Supplementary material for the article:

Ilić Đurđić, K.; Ostafe, R.; Prodanović, O.; Đurđević Đelmaš, A.; Popović, N.; Fischer, R.; Schillberg, S.; Prodanović, R. Improved Degradation of Azo Dyes by Lignin Peroxidase Following Mutagenesis at Two Sites near the Catalytic Pocket and the Application of Peroxidase-Coated Yeast Cell Walls. *Front. Environ. Sci. Eng.* **2020**, *15* (2), 19. https://doi.org/10.1007/s11783-020-1311-4.

Supporting Information

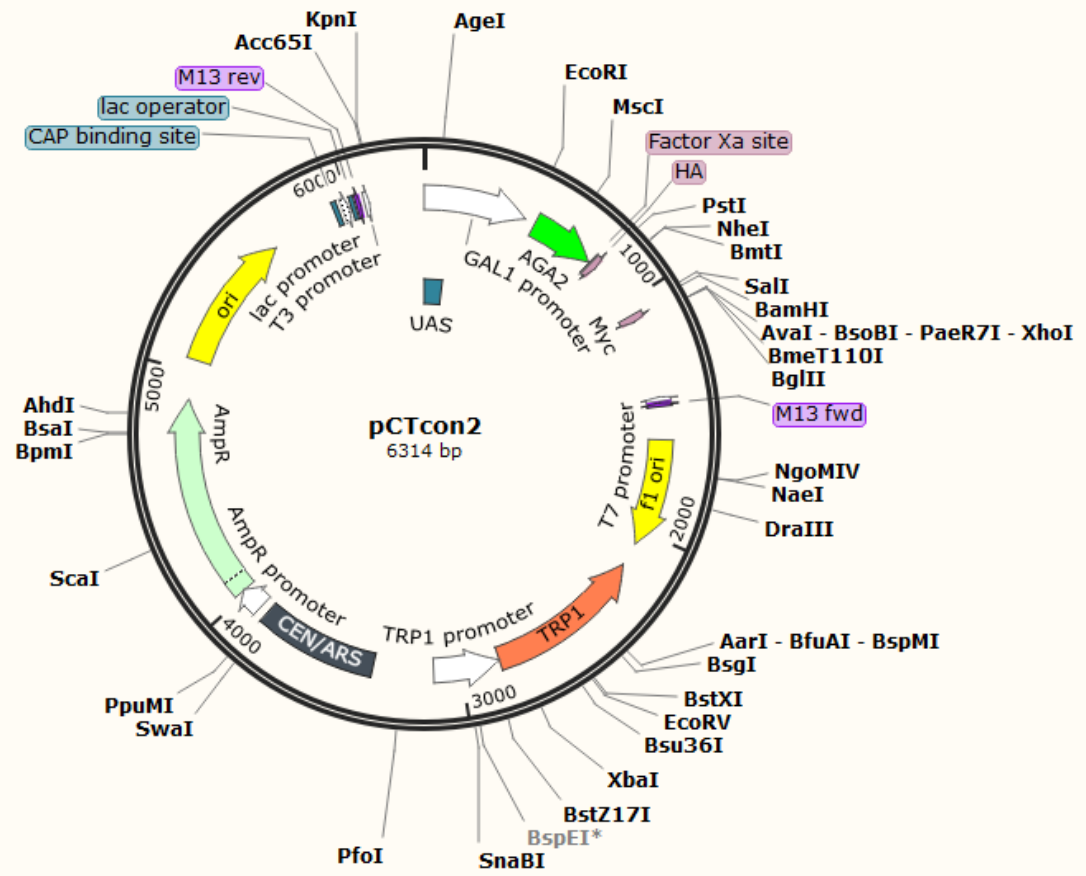

Figure S1. Map of pCTCON2 vector

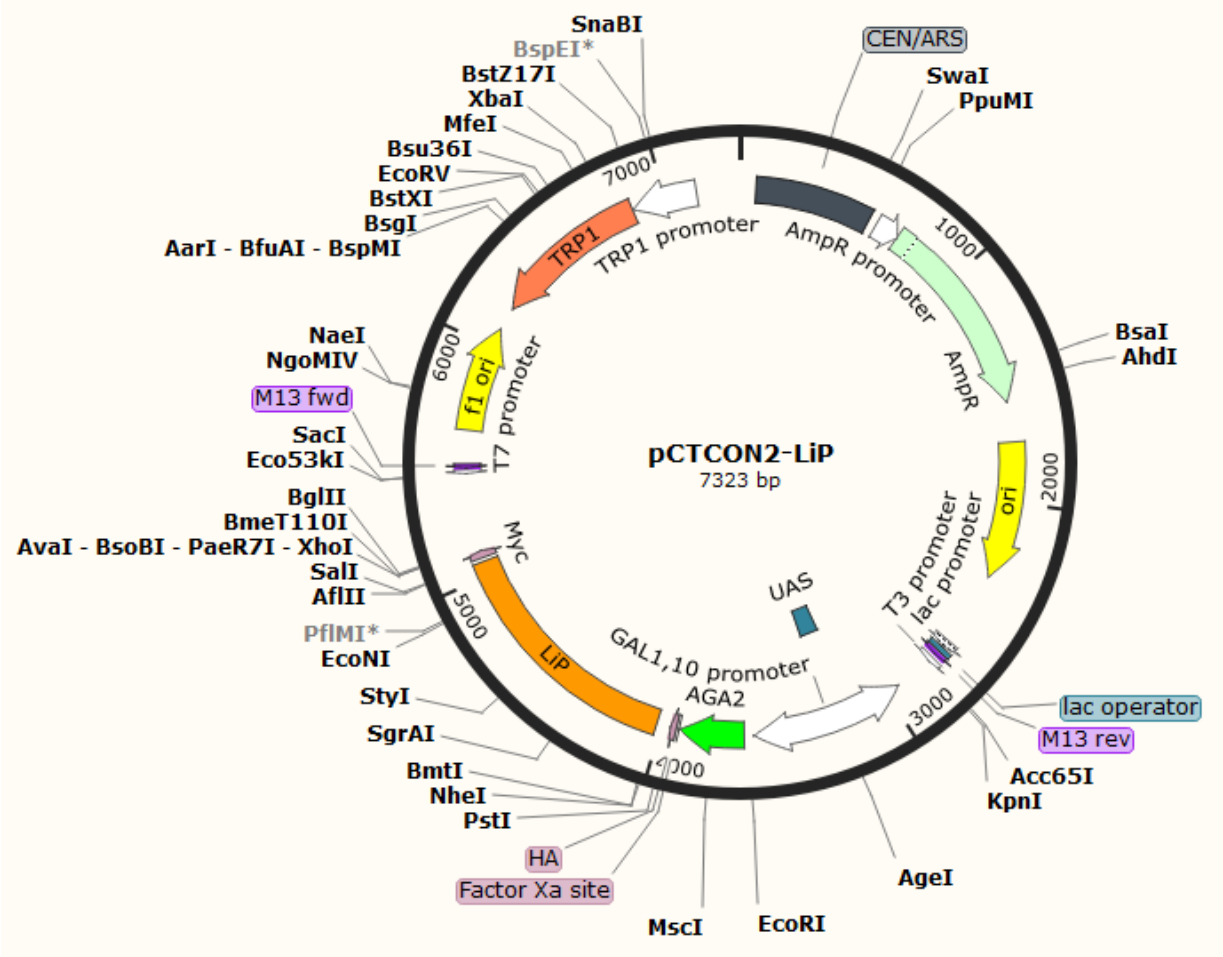

Figure S2. Schematic representation of LiP-pCTCON2 construct.

Table S1. Primers sequences

Application	Sequence (5' → 3')
Forward primer for cloning of LiP gene	AATGCTAGCGCAACCTGTGCTAATGGTAAG
Reverse primer for cloning of LiP gene	AATGTCGACTTAAGCCTTGTGTGGAGGTATTC
Forward primer for sequencing	CCCATACGACGTTCCAGACTACGC
Reverse primer for sequencing	GATCTCGAGCTATTACAAGTCCTCTTCAG
Primer for mutation of D165N	GCTGGAGAATTTNNNGAATTAGAATTGG
Primer for mutation of D264N	CAAAGTTGGTAGATNNNTTTCAATTCATTTTT

GCAACCTGTGCTAATGGTAAGACAGTAGGTGATGCTTCTTGTTGCGCCTGGTTCGATGTCTTAGATGAC
ATTCAAGCAAATATGTTTCACGGTGGTCAGTGTGGTGCCGAAGCTCACGAATCTATTAGATTGGTCTTT
CACGATTCTATAGCAATATCTCCTGCTATGGAGGCTAAAGGAAAGTTTGGTGGAGGTGGAGCCGATGG
TTCAATCATGATATTCGATACCATAGAAACCGCTTTCCACCCAAACATAGGATTGGATGAAGTAGTTGC
TATGCAAAAACCATTTGTCCAAAAACACGGAGTAACACCAGGTGACTTTATCGCTTTCGCCGGTGCCGT
CGCCTTATCTAATTGCCCTGGTGCTCCTCAGATGAACTTCTTTACCGGAAGGAAGCCAGCTACACAACC
AGCACCTGACGGTTTAGTACCAGAACCTTTTCATACTGTAGACCAAATTATAGCTAGAGTCAACGACGC
TGGAGAATTTGATGAATTAGAATTGGTATGGATGTTGTCTGCTCACTCTGTTGCAGCAGTCAATGACGT
TGATCCAACCGTCCAAGGATTACCTTTTGATTCAACTCCAGGAATTTTCGATTCTCAGTTTTTCGTTGAA
ACTCAATTCAGAGGAACTTTGTTTCCAGGTTCAGGAGGAAATCAAGGTGAAGTTGAGTCAGGTATGGC
TGGTGAAATCAGGATCCAGACAGATCATACATTGGCTAGAGATTCTAGGACTGCCTGTGAATGGCAAT
CTTTCGTCGGTAATCAATCAAAGTTGGTAGATGATTTTCAATTCATTTTTTTGGCTTTAACCCAGTTGGG
TCAAGATCCAAATGCAATGACAGATTGTTCTGATGTAATCCCATTATCAAAACCTATACCAGGTAATGG
TCCATTTTCATTCTTCCCTCCTGGTAAATCACATTCTGATATTGAACAGGCTTGTGCTGAGACTCCTTTT
CCATCTTTAGTTACTTTGCCAGGTCCAGCCACTTCAGTCGCAAGAATACCTCCACACAAGGCTTAAGTC
GA

Figure S3. Sequence of lignin peroxidase H8 gene (5' → 3').

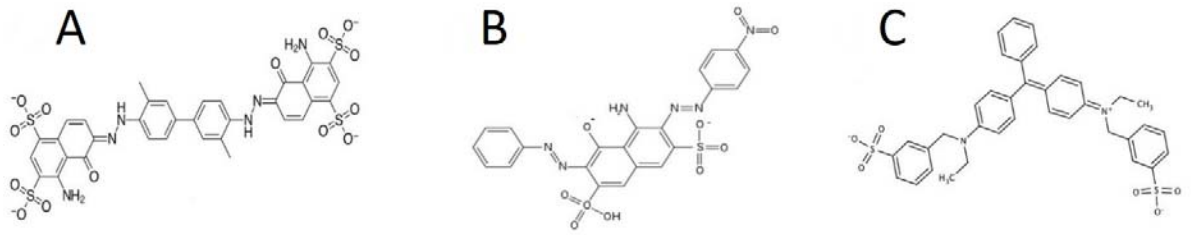

Figure S4: Structures of used azo dyes (A) Evans blue (B) Amido black 10B (C) Guinea green

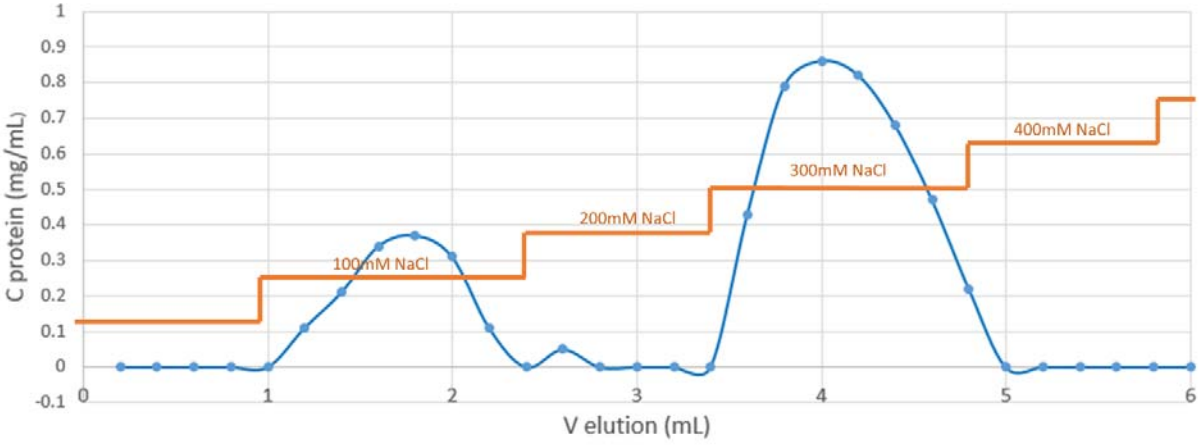

Figure S5. Chromatogram showing the elution of Aga2-wtLiP chimera using Vivapure mini spin columns with optimized NaCl step elution.

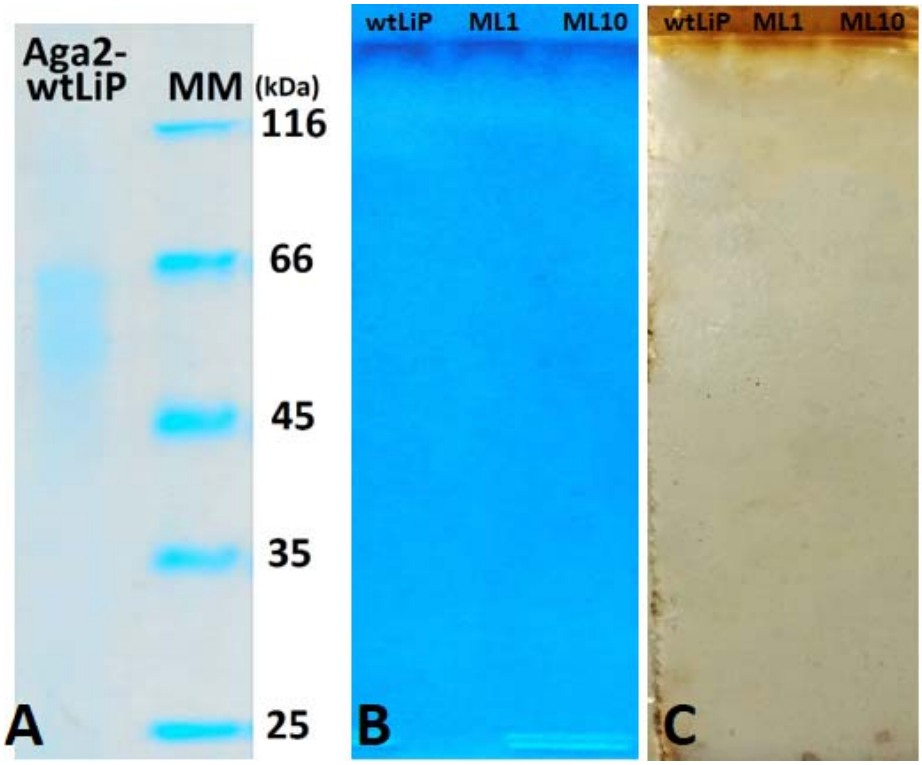

Figure S6. Polyacrylamide gel electrophoresis **A.** SDS-PAGE of purified aga2-wtLiP compared with molecular weight markers (MM) **B.** Native 12% polyacrylamide gel electrophoresis with protein bands after CBB R250 staining for Aga2-wtLiP and two selected mutants. **C.** Native 12% polyacrylamide gel electrophoresis with activity bands in the gel after incubation with 0.5 mM H_2O_2 and 9 mM guaiacol for Aga2-wtLiP and two selected mutants

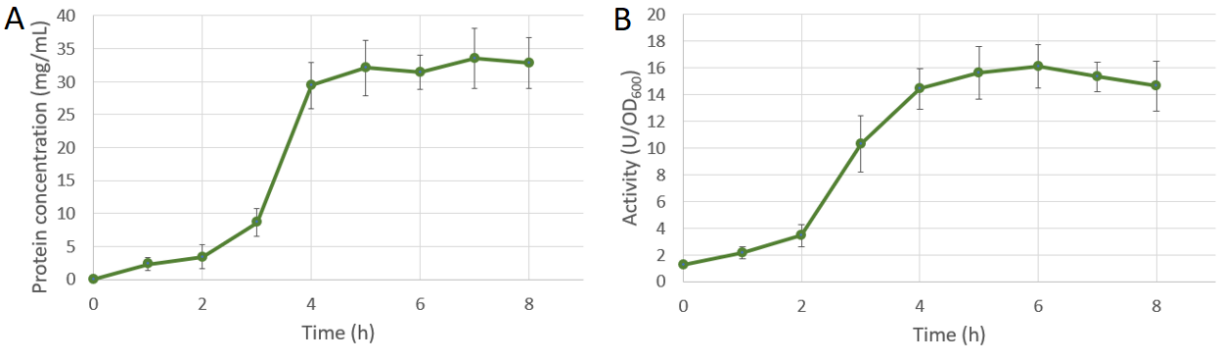

Figure S7. Concentration of intercellular proteins released from the cells during cell lysis and activity of wtLiP during cell lysis. (A) In order to optimize lysis of toluene-induced cell lysis we followed concentration of released proteins during 8 h using Bradford reagent. (B) Activity of wtLiP was followed during cell lysis with 2,4-DCP assay (0.2 mM DCP, 80mM 4-AAP and 1 mM H_2O_2). Data are means of triplicate experiments with error bars indicating standard deviations. Error bars are not visible when smaller than the symbol size.