Modulation of Nuclear Receptors through Ligand Architecture

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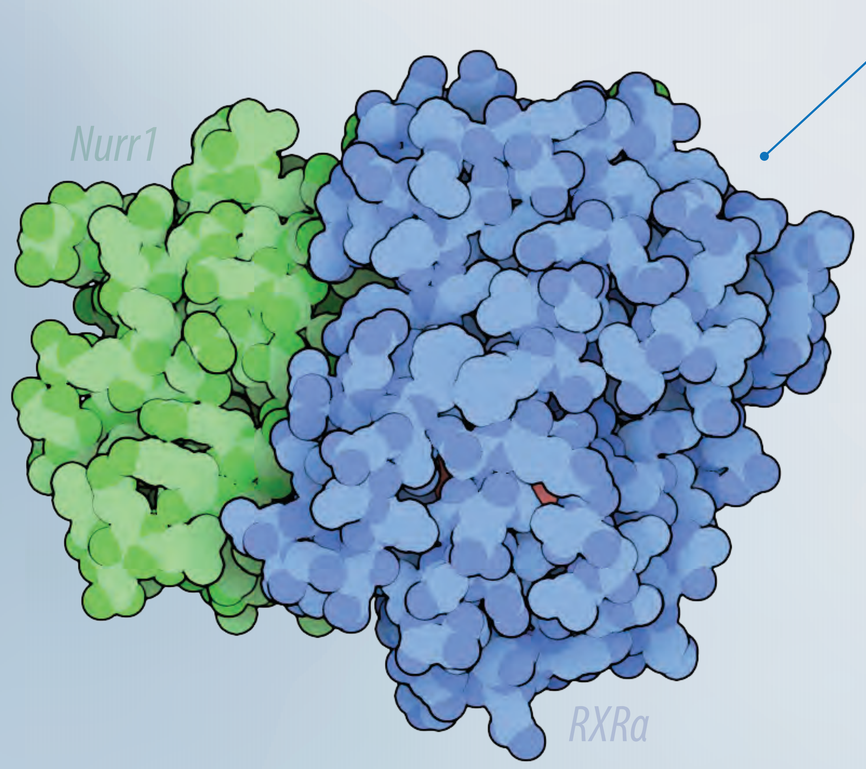
Nuclear receptors (NRs) have been one of the primary drug targets over the last decades for their ability to regulate gene expression. The traditional approach of modulating NRs is to design small synthetic molecules that interact with the ligand-binding domain (LBD) of the NR. Ligands can thereby either enhance or inhibit gene transcription. Apart from the effects on transcription, recent research shows that minor changes in the ligand scaffold can have a significant impact on the behavior of the NR. In this research, we show how small-molecules can change both the dimerization behavior of NRs and the recruitment of allosteric modulators. A detailed understanding of these ligand effects on a molecular level is essential for the design of more selective and effective drug molecules.



RXR-Nurr1 heterodimer model

Due to the absence of a crystal structure of the RXRα-Nurr1 heterodimer in literature, a model was made based on the full-length RXRα-PPARα crystal structure. Interestingly, H12 of Nurr1 is present at the dimerization interface, making contact with H7 and H11 of RXRα. The RXRα ligand is directly behind these helices. Therefore, we believe that ligands that introduce bulk towards H7 or H11, will abolish RXRα-Nurr1 heterodimerization.

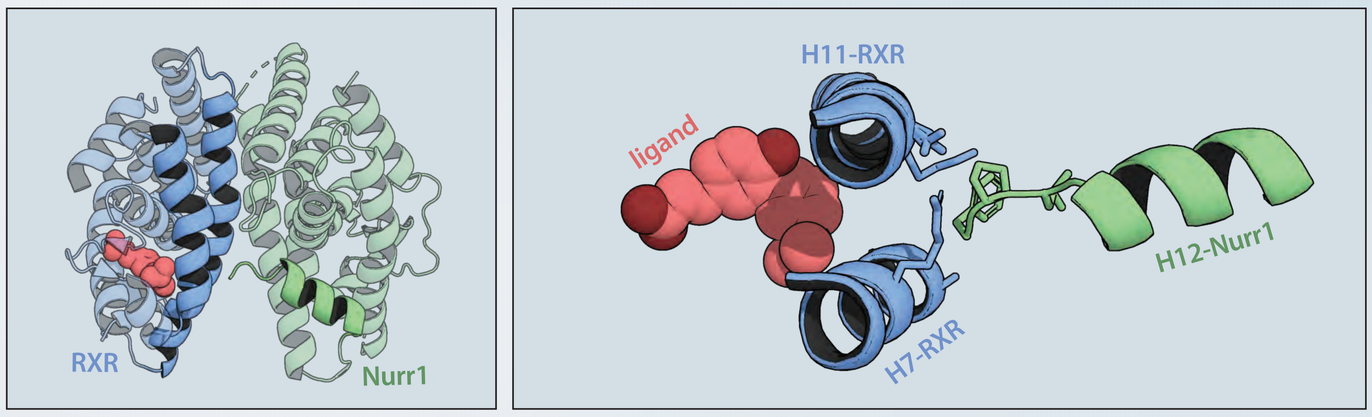
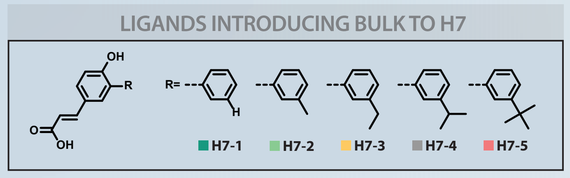
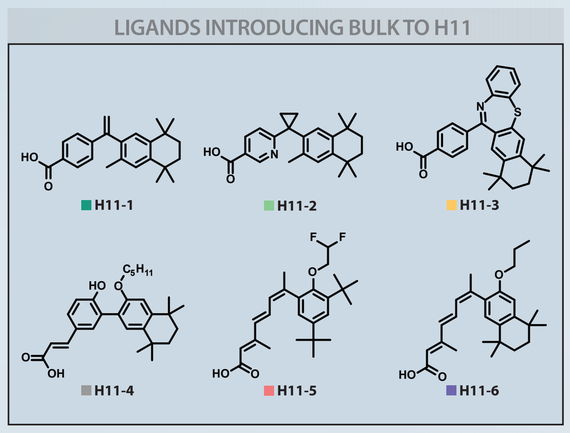


Figure 1 | Left. Structural model of the RXRα-Nurr1 heterodimer. Helices that are believed to be important for dimerization are highlighted. **Right.** Focussed view of the highlighted part, emphasizing the steric clash.

Quantification of RXR dimerization using the NanoBiT Technology

RXRα and Nurr1 LBD is functionalized with either small or large BIT from the NanoBIT system. Upon dimerization of the receptor, the SmBIT and LgBIT can associate, thereby producing a luminescent signal. Ligands promoting dimerization, therefore, enhance the luminescent signal. Ligands probing H7 and H11 of RXR were tested separately.





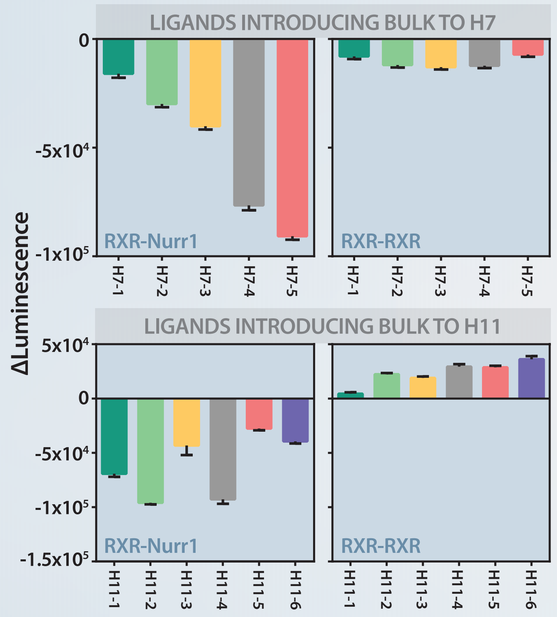
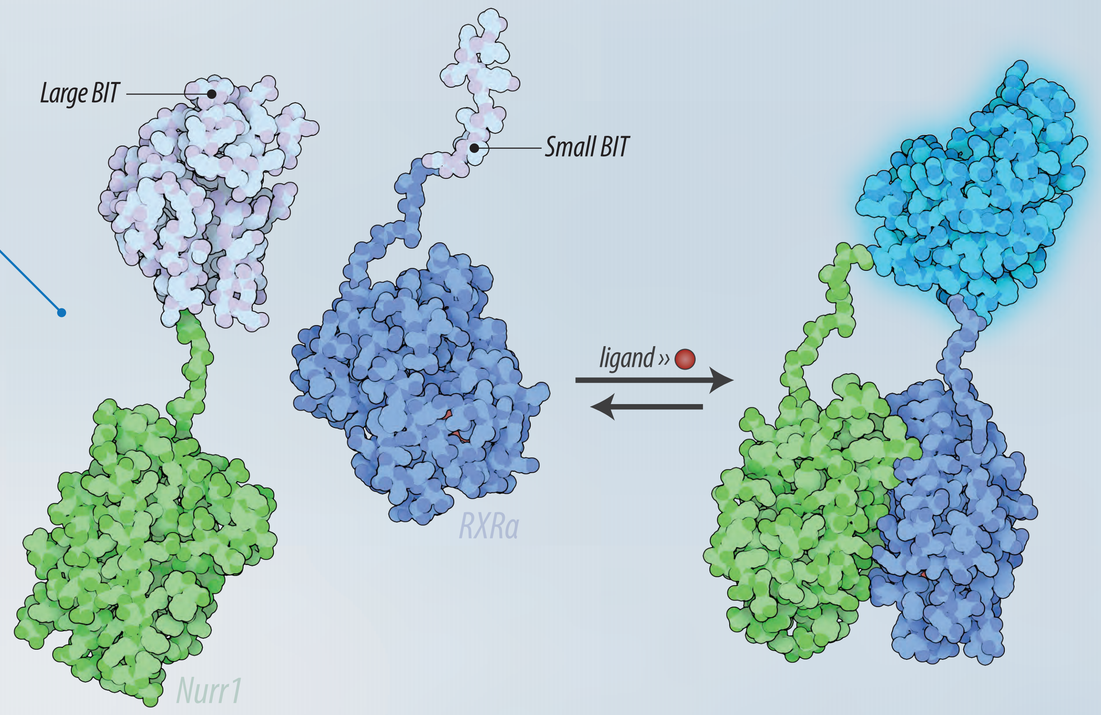


Figure 2 | Left. RXRα ligands used to investigate the effect of introducing bulk towards both H7 and H11. **Right.** Results of the NanoBiT complementation assay for both RXR-RXR homodimerization and RXR-Nurr1 heterodimerization. The luminescent signal was measured after equilibrium was reached and corrected to a DMSO control.



Crystal structures of RORγt with orthosteric and allosteric ligands

Recent research by our group shows that there is a cooperative binding of orthosteric and allosteric ligands in RORγt. To investigate how allosteric ligands influence the binding of the orthosteric ligand and vice versa, we crystallized RORγt in complex with both ligands. We successfully generated structures for four orthosteric and three allosteric ligands. Binding of the orthosteric ligand causes a conformational change (petrol color) in the protein, thereby "clamping" the allosteric ligand.

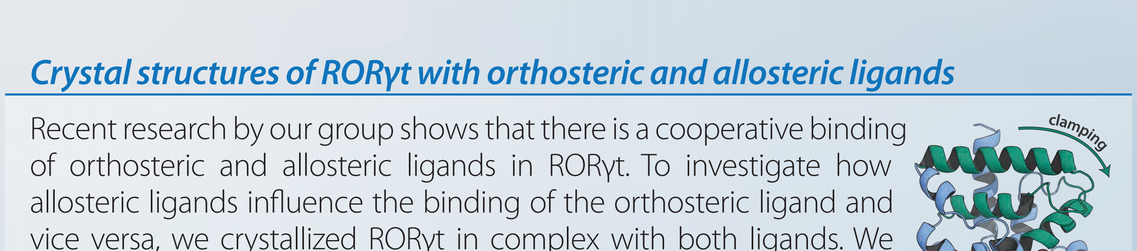


Figure 3 | Co-crystal structures of RORγt with multiple orthosteric and allosteric ligands.

Take-Home Message

Apart from their effect on transcription, the shape and chemical composition of the NR ligand shows to be important in other processes such as dimerization and allosteric modulator recruitment. The type of agonist or antagonist used in animal studies can, therefore, have a significant impact on the phenotypic readout.

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