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Evaluating the Heterodimerization of Nuclear Receptors and Their Ligand Affinity Using Flim-fret and Fcs Methods

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The vitamin D receptor (VDR) and liver X receptor (LXR) form heterodimers with retinoid X receptor (RXRα) in a ligand-dependent manner, regulating gene expression related to development and metabolism. Using FLIM-FRET microscopy, the interaction between VDR, LXR, and RXRα was analyzed, revealing FRET efficiencies of 8% for VDR-RXRα and 3.5% for LXR-RXRα without ligands, which increased to 12% and 4.5% upon treatment with their respective agonists (calcitriol and GW3965). This suggests that ligand activation enhances VDR and LXR interactions with RXRα.

A ligand sensor (EGFP-RXRα-LBD-mScarlet3) was created to detect RXRα agonist binding and measure affinity (Kd) through FRET efficiency changes due to conformational shifts. Fluorescence correlation spectroscopy (FCS) assessed the molecular brightness of EGFP, indicating the oligomerization state of RXR-LBD and related FRET efficiency changes. Cell lysates expressing the biosensor were purified using GFP-Trap Agarose beads and titrated with RXR agonists.

Overall, FLIM-FRET and FCS effectively studied RXRα interactions, with plans for further exploration of nuclear receptor associations and their co-factors using various fluorescence microscopy techniques.