Screening Platform from Marseille-Luminy - PCML

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(in alphabetical order)

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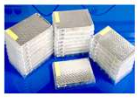
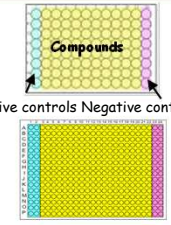
The emergence of viruses and the lack of therapeutic treatments directed against them is a growing worldwide threat. To address this problem, we have designed a viral genomic approach focused on the inhibition of viral targets. The combination of our experience in crystallography, enzymology and chemistry allowed us to develop a workstation devoted to the screening of chemical compounds (from commercial or academic sources) on recombinant and purified viral proteins. Rational chemical design will lead to potential new medicines. PCML offers its work experience and know-how to screening compounds on viral proteins, development of new technologies and providing chemical librairies according the project (ex : PPiChem library for the interaction protein/protein)



Chemical molecules Natural extracts (76.000)



In vitro Screening (96 and/or 384 wells)

SAR

Data base - LISM

Data analysis

Molecules hits

- **Biochemical characterization (% Inhibition, IC50…)**
- **Lead optimization**

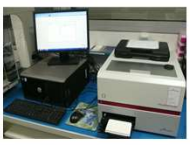
Orthogonal validation :
- Thermophoresis (Kd)
- Thermal-shift assay (Tm)
- Polarisation
- Inhibition mode

Beckman – Biomek I5

Beckman – Biomek 4000

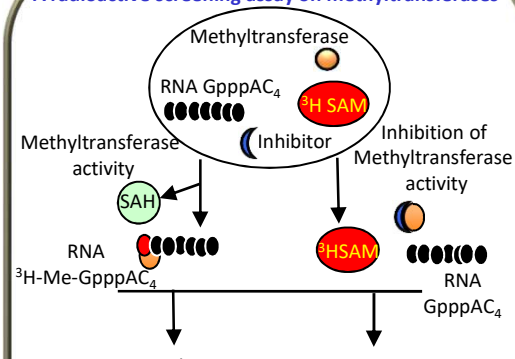
Perkin - Scintillation counter

BMG Labtech - Pherastar



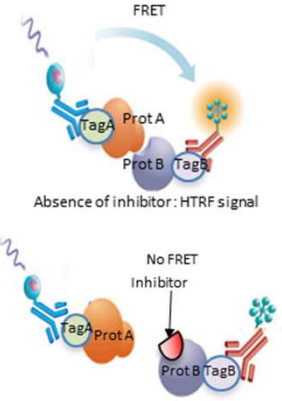
Tecan- Safire2

A radioactive screening assay on methyltransferases

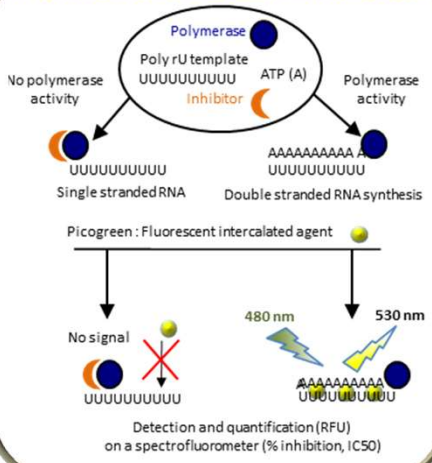


Transfert on DEAE membrane and radioactivity counting
(counts per min = cpm)
(% inhibition, IC50)

Protein-protein interactions : a screening method by HTRF

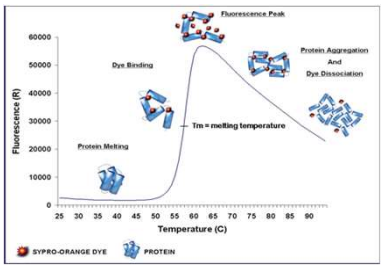


A fluorescent screening assay on polymerases



An orthogonal assay : specific fixation of compounds by 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

Our viral protein targets

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

In order to speed-up the discovery process of new antiviral agents, we have developed an integrated screening and drug design platform to discover, develop and characterize potential viral inhibitors starting from librairies of small organic molecules and natural extracts. PCML belongs to the Antiviral Drug Design Platform (AD2P) and to the Marseille Screening Center (MaSC). This collaborative network creates a dynamic interface appropriate for project development and optimization to extend the search for bioactive molecules.