Small molecule protein-protein interaction (PPI) modulators: challenges and opportunities for drug discovery & chemical biology

Emphasis on *in silico* approaches

Bruno O. Villoutreix, PhD

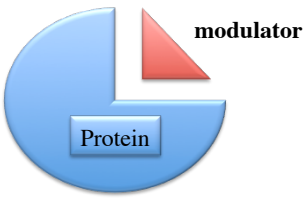
Strasbourg International Summer School in Chemoinformatics

23-27 June 2014

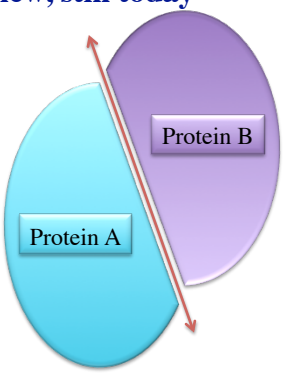
MTi

Modulation of PPIs with LMW cmpds ?

The traditional view, still today

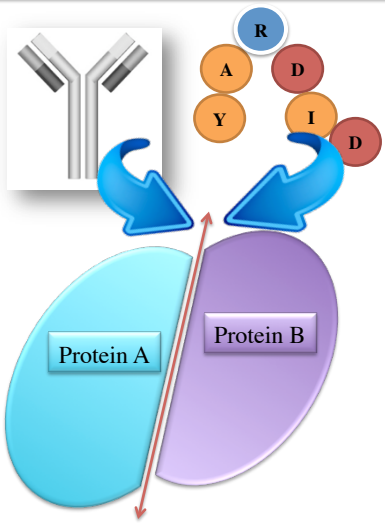


Enzymes, GPCRs..



In our mind:
PPIs → flat and large interfaces
→ can not be addressed by small cmpds

Modulation of PPIs with LMW cmpds ?



Thus, during many years, modulation of PPIs essentially with:

- ✓**Antibodies**
- ✓**Peptides**

Problems:
For some diseases, these molecules are very valuable… for others, it would be better to develop LMW PPI modulators

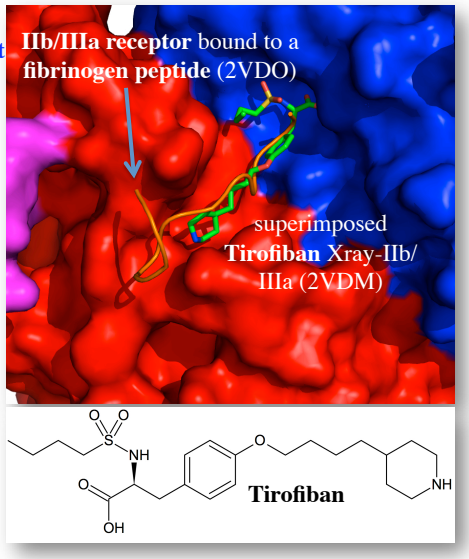
Modulation of PPIs with LMW cmpds ?

Indeed some PPI modulators are already on the market, but very few as of today

Tirofiban is a **reversible antagonist of fibrinogen binding to the GP IIb/IIIa receptor** (**also known as integrin $\alpha_{IIb}\beta_3$**), the major platelet surface receptor involved in **platelet aggregation**. It **prevents blood clotting**

Among the first drug whose origins can be traced back to a pharmacophore-based virtual screening design (1992) (RGD peptide mimic)

Hartzman et al. (1992), J Med Chem 35: 4640–4642



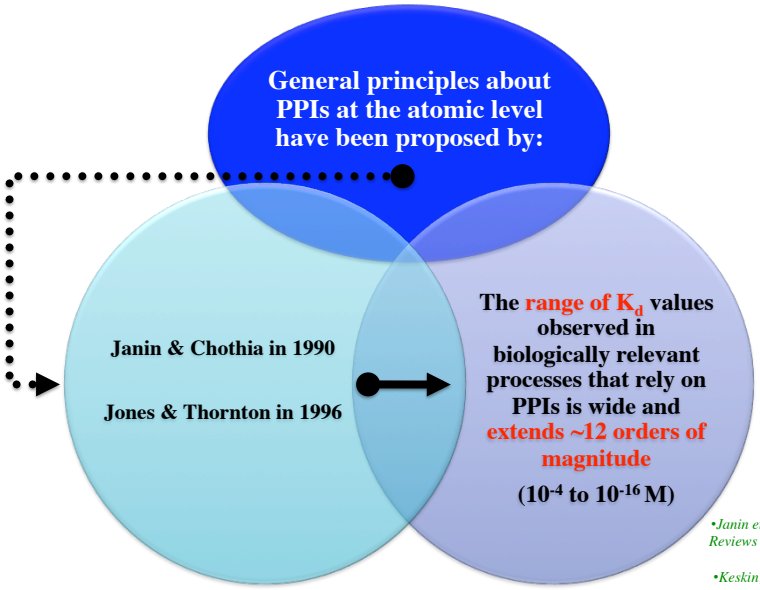
Outline for this presentation

Goal: try to summarize 30 years of worldwide PPI research in 30 min !!!

1. **Structural analysis of protein complexes**
2. **In silico "tools" to investigate PPIs**
3. **In silico design of LMW PPI inhibitors**
4. **Chemical probes acting on the anticoagulant protein C**
5. **Conclusions**

•Villoutreix et al., Molecular Informatics 2014
Thanks to Prof Schneider for the Strasbourg special issue

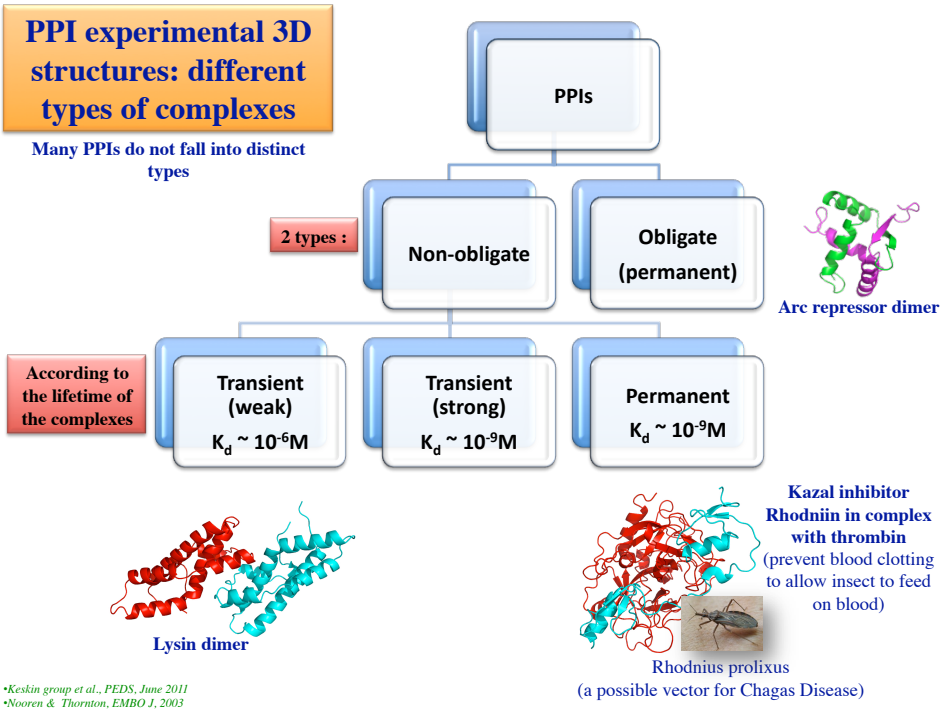
PPI experimental 3D structures



•Janin et al., Quaterly Reviews of Biophysics, 2008
•Keskin, Gursoy, Ma, Nussinov, Chem Rev 2008…

PPI experimental 3D structures: different types of complexes

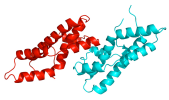
Many PPIs do not fall into distinct types



•Keskin group et al., PEDS, June 2011
•Nooren & Thornton, EMBO J, 2003

PPI experimental 3D structures: nature of the interface

Hotspots: an important information to design PPI modulators



•Within interface regions not all residues are equally important

•Concept of **hotspots**: the binding energy is not equally distributed among all amino acids participating in the interaction: **typically hotspots are defined as those residues contributing to around 1.5 to 2 kcal.mol^{-1} to the total binding energy of the complex. 9.5% of interfacial residues are hotspots**

•Clackson and Wells, Science. 1995 (human growth hormone and receptor)
•Moreira et al., Proteins, 2007, 68: 803

•Experimentally, often investigated by **Ala scan** (**possible in silico**) (warning, long-range effects can take place upon mutation to Ala)

•Morrow & Zhang, Curr Pharm Des 2012, 18:1255

•Hotspots: clusters of **conserved amino-acids** (evolutionary pressures)

•Hotspots often surrounded by a **O-ring** (Bogan & Thorn) that exclude solvent (**no bridging water** at the level of hotspots in general)

PPI experimental 3D structures: nature of the interface

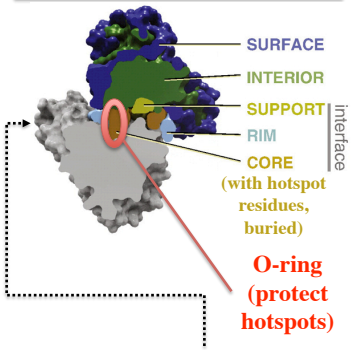
Transient interactions
The minimum protein surface that must be buried is ~1000 Å² (about 500 Å² by each protein, ~12 aa)
The interfaces are depleted in E, D, K & enriched in M, Y, W

The core comprises buried atoms and about 55% of all interface residues. This core region is enriched in aromatic residues and to a lesser extent, in aliphatic residues (but not Val, Ala and Pro). Arg residues can be present in both the core and the rim regions

The rim made of residues not fully buried, it has a composition close to the protein accessible surface

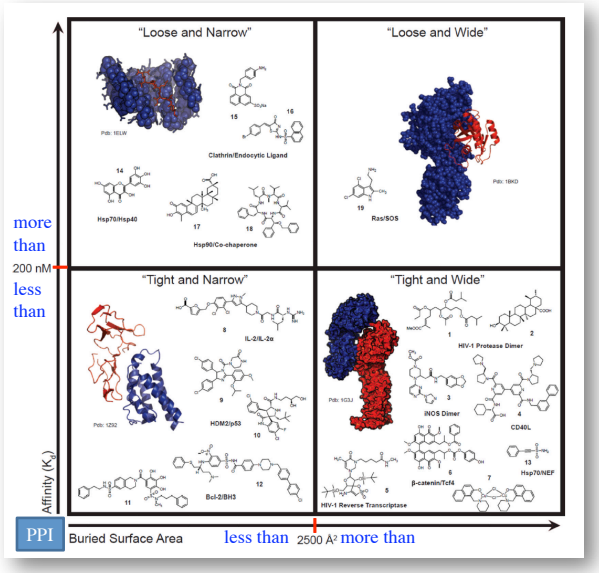
The so-called support zone seems similar in composition to the protein interior

•The core-rim-support model of protein-protein recognition & •The related O-ring model



•Levy, J Mol Biol, 2010
•Li et al., BMC Bioinformatics 2012, 13:51
•Bahadur….& Janin, Proteins, 2003
•Bogan & Thorn, J Mol Biol, 1998

PPI experimental 3D structures: druggable interface ? Structural analysis: different yet related opinions



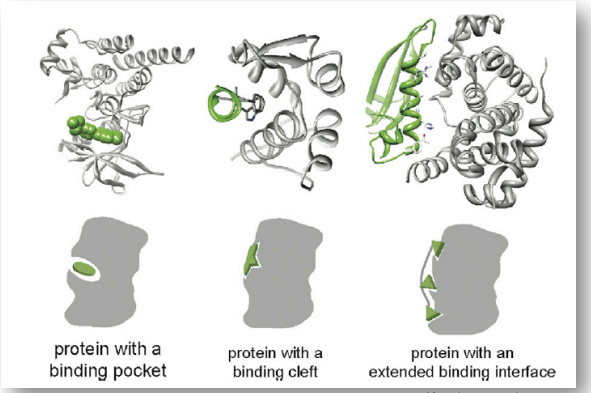
Relatively few examples of inhibitors in the "Loose and Wide" category

On average, compounds that inhibit "Tight and Narrow" PPIs have 10-fold better potency than those targeting "Loose and Narrow" contacts

•Matthew C. Smith and Jason E. Gestwicki, Expert Rev Mol Med. 14: e16. 2012. They use complexes from 2P2I and Timbal

PPI experimental 3D structures: druggable interface ?
Structural analysis: different yet related opinions

Secondary structure centric approach



The Gleevek/ tyrosine kinase (PDB code: 1XBB)	p53/MDM2 (PDB code: 1YCR)	cyclindependent kinase6/D-type viral cyclin (PDB code: 1G3N)
Regular targets	PPI	PPI

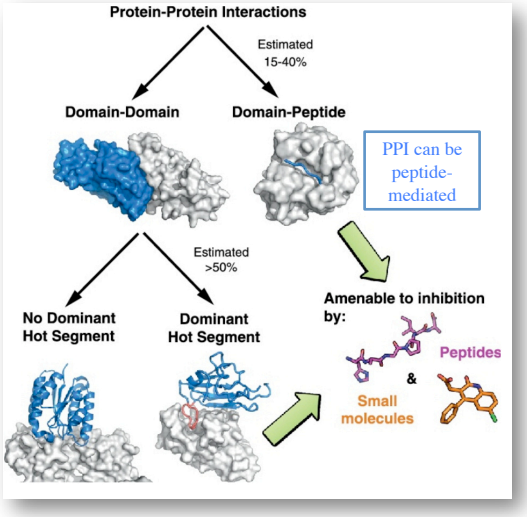
Helix-groove binders
The greatest success for HTS so far (presence of a clear pocket even if the cmpd collections are not yet optimized)

•Nero TL.. Parker MW Nature review Cancer 2014

•M Raj, BN. Bullock, PS. Arora Bioorganic & Medicinal Chemistry, 2012

PPI experimental 3D structures: druggable interface ?
Structural analysis: different yet related opinions

Hot segment



•London N, Raveh B, Schueler-Furman O. Curr Opin Chem Biol. 2013

1. Structural analysis of protein-protein complexes
2. **In silico "tools" to investigate PPIs**
3. In silico design of low molecular weight PPI inhibitors
4. Search for chemical probes: APC
5. Conclusions

www.vls3d.com

Store and organize tools collected during almost 15 years

•Villoutreix et al, Drug Discovery Today 2013

QR code

In silico tools that can be used to support the study of PPIs

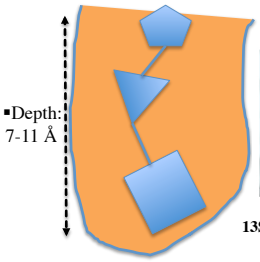
- **Prioritize a PPI target** (requires 3D in general)
  - **analyze** & **predict interface residues/regions**
  - find **hotspots**
  - predict the **3D structure of the complex**
  - investigate **flexibility** (and cryptic binding sites by MD or NMA)
  - find **binding pockets** and investigate **druggability**… pocket **similarity**
- **Prepare "ADME-Tox friendly PPI compound collections"**
- **Virtual screening "tuned for PPIs"**
- **Optimize** the molecules under different types of constraints

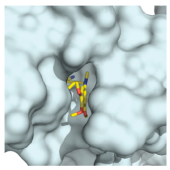
•Villoutreix et al., Molecular Informatics 2014

www.vls3d.com

In silico tools that can be used to support the study of PPIs: binding pockets

Cmpds have been essentially developed for regular pockets



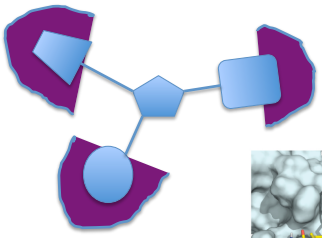


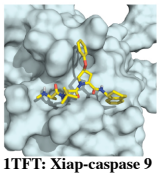
13SV: dihydrofolate reductase

Orthosteric (and somehow allosteric) pockets

- Hydrophobicity: 20-40% of polar surface area
- Surface area: 300-600Å^2
- Volume: ~500 Å^3

•Li et al, J Mol Graphics & Model, 2013
•Gao & Skolnick, Plos Comput Biol, Oct 2013
• Perot et al. DDT 2010



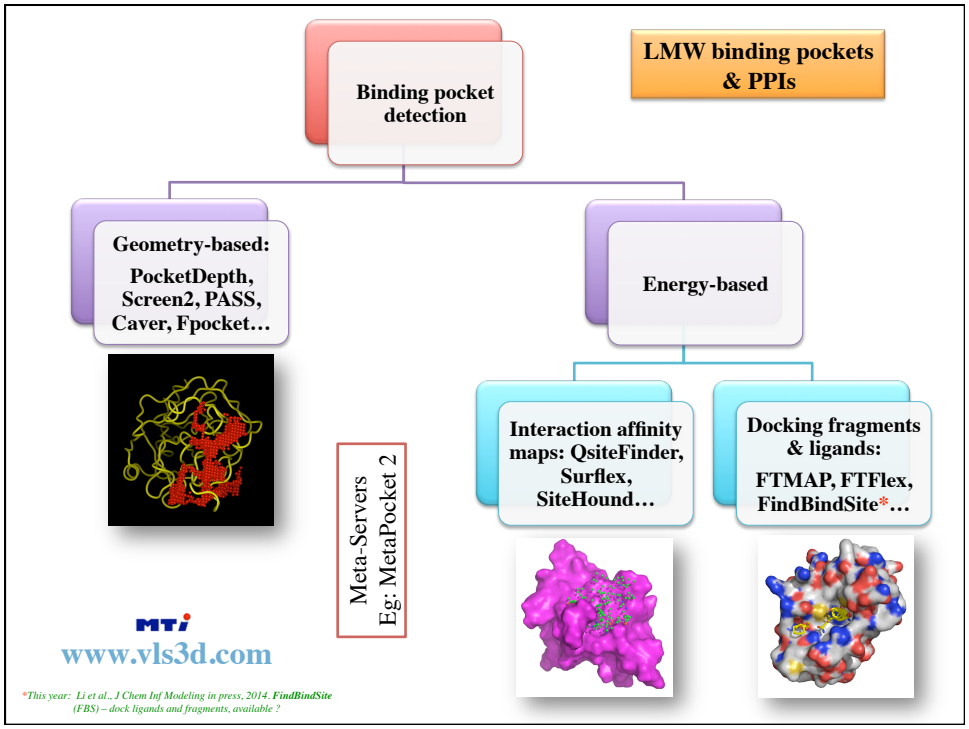


1TFT: Xiap-caspase 9

"PPI pockets"

- 3 to 5 subpockets, each ~ 54 Å^3

•Fuller et al., Jackson, DDT 2009



In silico tools that can be used to support the study of PPIs: binding pockets

The concept of **druggability**:

- It is important to find pockets but can they bind a LMW **drug-like molecule**?

- Complex because a system can be considered non druggable today but tomorrow, it might become druggable

- One can take **descriptors of binding pockets** (shape, size, hydrophobicity, polarity, depth, enclosure…) with co-crystallized drugs and pockets with ligands or only cavity, and search for differences, for instance use machine learning approaches to **develop a model that could predict "ligandability or druggability"**

- e.g., Volkamer et al., J Chem Inf Model 2012
- → **DoGSiteScorer (calibrated for regular targets/pockets)**

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	219.39	413.70	296.99	11.07	0.44
P1	180.54	423.27	207.11	10.45	0.36
P2	140.86	268.63	165.38	6.99	0.22
P3	124.61	261.51	162.02	11.33	0.35
P4	116.10	258.97	195.59	7.61	0.20
P5	113.22	270.07	117.47	7.40	0.21

http://dogsite.zbh.uni-hamburg.de/

PROF. DR. MATTHIAS RAREY

Center for Bioinformatics
University of Hamburg
Bundesstraße 43
20146 Hamburg
Germany

MTi

1. Structural analysis of protein-protein complexes
2. In silico tools to investigate PPIs
3. **In silico design of low molecular weight PPI inhibitors**
4. Search for chemical probes: APC
5. Conclusions

Some examples of PPI modulations

- **Orthosteric inhibition**: the LMW cmpd binds at a site that overlaps with the area interacting with the protein partner
- **Allosteric inhibition**: usually the cmpd binds away from the interface and induces changes (conformation, dynamics)
- **Interfacial binders**: the ligand binds to a pocket that is transiently formed and locks the complex in a nonproductive conformation
- **Stabilization** of PPIs (here also different mechanisms)

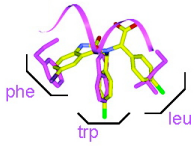
•Jin et al., Annual Rev Pharmaco Toxico, 54, 2014
•Ottmann et al., Angew. Chem. Int. Ed. 51, 2012 – 2018, 2012

PPI modulations: compound collections

- **HTS** is a key approach to find binders, but for PPIs **new compound collections** are needed (the hit rate for PPI is in general not as good as for regular targets). Presently, **compound collections contain essentially molecules for GPCRs, enzymes, ion channels,** even if some PPI dedicated collections are now available from some chemical vendors or from academia

•Macarron R et al. Impact of high-throughput screening in biomedical research. Nat Rev Drug Discov. 2011;10:188-95
•Sperandio et al., DDT 2010
•Zhang, Betzi, Morelli, Roche, Future Med Chem. 2014

For an easier PPI target (helix-groove) "p53/MDM2" HTS of 338,000 cmpds, 1216 hits, hit rate ~0.3



•Grasberger et al. J Med Chem 2005

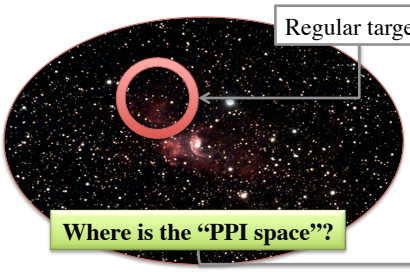
PPI modulations: regions of the chemical space?

- Observation of the cosmos suggest that there are about 10^{23} stars gathered into 10^{11} galaxies
- Do we have more LMW molecules < 500-600 than stars in the universe?
- Possibly yes

•Estimation of the size of drug-like chemical space based on GDB-17 data. Polishchuk PG, Madzhidov TI, Varnek A. J Comput Aided Mol Des. 2013; 27:675-9.

At present with 17 atoms and simple chemistry rules
→Virtual library of about 166 billion cmpds

•Ruddigkeit, van Deursen Ruud, Blum; Reymond. J. Chem. Inf. Model., 2012

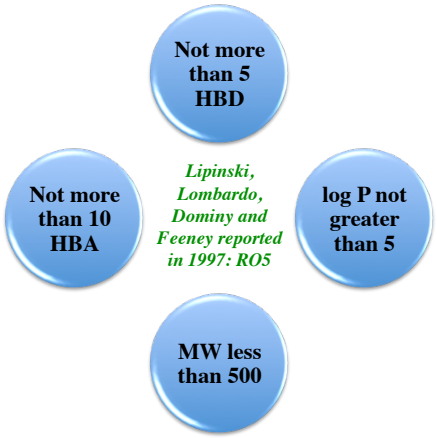


+

Warning, not all cmpds can be given to human

Need data to learn

PPI modulations: under ADMET restraints or constraints



These guidelines meant that **a molecule whose properties fell outside these boundaries would be less likely orally absorbed**
It was stated at that time that a compound with **two parameters out of range would be subject to an alert** but that the molecule should not be rejected without further investigations (**often now 1 violation or zero**). Some drugs do not follow these rules and are yet orally available but this does not prevent considering the rules as they have to be understood: **guidelines not rigid dogma**

PPI modulations: under ADMET restraints or constraints…

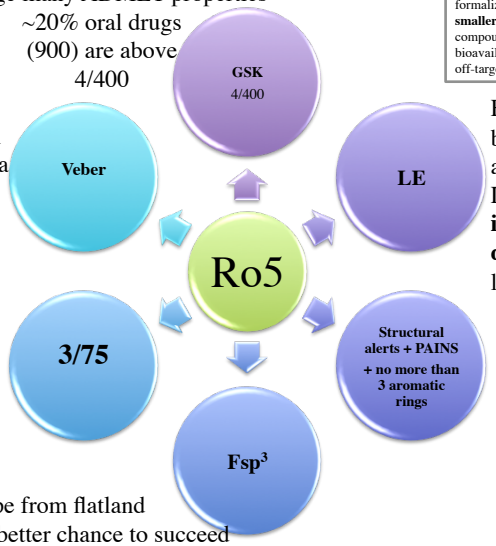
Too many rules kill the rules ? Where are iPPIs?

Damage many ADMET properties
~20% oral drugs (900) are above 4/400

Compounds that are **orally bioavailable** in rats (Polar Surface Area (tPSA) ≤ 140 Å^2 and no. rotatable bonds ≤ 10)

Sixfold increase in toxicity *in vivo* (24-fold for bases) when logP > 3 and tPSA < 75 Å^2

Escape from flatland (complexity), better chance to succeed

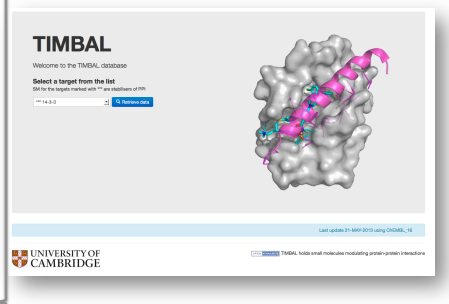


What many of these guidelines really formalize is that it is **better to start with a smaller and less lipophilic molecule** if the compound is intended to be orally bioavailable (but also to reduce binding to off-targets & anti-targets)

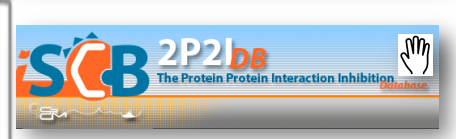
Free energy of binding per heavy atom…LLE…
Lead **optimization increases MW by 60 da** on average and log P

•Baell, J Med Chem 2010
•Lovering et al J Med Chem 2009
•Oprea J Chem Inf Comput Sci 2001
•Ritchie & Macdonald DDT 2009, …
•Kuntz et al., PNAS 1999
•Shultz, Bioorg Med Chem letters 2013

Need data to learn

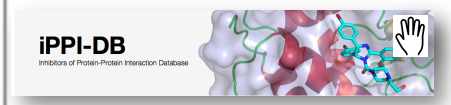


TIMBAL is a database containing small molecules that modulate protein-protein interactions. It was first created in 2008, by manually curating information extracted from relevant scientific publications. An analysis of the data was published in 2009, (*Higueruelo et al, 2009, Blundell Lab, Chem Biol & Drug Design*). The growth of data in the past years makes hand-curated databases a phenomenally time-consuming task. The maintenance of TIMBAL is done now through automated searches on the ChEMBL database (about 8000 cmpds)



2P2I$_{DB}$ is a hand-curated database dedicated to the structure of protein-protein complexes with known small molecule inhibitors. We have gathered from the PDB, about 200 small molecule inhibitors found at the interface

•Basse et al, NAR, 2013; Bourgeas et al, PlosOne 2010, (Morelli's lab)



iPPI-DB contains 1650 non-peptidic inhibitors (iPPI) across 13 families of Protein-Protein Interactions. The chemical structures, the physicochemical and the pharmacological profiles of these iPPI are manually extracted from the literature

•Labbé et al. Drug Discov Today 2013

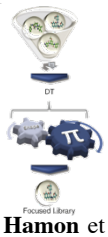
General trends: Higher MW, higher log P, more aromatic, more three-dimensional… Yet, some are "acceptable to start with" (60% from the iPPI-DB, about 950 molecules pass the RO5, 1 violation) & with new knowledge we can improve the physchem properties

PPI modulations: filters → focused collections

Learning from databases or datasets + silico modeling:

- 2007: **Neugebauer** et al, (25 iPPIs & 1135 drugs as non-PPIs). **Decision tree with 3 descriptors**: shape, ester function, 3D of the molecule

- 2010: **Reynes** et al., (66 diverses iPPIs & 557 regular oral drugs). **Decision tree**: Specific molecular shape and a critical number of 15 multiple bonds → Development of **PPI-HitProfiler** (available & online)



New more "ADMET"-friendly filters

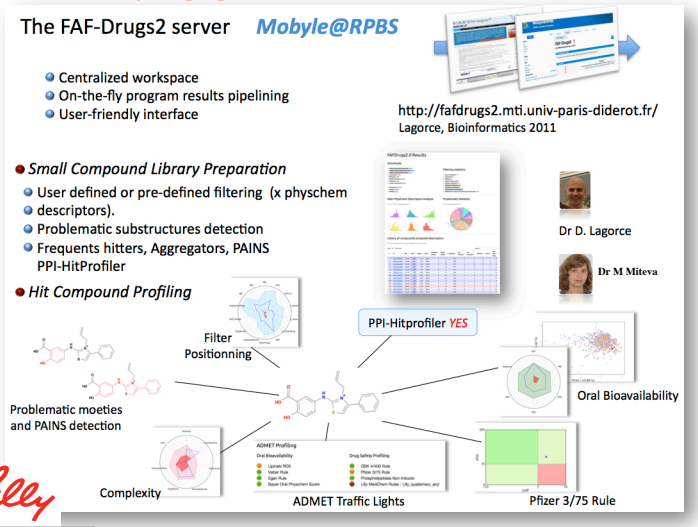
(see poster Kuenemann, Sperandio et al. 2014)

- 2013: **Hamon** et al., **2P2I$_{hunter}$**, **SVM** filter (40 diverse iPPIs & 1018 non-PPIs from the NCI diversity set, performed with 10 molecular descriptors)

•Neugebauer et al., J Med Chem 2007
•Higueruelo et al., Chem Biol Drug Des 2009
•Reynes et al. DDT 2010
•Sperandio et al. Plos Comput Biol 2010
•Villoutreix et al. Curr Pharm Des 2012
•Hamon et al, J R Soc Interface 2013
•Brooke Bullock Lao et al, JACS, helix mimics, 2014-2014

PPI modulations: users can prepare a collection online

For the time being we propose:

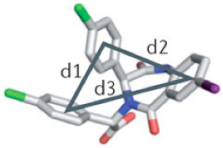


+ the E. Lilly filter

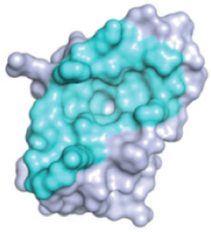
•The FAF-Drugs2 server: a multistep engine to prepare electronic chemical compound collections. Lagorce D, Maupetit J, Baell J, Sperandio O, Tufféry P, Miteva MA, Galons H, Villoutreix BO. Bioinformatics. 2011; 27:2018-20

PPI modulations: VLS

- **VLS screening approaches applied with some success to PPIs**

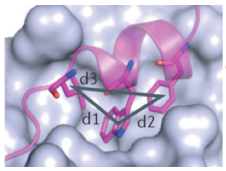


•**Compound-based pharmacophore** screen (NB: concept of pharmacophore first introduced in 1909 by Ehrlich)



•**Receptor-based virtual screening (drop of about 10% or more in success** when compared to docking-scoring on regular targets, as the **compounds are more at the surface and difficulties with scoring)**

•D. M. Kruger, G. Jessen and H. Gohlke, J Chem Inf Model, 2012
•Ragul Gowthaman, Eric J. Deeds and John Karanicolas. J Chem Inf Model 2013
•Falchi et al. Future Med Chem 2014



•**Receptor-based pharmacophore** screen

•Voet et al., Current Topics in Medicinal Chemistry, 2013, Vol. 13, No. 10
•Nero et al, Nat Rev Cancer 2014, 14: 248-62

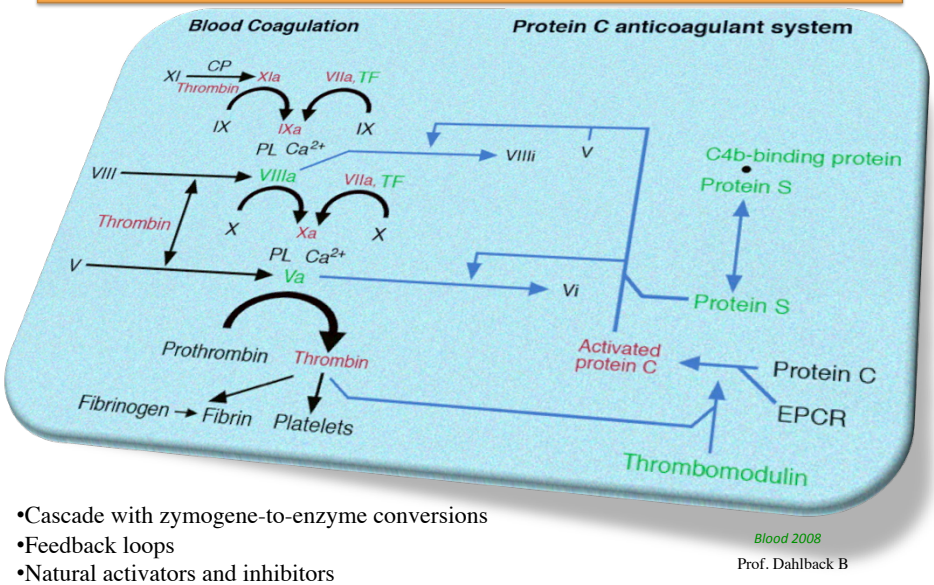
MTi

1. Structural analysis of protein-protein complexes
2. Other *in silico* tools to investigate PPIs
3. In silico design of low molecular weight PPI inhibitors
4. **Search for chemical probes: APC**
5. Conclusions

•Identification of novel small molecule inhibitors of activated protein C

Sperandio O; Wildhagen K; Schrijver R; Wielders S; Villoutreix B; Nicolaes G
Thrombosis Research 2014

Inhibiting protein-protein interaction: Anticoagulant protein C



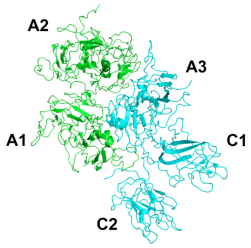
Prof. Dahlback B

- Cascade with zymogene-to-enzyme conversions
- Feedback loops
- Natural activators and inhibitors

Inhibiting protein-protein interaction: Anticoagulant protein C

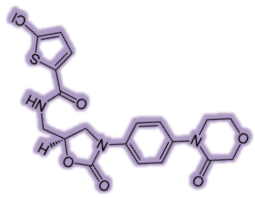
The therapeutic principle used for treatment of bleeding disorders such as **hemophilia** is to **supplement** the missing coagulation factor

E.g. Hemophilia A

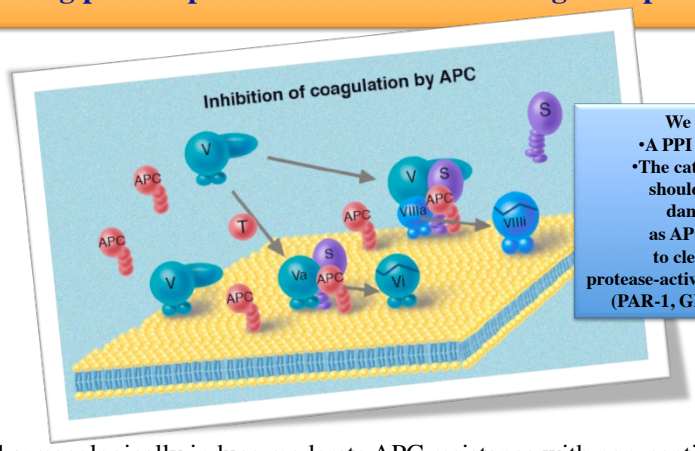


Whereas **inhibition of coagulation factors** is the dominating approach for treatment of **thrombosis**

E.g., Thrombosis



Inhibiting protein-protein interaction: Anticoagulant protein C



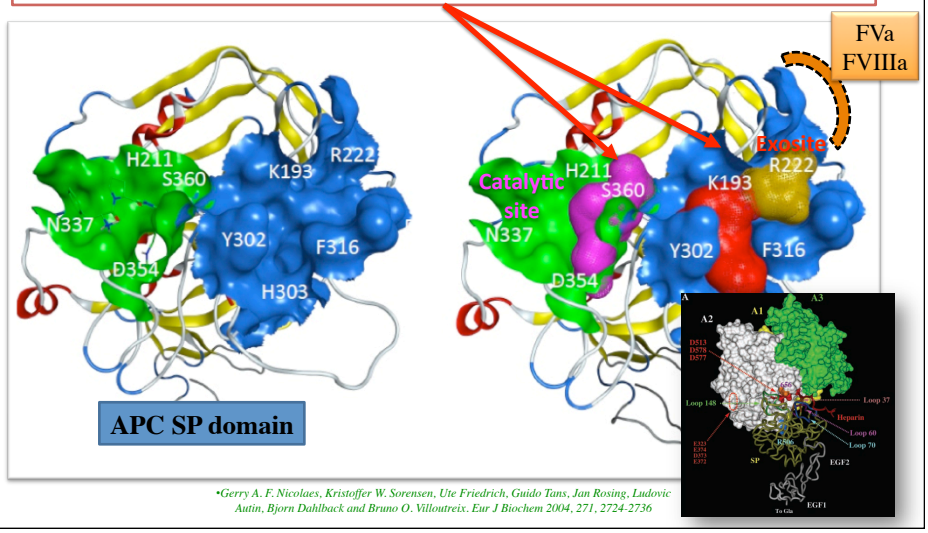
We need:
- **A PPI inhibitor**
- **The catalytic site should not be damaged as APC needs to cleave the protease-activated receptor 1 (PAR-1, GPCR related)**

Goal: pharmacologically induce moderate APC resistance with non-peptidic small molecules acting outside the catalytic site for **patients at risk of bleeding**.
Eg, reduce the inactivation of FVa by preventing PPI with APC. **May give rise to increased thrombin generation** (valuable in plasma from hemophiliacs, as antidote..)

•***Dahlbäck B, Villoutreix BO***. *Arterioscler Thromb Vasc Biol. 2005;* ***Dahlbäck B***. *Blood. 2008;* ***Griffin*** *et al, J Thromb Haemost 2007*

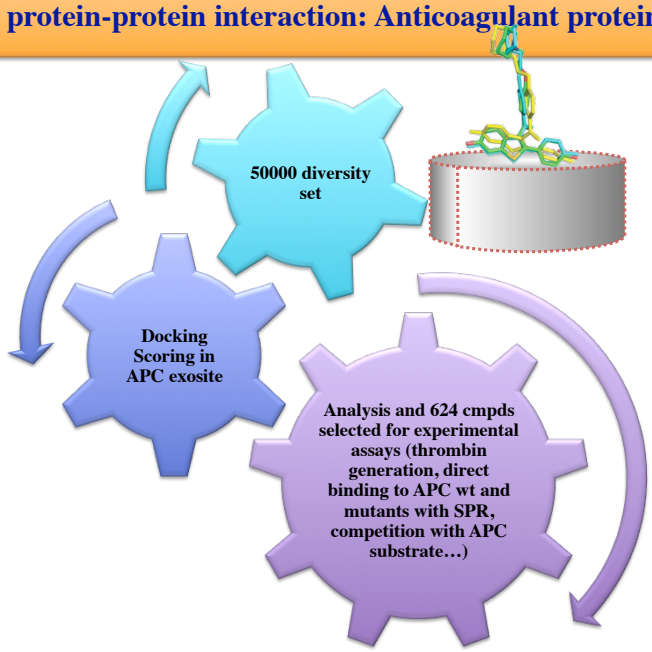
Inhibiting protein-protein interaction: Anticoagulant protein C

APC druggable pockets ? Docking, find cavities, probe mapping algorithms, fragment docking, simulation, side chain flexibility…

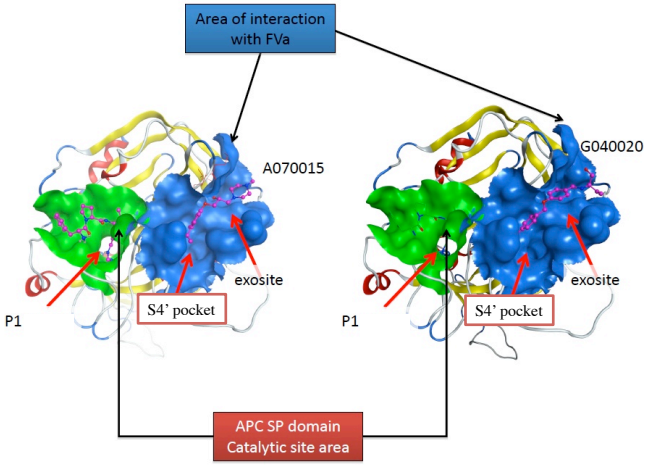


•*Gerry A. F. Nicolaes, Kristoffer W. Sorensen, Ute Friedrich, Guido Tans, Jan Rosing, Ludovic Autin, Bjorn Dahlback and Bruno O. Villoutreix. Eur J Biochem 2004, 271, 2724-2736*

Inhibiting protein-protein interaction: Anticoagulant protein C



Inhibiting protein-protein interaction: Anticoagulant protein C



Direct binding of the best hits to mutant APC (exosite) revealed that molecules presented a 100-500 fold lower affinity for the variant suggesting that these cmpds bind to this region of APC

Conclusions

- *In silico* tools have played a major role since the outset of interactomics, providing ways to **store**, **integrate**, **cure**, **visualize**, **analyze** and **predict** interactions
- *In silico* tools help to prioritize a PPI target (**analyze** & **predict interface regions**, find **hotspots**, predict the **3D structure of the complex**, investigate **flexibility**, find **binding pockets** and investigate **druggability**)
- *In silico* tools help to **design "PPI compound collections"**
- **Virtual screening can be used for PPIs, yet docking-scoring not as good as for regular targets**
- Combining in silico and experimental approaches → many success stories in term of "binders", **some starting hits for APC**
- Today some molecules are in advanced stages with about 20 LMW PPI cmpds in phase I to III clinical trials. **Expected sales worldwide (to start with) of over $800 million/each year within 5 years**, first in cancer & autoimmune diseases (to replace expensive mAbs and other biologics or to combine with other molecules)

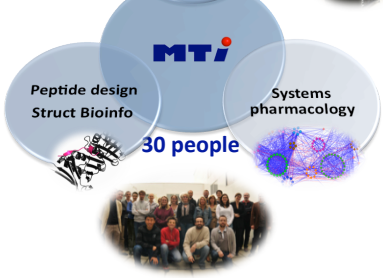
MTi Research Unit

Inserm

MTi

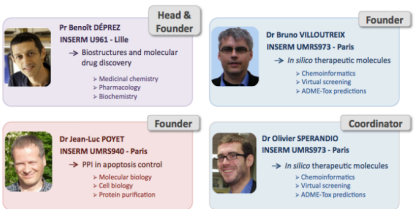
University Paris Diderot
650 m²

RPBS-MTI
958 64-Bits CPU core-linux computer cluster
2x15 Tb data storage facility



Sorbonne Paris Cité campus:
4 Universities + 4 Institutes in Paris 120,000 students
12,000 scientists in Life and Health Sciences
23 hospitals and 12,000 hospital beds

Doctoral School MTCI
Master Drug Design ISDD



CDithem Platform
www.CDithem.com

Acknowledgements

ADMET - PAINS

AUSTRALIA
Dr. **Baell** J

Coagulation

NETHERLANDS
Maastricht
Dr. **Nicolaes** G (and his group)
Wildhagen K; Schrijver R;
Wielders S

Coagulation

SWEDEN
Dr. **Dahlback** B

PPI & silico & applications

Dr. Miteva M' s team
Dr. **Lagorce** D
Dr. **Sperandio** O
Dr. Poyet JL
Dr. Bruzzoni H
Labbe C
Kuenemann M

PPI

Dr. **Laconde** G, Dr. **Reynes** C

Dr. **Galons** H

ADMET

The team of Dr. Tuffery
Dr Maupetit J
The team of Dr. Camproux &
Taboureau