

# INDUCED FIT AND VIRTUAL DOCKING APPLIED TO PPAR $\alpha$ AND PPAR $\gamma$ AGONISTS.

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## INTRODUCTION

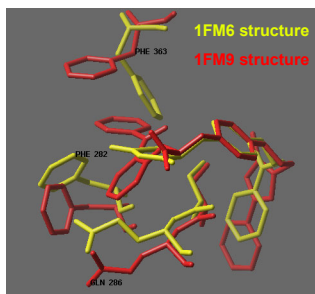
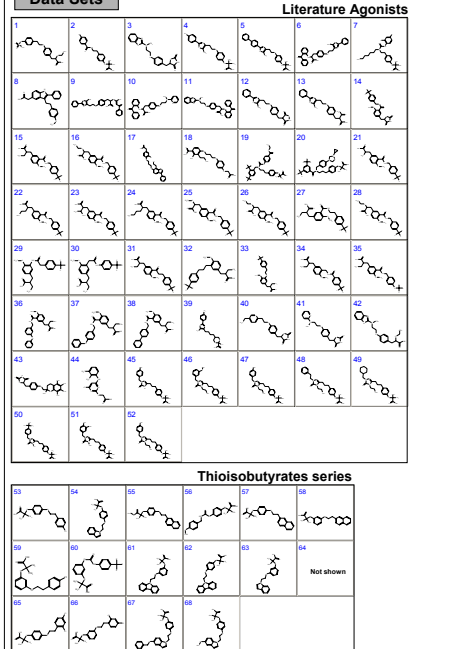
The peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) plays an important role in the metabolism of lipoproteins and fatty acids: its deregulation contributes to the pathogenesis of a variety of disease states, such as cardiac hypertrophy and hyperlipidaemia [1].

To better understand the structure-activity relationship, and to enhance alpha subtype selectivity for new substituted 2-phenylthio-2-(methyl)propanoic acid derivatives (thioisobutyrate), we have set up a virtual docking analysis using several literature agonists and the X-ray crystal structures of human PPAR $\alpha$  in complex with GW409544 (pdb accession code 1K7L) and PPAR $\gamma$  in complex with G1262570 and Rosiglitazone (pdb accession code 1FM9 and 1FM6).

Given the availability of x-ray structure of different sub-types of PPAR receptors (alpha and gamma), bounded to different ligands (Rosiglitazone, GW409544 and G1262570), this case study represents a perfect opportunity to test the performance of the new Schrödinger's Induced Fit Protocol.

## METHODS

### Data Sets



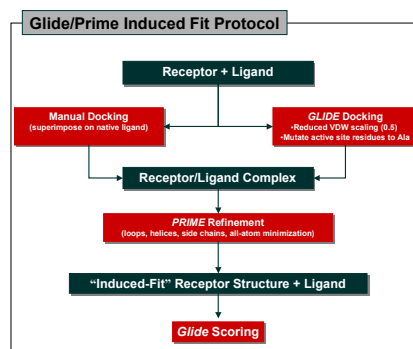
Induced fit has two main applications: (1) generation of the complex structure for a ligand known to be active but that cannot be docked in an existing (rigid) structure of the receptor, and (2) rescue of false negatives (poorly scored true binders) in virtual screening experiments, where instead of screening against a single conformation of the receptor, additional conformations obtained via induced fit are used.

Using the original x-ray structures of PPAR $\alpha$  (1K7L) and PPAR $\gamma$  (1FM6), we docked the most bulky literature agonist (G1262570) into the two active sites using a reduced vdW scaling and mutating the active site residues to Ala; the top 100 ranked poses were subsequently submitted to a refinement procedure, consisting in loops refinement, side chain prediction and structure minimization. The structure with the best Prime energy was chosen as new "induced fit" ligand-receptor structure, and then it was used to dock the set of literature ligands using the Glide SP scoring function.

A preliminary docking analysis with Glide [2], using agonists taken from literature and the available x-ray structures of PPAR $\alpha$  and PPAR $\gamma$ , give poor results in terms of docking accuracy for most of the molecules considered; this is probably due to the small dimension of the active site cavity in the x-ray structures, especially for the one that binds the smallest native ligand (Rosiglitazone, 1FM6). In fact, the crystal structure of PPAR $\gamma$  receptor bounded with G1262570 (1FM9) shows the capability of that protein to open an additional active site pocket, that could accommodate a bulky side-chain of a ligand molecule.

A superposition between the active site residues of 1FM6 and 1FM9 highlights that the fitting of G1262570 in the active site is promoted by a side chain displacement of residues 282, 286 and 363.

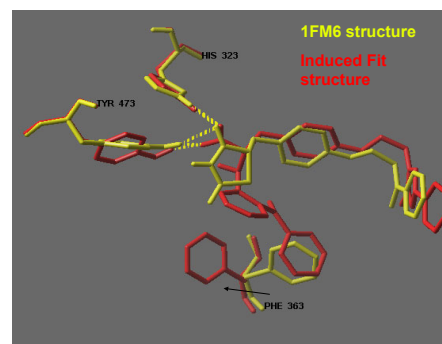
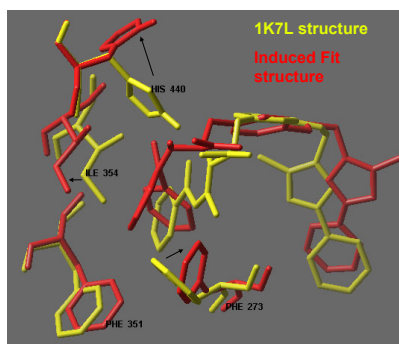
Starting from this observation, we have applied "The Glide/Prime induced fit protocol" developed by Schrödinger [2], that addresses this problem by "inducing" adjustments in the receptor structure to obtain an alternative structure that can accommodate ligands that would otherwise not fit into the original binding site.



## RESULTS

### Induced fit Structures

Using the procedure described above, we obtained two new 3D structures of the active site of the PPAR $\alpha$  and PPAR $\gamma$ ; in both cases, there is no significant movement in the backbone of the protein, but the side chain of some residues adopts a new conformation to permit a better fit of the new ligand. In particular, the flipping of aromatic moiety in Phe273 (alpha) and Phe363 (gamma) leads to an enlargement of the active site cavity, so that it becomes more conformed on the shape of G1262570. The importance of that structure's arrangement is clear especially for the PPAR $\gamma$  receptor, in which the movement of residue 363 avoids a steric clash, and moreover, forms a partial stacking interaction with the benzoylphenyl moiety of G1262570.



### Docking of Literature Agonists

Receptor	% agonist in good orientation	Spearman's Rank Correlation	EF' (70%)	EF (2%)
1K7L	53%	0.203	1.75	5.41
1FM6	50%	0.421	1.46	4.57
1FM9	51%	0.587	1.00	5.44
1K7L induced fit	55%	0.408	2.92	9.48
1FM6 induced fit	53%	0.405	2.08	5.22

A set of 52 known ligands of PPAR $\alpha$  and PPAR $\gamma$  coming from literature was docked into the new structures obtained from the induced fit protocol; the results were compared with those obtained from the original x-ray structures. The docking quality were evaluated considering: 1) Percentage of ligands oriented in agreement with the crystal binding mode of native ligand (docking accuracy); 2) The capability to identify active molecules within a database [4] of randomly selected drug-like compounds (database enrichment). Since direct correlation between interaction energy obtained from Glide and experimental activity was obtained limitedly to homologous subset of ligands [3], we have calculated the Spearman's rank order correlation coefficient [5] as an index of usefulness of docking experiments for qualitative activity prediction on the whole set.

The results shown in the table on the left clearly demonstrate an improvement of docking performance for the induced fit structures when compared with the results obtained using the x-ray structures; in particular, we have obtained a satisfactory increase in term of enrichment factor, both for EF'(70%), which measures the enrichment for recovering 70% of the known actives ("global enrichment"), and for EF(2%), which measures the enrichment for assaying just the top 2% of the ranked database ("early enrichment"). The best performance is obtained for PPAR $\alpha$  receptor.

### Docking of Thioisobutyrate series

The docking results for these molecules, using as target the two structures obtained with the induced fit protocol, are very similar to the ones obtained in a previous work [3], using the standard docking procedure.

Using some molecules in this set, we have tried to generate alternative structures of PPAR $\alpha$  and, respectively, PPAR $\gamma$  able to explain the difference in terms of  $\alpha/\gamma$  selectivity; unfortunately, probably due to the small number of molecules available, the present results are not already satisfactory. Further experimental data acquisition (new synthesized compounds and PPARs binding values) and docking refinement are still ongoing.

## CONCLUSIONS

In the light of new experimentally-determined receptor-agonist complexes, it is evident that molecules like G1262570 or GW409544, as examples, could not bind to the 1FM6 receptor structure due to unfavorable vdW contacts. As a consequence, if the 1FM6 was the only x-ray structure available, an active agonist could have not been discovered using virtual screening in which the receptor is held rigid.

This study has demonstrated that the induced fit protocol developed by Schrödinger can avoid that drawback, and can be used to improve the performance of virtual database screening using Glide. In this specific case, we obtained 2 alternative structures of PPAR receptors, in which the conformations of their active sites were adapted to fit a particular class of ligands. The new generated protein structures were also useful to better understand the binding mode and the structure activity relationships for the chosen set of molecules.

## REFERENCE

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