Volume 78 (2022)

Supporting information for article:

Structural basis for the transformation of the traditional medicine berberine by bacterial nitroreductase

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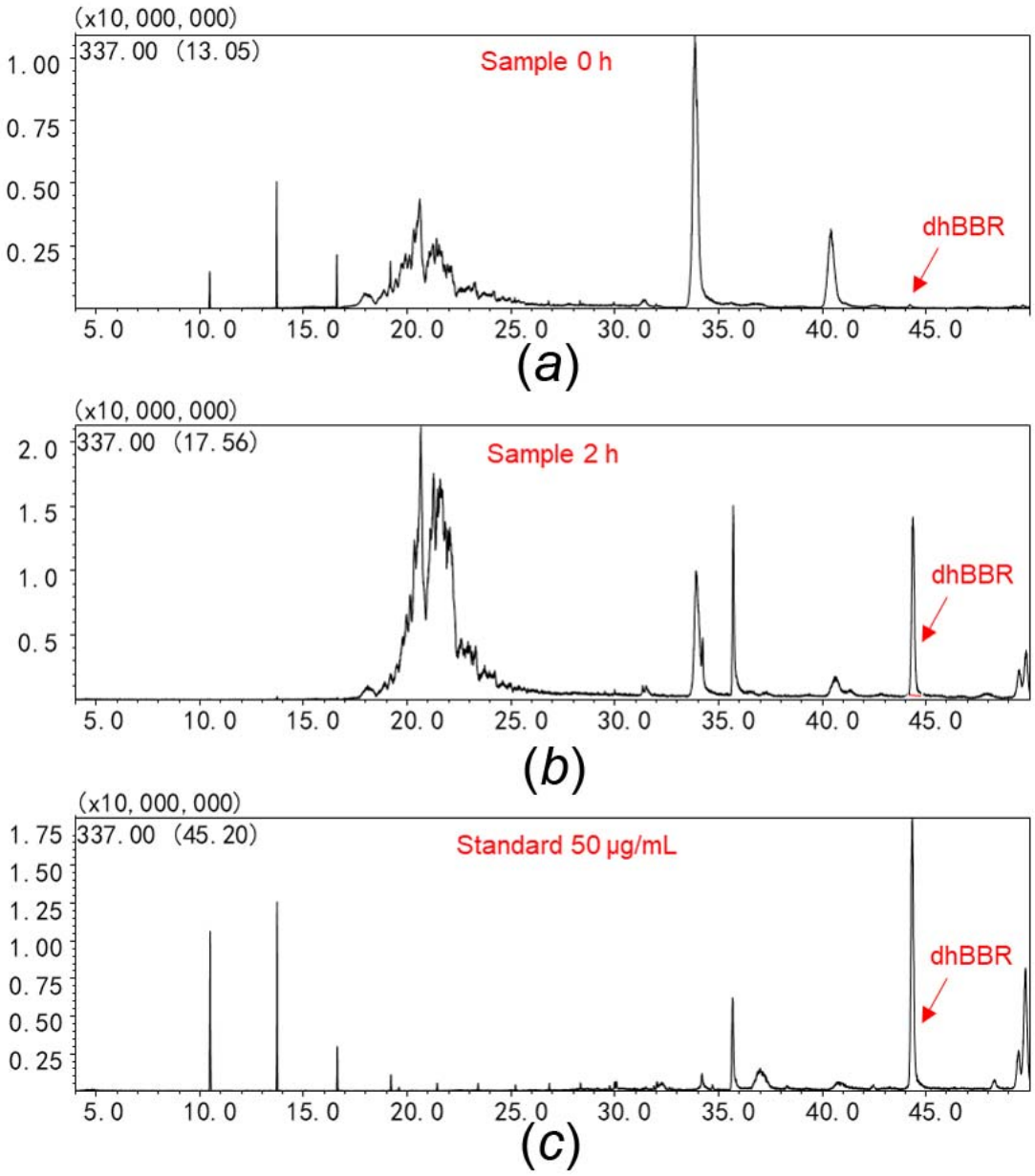

Figure S1 Detection of dhBBR by GC-MS in this study**.** The quantitative data of dhBBR at 0h (*a*), 2h (*b*) and standard solution (*c*) are shown using sim mode. The retention time of dhBBR is 44.28 min.

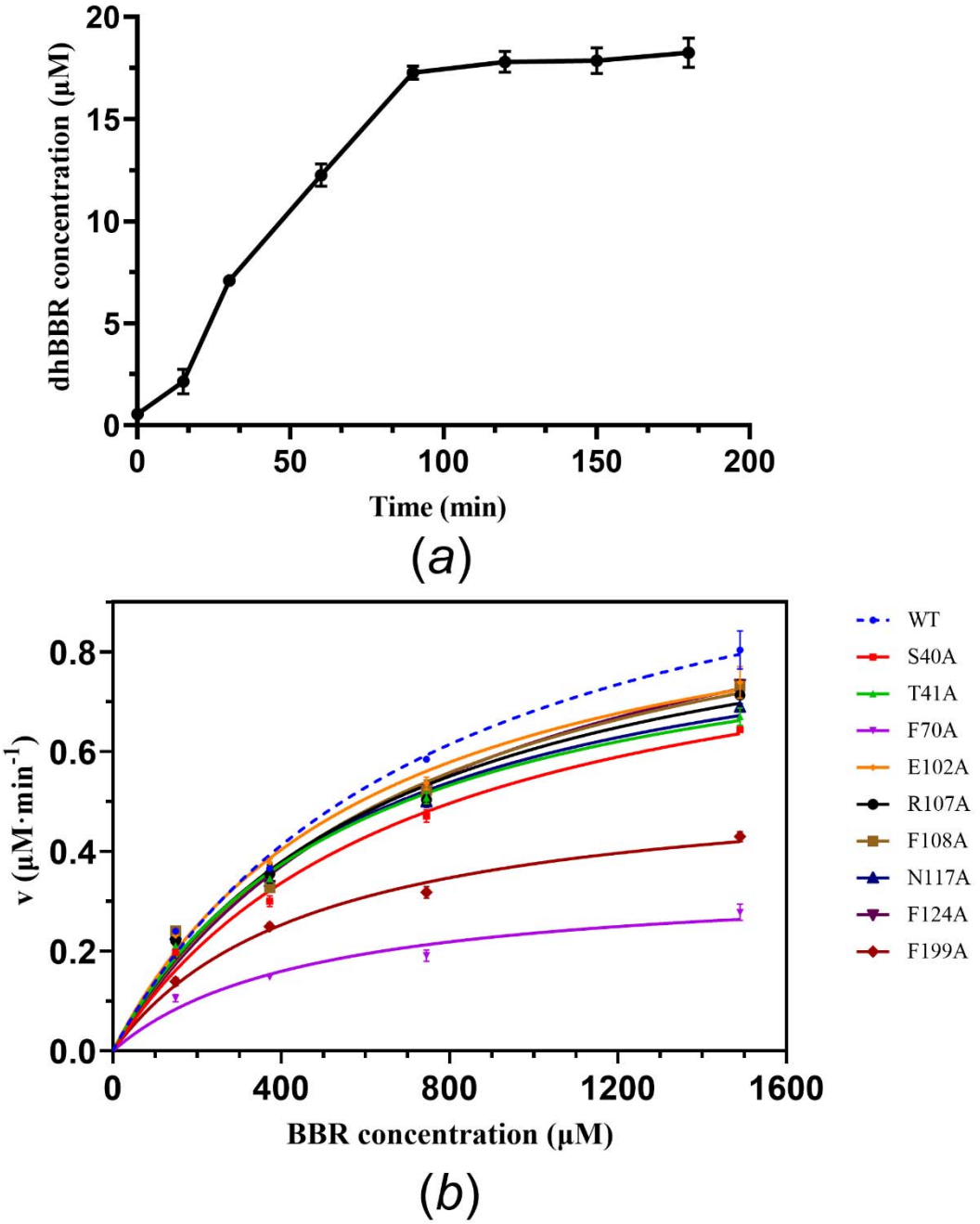

Figure S2 Steady-state kinetic data of EcNfsB for BBR-to-dhBBR conversion. (*a*) Time course analysis of dhBBR production catalyzed by wild-type EcNfsB for 180 in at the concentration of 750 μM BBR. The dhBBR concentration was determined by GC-MS at 0, 15, 30, 60, 90,120, 150 and 180 min, respectively. (*b*) The plots of initial rate (v) *versus* BBR concentration for wild-type and variants.

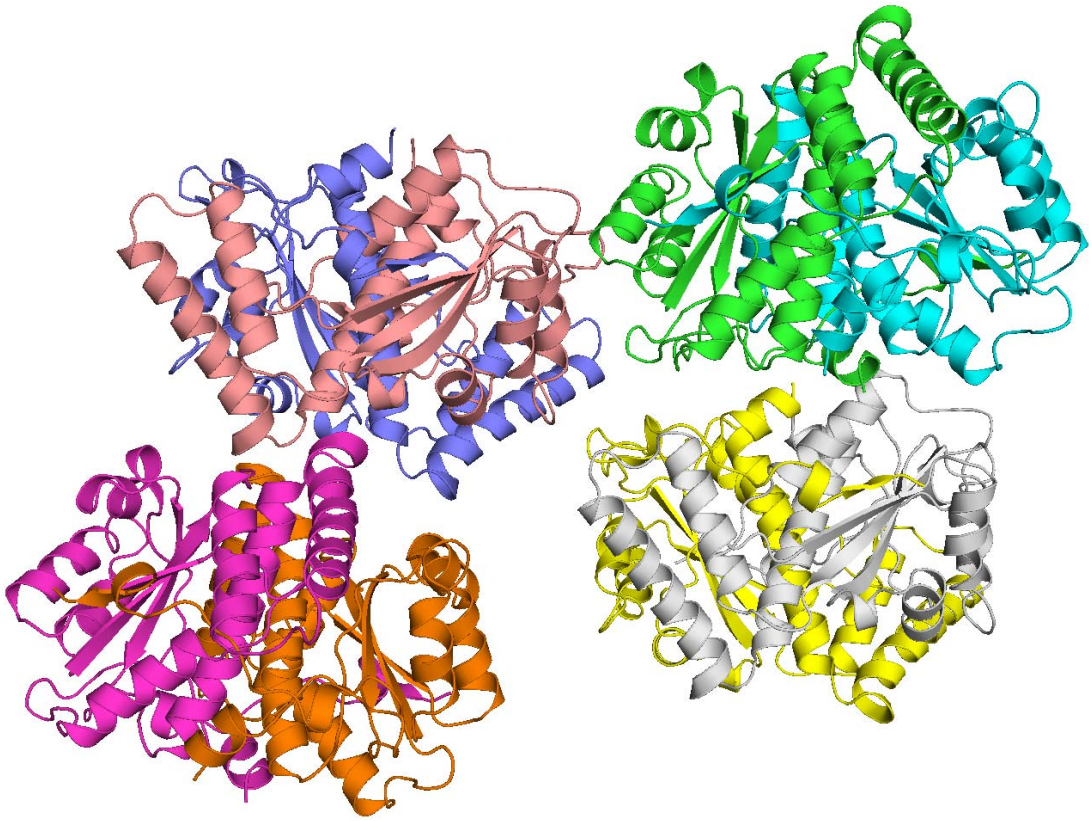

Figure S3 Crystal packing of EcNfsB molecules in the asymmetric unit. The eight protein molecules (chain A-H) are shown in different colors and can generate four homo-dimers. The FMN and BBR molecules binding to EcNfsB are not shown.

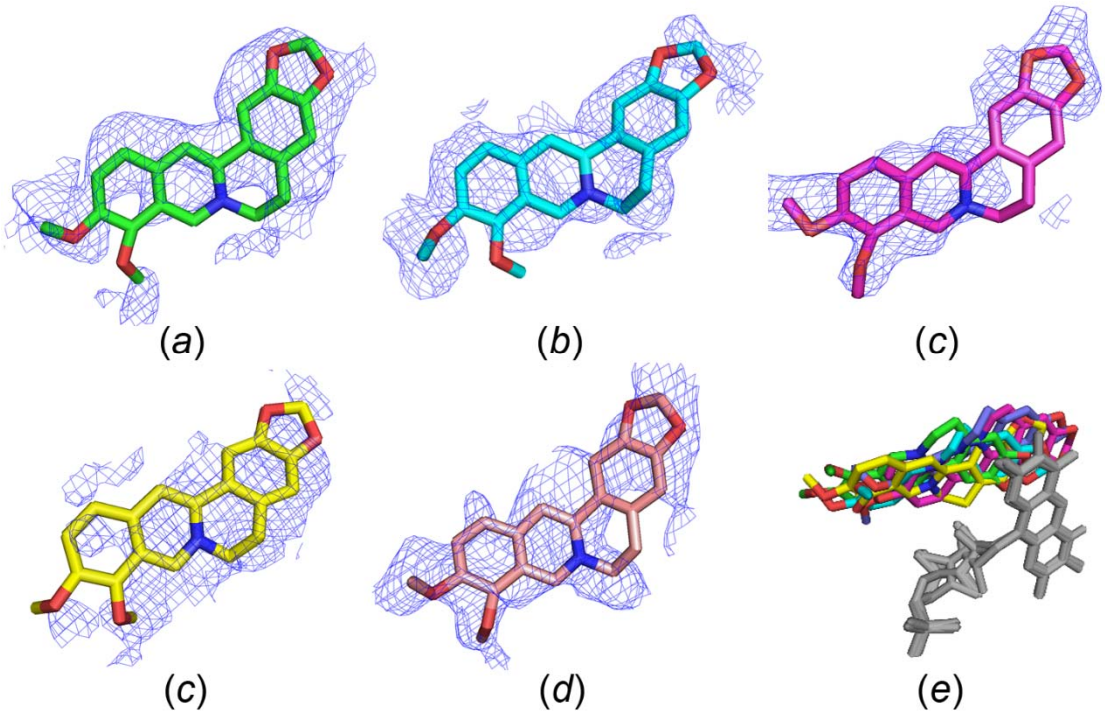

Figure S4 (*a*-*e*) The omit electron density maps of the modelled BBR molecules (in chains C, D, E, G and H, respectively) shown at a 2.0 sigma level. (*f*) Structural superposition of BBR molecules showed they have very similar orientations relative FMN (gray sticks).

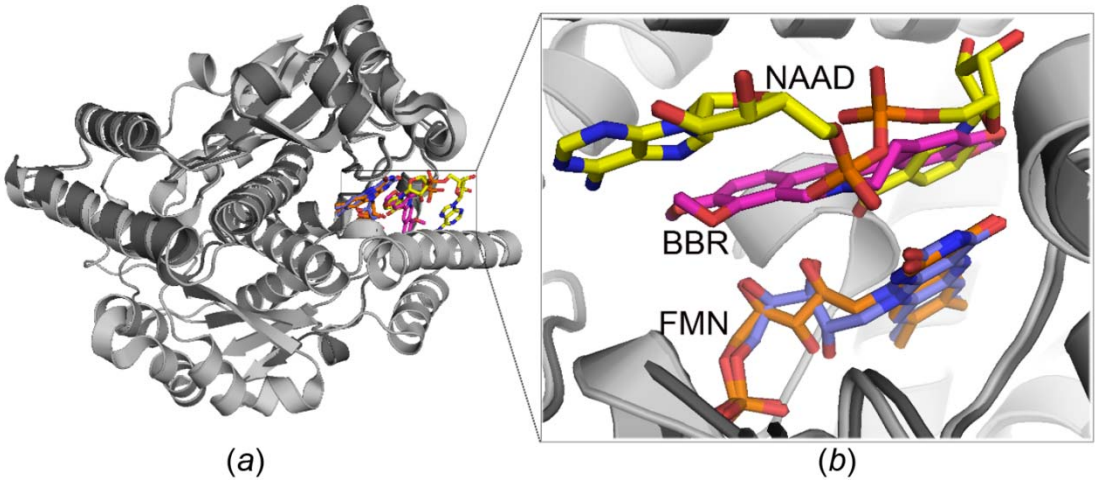

Figure S5 Structural comparison of EcNfsB-BBR with the NR from *Enterobacter cloacae*. (*a*) Structural superposition of EcNfsB with NAAD (NADPH analog)-binding NR (PDB ID: 5J8D) in light gray and dark gray, respectively. The BBR and NAAD are shown in magenta and yellow, respectively, and two FMN molecules are shown in purple and orange, respectively. (*b*) Close-up view of the active sits of the two NRs.

Table S1 The list of EcNfsB and EcNfsA (wild-type and mutants) primers in this study.

Primer	Sequence
EcNfsB	
Wild-type Forward	CGCGGATCCATGGATATCA TTTCTGTCGC
Reverse	CCGCTCGAGTTACACTTCGGTTAAGGTG
F70A Forward	CGGTAATTACGTGGCCAACGAGCGT
Reverse	GCCACGTAATTACCGGCAGCGGA
S40A Forward	AACGATAAAGGTGAGAAGTTCTTCGCTG
Reverse	CTCACCTTTATCGTTCGCGGCTTTC
T41A Forward	TAAAGGTCGCAAGGCCTTCGCTGATATGC
Reverse	GCCTTGCGACCTTTATCGTTCGCGG
F108A Forward	AGGTCGCAAGTTCGCCGCTGATATG
Reverse	GCGAACTTGCGACCTTTATCGTTCGC
F199A Forward	CAGCGTTGAAGATGCTAACGCTACGCTG
Reverse	CAGCGTTGAAGATGCTAACGCTACGCTG
E102A Forward	TGGTTGTTGACCAGGCAGATGCCG
Reverse	GCCTGGTCAACAACCAGCTTCAGC
R107A Forward	GATGCCGATGGCGCCTTTGCCACG
Reverse	GCGCCATCGGCATCTTCCTGGTCAAC
N117A Forward	GCGAAAGCCGCGGCCGATAAAGGTC
Reverse	GCCGCGGCTTTCGCTTCCGGC
F124A Forward	AGGTCGCAAGTTCAACGCTGATATG
Reverse	TTGAACTTGCGACCTTTATCGTTCGC
EcNfsA	
Wild-type Forward	CGCGGATCCATGACGCCAACCATTGAACT
Reverse	CCGCTCGAGTTAGCGCGTC GCCCAACCC