

Supplemental Information for

Molecular Mechanisms of Prolactin and its Receptor

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Supplemental Figure 1.

Amino Acid Sequence Alignment of Mature Mammalian Prolactins and Human Lactogenic Hormones. A: Sequence comparison of prolactins from various mammalian species: hPRL (human) P01236, prPRL (maquac) P055151, oPRL (ovine) P01240, bPRL (bovine) P01239, rbPRL (rabbit) Q28632, pPRL (porcine) P01238, rPRL (rat) P01237, and mPRL (mouse) P06879. B: Sequence comparison of human lactogenic hormones: hPRL (P01236), hGH (P01241), and hPL (P01243). Alignment was performed by the Clustal W (167) multiple alignment program in the UniProt suite (168).

Supplemental Figure 2.

Topology Diagram for Human Prolactin.

Supplemental Figure 3.

Amino Acid Sequence Comparisons of Mammalian Receptors. A. Sequence Comparisons of Mammalian Prolactin Receptors: hPRLr (human); P16471, oPRLr (ovine); O46561, bPRLr (bovine); Q28172, rbPRLr (rabbit); P14787, pPRLr (porcine); Q6JTA8, rPRLr (rat); P05710, mPRLr (mouse); Q08501 sequences. Numbering is for hPRL receptor. B. Sequence comparisons of the hPRL, hGH, and hEPO receptors. hPRLr; P16471, hGHr; P10912, and hEPOr; P19235. TM domains are underlined. Alignment was performed by the Clustal W (167) multiple alignment program in the UniProt suite (168).

Supplemental Figure 4.

Topology Diagram for the Extracellular Domain of the Human Prolactin Receptor .

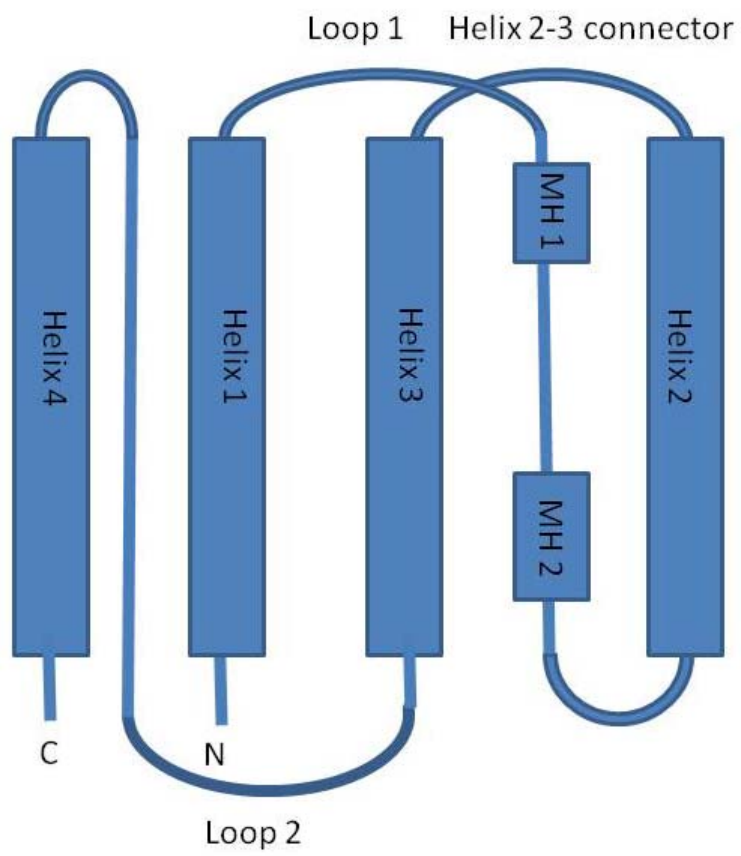
Supplemental Figure 1A.

	1	50
hPRL	LPICPGGAARCQVTLRDLFDRAVVL SHYIHNLSSEMFSEFDKRYTHGRGF	
prPRL	LPICPGGAARCQVTLRDLFDRAVVL SHYIHNLSSEMFSEFDKRYTHGRGF	
oPRL	TPVCPNGPGNCQVSLRDLFDRAVMVSHYIHNLSSEMFNEFDKRYAQGKGF	
bPRL	TPVCPNGPGNCQVSLRDLFDRAVMVSHYIHDLSSEMFNEFDKRYAQGKGF	
rbPRL	LPICPSGAVNCQVSLRDLFDRAVILSHHIHKL SSEMFNEFDKRYTQGRGF	
pPRL	LPICPSGAVNCQVSLRDLFDRAVILSHYIHNLSSEMFNEFDKRYAQGRGF	
rPRL	LPVCSGG - -DCQTPLPELFDQVVMVLSHYIHTLYTDMFIEFDKQYVQDREF	
mPRL	LPICSAG - -DCQTSLRELFDQVVILSHYIHTLYTDMFIEFDKQYVQDREF	
	1	48
	51	100
hPRL	ITKAINSCHTSSLATPEDKEQAQQMNQKDFLSLIVSILRSWNEPLYHLVT	
prPRL	ITRAINSCHTSSLPTPEDKEQAQQMNQKDFLSLIVSILRSWNEPLYHLVT	
oPRL	ITMALNSCHTSSLPTPEDKEQAQQTHHEVLMSLILGLLRSWNDPLYHLVT	
bPRL	ITMALNSCHTSSLPTPEDKEQAQQTHHEVLMSLILGLLRSWNDPLYHLVT	
rbPRL	ITKAINSCHTSSLSTPEDKEQAQQIHHEDLLNMVLRVLP SWNDPLYHLVT	
pPRL	ITKAINSCHTSSLSTPEDKEQAQQIHHVLLNLILRVLR SWNDPLYHLVT	
rPRL	IAKAINDCPTSSLATPEDKEQAQKVPPEVLLNLILSLVH SWNDPLFQLIT	
mPRL	MVKVINDCPTSSLATPEDKEQALKVPPEVLLNLILSLVQSSSDPLFQLIT	
	49	98
	101	150
hPRL	EVRGMQEAPDAILSKAVEIEEQTKRLL EGMELIVSQVHPETKENEIYPVW	
prPRL	EVRGMEEAPDAILSKAVEIEEQTKRLL EGMELIVSQVHPETKENEIYPVW	
oPRL	EVRGMKGVPDAILSRATIEIEEENKRLLEGMEMIFGQVIPGAKETEPYPVW	
bPRL	EVRGMKGAPDAILSRATIEIEEENKRLLEGMEMIFGQVIPGAKETEPYPVW	
rbPRL	EVRGMQEAPDAILSKATIEIEEQNRRLLEGM EKIVGQVHPGIKENEIYSVW	
pPRL	EVRGMQEAPDAILSRATIEIEEQNKRLLEGM EKIVGQVHPGIKENEVYSVW	
rPRL	GLGGIHEAPDAIISRATIEIEEQNKRLLEGI EKISQAYPEAKGNEIYLVW	
mPRL	GVGGIQEAPDAILSRATIEIEEQNKQLLEGVEKIISQAYPEAKGNGIYFVW	
	99	148
	151	199
hPRL	SGLPSLQMADEESRLSAYYNLLHCLRRDSHKIDNYLKLLKCR IHNNC	
prPRL	TGLPSLQMADEESRLSAYYNLLHCLRRDSHKIDNYLKLLKCR IHNNC	
oPRL	SGLPSLQTKDEDARHS AFYNLLHCLRRDSSKIDTYLKLLNCR IYNNC	
bPRL	SGLPSLQTKDEDARYS AFYNLLHCLRRDSSKIDTYLKLLNCR IYNNC	
rbPRL	SGLPSLQMADEDARLFAFYNLLHCLRRDSHKIDNYLKLLKCR IYDSNC	
pPRL	SGLPSLQMADEDTRLFAFYNLLHCLRRDSHKIDNYLKLLKCR IYDSNC	
rPRL	SGLPSLQGVDEESKDLAFYNNIRCLRRDSHKVDNYLKFLRCQIVHKNNC	
mPRL	SGLPSLQGVDEESKILSLRNTIRCLRRDSHKVDNFLKVLRCQIAHQNNC	
	149	197

Supplemental Figure 1B.

	1		49
hPRL	LPICPGGAARCQVTLRDLFDRAVVLSHYIHNLSSEMFSEFDKRYTHGRG-		
hGH	-----FPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQK		
hPL	-----VQTVPLSRLFDHAMLQAHRAHQLAIDTYQEFEEETYIPKDQK		
	1		41
	50		96
hPRL	---FITKAINSCHTSSLATPEDKEQAQOMNQKDFLSLIVSILRSWNEPLY		
hGH	YSFLQNPQTSLCFSESIPTPSNREETQOKSNLELLRISLLLIQSWLEP--		
hPL	YSFLHDSQTSFCFSDSIPTPSNMEETQOKSNLELLRISLLLIQSWLEP--		
	42		89
	97		146
hPRL	HLVTEVRGMQEAPAEILSKAVEIEEQTKRLL EGMELIVSQVHPETKENEI		
hGH	--VQFLRSVFANSLVYGASDSNVYDLLKDLE EGIQTLMGRL EDGSPRTGQ		
hPL	--VRFLRSMFANNLVYDTSDDYHLLKDLE EGIQTLMGRL EDGSRRTGQ		
	90		137
	147		199
hPRL	YPVWSGLPSLQMADEESRLSAYYNLLHCLRRDSHKIDNYL KLLKCRIIHNNNC-		
hGH	IFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF		
hPL	ILKQTYSKFDTNSHNHDALLKNYGLLYCFRKDMDKVETFLRMVQCRSVEGSCGF		
	138		191

Topology Diagram for Human Prolactin



Supplemental Figure 3A.

	1	50
hPRLr	QLPPGKPEIFKCRSPNKETFTCWWRPGTDGGLPTNYSPTYHREGETLMHE	
oPRLr	QSPPEKPKLIKCRSPGKETFTCWWEPEGADGGLPTNYTLTYRKEGETLIHE	
bPRLr	QSPPEKPKLVKCRSPGKETFTCWWEPEGADGGLPTNYTLTYHKEGETLIHE	
rbPRLr	QSPPGKPFIFKCRSPEKETFTCWWRPGADGGLPTNYTLTYHKEGETITHE	
pPRLr	QSPPGKPEIFKCRSPEKETFTCWKPGADGGLPTNYTLTYHKEGETFTHE	
rPRLr	QSPPGKPEIHKCRSPDKETFTCWNPNGTDGGLPTNYSLTYSKEGEKTTYE	
mPRLr	QSPPGKPEIHKCRSPDKETFTCWNPNGSDGGLPTNYSLTYSKEGEKNTYE	
	51	100
hPRLr	CPDYITGGPN SCHFGKQYTS MWRTYIMMVNATNQMGSSFSDELYVDVTYI	
oPRLr	CPDYKTGGPN SCYF SKKYTSIWKMYVITV SAINQMGISSSDPLYVDVTYI	
bPRLr	CPDYKTGGPN SCYF SKKHTSIWKMYVITV NAINQMGISSSDPLYVHVTYI	
rbPRLr	CPDYKTGGPN SCYF SKKHTSIWTIYIITV NAINQMGISSSDPRYVDVTYI	
pPRLr	CPDYKTGGPN SCYF NKKHTSIWTIYIITV NAINQMGISSSDPRYVDVTYI	
rPRLr	CPDYKTS GPN SCFFSKQYTSIWKIYIITV NAINQMGISSSDPLYVDVTYI	
mPRLr	CPDYKTS GPN SCFFSKQYTSIWKIYIITV NATNEMGSSTSDPLYVDVTYI	
	101	150
hPRLr	VQDP PLELAVEVKQPEDRKP YLWIKWSPPTLIDLKTGWFTLLYEIRLKP	
oPRLr	VEPEPPVNL TLELKH PEDRKP YLWIKWSPPTLTDVKSGWFSIQYEIRLKP	
bPRLr	VEPEPPANL TLELKH PEDRKP YLWIKWSPPTMTDVKSGWFIIQYEIRLKP	
rbPRLr	VEPDPPVNL TLEVKH PEDRKP YLWVKWLPPTLVDVRSGLTLQYEIRLKP	
pPRLr	VEPDPPVNL TLELKKPEDQKPYLWIKWLPPTLVDVRSGLTLQYEIRLQP	
rPRLr	VEPEPPRNL TLEVKQLKDKKTYLWVKWSPPTITDVKTGWFTMEYEIRLKP	
mPRLr	VEPEPPRNL TLEVKQLKDKKTYLWVKWLPPTITDVKTGWFTMEYEIRLKS	
	151	200
hPRLr	EKAAEWEIHFAGQQTQFKILSLHPGQKYLQV RCKPDHGYWSAWSPATFI	
oPRLr	EKATDWETHFAPKLTQLKIFNLYPGQKYLQV RCKPDHGYWSEWSPESFI	
bPRLr	EKATDWETHFTLKQTQLKIFNLYPGQKYLQV RCKPDHGYWSEWSPESSI	
rbPRLr	EKAAEWEIHFAGQQTQFKILSLYPGQKYLQV RCKPDHGFWSVWSPESSI	
pPRLr	EKTAEWEIHFAGQQTQFKILSLYPGQKYLQV RCKPDHGFWSWSPESSI	
rPRLr	EEAEWEIHF TGHQTQFKVFDLYPGQKYLQV TRCKPDHGYWSRWSQESSV	
mPRLr	EEAEWEIHF TGHQTQFKVFDLYPGQKYLQV TRCKPDHGYWSRWGQEKSI	
	201	250
hPRLr	QIPSDFTMNDTTVWISVAVLSAVICLIIVVAVALKGYS MVTCIFPPVPGP	
oPRLr	QIPNDFPVKDTSMWIFVGLSAVICLIMVVAVALKGYS MVTCILPPVPGP	
bPRLr	QIPNDFPVKDTSMWIFVAILSAVICLIMVVAVALKGYS MVTCILPPVPGP	
rbPRLr	QIPNDFTMKDITVWIFVAVLSTIICLIMVVAVALKGYS MVTCIFPPVPGP	
pPRLr	QIPNDFSMKDTTMMWIFVAILS AVVCLIMI VVAVALKGYS MVACILPPVPGP	
rPRLr	EMPNDFTLKDTT VWIIVAILS AVICLIMVVAVALKGYS MMTTCIFPPVPGP	
mPRLr	EIPNDFTLKDTT VWIIVAVLSAVICLIMVVAVALKGYS MMTTCIFPPVPGP	

251 300
 hPRLr KIKGFD AHLLEK GKSE ELLSALGCQDFPPTS DYEDLLVEY LEVDDSE DQH
 oPRLr KIKGFDIHLLEK GKSE ELLRALESQDFLPTS DCE DLLMEFIEVDDSE DQH
 bPRLr KIKGFDVHLLEK GKSE ELLRALESQDFPPTS DCE DLLMEFIEVDDCE DQ
 rbPRLr KIKGFDTHLLEK GKSE ELLSAFGCQDFPPTADCE DLLVEFLEVDDSE DQ
 pPRLr KIKGFDTHLLEK GKSE ELLSALGCQDFPPTS DCE DLLVEFLEVDDSE DQ
 rPRLr KIKGFDTHLLEK GKSE ELLSALGCQDFPPTS DCE DLLVEFLEVDDNE DER
 mPRLr KIKGFDTHLLEK GKSE ELLSALGCQDFPPTS DCE DLLVEFLEVDDNE DER

301 350
 hPRLr LMSVHSKEHPSQGMKPTYLDPD TDSGRGSCD SP SLLSEKCEEPQANPSTF
 oPRLr LMPHPSKEHMEQGVKPMHLDPD TDSGRGSCD SP SLLSEKCEEPQAYPSKF
 bPRLr LMPRPSKEHTEQGVKPMHLDLSDS GRGSCD SP SLLSEKCEEPQAHPSKF
 rbPRLr LMPAHSKEHSGPGMKPTDLDPD NDSGRGSCD SP SLLSEKCEEPQANPSTF
 pPRLr LMPAHSKEHPSQGRKPTHLPDSDS GRGSCD SP SLLSEKCEEP RANPPKF
 rPRLr LMPSHSKEYPGQGVKPTHLPDSDS GHGSYDSH SLLSEKCEEPQAYPPTL
 mPRLr LMPSHSKEYPGQGVKPTHLPDSDS GHGSYDSH SLLSEKCEEPQAYPPAF

351 397
 hPRLr YDPEVIEK PENP--ETTHTWDPQCISMEGKIPYFHAGGSKCSTWPLQP-
 oPRLr HIPEGPEKLEDPETNHTCLQAPQSTSGEGKIPYFLANGPKSSTWPFQPP
 bPRLr HTPEGPEKLENPETNL TCLQAPQSTSV EGKIPYFLANGPKSSTWPFQPP
 rbPRLr HTPEVIEQPEKPKANVTHTWDPQTISLVGKMPYLSVNGSKSSTWPLLQP-
 pPRLr HTPEGIEKPGDPETNLPRPQDPQSTSVESKLLYFHADGSKSSTWPLQPP
 rPRLr HIPEITEK PENPEANIPPTVDPQSTN-----PNFHVDAPKSSTWPLLPG-
 mPRLr HIPEITEK PENPEANIPPTPNPQNNT-----PNCHTDTSKSTTWPLPPG-

398 447
 hPRLr SQHNPRSSYHNI TDVCELAVGPAGAPATLLNEAGKDALKSSQTIKSREEG
 oPRLr SLYSPRYSYHNIADVCELALGMAGTTATLLDQTDQHAFKPSKTIETGGEG
 bPRLr SLYSPRYSYHNIADVCELALGMAGTTATSLDQTDQHALKASKTIETGREG
 rbPRLr GQHNTNSPYHNIADMCKLATS-----LDKIDKDALQSSKTEAAGEE
 pPRLr ILHDPGSSYHNLADVGQLVLGTAGATAALLEKTDRHAFNPPKTTETGGEG
 rPRLr -QHMPRSPYHSVADVCKLAGSPVNTLDSFLDKAEENVLKL SKALETGEE-
 mPRLr -QHTRRSPYHSIADVCKLAGSPGDTLDSFLDKAEENVLKL S--EDAGEE-

448 497
 hPRLr KATQQREVESFHSETDQDTPWLLPQEKTPFGSAKPLDYVEIHKVNKD GAL
 oPRLr KAAKQSESEGY SSEPDQDMAWPLLQDKTPLFS AKPLEYVEIHKVSQDGV L
 bPRLr KATKQRESEGCSSKPDQDTVWPRPQDKTPLISAKPLEYVEIHKVSQDGV L
 rbPRLr KATKQREVESSHSAEQDTGWLLPKEKPPFISP KPLDYVEIHKVNKD GAL
 pPRLr RATEQKESESFHSKTDQGTWVLLPQDKGPFVSPKPM EYVEIHKVSKD GAL
 rPRLr EVAEQKGAKSFP SD-KQNTPWPLLQEK SPTVYVKPPDYVEIHKVNKD GAL
 mPRLr EVAVQEGAKSFP SD-KQNTSWPPLLQEK GPIVYAKPPDYVEIHKVNKD GAL

498 547

hPRLr	SLLPKQRENSGKPKKPGTPENNKEYAKVSGVMDNNILVLPDPHAKNVAC
oPRLr	ALFPKQNE-----KVDAPETSKEYSKVSRVTDSNII LVLIPDLQAQNLT L
bPRLr	ALFPKQNE-----KFGAPEASKEYSKVSRVTDSNII LVLVPDPQAQNLT L
rbPRLr	SLLLKQKENG DQTGKAGTPETSKEYAKVSRVMDNNII LVLVQDPGAQNVAL
pPRLr	ALLPKQQENGDRPEKAGAPETSKEYAQVSRVMDNHI LVLVQDPRARNVAP
rPRLr	SLFPKQREN-NQTEKPGVPETSKEYAKVSGITDNNII LVLVPDSRAQNTAL
mPRLr	SLLPKQREN-HQTENPGVPETSKEYAKVSGVTDNNII LVLVPDSRAQNTAL

548

598

hPRLr	FEEAKEAPPSLEQNQAEKALANFTATSSKRLQLGGLDYLD PACFTHSFH
oPRLr	LEESAKKAPPALP-----
bPRLr	LEEPAKKAPPALP-----
rbPRLr	FEESTKEAPPSPSQNQAEKDLSSFSTAPSDCRLQQGGLDYLD PACFMHSLH
pPRLr	FEEPTKETPPSRPQNPAAKDLASFTTAPGHRHPLGGLDYLD PAGFMHSFQ
rPRLr	LEESAKKAPPSFEADQSEKDLASFTATSSNRRLQLGRLDYLDPTCFMHSFH
mPRLr	LEESAKKVPPSLEQNQSEKDLASFTATSSNCRLQLGRLDYLDPTCFMHSFH

Supplemental Figure 3B.

hPRLr 1	-----QLPPG--KPEIFKCRSPNKETFTCWWR	25
hGHr 1	SGSEATAAILSRAPWSLQSVNPLKTNSSKEPKFTKCRSPERETFSCHW	50
hEPOr 1	-----APPPNLPDPKFESKAALLAARGPEELLCFTERLEDLVCFWE	41
hPRLr 26	PGTDGGLPTN-----YSLTYHREGETLMHECPDYITGGPNSCHFGKQYT	69
hGHr 51	DEVHHGTKNLGPIQLFYTRRNTQEWQEWKECPDYVSAGENSCYFNSSFT	100
hEPOr 42	EAASAGVGPNG-YSFSYQLEDEPWKLCRLHQAPTARGAVRFWCSLPTADT	90
hPRLr 70	SMWRTYIMMVNATNQMGSSFSDELYVDVTYIVQDPDPLELAVEVK--QPE	117
hGHr 101	SIWIPYCIKLSN----GGTVDEKCFVDEIVQDPPIALNWTLLNVSLT	146
hEPOr 91	SSFVPLELRVTAAS---GAPRYHRVIHINEVLLDAPVGLVARLA-----	132
hPRLr 119	DRKPYLWIKWSPPTLIDLKTGWFTLLYEIRLKPEKAAEWEIHAFAGQQTEF	167
hGHr 147	GIHADIQVRWEAPRNADIQKGMVLEYLELQYKEVNETKWKMMDPILTTSV	196
hEPOr 133	DESGHVLRWLPPPETPMTS-HIRYEVDSAGNGAGSVQRVEILEGRTEC	182
hPRLr 169	KILSLHPGQKYLQVR---CKP-DHGYWSAWSPATFIQIP-- <u>SDFTMNDT</u>	211
hGHr 197	PVYSLKVDKEYEVRVR---SKQRNSGNYGEFSEVLYVTLPQMSQFTCEED	243
hEPOr 185	VLSNLRGRTRYTFAVRARMAEPSFGGFWSAWSEPVSLTLP-----SD	224
hPRLr 213	<u>T--VWISVAVLSAVICLIIVWAVALKG</u> -YSMVTCIFPPVPGPKIKGFDAH	258
hGHr 244	<u>FYFPWLLIIIFGIFGLTVMFLFVFLFSKQRIKMLILPPVPVKIKGIDPD</u>	293
hEPOr 225	<u>LDPLILTLSLILVILVLLTVLALLSHRRALKQKIWPGIPSESEFEGLF</u>	274
hPRLr 260	LLEKKGSEELLSALGCQD-FPPTSDYEDLLVEYLEVDDSE-DQHLMVSHS	306
hGHr 294	LLKEGKLEEVNTILAIHDSYKPEFHSDDSWVEFIELDIDEPDEKTEESDT	344
hEPOr 275	TTHKGNFQLWLQNDGCLWWSPTPFTE-----	301
hPRLr 308	KEHPSQGMKPTYLDP---DTDSGRGSCDSPSLLSEKCEE--PQANPSTFY	351
hGHr 345	DRLLSSDHEKSHSNLGVKDGDSGRTSCCEPDILETDFNANDIHEGTSEVA	394
hEPOr 302	-----DPPASLEVLSERCWGTMQAVEPGTDD	328
hPRLr 353	DPEVIEKPENPETHTTWDQPCISMEGKIPYFHAG----GSKCSTWPLPQP	397
hGHr 395	QPQRLKGEADLLCLDQKNQNSPYHDACPATQQPSVIAEKNKPQPLPTE	444
hEPOr 329	EGPLLEPVGSEHAQDTY-----LVLDKWLLPRN-	355
hPRLr 400	-SQHNPRSSYHNITDVCELAVGPAGAPATLLNEAGKDALKSSQTIKSREE	446
hGHr 445	GAESTHQAHIQLSNPSSLSNIDFYAQVSDITPAGSVVLSPGQKNKAGMS	494
hEPOr 356	----PPSEDLPGGGSVDIVAMDEGSEASSCSSALASKPSP-----E	394
hPRLr 450	GKATQQREVESFHSETDQDTPWLLPQEKTPFGSAKPLDYVEIHKVNKDGA	496
hGHr 495	QCDMHPPEMVSLCQENFLMDNAYFCEADAKKCI PVAPHIKVESHIQPSLNQ	543
hEPOr 395	GASAASFYITLDPSSQLLRPWTLCPELP---PTPHLKYLYLVVSDSGIS	441

hPRLr	500	LSLLPKQRENSGKPKKPGTPENNKEYAKVSGVMDNNILVLPDPHAKNVA	546
hGHr	544	EDIYITTESLTAAAGRPGTGEH-----VPGSEMPVPD	575
hEPOr	442	STDYSSGDSQGAQGG-----	455
hPRLr	550	CFEESAKEAPPSLEQNQA EKALANFTATSSKRLQLGGLDYLD PACFTHSFH	598
hGHr	576	YTSIHIVQSPQGLILNATALPLPDKEFLSSCGYVSTDQLNKIMP-----	619
hEPOr	458	-LSDGPYSNPYENSLIPAAEPLPPSYVACS-----	484

Topology Diagram for the Extracellular Domain of the Human Prolactin Receptor

