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2169-8

Conference on Molecular Aspects of Cell Biology: A Perspective from Computational Physics

11 - 15 October 2010

From in silico Demystification of GPCR Structure to the Design of New GPCR Ligands: The Human A3 Adenosine Receptor as a Key Study

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“I love adenosine receptors!”

Stefano Moro

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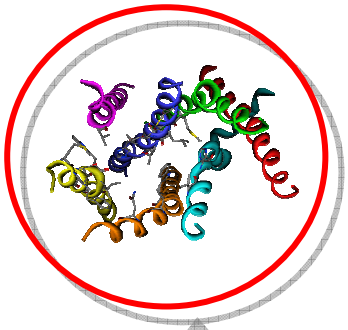
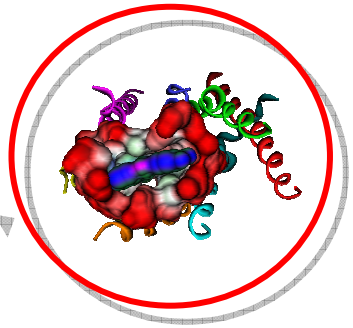
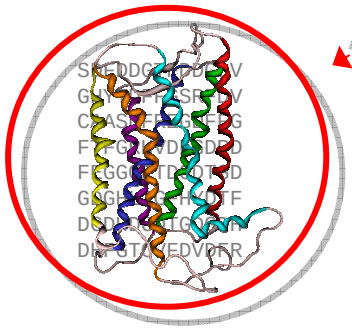
Menu del giorno (turistico):



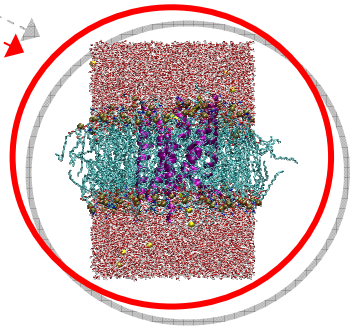
GPCR Mol Pharmacol



GPCR Lig Discovery



GPCR CompuBiology



GPCR Biophysics

"*Virtually every physical process in the body is controlled to one extent or another by G protein-coupled receptors, and about 50% of all medications work to modulate the activity of GPCRs*, " Krzysztof Palczewski, University of Washington, Seattle."

"*GPCRs represent the largest family of receptors for hormones and neurotransmitters, and therefore the largest group of targets for pharmaceutical therapeutics. These proteins are consequently very interesting from a physiologic perspective, and there is hope that a better understanding of GPCR structure will lead to more efficient development of drugs for a very broad spectrum of diseases.*" Brian Kobilka, Stanford University.

Basic GPCRs Principles

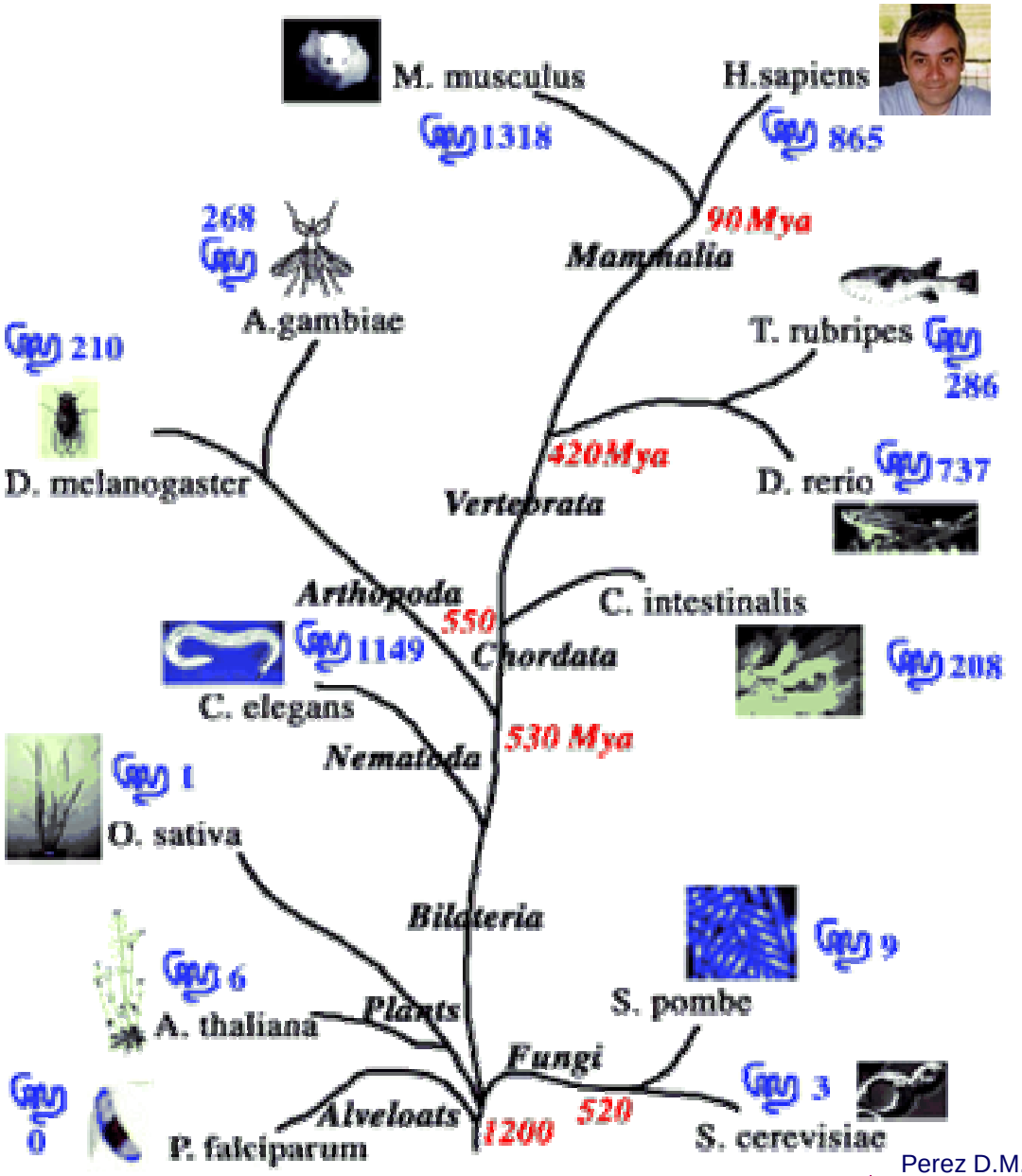
The GPCR super family includes several thousand (600 ÷ 800 into human cell) distinct but related *membrane* proteins.

They are found in a wide range of organisms and are involved in the transmission of signals across *membranes*.

Over 80% of all hormones signal using these types of receptors.

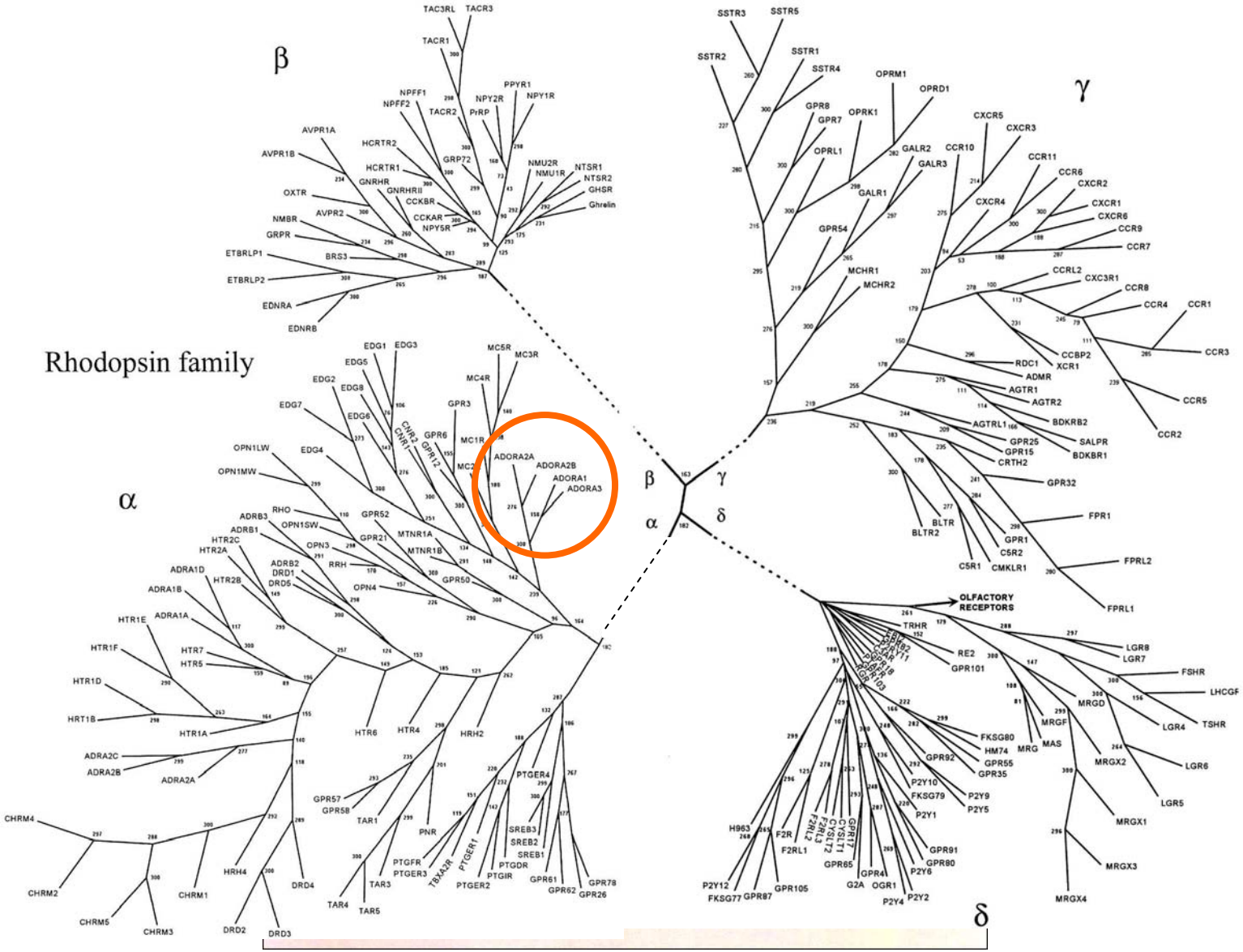
Although the receptors are *conserved* in structure, the ligands span a large range of vastly diverse entities from protein, peptides, small molecules, and light.

GPCR "Tree of life"

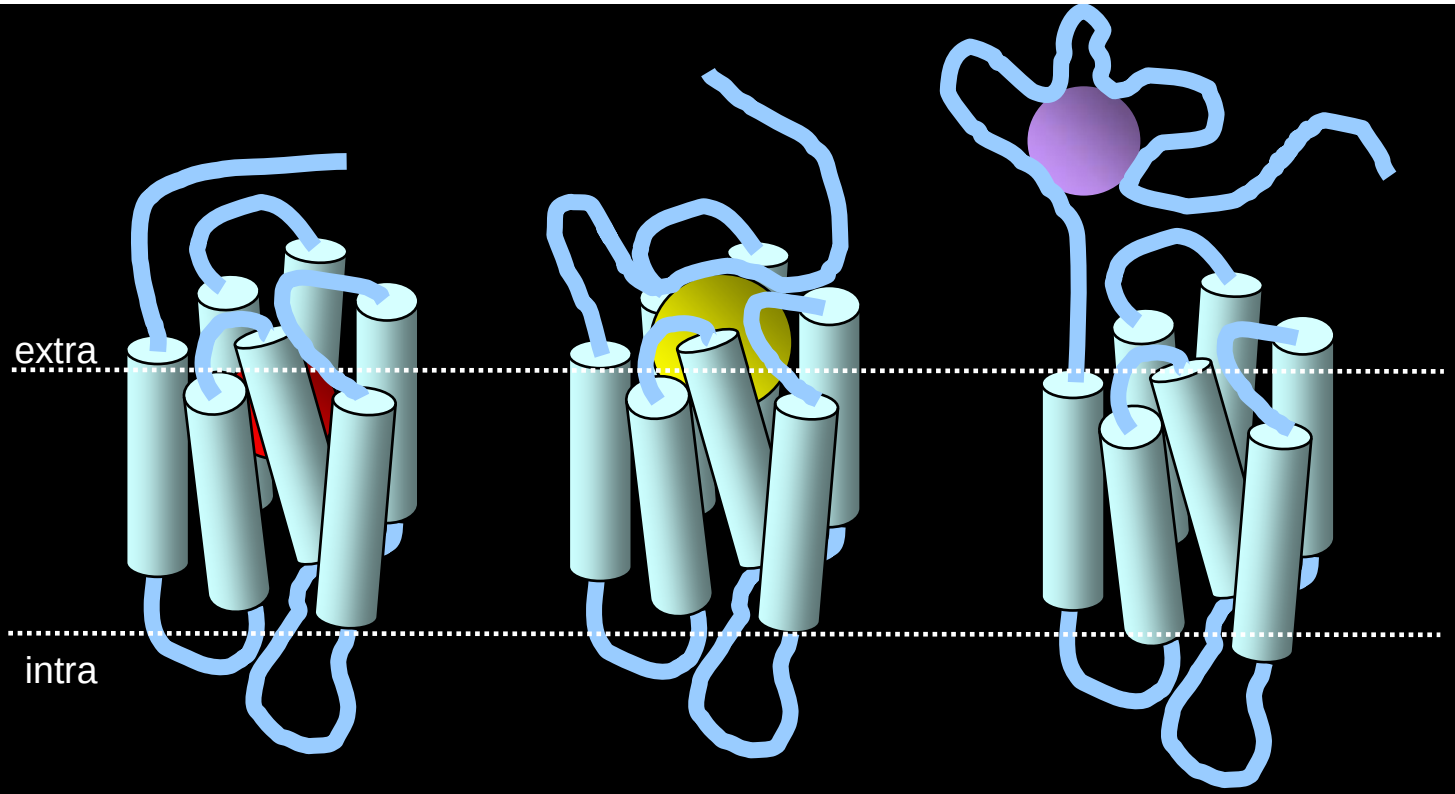


Perez D.M. Mol Pharmacol 67, 1383-1384 (2005)

GPCRs in the Human Genome



Fredriksson R. *et al Mol. Pharmacol 63,* 1256-1272 (2003)



Family A

Peptide/Proteins
- Small peptides (38)
- Large peptides (19)
- Small Proteins (33)
- Large proteins (4)

Small molecules
- Biogenic amines (41)
- Lipids (16)
- Nucleosides (13)
- Others (3)

Olfactory and taste (51)
Glycoprotein's (5)
Viral (19)
Orphans (236)

Family B

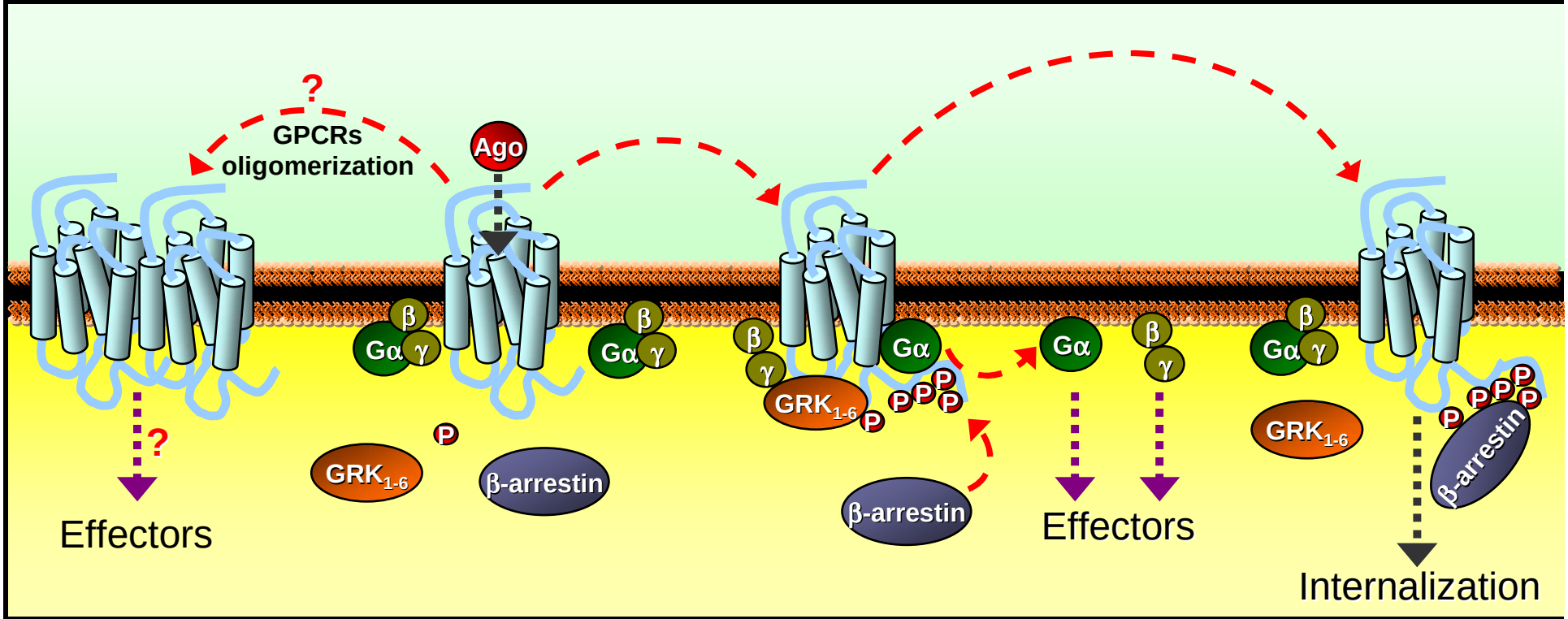
Peptide (3)
Protein (8)
Orphans (4)

Family C

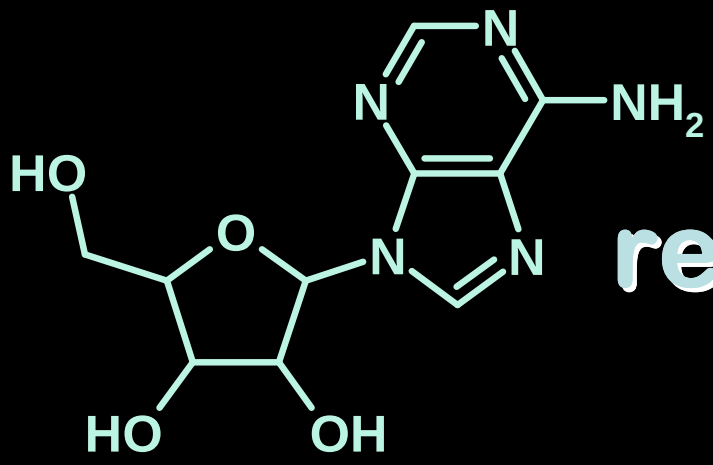
Glutamate (8)
Ca^{2+} (1)

The wonderful world of GPCR-land:

GPCRs have classically been assumed to exist and function as *monomeric* entities, and the paradigms of ligand binding and signal transduction were based on this hypothesis.



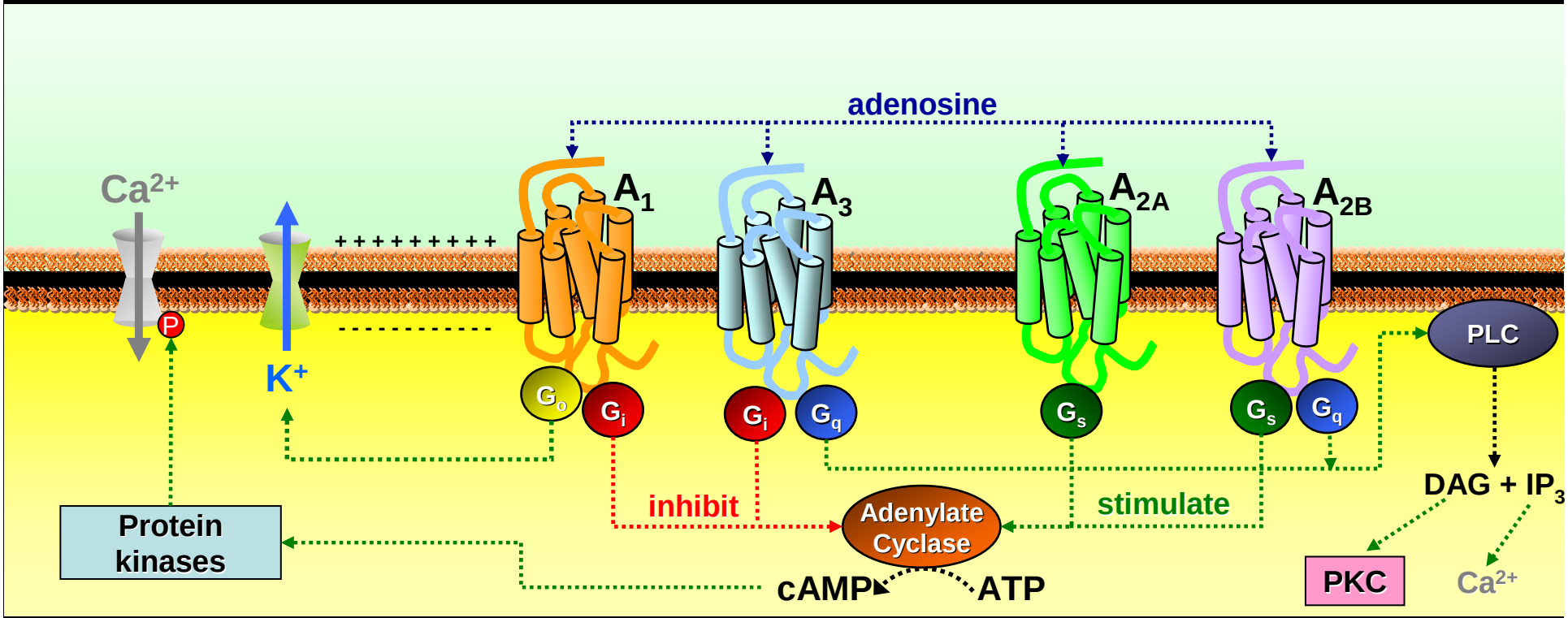
Moro S., Spalluto G., Jacobson K.A. *TIPS 26,* 44-51 (2005)



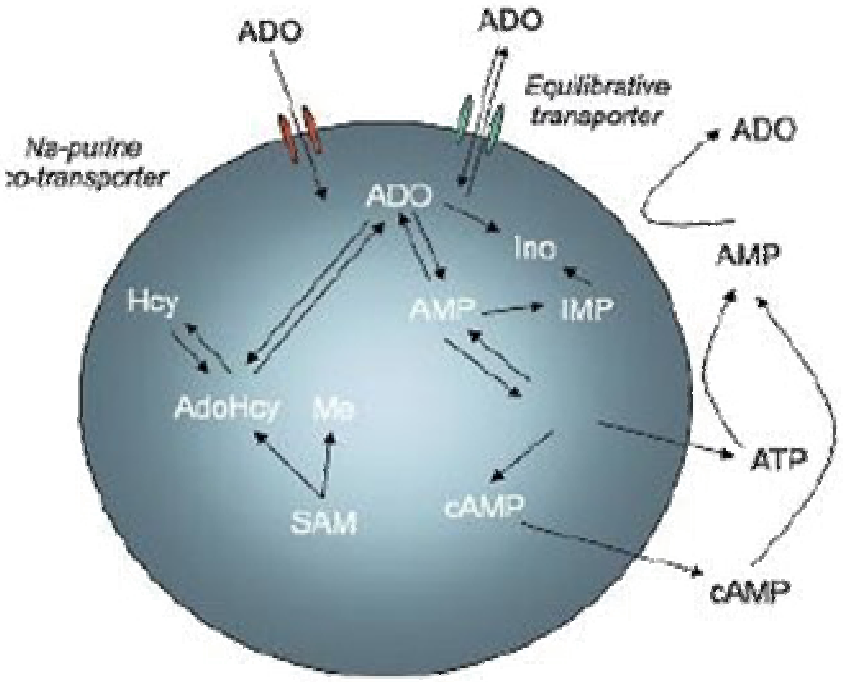
receptors

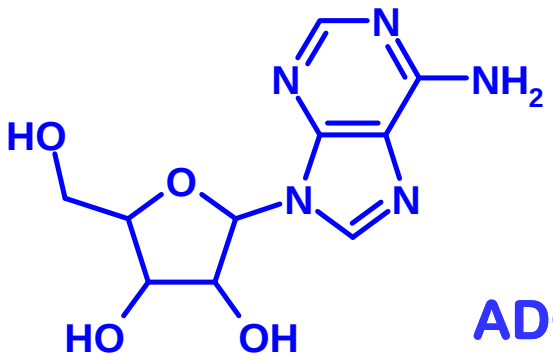
Adenosiland as a key study

Adenosine is an ubiquitous neuromodulator. It has a depressant action in the brain, heart, kidneys and other organs and is believed to mediate its effects via four adenosine receptor subtypes, termed A_1, A_{2A}, A_{2B}, and A_3.



Moro S., Spalluto G., Jacobson K.A. *TIPS 26,* 44-51 (2005)





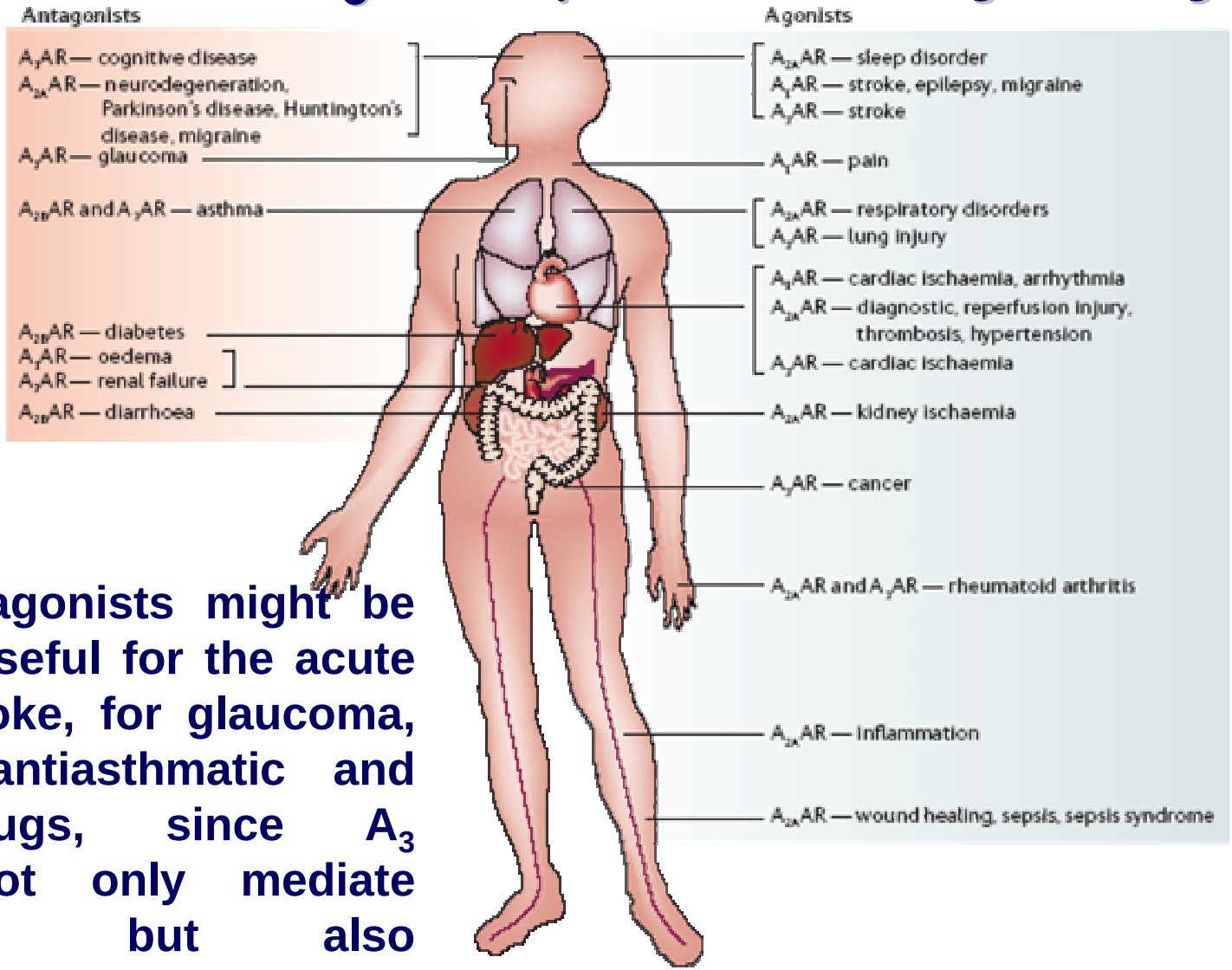
ADO

[ADO] ≅ 30 – 300 nM (*normal*)

[ADO] ≅ 10 μM (*hypoxia*)

Schematic of main intra- and extra-cellular metabolic pathways of adenosine. Adenosine is formed from AMP intracellularly by the enzymes ATP-ase, ADP-ase and 5′-nucleotidase and extracellularly by the respective ecto-enzymes. Adenosine kinase converts adenosine to AMP, while adenosine deaminase converts it to inosine. The third enzyme to metabolize adenosine is S-adenosylhomocysteine hydrolase, which converts adenosine to S-adenosylhomocysteine. Adenosine concentration between the intra- and extracellular spaces is equilibrated by nucleoside transporters.

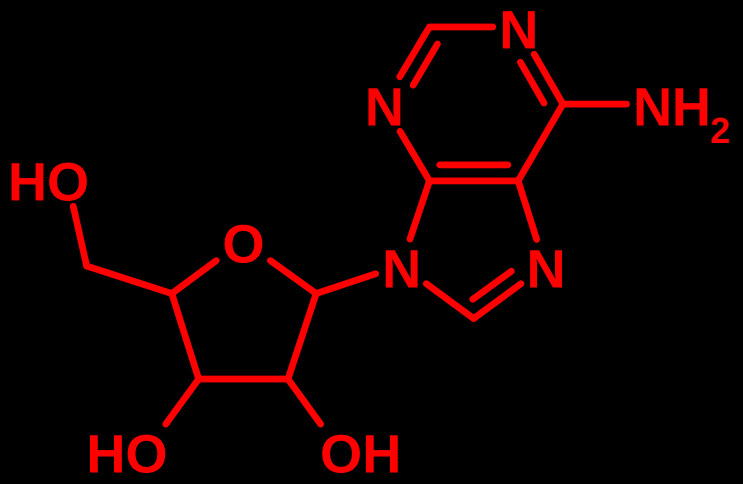
GPCRs: the human A_3 receptor as a key study



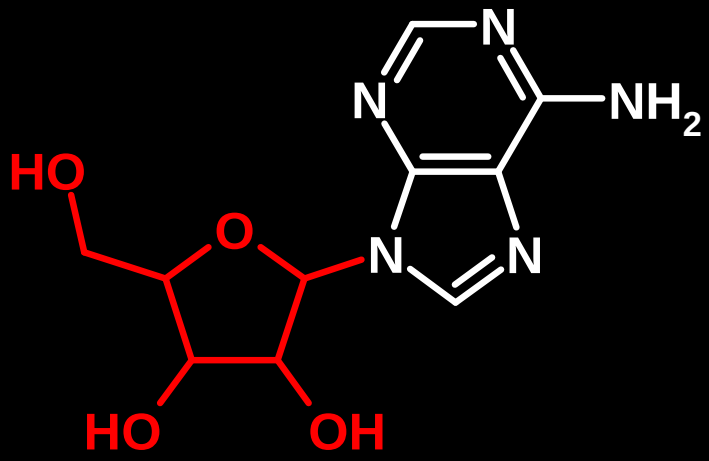
A_3 receptor antagonists might be therapeutically useful for the acute treatment of stroke, for glaucoma, and also as antiasthmatic and antiallergic drugs, since A_3 receptors cannot only mediate antiinflammatory, but also proinflammatory responses.

Jacobson K.A. et al. *Nature Drug Disc. 5,* 247-264 (2006)

Adenosiland: a flavor of SAR

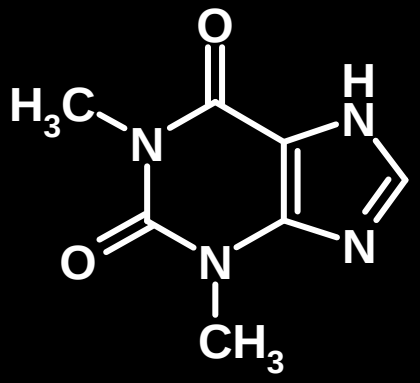


Adenosine

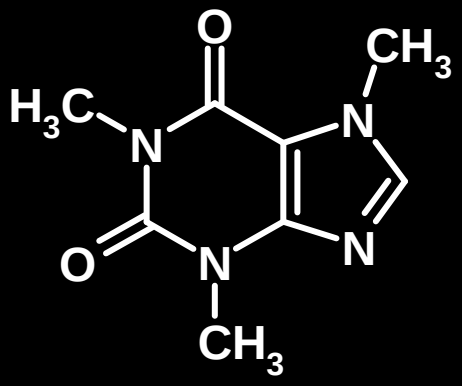


Ago/Anta

Prototypic antagonist of adenosine receptors:



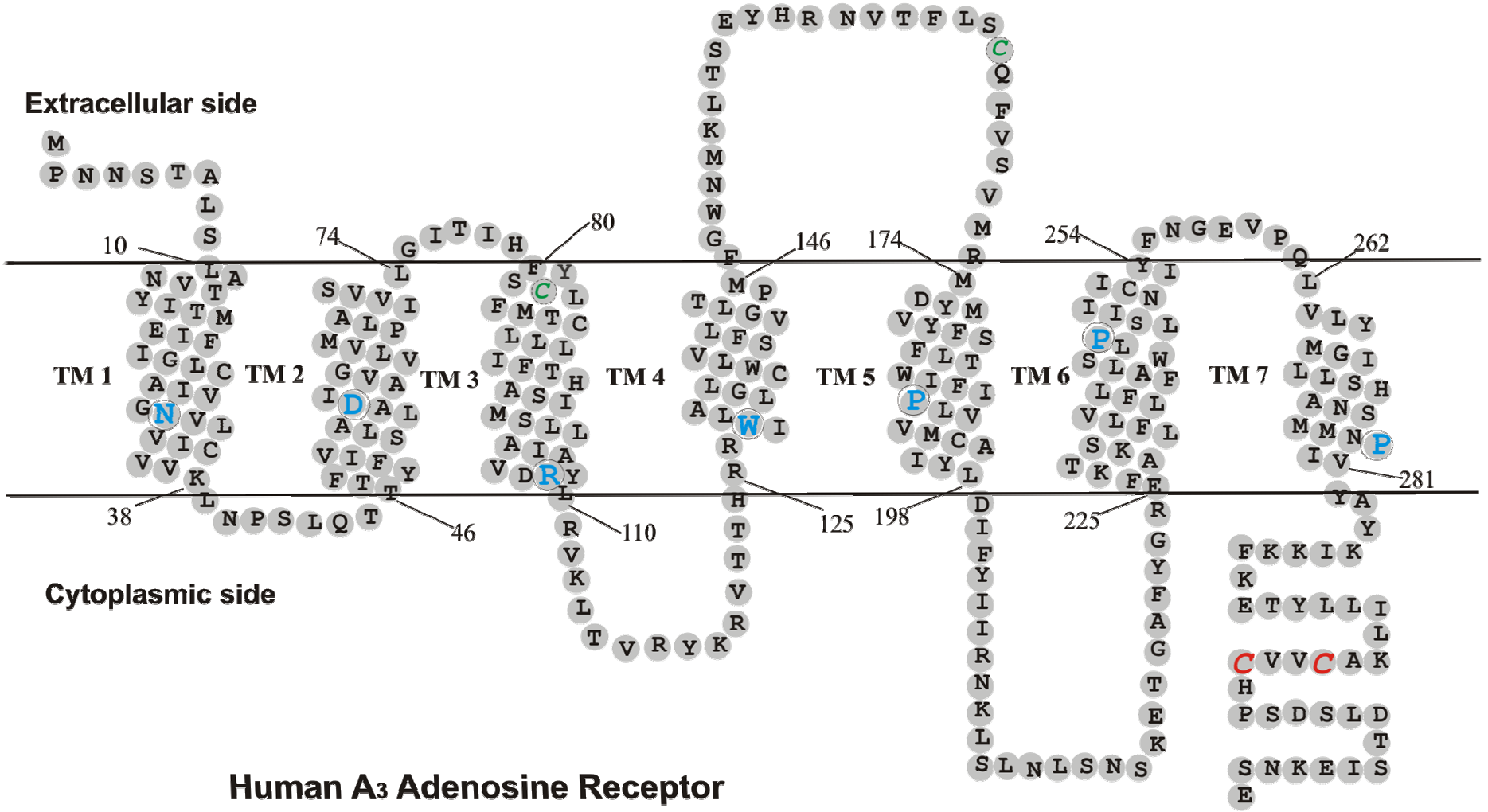
Theophylline



Caffeine

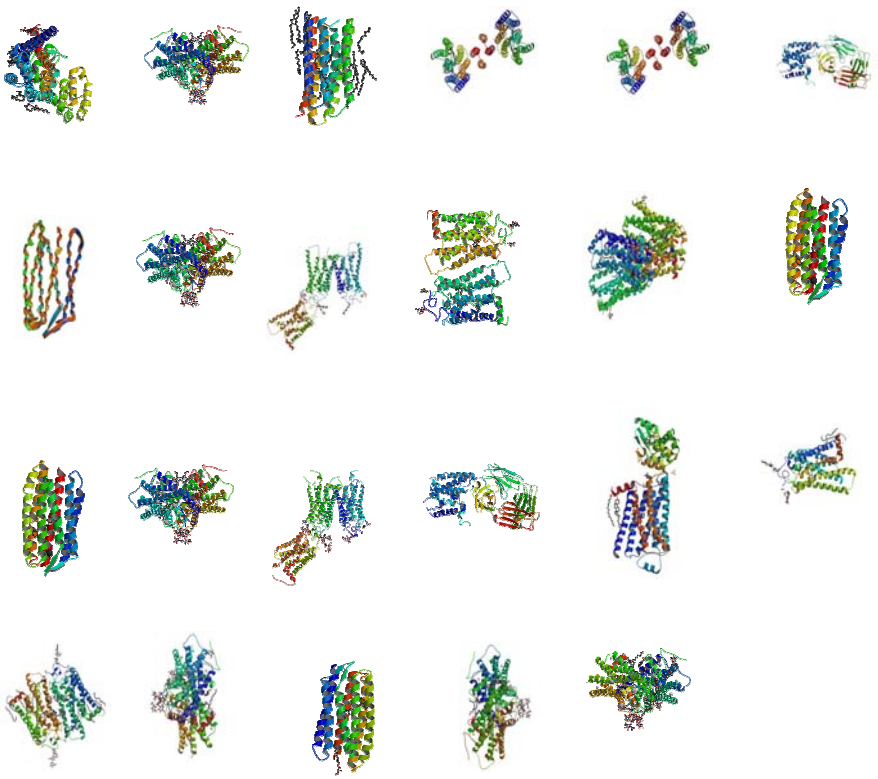
GPCRs: what about topology?

The huge amount of sequence information, ligand-binding, and mutation data is marked contrast to the scarcity of the three dimensional (3D) structural information.



Today available three dimensional (3D) structural information:

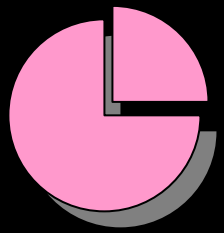
1F88	8/4/2000	2.80	Bovine Rhodopsin
1HZX	7/4/2001	2.80	Bovine Rhodopsin
1L9H	5/15/2002	2.60	Bovine Rhodopsin
1GZM	11/20/2003	2.65	Bovine Rhodopsin
1U19	10/12/2004	2.20	Bovine Rhodopsin
2HPY	8/22/2006	2.80	Bovine Rhodopsin
2G87	9/2/2006	2.60	Bovine Rhodopsin
2I35	10/17/2006	3.80	Bovine Rhodopsin
2I36	10/17/2006	4.10	Bovine Rhodopsin
2I37	10/17/2006	4.15	Bovine Rhodopsin
2J4Y	9/25/2007	3.40	Bovine Rhodopsin
2PED	10/30/2007	2.95	Bovine 9-cis-Rhodopsin
2RH1	10/30/2007	2.40	Human β2-Adrenergic Receptor
2R4R	11/6/2007	3.40	Human β2-Adrenergic Receptor
2ZIY	5/6/2008	3.70	Squid Rhodopsin
2Z73	5/13/2008	2.50	Squid Rhodopsin
3D4S	6/17/2008	2.80	Human β2-Adrenergic Receptor
3CAP	6/24/2008	2.90	Bovine Opsin
2VT4	6/24/2008	2.70	Turkey β1-Adrenergic Receptor
3DQB	9/23/2008	3.20	Bovine Opsin
3EML	10/14/2008	2.60	Human A2a Adenosine Receptor

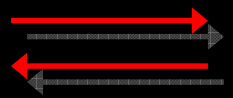


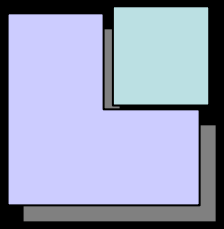
What available crystal structures can (hopefully) describe?

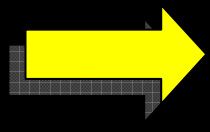
ANTAGONIST

AGONIST





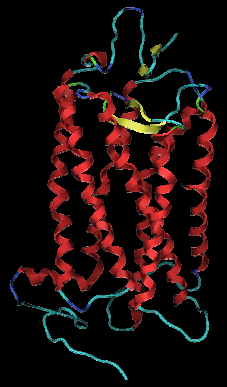


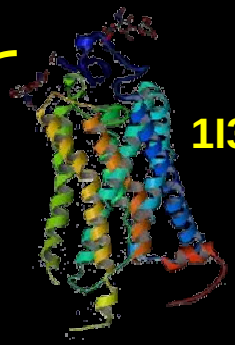


CELLULAR RESPONSE

INACTIVE (R)

ACTIVE (R*)





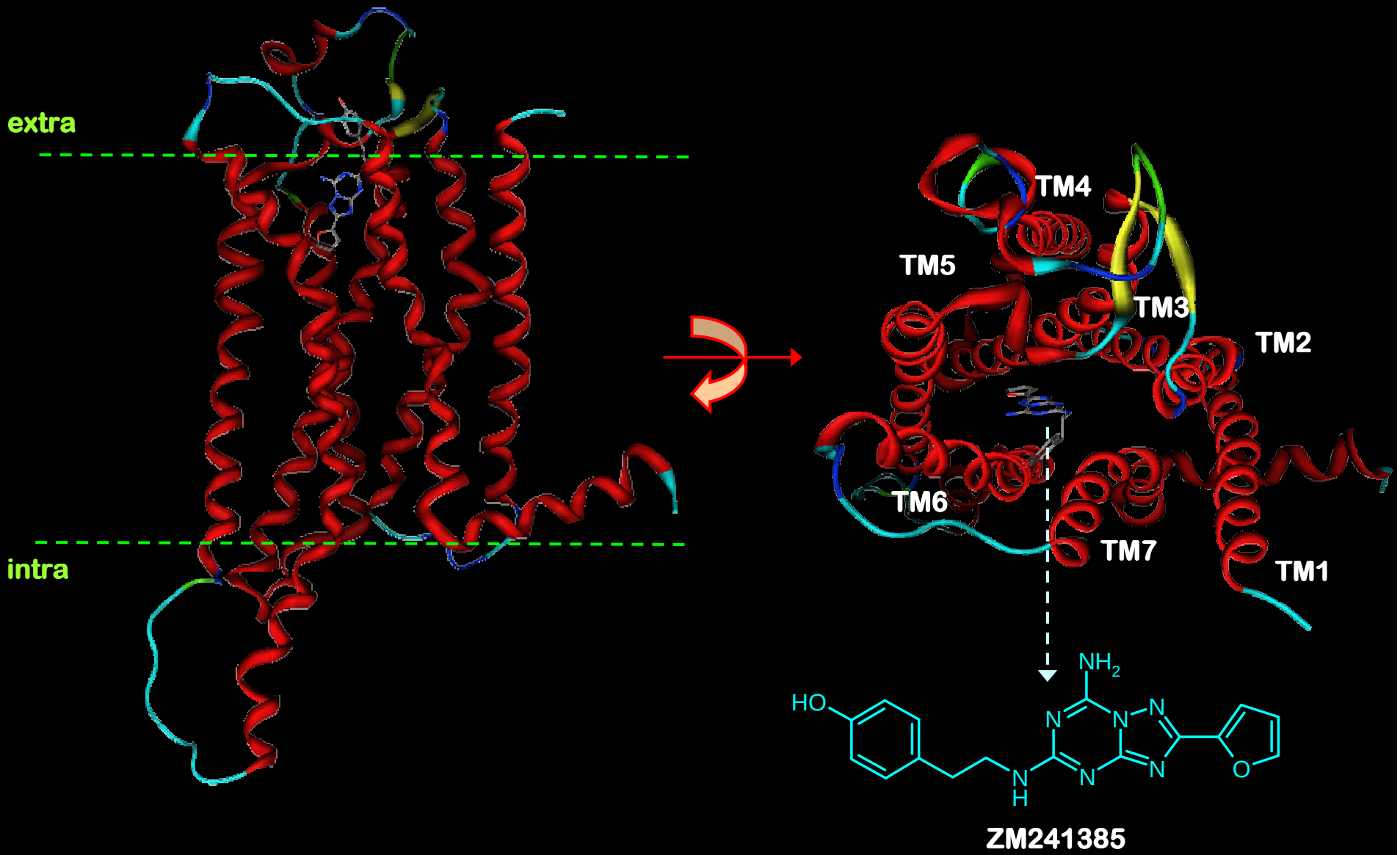
1l37, 4.15Å (2006)
??

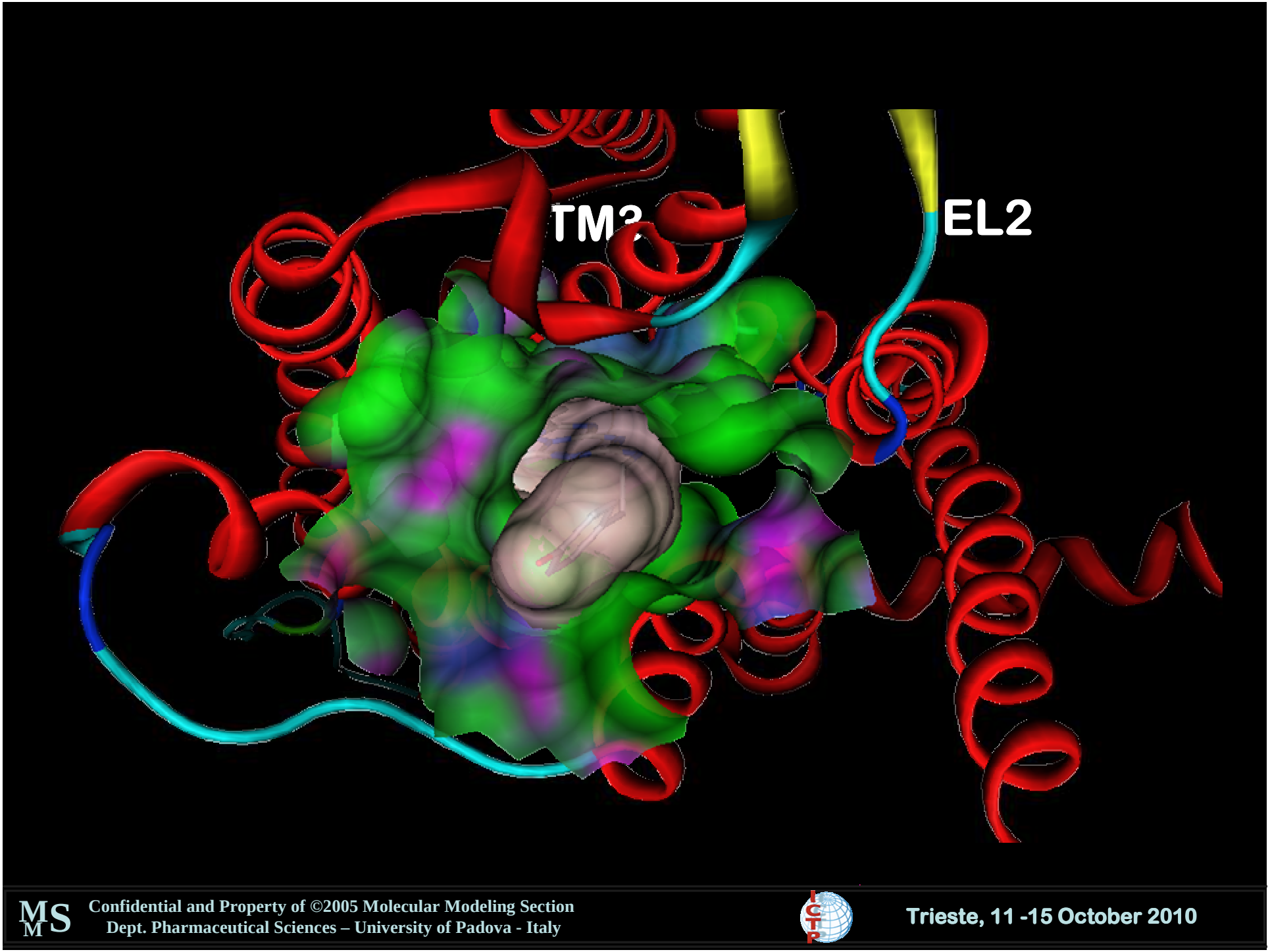
G-protein interaction (?)

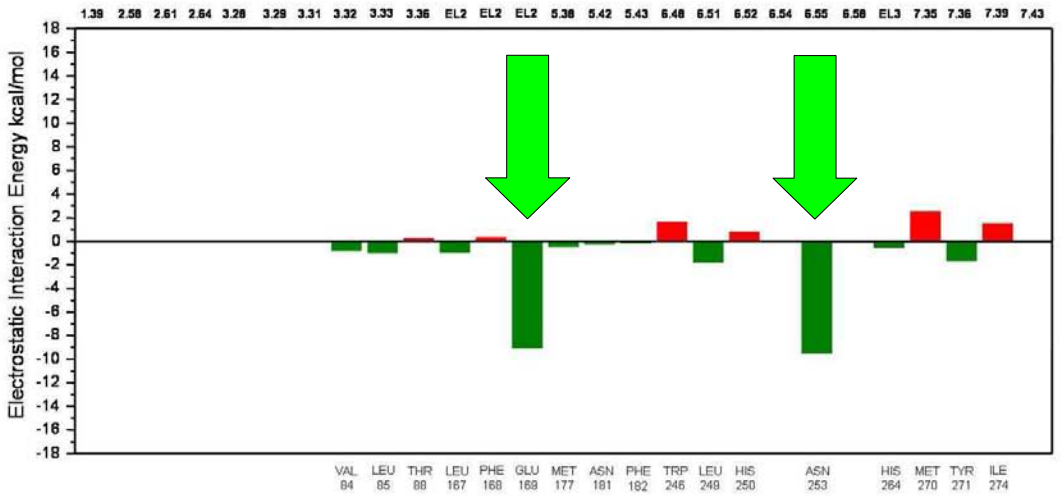
oligomerization (?)

XR-rhodopsin or β_2A or A_{2A} structures

Zooming into human A_{2A} receptor







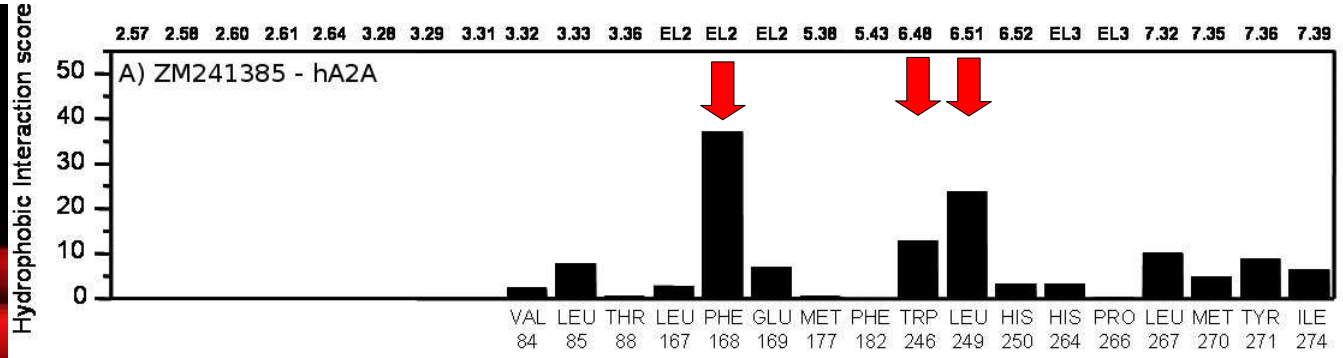
EL2

```
hA2A  (142-173):  -GWNNCGQPKEGKNHSQGCGEGQVACLFEDVVP
hA3   (148-173):  -GWNMKL------TSEYHRNVTFLSCQFVSVMR
```

TM6

```
hA2A  (225-258):LQKEVHAAKSLAIIVGLFALCWLPLHIINCFTFF
hA3   (222-255):YGREFKTAKSLFLVLFLFALSWLPLSIINCIIYF
```

Moro S., Paoletta S. et al *J. Med. Chem. 52, 7640-7652* (2009)



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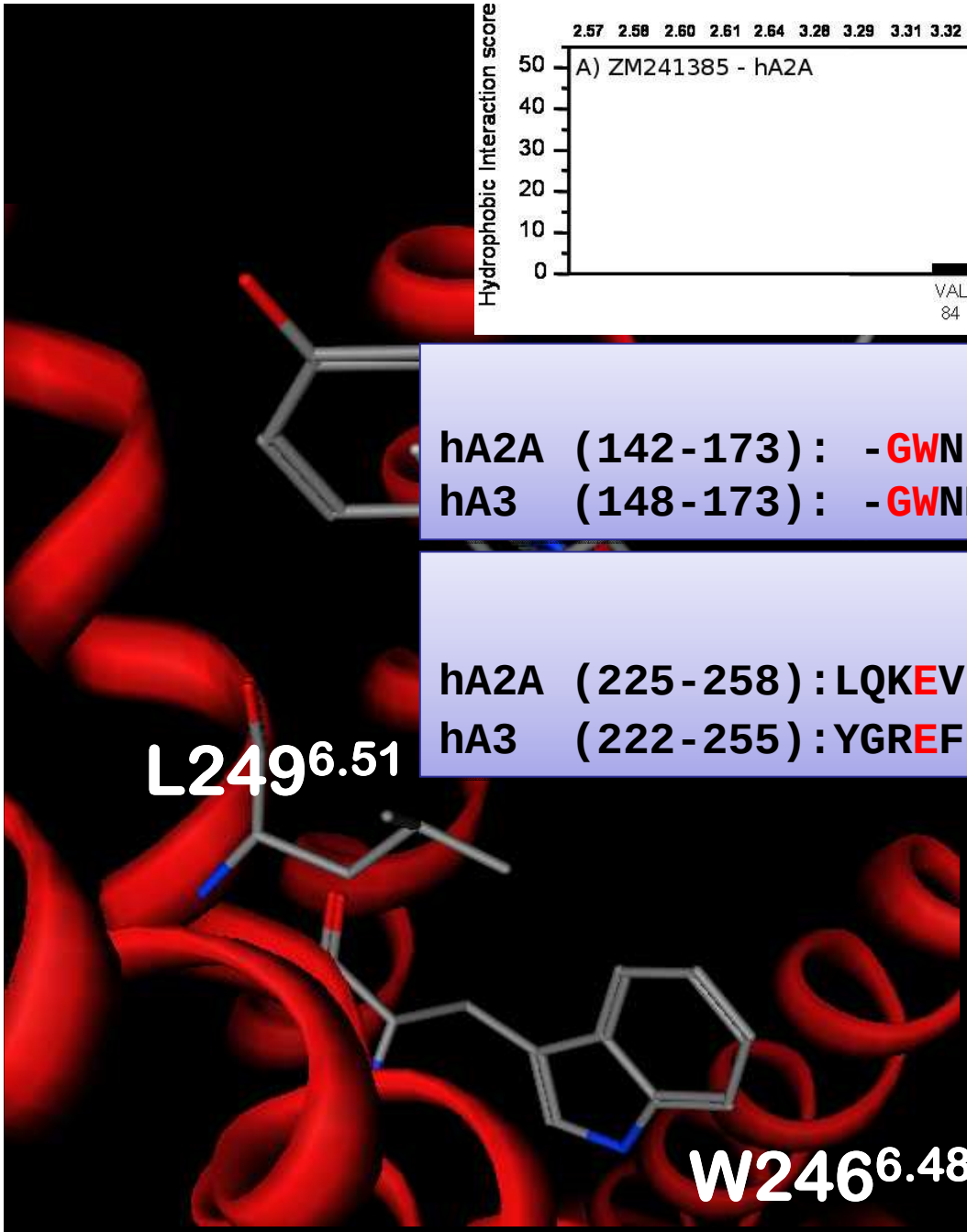

Moro S., Paoletta S. et al *J. Med. Chem. 52, 7640-7652* (2009)

Computational Biology

bRho or β_2A or A_{2A} structures

SDFDDGTFDDGDVGHYTDFFGSRFDVCAA
SDFREGDFFGFTFGRHVDFGDRDFEGGGE
TDGDTGDGDGHLIGFHYDTFDGDHDGDTG
DGDHDHFGTCVFDVDFRETHDGDTGDGBD
GCVCGDFDVDGEEDGDTDTDGDGTREFEG
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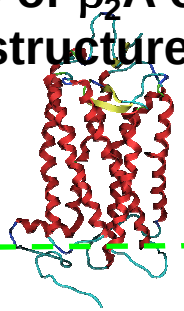
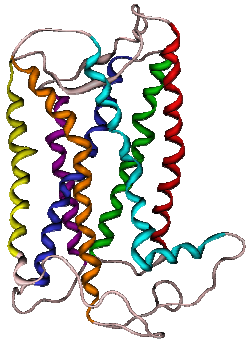
REWDFDRSFAWSWDWQRWEDSFRWFS



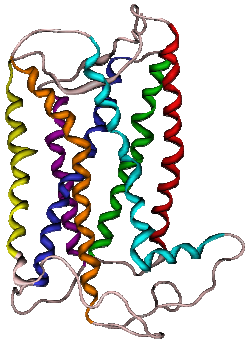
Receptor modeling



GPCR sequence

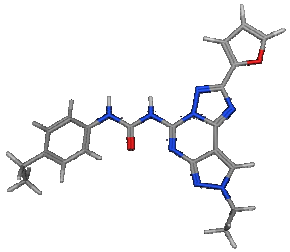
GPCR model

Computational MedChem



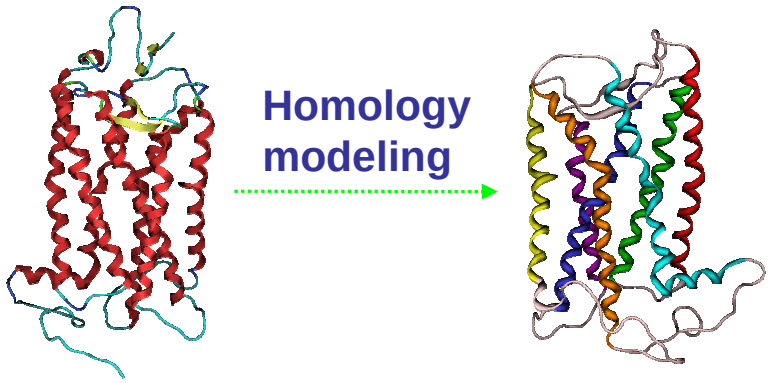
Receptor-driven Ligand Design

Ligand-driven Receptor Refinement



GPCR model

GPCR ligand



bRho or β_2A structure

GPCR model

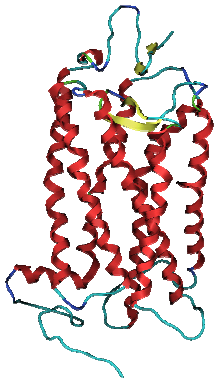
Two ugly questions:

- Is my homology model biophysically realistic?

- Is my homology model a good starting point to perform any kind of receptor-driven ligand design approach?

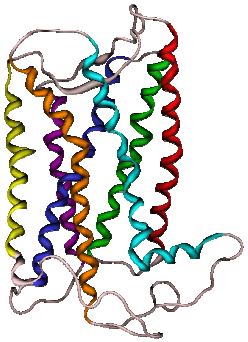
Crucial question in GPCR-SBDD: how can we choose the best template?

… and concerning adenosine receptors, is this still *crucial* now that we have the crystal structure of the human A_{2A}?



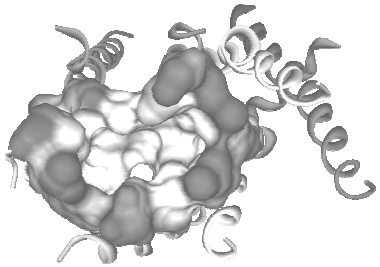
Template
X-Ray structure

Homology modeling



h_A_3R model

TM ligand cavity



h_A_3R binding site

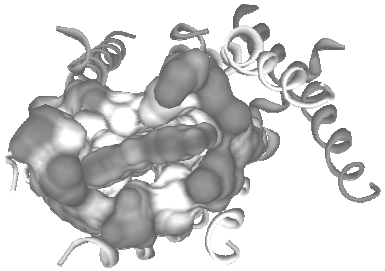
High throughput docking

Random Virtual Screening
(large known chemical dbases)

Focused Virtual Screening
(medium focused chemical dbases)

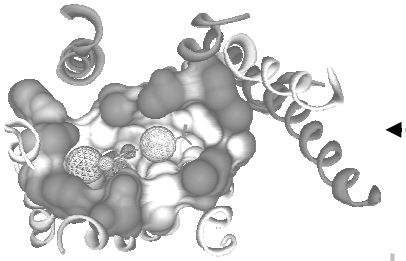
Virtual Libraries Screening

Classical MedChem Screening

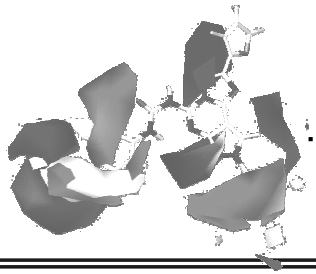


h_A_3R-ligand complexes

LBHM

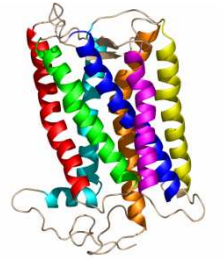


Receptor-based 3D-QSAR



Ligand scoring and selection

Homology Modeling: choosing the template



1F88	8/4/2000	2.80	Bovine Rhodopsin
1HZX	7/4/2001	2.80	Bovine Rhodopsin
1L9H	5/15/2002	2.60	Bovine Rhodopsin
1GZM	11/20/2003	2.65	Bovine Rhodopsin
1U19	10/12/2004	2.20	Bovine Rhodopsin
2HPY	8/22/2006	2.80	Bovine Rhodopsin
2G87	9/2/2006	2.60	Bovine Rhodopsin
2I35	10/17/2006	3.80	Bovine Rhodopsin
2I36	10/17/2006	4.10	Bovine Rhodopsin
2I37	10/17/2006	4.15	Bovine Rhodopsin
2J4Y	9/25/2007	3.40	Bovine Rhodopsin
2PED	10/30/2007	2.95	Bovine 9-cis-Rhodopsin
2RH1	10/30/2007	2.40	Human β2-Adrenergic Receptor
2R4R	11/6/2007	3.40	Human β2-Adrenergic Receptor
2ZIY	5/6/2008	3.70	Squid Rhodopsin
2Z73	5/13/2008	2.50	Squid Rhodopsin
3D4S	6/17/2008	2.80	Human β2-Adrenergic Receptor
3CAP	6/24/2008	2.90	Bovine Opsin
2VT4	6/24/2008	2.70	Turkey β1-Adrenergic Receptor
3DQB	9/23/2008	3.20	Bovine Opsin
3EML	10/14/2008	2.60	Human A2a Adenosine Receptor

Sequence Alignment - Percentage Identity

	h A2a AR	b Rho	h β2 AR
h A3 AR	30,6	14,1	18,9

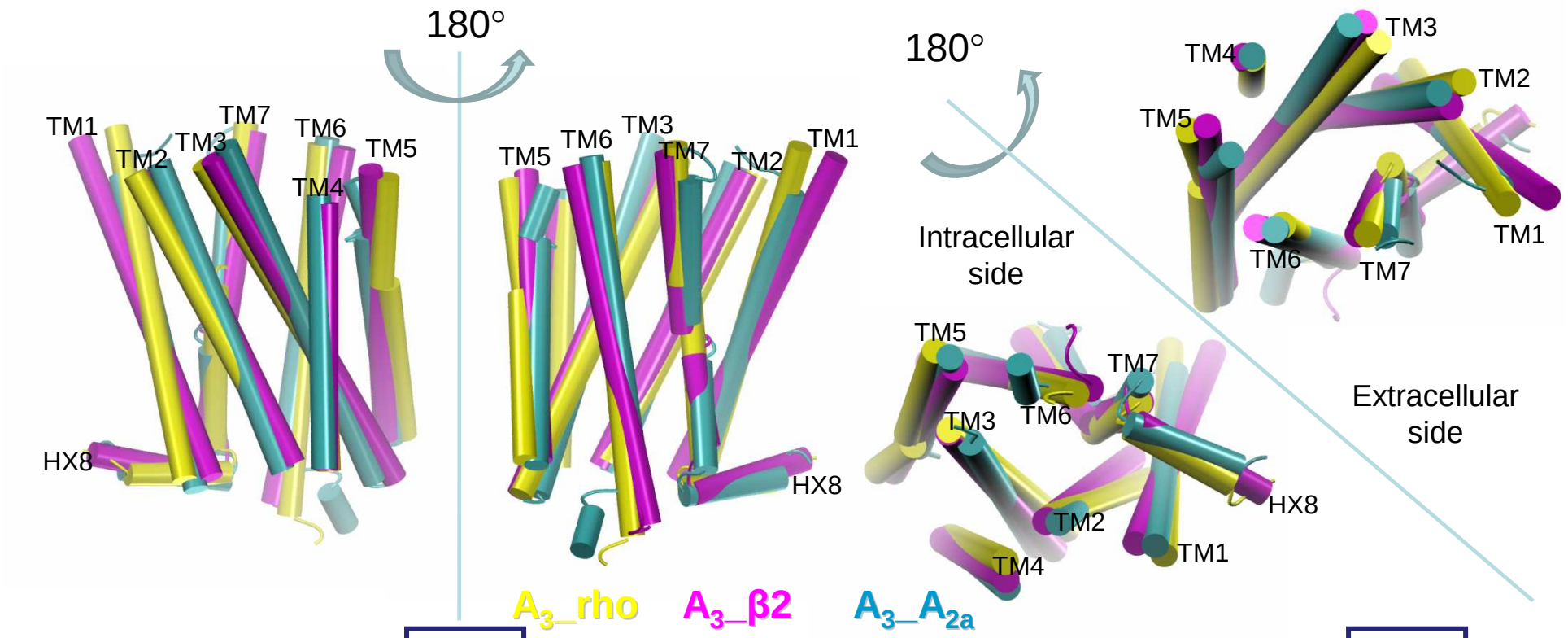
Sequence Alignment - Percentage Identity – TM region

	h A2a AR	b Rho	h β2 AR
h A3 AR	49,5	17,6	29,6

Sequence Alignment - Percentage Identity – EL2

	h A2a AR	b Rho	h β2 AR
h A3 AR	23,5	16,3	11,1

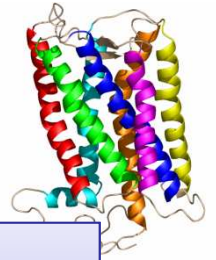
Homology Modeling: general overview



A_3_rho A_3_β2 A_3_A_{2a}

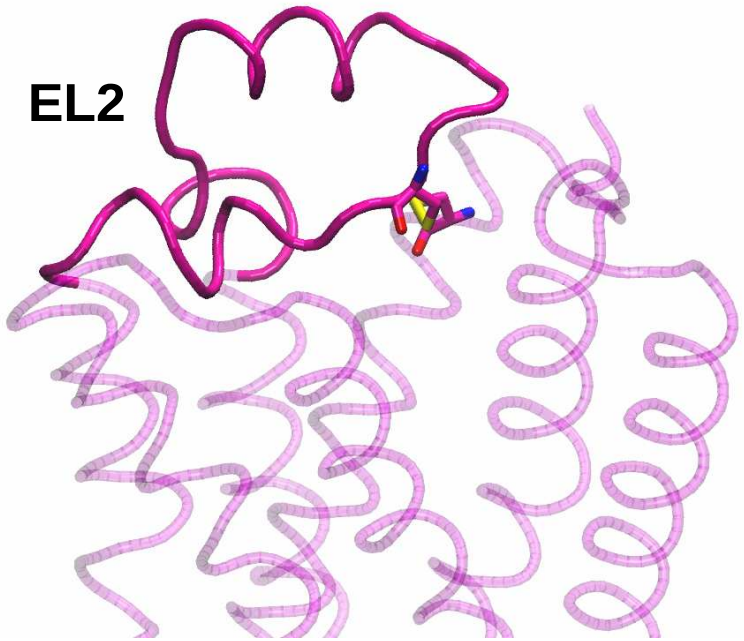
	all TM	all LOOPS	TM1	TM2	TM3	TM4	TM5	TM6	TM7	HX8	EL2
RMSD in Å with respect to hA3AR model from bovine rhodopsin (backbone)											
A3_beta2	2.29	10.86	2.82	2.12	1.98	2.01	2.07	2.19	1.85	3.73	11.44
A3_a2a	2.43	10.06	2.55	2.40	2.78	2.45	2.85	2.02	2.04	1.64	14.30
RMSD in Å with respect to hA3AR model from human β2 Adrenergic Receptor (backbone)											
A3_rho	2.29	10.86	2.82	2.12	1.98	2.01	2.07	2.19	1.85	3.73	11.44
A3_a2a	2.57	7.46	3.84	1.89	2.02	1.73	2.09	2.71	2.23	3.66	6.18
RMSD in Å with respect to hA3AR model from human A2 A Adenosine Receptor (backbone)											
A3_rho	2.43	10.06	2.55	2.40	2.78	2.45	2.85	2.02	2.04	1.64	14.30
A3_beta2	2.57	7.46	3.84	1.89	2.02	1.73	2.09	2.71	2.23	3.66	6.18

Homology Modeling: second extracellular loop

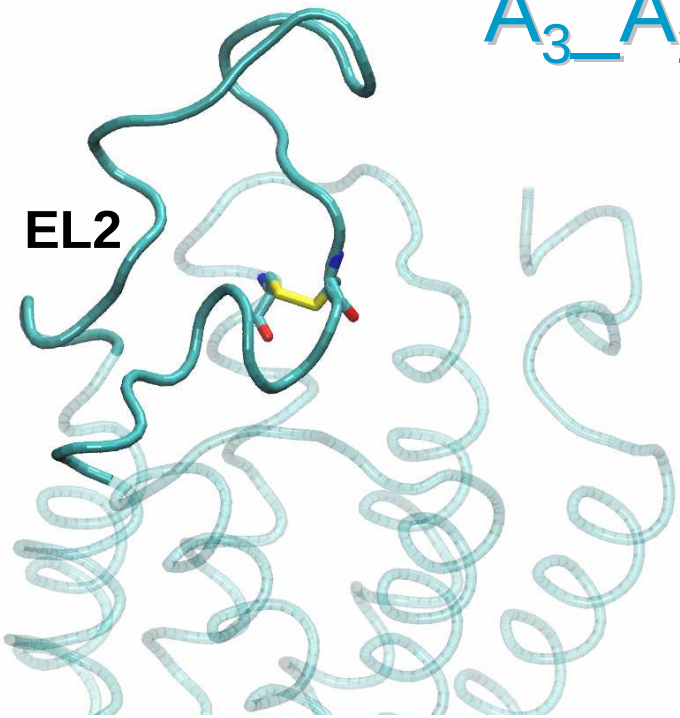


	EL2
hA2A (142-173):	-GWNNCGQPKEGKNHSQGCGEGQVACLFEDVVP
hA3 (148-173):	-GWNMKL------TSEYHRNVTFLSCQFVSVMR
hB2AR (171-195):	MHWYRAT-----HQEAINCYANETCCDFFT---

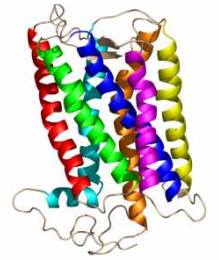
A_3_β2

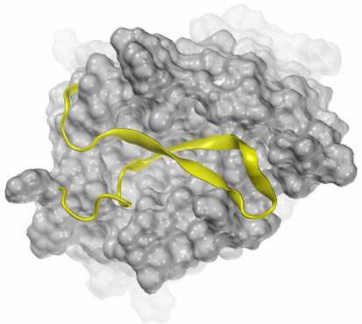


A_3_A_{2a}



Homology Modeling: second extracellular loop



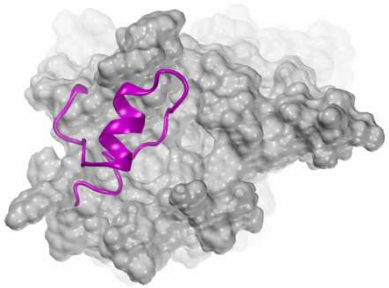


A_3_rhodopsin

Vol = 660 Å^3

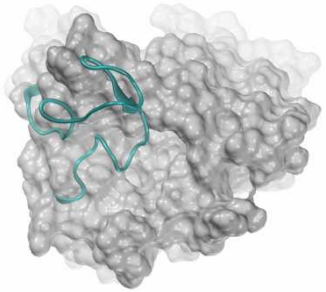
RMSD in Å – backbone EL2

	A_3_rho	A_3_β2	A_3_A_{2a}
A_3_rho		11.44	14.30
A_3_β2	11.44		6.18
A_3_A_{2a}	14.30	6.18	



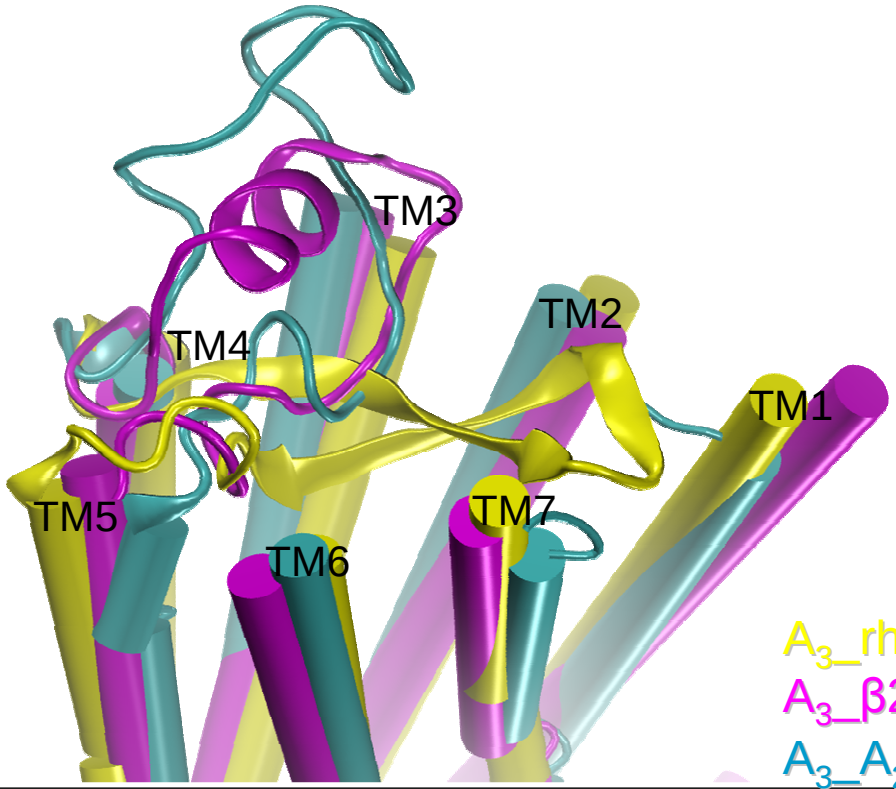
A_3_β2

Vol = 1620 Å^3



A_3_A_{2a}

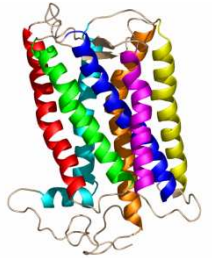
Vol = 1930 Å^3



Extracellular side view

First crucial question in SBDD: how can we test the biophysical reability of GPCR models?

Molecular Dynamics Simulations



Protein: 3255 atoms

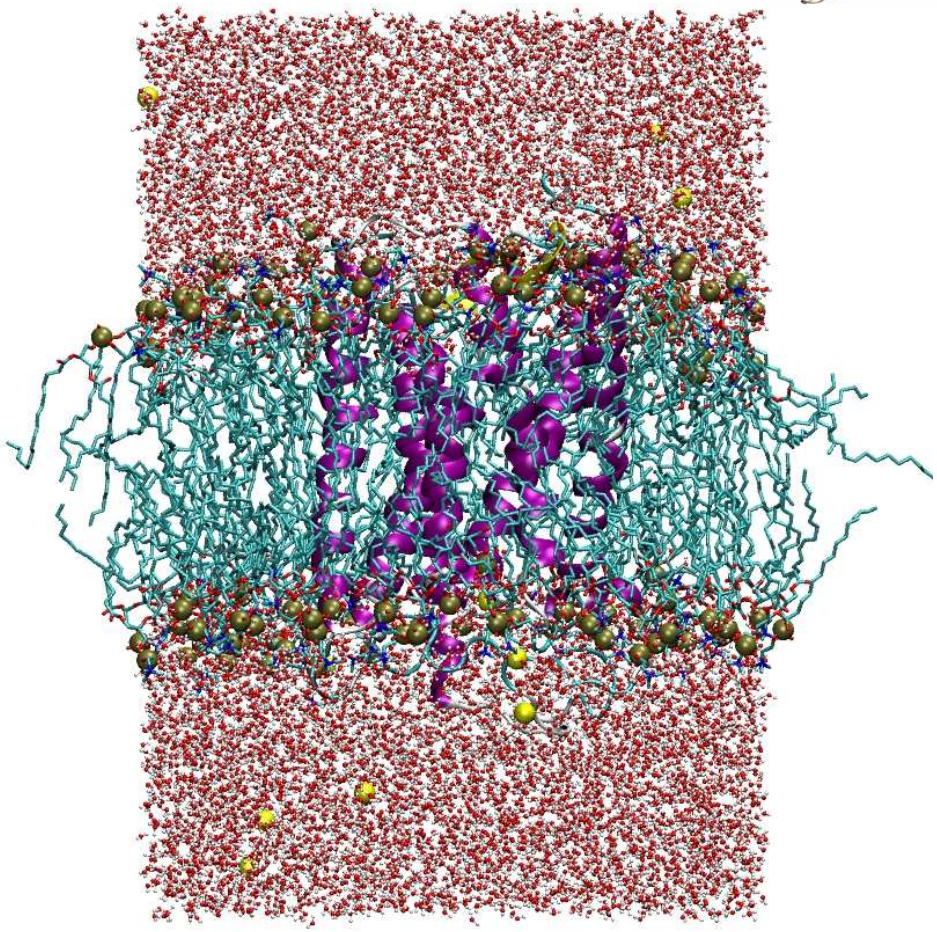
POPC: 6656 atoms

Water: 30289 atoms

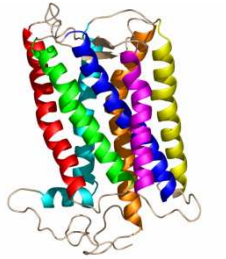
Ions: 10 atoms

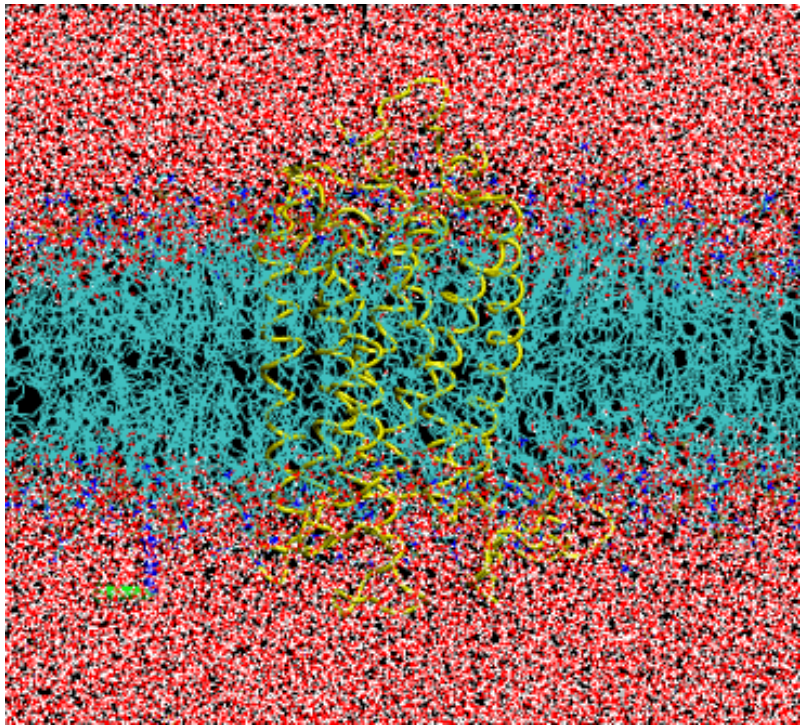
TOT: 40209 atoms

3 ns/day



Molecular Dynamics Simulations

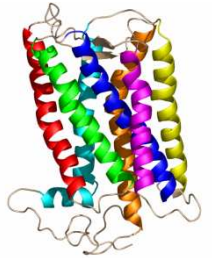




- 1-palmitoyl-2-oleoyl-phosphatidylcholine (**POPC**) membrane
- GROMACS 3.3 – GROMOS FF
- Periodic boundary conditions (PBCs)
- Ensemble – NPT (300 K)
- Pressure and temperature coupling
  - Berendsen coupling
- Time step: 2 fs
- Equilibration: 10 ns
- Run: 100 ns

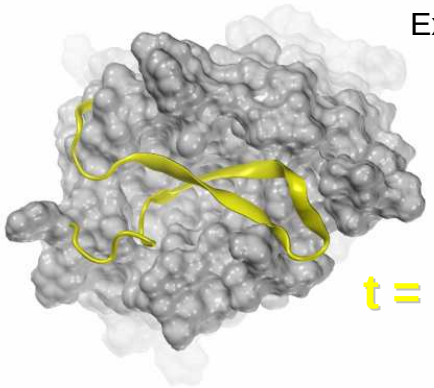
Morizzo E., Stockner T., Moro S., manuscript in preparation (2010)

Molecular Dynamics Simulations

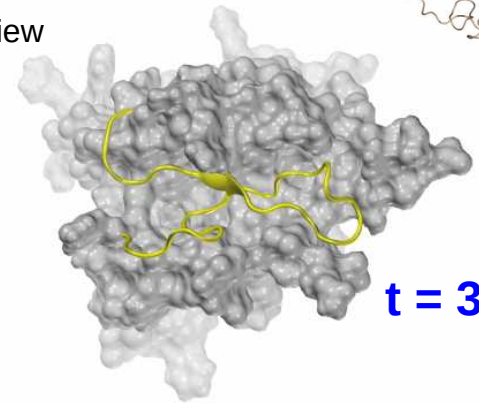


A_3_rhodopsin

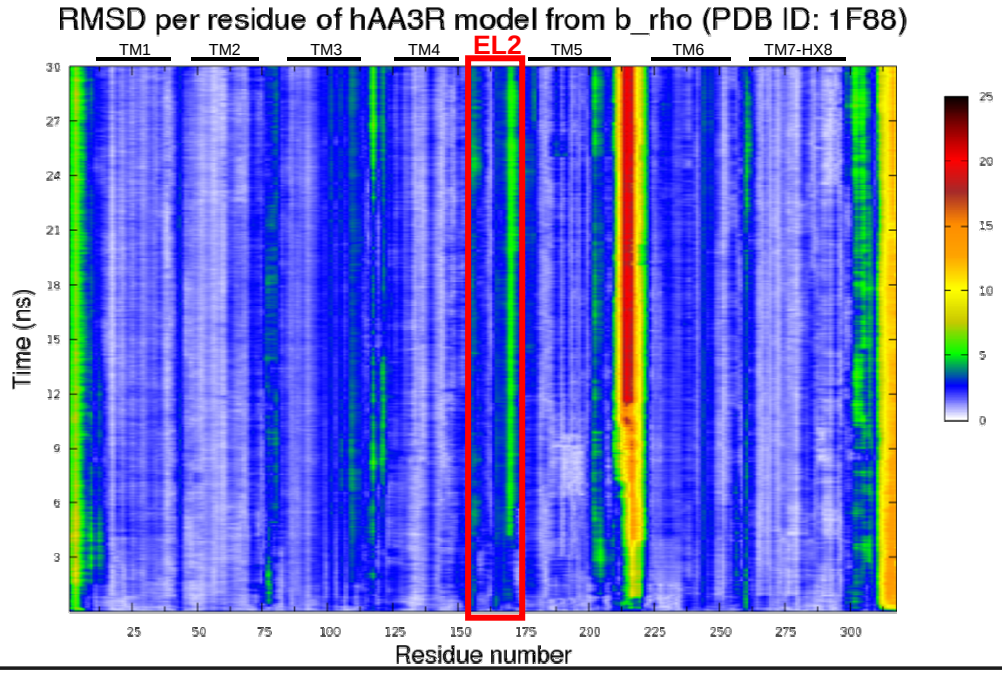
Extracellular side view

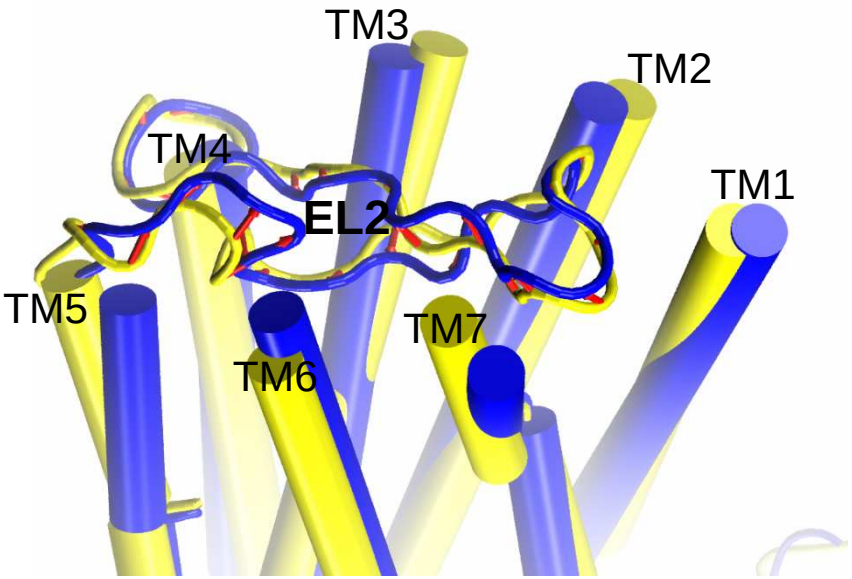


t = 0 ns



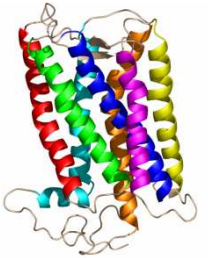
t = 30 ns



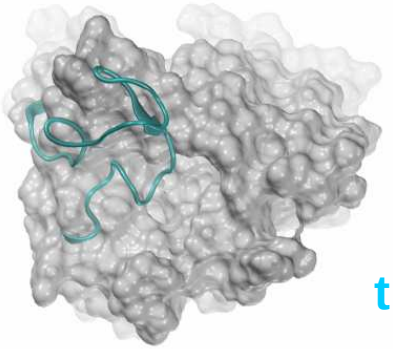


Morizzo E., Stockner T., Moro S., manuscript in preparation (2010)

Molecular Dynamics Simulations

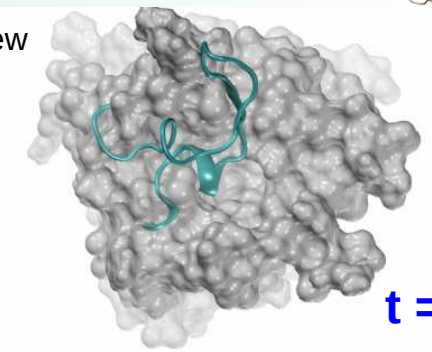


A_3_A_{2a}

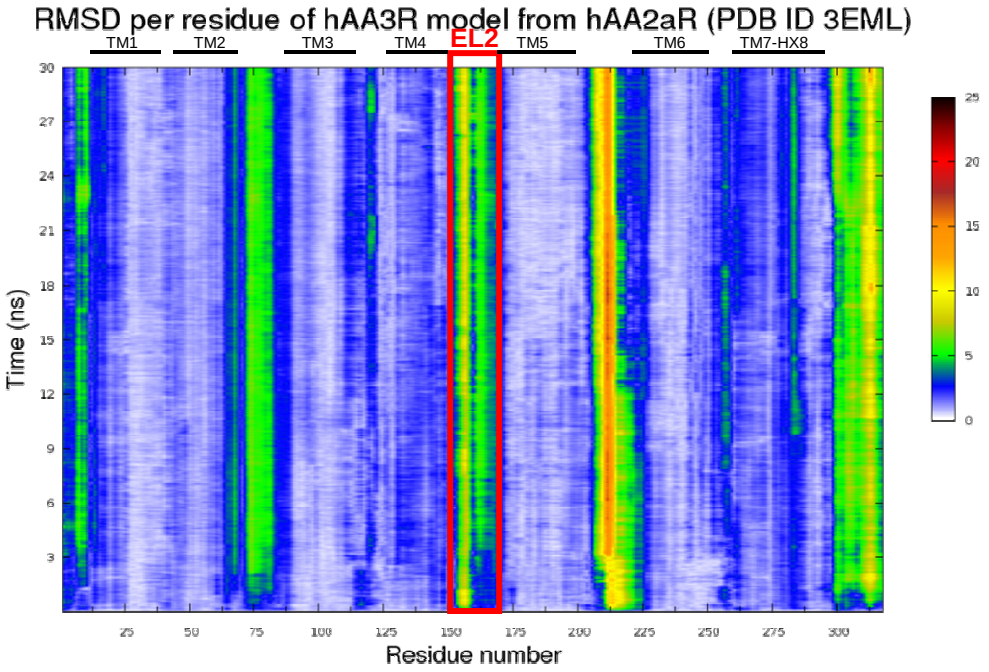


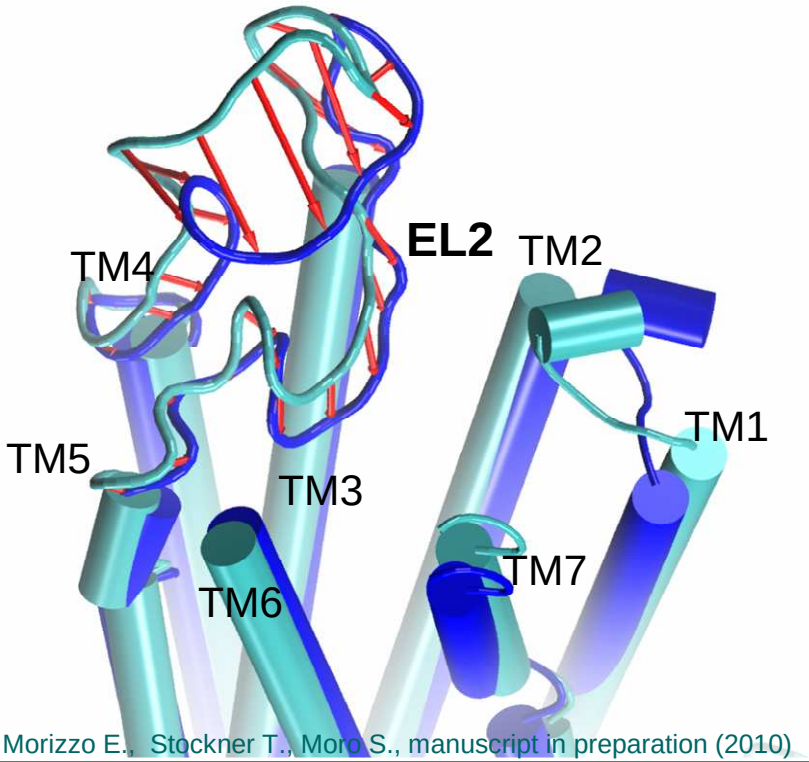
t = 0 ns

Extracellular side view



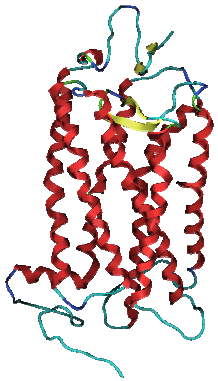
t = 30 ns





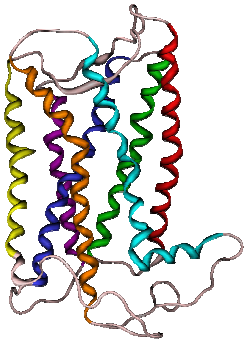
Morizzo E., Stockner T., Moro S., manuscript in preparation (2010)

Second crucial question in SBDD: how useful are GPCR models in scouting new ligands?



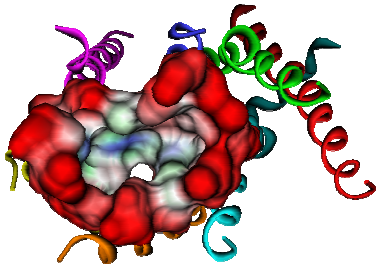
Template
X-Ray structure

Homology modeling



h_A_3R model

TM ligand cavity



h_A_3R binding site

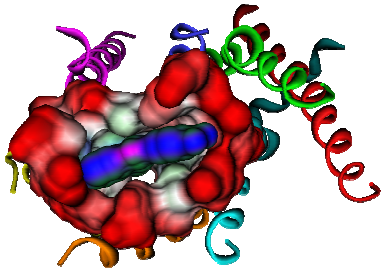
High throughput docking

Random Virtual Screening
(large known chemical dbases)

Focused Virtual Screening
(medium focused chemical dbases)

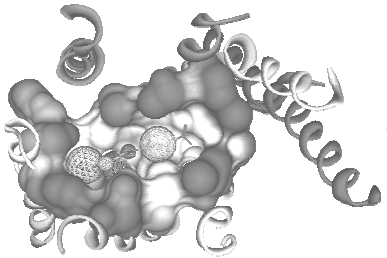
Virtual Libraries Screening

Classical MedChem Screening

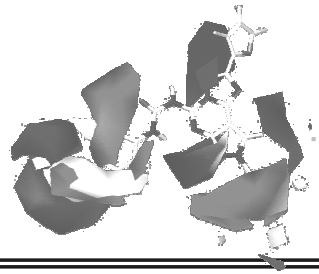


h_A_3R-ligand complexes

LBHM

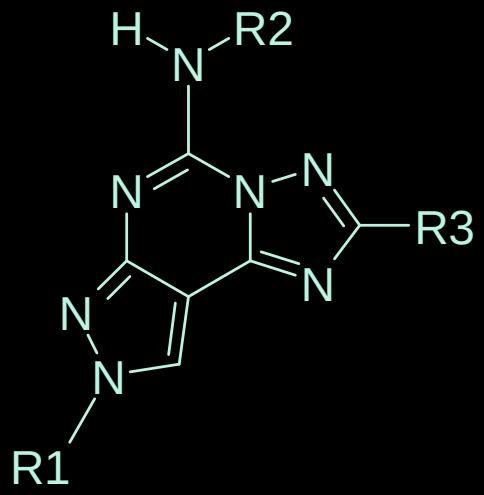


Receptor-based 3D-QSAR



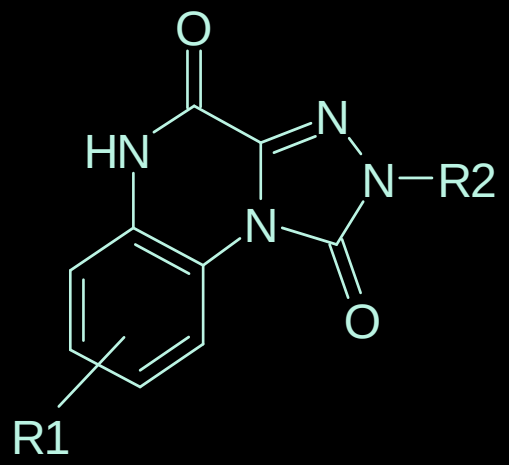
Ligand scoring and selection

Italian Chemical Probes



Pyrazolo-triazolo-pyrimidine

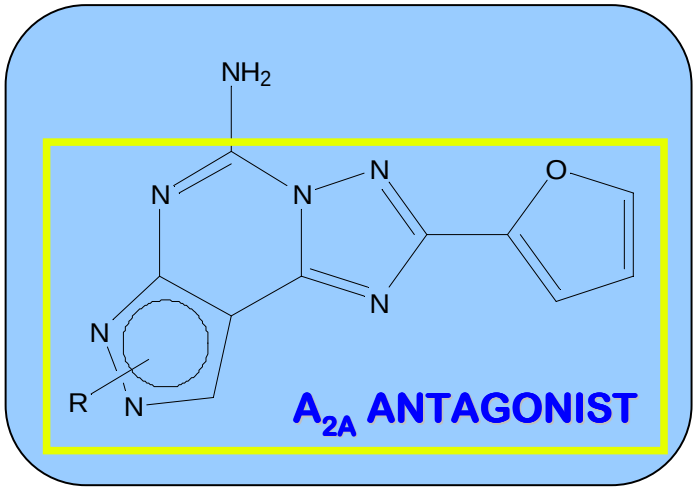
by prof. G. Spalluto
University of Trieste (Italy)

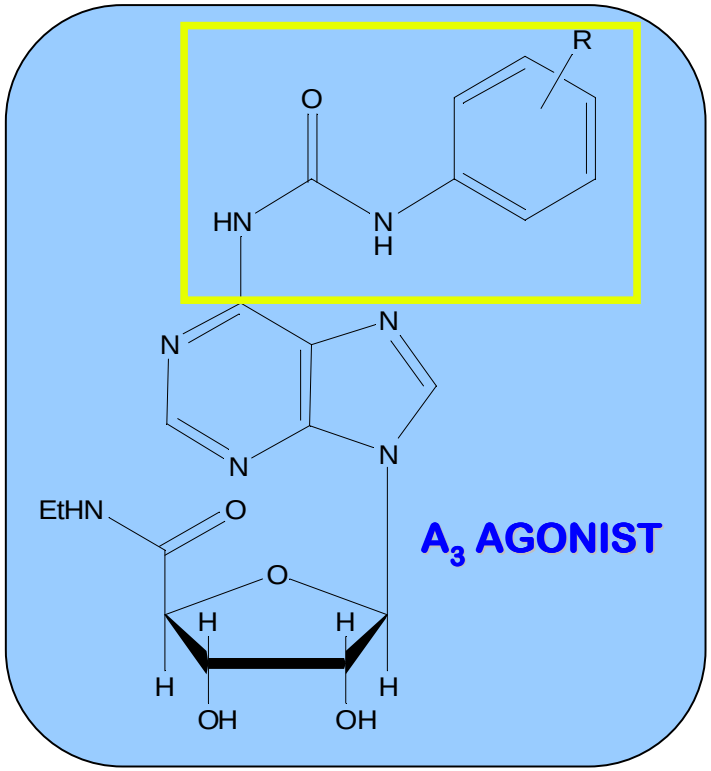


Triazolo-quinoxaline

by prof. V. Colotta
University of Florence (Italy)

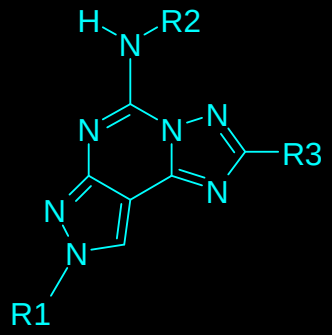
Molecular Hybridization Approach
(back to 1994… and to agonists!)





Moro S., Spalluto G. Baraldi P.G. et al *J. Med. Chem.* 45, 770-780 (2002)

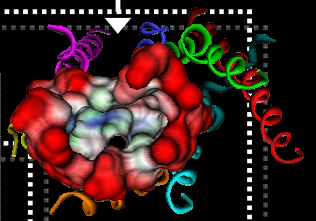
Receptor-driven ligand design



MOE-Dock, Glide, Gold…

Ligand hypothesis

consensus docking & scoring

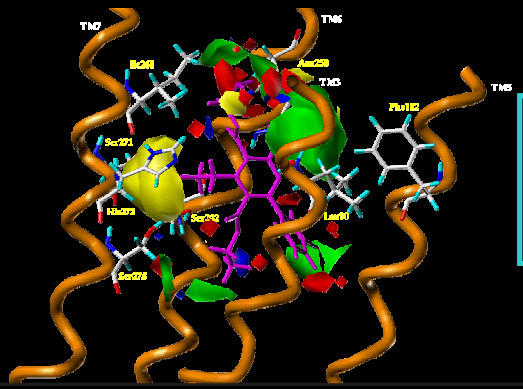


ranking and prioritize synthesis

biological assay

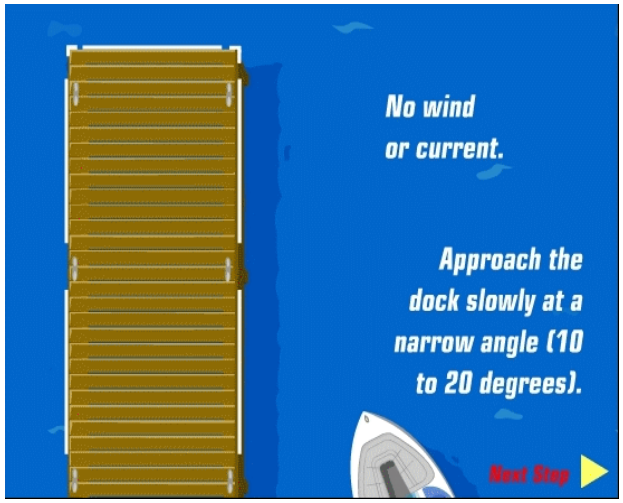
candidate selection

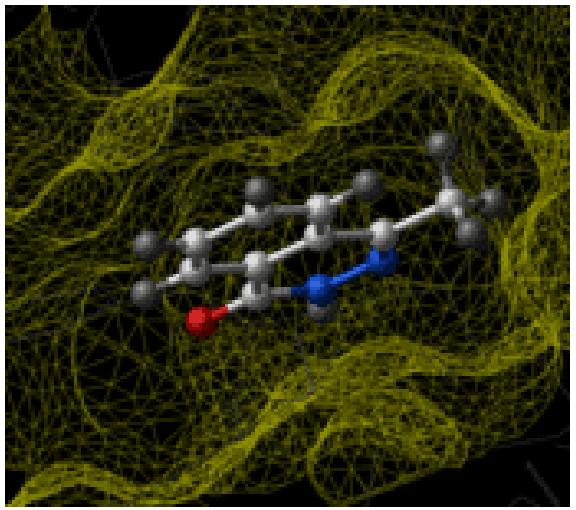
Receptor-driven 3D-QSAR

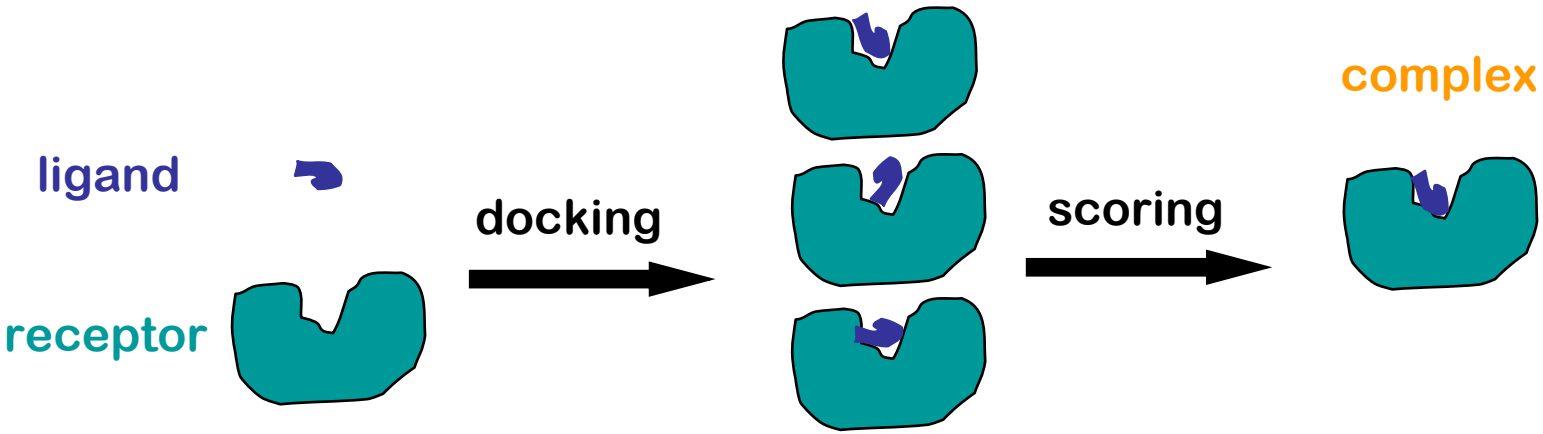


Moro S., Spalluto G., Jacobson K.A. *TIPS 26,* 44-51 (2005)

Molecular Docking: an useful tool to explore ligand-receptor complementarity



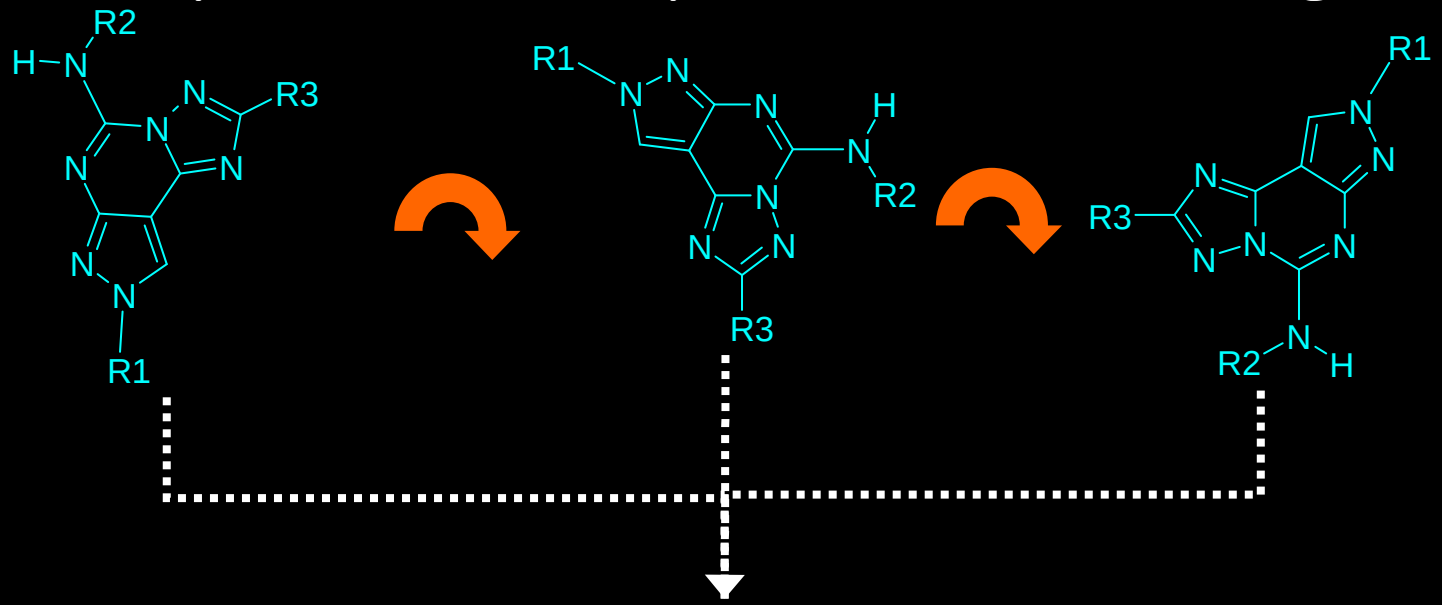


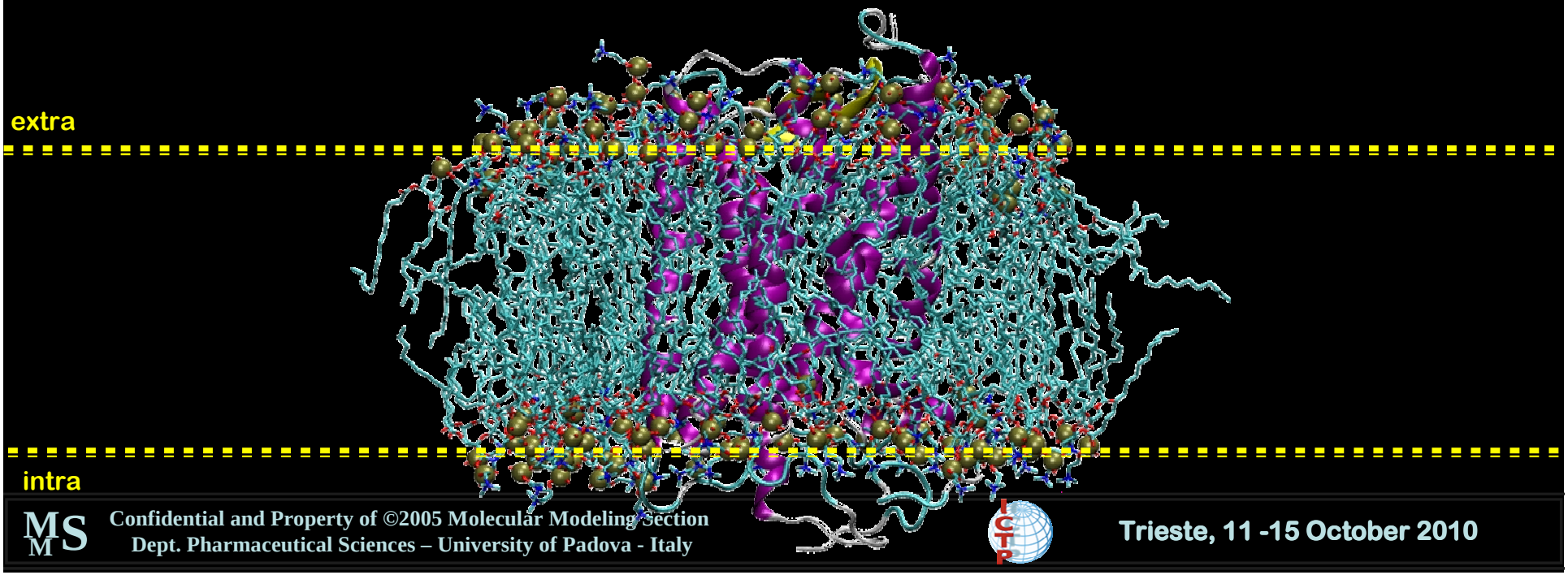


predict (x,y,z). etc

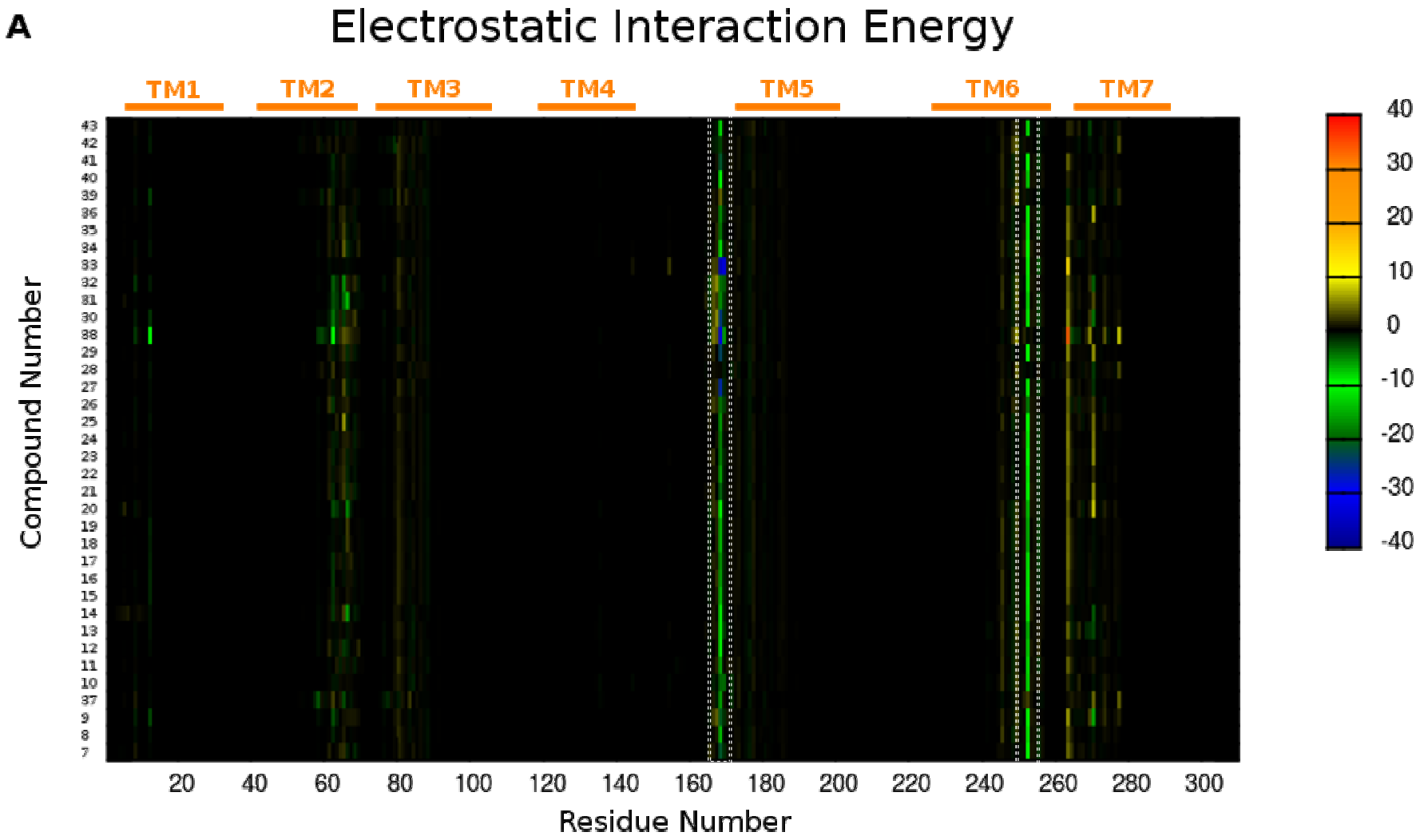
predict "ΔE_p"

Can we predict the possible binding motif?

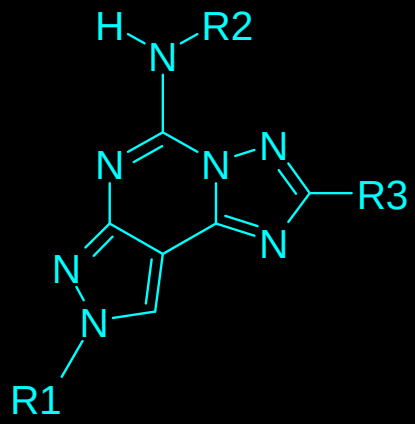




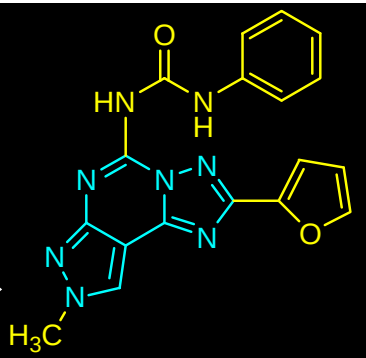
Can we predict the possible binding motif?

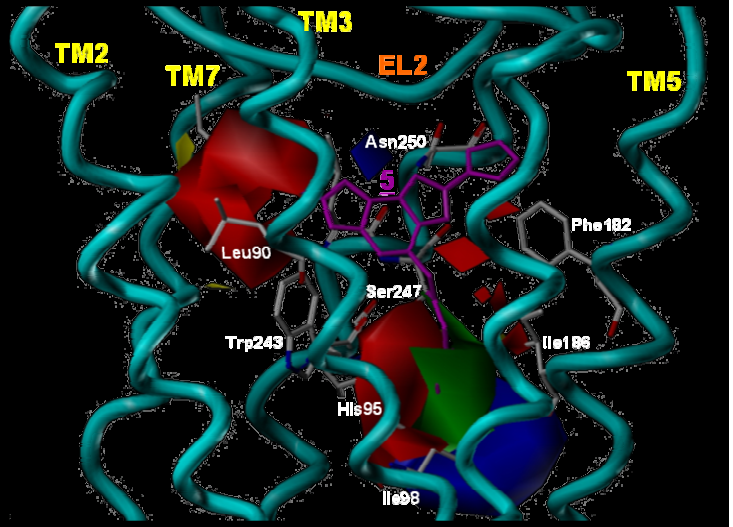


Moro S., Spalluto G., Paoletta S., Federco S. J Med Chem. (2010) submitted



Ki/selec optimization

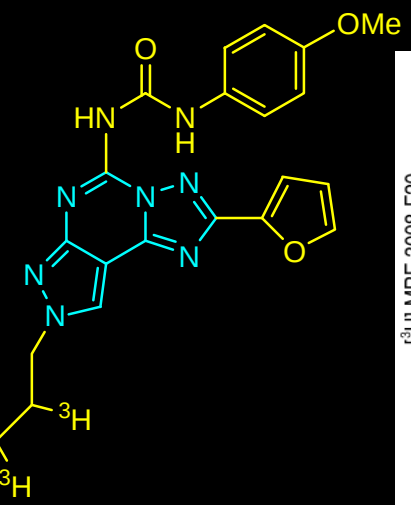


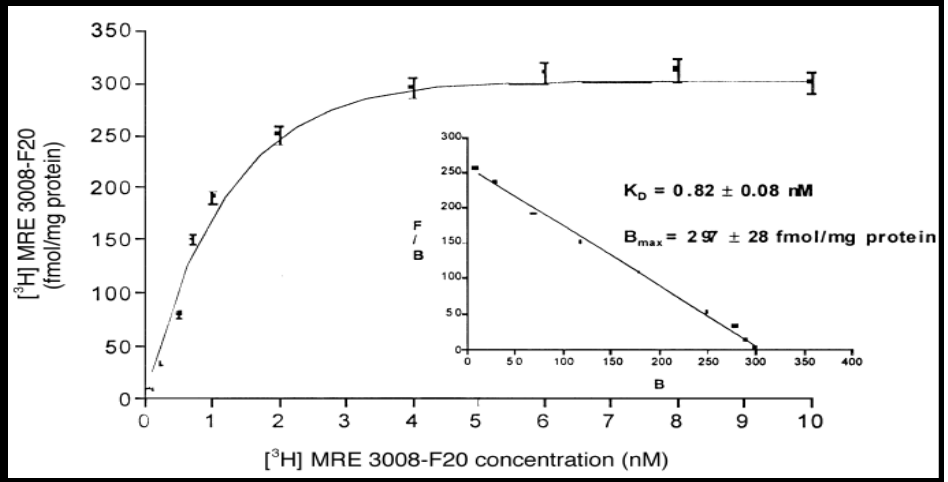


hA_1 = 594 nM
hA_{2A} = 381 nM
hA_{2B} = 222 nM
hA_3 = 0.16 nM

Moro S., Spalluto G. Baraldi P.G. et al *J. Med. Chem.* 45, 770-780 (2002)
Moro S., Spalluto G. Baraldi P.G., Jacobson K.A. et al *J. Med. Chem.* 45, 3579-3582 (2002)
Moro S., Spalluto G., Baraldi PG. et al *J. Med. Chem. 46, 4287-4296* (2003)

Tritiation

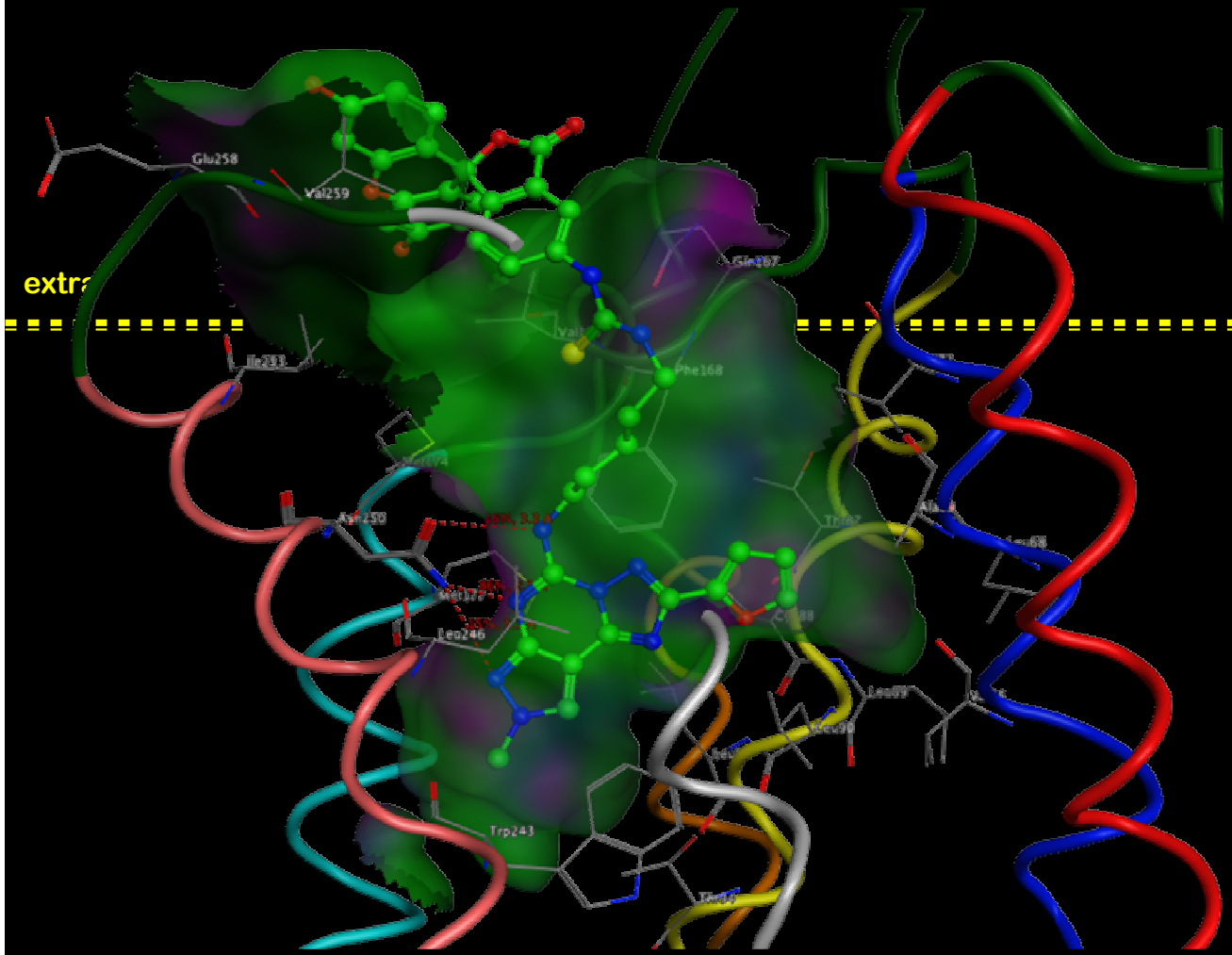


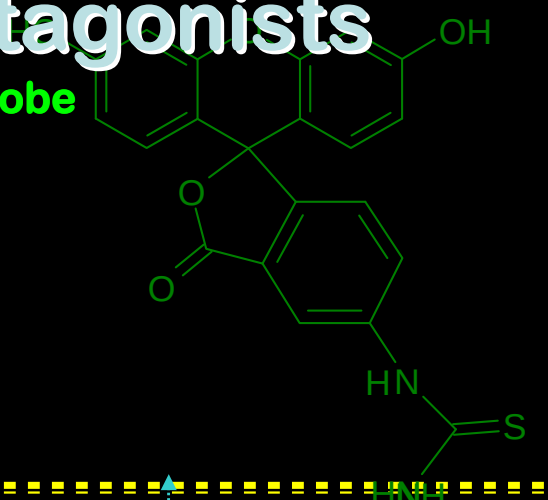


Moro S., Spalluto G. Baraldi P.G. et al *Biorg. Med. Chem. Lett.* 10, 209-211 (2002)

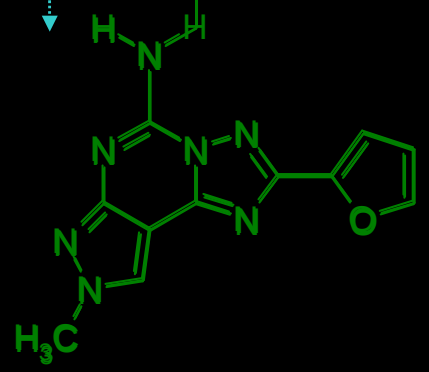
From radio- to fluo-A3 antagonists

fluo-probe



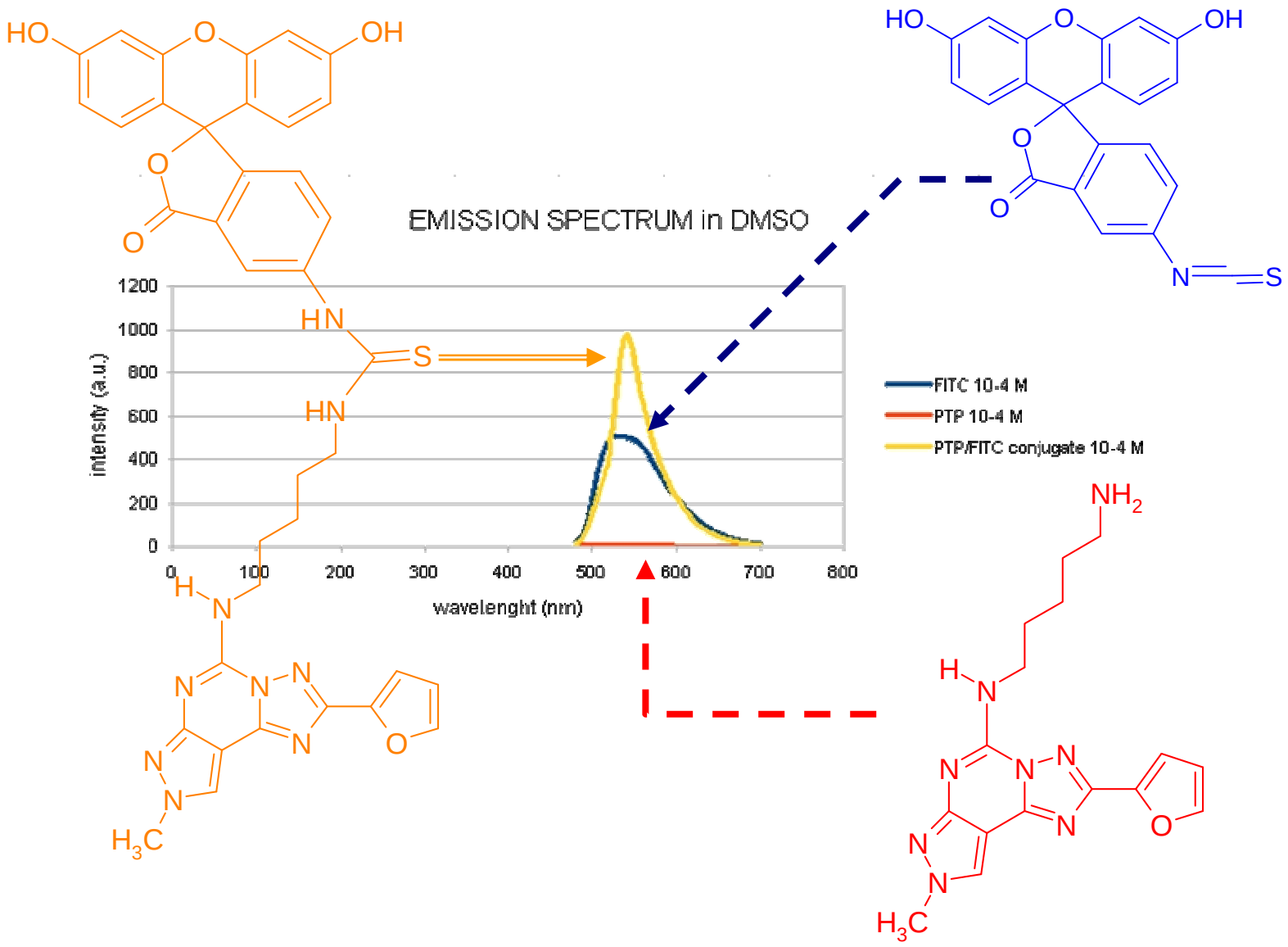


Spacer ≈ 8 Å



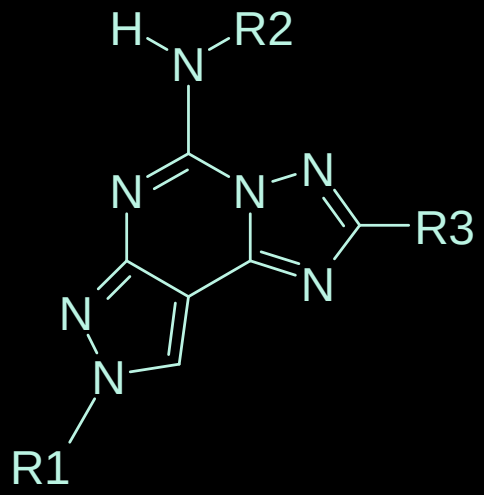
Scaffold

Moro S., Spalluto G., Paoletta S., Federco S. (2010) un published results



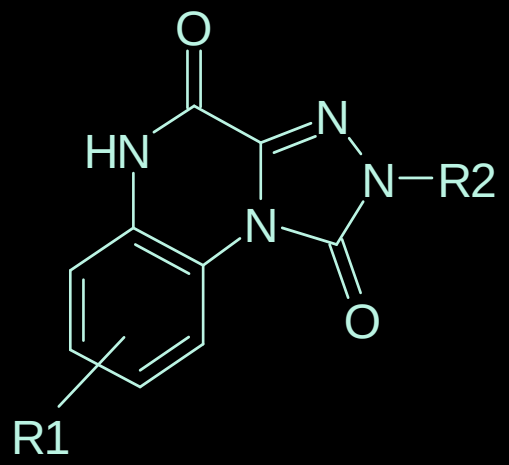
Moro S., Spalluto G., Paoletta S., Federco S. (2010) un published results

Italian Chemical Probes



Pyrazolo-triazolo-pyrimidine

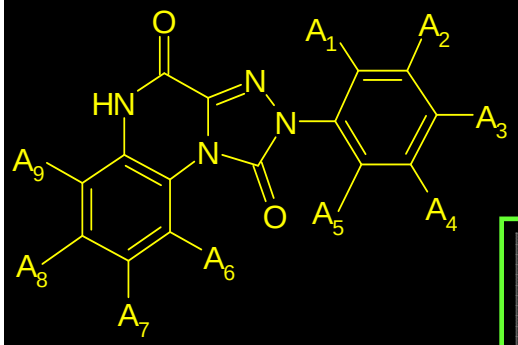
by prof. G. Spalluto
University of Trieste (Italy)



Triazolo-quinoxaline

by prof. V. Colotta
University of Florence (Italy)

Receptor-driven design of combinatorial libraries

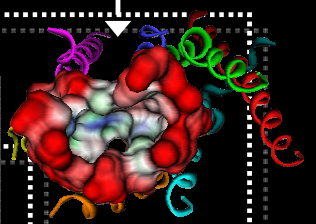


1385 compounds

MOE-Dock, Glide, Gold

in silico library

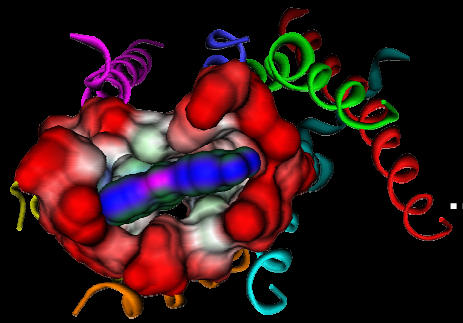
consensus docking & scoring



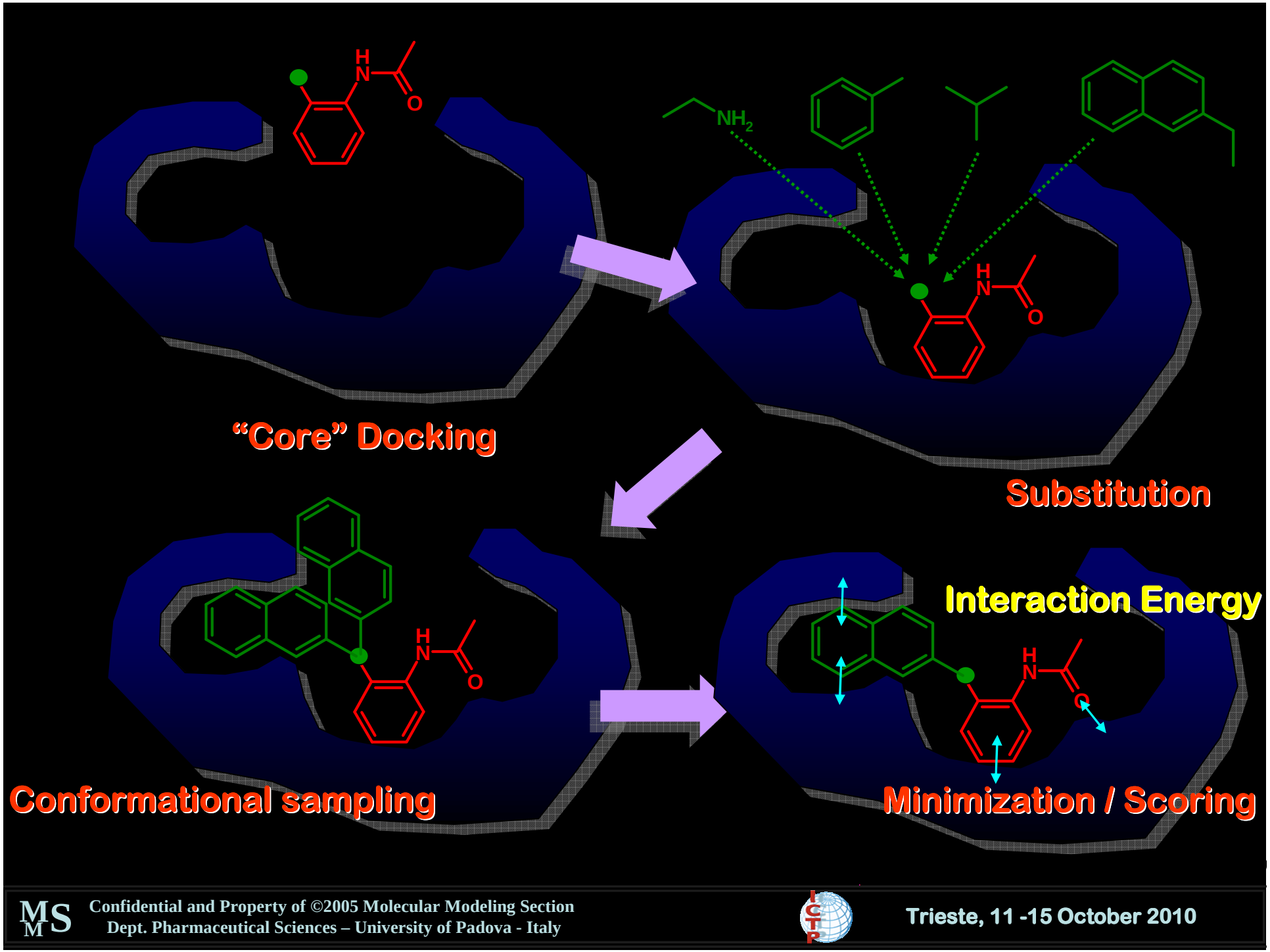
ranking and prioritize synthesis

biological assay

candidate selection

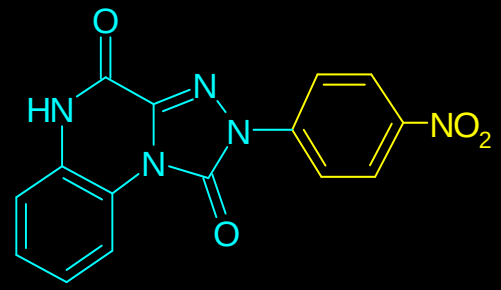


Moro S. Colotta V. *et al. J. Med. Chem.* 47, 3580-3590 (2004)

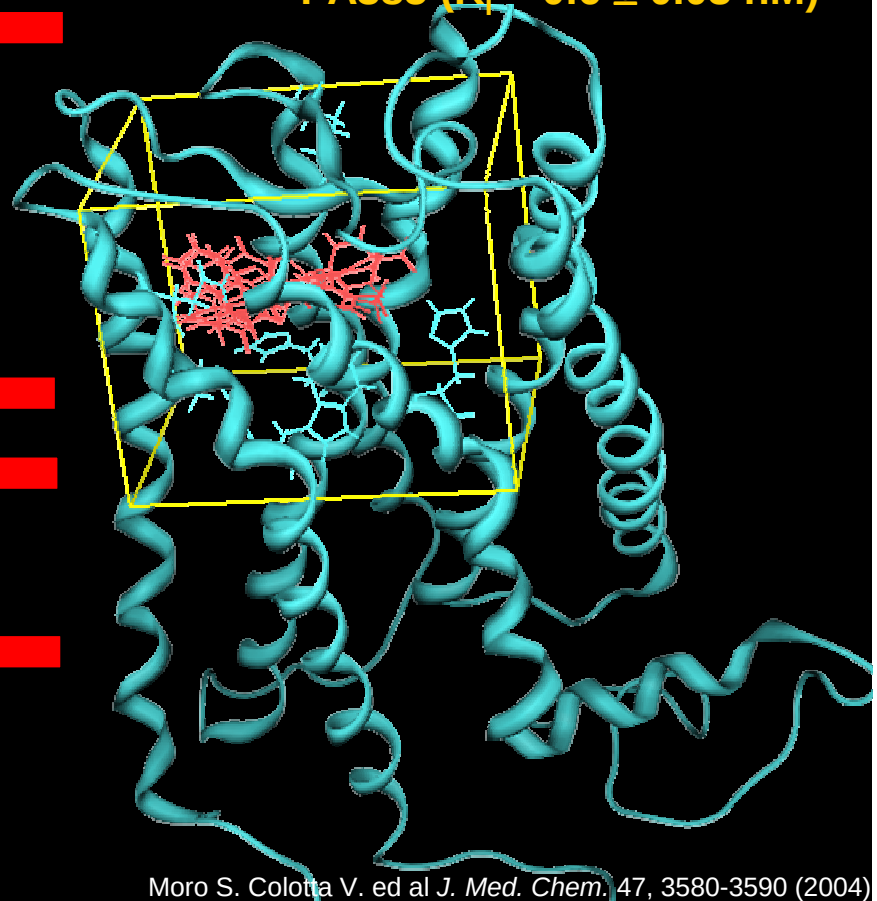


Receptor-based design of combinatorial libraries
… it seems to work:

	A_9	A_3	K_i (nM)
FA387	H	OH	47±3.9
FA386	H	OEt	175±15.3
FA373	H	NH_2	3600±264
FA381	H	NMe_2	429±35.9
FA385	H	NO_2	0.6±0.03
FA392	NO_2	OMe	4.7±0.52
FA417	NO_2	NO_2	24%
FA388	H	$OCOCH_3$	11.2±1.4
nn1	H	H	780±35
nn28	NO_2	H	279±21



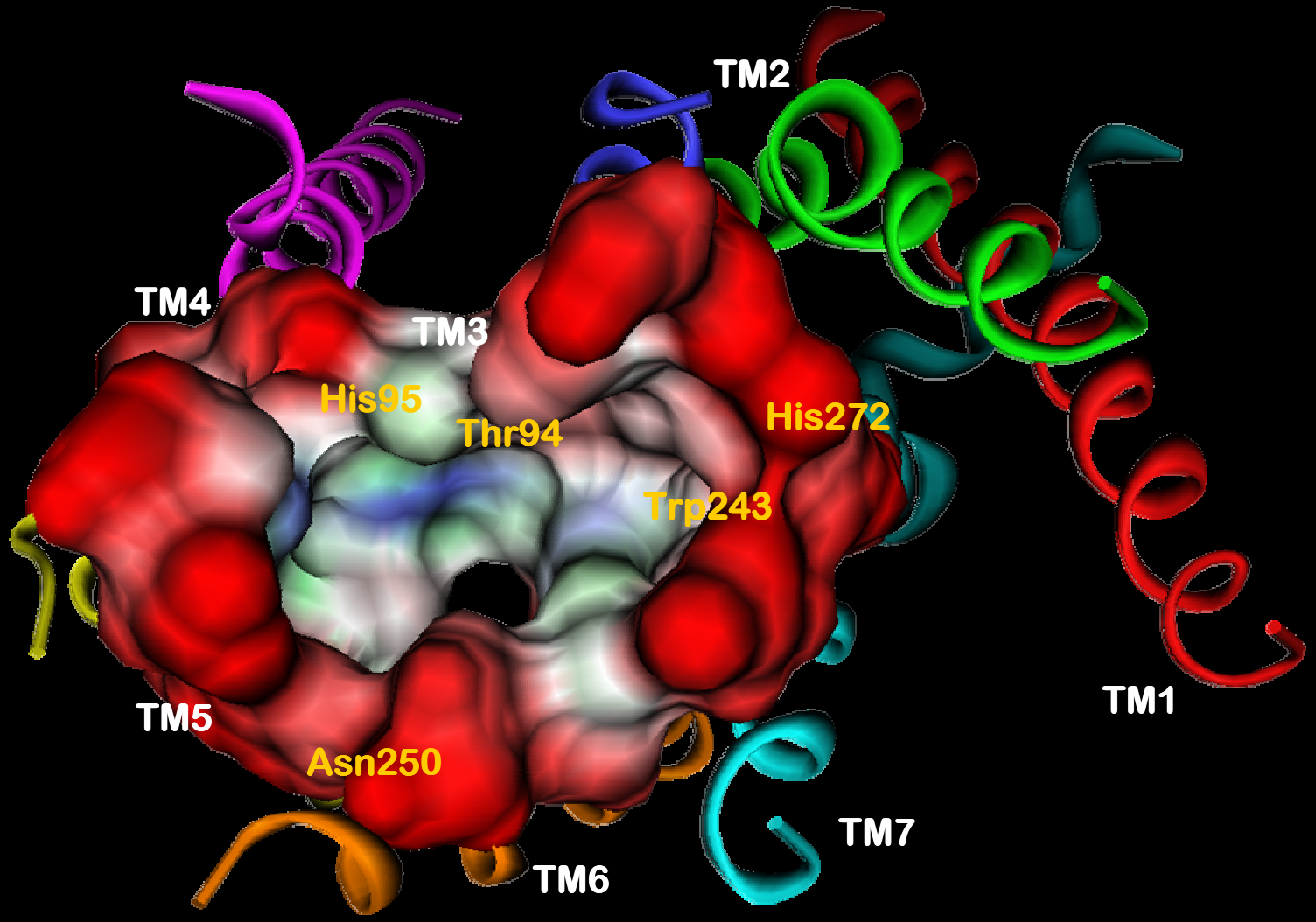
FA385 (K_i = 0.6 ± 0.03 nM)



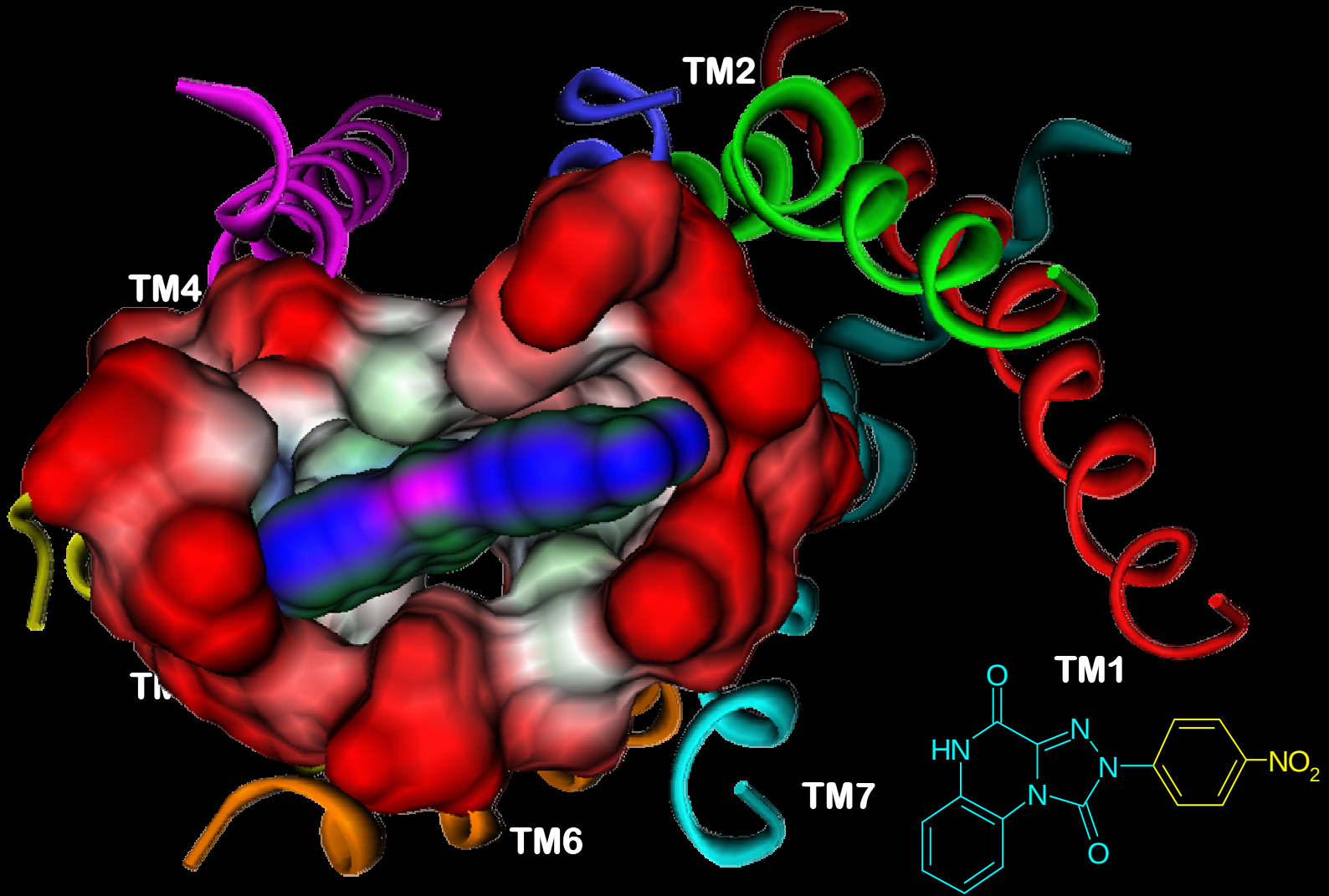
Moro S. Colotta V. ed al *J. Med. Chem.* 47, 3580-3590 (2004)

TMs domain topology

Human A_3 receptor



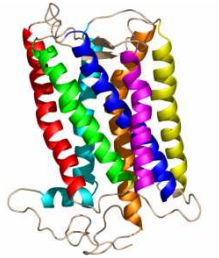
TMs ligand recognition domain



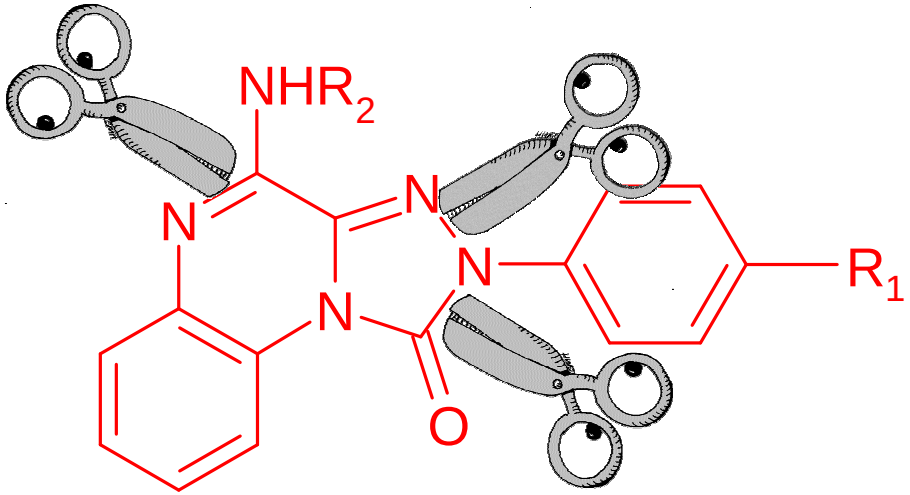
Moro S. Colotta V. *J. Med. Chem.* 47, 3580-3590 (2004)

FA385 (K_i = 0.6 ± 0.03 nM)

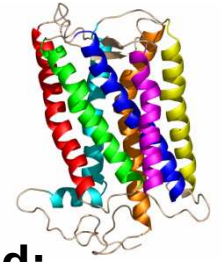
Molecular Simplification Approach



1. Reduce molecular complexity;
2. Simply synthetic route;
3. Improve water solubility;
4. Optimize chemical and/or metabolic stability.

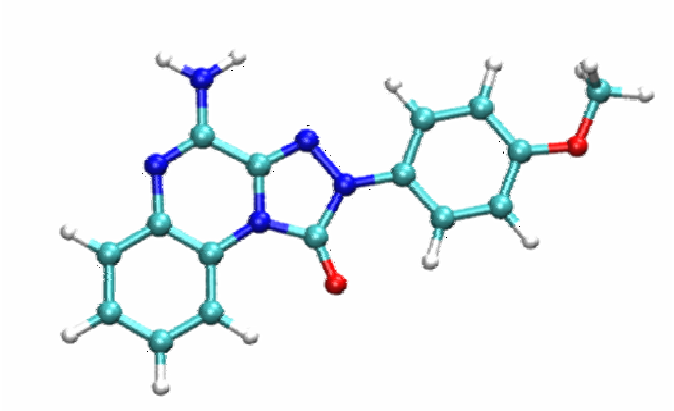


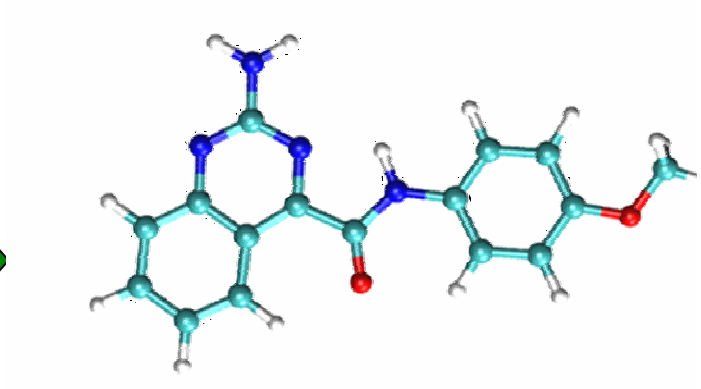
Molecular Simplification Approach

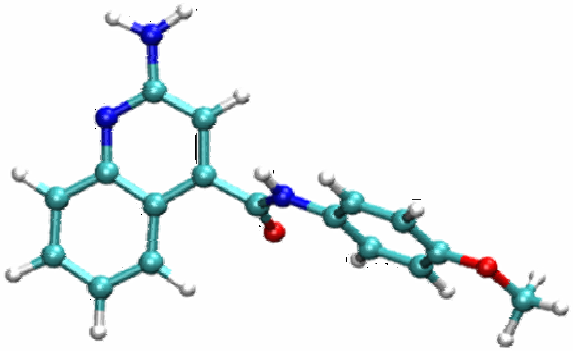


disconnection

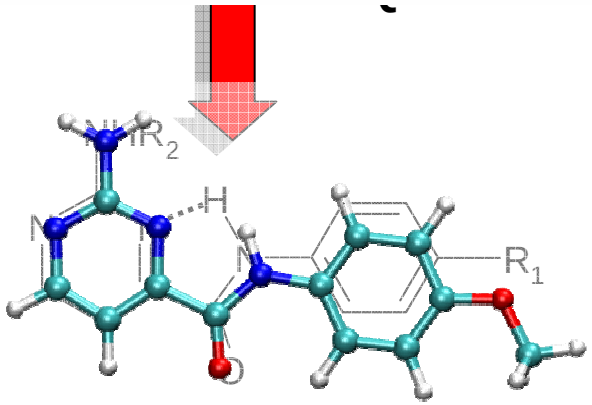
intra H-bond:
co-planarity is preserved





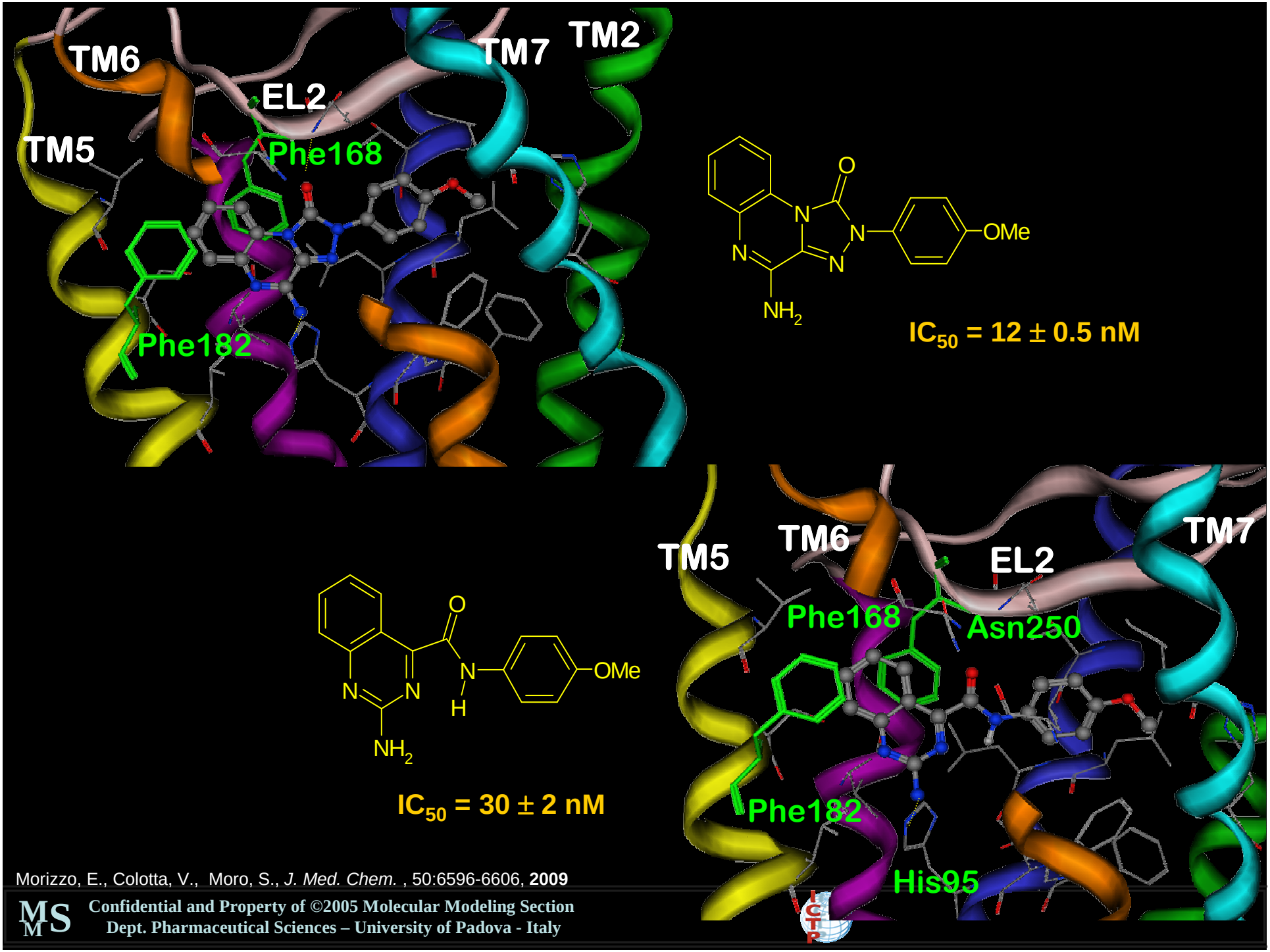


missing intra H-bond:
co-planarity is abolished

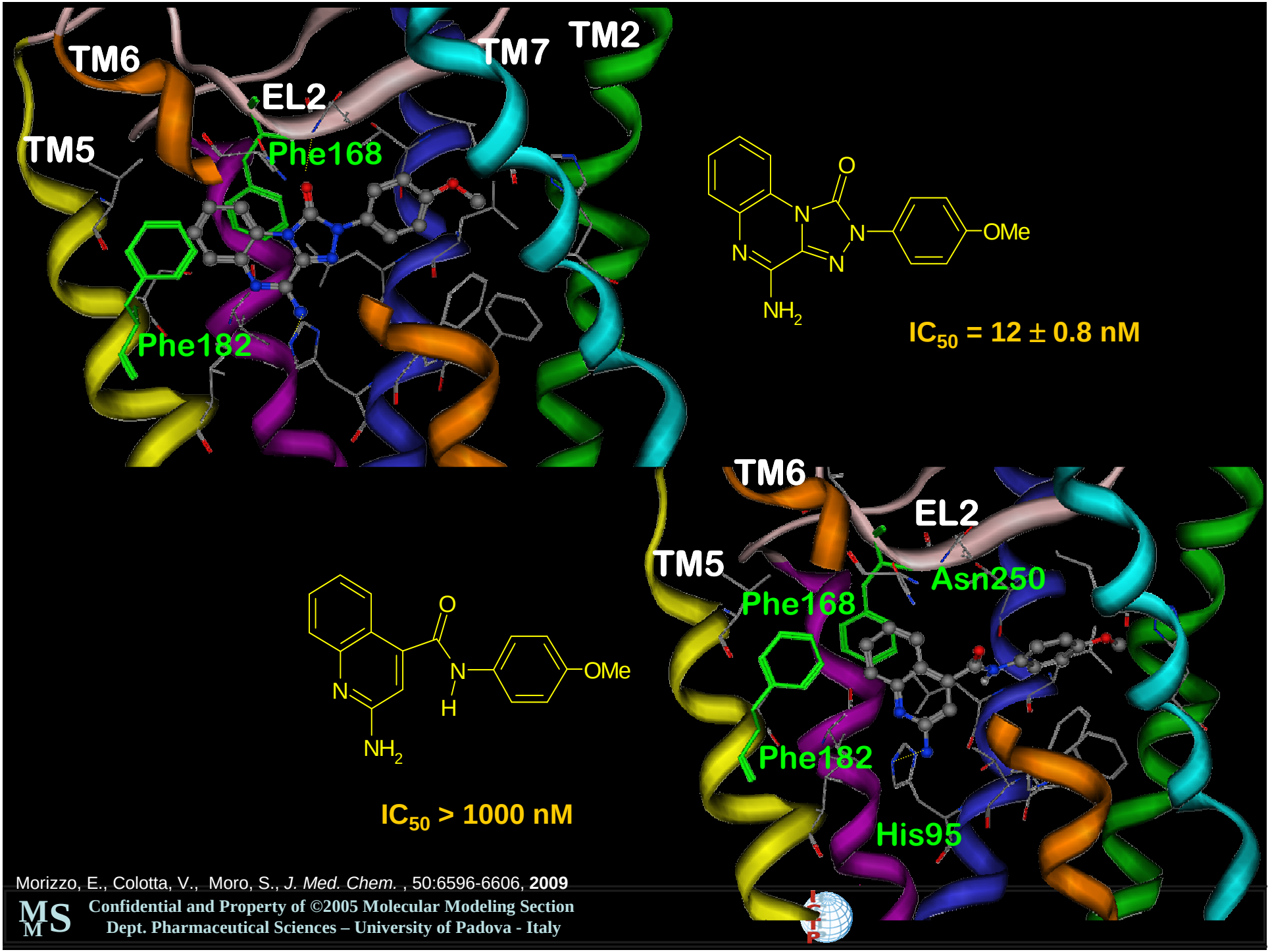


PYRM

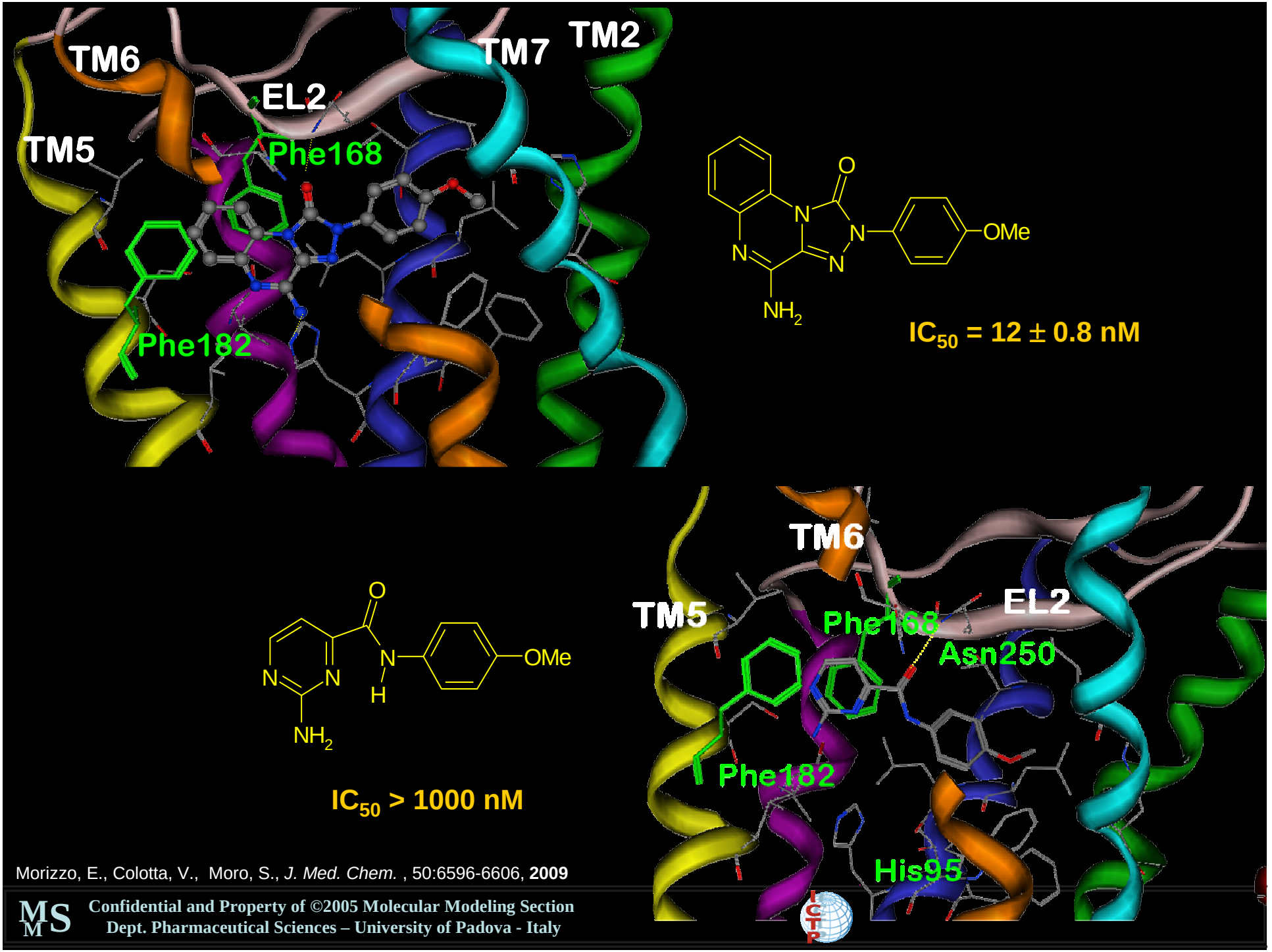
missing aromatic ring feature:
receptor complementarity is drastically reduced



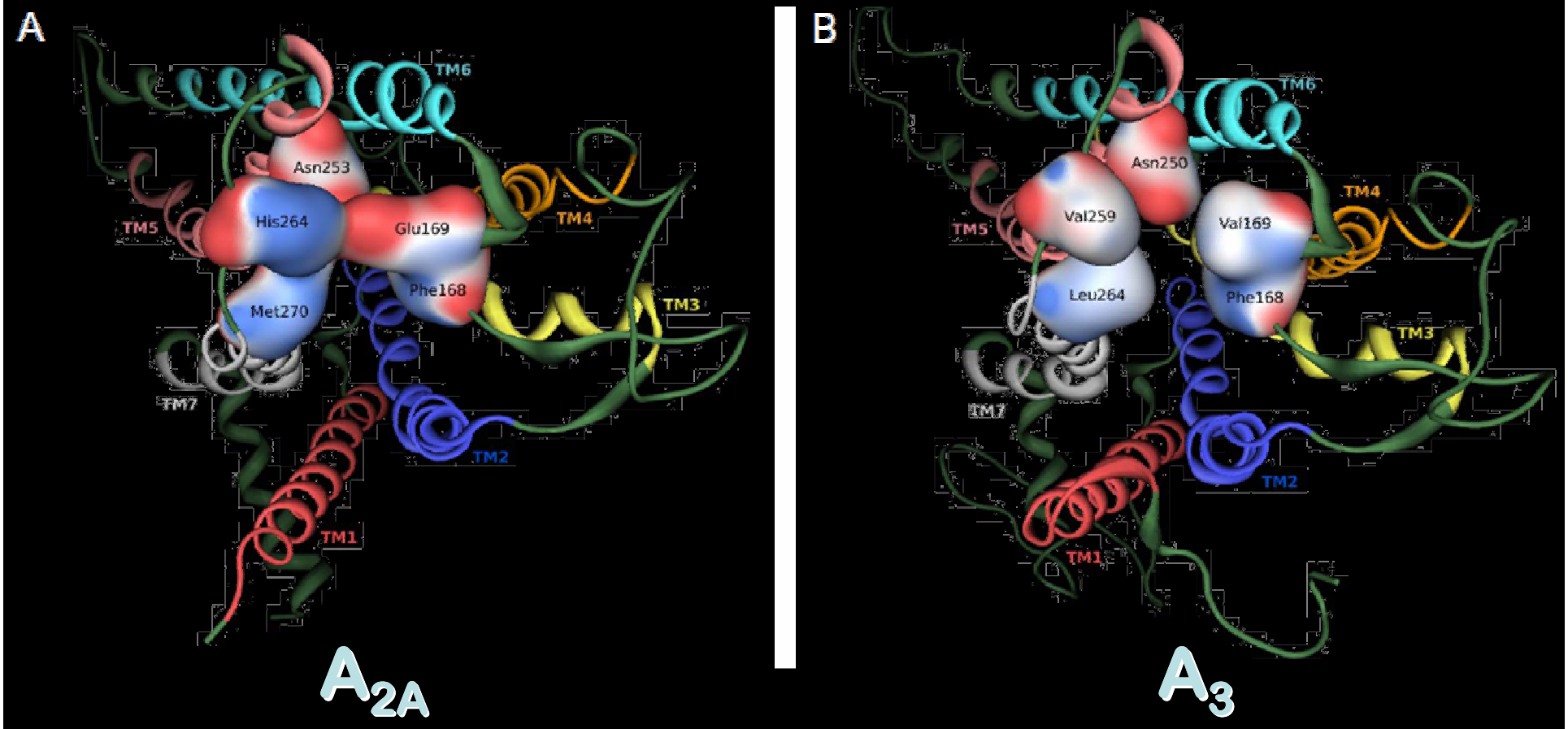
Morizzo, E., Colotta, V., Moro, S., *J. Med. Chem.* , 50:6596-6606, **2009**



Morizzo, E., Colotta, V., Moro, S., *J. Med. Chem.* , 50:6596-6606, **2009**


TM6
TM7
TM2
EL2
TM5
Phe168
Phe182
O
N
N
N
N
OMe
NH2
IC50 = 12 ± 0.8 nM
O
N
N
N
H
OMe
NH2
IC50 > 1000 nM
TM6
TM5
Phe168
EL2
Asn250
Phe182
His95
Morizzo, E., Colotta, V., Moro, S., J. Med. Chem. , 50:6596-6606, 2009
MS
Confidential and Property of ©2005 Molecular Modeling Section
Dept. Pharmaceutical Sciences – University of Padova - Italy

And, finally, what we have learned about A_{2A}/A_3 ARs selectivity until now:



A_{2A} A_3

Moro S., Paoletta S. et al *J. Med. Chem. 52, 7640-7652* (2009)

GPCRs: some crucial open questions ...at least in our mind!

Organization of the TMs;

Role of the ELs in ligand recognition;

Potency *versus* Selectivity;

Agonists *versus* Antagonists recognition;

GPCRs oligomerization;

GPCR/protein interaction;

"De-orphanize" orphan GPCRs…

Back to the future….

It is obvious that the emerging GPCR models will undoubtedly suffer in their quality from oversimplification, but, according to *E. Schrödinger*:

"***We can approach a complex understanding only when simple ideas, that can be perceived by the human brain as a whole, encompass the totality of what has been presented***".

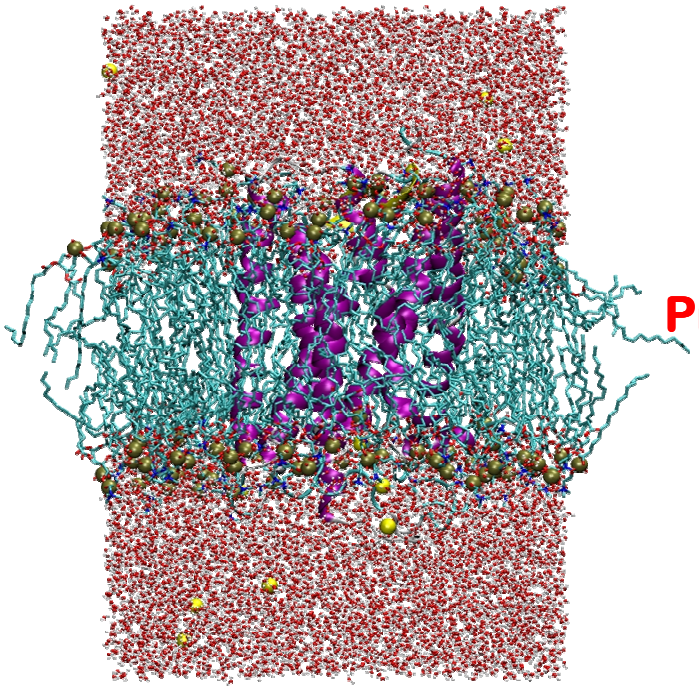
Personally, I prefer to envision the today's GPCR models exactly as such simple ideas, unifying the disciplines of medicinal chemistry, biophysics, and molecular biology in that these models display in their interdisciplinary potential.

“Honor and Pain”…

Synthesis:

Prof. Gianpiero Spalluto (Univ. of Trieste)

Prof. Vittoria Colotta (Univ. of Firenze)



Pharmacology Assays:

Dr. Kenneth Jacobson (NIH, Bethesda)

Prof. Karl-Norbert Klotz (Univ. of Würzburg)

Design… possibly rationale:

Molecular Modeling Section - MMS (Padova)