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

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Matricola: 1560711

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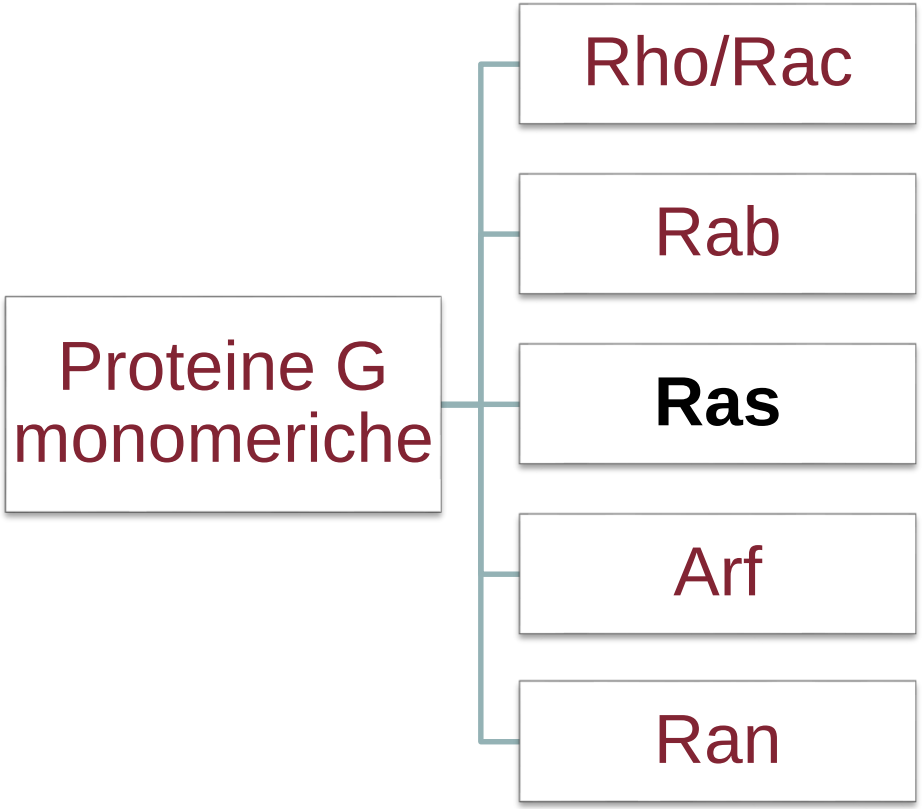
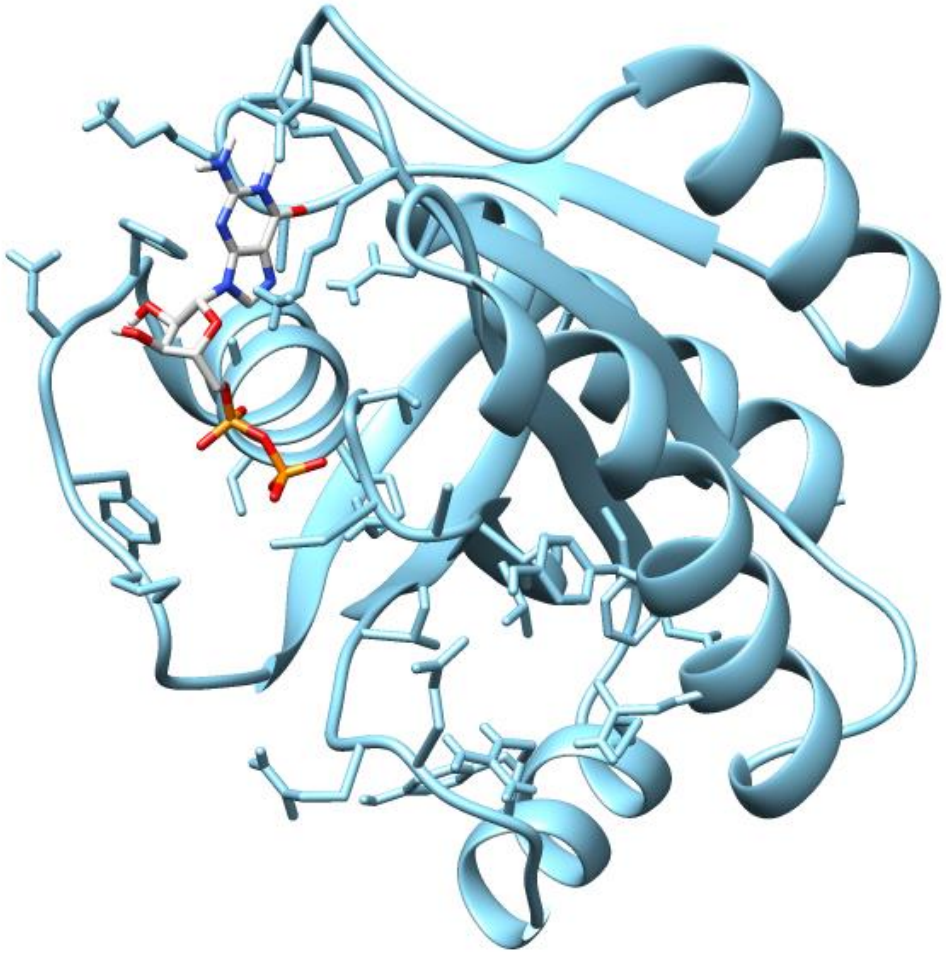
Superfamiglia proteine Ras



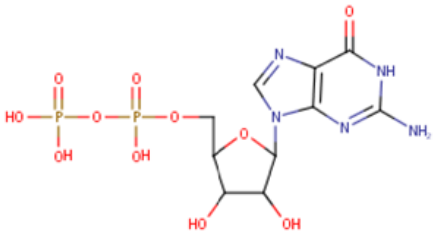
La deregolazione induce:

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

Le proteine Ras



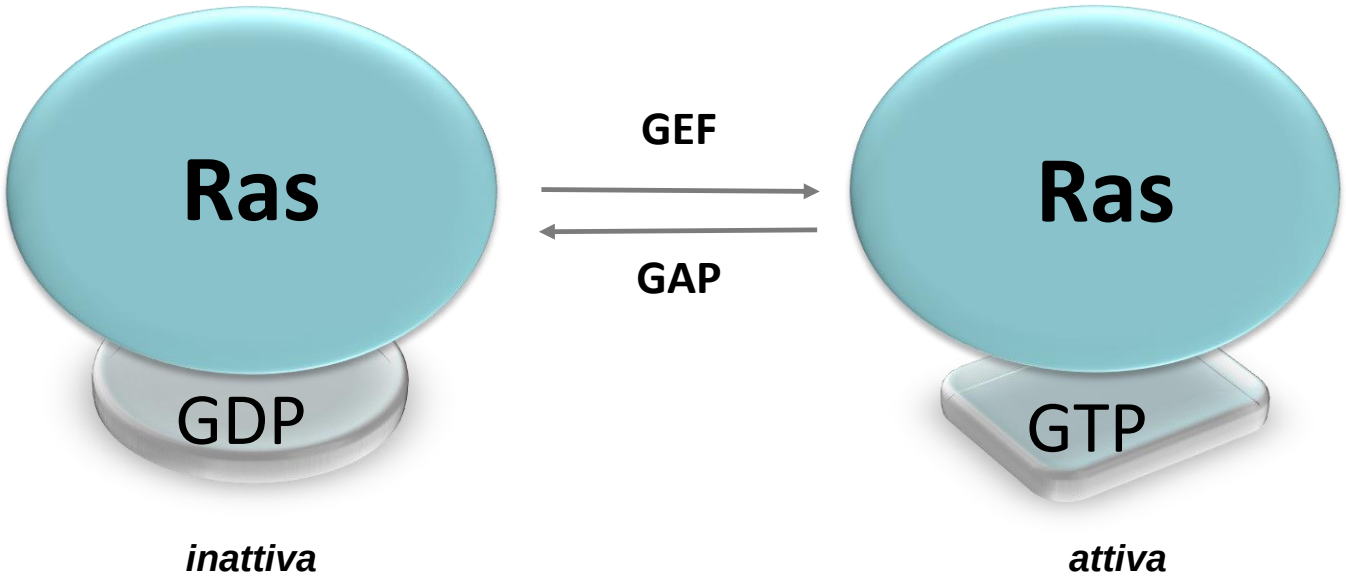
- GTPasi monomeriche
- 21 kDa
- Scambiatori molecolari



GDP

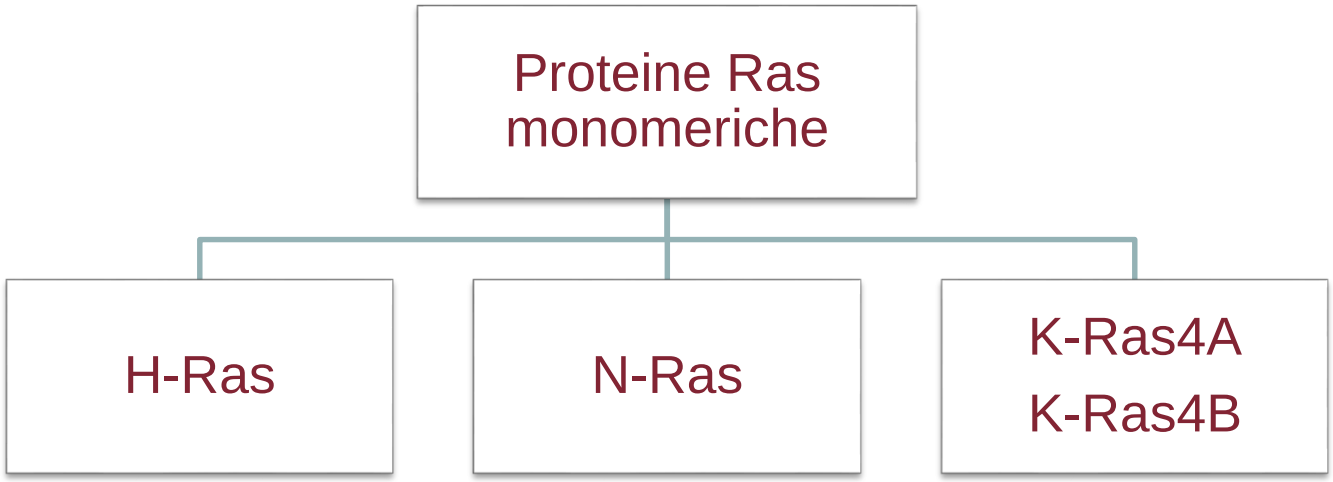
Le proteine Ras

Interruttori molecolari
"on/off"



Lo stato funzionale delle proteine Ras è regolato da:

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



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

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

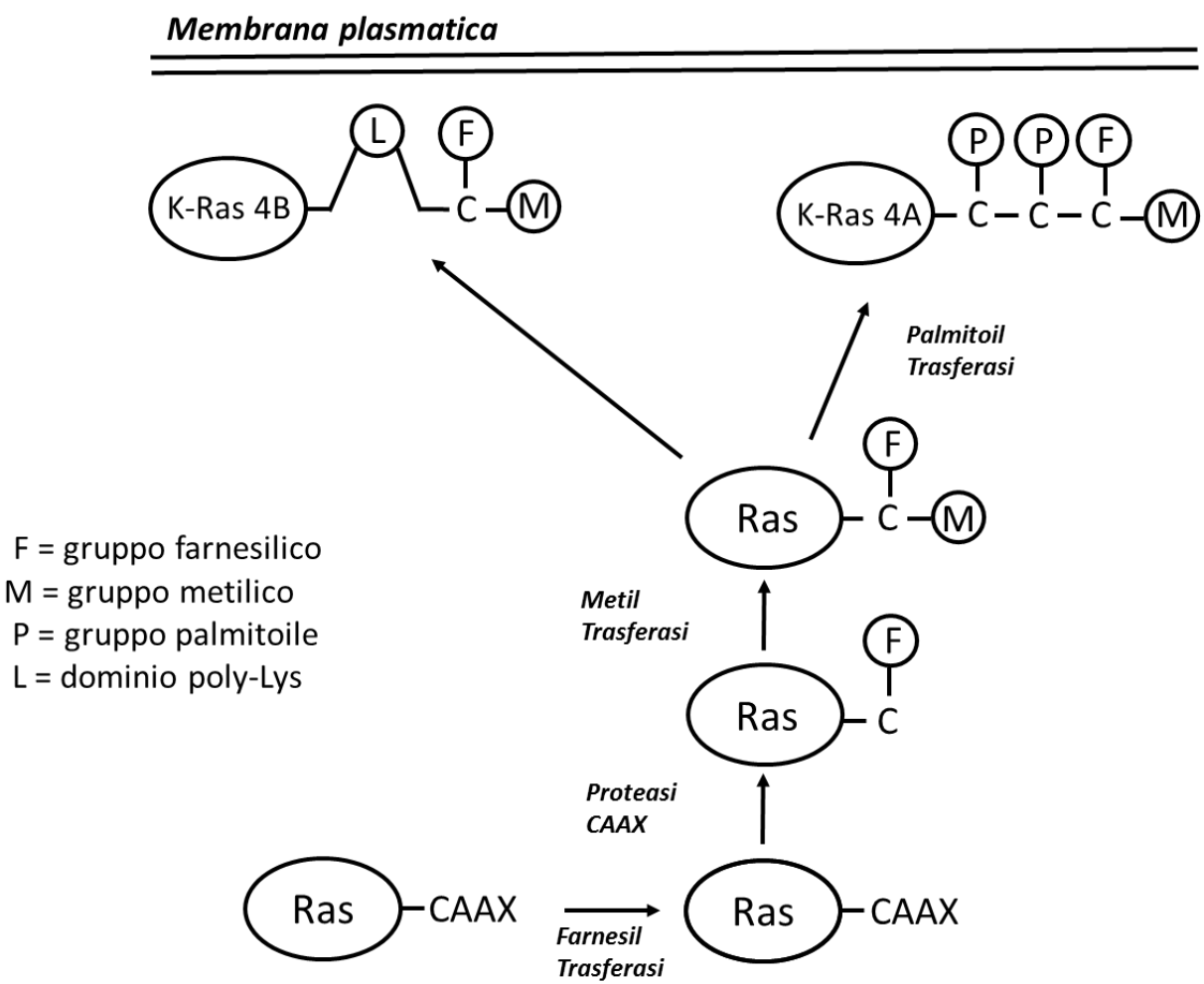
K-Ras4B } 189 aa

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

by www.RCMD.it



F = gruppo farnesilico
M = gruppo metilico
P = gruppo palmitoile
L = dominio poly-Lys

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

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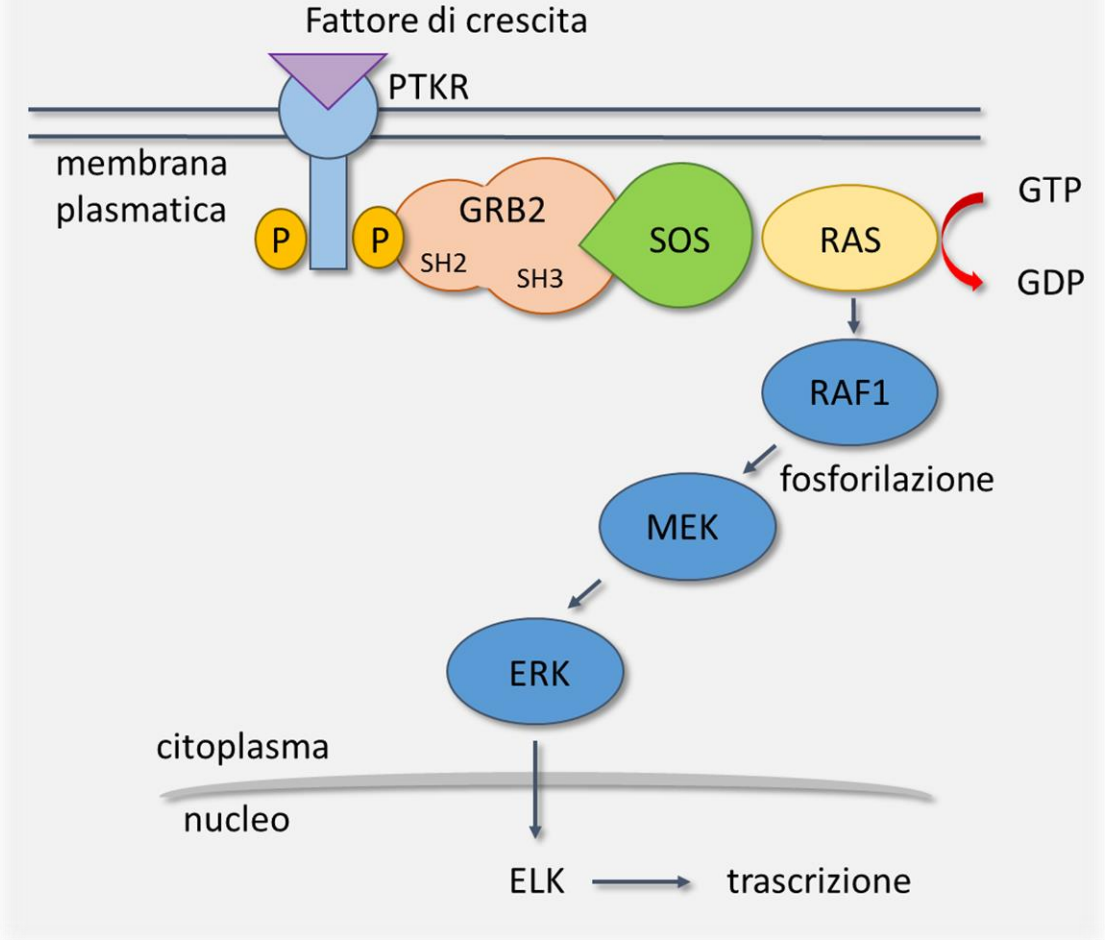
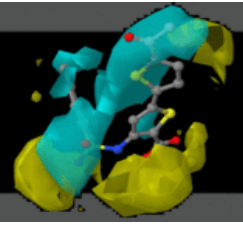
Pathway di K-Ras



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.



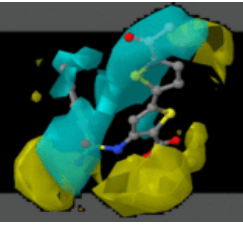
K-Ras e cancro

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

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

Codone 12

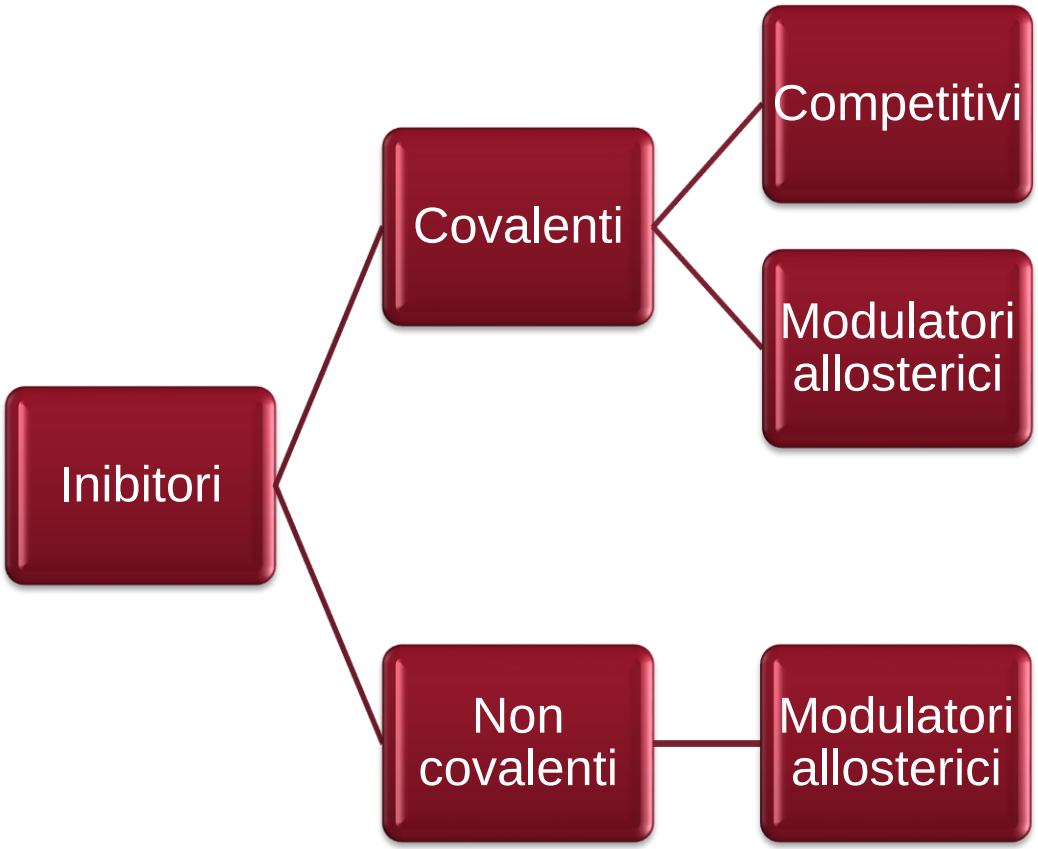
G → A
Gly (GGT) → Ser (AGT)
Gly (GGT) → Asp (GAT)
G→ T
Gly (GGT) → Cys (TGT)
Gly (GGT) → Val (GGT)
G → C
Gly (GGT) → Arg (CGT)
Gly (GGT) → Ala (GCT)

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)

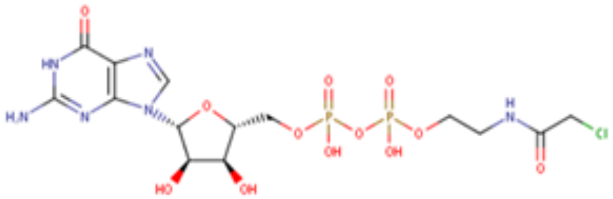
Altre mutazioni avvengono a carico dei codoni 59 e 61.

Inibitori delle K-Ras

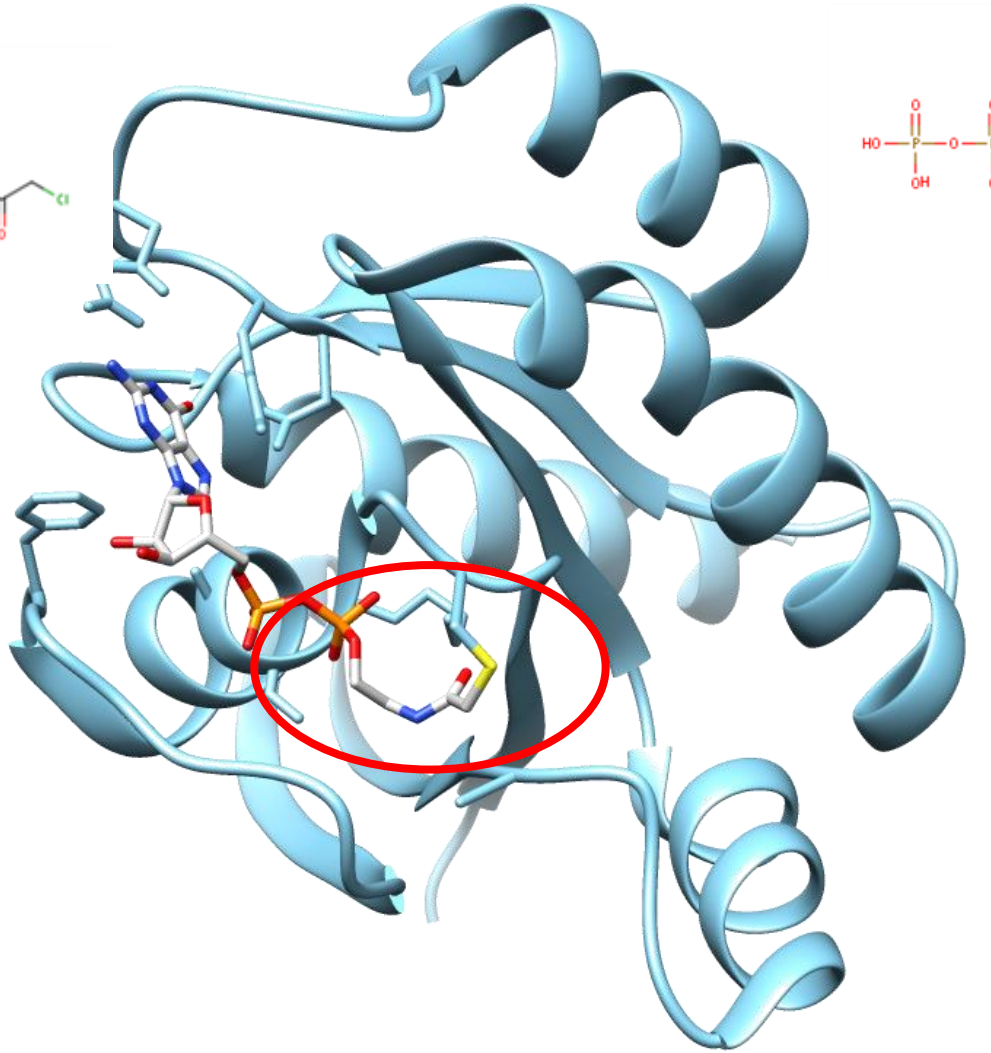


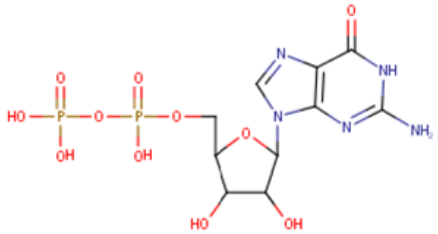
Cambiare la preferenza della proteina a favore del GDP e indebolire il legame con Raf.

Inibitori competitivi



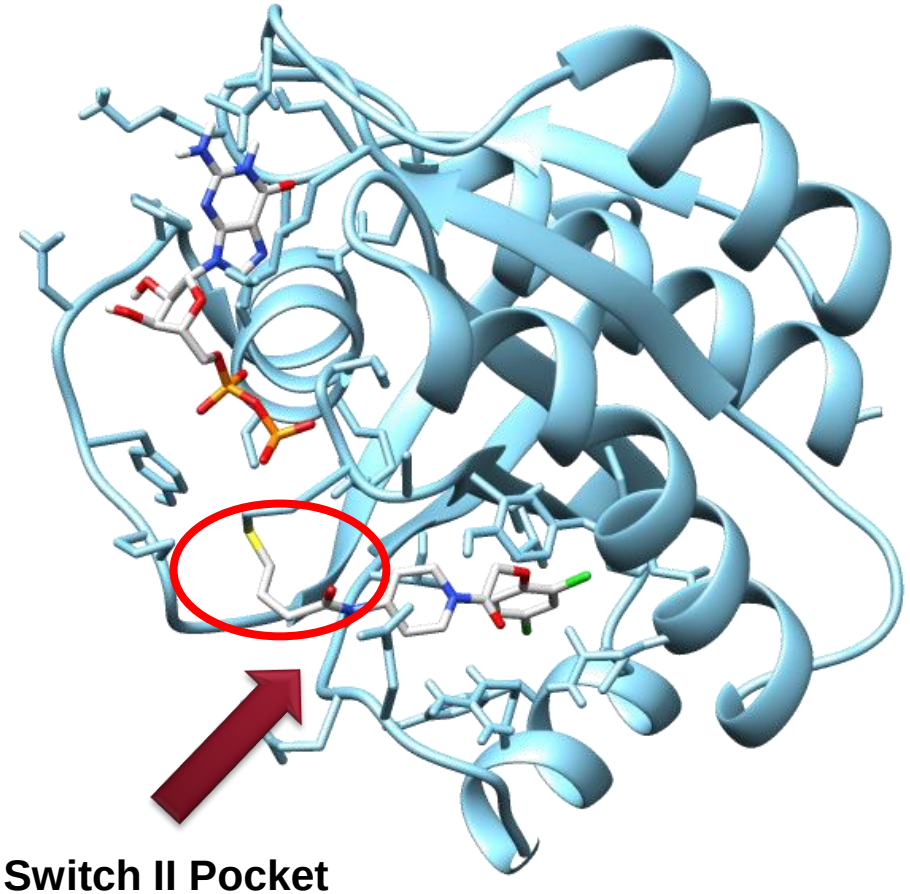
SML-83-71-9



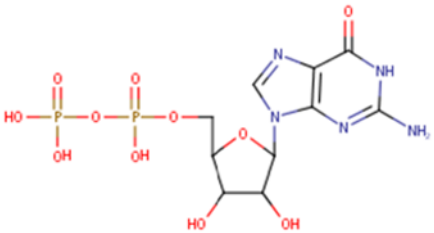


GDP

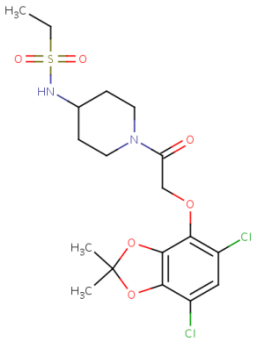
Inibitori covalenti delle K-Ras



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

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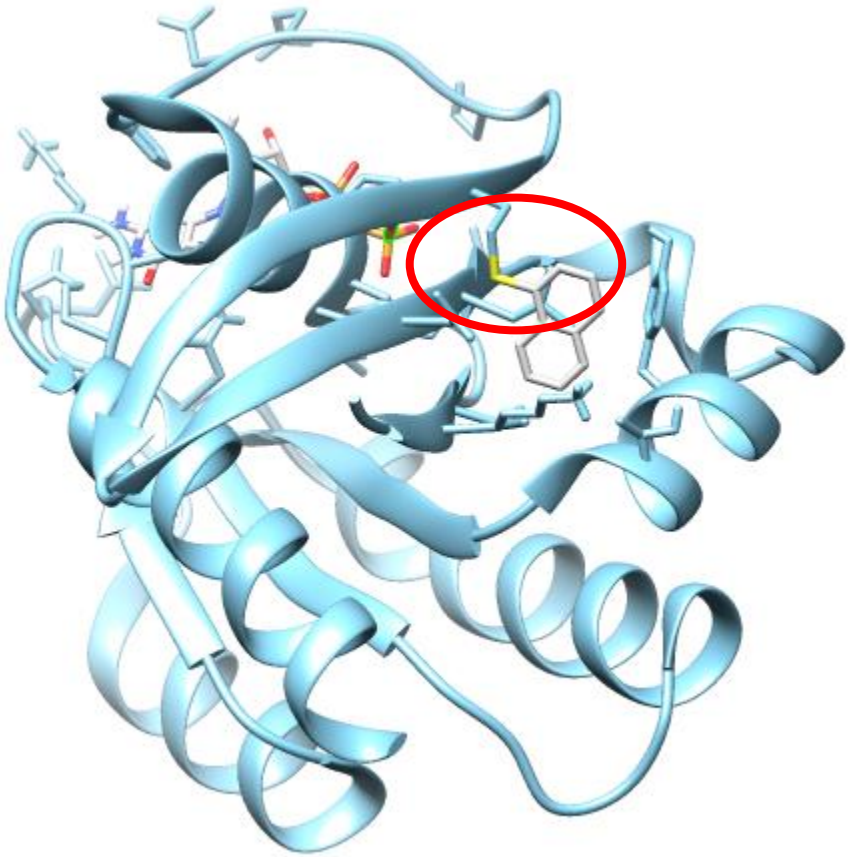
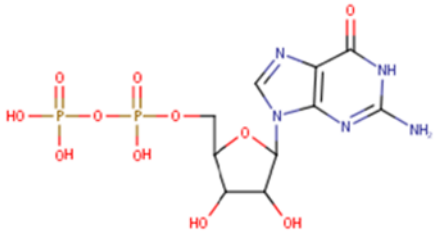
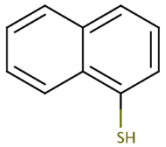


Tasca tra lo switch II ed il β-foglietto centrale.



GDP



4Q01

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

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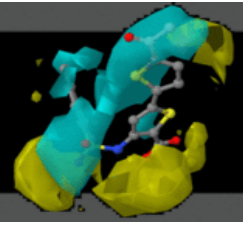
Scopo della tesi

16 complessi cristallizzati

- **Modeller**
- **Parametrizzazione**
- **Minimizzazione e allineamento**
- **Docking covalente**
- **3-D QSAR**
- **Docking reversibile**



DOCKING COVALENTE

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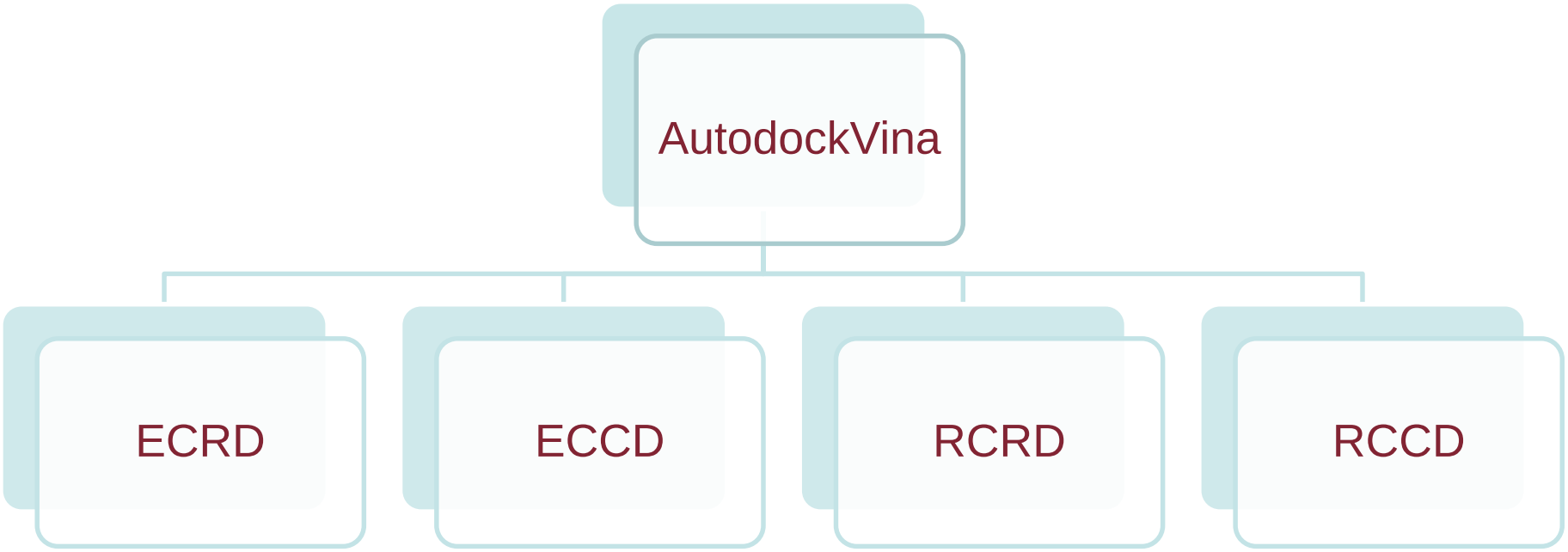
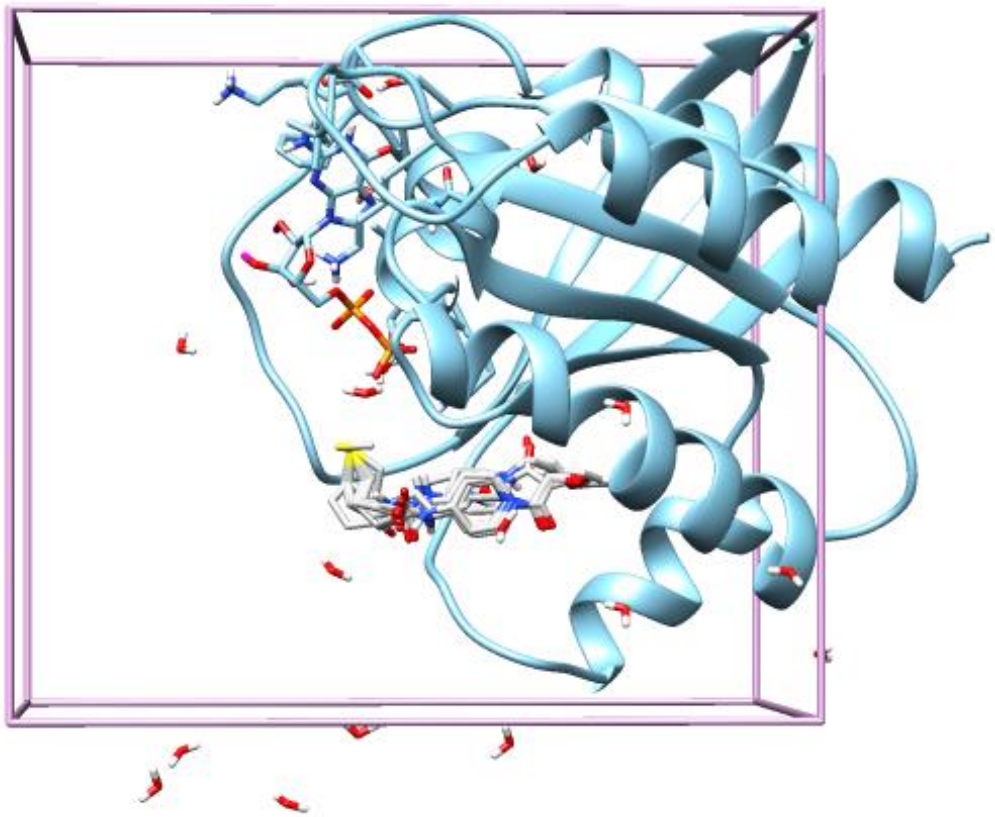
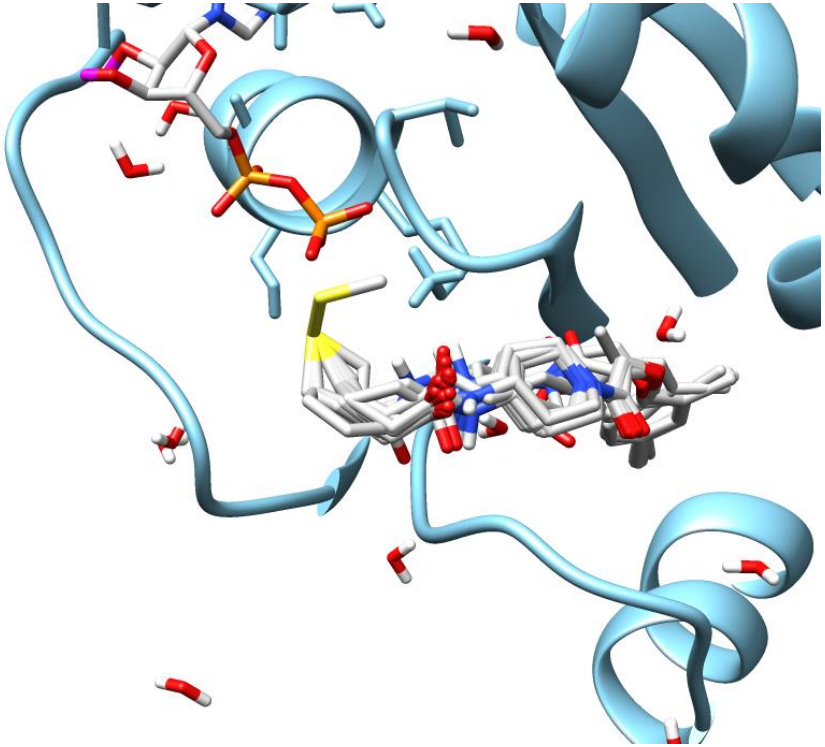
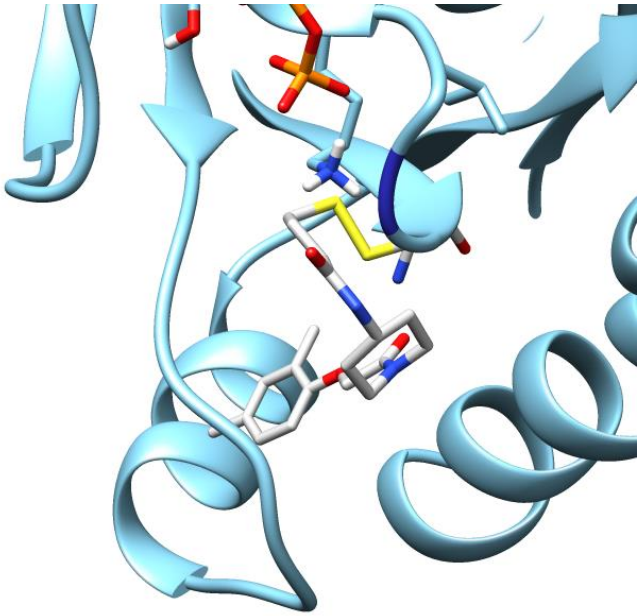
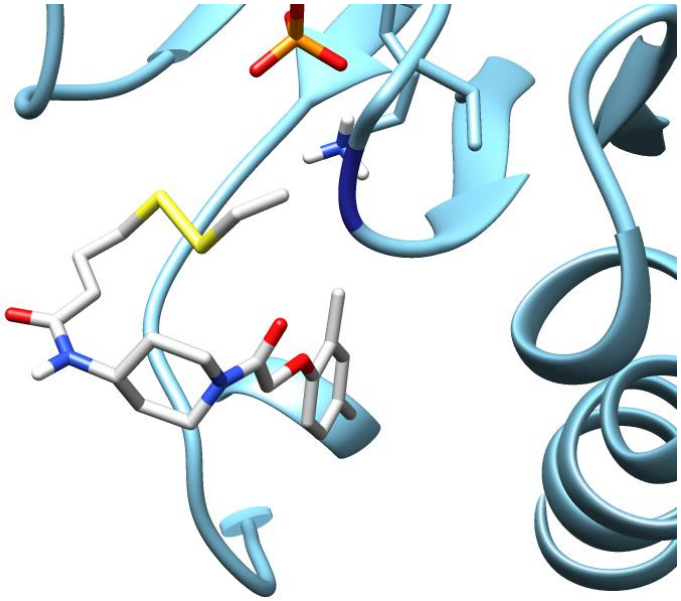










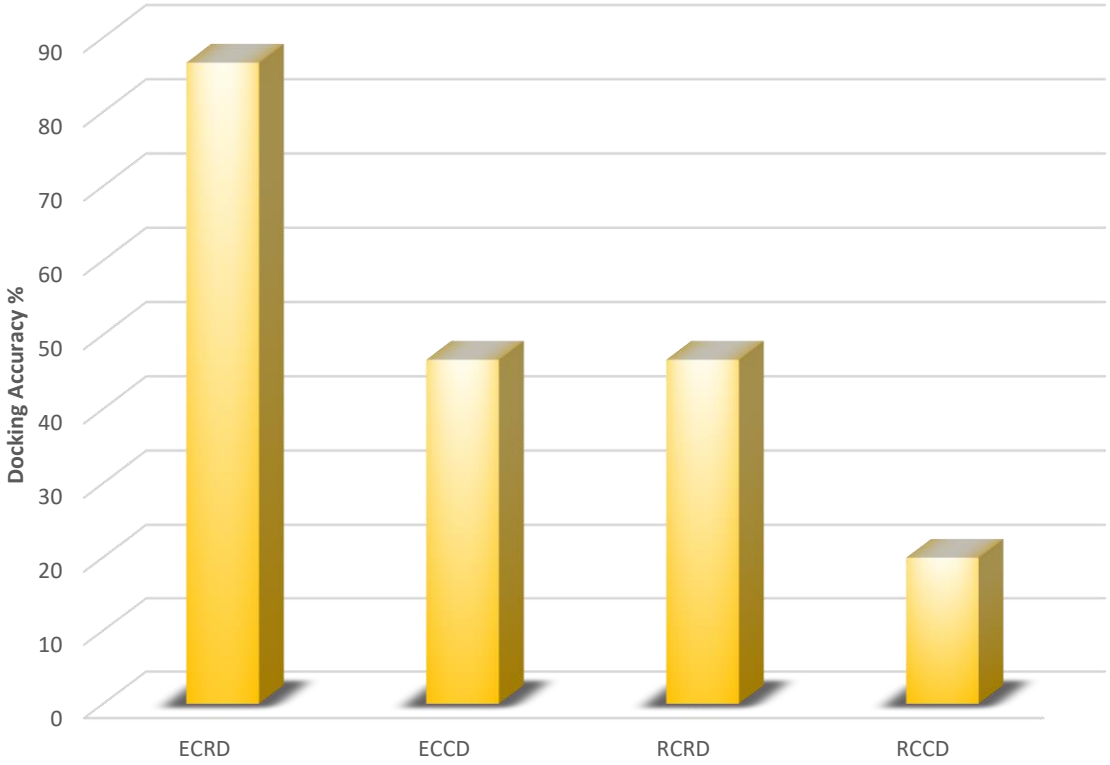
[NH3+] [C@H] (C=O) CS
(Cisteina)

Obconformer

SMART

Obfit

Docking covalente

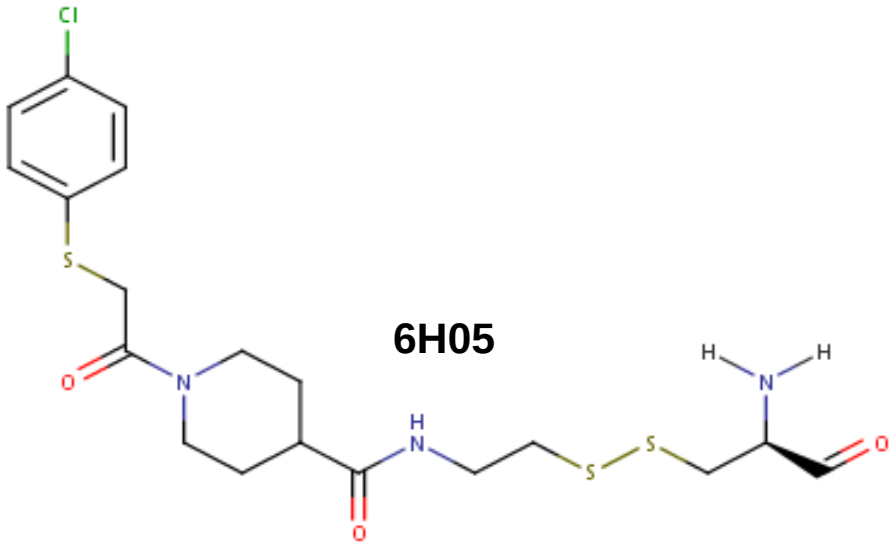


$$RMSD = \sqrt{\frac{\sum_{i=1}^{N} d_i^2}{N}}$$

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

Inibitori irreversibili

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

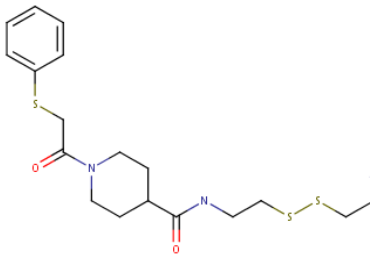


6H05

Docking covalente → **3-D QSAR**

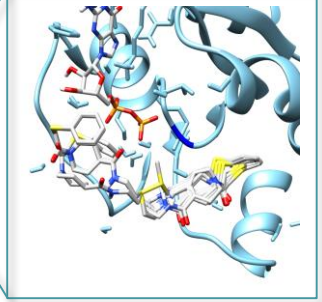
Inibitori irreversibili

Marvin Sketch

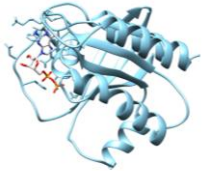


JO-148F

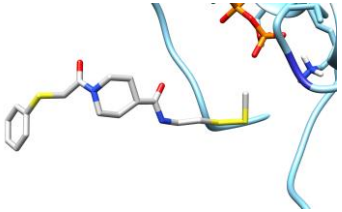
Crossdocking



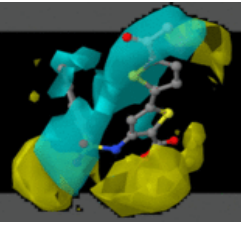
Obconformer



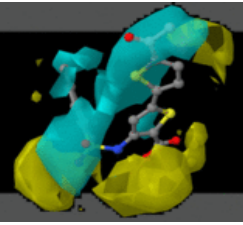
Obfit



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



Docking Covalente



3-D QSAR

by www.RCMD.it

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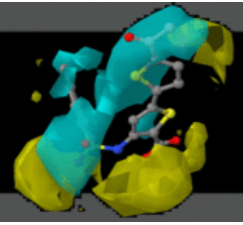
JO-148I	5.72
JO-148J	5.50

	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



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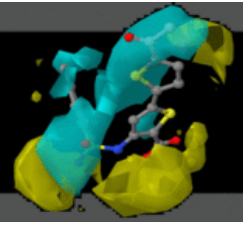

3-D QSAR: validazione interna LOO

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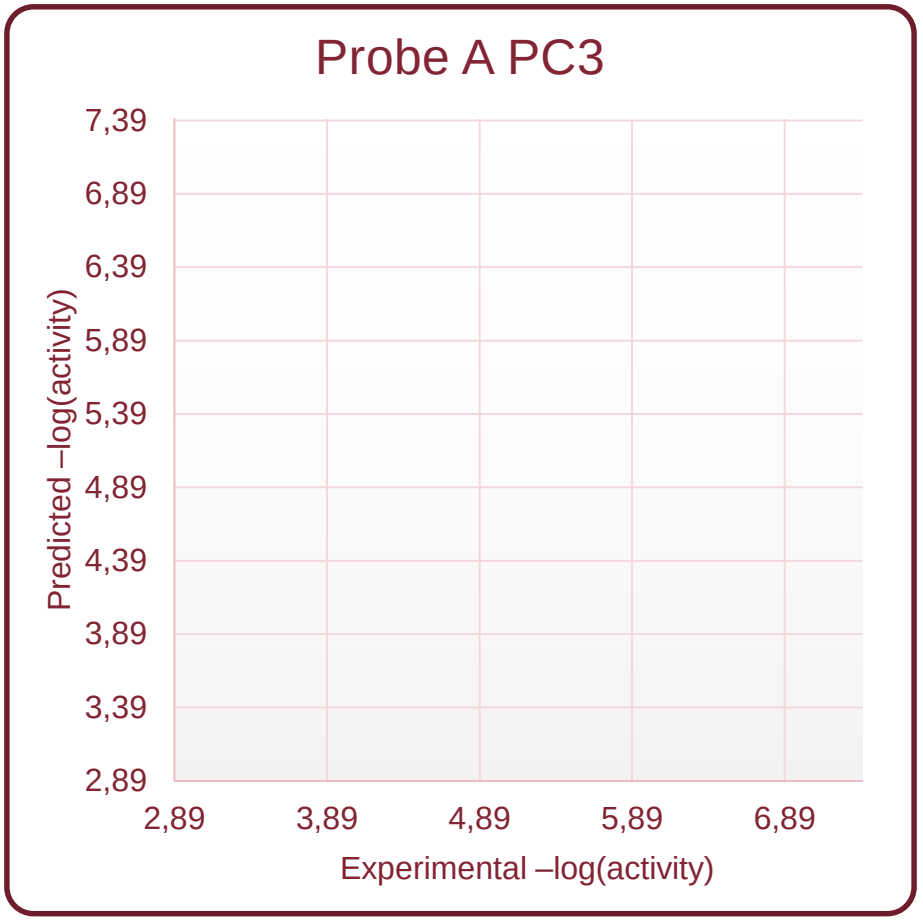
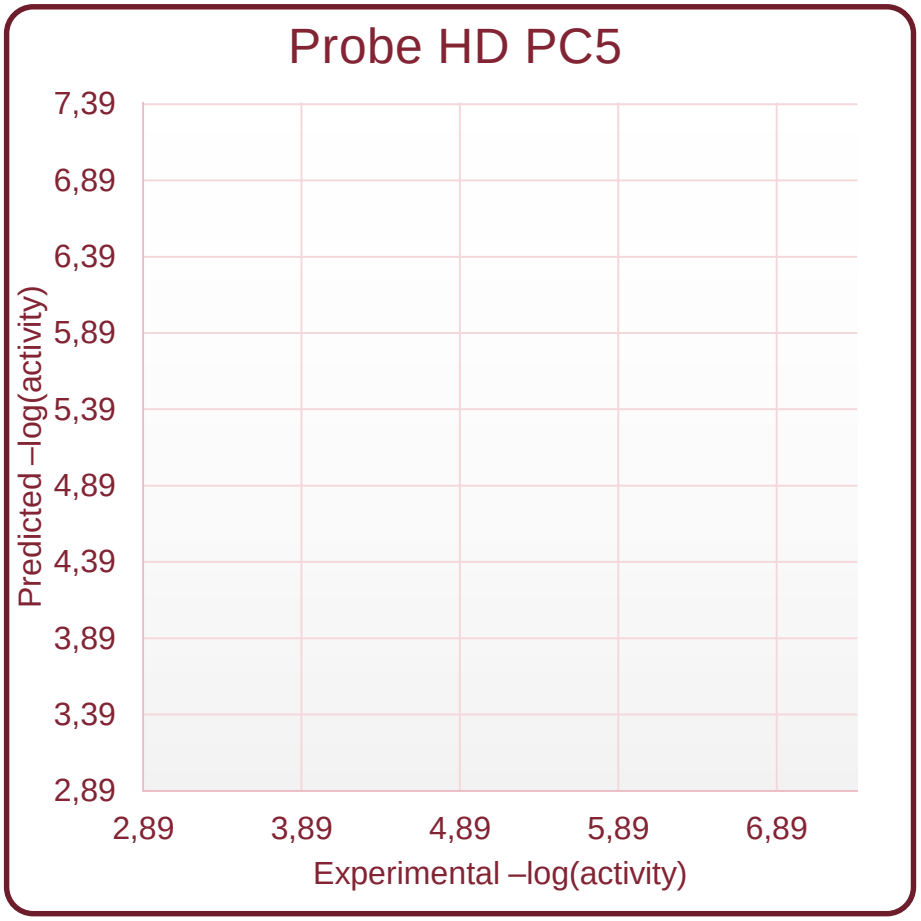
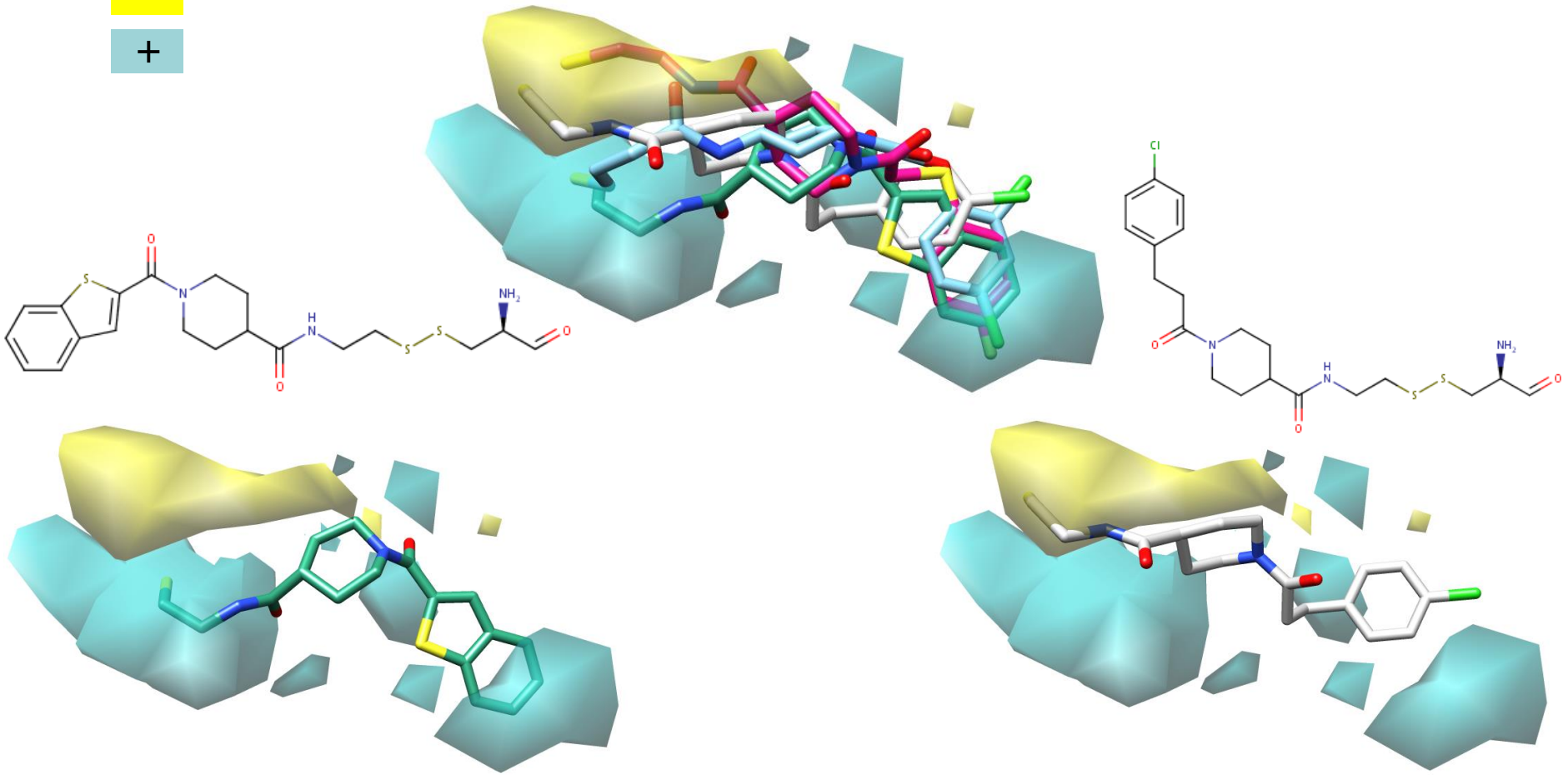




Probe A: *Average Activity Contribution Plot*

-

+



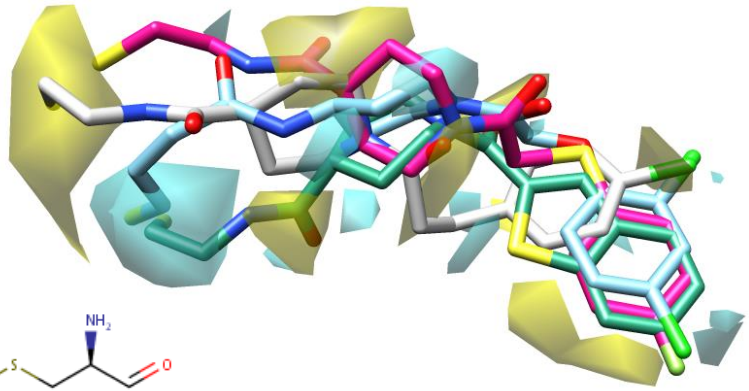
JO-148F pIC50= 6.40

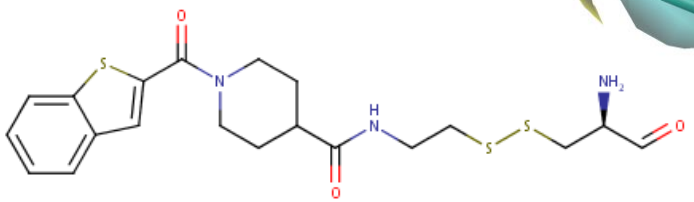
Compound1 pIC50= 3.89

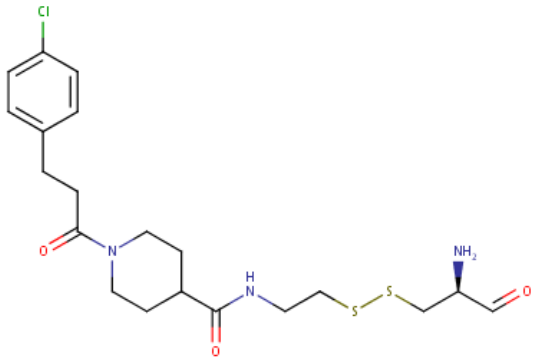
Probe HD: *Average Activity Contribution Plot*

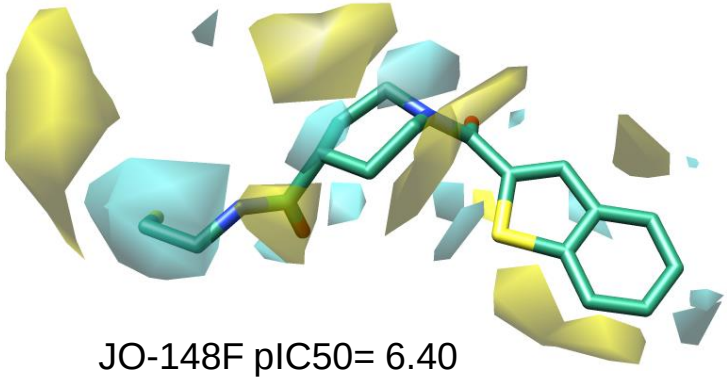
-

+

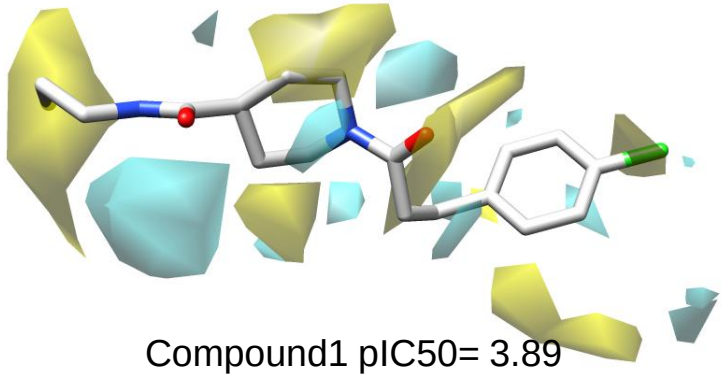








JO-148F pIC50= 6.40



Compound1 pIC50= 3.89

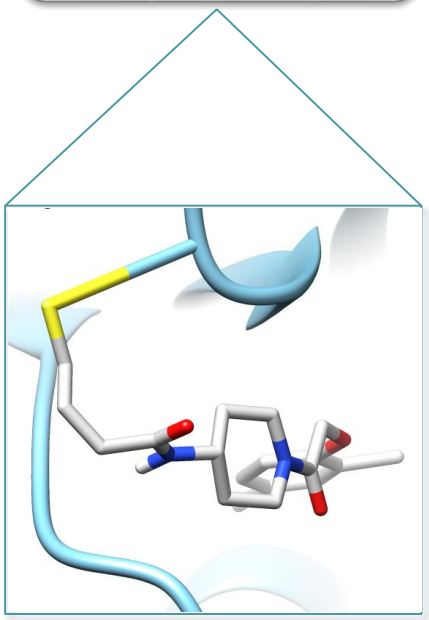
Formazione dei complessi reversibili

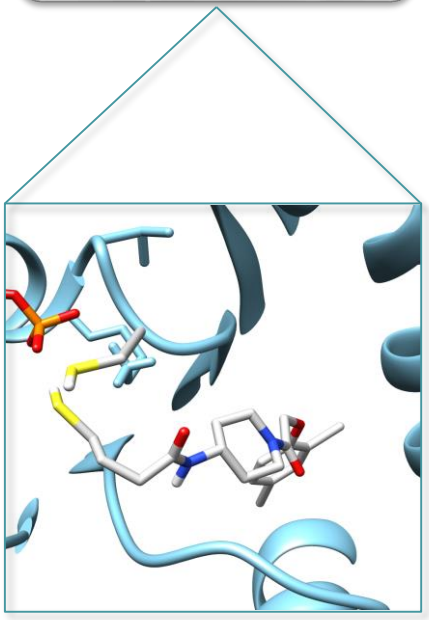
by www.RCMD.it

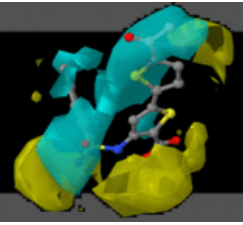
16 complessi covalenti

Rottura del legame covalente

Minimizzazione:
- 1000 step
- convergenza





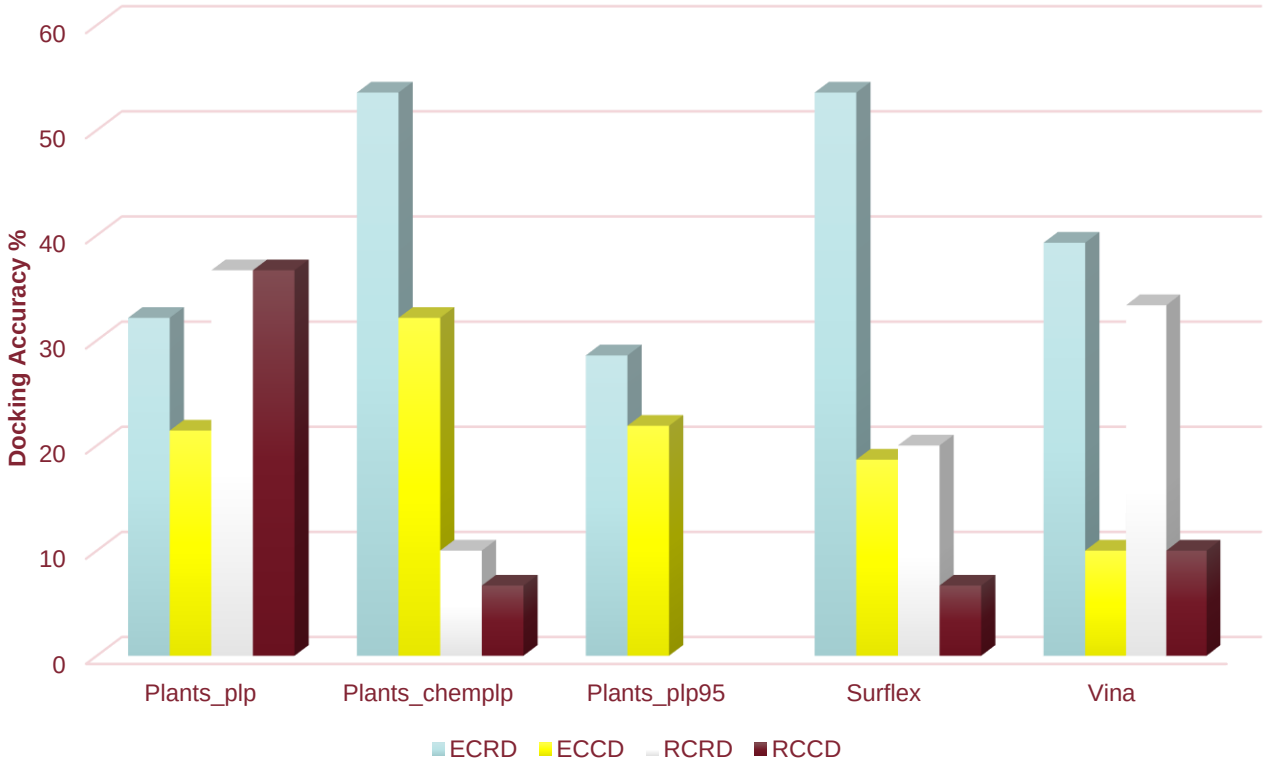


Blind Docking

Complessi minimizzati che hanno raggiunto la convergenza energetica

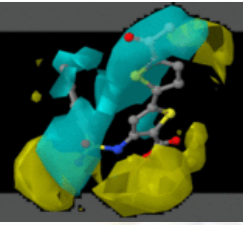
Blind docking

- AutodockVina
- Plants
  - PLP
  - CHEMPLP
  - PLP95
- Surflex



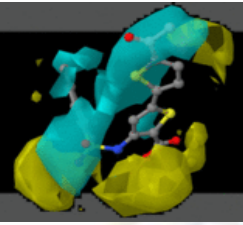
$$RMSD = \sqrt{\frac{\sum_{i=1}^{N} d_i^2}{N}}$$

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



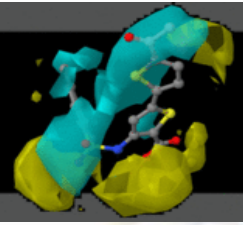
Conclusioni

- 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

- 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.