SUPPORTING INFORMATION

Turning a Hyperthermostable Metallo-Oxidase into a Laccase by Directed Evolution

Vânia Brissos[†], Maura Ferreira[†], Gregor Grass[‡] and Lígia O. Martins[†*]

[†]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av da República, 2780-157 Oeiras Portugal,* [‡]*Bundeswehr Institute of Microbiology, DZIF, Partner Site of German Center for Infection Research, Munich, Germany*

Address correspondence: Lígia O. Martins, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-15 Oeiras, Portugal, E-mail: lmartins@itqb.unl.pt

Table S1- Summary of primers used in the site-directed mutagenesis studies. Fwd indicates forward primers and Rev indicates reverse primers.

Name	Sequence
M449T Fwd	CGTAAACAACACGGGTACCTACCACCCCATGCACATAC
M449T Rev	GTATGTGCATGGGGTGGTAGGTACCCGTGTTGTTTACG
I441L Fwd	GGCGACGTGGTGATTTTAGAGTACGTAAACAACACG
I441L Rev	CGTGTTGTTTACGTACTCTTAAATCACCACGTCGCC
K245R Fwd	CCCTTATATGGACGTAGAGAGAAGGATTTACAGGTTCAG
K245R Rev	CTGAACCTGTAAATCCTTCTCTCTACGTCCATATAAGGG
R471G Fwd	GAAAGGAGCTTGGGACCTTTGGGGGCTACGGACCTCGG
R471G Rev	CCGAGGTCCGTAGCCCCCAAAGGTCCCAAGCTCCTTTC
P58S Fwd	CGGATACTTCCTTTTTTCCCGATGGACAGCGAGTAAG
P58S Rev	CTTACTCGCTGTCCATCGGAAAAAAGGAAGTATCCG
I199T Fwd	CTCGAATACGGAGTTACAGACATTCCGCTCATAATTCAGG
I199T Rev	CCTGAATTATGAGCGGAATGTCTGTAACTCCGTATTCGAG
Y172C Fwd	GGGTTATCAGGTTTACTGCGGTCTTGCGGGAATG
Y172C Rev	CATTCCCGCAAGACCGCAGTAAACCTGATAACCC
V19A Fwd	CCTTGGCTTTTCGGCTGGGGGACTTTCCCTCCTTTCC
V19A Rev	GGAAAGGAGGGAAAGTCCCCCAGCCGAAAAGCCAAGG
F55S Fwd	CAATATCCCCGGATACTCCCTTTTTCCCGATGGAC
F55S Rev	GTCCATCGGGAAAAAGGGAGTATCCGGGGATATTG
F17S Fwd	CTTTTCCGCCCTTGGCTCTTCGGTTGGGGGACTTTC
F17S Rev	GAAAGTCCCCCAACCGAAGAGCCAAGGGCGGAAAAG
F55SP58S Fwd	CAATATCCCCGGATACTCCCTTTTTTCCGATGGACAGCGAGTAAG
F55SP58S Rev	CTTACTCGCTGTCCATCGGAAAAAAGGGAGTATCCGGGGATATTG

Table S2 – Aromatic substrates used in this study.

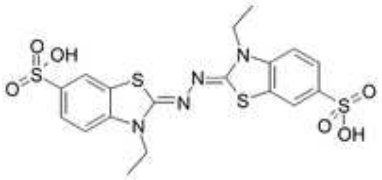
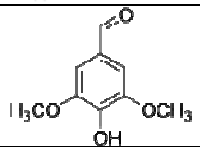
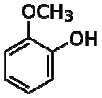
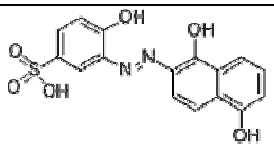
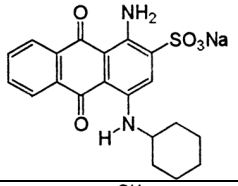
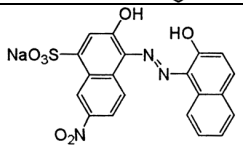
Aromatic substrates	
ABTS	
SGZ	
Guaiacol	
Mordant Black 9	
Acid Blue 62	
Acid Black 194	

Figure S1

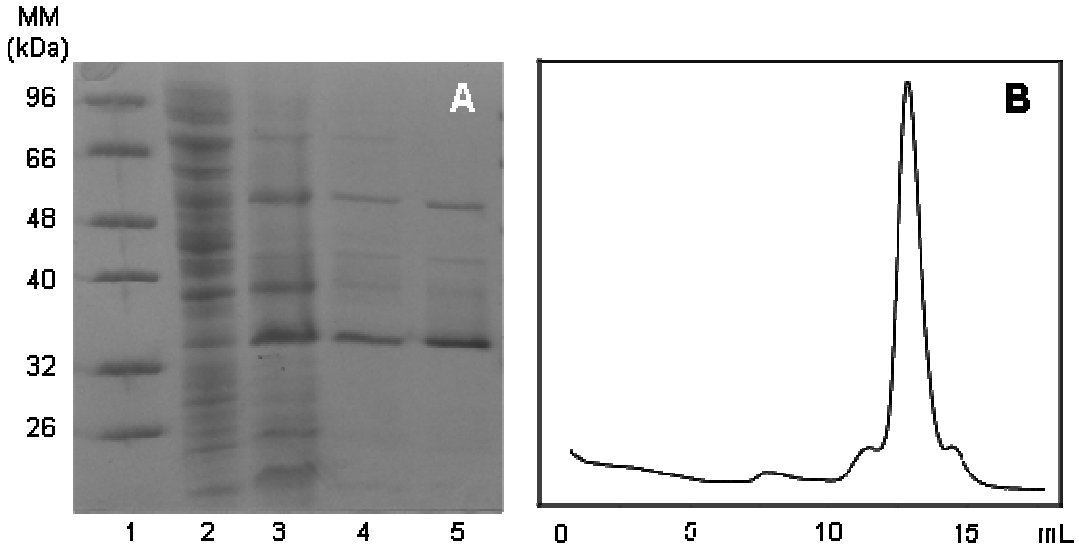


Figure S1 – (A) The purified recombinant 2B3 variant resulted in accumulation of two major bands of ~ 35 and ~ 59 kDa in the SDS-PAGE. Lane 1 – Standard molecular mass markers; Lane 2 – supernatant of a crude extract; Lane 3 – after incubation at 80°C for 20 min; Lane 4 – after a Q-Sepharose column, Lane 5 – after a Superdex 200 column. **(B)** Chromatogram of a purified preparation of the 2B3 variant using a size exclusion Superose 12HR 10/30 column showing a single peak with the MM of ~ 57 kDa, close to the theoretical value predicted from the *mcoA* gene sequence (59.5 kDa).

Figure S2

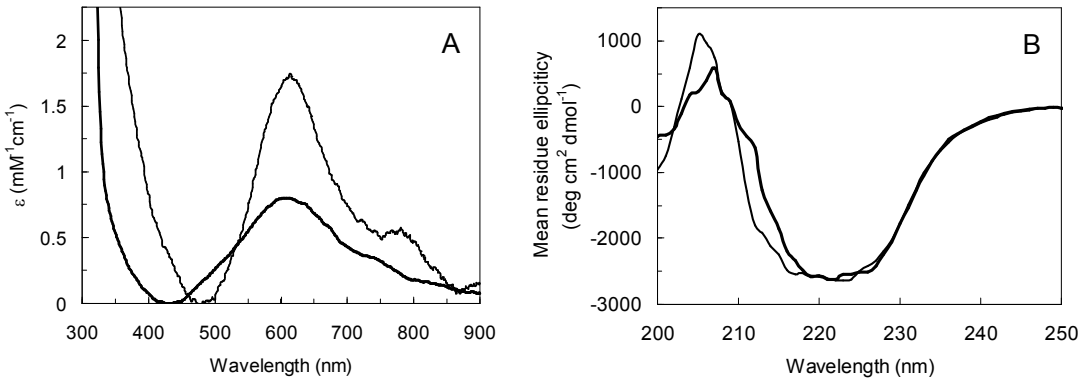


Figure S2 – (A) UV-visible and (B) CD spectra of McoA wild-type (thick line) and the evolved 2B3 variant (thin line) in 20 mM Tris-HCl buffer, pH 7.6.

Figure S3

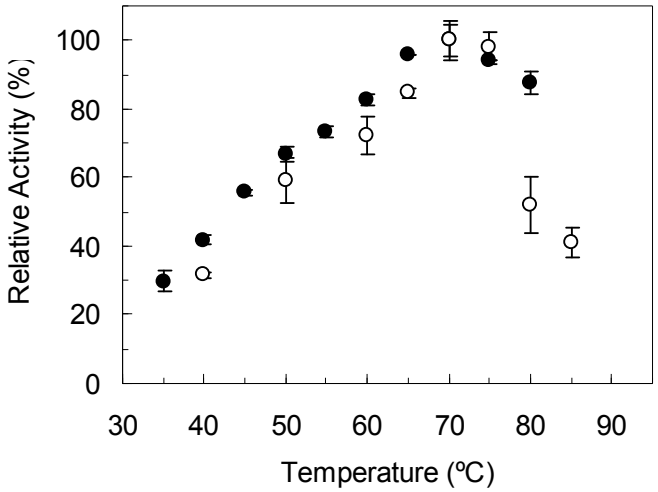


Figure S3 - Temperature dependence of McoA wild-type (open circles) and the evolved 2B3 variant (closed circles). Reactions were followed by monitoring ABTS (1 mM) oxidation in 100 mM acetate buffer, pH 4, at temperatures between 35 and 80°C.