

Studi computazionali su inibitori irreversibili della K-Ras (Kirsten Rat Sarcoma)

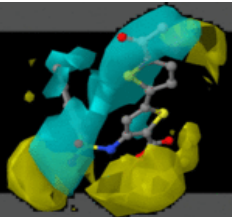


SAPIENZA
UNIVERSITÀ DI ROMA

Facoltà di Farmacia e Medicina
Corso di Laurea in Biotecnologie Farmaceutiche
Tesi Sperimentale in Chimica Farmaceutica
a.a. 2015/2016

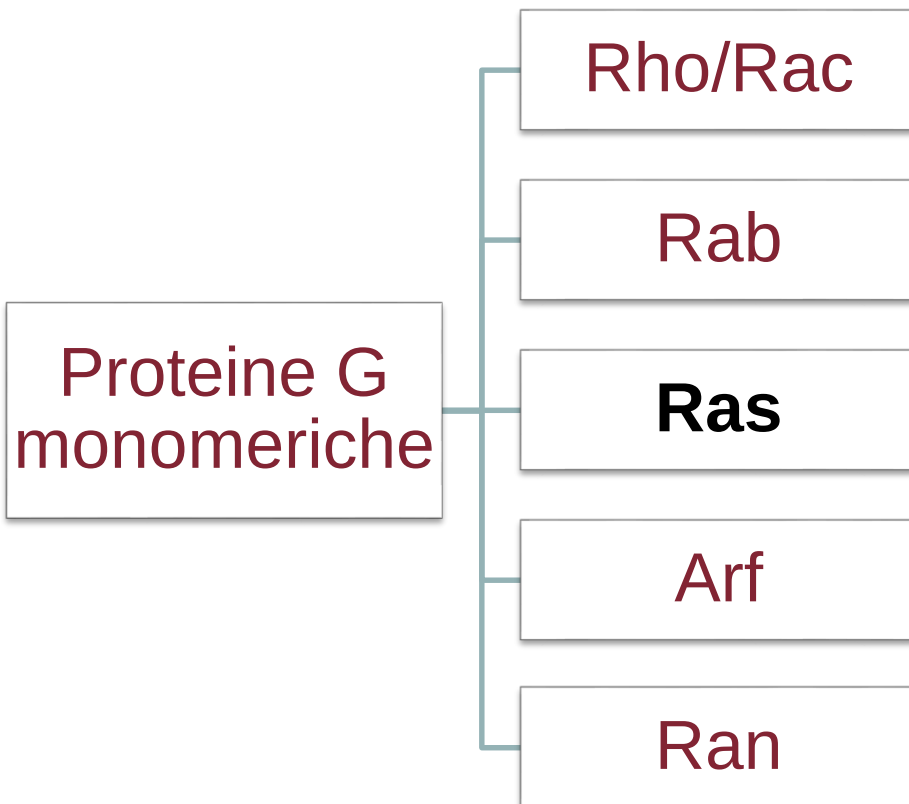
Laureanda: Claudia Trivisani
Matricola: 1560711

Relatore: prof. Rino Ragno



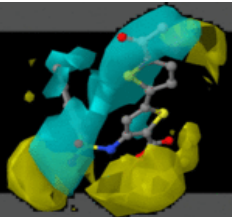
Superfamiglia proteine Ras

by www.RCMD.it



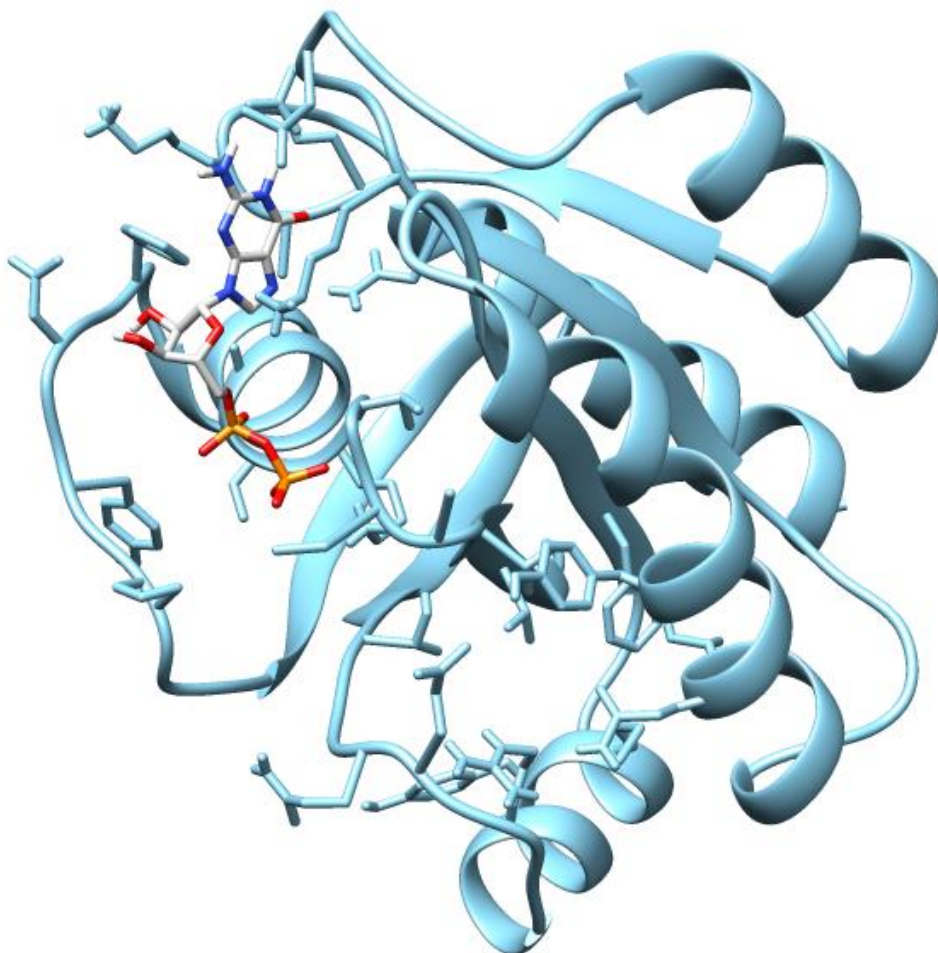
La deregolazione induce:

- Evasione dall'apoptosi
- Riprogrammazione del metabolismo cellulare
- Angiogenesi
- Metastatizzazione

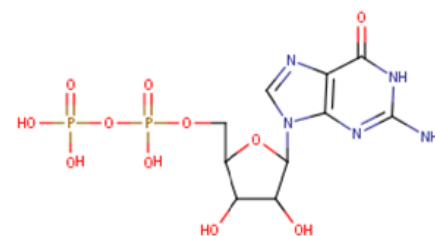


Le proteine Ras

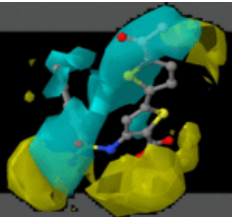
by www.RCMD.it



- GTPasi monomeriche
- 21 kDa
- Scambiatori molecolari

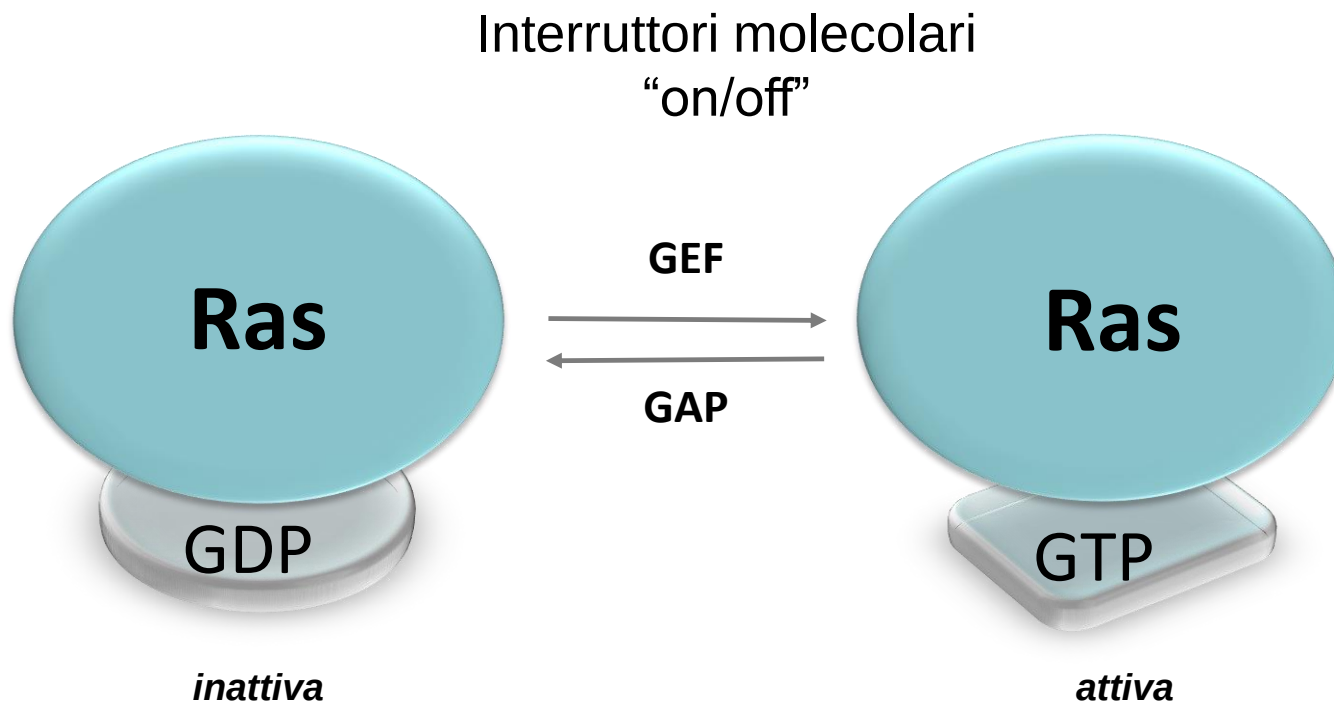


GDP



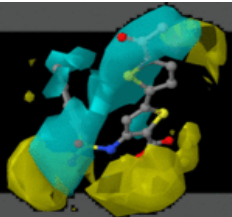
Le proteine Ras

by www.RCMD.it



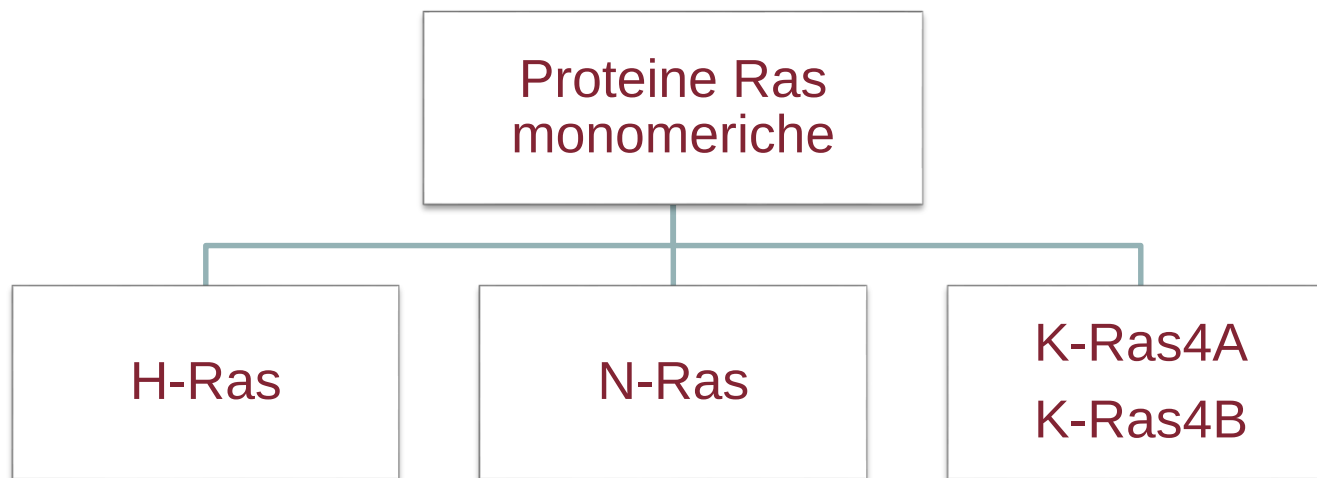
Lo stato funzionale delle proteine Ras è regolato da:

- **GEFs** → guanine nucleotide exchange factors
- **GAPs** → GTPases-activating proteins



Le proteine Ras

by www.RCMD.it

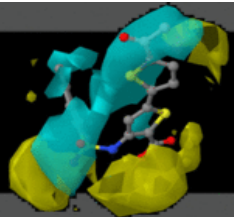


Presentano l'85% di similarità di sequenza.

Le mutazioni più frequenti avvengono a carico della

K-Ras

e bloccano la proteina in conformazione ATTIVA.



Struttura

by www.RCMD.it

H-Ras
N-Ras
K-Ras4A } 188 aa

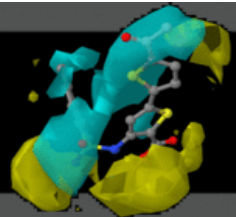
K-Ras4B } 189 aa

Aa da 1 a 165 altamente conservati.
Porzione C-terminale altamente variabile.



■ Sito di legame dell'effettore
■ Sito di legame del GTP
■ Dominio ipervariabile

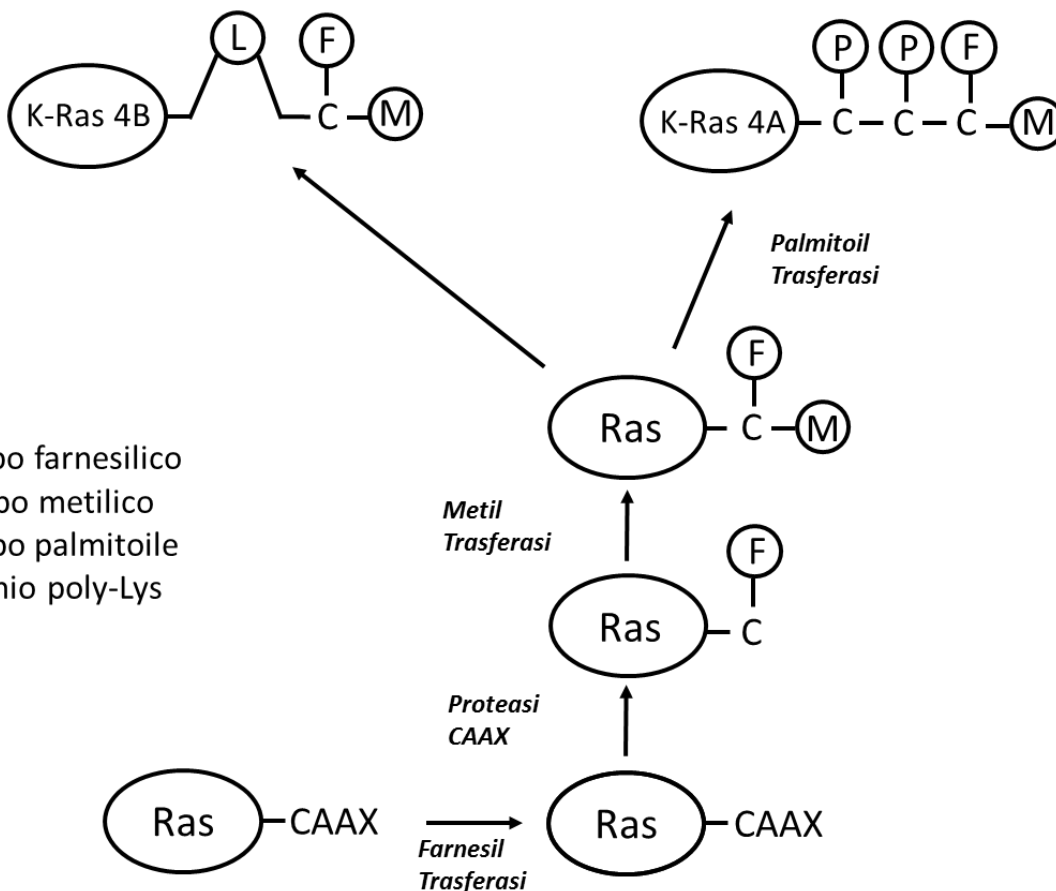
■ Switch I
■ Switch II



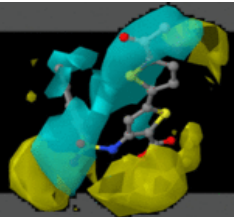
Modifiche post-traduzionali

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Membrana plasmatica

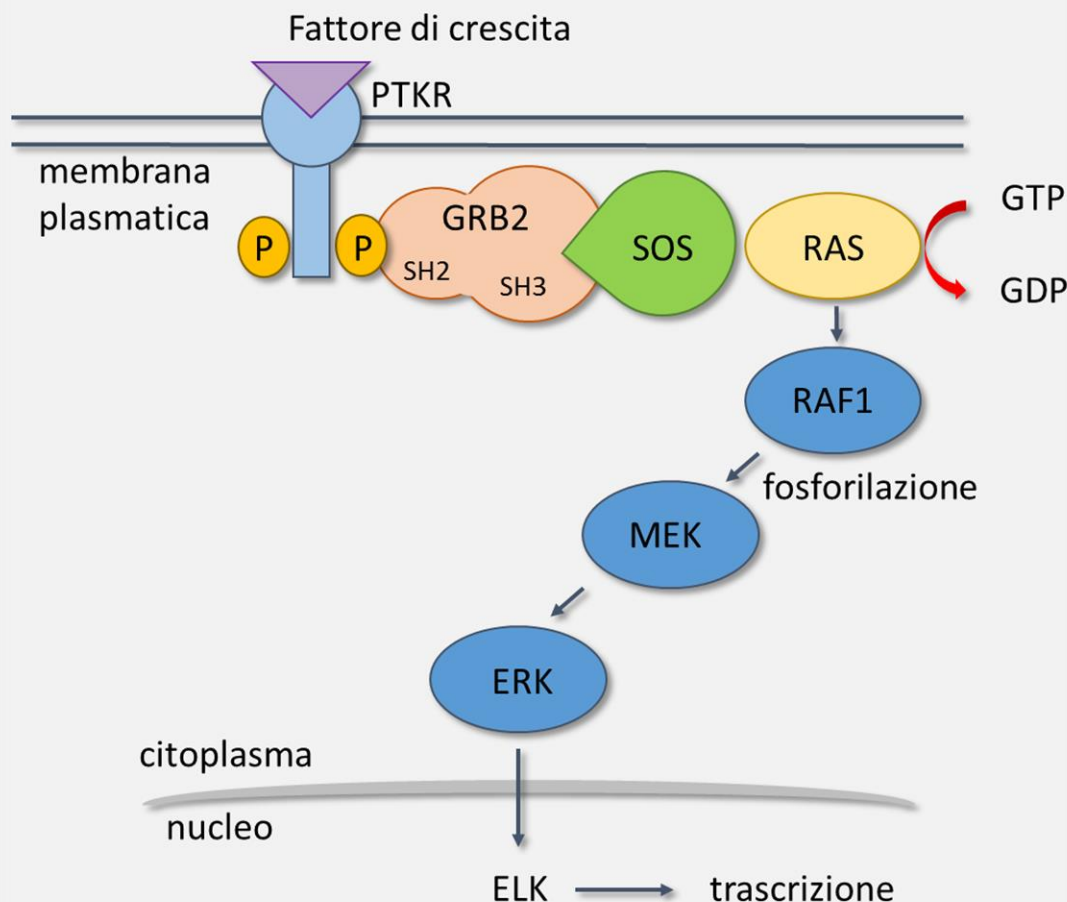


- C = Cys
- A = Leu, Ile, Val
- X = Met, Ser, Leu, Gln

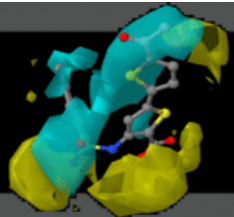


Pathway di K-Ras

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Nello stato attivo Ras attiva la cascata di fosforilazione delle MAPK (Proteine Chinasi Attivate da Mitogeni). Quando Erk viene fosforilata migra nel nucleo dove funge da regolatore trascrizionale.

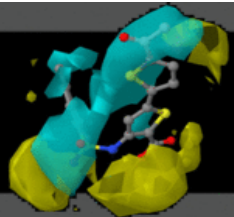


Le **mutazioni** attivanti le K-Ras si ritrovano:

- CARCINOMA PANCREATICO (70-90%)
- CARCINOMA DEL COLON-RETTO (30-50%)
- CARCINOMA DEL POLMONE (20-30%)

e sono associate a **resistenza** alle terapie con anti-EGFR effettuate con:

- Cetuximab
- Panitumumab.



Mutazioni in K-Ras

by www.RCMD.it

Le mutazioni bloccano la GTPasi in conformazione “on”.
Nel 97% dei casi riguardano i codoni 12 e 13.

Codone 12

G → A

Gly (GGT) → Ser (AGT)

Gly (GGT) → Asp (GAT)

G → T

Gly (GGT) → Cys (TGT)

Gly (GGT) → Val (GGT)

G → C

Gly (GGT) → Arg (CGT)

Gly (GGT) → Ala (GCT)

Codone 13

G → A

Gly (GGT) → Asp (GAC)

G → T

Gly (GGT) → Cys (TGT)

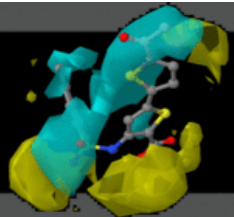
Gly (GGT) → Val (GGT)

G → C

Gly (GGT) → Arg (CGT)

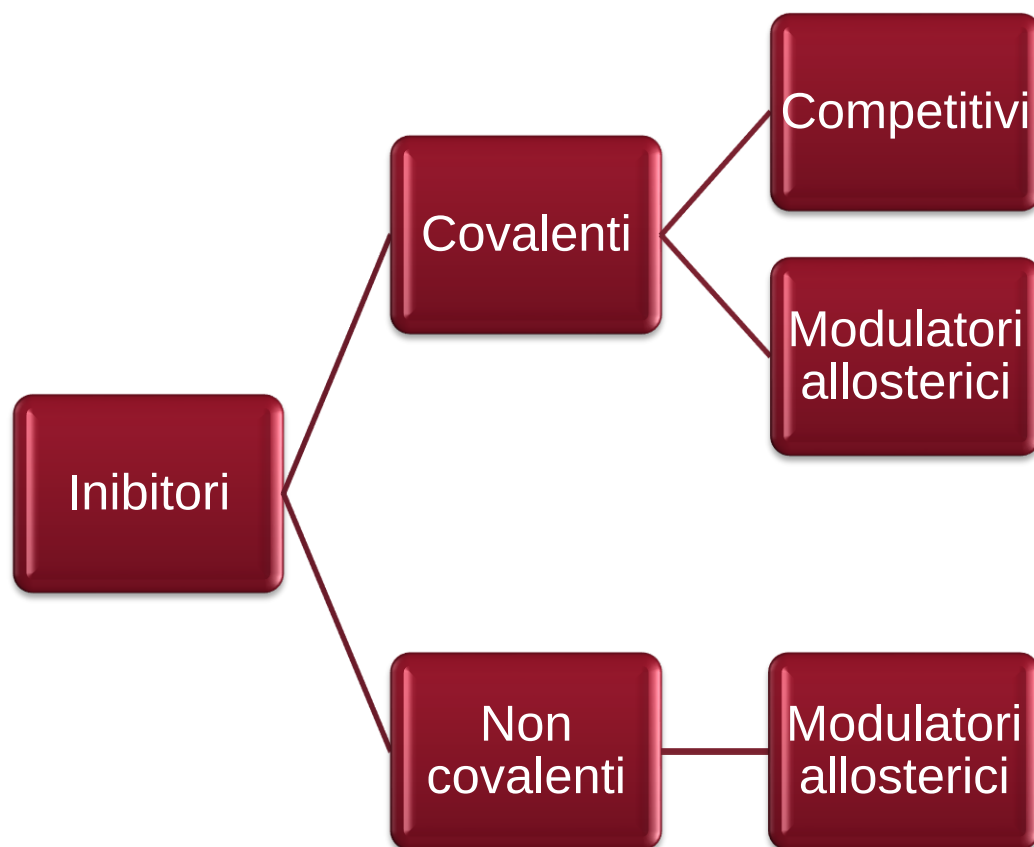
Gly (GGT) → Ala (GCT)

Altre mutazioni avvengono a carico dei codoni 59 e 61.

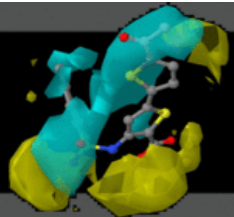


Inibitori delle K-Ras

by www.RCMD.it

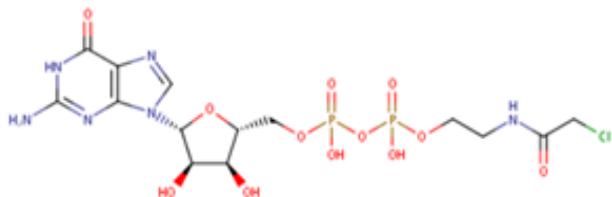


Cambiare la preferenza della proteina a favore del GDP e indebolire il legame con Raf.

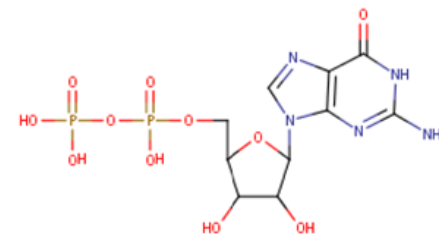


Inibitori competitivi

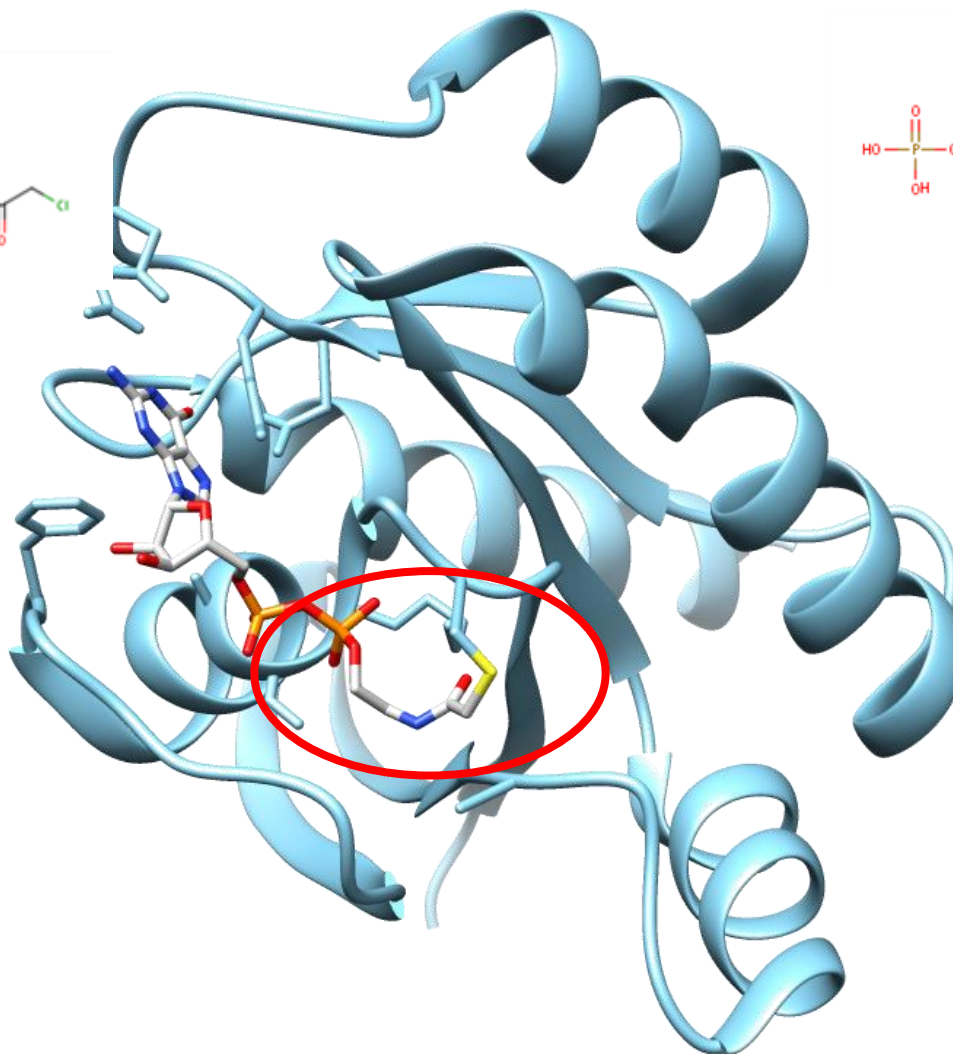
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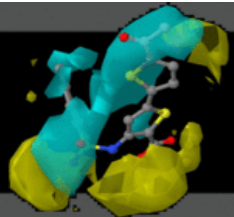


SML-83-71-9



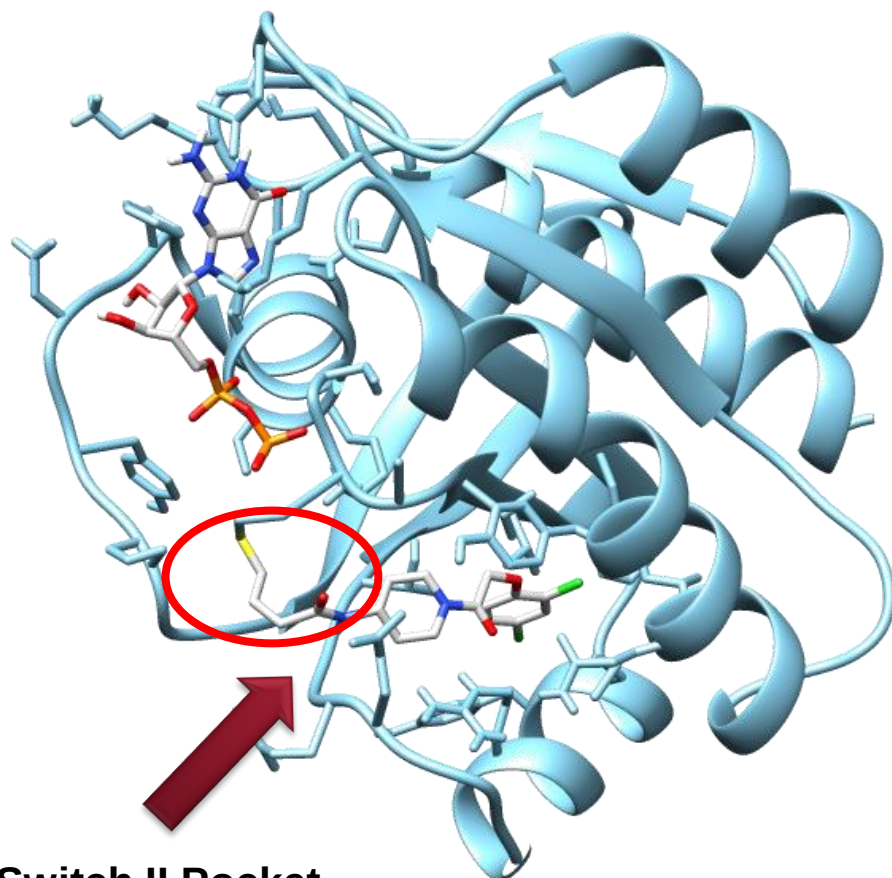
GDP



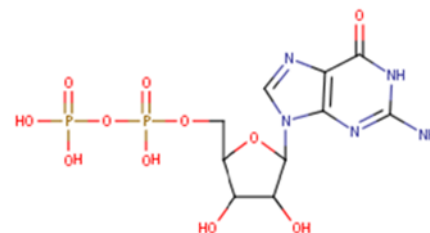


Inibitori covalenti delle K-Ras

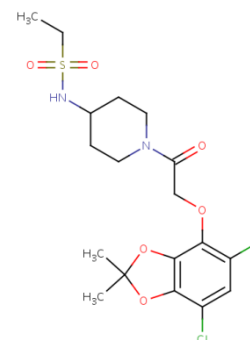
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Switch II Pocket



GDP



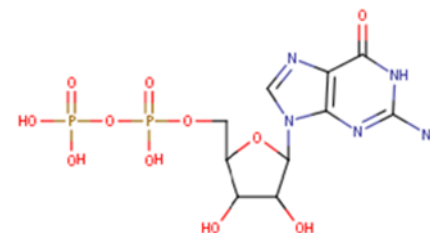
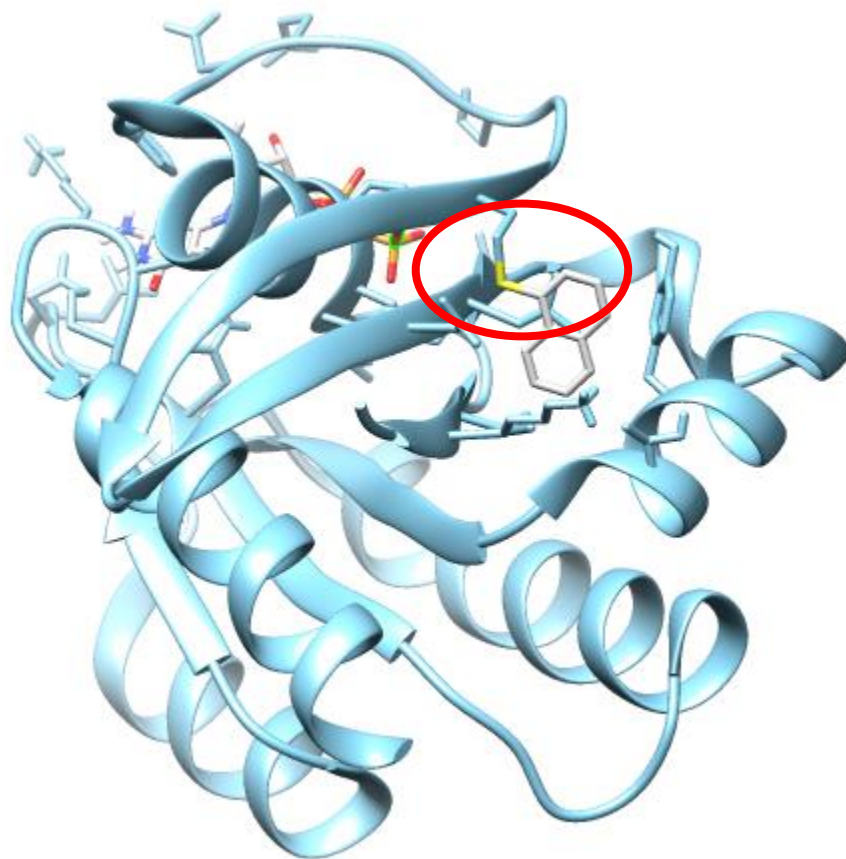
4LUC

Formano un legame covalente con la Cys12 portando la proteina in una conformazione aperta con Ras legata al GDP.

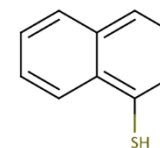


Inibitori covalenti del secondo sito di legame

by [www.RCMD.it](http://www.rcmd.it)



GDP



4Q01

Formano legami covalenti con la Cys39 o Cys 72 inibendo lo scambio nucleotidico mediato da Sos.

Tasca tra lo switch II ed il β -foglietto centrale.



Scopo della tesi

by **www.RCMD.it**

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An Information Portal to 120,252 Biological Macromolecular Structures

Search by PDB ID, author, macromolecule, sequence, or ligands

Advanced Search | Show by Annotations

Structure Summary 3D View Annotations Sequence Sequence Similarity Structure Similarity Experiment Literature

Biological Assembly 1

4LUC

Crystal Structure of small molecule disulfide 8 bound to K-Ras G12C

DOI: 10.2210/pdb4luc/pdb

Classification: [SIGNALING PROTEIN / INHIBITOR](#)

Deposited: 2013-07-25 Released: 2013-11-27

Deposition author(s): [Ostrem, J.M.](#), [Peters, U.](#), [Sos, M.L.](#), [Wells, J.A.](#), [Shokat, K.M.](#)

Organism: [Homo sapiens](#)

Expression System: [Escherichia coli](#)

Mutation(s): 10

Structural Biology Knowledgebase: 4LUC (2 models >14 annotations) [SARDB.org](#)

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 1.29 Å

R-Value Free: 0.169

R-Value Work: 0.152

wwPDB Validation

Metric	Percentile Ranks	Value
R-Value		0.174
Deviation		0
Bonds		0
Angles		0
Chiral		0
Planarity		0
Protein		2.4%

Full Report

View In 3D: JSmol or PV (in Browser)

Standalone Viewers

Simple Viewer Protein Workshop

Ligand Explorer Kiosk Viewer



**16 complessi
cristallizzati**

Modeller

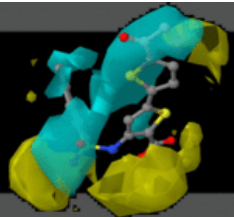
Parametrizzazione

**Minimizzazione e
allineamento**

Docking covalente

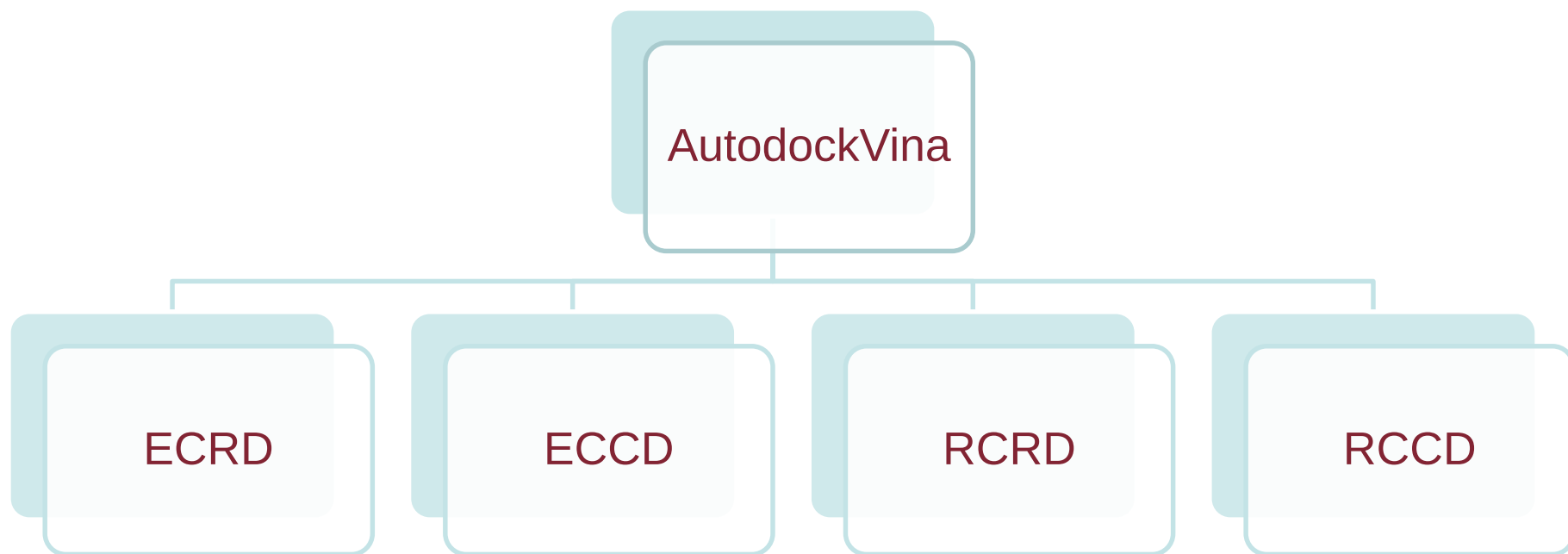
3-D QSAR

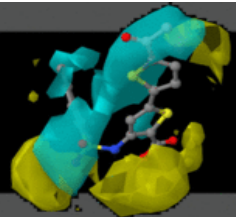
**Docking
reversibile**



DOCKING COVALENTE

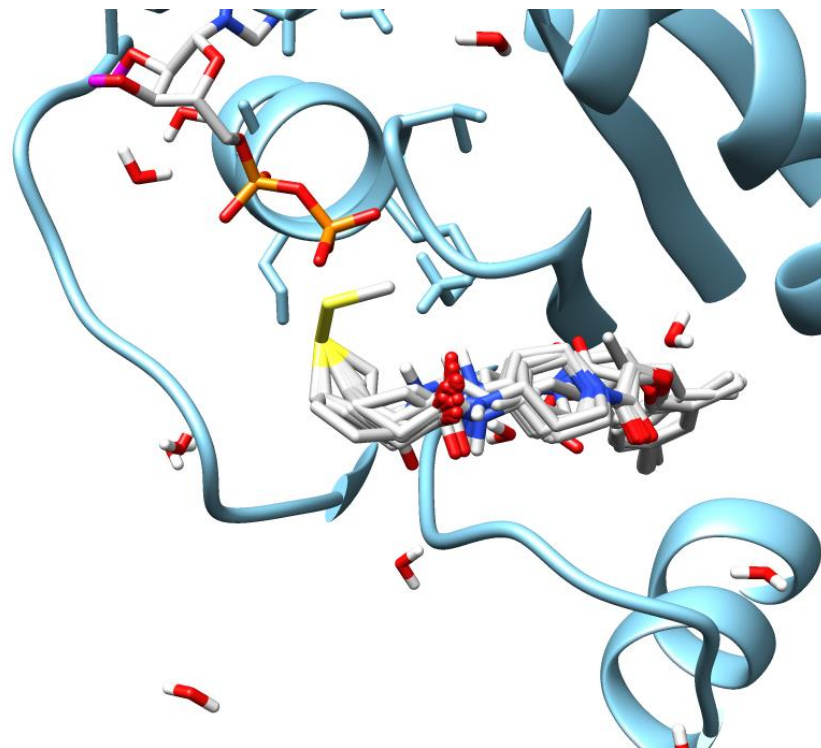
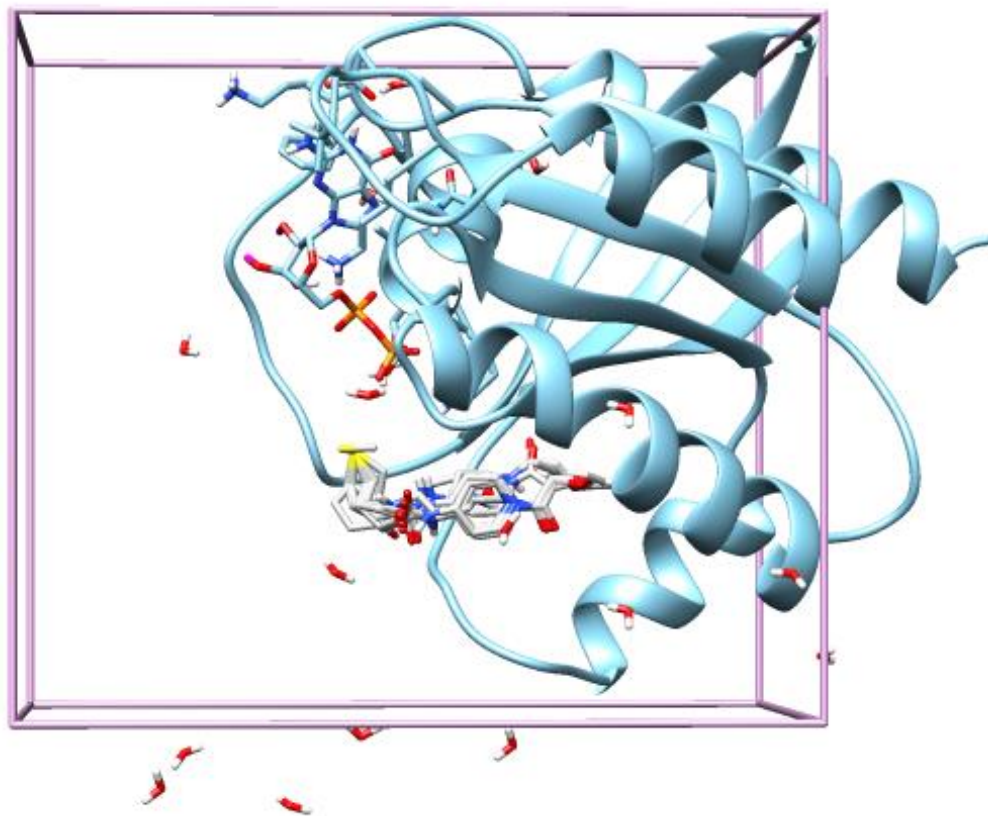
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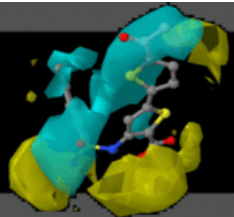




ECRD

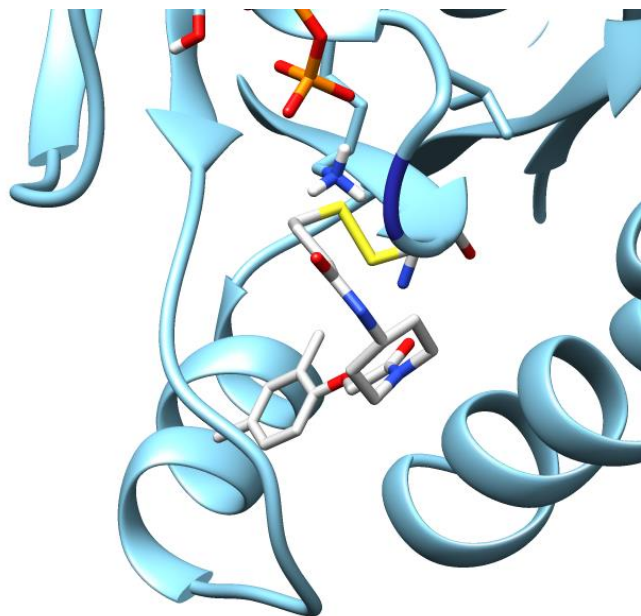
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RCRD e RCCD

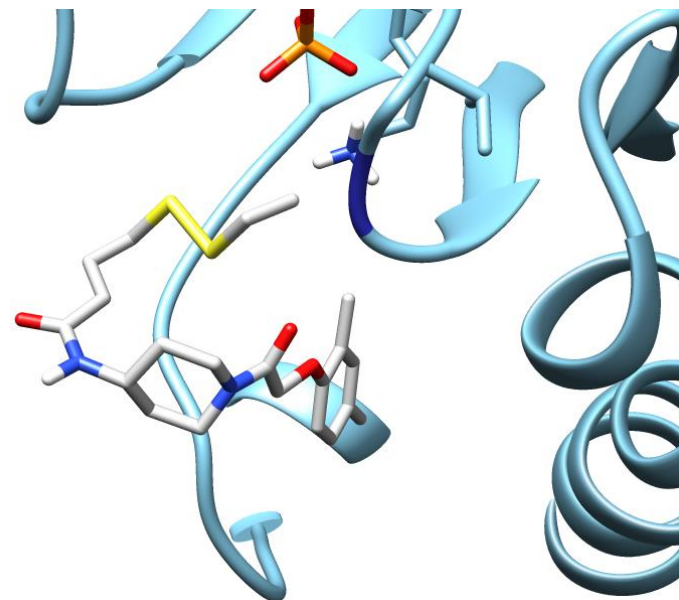
by www.RCMD.it



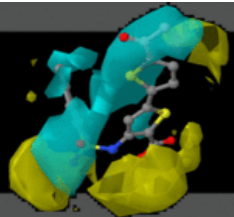
Obconformer

[NH3+] [C@H] (C=O) CS
(Cisteina)

SMART

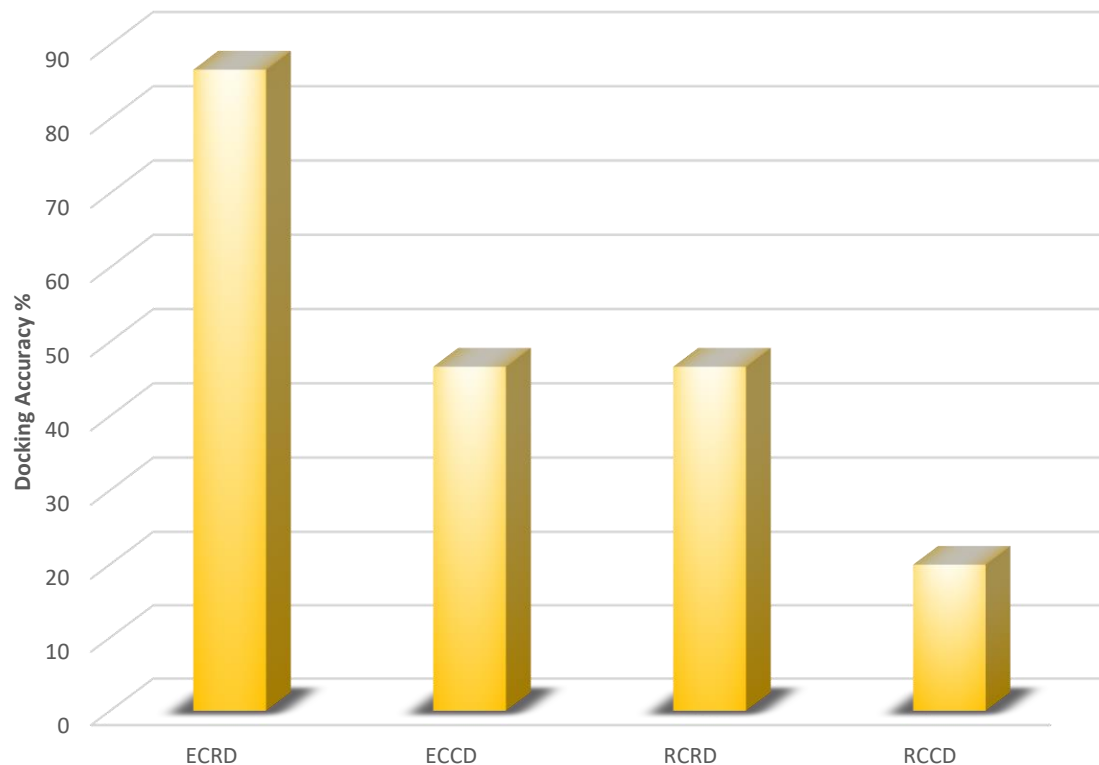


Obfit



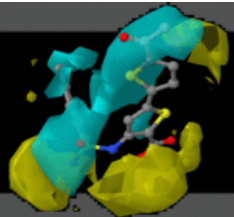
Docking covalente

by www.RCMD.it



$$RMSD = \sqrt{\frac{\sum_{i=1}^N d_i^2}{N}}$$

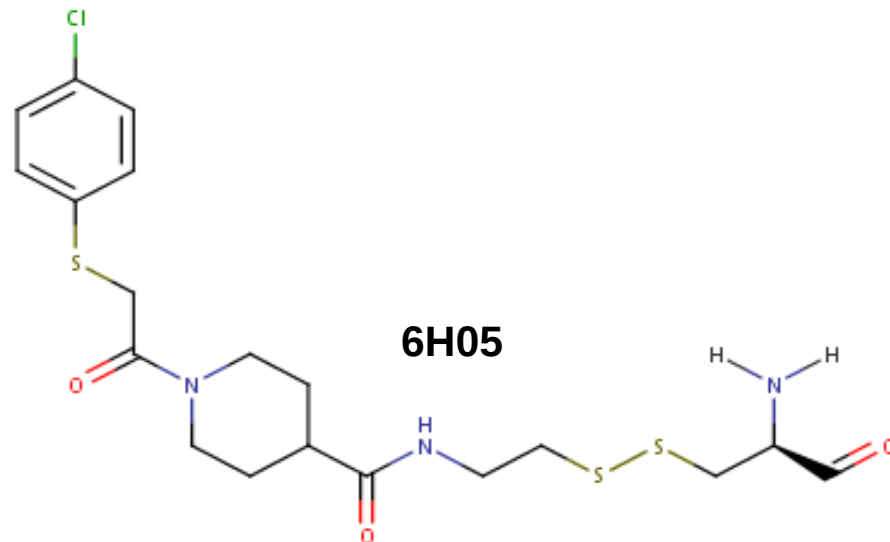
$$DA = 100 \frac{\sum RMSD_{(0-2)} + \frac{\sum RMSD_{(2-3)}}{2}}{N}$$



Inibitori irreversibili

by www.RCMD.it

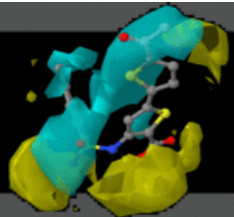
COMPOSTO	pIC ₅₀
6H05	5.19
compound1	3.89
compound2	4.71
compound5	5.59
JO-148A	5.13
JO-148B	5.72
JO-148C	5.75
JO-148D	5.57
JO-148E	6.40
JO-148F	6.40
JO-148H	5.70
JO-148I	5.72
JO-148J	5.50



Docking
covalente



3-D QSAR



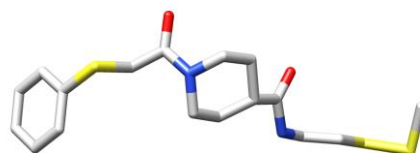
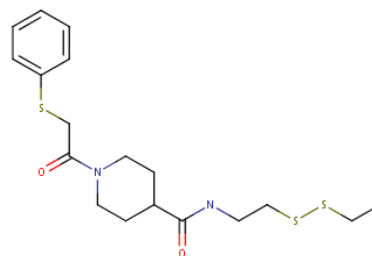
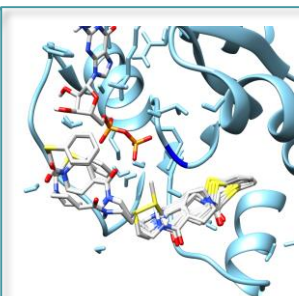
Docking Covalente

by www.rcmd.it

Inibitori
irreversibili

Marvin Sketch

Crossdocking

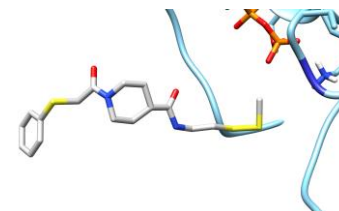


JO-148F

Obconformer

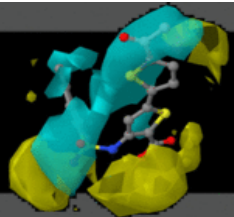


Obfit



SMART

[NH3+][C@H](C=O)CS



Docking Covalente

by www.RCMD.it



3-D QSAR

COMPOSTO	pIC ₅₀
4lucB	5.81
4lv6A	5.27
6H05	5.19
compound1	3.89
compound2	4.71
compound5	5.59
JO-148A	5.13
JO-148B	5.72
JO-148C	5.75
JO-148D	5.57
JO-148E	6.40
JO-148F	6.40
JO-148H	5.70
JO-148I	5.72
JO-148J	5.50



	PC	r ²	q ²
A	3	0.93	0.78
C	5	0.99	0.87
N	4	0.98	0.77
NA	4	0.99	0.83
HD	5	0.99	0.93
e	4	0.99	0.75
OA	4	0.97	0.81



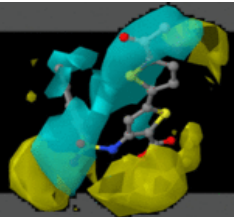
3-D QSAR

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JO-148A	5.13
JO-148B	5.72
JO-148C	5.75
JO-148D	5.57
JO-148E	6.40
JO-148F	6.40
JO-148H	5.70
JO-148I	5.72
JO-148J	5.50



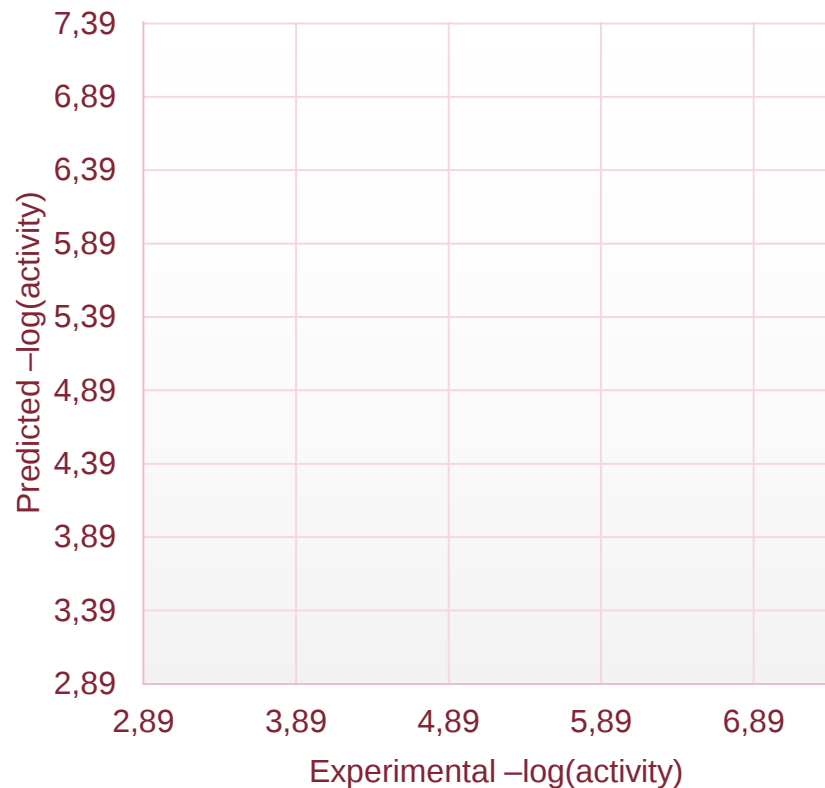
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C	5	0.99	0.87
N	4	0.98	0.77
NA	4	0.99	0.83
HD	5	0.99	0.93
e	4	0.99	0.75
OA	4	0.97	0.81



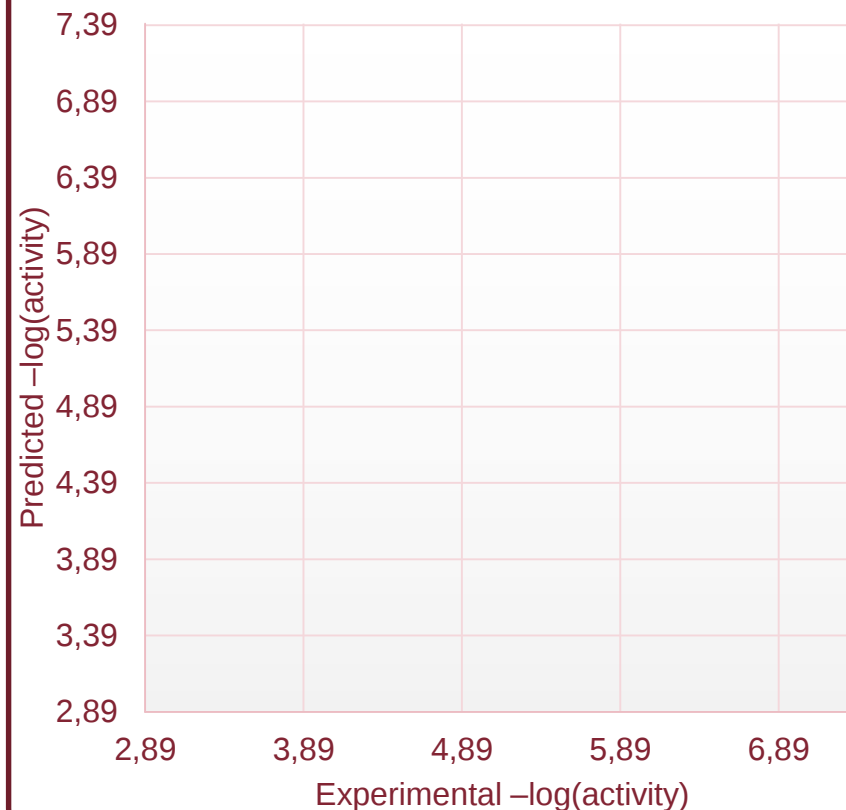
3-D QSAR: validazione interna LOO

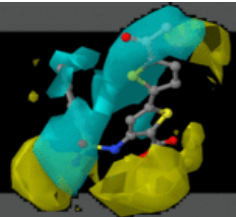
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Probe A PC3



Probe HD PC5

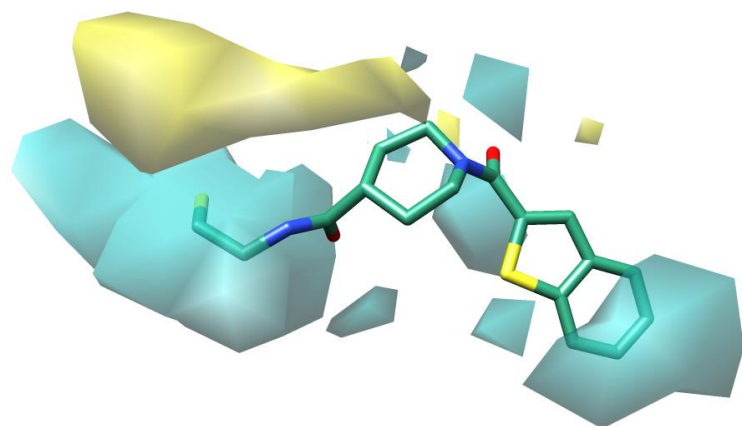
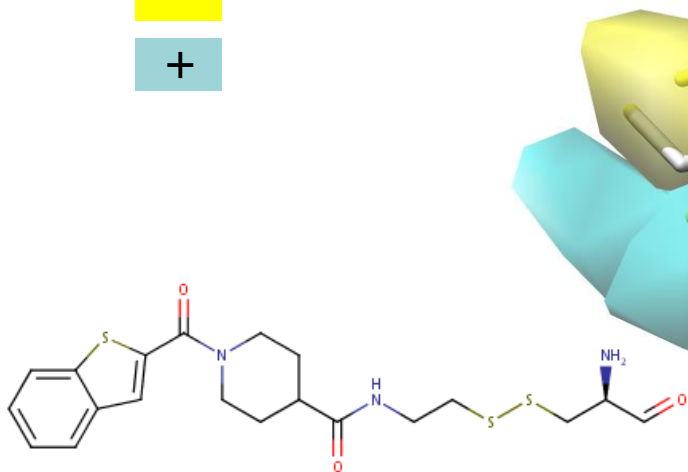
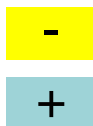




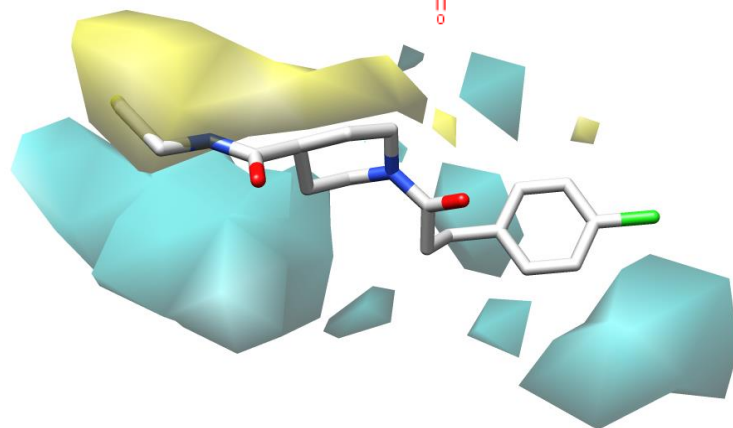
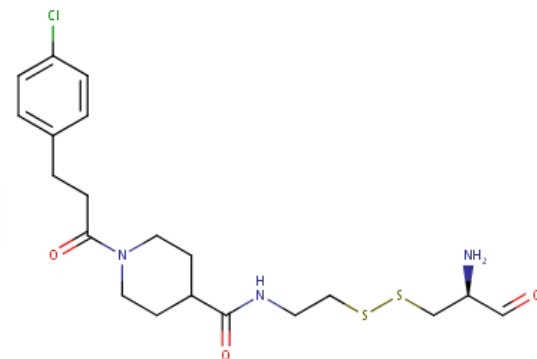
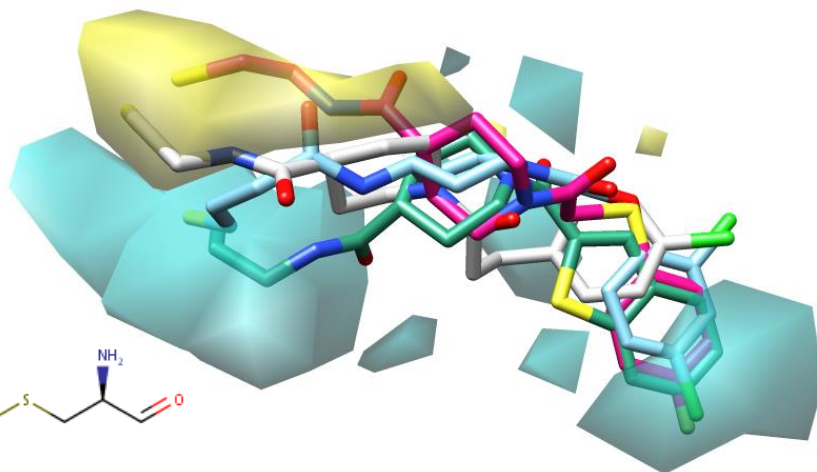
3-D QSAR: mappa COMFA2

by www.RCMD.it

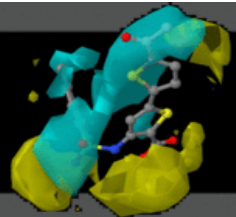
Probe A: Average Activity Contribution Plot



JO-148F pIC50= 6.40



Compound1 pIC50= 3.89



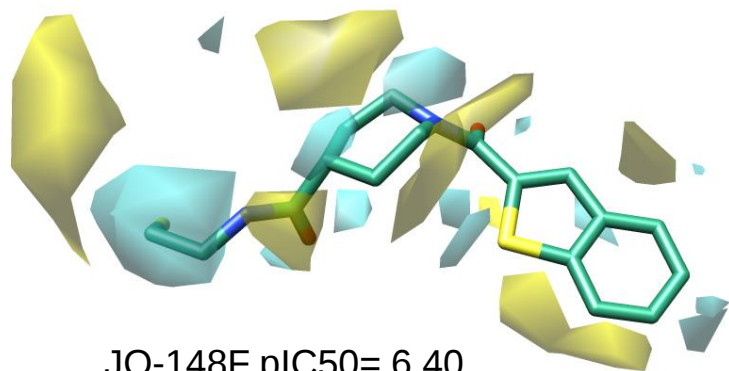
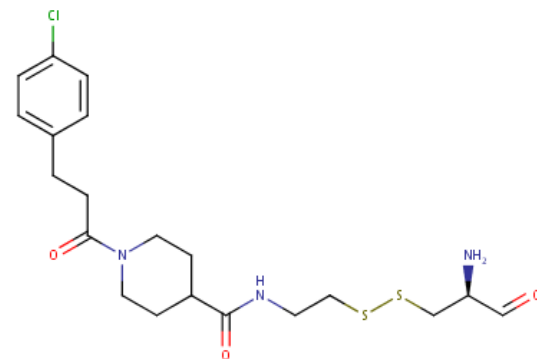
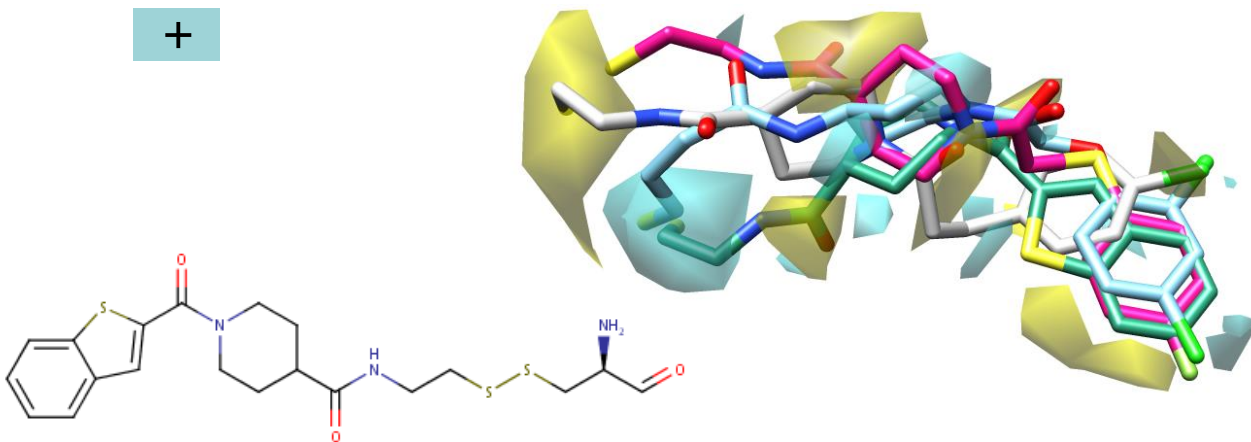
3-D QSAR: mappa COMFA2

by www.RCMD.it

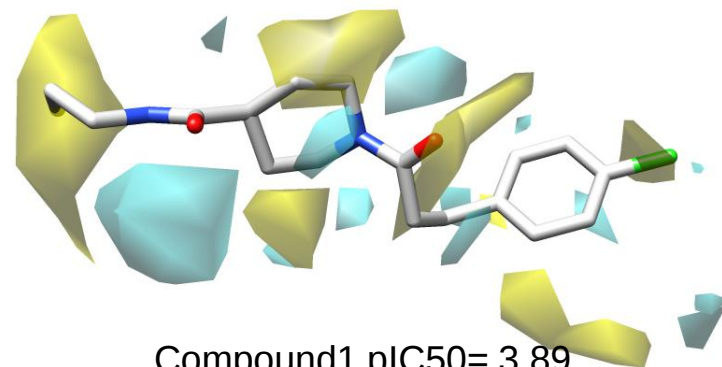
Probe HD: Average Activity Contribution Plot

-

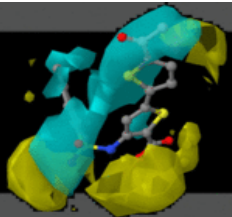
+



JO-148F pIC50= 6.40



Compound1 pIC50= 3.89



Formazione dei complessi reversibili

by www.RCMD.it

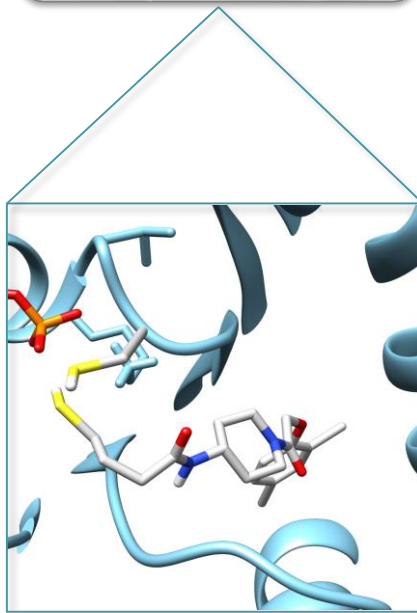
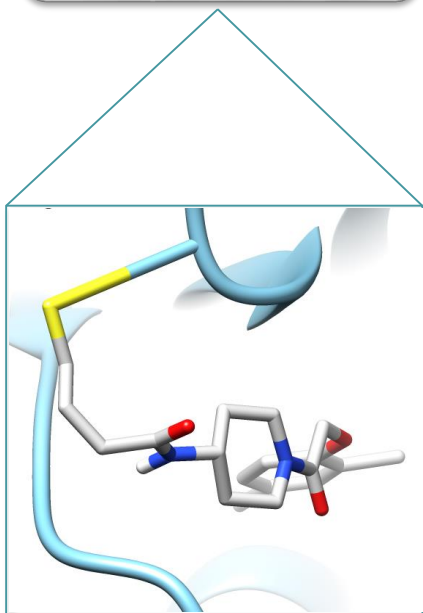
16 complessi
covalenti

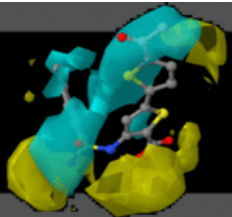


Rottura del
legame
covalente



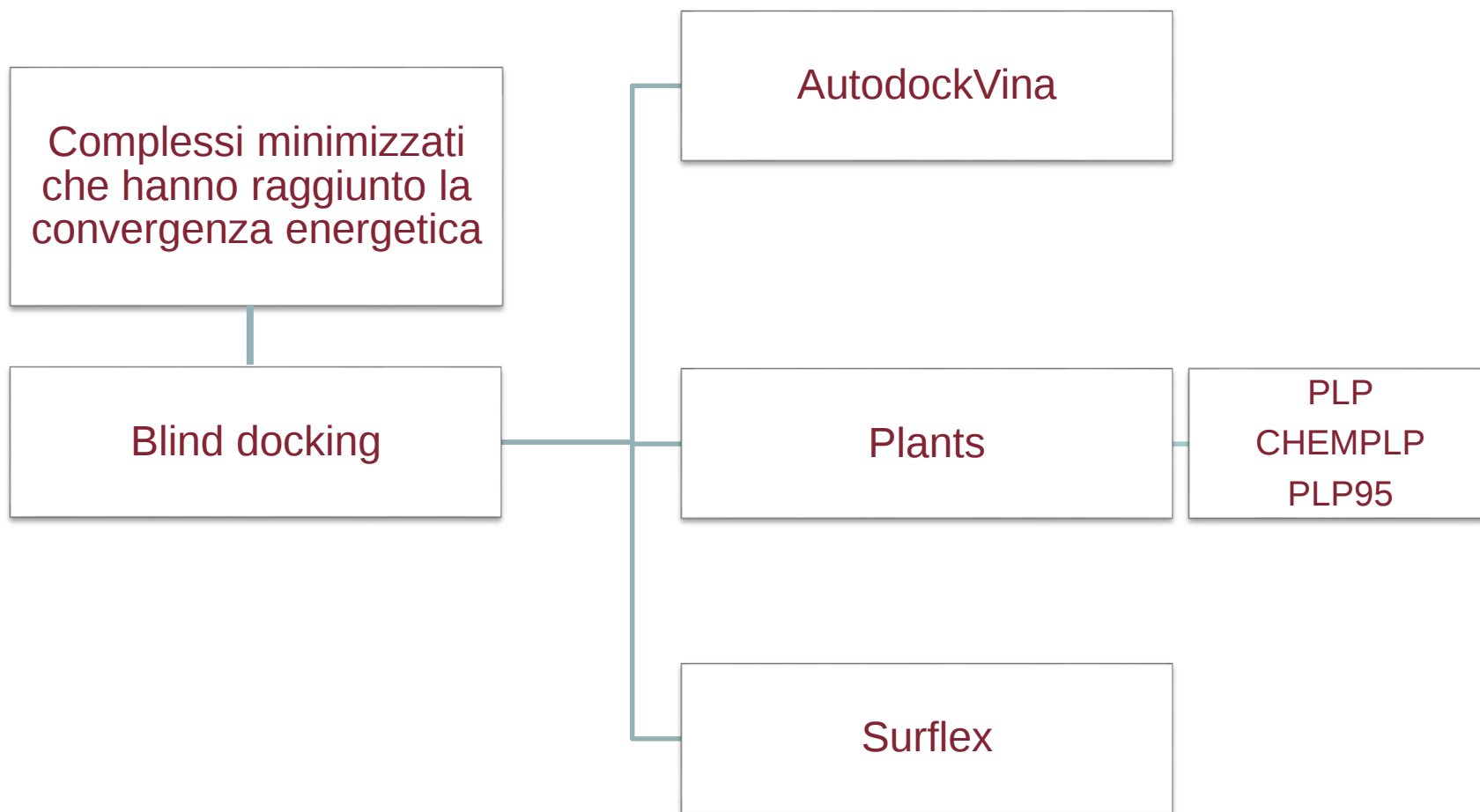
Minimizzazione:
- 1000 step
- convergenza

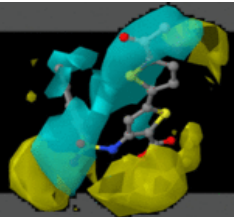




Blind Docking

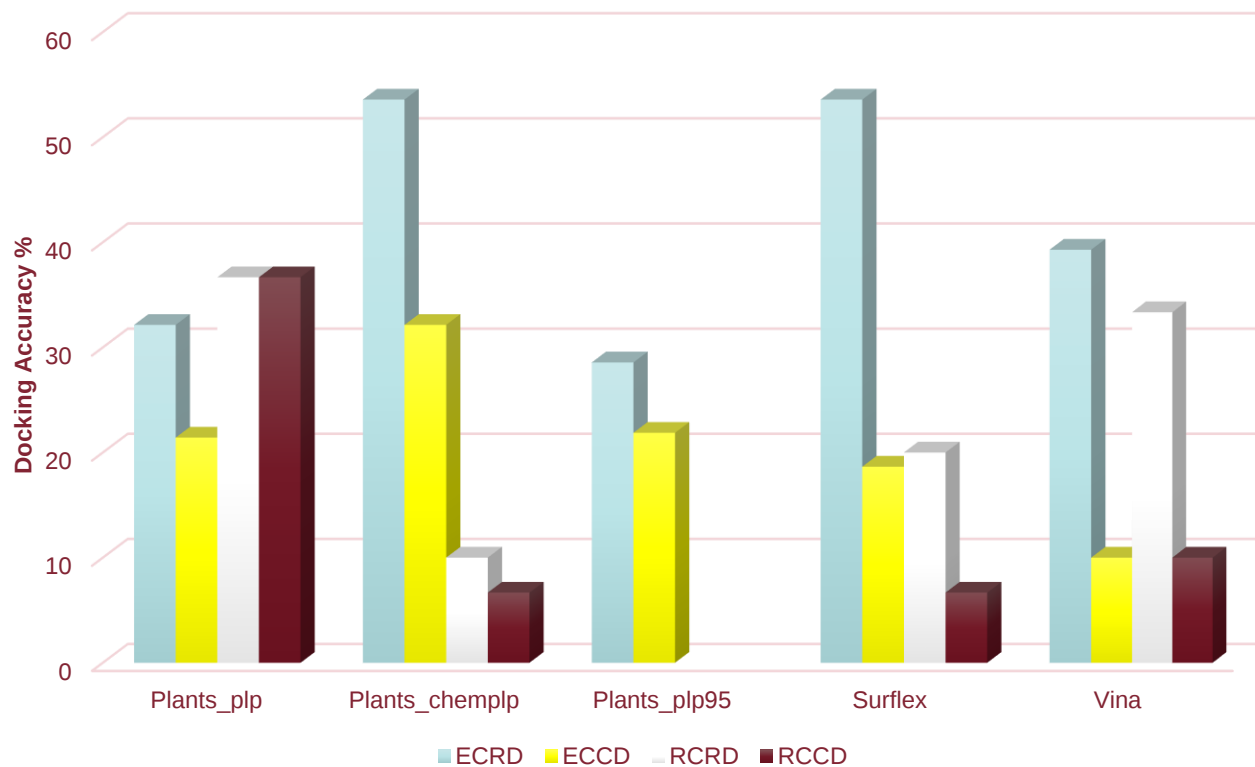
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ECRD

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$$RMSD = \sqrt{\frac{\sum_{i=1}^N d_i^2}{N}}$$

$$DA = 100 \frac{\sum RMSD_{(0-2)} + \frac{\sum RMSD_{(2-3)}}{2}}{N}$$



Conclusioni

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- Studio di docking covalente su inibitori irreversibili della K-Ras.
- Generazione di un modello 3-D QSAR .
- Predizione dell'attività di un set di molecole interne.
- Studio di docking reversibile a partire da inibitori covalenti della K-Ras.
- Informazioni utili per lo studio del binding mode e delle interazioni all'interno della tasca recettoriale.



Obiettivi futuri

by www.RCMD.it

- Docking reversibile sugli inibitori utilizzati per la generazione del modello 3-D QSAR.
- Utilizzo dei dati per effettuare un virtual screening.
- Progettazione nuovi inibitori.



*Grazie per la Vostra
attenzione.*