

Approcci Structure Based (SB) per la razionalizzazione delle interazioni proteina-inibitore applicate all' enzima MMP-2 e MMP-9



SAPIENZA
UNIVERSITÀ DI ROMA

Facoltà di Farmacia e Medicina
Corso di Laurea in Farmacia
Tesi Sperimentale in Chimica
Farmaceutica
a.a. 2014/2015

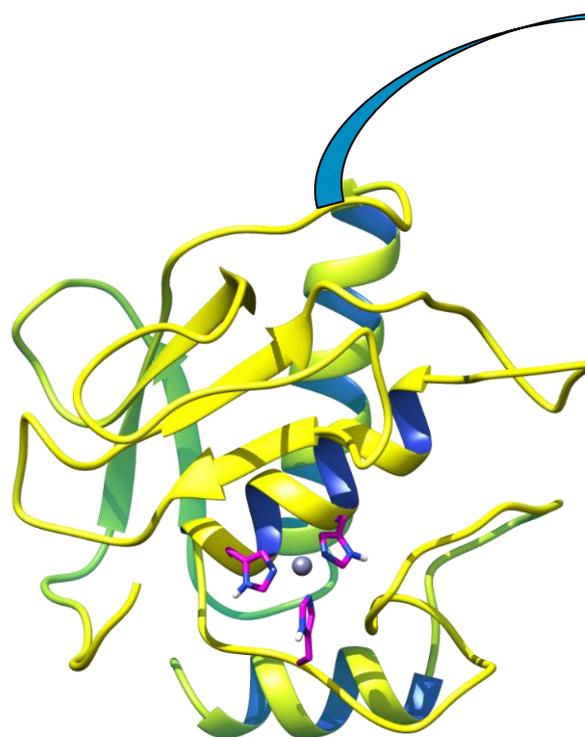
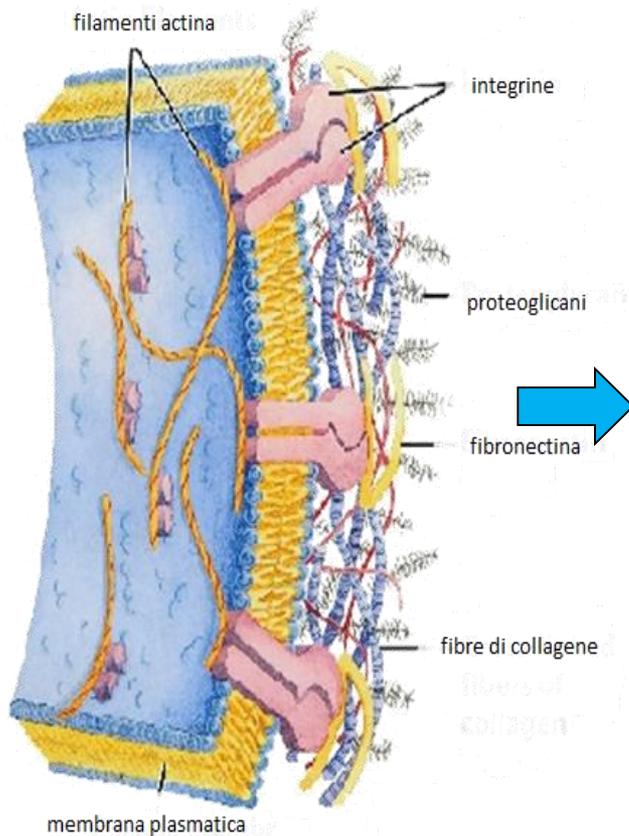
Laureanda: Maria Ilaria D' Andrea
Matricola: 1193112

Relatore: Prof. Rino Ragno



MMP

Matrix metalloproteinases (MMPs) sono delle endopeptidasi Zinco e Calcio dipendenti secreti nella matrice extracellulare (ECM).



Codice PDB: 20VZ

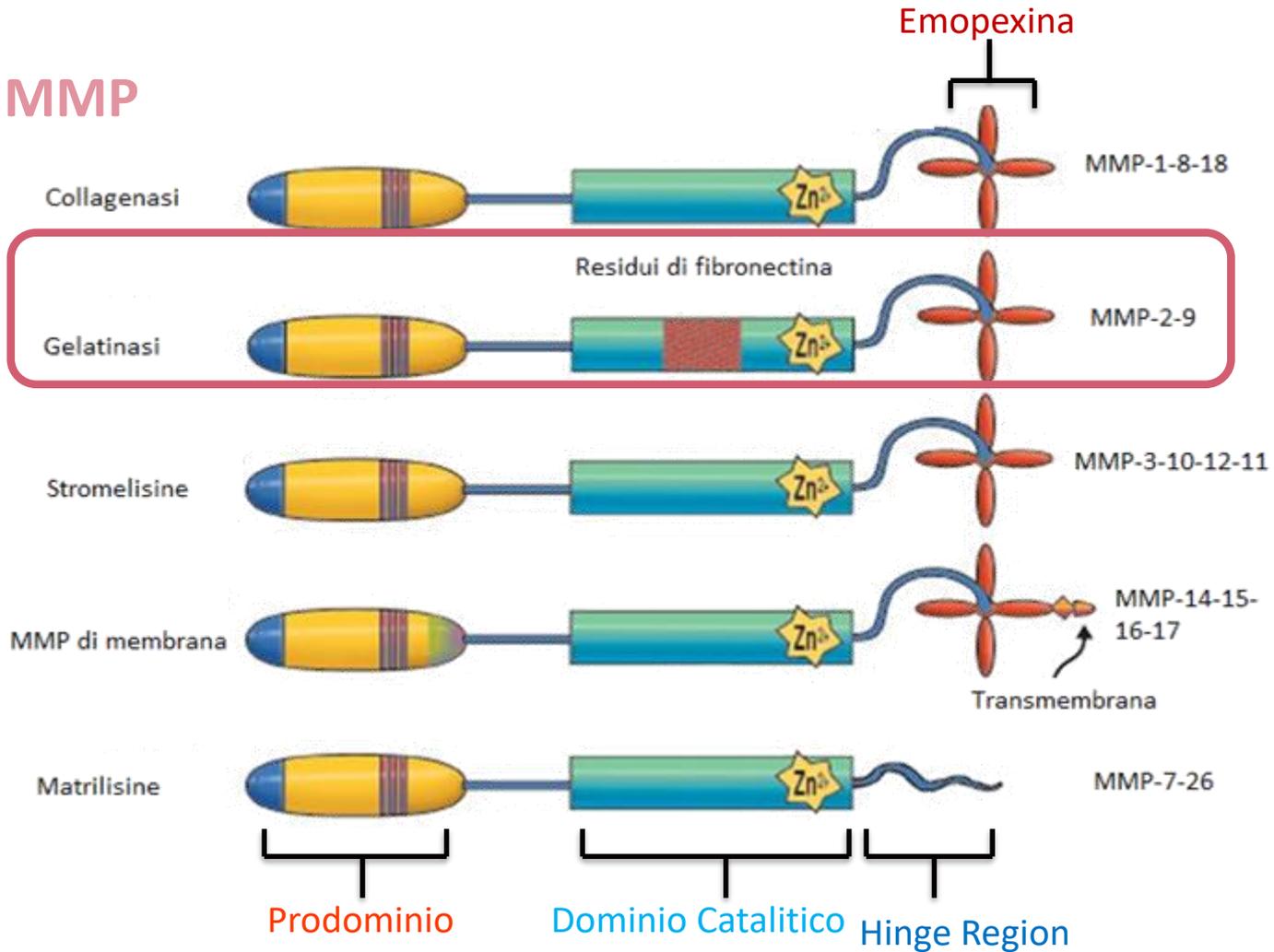
Degradazione della matrice

Rimodellamento tissutale



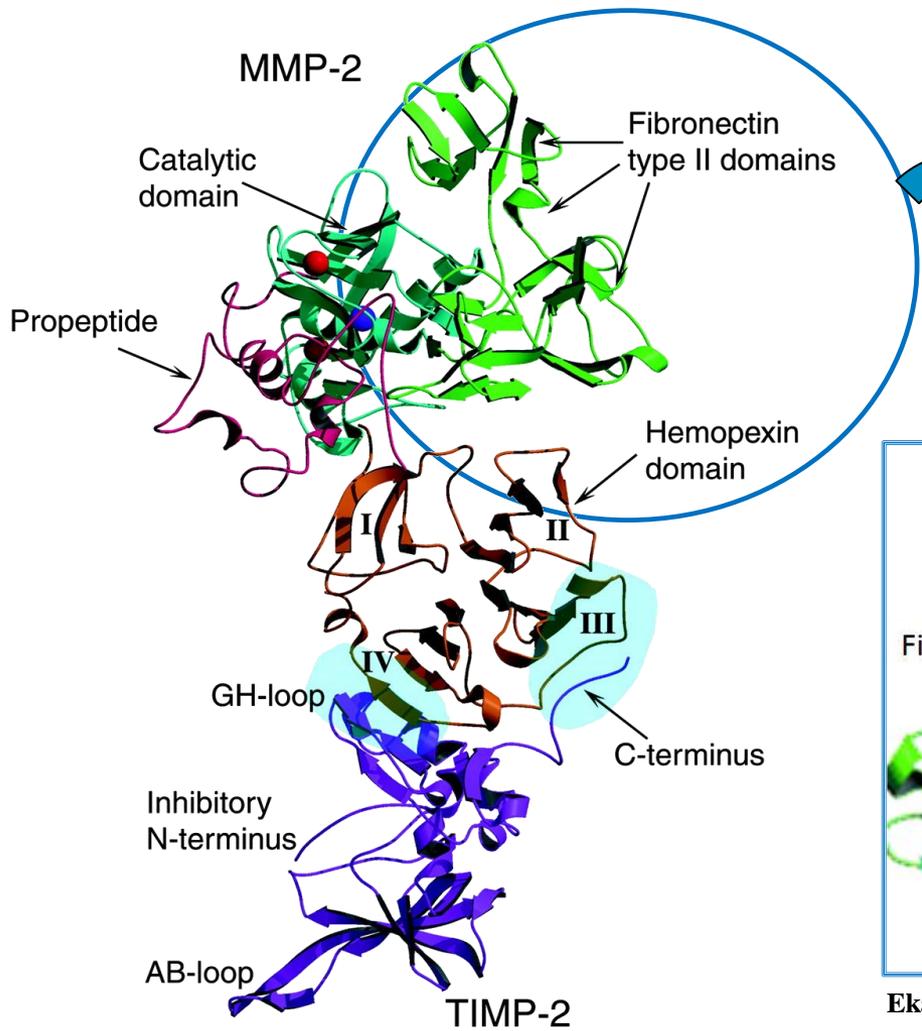
STRUTTURA E CLASSIFICAZIONE

➔ 24 MMP

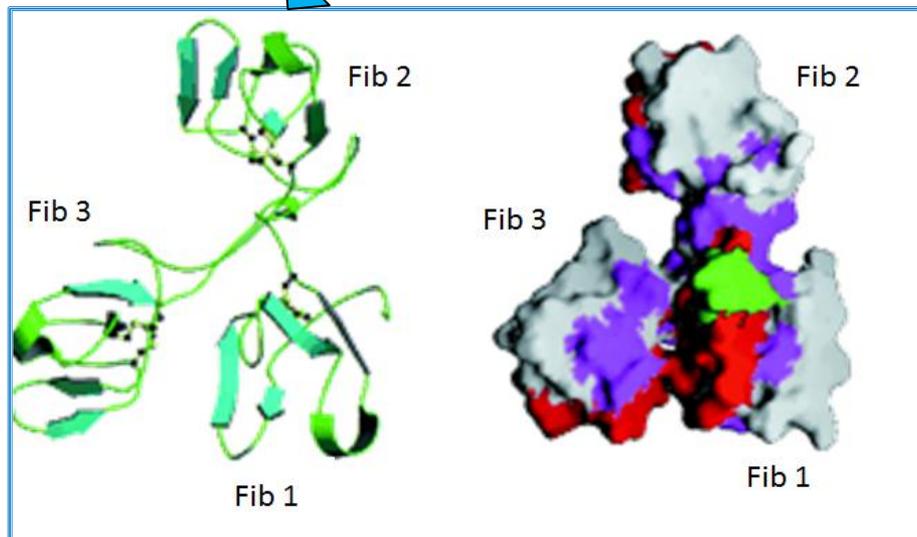




MMP-2 MMP-9



MMP-2 = 72 kDa
MMP-9 = 92 kDa

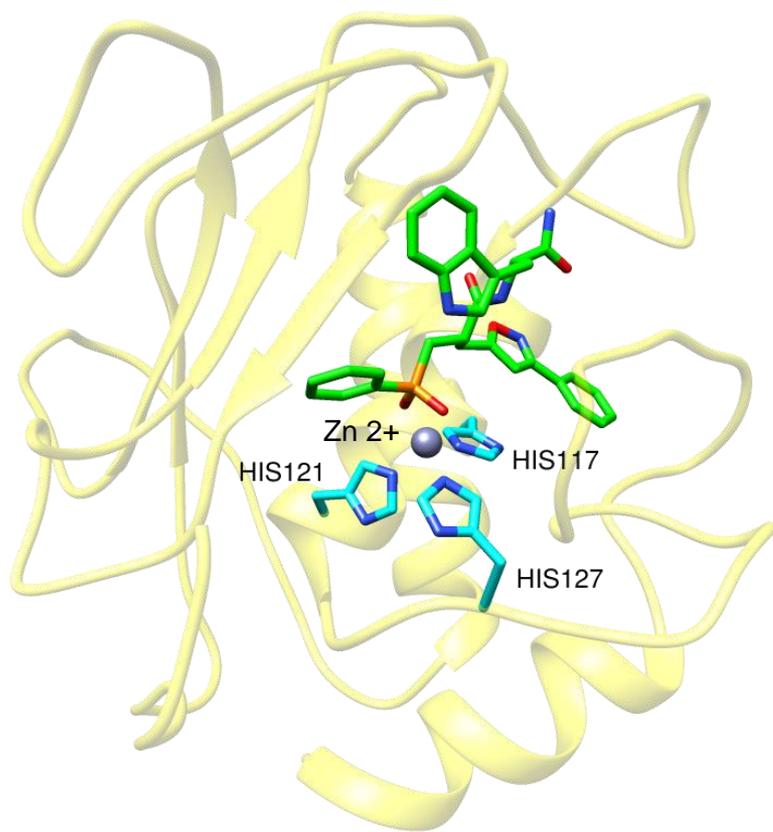


Ekaterina Morgunova et al. PNAS 2002;99:7414-7419



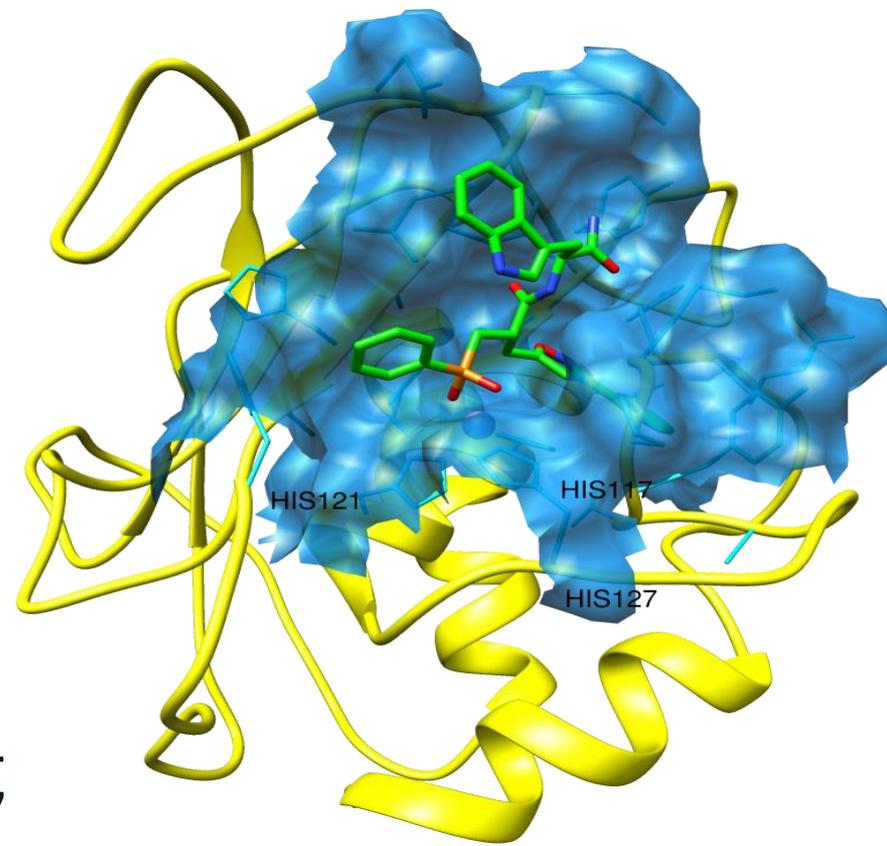
SITO ATTIVO

Consiste in 5 foglietti β (da SI a SV) e tre α -elica (da hA a hC)



Codice PDB: 20VZ

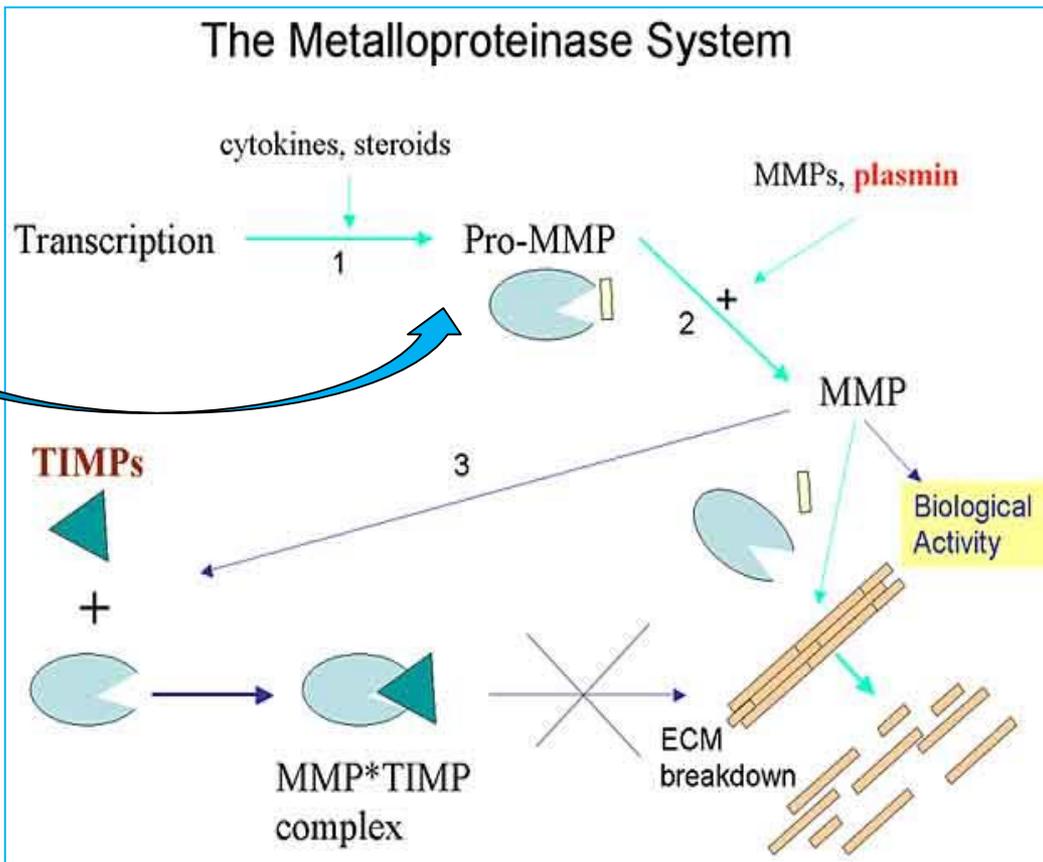
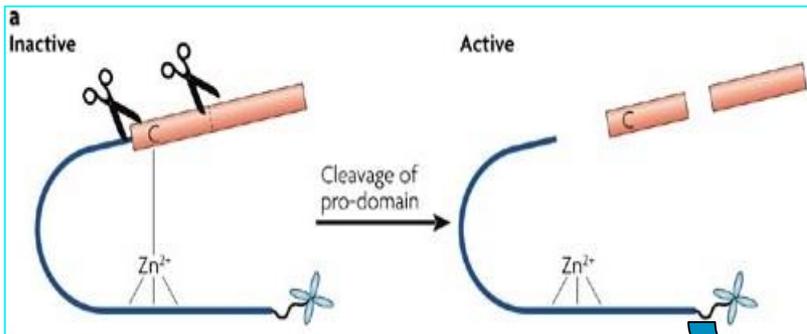
HIS 121
HIS 117
HIS 127



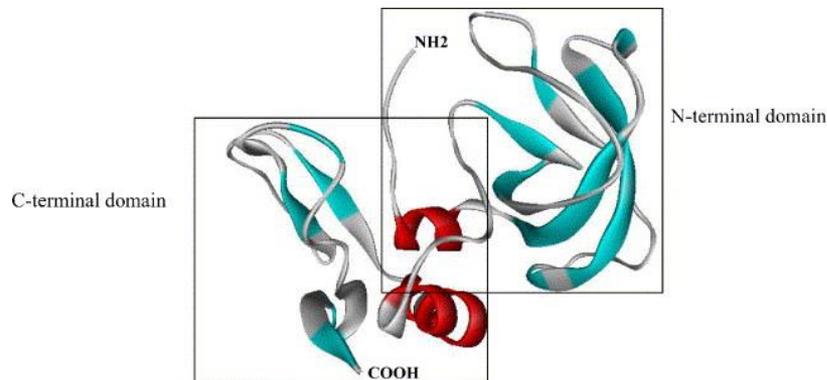
Codice PDB: 20VZ



REGOLAZIONE



Timp (Tissue Inhibitors MetalloProteinase)





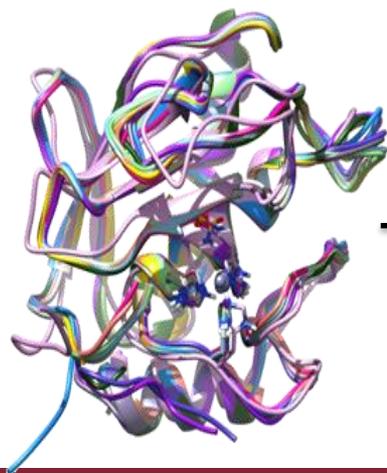
SCOPO DELLA TESI

Condizioni fisiologiche:

- Ovulazione
- Ciclo endometriale
- Gravidanza
- Guarigione dalle ferite
- Angiogenesi
- Rimodellamento osseo
- Eruzione dentaria

Condizioni patologiche:

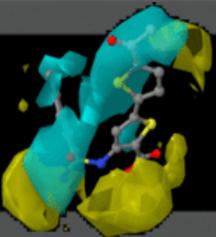
- Cancro
- Metastasi Tumorale
- Cardiomiopatia Dilatativa
- Fibrosi cistica
- Cirrosi epatica
- Glomerulonefrite
- Artrite reumatoide



Metodiche Computazionali



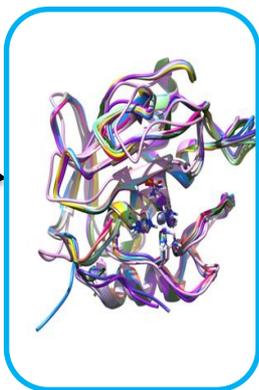
Progettazione
nuovi inibitori



RCSB
PDB
PROTEIN DATA BANK

Download
dei PDB

13 cplx



Complessi
Minimizzati ed
Allineati

Complessi
NON
Minimizzati ed
Allineati

DOCKING

3-D QSAR

Docking Assessment

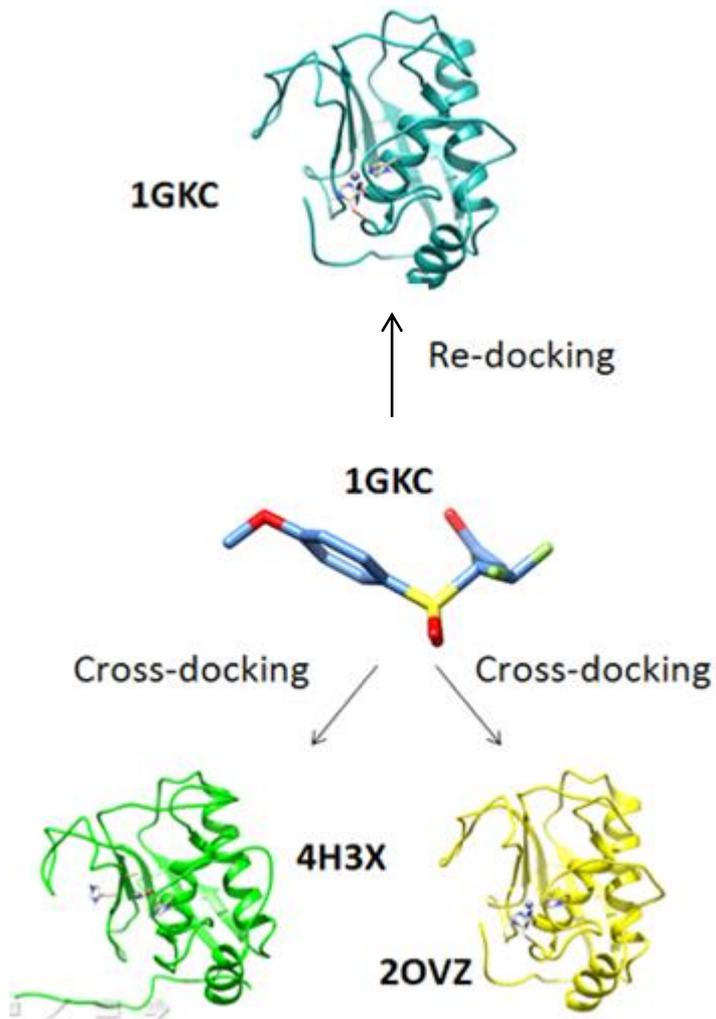
Applicazione del
docking

Consensus Docking

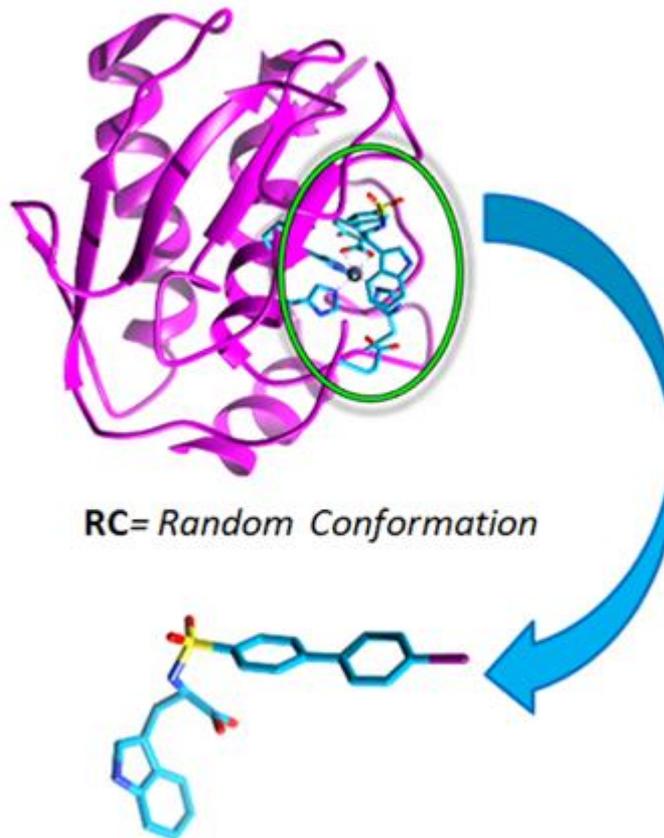
- Pulizia
- Separazione catene
- Add H
- Add Charge
- Minimizzazione → GROMACS



PROCEDURA

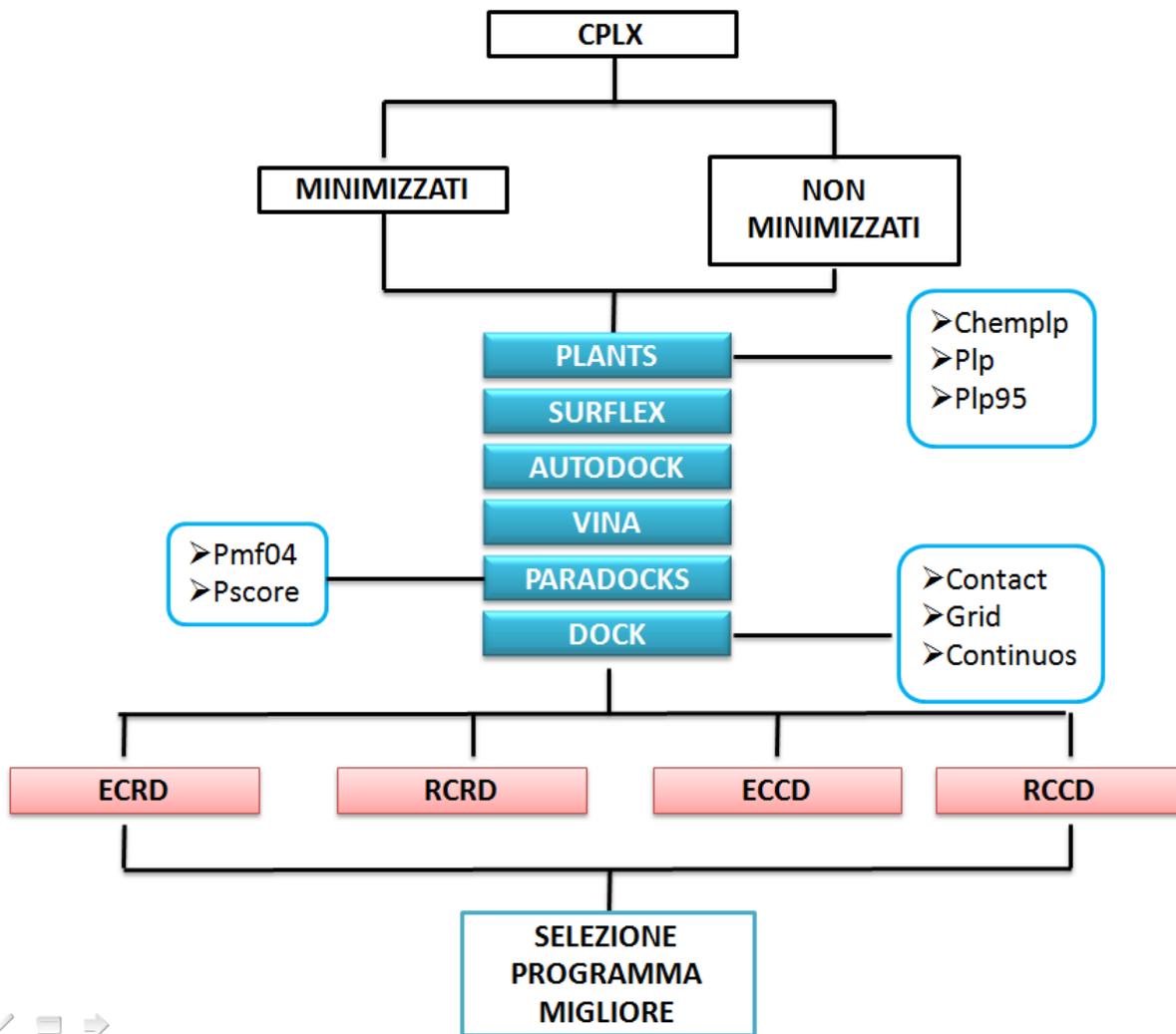


EC= *Experimental Conformation*





DOCKING ASSESSMENT



ECCD → *Experimental Conformation Cross-docking*

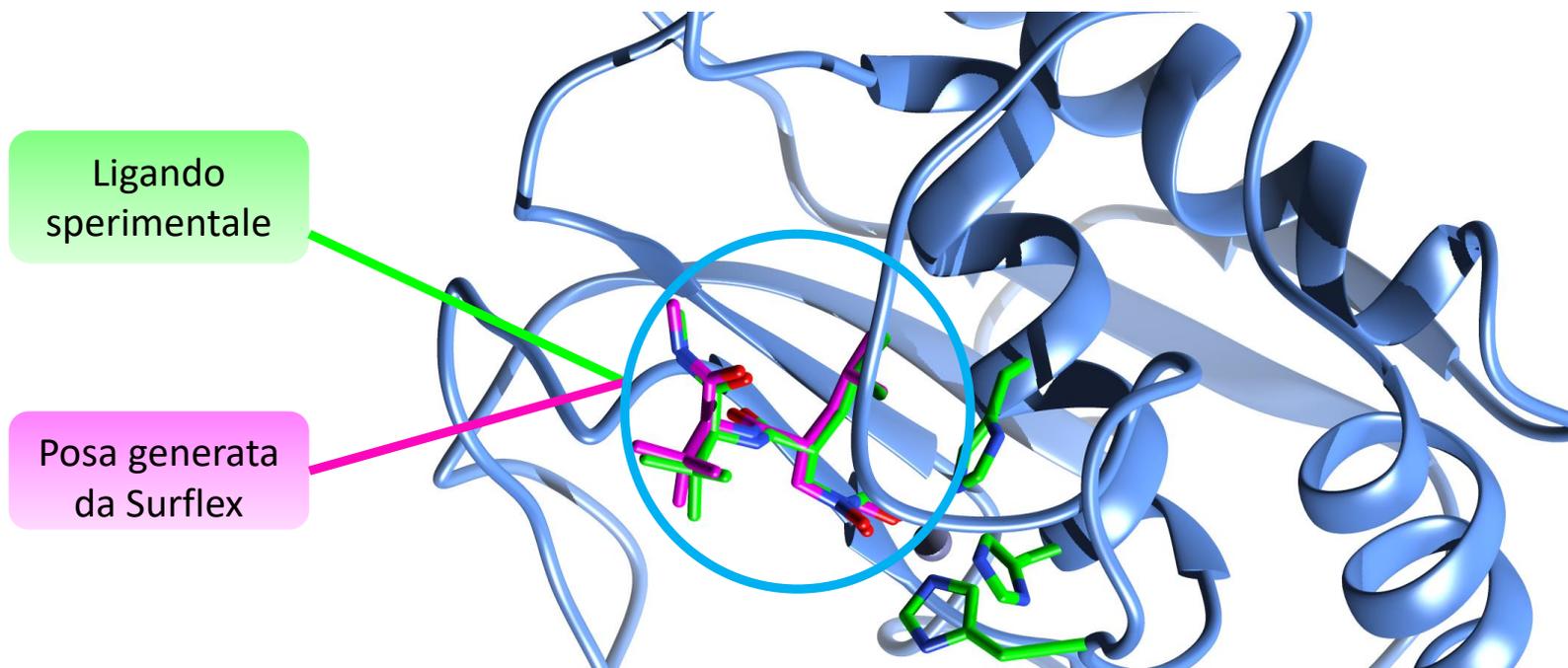
RCCD → *Random Conformation Cross-docking*

ECRD → *Experimental Conformation Re-docking*

RCRD → *Random Conformation Re-docking*



RMSD (*Root Mean Square Deviation*)



Codice PDB: 1GKC

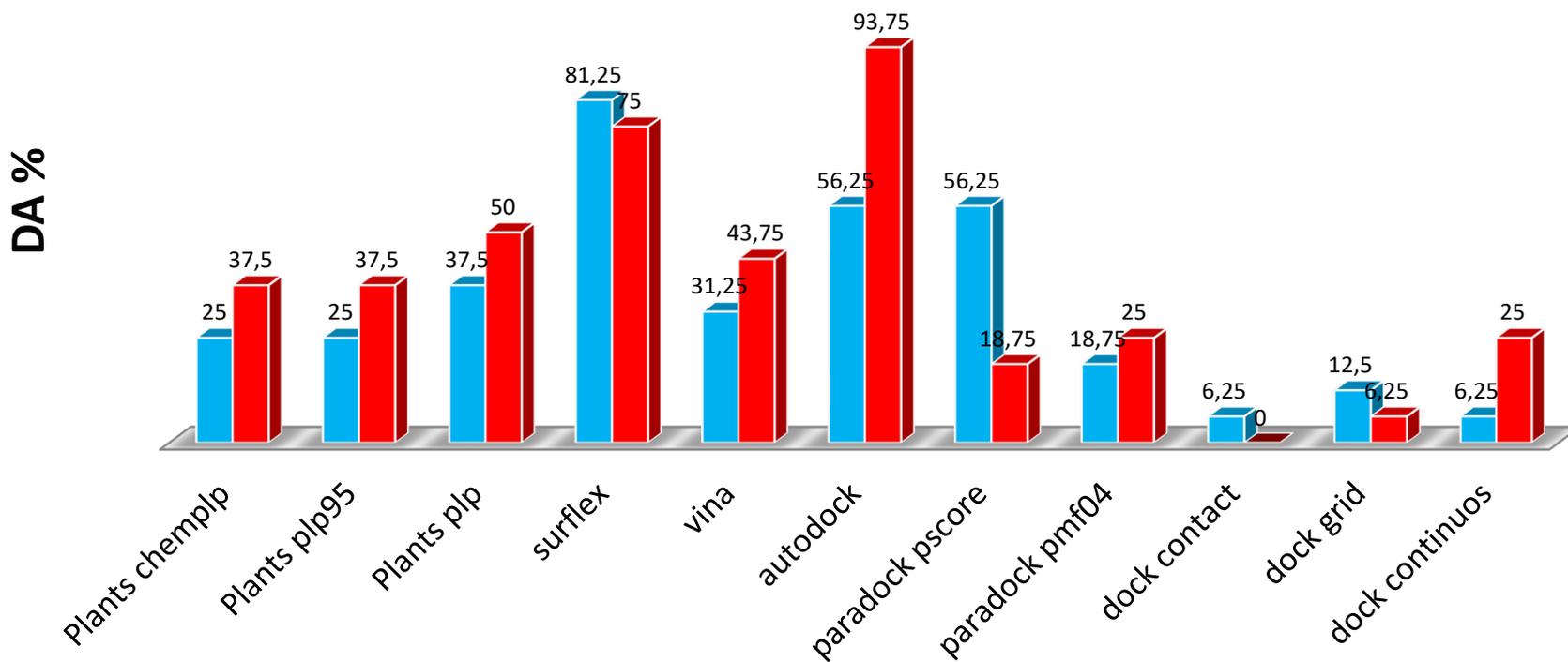
Docking Accuracy DA% \Rightarrow $DA = \text{frmsd} \leq a + 0.5$ ($\text{frmsd} \leq b - \text{frmsd} \leq a$)



RE-DOCKING

ECRD

■ NON MIN ■ MIN

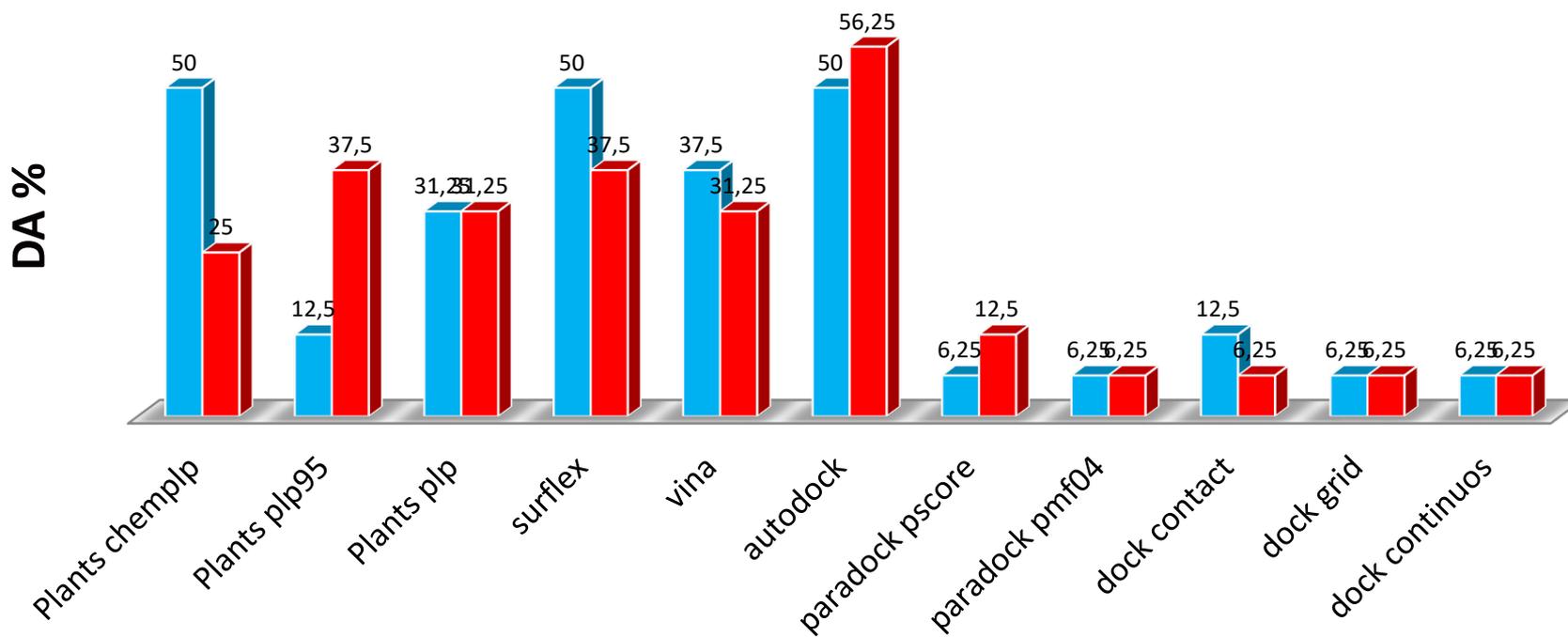




RE-DOCKING

RCRD

■ NON MIN ■ MIN

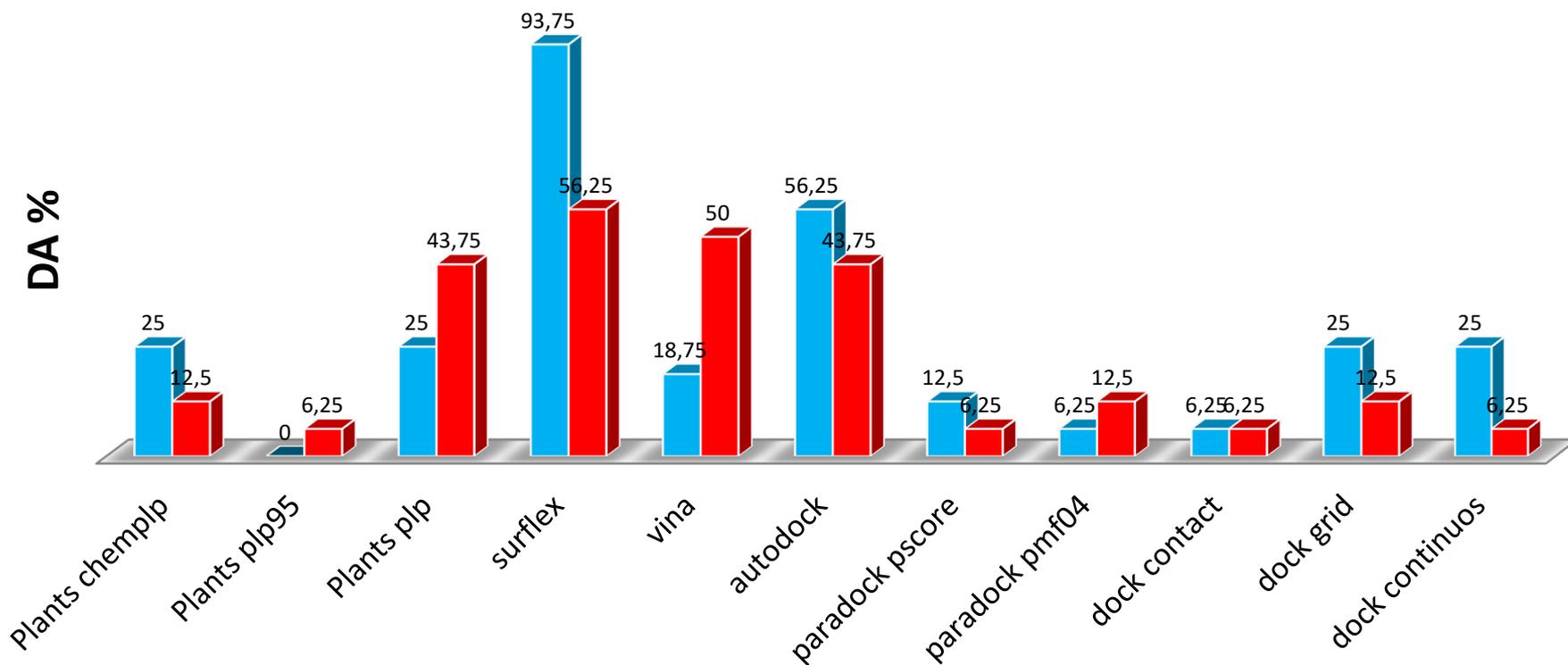


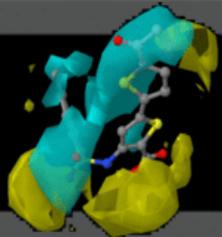


CROSS-DOCKING

ECCD

■ NON MIN ■ MIN

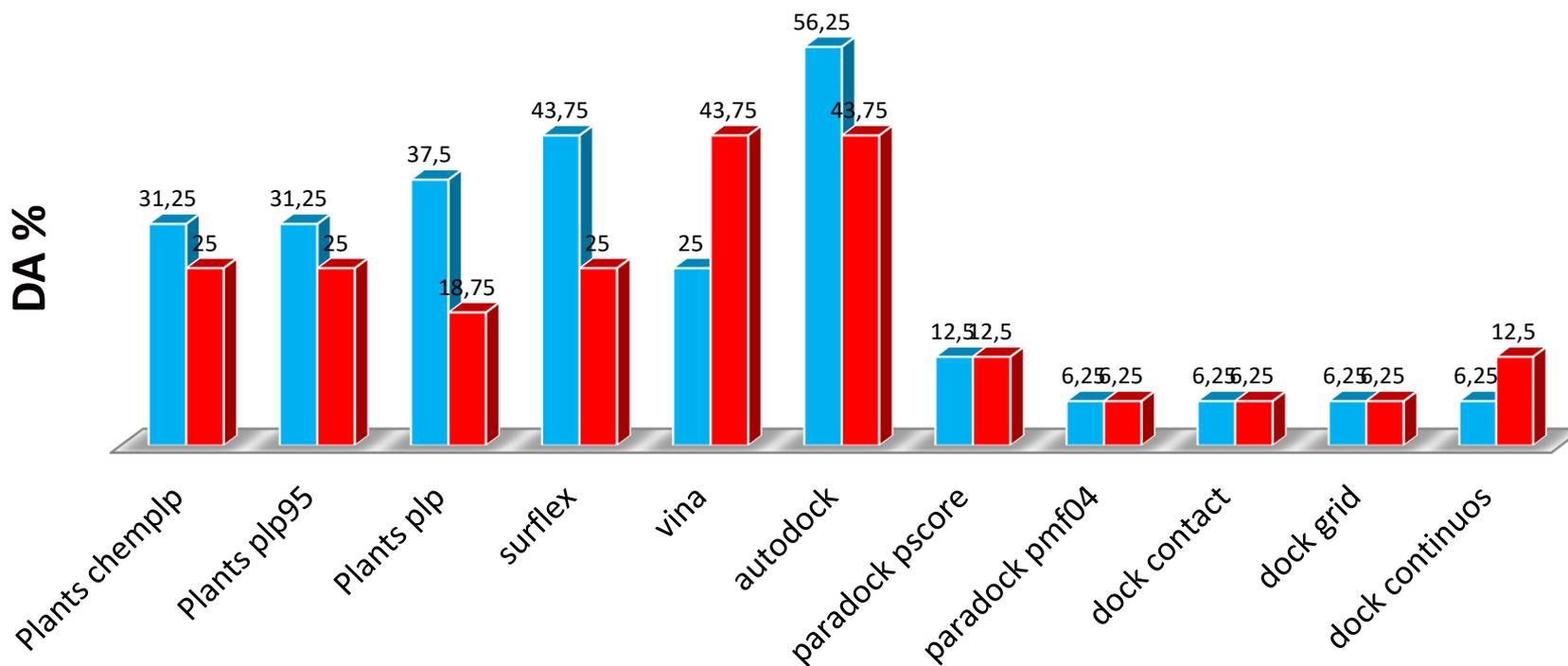




CROSS-DOCKING

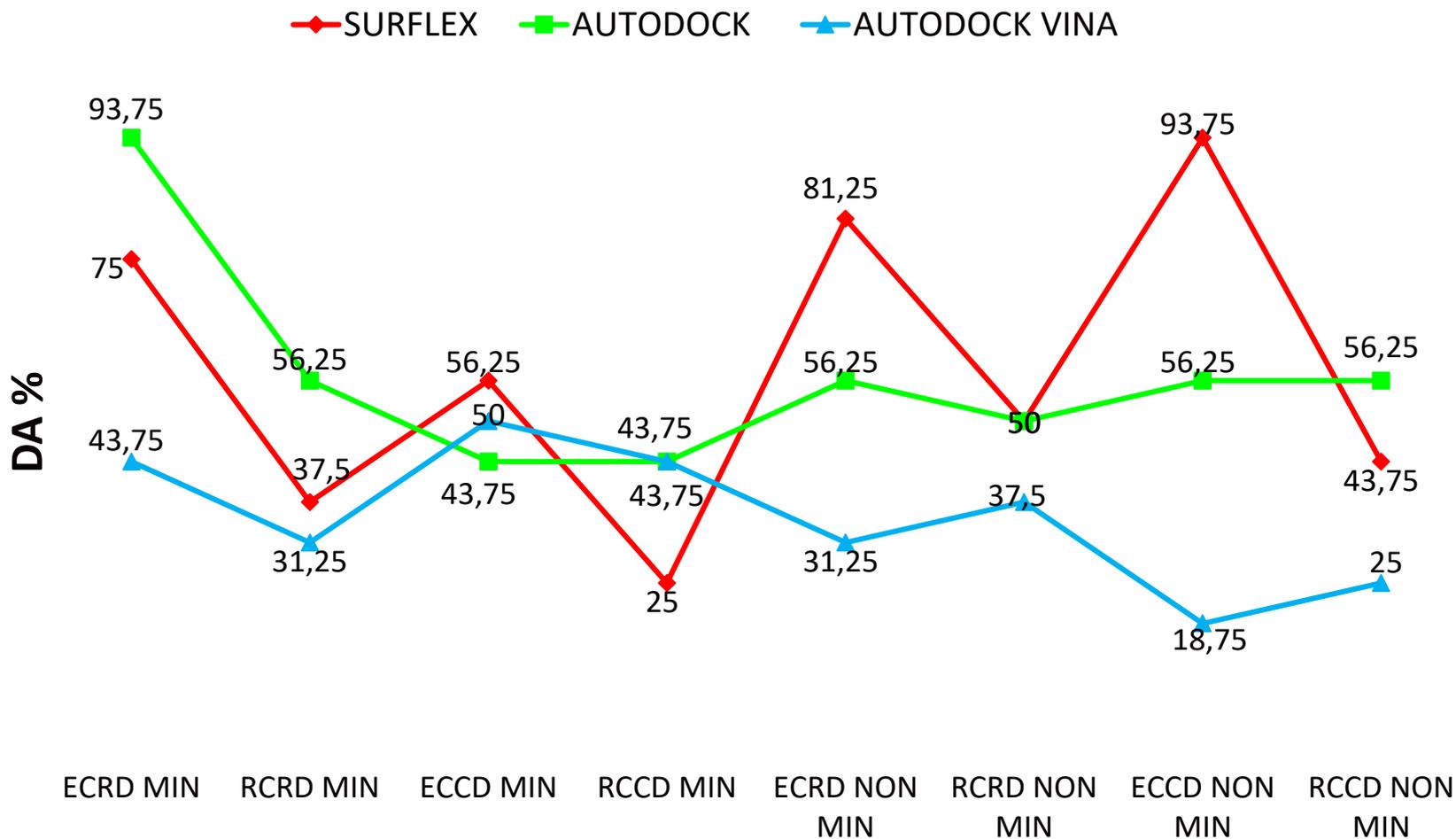
RCCD

■ NON MIN ■ MIN



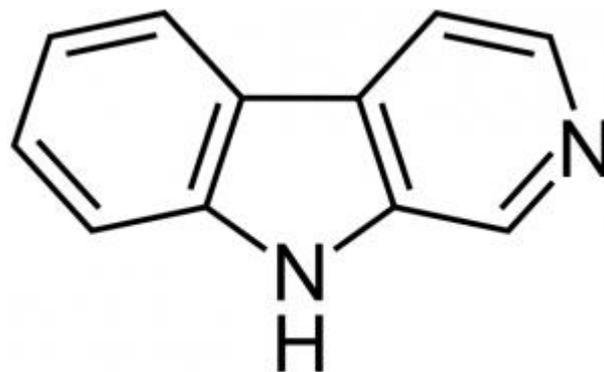
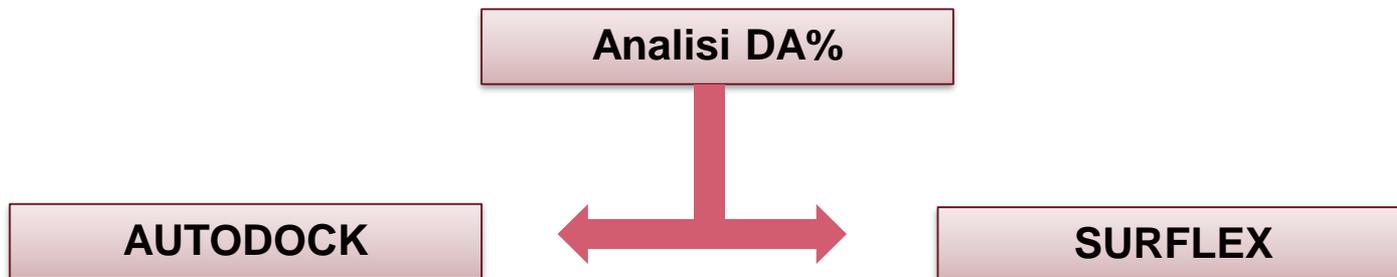


VALIDAZIONE METODO





APPLICAZIONE



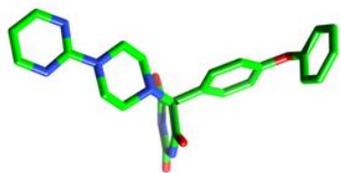
BETA-CARBOLINE



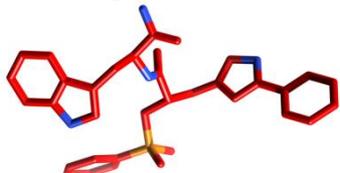


APPLICAZIONE DI DOCKING

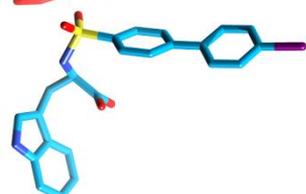
MMP-9 con attività nota



2ovx



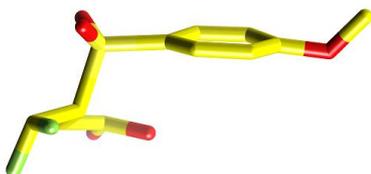
2ovz



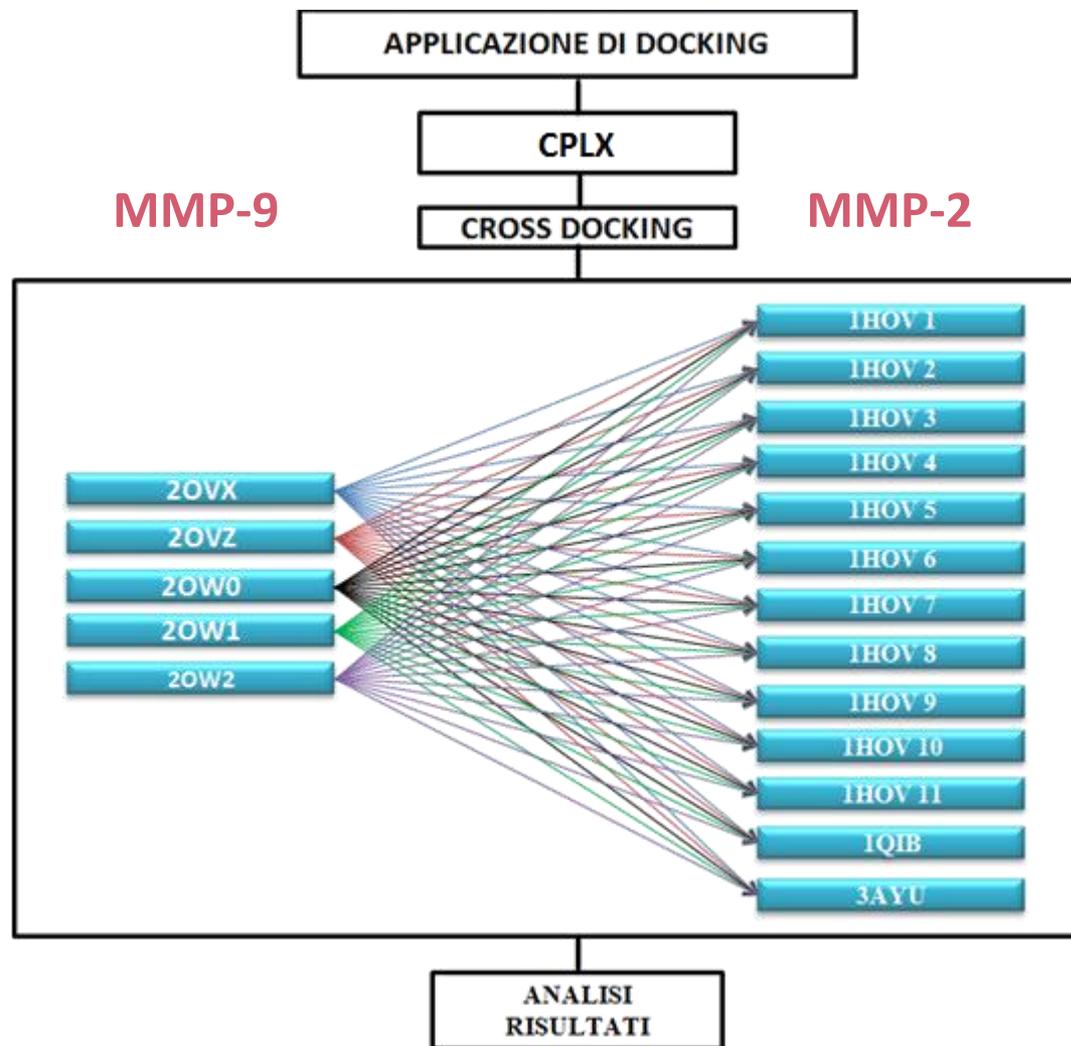
2ow0



2ow1



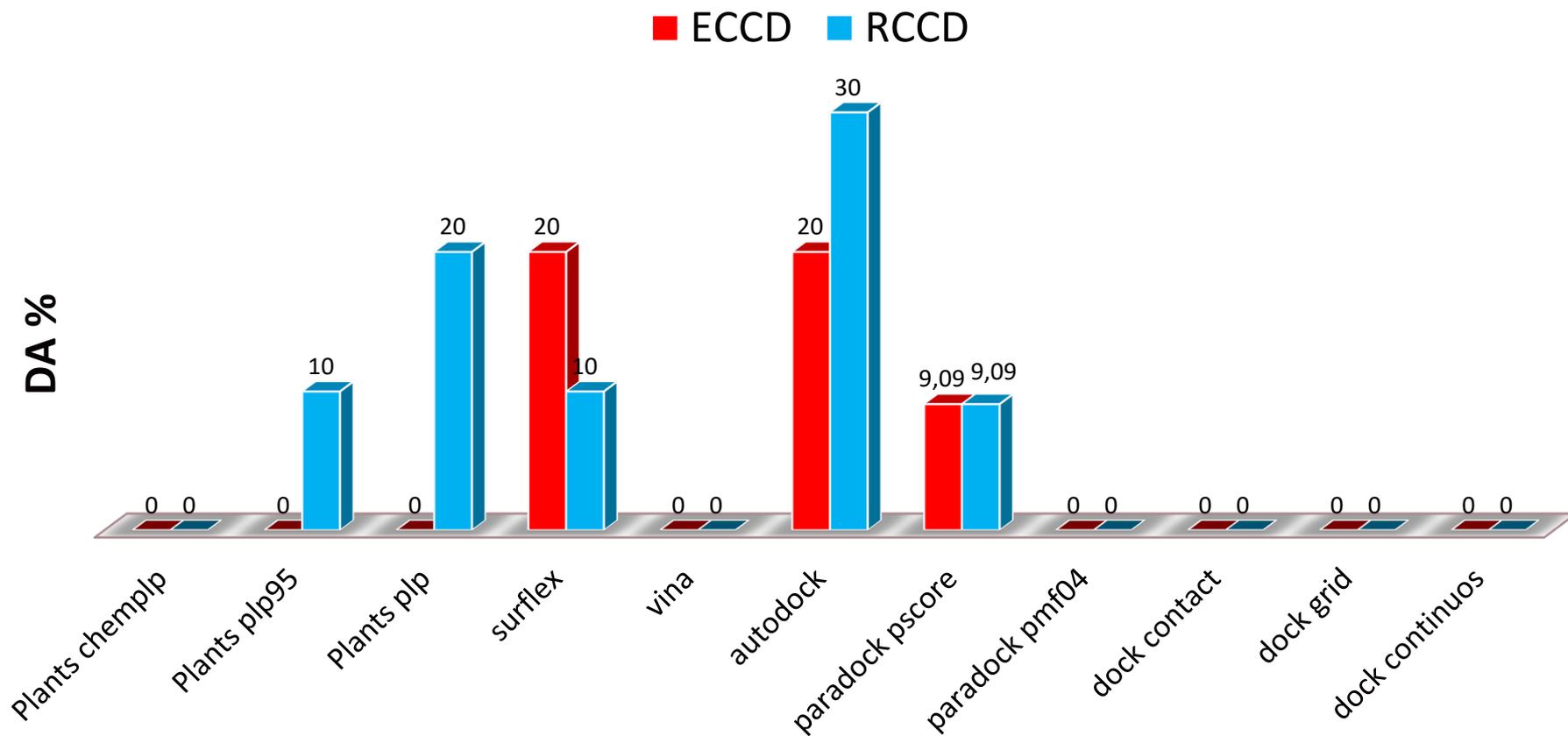
2ow2





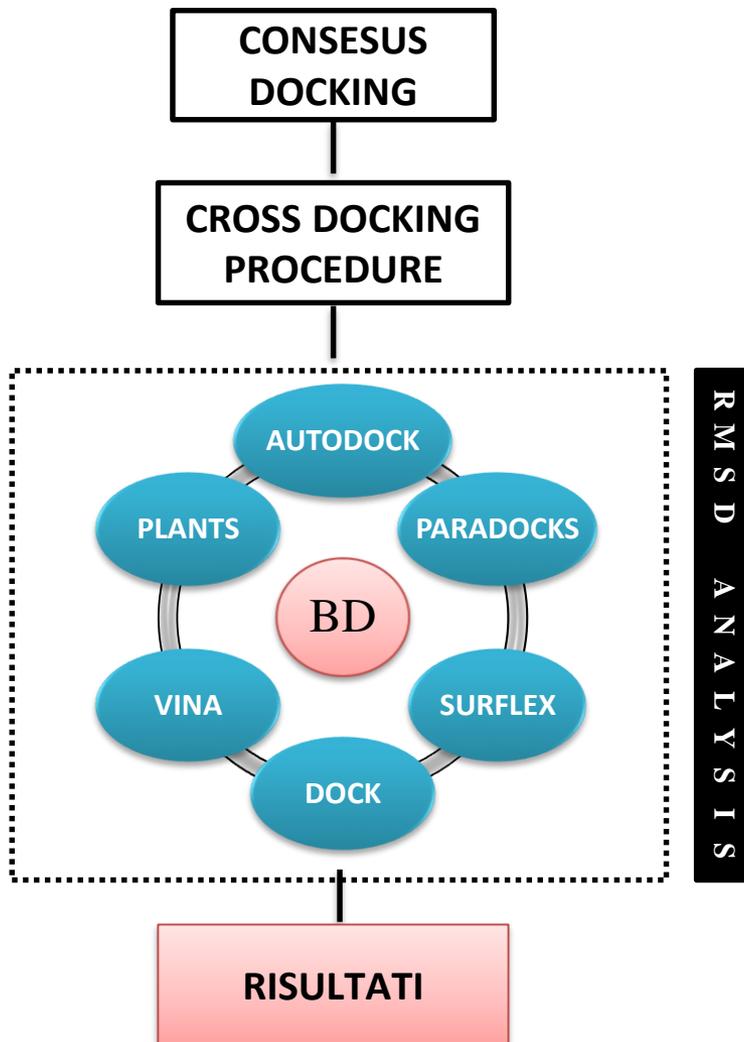
CROSS-docking

MMP9 MMP2





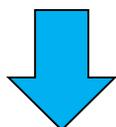
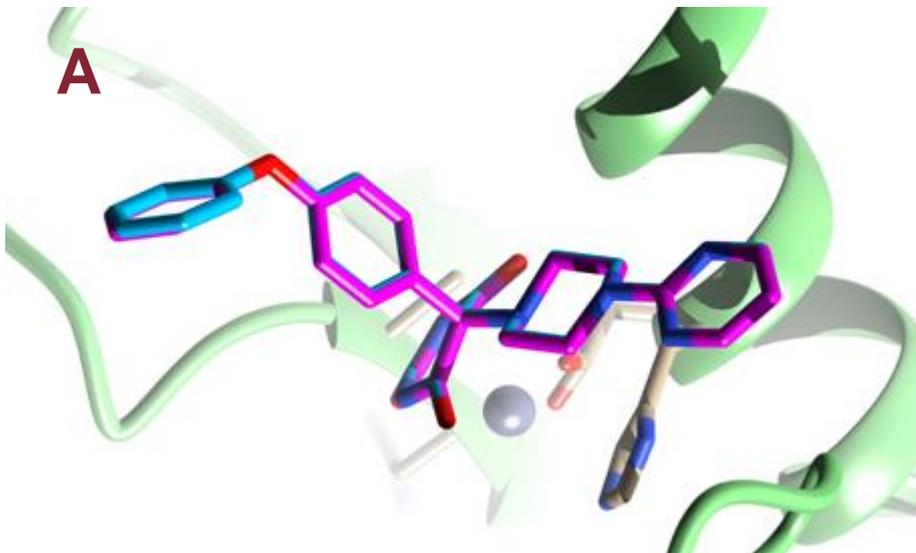
CONSENSUS-DOCKING





CONSENSUS-DOCKING

A

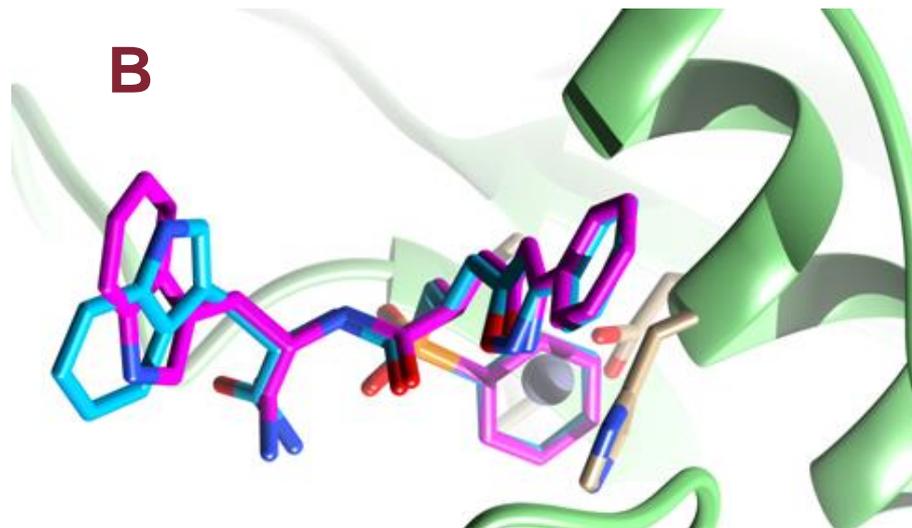


2OVX con i programmi Plants
plp95 e plp,
RMSD = 0,095.

2OVZ con i programmi
Plants plp95 e plp,
RMSD = 2,04.

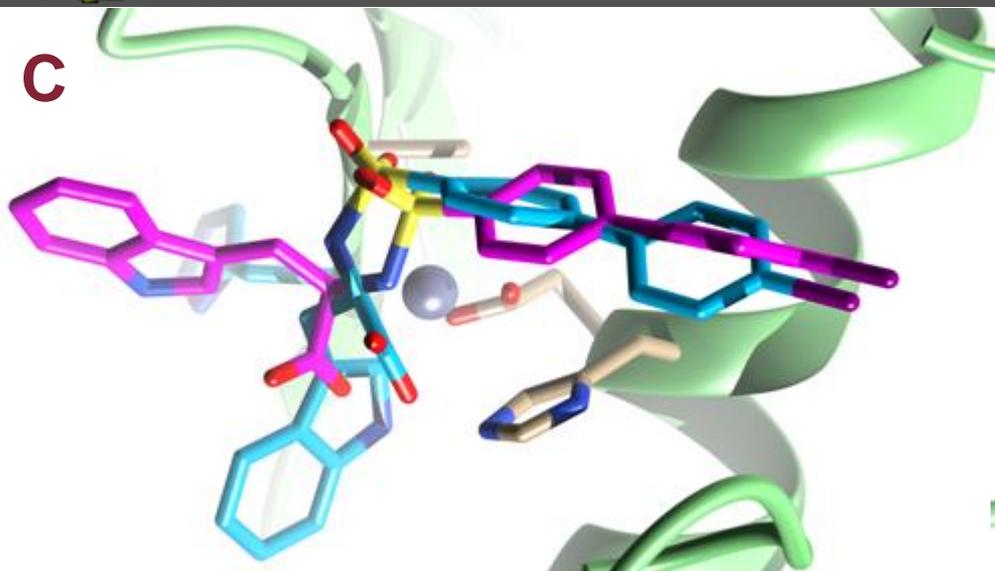


B

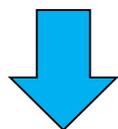




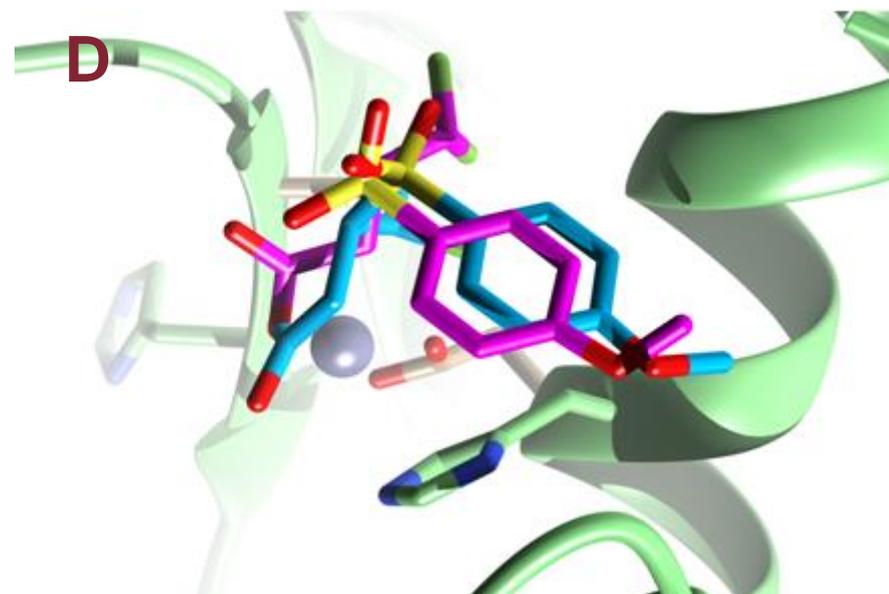
CONSENSUS-DOCKING



20W1 con i programmi
Plants chemplp e plp,
RMSD = 1,78

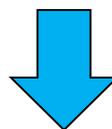
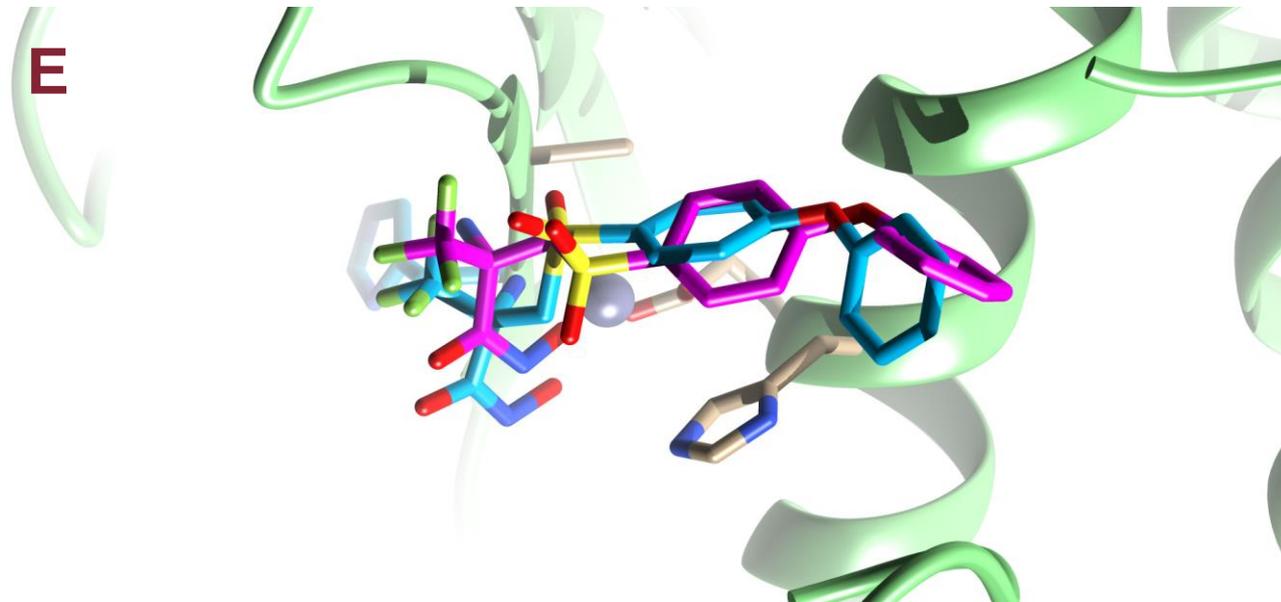


20W0 con i programmi
Plants plp95 e Autodock Vina,
RMSD = 1,48





CONSENSUS-DOCKING



2OW2 con i programmi
Surflex e Autodock,
RMSD = 2,56



3-D QSAR MMP-9

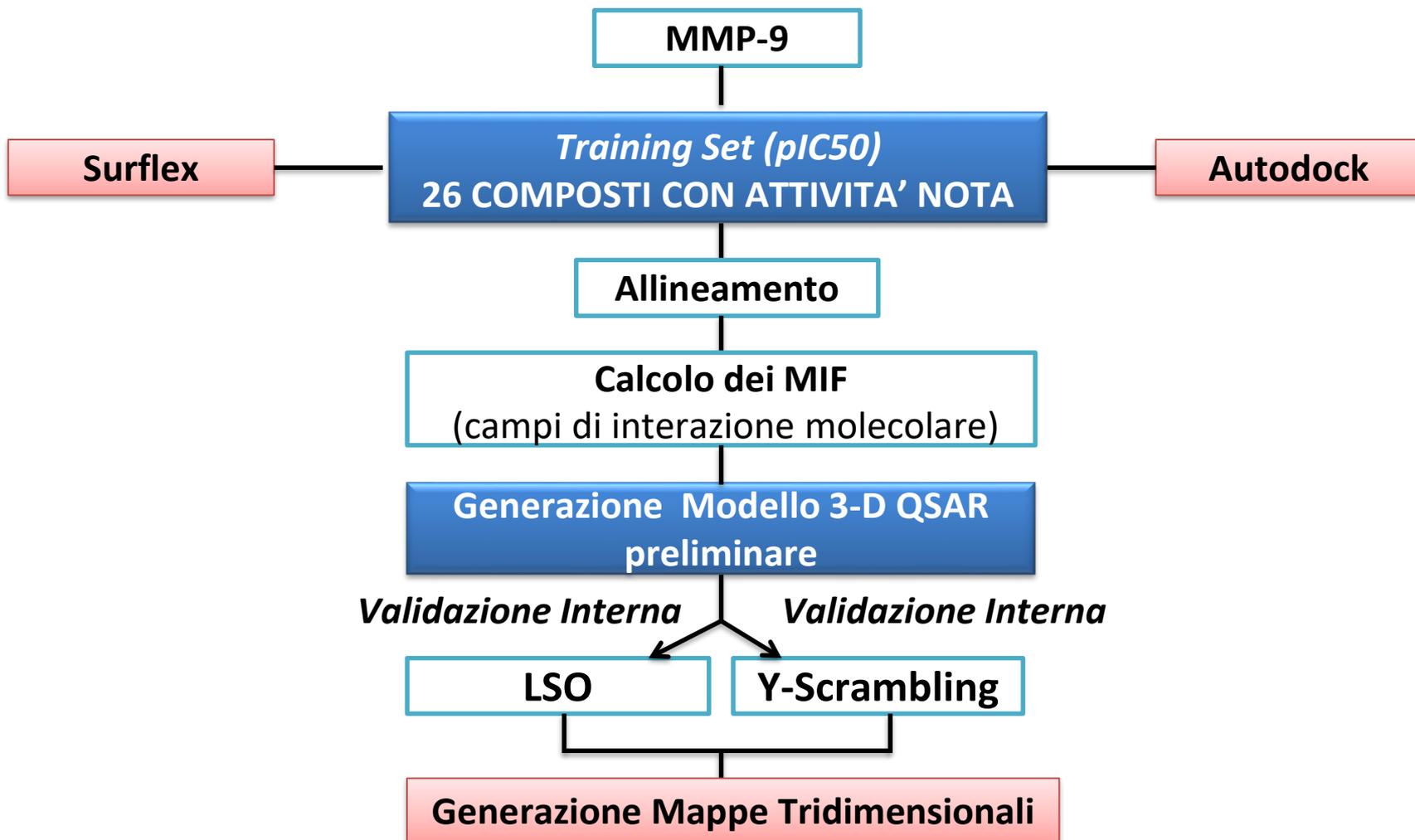




TABELLE 3-D QSAR

PROBE	PC	r^2	q^2 LSO	q^2 YS
A	2	0,792	0,086	-0,373
C	2	0,822	0,080	-0,358
d	3	0,543	0,053	-0,414
e	2	0,845	0,137	-0,438
HD	2	0,804	0,079	-0,376
N	2	0,793	0,078	-0,362
NA	2	0,793	0,078	-0,410
OA	2	0,795	0,091	-0,405

Tabella Surfex MMP9

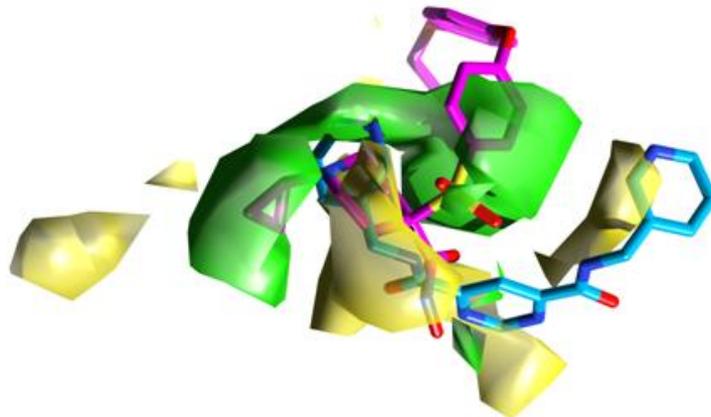
PROBE	PC	r^2	q^2 LSO	q^2 YS
A	1	0,404	0,036	-0,306
C	1	0,404	0,021	-0,371
d	1	0,287	0,124	-0,231
e	2	0,792	0,342	-0,393
HD	1	0,519	0,051	-0,349
N	1	0,418	0,020	-0,321
NA	1	0,407	0,017	-0,315
OA	1	0,439	0,005	-0,336

Tabella Autodock MMP9

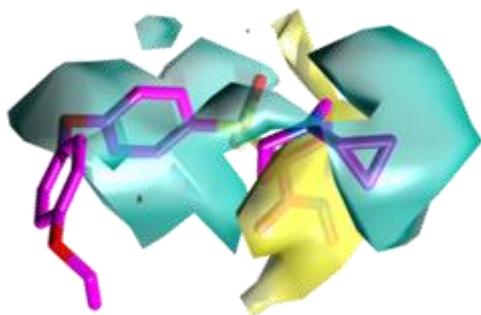


Surflex: Probe C

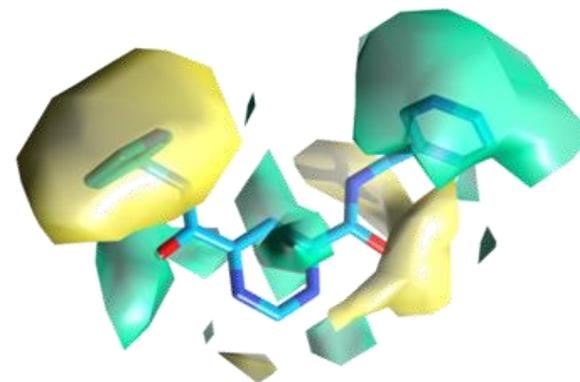
Comfa2



Activity Contribution Plot



Più Attiva

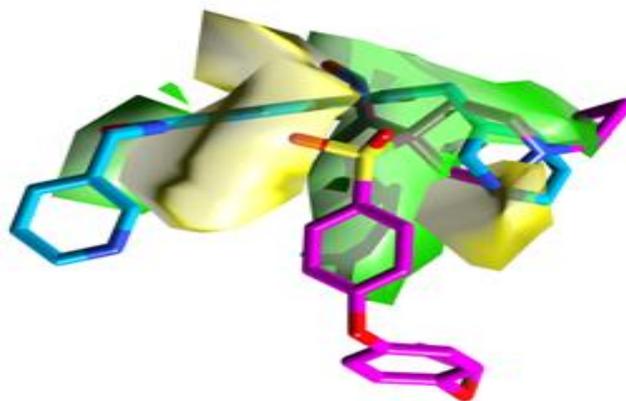


Meno Attiva

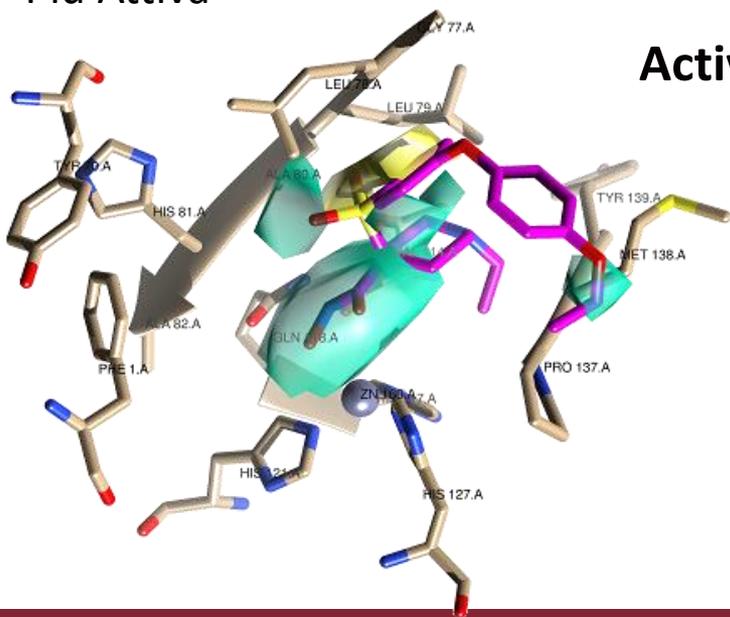


Surflex : Probe e

Comfa2

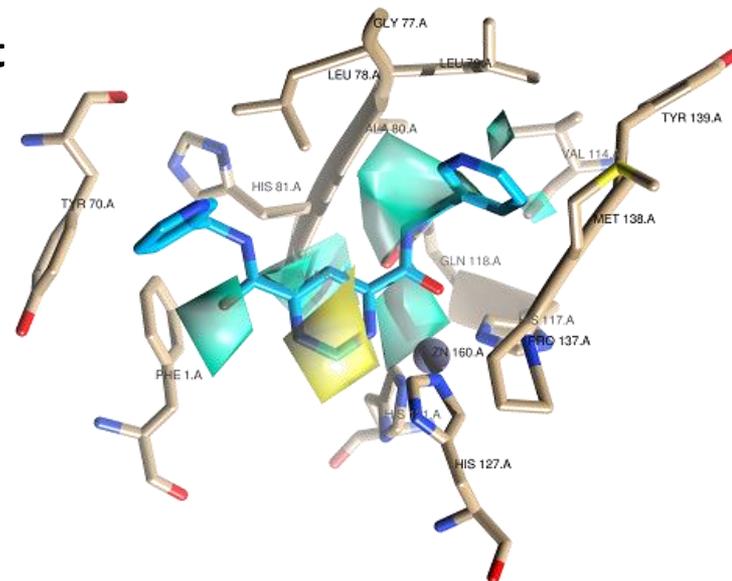


Più Attiva



Activity Contribution Plot

Meno Attiva





CONCLUSIONI

- Validazione della procedura di predizione del *binding mode* per un ligando capace di legare MMPs
- I programmi SURFLEX e AUTODOCK hanno dimostrato la migliore performance
- La procedura è stata usata per la progettazione di una nuova libreria di ligandi in collaborazione con il centro ricerche C4T (Tor Vergata) ma per motivi di segretezza non è stato riportato in questo lavoro
- Lo studio preliminare di 3-D QSAR ci ha dato informazioni sulla SAR per la progettazione di nuovi inibitori.



OBIETTIVI FUTURI

**AMPLIARE IL NUMERO
DI MOLECOLE DA
STUDIARE**

**CREARE MODELLI PIU'
GENERALIZZATI E
GLOBALI**

**PROGETTAZIONE DI
NUOVI INIBITORI**



GRAZIE PER L'ATTENZIONE

Quando si va verso un obiettivo, è molto importante prestare attenzione al cammino. È il cammino che ci insegna sempre la maniera migliore di arrivare, e ci arricchisce mentre lo percorriamo.

Il cammino di Santiago, Paulo Coelho