



Plate-forme de
Chimie Biologique
Intégrative de Strasbourg

UMS 3286 CNRS-UdS

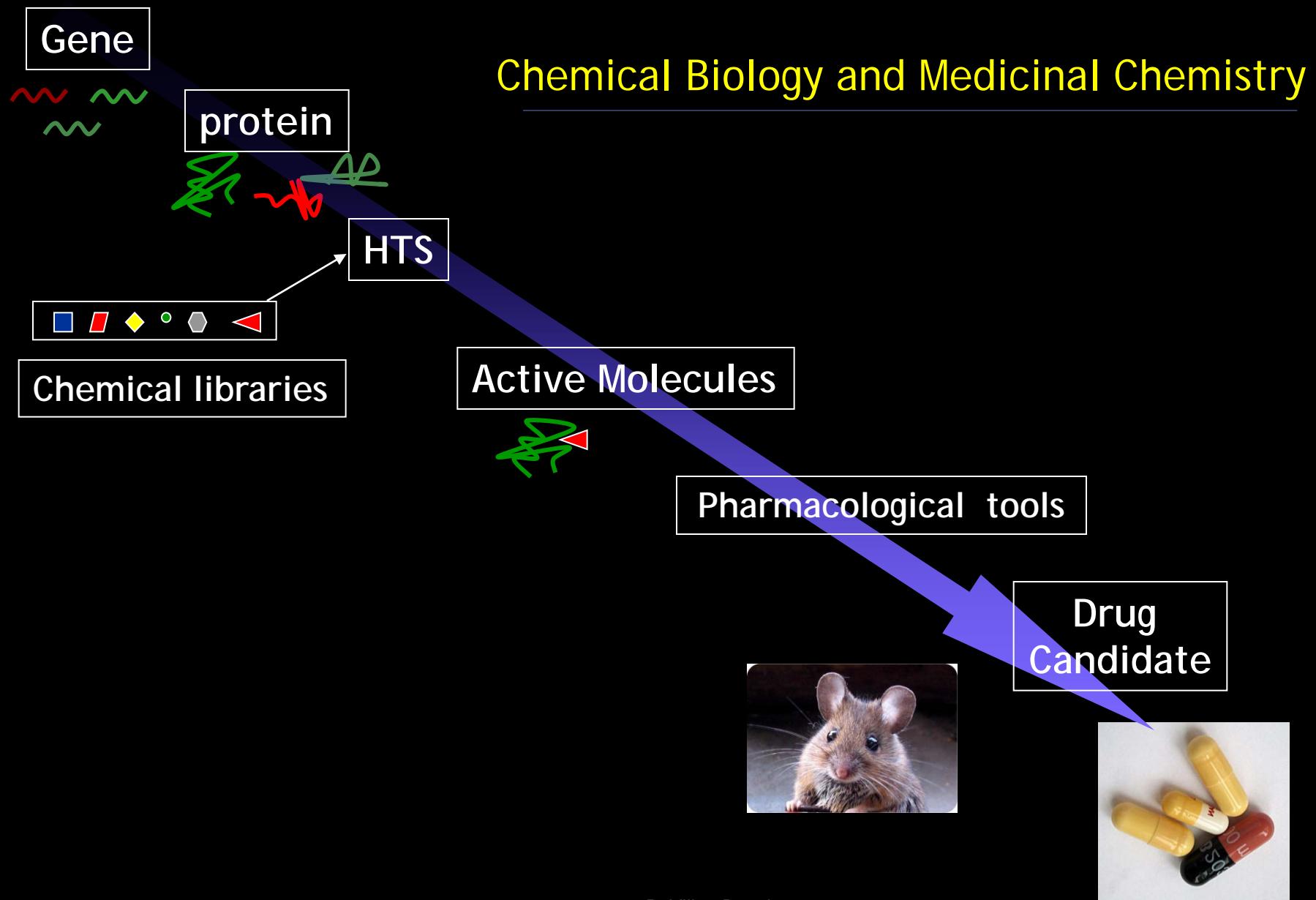


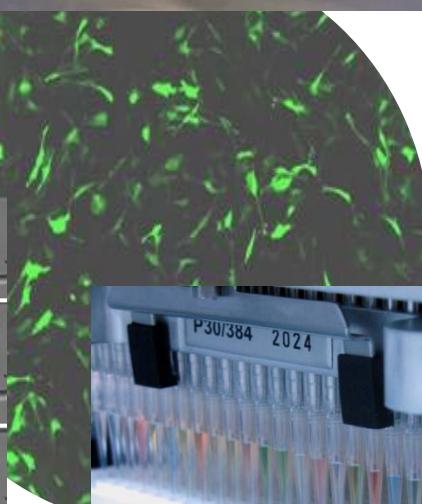
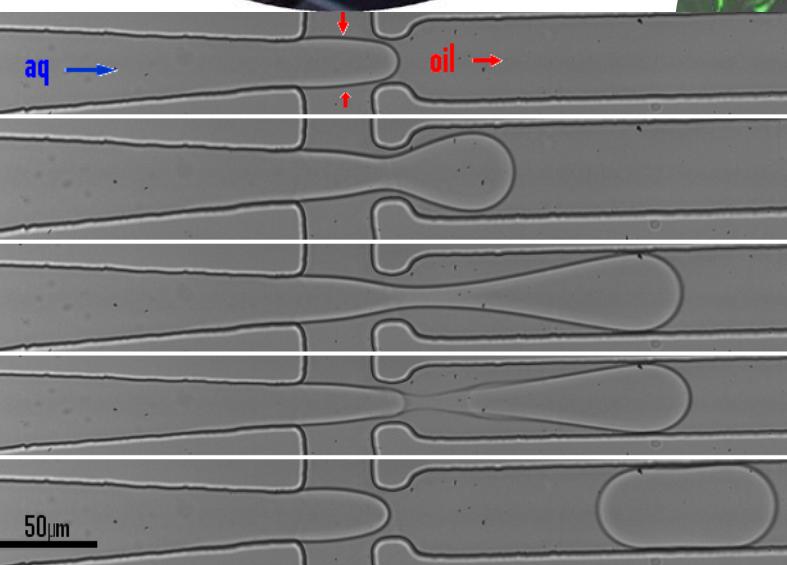
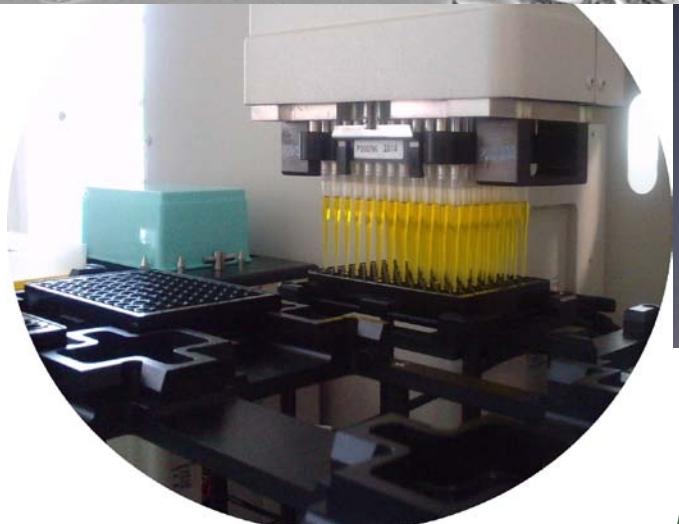
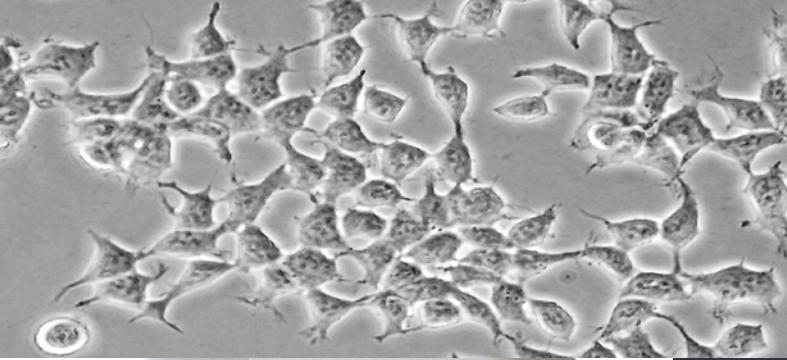
Contribution of HTRF technology in the search for kinase inhibitors and GPCR ligands: the use of KinEASE and Tag-Lite assays



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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 and 384
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

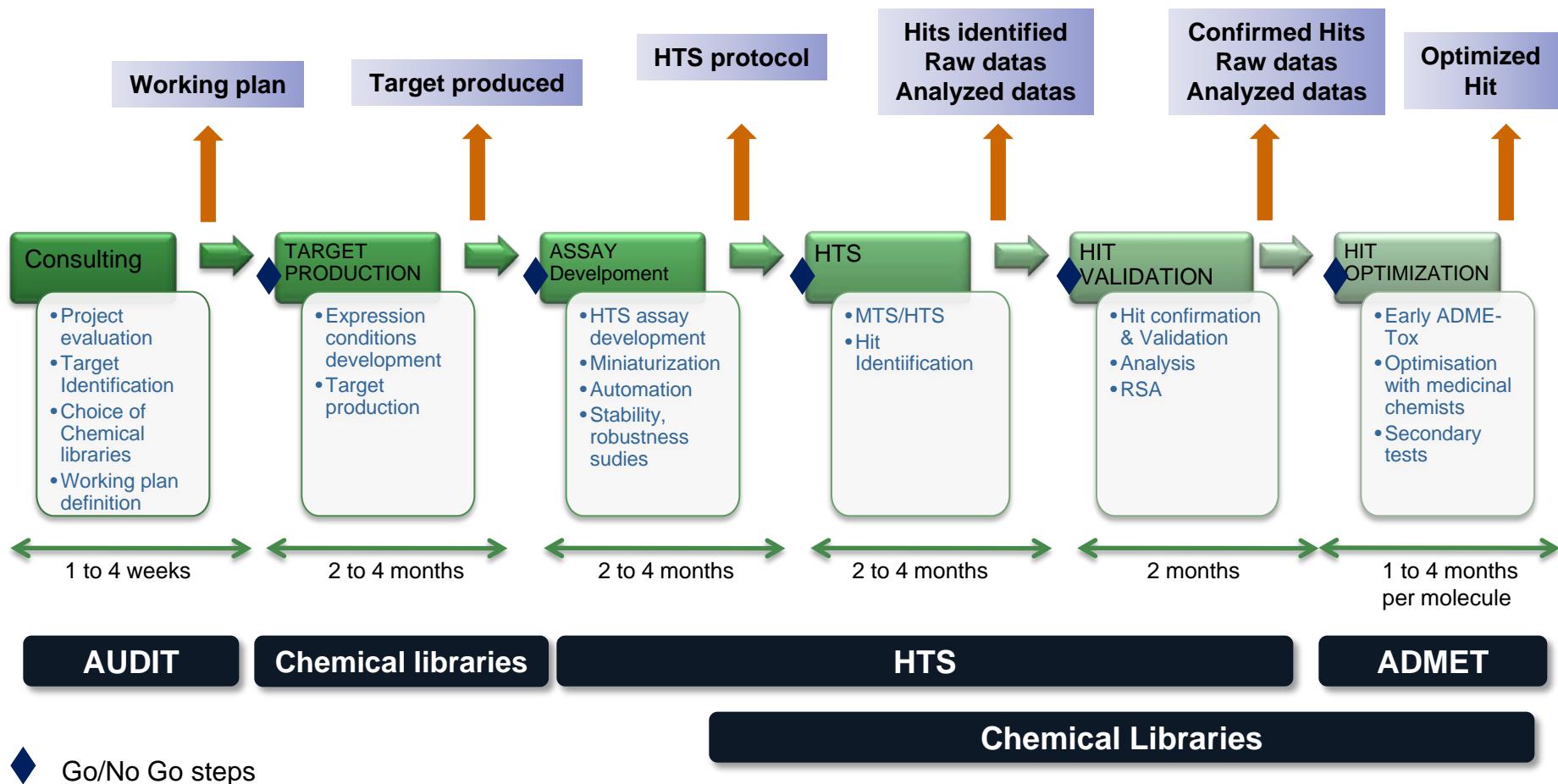
- Solubility
- Log D
- CHI
- pKa
- Chemical stability

In vivo Pharmacokinetics

- BBB
- Bioavailability

Cytotoxicity

MILESTONES





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

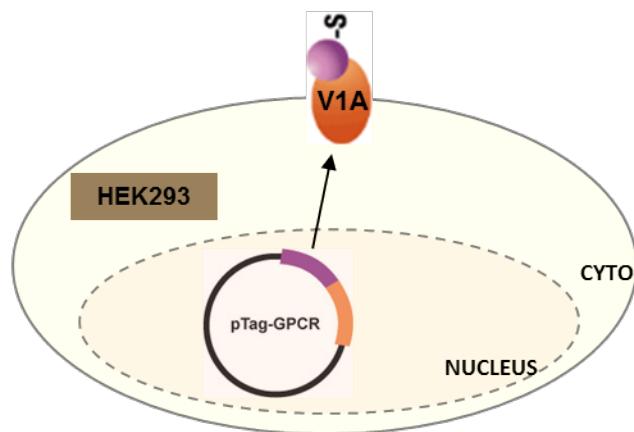


This document is subject to the provision on the reverse
1, boulevard Vivier Merle, 69443 Lyon cedex 03
This approval is carried out in accordance with the UKAS assessment and certification procedures and monitored by UKAS
International

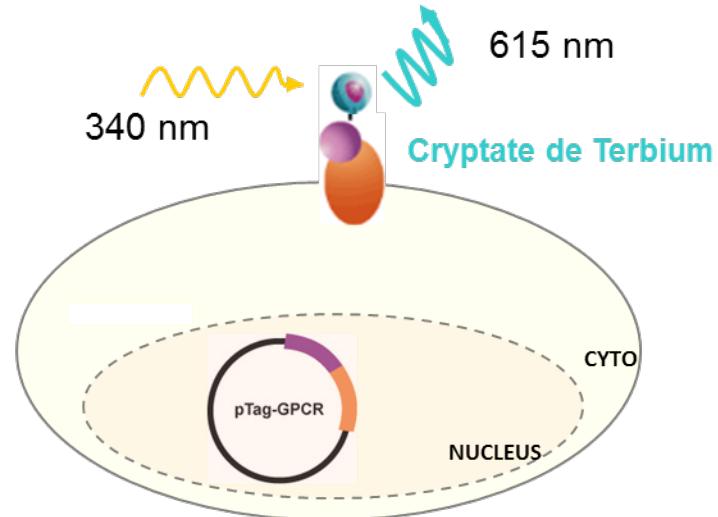
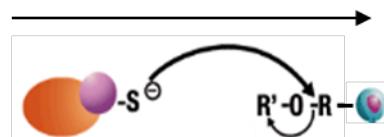
Search for Vasopressin ligands

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

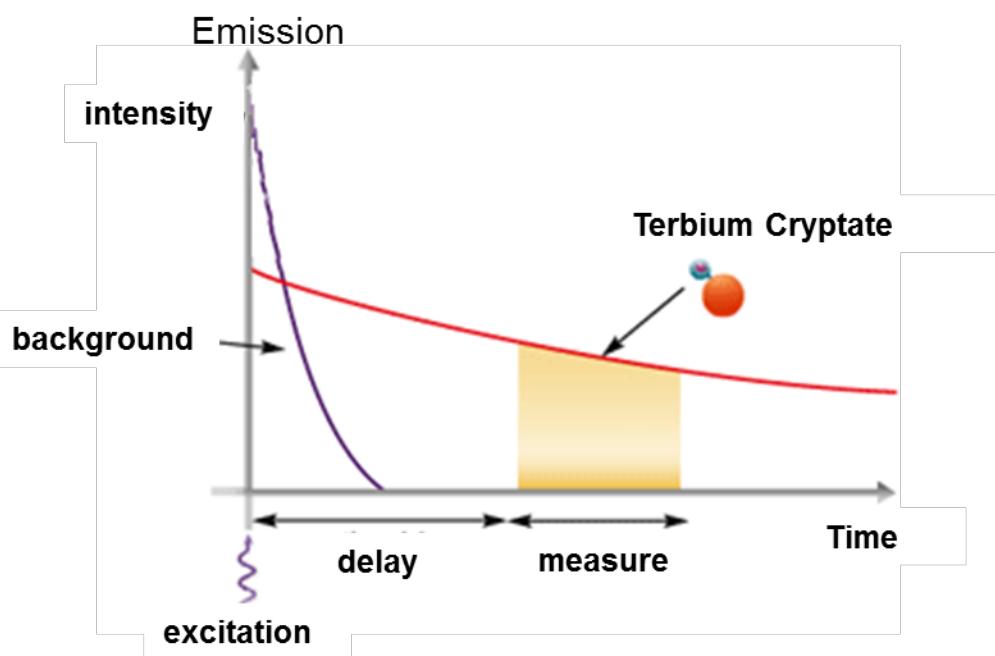
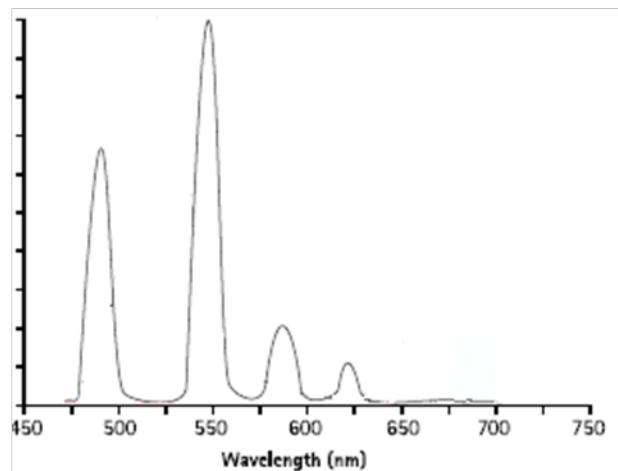
HEK 293 cells – V1A terbium cryptate labelled



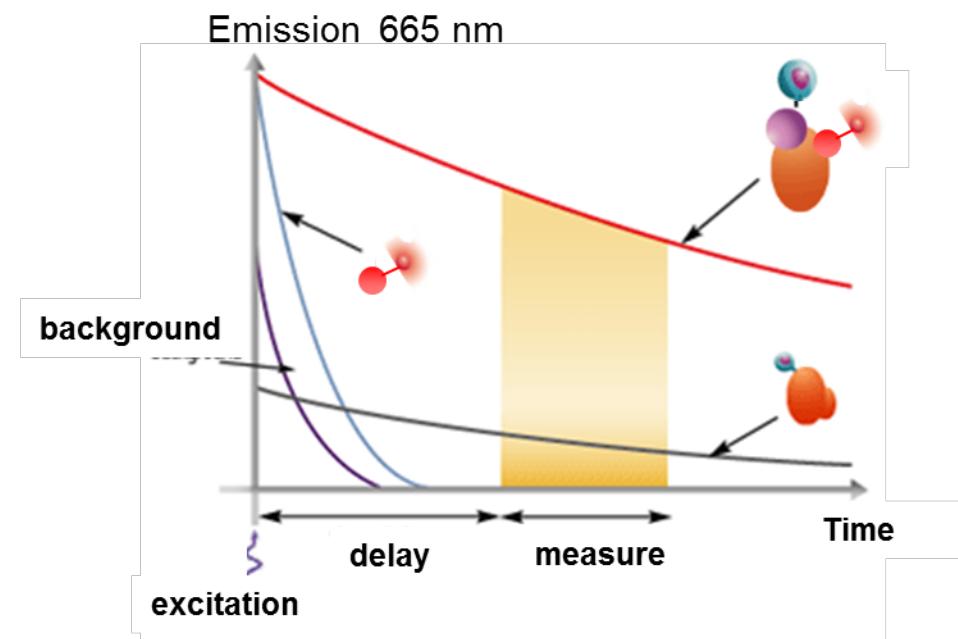
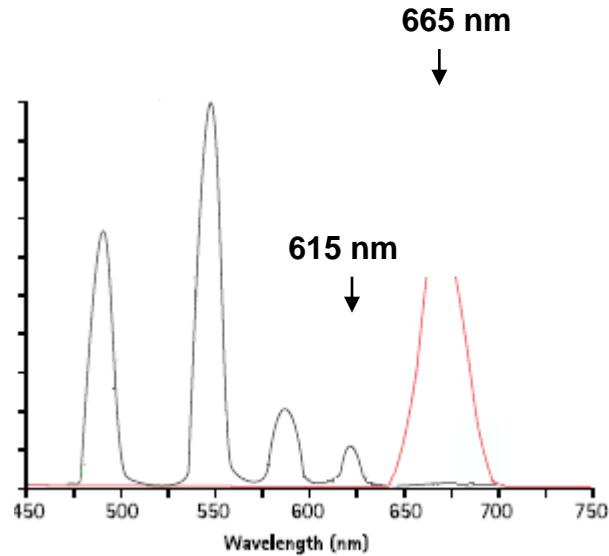
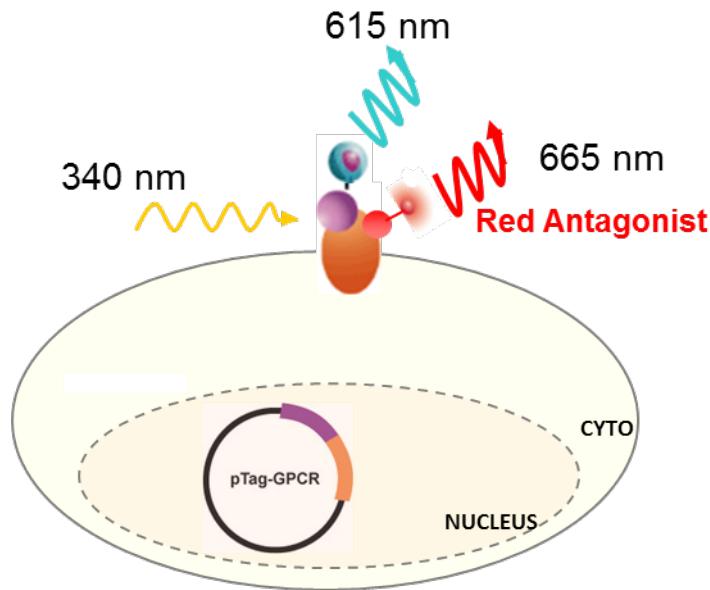
Terbium Cryptate



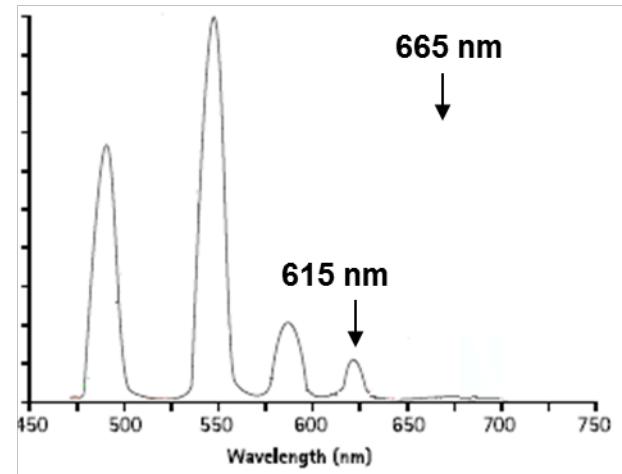
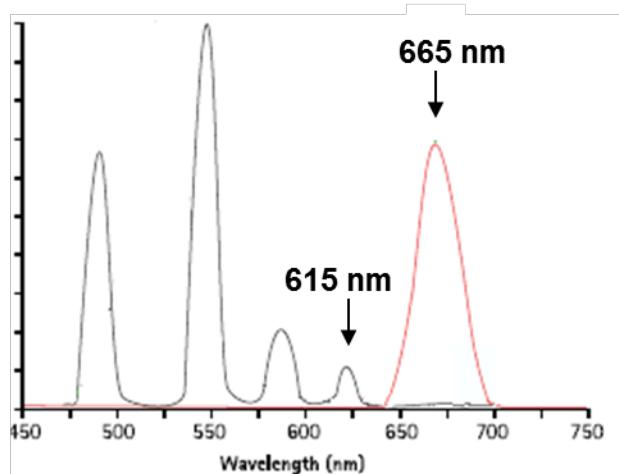
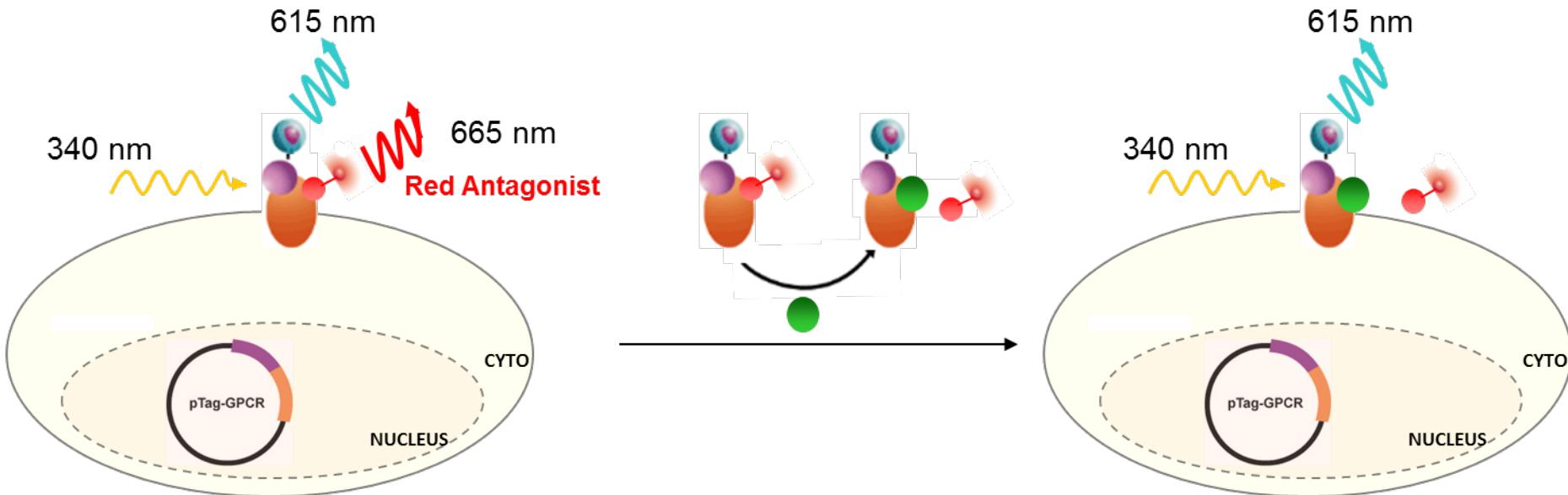
Terbium Cryptate



HTRF



Screening



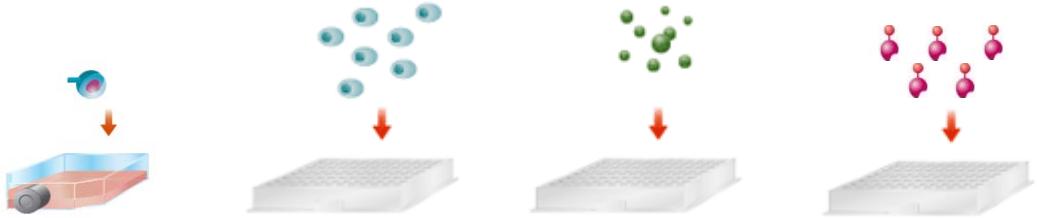
$$S = \frac{665}{615} \times 10$$

EXPERIENCE 1

Conditions :

- 384-well Plate (White ref 781075) & the same with low volume (784075)
- Different cellular concentrations (1.10^6 or 2.10^6 cells / ml)
- Kd checking : different con of fluorescent ligand
- Ki calculation of positive control (SR49059) in the presence of 1 nM of fluorescent ligand
- Incubation time : 30 min - 1 hour- 2 hours
- Duplicate
- Reading on Flexstation³

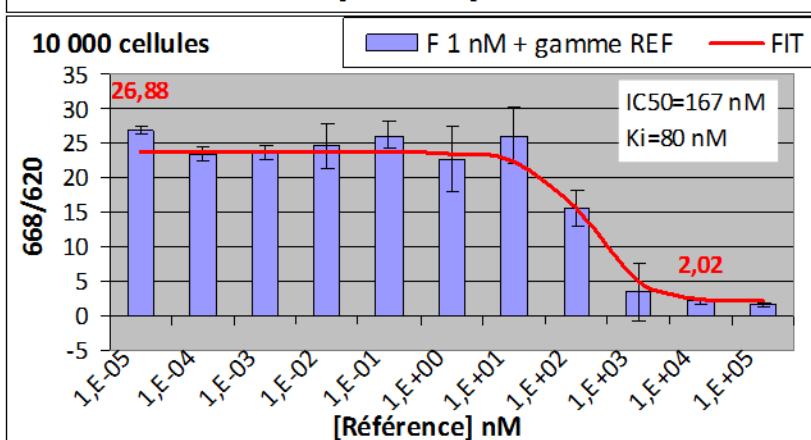
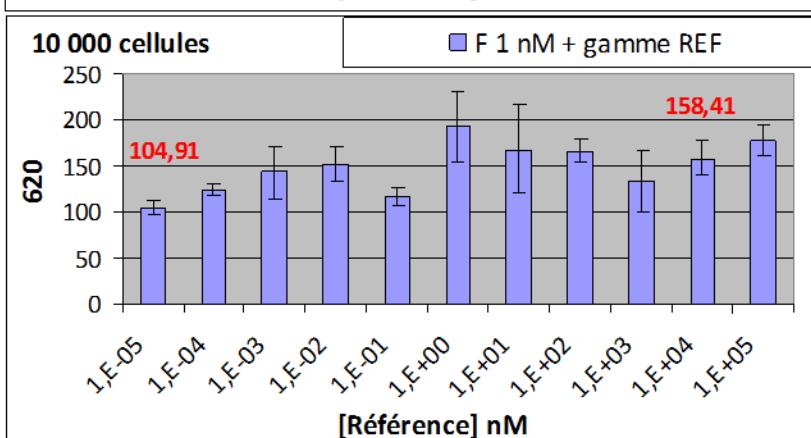
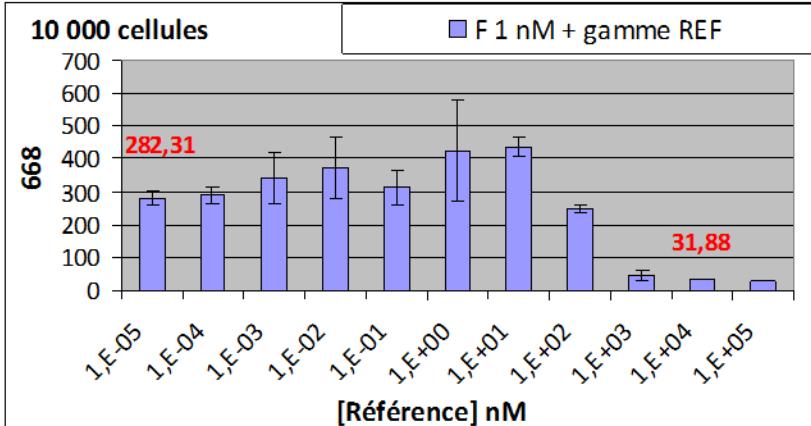
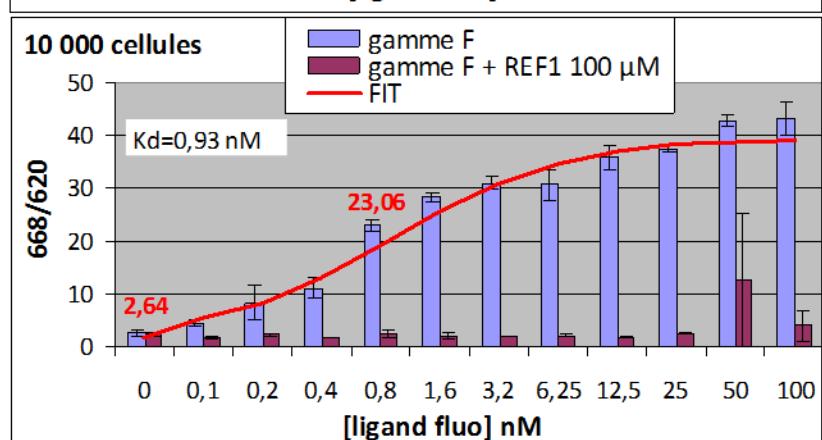
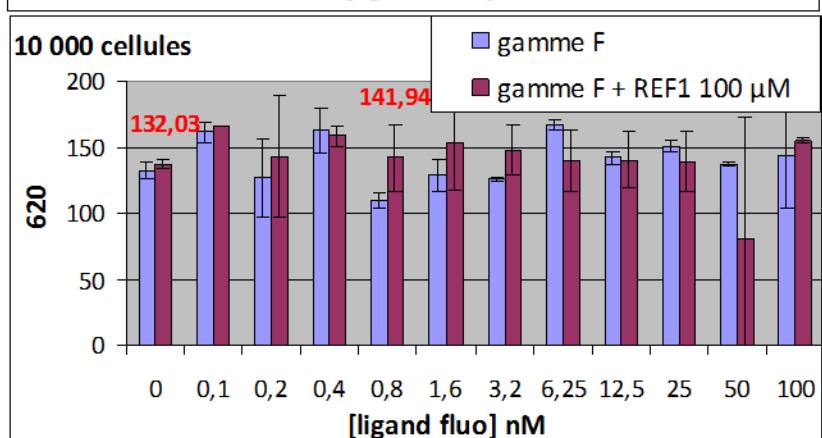
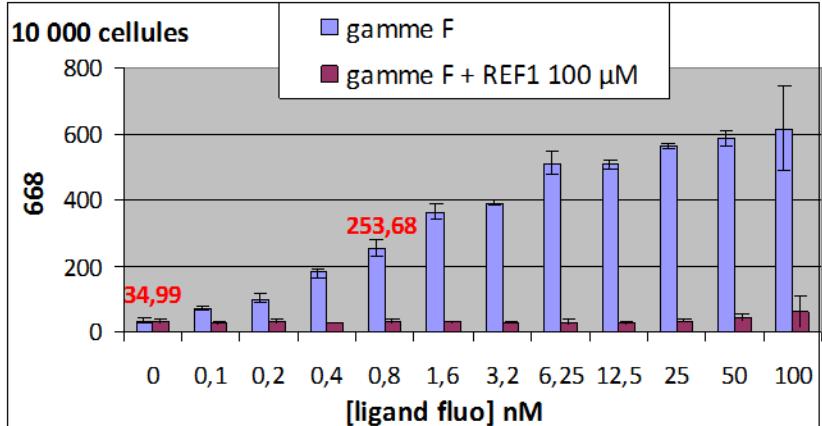
Sequence (manually):



- 1) Cell labelling with Lumi4-Tb
- 2) Seed cells (20 or 10 μ l)
- 3) Add compound SR49059 (10 μ l ou 5 μ l)
- 4) Add fluorescent ligand (10 μ l ou 5 μ l)

Ccl:

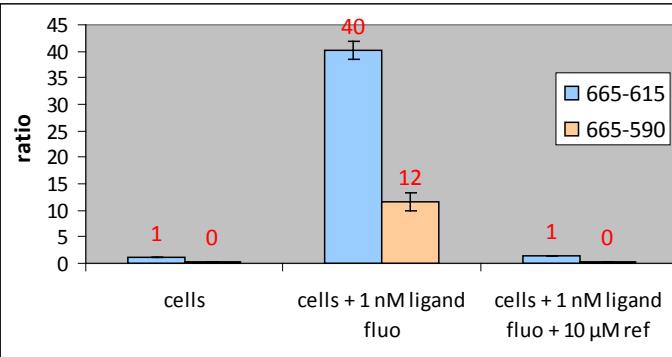
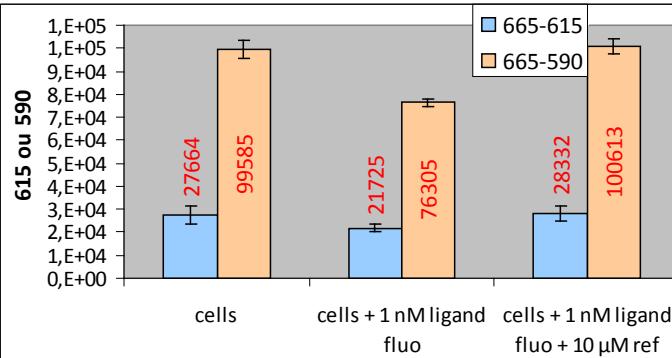
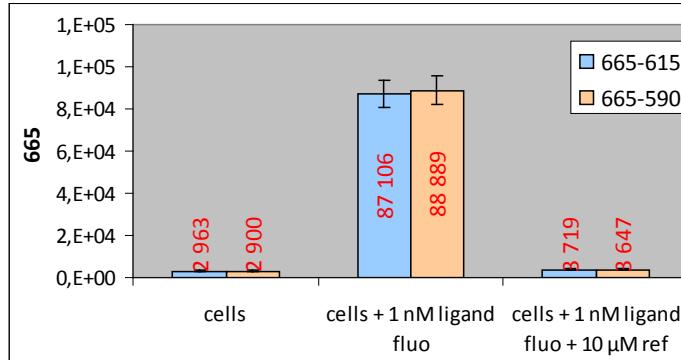
- low volume Plate
- 1.10^6 cells / mL \rightarrow 10 000 cells / well
- fluo ligand = 1 nM & SR49059 = 10 μ M
- Incubation 1h



Pourcentage d'inhibition

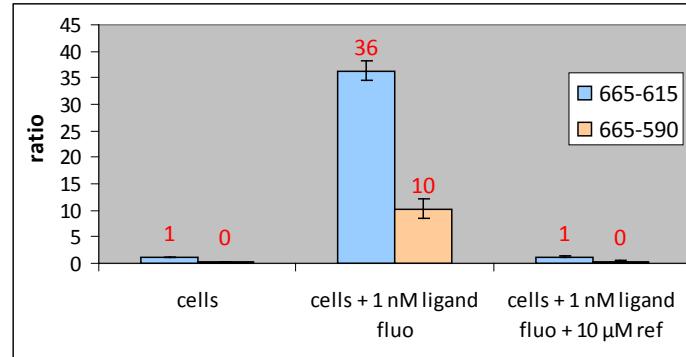
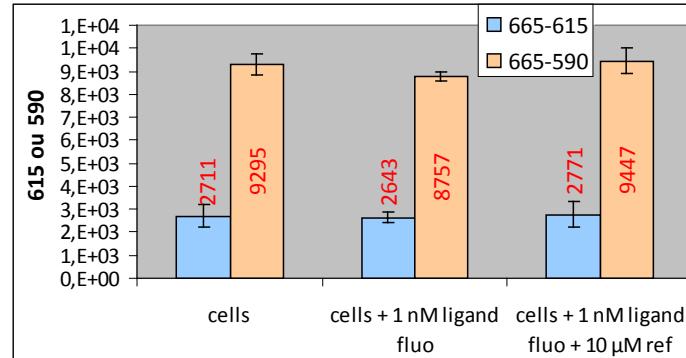
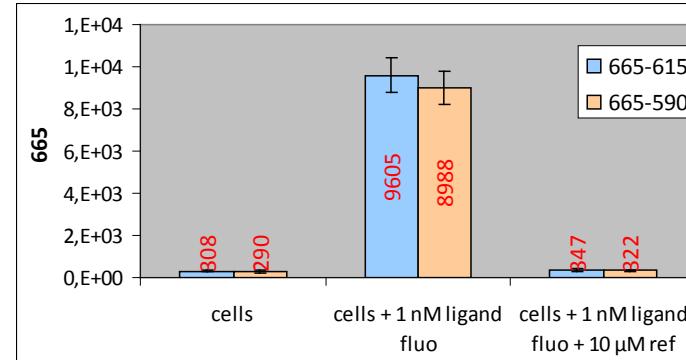
92 %

EXP 2 ENVISION (White plate)



Pourcentage d'inhibition	Z'
97 %	0,87
97 %	0,86

EXP 2 ENVISION (black plate)

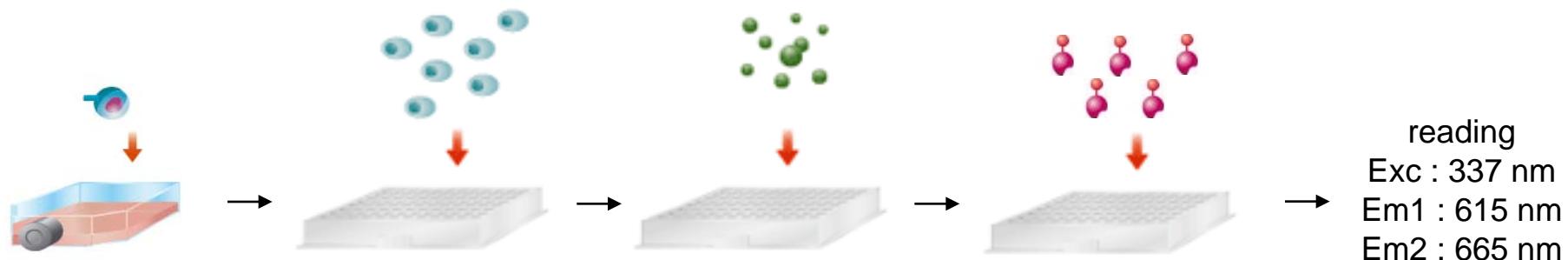


Pourcentage d'inhibition	Z'
97 %	0,83
97 %	0,82

CONCLUSION

- low volume Plate
- $1 \cdot 10^6$ cells / mL \rightarrow 10 000 cells / well
- fluo ligand = 1 nM & SR49059 = 10 μ M
- Incubation 1h (stable)
- Envision

METHOD



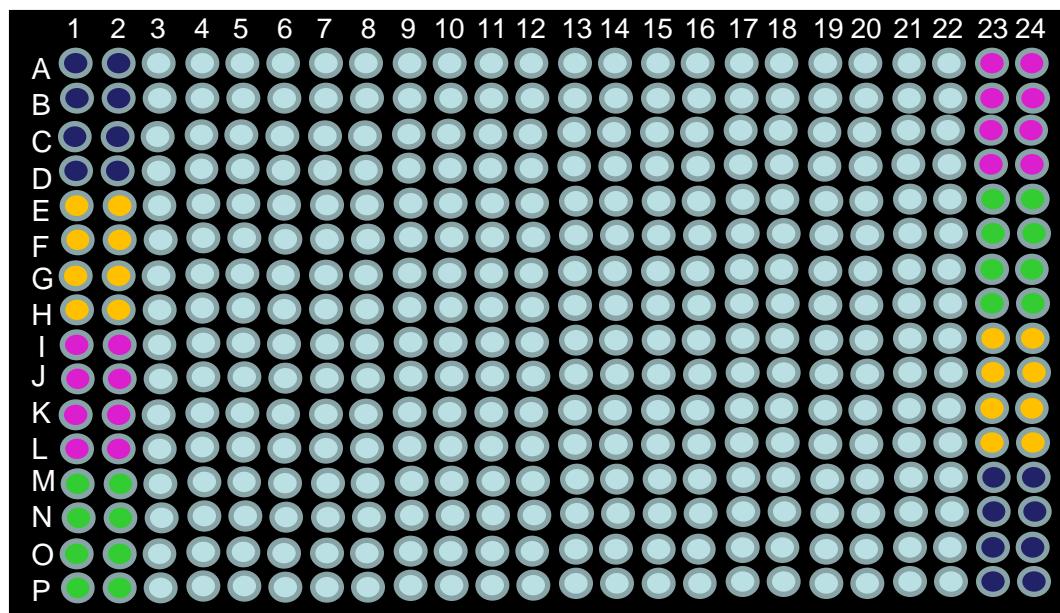
Cell labelling

Seeding into 384-well plates (10000 cells / well (10 μ l))

Compounds 10 μ M (5 μ l)

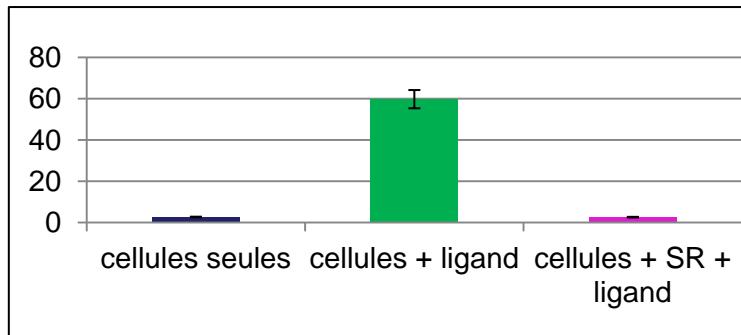
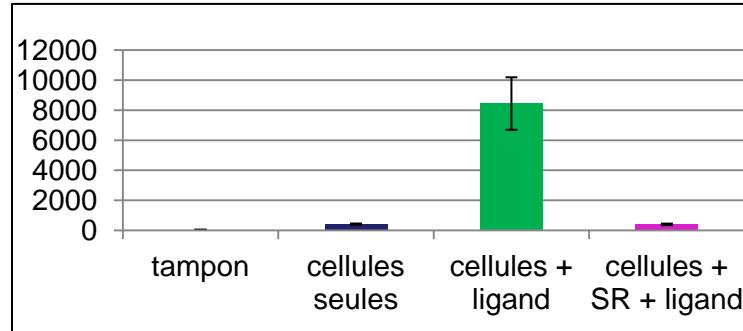
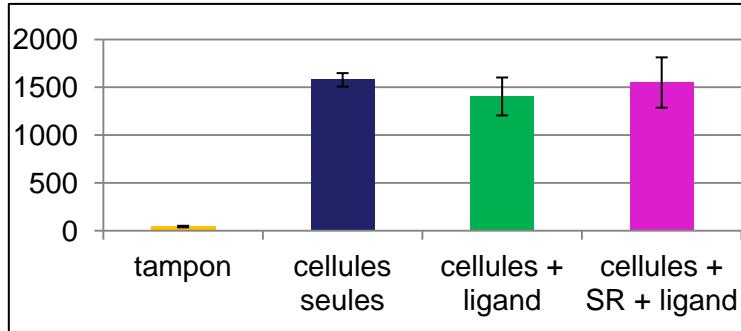
fluorescent ligand 1 nM (5 μ l)

reading
Exc : 337 nm
Em1 : 615 nm
Em2 : 665 nm



- Buffer + Buffer + Buffer
- Cells + Buffer + Buffer
- Cells + Buffer + f luo ligand
- Cells + SR49059 + fluo ligand

ANALYSIS



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

Exemple : 100 % 'inhibition with SR49059 (Plate 01)

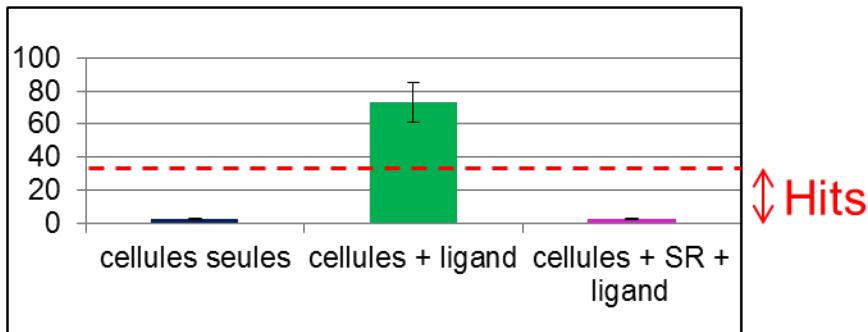
$$1 - \frac{3 \times [(\text{SD cells} + \text{ligand}) + (\text{SD cells} + \text{ligand} + \text{compound})]}{(\text{Mean cells} + \text{ligand}) - (\text{Mean cells} + \text{ligand} + \text{compound})}$$

Exemple : Z'=0,76 (Plate01)

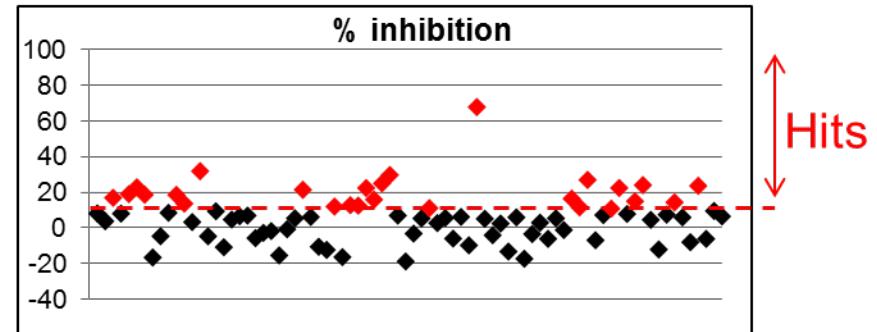
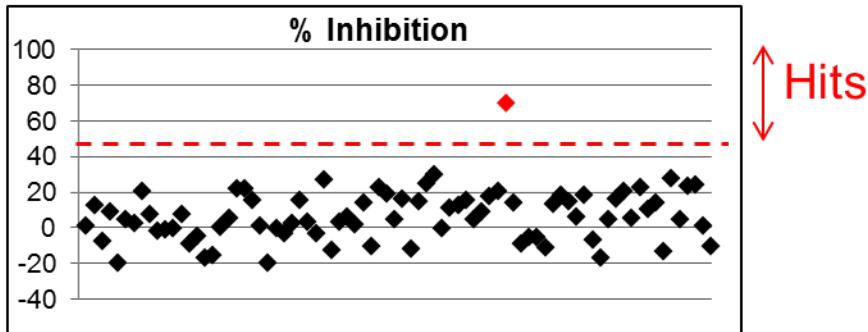
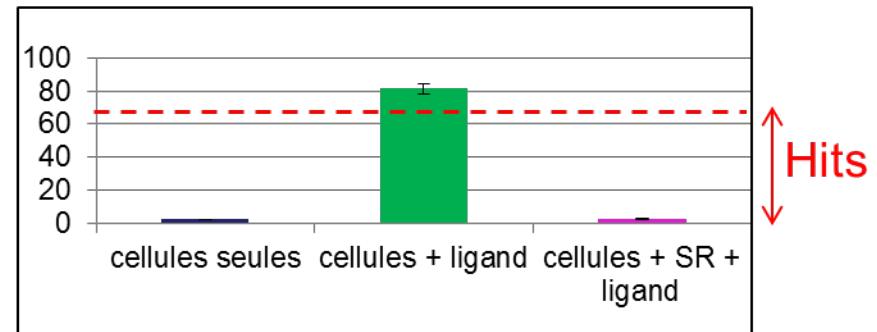
Hit Analysis

1) $(Cells + ligand) - 3 SD$

Ex n°1 (Plate 10, $Z'=0,47$)



Ex n°2 (plate 30, $Z'=0,89$)

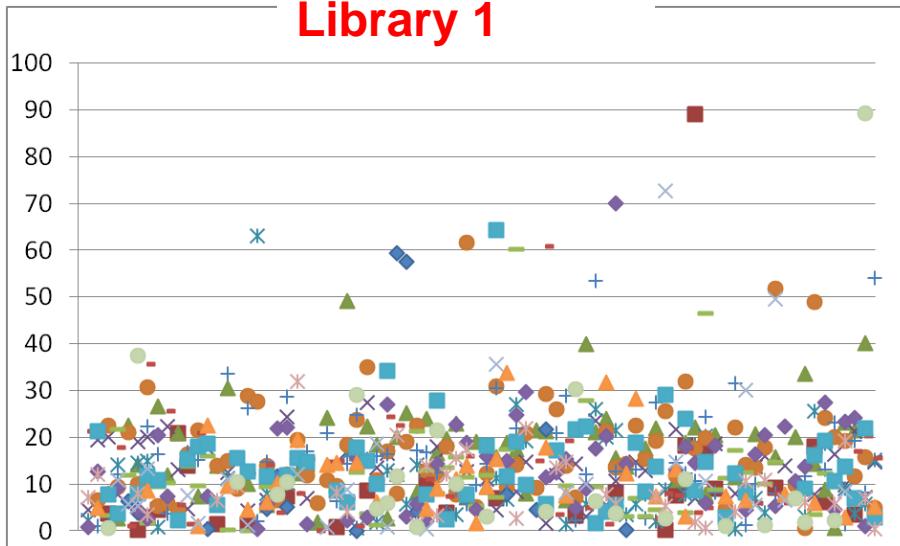


2) $(Cells + ligand) - 4 SD$

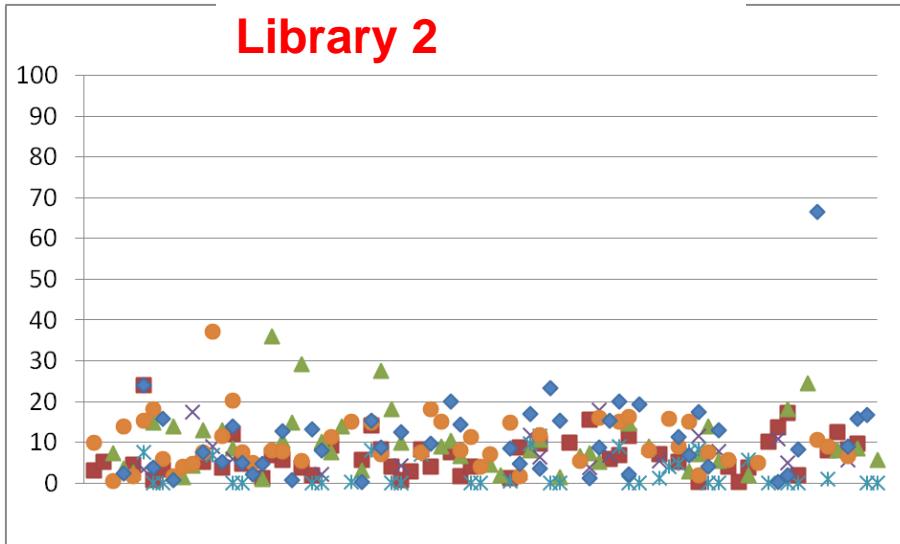
3) $(Cells + ligand) - 3 SD$ **AND** at least 30% inhibition

Result (6460 compounds)

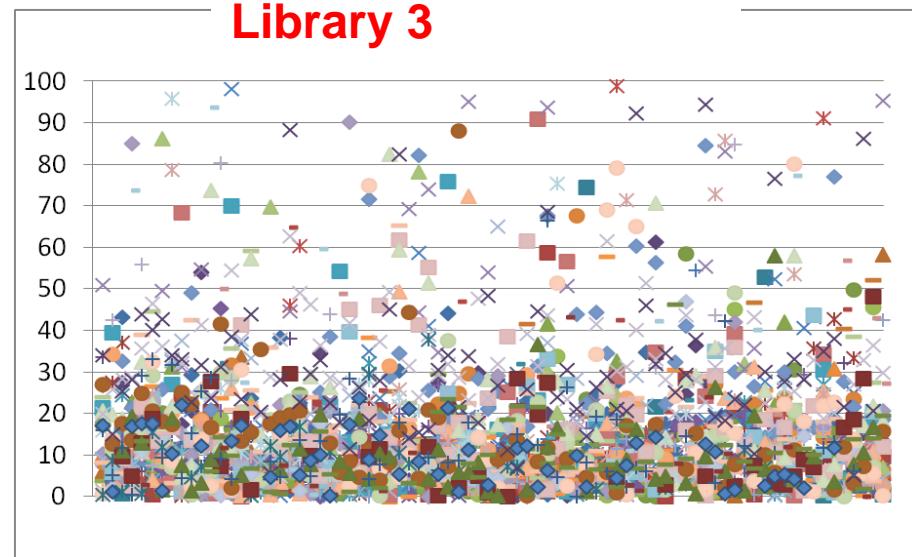
Library 1



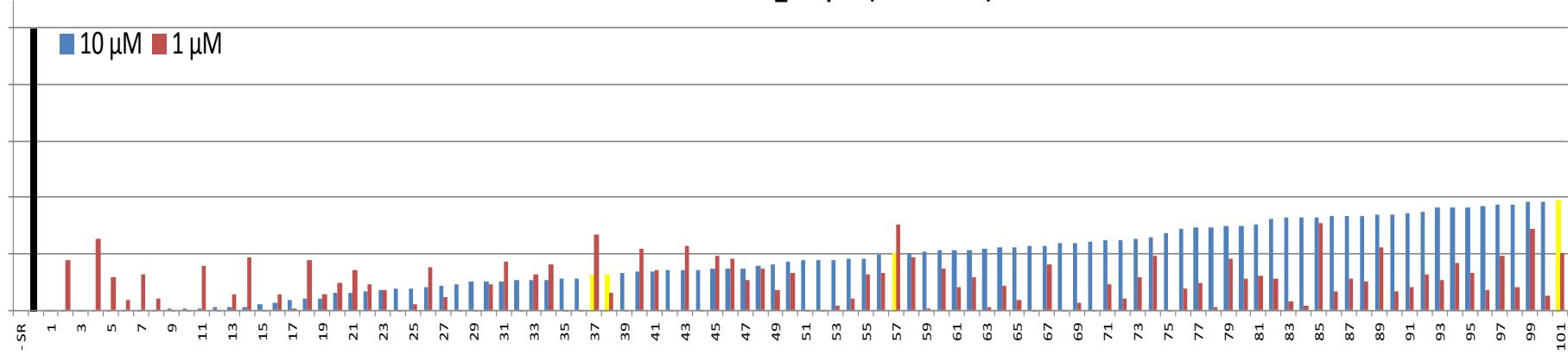
Library 2



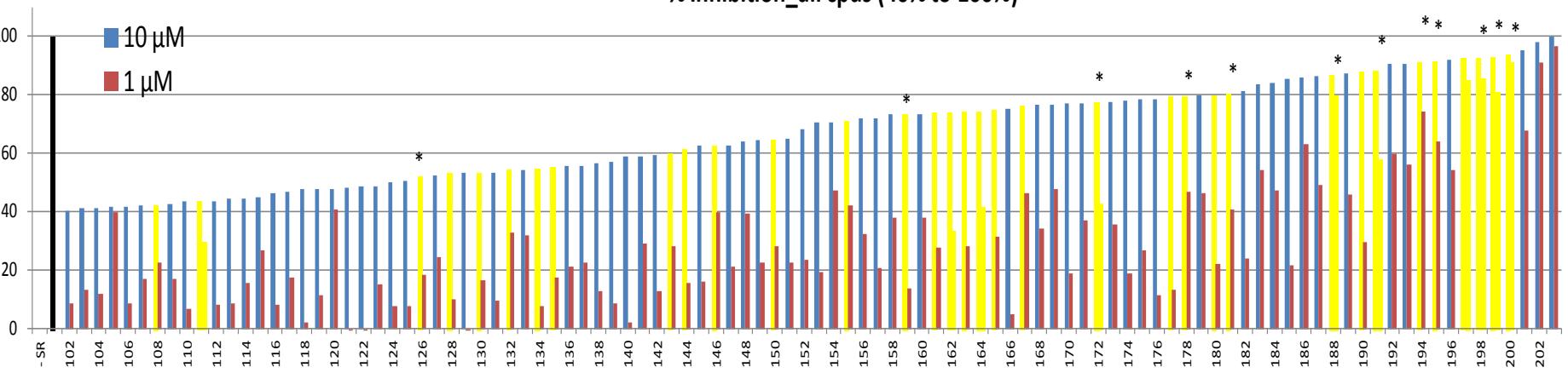
Library 3



% inhibition_all cpds (0% to 40 %)



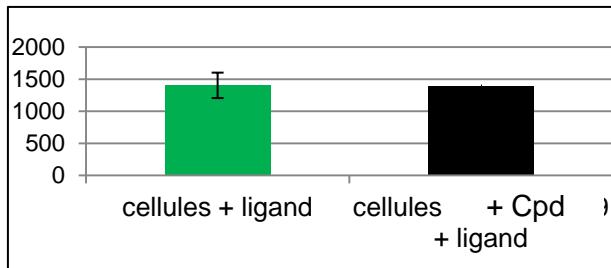
% Inhibition_all cpds (40% to 100%)



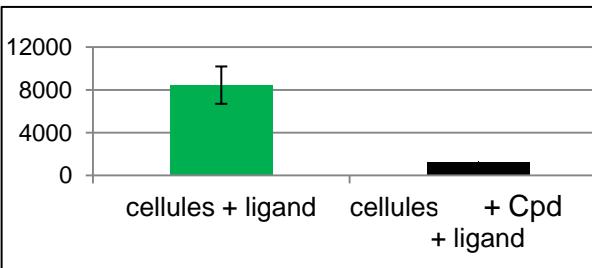
Real and false positives

Real positive (exemple Plate 1-F09)

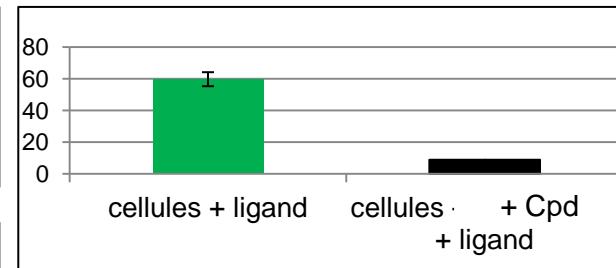
No variation at 615 nm



signal decreases at 665 nm

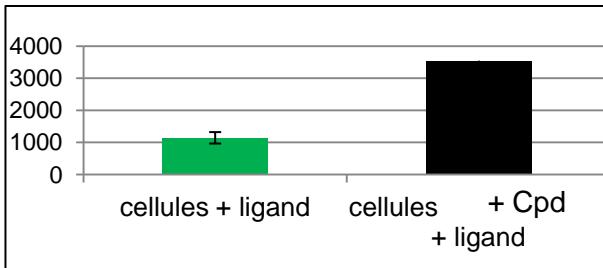


ratio decreases 665 nm / 615 nm

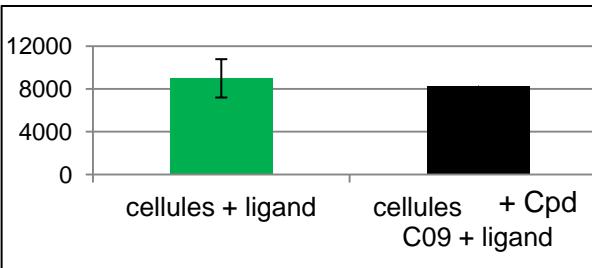


False positive (exemple Plate 13-C09)

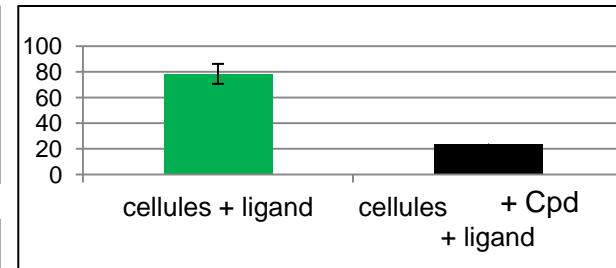
signal increases at 615 nm



no variation at 665 nm

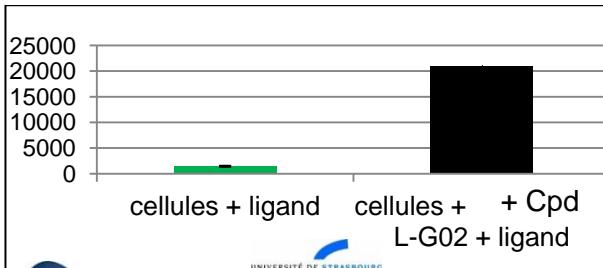


ratio decreases 665 nm / 615 nm

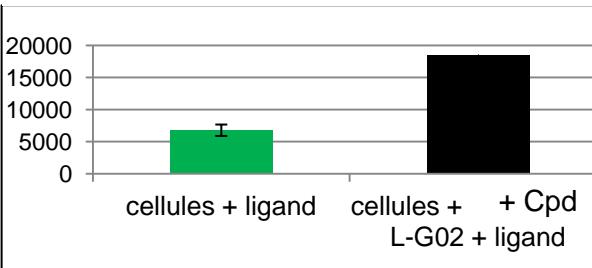


False positive (exemple plate 27-G02)

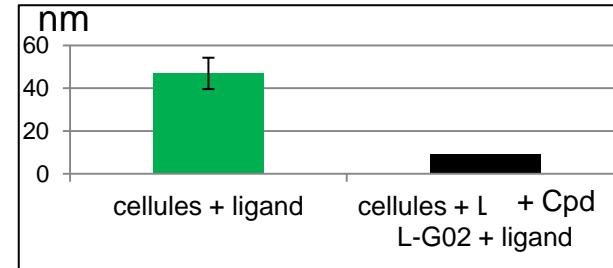
signal increases at 615 nm



signal increases at 665 nm



Ratio decreases 665 nm / 615



SUMMARY PRIMARY SCREEN

(Cells + ligand) – 3 SD AND at least an inhibition of 30%

compounds	hits	%	false positives	false positives % of hits	Real positives	Real positives % of total
6460	197	3,0%	47	23,9%	150	2,3%

CONFIRMATION

COMPOUNDS		I > 30%	I > 80%
203	10 µM	125	26
	1 µM	44	6

« strange » compounds		I > 30%	I > 80%
40 (12 also in another screen)	10 µM	35	12
	1 µM	19	4

Compounds with normal signal		I > 30%	I > 80%
163	10 µM	90	14
	1 µM	25	2

Ccl: → All antagonists

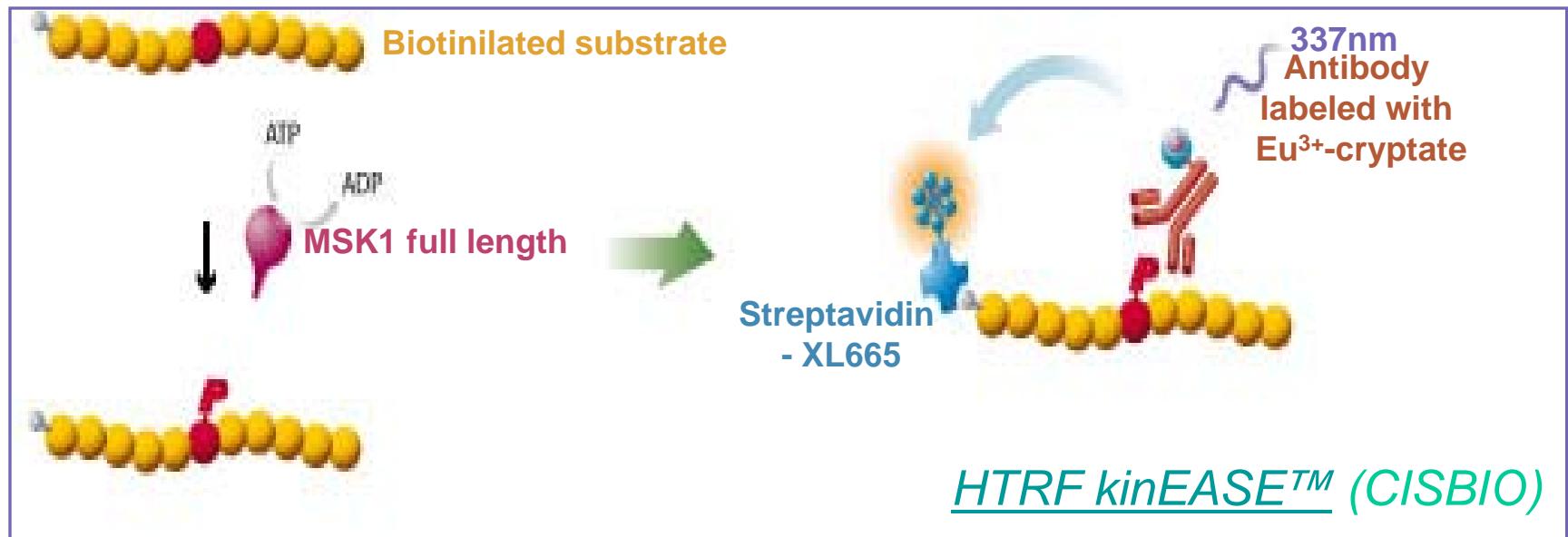
→ Next: screen more compounds

- primary screen was performed rapidly using Tag-Lite technology
 - + convenient, high throughput, robust, sensitive
 - false positives : fluorescent compounds
- hit validation was performed using a functional test (Calcium flux) (at lower throughput)

Search for Msk-1 inhibitors

- Enzymatic assay (kinase)
- Kit HTRF KinEASE

HTRF-MSK1

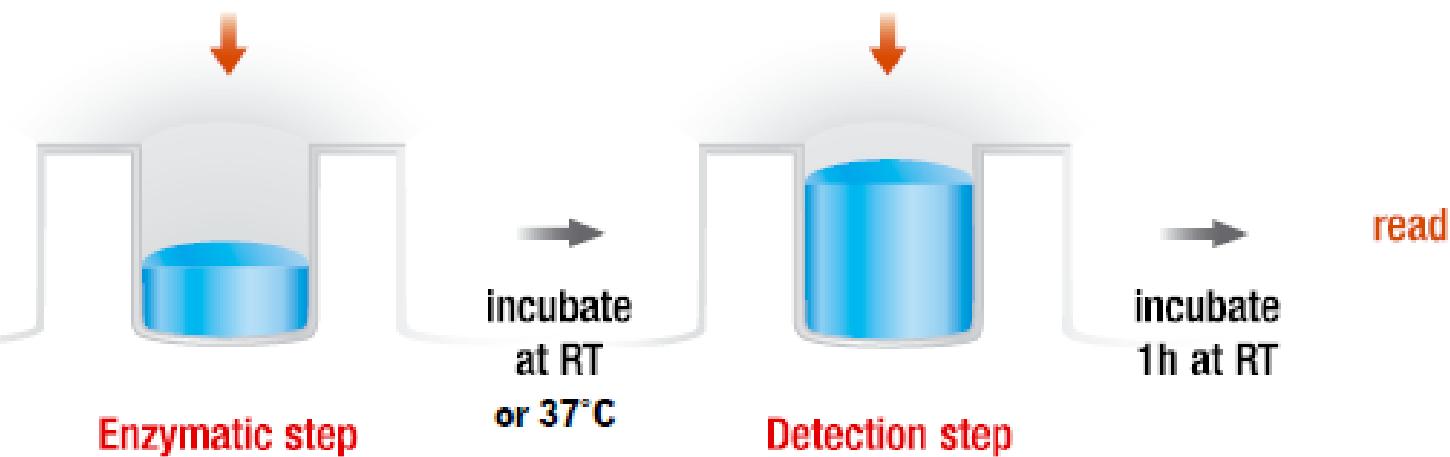
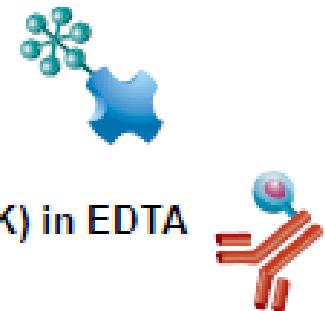


Choice of the substrate: STK S3

Kinase assay

4 µL Compounds (or kinase buffer)*
+ 2 µL Kinase
+ 2 µL STK Substrate-biotin
+ 2 µL ATP

5 µL Sa-XL665 in EDTA
+
5 µL STK Antibody-Eu(K) in EDTA



Search for Msk-1 inhibitors

- Relatively easy to set up for screening
- Excellent Z' ($> 0,75$)

Results

Tested compounds	Primary hits	Confirmed hits	Fluorescent	False positive	Confirmed (other technology)
7120	55	42	37	38	5
	0,77%	0,59%	88 % of confirmed hits	90 % of confirmed hits	0,12 % of confirmed hits

Ccl:

- **primary screen was performed rapidly with KinEASE kit**
 - + **convenient, high throughput, robust, very sensitive)**
 - **false positives (fluorescent compounds, biotin like compounds)**
12 identical false positives
- **hit confirmation was performed using a luminescent based technology (at lower throughput)**

V1A Project:

Pr Marcel HIBERT & Dr Dominique BONNET
(Laboratory of Therapeutic Innovation, Strasbourg)

Msk-1 project:

Dr Nelly FROSSARD & Simona NEMSKA (PhD student)
(Laboratory of Therapeutic Innovation, Strasbourg)

PCBIS:

Sophie Gioria (V1A)
Adeline Obrecht (Msk-1)



Thank you

