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Supporting information for article:

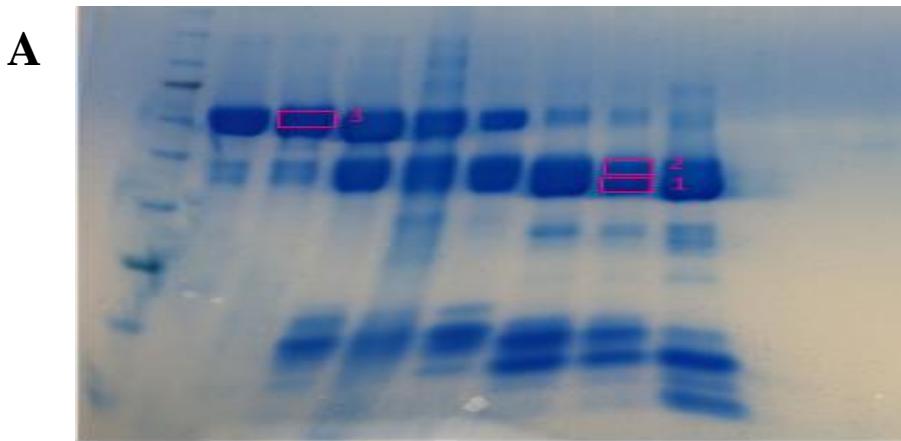
Crystal structures of the naturally fused CS and cytochrome *b*5 reductase domains of Ncb5or reveal an expanded CS fold, extensive CS–*b*5R interactions and productive binding of the NAD(P)⁺ nicotinamide ring

David R. Benson, Scott Lovell, Nurjahan Mehzabeen, Nadezhda Galeva, Anne Cooper, Philip Gao, Kevin P. Battaile and Hao Zhu

Table S1. A partial list of known structures of the CS domain and its partner. N/A: not available.

Protein	PDB #	Partner	Function	Reference
Ncb5or-CS (human)	6MV1, 6MV2	Ncb5or-b5R (human)	inter-domain (and inter-protein) electron transfer	This study
PIH1D1 (human)	6GXZ	RPAP3 (human)	HSP90 co-chaperone R2TP complex	(Henri <i>et al.</i> , 2018)
SHQ1 (human)	4PBD	Dyskerin (human)	H/ACA ribonucleoprotein particle assembly	(Singh <i>et al.</i> , 2015)
SHQ1 (yeast)	3EUD	Cbf5 (yeast)	H/ACA ribonucleoprotein particle assembly	(Singh <i>et al.</i> , 2009), (Singh <i>et al.</i> , 2015)
SGT1 (cress)	2JKI	Hsp90 (barley)	kinetochore complex	(Zhang <i>et al.</i> , 2008)
SGT1 (cress)	2XCM	RAR1 (cress)	regulation of innate immunity	(Zhang <i>et al.</i> , 2010)
p23/Hsp20 (human)	1EJF	N/A	heat shock (stress) response	(Weaver <i>et al.</i> , 2000)
NudC (human)	3QOR	N/A	maintenance of nuclear migration	(Zheng <i>et al.</i> , 2011)
NudC (mouse)	1WFI	N/A	maintenance of nuclear migration	N/A
NudCD2 (mouse)	2RH0	homo-dimer	maintenance of nuclear migration (?)	N/A
α B crystallin (human)	2WJ7	Hsp20 (rat)	heat shock (stress) response	(Bagneris <i>et al.</i> , 2009)
α A crystallin (bovine)	3L1E, 3LIF	α B crystallin (human)	heat shock (stress) response	(Laganowsky <i>et al.</i> , 2010)
USP19 (human)	1WH0	N/A	Ubiquitin Carboxyl-terminal Hydrolase	N/A
HspB2 (human)	6F2R	HspB3 (human)	heat shock (stress) response	N/A
Nuclear movement protein (E. cuniculi)	2O30	homo-dimer	nuclear movement	N/A
AgsA (S. typhimurium)	4ZJ9	homo-dimer	heat shock (stress) response	(Mani <i>et al.</i> , 2016)
KIAA1068 (human)	1WGV	N/A	unknown	N/A
Sgt1 (human)	1RL1	N/A	protein degradation	(Lee <i>et al.</i> , 2004)

Figure S1. **A)** SDS-PAGE of limited proteolysis of full-length Ncb5or with trypsin. Lanes (left-right): molecular marker, uncut, 0, 1, 2, 5, 24, 30, 48 hrs (digestion). Bands 1, 2 and 3 were excised from the gel and subjected to peptide mapping with chymotrypsin. The LC-MS/MS results of their products are listed in **B**).



B

Peptide Sequence	Begin Position	End Position	Precursor Mz	Precursor Charge	Peptide retention time	Sample 1 Ion peak area	Sample 2 Ion peak area	Sample 3 Ion peak area
NVPSQSFPAPR	10	21	600.312	2	14.61	0	27675	2236017
VASGGRSKVPL	25	36	535.8195	2	10.4	0	0	9834564
SGKDLTGL	50	58	395.7189	2	13.5	3981	0	2240638
ICIRGFVY	80	88	514.2733	2	25.77	38293	37061	2327473
ICIRGF	80	86	383.2074	2	21.12	0	0	40993764
VYNVSPY	86	93	421.2082	2	16.54	0	0	703489
DQVHRW	116	122	420.7092	2	9.69	0	0	461359
DQVHRWVNY	116	125	608.7965	2	17.99	0	0	3066170
ESMLKECL	125	133	505.2383	2	18.72	0	0	2762120
<u>EGPSYPSYDWF</u>	<u>170</u>	<u>181</u>	<u>674.28</u>	<u>2</u>	<u>31.95</u>	<u>4761810</u>	<u>1579519</u>	<u>0</u>
<u>DINLDSIIVDHQNDSFR</u>	<u>196</u>	<u>213</u>	<u>1000.987</u>	<u>2</u>	<u>28.3</u>	<u>424810</u>	<u>0</u>	<u>0</u>
<u>DINLDSIIVDHQNDSFR</u>	<u>196</u>	<u>213</u>	<u>667.6606</u>	<u>3</u>	<u>28.3</u>	<u>1795294</u>	<u>201435</u>	<u>0</u>
LIHIGLSHEVQEDF	223	237	818.9201	2	25.89	5930836	4460630	3302128
LIHIGL	223	229	333.2209	2	24.21	4296662	2649834	1914309
DFLGHPL	260	267	399.7109	2	23.22	25892176	14434548	17119236
CLMLPPSTHLQVPIQQHVY	301	320	1095.564	2	31.1	14668356	10471283	19999802
CLMLPPSTHLQVPIQQHVY	301	320	730.7115	3	31.1	20912006	17936324	31948456
CLMLPPSTHL	301	311	584.7963	2	24.08	2863936	500939	1053886
MLPPSTHLQVPIQQHVY	303	320	639.6732	3	23.98	1934978	611568	1671057

QVPIGQHVY	311	320	520.7798	2	15.31	15002936	8494890	7484312
QVPIGQHVYL	311	321	577.3218	2	21.68	7734609	4201766	3757868
LKLPITGTEIVKPY	320	334	786.474	2	26.59	1.58E+08	1.09E+08	160966512
LKLPITGTEIVKPY	320	334	524.6518	3	26.59	1.97E+08	1.48E+08	202504352
KLPITGTEIVKPY	321	334	729.932	2	21.88	64155364	46617284	73147008
KLPITGTEIVKPY	321	334	486.9571	3	21.88	53522968	40591712	58519704
TPVSGSLLSEF	334	345	568.7953	2	30.48	67067796	41762596	36763948
KEPVLPPNNKY	345	355	601.3324	2	13.04	6903187	4025524	2494419
KEPVLPPNNKY	345	355	401.224	3	13.04	2089857	1047379	620918
IKIYPTGLF	359	368	526.313	2	29.02	889565	454892	150452
TPELDRL	368	375	422.2322	2	17.86	1000109	412504	110493
QIGDFVSVSSPEGNF	375	390	791.8728	2	27.6	3125253	1734724	718776
VSVSSPEGNF	380	390	511.7431	2	18.21	1607199	949887	426350
ALTDIPSRL	419	428	493.2875	2	21.1	63188620	34123092	29151366
FNKTEDDIIW	434	444	640.8115	2	25.67	11851054	6974407	7392802
NKTEDDIIW	435	444	567.2773	2	23.42	46864804	26914894	28664094
VLSAPISEW	462	471	501.2688	2	27.07	13970847	5699884	10266745
NGKQGHISPALL	471	483	617.8488	2	17.3	2049715	709153	1163179
VCICGPVPF	497	506	524.7514	2	27.16	44645556	28843044	47993448
TEQGVRL	506	513	401.7245	2	12.2	8292545	6069676	8043911
TEQGVRL	506	514	458.2665	2	18.24	78778544	56141264	68987392
NFSKNEIHSF	517	527	611.7962	2	16.87	119918	46362	59720
SKNEIHSF	519	527	481.2405	2	10.88	3565085	2245790	1371276
SKNEIHSF	519	527	321.1628	3	10.88	643460	314515	171685
SKNEIHSFTA	519	529	567.2829	2	12.66	1436111	965453	2004352

Figure S2. Superposition of the CS domain of CS-b5R-NAD (red) with **A**) human chaperone protein p23 (gold; PDB entry 1EJF, (Weaver *et al.*, 2000)), **B**) nuclear movement protein from *E. cuniculi* GB-M1 (brown; PDB entry 2O30, <http://www.rcsb.org/structure/2O30>), **C**) CS domain of the essential baker's yeast H/ACA ribonucleoparticle assembly protein (lilac; PDB entry 3EUD (Singh *et al.*, 2009)), **D**) cress *Arabidopsis thaliana* SGT1-like protein (blue, 2XCM, (Zhang *et al.*, 2010)).

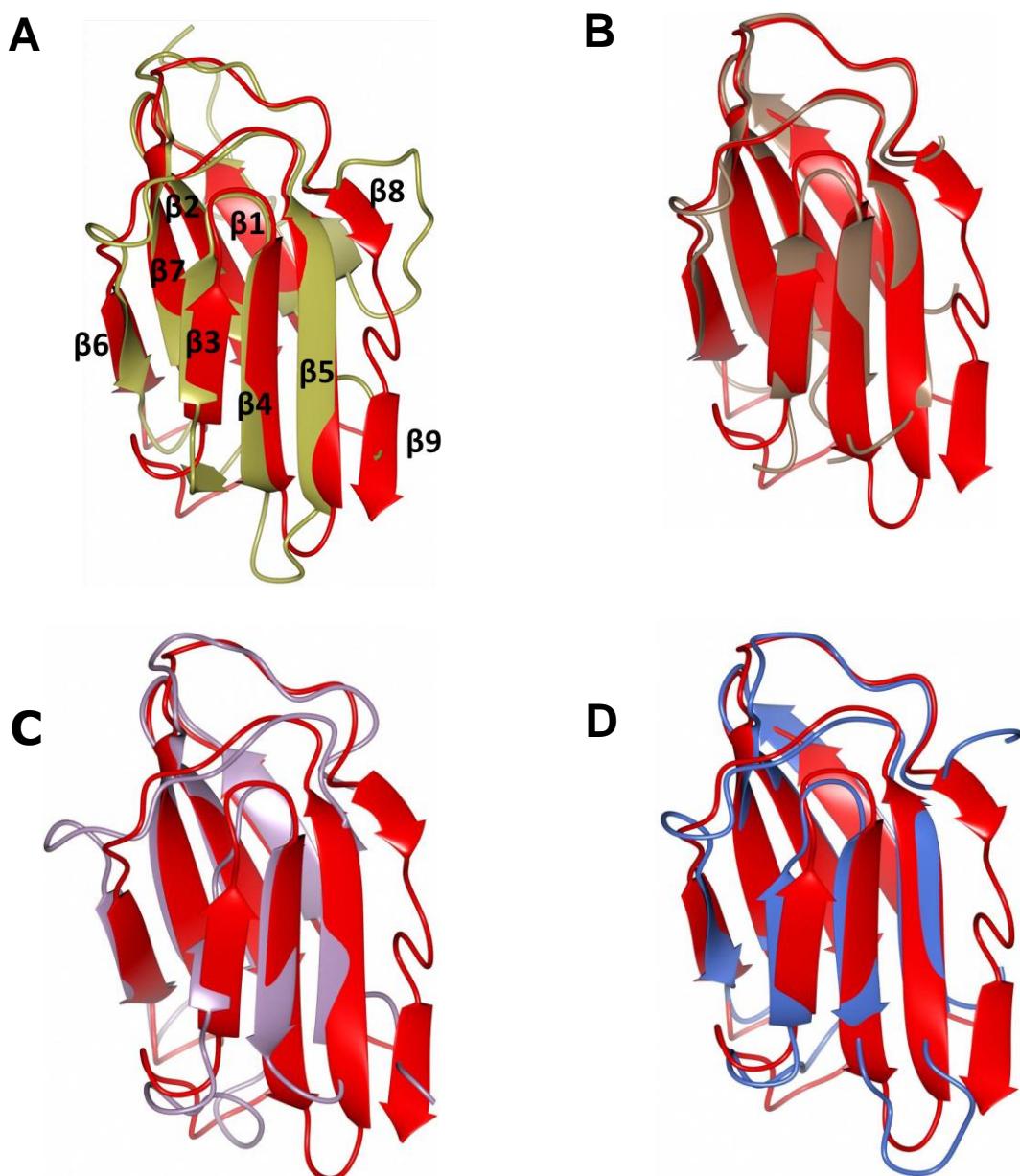


Figure S3. Superposition of the b5R domain of CS-b5R-NAD (magenta) with **A)** *P. polycephalum* b5R (2EIX, cyan); **B)** corn NADH dependent nitrate reductase (2CND, blue) (Lu et al., 1995), **C)** nitric oxide dioxygenase from *Vibrio cholerae* O1 biovar El Tor (4EH1, gold) and **D)** ferredoxin NADP reductase from *Neisseria gonorrhoeae* (5TR9, gold). The NAD and FAD sub-domains and the linker between strands β 9 and β 10 in CS-b5R-NAD are indicated in panel A.

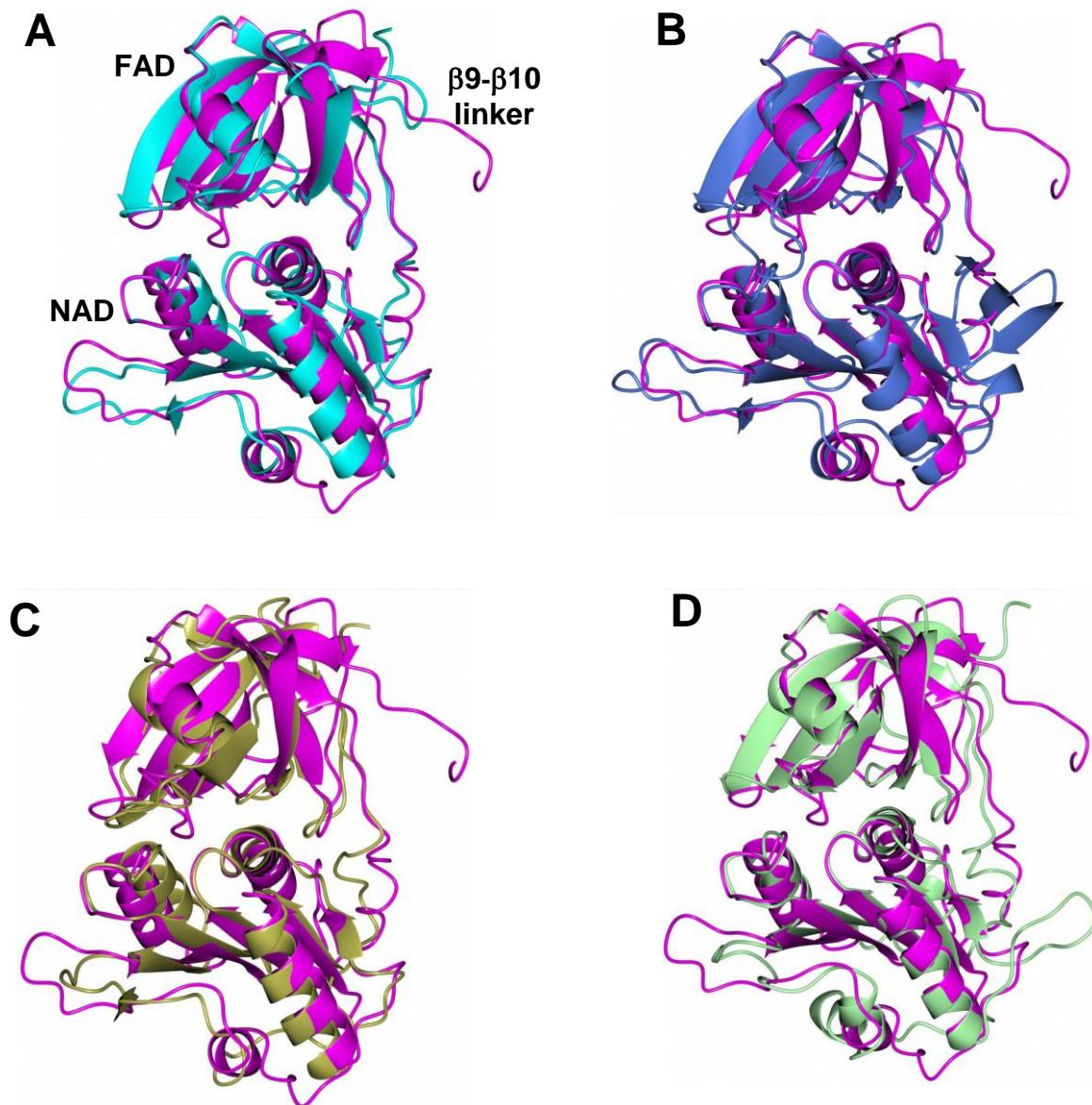
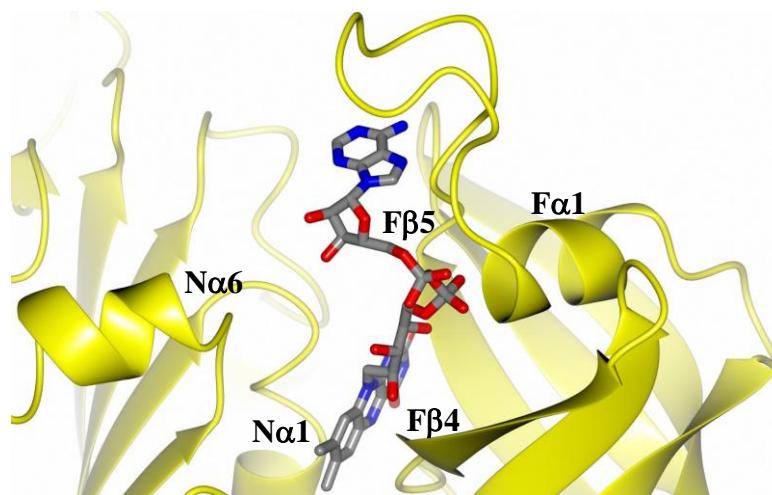


Figure S4. Comparison of the environment for the FAD adenine ring in human Cyb5R3 (1UMK, **A**) and CS-b5R (**B**).

A



B

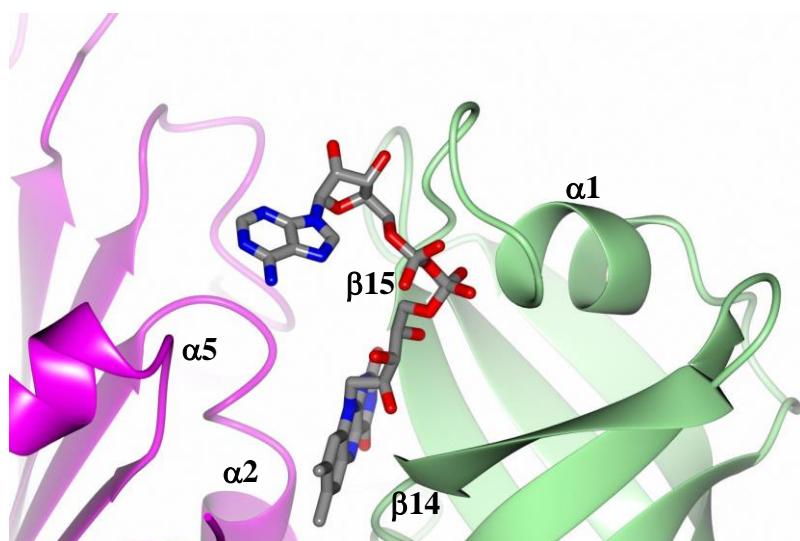
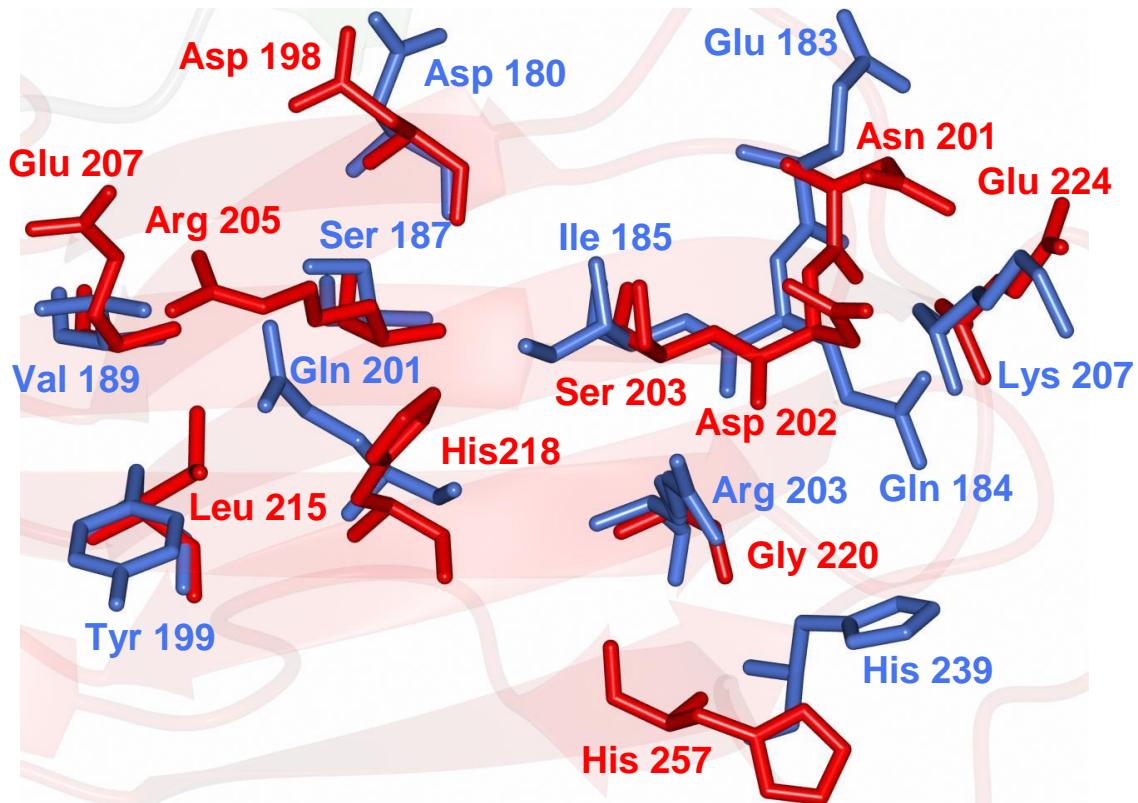


Figure S5. **A)** Comparison of the CS-domain surface residues of 2XCM (blue) and CS-b5R-NAD (red). **B)** Same as **A**) with the b5R domain shown.

A



B

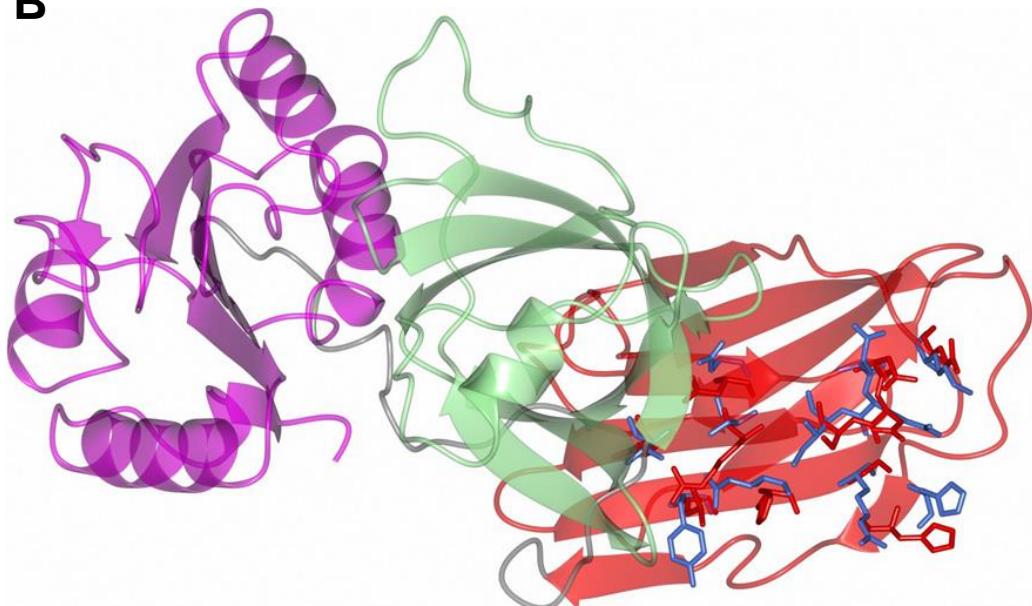
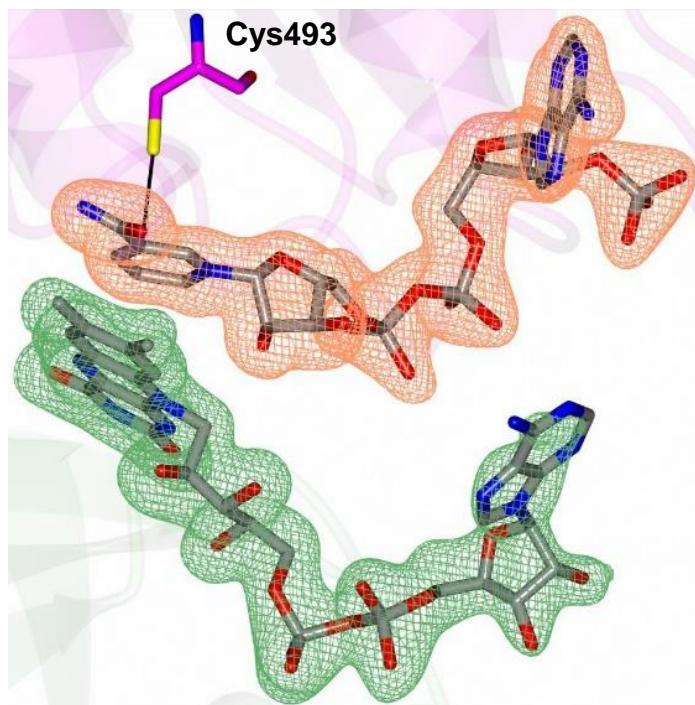


Figure S6. Interaction between FAD and NADP⁺ in CS-b5R-NADP. **A)** Electron density maps (Fo-Fc polder omit, Adams *et al.*, 2010) for FAD (green) and NADP⁺ (coral). **B)** Angle between the mean planes of the FAD (flavin) and NADP⁺ (nicotinamide) rings.

A



B

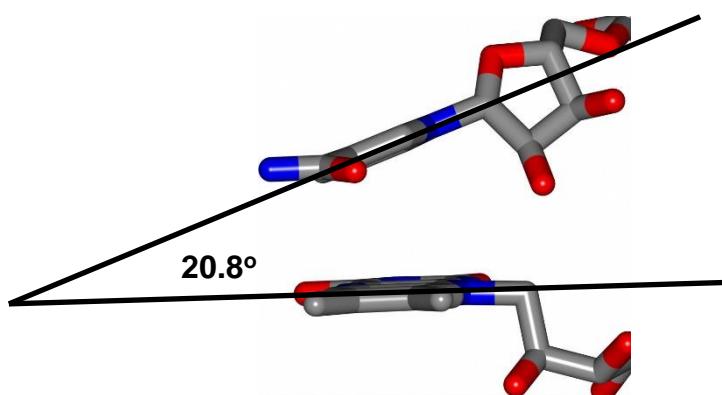


Figure S7. Two views of the structure of oxidized rat Cyb5R3 (PDB entry 1IB0) showing that the nicotinamide ring of bound NAD⁺ is outside of the active site. Panel B further highlights interactions between the NAD⁺ adenine ring and the protein (Bewley *et al.*, 2001)

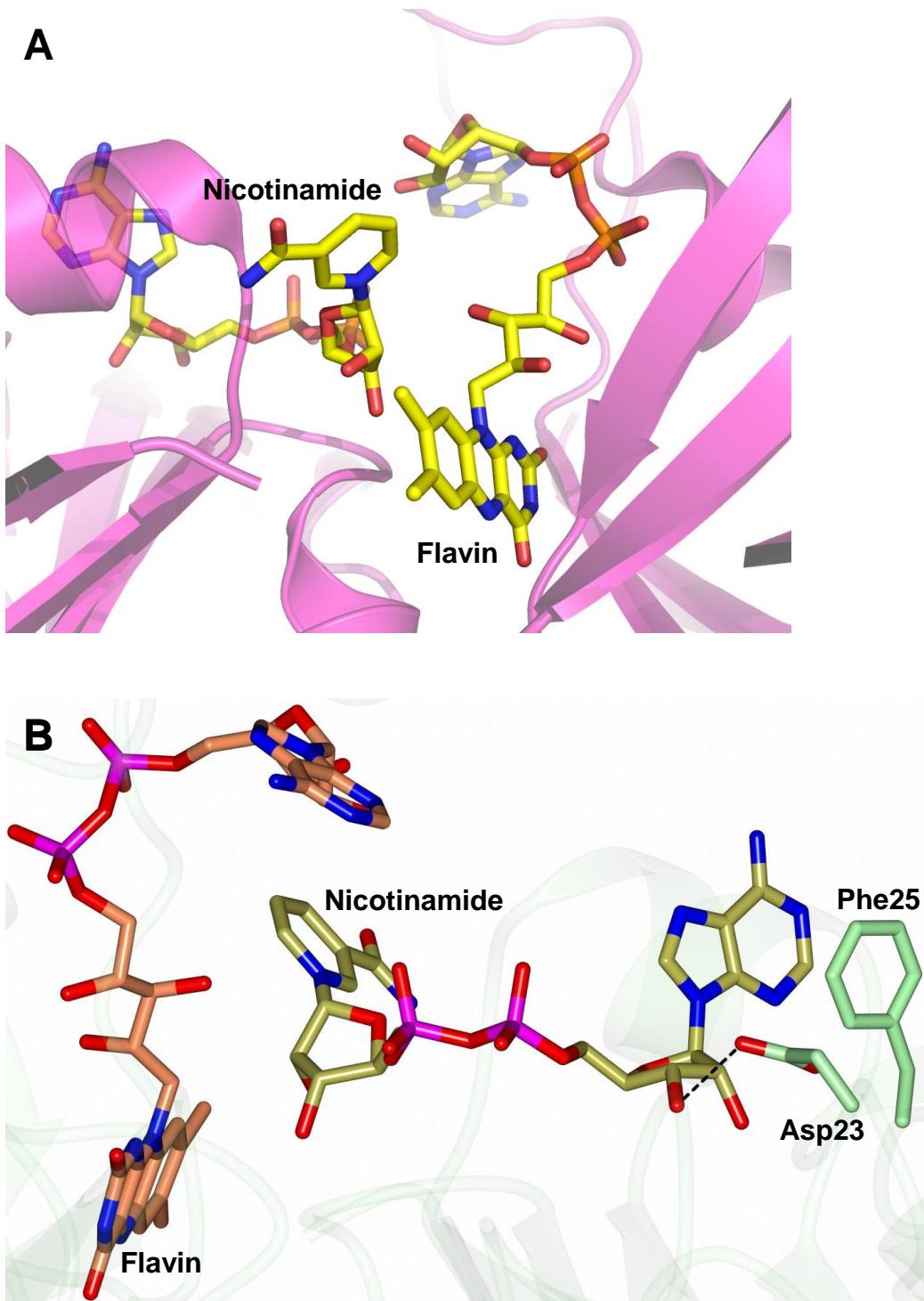


Figure S8. Side chain interactions with (A) NADP⁺ in CS-b5R-NADP; and (B) NAD⁺ in CS-b5R-NAD.

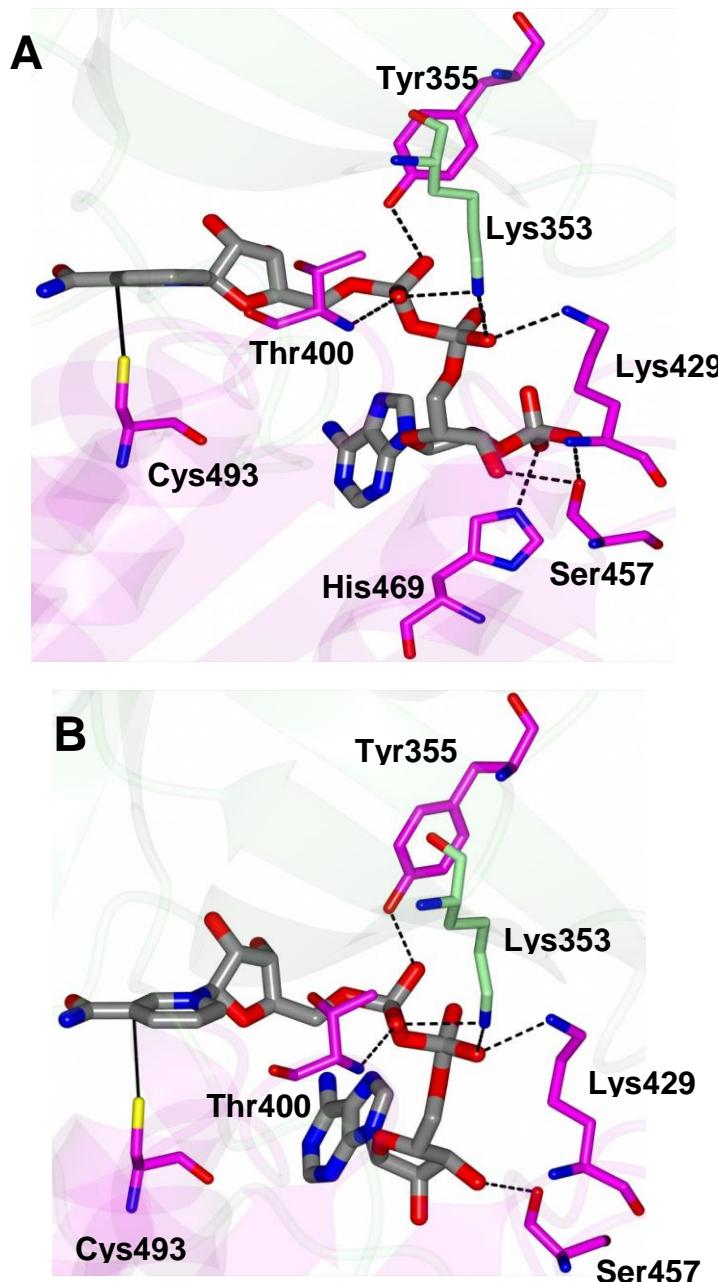


Figure S9. BlastP-guided sequence alignment of mammalian Cyb5R1-4 and homologous reductases from slime mold, plants and fungi. Gene Bank accession numbers are listed for all entries. Open-ended N-terminal sequences are indicated by ..., and C-terminal stop codon by *.

MNSRRREPITLQDPEAKYPLPLIEKEKISHNTRRFRFGLPSPDHVLGLPVGNYVQ
MSVKKKDITLQDPEAKYPLPLIEKEQISHNTRRFRFGLPSPDHVLGLPVGNYVH
MSVKKKDITLQDPEAKYPLPLIEKEQINHNTRRFRFGLPSPDHVLGLPVGNYVH
MGAQLSTLGHMVLFPWFLY SLLMKLFQRSTPAITLESPDIKYPLRLIDREIISHDTRRFRFALPPPQHQHILGLPVGQHIY
MGAQLSTLSHVVLSPWIFIY SLFMKLFQRSTPAITLENPDIKYPLRLIDKEVISPDTRRFRFALPSPQHQHILGLPIGQHIY
MGAQLSTLSRVVLSPWVFVY SLFMKLFQRSSPAITLENPDIKYPLRLIDKEIISHDTRRFRFALPSPQHQHILGLPIGQHIY
MDILTAPVLIQSVIVITVLYLFLKPAGSNSTPPPKPNKIPKALQDPVKYPLPLIEKEEINHDTKRFRGGLPSSHVGLPIGQHIY
MVENNTLIITGVVVVSSIAIYIYLKATNTCPF SLCKSKKRTLVDDSVKYPLPLIEKFIEISHDTRKFRFGLPSKDHLGLPIGQHVY
MGIQTSPVLLASLGVLVTLLGLAVGSYLVRRSRRPQVTLLDPNEKYLLRLLDKTTVSHNTKRFRFALPTAHHTLGLPVGKHY
MGIQPSPVLLASLGVLTLGLALGTYLVRSSRRPQVTLLDPNEKYLLRLLDKTTVSHNTRRFRFALPTAHHLGLPVGKHY
MGIQPSPVLLASLGVLFTLGLALGTYLVRSSRRPQVTLLDPNEKYLLRLLDKTTVSHNTRRFRFALPTAHHLGLPVGKHY
MSYAMSTTVAVTVGVVLVSTAGLLGYYYFNRKRKILITLIDPSEKYKLRLVDKEIISHDTRRFRFALPSPEHVGLPVGKHY
MVENNTLAITGGVVLISSVSLFLYRQLRAEKKSRTLEDDSVKYLPLIEKFIEISHNTRKFRFGLPSKDHLGLPIGHHVY
.HEVQEDFSVRVVESVGKIEIVLQKKENTSWDFLGHPLKNHNSLIPRKDTGLY **YRKCQLISKEDVTHDTRLFCMLPPSTHL** QVPIGNHJVY
.HEVQENFSVRVIENVGKIEIVLQKKESVSWQCLGDHLEKHDSFIPKKDTGLY **YRRCQLISKEDVTHDTRLFCMLPPSTHL** QVPVGQHVY
.HEVQENFSVRVIENVGKIEIVLQKKETVSWKCLGDPLEKHDSFIPKKDTGLY **YRQCQLISKEDVTHDTRLFCMLPPSTHL** QVPVGQHVY
 .. KREPALNPNEYKKFMLREKQIINHNTRLFRFNLHHPEDVVGGLPIGQHMS
 .. KGKQVEKALDPQEYRKFQLKEFKIVNHNTRIFRFALPNEDDILGLPIGQHIS
MDTEFLRTLDRQILLGVFVAvgagaAYFLTSSKKRVC LDOPENFKEFKLVKRHQLSHNVAKFVFEPLTSTVGLGLPIGQHIS
MEFSQSHRVEMISMAVALVAIVGGTAYYYVTKPKGCL DPENFKEFKLVKRTQLSHNVATFRFDLPTPKSVLGLPIGQHIS
MDFLQAQENQIILGVAVA VVAIGLGAVYLYSSKKTKGCLDPENFKEFKLIKKTQLSHNVARFKFALPTPSSVGLGLPIGQHIS
 .FPLLDKSFPTENLNPELLGHVVALVAVGFTAAYIYYRAKHPKGSLDPKNFKEFKLIKKTQLSHNVARFKFALPTPSSVGLGLPIGQHIS
 .. HGVYIPVFLIIFGTYIVKREWVGYAIVVAFSLGFHKFWRQRVRKVLSDKIQQFELSDKAVLNHTAIYRFRLPRANDVGLGLPIGQHLK
MAIDAQKLVVVIVVVPLLFKIIGPKTPVLPKRNDQSFPLV EKTFILTHNTSMYKFGGLPHADDVGLGLPIGQHIV
 .. CTGLLLNCVVTPLYFWKTQNGRIVVVVSLQFVVLYATAFISIGTDKSLYRNKVALPLSKTRISRNTSLYCFKLKYPFERLHIPMGYHLA
 .. YIPAALFVVGVAITTYMSGELKILQSLPILFMIIFVRAYSAYKRRRSLYPDRWTSLELEDQTIISKNATALYRFKLKTRLESLDIPAGHHVA
 .. PIKEITTPRPRQRKVALNSREKISCKLVSCTYISHNTRLFRFALPSEDQLLGLPVGKHIF
 .. RAPALSNPGRRIHCRLVAKKELSRDVRFLFRSLPSPDQVLGLGLPIGKHIF
 .. RTFVVREKQVESAYVTSF VLPADGGAVLDYQPGQYIG
 .. HHTPKLITFAISRPESY RFKAGQFSR

FAD/1

LLAKIDNELVVRAYTPVSSD
 LLAQINNELLVIRAYTPVSSD
 LLAQINNELLVIRAYTPVSSD
 LSARIDGNLVRPYTPISSD
 LSTRIDGNLVRPYTPVSSD
 LSTRIDGNLVRPYTPVSSD
 LSAKVNGLVVRAYTPVSSD
 LTANIDGKIIVRPYTPVSSD
 LSTRIDGNLVRPYTPVTS
 LSARIDGSLVIRPYTPVTS
 LSARIDGSQVIRPYTPVTS
 LSARIDGNLIVRPYTPVSSD
 LSANIGGKLIVRSYTPVSCD
LKLIPITGTEIVKPYTPVSGS **LLSEFKEPVLPNNKYIYFLIKIY**
LKLSVTGAEIVKPYTPVSDS **LLSDFKEPVLSPNKYIYFLIKIY**
LKLSVTGAEIVKPYTPVSE **LLSDFKEPVLSPNKYIYFLIKIY**
 VKATVDGKEIYRPYTPVSSD
 LRAVVGKGEVYRPYTPISSD
 CRGKDGQGEDVIKPYTPPTLD
 CRGKDSLGEVVKPYTPPTLD
 CRGKDAQGEEVIKPYTPPTLD
 ARGKDSQGEEVMRSYTPITLD
 VFVDVDGKEYRSYTPLSSD
 IKANINGKDIRTSYTPSLDG
 VRVTINGERLVRYYTPVNVP
 VRVPIDGKQEVRYNNPISSK
 LYATIGDKLCIRAYTPTSSV
 VCATIEGKLCMRAYTPTSMV
 IEVTPEGSDYREIRQYSL
 LGFYEGKGFIRWRAYSVVSA

FAD/2

DDRGFVDLIIKIYFKNVHPQYPEGGKMTQYLENMKIGETIFFR GPRGRLFYHGPGLNLGIR
 DDQGFVDLIIKIYFKNVHPKYPEGGKMTQYLENMKIGDTILFR GPTGRLFYNEPGTLLIK
 DDQGFVDLIIKIYFKNVHPKYPEGGKMTQYLENMKIGDTILFR GPTGRLFYNEPGTLLIK
 DDKGFVDLVIKVFKDTHPKFPAGGKMSQYLESMQIGDTIEFR **GPSGLLVYQGKGKFAIR**
 DDKGFVDLVIKVFKDTHPKFPAGGKMSQYLENMKIGDTIEFR **GPNGLLVYQGKGKFAIR**
 DDKGFVDLVIKVFKDTHPKFPAGGKMSQYLENMNIGDTIEFR **GPNGLLVYQGKGKFAIR**
 QDQGYVDLVIKVFKDTHPKFPAGGKMSQYLDNMKIGDTIDFR **GPNGLLVYQGKGKFAIR**
 EDLGFVDLVMKVYFKNTNEKFPEGGKMSQHLESIKIGDTINFR GPQGNIVYKGHGLFSIK
 EDQGYVDLVIKVFYKGVHPKFPEGGKMSQYLDLSLKVGDVVEFR **GPSGLLTYGKGHFN** IQ
 EDQGYVDLVIKVFYKGVHPKFPEGGKMSQYLDLSLKIGDMVEFW **GPSGLLSYAGKGNCNI** Q
 EDQGYVDLVIKVFYKGVHPKFSEGGKMSQYLDLSLKIGDVVEFR **GPSGLLSYAGKGNF** NIQ
 DDKGFVDLVIKVFYKGVHPKFPEGGKMSQYLESLRIGDVIDFR **GPGGLLEYKGAGRLD** IQ
 LDLGYVDLVMKVYFKNTHERFPDGGKMSQHLESIKIGDTSFR **GPHGSI** IYKGSGLFTVR
PTGLFTP ELDRLQIGDFVSVS **SPEGNFKI** SKFQELED
PAGLFTP ELDRLQIGDFISVS **GPEGNFKV** SKLQEVED
PAGLFTP ELDRLQIGDFVSVS **GPEGNFKV** SKLQEVED
 DEKGYFDLLIKVY
 EERGYFDLLIKVY
 SDVGRFELVIKMY
 TDVGYFELVVKMY
 SDVGHFELVIKMY
 SNIGYFELVVKMY
 ADKGYFDLLVKSY
 DTKGNFELLVKSY
 NTEGHLELVVKTY
 LESGYLDDLVVKAY
 DEVGFDFLLIKVYFKGVHPKYPNGGLMSQHLDLSIGSMLDV
 DEIGHFDLLVKVYFKNEHPKFPNGGLMTQYLDLSPVGSYIDVK
 HASNGREYRISVKREGVGS EYADTLEYFAVLI

Cyb5R2 / Human (NP_057313)
 Cyb5R2 / Mouse (NP_796190)
 Cyb5R2 / Rat (NP_001014266)
 Cyb5R3 / Human (NP_000389)
 Cyb5R3 / Mouse (NP_084063)
 Cyb5R3 / Rat (NP_620232)
 Cyb5R3 / Zebrafish (NP_001038825)
 Cyb5R3 / C.elegans (NP_504638)
 Cyb5R1 / Human (NP_057327)
 Cyb5R1 / Mouse (NP_082333)
 Cyb5R1 / Rat (NP_001013144)
 Cyb5R1 / Zebrafish (NP_956483)
 Cyb5R1 / C.elegans (NP_504639)
 Cyb5R4 / Human (NP_057314)
 Cyb5R4 / Mouse (NP_077157)
 Cyb5R4 / Rat (NP_596918)
 b5R/slime mold (Physarum, 2EIX)
 b5R/slime mold (Dictyostelium, XP_638259)
 b5R/Arabidopsis (NP_197279)
 b5R/Glycine (XP_003556838)
 b5R/Glycine (XP_003520929, 003554059)
 b5R/Glycine (XP_003531792)
 b5R/S.pombe (NP_587852)
 Cbr1p/S.cerevisiae (NP_012221)
 b5R/S.cerevisiae (NP_013623)
 b5R/S.cerevisiae (NP_013581)
 nitrate reductase/Glycine (XP_003544771)
 nitrate reductase/corn (2CND)
 nitric oxide dioxygenase (4EH1)
 ferredoxin reductase/Neisseria (5TR9)

FAD/1

LLAQINNELLVIRAYTPVSSD
 LLAQINNELLVIRAYTPVSSD
 LLAQINNELLVIRAYTPVSSD
 LSARIDGNLVRPYTPISSD
 LSTRIDGNLVRPYTPVSSD
 LSTRIDGNLVRPYTPVSSD
 LSAKVNGLVVRAYTPVSSD
 LTANIDGKIIVRPYTPVSSD
 LSTRIDGNLVRPYTPVTS
 LSARIDGSLVIRPYTPVTS
 LSARIDGSQVIRPYTPVTS
 LSARIDGNLIVRPYTPVSSD
 LSANIGGKLIVRSYTPVSCD
LKLIPITGTEIVKPYTPVSGS **LLSEFKEPVLPNNKYIYFLIKIY**
LKLSVTGAEIVKPYTPVSDS **LLSDFKEPVLSPNKYIYFLIKIY**
LKLSVTGAEIVKPYTPVSE **LLSDFKEPVLSPNKYIYFLIKIY**
 VKATVDGKEIYRPYTPVSSD
 LRAVVGKGEVYRPYTPISSD
 CRGKDGQGEDVIKPYTPPTLD
 CRGKDSLGEVVKPYTPPTLD
 CRGKDAQGEEVIKPYTPPTLD
 ARGKDSQGEEVMRSYTPITLD
 VFVDVDGKEYRSYTPLSSD
 IKANINGKDIRTSYTPSLDG
 VRVTINGERLVRYYTPVNVP
 VRVPIDGKQEVRYNNPISSK
 LYATIGDKLCIRAYTPTSSV
 VCATIEGKLCMRAYTPTSMV
 IEVTPEGSDYREIRQYSL
 LGFYEGKGFIRWRAYSVVSA

FAD/2

DDRGFVDLIIKIYFKNVHPQYPEGGKMTQYLENMKIGETIFFR GPRGRLFYHGPGLNLGIR
 DDQGFVDLIIKIYFKNVHPKYPEGGKMTQYLENMKIGDTILFR GPTGRLFYNEPGTLLIK
 DDQGFVDLIIKIYFKNVHPKYPEGGKMTQYLENMKIGDTILFR GPTGRLFYNEPGTLLIK
 DDKGFVDLVIKVFKDTHPKFPAGGKMSQYLESMQIGDTIEFR **GPSGLLVYQGKGKFAIR**
 DDKGFVDLVIKVFKDTHPKFPAGGKMSQYLENMKIGDTIEFR **GPNGLLVYQGKGKFAIR**
 DDKGFVDLVIKVFKDTHPKFPAGGKMSQYLENMNIGDTIEFR **GPNGLLVYQGKGKFAIR**
 QDQGYVDLVIKVFKDTHPKFPAGGKMSQYLDNMKIGDTIDFR **GPNGLLVYQGKGKFAIR**
 EDLGFVDLVMKVYFKNTNEKFPEGGKMSQHLESIKIGDTINFR GPQGNIVYKGHGLFSIK
 EDQGYVDLVIKVFYKGVHPKFPEGGKMSQYLDLSLKVGDVVEFR **GPSGLLTYGKGHFN** IQ
 EDQGYVDLVIKVFYKGVHPKFPEGGKMSQYLDLSLKIGDMVEFW **GPSGLLSYAGKGNCNI** Q
 EDQGYVDLVIKVFYKGVHPKFSEGGKMSQYLDLSLKIGDVVEFR **GPSGLLSYAGKGNF** NIQ
 DDKGFVDLVIKVFYKGVHPKFPEGGKMSQYLESLRIGDVIDFR **GPGGLLEYKGAGRLD** IQ
 LDLGYVDLVMKVYFKNTHERFPDGGKMSQHLESIKIGDTSFR **GPHGSI** IYKGSGLFTVR
PTGLFTP ELDRLQIGDFVSVS **SPEGNFKI** SKFQELED
PAGLFTP ELDRLQIGDFISVS **GPEGNFKV** SKLQEVED
PAGLFTP ELDRLQIGDFVSVS **GPEGNFKV** SKLQEVED
 EKGQMSQYIDHLPNGDFLQVR GPKGQFDY KPNMVKE
 EKGAMSGYVDNMFIGDSIEVK GPKGKFNY QPNMRKS
 PQGRMSHHFREMRVGDHLAVK GPKGRFKY QPGQFRA
 PQGRMSHHFREIREGDYMAVK GPKGRFKY QPNQVRA
 PQGRMSHHFREMRVGDYLSVK GPKGRFKY QPGEVRA
 PNGKMSHHFRQMKEGDFLAVR GPKGRFTY KPGQVRA
 PNGKVSKKFSELKIGDTIGV R GPKGNWKH RTGLARH
 PTGNVSKMIGELKIGDSIQIK GPRGNYHY ERNCRSH
 KHGVVSKYFDKLKIRQYVEFK GPLGELEY DQDTATE
 VDGKVSKEYFAGLNSGDTVDFK GPIGTLNY EPNSSKH
 DEVGFFDFLLIKVYFKGVHPKYPNGGLMSQHLDLSIGSMLDV
 DEIGHFDLLVKVYFKNEHPKFPNGGLMTQYLDLSPVGSYIDVK
 HASNGREYRISVKREGVGS EYADTLEYFAVLI

Cyb5R2 / Human (NP_057313)
 Cyb5R2 / Mouse (NP_796190)
 Cyb5R2 / Rat (NP_001014266)
 Cyb5R3 / Human (NP_000389)
 Cyb5R3 / Mouse (NP_084063)
 Cyb5R3 / Rat (NP_620232)
 Cyb5R3 / Zebrafish (NP_001038825)
 Cyb5R3 / C.elegans (NP_504638)
 Cyb5R1 / Human (NP_057327)
 Cyb5R1 / Mouse (NP_082333)
 Cyb5R1 / Rat (NP_001013144)
 Cyb5R1 / Zebrafish (NP_956483)
 Cyb5R1 / C.elegans (NP_504639)
 Cyb5R4 / Human (NP_057314)
 Cyb5R4 / Mouse (NP_077157)
 Cyb5R4 / Rat (NP_596918)
 b5R/slime mold (Physarum, 2EIX)
 b5R/slime mold (Dictyostelium, XP_638259)
 b5R/Arabidopsis (NP_197279)
 b5R/Glycine (XP_003556838)
 b5R/Glycine (XP_003520929, 003554059)
 b5R/Glycine (XP_003531792)
 b5R/S.pombe (NP_587852)
 Cbr1p/S.cerevisiae (NP_012221)
 b5R/S.cerevisiae (NP_013623)
 b5R/S.cerevisiae (NP_013581)
 nitrate reductase/Glycine
 nitrate reductase/corn (2CND)
 nitric oxide dioxygenase (4EH1)
 ferredoxin reductase/Neisseria (5TR9)

NADPH/1		
PDQTSEPKKTLADHLGMIAGGTGITPMLQLIRHITKD	PSDRTRMSLIFANQTEEDILVRKELEEIARTHPDQFDLWYTL	Cyb5R2 / Human (NP_057313)
ANKTSEPEKKLVHHLGMIAGGTGITPMLQLIRHITKD	TSDETRMSLLFANQTEEDILLRKELEEVATTHHKQFNLWYTL	Cyb5R2 / Mouse (NP_796190)
TDKTSEPEKKLVHHLGMIAGGTGITPMLQLIRHITKD	TSDGTRMSLLFANQTEEDILLRKELEEVATTHQNQFSLWYTL	Cyb5R2 / Rat (NP_001014266)
<u>PDKSNPII</u> RTVKSVGMIAGGTGITPMLQVIRAIMKD	<u>PDDHTVCHLLF</u> ANQTEKDILLRPELEELRNKHSARFKLWYTL	<u>Cyb5R3</u> / Human (NP_000389)
ADKSNPVPVRTVKSVGMIAGGTGITPMLQVIRAVLKD	PNDHTVCYLLFANQSEKDILLRPELEELRNEHSARFKLWYTV	<u>Cyb5R3</u> / Mouse (NP_084063)
ADKSNPVPVRTVKSVGMIAGGTGITPMLQVIRAVLKD	PNDHTVCYLLFANQSEKDILLRPELEELRNEHSARFKLWYTV	<u>Cyb5R3</u> / Rat (NP_620232)
PDKSEAEVRKFKHVAMIAAGGTGITPMLQLVRSITAD	SSDETVCSLIFANQTEKDILLRNELEDEVHRNHPSKLKLWYTL	<u>Cyb5R3</u> / Zebrafish (NP_001038825)
ADKAEPKNRVFKHLSMIAGGTGITPMLQVIAAILRD	PTDTQIRLLFANQTEDDILCRQELDDLAKEHPTRFRVWYTV	<u>Cyb5R3</u> / C.elegans (NP_504638)
PNKSPPEPRVAKKLGMIAGGTGITPMLQLIRAILKV	PEDPTQCFLFANQTEKDIIREDILEELQARYPNRFKLWFTL	<u>Cyb5R1</u> / Human (NP_057327)
PNKSPPELRVAKKLGMIAGGTGITPMLQLIRAILKV	PEDPTQCFLFANQTERDIILREDILEELQAQYPNRFKLWFTL	<u>Cyb5R1</u> / Mouse (NP_082333)
PNKSPPELRVAKKLGMIAGGTGITPMLQLIRAILKV	PEDPTQCFLFANQTEKDIIILREDILEELQAQYPIRFKLWFTL	<u>Cyb5R1</u> / Rat (NP_001013144)
ADKAPAETKTVKSLGLIAGGTGITPMLQLIRDITKN	PNDTTCSLLFANQTEKDILLKDELEEIQARHSDRFKLWFTV	<u>Cyb5R1</u> / Zebrafish (NP_956483)
MDKKAEPKNRFFKHLSMIAGGTGITPMLQVIAAILRD	PIDATQIRLLFANQTEDDILCRKELDELAEKHPTFRVWYTV	<u>Cyb5R1</u> / C.elegans (NP_504639)
LFLLAAGTGFTPMVKILNYALTD	IPSRLRKVKLMFNFNKTEDDIIWRSQLEKLFKD KRLDVEFVL	<u>Cyb5R4</u> / Human (NP_057314)
LFLLAAGTGFTPMVTVLNYALSH	MSSLRKVKLMFNFNKTEDDIIWRCQLEKLFALRE KRFDVEFVL	<u>Cyb5R4</u> / Mouse (NP_077157)
LFLLAAGTGFTPMVTVLNHALTH	MSSLRKVKLMFNFNKTEDDIIWRCQLEKLFALKD KRFHVEYVL	<u>Cyb5R4</u> / Rat (NP_596918)
MGMIAAGGTGITPMLQVARAIKRN	PKEKTIINLIFANVNEDDILLRTELDDMAKKY SNFKVYYVL	b5R/slime mold (Physarum, 2EIX)
IGMLAGGTGITPMLQVIKAILKN	PSDKETEISLVFGNITEEDILLKKELDELAEKHP QFKVYYVL	b5R/slime mold (Dictyostelium, XP_638259)
FGMLAGGSGITPMFQVARAILEN	PTDKTKVHЛИYANVTYDDILLKEELEGLTNTYPEQFKIFYVL	b5R/Arabidopsis (NP_197279)
LGMIAAGGTGITPMFQVTRAILEN	QQDKTNINLIYANVTFDDILLKEELDAFAIKFPNQFKVYYVL	b5R/Glycine (XP_003556838)
FGMLAGGSGITPMFQVVARAILEN	PNDRTKVKHЛИYANVTYEDILLKEELDGLASNYPDRFKIYYVL	b5R/Glycine (XP_003520929, 003554059)
FGMIAGGSGITPMFQLIRAILEN	PKDKTKVHLVYANVTVDDILLKEELDNFANKFAQRFEVYHVL	b5R/Glycine (XP_003531792)
FGMIAGGTGITPMLQIIRAVLSN	FEDPTEITLLYANVSEGDIVRDEIDALAKKDP RFTVHYVL	b5R/S.pombe (NP_587852)
LGMIAAGGTGIAPMYQIMKAIAMD	PHDTTKVSLVFGNVHEEDILLKKELEALVAMKPSQFKIVYYL	Cbr1p/S.cerevisiae (NP_012221)
LGIAGGSGITPVLQVQIIPSN	PEDLTHISLIYANETEDDILMKSQLDHMAKEYPHFKVHYVIH	b5R/S.cerevisiae (NP_013623)
LGIVAGGSGITPVLQIQLNEIITV	PEDLTKVSLLYANETENDILLKDELDEMAEKYPHFQVHYVVH	b5R/S.cerevisiae (NP_013581)
HGKQKFAKRLAMLAGGTGITPIYQVAQAILKD	PEDHTEMHLVYANHAEDDILLREELDAWAKTHCDRFKVWYVV	nitrate reductase/Glycine (XP_003544771)
NGKQRNARRLAMICGGSGITPMYQIIQAVLRDQ	PEDHTEMHLVYANRTEDDILLRDELDRAAEPDRLKVWYVI	nitrate reductase/corn (2CND)
LISAGVGATPMQAIHLTLAKQN	KSGVTLYACNSAKEHTFAGETAGLIA	nitric oxide dioxygenase (4EH1)
VMLCTGSGIAPFLSILEQPEIRQRFDTNLIVHSVSFPEELIFNDRLAALSEHPLVGEYGHSGRFVPVTT		ferredoxin reductase/Neisseria (5TR9)

NADPH/2	NADPH/3	
DRPIGWKYSSGFVTADMIKEHLPP	PAKSTLILVCGPPPLIQTAAHPNLEKLGYTQDMIFTY*	Cyb5R2 / Human (NP_057313)
DRPPSDWKYSSGFVSADMIKEHLPP	PGEDTLILVCGPPPLIQAAAHPSLEQLSYTKDMIFTY*	Cyb5R2 / Mouse (NP_796190)
DRPPSGWEYSSGFITADMIKEHLPP	PGEATLILVCGPPPLIQEEAHPSLEQLGYTKDMIFTY*	Cyb5R2 / Rat (NP_001014266)
DRAPEAWDYGQGFVNEEMIRDHLPP	PEEEPLVLMCGPPPMIQYACLPNLDHVGHPTERCFVF*	<u>Cyb5R3</u> / Human (NP_000389)
DKAPDAWDYSQGFVNEEMIRDHLRT	PGEEPLILMCGPPPMIQFACLPNLERVGHPKERCFTF*	<u>Cyb5R3</u> / Mouse (NP_084063)
DKAPDAWDYSQGFVNEEMIRDHLPP	PGEETLILMCGPPPMIQFACLPNLERVGHPKERCFTF*	<u>Cyb5R3</u> / Rat (NP_620232)
DRPSEGWKYSEGFVNAAMMKDHLP	ADSDVLLVMCGPPPAMIEKACLPNLLKLGYKKENIFAY*	Cyb5R3 / Zebrafish (NP_001038825)
DRPPVMWSYSSGFINDSMIKENLFP	PGDDSAVLLCGPPPMINFACIPNLEKLEYDPANRLLF*	Cyb5R3 / C.elegans (NP_504638)
DHPPKDWAYSKGFVTADMIREHLP	PGDDVLLVLCGPPPMVQLACHPNLDKLGYSQKMRFTY*	<u>Cyb5R1</u> / Human (NP_057327)
DSPPEDWTYSKGFTADMIQUEHLP	PAEDVLLLCGPPPMVQLACHPNLDKLGYSQKMRFTY*	<u>Cyb5R1</u> / Mouse (NP_082333)
DYPPEDWTYSKGFTADMIQUEHLP	PAEDVLLLICGPPPMVQLACHPNLDKLGYSQKMRFTY*	<u>Cyb5R1</u> / Rat (NP_001013144)
DRAPADWEYSQGFISAEMIQDHLP	PSDDSMILMCGPPPMIQFACNPNLTKLGYRQSQRFA*	<u>Cyb5R1</u> / Zebrafish (NP_956483)
SKASKDWRYSTGHINEEMIKEHLFP	SNEESAVLLCGPPPAMINCACIPNLTKLGHNSENYLIF*	<u>Cyb5R1</u> / C.elegans (NP_504639)
SAPISEWNGKQGHISPALSEFLKRNL	DKSKVLVCICGPVFTEQGV RLLHDLNFSKNEIHSGFTA*	<u>Cyb5R4</u> / Human (NP_057314)
SAPSPEWNGKQGHISRALLSEFLQRSS	ENSRAFLCICGPFTDEGI RLLHDLNFSDDEIHGFTA*	<u>Cyb5R4</u> / Mouse (NP_077157)
SAPSPEWNGKQGHVSALLSEFLQRSL	ENSKVFLCICGPFTDEGI RLLHDLNFSDDEIHGFTA*	<u>Cyb5R4</u> / Rat (NP_596918)
NNPPAGWTGGVGFVSADMIKQHFS	PSSDIKVMCGPPMMNKAMQ GHLETLGYTPEQWFIF*	b5R/slime mold (Physarum, 2EIX)
NNPKGWTQGVGFVSKEIIESRLPS	PSDQTMVIMCGPPMMNKAMT GHLETIGFNESENIFTF*	b5R/slime mold (Dictyostelium, XP_638259)
NQPPEVWDGGVGFVKEMIQTHCPA	PASDIQILRCGPPPMNKAMA ANLEALGYSPEMQFQF*	b5R/Arabidopsis (NP_197279)
NQPPEIWDGGVGFVKEMIQTDFPA	PASDIKILRCGPPPMNKAMA ANLEALGYSQMFAQF*	b5R/Glycine (XP_003556838)
NQPPEVWDGEGGFVSKEIQTTHCPA	PAQDIKILRCGPPPMNKAMA AHLEALGYASEMQFQF*	b5R/Glycine (XP_003520929, 003554059)
NKPPEQWNNGGIGFISKEIIKSHCPE	PAQDIQILRCGPPPMNKAMA THLDALGYTSNMQFEF*	b5R/Glycine (XP_003531792)
NNPENWKGSVGFVTQELIKAHPA	PSPETKVLICGPTPMVNSLR EATVALGYEKSRAISKLEDQVFV*	b5R/S.pombe (NP_587852)
DSPDREDWTGGVGYITKDVKEHLPA	ATMDNVQILICGPPAMVASR RSTVDLGFRRSKPLSKMEDQVFV*	Cbr1p/S.cerevisiae (NP_012221)
KPNGKNGDVGVTLEEMKRYLPK	QAEDHLLICGPPKMENML NYAKELGWSNGFHKGNGTDKVFVF*	b5R/S.cerevisiae (NP_013623)
YPSDRWTGDVGYITKDQMNRYLPE	YSEDNRLLICGPDGMNNLAL QYAKELGWVNSTRSSGDDQVFVF*	b5R/S.cerevisiae (NP_013581)
GIAKEGWQYSVGRITESIMREHLPK	SSSDALALTGPPPMIEFAVQPNLEKMGYDIKN DLLVF*	nitrate reductase/Glycine (XP_003544771)
DQVKRPEEGWKYSVGFTEAVLREHVP	GGDDTLALACGPPPMIQFAISPNEKMKYDmansfv*	nitrate reductase/corn (2CND)
QQGWMQQVWYRDESADDVILQGEMQLAELILPIEDGDFYLCGPIGFQYVVKQLLA LGVDKARIHYEVFGPHAQAA*		nitric oxide dioxygenase (4EH1)
RAANPSGLSGKRIPELLKNNSIEQALHTKLTP	ESTRFMICGNPEMVKDTFQTLLD MGYAMHRNRIPGGIMMENG*	ferredoxin reductase/Neisseria (5TR9)