SAPIENZA
UNIVERSITÀ DI ROMA

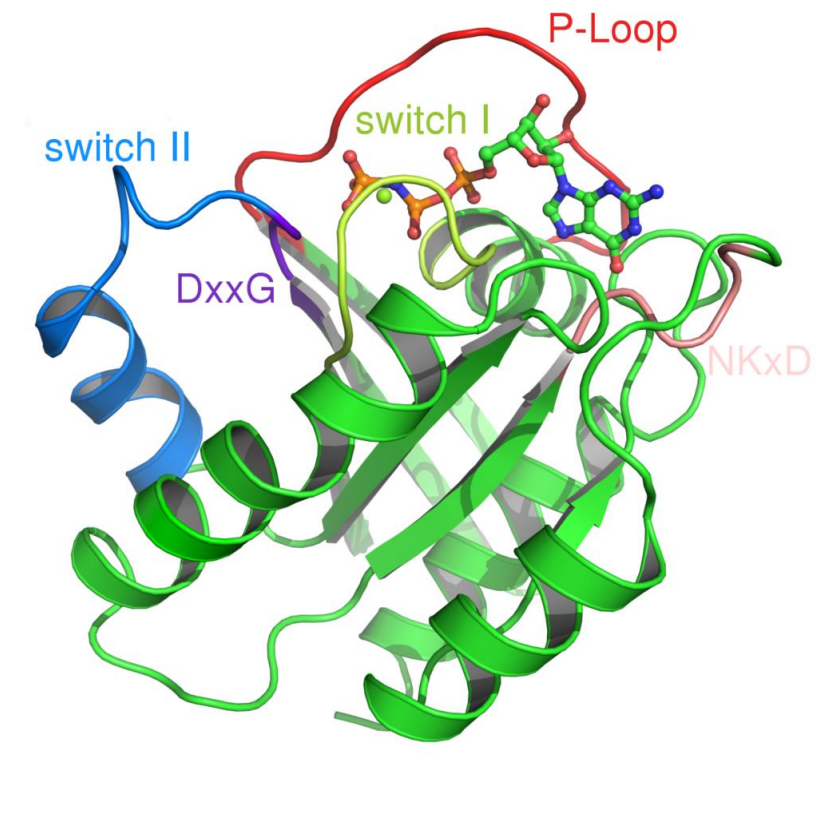
FACOLTÀ DI FARMACIA E MEDICINA

Approcci Structure-Based per la Razionalizzazione delle Interazioni Proteina-Ligando Applicate ai Recettori "Kras"

Relatore:
Chiar.mo Prof. Rino Ragno

Laureando:
Daniele Iovinelli

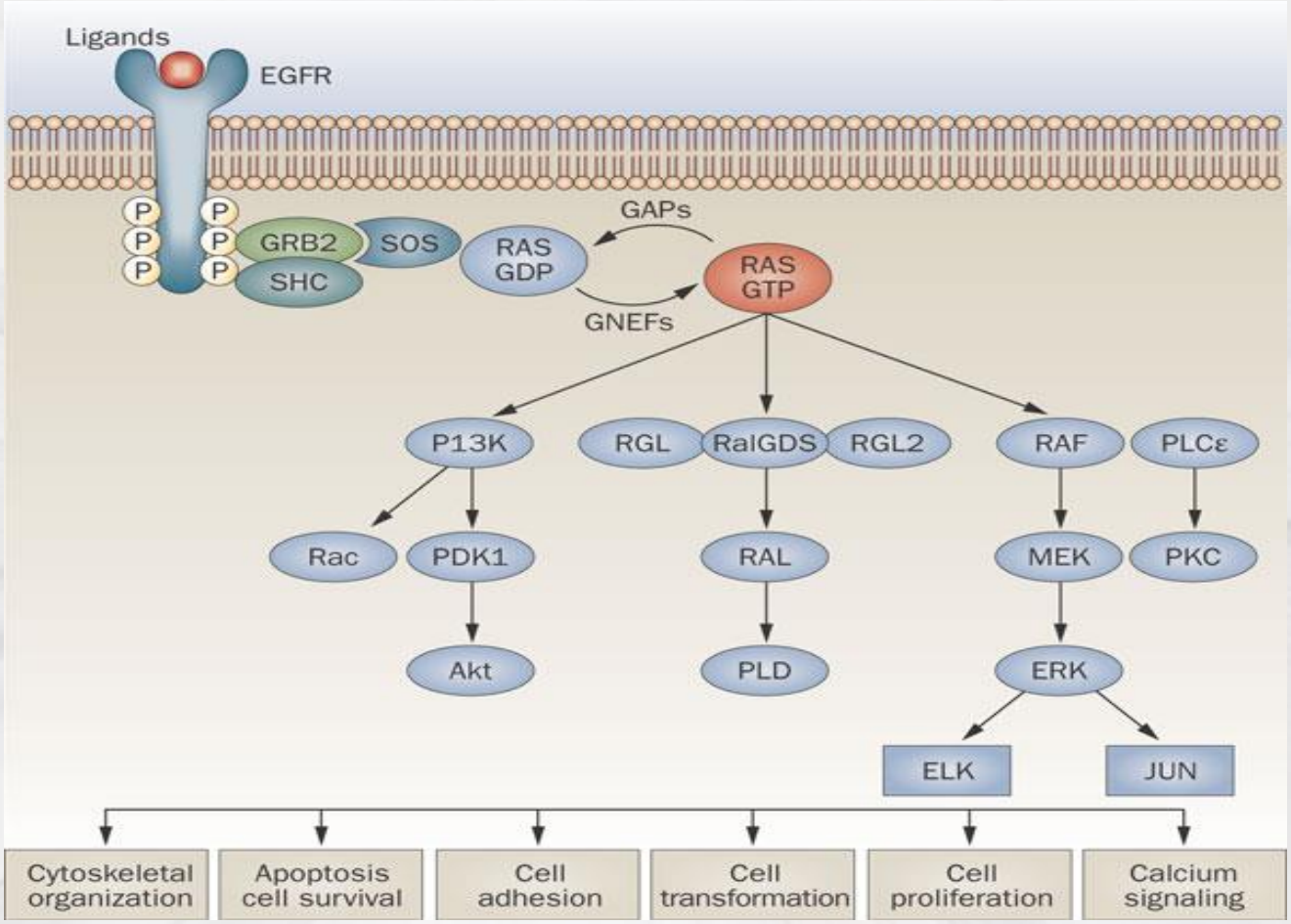
KRAS COME TARGET DI STUDIO



La Kras è una GTPasi conosciuta anche come V-Ki-RAS2 **Kirsten rat sarcoma viral oncogene homolog** o "KRAS" ,

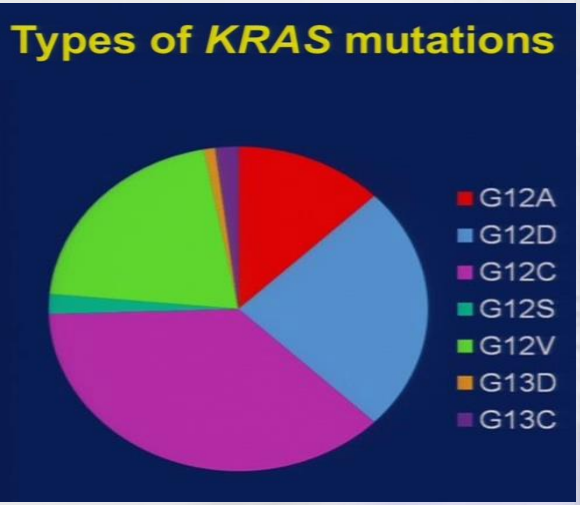
nell'uomo è codificata dal gene KRAS.

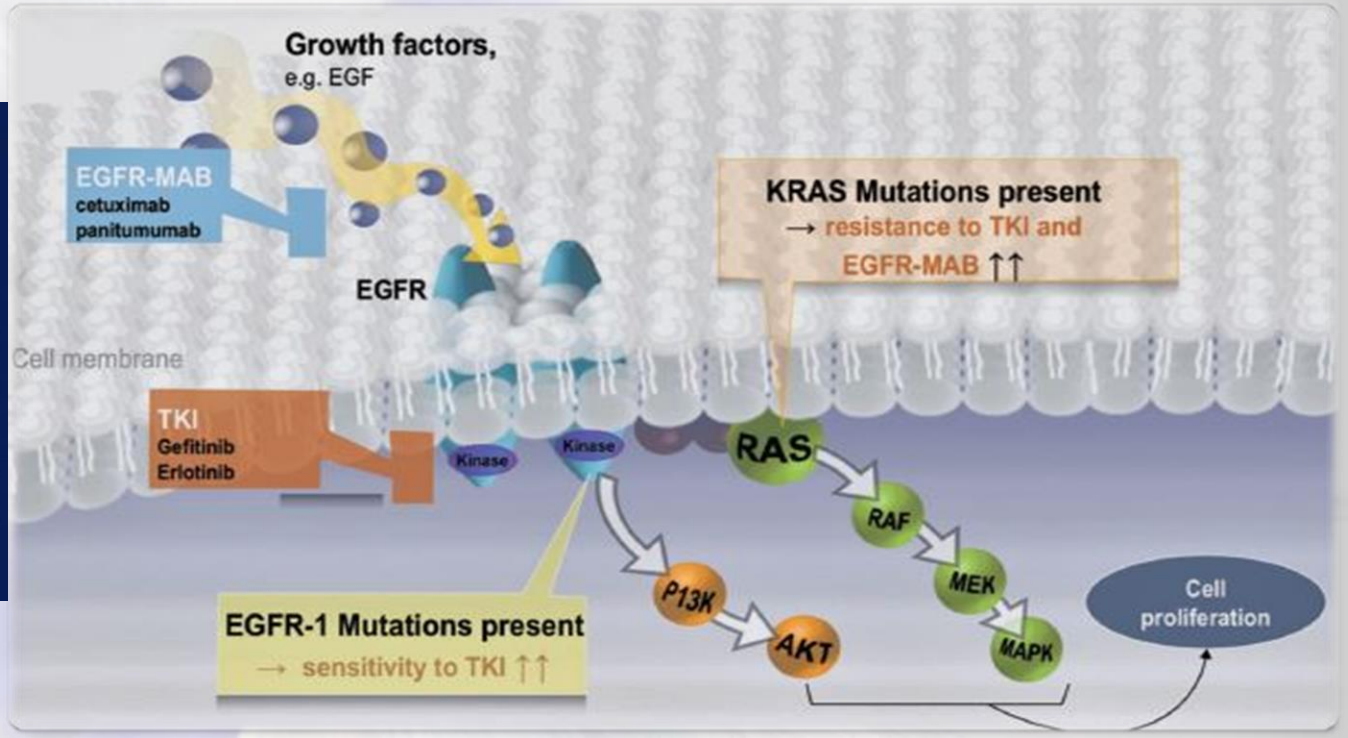
Meccanismo KRAS

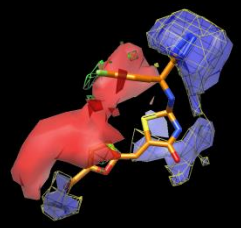


Normanno, N. *et al.* (2009), *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2009.111

Resistenza KRAS







Scopo del Lavoro

Studi di docking molecolare:

- definizione del protocollo
- automazione del processo
- validazione predittività dei binding modes

PROTEIN DATA BANK

PULIZIA COMPLESSI

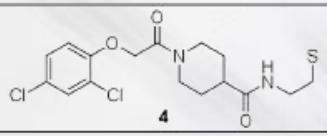
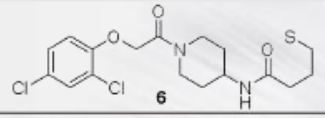
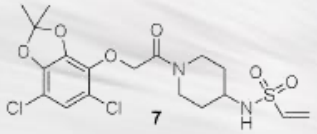
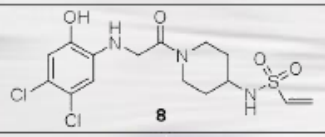
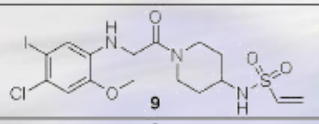
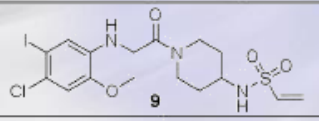
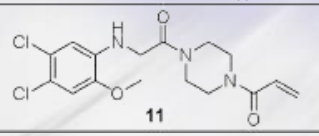
MODIFICA LIGANDI COVALENTI

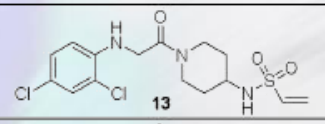
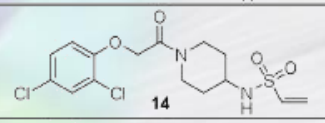
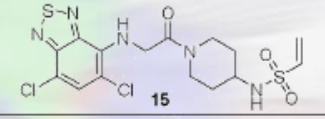
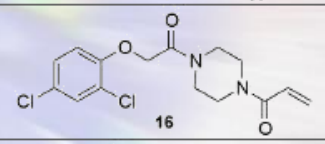
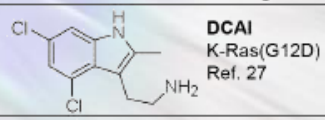
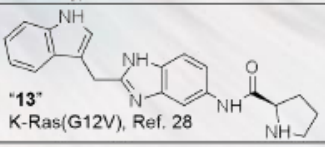
MINIMIZZAZIONE

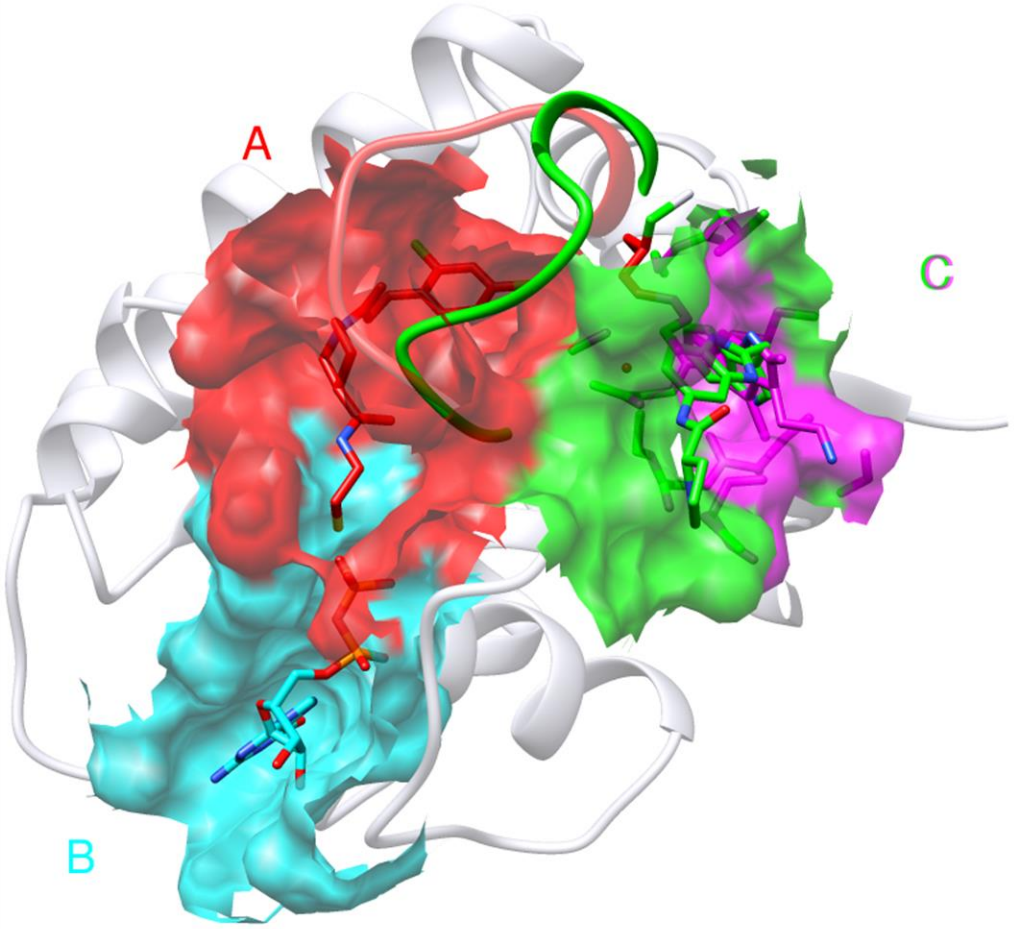
ALLINEAMENTO COMPLESSI

VALIDAZIONE DOCKING

Ligandi Co-Cristallizzati

Compound	PDB-code
 4	4LV6
 6	4LUC
 7	4M1O
 8	4LYF
 9	4LYH
 9	4LYJ
 11	4M21

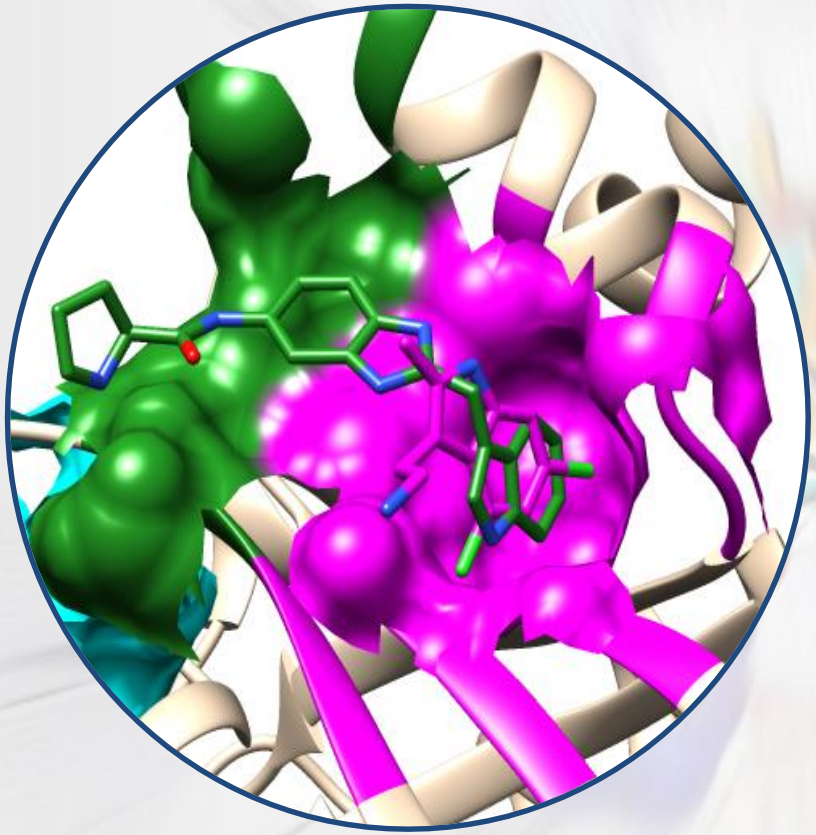
 13	4M1S
 14	4M1T
 15	4M1Y
 16	4M22
 **DCAI** K-Ras(G12D) Ref. 27	4DST
 "13" K-Ras(G12V), Ref. 28	4EPY

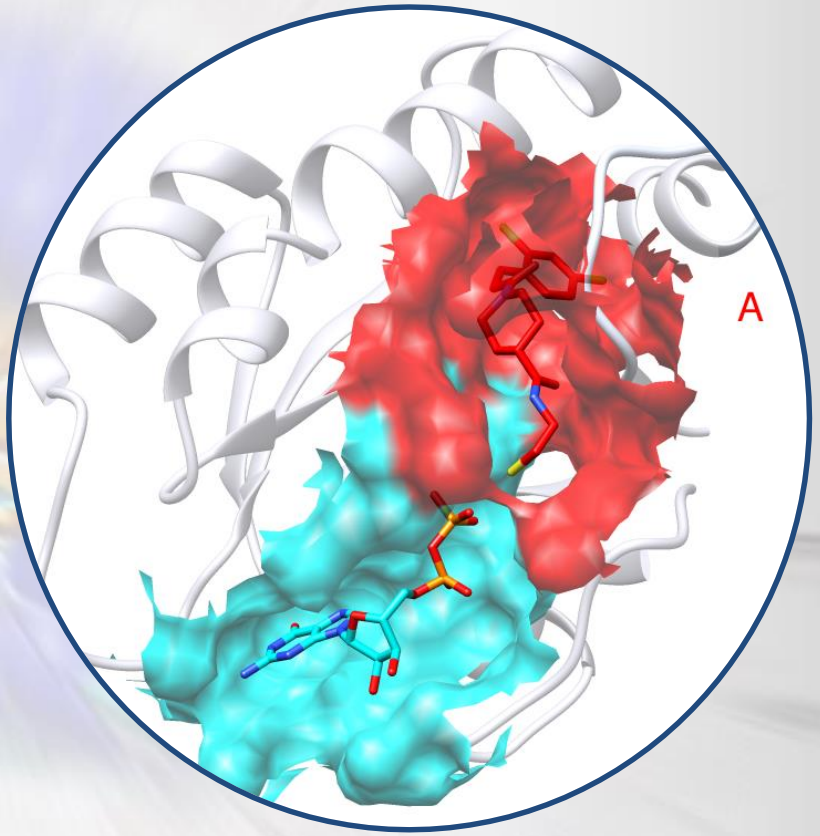


A:
Sito allosterico
"Covalente"

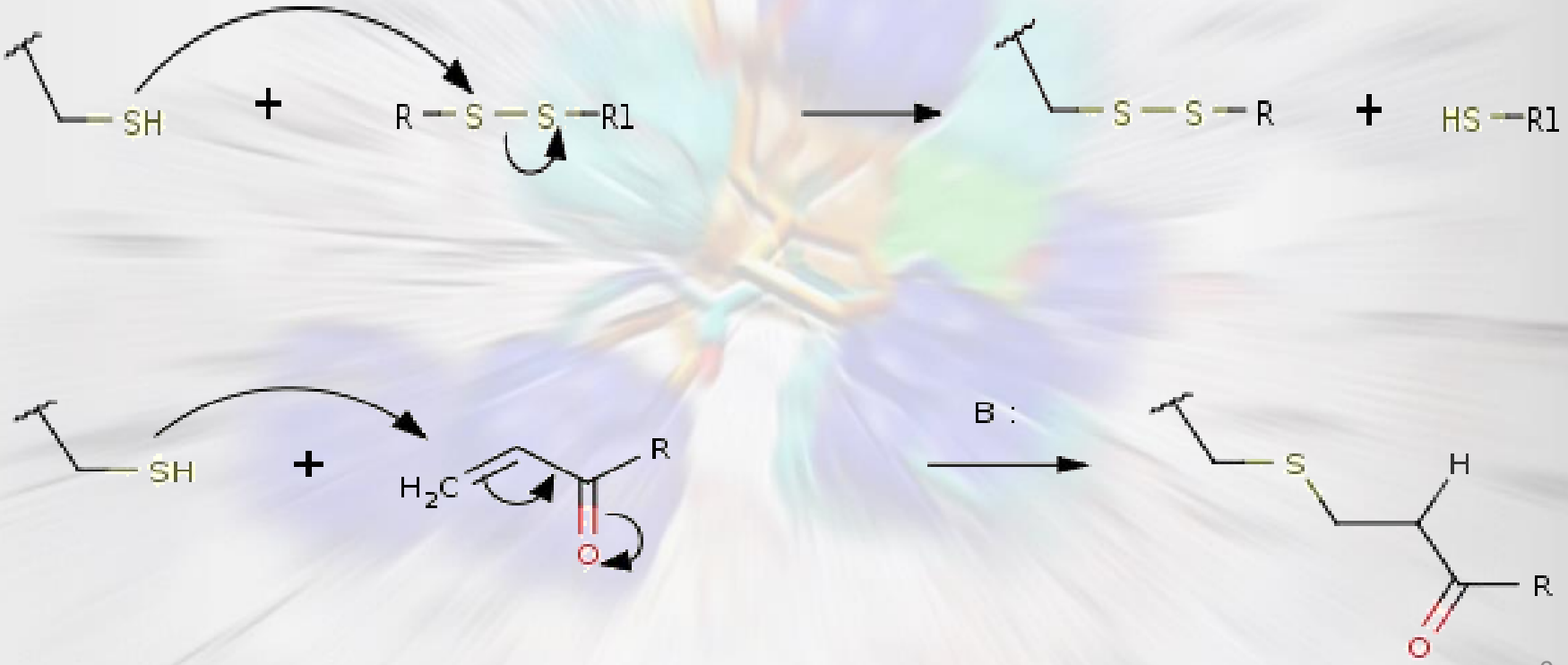
B:
Sito attivo (GDP)

C:
Sito Allosterico
"non Covalente"

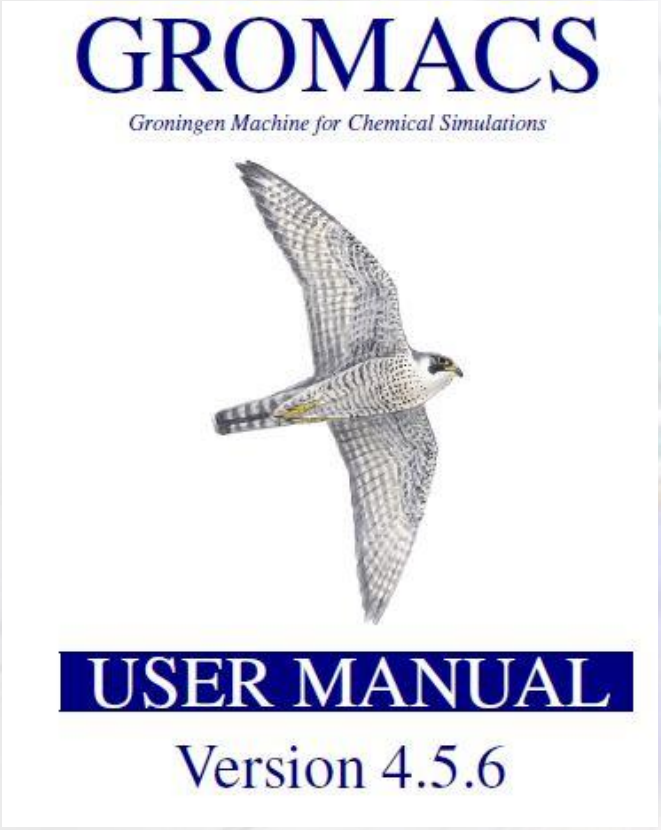



A

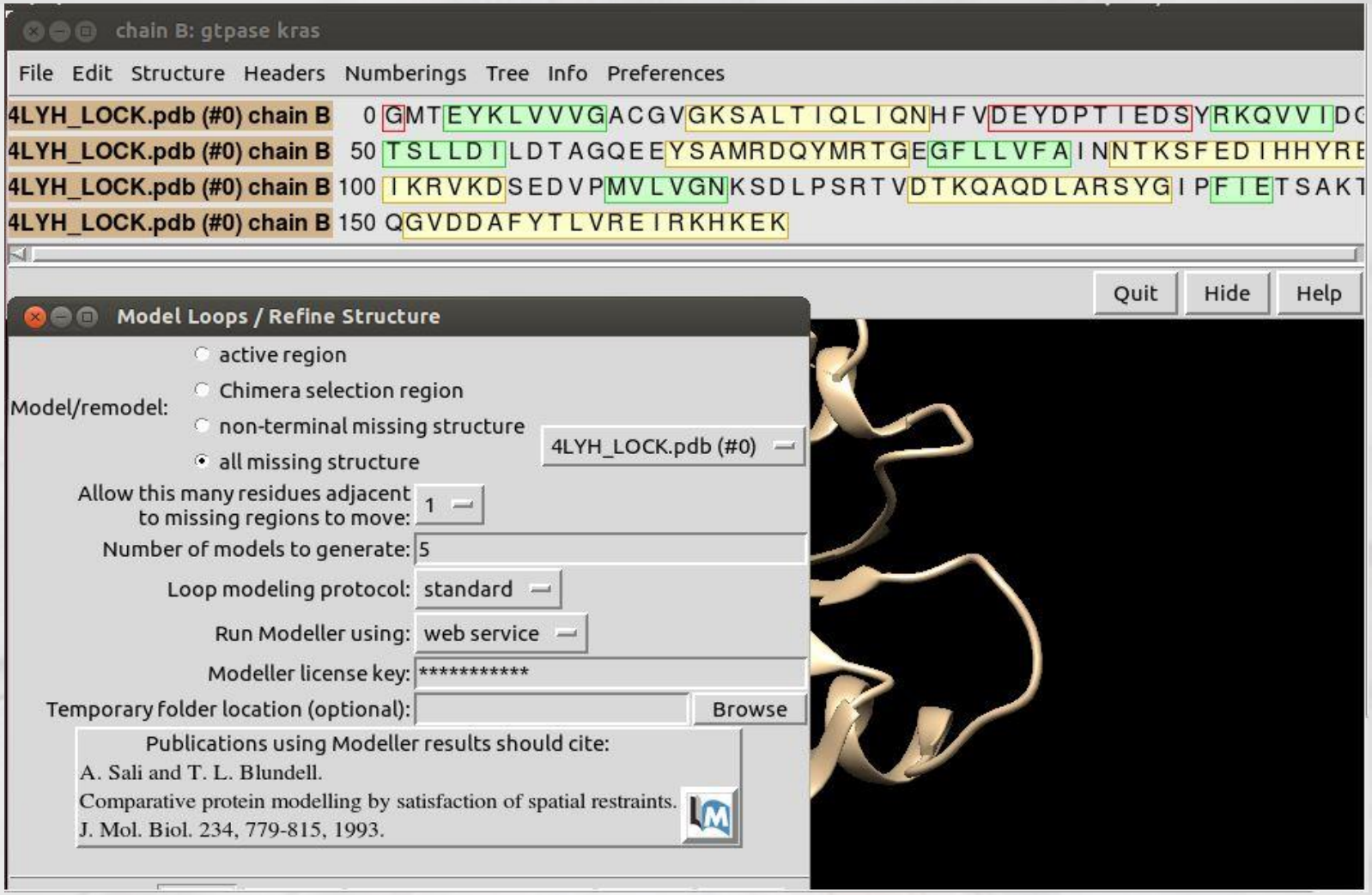
Meccanismo di Inibizione Covalente

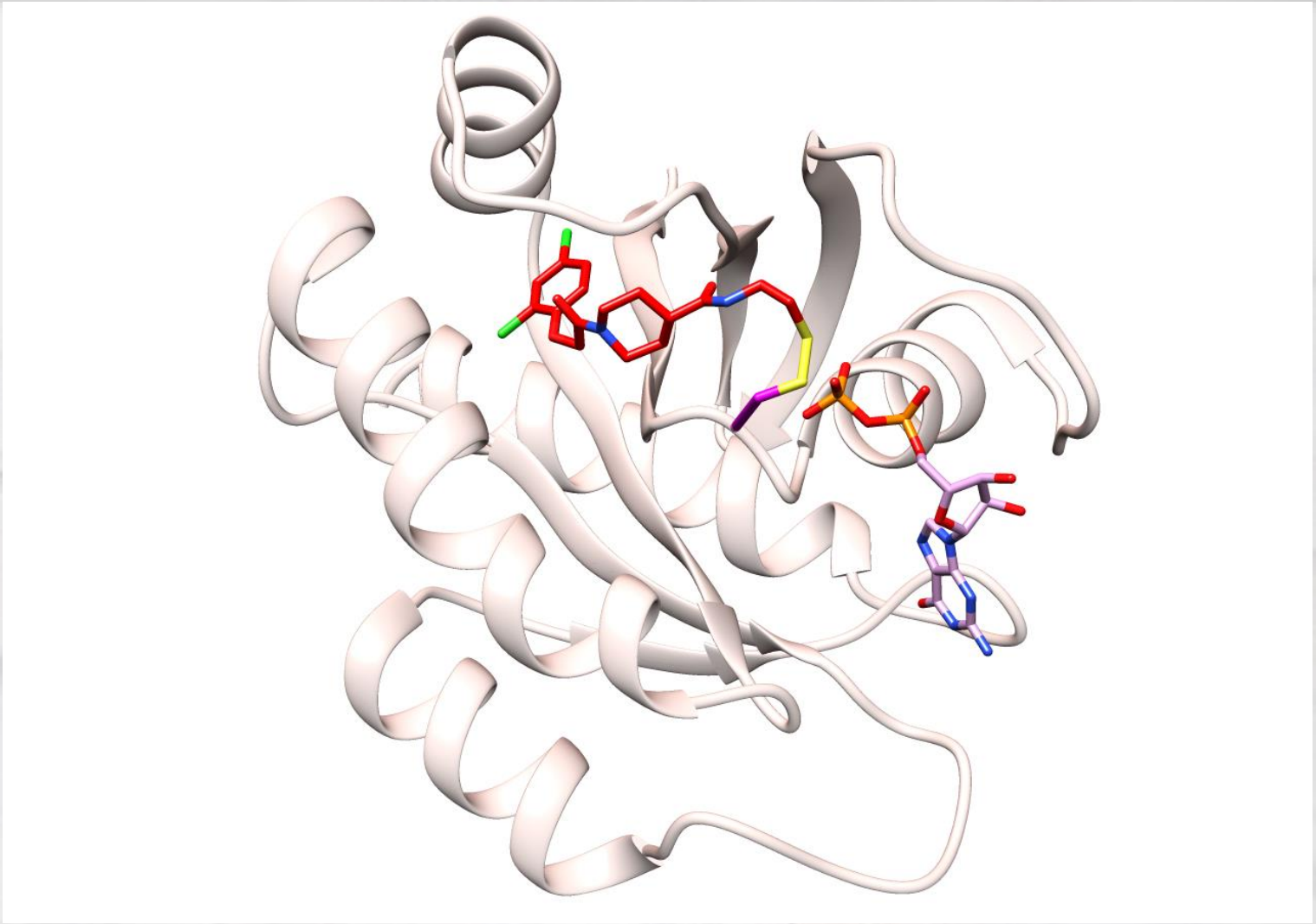


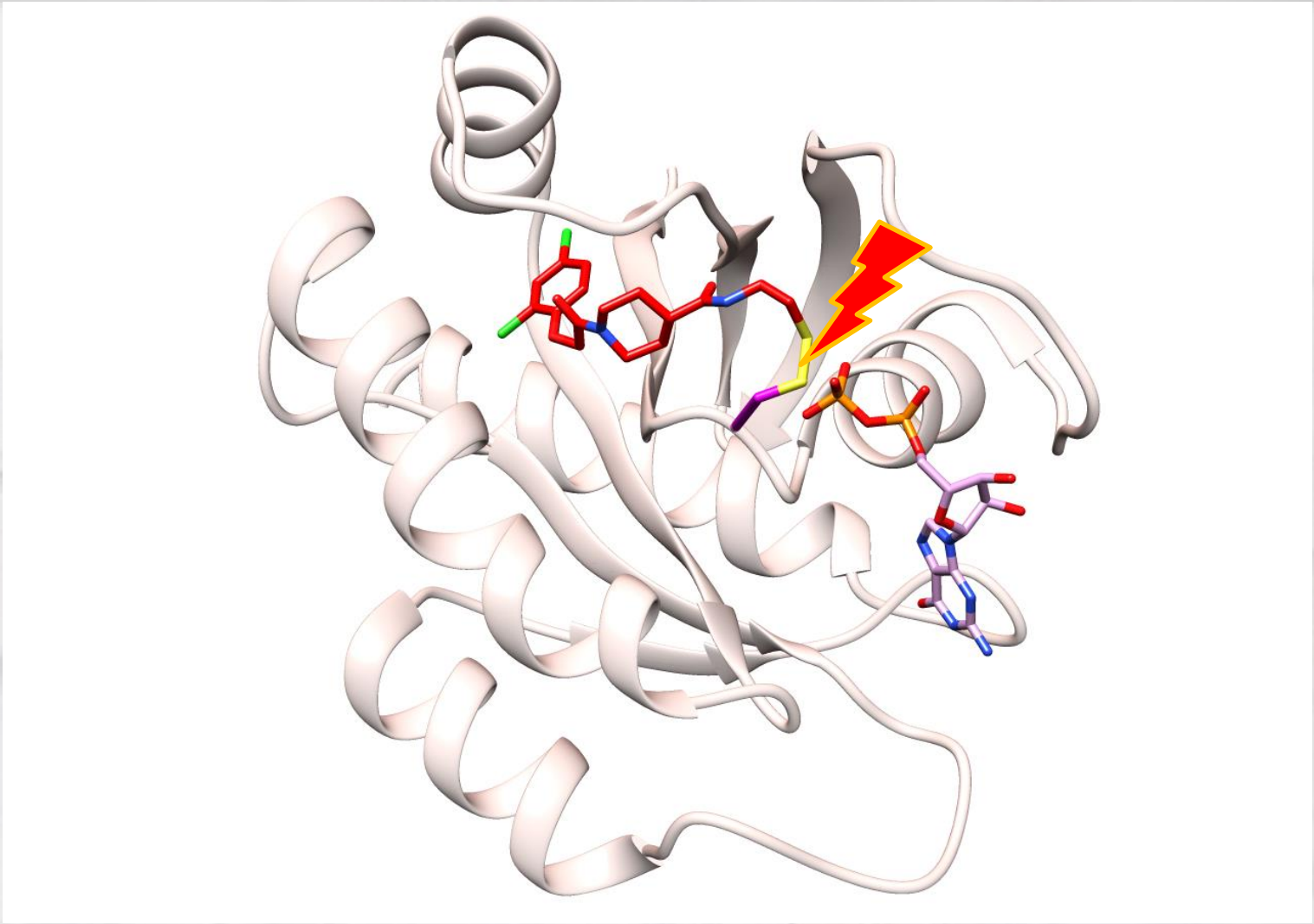
MINIMIZZAZIONE ENERGETICA

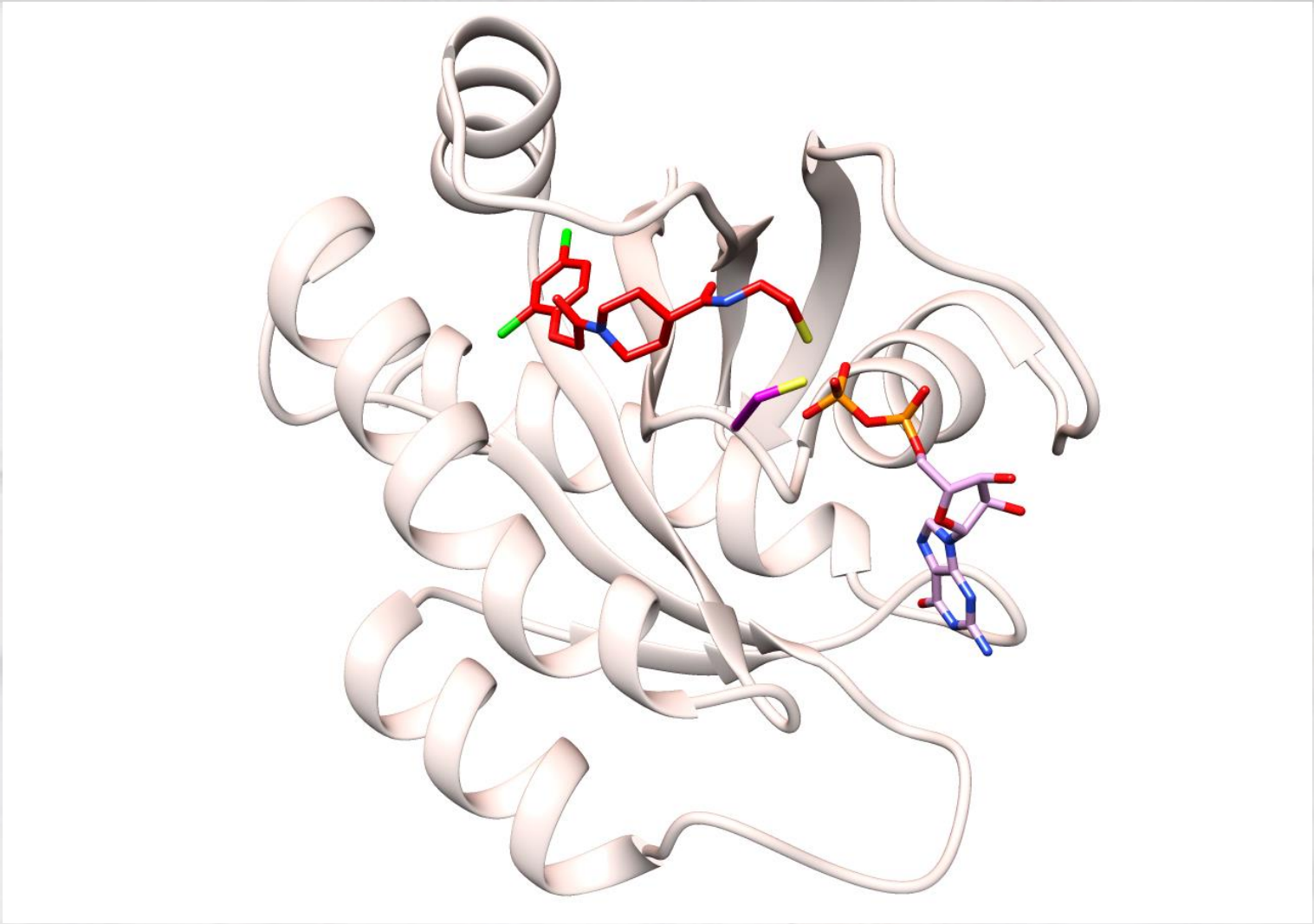


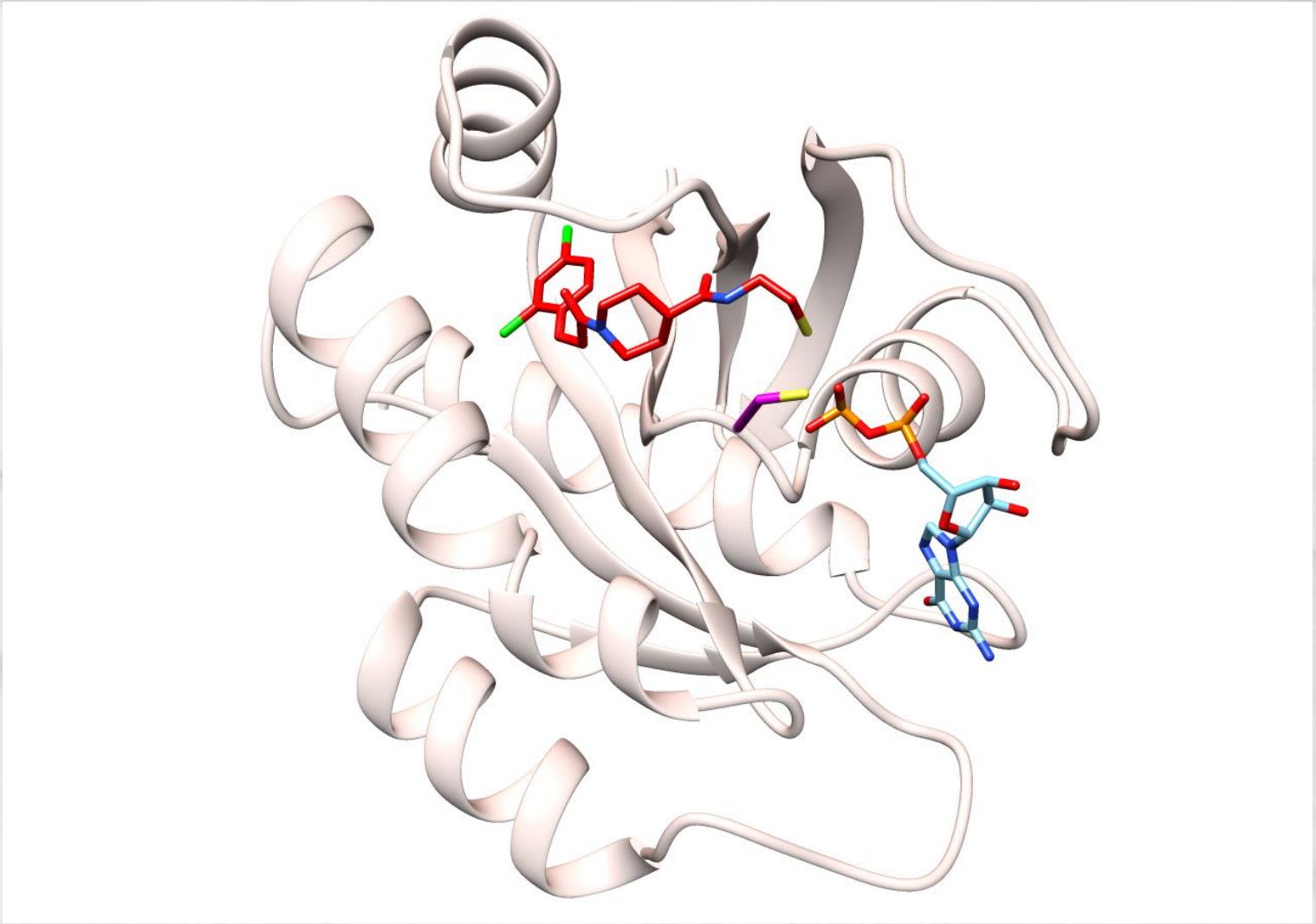
1. Nello studio della minimizzazione , il Force Field che è stato applicato è stato **AMBER99SB-ILDN**
2. Il modello di acqua utilizzato è stato **TIP4P TIP4-point**
3. Per creare la topolgia del ligando è stato utilizzato lo script *acpype.py* che utilizza **antechamber** (modulo del pacchetto AMBER)
4. Gli steps assegnati sono stati 5000

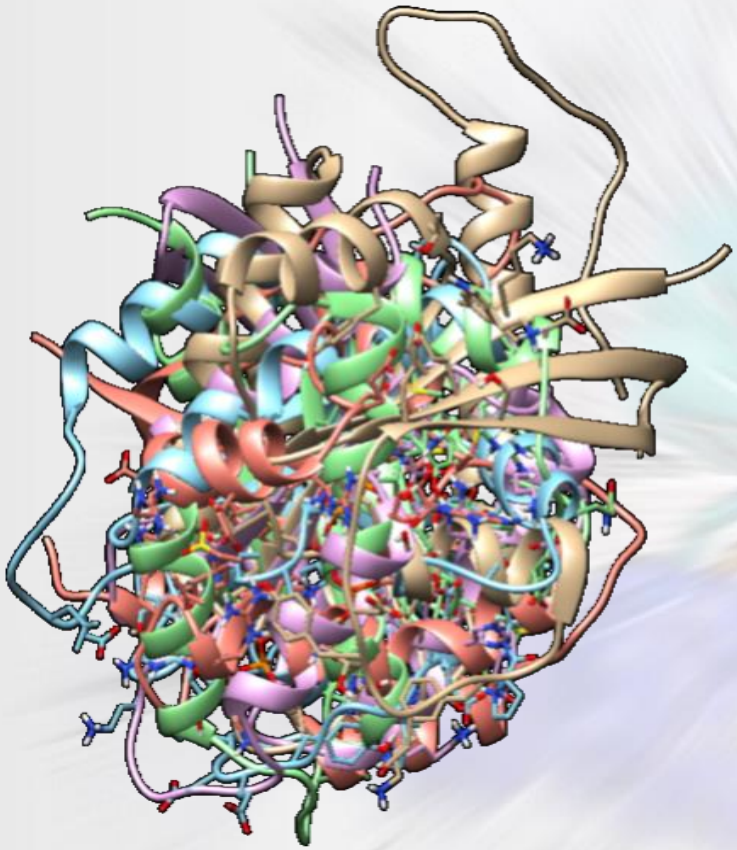

chain B: gtpase kras
File Edit Structure Headers Numberings Tree Info Preferences
4LYH_LOCK.pdb (#0) chain B 0 GMTEYKLVVVGACGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDG
4LYH_LOCK.pdb (#0) chain B 50 TSLLDILDTAGQEEYSAMRDQYMRTGEGFLLVFAINNTKSFEDIHHYRE
4LYH_LOCK.pdb (#0) chain B 100 IKRVKDSEDVPMVLVGNKSDLPSRTVDTKQAQDLARSYGIPFIETSAKT
4LYH_LOCK.pdb (#0) chain B 150 QGVDDAFYTLVREIRKHKEK
Quit
Hide
Help
Model Loops / Refine Structure
Model/remodel:
active region
Chimera selection region
non-terminal missing structure
all missing structure
4LYH_LOCK.pdb (#0)
Allow this many residues adjacent to missing regions to move: 1
Number of models to generate: 5
Loop modeling protocol: standard
Run Modeller using: web service
Modeller license key: **********
Temporary folder location (optional):
Browse
Publications using Modeller results should cite:
A. Sali and T. L. Blundell.
Comparative protein modelling by satisfaction of spatial restraints.
J. Mol. Biol. 234, 779-815, 1993.

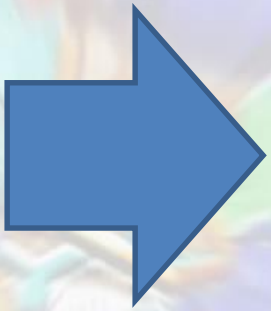


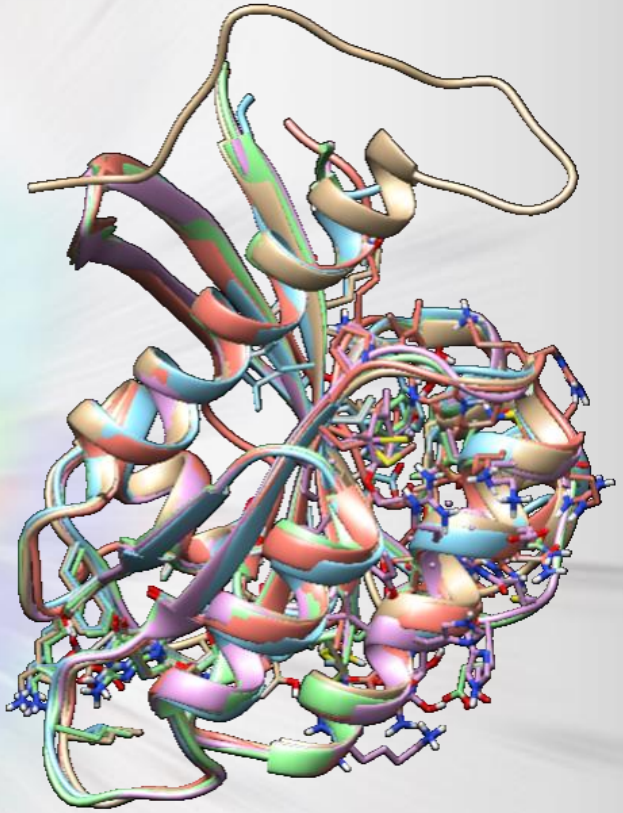




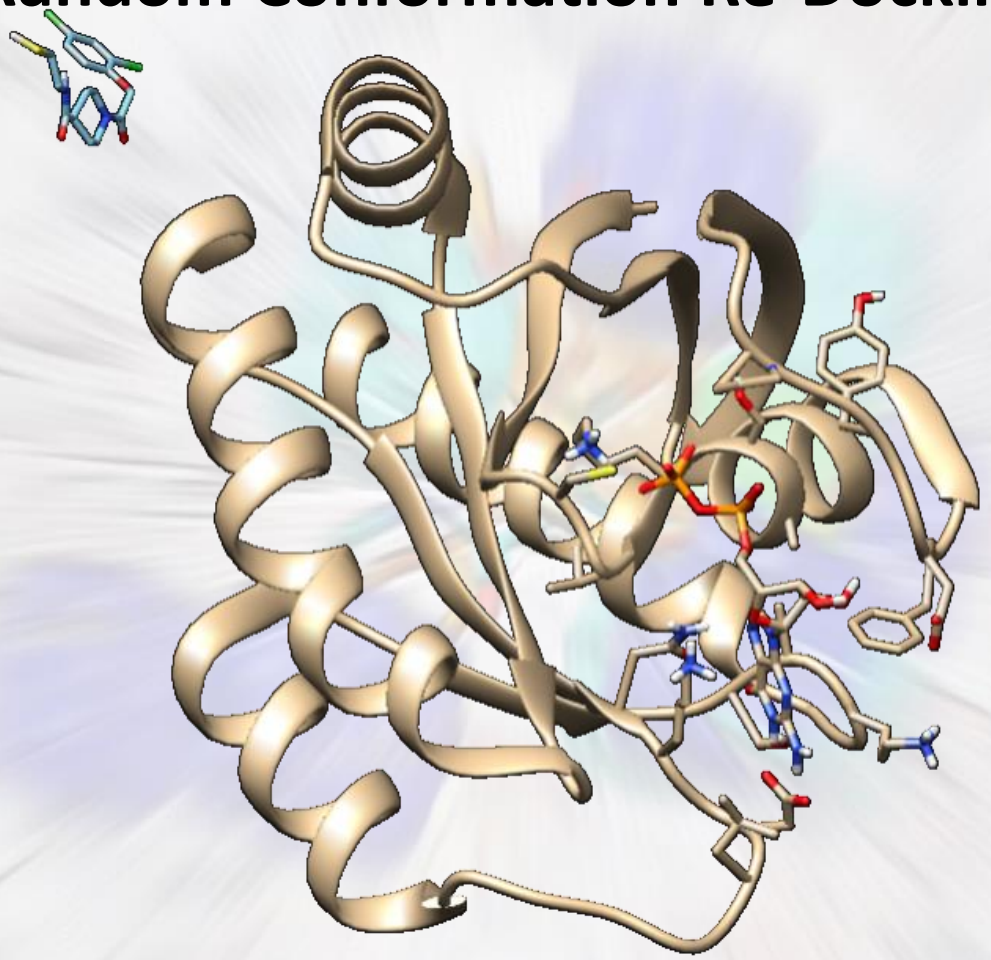








Random Conformation Re-Docking

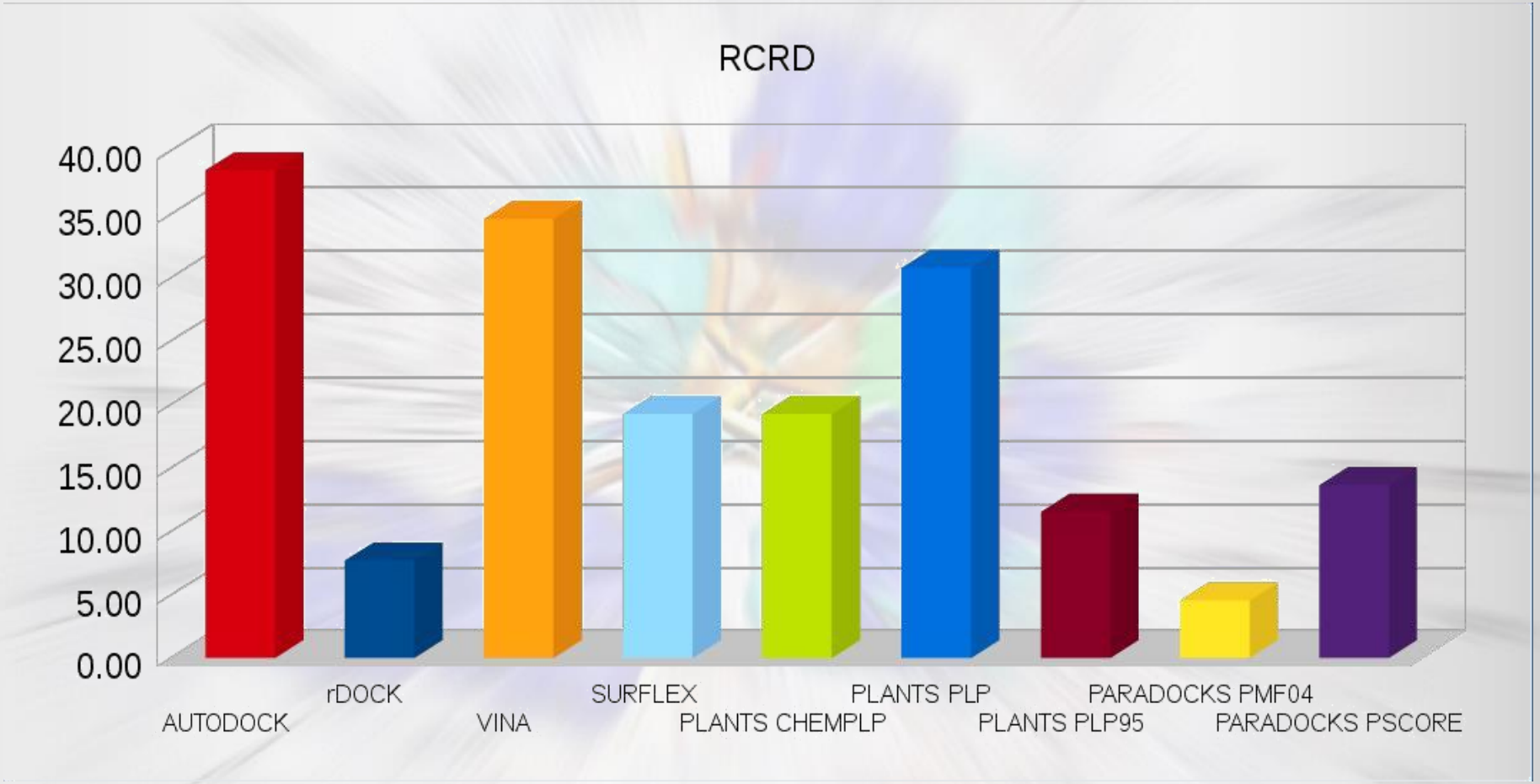


Root Mean Square Deviation

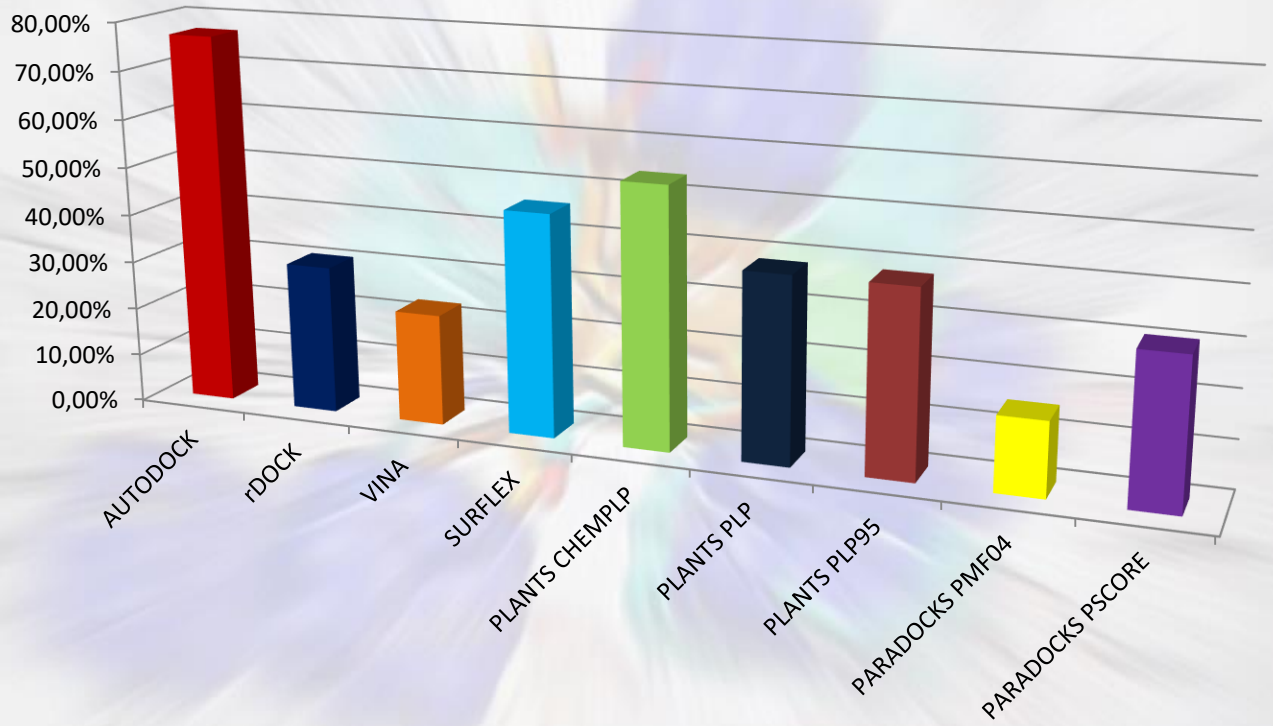
$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2}$$

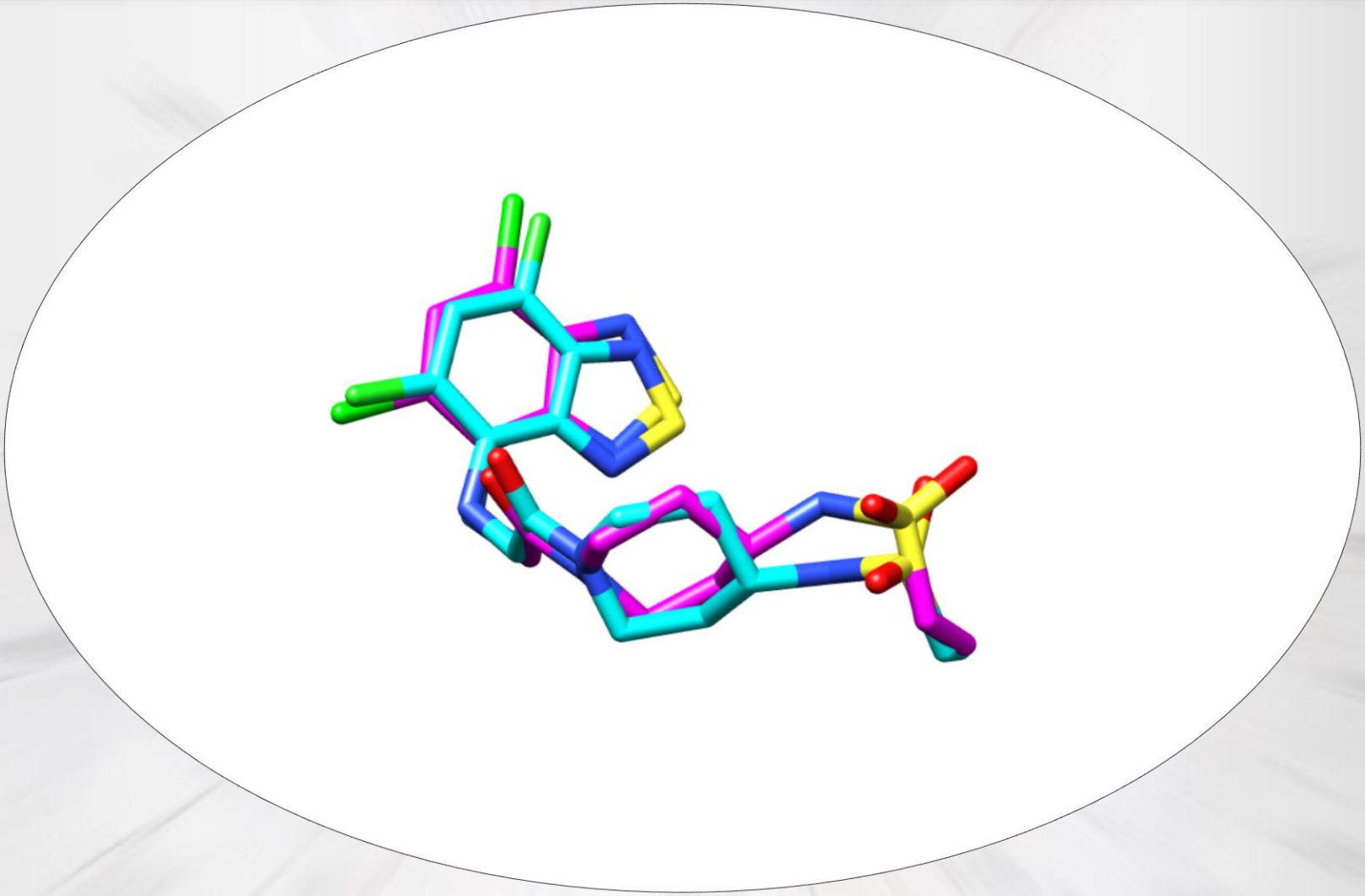
Docking **A**ccuracy

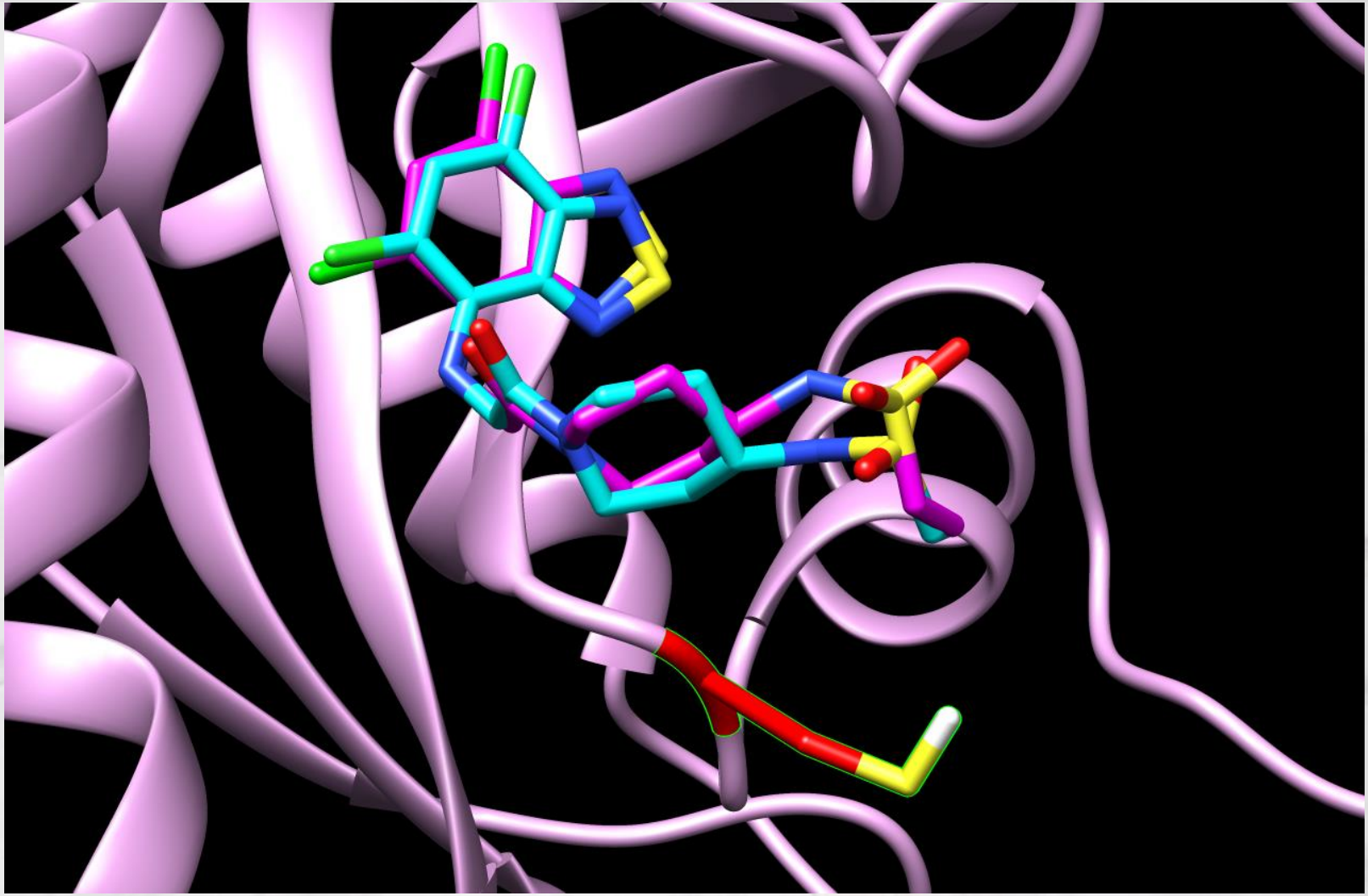
$$DA = f_{RMSD\leq 2} + 0.5\left(f_{RMSD\leq 3} - f_{RMSD\leq 2}\right)$$


RCRD
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
AUTODOCK
rDOCK
VINA
SURFLEX
PLANTS CHEMPLP
PLANTS PLP
PLANTS PLP95
PARADOCKS PMF04
PARADOCKS PSCORE

VALIDAZIONE DEL METODO

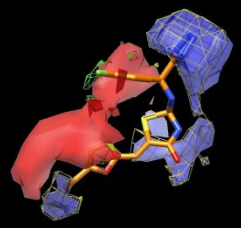






Conclusioni

1) È stato eseguito uno studio SB sui dati strutturali della KRAS attualmente disponibili
2) Su una serie di 9 programmi di docking molecolare, Autodock ha dimostrato di essere quello con la capacità predittiva migliore sia in termini di binding mode assoluto (RMSD) che per il giusto orientamento dei ligandi in studio.
3) Lo studio si è focallizzato sulle pose non legate covalentemente per la futura applicazione nella progettazione di nuovi agenti a meccanismo reversibile
4) Tecniche di cross-docking non si sono dimostrate efficaci indicando che per il design si dovranno usare i dati strutturali più simili alle molecole in studio.



Grazie dell'attenzione