Predicting estrogen receptor binding of chemicals using a suite of in silico methods: complementary approaches of (Q)SAR, molecular docking and molecular dynamics

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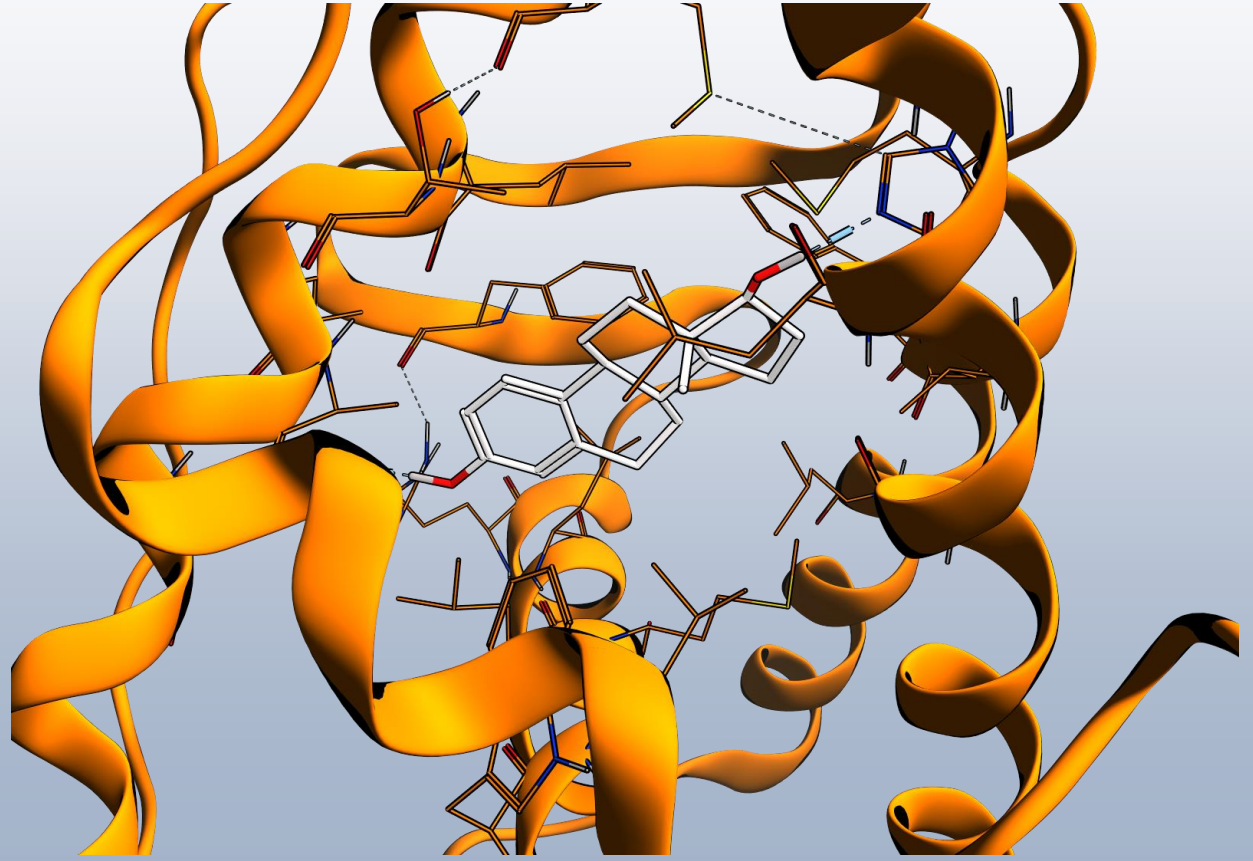
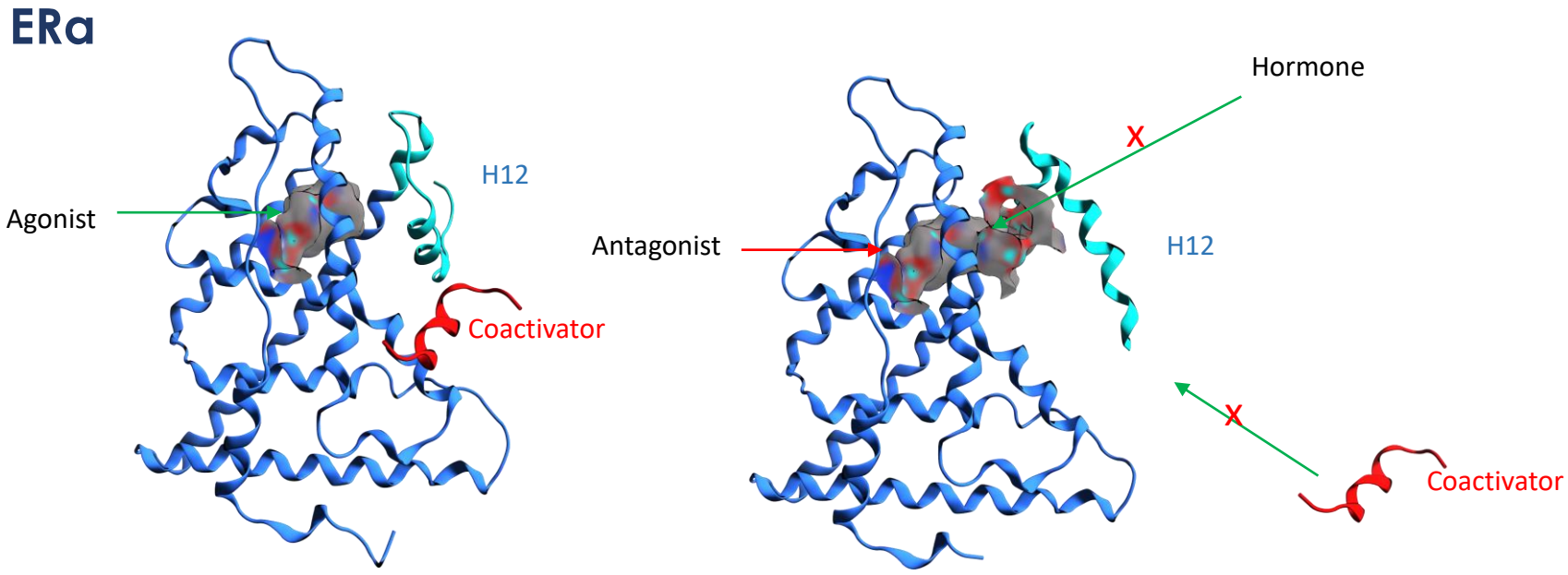


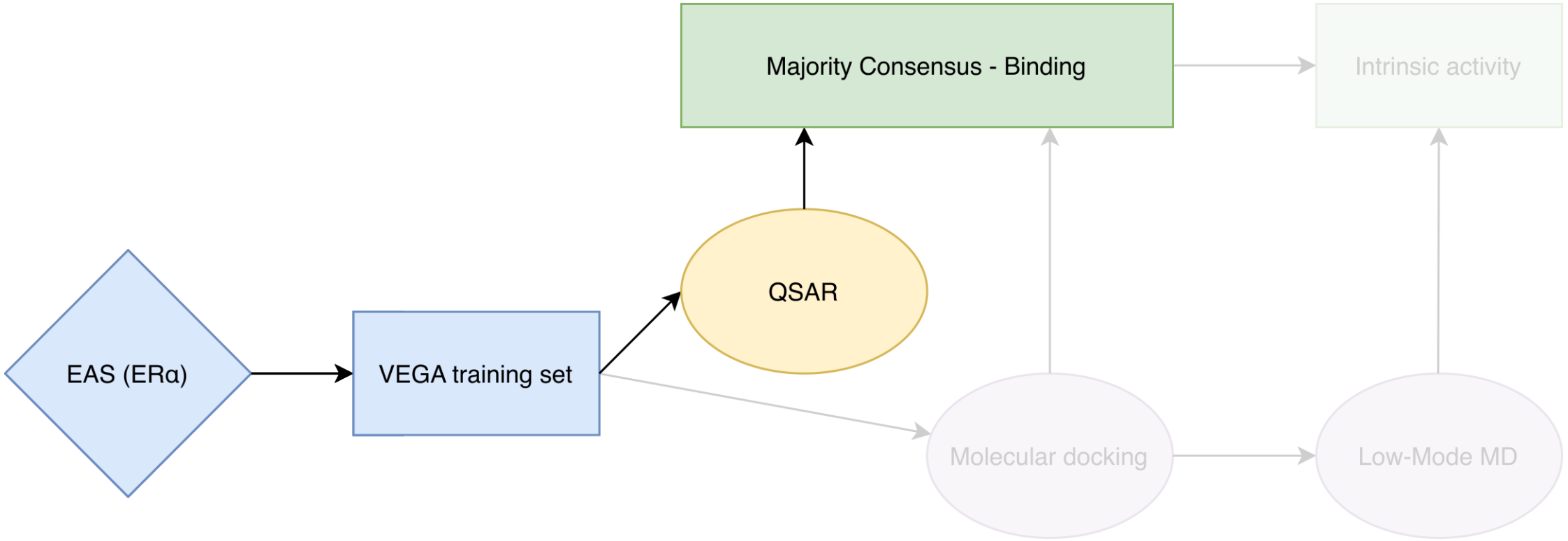
EuroMix

*The EuroMix project will deliver a **mixture test strategy and test instruments** using novel techniques as recently proposed by the Joint Research Centre (JRC) of the European Commission. The tests will result in data needed for refining **future risk assessment of mixtures relevant to national food safety authorities**, public health institutes, the European Food Safety Authority (EFSA), the European Chemical Agency (ECHA), industry, regulatory bodies and other stakeholders. Ultimately, this will provide information for future risk management decisions on the safety of chemicals in mixtures to be taken by the European Commission and the Codex Alimentarius.*

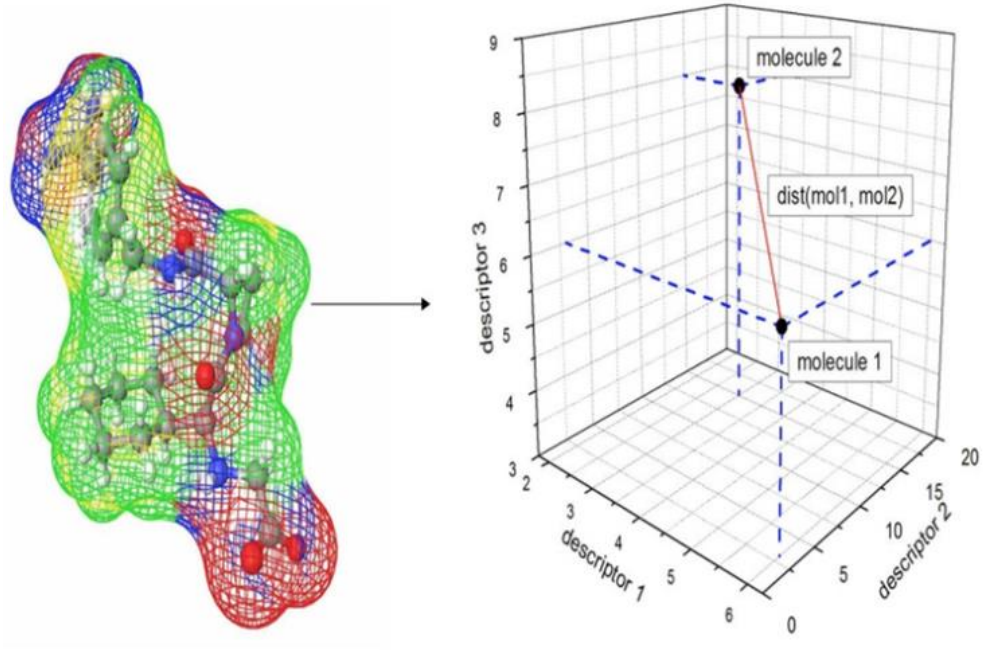
During this project, a screening protocol based on in silico techniques was developed to prioritize chemicals, considering different receptors/enzymes that are targets of the selected toxicological outcomes:

i. Endocrine interferences
ii. Developmental toxicity
iii. Liver toxicity (hepatic steatosis)





The VEGA database provides a qualitative description for Relative Binding Affinity (RBA) for endocrine disruptor screening. This database contains experimentally determined values of human ERa for both **receptor binding (RBA)** and **reporter gene (RA) assays**, expressed as percentage of activity using 17β-estradiol as reference. **To develop the dataset, any detectable activity was labelled active and no detectable activity was labelled inactive**.



A QSAR is a statistical model that relates a set of **structural descriptors** of a chemical compound to its **biological activity**.

The basic assumption of this methodology is that **similar molecules have similar activities**.

We used 7 freely-available model, specific for ERa, taking into account different molecular descriptors to categorise VEGA compounds in **Positive or Negative with respect to 17β-estradiol**, accounting VEGA experimental RBA.

The validity of the QSAR predictions was investigated by calculating the so-called Cooper Statistics below:

Sensitivity (true positive rate)= TP/TP+FN

Specificity (True negative rate) = TN/TN+FP

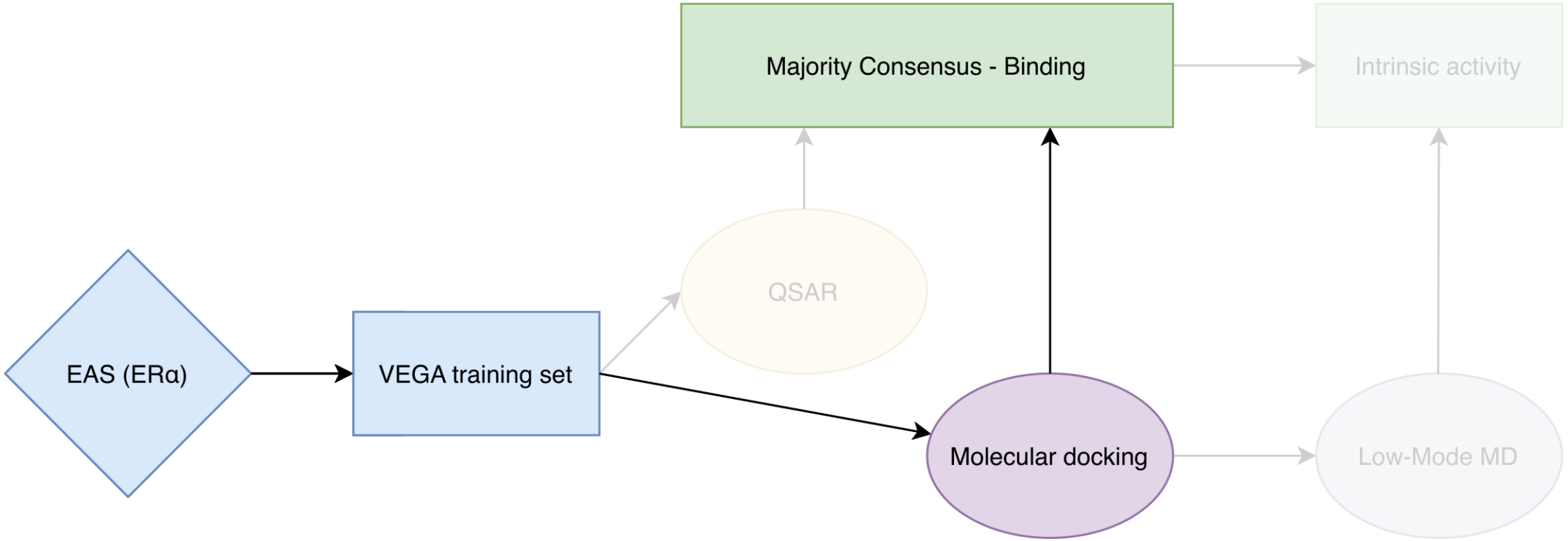
Accuracy = (TN+TP)/(TN+FP+FN+TP)

where TP = true positive, TN = true negative, FP = false positive and FN = false negative

Model	Sensitivity (True positive rate: TP/TP+FN)	Specificity (True negative rate: TN/TN+FP)	Accuracy (TN+TP)/(TN+FP+FN+TP)
COSMOS ER receptor model	0.85	0.40	0.55
DEREK Nexus	0.33	0.98	0.75
OCHEM estrogen receptor α agonists	0.88	0.51	0.66
OECD QSAR Toolbox alerts (Binding)	0.29	0.83	0.64
OECD QSAR Toolbox alerts (Alert)	0.75	0.64	0.68
VEGA - RBA	0.77	0.88	0.84
VEGA - CERAPP	0.73	0.68	0.70
Majority Consensus using 9 models	**0.77**	**0.82**	**0.80**

Among the 7 QSAR models, the **VEGA - RBA** model shows the highest accuracy and the best sensitivity, while the two models highlighted in red show low sensitivity values, but good values of specificity. **VEGA – RBA** model may be inflated, as although the validation set was not used to build the model.

We evaluate the QSAR method through a **majority consensus**: a compound was considered active if this is active in at least 4 QSAR models. The integration of these 7 QSAR models therefore shows **high Sensitivity, Specificity and Accuracy**, going beyond possible bias of building the models themselves.



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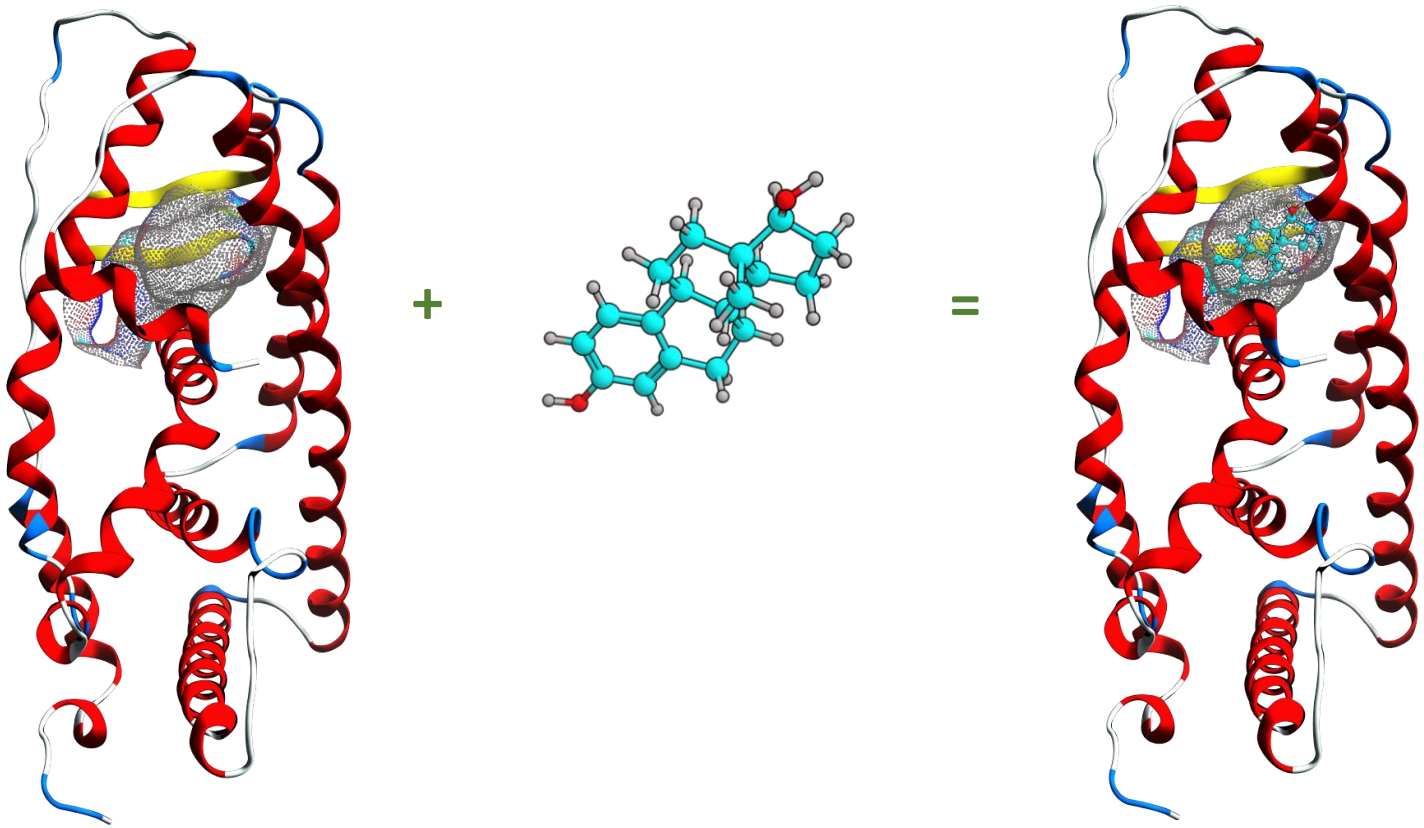
The 3D structure of ERa (PDB ID: 3UUD) ligand binding domain (LBD), co-crystallized with 17β-estradiol, was used for molecular docking simulations.
Binding free energy, expressed in kcal/mol, was computed for each complex to prioritize chemicals.

Two docking protocols were used in order to test the accuracy/computing speed ratio.



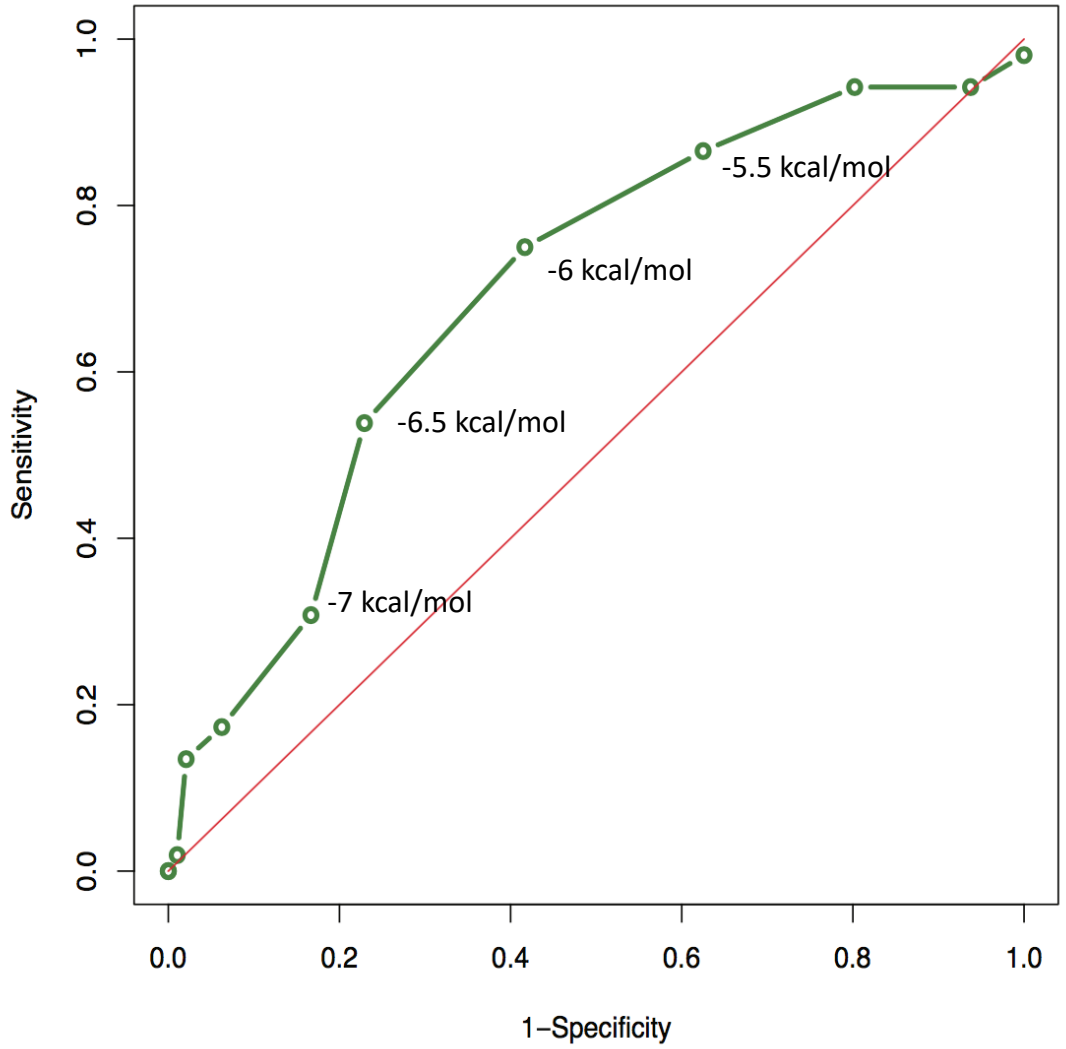
The validity of the molecular docking simulations was investigated by calculating the so-called Cooper Statistics below:

Sensitivity (true positive rate)= TP/TP+FN

Specificity (True negative rate) = TN/TN+FP

Accuracy = (TN+TP)/(TN+FP+FN+TP)

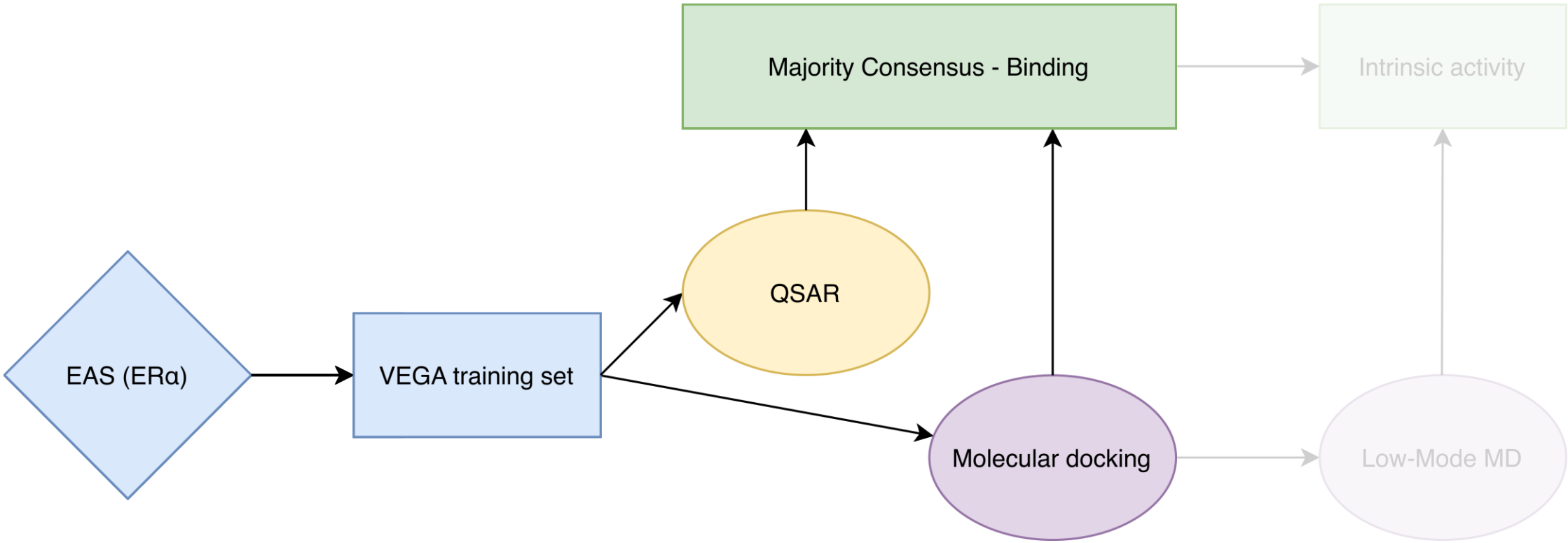
where TP = true positive, TN = true negative, FP = false positive and FN = false negative



Cooper statistics shows that accuracy of the molecular docking is comparable with the accuracy of the individual QSAR models.

Two different cut-off values were considered: the first of -6.5 kcal/mol shows the highest accuracy of the method, but a low sensitivity; the second of -6 kcal/mol, increases sensitivity, but slightly diminishes accuracy.

Cut-off (kcal/mol)	Sensitivity	Specificity	Accuracy
-5.5	0.87	0.38	0.55
-6	0.75	0.58	0.64
-6.5	0.54	0.77	0.69
-7	0.31	0.83	0.65


Majority Consensus - Binding
Intrinsic activity
QSAR
EAS (ERα)
VEGA training set
Molecular docking
Low-Mode MD

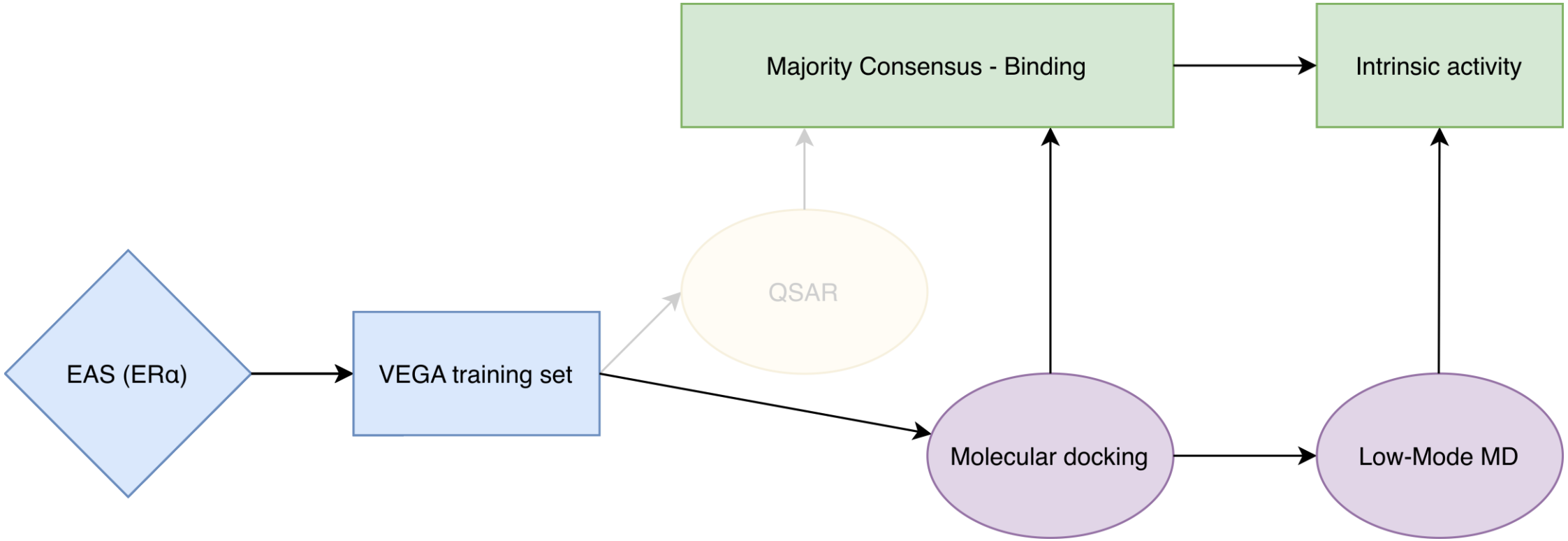
Cut-off (kcal/mol)	Sensitivity	Specificity	Accuracy	Note
Consensus including -6 cut off docking as one of models	0.87	0.63	0.71	Good accuracy and high Sensitivity
Consensus including -6.5 cut off docking as one of models	0.83	0.63	0.67	Good accuracy and balanced sensitivity and specificity
Consensus 4 or more QSAR models OR docking pos (-6 cut off)	0.94	0.49	0.65	
Consensus 4 or more QSAR models OR docking pos (-6.5 cut off)	0.87	0.63	0.71	Good if need to err on side of caution (87 % of true positives picked up, accuracy still reasonable).

QSAR Majority Consensus results were integrated with molecular docking one.

In particular, two different scenarios were considered:

i) the majority consensus was compiled assigning to molecular docking simulation result the same weight as the QSAR model;

ii) the majority consensus was compiled considering "positive" a compound if it is positive in at least 4 QSAR models or has a binding free energy lower than the considered cut off.



To evaluate the intrinsic activity, **10 compounds** were chosen from docking top scoring compounds for which QSAR models wrong predictions with respect to VEGA database. Among them, 5 are classified as active, 5 as inactive for VEGA RA. We added **17β-estradiol and 4-hydroxytamoxifen as reference** compounds for agonists and antagonists, respectively.

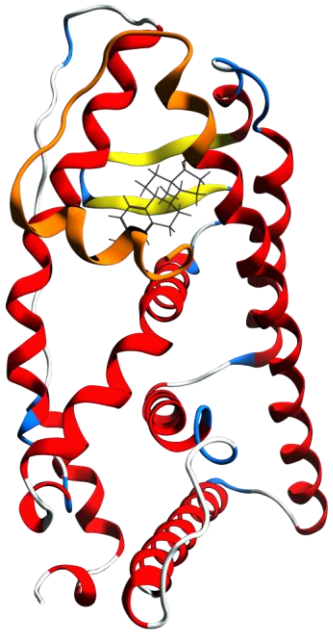
Moreover, we perform a structural alignment among agonist and antagonist to import molecular coordinates to ERa crystal structures with alpha helix H12 in closed conformation.

Low-Mode molecular dynamics simulation is a conformational search method that uses implicit vibrational analysis to focus a molecular dynamics trajectory along the low-mode vibrations.

This has the effect of searching for minima along the valleys and troughs on the potential energy surface and can be applied for studying the flexibility of certain structural regions of macromolecules such as protein loops.

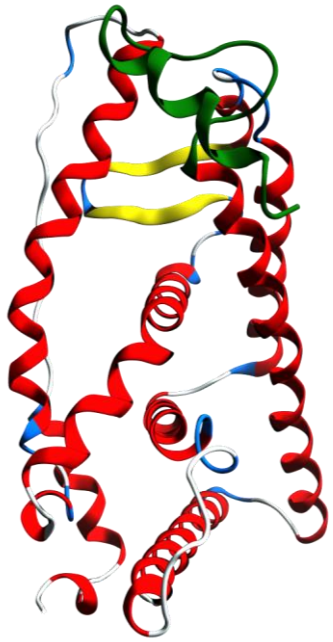
The closed conformation of a-helix 12 of reference 17β-estradiol::ERa complex was verified, while both the open and the free conformations of a-helix 12 of reference 4-hydroxytamoxifen::ERa and *apo*-ERa were simulated via LM.

17β-estradiol::ERa



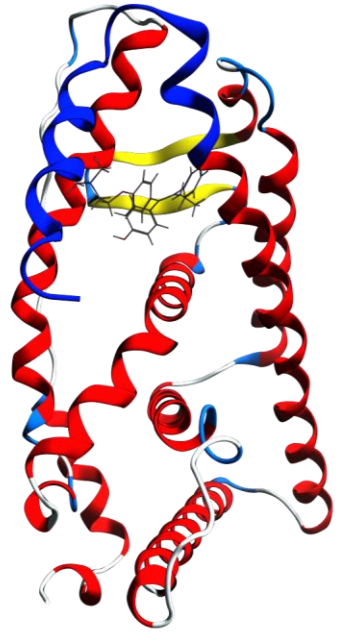
Closed

apo-ERa



Free

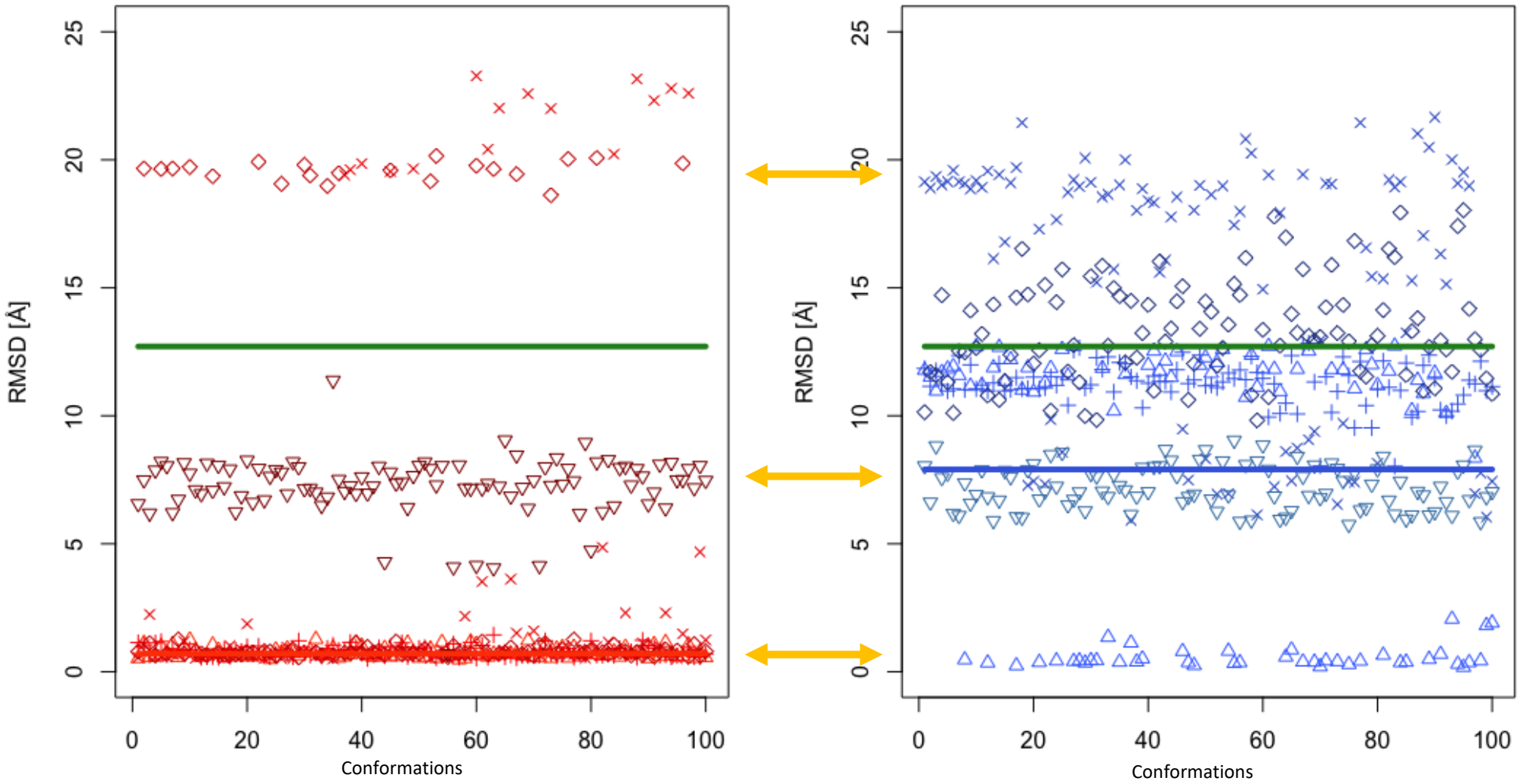
4-hydroxytamoxifen::ERa

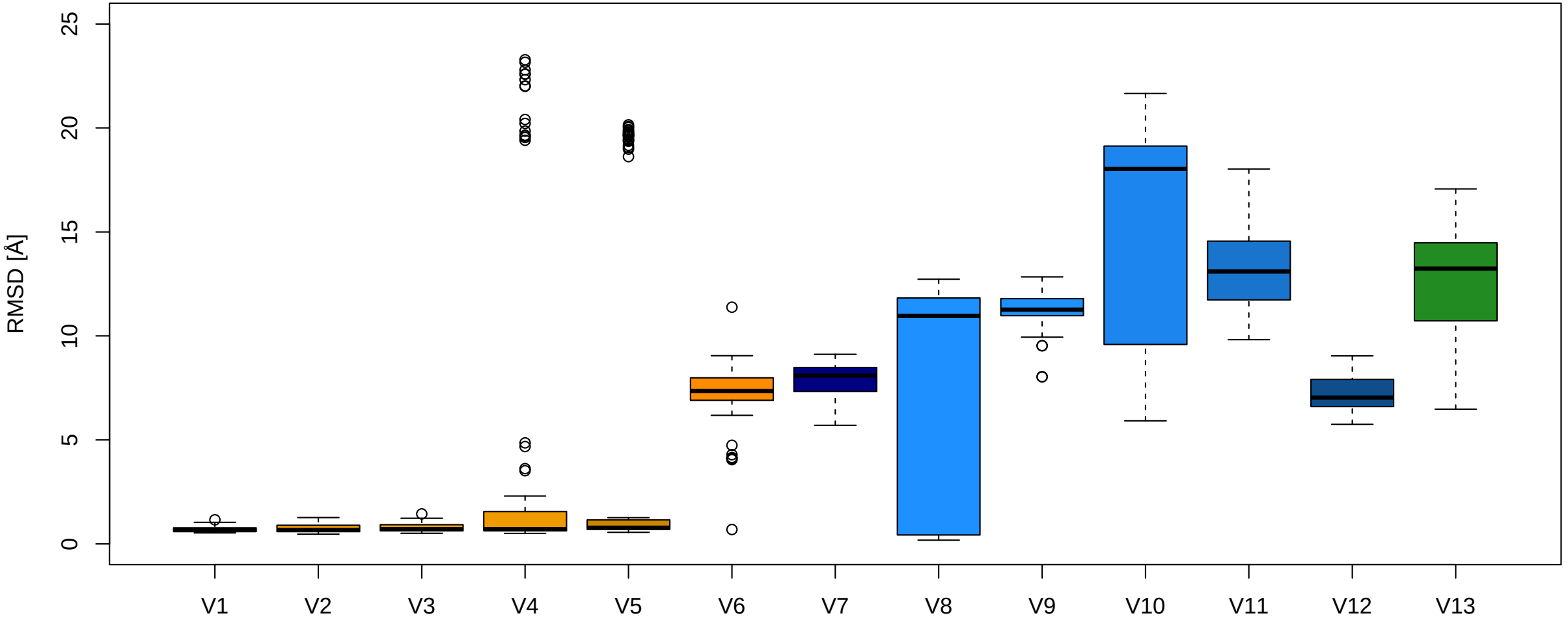


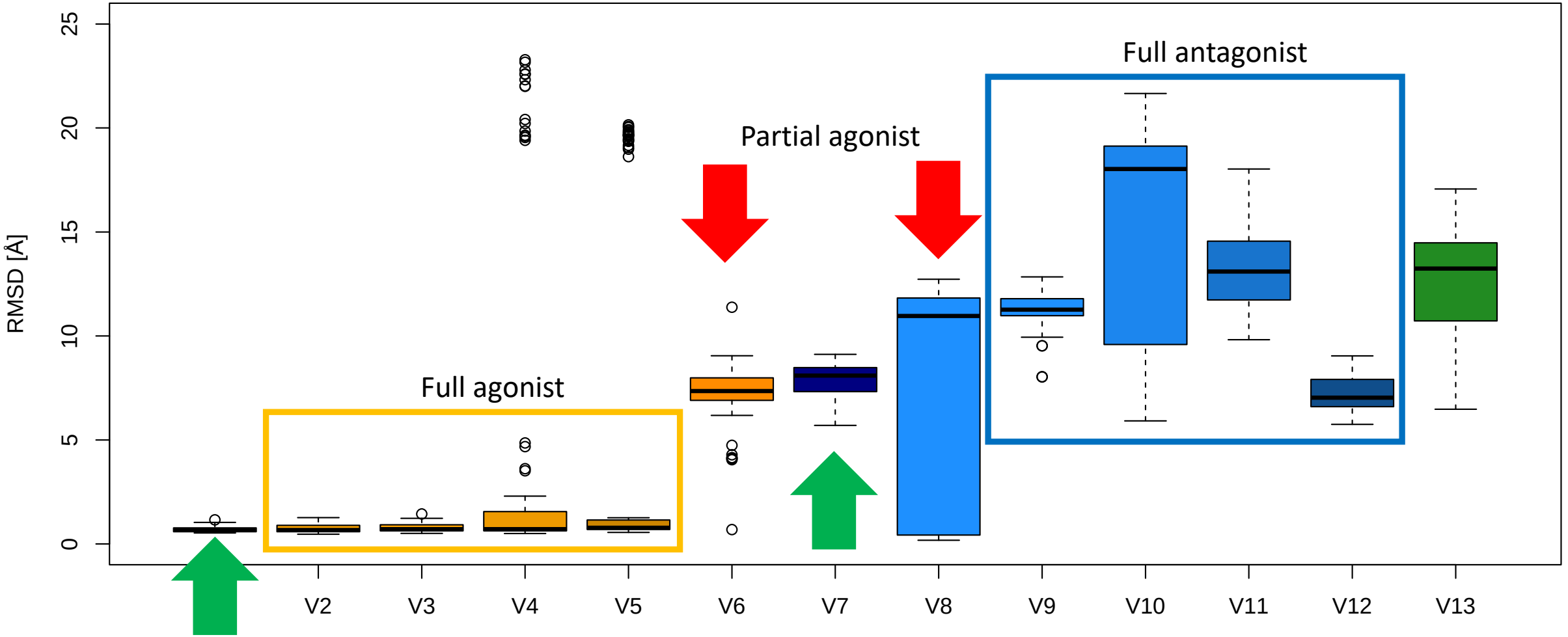
Open

Agonists

Antagonists




RMSD [Å]
0
5
10
15
20
25
V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
V13


RMSD [Å]
0
5
10
15
20
25
Full agonist
Partial agonist
Full antagonist
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
V13

We developed an integrated *in silico* pipeline to prioritize xenobiotics on the estrogen receptor alpha.

In the first step, two different methods were applied:

i) considering the QSAR models for ERa and computing the majority consensus among their prediction, it was obtained that this method is well balanced in Cooper Statistics with very high values (over 0.80);

ii) through the molecular docking simulation it was possible to compute the binding free energies for each compound with the estrogen alpha receptor, also evaluating the binding poses at the atomic level;

iii) through the general majority consensus of both methods it was possible to evaluate different scenarios, which are under discussion with the EUROMIX stakeholders;

iv) with the integration of low-mode it was possible to evaluate *in silico* the intrinsic activity of a small number of compounds.

EuroMix

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