Biochemistry 673
Feb. 8, 2005
Structural Biology of Nuclear Hormone Receptor Signalling

Reading for Thursday:
Picard and Yamamoto (1987), *EMBO J.*, **6**, pp. 3333-3340.
Picard et al. (1990), *Nature*, **348**, pp. 166-168.
Elbi et al. (2004), *PNAS*, **101**, pp. 2876-2881.

Structures of SHR's bound to DNA:
There are many nuclear hormone receptors, and their half-sites are quite similar. How are different sites recognized? We can address the recognition of spacing between half sites, direct vs. inverted repeat sites, and sequence-specific half-site recognition.

Sigler's group determined the X-ray crystal structure of the glucocorticoid receptor bound to DNA in Luisi et al., (Nature, 1991).
They made a symmetrized version of the GRE, hoping for ease in crystallization (the unit cell might contain just one monomer and half the helix).
Natural:
NAGAACANNNTGTTCTN
NTCTTCTNNNACAAGAN
Note inverted repeat.
Symmetrized:
CCAGAACATCGATGTTCTG
 GTCTTCTAGCTACAAGACC
4-base spacing
Turned out that the terminal C made a Hoogsteen triple with the last GC base pair (not uncommon) to help the molecule crystallize.
Surprisingly, the protein bound with one monomer forming specific interactions and one forming non-specific interactions: the protein dimerization interface dominated over sequence-specific DNA recognition. It bound DNA non-cooperatively: the decreased free energy for nonspecific binding compensated for the stabilization from the dimer interface. (PDB file 1R4O.pdb)
They also crystallized a complex with 3-bp spacing:
ICAGAACATCATGTTCTGA
 GTCTTCTAGTACAAGACTC in the paper
Vs.
TCAGAACATGATGTTCTCA
 GTCTTGTACTACAAGAGTC in the PDB file (1R4R.pdb, submitted 2003))
…I assume there's a story behind that but I don't know it.

The 3-base (natural) spacing leads to allosteric mutual stabilization of dimerization and DNA binding. Other nuclear receptors have different spacing.
There are two Zinc-binding modules with 4 Cys ligands. One forms sequence-specific DNA contacts, one does dimerization stabilized by phosphate contacts (protein alone is monomeric at NMR concentrations). Zn stabilizes structure as for classic zinc fingers.

Sigler's group also crystallized a chimeric GR/ER (estrogen receptor) with the key specificity-determining amino acids swapped. The residue/helix/domain swap is a classic experiment in molecular biology. They found that the non-cognate interactions of the ER residues with the GRE were mediated by a layer of water, whereas the cognate interactions are typical hydrogen bonding/van der Waals interactions.

Note that it is not known how the different homodimeric steroid hormone receptors recognize different binding sites! They see the same half-sites and spacing!

Spacing and dimerization specificity:
Mainly from Khorasanizadeh and Rastinejad, TIBS 2001.

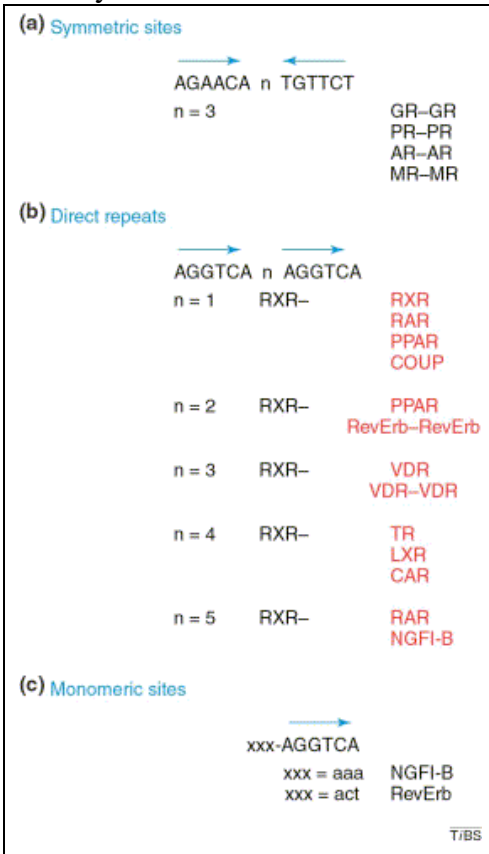

Fig 2. The types of DNA-response elements used by nuclear receptors. (a) Symmetric repeats using the consensus half-site 5′–AGAACA–3′ are used by the glucocorticoid receptor (GR), progesterone receptor (PR), androgen receptor (AR) and mineralocorticoid receptor (MR), each of which is a homodimer. The estrogen receptor (ER) binds similar symmetric sites but with consensus 5′–AGGTCA–3′ half-sites. (b) A '1–5 rule' specifies the use of direct-repeats with variable spacings by RXR and its many partners (depicted in red). Some receptors, such as the vitamin D receptor (VDR) or RevErb, can form homodimers as an alternative to heterodimers. The size of the inter-half-site spacing (n) can vary from one to five base-pairs.[Hence sites DR1-DR2-DR3-DR4-DR5. Note that RXR can also be downstream.] (c) Sites containing just one copy of 5′–AGGTCA–3′ flanked with specific 5′ sequences (xxx) are used by the nerve growth factor induced B (NGFI-B) receptor, RevErb and some other orphan receptors.

General architecture of nuclear hormone DBD's:

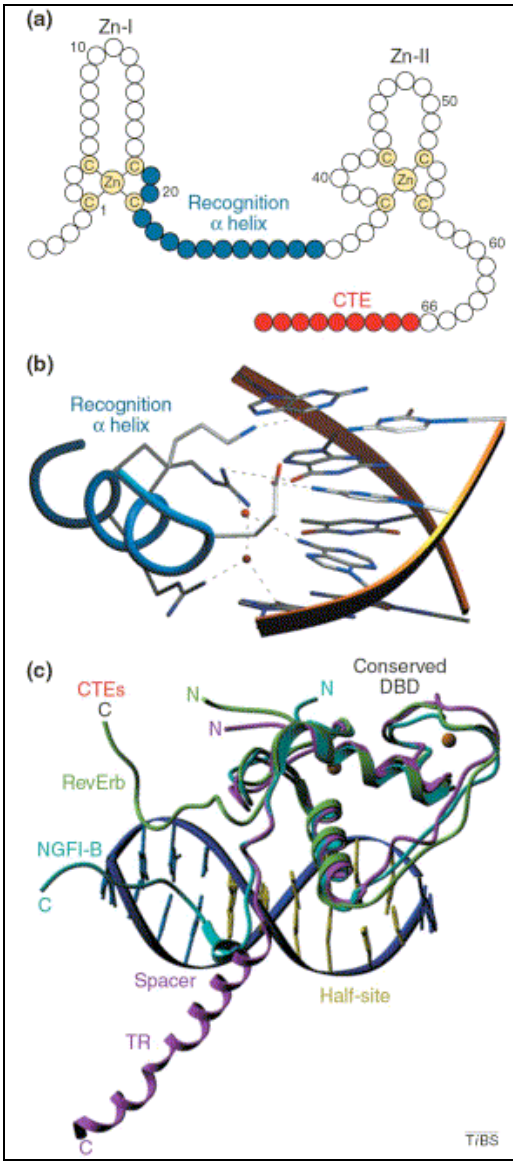

Fig. 4. (a) The DNA-binding domains (DBDs) in the nuclear receptor family contain a conserved recognition helix (shown in blue) and a variable C-terminal extension (CTE) that continues past the core 66-residue DBD into the hinge region. Each of these two elements provides a distinct DNA-binding surface [35]. (b)The recognition helix recognizes the major groove half-sites, with H2O bridging some of the protein–DNA interactions (water molecules are shown as dark circles). (c) By contrast, the CTEs of RevErb, NGFI-B and TR bind along the minor groove and backbone of DNA (Refs [32], [35], [36]).

Recognition of spacing variants:

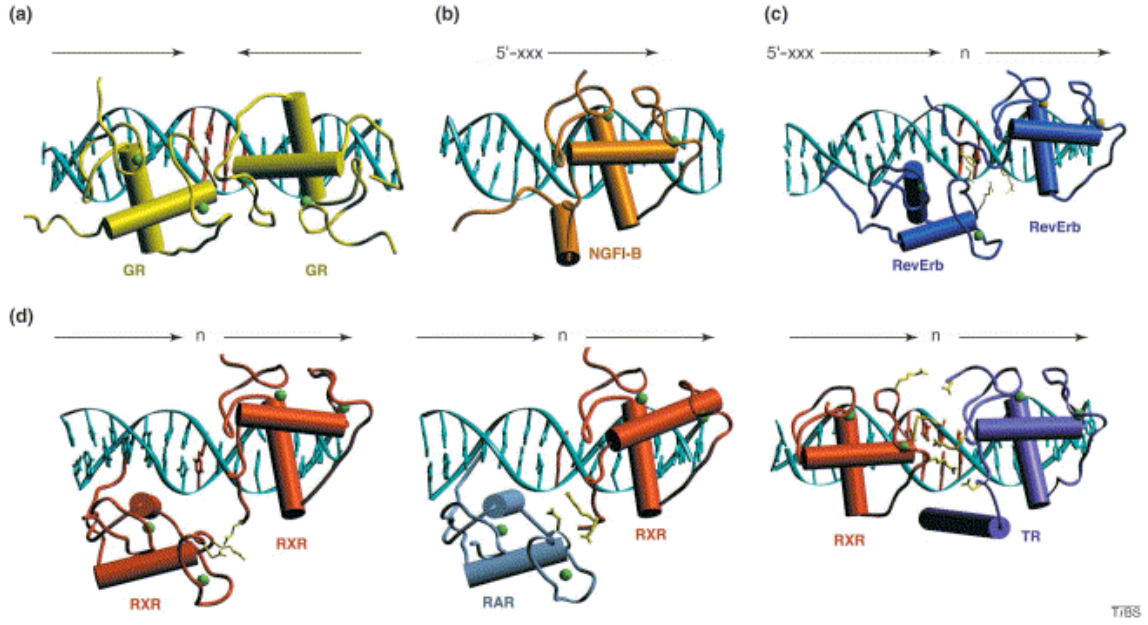

Fig. 3. Structures of complexes between receptor DNA-binding domains and their cognate DNA-response elements. (a) The GR homodimer bound to a symmetric target, (b)the NGFI-B bound to its extended monomeric site [30], (c) the RevErb homodimer bound to an extended direct-repeat element [35] and (d) three distinct RXR complexes [as a homodimer on DR1 (Ref. [33]), as a heterodimer with RAR on DR1 (Ref. [34]) and as a heterodimer with TR on DR4 (Ref. [32])]. Note that RXR binds only at the upstream half-site on DR4 (with TR), and only at the downstream half-site on DR1 (with RAR). The cylinders indicate helices, the half-site spacings are shown in red and protein side chains mediating intersubunit contacts are shown in yellow.

Spacing recognition is carried out via the RXR-partner dimer interface, which adjusts depending on RXR's protein partner and the spacing in question.
Propose that the "CTE's" or C-terminal extensions are molecular rulers that allow only one spacing. The binding sites for monomeric DBD's are too small to confer substantial affinity without some help from dimerization.

It is surprising how small the interaction interfaces are. The choice of what complexes assemble is undoubtedly a complicated function of what receptors are present, of possible interactions between ligand binding domains, of different coreceptor complexes, and so forth. As usual, biology operates through tuning many weak interactions.

The ligand binding domain
Good source: Nagy and Schwabe, 2004, TIBS 29, p. 317
Structures of Hsp90 bound to drugs are available, but not especially evocative.

We know a lot more about structural changes in the LBD and about how ligand binding changes the activity of the receptor.

Here the structures are larger and direct superpositions are more informative, so we will stick with published images.

Overall framework:
LBD's are all similar. Allows for heterodimerization, rapid evolution to adapt to new or no ligands. Primarily alpha-helical. Binding pocket is quite diverse, smaller pockets correspond to more specific receptors like the TR.

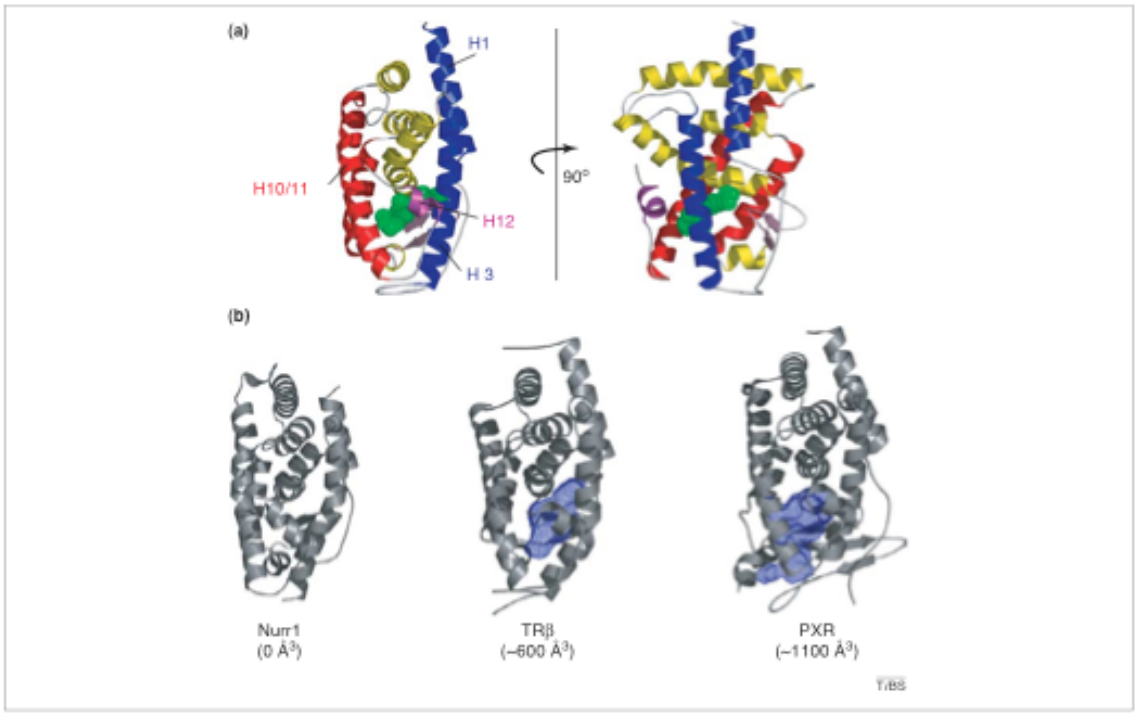

Figure 1. Structural framework of nuclear receptor ligand-binding domains (LBDs). **(a)** The LBD is formed from a three-layer α-helical sandwich. The layers are shown in blue, yellow and red. In the lower portion of the structure the central helical layer is absent, creating a mainly non-polar cavity in which the ligand (green) binds. Helix 12 and a β-strand (magenta) close the front and back of the ligand-binding cavity, as can be seen on the left. The structure shown is that of the retinoic acid receptor-γ (RARγ) bound to all-*trans* retinoic acid (PDB code 2LBD) [28]. **(b)** Structures of the LBD of Nurr1, the thyroid hormone receptor-β (TRβ) and the pregnane X receptor (PXR; PDB codes 1OVL, 1BSX and 1ILG, respectively) [18,22,26]. The ligand-binding cavity is illustrated by a blue mesh: Nurr1 has no cavity; the cavities in TRβ and PXR are about 600 and 1100 Å^3, respectively. Note that the size and position of the cavity is dependent upon the extent of opening up of the helices in the lower portion of the structure.

Conformational/dynamic changes upon ligand binding:
RXR alpha was an early example. Ligand binding displaces helix 11, which leads to a dramatic shift in the position of helix 12. However, may not actually be as dramatic as shown. (From Egea et al., (2000), EMBO J., 19, 2592).

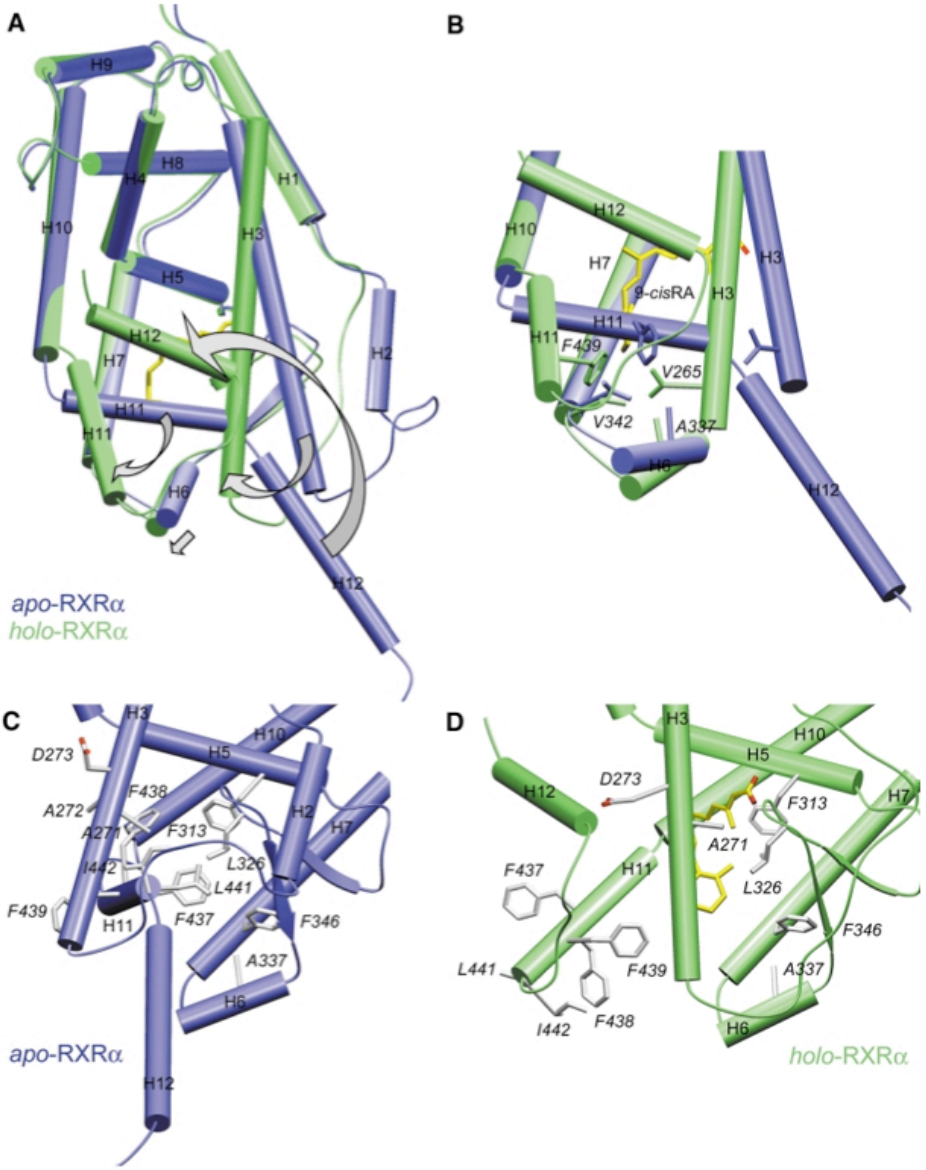

Fig. 4. The conformational changes induced by ligand binding in hRXRα. (**A**) Superimposition of unliganded (in blue) and liganded (in green) hRXRα monomers showing the main conformational differences affecting helices H3, H6, H11, H12 and the connecting helix H2. The arrows show the main structural changes affecting helices H3, H6, H11 and H12 upon ligand binding. The ligand is depicted in yellow and red for carbon and oxygen atoms, respectively. (**B**) Superimposition of helices H3, H6, H7, H11 and H12 regions between the *apo* (in blue) and *holo* (in green) forms of hRXRα. For the sake of clarity other regions have been omitted. The arrows in (A) emphasize the conformational changes occurring upon ligand binding, showing the binding site closure at the level of the β-ionone ring of the ligand molecule. Residues Val265 (H3), Ala337 (H6), Val342 (H7) and Phe439 (H11) involved in binding site closure are labelled. The ligand is depicted in yellow and red for carbon and oxygen atoms, respectively. (**C** and **D**) Comparison of the *holo* (in green) versus *apo* (in blue) hRXRα H3, H6, H11 and H12 regions. The same orientation is used in both views. In the *apo* form residues from helix H11 occupy the binding pocket. The ligand is depicted in yellow and red for carbon and oxygen atoms, respectively.

How do receptors interact with coactivators?

Typically corepressors bind to unliganded repressors (would like to have a structure…).
Agonist binding potentiates conformational change in position of helix 12 that allows for binding the LxxLL motif characteristic of coactivators.
Corepressors and coactivators bind to the same locations, allowing for a clear switchover of activity upon binding.
Inverse agonists stabilize the repressive state by leading to conformational changes that cause H12 to block the coactivator binding pocket or stabilize corepressor binding.
Again, the interacting surfaces are all surprisingly small.

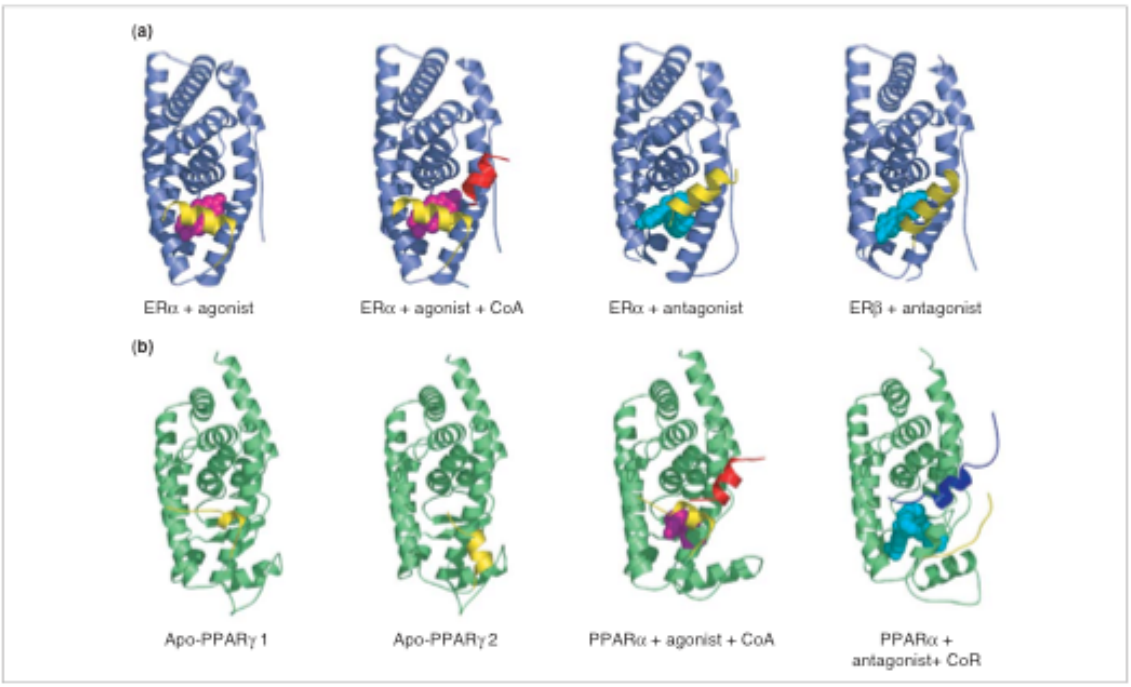

Figure 2. Effect of ligands and co-regulators on the position of helix 12. The top row of structures shows the oestrogen receptor (ER) bound to agonists and antagonists (PDB codes 1ERE, 1ERR, 1GWR and 1QKM) [19,63]. Note that in the antagonist-bound structures helix 12 adopts a position very similar to that of the co-activator in the agonist-bound structure. The bottom row of structures shows peroxisome proliferator-activated receptors (PPARs) in the absence of ligand (left two structures; PDB code 1PRG), bound to agonist and co-activator (PDB code 2PRG) and to antagonist and co-repressor (PDB code 1KKQ) [20,49]. Note that in the absence of ligand, helix 12 can adopt both the active position and an alternative position. In the presence of antagonist and co-repressor, helix 12 unravels. ERs and PPARs are shown in light blue and green, respectively; helix 12 in yellow; agonists and antagonists in magenta and cyan, respectively; and co-activator and co-repressor peptides in red and dark blue, respectively.

Suggest that dynamical properties of LBD's may be important in controlling conformational change:

Ligands make LBD more compact and more rigid. If general, helps to explain how ligand competes with Hsp90 for LBD binding.

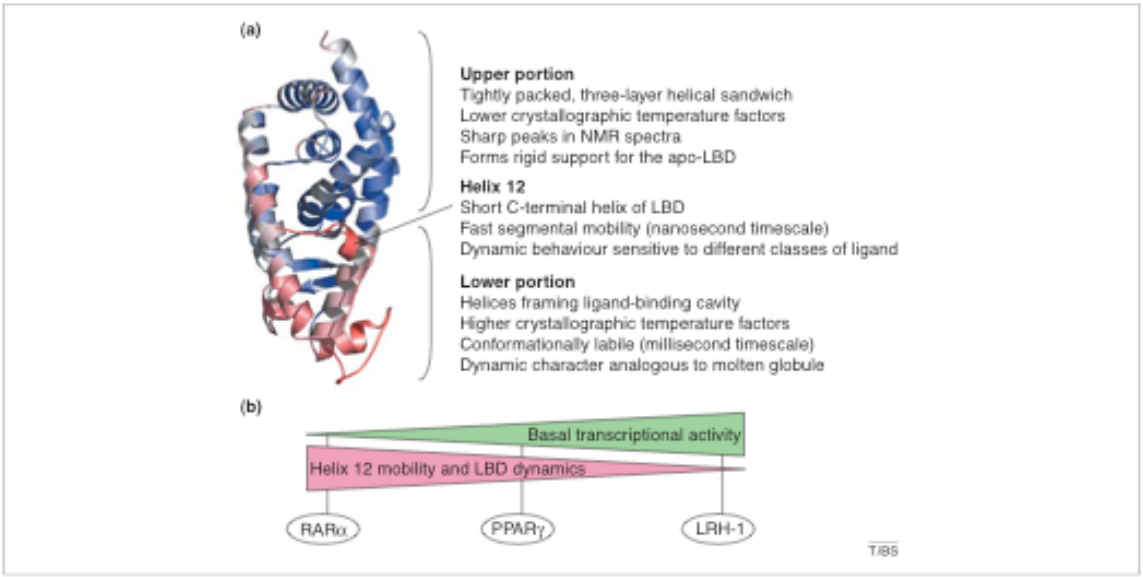

Figure 3. Nuclear receptor dynamics and activity. **(a)** Structure of a ligand-free peroxisome proliferator-activated receptor (PPARγ; PDB code 1PRG) [20] coloured according to crystallographic temperature factors and annotated according to our understanding of the dynamic properties of this molecule [53,56]. **(b)** Different nuclear receptors are likely to show different degrees of dynamic lability that correlate with their basal transcriptional activity. For example, liver receptor homologue-1 (LRH-1) has constitutive activity and is stable enough to be crystallized in the absence of ligand; retinoic acid receptor-α (RARα) is a repressor in the absence of ligand and crystallization attempts without ligand have been unsuccessful, which is consistent with the idea that the ligand-binding domain (LBD) is conformationally mobile; peroxisome proliferator-activated receptor-γ (PPARγ) adopts an intermediate position between these extremes.