Biochemistry Supporting Information

STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF ZHUI AROMATASE/CYCLASE FROM THE R1128 POLYKETIDE PATHWAY

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Figure S1. General route for Type II PKS polyketide chain formation and subsequent ketoreduction and/or cyclization leading to aromatic ring formation. Step 1 of the process involves priming KS with an acyl-CoA followed by decarboxylative condensation of malonyl units to produce a polyketide chain. Step 2 involves optional ketoreduction (in most cases specific for the C-9 carbonyl group), cyclization and aromatization. The three main classes of aromatase/cyclase (ARO/CYC) enzymes associated with a particular cyclization pattern and enzyme activity are illustrated, including TcmN [1-3], WhiE-ORFVI [4], RemI [5,6], ZhuI [7,8], MtmQ [9,10], ActVII [11], and Gris-ORF4 [12,13]. In general, class 1 are mono-domain proteins that promote dual cyclizations/aromatizations of an unreduced polyketide chain to yield regiospecific C9-C14 and C7-C16 bonds. Class 2 are mono- or di-domain proteins that also act on an unreduced polyketide chain but catalyze C7-C12 cyclization and aromatization of the first ring only. Class 3 are di-domain proteins that act following regiospecific ketoreduction (and presumably cyclization) to catalyze dehydration/aromatization of the first ring.

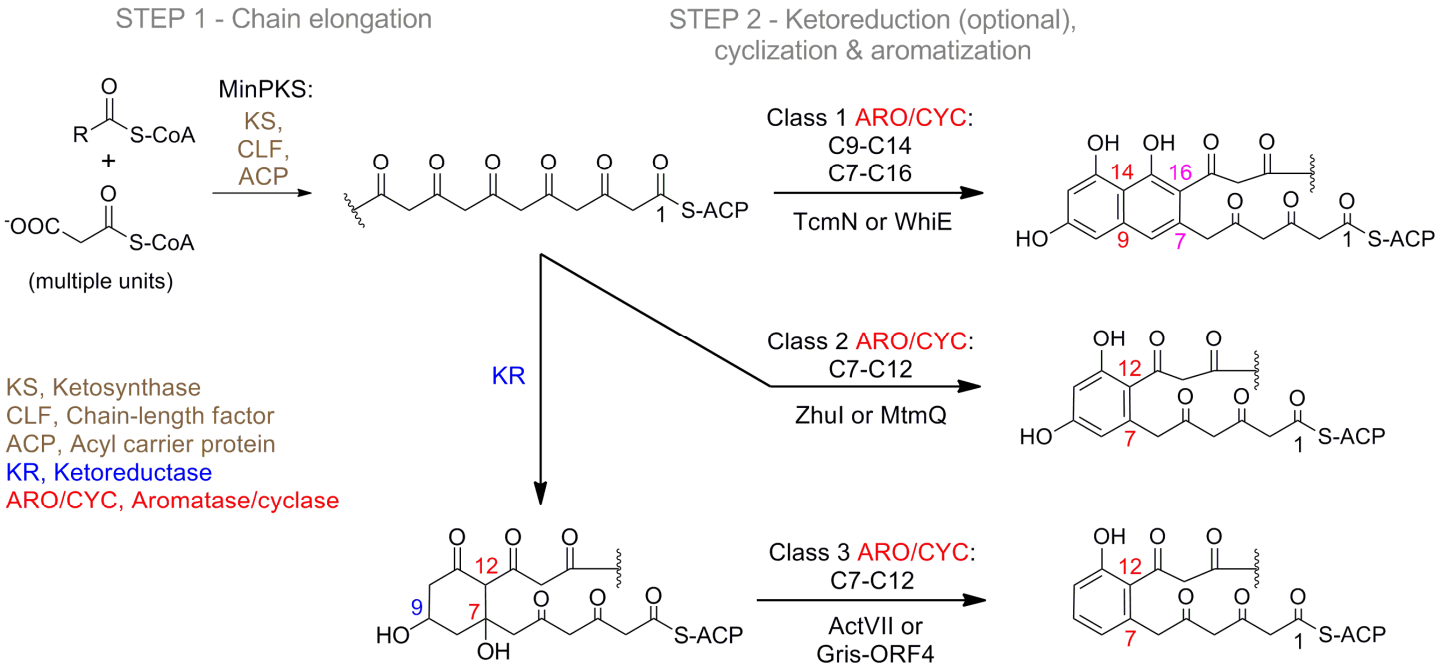

Figure S2. Multiple sequence alignment of select PKS ARO/CYC proteins. Sequences 1-11 are mono-domain proteins and sequences 12-22 are di-domain proteins. The sequence alignment was generated using ClustalW and colored using GeneDoc (red, green, and blue shading represent 100%, 80%, and 50% identity respectively). Numbering is according to the ZhuI sequence. Annotations included as part of the consensus line represent a conserved property at this position within a 3-residue exception limit (polar [%], hydrophobic [!], charged [#], aromatic [$]). An asterisk below the consensus line marks residues defining the interior pocket of ZhuI. Arrows indicate the three putative catalytic residues of ZhuI.

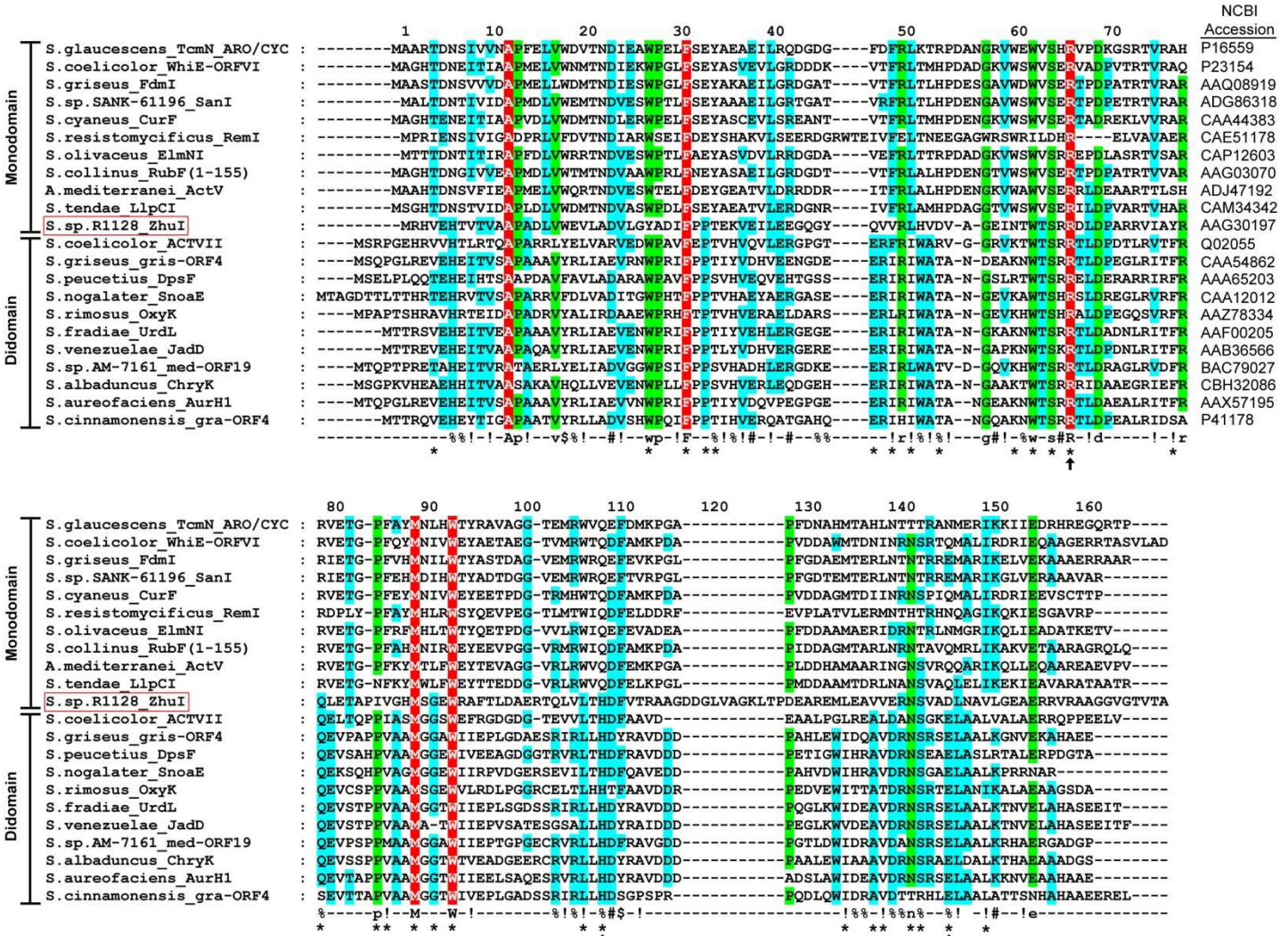

Figure S3. Size-exclusion chromatography studies illustrating that ZhuI ARO/CYC is dimeric in solution. (A) Elution profile of proteins used as standards to calibrate a Superdex 200 10/300 GL column (GE Healthcare): 1, ferritin (440 kDa); 2, catalase (232 kDa); 3, aldolase (158 kDa); 4, albumin (67 kDa); 5, ovalbumin (45 kDa); 6, chymotrypsinogen A (25 kDa); 7, ribonuclease A (13.7 kDa). Standard proteins were reconstituted to give a final concentration between 2.0-7.5 mg/mL then 20 µL of each sample was injected onto the column. The mobile phase for each run consisted of 20 mM sodium phosphate buffer, pH 7.0. The flow rate was 0.5 mL/min. (B) Elution profile of ZhuI ARO/CYC when 1 mL of protein at ~10 mg/mL (solid line), or ~2 mg/mL (dashed line), was injected and ran under the same conditions as described for (A). An asterisk marks aggregated protein eluting near the column void volume. (C) Calibration curve prepared by plotting K_{av} (the gel-phase distribution coefficient) vs. log molecular weight (in kDa). $K_{av} = (V_e - V_0)/(V_c - V_0)$, where V_e is the elution volume, V_0 is the column void volume (determined using blue dextran), and V_c is the column volume (taken as 24.5 mL). Linear regression analysis of the plotted standards was used to estimate the molecular weight of ZhuI to be 48 kDa (theoretical for dimer is 41 kDa).

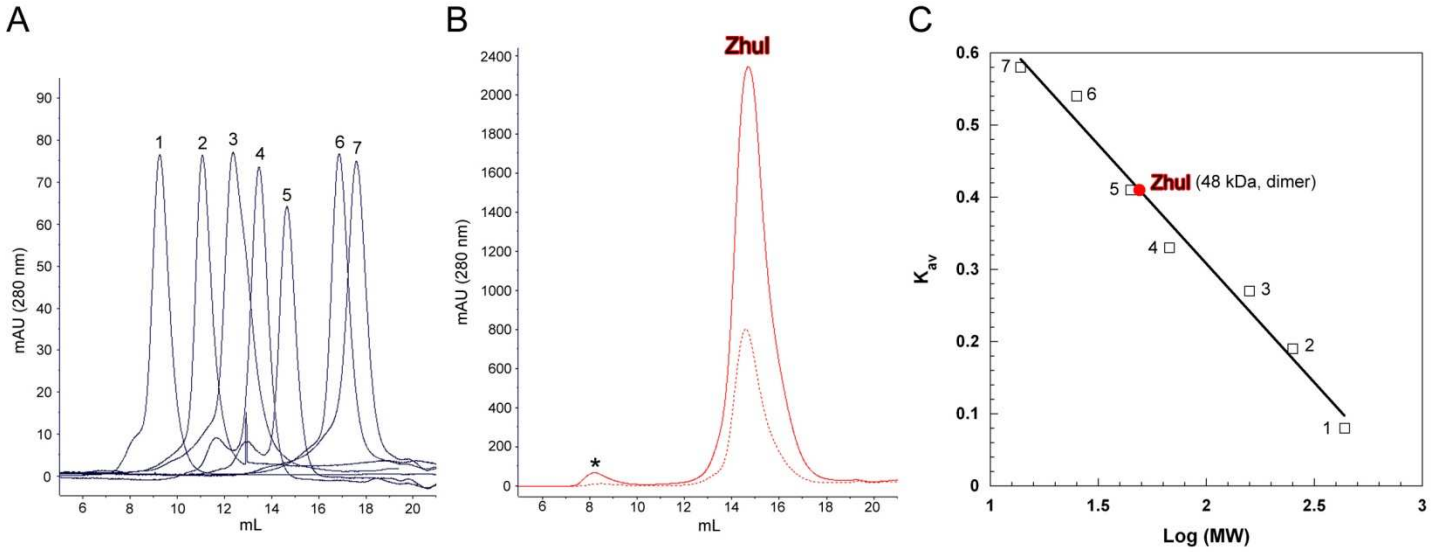

Figure S4. Dimeric structure of ZhuI ARO/CYC and comparison to other helix-grip fold (panels B and D) or hotdog fold (panel C) dimers. (A) ZhuI ARO/CYC from *Streptomyces* sp. R1128 (PDB ID, 3TFZ). (B) The Tcm ARO/CYC dimer resulting from the addition iodide (represented as purple spheres) to the crystallization drop (PDB ID, 2REZ). (C) The *Pseudomonas aeruginosa* fatty acid dehydratase FabZ 'double-hotdog' dimer (PDB ID, 1U1Z). (D) Dimeric structure of SMU.440 from *Streptococcus mutans* (PBD ID, 2B79).

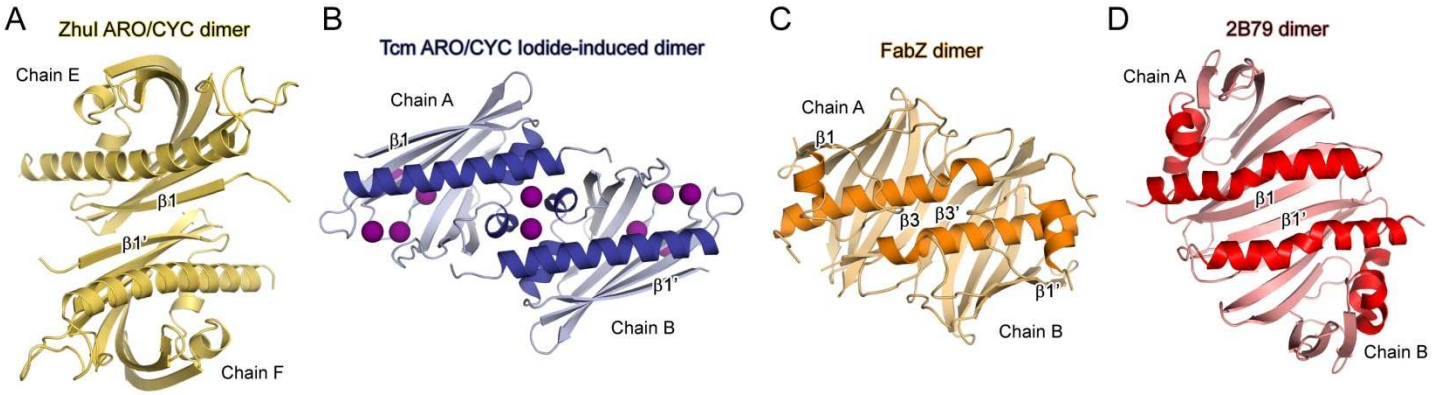

Figure S5. Stereoview images of the docking results described in the main text Figure 6 for ZhuI and TcmN ARO/CYC.

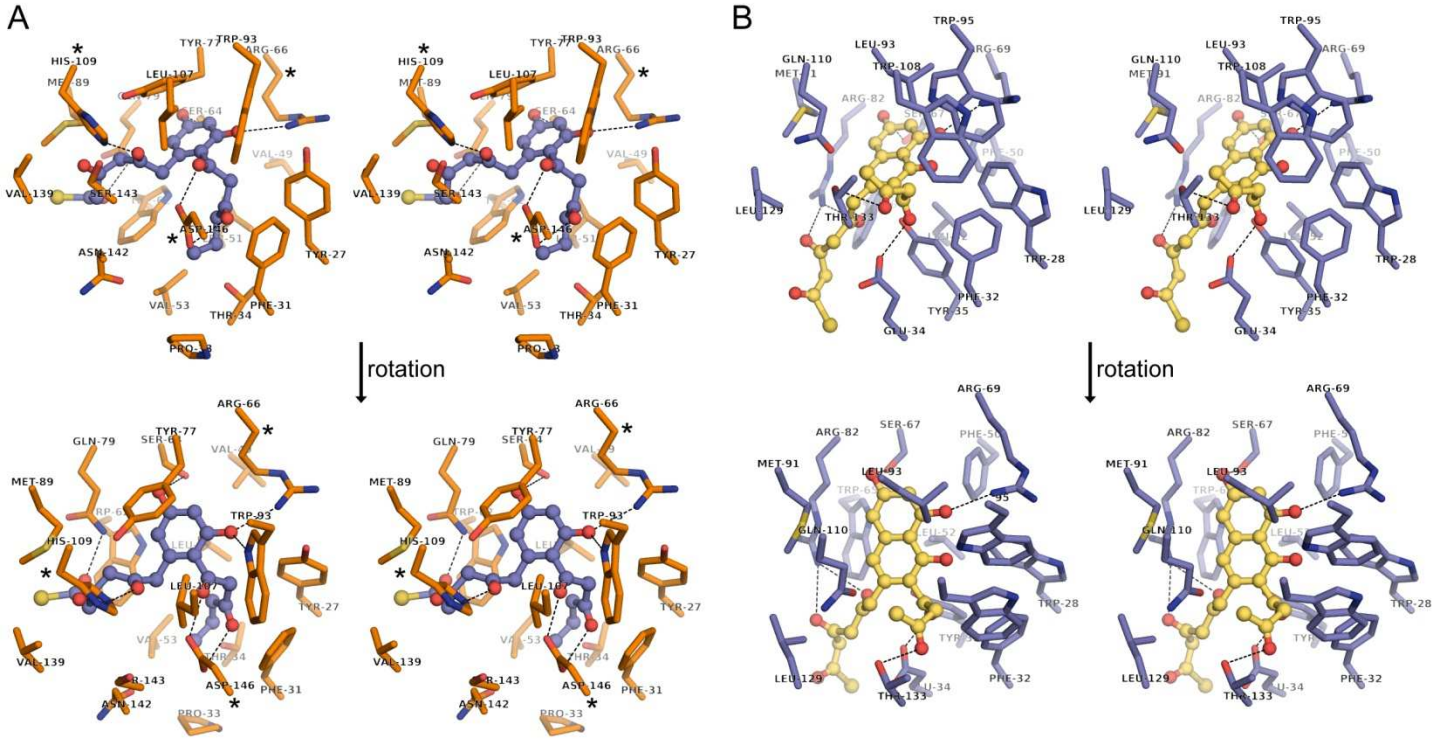

Figure S6. Circular dichroism (CD) data for WT and mutant ZhuI. All protein samples were prepared for CD by diluting the purified ZhuI (prepared for crystallization trials as described in the Materials and Methods section) to 15 µM with water and filtering (0.2 µm). Data were collected using a Jasco J-715 CD spectropolarimeter at 25 °C in a 0.1 cm path length quartz cuvette.