Biophysical and Structural Studies of the ERR-DNA complexes

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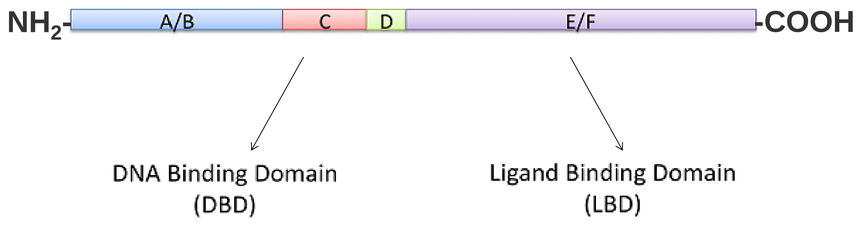
INTRODUCTION

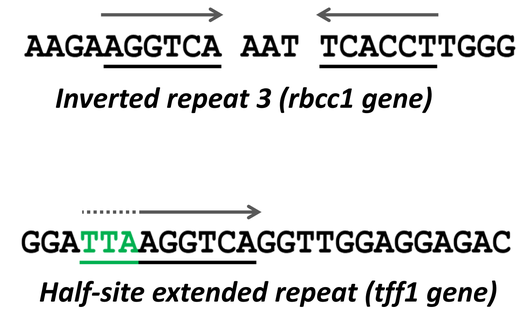
Nuclear receptors (NRs) act as transcriptional regulators through the binding to cis-acting response elements (REs) present in the gene promoters or enhancers.



General domain structure of nuclear receptors

The estrogen-related receptors are orphan nuclear receptors and exist in different isoforms : ERRα, ERRβ and ERRγ.

The ERRs, just like ER, can bind with high affnity inverted repeat 3 (IR3) response elements (REs). However most of the ERR natural REs sites found in the promoter of their target genes are extended half-sites (ERRE), composed of a single 6 bp half-site extended at its 5'-end by 3 bp.

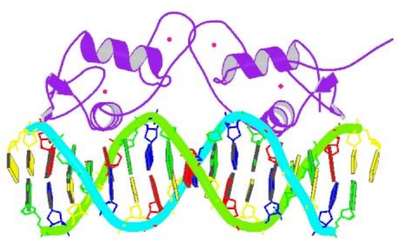


QUESTION : Is ERR a dimer or a monomer on DNA ?

ER-DBD & ERR-DBD ON RESPONSE ELEMENTS

Crystal structure of the Estrogen Receptor DBD-DNA complex

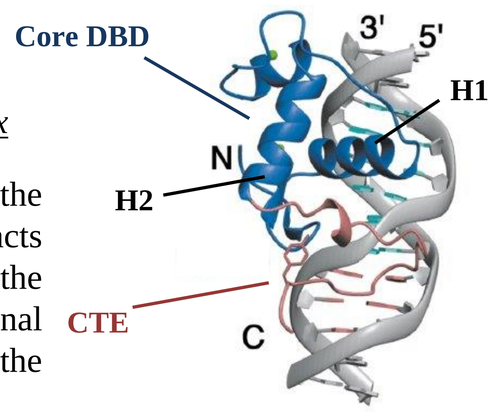
ER DBD binds as a symmetrical dimer to its palindromic binding site IR3. For each monomer, two main helices are arranged in a perpendicular way via hydrophobic interactions. The N-terminal helix triggers interaction with DNA thanks to specific contacts with the major groove.



5' CC**AGGTCA**CAG**TGACCT**G 3'
3' G**TCCAGT**GTC**ACTGGA**CC 5'

NMR solution structure of Estrogen-Related-Receptor DBD-DNA complex

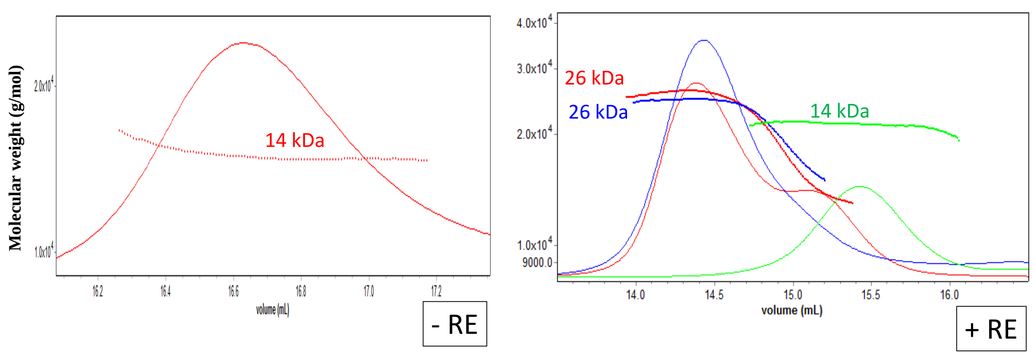
The CTE extension of the DBD crosses the phosphate backbone to make base-specific contacts within the minor groove in the 5' extension of the half site response element. These additional interactions allow the stabilization of the monomeric DBD onto DNA.



5' GCTCAAGGTCACG 3'
3' CGAGTTCCAGTGC 5'

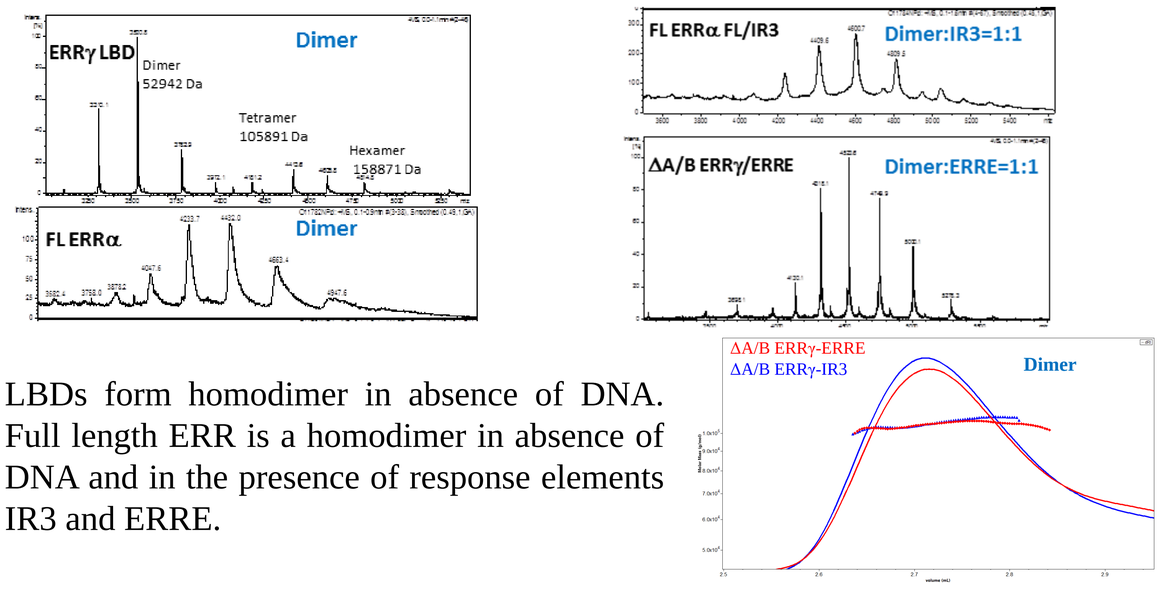
BIOPHYSICAL CHARACTERIZATION OF ERR-DBD ON DNA : MULTI-ANGLE LIGHT SCATTERING (MALS) & ESI-TOF MASS SPECTROSCOPY

ERRα-DBD is a monomer on DNA



Free ERRα-DBD is a 14kDa monomer (left panel). In complex with ERREs [ERRE (13bp, green), ERRE (21bp, blue)] and IR3 (21bp, red) the DBDα remains monomeric (right panel).

LBD and ERRα/γ full length are always dimeric

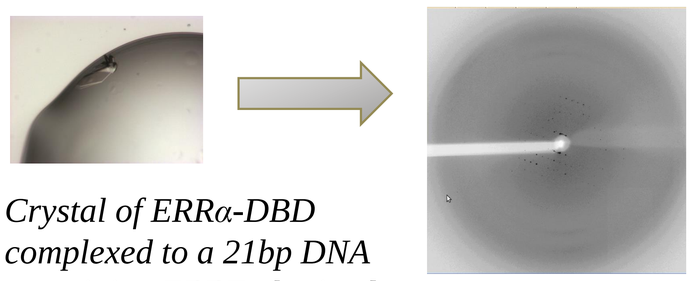


LBDs form homodimer in absence of DNA. Full length ERR is a homodimer in absence of DNA and in the presence of response elements IR3 and ERRE.

MALS and ESI-TOF data suggest that the dimerization surface between the DBDs is very weak and that the receptor dimerization is carried by the dimerization interface of LBDs.

CRYSTALLOGRAPHIC STUDIES AND SOLUTION STRUCTURE STUDIES OF ERR-DBD AND FULL LENGTH ERR IN COMPLEX WITH DNA

ERRα-DBD



Crystal of ERRα-DBD complexed to a 21bp DNA containing ERRE obtained with the PEGs screen

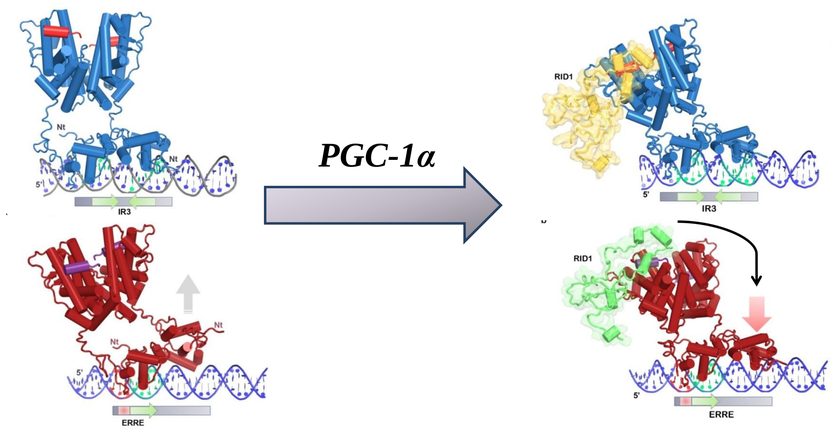
8 Å diffraction

5' GACCTGGATTA**AGGTCA**GGTT 3'
3' TGGACCTAATTCCAGTCCAAC 5'

Half-site extended repeat (ps2 gene)

SAXS studies show that full ERR bound to IR3 adopts one major asymmetric conformation. ERR bound to ERRE adopts a similar topology, but the SAXS solution structure suggests that the 3'-DBD is dynamic and not strongly bound to DNA.
Remarkably, upon interaction with the coactivator PGC-1α, ERR gets stabilized on its ERRE and adopts a conformation identical to that of PGC-1α/ERR-IR3.

ERRα/γ full length



PGC-1α stabilizes ERR on DNA response elements

SAXS data underline the importance of cofactors for the stabilization of ERR-DNA complexes which is an important issue in the crystallization process. The choice of DNA for crystallization is an other essential parameter. The length of the oligos, the structure adopted by the 3' and 5' ends and the base-to-base interactions are important features that play a key role in crystal formation (packing, contacts between asymetric units…).