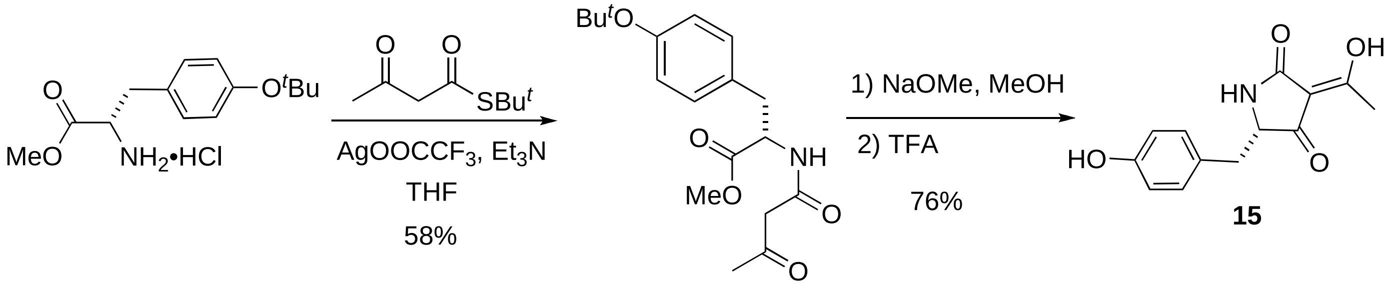
Table S1. Oligonucleotide lists for cloning *cpaD* from *A. oryzae* RIB40 and *A. flavus* NRRL3357

OLIGO No.	Sequence	Description
OLIGO_XL116	GATCGGATCCATGGAGATCTCCAAGAAAGCAGCAACAC	5' BamHI RIB40 cpaD long exon
OLIGO_XL105	GTCCACATCGGGATAGTAGGATGCCAAATCCGCTGG	3' RIB40 cpaD long exon
OLIGO_XL106	GATTTGGCATCCTACTATCCCGATGTGGACCTGCAGAC	5' RIB40 cpaD short exon
OLIGO_XL117	GATTGCGGCCGCCTAAGGATTGTGGCC	3' NotI RIB40 cpaD short exon
OLIGO_XL192	GATCGGATCCATGGCGAGTGCCGGCTATGATGTTC	5' BamHI NRRL3557 cpaD long exon
OLIGO_XL193	AGTCCACATCCGGATAGTACGATGCCAAATC	3' NRRL3357 cpaD long exon
OLIGO_XL194	GATTTGGCATCGTACTATCCGGATGTGGACTTAAATACTG	5' NRRL3357 cpaD short exon
OLIGO_XL195	GAGTGCGGCCGCTTAATGATTGTGGCCATC	3' NotI NRRL3357 cpaD short exon
OLIGO_XL196	GATCGGATCCATGGAGATCTCCAGGAAAGCAGC	5' BamHI NRRL3357 cpaD (**new sequence**) long exon

Table S2. Oligonucleotide lists for generating CpaD mutants.

CpaD mutant	Oligonucleotide sequence
CpaD E93L	CTTCGGACTTCCTTTCCTGCTGAGCTTCAATTAC
CpaD S95A	CTTCCTTTCGAGCTGGCGTTCAATTACTCCAAATC
CpaD N97L	TTCGAGCTGAGCTTCCTGTACTCCAAATCACTAC
CpaD R104E	CTCCAAATCACTACTAGAATTTGCATTCGAGCCC
CpaD D119L	CGGGAACGAAGGATCTGCCATTCAACACCCAG
CpaD D181L	GAACAAACTGGCAGCCCTGCTGGAGCCATCTGGC
CpaD K191E	GGCGATATTGTCTTGGAAACCTACATCTACCCG
CpaD T203V	GATCAAGTCGATCGCGGTTGGGACCCCAAAAGAG
CpaD D250L	CACTTTCTCTCATGCCTGTTGGTCAAGCCGTCC
CpaD R258E	CAAGCCGTCCGAGTCCGAAATCAAGGTCTACTG
CpaD K260E	CCGAGTCCCGAATCGAAGTCTACTGTATGGAAC
CpaD W33F	GACCACACAAAATGGTTCTATAGCACAGCTCCG
CpaD W33L	GACCACACAAAATGGCTGTATAGCACAGCTCCG
CpaD Y193F	ATTGTCTTGAAGACCTTCATCTACCCGCGGATC
CpaD Y193L	ATTGTCTTGAAGACCCTGATCTACCCGCGGATC
CpaD Y262F	CCCGAATCAAGGTCTTCCTGGCGTTCCATACA
CpaD W299F	CGCTGAGGGAGCTGTTCCAGCTATTGCCCGTC
CpaD W299L	CGCTGAGGGAGCTGCTGCAGCTATTGCCCGTC
CpaD Y346F	CCGAACCACAGATCTTCTTCCCTGCTTTTGGG
CpaD Y410F	CAAGGGGAAAAAACCGTTCATGAGTGTGTACCTC
CpaD Y410L	CAAGGGGAAAAAACCGCTGATGAGTGTGTACCTC
CpaD Y414F	CGTACATGAGTGTGTTCCTCCATACCTTCG

Scheme S1. Preparation of tyrosine-derived tetramic acid **15**.



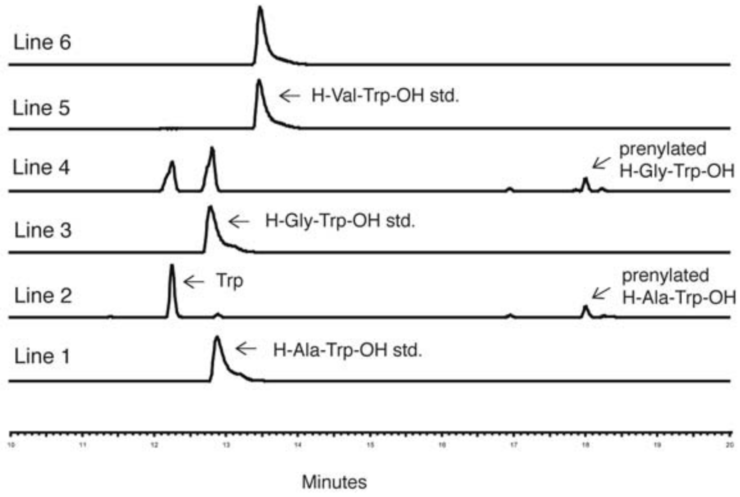


Figure S1. C-terminal tryptophan containing dipeptides are not good substrates for CpaD. HPLC traces Lane 1: H-Ala-Trp-OH standard; Lane 2: HPLC trace after incubating H-Ala-Trp-OH (250 µM), DMAPP (250 µM), CpaD (1µM) at 30 ºC for 15 h. Trace amount of prenylated H-Ala-Trp-OH was detected with L-tryptophan as the major product. Lane 3: H-Gly-Trp-OH standard; Lane 4: HPLC trace after incubating H-Gly-Trp-OH (250 µM), DMAPP (250 µM), CpaD (1µM) at 30 ºC for 15 h. Trace amount of prenylated H-Gly-Trp-OH was detected with L-tryptophan as the major product. Lane 5: H-Val-Trp-OH standard; Lane 6: HPLC trace after incubating H-Val-Trp-OH (250 µM), DMAPP (250 µM), CpaD (1µM) at 30 ºC for 15 h. No prenylated H-Ala-Trp-OH and L-tryptophan detected as products.

*A.oryzae*_CpaD MEISKKAATLLPKPFYVLSQALNLSNKDHTKWWYSTAPMFATMMAGAGYDVHAQYKFLCI 60
*A.flavus*_CpaD MEISRKAATELPKPFHVLSQALNLSNKDHAKWWYSTAPMFATMMASAGYDVHAQYKFLCI 60

*A.oryzae*_CpaD HREVIIPALGPYPEKGQPMHWKSHLTRFGLPFELSFNYSKSLLRFAFEPLGSLTGTKDDP 120
*A.flavus*_CpaD HREVIIPALGPYPEKGQPMHWKSHLTRFGLPFELSFNYSKSLLRFAFEPLGSLTGTEHDP 120

*A.oryzae*_CpaD FNTQAIRPVLQDLKAMVPGLDLEWFDHFTKALVVSEEEARTLLDRDIEIPVFKTQNKLAA 180
*A.flavus*_CpaD FNTQAIRPVLQDLKGIVPGLNLEWFDHFTKALVVSDEEAQALRDGDIEIPVFKTQNKLAA 180

*A.oryzae*_CpaD DLEPSGDIVLKTYIYPRIKSIATGTPKERLMFDAIKAADKFGKVATPLAILEEFIAERAP 240
*A.flavus*_CpaD DLEPSGDIVLKTYIYPRIKSIATGTPKERLMFDAIKAADKCGKITAPLAILKEFIAERAP 240

Aoryzae_CpaD TLLGHFLSCDLVKPSESRIKVYCMERQLDLASIEGIWTLNGRRNDPETLDGLDALRELWQ 300
*A.flavus*_CpaD TLLGHFLSCDLVKPSESRIKVYCMERQLDLASIEGIWTLNGRRNDPETLEGLDALRELWQ 300

*A.oryzae*_CpaD LLPVTEGLCPLPNCFYEPGTSPQEQLPFIINFTLSPKSALPEPQIYFPAFGQNDKTIAEG 360
*A.flavus*_CpaD LLPITEGLCPLPNCFYEPGTSPQEQLPFIINFTLSPKSPLPEPQIYFPAFGQNDRAIAEG 360

*A.oryzae*_CpaD LATFFESRGWGGLAKSYPADLASYYPDVDLQTANHLQAWISFSYKGKKPYMSVYLHTFEA 420
*A.flavus*_CpaD LATFFERRGWGGLAKTYPSDLASYYPDVDLNTANHLQAWISFSYKGKKPYMSVYLHTFEA 420

*A.oryzae*_CpaD FSAAAQEVAMCHDGHNP 437
*A.flavus*_CpaD FSGAAQEVSMCRDGHNH 437

Figure S2. Alignment of CpaD sequences from *A. oryzae* RIB40 and revised *A. flavus* NRRL3357 sequence. Amino acid residues shown in red indicate those missed from the predicted CpaD sequence as shown in the *Aspergillus* comparative database. (see http://www.broadinstitute.org/annotation/genome/aspergillus_group /GeneDetails.html?sp=S7000001155820388)

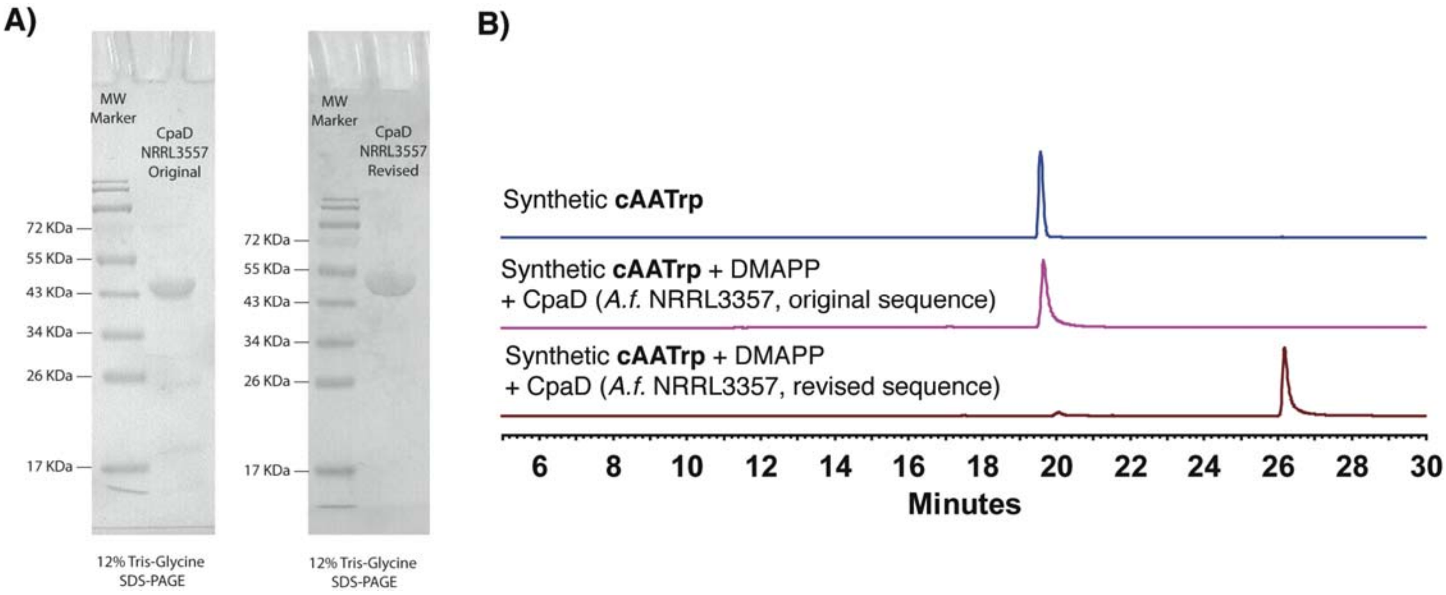


Figure S3. In vitro reconstitution of *A. flavus* NRRL3357 CpaD activities based on the original and revised protein sequences. (A) SDS-PAGE gel showing the heterelogously overexpressed and purified CpaDs based on the original and revised protein sequences. (B) HPLC assay showing the inactive and active natures of CpaDs overexpressed based on the original and revised protein sequences respectively.