Studi di docking molecolare e 3-D QSAR su inibitori della demetilasi istonica umana JMJD2A/KDM4A

Dipartimento di Farmacia dell'Università degli studi di Napoli Federico II
Facoltà di Farmacia e Medicina, Sapienza Università di Roma
Corso di Laurea in chimica e tecnologia farmaceutiche

Tesi Sperimentale in Modellistica Molecolare

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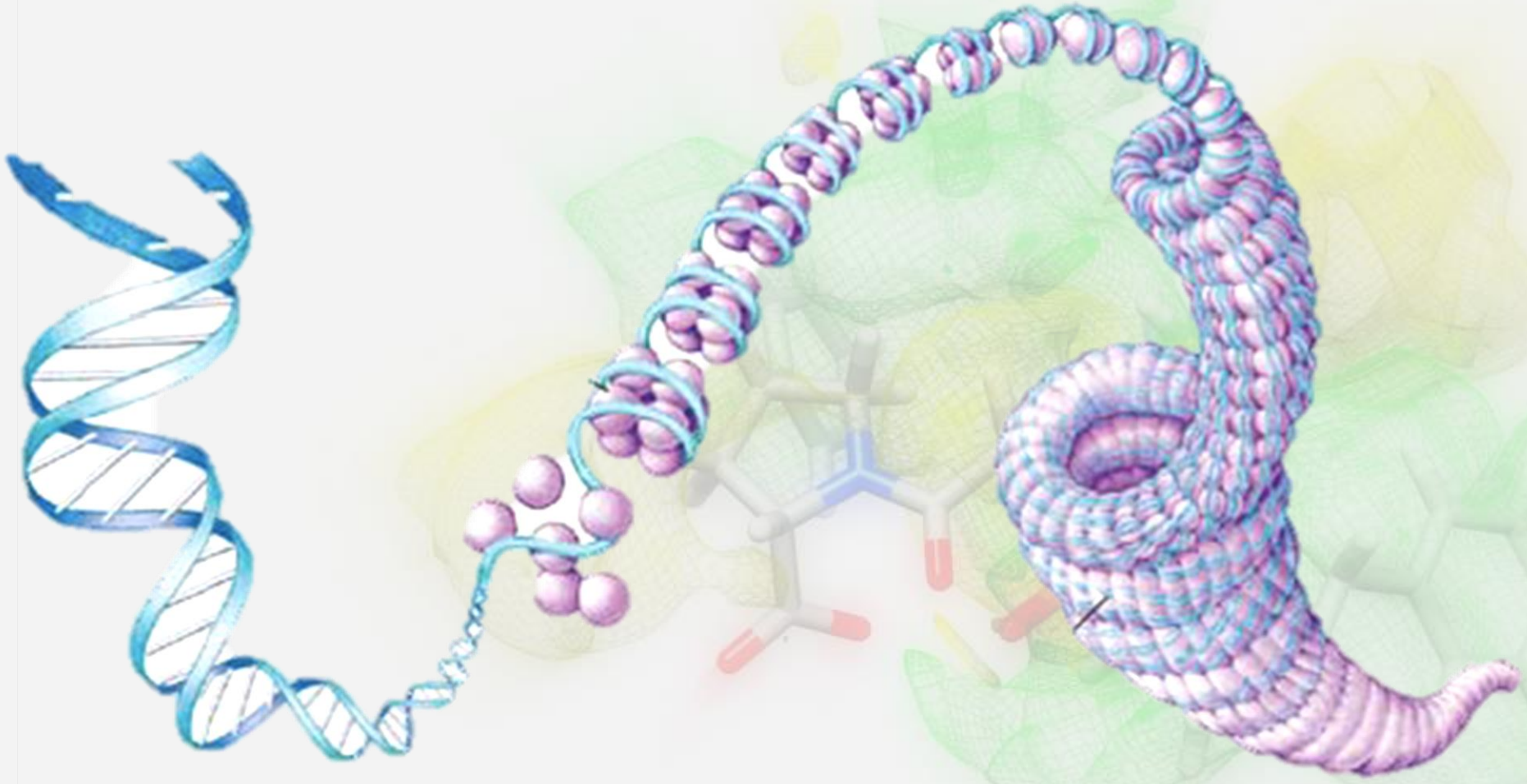
Candidato : Alfredo Gallo
Matricola: 512/1132

Relatori: Prof. Rino Ragno
Prof.ssa Caterina Fattorusso

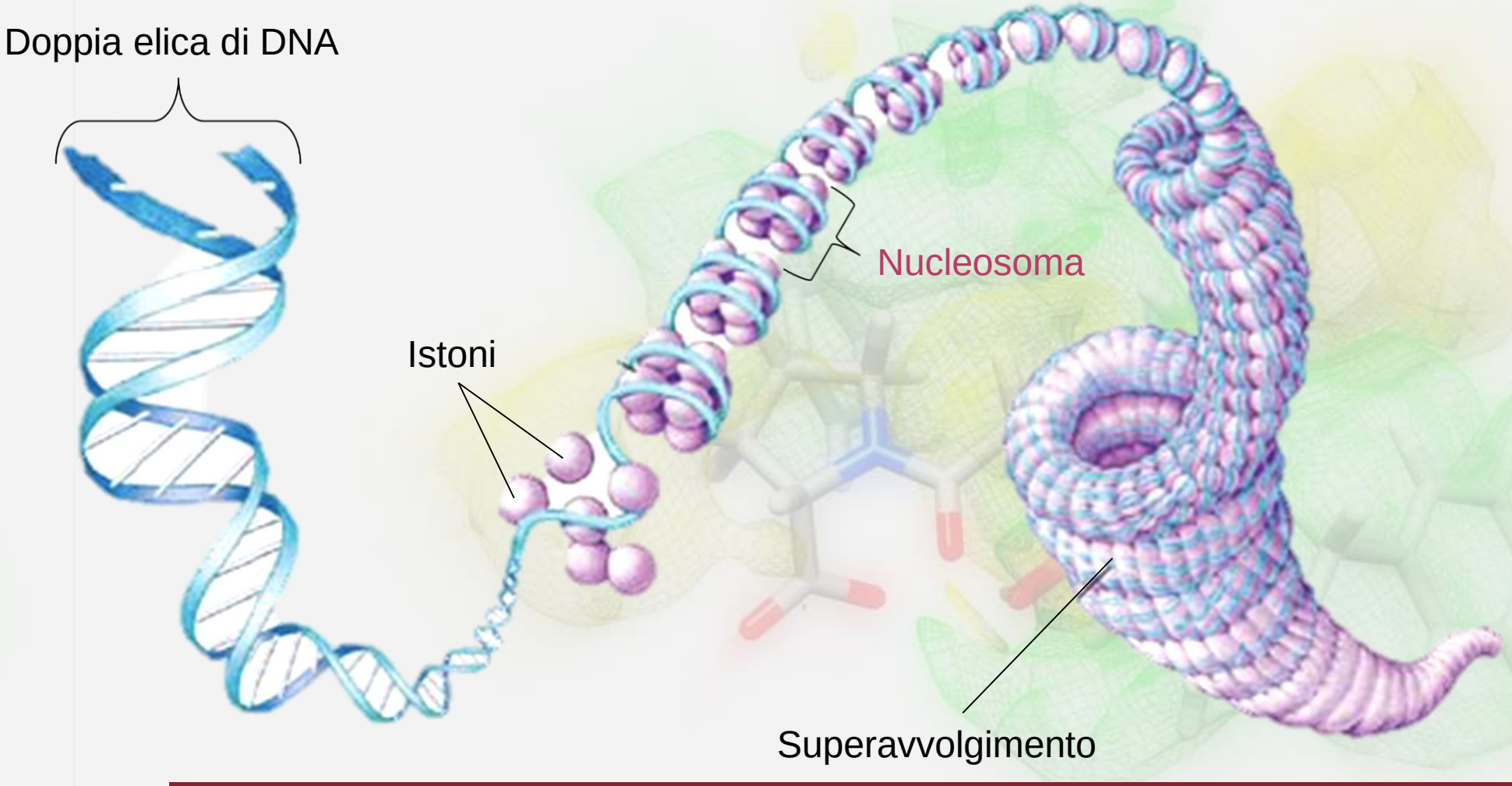
EPIGENETICA

Fenomeni che inducono una variazione nell'espressione genica in assenza di mutazioni vere e proprie del DNA

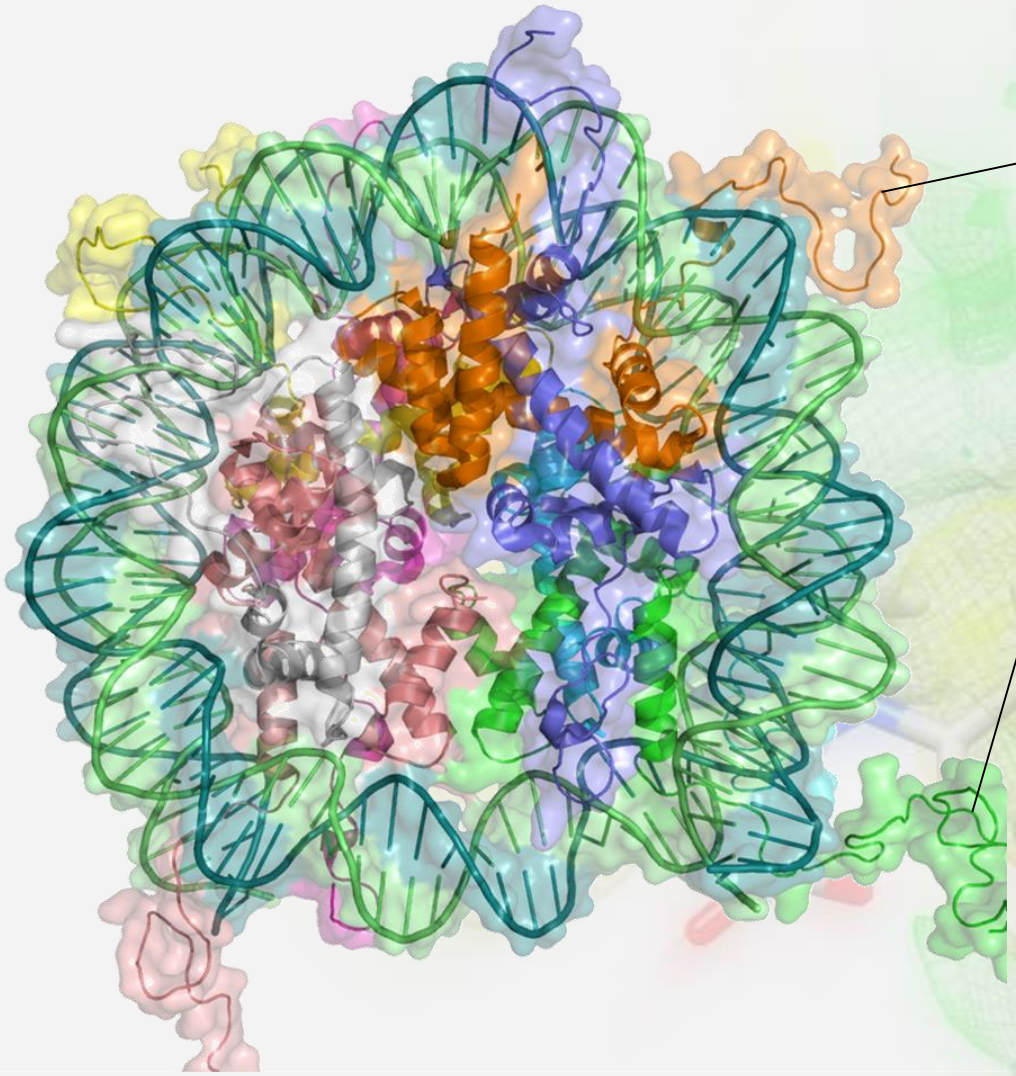


EPIGENETICA

Fenomeni che inducono una variazione nell'espressione genica in assenza di mutazioni vere e proprie del DNA

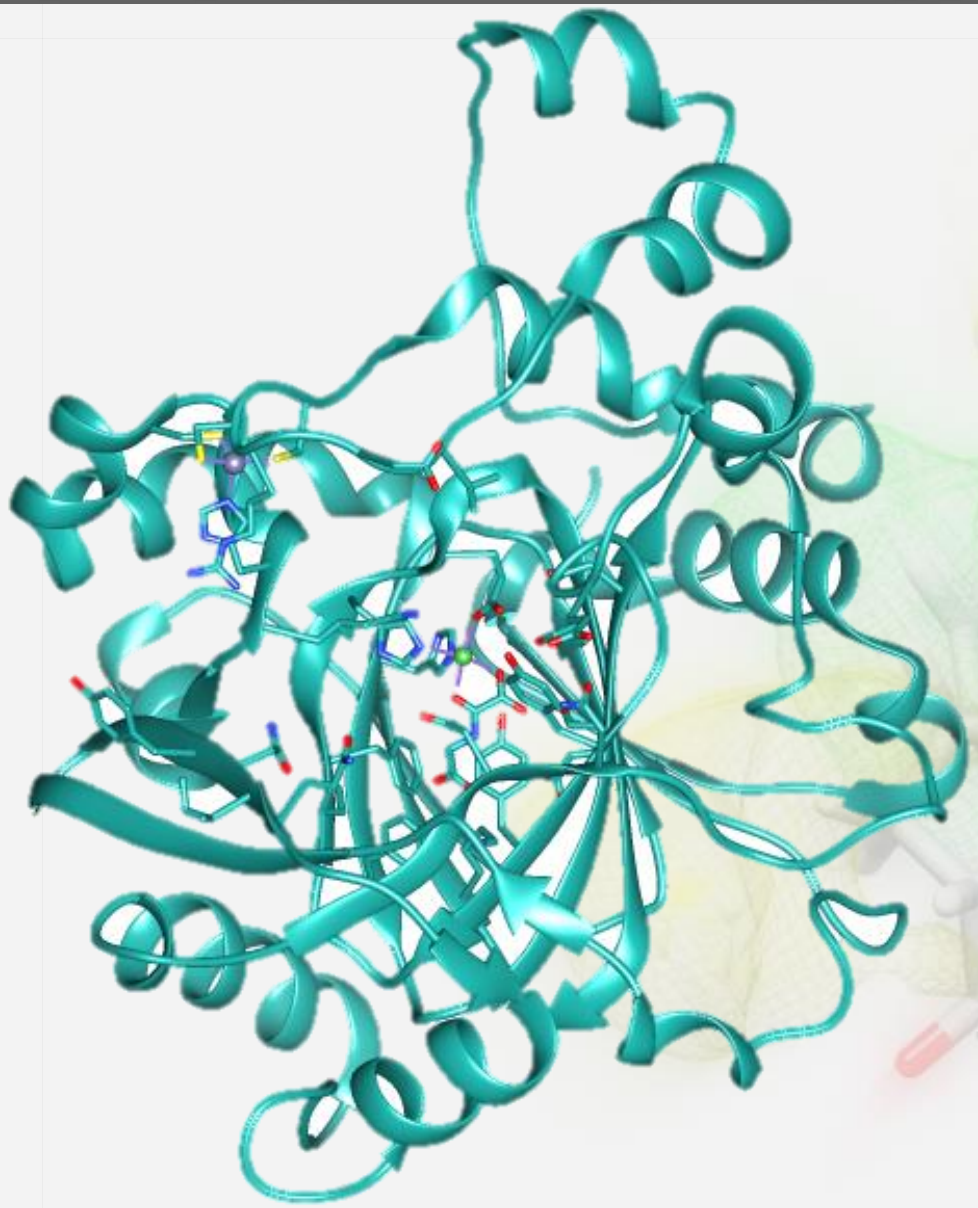


NUCLEOSOMA E PROTEINE ISTONICHE



Code istoniche

DEMETILASI ISTONICA (KDM4A)



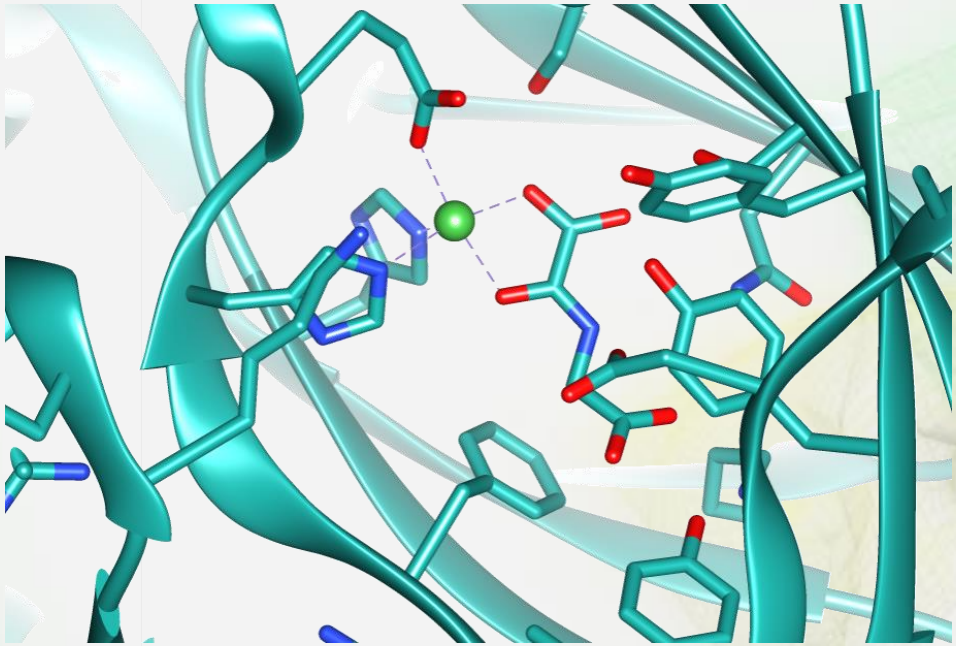
La sovraespressione delle HDM è stata riscontrata in forme tumorali a carico del:

- **Seno**
- **Polmone**
- **Prostata**
- **Colon**

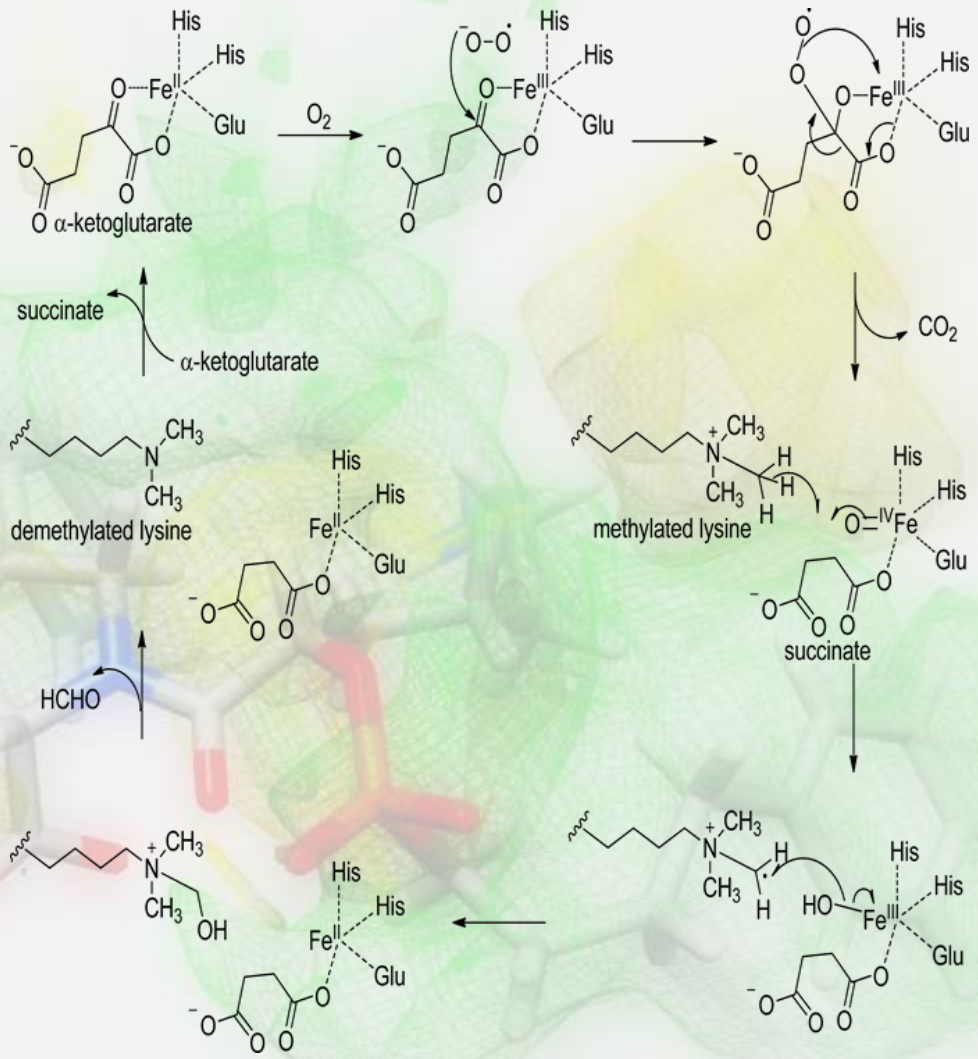
Tsukada Y et al. Histone demethylation by a family of JmjC domain-containing proteins. Nature 2006;439:811–816

DEMETILASI ISTONICA (KDM4A)

Dominio Jumomji-C ad attività diossigenasica Fe(II) e α-chetoglutarato dipendente con substrato H3K9 e H3K36



Meccanismo d'azione



Tsukada Y et al. Histone demethylation by a family of JmjC domain-containing proteins. Nature 2006;439:811–816

SCOPO DEL LAVORO

Analisi e studio mediante **metodiche computazionali** di molecole con attività inibitoria sulle KMD4A/JMJD2A, nel tentativo di chiarire le dinamiche d'interazione ligando-proteina e individuare un plausibile farmacoforo

APPROCCIO SPERIMENTALE

KDM4A dal PDB

Docking

Cross Docking

3-D QSAR

VALIDAZIONE DOCKING

KDM4A dal PDB

Docking

Cross Docking

3-D QSAR

- Surflex
- Dock
- ParaDockS
  - pmf04
  - p-score
- AutoDock
- Plants
  - plp
  - chemplp
  - plp95
- AutoDock Vina

VALIDAZIONE DOCKING

KDM4A dal PDB

Docking

Cross Docking

3-D QSAR

validazione

Re-Docking

- Experimental Conformation (ECRD)
- Random Conformation(RCRD)

Cross-Docking

- Experimental Conformation (ECCD)
- Random Conformation (RCCDE)

Surflex

Dock

ParaDockS
- pmf04
- p-score

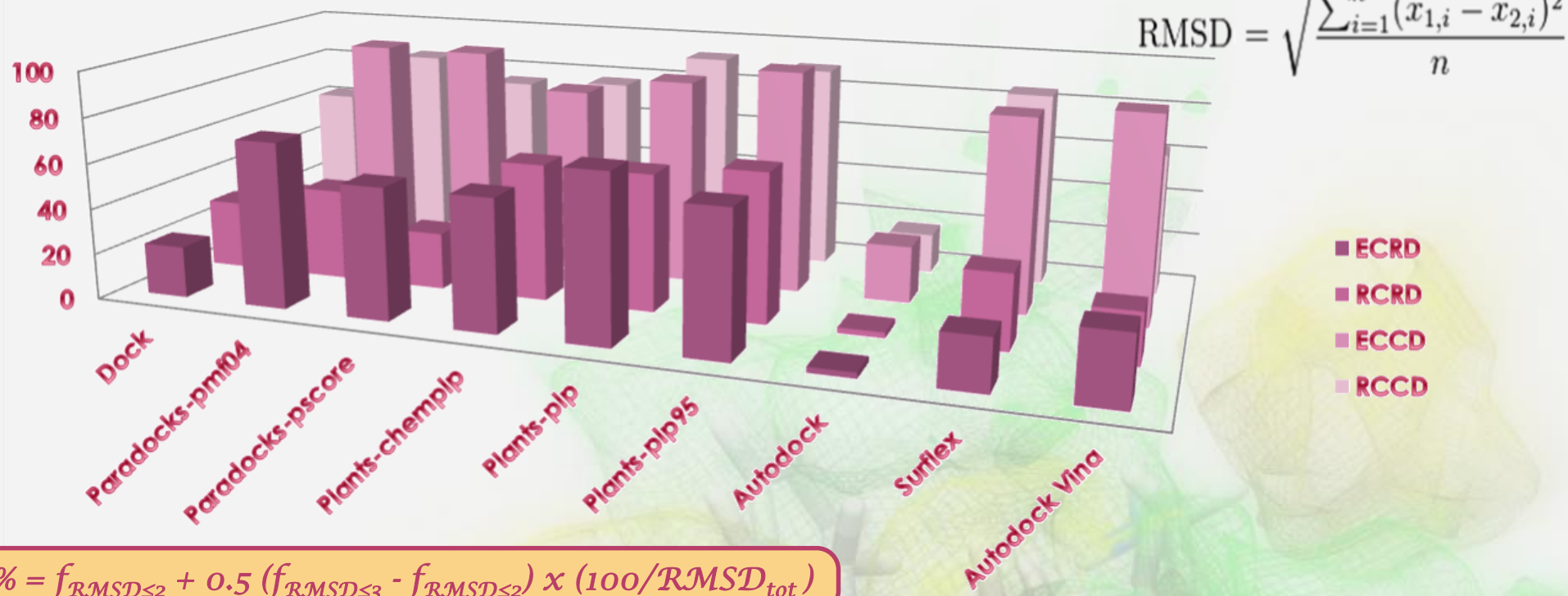
AutoDock

Plants
- plp
- chemplp
- plp95

AutoDock Vina

VALIDAZIONE DOCKING: DA%

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n}(x_{1,i} - x_{2,i})^2}{n}}$$



$$DA\% = f_{RMSD\leq 2} + 0.5\,(f_{RMSD\leq 3} - f_{RMSD\leq 2}) \times (100/RMSD_{tot})$$

PROGRAMMI	ECRD		RCRD		ECCD		RCCD	
	DA%	sd	DA%	sd	DA%	sd	DA%	sd
DOCK	22,5	1,29	30	1,44	22,5	1,17	62,5	1,02
PARADOCKS-pmf04	72,5	0,96	40	1,27	97,5	0,49	85	0,68
PARADOCKS-pscore	57,5	1,35	25	1,64	97,5	0,47	75	1,01
PLANTS-chemplp	57,5	1,57	60	1,24	82,5	1,02	77,5	1,1
PLANTS-plp	72,5	1,2	60	1,17	90	0,63	92,5	0,62
PLANTS-plp95	**62,5**	**1,4**	**65**	**1,08**	**97,5**	**0,55**	**90**	**0,55**
AUTODOCK	2,5	0,87	2,5	0,7	25	1,16	17,5	1,08
SURFLEX	22,5	1,98	32,5	1,4	85	0,8	85	0,72
VINA	30	1,54	22,5	1,38	90	0,58	62,5	0,9

CROSS DOCKING INIBITORI

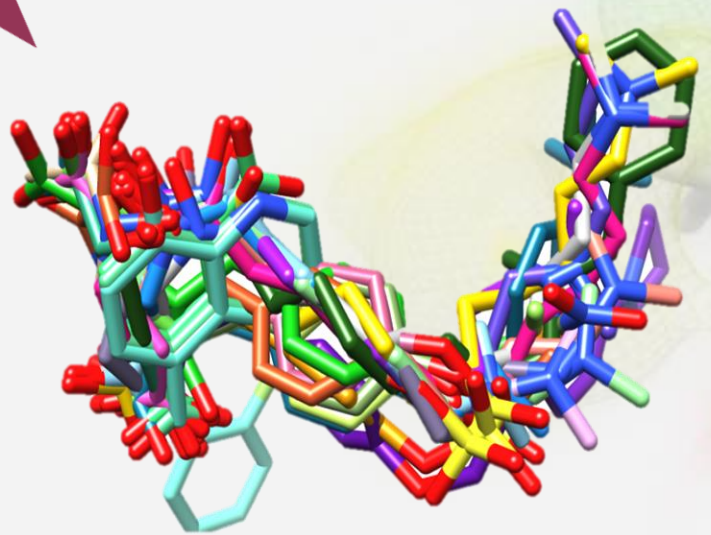
KDM4A dal PDB

Docking

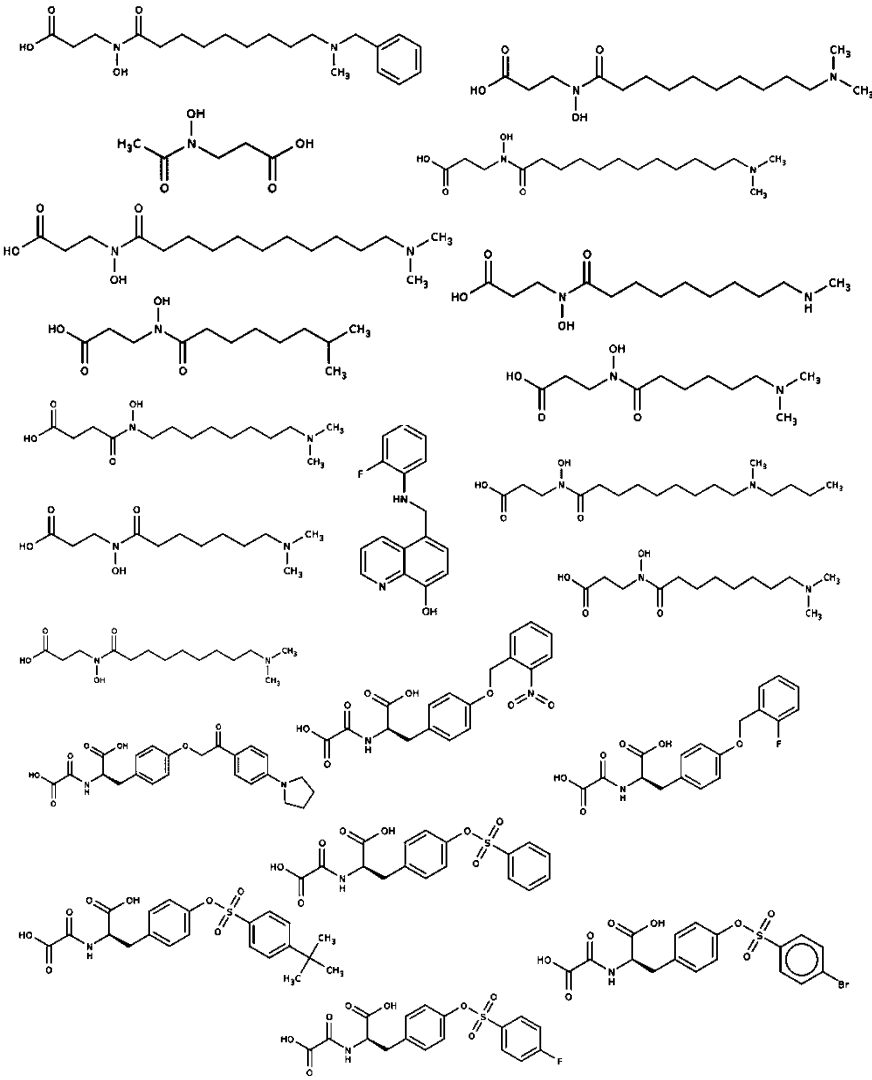
Cross Docking

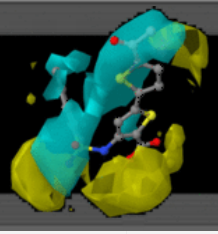
3-D QSAR

Plants – plp95



Inibitori ad attività biologica nota





3-D QSAR

KDM4A dal PDB → Docking → Cross Docking → **3-D QSAR**

- **3-D QSAutoGrid/R**

Scelta del *training set* e *test set*

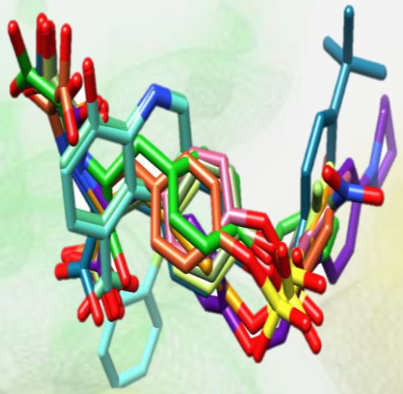
Calcolo campi di interazione molecolare (MIF)

Generazione del modello statistico

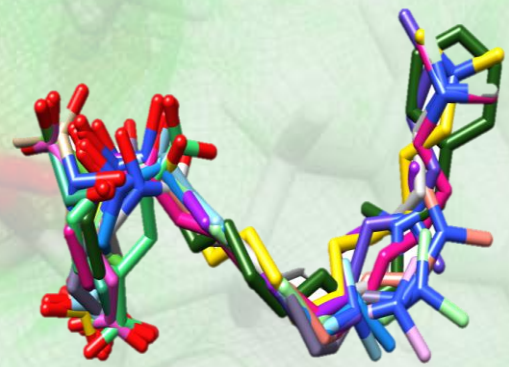
Validazione interna, esterna

Interpretazione risultati

✓ **Test set – saggio MALDI-TOF MS**



✓ **Training set – saggio FDH**



3-D QSAR

KDM4A dal PDB | Docking | Cross Docking | **3-D QSAR**

- **3-D QSAutoGrid/R**

Scelta del *training set* e *test set*

Calcolo campi di interazione molecolare (MIF)

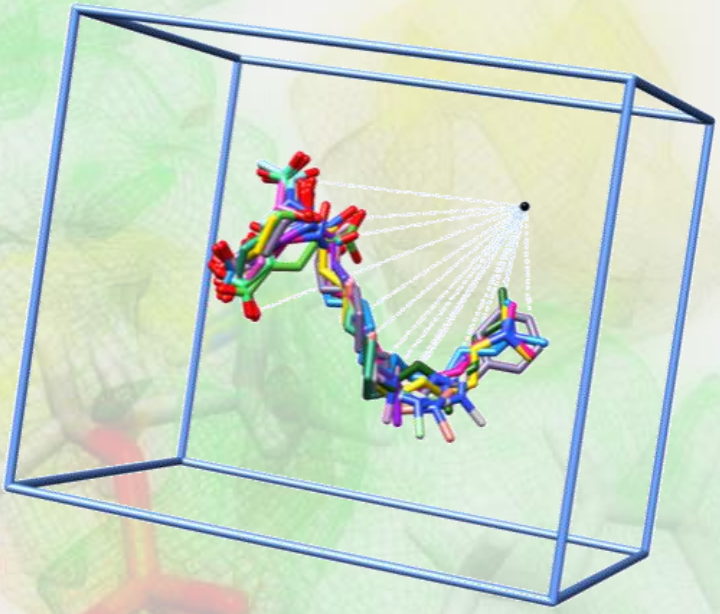
Generazione del modello statistico

Validazione interna, esterna

Interpretazione risultati

AutoGrid

✓ ***Creazione griglia 3-D attorno alle molecole allineate***



✓***Otto differenti Probe***
(A, C, HD, NA, N, OA, e, d)

3-D QSAR

KDM4A dal PDB

Docking

Cross Docking

3-D QSAR

- **3-D QSAutoGrid/R**

Scelta del training set e test set

Calcolo campi di interazione molecolare (MIF)

Generazione del modello statistico

Validazione interna, esterna

Interpretazione risultati

Analisi regressione linere (PLS)

✓Modello grezzo

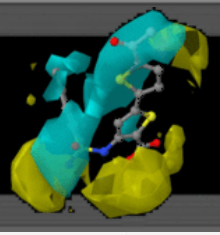
✓Modello Pretrattato

✓Modello Pretrattato Ottimizzato (GA)

$$r^2 = 1 - \frac{\sum (Y_i - Y_{icalc})^2}{\sum (Y_i - Y_{im})^2}$$

$$SDEC = \sqrt{\frac{\sum (Y_i - Y_{icalc})^2}{N}}$$

Creazione delle mappe di contorno



3-D QSAR

KDM4A dal PDB → Docking → Cross Docking → **3-D QSAR**

- **3-D QSAutoGrid/R**

1. Scelta del training set e test set
2. Calcolo campi di interazione molecolare (MIF)
3. Generazione del modello statistico
4. **Validazione interna ed esterna**
5. Interpretazione risultati

✓ ***Validazione interna***

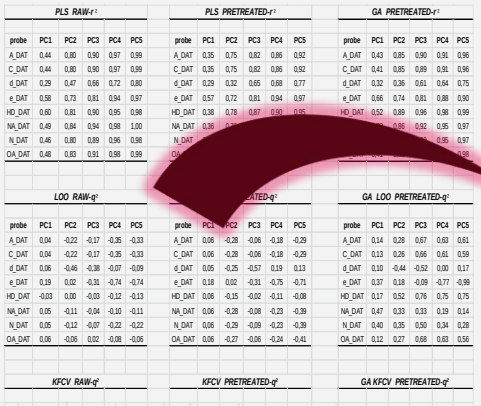
- ***Leave One Out CrossValidation (LOO)***
- ***K-Fold CrossValidation (KFCV)***

$$q^2 = 1 - \frac{\sum (Y_i - Y_{ipred})^2}{\sum (Y_i - Y_{im})^2}$$

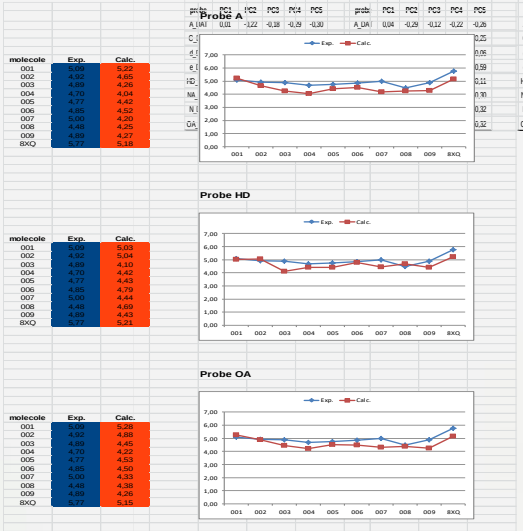
✓ ***Validazione esterna***

Test Set

VALIDAZIONE INTERNA

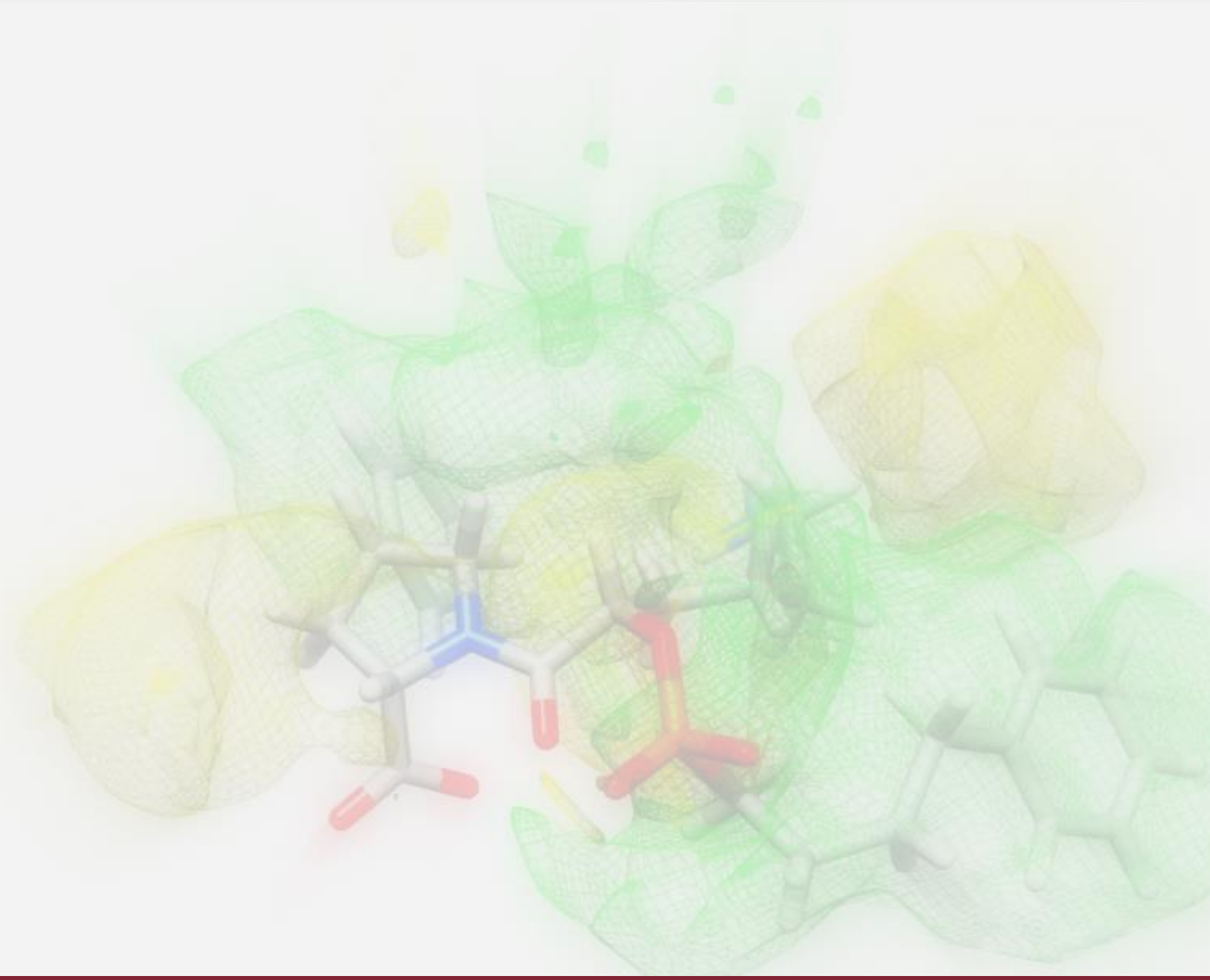


$$r^2 = 1 - \frac{\sum (Y_i - Y_{icalc})^2}{\sum (Y_i - Y_{im})^2} \qquad q^2 = 1 - \frac{\sum (Y_i - Y_{ipred})^2}{\sum (Y_i - Y_{im})^2}$$



PROBE	PC	r^2	q^2_{LOO}	q^2_{KFCV}
A	4	0,91	0,63	0,57
C	4	0,91	0,61	0,54
d	1	0,32	0,10	0,08
e	1	0,66	0,37	0,32
HD	4	0,98	0,75	0,67
NA	1	0,73	0,47	0,44
N	3	0,89	0,50	0,42
OA	4	0,94	0,63	0,56

VALIDAZIONE ESTERNA



INTERPRETAZIONE RISULTATI

Mappe Coefficiente PLS

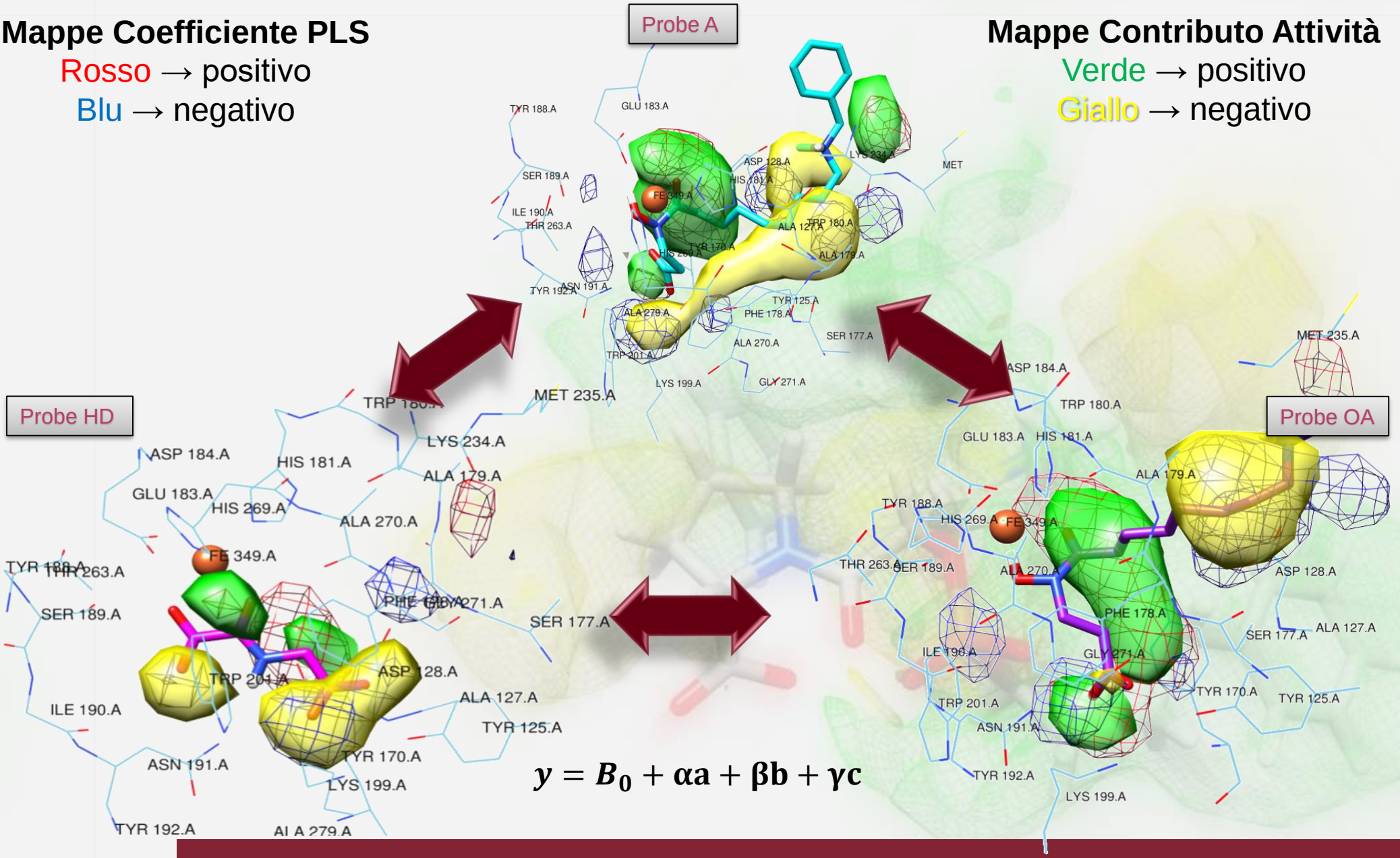
Rosso → positivo

Blu → negativo

Mappe Contributo Attività

Verde → positivo

Giallo → negativo

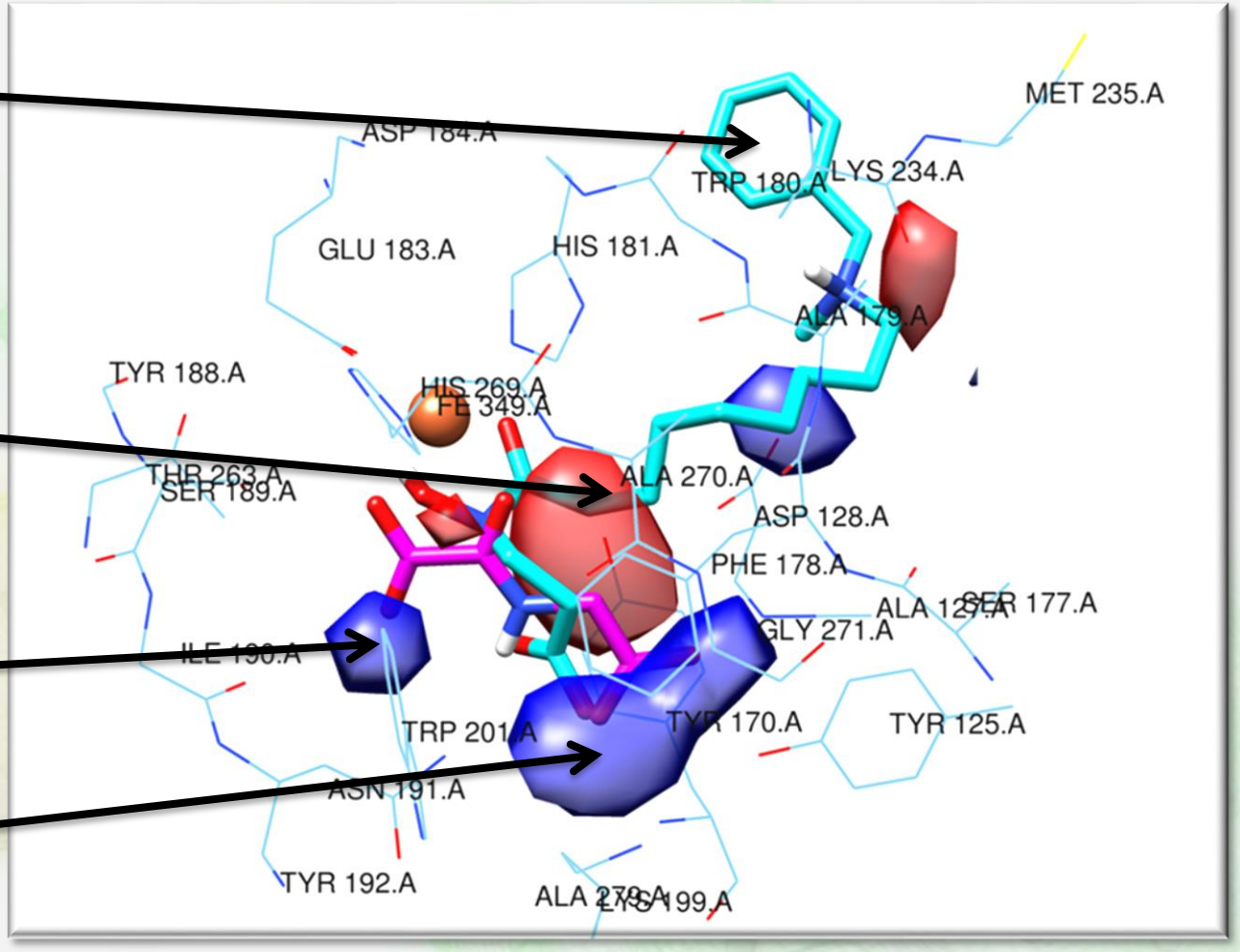


$$y = B_0 + \alpha a + \beta b + \gamma c$$

CONCLUSIONI

Gruppi che promuovono l'attività biologica:

- ✓ **Gruppo aromatico capace di creare interazioni di natura repulsiva nella tasca del sito attivo delimitata dai amminoacidi Arg309, Lys234 e Asp184**
- ✓ **La presenza di un ingombro sterico in α al gruppo responsabile dell'interazione col Fe**
- ✓ **Ossigeno capace di creare legami idrogeno con Asn191**
- ✓ **Un gruppo carbossilico capace di creare legami idrogeno con i residui Tys125 e Lys199**



www.3D-QSAR.com
Three-Dimensional Quantitative Structure-Activity Relationships Server

Sapienza Università di Roma

by www.RCMD.it

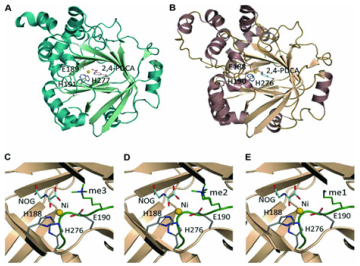
Combined 3D-QSAR Modeling and Molecular Docking Studies on Human Lysine-Specific Demethylase JMJD2A

Adele Pirolli[¥§], Alfredo Gallo[¥§] and Rino Ragno[¥§]

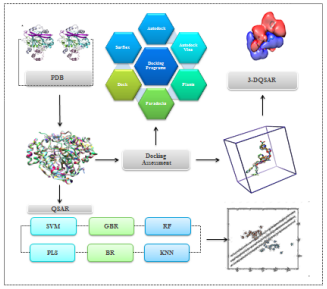
[§]Rome Center for Molecular Design, [¥]Dipartimento di Chimica e Tecnologie del farmaco, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy

ABSTRACT

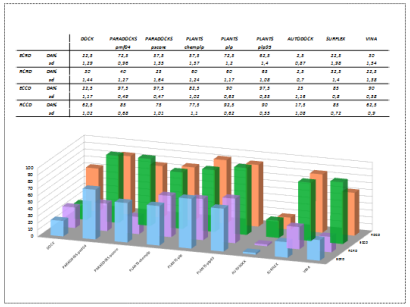
Post-translational histone modification plays a fundamental role in regulating gene function and it has been proposed that different combination of specific modification constitute a 'histone code' that dictates the epigenetic state of chromatine. There are several families of histone demethylases, which act on different substrates and play different roles in cellular function. The discovery of lysyl demethylases using Fe(ii) and 2-oxoglutarate or flavin (amine oxidases) as cofactors (2OG oxygenases) has changed the observation of methylation as a irreversible epigenetic marker. Human jumonji domain containing 2A (JMJD2A), also known as KDM4A, is a histone demethylase which is selective for di- and trimethyl Lys9 and Lys36 residues of Histone 3 tail (H3K9 and H3K36).



GENERAL PROCEDURE



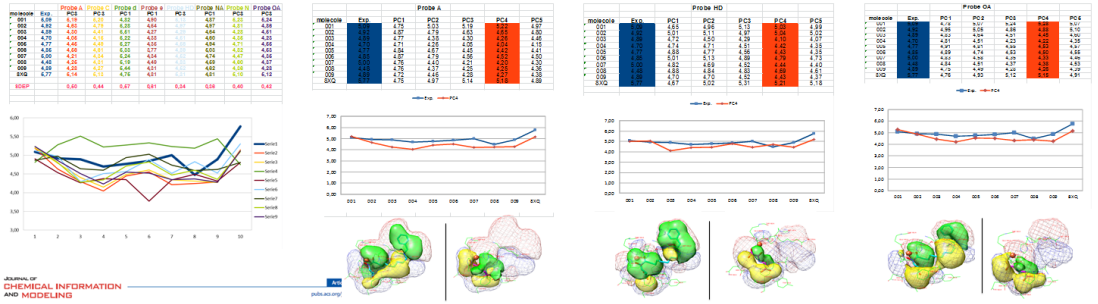
DOCKING ASSESSMENT



METHODS

In the present work, different docking approaches have been explored with the aim to find a scoring function that correctly ranks candidate ligands for KDM4A. Specifically, six molecular docking programs (AUTODOCK, AUTODOCK VINA, DOCK, PLANTS, PARADOCKS and SURFLEX) were evaluated using different settings. Docking assessment through re-docking and cross-docking on 20 unique ligand/KDM4A complexes, retrieved from PDB, revealed that PARADOCKS-pscore and PLANTS-plp95 showed the highest docking accuracy values.

3-DOSAR



JOURNAL OF CHEMICAL INFORMATION AND MODELING

3-D QSAutogrid/R: An Alternative Procedure To Build 3-D QSAR Models. Methodologies and Applications

Flavio Ballante[†] and Rino Ragno*[,†]

Probe A - PLS coefficient and Activity Contribution

Probe HD – PLS coefficient and Activity Contribution

Probe OA – PLS coefficient and Activity Contribution

N = Number of compounds in the training set; PC = Number of principal component analysis; r^2 = coventional square correlation coefficient; q^2 = cross-validation correlation coefficient; K3FCV = K-fold (5 Random group) CROSS VALIDATION METHOD.

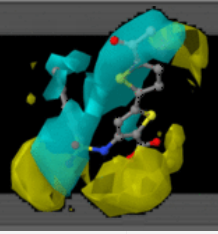
THERAPEUTIC IMPLICATION AND PERSPECTIVE

Many of the genes coding for HDMs are reported oncongenes and clearly methylation and demethylation is an important epigenetic event in both cancer development and progression, but also in other metabolic and central nervous system disorders epigenetic events may play a role .In the future, we intent to develop a COMBINE and a QSAR models, with the aim to perform in a second phase the screening of different libraries compounds available online.

References

Ballante, F.; Ragno, R. *J Chem Inf Model* **2012**, *52*, 1674-1685.

Elena, M., et al. "Investigation on the oxygen dependence of 2-oxaglutarate histone demethylase." Biochemical Journal 449.2**(2013)**:491-496.



GRAZIE PER LA CORTESE ATTENZIONRE