

Supplementary data

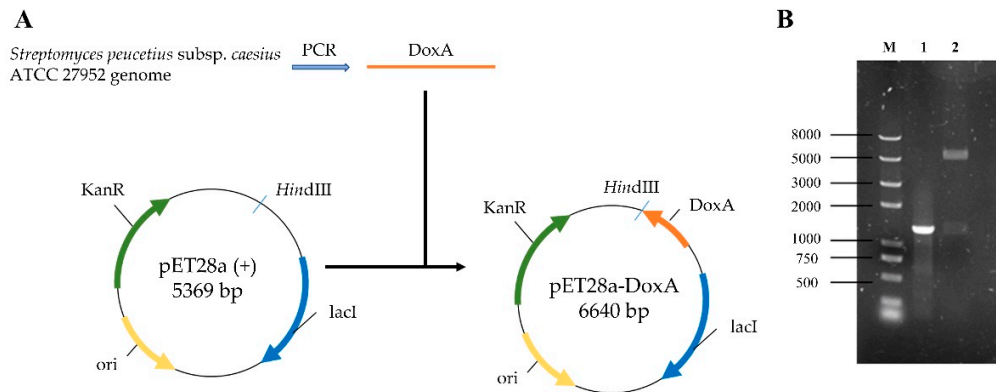


Figure S1. Schematic diagram of the recombinant plasmid pET28a-DoxA construction. (A): The construction of recombinant plasmid pET28a-DoxA; (B): DNA Electrophoresis profile of DoxA. Lane M: DNA marker, lane 1: *doxA* gene, lane 2: enzyme digestion of pET28a-DoxA.

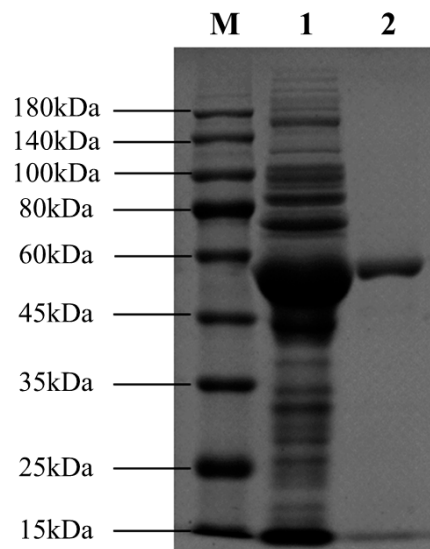


Figure S2. SDS-PAGE protein profile of DoxA. Lane M: protein marker, lane 1: the supernatant sample after cell fragmentation, lane 2: the purified DoxA.

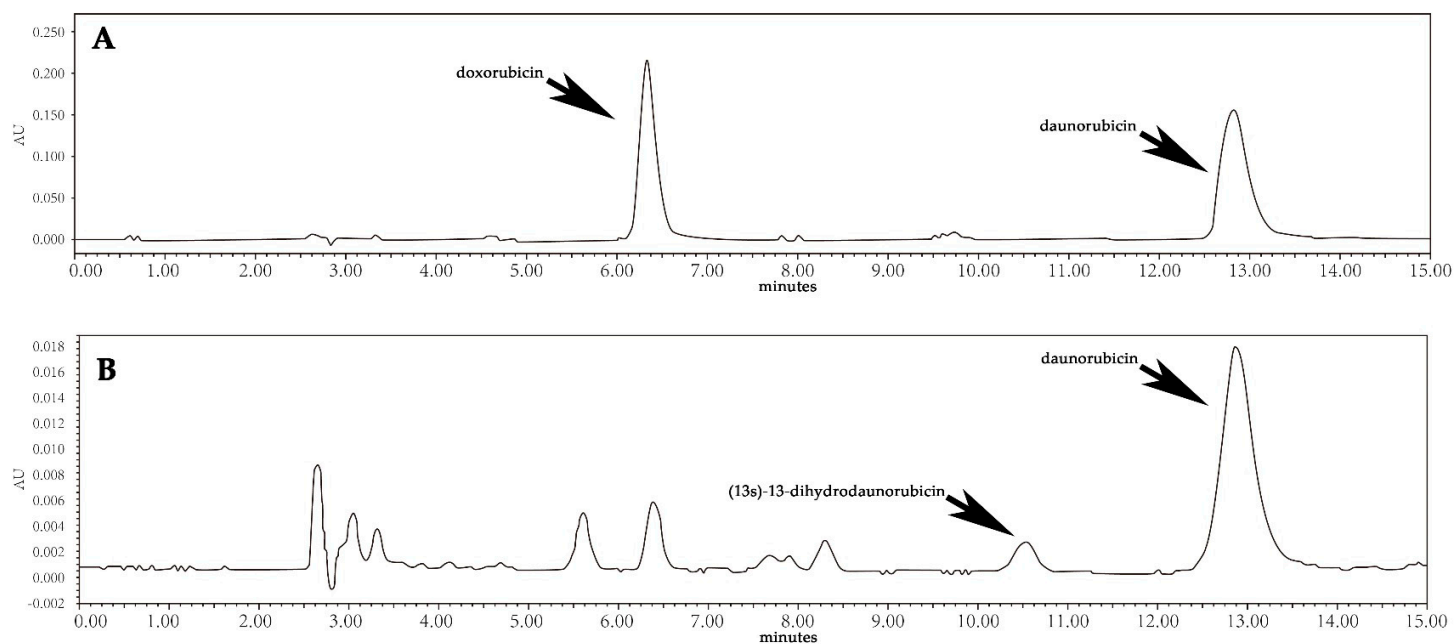
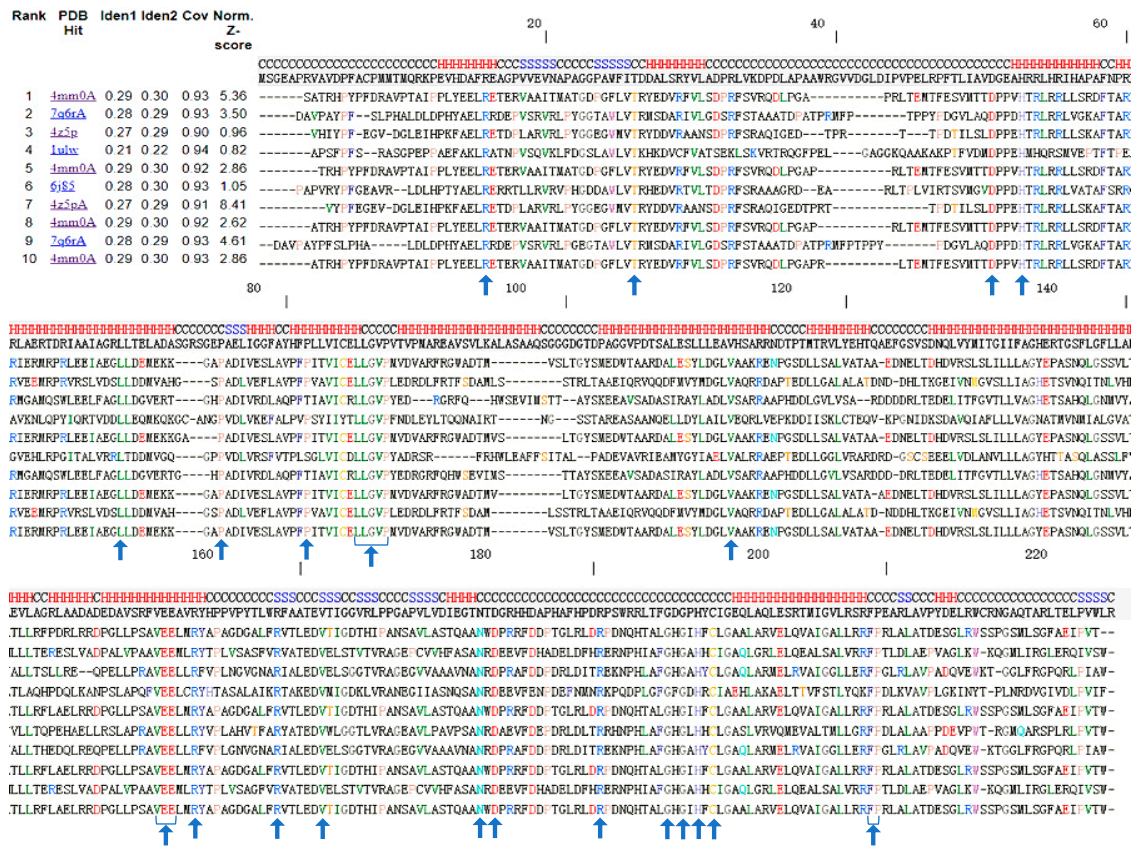


Figure S3. HPLC analysis of the catalytic products of daunorubicin by DoxA. **(A):** HPLC results of the doxorubicin/daunorubicin standards; **(B):** HPLC result of the sample.



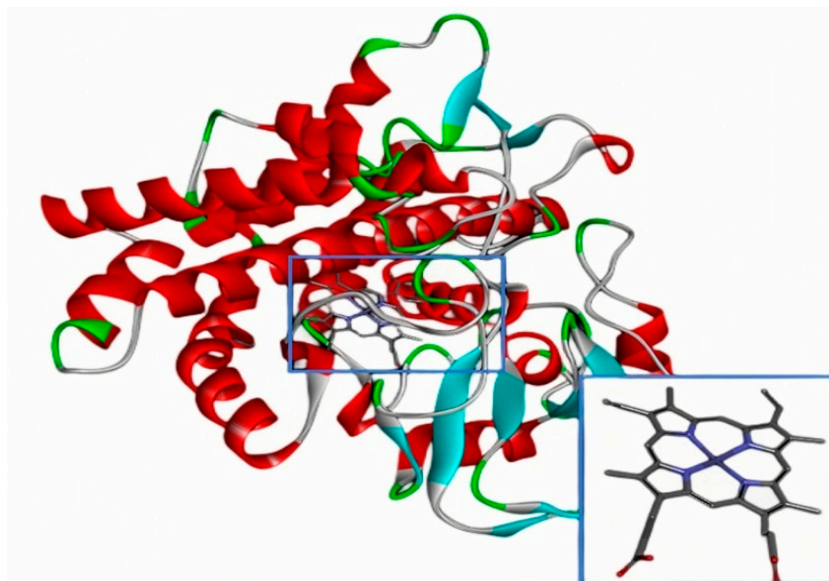


Figure S5. The complete DoxA-heme three-dimensional model.

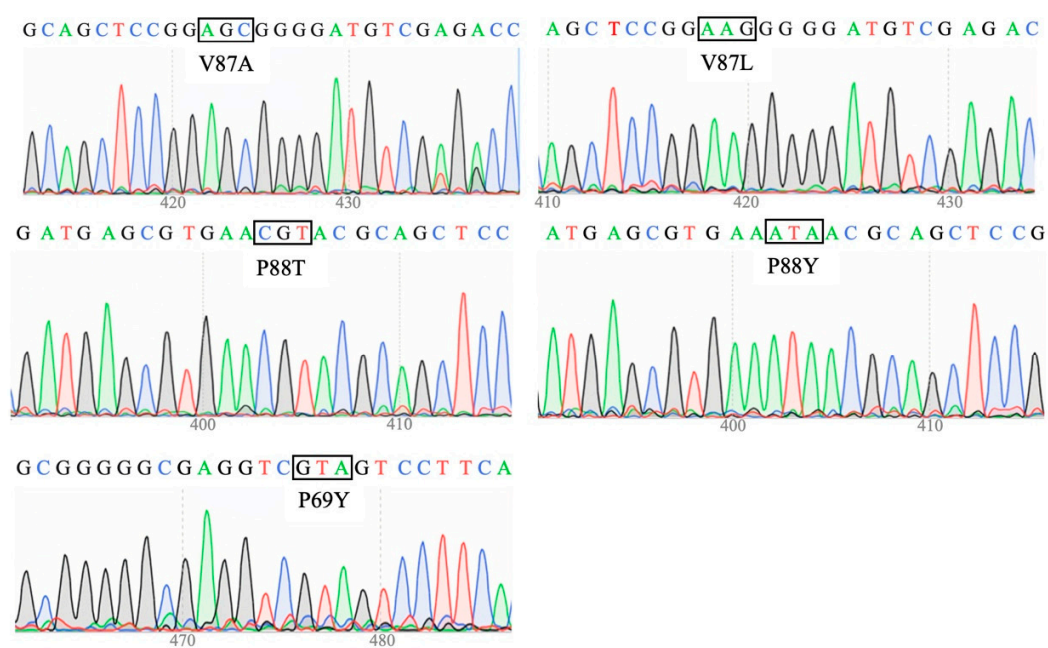


Figure S6. Sequence verification of the five plasmids containing DoxA mutants. (Notes: The black boxes were the mutant sites in the sequence of DoxA.)

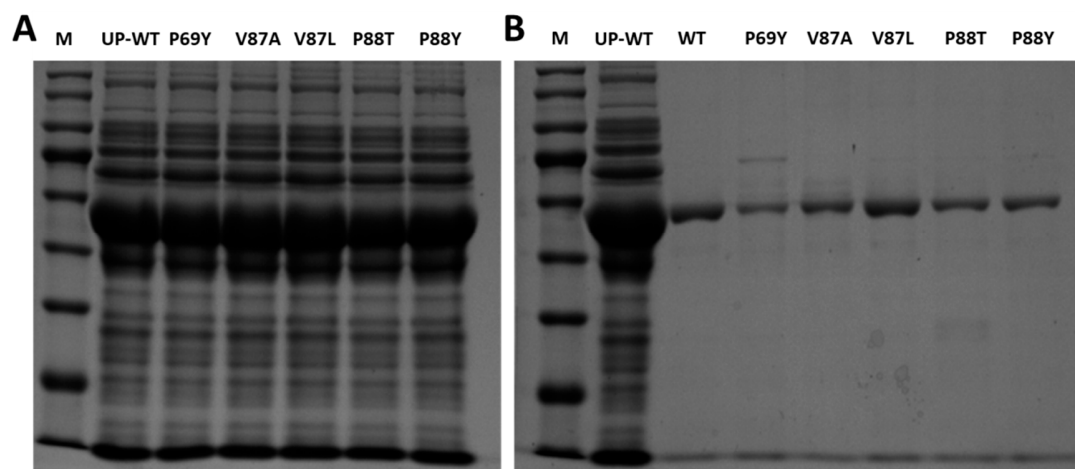


Figure S7. SDS-PAGE protein profile analysis of the mutants of DoxA. **(A):** the supernatant samples after cell fragmentation; **(B):** the purified proteins (Lane M: protein marker, lane UP-WT: unpurified wild-type DoxA, lane WT: purified wild-type DoxA).

Table S1. Free energy of DoxA-model with different protein conformations

Name	Score
doxA_I-TASSER_0001	-695.393
doxA_I-TASSER_0002	-672.837
doxA_I-TASSER_0003	-664.821
doxA_I-TASSER_0004	-708.753
doxA_I-TASSER_0005	-663.290
doxA_I-TASSER_0006	-706.663
doxA_I-TASSER_0007	-671.219
doxA_I-TASSER_0008	-700.996
doxA_I-TASSER_0009	-672.137
doxA_I-TASSER_0010	-669.950
doxA_I-TASSER_0011	-637.247
doxA_I-TASSER_0012	-702.992

doxA_I-TASSER_0013	-672.706
doxA_I-TASSER_0014	-644.515
doxA_I-TASSER_0015	-705.554
doxA_I-TASSER_0016	-701.584
doxA_I-TASSER_0017	-676.329
doxA_I-TASSER_0018	-658.026
doxA_I-TASSER_0019	-716.475
doxA_I-TASSER_0020	-672.129

Table S2. Primers used in this work

Primers	Sequence 5'-3'
DoxA-F	TCGAGTGCGGCCGCAAGCTTGCGCAGCCAGACGGGC
DoxA-R	CGAGCTCCGTCGACAAGCTATGAGCGGCGAGGCGC
PET-F	GATCTCGATCCCGCGAA
PET-R	GCAGCCAACTCAGCTTC
P69Y-F	GGCGGGGGCGAGGTCGTAGTCCTTCACG
P69Y-R2	TACGACCTCGCCCCGCGCCTGGCGGG
V87A-F	CGGACGCAGCTCCGGAGCGGGGATGTCTG
V87A-R	GCTCCGGAGCTGCGTCCGTTACGCTCA
V87L-F	CGGACGCAGCTCCGGAAGGGGGATGTCTG
V87L-R	CTTCCGGAGCTGCGTCCGTTACGCTCA
P88Y-F	GGCGATGAGCGTGAAATAACGCAGCTCC
P88Y-R	TATTTACGCTCATCGCCGTGGACGGCG
P88T-F	GGCGATGAGCGTGAACGTACGCAGCTCC
P88T-R	ACGTTACGCTCATCGCCGTGGACGGCG