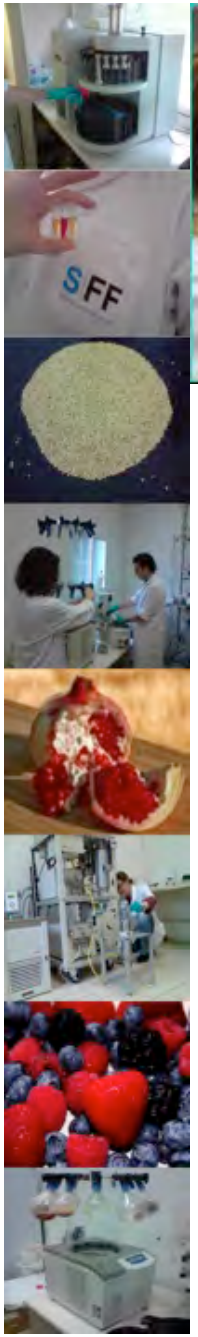
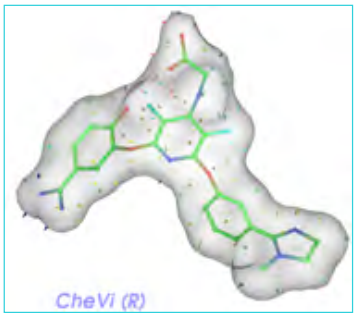
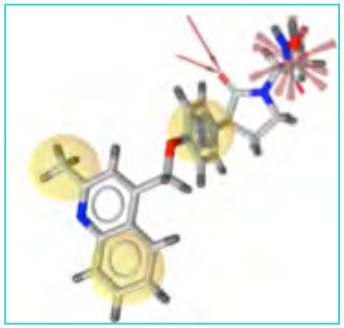
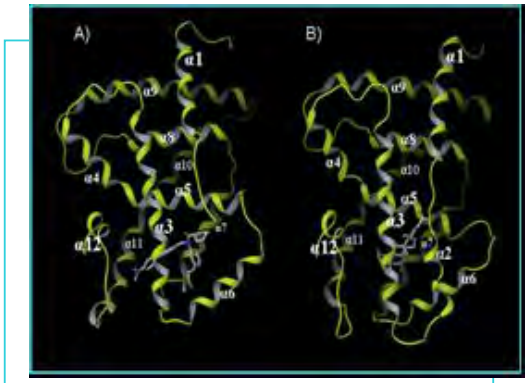


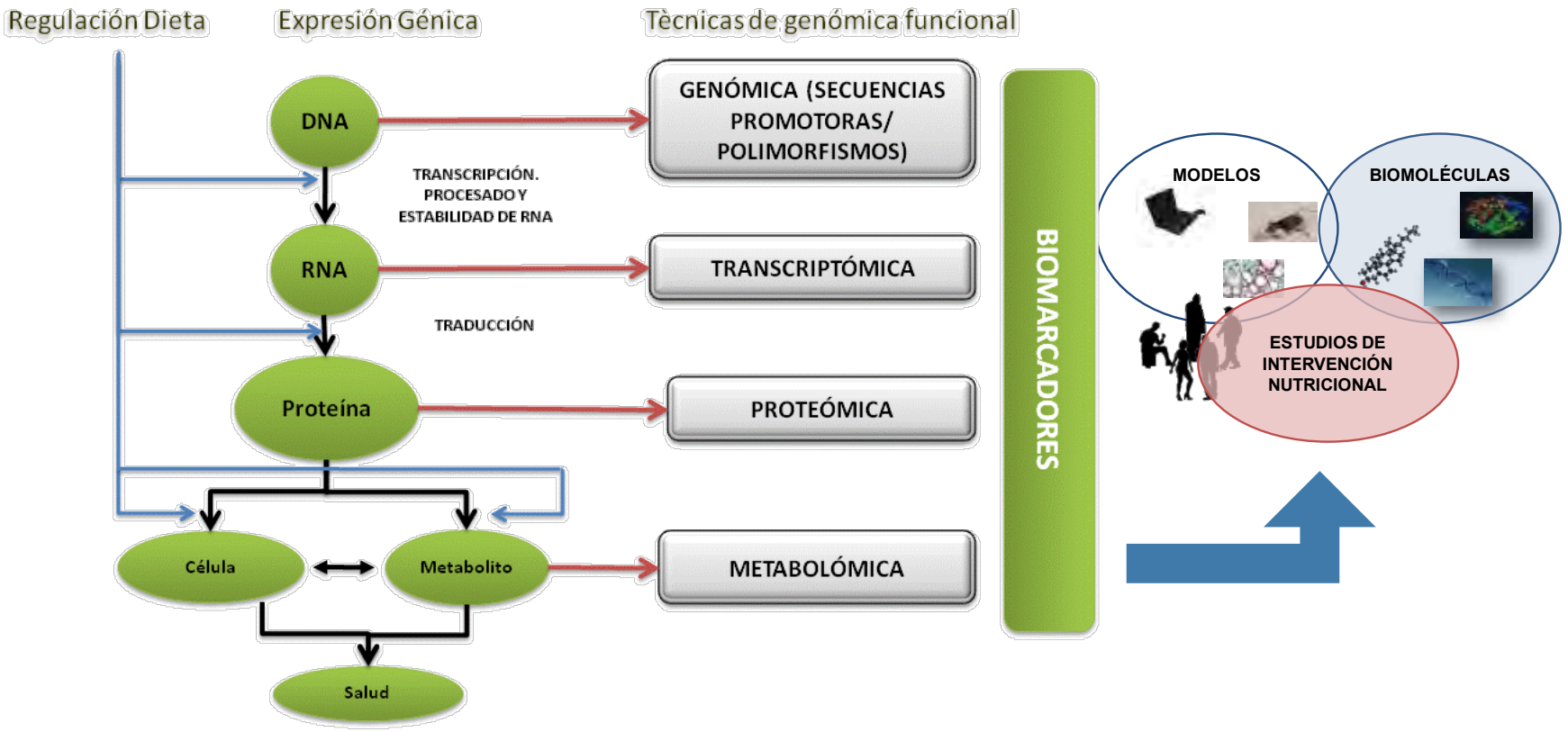
"Interés de la bioinformática en la sustanciación de declaraciones de propiedades saludables. Predicción de compuestos bioactivos frente a dianas predefinidas. Comprensión del mecanismo por el que los compuestos bioactivos mejoran las condiciones de salud y previenen enfermedades".







Action Mechanism+ Physiological Effect



ILSI Europe
Report Series

EMERGING TECHNOLOGIES FOR EFFICACY DEMONSTRATION

ILSI
International Life Sciences INSTITUTE

SUMMARY REPORT OF A WORKSHOP HELD IN FEBRUARY 2009

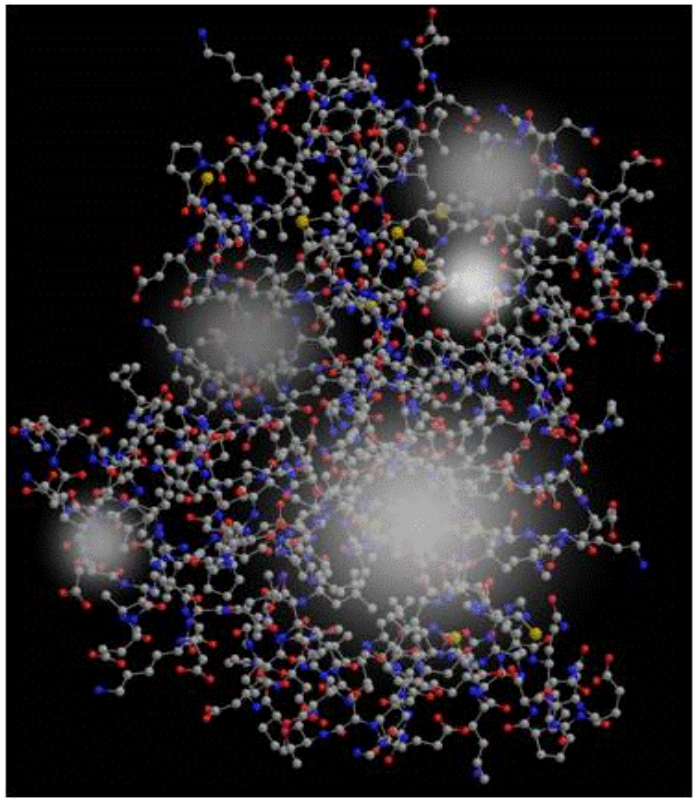
Organised by the ILSI Europe Emerging Technologies for Efficacy Demonstration Task Force

ILSI International Life Sciences Institute EUROPE

EMERGING TECHNOLOGIES TASK FORCE

Bioactive food ingredients are claimed to either reduce disease risks or to improve life quality by optimising and maintaining body functions. These cBackgroundMany laims have to be based on scientific substantiation. The project “Process for the Assessment of Scientific Support for Claims on Foods” (PASSCLAIM) developed a generic tool to assess the scientific support for health claims for foods. It also established criteria for markers and measurement techniques to be used to substantiate a claim. **However, today new technologies have been developed, such as the -omics technologies, which can serve as good tools to further strengthen the evidence of efficacy of specific bioactive food ingredients, detect new markers of efficacy which were not known up to date and/or generate reliable evidence in cases (i.e. calorie restriction) where this may be difficult for the classical biomarkers. Likewise, it is conceivable that imaging techniques derived from clinical diagnosis can provide evidence in humans, i.e. brain functions, which is very difficult to get with established biomarkers/tests.**

Objective



Bioinformatics Unit

Predicting which bioactive compounds would be the most effective one against a pre-defined protein target

Understanding how bioactive molecules in food can improve health conditions and prevent diseases like diabetes, obesity, cardiovascular pathologies …

Validation and Design of functional foods

Hypothesis:

Compounds are activators or inhibitors of one target depending on their binding mode

Summary

Natural compounds

- Characterization of ingredient
- (No ingredient defined)

Digestion process

- Bibliographic search of metabolites (papers and patents) and bioavailability studies.
- *In silico* biotransformations in order to generate metabolites

Metabolites

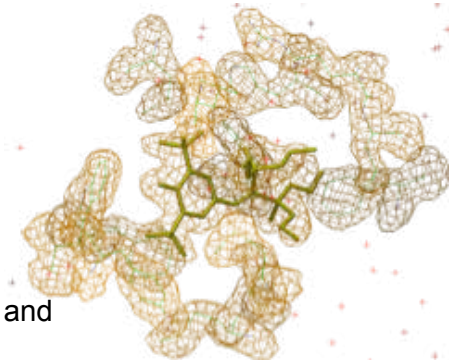
To fit metabolites to plasma conditions (pH, conformations, etc.)

Interaction

- *Ligand-based (3D-QSAR)*
- *Structure -based* (docking, homology modelling)

Targets

- EFSA
- Passclaim
- Scientific journals
- omics data



Natural compounds

Characterization of ingredient → To show plausible mechanism of action

soy

ASE
(accelerated
solvent extraction)

Rotary evaporation

Freeze dryer

EXTRACT

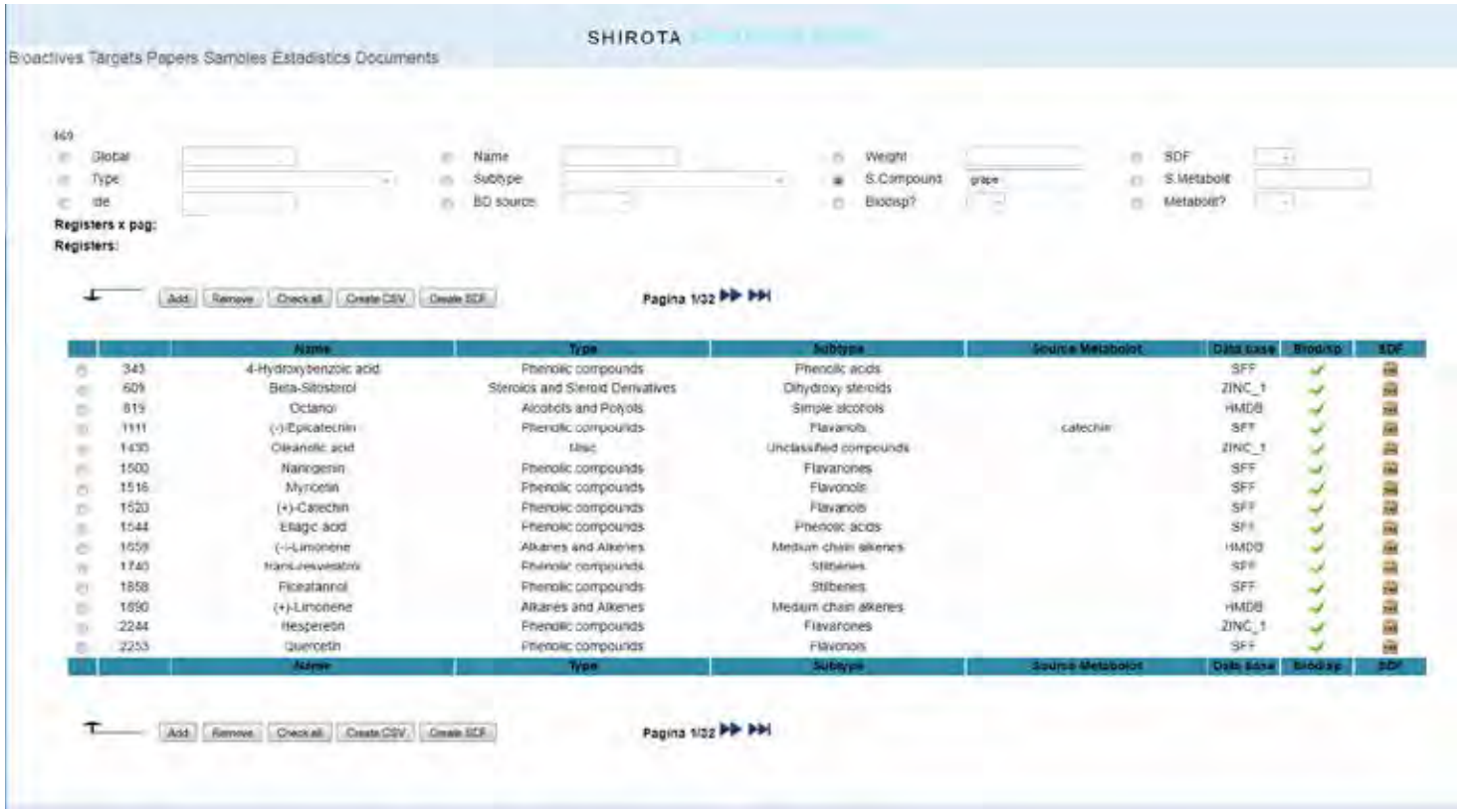
RRLC-DAD-TOF

HPLC-DAD-ITMS

Pico	Compuesto	t_R	TOF ESI(-) m/z	%	IT ESI(-) m/z	IT ESI(-) MS2 m/z	IT ESI(-) MS3 m/z
1	**Apigetrin** (Apigenin-7-O-glucoside)	3.2	431.101 545.0935 248.9629	12.8	**431.1 [M-H]**	**269.0 [M-H-162]**	225, 241, 213, 195
2	**Daidzin** (daidzein-O-glucoside)	3.35	461.1104 [M+HCOO] 253.0535 [M-H-162] 415.1057 [M-H] 529.0886	12.7	**461.1 [M+HCOO]** 253.0 [M-H-162]	415.0 [M-HCOO-H] **253.0 [M-H-162]**	225
3	**Glycitin** (glycitein-O-glucoside)	3.8	491.1216 [M+HCOO] 283.0634 [M-H-162] 559.1077	14.5	**491.1 [M+HCOO]** 283.1 [M-H-162]	445.0 [M-HCOO-H] **283.0 [M-H-162]**	268
4	**Genistin** (genistein-O-glucoside)	4.83	477.1061 [M+HCOO] 431.1006 [M-H] 269.0482 [M-H-162] 545.0934	17.0	**477.1 [M+HCOO]** 431.1 [M-H] 269.1 [M-H-162]	431.0 [M-HCOO-H] **269.0 [M-H-162]**	---
5	Unknown	4.8	609.1503 563.1445 677.1373 447.1081	17.9	**609.2 [M+HCOO]**	562.9 [M-HCOO-H] 269.0 429.1	
6	Unknown	5	609.1521 677.1392	18.3	**609.2 [M+HCOO]** 677.1	563.0 [M-HCOO-H] 429.0	
7	**Acetyldaidzin** Unknown	6.9	503.1245 [M+HCOO] 571.1117	25.8	**503.1 [M+HCOO]** 571.1	457.1 [M-HCOO-H] **253.0 [M-H-204]**	225,209,197
8	**Daidzein**	7.7	253.0536 [M-H]	25.5	**253.0 [M-H]**	225 [M-H-28] 209, 197	
9	**Glycitein**	8.1	283.0651 [M-H]	26.7	**283.0 [M-H]**	267.9 [M-H-15]	
10	**Dihydrogenistein**	8.4	271.0646 [M-H]	27.1	**271.0 [M-H]**	151.1 [M-H-120] 177.0 [M-H-94] 107.1	
11	**Genistein**	8.75	269.0481 [M-H]	28.3	**269.0 [M-H]**	241 [M-H-28] 225 [M-H-44] 201.0 181.1	

Natural compounds

No ingredient defined → to develop a new ingredient



300.000 compound which we know source (grapes, berries, etc)

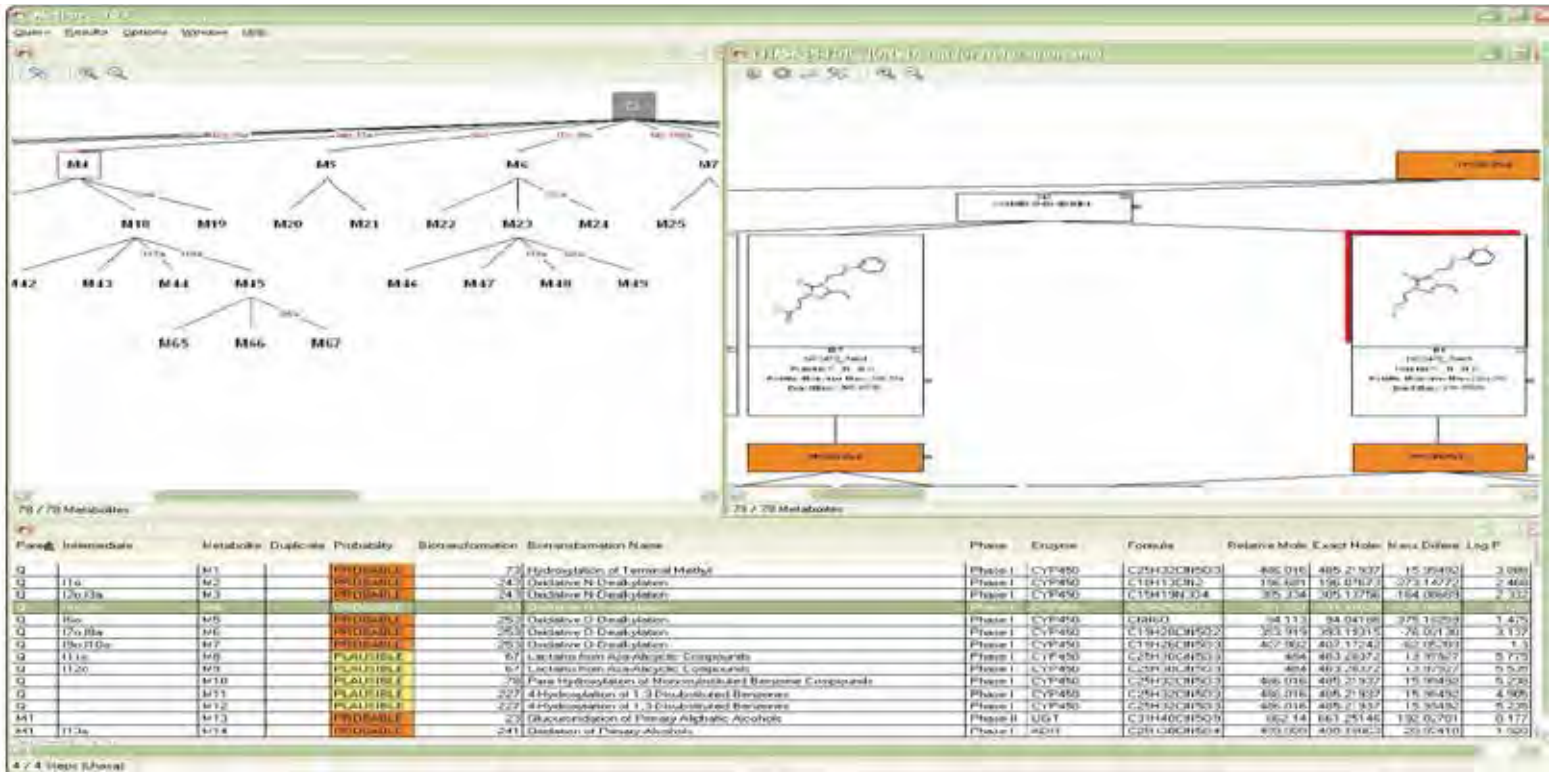
Digestion process

- Bibliographic search

  Urinary Excretion of Black Raspberry (Rubus occidentalis) Anthocyanins and Their Metabolites

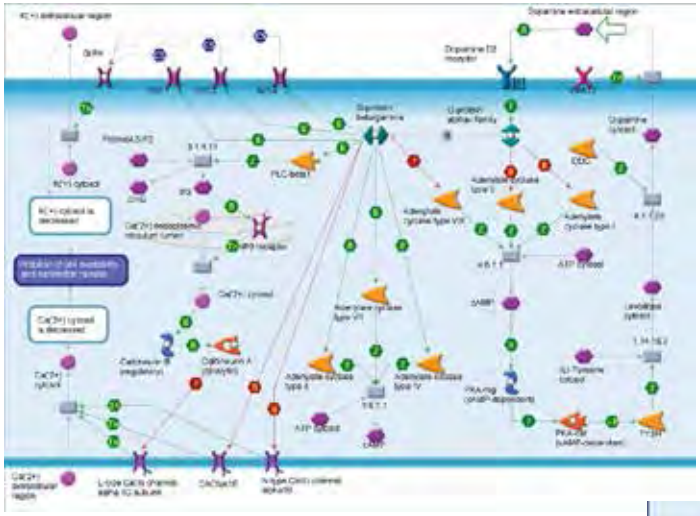
  Determination of procyanidins and their metabolites in plasma samples by improved liquid chromatography–tandem mass spectrometry

- *In silico* prediction



Targets

Pathway



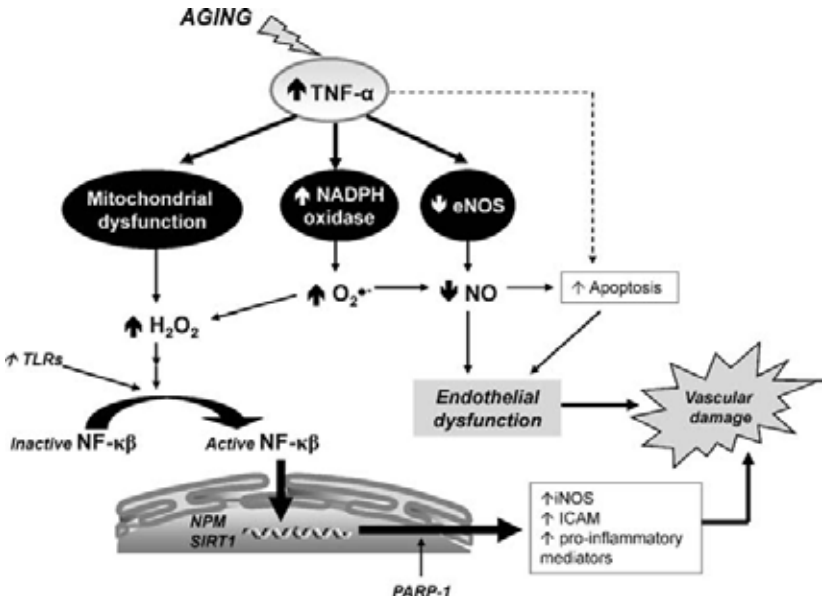
+

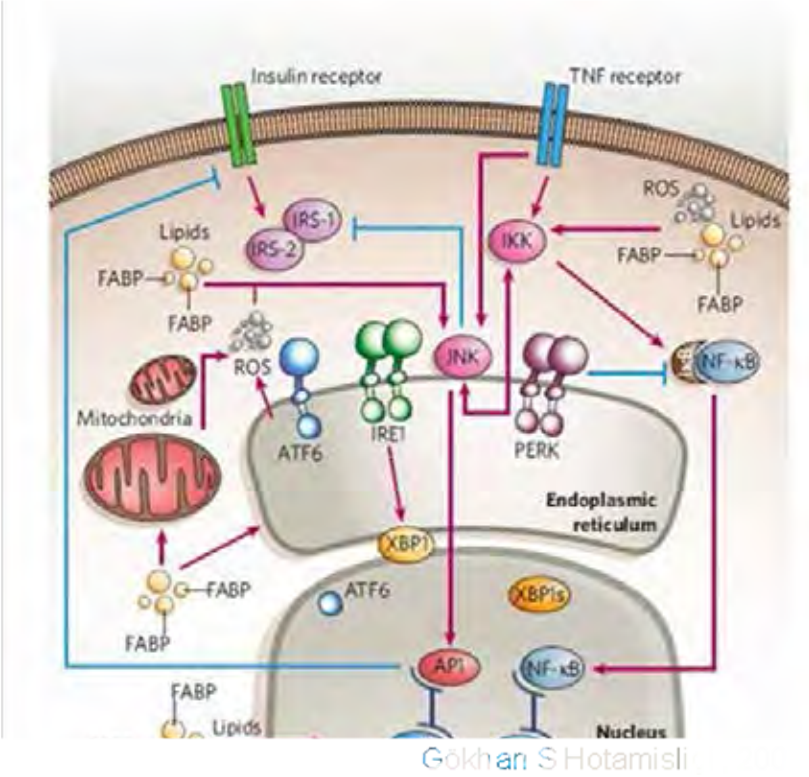
- EFSA
- Passclaim (biomakers)
-Scientific Journals
- omics data

List of targets

[…] In addition to these known kinds of markers, also markers for an early prediction of improvement (as early as possible) and special markers for demonstration of a claimed effect (validated markers to demonstrate a claimed effect of a substance) were suggested. Also a ranking of the markers into“light”and“hard”markers was proposed. The“light” markers (e.g., new markers which lack full validation) could be used for exploratory studies while the “hard”markers (e.g., markers with a long history of use) could be used for confirmatory studies. Further, **it might be a good suggestion to not only use markers describing a definite status (e.g. oxidised low- density lipoprotein (LDL) or LDL-cholesterol plasma levels as a recognised marker for cardiovascular disease risk) but to use a marker describing a dynamic effect** (e.g. Flow Mediated Dilation as a descriptor for coronary artery (cardiovascular) health).

Inflammatory protection



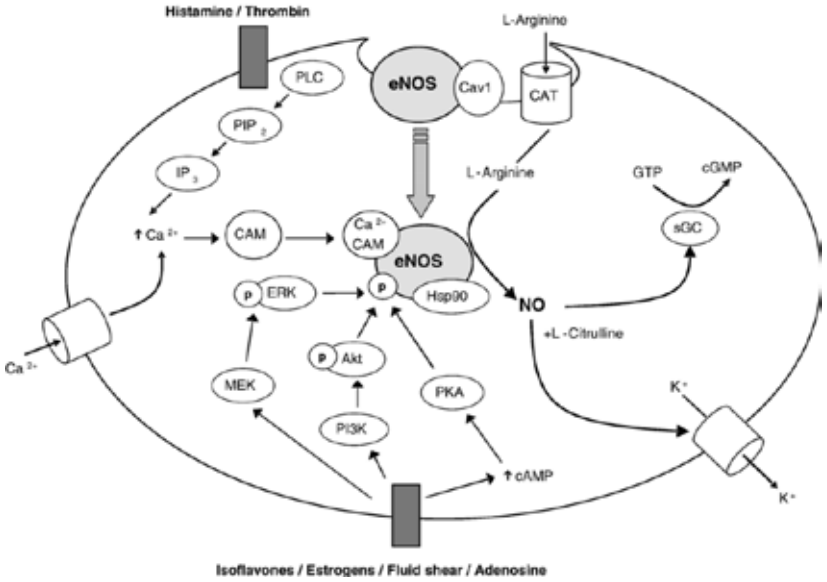


Gökhan S Hotamisli

TNF-a upregulates endothelial arginase in I/R, which causes a reduction of L-arginine availability to NOS, and leads to $O2^-$ production, thus impairing NO-mediated vasodilation (Xue Gao et al., 2007).

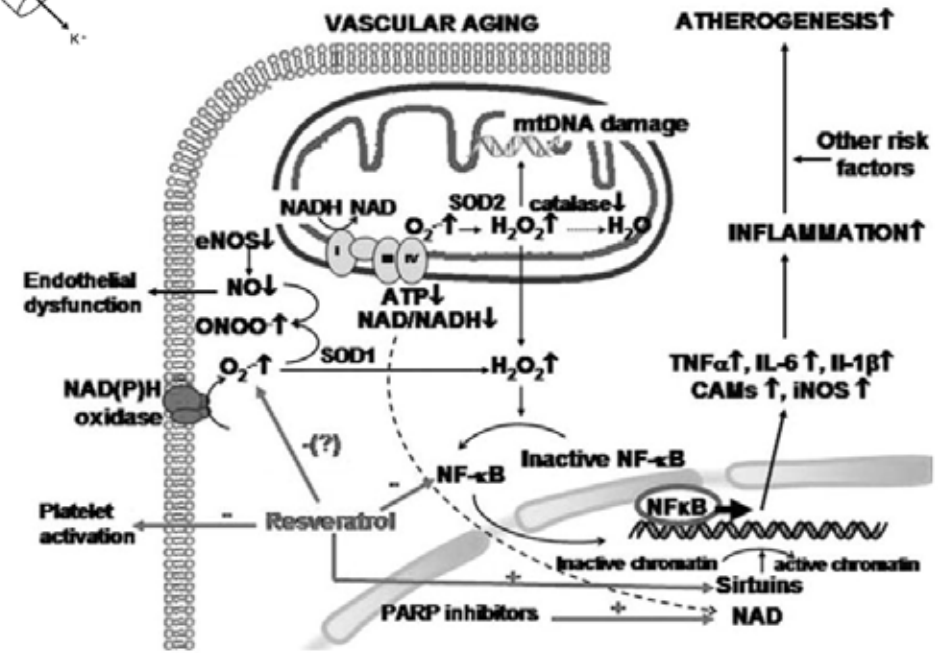
Typically, the canonical NF-kB signaling cascade is initiated by such stimuli as tumor necrosis factor a (TNFa), bacterial lipopolysaccharide (LPS), and genotoxic agents (Mia Rushe et al., 2008).

Endothelial Dysfunction (NO synthesis)

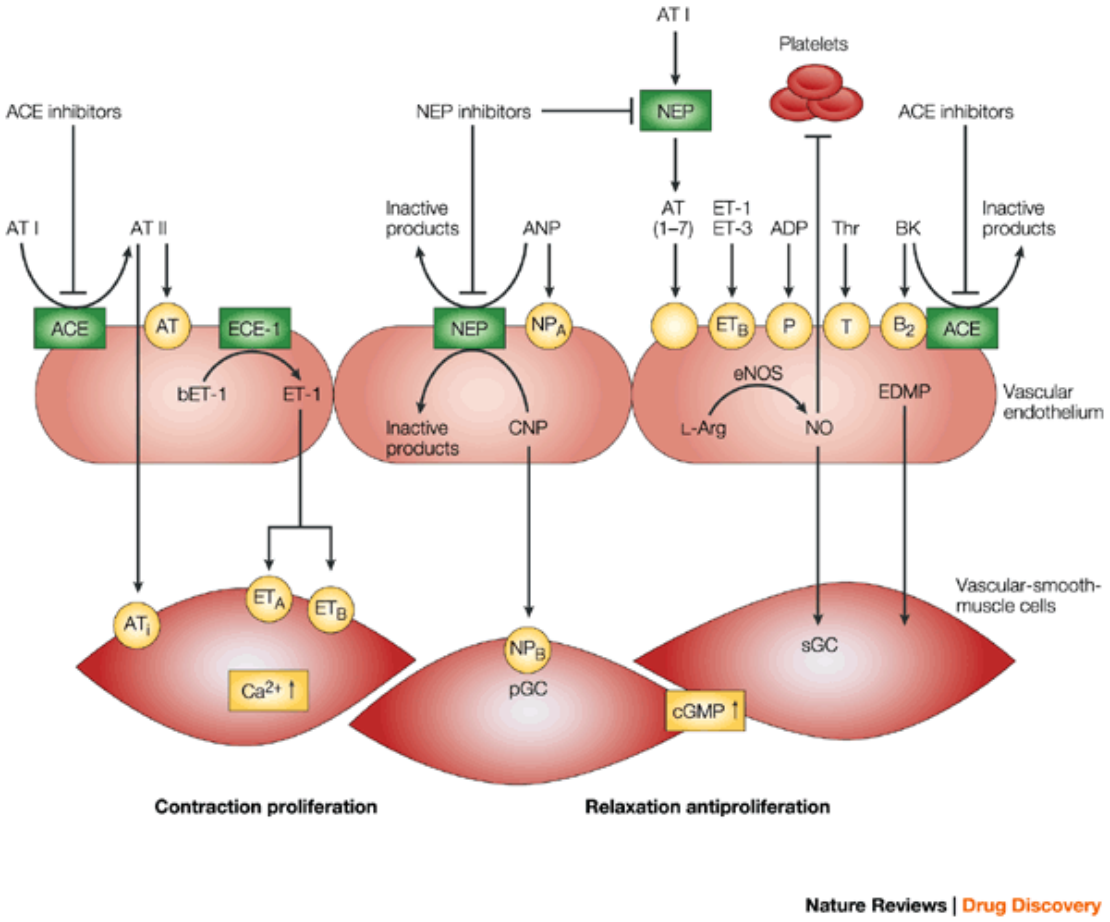


It is clear that eNOS has a key role in regulating the homeostasis between the endothelial cell surface, circulating cells and vasomotor function, and that it is also involved in pathological conditions where it can be activated posttranslationally (Giuseppe Cirino et al., 2003).

iNOS-produced NO appears to be involved in a broad range of inflammatory pathologies, such as septic shock, rheumatoid arthritis, and multiple sclerosis (Haitao Ji et al. 2007).

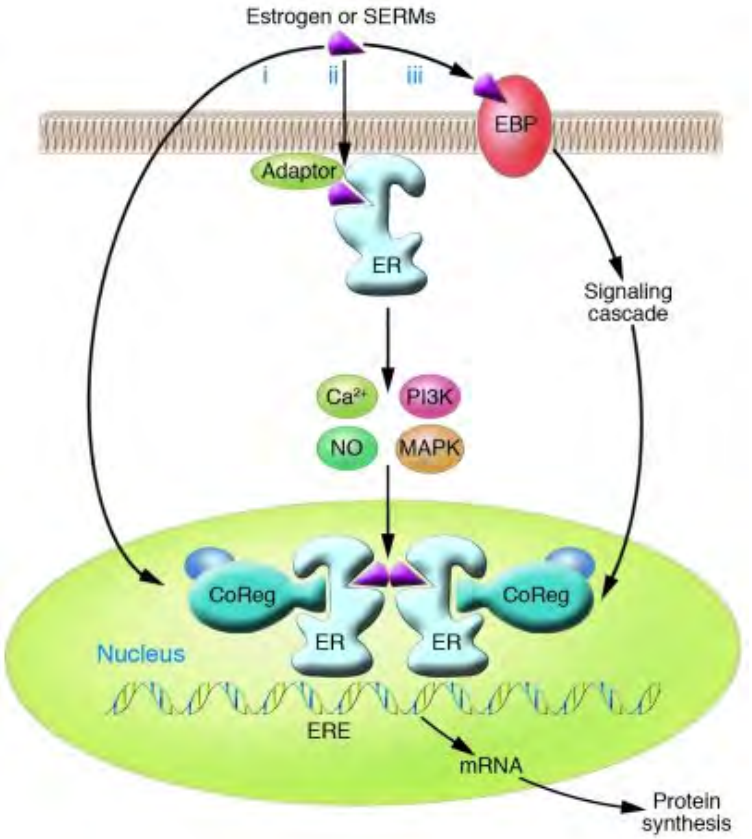


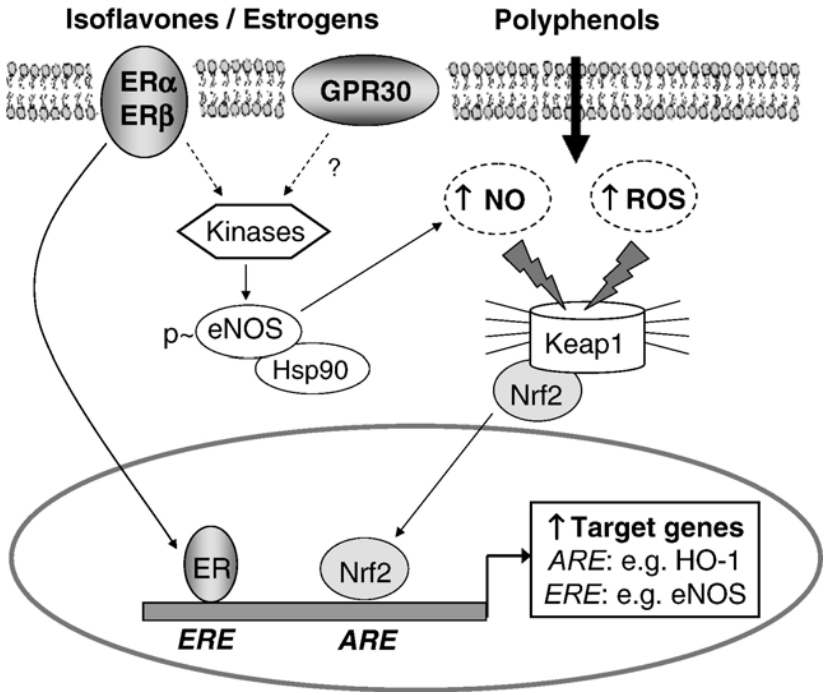
Vasodilatatory Effects



Antihypertensive treatments—such as ACE inhibitors, calcium channel blockers, and third generation β-blockers—reverse endothelial dysfunction in experimental animals and in hypertensive patients. Several effects of ACE inhibitors enhance NO release and bioactivity, including preventing the breakdown of endogenous bradykinin (a potent NO releaser). ACE inhibitors also protect NO bioavailability (Tang EHC & Vanhoutt PM, 2010).

Estrogenic Effects





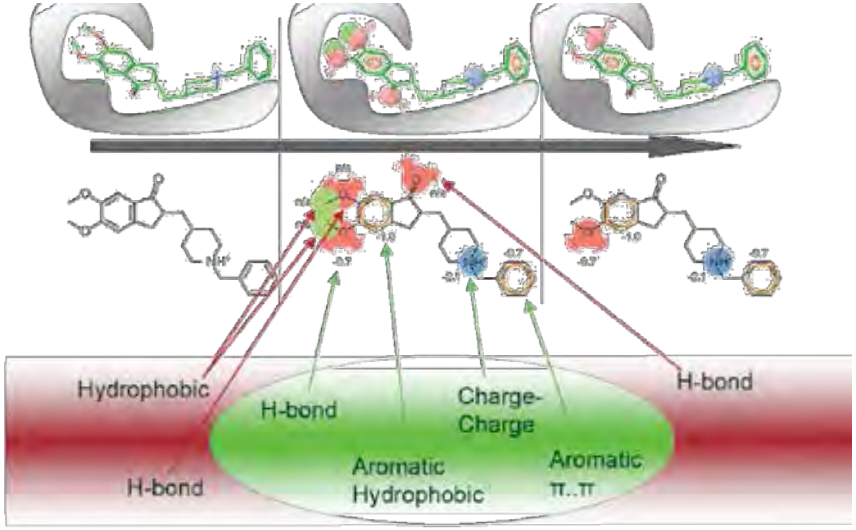
In this study, we have shown that the soy-derived isoflavones genistein and daidzein significantly protect vascular Endothelial cells against high-glucose- and H2O2-induced oxidative stress injury. This protective action is mediated by the regulation of Bcl-2/Bax expression, PI3K and Rho/ROCK signaling pathways, and ERβ expression, which suggests that soy isoflavones have multiple mechanisms of action against oxidative stress in vascular endothelial cells.
(Shang-Zhong Xu et al.,2009).

Methodology (structure-based)

1.- Preparation/selection of targets

2.- Definition of all the possible interaction models per target (training set)

- Docking



3.- Preparation of samples (sample set)

- Generation of metabolites
- Extraction of the interaction model (docking)

4.- Prediction of binding modes of samples

- Clustering

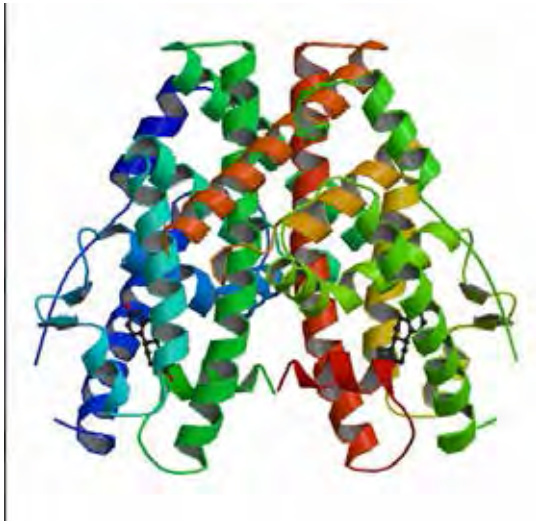
Methodology (structure-based)

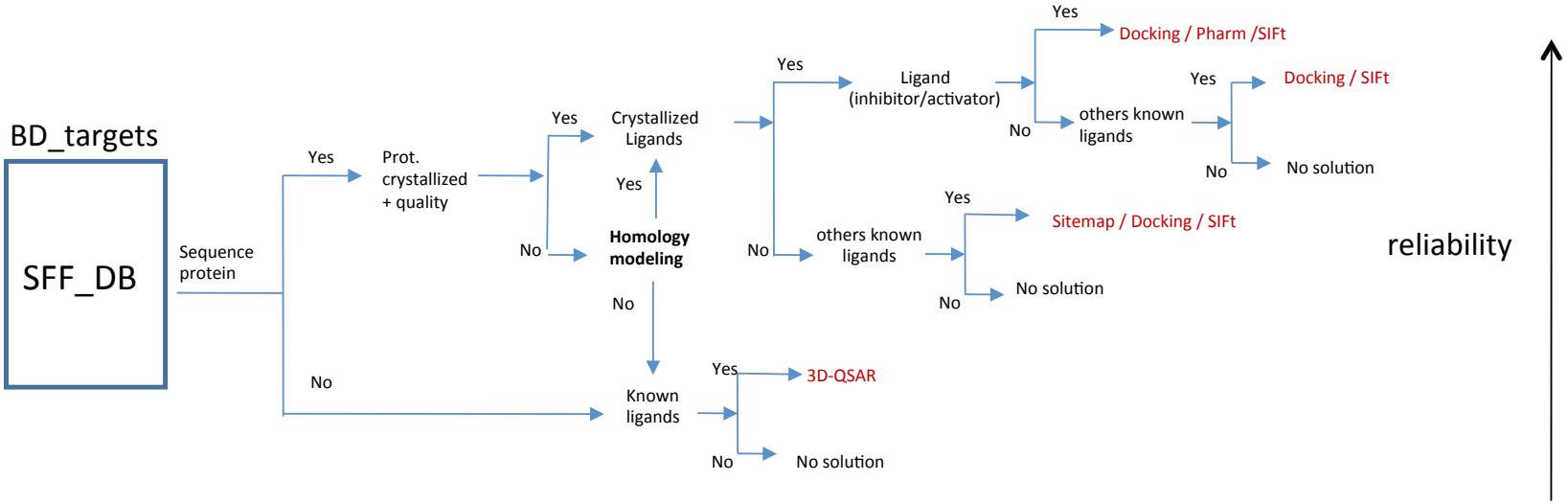
1.- Preparation/selection of proteins

i) Is the target crystallized? Protein Data Base structure, modelling …

ii) Is the crystallized ligand an activator or an inhibitor?

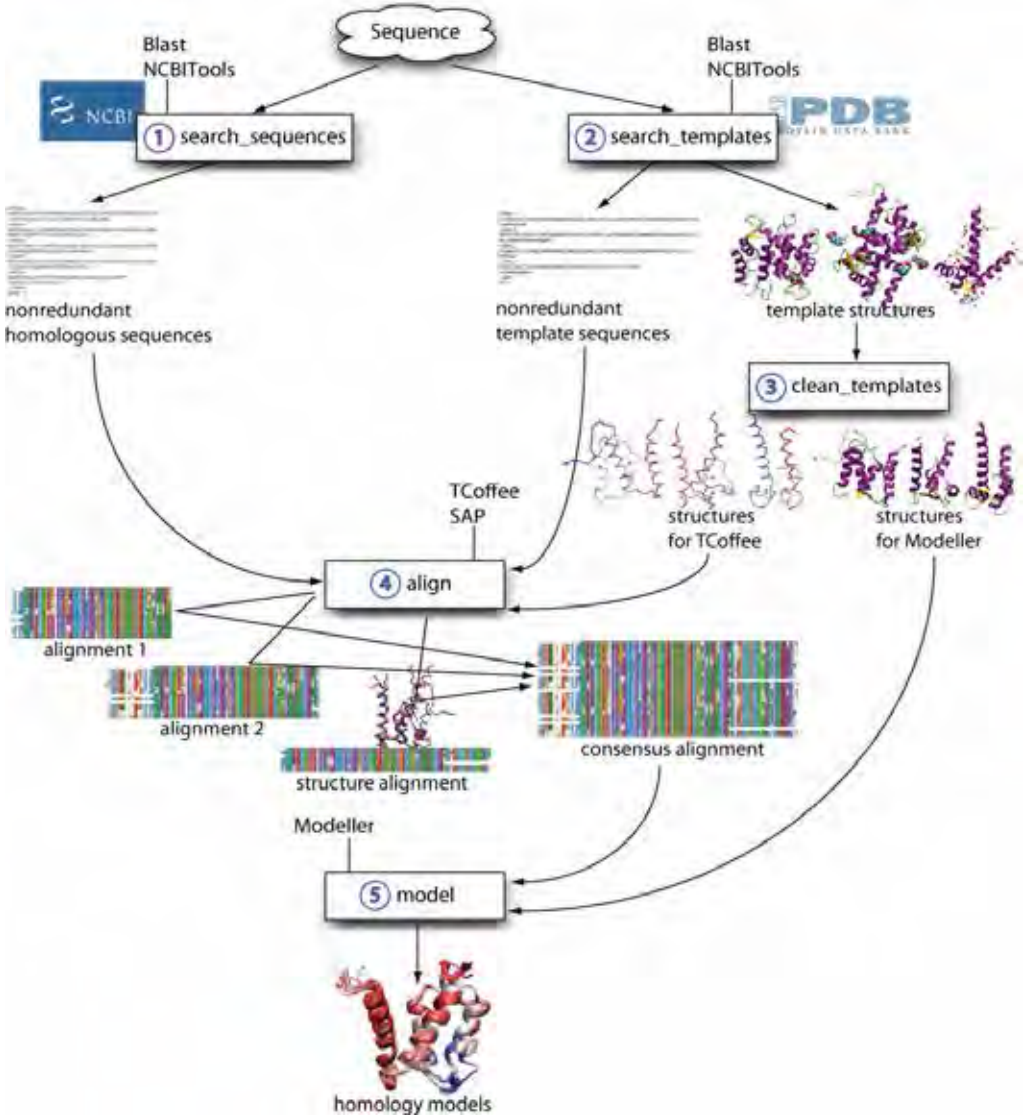
iii) Is the binding site well defined? Electron density





Methodology (structure-based)

Homology modeling



2.- Definition of all the possible interaction models

i) By known bioactive compounds

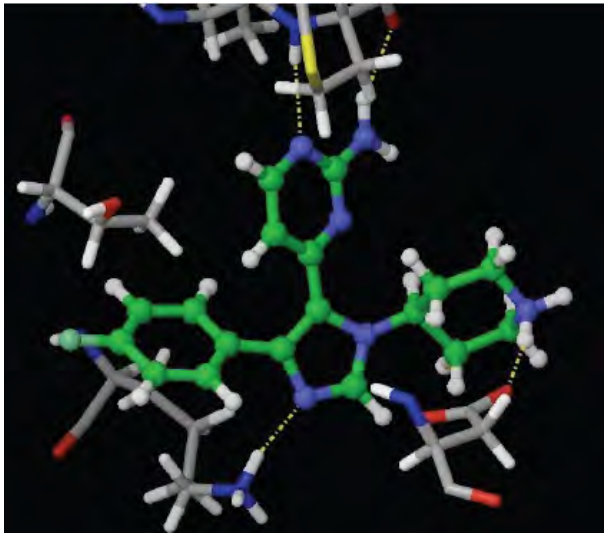
ii) By crystallized complex

} Training set

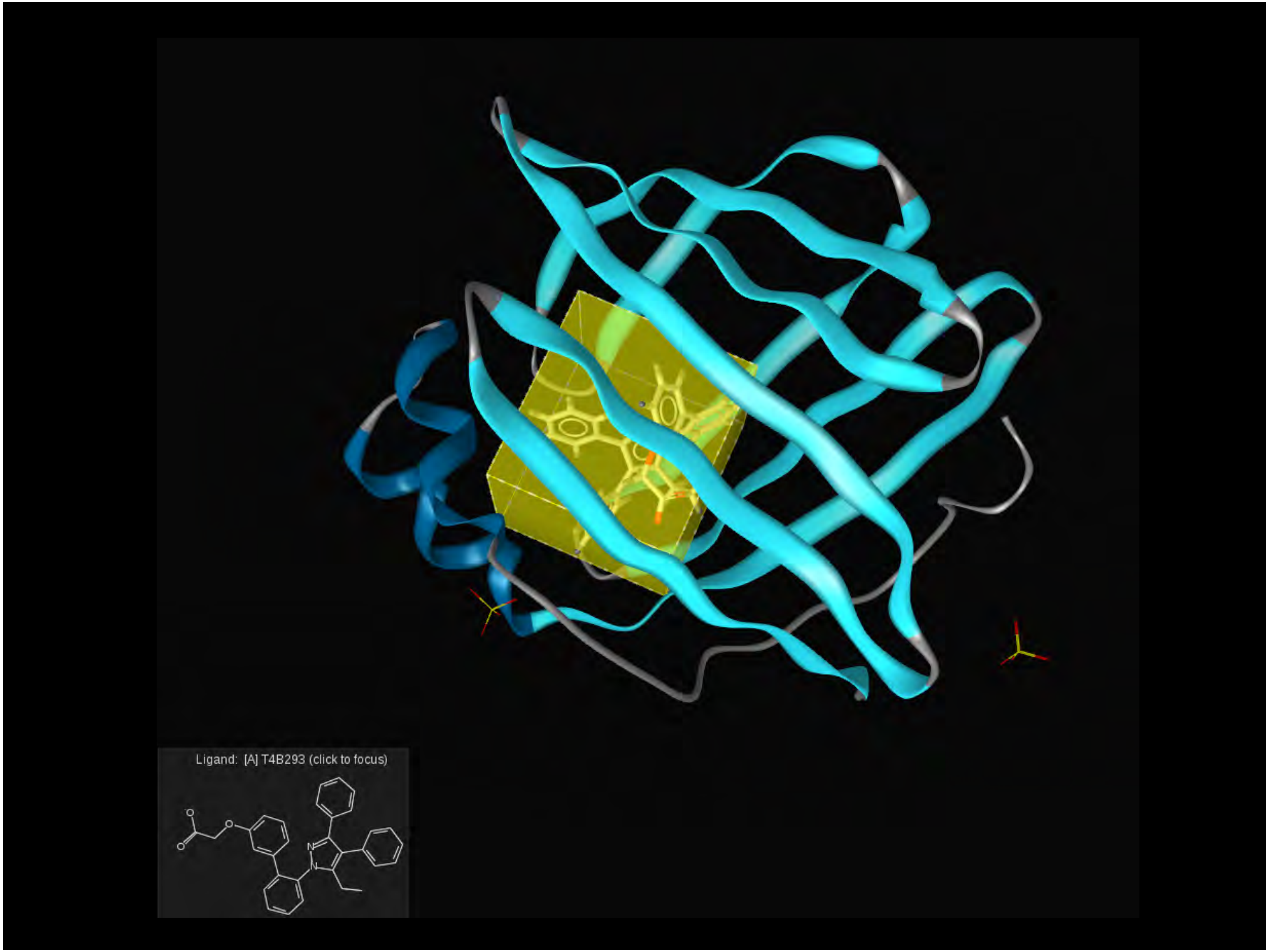
DOCKING reports the binding mode:

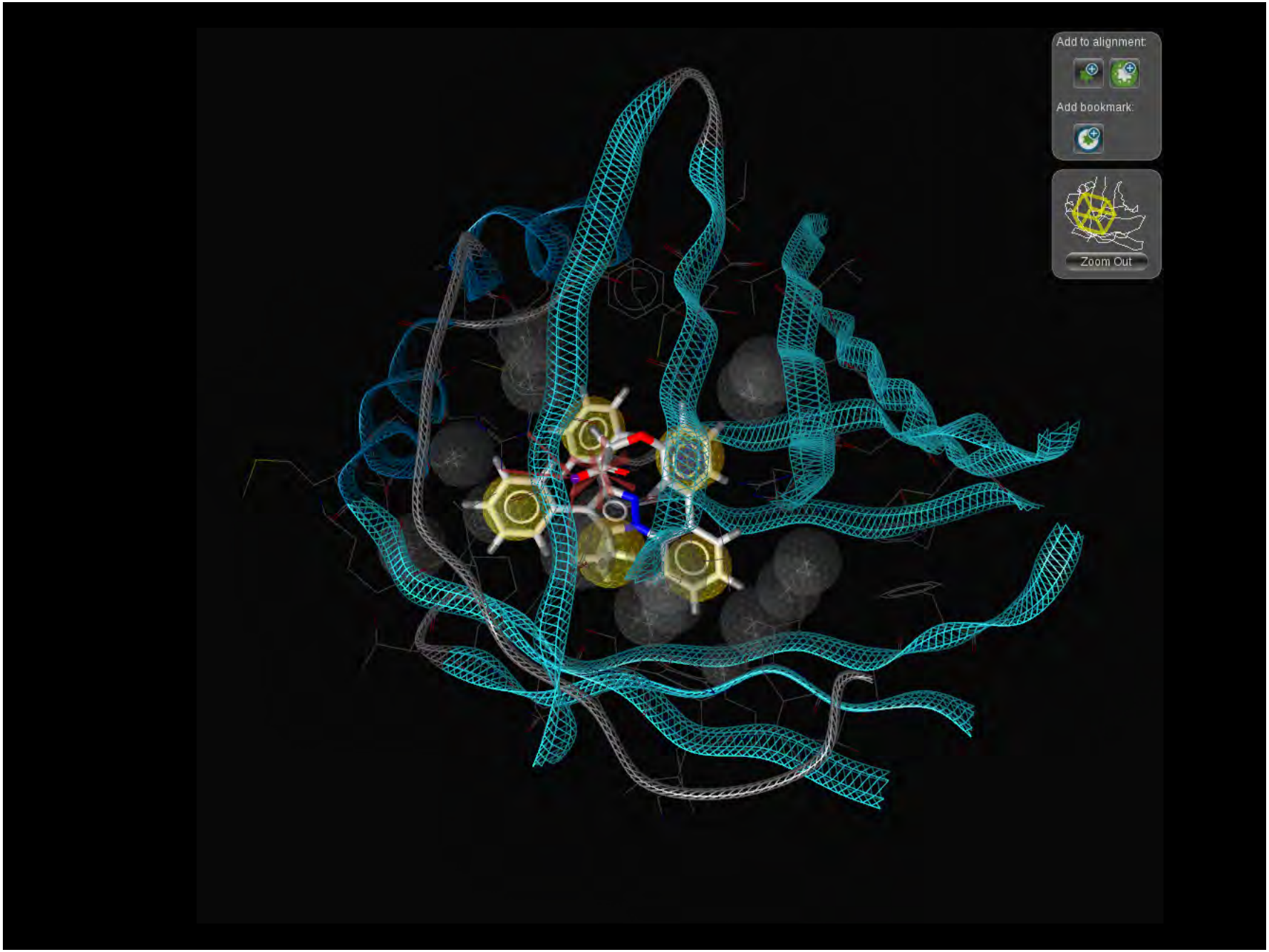
	Residue 1									Residue 2									Residue N								
compound 1	1	1	0	0	1	1	0	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	1	0
compound 2	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0
compound 3	1	1	0	1	0	1	0	1	0	1	1	0	1	0	0	0	0	0	1	1	0	1	0	1	0	1	0
⋮																											
compound n	1	0	0	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	1	0	1	0

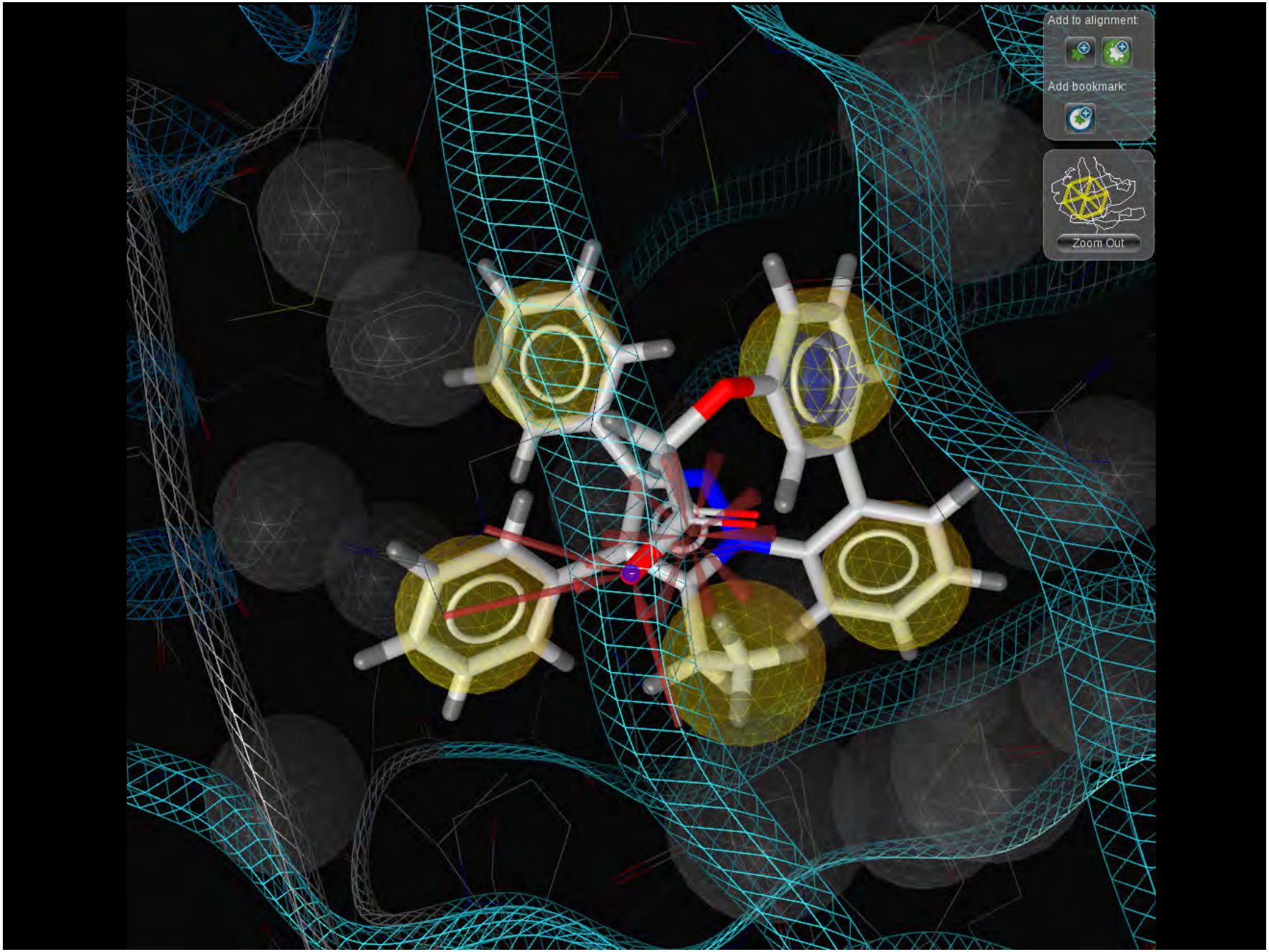
Data matrix



Bit 1 = whether it is in contact with the ligand.
Bit 2 = whether any main chain atom is involved in the contact.
Bit 3 = whether any side chain atom is involved in the binding.
Bit 4 = whether a polar interaction is involved.
Bit 5 = whether a non-polar interaction is involved.
Bit 6 = whether the residue provides hydrogen bond acceptor(s).
Bit 7 = whether it provides hydrogen bond donor(s).
Bit 8 = whether a aromatic interaction is involved.
Bit 9 = whether a charge interaction is involved.


Ligand: [A] T4B293 (click to focus)


Add to alignment:
Add bookmark:
Zoom Out


Add to alignment:
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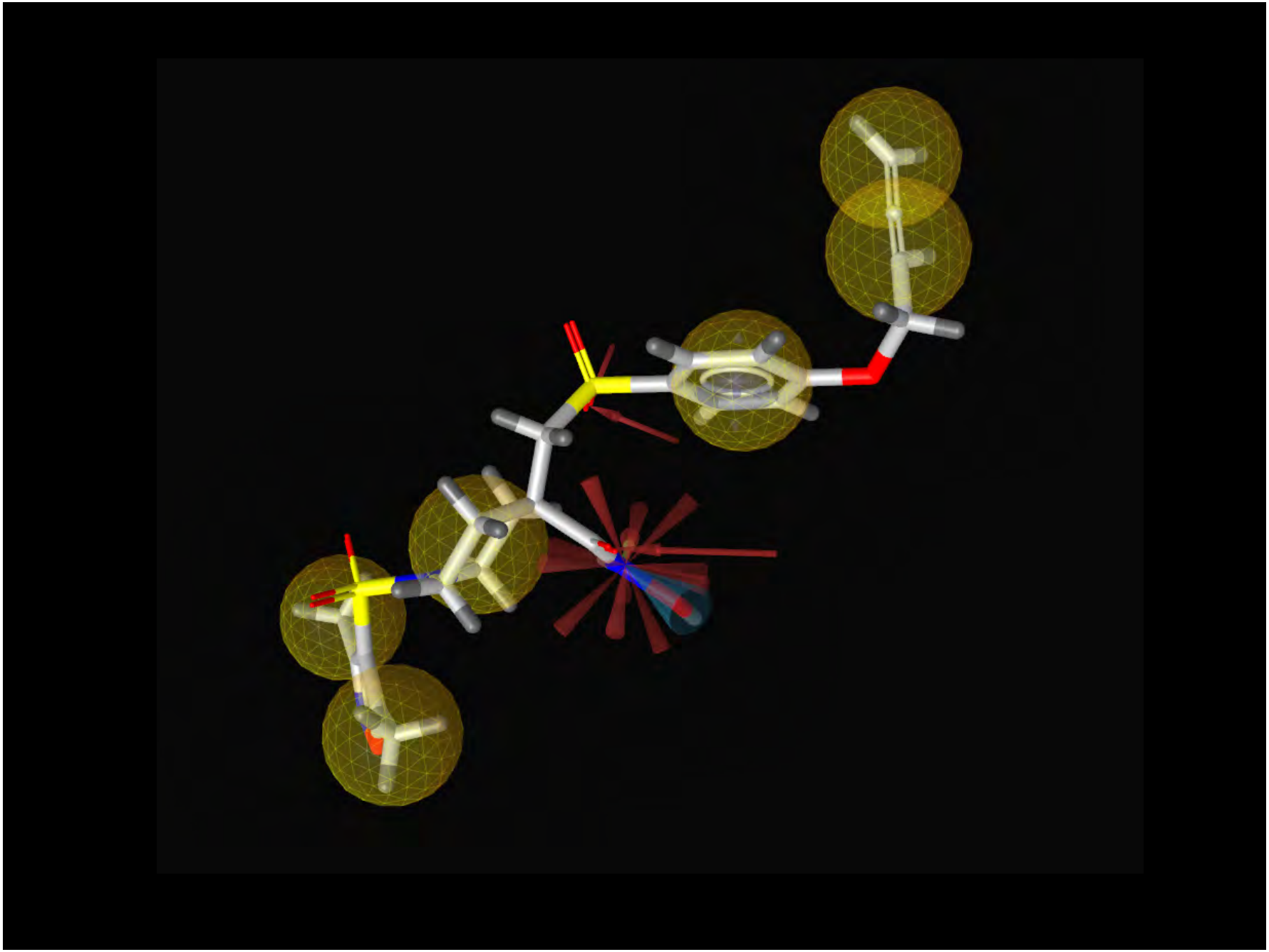
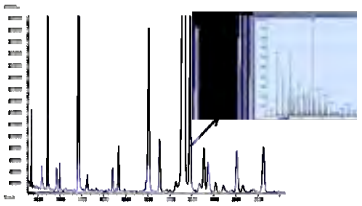


Methodology (structure-based)

3.- Preparation of samples (list of compounds to study)



1.- Characterization of the sample (Analytical Unit) — 24 comp.

2.- Generation of all the possible metabolites *in silico* — 1.000 comp.

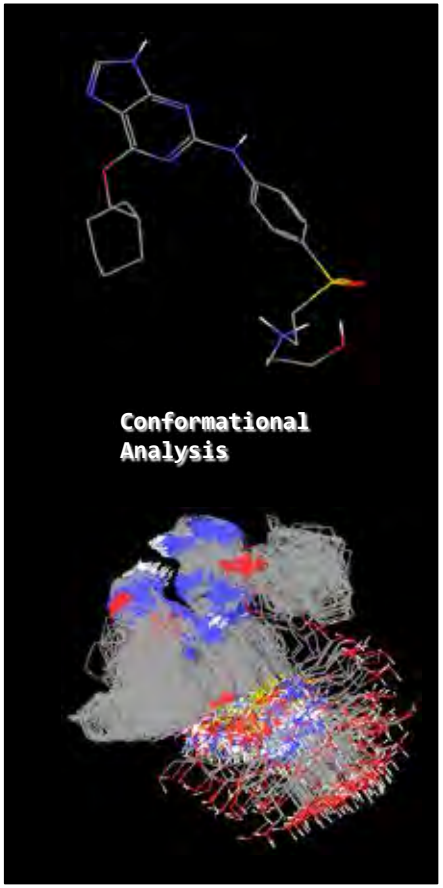
3.- Preparation of compounds — 3.000 comp.

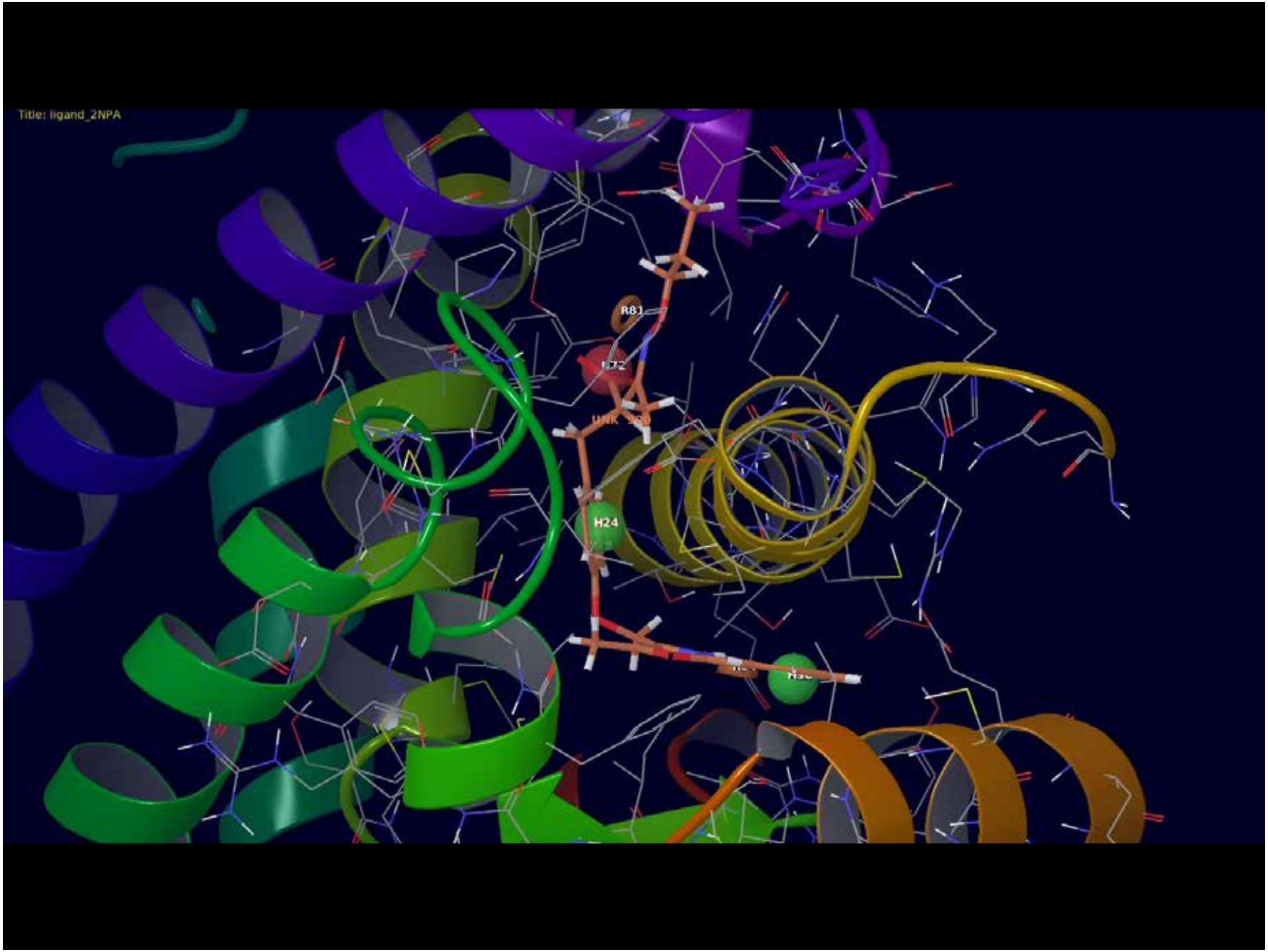
- Converting 2D structures in 3D
- Compounds at pH=7 ± 2
- Generation of tautomers
- Generation of stereoisomers

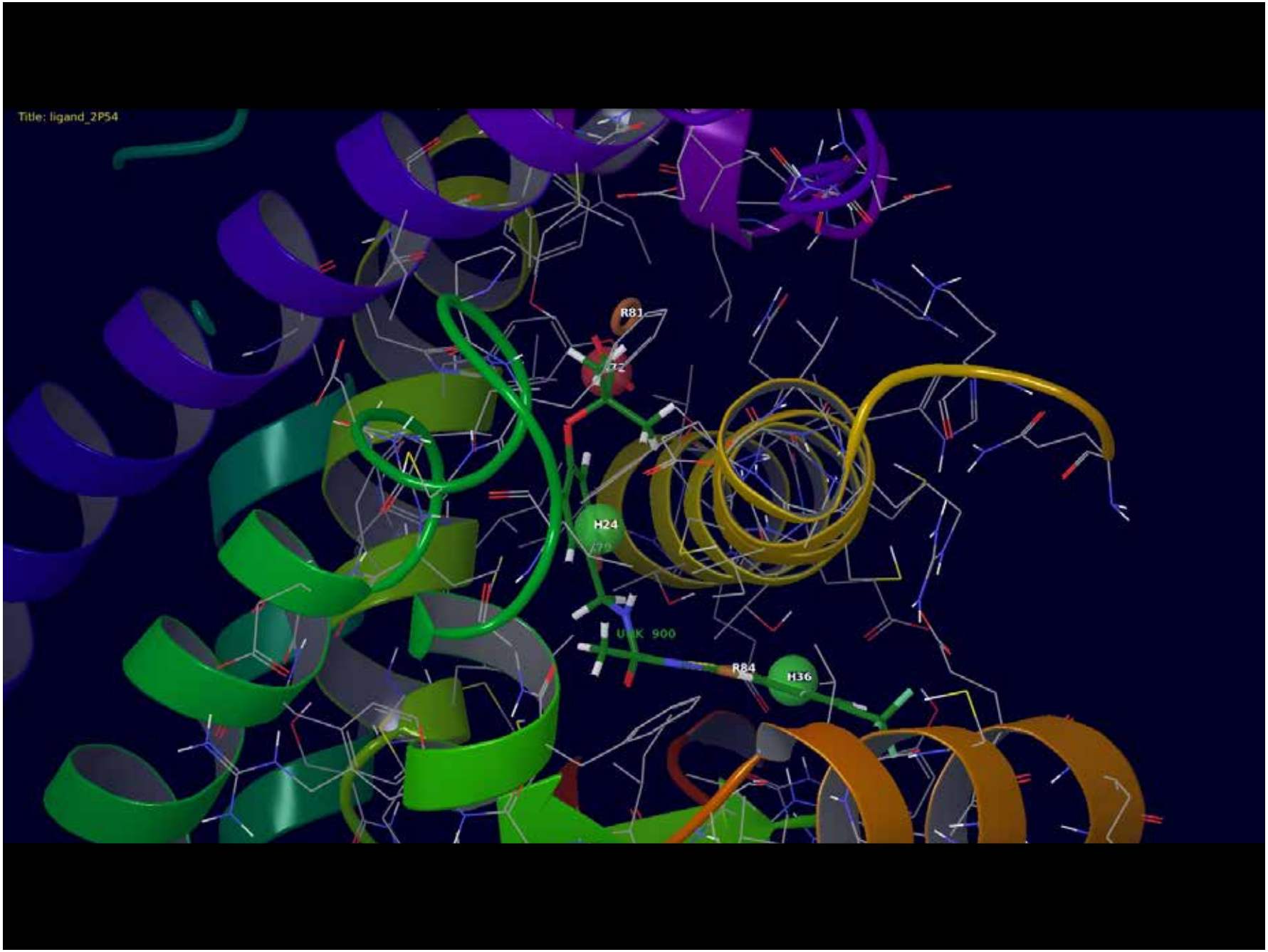
4.- Generation of all the possible conformers — 14.000 comp.

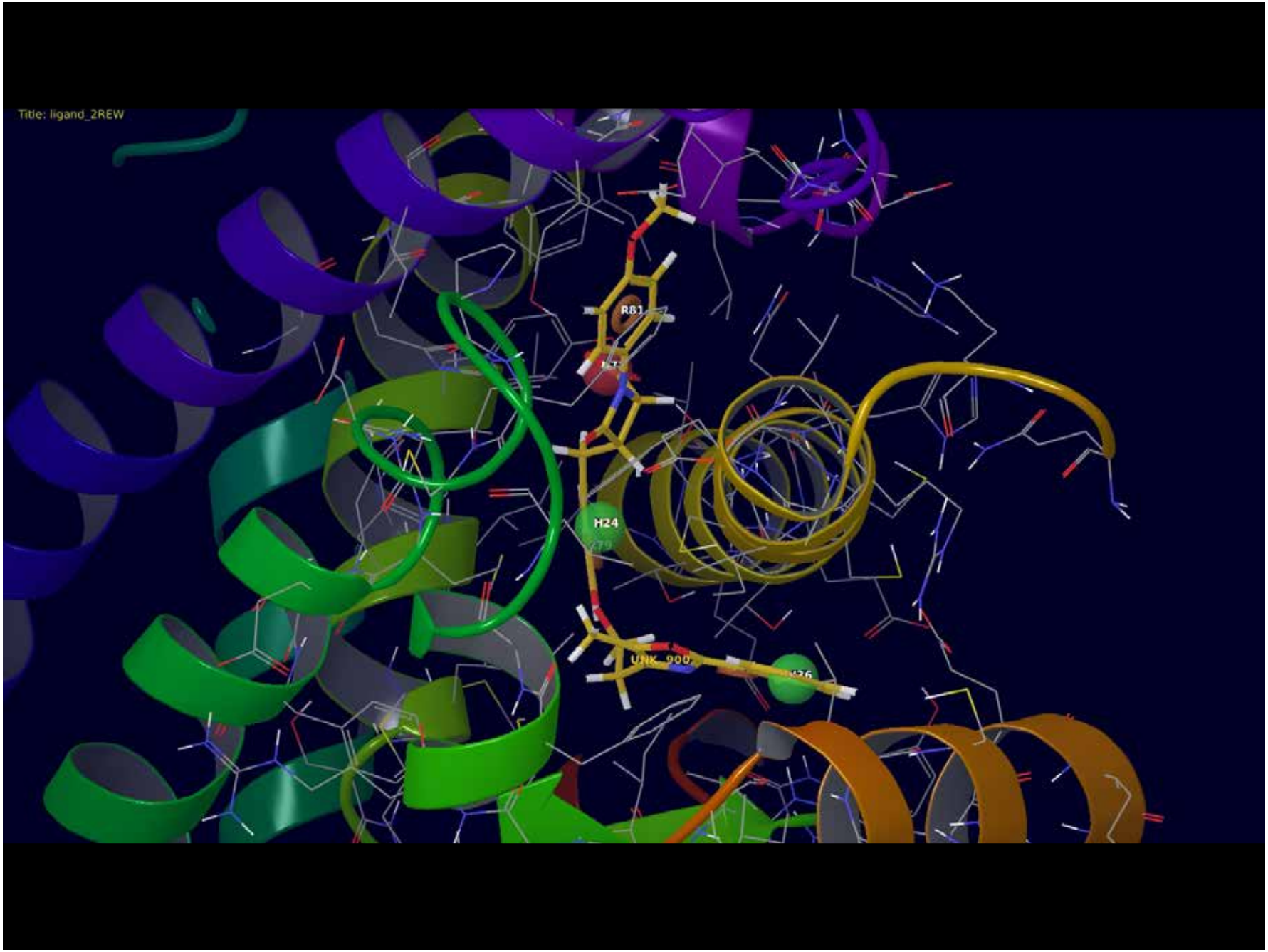
5.- Additional research of bibliographic metabolites is done as well.

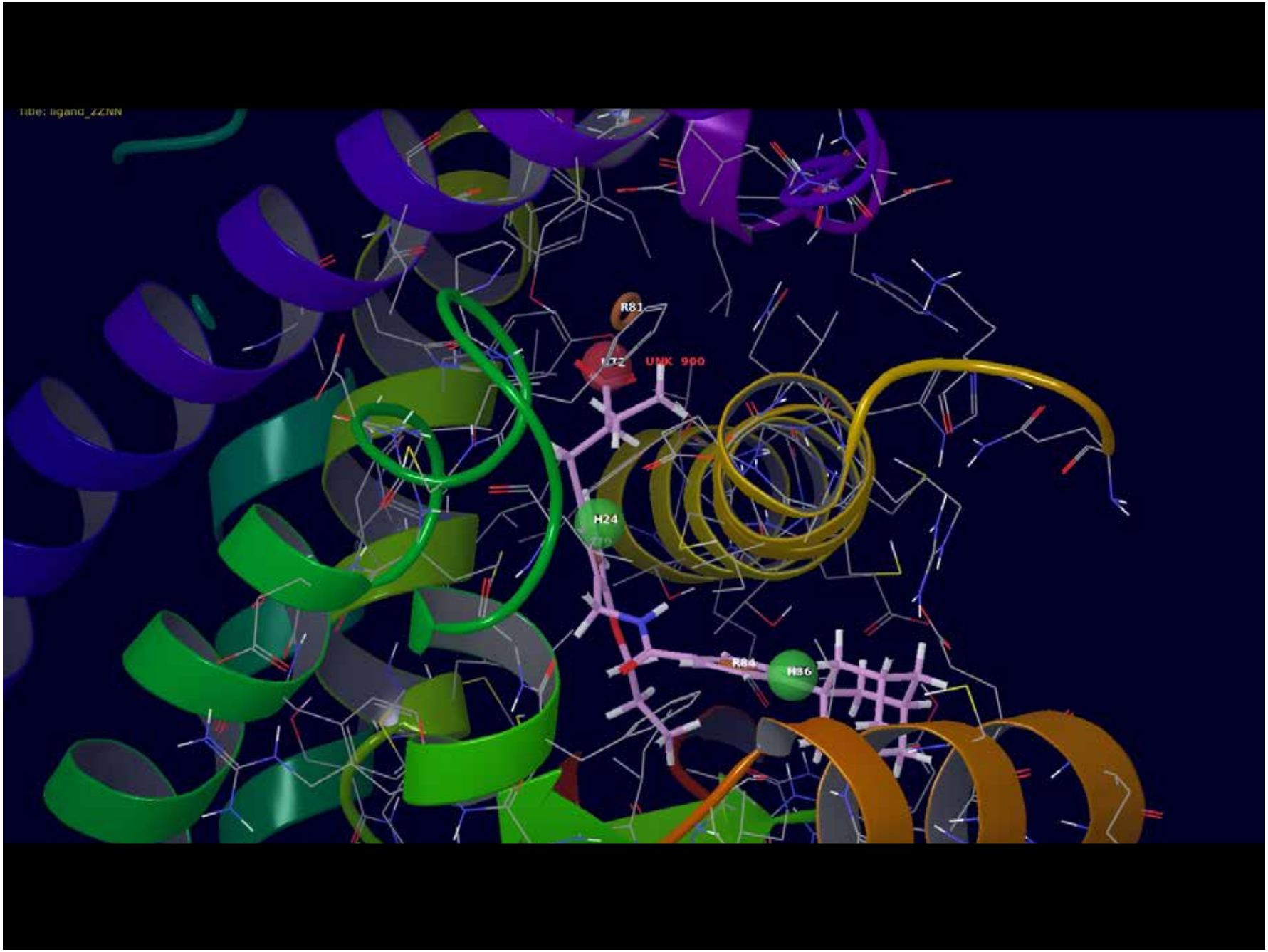
Finally, all the interaction models are generated by docking (sample).

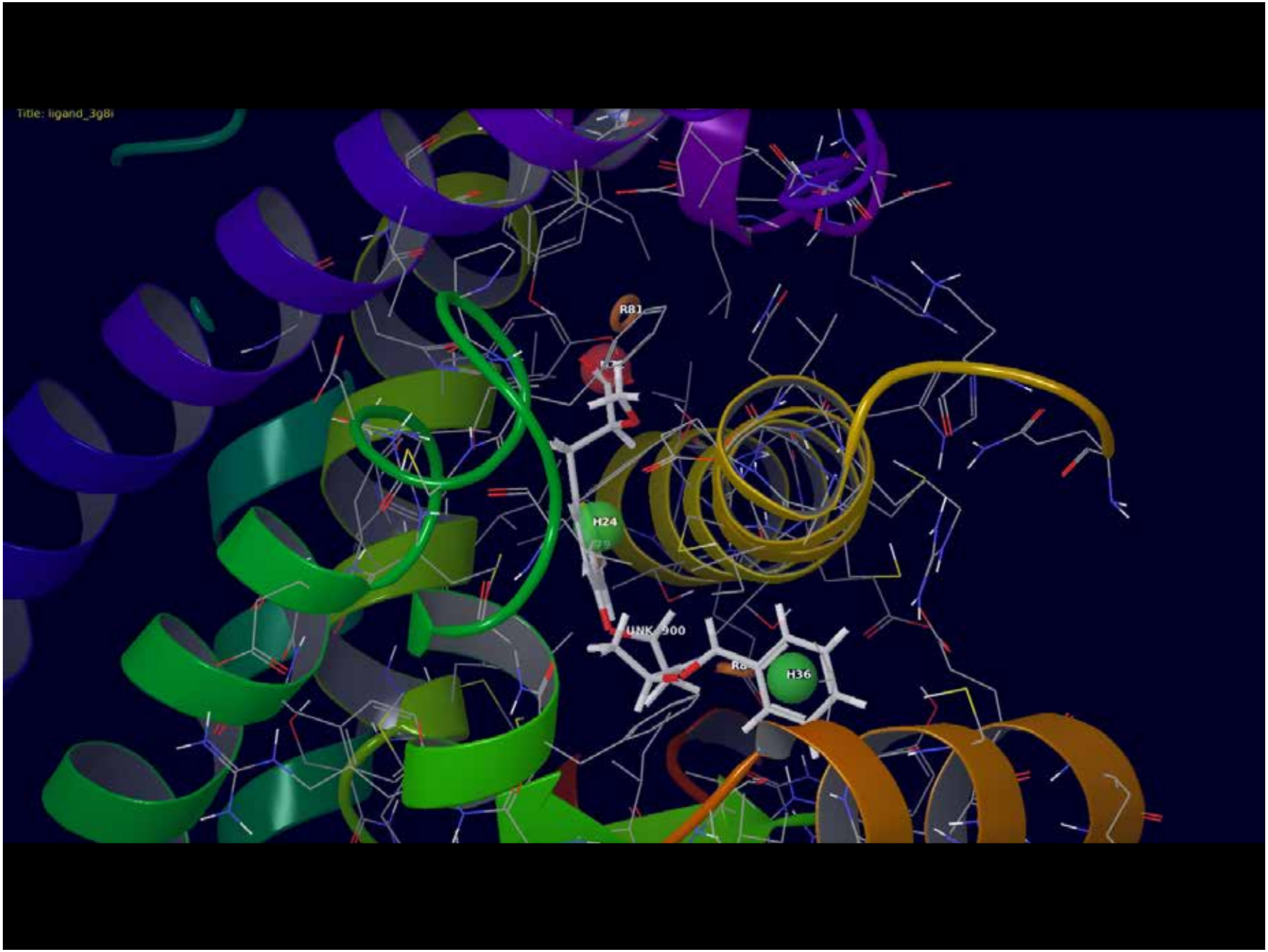



Title: ligand_2NPA
R81
H24


Title: ligand_2P54
R81
H24
R84
H36


Title: ligand_2REW
RB1
H24

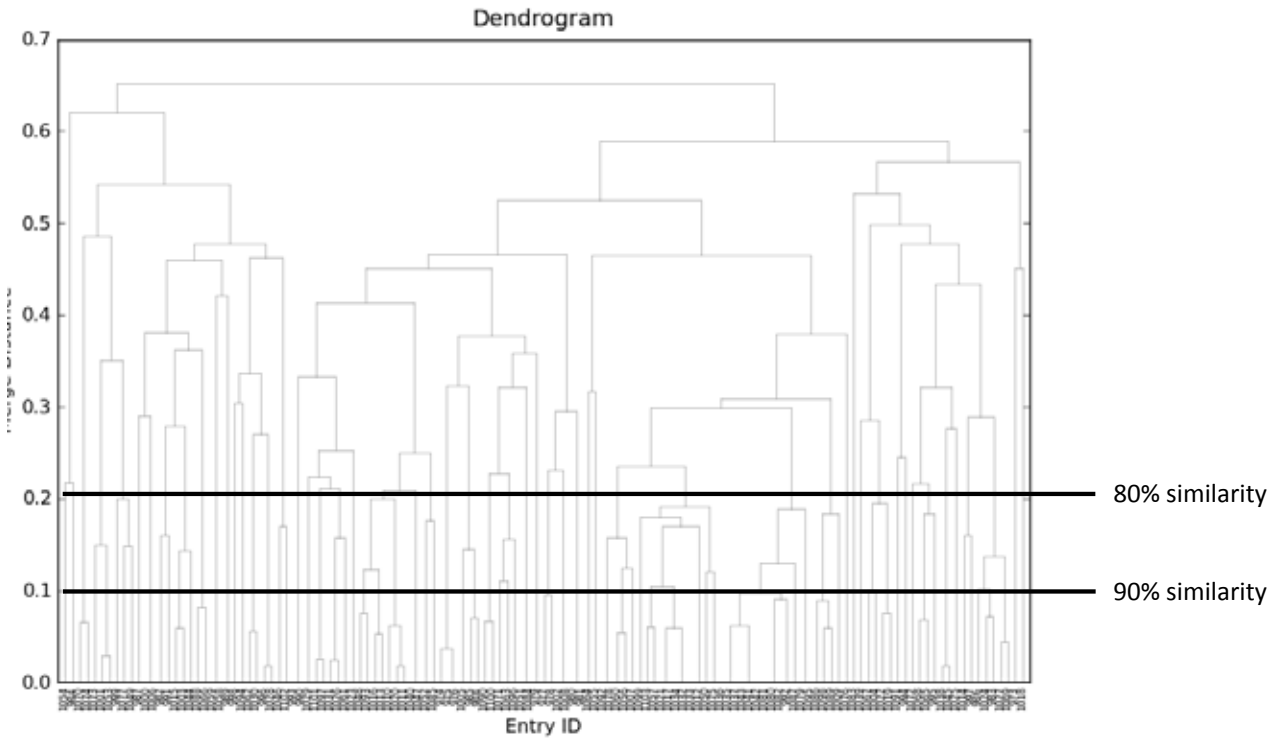

R81
UNK 900
H24
R84
H36


Title: ligand_3g8i
R81
H24
UNK 900
H36

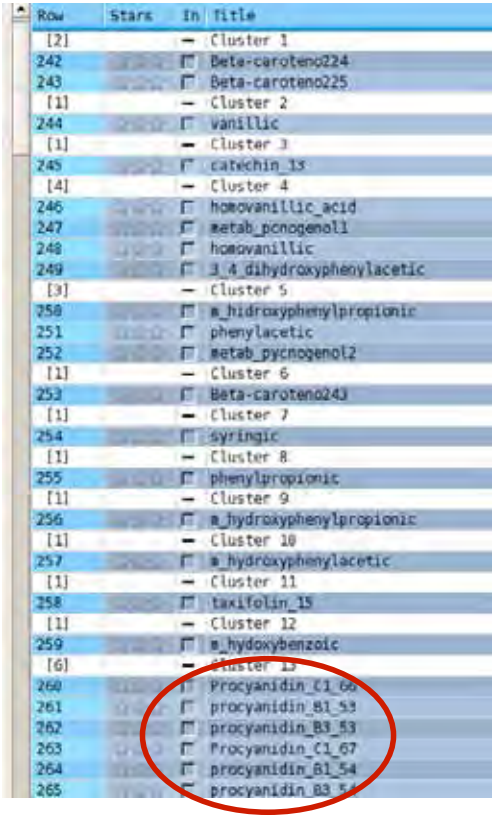
Methodology (structure-based)

4.- Prediction of our sample

- 14.000 compound vs targets.
- Compare binding mode of sample versus active compounds by clustering

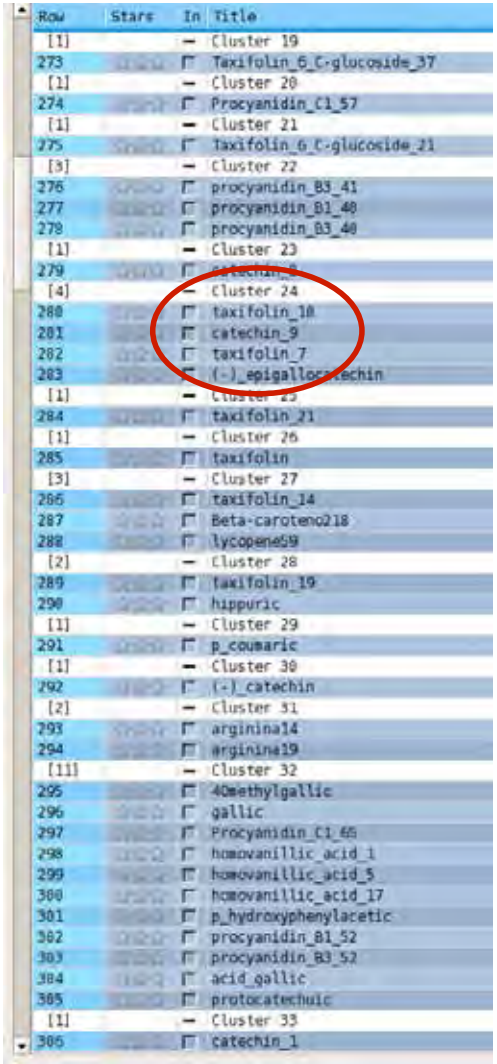


Methodology (structure-based)



Row	Stars	In	Title
[2]		–	Cluster 1
242		☐	Beta-caroteno224
243		☐	Beta-caroteno225
[1]		–	Cluster 2
244		☐	vanillic
[1]		–	Cluster 3
245		☐	catechin_13
[4]		–	Cluster 4
246		☐	homovanillic_acid
247		☐	metab_pcnogenol1
248		☐	homovanillic
249		☐	3_4_dihydroxyphenylacetic
[3]		–	Cluster 5
250		☐	m_hidroxyphenylpropionic
251		☐	phenylacetic
252		☐	metab_pycnogenol2
[1]		–	Cluster 6
253		☐	Beta-caroteno243
[1]		–	Cluster 7
254		☐	syringic
[1]		–	Cluster 8
255		☐	phenylpropionic
[1]		–	Cluster 9
256		☐	m_hydroxyphenylpropionic
[1]		–	Cluster 10
257		☐	m_hydroxyphenylacetic
[1]		–	Cluster 11
258		☐	taxifolin_15
[1]		–	Cluster 12
259		☐	m_hydoxybenzoic
[6]		–	Cluster 13
260		☐	Procyanidin_C1_66
261		☐	procyanidin_B1_53
262		☐	procyanidin_B3_53
263		☐	Procyanidin_C1_67
264		☐	procyanidin_B1_54
265		☐	procyanidin_B3_54

All these compounds will have a similar binding mode (90%)



Row	Stars	In	Title
[1]		–	Cluster 19
273		☐	Taxifolin_6_C-glucoside_37
[1]		–	Cluster 20
274		☐	Procyanidin_C1_57
[1]		–	Cluster 21
275		☐	Taxifolin_6_C-glucoside_21
[3]		–	Cluster 22
276		☐	procyanidin_B3_41
277		☐	procyanidin_B1_40
278		☐	procyanidin_B3_40
[1]		–	Cluster 23
279		☐	catechin_8
[4]		–	Cluster 24
280		☐	taxifolin_10
281		☐	catechin_9
282		☐	taxifolin_7
283		☐	(-)_epigallocatechin
[1]		–	Cluster 25
284		☐	taxifolin_21
[1]		–	Cluster 26
285		☐	taxifolin
[3]		–	Cluster 27
286		☐	taxifolin_14
287		☐	Beta-caroteno218
288		☐	lycopene59
[2]		–	Cluster 28
289		☐	taxifolin_19
290		☐	hippuric
[1]		–	Cluster 29
291		☐	p_coumaric
[1]		–	Cluster 30
292		☐	(-)_catechin
[2]		–	Cluster 31
293		☐	arginina14
294		☐	arginina19
[11]		–	Cluster 32
295		☐	4Omethylgallic
296		☐	gallic
297		☐	Procyanidin_C1_65
298		☐	homovanillic_acid_1
299		☐	homovanillic_acid_5
300		☐	homovanillic_acid_17
301		☐	p_hydroxyphenylacetic
302		☐	procyanidin_B1_52
303		☐	procyanidin_B3_52
304		☐	acid_gallic
305		☐	protocatechuic
[1]		–	Cluster 33
306		☐	catechin_1

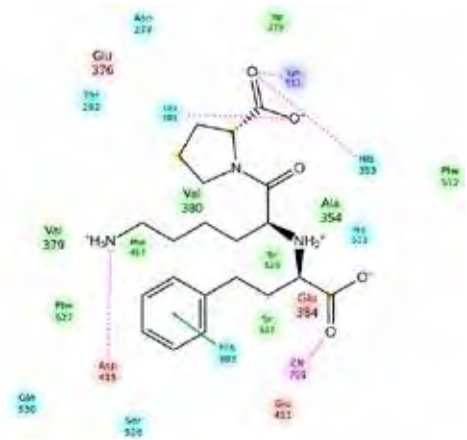
(-) epigallocatechin is a known inhibitor of FABP4 and taxifolin_10, taxifolin_7 and catechin_9 have similar binding mode (90%)

Taxifolin_10, taxifolin_7 and catechin_9 would have the same effect as (-) epigallocatechin

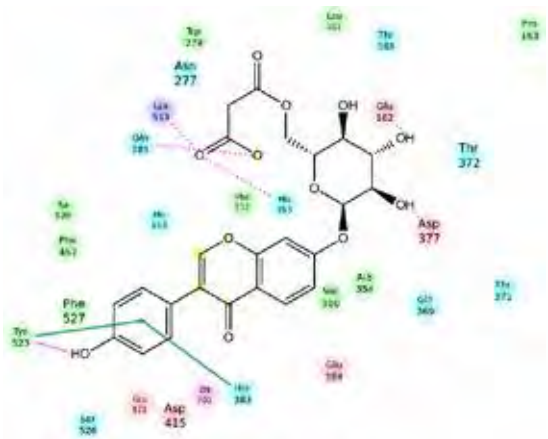
Results

ACE

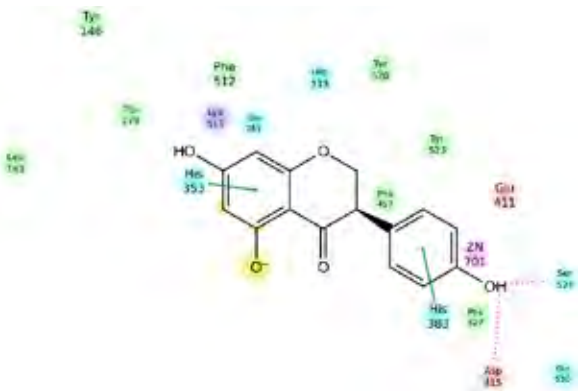
Clusters (dendograma)	nombre del compuesto	puro/metabolito	similitud %
clúster 30	Bioactivo del complejo cristalográfico 2C6N	--	100
	Malonyldaidzin	Puro (soja)	85.7
	(R)-Dihydrogenistein	Metabolito	79.4
	O-Desmethylangolensin	Metabolito	78.1
	(S)-Equol	Metabolito	75.7
	Daidzein	Puro (soja)	74.6
	Genistein	Puro (soja)	74.6
	Dihydroequol	Metabolito	73.1



a) Bioactivo del complejo cristalográfico 2C6N



b) Malonyldaidzin

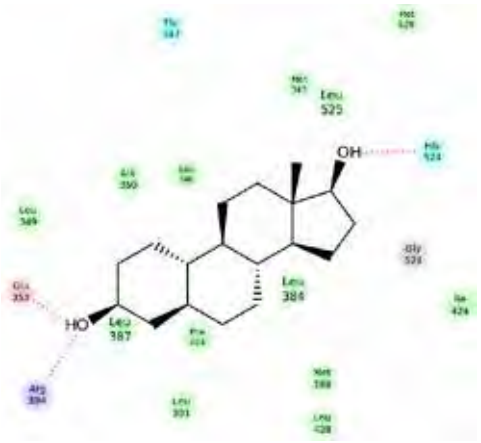


c) (R)-Dihydrogenistein

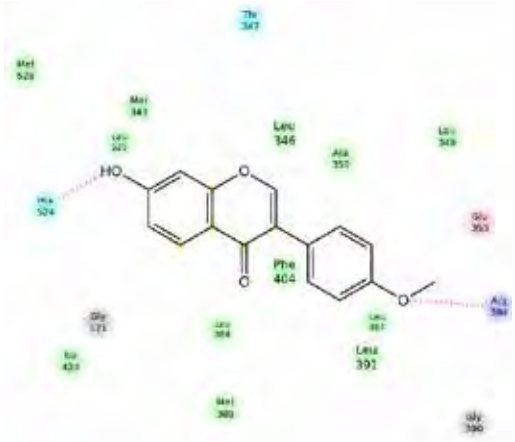
Results

ESRa

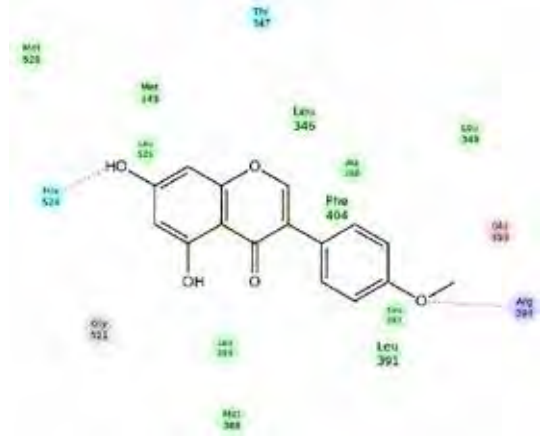
Clusters (dendograma)	nombre del compuesto	puro/metabolito	similitud %
clúster 249	Bioactivo del complejo cristalográfico 1GWQ	---	100
	O-desmethylangolensin	Metabolito	84
	Genistein	Puro (soja)	83
	(R)-dihydrodaidzein	Metabolito	83
clúster 488	Bioactivo del complejo cristalográfico 1L2I	---	100
	(R)-dihydrogenistein	Metabolito	79
	(S)-dihydrogenistein	Metabolito	78
clúster 485	Bioactivo del complejo cristalográfico 1GWR	---	100
	Formononetin	Puro (trébol rojo)	92
	Biochanin A	Puro (trébol rojo)	92
	(S)-dihydrodaidzein	Metabolito	78



a) Bioactivo del complejo cristalográfico 1GWR



b) Formononetin

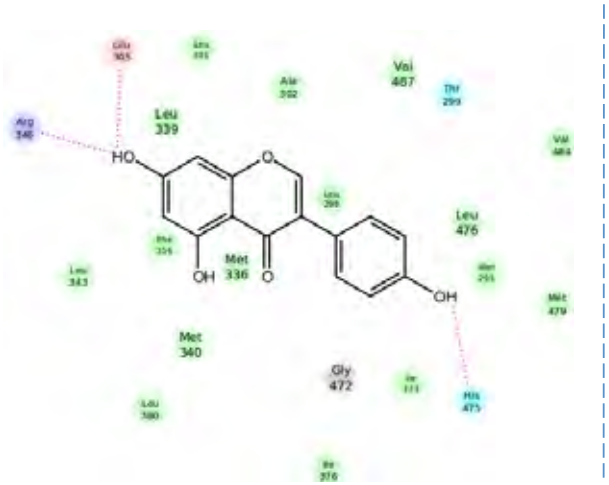


c) Biochanin A

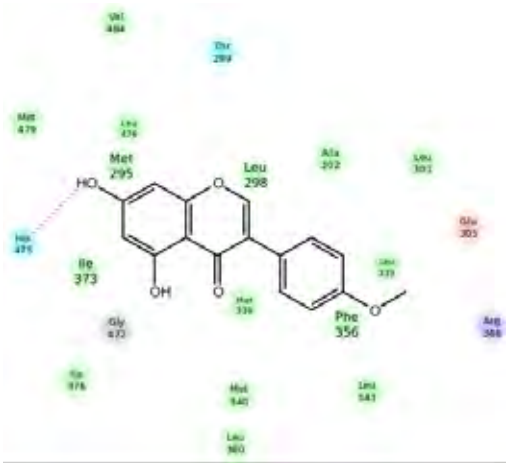
Results

ESRb

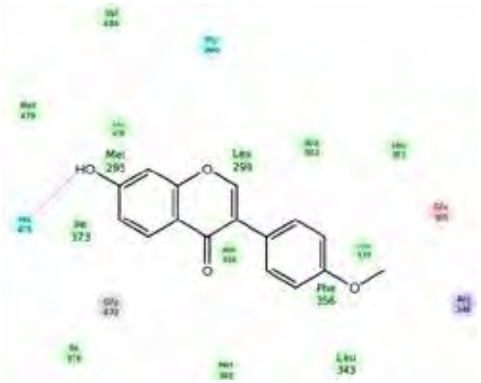
Clusters (dendograma)	nombre del metabol.	origen del metol.	similitud %
clúster 17	Bioactivo del complejo cristalográfico 1QKM	---	100
	Genistein	Puro (soja)	100
	O-Desmethylangolensin	Metabolito	96
	Biochanin A	Puro (trébol rojo)	88
	Formononetin	Puro (trébol rojo)	86
	(R)-Dihydrogenistein	Metabolito	80
	(S)-Dihydrogenistein	Metabolito	79
	(S)-Equol	Metabolito	77
	(R)-Equol	Metabolito	77
	(R)-Dihydrodaidzein	Metabolito	76
	Daidzein	Puro (soja)	75
	dihydroequol	Metabolito	72
	(S)-Dihydrodaidzein	Metabolito	72



a) Bioactivo del complejo cristalográfico 1qkm



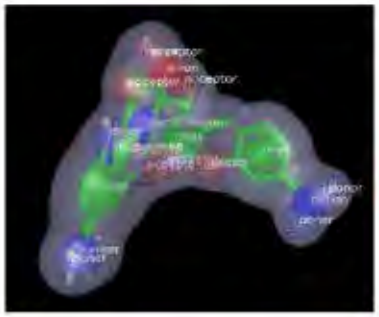
b) Biochanin_A



d) Formononetin

Methodology (ligand-based)

3D QSAR

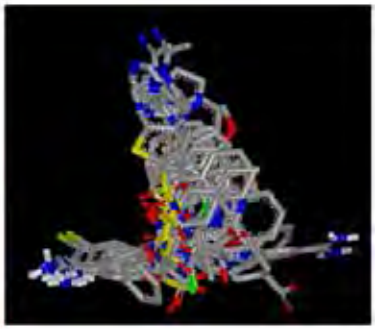


Active Compound(s)

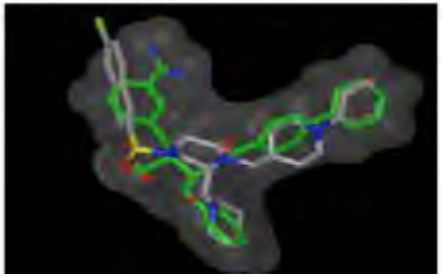
Query Creation

Query editing

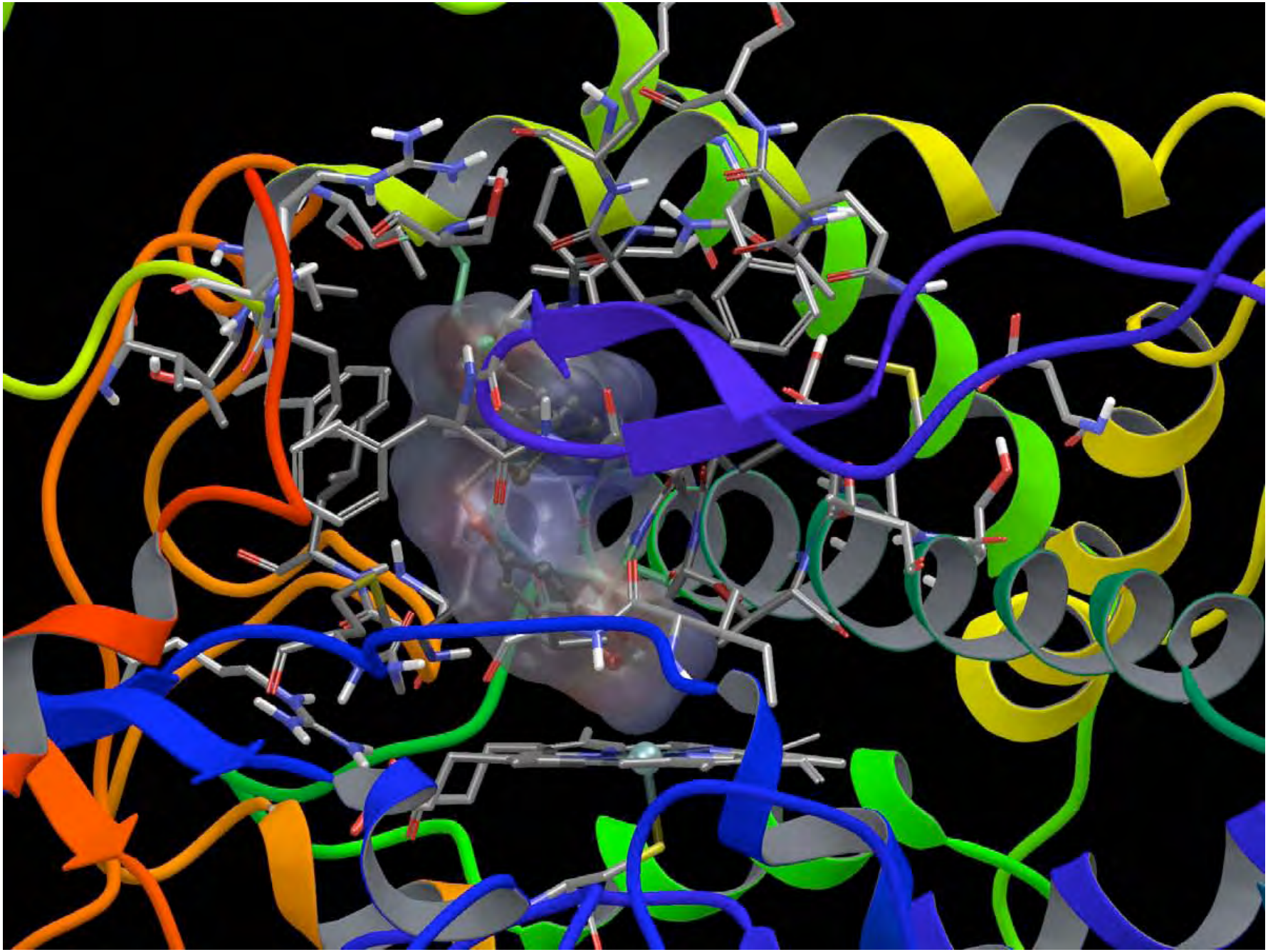
Query validation



- Compounds of the ingredient



- Shape similarity
- Electrostatic similarity



Muchas gracias

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