Supplementary Figures

Evolution of substrate specificity within a diverse family of β/α-barrel fold basic amino acid decarboxylases: X-ray structure determination of enzymes with specificity for L-arginine and carboxynorspermidine

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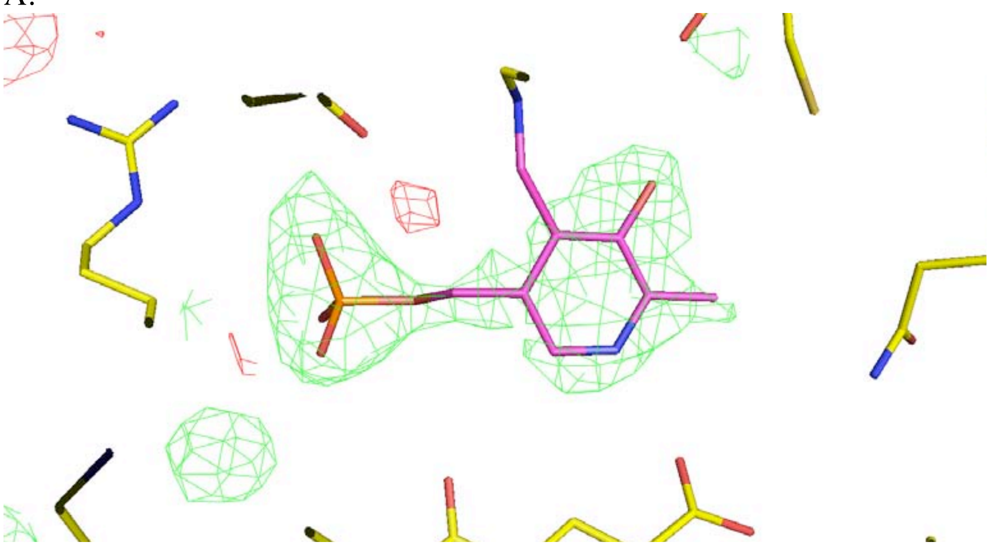
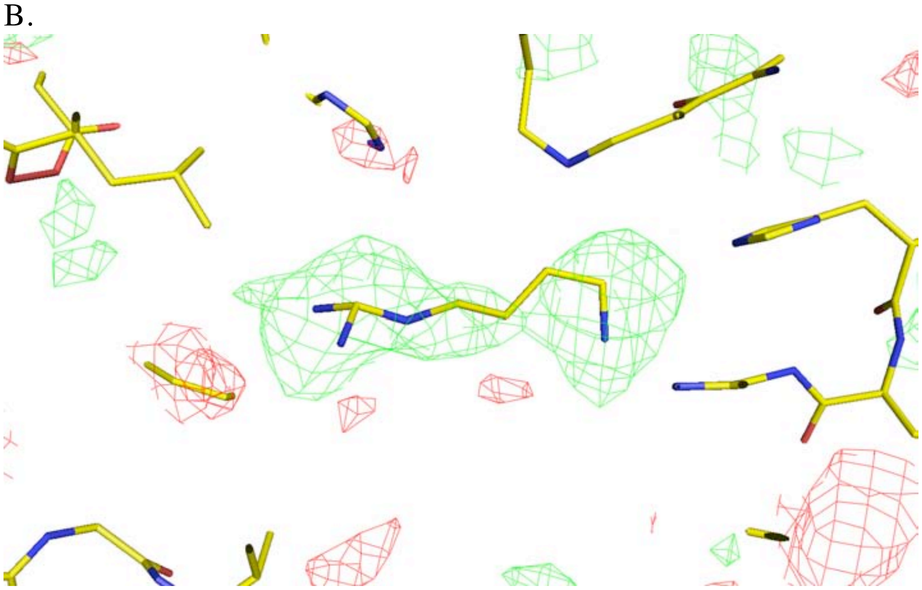
Figure 1S. Fo – Fc maps for *Vv*ADC and *Cj*CANSDC showing the PLP and ligand-binding sites. A) ADC PLP density, B) ADC agmatine density, C)CANSDC PLP density, D) CANSDC norspermidine density E) CANSDC glycerol density. The difference maps for PLP were contoured at ±3.0 sigma and for the other ligands at ±2.0 sigma. The coordinates shown are the final structure models. The maps are plotted before the ligands were built into the model. Positive density is shown in green, negative density in red. PLP is shown in pink, amino acid side chains and bound reaction products are shown in yellow, glycerol is shown in pink.

A.



B.



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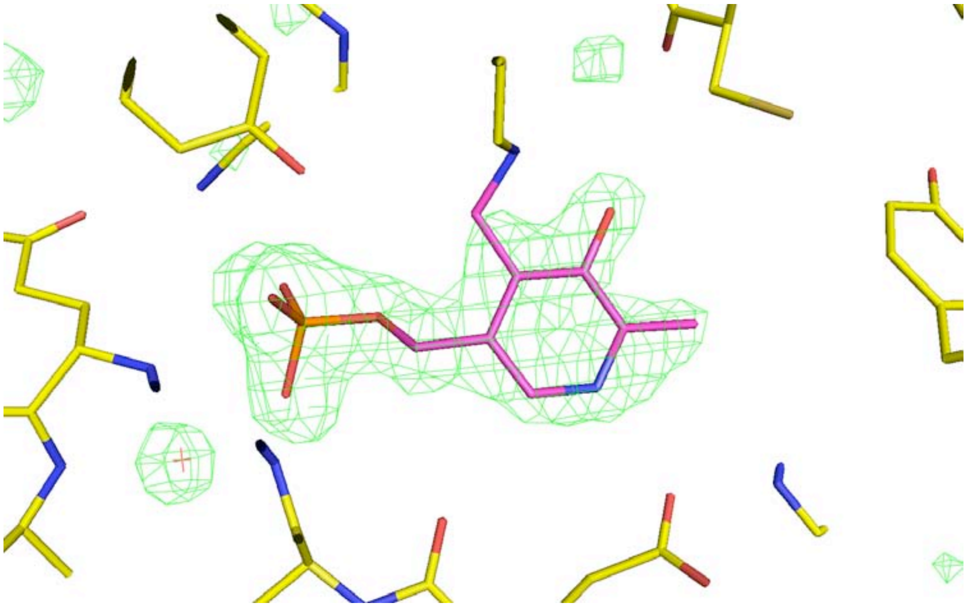

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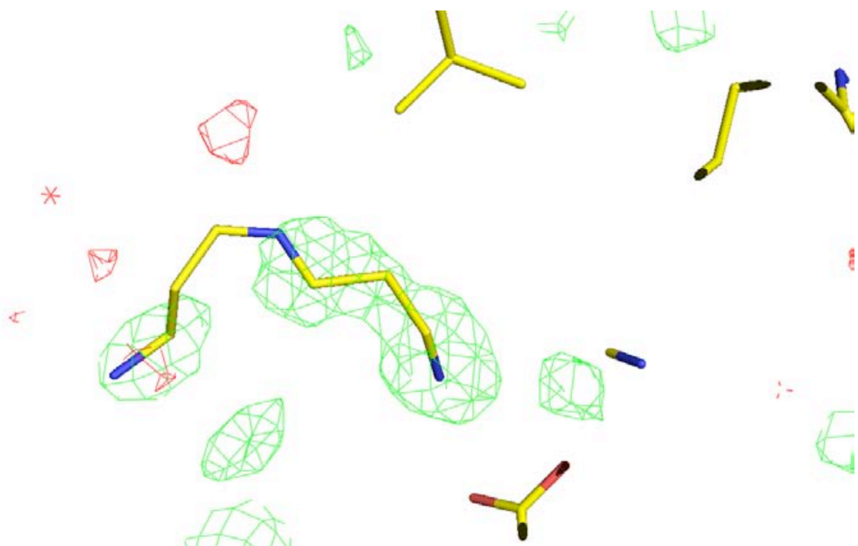

E.

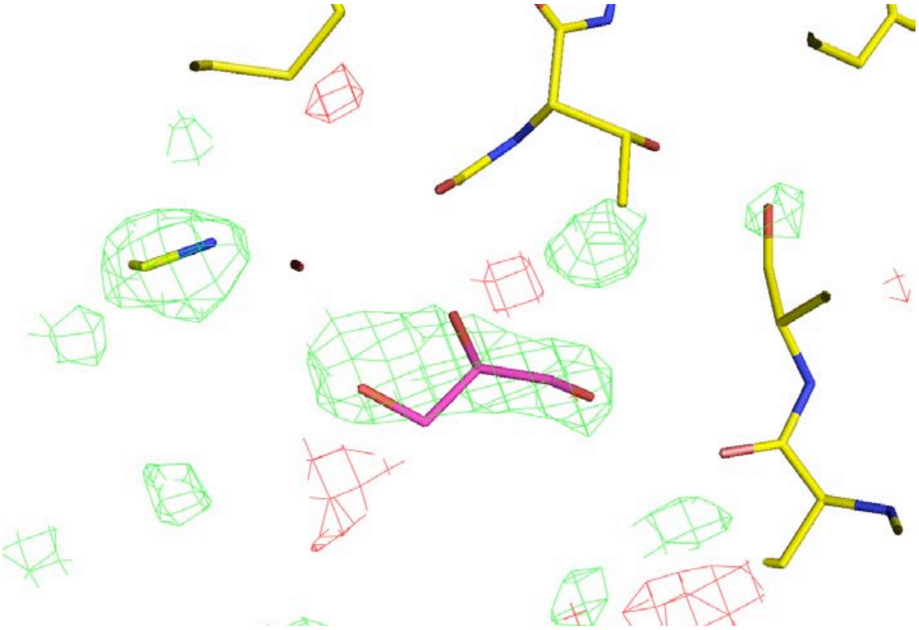

Figure 2S. Structural alignment of the major specificity groups within the basic amino acid β/α-barrel fold family enzymes. X-ray structures are available for all 6 sequences. Alignment was performed as described in Materials and Methods.

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                   α1       α2    β1           β2              α3             β3    α4
VvADC           KLDRVRADYNVHYWSQGFYG----IDDQGEMYVSPRSDNAHQIQLSKIVKQLEERQLNVPVLVRFPQILH   75
TbODC           --------------------RFLE------GFNT-------RDALCKKI-------SGDPFFVADLGDIV   49
CvADC           ---------------------------------M-------NSVVNNILKA---HPQTKSFYVSSPKIVE   28
VvLODC          ------------------SQSIFDIL----SAEE-------IHLIEASVE-----QFGAPLLLLDCDVIR   46
MjDAPDC         -----------MLGN-DTVE----IK-DGRFFID-------GYDAIELAE-----KFGTPLYVMSEEQIK   55
CjCANSDC        -----------------------------------------------FYE-----KIQTPAYILEEDKLR   19
                                                                              β1    α1

                                       β4    α(3-10)1   α5             β5      α6
VvADC           QRVHSICDAFNQAIEEYQYPNKYLLVYPIKVNQQREVVDEILASQAQLETKQLGLEAGSKPELLAVLAMA  145
TbODC           RKHETWKKCLP----------RVTPFYAVKCNDDWRVLGTLAALG-------TGFDCASNTEIQRVRGIG  102
CvADC           DLIDQWTILFP----------RVTPHYAVKCNNDEVLLKTMCDKN-------VNFDCASSSEIKKVIQIG   81
VvLODC          QQYRALKNALP----------NVTLHYALKPLPHPVVVRTLLAEG-------ASFDLATTGEVELVASEG   99
MjDAPDC         INYNRYIEAFKRWEE--ETGKEFIVAYAYKANANLAITRLLAKLG-------CGADVVSGGELYIAKLSN  116
CjCANSDC        KNCELLASVGEKS--------GAKVLLALKGFAFSGAMKIVGEYL-------KGCTCSGLWEAKFAKEYM   74
                                         β2    α(3-10)1 α2             β3        α3

                      β6          α7             β7          α8            β8
VvADC           QHASSVIVCN-GYKDREYIRLALIGEKLGHKVFIVLEKMSELDLVLREAKSLGVTPRLGIRIRLASQ---  211
TbODC           V-PPEKIIYANPCKQISHIRYARDSG----VDVMTFDCVDELEKVAKTHP----KAKMVLRISTDDRL--  166
CvADC           V-SPSRIIFAHTMKTIDDLIFAKDQG----VDIATFDSSFELDKIHTYHP----NCKMILRIRCDD----  138
VvLODC          V-PADLTIHTHPIKRDADIRDALAYG----CNVFVVDNLNELEKFKAYRD----DVELLVRLSF------  154
MjDAPDC         V-PSKKIVFNGNCKTKEEIIMGIEAN----IRAFNVDSISELILINETAKELGETANVAFRINPNVNPKT  181
CjCANSDC        D---KEIHTYSPAFKEDEIGEIASL-----SHHIVFNSLAQFHKFQSKTQ----KNSLGLRCNVEFS---  129
                        β4       α4              β5    α5   α(3-10)2      β6

                                           α9       α(3-10)2 β9              α10
VvADC           ---GAGKWQASGGEKSKFGLS--ASQVLNVISRLKKENQLDTLQLVHFHLGSQMANIRDVRNGVNESARF  276
TbODC           --------------SVKFGAK--VEDCRFILEQAKKLN--IDVTGVSFHVGSGSTDASTFAQAISDSRFV  218
CvADC           -PNATVQL------GNKFGAN--EDEIRHLLEYAKQLD--IEVIGISFHVGSGSRNPEAYYRAIKSSKEA  197
VvLODC          ---------------KKFGCS--PEQALVIIETAKEWN--IRIKGLSFHVGSQTTNPNKYVEAIHTCRHV  215
MjDAPDC         HPKISTGL-----KKNKFGLDVESGIAMKAIKMALEMEY-VNVVGVHCHIGSQLTDISPFIEETRKVMDF  245
CjCANSDC        ------------GRYSRLGIR--AKDFEN--VDL------NAIEGLHFHALCEE-SADALEAVLKVFEEK  186
                                        α6                   β7               α7
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                           β10                               α11                β11
VvADC      YCELRTLG-ANITYFDVGGGLAIDYDGTRSQSSNSMNYGLVEYARNIVNTVGDVCKDYKQPMPVIISESG  345
TbODC      FDMGTELG-FNMHILDIGGGFPGTRDA---------PLKFEEIAGVINNALEKHFP--PDLKLTIVAEPG  276
CvADC      FNEAISVG-HKPYILDIGGGLHADID----------GELSTYMSDYINDAIKDFFP---EDTVTIVAEPG  254
VvLODC     MEQVVERGLPALSTLDIGGGFPVNYTQQ--------VMPIDQFCAPINEALSLLP-----ETVHVLAEPG  272
MjDAPDC    VVELKEEG-IEIEDVNLGGGLGIPYYKDKQ------IPTQKDLADAIINTMLKYKD--KVEMPNLILEPG  306
CjCANSDC   FGKWI----GQMKWVNFGGGHHITK------------KGY--DVEKLIALCKNFSD--K-YGVQVYLEPG  235
            α3-10 3       β8                                    α8                β9

             α12     β12                          α13                   α14
VvADC      RSLTAHHAVLISNVIGTETYKPETVTEPEEDFPLLLNNMWRSWLNLHNGTDARALIEIYNDTQSDLAEVH  415
TbODC      RYYVASAFTLAVNVIAKKVT--------------------------------------------------  296
CvADC      RFFAEHYSVLATQVIGKRVR--------------------------------------------------  274
VvLODC     RFICAPAVTSVASVMGQAER--------------------------------------------------  292
MjDAPDC    RSLVATAGYLLGKVHHIKET--------------------------------------------------  326
CjCANSDC   EAVGWQTGNLVASVVDIIEN--------------------------------------------------  255
            α9          β10

                        α15                          α16           β13     α17   α18
VvADC      SQFATGVLTLEHRAWAEQTSLRIYYELNRLMSTKNRFHRPILDELSERLADKFFVNFSLFQSLPDSWGID  485
TbODC      ------------------------------------------------QSFMYYVNDGVYGSFNCILYDH  333
CvADC      -----------------------------------------------DGLYEYFFNESTYGGFSNVIFEK  297
VvLODC     -----------------------------------------------EGQIWYYLDDGIYGSFSGLMFDD  315
MjDAPDC    -----------------------------------------------PVTKWVMIDAG-MNDMMRPAMYE  348
CjCANSDC   ------------------------------------------------EKQIAILDTSSEAHMPDTIIMP  277
                                                           β11     α10    α11

              β14            α19    β15                β16  β17  β18          β19
VvADC      QVFPVLPL---------SGLQ-NAADRRAVMLDITCDSDGAIDAYVDGQGIESTLPV-PAWNEDEPYLMG  544
TbODC      AVVRPLPQ---------REPIPNEKLYPSSVWGPTCDGLDQIVE---------RYYL-PEMQV--GEWLL  382
CvADC      SVPTPQLL---------RDVPDDEEYVPSVLYGCTCDGVDVINH---------NVAL-PELHI--GDWVY  346
VvLODC     ARYPLTTI---------K---QGGELIPSVLSGPTCDSVDVIAE---------NILL-PKLNN--GDLVI  361
MjDAPDC    AYHHIINC---------K---VKNEKEVVSIAGGLCESSDVFGR---------DREL-DKVEV--GDVLA  394
CjCANSDC   YTSEVLNARILATRENEKISDLKENEFAYLLTGNTCLAGDVMG----------EYAFDKKLKI--GDKIV  335
                β12                     β13         β14                         β15
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                     α20     α3-10 3    β20          β21        α21         α22
VvADC      FFLVGAYQEILGDMHNLFGDTHSVVVNVGDQGEINIDFINEGDTVEDMMRYVHIDVDQIRKNYHSLVSQR  614
TbODC      FEDMGAYTVVGTSSFNGFQSPTIYYVV-------------------------------------------  409
CvADC      FPSWGAYTNVLTTSFNGFGEYDVYYI--------------------------------------------  372
VvLODC     GRTMGAYTSATATDFNFFKRAQTIALNEF-----------------------------------------  390
MjDAPDC    IFDVGAYGISMANNYNARGRPRMVLTSKK-----GVFLIRERETYADLIAKDIVPPHLL-----------  448
CjCANSDC   FLDQIHYTIVKNTTFNGIRLPNLMLLDHK----NELQMIR-EFSYKDYSLRN------------------  382
                    α3-10 4  α3-10 5    β16          β17        α12

                    α23
VvADC      VDQEEQQQILAELEQGLSGYTYLED  639
TbODC      -------------------------
CvADC      -------------------------
VvLODC     -------------------------
MjDAPDC    -------------------------
CjCANSDC   -------------------------
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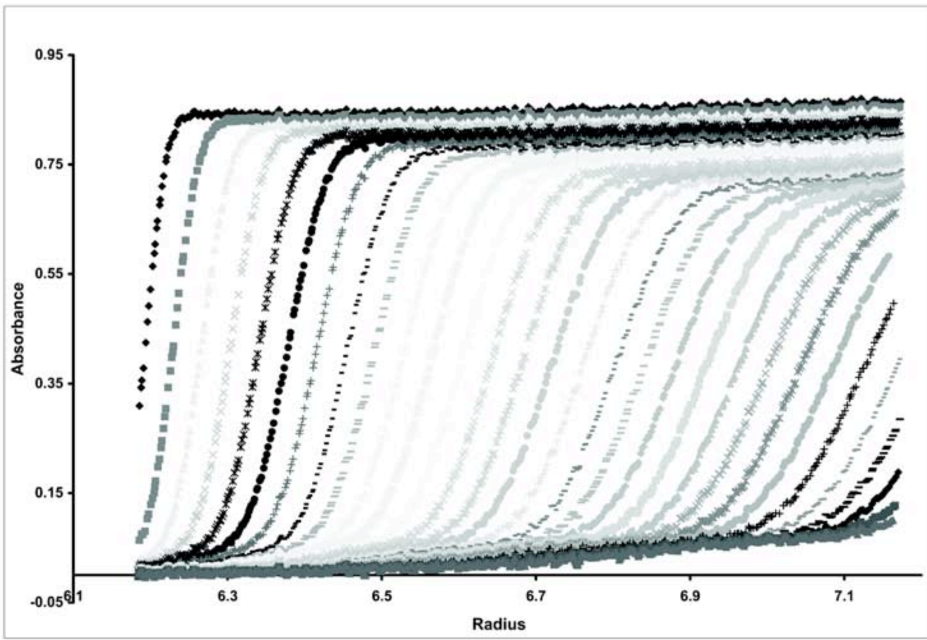
Figure 3. Sedimentation velocity analysis of *Vv*ADC. A. Velocity sedimentation was performed using a charcoal-filled dual sector centerpiece and scans are recorded at fixed intervals. An-50 Ti rotor was used at speed of 45,000 RPM and scans are displayed at 4 minute intervals (progressing from left to right on the plot). Three *Vv*ADC concentration of 0.1, 0.4 and 0.8 OD were used in the experiment. Data is only displayed for the 0.8 OD sample. B. Corresponding c(s) distribution. Model of continuous c(s) distribution and fitting option of Marquardt Levenberg were applied in the Sedfit analysis to determine the MW. The first 30 scans of *Vv*ADC were used in the calculation.

A.



B.

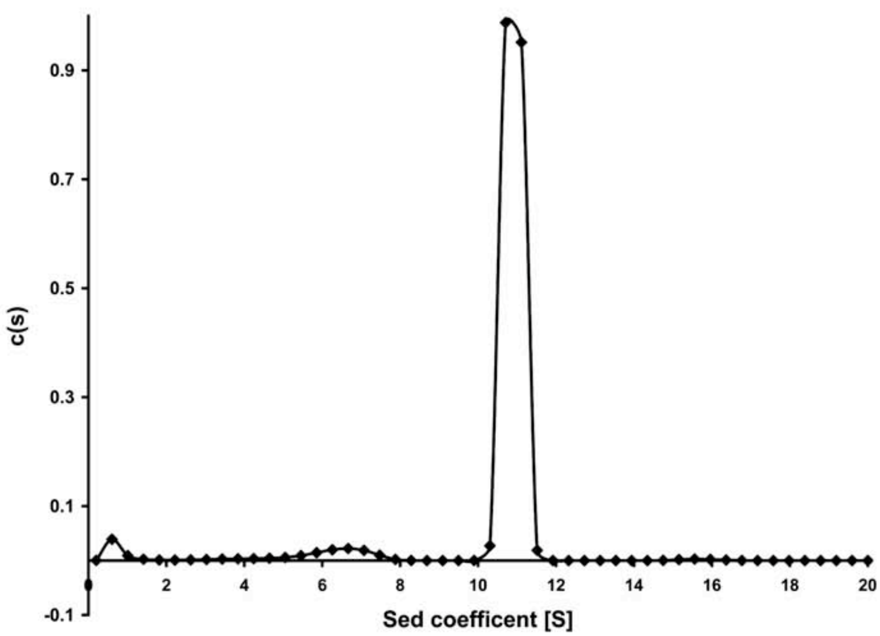


Figure 4S. Ribbon diagram of the *Cj*CANSDC structure aligned with *Tb*ODC shown from the back-side. The two *Cj*CANSDC monomers are shown in teal and light blue respectively, while the two *Tb*ODC monomers are in tan and grey. The C-terminal extension (C) that forms the novel dimer interface in *Cj*CANSDC is marked.