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REGULAR ARTICLE

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Automated docking of estrogens and SERMs into an estrogen receptor alpha and beta isoform using the PMF forcefield and the Lamarckian genetic algorithm

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Abstract A diverse set of estrogens and selective estrogen receptor modulators (SERMs) whose relative binding affinities (RBAs), with respect to 17β-estradiol are known, are automatically docked into a particular estrogen receptor alpha and beta (ERα and ERβ) in silico, utilizing the Lamarckian genetic docking algorithm and the potentials of mean force (PMF) function. After division into distinct classes (estrogens, SERMs), the ligands are ranked based upon the calculated ligand:receptor interaction energies, as well as experimental RBAs. Comparison of both rankings shows good agreement within the distinct ligand classes. The presented results indicate that the PMF may be applied to the estrogen receptor:ligand complexes, and the ranking of ligands within distinct classes is a very useful pre-screening tool for development of novel estrogen receptor ligands.

Keywords Estrogen receptor · PMF · Lamarckian genetic algorithm · SERM · Estradiols · Docking

1 Introduction

According to the National Cancer Institute, based on current rates, 13.2% of women born today will be diagnosed with breast cancer at some point of time in their lives. This estimate is based on cancer statistics for the years 2000 through 2002. Estimated lifetime risk of breast cancer has increased gradually over the past several decades [1]. According to the Centers for Disease Control and Prevention, breast cancer is the second most commonly diagnosed cancer among American women, and is second to lung cancer as the leading cause of cancer-related deaths among women. The estimates for 2004 included 215,990 new cases of invasive breast cancer being diagnosed, and 40,580 deaths reported. (American Cancer Society, "Cancer Facts and Figures", 2004).

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In 1993, a pathway was put forth as the model for potential breast cancer disease [2]. It was not until a few years later that earlier models were revised to include another molecule, a co-activator, that aided in allowing the complex to function as a transcription activating factor [3]. Since that time, we have come to understand that there are co-activators and also co-repressors. Taken together, this group of molecules is collectively known as co-regulators. At present, it is not clear how these molecules are discriminated in vivo.

Much work has been done on analytical methods to detect this myriad of complicated interactions leading to this disease. However, if we are going to fully explore the disease, and multiple target candidates for drugs, it seems sensible that an in silico screening method be evolved to examine potential protein:protein interactions along with potential drug candidates to derive thermodynamic data that will allow us to compare this to in vitro studies, and perhaps even use these in silico methods to design drug candidates for this disease to be used for in vitro studies. This can be accomplished by using computer modeling software that will allow for docking of small molecules into receptor proteins and deriving thermodynamic data as determined by the three-dimensional orientation, as well as many other parameters.

In this paper we present a validation model to show in silico studies match well with empirical relative binding affinity data, and show that much work may be done with this technique that has heretofore not been used to study this disease.

2 Materials and methods

I. General preparation

The crystal structure of estrogen receptor β (ERβ) complexed with genistein (1QKM) [4], estrogen receptor α (ERα) complexed with tetrahydroisoquinoline (1XQC) [5], and ERα in complexation with 17β-estradiol (1A52) [6], were extracted from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) [7]. Molecules with known physiological properties (Figs. 1, 2) were selected to be docked into the ligand binding domain of the

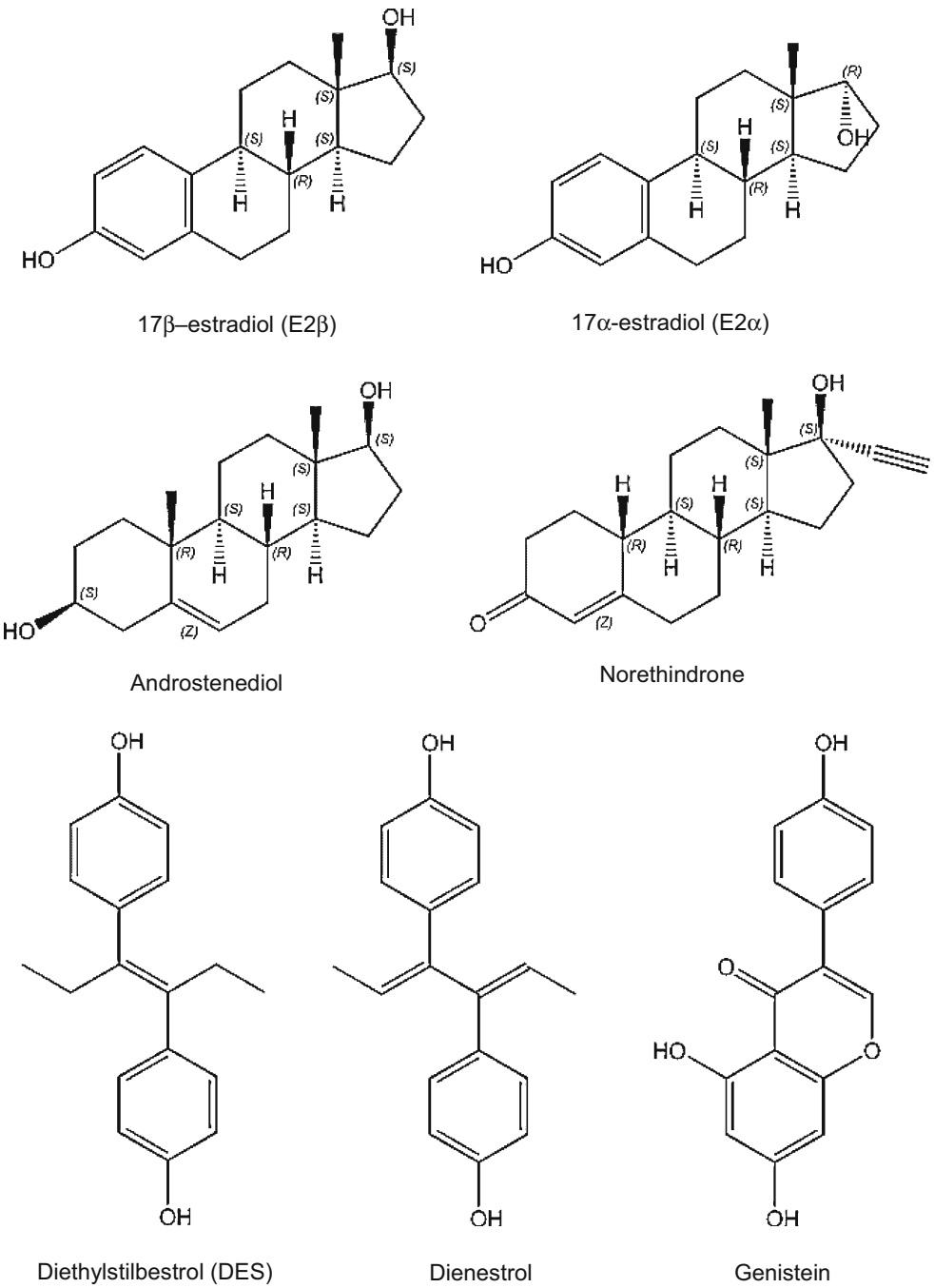


Fig. 1 Estrogen receptor agonists

three receptors. Subsequently, the selected ligands were divided into two categories (ligand subsets): estrogens (estrogen receptor agonists) and SERMs.

II. Preparation of protein structures

To prepare the structure for the calculations, hydrogens, missing amino acids and missing atoms were added to each structure. The added atoms and residues were optimized using molecular mechanics (MM2) [8, 9] with a convergence threshold of 0.0001 kcal/mol, keeping the remainder of the structure locked at their defined coordinates.

III. Preparation of ligand structures

The geometries were optimized using the Hartree Fock method (HF/6-31G**) in Gaussian 03 [10] followed by molecular dynamics (MD) simulations using CAChe WorkSystems Pro [11]. The molecular dynamics searches were done to perform conformational searches. The excited ligand struc-

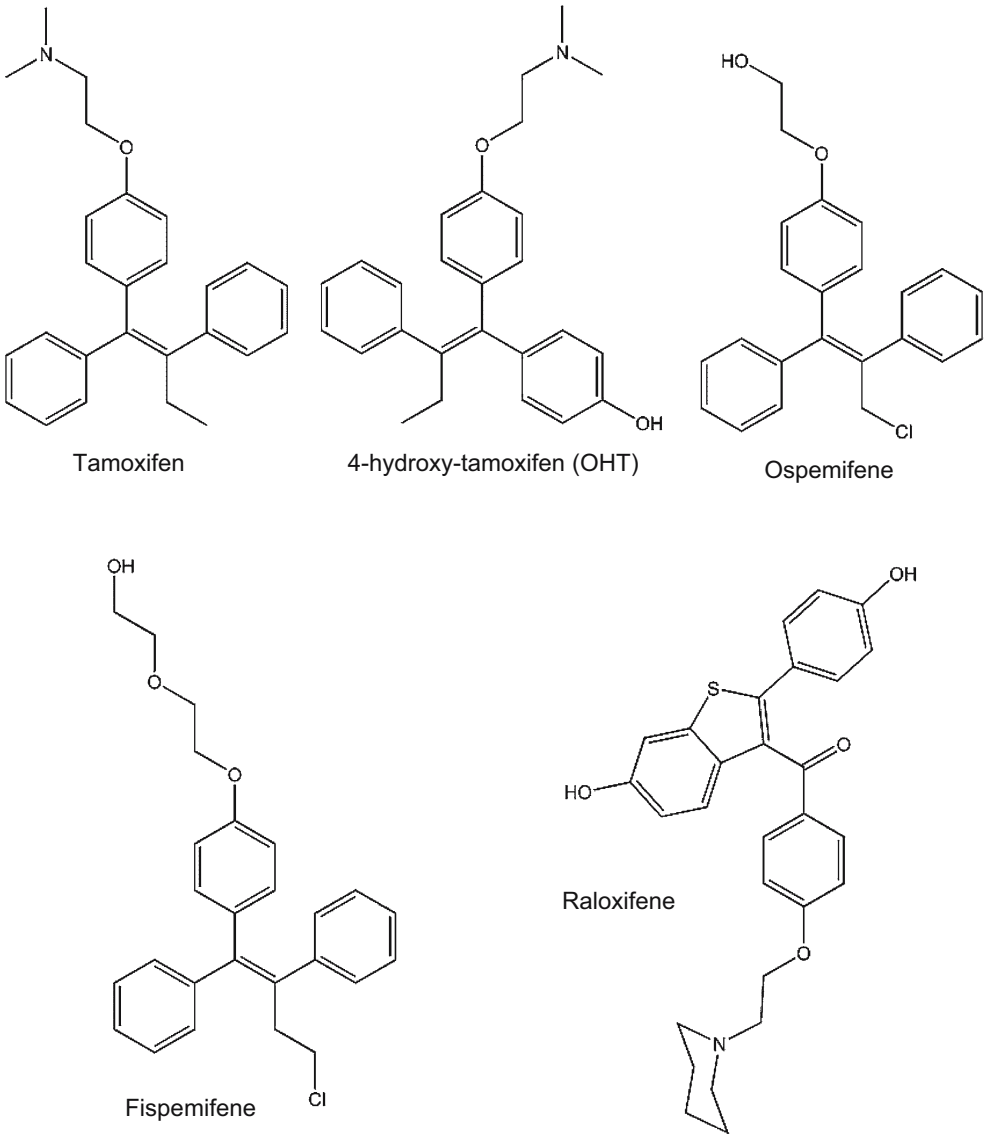


Fig. 2 Selective estrogen receptor modulators (SERMs)

tures obtained from the MD calculation were optimized with molecular mechanics (MM2) [8, 9], followed by Hartree Fock geometry optimization (HF/6-31G**).

IV. Trial docking

Trial dockings with various sets of genetic docking algorithm (GA) [12] parameters were done to determine the optimal parameter set for the GA. The criteria for an optimal parameter set included dock score reproducibility and dock score optimization.

V. Validation docking

Validation dockings were done for each of the receptor structures using CAChe WorkSystems Pro. The root mean square deviation between the native, co-crystallized ligand and its docked copy was calculated for each of the three validation dockings (Figs. 3–5).

VI. Docking

Amino acid residues within a distance of 10 Å of the original crystal structure ligand were selected as the active site. The ligands (Figs. 1 and 2) were docked into the receptor active site, using the Lamarckian genetic docking algorithm of the FastDock engine, implemented in CAChe WorkSystems Pro. Because of the stochastic nature of the algorithm, repeated docking cycles had to be performed to plot the energy landscape of protein:ligand interactions. Sixty copies were made from each ligand; each of which was subjected to a docking. The algorithm's probability to find a good orientation of the ligand in the binding pocket can be increased by re-docking

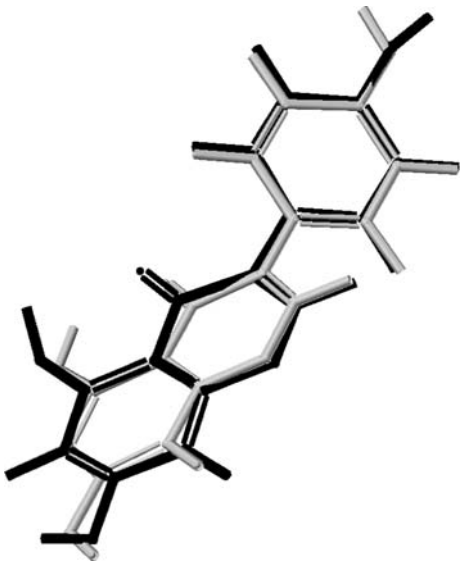


Fig. 3 Validation docking of 1QKM with genistein. The partial antagonist genistein is docked into the ligand binding pocket of the ERβ (PDB code 1QKM). Figure 4 shows the docked ligand (*gray*) and the ligand of the crystal structure (*black*) at their absolute positions in the binding pocket. The root mean square deviation between the two is calculated to be 3.277 Å

the ligand from its docked position. An example of this is shown with 17β-estradiol (E2β) (Fig. 6).

VII. Scoring

The PMF function [17] scores ligand orientations as they are docked. The real-time PMF output is input into the Lamarckian genetic algorithm. The complex with the best PMF re-docking score was geometry optimized using molecular mechanics (MM2) [8, 9] with a convergence threshold of 0.0001 kcal/mol, and the orientation of the ligand in the optimized protein:ligand structure was scored using the PMF scoring function.

VIII. Evaluation

Two rankings were performed. Initially, all docked ligands were ranked within a ligand subset according to the PMF score of the docking (ranking model A). Then the ligands were ranked according to the PMF score of the geometry optimized protein:ligand structure (ranking model B). The experimental RBAs of these ligands were obtained from the primary literature [12–14]. The PMF scores were plotted against the $\log_{10}$ of the experimental RBAs. The two ranking models are hereafter referred to as model A and model B.

2.1 Docking with the Lamarckian genetic docking algorithm

A GA is an optimization scheme that mimics the process of evolution. The individuals of a generation are represented by the configurations of a ligand in the search space. A fitness

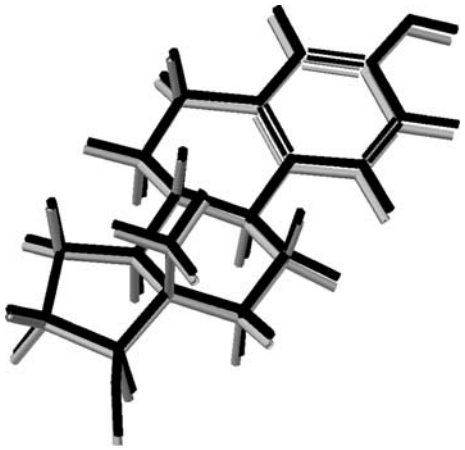


Fig. 4 Validation docking of 1A52 with 17β-estradiol. The agonist 17β-estradiol is docked into the ligand binding pocket of the ERα (PDB code 1A52). Figure 5 shows the docked ligand (*gray*) and the ligand of the crystal structure (*black*) at their absolute positions in the binding pocket. The root mean square deviation between the two is calculated to be 0.2532 Å

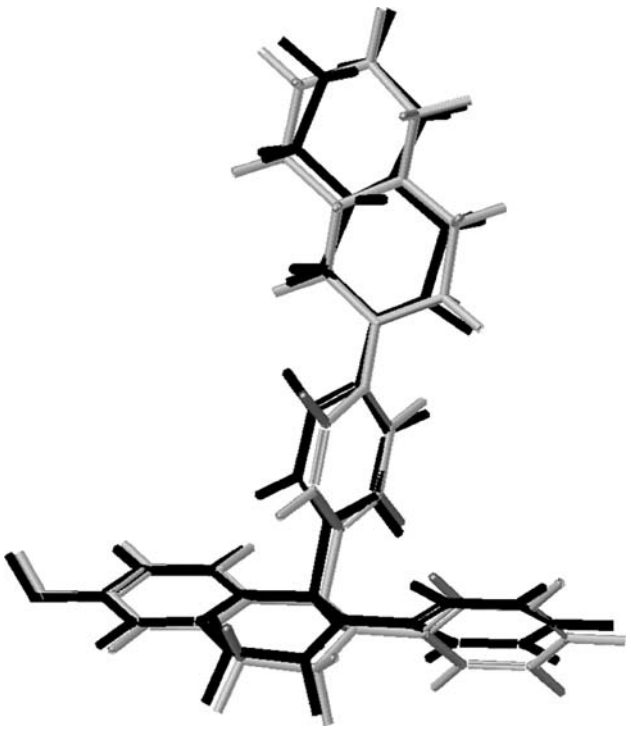


Fig. 5 Validation docking of 1XQC with tetrahydroisoquinoline (THIQ). The SERM tetrahydroisoquinoline is docked into the ligand binding pocket of the ERα (PDB code 1XQC). Figure 6 shows the docked ligand (*gray*) and the ligand of the crystal structure (*black*) at their absolute positions in the binding pocket. The root mean square deviation between the two is calculated to be 1.0078 Å

function is used to determine which individuals can repopulate. The selected offspring are used in the next iteration step of optimizing the orientations.

In this algorithm, a chromosome is assigned to each degree of freedom (translation, rotation, torsion) of a flexible

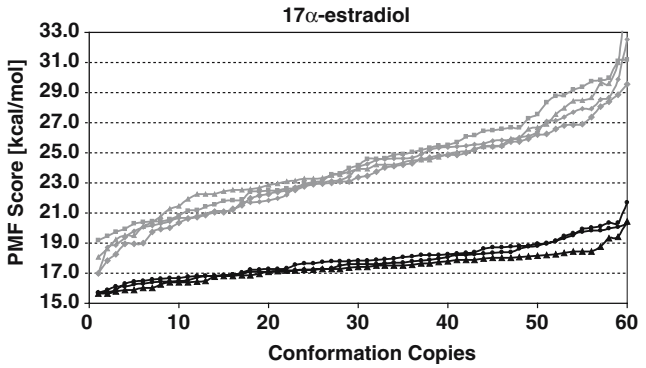


Fig. 6 PMF scores from 60 dockings, sorted in ascending order, and plotted for a diverse set of GA (genetic algorithm) parameters (docking scores in *grey*, redocking scores in *black*). Redocking a ligand from its optimized orientation results not only in a significant optimization of protein:ligand interactions (lowering of the PMF score), but also increases the overall reproducibility of the GA-based docking (*flattening* of the plotted curve). Roughly 25% of the re-docked ligands have a more favorable energy as the best ab initio docking

ligand. The evolutionary docking process is started by creating a random population of chromosomes (random position and random orientation of the ligand in the binding pocket). As in Charles Darwin's theory of evolution [16], each chromosome is scored by evaluating the PMF for fitness. Only the fittest (best orientations) are allowed to reproduce.

Several GA parameters, such as the population size, the number of generations, crossover rate, elitism and mutation rate, must be specified. These have to be applied in a manner as to ensure a maximum variety of conformations, such that a maximum number of local minima in the energy landscape (and preferably the absolute minima as well) of ligand:receptor interactions is searched in a minimal period of time.

The Lamarckian GA that is used by the FastDock compute engine in CAChe WorkSystems Pro is implemented in the program AutoDock [12]. In this specific GA, a minimization is performed so that individual ligand orientations adapt to the environment in which they are placed.

2.2 Scoring a ligand's orientation with PMF

The PMF is a general, knowledge-based function, that exploits structural information of known protein:ligand complexes, extracted from the RCSB PDB [7] and converts it into distance-dependent Helmholtz-free interaction energies of protein:ligand atom pairs [17]. In contrast to empirical scoring functions, the PMF constitutes a deductive approach. Given that the interactions between ligands and proteins are based on very complex and numerous interactions of forces, known protein:ligand structures (obtained from crystallographic and NMR measurements) are considered as the only reliable source of information.

The PMF are derived from a statistical analysis of protein and ligand atom occurrences at certain distances by using 697 protein:ligand complexes taken from the RCSB PDB [7]. Sixteen protein atom types and 34 ligand atom types are defined, resulting in 544 unique potentials.

The protein:ligand interaction-free energy PMF between a protein atom of type i and a ligand atom of type j can be calculated by equation 1 [17]

$$A_{ij}(r) = -k_{\mathrm{B}}T \ln \left[f^{j}_{\mathrm{Vol\_corr}}(r) \frac{\rho^{ij}_{\mathrm{seg}}(r)}{\rho^{ij}_{\mathrm{bulk}}} \right] \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and r is the atom pair distance. $f^{j}_{\mathrm{Vol\_corr}}(r)$ is a ligand volume correction factor (since intra-ligand interactions are not taken into account). $\rho^{ij}_{\mathrm{seg}}(r)$ is the number density of pairs of type ij in a structural database that occur in a certain radius range (indicated by seg). $\rho^{ij}_{\mathrm{bulk}}$ represents the distribution of i and j when no interaction between i and j occurs.

The PMF score is calculated as the sum over all protein:ligand atom pair (kl) interaction-free energies $A_{ij}(r)$ as a function of the atom pair distance r, and

$$\mathrm{PMF\_score} = \sum_{\substack{kl \\ ij \\ r<r^{ij}_{\mathrm{cutoff}}}} A_{ij}(r) \quad (2)$$

r^{ij}_{cutoff} is the cutoff radius for the atom type pair ij [17].

It is assumed that binding of a ligand to a protein is non-covalent. Therefore, non-bonded terms are added to the potential to keep atoms at typical non-bonded distance. The standard PMF implementation uses AMBER van-der-Waals potentials for this purpose [18]. The standard CAChe implementation uses specific 6–12 Lennard-Jones potentials for each pairwise interaction [11].

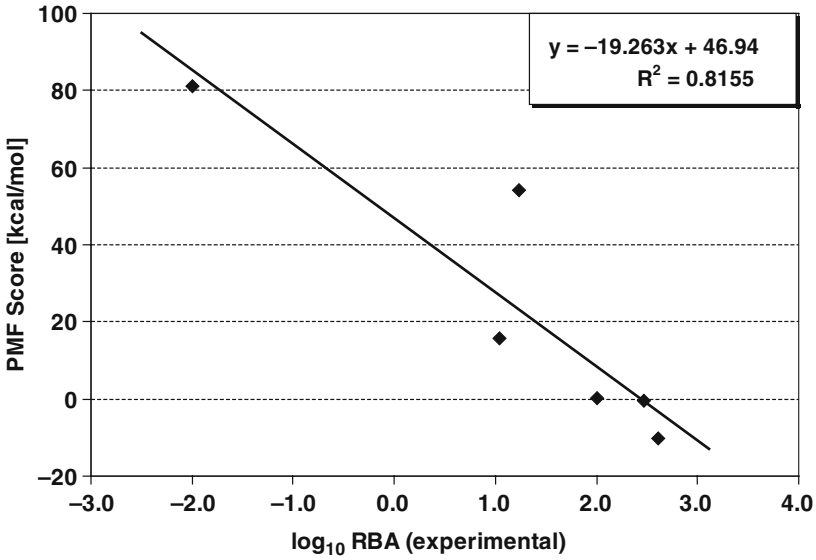
3 Results and discussion

Both the PMF rankings, one prior to geometry optimization (model A), and one after geometry optimization (model B), were performed for each ligand subset and were then compared to experimental RBAs which were determined by competitive radiometric binding assays [12–14]. The RBA of each ligand was calculated as the ratio of 17β-estradiol and competitor required to reduce the specific radioligand binding by 50% (ratio of IC_{50} values). The PMF scores were plotted against the $\log_{10}$ of the experimental RBAs and R^2 values were determined. The RBA predictability of each ranking was calculated as the ratio between the number of correctly ranked ligands and the total number of docked ligands.

3.1 1QKM docking

Six ligands were docked into the ligand binding pocket of the ERβ (1QKM).

Ranking model A (Fig. 7) is compared to experimental RBAs. Five out of six docked ligands are ranked in agreement to the experimental data. Only the relative ranking order of



Virtual Ranking (1QKM) (ERβ) (model A)

Ranking	Ligand	Score [kcal/mol]
1	◆ Dienestrol	–10.269
2	◆ DES	–0.607
3	◆ E2β	0.067
5	◆ Androstenediol	54.107
4	◆ E2α	15.628
6	◆ Norethindrone	81.166

Experimental Ranking (ERβ)

Ranking	Ligand	RBA	$\log_{10}$ RBA
1	Dienestrol	404	2.606
2	DES	295	2.470
3	E2β	100	2.000
4	Androstenediol	17	1.230
5	E2α	11	1.041
6	Norethindrone	0.01	–2.000

Fig. 7 1QKM docking (model A)

androstenediol and 17α-estradiol (E2α) is in disagreement with the experimental data. The resulting RBA predictability for model A of this ligand subset is: 83.33%. Figure 7 displays the correlation between PMF scores and the $\log_{10}$ of experimental RBAs (model A).

Ranking model B (Fig. 8) is also compared to experimental RBAs. Four out of six docked ligands are ranked in agreement with the experimental data. The relative ranking order of E2β and dienestrol is in disagreement with the experimental data. Diethylstilbestrol (DES) can be considered a false-negative and is therefore not taken into the linear regression calculation (Fig. 8). Docking scoring approaches used today tend to have a large number of false-positives and false-negatives. The false-positives are compounds with high scores but with no experimentally observable binding to the protein. The false-negatives are compounds with low scores but with high experimentally observable binding [19]. The resulting RBA predictability for model B of this ligand subset is: 66.67%. Figure 8 displays the correlation between the PMF scores and the $\log_{10}$ of experimental RBAs (model B).

3.2 1A52 docking

Seven ligands were docked into the ligand binding pocket of the ERα (1A52).

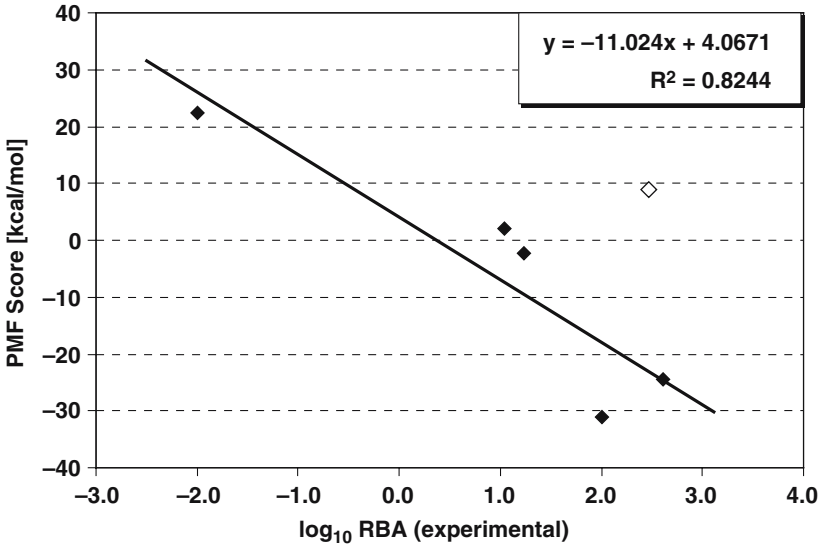
The ranking model A (Fig. 9) is compared to experimental RBAs. Four of the seven docked ligands are ranked in agreement with the experimental data. The relative ranking order of DES and dienestrol, as well as that of E2β and E2α is in disagreement to the experimental data. Genistein appears to be a false-positive and is therefore not taken into the regression calculation (Fig. 9) [19]. The resulting RBA predictability for model A of this ligand subset is: 57.14%. Figure 9 displays the correlation between the PMF scores and the $\log_{10}$ of experimental RBAs (model A).

The ranking model B (Fig. 10) is compared to experimental RBAs. Five of the seven docked ligands are ranked in agreement with the experimental data. The relative ranking order of androstenediol and E2α is in disagreement with the experimental data. Norethindrone appears to be a false-positive and is therefore not taken into the regression calculation (Fig. 10) [19]. The resulting RBA predictability for model B of this ligand subset is: 71.43%. Figure 10 displays the correlation between PMF scores and the $\log_{10}$ of experimental RBAs (model B).

3.3 1XQC docking

Five ligands were docked into the ligand binding pocket of ERα (1XQC).

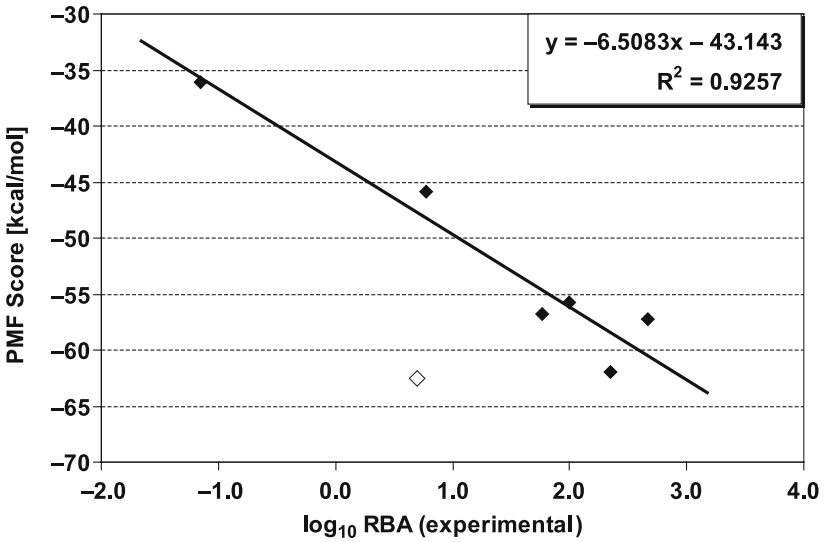
The ranking model A (Fig. 11) is compared to experimental RBAs. Four of the five docked ligands are ranked in agreement with the experimental data. Fispemifene appears to be a false-positive and is therefore not taken into the regression



Virtual Ranking (1QKM) (ERβ) (model A)		
Ranking	Ligand	Score [kcal/mol]
2	◆ Dienestrol	–24.501
5	◇ DES	8.999
1	◆ E2β	–31.139
3	◆ Androstenediol	–2.364
4	◆ E2α	2.066
6	◆ Norethindrone	22.496

Experimental Ranking (ERβ)			
Ranking	Ligand	RBA	$\log_{10}$ RBA
1	Dienestrol	404	2.606
2	DES	295	2.470
3	E2β	100	2.000
4	Androstenediol	17	1.230
5	E2α	11	1.041
6	Norethindrone	0.01	–2.000

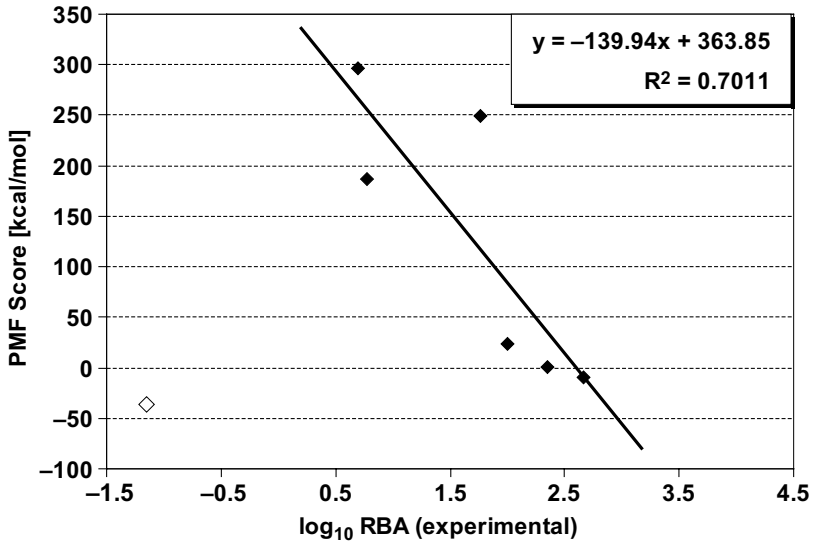
Fig. 8 1QKM docking (model B). DES is considered a false-negative and is therefore not taken into the regression calculation [19]



Virtual Ranking 1A52 (ERα) (model A)		
Ranking	Ligand	Score [kcal/mol]
3	◆ DES	–57.208
2	◆ Dienestrol	–61.957
5	◆ E2β	–55.745
4	◆ E2α	–56.727
6	◆ Androstenediol	–45.865
1	◇ Genistein	–62.510
7	◆ Norethindrone	–36.062

Experimental Ranking (ERα)			
Ranking	Ligand	RBA	$\log_{10}$ RBA
1	DES	468	2.670
2	Dienestrol	223	2.348
3	E2β	100	2.000
4	E2α	58	1.763
5	Androstenediol	6	0.778
6	Genistein	5	0.699
7	Norethindrone	0.07	–1.155

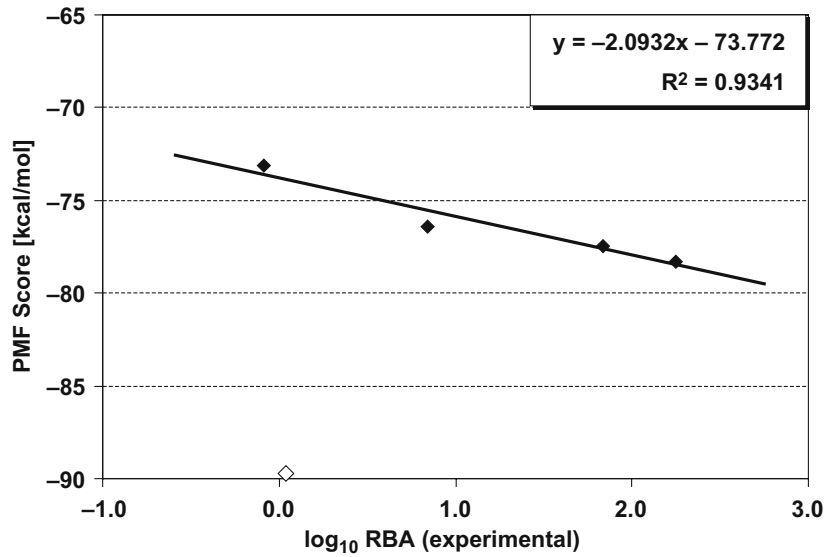
Fig. 9 1A52 Docking (model A). Genistein is considered a false-positive and is therefore not taken into the regression calculation [19]



Virtual Ranking 1A52 (ERα) (model B)		
Ranking	Ligand	Score [kcal/mol]
2	◆ DES	–9.683
3	◆ Dienestrol	0.712
4	◆ E2β	23.940
6	◆ E2α	248.688
5	◆ Androstenediol	187.384
7	◆ Genistein	296.389
1	◇ Norethindrone	–11.366

Experimental Ranking (ERα)			
Ranking	Ligand	RBA	$\log_{10}$ RBA
1	DES	468	2.670
2	Dienestrol	223	2.348
3	E2β	100	2.000
4	E2α	58	1.763
5	Androstenediol	6	0.778
6	Genistein	5	0.699
7	Norethindrone	0.07	–1.155

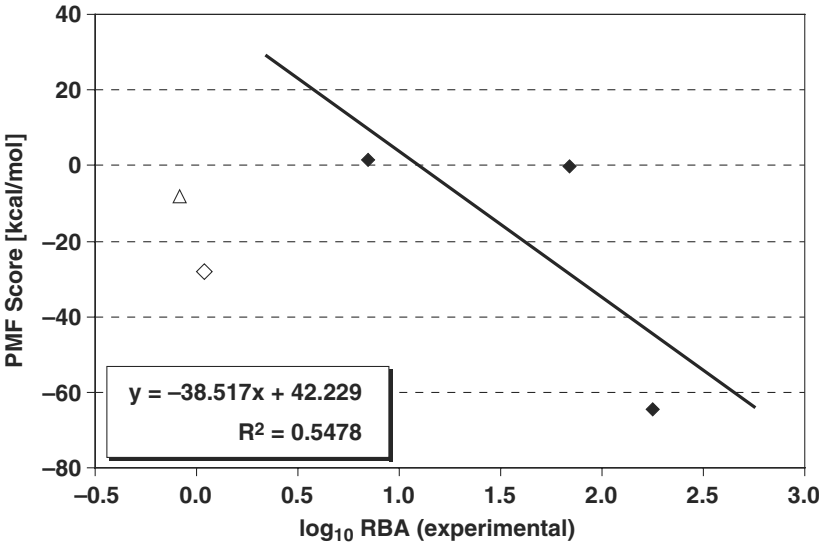
Fig. 10 1A52 Docking (model B). Norethindrone is considered a false-positive and is therefore not taken into the regression calculation [19]



Virtual Ranking (1XQC) (ERα) (model A)		
Ranking	Ligand	Score [kcal/mol]
2	◆ OHT	–78.284
3	◆ Raloxifene	–77.453
4	◆ Tamoxifen	–76.389
1	◇ Fispemifene	–89.696
5	◆ Ospemifene	–73.112

Experimental Ranking (ERα)			
Ranking	Ligand	RBA	$\log_{10}$ RBA
1	OHT	178	2.250
2	Raloxifene	69	1.839
3	Tamoxifen	7	0.845
4	Fispemifene	1.10	0.041
5	Ospemifene	0.82	–0.086

Fig. 11 1XQC docking (model A). Fispemifene is considered a false-positive and is therefore not taken into the regression calculation [19]



Virtual Ranking (1XQC) (ERα) (model B)		
Ranking	Ligand	Score [kcal/mol]
2	◆ OHT	–64.526
4	◆ Raloxifene	–0.206
5	◆ Tamoxifen	1.364
1	◇ Fispemifene	–28.122
3	△ Ospemifene	–8.204

Experimental Ranking (ERα)			
Ranking	Ligand	RBA	$\log_{10}$ RBA
1	OHT	178	2.250
2	Raloxifene	69	1.839
3	Tamoxifen	7	0.845
4	Fispemifene	1.10	0.041
5	Ospemifene	0.82	–0.086

Fig. 12 1XQC docking (model B). Fispemifene and Ospemifene are considered to be false-positives and are therefore not taken into the regression calculation [19]

calculation (Fig. 11) [19]. The resulting RBA predictability for model A of this ligand subset is: 80.00%. Figure 11 displays the correlation between the PMF scores and the $\log_{10}$ of experimental RBAs (model A).

The ranking model B (Fig. 12) is compared to experimental RBAs. Three of the five docked ligands are ranked in agreement with the experimental data. Fispemifene and ospemifene are considered to be false-positives and are therefore not taken into the regression calculation (Fig. 12) [19]. The resulting RBA predictability for model B of this ligand subset is: 60.00%. Figure 12 displays the correlation between the PMF scores and the $\log_{10}$ of experimental RBAs (model B).

4 Quantitative stucture activity relationships (QSARs)

Interactions of docked ligands with residues of the ligand binding pocket of the ER active site can be viewed at http://www.theallenlab.org/ECCC10.html. Comparison of the displayed figures shows that hydrogen bonds and steric interferences are key elements in the process of ligand recognition at a receptor's active site.

5 Conclusion

We have utilized the Lamarckian genetic docking algorithm to produce viable orientations of a variety of ligands, the scoring of which resulted in RBA predictabilities of 83.33 and 66.67% (1QKM docking model A and B, respectively), 57.14 and 71.43% (1A52 docking model A and B, respectively) as well as 80 and 60% (1XQC docking model A and B, respectively). In addition, R^2 values, ranging from 0.55 to 0.93, indicate a strong correlation between the virtual and experimental ranking.

This suggests the method presented here would be useful for a potential application in structure-based drug design as well as in the use as a pre-screening tool for the development of novel estrogen receptor ligands.

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