



# Nuclear receptors: *in vitro* and *in vivo* approaches

Parma, June 13-15 2017

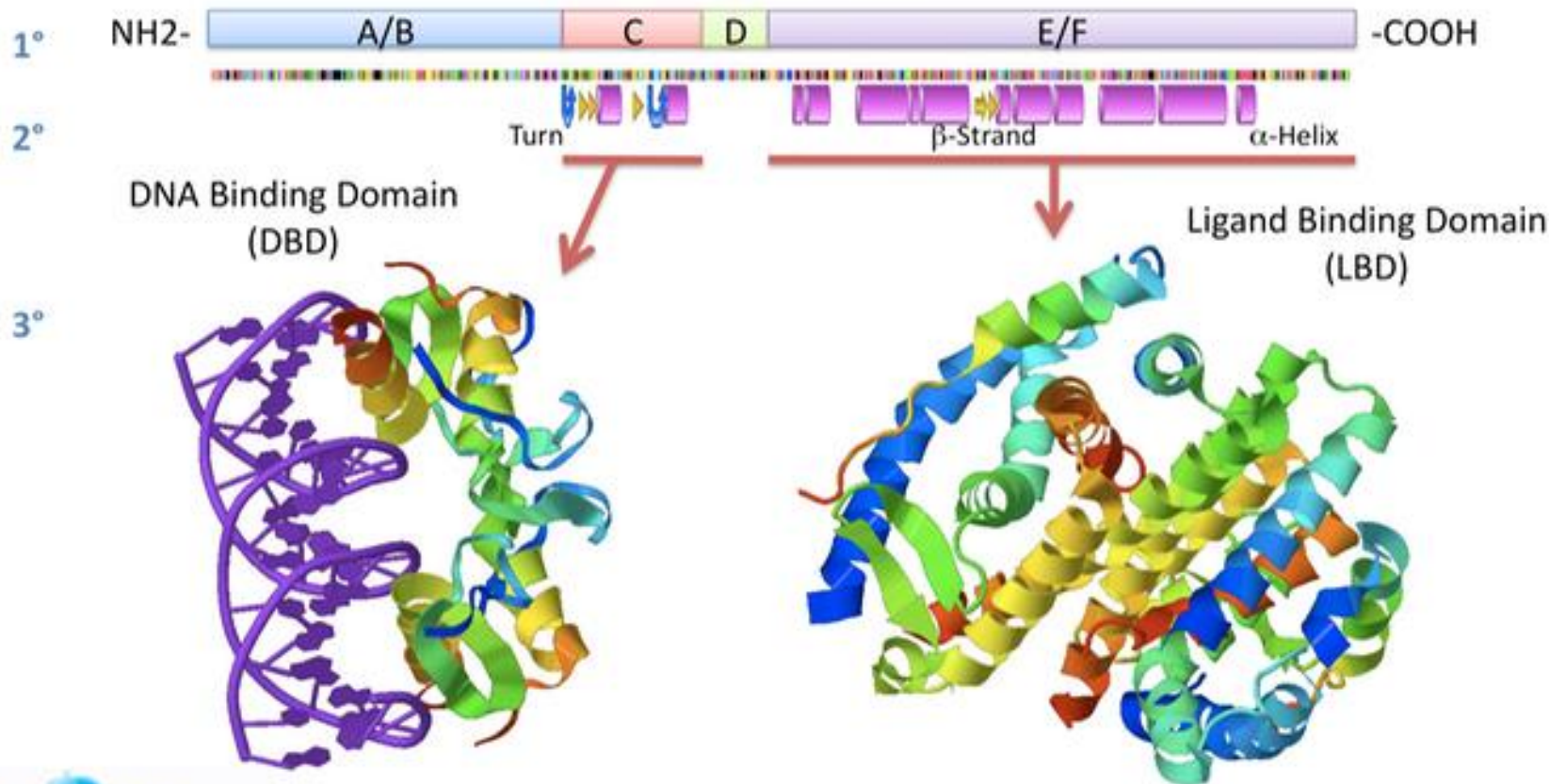
Adriana Maggi  
University of Milan  
[www.cend.unimi.it](http://www.cend.unimi.it)

# Intracellular Receptors, a family of 48 members in humans

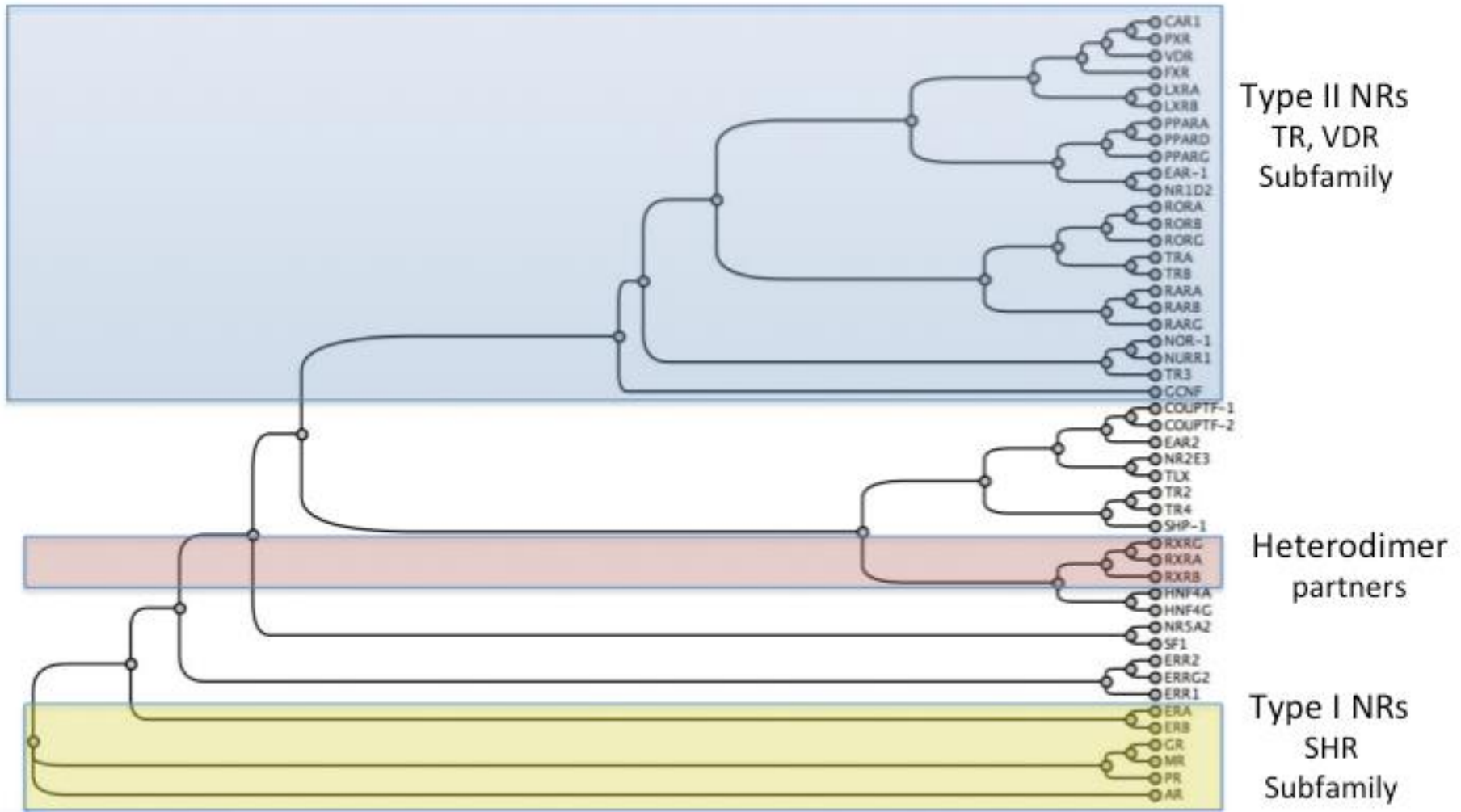
[Androgen Receptor \(AR; NR3C4\)](#)  
[Aryl Hydrocarbon Receptor \(AhR\)](#)  
[Constitutive Androstane Receptor \(CAR; NR1I3\)](#)  
[Estrogen Receptor Alpha \(ER \$\alpha\$ ; NR3A1\)](#)  
[Estrogen Receptor Beta \(ER \$\beta\$ ; NR3A2\)](#)  
[Estrogen Related Receptor Alpha \(ERR \$\alpha\$ ; NR3B1\)](#)  
[Estrogen Related Receptor Gamma \(ERR \$\gamma\$ ; NR3B3\)](#)  
[Farnesoid X Receptor \(FXR; NR1H4\)](#)  
[Glucocorticoid Receptor \(GR; NR3C1\)](#)  
[Liver Receptor Homolog-1 \(LRH-1; NR5A2\)](#)  
[Liver X Receptor Alpha \(LXR \$\alpha\$ ; NR1H3\)](#)  
[Liver X Receptor Beta \(LXR \$\beta\$ ; NR1H2\)](#)  
[Mineralocorticoid Receptor \(MR; NR3C2\)](#)  
[Peroxisome Proliferator-Activated Receptor Alpha \(PPAR \$\alpha\$ ; NR1C1\)](#)  
[Peroxisome Proliferator-Activated Receptor Delta \(PPAR \$\delta\$ ; NR1C2\)](#)  
[Peroxisome Proliferator-Activated Receptor Gamma \(PPAR \$\gamma\$ ; NR1C3\)](#)

[Pregnane X Receptor \(PXR; NR1I2\)](#)  
[Progesterone Receptor \(PGR; NR3C3\)](#)  
[Retinoic Acid Receptor Alpha \(RAR \$\alpha\$ ; NR1B1\)](#)  
[Retinoic Acid Receptor Beta \(RAR \$\beta\$ ; NR1B2\)](#)  
[Retinoic Acid Receptor Gamma \(RAR \$\gamma\$ ; NR1B3\)](#)  
[RAR-related Orphan Receptor Alpha \(ROR \$\alpha\$ ; NR1F1\)](#)  
[RAR-related Orphan Receptor Gamma \(ROR \$\gamma\$ ; NR1F3\)](#)  
[Retinoid X Receptor Alpha \(RXR \$\alpha\$ ; NR2B1\)](#)  
[Retinoid X Receptor Beta \(RXR \$\beta\$ ; NR2B2\)](#)  
[Retinoid X Receptor Gamma \(RXR \$\gamma\$ ; NR2B3\)](#)  
[Thyroid Hormone Receptor Alpha \(TR \$\alpha\$ ; NR1A1\)](#)  
[Thyroid Hormone Receptor Beta \(TR \$\beta\$ ; NR1A2\)](#)  
[Vitamin D Receptor \(VDR; NR1I1\)](#)

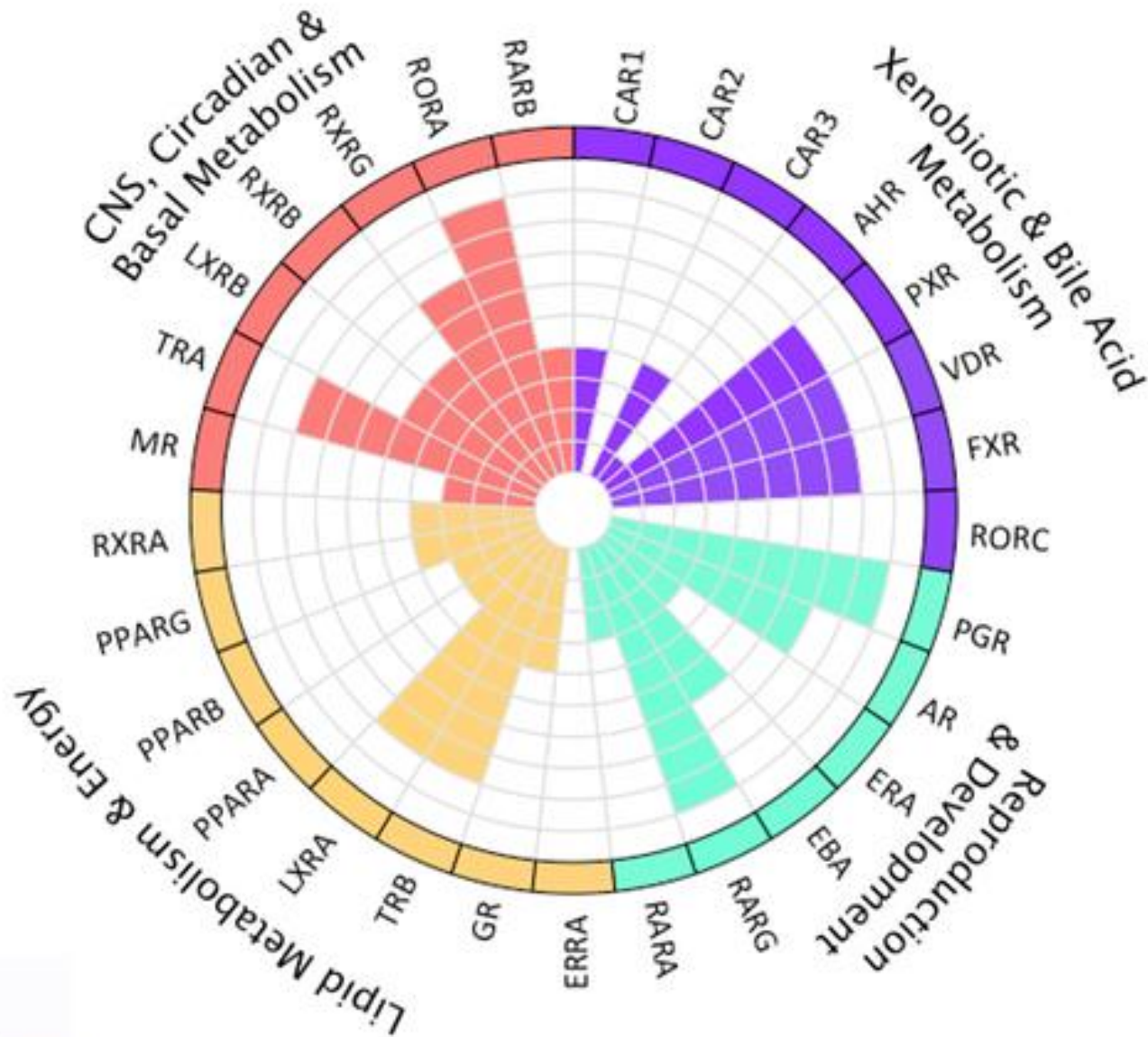
# Structure of NRs



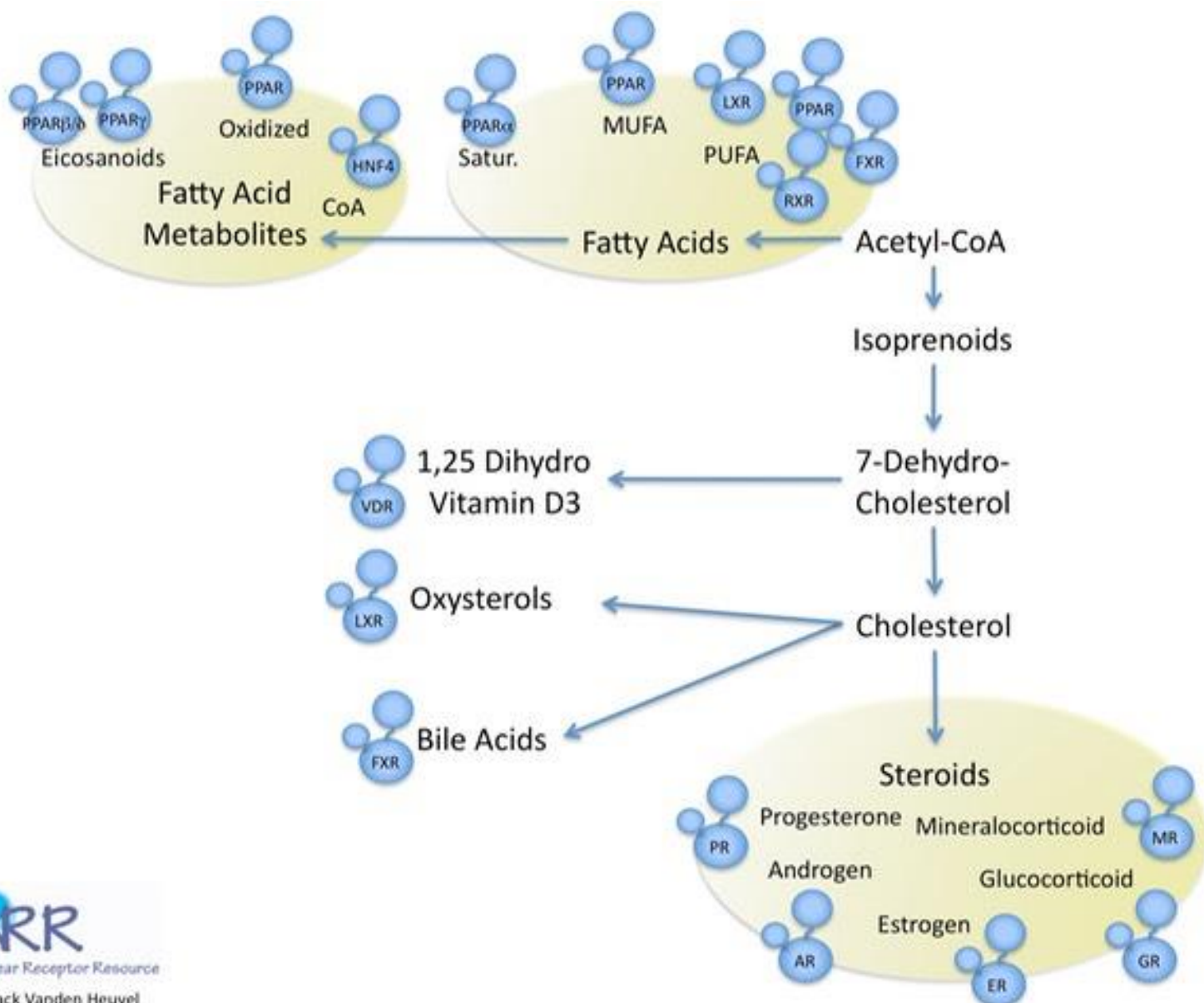
# NRs: Phylogeny



# NR Functional Classification



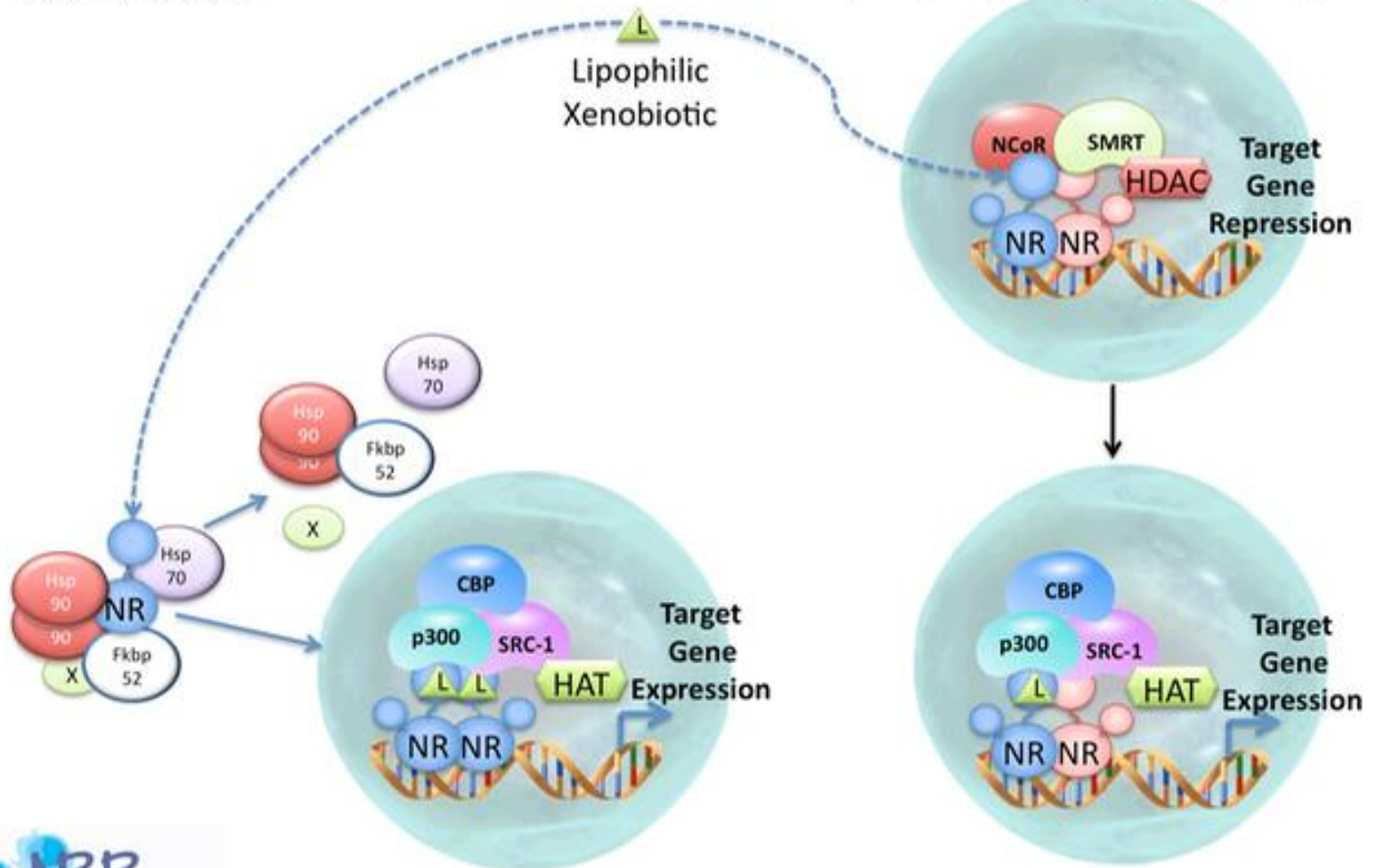
# The endogenous ligands for Intracellular Receptors



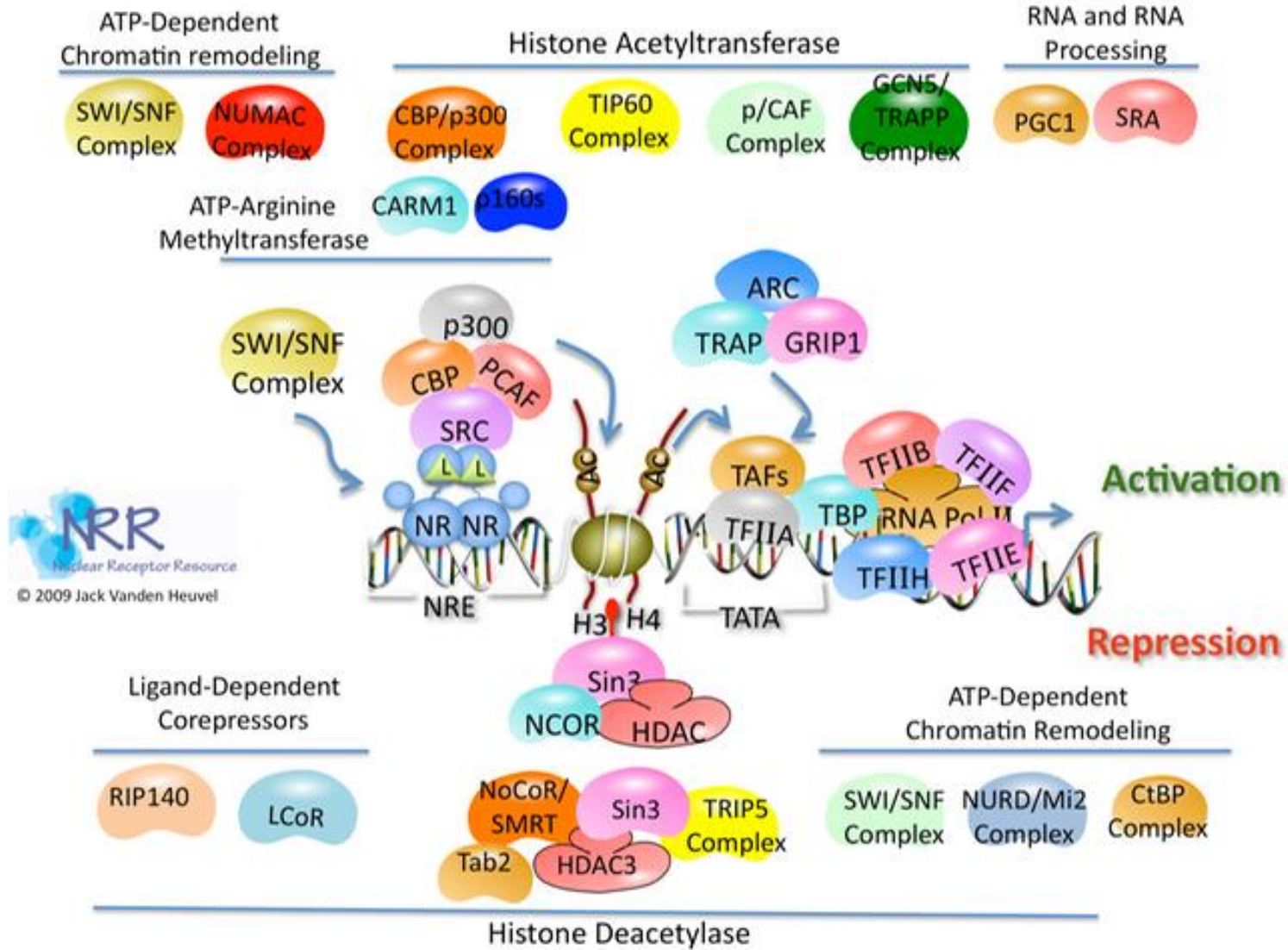
# MECHANISM OF ACTION 1.

A. Steroid hormone receptors  
(AR, ER, GR, MR)

B. Heterodimeric nuclear receptors  
(LXR, FXR, PPAR, RAR, PXR, VDR)

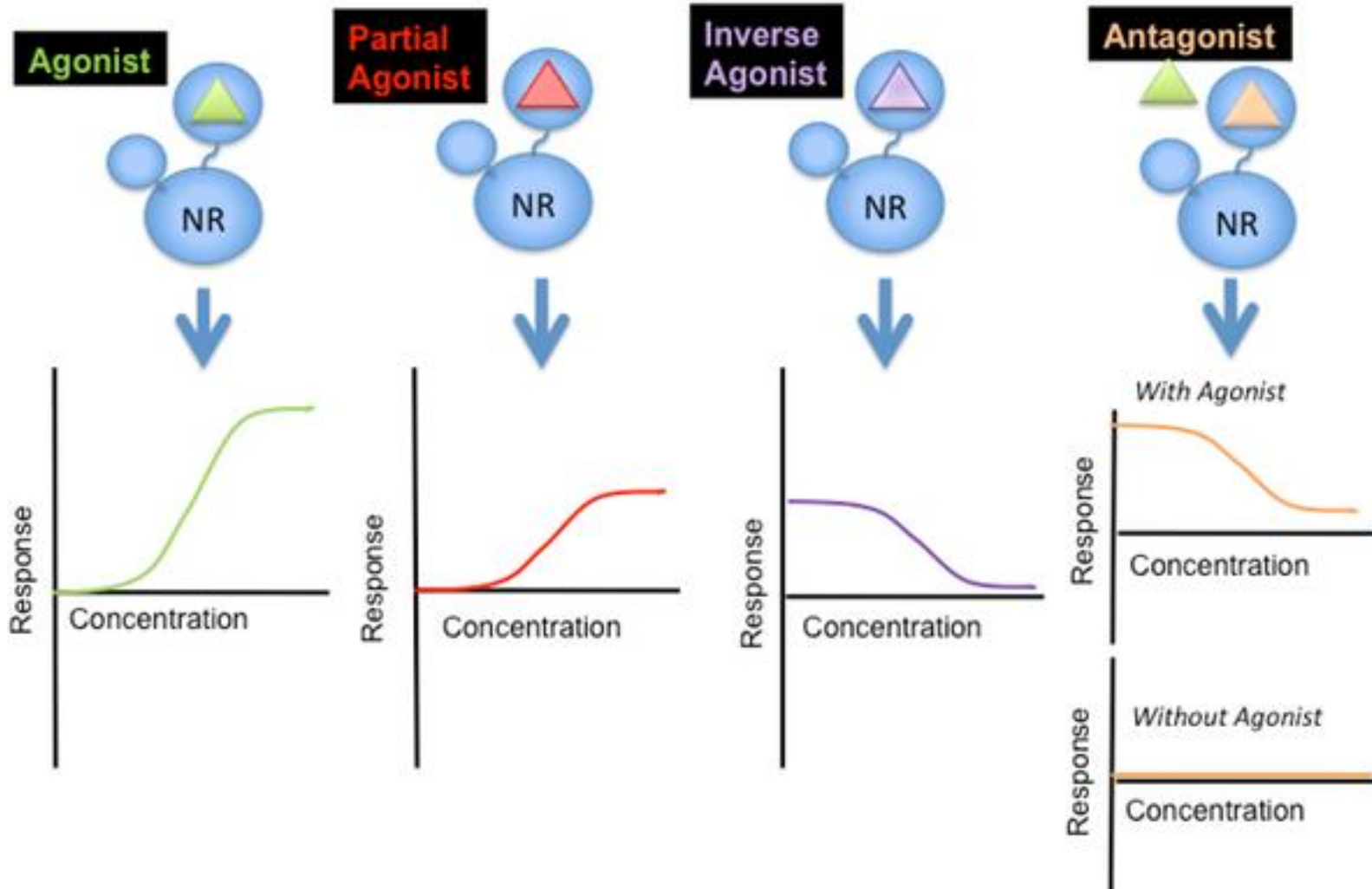


# MECHANISM OF ACTION 2.

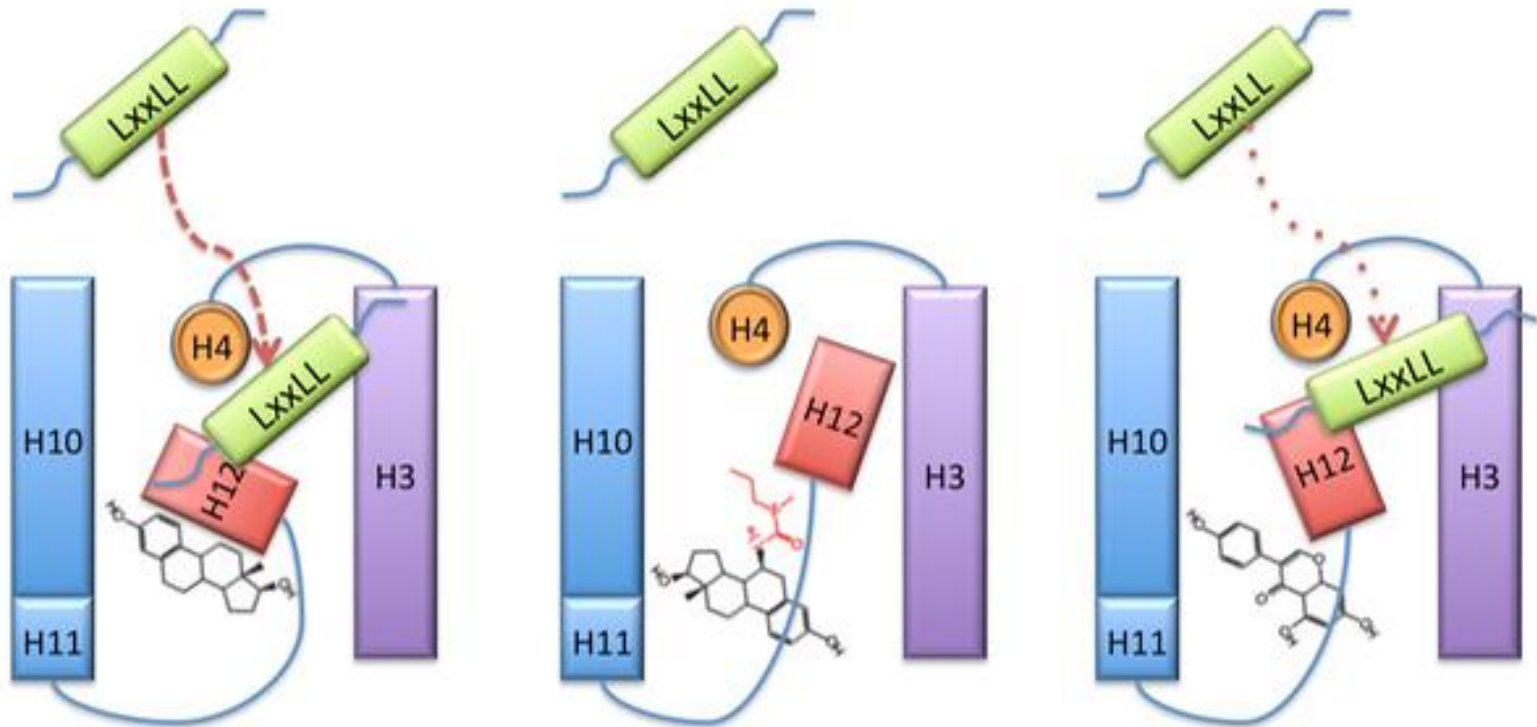




# THE PHARMACOLOGY OF INTRACELLULAR RECEPTORS



# THE PHARMACOLOGY OF INTRACELLULAR RECEPTORS 2.

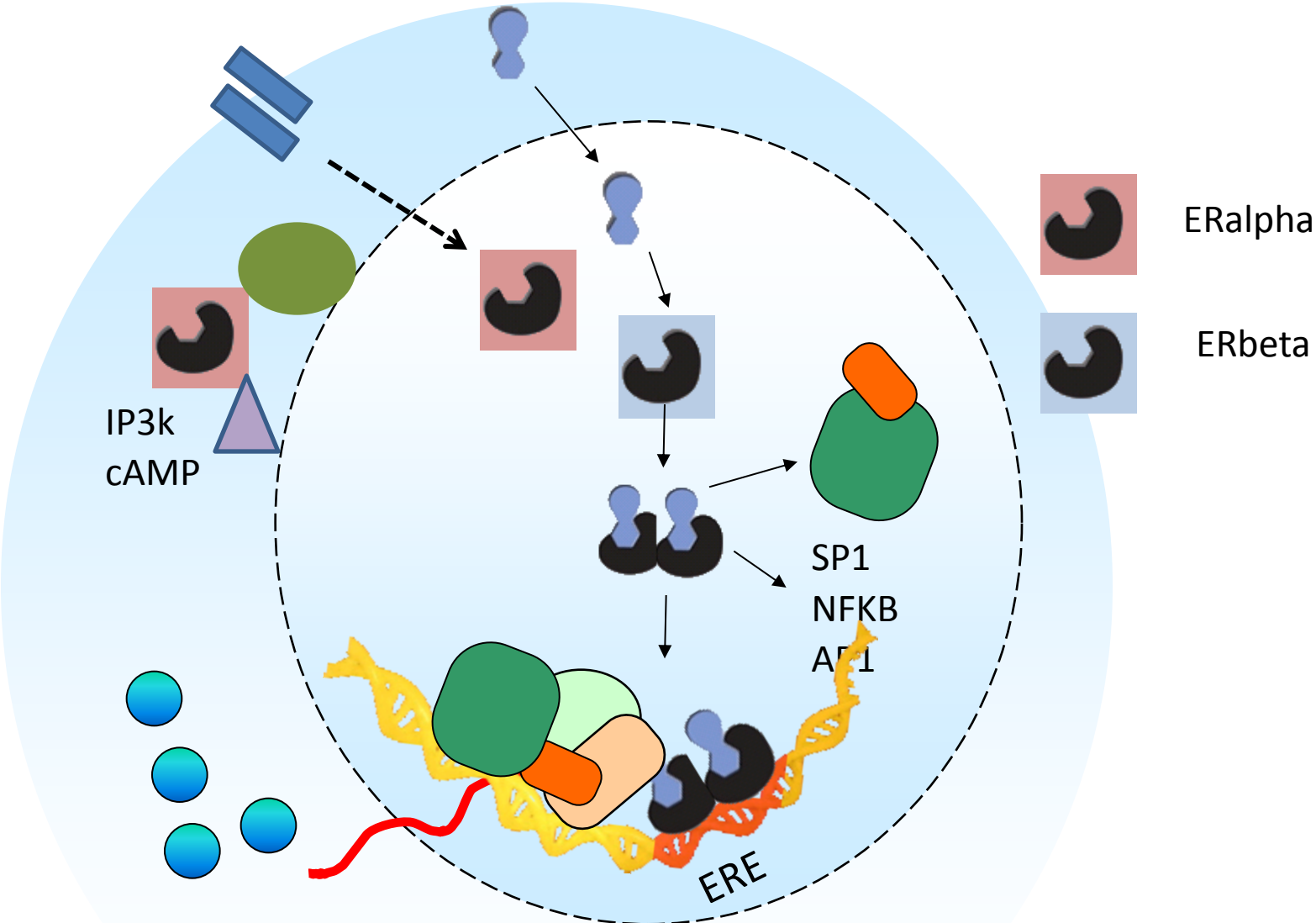


Agonist  
Estradiol

Antagonist  
ICI164384

Partial Agonist  
Genistein

# THE COMPLEXITY OF ESTROGEN ACTION

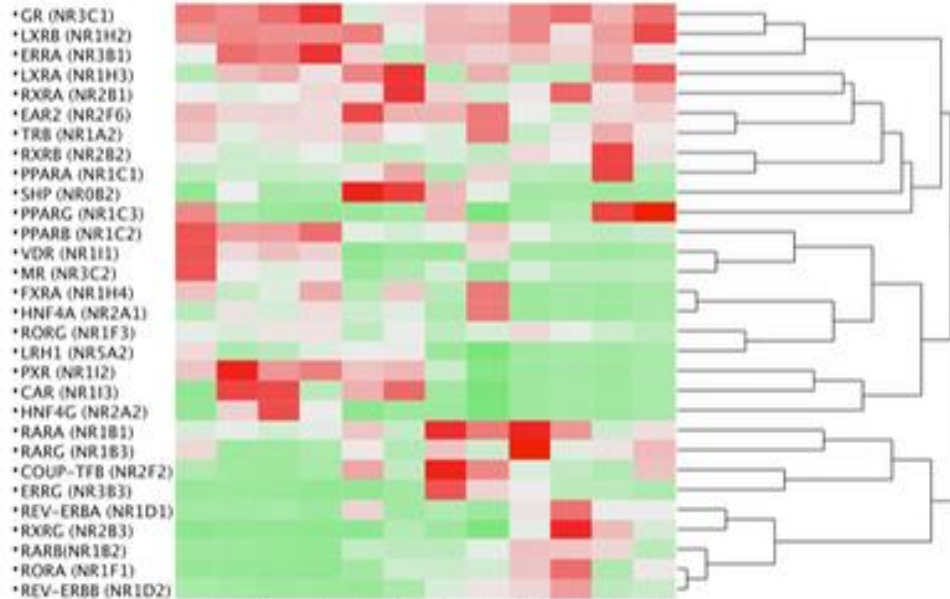


**THE TOOLS TO STUDY  
INTRACELLULAR RECEPTOR  
ACTIVITIES**

# INTRACELLULAR RECEPTORS : THE TOOLS

## GI and Metabolic Systems

### Tissue Expression



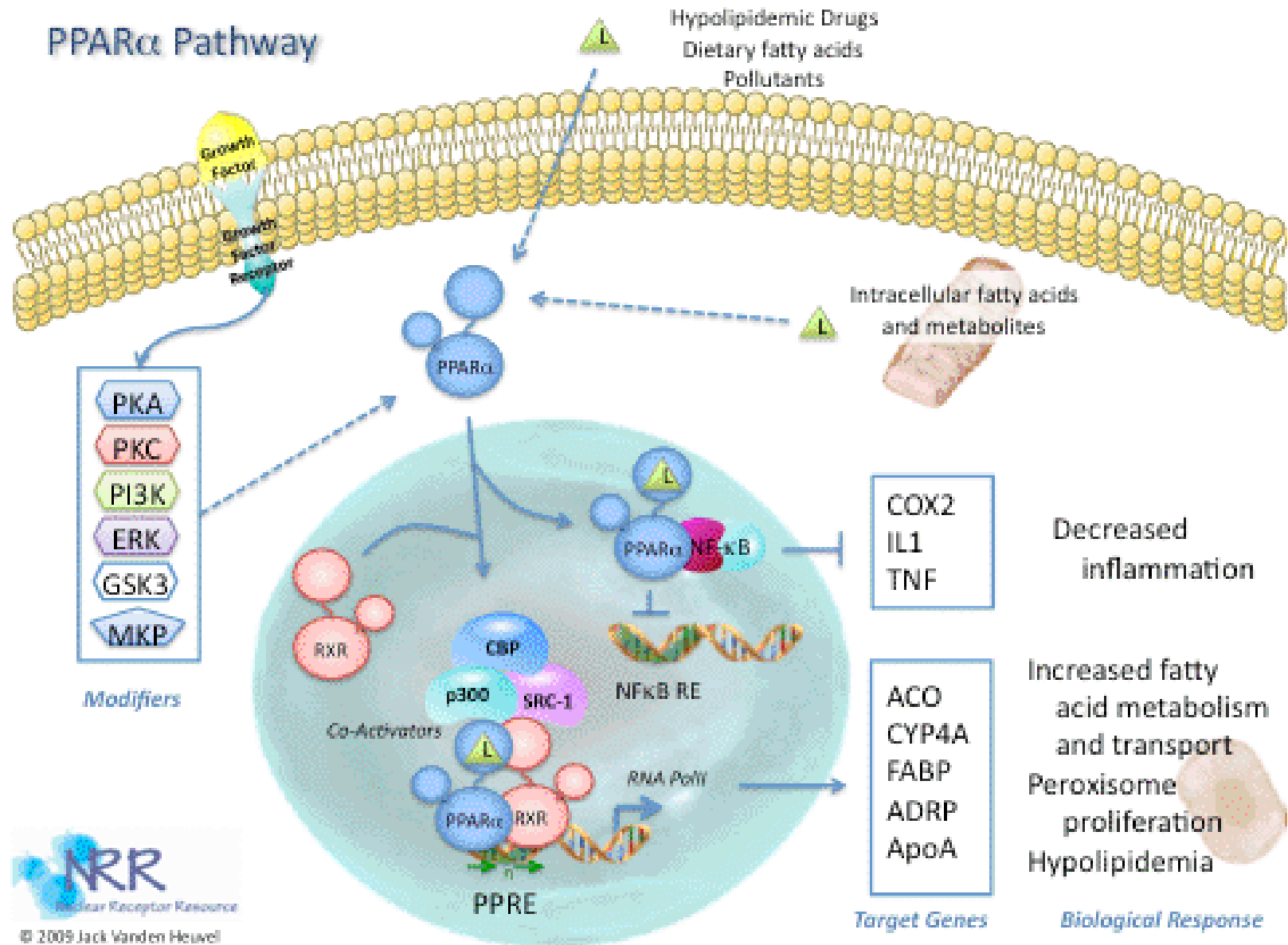
- GR (NR3C1)
- LXRβ (NR1H2)
- ERRα (NR3B1)
- LXRα (NR1H3)
- RXRA (NR2B1)
- EAR2 (NR2F6)
- TRβ (NR1A2)
- RXRE (NR2B2)
- PPARα (NR1C1)
- SHP (NR0B2)
- PPARγ (NR1C3)
- PPARβ (NR1C2)
- VDR (NR1I1)
- MR (NR3C2)
- FXRα (NR1H4)
- HNF4A (NR2A1)
- RORγ (NR1F3)
- LRH1 (NR5A2)
- PXR (NR1I2)
- CAR (NR1I3)
- HNF4G (NR2A2)
- RARA (NR1B1)
- RARG (NR1B3)
- COUP-TFβ (NR2F2)
- ERRγ (NR3B3)
- REV-ERBA (NR1D1)
- RXRG (NR2B3)
- RARB (NR1B2)
- RORA (NR1F1)
- REV-ERBB (NR1D2)

- Colon
- Duodenum
- Jejunum
- Ileum
- Gall Bladder
- Liver
- Stomach
- Kidney
- Tongue
- Skeletal Muscle
- Brown Adipose Tissue
- White Adipose Tissue

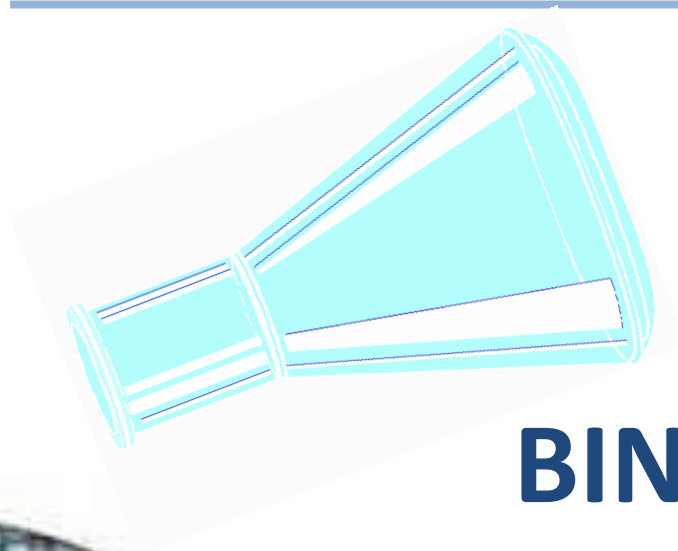
Data from [www.nursa.org/10.1621/datasets.02001](http://www.nursa.org/10.1621/datasets.02001)  
 Contributors: Bookout AL, Jeong Y, Downes M, Yu R, Evans RM and Mangelsdorf DJ  
 Funded by grant DK62434



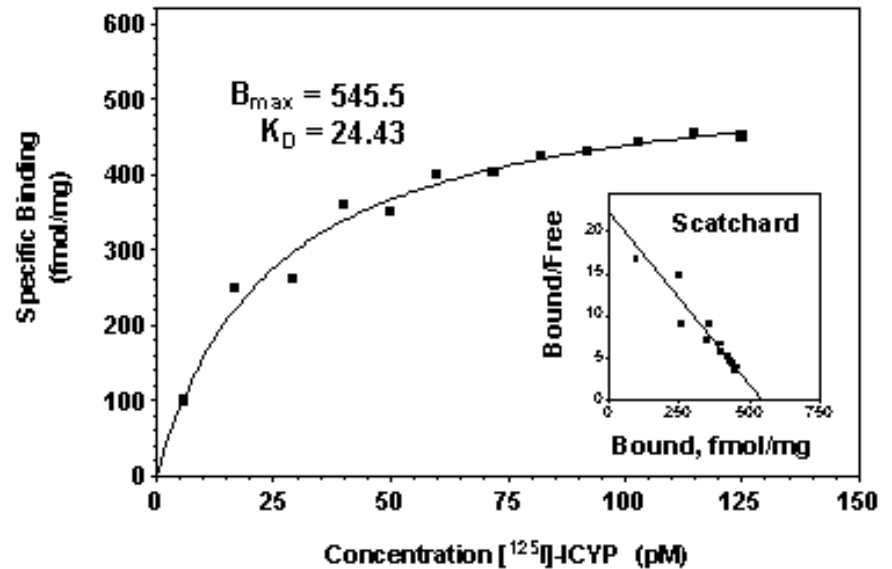
# INTRACELLULAR RECEPTORS : THE TOOLS



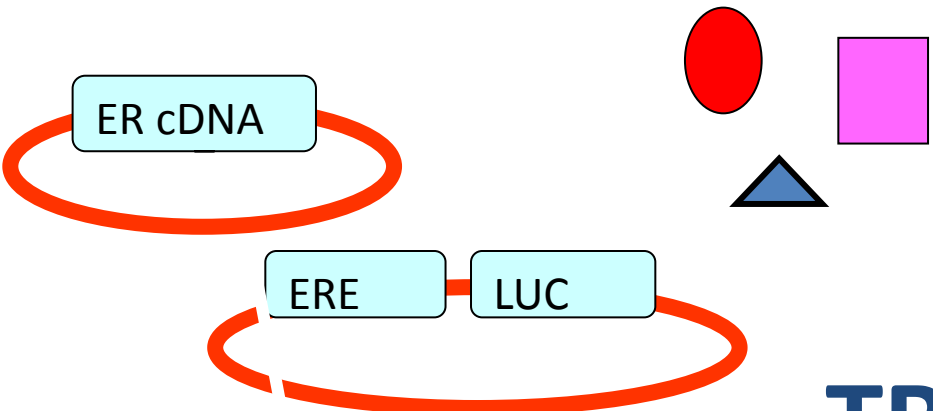
# FUNCTIONAL CHARACTERIZATION OF ER LIGANDS



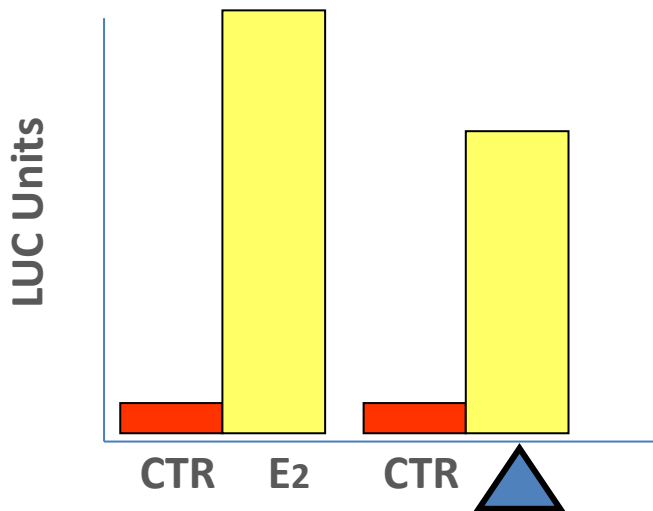
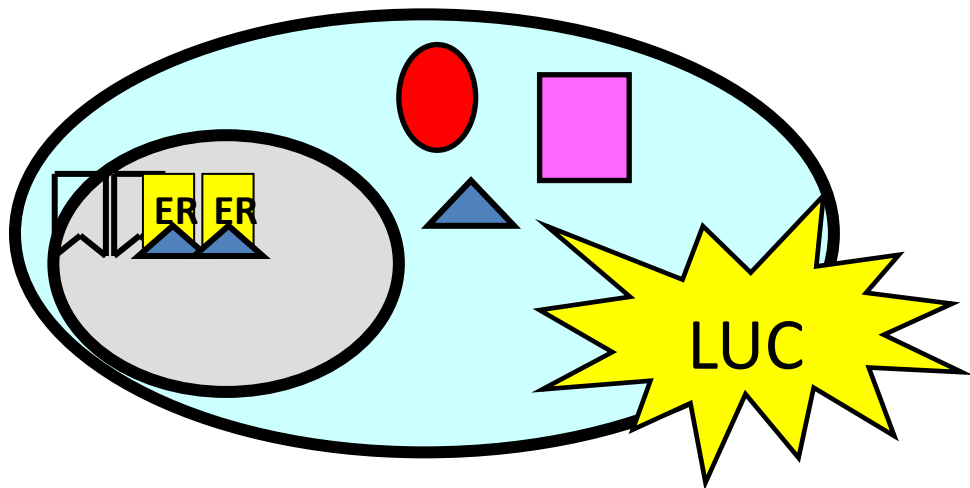
## BINDING ASSAY



# FUNCTIONAL CHARACTERIZATION OF ER LIGANDS



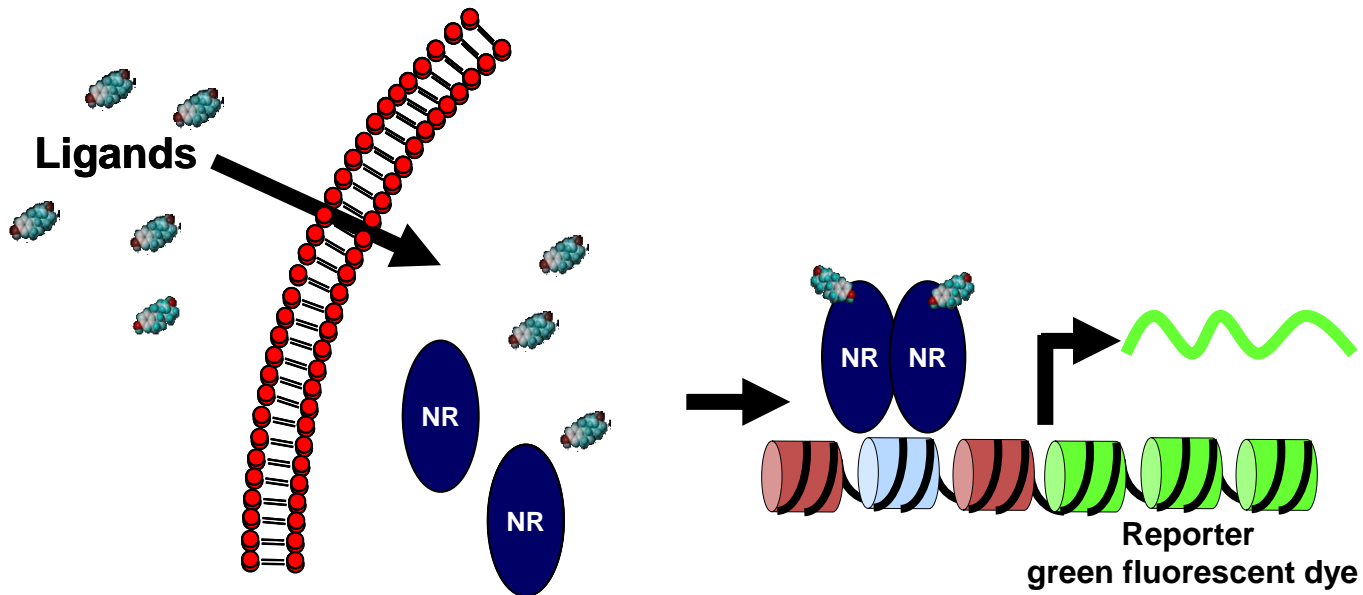
## TRANSFECTION ASSAY



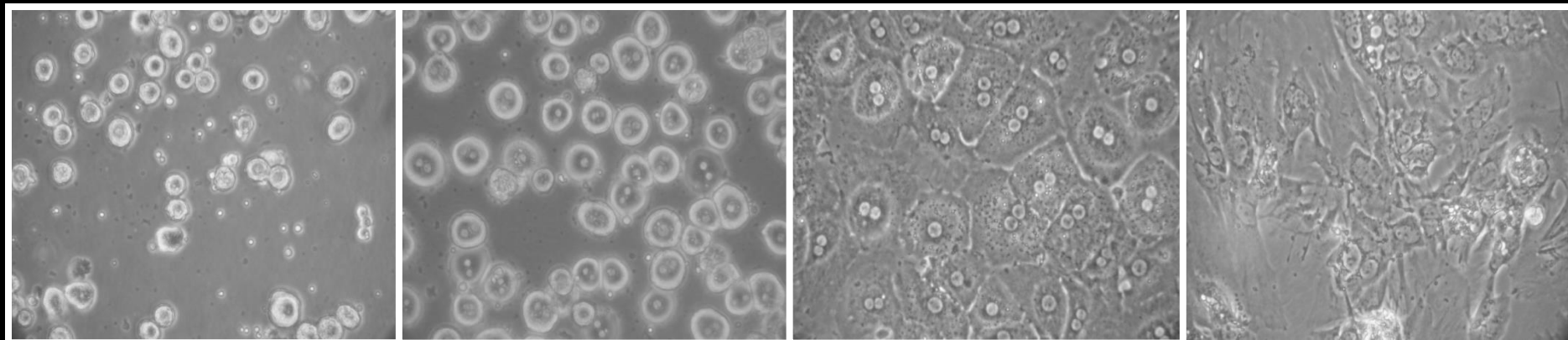


# FUNCTIONAL CHARACTERIZATION OF ER LIGANDS

## TRANSFECTION ASSAY



# ACTIVATION OF ERE-LUC REPORTER IN MOUSE HEPATOCYTES

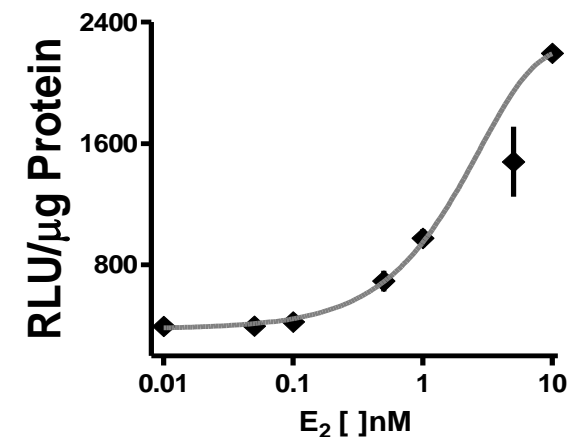
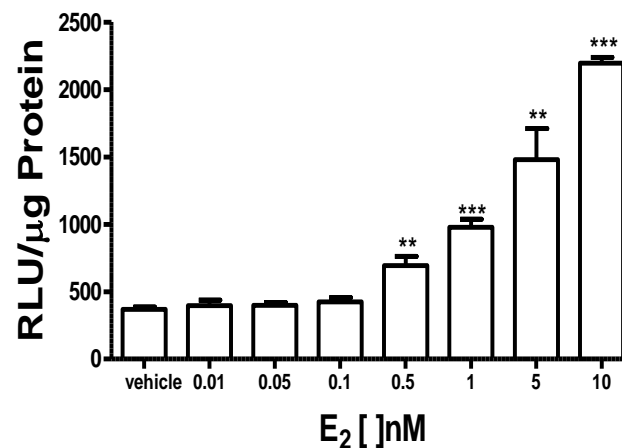
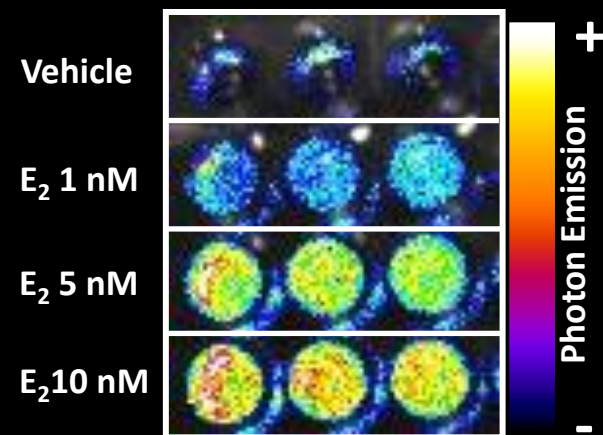


15 minutes

2 hours

18 hours

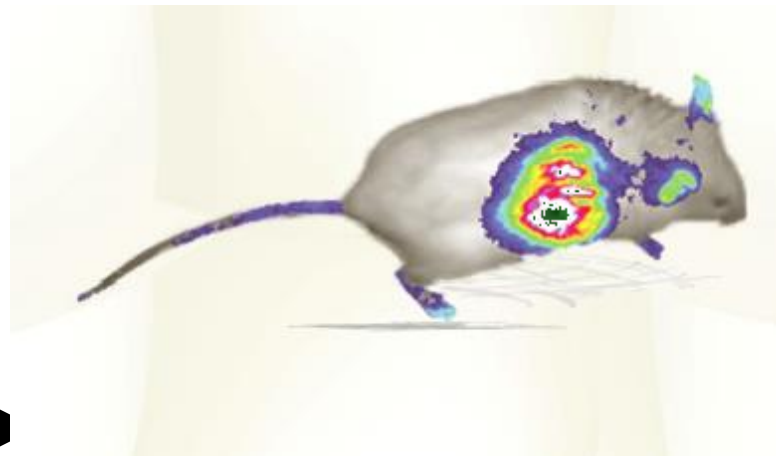
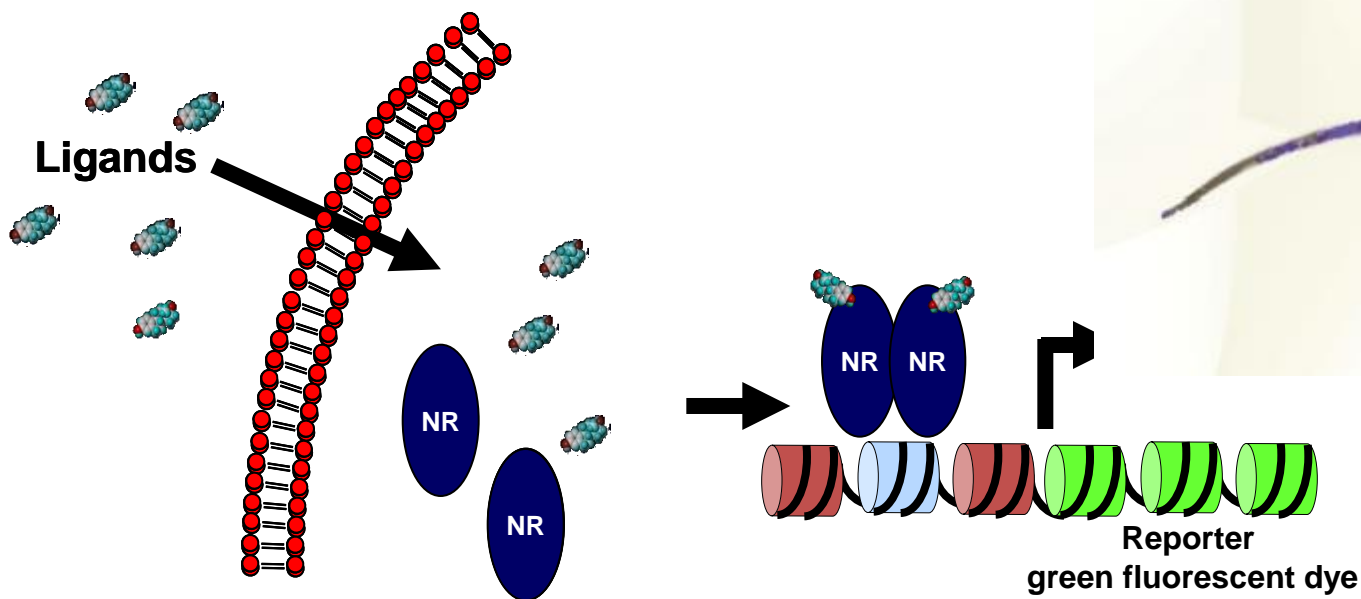
1 week



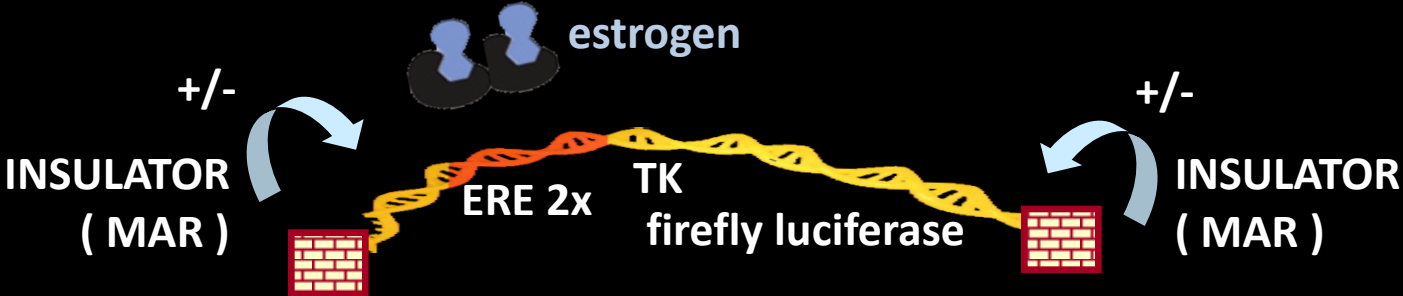
# FUNCTIONAL CHARACTERIZATION OF ER LIGANDS

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## REPORTER MICE, THE ULTIMATE FUNCTIONAL ASSAY



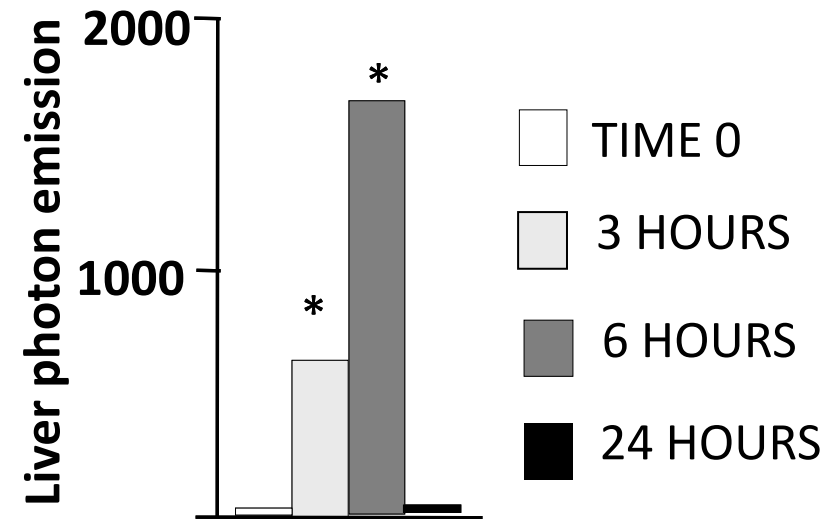
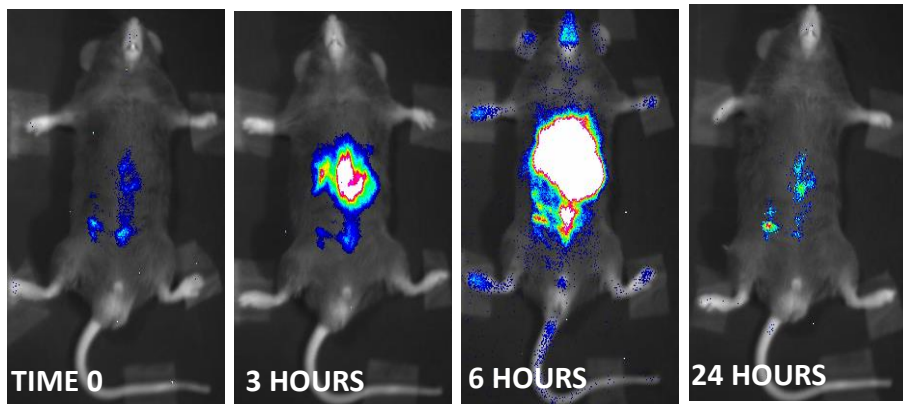
# *ERE-Luc reporter mice to study the dynamics of ER activity in living mice*



Ciana et al., Mol. Endocrinol. 2001  
Ciana et al., Nature Med. 2003

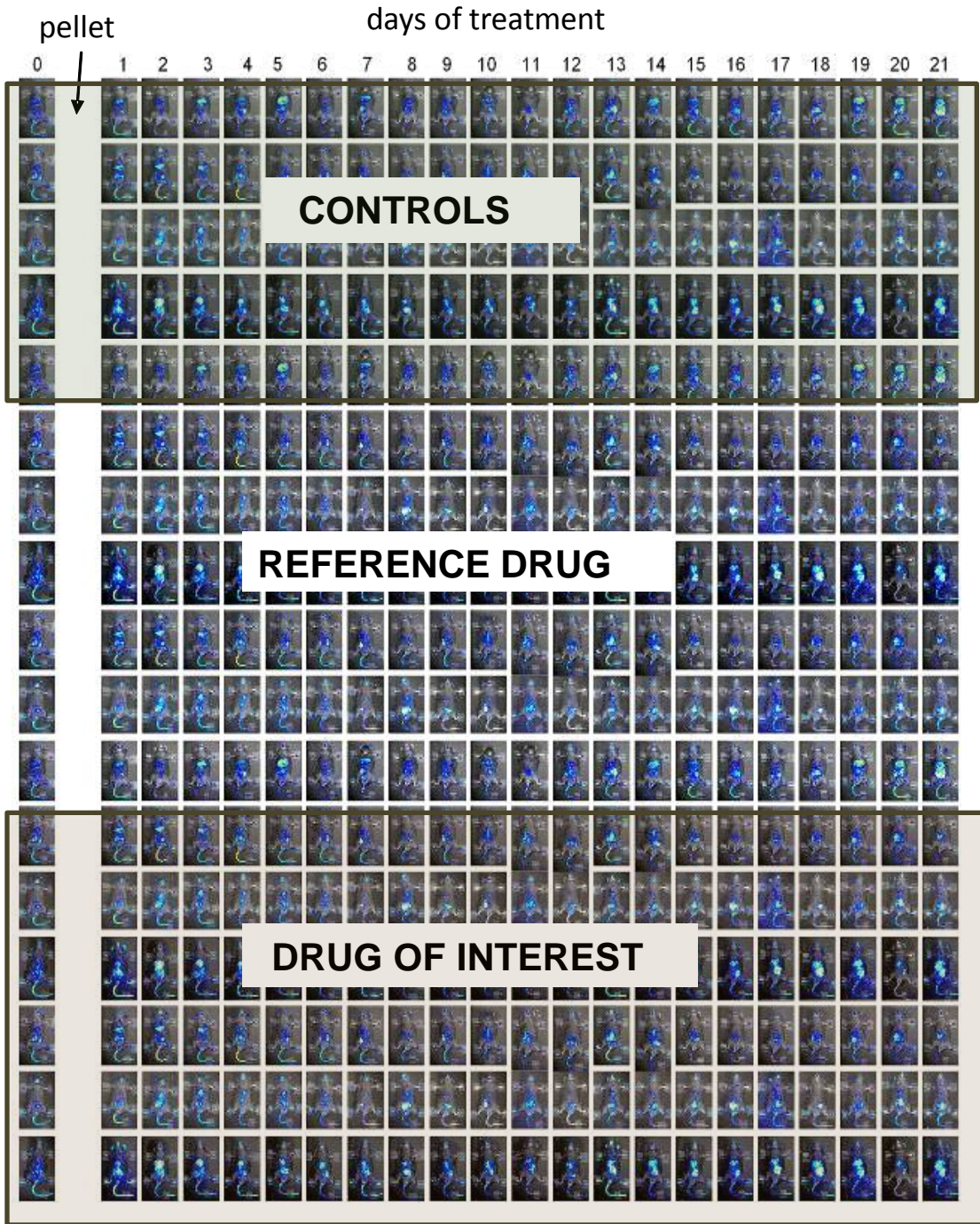
# SEMI-QUANTITATIVE ANALYSIS OF ER ACTIVITY I N THE ERE-LUC REPORTER MOUSE

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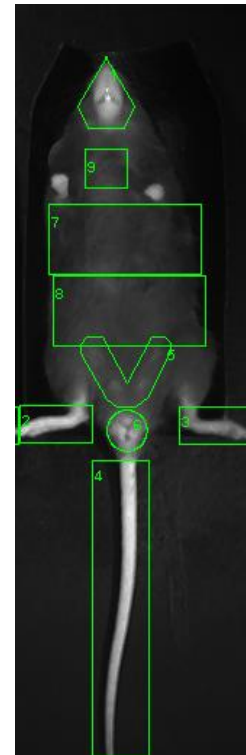
- Access to the dynamic of molecular events in complex organisms
- Access to the dynamic of drug effects
- 3Rs rule in drug development

**THE ERE-Luc REPORTER MOUSE  
A PHARMACOLOGICAL APPLICATION**

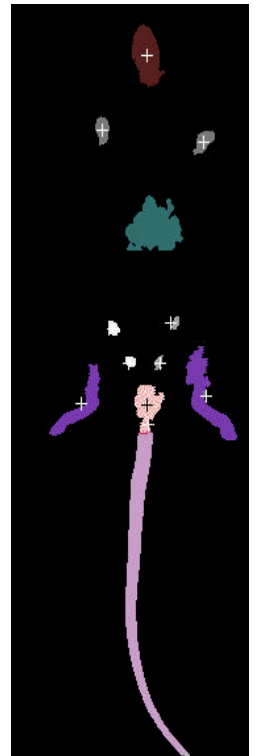


In vivo analysis of photon emission

Manual



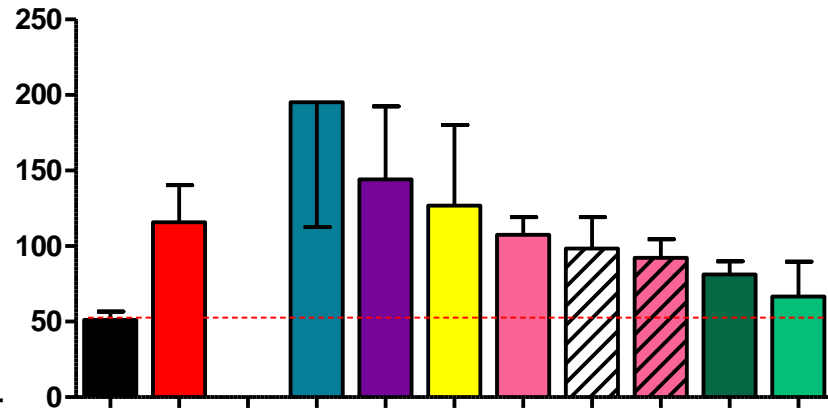
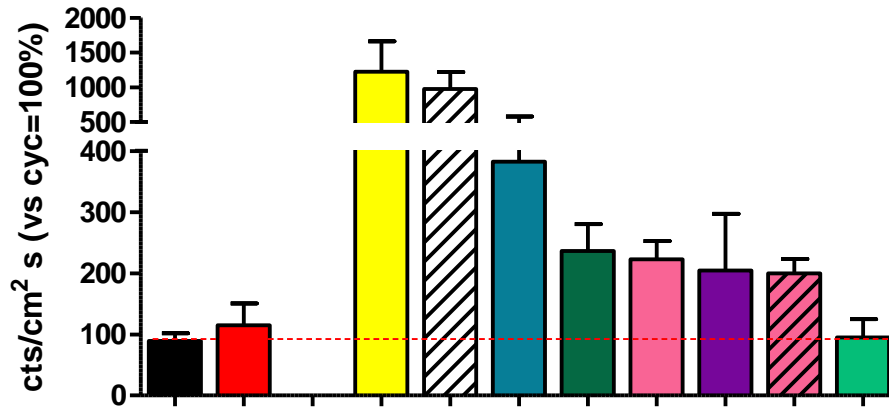
Automatic



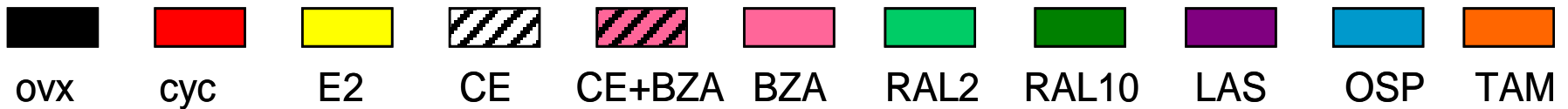
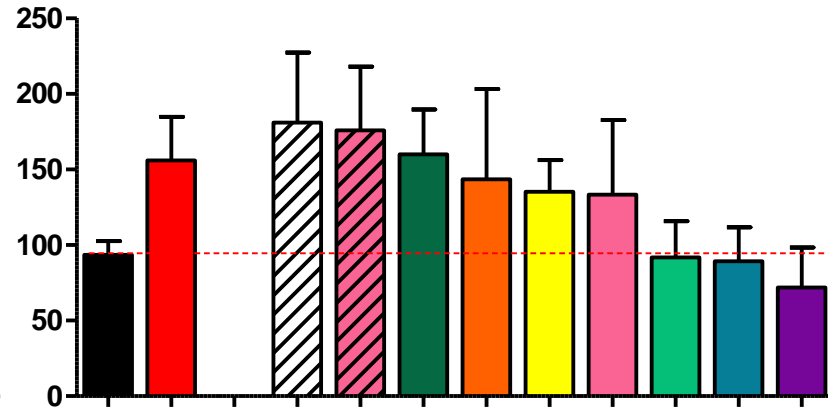
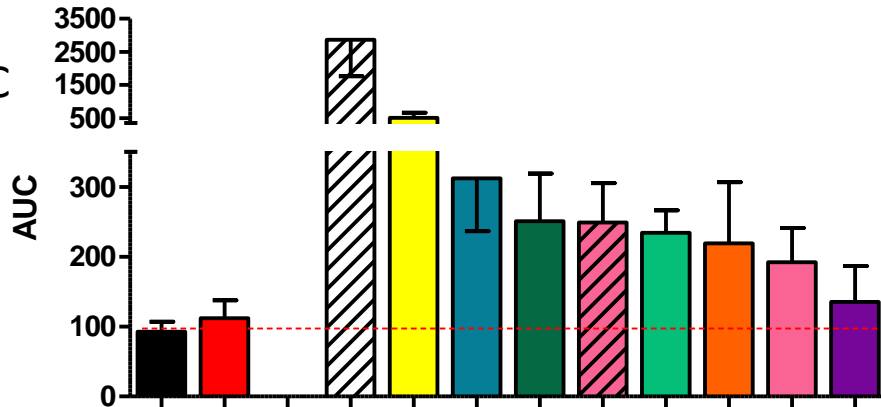
# HEPATIC AREA

# ABDOMEN

ACUTE

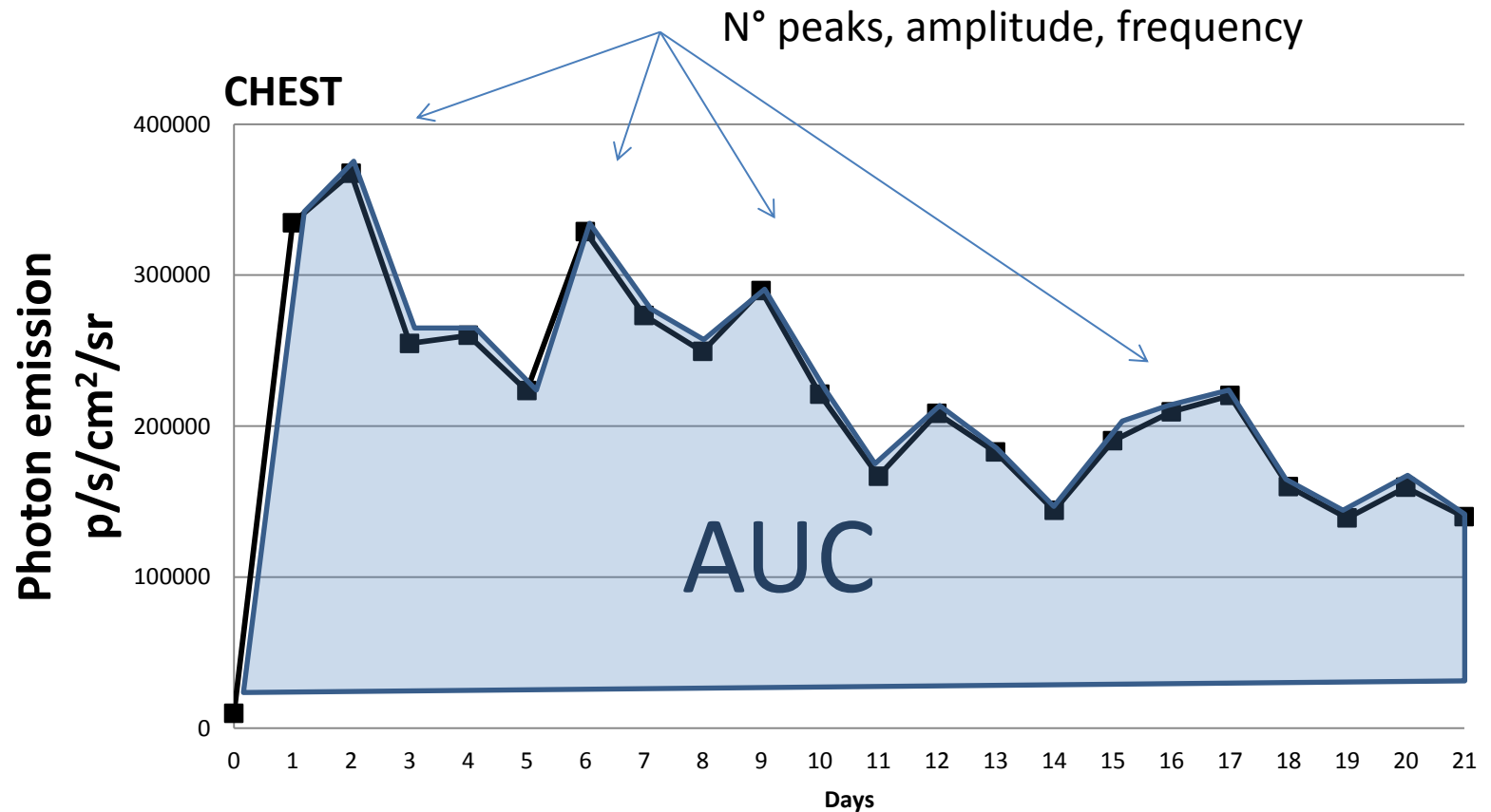


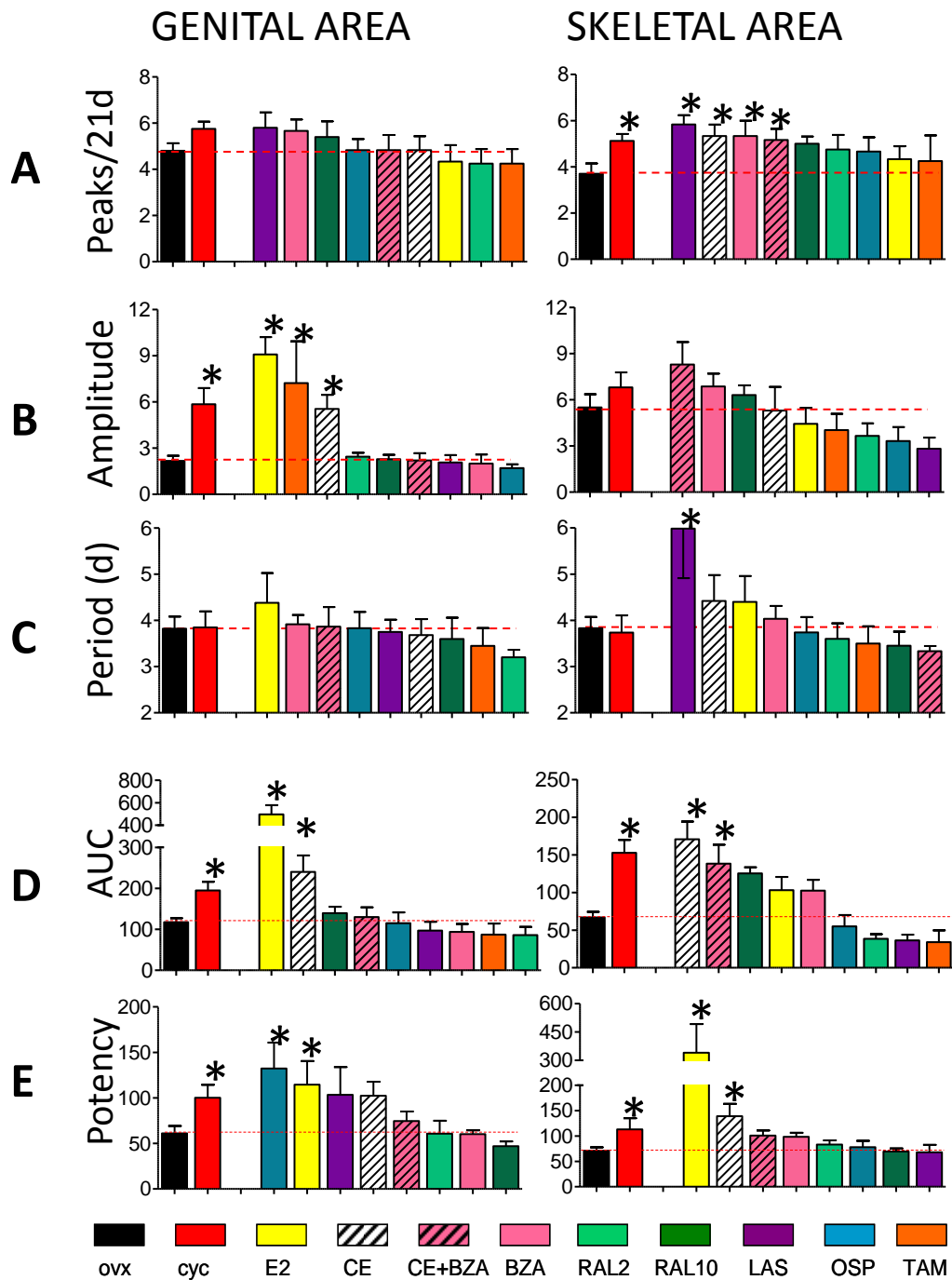
CHRONIC





# SERCHING FOR NOVEL MODALITIES TO MEASURE THE EFFICACY OF SERMs

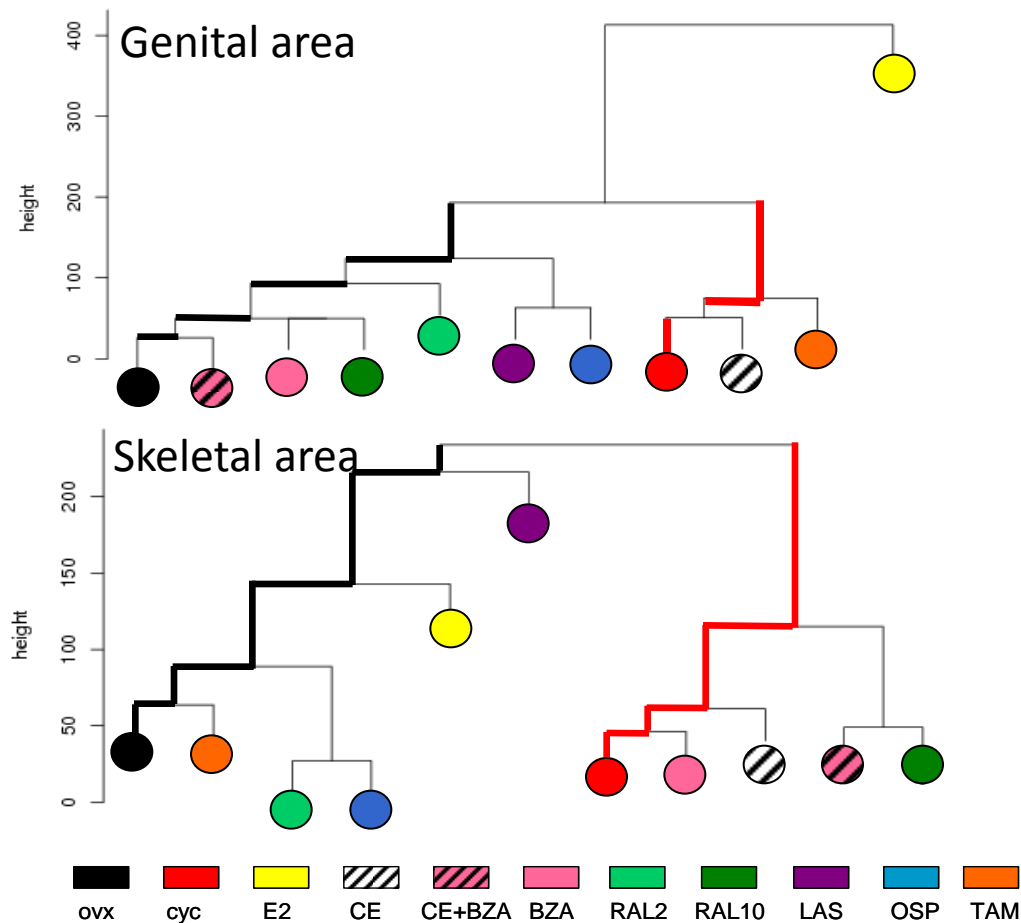


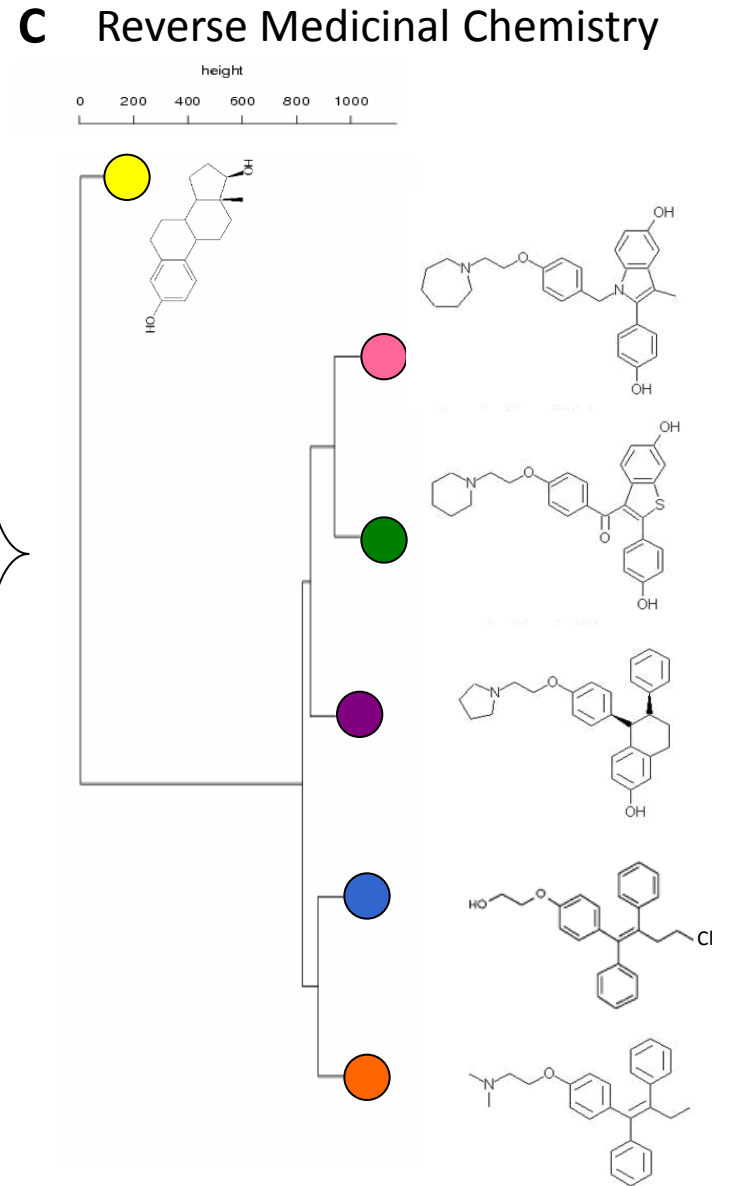
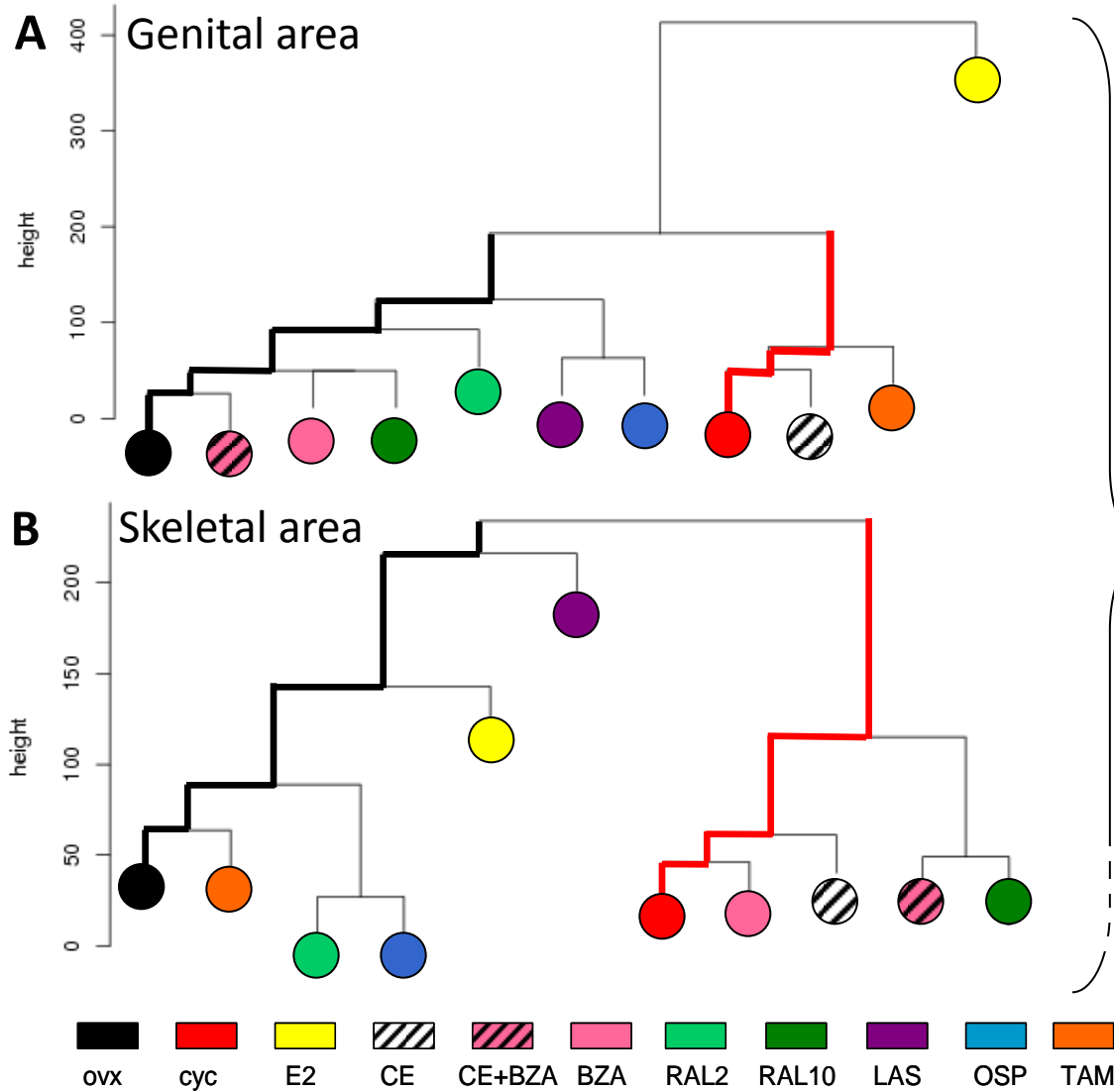


# **PHENETICS OF DRUG ACTION**

**THE APPLICATION OF AGGLOMERATIVE  
HIERARCHICAL CLUSTERING TO THE STUDY OF THE  
*IN VIVO* ACTION OF ESTROGENIC COMPOUNDS**

# clustering data to generate novel families of compounds





**Time and space:** the dimensions for a full understanding of the effect of drugs and pollutants ability to interfere with intracellular receptors activities

## **CONCLUSION 2**

**The ERE-Luc reporter mouse represents a novel tool to identify synthetic compounds and ED present in the environment or in the alimentary chain and to provide a comprehensive view on their activity on the whole body**

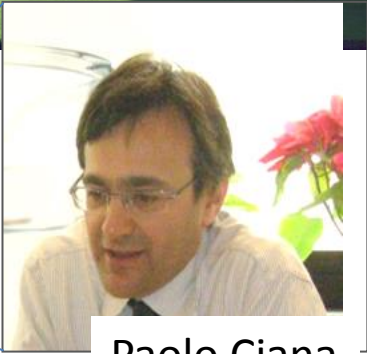


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Center of Excellence on  
Neurodegenerative Diseases

## Collaborators:

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Paolo Ciana



Paolo Ciana



Gianpaolo Rando

## Past Collaborators :

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Luisa Ottobrini  
Gianpaolo Rando

Valeria Benedusi  
Sara Della Torre  
Saba Khalilpour  
Federica Lolli  
Nicoletta Rizzi  
Alessandro Villa



Clara Meda



Sara Della Torre

Clara Meda  
Monica Rebecchi

## Funding:



fondazione  
cariplo

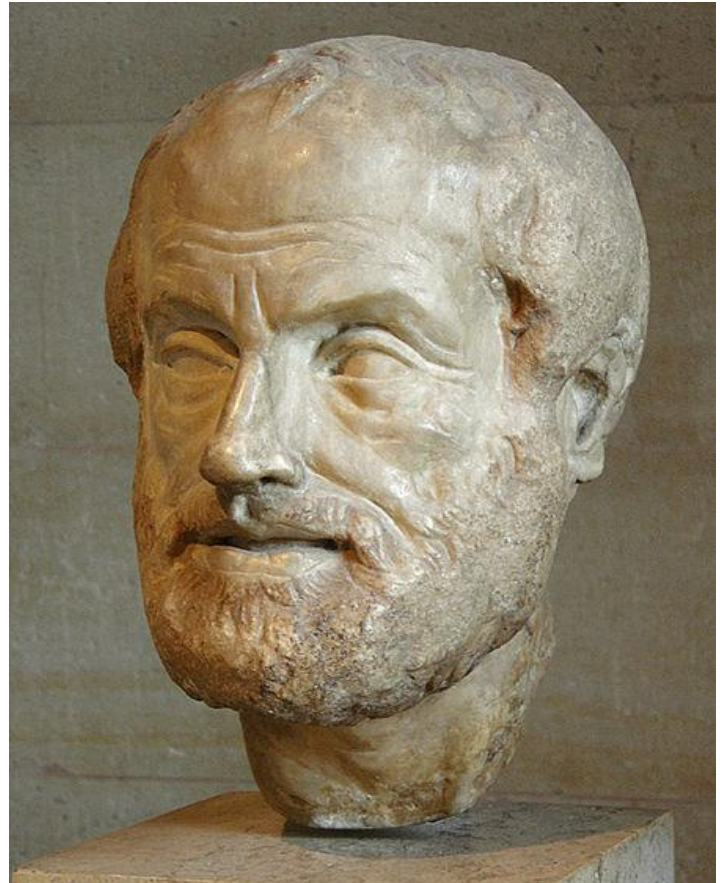




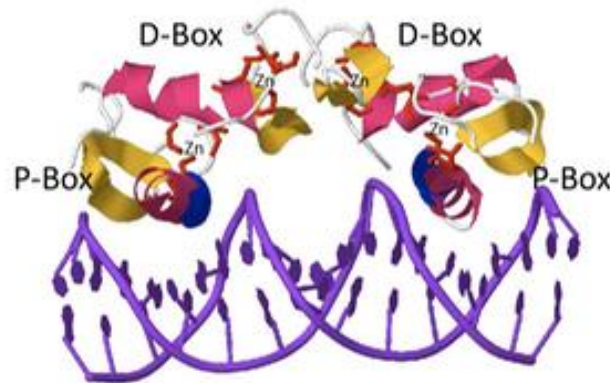
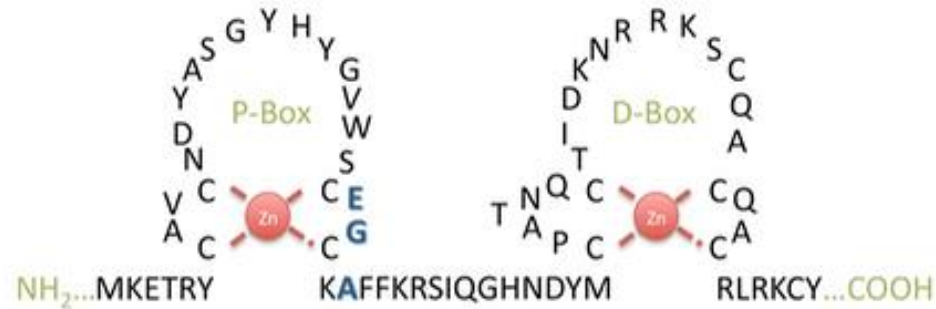
**“The whole is more  
than the sum of its  
parts “**

Aristotle (384 BC – 322 BC)

Metaphysics



# Intracellular Receptors, are transcription factors able to bind the DNA



RECEPTORS	P-BOX	HALF-SITE	RESPONSE ELEMENT
ER	cEGckA	AGGTCA	AGGTCA <sub>n</sub> nnTGACCT
GR, MR, PR, AR	cGSckV	TGTTCT	AGGACA <sub>n</sub> nnTGTCCT
PPAR, RAR, VDR, PPAR	cEGckG	AGGTCA	AGGTCA <sub>n</sub> AGGTCA