

Cancer Research Center of Marseille (CRCM)

Institut Paoli-Calmettes

Private non-profit Hospital
Network of 'Cancer Centers' (**UNICANCER**)

Breast, Pancreas, Hematology...

1200 employees/230 beds

Clinical trials (>30 Phases I)



CRCM

>350 Employees @ Research Center
19 Research Teams in Cancer Biology
17 Technological Platforms

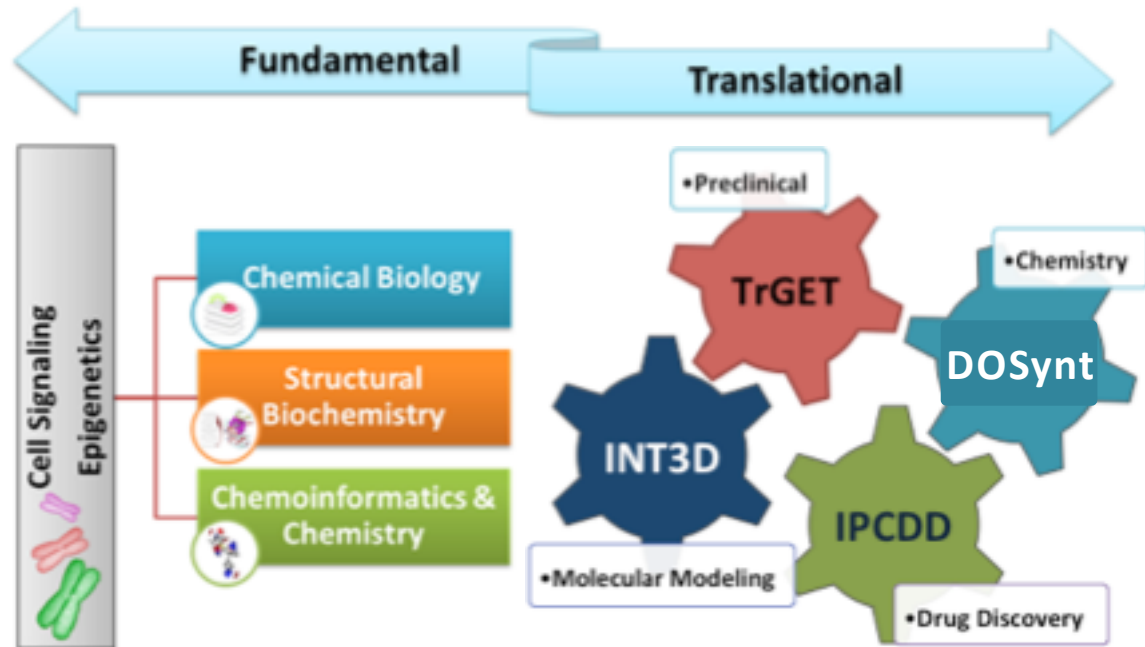


integrative Structural and Chemical Biology (iSCB)

Our Goals: develop “ Innovative approaches to tackle the refractory space of **Protein-Protein Interactions (PPIs)**, to define and target “druggable” PPIs, and to decipher molecular basis of oncogenesis.



<http://iscb.cnrs-mrs.fr/>

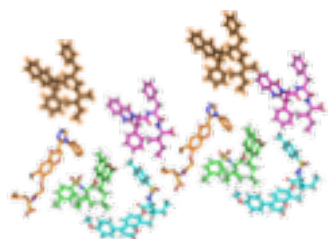


Co-Head : Drs. Xavier Morelli & Yves Collette

Strategies in PPI Drug Discovery

Knowledge-Based

Chemical Library Design

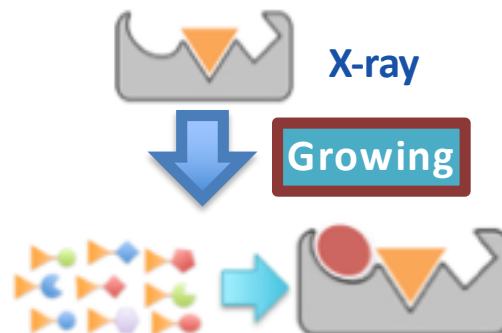


PPI-oriented Libraries

- Machine Learning
- SVM/RF

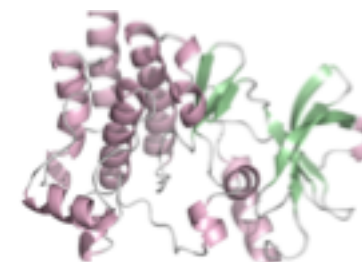
Structure-Based

Fragment Screening



- Diverse Focused Database
- Conformational Sampling

Virtual Screening



- Molecular Dynamics
- Docking
- Pharmacophore filtering

1

"Fr-PPIChem"
10K CPds

2

"DOTS"
integrated strategy

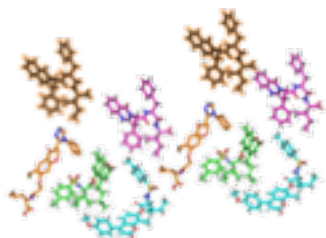
HTD Mc-Dso
Approach

Chemical Biology /
Drug Repositioning

Strategies in PPI Drug Discovery (PART I)

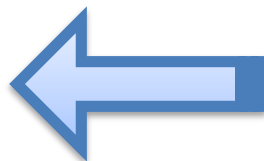
Knowledge-Based

Chemical Library Design



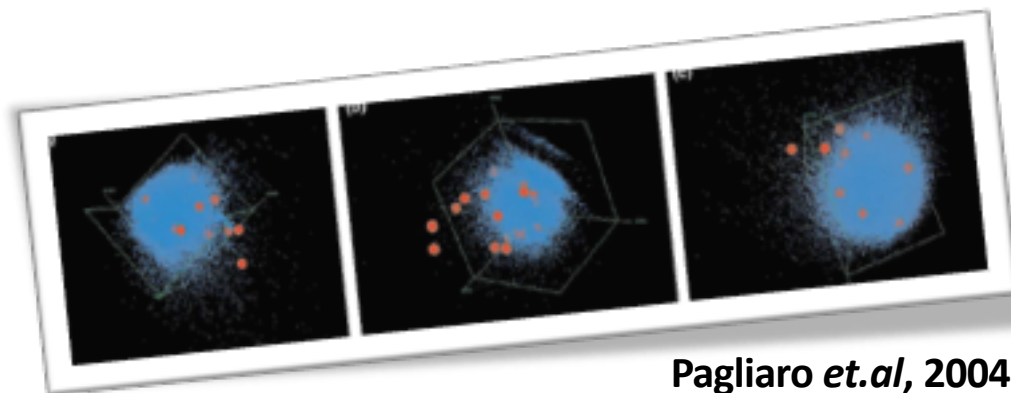
PPI-oriented Libraries

- Machine Learning
- SVM/Random Forest



Learn from
known inhibitors
to identify
new inhibitors

First Step



Pagliario *et.al*, 2004

Curr. Opin. Chem. Biol. 8, 442-9

2P2I_{DB}: A STRUCTURAL DATABASE



Since 2008...

<http://2p2idb.cnrs-mrs.fr>

2P2I_{DB}

**Structural Database dedicated to
orthosteric PPI modulators**



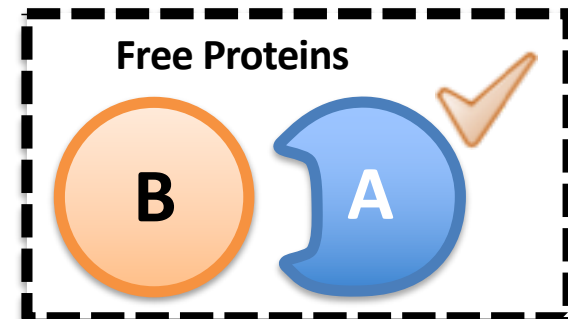
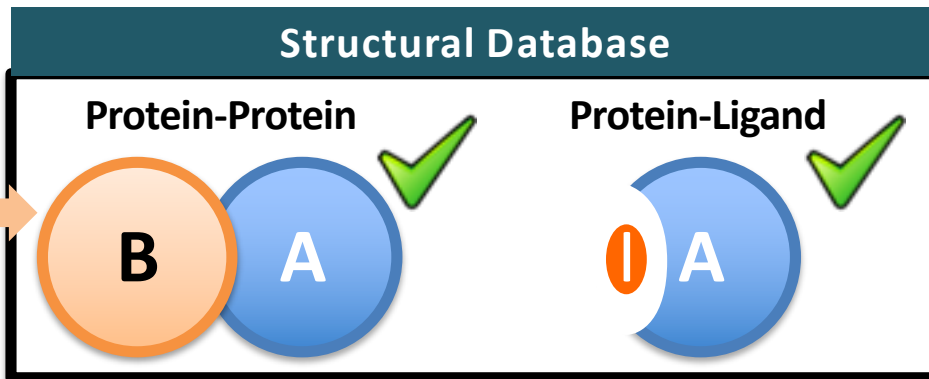
P Roche



MJ Basse



2P2I_{DB} : A Structural Database

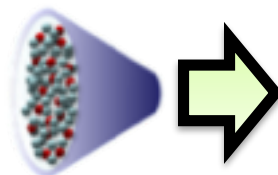
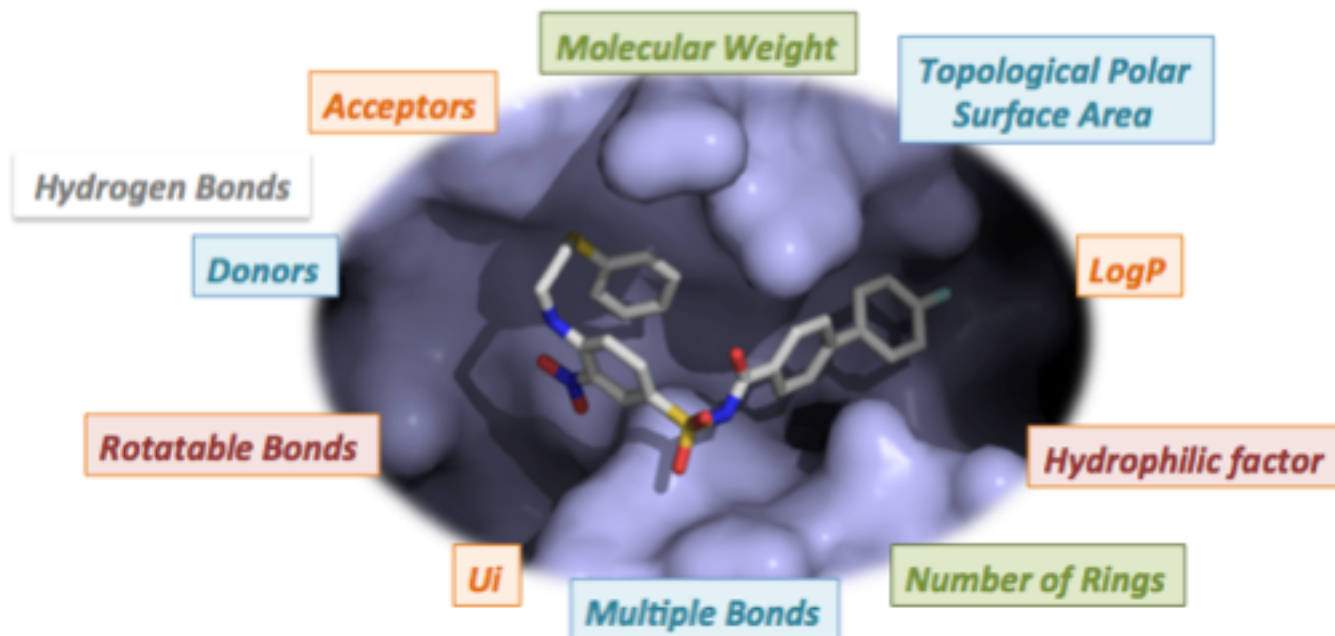


June 2018	Prot-Prot	Prot-Lig	Ligands
2P2I _{db}	32	742	659
Class 1	11	197	186
Class 2	9	152	138
BRD	11	393	335



Bourgeas, Basse, Morelli and Roche (2010) *PLoS ONE* 5:e9598
 Basse, Betzi, ..., Morelli and Roche (2013) *Nucleic Acid Research*, 41: D824-827
 Basse, Betzi, Morelli and Roche (2016) Database (Oxford).

2P2I Descriptors



In house PPI Chemical Library

2P2I_{3D}

1664 cpds



2P2I_{3D}: A PPI-Oriented Chemical Library



A Diverse & Privileged-based PPI-oriented Chemical Library

m/s Médecine/sciences 2015
Chemical libraries dedicated to protein-protein interactions
Sperandio O, Villoutreix B, Morelli X. and Roche P.
Med Sci (Paris), 31, 312-319.

MedChemComm 2013
2P2Ichem: focused chemical libraries dedicated to orthosteric modulation of protein-protein interactions
Véronique Hamon¹, Jean-Michel Brunel², Sébastien Combes², Marie-Jeanne Basse¹, Philippe Roche^{1,2*} and Xavier Morelli^{1,2*}

Future Medicinal Chemistry
Focused chemical libraries – design and enrichment: an example of protein-protein interaction chemical space
Xu Zhang, Stéphane Betzi, Xavier Morelli & Philippe Roche
Future Med. Chem. (2014) 6(11), 1291–1307

ACS chemical biology 2016
5 PPI Targets
Protein-Protein Interaction Inhibition (2P2I)-Oriented Chemical Library Accelerates Hit Discovery
Sabine Milhas^{1†}, Briggitt Raux¹, Stéphane Betzi¹, Carine Derviaux¹, Philippe Roche¹, Audrey Restouin¹, Marie-Jeanne Basse², Etienne Rebuffet¹, Adrien Lugari¹, Marion Badoir¹, Rudra Kashyap^{1†}, Jean-Claude Lissitzky¹, Cécilia Eydeux¹, Véronique Hamon¹, Marie-Edith Gourdel¹, Sébastien Combes¹, Pascale Zimmermann¹, Michel Aurand-Lions¹, Thomas Roux¹, Catherine Rogers^{1†}, Susanne Müller^{1†}, Stefan Knapp^{1†}, Eric Trinquet¹, Yves Collette¹, Jean-Claude Guillemot¹, and Xavier Morelli^{1†}

Journal of Medicinal Chemistry 2016
Bromodomains
Exploring Selective Inhibition of the First Bromodomain and Extra-terminal Domain (BET) Proteins
Briggitt Raux¹, Yulia Voltovich^{1†}, Carine Derviaux¹, Adrien Lugari¹, Etienne Rebuffet¹, Sabine Milhas^{1†}, Stéphane Prie¹, Thomas Roux¹, Eric Trinquet¹, Jean-Claude Guillemot¹, Stefan Knapp^{1†}, Jean-Michel Brunel¹, Alexey Yu. Fedorov¹, Yves Collette¹, Philippe Roche¹, Stéphane Betzi¹, Sébastien Combes¹, and Xavier Morelli¹

Proof of concept validated ✓

'Fr-PPICHEM' : a French National initiative

PPICHEM

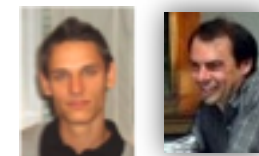


10,000 cpds

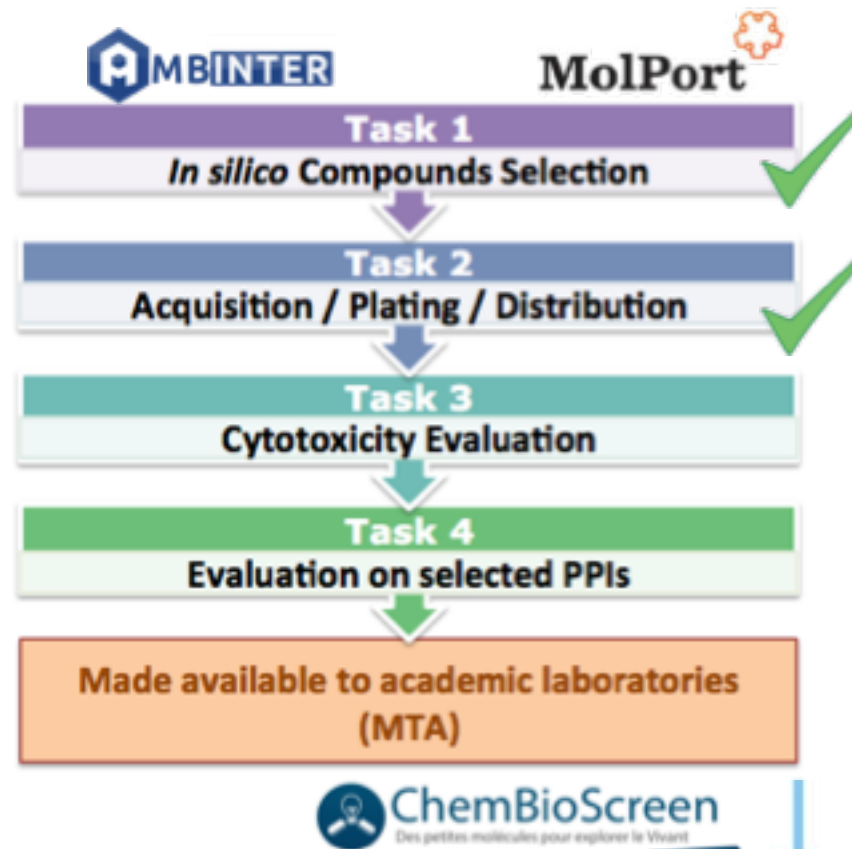
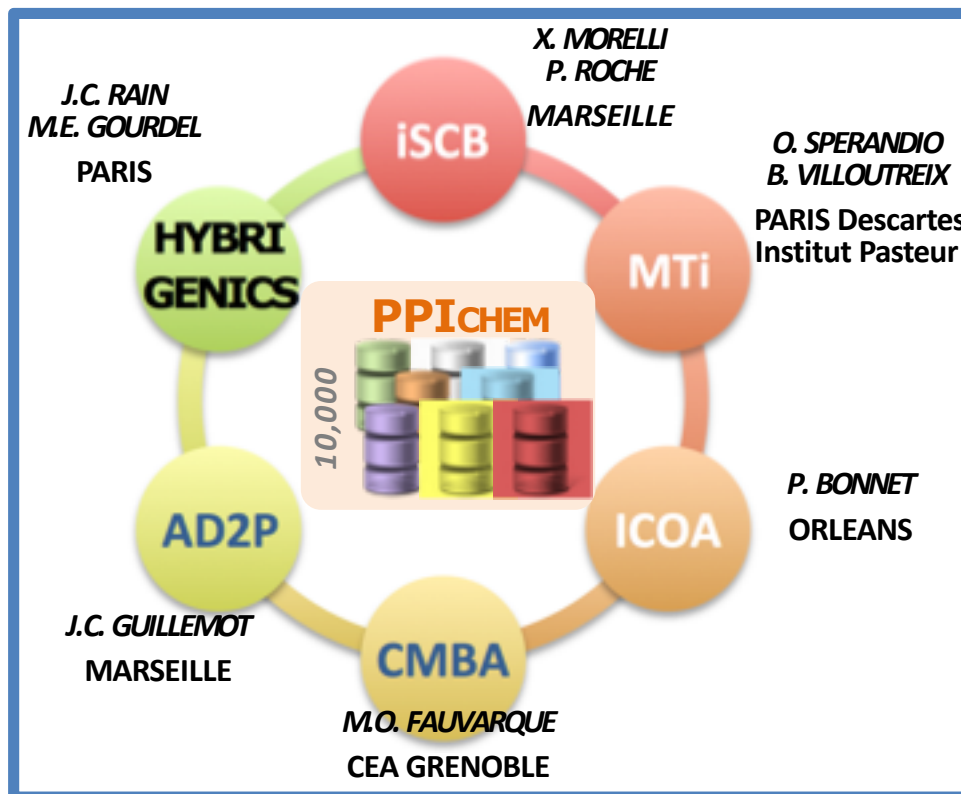
New Larger Version of 2P2I3D

v2.0 (MedChem) using updated algorithms, ADMET Filters

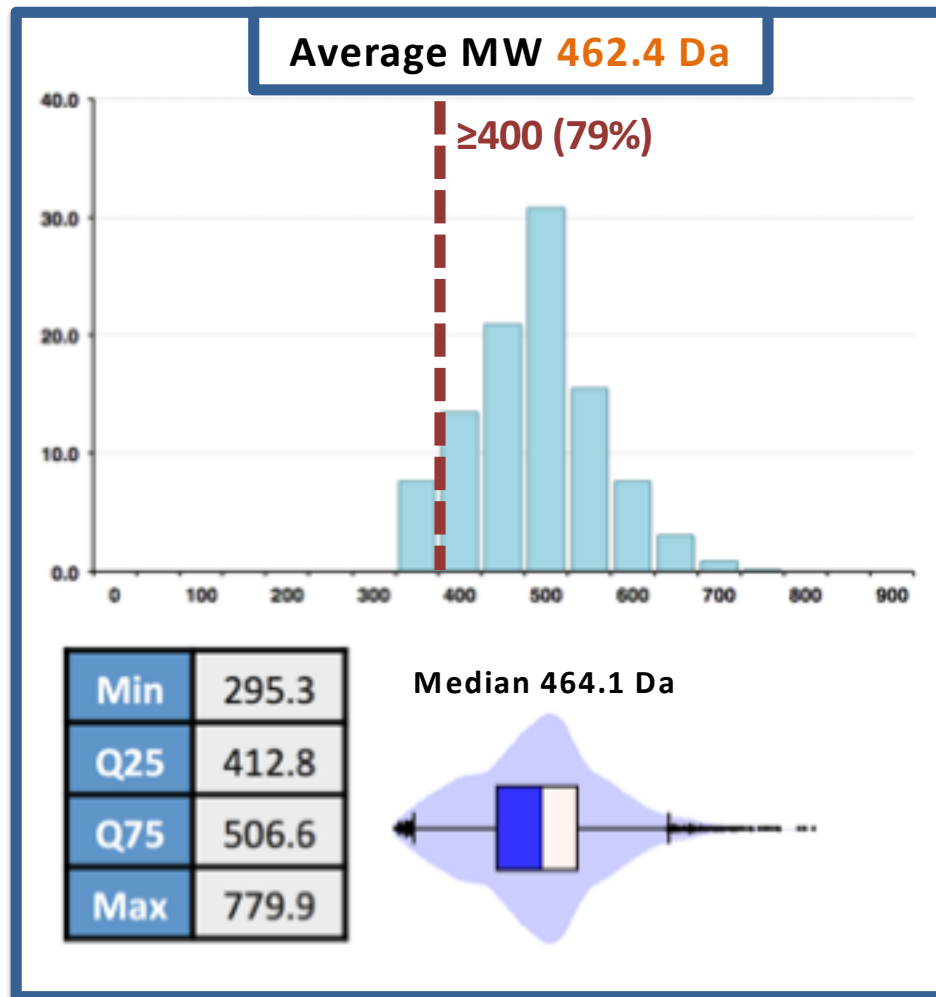
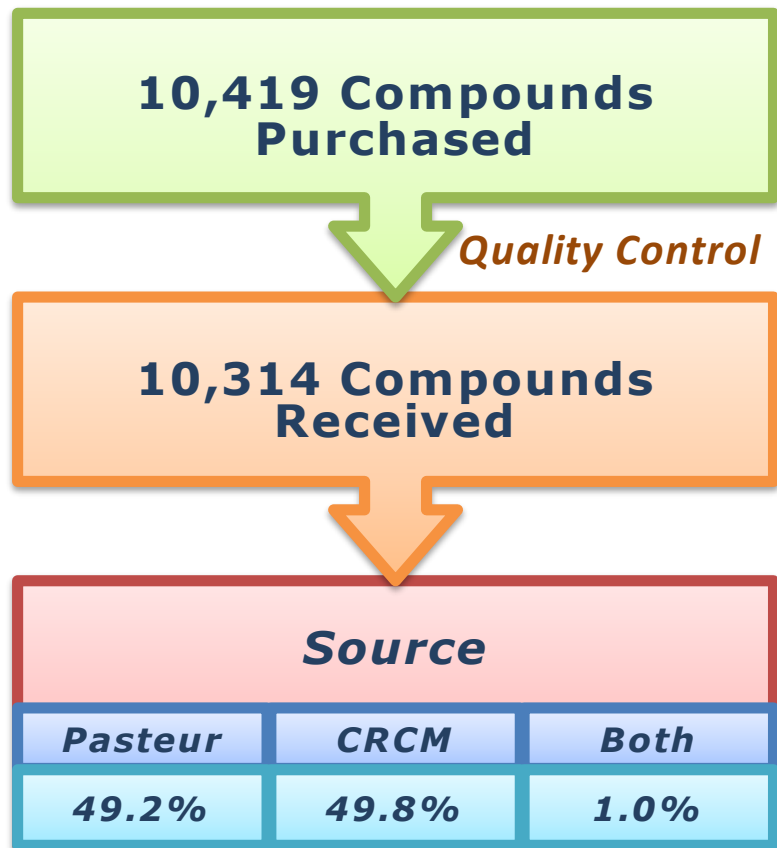
➤ National Program (ANR) => **10.000 Compounds PPI-Library**



C Muller P Roche



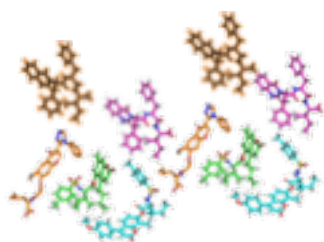
Fr-PPICHEM : Purchase & Properties



Strategies in PPI Drug Discovery (PART I)

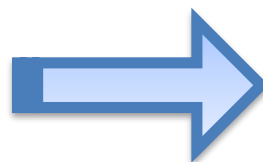
Knowledge-Based

Chemical Library Design



PPI-oriented Libraries

- Machine Learning
- SVM/Random Forest

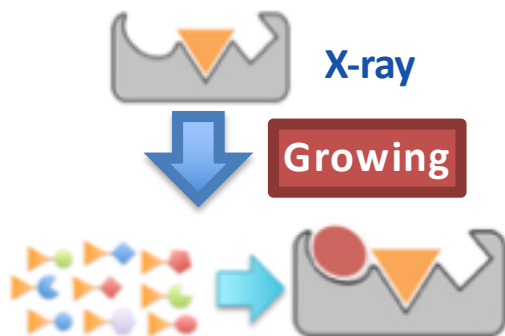


PPI-Oriented Chemical Library made of
10,000 compounds
Plated & available
for screening



Structure-Based

Fragment Screening



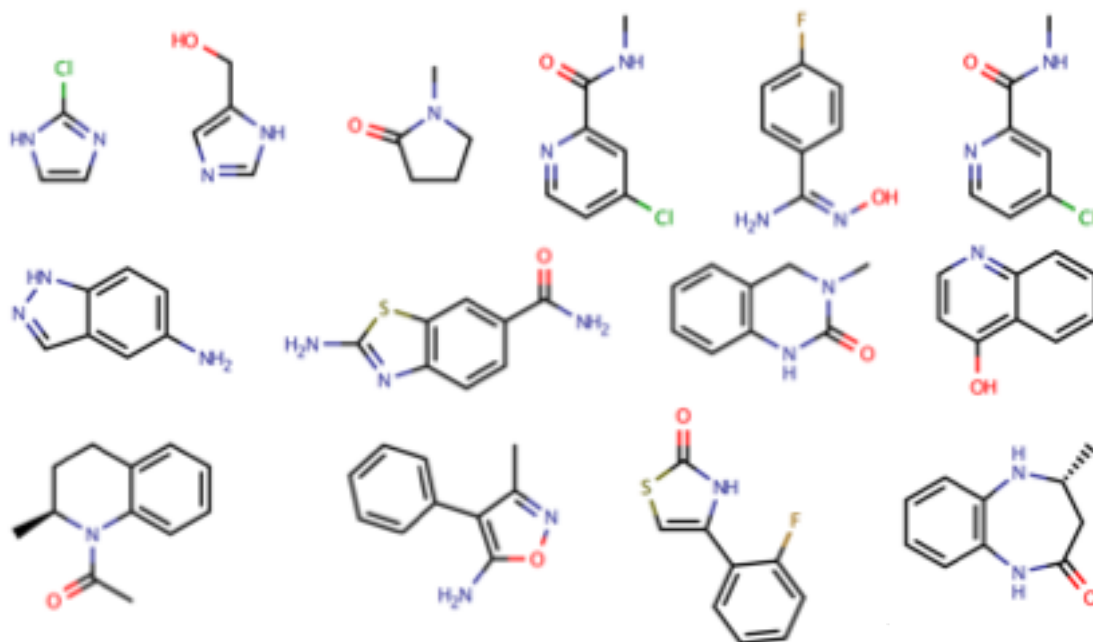
- Diverse Focused Database
- Conformational Sampling



Fragment-Based Strategy

CONCEPT

The fundamental concept of **fragment** screening is to **use simpler molecules** so that the **chemical space** can be sampled much more efficiently than is possible when using molecules of greater complexity.



Fragments :
MW ≤ 300g/mol

Why Fragments ?

Large Chemical Space represented with small number of fragments

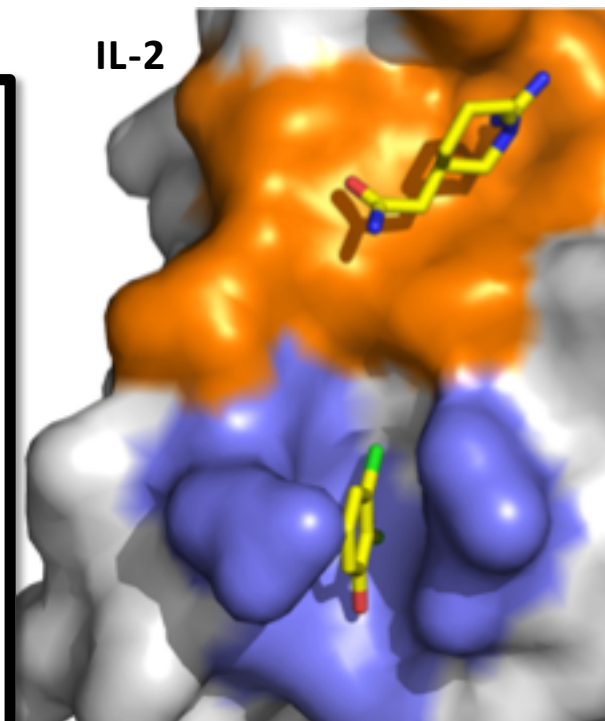


 **500-1000 fragments in a screening library**

Why Fragments ?

Fragments are well suited for PPI

IL-2



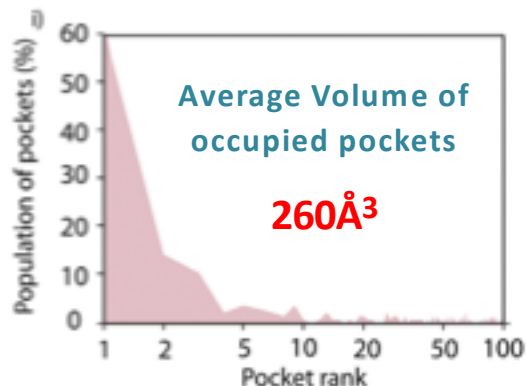
VENETOCLAX (BCL2)



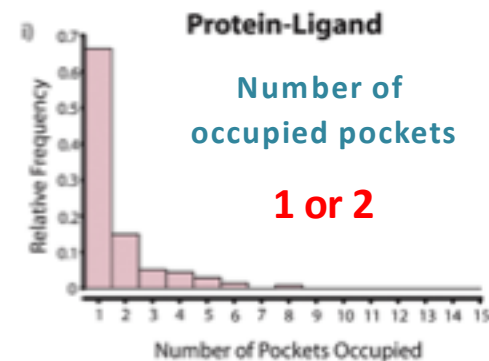
NEWS
CANCER HOPE
 VENETOCLAX HELPS SOME LEUKAEMIA PATIENTS
 3 Nov 2013 10:00 AM

Protein-Ligand Interactions

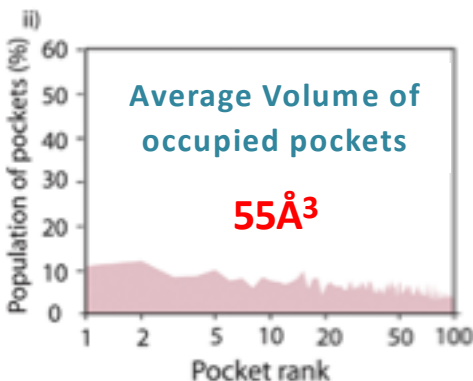
a) Percentage of proteins with occupied pockets



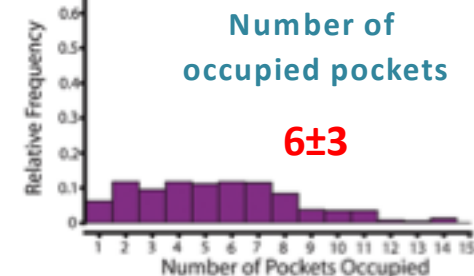
b) Number of occupied pockets per protein



Protein-Protein Interactions



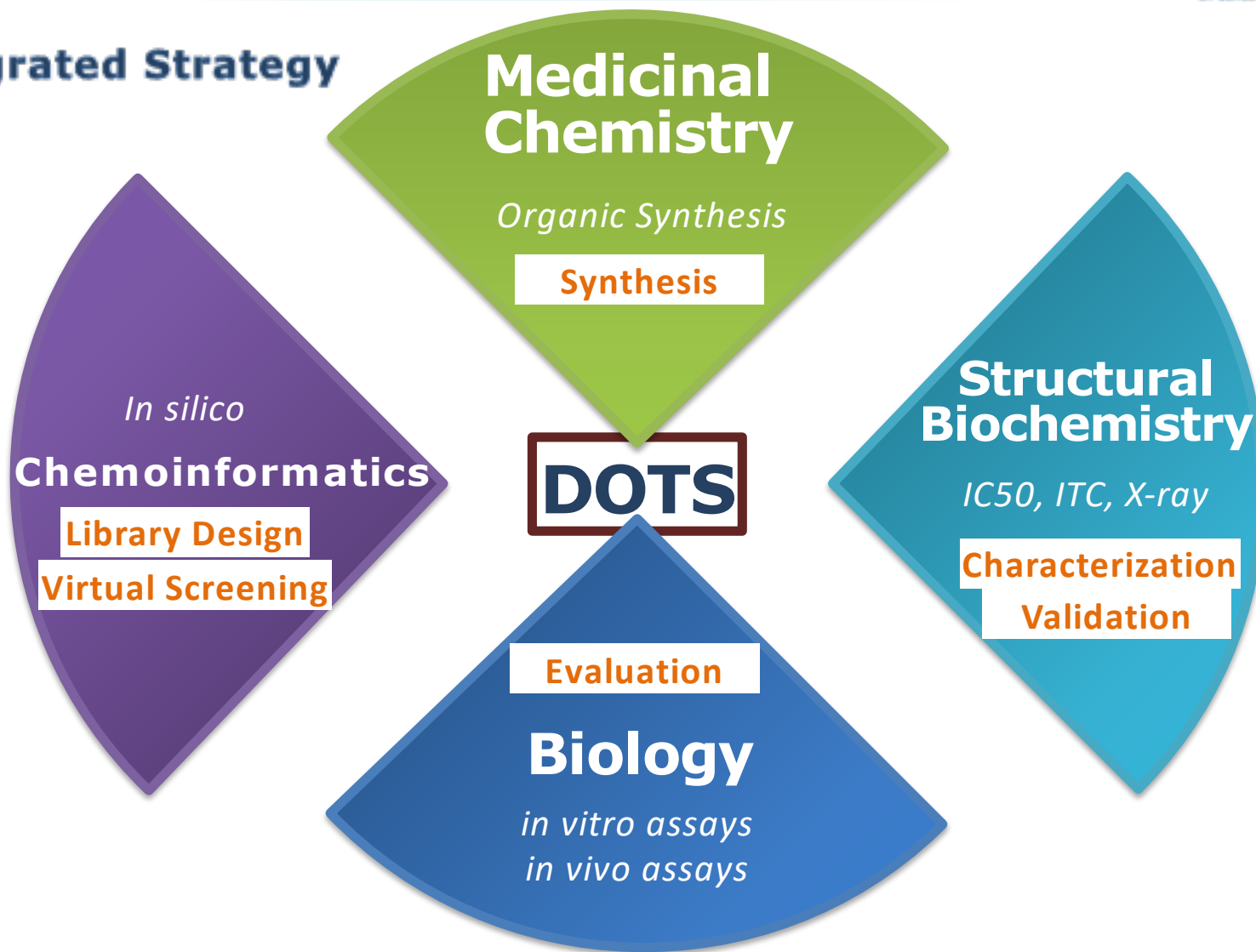
iii) Protein-Protein



Fuller et al (2009) *Drug Discov Today*. 14:155-61.

Diversity Oriented Target-focused Synthesis approach

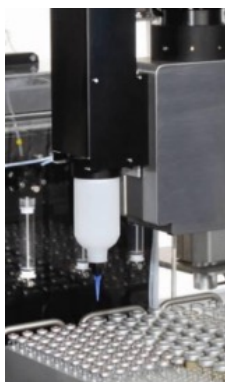
Integrated Strategy





DOSynth

Centre de Recherche
En Cancérologie de Marseille



Synthesis – ‘Hit2Lead’
Chemspeed Synthesizer SLTII

Purification

Analysis



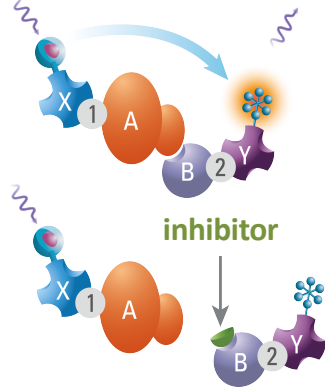
S Combes P Bremond

Evaluation and Characterization

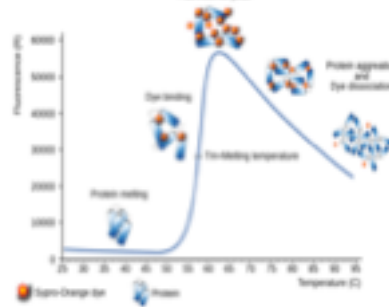
in vitro Methods

C Derviaux
Engineer

HTRF



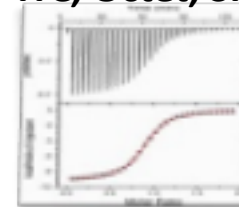
Thermal Shift (DSF)



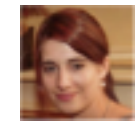
S Betzi
CR CNRS

Biophysical Methods

ITC, Octet, SPR

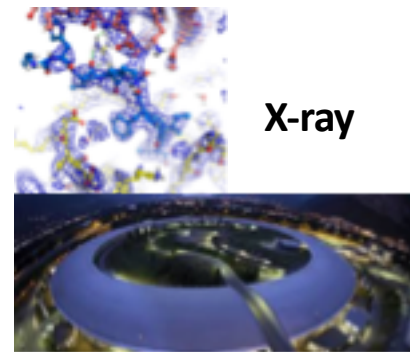


K Karrasco
PhD student



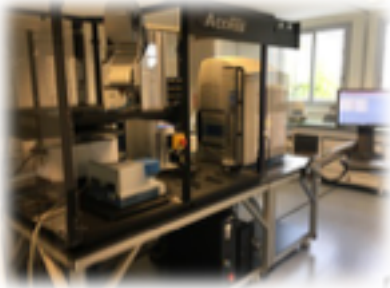
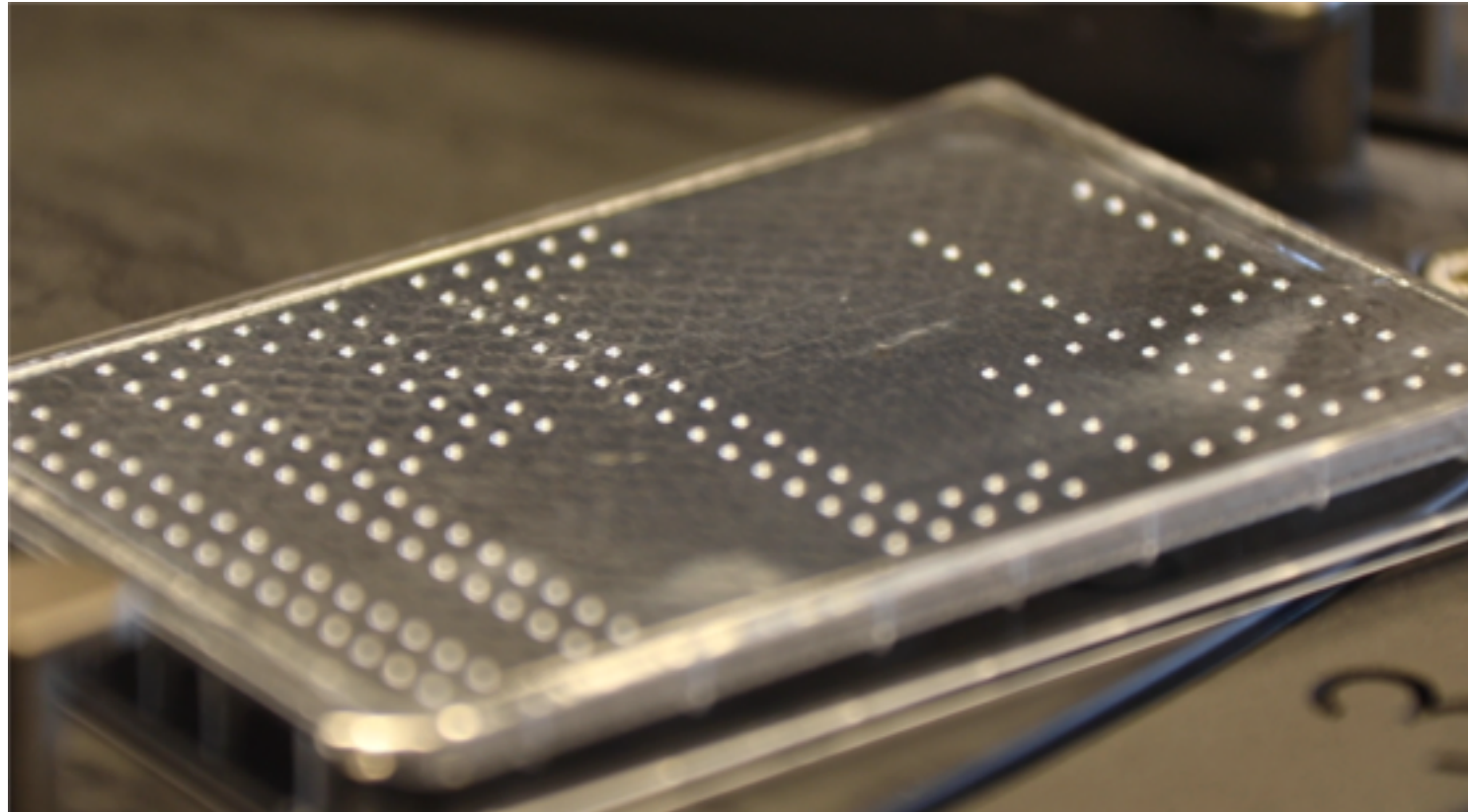
B Raux
PhD student

X-ray



**Access/Echo® Laboratory Workstation
PHERAstar (BMG LabTech)**

ACCESS / ECHO WORKSTATION



* Labcyte Echo 550 w/ ACCESS Workstation base (robot, gripper, access rack, centrifuge, sealer...)

* BMG PHERAstar

The Diversity Oriented Strategy (DOTS)



K Barral



S Betzi



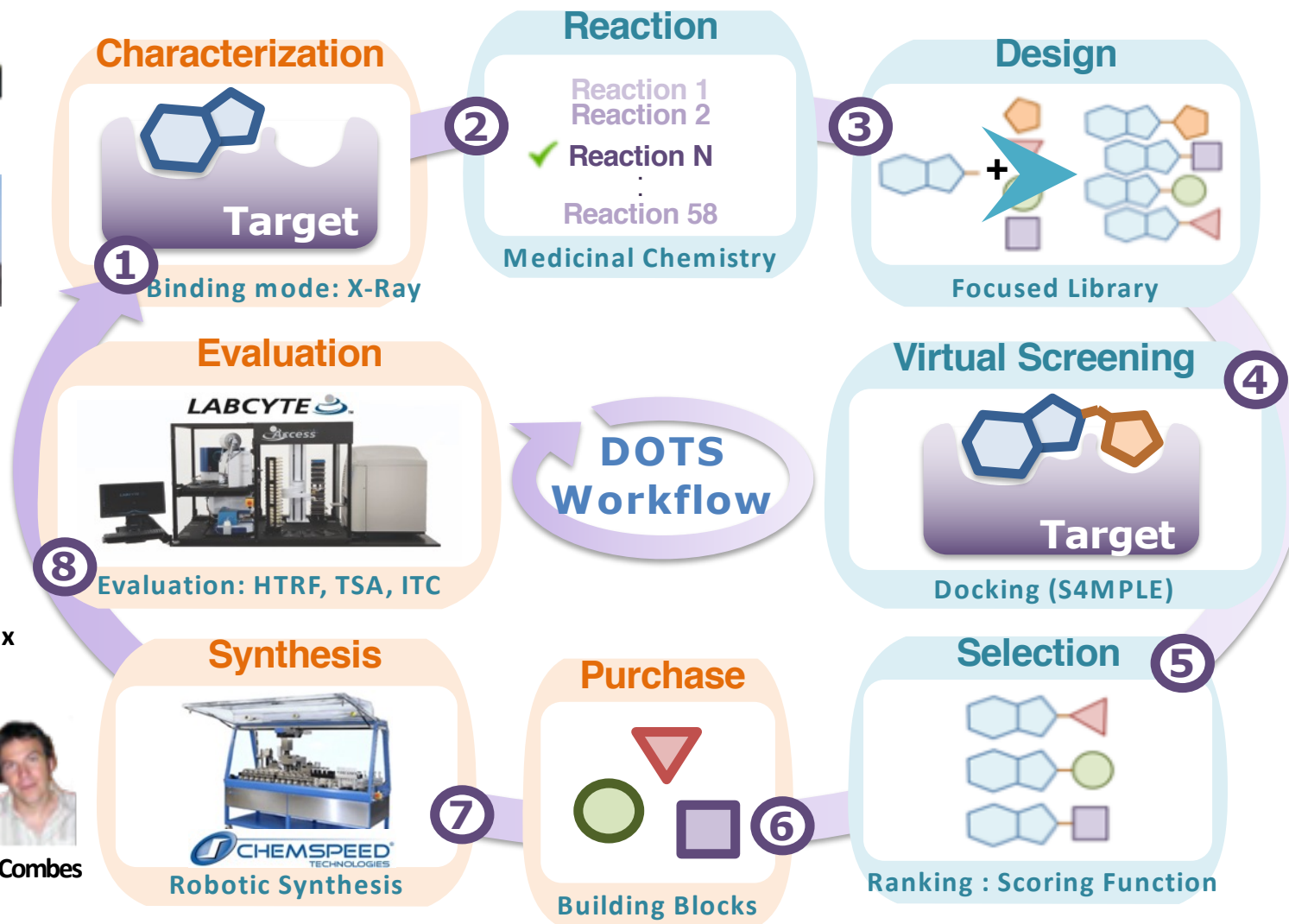
C Derviaux



P Bremond



S Combes



L Hoffer

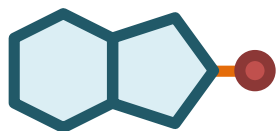
Collab.
A. Varnek
D. Horvath

Design

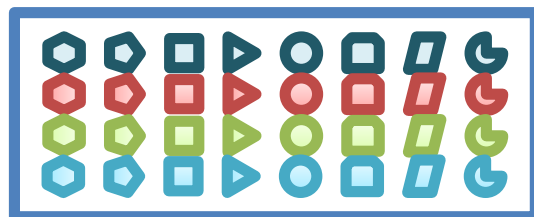


Focused Library

Virtual Focused Chemical Library



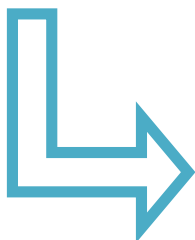
+



Building Blocks



Possible to generate a
very large collection
of **virtual** compounds



Not all relevant



Not all easy to synthesize



Not all compatible with MedChem developments

JOURNAL OF

CHEMICAL INFORMATION

AND MODELING A Collection of Robust Organic Synthesis Reactions for *In Silico*
Molecule Design

 NOVARTIS

Markus Hartenfeller,^{*,†} Martin Eberle,[†] Peter Meier,[†] Cristina Nieto-Oberhuber,[†] Karl-Heinz Altmann,[†]
Gisbert Schneider,[‡] Edgar Jacoby,[†] and Steffen Renner[†]

Collection of 58 reactions for computer-based molecule construction

- Inspired by real-world chemistry
- Compiled with medicinal chemists
- Commonly used in hit-to-lead stages (**pharmaceutical industry**)
- Easy amenable to real chemical synthesis (**high yields**)
- Mainly one-step reactions



Hartenfeller et al (2011) *J Chem Inf Model*. 2011 51:3093-8.

Design

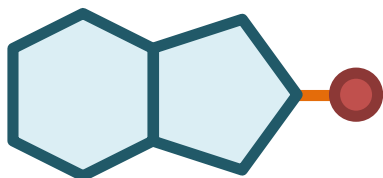
Virtual Focused Chemical Library



Focused Library

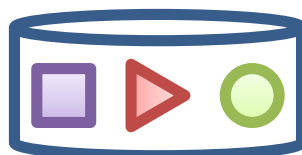
*Set of Encoded
Chemical Reactions*

Activated Fragment



+

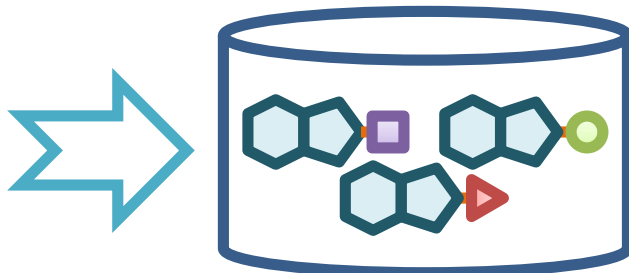
Building
Block Library



+

- Rule 01
- Rule 02
- ...
- ...
- Rule 58

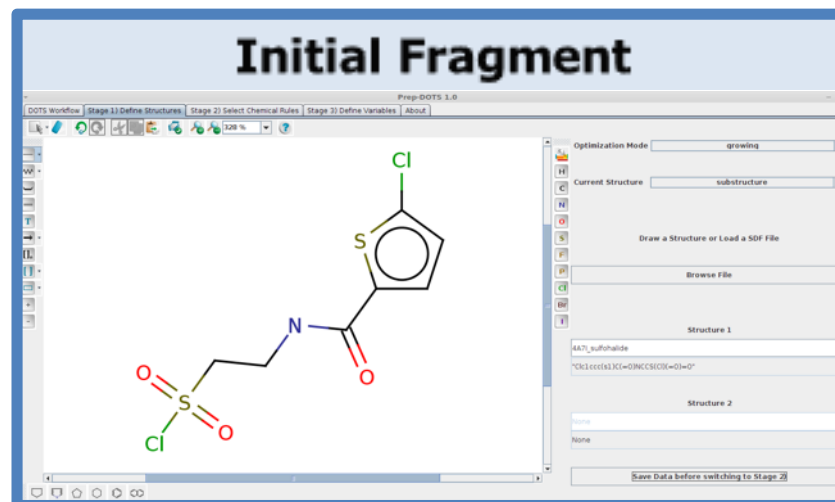
Virtual Focused Library



**All Compounds generated
should be amenable to
organic synthesis**

DOTS Java applet

**A standalone Java Applet
has been developed to
automatically generate the
virtual Library**

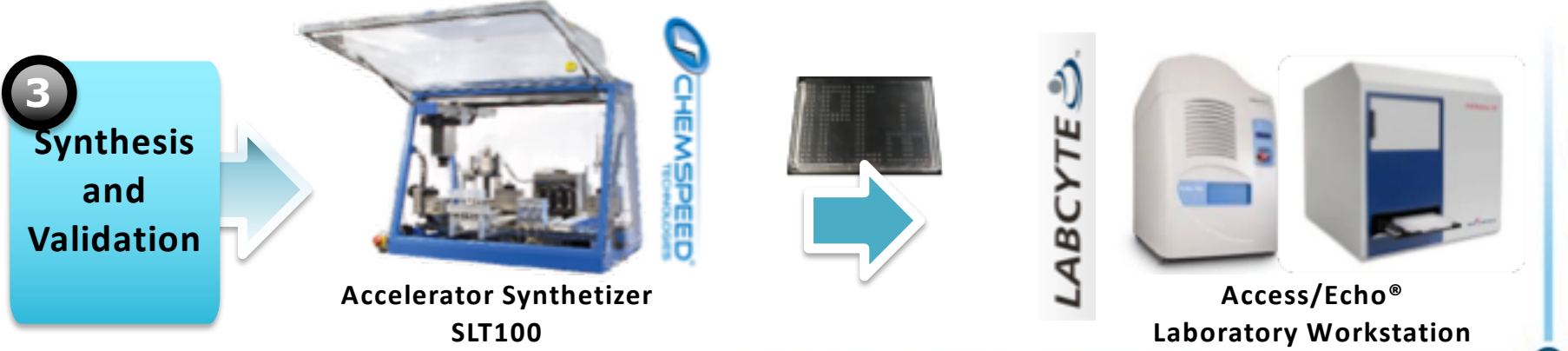


Chemical Rules Selection

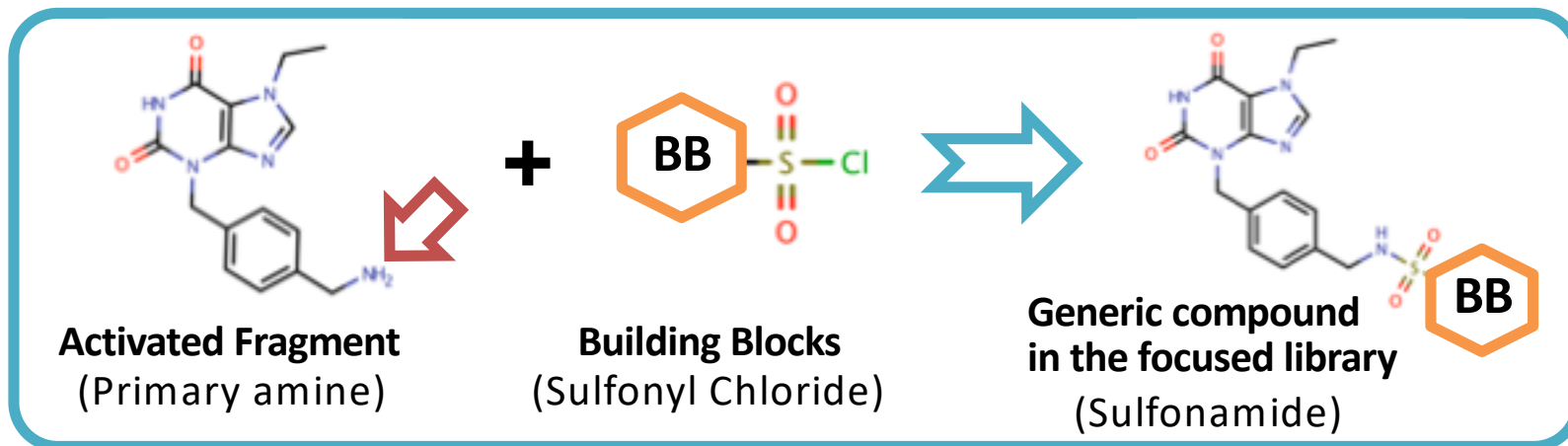
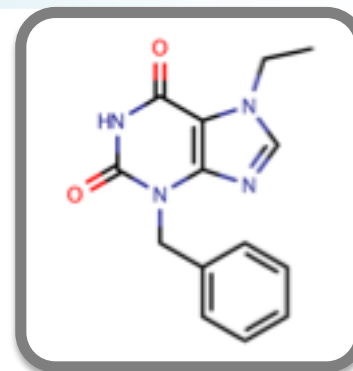
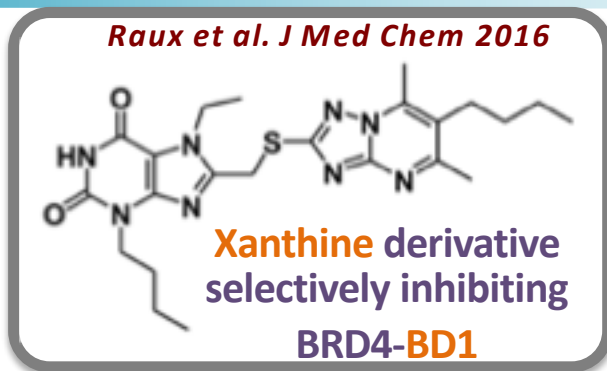
Rule ID	Rule Name	Status
34	rule_34_imide_mitsunobu	skipped
35	rule_35_phenol_ether_mitsunobu	skipped
36	rule_36_sulfonamide_mitsunobu	skipped
37	rule_37_tetrazole_mitsunobu-1	skipped
38	rule_38_tetrazole_mitsunobu-2	skipped
39	rule_39_tetrazole_mitsunobu-3	skipped
40	rule_40_tetrazole_mitsunobu-4	skipped
41	rule_41_vinyl_chem_heck	skipped
42	rule_42_vinyl_heck	skipped
43	rule_43_stille_add_step_organostannane	skipped
44	rule_44_carbonyl_grignard_add_step_magnesium-halide	skipped
45	rule_45_alcohol_grignard_add_step_magnesium-halide	skipped
46	rule_46_alkyne_sonogashira	skipped
47	rule_47_amide_schotten-baumann_add_step_acyl-chloride	skipped
48	rule_48_sulfonamide	skipped
49	rule_49_p-arylation_heterocycles	skipped
50	rule_50_wittig_add_step_ylide_ethylphosphine	skipped
51	rule_51_amination_buchwald-hartwig	skipped
52	rule_52_imidazole	skipped
53	rule_53_decarboxylative-coupling	skipped
54	rule_54_amination_nucleophilic_subst_heteroatomic	skipped

Variable Parameters

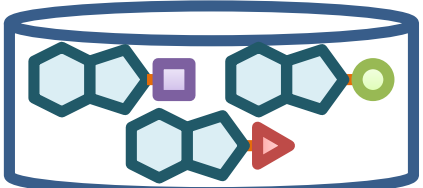
DOTS Hit Explosion Strategy (3 Steps)



Proof of Concept on BRD inhibitors

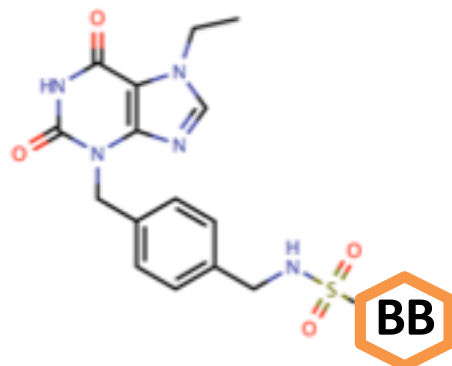


Focused Library



576 Compounds

Virtual Screening



576 Compounds

Virtual Screening



R1

 #1	 #2	 #3
 #4	 #5	 #6
 #7	 #8	 #9
 #10	 #11	 #12
 #13	 #14	 #15
 #16	 #17	

**17 Compounds
selected**

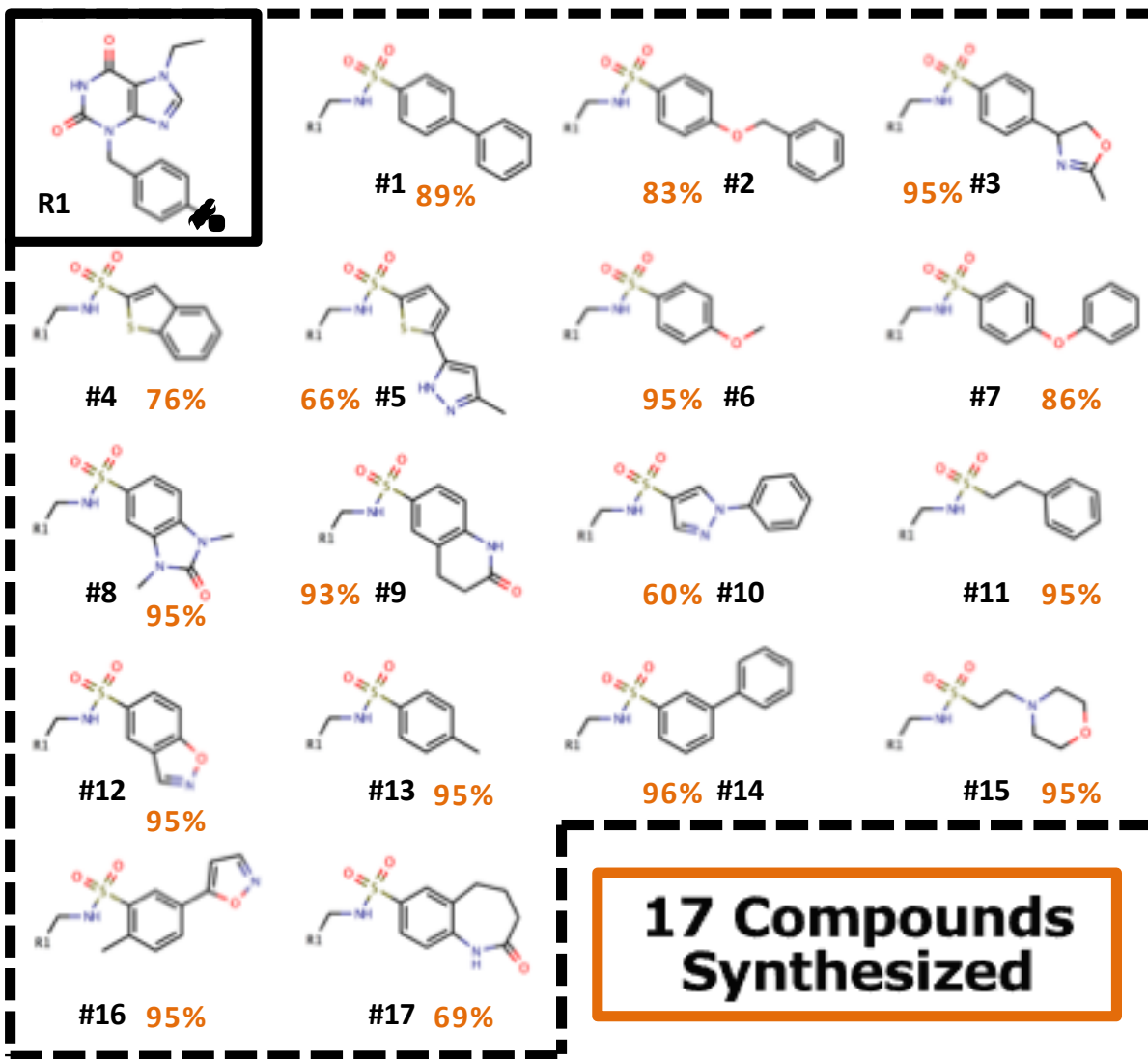
Robotic Organic Synthesis



S Combes

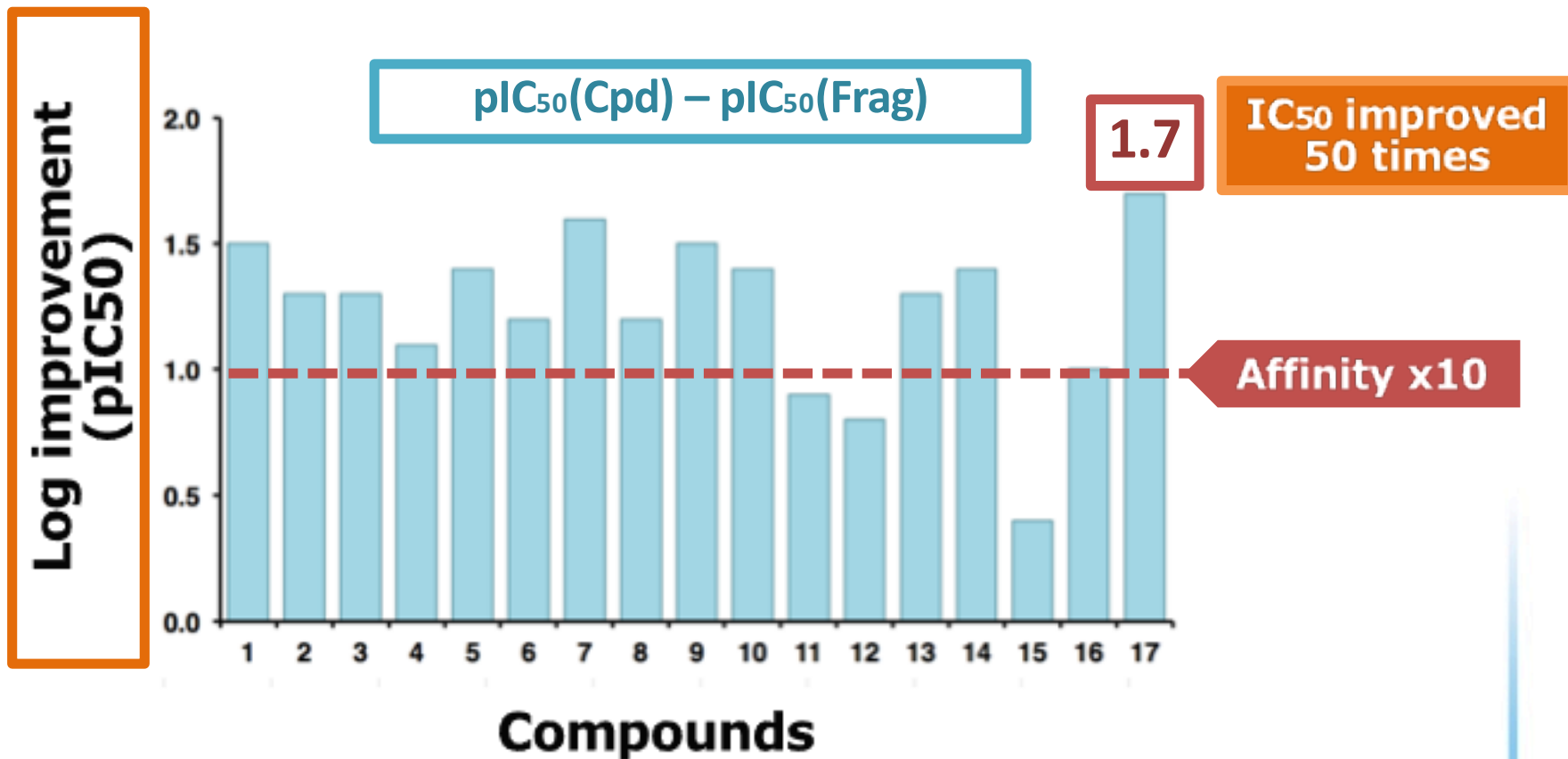


Y Voitovich



In vitro Evaluation (HTRF)

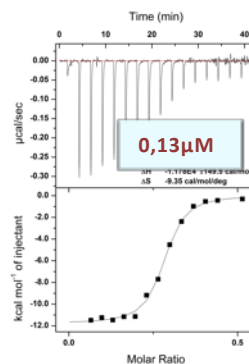
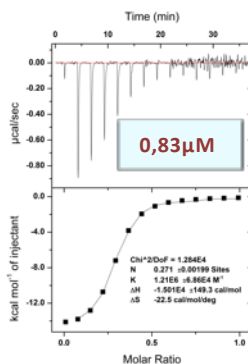
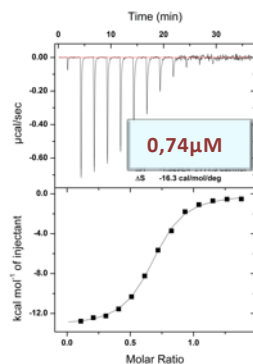
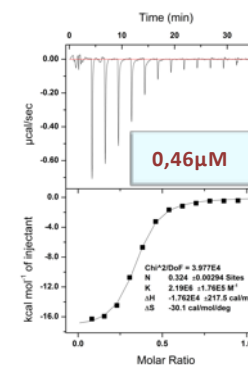
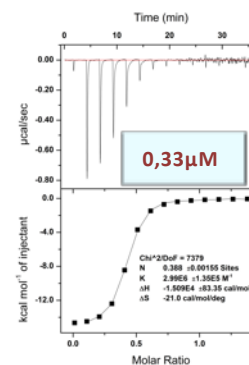
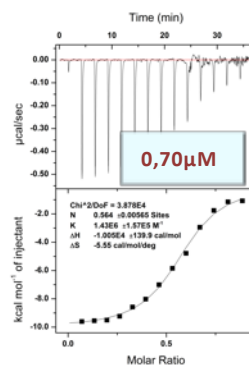
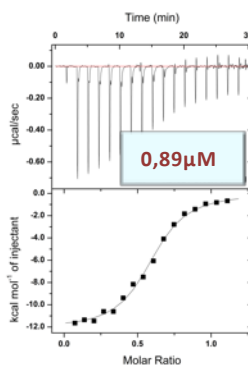
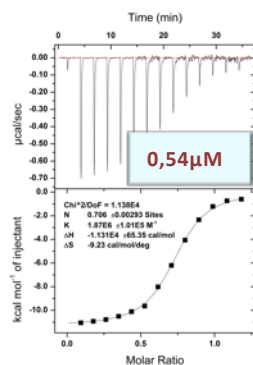
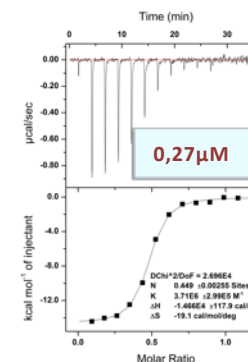
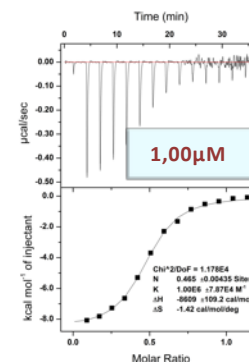
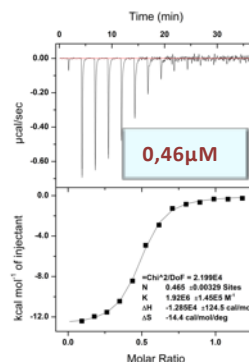
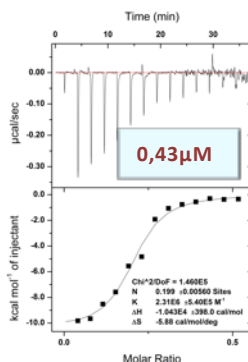
All 17 Compounds show improved
pIC₅₀ values (**HTRF**)



Orthogonal Validation (ITC)



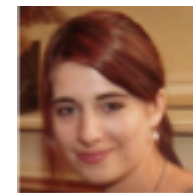
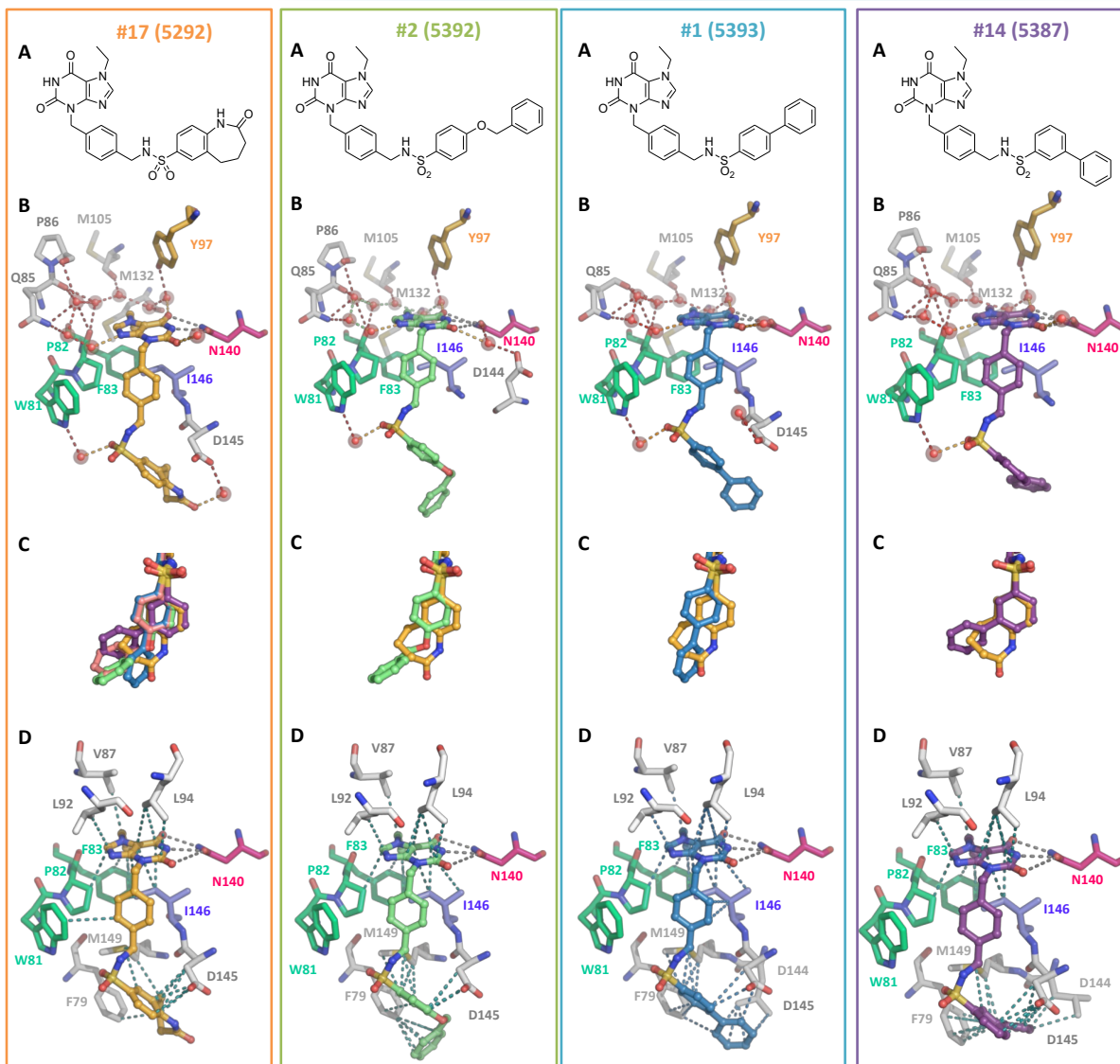
K Karrasco



**All Compounds
validated by ITC or TSA**

**Best Compound :
K_D ≈ 180 nM; ΔT_m 5.4 °C**

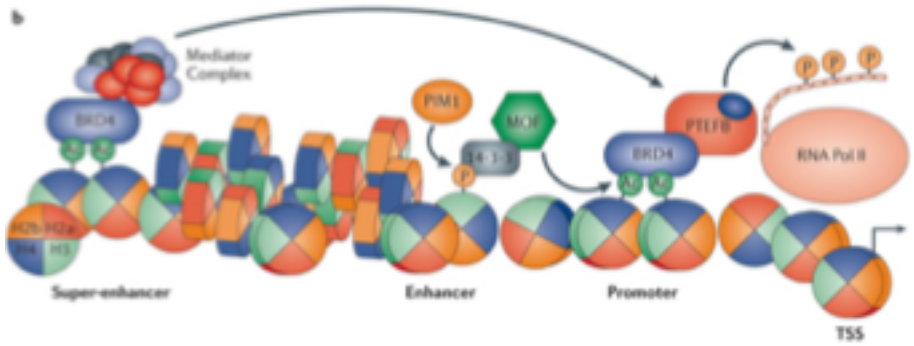
3D Structures



B Raux

**Binding Mode
of optimized
probes is
Conserved**

J. Med. Chem. 2018 Jun 22.
doi: 10.1021/acs.jmedchem.8b00653

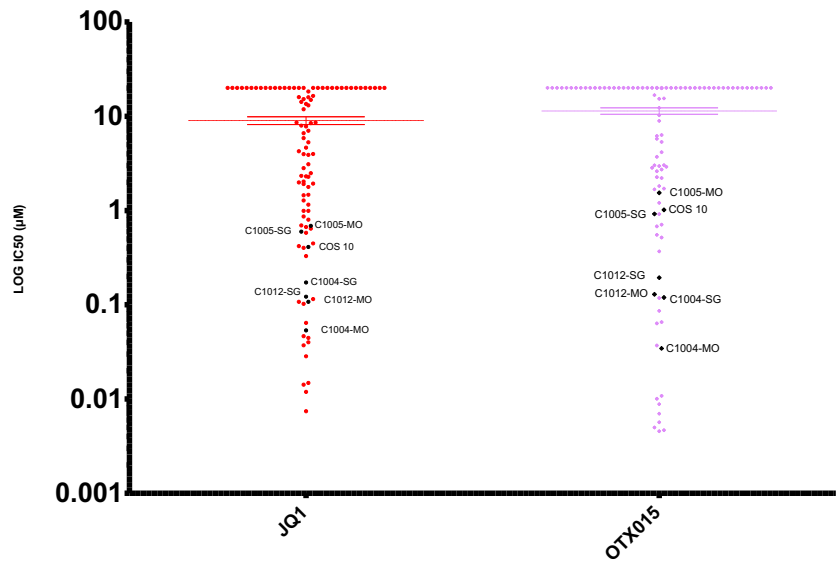


Perspectives :

➤ Developing a set of selective probes (intra-BET, BD1 vs. BD2), to decipher specific (transcriptional) functions?

➤ Therapeutic interest (selectivity, resistance?)

➤ Biomarkers with clinical value?

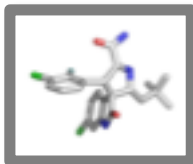


Clinical Trial "HematoBio" on going (N. Vey)



Conclusions & Perspectives

Design of PPI-Focused Chemical Libraries



- 2P2IDB structural database
- Profile of orthosteric inhibitors characterized
- 2P2I3D a PPI-focused library (*proof-of-concept*)

Fr-PPICHEM : New Larger Version of 2P2I3D



- v2.0 (MedChem) using new data from 2P2IDB , ADMET Filters
- 'Fr-PPI-Chem' a French National initiative (*available through MTA*):
 - *National Program (ANR) => 10.000 Compounds PPI-Library*

Fragment-Based integrated Protocol (DOTS)



- Hit-to-Lead Optimization via Hit explosion
 - *MedChem virtual Library /SAMPLE combined with robotic platforms*
- Proof-of-concept **validated** on a BRD fragment
 - Several improved inhibitors identified
 - Validated (HTRF, ITC, TSA, X-ray): Lead on going...

On going applications of DOTS

- 2 Fragment libraries available @iSCB (1000 cpds)
- DOTS applied to several CRCM projects

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