UEM919 – Cours 4

Défis dans la découverte d'inhibiteurs d'interactions protéine-protéine : application dans le traitement du cancer et autres pathologies

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Loaboratoire : **Molécules fluorées et peptides d'intérêt thérapeutique (FLUOPEPIT)**

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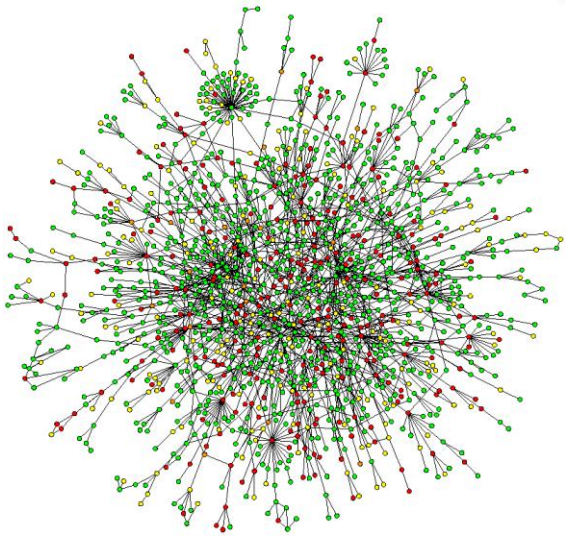
Generalities on Protein–Protein interactions (PPIs)

- Introduction
- Biophysical identification of PPI

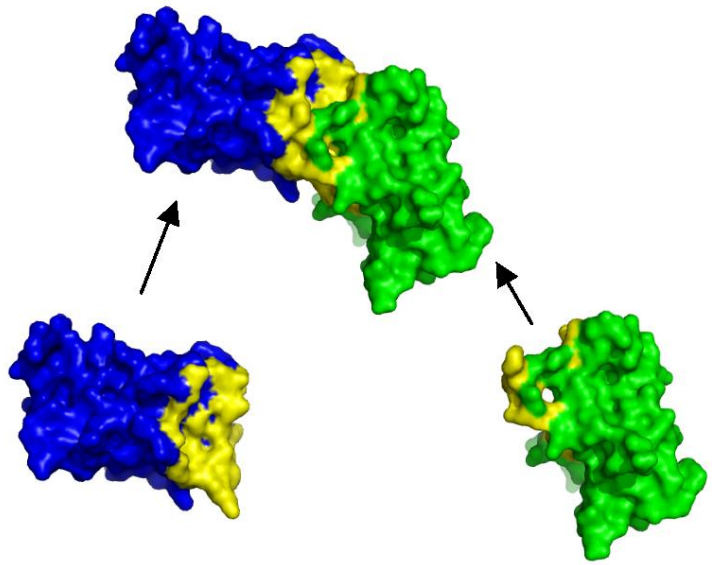
Protein–protein interactions (PPIs)

- Cell homeostasis depends on a fine-tuned network of protein–protein interactions (PPIs).
- Human **interactome** involves between 130 000 and 600 000 PPIs
- Around 9 000 structures of protein–protein complexes involving 13 000 proteins have been reported in the Protein Data Bank

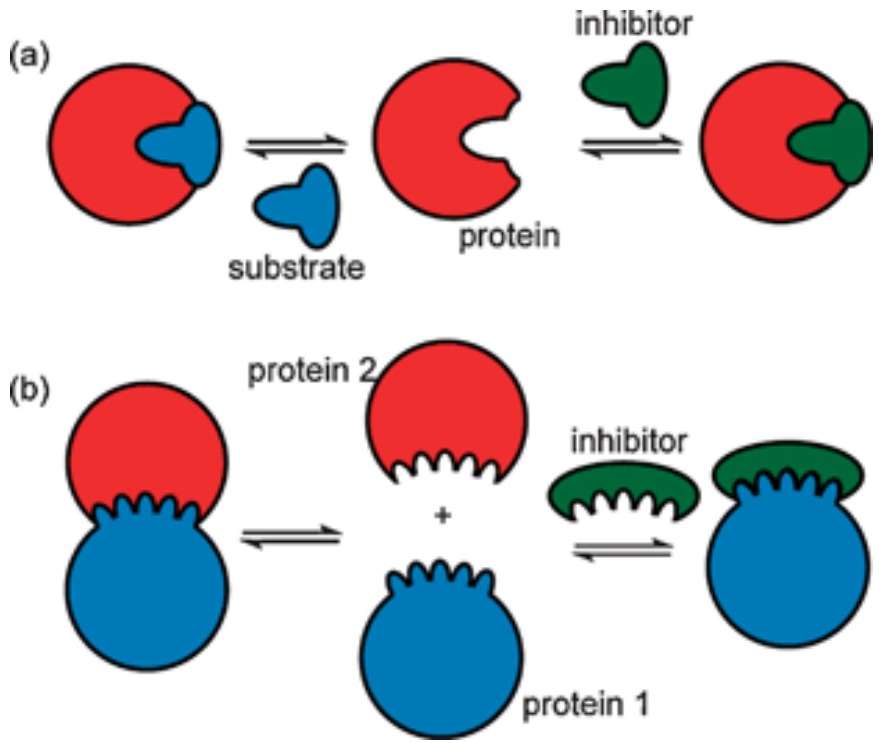


- Biological or aberrant, **associated to a large number of pathologies : cancer, infections, neurological disorders, heart failure, inflammation, oxidative stress**
- Twenty years ago, PPIs were commonly regarded to as ‘undruggable’

- **Small protein fragments** are involved in interactions : “hot-spot”
- **Generally well defined secondary structure**



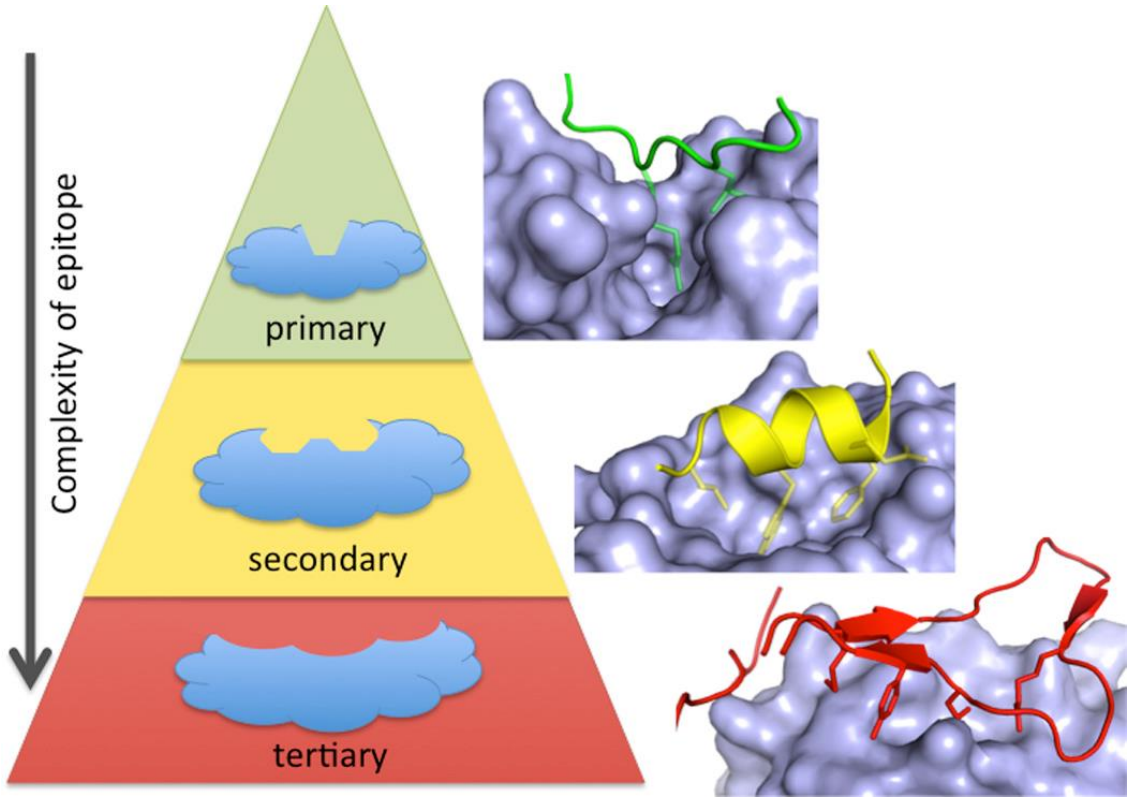
Protein–protein interactions (PPIs)



Protein interfaces in traditional targets, such as enzyme active sites : 300–500 A $^{\circ 2}$

Protein interfaces in PPIs : large (1000–2000 A $^{\circ}$2) and flat interfacial areas

Protein–protein interactions (PPIs)



Classified by whether one side of the interface consists of a primary (linear) protein sequence (green), a single region of secondary structure (such as an alpha helix, yellow), or multiple sequences requiring tertiary structure (red).

Structures shown are BRDt/histone (green, 2WP1), MDM2/p53 (yellow, 1YCR), and IL-2/IL-2Ra (red, 1Z92)

Règles de Lipinski

Capacité d'absorption souvent quantifiée par la **biodisponibilité orale**. Définie comme le rapport dans le sang, entre la concentration la plus élevée atteinte par un composé administré oralement, et la concentration atteinte par la même dose du composé injecté directement par voie intraveineuse.

La biodisponibilité peut varier considérablement d'une espèce à l'autre. Les résultats obtenus chez l'animal sont difficiles à appliquer à l'homme.
En dépit de cette variabilité, **quelques généralisations :**

- Poids moléculaire < 500 (opt ~= 350)
- Nbre de liaisons H accepteurs < 10 (opt ~=5)
- Nbre de liaisons H donneurs < 5 (opt ~=2)
- -2 < clogP < 5 (opt ~= 3)
- Nbre d'angles de rotations =< 5

Lipinski et al, Adv. Drug. Del. Rev., 23, 3-25 (1997)

Règles de plus en plus controversées (J. Med. Chem. 2019, 62, 1701−1714)
Règles difficiles à appliquer pour inhiber des interactions protéine-protéine!

Current strategies for PPI characterization

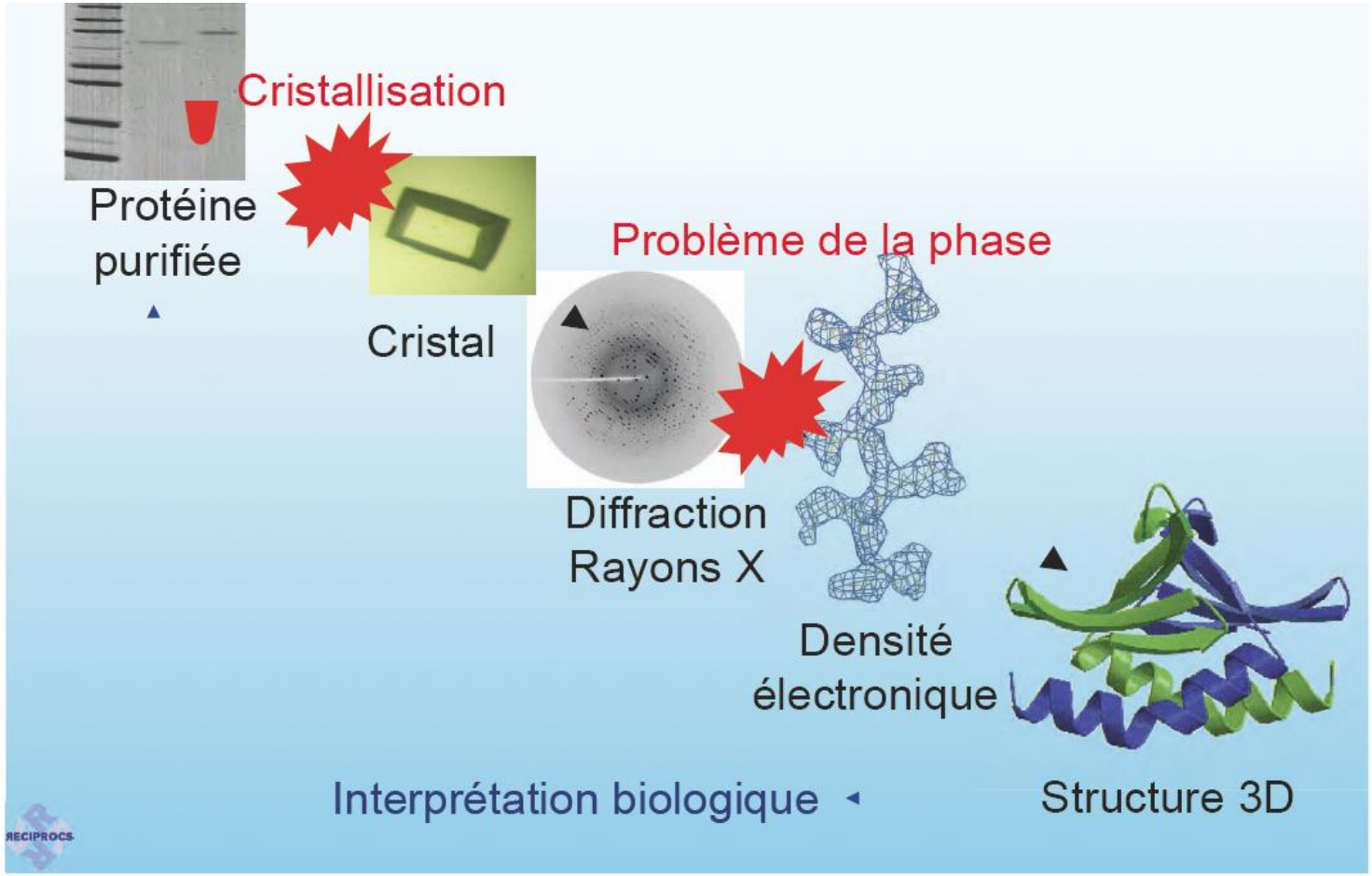
Around 9 000 structures of protein–protein complexes involving 13 000 proteins have been reported in the Protein Data Bank

- **X-ray crystallography** : to determine, in isolation or in interaction, globular domain structures
- **NMR** : can also investigate transient and weak PPIs
- **cryo-electron microscopy** (cryo-EM) : to determine PP complexes
- **small-angle X-ray scattering** (SAXS) : low resolution structural technique
- **in silico** approaches

- Förster/**Fluorescence resonance energy transfer** (FRET)
- **Gel Filtration Chromatography**
- **Surface plasmon resonance** (SPR)
- Mass spectroscopy techniques

Review : An Overview of Current Methods to Confirm Protein Protein Interactions. Protein & Peptide Letters, **2018**, *25*, 728.

X-ray crystallography

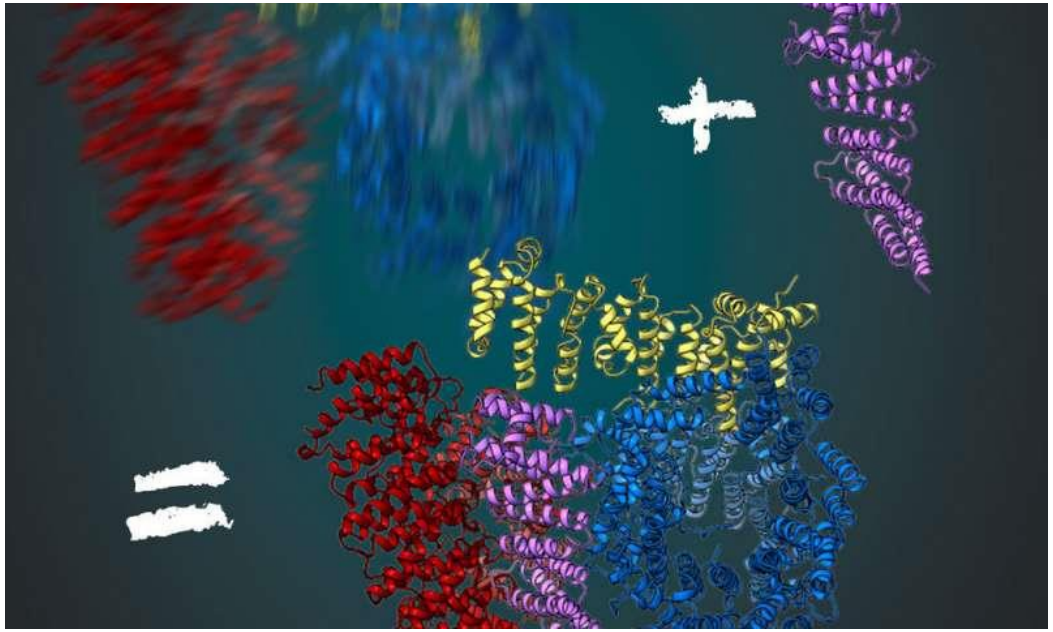


Cryo-electron microscopy

Transmission electron microscope

A solution of the purified protein is frozen (no crystals)

Several thousand similar images are acquired and computed to reconstruct the original structure with high resolution.



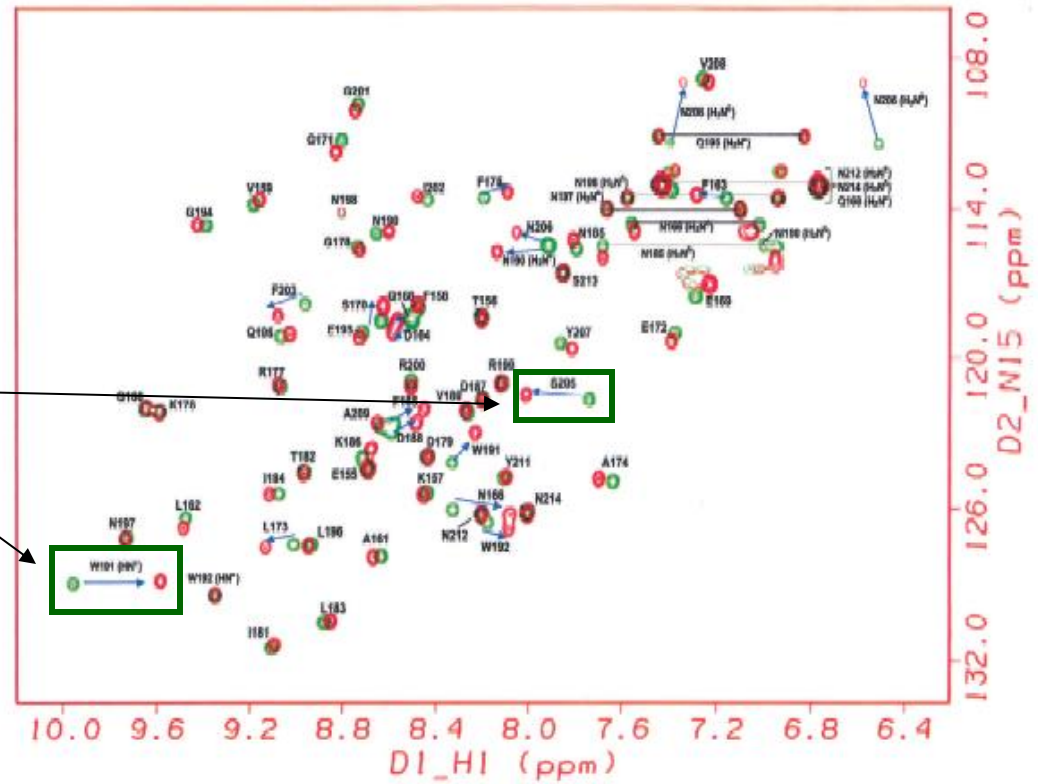
The cryo-electron microscopy structure of huntingtin : three flexible regions (red, yellow and blue).
In interaction with HAP40 (purple)

NMR Analysis of Protein-Ligand Interactions

Protein 1 Chemical Shift Changes Upon Ligand or Protein 2 Binding

- Assigned 2D ^{1}H-^{15}N HSQC NMR Spectra
  - overlay spectra in presence/absence of ligand
  - changes in peak position indicate binding
  - identity of peaks that change identifies binding site on protein surface

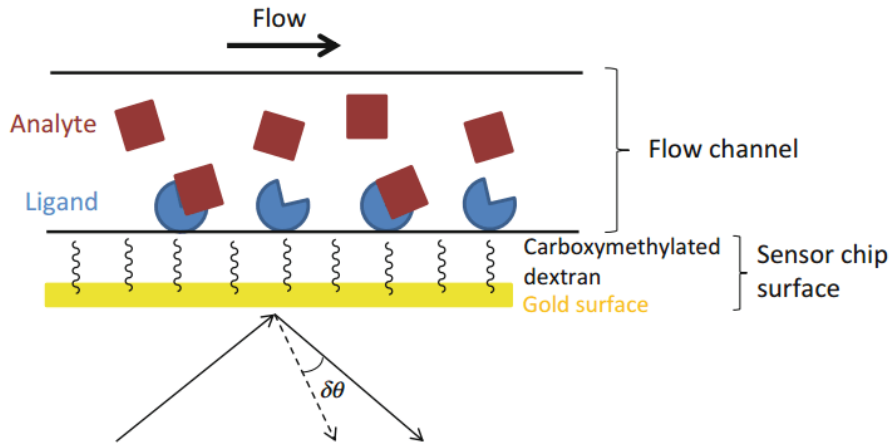
Peptide Binding to C-terminal SH3 domain of Sem-5 induces chemical shift changes

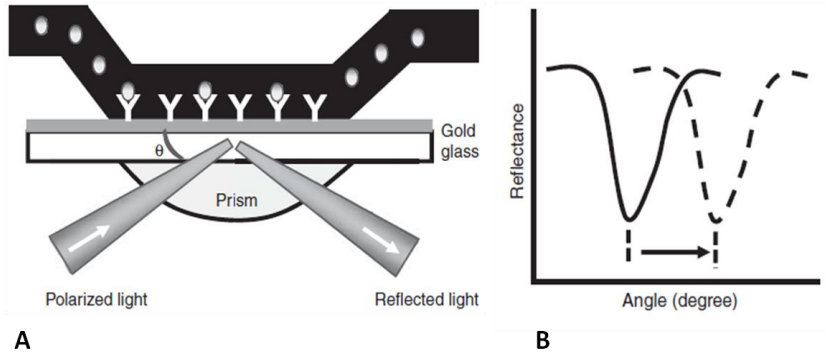


Protein Science (2003), 12:982–996.

Surface plasmon resonance (SPR)

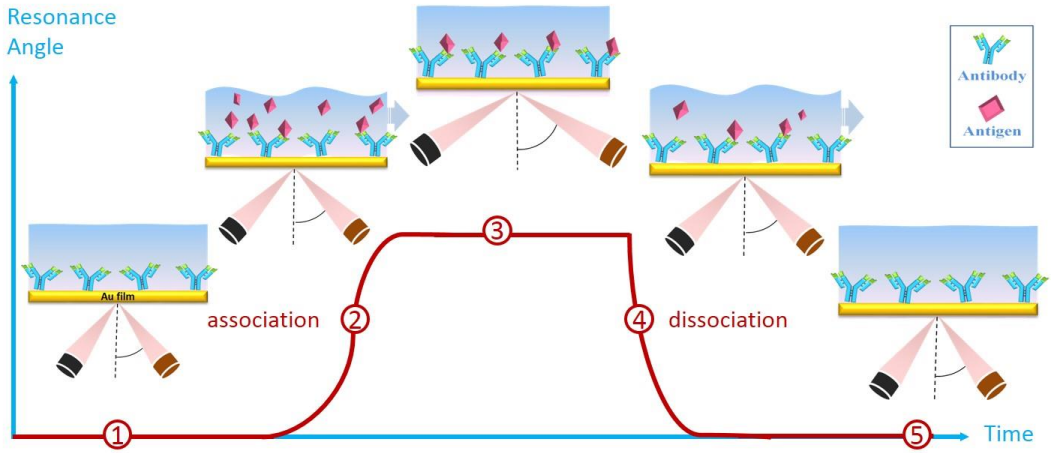
- **Most popular** label-free ligand binding assay
- **Analyze the binding of ligands/proteins to the receptors/proteins linked to a gold surface** (or binding of biological targets to ligands linked to the gold surface).
- SPR is used to study binding kinetics





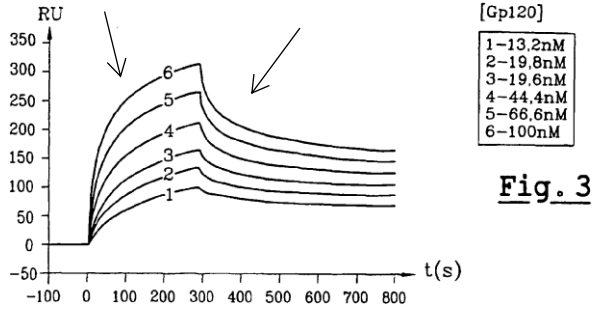
Surface plasmon resonance (SPR)

- follow the association and dissociation mechanisms in real time by creating a sensorgram which detects the changes in wavelength.
- **Quantitative method** since these changes are related to the number of bound ligand molecules



Association

Dissociation



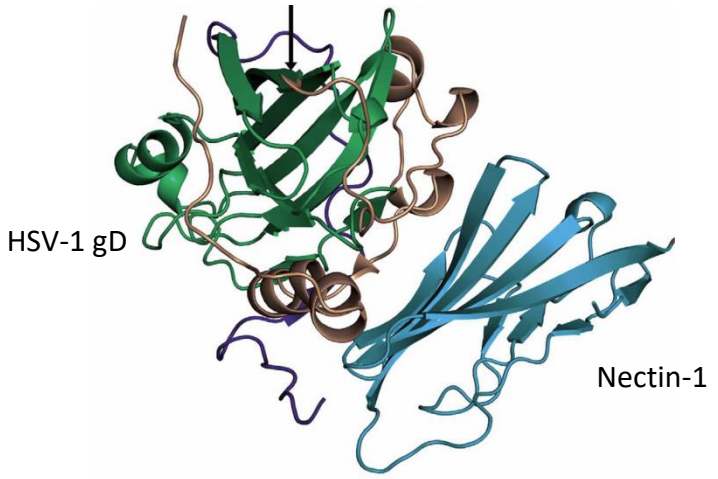
[Gp120]
1–13,2nM
2–19,8nM
3–19,6nM
4–44,4nM
5–66,6nM
6–100nM

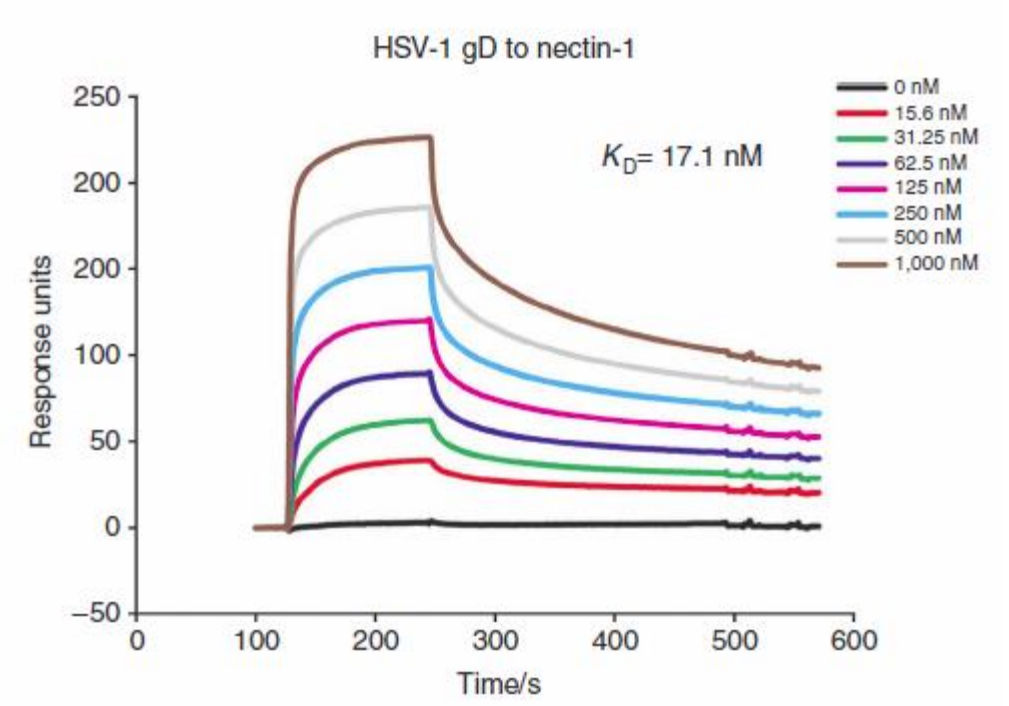
Fig. 3

$$A + L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AL \quad \text{Affinity :} \quad K_D = \frac{k_d}{k_a}$$

Surface plasmon resonance (SPR)

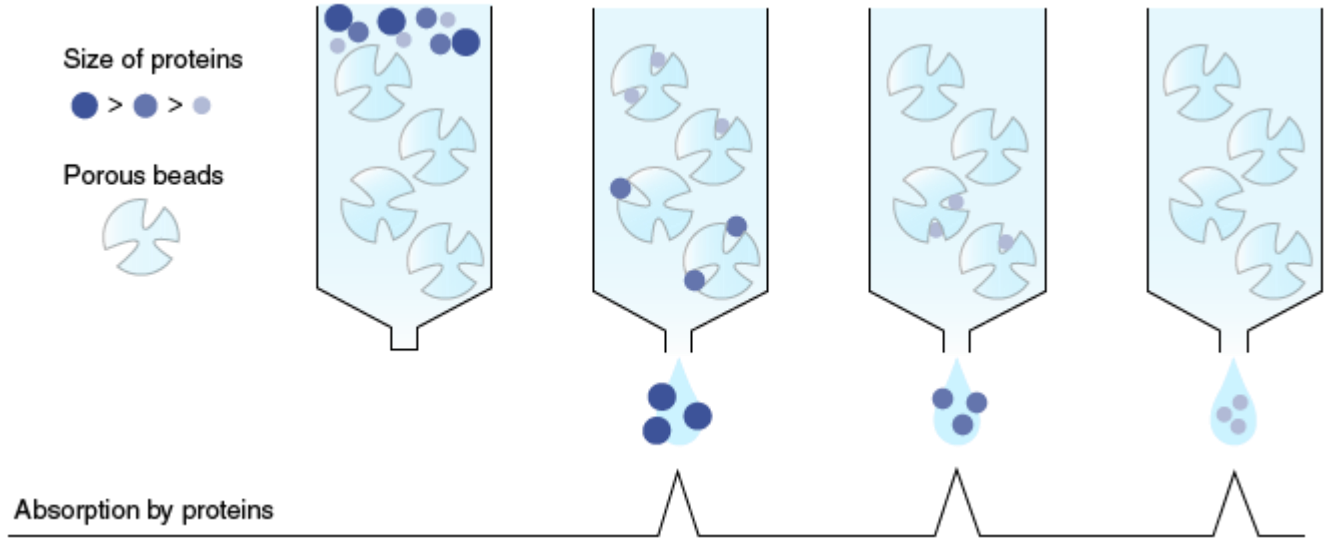
Exemple of protein-protein interactions





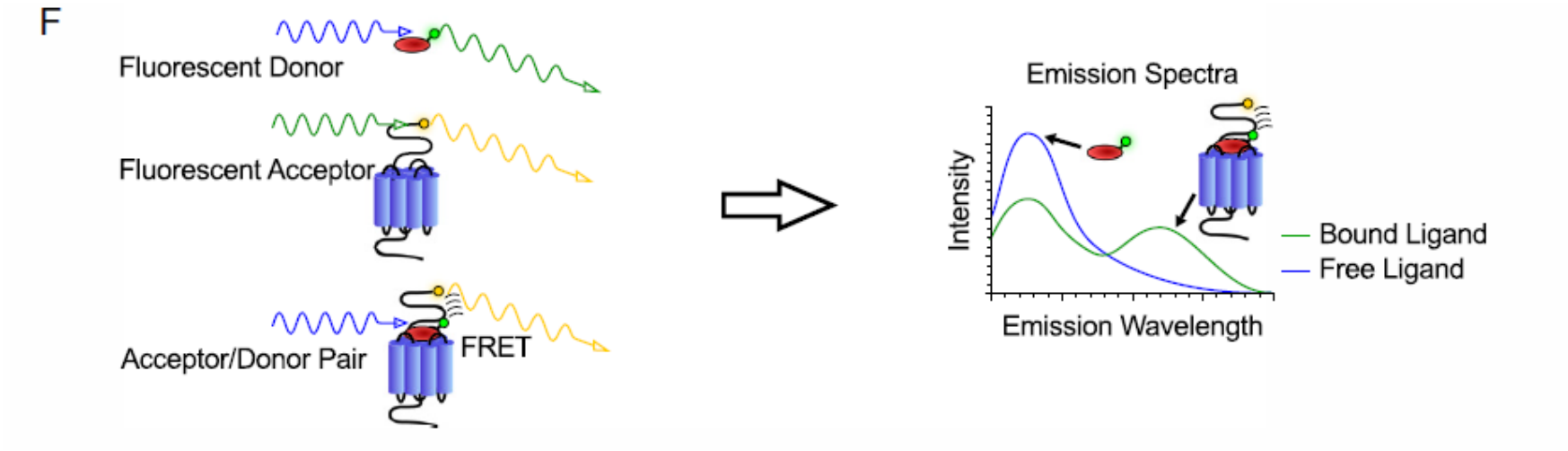
Gel Filtration Chromatography

Technique to separate molecules dissolved in a solution based on **differences in mobility owing to their respective molecular weights and three-dimensional shape**. Smaller molecules diffuse further into the pores of the beads and therefore move through the bed more slowly, while larger molecules enter less or not at all and thus move through the bed more quickly.



PPIs : a peak is detected at the retention time corresponding to the molecular weight that totals each protein.
It is possible to purify the protein complex

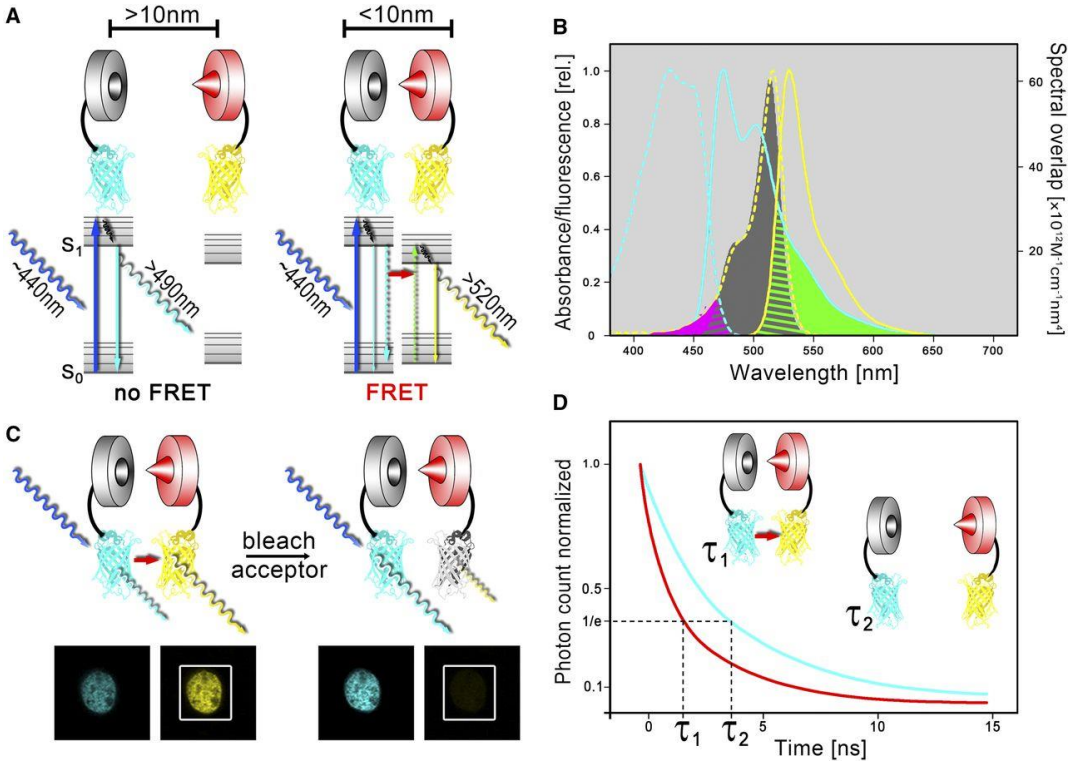
Fluorescence Resonance Energy Transfer (FRET)

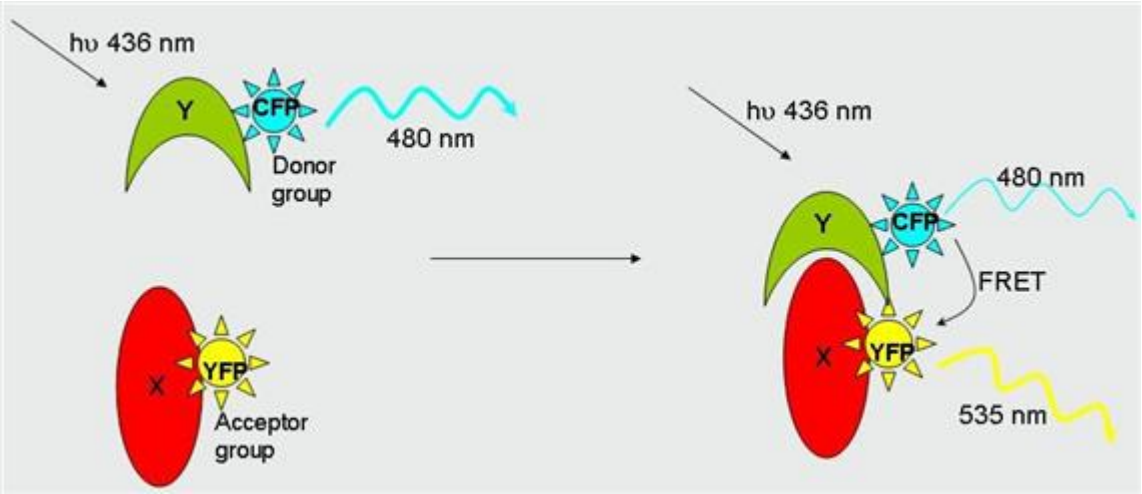


Fluorescence Resonance Energy Transfer (FRET) between a fluorescent protein1 and a fluorescently labeled protein2.
Binding of protein1 to protein2 leads to **transfer of fluorescence from donor to acceptor** that can be detected via a **decrease in donor emission or an increase in acceptor emission**.

The strong ($1/r^6$) distance dependence of the interaction makes it useful for **measuring distances** or determining whether **the labels are in close proximity on the scale of 1–10 nm**

Fluorescence Resonance Energy Transfer (FRET)

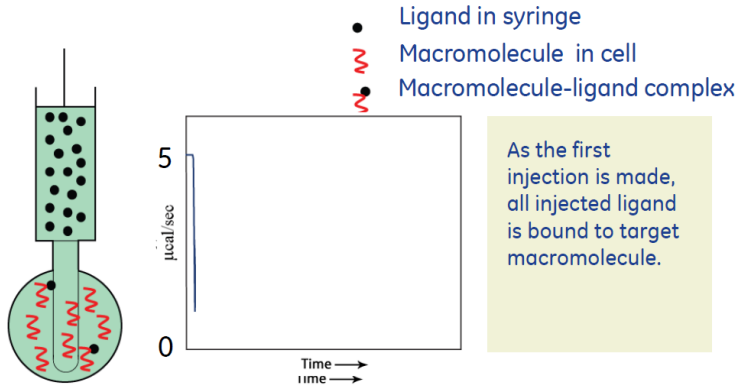


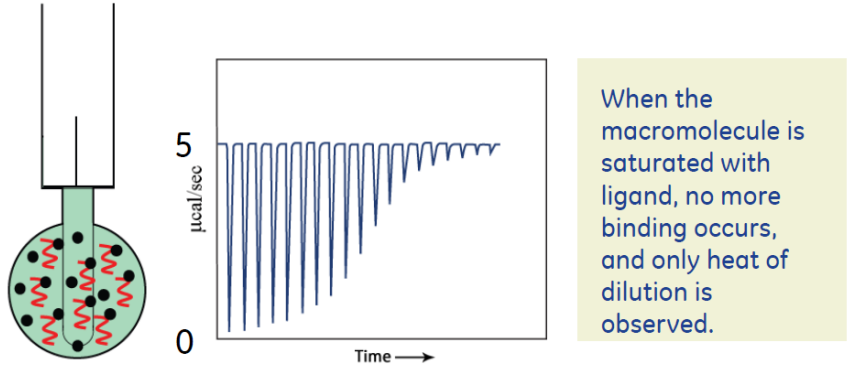


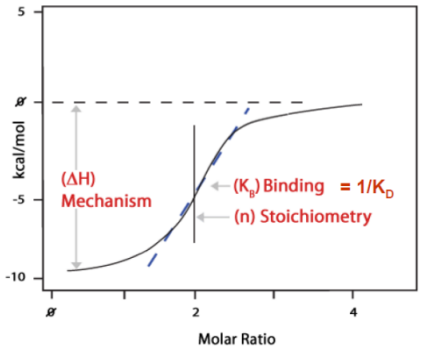
Thermodynamic binding assays

❑ **Isothermal titration calorimetry (ITC):**

- ITC measures the **binding enthalpy variation by sensing the heat caused by the binding reaction**
- Informations on stœchiometry, enthalpy, entropy and dynamic of the bond between the two proteins in interaction
- Low throughput and sensitivity, and requires large sample volumes
- For PPIs using Microcal ITC





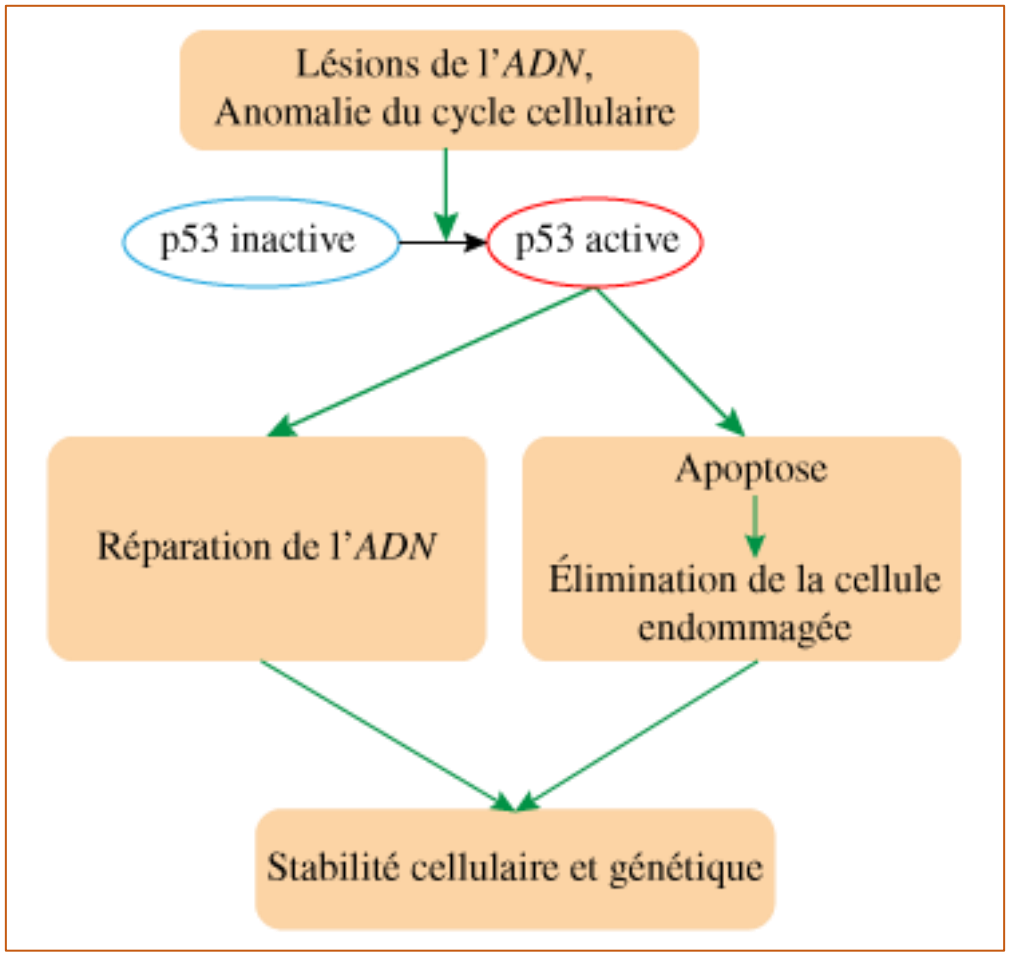


Protein–Protein interactions (PPIs)

- Diseases in PPIs
- Identification of inhibitors of PPI

PPIs in Cancer

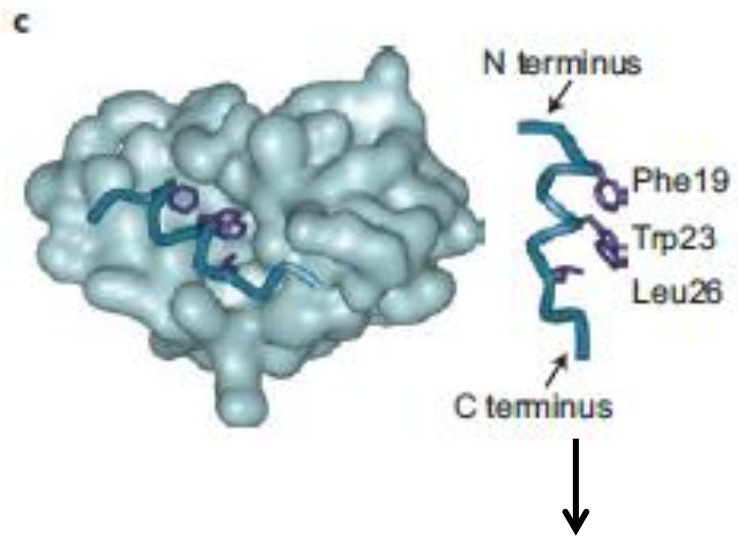
Importance de la protéine p53 pour induire l'apoptose des cellules tumorales



Protein–protein interactions (PPIs) in Cancer

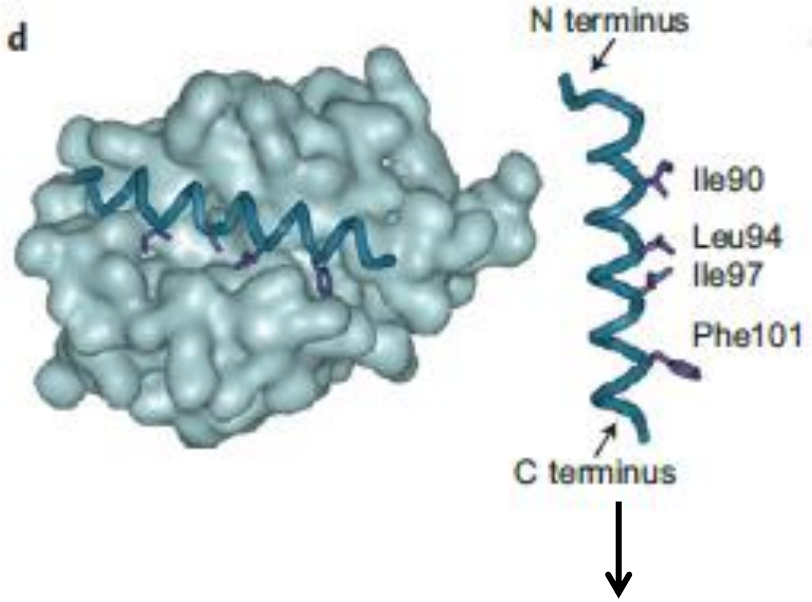
Efficiency of proapoptotic proteins decreases because of deleterious PPIs

hDM2 (atiapoptotic)/P53 (proapototic) interaction facilitate p53 degradation



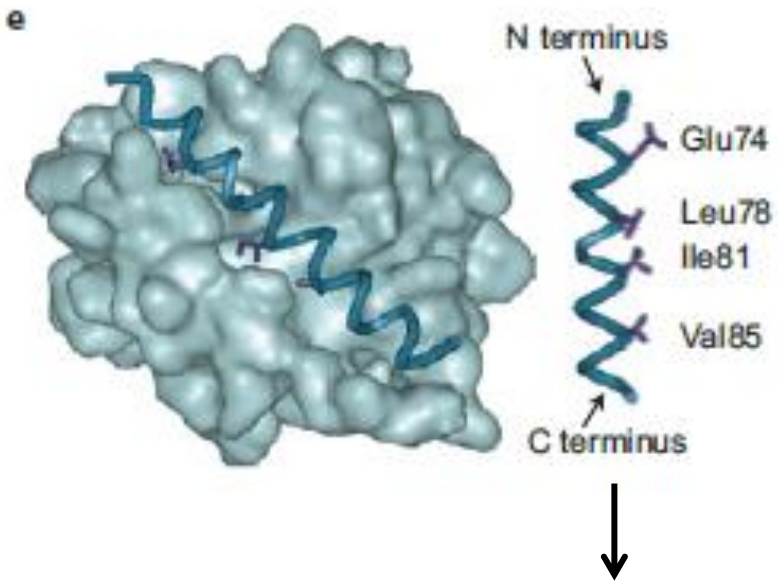
isolated helical segment of p53

Bcl-XL (antiapoptotic)/BIM (proapoptotic) interaction



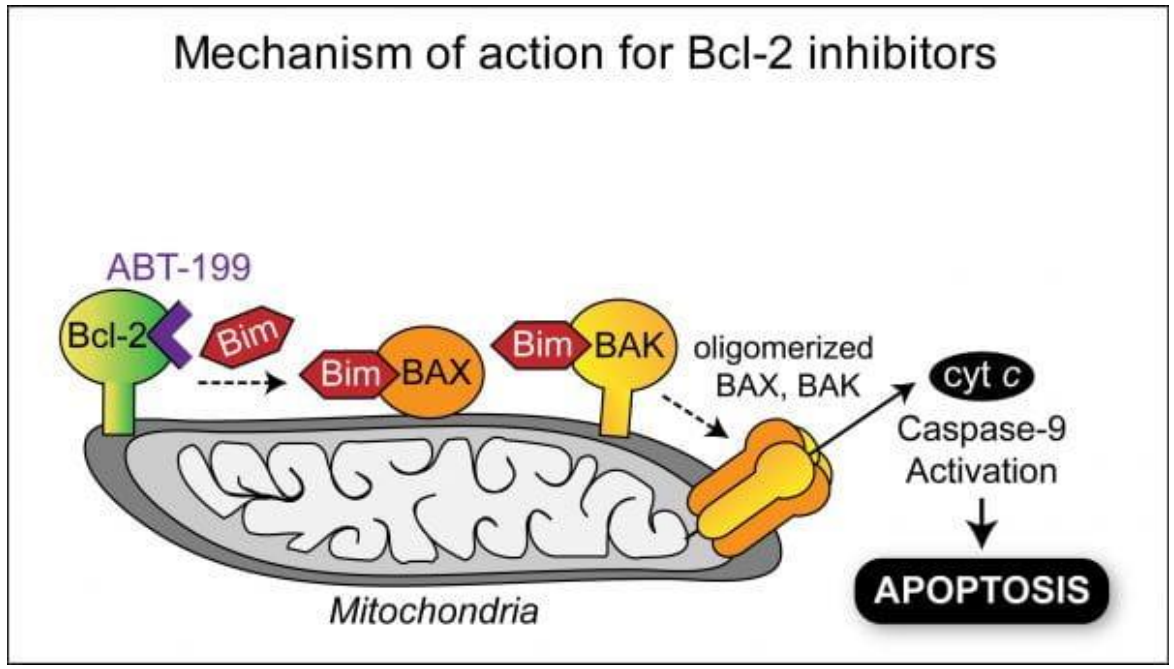
isolated helical segment of BIM

Mcl-1 (antiapoptotic)/NOXA B (proapoptotic) interaction

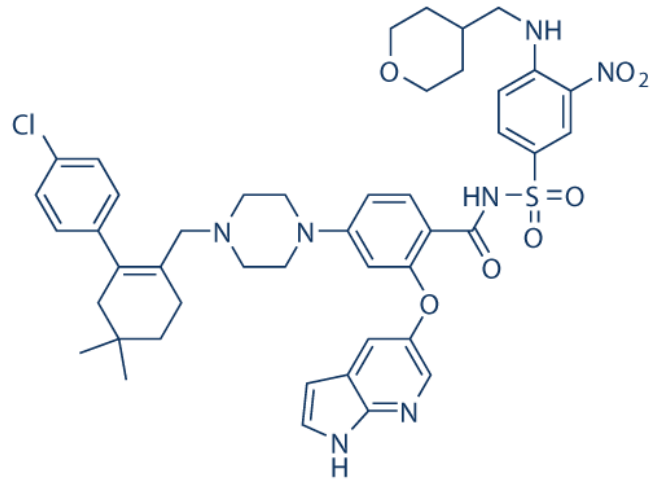


isolated helical segment of NOXA B

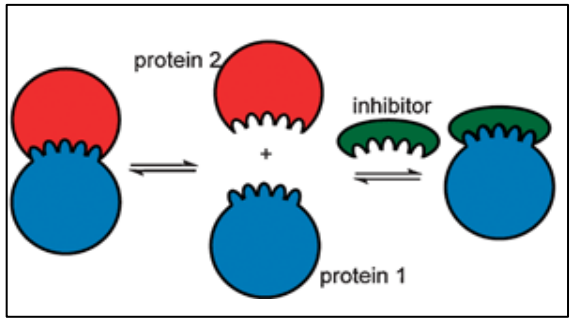
Protein–protein interactions (PPIs) in Cancer

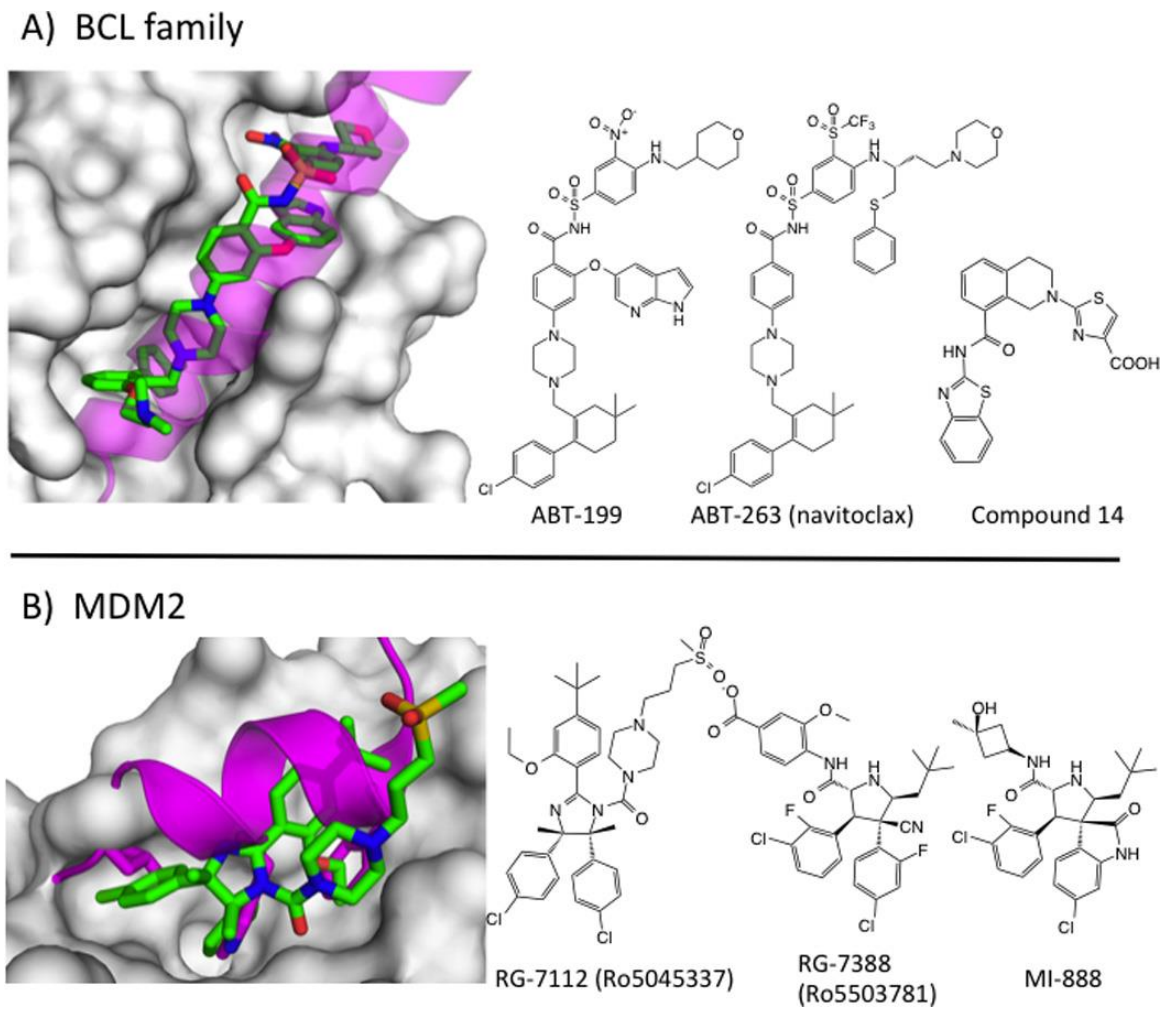


2016, approval of the Bcl-2 inhibitor, Venclexta (ABT-199)
Orally active for treatment of chronic lymphocytic leukemia



Small molecules inhibitors of Protein–protein interactions (PPIs)





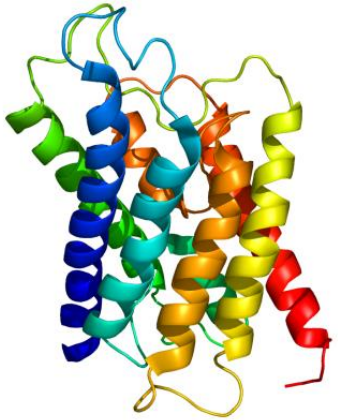
A) Structure of BCL-2 (white surface) bound to ABT-199 (green sticks, PDB: 4MAN) with overlaid BAX BH3 peptide (magenta cartoon, PBD: 2XA0). Chemical structures of selected BCL-2 and BCL-xL inhibitors.

B) Structure of MDM2 (white surface) bound to RG-7112 (green sticks, PDB: 4IPF) with overlaid p53 peptide (magenta cartoon, PDB: 1YCR). Chemical structures of compounds in clinical testing or their closest published analogs.

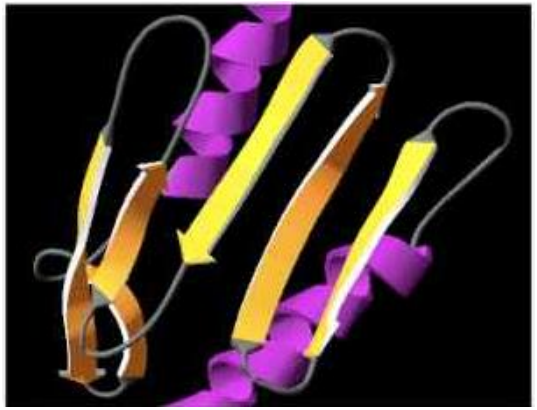
Secondary structures mimics

- **Small protein fragments** are involved in interactions : “hot-spot”
- **Well defined secondary structure**

α-Helices

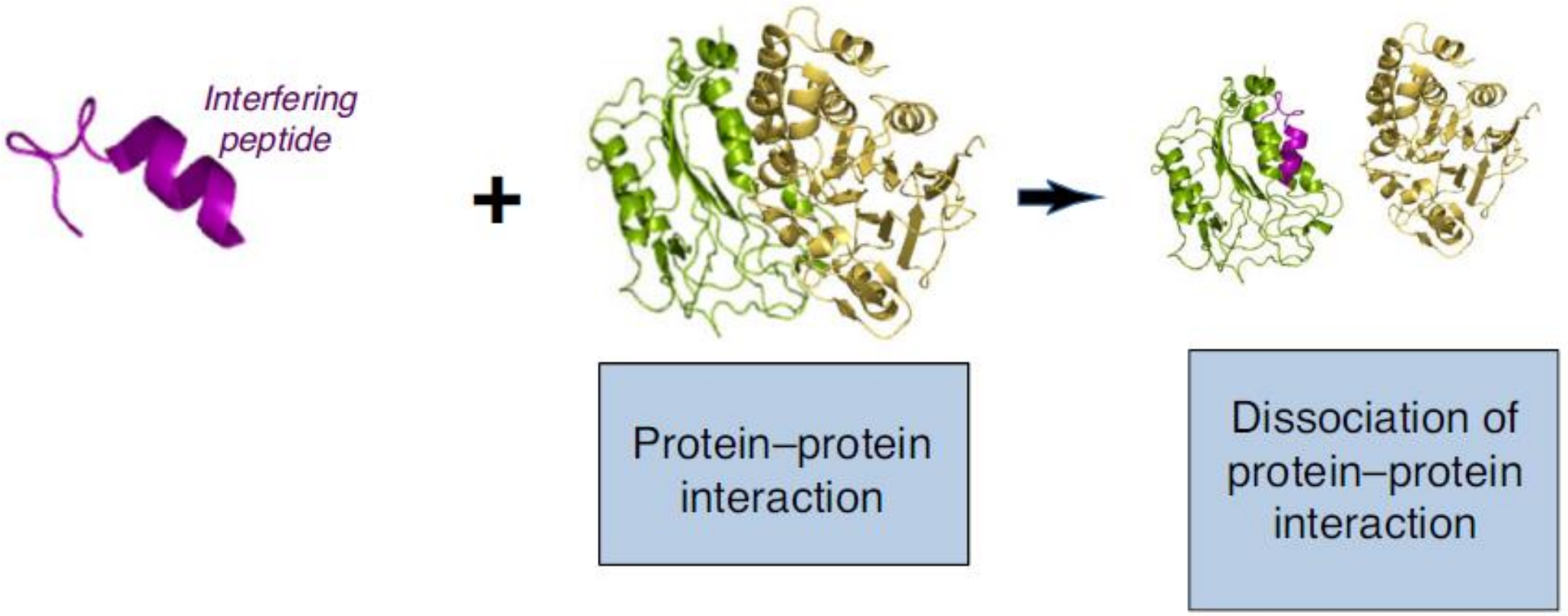


β-Sheet



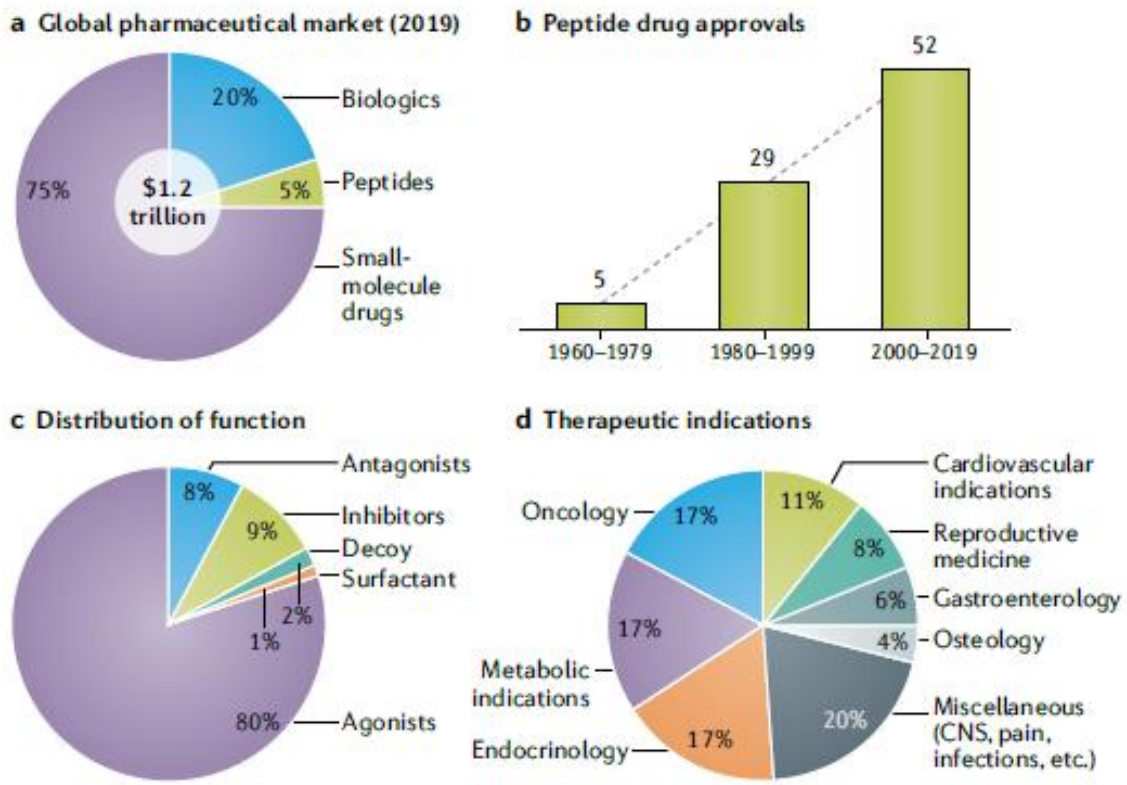
Turn

Interfering Peptides / Secondary structures mimics

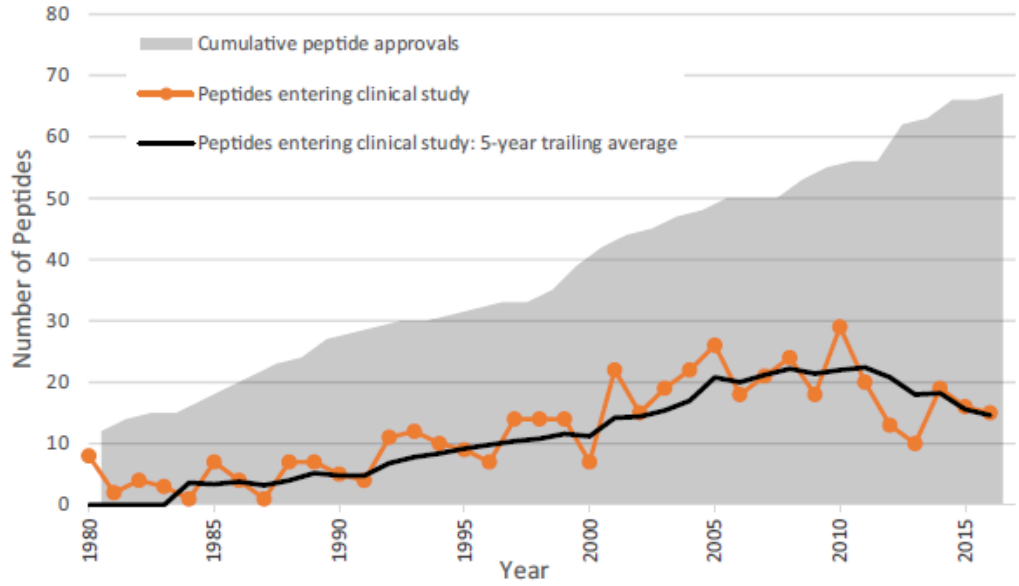


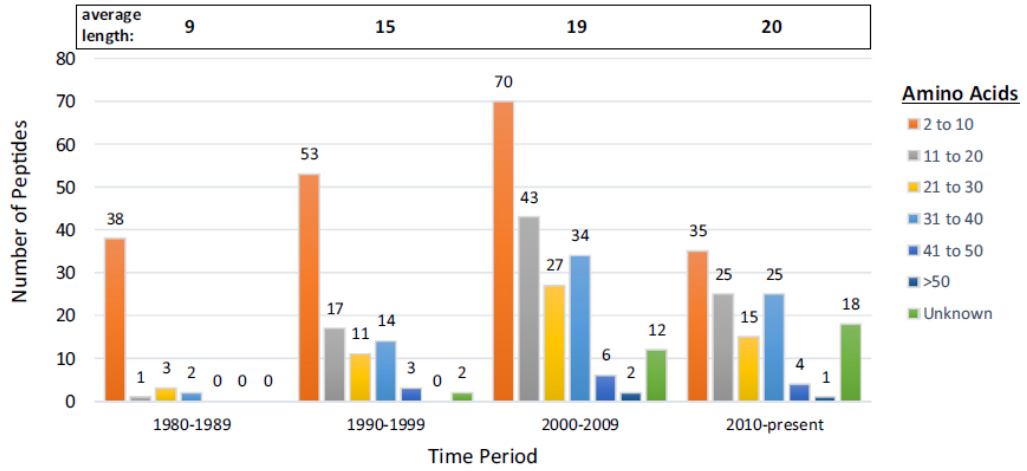
Therapeutic peptides

growing interest in the pharmaceutical industry



Nature Reviews, 2021, *20*, 309





Biorg. Med. Chem. 2017, *17*, 31022

Peptides Thérapeutiques

Avantages:

- **Puissance supérieure**; médicaments peptidiques sont généralement très actifs sur leur récepteur cible, ont un effet élevé à faibles doses

- **Sélectivité supérieure**; peptides ont un ajustement très serré à leurs récepteurs, beaucoup plus sélectifs que les petites molécules. Les peptides ont tendance à se lier uniquement à leur récepteur cible. Effets secondaires moins importants

- Sont produits biologiques d'origine naturelle avec **une meilleure sécurité**: Les peptides sont naturellement dégradés dans le sang par des enzymes en leurs acides aminés constitutifs. Moins accumulation dans le tissu corporel et moins de toxicité (également en raison de leurs faibles doses)

- **Impact environnemental**

Inconvénients :

- **Courte durée**: Beaucoup de peptides naturels ont une demi-vie très courte, 2-30 minutes. Pour une utilisation thérapeutique, peptides natifs nécessiteraient une administration constante.

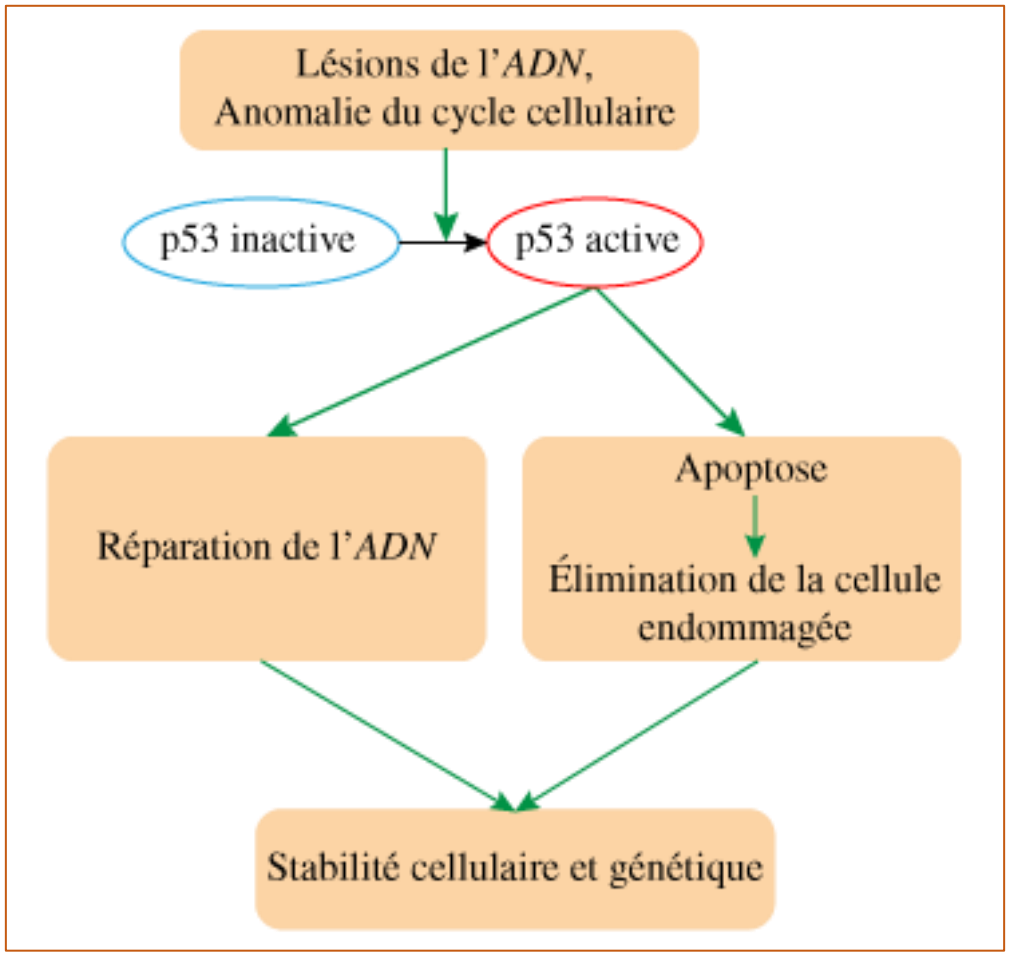
- **Ne peuvent pas être administrés par voie orale**: La plupart des peptides doivent être administrés par injection, car dégradation par le système digestif

- **N'adopte pas (ou peu) de structure secondaire**

Drug discovery today, 2015, 20, 122; Frontiers in Chemistry , 2014, 2, Article,62, 1; Nature Reviews, 2021, 20, 309

PPIs in Cancer

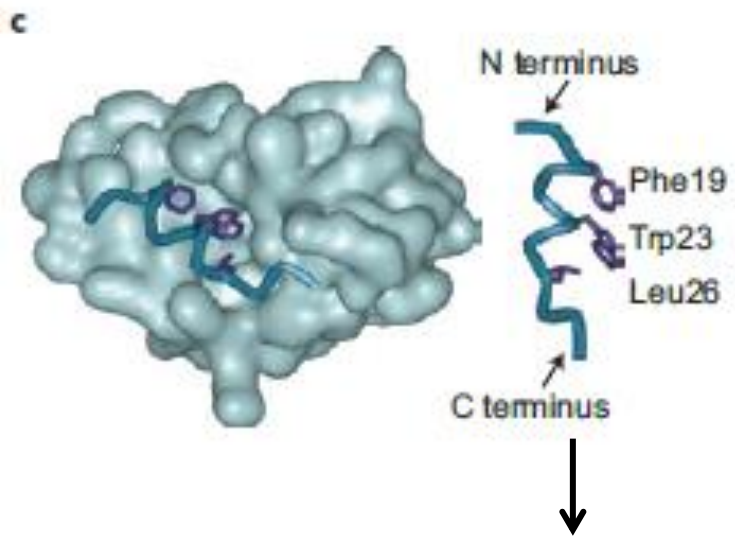
Importance de la protéine p53 pour induire l'apoptose des cellules tumorales



PPIs in Cancer

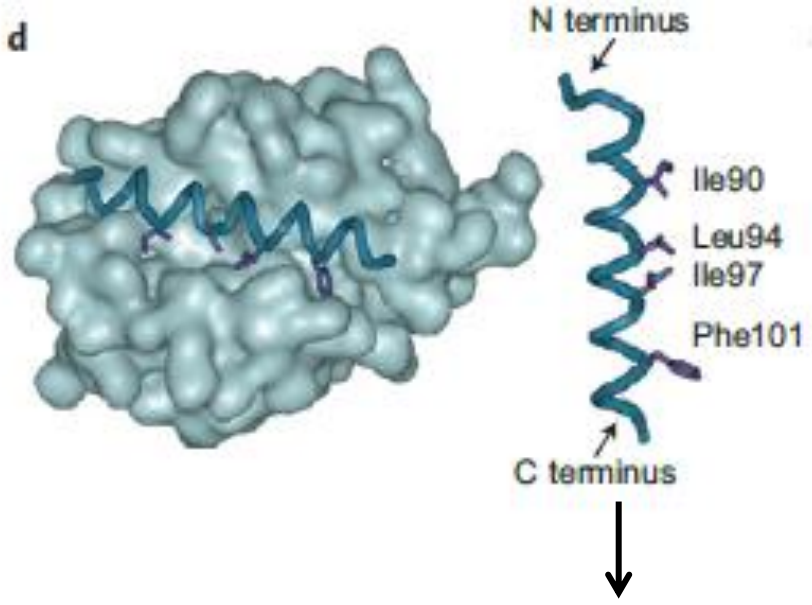
Release proapoptotic proteins to treat cancer

hDM2/P53 (proapototic) interaction facilitate p53 degradation



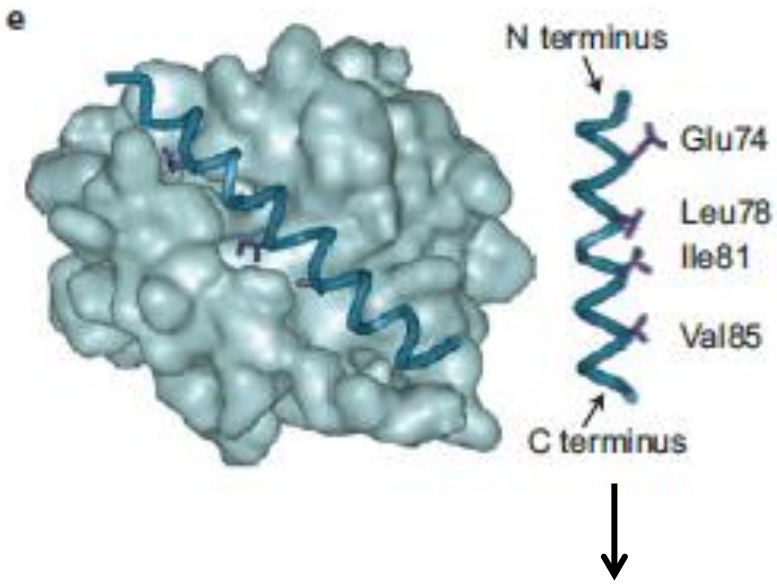
isolated helical segment of p53

Bcl-XL (antiapoptotic)/BIM (proapoptotic) interaction



isolated helical segment of BIM

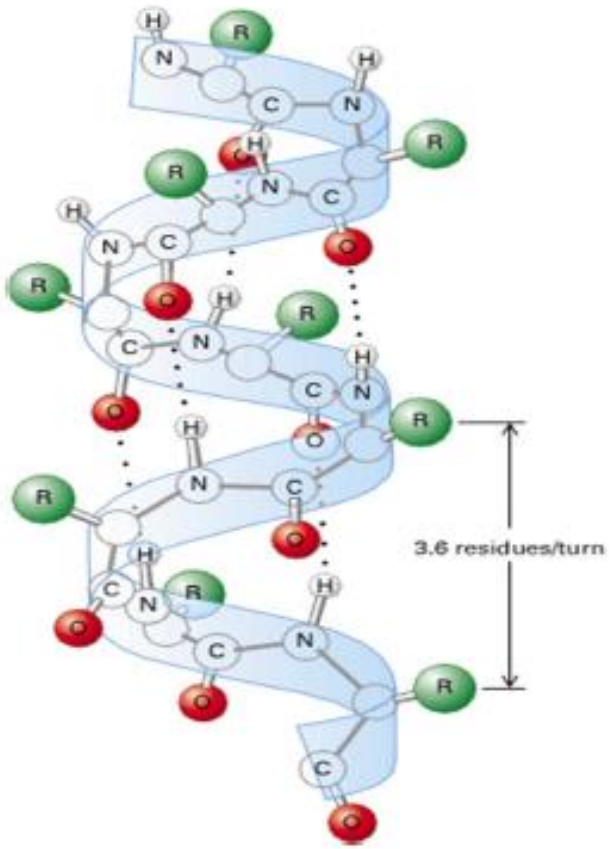
Mcl-1 (antiapoptotic)/NOXA B (proapoptotic) interaction

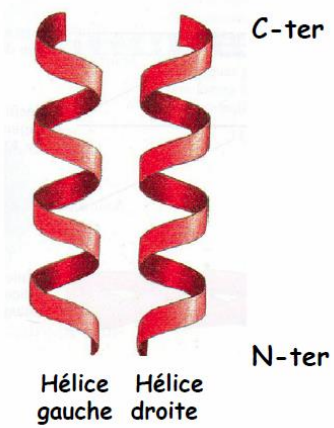


isolated helical segment of NOXA B

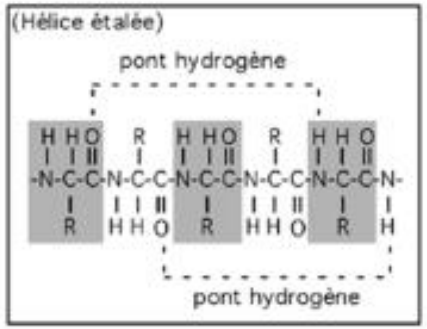
Design of stapled peptide to mimic alpha helix

Hélice alpha





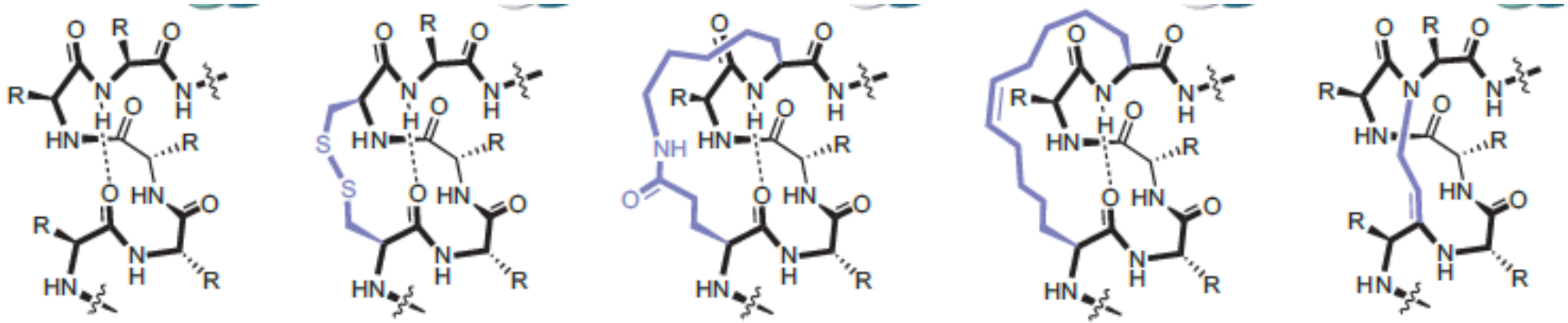
LH entre CO_i et NH_{i+4}



STAPLED peptide : lien covalent entre résidus i et i+4 ou i et i+7

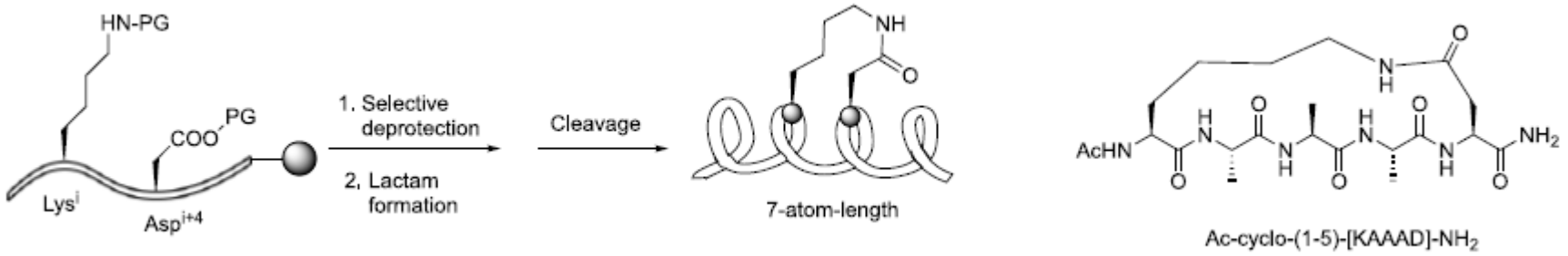
Helix mimics

Covalent helix stabilization of small peptides : Stapled Helix



α-helix | Cys–Cys disulfide bridge α-helix | Glu–Lys lactam bridge α-helix | Hydrocarbon-stapled α-helix | HBS α-helix

- Classical Lys/Asp Lactam stapling

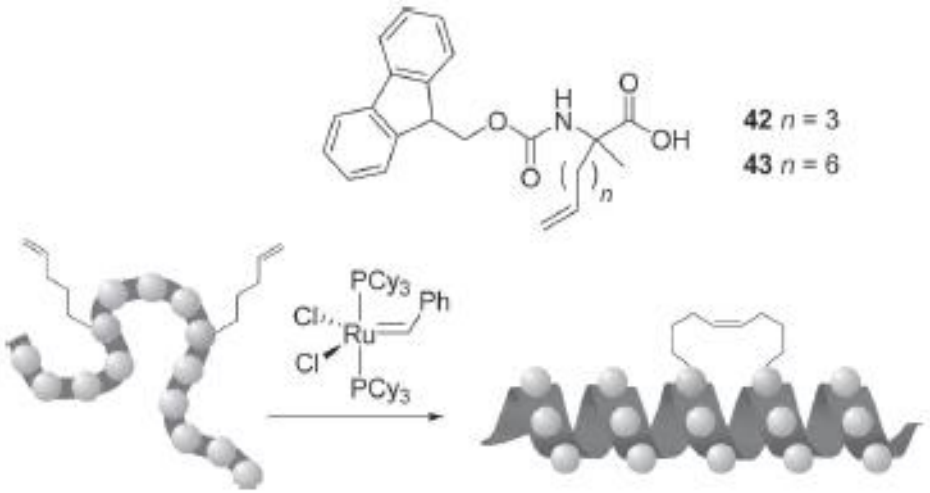


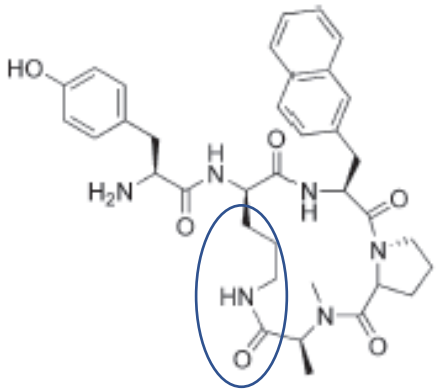
Ac-cyclo-(1-5)-[KAAAD]-NH$_2$

Cyclization

Backbone to side-chain

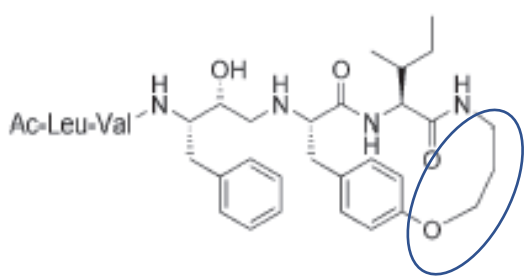
Covalent helix stabilization of small peptides



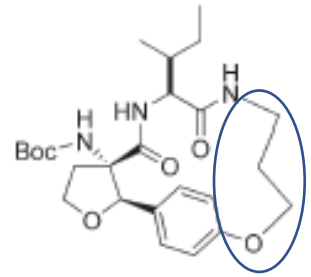


44

Backbone to side-chain



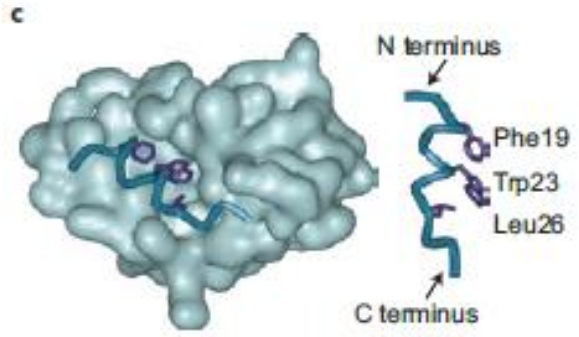
45



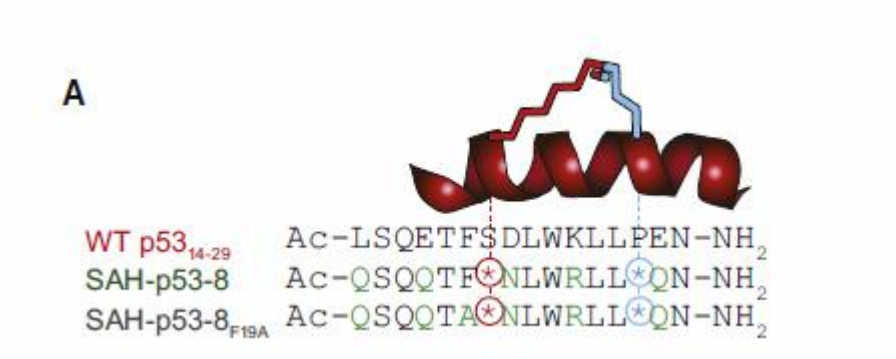
46

Helix mimics of p53 inhibitor of p53/hDM2 interaction

P53 (proapototic)/hDM2 (facilitate p53 degradation)

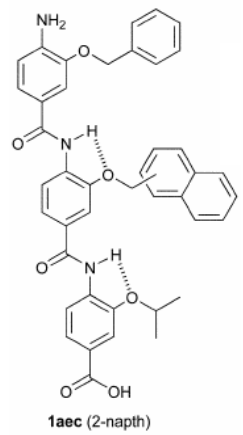


Stapled helix inhibitor



Cancer Cell , 2010,18, 411–422

Oligoamide inhibitor



1aec (2-napth)

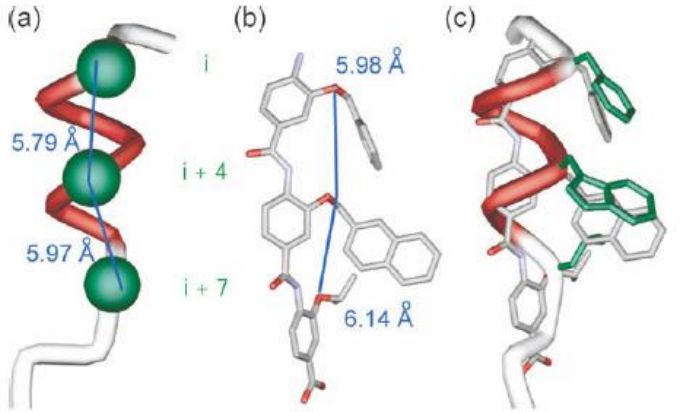


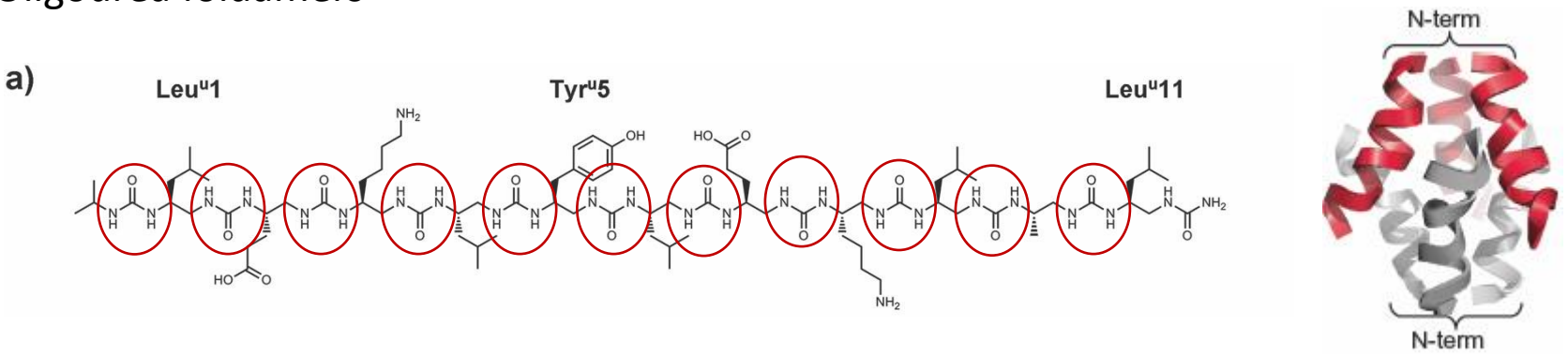
Fig. 2 (a) p53 helix depicting key side chains (in green); (b) minimised structure of an aromatic oligoamide with R^3 = Bn, R^2 = Me-2-Napth and R^3 = ^{i}Pr (carbon in grey, oxygen in red and nitrogen in light purple); (c) p53 α-helix superimposed onto minimised aromatic oligoamide.

Chem. Commun., 2009, 5091-5093

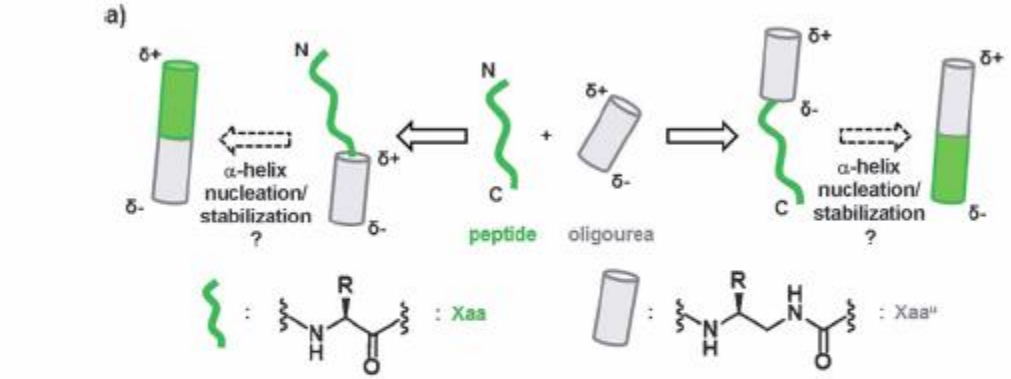
PDB : 3V3B
Structure of the Stapled p53 Peptide Bound to Mdm2

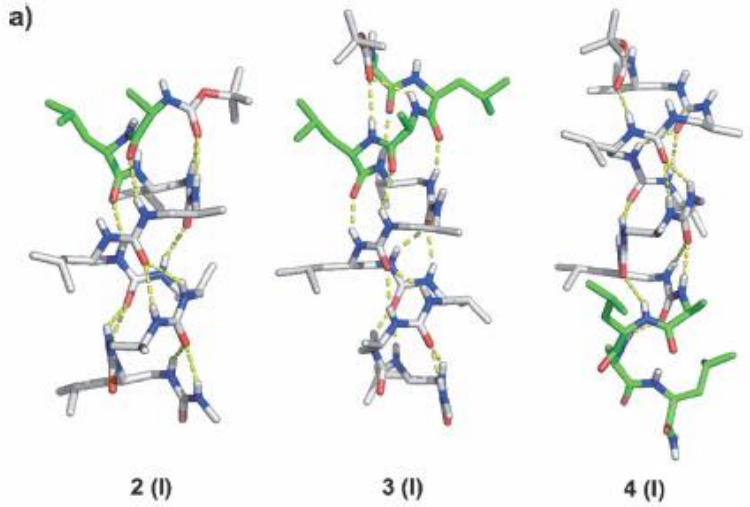
Helix peptidomimetics

Oligourea foldamers



Self-assembly of Amphiphilic Non-peptide Helical Foldamers
Nature Chemistry , 2015, **7**, 871–878

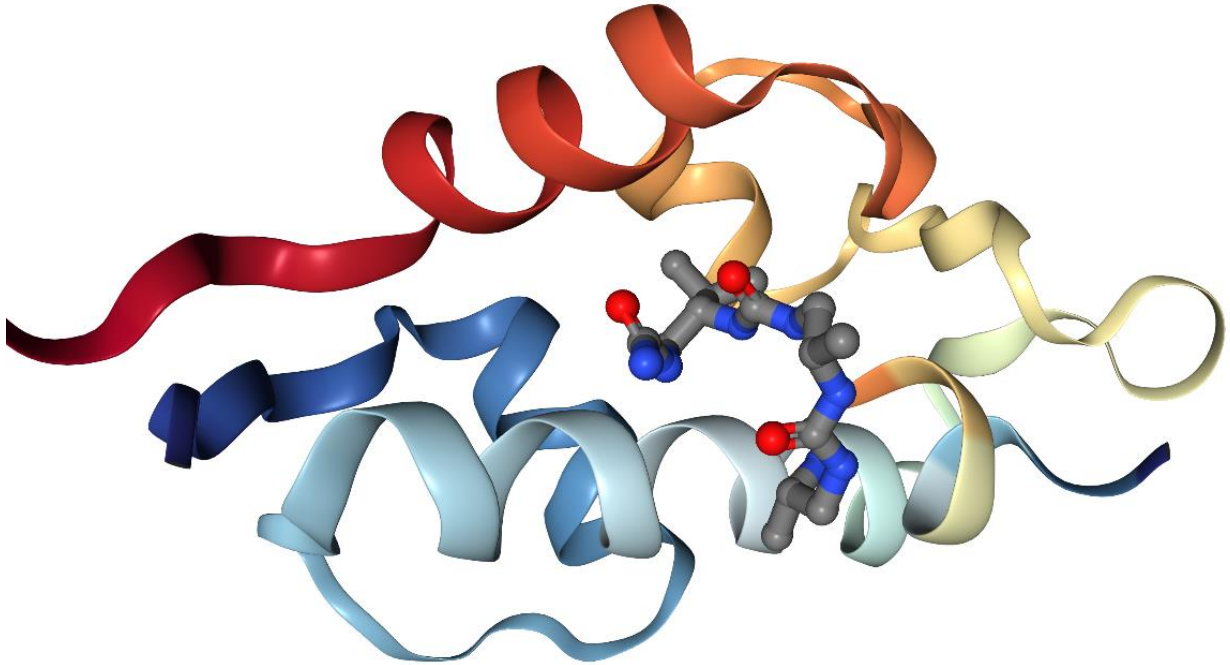




Angew. Chem. Int. Ed. 2015, 54, 9816 –9820

Helix peptidomimetics

Oligourea foldamers

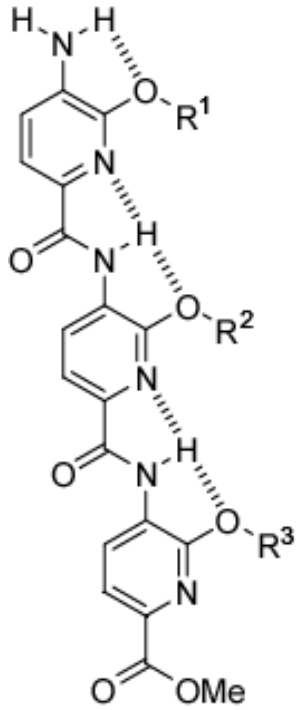


6HFA

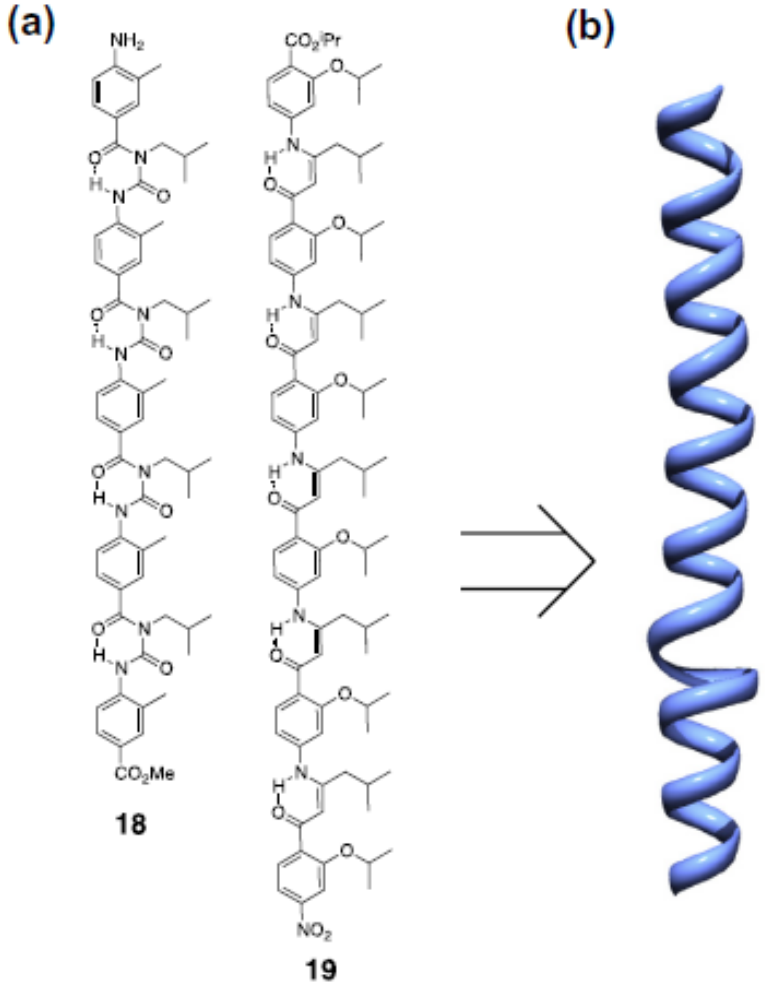
Crystal structure of hDM2 in complex with a C-terminal triourea capped peptide chimera foldamer.

Aromatic Foldamers as Helix mimics

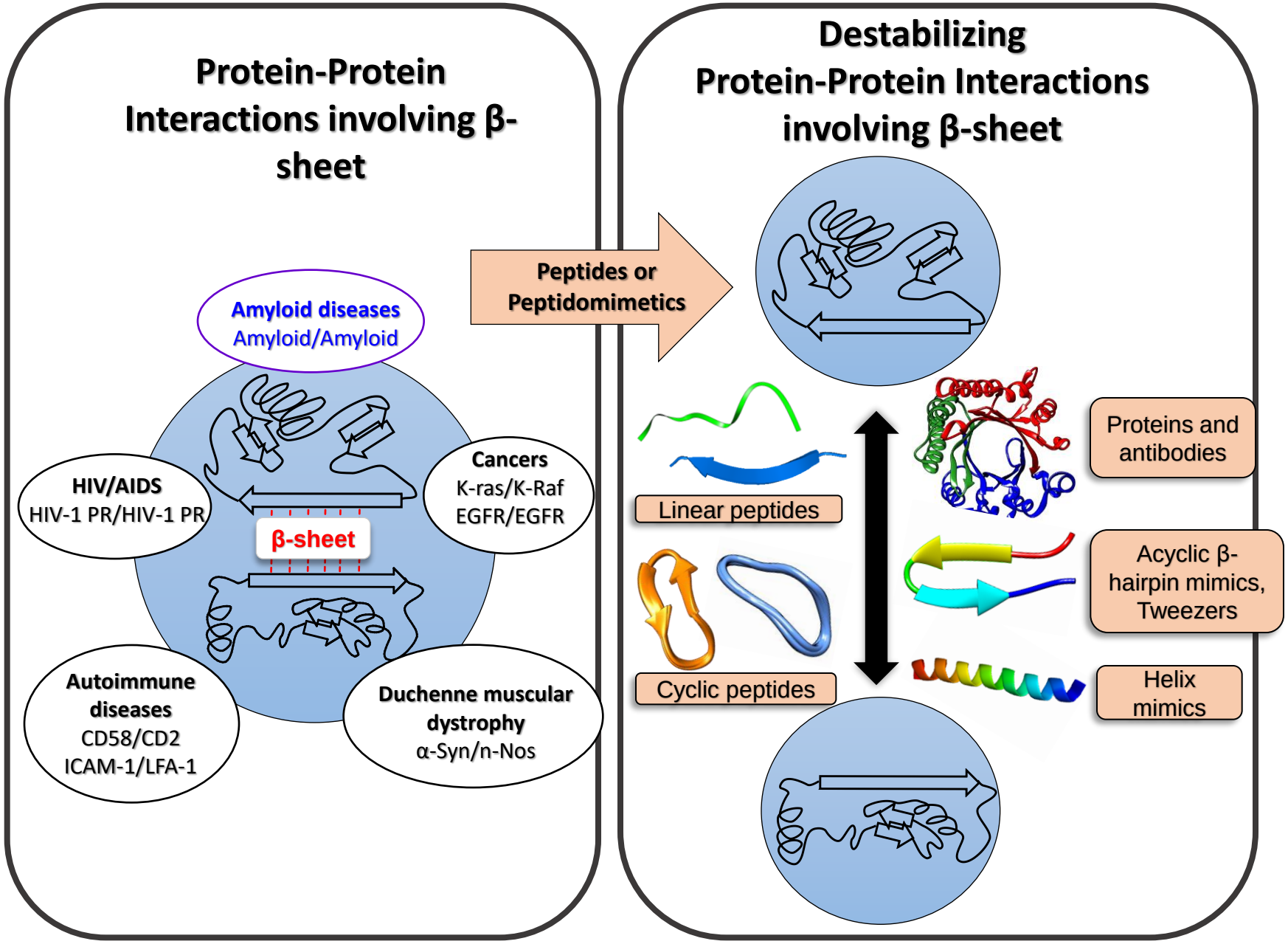
Foldamer : any oligomer with a propensity to fold into a well-defined three-dimensional structure in a process guided by intramolecular hydrogen bonds or electrostatic interactions (Gellman, S. H. Acc. Chem. Res., **1998**, 31, 173)



Pyridyl-carboxamide α-helix mimetic



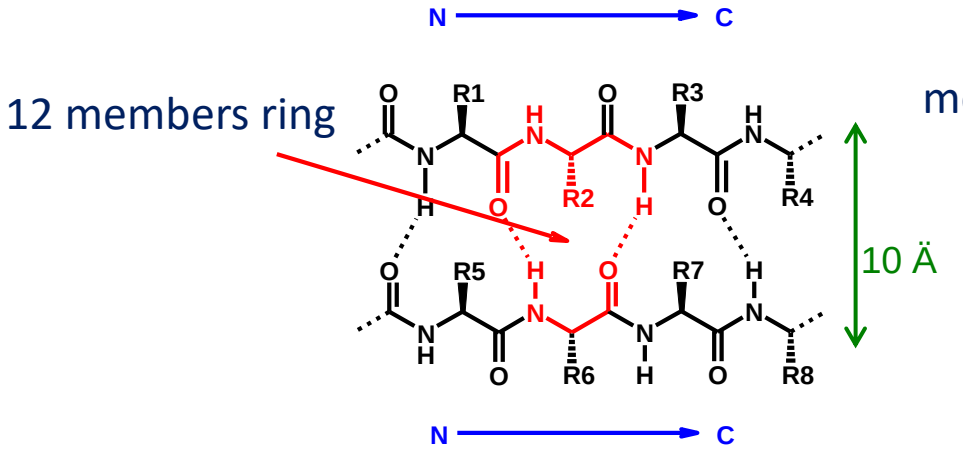
Benzoylurea and enaminone α-helix mimetic



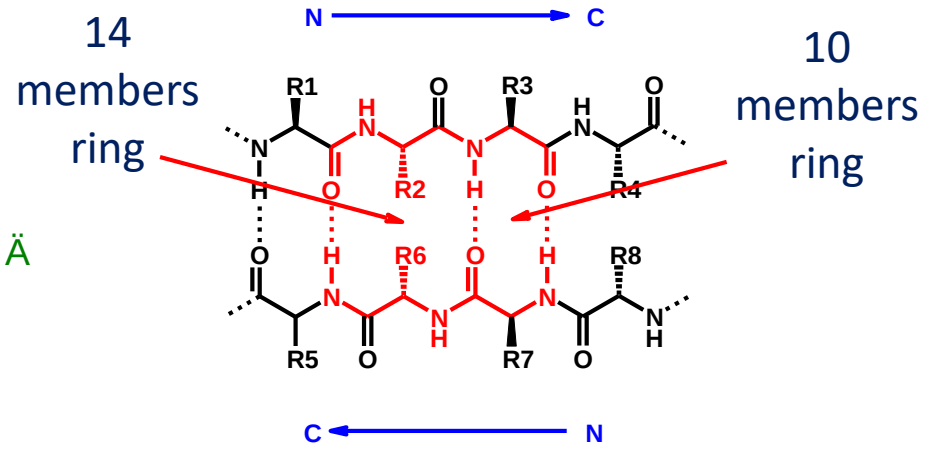
J. Laxio Arenas, J. Kaffy, S. Ongeri **2019**, *Curr. Opin. Chem. Biol. 52,* 157-167

β-strands/ β-sheets mimics

β-Sheet : Hydrogen bonds + Hydrophobic interactions

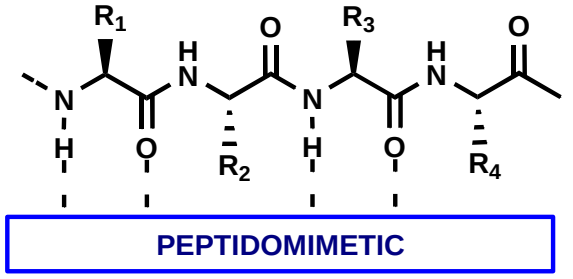


Parallel β-sheet



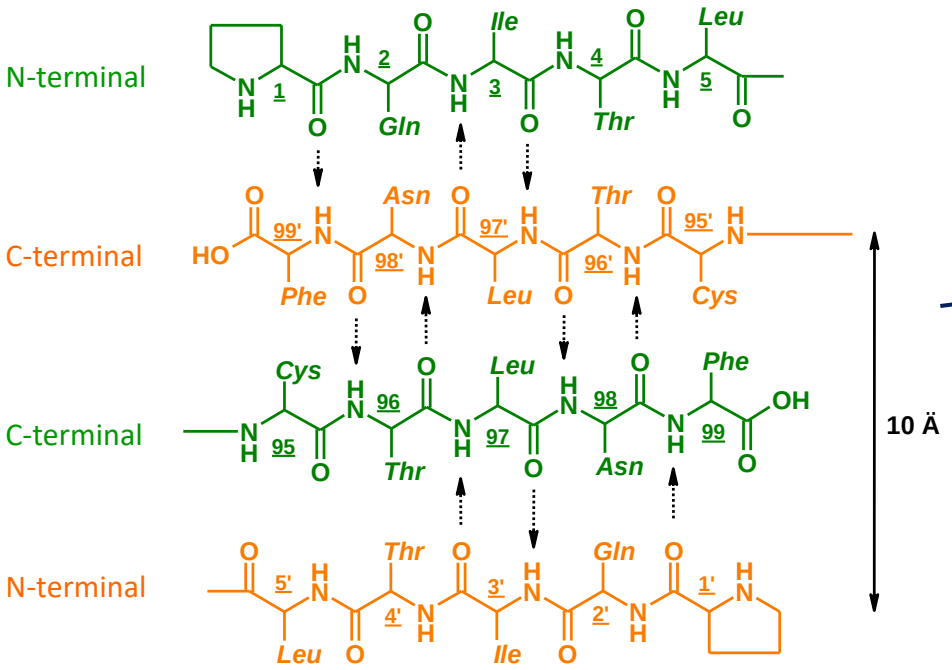
Antiparallel β-sheet

Design peptidomimics of β-strands and β-sheets to modulate protein-protein interactions

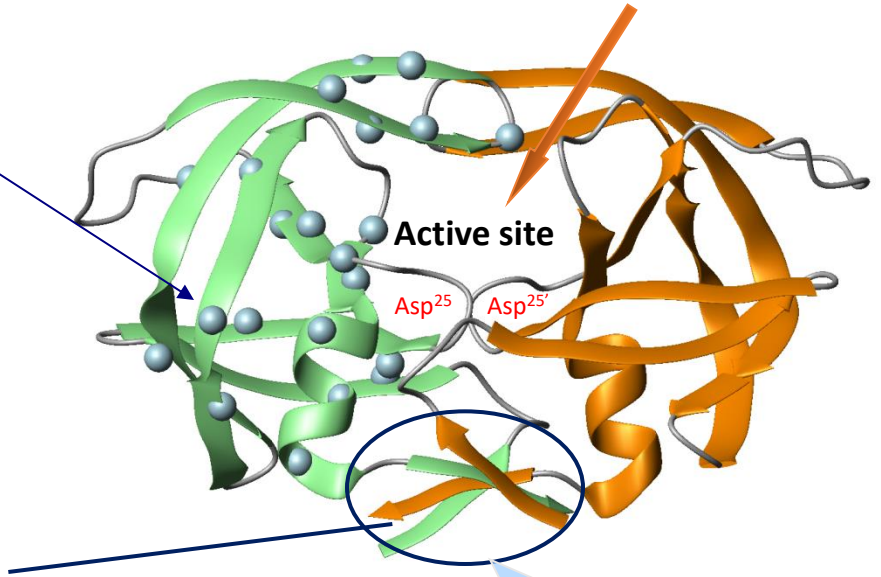


Structure of the HIV-1 PROTEASE

PR is active only in its dimeric form.



Major mutations

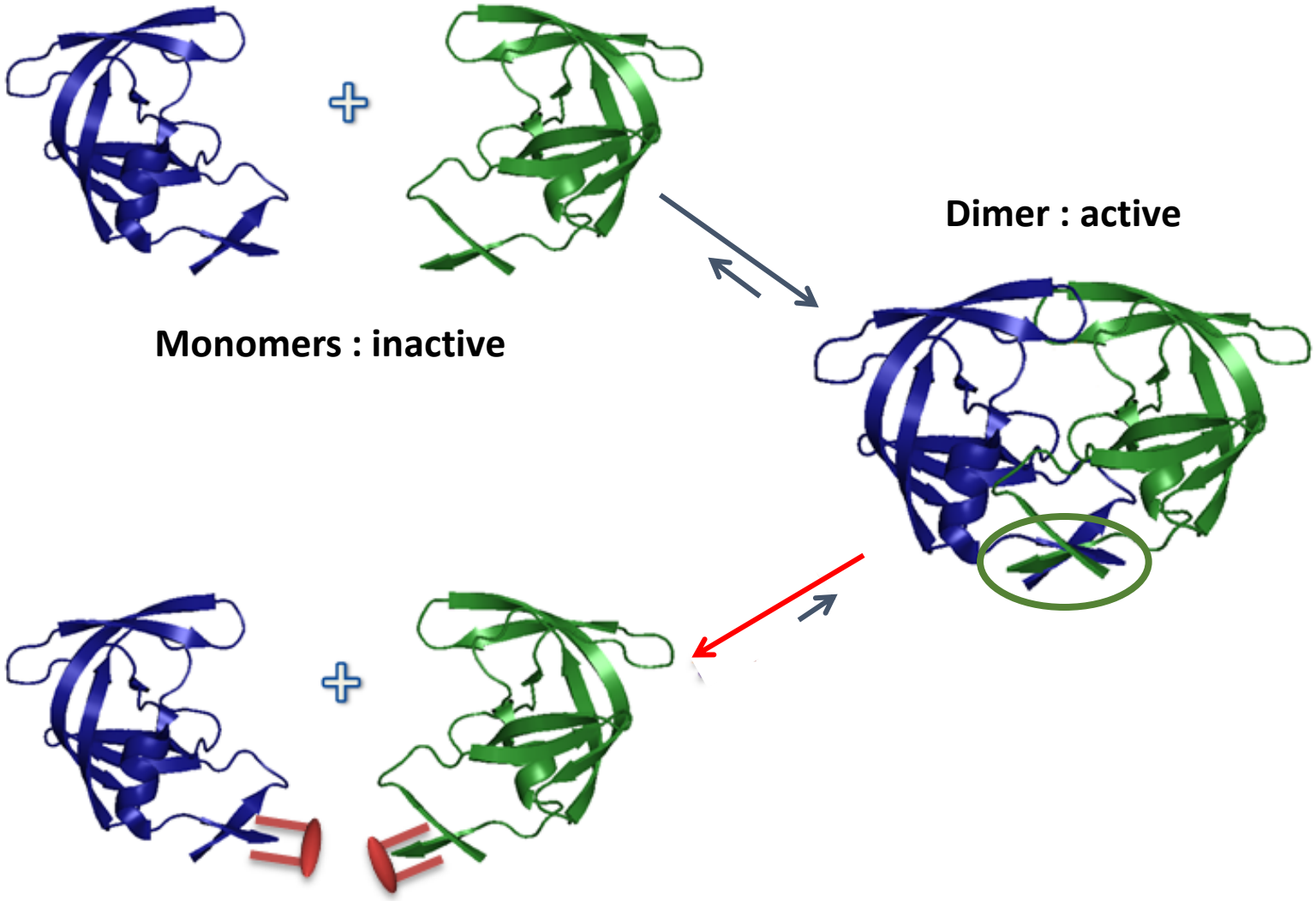


Therapeutic antiproteases PIs

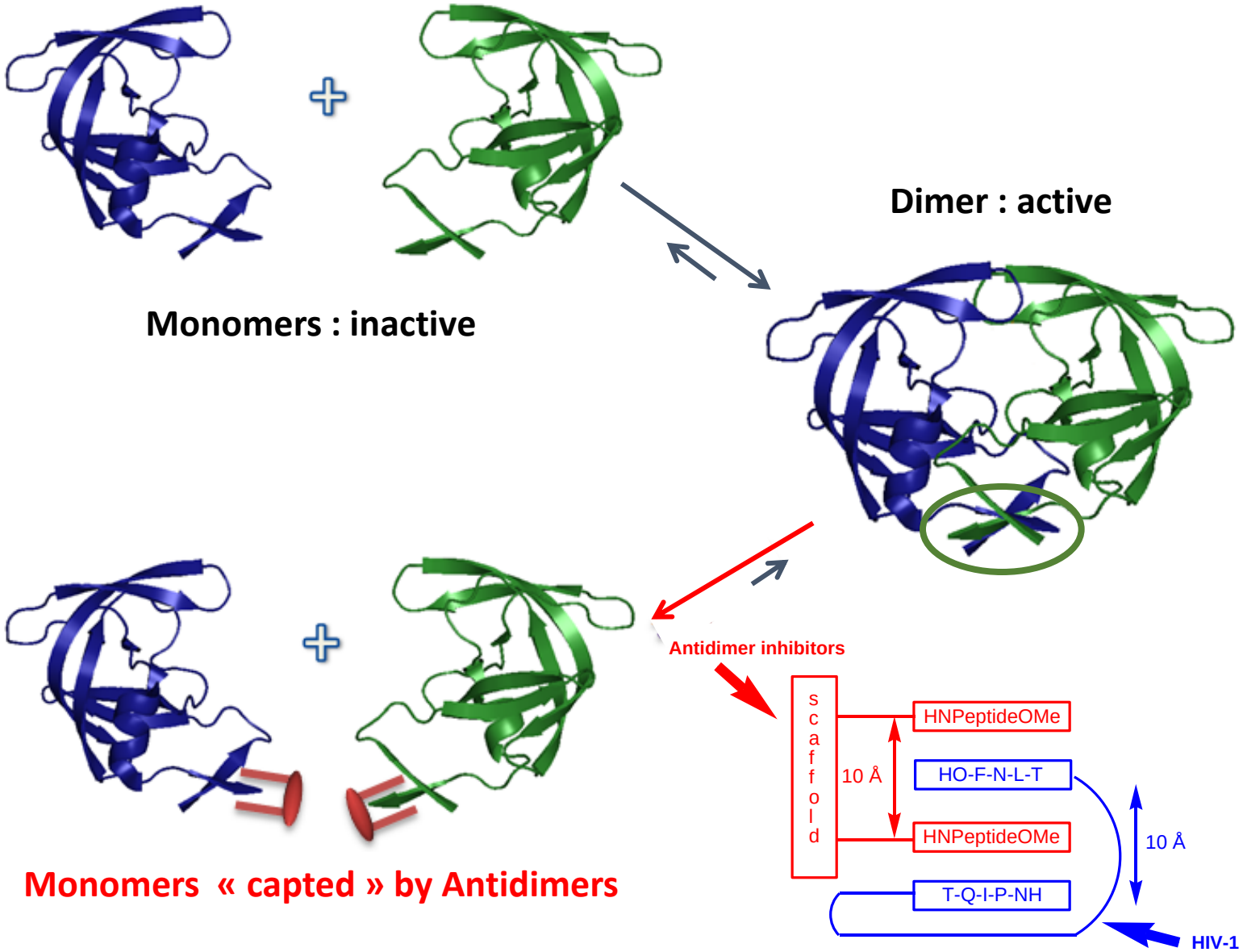
Dimerization inhibitors target the antiparallel β-sheet

This antiparallel β-sheet provides 75% of the dimerization stabilization energy.

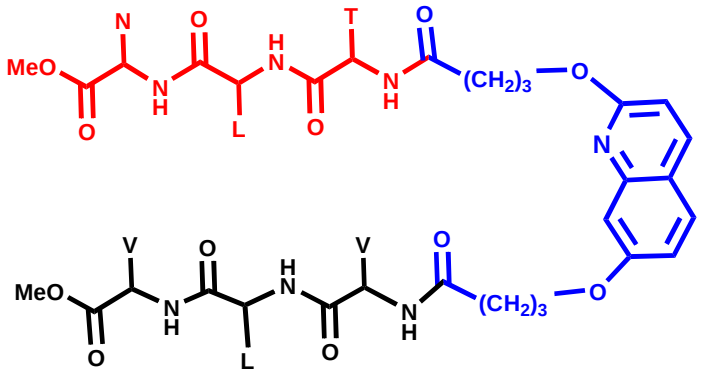
DIMERIZATION INHIBITORS OF HIV-1 PROTEASE



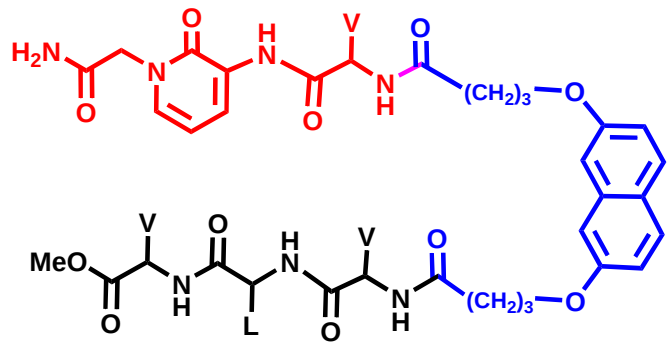
DIMERIZATION INHIBITORS OF HIV-1 PROTEASE



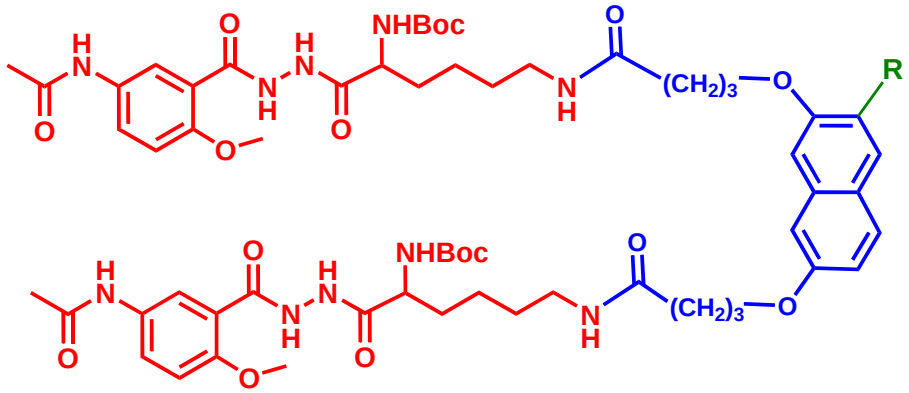
Molecular tongs : DIMERIZATION INHIBITORS OF HIV-1 PROTEASE



J. Med. Chem. **2004**, *47*, 6392

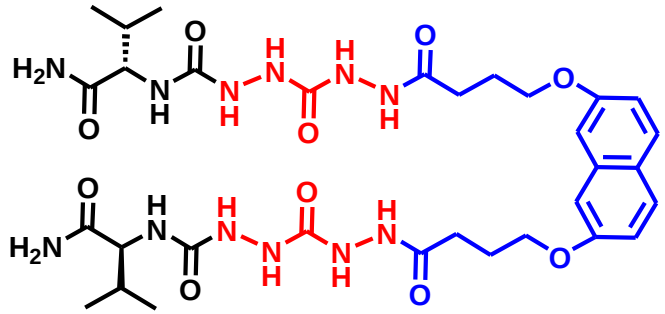


J. Med. Chem. **2006**, 49, 4657



R = H *ChemMedChem,* **2010**, *1899*

R = Polar group *Medchemcom,* ***2014***, DOI: 10.1039/C4MD00032C



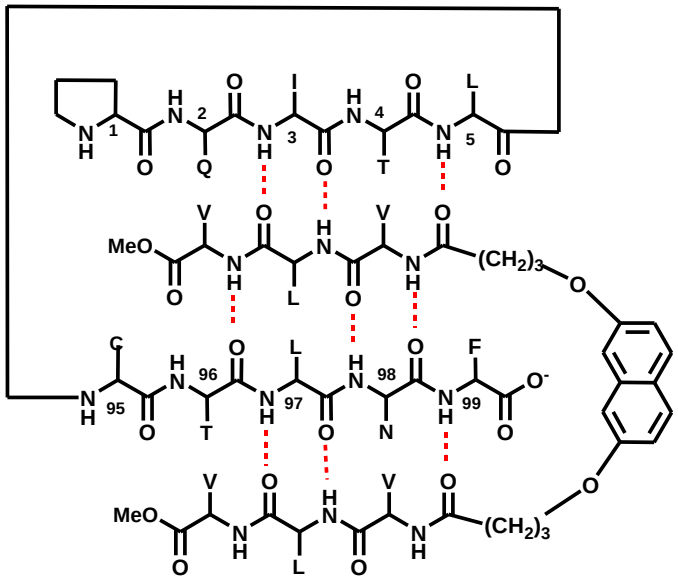
Solubility in water : 0.3 mg/ml

J. Med. Chem. **2012**, 55, 6762

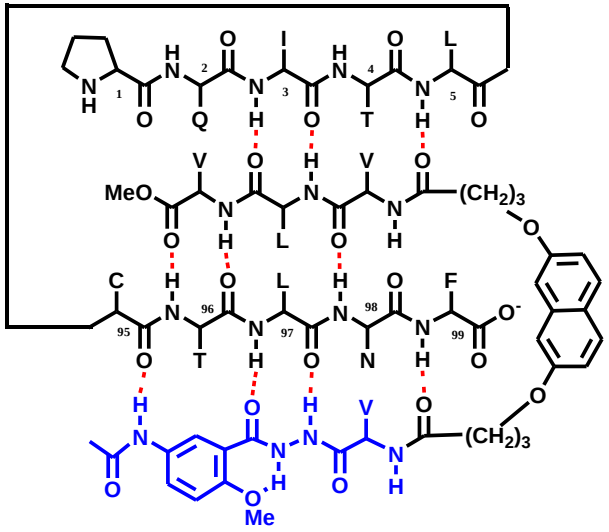
- ✓ K_{iD} up to 50 nM
- ✓ Efficient on mutated HIV-1 proteases

Molecular tongs /HIV-1 PROTEASE : Design

HIV-1 PR monomer

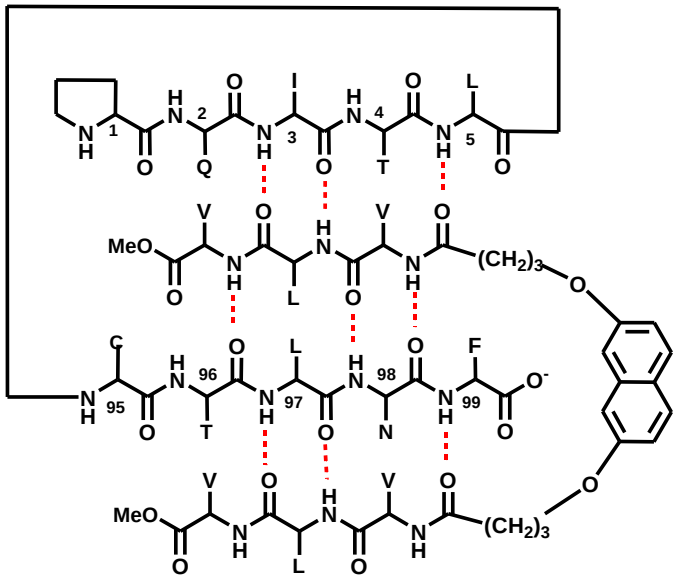


HIV-1 PR monomer

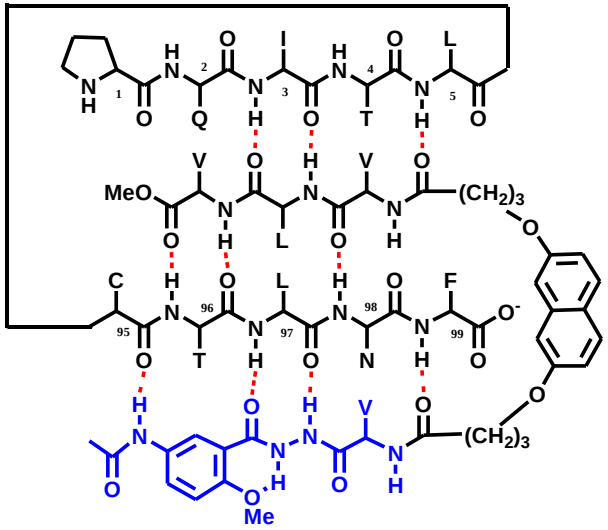


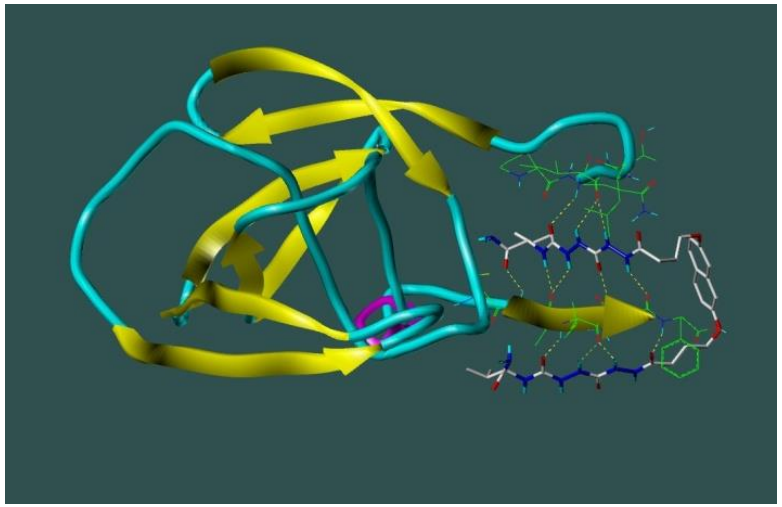
Molecular tongs /HIV-1 PROTEASE : Design

HIV-1 PR monomer

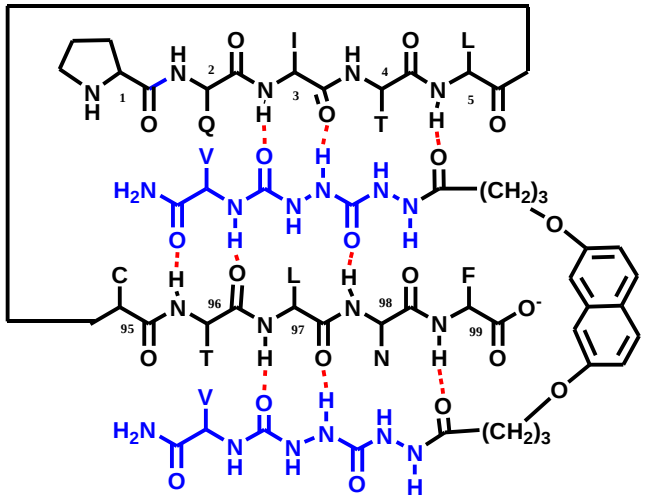


HIV-1 PR monomer



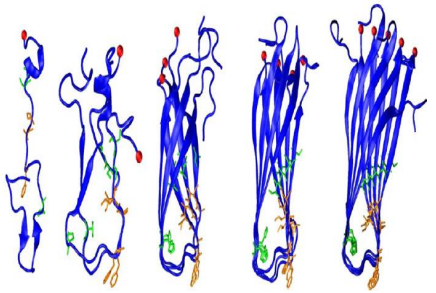


HIV-1 PR monomer



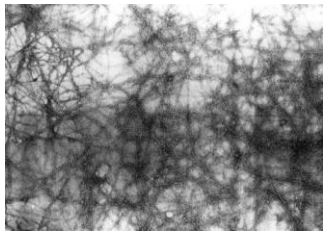
Protein misfolding and Amyloid protein aggregation

- **Misfolded structures alter protein proper configuration : β-sheet rich**



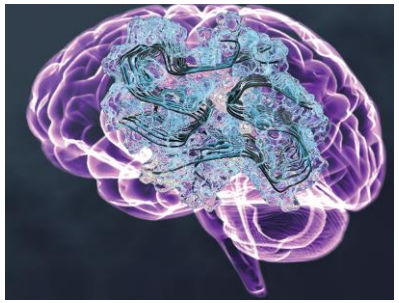
- **Erroneous protein-protein interactions – aggregation**

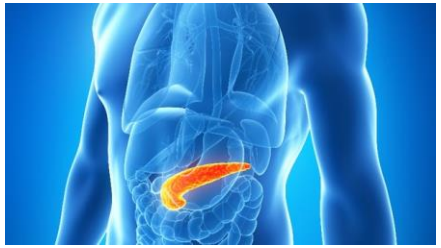
- Abnormal accumulation of amyloid fibrils in organs can lead to **amyloidosis**



- Hallmarks **of more than 20 serious human diseases**,

Alzheimer's disease (Aβ peptide, tau protein), **Diabetes mellitus type 2 (IAPP)**, Prion disease (PrPres), Parkinson's disease (synuclein), amyloid cardiopathies….





a) Ke, P. C. et al. *Chem. Soc. Rev.* **2017**, *46*, 6492; b) Eisele, Y. S. et al. *Nat. Rev.* **2015**, *14*, 759; c) McDade, E. et al. *Nature* **2017**, *457*, 153.

Amyloid proteins aggregation linked to 20 serious diseases called amyloidosis

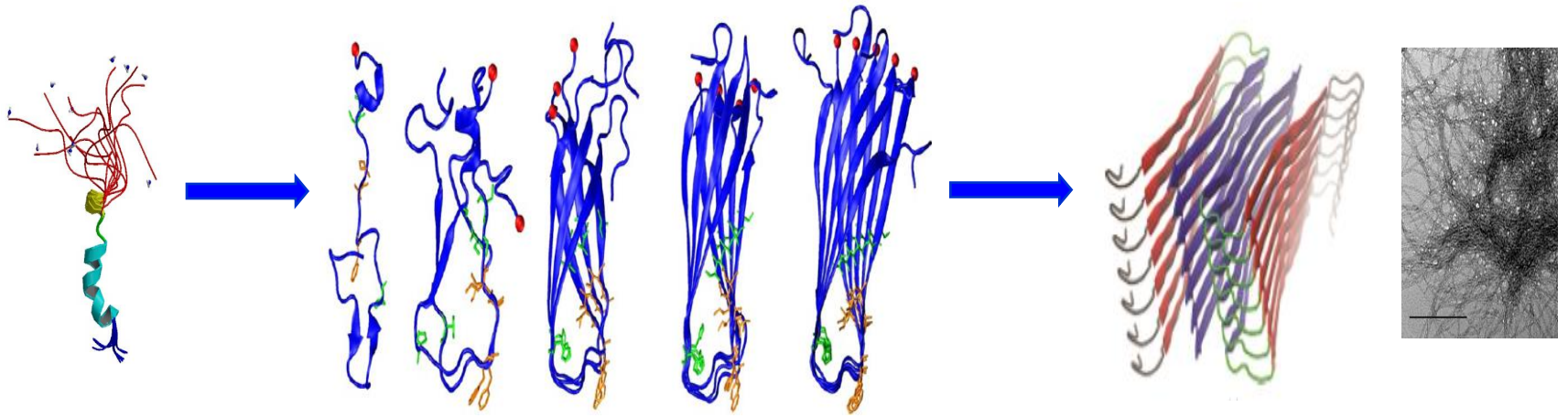
$A\beta_{1-42}$ in Alzheimer's disease / hIAPP in type 2 Diabetes

Aggregation process

Monomers
Non toxic

Oligomers
- Highly toxic
- Target membrane neurones/β-cells
- Disturb ionic homeostasis/RE stress…

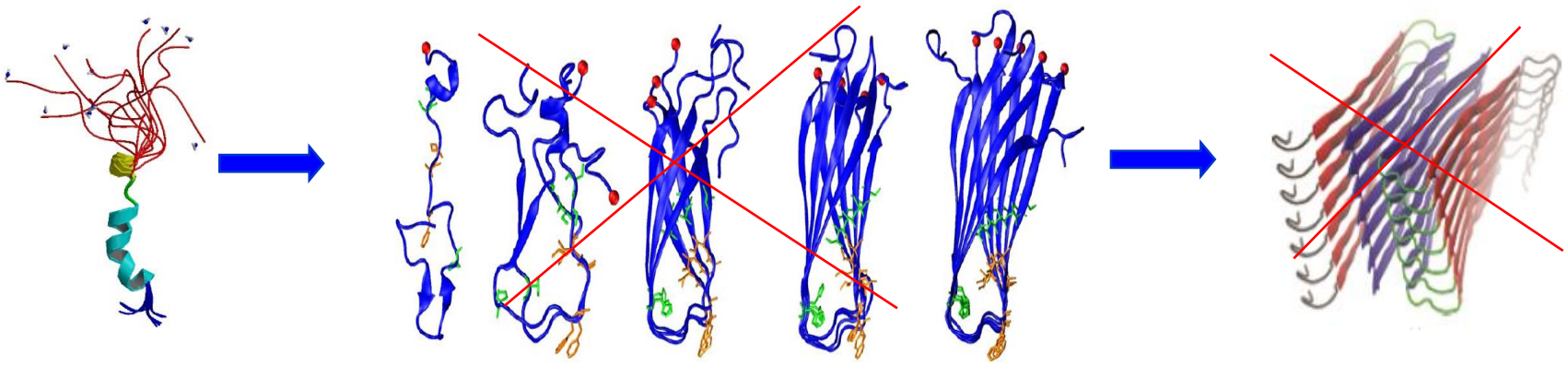
Fibrils
- ROS production
- Promote nucleation of toxic oligomers



From α-helix to…….. β-sheet rich aggregates

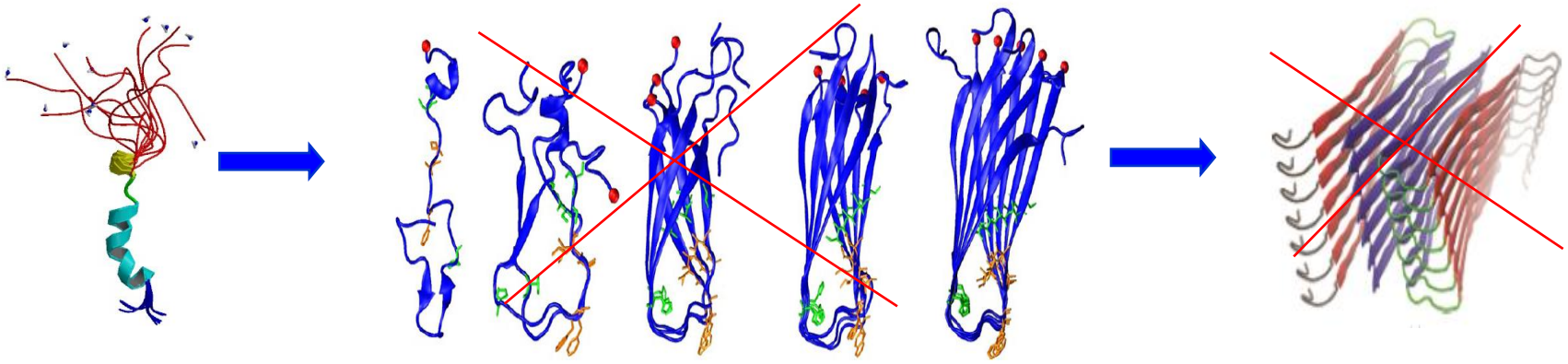
Preserving the non toxic hIAPP/Aβ1-42 monomer
Preventing oligomerization and fibrillization

From α-helix to…….β-sheet

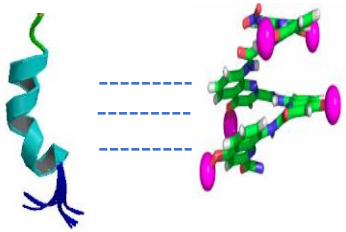


Preserving the non toxic hIAPP/Aβ1-42 monomer
Preventing oligomerization and fibrillization

From α-helix to…….β-sheet

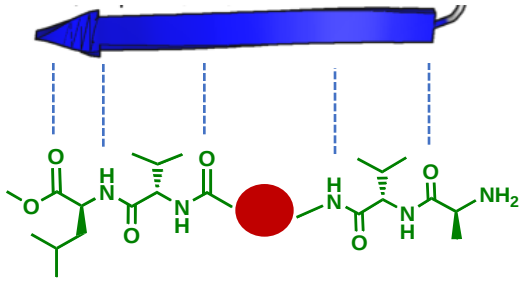


❑ **mimics of helix**



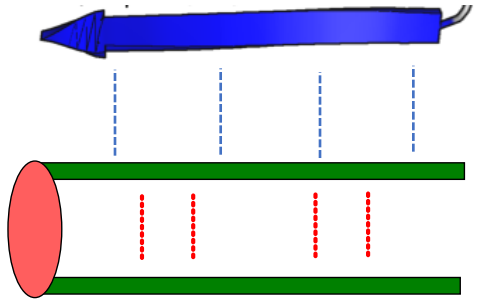
stabilizing monomer helical structure

❑ **β-sheet breakers**



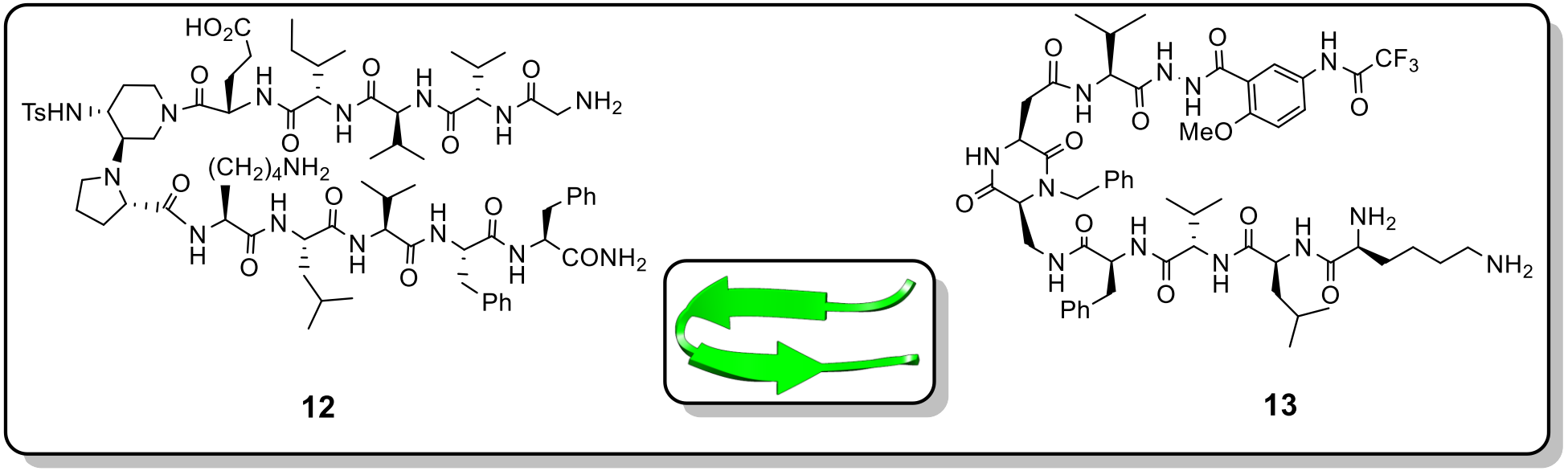
polar and/or flexible groups disrupting hydrogen bonds and hydrophobic interactions

❑ **β-hairpin mimics**



disrupting intramolecular and intermolecular interactions

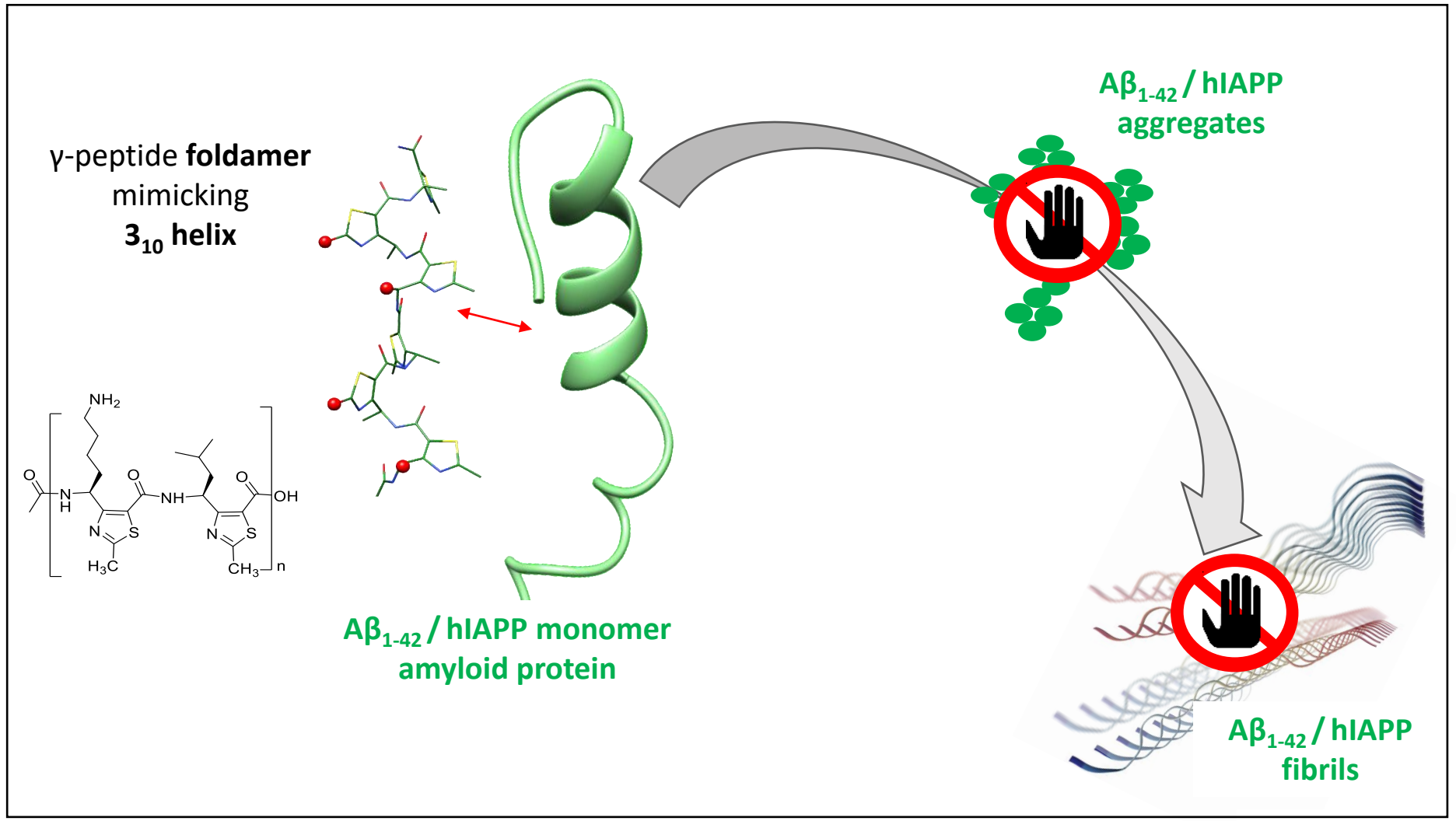
Acyclic β-hairpin mimics inhibiting PPIs in Alzheimer and Diabetes



S. Ongeri et al. *Chem.Sci.* **2017,** *8*, 1295

S. Ongeri et al. *Eur. J. Org. Chem.* **2017**, 2971

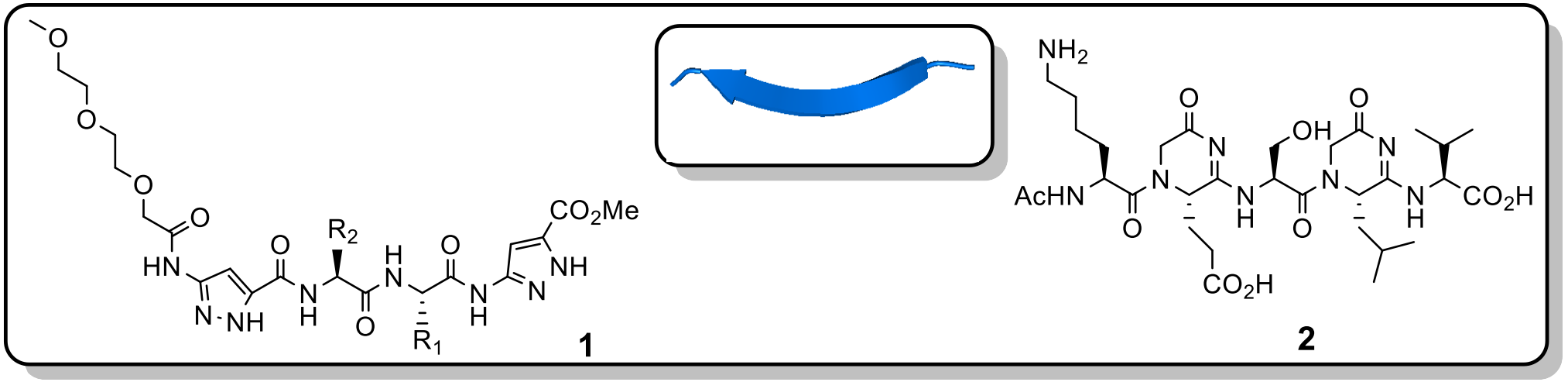
Helix mimics inhibiting PPIs in Alzheimer and Diabetes



S. Ongeri et al. *Chem. Eur. J.* **2020**, *26*, 14612

Linear peptides inhibiting PPIs involving β-sheet

β-Strand mimics



Aβ ligand inhibits aggregation
Alzheimer's disease

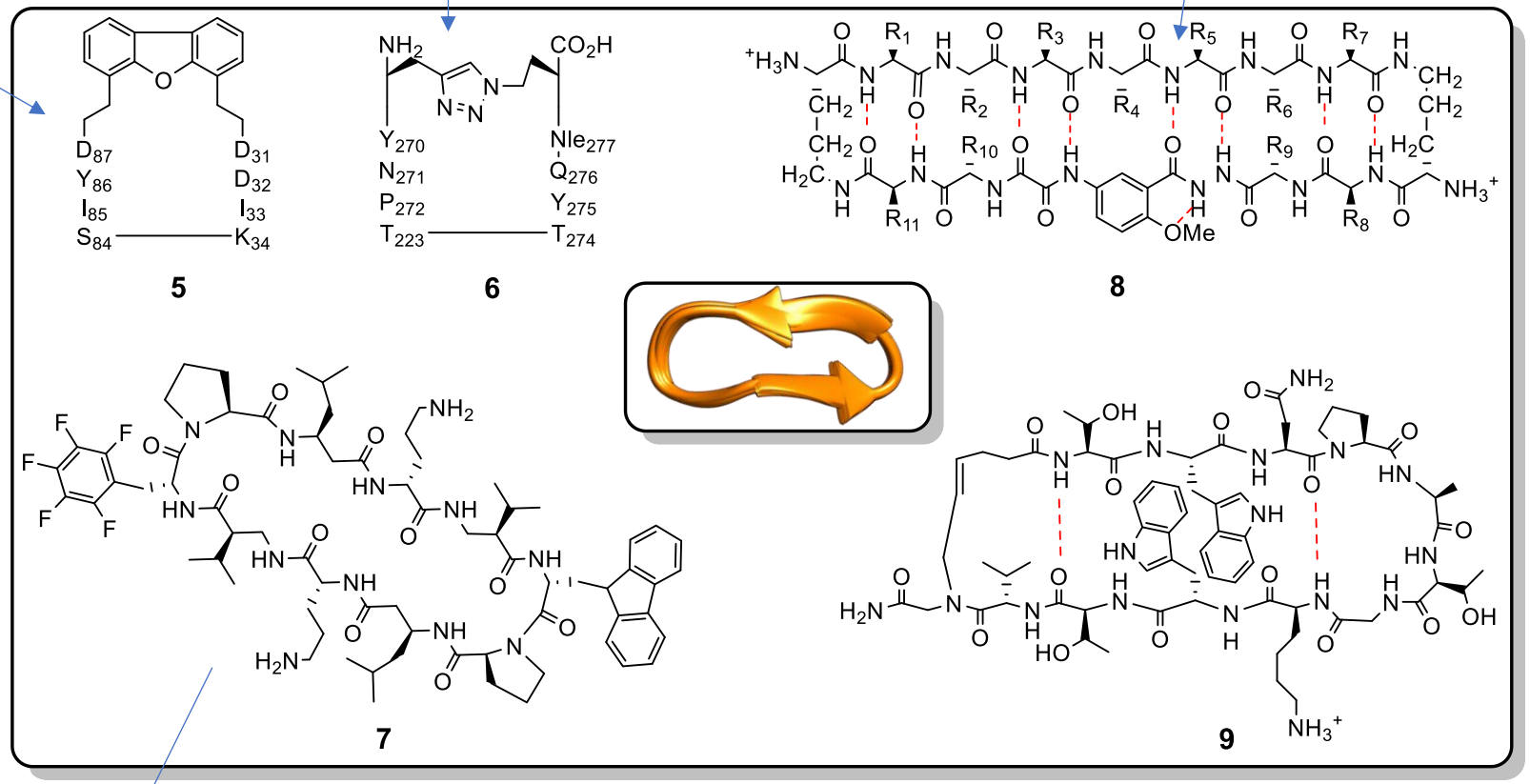
inhibit the interaction of the PDZ domain of α-1 syntrophin with nNOS (Duchenne muscular dystrophy)

Cyclic β-hairpin mimics inhibiting PPIs involving β-sheet

β-strand sequences of T-cell glycoprotein CD2 bind CD58 of epithelial cells autoimmune diseases (rheumatoid arthritis)

inhibition of EGFR dimerization (carcinomas)

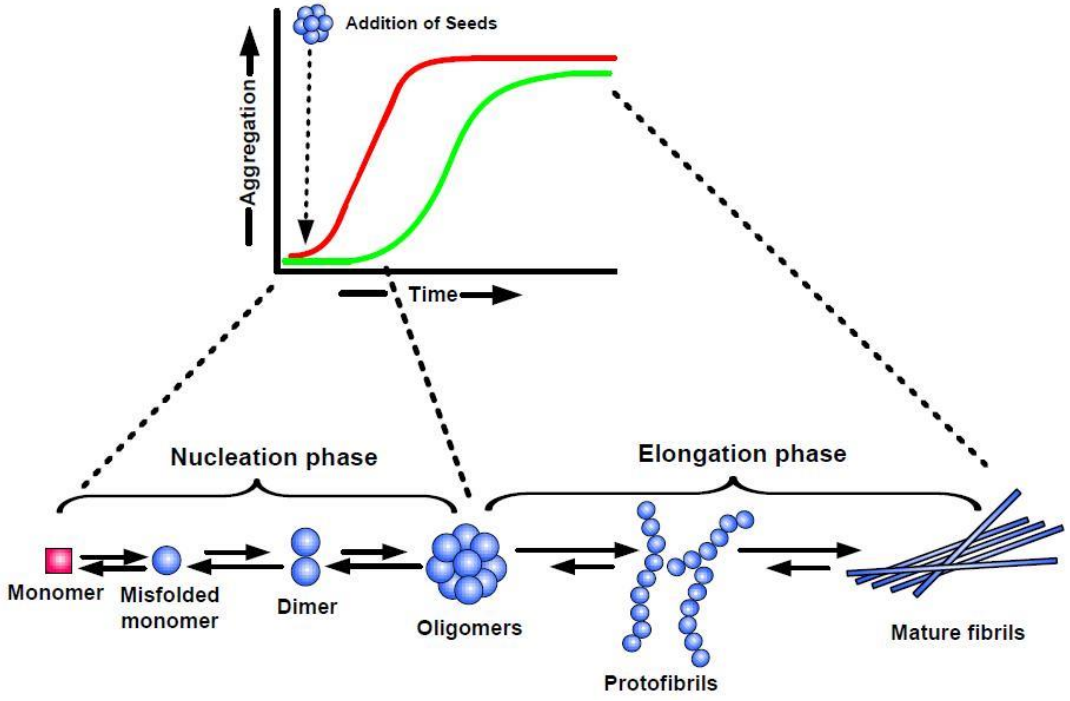
Inhibition of amyloid proteins aggregation Alzheimer's disease, Type 2 Diabetes

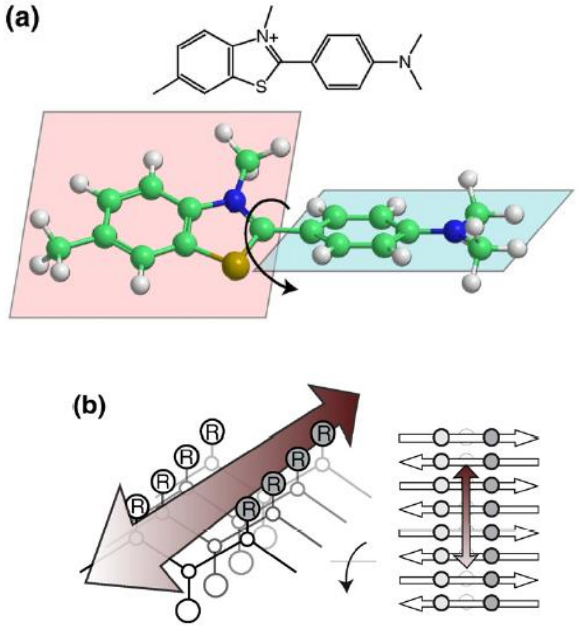


Aβ ligand inhibits aggregation Alzheimer's disease

Evaluation of amyloid proteins aggregation/fibrillization kinetics

- **Thioflavin T Fluorescence : classical method**



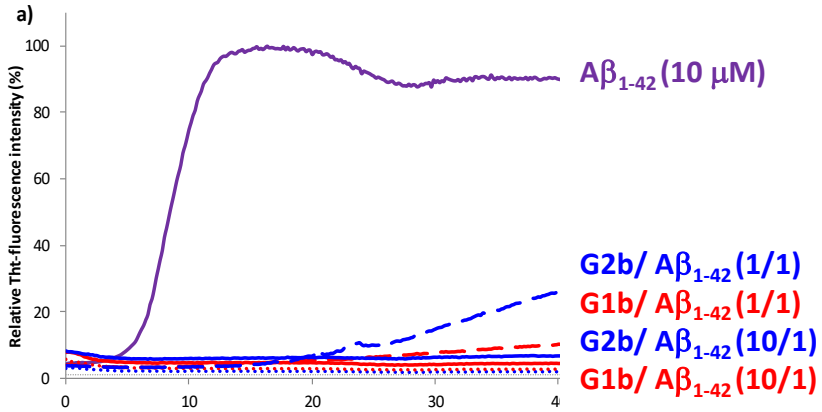


When ThT is bound to amyloid fibrils, internal rotation in the dye molecule is blocked due to steric hindrance
→ high quantum yield of fluorescence

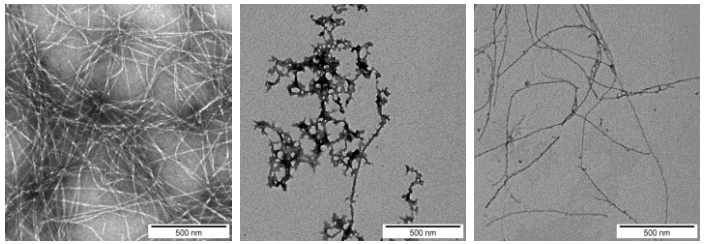
Biancalana *et al.Biochimica et Biophysica Acta,* 1804, **2010,** 1405–1412

❑ Inhibition of Fibrillization : two classical methods

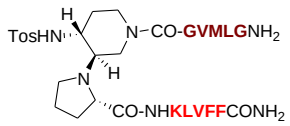
✓ ThT Fluorescence Spectroscopy of Aβ1-42



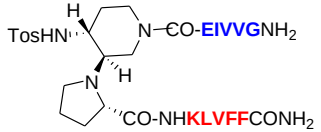
✓TEM of Aβ1-42



$Aβ_{1-42}$ **$Aβ_{1-42}$/G1b: 1/1**

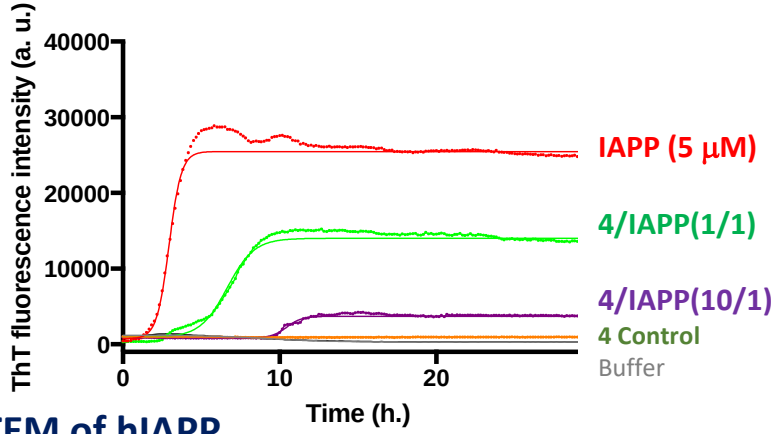


$Aβ_{1-42}$/G2b: 1/1

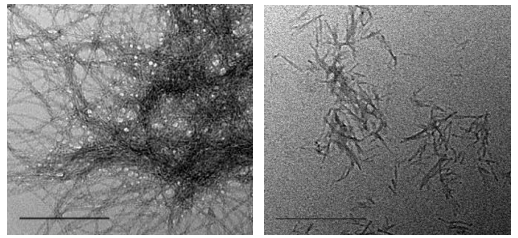


S. Pellegrino, N. Tonali, S. Ongeri et al. *Chem.Sci.*, **2017,** *8*, 1295

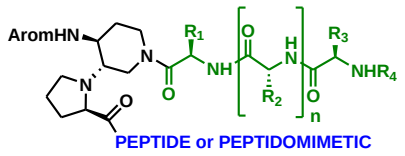
✓ ThT Fluorescence Spectroscopy of hIAPP



✓TEM of hIAPP



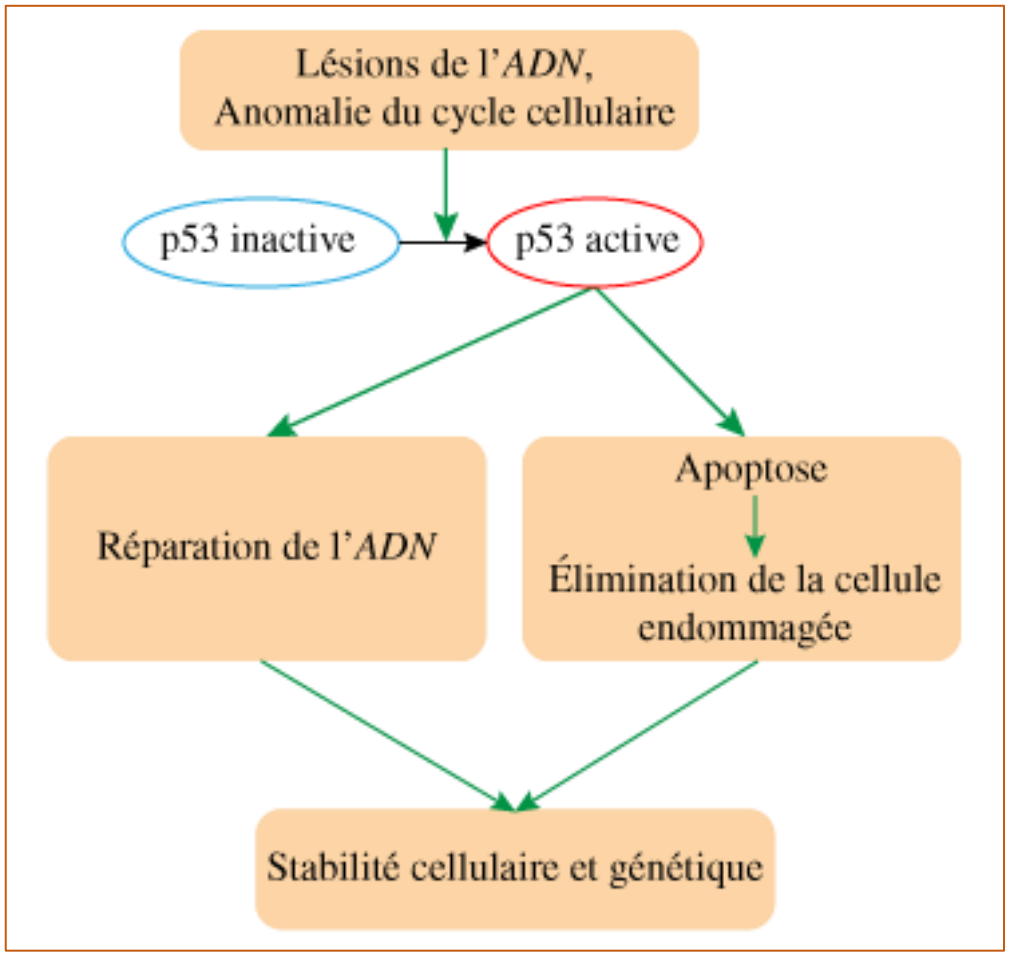
hIAPP **$Aβ_{1-42}$/4** 1/10



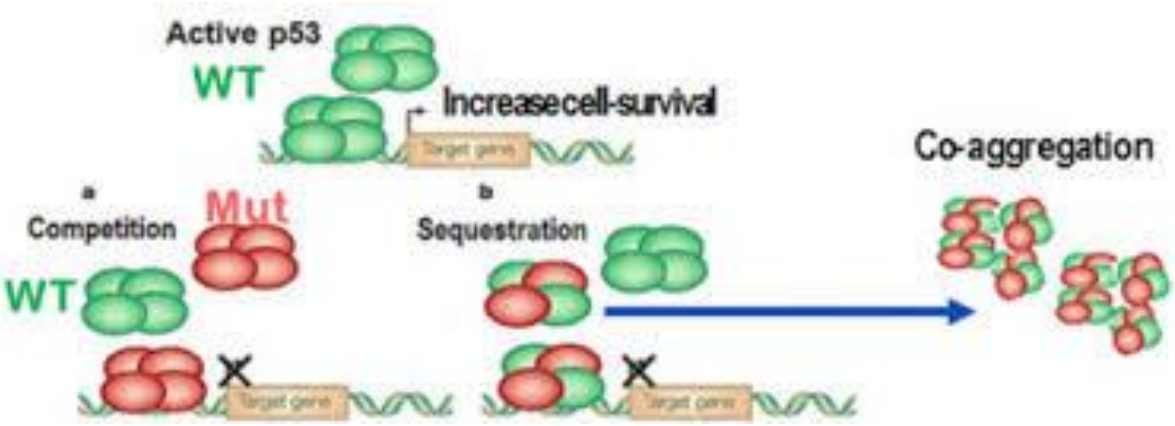
J. Lesma, J. Kaffy, S. Ongeri et al. *Front. Cell Dev. Biol.,* **2021**, *9*, 729001

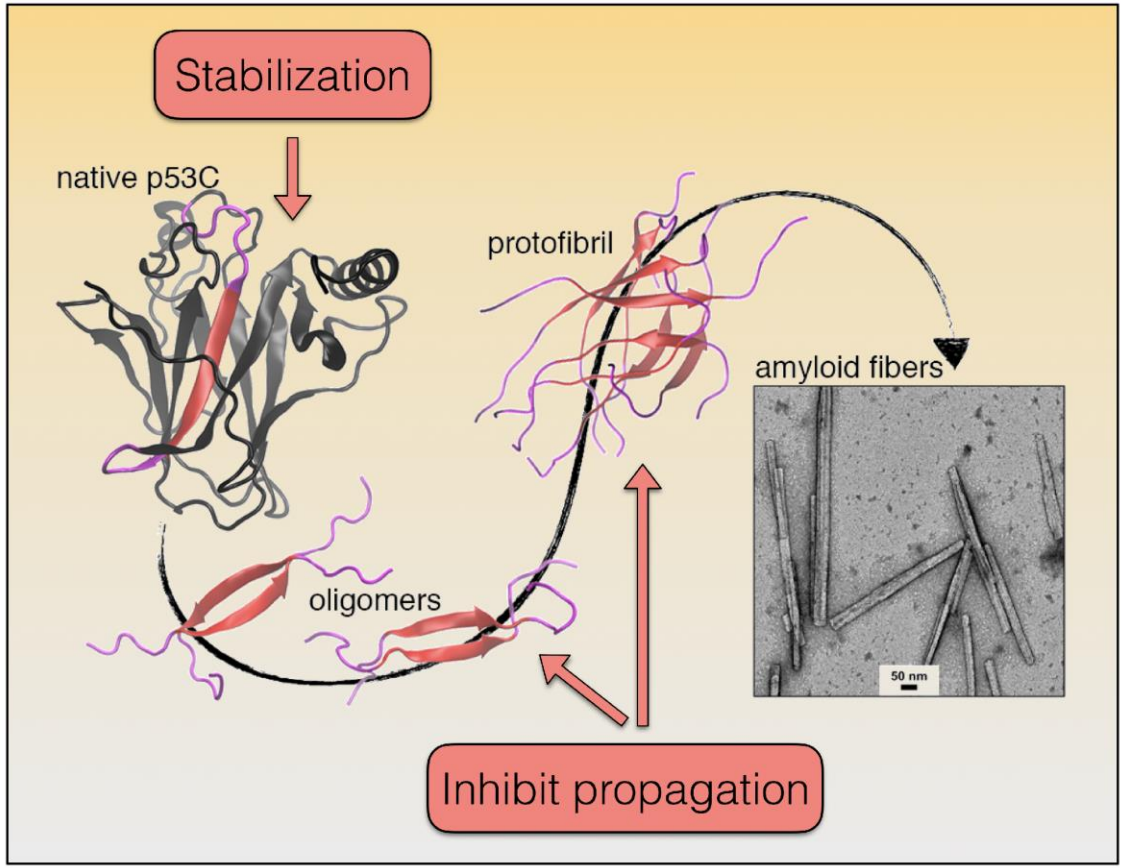
PPIs in Cancer

Importance de la protéine p53 pour induire l'apoptose des cellules tumorales

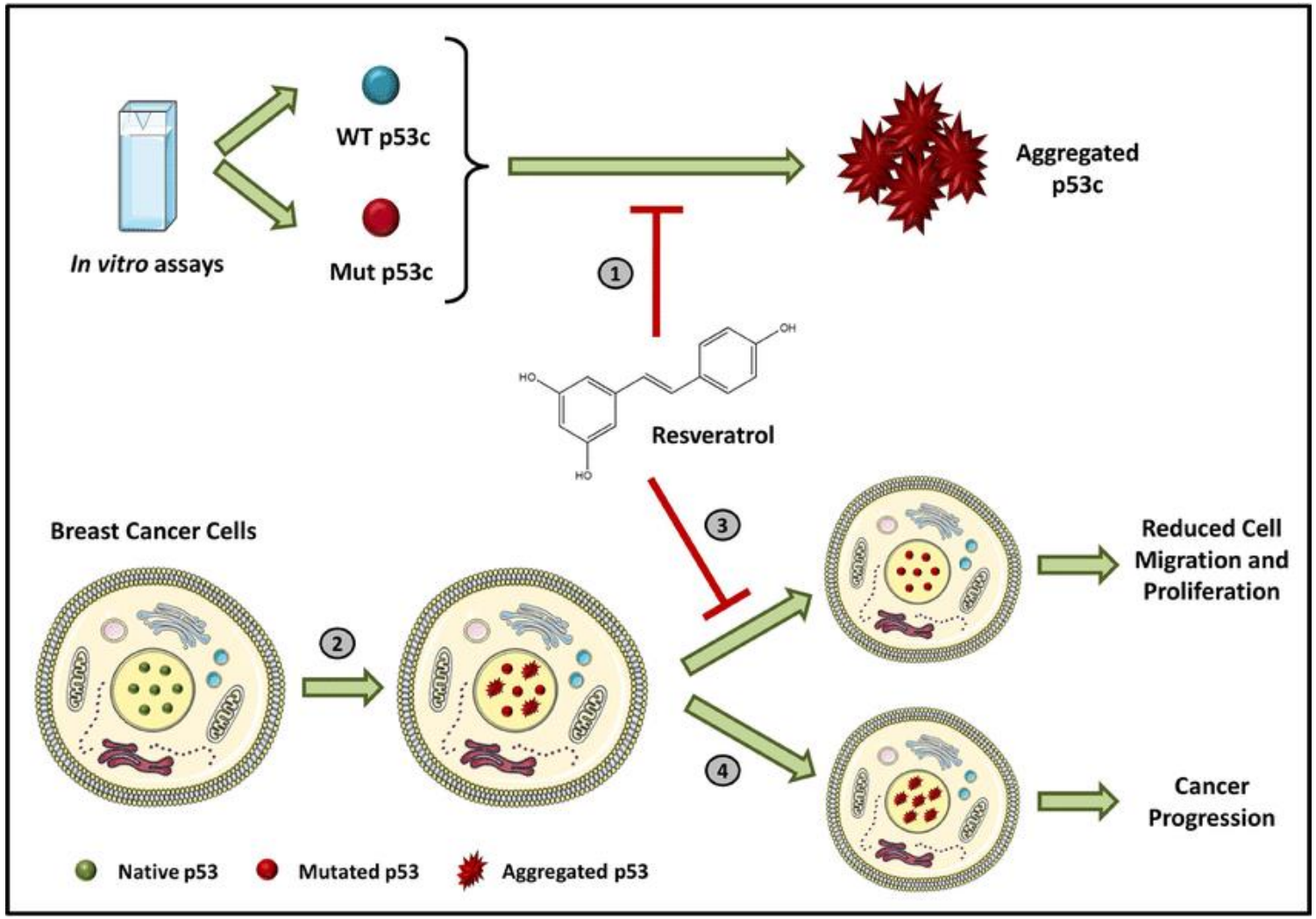


Cancer is a p53 protein aggregation disease





Inhibiting mutated p53 protein aggregation to treat cancer



Oncotarget. 2018 Oct 2; 9(77): 34455–34456.

CONCLUSION

- **Intercommunication between proteins crucial** in both biological and pathological processes
- **Modulators of PPIs** current promising strategy towards **next-generation drugs**
- Still considered as a challenging issue because PPIs involve rather **flat and large proteins areas**
- The **hot-spot** residues generally adopt secondary structures, using **compounds mimicking secondary structures** relevant strategy to modulate PPIs
- Peptide derivatives and analogues mainly described to target PPIs involving helix structures
- PPIs involving β-sheet secondary structures probably still underestimated, however, already questioned in many diseases that crucially need therapeutics
- New synthetic strategies to improve productivity and to increase the half-life and bioavailability of peptides and peptidomimetics, along with alternative routes of administration, have allowed a **larger number of peptide-based drugs to be now marketed**