Almost Ready to Submit

3D model of lamprey estrogen receptor with estradiol and 15α-hydroxy-estradiol

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Abstract. Lamprey, a basal vertebrate, contains orthologs of the estrogen receptor [ER], progesterone receptor and corticoid receptor. A perplexing property of lamprey is that 15α-hydroxy-steroids are active steroids. For example, 15α-hydroxy-estradiol [15α-OH-E2] is the estrogen, instead of estradiol [E2]. To investigate how 15α-OH-E2 binds lamprey ER, we constructed a 3D model of the lamprey ER with E2 and 15α-OH-E2. Our 3D model shows that Sδ on Met-409 can form a hydrogen bond with the 15α-hydroxyl on 15α-OH-E2. In human ER α, the corresponding residue Ile-424 has a van der Waals contact with 15α-OH-E2. BLAST analysis of GenBank indicates that among vertebrate ERs, only lamprey ER contains a methionine at this position. Thus, the contact between Sδ on Met-409 and 15α-OH-E2 is unique. Our 3D model of lamprey ER should prove useful in virtual screening of chemical libraries to

identify compounds for controlling reproduction in sea lamprey, an environmental pest in Lake Michigan.

Running title: 3D model of Lamprey Estrogen Receptor

Key words: lamprey estrogen receptor; estrogen receptor evolution, control of sea lamprey

Introduction.

Lamprey and hagfish, two jawless fish, are at the base the vertebrate line, which has motivated studies of these fish to understand early events in vertebrate evolution . In particular, sea lamprey (*Petromyzon marinus*) is of interest for understanding the origins of adrenal and sex steroid signaling because orthologs of vertebrate estrogen receptor [ER], progesterone receptor [PR] and a corticoid receptor [CR] have been cloned from sea lamprey . A puzzle about sea lamprey is that the principal estrogens, androgens and progestins in its serum differ from that in humans . For example, lamprey serum contains 15α-hydroxy-estradiol [15α-OH-E2] and 15α-hydroxy-estrone [15α-OH-E1] and low levels of estradiol [E2], which is the main estrogen in land vertebrates and bony fish. Lamprey serum also contains 15α-OH-progesterone and 15α-OH-testosterone and low levels of progesterone and testosterone. These data suggest that 15α-hydroxy-steroids are the active steroids in lamprey.

To determine if there is a structural basis in lamprey ER for the recognition of 15α-OH-E2, we constructed a 3D model of lamprey ER complexed with E2 and 15α-OH-E2. This 3D model shows that Sδ on Met-409 in lamprey ER can have a hydrogen bond with 15α-hydroxyl on 15α-OH-E2. In human ERα, the corresponding residue is Ile-424, which has a van der Waals contact with 15α-OH-E2. A BLAST search of GenBank found that almost all other vertebrate ERs contain an isoleucine and none contain a methionine at this position. The uniqueness of lamprey Met-409 and its stabilizing interaction with the 15α-hydroxyl on 15α-OH-E2 suggests that it may be possible to find chemicals that selectively inhibit lamprey ER by using our 3D model of lamprey ER as a template for virtual screening of chemical libraries. Such chemicals

could be used to control reproduction of *P. marinus*, which is a pest in the Great Lakes in the USA.

Methods

Construction of 3D Models

The 3D structure of human ERα [PDB: 1G50] was used as a template for constructing the 3D model of lamprey ER. The sequences of the steroid-binding domain of lamprey ER and human ERα are 57% identical without any gaps [Figure 1]. This strong similarity between

lamprey ER and its template gives us confidence in the accuracy lamprey 3D model.

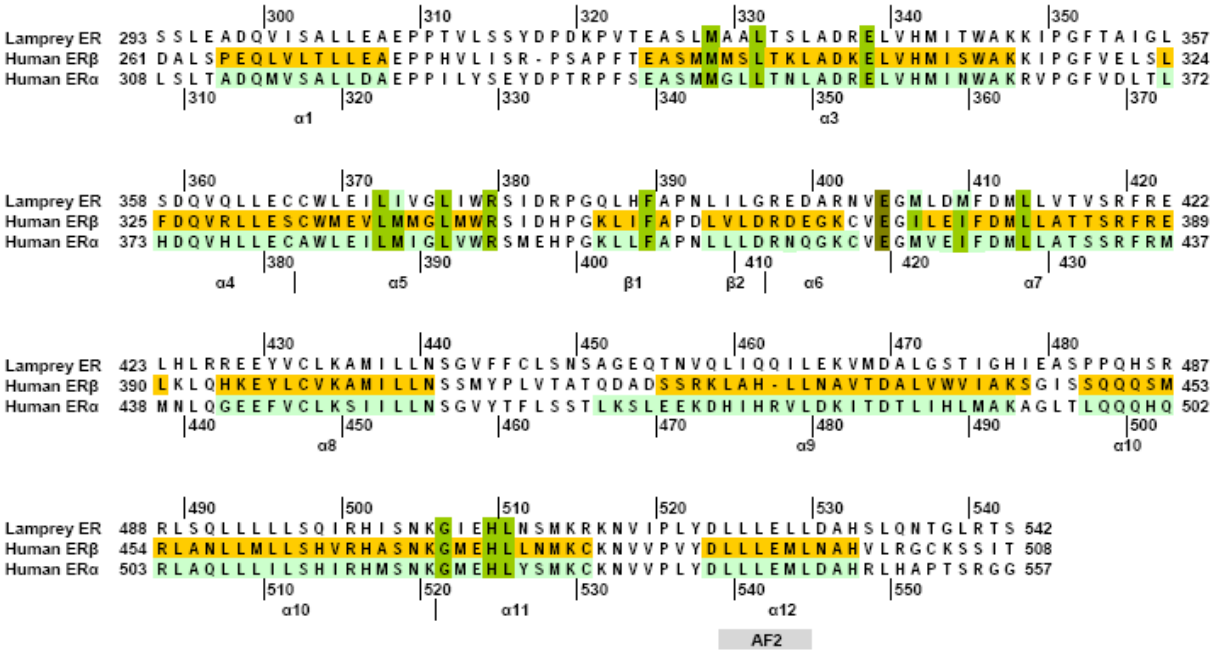

Figure 1. Alignment of lamprey ER with human ERα and human ERβ. α-helices and β-strands from the crystal structures of ERα and ERβ are shaded in each sequence and notated

below the alignment. Residues in human ERα involved in binding of estradiol are shown in green. Glu-419, which stabilizes His-524 is shaded in brown. Crystal structure accessions are human ERα [PDB: 1G50], human ERβ [1QKM].

We used the Multiple Mapping Method (MMM) software to construct the 3D model of lamprey ER. We selected three alignment algorithms Muscle, Align2D and ClustalW to align the target sequence [lamprey ER] and the human ERα template [1G50]. MMM takes each alignment and constructs a composite alignment, which is then used by Modeller to construct the 3D model of lamprey ER.

After we obtained the apo-3D model of lamprey ER, we inserted E2 into lamprey ER, by overlapping lamprey ER with human ERα. E2 was extracted from human ERα and inserted into lamprey ER using the Biopolymer option in Insight II. Builder from Insight II was used to add the 15α-hydroxyl group to E2 for analysis in lamprey ER and human ERα.

We refined the structure of lamprey ER with E2 and 15α-OH-E2 and human ERα with 15α-OH-E2 using Discover 3 in Insight II. For this energy minimization step, Discover 3 was run for 10,000 iterations, using a distant dependent dielectric constant of 2.

Results

Figure 2 shows that our 3D model of lamprey ER and the crystal structure of human ERα overlap nicely. The root mean square deviation [RMSD] of their Cα chains is 1.4Å.

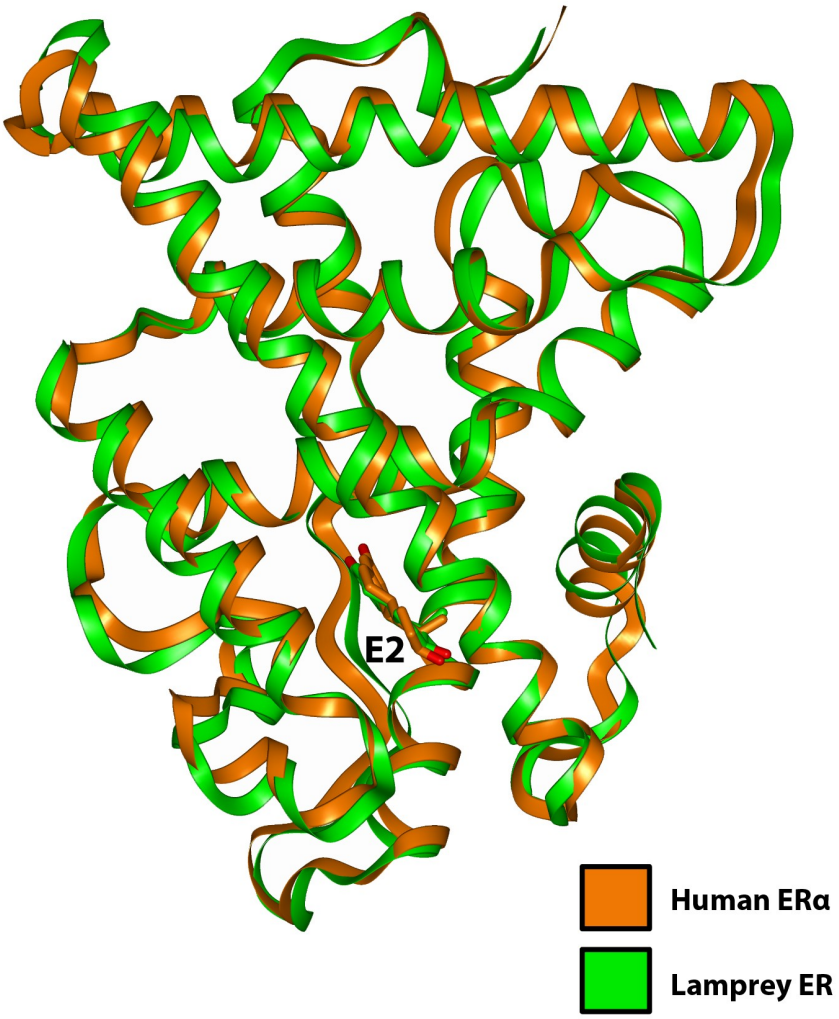

Figure 2. Overlap of 3D models of lamprey ER with human ERα. The 3D model of lamprey ER with estradiol was superimposed on human ERα. There is excellent overlap. The root mean square deviation between the Cα backbone of human ERα and lamprey ER is 1.4Å.

In Figure 3A and 3B, we show the interaction of E2 with eight residues from human ERα and lamprey ER.

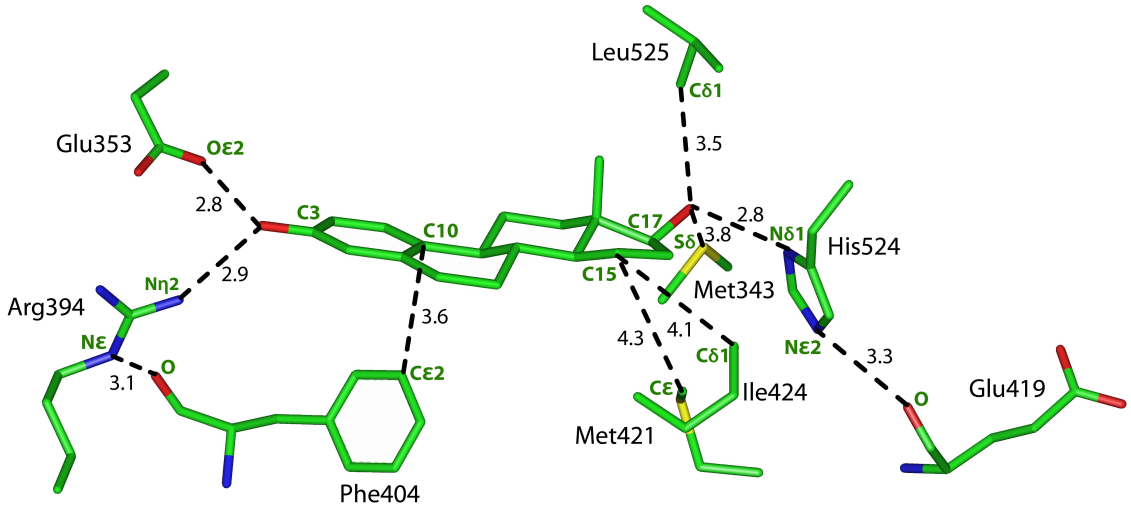

Figure 3A

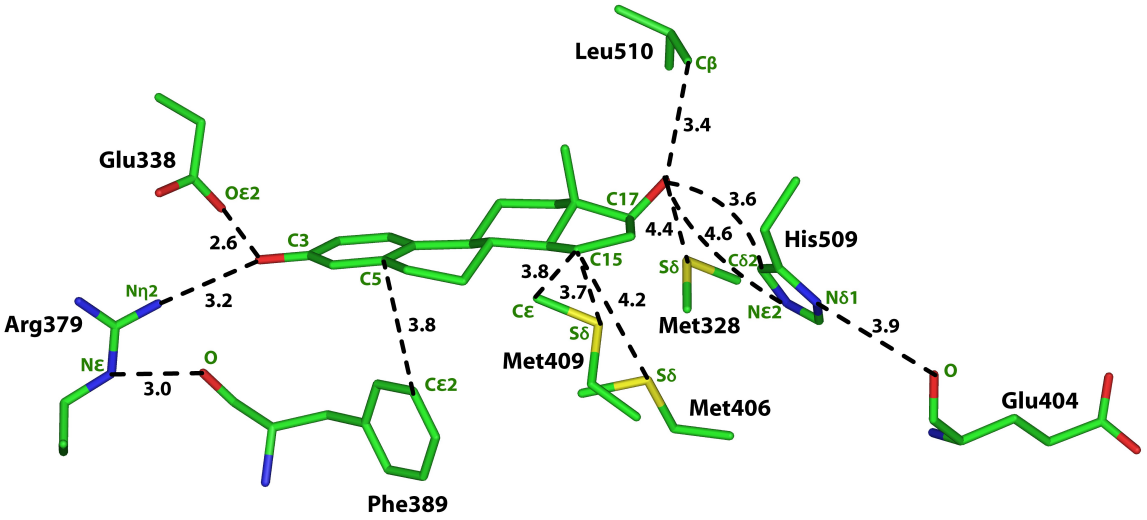

Figure 3B

Figure 3. Interaction of E2 with human ERα and the 3D model lamprey ER.
A. Interaction between E2 and human ERα.
B. Interaction between E2 and the 3D model of lamprey ER. Lamprey ER has stabilizing interactions with the A ring of E2 similar to those in human ERα. His-509 has rotated and does not have a hydrogen bond with the C17-hydroxyl on E2. Instead, Cδ2 has a van der Waals contact with the C17-hydroxyl on E2. Also, Cε and Sδ on Met-409 have stabilizing contacts with C15 on E2.

Previous analyses have shown that these residues stabilize E2 in human ER . Three of these amino acids, Arg-394, Glu-353 and Phe-404 in human ERα, correspond to functionally important residues in the PR , GR , AR and MR . These steroid receptors contain corresponding arginine and phenylalanine residues, and a glutamine, which is a conservative replacement of glutamic acid.

We selected His-524 because it has a hydrogen bond with the 17β-hydroxyl on the D ring of E2. This hydrogen bond between a substituent on the D ring in E2 and human ERα is not found in other adrenal and sex steroid receptors . Met-343 and Leu-525 also stabilize the 17β-hydroxyl on E2. Met-421 and Ile-424 have contacts with the 15α-hydroxyl on 15α-OH-E2. We also show an important stabilizing interaction between the backbone oxygen of Glu-419 and His-524 .

Comparison of estradiol binding to human ERα and lamprey ER.

As shown in Figure 3A, human ERα has stabilizing hydrogen bonds with the A ring of E2. The phenolic hydroxyl on E2 is 2.8Å from Oε2 on Glu-353 and 2.9Å from Nη2 on Arg-394. The side chain on Arg-394 is stabilized further through a hydrogen bond between Nε and the backbone oxygen on Phe-404. Cε2 on Phe-404 also has a stabilizing van der Waals contact with C10 on estradiol.

Figure 3B shows that lamprey ER has similar stabilizing hydrogen bonds with the A ring of E2 as found with human ERα. The C3-hydroxyl on E2 is 2.6Å from Oε2 on Glu-338 and

3.2Å from Nη2 on Arg-379. Nε on Arg-379 is 3Å from the backbone oxygen on Phe-389. Cε2 on Phe-389 has a van der Waals contact with C5 on E2.

Comparison of Figure 3A and 3B reveals significant differences in the interaction between the D ring of E2 and human ERα and lamprey ER. In human ERα, Nδ1 on His-524 is 2.8Å from the 17β-hydroxyl on the D ring of E2. In addition to this conserved hydrogen bond between His-524 and E2, Cε1 on His-524 has a van der Waals contact with the 17β-hydroxyl, which is 3.4Å from Cε1. His-524 is stabilized by an interaction with the backbone oxygen on Glu-419, which is 3.3Å from Nε2 on His-524 [Figure 3A]. The 17β hydroxyl on E2 is 3.8Å from Sδ of Met-353 and 3.5Å from Cδ1 of Leu-525. These interactions also stabilize E2 in ERα

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Figure 3B shows that in lamprey ER, His-509 has rotated so that Nε2 is 4.6Å from the 17 β-hydroxyl on E2, which is too distant for a hydrogen bond. As a result of this rotation, Cδ2 on His-509 has van der Waal contacts with the 17β-hydroxyl, C17 and C16 on E2, which are 3.6Å, 3.9Å and 3.5Å distant, respectively, from Cδ2. The 17β-hydroxyl on E2 is 4.4Å from Sδ on Met-328 and 3.4Å and 3.5Å, respectively, from Cβ and Cδ1on Leu-510. The backbone oxygen of Glu-404 is 3.9Å from Nδ1 on His-509. These distances between lamprey ER and E2 are not as favorable for stabilizing E2 binding as found in human ERα. There are, however, other unique stabilizing contacts between Met-409 on lamprey ER and C15 on E2, which could compensate for the loss of the hydrogen bond between His-509 and the 17β-hydroxyl on E2. Sδ and Cε on Met-409 are 3.7Å and 3.8Å, respectively from C15 on E2. For comparison, in human ERα, Cδ1on Ile-424 is 4.07Å from C15.

Comparison of 15α-hydroxy-estradiol binding to human ERα and lamprey ER.

Figure 4A shows that the stabilizing interactions between 15α-OH-E2 and human ERα are similar to that shown in Figure 3A for E2 and human ERα. However, as found for E2 binding to human ERα and lamprey ER, there are important differences in the interaction between the D ring of 15α-OH-E2 and human ERα [Figure 4A] and lamprey ER [Figure 4B].

The 17β-hydroxyl on E2 still has favorable interactions with His-524, Met-343 and Leu-525 in human ERα. Also, Cε on Met-421 and Cγ2 on Ile-524 are 3.8Å and 3.2Å, respectively, from the 15α-hydroxyl on 15α-OH-E2. The backbone oxygen on Glu-419 is 3Å from Nε2 of His-524, which stabilizes His-524.

The 3D model of lamprey ER with 15α-OH-E2 reveals that His-509 and Met-328 do not have the same stabilizing interactions found in the corresponding residues in 3D model of human ERα with 15α-OH-E2. As shown in Figure 4B, in lamprey ER, Nε2 on His-509 and Sδ on Met-328 are 4.5Å and 5.0Å, respectively from the 17β-hydroxyl on 15α-OH-E2. These distances are too far for the formation of a hydrogen bond. There are, however, van der Waals contacts between His-509 and the D ring on 15α-OH-E2. Thus, Cδ2 on His-509 is 3.6Å, 3.9Å and 3.5Å from the 17β-hydroxyl, C17 and C16, respectively. Leu-510 still stabilizes the 17β-hydroxyl on 15α-OH-E2. Cβ and Cδ1 on Leu-510 are 3.6Å from the C17-hydroxyl on 15α-OH-E2. The backbone oxygen of Glu-404 is 3.6Å from Nε2 on His-509. There also are unique stabilizing contacts between the 15α-hydroxyl on 15α-OH-E2 and Met-406 and Met-409. Cε and Sδ on Met 406 and Sδ on Met-409 are 3.6Å, 3.5Å and 2.9Å, respectively, from the C15 hydroxyl on 15 α-OH-E2.

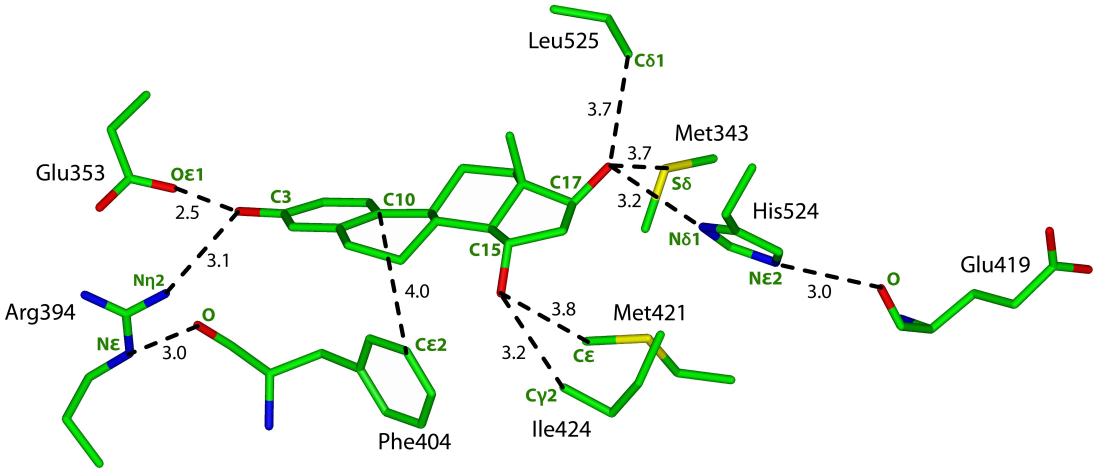

Figure 4A

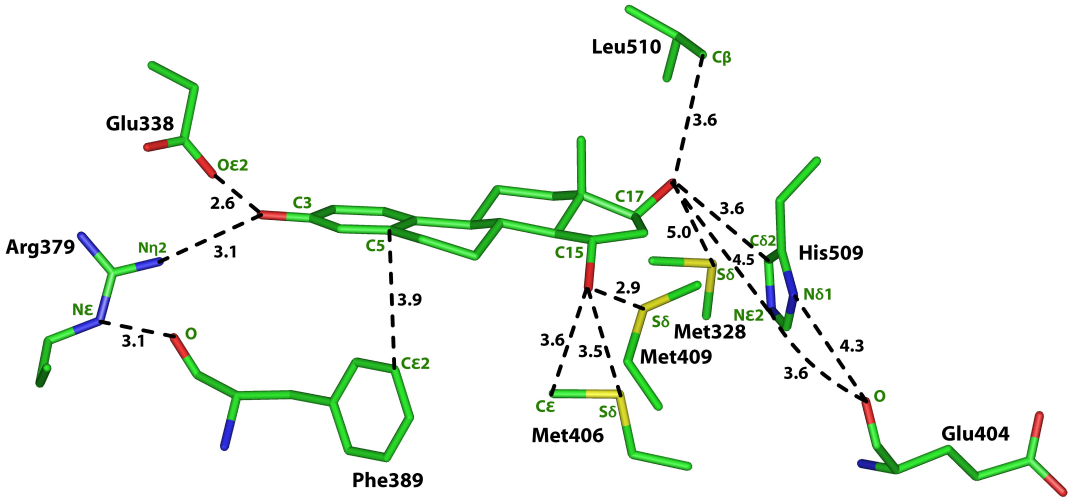

Figure 4B

Figure 4. Interaction of 15α-OH-E2 with human ERα and the 3D model lamprey ER.
A. In human ERα, Cγ2 on Ile-424 and Cε on Met-421 have van der Waals contacts with 15α-OH-E2.
B. Lamprey ER has stabilizing interactions with the A ring of 15α-OH-E2 that are similar to those in human ERα. His-509 has rotated and does not form a hydrogen bond with the C17-hydroxyl on E2. Cδ2 on His-509 has a van der Waals contact with the C17-hydroxyl. Sδ on Met-406 and Met-409 stabilize 15α-OH-E2.

Met-409 lamprey ER is unique among vertebrate ERs.

A BLAST search of GenBank, which contains over 500 ERs from a variety of vertebrates, found that almost all ERs contain an isoleucine corresponding Ile-424 found in human ERα and ERβ [Figure 1]. There were no vertebrate ERs with a methionine at this position.

Interestingly, at this position in ERβ, there is a valine, instead of an isoleucine, in five New world monkeys: *Cebus apella* (brown capuchin) [GenBank: **ABY64736**], *Callithrix jacchus* (white-tufted-ear marmoset) [GenBank: **Q95171**], *Ateles paniscus* (black spider monkey) [GenBank: **ABY64735**], *Pithecia pithecia* (white-faced saki) [GenBank: **ABY64737**],

Callicebus donacophilus (Bolivian titi) [GenBank: **ABY64738**] and a fish *Acipenser schrenckii* (Amur sturgeon) [GenBank: **BAG82652**] . Valine is a conservative replacement of isoleucine. ERα in the above vertebrates contains the conserved isoleucine.

Discussion

We have constructed a 3D model of lamprey ER using the crystal structure of human ER α as a template. There is excellent conservation in the structures of human ERα and our 3D model of lamprey ER, as seen in the RMSD of 1.4Å between their Cα chains [Figure 2].

Comparison of lamprey ER and human ERα in Figures 3 and 4 reveals a conservation of interactions of the A ring of E2 and 15α-OH-E2 with lamprey ER and human ER [Figures 3 and 4]. It is in the interaction of human ERα and lamprey ER with the D ring on E2 and 15α-OH-E2 that we find a key difference. There is a unique hydrogen bond between Sδ on Met-409 in lamprey ER and 15α-hydroxyl on 15α-OH-E2 [Figure 4B]. In contrast, Ile-524 in human ERα has a van der Waals contact with the 15α-hydroxyl group [Figure 4A]. In lamprey ER, His-509 does not have a stabilizing hydrogen bond with the 17β-hydroxyl on E2 or 15α-OH-E2. There are, however, van der Waals contacts between Cδ2 on His-509 and the D ring of E2 and 15α-OH-E2 [Figure 4B]. These van der Waals contacts and the unique interaction between Sδ on Met-409 and E2 and 15α-OH-E2 appear to compensate for the loss of the hydrogen bond between His-509 and the 17β-hydroxyl on E2 and 15α-OH-E2 [Figures 3B and 4B]. These additional stabilizing interactions may explain the data of Paris et al , who found that lamprey ER is activated by E2.

Evolutionary Implications

BLAST analysis of GenBank did not find any other ERs with a methionine at the position corresponding to Ile-424 inhuman ERα. The uniqueness of Met-409 in lamprey ER and the strong conservation of Ile at the corresponding position in human ERα and ERβ and in almost all

other ERs in GenBank suggest a functional role for Met-409 in lamprey ER and Ile-424 in human ERα and the corresponding isoleucine in other ERs.

Interestingly, ERβ in five New World monkeys and a sturgeon have a valine instead of isoleucine at the position corresponding to Ile-424 in human ERα. Valine is a conservative replacement of Ile and would have similar van der Waals contacts with 15α-OH-E2, in contrast to the hydrogen bond between Sδ on Met-409 in lamprey ER and 15α-OH-E2. The strong conservation of isoleucine at this position in vertebrate ERα and ERβ suggests that replacement of isoleucine by valine in some New World primates and in a sturgeon may be functionally important.

Environmental implications

Sea lamprey is a pest in the Great Lakes, where lamprey consumes trout and other valuable fish. Our 3D model of lamprey ER identifies a unique structure that interacts with the D ring on E2. This difference from other vertebrate ERs could be exploited to find compounds that selectively inhibit lamprey ER by virtual screening of chemical libraries for binding to our 3D model of lamprey ER. Such contraceptives would provide a means to control sea lamprey.

References