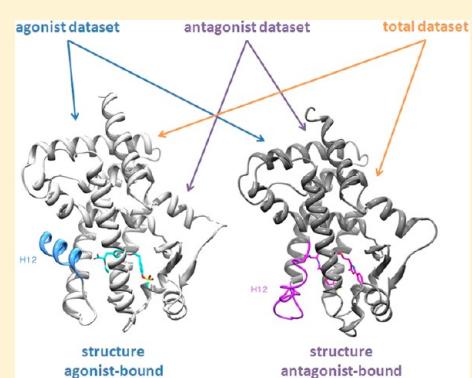


# Importance of the Pharmacological Profile of the Bound Ligand in Enrichment on Nuclear Receptors: Toward the Use of Experimentally Validated Decoy Ligands

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**ABSTRACT:** The evaluation of virtual ligand screening methods is of major importance to ensure their reliability. Taking into account the agonist/antagonist pharmacological profile should improve the quality of the benchmarking data sets since ligand binding can induce conformational changes in the nuclear receptor structure and such changes may vary according to the agonist/antagonist ligand profile. We indeed found that splitting the agonist and antagonist ligands into two separate data sets for a given nuclear receptor target significantly enhances the quality of the evaluation. The pharmacological profile of the ligand bound in the binding site of the target structure was also found to be an additional critical parameter. We also illustrate that active compound data sets for a given pharmacological activity can be used as a set of experimentally validated decoy ligands for another pharmacological activity to ensure a reliable and challenging evaluation of virtual screening methods.



## INTRODUCTION

Virtual screening is now widely accepted as a complement to bioactivity screening,<sup>1</sup> and structure-based (SBVLS) and ligand-based (LBVLS) virtual ligand screening methods are commonly integrated into drug discovery processes.<sup>2</sup> High-quality benchmarking data sets are needed to warrant robust evaluation of these methods and ensure their reliability. Different benchmarking data sets have been developed over the years,<sup>3–6</sup> among which the Directory of Useful Decoys (DUD)<sup>7</sup> and its enhanced version (DUD-E)<sup>8</sup> are considered as the current gold standard benchmarking databases. These databases include respectively eight and 11 nuclear receptors (NRs), but only estrogen receptor alpha (ER\_alpha) in the DUD is presented with two distinct data sets according to the ligand agonist or antagonist pharmacological profile. Taking into account this profile information should improve the quality of the benchmarking data sets since ligand binding can induce conformational changes in the nuclear receptor structure and such changes may vary according to the agonist or antagonist ligand profile.<sup>9</sup> The recently released Nuclear Receptors Ligands and Structures Benchmarking DataBase (NRLiSt BDB)<sup>10</sup> comprises 27 NRs for which more than one agonist and one antagonist ligand and at least one experimental structure are available, as well as two separate agonist and antagonist data sets. These data sets regroup experimentally characterized agonist and antagonist ligands, all corresponding to human holo structures available in the Protein Data Bank (PDB), and their corresponding decoys as provided by the DUD-E decoy generation tool.<sup>8</sup>

In the present work, we studied the impact on enrichment of different features of the NRLiSt BDB using an SBVLS method, Surflex-Dock (SF). We evaluated the influence of

using separate data sets by comparing SF performances in terms of enrichment when considering agonist ligands and antagonist ligands split into two distinct data sets or mixed altogether in a single one as in the DUD-E. Since the choice of the structure of reference is of major importance in terms of enrichment and docking accuracy,<sup>11–14</sup> we decided to assess the influence of the pharmacological profile (agonist or antagonist) of the ligand bound in the binding site of the reference structure on enrichment. Also, for those NRs for which sufficient data were available, we explored the impact of using experimentally confirmed decoy ligands instead of putative DUD-like decoys on enrichment. This work brings up several guidelines that could be used to enhance the quality of benchmarking studies by taking into account the pharmacological profiles of the ligands and using experimentally validated decoy ligands.

## EXPERIMENTAL METHODS

**NRLiSt BDB.** The NRLiSt BDB is a public benchmarking data set designed for evaluation of both structure-based and ligand-based methods. The NRLiSt BDB is dedicated to the NRs and contains an agonist data set and an antagonist data set for the 27 targets (out of the 48 known NRs) for which more than one agonist ligand, one antagonist ligand, and at least one experimental structure are available. Each data set consists of three elements: all of the available human holo PDB structures (except for RXR\_gamma, for which only one apo structure was available), all of the ligands found to be agonists or antagonists

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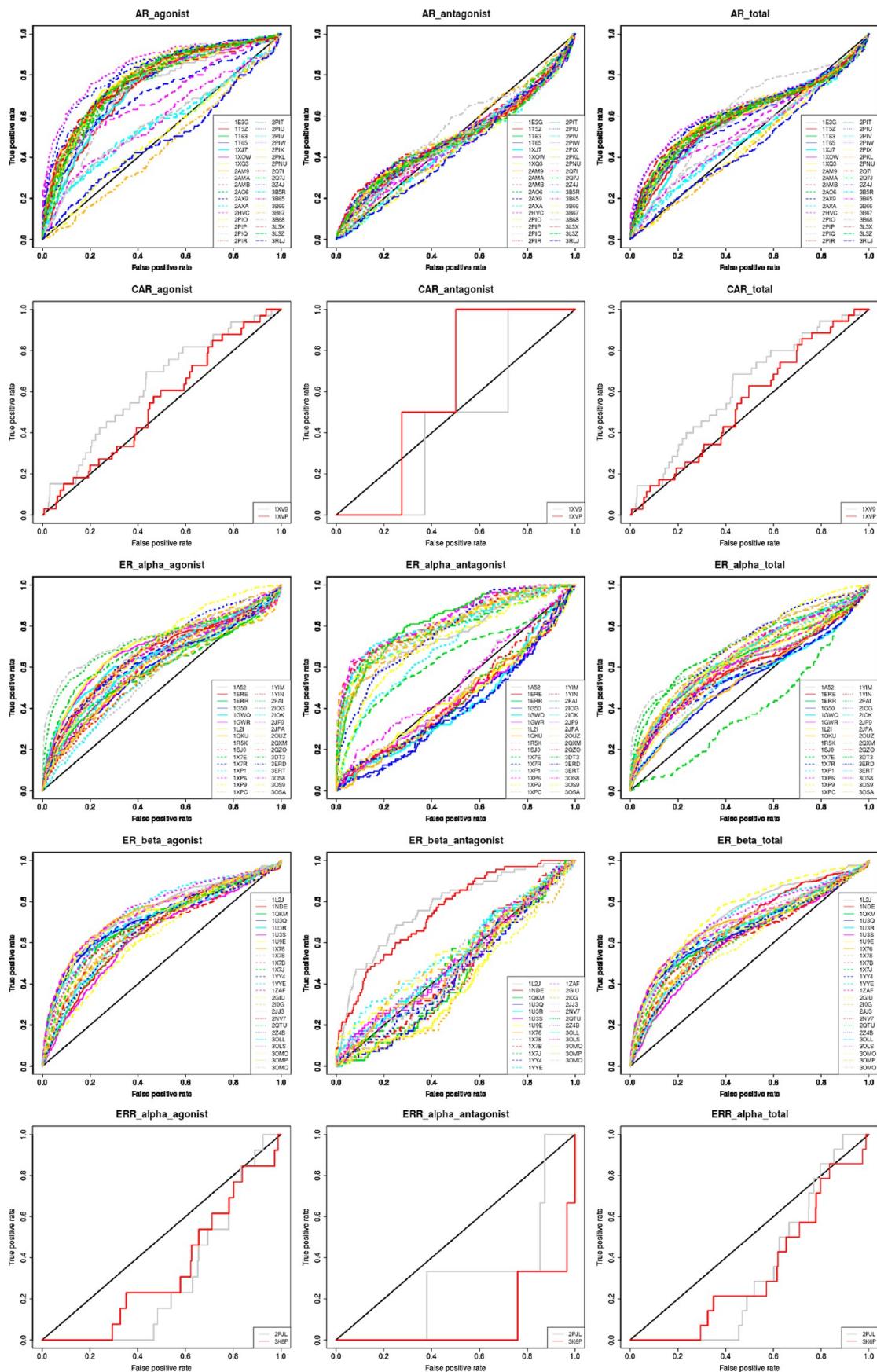


Figure 1. continued

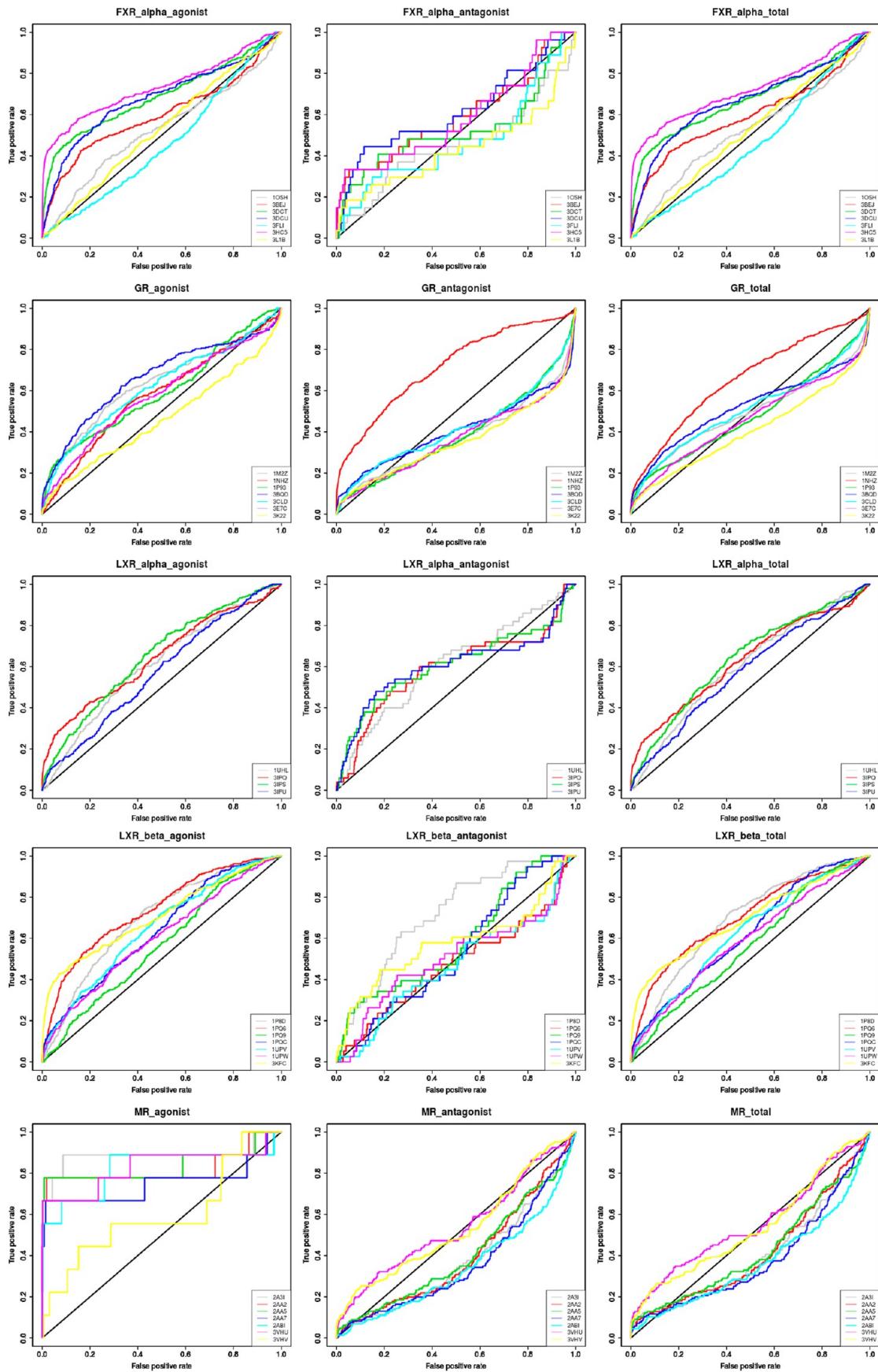
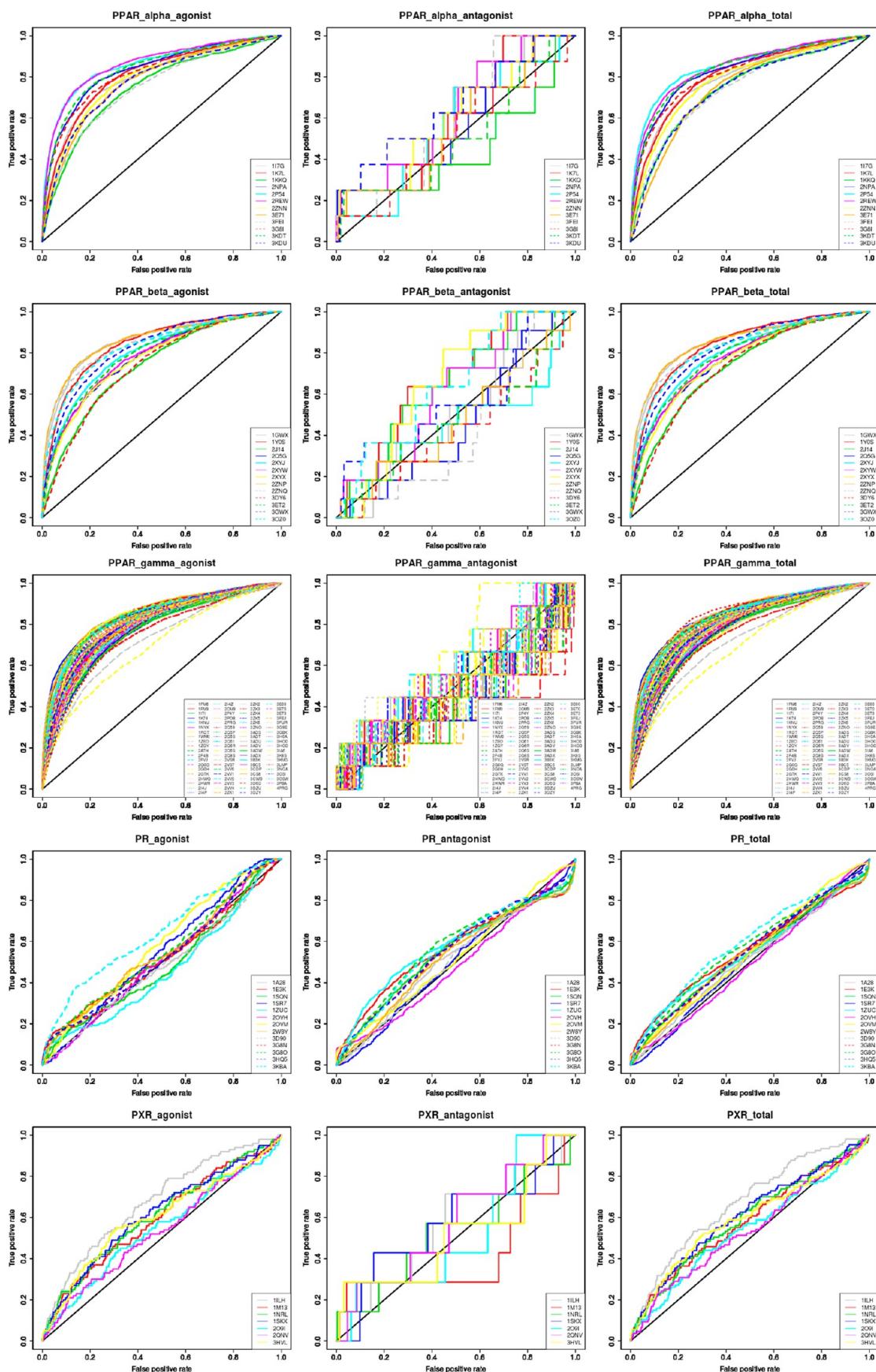


Figure 1. continued



**Figure 1.** continued

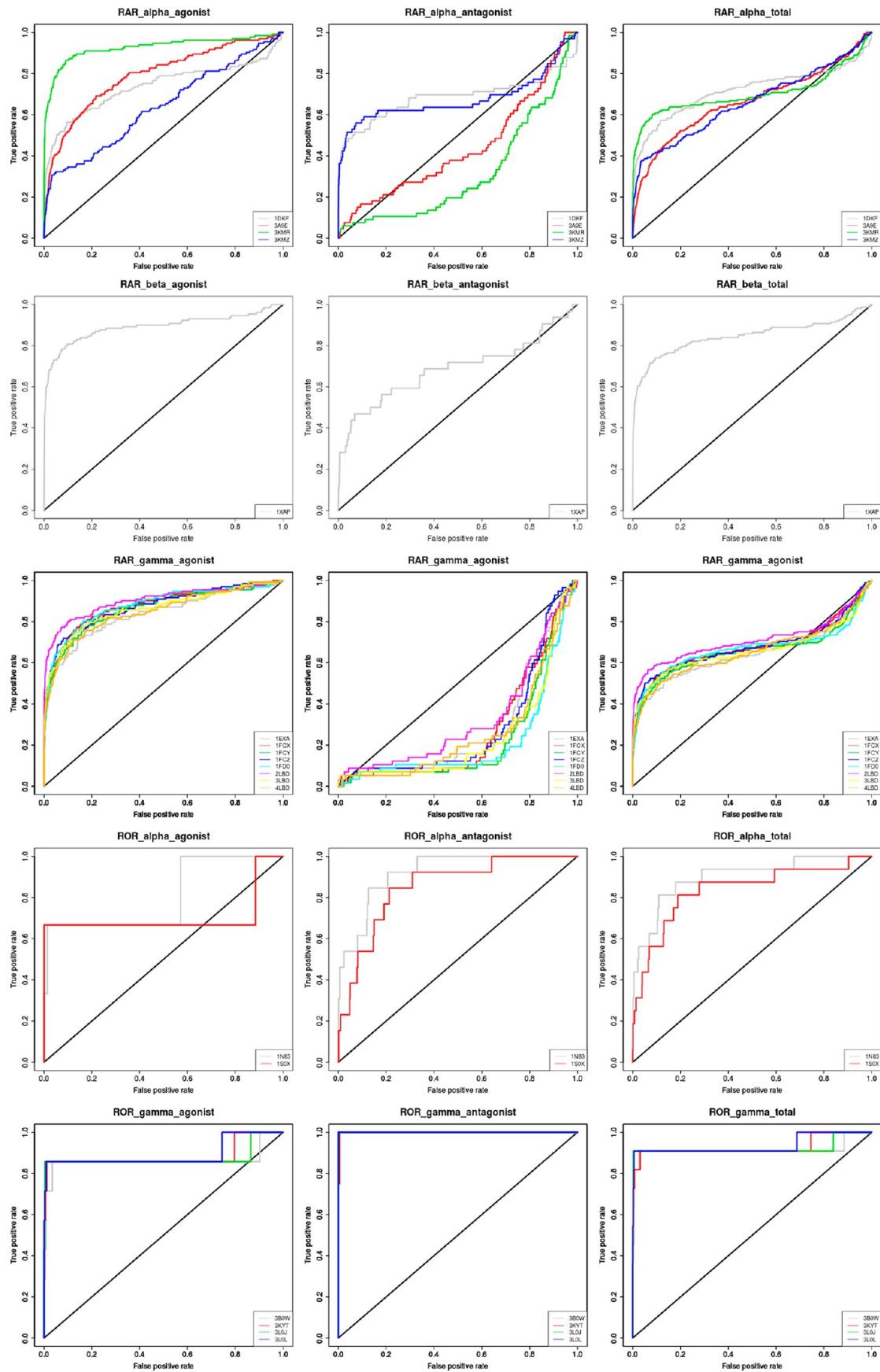
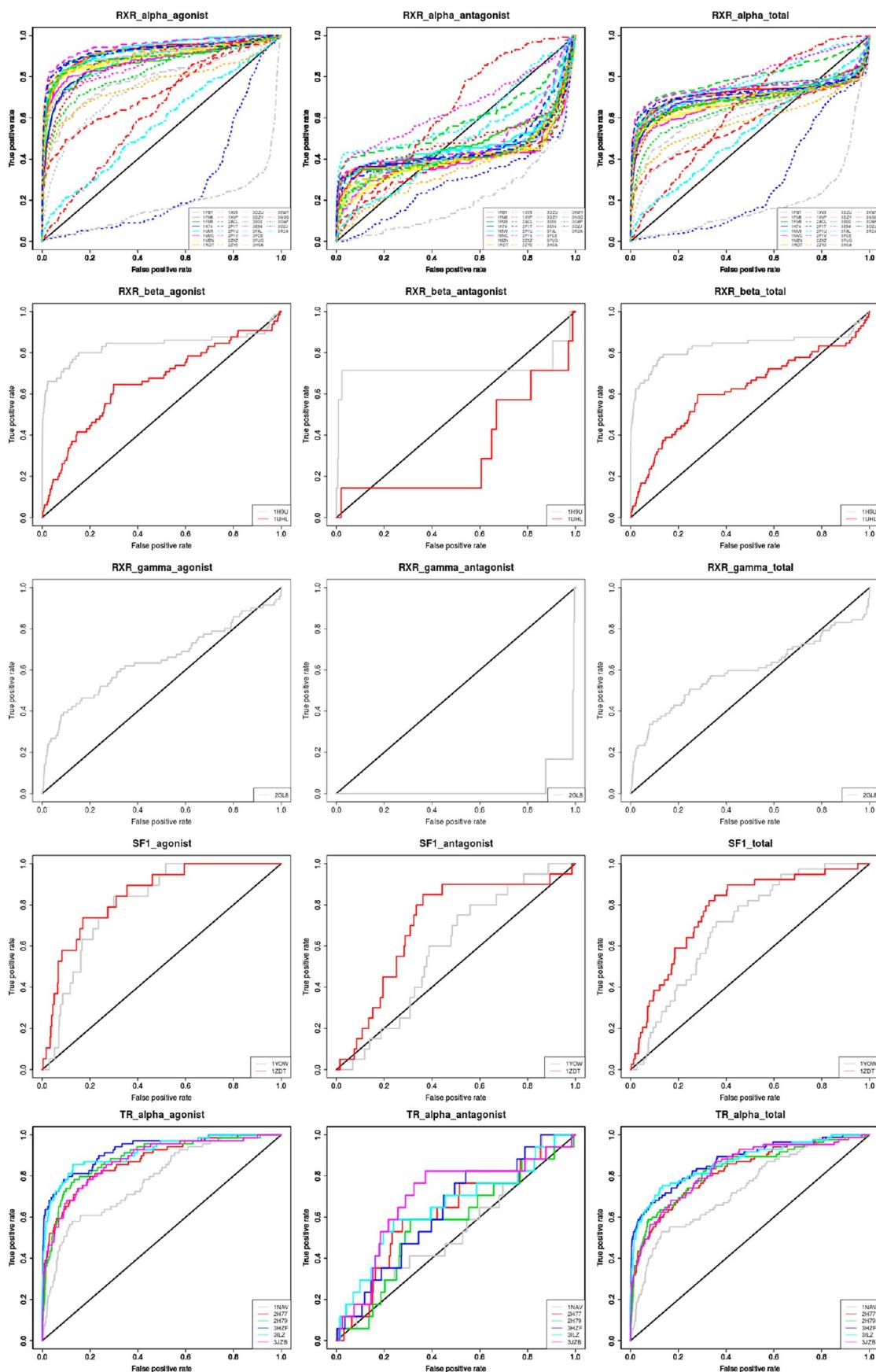
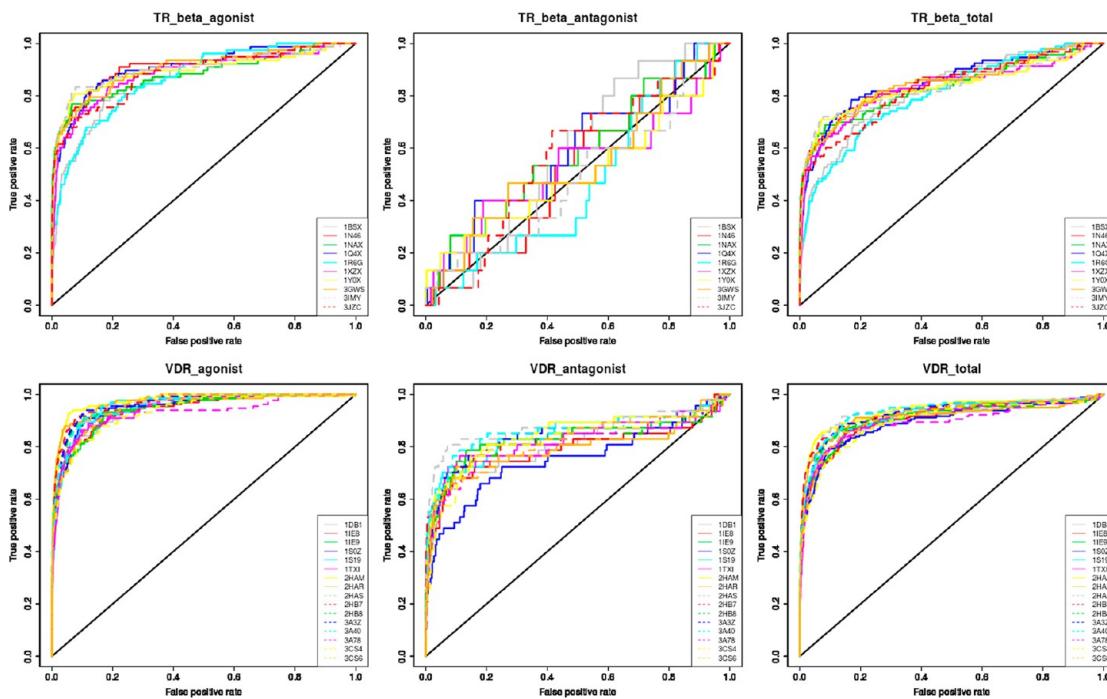


Figure 1. continued



**Figure 1.** continued



**Figure 1.** ROC curves obtained using Surflex-Dock with the agonist data set, the antagonist data set, or the total data set of a given NR on all structures composing that NR data set.

in the scientific literature, and their corresponding computed decoys obtained using the DUD-E decoy generation tool.<sup>8</sup> The NRLiSt BDB comprises 7853 actives, 458 981 decoys, and 339 structures divided into 54 data sets of various sizes in terms of active ligands (ranging from two ligands for CAR antagonists to 1820 ligands for PPAR\_gamma agonists) and available structures (from one for RAR\_beta and RXR\_gamma to 80 for PPAR\_gamma). The NRLiSt BDB was downloaded from the Web site <http://nrlist.drugdesign.fr>.

**Preparation of Ligands and Decoys.** All of the ligands and decoys provided in MOL2 format in the NRLiSt BDB were used in this study. Agonists and antagonists and their corresponding decoys were considered in two separate data sets. A third data set, named the “total” data set, was constructed for each NR by gathering together all of ligands (either agonist or antagonist) and their corresponding decoys.

**Surflex-Dock.** SF is based on a modified Hammerhead fragmentation/reconstruction algorithm to dock compounds flexibly into the binding site.<sup>15</sup> The query molecule is decomposed into rigid fragments that are superimposed on the Surflex-protomol, i.e., molecular fragments covering the entire binding site. The docking poses are evaluated by an empirical scoring function. For each structure, the binding site has been defined as 4 Å around the cocrystallized ligand. Surflex minimization options were used before docking (+premin) on the ligands and after docking (+remin) on all of the atoms of the binding site. In this study, Surflex-Dock version 2.5 was used for all of the calculations.

**Performance Metrics.** All of the enrichment graphs were produced with the statistical and graphical tool R (<http://www.r-project.org/>). The ROCR package<sup>16</sup> was used to plot receiver operating characteristic (ROC) curves, and the Wilcoxon–Mann–Whitney algorithm was used for the ROC area under the curve (AUC) calculations. Enrichment factors (EFs) were computed as follows:

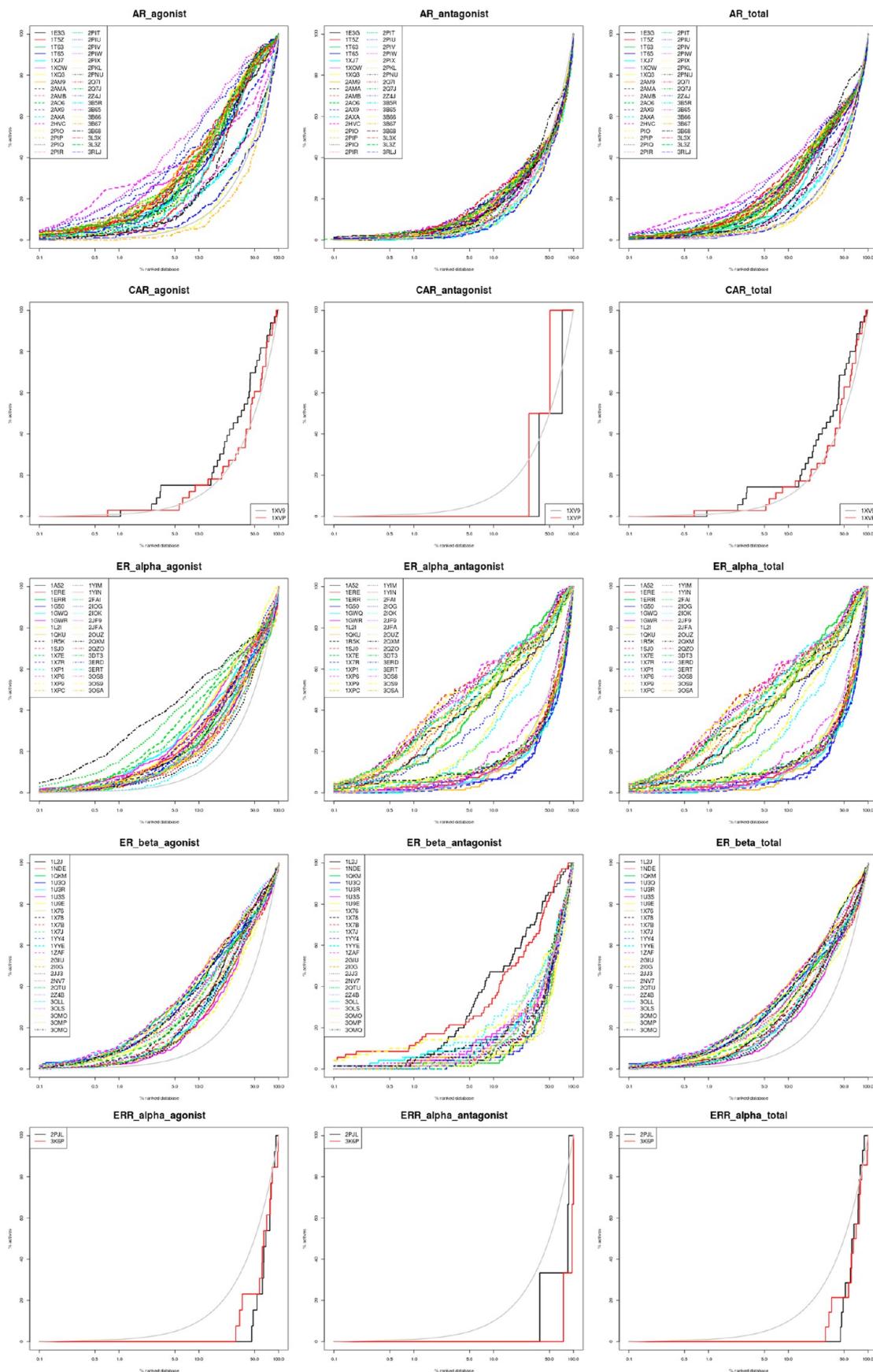
$$EF_x\% = \frac{N^{x\%}_{\text{experimental}}}{N^{x\%}_{\text{actives}}}$$

## RESULTS

### Impact of Using Separate Data Sets on Overall Enrichment.

DUD and its enhanced version DUD-E are the current standard benchmarking databases. Interestingly, one constitutional change between these two versions is that the two original ER\_alpha data sets (ER\_alpha\_agonist and ER\_alpha\_antagonist) of the first release have now been merged into a single ER\_alpha data set. The NRLiSt BDB is composed of two separate data sets for each NR, one agonist data set and one antagonist data set. To evaluate the relevance of this choice, for each NR we analyzed the performance on enrichment of a structure-based virtual screening method, Surflex-Dock (SF), on all of the experimental structures composing the data set, using the two separated agonist and antagonist data sets and the “total” data set gathering both the agonist and antagonist data sets together (Figures 1 and 2 and Table 1).

For 10 out of the 27 NRs (AR, ER\_beta, GR, MR, RAR\_beta, RAR\_gamma, ROR\_gamma, RXR\_alpha, RXR\_beta, and VDR), the mean AUC obtained by screening a separate data set on all of the structures available for a given NR (the agonist data set for all but VDR and the antagonist data set for VDR) was significantly superior to the mean AUC obtained with the total data set according to a Wilcoxon test.<sup>17</sup> This trend was strongly confirmed when focusing on single AUC values. For every NR, the best signal was always associated with a separate data set (the agonist data set for 21 NRs and the antagonist data set for six NRs). For 17 out of the 25 NRs for which more than one experimental structure of the protein was available, the structure that provided the best AUC was the same using separate data sets or the total data set. The separate data sets provided



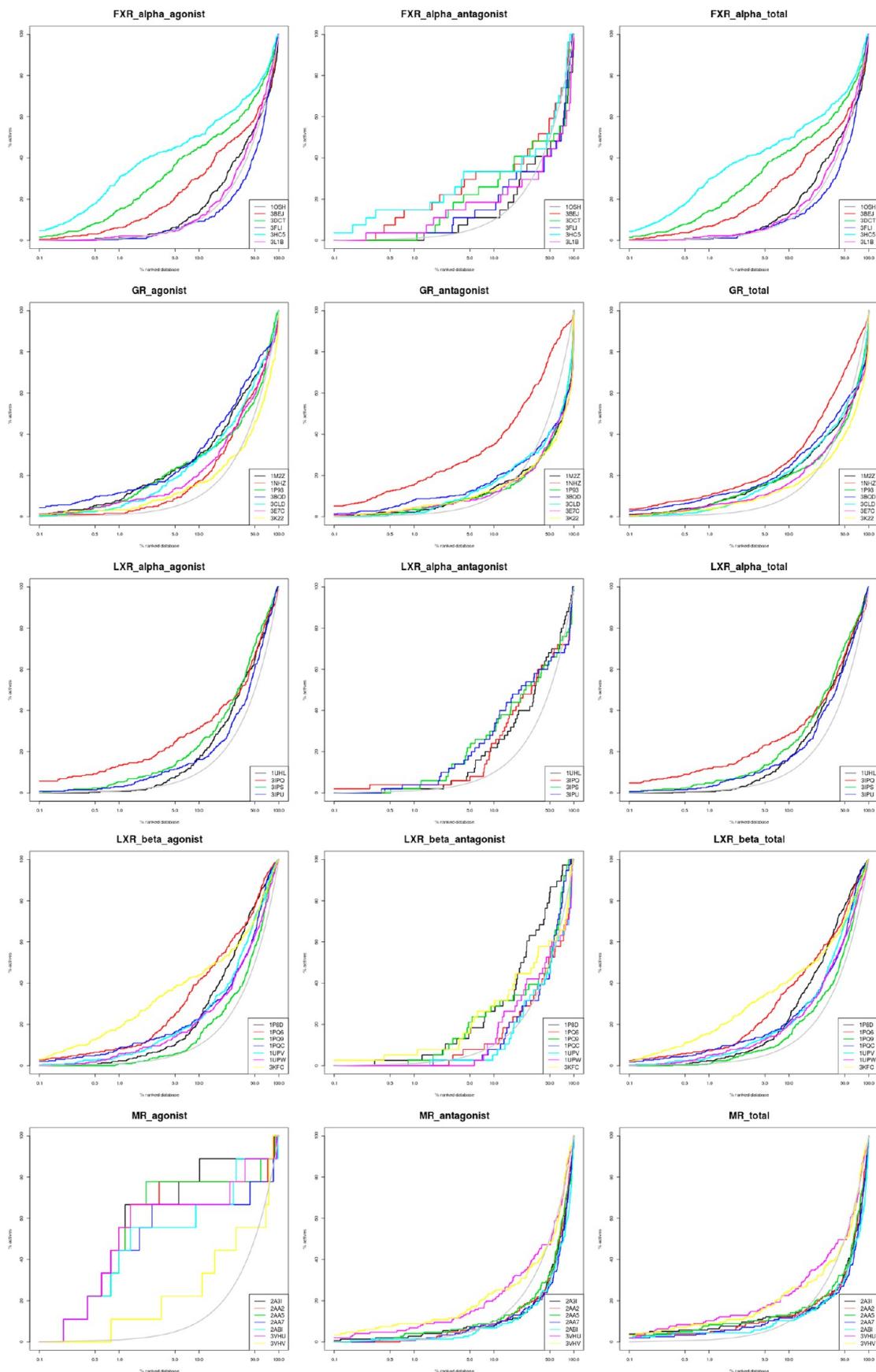
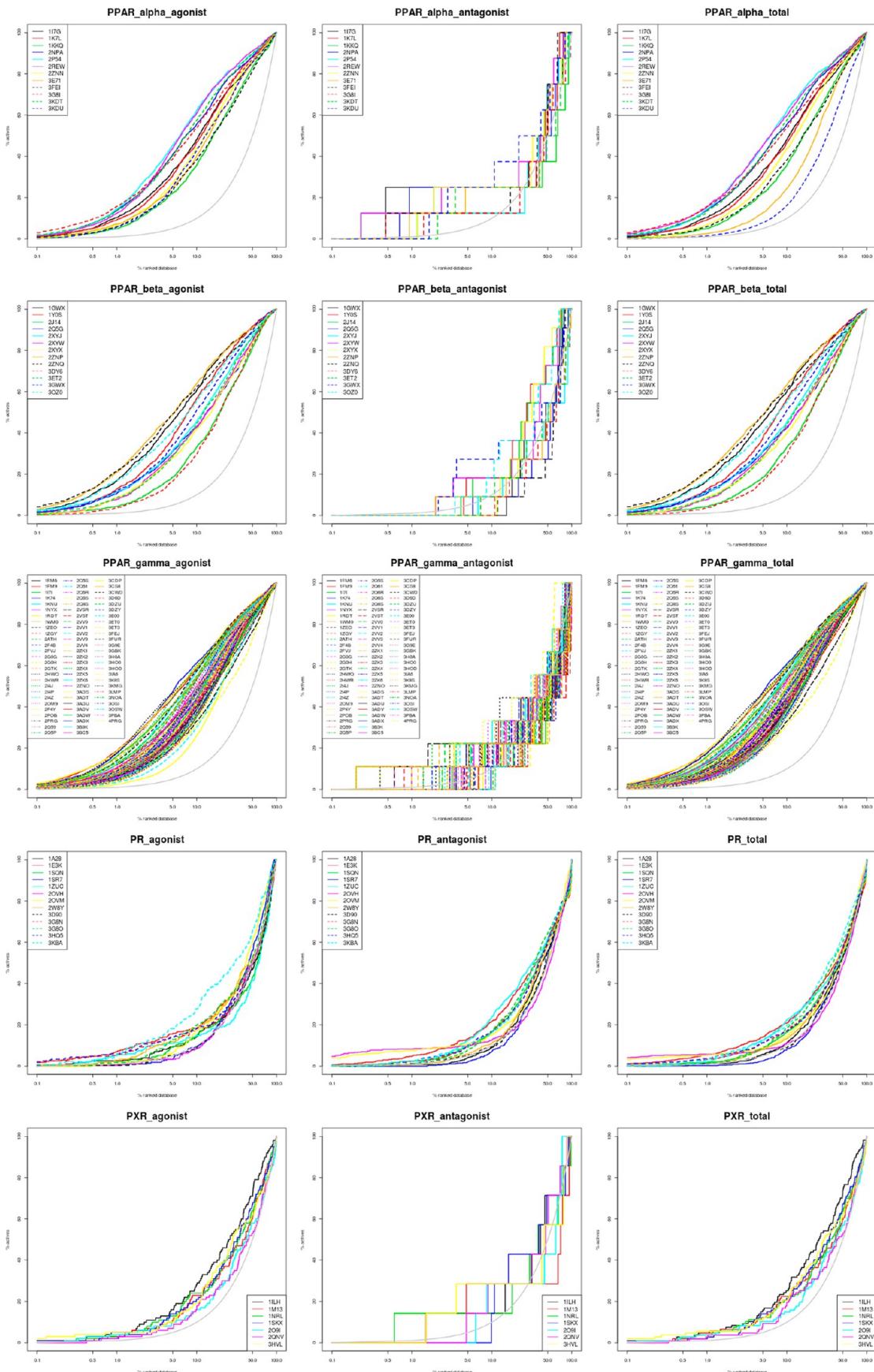


Figure 2. continued



**Figure 2.** continued

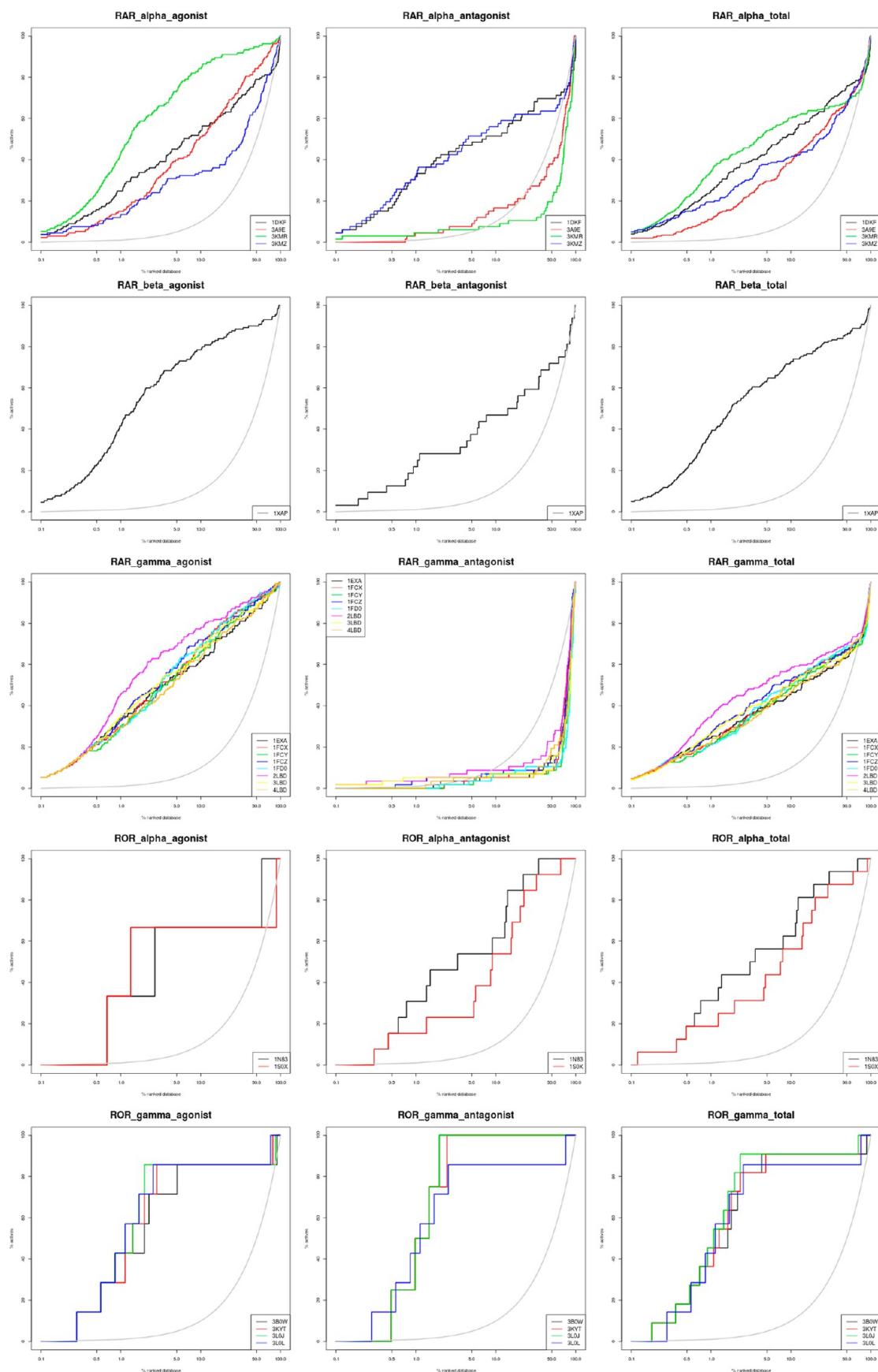
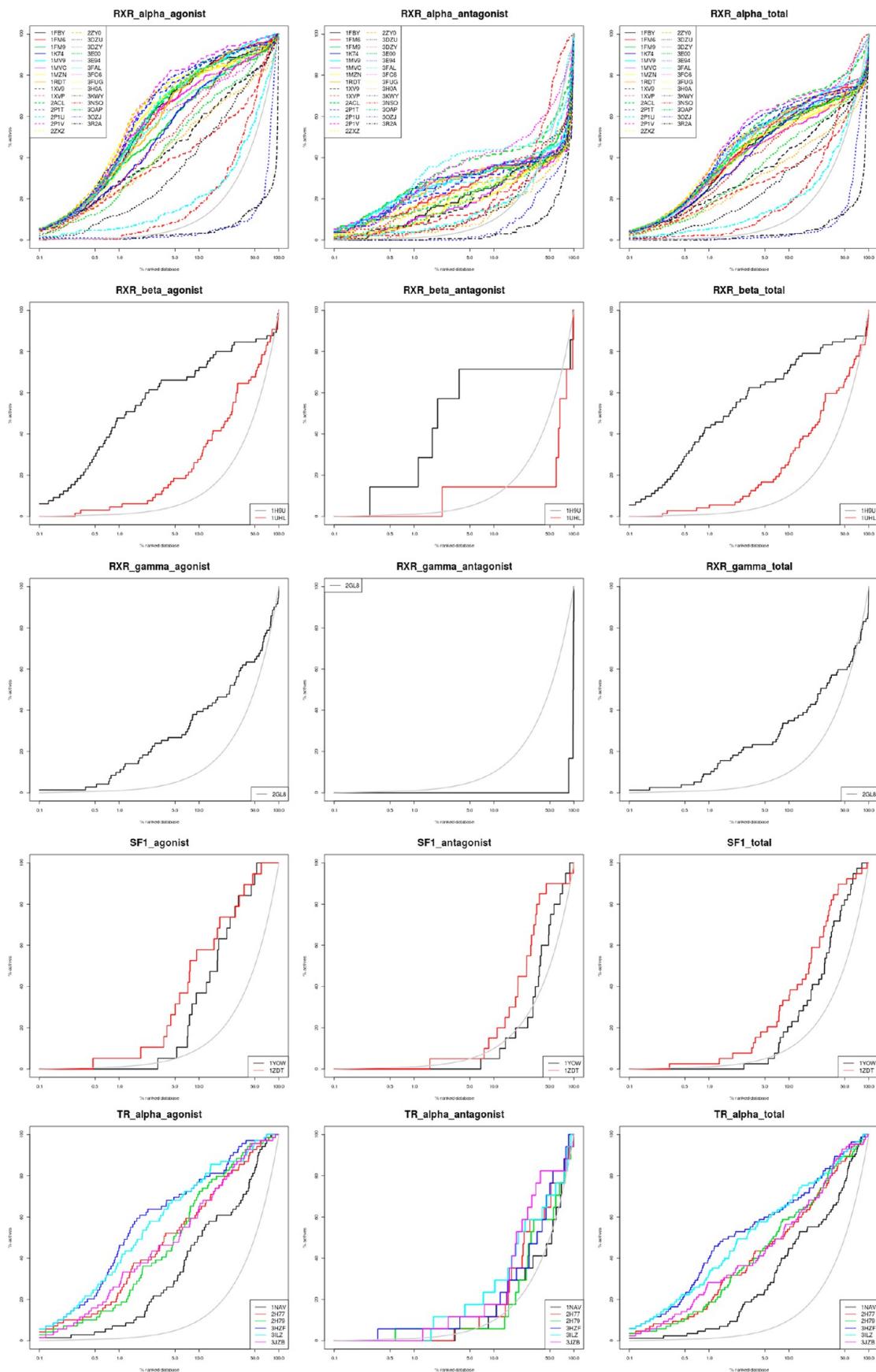
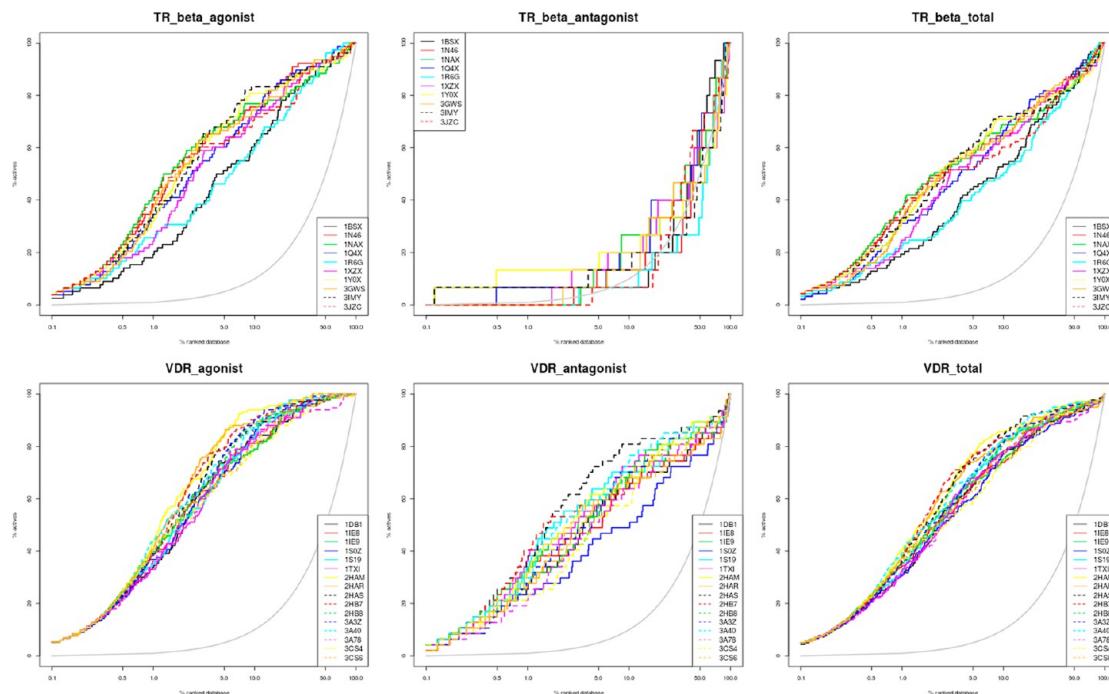


Figure 2. continued





**Figure 2.** Enrichment graphs obtained using Surfflex-Dock with the agonist data set, the antagonist data set, or the total data set of a given NR on all structures composing that NR data set.

the best AUC for 317 out of the 339 structures of the NRLiSt BDB. For seven out of the 27 NRs, all of the AUC values obtained with one of the separate data sets were superior to the best AUC obtained with the total data set. For 18 out of the 27 NRs, for at least 50% of the structures, the AUC values obtained with one of the separate data sets were superior to the best AUC obtained with the total data set for that target.

**Impact of Using Separate Data Sets on Early Enrichment.** Similarly, for 24 out of the 27 NRs, the best early enrichment was obtained using a separate data set (the agonist data set for 18 NRs and the antagonist data set for six NRs). For only one NR (ERR\_alpha) was there no early enrichment with either the separate data sets or the total data set. For 16 out of the 25 NRs with more than one structure, the structure that provided the best early enrichment was the same using one of the separate data sets or using their combination. Over the 339 experimental structures tested in this study, the data set leading to the best early enrichment was one of the two separate data sets in 289 cases and their combination in 46 cases.

**Importance of the Bound Ligand.** We wanted to investigate the importance of the pharmacological profile of the cocrystallized ligand in the binding site of the structure on the performances in enrichment using SF with separate data sets. For this part of the interpretation, we focused on the NRs for which at least one agonist-bound and one antagonist-bound structure were available (ER\_alpha, ER\_beta, GR, MR, PPAR\_alpha, PPAR\_gamma, PR, RAR\_alpha, ROR\_gamma, RXR\_alpha). We thus compared the performances obtained with SF in enrichment using the agonist data set and the antagonist data set separately on each structure. We compared the enrichments associated with the best agonist-bound structure and the best antagonist-bound structure for these 10 NRs (see Figures 3 and 4). For all of the NRs, the structure that gave the best enrichment was an agonist-bound structure when the agonist data set was used. In regard to the antagonist data sets, the structure that gave the best performance in

enrichment was an antagonist-bound structure for six out of the 10 NRs tested. As shown in Figure 5, there is a clear separation of the score distribution profiles between the agonist-bound structures and the antagonist-bound ones using the agonist data sets (for seven NRs out of 10) and using the antagonist data sets (for seven NRs out of 10).

#### Impact of Using Experimentally Confirmed Decoy Ligands.

The decoys provided in the NRLiSt BDB were generated using the DUD-E automated tool. As it is often the case in benchmarking databases, these decoys are assumed to be inactive with no experimental confirmation. Therefore, we decided to use the antagonist data sets as decoy ligand data sets for the agonist data sets and, reciprocally, to use the agonist data sets as decoy ligand data sets for the antagonist data sets if possible (i.e., when a sufficient number of ligands and the appropriate bound structure were available). We thus evaluated the enrichment obtained with the agonist data sets of AR, GR, MR, and PR and with the antagonist data sets of ER\_alpha, ER\_beta, PPAR\_alpha, PPAR\_gamma, RAR\_alpha, and RXR\_alpha using the corresponding antagonist or agonist data sets as their decoys (i.e., using experimentally confirmed decoy ligands). The results are shown in Table 2.

For the agonist data sets (Figure 6A), overall good performances were obtained, except on PR, for which the ROC curve obtained was close to the random one. Similarly, on the antagonist data sets (Figure 6B), overall good performances were associated with ER\_alpha, ER\_beta, and RXR\_alpha, while poor performances were obtained on PPAR\_alpha and PPAR\_gamma and, to a lesser extent, on RAR\_alpha. Similar trends were found when focusing on early enrichments (Figure 7). These results could be correlated with the ratio of the number of active compounds to the number of decoy ligands in the data set. Indeed, when the number of decoy ligands was too large (PPAR\_alpha and PPAR\_gamma) relative to the number of active compounds, the corresponding performances in enrichment were impacted. On the targets for which the number of

**Table 1.** Areas under the ROC Curve (AUC) and Enrichment Factors (EF) at 1% and 10% Obtained by Docking with Surflex-Dock with the 339 NR Structures Contained in the NRLiSt BDB and Used in This Study

structure	agonist data set			antagonist data set			total data set		
	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
AR									
1E3G	0.742	9.76	3.78	0.488	2.20	1.98	0.594	6.07	3.10
1TSZ	0.727	3.80	2.76	0.494	2.65	1.41	0.592	2.67	2.23
1T63	0.777	8.14	3.89	0.495	2.21	1.67	0.612	5.34	2.74
1T65	0.746	7.05	3.30	0.483	1.32	1.54	0.593	4.37	2.50
1XJ7	0.744	8.68	2.70	0.485	0.88	0.75	0.592	3.64	1.87
1XOW	0.769	11.93	4.16	0.491	1.76	1.89	0.605	8.01	2.89
1XQ3	0.770	11.93	4.11	0.494	1.32	1.76	0.609	7.53	2.99
2AM9	0.782	8.68	4.49	0.497	2.65	1.81	0.615	5.83	3.01
2AMA	0.752	8.68	3.46	0.485	1.76	1.67	0.597	4.86	2.60
2AMB	0.763	9.76	3.95	0.510	1.76	1.63	0.612	6.07	2.91
2AO6	0.748	7.59	3.51	0.501	1.76	1.67	0.601	4.37	2.70
2AX9	0.698	5.97	3.73	0.500	4.41	2.07	0.585	6.31	2.94
2AXA	0.567	4.34	2.27	0.453	2.20	1.06	0.504	3.16	1.60
2HVC	0.696	25.49	3.95	0.470	4.85	1.59	0.568	14.32	2.74
2PIO	0.770	7.59	3.95	0.492	0.88	1.59	0.609	3.16	2.74
2PIP	0.778	11.93	3.73	0.507	2.65	1.54	0.617	6.31	2.50
2PIQ	0.759	5.97	3.57	0.495	0.88	1.41	0.606	2.67	2.40
2PIR	0.775	12.48	3.89	0.478	1.76	1.37	0.600	6.56	2.57
2PIT	0.766	13.02	3.68	0.477	0.88	1.15	0.596	5.10	2.45
2PIU	0.824	18.98	5.51	0.509	3.53	2.11	0.638	11.17	3.79
2PIV	0.759	5.42	3.57	0.484	1.76	1.37	0.599	3.40	2.55
2PIW	0.845	21.15	5.68	0.519	3.97	1.89	0.650	12.14	3.79
2PIX	0.781	4.34	3.46	0.504	0.88	1.76	0.614	2.67	2.69
2PKL	0.782	9.22	4.27	0.488	2.21	1.94	0.611	7.28	3.16
2PNU	0.739	4.34	2.32	0.521	2.21	1.01	0.609	2.19	1.38
2Q7I	0.765	10.85	4.11	0.490	2.20	1.50	0.603	6.55	2.84
2Q7J	0.760	7.59	3.68	0.501	3.97	1.76	0.608	6.07	2.79
2Z4J	0.804	16.27	4.54	0.502	1.76	1.63	0.627	8.98	3.11
3B5R	0.560	4.34	2.32	0.458	0.88	0.75	0.503	2.43	1.46
3B65	0.579	3.25	2.27	0.463	0.88	1.15	0.513	1.70	1.72
3B66	0.500	2.71	0.76	0.441	2.21	0.75	0.469	2.43	0.78
3B67	0.453	0.00	0.59	0.483	3.97	1.10	0.475	2.67	0.87
3B68	0.566	2.71	2.27	0.489	3.97	1.50	0.523	3.64	1.80
3L3X	0.782	9.22	4.43	0.502	3.53	2.38	0.619	5.83	3.37
3L3Z	0.785	10.85	4.16	0.493	3.53	2.25	0.615	7.28	3.23
3RLJ	0.483	2.17	1.35	0.439	0.88	0.88	0.461	0.97	1.07
CAR									
1XV9	0.630	0.00	1.52	0.545	0.00	0.00	0.622	3.00	1.44
1XVP	0.544	3.09	1.52	0.613	0.00	0.00	0.548	3.00	1.44
ER_alpha									
1A52	0.634	5.08	2.41	0.755	17.02	5.04	0.657	9.40	2.93
1ERE	0.656	5.77	2.97	0.438	14.06	5.04	0.592	5.41	2.59
1ERR	0.586	3.23	2.41	0.822	2.98	1.10	0.637	7.14	3.13
1G50	0.596	3.46	2.12	0.410	0.74	0.66	0.543	3.13	1.86
1GWQ	0.647	7.84	3.56	0.423	0.74	1.10	0.577	7.31	3.06
1GWR	0.690	8.07	3.56	0.445	1.48	1.10	0.618	6.79	3.25
1L2I	0.700	5.77	3.61	0.457	5.92	1.24	0.630	5.75	3.18
1QKU	0.594	4.38	2.00	0.448	0.74	1.02	0.551	3.66	1.68
1RSK	0.668	5.08	2.71	0.812	25.90	5.62	0.693	10.45	3.11
1SJ0	0.604	3.92	2.32	0.823	28.12	6.35	0.650	13.06	3.06
1X7E	0.701	10.29	4.44	0.559	5.18	1.39	0.627	10.10	3.84
1X7R	0.639	6.46	3.17	0.401	0.74	0.66	0.568	5.57	2.73
1XP1	0.615	4.15	2.00	0.799	25.90	5.48	0.648	11.49	2.71
1XP6	0.596	2.77	2.14	0.837	31.07	6.43	0.650	11.67	3.00
1XP9	0.594	3.39	2.00	0.806	31.08	5.55	0.638	12.89	2.83
1XPC	0.557	3.69	1.98	0.792	22.94	5.26	0.608	10.62	2.74
1YIM	0.555	3.23	1.68	0.811	26.64	5.91	0.611	13.06	2.59

Table 1. continued

structure	agonist data set			antagonist data set			total data set		
	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
ER_alpha									
1YIN	0.580	2.77	1.84	0.813	17.76	5.99	0.623	10.62	2.80
2FAI	0.731	14.86	4.97	0.556	3.73	1.25	0.647	13.61	4.23
2IOG	0.674	3.69	2.83	0.771	12.58	3.87	0.691	6.44	2.81
2IOK	0.572	1.15	1.43	0.704	4.44	3.14	0.597	3.66	1.93
2JF9	0.611	3.92	2.32	0.836	27.38	6.28	0.659	11.15	3.09
2JFA	0.615	5.77	2.71	0.810	22.20	5.77	0.653	10.80	3.11
2OUZ	0.615	4.15	2.16	0.796	20.72	5.26	0.648	10.27	2.87
2QXM	0.738	24.23	5.44	0.500	6.05	1.42	0.661	21.35	4.54
2QZO	0.655	7.84	3.20	0.466	2.22	1.24	0.594	6.79	2.69
3DT3	0.699	9.46	4.02	0.792	25.16	5.62	0.704	12.02	3.89
3ERD	0.627	4.15	3.12	0.415	1.48	1.31	0.568	3.66	2.78
3ERT	0.630	4.84	2.69	0.810	14.80	5.91	0.660	10.27	3.06
3OS8	0.661	7.15	2.85	0.528	3.70	1.97	0.617	5.40	2.57
3OS9	0.688	3.23	2.71	0.717	4.44	3.58	0.676	4.18	2.40
3OSA	0.680	4.84	3.01	0.774	13.32	5.04	0.696	8.53	3.32
ER_beta									
1L2J	0.710	2.81	3.08	0.773	5.74	4.72	0.717	3.67	3.11
1NDE	0.656	3.32	2.32	0.752	11.48	3.44	0.667	4.75	2.44
1QKM	0.711	9.18	4.15	0.443	0.00	0.29	0.658	9.51	3.78
1U3Q	0.723	8.16	4.15	0.454	4.31	0.57	0.670	7.81	3.80
1U3R	0.672	3.06	2.55	0.472	5.74	1.43	0.644	3.25	2.51
1U3S	0.634	2.30	2.32	0.500	2.87	1.72	0.610	2.16	2.25
1U9E	0.728	7.65	4.30	0.417	1.44	0.57	0.669	6.92	3.74
1X76	0.748	10.46	4.56	0.504	4.31	1.15	0.701	9.72	4.13
1X78	0.685	5.10	3.05	0.486	0.00	0.72	0.645	4.54	2.64
1X7B	0.661	4.34	3.11	0.491	2.91	0.87	0.627	3.90	2.84
1X7J	0.698	6.38	3.36	0.512	2.87	0.86	0.662	5.62	3.13
1YY4	0.715	10.71	4.53	0.469	0.00	1.43	0.669	9.29	4.02
1YYE	0.707	10.46	4.07	0.520	0.00	1.29	0.670	10.15	3.65
1ZAF	0.745	12.24	4.56	0.507	1.44	1.29	0.698	11.45	4.02
2GIU	0.640	2.55	1.99	0.561	10.05	2.00	0.751	4.43	3.10
2I0G	0.672	4.34	2.85	0.529	1.44	1.15	0.647	3.89	2.64
2JJ3	0.688	2.30	2.62	0.523	1.44	0.86	0.655	2.16	2.40
2NV7	0.688	8.67	3.77	0.452	0.00	0.86	0.642	7.78	3.39
2QTU	0.653	3.06	2.52	0.454	0.00	1.00	0.617	2.59	2.29
2Z4B	0.657	4.85	2.77	0.468	0.00	0.86	0.623	4.32	2.51
3OLL	0.745	8.16	3.77	0.568	5.74	2.29	0.712	7.78	3.50
3OLS	0.745	9.95	3.67	0.524	4.31	1.57	0.703	9.07	3.39
3OMO	0.664	6.38	3.54	0.419	1.44	0.72	0.621	6.48	3.30
3OMP	0.680	4.85	3.21	0.408	0.00	0.44	0.631	4.33	3.01
3OMQ	0.739	8.93	3.97	0.489	2.87	1.43	0.692	7.99	3.69
ERR_alpha									
2PJL	0.297	0.00	0.00	0.298	0.00	0.00	0.339	0.00	3.42
3K6P	0.342	0.00	0.00	0.091	0.00	0.00	0.337	0.00	2.37
FXR_alpha									
1OSH	0.524	1.26	1.38	0.455	0.00	1.12	0.518	1.15	1.36
3BEJ	0.598	6.28	3.06	0.593	16.13	3.36	0.596	6.92	3.14
3DCT	0.688	15.07	4.50	0.525	0.00	2.61	0.675	14.42	4.35
3DCU	0.679	4.52	3.13	0.615	4.03	3.73	0.667	4.90	3.78
3FLI	0.460	0.63	0.94	0.471	4.04	1.49	0.461	0.58	1.01
3HCS	0.735	30.13	5.06	0.585	16.15	3.36	0.722	29.98	4.93
3L1B	0.526	2.20	1.09	0.434	4.04	1.87	0.519	2.31	1.15
GR									
1M2Z	0.636	7.84	2.98	0.376	2.18	1.41	0.494	5.89	2.05
1NHZ	0.579	1.71	1.73	0.717	15.32	3.50	0.655	10.30	2.72
1P93	0.596	6.82	2.95	0.387	3.54	1.17	0.483	5.28	2.02
3BQD	0.661	11.53	3.15	0.407	8.16	1.77	0.520	9.21	2.40
3CLD	0.619	4.41	2.88	0.419	1.91	1.68	0.510	3.32	2.20

Table 1. continued

structure	agonist data set			antagonist data set			total data set		
	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
GR									
3E7C	0.578	7.16	2.10	0.369	4.35	1.17	0.464	5.13	1.61
3K22	0.464	3.39	1.66	0.357	4.08	1.25	0.410	3.62	1.45
LXR_alpha									
1UHL	0.612	0.77	1.66	0.611	2.03	2.20	0.608	0.65	1.68
3IPO	0.643	13.52	3.13	0.591	4.06	2.40	0.631	11.99	2.85
3IPS	0.651	5.43	2.28	0.608	2.03	3.01	0.640	4.86	2.20
3IPU	0.574	3.09	1.62	0.604	4.06	3.01	0.584	3.24	1.65
LXR_beta									
1P8D	0.693	2.41	2.27	0.717	2.64	2.64	0.691	2.44	2.28
1PQ6	0.736	8.25	4.16	0.478	0.00	1.05	0.702	7.32	3.80
1PQC	0.637	8.59	2.27	0.535	0.00	0.79	0.624	7.93	2.13
1PQ9	0.564	1.03	1.31	0.591	2.64	2.90	0.566	0.91	1.43
1UPV	0.648	4.47	2.20	0.470	0.00	0.53	0.623	3.97	1.98
1UPW	0.604	5.84	2.27	0.508	0.00	1.05	0.592	5.18	2.01
3KFC	0.708	18.57	4.40	0.580	5.27	2.90	0.694	15.58	4.14
MR									
2A3I	0.887	56.00	7.84	0.380	2.74	0.75	0.401	6.47	1.23
2AA2	0.821	44.80	7.84	0.395	1.39	0.89	0.413	4.53	1.29
2AAS	0.834	44.80	7.84	0.401	4.11	1.03	0.420	8.40	1.36
2AA7	0.750	44.80	6.72	0.361	0.69	0.82	0.378	4.53	1.16
2ABI	0.822	44.80	6.72	0.345	1.37	0.69	0.367	4.53	1.03
3VHU	0.829	56.00	6.72	0.541	6.94	2.06	0.552	10.34	2.26
3VHV	0.600	11.20	2.24	0.534	8.92	2.47	0.537	9.05	2.45
PPAR_alpha									
1I7G	0.797	10.44	4.81	0.602	26.75	2.55	0.795	10.53	4.80
1K7L	0.807	9.37	4.70	0.595	13.38	2.55	0.805	9.39	4.69
1KKQ	0.748	4.94	3.56	0.447	0.00	2.55	0.746	5.06	3.55
2NPA	0.833	13.74	5.58	0.556	26.75	2.55	0.831	13.81	5.56
2PS4	0.858	14.59	6.23	0.565	13.38	1.27	0.855	14.51	6.20
2REW	0.863	12.54	6.10	0.624	13.38	2.55	0.847	15.52	5.97
2ZNN	0.786	6.80	4.39	0.602	0.00	2.55	0.784	6.90	4.38
3ET1	0.777	7.23	4.04	0.556	13.38	2.55	0.749	2.58	2.44
3FEI	0.746	5.94	3.83	0.599	13.38	1.27	0.745	5.98	3.82
3G8I	0.821	15.81	5.40	0.517	0.00	1.27	0.819	15.80	5.37
3KDT	0.839	13.81	5.75	0.507	0.00	2.55	0.838	13.52	5.76
3KDU	0.776	5.72	4.28	0.653	0.00	2.55	0.742	1.54	1.63
PPAR_beta									
1GDX	0.857	16.85	6.06	0.516	0.00	0.00	0.852	16.99	6.02
1Y0S	0.844	12.89	5.68	0.667	0.00	1.84	0.842	12.85	5.65
2J14	0.736	4.29	3.31	0.645	0.00	1.84	0.735	4.35	3.32
2QSG	0.777	10.68	4.29	0.457	0.00	0.92	0.774	40.67	4.24
2XYJ	0.798	11.45	4.57	0.446	0.00	0.92	0.794	11.54	4.53
2XYW	0.779	7.82	4.29	0.614	0.00	1.84	0.776	7.73	4.28
2XYX	0.771	8.70	4.17	0.662	0.00	0.00	0.768	8.93	3.98
2ZNP	0.861	21.04	6.39	0.476	0.00	0.92	0.856	20.91	6.35
2ZNQ	0.842	21.50	6.27	0.416	0.00	0.00	0.837	21.37	6.19
3DY6	0.736	3.63	2.97	0.428	0.00	0.00	0.733	3.70	2.96
3ET2	0.788	8.59	4.45	0.470	0.00	0.92	0.785	8.49	4.41
3GDX	0.823	11.79	5.09	0.570	0.00	2.76	0.820	11.76	5.09
3OZ0	0.832	17.09	5.56	0.651	0.00	1.84	0.829	16.89	5.54
PPAR_gamma									
1FM6	0.795	12.10	4.79	0.474	0.00	2.24	0.793	11.93	4.78
1FM9	0.848	18.64	5.87	0.529	0.00	2.24	0.846	18.60	5.87
1I7I	0.807	16.17	5.38	0.460	11.18	2.24	0.807	15.87	5.38
1K74	0.851	17.49	6.12	0.443	0.00	2.24	0.850	17.73	6.08
1KNU	0.836	12.43	5.70	0.555	0.00	2.23	0.835	12.42	5.68
1NYX	0.808	7.64	4.44	0.450	0.00	1.12	0.807	7.82	4.47
1RDT	0.853	18.42	5.94	0.520	0.00	1.12	0.850	18.77	5.93

Table 1. continued

structure	agonist data set			antagonist data set			total data set		
	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
PPAR_gamma									
1WM0	0.764	8.47	3.81	0.538	0.00	1.12	0.760	7.88	3.78
1ZEO	0.841	13.97	5.77	0.454	0.00	2.24	0.839	13.95	5.76
1ZGY	0.807	9.68	4.96	0.413	11.18	2.24	0.807	9.68	4.96
2ATH	0.802	7.15	4.66	0.441	0.00	1.12	0.801	7.22	4.68
2F4B	0.785	5.33	4.10	0.529	0.00	2.24	0.784	5.20	4.09
2FVJ	0.776	5.50	3.91	0.473	0.00	2.24	0.798	4.38	3.60
2G0G	0.790	9.13	4.32	0.453	0.00	1.12	0.788	9.14	4.30
2G0H	0.812	9.02	4.67	0.468	11.18	1.12	0.811	9.90	4.79
2GTK	0.833	14.85	5.52	0.449	0.00	2.24	0.831	14.83	5.51
2HWQ	0.765	5.00	3.69	0.521	0.00	2.24	0.764	4.98	3.68
2HWR	0.836	9.13	5.33	0.462	0.00	1.12	0.846	5.56	4.35
2I4J	0.820	9.62	5.28	0.517	11.18	2.24	0.819	9.63	5.27
2I4P	0.802	8.52	4.68	0.480	0.00	1.12	0.800	8.54	4.67
2I4Z	0.835	10.34	5.44	0.506	0.00	2.24	0.834	10.29	5.45
2OM9	0.820	9.40	5.15	0.599	0.00	3.35	0.819	9.30	5.13
2P4Y	0.763	3.90	3.33	0.460	0.00	1.12	0.761	3.89	3.32
2POB	0.838	9.67	5.48	0.513	0.00	1.12	0.836	9.57	5.47
2PRG	0.765	5.83	3.89	0.464	0.00	2.24	0.764	5.80	3.89
2Q59	0.801	10.17	4.85	0.491	0.00	1.12	0.801	10.18	4.86
2Q5P	0.787	6.60	4.10	0.420	11.18	1.12	0.785	6.62	4.12
2Q5S	0.774	6.65	3.84	0.465	0.00	1.12	0.773	6.73	3.85
2Q61	0.771	5.39	3.65	0.533	0.00	1.12	0.770	5.09	3.60
2Q6R	0.796	6.87	4.43	0.493	0.00	1.12	0.794	6.84	4.42
2Q6S	0.794	7.26	4.34	0.458	11.18	2.24	0.793	7.28	4.35
2Q8S	0.798	8.63	4.74	0.448	0.00	2.24	0.797	8.59	4.73
2VSR	0.717	6.93	3.33	0.564	11.18	2.24	0.715	6.95	3.31
2VST	0.771	5.66	3.83	0.356	0.00	1.12	0.769	5.64	3.82
2VV0	0.836	12.98	5.47	0.485	0.00	2.24	0.834	12.91	5.45
2VV1	0.788	6.66	4.05	0.448	0.00	1.12	0.792	7.11	4.25
2VV2	0.801	6.65	4.40	0.437	0.00	2.24	0.799	6.62	4.39
2VV3	0.795	6.27	4.17	0.542	11.18	2.24	0.798	6.67	4.32
2VV4	0.794	6.43	4.20	0.570	0.00	1.12	0.796	6.84	4.44
2ZK1	0.774	5.73	3.79	0.509	0.00	1.12	0.777	6.19	3.93
2ZK2	0.760	6.01	3.84	0.507	0.00	2.24	0.776	10.01	4.40
2ZK3	0.751	8.52	3.90	0.454	0.00	1.12	0.749	8.48	3.89
2ZK4	0.803	7.26	4.41	0.444	11.18	1.12	0.801	7.11	4.39
2ZK5	0.764	6.70	3.90	0.545	0.00	1.12	0.768	6.95	4.01
2ZK6	0.772	6.10	3.80	0.507	0.00	1.12	0.770	6.07	3.78
2ZNO	0.797	4.57	3.69	0.498	0.00	2.24	0.776	4.92	3.85
3ADS	0.815	9.67	4.68	0.469	0.00	2.24	0.815	10.78	4.83
3ADT	0.779	5.39	4.05	0.408	0.00	0.00	0.777	5.36	4.03
3ADU	0.777	6.27	3.88	0.530	0.00	2.24	0.779	6.29	3.92
3ADV	0.797	4.90	3.56	0.464	0.00	1.12	0.773	6.29	3.86
3ADW	0.761	4.56	3.53	0.410	0.00	1.12	0.767	5.47	3.91
3ADX	0.767	5.28	3.70	0.470	0.00	1.12	0.765	5.25	3.71
3B3K	0.841	7.75	4.89	0.560	11.18	2.24	0.837	13.51	5.52
3BC5	0.810	10.73	4.95	0.560	11.18	2.24	0.821	13.13	5.27
3CDP	0.831	2.57	2.60	0.451	11.18	2.24	0.820	8.65	5.03
3CDS	0.810	7.37	4.76	0.475	11.18	1.12	0.814	8.32	4.94
3CS8	0.797	6.05	3.88	0.447	11.18	1.12	0.790	9.08	4.25
3CWD	0.836	10.12	5.35	0.461	0.00	1.12	0.834	10.18	5.34
3D6D	0.807	8.74	4.64	0.507	0.00	2.24	0.817	10.72	4.91
3DZU	0.797	8.14	4.34	0.460	0.00	1.12	0.795	7.99	4.32
3DZY	0.786	3.50	3.03	0.538	0.00	2.24	0.814	12.25	4.80
3E00	0.794	6.21	4.23	0.468	0.00	1.12	0.793	6.18	4.22
3ET0	0.689	8.30	3.12	0.607	11.18	1.12	0.688	8.21	3.11
3ET3	0.827	12.15	5.45	0.460	0.00	2.24	0.826	12.09	5.43
3FEJ	0.823	17.92	5.85	0.431	0.00	2.24	0.822	17.45	5.91

Table 1. continued

structure	agonist data set			antagonist data set			total data set		
	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
PPAR_gamma									
3FUR	0.764	4.40	3.58	0.454	0.00	1.12	0.763	4.38	3.57
3G9E	0.845	13.97	6.00	0.504	0.00	2.24	0.843	13.84	5.98
3GBK	0.819	7.20	4.87	0.433	11.18	1.12	0.817	7.11	4.85
3H0A	0.829	8.86	5.42	0.546	0.00	1.12	0.827	8.81	5.41
3HO0	0.810	7.92	4.64	0.501	0.00	2.24	0.809	7.88	4.62
3HOD	0.808	8.69	4.93	0.543	0.00	3.35	0.807	8.65	4.92
3IA6	0.824	15.78	5.89	0.402	11.18	2.24	0.820	15.60	5.83
3K8S	0.777	7.04	4.01	0.468	0.00	2.24	0.778	7.22	4.01
3KMG	0.787	7.48	4.23	0.392	0.00	1.12	0.785	7.44	4.21
3LMP	0.764	7.09	3.80	0.473	0.00	0.00	0.763	7.00	3.81
3NOA	0.807	8.91	4.73	0.434	0.00	1.12	0.806	8.87	4.72
3OSI	0.788	5.61	3.81	0.530	0.00	0.00	0.786	5.58	3.80
3OSW	0.780	5.78	3.91	0.484	0.00	2.24	0.779	5.69	3.93
3PBA	0.782	4.40	3.78	0.466	0.00	2.24	0.781	4.38	3.77
4PRG	0.788	7.53	4.31	0.538	0.00	2.24	0.786	7.33	4.32
PR									
1A28	0.494	2.61	1.41	0.511	0.76	0.91	0.509	1.38	1.15
1E3K	0.524	7.07	1.85	0.566	4.74	2.17	0.555	5.52	2.09
1SQN	0.488	0.37	1.44	0.550	2.46	1.66	0.533	1.88	1.64
1SR7	0.547	0.74	0.96	0.498	0.00	0.70	0.513	0.25	0.81
1ZUC	0.463	5.58	1.48	0.574	1.14	2.38	0.541	2.13	2.24
2OVH	0.509	0.37	0.85	0.486	8.33	1.27	0.492	5.77	1.11
2OVM	0.579	1.12	1.70	0.526	7.57	1.51	0.542	5.52	1.45
2W8Y	0.545	2.98	1.85	0.522	0.57	1.12	0.532	1.13	1.34
3D90	0.486	0.37	0.89	0.500	1.33	0.93	0.498	1.00	0.90
3G8N	0.526	5.58	1.82	0.554	2.27	1.64	0.547	3.26	1.69
3G8O	0.549	1.86	1.96	0.570	1.14	1.66	0.566	1.25	1.83
3HQ5	0.528	5.96	1.82	0.557	2.46	1.66	0.550	3.64	1.71
3KBA	0.644	5.21	2.78	0.565	2.27	1.64	0.592	3.26	1.98
PXR									
1ILH	0.677	5.06	2.80	0.568	0.00	1.45	0.671	4.67	2.52
1M13	0.594	3.03	2.30	0.412	0.00	2.89	0.581	4.67	2.24
1NRL	0.606	3.03	2.40	0.529	15.90	1.45	0.592	3.74	2.15
1SKX	0.613	2.02	2.10	0.577	0.00	1.45	0.608	1.87	2.15
2O9I	0.543	4.05	1.60	0.516	0.00	2.89	0.540	3.74	1.50
2QNV	0.535	2.02	1.60	0.573	0.00	2.89	0.540	2.80	1.96
3HVL	0.599	5.06	1.90	0.518	0.00	2.89	0.591	5.61	2.06
RAR_alpha									
1DKF	0.738	25.12	5.42	0.686	32.38	5.16	0.717	25.14	5.23
3A9E	0.792	15.23	5.12	0.439	4.63	1.67	0.663	11.06	3.87
3KMR	0.927	43.40	8.42	0.317	4.63	0.76	0.705	34.19	6.03
3KMZ	0.639	12.94	3.39	0.678	32.38	5.62	0.660	19.61	4.12
RAR_beta									
1XAP	0.892	42.02	7.85	0.687	22.08	4.70	0.846	38.30	7.29
RAR_gamma									
1EXA	0.836	34.17	6.22	0.274	0.00	0.53	0.650	24.40	4.55
1FCX	0.875	29.61	6.82	0.270	0.00	0.53	0.674	21.21	5.19
1FCY	0.862	30.37	6.59	0.233	0.00	0.70	0.659	21.74	4.82
1FCZ	0.873	33.41	7.20	0.283	3.52	0.70	0.680	27.05	5.24
1FD0	0.878	29.61	6.97	0.217	0.00	0.70	0.667	21.21	5.08
2LBD	0.902	45.55	7.73	0.329	3.52	0.88	0.711	34.47	5.82
3LBD	0.859	34.93	6.82	0.241	5.28	0.70	0.660	25.99	5.03
4LBD	0.847	29.61	6.37	0.261	0.00	0.53	0.655	22.27	4.60
ROR_alpha									
1N83	0.805	50.00	6.67	0.921	33.95	6.17	0.901	31.72	6.27
1SOX	0.705	50.00	6.67	0.852	16.97	5.40	0.836	19.03	5.64
ROR_gamma									
3B0W	0.864	50.71	8.69	1.000	51.00	10.20	0.915	50.64	9.21
3KYT	0.883	33.81	8.69	0.999	51.00	10.20	0.928	40.51	9.21

Table 1. continued

structure	agonist data set			antagonist data set			total data set		
	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
ROR_gamma									
3L0J	0.875	50.71	8.69	1.000	51.00	10.20	0.923	40.51	9.21
3L0L	0.892	50.71	8.69	1.000	51.00	10.20	0.936	50.64	9.21
RXR_alpha									
1FBY	0.902	35.71	8.05	0.426	9.96	2.58	0.696	25.79	6.00
1FM6	0.919	36.67	8.29	0.433	16.09	3.33	0.717	27.26	6.23
1FM9	0.865	35.24	7.24	0.480	25.28	3.49	0.701	29.60	5.85
1K74	0.874	27.37	7.34	0.451	22.22	3.56	0.697	24.03	5.88
1MV9	0.933	38.10	8.29	0.476	22.99	3.56	0.739	30.48	6.38
1MVC	0.878	40.81	7.67	0.390	9.96	2.50	0.669	29.31	5.47
1MZM	0.894	42.73	7.96	0.398	7.66	2.27	0.677	30.48	5.67
1RDT	0.912	35.24	8.10	0.436	21.45	3.56	0.713	28.43	6.35
1XV9	0.793	28.57	5.76	0.416	7.66	2.50	0.632	19.64	4.42
1XVP	0.727	24.49	4.77	0.376	7.66	1.67	0.574	17.00	3.51
2ACL	0.922	38.41	8.48	0.579	25.28	4.17	0.796	30.48	6.79
2P1T	0.928	40.48	8.43	0.457	19.16	3.18	0.721	31.07	6.44
2P1U	0.918	33.81	8.14	0.462	21.45	2.96	0.712	28.72	6.14
2P1V	0.951	44.76	8.71	0.494	23.76	3.64	0.749	34.00	6.73
2ZXZ	0.906	46.57	8.20	0.415	16.86	2.80	0.695	34.29	5.97
2ZY0	0.904	45.13	8.15	0.428	13.79	3.03	0.698	33.41	5.97
3DZU	0.749	12.38	4.62	0.443	3.83	1.44	0.601	7.91	3.42
3DZY	0.870	27.14	6.71	0.496	15.32	3.56	0.712	21.69	5.53
3E00	0.824	22.09	6.29	0.402	8.43	2.58	0.648	17.58	4.83
3E94	0.277	0.95	0.33	0.287	0.00	0.30	0.285	0.59	0.20
3FAL	0.888	38.10	7.33	0.614	27.58	4.39	0.785	30.78	6.17
3FC6	0.873	39.85	7.10	0.647	22.21	4.32	0.791	29.30	5.94
3FUG	0.880	34.09	7.58	0.404	11.49	2.73	0.674	23.45	5.50
3H0A	0.764	23.33	5.86	0.361	3.06	1.44	0.586	14.65	4.01
3KWy	0.177	0.48	0.33	0.207	0.00	0.23	0.183	0.00	0.29
3NSQ	0.634	0.48	1.67	0.631	3.83	1.97	0.647	2.34	1.76
3OAP	0.896	40.81	8.15	0.424	13.79	2.65	0.692	29.59	5.94
3OZJ	0.923	40.95	8.52	0.445	25.28	3.64	0.725	31.94	6.55
3R2A	0.569	5.76	2.10	0.448	3.83	1.59	0.529	5.28	1.87
RXR_beta									
1H9U	0.836	48.17	7.09	0.725	16.90	7.24	0.833	43.74	7.10
1UHL	0.656	4.66	2.78	0.326	0.00	1.45	0.622	4.23	2.64
RXR_gamma									
2GL8	0.646	10.09	3.95	0.027	0.00	0.00	0.596	9.27	3.51
SF1									
1YOW	0.807	0.00	3.71	0.585	0.00	0.50	0.701	0.00	2.06
1ZDT	0.844	5.63	5.83	0.707	0.00	1.50	0.776	2.66	3.35
TR_alpha									
1NAV	0.781	7.32	5.08	0.541	0.00	1.19	0.739	5.99	4.48
2H77	0.872	23.44	6.24	0.618	0.00	1.19	0.823	19.18	5.41
2H79	0.888	17.58	7.11	0.570	6.30	0.59	0.829	17.97	6.01
3HZF	0.926	45.40	7.69	0.635	6.31	1.19	0.874	40.74	6.72
3ILZ	0.915	38.09	7.69	0.651	0.00	2.38	0.868	32.36	6.71
3JZB	0.877	26.36	6.53	0.695	0.00	1.78	0.836	28.76	5.66
TR_beta									
1BSX	0.865	19.37	6.16	0.585	6.75	0.67	0.821	19.48	5.38
1N46	0.910	37.73	7.58	0.527	0.00	1.33	0.848	33.56	6.45
1NAX	0.883	42.61	7.70	0.575	0.00	2.67	0.832	36.80	6.88
1Q4X	0.906	34.85	7.19	0.583	6.75	2.00	0.854	31.38	6.68
1R6G	0.867	25.82	6.04	0.471	0.00	0.67	0.803	22.73	5.06
1XZX	0.885	24.52	7.19	0.532	0.00	2.00	0.830	24.89	6.36
1Y0X	0.897	32.26	8.09	0.509	13.50	2.00	0.835	32.46	7.11
3GWS	0.904	37.42	7.44	0.539	0.00	1.33	0.847	33.54	6.36
3IMY	0.905	34.86	8.35	0.491	6.75	1.33	0.837	32.47	7.20
3JZC	0.885	40.02	7.19	0.565	0.00	0.67	0.828	36.80	6.02

Table 1. continued

structure	agonist data set			antagonist data set			total data set		
	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
VDR									
1DB1	0.945	34.13	8.36	0.810	26.44	6.39	0.902	30.84	7.84
1IE8	0.942	39.44	8.13	0.789	33.05	6.81	0.901	34.77	7.39
1IE9	0.944	40.20	7.91	0.831	33.05	6.81	0.910	35.89	7.50
1SOZ	0.952	37.17	8.51	0.753	24.24	4.90	0.891	30.84	7.73
1S19	0.951	35.65	8.36	0.849	35.24	7.02	0.919	32.52	8.23
1TXI	0.944	33.37	8.28	0.830	37.46	7.24	0.909	32.53	7.78
2HAM	0.976	43.99	9.41	0.850	33.05	6.81	0.936	40.94	8.56
2HAR	0.965	40.95	8.88	0.797	28.65	6.81	0.914	38.13	8.23
2HAS	0.962	40.19	8.73	0.874	37.46	8.09	0.935	37.57	8.45
2HB7	0.968	38.68	9.03	0.821	41.87	6.60	0.923	35.89	8.34
2HB8	0.951	39.44	8.81	0.818	28.65	6.81	0.910	36.45	8.12
3A3Z	0.965	39.44	9.03	0.834	28.65	7.03	0.923	36.45	8.17
3A40	0.965	43.99	8.73	0.853	30.84	7.45	0.931	39.25	8.28
3A78	0.930	36.40	8.21	0.800	22.03	6.39	0.889	31.40	7.72
3CS4	0.933	40.96	8.06	0.821	22.03	5.75	0.897	37.01	7.45
3CS6	0.955	40.20	8.06	0.818	28.65	6.60	0.914	37.57	7.73

decoy ligands outmatched the number of active compounds (MR\_agonist, ER\_alpha\_antagonist, ER\_beta\_antagonist, and RXR\_alpha\_antagonist), the best performances in enrichment were observed in terms of both AUC and EF<sub>1%</sub>.

## DISCUSSION

In the present study, we used an exhaustive NR-focused benchmarking database, the NRLiSt BDB, to study on the one hand the importance of distinguishing agonist and antagonist ligand data sets for the quality of benchmarking databases and on the other hand whether the pharmacological profile of the cocrystallized ligand could guide the query structure choice for docking methods.

**Impact of Using Separate Data Sets on the Overall Enrichment.** Numerous benchmarking databases have been proposed over time,<sup>3–8,18</sup> but the DUD<sup>7</sup> and DUD-E<sup>8</sup> databases offer the highest-quality features for evaluation methods to date and are thus considered as the gold standard. In light of a previous study,<sup>11</sup> we assumed that the quality of benchmarking data sets could be further improved, especially by providing separate data sets according to ligand pharmacological profiles, as already made in a GPCR ligands database.<sup>18</sup>

To test this hypothesis, for each of the 27 NRs of the NRLiSt BDB, we used three data sets, the agonist and antagonist data sets as provided in the NRLiSt BDB and a total data set constructed by combining the agonist and antagonist data sets. We wanted to study the impact of this choice on the performance in enrichment after a retrospective structure-based virtual screening using Surflex-Dock.

For each NR, the data set that provided the best enrichment was always the agonist data set or the antagonist data set, regardless of the structure used for screening. Moreover, for 97% and 89% of the structures used in this study, respectively, the best AUC and the best early enrichment were obtained using one of the two separate data sets. It thus appears that the choice to build separate data sets is relevant in view of the enhanced performances obtained. Another point is that the structure associated with the best AUC or best early enrichment for a given NR was in the majority of cases the same when one of the separate data sets or the total data set was used. This observation confirms that the choice of a more

appropriate query impacts the docking performance.<sup>11–14</sup> However, for 26% of the targets, the AUC value associated with the agonist or antagonist data set for each structure was always superior to the AUC value obtained for the best-performing structure with the total data set. Furthermore, for 67% of the NRs, for at least 50% of their structures the AUC value obtained with one of the separate data sets was superior to the best AUC value obtained with the total data set for that target. It seems that when agonist ligands and antagonist ligands are pooled for a given NR (i.e., in the case of the total data set), there could be a bias with the evaluation of structure-based methods due to the pharmacological profiles of the ligands. Hence, separating the data sets depending on their pharmacological activity (agonist or antagonist) seems to be a better option to ensure a benchmarking database of the best quality.

## Importance of the Bound Ligand in the Structure Used for Screening.

It is currently accepted that the quality of the enrichment depends on the query conformation,<sup>11–14</sup> and the results presented here again confirm this point. We studied the influence of the pharmacological profile of the ligand cocrystallized in the binding site on the performance in enrichment of Surflex-Dock, since conformational changes occur in protein binding sites upon ligand binding. For all of the NRs studied, the structure that led to the best enrichment was always agonist-bound when the agonist data set was used, and an antagonist-bound structure was most often better with the antagonist data set. This observation was confirmed with the score distributions of agonist ligands and antagonist ligands depending on the bound ligand in the original structure. The structural basis of agonist and antagonist action was previously reported,<sup>9</sup> underlying the different conformational changes occurring upon agonist or antagonist binding. The NR apo structures present a fold constituted of the association of 12  $\alpha$ -helices and a short  $\beta$ -turn in an antiparallel “ $\alpha$ -helical sandwich”. Agonist binding induces the repositioning of helices H11, H12, and H3 of the receptor and generates a surface requisite for coactivator recruitment.<sup>9</sup> Conformational changes induced by antagonist ligand binding affect the conformation of helix H12, which is no longer able to adapt its holo position, preventing the formation of the coactivator recruitment surface.

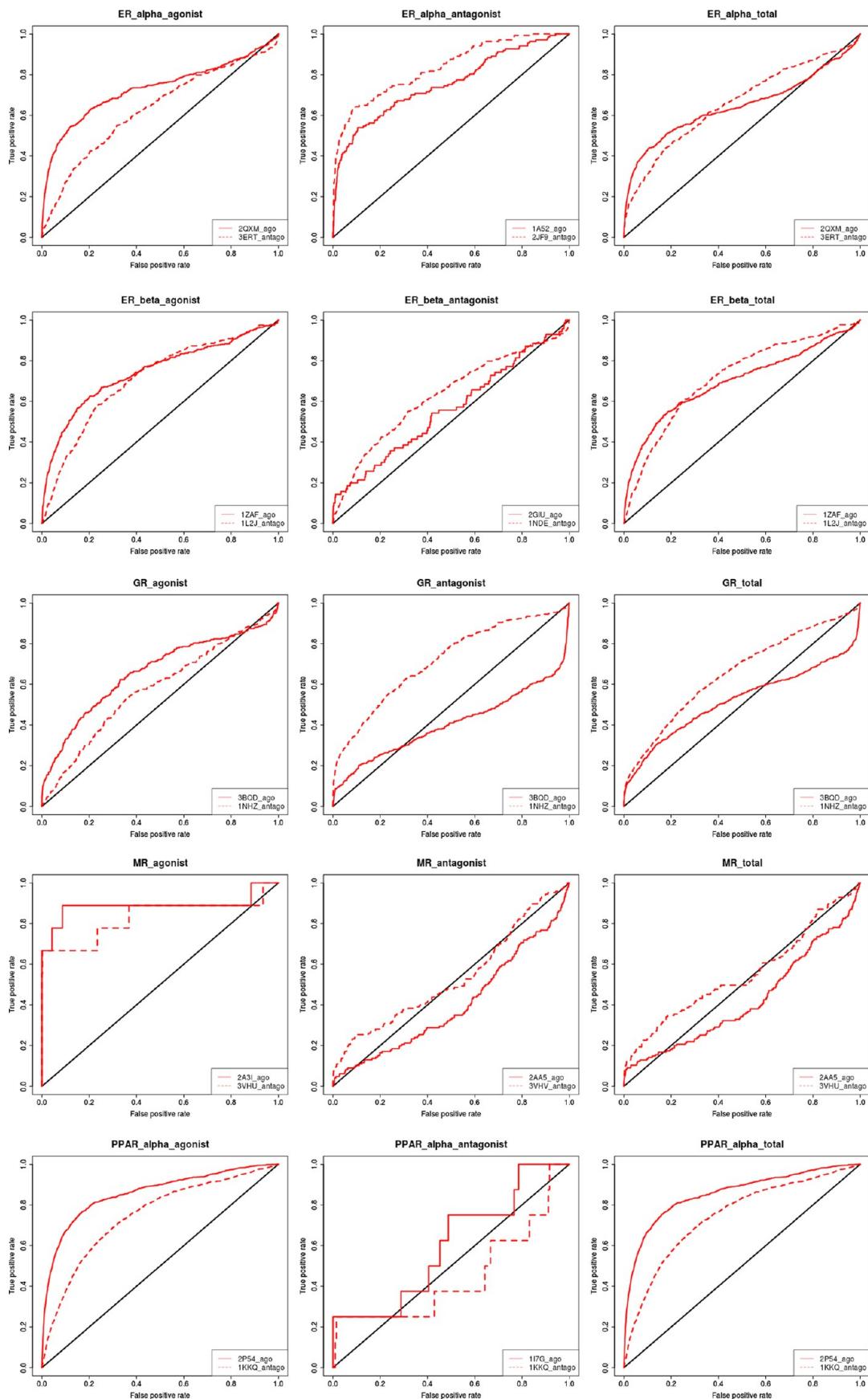
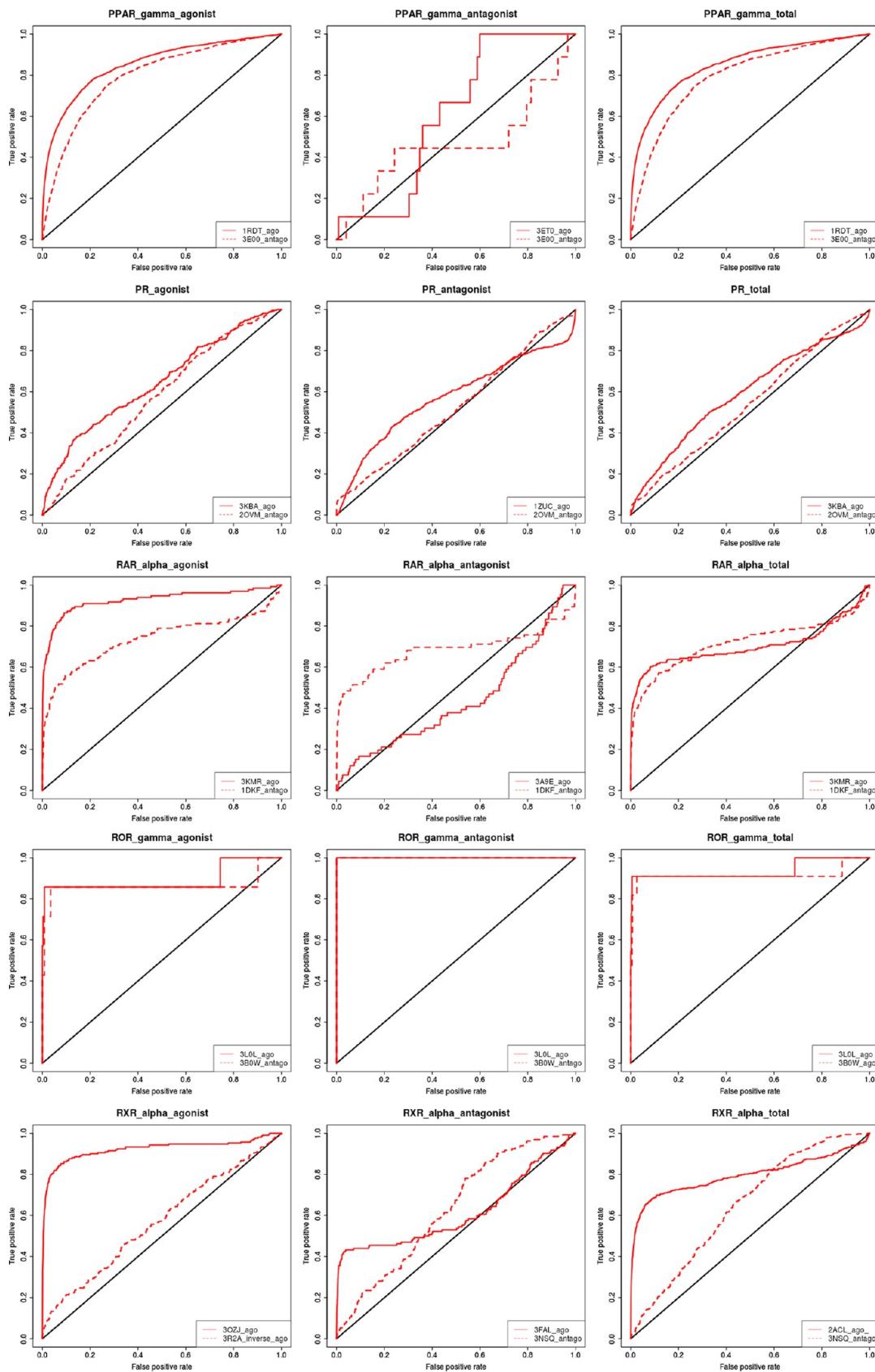


Figure 3. continued



**Figure 3.** ROC curves obtained using Surfflex-Dock with the agonist data set, the antagonist data set, or the total data set of a given NR on the best agonist-bound structure (solid lines) and the best antagonist-bound structure (dashed lines) for the 10 NRs presenting at least one agonist-bound structure and one antagonist-bound structure.

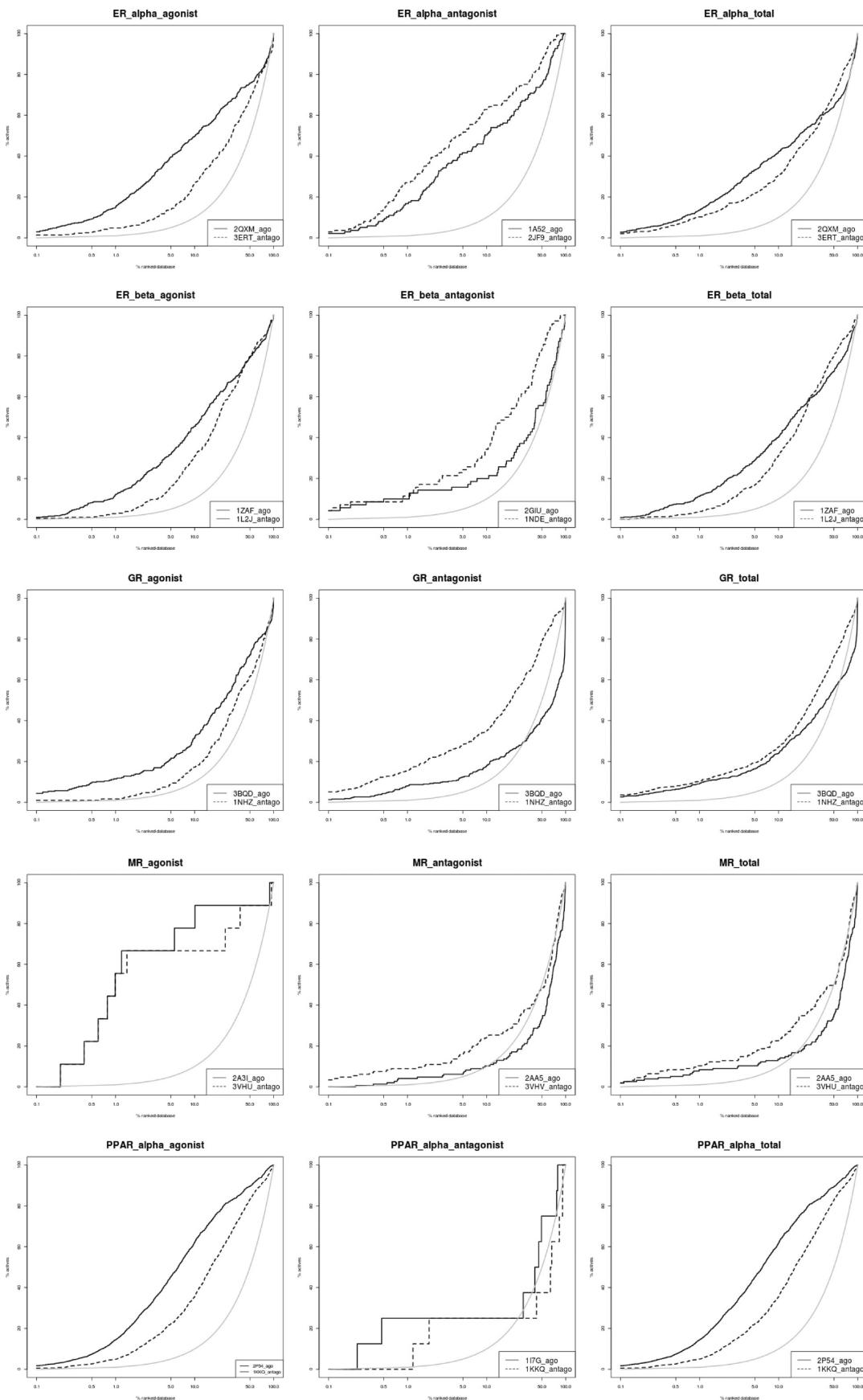
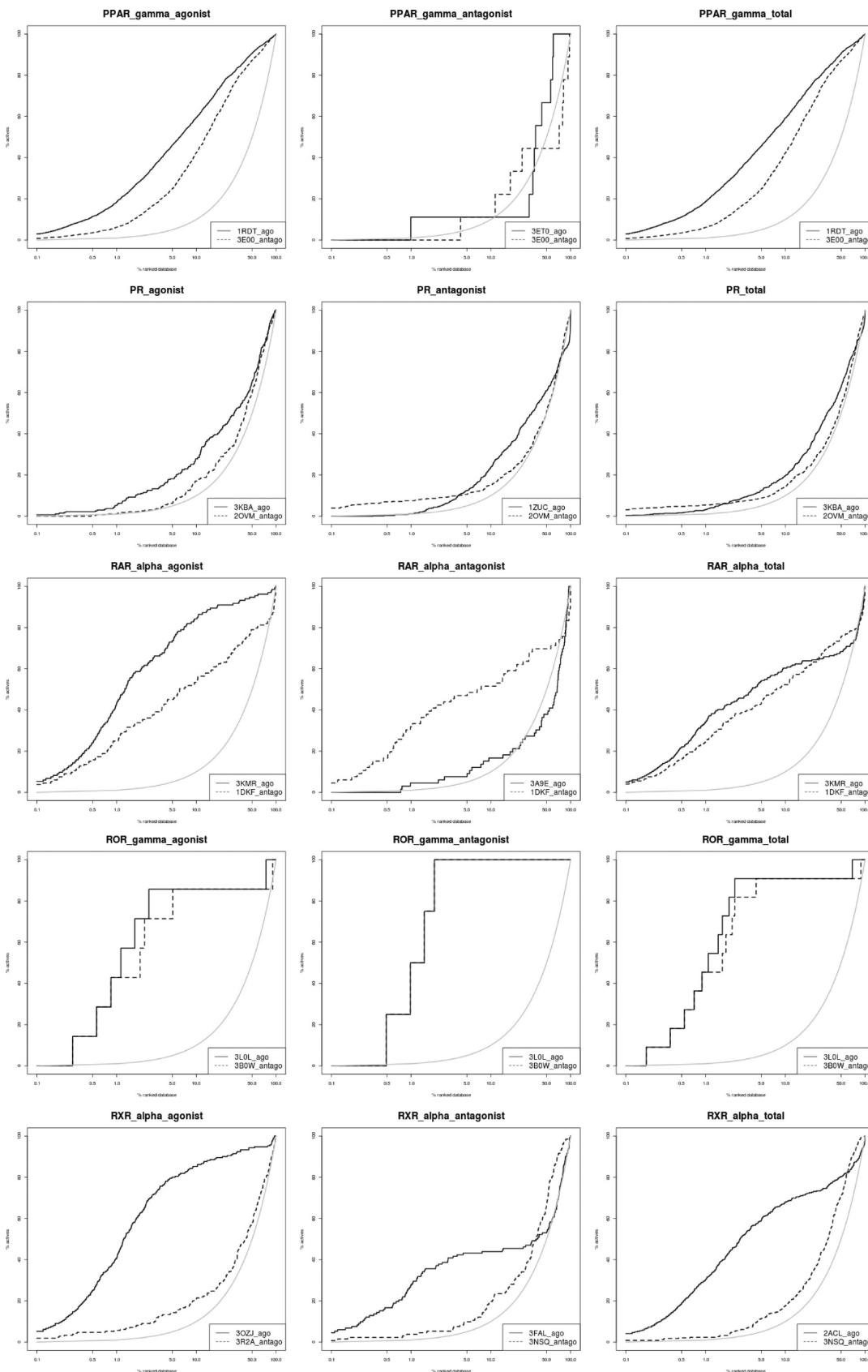


Figure 4. continued



**Figure 4.** Enrichment graphs obtained using Surfflex-Dock with the agonist data set, the antagonist data set, or the total data set of a given NR on the best agonist-bound structure (solid lines) and the best antagonist-bound structure (dashed lines) for the 10 NRs presenting at least one agonist-bound structure and one antagonist-bound structure.

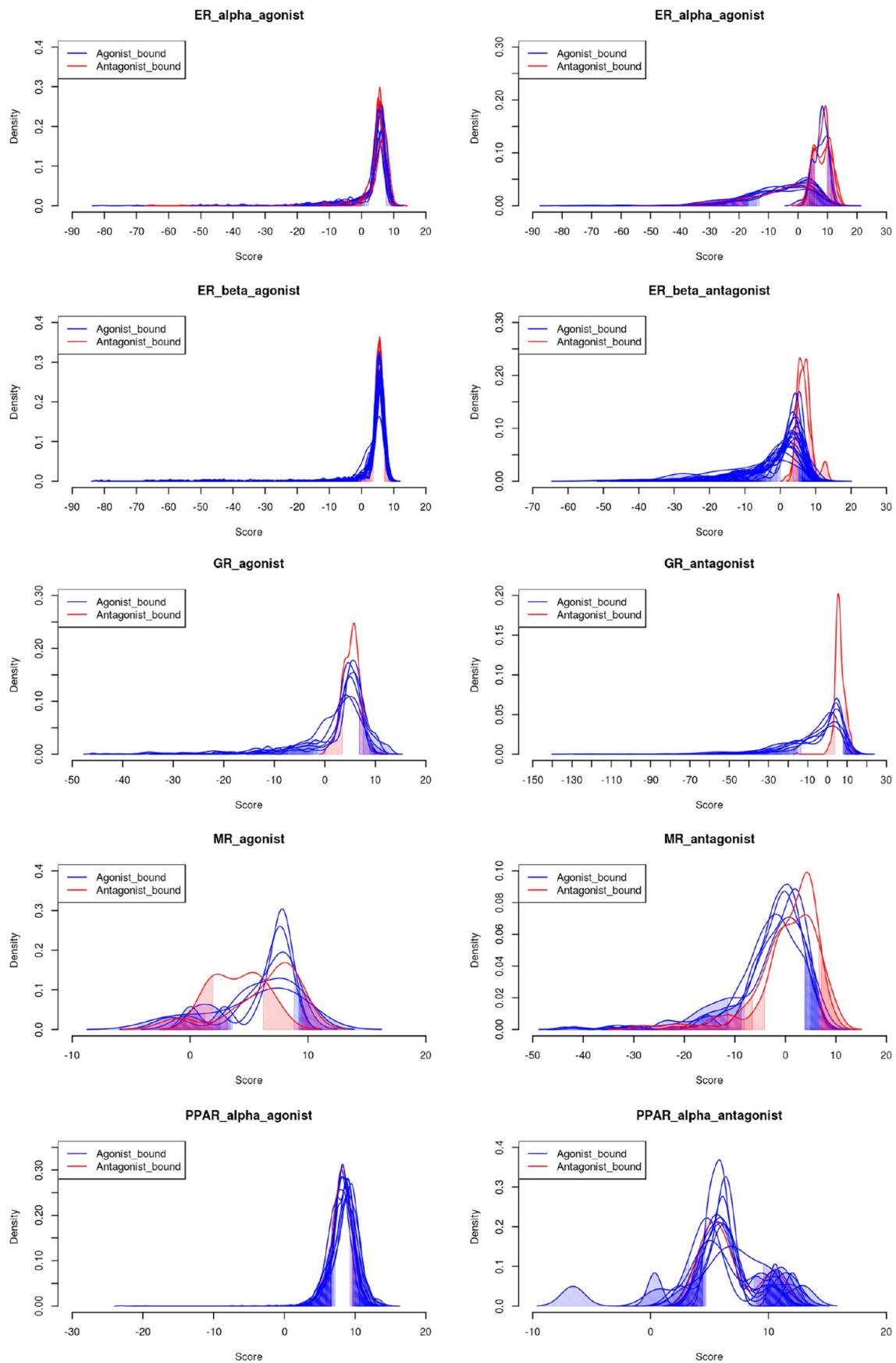
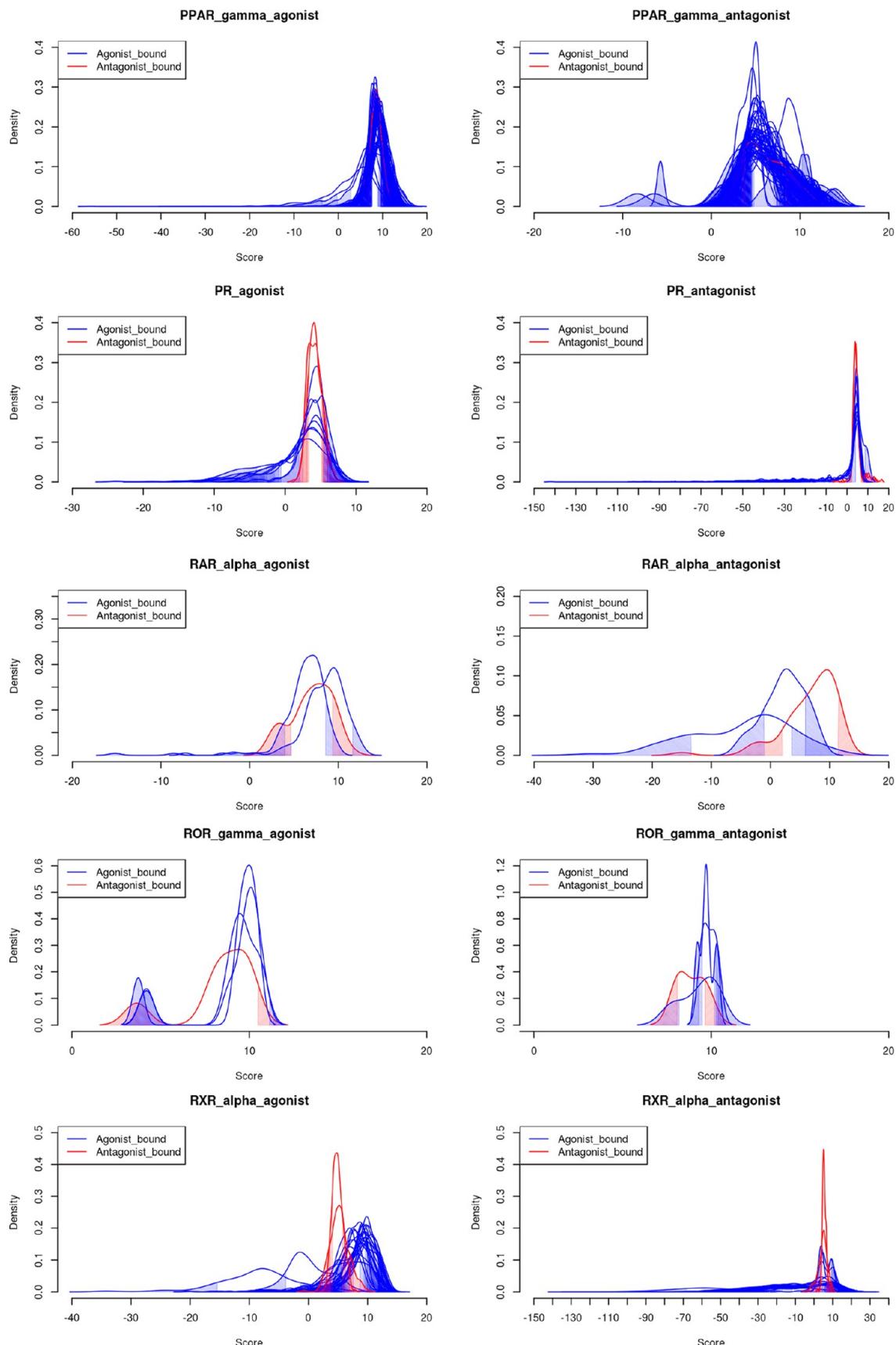
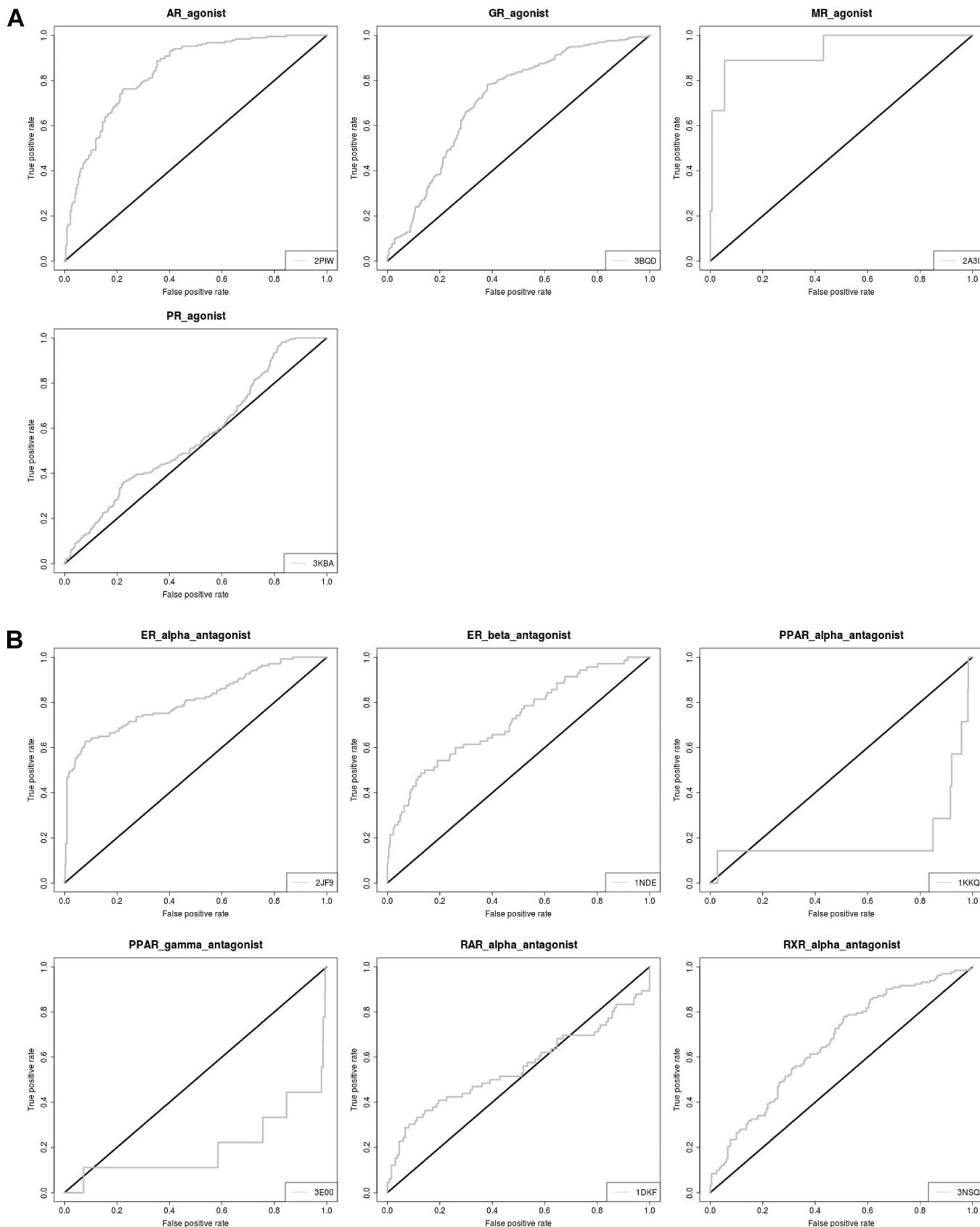


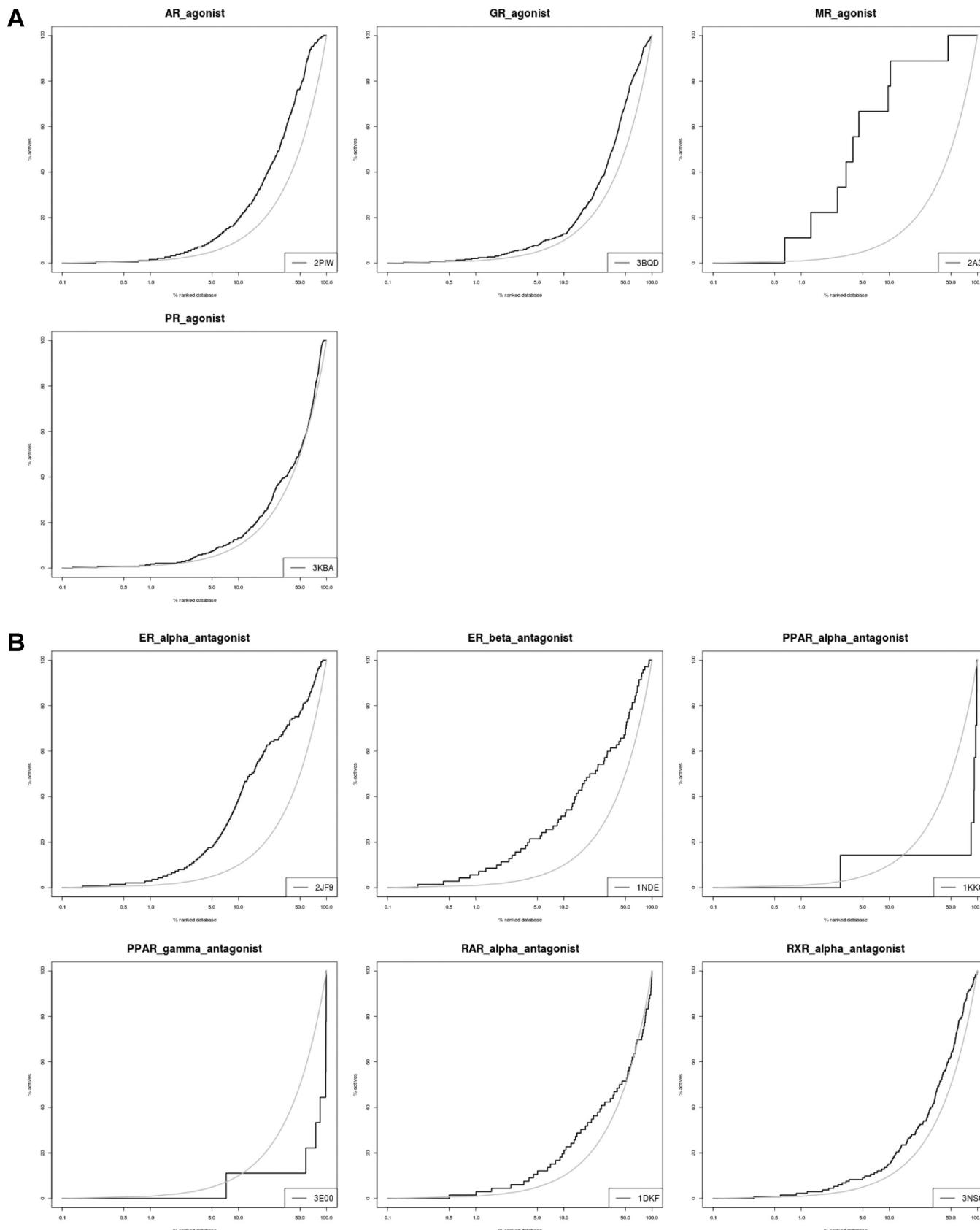
Figure 5. continued



**Figure 5.** Score distribution curves obtained using Surflex-Dock with the agonist data set and the antagonist data set on all agonist-bound structures (blue) and all antagonist-bound structures (red) for the 10 NRs presenting at least one agonist-bound structure and one antagonist-bound structure.



**Figure 6.** ROC curves obtained with (A) the agonist data sets of AR, GR, MR, and PR using the corresponding antagonist data sets as experimentally confirmed decoys and (B) the antagonist data sets of ER\_alpha, ER\_beta, PPAR\_alpha, PPAR\_gamma, RAR\_alpha, and RXR\_alpha using the corresponding agonist data sets as experimentally confirmed decoys.



**Figure 7.** Enrichment graphs obtained with (A) the agonist data sets of AR, GR, MR, and PR using the corresponding antagonist data sets as experimentally confirmed decoys and (B) the antagonist data sets of ER\_alpha, ER\_beta, PPAR\_alpha, PPAR\_gamma, RAR\_alpha, and RXR\_alpha using the corresponding agonist data sets as experimentally confirmed decoys.

**Table 2.** Areas under the ROC Curve (AUC) and Enrichment Factors (EF) at 1% and 10% Obtained with Surflex-Dock on (1) the Agonist-Bound Structures Previously Found To Be Associated with the Best Performances for AR, GR, MR, and PR Using the Agonist Data Sets as Active Compounds and the Antagonist Data Sets as Decoy Ligands and (2) the Antagonist-Bound Structures Previously Found To Be Associated with the Best Performances for ER\_alpha, ER\_beta, PPAR\_alpha, PPAR\_gamma, RAR\_alpha, and RXR\_alpha Using the Antagonist Data Sets as Actives and the Agonist Data Sets as Ligand Decoys (A/D Is the Ratio of the Number of Active Compounds to the Number of Decoy Ligands for a Given Data Set)

NR (structure)	agonist data set				antagonist data set			
	A/D	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>	A/D	AUC	EF <sub>1%</sub>	EF <sub>10%</sub>
AR (2PIW)	1/1.23	0.840	1.67	1.96				
ER_alpha (2JF9)					1/3.17	0.809	3.36	3.91
ER_beta (1NDE)					1/5.60	0.724	6.61	3.16
GR (3BQD)	1/1.25	0.716	2.25	1.29				
MR (2A3I)	1/16.22	0.937	17.22	8.04				
PPAR_alpha (1KKQ)					1/200.14	0.195	0.00	1.43
PPAR_gamma (3E00)					1/202.22	0.200	0.00	1.11
PR (3KBA)	1/1.96	0.564	1.69	1.35				
RAR_alpha (1DKF)					1/2.02	0.554	3.02	2.06
RXR_alpha (3NSQ)					1/11.83	0.664	2.59	1.52

These clear conformational differences may explain the diverse performances obtained using agonist- or antagonist-bound structures. It appears wise to use agonist-bound structures for the screening of agonist ligands and antagonist-bound structures for the screening of antagonist ligands, if available. In a previous study using only one receptor (ER), Liebeschuetz obtained similar results and conclusions.<sup>19</sup>

**Exploring Better Decoys.** The quality of a benchmarking database depends not only on the good selection of actives and structures: decoys play a central role in the evaluation process. Ideally, inactive compounds should be included, just as active compounds, on the basis of experimental data. Unfortunately, the compounds found to be experimentally inactive for a given target, often called “negative” data, are seldom documented or are insufficient to constitute benchmarking data sets. Thus, compounds presumed to be inactive for a given target, called decoys, have been selected on the basis of their nonsimilarity with known active compounds.<sup>20</sup> However, there is no evidence that some compounds selected as decoys are inactive, and the use of true inactive compounds instead of presumed decoys should enhance the quality of the evaluation. Using the NRLiSt BDB, we performed such a study by using antagonist compounds as decoy ligands for agonist activity and reciprocally. A compound that can be both an agonist and an antagonist is a modulator, and modulators were not included in the NRLiSt BDB.<sup>10</sup> Since we wanted to have at least as many decoy ligands as active ligands, we performed the analyses on the 10 targets of the NRLiSt BDB that presented a sufficient number of ligands in each data set and an appropriate structure. We found that despite the difficulty due to experimental decoys, the performances in enrichment obtained using Surflex-Dock were still acceptable for a majority of the selected NRs. It is worthy of note that the observed performance depended on the ratio of the number of active compounds to the number of decoy ligands, as highlighted in the literature for putative decoys.<sup>7,13</sup>

## CONCLUSION

In the present work, we used the NRLiSt BDB, a new benchmarking database dedicated to NRs, to study the impact of the choices regarding its construction on enrichment with a SBVLS method, Surflex-Dock. We found that distinguishing agonists from antagonists actually enhances the quality of the

evaluation since the best docking performances were obtained with the separate data sets rather than with the total data sets. Furthermore, the choice of the structure used for both docking method evaluation and virtual ligand screening studies is of high importance, and the pharmacological profile of the ligand bound in the binding site was found to be a critical parameter. We finally have shown that the NRLiSt BDB active compound data set for a given target/pharmacological activity can be used as a set of decoy ligands for the other pharmacological activity to ensure a reliable and challenging evaluation of virtual screening methods. On the basis of this work, we have shown that (1) the NRLiSt BDB constitutes a high-quality and reliable benchmarking data set that can be used for the evaluation of virtual screening methods, (2) the rationale used to construct databases such as the NRLiSt BDB could become a reference for developing better benchmarking data sets, and (3) the use of active ligand data sets for a given target and for a given pharmacological activity as experimentally validated decoy ligand data sets for the same target but for another pharmacological activity could ensure a more robust and challenging evaluation.

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### Notes

The authors declare no competing financial interest.

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