Supporting Information

The missing linker: a dimerization motif located within polyketide synthase modules

Jianting Zheng[1*], Christopher D. Fage[1*], Borries Demeler[2],
David W. Hoffman[1] & Adrian T. Keatinge-Clay[1]

[1]Department of Chemistry & Biochemistry, The University of Texas at Austin,
1 University Station A5300, Austin, TX 78712
[2]Department of Biochemistry, The University of Texas Health Science Center at San Antonio,
7703 Floyd Curl Drive, San Antonio, TX 78229

*These authors contributed equally to this work.

Corresponding author: Adrian Keatinge-Clay, Ph.D.
The University of Texas at Austin, Department of Chemistry & Biochemistry, 1 University Station A5300, Austin, TX 78712
(512) 471-2977, adriankc@utexas.edu

Supplementary Figure 1. The pseudo-twofold symmetry of the DE dimer. **a)** A stereodiagram shows the superposition of the two DE dimers in the asymmetric unit (as seen looking down their pseudo-twofold axes from the KS dimer). The third helix of DE, α3, bends significantly in the "bent" monomers, resulting in a slight asymmetry within each DE dimer. **b)** A stereodiagram shows the two principal DE conformations through a superposition of the four DE+KR monomers in the asymmetric unit. **c)** A stereodiagram reveals that the bend after the third turn of α3 is the principal difference between the four DE monomers in the asymmetric unit.

Supplementary Figure 2. Size-exclusion chromatography of a DE+KR fragment. **a)** The migration of Spn(DE+KR)3 through a Superdex 200 gel filtration column is compared to standards. **b)** The observed molecular weight was calculated to be ~90 kDa (60 kDa expected for the monomer; 120 kDa expected for the dimer).

Supplementary Figure 3. Analytical ultracentrifugation of Amp(DE+KR)2. The van Holde–Weischet integral sedimentation coefficient distributions obtained from [Amp(DE+KR)2] = 3.8 μM and 11.5 μM each indicate a homogeneous species with similar sedimentation coefficients. The calculated molecular weight (~56 kDa) is in excellent agreement with predicted monomer molecular weight (56 kDa).

Supplementary Figure 4. Sequence alignment of linkers. **a)** The average linker between the KS+AT didomain and DE is 14 residues. The shortest linker is 9 residues (Gdm3 and Hbm3). **b)** The average linker between the KR domain and helix 0 of ACP is 14 residues. The shortest linker is 9 residues (Spn7). **c)** The average linker between the ACP domain and KS is 19 residues. The shortest linker is 16 residues (Spn6). All sequences were obtained from the MAPSI database and aligned with the program ClustalX. Amp, amphotericin; Bor, borrelidin; Con, concanamycin; FR008, FR008; Gdm, geldanamycin; Hbm, herbimycin; Nys, nystatin; Ole, oleandomycin; Rif, rifamycin; Spn, spinosyn. The number after the PKS acronym indicates from which module the sequence was obtained. Accession codes (GI): Amp1 and Amp2, 14794905; Amp10 - Amp14, 14794893; Bor4, 39725434, Con3 and Con4, 74026478; Con6, 74026479; Con10 and Con11, 74026481; FR008_2, 34766452; FR008_12-FR008_16, 34766455; FR008_19, 34766454; Gdm3, 28192601; Gdm5, 28192602; Hbm3, 63033851; Nys1 and Nys2, 8050849; Nys10 – Nys12, 8050840; Ole2, 9049535; Ole3, 9049536; Ole5, 2492659; Rif3, 300782548; Spn1, 348173395; Spn3, 348173392; Spn4, 348173391; Spn6 and Spn7, 348173389.