



Plate-forme de
Chimie Biologique
Intégrative de Strasbourg

UMS 3286 CNRS-UdS



Can HTRF technology answer to different scientific
questions ?

An academic with industrial standards point of view



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1997



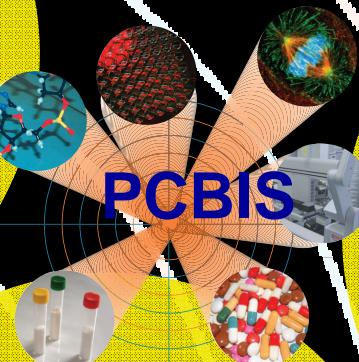
Marcel
HIBERT



Jacques
HAIECH



Jean-Luc
GALZI

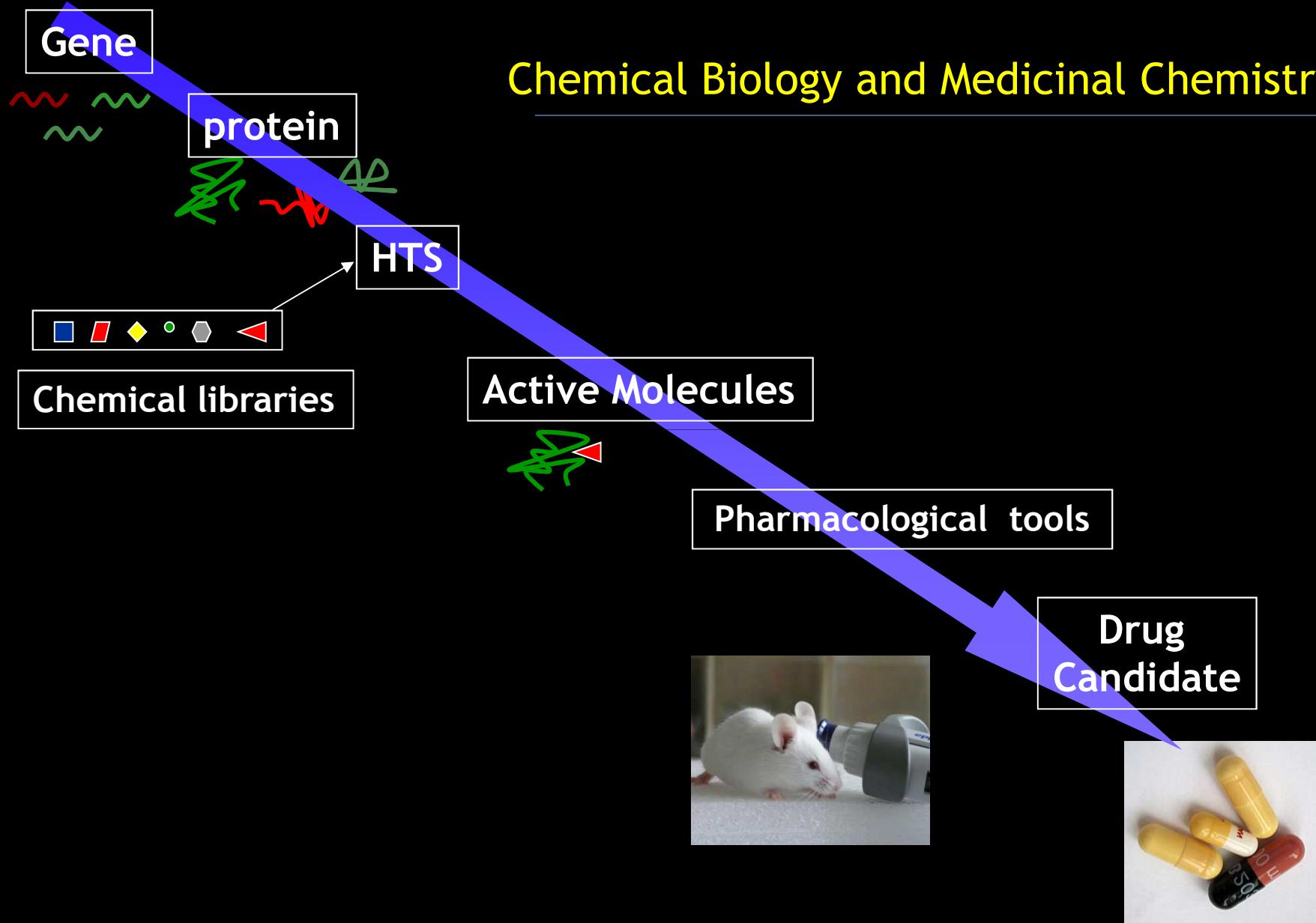


P. Villa / Illkirch
27 juin 2012



Plate-forme de chimie
biologique intégrative
de Strasbourg

Chemical Biology and Medicinal Chemistry



QUALITY ?

ISO 9001



CERTIFICATE OF APPROVAL

This is to certify that the Quality Management System of:

PCBIS
(Platform of integrative chemical biology of Strasbourg)
CNRS-Université de Strasbourg
Illkirch, France

has been approved by Lloyd's Register Quality Assurance France SAS
to the following Quality Management System Standards:

ISO 9001:2008

The Quality Management System is applicable to:

**High throughput screening, chemical libraries, target
libraries, ADME-Tox.**

Approval
Certificate No: FQA 4000448

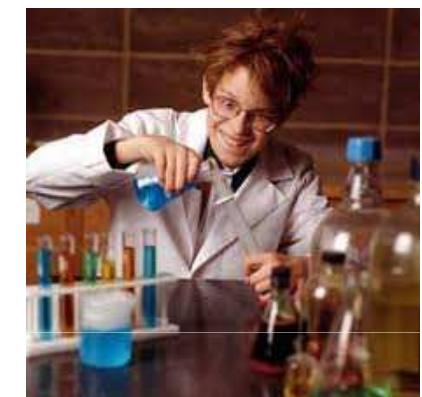
Original Approval: 16th January 2007
Current Certificate: 16th January 2010
Certificate Expiry: 15th January 2013


Issued by: Lloyd's Register Quality Assurance France SAS



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1, boulevard Vivier Merle, 69443 Lyon cedex 03
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Document 11

Bridge ?



Biology

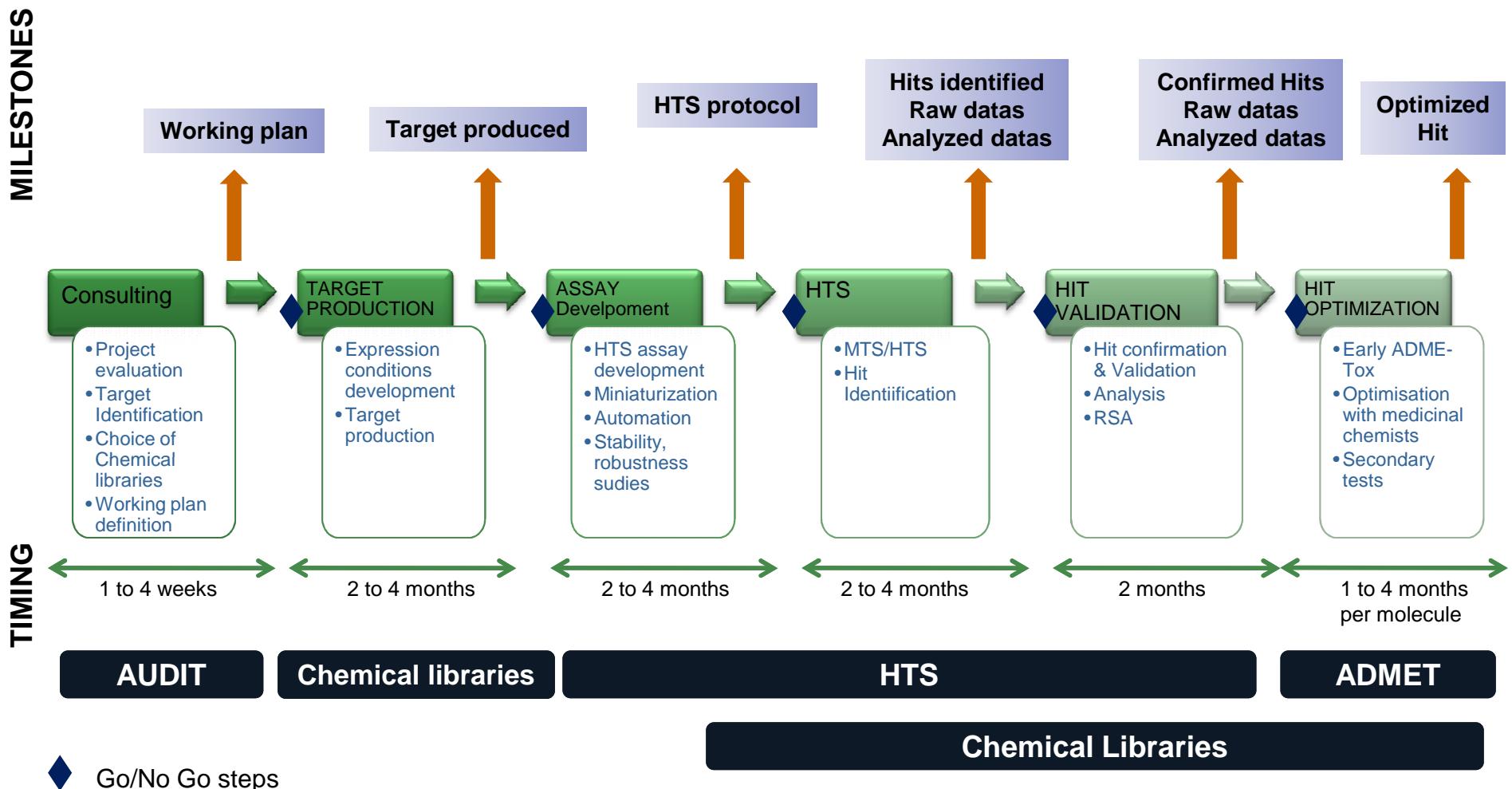


Chemistry

P. Villa, Avignon
2013 Apr 26

Bridge !





Assay	Model	Technology	Format
Cytokine secretion (TNF α , IL-1,6,8...)	Peripheral blood mononuclear cells and other cell types	ELISA HTRF	96
Nitrite oxide (NO) production	RAW cells	Absorbance	96
Kinase activity detection	Ser/thr kinase	HTRF/Luminescence	384
Cell growth	Bacteria (E. coli) Eucaryotic cells	Absorbance Confluence measurement	96 96
Cell survival	HEK293, HepG2, MCF7, Caco2, RAW, HeLa, HL-60, U937, your cells...	Absorbance Luminescence	96 / 384 /1536
Cell death	Caspase activity	luminescence	96 and 384
- Aggregation - Size measurement	Soluble protein	Dynamic Light Scattering (DLS)	96 and 384
Calcium flux	GPCR Calmodulin	Fluorescence	96 and 384
Cyclic AMP	Cells	Luminescence HTRF	96 and 384
Tissue regeneration (scratch wound)	Cell lines	Confluence measurement	24
Membrane receptor Binding	GFP fused GPCR expressing HEK293	FRET HTRF	96
Molecular Binding	Soluble protein	Anisotropy	96 and 384
Permeability	Caco2	Cell monolayer	24
Your favorite assay	Your model	Compatible with our apparatus	96-384

TechMed

Preclinical ADME

- **permeability**
- **plasmatic protein binding**
- **metabolism: plasma, liver**

Physicochemical Properties

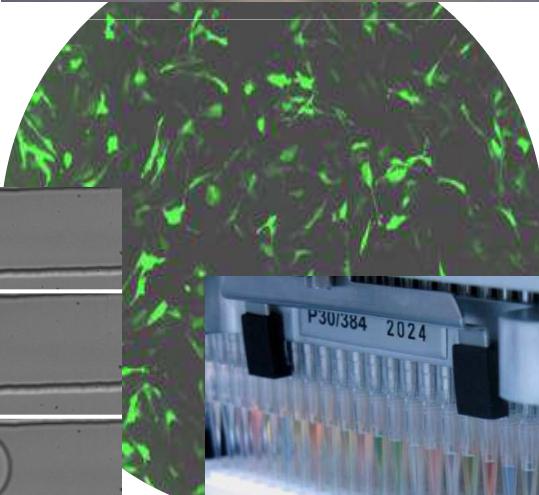
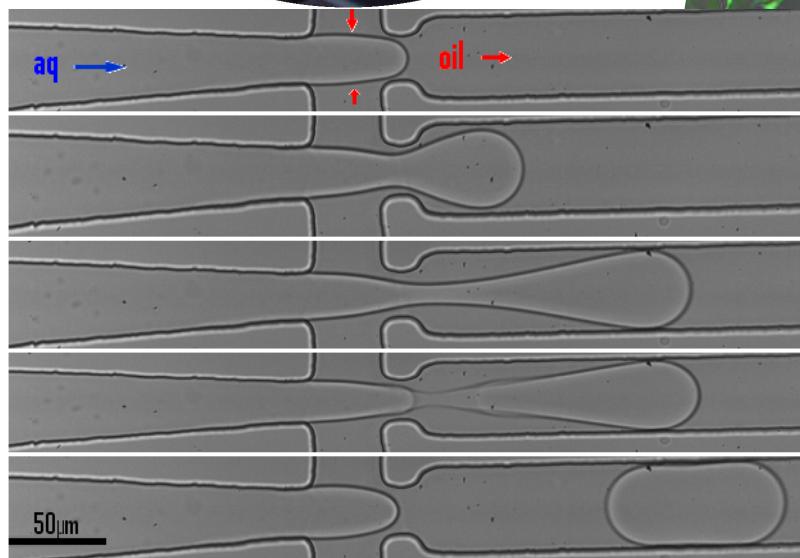
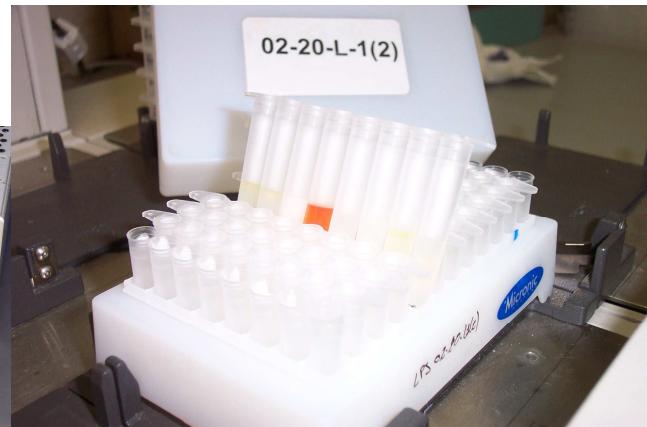
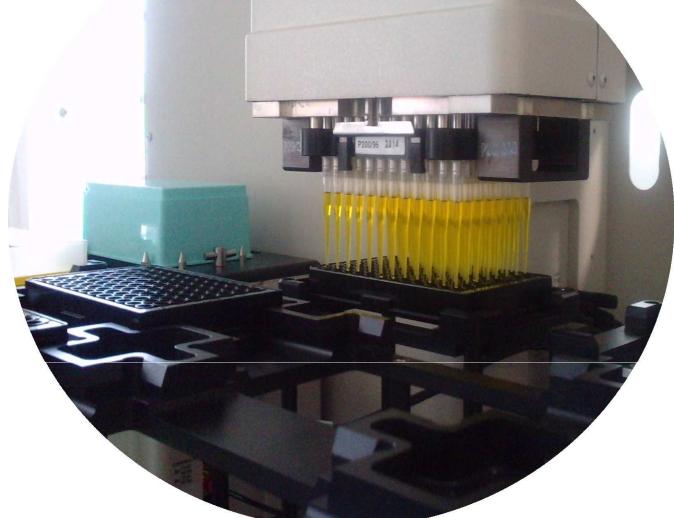
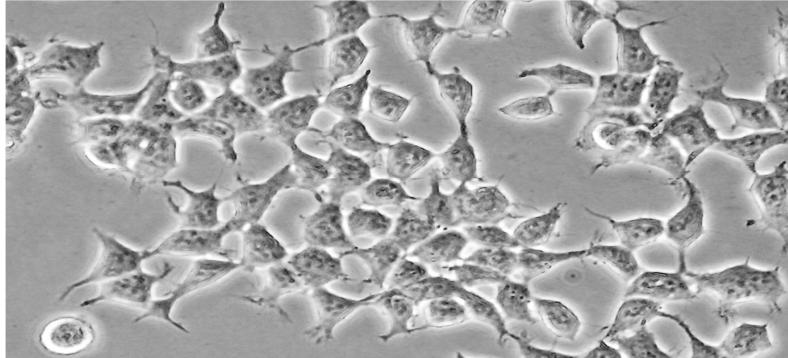
- **Solubility**
- **Log D**
- **CHI**
- **pKa**
- **Chemical stability**

In vivo Pharmacokinetics

- **BBB**
- **Bioavailability**

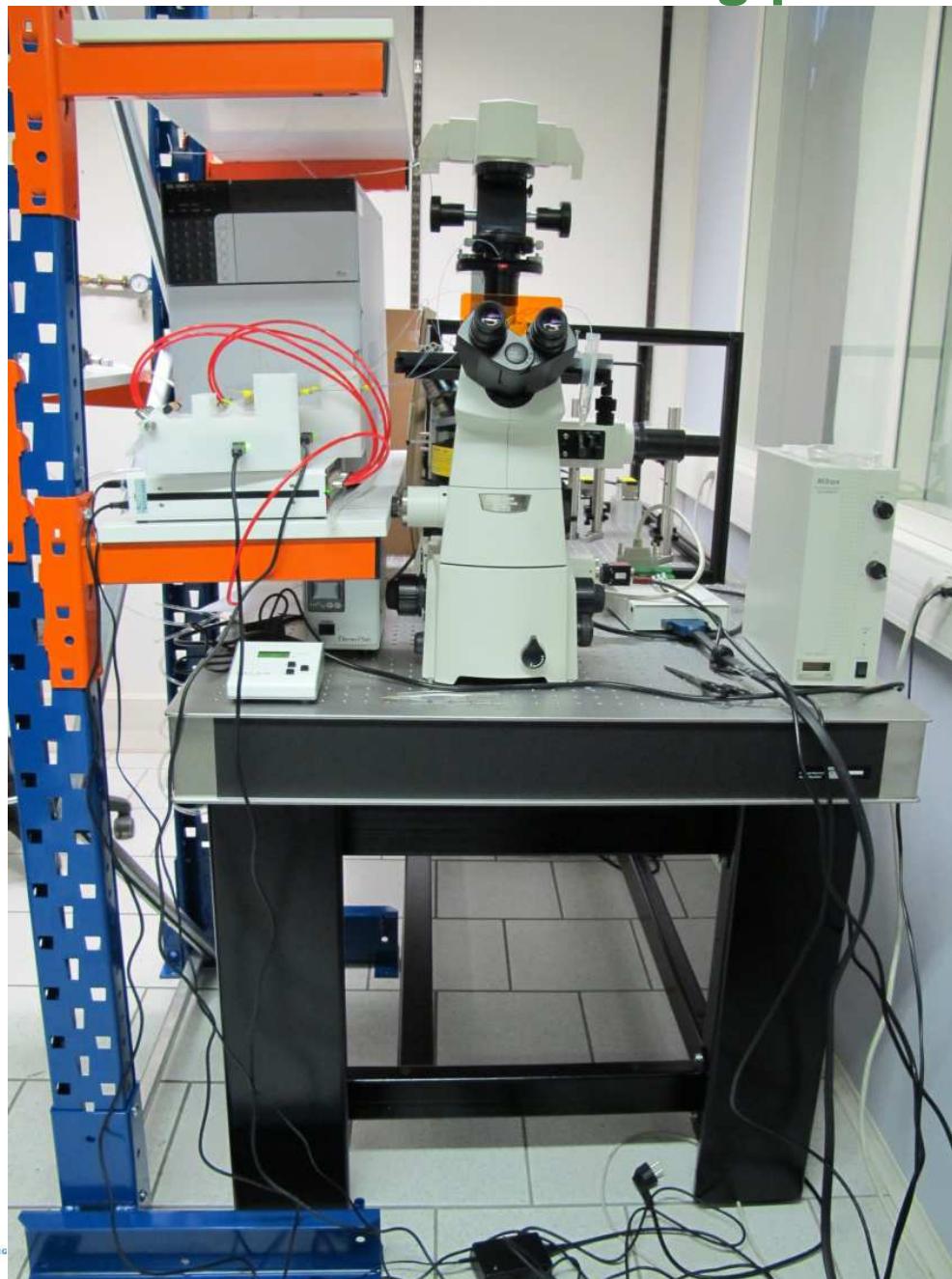
Cytotoxicity







Microfluidic screening platform



Quantitative HTS

Current and future HTS processes



Compound Library

Primary HTS
1 Point, 1 Concentration

Confirm Positives
2 Points, 1-2
Concentrations

Measure IC50s
2 Points, 8 Concentrations

Lead Chemical Series
Enter Compound
Optimisation Programs

Quantitative HTS

Current and future HTS processes



Compound Library

Primary HTS

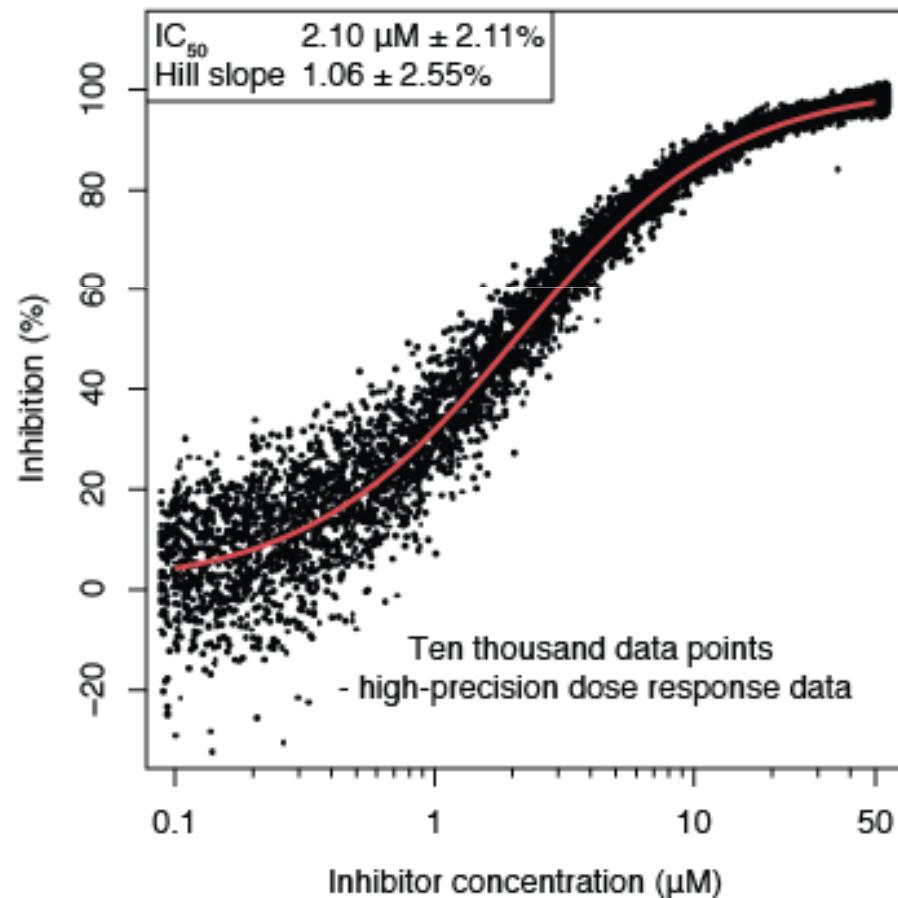
1 Point, 1 Concentration

Confirm Positives
2 Points, 1-2
Concentrations

Measure IC₅₀s
2 Points, 8 Concentrations

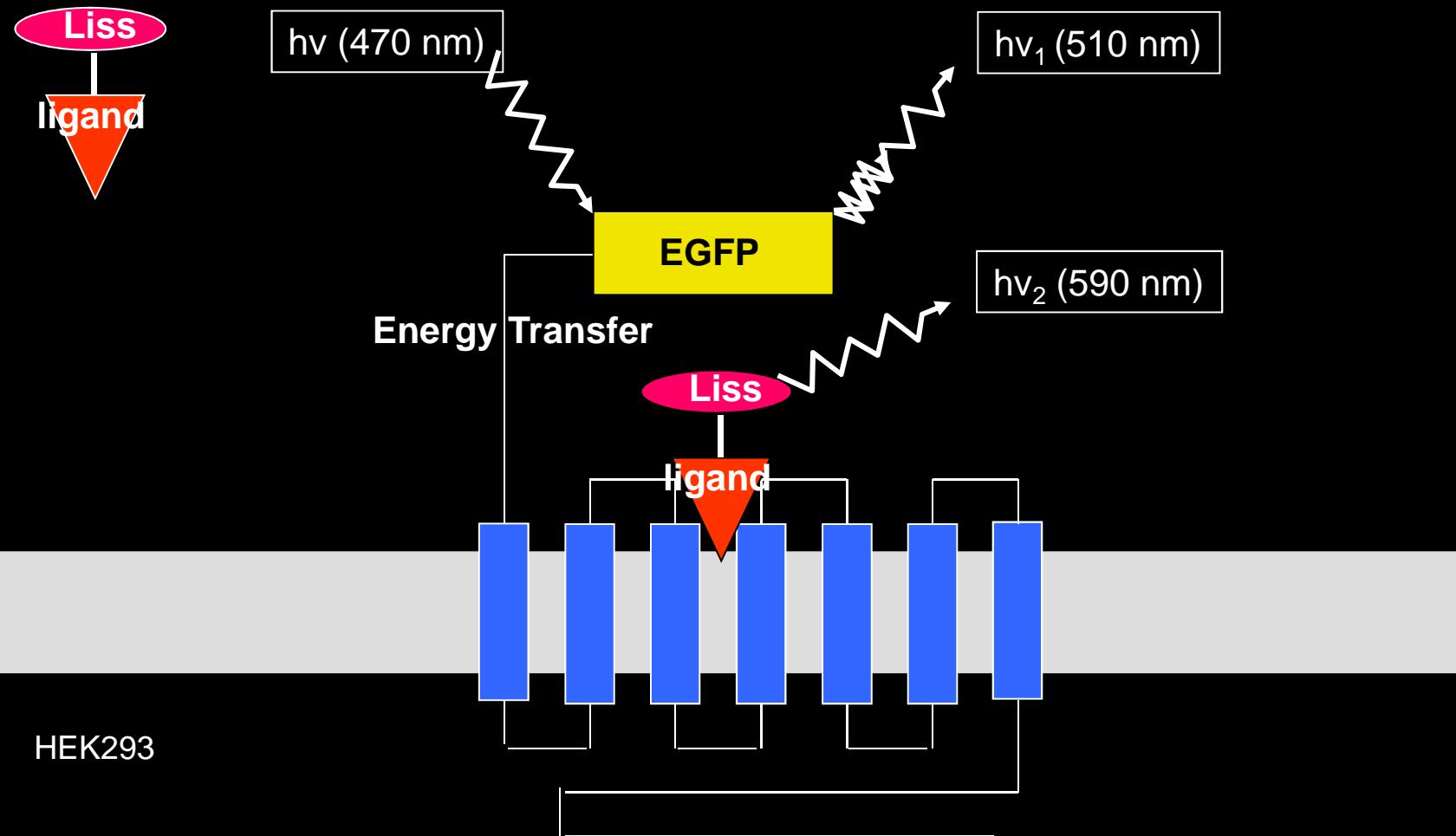
Lead Chemical Series
Enter Compound
Optimisation Programs

High-resolution dose-response curve



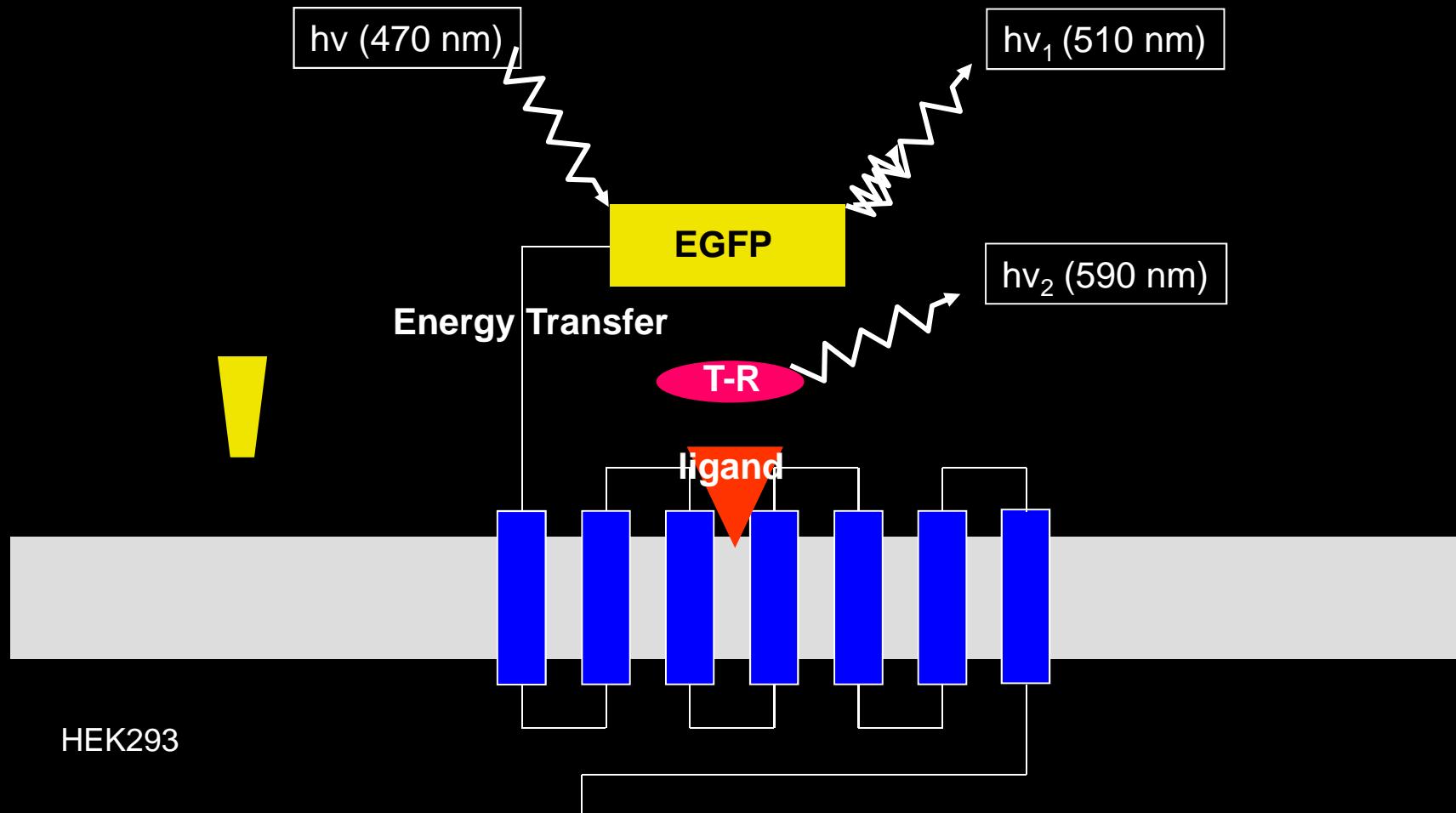
Generic assay

FRET-based binding assay



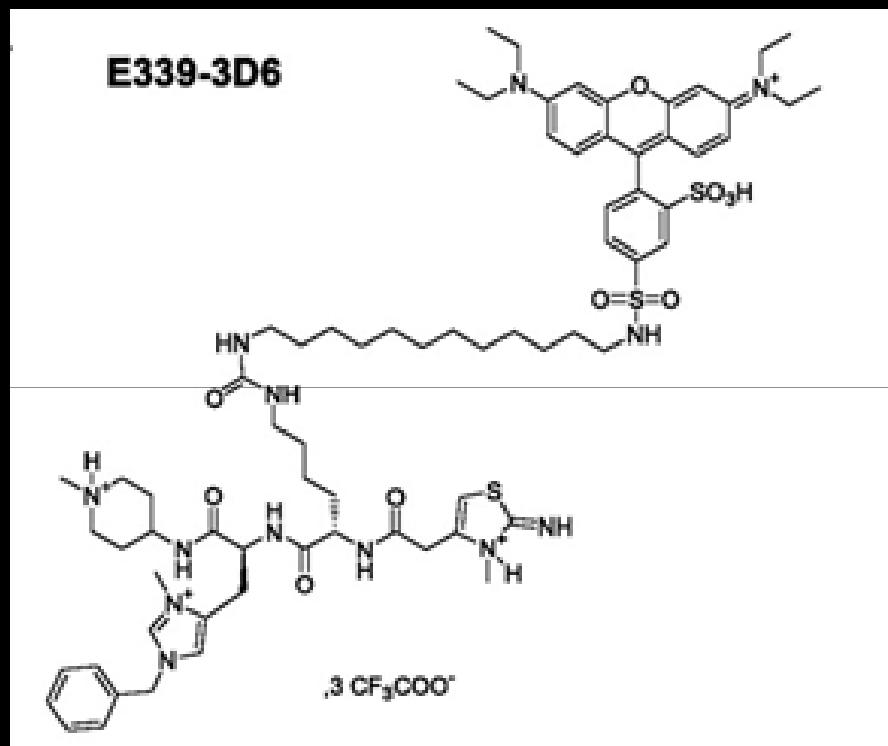
Generic assay

FRET-based binding assay

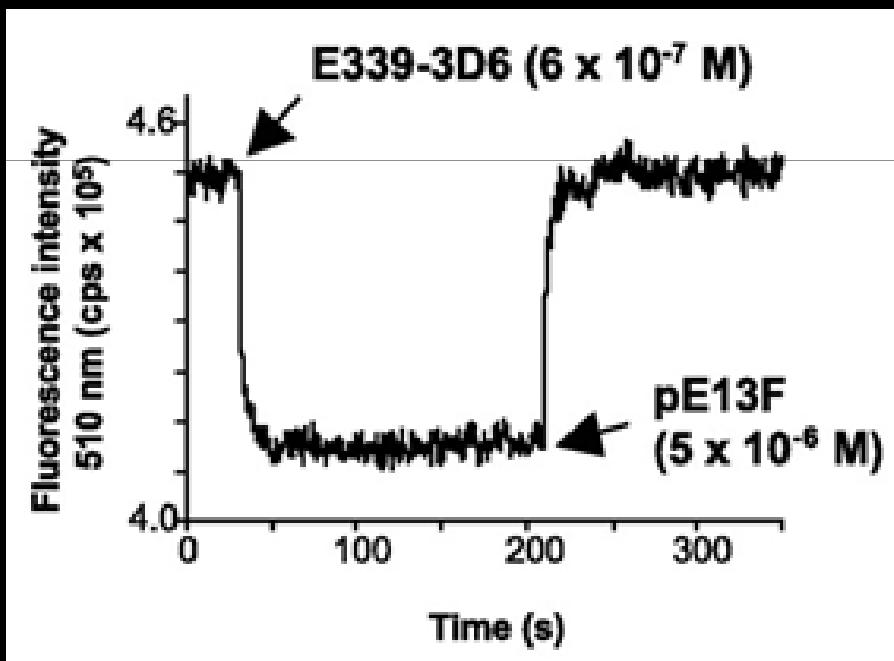
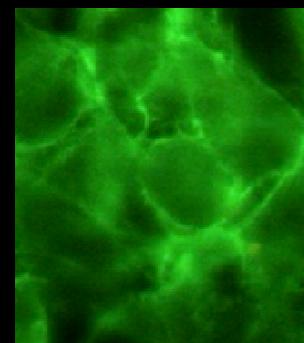


Example : orphan RCPG

JL Galzi
M Hibert
D Bonnet



APJ



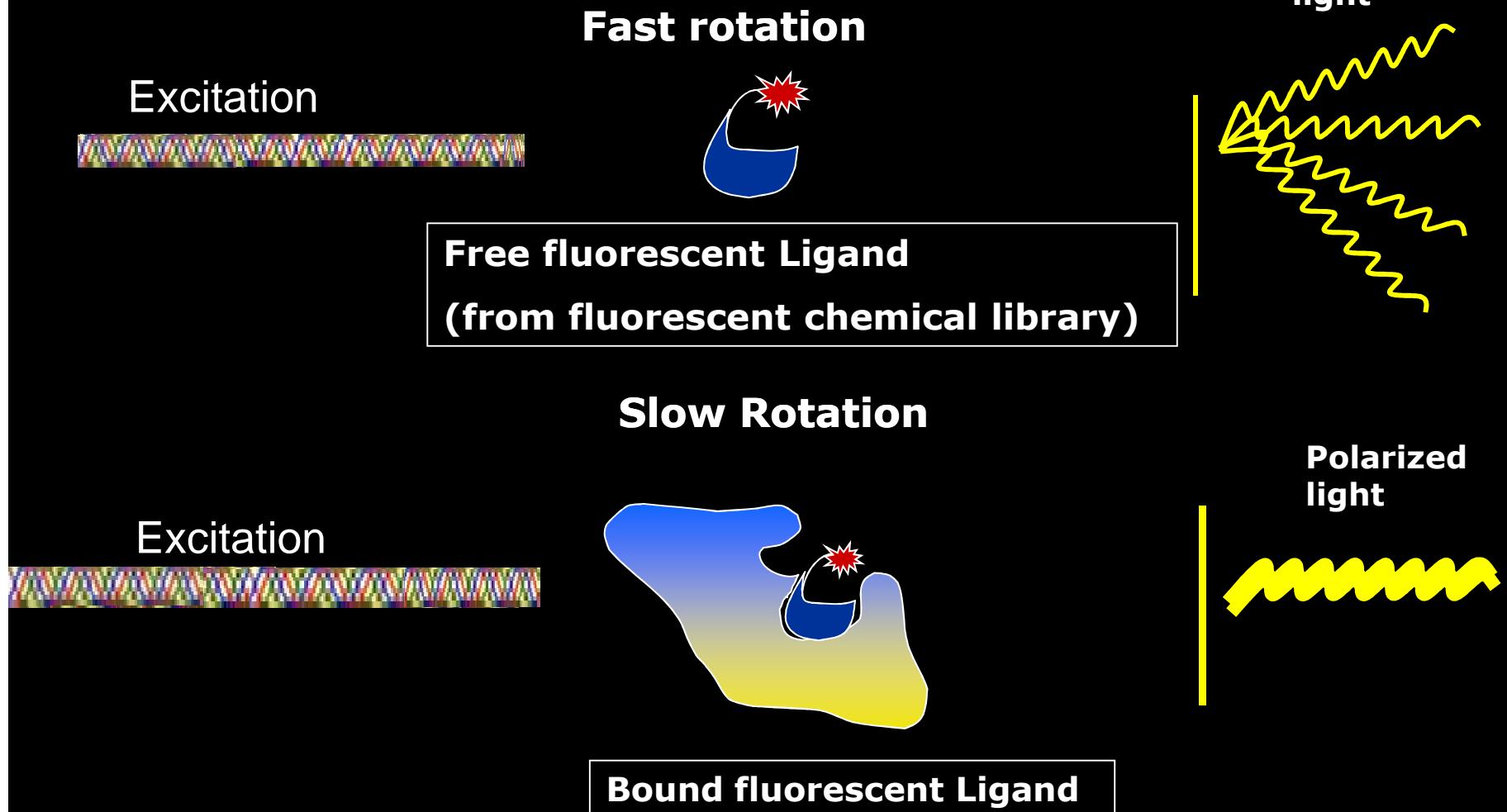
Collaboration C Llorens-Cortes, Collège de France

Iturrioz et al., FASEB 2010



Binding assay for soluble protein

Fluorescence polarization



Strategy

- Step 1: assay development :

soluble Protein + fluorescent chemical library → fluorescent probe

- Step 2: using the assay (HTS: competition)

Protein + fluorescent probe + chemical library

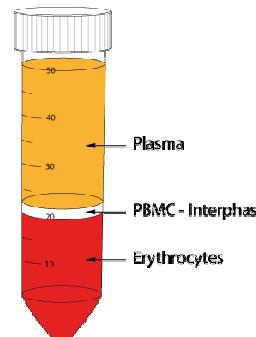
→ Hit



Blood from donors



PBMC purification



PBMC
+/- LPS



compounds



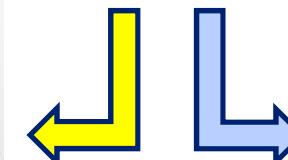
Cytokine detection



Cell Supernatant



Cell culture 24h



Cell survival test
(toxicity)



Active
compounds

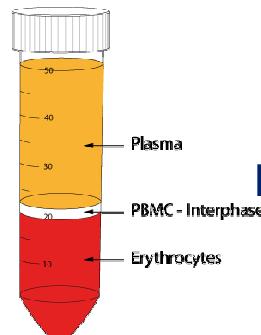
non-toxic
compounds

Hits

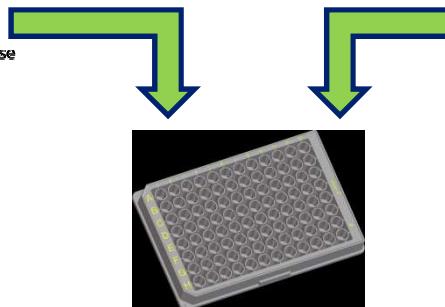
Blood from donors



PBMC purification



PBMC
+/- LPS



compounds



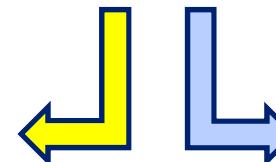
HTRF kit



Cytokine detection



Cell culture 24h



Cell survival test
(toxicity)



Cell Supernatant



Active
compounds

non-toxic
compounds

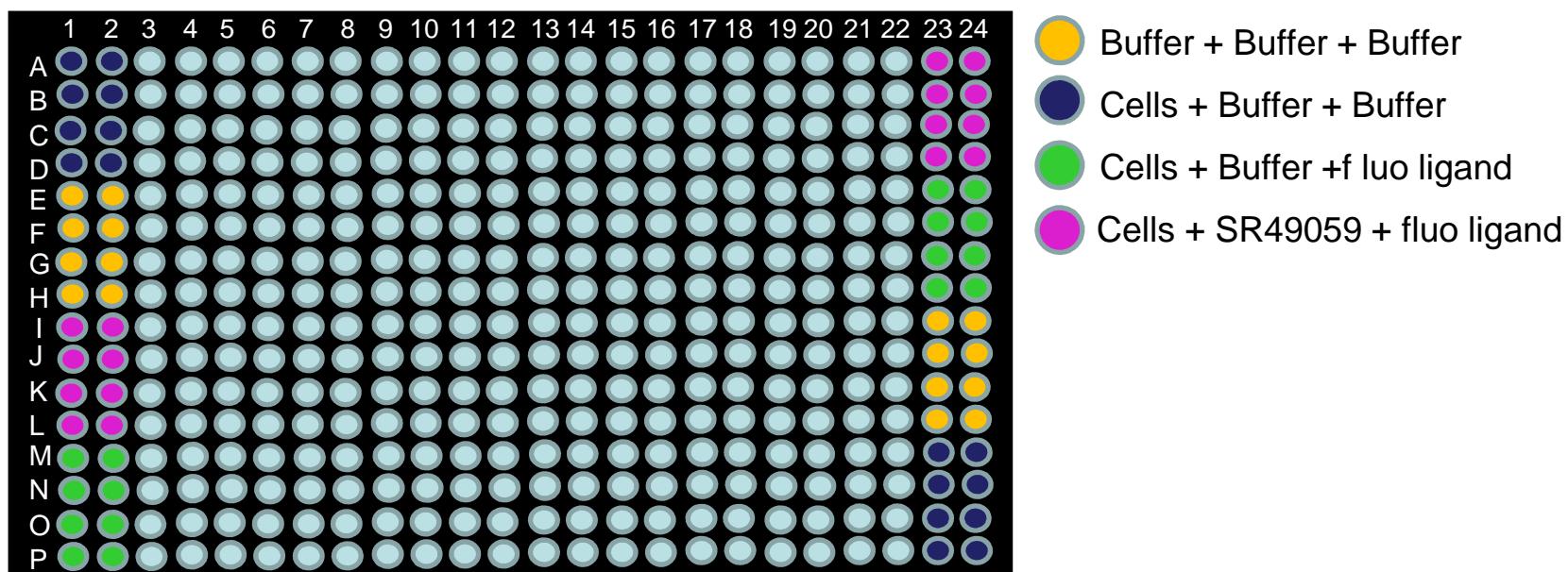
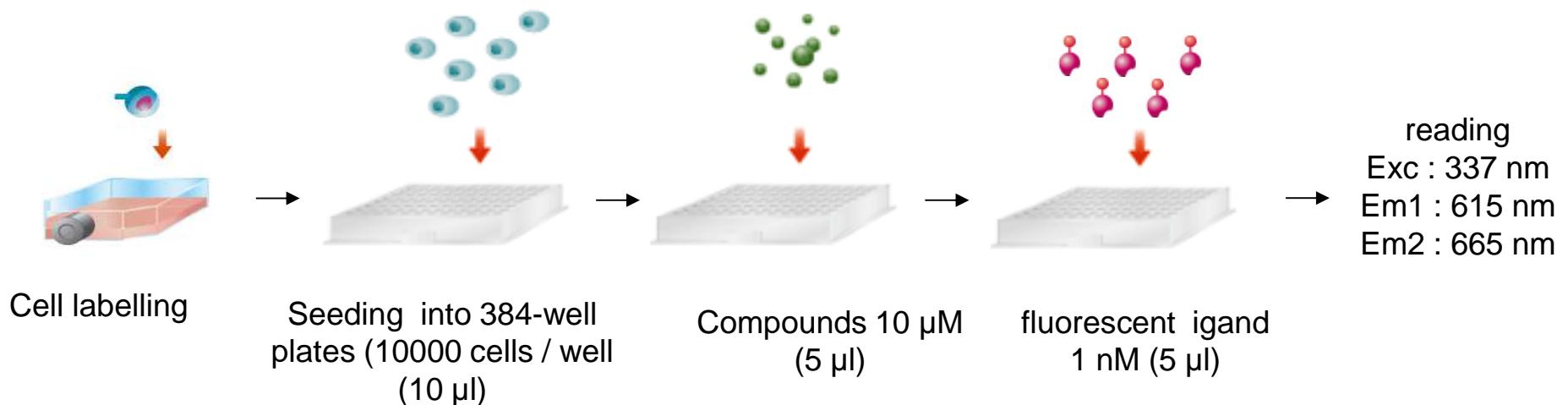
Hits

Method	Classical / ELISA	HTRF	Gain obtained
Time	3 days	1,5 day	Half time
Throughput	160 cpds	> 1000 cpds	X 5

Search for Vasopressin agonists

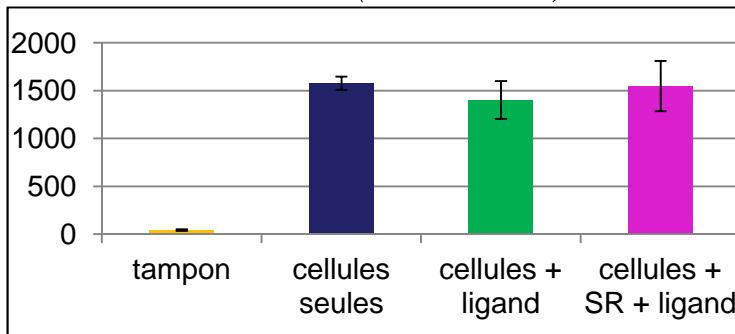
- GPCR (Vasopressin 1A receptor)
- HTFR: Tag-Lite (Cisbio)

METHOD: HEK cells; V1A terbium cryptate labelled

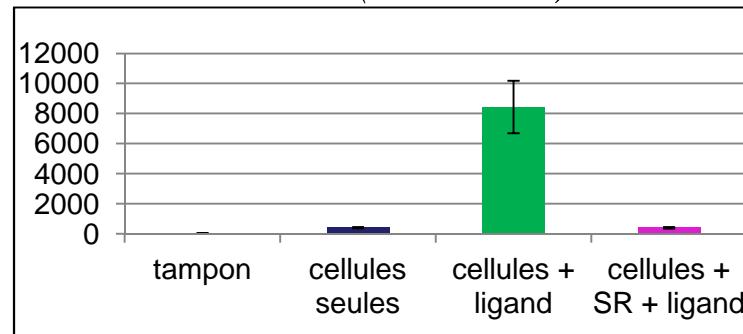


ANALYSIS

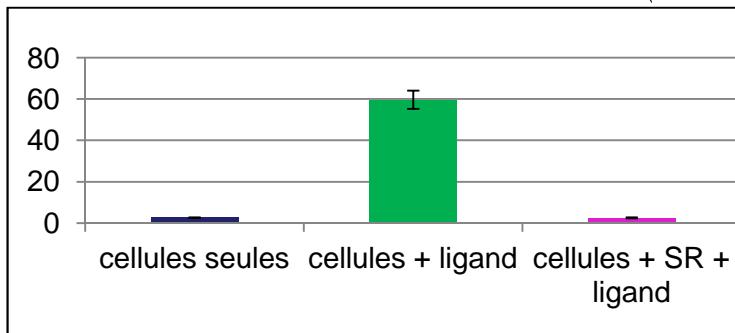
Emission 615 nm (Plate 01)



Emission 665 nm (Plate 01)



Ratio Em 665 nm / Em 615 nm (Plate 01)



$$\frac{(\text{cells} + \text{ligand}) - (\text{cells} + \text{ligand} + \text{compound})}{(\text{cells} + \text{ligand}) - (\text{cells})} \times 100$$

Exemple : 100 % inhibition with SR49059 (Plate 01)

$$1 - \frac{3 \times [(\text{SD cells} + \text{ligand}) + (\text{SD cells} + \text{ligand} + \text{compound})]}{\sqrt{(\text{Mean cells} + \text{ligand}) - (\text{Mean cells} + \text{ligand} + \text{compound})}}$$

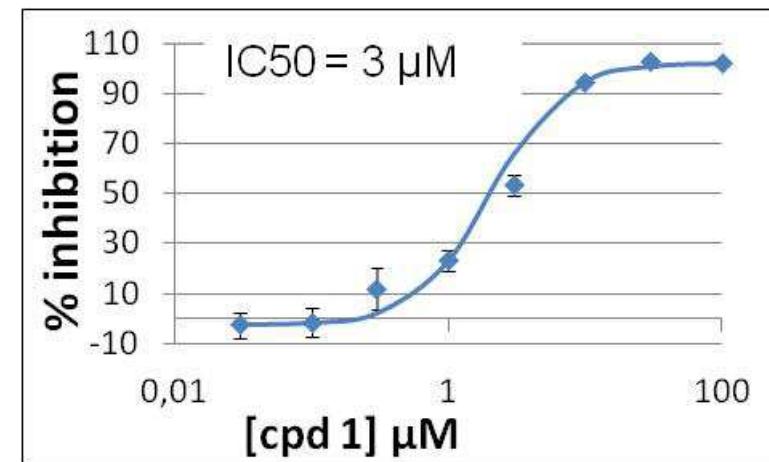
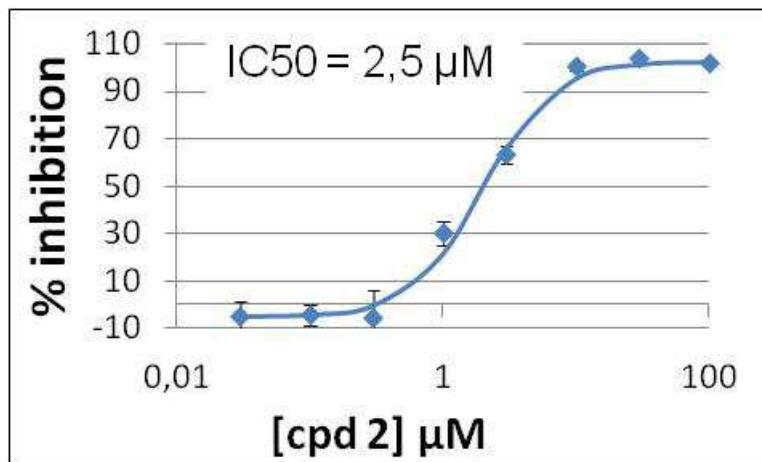
Exemple : Z'=0,76 (Plate01)

Results

6508 molecules tested: 121 hits ($Z' > 0,7$)

37 hits showed antagonist activity with more than 50% of inhibition of the Ca^{2+} response at 10 μM .

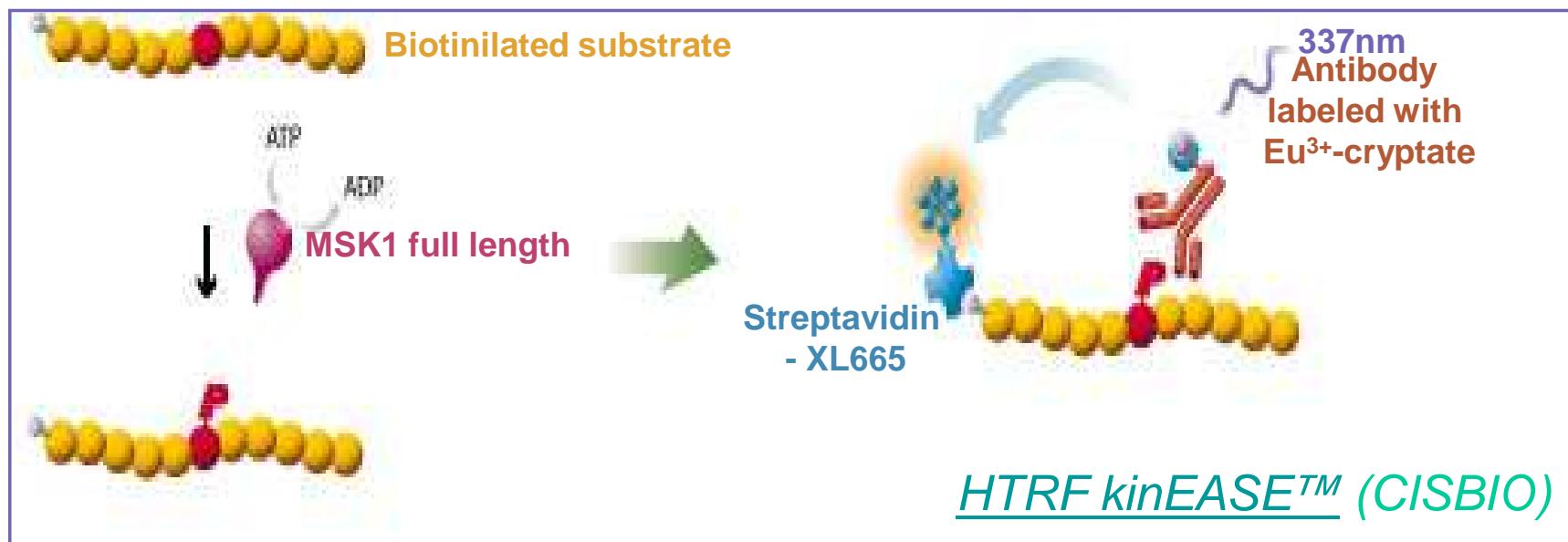
We focused on 6 out of them and performed dose-response validation curves.



Search for Msk-1 inhibitors

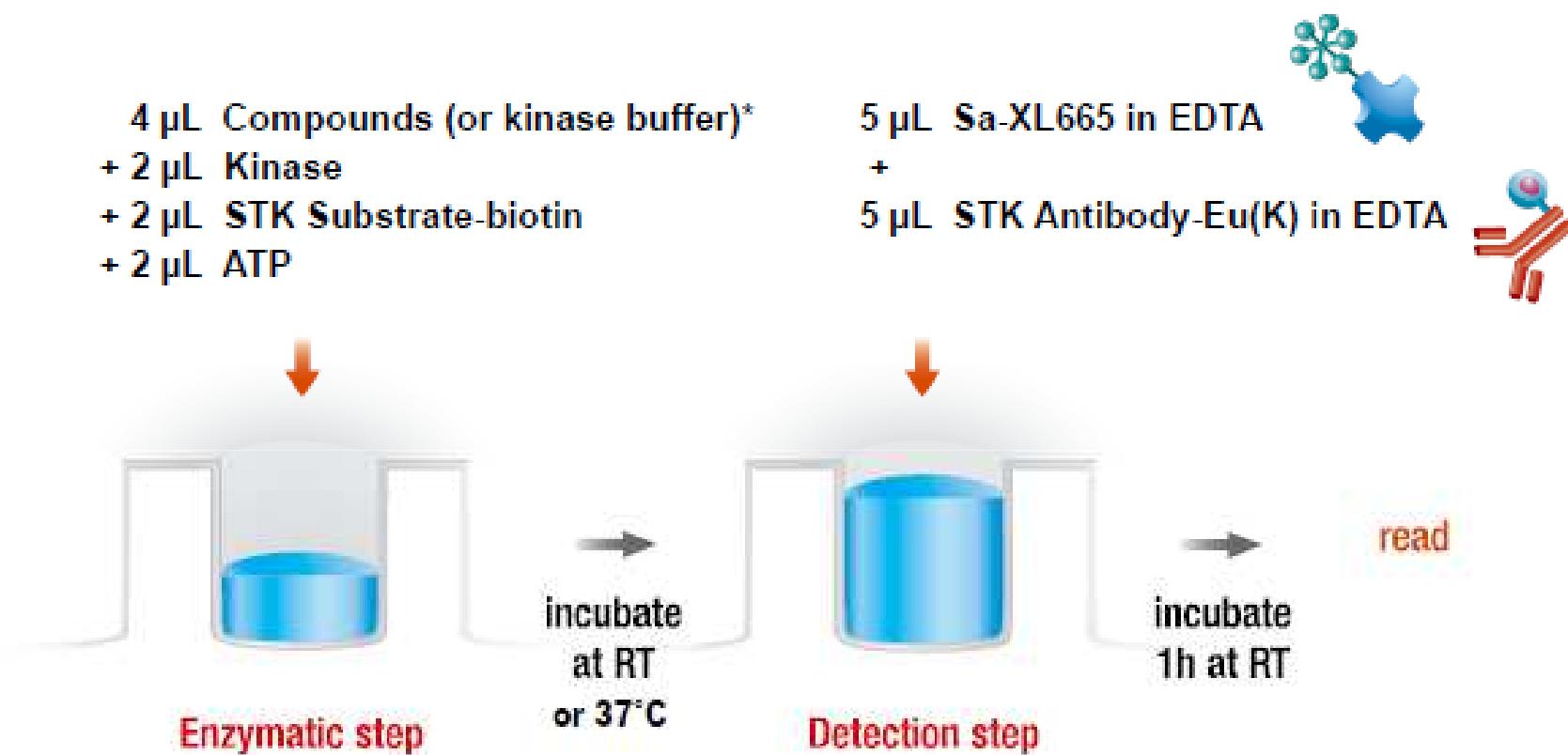
- Enzymatic assay (kinase)
- Kit HTRF KinEASE

HTRF-MSK1



Choice of the substrate: STK S3

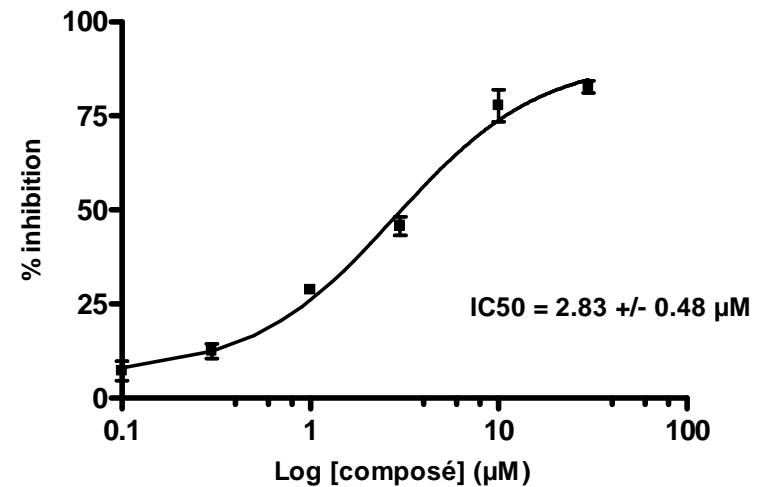
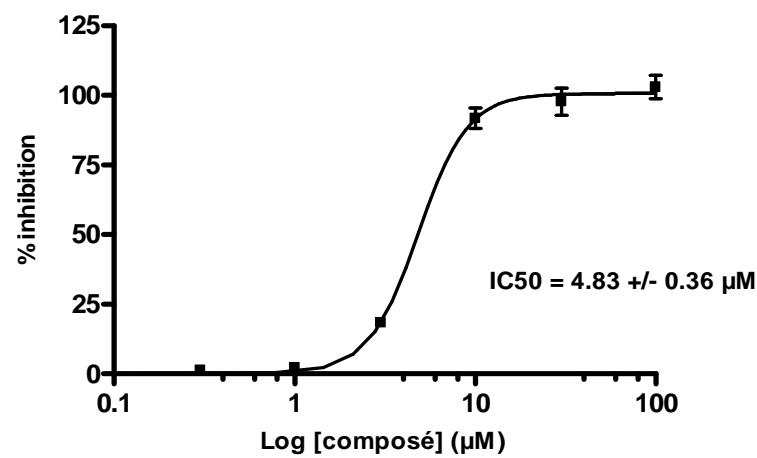
Kinase assay



Results

7120 compounds tested: 55 hits ($Z' > 0,75$)

5 hits showed inhibitory activity with validated with another technology (luminescence-based).



**Platform of Chemical Biology
(UMS 3286 CNRS-UdS):**

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Christel VALENCIA
Raphael CALBRIX
Yannick BECK
Annick MARIN

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Claire MARSOL (& LIT)
Pascale BUISINE (& LIT)

ADME

Patrick GIZZI (& BSC)
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Biology

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Simona NEMSKA

Medalis project (Cancer & inflammation)

Andrew GRIFFITHS
Raphael CALBRIX
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Jean-Luc GALZI





P. Villa, Avignon
2013 Apr 26

Palace of Popes

François 1^{er} from Cisbio

