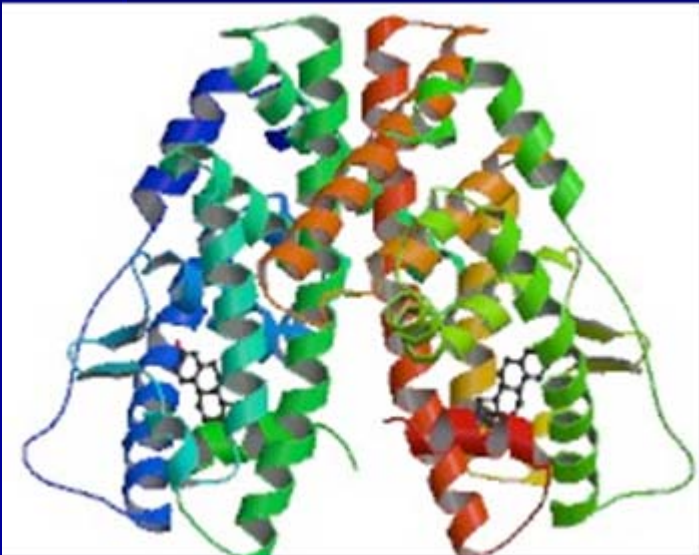
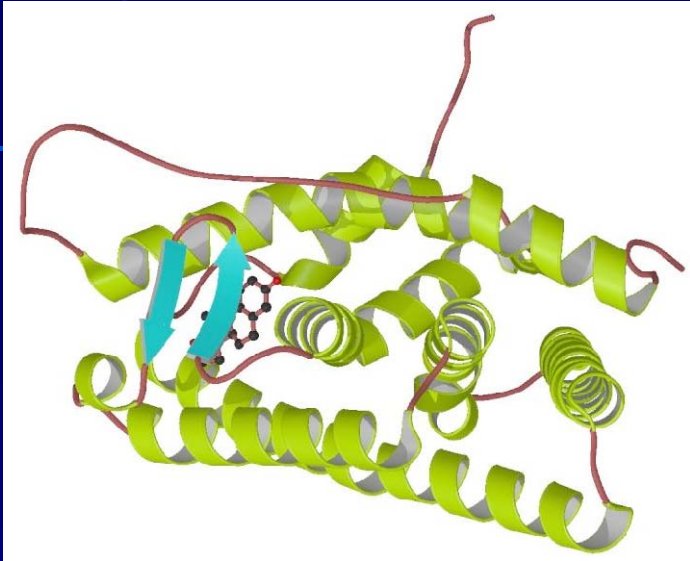


Estrogen Receptor Project

S. Luft '08

- Manmade materials such as pesticides and plasticizers have been implicated in human pathologies such as cancer and autism.
- *Can these compounds bind to and interfere with steroid receptors such as the estrogen receptor? Are the **xenoestrogens**?*
- We'll use polarization anisotropy to test.

Estrogen Receptor alpha



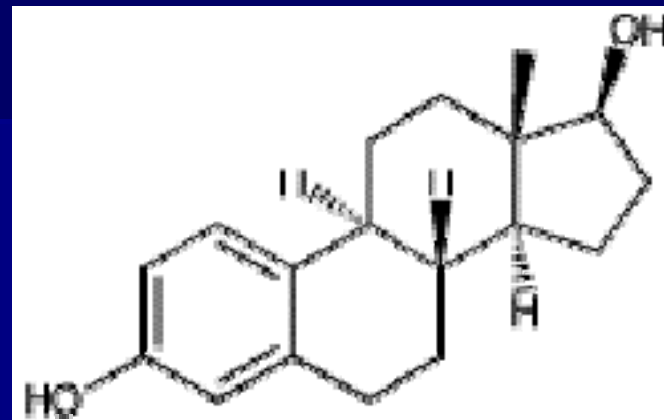
- Ligand binding domain, DNA binding domain, 2 transcription activating domains
- Two wedges, each of 12 alpha helices, form a dimer with the LBD in between at the narrow end
- Can bind to a range of ligands because the LBD is so large
- ER + ligand moves from cytosol to nucleus and binds to the estrogen response element (ERE) section of DNA

http://www.ebi.ac.uk/msd-srv/msdtarget/strs_images/1qkt.jpeg

http://www.pdb.org/pdb/images/1g50_bio_r_250.jpg?bioNum=2

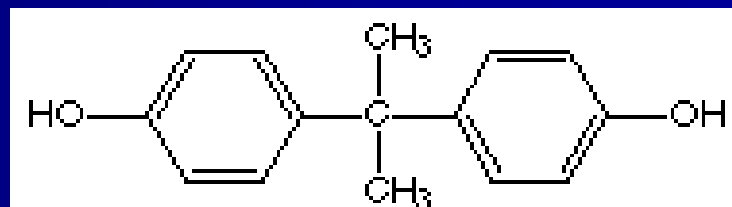
Xenoestrogens mimic estrogen

- Estradiol- primary biological substrate
- xenoestrogens have the common feature of an aromatic ring with an electronegative atom on the ring (DDT and bisphenol A)



Estradiol

<http://www.3dchem.com/imagesofmolecules/Estradiol.gif>



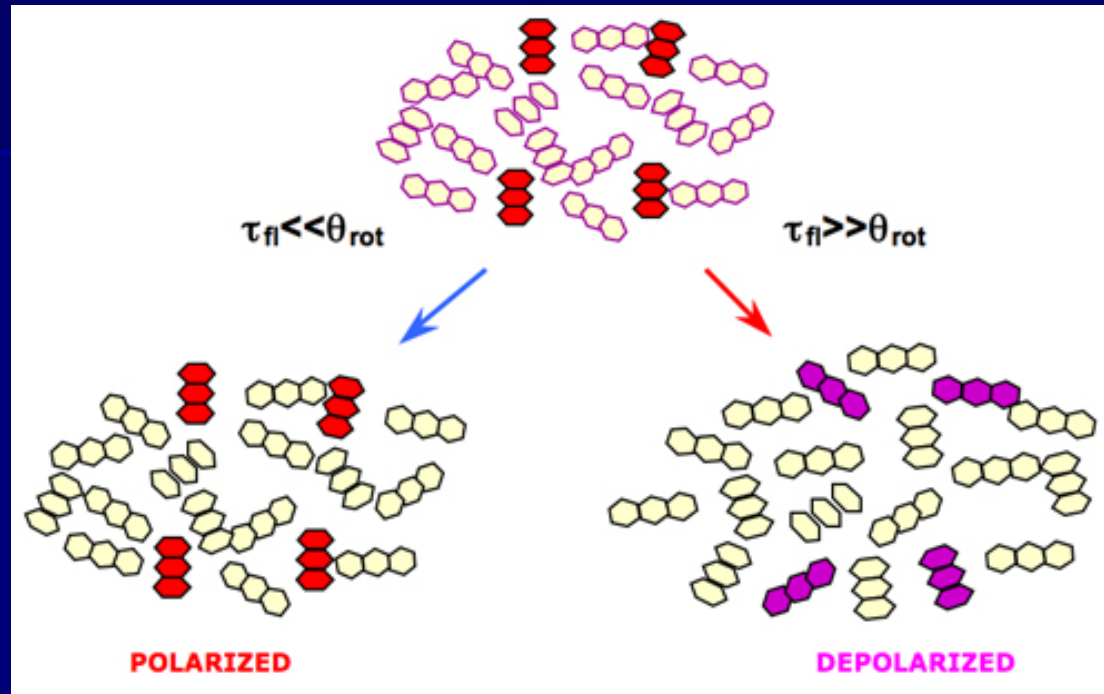
Bisphenol A

http://courses.washington.edu/z490/ed/Other%20Risks%20for%20People_files/image002.gif

Fluorescence Polarization

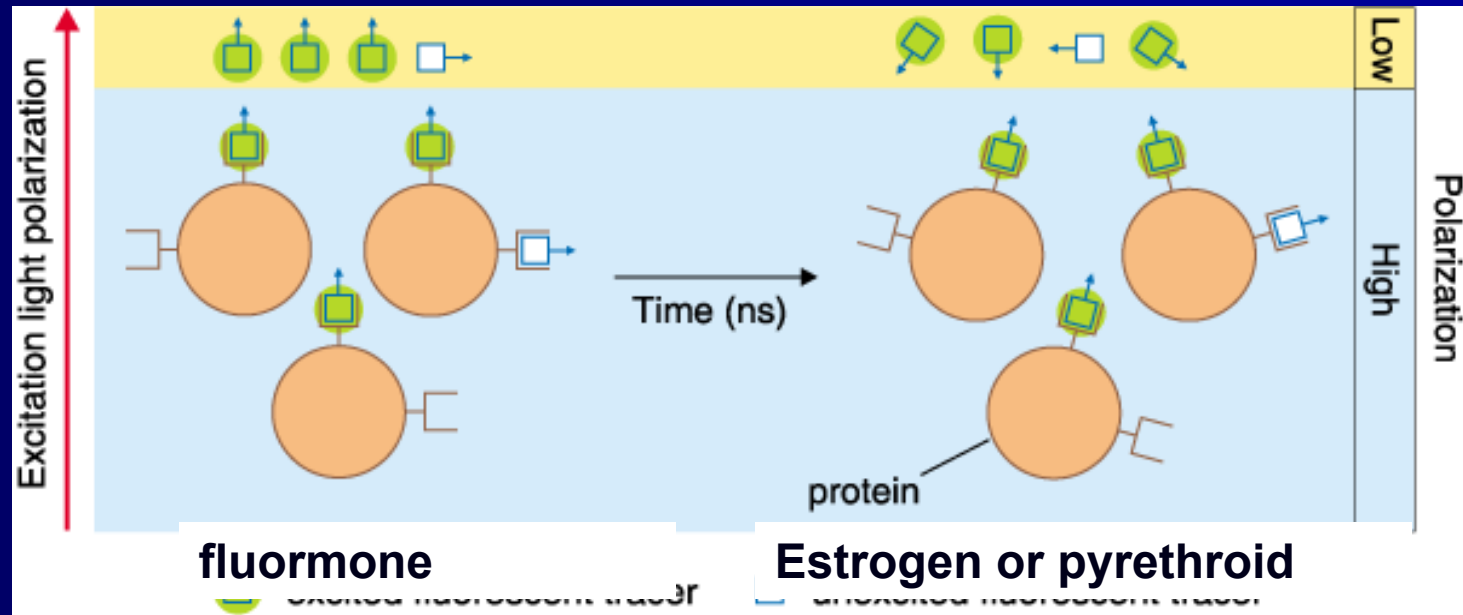
Fluorescent Lifetime = τ_{fl}

Rotational correlation time = θ_{rot}



- If $\tau_{fl} \ll \theta_{rot}$ (bound fluorophore) molecule doesn't rotate during excited state lifetime and emitted light will be polarized.
- If $\tau_{fl} \gg \theta_{rot}$ (free fluorophore) molecule doesn't rotate during excited state lifetime and emitted light will be depolarized.
- A fluorescent tagged molecule is displaced from the receptor by a competitor molecule (estrogen or xenoestrogen)
- When this occurs, the released tagged molecule rotates more rapidly in solution, and thus polarization decreases.

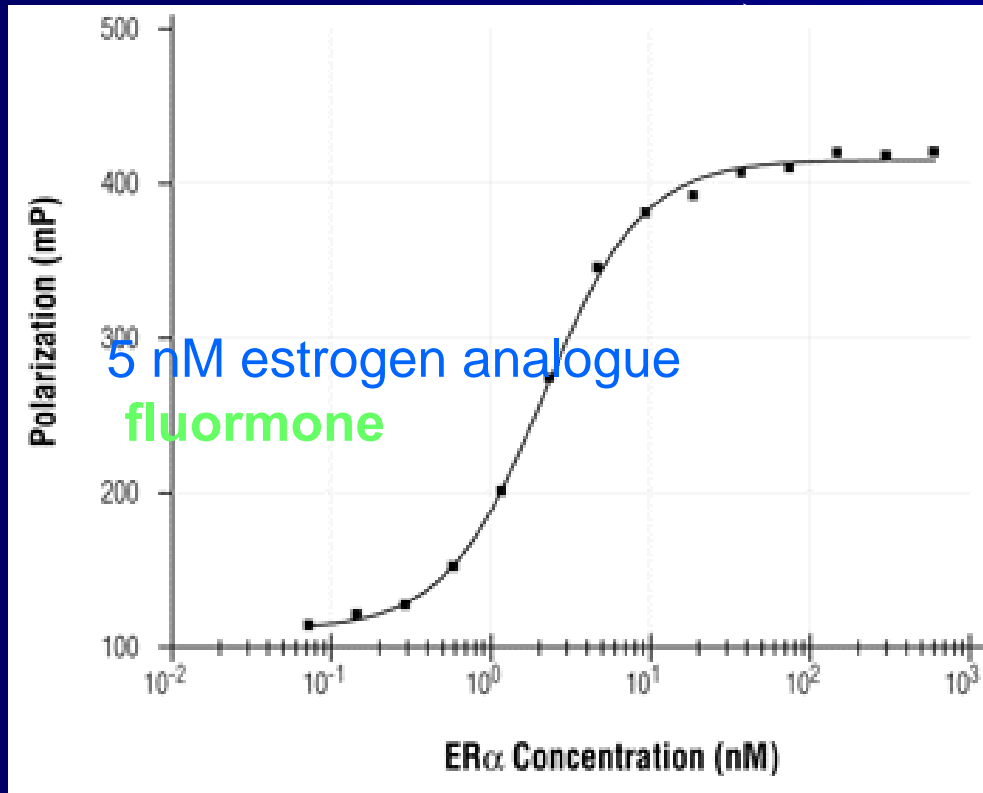
Xenoestrogen Project



Polarization Measures Binding

Increasing amounts of estrogen receptor

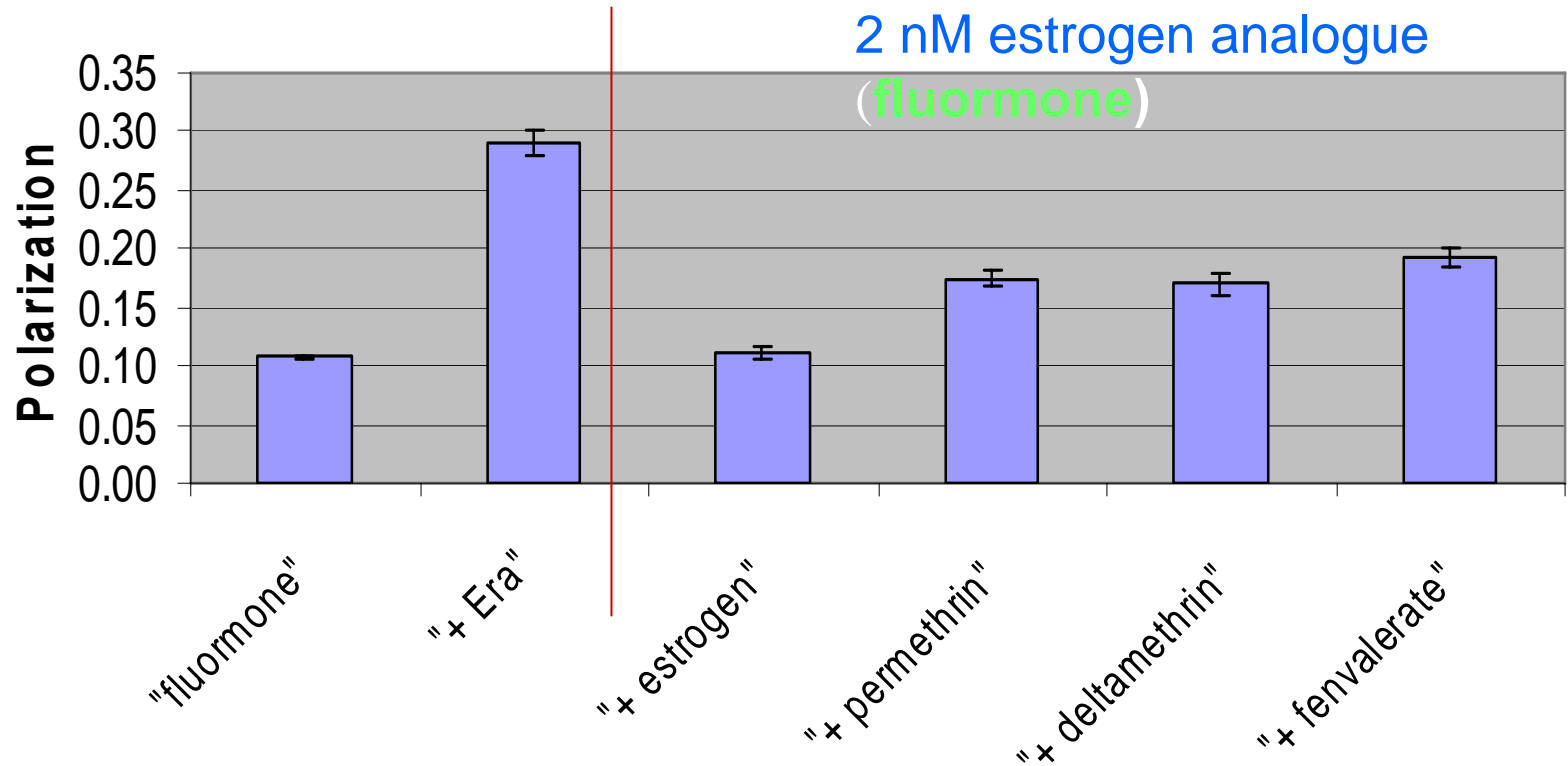
- When small molecules which tumble freely (low polarization) bind to much larger molecules which don't tumble as easily (high polarization), there is a concomitant increase in polarization



Fluormone binding to Estrogen Receptor

Polarization Measures Competition

Pyrethrins Bind to Estrogen Receptor



- Pyrethrins tested ARE able to mimic estrogen in the displacement of fluormone from the ER