6 APPENDIX

Collection of Proline-Kinked Transmembrane Helices

Because of influences on transmembrane helices by different proline insertions, we collected structural data based on transmembrane helices with proline-kinks. To decide, which structure (first four alphanumeric letters of entries represent entries of Protein Data Base, PDB) finally should be used for modeling our transmembrane bundle based on rhodopsin template, the following sequences were aligned against the respective sequence from our receptors.

CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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                                        *
    1a87 ----------------------WGPLLLEVESWII                                  13
 1aij_1_1 ----------------------HIPFAFAFAILAYLTLVLFRPVM                        23
 1aij_1_2 ----------------------HYNPAHMIAISFFFTNALALALHGALVLSAA               31
 1aij_1_3 ---------------------MRTPDHEDTFFRDL                                 14
 1aij_1_4 ----------------------GWNPAVFLR                                     9
 1aij_1_5 ----------------------IRPIL                                         5
 1aij_1_6 ----------------------FYNPFHGLSIAFLYGSALLFAMHGATILA                 29
 1aij_1_7 ----------------------GFPHAPTGDPMK                                  12
 1arl_a_1 ----------------------VIPALFGGFGNYFMPLH                             17
 1arl_a_2 ----------------------AFPRLNNLSYWMYVCGVALGVASLL                     25
 1arl_a_3 ---------------------LSLPVLAGAITMLLMD                               16
 1arl_a_4 ----------------------GDPVLYQHILWFFGHPEVYIIILPGFGIISHVIST           35
 1arl_a_5 ----------------------YLPMVLAMAAIGIL                               14
 1arl_a_6 ----------------------AVPTGIKVFSWIAT                               14
 1arl_a_7 ----------------------KIPMLWAFGFLFLFTVGGVTGVVL                     24
 1bcc_c_1 ----------LTRFFALHFLLPFAIAGITIIHLTFLHE                             28
 1bcc_c_2 ----------------------DKIPFHPYYSFKDILGLTLMLTPFLTLA                 28
 1bcc_c_3 ------------------LILFLIPFLH                                        10
 1bcc_c_4 ----------------------FRPLSQTLFWLLVANLLILTWIGSQPVEHPFIIIGQMASL     40
 1bcc_c_5 ----------------------LFPTIGTLENKM                                  12
   1bcc_e ----------------------ESDPSRKGFSYLVTAVTTLGVAYAAKNVVTQFVSSM         36
 1bcc_g_1 ----------------------GVPNVW                                        6
 1bcc_g_2 ----------------------VAPPFLAFYLLYTWGTQEF                          19
 1bgy_c_1 ----------------------FILPFIIMAIAMVHLLFL                           18
 1bgy_c_2 ----------------------DKIPFHPYYTIKDILGALLL                         20
 1bgy_c_3 ----------------------LIPLL                                         5
 1bgy_c_4 ----------------------GGQPVEHPYITIGQLASVLYFLLILVLMPTAGTIENKL     38
   1bgy_d ----PEHDHRKRMGLKMLLMMGLLLPLVYAMKRHKWSVLKS                          37
   1bgy_f ----------------------YLEPYLKEVIRERKEREEW                          19
 1bgy_g_1 --------------------YFSKGIPNVLR                                     11
 1bgy_g_2 -------------------ILRVAPPFVAFYLVYTWGTQEFE                          23
 1c3w_a_1 ----------------------SDPDAKKFYAITTLV                              15
 1c3w_a_2 ----------------------TTPLLLLDLALL                                 12
 1c3w_a_3 ----------------------MRPEVASTFKVLRNVTVVLWSAYPVVWL                 28
    1cii ----------------------WRPLFVKTE                                      9
   1col_a ----------------------WGPLMLEVESWVL                                 13
   1dxr_a -EKMTLSISVLLSLTVFLLVIVELIPST                                        27
 1el2_a_1 ----------------------GRPRLIWGATLMIPLVSISSYLGLLS                    26
 1el2_a_2 ----------------------STPMILLALGLL                                  12
 1el2_a_3 -------AEIFDTLRVLTVVLWLGYPIVWAV                                     24
   1ezv_d ----------------------EPEHDERKRLGLKTVIILSSLYLLSIWVKKFKWAGIK       37
 1ezv_f_1 -----------------VLSKLCVPVANQFINLA                                  17
 1ezv_f_2 ----------------------ENPIMQTALR                                    10
 1ezv_f_3 ----------------------LLPYILEAEAAAKEKDELD                           19
   1ezv_g -------------------FLYVLIPAGIYWYWWKNGNEYNEFL                        25
 1fx8_a_1 ----------------------LNPAVTIALWL                                   11
 1fx8_a_2 ----------------------VIPFIVSQVAGAFCAAALVYGL                        22
 1fx8_a_3 --------------------GNGVPRGPLAPLLIGLLIAVIGASMGPLT                  29
 1fx8_a_4 ----------------------MNPARDFGPKVFAWL                               15
 1fx8_a_5 ----------------------FGPIVGAIVGAFAYRK                              16
   1ih5_a ----------------------VGPFIGGAL                                     9
 1jb0_a_1 ----------------------SLPINKLL                                      8
 1jb0_a_2 ----------------------DIPLPHEFILNPSLMAE                             17
 1jb0_a_3 ----------------------TDYPTQLSLFTHHMWIGGFLVVGGAAHGAIFMV            33
 1jb0_b_1 ----------------------AIPES                                         5
 1jb0_b_2 ----------------------STMPHPAGLAPFF                                 13
 1jb0_b_3 --------------------GGFHPQTES                                       9
 1jb0_b_4 ----------------------WLPGWLDA                                      8
 1jb0_b_5 ----------------------IGPGDFLVHHAIALGLHTTTLILVKGAL                 28
 1jgj_a_1 ----------------------FVPRYIDWILTTPLIVYFLGLLA                       23
 1jgj_a_2 ----------------------VGPMTES                                       7
 1jgj_a_3 ----SGIKSLYVRLRNLTVVLWAIYPFIWL                                      26
 1jgj_a_4 ----------------------LTPTVDVALIVYLDLV                              16
   1kzu_a --------------NQGKIWTVVNPAIGIPALLGSVTVIAILVHLAILSHTTWFPAYWQGGV     48
   1lgh_a ----------------------SNPKDDYKIWLVINPSTWLPVIWIVATVVAIAVHAAVLAAPGFNWIALGAAKSAAK 56
 1prc_l_1 ----------------------SQGPTWDPFAI                                   11
 1prc_l_2 ----------------------HVPLAPCVPIFMFCVLQVFRPLL                       23
 1prc_l_3 ----------------------HYNPGHMSSVSFLFVNAMALGLHGGLILSV               30
 1prc_l_4 ----------------------GWPEW                                         5
 1prc_l_5 ----------------------CIHPTLV                                       7
 1prc_l_6 ----------------------GVPFGIWPHIDWLTAFSIR                           19
 1prc_l_7 ----------------------YYCPWHGFSIGFAYGCGLLFAAHGATILA                29
 1prc_l_8 ----------------------EGAPLQPTGNPLV                                 13
    ruler 1.......10........20........30........40........50........60........70.........
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CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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                                     *
  1prc_l_9 ---------------------LYATPERA                                                        8
  1qle_c_1 -----------------------IWPFFGAI                                                      8
  1qle_c_2 ----------------------EHTPVVRIGLQYGFILFIMSEVMFFVAWFW                               30
  1vsg_a_1 --------------------QAFWQPLCQVSEELDDQPKGALFTLQAAASKIQKMRDAALRASIYAEI               48
  1vsg_a_2 -----------------------HQPWIDAWKAKK                                                 12
  2occ_a_1 -----------------------VMPIM                                                         5
  2occ_a_2 -----------------------AFPRMNNMSFWLLPPSFLLLLAS                                     23
  2occ_a_3 -----------------------SLPVLAAG                                                      8
  2occ_a_4 ---------------------GGGDPILYQHLF                                                   12
  2occ_a_5 -----------------------GHPEVYILILPGFGMISHIVTY                                      22
  2occ_a_6 -----------------------AIPTGVKVFSWLATL                                             15
  2occ_a_7 -----------------------WSPAMMWALGFIFLFTVGGLTGIV                                    24
  2occ_a_8 -----------------------WFPLF                                                         5
  2occ_c_1 -----------------------VNPSPWPLTGALSALLMTSGLTMWF                                   25
  2occ_c_2 ----------------------HHTPAVQKGLRYGMILFIISEVLFFTGFFWAFYHSS                         36
  2occ_c_3 -----------------------IHPLNPLEVPLLNTSVLLA                                         19
    2vsg_a -----------------------WQPECELTAEL                                                 11
  1c17_a_1 ------LIPLLRTQFFIVMGLVDAIPMIAVGLGLYVMFAVA                                          35
 1c17_m_1a -------------------KSKLIAPLALTIFVWVFLMNLMDLLPIDLLP                               31
 1c17_m_1b KSKLIAPLALTIFVWVFLMNLMDLLPIDLLP                                                   31
  1c17_m_2 -----------------GLPALRVVPSADVNVTLSMALGVFILILFYSI                                 32
  1c17_n_1 --------------------SLLSKPVSLGLRLFGNMYAGELIFILIAGL                                30
  1c17_n_2 -------------------QWILNVPWAIFHILIITLQAFIFMVLTIVYLS                               32
     ruler 1.......10........20........30........40........50........60........70.........
```

Multiple Sequence Alignment of Endothelin Receptors by Subtypes

Endothelin receptor subtypes ETA and ETB from different species were sorted by subtype, and were aligned using typical transmembrane arrangement of G protein-coupled receptors.

et1r – endothelin receptor subtype ETA
etbr – endothelin receptor subtype ETB
OPSD – rhodopsin

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CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT
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          etlr_bovin ME---TFWLRLSFWVALVGGVISDNPESYSTNLSIHVDSVATFHGTELSF    47
        etlr_chicken ME---ALYLRVSSLLLLLGFVLCDSSDKYATNWSDVGIYLPTSSGIESSL    47
etlr_mouse_fragment ----------------------------------------------------
           etlr_pig ME---TFCFRVSFWVALLGCVISDNPESHSTNLSTHVDDFTTFRGTEFSL    47
           etlr_rat MG---VLCFLASFWLALVGGAIADNAERYSANLSSHVEDFTPFPGTEFNF    47
etlr_rabit_fragment ---VISDNPERYSTNLSNHMDEFTTFHGPELNLLV------TTHRPTNLV    41
         etlr_sheep ME---TFWLRVSFWVALVGGVISDNPESYSTNLSIHVDSVTTFRGTELSF    47
         etlr_human ME---TLCLRASFWLALVGCVISDNPERYSTNLSNHVDDFTTFRGTELSF    47
       etar_selectiv TKTSATAQYFGRFDHNDFFSNQLISFVPFEQHTNATSEQDGCINNSRSFK    50
       etbr_selectiv IEKETLSKHYEDEVASKKWLVDTMSYLIHPVQTAQKTSIFRSQNLLQNPL    50
          etbr_human MQPPPSLCGRALVALVL-ACGLSRIWGEERGFPPDRAT-PLLQTAEIMTP    48
          etbr_bovin MQPLPSLCGRALVALIL-ACGVAGIQAEEREFPPAGATQPLPGTGEMMET    49
          etbr_canfa MQPPPSLCGRALVALVL-ACGLSRIWGEERGFPPDRAT-PLLQTAEIMTP    48
          etbr_horse MQPLPTLCGRVLVALIL-ACGVAGVQGEERRFPPARATPPLLGFEEIMTP    49
          etbr_mouse MQSPASRCGRALVALLL-ACGFLGVWGEKRGFPPAQATLSLLGTKEVMTP    49
            etbr_pig MQPLRSLCGRALVALIF-ACGVAGVQSEERGFPPAGATPPALRTGEIVAP    49
            etbr_rat MQSSASRCGRALVALLL-ACGLLGVWGEKRGFPPAQATPSLLGTKEVMTP    49
etbr_cotja_fragment -----EIKETFKYINTVVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-N    43
etbr_macfa_fragment -------P-----FGAEMCKLVPFIQKASVGITVLSLCALSIDRYRAVAS    38
          etbr_rabit MQPPPSLCGLALLALVL-ACGMAEVWGEEREMPSAPATPPLLGASEILTP    49
          et_r_oryla -MRASVLLLCVFMVTEVGTSRLVRDSQSAP----EALEVSETNSSVQLPL    45
                     --------SSTM-TMTMTM-----SSTMTMTMH                     19
                OPSD ----------------------------------------------------
               ruler 1.......10........20........30........40........50

          etlr_bovin VV------TTHQPTNLALPSNGSMHN------------------------    67
        etlr_chicken LP------TTRRPP----VSNQTVK-------------------------    62
etlr_mouse_fragment ------------------------------------ATLLRIIYQNK    11
           etlr_pig VV------TTHRPTNLALPSNGSMHN------------------------    67
           etlr_rat LG------TTLQPPNLALPSNGSMHG------------------------    67
etlr_rabit_fragment LPSNGSRHN-----------------------YCPQQTKITSAFKYINT    67
         etlr_sheep VV------TTHQPTNLALPSNGSMHN------------------------    67
         etlr_human LV------TTHQPTNLVLPSNGSMHN------------------------    67
       etar_selectiv EMKFQ                                              55
       etbr_selectiv K                                                  51
          etbr_human PT------KTLWP----KGSNASLARSLAPAEVPKGDRTAGSPPRTISPP    88
          etbr_bovin PT------ETSWP----GRSNASDPRSSATPQIPRGGRMAGIPPR--TPP    87
          etbr_canfa PT------KTLWP----KGSNASLARSLAPAEVPKGDRTAGSPPRTISPP    88
          etbr_horse PT------KTSWP----TGSNASVPRLSAPPQMPKAGRTAGAQRRTLPPP    89
          etbr_mouse PT------KTSWT----RGSNSSLMRSSAPAEVTKGGRGAGVPPRSF-PP    88
            etbr_pig PT------KTFWP----RGSNASLPRSSSPPQMPKGGRMAGPPARTLTPP    89
            etbr_rat PT------KTSWT----RGSNSSLMRSSAPAEVTKGGRVAGVPPRSF-PP    88
etbr_cotja_fragment ILIASLALGDLLHIIIDIPISVYKLLAEDWP-----FGVEMCKLVPFIQK    88
etbr_macfa_fragment WSRIKGIGIPKWTAVEIVLIWVVSVVLAVPEAIGFDMITMDYKGSYLRIC    88
          etbr_rabit ST------KTSWP----RDSNASLPRSSAPAEIPKEGRTAGAPRRT--PP    87
          et_r_oryla PQPGR-----PRSPPPPMCVKPTEIKDAFKYVNTIISCLIFVVGIIGNST    90
                                                                           19
                OPSD ----------------------------MNGTEGPNFYVPFSNKTGVVRSPF    24
               ruler .......60........70........80........90.......100
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CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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```
          etlr_bovin YCPQQTKITSAFKYINTVISCTIFIVGMVGNATLLRIIYQNKC-MRNGP-   115
        etlr_chicken -CSQQTKIAETFKYINTVVSCAIFIVGMVGNATLLRIIYQNKC-MRNGP-   109
etlr_mouse_fragment C-MRNGP-NALIASLALGDLIYVVIDLPINVFKLLAGRWPFDHNDFGVFL    59
           etlr_pig YCPQQTKITSAFKYINTVISCTIFIVGMVGNATLLRIIYQNKC-MRNGP-   115
           etlr_rat YCPQQTKITTAFKYINTVISCTIFIVGMVGNATLLRIIYQNKC-MRNGP-   115
etlr_rabit_fragment VISCTIFIVGMVGNATLLRIIYQNKC-MRNGP-NALIASLALGDLIYVVI   115
         etlr_sheep YCPQQTKITSAFKYINTVISCTIFIVGMVGNATLLRIIYQNKC-MRNGP-   115
         etlr_human YCPQQTKITSAFKYINTVISCTIFIVGMVGNATLLRIIYQNKC-MRNGP-   115
       etar_selectiv                                                      55
       etbr_selectiv                                                      51
          etbr_human PCQGPIEIKETFKYINTVVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   136
          etbr_bovin PCDGPIEIKETFKYINTVVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   135
          etbr_canfa PCEGSIEIKETFKYINTVVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   136
          etbr_horse PCERTIEIKETFKYINTVVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   137
          etbr_mouse PCQRNIEISKTFKYINTIVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   136
            etbr_pig PCEGPIEIKDTFKYINTVVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   137
            etbr_rat PCQRKIEINKTFKYINTIVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   136
etbr_cotja_fragment ASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVISVVL   138
etbr_macfa_fragment LLHPVQKTAFM                                          99
          etbr_rabit PCQRPTEIKDTFKYINTVVSCLVFVLGIIGNSTLLRIIYKNKC-MRNGP-   135
          et_r_oryla LLRIIYRNKC-MRNGP-NVLIGSLALGDLLYIIIAIPINVYKLIAEDWP-   137
                                                                           19
                OPSD EAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKK-LRT-PL    72
               ruler .......110.......120.......130.......140.......150
```

```
          etlr_bovin NALIASLALGDLIYVVIDLPINVFKLLAGRWPFEQNDFGVFLCKLFPFLQ   165
        etlr_chicken NALIASLALGDLIYIVIDIPIIVYKLLAQKWPFGDSEFGQFLCKFLPFIQ   159
etlr_mouse_fragment CKLFPFLQKSSVGITVLNLCALSVDRYRAVASWSRVQGIGIPLITAIEIV   109
           etlr_pig NALIASLALGDLIYVVIDLPINVFKLLAGRWPFENHDFGVFLCKLFPFLQ   165
           etlr_rat NALIASLALGDLIYVVIDLPINVFKLLAGRWPFDHNDFGVFLCKLFPFLQ   165
etlr_rabit_fragment DLPINVFKLLAGRWPFDHNDFGVFLCKLFPFLQKSSVGITVLNLCALSVD   165
         etlr_sheep NALIASLALGDLIYVVIDLPINVFKLLAGRWPFEQNDFGVFLCKLFPFLQ   165
         etlr_human NALIASLALGDLIYVVIDLPINVFKLLAGRWPFDHNDFGVFLCKLFPFLQ   165
       etar_selectiv                                                      55
       etbr_selectiv                                                      51
          etbr_human NILIASLALGDLLHIVIDIPINVYKLLAEDWP-----FGAEMCKLVPFIQ   181
          etbr_bovin NILIASLALGDLLHIIIDIPINTYKLLAKDWP-----FGVEMCKLVPFIQ   180
          etbr_canfa NILIASLALGDLLHIIIDIPITVYKLLAEDWP-----FGVEMCKLVPFIQ   181
          etbr_horse NILIASLALGDLLHIIIDIPINVYKLLAEDWP-----FGVEMCKLVPFIQ   182
          etbr_mouse NILIASLALGDLLHIIIDIPINTYKLLAEDWP-----FGAEMCKLVPFIQ   181
            etbr_pig NILIASLALGDLLHIIIDIPINVYKLLAEDWP-----FGVEMCKLVPFIQ   182
            etbr_rat NILIASLALGDLLHIIIDIPINAYKLLAGDWP-----FGAEMCKLVPFIQ   181
etbr_cotja_fragment AVPEAIAFDMITMEYRGKDLRICLLHPTQKTSFMMFYKQAKDWWLFSFYF   188
etbr_macfa_fragment                                                       99
          etbr_rabit NILIASLALGDLLHIIIDIPINVYKLLAEDWP-----FGAEMCKLVPFIQ   180
          et_r_oryla ----FGVYICKLMPFIQKASVGITVLSLCALSIDRYHAVTSWSRVKGMGI   183
                                                                           19
                OPSD NYILLNLAVADLFMVFGGFTTTLYTSLHGYFV-----FGPTGCNLEGFFA   117
               ruler .......160.......170.......180.......190.......200
```

CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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```
          etlr_bovin KSSVGITVLNLCALSVDRYRAVASWSRVQGIGIPLVTAIEIVSIWILSFI   215
        etlr_chicken KASVGITVLNLCALSVDRYRAVASWSRVQGIGIPMITAIEIFSIWLLSFI   209
etlr_mouse_fragment SIWILSFILAIPEAIGFVMVPFEYKGELHRTCMLNATSK--FMEFYQDVK   157
            etlr_pig KSSVGITVLNLCALSVDRYRAVASWSRVQGIGIPLVTAIEIVSIWILSFI   215
            etlr_rat KSSVGITVLNLCALSVDRYRAVASWSRVQGIGIPLITAIEIVSIWILSFI   215
etlr_rabit_fragment RYRAVASWSRVQGIGIPLITAIEIVSIWILSFILAIPEAIGFVMVPFEYR   215
          etlr_sheep KSSVGITVLNLCALSVDRYRAVASWSRVQGIGIPLVTAIEIVSIWILSFI   215
          etlr_human KSSVGITVLNLCALSVDRYRAVASWSRVQGIGIPLVTAIEIVSIWILSFI   215
       etar_selectiv                                                     55
       etbr_selectiv                                                     51
          etbr_human KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVV   231
          etbr_bovin KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVV   230
          etbr_canfa KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVV   231
          etbr_horse KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVV   232
          etbr_mouse KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVV   231
            etbr_pig KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVV   232
            etbr_rat KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVV   231
etbr_cotja_fragment CLPLAITALFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVLV   237
etbr_macfa_fragment                                                    99
          etbr_rabit KASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVI   230
          et_r_oryla PLWKAVEVTLIWLVAVVLAVPEALAFDMLEMPYRGNKLRICLLHPEQPTV   233
                                                                         19
                OPSD TLGGEIALWSLVVLAIERYVVVCKPMSNF-RFGENHAIMGVAFTWVMALA   166
               ruler .......210.......220.......230.......240.......250
```

```
          etlr_bovin LAIPEAIGFVMVPFEYKGAQHRTCMLNATSK--FMEFYQDVKDWWLFGFY   263
        etlr_chicken LAIPEAIGFAVVPFRYKDESYVTCMLNPTNK--FMLFYKDAKDWWLFGFY   257
etlr_mouse_fragment DWWLFGFYFCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRREVA   207
            etlr_pig LAIPEAIGFVMVPFEYKGEEHKTCMLNATSK--FMEFYQDVKDWWLFGFY   263
            etlr_rat LAIPEAIGFVMVPFEYKGEQHRTCMLNATTK--FMEFYQDVKDWWLFGFY   263
etlr_rabit_fragment GEQHKTCMLNATSK--FMEFYQDVKDWWLFGFYFCMPLVCTAIFYTLMTC   263
          etlr_sheep LAIPEAIGFVMVPFEYKGAQHRTCMLNATSK--FMEFYQDVKDWWLFGFY   263
          etlr_human LAIPEAIGFVMVPFEYRGEQHKTCMLNATSK--FMEFYQDVKDWWLFGFY   263
       etar_selectiv                                                     55
       etbr_selectiv                                                     51
          etbr_human LAVPEAIGFDIITMDYKGSYLRICLLHPVQKTAFMQFYKTAKDWWLFSFY   281
          etbr_bovin LAVPEAVGFDIITSDHIGNKLRICLLHPTQKTAFMQFYKTAKDWWLFSFY   280
          etbr_canfa LAVPEAVGFDMITIDYKGRYLRICLLHPTQKTAFMQFYKTAKDWWLFSFY   281
          etbr_horse LAVPEAVGFDMITADYKGSYLRICLLHPTQKTAFMQFYKNAKDWWLFSFY   282
          etbr_mouse LAVPEAIGFDMITSDYKGKPLRVCMLNPFQKTAFMQFYKTAKDWWLFSFY   281
            etbr_pig LAVPEALGFDMITTDYKGNRLRICLLHPTQKTAFMQFYKTAKDWWLFSFY   282
            etbr_rat LAVPEAIGFDVITSDYKGKPLRVCMLNPFQKTAFMQFYKTAKDWWLFSFY   281
etbr_cotja_fragment FALCWLPLHLSRILKLTIYDQKDPNRCELLSFFLVMDYIGINMASLNSCI   287
etbr_macfa_fragment                                                    99
          etbr_rabit LAVPEAIGFNLVTIDYKGSYLRICLLNPTQKTAFMQFYKTAKDWWLFSFY   280
          et_r_oryla YMKFYQEAKDWWLFGFYFCLPLACTGIFYTLMTCEMLSRKKG-MRIALND   282
                                                                         19
                OPSD CAAPPLVGW-----SRYIPEGMQCSCGIDYYTPH--EETNNESFVIYMFV   209
               ruler .......260.......270.......280.......290.......300
```

CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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```
         etlr_bovin FCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRREVAKTVFCLVV  313
       etlr_chicken FCMPLACTAIFYTLMTCEMLNRRNSNLRIALSEHLKQRREVAKTVFCLVV  307
etlr_mouse_fragment KTVFCLVVIFALCWFPLHLSRILKKTVYDEMDKNRCELLSFLLLMDYIGI  257
           etlr_pig FCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRREVAKTVFCLVV  313
           etlr_rat FCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRREVAKTVFCLVV  313
etlr_rabit_fragment EMLNRRNGSLRIALSEHLKQRREVAKTVFCLVVIFALCWFPLHLSRILKK  313
         etlr_sheep FCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRREVAKTVFCLVV  313
         etlr_human FCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRREVAKTVFCLVV  313
      etar_selectiv                                                    55
      etbr_selectiv                                                    51
         etbr_human FCLPLAITAFFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  330
         etbr_bovin FCLPLAITALFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  329
         etbr_canfa FCLPLAITAFFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  330
         etbr_horse FCLPLAITAFFYTLETCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  331
         etbr_mouse FCLPLAITAVFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  330
           etbr_pig FCLPLAITAFFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  331
           etbr_rat FCLPLAITAIFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  330
etbr_cotja_fragment NPIALYLVSKRFQNCFKSCLCCWC-QS-KDLLSLEERQSCLKFKANDHGY  335
etbr_macfa_fragment                                                    99
         etbr_rabit FCLPLAITAFFYTLMTCEMLRKKSG-MQIALNDHLKQRREVAKTVFCLVL  329
         et_r_oryla HMKQRREVAKTVFCLVLIFAFCWLPLHLSRILKKTVYNENDPNRCELLSF  332
                                                                        19
               OPSD VHFIIPLIVIFFCYGQLVFTVKE--AAAQQQESATTQKAEKEVTRMVIIM  257
              ruler .......310.......320.......330.......340.......350
```

```
         etlr_bovin IFALCWFPLHLSRILKKTVYDEMDTNRCELLSFLLLMDYIGINLATMNSC  363
       etlr_chicken IFALCWFPLHLSRILKKMVYNERDPGRCELLSFLLPLDYISINLATMNSC  357
etlr_mouse_fragment NLATMNSCINPIA                                      270
           etlr_pig IFALCWFPLHLSRILKKTVYDEMDKNRCELLSFLLLMDYIGINLATMNSC  363
           etlr_rat IFALCWFPLHLSRILKKTVYDEMDKNRCELLSFLLLMDYIGINLATMNSC  363
etlr_rabit_fragment TVYDEMDKNRCELLSFLLLMDYIGINLATMNSCINPIALYFVSKKFKNCF  363
         etlr_sheep IFALCWFPLHLSRILKKTVYDEMDTNRCELLSFLLLMDYIGINLATMNSC  363
         etlr_human IFALCWFPLHLSRILKKTVYNEMDKNRCELLSFLLLMDYIGINLATMNSC  363
      etar_selectiv                                                    55
      etbr_selectiv                                                    51
         etbr_human VFALCWLPLHLSRILKLTLYNQNDPNRCELLSFLLVLDYIGINMASLNSC  380
         etbr_bovin VFALCWLPLHLSRILKLTLYDQHDPRRCEFLSFLLVLDYIGINMASLNSC  379
         etbr_canfa VFALCWLPLHLSRILKLTIYDQNDPNRCELLSFLLVLDYIGINMASLNSC  380
         etbr_horse VFALCWLPLHLSRILKHTLYDQNDPHRCELLSFLLVLEYIGINMASLNSC  381
         etbr_mouse VFALCWLPLHLSRILKLTLYDQSNPHRCELLSFLLVLDYIGINMASLNSC  380
           etbr_pig VFALCWLPLHLSRILKLTLYDQNDSNRCELLSFLLVLDYIGINMASLNSC  381
           etbr_rat VFALCWLPLHLSRILKLTLYDQSNPQRCELLSFLLVLDYIGINMASLNSC  380
etbr_cotja_fragment DNFRSSNKYSSS                                       347
etbr_macfa_fragment                                                    99
         etbr_rabit VFGLCWLALHLSRILKLTLYDQNDPNRCELLSFLLVLDYIGINMASLNSC  379
         et_r_oryla LLVMDYIGINMASLNSCINPIALYFVSQKFKNCFQSCLCCWC-Y---RTS  378
                                                                        19
               OPSD VIAFLICWLPYAGVAFYIFTHQGSD---------FGPIFMTIPAFFAKTS  298
              ruler .......360.......370.......380.......390.......400
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CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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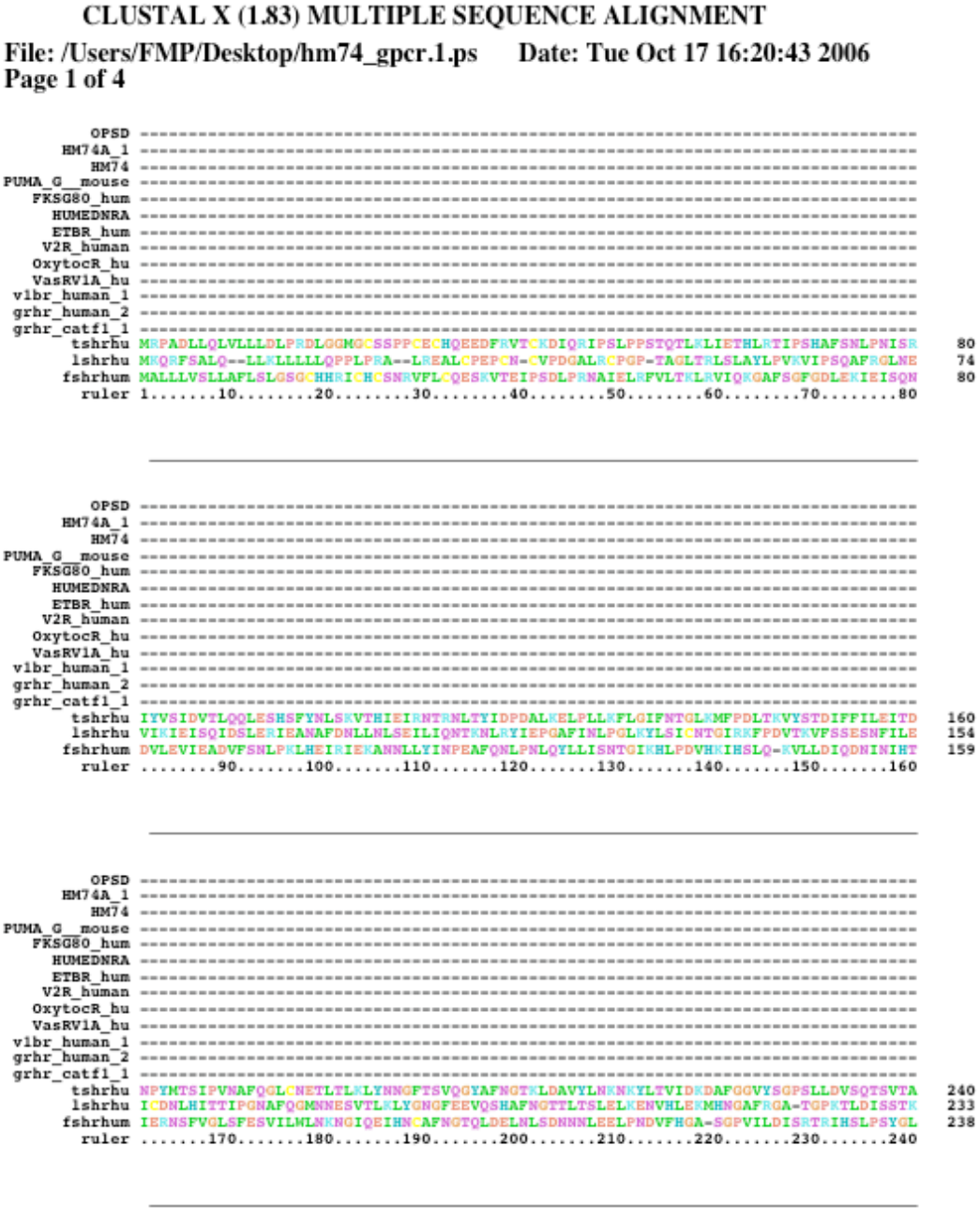
         etlr_bovin INPIALYFVSKKFKNCFQSCLCCCCYQSKSLMTSVPMNGTSIQWKNHEQN   413
       etlr_chicken INPIALYFVSKKFKNCFQSCLCCCCSQSKSLATSVPMNGTSIQWKNQELN   407
etlr_mouse_fragment                                                     270
          etlr_pig INPIALYFVSKKFKNCFQSCLCCCCYQSKSLMTSVPMNGTSIQWKNHEQN   413
          etlr_rat INPIALYFVSKKFKNCFQSCLCCCCHQSKSLMTSVPMNGTSIQWKNQEQ-   412
etlr_rabit_fragment QSCLCCCCHQSKSLMTSVPMNGTSIQWKNH                     393
        etlr_sheep INPIALYFVSKKFKNCFQSCLCCCCYQSKSLMTSVPMNGTSIQWKNPEQN   413
        etlr_human INPIALYFVSKKFKNCFQSCLCCCCYQSKSLMTSVPMNGTSIQWKNHDQN   413
      etar_selectiv                                                      55
      etbr_selectiv                                                      51
         etbr_human INPIALYLVSKRFKNCFKSCLCCWC-QSFEEKQSLEEKQSCLKFKANDHG   429
         etbr_bovin INPIALYLVSKRFKNCFKSCLCCWC-QSFEEKQSLEEKQSCLKFKANDHG   428
         etbr_canfa INPIALYLVSKRFKNCFKSCLCCWC-QSFEEKQSLEEKQSCLKFKAN     426
         etbr_horse INPIALYLVSKRFKNCFKWCLCCWC-QSFEEKQSLEDKQSCLKFKANDHG   430
         etbr_mouse INPIALYLVSKRFKNCFKSCLCCWC-QTFEEKQSLEEKQSCLKFKANDHG   429
           etbr_pig INPIALYLVSKRFKNCFKSCLCCWC-QSFEEKQSLEEKQSCLKFKANDHG   430
           etbr_rat INPIALYLVSKRFKNCFKSCLCCWC-QTFEEKQSLEEKQSCLKFKANDHG   429
etbr_cotja_fragment                                                     347
etbr_macfa_fragment                                                      99
         etbr_rabit INPIALYLVSKRFKNCFKSCLCCWC-QSFEEKQSLEEKQSCLKFKANDHG   428
         et_r_oryla PLDERGSGGRWKGSCQVNGLDRTSSR-SS                       406
                                                                         19
               OPSD AVYNPVIYIMMNKQFRNCMVTTLCC-----GKNP-LGDDEASTTVSKTET   342
              ruler .......410.......420.......430.......440.......450
```

```
         etlr_bovin NHNTERSSHKDSIN   427
       etlr_chicken NHNTDRSSHKDSIN   421
etlr_mouse_fragment                  270
          etlr_pig NHNTERSSHKDSIN   427
          etlr_rat NHNTERSSHKDSMN   426
etlr_rabit_fragment                  393
        etlr_sheep NHNTERSSHKDSIN   427
        etlr_human NHNTDRSSHKDSMN   427
      etar_selectiv                   55
      etbr_selectiv                   51
         etbr_human YDNFRSSNKYSSS    442
         etbr_bovin YDNFRSSNKYSSS    441
         etbr_canfa                  426
         etbr_horse YDNFRSSNKYSSS    443
         etbr_mouse YDNFRSSNKYSSS    442
           etbr_pig YDNFRSSNKYSSS    443
           etbr_rat YDNFRSSNKYSSS    442
etbr_cotja_fragment                  347
etbr_macfa_fragment                   99
         etbr_rabit YDNFRSSNKYSSS    441
         et_r_oryla                  406
                                      19
               OPSD SQVAPA           348
              ruler .......460....
```

Multiple Sequence Alignment of Nicotinic Acid Receptors GPR109A/B

Because of the very recent de-orphanization of GPR109A only few experimental data were available. As a result, GPR109A and GPR109B were aligned against GPCRs that are intensively investigated.

HM74A – GPR109A OPSD – rhodopsin EDNRA – ETA receptor
HM74 – GPR109B OxytocR – Oxytocin receptor ETBR – ETB receptor
TSHR – Thyroid-stimulating hormone receptor LSHR – Luteinizing hormone receptor
FSHR – Follicle-stimulating hormone receptor etc.



CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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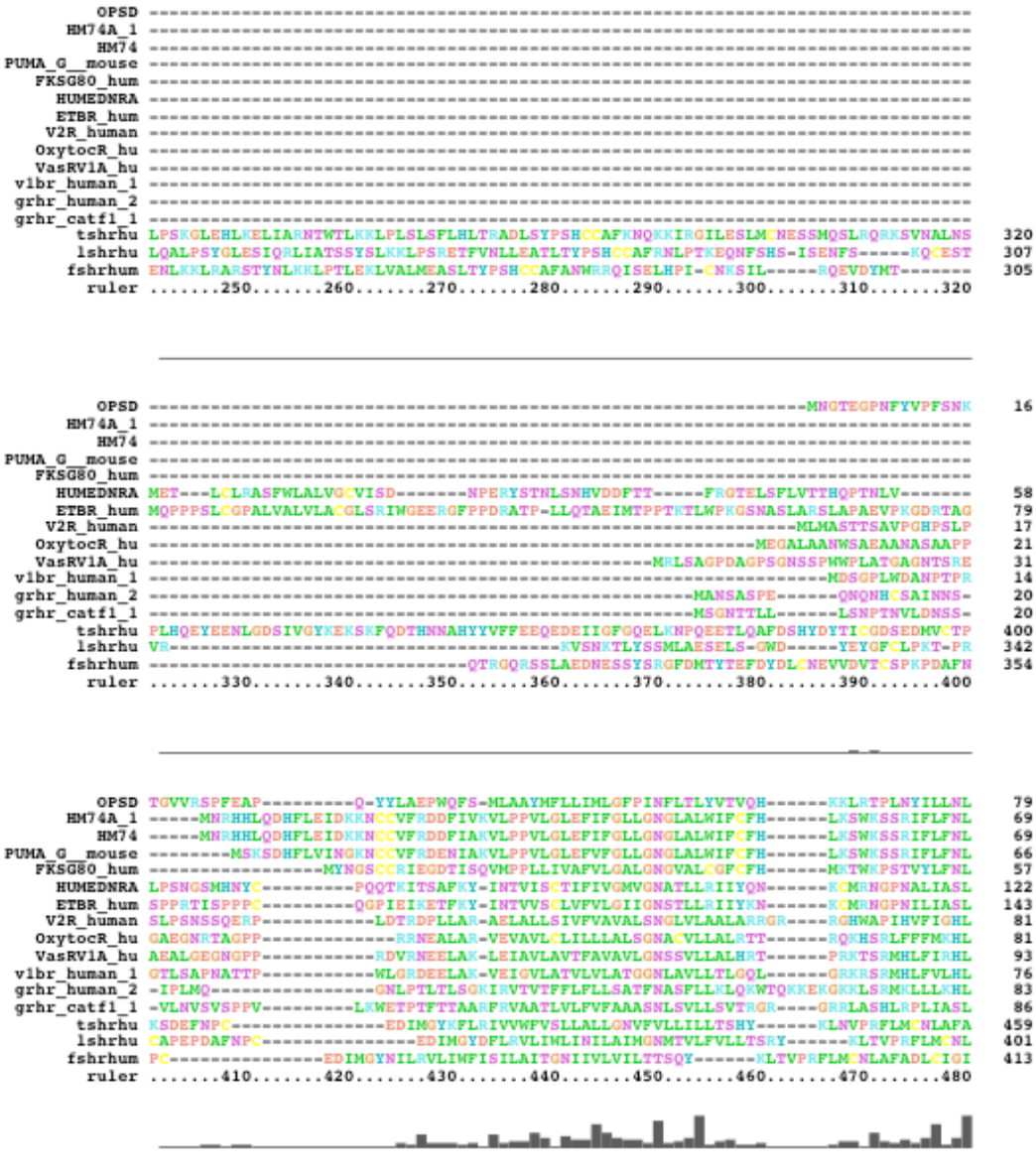

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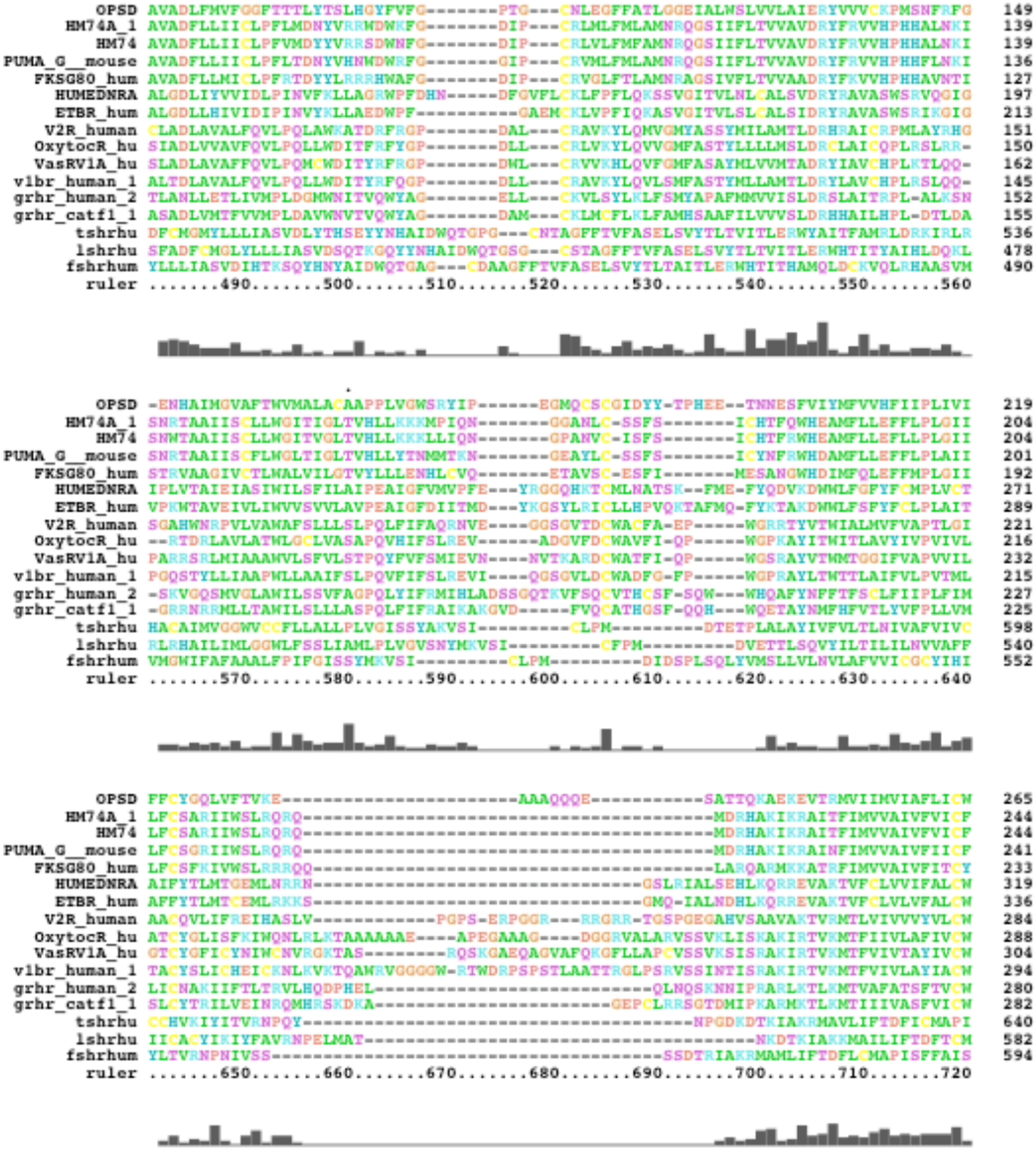

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          OPSD LPYAGVAFYIF------THQGSDFGP---IFMTIPAFFAKTSAVYNPVIYIMMNKQFRNCMV-TTLCC-GKNP---LG-D  330
       HM74A_1 LPSVVVRIRIFWLLHTSGTQNCEVYRSVDLAFFITLSFTYMNSMLDPVVYYFSSPSFPNFFSTLINRCLQRKMTGEPDNN  324
          HM74 LPSVVVRIRIFWLLHTSGTQNCEVYRSVDLAFFITLSFTYMNSMLDPVVYYFSSPSFPNFFSTLINRCLQRKMTGEPDNN  324
PUMA_G__mouse LPSVAVRIRIFWLLYKYNVRNCDIYSSVDLAFFTTLSFTYMNSMLDPVVYYFSSPSFPNFFSTCINRCLRKKTLGEPDNN  321
   FKSG80_hum LPSVSARLYFLMTVPSS-----ACDPSVHGALHITLSFTYMNSMLDPLVYYFSSPSFPKFYNKLNICSLKPKQPGHSKTQ  308
      HUMEDNRA FPLHLSRILKKTVYNEMDKNRCELLSFLLLMDYIGINLATMNSCINPIALYFVSKKFKNCFQSCLCCCC-YQSKSLMTSV  398
       ETBR_hum LPLHLSRILKLTLYNQNDPNRCELLSFLLVLDYIGINMASLNSCINPIALYLVSKRFKNCFKSCLCCWCQSFEEKQS-LE  415
       V2R_human APFFLVQLWAAN------DPEAP---LEGAPFVLLMLLASLNSCTNPWIYASFSSSVSSEL-RSLLCCARGRTPPSLGPQ  354
     OxytocR_hu TPFFFVQMWSVW------DANAP---KEASAFIIVMLLASLNSCCNPWIYMLFTGHLFHELVQRFLCCSASYLKGRRLG-  358
     VasRV1A_hu APFFIIQMWSVW------DPMSVWTESENPTITITALLGSLNSCCNPWIYMFFSGHLLQDCVQSFPCCQNMKEKFNKEDT  378
   v1br_human_1 APFFSVQMWSVW------DKNAPDEDSTNVAFTISMLLGNLNSCCNPWIYMGFNSHLLPRPLRHLACCGGPQPRMRRRLS  368
   grhr_human_2 TPYYVLGIWYWF------DPEML-NRLSDPVNHFFFLFAFLNPCFDPLIYGYFSL                            328
   grhr_catf1_1 TPYYLLGIWYWF------QPQML-HVIPDYVHHVFFVFGNLNTCCDPVIYGFFTPSFRADLSRCFCWRNQNASAKSLPHF  355
         tshrhu SFYALSAILNK---------PLITVSNSKILLVLFYPLNSCANPFLYAIFTKAFQRDVFILLSKFGICKRQAQAYRGQRV  711
         lshrhu APISFFAISAAFKV---------PLITVTNSKVLLVLFYPINSCANPFLYAIFTKTFQRDFFLLLSKFGCCKRRAELYRR  653
        fshrhum ASLKV---------PLITVSKAKILLVLFHPINSCANPFLYAIFTKNFRRDFFILLSKCGCYEMQAQIYRTETSSTVHNT  665
          ruler .......730.......740.......750.......760.......770.......780.......790.......800
```

```
          OPSD DEASTTVS---KTETSQVAPA                                                  348
       HM74A_1 RSTSVELTGDPNKTRGAPEALMANSGEPWSPSYLGPTSP                                363
          HM74 RSTSVELTGDPNKTRGAPEALMANSGEPWSPSYLGPTSNNHSKKGHCHQEPASLEKQLGCCIE       387
PUMA_G__mouse RSTSVELTGDPSTTRSIPGALMADPSEPGSPPYLASTSR                                360
   FKSG80_hum RPEEMPISNLGRRSCISVANSFQSQSDGQWDPHIVEWH                                 346
      HUMEDNRA PMNGTSIQWKNHDQNNHNTDRSSHKDSMN                                         427
       ETBR_hum EKQSCLKFKANDHGYDNF-RSSNKYSSS                                         442
     V2R_human DESCTTASSSLAKDTSS                                                    371
     OxytocR_hu ETSASKKSNSSSFVLSHRSSSQRSCSQ----PSTA                                 389
     VasRV1A_hu DS-MSRRQ-TFYSNNRSPTNSTGM-WKDSPKSSKSIKFIPVST                         418
   v1br_human_1 DSLSSRHTTL                                                         378
   grhr_human_2                                                                    328
   grhr_catf1_1 SGHRREVSGEAESDLGSGDQPSGQ                                           379
         tshrhu PPKNSTDIQVQKVTHDMRQGLHNMEDVYELIENSHLTPKKQGQISEEYMQTVL               764
         lshrhu KDFS-AYTSNCKNGFTGSNKPSQSTLKLSTLHCQGTALLDKTRYTEC                     699
        fshrhum HPRNGHCSSAPRVTNGSTYILVPLSHLAQN                                      695
          ruler .......810.......820.......830.......840.......850.......860...
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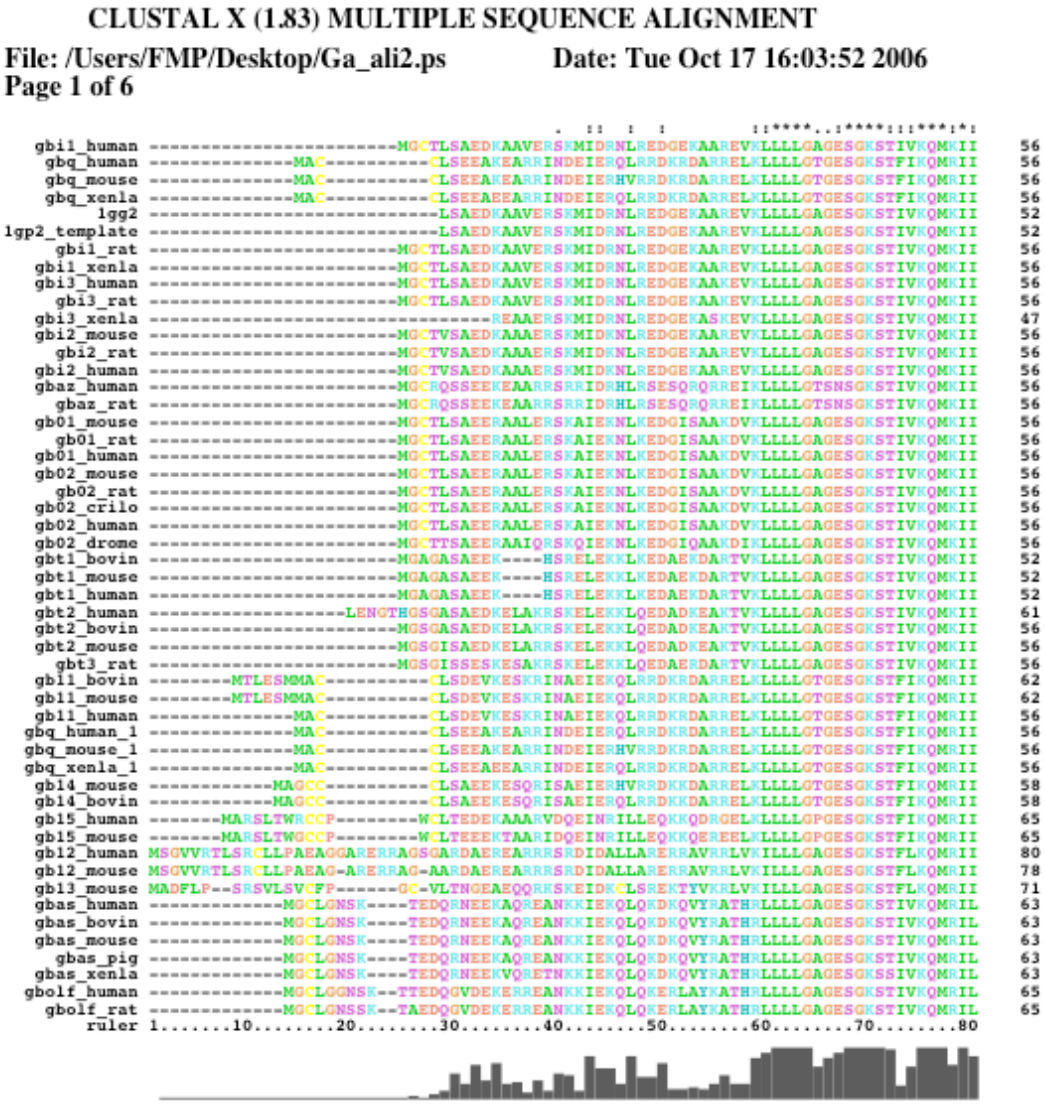
Multiple Sequence Alignment of Gα Proteins

Investigations of selectivity patterns for G protein interaction have been done by using sequence and structural data of several G protein families. The codes on the left resemble the entries of SwissProt database.



CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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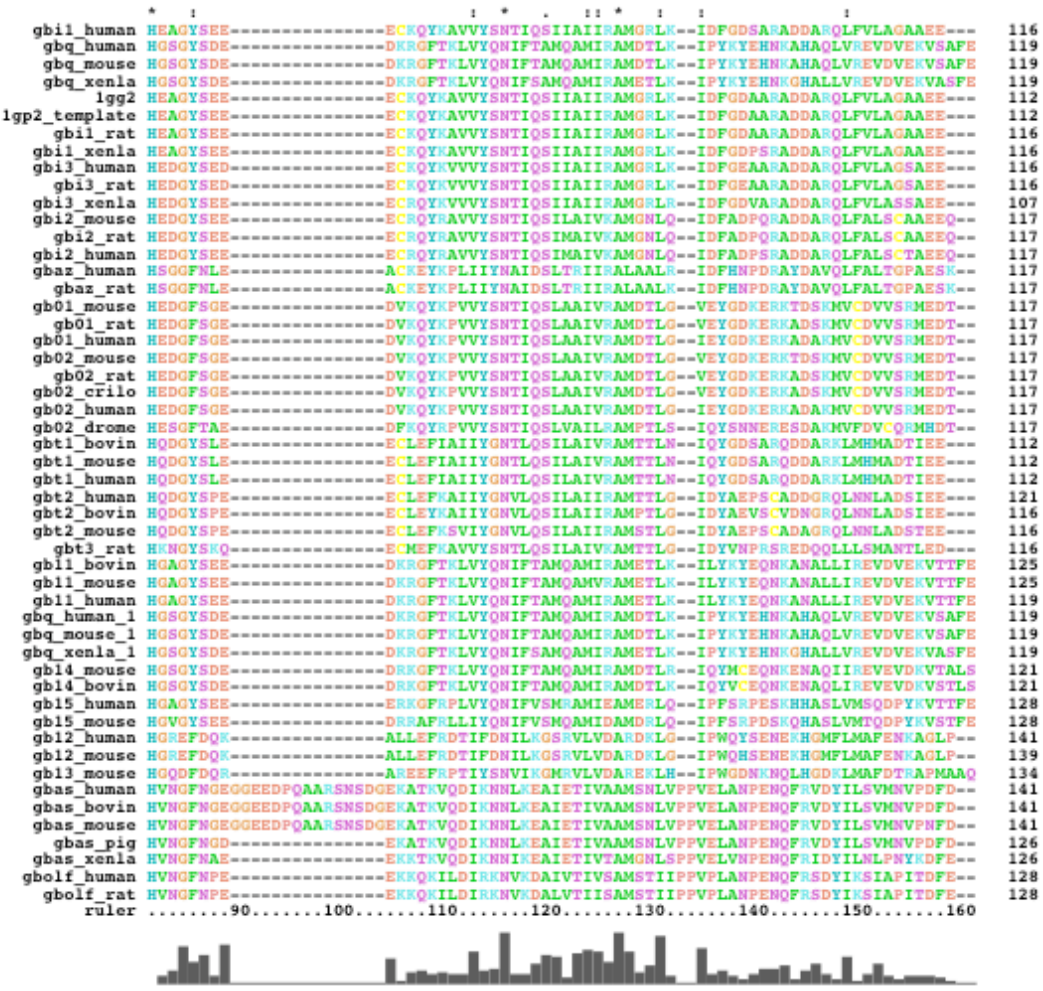

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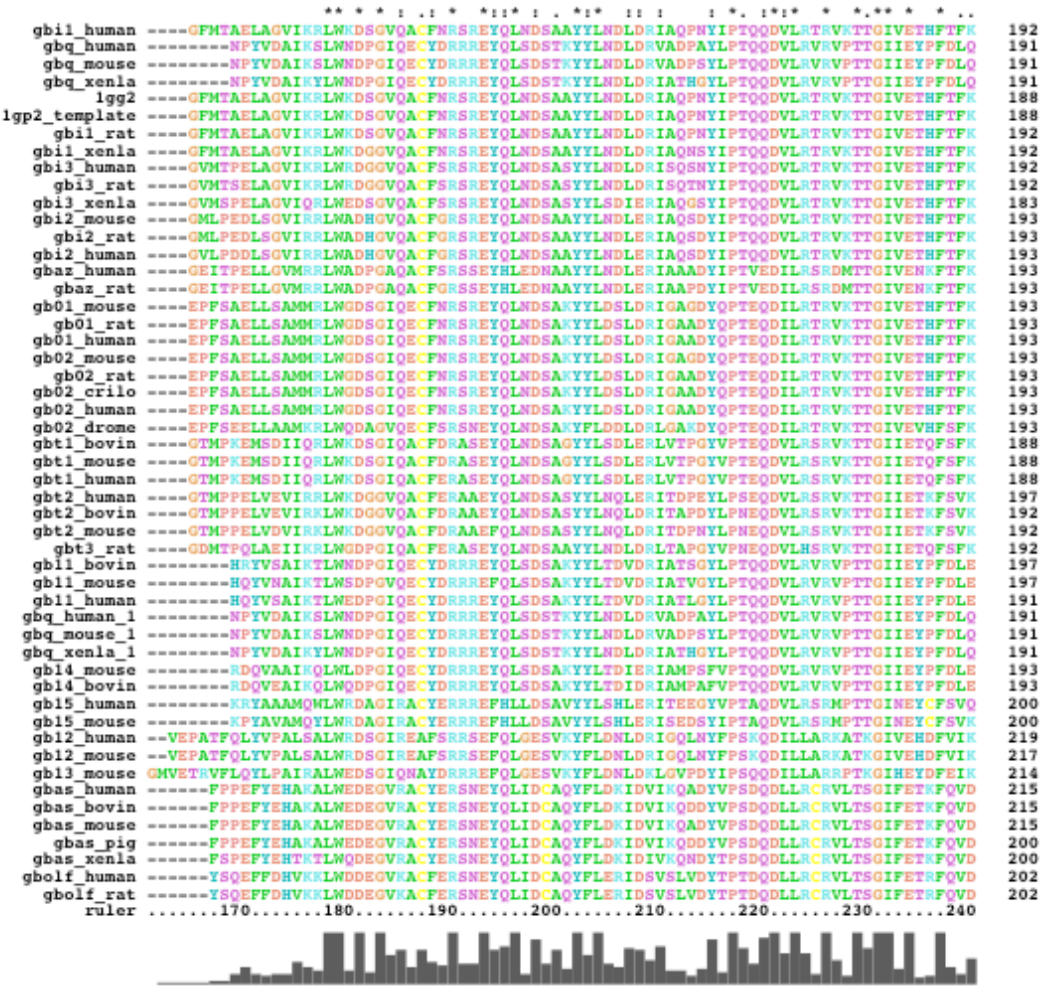

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                 .  :::.***.*:.:*::*:.**:.: .::: .  * ::  : *    .*: *   :* :*     :   *::***** *
   gbi1_human DLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  272
    gbq_human SVIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  271
    gbq_mouse SVIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  271
    gbq_xenla SVIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  271
         1gg2 DLHFKMFDVGAQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  268
1gp2_template DLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  268
    gbi1_rat DLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  272
   gbi1_xenla DLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  272
   gbi3_human DLYFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTETSIILFLNKKD  272
    gbi3_rat ELYFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  272
   gbi3_xenla DLYFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLLLAEDEEMNRMHEESMKLFDSICNNKWFIDTSIILFLNKKD  263
   gbi2_mouse DLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSAYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  273
    gbi2_rat DLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSAYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  273
   gbi2_human DLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSAYDLVLAEDEEMNRMHEESMKLFDSICNNKWFTDTSIILFLNKKD  273
   gbaz_human ELTFKMVDVGGQRSERKKWIHCFEGVTAIIFCVELSGYDLKLYEDNQTSRMAESLRLFDSICNNNWFINTSLILFLNKKD  273
    gbaz_rat ELTFKMVDVGGQRSERKKWIHCFEGVTAIIFCVELSGYDLKLYEDNQTSRMAESLRLFDSICNNNWFINTSLILFLNKKD  273
   gb01_mouse NLHFRLFDVGGQRSERKKWIHCFEDVTAIIFCVALSGYDQVLHEDETTNRMHESLMLFDSICNNKFFIDTSIILFLNKKD  273
    gb01_rat NLHFRLFDVGGQRSERKKWIHCFEDVTAIIFCVALSGYDQVLHEDETTNRMHESLMLFDSICNNKFFIDTSIILFLNKKD  273
   gb01_human NLHFRLFDVGGQRSERKKWIHCFEDVTAIIFCVALSGYDQVLHEDETTNRMHESLMLFDSICNNKFFIDTSIILFLNKKD  273
   gb02_mouse NLHFRLFDVGGQRSERKKWIHCFEDVTAIIFCVALSGYDQVLHEDETTNRMHESLKLFDSICNNKWFTDTSIILFLNKKD  273
    gb02_rat NLHFRLFDVGGQRSERKKWIHCFEDVTAIIFCVALSGYDQVLHEDETTNRMHEFLKLFDSICNNKWFTDTSIILFLNKKD  273
   gb02_crilo NLHFRLFDVGGQRSERKKWIHCFEDVTAIIFCVALSGYDQVLHEDETTNRMHESLKLFDSICNNKWFTDTSIILFLNKKD  273
   gb02_human NLHFRLFDVGGQRSERKKWIHCFEDVTAIIFCVALSGYDQVLHEDETTNRMHESLKLFDSICNNKWFTDTSIILFLNKKD  273
   gb02_drome NLNFKLFDVGGQRSERKKWIHCFEDVTAIIFCVAMSEYDQVLHEDETTNRMQESLKLFDSICNNKWFTDTSIILFLNKKD  273
   gbt1_bovin DLNFRMFDVGGQRSERKKWIHCFEGVTCIIFIAALSAYDMVLVEDDEVNRMHESLHLFNSICNHRYFATTSIVLFLNKKD  268
   gbt1_mouse DLNFRMFDVGGQRSERKKWIHCFEGVTCIIFIAALSAYDMVLVEDDEVNRMHESLHLFNSICNHRYFATTSIVLFLNKKD  268
   gbt1_human DLNFRMFDVGGQRSERKKWIHCFEGVTCIIFIAALSAYDMVLVEDDEVNRMHESLHLFNSICNHRYFATTSIVLFLNKKD  268
   gbt2_human DLNFRMFDVGGQRSERKKWIHCFEGVTCIIFCAALSAYDMVLVEDDEVNRMHESLHLFNSICNHKFFAATSIVLFLNKKD  277
   gbt2_bovin DLNFRMFDVGGQRSERKKWIHCFEGVTCIIFCAALSAYDMVLVEDDEVNRMHESLHLFNSICNHKFFAATSIVLFLNKKD  272
   gbt2_mouse DLNFRMFDVGGQRSERKKWIHCFEGVTCIIFCAALSAYDMVLVEDDEVNRMHESLHLFNSICNHKFFAATSIVLFLNKKD  272
    gbt3_rat DLNFRMFDVGGQRSERKKWIHCFEGVTCIIFCAALSAYDMVLVEDEEVNRMHESLHLFNSICNHKYFATTSIVLFLNKKD  272
   gb11_bovin NIIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIVTYPWFQNSSVILFLNKKD  277
   gb11_mouse NIIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  277
   gb11_human NIIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  271
 gbq_human_1 SVIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  271
 gbq_mouse_1 SVIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  271
 gbq_xenla_1 SVIFRMVDVGGQRSERKKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKD  271
   gb14_mouse NIIFRMVDVGGQRSERKKWIHCFESVTSIIFLVALSEYDQVLAECDNENRMEESKALFRTIITYPWFLNSSVILFLNKKD  273
   gb14_bovin NIIFRMVDVGGQRSERKKWIHCFESVTSIIFLVALSEYDQVLAECDNENRMEESKALFKTIITYPWFLNSSVILFLNKKD  273
   gb15_human KTNLRIVDVGGQKSERKKWIHCFENVIALIYLASLSEYDQCLEENNQENRMKESLALFGTILELPWFKSTSVILFLNKTD  280
   gb15_mouse KTKLRIVDVGGQRSERKKWIHCFENVIALIYLASLSEYDQCLEENDQENRMEESLALFSTILELPWFKSTSVILFLNKTD  280
   gb12_human KIPFKMVDVGGQRSQRQKWFQCFDGITSILFMVSSSEYDQVLMEDRRTNRLVESMNIFETIVNNKLFFNVSIILFLNKMD  299
   gb12_mouse KIPFKMVDVGGQRSQRQKWFQCFDGITSILFMVSSSEYDQVLMEDRRTNRLVESMNIFETIVNNKLFFNVSIILFLNKMD  297
   gb13_mouse NVPFKMVDVGGQRSERKRWFECFDSVTSILFLVSSSEFDQVLMEDRQTNRLTESLNIFETIVNNRVFSNVSIILFLNKTD  294
   gbas_human KVNFHMFDVGGQRDERRKWIQCFNDVTAIIFVVASSSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVILFLNKQD  295
   gbas_bovin KVNFHMFDVGGQRDERRKWIQCFNDVTAIIFVVASSSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVILFLNKQD  295
   gbas_mouse KVNFHMFDVGGQRDERRKWIQCFNDVTAIIFVVASSSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVILFLNKQD  295
    gbas_pig KVNFHMFDVGGQRDERRKWIQCFNDVTAIIFVVASSSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVILFLNKQD  280
   gbas_xenla KVNFHMFDVGGQRDERRKWIQCFNDVTAIIFVVASSSYNMVIREDNHTNRLQEALNLFKSIWNNRWLRTISVILFLNKQD  280
  gbolf_human KVNFHMFDVGGQRDERRKWIQCFNDVTAIIYVAACSSYNMVIREDNNTNRLRESLDLFESIWNNRWLRTISIILFLNKQD  282
   gbolf_rat KVNFHMFDVGGQRDERRKWIQCFNDVTAIIYVAACSSYNMVIREDNNTNRLRESLDLFESIWNNRWLRTISIILFLNKQD  282
       ruler .......250.......260.......270.......280.......290.......300.......310.......320
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CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

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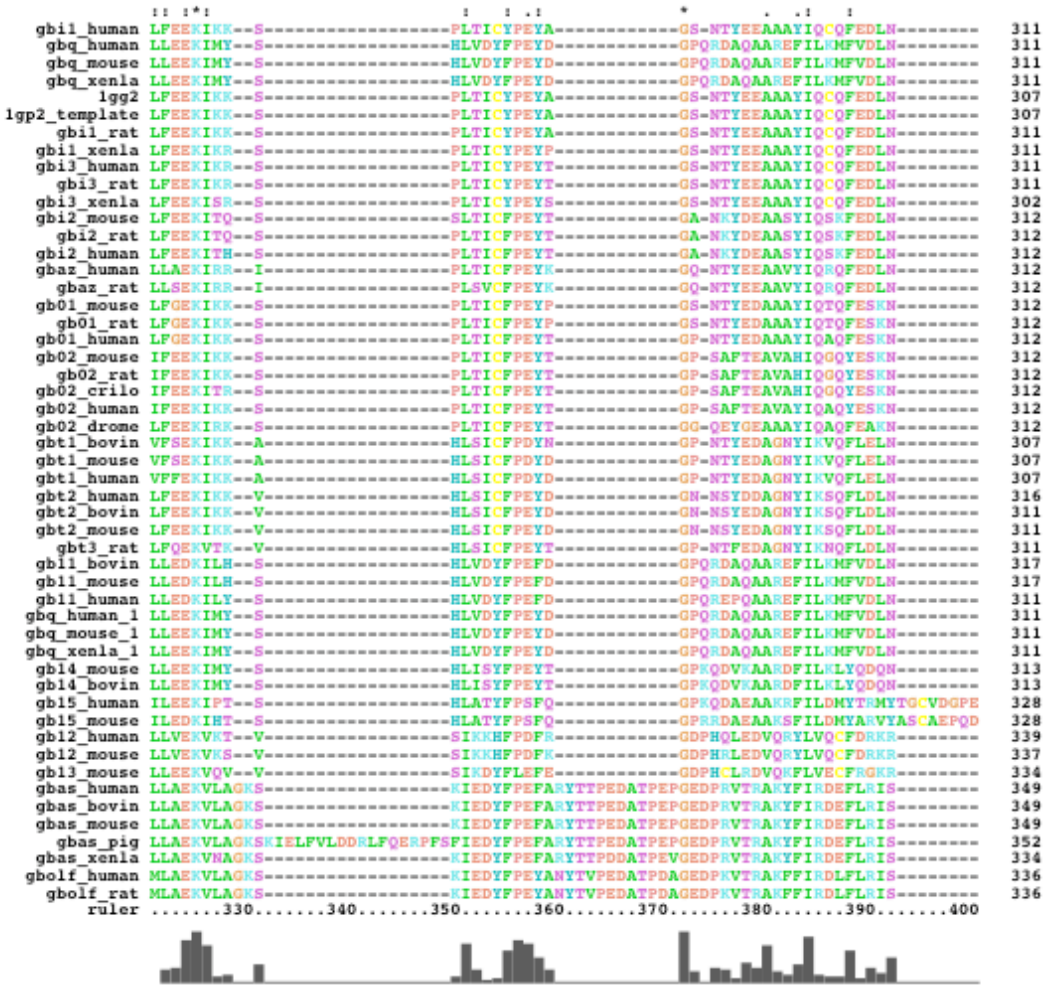

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    gbq_human ----PDSDKIIYSHFTCATDTENIRFVFAAVKDTILQLNLKEYNAV   353
    gbq_mouse ----PDSDKIIYSHFTCATDTENIRFVFAAVKDTILQLNLKEYNLV   353
    gbq_xenla ----PDSDKIIYSHFTCATDTENIRFVFAAVKDTILQLNLKEYNLV   353
         1gg2 ---KRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIK            341
1gp2_template ---KRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKNNL        344
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   gbi1_xenla ---KRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF   354
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     gbi3_rat ---RRKDTKEVYTHFTCATDTKNVQFVFDAVTDVIIKNNLKECGLY   354
   gbi3_xenla ---RRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKSNLMECGLY   345
   gbi2_mouse ---KRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF   355
     gbi2_rat ---KRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF   355
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   gb02_crilo ---KSA-HKEIYTHFTCATDTNNIQFVFDAVTDVIIAKNLRGCGLY   354
   gb02_human ---KSA-HKEIYSHVTCATDTNNIQFVFDAVTDVIIAKNLRGCGLY   354
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   gbt1_mouse ---MRRDVKEIYSHMTCATDTQNVKFVFDAVTDIIIKENLKDCGLF   350
   gbt1_human ---MRRDVKEIYSHMTCATDTQNVKFVFDAVTDIIIKENLKDCGLF   350
   gbt2_human ---MRKDVKEIYSHMTCATDTQNVKFVFDAVTDIIIKENLKDCGLF   359
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   gbl4_bovin ----PDKEKVIYSHFTCATDTENIRFVFAAVKDTILQLNLREFNLV   355
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   gbl5_mouse GGRKGSRARRFFAHFTCATDTQSVRSVFKDVRDSVLARYLDEINLL   374
   gbl2_human --RNRS--KPLFHHFTTAIDTENVRFVFHAVKDTILQENLKDIMLQ   381
   gbl2_mouse --RNRS--KPLFHHFTTAIDTENIRFVFHAVKDTILQENLKDIMLQ   379
   gbl3_mouse --RDQQQ-RPLYHHFTTAINTENIRLVFRDVKDTILHDNLKQLMLQ   377
   gbas_human -TASGDGRHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL   394
   gbas_bovin -TASGDGRHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL   394
   gbas_mouse -TASGDGRHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL   394
     gbas_pig -TASGDGRHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL   397
   gbas_xenla -TASGDGRHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL   379
  gbolf_human -TATGDGKHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLKQYELL   381
    gbolf_rat -TATGDGKHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLKQYELL   381
        ruler .......410.......420.......430.......440......
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Summary

Using homology of GPCR subtypes as well as their differences in biological function, we characterized ligand binding for endothelin receptors ETA and ETB.
For the first time, endothelin receptor-selective peptide ligands were subdivided into 4 regions (addressor, hook, core, modulator), explaining all existing data in literature as well as data from our co-operation partners. This is based on interactions with 4 complementary regions (gateway, edge, neck, binding cleft), found at the endothelin receptors. The pairwise interactions of addressor and gateway, hook and edge, core and neck, as well as modulator and binding cleft accurately explain the peptide ligands' selectivity for endothelin receptors and endothelin receptor subtypes. We impressively demonstrated this by the design and experimental validation of a new ETB-selective peptide ligand. Additionally, for the first time we described the necessities and differences in activation and in inhibition of both endothelin receptors. According to our models the molecular differences of peptide ligand-induced receptor activation and inhibition can be explained by the existence and absence of negatively charged residues at the peptide ligand's hook region, as counterpart of the receptor's edge region. In the case of receptor activation we suggest a scenario where the ligand's hook region catches the receptor's edge region. In succession, the ligand's modulator region is restrained in a position where its C-terminus orients in between transmembrane helices TMH3, TMH6 and TMH7 and induces reorientation of several side chains, finally leading to receptor activation. In the case of inhibition, the ligands lack the charged hook region as well as bulky moieties at the N-terminal portion. Such antagonistic peptides slide beyond the edge down into the transmembrane binding cleft and additionally constrain the existing interactions between the transmembrane helices of the inactive state, leading to inhibition. One of the most impressive results is the orientation of the last two residues of the antagonist's C-terminal modulator region at the same site of the inverse agonist 11-cis-retinal in inactive rhodopsin, demonstrating conserved mechanisms in many GPCRs.
Additionally, our models explain the so far unclear formation of the super-stable complex between ET-1 and ETB occurring in many mammals. A lid-like mechanism on this receptor's N-terminus, which is different to ETA in sequence and structure, covers ET-1 and restrains it tight into the binding site.

In a second project, we utilized the high degree of sequence homology between the human nicotinic acid receptor GPR109A and its homologue GPR109B, which has only low affinity for nicotinic acid. For binding of nicotinic acid to GPR109A we could identified crucial residues. The binding site is positioned between transmembrane helices TMH2, TMH3 and TMH7, and is therefore an interaction site different than most other rhodopsin-like GPCRs, where the binding site is located between TMH4, TMH5 and TMH6. Validated experimental data on predicted aromatic residues within TMH5 and TMH7, as well as at ECL2, clearly confirm this result. Combining mutagenesis data and comparative structural modeling allowed us to identify five residues located in close spatial proximity to the main interaction points for nicotinic acid. The characterization of the structural determinants and complementary pharmacophoric patterns for nicotinic acid binding in GPR109A is of general importance for understanding the binding mechanism of small molecule ligands to GPCRs. It is also important in the design and the development of new drugs, acting via GPR109A to treat dyslipidemic disorders. Comparisons of both binding sites explained the differences in ligand selectivity. Furthermore, they led to the proposal of 2-oxo-octanoic acid as a ligand selectively for GPR109B but not GPR109A, which was experimentally proven by our collaborators. Necessary interaction features, which were identified for 2-oxo-octanoic acid at GPR109B, could be unexceptionally applied to the recently published family of GPR109B-compounds based on benzotriazole-5-carboxylic

acid.
Taken together, nicotinic acid possesses the optimal hydrogen-bond patterns to bind to the rather hydrophilic patterns of the small binding site in GPR109A, whereas 2-oxo-octanoic acid possesses optimal hydrophobic patterns to bind to the additional specific hydrophobic cleft of GPR109B. Both receptor sites consist of hydrophilic and hydrophobic patterns, and are in spatial proximity. The binding of Acifran, which is an unselective ligand for both receptors, possesses the hydrophobic patterns (benzene, methyl) to bind to the hydrophobic binding cleft of GPR109B. Acifran also contains hydrophilic patterns (furan ring oxygen and carbonyl oxygen), allowing the interaction with the hydrophilic site in the GPR109A.

In a third approach, investigations on interactions of G proteins with their small-, medium- and large-sized ligands identified a common pattern for recognition and/or interaction, namely the interaction of negative and positive charges on G proteins and their ligands. This pattern was clearly identified by studies of small-sized alkyl-substituted amino acid derivatives (so called lipoamines) as well as by medium-sized secretagogues mastoparan-X and mastoparan-S. It contains specific distances of positive charges within G protein-ligands, recognized by negatively charged residues of complementary distances in G proteins. Investigations of four different G protein-subtypes (Gαi, Gαo, Gαq, Gαs) clearly showed different localizations and, therefore, distances of negatively charged residues in close proximity to the C-terminus (below 12 Å for Gαi, around 15Å for Gαq, and greater than 18 Å for Gαs). As a result, it was possible to design a new compound (FU244) that exclusively interacts with Gαi but not with Gαq or Gαs. The application of these patterns to the interaction interface of GPCRs and G proteins resulted in identification of two residues within ICL2 that are required for Gαi selectivity of ETA and ETB. This way, we demonstrated that also in GPCRs complementary charge patterns determine G protein-selectivity.

Zusammenfassung

In der hier vorliegenden Studie wurden die Interaktionsmechanismen und Hintergründe für selektive Wechselwirkungen zwischen G-Protein gekoppelten Rezeptoren, deren Liganden und G-Proteinen untersucht. Ausgangspunkt dafür waren homologe Rezeptorsubtypen, die sich durch Unterschiede in ihren Funktionen auszeichnen.
Die Ursachen für Selektivität und hochaffine Ligandenbindung der Endothelin-Rezeptorsubtypen ETA und ETB war vor der Arbeit nicht aufgeklärt. Basierend auf unseren Untersuchungen, präsentieren wir hier erstmals Liganden-Rezeptor-Wechselwirkungen, die auf vier unterschiedlichen aber zueinander komplementären Erkennungsregionen in Liganden und Rezeptoren beruhen. Vergleiche von Sequenz- und Funktionsinformationen von endothelin-rezeptorselektiven Peptiden führten schlussendlich zur Unterteilung der Peptidliganden in vier Regionen (*Addressor, Hook, Core, Modulator*), welche die existierenden Daten aus der Literatur und von unseren Kooperationspartnern erklären. Gleichsam zu diesen vier Regionen an Peptidliganden wurden komplementäre Erkennungsregionen an beiden Endothelin-Rezeptorsubtypen identifiziert: *Gateway, Edge, Neck, Binding Cleft*. Paarweise Wechselwirkungen von *Addressor* und *Gateway*, *Hook* und *Edge*, *Core* und *Neck* sowie *Modulator* und *Binding Cleft*, erklären dabei vollständig die Ursachen und Hintergründe der Selektivität für Endothelin-Rezeptoren sowie deren Subtypen, wie wir eindrucksvoll durch die Entwicklung eines neuen ETB-selektiven Peptidliganden zeigen konnten.
Weiterhin war es uns dadurch möglich, die Notwendigkeiten und Unterschiede für Aktivierung und Inhibierung beider Endothelin-Rezeptorsubtypen aufzuzeigen. Dabei liegt der Hauptunterschied zwischen peptidligandenabhängiger Aktivierung und Inhibierung am Vorhandensein negativer Ladungen innerhalb der *Hook*-Region der Peptidliganden. Sind solcherart Ladungen innerhalb des *Hook* vorhanden, können sie mit entsprechenden Ladungen der *Edge*-Region des Rezeptors wechselwirken, wodurch der aktivierende Ligand nicht weiter in die *Binding Cleft* zwischen den Transmembran-Helices TMH3, TMH6 und TMH7 hinabtaucht. Dadurch wird eine Neuorientierung wichtiger Rezeptorseitenketten innerhalb des Transmembran-Helixbündels hervorgerufen, die den Rezeptor in den aktiven Zustand überführt.
Fehlen negative Ladungen innerhalb der *Hook*-Region, wie dies bei Inhibitoren der Fall ist, gleitet der Ligand tief in die *Binding Cleft* und sorgt dort durch zusätzliche Interaktionen mit Rezeptorseitenketten zu einer Stabilisierung des inaktiven Zustands des Rezeptors – der daraufhin inhibiert wurde.
Eins der am beeindruckendensten Ergebnisse dieser Untersuchungen ist die Orientierung der beiden letzten Aminosäuren des C-Terminus (innerhalb des *Modulator*) von Inhibitoren, die in identischer Position zu der bekannten Struktur von 11-cis-Retinal in Rinderrhodopsin zu liegen kommen. Dadurch lässt sich eine Konserviertheit dieses Mechanismus in vielen G-Protein gekoppelten Rezeptoren vermuten.
Ferner war es uns durch unsere Modelle möglich, die bisher unverstandene Formierung des superstabilen Komplexes zwischen Endothelin-1 und Endothelin-Rezeptorsubtyp ETB zu erklären. Während der Ligandenbindung an ETB werden Sequenz- und Strukturbereiche des N-Terminus dieses Rezeptors genutzt, um ET-1 wie mit einem Deckel zu bedecken. Da diese N-terminalen Bereiche in ETA anders formiert und gestalten sind, ist dort dieser Mechanismus und damit auch die Bildung eines superstabilen Komplexes nicht möglich.

Desweiteren wurde die Ligandenbindung an Nikotinsäure-Rezeptoren GPR109A und GPR109B untersucht. Obwohl beide Rezeptoren einen hohen Grad an Sequenz- und Strukturhomologie aufweisen, bindet GPR109B Nikotinsäure mit sehr geringer Affinitität, während GPR109A für die Bindung dieses Liganden optimiert ist.

Durch die Nutzung der hohen Homologie und der funktionellen Unterschiede war es uns möglich, Aminosäuren mit kritischer Bedeutung für die Ligandenbindung an beiden Rezeptoren zu identifizieren. Die Bindungsstelle befindet sich zwischen den Transmembran-Helices TMH2, TMH3 und TMH7 und ist somit unterschiedlich zu den meisten anderen rhodopsin-ähnlichen Rezeptoren, deren Bindungstaschen zwischen TMH4, TMH5 und TMH6 liegt. Dass diese Bindungsstelle dennoch richtig identifiziert wurde, bestätigen experimentelle Ergebnisse von mutierten Aromatenresten in TMH5 und TMH7, die aufgrund unserer Vorschläge gemacht wurden.
Durch die korrekte Identifizierung der Bindungstasche von Nikotinsäure konnten wir ebenfalls komplementäre pharmakophore Muster zwischen Rezeptor und Ligand ableiten, die nun für die Identifizierung und Entwicklung weiterer Medikamente genutzt werden können. Dass dies so möglich ist, wurde von uns und den experimentellen Daten unserer Kooperationspartner mit der Identifizierung von 2-Oxo-oktansäure als selektiven Liganden für GPR109B und nicht für GPR109A bestätigt.
Ferner sind die für 2-Oxo-oktansäure identifizierten pharmakophoren Muster ebenso auffindbar in den ebenso GPR109B-selektiven Verbindungen basierend auf Benzotriazol-5-carbonsäure, welche vor kurzem veröffentlicht wurden.

In einem dritten Projekt wurden die Wechselwirkungen von G-Protein sowie deren klein-, mittel- und großmolekularen Interaktionspartnern untersucht. Dabei identifizierten wir ein generelles Muster für G-Protein-Selektivität, welches auf komplementären Ladungsabständen beruht. Diese Muster wurden klar für die Wechselwirkungen der alkyl-substituierten Aminosäurederivate (auch Lipoamine genannt) und der sekretierten Peptide (z.B. Mastoparan-X und Mastoparan-S) herausgearbeitet.
Im Allgemeinen interagieren positive Ladungen von G-Protein-Liganden an negativen Ladungen vergleichbarer Abstände an benachbarten Strukturen des C-terminus von G-Proteinen. Unsere Untersuchungen von vier verschiedenen G-Protein-Subtypen (Gαi, Gαo, Gαq, Gαs) identifizierten verschiedene Verteilungen und Ladungsabstände negativer Aminosäuren in diesen Regionen (unter 12 Å für Gαi, etwa 15 Å für Gαq, und über 18 Å für Gαs). Basierende auf diesen Erkenntnissen entwickelten wir einen neues Lipoamin mit optimierten Ladungsabständen für Gαi. Tatsächlich wurde für die entsprechende Struktur FU244 experimentell gezeigt, dass dieser Ligand mit Gαi interagiert, nicht aber mit Gαq oder Gαs.
Anwendung dieser Ladungsmuster auf die Interaktionen zwischen G-Proteinen und G-Protein gekoppelten Rezeptoren resultierte schlussendlich in der Identifizierung von zwei Aminosäuren innerhalb des intrazellulären Loops ICL2 an ETA und ETB, die absolut notwendig sind für die Wechselwirkung mit Gαi.
Daraus schliessen wir, dass distanzabhängige Ladungsmuster sowohl für nieder- und mittelmolekulare G-Protein-Liganden, als auch für hochmolekulare Interaktionspartner wie G-Protein gekoppelte Rezeptoren zur Bestimmung für G-Protein-Selektivität notwendig sind.

Zusammenfassend lässt sich festhalten, dass die Kombination aus Computer-Untersuchungen homologer Rezeptor Subtypen unterschiedlicher, biologischer Funktion und experimenteller Absicherung zu stimmigen Hypothesen und Theorien über die Mechanismen von G-Protein gekoppelten Rezeptoren führt. Dadurch stellt die hier vorgestellte Methode ein wertvolles Werkzeug in der Charakterisierung von GPCRs, deren Ligandenbindung und G-Proteinkopplung dar.

Publications

Publications in Journals

Kleinau G, Jäschke H, Neumann S, **Lättig J**, Paschke R and Krause G (2004), Identification of a Novel Epitope in the TSH Receptor Ectodomain Acting as Intramolecular Signalling Interface. *J Biol Chem.* 279 (49): 51590-51600

Tunaru S, **Lättig J**, Kero J, Krause G and Offermanns S (2005), Characterization of Determinants of Ligand Binding in the Nicotinic Acid Receptor GPR109A (HM74A/PUMA-G). *Mol. Pharmacol.* 68 (5): 1271-1280

Lättig J, Böhl M, Fischer P, Tischer S, Tietböhl C, Menschikowski M, Gutzeit HO, Metz P and Pisabarro MT (2007), Mechanism of Inhibition of Human Secretory Phospholipase A2 by Flavonoids – Rationale for Lead Design. *J Comput Aided Mol Des.* 21 (8): 473-483

Lättig J, Furkert J, Oksche A and Krause G, Structural Determinants for Ligand Selectivity at EtA- and EtB-Receptors. *Biophys J.* (submitted)

Lättig J, Böhl M, Böhl J, Pisabarro MT and Gutzeit HO, An experimental and theoretical approach to study inhibitor binding to phosholipase A2. *J Comput Aided Mol Des.* (in preparation)

Lättig J, Tunaru S, Kero J, Krause G and Offermanns S, Selectivity Patterns to Differentiate Between Nicotinic Acid Receptor GPR109A and Related Receptor GPR109B: First Ligand for GPR109B. (in preparation)

Lättig J, Furkert J, Oksche A and Krause G, Residues of the 2nd Intracellular Loop of Endothelin Receptor Subtypes A and B Selectively for Interactions with G-protein Subunit Gαi. (in preparation)

Contributions to Books

Lättig J, Schunack W, Nürnberg B and Krause G (2003), Towards 3D-patterns for G-protein Specificity of GPCRs by G-protein Interacting Small Molecules. Designing Drugs and Crop Protectants: Processes, Problems and Solutions. Editors: M Ford, D Livingston, J Dearden, H van de Waterbeemd Blackwell publishing Inc., Oxford, UK

Lendeckel U, Bukowska A, **Lättig J** and Brandt W, Alanyl-Aminopeptidase in Human T Cells: Structure and Function. Aminopeptidases in Biology and Disease Editors: Hopper NM, Lendeckel U Kluwer Academic/Plenum Publishers, New York, USA

Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Referenzen und Hilfsmittel verfasst habe.

Ich habe diese Arbeit bisher noch an keiner Universität vorgelegt.

Ich erkläre weiter, dass ich mich bisher nicht an einer anderen Einrichtung um einen Doktorgrad beworben habe und ebenso keine derartigen Titel besitze.

Berlin, 20. Juli 2007 Jens Lättig