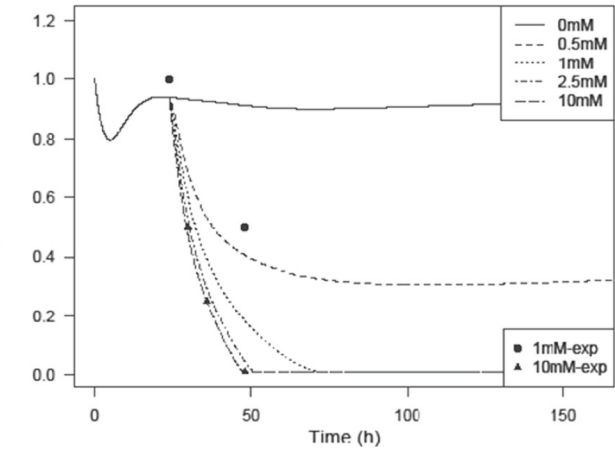












Leclerc et al; tox in vito , 2014
Leclerc et al. Cell bio tox, 2015
Leclerc et al., J applied tox, 2016

cnrs advancing the frontiers

LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820

Collab chaire Tox Pred. UTC; INERIS

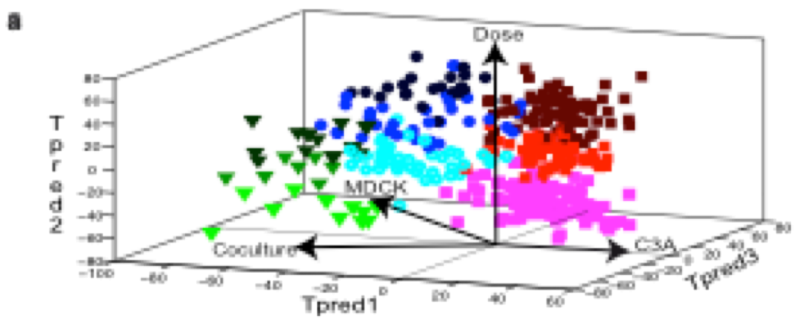
INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

東京大学 THE UNIVERSITY OF TOKYO

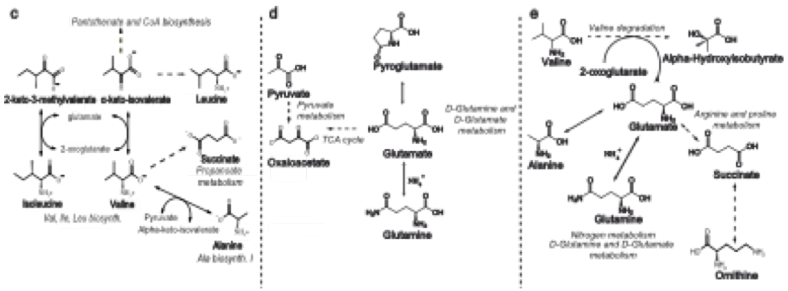
Metabonomic dose response analysis of chemical toxicity

Microfluidic signature database:

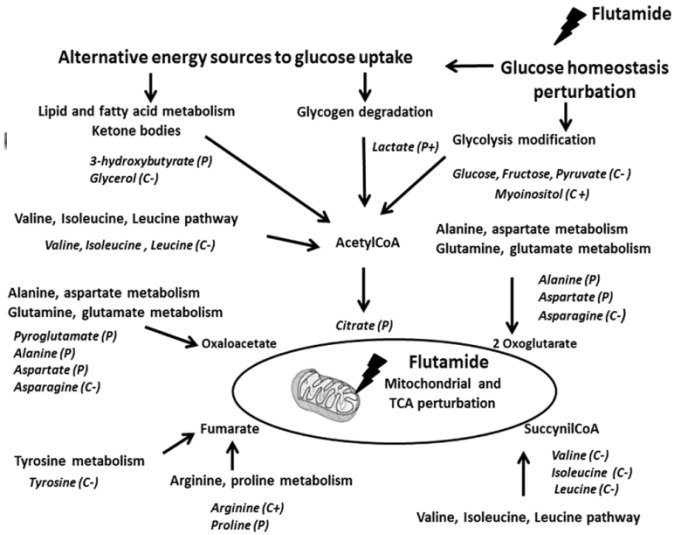
Liver, kidney, liver – kidney cocultures, Cell lines, primary cells, Petri , biochips
Molecules : APAP, Flutamine, Hydroxy flutamine, DMSO, NH3, MeOH, mixtures



Dose-trajectory model: dose & compound OPLSDA model



Specific Molecules signatures



Mechanistic interpretation

Coherence with in vivo reports ????
*Glucose homeostasis perturbation
*Mitochondrial damage

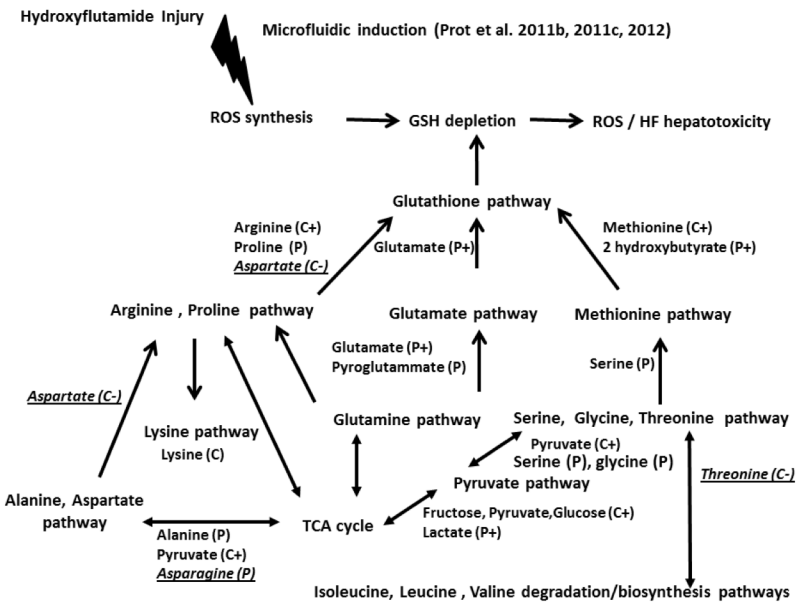
Shintu et al, anal chem 2012; Choucha et al., Tox Sci. 2013

Shintu et al., Anal Chem 2012

Collab CRMN Lyon

Biomarkers of GSH depletion in HepG2/C3a

Hydroxyflutamide related GSH Depletion



Choucha et al., Tox Sci. 2013

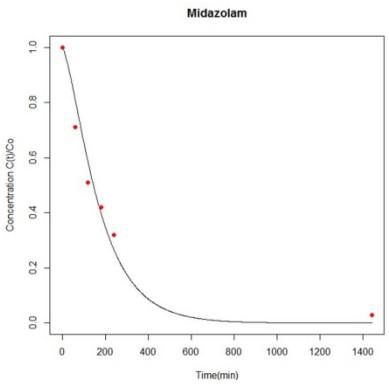
Hydroxyflutamide /APAP common biomarkers of GSH Depletion

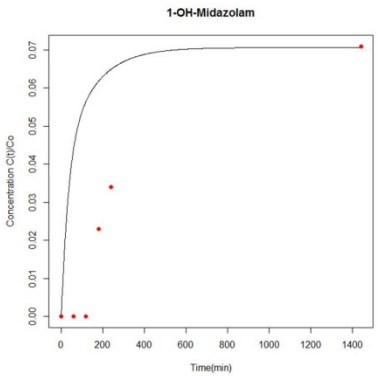
Compounds	Variations	α_s (9 control *vs* 6 HF biochips)	α_s (9 control *vs* 11 APAP biochips)
2-Hydroxybutyrate	**P+**	3.61×10^{-2}	$3.\times 10^{-3}$
Alanine	**P+**	3×10^{-1}	3×10^{-4}
Arginine	**C+**	7×10^{-3}	3×10^{-3}
Fructose	**C+**	2×10^{-3}	1×10^{-3}
Glucose	**C+**	2×10^{-3}	5.5×10^{-3}
Glutamate	**C+**	4×10^{-2}	2×10^{-2}
Lactate	**P+**	5×10^{-2}	9×10^{-3}
Lysine	**C/C+**	1×10^{-2}	5×10^{-3}
Methionine	**C+**	7×10^{-4}	5×10^{-2}
Proline	**P**	5×10^{-2}	2×10^{-2}
Pyroglutamate	**P**	4.61×10^{-3}	6×10^{-2}
Pyruvate	**C+**	6×10^{-1}	6×10^{-4}
Serine	**P**	2×10^{-3}	2×10^{-2}

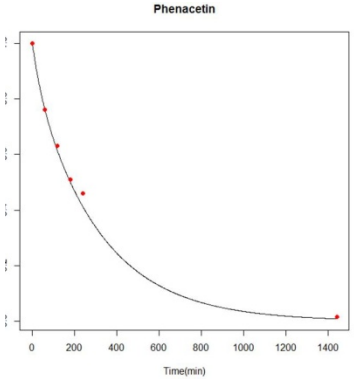
24h-10µM-hydroxyflutamide (HF) 72h-1mM-acetaminophen (APAP)
Both conditions lead to hepatotoxicity *via* cell death when compared to controls.

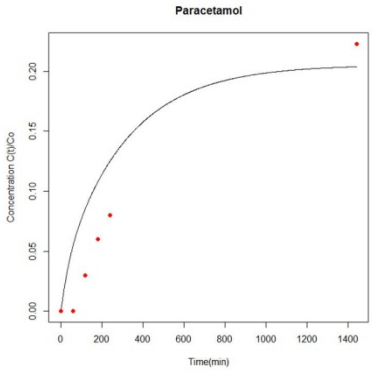
cnrs advancing the frontiers
LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820
INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO
東京大学 THE UNIVERSITY OF TOKYO

Drug clearance and extrapolation *in vitro in vivo* in rat









$$\frac{dQ_{HepB}(t)}{dt} = F \times \left(C_{R1}(t) - C_{HepB}(t)\right) - fu_{med} CL_{\text{int},invitro} C_{HepB}(t)$$

Estimated clearance via in vitro PK

$$CLh = \frac{Q_H \times \frac{fu_b}{fu_{med}} \times CL_{int,\ in\ vitro} \times SF}{Q_H + \frac{fu_b}{fu_{med}} \times CL_{int,\ in\ vitro} \times SF}$$

Predicted in vivo clearance via organ model

	Models predictions (ml/min/kg of BDW)	Literature data (ml/min/kg of BDW)
	$CLh_{\ in\ vivo}$	$CLh_{\ in\ vivo}$
Phenacetin	72	84[b]
Paracetamol-Phe	41	23.8[d]
Paracetamol	30	23.8[d]
Propranolol	85	100[b]
Tolbutamide	0.1	0.48[b]

	Models predictions (ml/min/kg of BDW)	Literature data (ml/min/kg of BDW)
	$CLh_{\ in\ vivo}$	$CLh_{\ in\ vivo}$
Caffeine	13.08	12[b]
Paraxanthine	4.2	15[g]
Dextromethorphan	61	80[b]
Dextrorphan	9.5	---
Midazolam	44.1	44[b]
1 OH-Midazolam	21	62.2[l]

Baudoin et al; J of Pharm Sci. , 2014; Legendre et al, J Pharm Sci, 2013

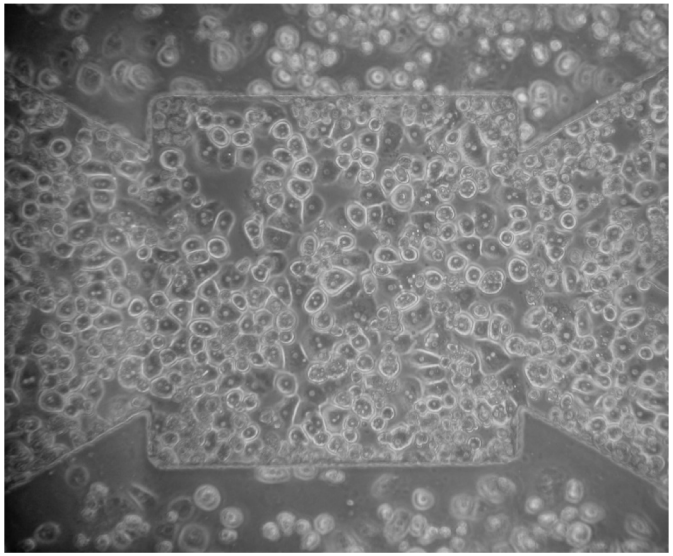
cnrs advancing the frontiers

LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820

INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

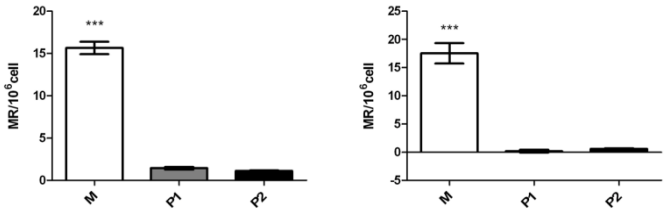
東京大学 THE UNIVERSITY OF TOKYO

Higher metabolic performance in biochips versus Petri for human primary cryopreserved hepatocytes



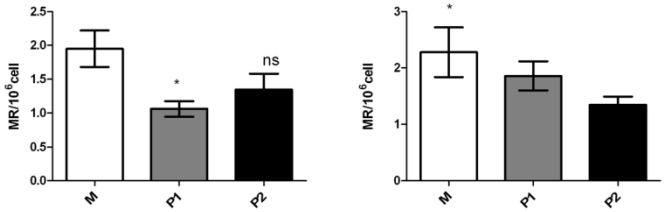
Gene name	post decongelation n=4	Dynamic biochips n=7	Static biochips n=3
HNF4a	100	85±2	6±1
PXR	100	54±1	5±1
CYP1A2	100	80±3	6±1
CYP2B6	100	27±1	1.5±0.2
CYP3A4	100	53±2	0.7±0.2
OATP2	100	98±2	3±0.5
Pgp	100	74±4	74±8

Dextromethorphan



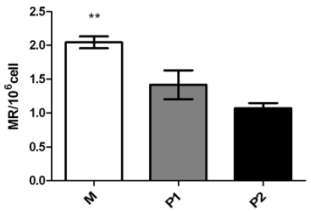
Transformation in Dextrorphan/ 3-Methoxymorphinan

Omeprazole



Transformation in 5-OH-omeprazole/ Omeprazole-sulfone

Acétaminophène



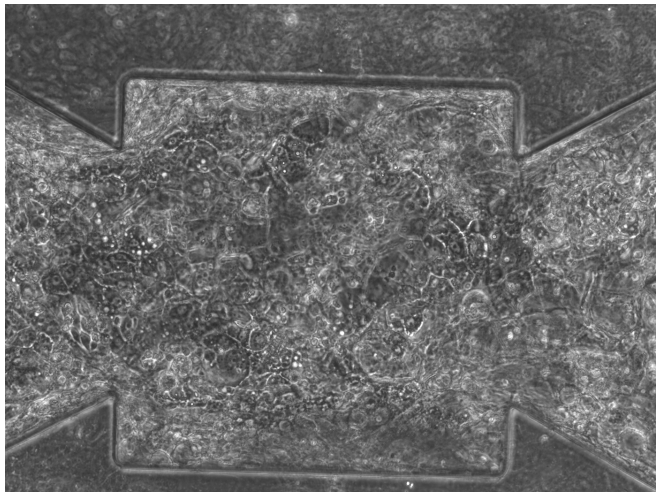
Transformation in Acetaminophen-glucuronide

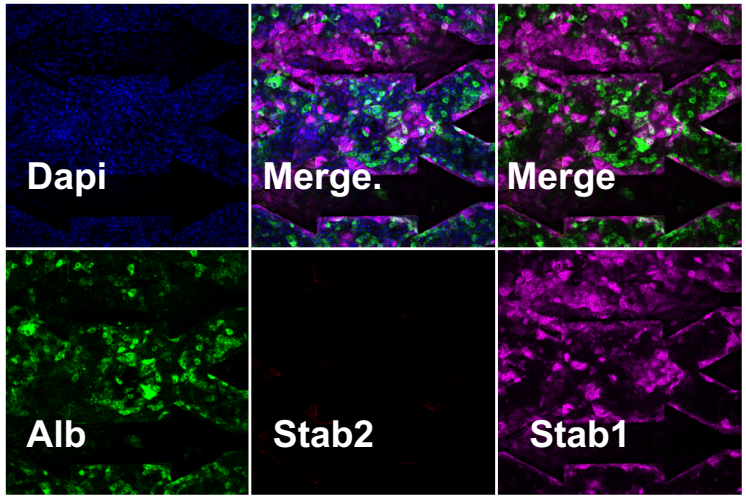
Prot et al, Int J Pharm, 2011

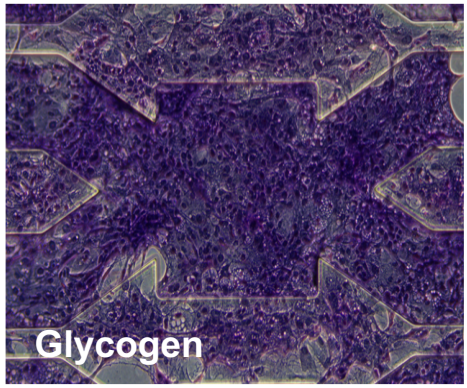
Laboratory for Integrated Micro-Mechatronic Systems
LIMMS/CNRS-IIS UMI 2820

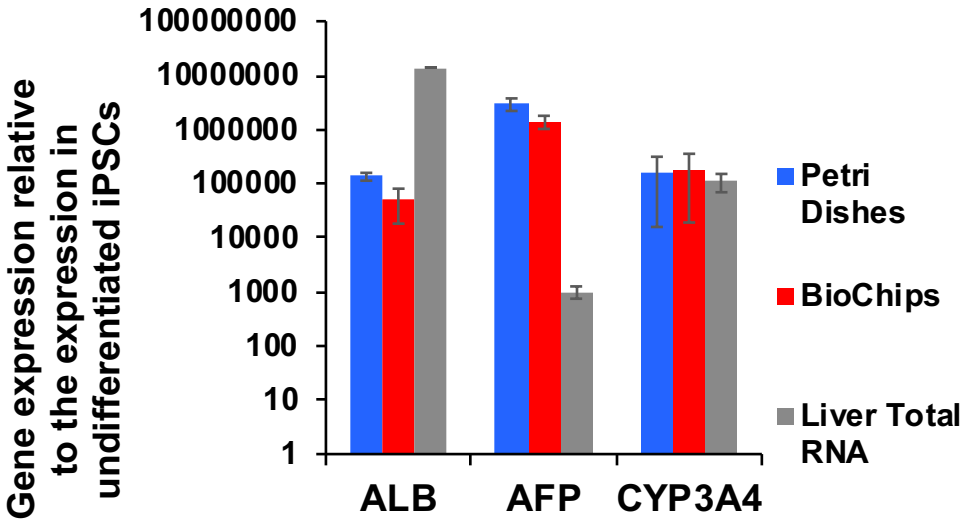
THE UNIVERSITY OF TOKYO

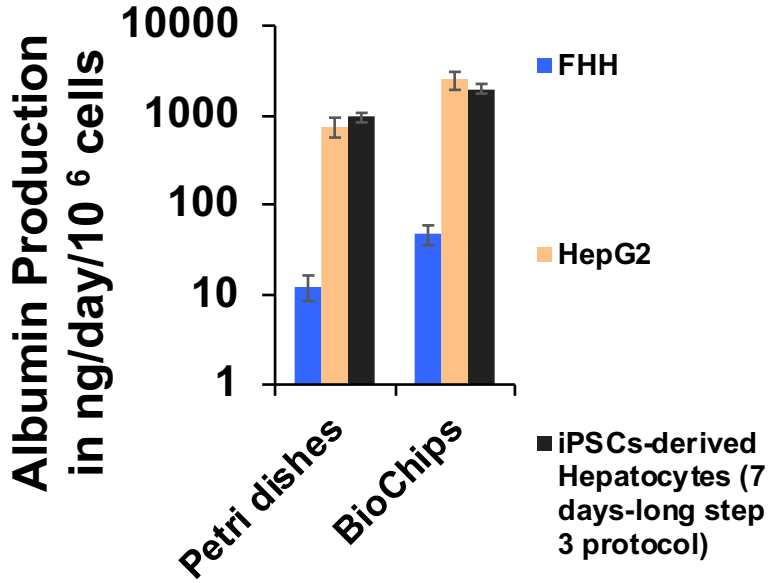
Toward personnalised medicine using iPSC











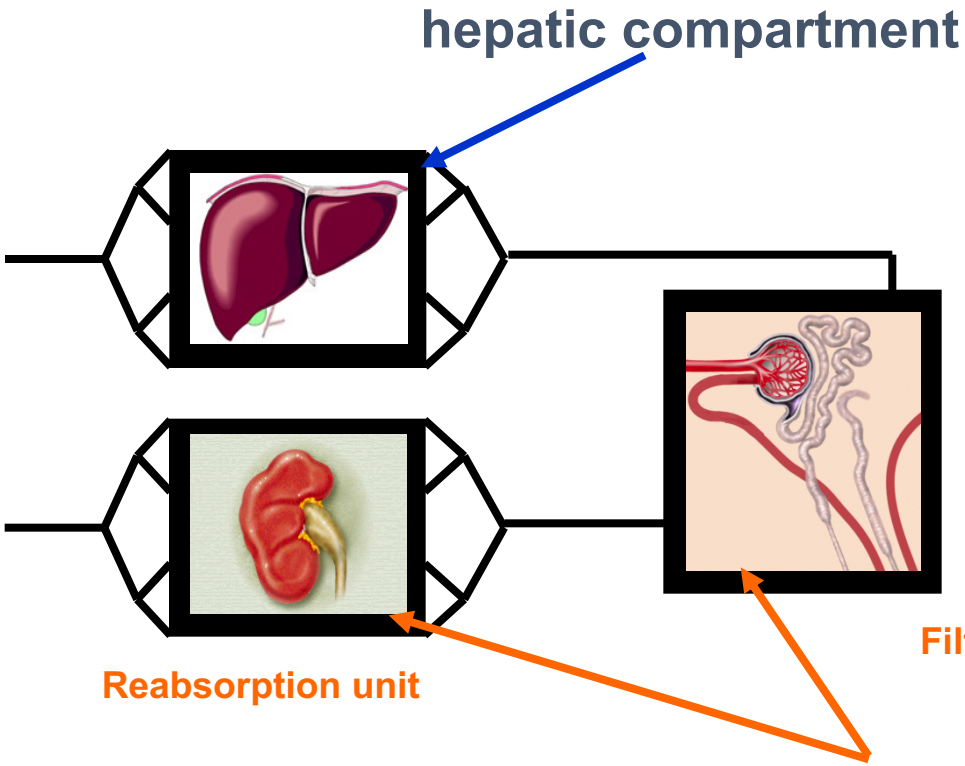
cnrs advancing the frontiers

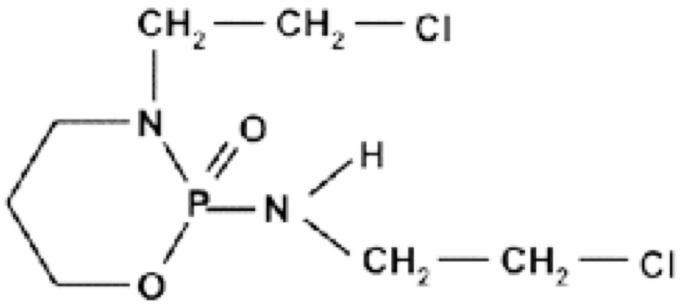
LIMMS
Laboratory for Integrated Micro-Mechatronic Systems
LIMMS/CNRS-IIS UMI 2820

INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

東京大学
THE UNIVERSITY OF TOKYO

Liver kidney organs interactions





Ifosfamide

Antinéoplasic drug →Solid tumors

Nitogen mustard:

Alkylation: crosslink with DNA

Apoptosis of cancerous cells

ANR PCV2007 µHepaReTox :
UMR CNRS 7338, INERIS (METO), INSERM (991), CNRS UMR 8029 (SATIE BioMIS)

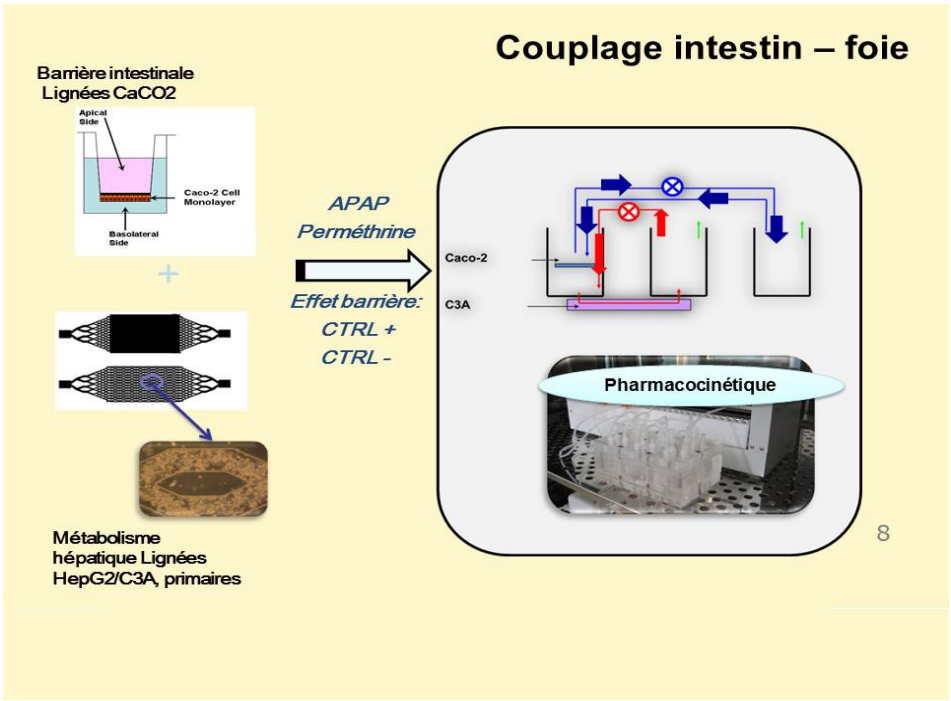
cnrs advancing the frontiers

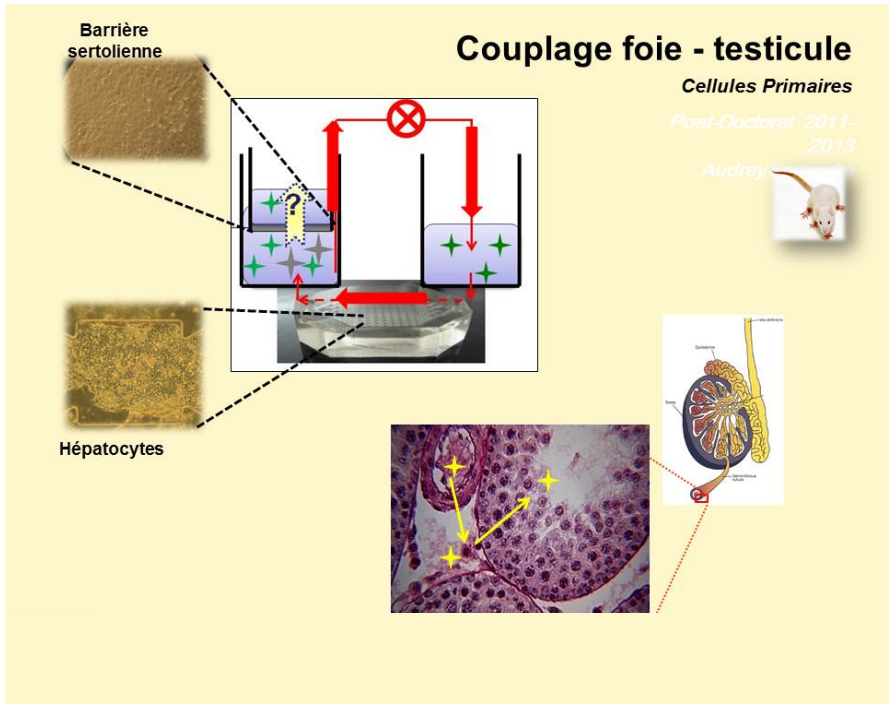
LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820

INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

東京大学 THE UNIVERSITY OF TOKYO

Integration of barrier in organ on chip module





Under going organ to organ interactions:

- intestine liver = first pass
- liver testis = site of toxicity

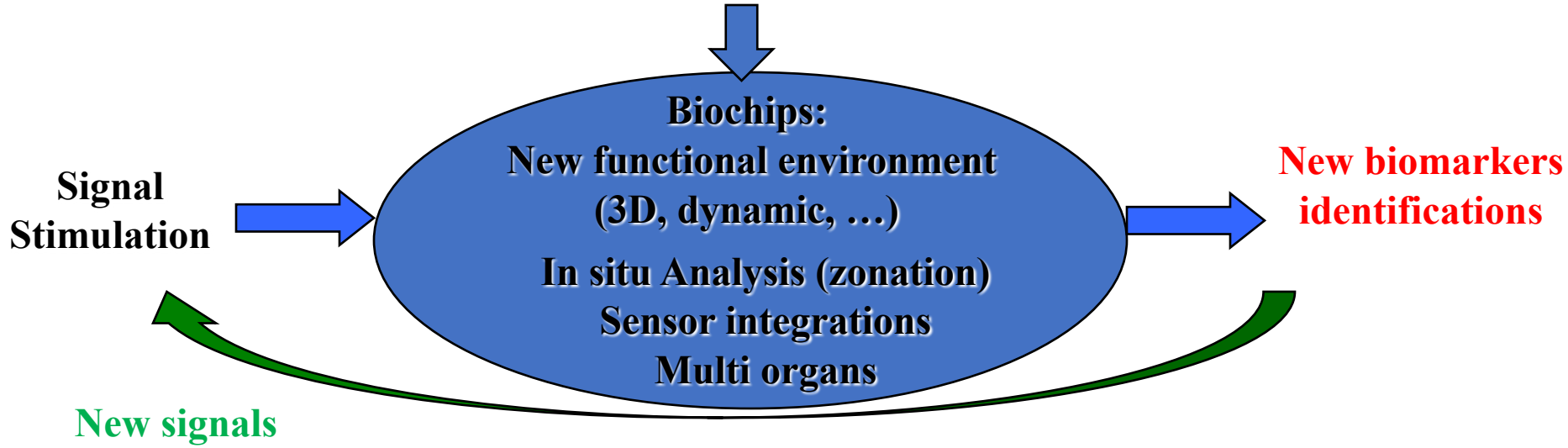
Project Fondation/UTC
pesticides, drugs

Microfluidic integrative approach

Objectives: Understanding of biological systems

Problems : Pertinence of actual model

Conventional and new type of bio analysis



Solutions:

On chip system biology
New signals= new responses

→ Identification of biological processes

Remerciements

Universite de Technologie de Compiegne:

- C. Legallais (Artificial organs)
- R. Baudoin, A Legendre, J.M. Prot, P.E Poleni, T. Bricks (Bioartificial microchip)
- L Choucha Snouber, C Ramello, C Desrousseaux (Bioartificial microchip, Kidney data)
- P. Paullier, R Jellali (filtration expriment, biochip design, material sciences)
- F. Merlier (HPLC-MS)

Collaborations with

University of Tokyo: Profs Fujii Sakai, Miyajima, Okitsu

Riken : S Poulain, C Plessy

INSERM U991: A. Corlu, C. Aninat, C Guguen Guillouzo (Liver biologists)

Institut Cochin: F. Letourneur and P. Chafey (Transcriptome and Proteome data)

CEA Saclay: E. Ezan, G. Madalinski, Henri Benech, Gregory Nicolas (MS, CIME)

CRMN: M. Dumas, P Toulhoat, L . Shintu, B Helena-Herrnann, A Bunescu (RMN)

INERIS: A Pery, C Brochot, F Bois , D Ouattara, J Hamon (in silico)

Grants: Région Picardie, Fond Social Européen, Fondation UTC

ACI jeune chercheur, ANR PCV, CP2D, émergence, RHU iLite

cnrs advancing the frontiers

LIMMS Laboratory for Integrated Micro-Mechatronic Systems LIMMS/CNRS-IIS UMI 2820

INSTITUTE OF INDUSTRIAL SCIENCE THE UNIVERSITY OF TOKYO

東京大学 THE UNIVERSITY OF TOKYO

Merci de votre attention