

Supplementary data for article:

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Supporting information

Saturation mutagenesis to improve the degradation of azo dyes by versatile peroxidase and application in form of VP-coated yeast cell walls

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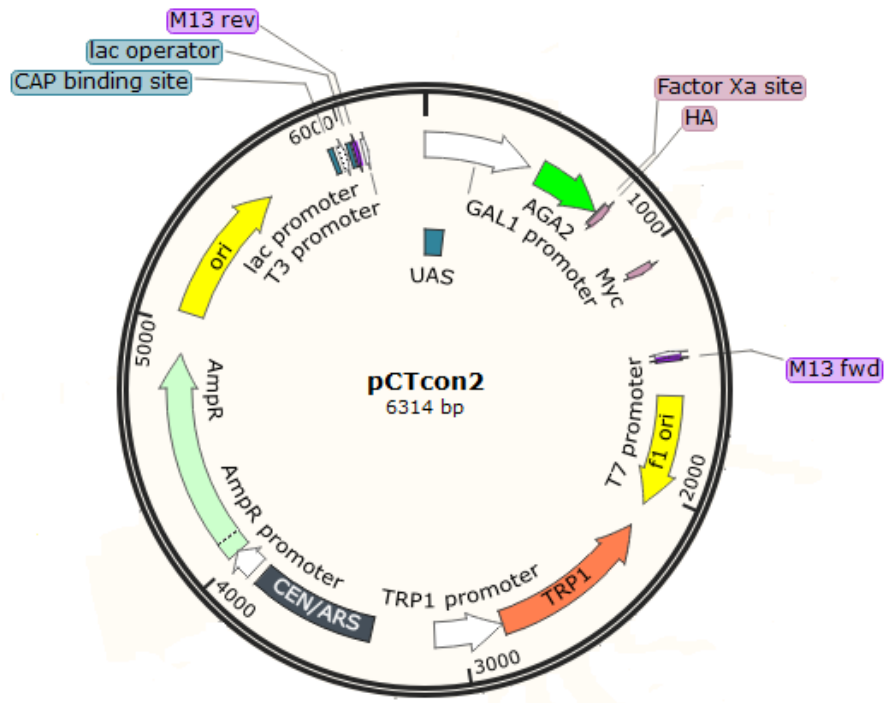


Figure S1. Map of pCTCON2 vector

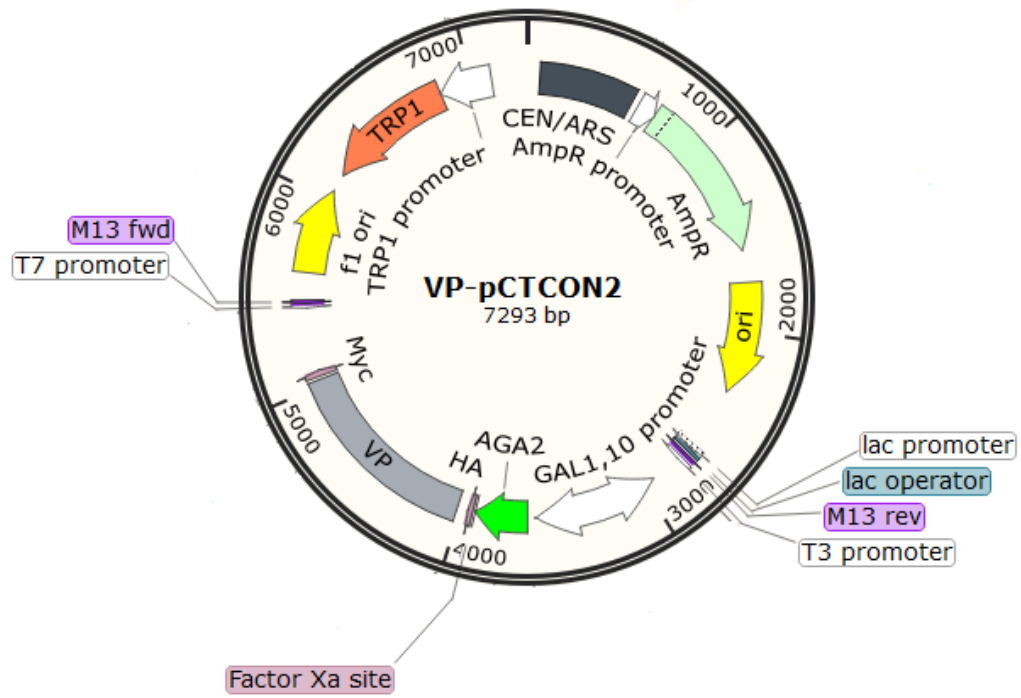


Figure S2. Map of VP-pCTCON2 construct

atggctacttggatgacggtagaacaactgcaaagccgcatgtgcatcctattccaatcttgacgatattcaggagaactgtttgatggctcaatgcggtgaagag
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ctgctacccattccagccttaacagctgacctggtcctgtaacatctgtccaccagttccaggtagctcagtaa

Figure S3. Versatile peroxidase sequence

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CLUSTAL format alignment by MAFFT (v7.452)

Lignin|PDBID|CH AAVIEKRATCSNGKTVGDASCCAWFDVLDLDDIQNLFHGGQCGAEAHESIRLVFHDSIAIS
Versatile|PDBID -----ATCDDGRTTANAACCILFPILDDIQENLFDGANCGEVHESLRLTFHDAIGFS
                ***.:*:*..:*** * :*****:***.:** *.***:*.***:*.:*

Lignin|PDBID|CH PAMEAQGKFGGGGADGSIMIFDDIETAFHPNIGLDEIVKLQKPFVQKHGVTPGDFIAFAG
Versatile|PDBID PTL-----GGGGADGSIIAFDTIETNFPANAGIDEIVSAQKPFVAKHNISAGDFIQFAG
*: : *****: ** ** * .* *:***. ***** **.:***** **

Lignin|PDBID|CH AVALSNCPGAPQMFFFTGRAPATQPAPDGLVPEPFHTVDQIINRVNDAGEFDELELVWML
Versatile|PDBID AVGVSNCPGGVRIPFFLGRPDVAASPDHLVPEPFDSVDSILARMGDAG-FSPVEVVWLL
**.:*****. : : ** ** . * . :** *****.:**.*: *:.*** * . :***:*

Lignin|PDBID|CH SAHSVAAVNDVDPTVQGLPFDSTPGIFDSQFFVETQLRGTAFPGSGGNQGEVESPLPGEI
Versatile|PDBID ASHSIAAADKVDPSIPGTPFDSTPEVFDSQFFIETQLKGRLFPGTADNKGEAQSPLQGEI
:***:***.:***: : * ***** :*****:*****:* ***:..*:***:*** **

Lignin|PDBID|CH RIQSDHTIARDSRTACEWQSFVNNQSKLVDDFQFIFLALTQLGQDPNAMTDCSDVIPQSK
Versatile|PDBID RLQSDHLLARDPQTACEWQSMVNNQPKIQNRFAATMSKMALLGQDKTKLIDCSDVIPTPP
*:*** :***.:*****:***.*: : * : : : *** . : ***** .

Lignin|PDBID|CH PIPGNLPFSFFPAGKTIKDVEQACAETPFPTLTTLPGPETSQVRIPPPPGA
Versatile|PDBID ALVGA---AHLPAAGFSLSDVEQACAATPFPAALTADPGPVTSV---PPVPGS
.: * :.:*** :.:***** *****:***: *** ** ** ***:

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Figure S4. Multiple alignment of VP form *P. eryngii* and LiP form *P. chrysosporium* performed using MAFFT algorithm.

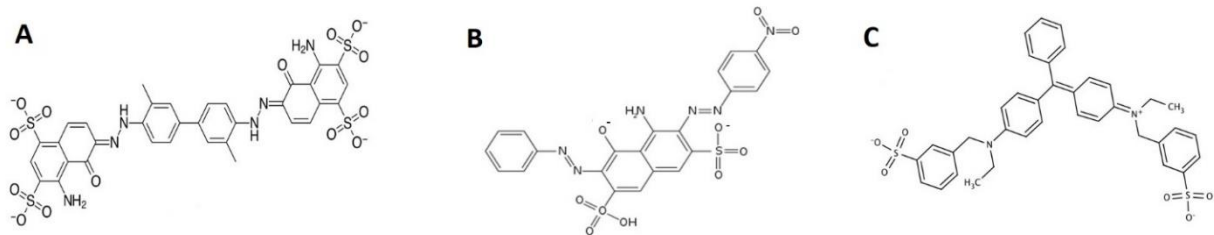


Figure S5. Structures of tested azo-dyes (A) Evans blue (B) Amido black 10B (C) Guinea green

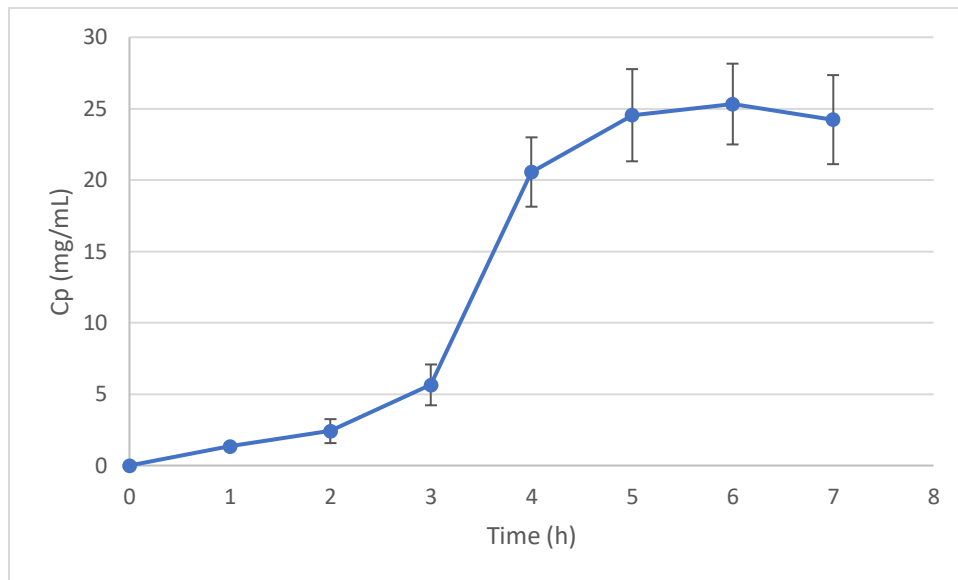


Figure S6. Protein concentration during cell lysis. Before and after every hour of toluene-induced cell lysis process 100 μ L aliquots were taken and centrifuged. Protein concentration was determined in supernatant using Bradford reagent. Data are means of triplicate experiments with error bars indicating standard deviations. Error bars are not visible when smaller than the symbol size.

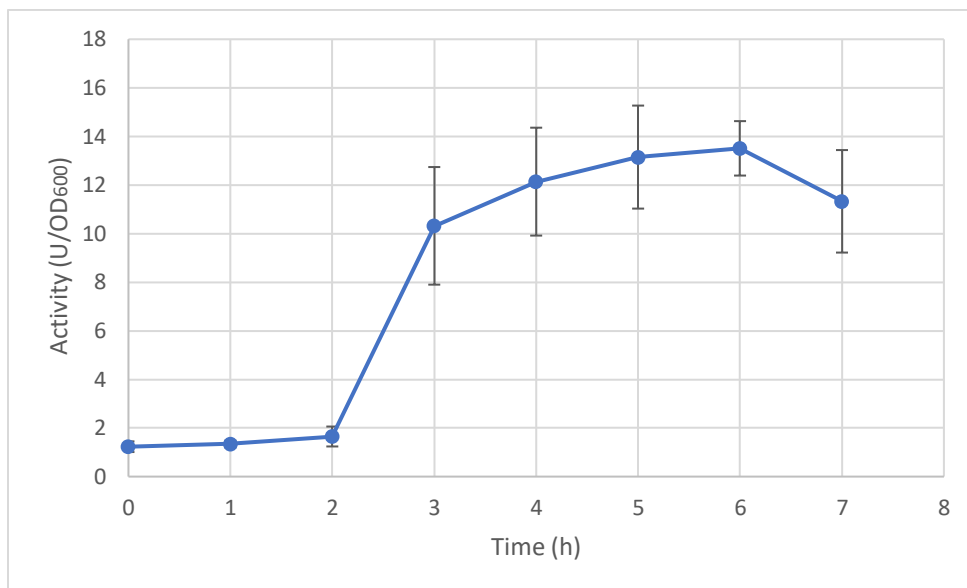


Figure S7. Activity of VP during lysis. Before and after every hour of lysis process 100 μ L aliquots were taken and centrifuged. After 3 washing steps cells/cell walls were resuspended to final OD₆₀₀ = 0.1. Activity of VP was measured with ABTS assay (2 mM ABTS, 0.5 mM H₂O₂ in 100 mM Na-tartrate buffer pH 3.5) by monitoring absorbance at 405 nm. Data are means of triplicate experiments with error bars indicating standard deviations. Error bars are not visible when smaller than the symbol size.

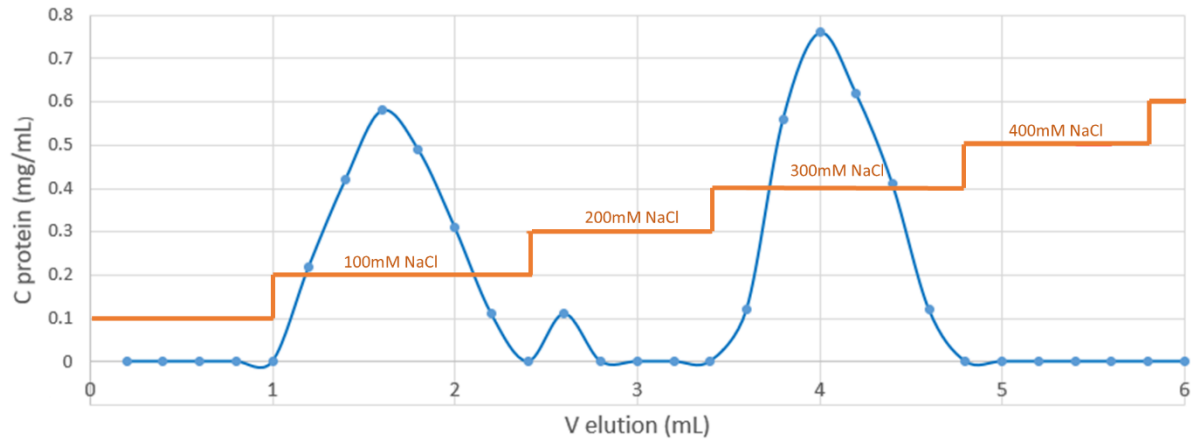


Figure S8. Chromatogram showing the elution of Aga2-wtVP chimera using Vivapure mini spin columns with optimized NaCl step elution.

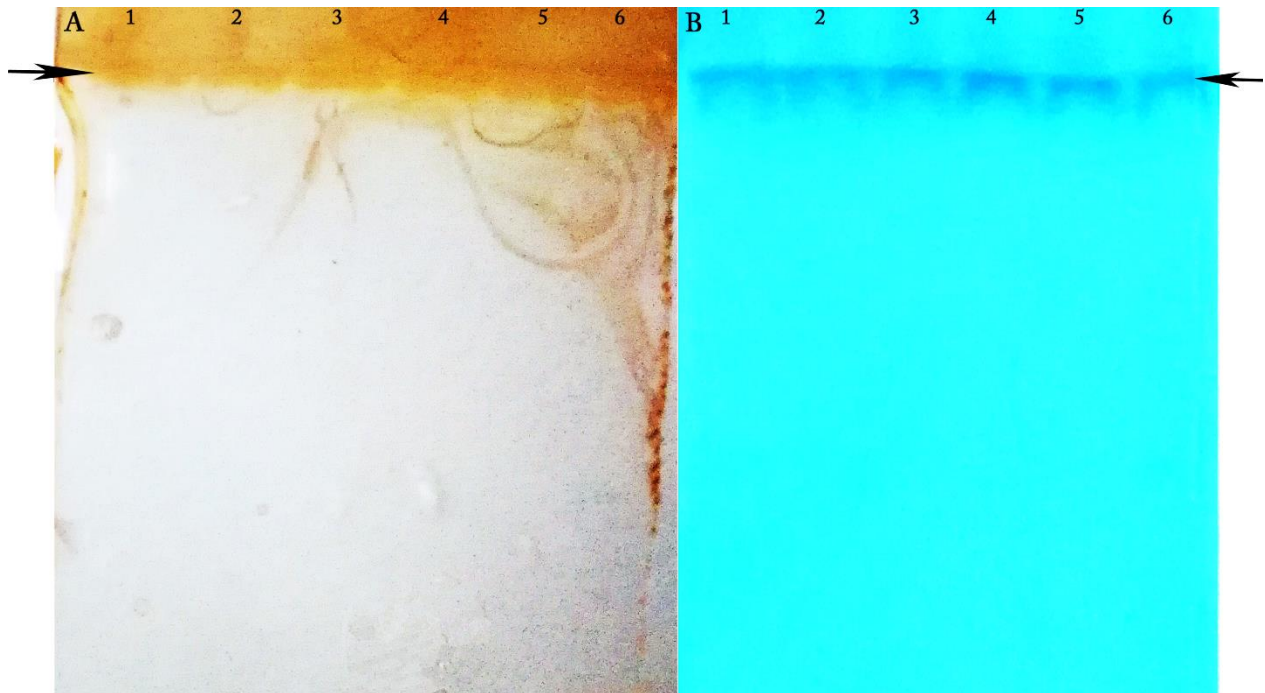


Figure S9. Native 12% polyacrylamide gel electrophoresis. (1) Aga2-wtVP (2) Aga2-MVP1 (3) Aga2-MVP2 (4) Aga2-MVP3 (5) Aga2-MVP4 (6) Aga2-MVP5 A. Activity bands in the gel after incubation with 0.5 mM H₂O₂ and 9 mM guaiacol (arrow pointing at them). B. Protein bands after CBB R250 staining (arrow pointing at them).

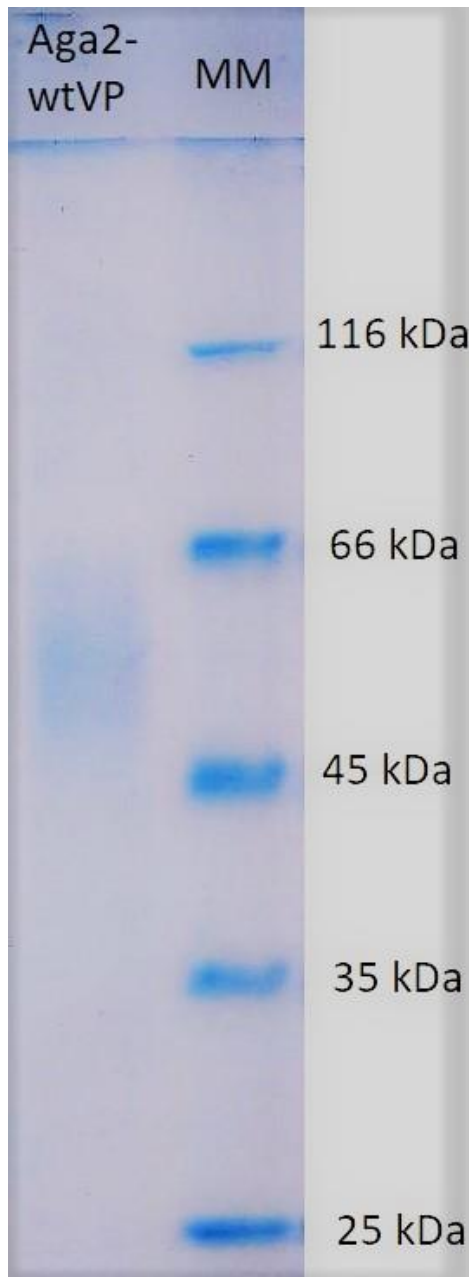


Figure S10. SDS-PAGE of purified aga2-wtVP compared with molecular weight markers (MM)

Table S1. Primers sequences

| Application | Sequence |
|---------------------------------------|--|
| Forward primer for cloning of VP gene | ATCGATGCTAGCATGGCTACTTGTGATGACGGTAGAACAACTGCAAATGCCGC |
| Reverse primer for cloning of VP gene | TACTAGTGGATCCCTCGAGACTACCTGGAAGTGGTGGGAAGAGATGTTACAGGACC |
| Forward primer for sequencing | CCCATACGACGTTCCAGACTACGC |
| Reverse primer for sequencing | GATCTCGAGCTATTACAAGTCCTCTTCAG |
| Primer for mutation of A160N | CTGGTTTTAGTCCTNNNGAAGTTGTATGG |
| Primer for mutation of V260N | CAAAACAGATTCNNNGCAACTATGTTCG |

Table S2. Kinetic parameters for wtVP and MV1-5 for evans blue, amido black 10B and guinea green determined for Aga2-VP fusion proteins. Data are means of triplicate experiments and error bars indicate standard deviation.

| Variant | k_{cat} (s^{-1}) | K_{m} (μM) | $k_{\text{cat}}/K_{\text{m}}$ ($\text{mM}^{-1}\text{s}^{-1}$) |
|-----------------|--------------------------------------|----------------------------------|---|
| Evans blue | | | |
| WT VP | 1.17±0.23 | 27.52±2.95 | 42.51 |
| MV1 | 1.95±0.19 | 7.74±0.95 | 251.94 |
| MV2 | 2.95±0.09 | 6.54±0.93 | 451.07 |
| MV3 | 2.03±0.64 | 37.32±7.44 | 54.39 |
| MV4 | 1.30±0.23 | 9.92±1.10 | 110.89 |
| MV5 | 1.58±0.05 | 8.15±0.45 | 193.86 |
| Amido black 10B | | | |
| WT VP | 1.05±0.40 | 15.76±2.73 | 66.62 |
| MV1 | 5.14±0.79 | 6.91±0.94 | 743.85 |
| MV2 | 4.64±0.82 | 17.53±1.44 | 260.47 |
| MV3 | 6.34±0.90 | 5.88±0.94 | 1078.23 |
| MV4 | 1.99±0.19 | 11.23±2.24 | 177.20 |
| MV5 | 0.50±0.29 | 19.43±1.94 | 25.57 |
| Guinea green | | | |
| WT VP | 0.81±0.16 | 31.28±5.42 | 25.89 |
| MV1 | 3.24±0.25 | 19.91±2.23 | 162.73 |
| MV2 | 0.33±0.12 | 40.15±2.32 | 82.19 |
| MV3 | 0.53±0.14 | 38.34±1.94 | 13.82 |
| MV4 | 5.14±0.40 | 8.23±1.02 | 624.54 |
| MV5 | 3.96±0.82 | 11.52±3.12 | 343.75 |