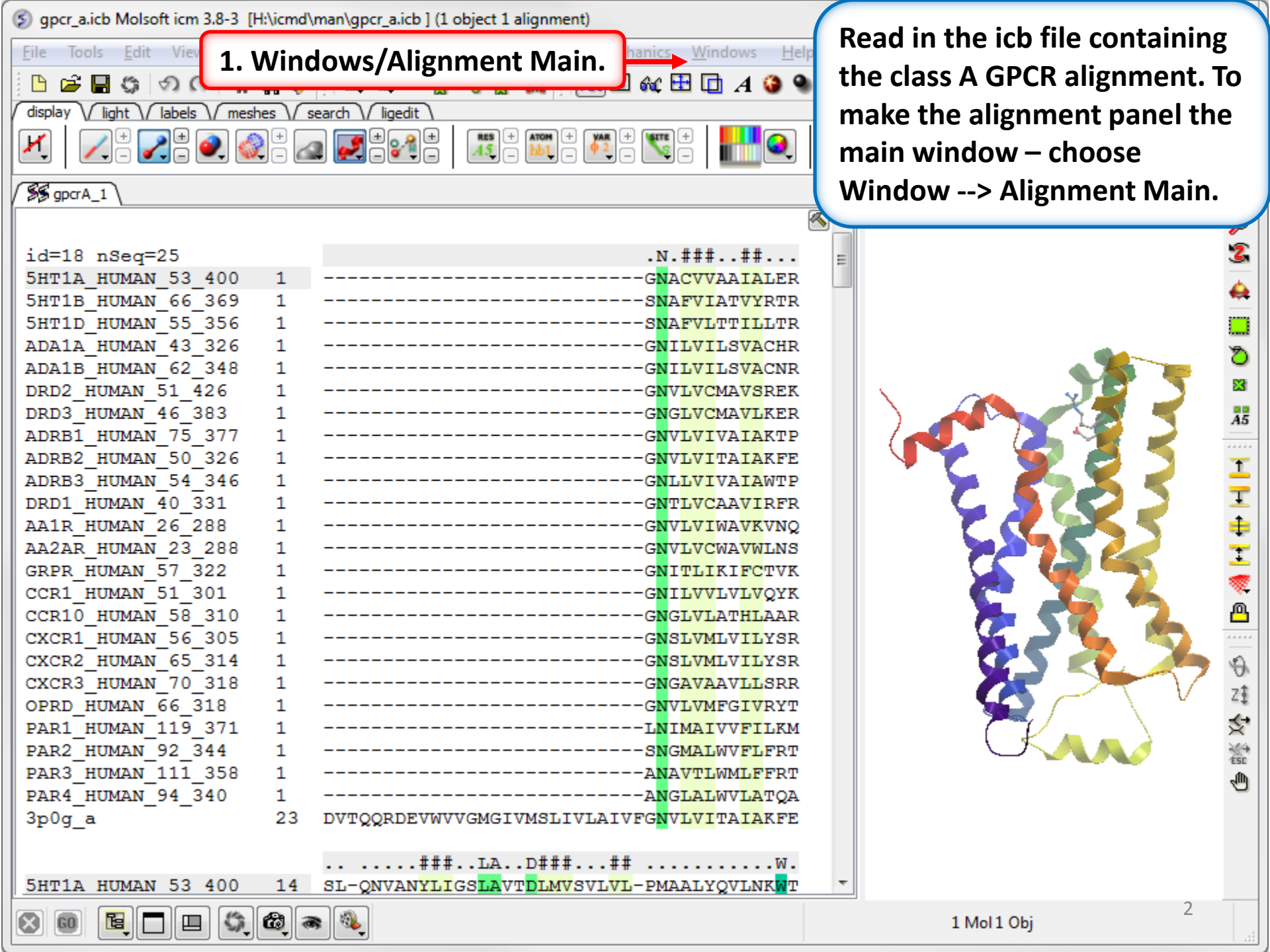
Alignment Annotation

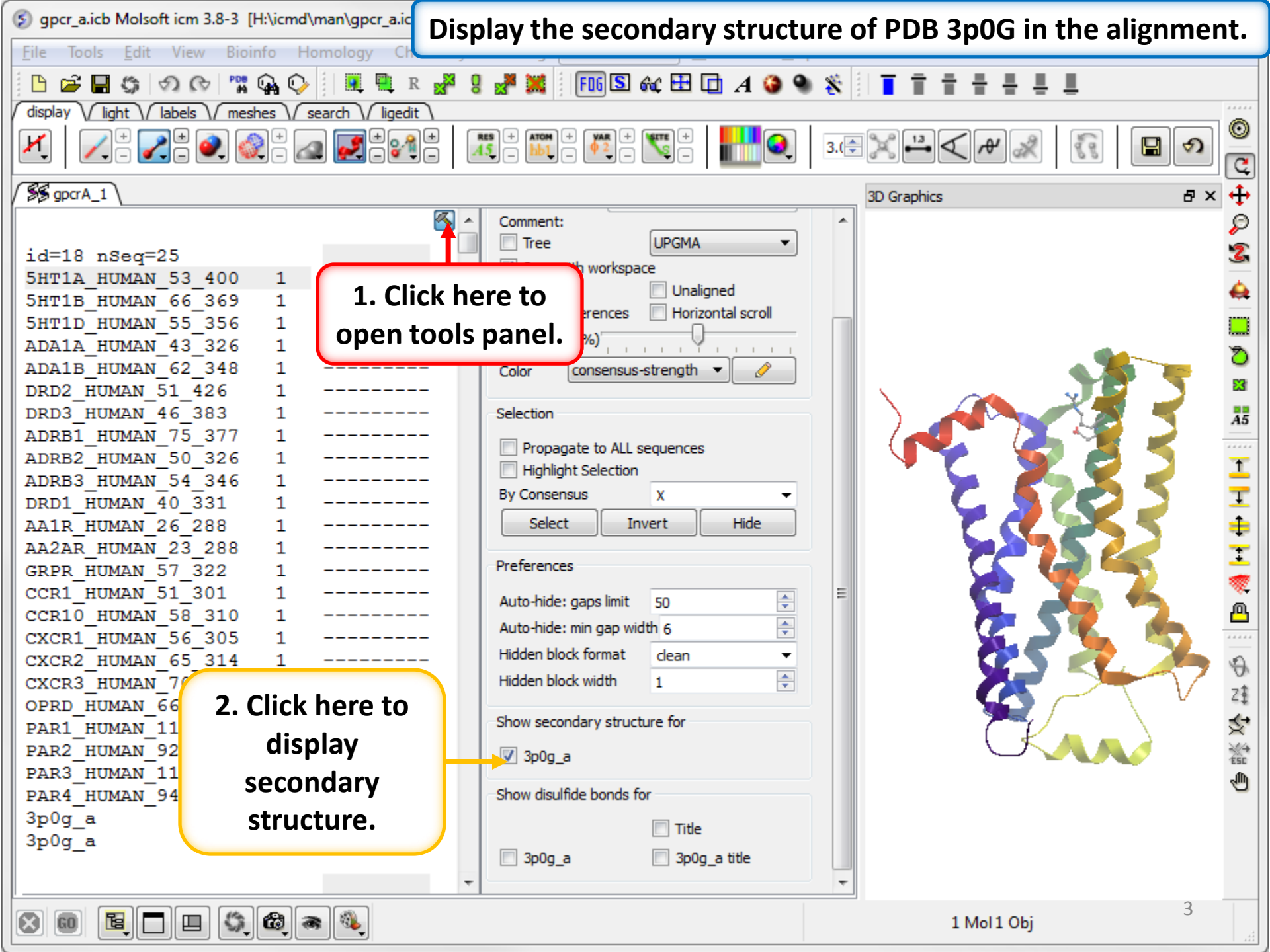
G-Protein Coupled Receptors (GPCRs) all share a common structural core of seven transmembrane helices but they lack significant sequence homology between subfamilies. When modeling GPCRs it is important to get a good alignmnent between the query and template structure. Each helix has one or more conserved motifs:

Helix 1: GX_3**N** or G**N**
Helix 2: N(S,H)LX_3**D**$X_{7,8,9}$P
Helix 3: SX_3LX_2IX_2D(E,H)**R**Y
Helix 4: **W**X_8,9P
Helix 5: FX_2**P**X_7Y
Helix 6: FX_2CW(Y,F)X**P**
Helix 7/Helix 8: LX_3NX_3N(D)**P**X_2Y$X_{5,6}$F

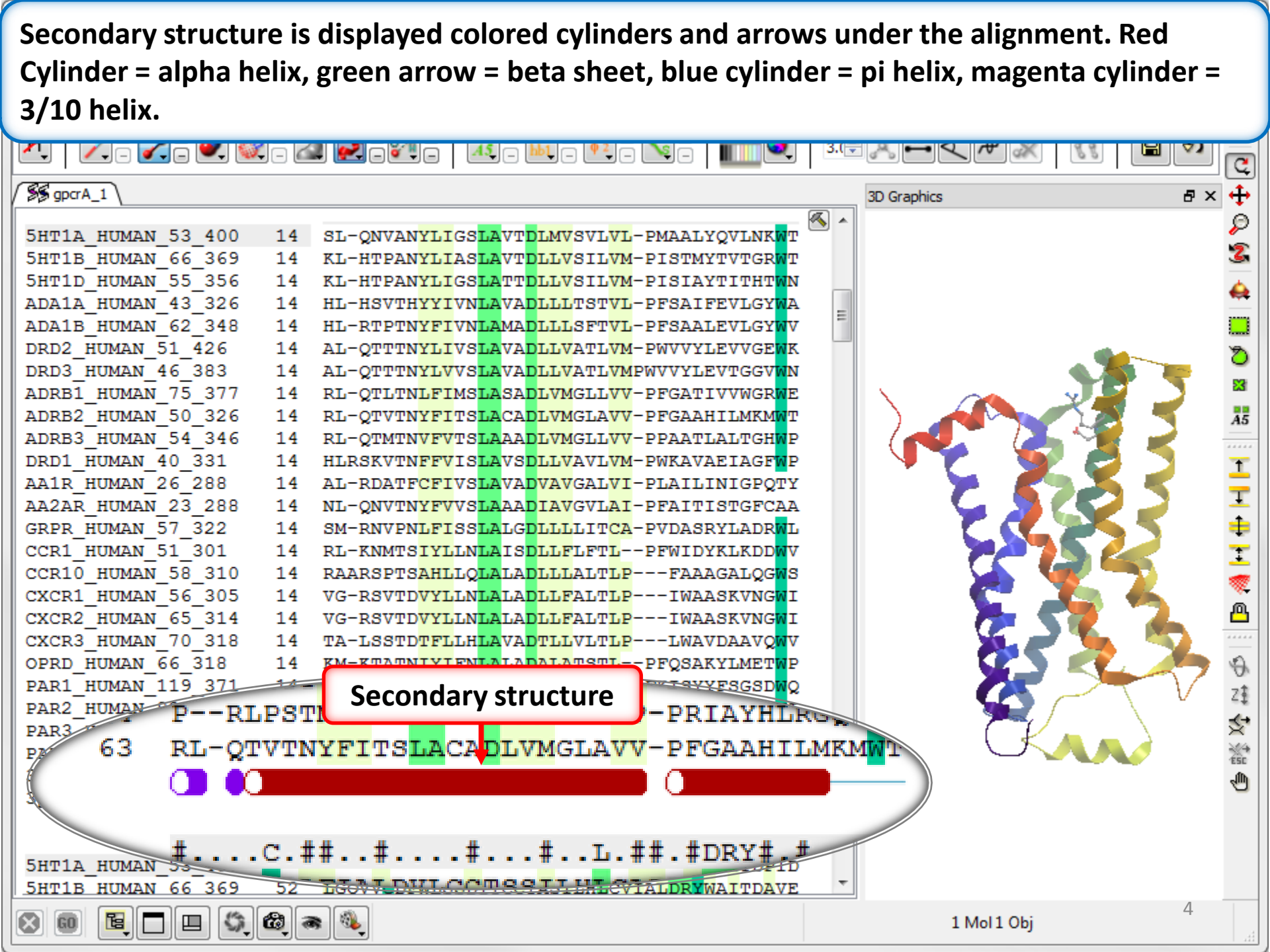
The ProSite class A alignment http://prosite.expasy.org/PDOC00210 can be used to guide GPCR alignments.

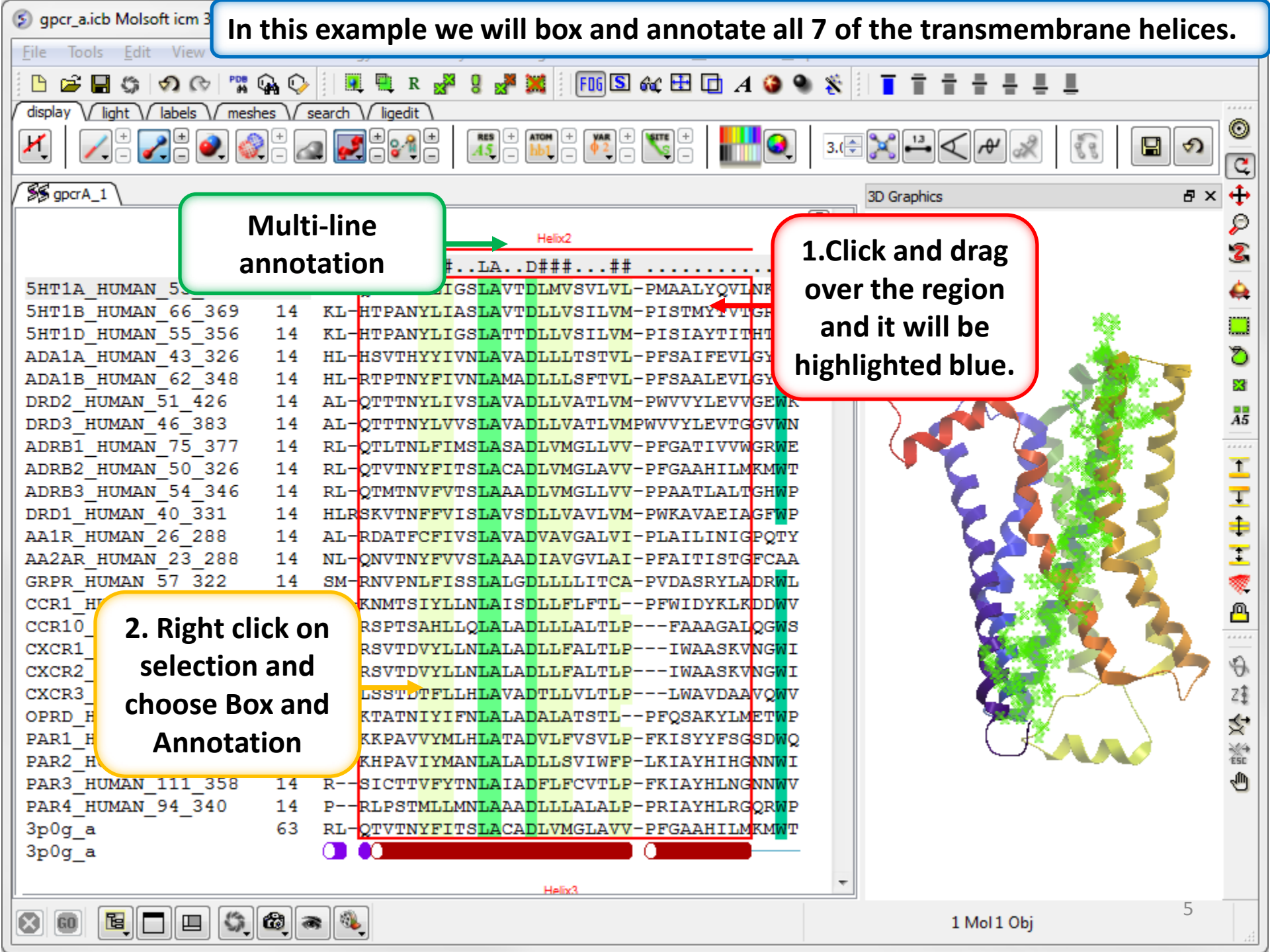

gpcr_a.icb Molsoft icm 3.8-3 [H:\icmd\man\gpcr_a.icb] (1 object 1 alignment)
1. Windows/Alignment Main.
Read in the icb file containing the class A GPCR alignment. To make the alignment panel the main window – choose Window --> Alignment Main.
File Tools Edit Windows Help
display light labels meshes search ligedit
gpcrA_1
id=18 nSeq=25 .N.###..##...
5HT1A_HUMAN_53_400 1 ----------GNACVVAAIALER
5HT1B_HUMAN_66_369 1 ----------SNAFVIATVYRTR
5HT1D_HUMAN_55_356 1 ----------SNAFVLTTILLTR
ADA1A_HUMAN_43_326 1 ----------GNILVILSVACHR
ADA1B_HUMAN_62_348 1 ----------GNILVILSVACNR
DRD2_HUMAN_51_426 1 ----------GNVLVCMAVSREK
DRD3_HUMAN_46_383 1 ----------GNGLVCMAVLKER
ADRB1_HUMAN_75_377 1 ----------GNVLVIVAIAKTP
ADRB2_HUMAN_50_326 1 ----------GNVLVITAIAKFE
ADRB3_HUMAN_54_346 1 ----------GNLLVIVAIAWTP
DRD1_HUMAN_40_331 1 ----------GNTLVCAAVIRFR
AA1R_HUMAN_26_288 1 ----------GNVLVIWAVKVNQ
AA2AR_HUMAN_23_288 1 ----------GNVLVCWAVWLNS
GRPR_HUMAN_57_322 1 ----------GNITLIKIFCTVK
CCR1_HUMAN_51_301 1 ----------GNILVVLVLVQYK
CCR10_HUMAN_58_310 1 ----------GNGLVLATHLAAR
CXCR1_HUMAN_56_305 1 ----------GNSLVMLVILYSR
CXCR2_HUMAN_65_314 1 ----------GNSLVMLVILYSR
CXCR3_HUMAN_70_318 1 ----------GNGAVAAVLLSRR
OPRD_HUMAN_66_318 1 ----------GNVLVMFGIVRYT
PAR1_HUMAN_119_371 1 ----------LNIMAIVVFILKM
PAR2_HUMAN_92_344 1 ----------SNGMALWVFLFRT
PAR3_HUMAN_111_358 1 ----------ANAVTLWMLFFRT
PAR4_HUMAN_94_340 1 ----------ANGLALWVLATQA
3p0g_a 23 DVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAIAKFE
.. ..###..LA..D###...## ....W.
5HT1A_HUMAN_53_400 14 SL-QNVANYLIGSLAVTDLMVSVLVL-PMAALYQVLNKWT
1 Mol 1 Obj
2

Display the secondary structure of PDB 3p0G in the alignment.



Secondary structure is displayed colored cylinders and arrows under the alignment. Red Cylinder = alpha helix, green arrow = beta sheet, blue cylinder = pi helix, magenta cylinder = 3/10 helix.




gpcr_a.icb Molsoft icm 3
In this example we will box and annotate all 7 of the transmembrane helices.
File Tools Edit View
display light labels meshes search ligedit
gpcrA_1
Multi-line annotation
Helix2
1.Click and drag over the region and it will be highlighted blue.
2. Right click on selection and choose Box and Annotation
3D Graphics
Helix3
1 Mol 1 Obj

gpcr_a.icb Molsoft icm 3.8-3 [H:\icmd\man\gpcr_a.icb *] (1 object 1 alignment)

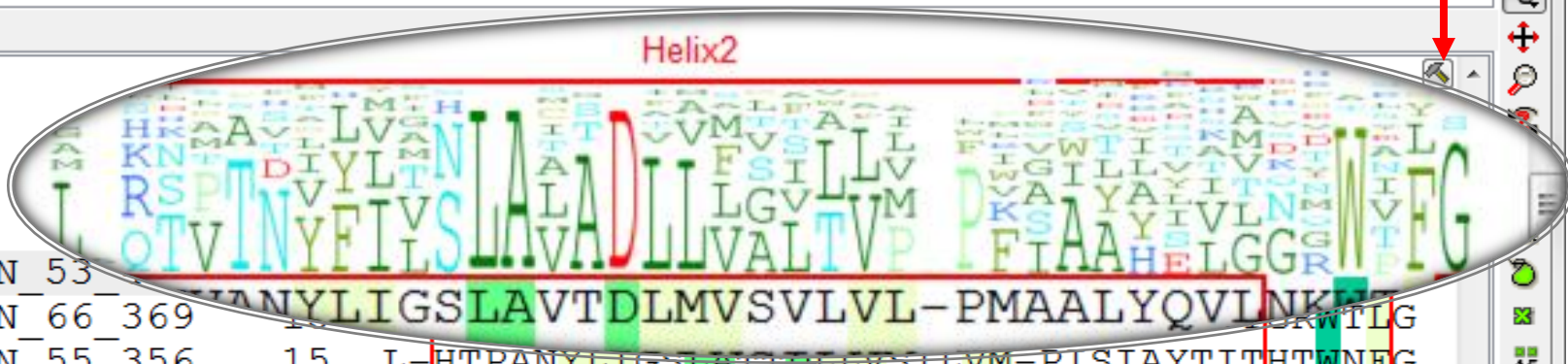
Display the sequence profile.

File Tools Edit View Bioinfo Homology Chemistry Docking MolMechanics Windows Help

display light labels meshes search ligedit

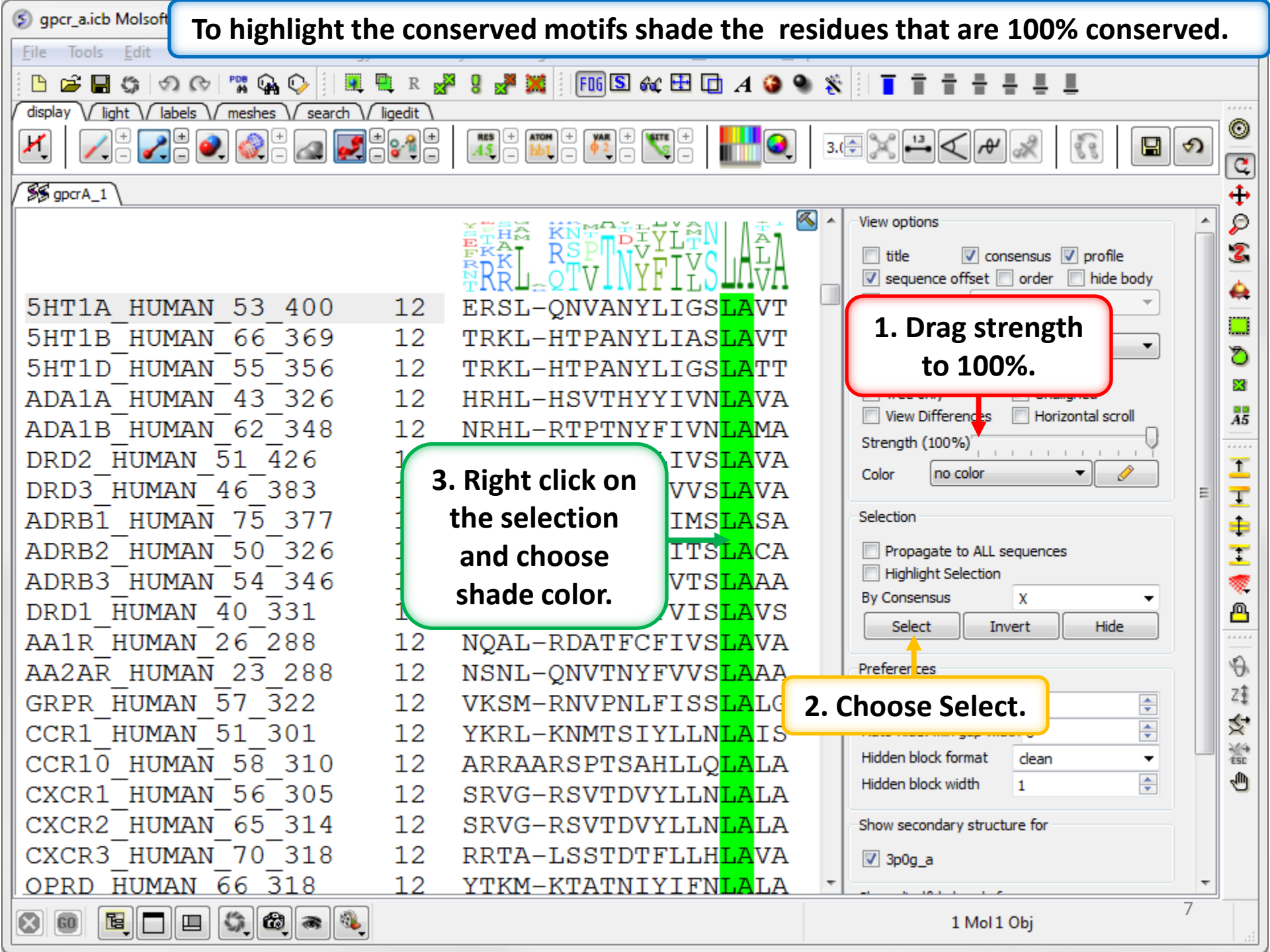
1. Open the tools panel and choose profile.

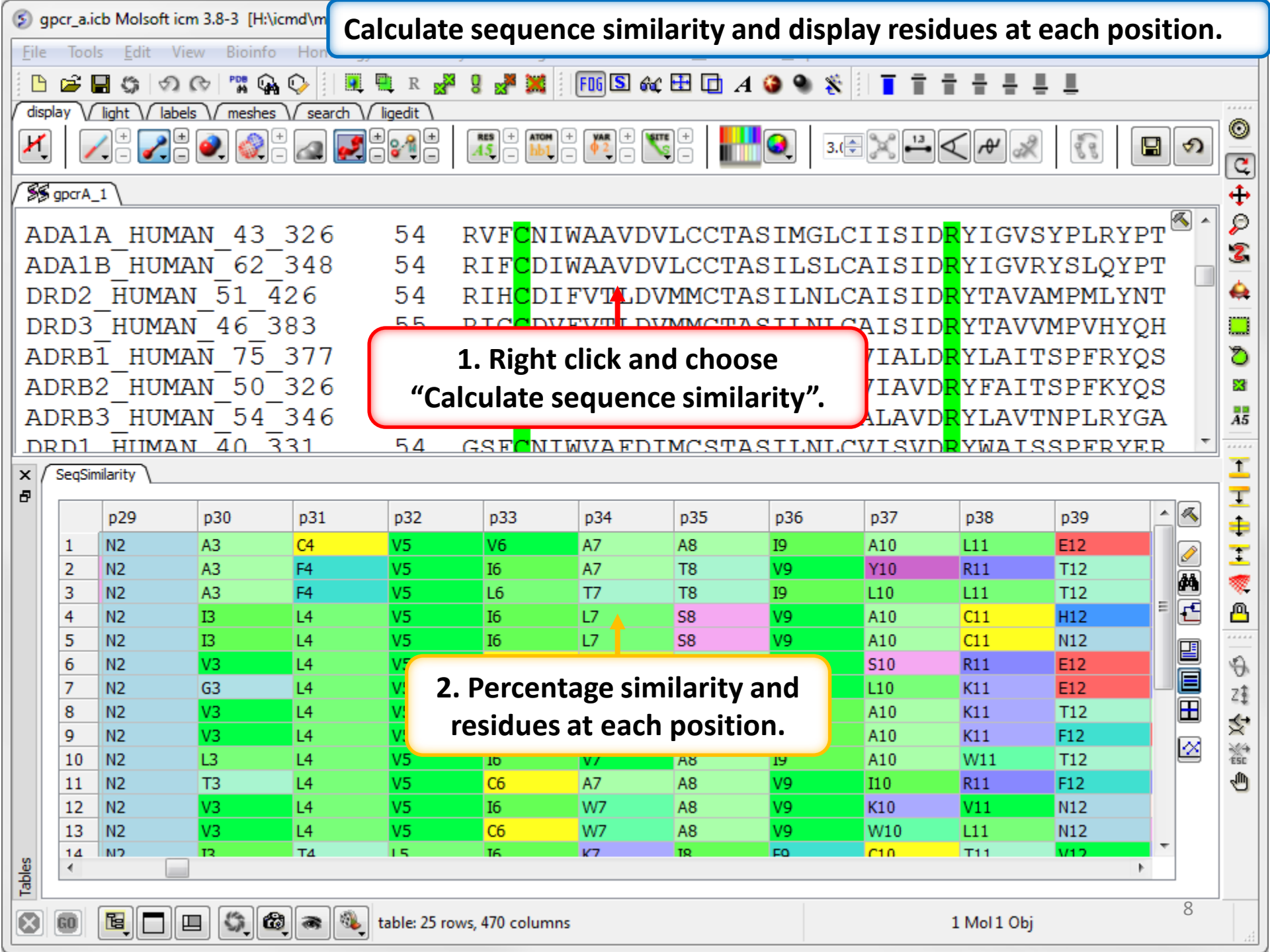
gpcrA_1




```
5HT1A_HUMAN_53_                 ANYLIGSLAVTDLMVSVLVL-PMAALYQVLNKWTLG
5HT1B_HUMAN_66_369              15  L-HTPANYLIGSLAVTDLLVSILVM-PISIAYTITHTWNFG
5HT1D_HUMAN_55_356              15  L-HSVTHYYIVNLAVADLLLTSTVL-PFSAIFEVLGYWAFG
ADA1A_HUMAN_43_326              15  L-RTPTNYFIVNLAMADLLLSFTVL-PFSAALEVLGYWVLG
ADA1B_HUMAN_62_348              15  L-QTTTNYLIVSLAVADLLVATLVM-PWVVYLEVVGEWKFS
DRD2_HUMAN_51_426               15  L-QTTTNYLVVSLAVADLLVATLVMPWVVYLEVTGGVWNFS
DRD3_HUMAN_46_383               15  L-QTLTNLFIMSLASADLVMGLLVV-PFGATIVVWGRWEYG
ADRB1_HUMAN_75_377              15  L-QTVTNYFITSLACADLVMGLAVV-PFGAAHILMKMWTFG
ADRB2_HUMAN_50_326              15  L-QTMTNVFVTSLAAADLVMGLLVV-PPAATLALTGHWPLG
ADRB3_HUMAN_54_346              15  LRSKVTNFFVISLAVSDLLVAVLVM-PWKAVAEIAGFWPF-
DRD1_HUMAN_40_331               15  L-RDATFCFIVSLAVADVAVGALVI-PLAILINIGPQTYF-
AA1R_HUMAN_26_288               15  L-QNVTNYFVVSLAAADIAVGVLAI-PFAITISTGFCAAC-
AA2AR_HUMAN_23_288              15  M-RNVPNLFISSLALGDLLLLITCA-PVDASRYLADRWLFG
GRPR_HUMAN_57_322               15  L-KNMTSIYLLNLAISDLLFLFTL--PFWIDYKLKDDWVFG
CCR1_HUMAN_51_301               15  AARSPTSAHLLQLALADLLLALTLP---FAAAGALQGWSLG
CCR10_HUMAN_58_310              15  G-RSVTDVYLLNLALADLLFALTLP---IWAASKVNGWIFG
CXCR1_HUMAN_56_305              15  G-RSVTDVYLLNLALADLLFALTLP---IWAASKVNGWIFG
CXCR2_HUMAN_65_314              15
```

1 Mol 1 Obj


gpcr_a.icb Molsoft
To highlight the conserved motifs shade the residues that are 100% conserved.
File Tools Edit
display light labels meshes search ligedit
gpcrA_1
5HT1A_HUMAN_53_400 12 ERSL-QNVANYLIGSLAVT
5HT1B_HUMAN_66_369 12 TRKL-HTPANYLIASLAVT
5HT1D_HUMAN_55_356 12 TRKL-HTPANYLIGSLATT
ADA1A_HUMAN_43_326 12 HRHL-HSVTHYYIVNLAVA
ADA1B_HUMAN_62_348 12 NRHL-RTPTNYFIVNLAMA
DRD2_HUMAN_51_426 IVSLAVA
DRD3_HUMAN_46_383 VVSLAVA
ADRB1_HUMAN_75_377 IMSLASA
ADRB2_HUMAN_50_326 ITSLACA
ADRB3_HUMAN_54_346 VTSLAAA
DRD1_HUMAN_40_331 VISLAVS
AA1R_HUMAN_26_288 12 NQAL-RDATFCFIVSLAVA
AA2AR_HUMAN_23_288 12 NSNL-QNVTNYFVVSLAAA
GRPR_HUMAN_57_322 12 VKSM-RNVPNLFISSLALG
CCR1_HUMAN_51_301 12 YKRL-KNMTSIYLLNLAIS
CCR10_HUMAN_58_310 12 ARRAARSPTSAHLLQLALA
CXCR1_HUMAN_56_305 12 SRVG-RSVTDVYLLNLALA
CXCR2_HUMAN_65_314 12 SRVG-RSVTDVYLLNLALA
CXCR3_HUMAN_70_318 12 RRTA-LSSTDTFLLHLAVA
OPRD_HUMAN_66_318 12 YTKM-KTATNIYIFNLALA
View options
title consensus profile
sequence offset order hide body
View Differences Horizontal scroll
Strength (100%)
Color no color
1. Drag strength to 100%.
3. Right click on the selection and choose shade color.
Selection
Propagate to ALL sequences
Highlight Selection
By Consensus X
Select Invert Hide
2. Choose Select.
Preferences
Hidden block format clean
Hidden block width 1
Show secondary structure for
3p0g_a
1 Mol 1 Obj
7



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2	N2	A3	F4	V5	I6	A7	T8	V9	Y10	R11	T12
3	N2	A3	F4	V5	L6	T7	T8	I9	L10	L11	T12
4	N2	I3	L4	V5	I6	L7	S8	V9	A10	C11	H12
5	N2	I3	L4	V5	I6	L7	S8	V9	A10	C11	N12
6	N2	V3	L4	V5					S10	R11	E12
7	N2	G3	L4	V5					L10	K11	E12
8	N2	V3	L4	V5					A10	K11	T12
9	N2	V3	L4	V5					A10	K11	F12
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