Determine basis of RXR and DIOXIN binding utilizing crystallography

1. Binding affinity of hRXRα and Dioxin. The affinity of DIOXIN for *h*RXRα was determined determined by intrinsic tryptophan fluorescence quenching spectroscopy. There is a dose-dependent quenching of *Trp* fluorescence when titrated with DIOXIN (**Fig. 1**). The binding affinity (K_d) of the *h*RXRα(DIOXIN) complex, K_d = 17.5 ± 2.7 nM. The comparable binding affinity to 9 cis-RA suggests a a tight complex of *h*RXRα and DIOXIN that can be isolated and crystallized.

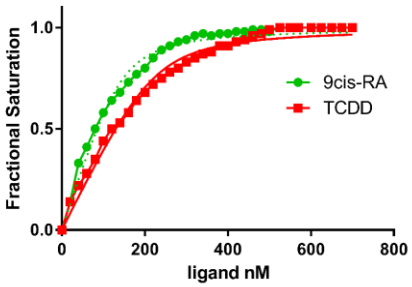
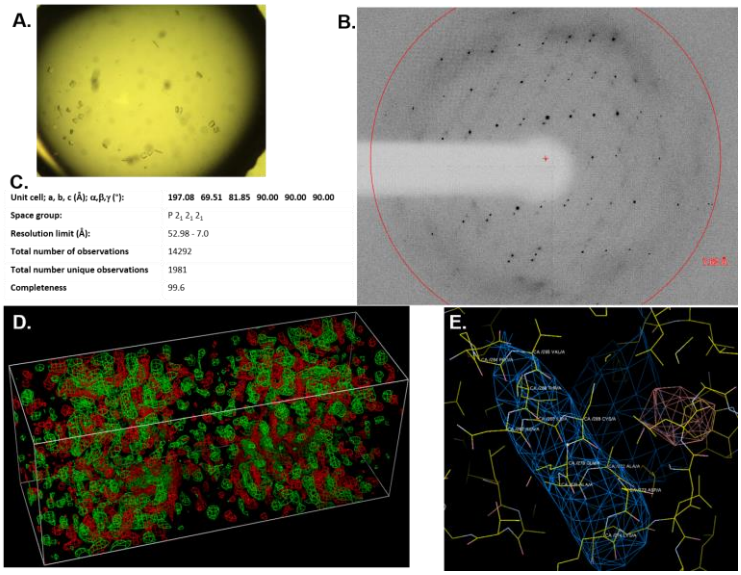


Fig. 1. *Trp fluorescence quenching by DIOXIN and 9 cis-RA.* *h*RXRα(9cis-RA) K_d = 13.3 ± 2.0 nM; *h*RXRα(DIOXIN) K_d = 17.5 ± 2.7 nM. Titrations in triplicate.

2 Crystallization of the hRXRα(dioxin) complex. Crystallization conditions for *h*RXRα(DIOXIN) were determined from a sparse matrix screen of multiple precipitant and buffer conditions (PMID 1656050). The *h*RXRα LBD was prepared heterologously from *E. coli* and purified by Strep-affinity, anion-exchange and size-exclusion chromatography and concentrated to approximately 7.0 mg/ml. *h*RXRα LBD was incubated with 3-times molar excess of DIOXIN at room temperature for 1h before screening for crystallization. One such condition was identified and subsequent fine-screens yielded crystals that are approximately 100 μM on the longest and 10 μM on the shortest dimensions, respectively (**Fig. 2**). However, these crystals are fragile.



F.

Unit cell; *a*, *b*, *c* (Å); α,β,γ (°)	197.08, 69.51, 81.85; 90.00, 90.00, 90.00
Space group:	P 2_1 2_1 2_1
Resolution limit (Å):	52.98 - 7.0
Total observations	14292
Unique observations	1981
Completeness	99.6

Fig 2. *Crystallography of hRXRα(DIOXIN).* **A.** crystals of *h*RXRα(DIOXIN) complex. **B.** Single diffraction image of *h*RXRα(DIOXIN) at APS. **C.** Cell constants for *h*RXRα(DIOXIN). **D.** molecular replacement solution and crystal packing. **E.** Zoomed-in view of electron density at 7.0 Å. **F.** X-ray Diffraction Data collection statistics.

3. X-ray Diffraction, processing and structure determination. Diffraction data was collected at the LS-CAT beamline of the Advance Photon Source, Argonne, IL. Approximately, 100 crystals were tested for diffraction. The highest diffraction detected is at a resolution of 7.0 Å (**Fig. 2B&F**). Data was processed with Mosflm in the CCP4i package (PMID: 21460445). The space group is $P2_12_12_1$ with 4 molecules of *h*RXRα(DIOXIN) within the crystallographic asymmetric unit (Matthews coefficient = 2.4). Data collection and processing statistics are in **Fig 2F**.

The structure was determined by molecular replacement using the *h*RXRα LBD structure (PMID 10835357) as the search model. The molecular packing shows no steric clashes and electron density shows elements of secondary structure (**Fig 2D**).