

Supplementary Material

Figure S1: Alignment of known anthocyanin malonyltransferases. From top to bottom: *Glandularia × hybrida* Vh3Mat1 (AAS77402.1), *Lamium purpureum* Lp3Mat (AAS77404.1), *Nicotiana tabacum* NtMat1 (BAD93691.1), *Salvia splendens* Ss5Mat (AAL50566.1), *Dahlia pinnata* Dv3Mat (Q8GSN8.1), *Pericallis cruenta* Sc3Mat (AAO38058.1), *Perilla frutescens* Pf5Mat (AAL50565.1), *Chrysanthemum × morifolium* Dm3Mat1 (AAQ63615.1), *Chrysanthemum × morifolium* Dm3Mat2 (AAQ63616.1), *Chrysanthemum × morifolium* Dm3Mat3 (BAF50706.1), *Oryza sativa* OsMat1 (NP_001046855.1), *Zea mays* ZmAat1 (NP_001148286.2). Motifs 1 to 3 are indicated above their respective amino acids.

Figure S2: Purification of Recombinant Aat1. 1) Uninduced culture, 2) resuspended culture before sonication, 3) soluble protein fraction after sonication, 4) flow-through of the Ni-NTA column, 5) purified Aat1 with an arrow indicating the protein band.

Figure S3: Michaelis-Menten Plots. Dots indicate measured velocity over initial substrate concentration. Velocity was determined as micromoles of product formed over time. Red lines represent the fitted model as determined by the Michael-Menten equation modified by K. A. Johnson (2019) [1].

References

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gb|AAS77402.1| 1 ----MA TTRK-----TVRRCG---AFADAGEAVDORPITFDITWVDFEQRLLFYQYFCSKHFVHIVPNLRKSLKOTLHYEYAGKLRFPV---SG---MF
gb|AAS77404.1| 1 ----MNSAKK-----NVLEHGAFAPAAA---DEVAQRORLPLTFDHVLWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
dbj|BAD93691.1| 1 ----MA-----VLEQCC---VP---SGSATHEPLPTFDHVLWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
gb|AAL50566.1| 1 ----MTTT-----TLELCH---EFP---PPAANLISPLFFDKWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
sp|Q8GSN8.1| 1 ----VDNIP-N-----TLEHRS---SGLGHRSLPLTFFDQWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
gb|AAO38058.1| 1 ----MDIP-C-----NLEHAR---SGLGHRSLPLTFFDQWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
gb|AAL50565.1| 1 ----MT-T-----TLEHCR---TLE---GHRSLPLTFFDQWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
gb|AAQ63615.1| 1 ----MANS-I-----TLEOCR---SGLGHRSLPLTFFDQWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
gb|AAQ63616.1| 1 ----MNP-I-----TLEHRS---SGLGHRSLPLTFFDQWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
dbj|BAF50706.1| 1 ----MALP-I-----TVLEQC---SGLGHRSLPLTFFDQWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F
ref|NP_001046855.1| 1 MAPATQMAAPPRA---RGGSRVTRTARAP---SSDGVPMVERALPLTFLDANLTPPPVDRFVYLG-ADDGGAHVSPLADLSRALVVEYAGKLRFPVLDGSGF---F
ref|NP_001148286.2| 1 MAAATAAATEAAGHHQEQQRFRVDTALAP-AAA---PALPERSPLTFFDQWVDFEQRLLFYQYFCSAHFVQTIIPNLRKSLKOTLHYEYAGKLRFPVLDGSGF---F

gb|AAS77402.1| 96 ELRYVPGDSVSVTFAEN-NGDFDFH-----TGNHVRDSLEFYFASDLEPEVTEPDFGFTVPLFAOVVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
gb|AAS77404.1| 96 ELRYVPGDSVSVTFAEN-TEADEFN-----TGDQARVALRHHFVDFPHKIDSDSGFRITPLFAOVVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
dbj|BAD93691.1| 96 ELRYVPGDSVSVTFAEN-DLDF-N-----ICGHRNTKDFYHVFVDFEAPKADP-GVQLAFLFAOVVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
gb|AAL50566.1| 91 QRYVAGDSVSVTFAEN-NSDFE-S-----TGNHVRDADQYDFVDFEPIEES-NWKLNAVOVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
sp|Q8GSN8.1| 97 ELRYVPGDSVSVTFAEN-TLDF-N-----TGNHPRKCCNGYPLVDFEIGNAVKES-VVTVFESVOVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
gb|AAO38058.1| 99 ELRYVPGDSVSVTFAEN-CLDF-N-----TGNHPRKCCNGYPLVDFEIGNAVKES-VVTVFESVOVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
gb|AAL50565.1| 89 ELRYVAGDSVSVTFAEN-TDFD-M-----TGNHVRDADQYDFVDFEPIAEFF-CKIKPLSVOVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
gb|AAQ63615.1| 99 ELRYVPGDSVSVTFAEN-SLDF-N-----TGNHPRKCCNGYPLVDFEIGNAVKES-VVTVFESVOVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
gb|AAQ63616.1| 99 ELRYVPGDSVSVTFAEN-CLDF-N-----TGNHPRKCCNGYPLVDFEIGNAVKES-VVTVFESVOVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
dbj|BAF50706.1| 94 ELRYVPGDSVSVTFAEN-NLIL-N-----TGNHPRKCCNGYPLVDFEIGNAVKES-VVTVFESVOVLFPEV-GISGISTNHHSAGDASIGERKMSMVARK-GCEILAQKN
ref|NP_001046855.1| 109 ELRYVPGDSVSVTFAEN-DDGVGVDFE-AADD-PRVAKIAPLVDFEIGDGAAL-AVAVHPLPARRAAGVHHAACLSSTHEHTHTAAACA-----GAALVP
ref|NP_001148286.2| 110 ELRYVPGDSVSVTFAEN-NLID-HI-----ADDSVGVQVALAPLAPVDFEIGDGAAL-AVAVHPLPARRAAGVHHAACLSSTHEHTHTAAACA-----GAALVP

gb|AAS77402.1| 208 E---LHEDRDKKESGRADLWNRQ-----QIGSDHSEFNT--FRATELRHREHLKNI/AEKKGSH-----SSEFTVTSYWSCVAKSAESGEVDTLPPY
gb|AAS77404.1| 205 DR---PDRDKKESGKRANFNQRAQ-----IPTSPNPPSN--VAKTELOSKIKTKLDIVAREANFSY-----SSEFTVLAHWAKSAESAEGEVDLAPPY
dbj|BAD93691.1| 197 F---IEHEDRDKKDPNGKMSWNEKK-----KHMKMSVYVPPDKVRETEFRHREHLKNI/VTRFQTH-----SSEFTVTCAYWSCVAKSAEATGEVDLNGEF
gb|AAL50566.1| 199 DLSLELDRFIN-DPNKIDA-FWKVNRNI-----PLKTASFLPLN-IVVSTPFRHREHLKNTAT--KS-PA-----SKSDEN-APFL
sp|Q8GSN8.1| 205 E---PVVDRFNIENRNOTRLEGT-----IQAPSVSDVRAVTEJARRHREHLKNI/VTOLNEM-----SSEFTVTCYWSCVAKSLVNMKKGKGDLEQ
gb|AAO38058.1| 207 E---PVVDRFNIENRNOTRLEGT-----IQAPSVSDVRAVTEJARRHREHLKNI/VTOLNEM-----SSEFTVTCYWSCVAKSLVNMKKGKGDLEQ
gb|AAL50565.1| 197 GESL-LVDRDKKDEIDT-FWKVNRNI-----PLKPSFLPLN-IVVRAVTEJARRHREHLKNI/VTOLNEM-----SSEFTVTCYWSCVAKSLVNMKKGKGDLEQ
gb|AAQ63615.1| 207 E---LVVDRFNVDFDEYRHRHTELE-----IKPSSVPLN-DKVRATEJARRHREHLKNI/VTOLNEM-----SSEFTVTCYWSCVAKSLVNMKKGKGDLEQ
gb|AAQ63616.1| 208 E---RVVDRFDIENRNOTRLEGT-----IQAPSVSDVRAVTEJARRHREHLKNI/VTOLNEM-----SSEFTVTCYWSCVAKSLVNMKKGKGDLEQ
dbj|BAF50706.1| 203 K---RVDRIKYVDEAYKRAKLEENEDVYQSLAGS-DKLRATPLTRALNQLKDRVLA---QLLLEFY-----SSEFTVTCYWSCVAKSLVNMKKGKGDLEQ
ref|NP_001046855.1| 208 K---PVVDRFREREDLYD---MVSRTESDTRSP-NP--K-LATFTEGELISKDRVAGVAARRGKSPPRCSVVAFAIWHIYALGVDADNKHGGRH
ref|NP_001148286.2| 214 P---PVVDRFPEERGLYDYLRSMPMVSQDDEFFVLGKPKQDFED--KALATELQQLSLSA/AHEAARRGMTPPRCSILAFVWSCVAKSAAGAAAAA---ERSY

gb|AAS77402.1| 309 GFADARHEDPEAFAAVFGNCLFVYETHGKAG---GSEFTVTPSEIENKNNKNELEDAHEAVKVGPIGHGLGVAGSPKDLVDFDFGMCNENRVSISIND
gb|AAS77404.1| 306 SIAADARSDPEFPATYFGNCLTAAAESRGRKCG---KGEFAALFGLVSKRWNKAGELEDAHEAVKVA-PLFSHFYVGSSEPKDLVDFDFGMCNENRVSISIND
dbj|BAD93691.1| 300 ECADARSAQGFPEFPATYFGNCLVGVARTQVDLAK---KGEFAALFGLVLRERDPEWLLSGS--NKEKD-KVDARPSVAGSPKDLVDFDFGMCNENRVSISIND
gb|AAL50566.1| 294 FIPVPAARSDPPFENYFGNCLVSSVAQERKQIA---GFAFAEATGEBGRKNNLELLIGANNLSDIF-KCFGMSVSVSSEPKDLVDFDFGMCNENRVSISIND
sp|Q8GSN8.1| 306 FIPVPAARSDPPFENYFGNCLPCVLTNGLDAG---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND
gb|AAO38058.1| 307 FIPVPAARSDPPFENYFGNCLPCVLTNGLDAG---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND
gb|AAL50565.1| 293 FIPVPAARSDPPFENYFGNCLVGVVKEHEKKA---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND
gb|AAQ63615.1| 307 FIPVPAARSDPPFENYFGNCLPCVLTNGLDAG---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND
gb|AAQ63616.1| 308 FIPVPAARSDPPFENYFGNCLPCVLTNGLDAG---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND
dbj|BAF50706.1| 298 EFPVPAARSDPPFENYFGNCLPCVLTNGLDAG---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND
ref|NP_001046855.1| 314 EFPVPAARSDPPFENYFGNCLPCVLTNGLDAG---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND
ref|NP_001148286.2| 324 EFPVPAARSDPPFENYFGNCLPCVLTNGLDAG---NENVAALFGLVSKRWNKAGGLEIYARVDGK---PARKVSPKNNYDFDFGMCNENRVSISIND

gb|AAS77402.1| 423 E---SLCHSDFESGLEIGSL---KKMEASVADR-----HGLKI-----
gb|AAS77404.1| 420 SYSL---SLCHSDFESGLEIGSL---ERMAARQAIDRL---GLKI-----
dbj|BAD93691.1| 412 D-GHMSLSKRSKSGDGLDGLSL---KTMARAAAT---RGSFL-----
gb|AAL50566.1| 408 NHS---SLCHSDFESGLEIGSL---RMEARAEERASIMAASPARSPALVEPL-----
sp|Q8GSN8.1| 418 E---SL---SLSACKSAQDFEIGCF---SMOMEASGRIN---GDELAIAS-----
gb|AAO38058.1| 419 T-SL---SLNASKSAQDFEIGLSL---SMOMEASSTED---GDEQVSL-----
gb|AAL50565.1| 407 KYR---SLCHS---DGLDGLSL---GEMEABAAI---AGDAKLDS-----
gb|AAQ63615.1| 419 E---SL---ANNASKESQDFEIGLCS---NMOMEABADIN---GDEEI-----
gb|AAQ63616.1| 420 E---SL---ANNASKESQDFEIGLCS---NMOMEABADIN---GDEEIV-----
dbj|BAF50706.1| 414 E---AL---SINSKESNEDLEIGLCS---ATQMDVAVHED---DQKAYL-----
ref|NP_001046855.1| 430 E---AL---SAAEG---RGGTEIGLCS---PEMERRCAD---AVAVSPSRPVTRDMDRSAPGHPA-----
ref|NP_001148286.2| 439 E---AL---SADAEVGGGPEIGLCS---VASGDMNRQSVAD---GMEVRL-----

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Figure S2: Purification of Recombinant Aat1. 1) Uninduced culture, 2) resuspended culture before sonication, 3) soluble protein fraction after sonication, 4) flow-through of the Ni-NTA column, 5) purified Aat1 with an arrow indicating the protein band.

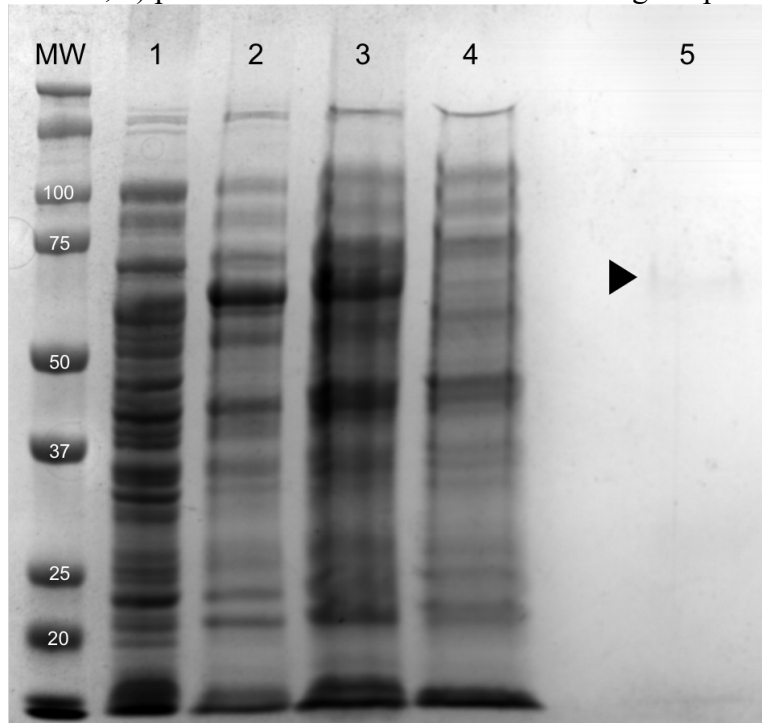
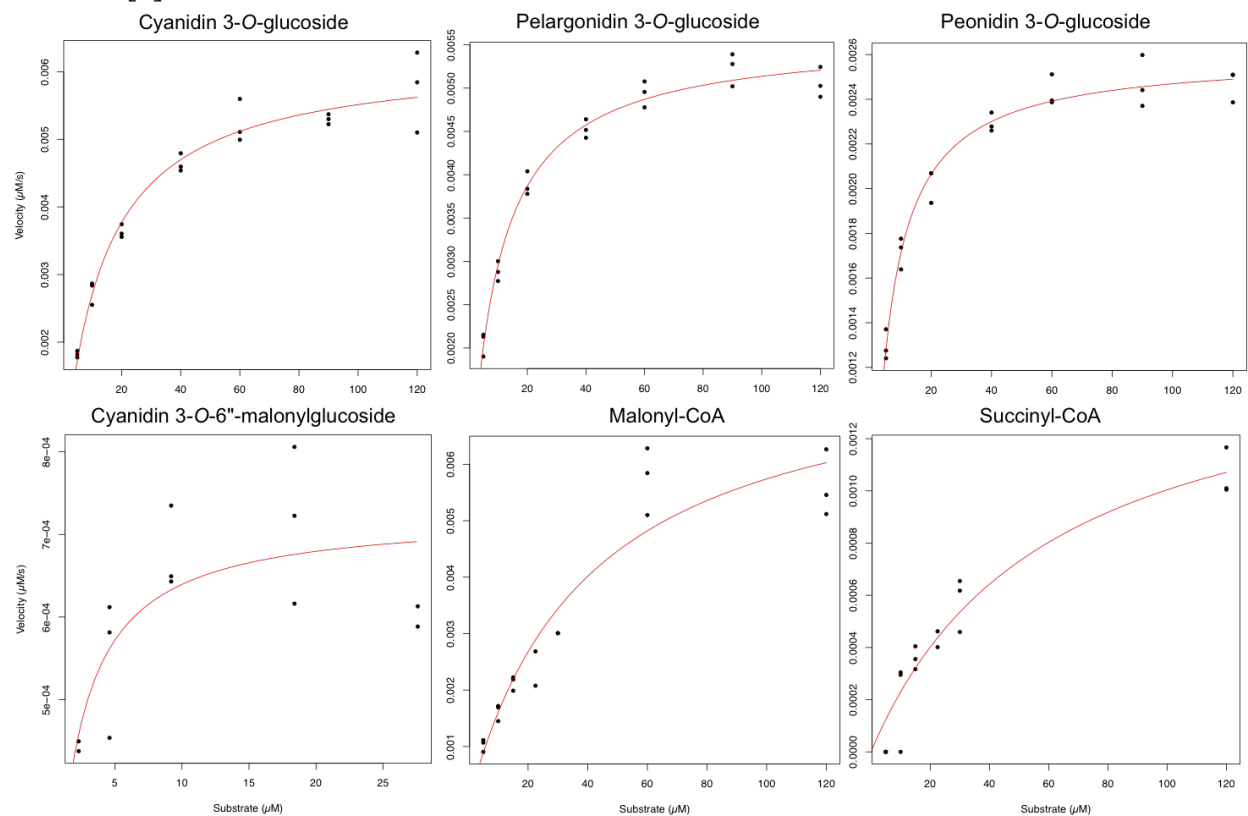


Figure S3: Michaelis-Menten Plots. Dots indicate measured velocity over initial substrate concentration. Velocity was determined as micromoles of product formed over time. Red lines represent the fitted model as determined by the Michael-Menten equation modified by K.A. Johnson [1].



References:

1. Johnson, K.A. New Standards for Collecting and Fitting Steady State Kinetic Data. *Beilstein J. Org. Chem.* 2019, 15, 16–29, doi:10.3762/bjoc.15.2.