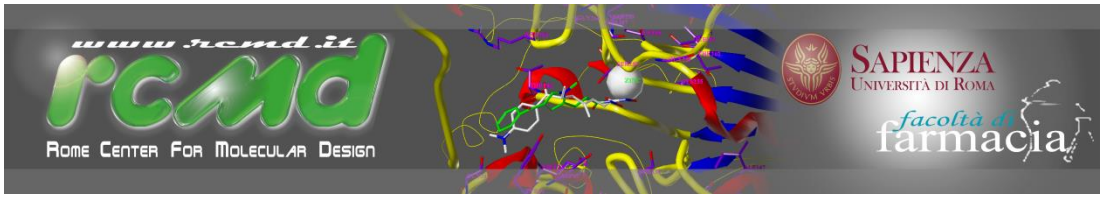


Beta-Secretasi: Relazioni Quantitative Struttura-Attività di Tipo Tridimensionale (3-D QSARs) e Valutazione della Capacità Predittiva

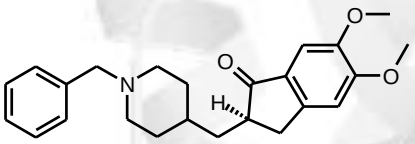
Relatore: Dr. Rino Ragno

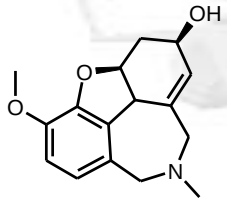
Laureando: Alberto De Petris

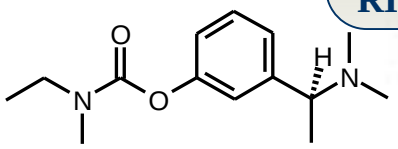
La Malattia di Alzheimer (Alzheimer Disease)

"Progressiva perdita della memoria e della capacità cognitiva accompagnata da afasia." Alois Alzheimer (1906)

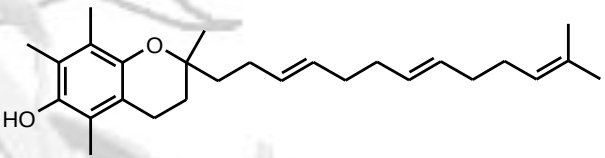
AD: terapia attuale

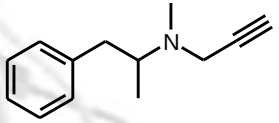






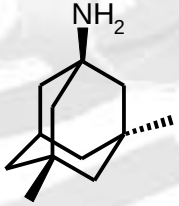
INIBITORI DELL'ACETIL-COLINESTERASI
Es. DONEZEPIL, GALANTAMINA, RIVASTIGMINA





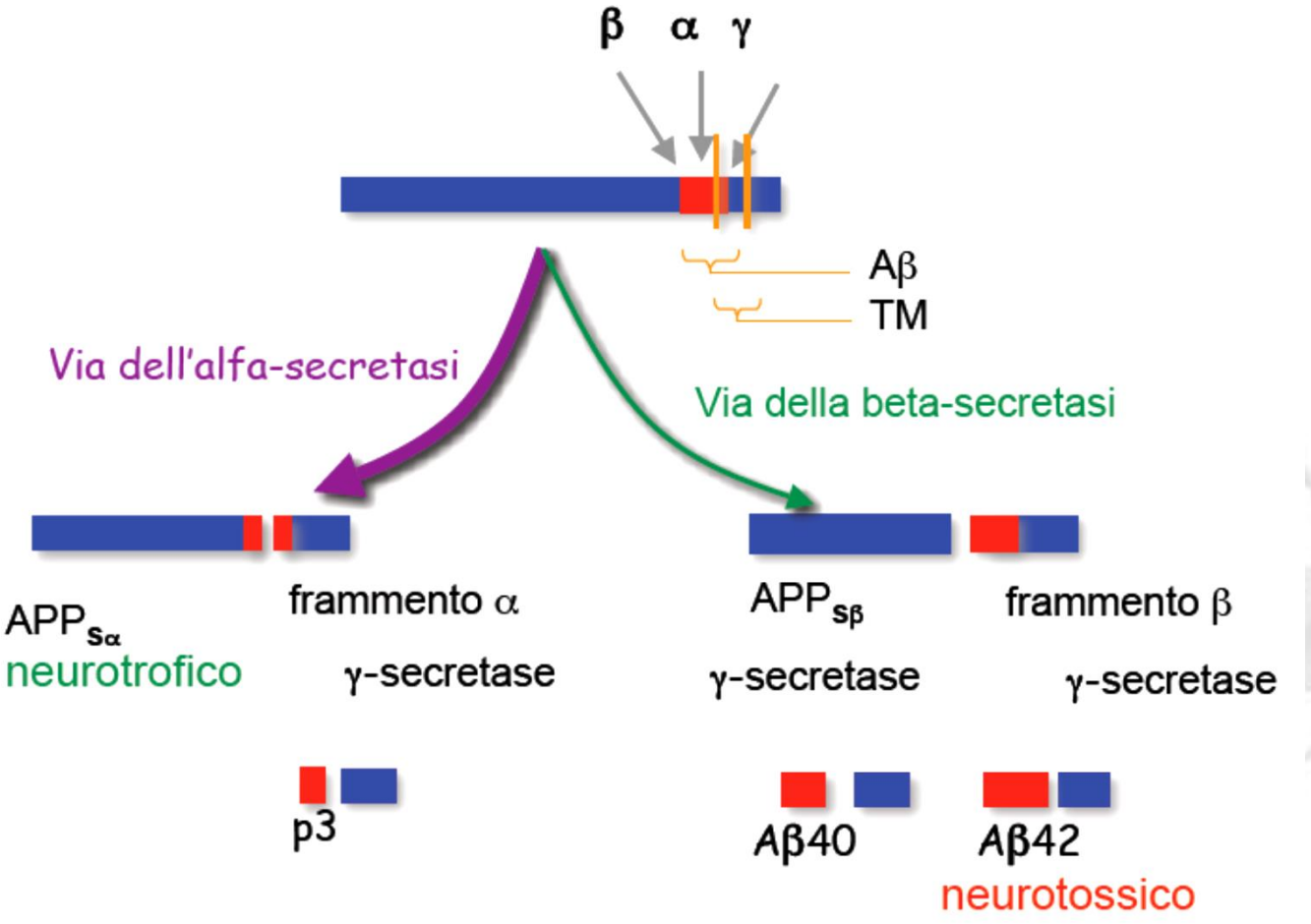
ANTIOSSIDANTI
Es. SELEGILINA, VITAMINA E

MEMANTINA
FDA 2003

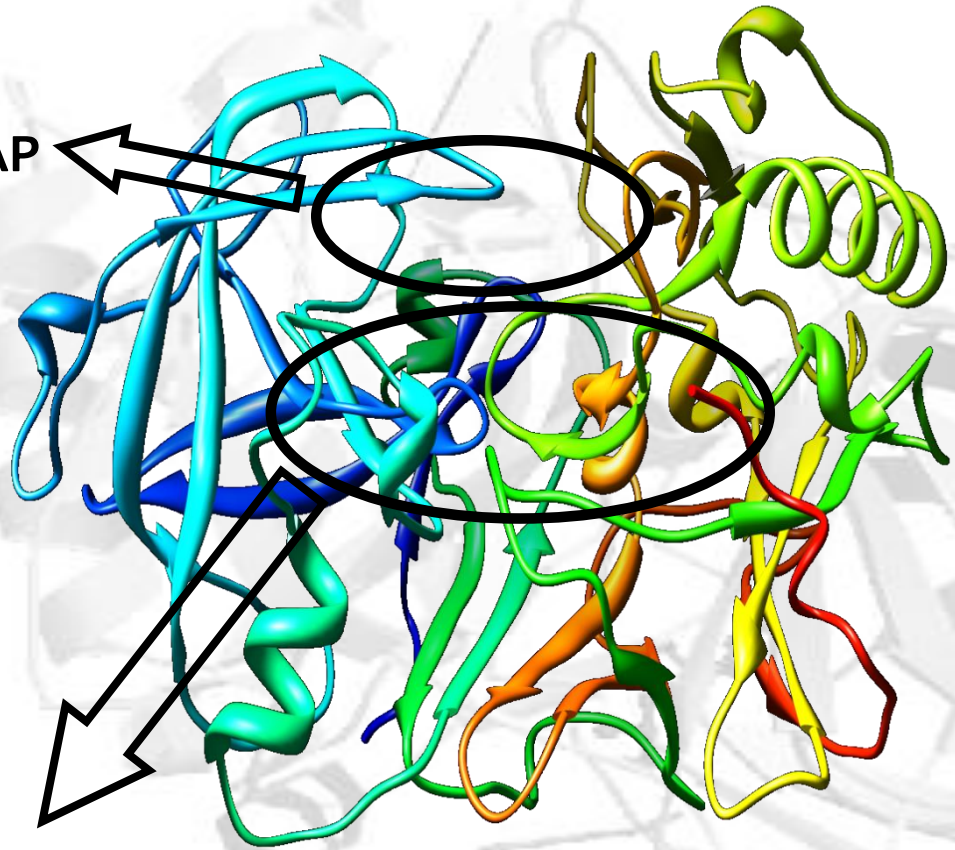


NECESSITÀ DI NUOVI INIBITORI POTENTI

BACE-1 *(β site of APP cleaving enzyme)*

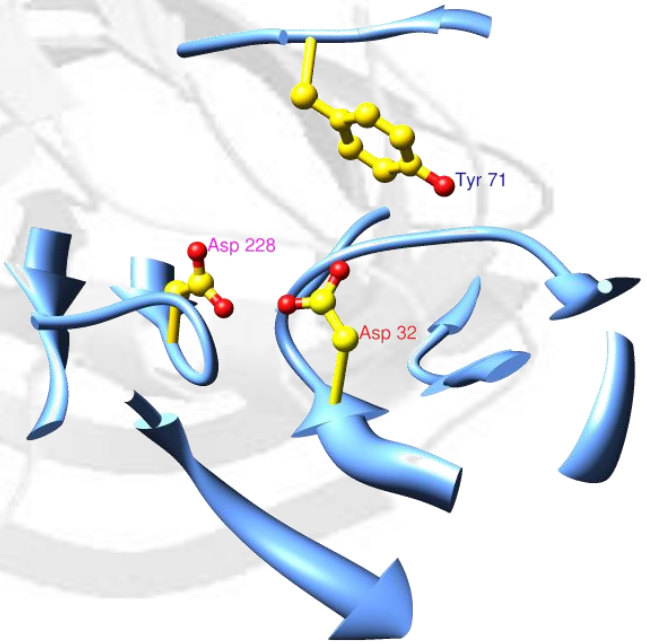


BACE-1



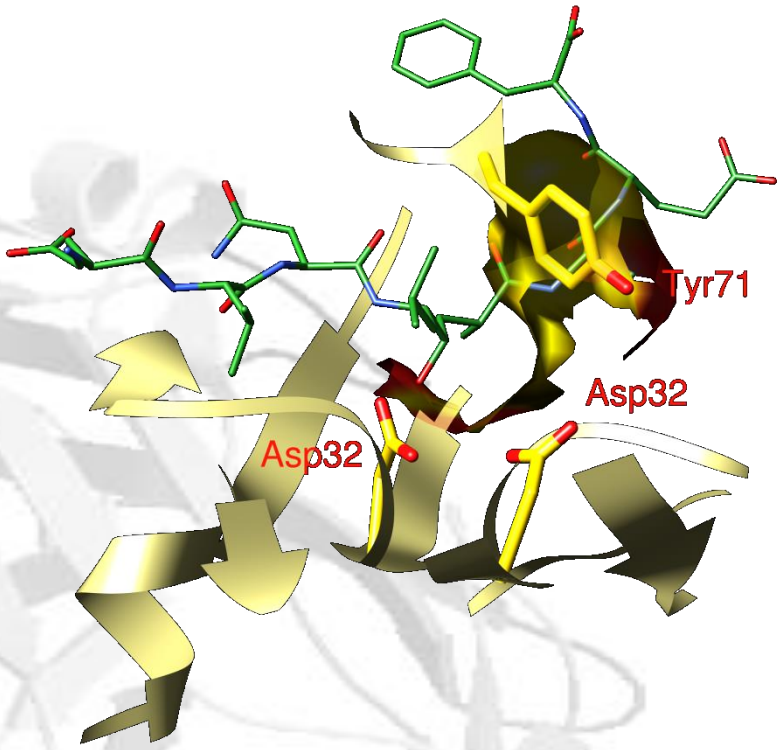
Polipeptide (391 residui)

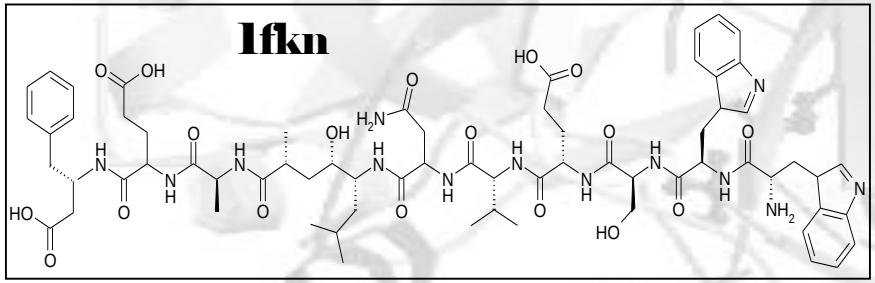
BACE-1
sito attivo

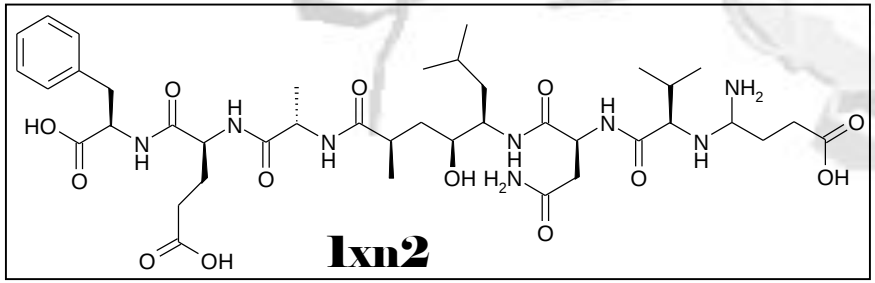


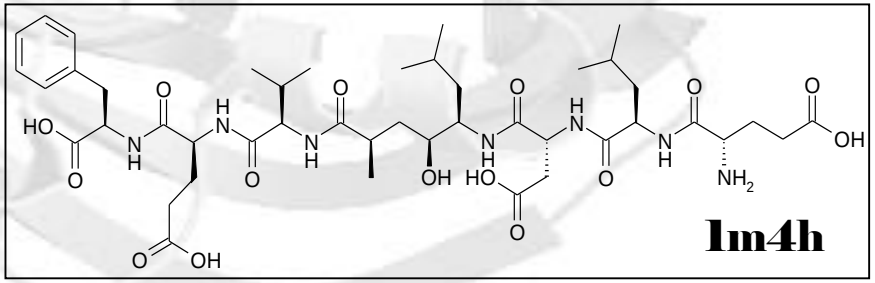
Inibitori Pseudopeptidici ad alto Peso Molecolare

- **AGISCONO SUL SITO ATTIVO IN MODO COMPETITIVO**
- **MIMANO LA STRUTTURA PEPTIDICA DELL'APP**
- **INIBIZIONE DEL TAGLIO PROTEOLITICO DELLA β-secretasi**



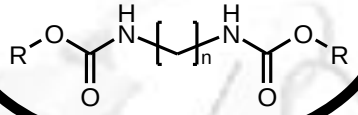




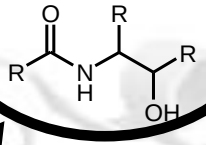


Inibitori Pseudopeptidici di medio Peso Molecolare

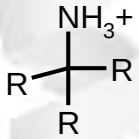
Derivati uretanici



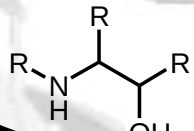
Derivati ammidici



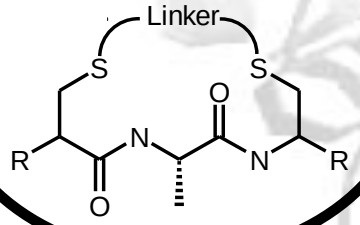
Derivati amminici



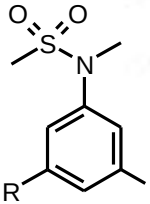
Derivati idrossietilamminici

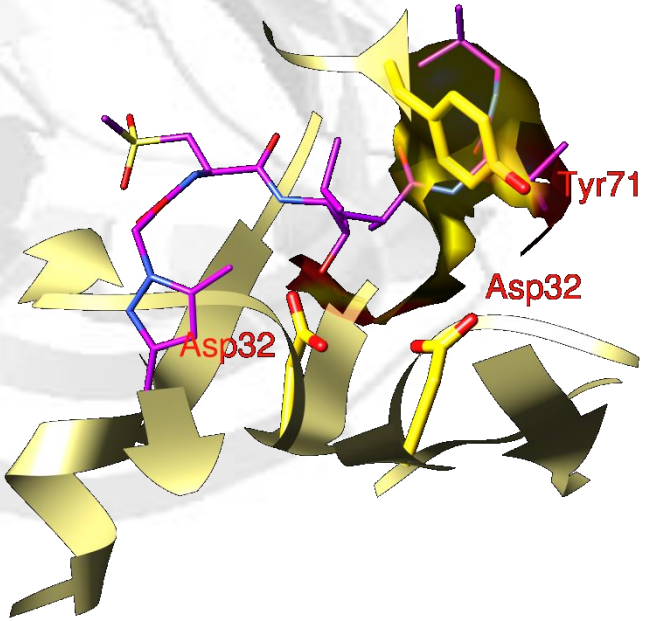


Macroeterocicli



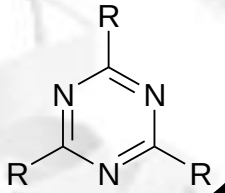
Derivati Solfonammidici



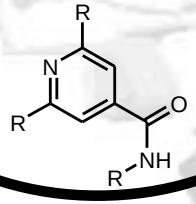


Inibitori Organici Azotati di Sintesi

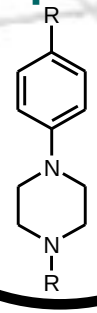
Derivati triazinici



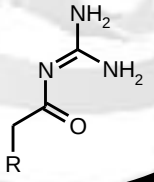
Derivati isonicotinamidici

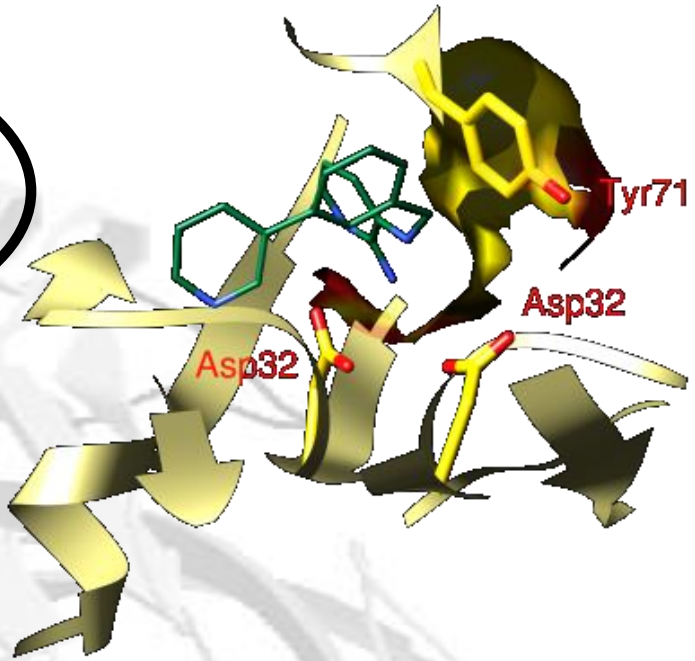


Derivati Fenilpiperazinici

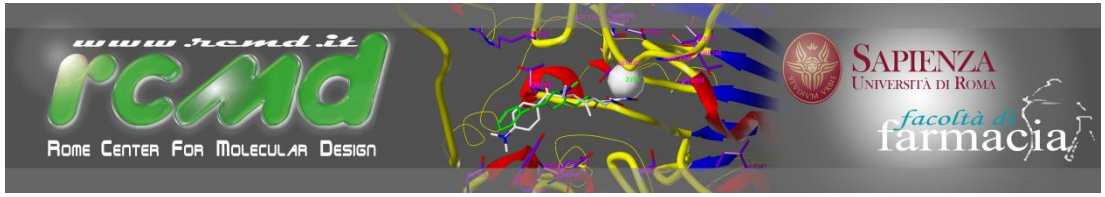


Derivati acilguanidinici



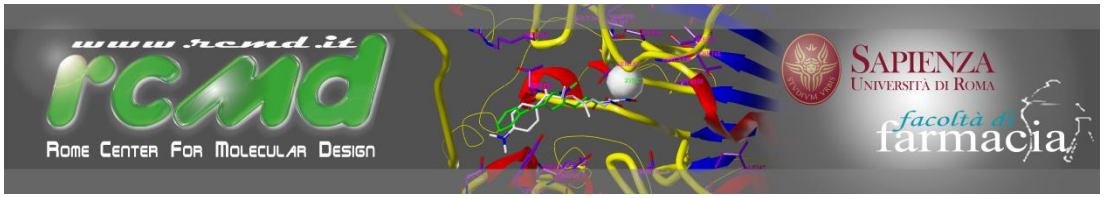


Overview



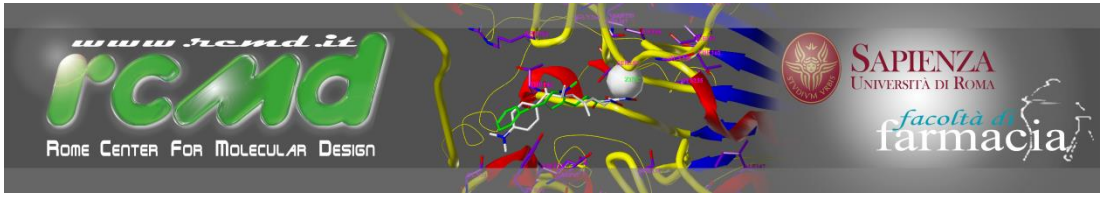
- Strutture ai raggi X
- Caratterizzazione Binding Site
- Selezione TrainingSet Exp
  - Structure-Based 3-D QSAR
    - N composti ad attività nota
      - Validazione Esterna
        - *Ligand Based*
          - Cross-Docking modeled
        - *Structure Based*
          - Cross-Surflex
  - Regole di allineamento
    - *Ligand Based*
      - Surflex
        - Re-surflex
        - Cross-Surflex
    - *Structure Based*
      - Autodock
        - Re-Docking
          - Modeled
          - Sperimentale
        - Cross-Docking
          - Modeled
          - Sperimentale

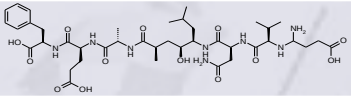
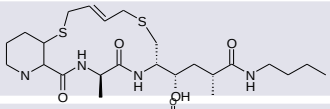
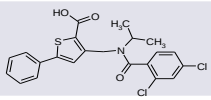
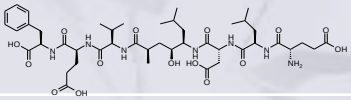
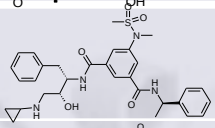
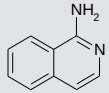
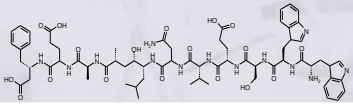
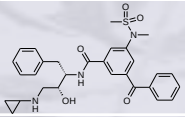
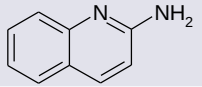
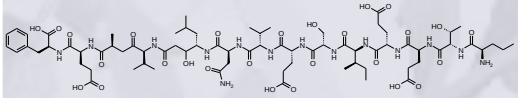
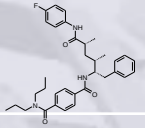
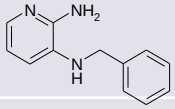
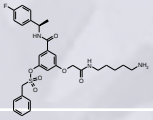
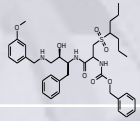
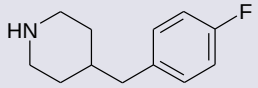
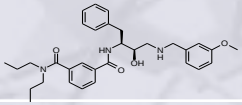
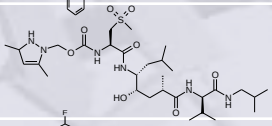
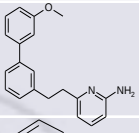
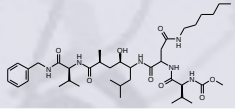
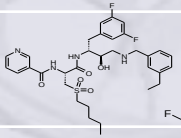
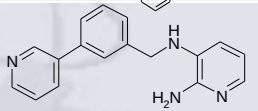
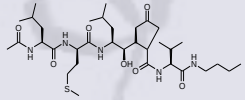
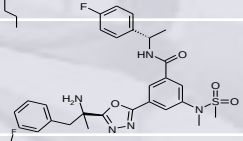
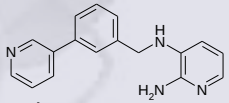
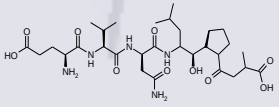
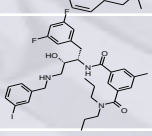
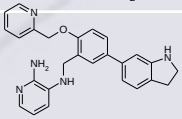
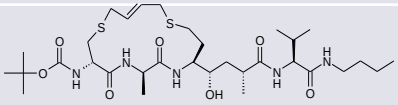
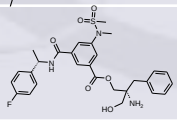
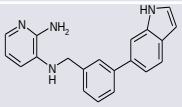
Costruzione del Modello 3-D QSAR



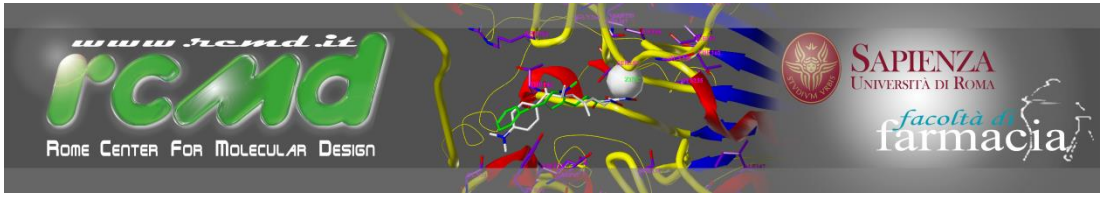
- Scelta del Training Set
- Costruzione dei modelli molecolari
- Allineamento delle molecole
- Calcolo dei campi di interazione molecolare (MIF)
- Elaborazione statistica dei modelli 3-D QSAR (PLS/GOLPE)
- Interpretazione dei risultati
- Validazione del modello 3-D QSAR

Scelta del Training Set



1fkn		2f3f		2of0	
1m4h		2b8l		2ohk	
1xn2		2b8v		2ohl	
1xn3		2fdp		2ohm	
1tqf		2hiz		2ohn	
1w51		2g94		2ohq	
1xs7		2hm1		2ohr	
1ym2		2irz		2ohs	
1ym4		2iqg		2oht	
2f3e		2is0		2ohu	

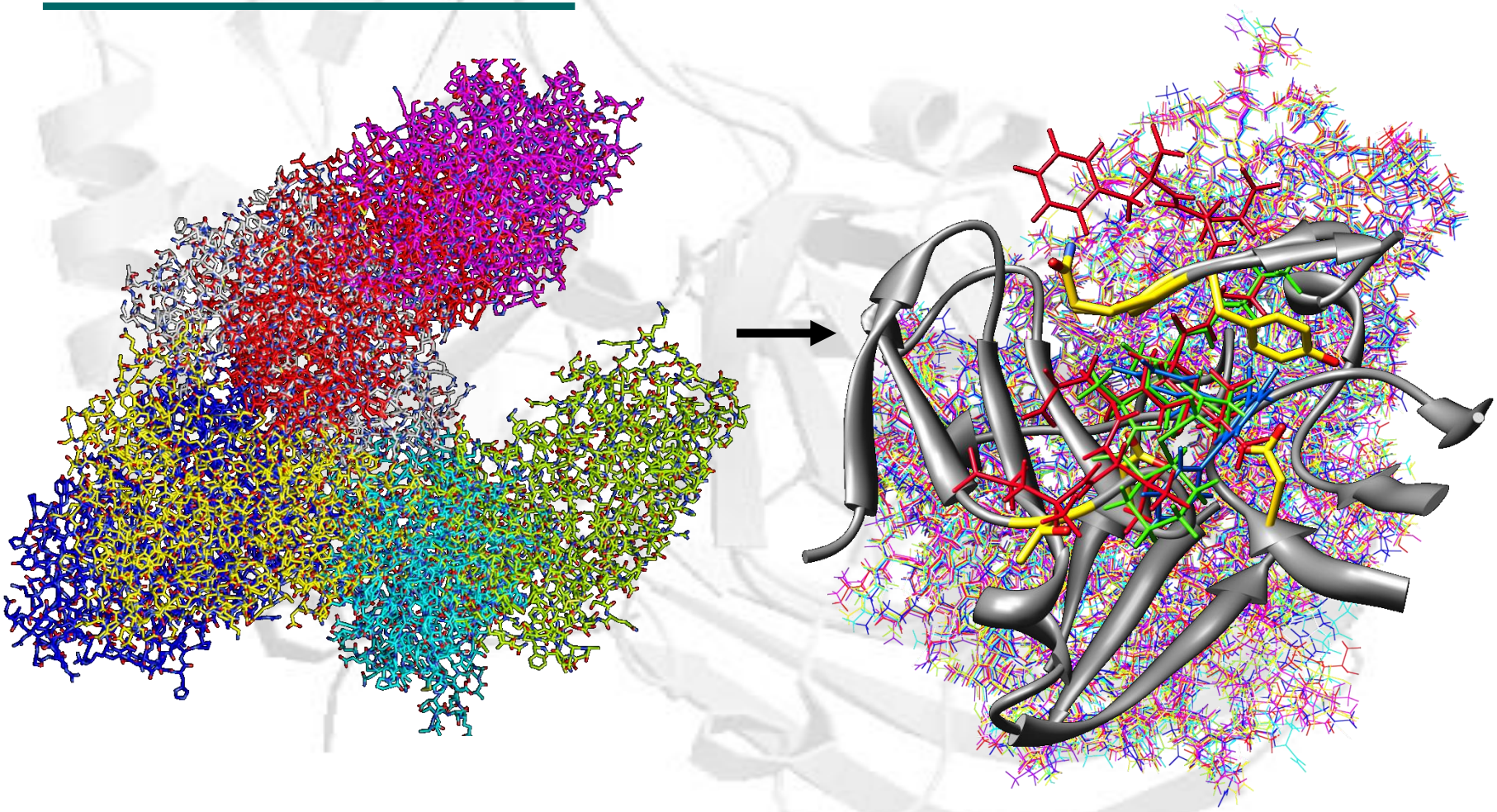
Costruzione dei modelli molecolari



MINIMIZZAZIONE (AMBER9)

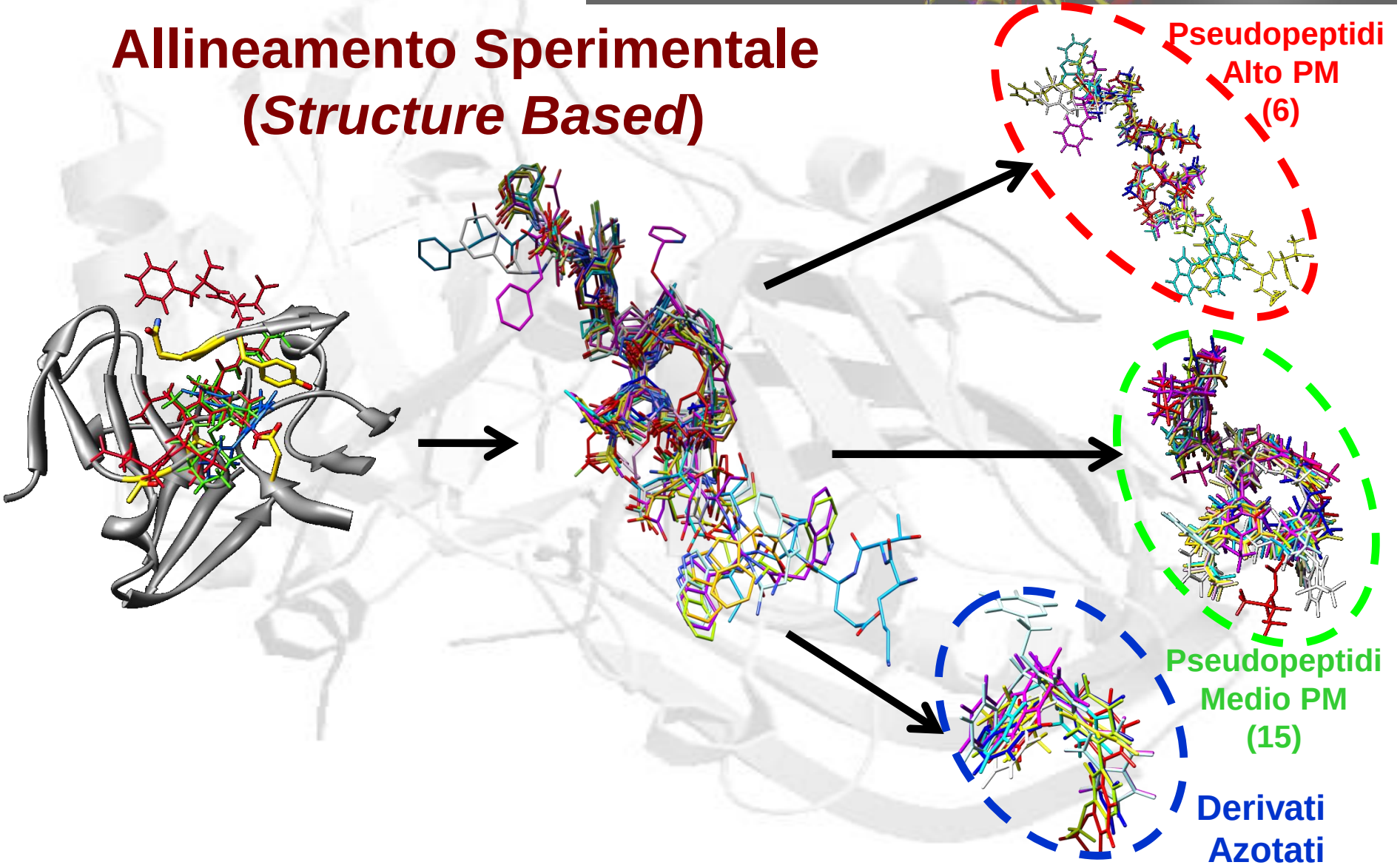
COMPLESSI OTTIMIZZATI GEOMETRICAMENTE E STRUTTURALMENTE

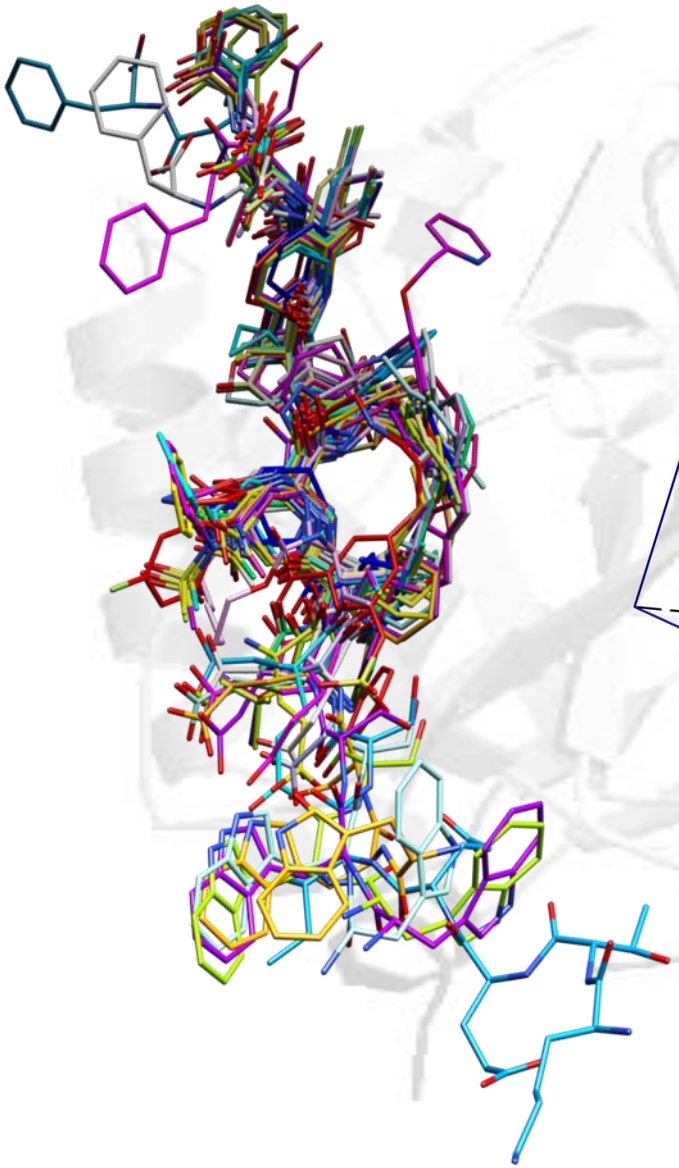
Allineamento structure-based



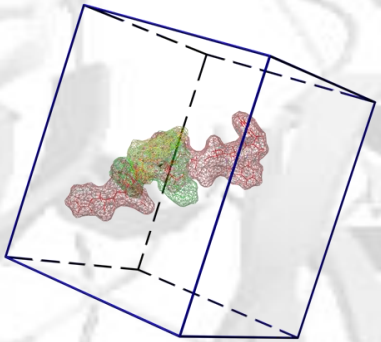
COMPLESSI BACE1/INIBITORI DA PDB **COMPLESSI ALLINEATI**

Allineamento Sperimentale (*Structure Based*)





Training Set Allineato
(30 molecole)

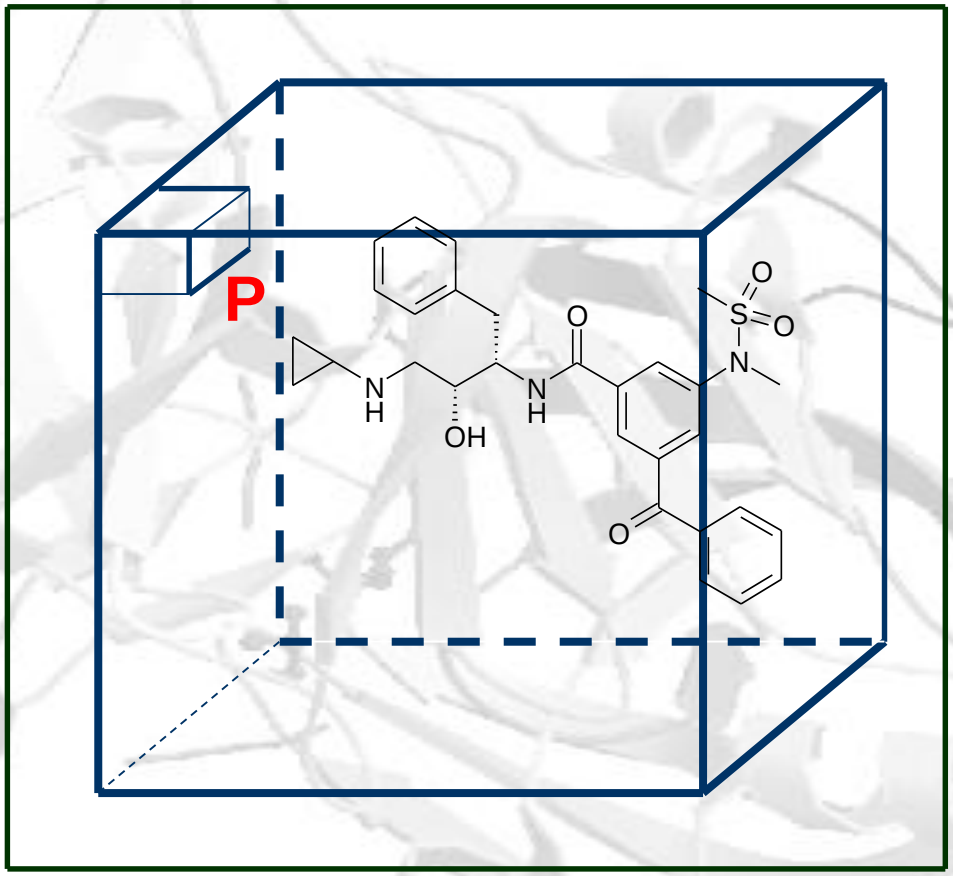


MIF

PLS

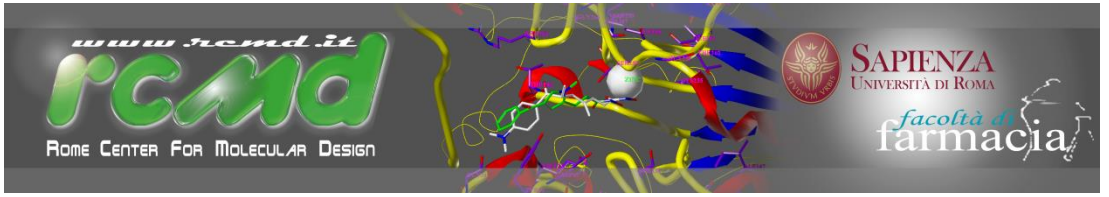
Modello 3-D QSAR

Calcolo dei Campi di Interazione Molecolare (MIF)



GRID: Interazioni energetiche *probe-target* per ogni punto della griglia costruita attorno al target

Elaborazione Statistica dei Modelli 3-D QSAR



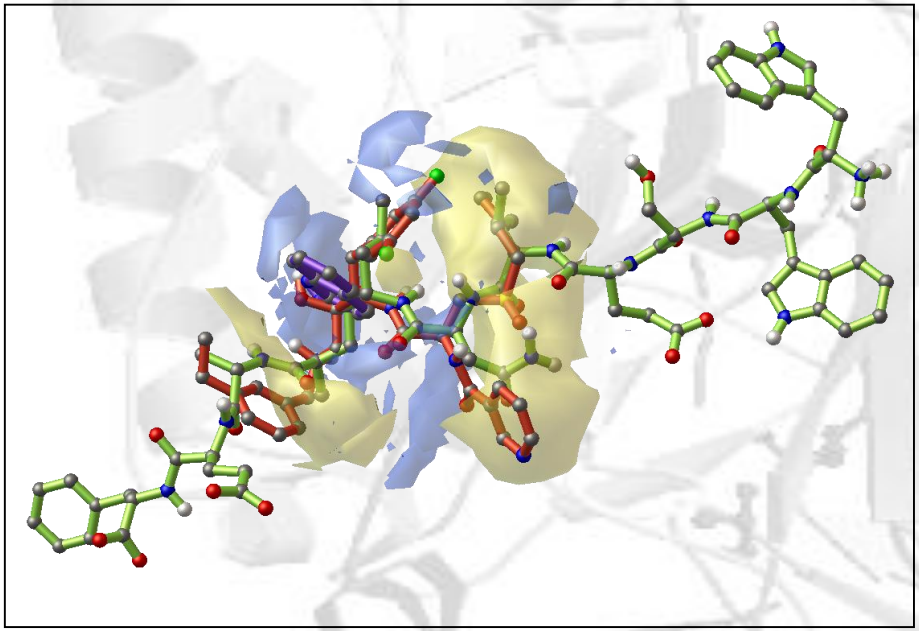
PLS/GOLPE

-I dati ottenuti da GRID vengono importati su GOLPE → relazione x/y

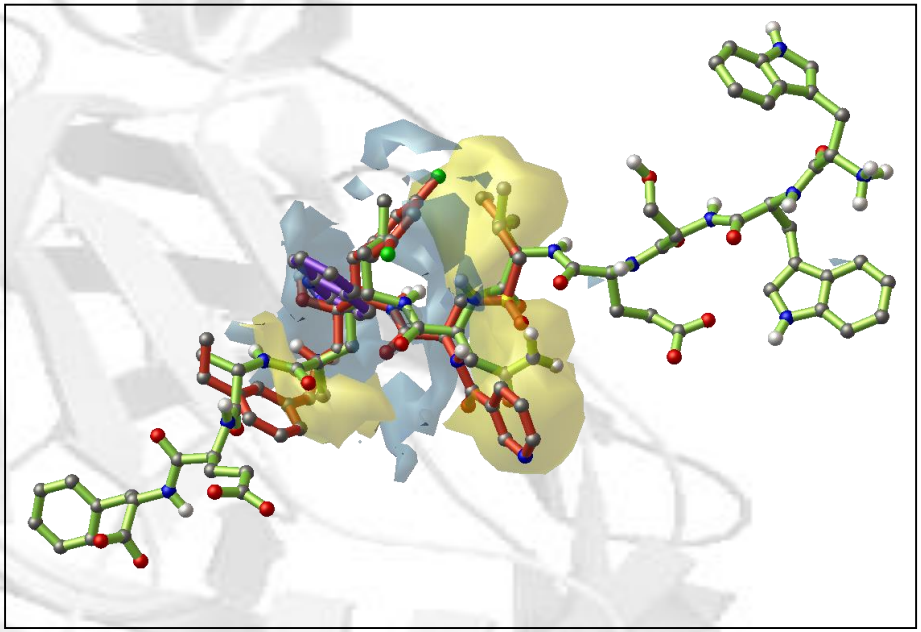
-Selezione delle variabili con pretrattamento dei dati → eliminate informazioni poco significative o ripetitive

PROBE	Comp	VAR.	r^2	SDEP	q^2
DRY	3	10214	0,99	1,48	0,52
N3+	3	277652	0,92	1,07	0,74
OH	3	277674	0,98	0,99	0,78
O::	3	277675	0,97	1,02	0,77
O	3	277684	0,97	0,97	0,79
N1	3	277673	0,98	0,96	0,80
O1	3	263119	0,98	1	0,78
C1=	3	29852	0,98	0,90	0,82

Mappe di correlazione

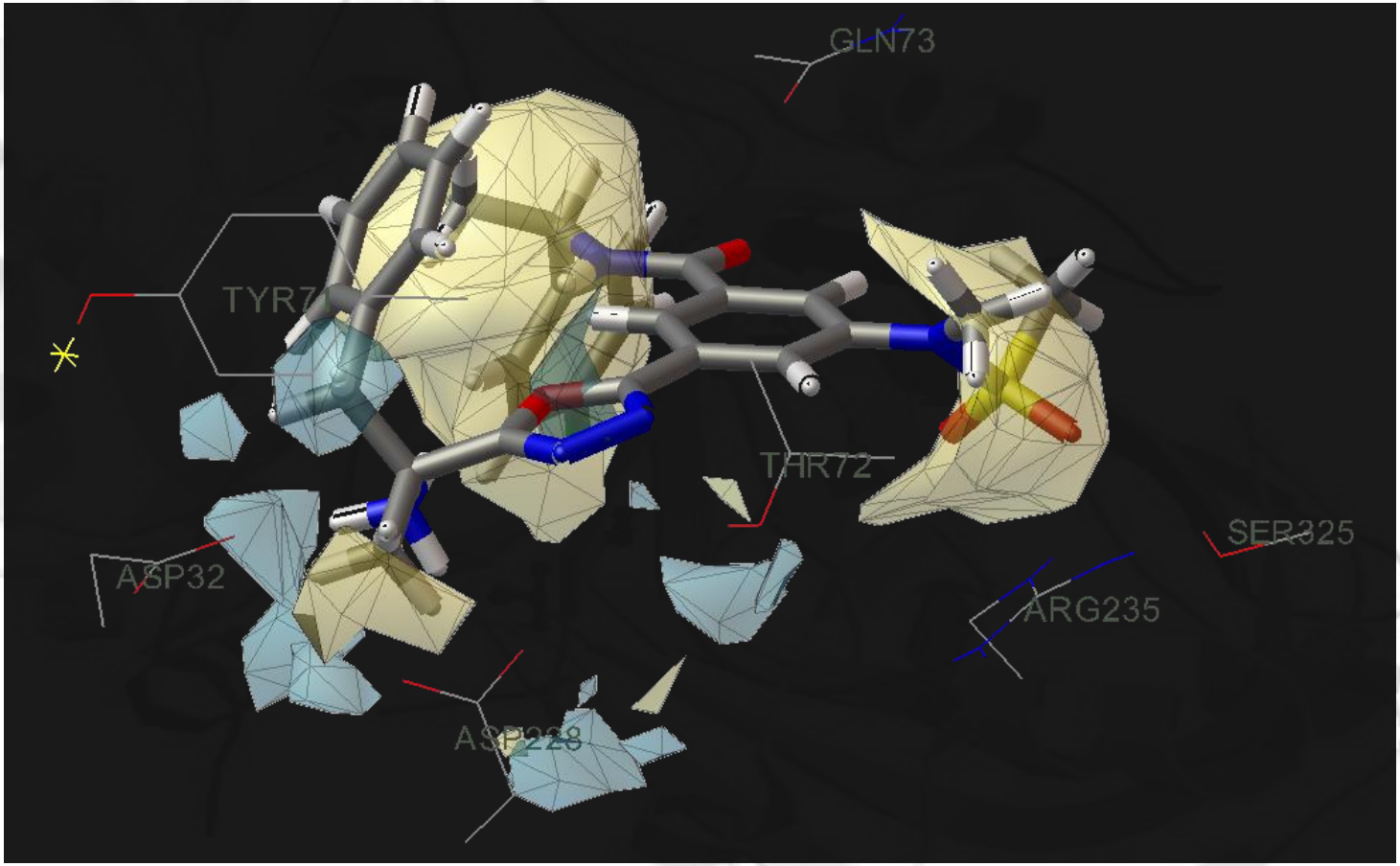


Coefficienti PLS ottenuti dal modello utilizzando il probe C1=

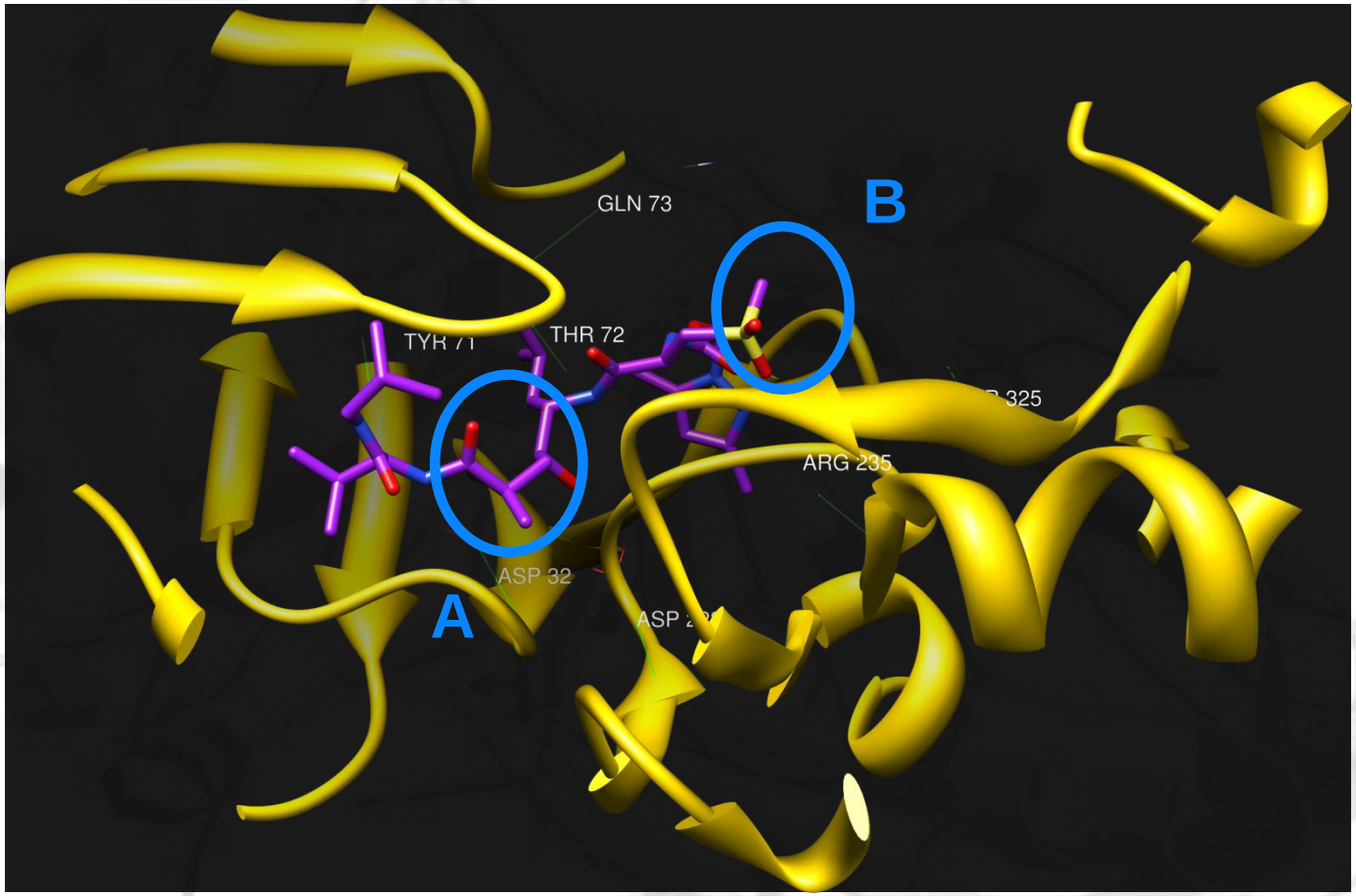


Coefficienti PLS ottenuti dal modello utilizzando il probe O carbonilico

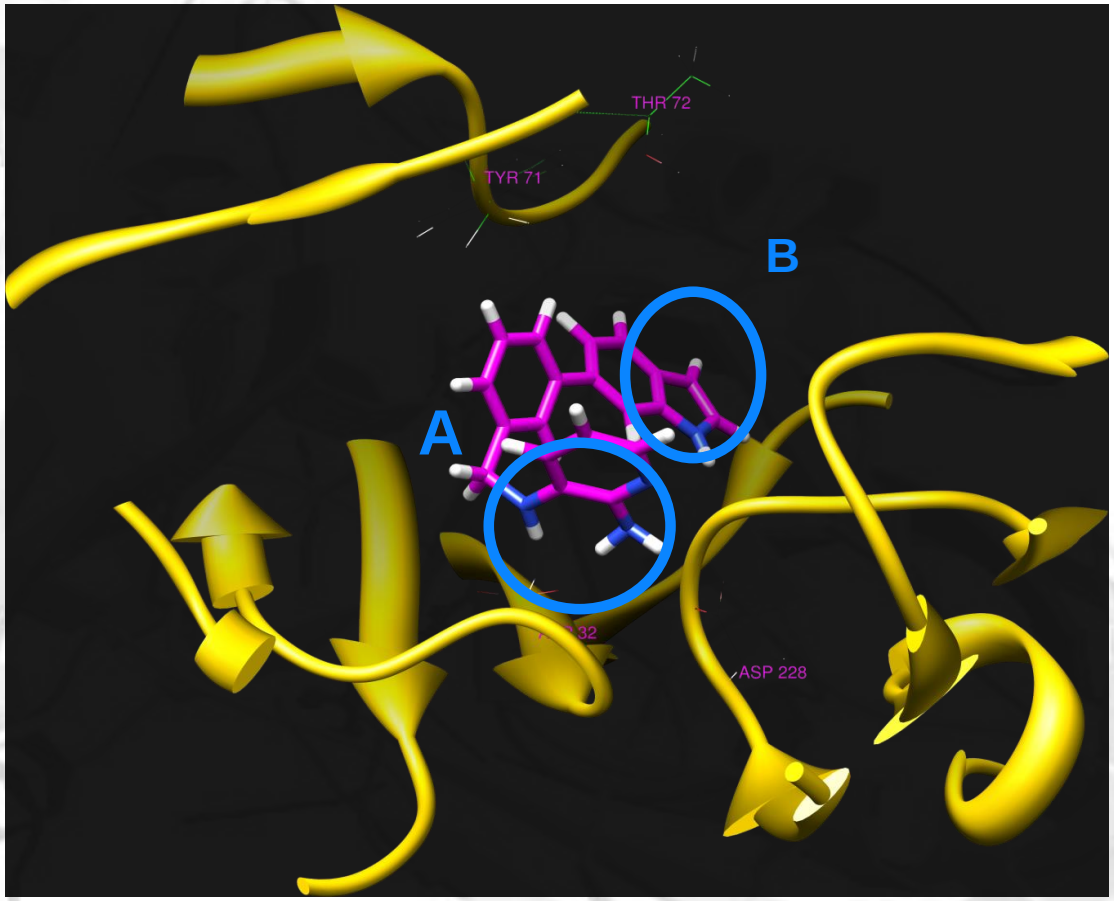
GRID-GOLPE Activity Contribution Plot



Mappa dei Contributi all'Attività ottenuta utilizzando il probe C1=

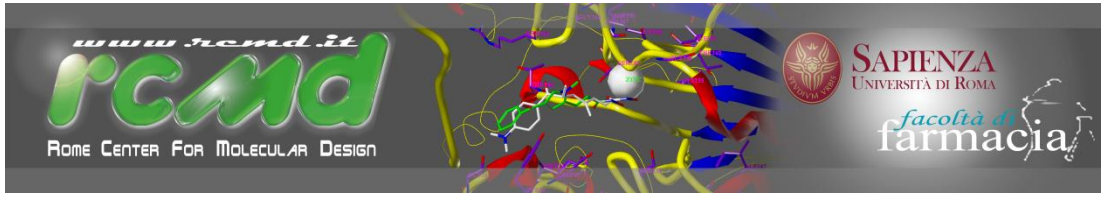


Molecola più attiva degli pseudopeptidi in cui sono evidenziate le possibili zone di maggiore interesse biologico



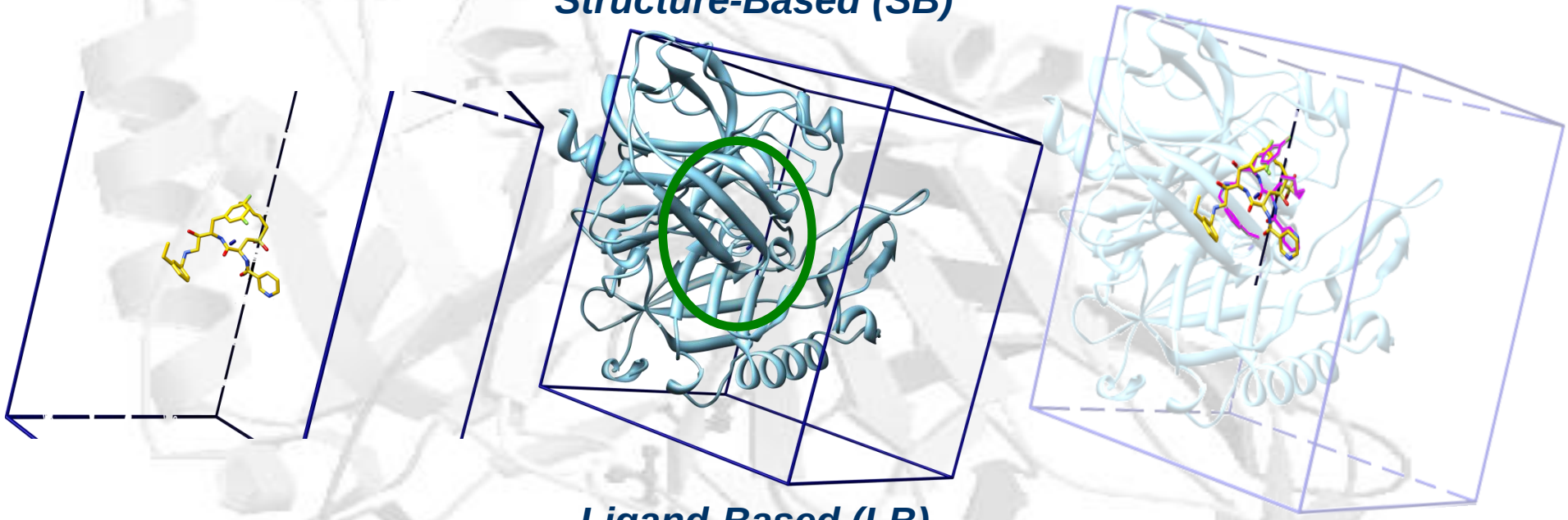
Molecola più attiva dei derivati azotati in cui sono evidenziate le possibili zone di maggiore interesse biologico

Regole di Allineamento

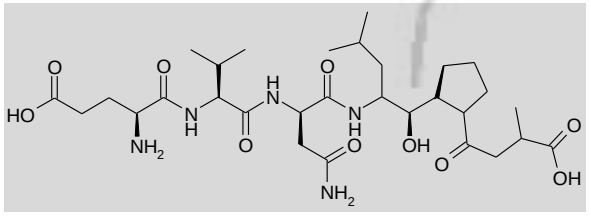


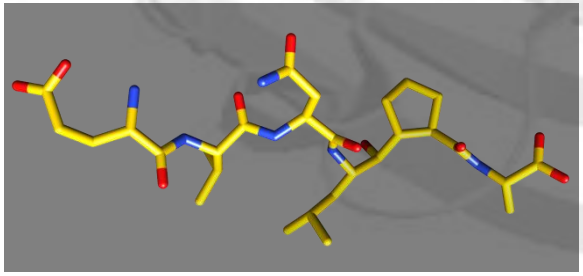
1 → **2** → **3**

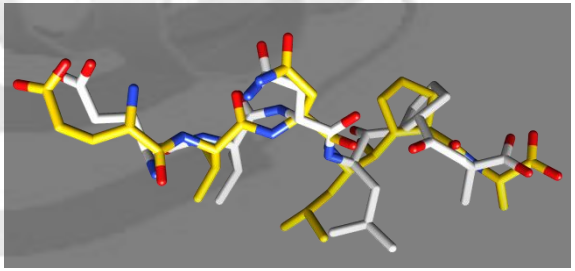
Structure-Based (SB)



Ligand-Based (LB)

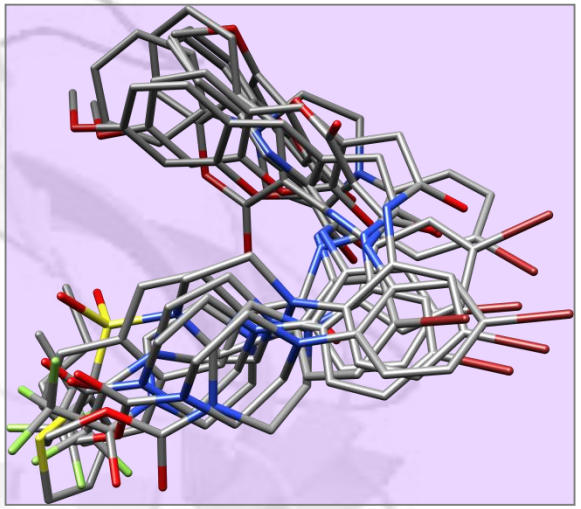






Validazione della Capacità Predittiva

- **TEST SET ESTERNI** → **26 Test Set (323 molecole)**



- **Allineamento LB (SURFLEX)** →

- **Allineamento SB via Cross-Docking (AUTODOCK 4.0.1.)** → **Best Cluster**

Validazione della Capacità Predittiva

Nome	Nr. Comp. Test Set	C1=	DRY	N1	N3+	O::	O	O1	OH
BMCL_1016	16	[illegible]	0.98	1.03	1.00	1.20	[illegible]	1.00	1.12
BMCL_1021	28	0.74	0.90	0.83	1.00	0.82	0.77	0.83	0.82
BMCL_1026	19	[illegible]	1.42	1.35	1.25	1.25	[illegible]	1.35	1.36
BMCL_1121	2	0.96	1.19	1.12	1.13	1.06	1.01	1.12	1.12
BMCL_15	8	0.68	[illegible]	0.82	1.08	1.03	0.82	0.94	0.91
BMCL_1629	11	0.93	0.25	0.67	1.49	1.03	1.15	0.50	0.70
BMCL_1788	15	1.45	[illegible]	1.87	1.32	1.69	1.71	1.87	2.01
BMCL_1999	16	0.45	0.48	0.49	0.73	0.49	0.50	0.52	0.49
BMCL_2386	18	1.29	1.50	1.49	2.18	1.41	1.35	1.65	1.51
BMCL_4359	22	1.16	1.27	1.45	1.68	1.42	1.30	1.46	1.50
BMCL_4551	19	1.49	1.25	1.19	1.35	1.31	1.26	1.48	1.13
BMCL_644	15	[illegible]	0.83	0.88	1.16	1.04	1.00	0.82	0.85
BMCL_77	4	0.60	0.97	0.78	1.00	0.72	0.66	0.84	0.84
BMCL_7881	28	[illegible]	1.81	2.04	1.68	1.76	1.68	2.04	2.10
JACS_33	8	1.21	1.92	0.82	0.39	0.97	1.12	0.73	0.80
JACS_42	6	2.23	1.24	1.74	1.36	1.65	[illegible]	1.67	1.66
JMC_164	9	0.96	0.93	0.89	1.08	0.85	0.80	0.87	0.87
JMC_2082	11	1.60	[illegible]	1.50	1.47	1.43	[illegible]	1.52	1.47
JMC_4567	29	1.13	0.90	1.12	1.18	1.13	1.14	1.17	1.17
JMC_49_3	7	0.97	[illegible]	1.06	1.05	1.00	0.99	1.03	1.09
JMC_6158	5	1.43	0.64	1.49	2.01	1.42	1.34	1.59	1.50
JMC_5111	4	1.16	[illegible]	0.87	1.19	1.00	1.01	0.87	0.79
JMC_6119	5	[illegible]	1.56	1.63	1.42	1.70	1.73	1.48	1.46
JMC_6450	9	0.60	1.16	0.84	1.01	0.83	0.80	0.91	0.94
JMC_7273	7	[illegible]	1.53	1.44	1.41	1.38	1.41	1.52	1.54
JMC_781	6	0.40	0.72	0.42	0.57	0.59	0.48	0.42	0.44
SDEP		**1.12**	**1.19**	**1.15**	**1.24**	**1.16**	**1.14**	**1.16**	**1.16**

CONCLUSIONI

- Costruzione del modello 3-D QSAR
- Interpretazione delle mappe di correlazione e analisi delle zone di interesse
- Messa a punto dei metodi di allineamento LB e SB
- Validazione del metodo con oltre 300 molecole
- E' in corso l'applicazione del nuovo modello per lo sviluppo di nuovi composti ad attività BACE-inibitoria