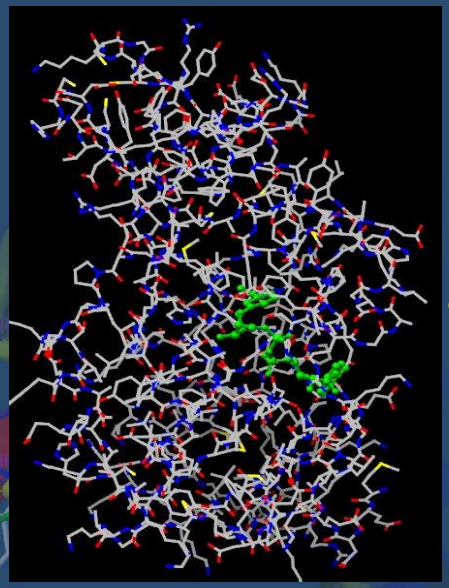

# Relazioni quantitative struttura-attività di tipo tri-dimensionale mediante l'utilizzo di informazioni strutturali del recettore: applicazione agli inibitori delle sirtuine

**Laureando:**
**Girolamo Capezzera**

**Relatore:**
**Chiar.mo dott. Rino Ragno**

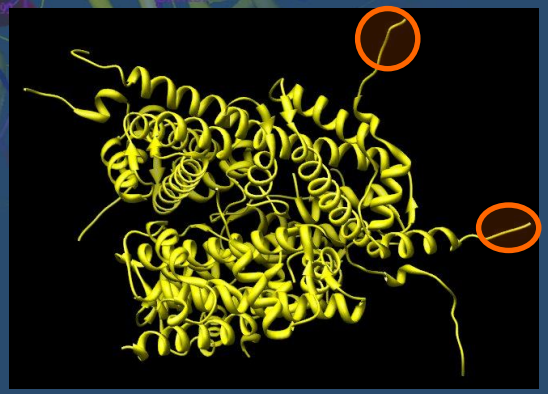
# Sirtuine



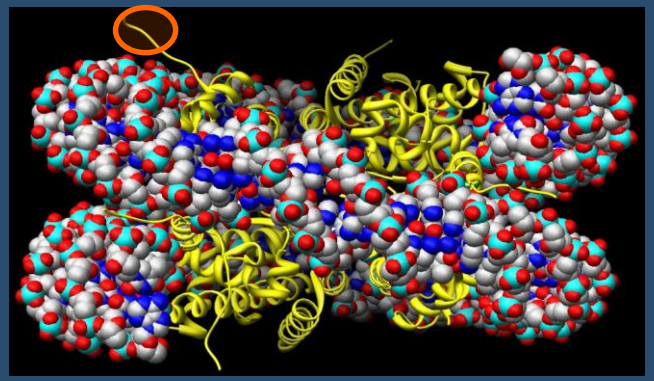
SIRT2

piccolo dominio flessibile

dominio più grande Rossmann fold



Ottamero istonico

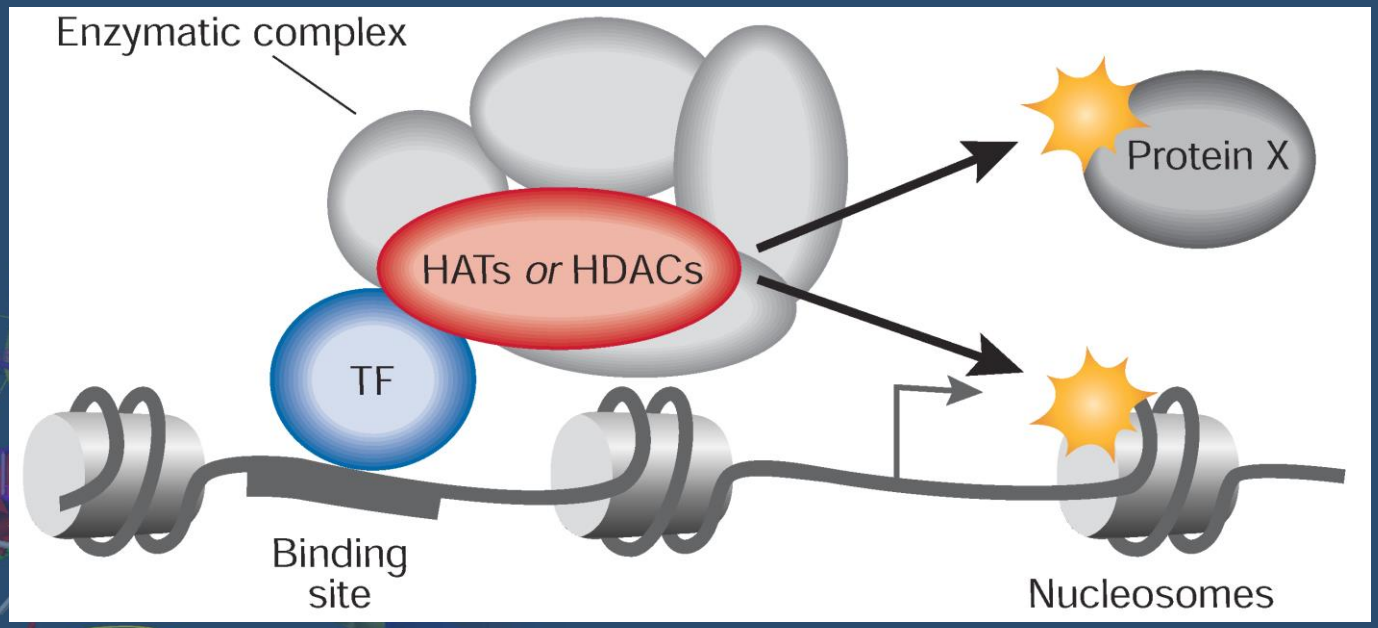


Nucleosoma

# Sirtuine


# CANCEROGENESI

# Sirtuine



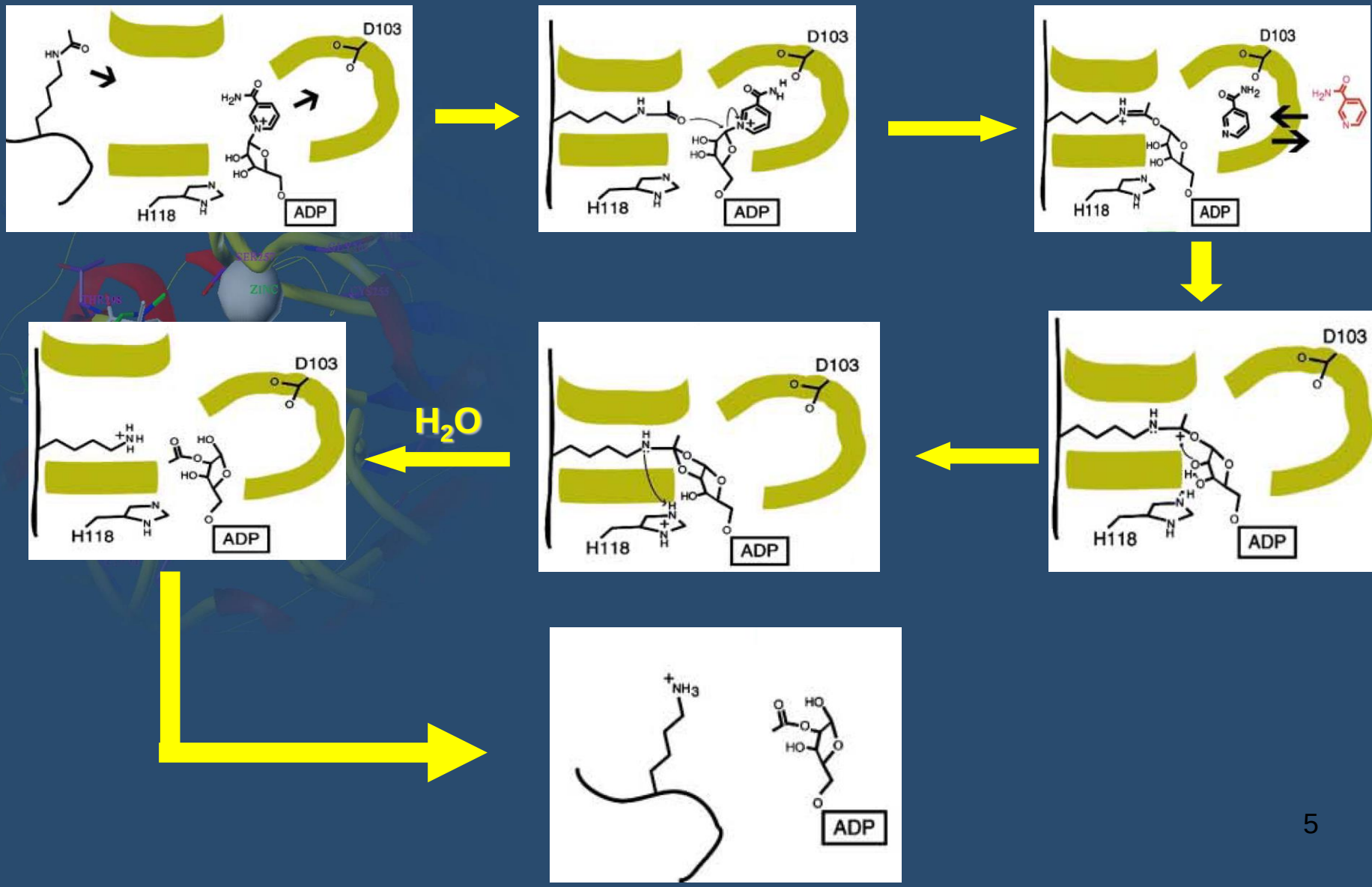

**Influenze extraistoniche**

- **Regolazione del ciclo cellulare**
- **Controllo dell'apoptosi**
- **Controllo delle risposte cellulari allo stress**

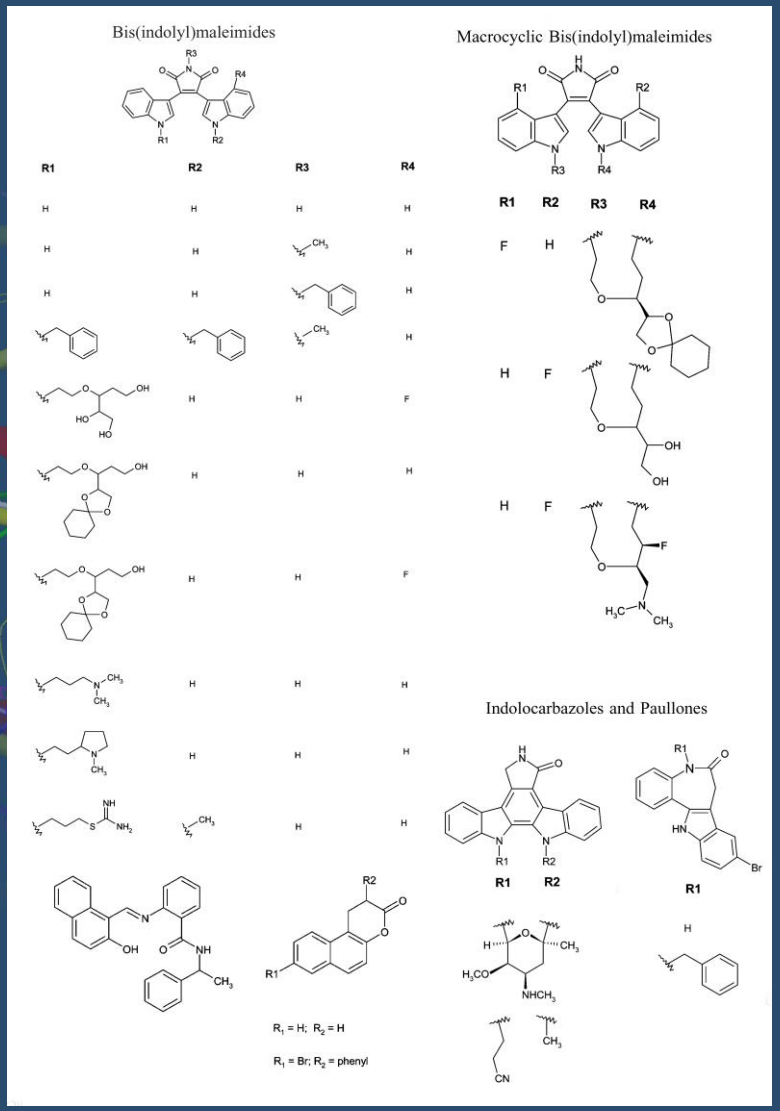
**Influenze istoniche**

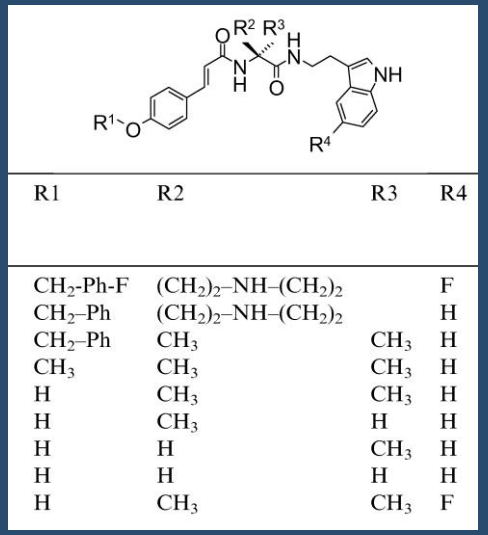
- **Substrati istonici non trascrizionali**
- **Regolazione della trascrizione genica**

# Meccanismo Catalitico

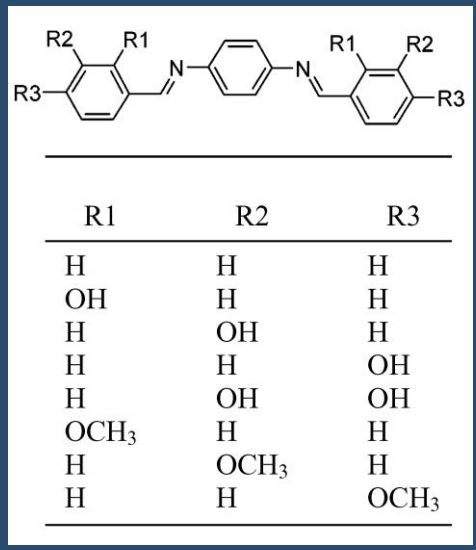


# Inibitori delle Sirtuine



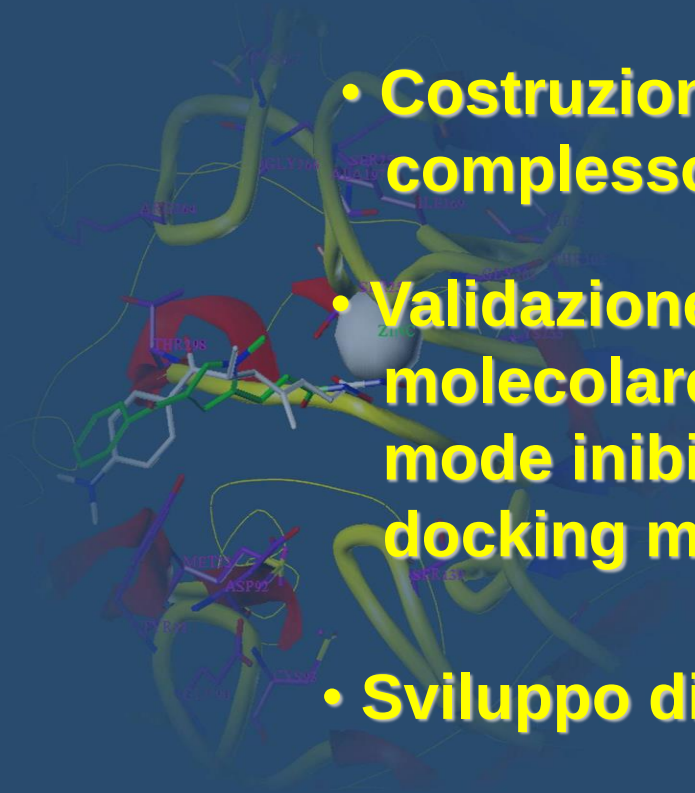
| R1 | R2 | R3 | R4 |
|---|---|---|---|
| $CH_2$-Ph-F | $(CH_2)_2$–NH–$(CH_2)_2$ | | F |
| $CH_2$–Ph | $(CH_2)_2$–NH–$(CH_2)_2$ | | H |
| $CH_2$–Ph | $CH_3$ | $CH_3$ | H |
| $CH_3$ | $CH_3$ | $CH_3$ | H |
| H | $CH_3$ | $CH_3$ | H |
| H | $CH_3$ | H | H |
| H | H | $CH_3$ | H |
| H | H | H | H |
| H | $CH_3$ | $CH_3$ | F |



| R1 | R2 | R3 |
|---|---|---|
| H | H | H |
| OH | H | H |
| H | OH | H |
| H | H | OH |
| H | OH | OH |
| $OCH_3$ | H | H |
| H | $OCH_3$ | H |
| H | H | $OCH_3$ |


- J. Trapp; J.Med.Chem., 2006, 49, 7307-7316.
- H. Kiviranta; Bioorganic & Medicinal Chemistry Letters, 2007, 2448-2451.
- J. Leppanen; J. Med. Chem. 2006, 49, 7907-7911.

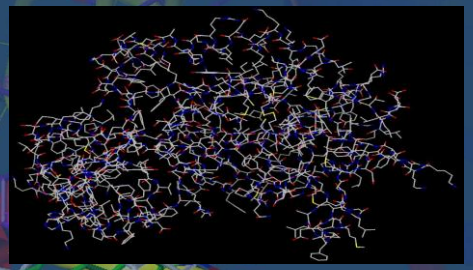
# Approccio Sperimentale



- Costruzione e minimizzazione del complesso ternario SIRT2/ALY/$NAD^+$
- Validazione del programma di docking molecolare Autodock4 ed analisi del binding mode inibitori SIRT2 attraverso studi di docking molecolare
- Sviluppo di modelli 3-D QSAR

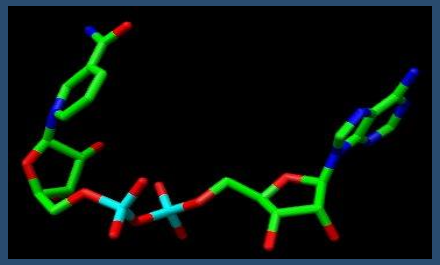
# Costruzione e miminizzazione del complesso ternario SIRT2/ALY/NAD+

*HOMO SAPIENS*



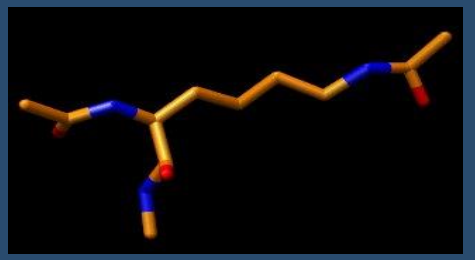
SIRT2

*Archaeoglobus Fulgidus*



NAD+

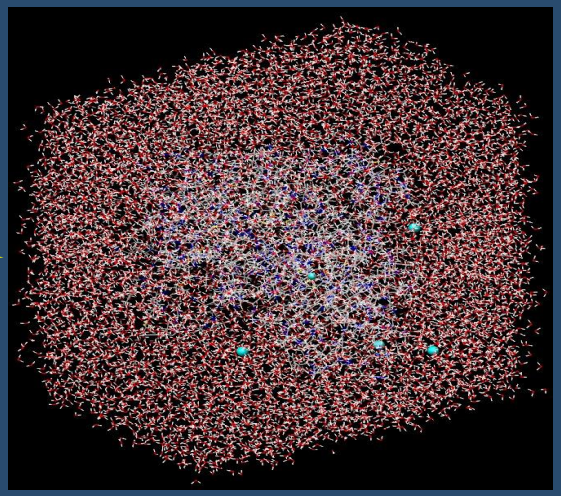
*Thermotoga Maritima*



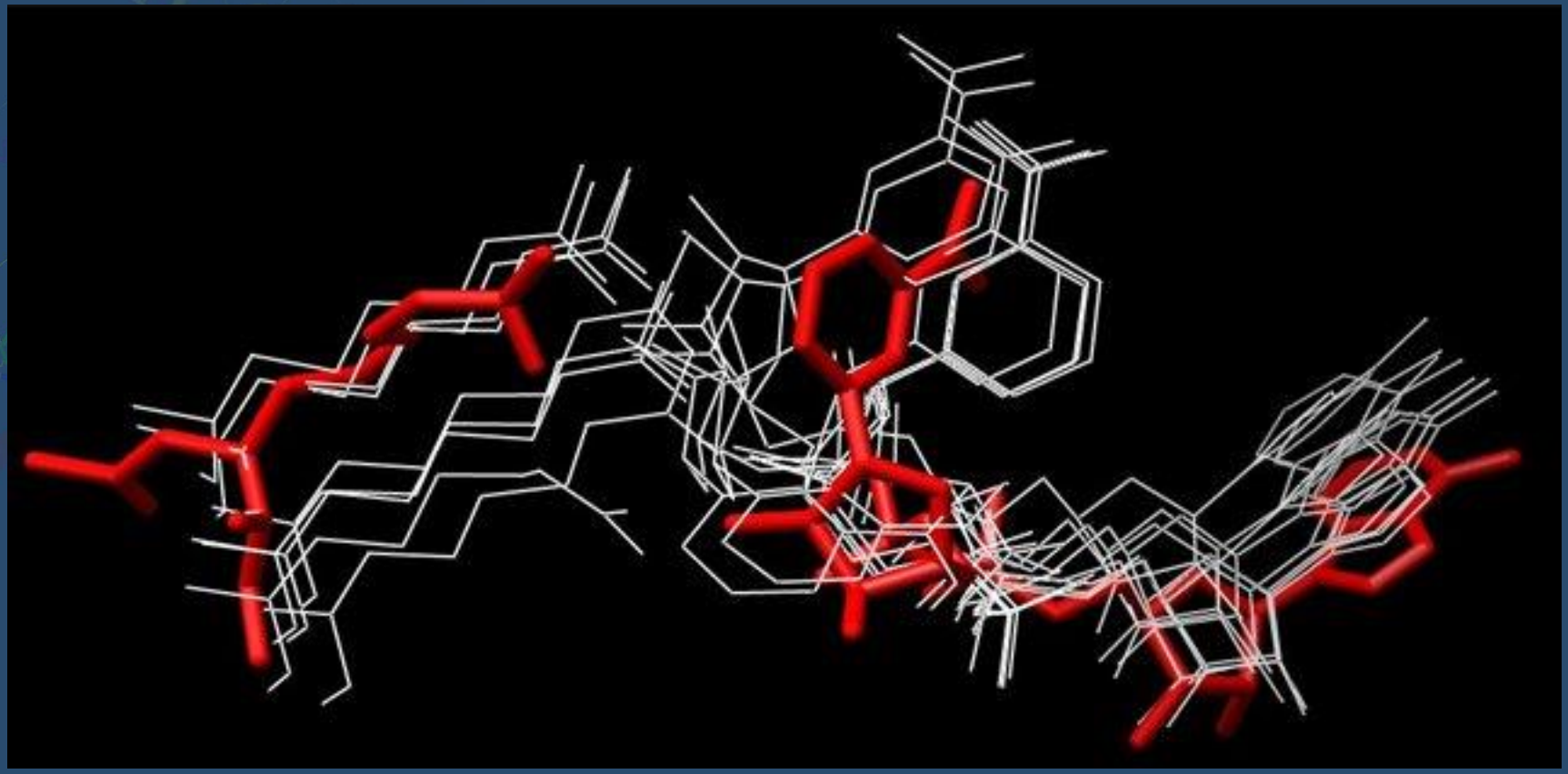
ALY

minimizzazione (AMBER 8)

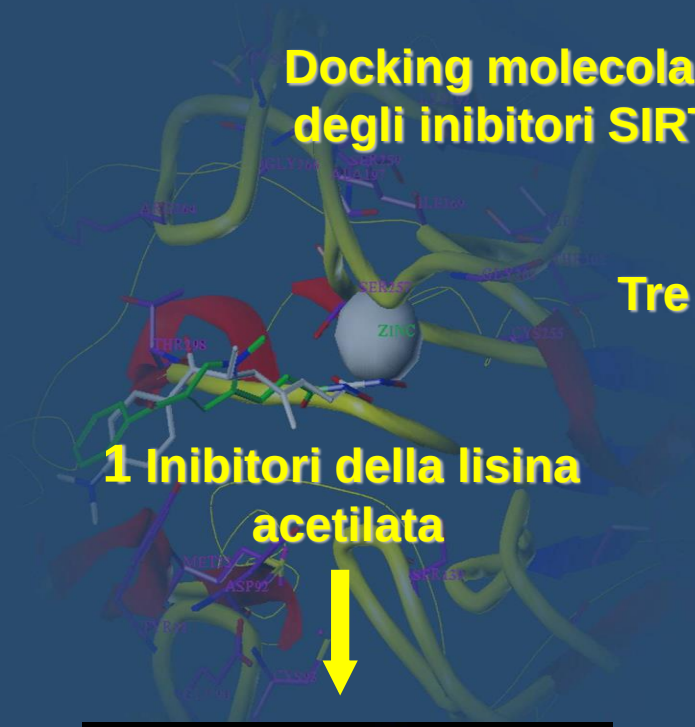
Complesso ternario minimizzato



# Validazione del programma di docking molecolare Autodock4



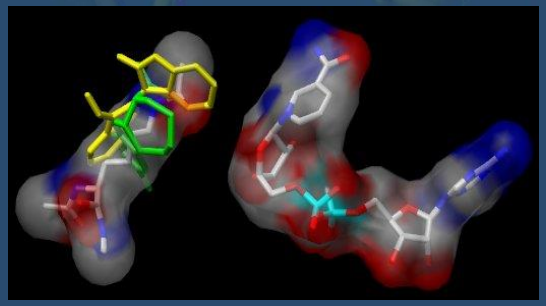
# Analisi del binding mode dei 72 inibitori SIRT2 attraverso studi di docking molecolare



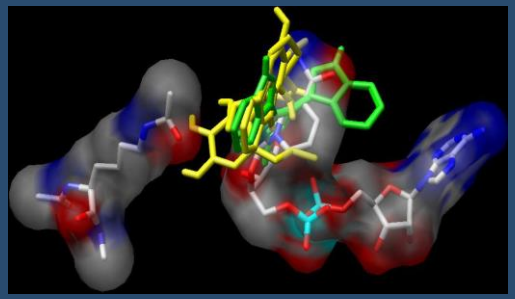
**Docking molecolare degli inibitori SIRT2** → **Valutazione del binding mode**

**Tre possibili siti di inibizione:**

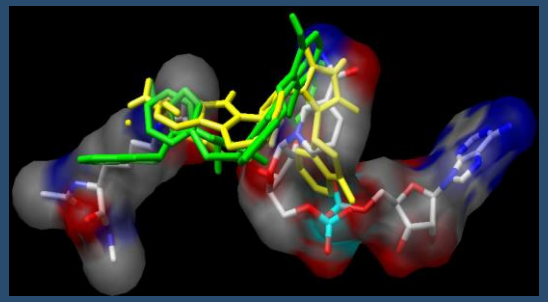
**1** Inibitori della lisina acetilata



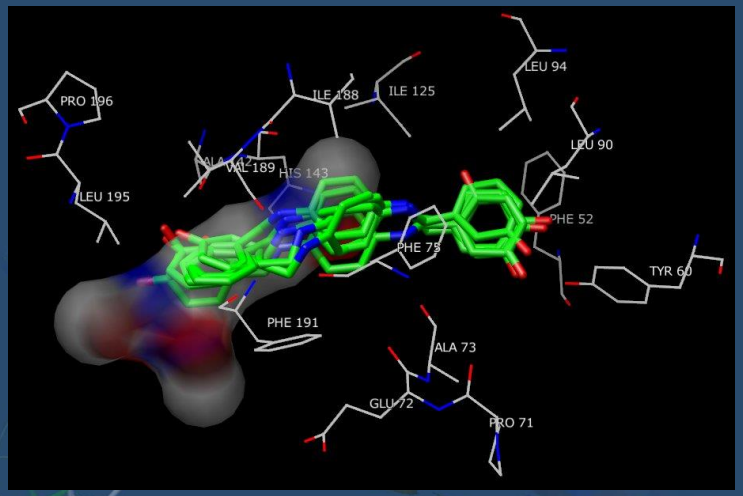
**2** Inibitori del cofattore $NAD^+$



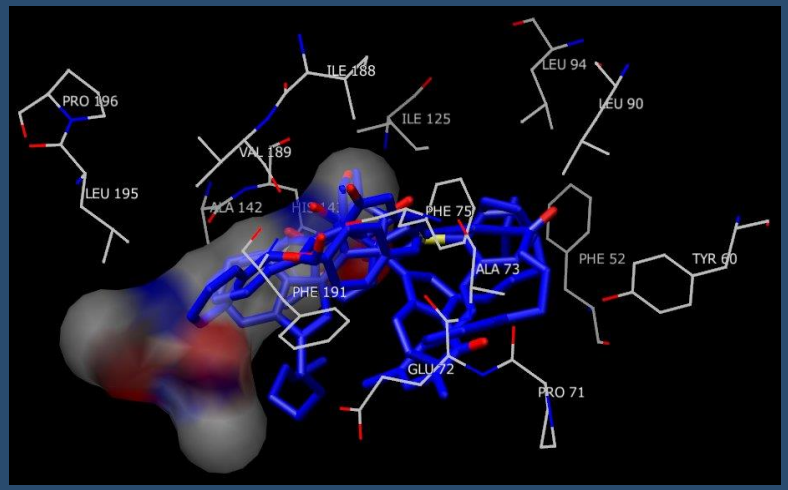
**3** Inibitori del sito intermedio



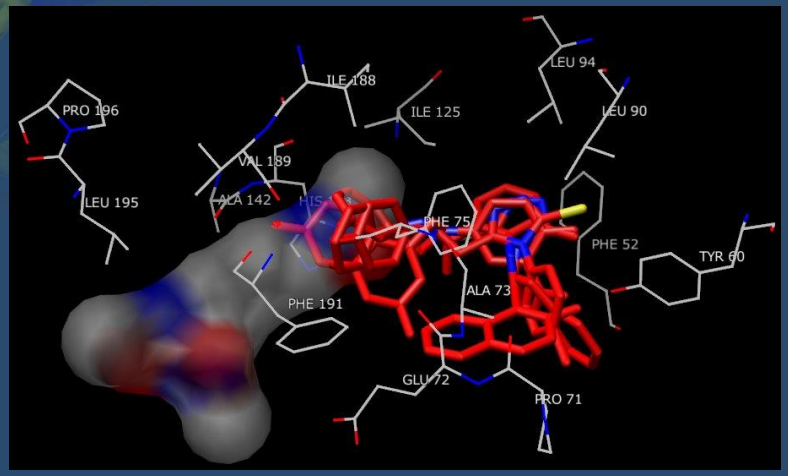
# Analisi del binding mode degli inibitori della lisina acetilata




Strutture attive ($IC_{50}$ = 0,8-5 μM)

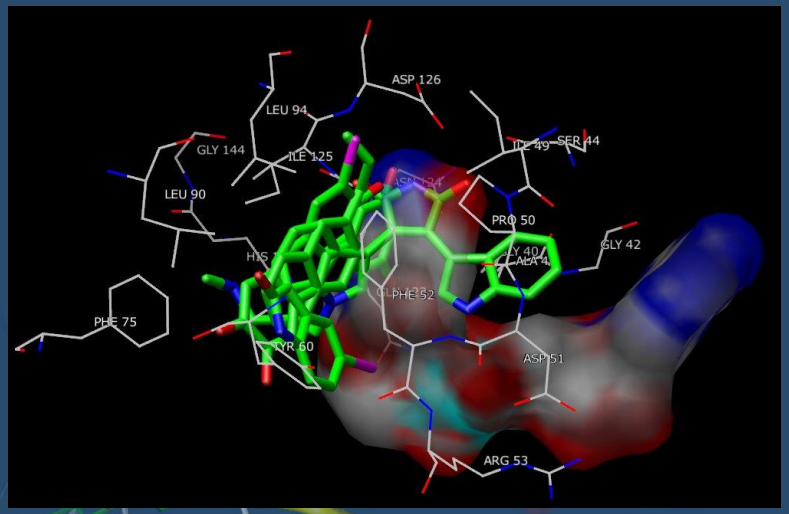



Strutture mediamente attive ($IC_{50}$ = 100 μM)

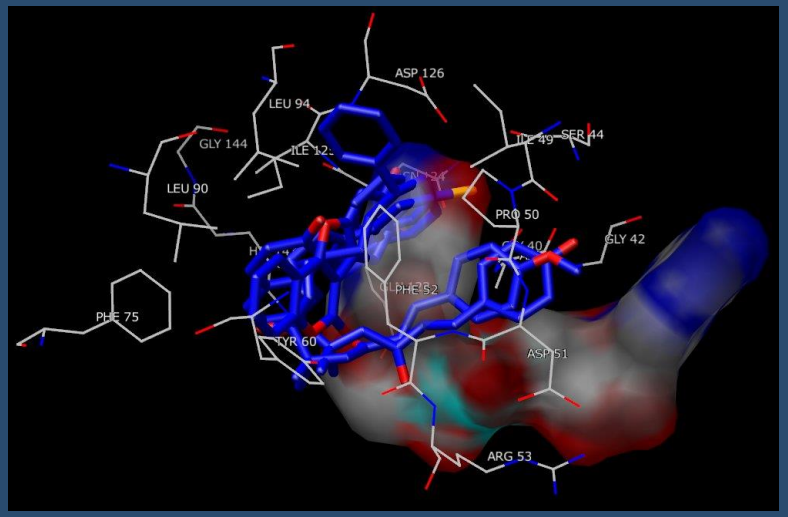



Strutture poco attive ($IC_{50}$ > 300 μM)

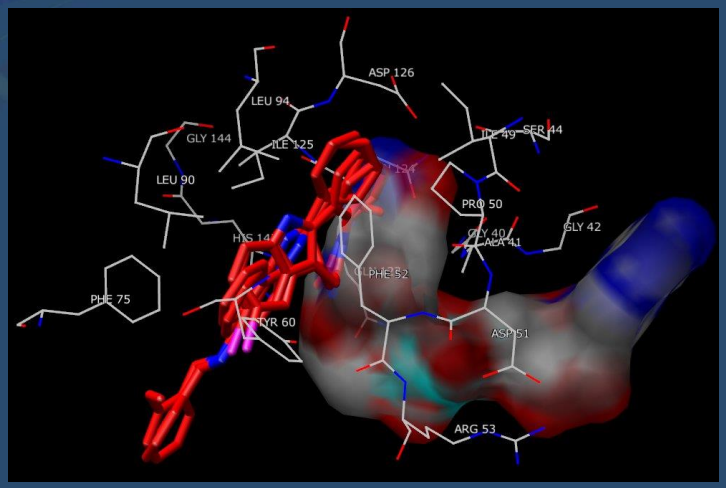
# Analisi del binding mode degli inibitori del cofattore $NAD^+$




Strutture attive ($IC_{50}$ = 12-35 μM)

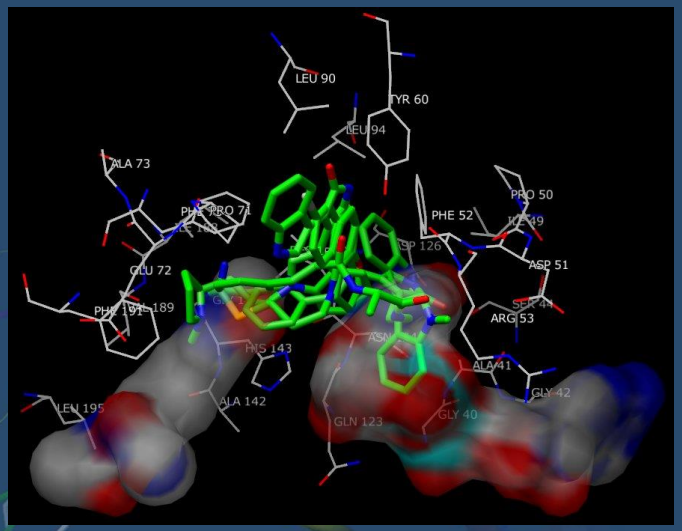



Strutture mediamente attive ($IC_{50}$ ~140 μM)

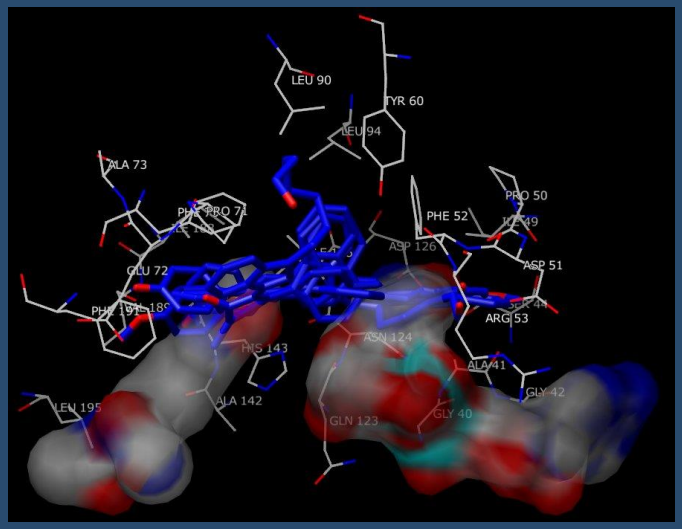



Strutture poco attive ($IC_{50}$ = 300 μM)

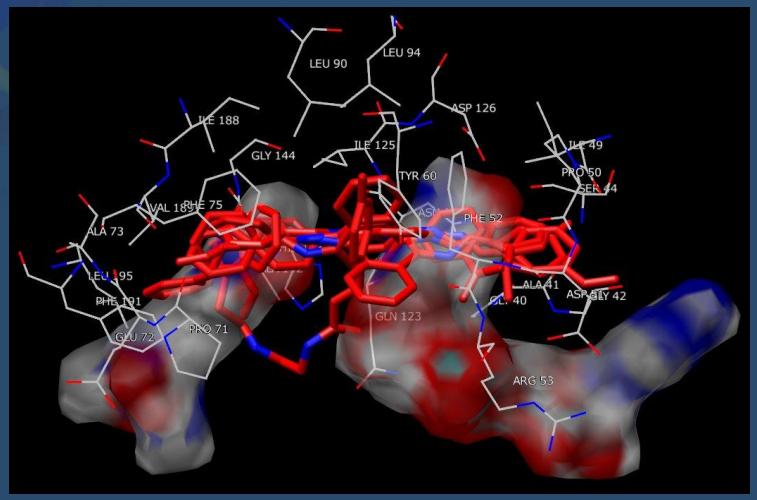
# Analisi del binding mode degli inibitori con disposizione intermedia




Strutture attive ($IC_{50}$ ~ 10 μM)



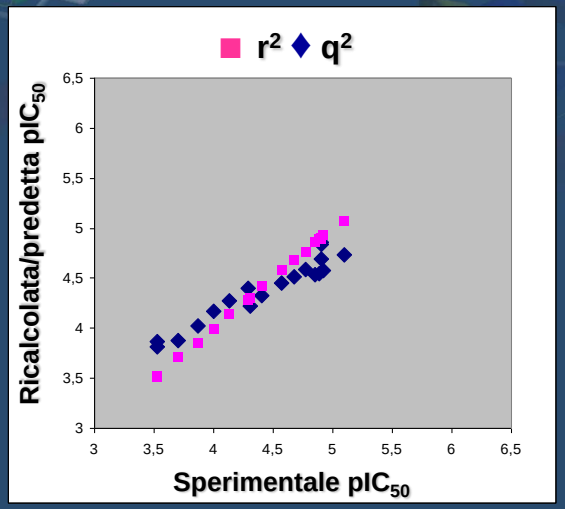

Strutture mediamente attive ($IC_{50}$ ~140 μM)

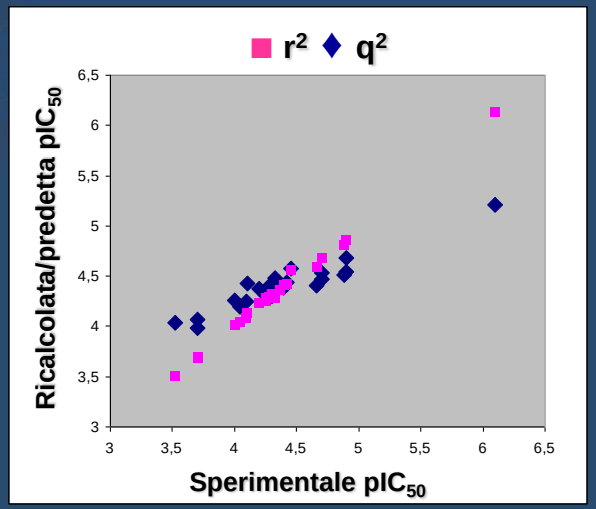


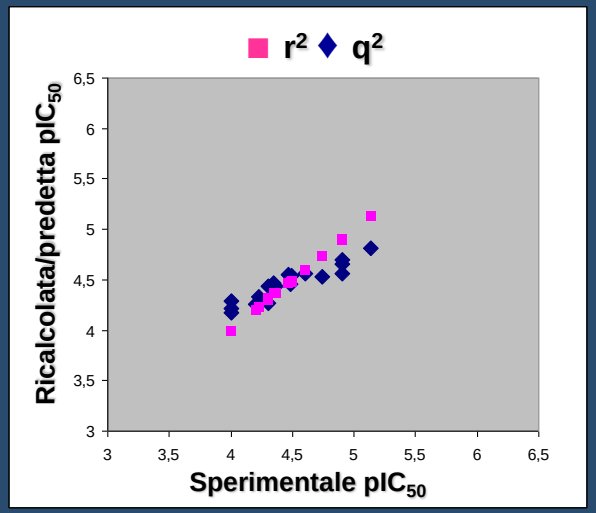

Strutture poco attive ($IC_{50}$ > 300 μM)

# Sviluppo di modelli 3-D QSAR

| Probabile sito di inibizione | Allineamento esaminato | Strutture | *Probe* | FFD | N | P.C. | q2 | r2 | $SDEP_{CV}$ |
|---|---|---|---|---|---|---|---|---|---|
| Lisina acetilata | Best Cluster | 19 | DRY-OH | II | 782 | 5 | 0,91 | 0,99 | 0,15 |
| Sito Intermedio | Best Cluster | 24 | DRY-OH | II | 1118 | 3 | 0,66 | 0,99 | 0,27 |
| Cofattore $NAD^+$ | Best Cluster | 18 | DRY-OH | II | 1064 | 5 | 0,68 | 0,99 | 0,19 |



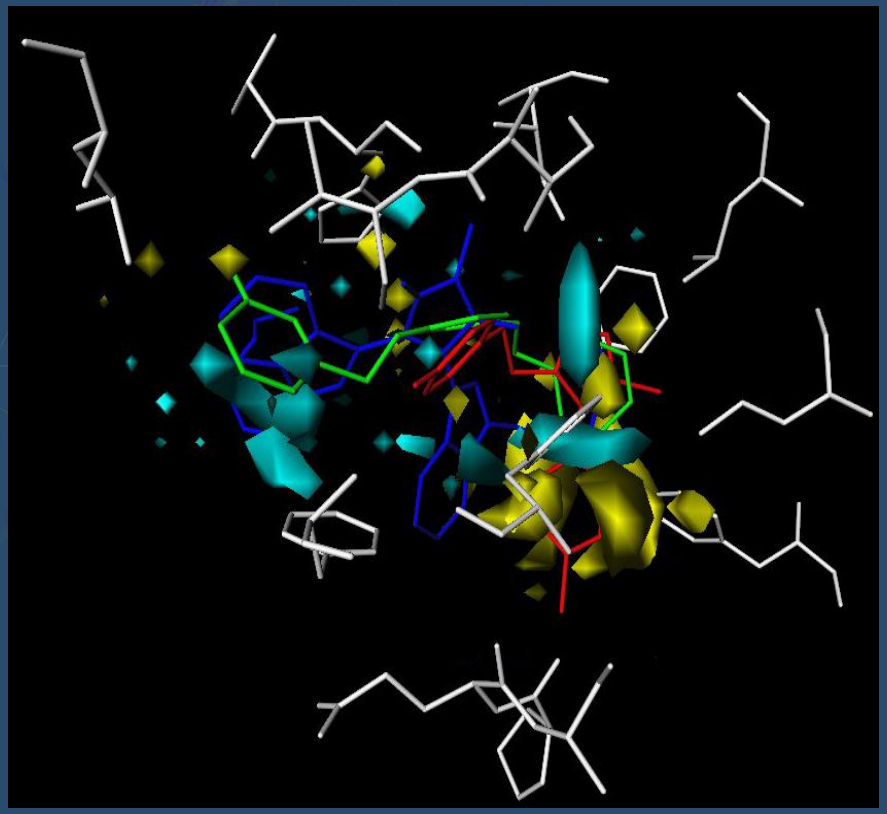

**Inibitori lisina acetilata**

**Inibitori intermedi**

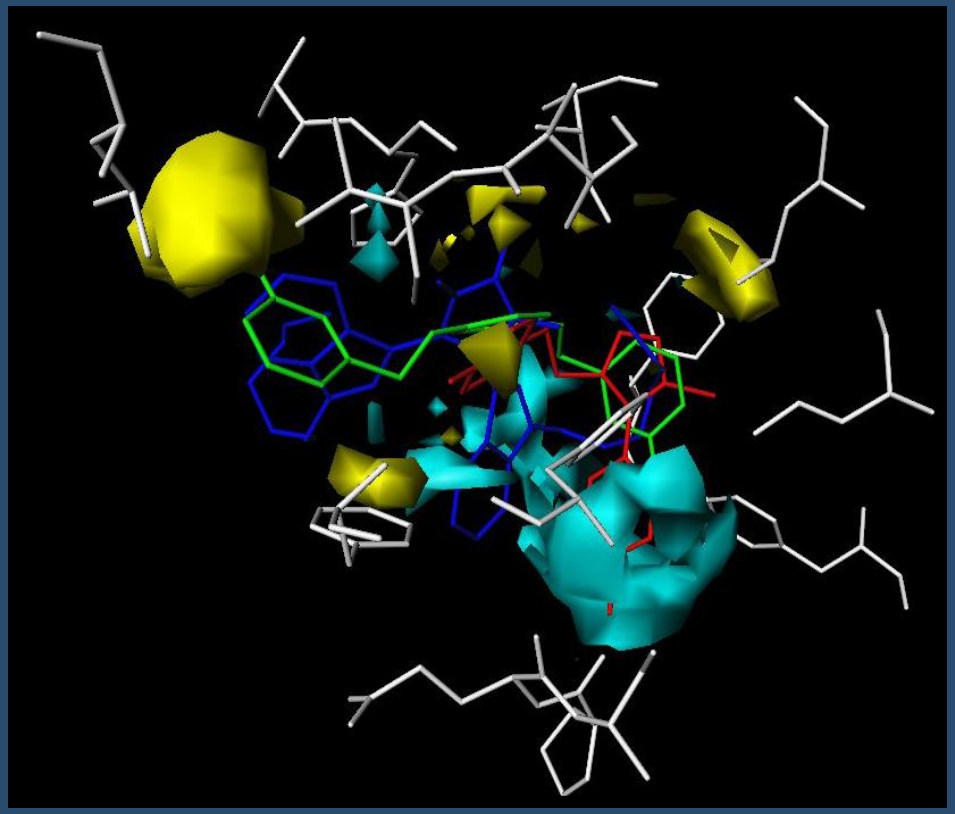
**Inibitori cofattore $NAD^+$**

# 3-D QSAR: Analisi delle mappe

## Inibitori della lisina acetilata



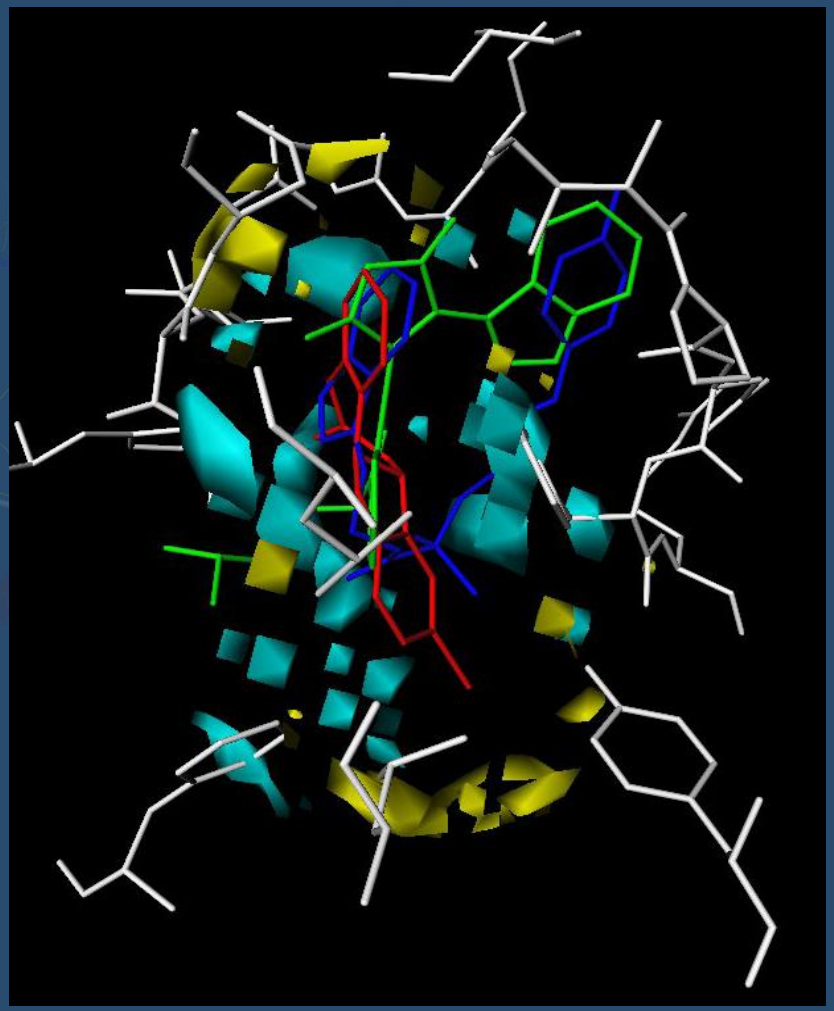
PLS Coefficient atomo sonda DRY



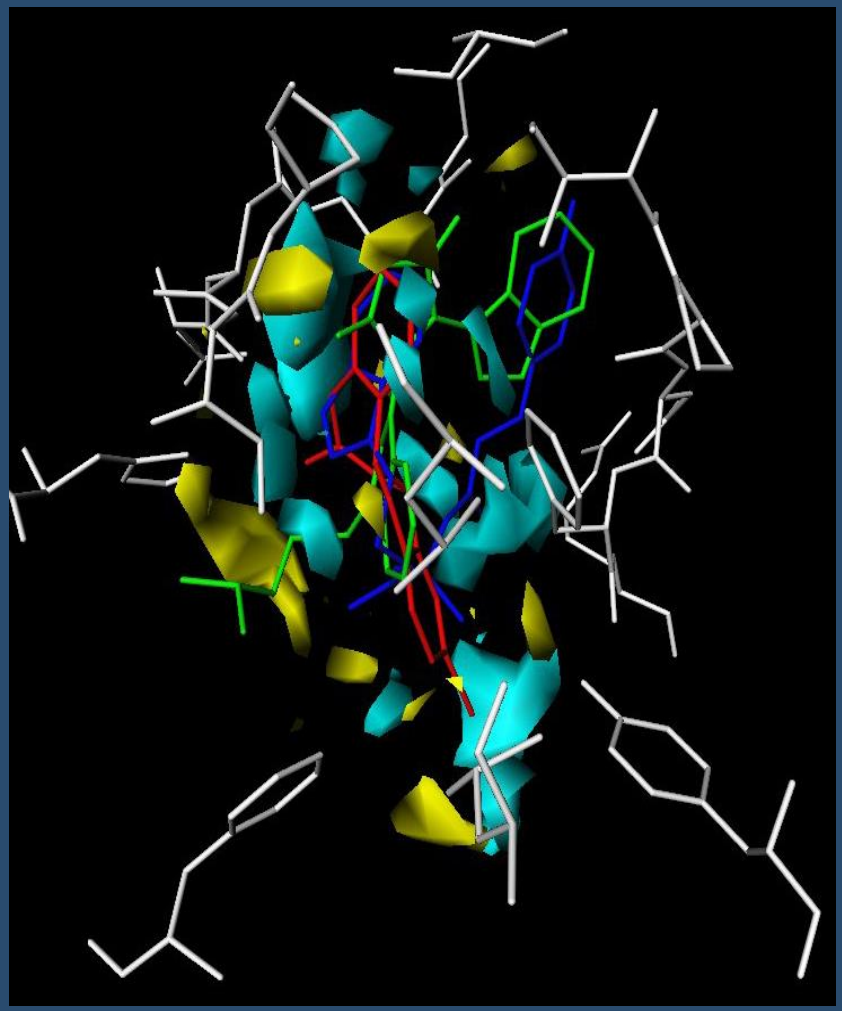
PLS Coefficient atomo sonda OH

# 3-D QSAR: Analisi delle mappe

## Inibitori del cofattore $NAD^+$



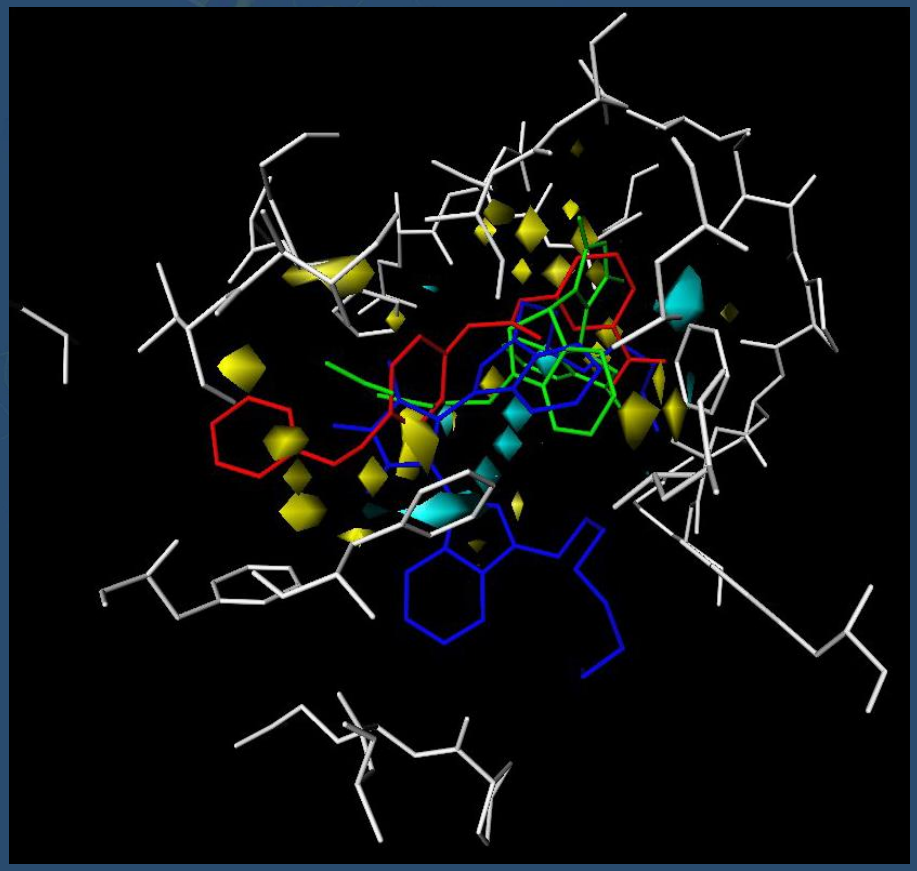
PLS Coefficient atomo sonda DRY



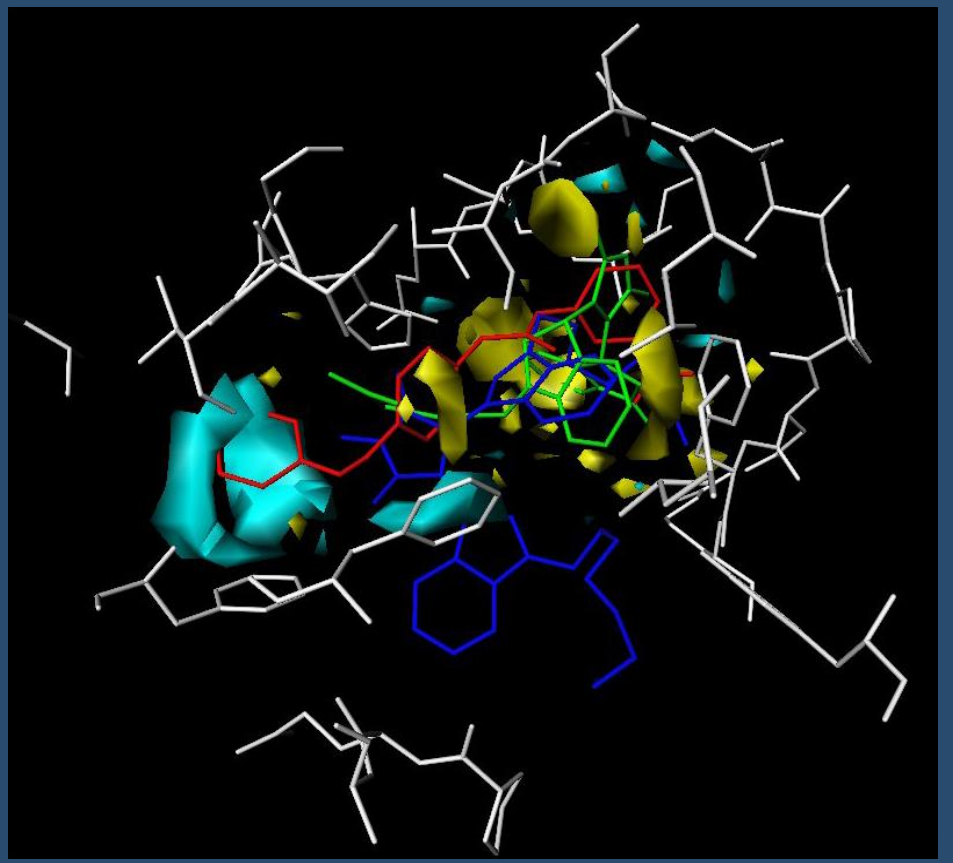
PLS Coefficient atomo sonda OH

# 3-D QSAR: Analisi delle mappe

## Inibitori con disposizione intermedia



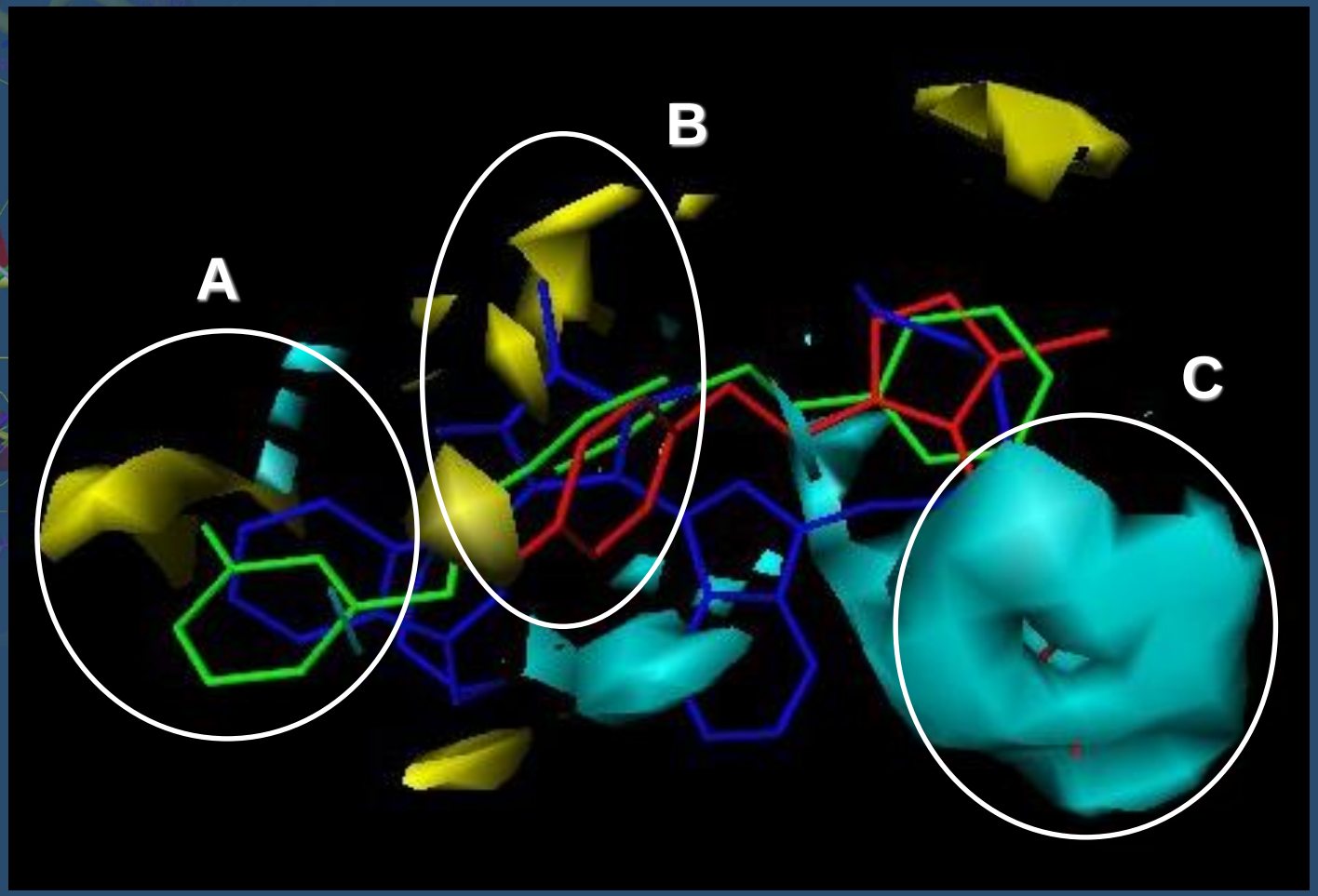
PLS Coefficient atomo sonda DRY



PLS Coefficient atomo sonda OH

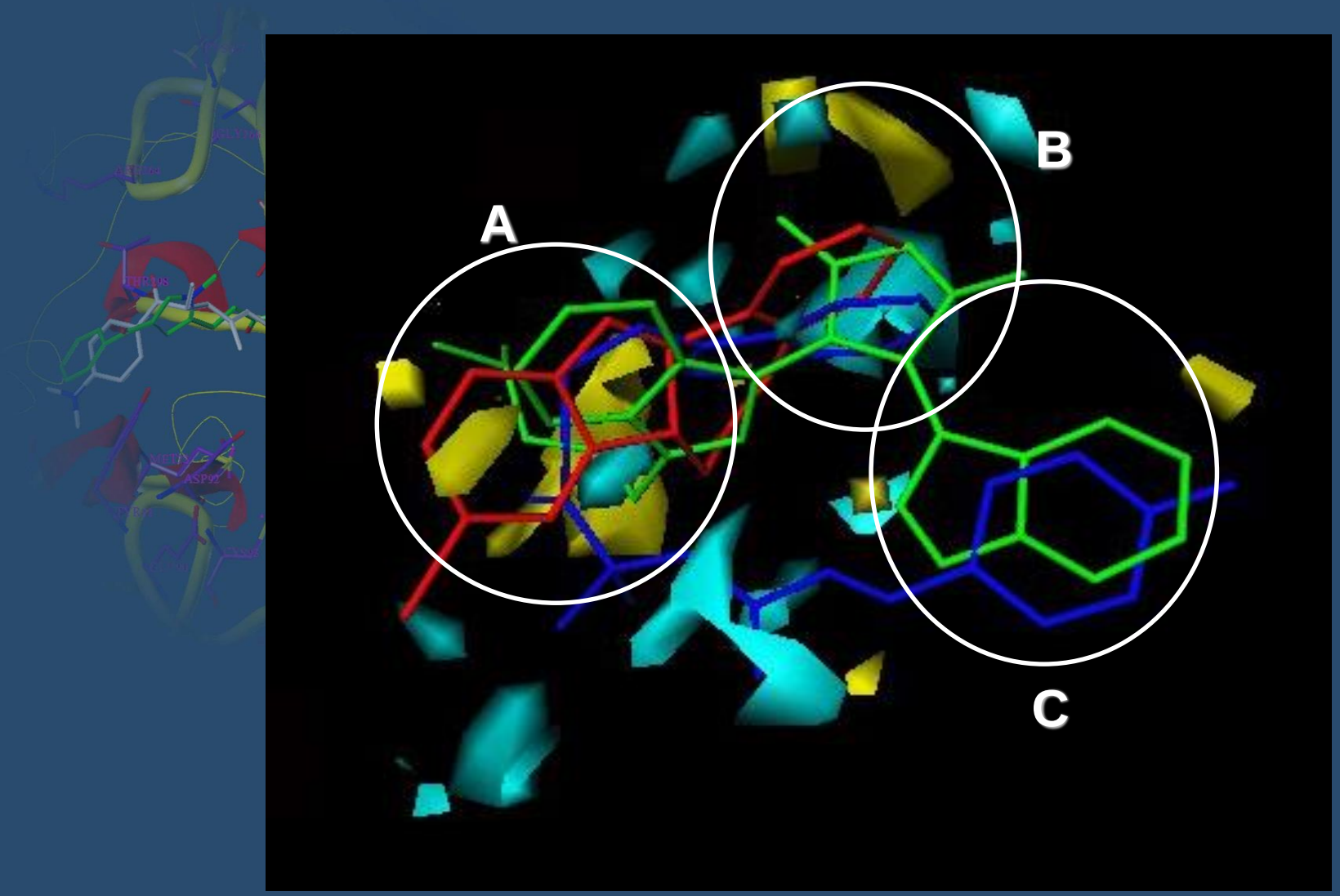
# 3-D QSAR: Analisi delle mappe

## Inibitori della lisina acetilata



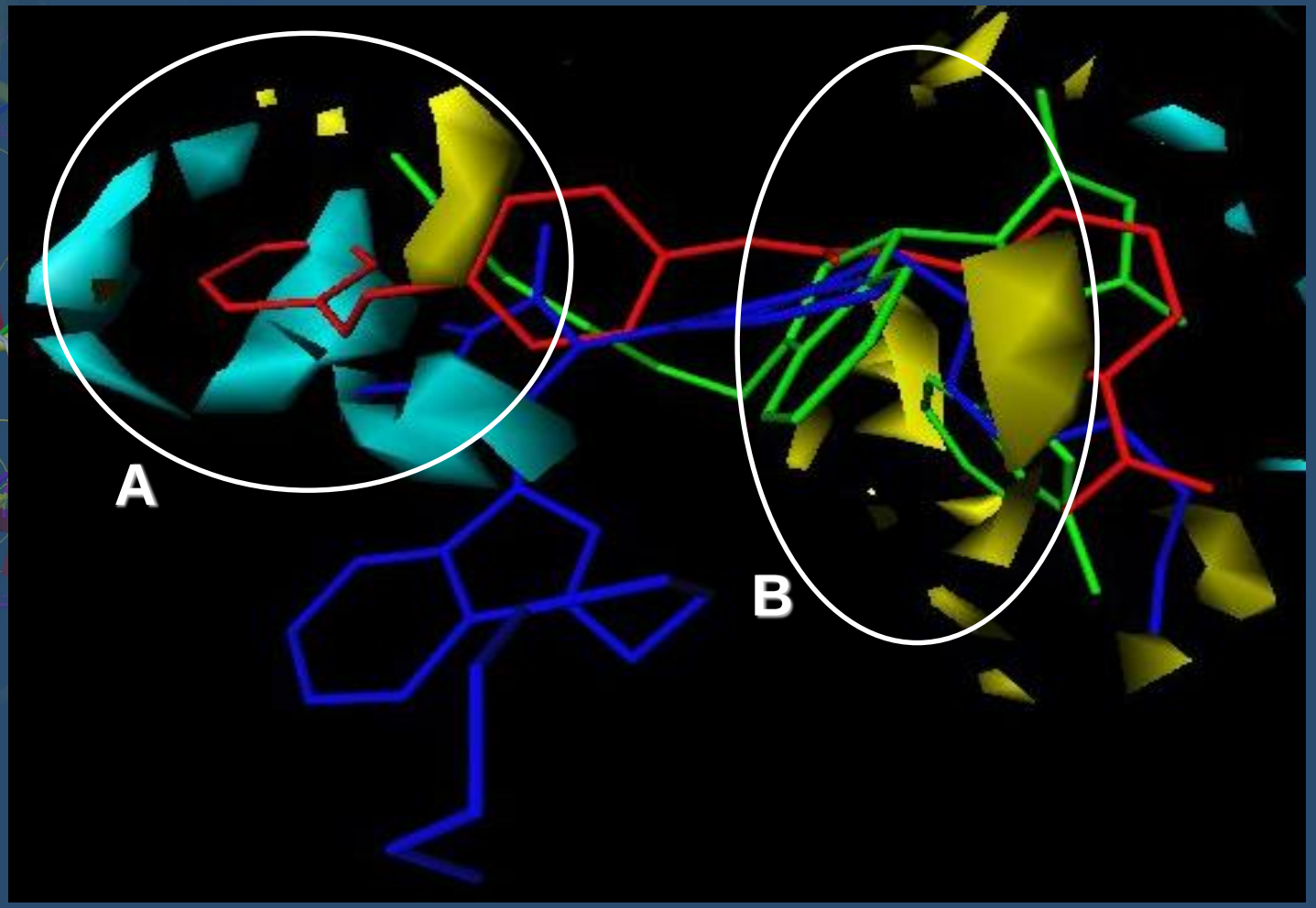
# 3-D QSAR: Analisi delle mappe

## Inibitori del cofattore $NAD^+$

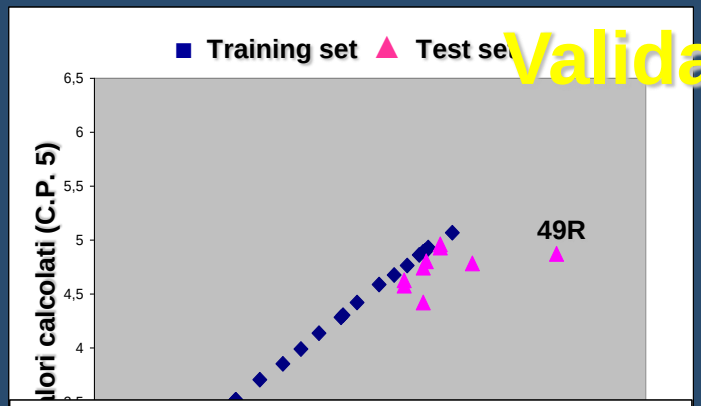


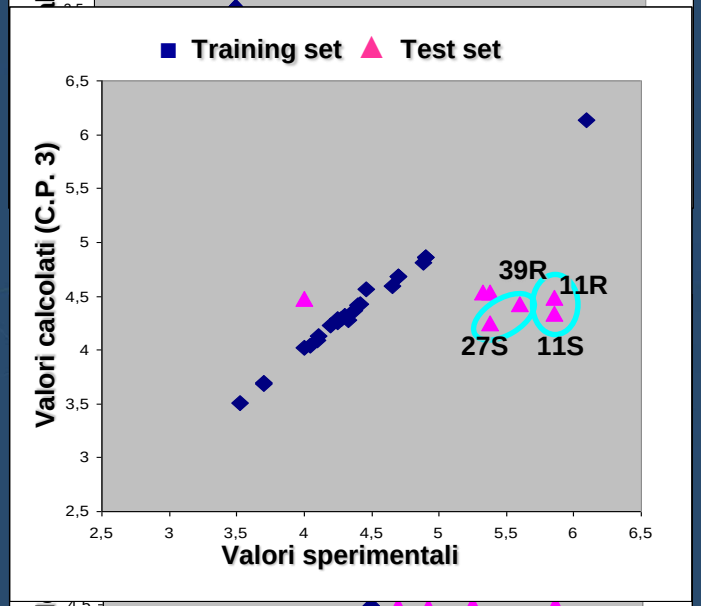
# 3-D QSAR: Analisi delle mappe

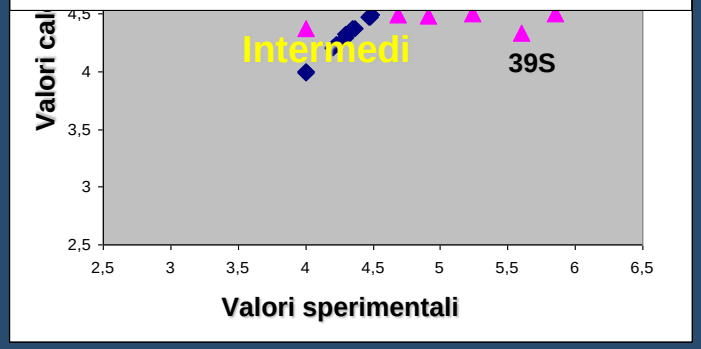
## Inibitori con disposizione intermedia



# Validazione dei modelli




**Cofattore**

| Probabile sito di inibizione | All. | T. s. | Test set esterno | $SDEP_{EXT}$ | P.C. |
|---|---|---|---|---|---|
| Lisina acetilata | BC | 19 | 6S, 7S, 8S, 9S, 10S, 6R, 7R, 8R, 49R | 0,4 | 5 |
| Sito Intermedio | BC | 24 | 11S, 27S, 46S, 11R, 27R, 31R, 39R | 1,09 | 3 |
| Cofattore $NAD^+$ | BC | 18 | 31S, 31S, 49S, 9R, 10R, 46R | 0,85 | 5 |

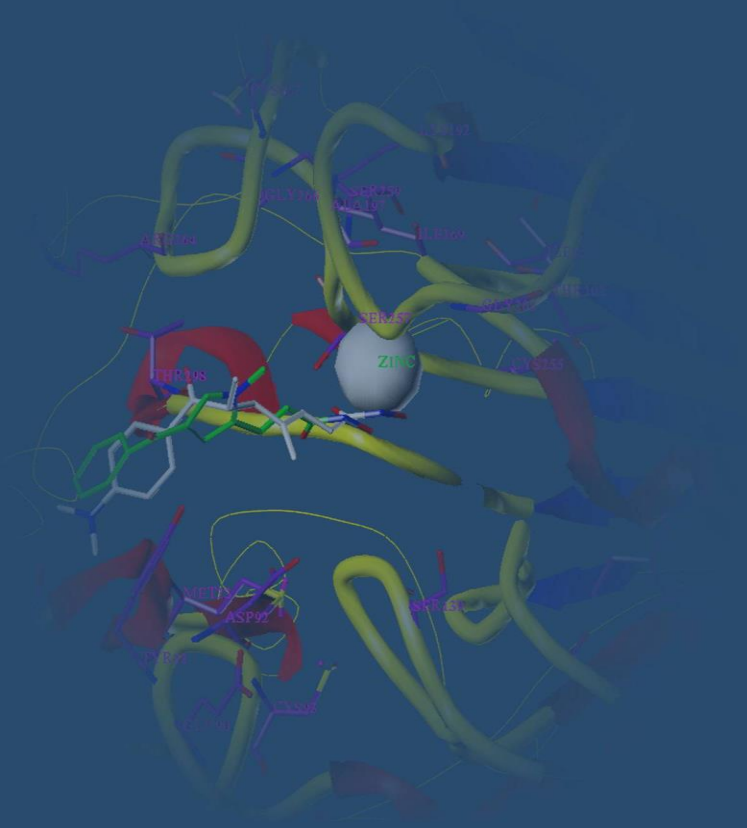
| Struttura | Sito di inibizione | Attivita Sperimentale | Attivita Calcolata | Errore di Predizione |
|---|---|---|---|---|
| 46S | INTERMEDI | 4 | 4,47 | -0,47 |
| 46R | NAD+ | 4 | 4,37 | -0,37 |
| 8R | ALY | 5,01 | 4,96 | 0,05 |
| 8S | ALY | 5,01 | 4,93 | 0,08 |
| 10S | ALY | 4,91 | 4,81 | 0,1 |
| 6S | ALY | 4,75 | 4,63 | 0,12 |
| 7S | ALY | 4,89 | 4,75 | 0,14 |
| 6R | ALY | 4,75 | 4,57 | 0,18 |
| 31S | NAD+ | 4,68 | 4,49 | 0,19 |
| 10R | NAD+ | 4,91 | 4,48 | 0,43 |
| 9S | ALY | 5,24 | 4,78 | 0,46 |
| 7R | ALY | 4,89 | 4,42 | 0,47 |
| 9R | NAD+ | 5,24 | 4,5 | 0,74 |
| 31R | INTERMEDI | 5,33 | 4,54 | 0,79 |
| 27R | INTERMEDI | 5,38 | 4,53 | 0,85 |
| 49R | ALY | 5,85 | 4,87 | 0,98 |
| 27S | INTERMEDI | 5,38 | 4,25 | 1,13 |
| 39R | INTERMEDI | 5,6 | 4,43 | 1,17 |
| 39S | NAD+ | 5,6 | 4,33 | 1,27 |
| 49S | NAD+ | 5,85 | 4,5 | 1,35 |
| 11R | INTERMEDI | 5,86 | 4,49 | 1,37 |
| 11S | INTERMEDI | 5,86 | 4,33 | 1,53 |

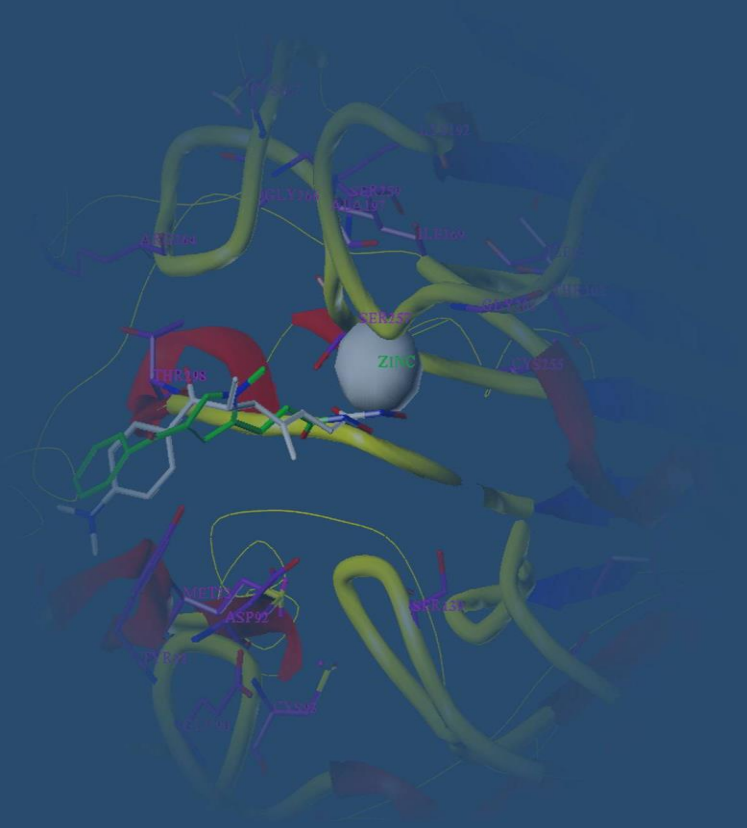
**70%**

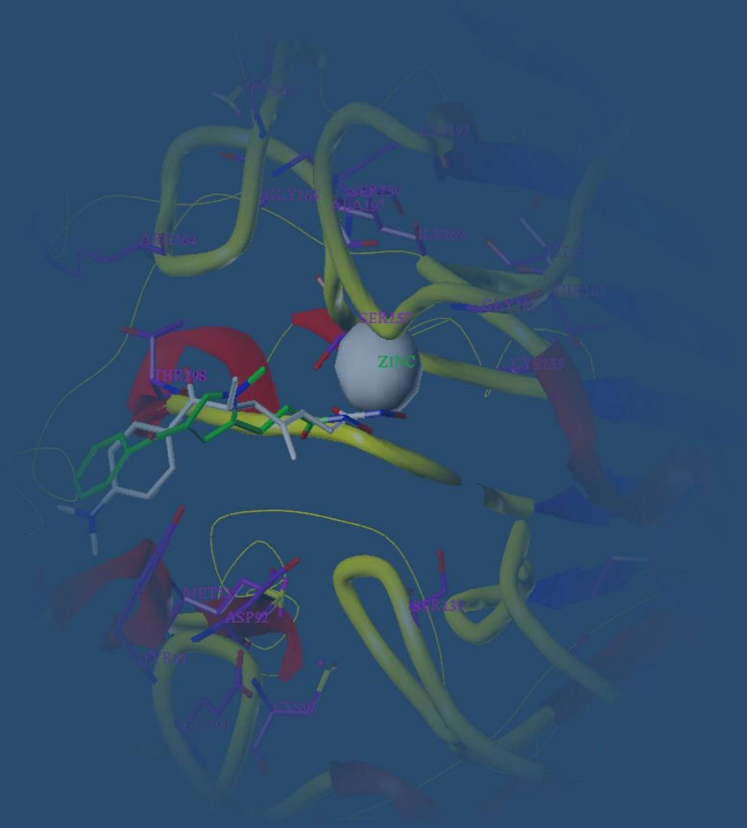
**30%**

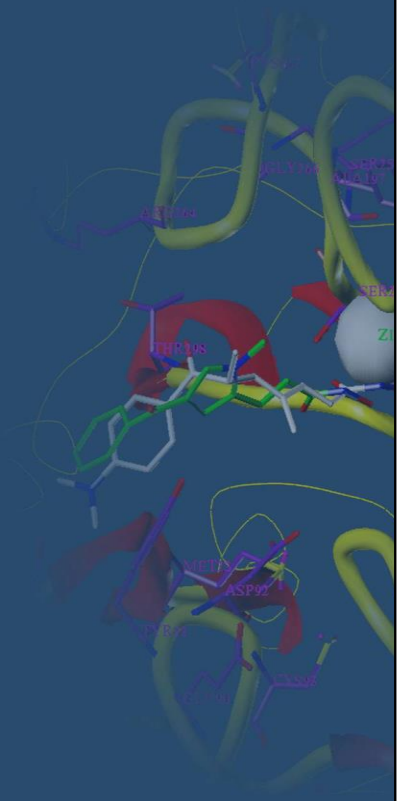
# CONCLUSIONI

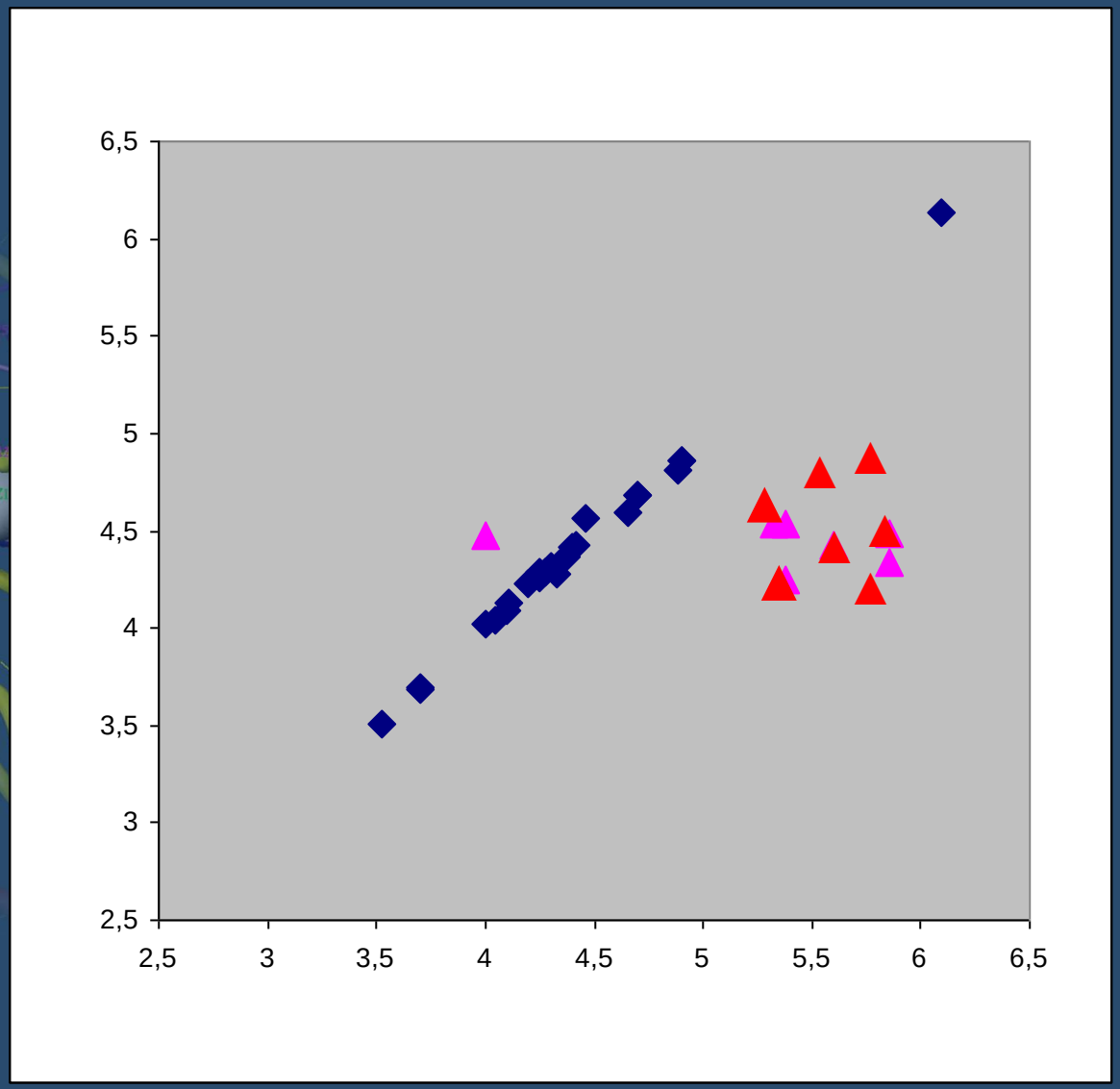
- Docking (Autodock4) di 72 strutture di inibitori SIRT2
- Binding Mode: Tre possibili siti di inibizione per SIRT2
- GRID/GOLPE: individuazione dei gruppi farmacoforici più importanti per l'attività biologica
- Informazioni strutturali su possibili nuovi inibitori SIRT2


SER257
ZINC
TRP109
ASP92


SER257
ZINC
TRP109
ASP92


SER257
ZINC
TRP109
ASP92

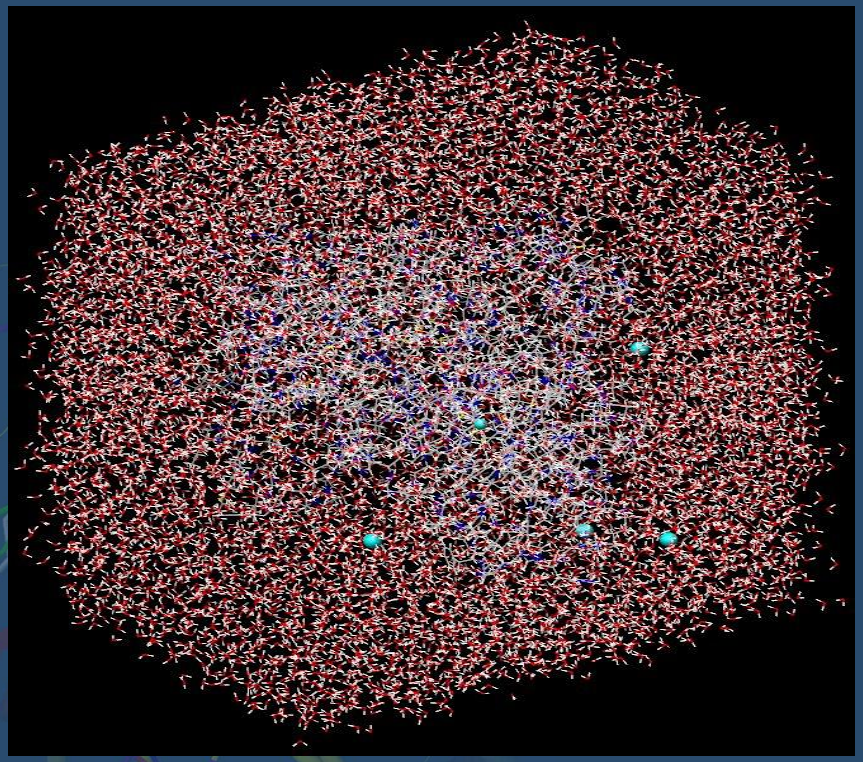



6,5
6
5,5
5
4,5
4
3,5
3
2,5
2,5
3
3,5
4
4,5
5
5,5
6
6,5

# Scelta modelli 3-DQSAR

| sito | All. | n° S. | Ster. | *Probe* | FFD | N | P.C. | $q^2$[a] | r2 | $SDEP_{CV}$[a] | | L(ODS)O | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | S.G. | $q^2$[b] | $SDEP_{CV}$[b] |
| Lisina acetilata | BC | 24 | S | DRY-OH | II | 795 | 4 | 0,91 | 0,99 | 0,15 | 7 | 0,89 | 0,17 |
| Sito Intermedio | BC | 27 | S | DRY-OH | II | 815 | 4 | 0,77 | 0,99 | 0,28 | 6 | 0,74 | 0,3 |
| Cofattore $NAD^+$ | BC | 21 | S | DRY-OH | II | 886 | 5 | 0,78 | 0,99 | 0,22 | 4 | 0,67 | 0,28 |
| Lisina acetilata | BC | 23 | R | DRY-OH | II | 627 | 5 | 0,86 | 0,99 | 0,28 | 7 | 0,86 | 0,21 |
| Sito Intermedio | BC | 28 | R | DRY-OH | II | 893 | 5 | 0,77 | 0,99 | 0,3 | 6 | 0,67 | 0,35 |
| Cofattore $NAD^+$ | BC | 21 | R | DRY-OH | II | 1028 | 5 | 0,68 | 0,99 | 0,21 | 5 | 0,7 | 0,2 |
| Sito Intermedio | BD | 36 | S | DRY-OH | II | 1072 | 4 | 0,65 | 0,99 | 0,32 | 7 | 0,72 | 0,28 |
| Cofattore $NAD^+$ | BD | 34 | S | DRY-OH | II | 897 | 5 | 0,7 | 0,98 | 0,3 | 5 | 0,67 | 0,31 |
| Sito Intermedio | BD | 36 | R | DRY-OH | II | 939 | 4 | 0,62 | 0,94 | 0,3 | 6 | 0,72 | 0,28 |
| Cofattore $NAD^+$ | BD | 33 | R | DRY-OH | II | 945 | 5 | 0,57 | 0,98 | 0,32 | 5 | -0,05 | 0,36 |

# Minimizzazione del complesso ternario



→ AMBER8

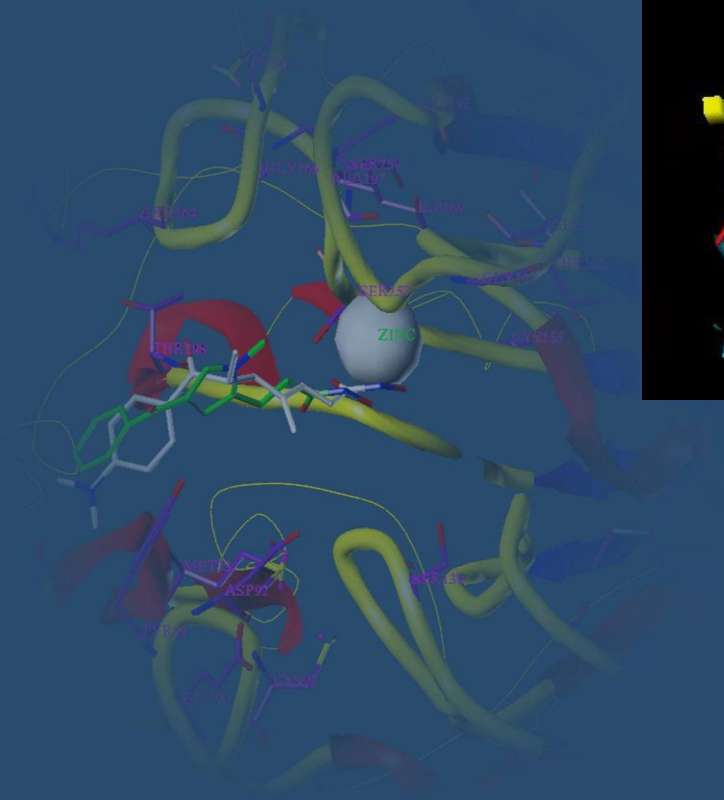
**Minimizzazione solvente acquoso**

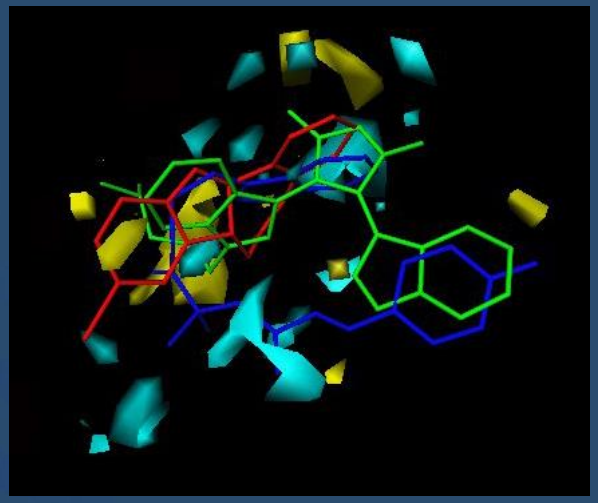
**Minimizzazione complesso ternario**

```
A
Minimizzazione complesso: solo H20
&cntrl
  imin   = 1,
  maxcyc = 1000,
  ncyc   = 250,
  ntb    = 1,
  ntr    = 1,
  cut    = 10
 /
Hold the Everything fixed except the protein
500.0
RES 1 356
END
END
```

```
B
Minimizzazione di tutto il sistema mantenendo fermo il backbone
&cntrl
  imin   = 1,
  maxcyc = 5000,
  ncyc   = 1000,
  ntb    = 1,
  ntr    = 1,
  cut    = 10
 /
Hold the backbone fixed
100.00
FIND
* * M *
SEARCH
RES 1 356
END
END
```

# Analisi degli inibitori del $NAD^+$

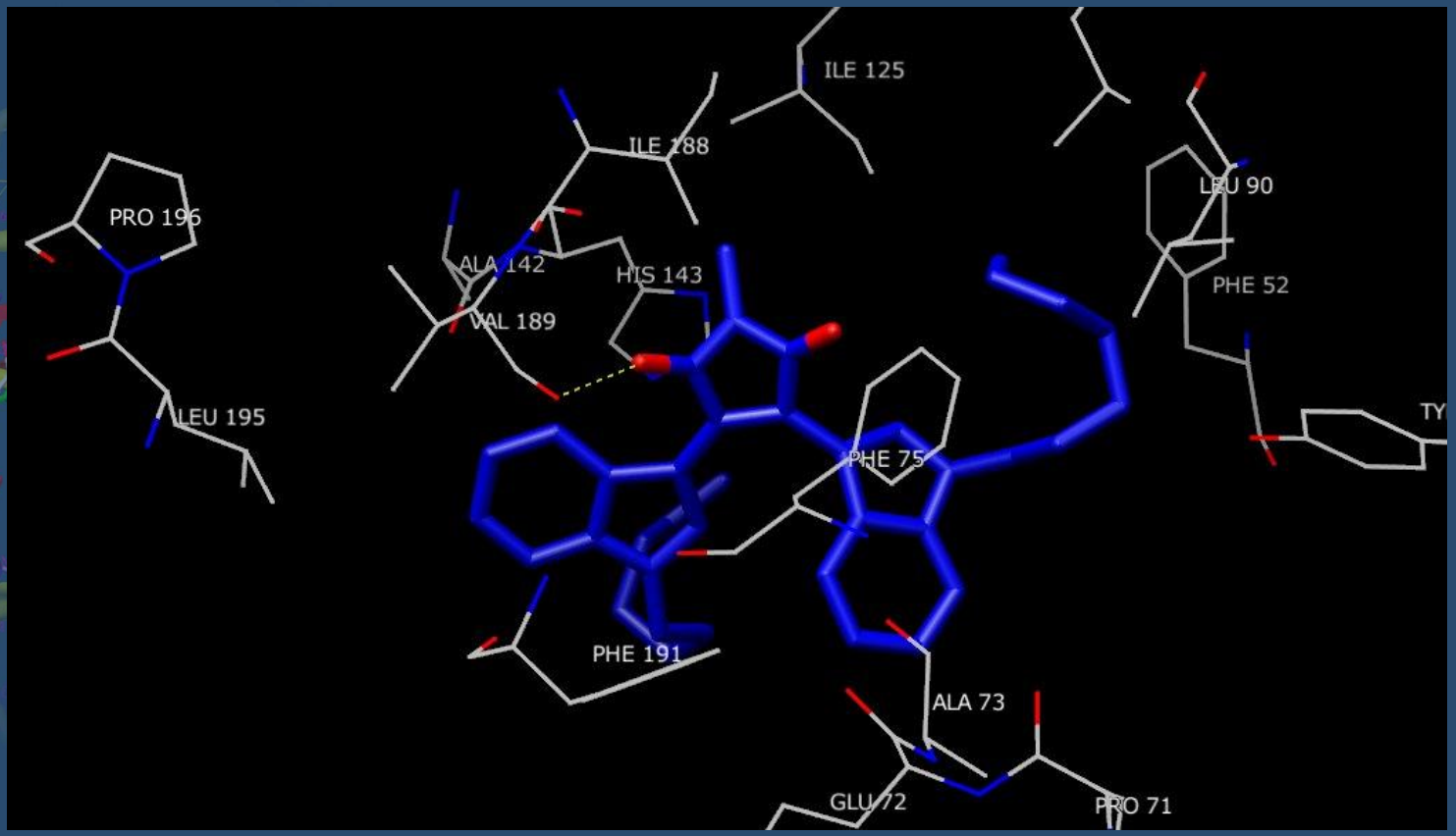


Atomo sonda
OH

# Analisi del binding mode degli inibitori della lisina acetilata

## Strutture mediamente attive

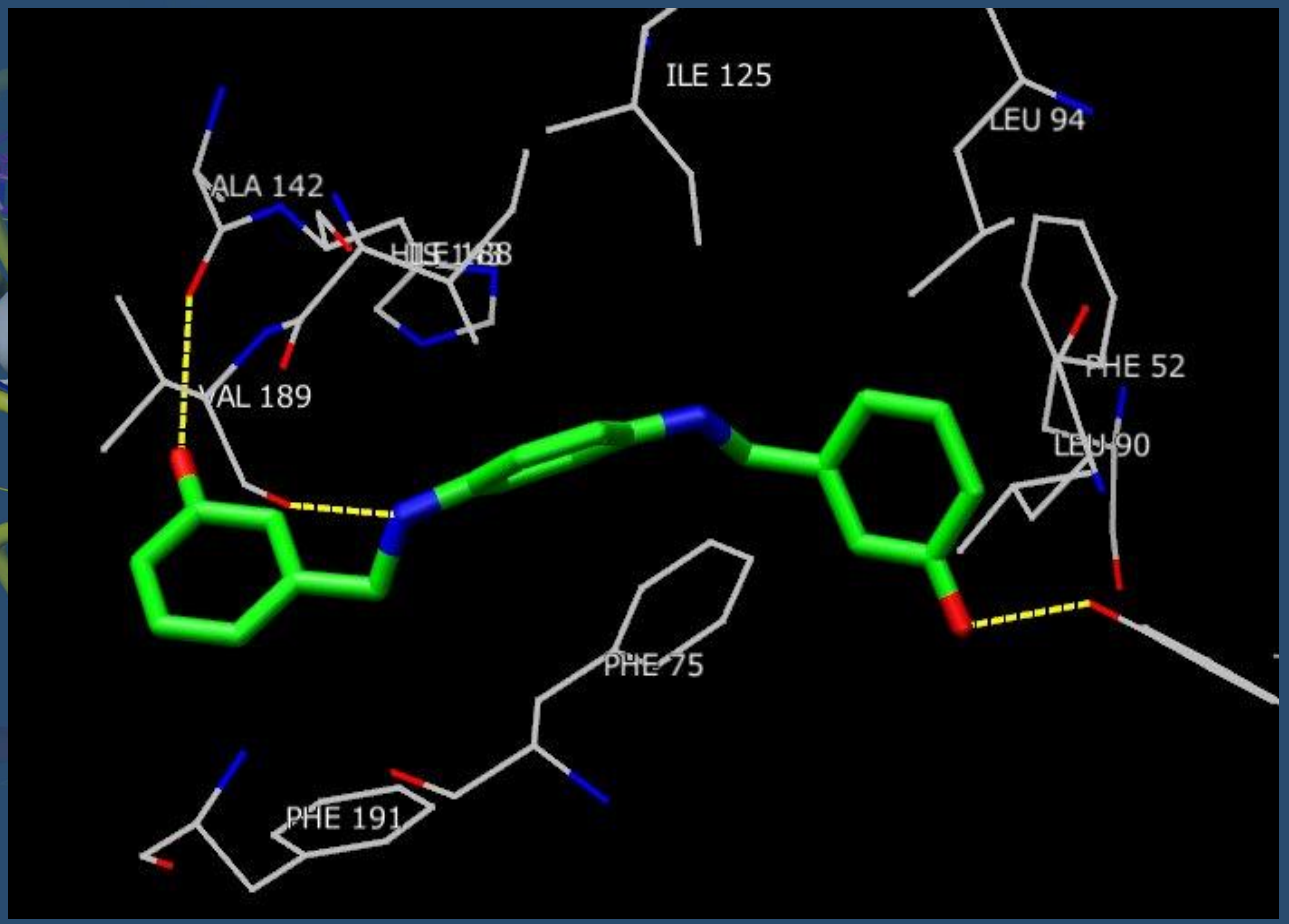



**Derivati bisindolmaleimmidici**

**29** $IC_{50}$= 100 µmoli

# Analisi del binding mode degli inibitori della lisina acetilata

## Strutture più attive

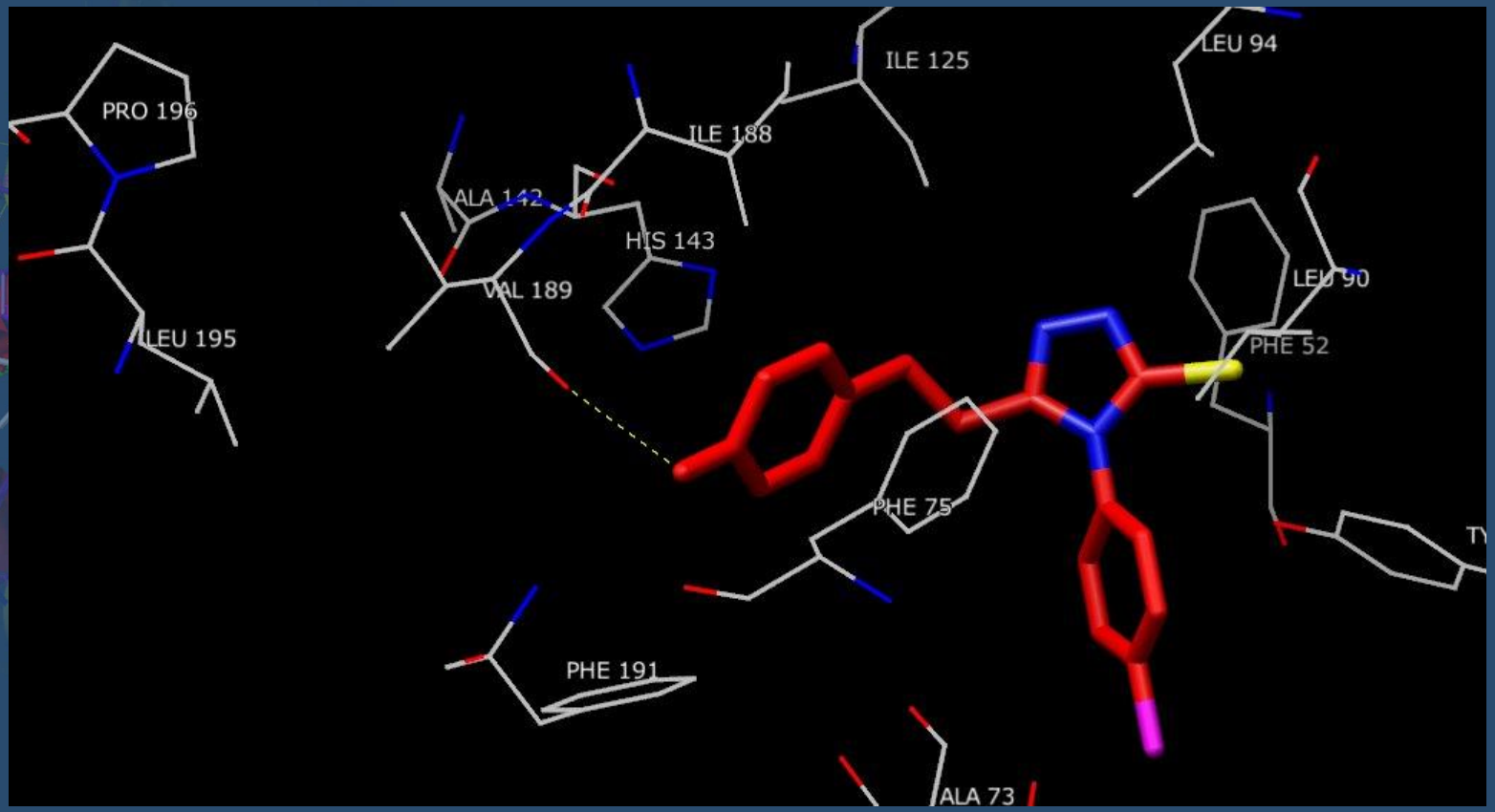



**Derivati N-N'-bisbenzilidenbenzenici**

**64** $IC_{50}$ = 8 µmoli

# Analisi del binding mode degli inibitori della lisina acetilata

## Strutture meno attive

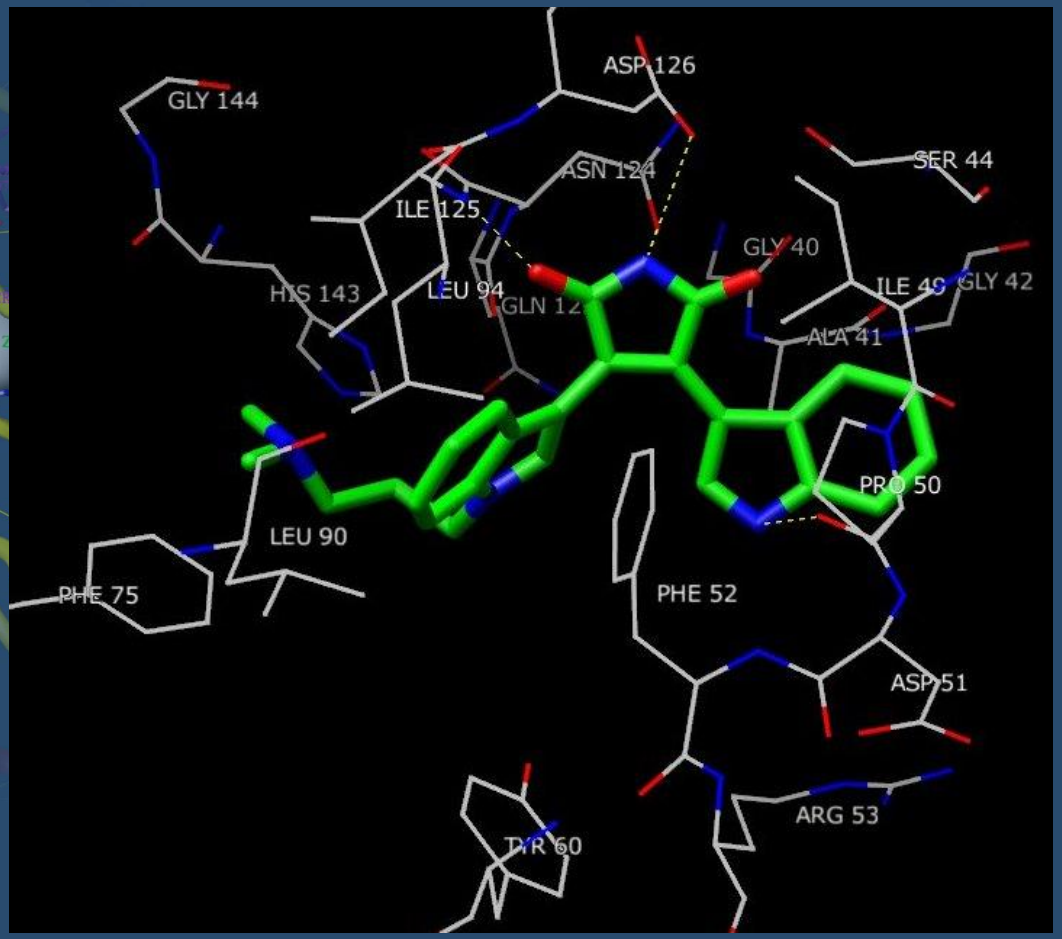



## Derivati triazolici

**1** $IC_{50}$ > 300 µM

# Analisi del binding mode degli inibitori del cofattore $NAD^+$

## Strutture più attive

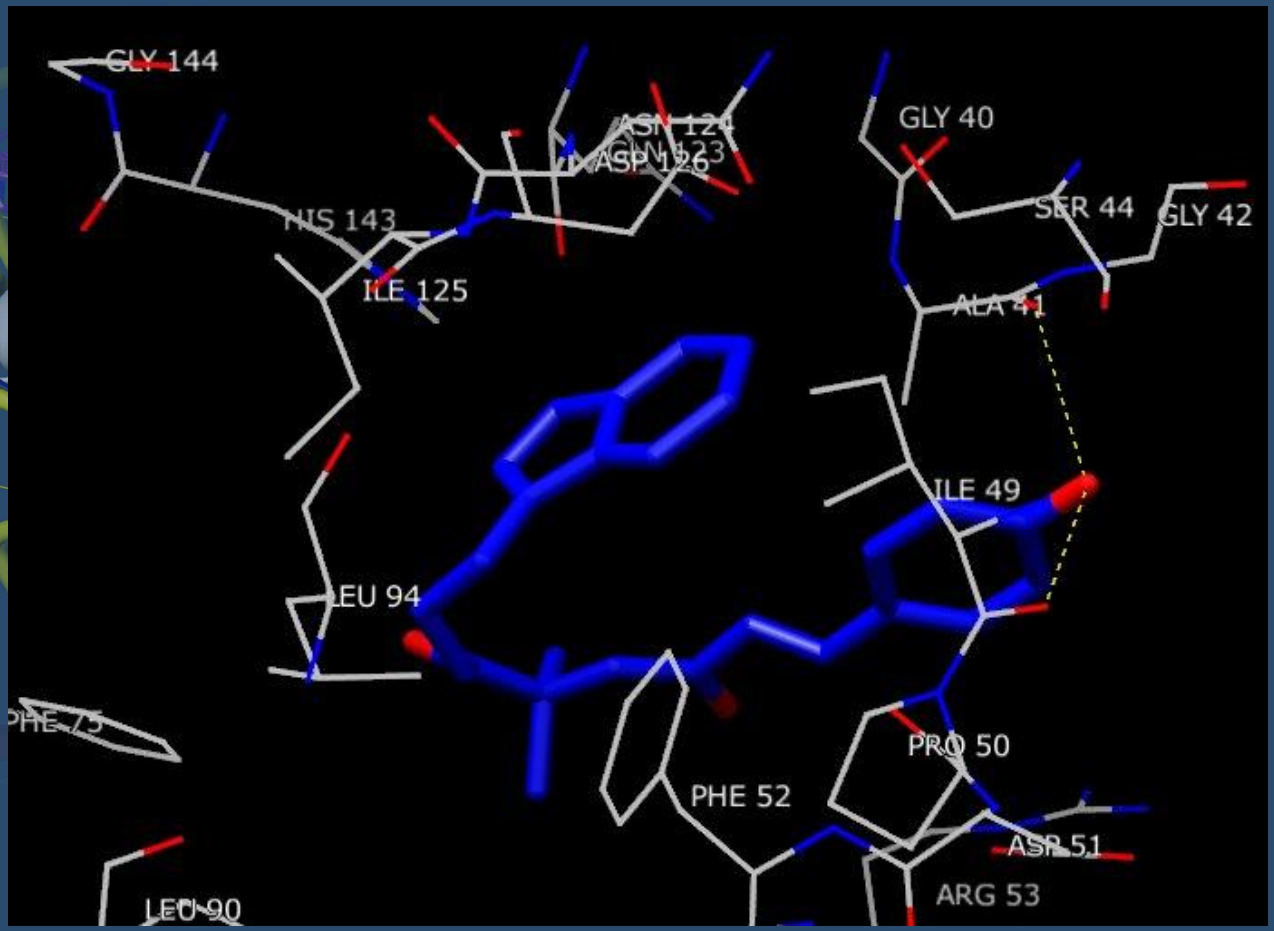



## Derivati bisindolmaleimmidici

**26 $IC_{50}$= 7,3 µM**

# Analisi del binding mode degli inibitori del cofattore $NAD^+$

## Strutture mediamente attive

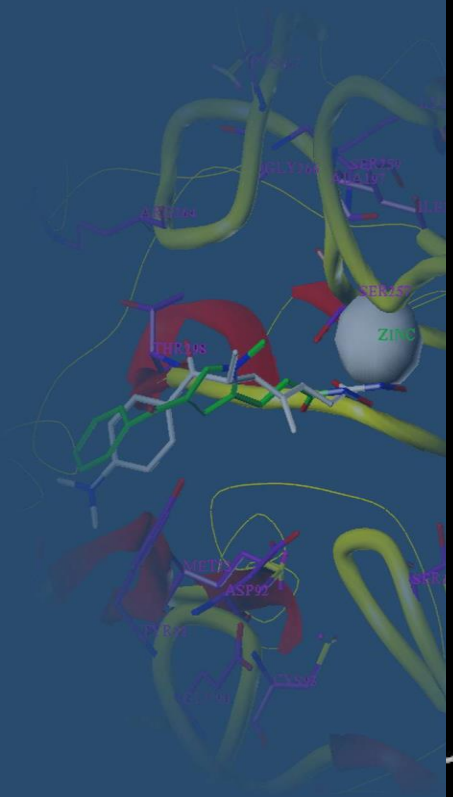


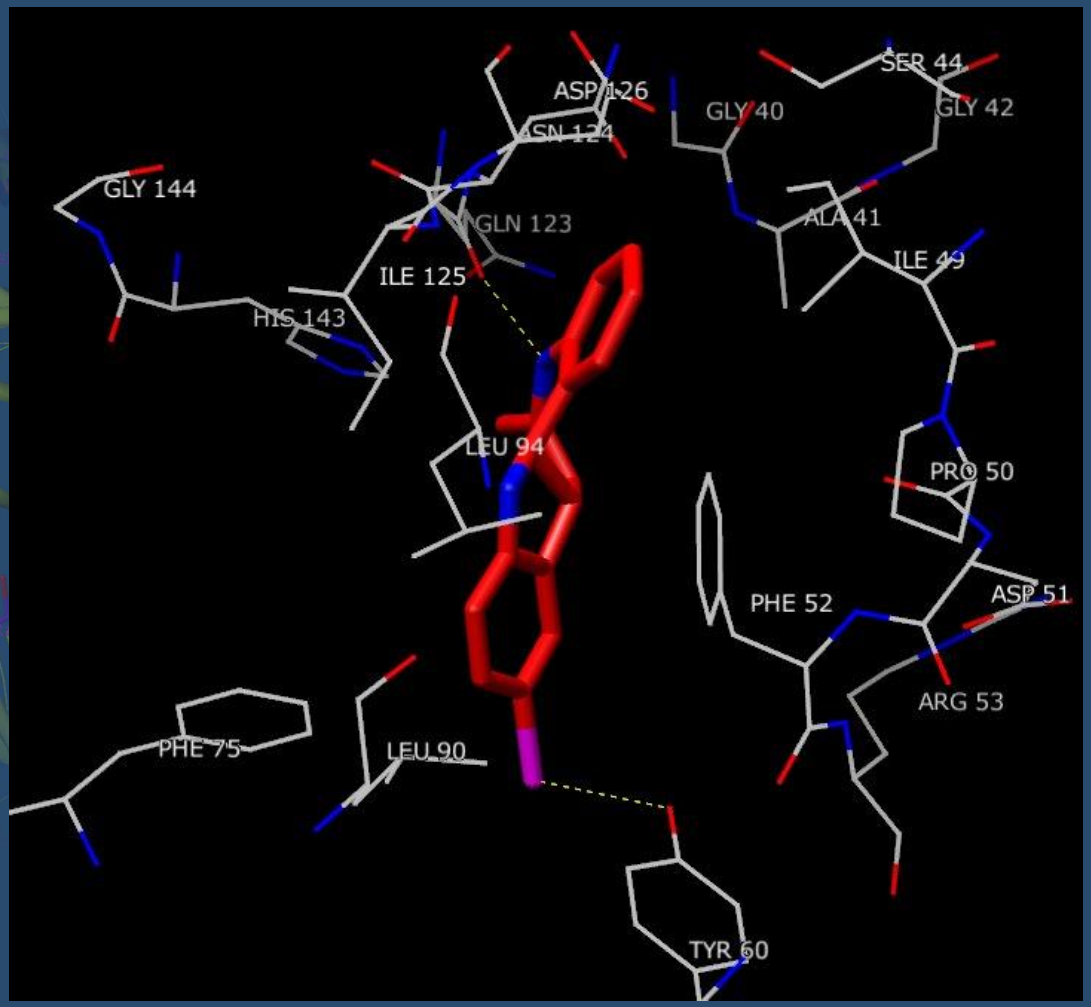

## Derivati triptamidici

**15** $IC_{50}$ **= 50 µM**

# Analisi del binding mode degli inibitori del cofattore NAD+

## Strutture poco attive



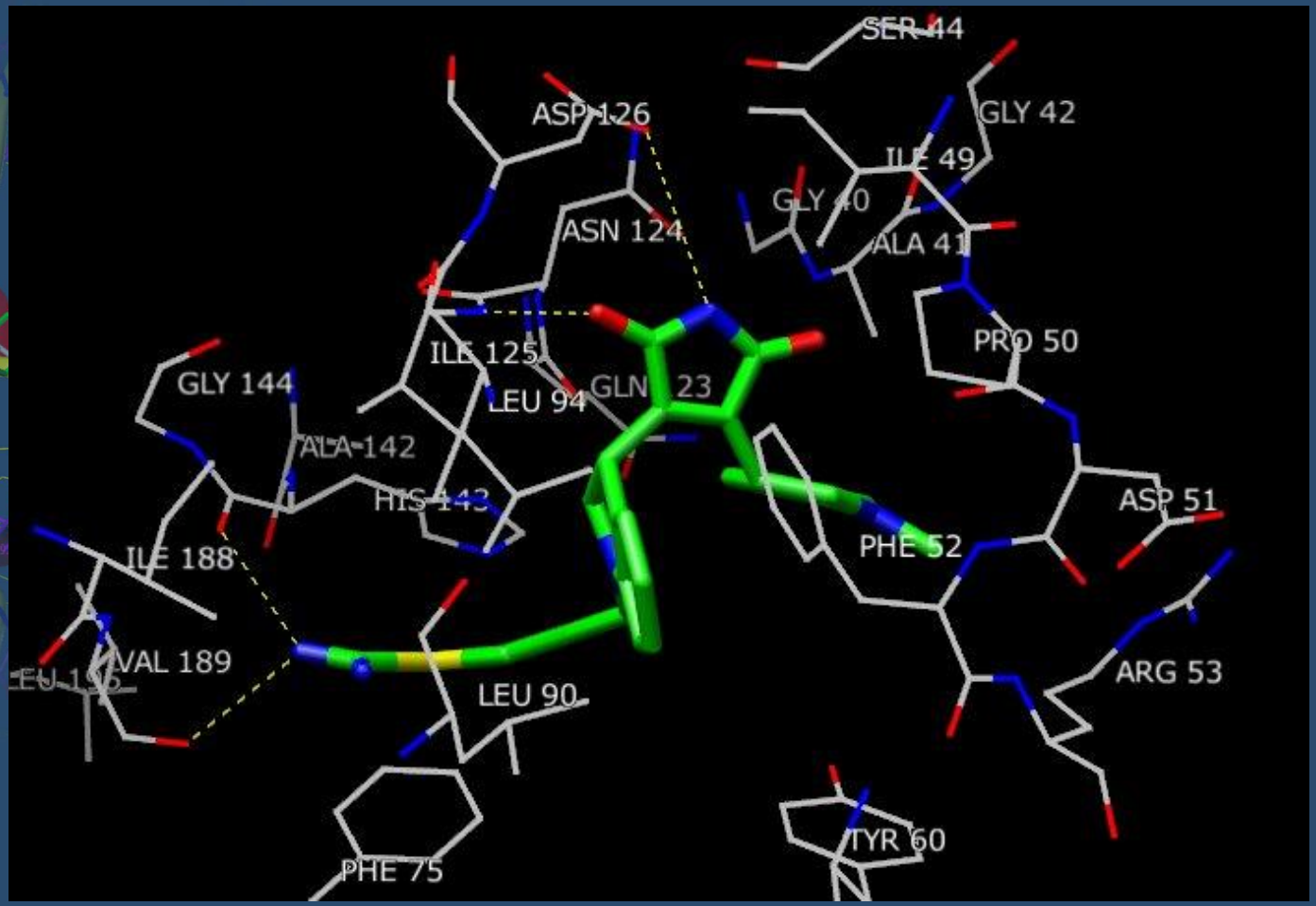



## Derivati paullonici

**35 $IC_{50}$= 100 µmoli**

# Analisi del binding mode degli inibitori intermedi

## Strutture più attive

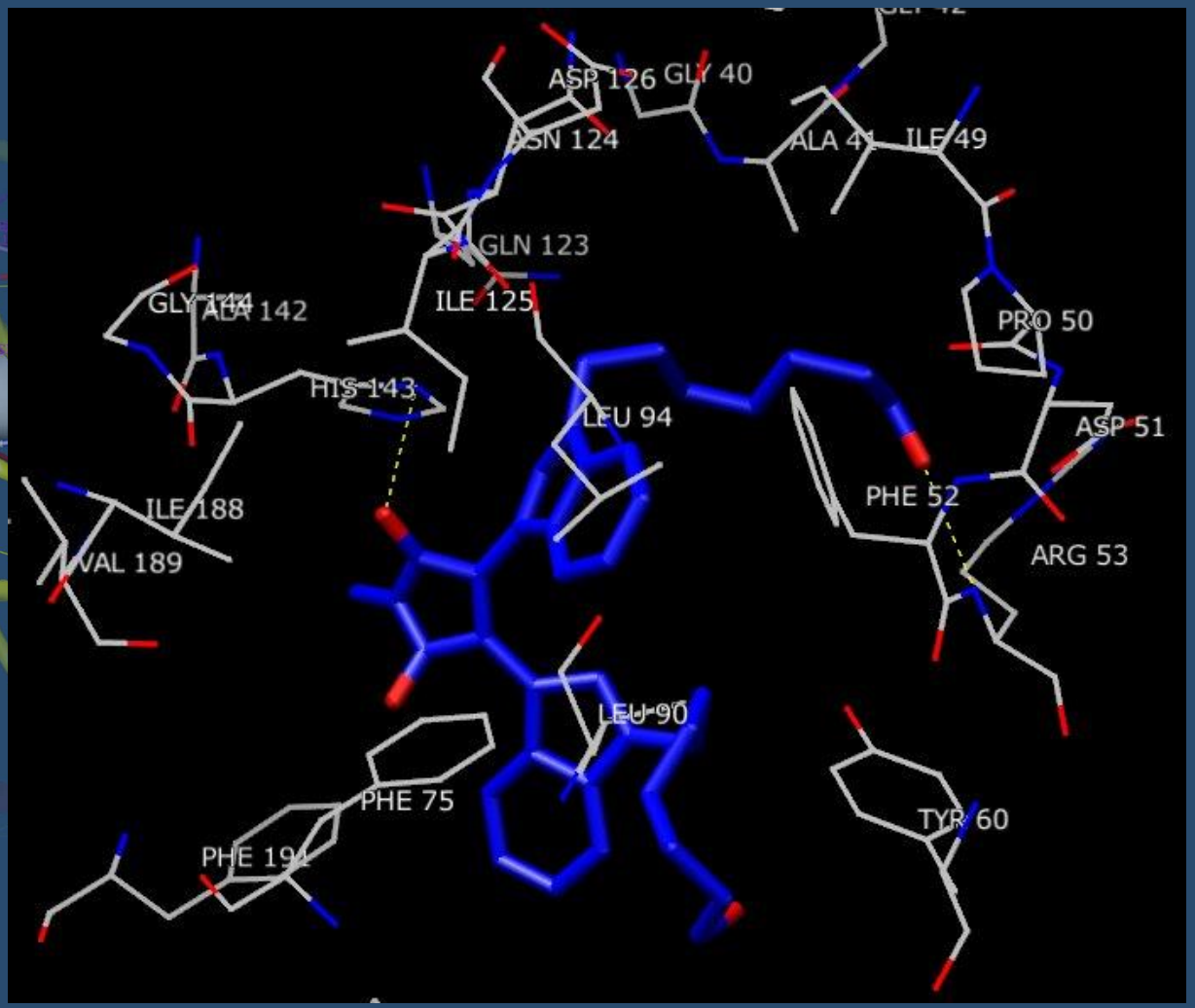



## Derivati bisindolmaleimmidici

**28** $IC_{50}$= 0,8 µmoli

# Analisi del binding mode degli inibitori intermedi

## Strutture mediamente attive

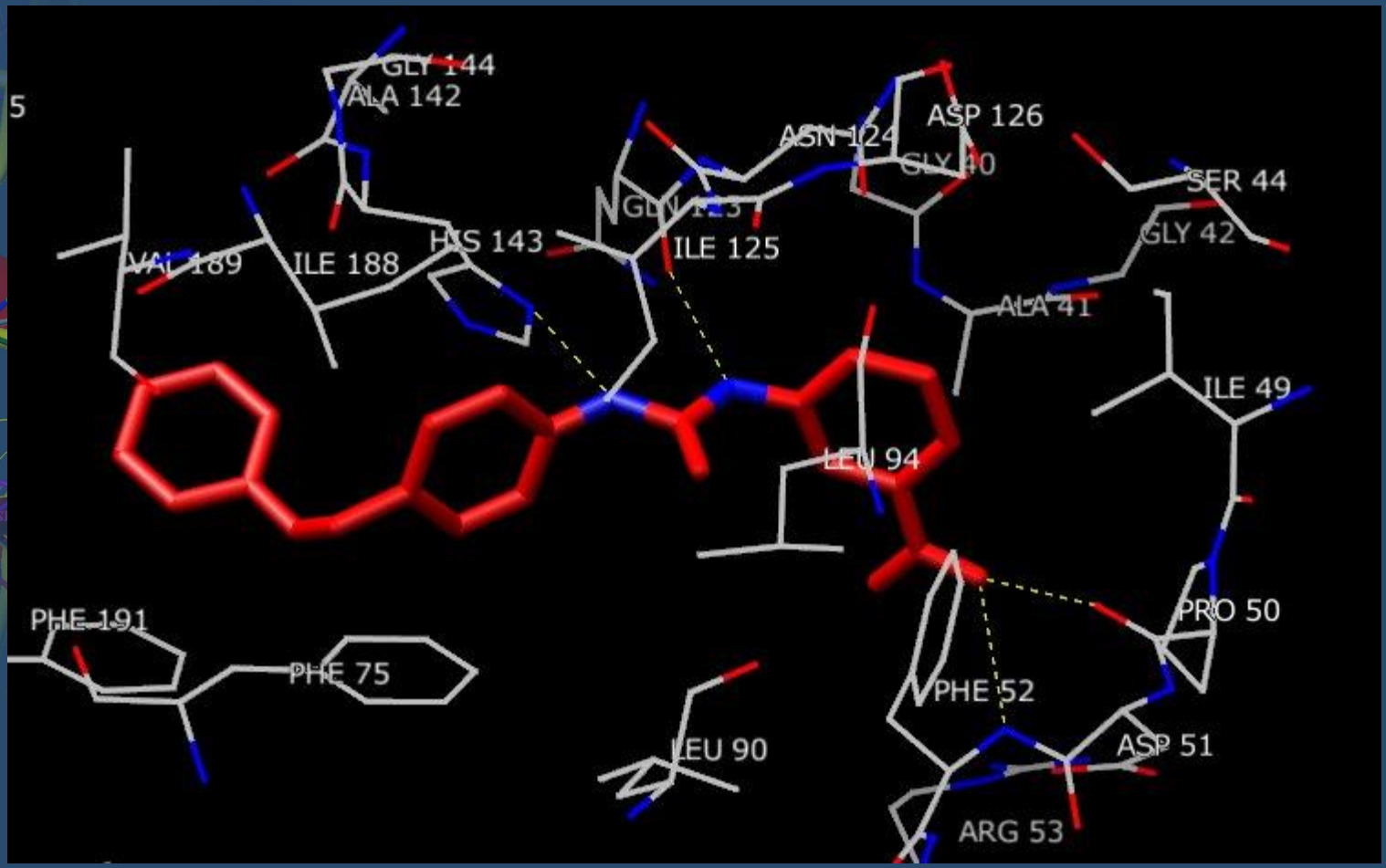



## Derivati bisindolmaleimmidici

28 $IC_{50}$= 100 µmoli

# Analisi del binding mode degli inibitori intermedi

## Strutture poco attive




## Strutture bisfenilammidiche

**2** $IC_{50}$ > 300 µmoli