

Applicazione di metodiche computazionali ad inibitori delle proteine Bcl-2/Bcl-xL per lo sviluppo di modelli "Structure-Based"

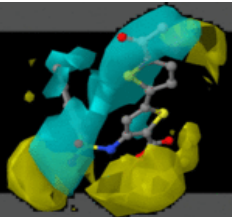


SAPIENZA
UNIVERSITÀ DI ROMA

Facoltà di Farmacia e Medicina
Corso di Laurea in Chimica e Tecnologie Farmaceutiche
Tesi Sperimentale in Chimica Farmaceutica
a.a. 2017/2018

Laureando: Sonia Vellucci
Matricola: 1380492

Relatore: prof. Rino Ragno

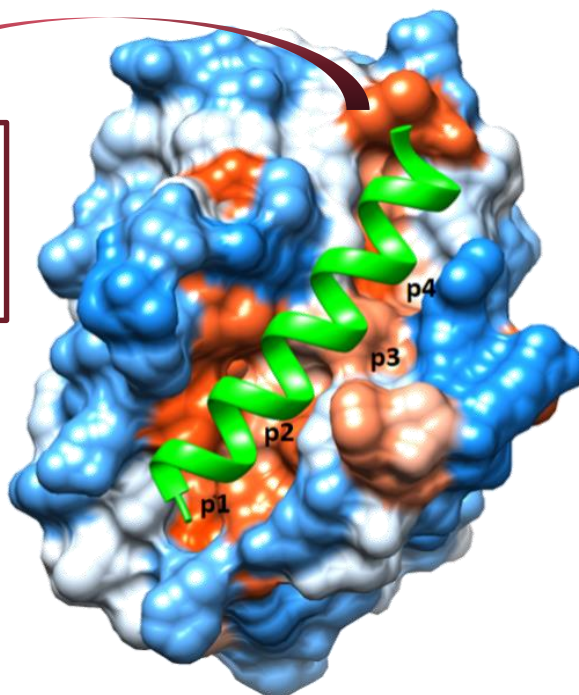


Bcl-2 : classificazione e struttura

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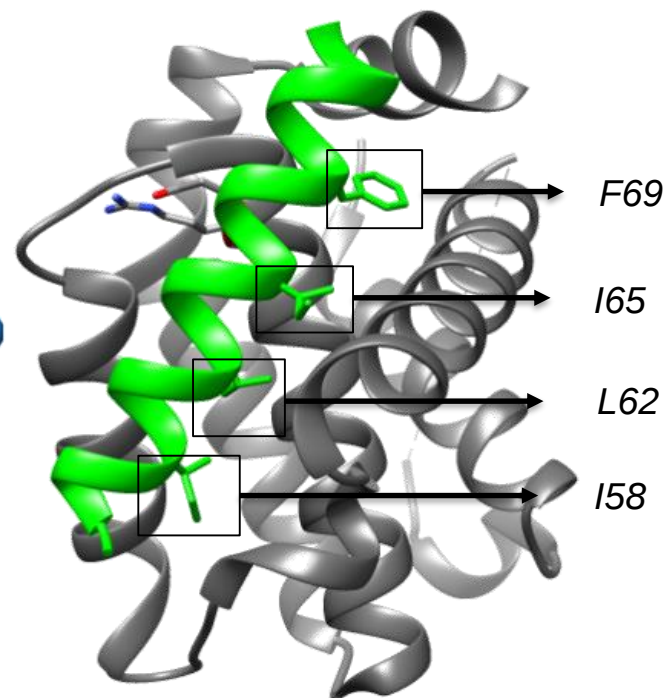
Proteine proapoptotiche:

Bax, Bak, Diva, Bcl-Xs,
Bim, Bik, Bad, Bid, Egl-1

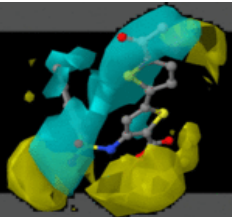


Proteine antiapoptotiche:

Bcl-2, Bcl-xL, Mcl-1, CED-9, A1, Bfl-1



Residui idrofobici conservati
nelle posizioni
i, i+4, i+7, i+11 dell' α -elica
BH3.

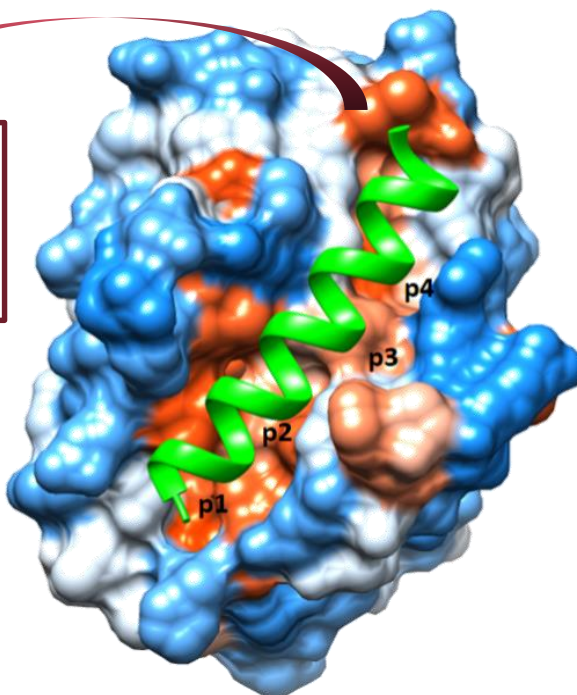


Bcl-2 : classificazione e struttura

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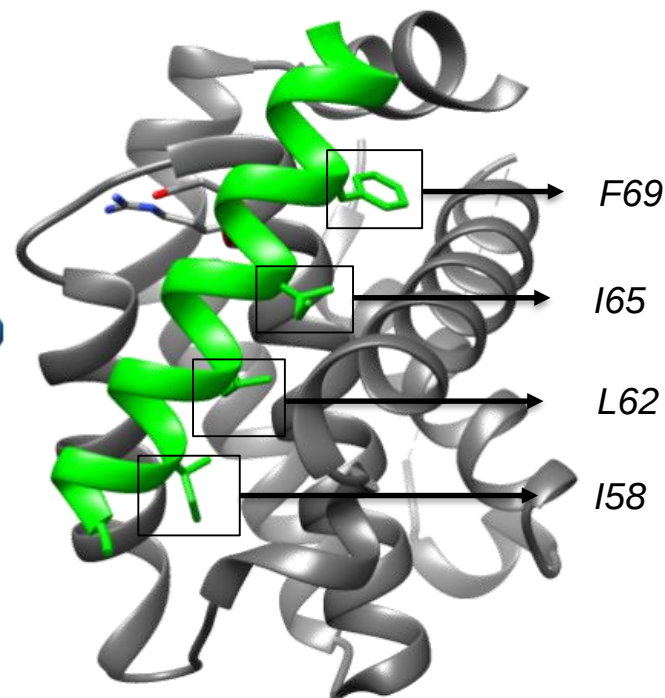
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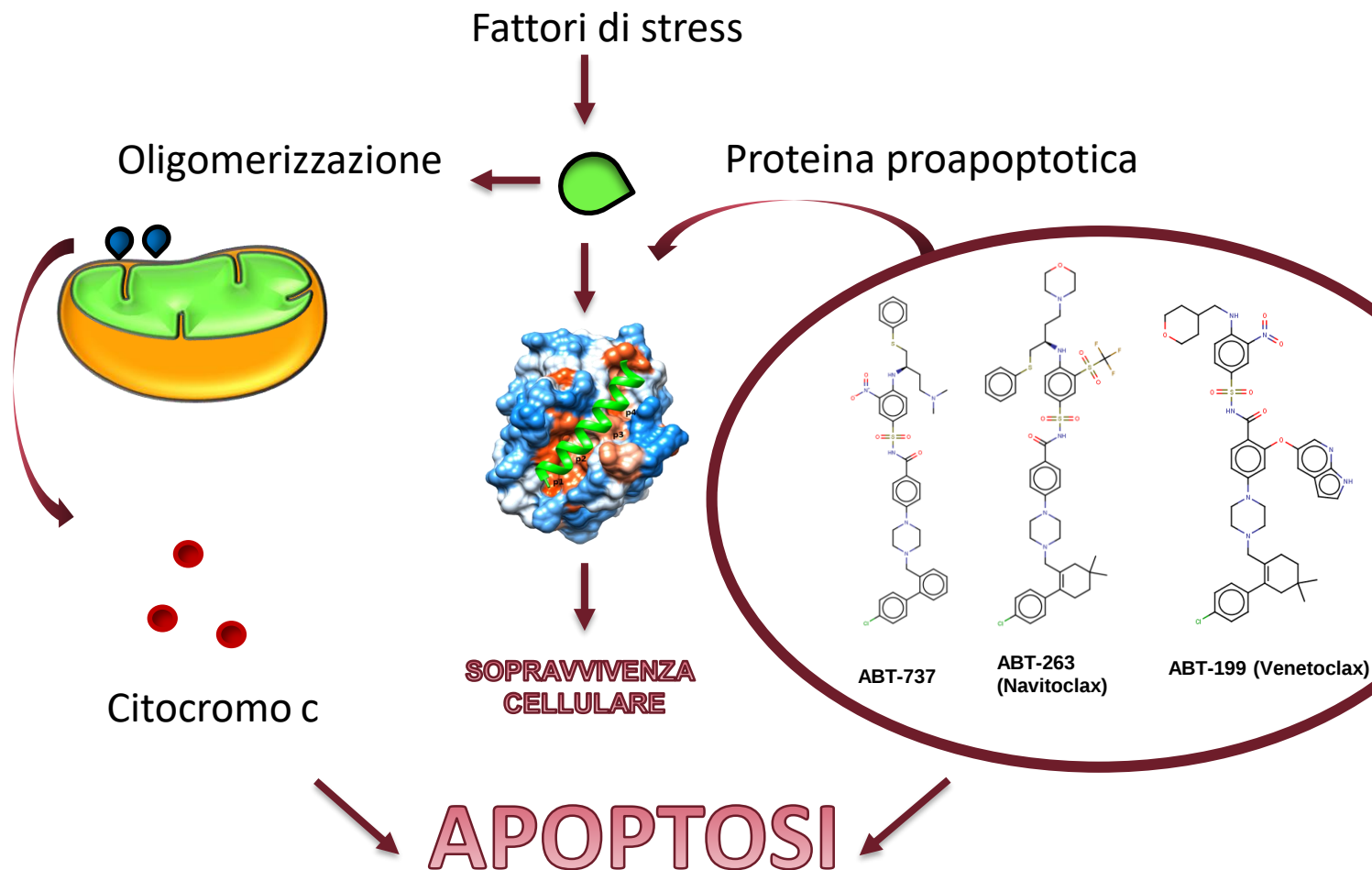


Residui idrofobici conservati
nelle posizioni
i, i+4, i+7, i+11 dell' α -elica
BH3.



Il ruolo delle Bcl-2

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Scopo

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AmberTools

Modello
matematico in
grado di predire
l'attività di inibitori
Bcl-2/Bcl-xL

OpenMM

MarvinSketch





Fasi del lavoro

by www.RCMD.it

Studio della
letteratura
relativa al
target

Preparazione
del training
set

Sviluppo del
modello
COMBINE

Preparazione
del test set
esterno

Validazione
del modello
COMBINE

Analisi dei
risultati



Screening e pulizia dei dati

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27 Bcl-xL
14 Bcl-2

Complessi iniziali

11 Bcl-xL
6 Bcl-2

Complessi aventi:

- Ligandi *small molecules* (non peptidici)
- Ligandi con atomi parametrizzabili
- Proteine umane
- Ligandi sottoposti a saggi enzimatici

8 Bcl-xL
5 Bcl-2

Complessi aventi:

- Ligandi saggiati su entrambe le proteine



Screening e pulizia dei dati

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27 Bcl-xL
14 Bcl-2

Complessi iniziali

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6 Bcl-2

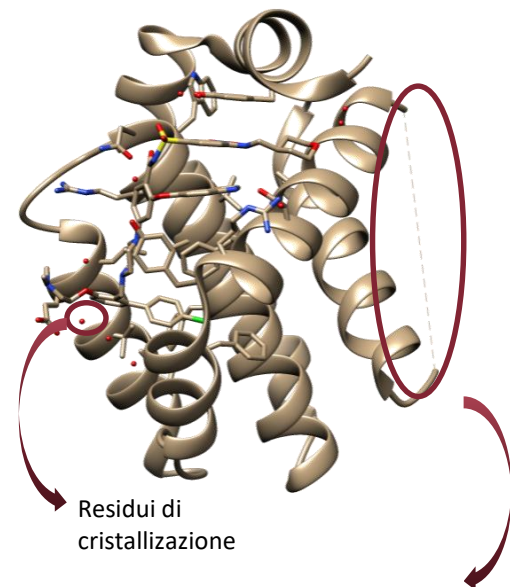
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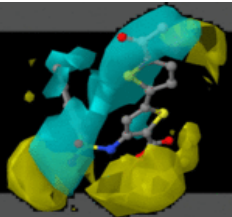
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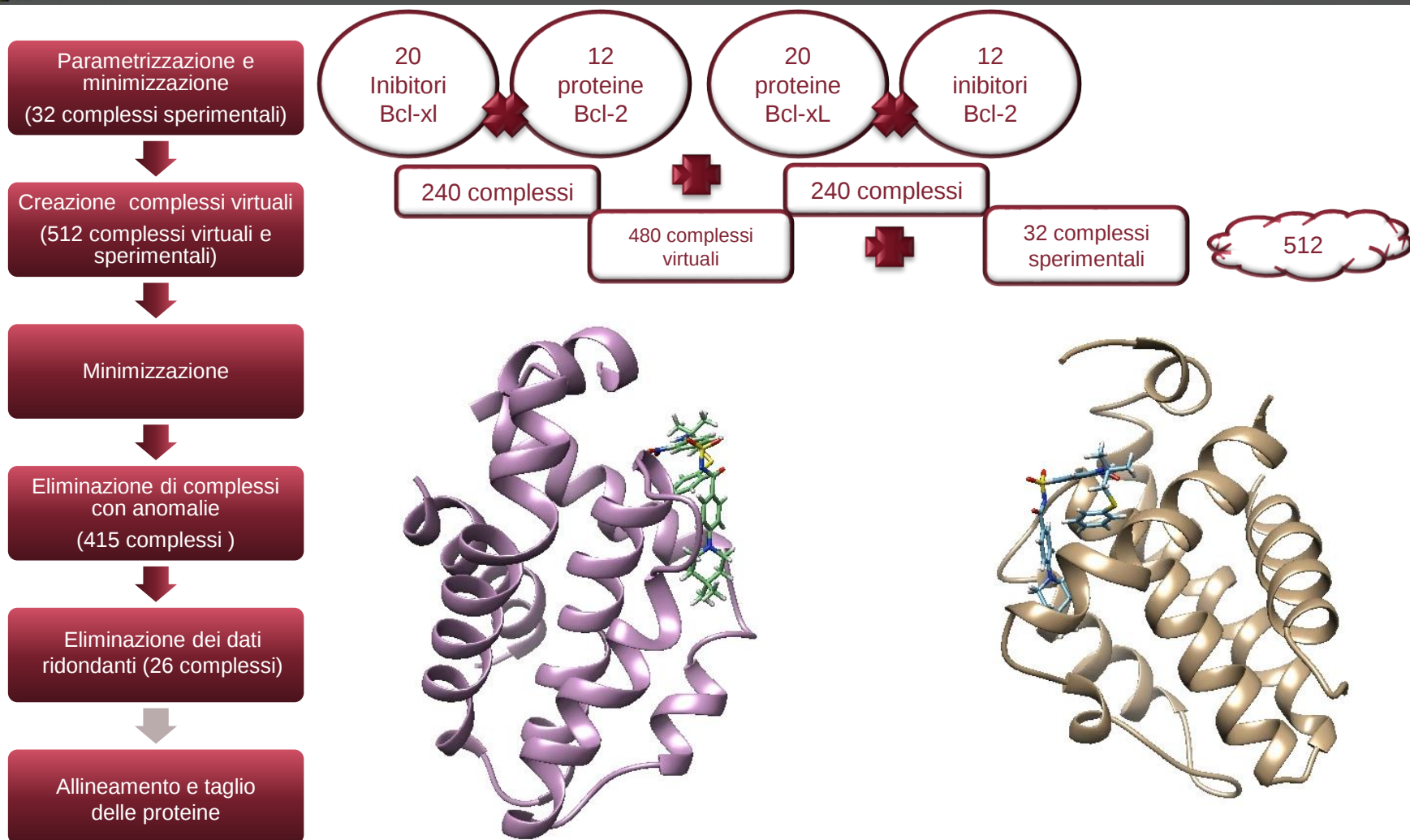
Modeller





Metodologia sperimentale

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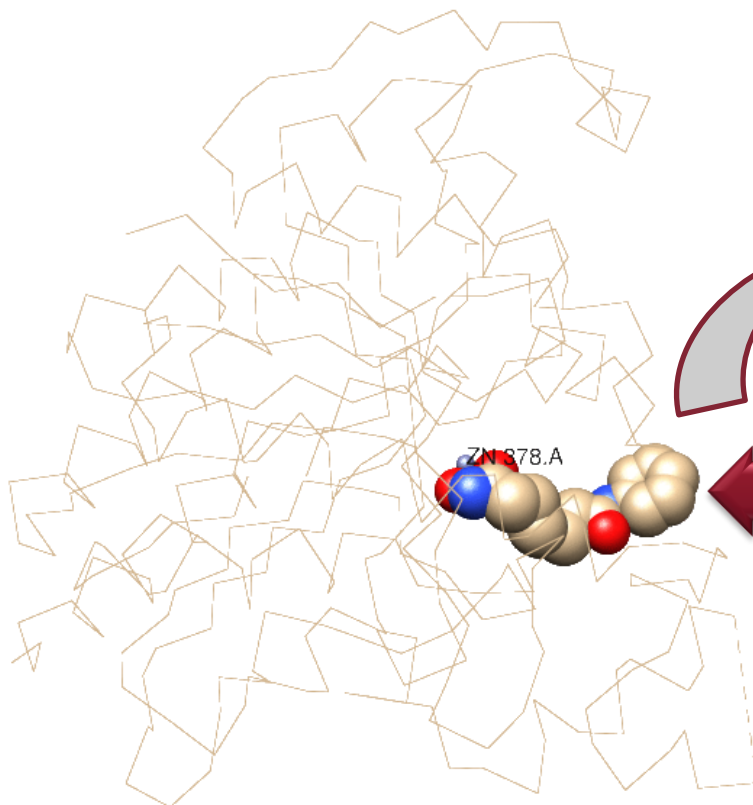
Journal of
Medicinal Chemistry

Subscriber access provided by UNIVERSITA DI ROMA LA SAPIENZA

Prediction of Drug Binding Affinities by Comparative Binding Energy Analysis

Angel R. Ortiz, M. Teresa Pisabarro, Federico Gago, and Rebecca C. Wade

J. Med. Chem., 1995, 38 (14), 2681-2691 • DOI: 10.1021/jm00014a020 • Publication Date (Web): 01 May 2002



SAHA

Diagram illustrating the PLS regression model structure. It shows a matrix of predictor variables (X) with columns Res1, Res2, Res3, ..., Res-n and rows Cplx 1, Cplx 2, Cplx 3, ..., Cplx m. The values in the X matrix are X1, X2, X3, ..., Xn. To the right of the X matrix is a vector of response variables (Y) with values Y1, Y2, Y3, ..., Ym. A double-headed arrow labeled 'PLS' connects the X matrix and the Y vector, indicating the relationship between the predictor and response variables in the PLS model.

PLS

COMBINE Model

$$\Delta U = \sum_{i=1}^{n_l} \sum_{j=1}^{n_r} u_{ij}^{\text{VDW}} + \sum_{i=1}^{n_l} \sum_{j=1}^{n_r} u_{ij}^{\text{ELE}}$$

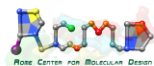


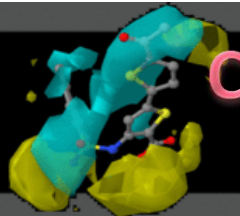
La generazione dei modelli COMBINE

by **RCMD**.it



Fields	r^2	q^2	$q^2(\text{YS})$	SA	Scaling	Max N Components	Score Func
STE.ELE.DRY	0.909 (5)	0.203 (5)	-0.28	False	True	8	LOO
STE.DRY	0.509 (1)	0.209 (1)	-0.409	False	True	8	LOO
STE.ELE.DRY.HB	0.707 (2)	0.101 (2)	-0.501	False	True	8	LOO
STE.ELE	0.873 (6)	0.293 (6)	-0.792	False	True	8	LOO
STE.HB	0.676 (3)	0.155 (3)	-0.956	False	True	8	LOO
STE	0.707 (3)	0.390 (3)	-1.032	False	True	8	LOO
STE.DRY.HB	0.626 (2)	0.119 (2)	-1.614	False	True	8	LOO
STE.ELE.HB	0.703 (2)	0.092 (2)	-1.775	False	True	8	LOO

Py-ComBinE by  ROSE CENTER FOR MOLECULAR DESIGN



Crossdocking: test set per la validazione del modello

by www.rcmd.it



23 inibitori



23 inibitori x 415 proteine

9.545 complessi



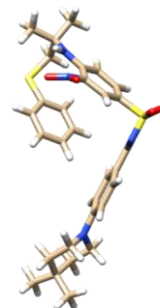
9.545 complessi x 20 conformazioni possibili di binding

190.900 complessi

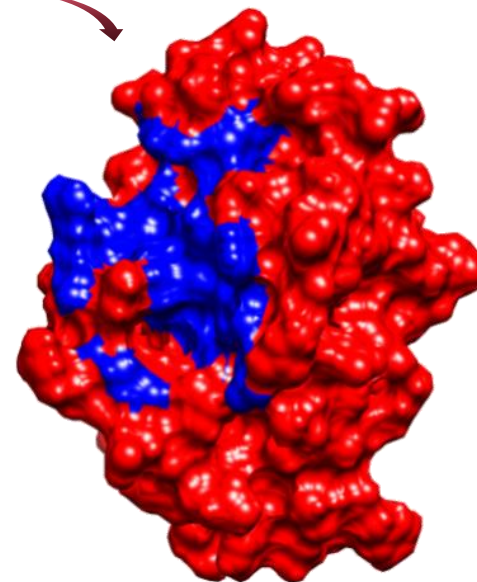


23 Bcl-xL

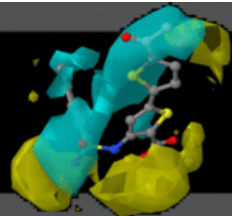
23 Bcl-2



ChemAxon



415 proteine



Scelta del modello COMBINE

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			ATTIVITA' PREDETTE							
N°	Proteina	Exp	STE.ELE.DRY	STE.DRY	STE.ELE.DRY.HB	STE.ELE	STE.HB	STE	STE.DRY.HB	STE.ELE.HB
18	Bcl-xl	10.50	11.42	10.62	10.87	10.71	10.17	11.37	11.07	10.20
1	Bcl2	9.50	10.50	8.74	9.01	9.94	8.95	9.42	8.29	9.11
1	Bcl-xl	9.50	9.10	8.38	9.17	9.30	9.11	9.65	9.01	8.99
2	Bcl2	9.41	10.43	9.19	9.23	8.64	8.67	8.68	8.40	9.00
3	Bcl2	9.31	9.68	8.97	8.79	8.24	8.88	8.71	8.55	8.72
3	Bcl-xl	9.31	10.29	7.94	8.21	9.63	8.40	9.16	8.03	8.41
2	Bcl-xl	9.23	7.33	8.38	8.73	6.84	8.91	8.10	8.51	8.94
4	Bcl-xl	9.22	9.51	8.24	8.16	9.46	8.25	9.27	8.17	8.04
19	Bcl-xl	9.10	9.66	9.40	9.37	9.13	9.39	10.27	9.20	9.14
21	Bcl-xl	9.01	12.70	8.57	8.95	11.92	8.46	10.23	8.67	8.58
5	Bcl-xl	8.76	8.47	7.68	7.93	8.98	8.64	9.47	7.61	8.54
4	Bcl2	8.70	8.58	7.78	8.01	8.20	8.15	8.03	7.50	8.37
5	Bcl2	8.67	5.58	6.01	6.14	6.16	6.24	5.99	5.93	6.35
6	Bcl2	8.62	7.43	6.63	6.89	7.54	6.99	7.72	6.71	7.07
15	Bcl-xl	8.40	8.60	8.67	8.24	9.15	8.78	10.13	8.87	8.19
7	Bcl-xl	8.36	9.11	8.74	8.76	9.89	8.76	10.33	8.56	8.57
7	Bcl2	8.34	9.69	8.86	8.82	9.98	8.91	9.77	8.63	8.54
8	Bcl2	8.31	4.12	5.26	5.29	4.59	5.59	5.64	5.27	5.86
9	Bcl2	8.29	8.52	8.37	8.42	9.43	8.80	9.46	8.26	8.56
10	Bcl2	8.25	7.97	7.59	6.82	7.53	7.98	9.22	7.59	6.81
9	Bcl-xl	8.20	9.40	9.22	9.08	9.84	8.97	10.18	9.03	8.82
11	Bcl2	8.10	7.29	8.33	8.69	7.67	9.25	8.96	8.48	9.00
12	Bcl2	8.10	7.67	8.60	8.03	8.41	8.75	9.70	8.61	7.58
13	Bcl2	8.00	5.11	5.21	5.42	5.11	5.90	6.42	5.33	6.22
12	Bcl-xl	7.70	9.50	9.77	9.84	9.73	9.89	9.94	9.53	9.71
14	Bcl2	7.68	7.41	8.33	8.82	8.48	9.04	9.15	8.34	9.03
16	Bcl-xl	5.15	7.16	6.63	6.83	7.31	6.96	7.98	6.88	7.01
22	Bcl2	4.51	6.79	7.57	7.35	7.04	7.65	8.35	7.49	7.70
23	Bcl2	4.51	4.51	5.00	4.97	5.22	5.14	5.61	5.04	5.62
23	Bcl-xl	4.03	7.52	7.74	7.35	7.41	7.33	8.35	7.75	6.92
			21.00	7.00	11.00	23.00	7.00	25.00	7.00	7.00
			9.00	3.00	5.00	7.00	3.00	7.00	3.00	4.00
			42.86	42.86	45.45	30.43	42.86	28.00	42.86	57.14

Percentuali di predizione



La selettività del modello STE.ELE.HB

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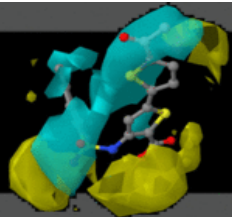
N°	Proteina	COMBINE		SELETTIVITA' Bcl-2/Bcl-xL	
		Exp	STE.ELE.HB	Exp	STE.ELE.HB
18	Bcl-xl	10.50	10.20	0.65	0.84
1	Bcl2	9.50	9.11	1.00	1.01
1	Bcl-xl	9.50	8.99	1.00	1.01
2	Bcl2	9.41	9.00	1.02	1.01
2	Bcl-xl	9.23	8.94	1.02	1.01
19	Bcl-xl	9.10	9.14	0.73	1.00
11	Bcl2	8.10	9.00	1.17	1.07
12	Bcl2	8.10	7.58	1.05	0.78
12	Bcl-xl	7.70	9.71	1.05	0.78
14	Bcl2	7.68	9.03	1.49	1.19
11	Bcl-xl	6.94	8.43	1.17	1.07
18	Bcl2	6.85	8.54	0.65	0.84
19	Bcl2	6.80	8.77	0.75	0.96
14	Bcl-xl	5.15	7.57	1.49	1.19



La selettività del modello STE.ELE.HB

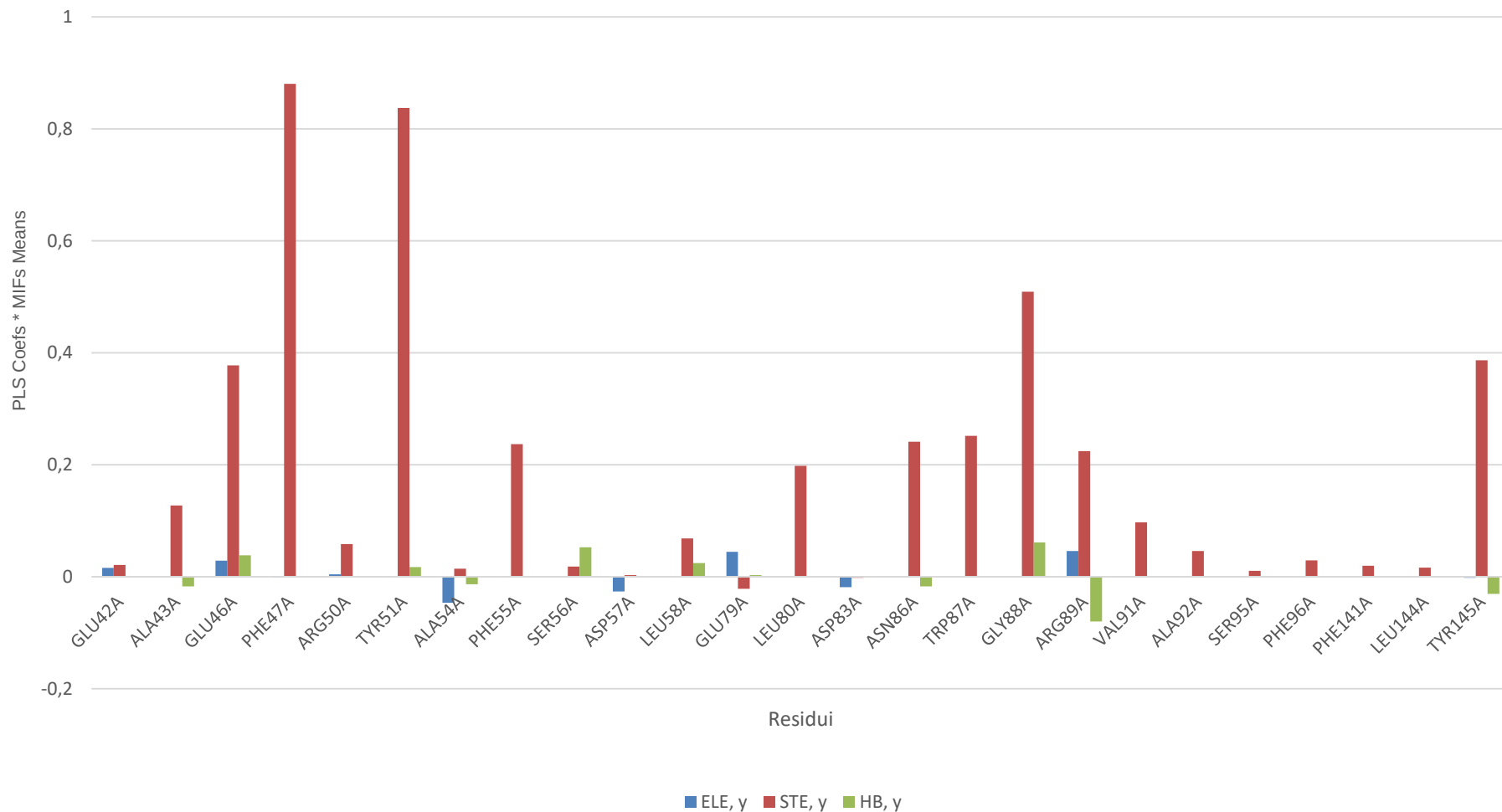
by www.RCMD.it

N°	Proteina	COMBINE		SELETTIVITA' Bcl-2/Bcl-xL	
		Exp	STE.ELE.HB	Exp	STE.ELE.HB
18	Bcl-xl	10.50	10.20	0.65	0.84
1	Bcl2	9.50	9.11	1.00	1.01
1	Bcl-xl	9.50	8.99	1.00	1.01
2	Bcl2	9.41	9.00	1.02	1.01
2	Bcl-xl	9.23	8.94	1.02	1.01
19	Bcl-xl	9.10	9.14	0.73	1.00
11	Bcl2	8.10	9.00	1.17	1.07
12	Bcl2	8.10	7.58	1.05	0.78
12	Bcl-xl	7.70	9.71	1.05	0.78
14	Bcl2	7.68	9.03	1.49	1.19
11	Bcl-xl	6.94	8.43	1.17	1.07
18	Bcl2	6.85	8.54	0.65	0.84
19	Bcl2	6.80	8.77	0.75	0.96
14	Bcl-xl	5.15	7.57	1.49	1.19



Contributo medio dell'attività

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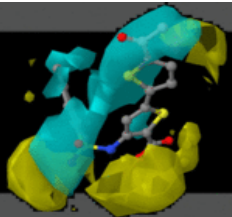


Selettività Bcl-2

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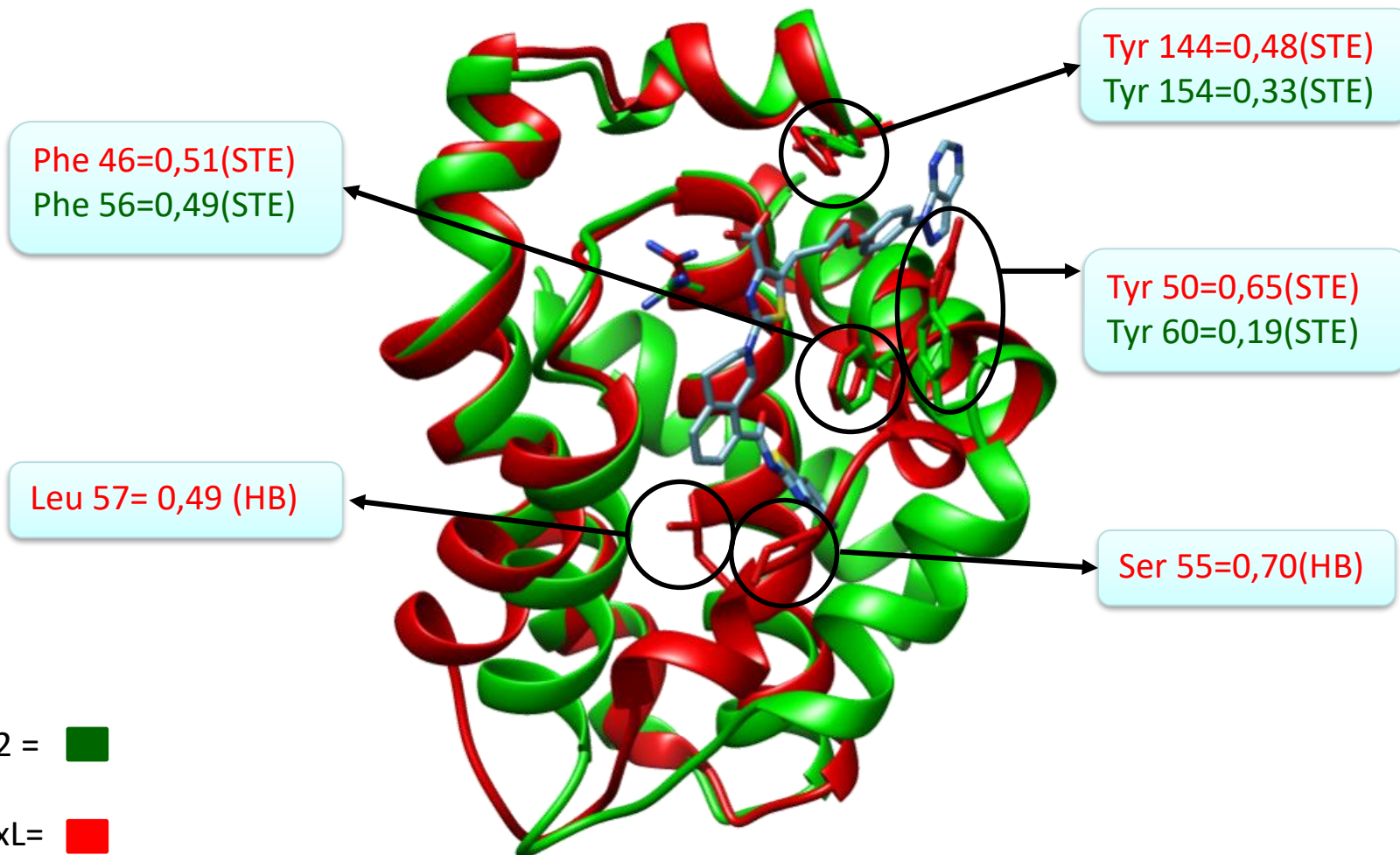
RESIDUI BCL-2	ELE	STE	HB
GLN51	0.00	0.03	0.00
ALA52	0.00	0.12	0.00
ASP55	-0.01	0.27	0.00
PHE56	0.00	0.49	0.00
ARG59	0.00	0.01	0.00
TYR60	0.00	0.19	0.01
ASP63	0.03	0.00	0.00
PHE64	0.00	0.18	0.00
GLU66	0.03	0.00	0.00
MET67	0.00	0.05	0.00
GLU88	-0.04	-0.02	0.00
LEU89	0.00	0.18	0.00
ASP92	0.21	0.00	0.00
ASN95	0.01	0.15	-0.09
TRP96	0.00	0.04	0.00
GLY97	0.00	0.26	0.00
ARG98	0.38	0.30	-0.33
VAL100	0.00	0.05	0.00
ALA101	0.00	0.04	0.00
PHE105	0.00	0.03	0.00
TYR154	0.00	0.33	0.00

RESIDUI BCL-XL	ELE	STE	HB
ALA42	0.00	0.10	0.00
GLU45	0.00	0.23	0.00
PHE46	0.00	0.51	0.00
TYR50	0.00	0.65	0.00
PHE54	0.00	0.39	0.00
SER55	0.00	0.05	0.70
ASP56	0.02	0.01	0.00
LEU57	0.00	0.09	0.49
GLU78	-0.04	-0.01	0.00
LEU79	0.00	0.18	0.00
ASP82	0.10	0.00	0.00
ASN85	0.01	0.12	-0.07
TRP86	0.00	0.05	0.00
GLY87	0.00	0.27	0.00
ARG88	0.32	0.28	-0.60
VAL90	0.00	0.07	0.00
ALA91	0.00	0.04	0.00
PHE95	0.00	0.03	0.00
LEU143	0.00	0.02	0.00
TYR144	0.00	0.48	0.00



Selettività Bcl-2

by www.RCMD.it



Bcl-2 = ■

Bcl-xL= ■

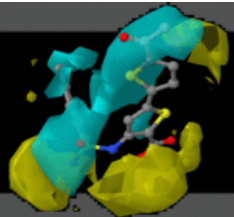


Selettività Bcl-xL

by www.RCMD.it

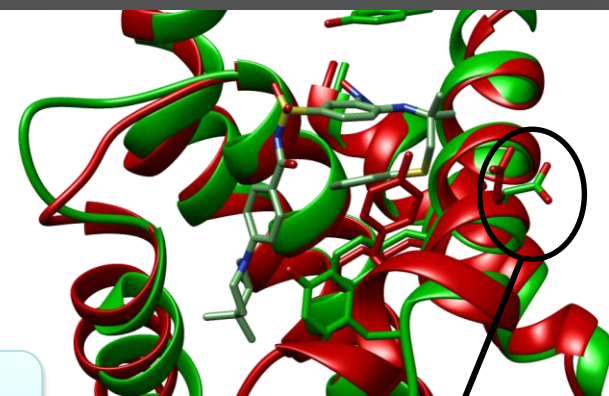
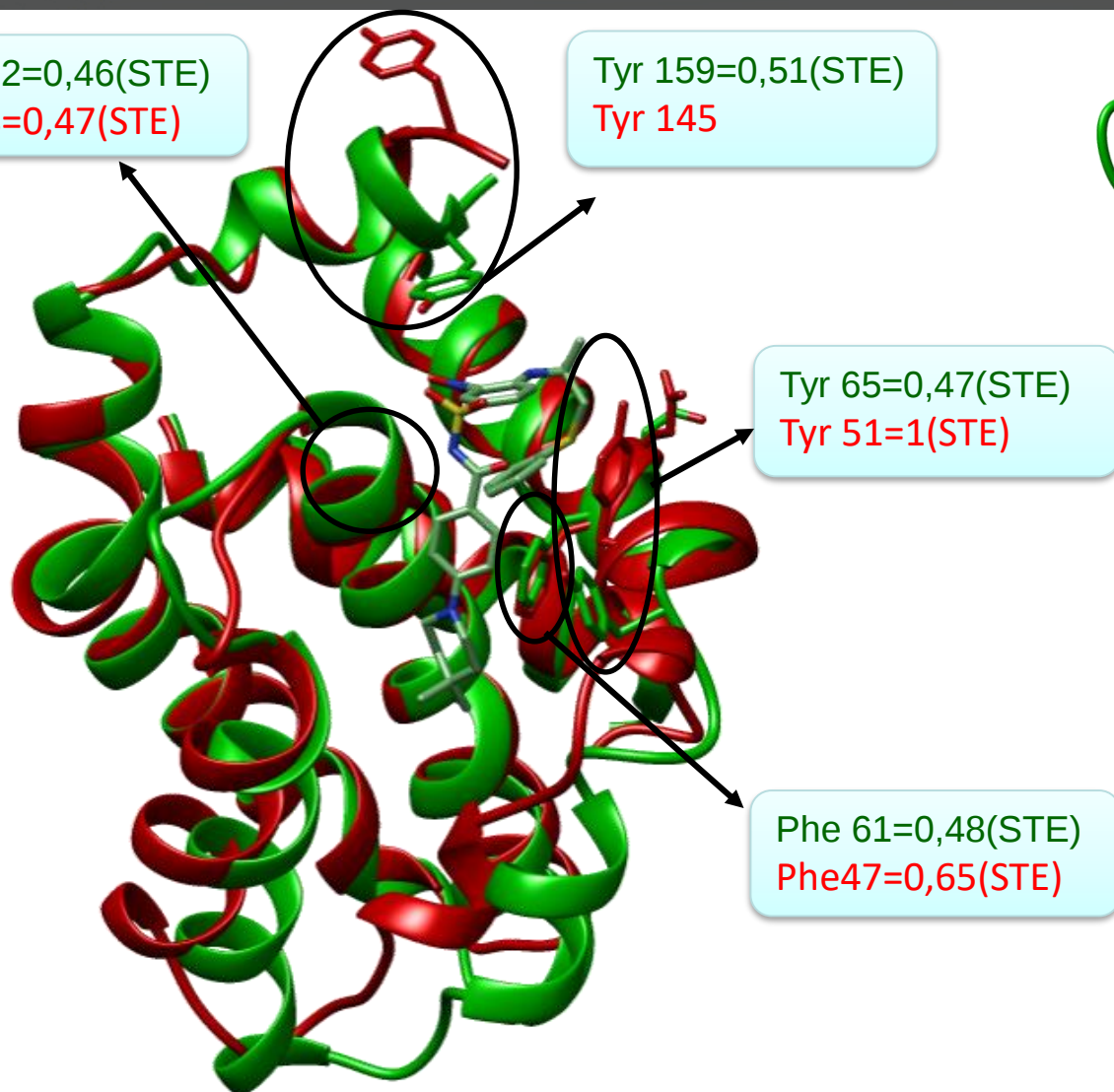
RESIDUI BCL-XL	ELE	STE	HB
GLU42A	0.00	0.01	0.00
ALA43A	0.00	0.14	0.00
GLU46A	0.00	0.37	0.00
PHE47A	0.00	0.65	0.00
ARG50A	0.00	0.01	0.00
TYR51A	0.00	1.00	0.00
PHE55A	0.00	0.16	0.00
SER56A	0.00	0.01	0.00
LEU58A	0.00	0.02	0.00
LEU80A	0.00	0.07	0.00
ASN86A	0.00	0.18	-0.02
TRP87A	0.00	0.22	0.00
GLY88A	0.00	0.47	0.00
ARG89A	-0.01	0.10	0.00
VAL91A	0.00	0.13	0.00
ALA92A	0.00	0.03	0.00
PHE141A	0.00	0.01	0.00

RESIDUI BCL-2	ELE	STE	HB
ALA57A	0.00	0.05	0.00
ASP60A	0.00	0.05	0.00
PHE61A	0.00	0.48	0.00
ARG64A	0.00	0.08	0.00
TYR65A	0.00	0.47	0.00
ASP68A	0.01	0.00	0.00
PHE69A	0.00	0.01	0.00
GLU93A	-0.01	0.00	0.00
LEU94A	0.00	0.06	0.00
ASN100A	0.00	0.13	0.00
TRP101A	0.00	0.32	0.00
GLY102A	0.00	0.46	0.00
ARG103A	-0.03	0.10	0.00
VAL105A	0.00	0.08	0.00
ALA106A	0.00	0.02	0.00
PHE155A	0.00	0.01	0.00
TYR159A	0.03	0.51	0.00



Selettività Bcl-xL

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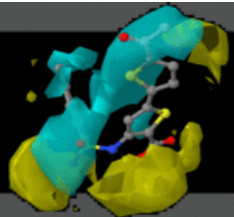
Asp 60
Glu 46=0,37(STE)

Bcl-2 =



Bcl-xL=



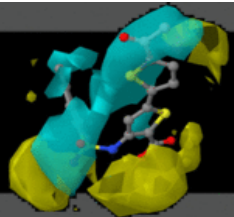


Conclusioni

by www.RCMD.it

- Sono stati elaborati modelli COMBINE attraverso il portale www.3d-qsar.com;
- Il docking con Plants ci ha permesso di creare il test set esterno per la validazione del modello;
- È stato possibile individuare un modello COMBINE in grado di predire l'attività e la selettività dell'inibitore.





Prospettive future

by www.RCMD.it

Il modello potrà essere utilizzato per progettare inibitori potenti e selettivi. Questo potrà permettere la creazione di nuovi farmaci con minori effetti collaterali.

