Study of the Individual Cytochrome b_5 and Cytochrome b_5 Reductase Domains of Ncb5or Reveals a Unique Heme Pocket and a Possible Role of the CS Domain

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Running Head: Unique heme pocket and possible role of CS in human Ncb5or

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SUPPELMENTAL DATA

FIGURE LEGENDS

Supplemental Figure 1. Sequence alignment of Cyb5A from vertebrates. Residue numbers and helices of human Cyb5A corresponding to those in Figure 1B are labeled. Conserved amino acid residues in each group are marked by "*" (identical, heme-ligands in red) or "-" (chemically similar). All are annotated from genomic sequences except those from human, mouse, rat, bovine, horse, pig, rabbit, Xenopus and Zebrafish are confirmed by transcript or protein sequences. GenBank accession numbers are: Human (NP_683725), Chimpanzee (XP_001135885), Monkey (XP_001083378), Orangutan (ENSPPYP00000010369), Guinea pig (ENSCPOP00000010893), Mouse (NP_080073), Rat (NP_071581), Bovine (NP_776458), Horse (NP_001153204), Pig (NP_001001770), Goat (ABQ12619), Dog (XP_533373), Rabbit (P00169), Opossum (XP_001373630), Zebrafinch (XP_002195467), Chicken (NP_001001748), Xenopus laevis (NP_001086707), Xenopus tropicalis (NP_001015979), Rice fish (ENSORLT00000004278), Zebrafish (NP_998300), salmon (ACI66502), pufferfish (ENSTNIP00000008519), Fugu (ENSTRUP00000013474), Stickleback (ENSGACP00000002650), C. Armatus (CAE75863), Rainbow smelt (ACO09548). A 92% identity or a 96% similarity is found for human and rat Cyb5A sequences.

Supplemental Figure 2. Sequence alignment of Ncb5or-b_5 core from animals. Residue numbers and helices of human Ncb5or-b_5 corresponding to those in Figure 1B are labeled. Conserved amino acid residues in each group are marked by "*" (identical, heme-ligands in red) or "-" (chemically similar). All sequences are annotated from genomic sequences except sequences of human, mouse and rat have been confirmed by cloned cDNAs. Ncb5or is also named Cyb5R3, b5/b5R, and b5+b5R. GenBank accession numbers are: Human (NM_016230), Chimpanzee (XM_518614), Monkey (XM_001085835), Guinea pig

(EB369672), Mouse (NM_024195), Rat (NM_133427), Bovine (NM_001038159), Horse (XM_001499913), Sheep (DY519717), Dog (XM_532219), Squirrel (CO734025), Platypus (XM_001512881), Opossum (XM_001375853), Zebrafinch (XM_002188637), Chicken (XM_001233870), Xenopus laevis (NM_001016756), Little skate (CV222314), Rice fish (AM140533), Zebrafish (NM_001020660), Salmon (DY707237), Stickleback (DN714575), Pimephales (DT192112), Pea aphid (XM_001948299), Beetle (XM_963135), Honeybee (XM_394412), Jewel Wasp (XM_001601866), Drosophila (NM_137575), Louse (XM_002428283), Deer tick (XM_002401084), Mosquito (XM_001650886), Tunicate (XR_053035), Lancelet (XM_002603916), C.elegans (NM_001026613), Teladorsagia (CB038604), Trichuris (BM277379), Sea urchin (CD307914), Coral (EZ038676), Sea anemone (DV081463), Trichoplax (XM_002112883), Hydra (XM_002165807). A 93% identity or a 100% similarity is found for human and rat Ncb5or-b5 sequences.

Supplemental Figure 3. 2Fo-Fc electron density map contoured at 1σ showing the two orientations of the heme molecules in Ncb5or-b_5 associated with subunit A (left) and subunit B (right).

Supplemental Figure 4. Thermostability of wild-type Ncb5or-b_5 and its two mutants, b_5W114A and b_5R113A. The percent of heme loss (Y axis) was monitored by the decrease in A413 and plotted as a function of temperature. Thermal denaturation experiments were performed on a Varian Carey 100 Bio UV/Visible spectrophotometer equipped with a Peltier-thermostated multiple cell holder and a dedicated temperature probe accessory (± 0.1 °C). Solutions were buffered to pH 7.0 using 50 mM potassium phosphate. Experiments were performed in quartz cuvettes of 1 cm path length and 1 mL sample volume, equipped with tight-fitting PTFE lids. The temperature was increased in increments of 2 °C, and samples were equilibrated for 5 min after reaching each desired temperature. Thermal denaturation midpoints (T_m values) were obtained by fitting plots of absorbance at the Soret band λ_{max} (412 nm) vs. temperature to a previously described equation describing a two-state equilibrium (1). Tm (mid-point temperature) = 72.0 (Ncb5or-b_5), 73.4 (Ncb5or-b_5R113A), 71.5 (Ncb5or-b_5W114A), 73.5 (human Cyb5A).

Supplemental Figure 5. Circular dichroism (CD) spectra of human Ncb5or-b_5. (A) Far UV (190 – 250 nm) and (B) visible (350 – 500 nm) spectra of wild-type Ncb5or-b_5 and its two mutants, b_5W114A and b_5R113A, monitored at a concentration of 17.4, 14.5, and 15.3 μM heme, respectively. CD spectra were recorded on a JASCO-815 spectropolarimeter equipped with a Peltier thermostated cell holder.(2) Solutions were buffered to pH 7.0 using 10 mM sodium phosphate. All spectra were obtained at 0.1 nm with a response time of 4 sec and a scan rate of 50 nm/min at room temperature. Final spectra represent the average of at least five scans. Background correction was accomplished by subtraction of a spectrum recorded at the same temperature and containing only buffer. Far-UV spectra are reported in terms of mean residue ellipticity ([θ], in deg·cm^2·dmol^{-1}), calculated as $[\theta] = [\theta]_{obs}[MRW/(10lc)]$ where $[\theta]_{obs}$ is the ellipticity measured in millidegrees, MRW is the polypeptide mean residue molecular weight (molecular weight divided by the number of amino acids), c = sample concentration in mg/mL, and l = optical path length of the cell in cm. Near-UV and visible region spectra are reported in terms of molar ellipticity ([θ], in deg·cm^2·dmol^{-1}), calculated as $[\theta]= [\theta]_{obs}[MW/(10lc)]$ where MW is the molecular weight of the polypeptide.

Supplemental Table 1. Crystallographic data for human Ncb5or-b_5 refined to 1.25Å resolution.

Data Collection	
Unit-cell parameters (Å, °)	a = 38.48, b = 56.37, c = 42.56, β = 100.67
Space group	$P2_1$
Resolution (Å)[1]	30.0-1.25 (1.28-1.25)
Wavelength (Å)	1.0000
Temperature (K)	100
Observed reflections	185,062
Unique reflections	49,981
$\langle I/\sigma(I) \rangle$[1]	13.0 (1.9)
Completeness (%)[1]	99.0 (93.6)
Redundancy[1]	3.8 (3.1)
R_{sym} (%)[1, 2]	5.8 (72.4)
Refinement	
Resolution (Å)	30.0-1.25
Reflections (working/test)	46,492 / 2,489
R_{factor} / R_{free} (%)[3]	14.7 / 17.3
No. of atoms (protein (A:B) / Heme / sulfate /water)	742:743 / 172 / 10 / 145
Model Quality	
R.m.s deviations	
Bond lengths (Å)	0.013
Bond angles (°)	1.477
Average B factor (Å^2)	
All Atoms	15.9
Protein (chain A/B)	15.2 / 15.3
Heme	11.0
Sulfate	27.7
Water	28.0
Coordinate error based on R_{free} (Å)	0.043
Ramachandran Plot (chain A/B)	
Most favored (%)	97.1
Additionally allowed (%)	2.9

1) Values in parenthesis are for the highest resolution shell.

2) $R_{sym} = \Sigma_{hkl}\Sigma_i |I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the intensity measured for the ith reflection and $\langle I(hkl)\rangle$ is the average intensity of all reflections with indices hkl.

3) $R_{factor} = \Sigma_{hkl} ||F_{obs}(hkl)| - |F_{calc}(hkl)|| / \Sigma_{hkl} |F_{obs}(hkl)|$; Rfree is calculated in an identical manner using 5% of randomly selected reflections that were not included in the refinement.

Supplemental Table 2. Summary of interplanar dihedral angles and EPR signals of Ncb5or and other members of the cytochrome b_5 superfamily. Interplanar dihedral angles of bis-histidine ligands were calculated with Mercury (http://www.ccdc.cam.ac.uk/products/mercury/). N/A: not available.

Protein Name (source)	PDB #	g_{max} or g_z	Interplanar dihedral angles	Reference
Ncb5or (human)	3LF5	3.56	83.2° / 81.3° (His^{89},His^{112})	This study
Ncb5or (rat)	N/A	3.54	N/A	(3)
Cyb5A (human)	N/A	3.09	N/A	This study
Cyb5A (bovine)	1CYO	3.1	21.2° (His^{44},His^{68})	(4,5)
Cyb5A (rat)	N/A	3.05	N/A	(3)
Cyb5B (rat)	1B5M	3.03	11.9° (His^{42},His^{66})	(6,7)
Cytochrome b_5 (M. domestica)	2IBJ	3.07	23.8° (His^{39},His^{63})	(2,8)
Cytochrome b_5 (A. suum)	1X3X	N/A	46.1° (His^{38},His^{62})	(9)
Cytochrome b558 (E. vacuolata)	1CXY	N/A	43.9° (His^{42},His^{70})	(10)
Sulfite oxidase (chicken)	1SOX	2.93	9.6° (His^{40},His^{65})	(11,12)
Cytochrome b_2 (yeast)	1LTD	2.99	42.3° (His^{43},His^{66})	(13,14)
Nitrate reductase (spinach)	N/A	2.98	N/A	(15)

Supplemental Table 3. Kinetic properties of Cyb5A mutants tested for electrostatic interaction with Cyb5R3. Heme ligands are His^{44} and His^{68} in Cyb5A, corresponding to His^{89} and His^{112} in Ncb5or, respectively. [a] Both k_{cat} and K_M values are the ratio against that of wild-type Cyb5A. [b] N116 is displaced relative to D71 given the difference in local secondary structure, but it is the closest residue spatially and is pointed generally in the same direction.

Cyb5A	k_{cat} [a]	K_M [a]	Helix	Residue in Ncb5or	Ref.
E42A	1.0	1.2	α2	E87	(16)
E43A	1.0	3.1	α2	Y88	(16)
E48A	1.3	1.7	α3	E93	(17)
E49A	0.8	0.6	α3	D94	(17)
E48A/E49A	1.3	1.1	α3	-	(17)
E53A	1.2	1.2	α3	R98	(17)
E61A	1.3	1.4	α4	E106	(17)
E64A	1.0	1.0	α4	D109	(16)
D65A	1.0	1.5	α4	Q110	(17)
D71A	0.7	6.4	α5	N116 [b]	(16)
E74A	1.1	1.8	α5	S119	(16)
E48/E49/E53/E61/D65→A	0.9	1.4	α3,4	-	(17)
E42/E43/E48/E49/E53/D58/E61/E64/D65→A	0.9	6.1	α2,3,4	-	(17)

Supplemental Figure 1.

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                                        __α1__                  __α2__    __α3___     __α4__    ____α5___       __α6___
Human                         6 DEAVKYYTLEEIQKHNHSKSTWLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDAREMSKTFIIGELHPDDRPKL 92
Chimpanzee                      DEAVKYYTLEEILKHNHSKSTWLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDAREMSKTFIIGELHPDDRPKL
Monkey                          DEAVKYYTLEEIQKHNHSKSTWLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDAREMSKTYIIGELHPDDRPKL
Orangutan                       DEAVKYYTLEEIQKHNHNKSTWLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDAREMSKTFIIGELHPDDRPKL
Guinea pig                      EQAVKYYTLEEIEKHKDSKSTWVILHHKVYDLTRFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTFIIGEVHPDDRPKL
Mouse                           DKDVKYYTLEEIQKHKDSKSTWVILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTYIIGELHPDDRSKI
Rat                             DKDVKYYTLEEIQKHKDSKSTWVILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTYIIGELHPDDRSKI
Bovine                          SKAVKYYTLEEIQKHNNSKSTWLILHYKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTFIIGELHPDDRSKI
Horse                           DKAVKYYTLEEIKKHNHSKSTWLILHHKVYDLTKFLDEHPGGEEVLREQAGGDATENFEDIGHSTDARELSKTFIIGELHPDDRSKI
Pig                             DKAVKYYTLEEIQKHNNSKSTWLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTFIIGELHPDDRSKI
Goat                            SKPVKYYTLEEIQKHNHSKSTWLILHYKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTFIIGELHPDDRSKI
Dog                             DQTVKYYTLEEIQKHNHSKSTWLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTFIIGELHPDDRAKI
Rabbit                          DKDVKYYTLEEIKKHNHSKSTWLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTFIIGELHPDDRSKL
Opossum                         GEKVKYYTLEEIQKHNHSKSTWLILHHQVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARELSKTYIIGELHPDDRDKI
Conserved in mammals (14)          ********* **   ****-***  *****-**-***********************-********-***-****-***** *-
Zebrafinch                      PWRGRYYRLEEVQKHNNSQSTWIIIHNRIYDVTKFLDEHPGGEEVLREQAGGDATENFEDVGHSTDARTLSESFIVGELHPDDRSKL
Chicken                         AWRGRYYRLEEVQKHNNSQSTWIIVHHRIYDITKFLDEHPGGEEVLREQAGGDATENFEDVGHSTDARALSETFIIGELHPDDRPKL
Xenopus laevis                  QKEVKYYTLEEIKQHNHSKSTWILLHNKVYDVTKFLEEHPGGEEVLREQAGGDATETFEDIGHSTDARNMSKEFVIGELHPDDLSKI
Xenopus tropicalis              QKEVKYYTLEEIKQHNHSKSTWILIHNKVYDVTKFLEEHPGGEEVLREQAGGDATETFEDIGHSTDARNMSKEFIIGELHPDDRSKI
Rice fish                       PEGVKYYRLSEIEAQNSFKSTWIIIHHKVYDVTKFLDEHPGGEEVLREQAGGDATESFEDVGHSTDAREMASGMVIGELHPDDRHKI
Zebrafish                       GNGVKYYRLSEVEERNSFKSTWIIIHNKVYDVTKFLEEHPGGEEVLREQAGGDATESFEDVGHSTDAREMASSMLIGEVHPDDRDKI
Salmon                          GQAVKYYRLSEIEEQNTFKSTWIIINFNVYDVTKFLEEHPGGEEVLREQAGGDATESFEDVGHSSDAKEMAANMIIGELHPDDRPKM
pufferfish                      SDGVKYYRLSEIEEHKTVKSTWIIINFKVYDVTKFLEEHPGGEEVLREQAGGDGTESFEDVGHSTDAREMAAEMMIGELHPEDRHKI
Fugu                            PSGVKYFRLSEIEEQKSIKSTWIIINYKVYDVTKFLEEHPGGEEVLREHAGGDATESFEDVGHSTDAREMAGGLLMGELHPDDRHKI
Stickleback                     PAGVKYFRLSEIEQQNTFKSTWIIIHHKVYDVTKFLEEHPGGEEVLREQAGGDATESFEDVGHSSDAREMSVAMVIGELHPDDRHKI
C. Armatus                      KDAVKYYRLSEVEKQNTFKSTWIIINNKVYDVTQFLEEHPGGEEVLREQAGGDATESFEDVGHSRDAREMAAEMLIGEVHPEDRPKL
Rainbow smelt                   GHPVKYYRLSEIEERNTFKCTWIIIHHNIYDVTKFLEEHPGGEEVLREQGGGDATESFEDVGHSSDAREMAASMVIGELHPDDRPKI
Conserved in vertebrates (26)       -*- * *-        **---   -**-* **-*********** -***-** ***-*** **- -    --**-**-*  *-
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Supplemental Figure 2.

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                                       __α1__                  __α2__   __α3__      __α4__        __α5__
Human                       51 KGRLIEVTEEELKKHNKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTELFDQVHRWVNYESMLKECLVGRMAIKPAVLK 137
Chimpanzee                     KGRLIEVTEEELKKHNKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTELFDQVHRWVNYESMLKECLVGRMAIKPAVLK
Monkey                         KGRLIEVTEEELKKHNKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTELFDQVHRWVNYESMLKECLVGRMAIKPAVLK
Guinea pig                     KGRLIEVTEEELKKHNKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTDLFNQVHRWVNYESMLKECLVGRMAVKPAVPK
Mouse                          KGGLIEVTEEELKKHNKKEDCWICIRGFVYNVSPYMEYHPGGEDELMRAARADGTDLFNEVHRWVNYESMLKECLVGRMAVKPAVPK
Rat                            KGGLIEVTEEELKKHNKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGADGTDLFNEVHRWVNYESMLKECLVGRMAVKPAVPK
Bovine                         KGRLIEVTEEELKKHNKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTDLFDQVHRWVNYESMLKECLVGRMAMKPALPK
Horse                          KGRLIEVTEEELKKHDKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTDLFDQVHRWVNYESMLKECLVGRMAVKPAAPK
Sheep                          KGRLIEVTEEELKKHNKKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTDLFDQVHRWVNYESMLKECLVGRMAMKPALPK
Dog                            KGRLIEVTEEELKRHNTKDDCWICIRGFVYNVSPYMEYHPGGEDELMRAAGSDGTDLFDQVHRWVNYESMLKECLVGRMAIKPTIPK
Squirrel                       KGRLIDVTEEELKKHNKKEDCWICIRGFVYNVSSYMEFHPGGEDELMRAAGSDGTDLFDQVHRWVNYESMLKECLVGRMAVKPAIPK
Platypus                       RGRLIEVTEEELAKHNKKDDCWVCIRGLVYNVSPYMEYHPGGEDELMRAAGTDGTDLFDQVHRWVNYESMLKECLIGRMAAKPVAAA
Opossum                        KGRLIEVTEEELIRHNKKDDCWVCIRGLVYNVSPYMEYHPGGEDELMRAAGSDGTDLFDQVHRWVNYESMLKECLVGRMAVKPVAVA
Conserved in mammals (13)      -* **-****** -* **-***-****-***** ***-************* ***-**  ***************-****-** -
Zebrafinch                     KGRLIEVTEDELSKHNRKEDCWICIRGFVYNVTPYMEYHPGGEDELMKAAGTDGTDLFDQVHRWVNYESMLKECLVGRMAVKPVAIS
Chicken                        KGRLIEVTEDELAKHNKKEDCWICIRGLVYNVTPYMEYHPGGEDELMKAAGADGTDLFDQVHRWVNYESMLKECLVGRMAFKPIAAP
Xenopus laevis                 KGRLIDVTEEELAQHNKKEDCWICIRGMVYNITPYMEYHPGGEEELMKAAGRDGTDLFDQVHRWVNYESMLKECLIGRMAIKHVSIS
Little skate                   KGKLLRVTPEELAKHKEKTDCWICIRGMVYNVTPYLEYHPGGEDELMRGAGADATELFDQIHSWVNYDSILKECLVGRMTSKPFPVA
Rice fish                      RGRLIDVTPEELEKHNRRDDCWTCIRGMVYNVTPYIDYHPGGEDELMKAAGIDGTDLFDQVHRWVNYESMLKECLVGRMALKTPAAV
Zebrafish                      RGRLIEVSQEELRKHNNRDDCWTCIRGLVYNLSAYMDFHPGGEEELMRAAGIDSTDLFDEVHRWVNYESMLKECLVGRMAVKPSPAL
Salmon                         RGRLIDVTEEELQKHNTRHDCWTCIRGMVYNVSPYMDFHPGGEEELMKAAGIDGTDLFDQVHRWVNYESMLKECLVGRMAVKASTA
Stickleback                    RGRLIEVTQEELQRHNTRNDCWTCIRGMVYNVTPYMDYHPGGEEELMKAAGIDGTDLFDQVHRWVNYESMLKECLVGRMATAVIKAA
Pimephales                     RGKLIEVTEDELKKHNTRNDCWTCIRGMVYNVSAYMDFHPGGEAELMRAAGIDGTDLFDQVHRWVNYESMLKECLVGRMAVKLASAP
Conserved in vertebrates (22)  -* **-*- -**  *  - *** **** ***-- *---***** ***---* *-****  -* ************-***
Pea aphid                      GGKMLSISKSELAKHNKRTDAWLAIRGTVYNVTQYMDFHPGGVDELVRGIGTDATKLFSEIHAWVNYESILQKCVVGRLVNEELFKL
Beetle                         QAGNLSVTPSELALHNKETDAWLCIRGRVYNVTAYLPFHPGGPEQLMKGAGKDATRLFEEVHPWVNFDQILTKCYVGKLKKNAPPPP
Honeybee                       GGVPRIVTLSELASHNKQNDAWIAIRGIVFNVTRYMDFHPGGISELMRGVGKDATKLFESVHAWVNYQSILQKCVVGRLSRGSITGS
Jewel wasp                     GGKLLSVTPAELAKHNTENDAWIAIRGIVFNVSRYMSFHPGGVPELMKGAGKDATKLFDDVHAWVNYQSILQKCVVGRLERSAGAEL
Drosophila                     GGRLVPVSRTELARHNKIDDAWMAIRGRVFNVTRYMDFHPGGVDELMRGVGRDATKLFDEVHAWVNYPQLLGKCYVGPLKDNETKPA
Louse                          GGVLKDVPLDELAEHNSKENAWISLRGKVYNVSHYMNFHPGGVPELMKGVGKDATKLFTEIHPWVNYESILQKCLVGRLVKNNPTAT
Deer tick                      GGKVLDVNPEELARHSRRDDAWICLKGRVYNVTPYMEFHPGGEEELMRGVGKDATDLFIQVHKWVNFESMLEKCLVGRLVGPPANSI
Mosquito                       GGRIVPVSHAELAKHDRAEDAWMAIRGKVYNVTRYMNFHPGGADELMRGAGKDATRLFEEVHAWVNYESLLAKCYIGPLRNTDTTDT
Tunicate                       GPRPLKISMEELAKHNTRDDAWMAIRGYVFNVTRYMEYHPGGESELMRGVGKDATSLFEEVHRWVNFESMLKACFIGTLIPDFTIPK
Lancelet                       GGRLQNVTPEELAKHDKEGNVWTAIRGKVYNVTAYAEYHPGGAEELMRAAGKDGTDLFNEVHRWVNYESMLESCLVGRLQEASSKND
C.elegans                      KRVSGGVDHVELMKHNTKDDCWVHLFGIVYDVTKYLDFHPGGIPELLRGAGRDATPLFNQYHAWVNYESMLKACVVGPFIGDLTKLP
Teladorsagia                   AKKRMSVDHEELMKHNQRDDCWVHIFGQVYDVTSYLEFHPGGIPELMRAAGTDATDLFNQFHAWVNYENMLKSCLVGRFTGDLSKLP
Trichuris                      GGRILKVTAKDLREHSTADNLWILLDGRVYNVTHYLPFHPGGRDALLIFIGQDGAPAY-QYHPWVNCHSVLERCLVGYFVDQRSVDE
Sea urchin                     QGQKMDVSEEELAKHNKIDDGWLAIRGQVYNVTPYMEYHPGGAEELMKGLGIDATDLFNEVHRWVNVESMLEKCHIGPLQKGDPLAF
Coral                          GGIVRPITEEELSQHNTQSDAWICIRGRVYNITPYLEYHPGGIPEIMRGVGKDGTDLFDETHKWVNAESMLEKCFIGPLKRDIAKVK
Sea anemone                    NGQHRPVTFEELDKHTSEEDCWIAIRGKVYNLTPYLEYHPGGIPEIMKGAGKDGTQLFDEVHKWVNAESMMEKCFIGPLVGVPQMGP
Trichoplax                     GGIIRKITNDELARHNTETDAWTCIRGKVYNITPYMKFHPGGIDELMRSVGCDGTDLFDEIHRWVNVESMLAKCLVGYIIADGVDGK
Hydra                          EGRNGSITLEELANHNTVKDCWTAIRGKVYNITPYLEFHPGGIEELMKSAGIDATTIFDQVHRWVNIESMLAKCYLGPLKVNIEKYL
Conserved in all (40)                -   -*  *      *  - * *- -- *- -****   --  -* *-   --  * ***    --  * -* -
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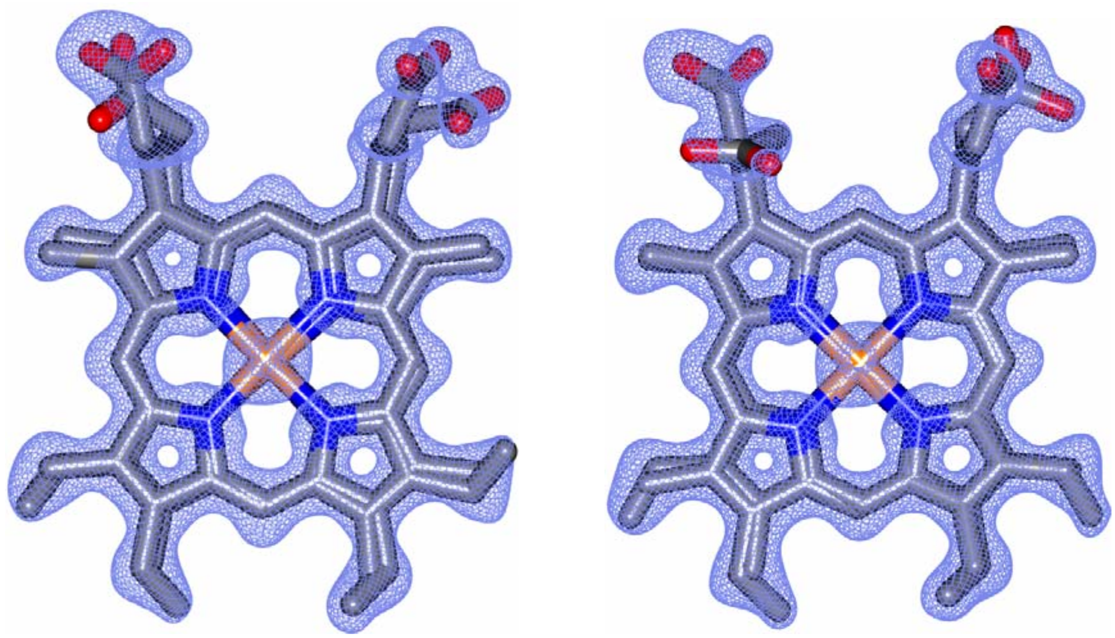
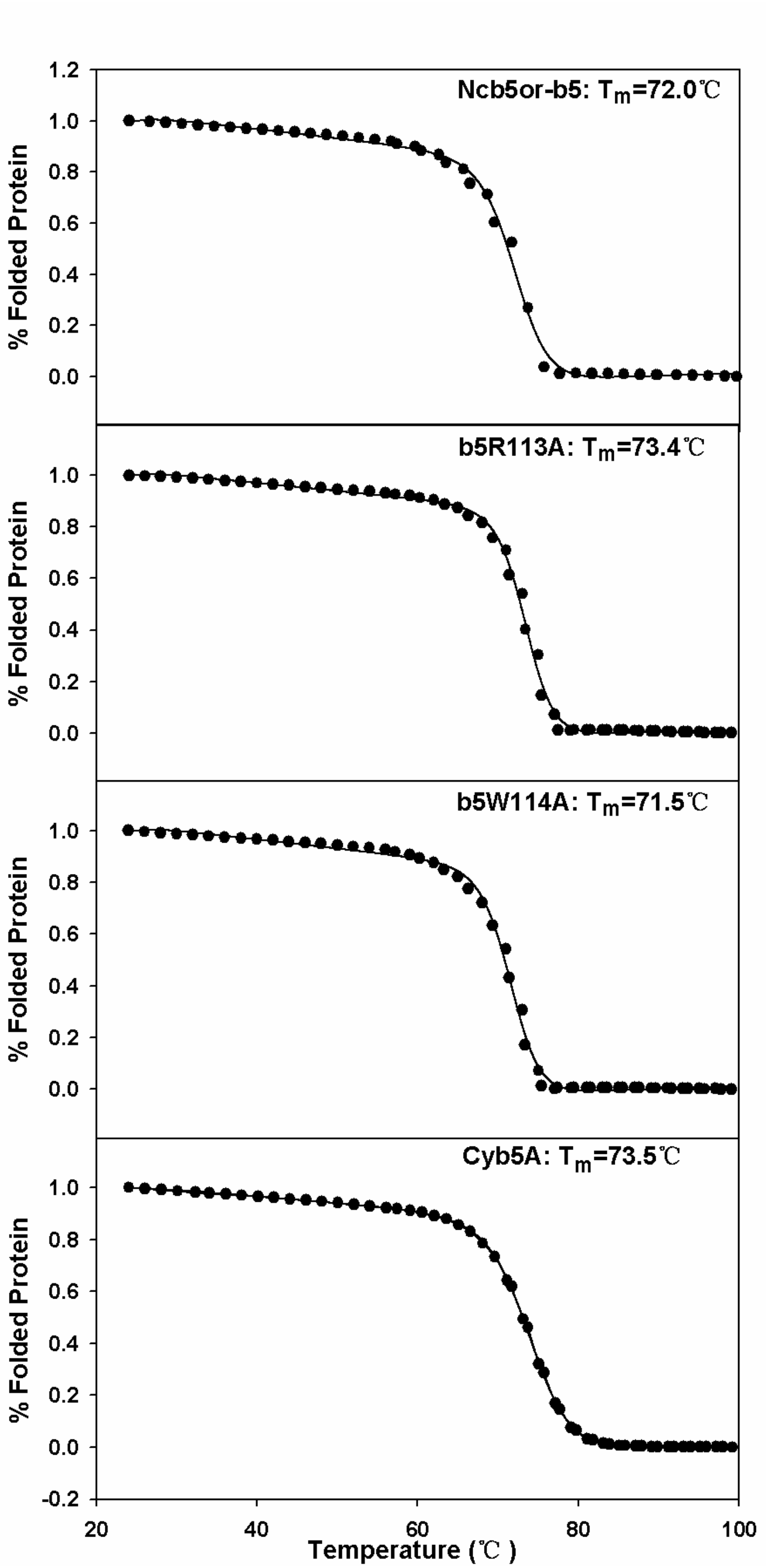
Supplemental Figure 3.

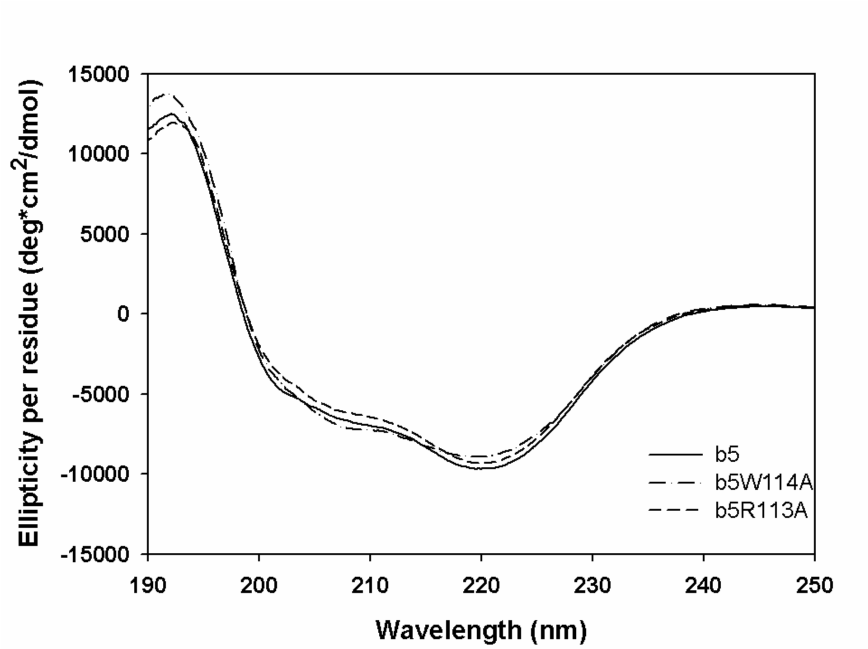


Supplemental Figure 4.

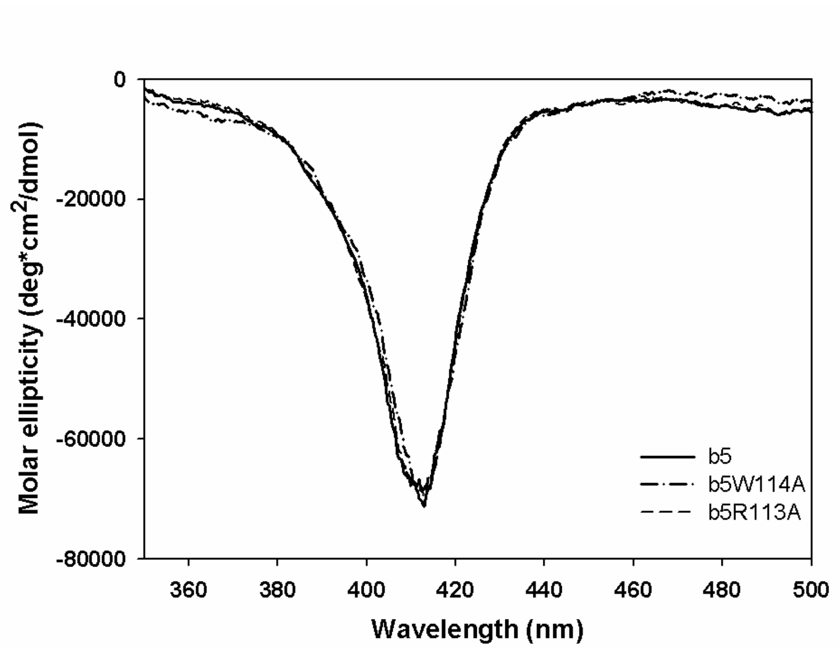


Supplemental Figure 5.

A.



B.



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