

Crystal structure of the prolyl-acyl carrier protein oxidase involved in the biosynthesis of the cyanotoxin anatoxin-a

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Supporting Information. Figures S1 to S10.

Figure S1. Sequence of wild-type AnaB and of the recombinant histidine-tagged AnaB and their calculated average molecular weight. The N-terminal sequence that has been added after cloning into the pET-28 vector is in bold type.

>AnaBwt

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MDFAWNSQQIQFRKKVIQFAQQSLISDLIKNDKEEIFNRDAWQKCSEFGV 50
HGWPIPARYGGQELDILTTAYALQGLGYGCKDNGLIFAMNAHIWACEMPL 100
LTFGTEEQKEKYLPLLCRGGWIASHAATEPQAGSDIYSLKTTAQKDGDKY 150
ILNGYKHYVTNGTIADLFIIIFATIDPSLGKEGLTTFMIEKDTPGLILSKP 200
ISKMGMRTAEVPELRLLENCEVSAANRLGEEGTGLAIFNHSMEWERGFILA 250
AAVGTMERLLEQSIRYARSHKQFGQAIGKFQLVANKLVEMKLRLLENAKAY 300
LYKVAWMKENKQMALLEASMANLYISEAWVQSCLEAIEIHGAYGYLTNTE 350
LERELRDAIASKFYSGTSEIQRVVIAKFLGL 381
```

Mw (average mass): 42 859.30 Da

>H6AnaB

```
MGSSHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFDFAWNSQQIQFRKK 50
VIQFAQQSLISDLIKNDKEEIFNRDAWQKCSEFGVHGWPIPARYGGQELD 100
ILTTAYALQGLGYGCKDNGLIFAMNAHIWACEMPLLTFGTEEQKEKYLPL 150
LCRGGWIASHAATEPQAGSDIYSLKTTAQKDGDKYILNGYKHYVTNGTIA 200
DLFIIIFATIDPSLGKEGLTTFMIEKDTPGLILSKPISKMGMRTAEVPELR 250
LENCEVSAANRLGEEGTGLAIFNHSMEWERGFILAAAVGTMERLLEQSIR 300
YARSHKQFGQAIGKFQLVANKLVEMKLRLLENAKAYLYKVAWMKENKQMAL 350
LEASMANLYISEAWVQSCLEAIEIHGAYGYLTNTELERELRDAIASKFYS 400
GTSEIQRVVIAKFLGL 416
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Mw (average mass): 46 417.10 Da

Figure S2. AnaB elution on a calibrated gel filtration column. A: AnaB was first purified on a nickel affinity column, concentrated by ultrafiltration and loaded (2 mg) on a Superdex S200 column (see the Materials and Methods section for details). B: the column was calibrated with commercial molecular weight markers and the logarithm of the molecular weight was plotted against the constant K_{av} . C: the Stokes radius was plotted against the constant K_{av} . The apparent molecular weight of AnaB was 151 kDa and its apparent Stokes radius was 4.67 nm. The molecular weight markers were the followings:

Protein	Mw (kDa)	R_s (nm) ^a
Ferritin	440	6.10
Aldolase	158	4.81
Conalbumin	75	3.64
Ovalbumin	43	3.05
Carbonic anhydrase	29	2.30
Ribonuclease	13.7	1.64

^aData taken from: Erickson, H. P. (2009) Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. *Biol. Proced. Online* 11, 32-51.

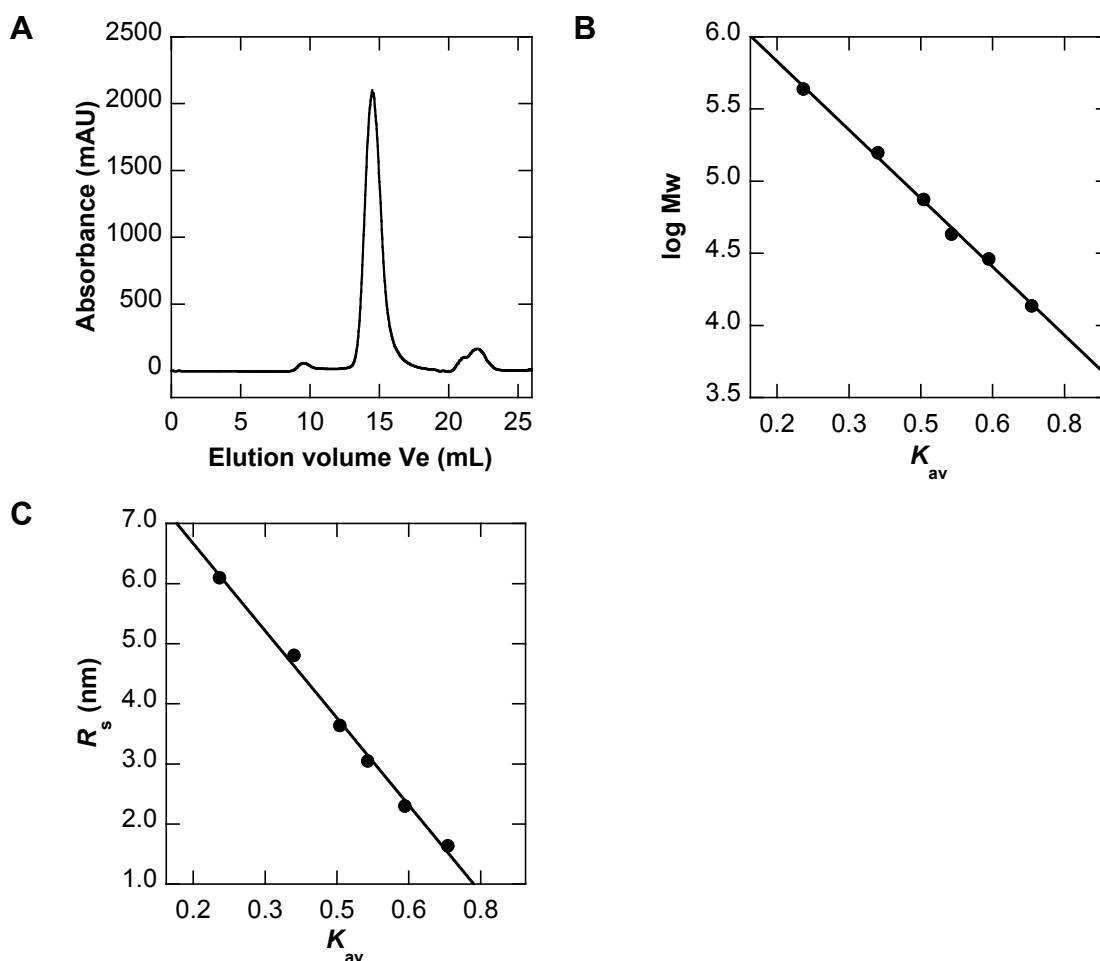


Figure S3. Multiple alignment (CLUSTAL X)¹ of the sequence of AnaB with that of its homologs for which a three-dimensional structure has been published (the sequences were downloaded from the PDB website). A star indicates identity, a colon strong similarity, and a dot weak similarity. The corresponding dendrogram is shown (drawn using NJPlot).² The enzyme names and sources are collected in a Table, below.

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3MKH	-----MAIDFHLSASQK	12
3D6B	-----MAAATFH--WDDPLLLDQQLADDER	23
1SIQ	-----EFD--WQDPLVLEEQLTDEI	19
2IX6	MAVLSSADRASNEKKVKSSYFDLPPMEMSVAFPOATPASTFPPCTSDYYHFNDLLTPEEQ	60
1IVH	-----HSLLPVDDAINGLSEEQR	18
AnaB	-----MDFAWN---SQOI	10
1RX0	-----MVQTGHRSLTSCIDPSMGLNEEQK	24
3MDD	-----GFSFELTEQOK	11
1JQI	-----LHSVYQSVLPEHQ	15
1BUC	-----MDFNLTDIQQ	10
3B96	-----ESKSFVGMFKGQLTTDQVFPYPSVLNEEQT	31
3MPI	-----MDFNLSKELQ	10
1W07	-----MEGIDHLADERN	12
2FON	-----MRGSHHHHHGSACELVREMEGVLDYLERK	31
1R2J	-----MP	2
3DJL	-----MHWQTHTVFNQPIPLNNSNLYLSDGALCEAVTREGAGWDSDFLASIGQQ	49
3D9G	EARRHAQAFAN--TVLTKASAEYSTQK---DQLSRFQATRPFYREAVRHGLIKAQVPIPL	65
3MKH	GTYYAARSLAR--NLLMPARQTYLQHPP--NSPLRFQSTQPTYAAAVSAGILKQGISPAH	68
3D6B	MVRDAAHAYAQ--GKLAPRVTEAFRHE-----TTD--AAIFREMGEIGLLGPTIPEQY	72
1SIQ	LIRDTRFTYCQ--ERLMPRILLANRNE-----VFH--REIISEMGELGVLGPTIKG-Y	67
2IX6	AIRKKVRECME--KEVAPIMTEYWEKA-----EFP--FHITPKLGAMGVAGGSIKG-Y	108
1IVH	QLRQTMAKFLQ--EHLAPKAQEIDRSN-----EFKNLREFWKQLGNLGLGITAPVQY	69
AnaB	QFRKKVIQFAQ--QSLISDLIKNDKEE-----IFN--RDAWQKCSEFGVHGWPPIPARY	59
1RX0	EFQKVAFDFAA--REMAPNMAEWDQKE-----LFP--VDVMRKAQQLGFGGVYIQTDV	73
3MDD	EFQATARKFAR--EEIIPVAAEYDRTG-----EYP--VPLLKRAWELGLMNTHIPESF	60
1JQI	MLRQTCRDFAE--KELVPIAAQLDKEH-----LFP--TSQVKKMGELGLLAMDVPEEL	64
1BUC	DFLKLAHDFGE--KKLAPTVTTERDHKG-----IYD--KELIDELLSLGITGAYFEEKY	59
3B96	QFLKELVEPVSRFFEEVNDPAKNDALD-----MVE--ETTWQGLKELGAFGLQVPEL	82
3MPI	MLQKEVRNFVN--KKIVPFADQWDNEN-----HFP--YEEAVRPMGELGFFGTVIPEEY	60
1W07	KAEPDVEDMKIVWAGSRHAFVSDRIAR-----LVASDPVFEKSNRRLSRKELFKSTL	66
2FON	KAGFDVDEMKIVWAGSRHDFELTDRI SK-----LVASDPGFSKEGRMTLPRKELFKNTL	85
1R2J	ERDALLTDLVG-----DRAAEWDTSG-----ELP--RDLLVRLGADGLLCAEVAAEH	47
3DJL	LGTAESELELGRLANVNPPELLRYDAQRRLLDVRFHFAWHLMLQALCTNRVHNLAWEEEDA	109
3D9G	GGTM---ESLVHESIILEELFAVEPATSIITIV---ATALGLMPVILCDSPSLQEKFLKPF	119
3MKH	GGTG---GTLIESAILVEECYSVEPSAALTIF---ATGLGLTPINLAAGP-QHAEFLLAPF	121
3D6B	GGPG---LDYVSYGLIAREVERVDSGYRSMS--VQSSLVMVPIFEFGSDAQKEKYLKPL	127
1SIQ	GCAG---VSSVAYGLLARELERVDSGYRSAMS--VQSSLVMHPIYAYGSEEQRQKYLPL	122
2IX6	GCPG---LSITANAIATAEIRVDASCSTFIL--VHSSLGMLTIALCGSEAQKEKYLPSL	163
1IVH	GGSG---LGYLEHVLVMEEISRASGAVGLSYG--AHSNLCINQLVRNGNEAQKEKYLKPL	124
AnaB	GGQE---LDILTAYALQGLGYGCKDNGLIFAMNAHIWACEMPLLTFGTTEEQKEKYLPL	116
1RX0	GGSG---LSRLDTSVIFEALATGCTSTTAYIS--IHN-MCAWMIDSFNNEEQRHKFCPPL	127
3MDD	GGLG---LGIIDSLITEELAYGCTGVQTAIE--ANT-LGQVPLIIGGNYQQQKYLGRM	114
1JQI	SGAG---LDYLAYSIALEEISRGCASTGVIMS--VNNSLYLGPILKFGSSQQQOWITPF	119
1BUC	GGSGDDGDVLSYIILAVEELAKYDAGVAITLS--ATVSLCANPIWQFTEAQKEKFLVPL	117
3B96	GGVG---LCNTQYARLVEIVGMHDLGVGITLG--AHQSIGFKGILLFGTKAQKEKYLKPL	137
3MPI	GGEG--MDQGWLAAMIVTEEIIARGSSALRVQLN--MEVLGCAYTILTYGSEALKKKYVPKL	117
1W07	RKCAHAFKRI IELRLNEEEAGRLRHFIQPAYVDLHWGMFVPAIKGQGTEEQKWLKSLA	126
2FON	RKAAAYAWKRI IELRLSQEEATMLRRYVDEPAFTDLHWGMFIPAIKGGQTDKQEKWPLA	145
1R2J	GGLG---LGSRENGETAHVGLSCSSLRVMT---SQGMAAWTVQRLGDAGQRATFLKEL	101
3DJL	RSGAFVARAARFMLHAQVEAGSLCPIITMTFAATPLLLQMLPAPFQDWTTPLLSDRYDSSL	169

3D9G ISGEGE--PLASLMHSEPNGTANWLQKGGPGLQTTARKVGNVNEWVISG-----EKLWPSNS 172
3MKH LSGEVS--PLASLVFSEPPGVANALEKGGPGLQTTARLEGDEWVING-----EKMWATNC 174
3D6B ATGE----WLGCFGLTEPNHGS---DPGSMVTRARKVPG--GYSLSG-----SKMWITNS 173
1SIQ AKGE----LLGCFGLTEPNHGS---DPSSMETRAHYNSSNKSYSYTLNG-----TKTWITNS 170
2IX6 AQLN---TVACWALTEPDNGS---DASGLGTATKVEG--GWKING-----QKRWIGNS 209
1IVH ISGE----YIGALAMSEPNAGS---DVVSMKLAKE--KKGNYHILNG-----NKFWITNG 170
AnaB CRGG----WIASHAATEPQAGS---DIYSLKTTAQ--KDGDKYILNG-----YKHVVTNG 162
1RX0 CTME----KFASYCLTEPSSGS---DAASLLTSAK--KQGD--HYILNG-----SKAFISGA 173
3MDD TEEP----LMCAYCVTEPAGS---DVAGIKTKAE--KKGD--EYIING-----QKMWITNG 160
1JQI TNGD----KIGCFALSEPNGS---DAGAASTAR--EEGD--SWVLNG-----TKAWITNS 165
1BUC VEGT----KLGAFGLTEPNAGT---DASGQQTAT--KNDDGTYTLNG-----SKIFITNG 164
3B96 ASGE----TVAAFCLTEPSSGS---DAASIRTSAPVSPCGKYITLNG-----SKLWISNG 185
3MPI SSAE----FLGGFGITEPDAGS---DVMMSSTAE--DKGDHLLNG-----SKTWISNA 163
1W07 NKMQ----IIGCYAQTELGHGS---NVQGLETTATLDPKTDDEFVHPTPTQASKWPPGGL 179
2FON YKMQ----IIGCYAQTELGHGS---NVQGLETTATLDPKTDDEFVHPTPTLSSKWWPPGGL 198
1R2J TSGK----LAAVGFSEPOAGS---DLSAMRTRVR--LDGDTAVVDG-----HKVWTAA 146
3DJL LPGAQKRGLLIGMGMTEKQGGG---DVMSNTTRAERLEDG--SYRLVG-----HKWFFSV 219

. : * : : . . : : :

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3MKH AGWDFKGCDDLACVVCVCR---DATTPLEEGQDPENKVMILVTRADLDRNGEGS--FEVLRHV 230
3D6B P-----IADVFVWAKLD-----EDGRDEIRGFIELEKCKG-----LSAPAIH 211
1SIQ P-----MADLFVWVWARC-----EDG--CIRGFLEKGMRG-----LSAPRIQ 205
2IX6 T-----FADLLIIFARNT-----TTN--QINGFIVKDDAPG-----LKATKIP 245
1IVH P-----DADVLIVYAKTDL---A---AVPASRGITAFIVEKGMPPG-----FSTSKKL 211
AnaB T-----IADLFIIIFATIDP---S---LG--KEGLTFMIEKDTPG-----LILSKPI 201
1RX0 G-----ESDIYVVMCIRTG-----GPGPKGISCIIVVEKGTTPG-----LSFGKKE 211
3MDD G-----KANWYFLLARSDPPD-----KAPASKAFVGFIVEADTPG-----VQIGRKE 202
1JQI W-----EASATVVFASD-----RSRQNKGISAFVLPMPPTPG-----LTLGKKE 204
1BUC G-----AADIYIVFAMTD-----KSKGNHGI TAFIILEDGTPG-----FTYGKKE 203
3B96 G-----LADIFTVFAKTPVTDPA---TGAVKEKIFAFVVERGFVG-----ITHGPPE 229
3MPI A-----QADVLIYYAYTDK-----AAGSRGLSAFVIEPRNFP-----GIKTSNL 202
1W07 G-----KVSTHAVVYARLIT-----NGKDYGIHGFIVQLRSLEDHSPNITVGDIG 226
2FON G-----KVSTHAVVYARLIT-----DGKDYGVNGFIVQLRSLEDHKLPLPGVTVGDIG 245
1R2J A-----YADHLVVVFLQEQ-----DGSAGVVVVPADTPG-----VRVERVP 181
3DJL P-----QSDAHLVLAQTAG-----GLSCFFVPRFLPD--GQRNAIRLERLK 258

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3D9G ELAG---HITTSGPHTRFTEFHVPHENLLCTPGLKAQGLVETA-----FAMSAA 277
3MKH ATPG---HTSVSGPHVRYTNRVPTKNVLCVAGQGA--VAFGA-----FDGSAV 275
3D6B GKVG---LRASITGEIVLDEAFVPEENILPHVKG--LRGPFTC-----LNSARY 255
1SIQ GKFS---LRASATGMIIMDGVEVPEENVLPGASS--LGGPFGC-----LNNARY 249
2IX6 NKIG---LRMVQNGDILLQNVFPDEDRLPVNS--FQDTSKV-----LAVSRV 289
1IVH DKLK---MRGNTCELIFEDCKIPAANILGHENKGVVVLMSG-----LDLERL 256
AnaB SKMG---MRTAEVPELRELENCEVSAANRLGEEGTGLAIFNHS-----MEWERG 246
1RX0 KKVQ---WNSQPTRAVIFEDCAVPVANRIGSEGGFLIARV-----LNGGRI 256
3MDD INMG---QRCSDRGIVFEDVRVPKENVLTGEGAGFKIAMGT-----FDKTRP 247
1JQI DKLK---IRASSTANLIFEDCRIPKENLLGEPGMGFKIAMQT-----LDMGRI 249
1BUC DKMG---IHTSQTMELVFQDVKVAENMLGEEGKGFKIAMMT-----LDGRI 248
3B96 KKMV---IKASNTAEVFFDGVRVPSENVLGEVGSFGKAMHI-----LNNGRF 274
3MPI EKLK---SHASPTGELFLDNVVKPENILGKPGD GARIVFGS-----LNHTRL 247
1W07 TKMNGAYNSMDNGFLMFDHVRIPRQMLMRLSKVTREGEVYVSDVPKQLVYGTMVYVRQ 286
2FON MKFGNGAYNSMDNGVLSFDHVRIPRQMLMRVSVQTKGKYVQSDIPRQLLYGTMVYVRQ 305
1R2J KPSG---CRAAGHADLHLDQVRVPAGAVLAGSGASLPMVAAS-----LAYGRK 227
3DJL DKLK---NRSNASCEVEFQDAIG---WLLGLEGEGIRLILKMG-----GMTRF 300

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3D9G LVGAMAIGTARAFAFEALVFAKSDTRGGS-----KHIEHQSVADKLIDCKIRLETSRL 331
3MKH LVGAMGVGLMRAAFDAALKFAKEDNRGGA-----VPLLERQAFADLLSGVKIQTEAARA 329
3D6B GIAWGALGAAESCWHIARQVYVLDKQFG-----RPLAANQLIQKKLADMQTEITLGLQ 308
1SIQ GIAWVGLGASEFCLHTARQYALDRMQFG-----VPLARNQLIQKKLADMLTEITLGLH 302
2IX6 MVAWQPIGISMGIYDMCHRYLKERKQFG-----APLAAFQLNQKLVQMLGNVQAMFL 342
1IVH VLAGGFLGLMQAVLDHTIPYLHVREAFG-----QKIGHFQLMQGMADMYTRLMACRQ 309
AnaB FILAAAVGTMERLLEQSIYARSHKQFG-----QAIGKFLVANKLVEMKLRLENAKA 299
1RX0 NIASCSLGAHAHVSILTRDHLNVRKQFG-----EPLASNQYLQFTLADMATRLVAARL 309
3MDD PVAAGAVGLAQRALDEATKYALERKTFG-----KLLAEHQGISFLLADMAMKVELARL 300
1JQI GIASQALGIAQASLDCAVKYAENRHAFG-----APLTKLQNIQFKLADMALALESARL 302
1BUC GVAAQALGIAEAALADAVEYSKQVQFG-----KPLCKFQISIFKLADMAMKQIEAARN 301
3B96 GMAAALAGTMRGIIAKAVDHATNRQFG-----EKIHNFLIQEKLARMVLMQYVTE 327
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1W07 TIVADASNLSRAVCIATRYSAVRRQFGAHNGGIETQVIDYKTOQNRLFPLASAYAFRF 346
2FON SIVADASLMSRAVCIATRYSAVRRQFGSQQNGQETQVIDYKTOQNRLFPLASAYAFRF 365
1R2J SVAWCVGILRACRTAAVAHARTREQFG-----RPLGDHQLVAGHIADLWTAEQIAAR 280
3DJL DCALGSHAMRRRAFLAIYHAHQHVFG-----NPLIQQLMRHVLSRMALQLEGQTA 353

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3MKH LTKWAAHAMENPG-----DYDARRELALAAKVFCEAAVKACTDVINAVGISAYDL 381
3D6B GVLRLGRMKD-----EGTAAVEITSIMKRNSCGKALDIARLARMDLGGNGISD 356
1SIQ ACLQLGRLKD-----QDKAAPMVSLLRNCGKALDIARQARDMLGGNGISD 350
2IX6 MGWRLCKLYE-----TGQMPGQASLGKAWISSKARETASLGRELLGGNGILA 390
1IVH YVYNVAKACD-----EGHCTAKDCAGVILYSAECATQVALDGIQCFGGNGYIN 357
AnaB YLYKVAWMKE-----NKQMALLEASMANLYISEAWVQSCLEAIEIHGAYGYLT 347
1RX0 MVRNAAVALQE-----ERKDAVALCSMAKLFATDECFAICNQALQMHGGYGYLK 358
3MDD SYQRAAWEIDS-----GRR-NTYYASIAKAYAADIANQLATDAVQVFGGNGFNT 348
1JQI LTWRAAMLKDN-----KKP-FTKESAMAKLAASEAATAISHQAIQILGGMGYVT 350
1BUC LVYKAACKQE-----GKP-FTVDAAIKRVASDVAMRVTEAVQIFGGYGYSE 349
3B96 MAYMVSANMDQ-----GATDFQIEAAISKIFGSEAAWKVTDCEIQIMGGMGFMK 376
3MPI LAYKAAAAKDE-----GRLNGLDVAMAKYAAGEAVSKCANYAMRILGAYGYST 349
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1R2J VCEYASDHWDE-----GSPMVPATILAKHVAERAAGAATAAQVVLASAGARE 329
3DJL LLFRLARAWDRR-----ADAKEALWARLFTPAAKFVICKRGMFPVAEAMEVLGGIGYCE 407

3D9G DMSFPRLLNEVMCYPLFDGGNIGLRRRQMQRVMALEDYEPWAATYGSSEKVDKSRLL----- 438
3MKH QRPFDLLNTAVVLP IFDGGNVGIRRRHLQQLMLKPTYDAWSSTYGSFPGSHHHHHH--- 438
3D6B EFGVARHLVNLVNTYEGTHDIHALILGRAQTGQIAFF----- 395
1SIQ EYHVIRHAMNLEAVNTYEGTHDIHALILGRAITGQIAFTASK----- 392
2IX6 DFLVAKAFCDLEPIYTYEGTYDINTLVTGREVTGIA SFKPA TRSRLLDDDGKLEHHHHHHH- 449
1IVH DFPMPGRFLRDAKLYEIGAGTSEVRRLVIGRAFNDHF----- 394
AnaB NTELERELRDAIASKFYSGTSEIQRVVIAKFLGL----- 381
1RX0 DYAVQYVVRDSRVHQILEGSNEVMRILISRSLLOE----- 393
3MDD EYPVEKLMRDAKIYQIYEGTAQIQRI IAREHIGRYK----- 385
1JQI EMPAERYRDA RITEIYEGTSEIQRLVIAGHLLRSYRS----- 388
1BUC EYPVARHMRDAKITQIYEGTNEVQLMVTGGALLR----- 383
3B96 EPGVERVLRDLRIFRIFEGTNDILRFLVQLQCGMDKKGKELSGLSALKNPFGNAGLLGE 436
3MPI EYPVARFYRDAPTYMVEGSANICKMI IALDQLGVRKANRKGHHHHHHH----- 397
1W07 CSGLPELFAVYVPACTYEGDNVVLQQLQVARFLMKTVAQLGSGKVPVGTAYMGRAAHLQ 466
2FON SSGLPPELFAVYVPACTYEGDNVVLQQLQVARFLMKTISQLGTGKPKVGTVSYMGRIEHLMQ 485
1R2J GHVVERAYRDAKLMEIEGSSEMCRVMLAQHALALPA----- 366
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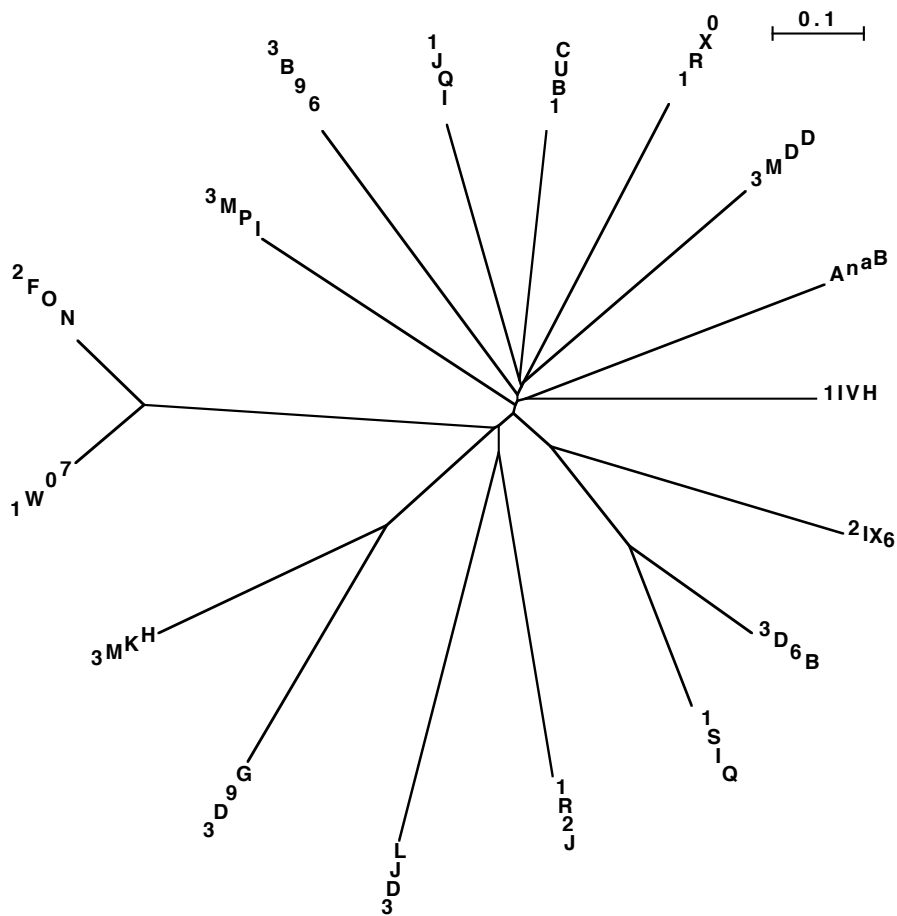
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3D9G -----
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1SIQ -----
2IX6 -----
1IVH -----
AnaB -----
1RX0 -----
3MDD -----
1JQI -----
1BUC -----
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3MPI -----
1W07 CRSGVQKAEDWLNPDVVLVLEAFPEARALRMAVTCANLSKFENQEQGFQELLADLVEAAIAH 526
2FON CRSDVKQAEADWLKPSAVLEAFEARARMVACANLSKFENQEEGFAELAADLVEAAVAH 545
1R2J -----
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3D9G -----
3MKH -----
3D6B -----
1SIQ -----
2IX6 -----
1IVH -----
AnaB -----
1RX0 -----
3MDD -----
1JQI -----
1BUC -----
3B96 LQRLADGAILDYAMVVVLSRASRSLSSEGHPTAQHEKMLCDTWCIEAAARIREGMAALQSD 556
3MPI -----
1W07 CQLIVVSKFIKLEQDIGGKGVKKQLNLCYIYALYLLHKKHLDGFLSTNCITPKQASLAN 586
2FON CQLIVVSKYIEKLOQNI PGKGVKQQLVLCGIYSLFILHKKHGDFLGTGYITSKQGSAN 605
1R2J -----
3DJL QNDLLLRATGGVCV----- 541

3D9G	-----	
3MKH	-----	
3D6B	-----	
1SIQ	-----	
2IX6	-----	
1IVH	-----	
AnaB	-----	
1RX0	-----	
3MDD	-----	
1JQI	-----	
1BUC	-----	
3B96	PWQQELYRNFKSIKALVERGGVVTSNPLGF-----	587
3MPI	-----	
1W07	DQLRSLYTQVRPNAVALVDAFNYYTDHYLNSVLGRYDGNVYPKLFEEALKDPLNDSVVPDG	646
2FON	DQLRALYSQLRPNAVSLVDAFNYYTDHYLGSILGRYDGNVYPKLYEAAWKDPLNKSDIADG	665
1R2J	-----	
3DJL	-----	

3D9G	-----	
3MKH	-----	
3D6B	-----	
1SIQ	-----	
2IX6	-----	
1IVH	-----	
AnaB	-----	
1RX0	-----	
3MDD	-----	
1JQI	-----	
1BUC	-----	
3B96	-----	
3MPI	-----	
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3DJL	-----	

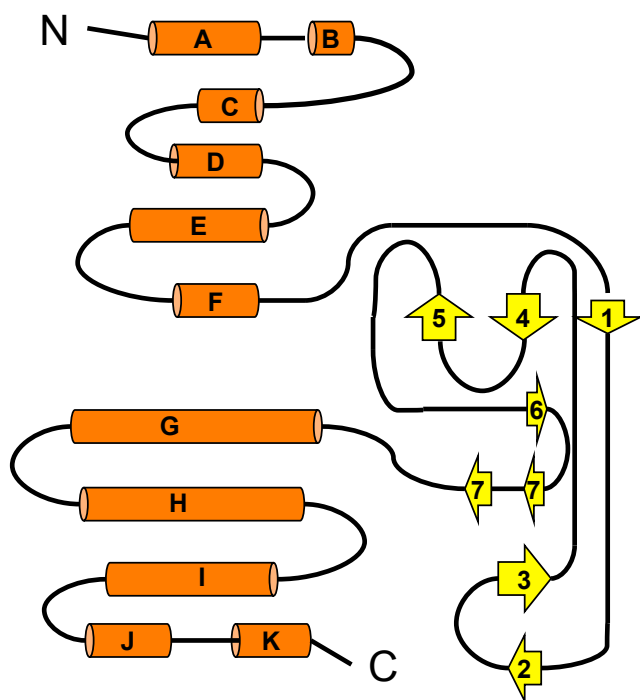


PDB accession	Enzyme	Enzyme class	Source
3D9G	nitroalkane oxidase	NAOX	<i>Fusarium oxysporum</i>
3MKH	nitroalkane oxidase	NAOX	<i>Podospora anserina</i>
3D6B	glutaryl-CoA dehydrogenase	GCD	<i>Burkholderia pseudomallei</i>
1SIQ	glutaryl-CoA dehydrogenase	GCD	<i>Homo sapiens</i>
2IX6	short-chain acyl-CoA oxidase	ACOX4	<i>Arabidopsis thaliana</i>
1IVH	isovaleryl-CoA dehydrogenase	IVD	<i>Homo sapiens</i>
4IRN	prolyl-acyl carrier protein oxidase	AnaB	<i>Oscillatoria sp. PCC 6506</i>
1RX0	isobutyryl-CoA dehydrogenase	IBD	<i>Homo sapiens</i>
3MDD	medium-chain acyl-CoA dehydrogenase	MCAD	<i>Sus scrofa</i>
1JQI	short-chain acyl-CoA dehydrogenase	SCAD	<i>Rattus norvegicus</i>
1BUC	butyryl-CoA dehydrogenase	BCAD	<i>Megasphaera elsdenii</i>
3B96	very long-chain acyl-CoA dehydrogenase	VLCAD	<i>Homo sapiens</i>
3MPI	glutaryl-CoA dehydrogenase	GCD	<i>Desulfococcus multivorans</i>
1W07	acyl-CoA oxidase 1	ACOX1	<i>Arabidopsis thaliana</i>
2FON	acyl-CoA oxidase 1	ACOX1	<i>Solanum lycopersicum</i>
1R2J	acyl-ACP dehydrogenase, FkbI	ACAD	<i>Streptomyces hygroscopicus</i>
3DJL	alkylation response protein, AidB	ACAD	<i>Escherichia coli</i>

(1) Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, *Nucleic Acids Res.* 22, 4673-4680.

(2) Perrière, G., and Gouy, M. (1996) WWW-query: An on-line retrieval system for biological sequence banks, *Biochimie* 78, 364-369.

Figure S4. An artist view of the topology of AnaB secondary structure. α -Helices are represented by cylinders, and β -strands by arrows, at approximate scale. The secondary structure elements are labeled using the nomenclature proposed for MCAD (PDB accession: 3MDD).¹



(1) Kim, J. J., Wang, M., and Paschke, R. (1993) Crystal structures of medium-chain acyl-CoA dehydrogenase from pig liver mitochondria with and without substrate, *Proc. Natl. Acad. Sci. U. S. A.* 90, 7523-7527.

Figure S5. Stereo view of Figure 1D. Superposition of AnaB and human IVD monomers. AnaB is colored as in Figure 1A, IVD chain in grey, and the FAD as in Figure 1A. Helices A, B, D, E and G where the main differences between AnaB and IVD fold are visible, are highlighted while the rest of the chains are shown in transparent cartoons.

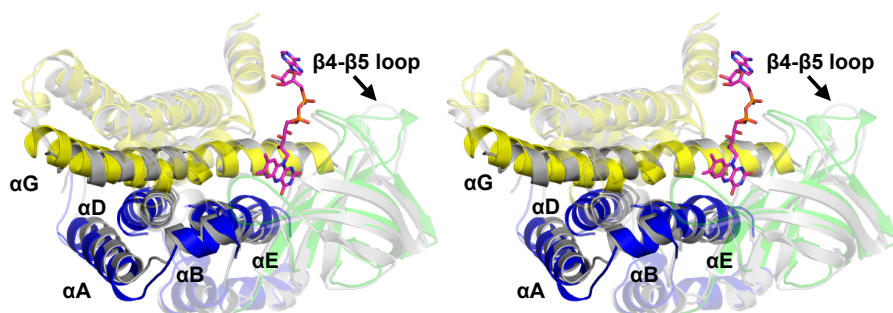


Figure S6. Stereo view of the superposition of the α -E α -G region in the structure of AnaB with that of MCAD. A: Helices E and G (with the catalytic base E244) of AnaB are represented in blue and yellow, respectively, while helices J and K with their connecting loop are colored in orange. The superimposed structure of MCAD is displayed in grey (with the catalytic base E376). The residues responsible for making the substrate pocket shallower in AnaB and the corresponding residues in MCAD are labeled and shown in sticks. The distance between the C α atoms of I248 and N90 in AnaB is 5.4 Å, and the corresponding distance in MACD (between and V259 and E99) is 8.0 Å. For clarity the ligands are not represented in A. B: The cofactor FAD of AnaB and the octanoyl-CoA substrate of MCAD are displayed in magenta and grey lines, respectively.

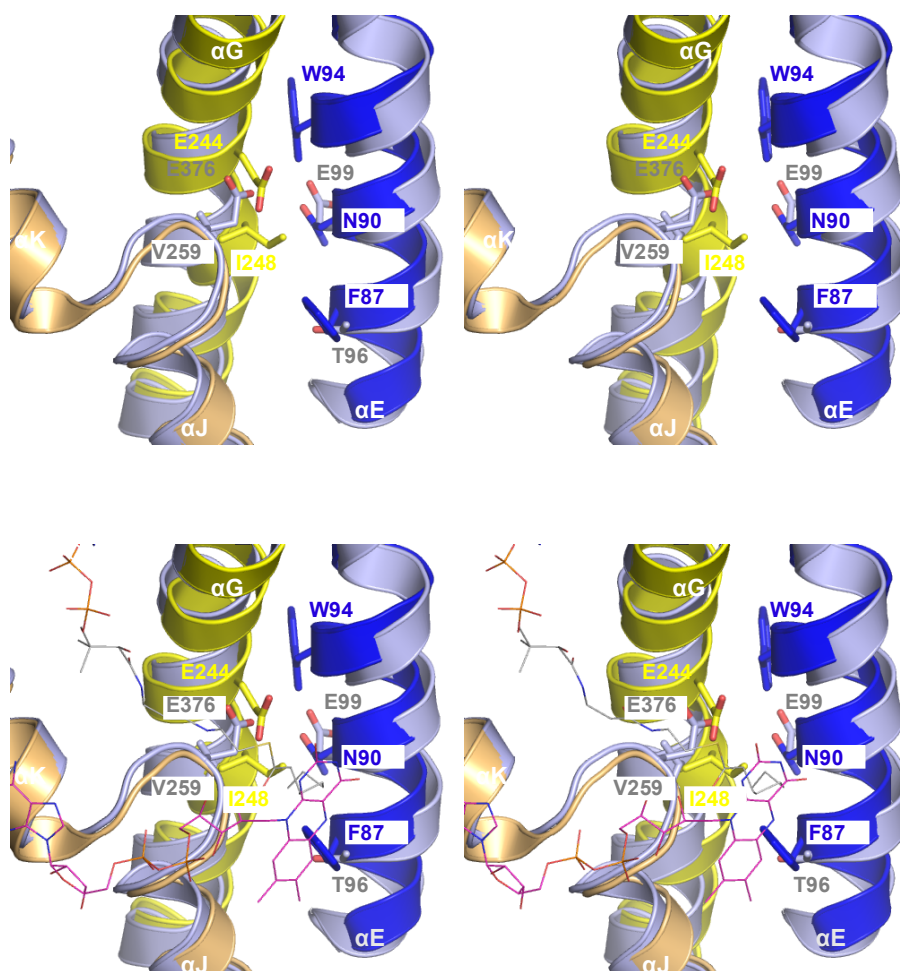


Figure S8. Stereo view of the superposition of AnaB (yellow) and IVD (cyan) monomers in the region surrounding the adenosine moiety of the CoA persulfide ligand (magenta) of IVD. FAD, CoASSH, and important residues are displayed as sticks. Hydrogen bonds are shown as dotted lines. For clarity, the full-length proteins are shown as transparent cartoons. There are two residues of IVD, Ser190 and Arg191 that form hydrogen bonds with the ribose 3'-phosphate of the CoASSH ligand. The corresponding residues in AnaB, Lys180 and Glu181, are strikingly different, and, in the overlaid structure, seem to make steric or charge repulsions, with the adenosine part of CoASSH. In addition, the presence of the bulky residue Tyr137 unique to AnaB when compared to all others ACADs, seems to be incompatible with the binding of the adenosine CoA moiety.

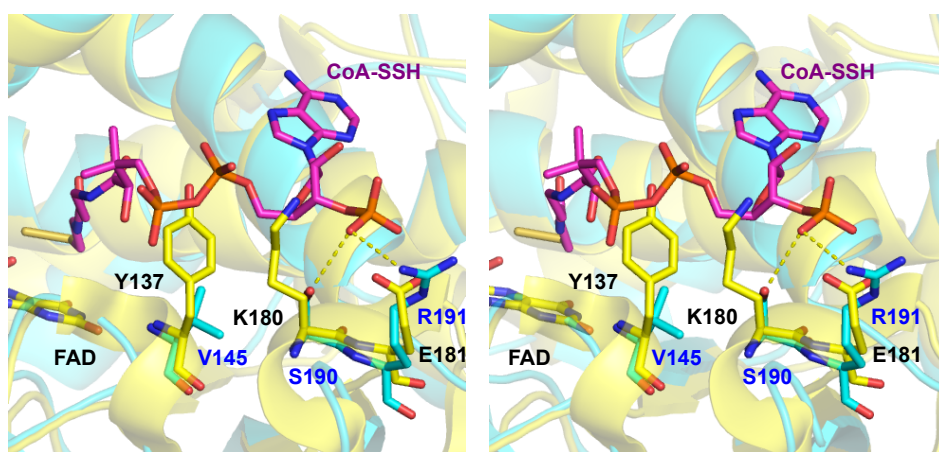


Figure S9. Comparison of the location of His residues near the flavin N5-C4a locus, in AnaB (yellow) and in glucose oxidase (grey, pdb 1GAL). Stereo view.

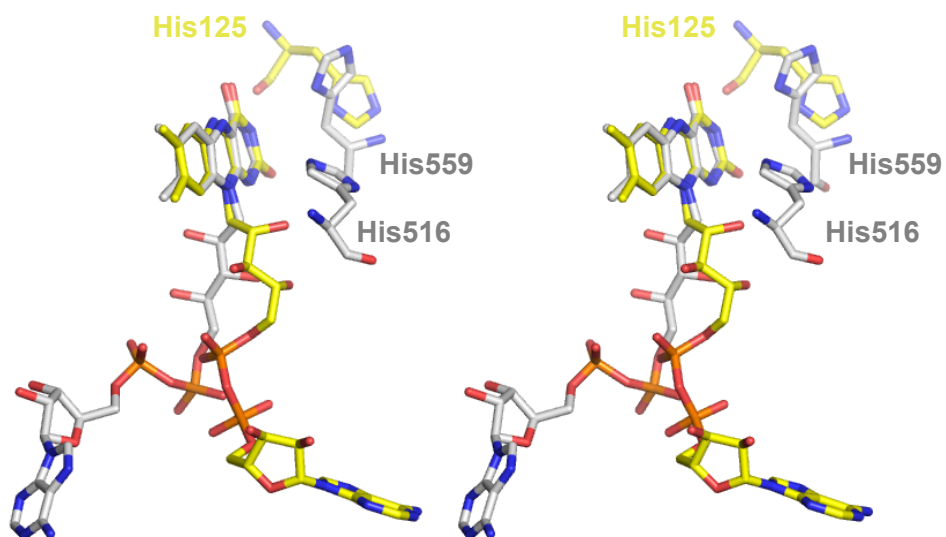
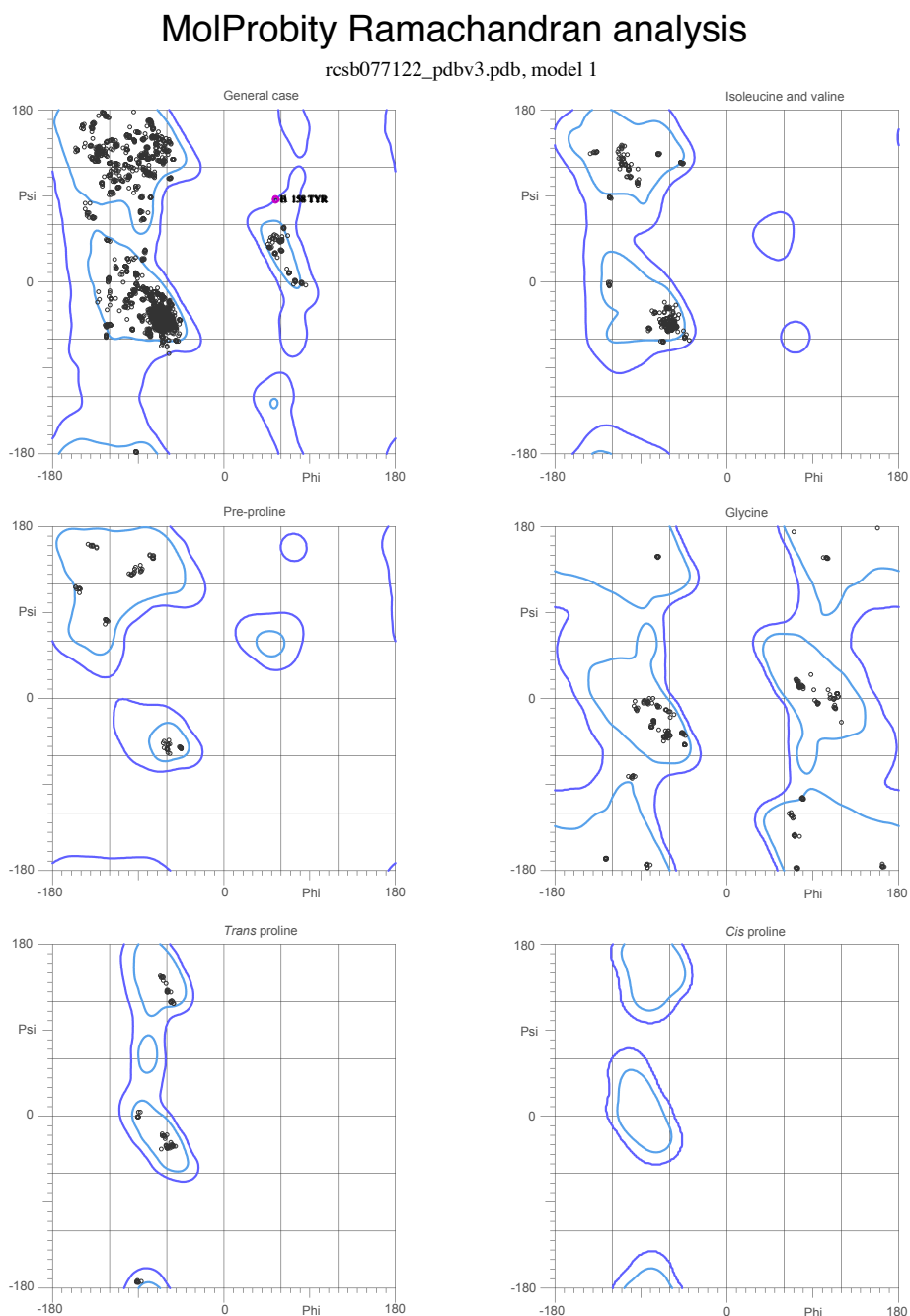


Figure S10. Ramachandran plot for AnaB.



95.6% (2877/3008) of all residues were in favored (98%) regions.
99.8% (3002/3008) of all residues were in allowed (>99.8%) regions.

There were 6 outliers (phi, psi):

- A 158 TYR (55.0, 87.9)
- B 158 TYR (54.8, 87.9)
- D 158 TYR (55.0, 88.0)
- F 158 TYR (55.1, 87.4)
- G 158 TYR (54.8, 87.0)
- H 158 TYR (54.7, 88.0)

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Lovell, Davis, et al. Proteins 50:437 (2003)