

Cellular, molecular and structural views of Endocrine Disruptor Compounds / Nuclear Receptor interactions

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Hormonal Signalling and Cancer

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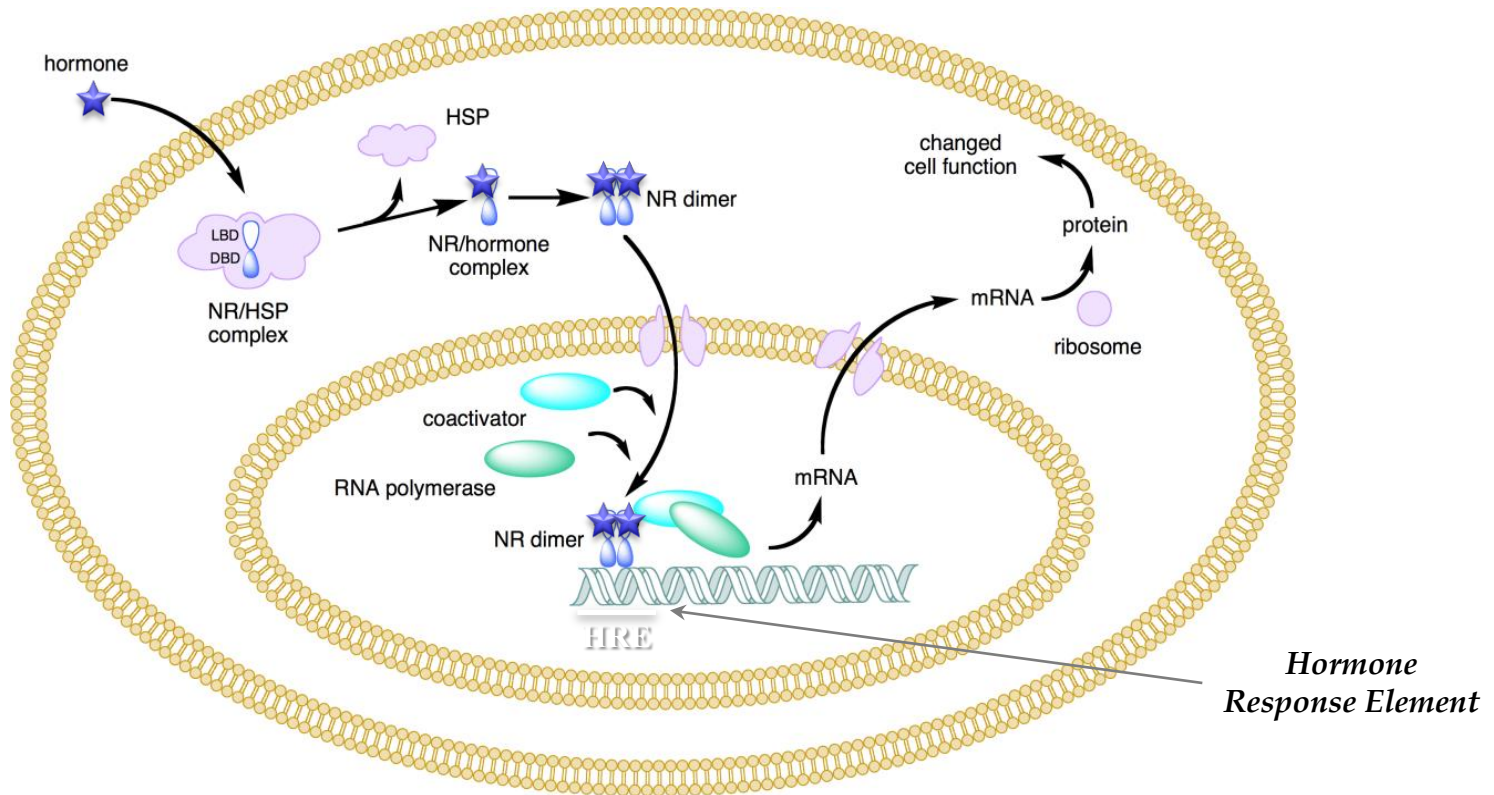
INSERM U896 - UM1 - CRLC Val d'Aurelle

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• Nuclear hormone receptors – Biological function

- ⇒ NHRs are ligand-dependent transcription factors: ligand binding induces conformational changes and allows the recruitment of transcriptional co-regulators in the promoter region.



- ⇒ Misregulation of the endocrine system can cause diseases such as cancers, infertility, diabetes, obesity, ...

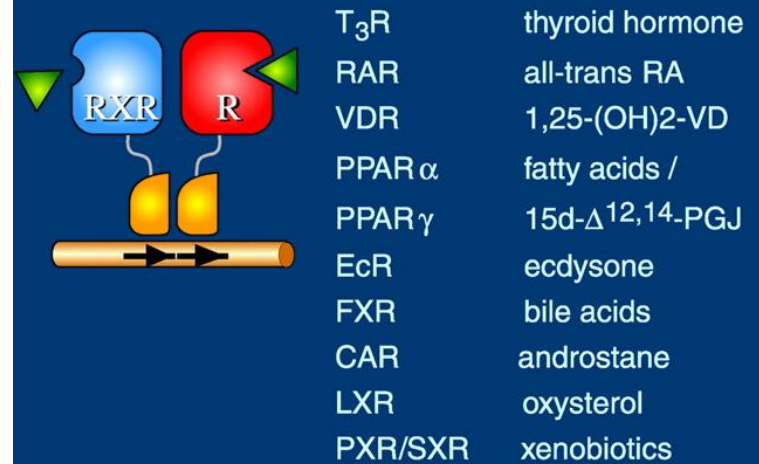
• Nuclear hormone receptors - Classification

⇒ 48 human NHRs: different ligands and different functions
differential distribution in tissues

Steroid Receptors



RXR Heterodimers



Dimeric Orphan Receptors



Monomeric / Tethered Orphan Receptors

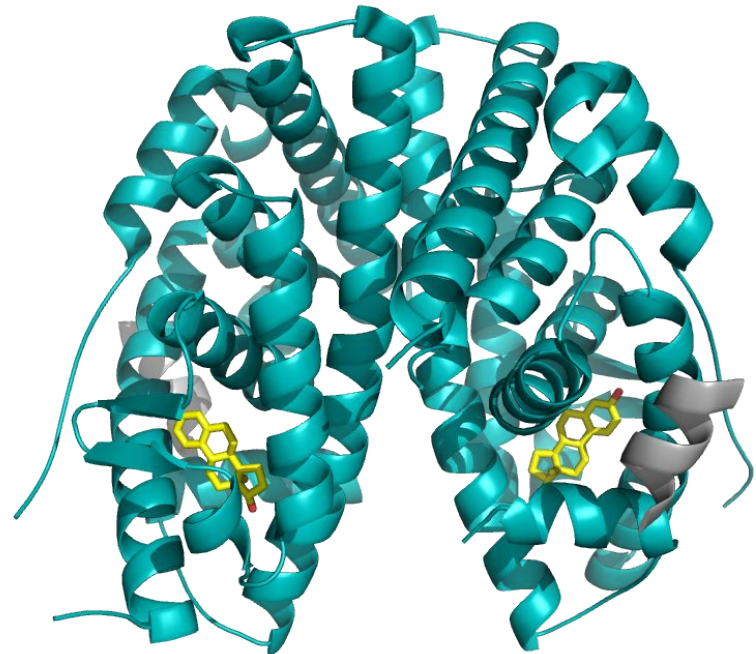
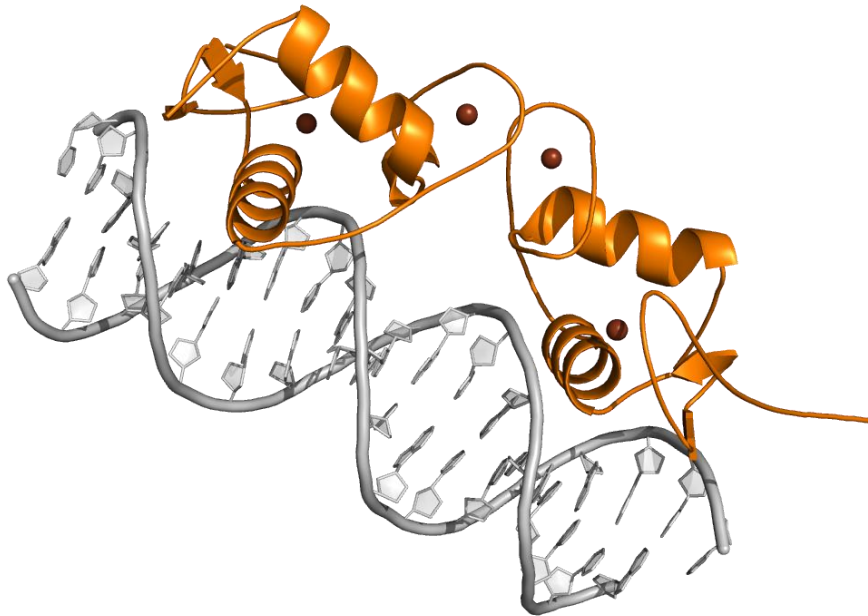


• Nuclear hormone receptors - A conserved structure

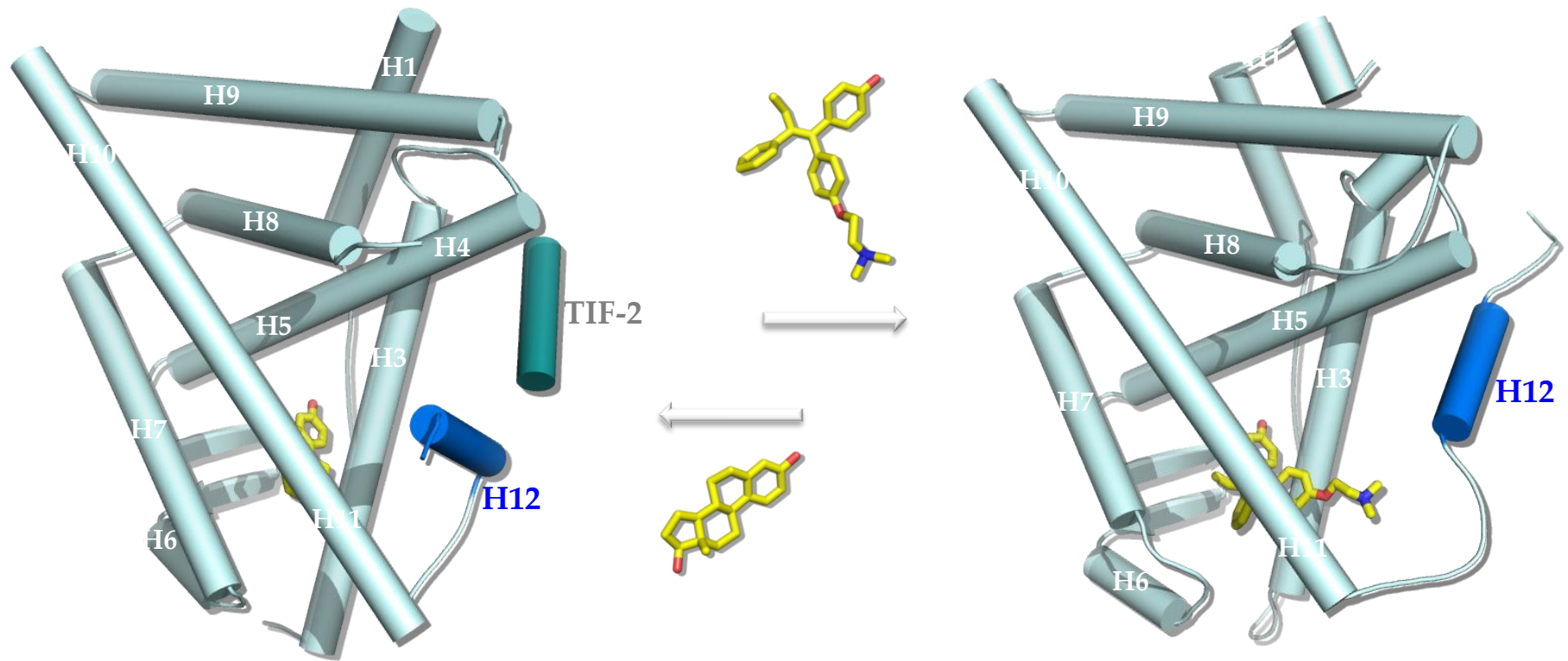


Activation function (AF-1)

Activation function (AF-2)



• Nuclear hormone receptors - Structure of the LBD

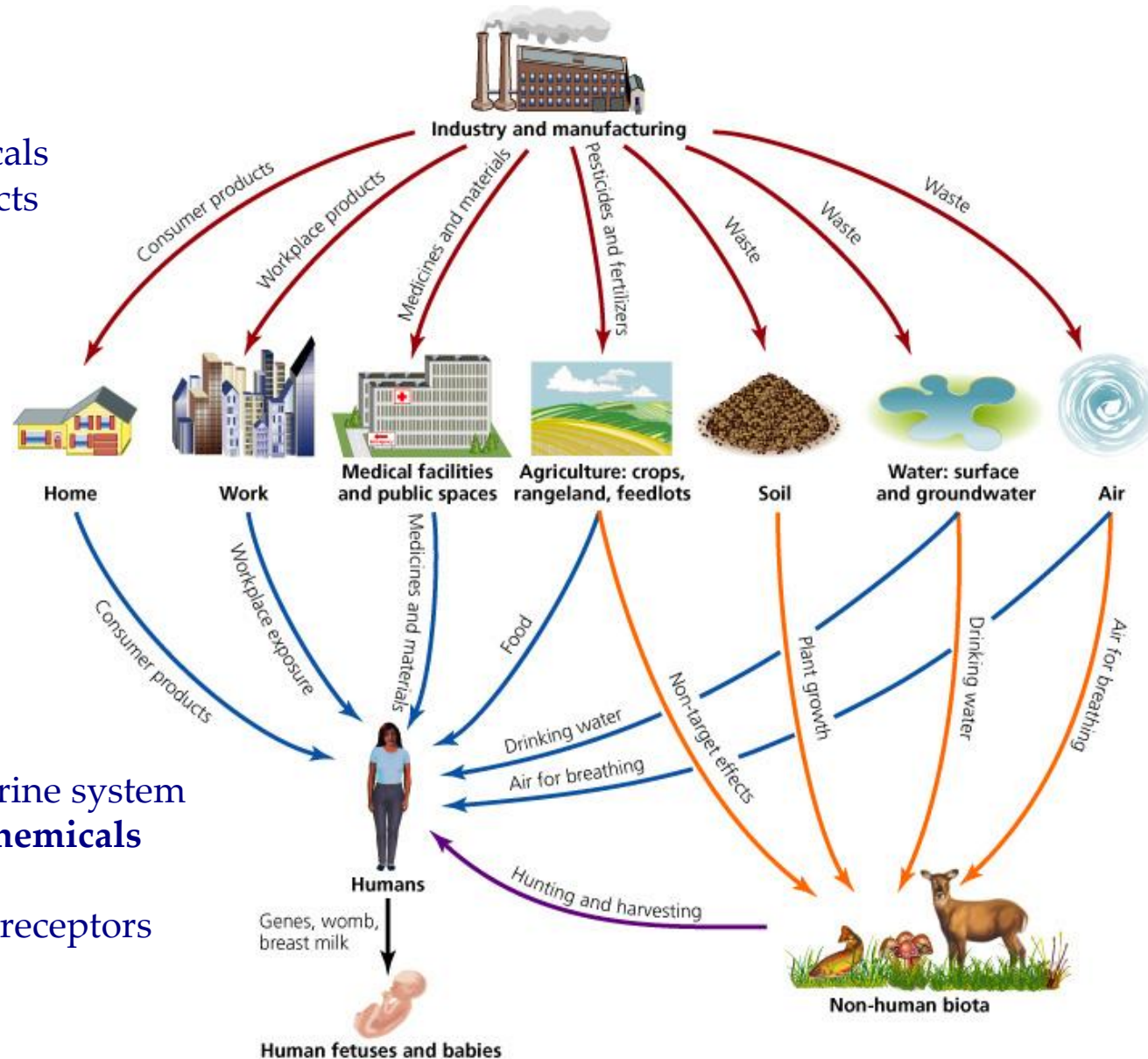


The hydrophobic docking groove formed by helices H3, H4 and H11 accommodates the LxxLL motif exhibited by the coactivator or the H12 helix.

• A number of environmental contaminants act as EDCs

≈150,000 synthetic chemicals
used in consumer products

Dissemination into
the environment



Adverse effects on the endocrine system

➤ **endocrine disrupting chemicals (EDCs)**

➤ interaction with nuclear receptors

Representative EDCs and their NHR targets

Model compounds

Bisphenols

A (BPA), B, C, E, F

- BPA: highest volume produced

- Adipogenesis

Concerns



- Surfactants

- Frequent contaminants of

ma



- Phthalates

- Adipogenesis



- Pesticide prohibited for over 10

- High levels are still detectable

- Associated with

- Fl

- TBBPA

- Obese

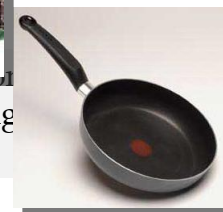


- Manufacture of fluoropolymers (used in cookware, weather

- Increased c



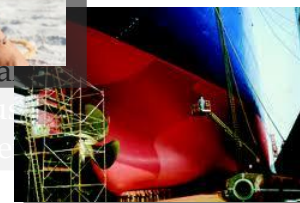
- UV



- Biocides in many industrial

- Imposed, obesogens (su

- Induction of adipoge



NHRs (known or suspected)

ERs, ERR
AR

ERs, ERR γ
AR

ERs, PPARs
AR

ER α
ER β

PPAR γ

PPARs, ERs
AR

ERs
AR

PPARs
RXRs

Alkylphenols

4-tert-octylphenol, 4n-nonylphenol

Phthalates

MEHP, DHEP, BBP, DBP

Chlordecone

(Kepone)

Halogenated BPA

TetraBromo/Chloro-BPA (TBBPA, TCBPA)

Perfluoroalkyl compounds

(PFCs; PFOA et PFOS)

Benzophenones

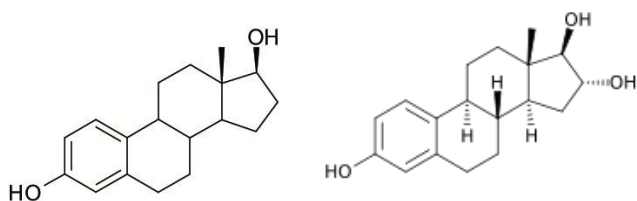
1, 2, BP3, THB

Organotins

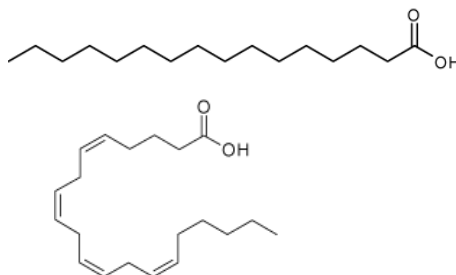
*TriButylTin (TBT), TriPropylTin (TPT),
TriPhenylTin (TphT), DiButylTin (DBT)*

• EDCs and natural hormones are structurally unrelated

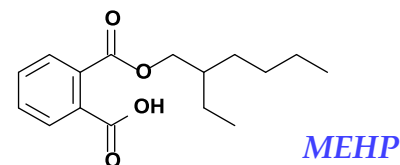
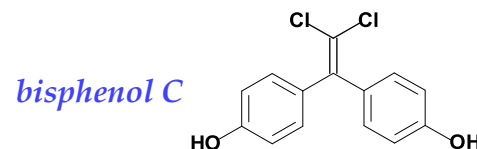
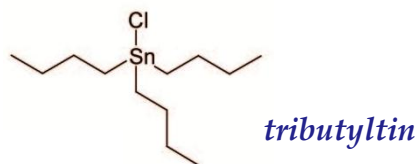
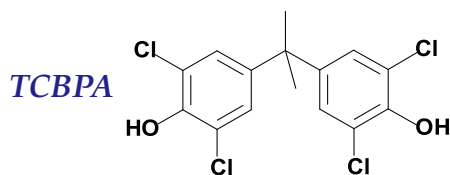
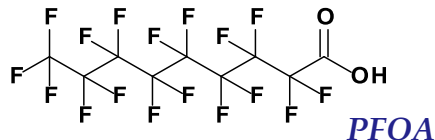
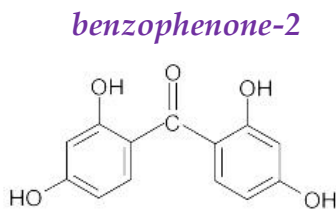
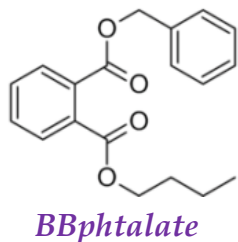
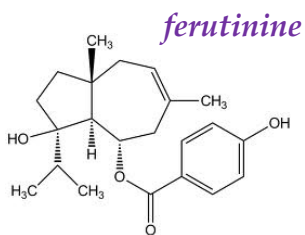
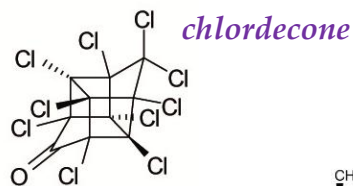
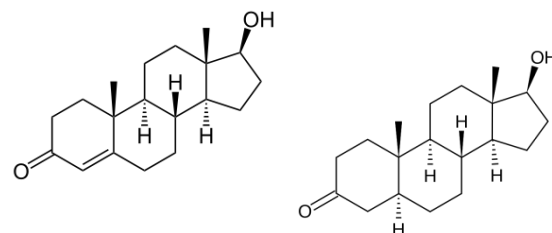
Estrogen Receptor



Peroxisome Proliferator Activated Receptor



Androgen Receptor



Methods to monitor EDCs in environmental samples

In vitro methods

Binding to nuclear receptor (radioactive or fluorescent detection)

Interaction of coactivator peptide (agonists) or corepressor peptide (antagonists) to nuclear receptor (fluorescent detection)

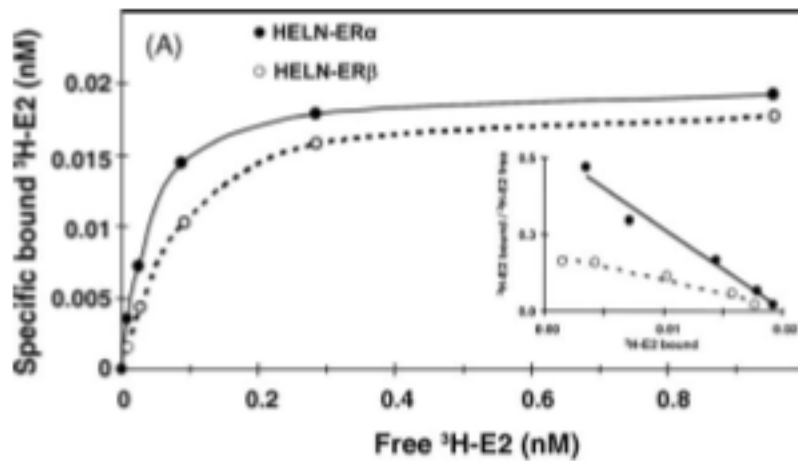
Cellular *methods*

Endogenous genes (pS2, vitellogenin, EROD) detection or cell growth measurement (E and A-Screen)

Reporter gene expression (β -galactosidase, luciferases)

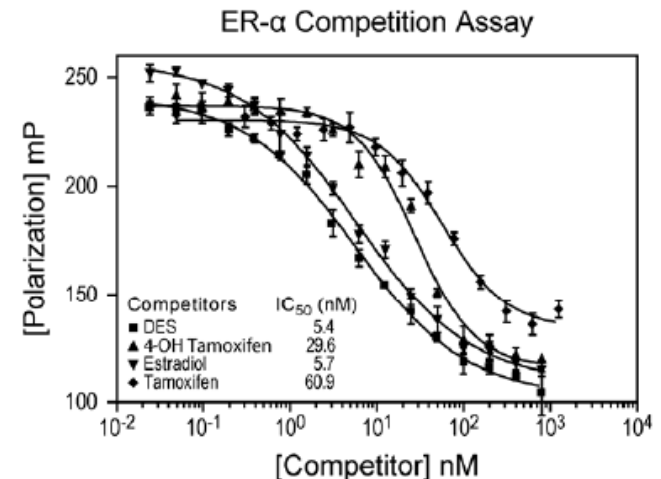
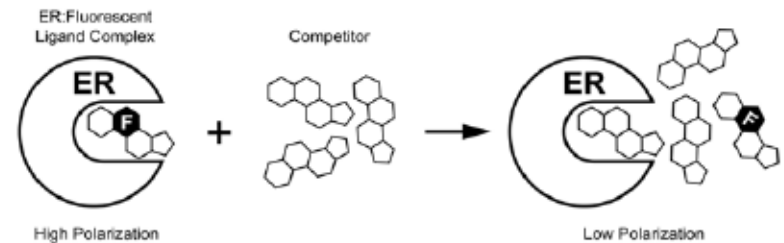
Radioactive ligand binding

- Recombinant ER α
- Radioactive E2 (perkin elmer)
- Non radioactive E2 and ligand
- Measurement of an relative affinity (compared to E2)



Fluorescent ligand binding

- Polarscreen
ER α , AR, GR, PR, PPAR γ



Gene reporter technology

Vit (ERE) promoter

Structural Vitellogenin gene



Natural protein

Agonists

ER α

Antagonists

Vit (ERE) promoter

Structural reporter gene



Reporter protein

ERE-TATA

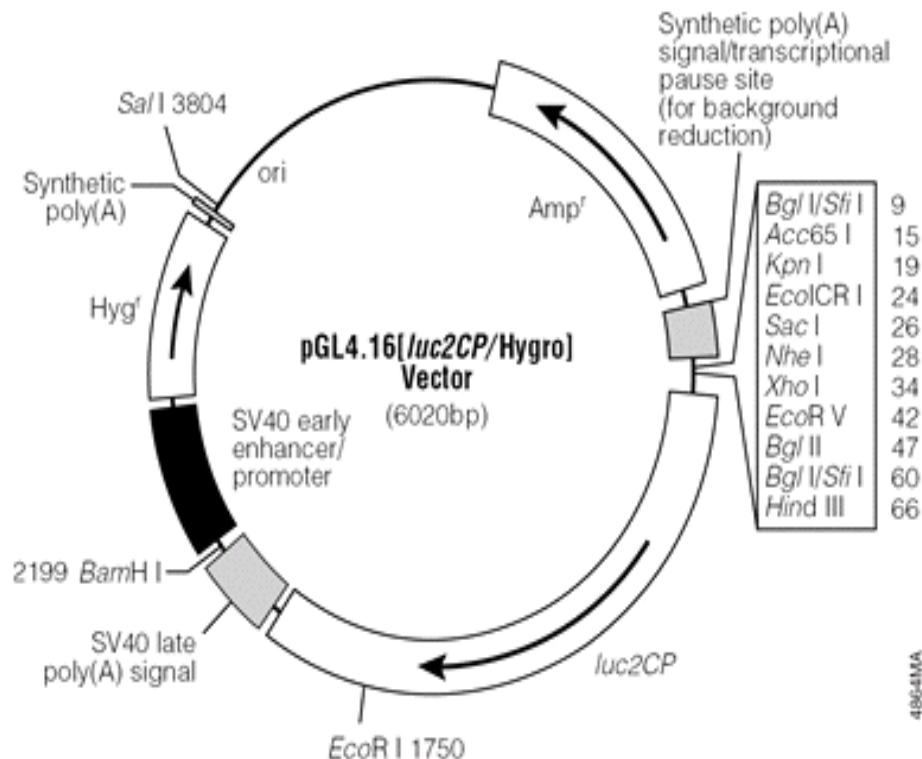
Structural reporter gene



Reporter protein

Luciferase plasmid construction

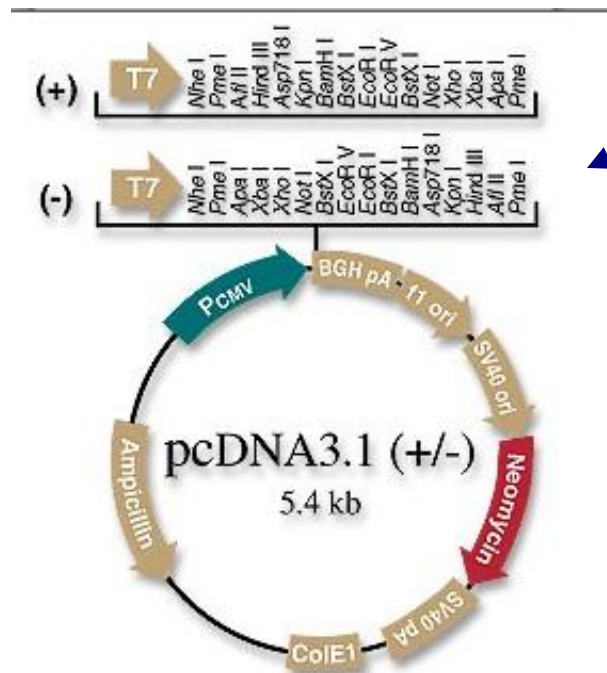
- Insert the promoteur or the ERE in front of the luciferase gene in a luciferase plasmid



AGGTCAnnnTGACCT

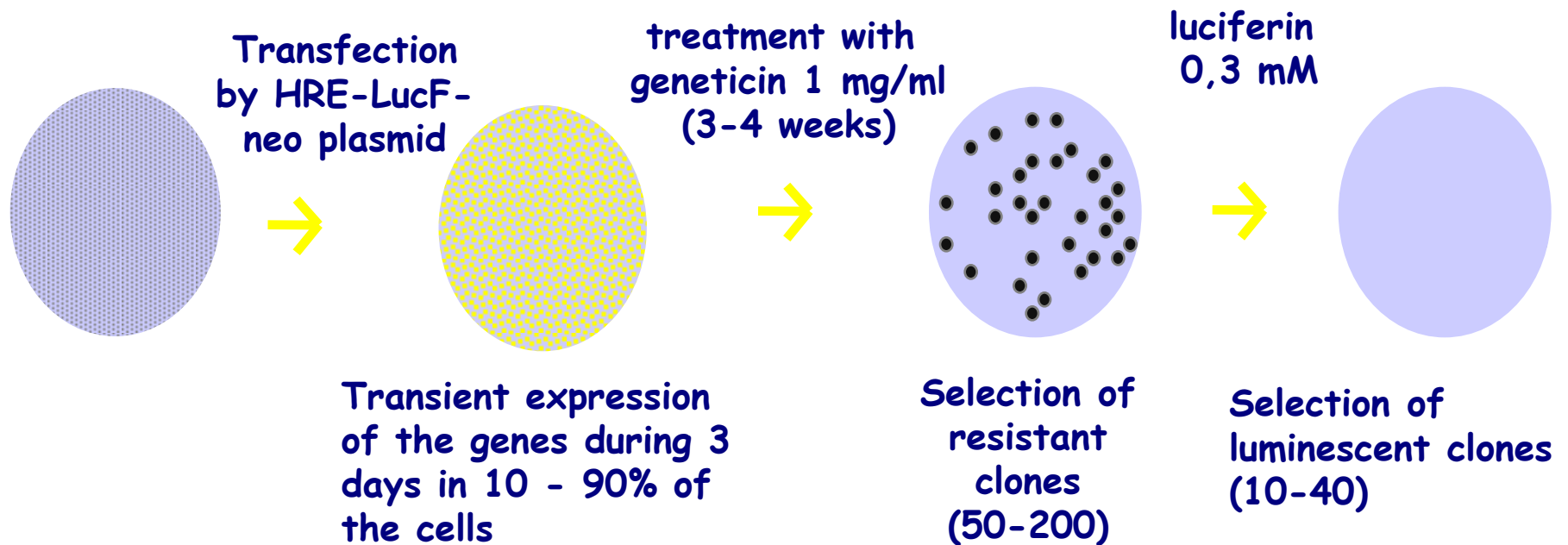
Nuclear receptor plasmid construction

- insert the coding sequence of the receptor behind a strong promoter in a expression plasmid

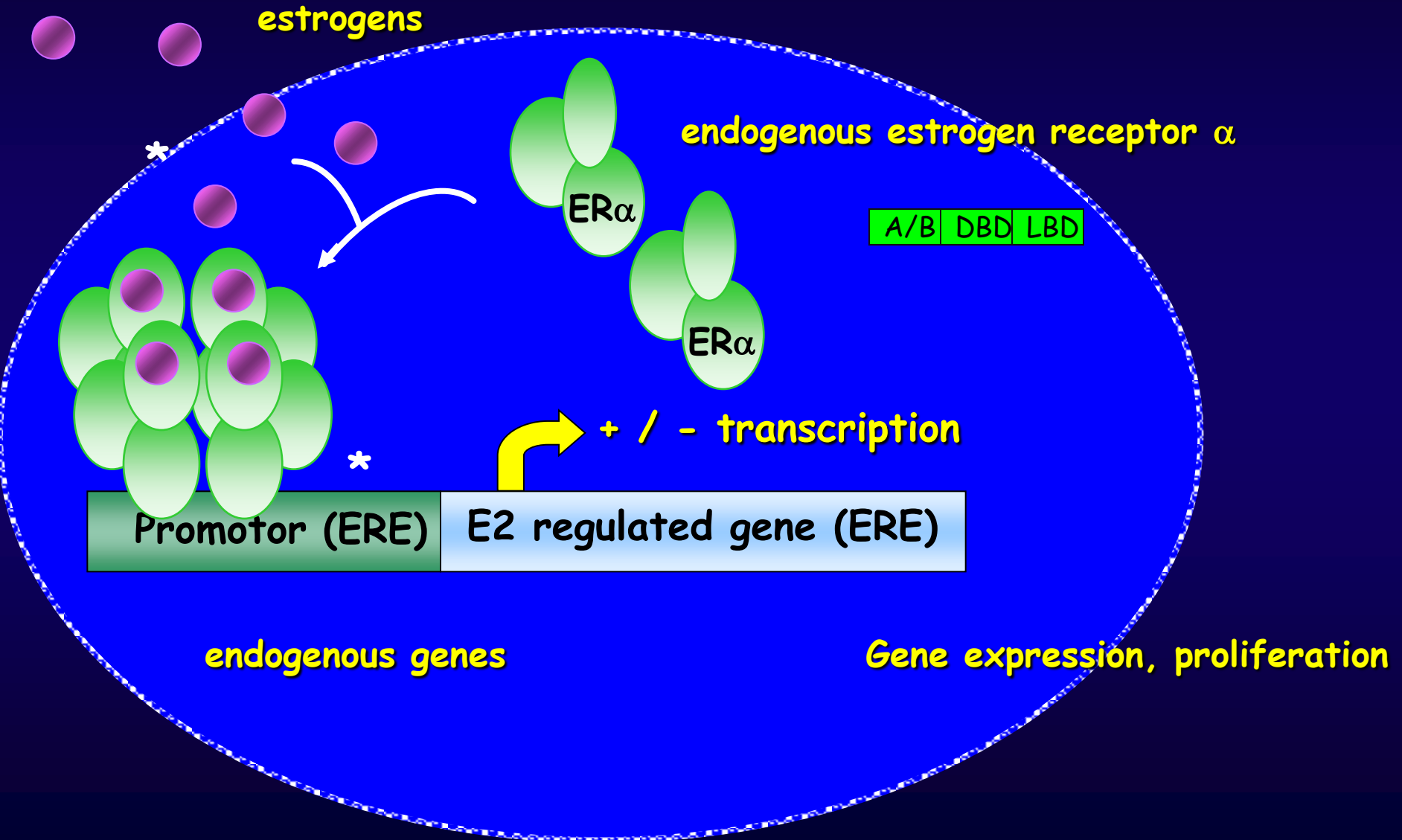


nuclear receptor sequence
(1500 à 3000 nucleotides)
obtained by gene synthesis
(Millegen ou MWG ; 0,35€/base)

Stable transfection procedure



MCF-7 cell line



MELN cell line

MCF-7

estrogens

endogenous estrogen receptor α

ER α

ER α

A/B DBD LBD

+ / - transcription

ERE

β globine

LucF

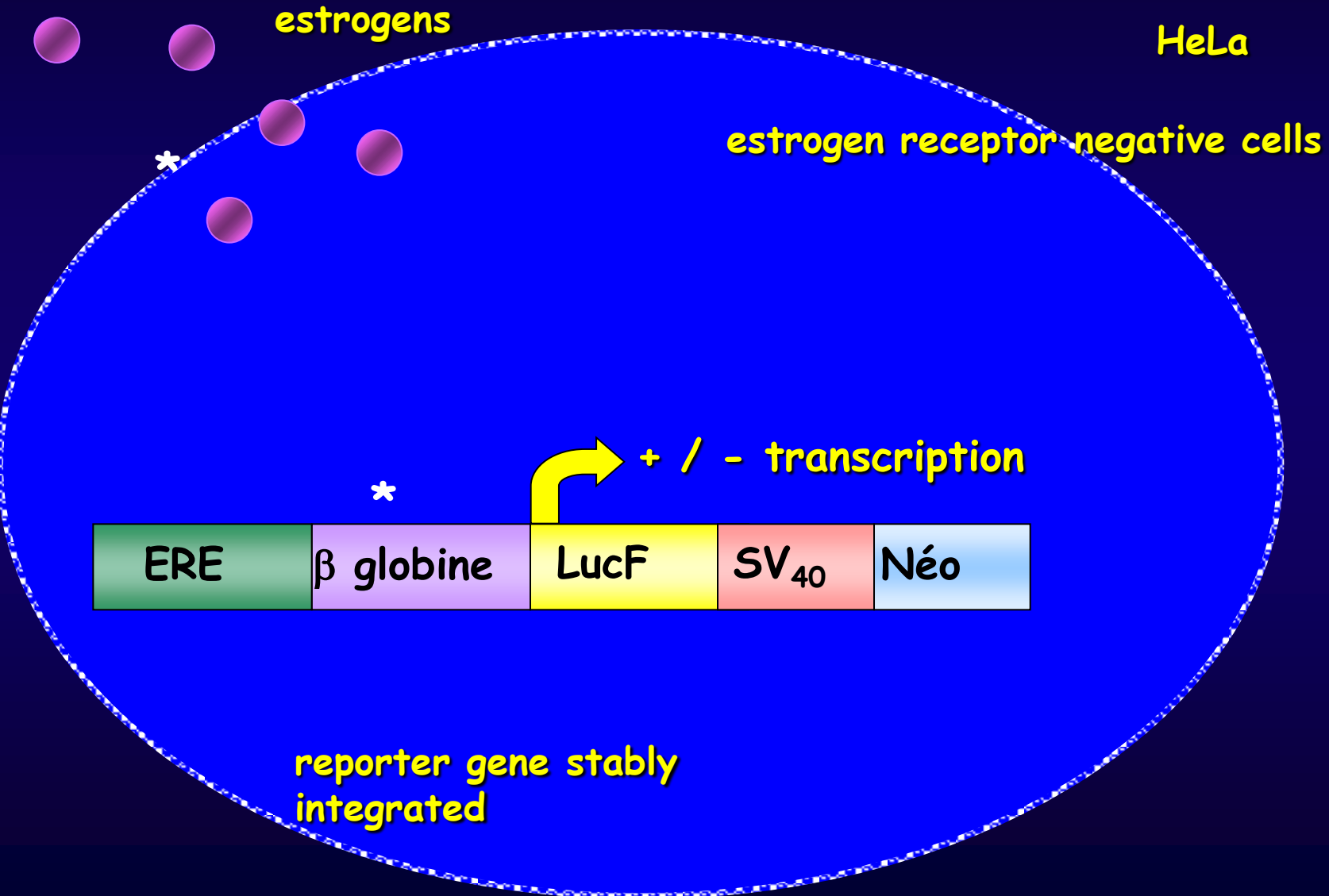
SV₄₀

Néo

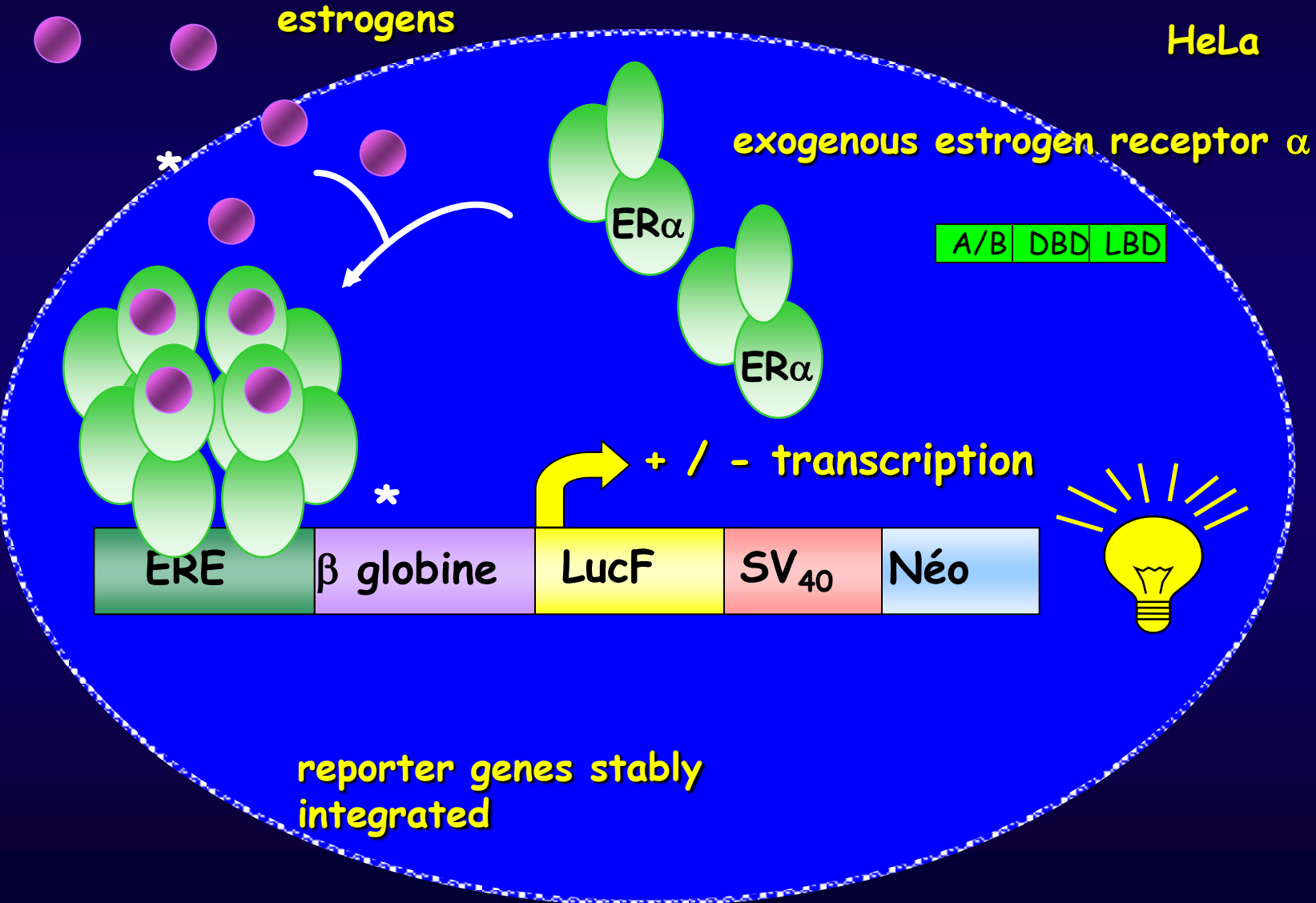
luciferase gene stably integrated



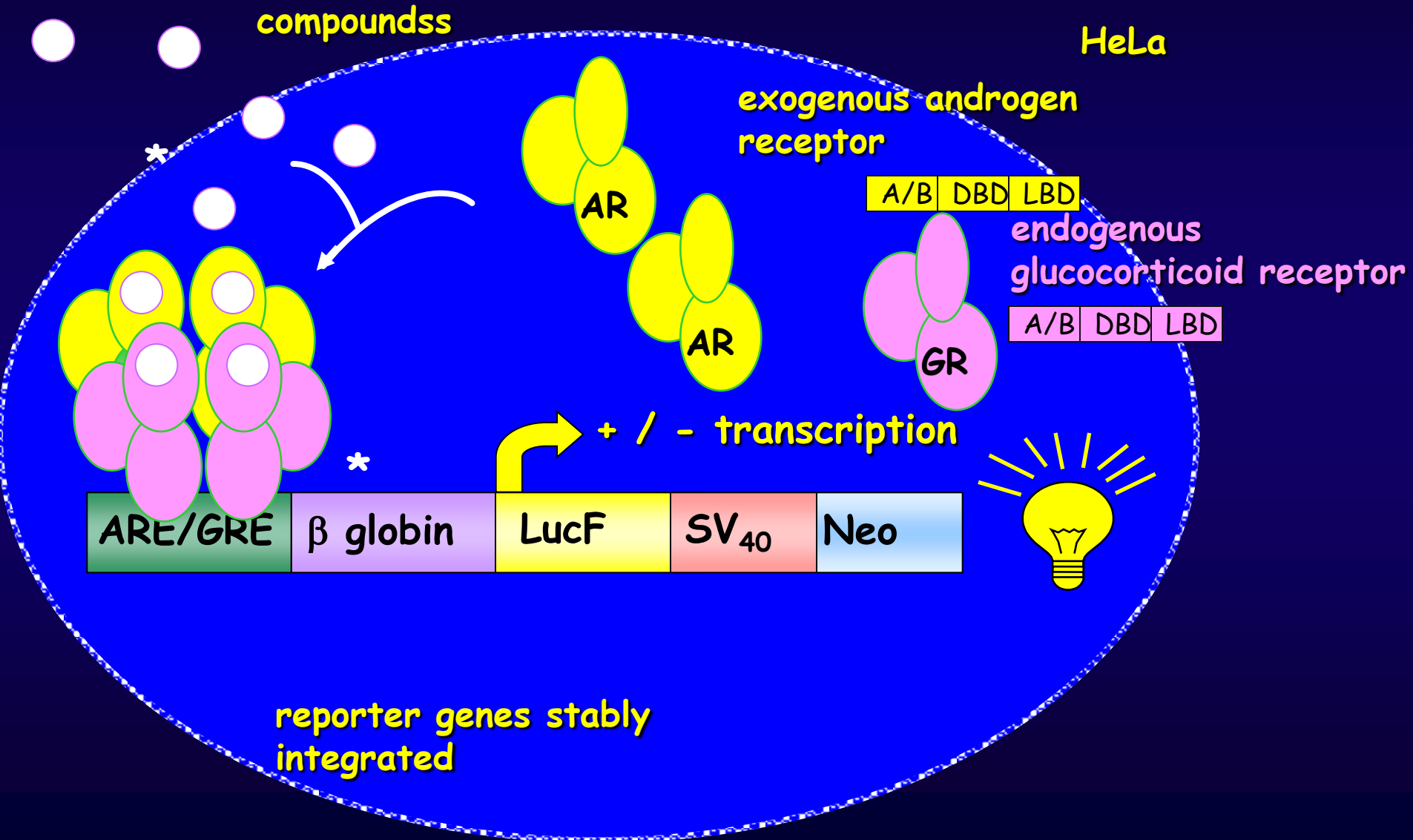
HELN ER α (or ER β) cell lines (1)



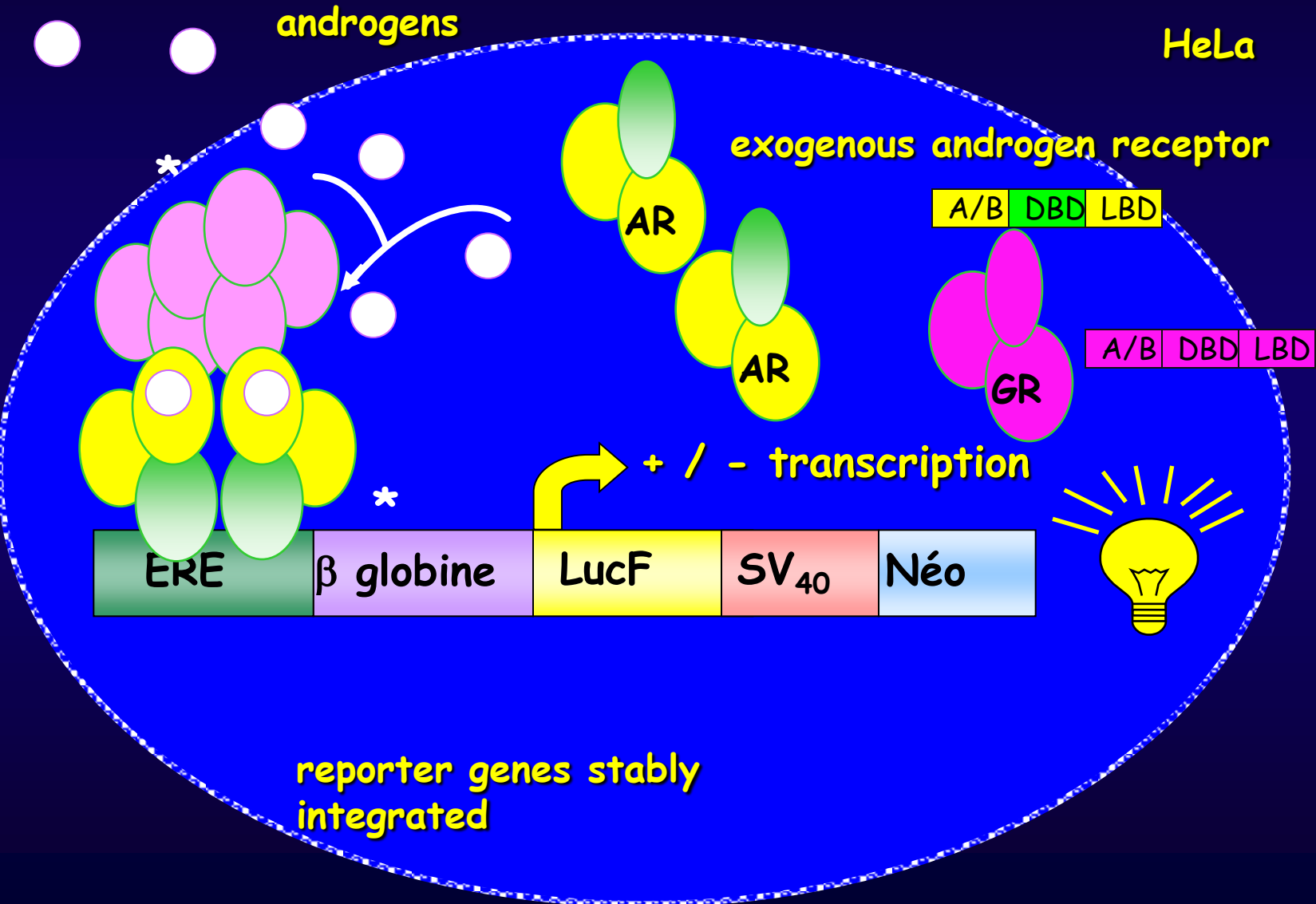
HELN Er α or ER β cell lines (2)



Androgenic cell lines (PALM, MDA-Kb2)



HELN AR (ER α DBD) cell line

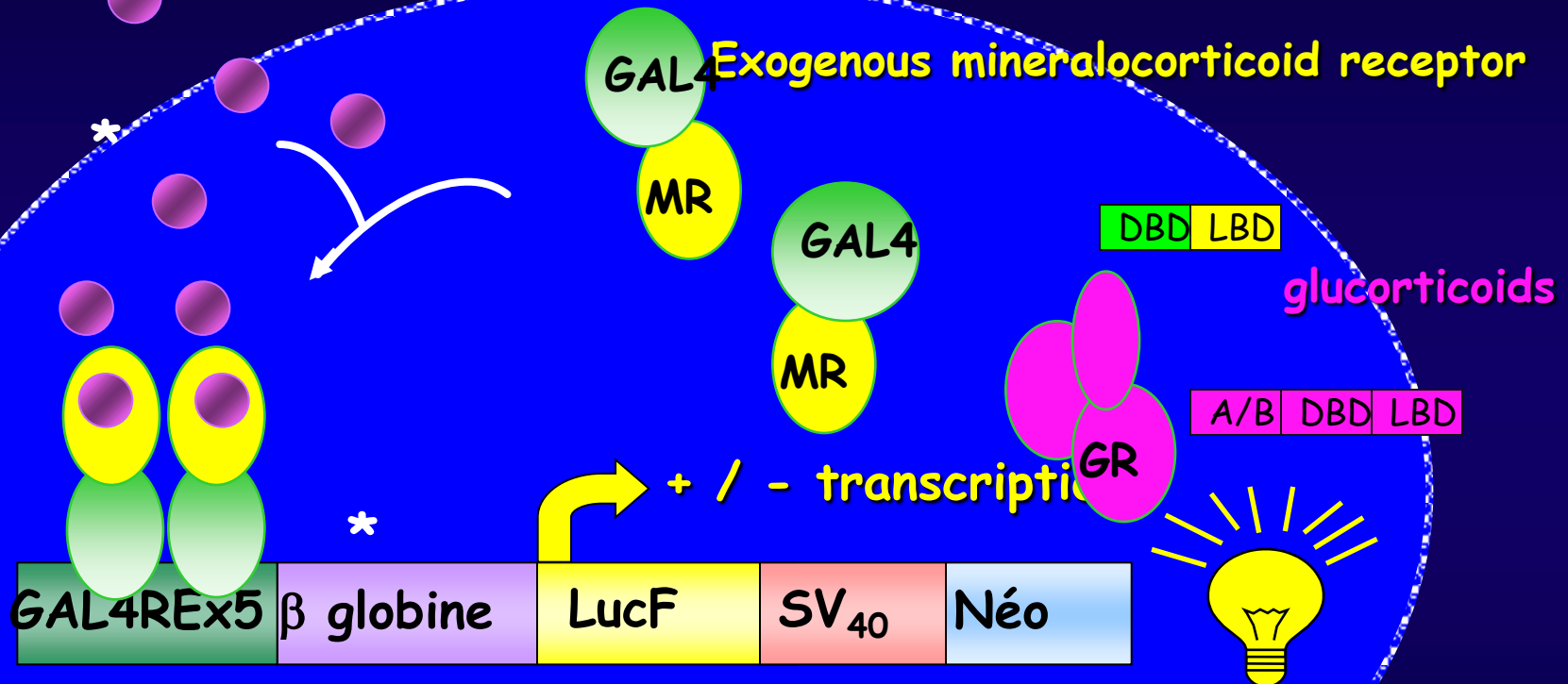


HG5LN GAL4(DBD)-MR(LBD) cell lines

mineralocorticoids

HeLa

Exogenous mineralocorticoid receptor

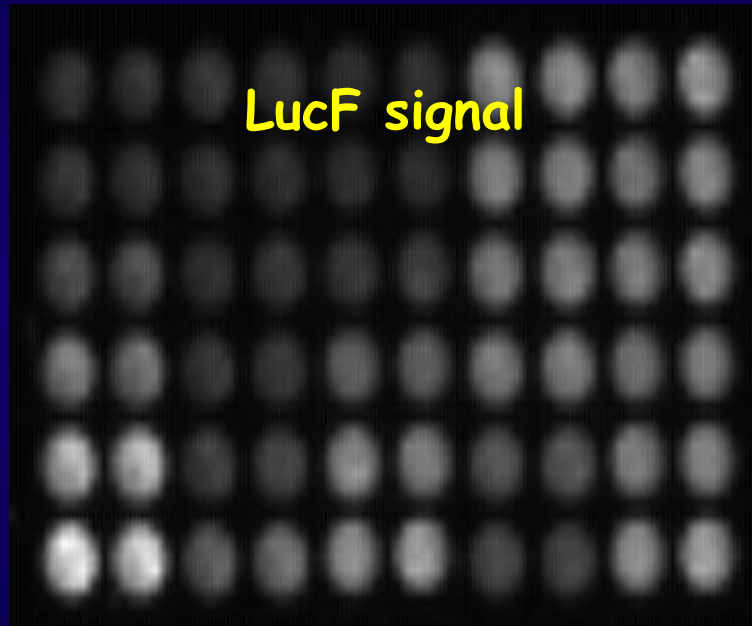


reporter genes stably integrated

Bioluminescent cellular models

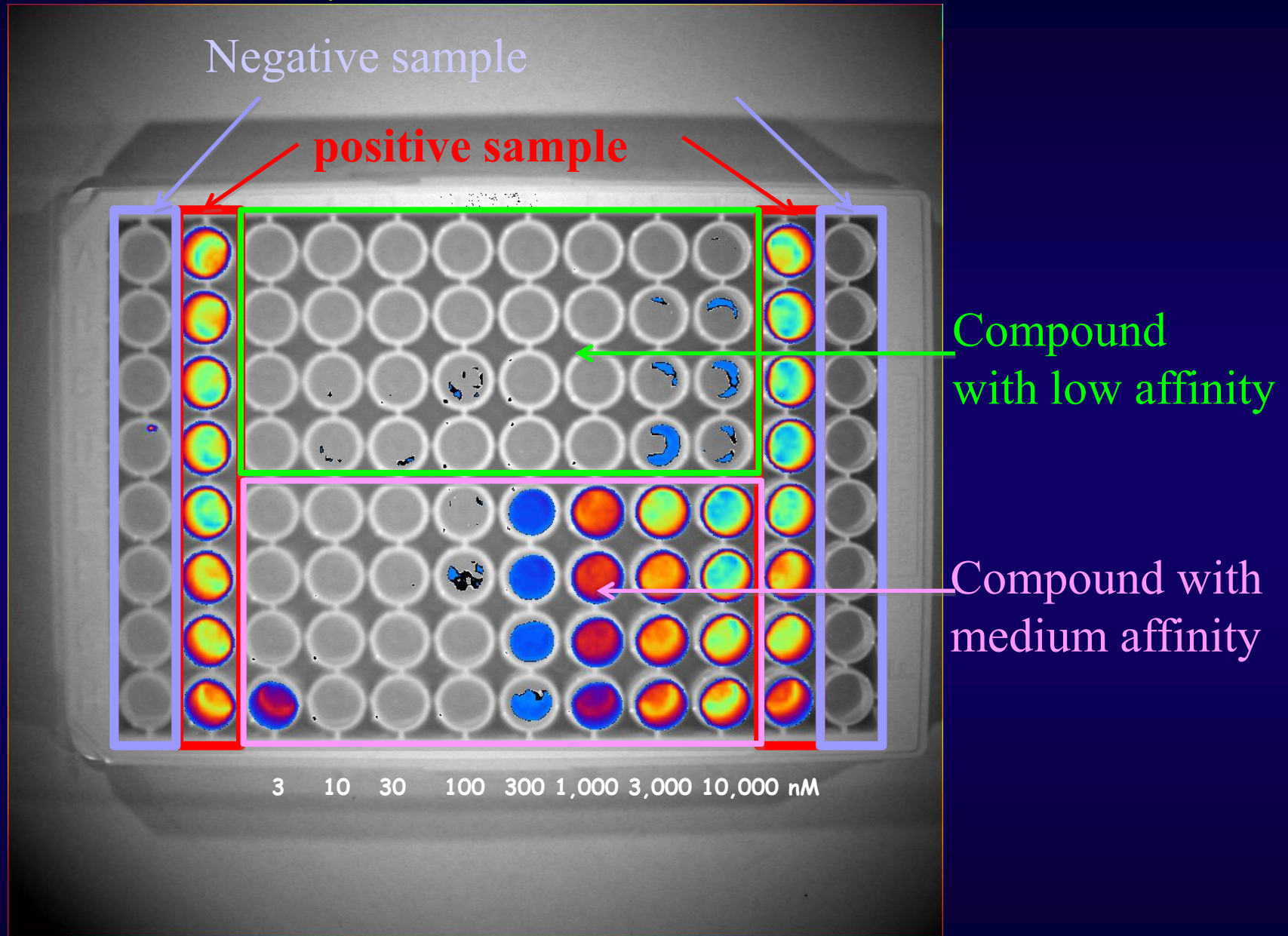
nuclear receptor	species	cell model	receptor
ER α , β	h	HeLa, MCF-7, HepG2, LS174T	WT
PR	h	HeLa	WT, Gal ₄ , ER α DBD
GR	h	HeLa	WT, Gal ₄
AR	h	HeLa, U2OS, PC ₃	WT, ER α DBD
MR	h	HeLa	Gal ₄ , ER α DBD
PPAR α , δ , γ	h	HeLa	Gal ₄
RAR α , β , γ	h	HeLa	WT, Gal ₄ , ER α DBD
T ₃ R α , β	r	HeLa	Gal ₄
VDR	h	HeLa, MCF-7	WT
PXR	h	HeLa, HepG2, LS174T	Gal ₄
CAR	h	HeLa	Gal ₄
AhR	h	HeLa, MCF-7, HepG2, LS174T, ZFL	WT

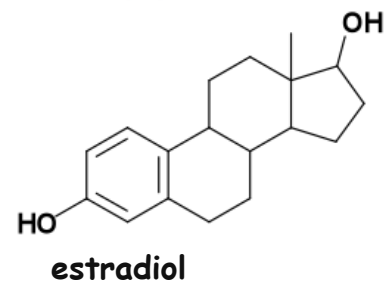
Firefly Luciferase activity detection



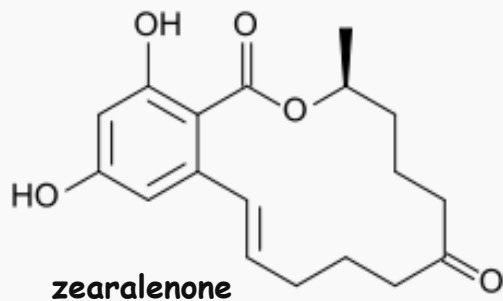
- T 0h : cell seeding (150 μ l, 20000 celluless)
- T 8h : sample addition (50 μ l)
- T 24h : luciferin addition (0,3 mM)
- luminescence measurement (firefly luciferase)

Luciferase activity detection

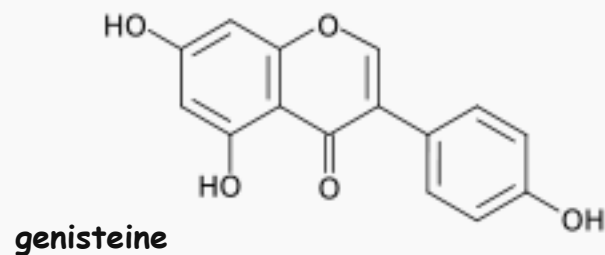




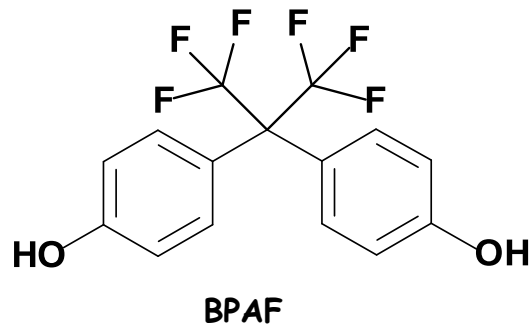
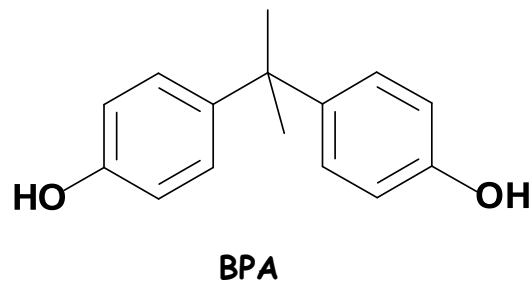
Agonist ER α , ER β



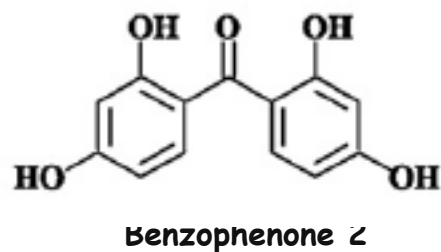
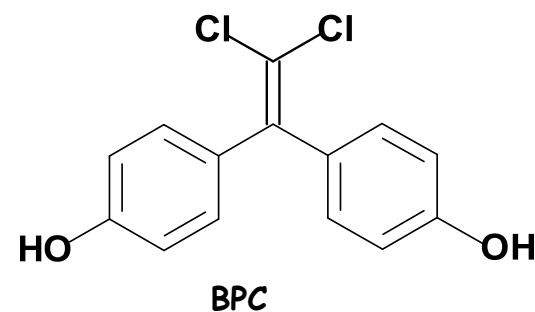
Agonist ER α and ER β , antagonist AR



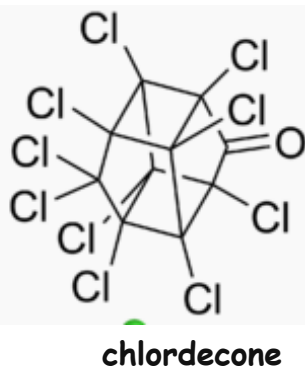
Agonist ER α and ER β



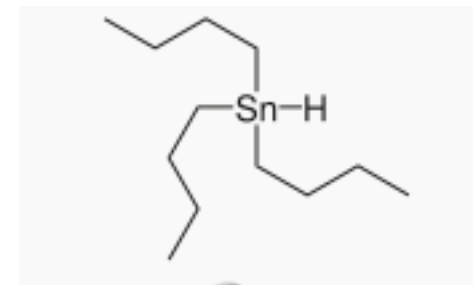
Agonist ER α and ER β , antagonist AR, agonist ERR γ , agonist PXR



Agonist ER α and ER β , antagonist AR



Agonist ER α , antagonist ER β

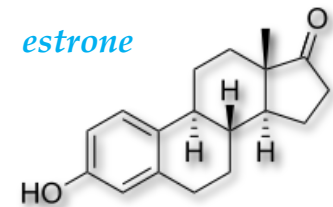
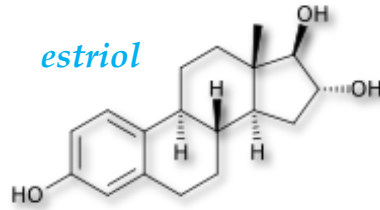
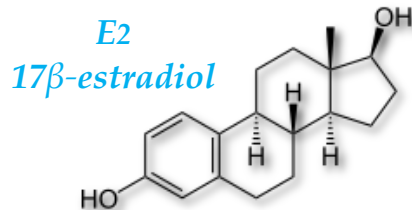


Agonist RXRs, partial agonist PPAR γ

• The estrogen receptors

⇒ ERs' functions

- key roles in development and maintenance of sexual and reproductive functions
- roles in morphogenesis of the uterus, ovary and mammary glands, prostate, lung and brain
- roles in skeletal, cardiovascular, and central nervous systems

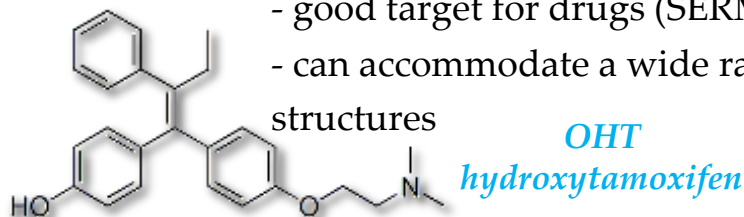


⇒ Two ER subtypes: ER α and ER β

- high degree of sequence homology except in their N-terminal domains
- bind to the same DNA response elements
- similar affinities for E2

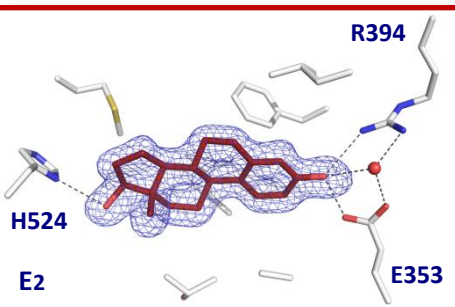
⇒ ERs' ligand cavity appears generous in size

- good target for drugs (SERM, antiestrogens)
- can accommodate a wide range of compounds with diverse structures

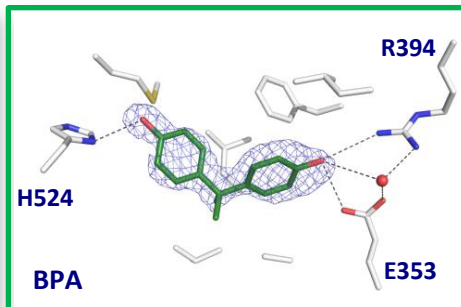


➡ EDCs

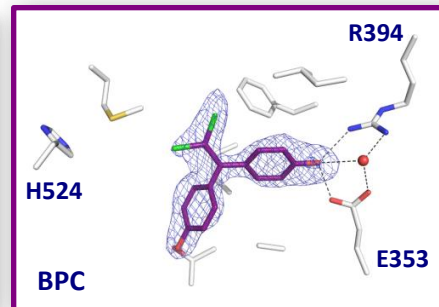
● Binding modes of endocrine disruptors in ER



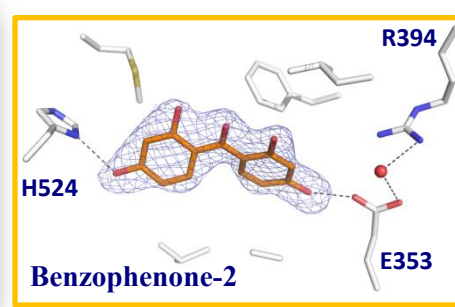
(17 β -estradiol)



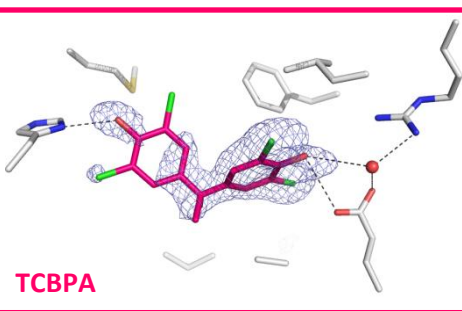
(plastics)



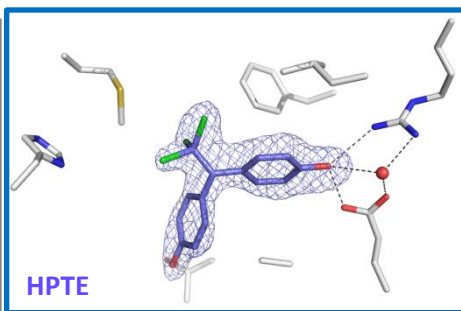
(plastics)



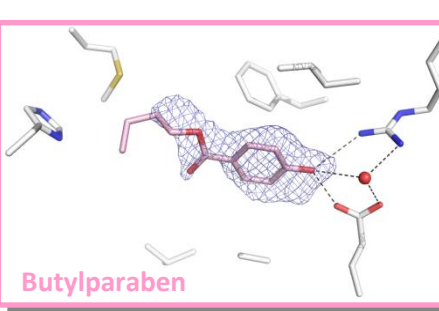
(UV filter)



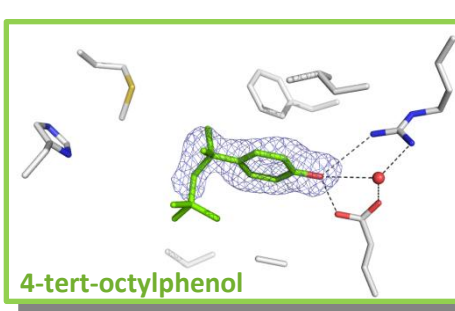
(flame retardant)



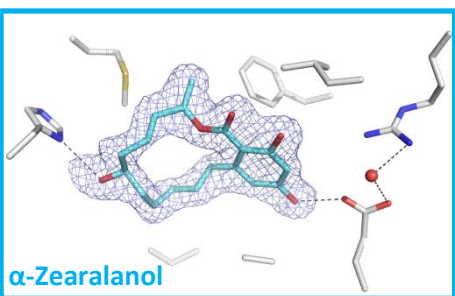
(methoxychlor metabolite)



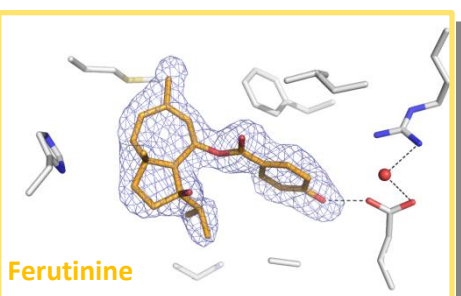
(preservatives)



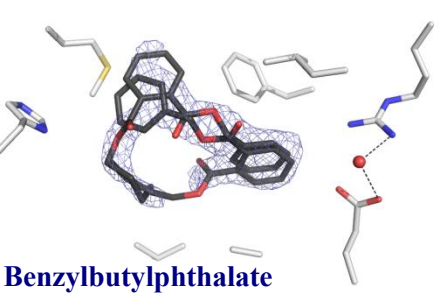
(surfactant)



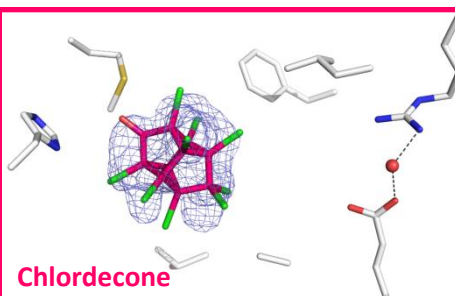
(mycoestrogen)



(phytoestrogen)

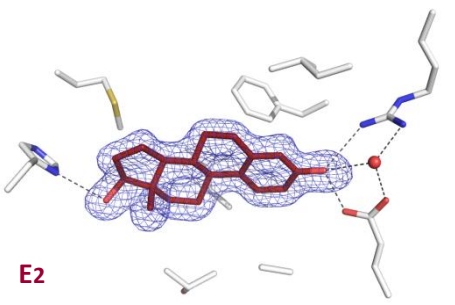


(plastics)

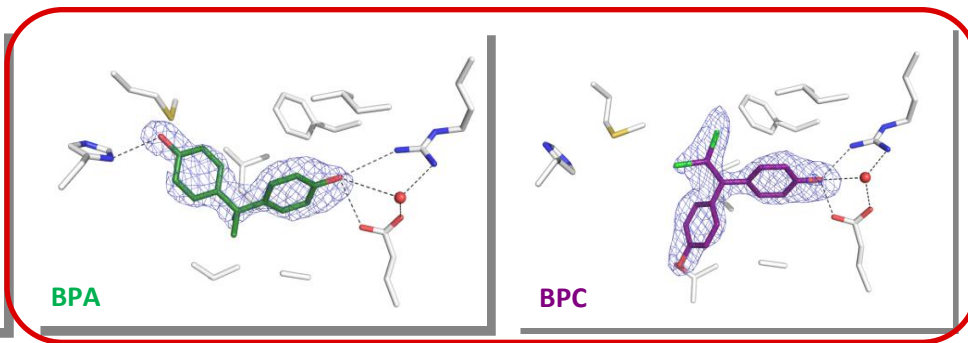


(pesticide)

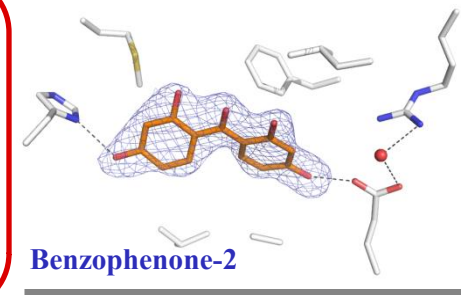
● Binding modes of endocrine disruptors in ER



E2
(17 β -estradiol)

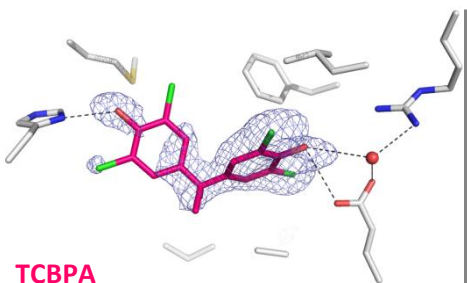


BPs = compounds involved in the manufacture of polycarbonates.



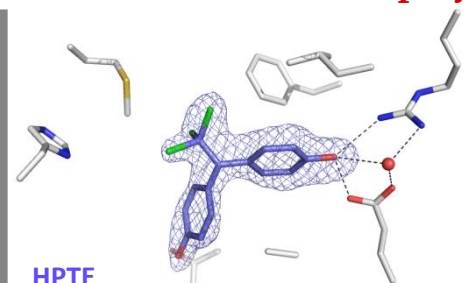
Benzophenone-2

filter



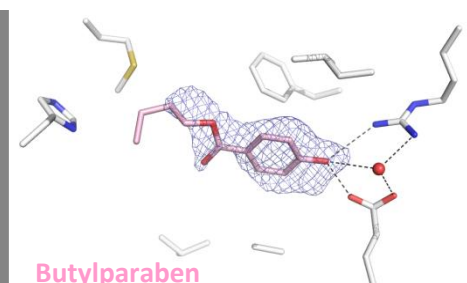
TCBPA

flame



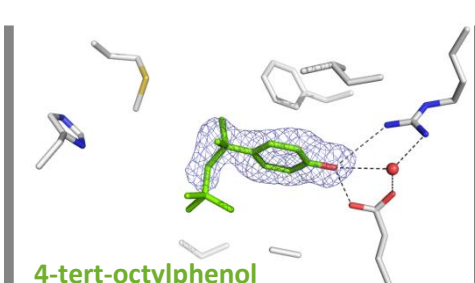
HPTE

metabolite



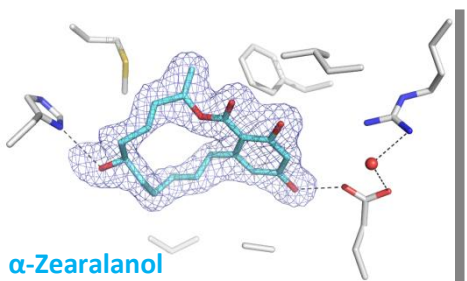
Butylparaben

preservatives



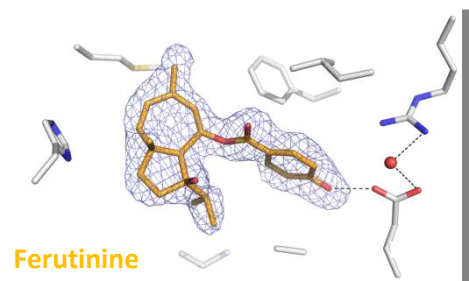
4-tert-octylphenol

surfactant



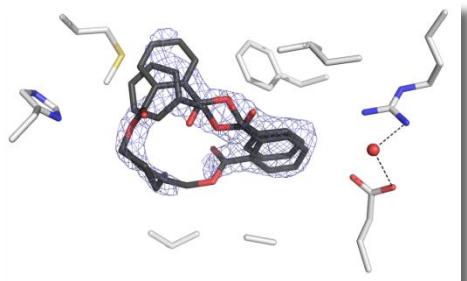
α -Zearalanol

mycoestrogen

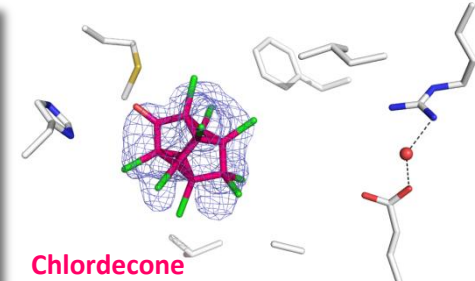


Ferutinine

phytoestrogen



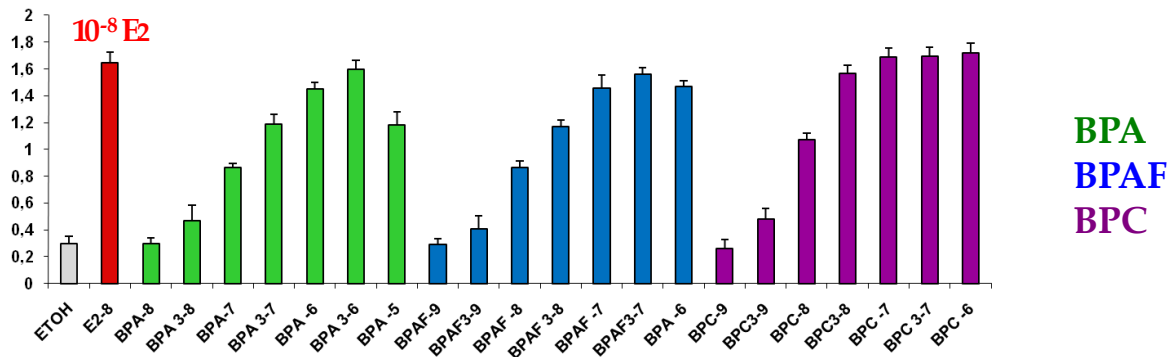
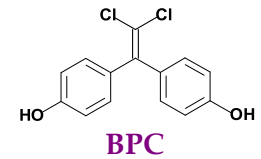
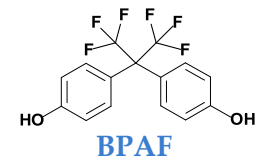
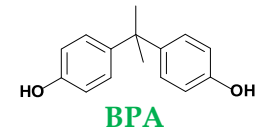
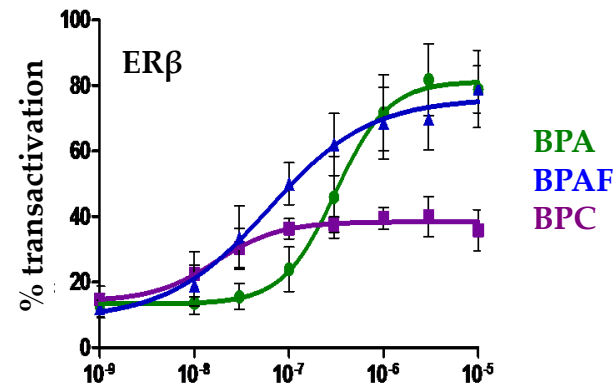
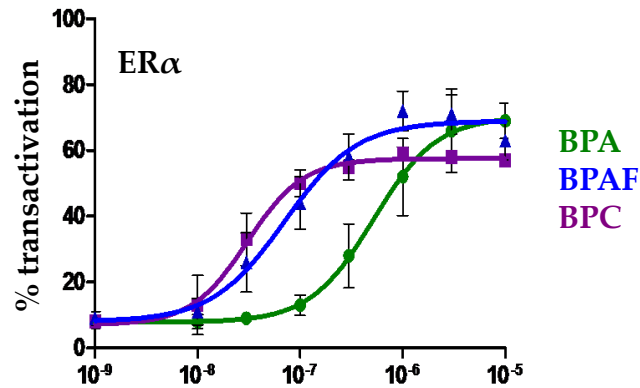
plastics



Chlordecone

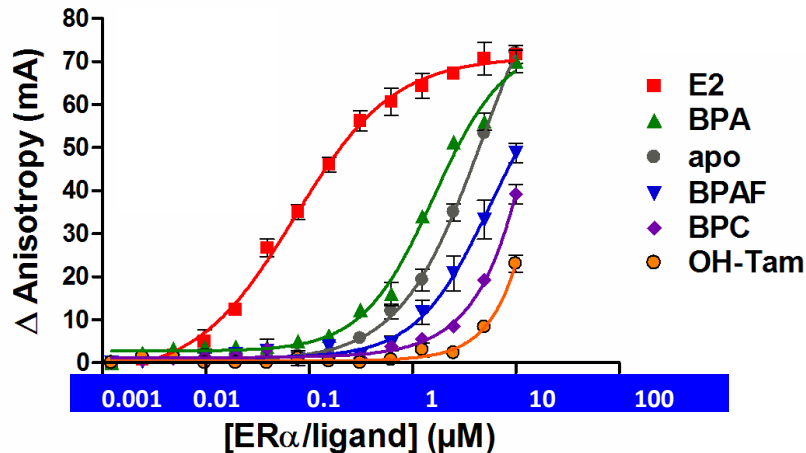
pesticide

Bisphenols are selective estrogen receptor modulators



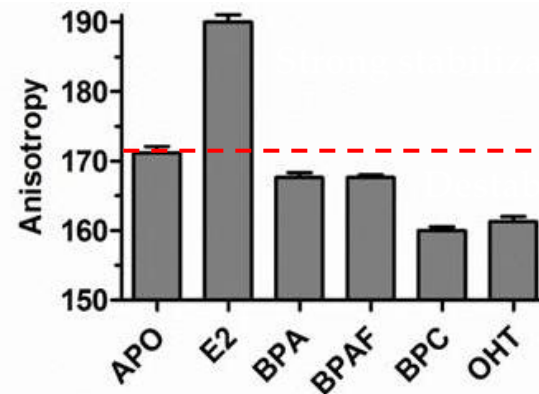
Ability of BPs to promote the recruitment of a coactivator

Measuring the interaction between the ER α -LBD and a fluorescent CoA peptide by fluorescence anisotropy



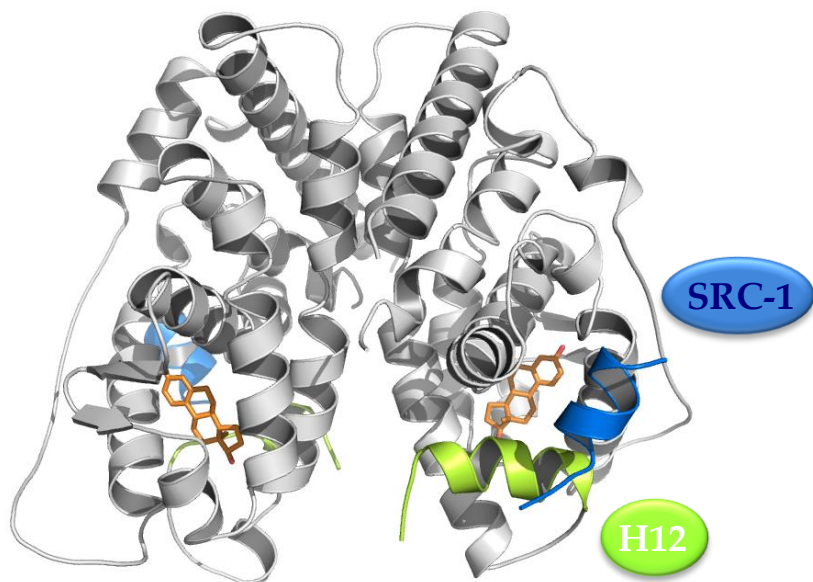
BP s poorly increased (BPA) or even weakened (BPAF and BPC) the basal interaction of ER α with the LxxLL motif

Measuring the dynamic of helix H12 by fluorescence anisotropy (fluorophore on H12)

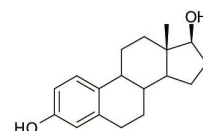


The dynamic of the C-terminal H12 helix is higher in the presence of bisphenols
BPC > BPA \approx BPAF \gg E2

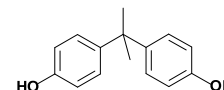
Structures of ER α / bisphenol complexes



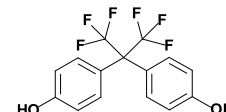
Agonist conformation (with CoA peptide)



estradiol

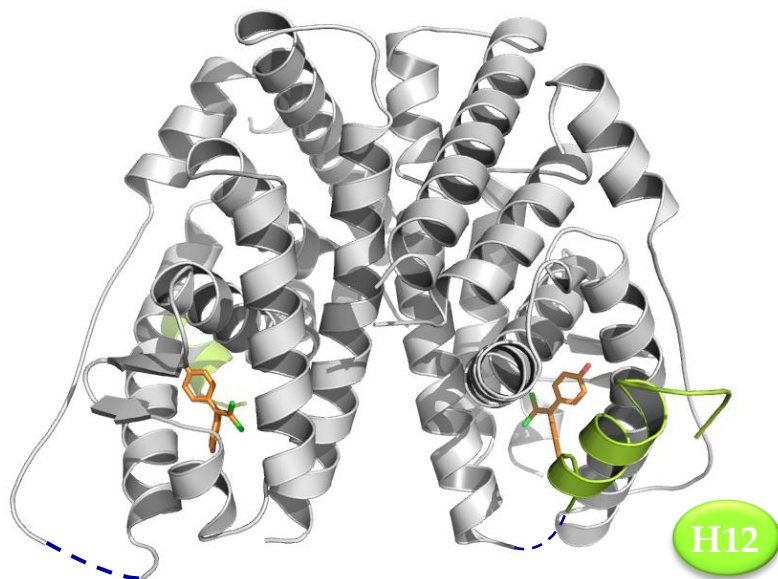


BPA

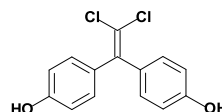


BPAF

Reso.	1.60 Å	2.20 Å	2.05 Å
R / R_{free}	0.166 / 0.195	0.206 / 0.245	0.189 / 0.231



Antagonist conformation (no peptide)



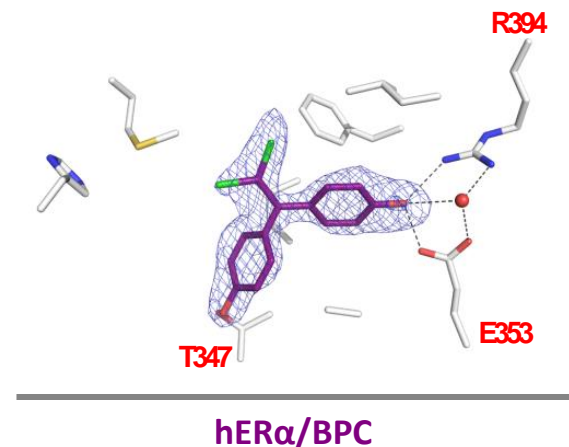
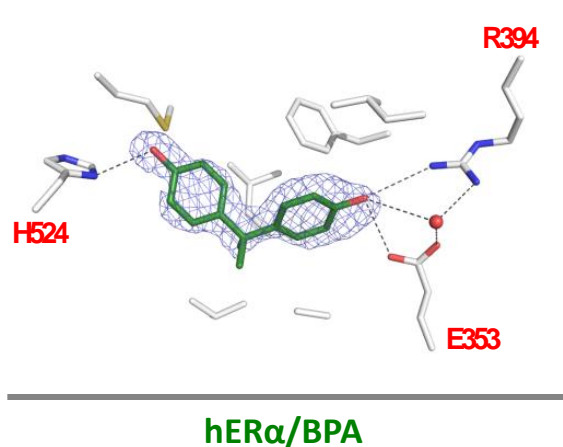
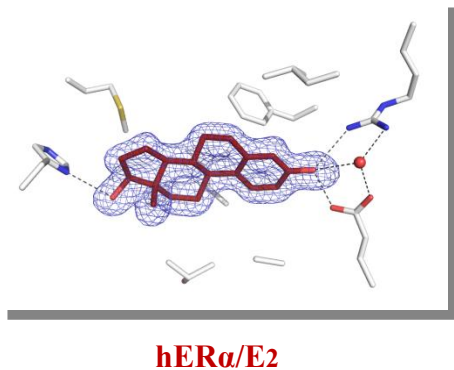
BPC

wt-ER α

Reso.	2.10 Å
R / R_{free}	0.214 / 0.255

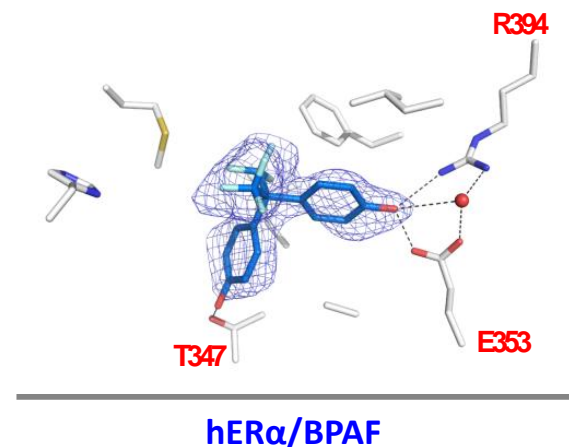
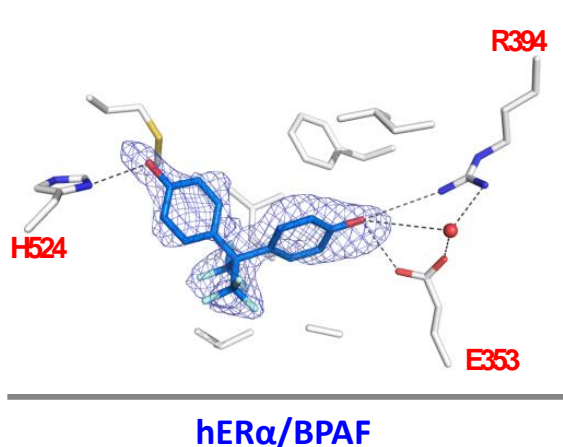
(Delfosse et al., PNAS 2012)

• Bisphenol binding modes

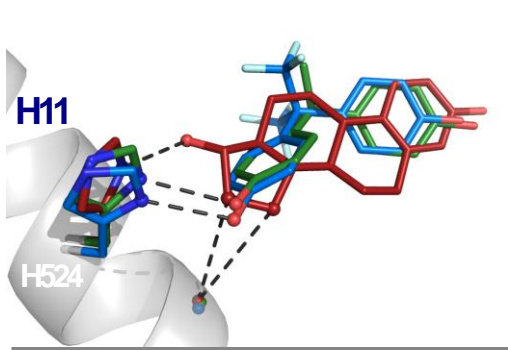


→ 3 key H-bonds in the agonist-bound conformation
E353 - R394 - H524

→ One new H-bond in the antagonist-bound conformation
T347

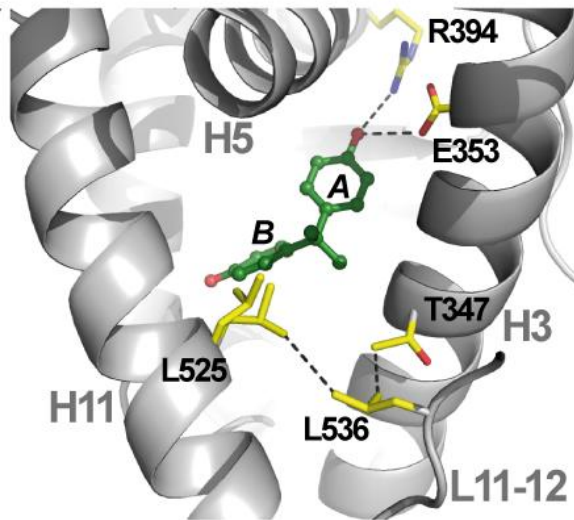
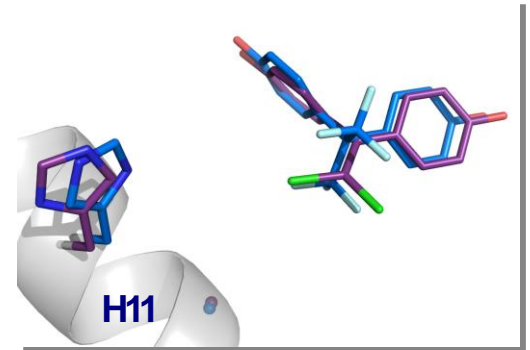


• Structural basis for bisphenol action

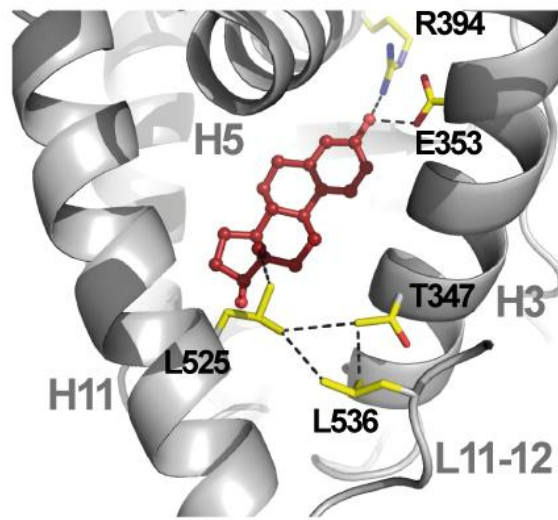


Hydrophobic interactions with Gly521 and Leu525 are disrupted

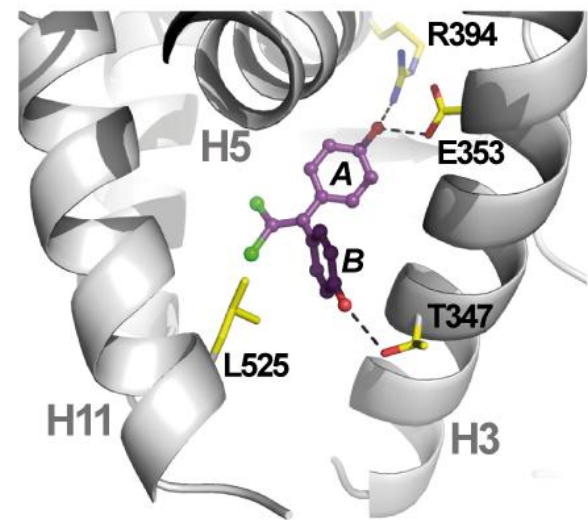
Reorientation of the His524 imidazole ring



hER α /BPA



hER α /E2

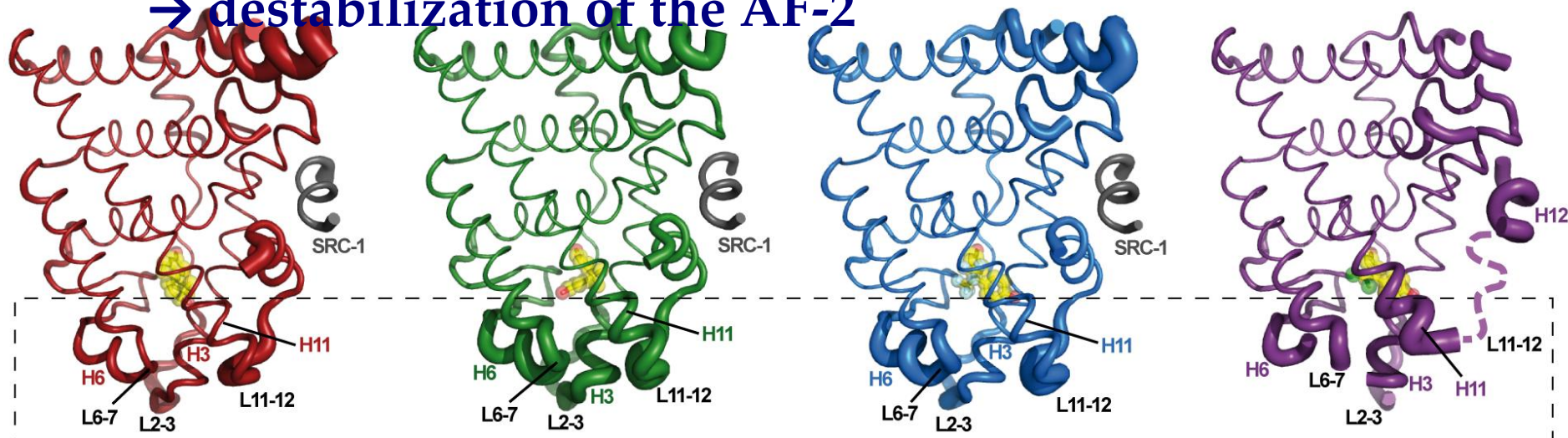


hER α /BPC

• Structural basis for bisphenol action

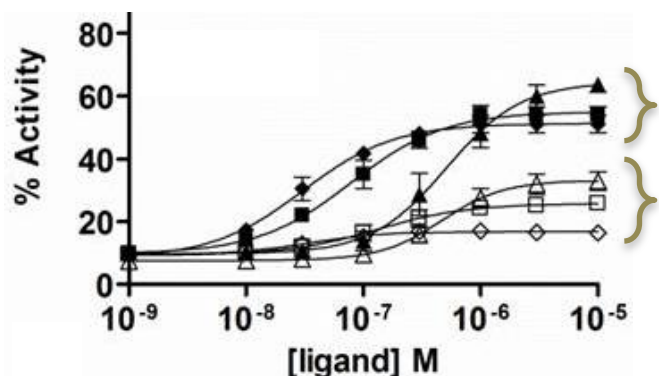
→ B factors in the “lower part of the LBD” are increasing when BPs are bound

→ destabilization of the AF-2



Bisphenols perturb the secondary structures of the LBP, helices H3 and H11, and the loop L11-12, which are important for the docking of helix H12 in its active position.

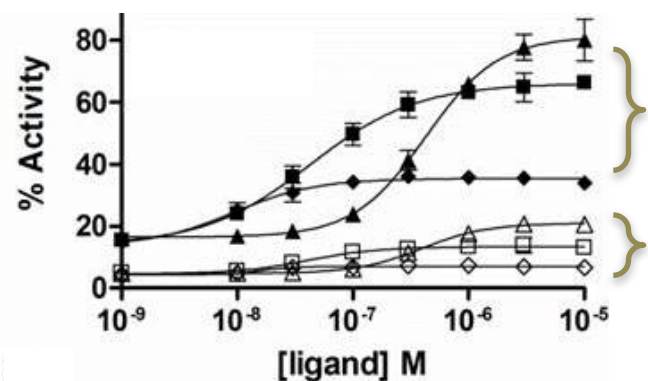
• The activity of bisphenols on hERs relies mostly on the AF1



ER α

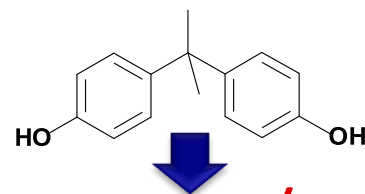
Δ AB-ER α)

△ BPA
□ BPAF
◇ BPC



ER β

Δ AB-ER β)



+++

+/-

(AF-1) A/B

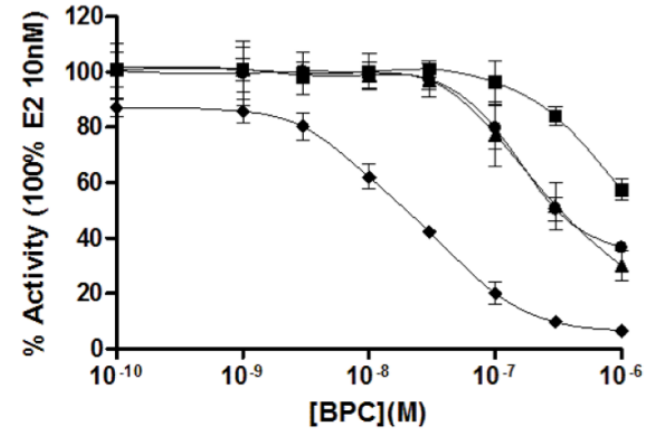
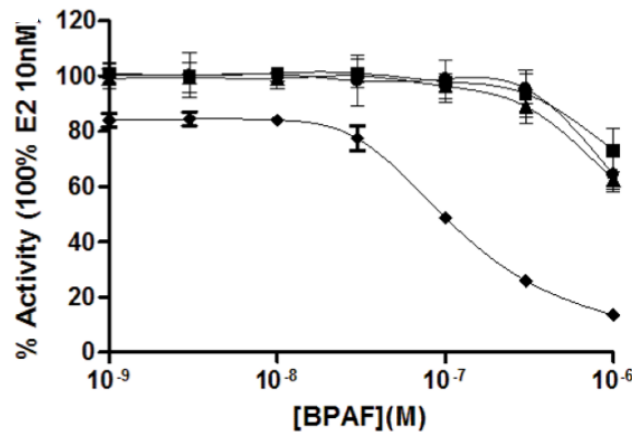
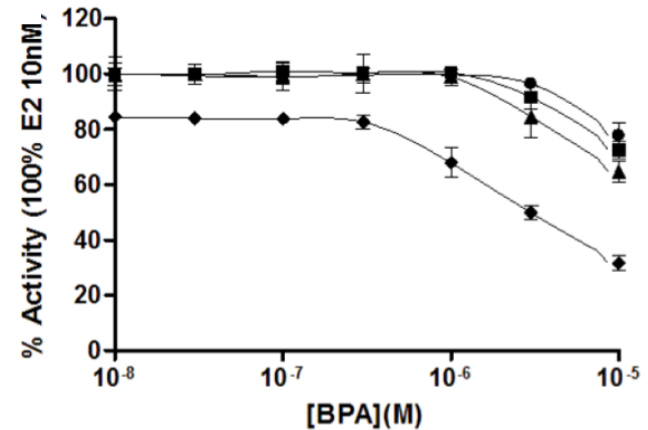
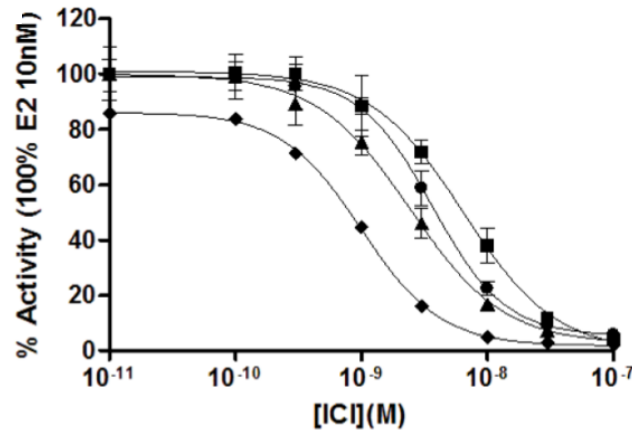
DBD/C

D

(AF-2)

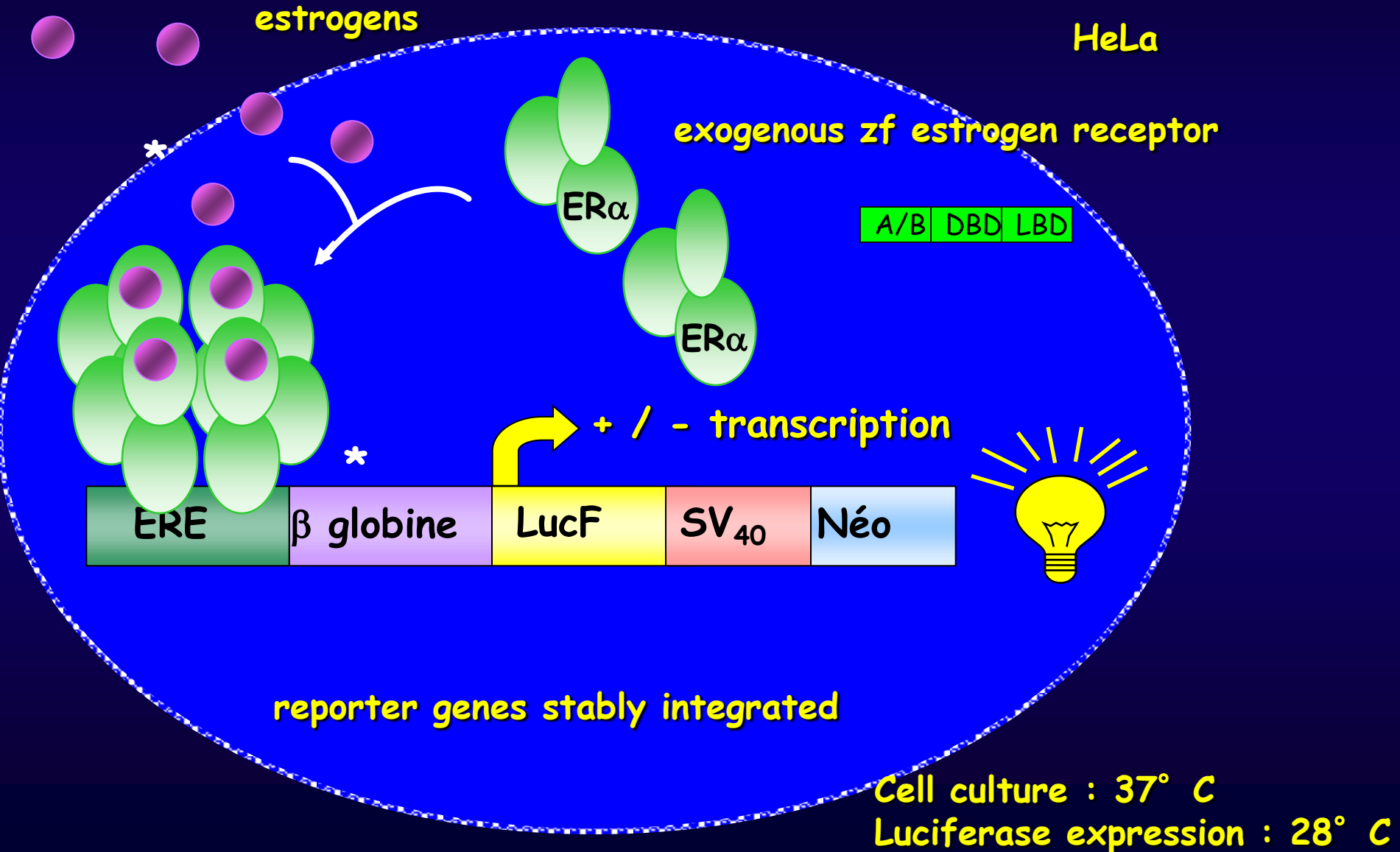
F

● The activity of bisphenols on hERs relies mostly on the AF1

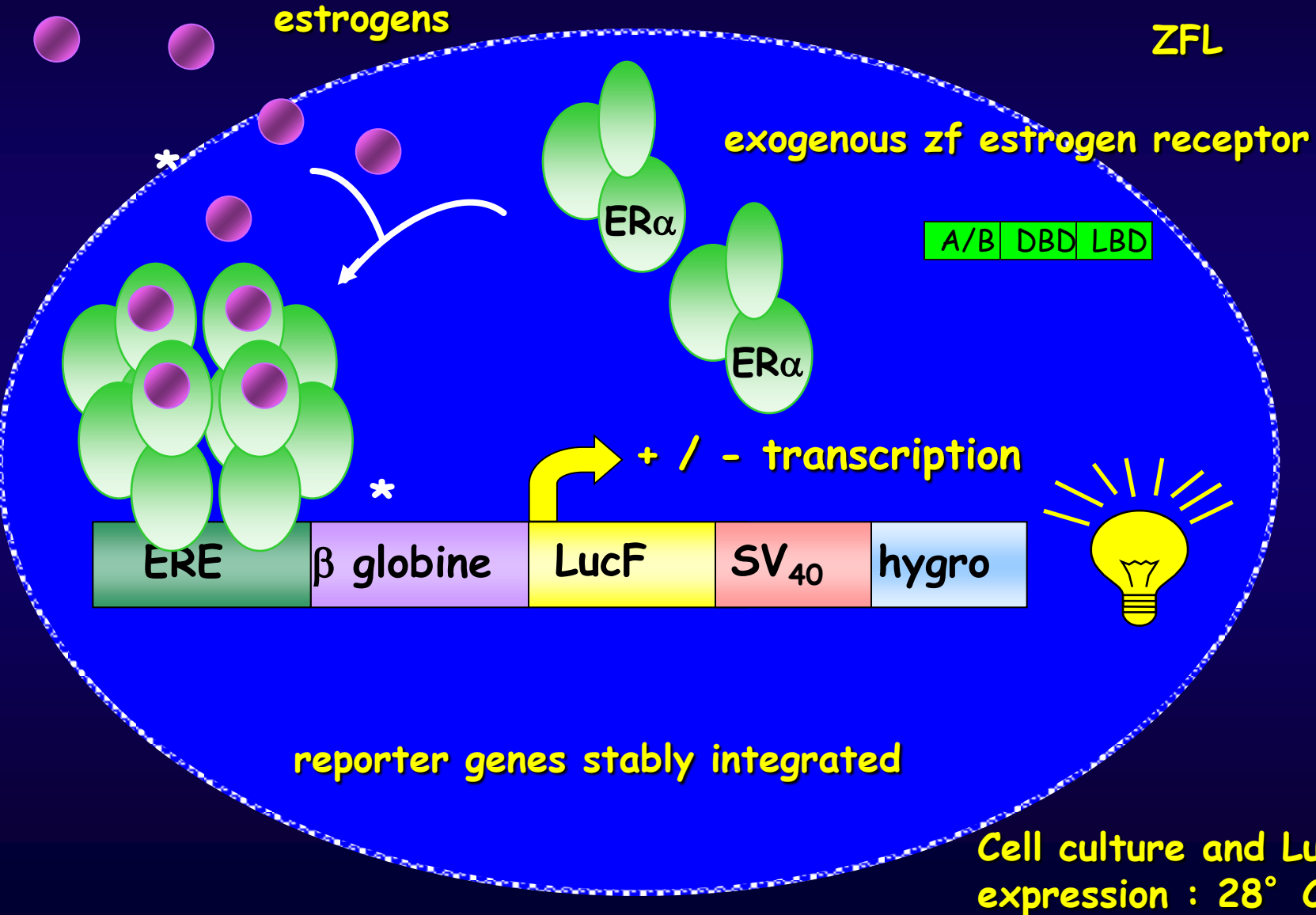


• The activity of bisphenols on zfNRs

HELN zfER α , ER β 1 or ER β 2 cell lines (human cellular context)



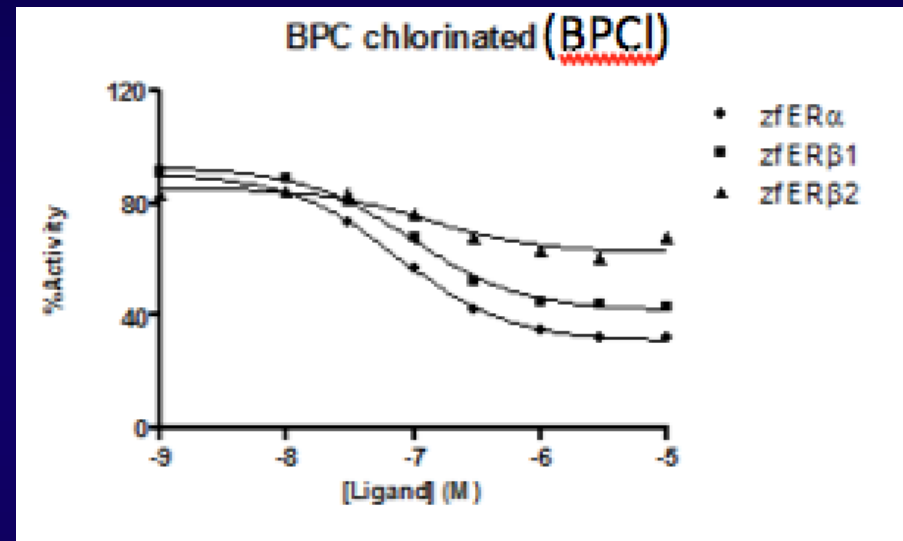
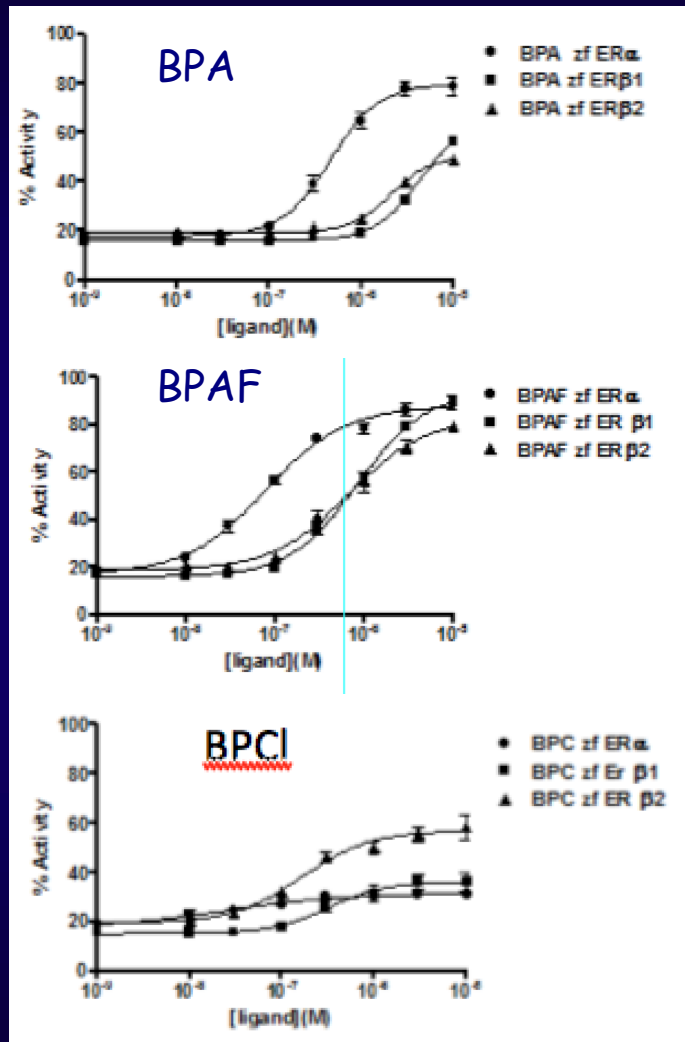
ZFL zfER α , ER β 1 or ER β 2 cell lines (zebrafish cellular context)



Bioluminescent cellular models expressing zf nuclear receptors

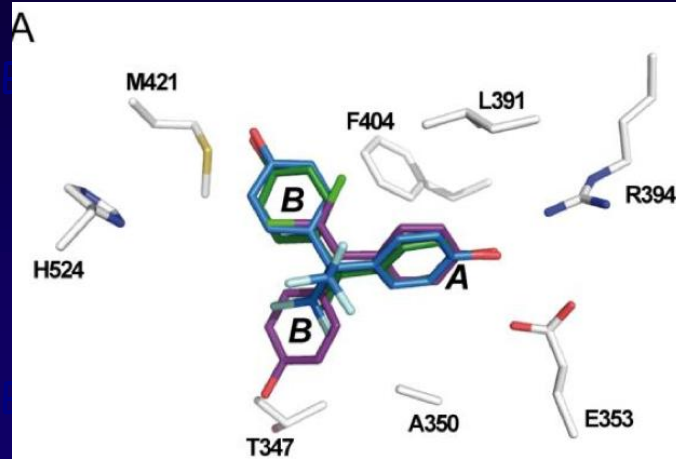
nuclear receptor	species	cell model	receptor
ER α , β	h,zf	HeLa, <i>ZFL</i>	WT
PR	h,zf	HeLa	WT, ER α DBD
GR	h,zf	HeLa	WT, Gal ₄
AR	h,zf	HeLa, U2OS	WT, ER α DBD
MR	h,zf	HeLa	ER α DBD
ERR γ	h,zf	HeLa	Gal ₄
PPAR γ	h,zf	HeLa	Gal ₄
AhR	h,zf	HeLa, <i>ZFL</i>	WT

Bisphenols activity on zfERs

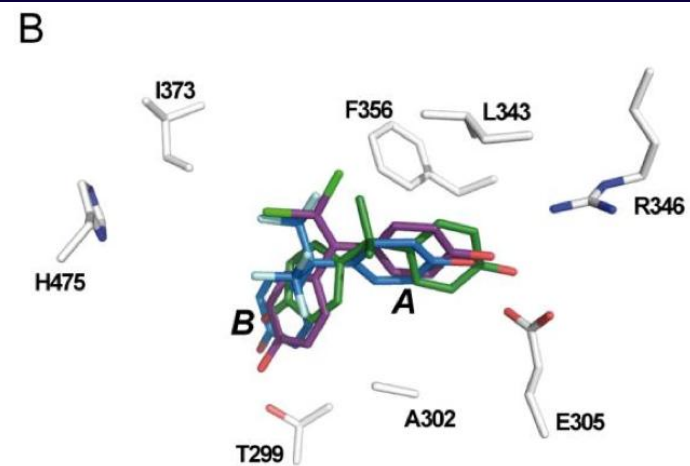


Bisphenols activity on other human nuclear receptors

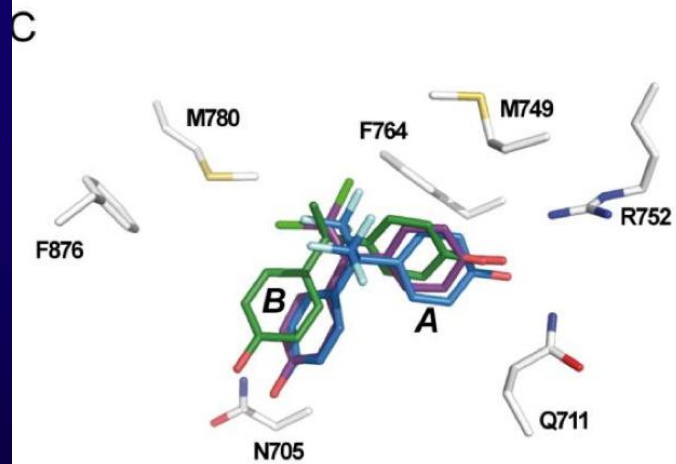
ER α



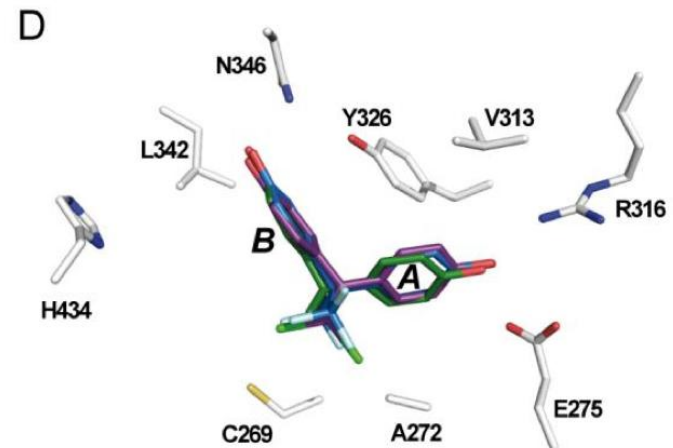
ER β



AR



ERR γ

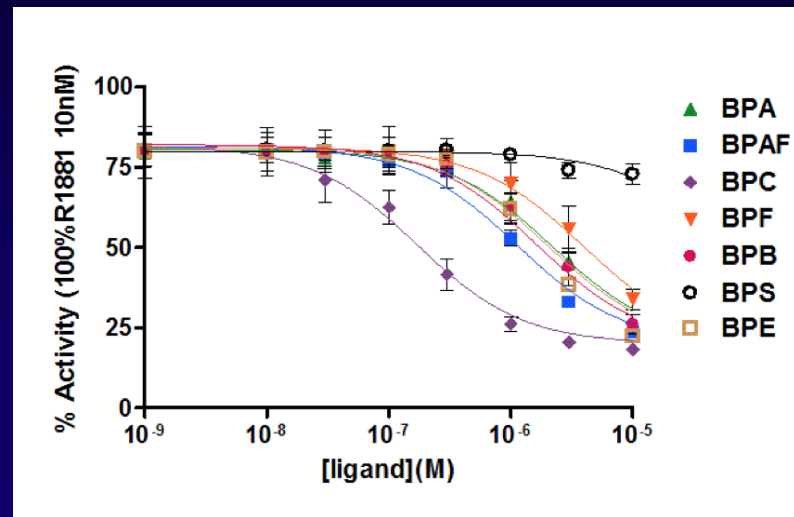


Bisphenols activity on other human nuclear receptors

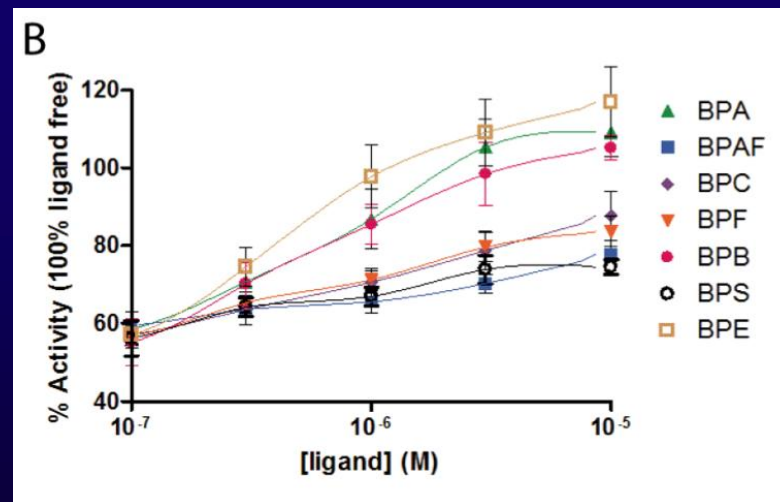
BPA

AR

BPAF



ERR γ



BPS is the less active on the nuclear receptors



Cancer Research Institute, Montpellier

Functional studies, validation & screening

CBS members

Structural analysis



P. Balaguer

M. Grimaldi



W. Bourguet

A. le Maire



A. Boulahtouf



Sélim
François
Nicolas
Jean-Marc



V. Delfosse