

Supplementary Table and Figures for “Structural Basis for the Enantiospecificities of *R*- and *S*-Specific Phenoxypropionate/ α -Ketoglutarate Dioxygenases” by Tina A. Müller, Maria I. Zavodszky, Michael Feig, Leslie A. Kuhn, and Robert P. Hausinger

Table S1. Sequences of mutagenesis primers used to create altered *rdpA*- and *sdpA* genes

| original gene | plasmid | mutant | primer sequence ^a |
|---------------|---------|-------------|--|
| <i>rdpA</i> | pMec151 | V80A | 5'-CCC CGT CGA TCC CGC ACC CCT GCT CAA GA-3' |
| | pMec152 | Q93A | 5'-AGG GTA TCC AGA GGT GGC AAT GAT CCG CCG CGA AG-3' |
| | pMec153 | Y221A | 5'-TGT ACG TGA ACC AGG TCG CAT GCC AGC GCA TCG AG-3' |
| | pMec154 | R285A | 5'-CCG CTA CCT GAC GGC CAC CAC GGT CGG TG-3' |
| | pMec155 | I106G/G107I | 5'- GAA AGC GGG CGT GTG GGC ATA GAT GAC TGG CAC A-3' |
| | pMec156 | I106G/107N | 5'- GAA AGC GGG CGT GTG GGC AAT GAT GAC TGG CAC A -3' |
| | pMec157 | L83A | 5'-CCC GTG CCC CTG GCG AAG AGC ATC GAA G-3' |
| | pMec158 | I106A | 5'-GCG GGC GTG TGG CAG GTG ATG ACT G-3' |
| | pMec160 | F171A | 5'-CCA CGC GTG TGG CGG GCT CGC TCT AC-3' |
| | pMec161 | F171Q | 5'-CCA CGC GTG TGC AAG GCT CGC TCT AC-3' |
| <i>sdpA</i> | pMec191 | E69A | 5'-GGC CAC TCG AGT TCG CAA TGG CCG CGA TCA G-3' |
| | pMec192 | H208A | 5'-TGC TGA TCG GCC GCG CAG CCC ACG CCA TTC C-3' |
| | pMec193 | H272A | 5'-GAA GCG CAT CAT GTG GGC AAG CCG CAT CGC CGG-3' |
| | pMec194 | R274A | 5'-CAT CAT GTG GCA CAG CGC AAT CGC CGG CGA CCC-3' |
| | pMec195 | G97N/N98G | 5'- CAT GAT GAA GAT CCT GAA GAA CGG CAT GGG CTG GCA T-3' |
| | pMec196 | G97I/N98G | 5'- CAT GAT GAA GAT CCT GAA GAT CGG CAT GGG CTG GCA T-3' |
| | pMec197 | Q162F | 5'-CTG CAT TAC AGC TTT TCG AAG CTC GGC-3' |
| | pMec198 | R207A | 5'-CTG CTG ATC GGC GCC CAC GCC CAC G-3' |
| | pMec199 | R207V | 5'-CTG CTG ATC GGC GTG CAC GCC CAC GCC-3' |

^a The reverse primer used for the creation of all mutants was the complement of the forward primer.

Figure S1. Amino acid sequences of RdpA (A) and SdpA (B) aligned to TauD. The predicted secondary structural elements for the query proteins are given above the sequences, and the secondary structure found in the TauD crystal structure is shown below. The letter H indicates helical structure and E stands for β sheet. Identical residues are depicted in white against a black background and similar residues are highlighted by gray boxes.

Figure S2. Homology models of RdpA (A) and SdpA (B). Ribbon diagrams (colored blue at the *N*-termini to red at the *C*-termini) are shown for the RdpA and SdpA monomers, in each case revealing the typical jellyroll motif of α KG dependent dioxygenases. Also shown as spheres are the bound iron atoms, and the side chains of the Fe^{II} binding ligands along with α KG are depicted in stick form.

Figure S3. Representative purification of variant forms of SdpA as analyzed by SDS-PAGE. Proteins were resolved by using 12% polyacrylamide gels and stained with Coomassie brilliant blue. For each sample derived from BL21(DE3) cells containing the indicated plasmid, lane 1 contains cell extracts and lane 2 contains the proteins eluted from Ni-NTA resin. The final lane contains molecular weight markers (indicated in kDa).

Figure S1

A

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- EEEEE- - EEEEEEE- - - - - HHHHHHHHHHHHH- - EEEEE- - - - HHHHHHHHHH
RdpA 1 ERIVAQPLTGVLGAETITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAFSR
TauD 1 .RLSITPLGPYIGAQISGADLTRPLSDNQFEQLYHAVLRHQVVFLRDQAITPQQRALAQ
- - - EEEE- - - EEEE- - - - - HHHHHHHHHHHHH- - EEEE- - - - HHHHHHHHHH

H- - - - - EEEEE- - - - - EEEEEEE- - - - -
RdpA 61 RFGPVDPVPLLKSIEGYPEVQMIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIDVP
TauD 60 RFGELHIHPVYPHAEGVDEIIVLDTHNDNPPD. .NDNWHTDVTFIETPPAGAILAAKELP
HH- - E- - - - - EE- - EEEEEEEEEE- - E- - - - - E- - - - - EEEEEEEEEE- -

- - - - - HHHHHHHHHHH- - HHHHHHH- - EEEE- - - - - EEEE- - - - -
RdpA 121 EHGGDTGFLSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDV
TauD 118 STGGDTLWTSGIAAYEALSVPFRQLLSGLRAEHDFRKSFP. .EYKYRKTEEEHQRWREA
- - - - - EEEEEHHHHHH- - HHHHHHH- - EEEE- HHHH- - - - - HHH- - - - - HHHHHHHH

- - - - - EEEE- - - - - EEEEE- - EEEEE- - - - - HHHHHHHHHHH- - - EEEE
RdpA 181 DAGDRETVHPLVVTHTPGSRKGLYVNQVYCQRIEGMTDAESKPLLQFLYEHAATRFDFTCR
TauD 175 VAKNPPLLHPVVRTHPVSQKALFVNEGFTTRIVDVSEKSEALLSFLFAHITKPEFQVR
HHH- - EEEE- EEE- - - - - EEE- - - - - EEEE- - - - - HHHHHHHHHHHHHHH- - HHHEEE

EE- - - - - EEEEE- - HHH- - - - - EEEEEEE- - - - -
RdpA 241 VRWKKDQVLVWDNLCTMHRVAVPDYAGKFRYLTRITVGGVRRPAR
TauD 235 WRWQPNDAIWDNRVTQHYANADYLPQRRIMHRATILGDKPFY
E- - - - - EEEEE- - EEEE- - - - - EEEEEEEEEE- - - E-

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B

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- - EEEEE- - - EEEEE- - - - - HHHHHHHHHHHHH- - EEEEE- - - - HHHHHHHHHH
SdpA 1 PAFDIAPLDATFGAVVTGVKLDLDDAGWLDLQAAWLEYALLVFPDQHLTREQQIAFARR
TauD 1 .RLSITPLGPYIGAQISGADLTRPLSDNQFEQLYHAVLRHQVVFLRDQAITPQQRALAQR
- - - EEEE- - - EEEE- - - - - HHHHHHHHHHHHH- - EEEE- - - - HHHHHHHHHH

H- - - - - EEEE- - - - - EEEEEEE- - - - -
SdpA 61 FGPLEFEMAAISNVRPDGSLRVEVDNDDMMKILKGNMGWHADSTYMPVQAKGAVFSAEVV
TauD 60 FGELHIHPVYPHAEGVDEIIVLDTHND- - - - NPPDNDNWHTDVTFIETPPAGAILAAKELP
H- - E- - - - - EE- - EEEEEEEEEE- - - - - E- - - - - E- - - - - EEEEEEEEEE- -

- - - - - HHHHHHHHHHH- - HHHHHHHHH- - - - - HHHH- - - - -
SdpA 121 PSVGGQTGFADMRAAYDALDEDLKARVETLQARHSLHYSQSKLGHQTKAADGEYSYGLH
TauD 116 PSTGGDTLWTSGIAAYEALSVPFRQLLSGLRAEHDFRKSFPPEYKYRKTEEEHQRWREAVA
- - - - - EEEEEHHHHHH- - HHHHHHH- - EEEE- HHHH- - HHH- - - - - HHHHHHHHHH

- - - - - EEEE- - - - - EEEE- - - - - HHHHHHHHHHHHHHH- - - EEEEE-
SdpA 181 DGPVPLRPLVKIHPETGRKSLIIGRHAHAIPGLEPAESERLLQQLIDFACQPPRIYHHDW
TauD 176 KNPPPLLHPVVRTHPVSQKALFVNEGFTTRIVDVSEKSEALLSFLFAHITKPEFQVRWRW
H- - EEEE- EEE- - - - - EEE- - - - - EEEE- - - - - HHHHHHHHHHHHHHH- - HHHEEEE- -

- - - - - EEEEE- - HHHH- - - - - EEEEEEE- - - - -
SdpA 241 APGDAVLWDNRCLLHQATPDMTQKRIMWHSRIAGDPASET
TauD 235 QPNDAIWDNRVTQHYANADYLPQRRIMHRATILGDKPF
- - - EEEEE- - EEEE- - - - - EEEEEEEEEE- - - - -

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Figure S2

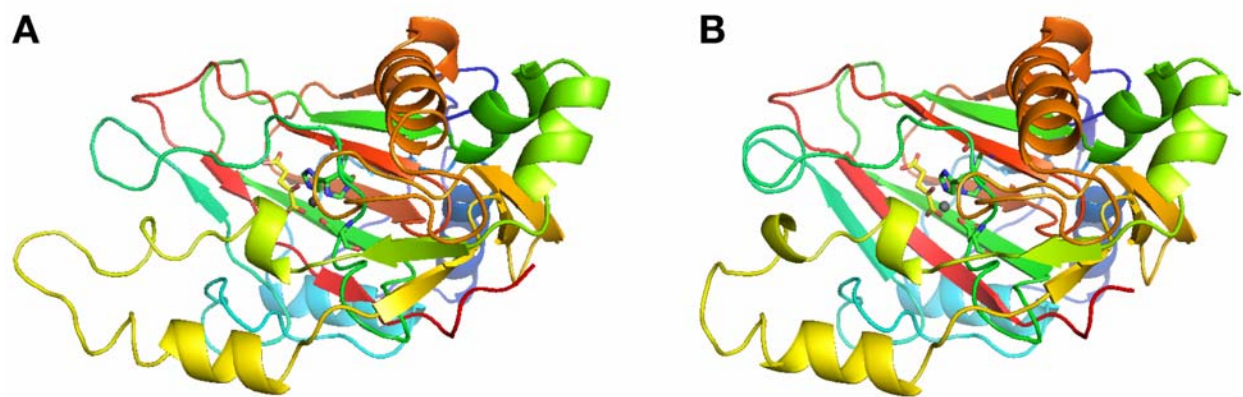


Figure S3

