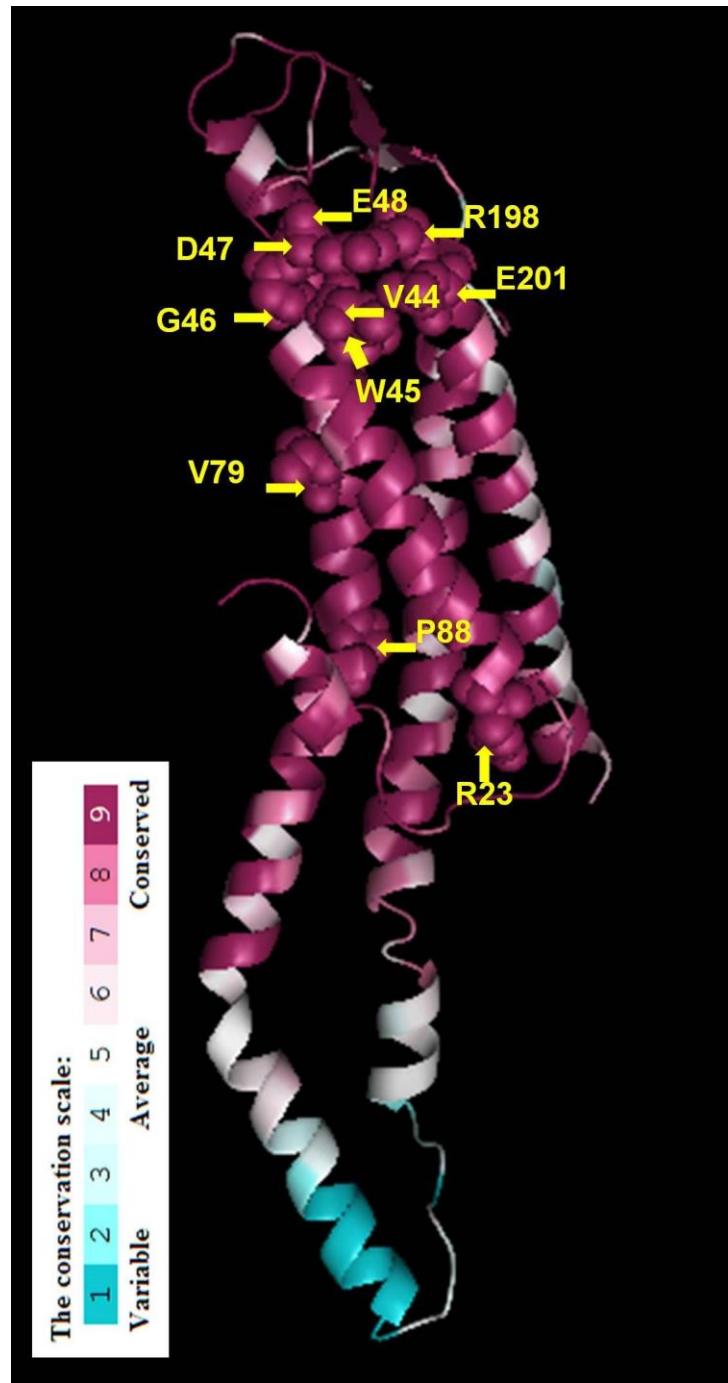
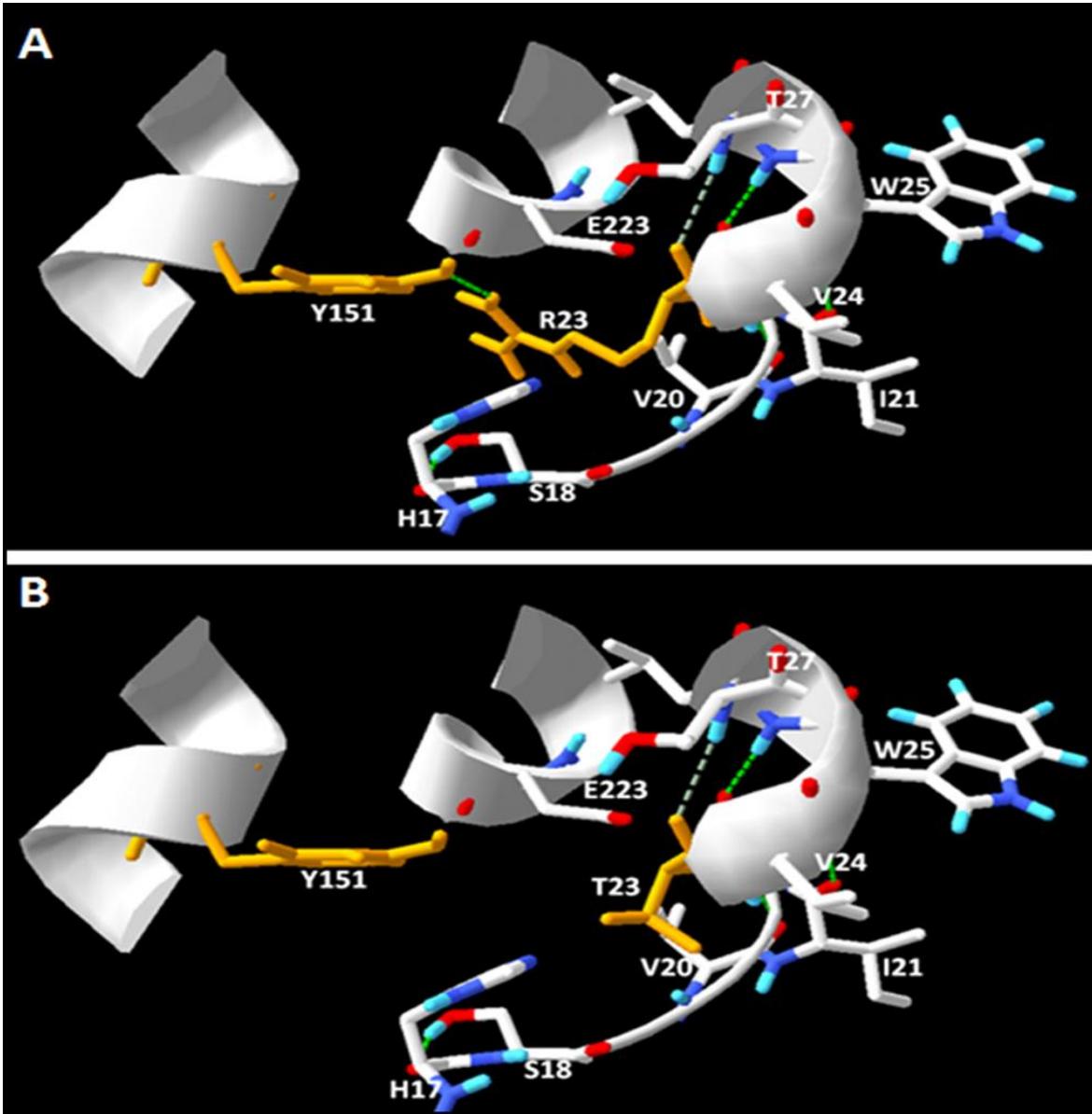


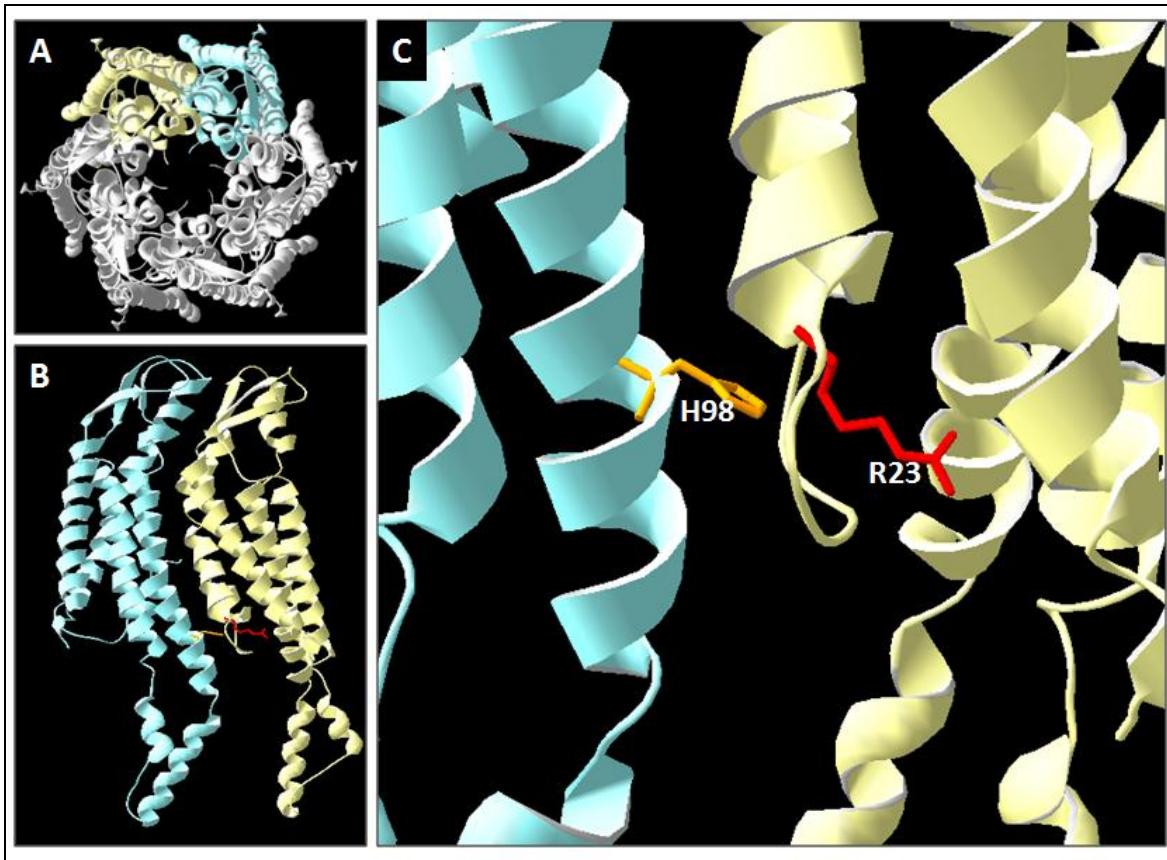
SUPPLEMENTARY FIGURES:



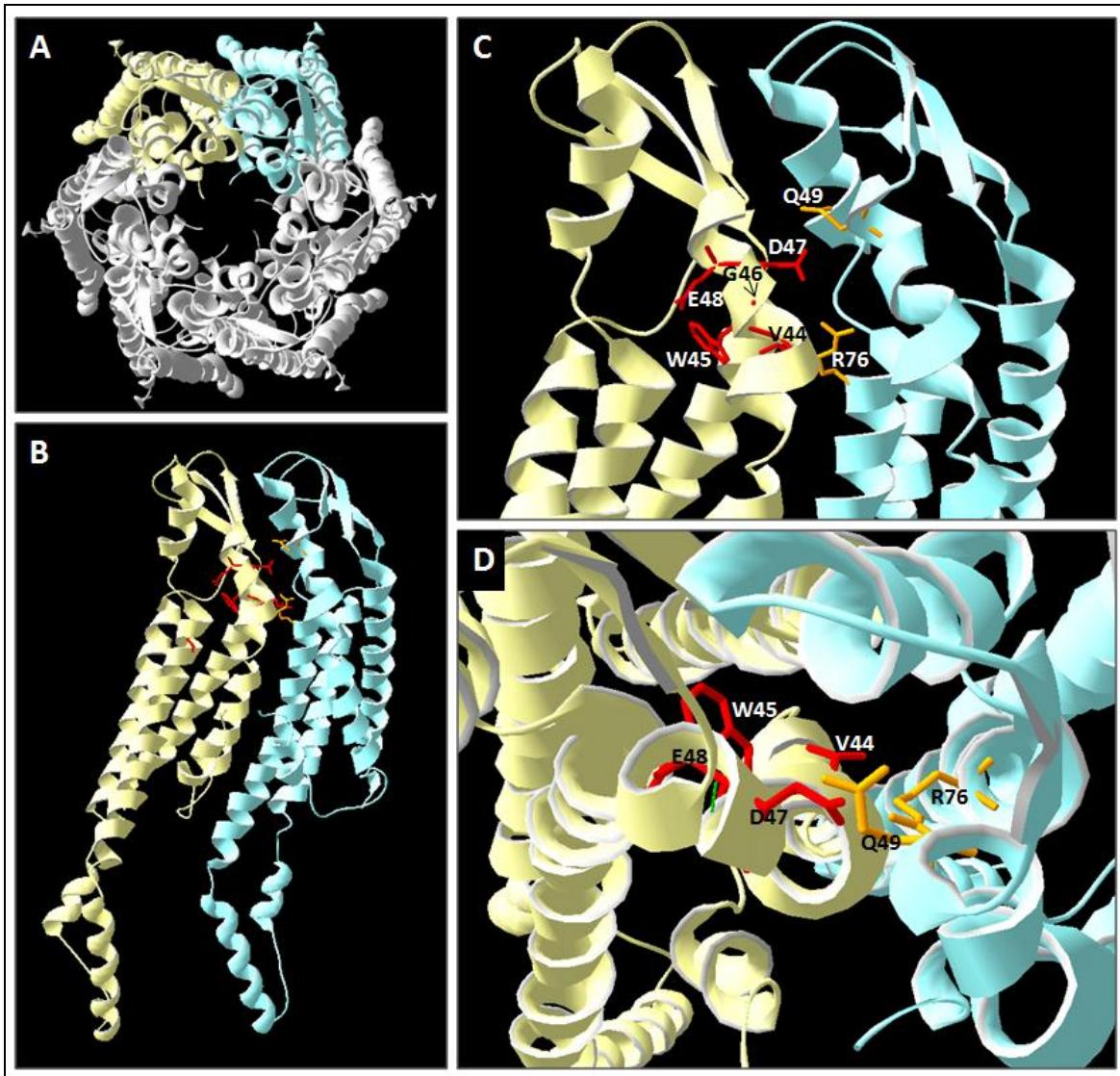
Supplemental Figure 1.



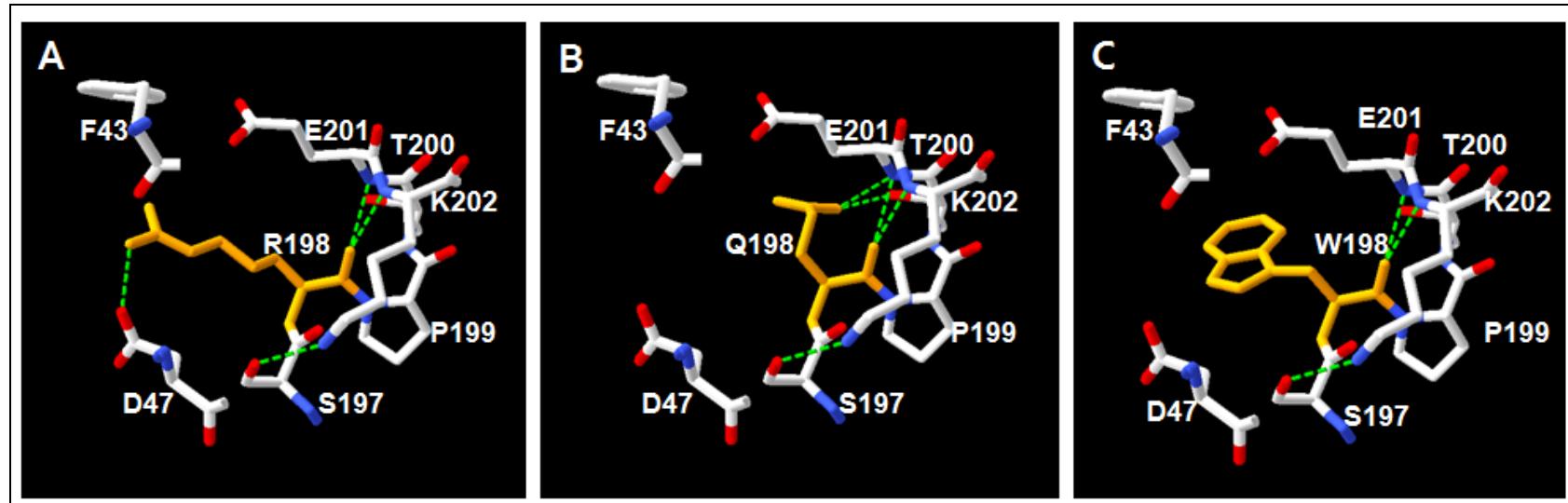
Supplemental Figure 2.



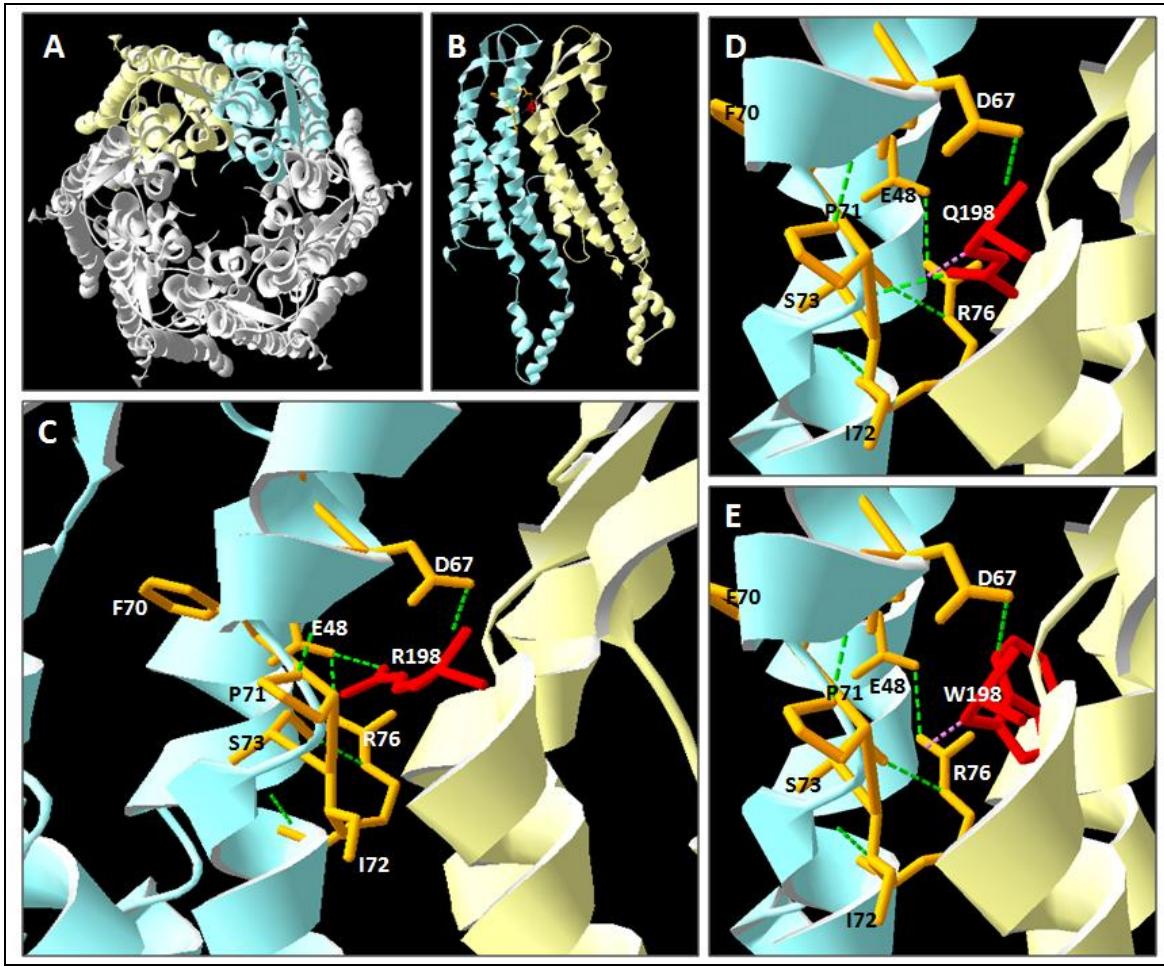
Supplemental Figure 3.



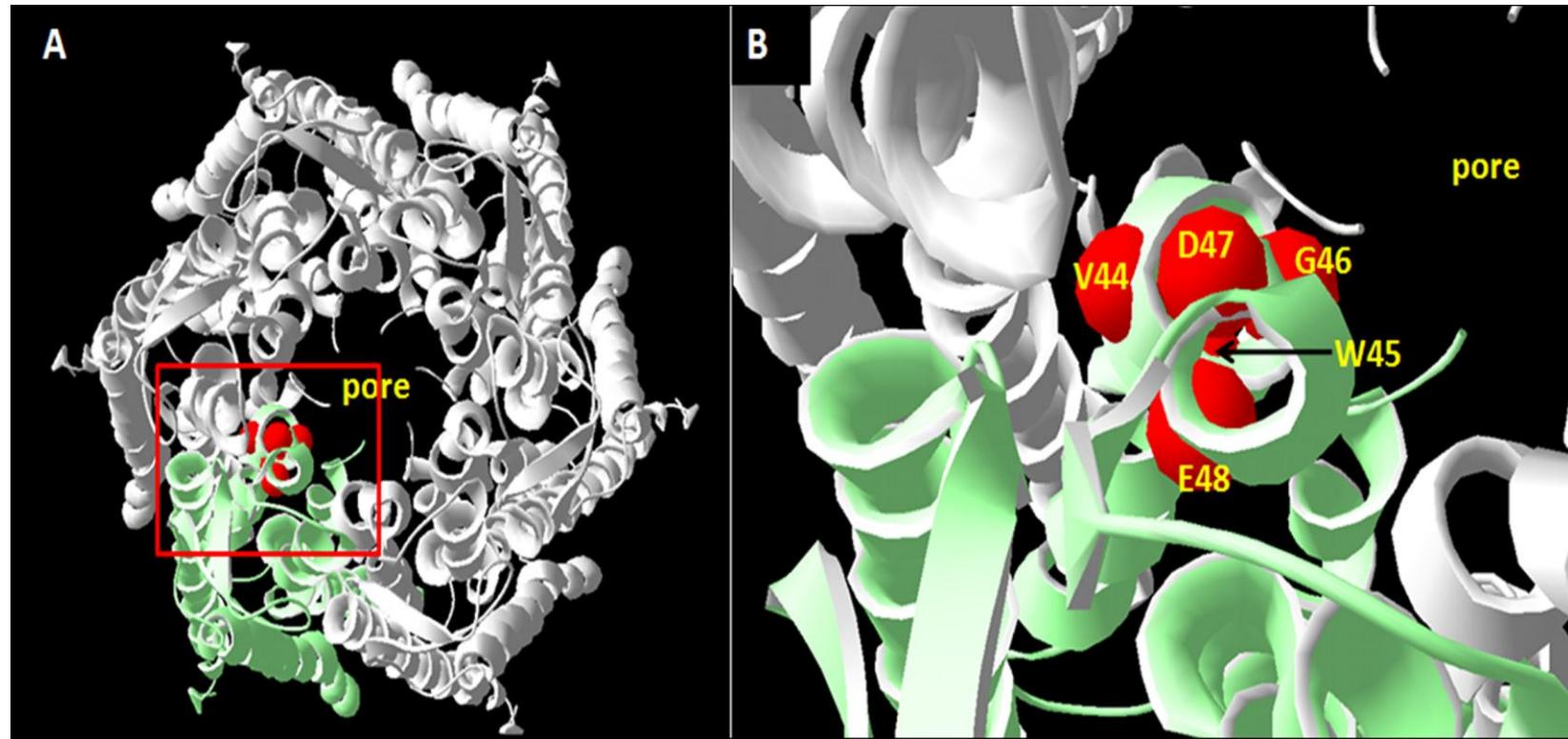
Supplemental Figure 4.



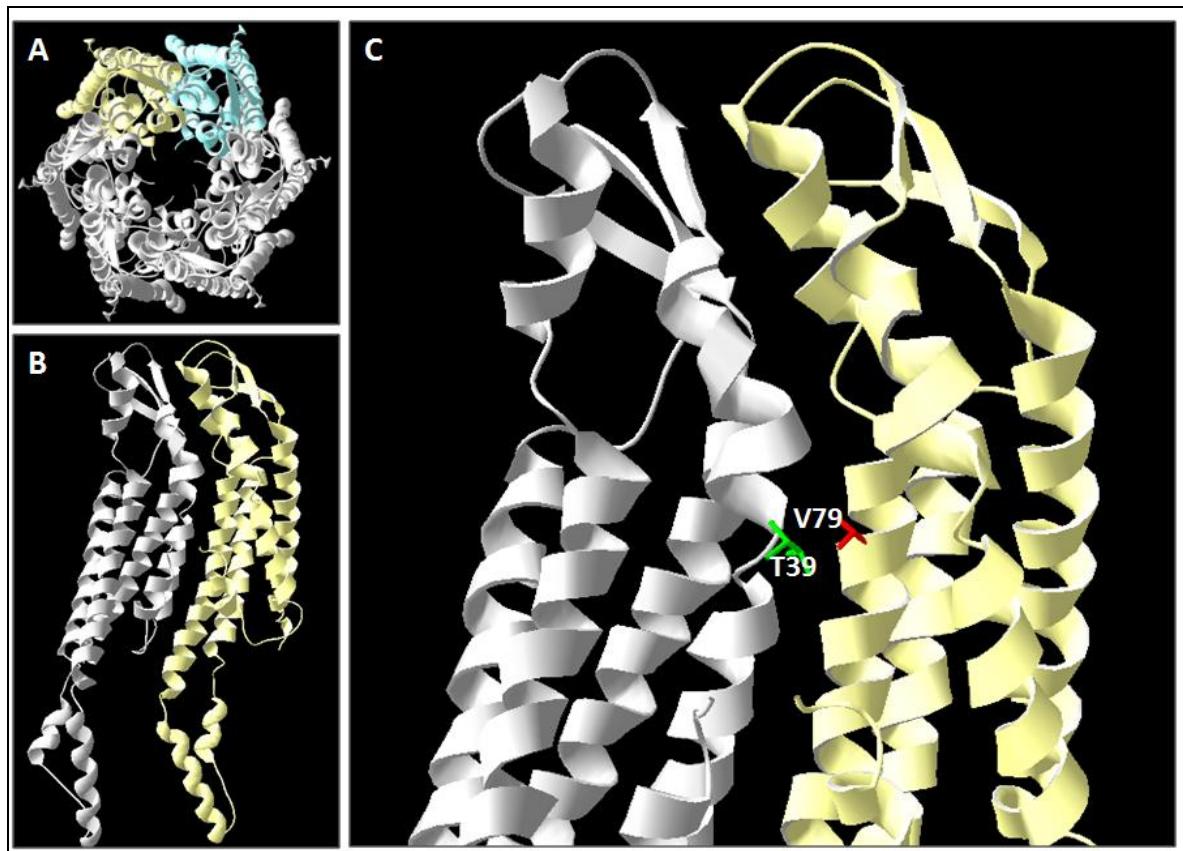
Supplemental Figure 5.



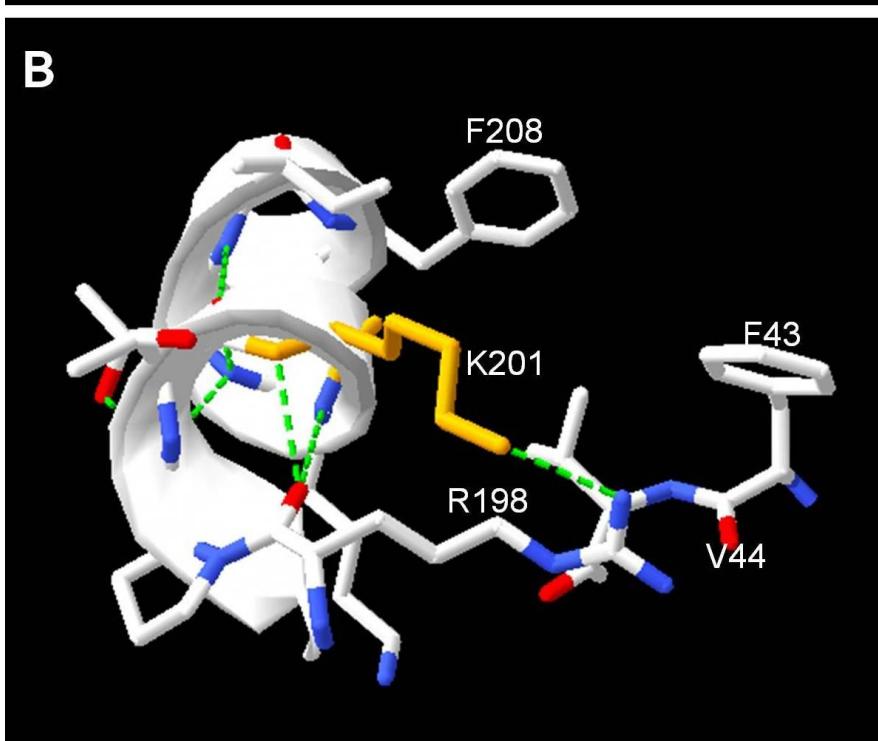
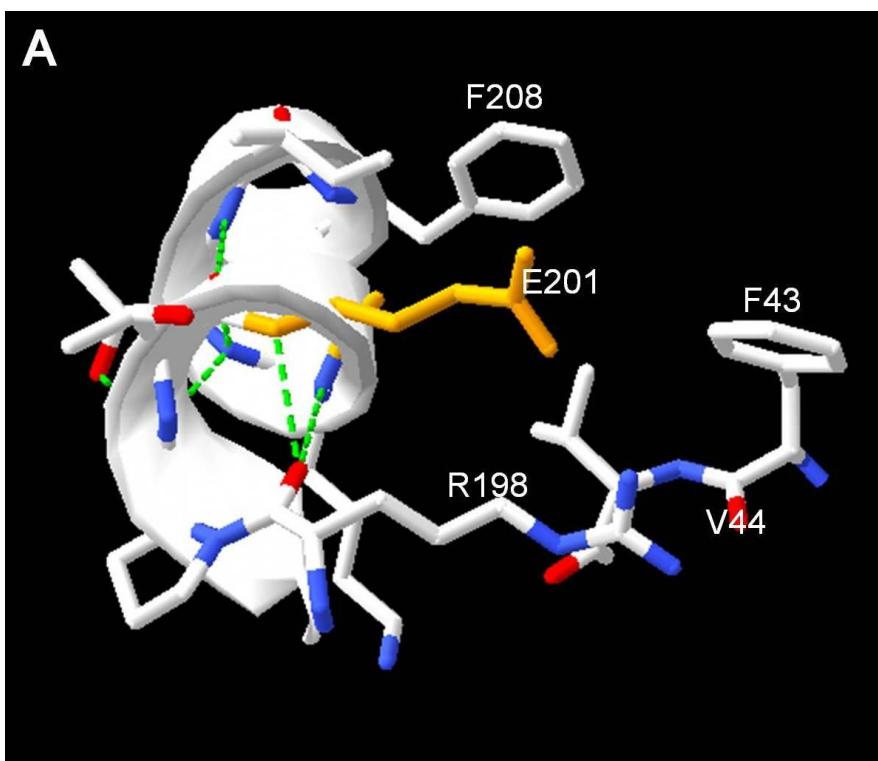
Supplemental Figure 6.



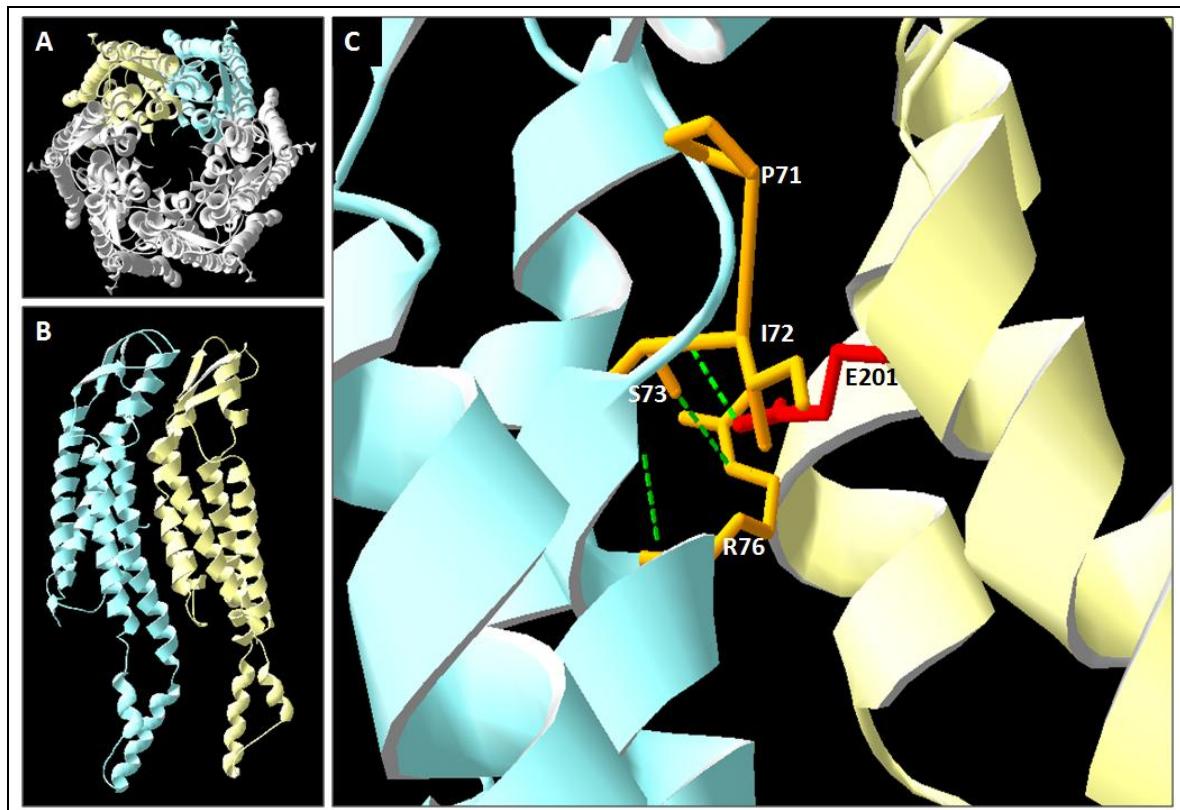
Supplemental Figure 7.



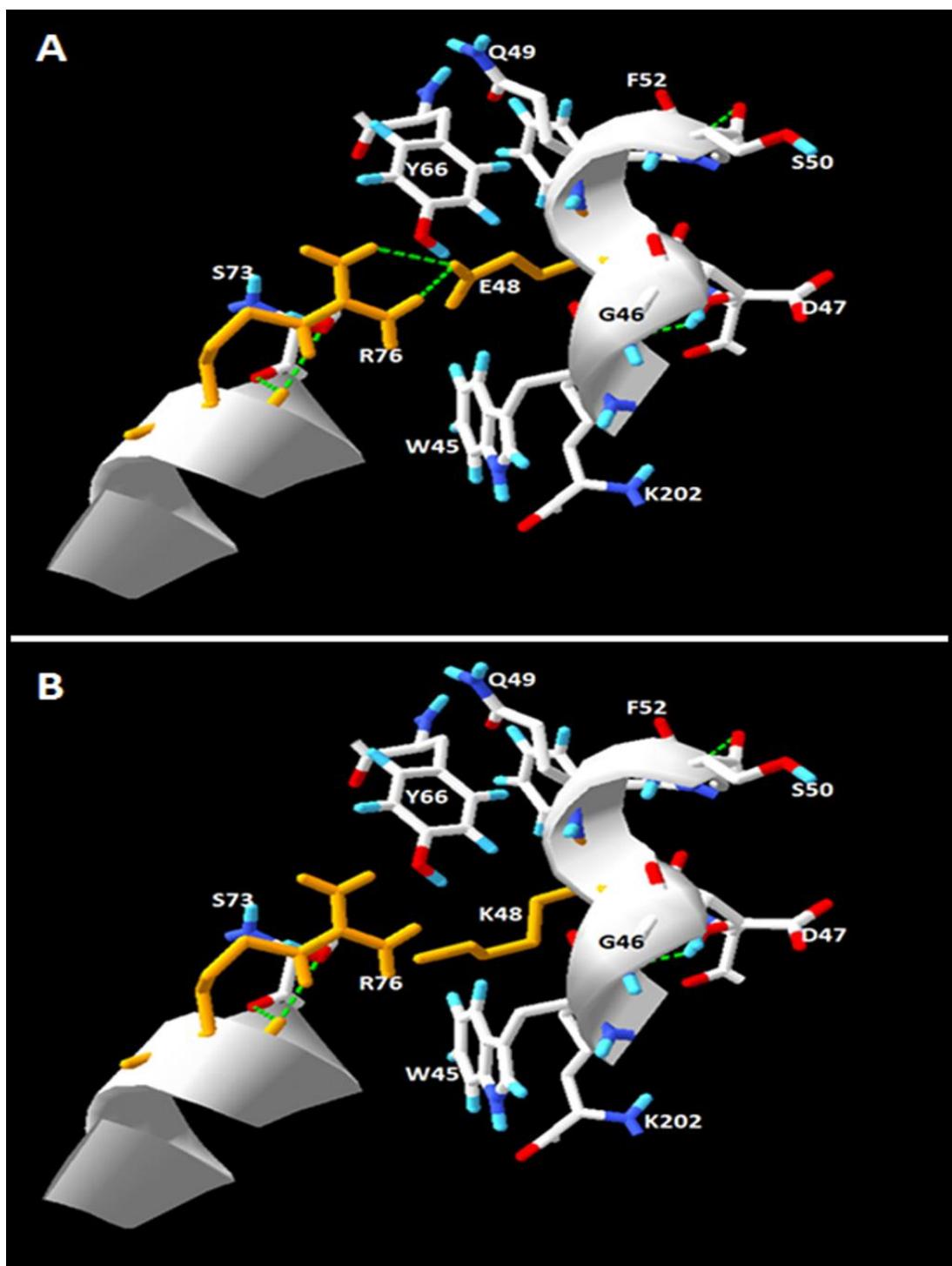
Supplemental Figure 8.



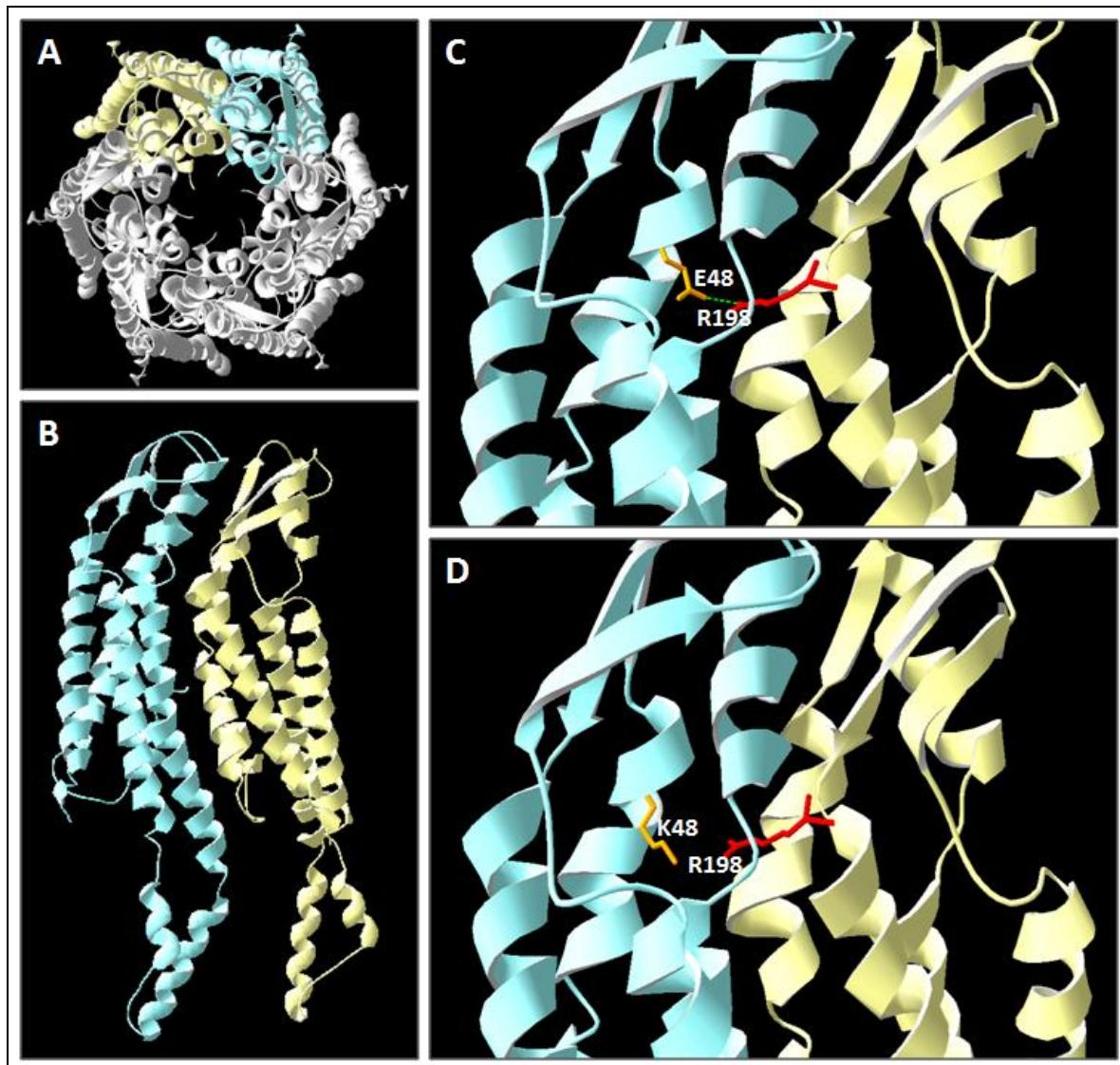
Supplemental Figure 9.



Supplemental Figure 10.



Supplemental Figure 11.



Supplemental Figure 12.

SUPPLEMENTARY FIGURE LEGENDS:

Supplemental Figure 1. The homology model of Cx50 color-coded according to evolutionary conservation.

Ribbon view of the Cx50 homology model is shown with the mutation-involving residues plotted on it. The structure is color-coded according to evolutionary conservation using the ConSurf server, with turquoise-through-maroon corresponding to variable-through-conserved positions (see color bar). PyMOL was used for visualization. All of the functionally characterized Cx50 mutations involve highly conserved residues. *Check supplementary text for multiple sequence alignment.*

Supplemental Figure 2. The 3-dimensional neighborhood (surrounding 4Å space) of R23 in normal Cx50 protein (homology model).

A. Residues lying within surrounding 4Å space of R23 are shown. R23 (TM1) has inter-helix H-bonding with side-chain of Y151 (TM3). B. The H-bond of R23 with Y151 is lost in the mutant R23T. Green and grey dotted lines indicate potential H-bonds (strong and weak, respectively) - computed using the SwissPDBViewer.

Supplemental Figure 3. The 3-dimensional neighborhood of R23 in the oligomeric complex.

A. Docked model of hemichannel – viewed from the top. Two adjacent Cx50 subunits are colored. B. Two adjacent Cx50 subunits are shown to be arranged in similar relation as in the hexameric complex – viewed from outer lipid-facing side. C. Mutation-involving residue R23 (red) in one subunit (yellow) and H98 (orange) of adjacent subunit (blue) lie within 4Å space.

Supplemental Figure 4. The 3-dimensional neighborhood of V44, W45, G46, D47 and E48 in the oligomeric complex.

A. Docked model of hemichannel – viewed from the top. Two adjacent Cx50 subunits are colored. B. Two adjacent Cx50 subunits are shown to be arranged in similar relation as in the hexameric complex – viewed from pore side. C. Mutation-involving residues 44-48 are shown (red) in one subunit (yellow); two residues (R76 & Q49 - orange) in adjacent subunit (blue) within surrounding 4Å space of the mutation-involving residues are shown. D. The intermolecular interface (involving residues 44-48 of one chain and R76 & Q49 of adjacent chain) is shown – viewed from the top. Side-chains of V44 & D47 point outwards, facing adjacent subunit; side-chains of W45 & E48 are directed towards the subunit. There is no inter-subunit H-bonding involving the residues.

Supplemental Figure 5. The 3-dimensional neighborhood (surrounding 4Å space) of R198 in normal Cx50 protein (homology model).

A. Residues lying within surrounding 4Å space of R198 are shown. B. R198Q leads to loss of 1 H-bond with D47 and gain of another H-bond with T200/E201. C. R198W leads to a loss of 1 H-bond with D47. Green lines indicate potential H-bonds - computed using the SwissPDBViewer.

Supplemental Figure 6. The 3-dimensional neighborhood of R198 in the oligomeric complex.

A. Docked model of hemichannel – viewed from the top. Two adjacent Cx50 subunits are colored. B. Two adjacent Cx50 subunits are shown to be arranged in similar relation as in the hexameric complex – viewed from outer lipid-facing side. C. Neighbouring residues in adjacent subunit (orange) that lie within surrounding 4Å space of R198 (red) are shown. R198 might form H-bond with E48 of adjacent subunit. Green dotted lines indicate potential H-bond - computed using the SwissPDBViewer. D. R198Q – the H-bond with E48 is

replaced with a new H-bond with S73; steric clash induced (pink dotted line). E. R198W – the H-bond with E48 is lost; steric clash induced.

Supplemental Figure 7. The 3-dimensional neighborhood of G46 in the oligomeric complex.

A. Docked model of hemichannel (constructed by SymmDock server) formed by symmetric docking of Cx50 subunits (Cx50 homology models). A single Cx50 subunit is colored light-green. Mutation-involving residues (red spheres) in the junction of TM1 and EC1 are localized in the docked model. B. Zoomed view of the residue-positions. G46 is a pore-exposed residue.

Supplemental Figure 8. The 3-dimensional neighborhood of V79 in the oligomeric complex.

A. Docked model of hemichannel – viewed from the top. Two adjacent Cx50 subunits are colored. B. Two adjacent Cx50 subunits are shown to be arranged in similar relation as in the hexameric complex – viewed from pore side. C. Mutation-involving residue V79 (red) in one subunit (yellow) and T39 (green) of adjacent subunit (grey) lie within 4 \AA space.

Supplemental Figure 9. The 3-dimensional neighborhood (surrounding 4 \AA space) of E201 in normal Cx50 protein (homology model).

A. Residues lying within surrounding 4 \AA space of E201 are shown. B. A new H-bond with side-chain of R198 is formed by the mutation E201K. Green dotted line indicates potential H-bond - computed using the SwissPDBViewer.

Supplemental Figure 10. The 3-dimensional neighborhood of E201 in the oligomeric complex.

A. Docked model of hemichannel – viewed from the top. Two adjacent Cx50 subunits are colored. B. Two adjacent Cx50 subunits are shown to be arranged in similar relation as in

the hexameric complex – viewed from outer lipid-facing side. C. P71, I72, S73 and R76 (orange) of adjacent subunit (blue) lie within surrounding 4Å space of E201 (red). E201 forms inter-molecular H-bonding with S73 of adjacent subunit.

Supplemental Figure 11. The 3-dimensional neighborhood (surrounding 4Å space) of E48 in normal Cx50 protein (homology model).

A. Residues lying within surrounding 4Å space of E48 are shown. E48 (TM1) has inter-helix H-bonding with R76 (TM2). B. The H-bond of E48 with R76 is lost in the mutant E48K. Green dotted lines indicate potential H-bonds H-bonds - computed using the SwissPDBViewer.

Supplemental Figure 12. The 3-dimensional neighborhood of E48 in the oligomeric complex.

A. Docked model of hemichannel – viewed from the top. Two adjacent Cx50 subunits are colored. B. Two adjacent Cx50 subunits are shown to be arranged in similar relation as in the hexameric complex – viewed from outer lipid-facing side. C. R198 (red) of adjacent subunit (yellow) lies within surrounding 4Å space of E48 (orange). E48 has inter-molecular H-bonding with R198 of adjacent subunit. Green dotted line indicates potential H-bond - computed using the SwissPDBViewer. D. The H-bond of E48 with R198 is lost in the mutant E48K.

SUPPLEMENTARY TEXT:

CLUSTAL 2.1 multiple sequence alignment constructed by CLUSTALW2 server – 52% sequence similarity between human Cx50 protein sequence and human Cx26 protein sequence, which was used as template (*PDB: 2ZW3*) for homology modeling

2ZW3	-MDWGTIQTILGGVNKHSTSIGKIWLTVLFIFRIMILVVAKEVGDEQADFVCNTLQPG	59
Cx50	MGDWSFLGNILEEVNEHSTVIGRVWLTVLFIFRILILGTAAEFVGDEQDFVCNTQQPG	60
	*** . * . ** * : *** * : * : * : * : * : * : * : * : * : * : * : * :	
2ZW3	CKNVCYDHYPISHIRLWALQLIFVSTPALLVAMH-VAYRRHEKKRKFIKGEIKSEFK--	116
Cx50	CENV CYDEAFPISHIRLWVLQIIFVSTPSLMYVGHAVHYVRMEEKRKSREAEELGQQAGT	120
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
2ZW3	----DIEEIK-----TQKVRIEGSLWWTYTSSIFFRVIFEAAFMYVFYVMDGFSMQR	165
Cx50	NGGPDQGSVKSSGSKGKFRLEGTLRLTYICHIIFKTLFEVGFIVGHYFLYGRILP	179
	* . : * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
2ZW3	LVKCNAWPCPNTVDCFVSRPTEKT VFTVFMIAVSGICILLNVTELCYLLIRYCSGKSKKP	225
Cx50	LYRCSRWP CPNVVDCFVSRPTEKTIFLFMLSVASVSLFLNVMELGHLGLKGIRSALKRP	239
	* : * . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
2ZW3	V 226	
Cx50	V 240	
	*	

CLUSTAL 2.0.10 multiple sequence alignment constructed by UNIREF90 – 150 non-redundant sequences homologous to human Cx50 were selected by PSI-BLAST and aligned

Input_protein_seq denotes human Cx50 protein sequence (NP_005258.2)

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UniRef90_G1U3S9_1_407	G-QAL-----PTTEQNWAFTP-----VA
UniRef90UPI00032AEFC1_1_413	P-PAG-----DSTEVALVAEPEKGRL-----LPTS
UniRef90UPI00034F30A8_1_402	PGAPL-----PTADFRVQAQG-----EAHG
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UniRef90_F7G5V2_1_446	PGAPP-----QPTEFRMVTLP-----EERS
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-----E-----
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-----ADQGAESE-----ERAQV-----
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-----EKVEAGEEEQ-----EP-----
-----EKVKAAEEEEEE-----QEEEQQAP-----
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--KPEEH--NGASPGH
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--KPDVP--HGVSSGH
--KPDVP--SGVSSGH
--GPEMA--NGVPNAH
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--NPEVR--NNASP-
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--SEMTP--STPSTS
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-----ATEGQEVPVAEGERKVTAGVQESEKDELLAEKASKQGL
-----APEGQETVAVPDRERVETPGVGKEDKEELQAEVTKQGL
-----TLEVQEAIPEGERVETPGVGKEGEKEELQIEVKVTQGL
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-----NGNG-----HSLGESELLAVTPDQ-GVPAVTSPV
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-----GAAG-----SSGDS-----D-GEGAVT-AV
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-----DRSG-----SSLEESALAVTPEEEE-QALTTA
-----VEGSG-----SSLEDSALAVTPEEEEQAVTTAA
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-----GRAG-----NRVLLVNGNGRSSADTPDAGEFAINTNI
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-----LVNGGS-----LGGGSGGEAEAG-EEWPGTRV
-----NGGS-----LGGGSGGEAEAG-EEWPGTRV
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-----WGGGTN-----EQEGRRV-----STRV
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RLPHC-----YQNDKPRLSK----ASSKARSDDLGV
SLPHG-----YQSDKRRLSK----ASSKARSDDLGV
RLPHG-----YHSDKRRLSK----ASSKARSDDLGV
RLPHG-----YHSDKRRLSK----ASSKARSDDLGV
RLPHG-----YHSDKRRLSK----ASSKARSDDLGV
RLPQG-----YPSDKRRLSK----ASSKARSDDLGV
RLPQA-----YHGDKRRPSK----ASSKARSDDLGV
RPPQG-----FGGDKRRLSK----ASSKARSDDLGV
RLPHS-----YQSDKRRLSK----ASSKARSDDLGV
PNPHG-----YQSDKRRLSK----ASSKARSDDLGV
HPTHG-----YHSDKRRLSK----ASSKARSDDLGV
RLPPG-----YHGDKRRLSK----ASSKARSDDLGV
RLPQG-----YHSDKRRLSK----ASSKARSDDLGV
RLPQG-----YHSDKRRLSK----ASSKARSDDLGV
CLPHG-----CHSEKRRLSK----TSSKARSDDLGV
RLPHG-----YYSEKRRLSK----TSSRARSDDLGV
RLPHS-----YHTDKRRLSK----ASSKARSDDLGV
AFPEN-----YFNEKRRLSKAS--RASSKARSDDLGV
ALPES-----YFNEKRRFSKAS--RASSKARSDDLGV
LLSNG-----YLPDKRRRLSKTS--HASSKARSDDLGV
QVSNG-----YFNEKRRRLSKTS--HASSKARSDDLGV
SHPG-----FFRDKRRRLSKTS--TSSNRLRPSDLAV
CSPDGL-----LVKDKRRFSKASG--TSS-RTRADDLSV
PAEKTPSLCPEL-----TTDDARPLSRLS--KASSRARSDDLTV
PAEKTPSLCPEL-----TTDDARPLSRLS--KASSRARSDDLTV
PAEKAPSLCPEL-----TADDARPLSRLS--KASSRARSDDLTI
SAEKAPSLCPEL-----TTDDNRPLSRLS--KASSRARSDDLTI
SAEKAPSLCPEL-----TADDTRPLSRLS--KASSRARSDDLTI
PVEKSPSLCPDP-----SRDDTRPLSRLS--KTSSRARSDDLTV
PAEKAPSLCPEL-----TGDDTRPLSRLS--KASSRARSDDLTV
PAEKAPSLCPEL-----SGDDTRPLSRLS--KASSRARSDDLTI
TPEKAPSLCAEL----PGEDTRPLSRLS--KASSRARSDDLTV
PAEKAPALCAES----TSDDTRPLSRLS--KTSSRARSDDLTV
PAEKAPSLCAEV----TTDETRPLSRLS--KASSRARSDDLTV
SAEKAPSLCPEL-----TAEEARPLSRLS--KASSRARSDDLTI
PVEKAP-LCPEL-----AADDSPRLSRLS--KASSRARSDDLTV
PVEKIP-LCPEL-----AADDTRPLSRLS--KASSRARSDDLTV
-----LLCAEL-----AADDPRALSRLS--KASSRARSDDLTV
-----AET--DTRPPSRLS--KTSSRARSDDLTV
-----VELTADTRPPSRLS--KASSRART----
-----AELATDVRSLSRLS--KASSRARSDDLTV
-----DTIDDSRPLSRLS--KASSRARSDDLTI
-----ETIEDNRPLSRLS--KASSRARSDDLTV
-----DTIEDTRPLSRLS--KASSRARSDSL--
-----DTIEDTRPLSRLS--KASSRARSDDLNV
-----ETIEDTRPLSSLS--KASSRARSDDLTV
PSNSATPERVPSRAAS--EHAPYSRSLSRLS--KASSRARSDDLTV
PSNSATPERVPSRAAS--EHAPLDAPSRAASEHAPSDRAPS
ALEPDIVEYEPLGPMPMLNLEFIPDSRSLSRLS--KASSRARSDDLTV
-----PLSRLS--KASSRARS----
-----MEDEQEPLGGNV--ALPSTADTRP--
-----EVDSLKTPTVLPPEVLEEHSEGESVEE--
PAEKAPSPGAEL----AGDDTRPLSRLS--KASSRARSDDLTV
EMHAPPPLPTD-----PGRSSKASKSSG----SRARPDDLAI
EMHAPPPLPTD-----PGRSSKASKSSG----SRARPDLAI
AMHAPPPLPTD-----PGRSSKASKSSA----GHARPDDLAV
EMHVPPPLPAD--PGRSSKASKSSG----GRARPNDLAI
EMHMPPPLPTD-----PRRSSKASKASN----SQARPNDLAI
EMHMPPPLPTD-----PRRSSKASKASN----SQARPNDLAI
DMHAPPPLLPAE----PRRSSTASKASG----GRARPSDLAI
ELHAPPPEPAD--PGRSSKASKSSG----GRARASDLAI
ELHAPPPEPAD--PGRSSKASKSSG----GRARAGDLAI
ETHAPPPLPED--AGRLSKASRASG----GRARPNDLPI
QMHQPPPLPLGD--PGRASKASRASS----GRARPEDLAI
QMHQPPPLLLGD--PGRASKASRASS----GRARPEDLAI

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ELHTPPLLVE-----PGRASKASKASS---GRARPGDLTI
EMHSPLVLLD-----PGRSSKS---SN----GRARPGDLAI
EMHSPLVLLD-----PGRSSKA---SS----GRARPGDLAI
EMHNPLLLD-----PGRSSKA---SN----RRAKPGDLAI
EMHEPPLLTD-----PGRSSKA---SS----GRARPGDLAI
EMHVQPSEPADPAPDP---VDPGRSSKA---SS----GRARPDDLAI
EMHAPPLEAD-----AGRCSKAS---G----GRARPSDLAI
EMHAPPSLSAD-----AGRCSKAS---SS----GRARPSDLAI
QVEAPALLGD-----TGRSSKAS---S----GRAKPRDLAI
-VEAPALLPED-----PRRSSKAS---S----GRAKPGDLAI
DMHVPPLLGD-----VGRASKASKSSS----GWARADDLAI
DMHVPPLLGD-----VGRASKASKSSS----GWARADDLAI
DTHMPPPLLGD-----VGQASKASKLSS----GRPRADDLAI
DMHRPPLLGD-----PGRASKTSRASSS----GRLRADDLAI
ELHRPPLLRLD-----AGQSGKAS---SN----RARPNDLAI
EMHAPPPLLVD-----SRLLSKASKASS---GRARSDDLAI
EMHAPPLLID-----TRRLSKASKSSS----GRARSDDLAV
EIHESPLLLD-----LRLLSKASKASS---CRARSDDLAI
EIHESPLLLD-----LRLLSKASKASS---CRARSDDLAI
EMHEPPVMVSTD-----PRRLSRASKSSS----IRARPSDLAV
EMHEPPVMVSTD-----PRRLSRASKSSS----IRARPSDLAV
EMHEPPVTVRTD-----PRRLSRSTS----IRTRPSDLAV
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EMHEPPATAGVD-----PRRLSRASKSSS----VRARPSDLAV
EMHEPPATAGVD-----PRRLSRASKSSS----VRARPSDLAV
EMHDPPPAPGVD-----PRRLSRASKSSS----VRARPSDLAV
EMHEPP-VIFD-----ARRLSRASKASS---VRARPNDLAV
EMHWPPASATT-----DIRKPSRASKCS---VRARPDDLAV
HSMFPTLPRHTPL-----YALTTPPSRASKCS---VRARPDDLAV
EMHWPPASAAT-----DIRKPSRASKSSS----VRARPDDLAV
EMHQPPAAAGT-----DNRKPSRASKSGG---GRARPDDLAV
DATAEMHPPA-----DTRRPSRACKYSG---ARARPDDLEV
EMHEPPLLID-----
EMHEPPQLID-----
EMHEPPLLVDTR-----RLSRASKSSS----CRARSDDLAV
EMHEPPVPTDTR-----RL-----
EMHQPP-----
EGSADSQRNNS-----QSSKHS-----
EMSIENQRNNS-----QSSKHS-----
EAPAESQRNNS-----QSSKHS-----
SSPSDNQRSSS-----RSTKHGNKAKP---ED-----
EKQRLPSRNSR-----HSSNRSRPG-----
GSP--TQPRPG-----YSTKRS-----
QLHDPPLHSGGEH-----DIDLRLLSRASS----RVRSDQLQV-----
ECGSSEYPTLPAVDTSSCPALSGIVRKSRVSP-----
ERARSEYPTLPVSDASSSTVLSGITRKTRR-----
GCTSSEYPTLPV--ASSCATMSGAARKSRRVSP-----
-LVTSEYPTLPVADATSCPTLSGITRKSR-----
VRERRENFGDKDSERNHYSRG-----VTVDLENHMRQSPPKVFSLPANC
VKERRENFGDKDSERNHYSRG-----VSVDLENHVSQSPQKVFSLPANC
LMEKRETEGDKDSKRNYYSRGHSIPGVIAIDGENNMQRQS PQT-----
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KQENTTTGHQANFEAAVHLGYR--PEMPLGAALYPSLQPEMTFSPPTDCT
PGTTTTS--RHCPAYAVGTWKQSQDLEPLGEPLTDLHSDSSVRESGGWV
P-DTAS--RHCPTYAAGTWKQSQDLEPSGEPATDLHS--YCRDSDSSV
LGTTTAP--RHCPSYTIGTWEQPOQHPRSSGEPLTDFHS--HCRDSDGSV
LGSTTAP--RHCPSYAI-----
LGTTTAP--RNCPSFAVGTWEQSQDPEPSGEPLTDLHS--HCRDSEGS
LGKMTAS--RHCPSYAIGTLEKSQDLELSGEPLTDLHS--HC-----
LGATVAS--QHCPSYAIGTWEQSQDLESSGEPLTDMRS--HFKGSDGSV
LGETTAP--RHCPSYAIGTWEQSYDLQPSEEPLTDFHN--HCRDSNGSM
LGTKMAP--RHCPSHTTGWPWEQSQDLPQSGEPLADLHS--HCRHSDGS
LGTTMAP--RHCPSRTTGWPWEQSQDLPQSGEPLTDLHS--HCRHSDGS
LGTTMAS--RHCLSHTTGPWERPQDLKPSGEPLTDLHS--HCRDSNGSR
LGTEIAP--RHCPSYAIGTWEQSQDRRPSGEALTDLNS--KCRDSDGSV
LERTTAP--EHCPSYVPGTWEPLPQDLKPPGEPLTDLHS--HCKDSDGSV
LGTMAS--PHCPPSTLGTWERSHGQDASGKPLSDLQS--H-----

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WKPKWPRATRGPSAESENQALPP--

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RTARSLSDPWNGSTGGIQLQSRGSPPRGNLRRQSRGSTGRPRALSRAD--
DKSR----PGSRKASFLSR--
R--

RESGVWIDRSRPGSRKASFLSR--

R--
RESGVWTDRSCSGSRKASFLSR--
TDSR--
RESRAQTD--
RESRVQTD--

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RKHSR-----

SSPSDSTD-----
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