



Screening Platform of Marseille-Luminy (PCML)



PCML is involved in MaSC

Platforms bringing together 3 sites specializing in screening



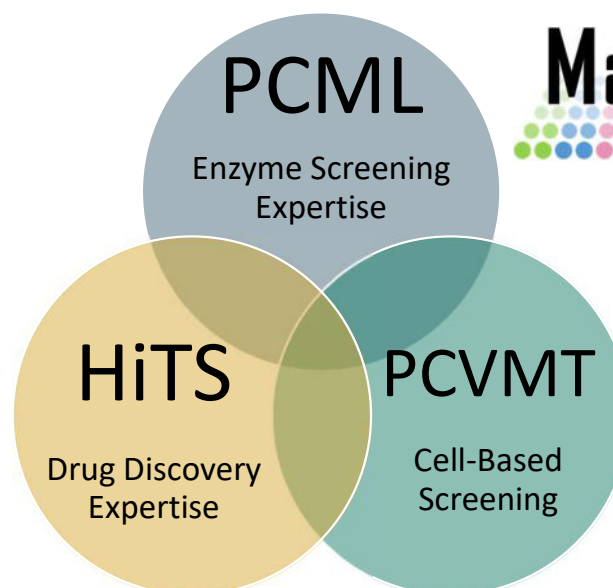
MaSC

Marseille Screening Center

<https://masc.univ-amu.fr>



PLATEFORME
TECHNOLOGIQUE
AIX-MARSEILLE



PCML : People



Jean-Claude Guillemot
Professeur

Scientific manager



Cécilia Eydoux
Ingénieure de recherche

Operational manager



Tom Malaval
Ingénieur d'études

Production-Purification of
proteins – Assays developments
(AMIDEX/CISAM+ funds)



Barbara Selisko
Ingénieure de recherche

Coordination - Logistic, administration

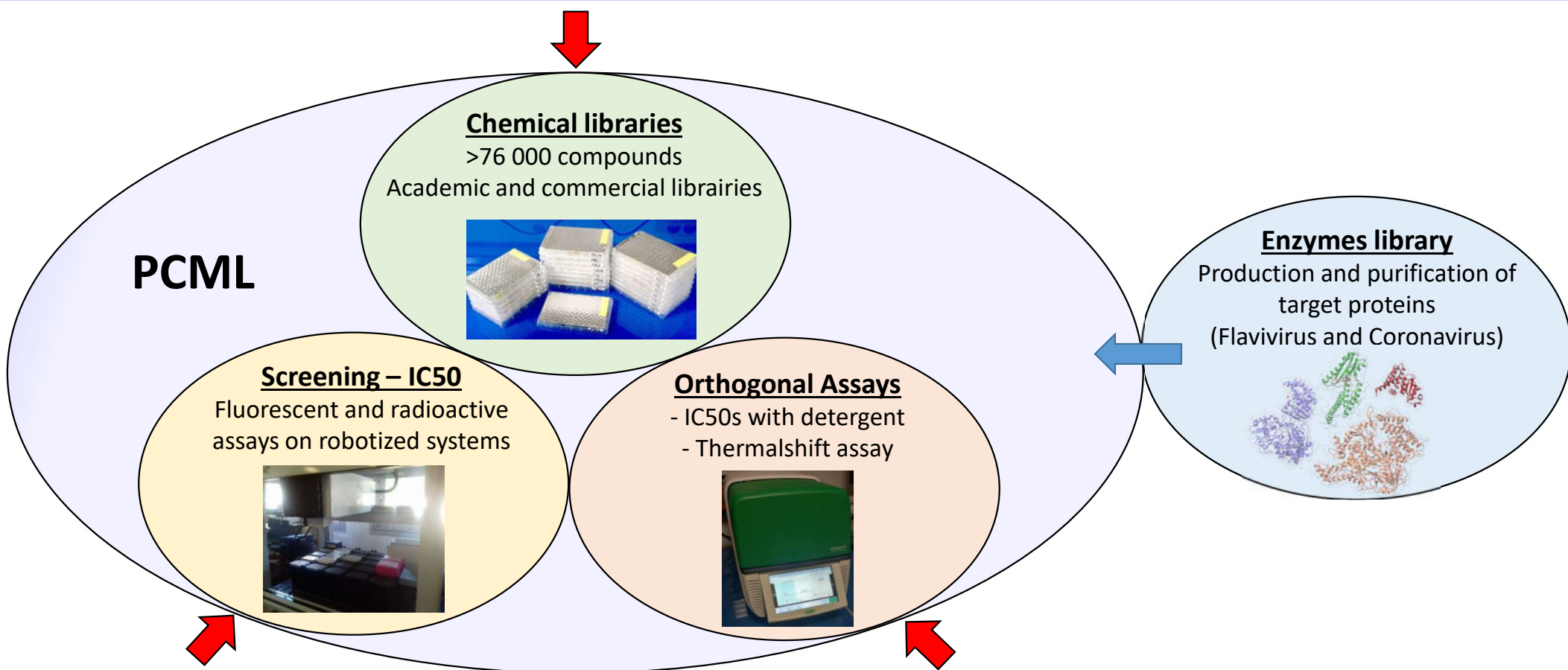


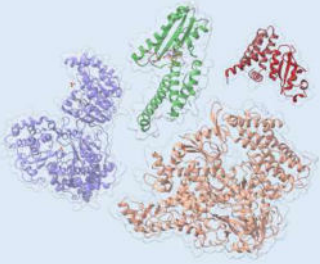
Bruno Canard
Directeur de recherche

Scientific committee member
Internal expert

<https://www.afmb.univ-mrs.fr/facility/plateforme-de-criblage-marseille-luminy/>

Expertise and know-how of PCML





Enzyme library



Mikael Feracci
Postdoctoral fellow



Tom Malaval
Ingénieur d'études

Flavivirus

18* NS5 full

6* MTase

15* RdRp

PCML

Medium to High
throughput assays

Coronavirus (1&2)

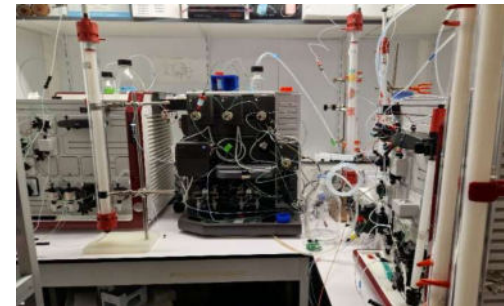
Nsp12

Nsp8

Nsp8L7 (CoV2)

Nsp7L8 (CoV1)

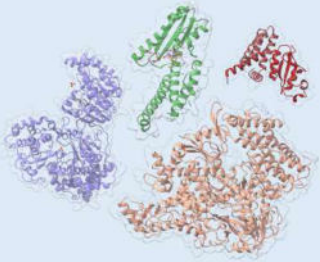
Nsp13 (CoV2)



NS5, RdRp and MTase proteins & assays availables

PROTOTYPIC target		Specificity (Pan – target)		Production / purification			Picogreen assay			TSA assay		
				NS5	RdRp	MTase	NS5	RdRp	MTase	NS5	RdRp	MTase
	MODV	Modoc virus		X	X					<i>not possible</i>		
	PWV	Powassan virus		X	X		X	X		X	X	
	KFDV	Kyasanur Forest Disease virus		X						X		
	OCHFV	Omsk Hemorrhagic Fever virus		X	X		X			X		
	LIV	Louping-ill virus		X	X		X			X	<i>not possible</i>	
	TBEV	Tick-Borne Encephalitis virus		X	X							
	YFV	Yellow Fever virus		X	X		X	X		X	X	
	EHV	Edge Hill virus		X	X		X	X		X	X	
	SABV	Saboya virus		X						X		
	DENV4	Dengue virus serotype_4		X	X	X	X	X		X	X	X
	DENV1	Dengue virus serotype_1		X	X	X	X	X		X	X	X
	DENV3	Dengue virus serotype_3		X	X	X	X	X	X	X	X	X
	DENV2	Dengue virus serotype_2		X	X	X	X	X		X	X	X
	ZKV	Zika virus		X	X	X	X	X		X	X	X
	ITV	Israel Turkey Meningoencephalitis virus		X	X		X			X		
	SLEV	Saint Louis Encephalitis virus		X			X			X		
	JEV	Japanese Encephalitis virus		X	X		X	X		X	X	
	WNV	West Nile virus		X	X	X	X	X		<i>not possible</i>	X	

Clustal-Ω sequence alignment based on the RdRp domain.



Enzyme library



Bunyavirales

(*LCMV, Lassa, Toscana, Rift valley fever virus...*)

11* EndoN

3* ExoN

L – protein

Retro-Virus (*HIV*)

Reverse transcriptase

Mononegavirales (*RSV*)

RdRp, Mtase

Alpha-like Viruses (*VEEV-HEV-Chik*)

Nsp1

ORF-1 : Hel, Macro, Mtase

**Viral
team**

Low
throughput assays
(characterization,
mode of action)

Coronavirus (1&2) (*MERS,SARS*)

Nsp14 (ExoN)

Nsp7

Nsp15 (EndoU)

Nsp9

Nsp3 (Macro and Protease)

Arteriviridae (*EAV*)

Nsp9

Nsp6 , Nsp 7

Picornaviridae (*Cox,Polio*)

RdRp, Helicase

Others Flaviviridae (*HCV, BVDV*)

NS5b



Chemical libraries

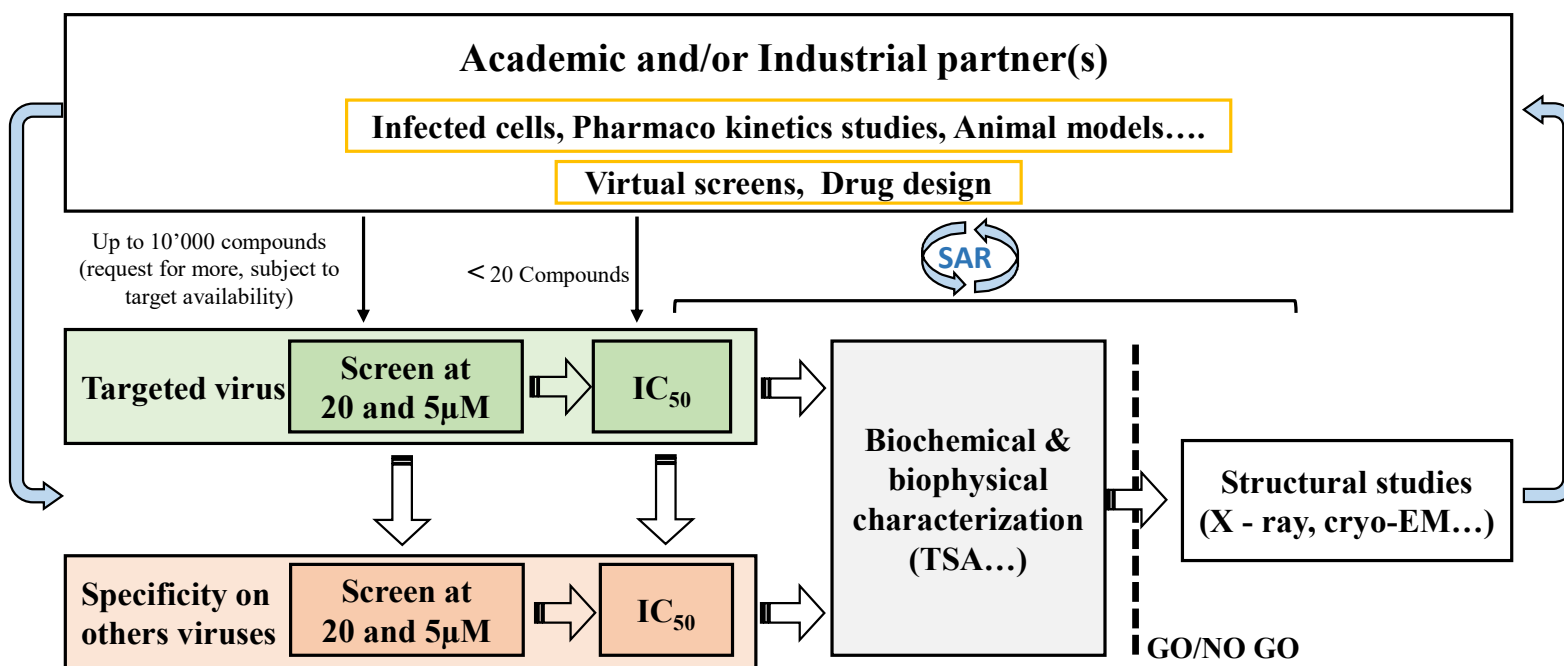
More than 76 000 compounds are available from academic and commercial libraries

- Prestwick library (chemical, natural, pyridazin)
- National Cancer Institute diversity (NCI) library
- Chembridge™ « Diversity set » library
- French National Library divided into :
 - Strasbourg library
 - Curie Institute library
 - Gif sur Yvette ICSN library
 - Caen library
- Active sight™ fragment based library
- Medecines for Malaria Venture (MMV) library (Pandemic and GHP)
- Libraries dedicated to the inhibition of the interaction protein/protein or protein/peptide :
 - 2P2I3D
 - Fr-PPICChem
 - Life chemical rule of four
 - Part of the ChemDiv Eccentric
 - Fragments library

Plates size :

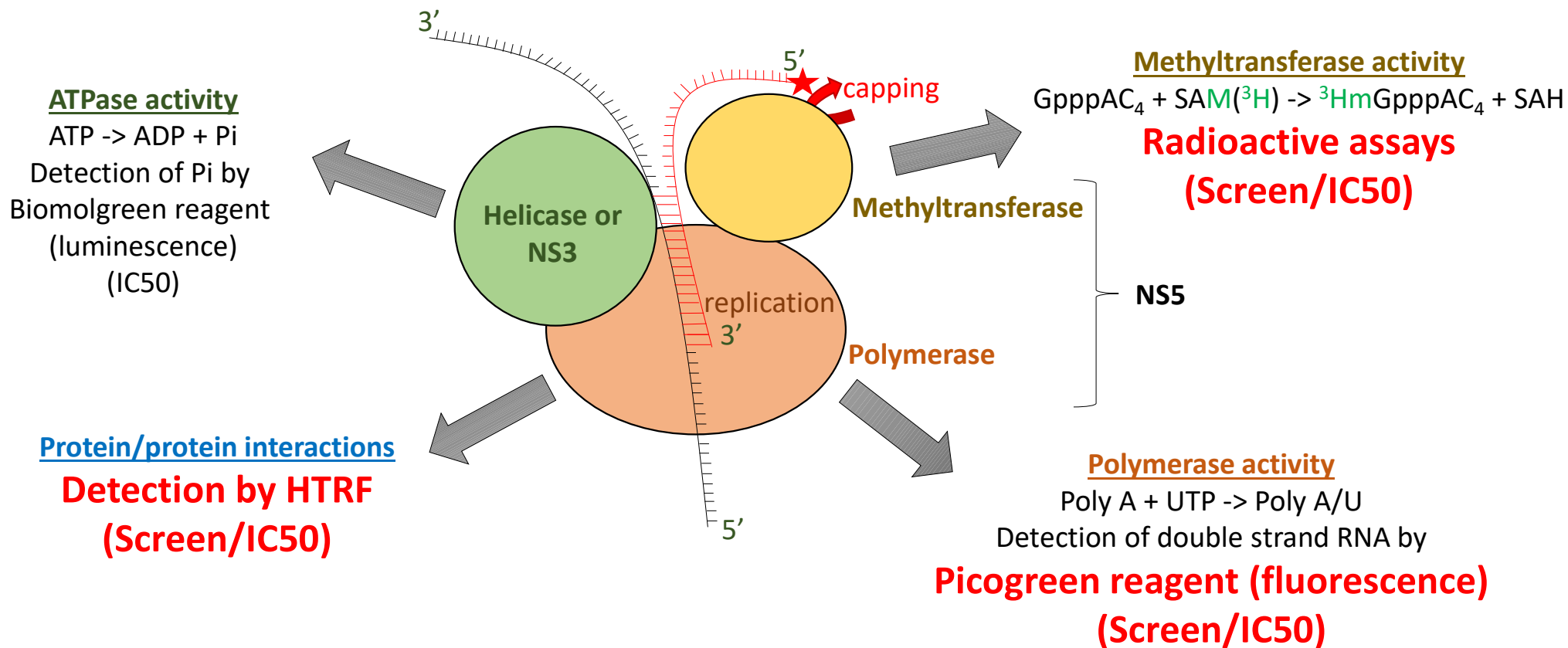
- 96 wells
- **384 wells**
- **1536 wells** (NS5 DV, Sars CoV)

Workflow on PCML





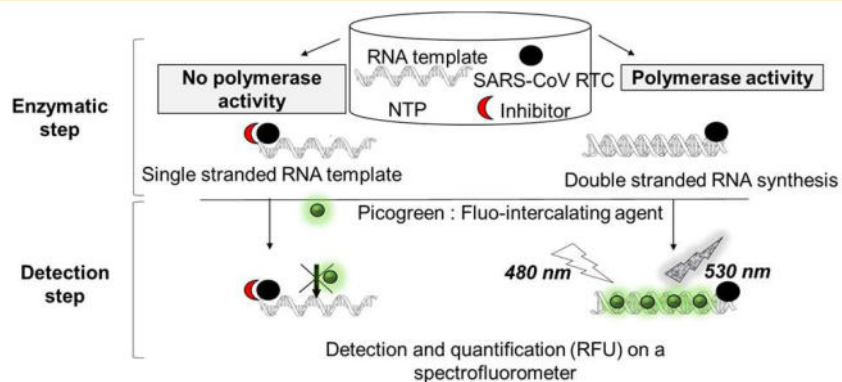
Screening – IC50





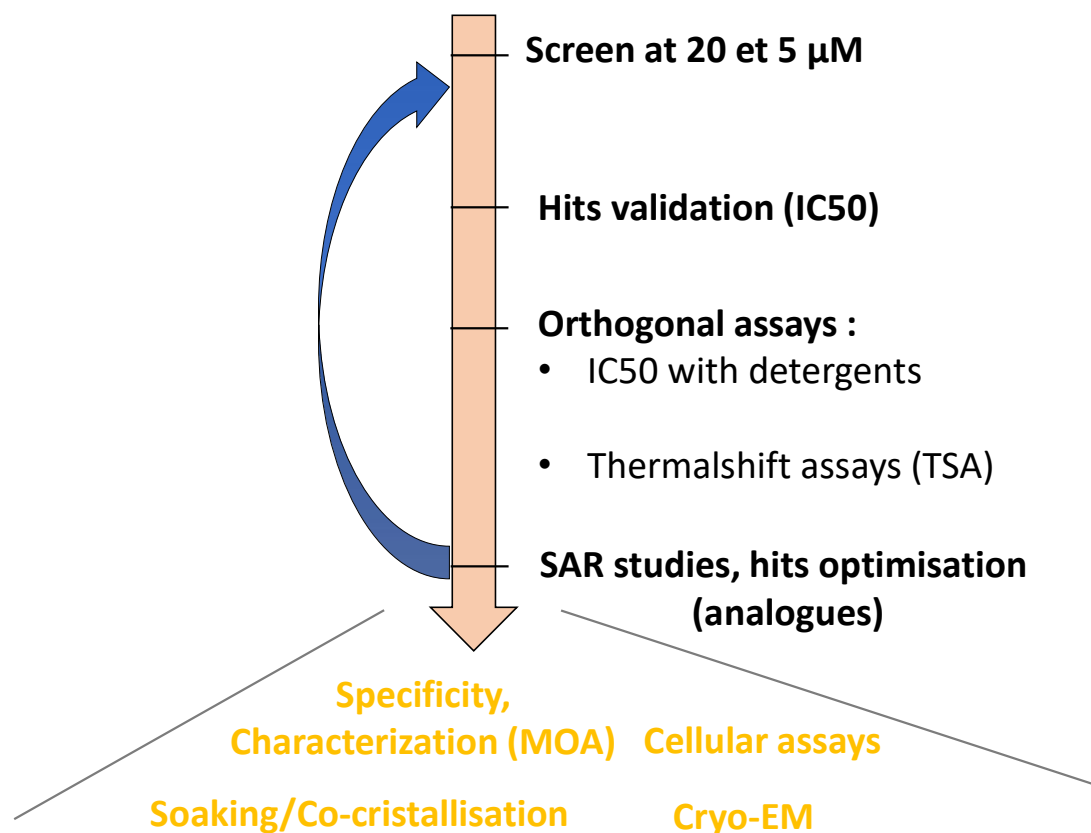
Screening – IC50

Example on SARS Cov-2 replication complex



Eydoux C, Fattorini V, Shannon A, Le TT, Didier B, Canard B, Guillemot JC.
J Virol Methods. 2021 Feb;288:114013. PMID: 33166547

	ID	Number of Compounds	Hit rate	Z'
Prestwick	Chemical	1520	3.02%	0,8
	Natural	320	9.7%	0,75
	Pyridazine	400	2.2%	0,78
Academic	PPI-Chem	10 314	0.75%	0,85
	CNE	1040	3.4 %	0,8
	Virtual screen	618	0,30%	0,84
Pharma Y	Virtual screen	150	0%	0,8
Pharma J	virtual screen	129+2839	0 to 1,2%	0,75
Pharma P	Virtual screen	4996	(0,1 to 0,04%)	0,78
		22 326		



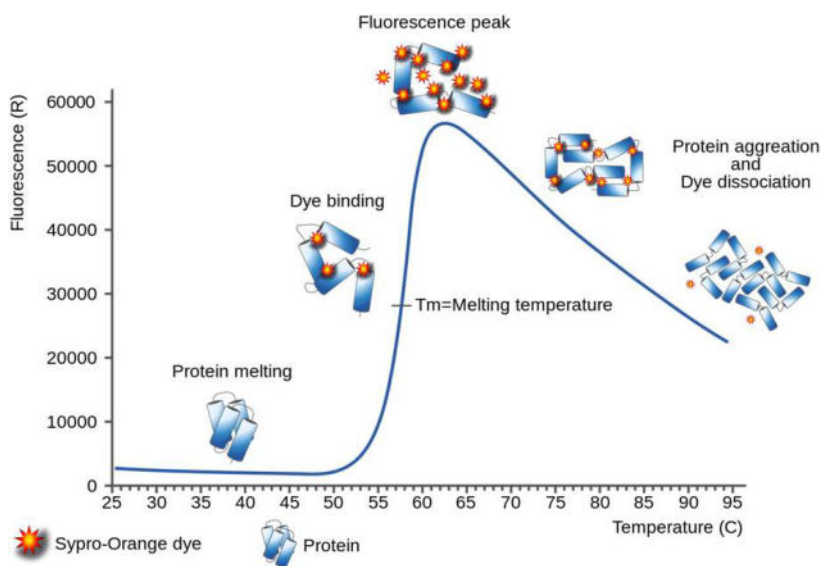


Orthogonal assays



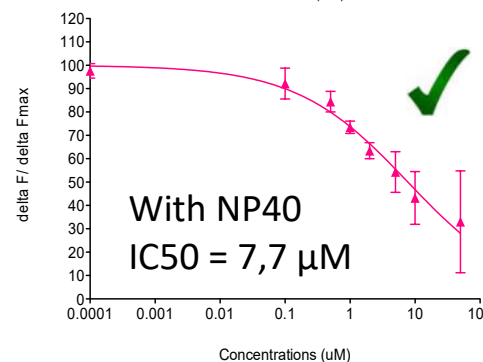
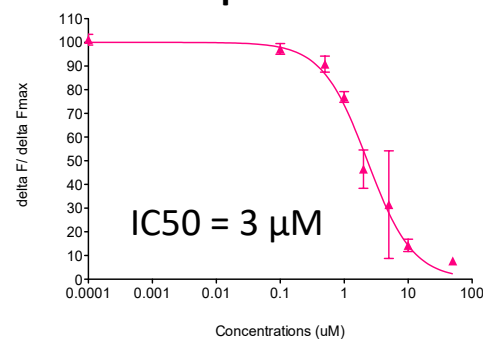
Thermal shift assay (TSA)

Validation of the compound/protein interaction ($K_{d_{app}}$)
Help to cristalogenesis, cryo-EM...

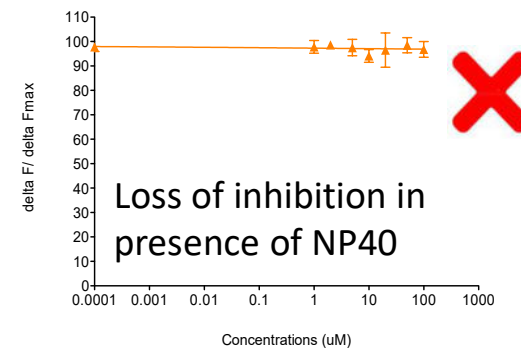
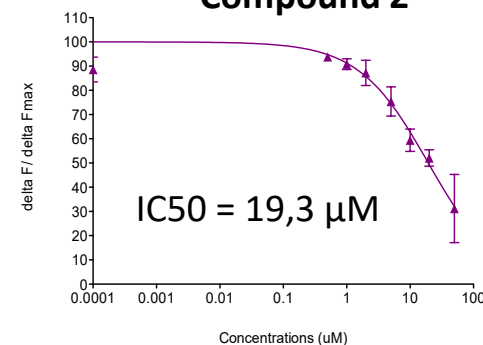


IC₅₀ with and without detergent
(agregator,unspecific binding...)

Compound 1



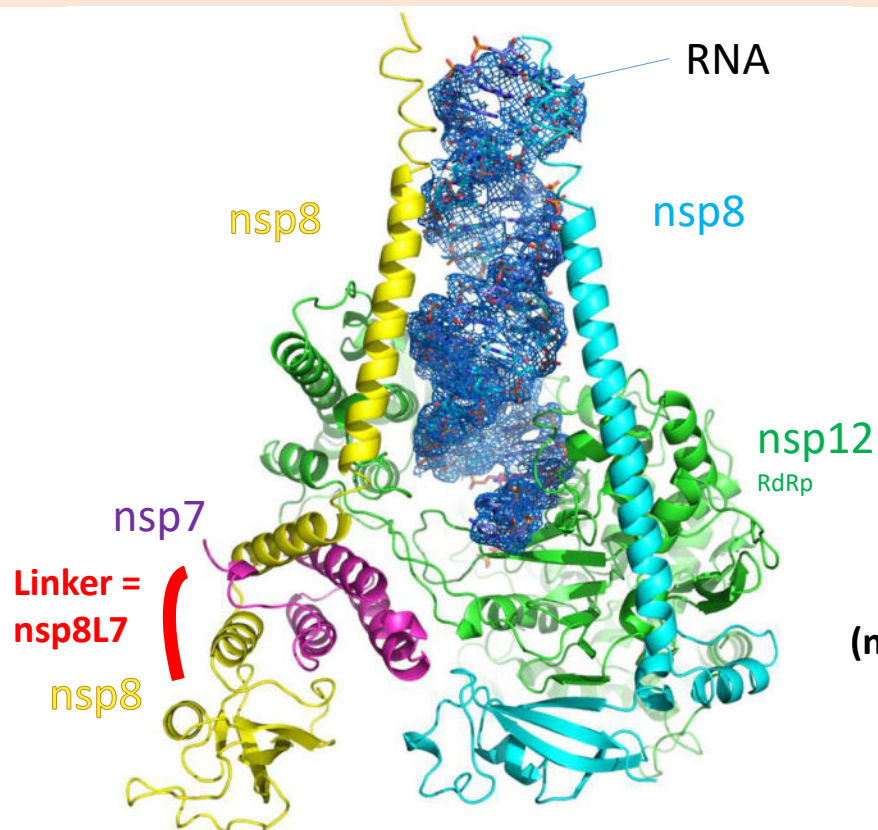
Compound 2





Orthogonal assays

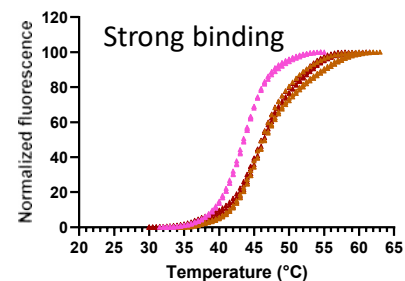
Simplify mapping of hits on the SARS-CoV2 RTC by TSA assay



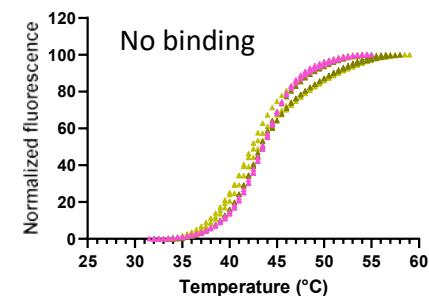
nsp12

(nsp12 + nsp8L7)
complex

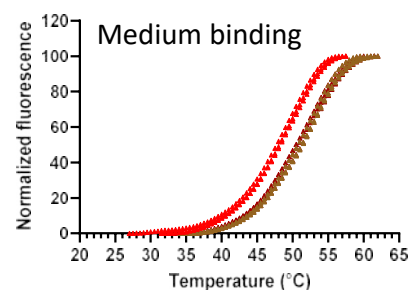
Hit A



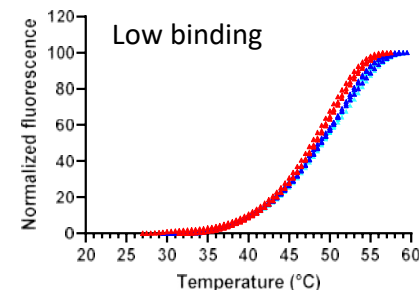
Hit B



Medium binding



Low binding



-> Hit A : Cryo-EM and co-crystalization are on going