afmb
architecture et fonction
des macromolécules biologiques

Screening platform of Marseille-Luminy

Biochemical assays and technical developments

Contact : Jean-Claude.Guillemot@univ-amu.fr

Région Provence Alpes Côte d'Azur

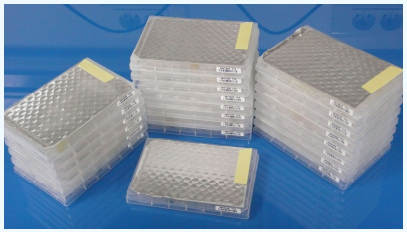
Ville de Marseille www.marseille.fr

Plateforme technologique Aix-Marseille

Aix*Marseille université

cnrs advancing the frontiers

The screening platform of Marseille-Luminy (PCML)



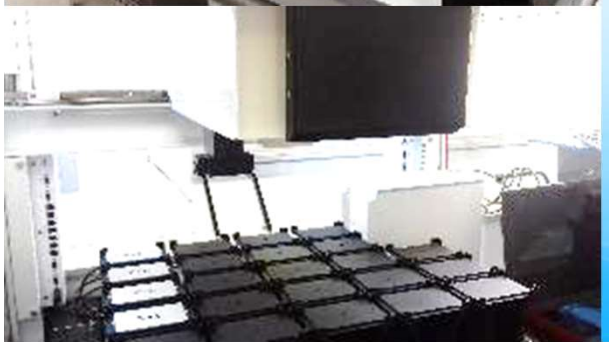
Chemical Libraries

Modul-Bio
High-throughput solutions

Databases

Radioactive or fluorescent screening
HTRF

Robotics



Reading

Scintillation

Fluorescence

Results

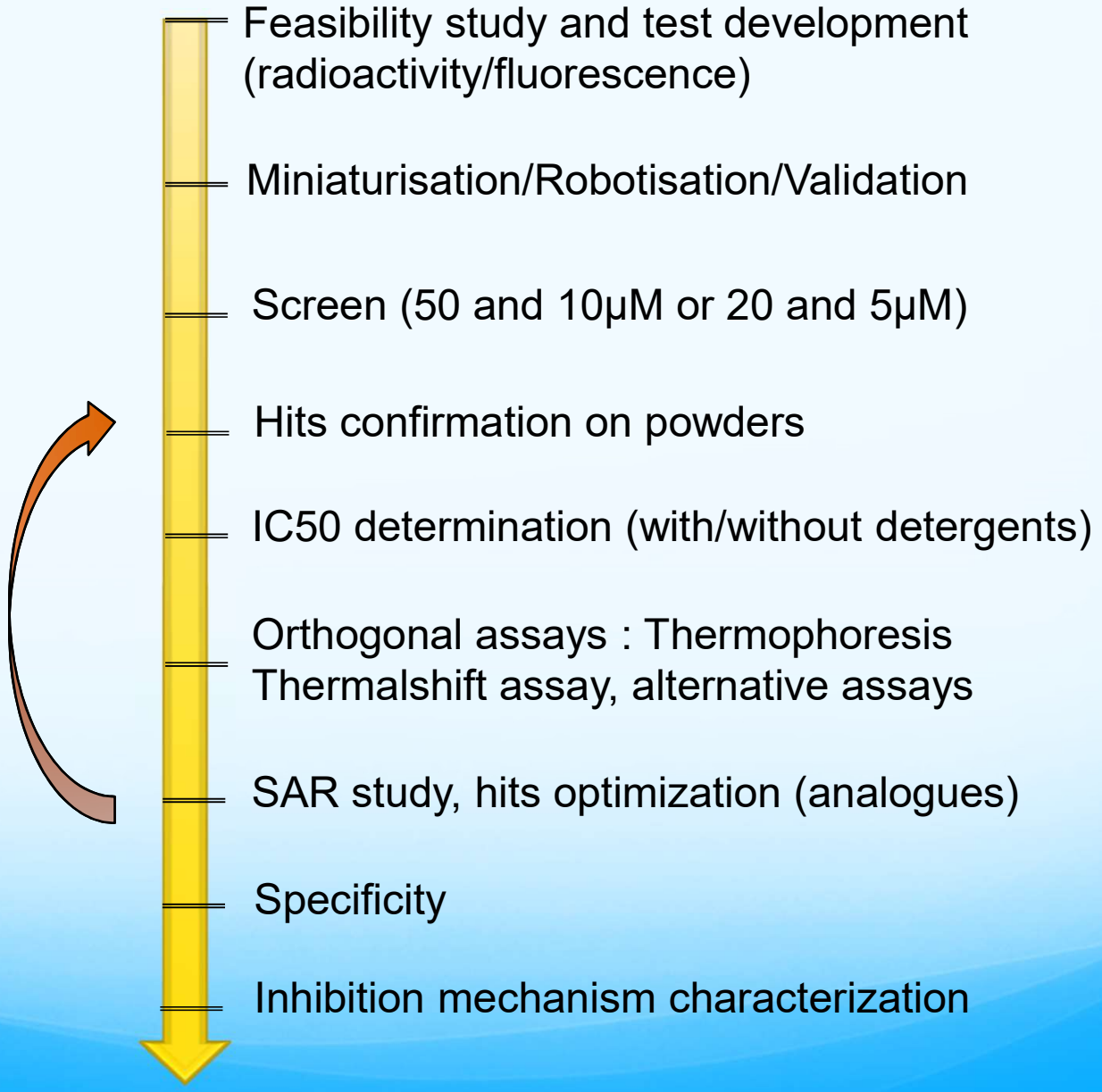
Data analysis
- % inhibition
- IC_{50}

PCML Library in 2022

About 76,000 molecules from various origins are in stock :

- National cancer institute diversity library (NCI) (2000)
- Chembridge™ "Diversity Set" library (30,000)
- The “Chimiothèque Nationale Essentielle”(CNE), a representative subset of the Chimiothèque Nationale” (1040)
- The “Chimiothèque Nationale” (21,000) divided into :
    - Strasbourg Library (5000)
    - Curie Institute Library (8000), chemicals (3000) and natural extracts (5000) library,Gif-sur-Yvette ICSN
    - Caen Library (400)
- Active sightTM fragment based library (360)
- Compounds developed in-house by our chemists team (400) or molecules from collaborative studies and commercial providers (>2000) are regularly added to this base.
- Libraries focus on the inhibition of protein-protein or protein-peptide interactions : $2P2I_{3D}$ (1664), Life chemical rule of four (4300), subset ChemDiv Eccentric (515), PPICHem (10 314)
- Prestwick library (2240) : chemical (1520), natural (320), pyridazine (400)

From screen to hits



Screening results on Dengue polymerase

17081 Compounds

French National Library
(Strasbourg, Curie institut, ICSN, Caen libraries)
Prestwick library
National cancer institute diversity (NCI)

Screen at 50 µM
Threshold 80% inhibition

(8,4%) 1432 compounds selected

Screen at 10 µM
Threshold 80% inhibition

(0,87%) 149 compounds selected

Hits powder confirmation (10µM)

(0,35%) 60 compounds selected

IC50 determination, biochemical characterization

4 developed molecules,
including the original hit 3438

Production/Purification of enzymes of interest

1) Production

- Bacterial transformation (E.coli)
- pre-cultures and cultures (1 to 10L /protein)

2) Purification

- Bacterial lysis
- 1° step of purification : Affinity chromatography (Cobalt or Nickel) using histidin tag
- 2° step of purification : chromatography by ionic exchange ou size exclusion
- Appropriate storage according stability and future use of the protein
- Yield : from 0,3 mg to 2 mg/L

Available proteins on PCML

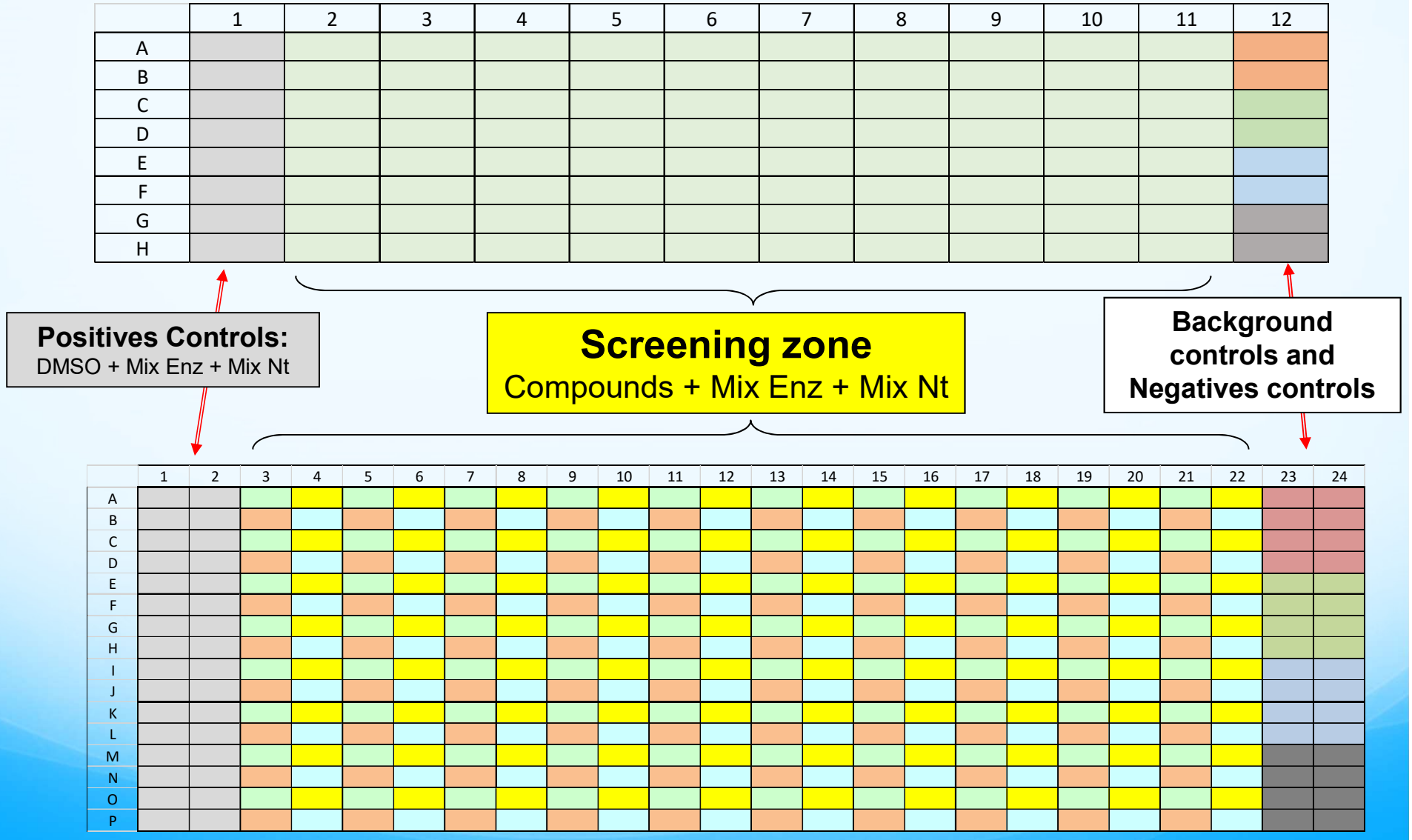
NS5 from
- Dengue virus serotypes 1,2,3 and 4
- Zika virus
- West-Nile virus

SARS replication complex :
- nsp12 Cov-1 and Cov-2
- nsp8 Cov-1 and Cov-2
- nsp8L7 Cov-1 and Cov-2
- nsp7 Cov-1 and Cov-2

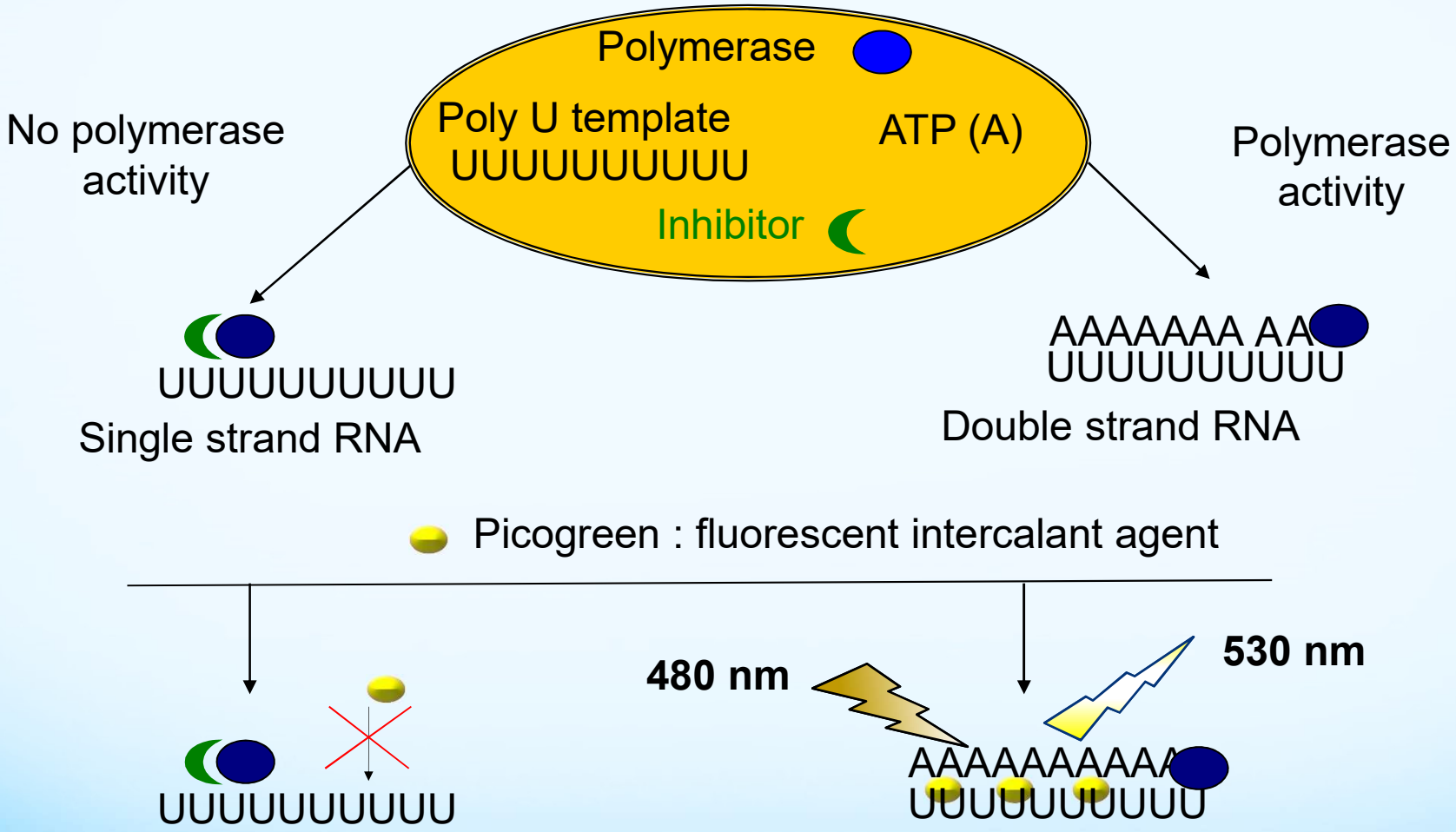
Polymerases from
- Dengue virus serotypes 2,3,4
- West-Nile virus
- HCV virus

Methyltransferases from
- Dengue virus serotypes 1,2,3 and 4
- Zika virus
- West-Nile virus

Experimental screening plate pattern
96 and 384 wells plates

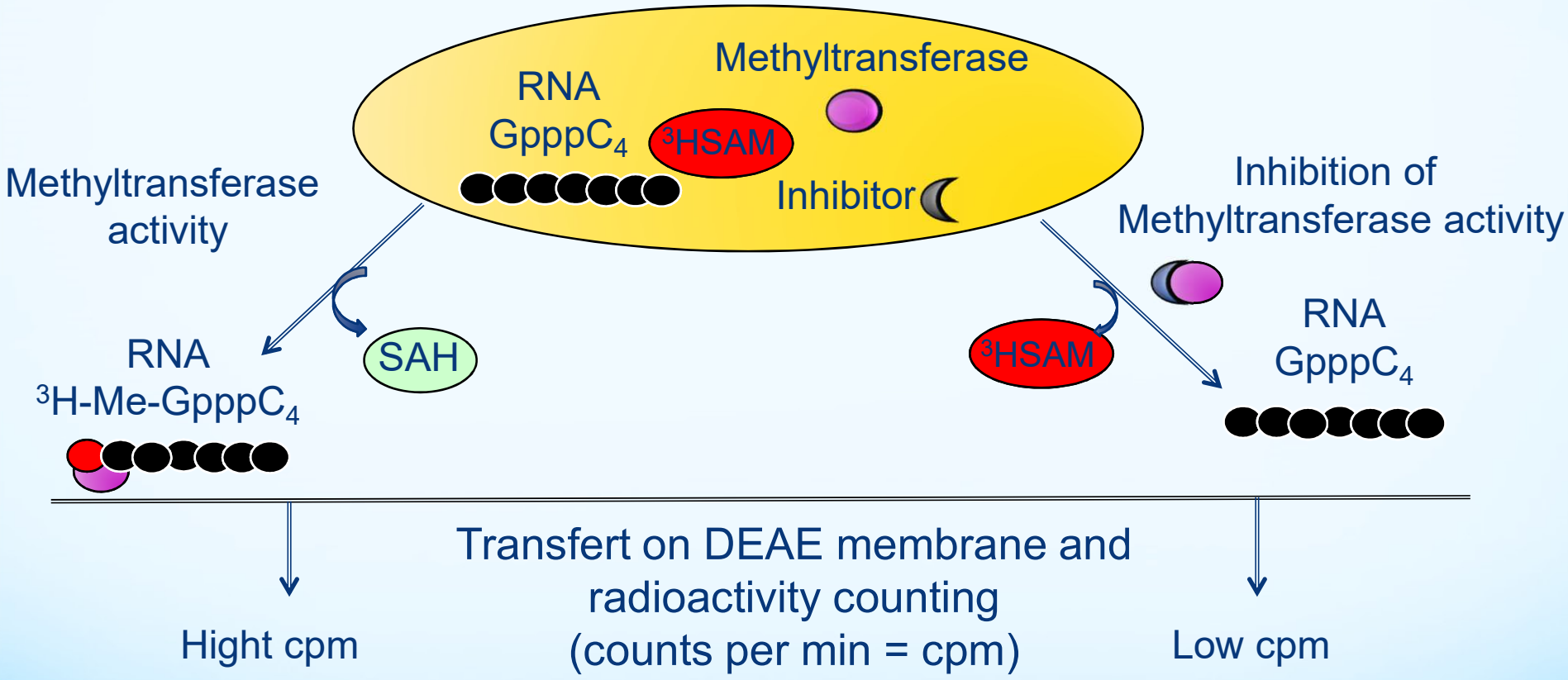


A screening polymerase assay based on fluorescence



Detection and quantification (RFU)
with a Tecan Safire^2 spectrofluorometer
% inhibition – IC50

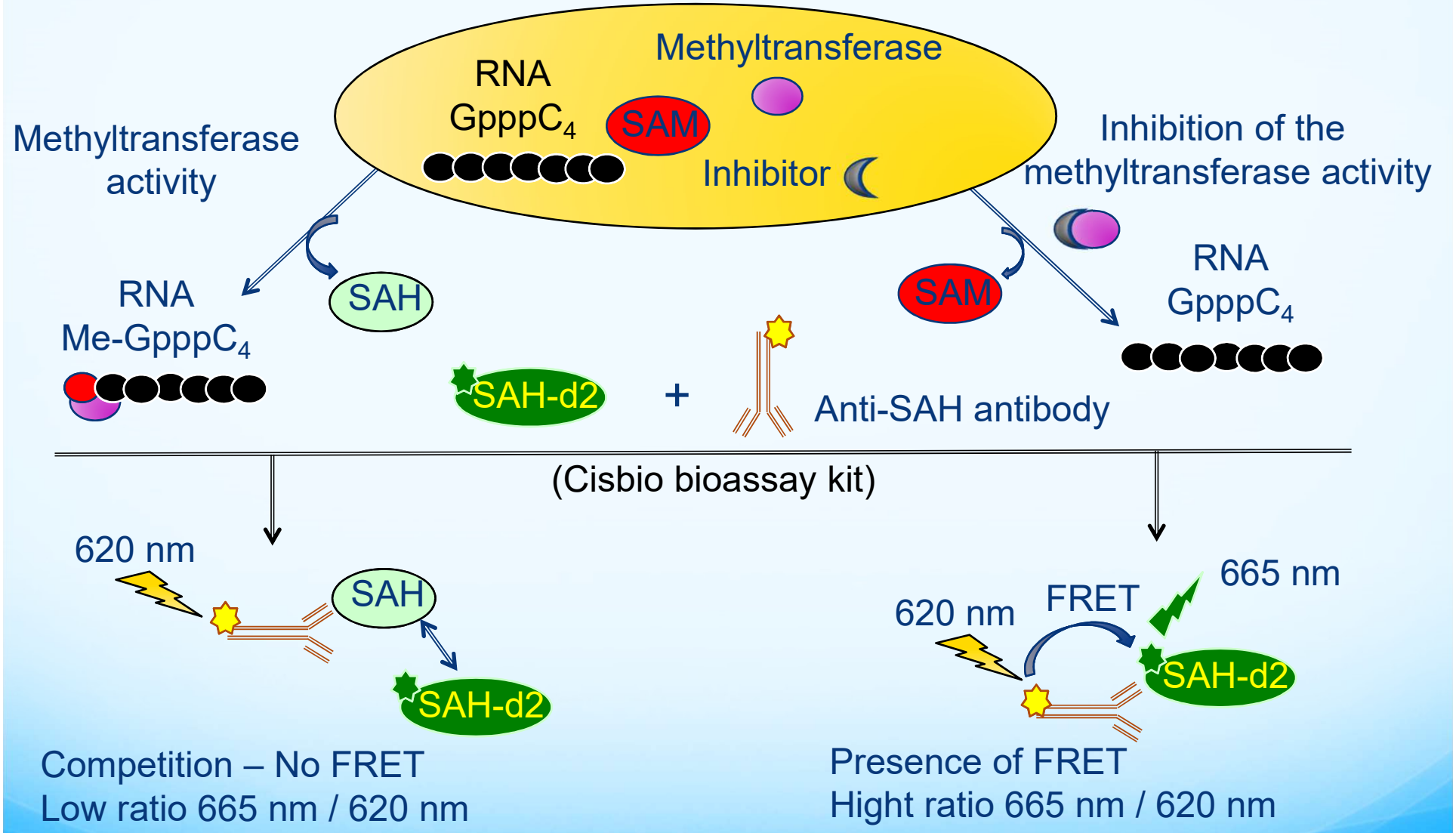
Determination of methyltransferase activity on radioactive assay



<u>Using :</u>

- Enzymes stocks validation
- Inhibitor potency evaluation of compounds (screens, IC50…)

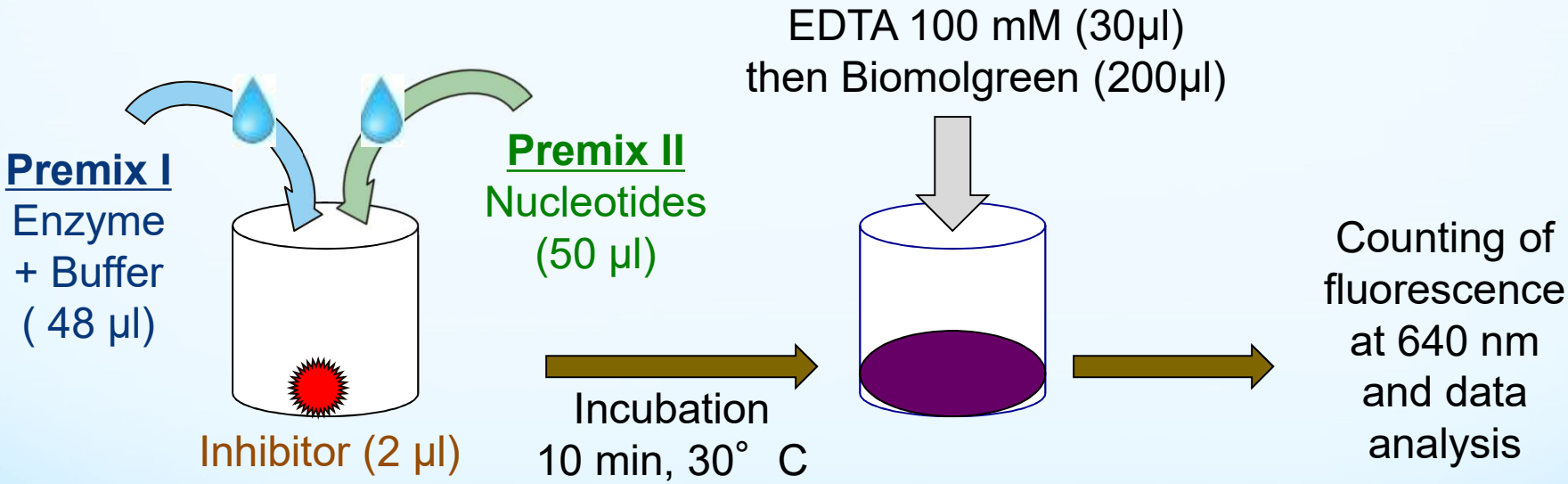
Determination of methyltransferase activity on HTRF assay



Using :

- Inhibitor potency evaluation of compounds (screens, IC50)

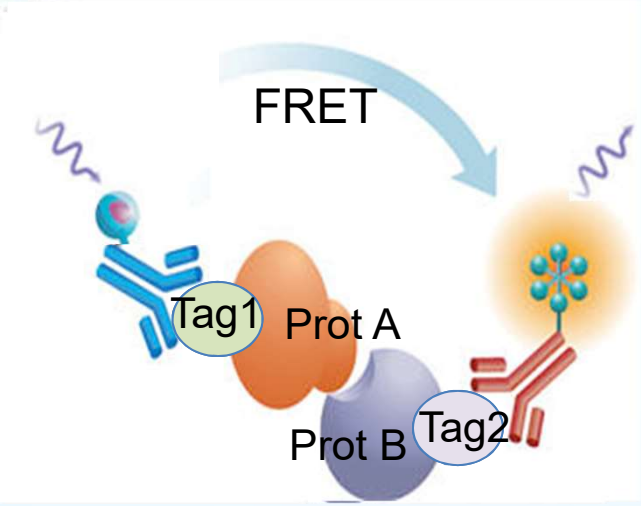
Determination of ATPase activity on fluorescent assay



Using :

- Inhibitor potency evaluation of compounds (IC50)
- Available on Dengue 3 NS3 and Helicase domain

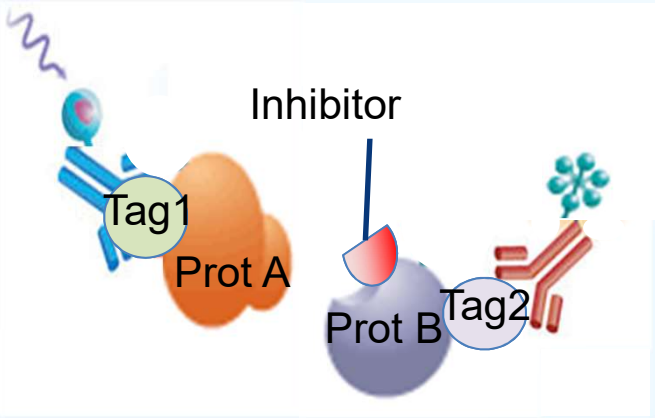
Protein-Protein interactions : screening method by HTRF



Absence of inhibitor

- HTRF signal

No FRET



Presence of inhibitor

- no HTRF signal

- Robotized assay (384-wells)
- Avalaible on Dengue NS3 – NS5MTase
- Screening on PPICHem library

Thermalshift assay (TSA)

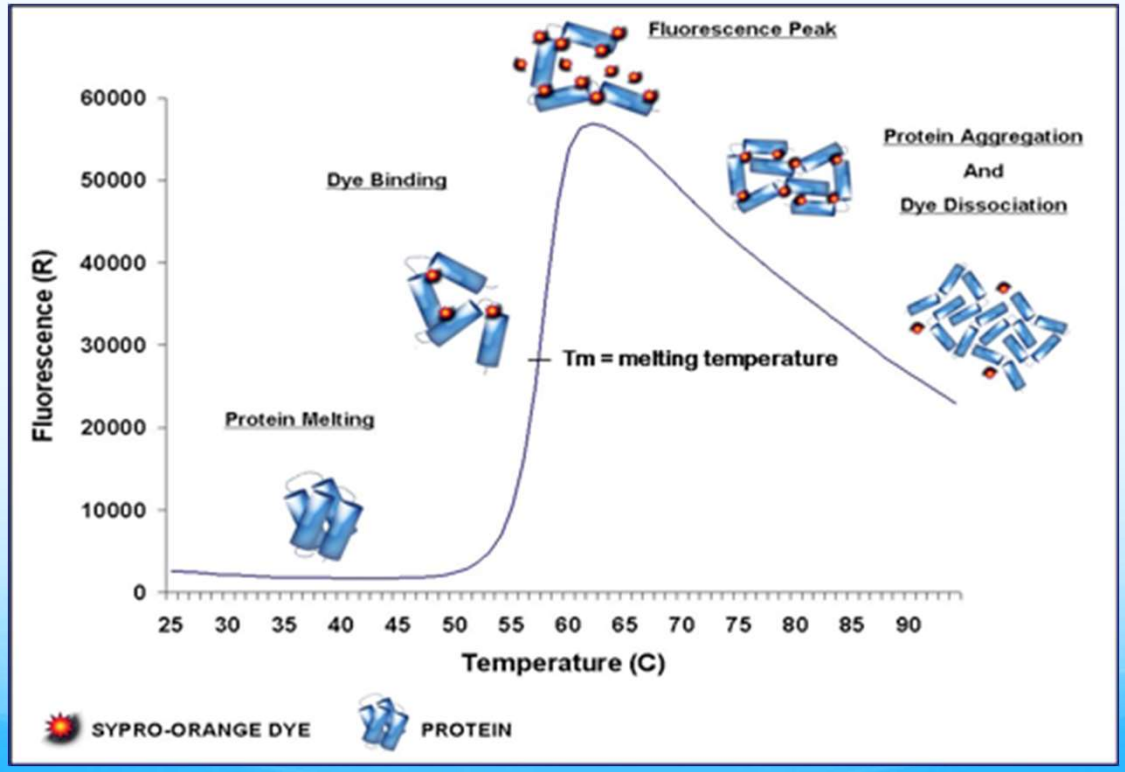
- Determination of the protein of interest Tm (melting temperature) by using a fluorescent probe (Sypro-orange)
- Tm measurement with compound and and determination of the variation (ΔTm)

ΔTm > 0 : the compound stabilises the protein

ΔTm <0 : the compound destabilises the protein

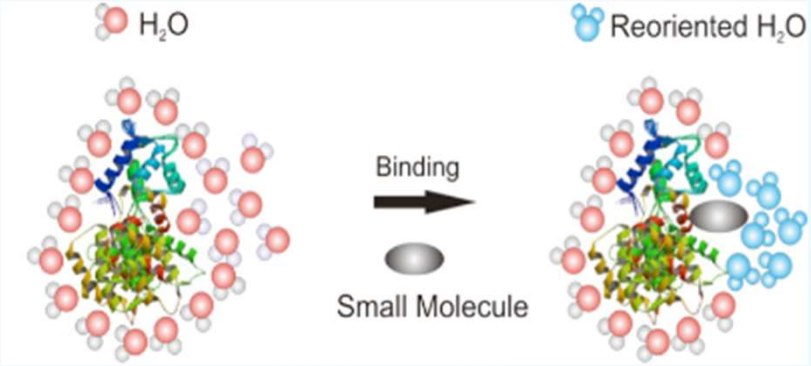
-> validation of the compound/protein interaction

-> help to cristallogenesis



Affinity constant (Kd) determination by thermophoresis

Principle :
Following of water molecules re-orientation in a temperature gradient after binding a ligand to a fluorescent labeled substrate



Substrate : Dengue Polymerase or Dengue NS5
Ligand : Ions, Nucleotides or Inhibitors

1) Development of the assay:
- Fluorescent labelling conditions
- Choice of capillary (standard/hydrophilic/ hydrophobic)
- Buffer composition and protein concentration
- Stability and reproductibility

2) Kd determination
16 concentrations of each inhibitor / assay

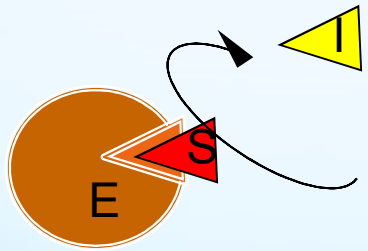
Biochemical characterization of a hit : Inhibition mode

Effect of increasing concentrations of inhibitor throught a range of substrate (Template or Nucleotide)

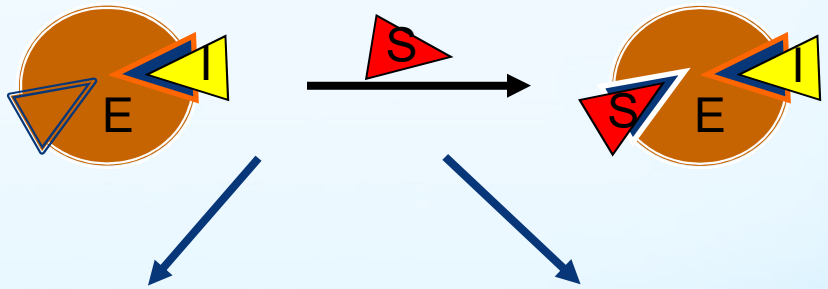
Determination of :
- inhibition mode (competitive / uncompetitive / non competitive or mixte)
- inhibition constant : Ki and Ki'

Fixation in the active site

<u>COMPETITIVE Inhibitor</u>



Fixation in an allosteric site



<u>NON COMPETITIVE Inhibitor</u>

Fixation both on ES complex and free E

<u>UNCOMPETITIVE Inhibitor</u>

Fixation only on ES complex

S : Substrate
I : Inhibitor
E : Enzyme
ES : Enzyme + substrate

Limitation : Only possible on the best compounds
Few months/compounds