

Supplementary Data

Modular Assembly of Phosphite Dehydrogenase and Phenylacetone Monooxygenase for Tuning Cofactor Regeneration

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Table S1. Oligonucleotide primer for PTDH and PAMO ORF amplification.

Primer	Primer (direction 5'-3' / sequence)
PTDH For	CCTGAAGGTCTCGTGGTATGCTGCCAACTGGTCATCACCCATC
PTDH Rev	CCCGGTCTCTCACCGTCAGCCGCAGGGTTGGCTTCG
PAMO for	CCTGAAGGTCTCGTGGTATGCCGGCAGACGACTGTC
PAMO rev	CCCGGTCTCTCACCGTGAGGACGAAACCTCGTA
PAMO Bsa mut for	CTGCGCTCAGCAACATGCTGGTGTCTATC
PAMO Bsa mut rev	CACGTGCTGTTCGATAGACACCAGCATGTTG

Table S2. Synthetic gene constructs.

Synthetic genes	Base sequences
RIAD	CCTGAAGGTCTCGGGTGGTGGTAGCGGTGGCGGTGGTCAG
RIAD-RIAD	CGGGCGGTGGTTGCAGAGACCGCTAAAGTTGCGGTCTTTGCG GTCTGGAACAGTATGCAAATCAGCTGGCAGATCAGATTATCAA GAAGCAACCGAAGGTTGCTAACTTGAGAGACCGGAAATGAAGG TCTCGTTGCGGTAGCCTCGTGAATGTGAACGTGATGTTCAGAAA CATATAATTAGGCCCTGCTGAAAGATAGCATTGTTCAGCTGTG ACCGCACGTCCCGAACGTCCGATGGCATTCTGCGCGAATATT GAACGTCTGGAAAAAGAAGAAGCCAATAACTTGAGAGACCGG AC
RIDD	CAAAGGTCTCATTGCGGTCTGGAACAGTATGCAAATCAGCTGGC AGATCAGATTATCAAAGAACGAAACCGAAGGTTGCGGCAGGTGGT GTAGCGGTGGCGGTGGTTCAAGGCAGGCGGTGGTTGTGGCCTGGAA CAGTACCGAATCAGCTGGCCGATCAGATCATCAAAGAACGAC CGAAGGCTGTTAACTTGAGAGACCAATGAAAGACGTTCGATAT GCTATAGCTATTAGACTATGGATATGCTATCGATAGATCGAT CGGATATCGATTAAATTGATTAAAATTATTGCTATAGGCT ATAG

Table S3. Molecular weights of size exclusion chromatography standards.

Standard Protein	MW	Log MW	Elution Vol (mL)
Thyroglobulin	670000	5.83	9.48
γ -globulin	158000	5.20	12.5
Ovalbumine	44000	4.64	15.0
Myoglobin	17000	4.23	16.82
Vitamin B12	1350	3.13	19.75

Table S4. Conversion of bicyclo[3.2.0]hept-2-en-6-one. Conversion was measured by GC-MS analysis, based on product peak areas.

Enzymes complexes	Time (hours)	Conversion (%)
PTDH _A -PAMO _I	5	59±4.2
	24	88±0.2
PTDH _{A2} -PAMO _I	5	60±0.7
	24	94.5±2.6
PAMO _A -PTDH _I	5	60±5.6
	24	78.5±0.7
PAMO _{A2} -PTDH _I	5	61.5±0.2
	24	77.1±2.6
PTDH-PAMO	5	68±2.8
	24	94.5±2.1

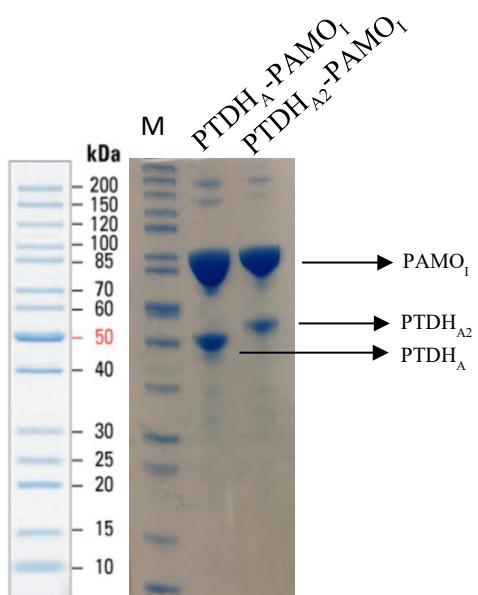
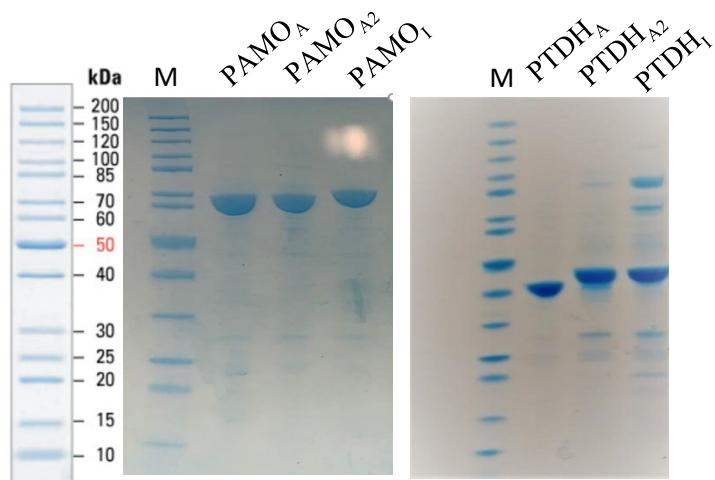
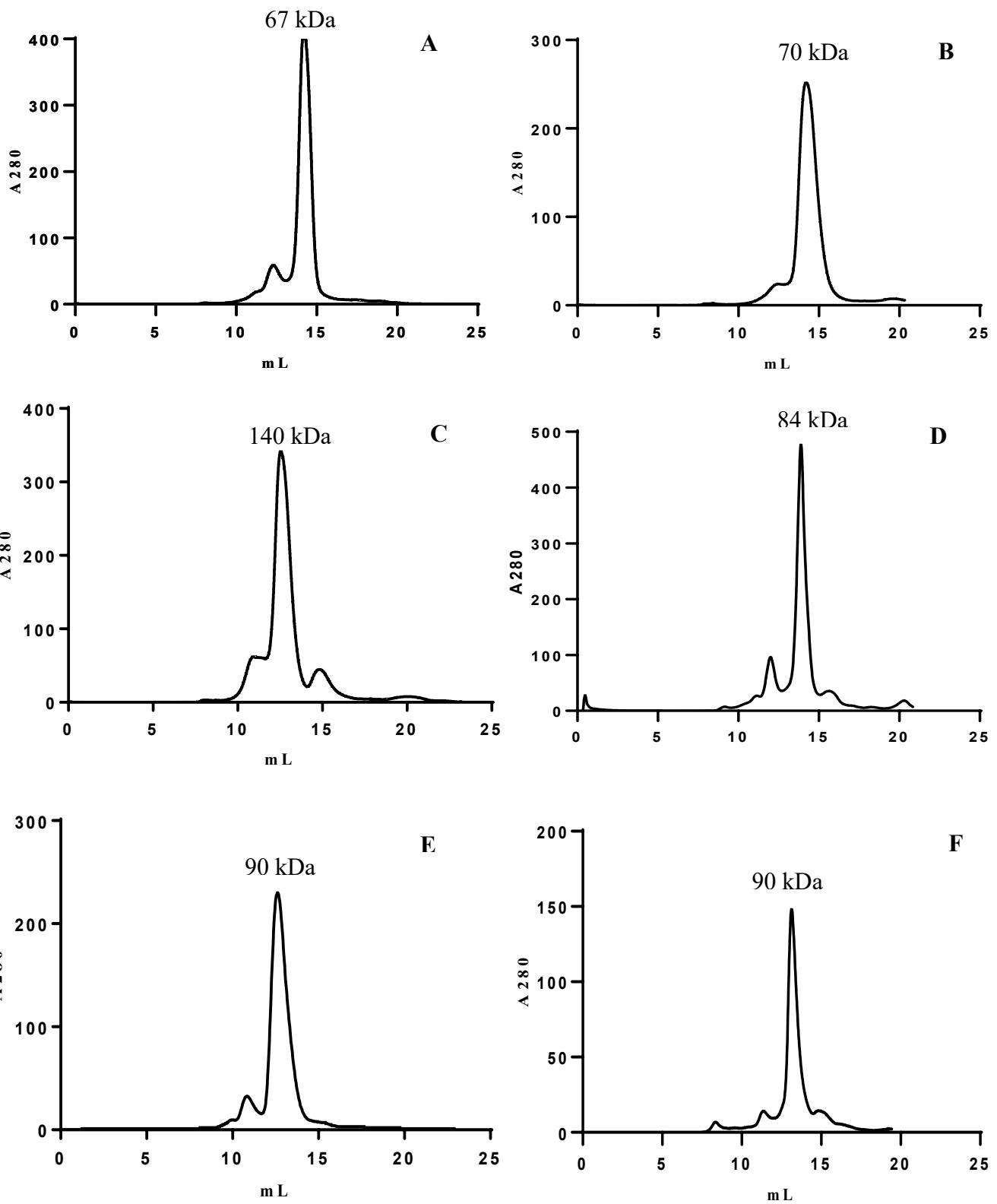


Figure S1. SDS-PAGE analysis. A: analysis of purified tagged PAMO variants; B: analysis of purified tagged PTDH variants; C: complex PTDH_A-PAMO_I and PTDH_{A2}-PAMO_I.



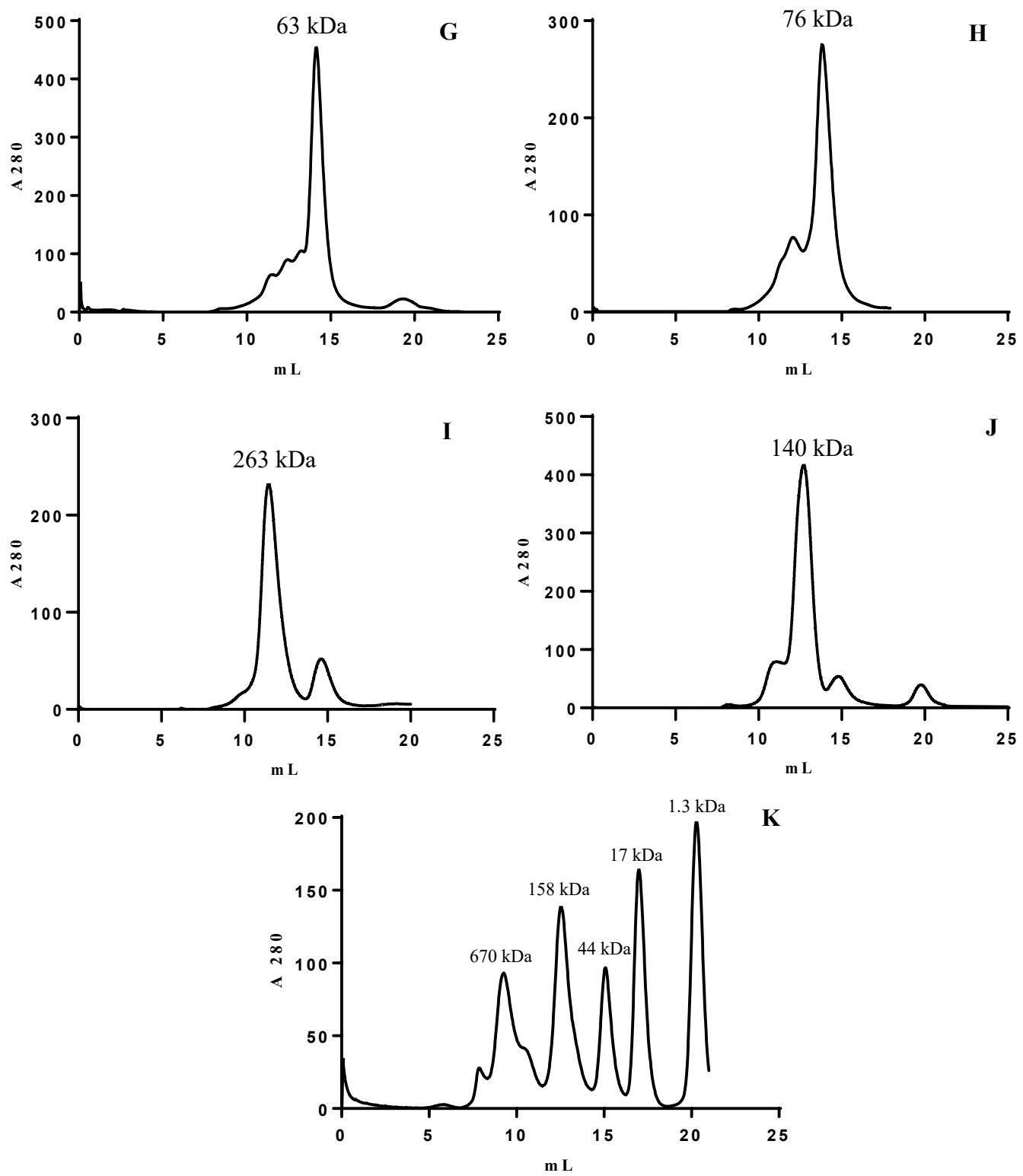


Figure S2. Size exclusion chromatogram of (A) PAMO_A, (B) PAMO_{A2}, (C) PAMO_I, (D) PTDH_A, (E) PTDH_{A2}, (F) PTDH_I, (G) PAMO_A-PTDH_I, (H) PAMO_{A2}-PTDH_I, (I) PAMO_A-PAMO_I, (J) PAMO_{A2}-PAMO_I, (K) size exclusion protein standards.