Peptide-based chemical tools and PROTACS to address biological challenges

Muriel Amblard

Pôle de Biologie-Santé - Symposium des jeunes scientifiques

Institut of Biomolecules Max Mousseron (IBMM)

6 Departments : Essential Biomolecules for Life

- Saccharides
- Nucleosides & Oligonucleotides
- Biopolymers
- Lipids
- Peptides & Proteins

Chemistry/Biology/Health

Team Amino acids, Peptides and Proteins


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(IE CNRS, 2017)

http://ibmmpeptide.com/
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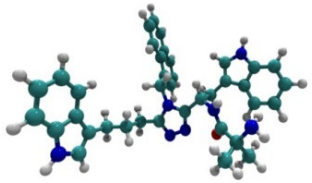
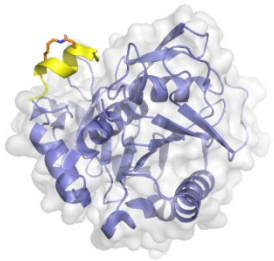
FIELDS OF EXPERTISES

Peptide science

Bioorganic and Medicinal Chemistry
Heterocycles, Drug design



Structural studies



MAJOR AREAS OF RESEARCH

- **Ligands based-drug design : Receptors and Enzymes**
- **Structured oligomers for biological applications and catalysis**
- **Peptide-Based Polymers and Materials**

APPLICATIONS

- **Drugs** (metabolism, cancer, antimicrobial, ….)
- **Drug delivery systems**
- **Diagnosis**
- **Tissue engineering**
- **Medical Devices**

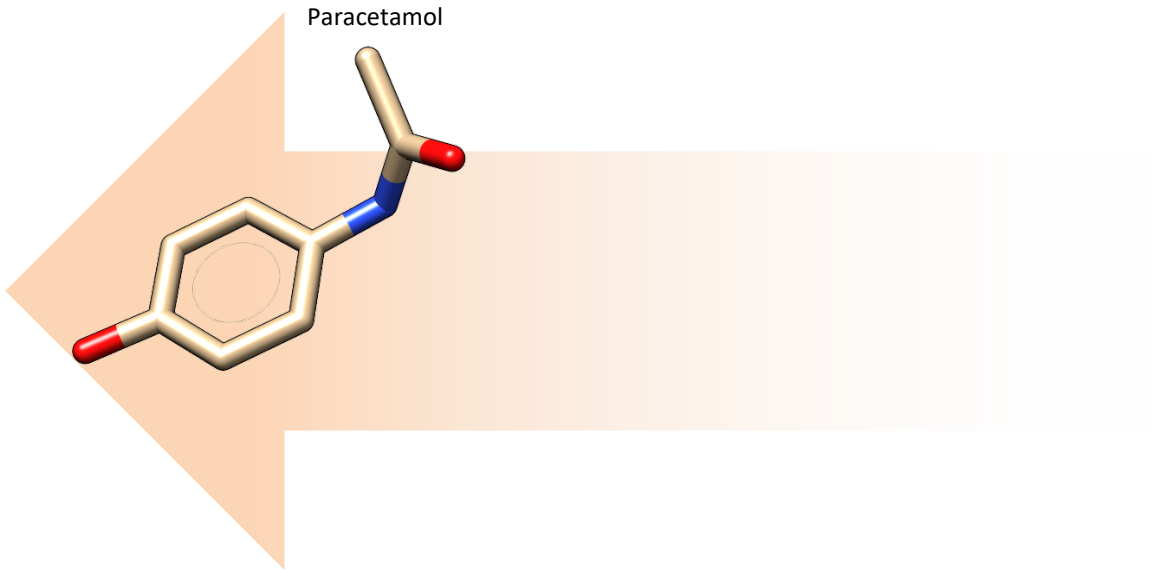
Drug discovery: different classes of therapeutics

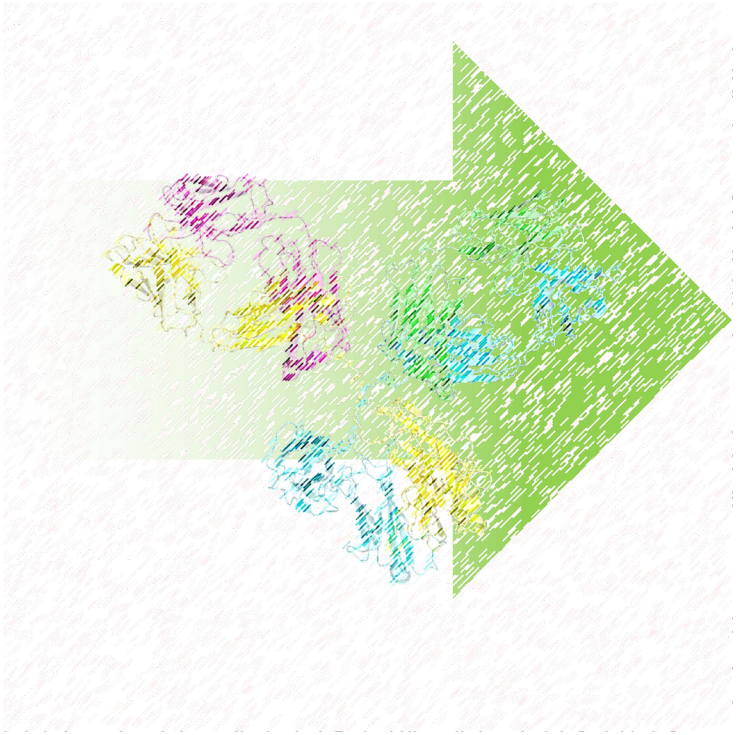
For decades

< 500 Da

Modulation of protein function
Small molecules that can fit into enzyme or receptor pockets to activate or inhibit their function

Paracetamol





- ✓ ***Oral bioavailability***
- ✓ ***Stability***

Small molecules → Can't modulate all biological targets

Drug discovery: different classes of therapeutics

End of the 2000s: limitation of small molecules → the rise of biologics (antibodies) and siRNA

< 500 Da

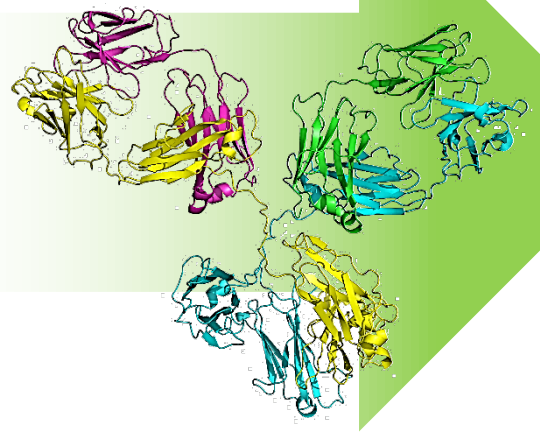
Paracetamol

✓ *Oral bioavailability*
✓ *Stability*

Small molecules

> 5000 Da

Antibody, Insulin, growth factor, etc



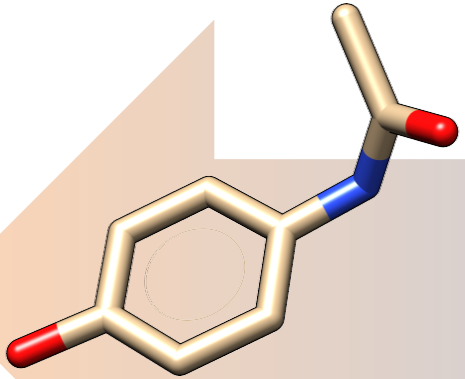
✓ ***High specificity***

Size
Membrane permeability
Delivery
Stability
Off-target
cost

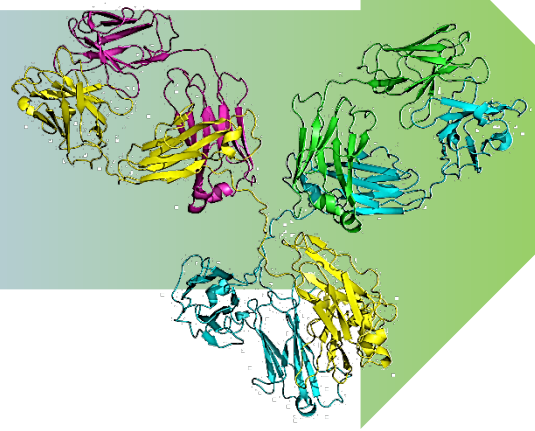
Biologics

Targeting “Undruggable” proteins

Paracetamol



Antibody, Insulin, growth factor, si-RNA, etc



“Undruggable” targets

- ✓ ***Oral bioavailability***
- ✓ ***Stability***

Small molecules

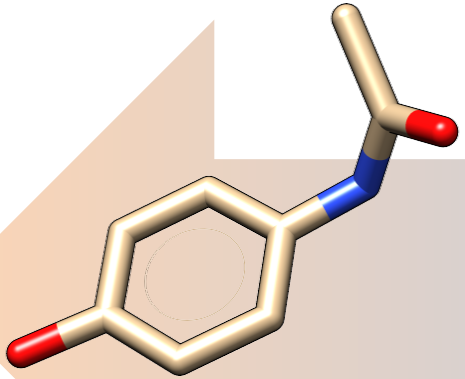
80% of disease-causing proteins cannot be targeted by currently available therapeutics

- ✓ ***High specificity***

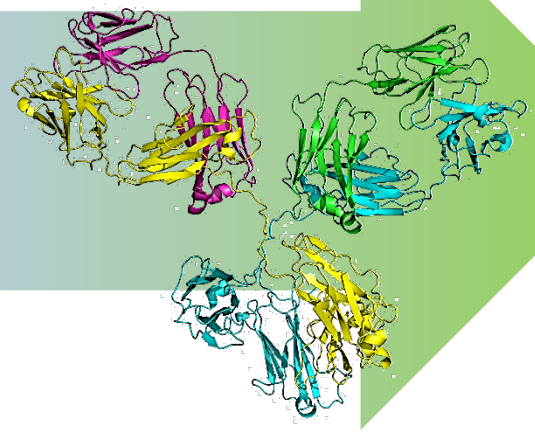
Biologics
SiRNA

Peptides to fill the gap

Paracetamol



Antibody, Insulin, growth factor, si-RNA, etc



Peptides

- ✓ ***Oral bioavailability***
- ✓ ***Stability***

- ✓ ***High specificity***

Peptide derivatives

Small molecules

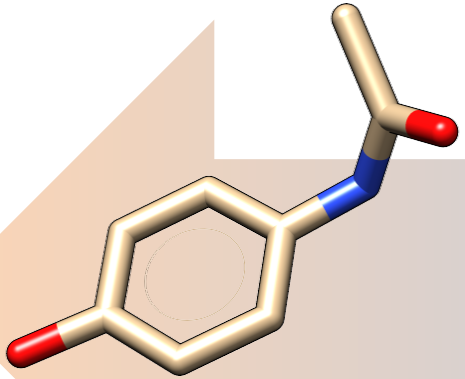
Bioavaibility of small molecules

Specificity of proteins

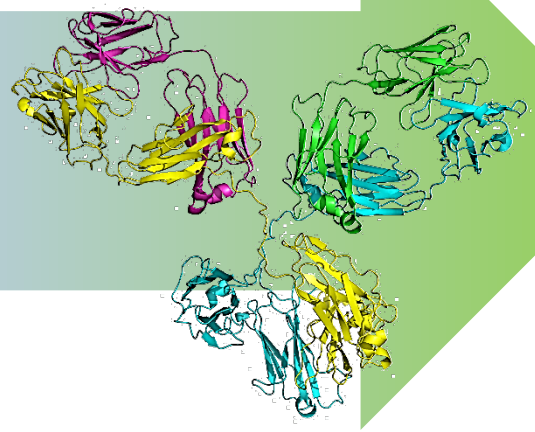
Biologics SiRNA

Peptides to fill the gap

Paracetamol



Antibody, Insulin, growth factor, si-RNA, etc



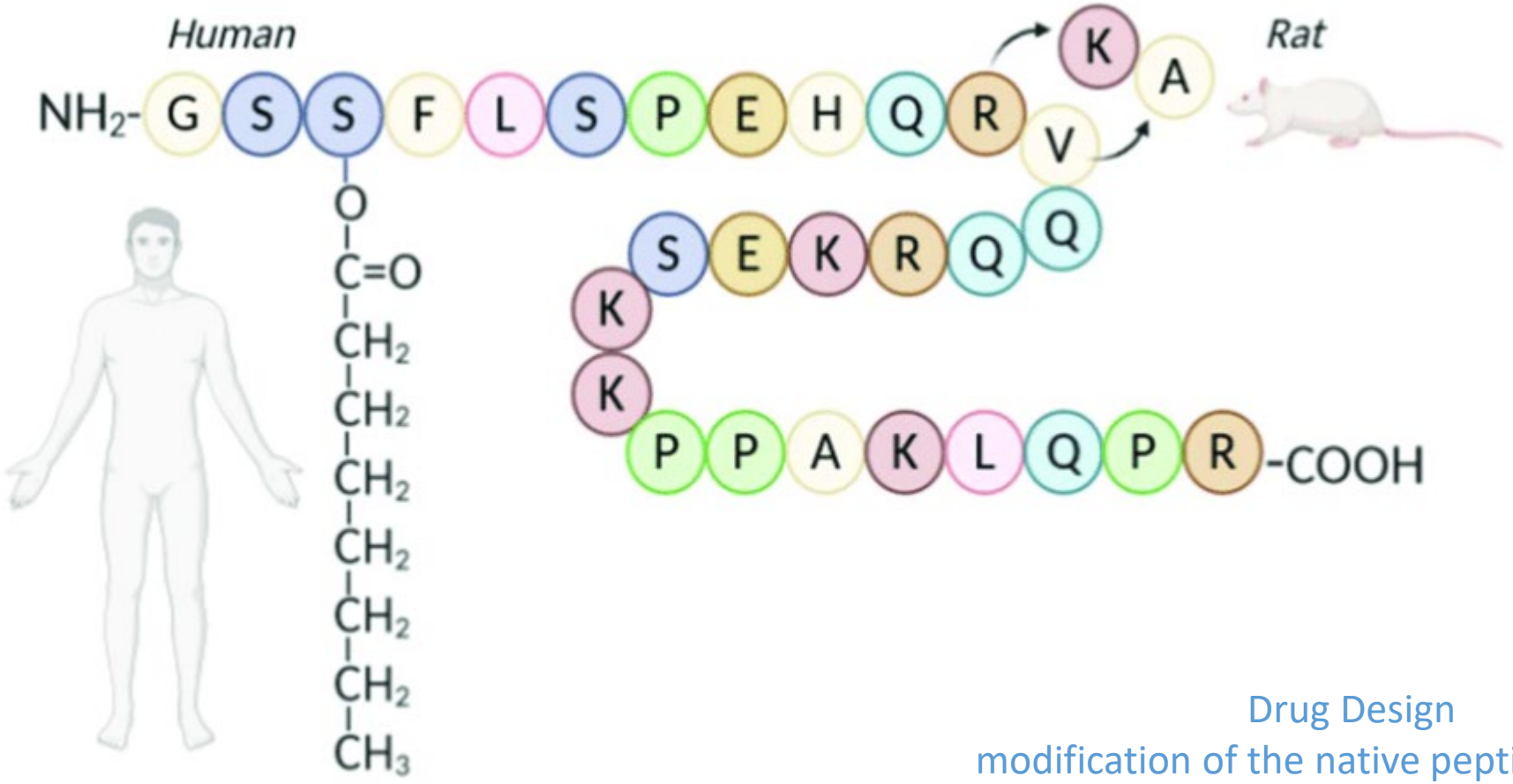
140 peptides in clinical development in 2019 (Drug Deliv. Ther. 2019,9, 606)

Trends in peptide drug discovery, *Nature Reviews Drug Discovery* , 20, 309–325 (2021)

GPCR Ligands: drug design and receptor's study

Ghrelin: a peptide that increases appetite and stimulates the release of growth hormone
Ghrelin implicated in many addictions (alcohol, drugs, …)

Jean Martinez and
Jean-Alain Fehrentz



Structure of ghrelin in a human and rat.

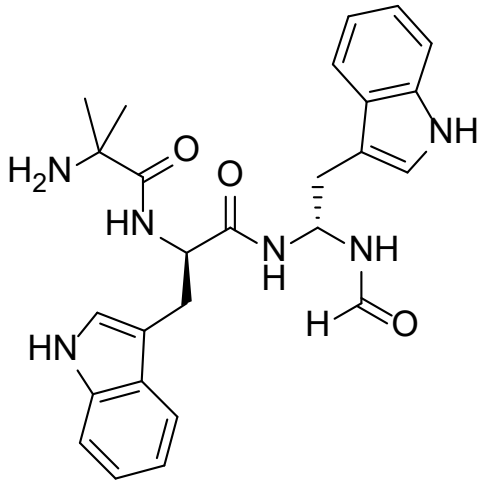
Drug Design
modification of the native peptide sequence

GPCR Ligands: drug design and receptor's study

Ghrelin: 800 analogues (agonists, antagonists and inverse agonists)

Ghrelin increases appetite and stimulates the release of growth hormone

JMV 1843



AGHDiagnose Kit

Macrilen

Æterna Zentaris

The first orally-approved drug (USA 2017 and Europe 2018) for the Diagnosis of adult growth hormone deficiency

WO 01/96300

Peptide-based polymers and materials

Objective:

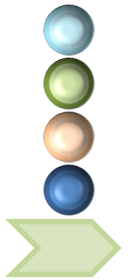
Design of new materials with tunable properties
afforded by a bioorganic unit (a well defined peptide)

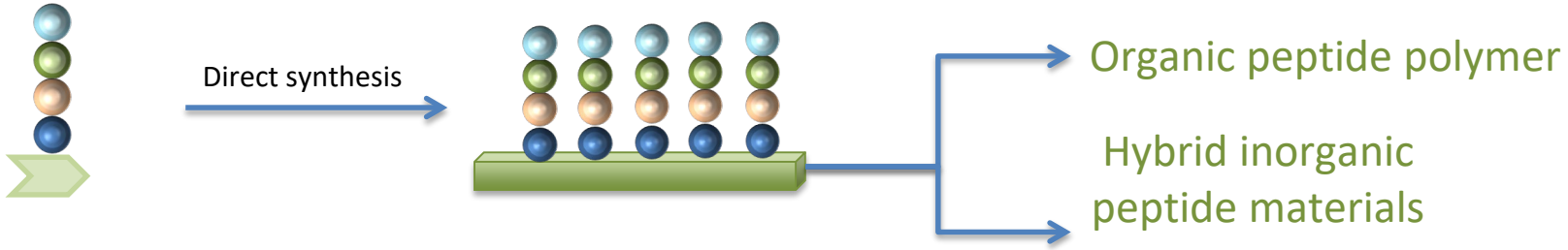
Strategy:

Synthesis of building block displaying :

-a peptide sequence

-one (or more) reactive groups for material assembly (polymerization, condensation)

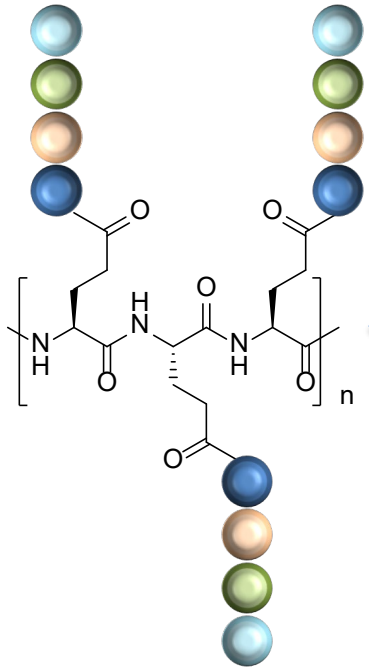




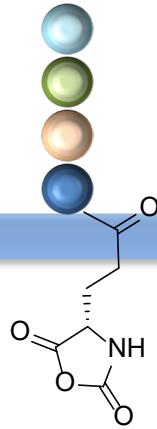
A bottom-up approach : the peptide block itself may polymerize to obtain materials and polymers

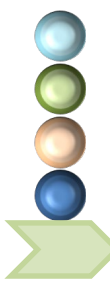
Peptide-based polymers and materials

Organic peptide polymer

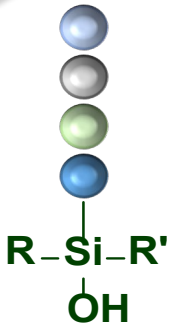


ROP



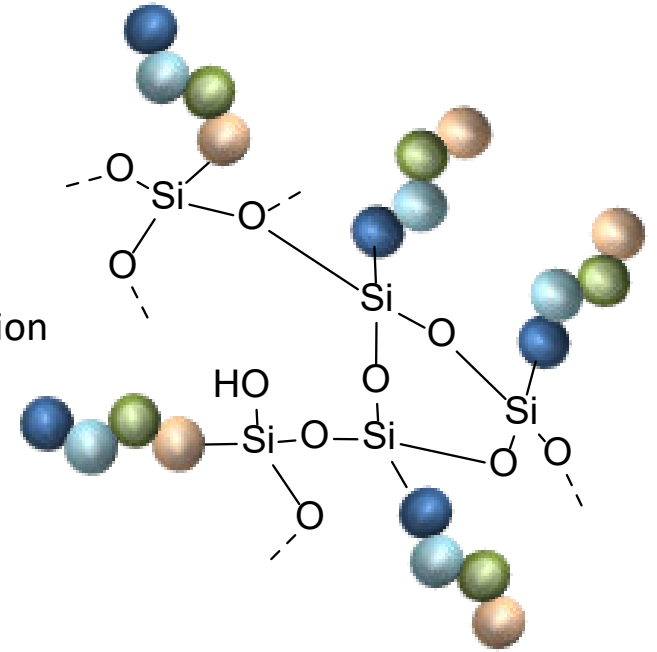


Sol-Gel

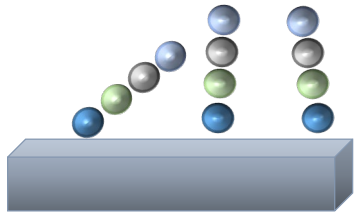


1) Hydrolysis
2) Condensation

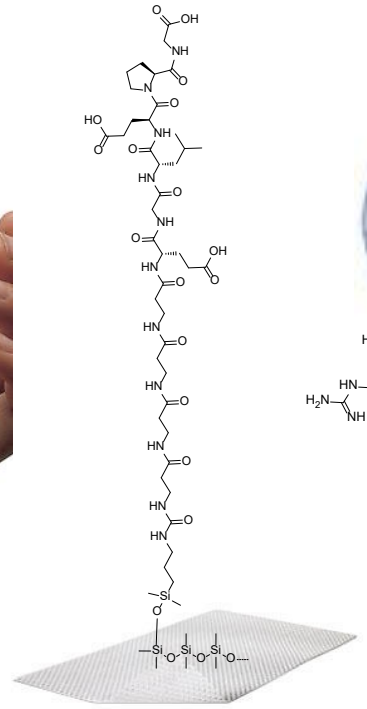
3D Hybrid peptide material

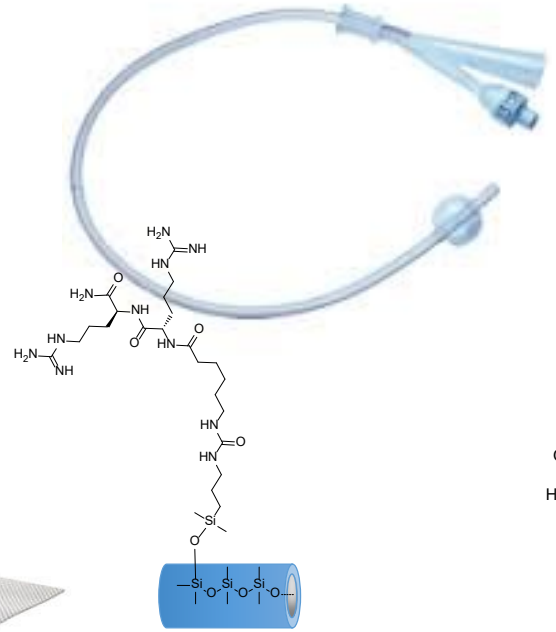


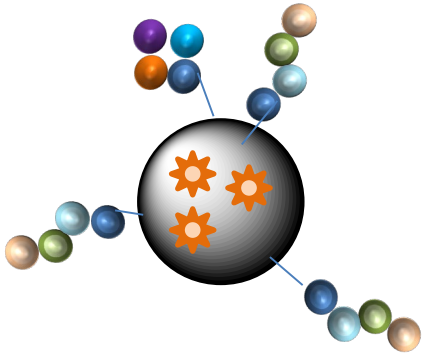
Surface functionalization



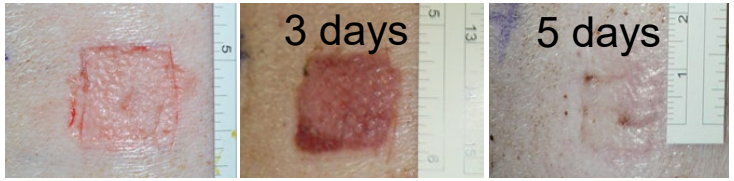
Surface Functionalization







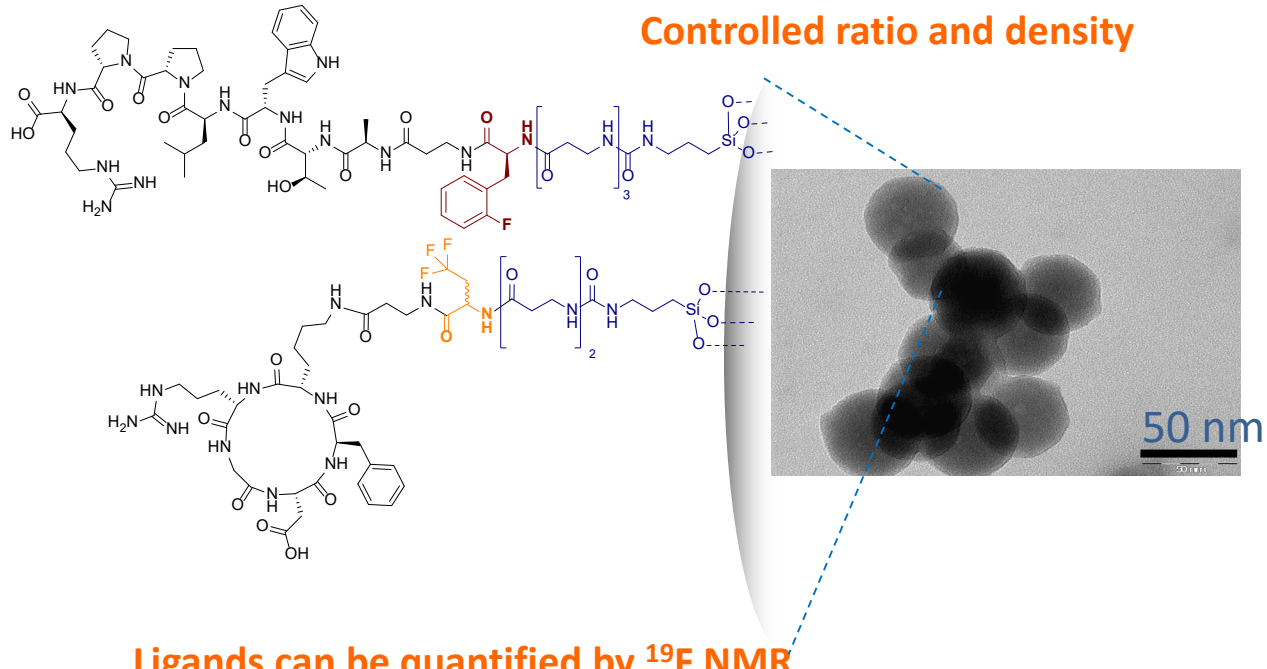
Wound-healing or antibacterial peptide



80% inhibition S. Aureus
4 weeks
>> Ag+ catheter

C. Pinese, et al., *Materials Today Chemistry* **2017**, *4*, 73–83.
C. Pinese, et al. *Adv. Healthcare Mater.* **2016**, *5*, 3067–3073.
J. Ciccione, et al. *Chem. Mater.* **2016**, *28*, 885–889.

Controlled ratio and density



Ligands can be quantified by ^{19}F NMR

J. Ciccione et al ***Chem. Mater.*** **2016**, *28*, 885–889.
T. Jia et al. ***Biomaterials*** **2018**

A la carte' multifunctional Hybrid hydrogels

Hybrid block for the network

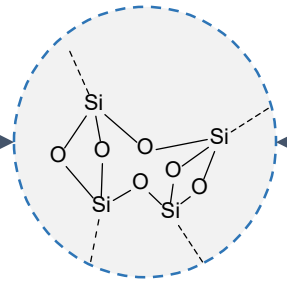
$(RO)_3Si$- -$Si(RO)_3$

Hybrid peptides for adhesion
Linked to the network

-$Si(RO)_3$

Growth factors
Not linked to the network to be released

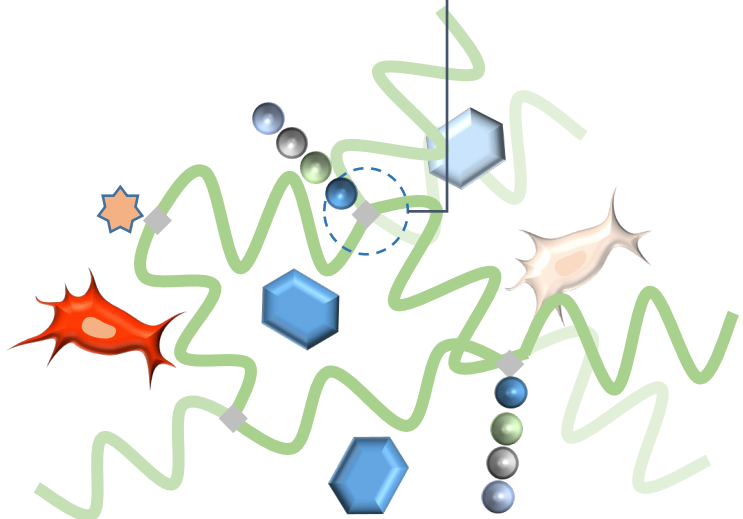
Cells



Si-O-Si bond formation

37°C pH 7.4

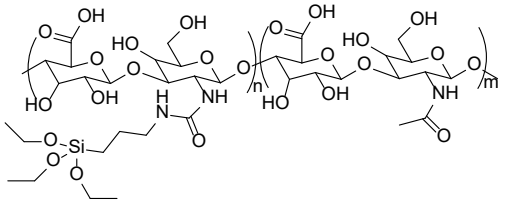
sol-gel

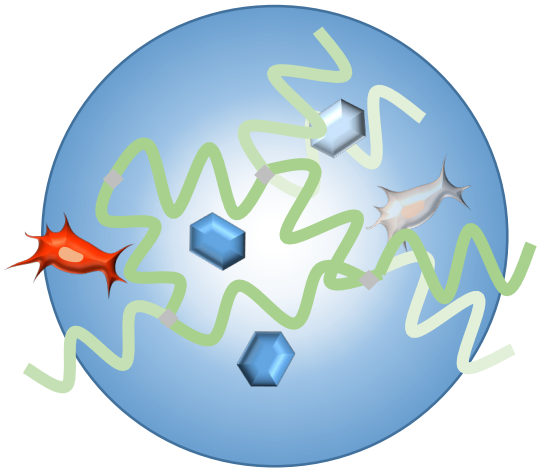


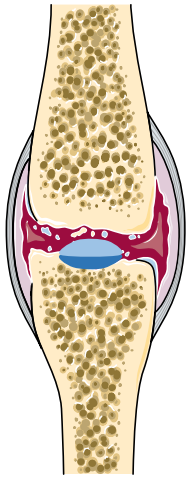
Multifunctional hydrogel

Biomimetic matrix for 3D printing

Biomimetic hydrogel (hyaluronic acid, collagen, growth factors, ligands, MSCs …)





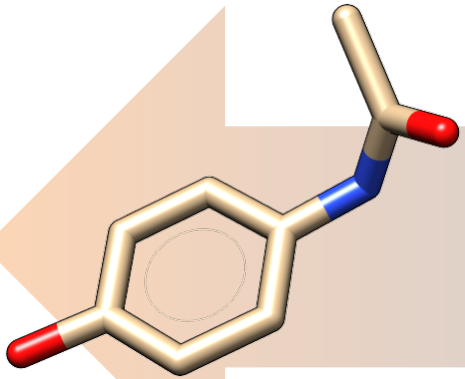


3D Bioprinting with hybrid biomimetic bioink containing cells

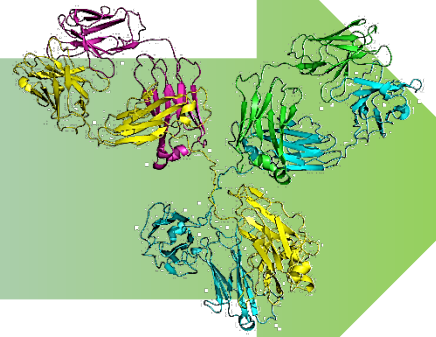
Bi-layer (bone/cartilage) Implants

Dr. Danièle Noel *group Stem cells, regenerative medicine, biotherapies, immunotherapies*, IRMB Montpellier

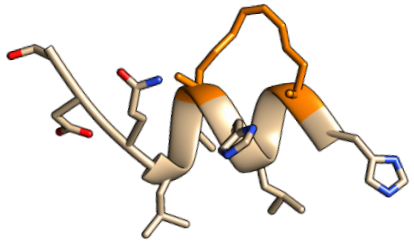
Alternative strategies for therapeutic tool development



Antibody, Insulin, growth factor,
si-RNA, etc



Secondary structure mimics / stabilization



- ✓ ***Oral bioavailability***
- ✓ ***Stability***

Small molecules

- ✓ ***High specificity***

Biologics

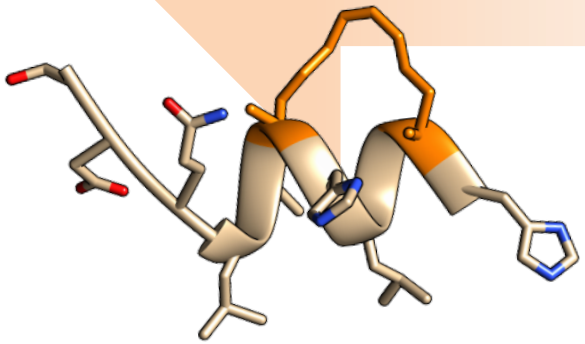
From the control of the molecular shape to the control of functions

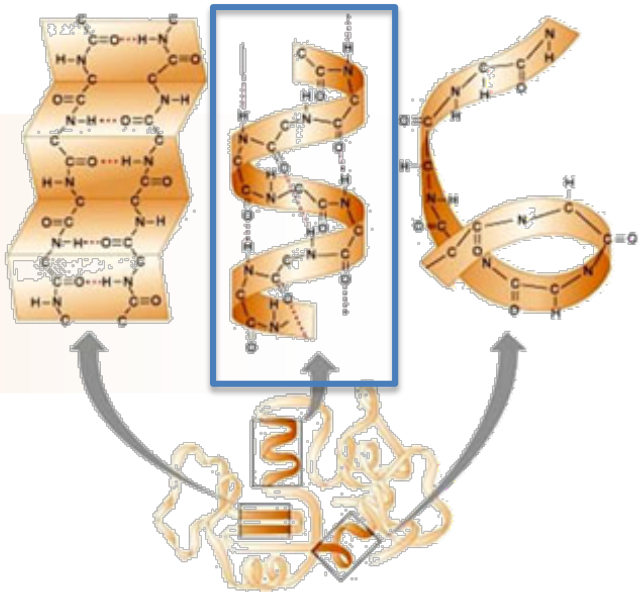
Controlled shape

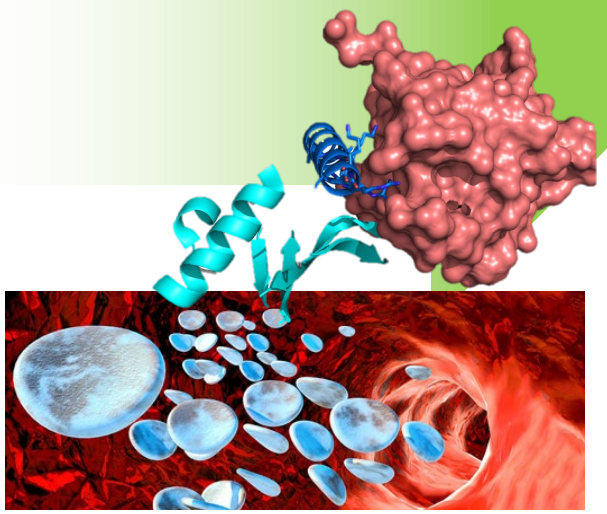
Secondary structures of proteins

Biological functions

Mimic or stabilize



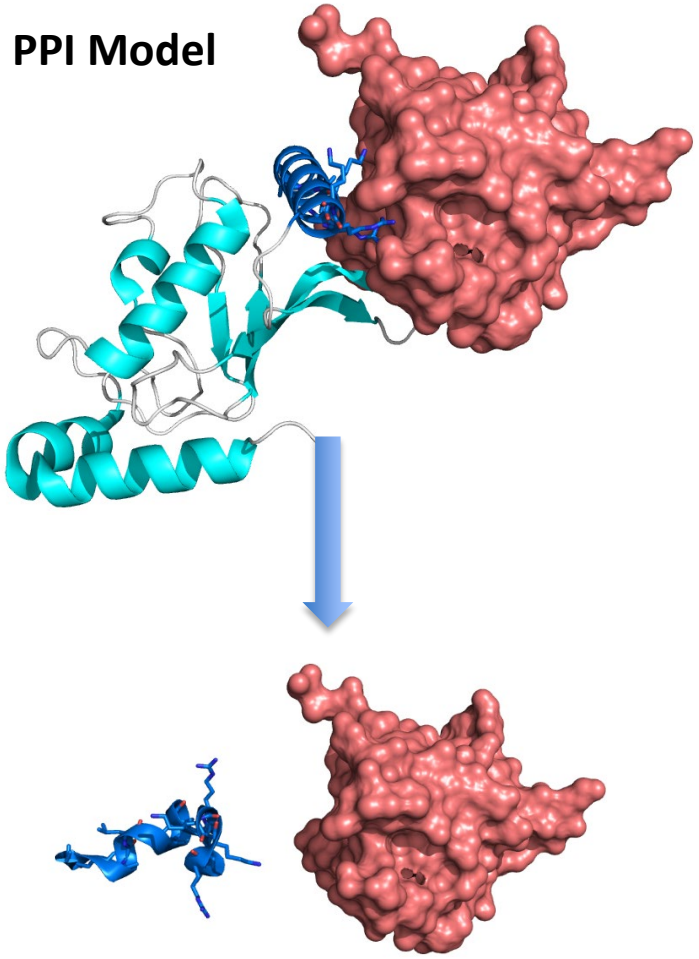




Implication in many molecular recognition process
(protein–protein, protein/DNA, RNA interaction, etc)

Alternative strategies for therapeutic tool development

PPI Model



Peptide Sequences
helix

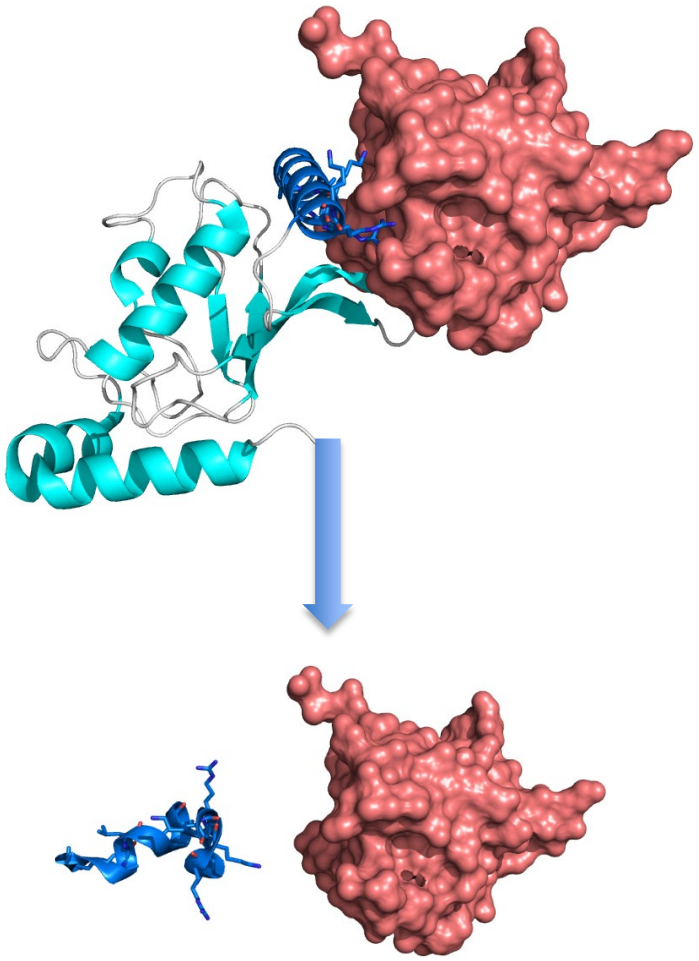
Do not maintain their structure when removed from the context of the protein

Low bioavailability
degradation, elimination,
low cell membrane permeability

Research strategy

Mimic or stabilize the secondary structure of proteins

Secondary structure mimetics – α-Helix



Peptide Sequences helix

Do not maintain their structure when removed from the context of the protein

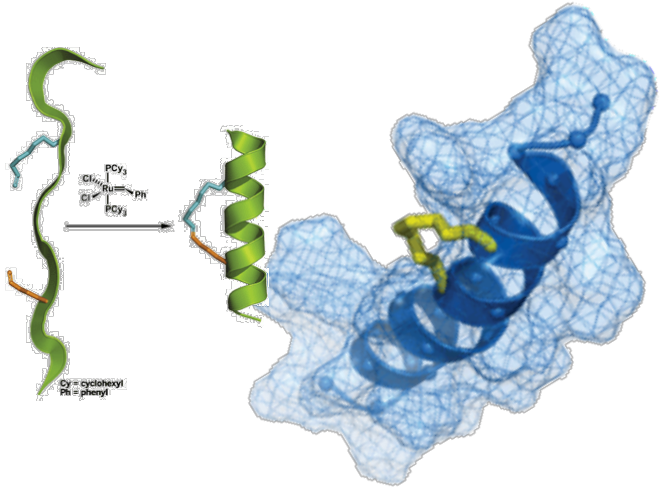
Low bioavailability
degradation, elimination,
low cell membrane permeability

Research strategy

Stapled Peptides and Foldamers

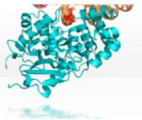
Mimicry approach: stapled peptides

✓ **Stapled peptides** : stabilized α-helical structures for targeting 'undruggable' proteins



Locking peptides into their bioactive α-helical conformation through site-specific introduction of a chemical brace

Verdine Laboratory
Department of Chemistry and Chemical Biology
Harvard University



and Aileron therapeutics

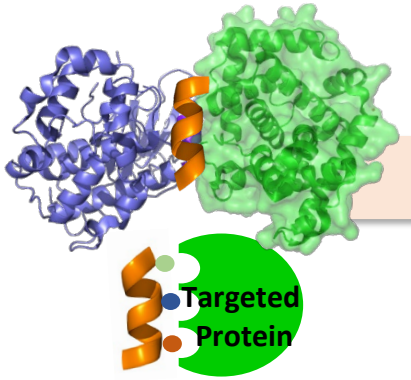
Verdine technology
Nature **2009**, *462*, 182
Nat. Med. **2008**, *14,* 144.
Science **2004**, *305*, 1466

Stapled peptides in clinical trials

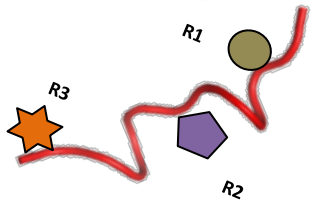
Stapled, Computational and Structural Biotechnology Journal, 2019, 17, 263
Bluntzer, M. T. et al. Designing Stapled Peptides to Inhibit Protein-Protein Interactions: An Analysis of Successes in a Rapidly Changing Field. *Peptide Science* **2021**, *113* (1)

Mimicry approach: stapled peptides

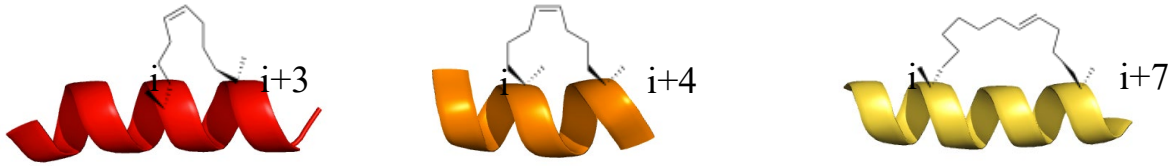
✓ **Stapled peptides** for the stabilization of α-helical structures for targeting 'undruggable' proteins

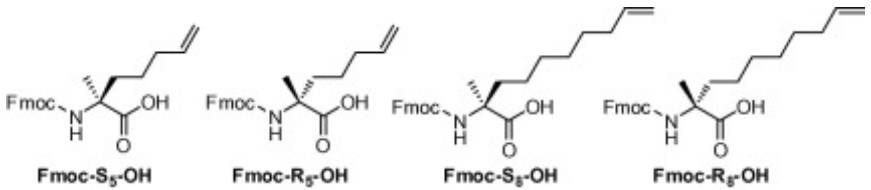


Wild peptide sequences

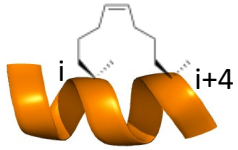


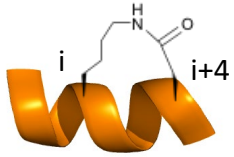
Folded peptides by α-helix stabilization
Stapling



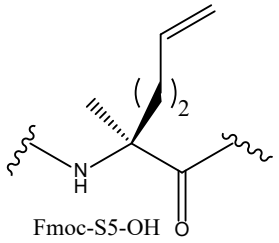


Stapled peptides





Staples



« Verdine »

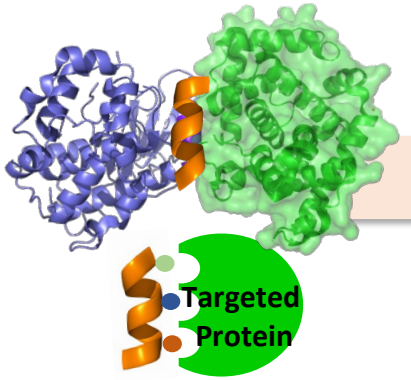
Lys/Asp

Lactame
« Fairlie Cyclisation »

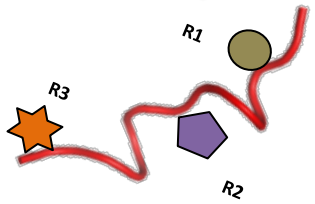
Verdine technology
Nature **2009**, *462*, 182
Nat. Med. **2008**, *14*, 144.
Science **2004**, *305*, 1466

Mimicry approach: stapled peptides

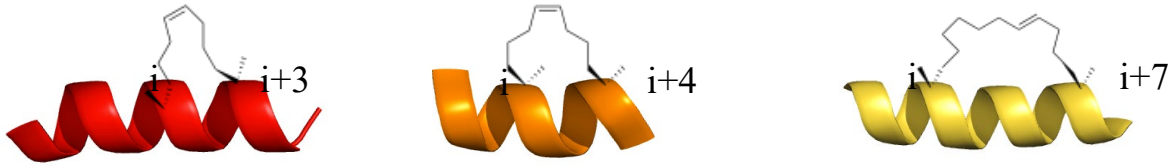
✓ **Stapled peptides** for the stabilization of α-helical structures for targeting 'undruggable' proteins

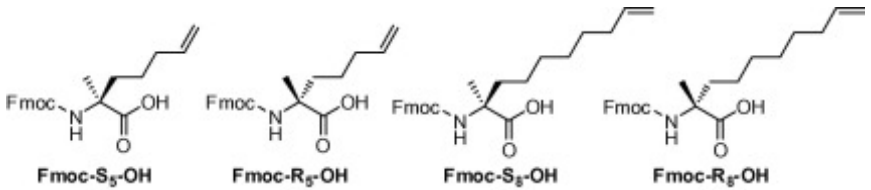


Wild peptide sequences

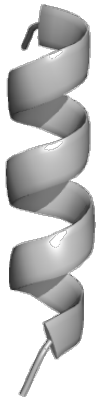


Folded peptides by α-helix stabilization
Stapling





Stapled Peptides



Conformational stability

Enzymatic degradation stability

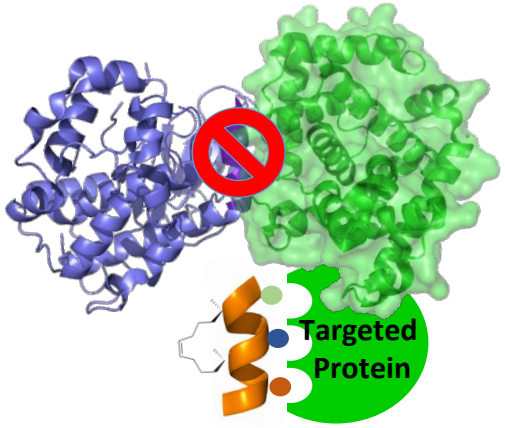
Ability to cross the cell membranes

Verdine technology
Nature **2009**, *462*, 182
Nat. Med. **2008**, *14*, 144.
Science **2004**, *305*, 1466

Control of the molecular shape : A Window for Drug Discovery

✓ PPI inhibitors

Inhibition of challenging Proteins



CDK4/Cycline D: KRAS-Mutant lung cancer

- ✓ **Identification of a stapled peptide inhibitor that mimic CDK4 helix**
- ✓ **Antiproliferative effect *in vitro*, *in vivo***

Collaboration May Morris, IGMM – (INCA)

Theranostics 2020; 10(5):2008-2028

PCSK9/LDLR: cholesterol uptake

- ✓ **Identification of a potent PCSK9/LDLR interaction inhibitor - Restore LDL uptake**

Collaboration Sanofi

J. Med. Chem. *2021,* 12;64(15):10834

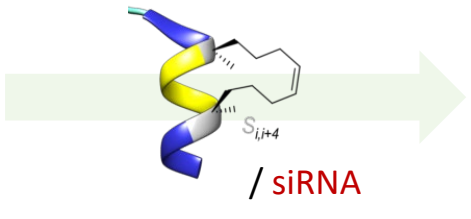
PPI involved in the SUMOylation process

- ✓ **SUMOylation and Acute Myeloid Leukemia** resistance to chemotherapy

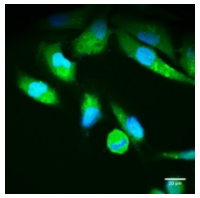
Collaboration Guillaume Bossi, IGMM - (MUSE)

✓ Cell-penetrating compounds

Cellular uptake
siRNA delivery

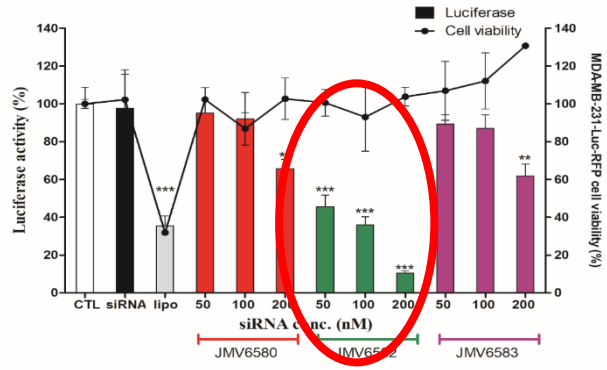


/ siRNA



Fluo-Ahx-S_5-III-S_5-RRC-NH_2

Inhibition of luciferase activity in MDA-MB-231-RFP-Luc cells



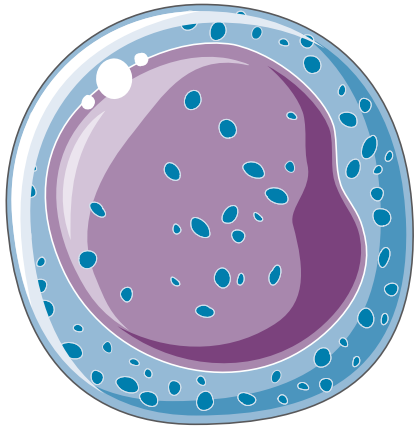
Nanomaterials, 2020,10(12):E2334

SUMOylation and Acute Myeloid Leukemia

Inhibition of the SUMOylation constitutes a relevant therapeutic approach in Acute Myeloid Leukemias

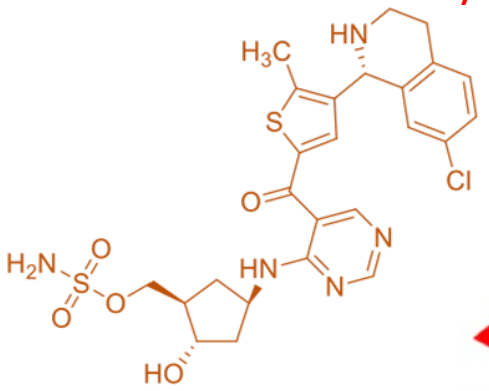
G.BOSSIS

Chemotherapy
Epigenetic therapy
Differentiation therapy

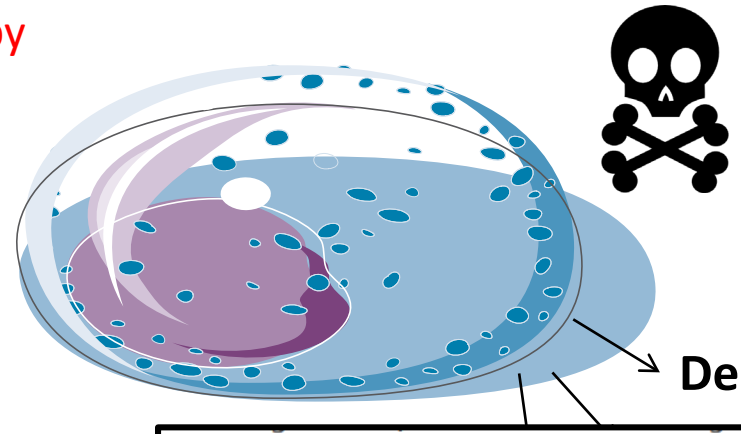


Leukemia cells

Inhibition of the SUMOylation



TAK981



Dedifferentiation

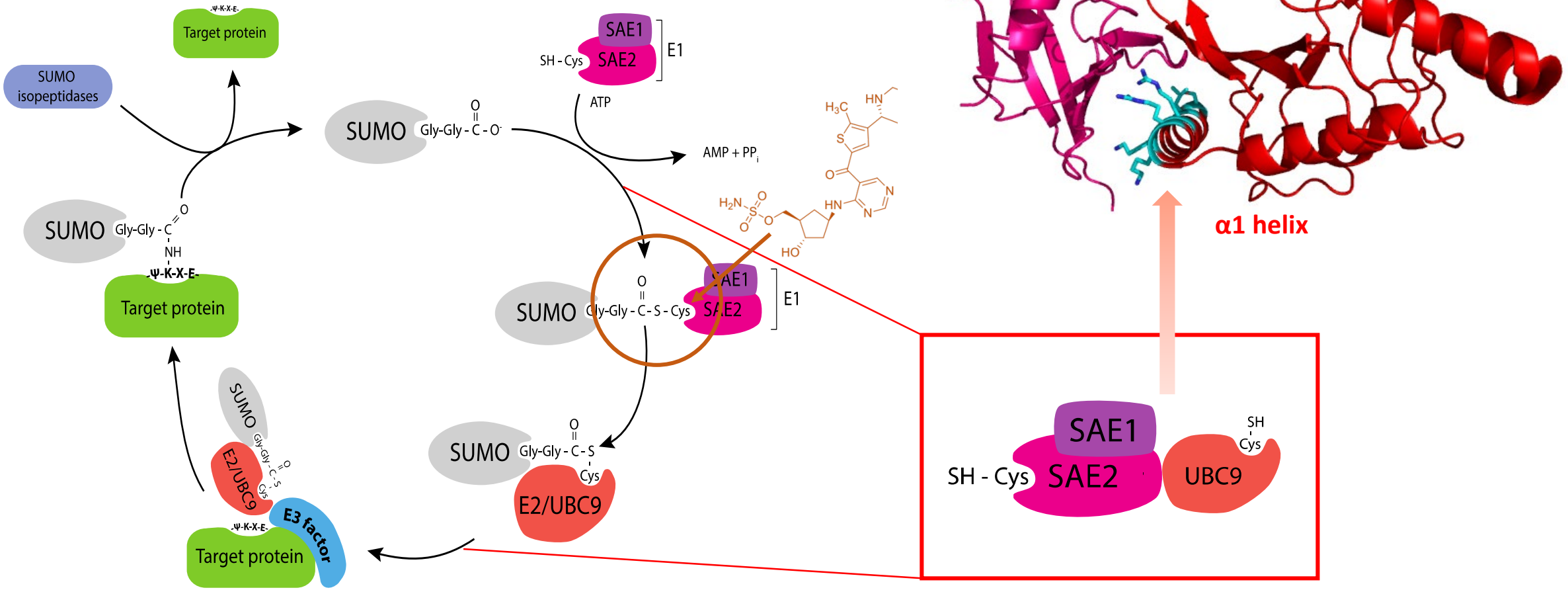
DEVELOPMENTAL THERAPEUTICS—IMMUNOTHERAPY

Phase 1/2 study of the novel SUMOylation inhibitor TAK-981 in adult patients (pts) with advanced or metastatic solid tumors or relapsed/refractory (RR) hematologic malignancies.

Takeda

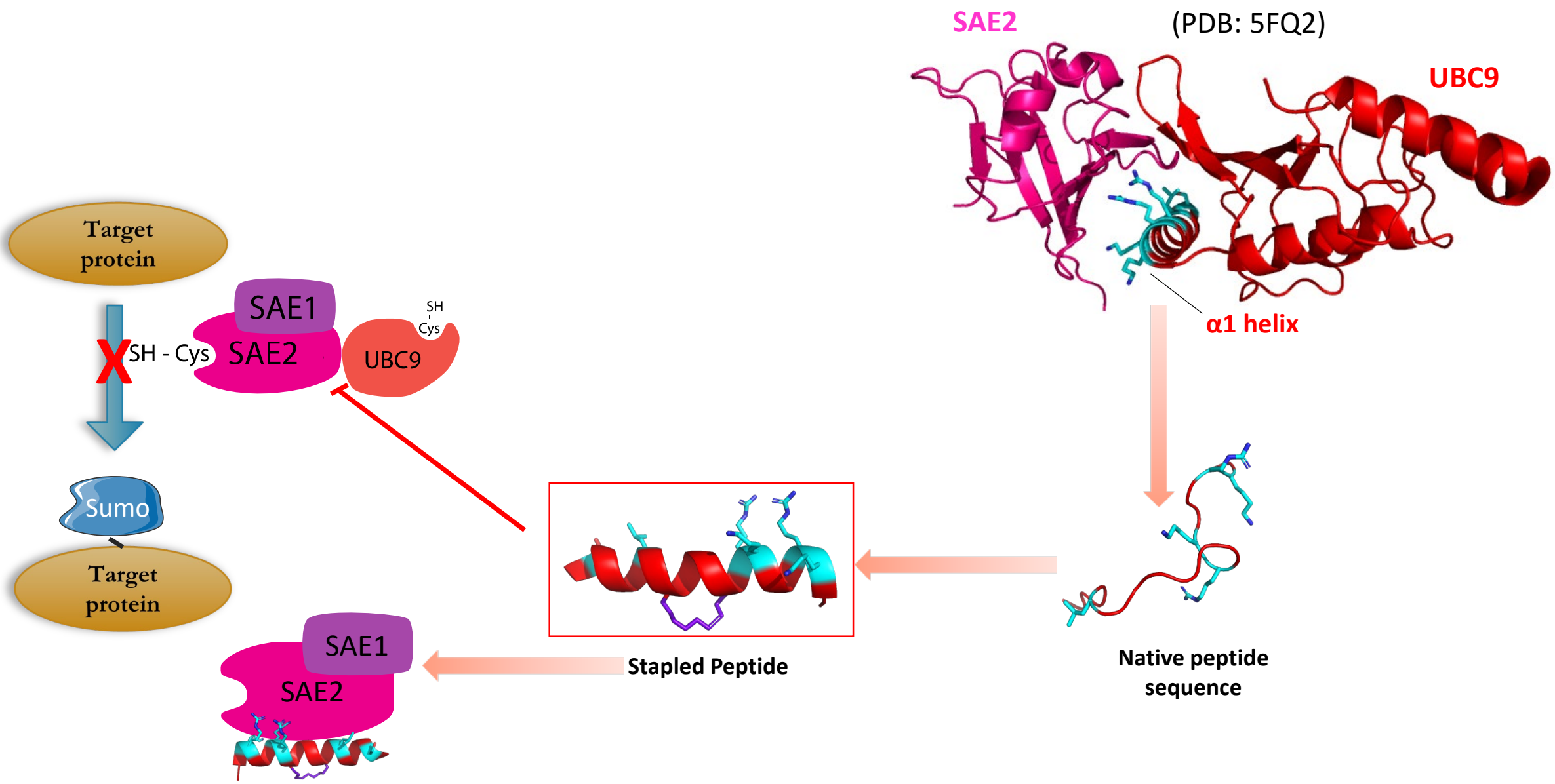
Bossis et al, *Cell Reports*, 2014
Baik et al, *Cancer Research*, 2018
Gâtel et al, *Life Science Alliance*, 2020

SUMOylation : Design of PPI Inhibitors



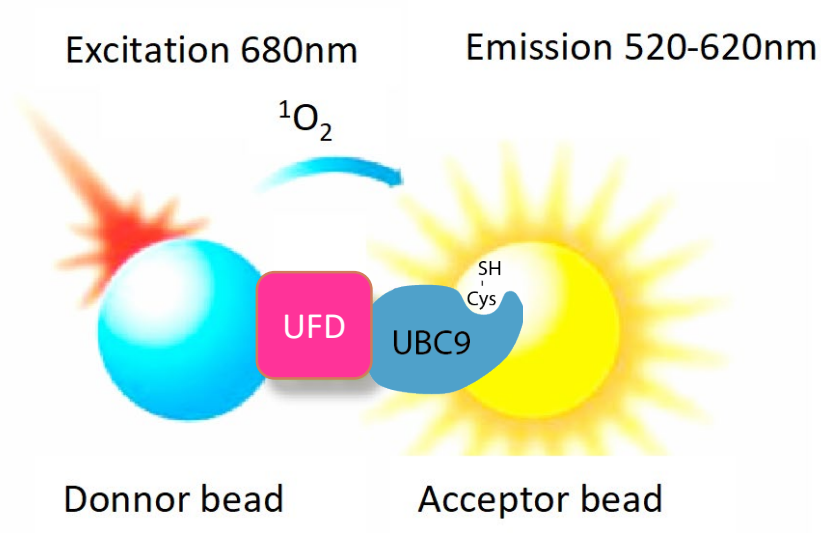
MUSE/SATT

Inhibition of the SUMOylation : Stapled Peptide Inhibitors

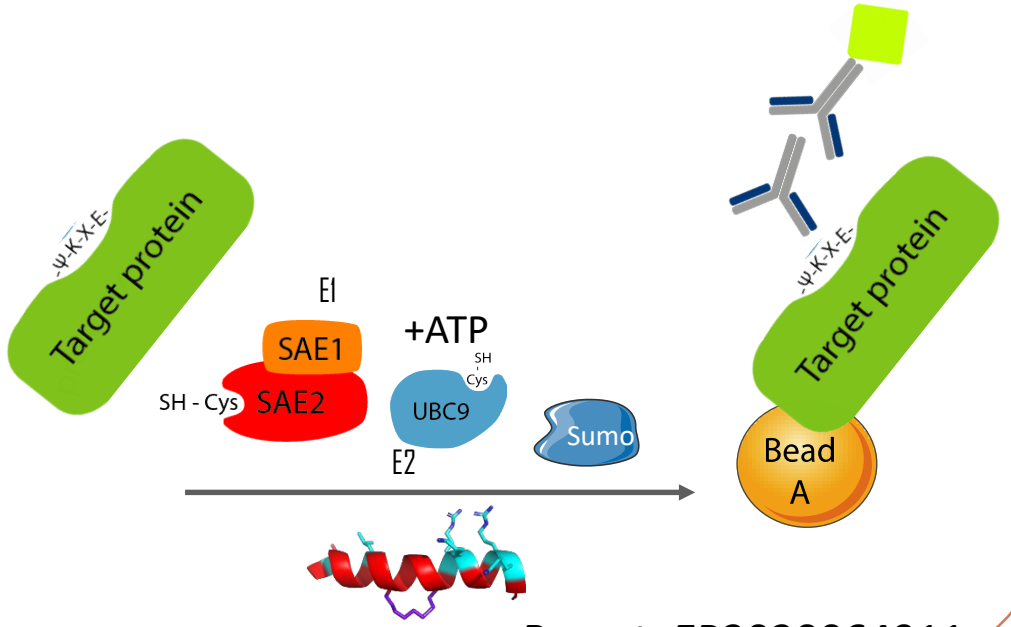


Stapled peptides Evaluation : Affinity and Sumoylation Activity

Alphascreen: interaction SAE2/UBC9

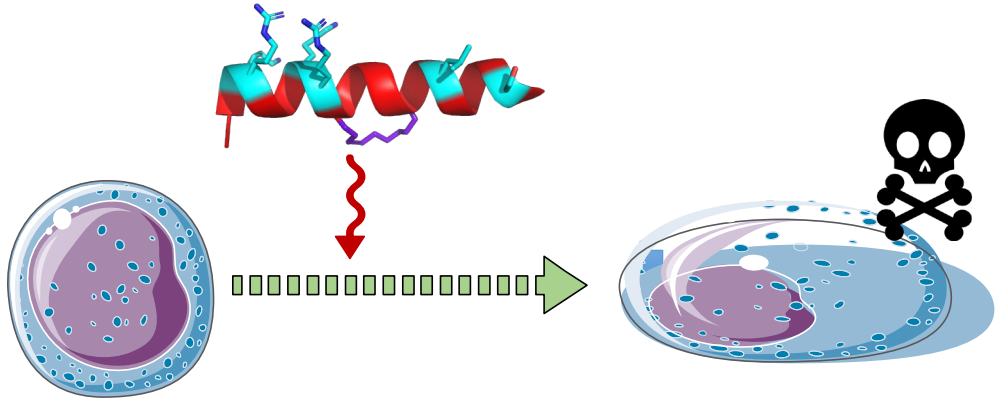


Luminex: SUMOylation Activity

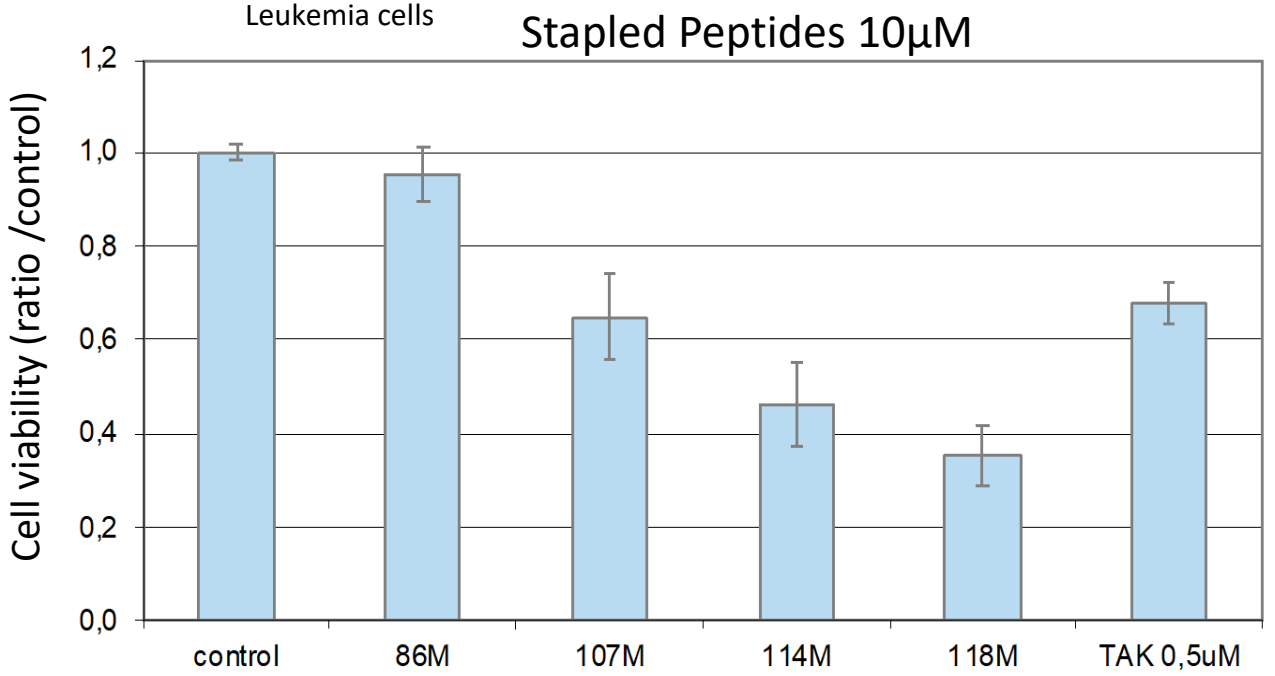


Brevet: EP2020064911

Toxicity of the stapled peptides



Leukemia cells

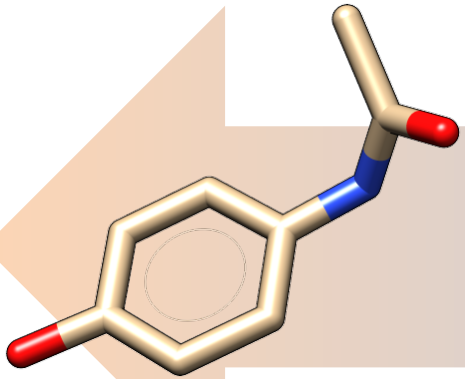


Cp	Interaction SAE2/UBC9 IC50 (α-screen)	SUMOylation Activity Inh. SUMO (50 µM) (residual activity)
control	102 uM	94%
86M	No inhibition/ 107 uM	125%
107M	11,24 uM	26%
114M	3,89 µM	32 %
118M	6,5/11uM	37 %

Identification of Hits

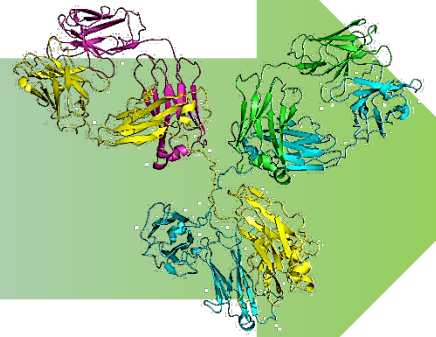
Optimization process (ANR PRC 2021, SUMOTarg)

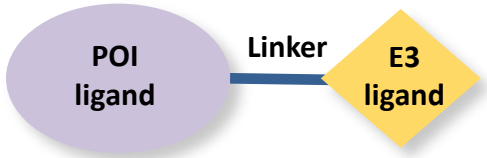
Alternative strategies for therapeutic tool development



PROTAC molecules

Antibody, Insulin, growth factor,
si-RNA, etc





- ✓ ***Oral bioavailability***
- ✓ ***Stability***

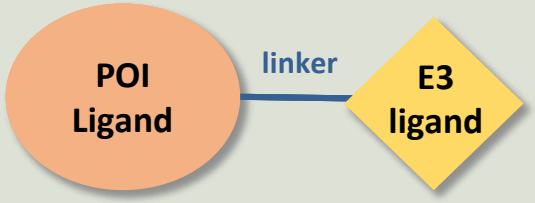
Small molecules

- ✓ ***High specificity***

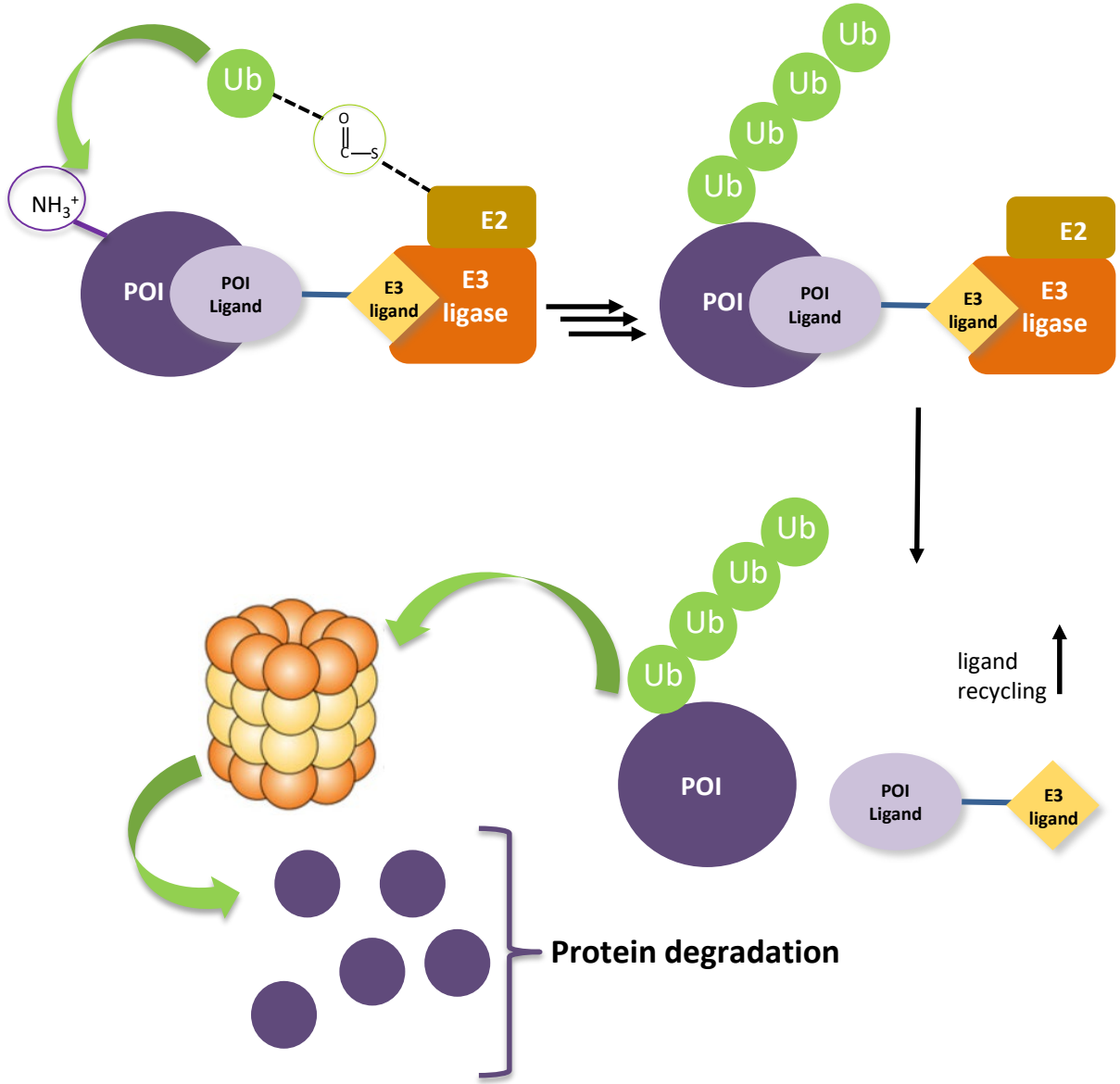
Biologics

PROTAC: Proteolysis Targeting Chimera

What is a PROTAC



Heterobifunctional molecule



Sakamoto et al, PNAS, 2001
J. Salami, C. M. Crews, Science 2017, 355, 1163
A. C. Lai, C. M. Crews, Nat. Rev. Drug Discov. 2017, 16, 10

Advantages of PROTAC-mediated Protein Degradation

New paradigm in drug discovery

Degradation of the target protein instead of its inhibition

Protacs hijacks the ubiquitin-proteasome system to promote the destruction of target protein

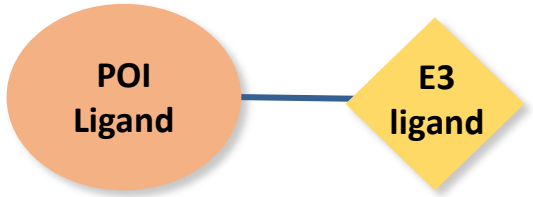
PROTAC concept was pioneered in 2001 by C. Crews et al (Yale University)
PROTACs over 400 research articles and 170 patents
In 2019, 2 PROTACs entered phase 1 and 1/2 clinical trials for the treatment of metastatic castration resistant prostate cancer (NCT03888612) and locally adv./metastatic breast cancer (NCT04072952)

Any unique part of the protein surface can be targeted

Can target non-enzymatic, structural and regulatory protein

PROTAC may reduce the risk of resistance

PROTAC: Proteolysis Targeting Chimera



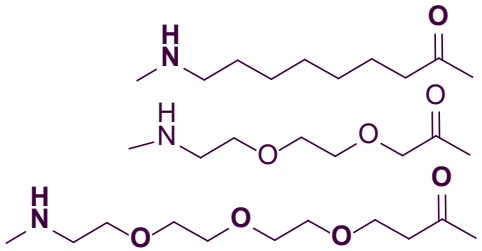
POI recruiting moiety

Well-characterized ligands (inhibitor/antago/agonist)

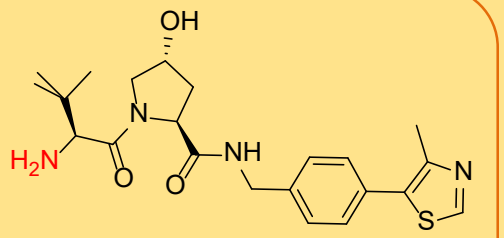
Peptide modulators (stapled peptides)

Low affinity POI ligands (cooperativity effect)

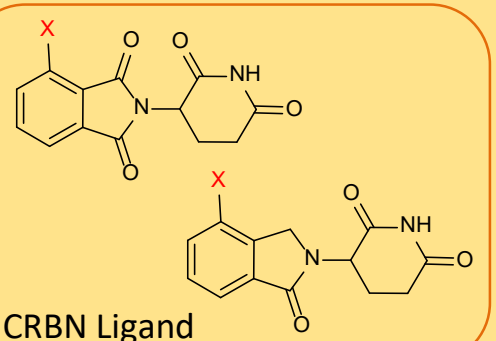
Linker



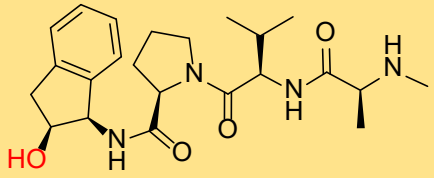
E3 Ligase Ligands



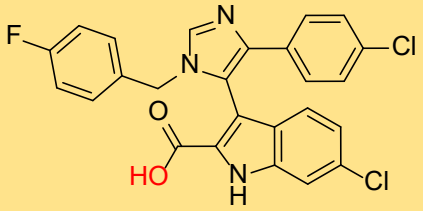
VHL Ligand



CRBN Ligand



IAP Ligand



MDM2 Ligand

Our Target: Nuclear Receptor PXR

PXR is involved in cancer cells resistance to chemotherapy

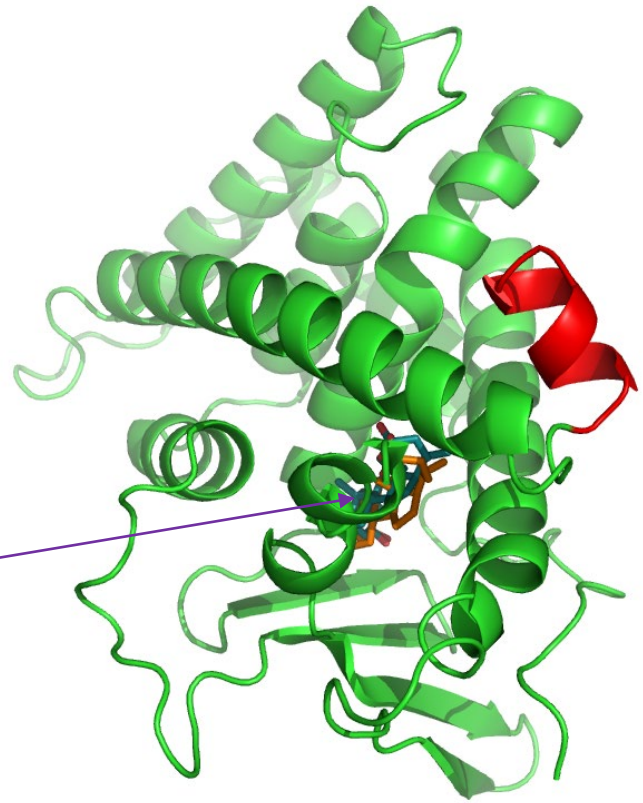
PXR promotes cancer stem cells-mediated relapse and correlate with poor survival probability in patients

→ Target genes: **ALDH1A1, CYP3A4**

siRNA → strongly decrease tumor relapse and tumor initiation potential after chemotherapy

PXR knock-out mice → viable

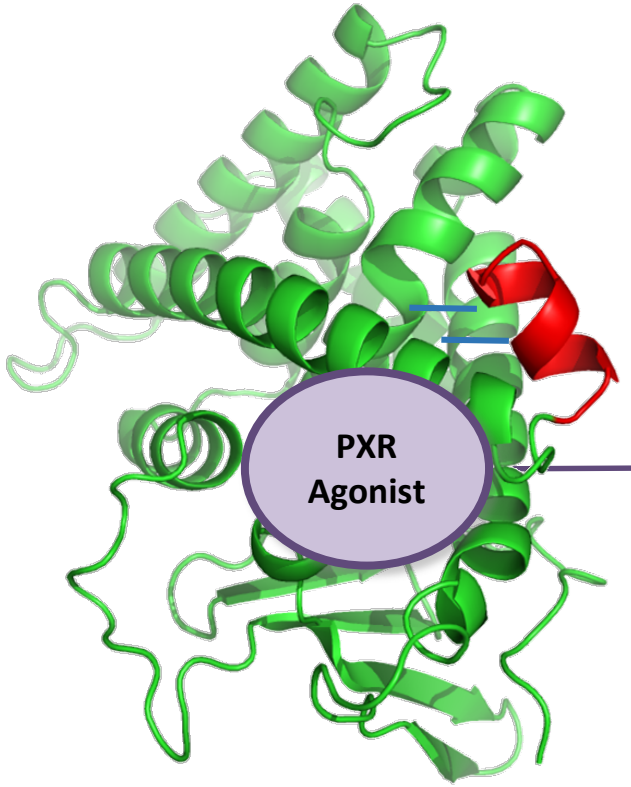
Existence of a ligand binding domain : LBD



Impossible to identify PXR antagonists

Collaboration: Jean-Marc Pascussi, Julie Pannequin, Lucile Bansard (IGF)
CNRS and SATT maturation project

Turn agonist into a target degrader



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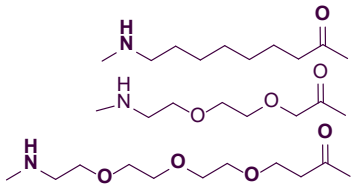
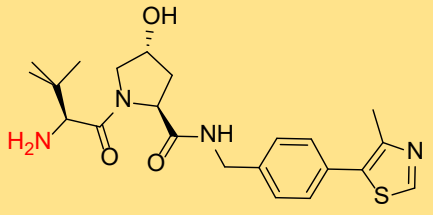
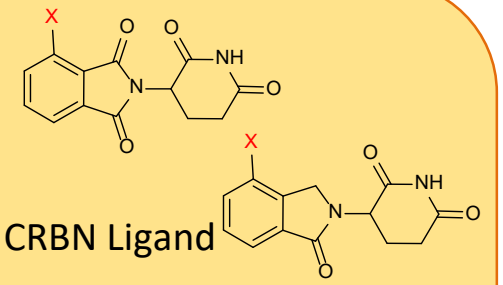


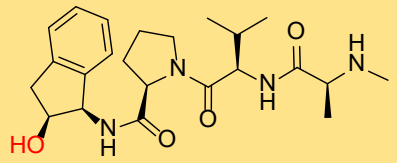
Ligand E3 ligases



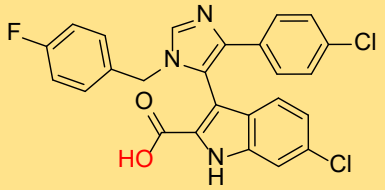
VHL Ligand



CRBN Ligand



IAP Ligand



MDM2 Ligand

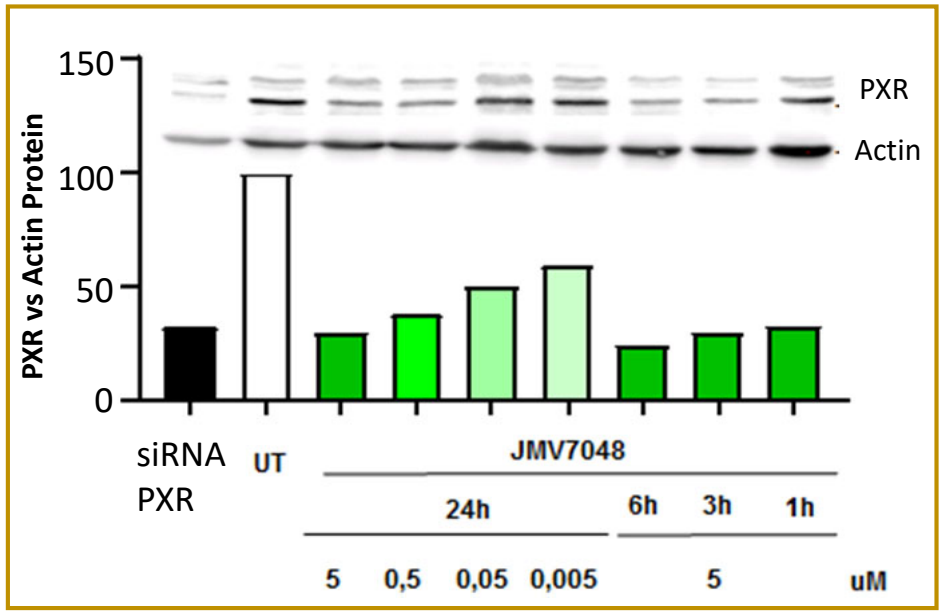
“Trial and error” approach for linker position attachment

Affinity ≤ 100 nM and without any cellular toxicity at 10µM

Collaboration : William Bourguet, Vanessa Delfosse (CBS)

JMV 7048 : PROTAC ?

PXR levels : Kinetic and dose-dependant effect

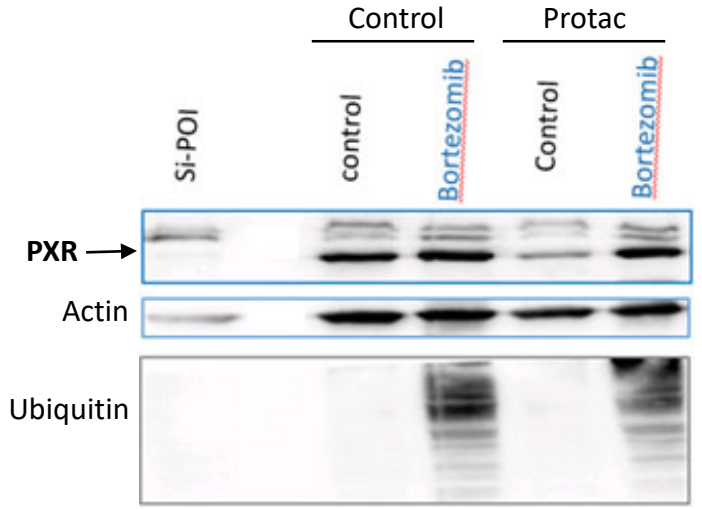


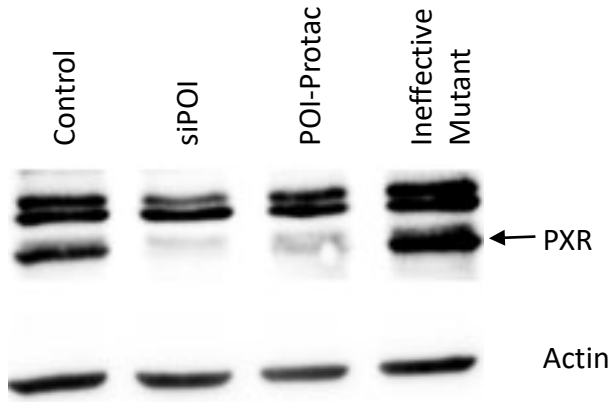
Colon cancer cell line (LS174T)

Identification of a PROTAC molecule (JMV 7048)

Proteasome Activity control

Inhibition of the proteasome (Bortezomib)



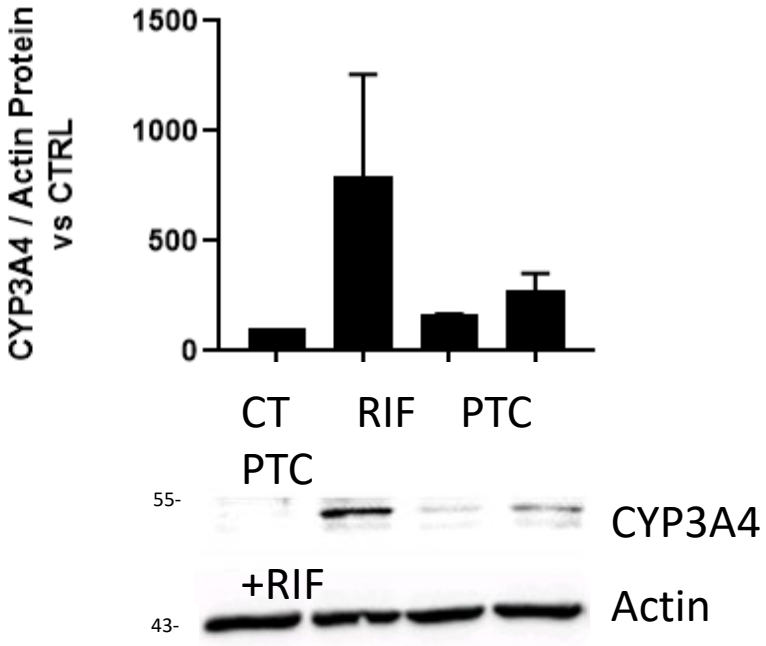


JMV 7048 : activity on CYP3A4, Aldefluor Expression and Tumorsphere formation

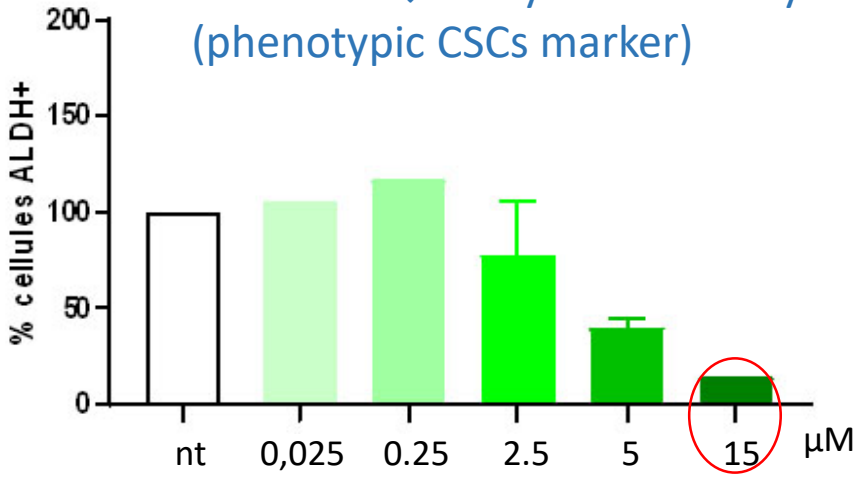
Rifampicin: Standard agonist of PXR → induction of target gene expression

- ✓ **PXR-signalling pathway in colon cancer cells (CYP3A4)**

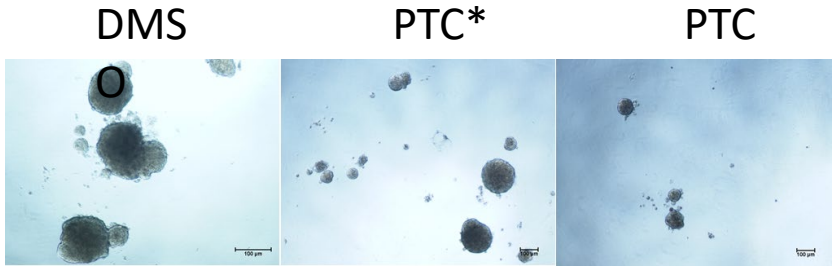
LS174T with stable PXR over expression

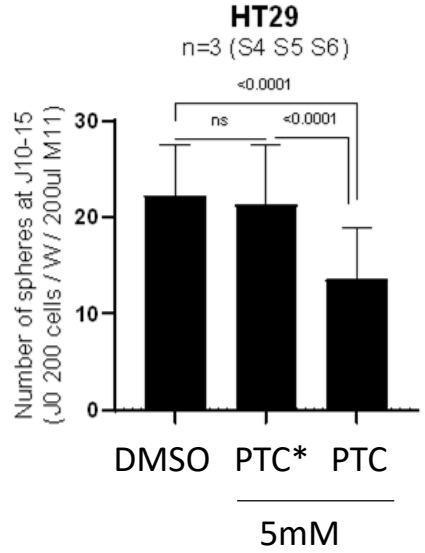


Aldefluor → enzymatic activity (phenotypic CSCs marker)



% TFS = Tumorsphere forming cells (phenotypic CSCs marker)





Conclusions

Nuclear Receptor PXR

✓ **Proof of concept *in vitro***

CNRS INNOVATION

aXLR SATT

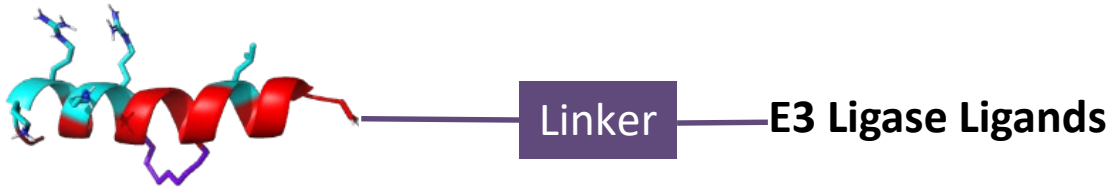
Patent deposit : PXR Receptor Based Protac Compounds and Associated Methods Of Use

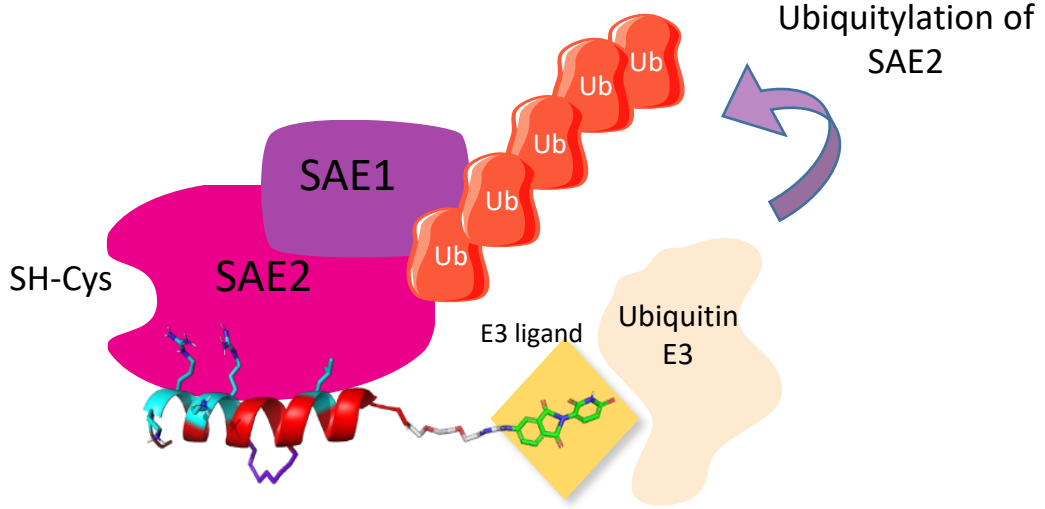
✓ ***In vivo* evaluation of the PROTAC**

✓ **Adjuvant to chemotherapy ?**

SUMOTarg

✓ **Indentification of SAE2 binders → PROTACs design**





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