Supplementary Material

Protein Preparation, Crystallization, Data collection and Structure Resolution

Human estrogen receptor alpha-ligand binding domain (ERα-LBD, Ser301 to Thr553) with three Cys to Ser substitutions (C381S, C417S, C530S) was cloned and expressed in *Echerichia coli*. Protein purification was done as described previously.[1] The ERα-LBD in 50 mM Tris-HCl buffer (pH 8.0,) 50 mM NaCl, 2 mM DTT (1,4-Dithio-DL-threitol) and 2 μM **29,** was concentrated to 10 mg/mL prior to crystallization.

Crystals were grown in a hanging drop setup (Linbro plate) at room temperature (20 °C). The reservoir buffer contained 100 mM Bicine buffer (pH = 9.0), 28% PEG-550 MME and 100 mM NaCl. The hanging drop was made by mixing 1 μL protein solution with 1 μL reservoir solution.

Crystals were cryoprotected by adding 1 μL reservoir solution to the hanging drop (30 s equilibration time), then mounted in a cryo-loop and frozen directly at 100 K in the nitrogen stream. The first diffraction data set ("data set I", c.f. Table 1) was collected to 2.05 Å from a single frozen crystal on a MAR345 imaging plate system (150 μm pixel size, 240 mm plate) at the Swiss Norwegian Beam line of the ESRF (Grenoble, F). 215 Images were collected with 1.0° rotation each, using an exposure time of 30 s per frame and a crystal-to-detector distance of 235 mm. Because of incompleteness (due to overloads) in the low resolution range of data set I, a second data set ("data set II", c.f. Table 1) using a second crystal was collected in house to 2.4 Å on an EnrafNonius FR591 rotating Cu-anode generator (operating at 45 kV, 90 mA) equipped with a MacScience DIP2030 imageplate system. A total of 326 diffraction

images were recorded at 100 K with an exposure time of 2000 s for 1.0° rotation per image at a crystal-to-detector distance of 195 mm. The two data sets were processed and subsequently merged with the HKL program suite version 1.6.1 [2] and structure factor amplitudes were generated with the CCP4 4.0-package [3,4] . Data processing and merging statistics are shown in Table 1. The space group is $P2_1$ with unit cell parameters a = 55.1 Å, b = 156.6 Å, c = 59.0 Å and β = 90.4°. There are four complexes of ERα LBD/**29** (forming two dimers) per asymmetric unit. The estimated B-factor by Wilson plot is 31.9 $Å^2$.

Table 1. Data processing and merging statistics for data sets I+II

X-ray source (data set I)	SNBL Grenoble ($\lambda = 0.8727$Å)
X-ray source (data set II)	CuKα ($\lambda = 1.5418$Å)
Temperature of data collection	100K
Number of crystals	2
Space group	$P2_1$
Unit cell dimensions	a = 55.1 Å, b = 156.6 Å, c = 59.0 Å, $\beta = 90.4^{\circ}$
Number of monomers / asymmetric unit	4
Matthews coefficient (Vm)	2.2 Å^3/Da
Solvent content	44 %
Overall B-factor from Wilson plot	31.9 Å^2
Resolution range (last shell)	10.0 - 2.05 Å (2.12 - 2.05 Å)
Number of observations	403555
Number of unique reflection	59332
Mosaicity	0.61°
Data redundancy (last shell)	6.8 (4.9)
Data completeness (last shell)	95.2 % (89.9 %)
$< I/\sigma(I) >$ (last shell)	22.3 (1.8)
R_{merge} on I (last shell)	9.5 % (42.2 %)

The structure was determined by molecular replacement using the coordinates of the ligand binding domain (LBD) of human ERα (PDB accession code 1UOM[1]) as a starting model. Molecular replacement was performed with MOLREP[4], using all measured data between 10 and 3 Å. A clear solution was found with a correlation-coefficient of 54.3%, an R-factor of 46.5%, having 4 molecules in the asymmetric unit. The program REFMAC version 5.0[5] was used for refinement. Bulk solvent

correction, an initial anisotropic B factor correction and restrained isotropic atomic B-factor refinement were applied. The refinement target was the maximum-likelihood target using amplitudes. No sigma cut-off was applied on the structure factor amplitudes and NCS-restraints were not applied. Cross-validation was used throughout refinement using a test set comprising 5.1% (2992) of the unique reflections. Water molecules were identified with the program ARP/wARP[6] and selected based on difference peak height (greater than 3.0σ) and distance criteria. Water molecules with temperature factors greater than 70Å^2 were rejected. The program O version 7.0[7] was used for model rebuilding and the quality of the final refined model (**Table 2**) was assessed with the programs PROCHECK version 3.3[8] and REFMAC version 5.0[6]. The final model contains residues 307-330, 341-461, 464-528, 535-548 for chains A, C and residues 307-330, 341-461, 464-525, 535-548 for chains B, D. For the superposition (using the lsq-option in O[7]) of the complex ERα–LBD/**29** (chain identifiers A/L) with ERα–LBD/raloxifene (PDB accession code 1ERR[9]), the Cα coordinates of the respective residues 307-330, 341-419, 421-459, 470-525 and 535-546 were used, yielding a root mean square deviation (rmsd)-value of 0.56 Å. A superposition with the complex of ERα–LBD containing estradiol (PDB accession code 1QKT[10]) using the same Cα coordinates yielded an rmsd-value of 0.66 Å. A superposition with the complex of ERα–LBD containing estradiol (PDB accession code 1UOM[10]) using the same Cα coordinates yielded an rmsd-value of 0.69 Å. Pictures were made with O[7].

Structure factors and coordinates for the refined structure were deposited in the protein structure database (PDB ID code: 1XQC).

Table 2: Refinement statistics

Data used in refinement:	
- resolution range (last shell)	10.0 - 2.05 Å (2.10 - 2.05 Å)
- intensity cutoff (Sigma(F))	None
- number of reflections	55974
- completeness [working + test set] (last shell)	95.3 % (89.9 %)
- test set	5.1 % (2992 reflections)
Fit to data used in refinement:	
- overall R_{cryst} (last shell)	21.1 % (32.4 %)
- overall R_{free} (last shell)	25.4 % (35.2 %)
Number of non-hydrogen atoms used in the refinement:	
- protein atoms (total of chains A, B, C, D)	7116
- ligand atoms (total of chains L, M, N, O)	132
- water molecules (chain W)	374
Mean temperature factors for protein chains A, B, C, D	43.2 Å^2, 46.6 Å^2, 45.5 Å^2, 44.4 Å^2
Mean temperature factors for ligand chains L, M, N, O	33.2 Å^2, 35.1 Å^2, 32.6 Å^2, 36.6 Å^2
Mean temperature factors for water molecules chain W	47.0 Å^2
Deviations from ideal geometry:	
- bond length	0.018 Å
- bond angles	1.55°
- esu based on R-value	0.245 Å
- esu based on maximum likelihood	0.169 Å
- Ramachandran plot outliers	none
- G factor from PROCHECK	0.10

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