PPI Modulators
-Toward next generation therapeutics-

Literature Seminar

2014.5.22 (Thu.)

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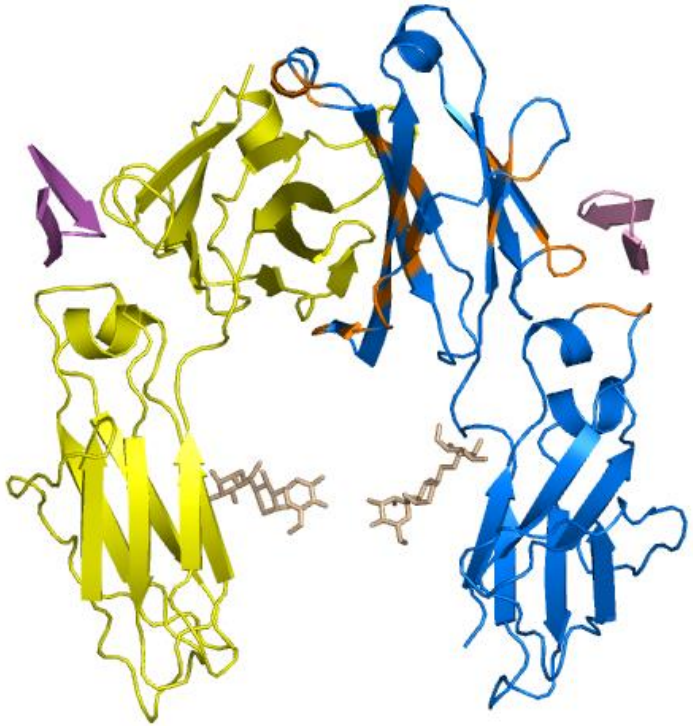
Protein-protein interactions (PPI)

Many cellular process depend upon enzymatic reactions
However, proteins rarely act alone and protein-protein interactions (PPIs) mediate a large number of important regulatory pathways

e.g.

- signal transduction
- transport across membranes
- cell metabolism
- muscle contraction



Definition of PPI

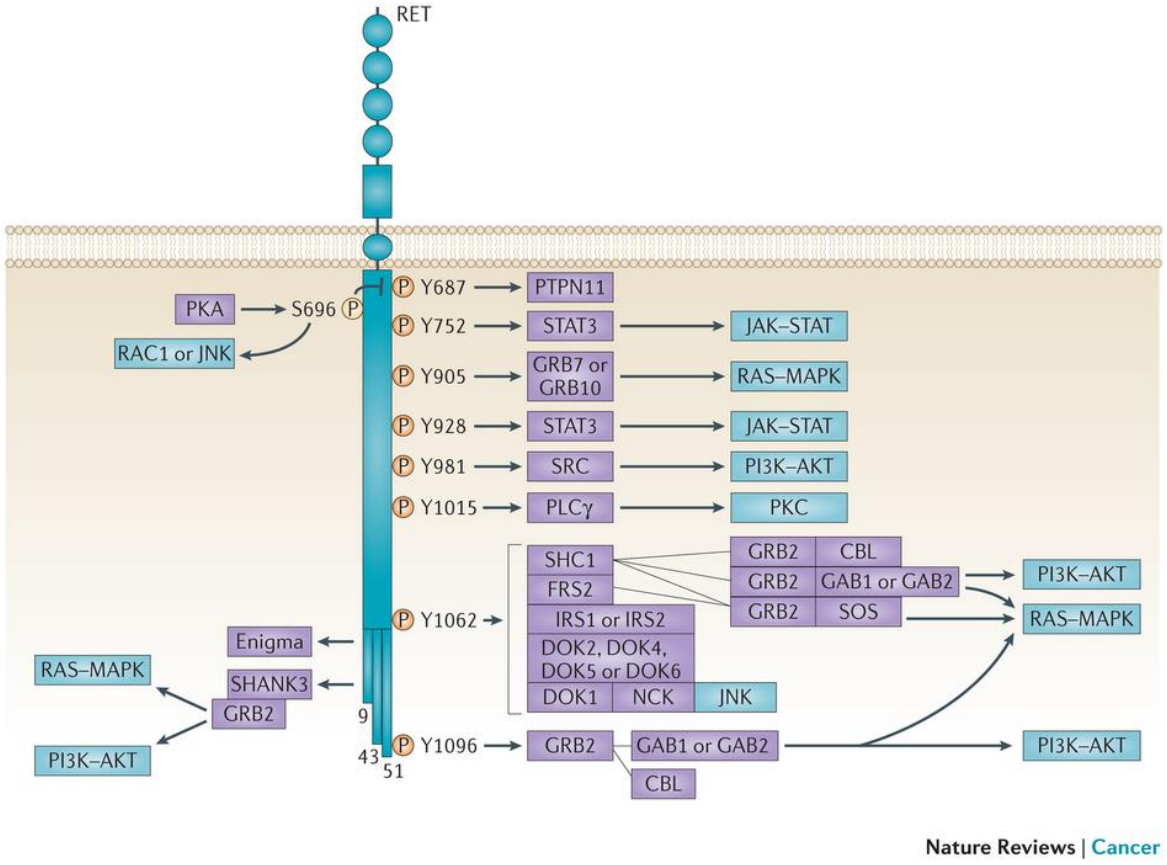
Protein-protein interactions is physical contacts established between two or more proteins

We have to consider that…

- **The interaction interface is intentional and not accidental**
  i.e., the result of specific selected biomolecular events/forces

- **The interaction interface is non-generic, evolved for a specific purpose distinct from totally generic functions such as protein production and degradation**

PPI in signal transduction



PPI is central to biological processes

<u>Complete mapping of PPI</u> is one of main scopes of current biological research

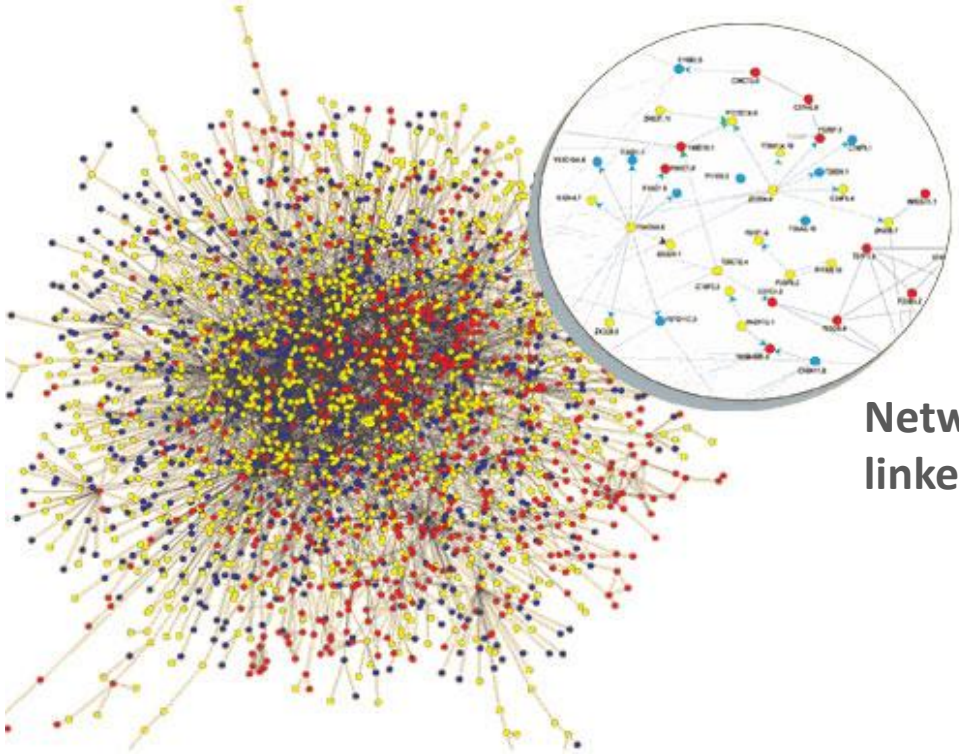
II

Interactome

Nature Reviews Cancer, **2014**, 14, 173

Interactome

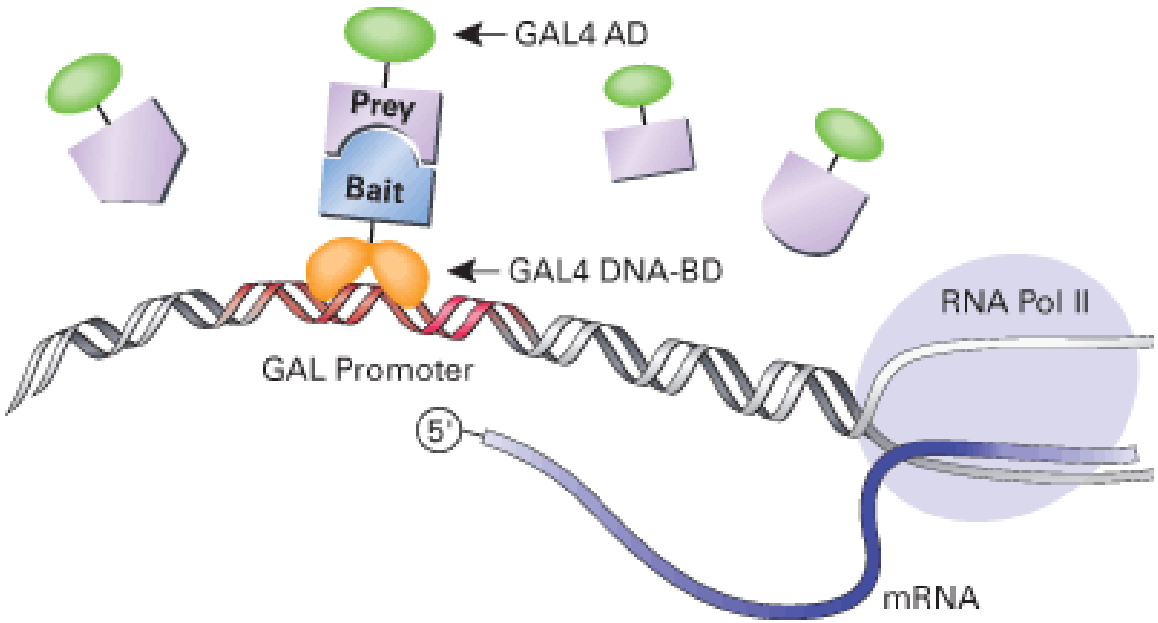
Finding interaction partners for a protein can reveal its function
Building entire PPI network is the next step after the “Human Genome Project”



Network is represented as protein “nodes” linked by interaction “edges”

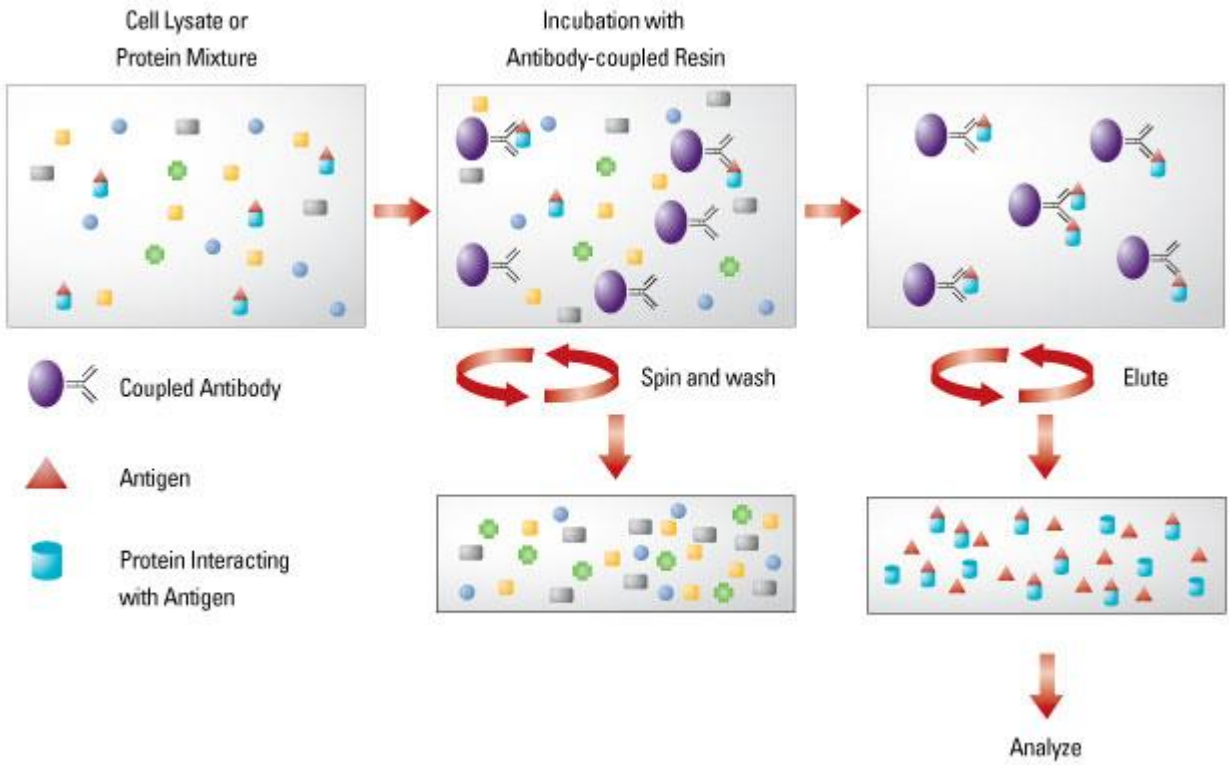
PPI Determination

Yeast two-hybrid (Y2H)

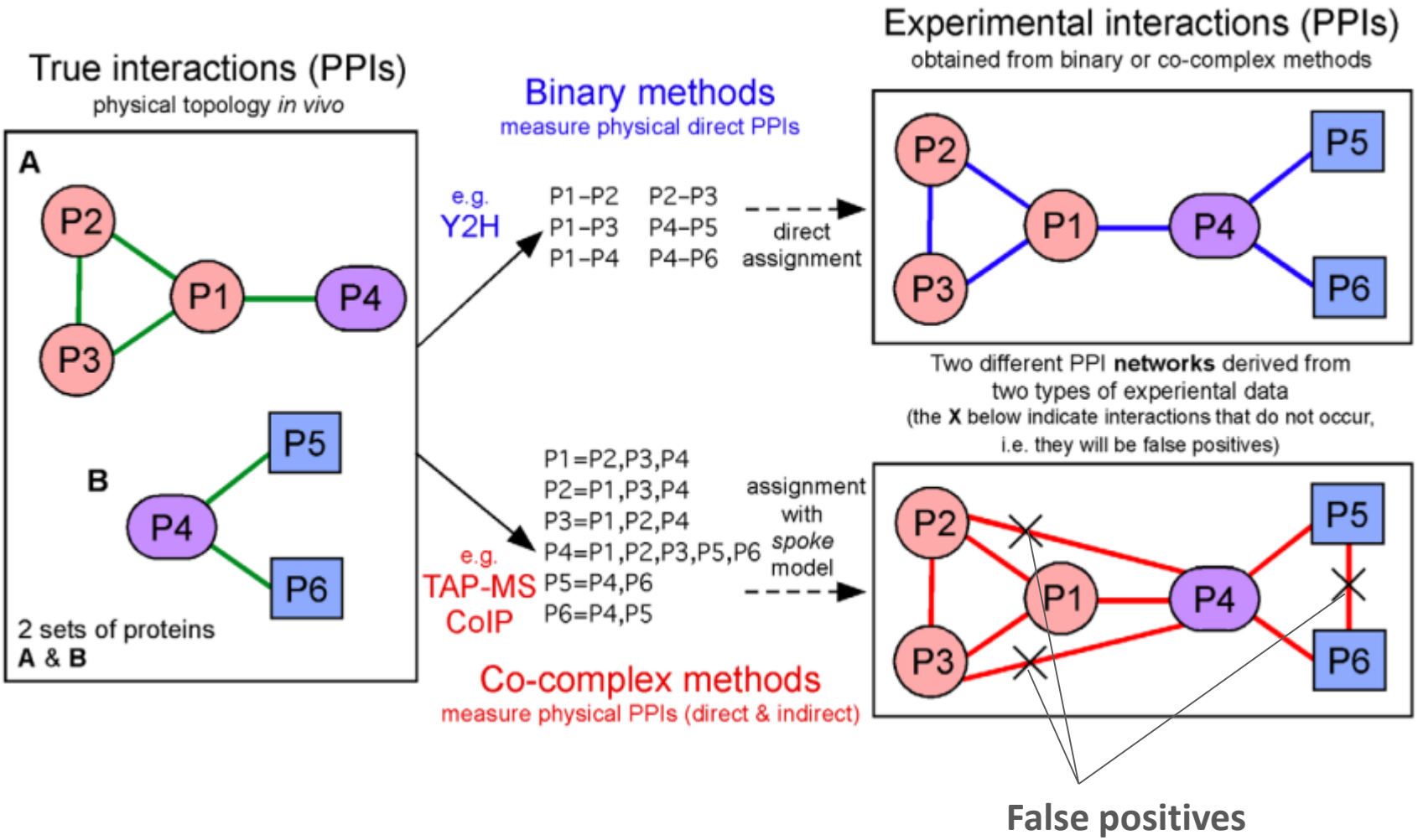


PPI Determination

Co-immunoprecipitation (CoIP)



PPI Determination

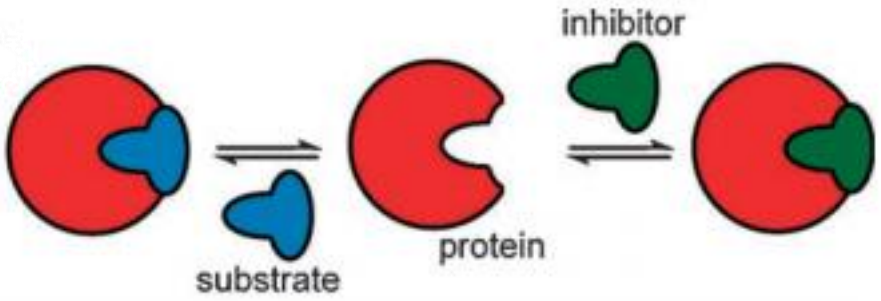


PPI databases

Acronym	Database Full Name and URL	PPI Sources	Type of MI	Species	*n* Proteins (Dec. 2009)	*n* Interactions (Dec. 2009)
Primary Databases: PPI experimental data (curated from specific SSc & LSc published studies)						
BIND	Biomolecular Interaction Network Database, http://bond.unleashedinformatics.com/	Ssc & Lsc published studies (literature-curated)	PPIs & others	All	[31,972]	[58,266]
BioGRID	Biological General Repository for Interaction Datasets, http://www.thebiogrid.org/	Ssc & Lsc published studies (literature-curated)	PPIs & others	All	[28,717]	[108,691]
DIP	Database of Interacting Proteins, http://dip.doe-mbi.ucla.edu/dip/	Ssc & Lsc published studies (literature-curated)	Only PPIs	All	20,728	57,683
HPRD	Human Protein Reference Database, http://www.hprd.org/	Ssc & Lsc published studies (literature-curated)	Only PPIs	Human	27,081	38,806
IntAct	IntAct Molecular Interaction Database, http://www.ebi.ac.uk/intact/	Ssc & Lsc published studies (literature-curated)	PPIs & others	All	[60,504]	[202,826]
MINT	Molecular INTeraction database, http://mint.bio.uniroma2.it/mint/	Ssc & Lsc published studies (literature-curated)	Only PPIs	All	30,089	83,744
MIPS-MPact	MIPS protein interaction resource on yeast, http://mips.gsf.de/genre/proj/mpact/	Derived from CYGD	Only PPIs	Yeast	1,500	4,300
MIPS-MPPI	MIPS Mammalian Protein-Protein Interaction Database, http://mips.gsf.de/proj/ppi	Ssc published studies (literature-curated)	Only PPIs	Mammalian	982	937
Meta-Databases: PPI experimental data (integrated and unified from different public repositories)						
APID	Agile Protein Interaction DataAnalyzer, http://bioinfow.dep.usal.es/apid/	BIND, BioGRID, DIP, HPRD, IntAct, MINT	Only PPIs	All	56,460	322,579
MPIDB	The Microbial Protein Interaction Database, http://www.jcvi.org/mpidb/	BIND, DIP, IntAct, MINT, other sets (exp & lit.-curated)	Only PPIs	Microbial	7,810	24,295
PINA	Protein Interaction Network Analysis platform, http://csbi.ltdk.helsinki.fi/pina/	BioGRID, DIP, HPRD, IntAct, MINT, MPact	Only PPIs	All	[?]	188,823
Prediction Databases: PPI experimental and predicted data ("functional interactions", i.e., interactions *lato sensu* derived from different types of data)						
MiMI	Michigan Molecular Interactions, http://mimi.ncibi.org/MimiWeb/	BIND, BioGRID, DIP, HPRD, IntAct, & nonPPI data	PPIs & others	All	[45,452]	[391,386]
PIPs	Human PPI Prediction database, http://www.compbio.dundee.ac.uk/www-pips/	BIND, DIP, HPRD, OPHID, & nonPPI data	PPIs & others	Human	[?]	[37,606]
OPHID	Online Predicted Human Interaction Database, http://ophid.utoronto.ca/	BIND, BioGRID, HPRD, IntAct, MINT, MPact, & nonPPI data	PPIs & others	Human	[?]	[424,066]
STRING	Known and Predicted Protein-Protein Interactions, http://string.embl.de/	BIND, BioGRID, DIP, HPRD, IntAct, MINT, & nonPPI data	PPIs & others	All	[2,590,259]	[88,633,860]
UniHI	Unified Human Interactome, http://www.mdc-berlin.de/unihi/	BIND, BioGRID, DIP, HPRD, IntAct, MINT, & nonPPI data	PPIs & others	Human	[22,307]	[200,473]

Large scale identification of PPIs generated hundreds of thousands interactions, and they are collected together in specialized biological databases

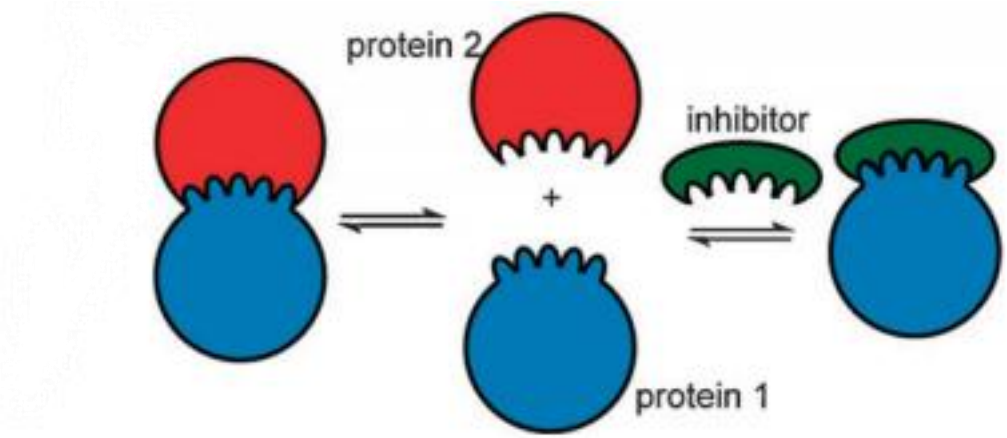
Traditional inhibitor of protein function



Inhibitors bind to enzyme active site which is

- **well-defined**
- **deep amd less accessible to bulk solvent**
- **relatively small (100 – 600 Å^2)**

PPI inhibitors



PPIs are difficult, unconventional drug target due to

- **Large binding interface areas(600-1300 Å^2)**
- **Shallow solvent-exposed surface**
- **Surfaces differ from small-molecule binding site in shapes and amino acid residue composition**

Traditional medicinal chemistry is less effective for PPIs

PPI inhibitors

PPI inhibitors approved for clinical applications today are usually based on humanized monoclonal antibodies (e.g., Tocilizmab, Bevacizumab)

However, antibodies are expensive and often seriously hindered by solubility, route of administration, distribution, and stability problems as well as by the possibility of a strong immune response

Small-molecule inhibitors are the ideal drug for targeting PPIs

Constrained Peptides

It was envisioned to use peptides directly derived from binfing epitopes of target PPI

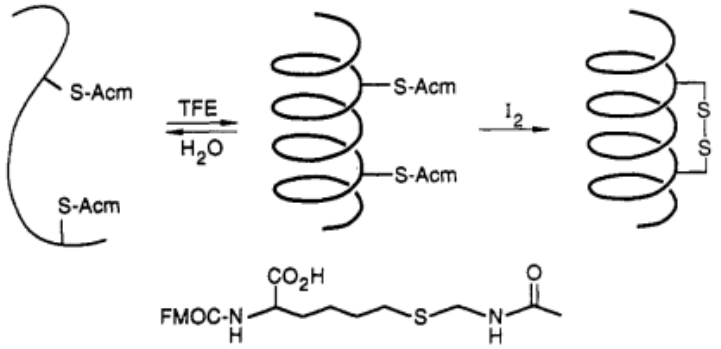
However...

- **These peptides adopt only random coil or become structually less defined**
- **Increased susceptibility to proteolytic degradation**
- **Reduction in cell wall permeability**

Research has focused on generating conformationally robust peptide

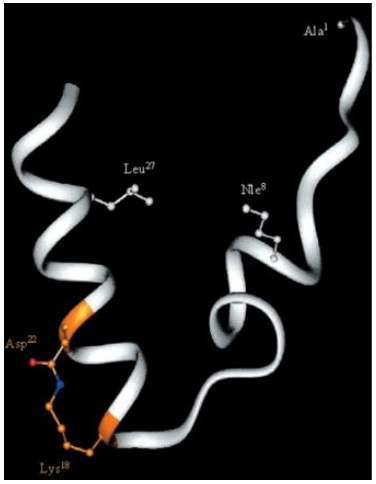
Constrained secondary structures

Disulfide

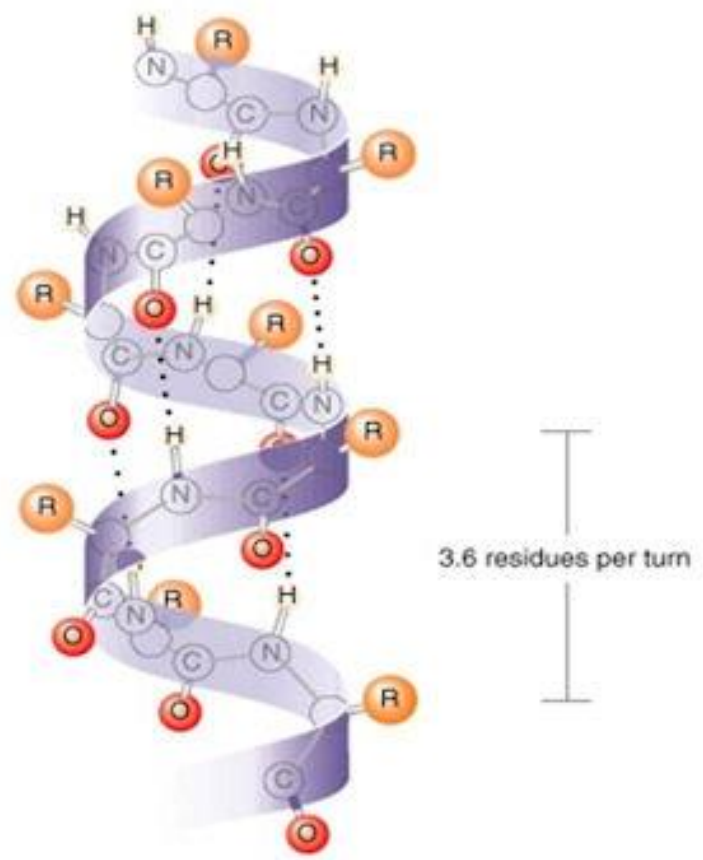


J. Am. Chem. Soc. **1991**, 113, 9391

Lactam



J. Am. Chem. Soc. **2000**, 122, 3007



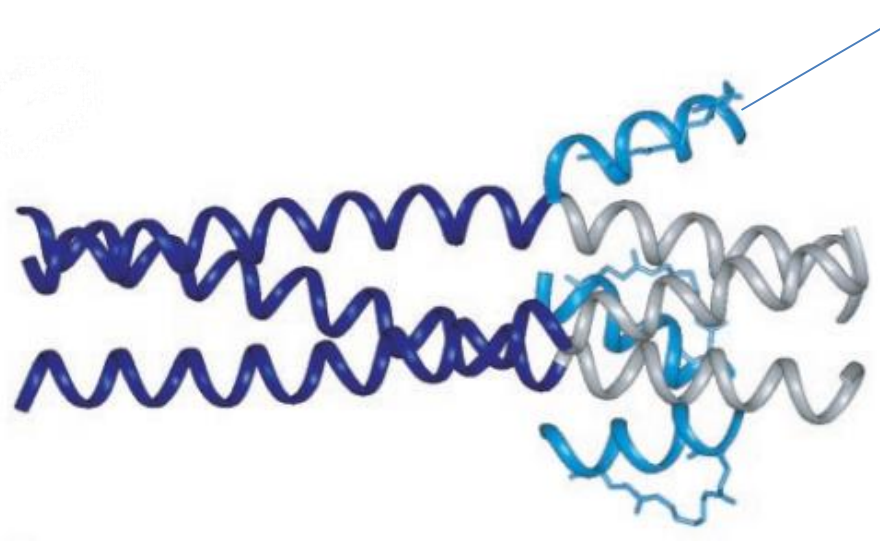
Covalent link between i, i+3, i+7
Stabilize α-helix

Stabilized α-helix

C14linkmid

suc-MTWQEWDREIQNYT-am

HIV-1 entry inhibitor



IC50 = 35 µM

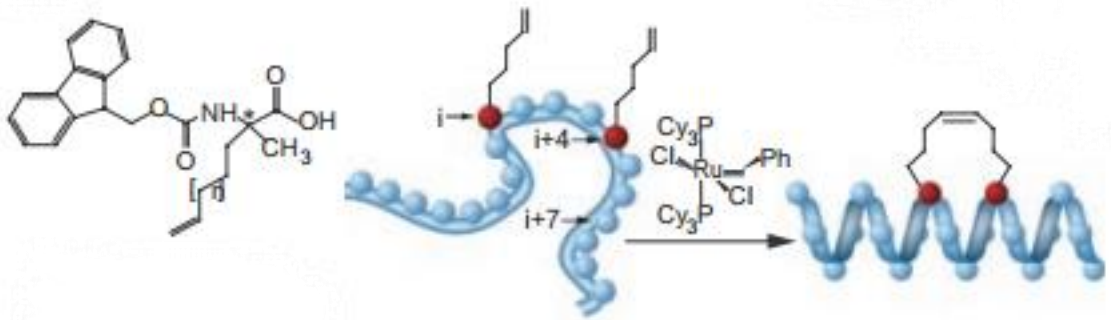
C14linkmid successfully disrupted the assembly of gp41 core

PNAS. **2002**, 99, 14644

Hydrocarbon stapling

**while disulfide and lactam bridges are effective in stabilizing α-helix,
such mimetics are not always stable in cells and are generally susceptible to degradation**

Stabilized alpha-helix of BCL-2 domains (SAHBs)



K_D = 38.8 nm

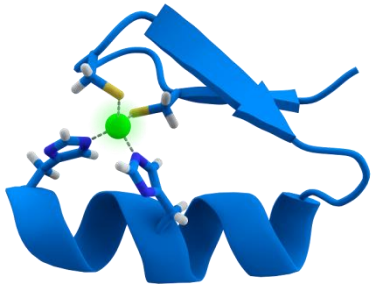
All hydrocarbon cross-links was introduced via ring-closing metathesis
Metabolically stable α-helix worked as Bcl-2 protein inhibitor

Science, **2004**, 305, 1466

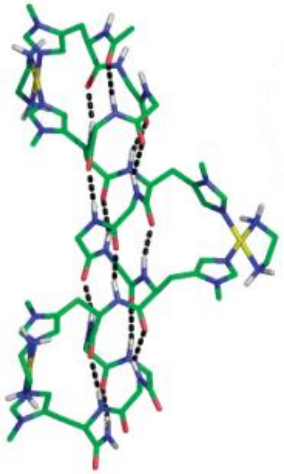
Metallopeptide

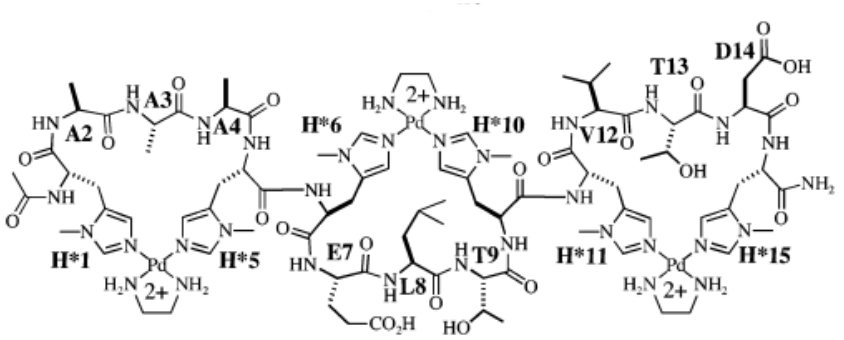
Metal ions play an important role in stabilizing α-helices in nature

e.g. Zinc finger



Pd^{2+} clip stabilized α-helix in DMF but low helicity (<40%) in water

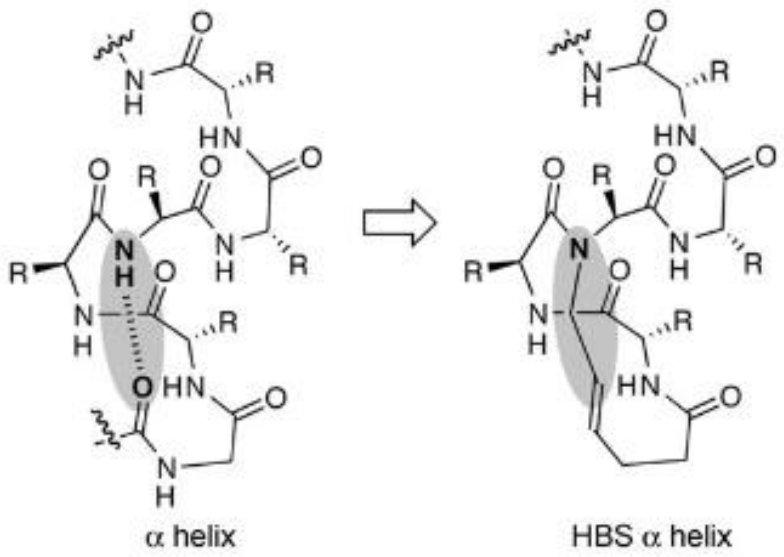




J. Am. Chem. Soc. **2004**, 126, 4828

Hydrogen bond surrogate

gp41-mediated cell fusion inhibitor

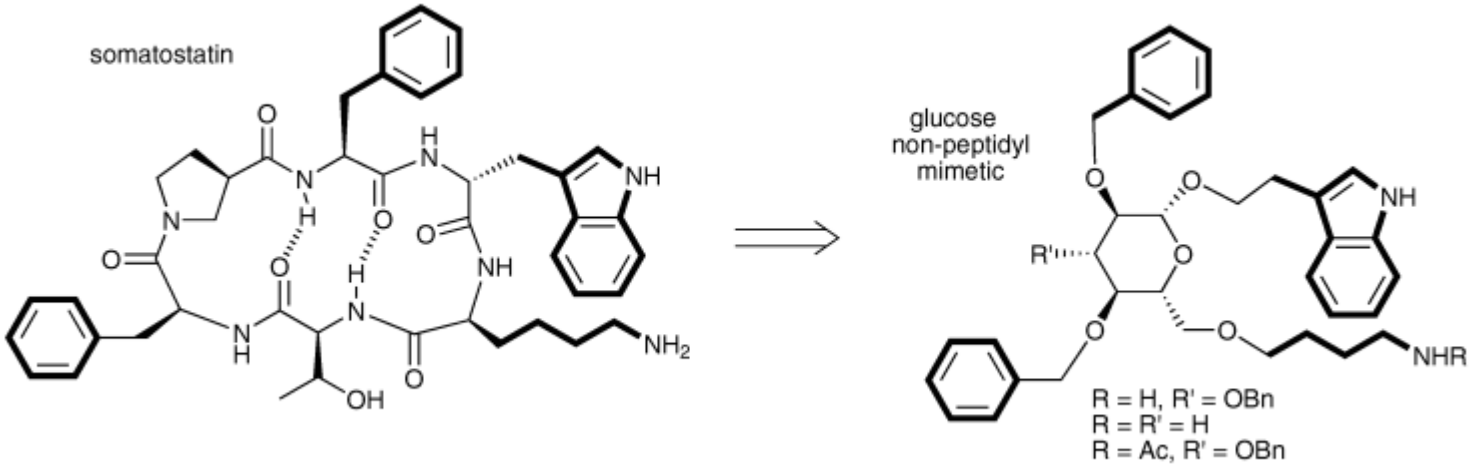


K_D < 5μM

Replacing hydrogen bond into covalent linkage stabilized α-helix

Angew. Chem. Int. Ed. **2008**, 47, 1879

Secondary structure mimetics



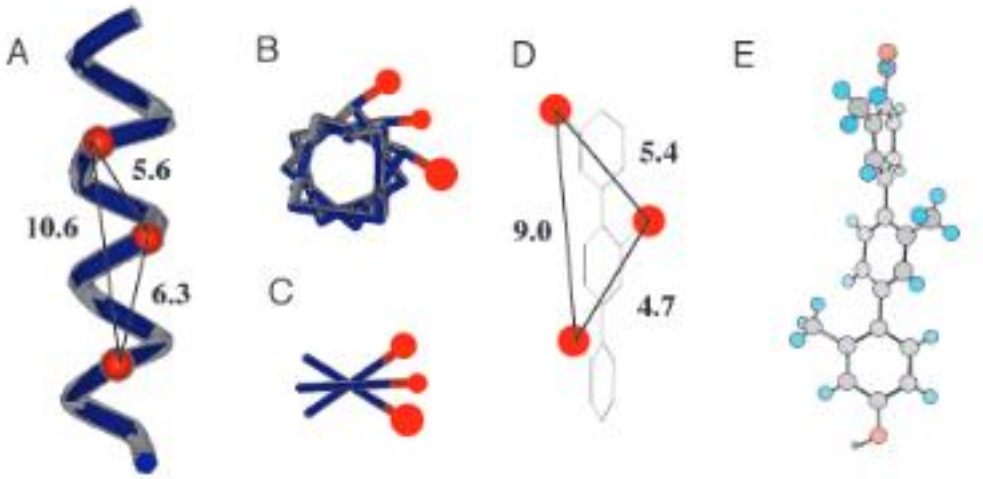
First non-peptidyl peptidomimetics

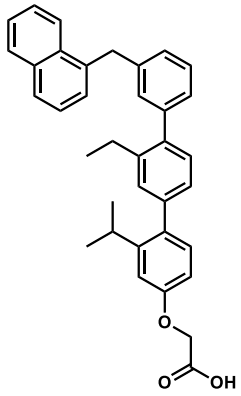
**Mimetic work as Somatostatin receptor agonist,
although with reduce activityrelative to natural hormone**

J. Am. Chem. Soc. **1992**, 114, 9217

α-helix mimetic

Terphenyl scaffold reasonably mimic the surface of α-helical peptide



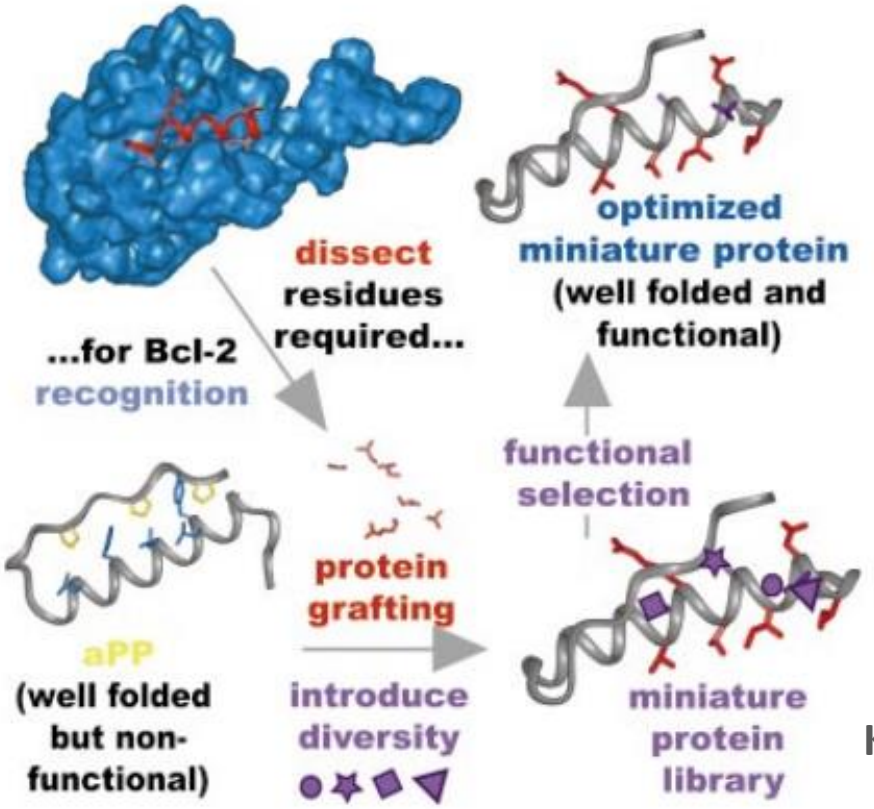


The mimetic inhibited calmodulin-smMLCK interaction

IC_{50} = 9 nM

J. Am. Chem. Soc. **2001**, 123, 5382

Protein grafting



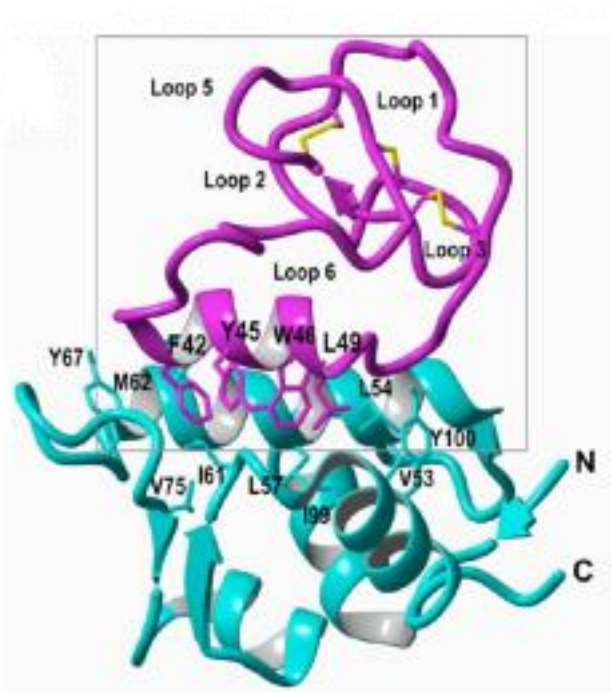
$K_D = 52 \pm 5\ \mu M$

Grafting Bak residues to stable aPP gave miniature protein inhibitor

Angew. Chem. Int. Ed. **2001**, 40, 3806

Cyclotide

Plant-derived disulfide-rich miniprotein with an intringuing circular cysteine knot (CCK)



Enhanced metabolic stability
Cell penetrating properties
Tolerance to substantial sequence varidation

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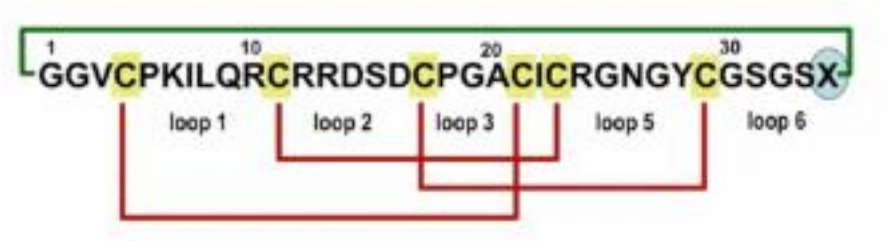
Good scaffold for peptide grafting



p53-Hdm2/HdmX inhibitor

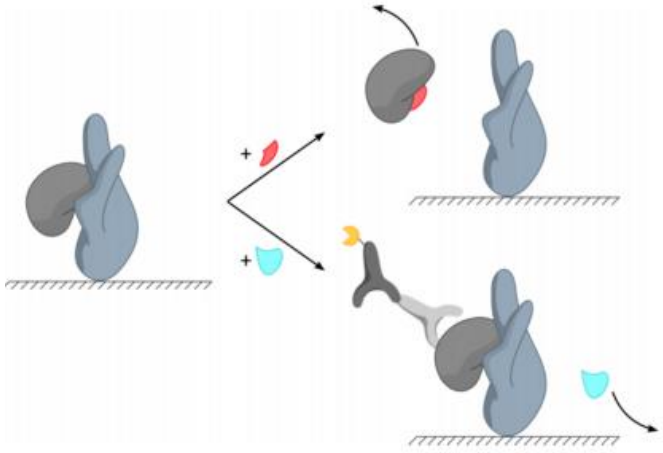
K_{D_Hdm2} = 2.3 ± 0.1 nM

K_{D_HdmX} = 9.7 ± 0.9 nM

J. Am. Chem. Soc. **2013**, 135, 11623

Approach to small-molecule PPI inhibitors

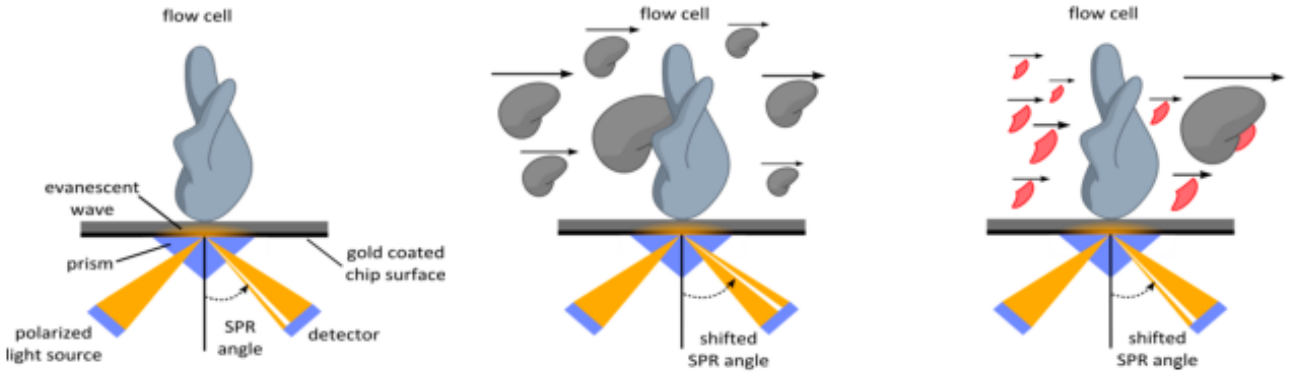
Enzyme-linked immunosorbent assay (ELISA)



PPI inhibition

No inhibition

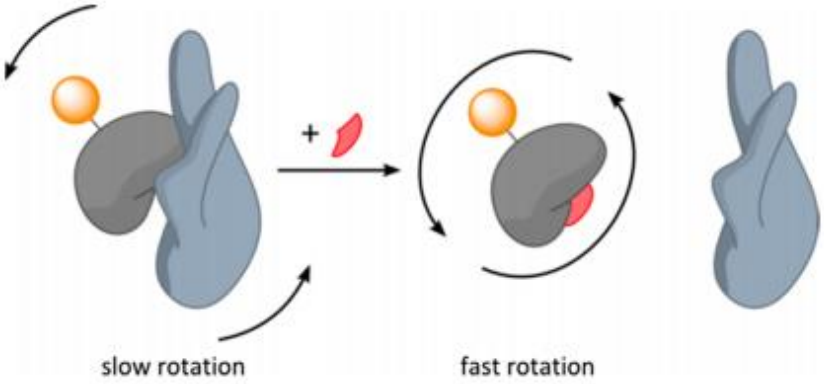
Surface Plasmon Resonance (SPR)



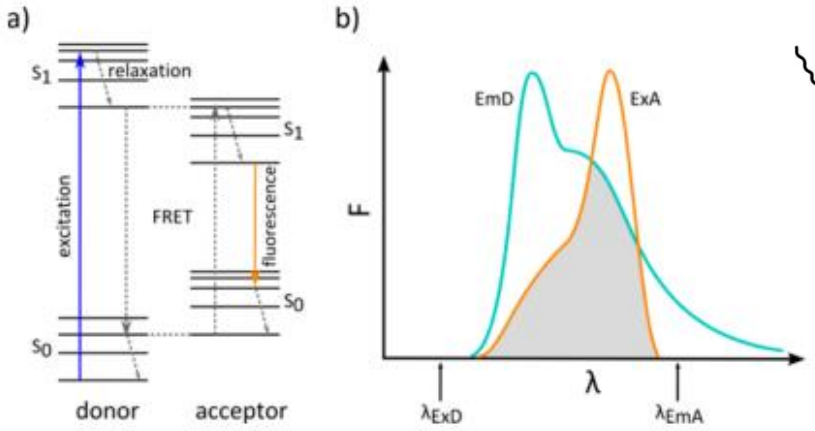
PPI inhibition

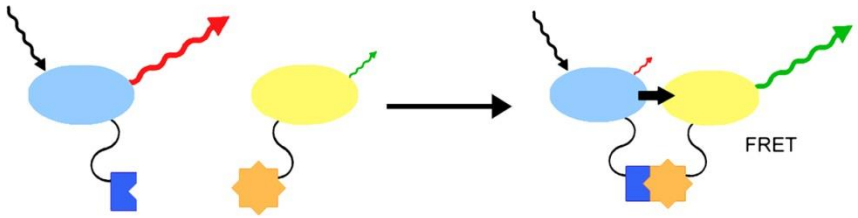
Approach to small-molecule PPI inhibitors

Fluorescent Polarization (FP)

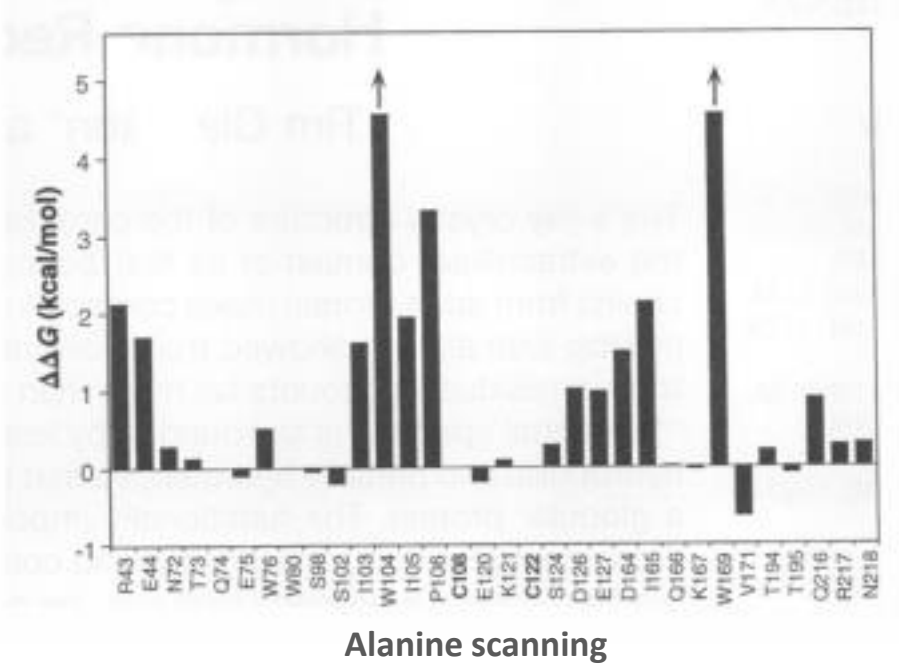


Fluorescence Resonance Energy Transfer (FRET)

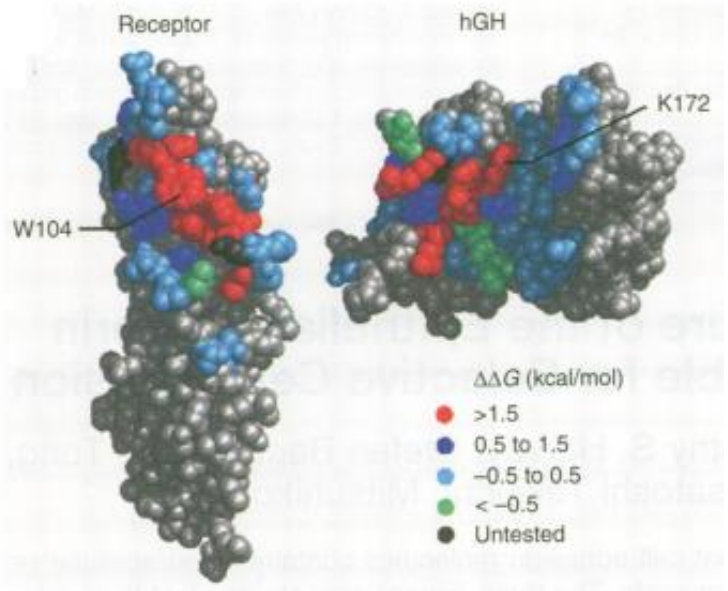




Hot spot theory



Alanine scanning



Only a few strong interactions are important for the binding free energy

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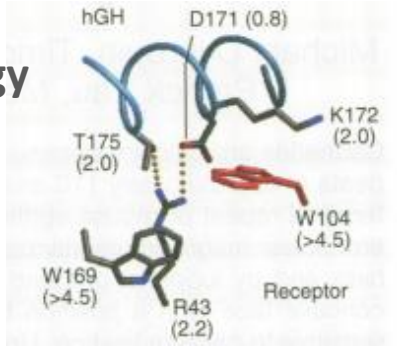
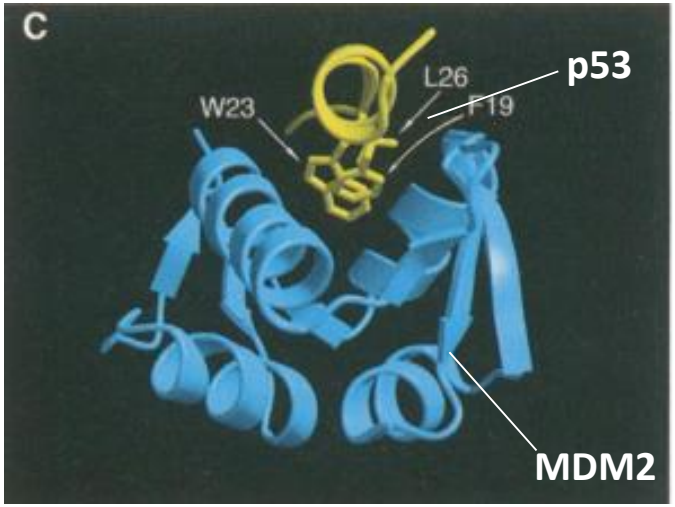
Hot spot

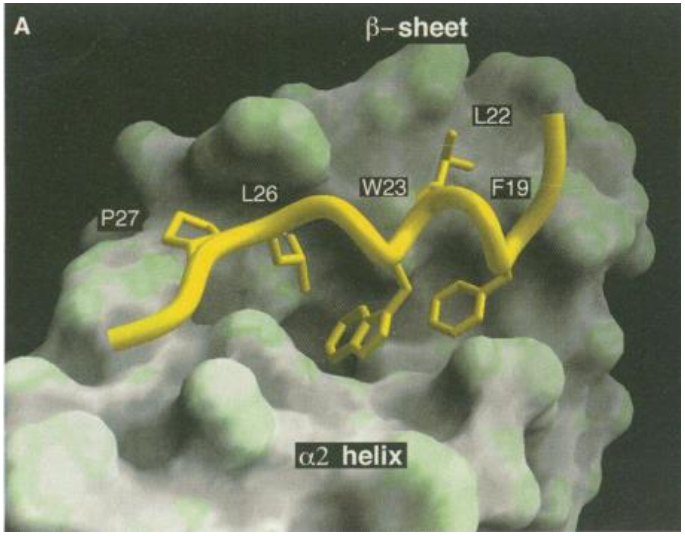


A mimic of the smaller functional epitope may suffice for modulators

Science, **1995**, 267, 383

Small-molecule PPI inhibitor





Crystal structure of p53-MDM2 complex revealed that

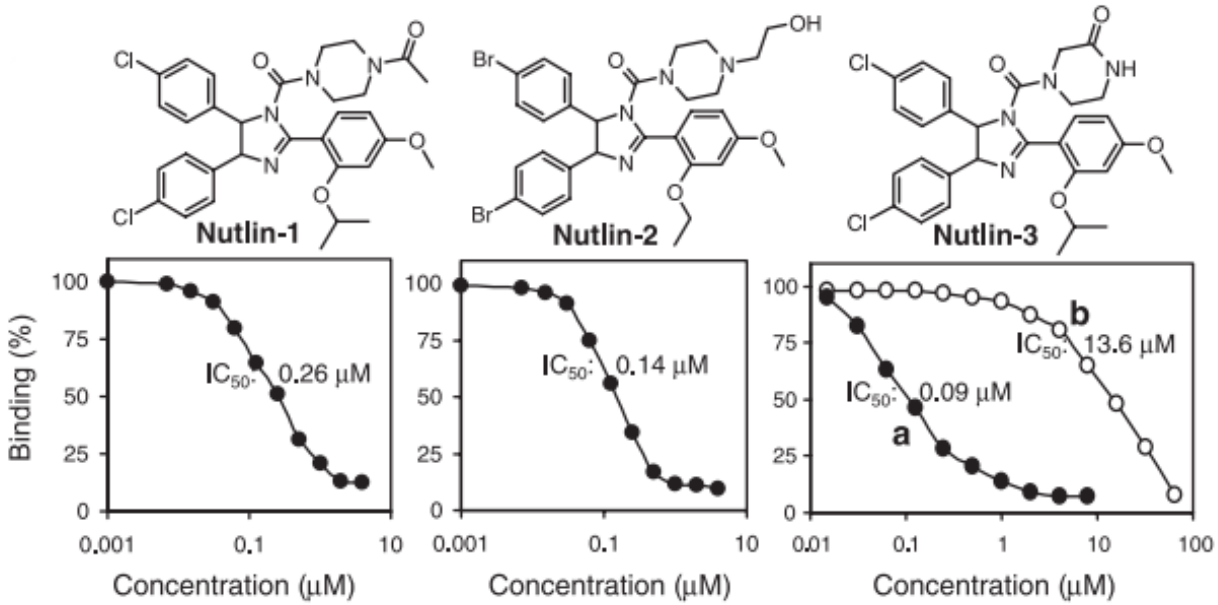
- **MDM2 possesses a relatively deep hydrophobic pocket**
- **15-residue α-helical transactivation domain of p53 insert into the hydrophobic cleft**
- **In particular, a triad of p53 amino acids (Phe^{19}, Trp^{23}, and Leu^{26}) is important**

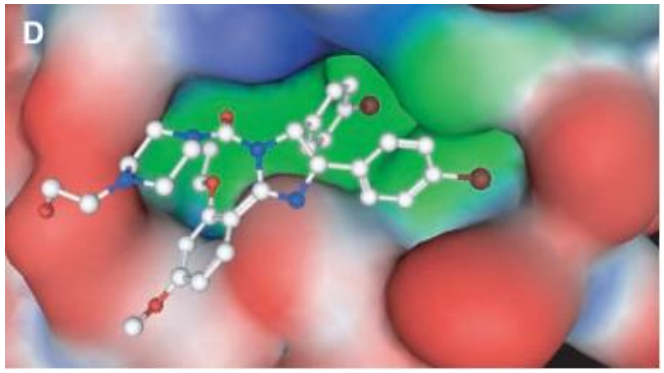
These facts raised expectation for small-molecule PPI inhibitor

Science, **1996**, 274, 948

High throughput screening (HTS)

Discovery of Nutlins (MDM2 inhibitor) through HTS (Roche)

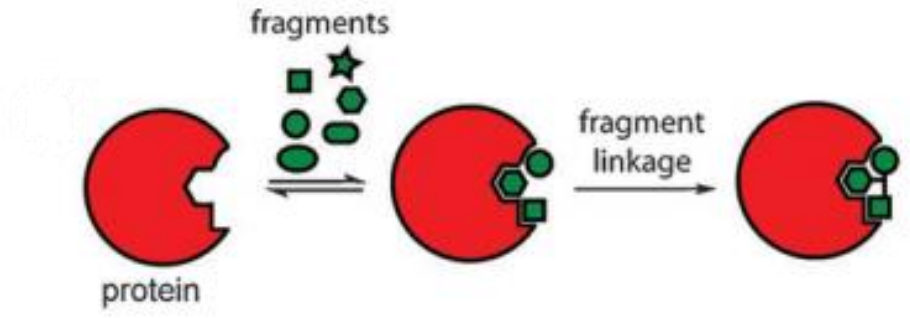




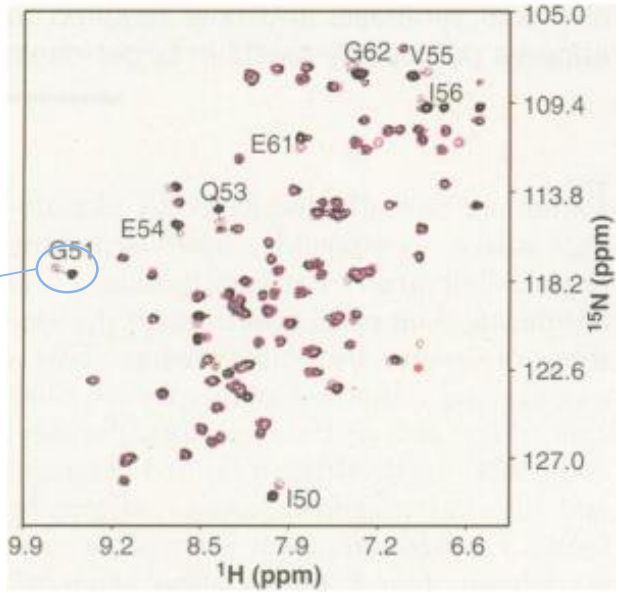
cis-imidazoline

Science, **2004**, 303, 844

Fragment based drug discovery (FBDD)



Structure-Activity Relationship (SAR) by NMR



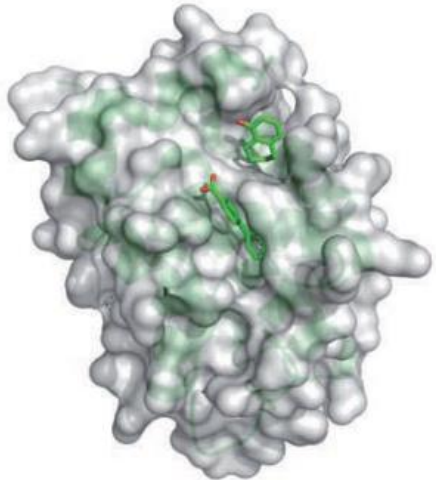
Significant change is observed

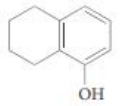
^{15}N-heteronuclear singlequantum correlation (^{15}N-HSQC)

Science, **1996**, 274, 1531

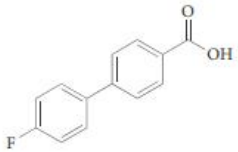
Fragment based drug design

Discovery of Bcl-2 family protein inhibitor ABT-737 (Abbott)

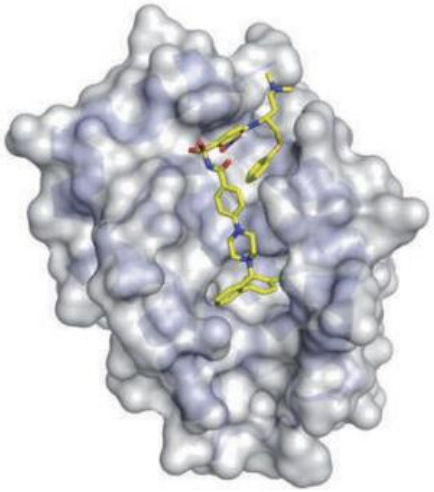


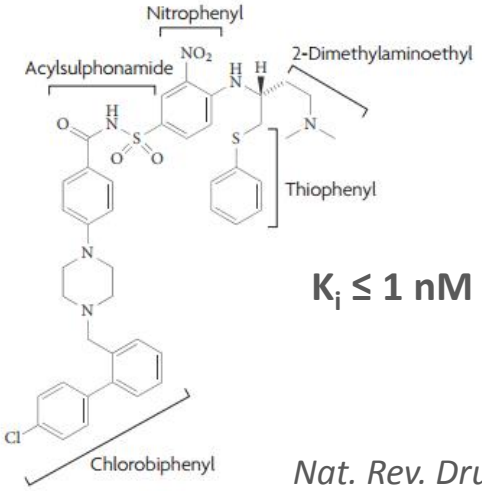


K_d = 4.3 ± 1.6 mM



K_d = 0.30 ± 0.03 mM

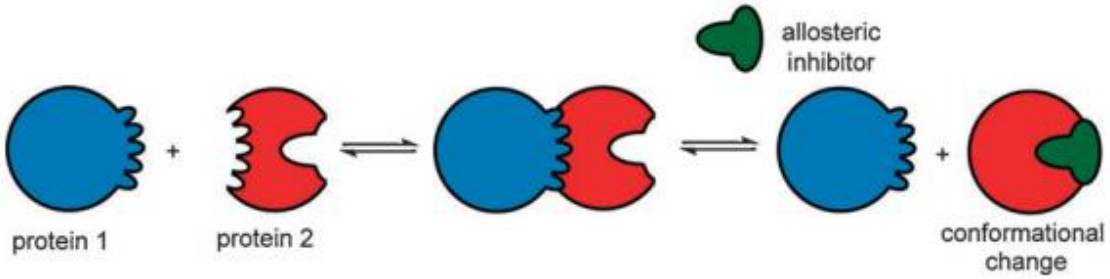


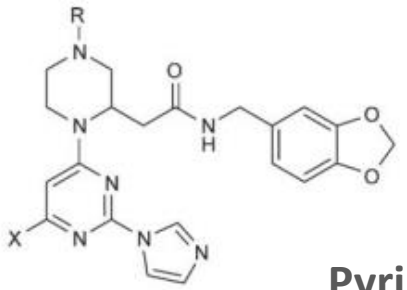


$K_i \leq 1$ nM

Narure, **2005**, 435, 677
Nat. Rev. Drug. Discov. **2008**, 7, 989

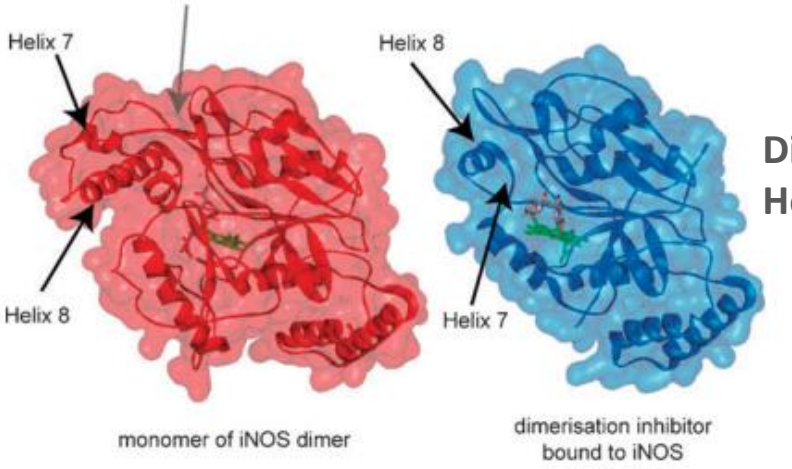
Allosteric inhibitors





R = H, X = Cl
R = CO_2CH_3, X = H
R = $COCH_2CH_3$, X = H

Pyrimidine-imidazole compound allosterically inhibits dimerization of iNOS



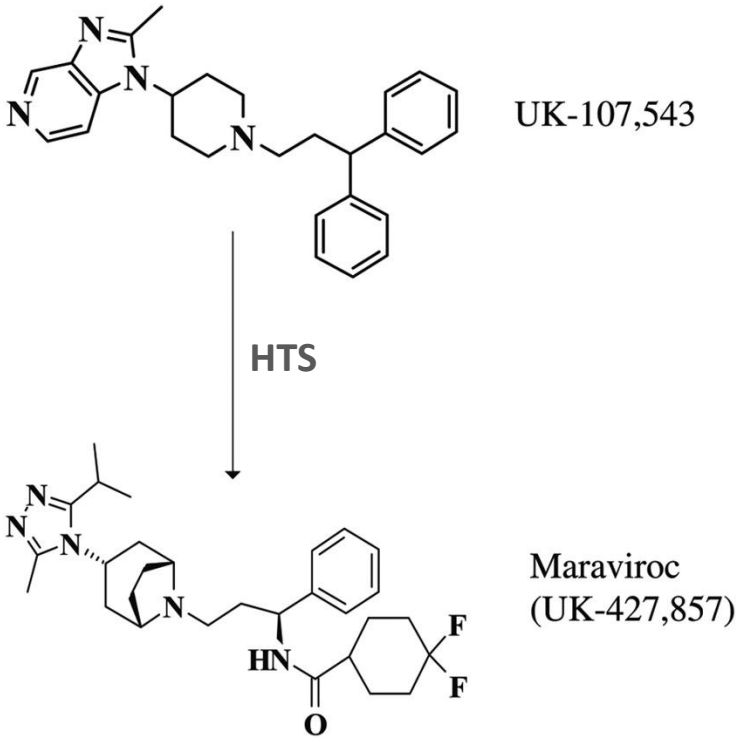
Disrupted helix 7 leads to disorder in helix 8
Helix 8 is part of the dimer interface

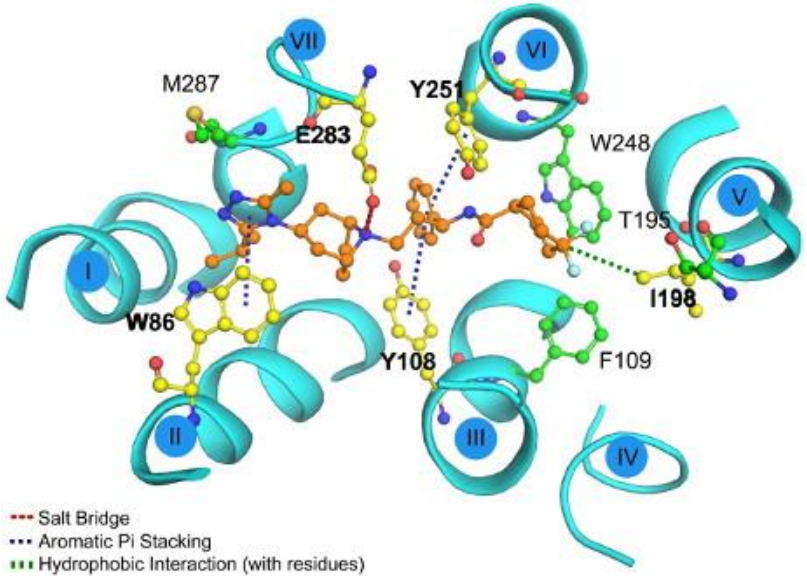
PNAS, **2000**, 97, 1506

Allosteric inhibitors

Maraviroc (Selzentry®, Pfizer): HIV-1 entry inhibitor

A marketed small-molecule PPI inhibitor

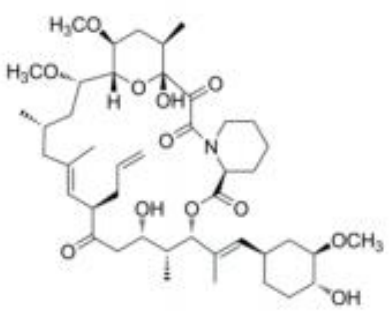




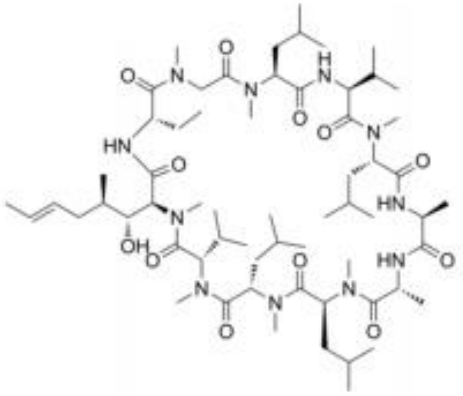
Maraviroc allosterically inhibits CCR5

Mol. Pharmacol. **2008**, 73, 789

PPI stabilizers



FK506



Cyclosporin A

FK506 and Cyclosporin A (CsA) are known as immunosuppressive natural products

Their binding proteins were identified and named FKBP and Cyclophilin A (CyPA)

Science, **1984**, 226, 544
Nature, **1989**, 341, 755

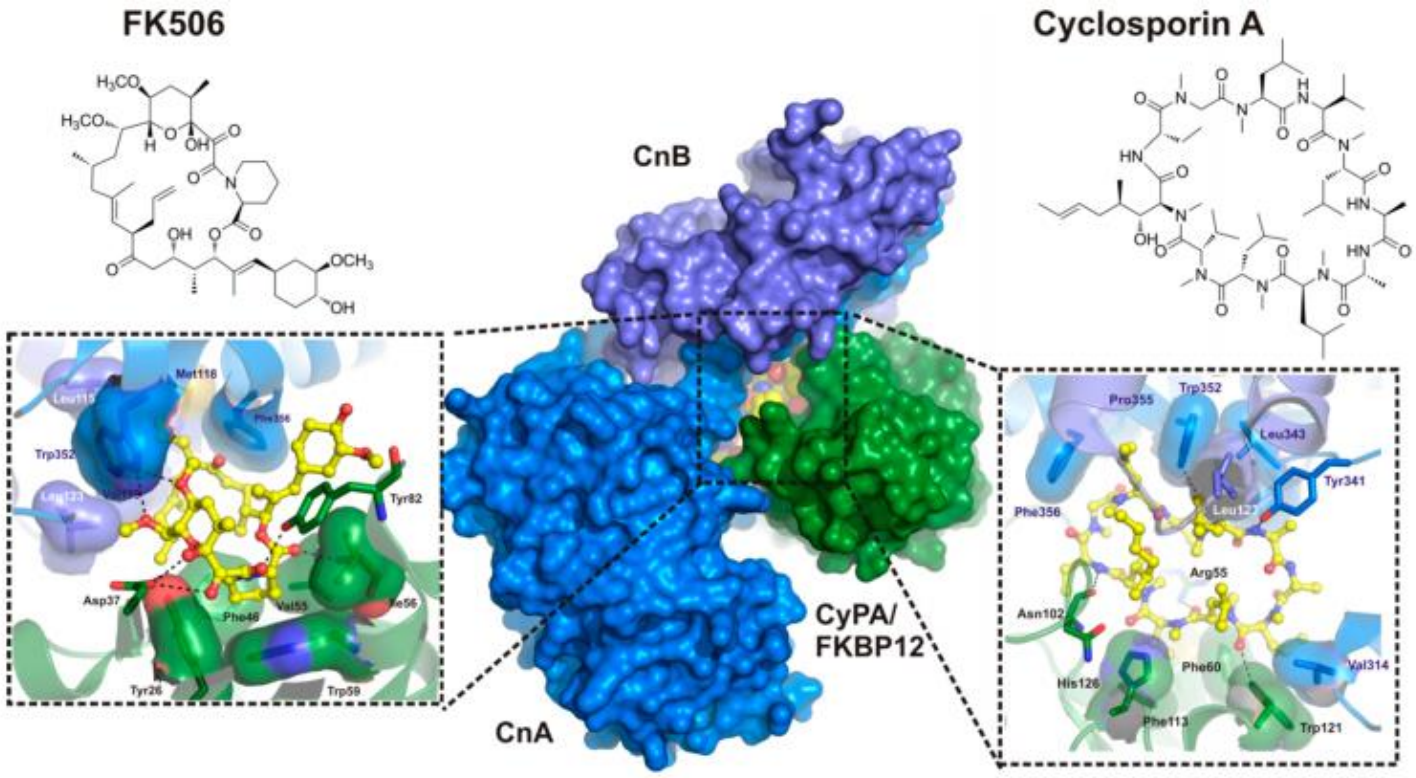
Crystal structure of complex revealed they occupy enzyme active sites

Science, **1991**, 252, 839
Nature, **1993**, 361, 91

However, inhibition of enzymatic activity alone couldn't explain immunosuppression

PPI stabilizers

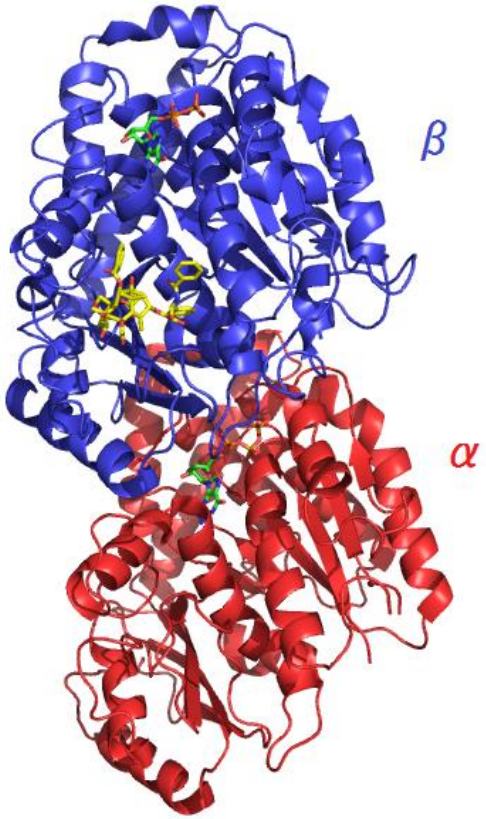
CyPA-CsA (FKBP-FK506) binds to Calcineurin subunits CnA and CnB
CsA and FK506 stabilize PPI and inhibit Cn phosphatase activity

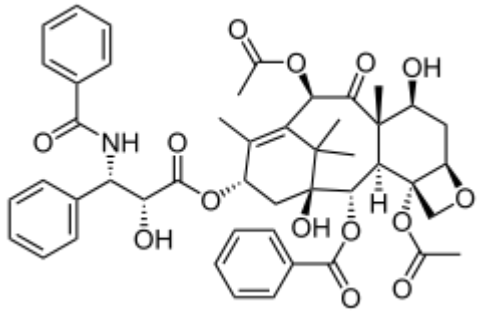


Cell, **1991**, 66, 807

Natural PPI stabilizer

Paclitaxel: isolated from *Taxus brevifolia*





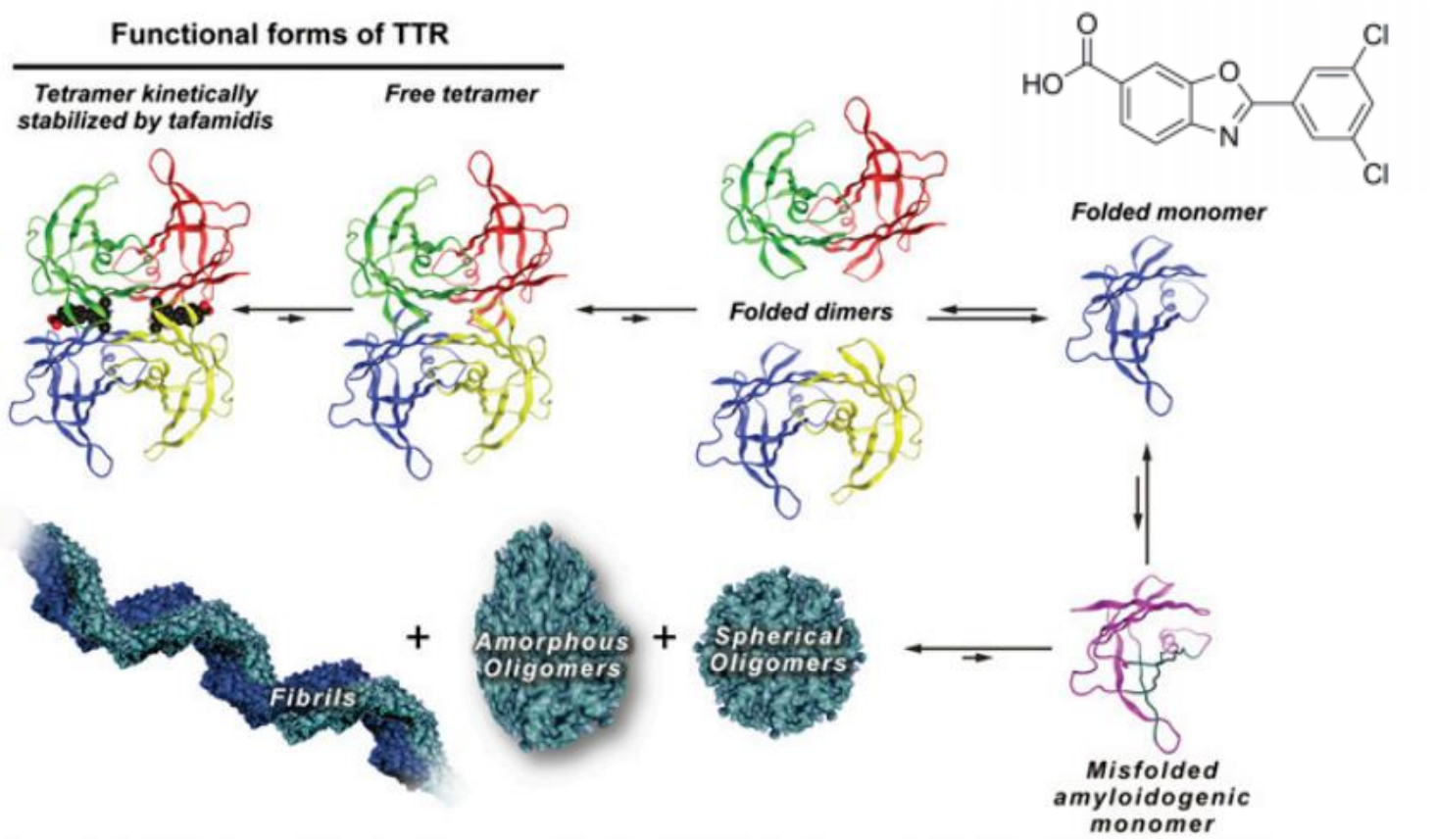
Paclitaxel allosterically stabilize tubulin heterodimer
Suppressed dynamicity of tubulins induces abnormal mitosis and leads to cell death

Paclitaxel works as anticancer drug

PNAS, **2006**, 103, 10166

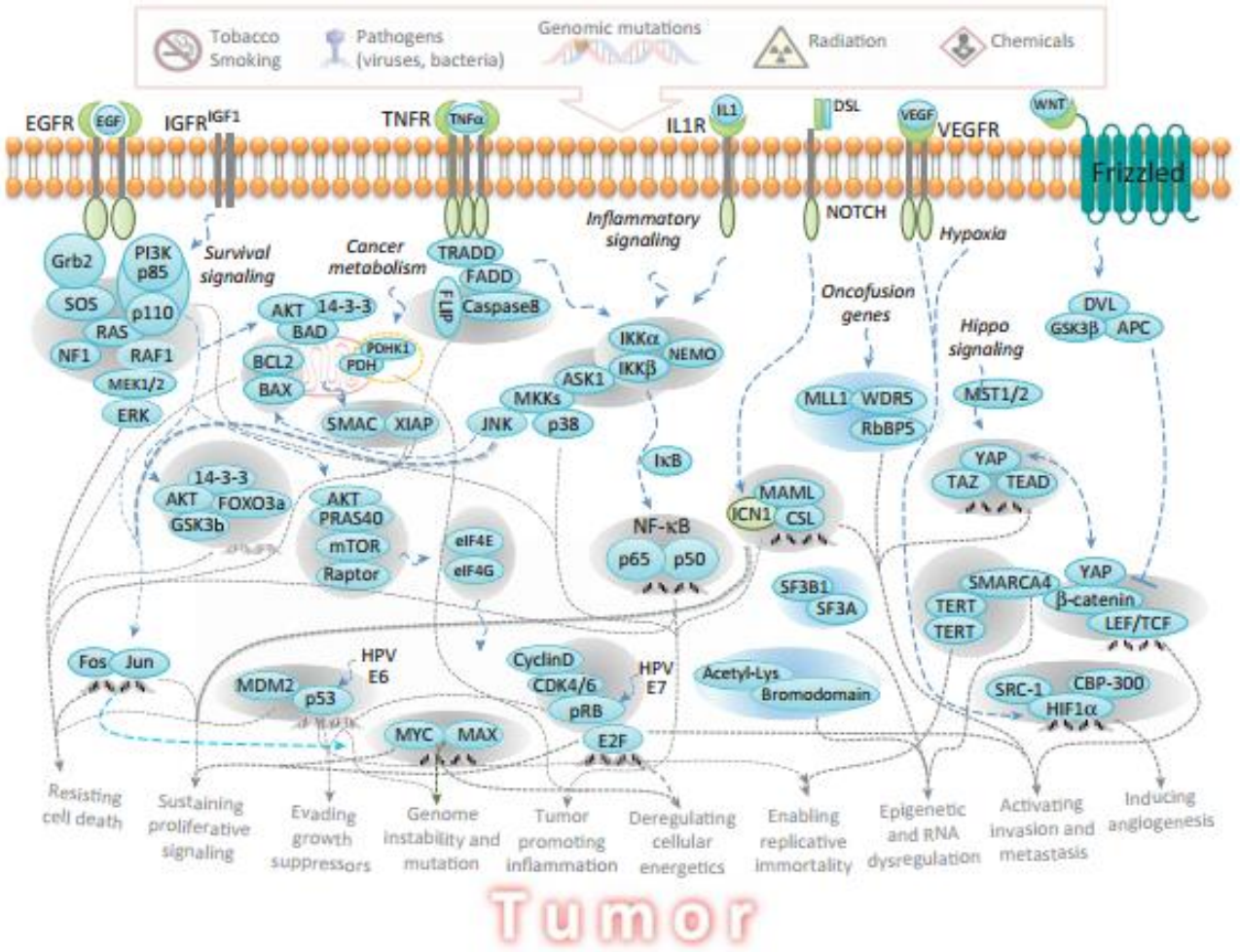
Small-molecule PPI stabilizer

Tafamidis (Vyndaqel®, Pfizer): first-in-class transthyretin stabilizer to treat ATTR
Approved by the European Medicines Agency in 2011 (Japan in 2013)



PNAS, **2012**, 109, 9629

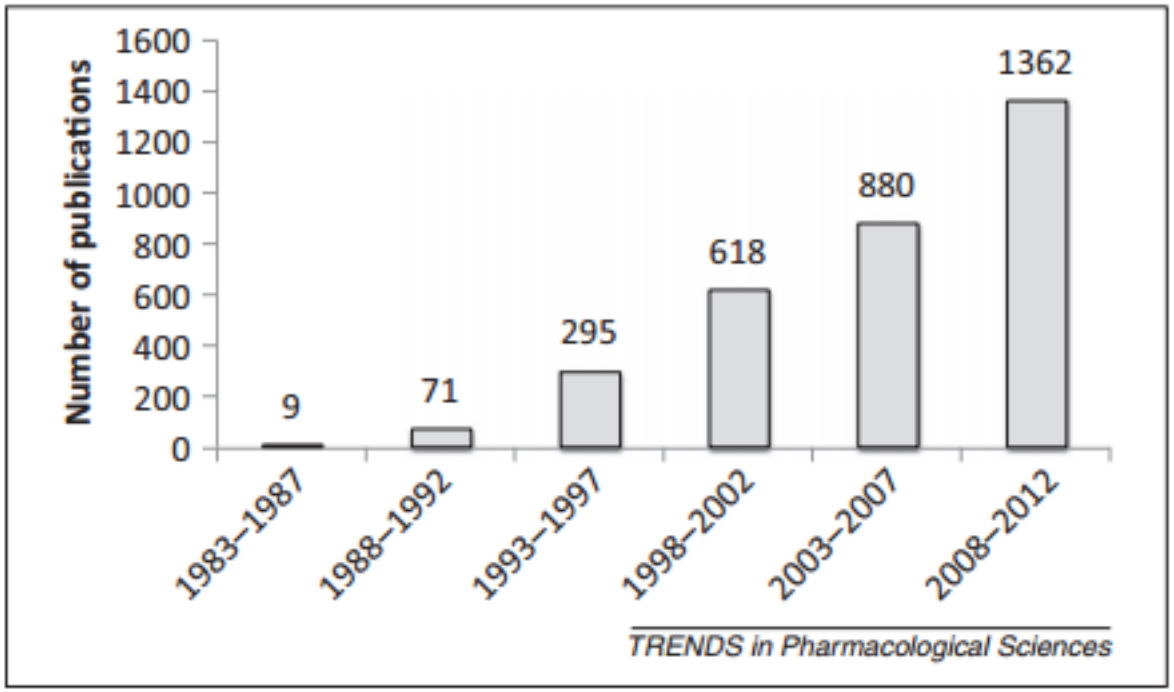
PPIs in oncogenic signaling networks



PPIs play essential roles in relaying oncogenic signals

Trends. Pharmacol. Sci. **2013**, 34, 393

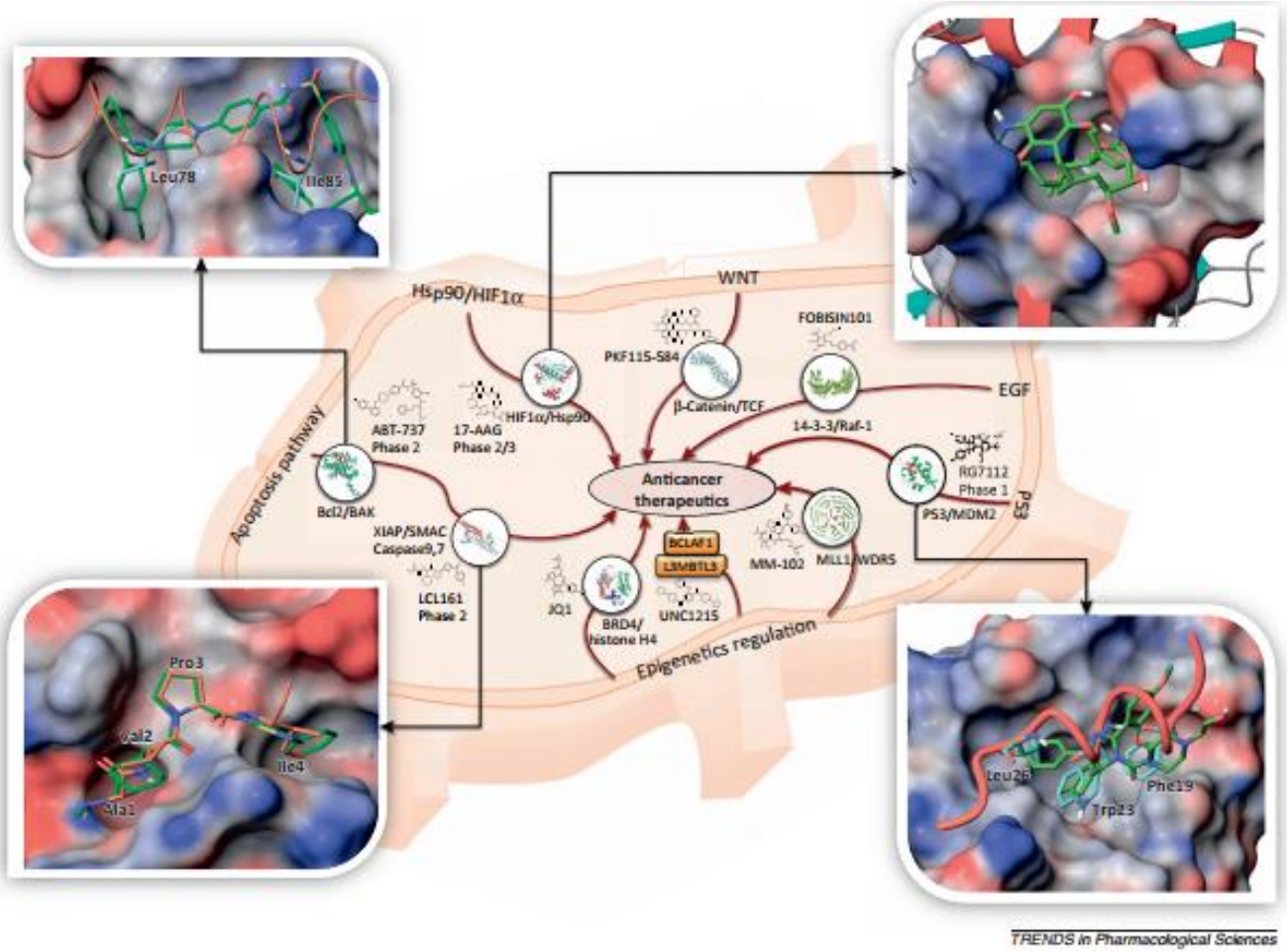
Rising interest in targeting PPIs



Interest in targeting PPIs as anticancer strategies has increased
PPI is a higly promising target for anticancer therapeutics

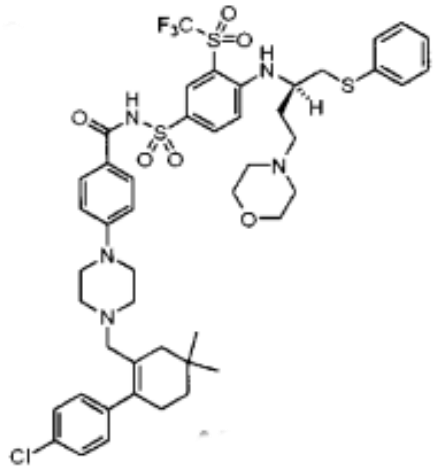
Trends. Pharmacol. Sci. **2013**, 34, 393

Approaches toward anticancer therapeutics



Trends. Pharmacol. Sci. **2013**, 34, 393

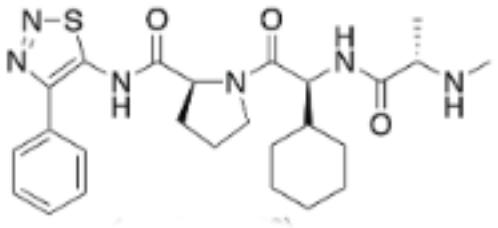
Clinical anticancer PPI modulators



Bcl-2 proteins inhibitor

Navitoclax

Phase Ⅱ for CLL treatment

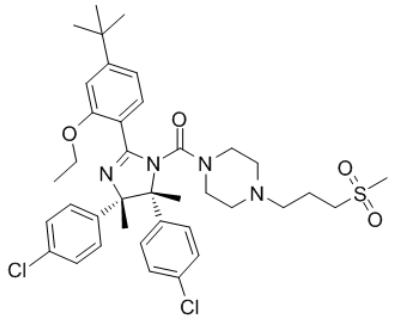


XIAP inhibitor

GDC-0152

Completed Phase Ⅰ

safety/pharmacokinetic evaluation

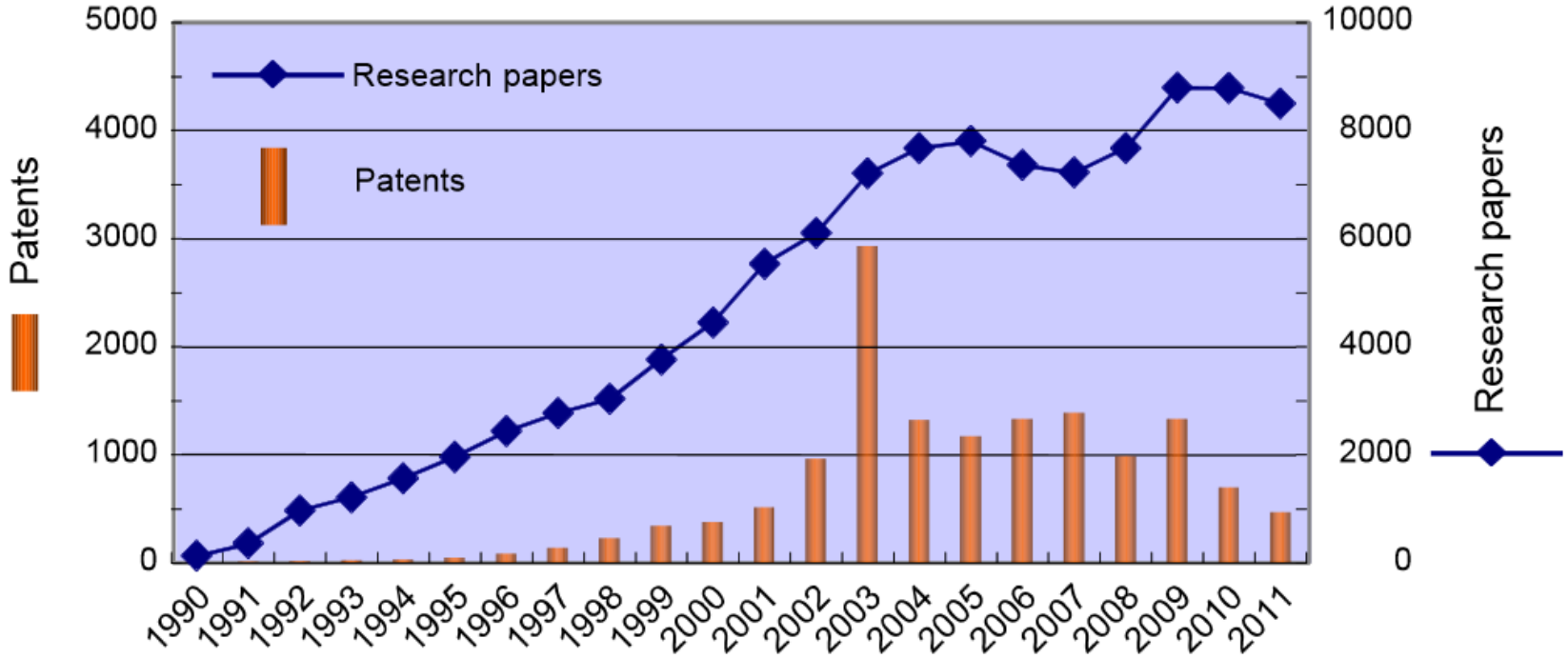


MDM2 inhibitor

RG-7112

Phase Ⅰ for both solid and hematologic malignancies

Progress in PPI researches



The last 10-15 years has been significant progress in PPIM development

Bio ventures focused on PPIM development

Interprotein

Interprotein Corporation is drug discovery company, especially we are involving with early stage drug discovery. And we are focusing on the discovery research of synthetic small-molecule based on *in silico* drug design (INTENDD) and synthetic peptide based on helix-loop-helix micro antibodies technology for targeting protein-protein interaction (PPI) modulations as innovative therapeutics to meet high unmet medical needs.

http://www.interprotein.com/

http://www.prismbiolab.sakura.ne.jp/

Bio ventures focused on PPIM development

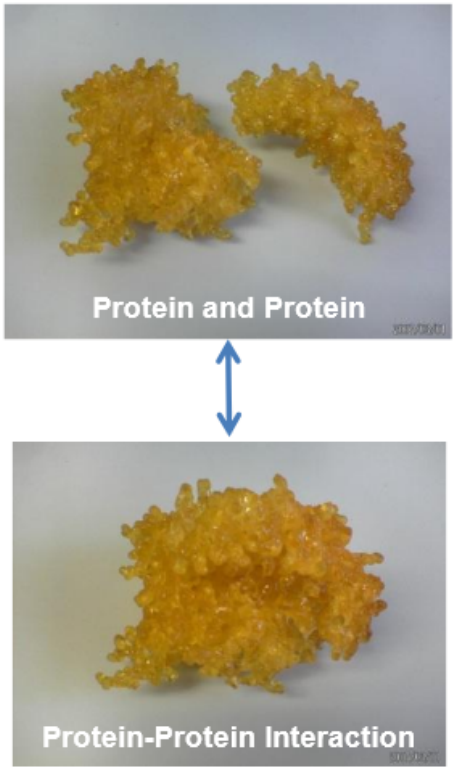
2012年7月	免疫生物研究所	IBL-International（独）	ライセンス契約及び共同開発契約	アルツハイマー病に関与する「アミロイドbタンパク質」測定診断薬の共同開発契約，原料抗体および測定キット製造のためのノウハウをライセンスアウト
	アンジェスMG	田辺三菱製薬	ライセンス契約（基本合意）	HGF遺伝子治療薬の米国における末梢性血管疾患
	ペプチドリーム	第一三共	マルチターゲット探索契約	ペプチド医薬の候補化合物を創製
2012年6月	ツーセル	中外製薬	ライセンス契約（優先交渉権）	滑膜由来MSCを用いた軟骨再生医療，株式引受契約
	ノーベルファーマ	Pfizer	ライセンス契約	シロリムス（技術導入）
2012年3月	ナノキャリア	エーザイ	共同研究開発	ミセル化ナノ粒子技術
	オンコセラピー・サイエンス	塩野義製薬	ライセンス契約	5種の「オンコアンチゲン」由来のペプチドワクチン（ペプチドカクテルワクチン）（2009/2/2～）適用拡大と権利譲渡
	セルシード	テルモ	基本合意	ヒト骨格筋筋芽細胞シートを用いた心筋再生治療の実用化を目指した基本合意書を取り交わす
2012年1月	免疫生物研究所	タカラバイオ	販売契約	日本国内における研究用試薬製品の販売および抗体作製などの受託サービスの提供，販売契約を締結
	ノーベルファーマ	アストラゼネカ	製造販売承認	抗ウイルス化学療法剤ホスカビル®注（技術導入）
2011年12月	インタープロテイン	武田薬品工業	分子設計に関する契約	INTENDD（Interprotein's Engine for New Drug Design）を用い，タンパク質間相互作用を制御する低分子化合物設計
2011年11月	リプテック	ヤクルト本社	独占的オプション契約	ヒト化モノクローナル抗体プログラム「LIV-2008」について，オプション権を行使する場合は，別途両社でライセンス契約を締結（全世界における独占的な開発・製造・販売権を取得）
				ヒトLIFを封入したプロテインビーズ胚性幹細胞や人工多能性

Bio ventures focused on PPIM development

INTENDD Interprotein's Engine for New Drug Design

I. Identify ligand binding sites and establish design strategy
   i. Use of **"real space 3D models"** in determining protein's ligand binding sites
   ii. Precise **3D model** plays a key role to find binding pockets for protein-protein interaction inhibitors.

II. SBSG (Structure-Based Scaffold Generation) method as original *de novo* drug design
   i. Formation of ligand skeletons search of ring positions SBSG provides high-quality population containing large number of hit compounds.
   ii. Promising compounds are selected by clustering and filtering process based on binding structure based mechanism (but not energy).
   iii. SBSG method achieves real *de novo* drug design.



http://www.interprotein.com/

Future Outlook

Effort aimed at developing PPIM will be accelerated by a number of recent advances

e.g., HTS, PPI-focused library, fragment discovery, *in silico* screening

Further understanding of PPI networks will lead to therapeutics for at present undruggable targets

e.g., Creutzfeldt-Jakob disease, Alzheimer's disease