

Supplementary information for

“Activation of RXR/PPAR heterodimers by organotin environmental endocrine disruptors”

Albane le Maire, Marina Grimaldi, Dominique Roecklin, Sonia Dagnino, Valérie Vivat-Hannah, Patrick Balaguer and William Bourguet

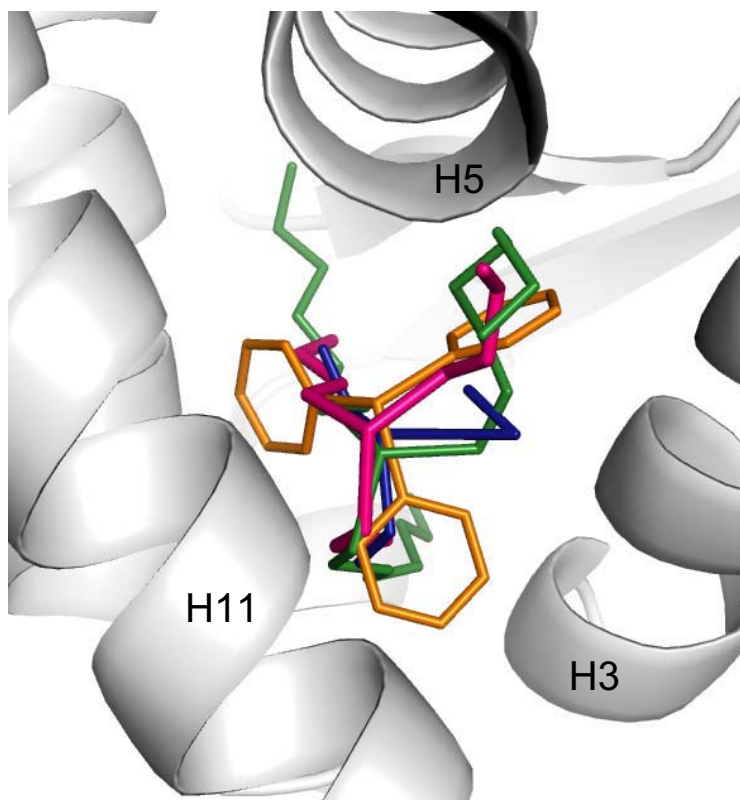


Fig S1 Comparison of the interaction between RXR α LBD and organotin. Superposition of TBT-A (pink), TPT (triphenyltin, orange), TET (triethyltin, blue) and TOT (trioctyltin, green) in stick representation in the TBT binding pocket shown in white.

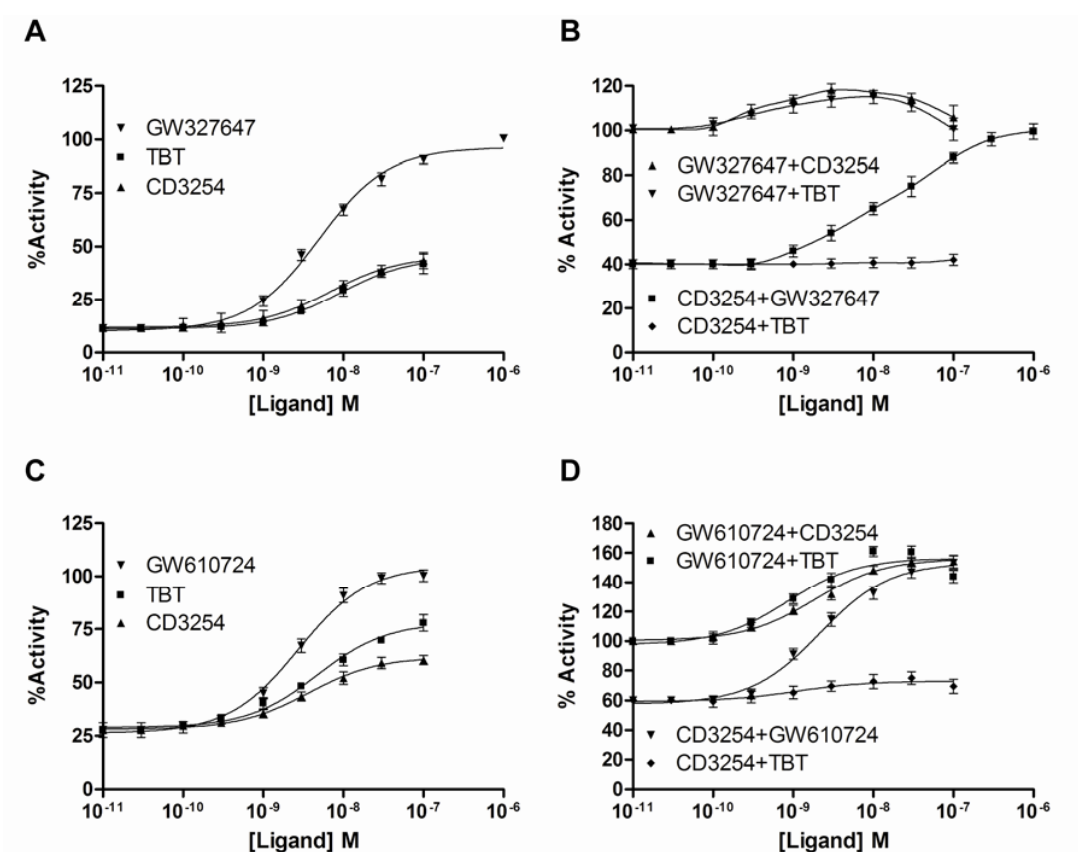


Fig S2 TBT activates RXR/PPAR α and RXR/PPAR δ heterodimers. **(A)** Stably transfected HELN PPAR α cells were incubated with increasing concentrations of GW327647, CD3254 or TBT to measure their effect on the transcriptional activity of the heterodimer formed by Gal4-PPAR α and endogenous RXR. **(B)** To measure the specific effect of the organotin on PPAR α and RXR, GW327647, CD3254 or TBT were also tested in the presence of saturating concentrations of RXR (CD3254; 0.1 μ M) or PPAR α (GW327647; 1 μ M) specific ligands. 100% activity corresponds to the activity obtained with 1 μ M GW327647. **(C)** and **(D)** Same experiments with HELN PPAR δ cells and GW610724 as the PPAR δ specific agonist. To measure the specific effect of the organotin on PPAR δ and RXR, GW610724, CD3254 or TBT were tested in the presence of saturating concentrations of RXR (CD3254; 0.1 μ M) or PPAR δ (GW610724; 0.1 μ M) specific ligands.

Table S1 Data collection and refinement statistics

Data collection	
Space group	P43212
Cell dimensions a, b, c (Å)	64.03, 64.03, 111.88
Resolution range (Å)	30.77-1.90 (2.00-1.90) *
R _{sym}	0.062 (0.455) *
I/σI	25.9 (4.3) *
Completeness, %	98.0 (97.2) *
Redundancy	8.8 (9.2) *
Refinement	
Resolution range (Å)	28.63-1.90
Reflections used in refinement	17, 579
R (%) /R _{free} (%)	19.0 / 22.5
Total number of atoms	1,960
No. protein atoms	1,676
No. ligand atoms	26
No. water molecules	166
Average B-factor (Å ²)	25.2
Protein B-factor (Å ²)	24.2
Ligand B-factor (Å ²)	21.6
Water B-factor (Å ²)	33.2
Rmsd from ideality	
Bond lengths (Å)	0.011
Bond angles (°)	1.206
Ramachandran plot	
Most-favored regions (%)	95.4
Additionally allowed regions (%)	4.1
Generously allowed regions (%)	0.5
Disallowed regions (%)	0.0

*Values in parentheses are for highest resolution shell.

Table S2 Comparison of the binding pocket properties in RXR α -agonists (9cis-RA and CD3254) and RXR α -organotins (TBT, TPT, TET and TOT) complexes and comparison of the agonist ligands and organotin compounds ability to bind and activate RXR α . Ligand volumes were calculated using Vega ZZ (Pedretti et al., 2004). Organotins were manually docked in the TBT binding pocket and the complexes were energy-minimized using REFMAC5 from the CCP4 suite (CCP4, 1994). Protein ligand contacts (cutoff 4.2Å) were evaluated using CONTACT (CCP4, 1994).

Ligand	Ligand volume (Å ³)	Total contact numbers	Type of contacts (covalent/vdw /polar)	Activation and Binding	
				EC ₅₀ of GAL-RXR activity (nM)	IC ₅₀ for competition with [³ H]9cis-RA (μM)
TBT ^a	233	49	1 / 49 / 0	14.4 ^e	0.468 ^e
9cis-RA ^b	319	74	0 / 69 / 5	10.2 ^e	0.107 ^e
CD3254 ^c	352	107	0 / 100 / 7	10.0 ^f	-
TPT ^d	245	82	1 / 81 / 0	13.9 ^e	0.527 ^e
TET ^d	131	21	(1) / 20 / 0	not detectable ^e	6.76 ^e
TOT ^d	436	103	(1) / 102 / 0	not detectable ^e	>10 ^e

^a The RXR α -TBT complex is described in this study.

^b PDB code 1FBY (Egea et al., 2000).

^c The structure of RXR α -CD3254 has been solved but not yet published (our results).

^d These complexes were modeled from the structure of RXR α -TBT complex. Number and type of contacts are in italics to emphasize that they are only estimations.

^e Nakanishi et al., 2005

^f Nahoum et al., 2007

Supplementary METHODS

Preparation of stable PPAR cell lines. HGELN hPPAR α , hPPAR δ and hPPAR γ cell lines were generated in two steps. Firstly, we generated the HGELN cell line by transfecting HeLa cells with the pGAL4RE-ERE- β Glob-Luc-SVNeo plasmid. Secondly, we transfected HGELN cells with pGAL4-PPAR-puro plasmids. pGAL4RE-ERE- β Glob-Luc-SVNeo contains a luciferase gene driven by a yeast activator GAL4 and an estrogen receptor binding site in front of the β -globin promoter and a neomycin phosphotransferase gene under the control of the SV40 promoter. In pGAL4-PPAR-puro (Seimandi et al, 2005), the encoded chimeric GAL4-PPAR proteins consists of 1-147 GAL4 amino acids followed by the human PPAR LBDs. (Puromycin N-acetyl transferase selection marker expression confers resistance to puromycin.

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