SUPPLEMENTARY MATERIAL

FUNCTIONAL DIFFERENCES OF INVARIANT AND HIGHLY CONSERVED RESIDUES IN THE EXTRACELLULAR DOMAIN OF THE GLYCOPROTEIN HORMONE RECEPTORS

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The material presented in this Supplement refer to: (a) structural information on the FSH-FSHR ECD complex (1), (b) our results from molecular modeling of LH, TSH and the complexes of CG and TSH with the LHR ECD and TSHR ECD, respectively.

RESULTS

Information on the structure of the FSH-FSHR ECD complex- The interactions of the six amino acid residues of FSHR shown to be in contact with the FSH α-subunit (1) are given in Supplemental Table 1. These six residues interact with seven sites on the hormone. Panels A-C of Supplemental Fig. 1 show the structures of β-strands 2, 4 and 5, and the detailed interactions of FSHR K^{74}, Y^{124}, N^{129}, D^{150} and D^{153} with the α-subunit are presented in panel D.

Supplemental Figure 1. Schematic representations of the FSHR ECD residues studied. Residues that comprise β-strands 2 (A), 4 (B) and 5 (C), respectively, of the FSHR ECD (1) are shown. Interacting residues are protruding from the concave side of the ECD (pointing to the right) while hydrophobic residues point inwards (to the left) and form the hydrophobic core typical for a LRR domain. A schematic LIGPLOT (2) presentation of the interactions between the FSH α-subunit (α) and the FSHR ECD (R) relevant for this study is shown in D.

References

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2. Reynolds C, Damerell D, Jones S (2009) *Bioinformatics* **25**, 413-414
3. Laskowski RA (1995) *J. Mol. Graph.* **323-330**, 307-308
4. DeLano WL (2002) The PyMOL Molecular Graphics System. DeLano Scientific, Palo Alto, CA, USA (http://www.pymol.org)
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Supplemental Table 1. Interactions between FSHR ECD and FSH α-subunit.

Hydrogen bonds and salt bridges				
FSHR ECD		FSH α-subunit		Distance (Å)
K^{74}	Nζ	S^{85}	Oγ	$3.4^{1}/3.1^{2}$
	Nζ	T^{86}	Oγ1	$3.2^{1}/3.4^{2}$
N^{129}	Oδ1	L^{48}	N	$3.3^{1}/3.2^{2}$
	Oδ1	V^{49}	N	$3.1^{1}/3.0^{2}$
D^{150}	Oδ2	K^{91}	Nζ	$3.1^{1}/2.8^{2}$
D^{153}	$Oδ2^{1}/Oδ2^{2}$	K^{51}	Nζ	$3.4^{1}/3.2^{2}$
	$Oδ1^{2}$	K^{51}	Nζ	3.2^{2}

Hydrophobic (carbon-carbon) contacts		
Y^{124}	Y^{88}	$\leq 4.0^{1,2}$
T^{130}	L^{48}	$\leq 4.0^{1,2}$
D^{153}	V^{49}	$\leq 4.0^{1,2}$

No interactions
S^{78}
D^{224}
S^{226}

[1,2]Distances are given between the FSHR ECD and FSH α-subunit residues indicated, for each of the two slightly different hormone-receptor complexes (1).

Supplemental Table 2. PROTORP output of hormone-receptor interfaces in the three complexes

	FSH-FSHR[1]			FSH-FSHR[2]			CG-LHR			TSH-TSHR		
	FSHα	FSHβ	ECD	FSHα	FSHβ	ECD	CGα	CGβ	ECD	TSHα	TSHβ	ECD
Interface accessible surface area (Å^2)	817.68	617.40	744.03	859.69	605.67	786.86	813.37	514.28	777.11	757.65	575.23	672.40
% Interface accessible surface area	10.96	7.61	6.52	12.27	7.20	7.29	11.80	6.49	6.86	10.27	6.86	5.72
Atoms in interface	59	49	75	63	45	83	64	42	80	64	49	74
% Polar atoms contribution to interface	35.87	34.67	43.92	33.87	36.71	42.38	34.54	44.46	47.50	33.73	42.96	32.32
% Non-polar atoms contribution to interface	45.84	48.09	28.94	46.92	55.99	28.96	47.58	27.72	23.65	51.08	48.47	37.38
% Neutral atoms contribution to interface	17.70	16.61	25.87	18.74	6.55	27.90	17.34	26.89	27.54	15.03	7.95	28.80
Residues in interface	17	17	27	17	15	28	16	16	27	19	17	24
% Polar residues in interface	52.94	29.41	44.44	58.82	26.67	39.29	50.00	18.75	44.44	57.89	29.41	37.50
% Non-polar residues in interface	17.65	35.29	22.22	17.65	40.00	21.43	18.75	43.75	14.81	15.79	41.18	29.17
% Charged residues in interface	29.41	35.29	33.33	23.53	33.33	39.29	31.25	37.50	40.74	26.32	29.41	33.33
Residues on surface	259	275	259	245	269	245	255	275	255	269	287	269
% Polar residues on surface	42.47	42.18	42.47	43.27	41.64	43.27	41.57	37.45	41.57	43.49	42.86	43.49
% Non-polar residues on surface	31.66	30.18	31.66	31.84	31.23	31.84	35.29	39.64	35.29	35.32	35.54	35.32
% Charged residues on surface	25.87	27.64	25.87	24.90	27.14	24.90	23.14	22.91	23.14	21.19	21.60	21.19
Planarity (Å)	2799	1519	2930	2622	1971	3002	2929	1826	3130	2945	2180	2779
Eccentricity	0.898	0.935	0.774	0.876	0.920	0.720	0.891	0.949	0.807	0.871	0.952	0.745
Secondary structure in interface	Coil	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta
% Alpha character in interface	5.88	11.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.26	17.65	0.00
% Beta character in interface	17.65	47.06	51.85	29.41	53.33	35.71	37.50	56.25	40.74	26.32	41.18	37.50
Secondary structure on surface	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta	Beta
% Alpha character on surface	2.70	2.55	2.70	0.00	0.00	0.00	2.75	2.91	2.75	2.97	2.79	2.97
% Beta character on surface	49.81	50.91	49.81	51.02	49.81	51.02	50.59	50.91	50.59	49.44	50.17	49.44
Hydrogen bonds	7	3	7	13	5	13	6	0	6	2	2	2
Salt bridges	31	29	20	38	27	29	32	18	16	20	21	11
Gap volume (Å^3)	7475.62	8012.25	7475.62	8096.62	7809.75	8096.62	8575.88	7519.50	8575.88	8211.38	8265.38	8211.38
Gap volume index (Å)	4.57	6.49	5.02	4.71	6.45	5.14	5.27	7.31	5.52	5.42	7.18	6.11

PROTORP (2) (http://www.bioinformatics.sussex.ac.uk/protorp/params.html) calculates the properties for one subunit-subunit interaction at the time and the table therefore lists the interface of the α-subunit with the ECD, the interface of the β-subunit with the ECD, and the ECD interface with the α-subunit. The accessible surface area (ASA) is calculated using the NACCESS algorithm. Residues that loose ≥1.0 Å2 of ASA upon complex formation are defined as being part of the interaction site. The terms used to define the shape of the interaction site as well as the gap volume are calculated using the SURFNET package (3). The gap volume is often used to indicate the complementarity and closeness of the two interacting surfaces. The indices 1 and 2 indicate the two slightly different FSH - FSHR complexes found in the PDB deposited by Fan and Hendrickson (1).

Supplemental Table 3. Qualitative comparison between experimental and modeled structures. RMSD values after C-α superposition, percentage sequence alignment for superpositioned structures and Ramachandran values.

	Root-mean-square error of superposition in Angstrom of all Equivalent C-α atoms (relative cover of query vs target length)							
GpHs	**FSH[1]**	**FSH[2]**	**1FL7[1]**	**1FL7[2]**	**1HCN**	**LH**	**TSH**	**CG**
FSH[1]	x	0.7 (99%)	1.0 (95%)	1.6 (95%)	1.5 (88%)	0.4 (100%)	0.3 (99%)	0.7 (98%)
FSH[2]		x	1.0 (96%)	1.5 (97%)	1.6 (89%)	0.7 (98%)	0.7 (98%)	0.8 (96%)
1FL7[1]			x	1.2 (97%)	1.7 (89%)	1.2 (97%)	1.0 (96%)	0.8 (93%)
1FL7[2]				x	1.6 (88%)	1.7 (97%)	1.6 (95%)	1.3 (93%)
1HCN					x	1.5 (88%)	1.6 (89%)	1.8 (88%)
LH						x	0.4 (97%)	0.7 (98%)
TSH							x	0.6 (98%)
CG								x

	Sequence identity of query and target in aligned regions in percentage							
GpHs	**FSH[1]**	**FSH[2]**	**1FL7[1]**	**1FL7[2]**	**1HCN**	**LH**	**TSH**	**CG**
FSH[1]	x	100	99	99	69	67	70	68
FSH[2]		x	99	99	69	66	70	69
1FL7[1]			x	100	69	65	69	67
1FL7[2]				x	68	65	69	67
1HCN					x	94	71	100
LH						x	69	92
TSH							x	69
CG								x

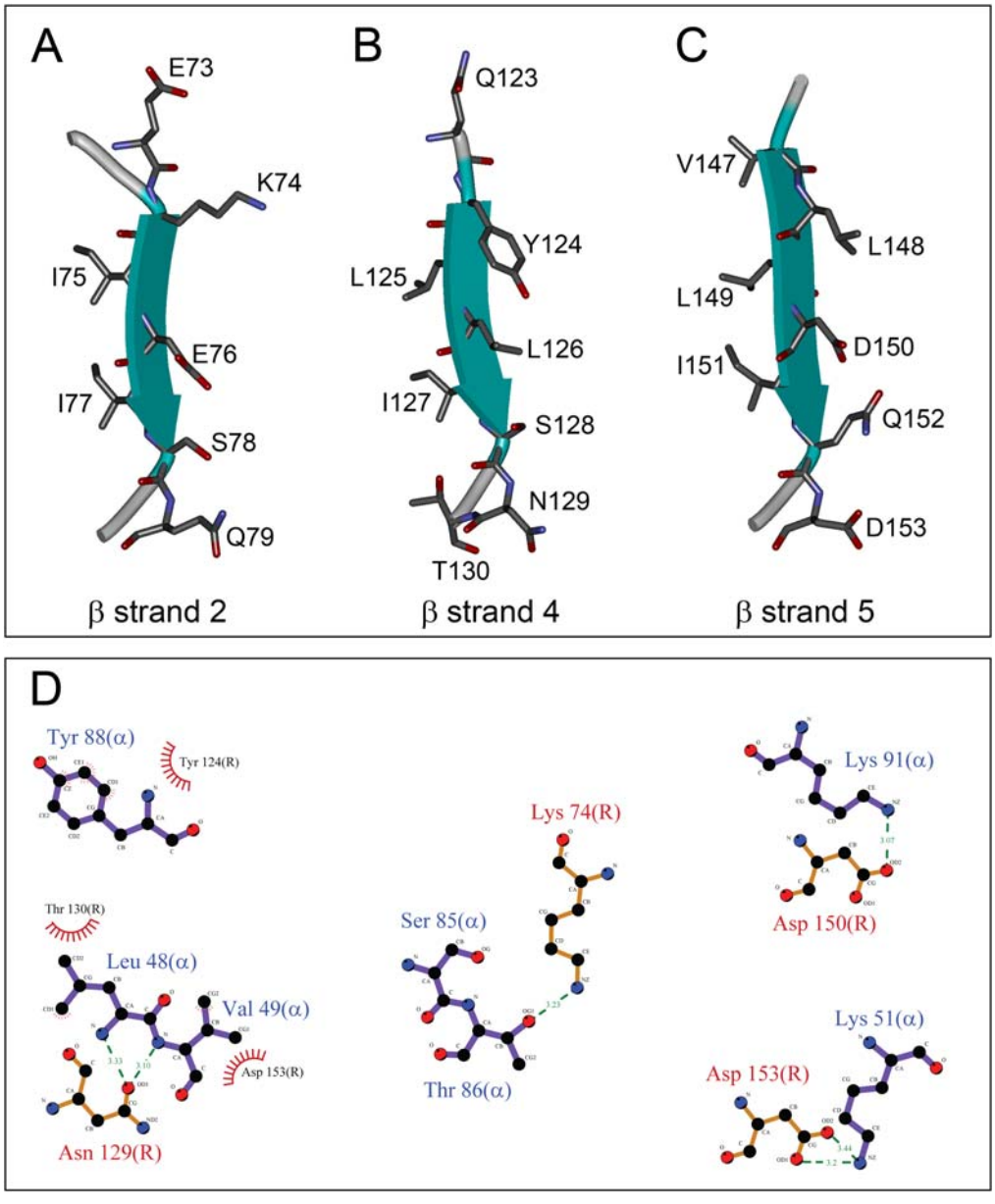
ECDs	**1XWD[1]**	**1XWD[2]**	**LHR**	**3G04**
1XWD[1]	x	0.7 (95%)	0.4 (98%)	1.1 (91%)
1XWD[2]		x	0.5 (91%)	1.2 (95%)
LHR			x	1.1 (93%)
3G04				x

ECDs	**1XWD[1]**	**1XWD[2]**	**LHR**	**3G04**
1XWD[1]	x	100	47	40
1XWD[2]		x	47	40
LHR			x	46
3G04				x

Residues in Ramachandran regions			
ID	**favoured**	**allowed**	**outliers**
FSH[1]	177	12	2
FSH[2]	179	8	1
1FL7[1]	156	20	12
1FL7[2]	173	10	7
1HCN	177	7	7
LH	184	7	4
TSH	182	7	2
CG	169	10	7
1XWD[1]	215	21	4
1XWD[2]	207	23	1
LHR	215	12	5
3G04	212	11	4

% Occupancy of Ramachandran regions			
ID	**favoured**	**allowed**	**outliers**
FSH[1]	92.7	6.3	1.0
FSH[2]	95.2	4.3	0.5
1FL7[1]	83.0	10.6	6.4
1FL7[2]	91.1	5.3	3.7
1HCN	92.7	3.7	3.7
LH	94.4	3.6	2.1
TSH	95.3	3.7	1.0
CG	90.9	5.4	3.8
1XWD[1]	89.6	8.8	1.7
1XWD[2]	89.6	10.0	0.4
LHR	92.7	5.2	2.2
3G04	93.4	4.8	1.8

The initial superposition was done using PyMol (4) but in order to get the numbers of the C-α superposition we used the web-based server TopMatch (5). Both programs agreed on the RMSD values. Ramachandran plots were generated using RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php). See Table 9 (main text) for additional information.



Supplemental Figure 1