Summary of *Investigating the Effects of Estrogen Cofactors on Ligand Binding Affinity*
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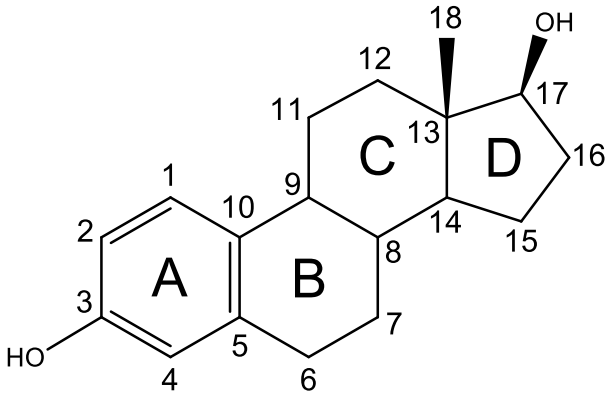
Introduction

Estrogens form a class of steroid hormones with important systemic effects. They promote cell growth, reproductive function and sexual differentiation, exert neuro- and vascular protective effects, and are important in bone growth and maintenance. Abnormal estrogenic function is implicated in pathologies including breast, endometrial and prostate cancers, infertility and osteoporosis. Many exogenous chemicals, including compounds found in soy, plastics and pharmaceuticals, exert physiologically significant estrogenic effects.



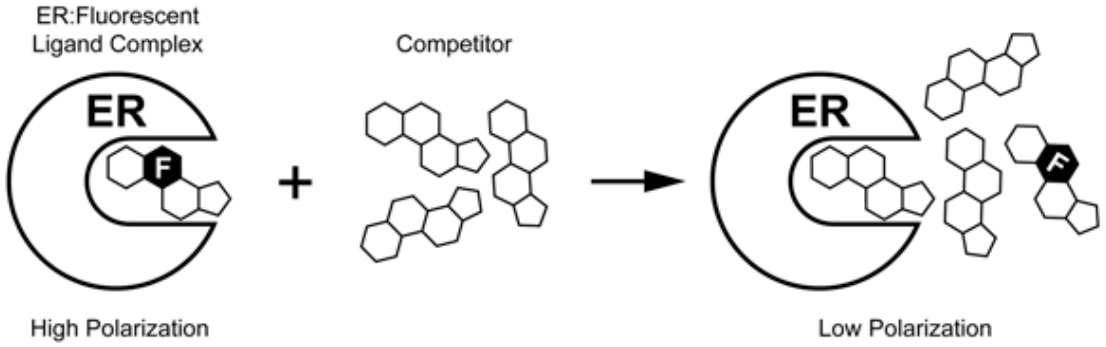
The structure of 17β-Estradiol, the most common and most potent endogenous estrogen, with carbons numbered and rings lettered.

The effects of estrogen are mediated through the estrogen receptors (ER-α and –β), which bind hormone, dimerize, bind to DNA, recruit other transcription factors, and induce transcription of downstream genes. Steroid Receptor Coactivators (SRC) 1 and 3 are ER cofactors recruited into the transcription complex. They bind the receptors with high affinity, change ligand-receptor dissociation half-lives and are recruited in a ligand-specific manner. The present work uses a fluorescence polarization competitive binding assay established through previous work in the O'Hara lab and elsewhere to test whether SRC-1 changes the binding affinity of estradiol for the ERs.

Materials and Methods

In a solution of constant temperature and viscosity, the rate of rotation of a molecule as a whole, called tumbling, is correlated with its molecular weight. The binding of a low MW fluorescent "tracer" molecule (MW < 1000 Da) to receptor proteins like ER (MW > 60 kDa) yields a significant change in the tumbling rate of the tracer. Because fluorophores always absorb and emit only light with an electric vector parallel to the transition dipole moment of the fluorophore, a change in the tumbling rate of a fluorophore can be detected as a

change in the ratio of the intensity of emitted light polarized parallel and perpendicular to the exciting light. This calculated ratio, the difference in intensities over the sum, is called the fluorescence polarization. Unbound fluorophore should yield a low polarization value because the low weight fluorophore rotates quickly in solution; in the fluorophore-receptor complex, the effective molecular weight of the fluorophore is much higher, yielding a lower rate of tumbling and a higher polarization. A competitor (estrogen) is titrated into a solution containing at least the receptor and tracer; the resulting polarizations are fitted to a sigmoidal dose-response curve.



Schematic of a fluorescence polarization competitive binding assay <http://tools.invitrogen.com/content/sfs/manuals/polarscreen_ERbeta_Green_man.pdfa>. When the fluorescent tracer is mostly bound to the receptor, it will return a high value of polarization; when it is unbound, the polarization value will be low. By tracking the competitor dose-dependency of depolarization, the binding affinity of the competitor molecule can be tracked.

The vertical midpoint of this curve is the half-maximal inhibition constant (IC50) and, with the known dissociation constant (Kd) and concentration ([S]) of the tracer, can be used in the Cheng-Prusoff equation to calculate the inhibition constant (Ki) of the competitor.

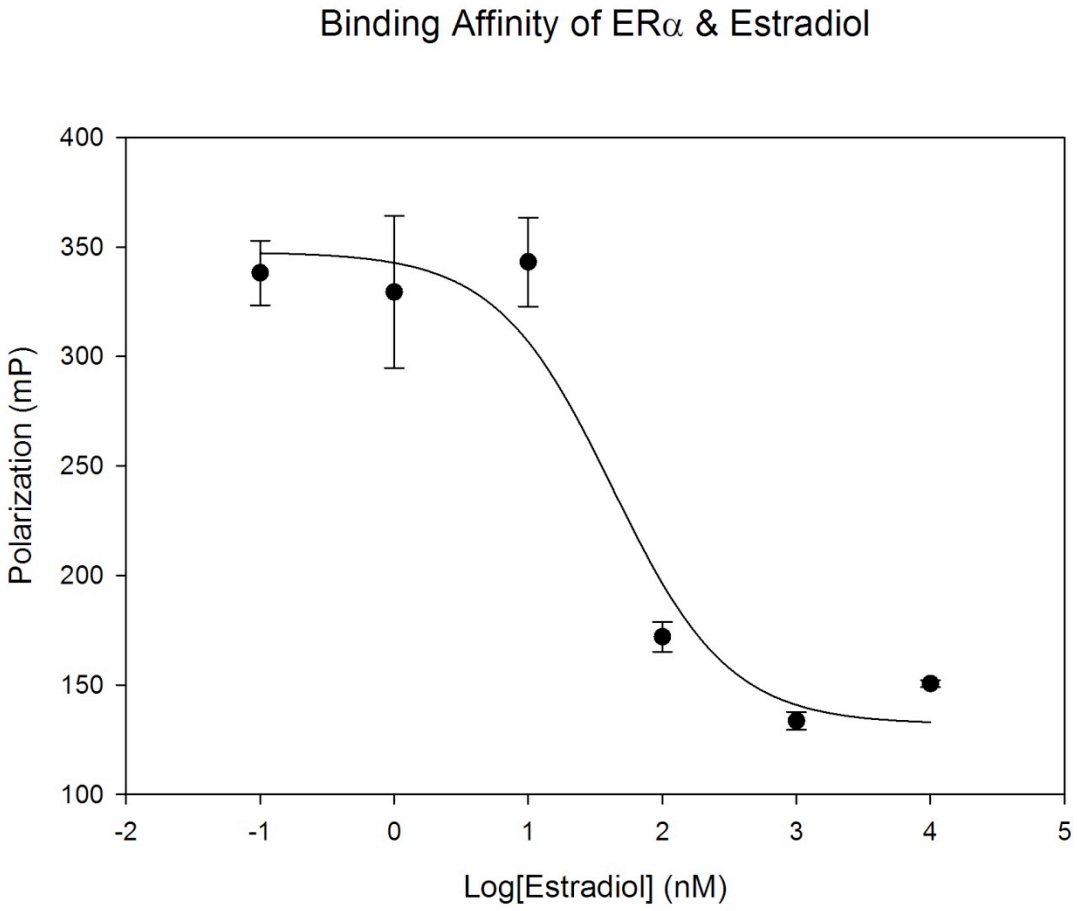
$$K_i = \frac{IC_{50}}{1 + \frac{[tracer]}{K_d}}$$

The Cheng-Prusoff Equation for competitive binding assays. Ki is the inhibition constant of the competitor; IC50 is the half-maximal inhibition constant; [tracer] is the concentration of tracer; Kd is the dissociation constant of the tracer for the receptor.

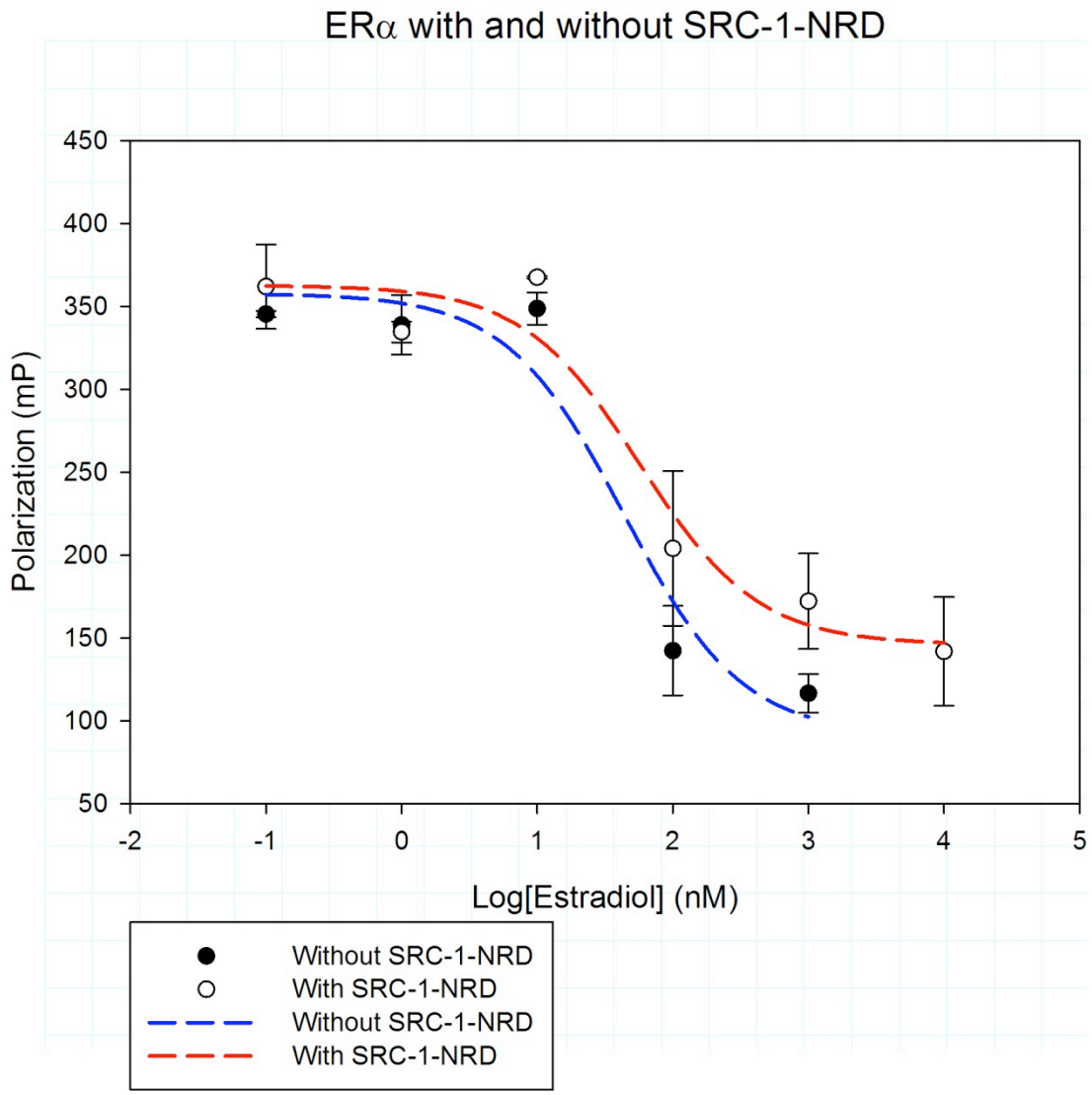
Results & Conclusions

Plasmids encoding the ligand binding domains (LBDs) of the ERs and the nuclear receptor interacting domains of SRC-1 and -3 were obtained and their encoded proteins were expressed in *E.* coli and purified by nickel-affinity chromatography. Fluorescence

polarization competitive binding assays using these reagents as well as those purchased from Invitrogen did not reproduce the data from Invitrogen and were consistently just barely within the margin of error of the results of CT Knuff. Initial experiments comparing ligand-receptor binding affinities in the presence or absence of SRC-1 showed no significant difference.



Plot of ER-α & Estradiol Competitive Binding Assay, with Dose-Response Curved Fitted. This is within error of CT Knuff's results, but a log-fold higher than those of Invitrogen.



Binding Affinity of Estradiol & ER-α in the Presence and Absence of SRC-1-NRD. No significant difference was found.

Further work is necessary to validate this assay. The purification process for the ER LBD peptides must be improved. The precise conditions of the assay need to be optimized to reduce variability and improve reproducibility.