DOCTORAL THESIS

Measuring binding kinetics of ligands with tethered receptors by fluorescence polarization complemented with total internal reflection fluorescence microscopy

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Measuring Binding Kinetics of Ligands with Tethered Receptors by Fluorescence Polarization Complemented with Total Internal Reflection Fluorescence Microscopy

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Abstract

The study of the binding between estrogen receptors (ER) and their ligands *in vitro* has long been of interest mainly because of its application in anti-estrogen drug discovery for breast cancer treatment as well as in the screening of environmental contaminants for endocrine disruptors. Binding strength was conventionally quantified in terms of equilibrium dissociation constant (K_D). Recently, emphasis is shifting towards kinetics rate constants, and off-rate (k_{off}) in particular. This thesis reported a novel method to measure such binding kinetics based on fluorescence polarization complemented with total internal reflection fluorescence (FP-TIRF). It used tethered receptors in a flow cell format. For the first time, the kinetics rate constants of the binding of full-length human recombinant ERα with its standard ligands were measured. k_{off} was found to range from 1.3×10^{-3} to 2.3×10^{-3} s^{-1}. k_{on} ranged from 0.3×10^{5} to 11×10^{5} M^{-1} s^{-1}. The method could also be used to screen potential ligands. Motivated by recent findings that ginsenosides might be functional ligands of nuclear receptors, eleven ginsenosides were scanned for binding with ERα and peroxisome proliferator-activated receptor gamma (PPARγ). None of the ginsenosides showed significant binding to ERα, but Rb1 and 20(S)-Rg3 exhibited significant specific binding with PPARγ.

Table of Contents