Supporting Information for

# Molecular Recognition between Ketosynthase and Acyl Carrier Protein Domains of the 6-Deoxyerythronolide B Synthase

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## SI Text

**SI Materials and Methods.** ***Reagents and chemicals*****.** DL-[2-Methyl-$^{14}$C]-methylmalonyl-CoA was purchased from American Radiolabeled Chemicals. [1-$^{14}$C]-(2*S*,3*R*)-2-Methyl-3-hydroxypentanoyl-*N*-acetylcysteamine thioester ([$^{14}$C]-NDK-SNAC) was prepared by custom synthesis by Amersham Pharmacia. Unlabeled NDK-SNAC was prepared by established methods (1-3). All other chemicals were from Sigma. Thin-layer chromatography (TLC) plates were from J. T. Baker. SDS PAGE-gradient gels were from BioRad. Ni-NTA affinity resin was from Qiagen. HiTrap-Q anion exchange column was from Amersham Pharmacia.

***Construction and expression of fusion proteins derived from DEBS ACP3/ACP6 and ACP2/ACP4.*** The construction of genes encoding DEBS ACP3 (pVYA5) (4), ACP6 (pPK223) (4), ACP2(2) (pNW6) (5), and ACP4(4) (pNW8) (6) has been described previously. Residue numbering was based on the NMR structure of DEBS ACP2 (7), with Ser54 being the conserved site of phosphopantetheine attachment. Fusion proteins were prepared using the gene SOE method (8). For each fusion construct, the first round of PCR was used to produce two fragments, one encoding the DNA fragment upstream of the junction site (using the "Upstream" PCR primers in Table S1, S2 and S3), and another encoding the DNA fragment downstream of the junction site (using the "Downstream" PCR primers in Table S1, S2 and S3). For SHIV63-65 the desired upstream fragment was obtained by heat annealing chemically synthesized forward and reverse strands. The two PCR products, with 16- to 18-base-pair overlap, then primed each other in a second round of PCR, generating the gene encoding the corresponding

fusion protein. The fusion gene was then introduced into pET28 as an *Nde*I-*Eco*RI fragment. The schematics of the fusion proteins are described in Fig. 2 and Fig. 5. The resulting plasmids were transformed into *E. coli* BAP1 (9) to produce the desired *holo*-ACP proteins (10). The proteins were expressed and purified using a previously described protocol (11). The expression and purification of DEBS [KS3][AT3] (AYC02) and [KS6][AT6] (AYC11) has been described previously (11).

SHIV 25, 22, 26, 29 and 20 contain a His (residue 26 on ACP6) to Ala (corresponding residue in ACP3) mutation in $H_I$. We observed that all constructs containing $H_I$ from ACP6 and $L_I$ from ACP3 did not yield soluble ACP proteins (specifically AYC71, 81, 82 and SHIV12 the parent plasmids of SHIV25, 22, 26 and 20, respectively), regardless of the remaining sequence of the ACP. Careful *in silico* analysis (12) of homology models of ACP3, ACP6 and a representative inactive chimera identified a cluster of close contacts between $H_I$ (H26) and $L_I$ (V41-G42-Q43) in ACP6 that were absent in ACP3 (whose corresponding residues were A26 and, I41-N42-V43 respectively). We therefore hypothesized that an unfavorable interaction between H26 and N42-V43 was the source of the problem. The H26A mutants of AYC71, 81, 82 and SHIV12 (SHIV25, 22, 26 and 20, respectively) were constructed and purified as active ACP proteins in yields of 5-8 mg per liter culture. For this reason, SHIV29 also included the H26A mutation, and was successfully expressed as an active protein (4 mg per liter culture).

***Construction and expression of DEBS ACP3 single or double mutants***

A plasmid encoding DEBS ACP3 (pVYA5) was used as the template. The PCR primer sequences used to construct the single or double mutants (K62N, R63Q, R71A, K62N/R71, R63Q/R71A) are tabulated in Table S4. The resulting plasmids were transformed into *E. coli* BAP1, expressed and purified as described above for the fusion constructs to obtain the desired *holo*-ACP. Residue numbering is consistent with Fig. 2.

***Construction and expression of Module 3 mutant proteins.*** The R1420A+R1421A Module 3 mutant described in Fig. S6 was obtained by utilizing primers 5'-GCCGAGATCAACGTGGCCGCGGCGTTCAGC GAGCTCGG-3' and 5'-CCGAGCTCGCTGAACGCCGCGGCCACGTTGATCTCGGC-3'. The resulting plasmids were introduced into *E. coli* BAP1 (9) by transformation to produce the desired *holo*-proteins (10). The proteins were expressed and purified using a previously described protocol (11).

***Kinetic analyses of Module 3 mutant.*** Triketide lactone formation assay (Fig. S6) was performed as follows:

Each time point was set up in a reaction volume of 10 μL. Module 3 construct (10 μM of wild-type or R1420AR1421A mutant) was incubated with unlabeled NDK-SNAC (1 or 5 mM), TCEP (2.5 mM) and DL-[2-methyl-$^{14}$C]-methylmalonyl-CoA (200 μM ) at room temperature in 100 mM phosphate buffer at pH 7.2. At each time point (5 - 30 min) the reaction was quenched and processed as described above for the chain elongation assay.

***Circular dichroism spectroscopy.*** The proteins were diluted in 20 mM potassium phosphate buffer (pH 7.2). All of the proteins had a concentration of 0.25 mg/ml; circular dichroism ellipticity was recorded with a JASCO J-815 instrument (JASCO, Easton, MD). Spectra from 260 to 195. nm were scanned at a step of 1 nm at 25°C in a 0.1 cm cuvette, with three repeats and an averaging time of 1 s.

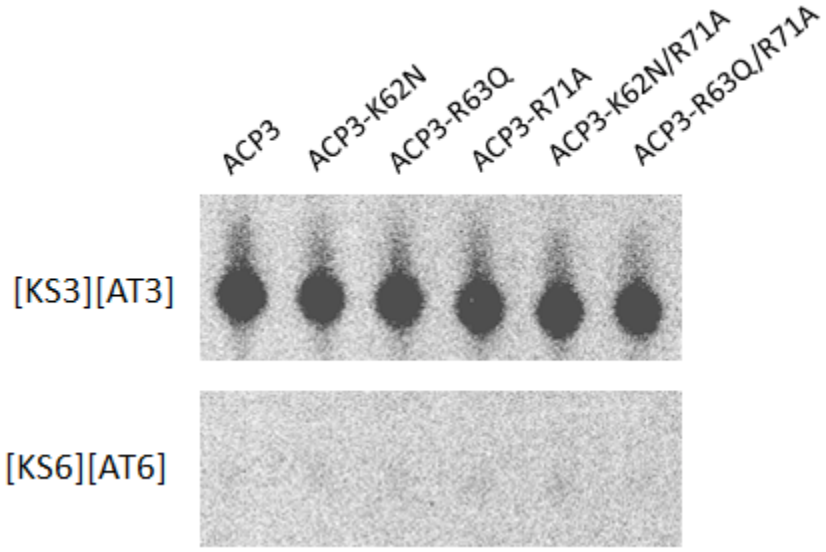



**Fig. S1.** Evaluation of ACP3 mutants designed to manipulate surface electrostatic features. Radio-TLC assays for chain elongation activity by the wild type and mutants of ACP3 in the presence of [KS3][AT3] or [KS6][AT6]. All mutants displayed identical specificity for KS3 over KS6 as wild-type ACP3. Refer to the '$L_I$ is the principal determinant of KS-ACP recognition during polyketide chain elongation.' section in the main text and Fig. 2D for interpretation of results. Refer to *Materials and Methods* section and Fig. S2A for a description of the assay. Residue numbering is as per Fig. 2.

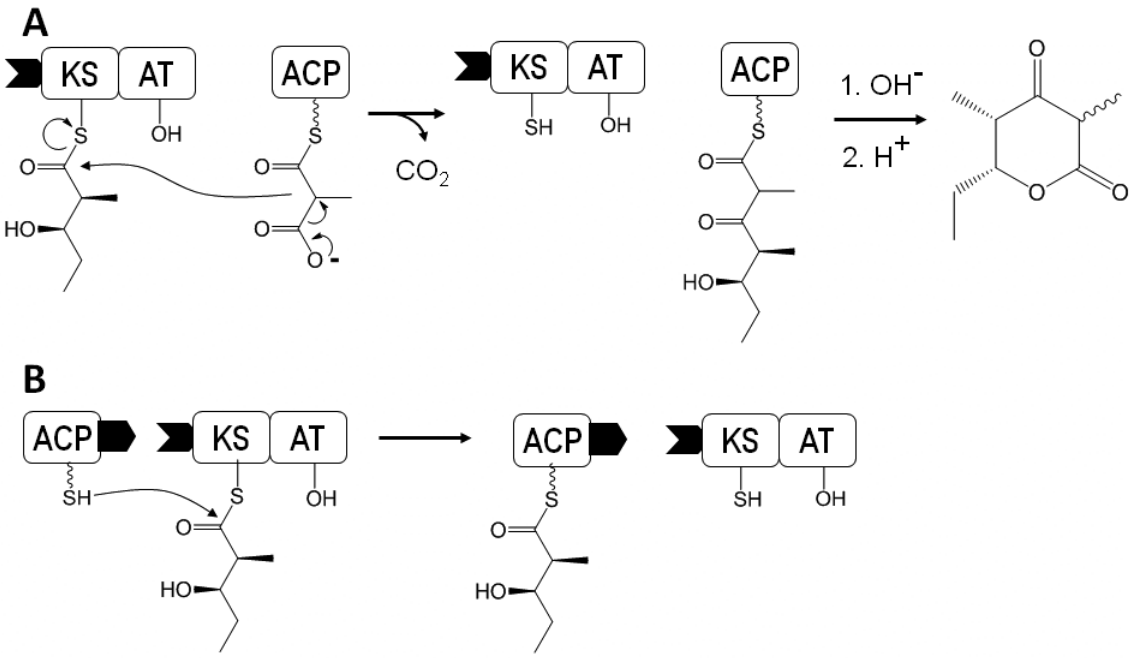



**Fig. S2.** Kinetic analyses of dissociated [KS][AT] didomains from DEBS modules 3 or 6. Schematic representation of the assays described in *Materials and Methods* illustrating (A) Chain elongation catalyzed by KS (primed with NDK derived from the corresponding SNAC thioester) and ACP (primed with a methylmalonyl extender unit derived from the corresponding CoA). The ACP is primed by the action of the AT domain. This is a single turnover assay and the lactone is released during the workup. (B) Chain transfer of NDK from the active site Cys to the ACP. This is the microscopic reverse of the intermodular chain transfer reaction in a natural assembly line. The KS is primed by self-acylation with NDK derived from the corresponding SNAC thioester. Interprotein linkers that are known to facilitate intermodular chain transfer (5, 13) are shown as matching solid tabs (black). The use of the simpler polyketide substrate (NDK) relative to the natural substrates for module 3 and 6 is motivated by the observation of a poor correlation between the rates for the optimal substrate and the natural substrate for these modules (14). As noted previously, the hypothesis that the unnatural or simpler substrates lack the complex recognition features of the natural substrate and may consequently have attenuated turnover is unlikely to be true at least for modules 3 and 6, since the reported $k_{cat}$ for NDK is comparable to the reported turnovers for multi-modular assembly lines (14).

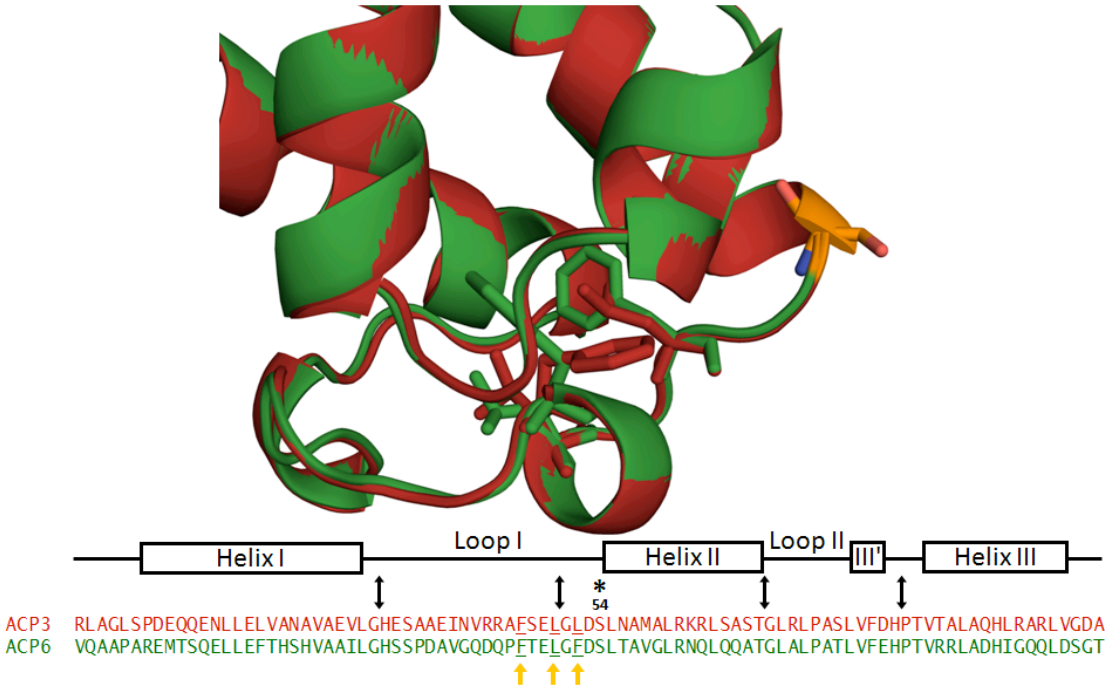



**Fig. S3.** Alternative packing of the hydrophobic core in ACP3 and ACP6. A relatively conserved hydrophobic triad (F47, L50 and L52 in ACP3, and F47, L50 and F52 in ACP6, orange arrows) that lies at the $L_I$ – $H_{II}$ fusion junction was predicted to adopt significantly different conformations in homology models for ACP3 and ACP6. In particular, F47 was oriented differently in the two cases while the plane of the aromatic ring of F52 in ACP6 was rotated 90° relative to L52 in ACP3. The conserved S54 residue at the N-terminus of $H_{II}$ is highlighted in orange.

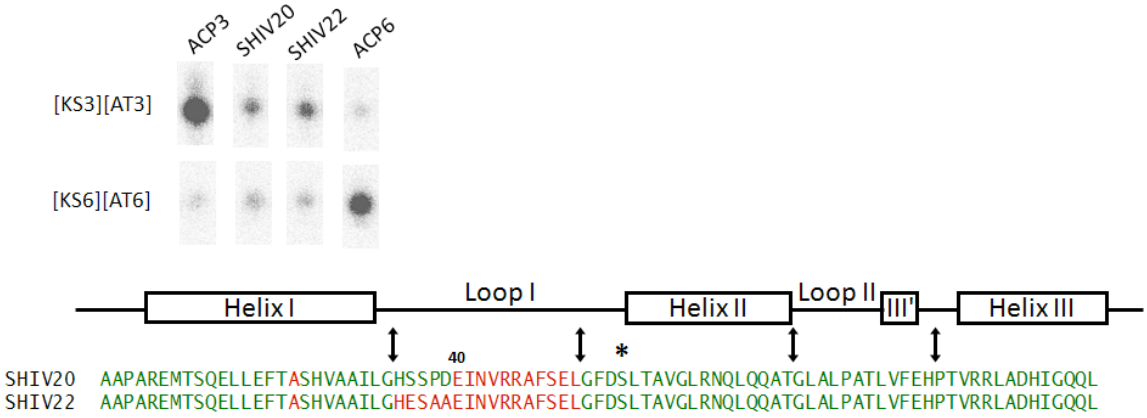



**Fig. S4.** Evaluation of chimeric ACP proteins in an assay for polyketide chain elongation. SHIV20 showed a preference for [KS3][AT3] over [KS6][AT6], similar to that observed for SHIV22. The color scheme is similar to Figure 2 (red = ACP3, green = ACP6).

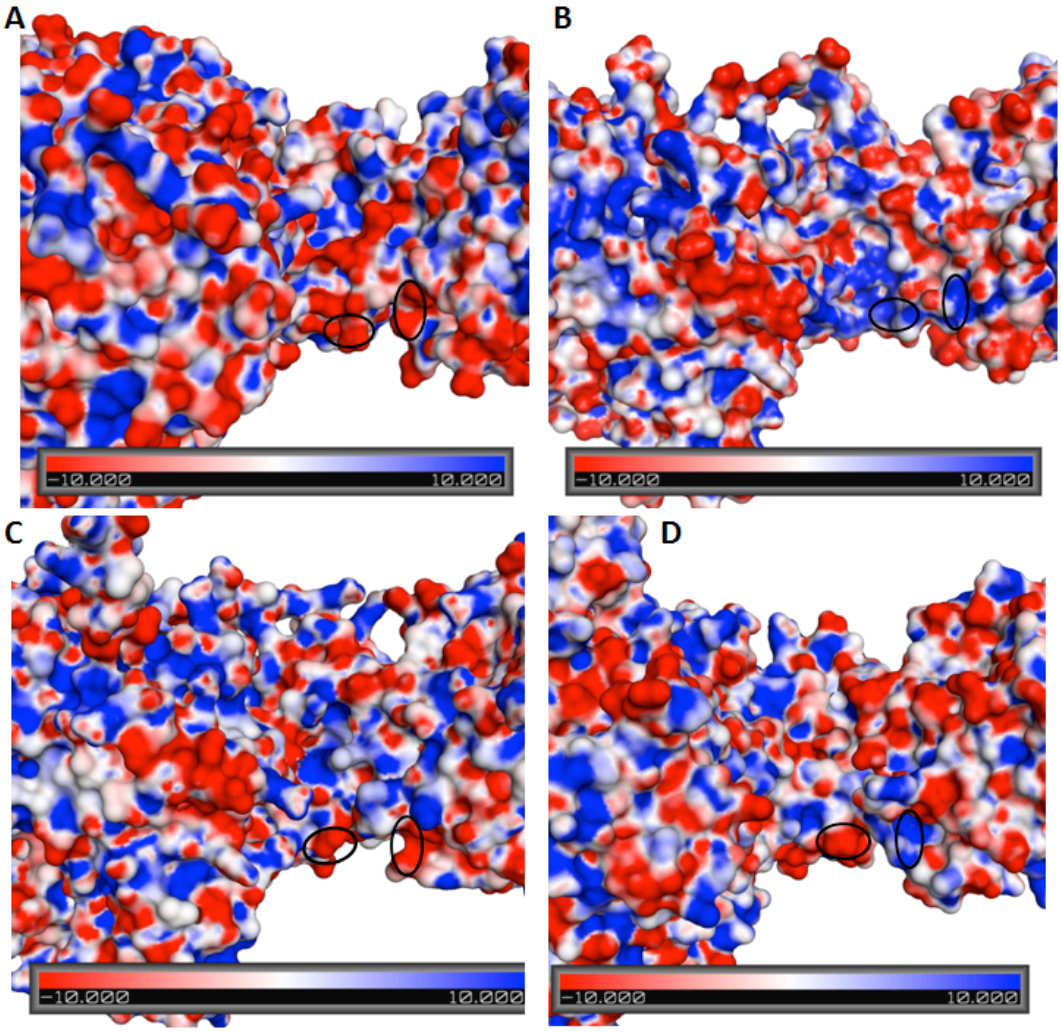
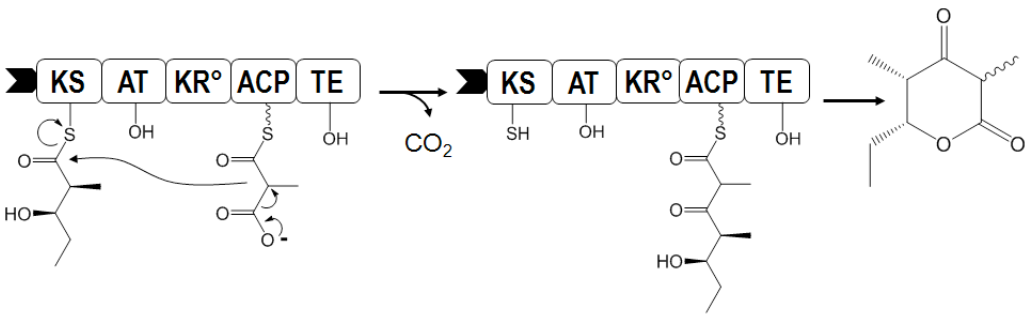



**Fig. S5.** Electrostatic surface maps of [KS][AT] didomains. Two charged regions circled on each surface map correspond to region II (left oval) and region I (right oval) for [KS][AT] didomains from DEBS module 3(A), 6(B), 4(C), and 5(D). Docking models reveal that these patches interact with residues 45 and 44, respectively, of the ACP domain. Blue and red indicate positive and negative potentials, respectively (scaled from ± 10 kT/e). The potentials in each of these two regions are complementary to those of the side chains of ACP3 at positions 44 (R) and 45 (R), of ACP6 (44D, 45Q), ACP4 (44R, 45K) and ACP5 (44D, 45R). The residue level definitions of region I and region II are as follows (numbering for module 3 (PDB ID code: 2QO3), 4 and 6 is as in reference (15) and for module 5 (PDB ID code: 2HG4)as in reference (16)):
Module 3: region I (823D + 850D); region II (517E + 553E + 555E)
Module 6: region I (822H); region II (519R + 555A + 556G)
Module 4: region I (821G + 823D + 850E); region II (517D + 551T + 552S)
Module 5: region I (548R + 549T); region II (514E + 546Q)
In some cases more than one residue exhibiting electrostatic complementarity was present in close proximity which precluded definitive identification of the specific residue(s) involved. All possible residues are reported for those cases.

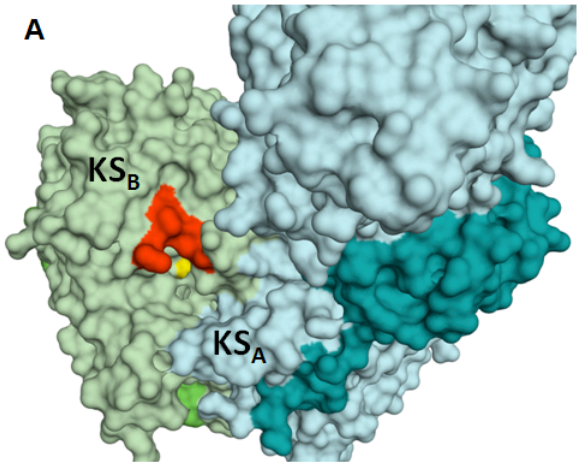



| Protein | Rate of polyketide product formation |
|---|---|
| wt M3TE | 100 |
| R1420A+R1421A[1] | 10 |

1) R1420 and R1421 in M3TE are equivalent to R44 and R45 in a stand alone ACP domain.

**Fig. S6.** Kinetic analyses of the complete module 3 fused to a thioesterase (TE) domain. Schematic representation of the assay performed in this study, illustrating chain elongation catalyzed by KS (primed with NDK derived from the corresponding SNAC thioester) and ACP (primed with a methylmalonyl extender unit derived from the corresponding methylmalonyl-CoA). The ACP is primed by the action of the AT domain. The TE domain catalyzes lactone formation and product release. The R1420A+R1421A mutant showed a marked reduction in the rate of polyketide product formation relative to the wild type (wt).

A




B

| Module | KS Hydrophobic patch residues | ACP partner |
|---|---|---|
| 1 | AP ~ LG ~ I | L |
| 2 | AP ~ LG ~ V | V |
| 3 | AP ~ LG ~ I | M |
| 4 | AP ~ LG ~ I | V |
| 5 | AP ~ LG ~ I | V |
| 6 | AP ~ LG ~ V | V |

**Fig. S7.** A conserved hydrophobic interaction between the KS and ACP. All KS domains bear a conserved hydrophobic patch (red, Fig. S7A) near the entrance to the buried active site (yellow, Fig. S7A). Panel B: This hydrophobic patch comprises of residues on a single KS subunit ($KS_B$ in Fig. S7A). The Ser residue on the ACP to which the pantetheinyl arm is attached is presumably positioned at the entrance to the active site pocket. In all models thus constrained, the residue which is the Ser+4$^{th}$ residue on helix II of the ACP is well positioned to interact with this patch. The variable residue (I in KS3 and V in KS6) in the KS patch presumably confers specificity to this interaction (Ser+4$^{th}$ residues for ACP3 and ACP6 are M and V, respectively). The coloring and nomenclature scheme for the didomain protein is the same as in Fig. 4.

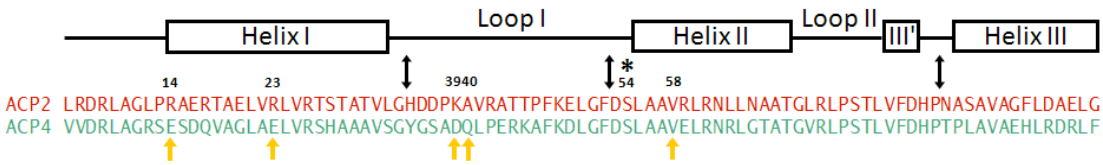



**Fig. S8.** Structure-based hybrid ACP protein construction. Sequence alignment of DEBS ACP2 and ACP4. The black arrows indicate the fusion sites utilized to recombine the secondary structure elements of ACP2 and ACP4. The asterisk indicates the conserved Ser54 phosphopantetheine attachment site. Additional fusion sites used to generate chimeras of ACP2 and ACP4 detailed in Fig. 5 are indicated by residue number and highlighted by orange arrows.

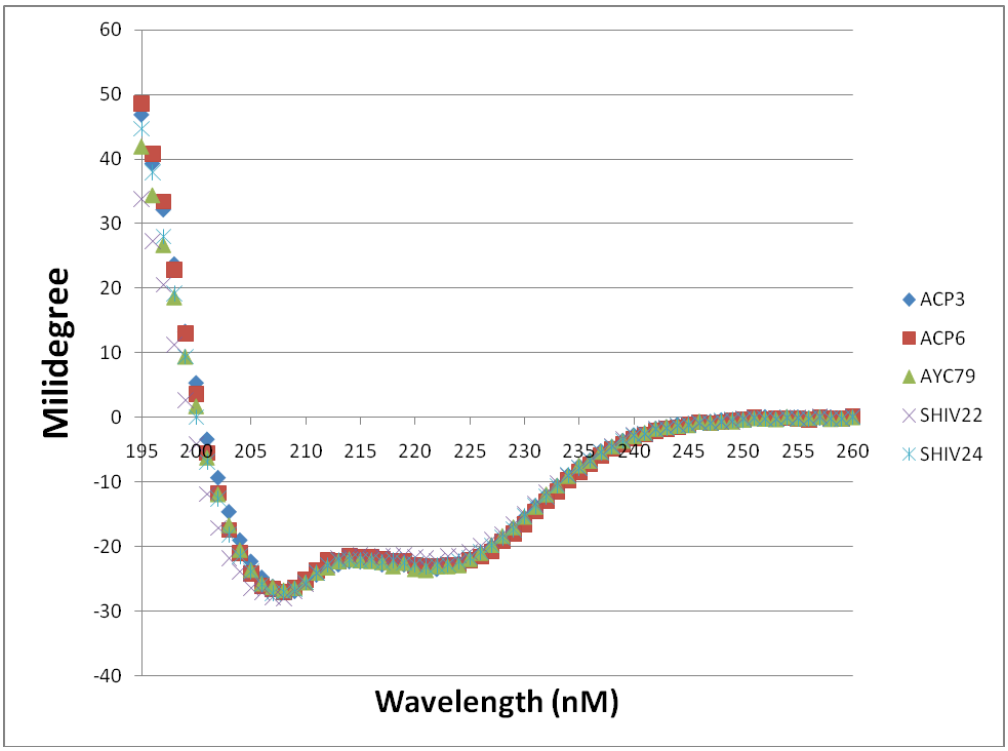



**Fig. S9.** Circular dichroism spectra of representative ACP proteins. Spectra were recorded for representative proteins in the chain elongation assay (ACP3, ACP6, AYC79, SHIV22, and SHIV24).

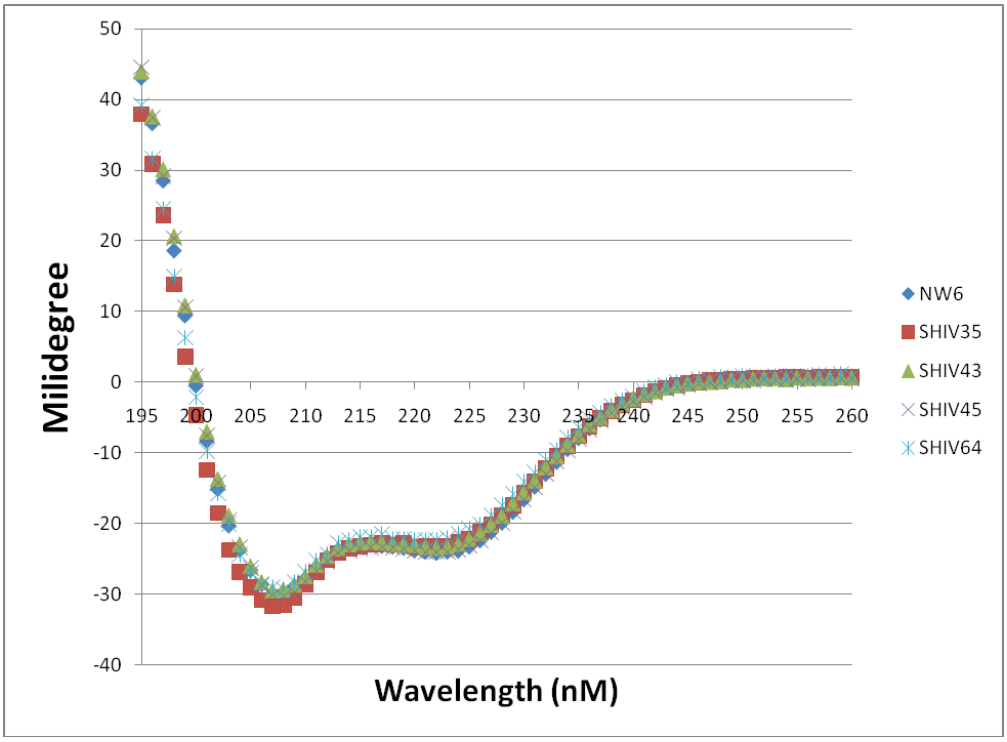



**Fig. S10.** Circular dichroism spectra of representative ACP proteins. Spectra were recorded for representative proteins in the chain transfer assay (NW6 (wild type ACP2(2)), SHIV43, SHIV45, and SHIV35).

**Table S1.** Primers and templates used for DEBS ACP3/6 fusion construction.

| Plasmid | Fragment | PCR primers | PCR template |
|---|---|---|---|
| pAYC67 | Upstream | 5'-AAAAAACATATGCGGCTCGCGGGGCTTTCCCCG-3'<br>5'-CGTCCGGGCTGGAATGGCCAAGCACCTCGGCAAC-3' | pVYA5 |
| | Downstream | 5'-GTTGCCGAGGTGCTTGGCCATTCCAGCCCGGACG-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pPK223 |
| pAYC68 | Upstream | 5'-AAAAAACATATGCGGCTCGCGGGGCTTTCCCCG-3'<br>5'-GTCAGCGAGTCGAAGCCGAGCTCGCTGAACGCGC-3' | pVYA5 |
| | Downstream | 5'-GCGCGTTCAGCGAGCTCGGCTTCGACTCGCTGAC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pPK223 |
| pAYC69 | Upstream | 5'-AAAAAACATATGCGGCTCGCGGGGCTTTCCCCG-3'<br>5'-GCGGGCAGCGCGAGCCCGGTGCTCGCCGACAGGC-3' | pVYA5 |
| | Downstream | 5'-GCCTGTCGGCGAGCACCGGGCTCGCGCTGCCCGC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pPK223 |
| pAYC70 | Upstream | 5'-AAAAAACATATGCGGCTCGCGGGGCTTTCCCCG-3'<br>5'-CAACCTGCGGACCGTGGGGTGGTCGAACACCAGC-3' | pVYA5 |
| | Downstream | 5'-GCTGGTGTTCGACCACCCCACGGTCCGCAGGTTG-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pPK223 |
| pAYC71 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-CGGCGGCGGACTCGTGCCCGAGGATCGCCGCG-3' | pPK223 |
| | Downstream | 5'-CGCGGCGATCCTCGGGCACGAGTCCGCCGCCG-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pVYA5 |
| pAYC72 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GTTGAGCGAGTCGAGTCCGAGCTCGGTGAACGGC-3' | pPK223 |
| | Downstream | 5'-GCCGTTCACCGAGCTCGGACTCGACTCGCTCAAC-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pVYA5 |
| pAYC73 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GCGGGCAGCCGCAGGCCGGTGGCCTGCTGGAGC-3' | pPK223 |
| | Downstream | 5'-GCTCCAGCAGGCCACCGGCCTGCGGCTGCCCGC-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pVYA5 |
| pAYC74 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GCGCGGTGACGGTGGGGTGCTCGAACACCAGGG-3' | pPK223 |
| | Downstream | 5'-CCCTGGTGTTCGAGCACCCCACCGTCACCGCGC-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pVYA5 |
| pAYC79 | Upstream | 5'-AAAAAACATATGCGGCTCGCGGGGCTTTCCCCG-3'<br>5'-GTTGAGCGAGTCGAGTCCGAGCTCGGTGAACGGC-3' | pAYC67 |
| | Downstream | 5'-GCCGTTCACCGAGCTCGGACTCGACTCGCTCAAC-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pAYC72 |
| pAYC80 | Upstream | 5'-AAAAAACATATGCGGCTCGCGGGGCTTTCCCCG-3'<br>5'-GCGGGCAGCCGCAGGCCGGTGGCCTGCTGGAGC-3' | pAYC67 |
| | Downstream | 5'-GCTCCAGCAGGCCACCGGCCTGCGGCTGCCCGC-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pAYC73 |
| pAYC81 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GTCAGCGAGTCGAAGCCGAGCTCGCTGAACGCGC-3' | pAYC71 |
| | Downstream | 5'-GCGCGTTCAGCGAGCTCGGCTTCGACTCGCTGAC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pAYC68 |
| pAYC82 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GCGGGCAGCGCGAGCCCGGTGCTCGCCGACAGGC-3' | pAYC71 |
| | Downstream | 5'-GCCTGTCGGCGAGCACCGGGCTCGCGCTGCCCGC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pAYC69 |

**Table S2.** Primers and templates used for DEBS ACP3/6 fusion construction.

| Plasmid | Fragment | PCR primers | PCR template |
|---|---|---|---|
| pSHIV12 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-CGGCGCACGTTGATCTCGTCCGGGCTGGAATGCC-3' | pAYC72 |
| | Downstream | 5'-GGCATTCCAGCCCGGACGAGATCAACGTGCGCCG-3'<br>5'- TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG -3' | pAYC68 |
| pSHIV20 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GATCGCCGCGACGTGCGACGCCGTGAACTCCAGCAACTC-3' | pSHIV12 |
| | Downstream | 5'-GAGTTGCTGGAGTTCACGGCGTCGCACGTCGCGGCGATC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pSHIV12 |
| pSHIV22 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GATCGCCGCGACGTGCGACGCCGTGAACTCCAGCAACTC-3' | pAYC81 |
| | Downstream | 5'-GAGTTGCTGGAGTTCACGGCGTCGCACGTCGCGGCGATC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pAYC81 |
| pSHIV24 | Upstream | 5'-AAAAAACATATGCGGCTCGCGGGGCTTTCCCCG-3'<br>5'-CAGGCGCTTGCGCAGGGCGACCGCGGTCAGCGAGTC-3' | pAYC67 |
| | Downstream | 5'-GACTCGCTGACCGCGGTCGCCCTGCGCAAGCGCCTG-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pAYC72 |
| pSHIV25 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GATCGCCGCGACGTGCGAcgcCGTGAACTCCAGCAACTC-3' | pAYC71 |
| | Downstream | 5'-GAGTTGCTGGAGTTCACGgcgTCGCACGTCGCGGCGATC-3'<br>5'-TTTTTTGAATTCGCGGCGTCACCGACGAGCCGG-3' | pAYC71 |
| pSHIV26 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GATCGCCGCGACGTGCGAcgcCGTGAACTCCAGCAACTC-3' | pAYC82 |
| | Downstream | 5'-GAGTTGCTGGAGTTCACGgcgTCGCACGTCGCGGCGATC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG -3' | pAYC82 |
| pSHIV29 | Upstream | 5'-AAAAAACATATGGCGGCCCCGGCGCGGGAGATG-3'<br>5'-GAGCTGGTTGCGCAGCCCCATCGCGTTGAGCGAGTC-3' | pSHIV26 |
| | Downstream | 5'-GACTCGCTCAACGCGATGGGGCTGCGCAACCAGCTC-3'<br>5'-TTTTTTGAATTCGCGAGCTGCTGTCCTATGTGG-3' | pAYC68 |

**Table S3.** Primers and templates used for DEBS ACP2/4 fusion construction.

| Plasmid | Fragment | PCR primers | PCR template |
|---|---|---|---|
| pSHIV32 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGC-3'<br>5'-CGCCTTCGGGTCGTCGTGCCCGGAGACCGCCGCCGC-3' | pNW8 |
| | Downstream | 5'-GCGGCGGCGGTCTCCGGGCACGACGACCCGAAGGCG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pNW6 |
| pSHIV34 | Upstream | 5'-AAAAAACATATGCTGCGCGACCGGCTGGC-3'<br>5'-CACCGCCGGTGAGGCCGCGCCGAGCTCGGCGTCGAG-3' | pNW6 |
| | Downstream | 5'-CTCGACGCCGAGCTCGGCGCGGCCTCACCGGCGGTG-3'<br>5'-TTTTTTGAATTCCTACAGGTCCTCTCCCCC-3' | pNW8 |
| pSHIV35 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGC-3'<br>5'-CTCCCCCCGGACCTCGGTGAACAGCCTGTCCCGCAG-3' | pNW8 |
| | Downstream | 5'-CTGCGGGACAGGCTGTTCACCGAGGTCCGGGGGGAG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pNW6 |
| pSHIV36 | Upstream | 5'-AAAAAACATATGCTGCGCGACCGGCTGGC-3'<br>5'-CTTGCGCTCGGGGAGCTGCTTCGGGTCGTCGTGGCC-3' | pNW6 |
| | Downstream | 5'-GGCCACGACGACCCGAAGCAGCTCCCCGAGCGCAAG-3'<br>5'-TTTTTTGAATTCCTACAGGTCCTCTCCCCC-3' | pNW8 |
| pSHIV37 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGC-3'<br>5'-GGCGTGGTCGCGCGCACCGCGTCGGCCGAGCCGTACCCG-3' | pNW8 |
| | Downstream | 5'-CGGGTACGGCTCGGCCGACGCGGTGCGCGCGACCACGCC-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pNW6 |
| pSHIV42 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGC-3'<br>5'-CTCCACCGCGGCCAGCGAGTCGAACCCGAGCTCCTTG-3' | pSHIV32 |
| | Downstream | 5'-CAAGGAGCTCGGGTTCGACTCGCTGGCCGCGGTGGAG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pSHIV35 |
| pSHIV43 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGC-3'<br>5'-CTCCACCGCGGCCAGCGAGTCGAACCCGAGCTCCTTG-3' | pSHIV37 |
| | Downstream | 5'-CAAGGAGCTCGGGTTCGACTCGCTGGCCGCGGTGGAG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pSHIV35 |
| pSHIV45 | Upstream | 5'-AAAAAACATATGCTGCGCGACCGGCTGGC-3'<br>5'-CTCCACCGCGGCCAGCGAGTCGAACCCGAGCTCCTTG-3' | pSHIV34 |
| | Downstream | 5'-CAAGGAGCTCGGGTTCGACTCGCTGGCCGCGGTGGAG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pSHIV42 |
| pSHIV47 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGC-3'<br>5'-GCGGAGTCGGGTGGTCGAAGACCAGCGTCGACGGC-3' | pSHIV44 |
| | Downstream | 5'-GTCGACGCTGGTCTTCGACCACCCGACTCCGCTG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pSHIV45 |
| pSHIV62 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGC-3'<br>5'-CTCCCCCCGGACCTCGGTGAACAGCCTGTCCCGCAG -3' | pSHIV36 |
| | Downstream | 5'-CTGCGGGACAGGCTGTTCACCGAGGTCCGGGGGGAG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pNW6 |
| pSHIV63 | Upstream | 5'-AAAAAACATATGCTGCGCGACCGGCTGGCGGGTCTGCCGCGTGCC<br>GAGCGGACGGCGGAGCTGGTGCGCCTGGTGCGTTCACACGCGGCGGCG<br>GTCTCC-3'<br>5'-GGAGACCGCCGCCGCGTGTGAACGCACCAGGCGCACCAGCTCCGC<br>CGTCCGCTCGGCACGCGGCAGACCCGCCAGCCGGTCGCGCAGCATATG<br>TTTTTT-3' | - |
| | Downstream | 5'-GGTGCGTTCACACGCGGCGGCGGTCTCC-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pSHIV35 |

| | | | |
|---|---|---|---|
| pSHIV64 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGCCGGTCGGTCCCGTGCC<br>GAGCGGACGGCGGAGCTGGTGCGCCTGGTGCGTTCACACGCGGCGGCG<br>GTCTCC-3'<br>5'-GGAGACCGCCGCCGCGTGTGAACGCACCAGGCGCACCAGCTCCGC<br>CGTCCGCTCGGCACGGGACCGACCGGCGAGCCGGTCGACCACCATATG<br>TTTTTT-3' | - |
| | Downstream | 5'-GGTGCGTTCACACGCGGCGGCGGTCTCC-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pSHIV35 |
| pSHIV65 | Upstream | 5'-AAAAAACATATGGTGGTCGACCGGCTCGCCGGTCGGTCCCGTGCC<br>GAGCGGACGGCGGAGCTGGTGCGCCTGGTCCGCACCAGCACCGCGACC<br>GTGCTGGGCTACGGCTCGGCCGACCAG-3'<br>5'-CTGGTCGGCCGAGCCGTAGCCCAGCACGGTCGCGGTGCTGGTGCG<br>GACCAGGCGCACCAGCTCCGCCGTCCGCTCGGCACGGGACCGACCGGC<br>GAGCCGGTCGACCACCATATGTTTTTT-3' | - |
| | Downstream | 5'-CACCGCGACCGTGCTGGGCTACGGCTCGGCCGACCAG-3'<br>5'-TTTTTTGAATTCTCAATCGCCGTCGAGCTCC-3' | pSHIV35 |

**Table S4.** Primers and templates used for DEBS ACP3 mutant construction.

| Plasmid | Mutation | PCR primers | PCR template |
|---|---|---|---|
| pAYC53 | ACP3 K62N | 5'-CGATGGCCCTGCGCAACCGCCTGTCGGCGAGC-3'<br>5'-GCTCGCCGACAGGCGGTTGCGCAGGGCCATCG-3' | pVYA5 |
| pAYC54 | ACP3 R63Q | 5'-GGCCCTGCGCAAGCAGCTGTCGGCGAGCACCGG-3'<br>5'-CCGGTGCTCGCCGACAGCTGCTTGCGCAGGGCC-3' | pVYA5 |
| pAYC55 | ACP3 R71A | 5'-CGAGCACCGGCCTGGCGCTGCCCGCGTCGCTGG-3'<br>5'-CCAGCGACGCGGGCAGCGCCAGGCCGGTGCTCG-3' | pVYA5 |
| pAYC65 | ACP3 K62N/R71A | 5'-CGAGCACCGGCCTGGCGCTGCCCGCGTCGCTGG-3'<br>5'-CCAGCGACGCGGGCAGCGCCAGGCCGGTGCTCG-3' | pAYC53 |
| pAYC66 | ACP3 R63Q/R71A | 5'-CGAGCACCGGCCTGGCGCTGCCCGCGTCGCTGG-3'<br>5'-CCAGCGACGCGGGCAGCGCCAGGCCGGTGCTCG-3' | pAYC54 |

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