Supplementary Information

Broadening the biocatalytic toolbox – Screening and expression of new unspecific peroxygenases

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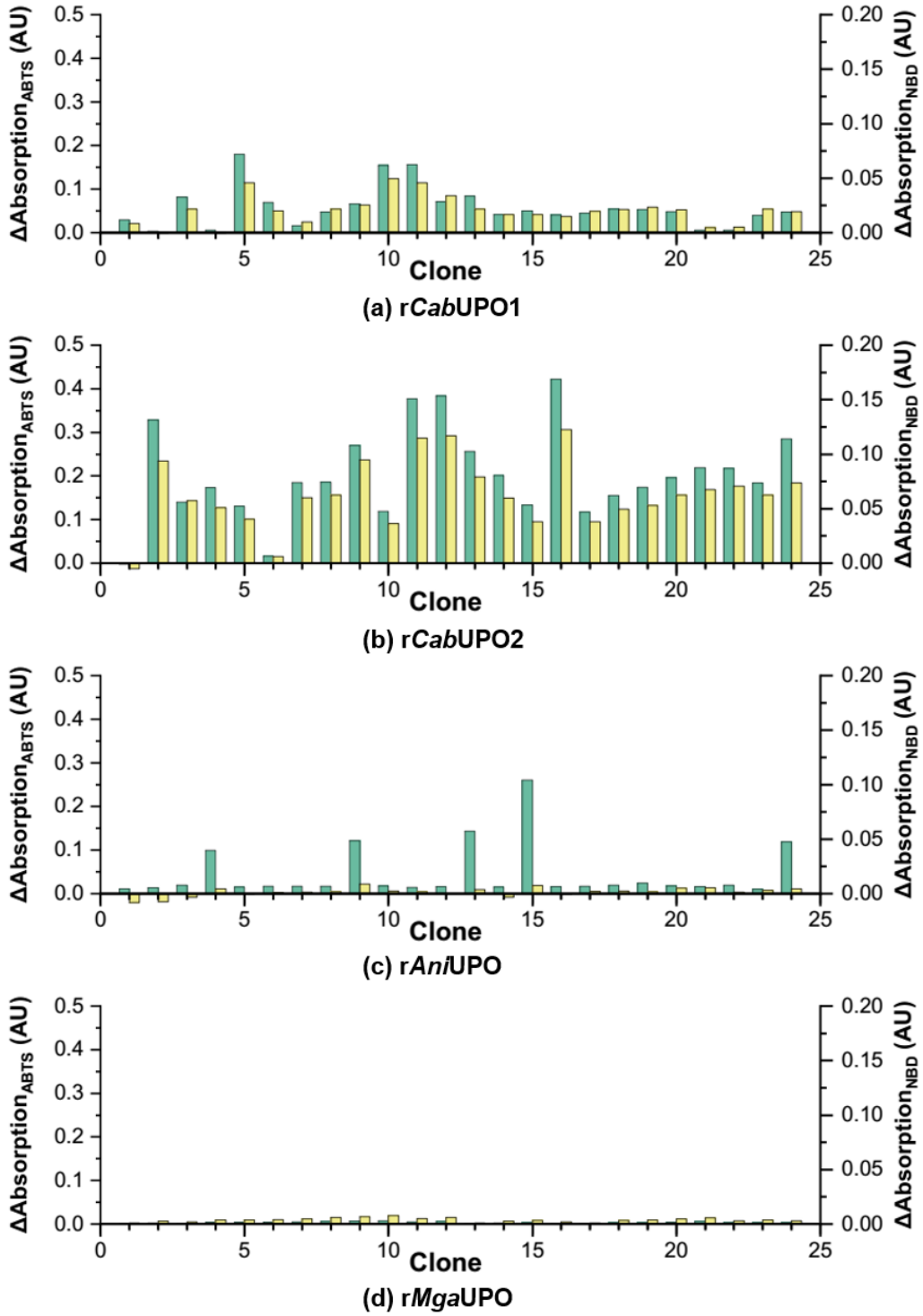
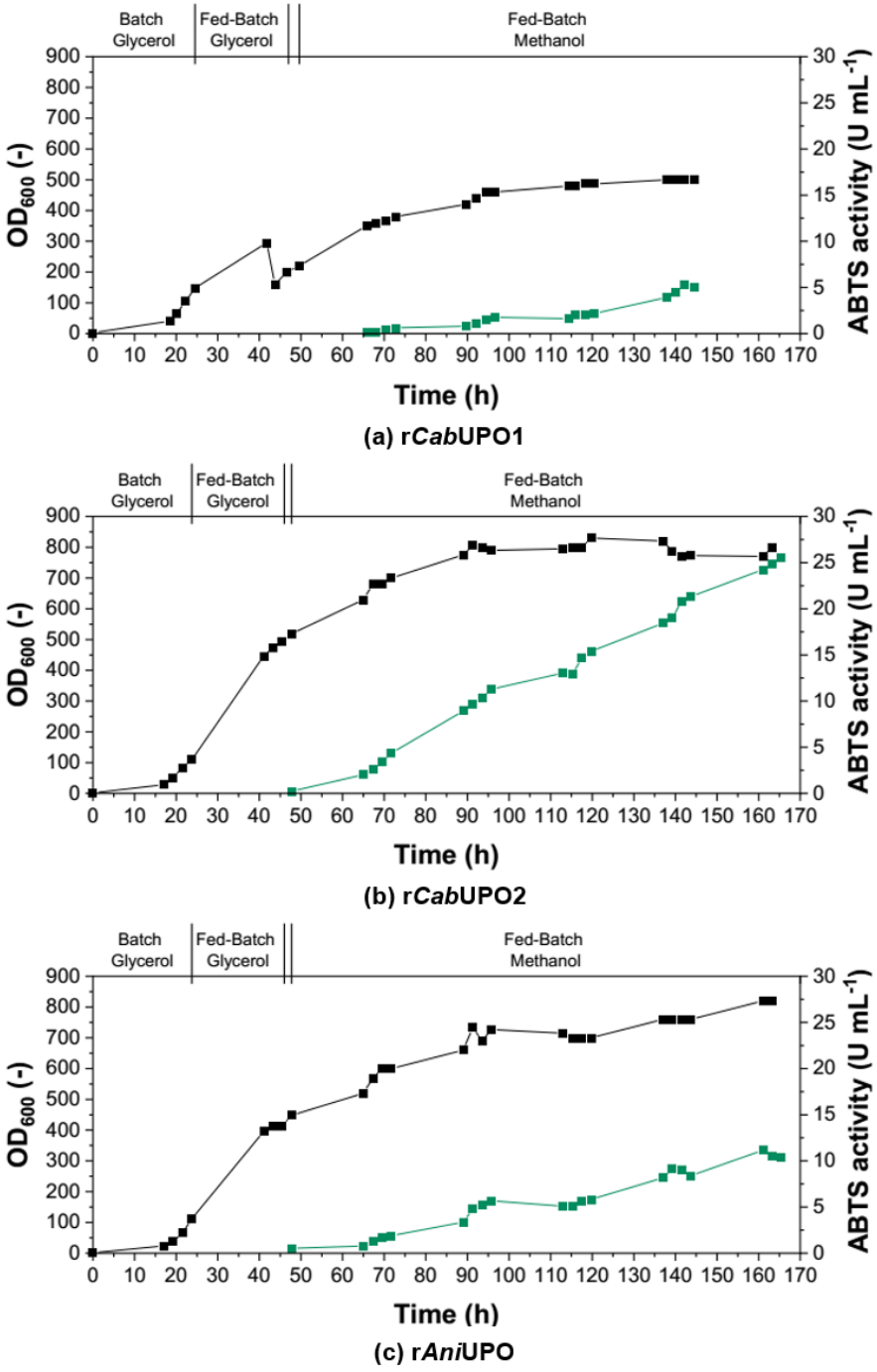
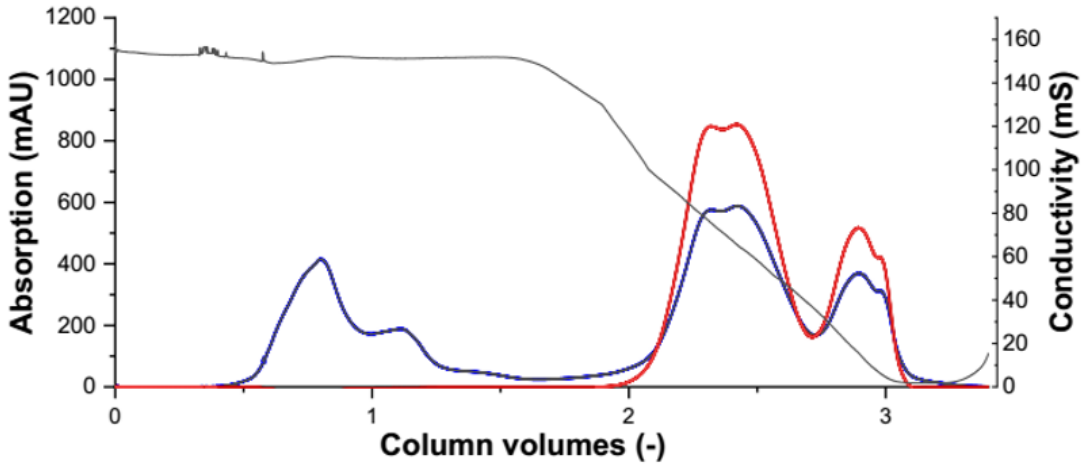
Supplementary Results



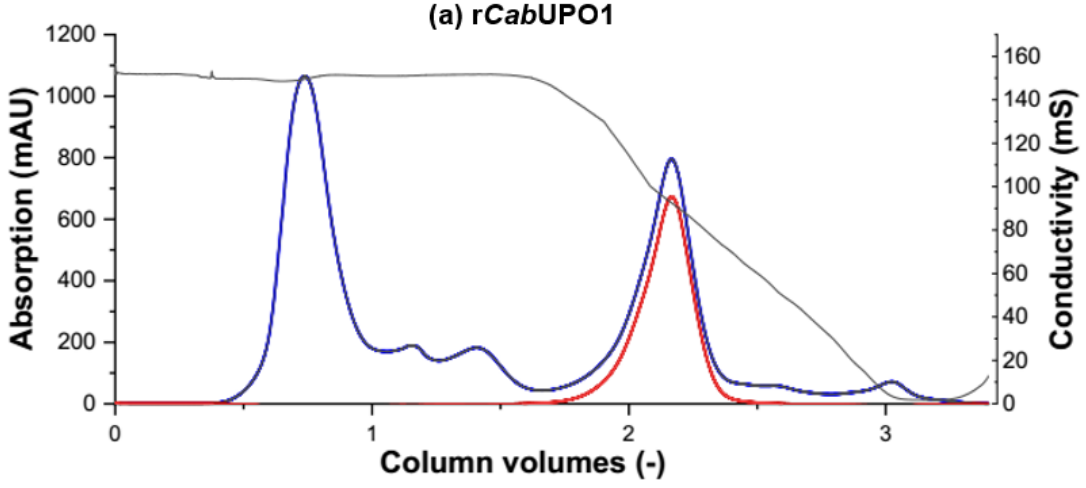
SI Figure S1: Screening of supernatant of *P. pastoris* clones transformed with UPO expression plasmids for activity using ABTS (■) and NBD (■) assays after cultivation in deep well plates for 94 h. The activity was determined by means of an endpoint measurement after 30 min of reaction time. r*Mga*UPO (d) exemplarily depicts a screening yielding no active clones. Clones chosen for subsequent larger scale production were: r*Cab*UPO 1, clone 11, r*Cab*UPO 2, clone 16, r*Ani*UPO, clone 15.



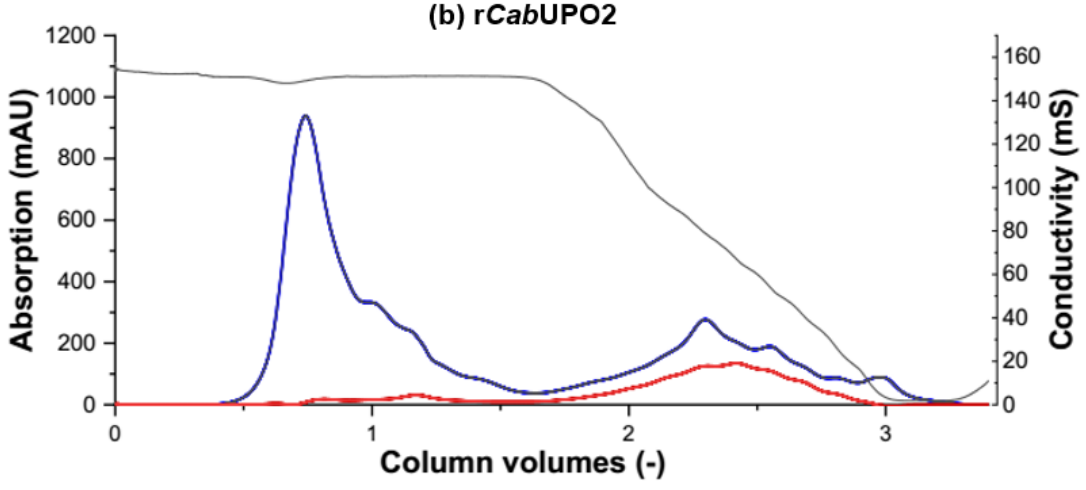
SI Figure S2: Production of recombinant UPOs with *P. pastoris* X33 in a bioreactor. Phases of the glycerol and methanol (fed)-batch process are indicated. A double bar marks the starvation phase preceding the methanol fed-batch. Cultivation was monitored by measurement of OD_{600} (■) and enzyme production by determination of the ABTS activity (■). The drop in OD at 40 h for r*Cab*UPO 1 was the result of losses of culture broth due to overpressure in the reactor. The loss of culture broth was compensated by aseptical addition of fresh medium, which lead to this drop in cell density.



(a) r*Cab*UPO1

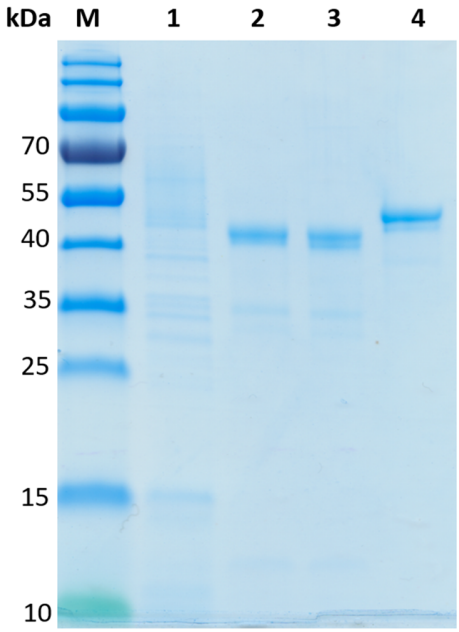


(b) r*Cab*UPO2

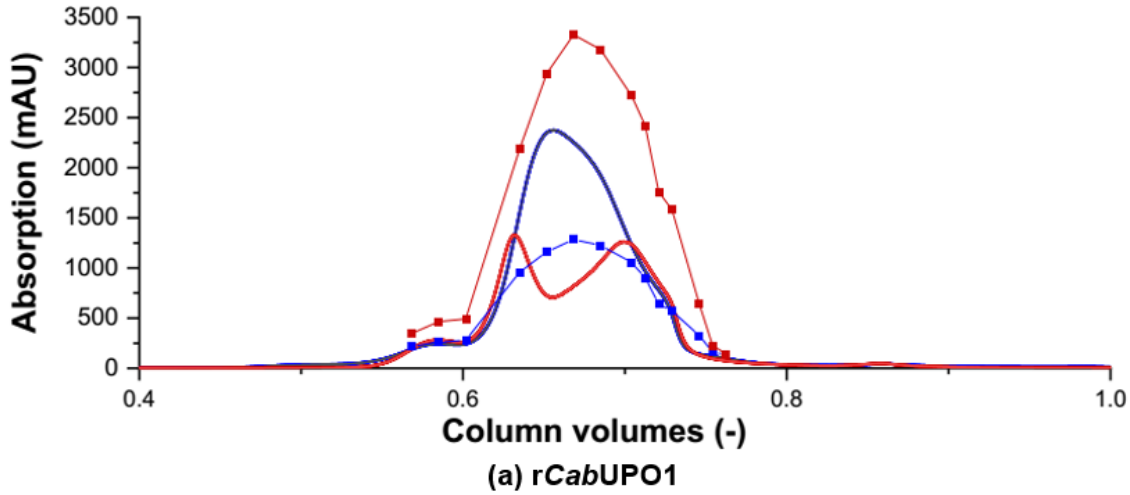


(c) r*Ani*UPO

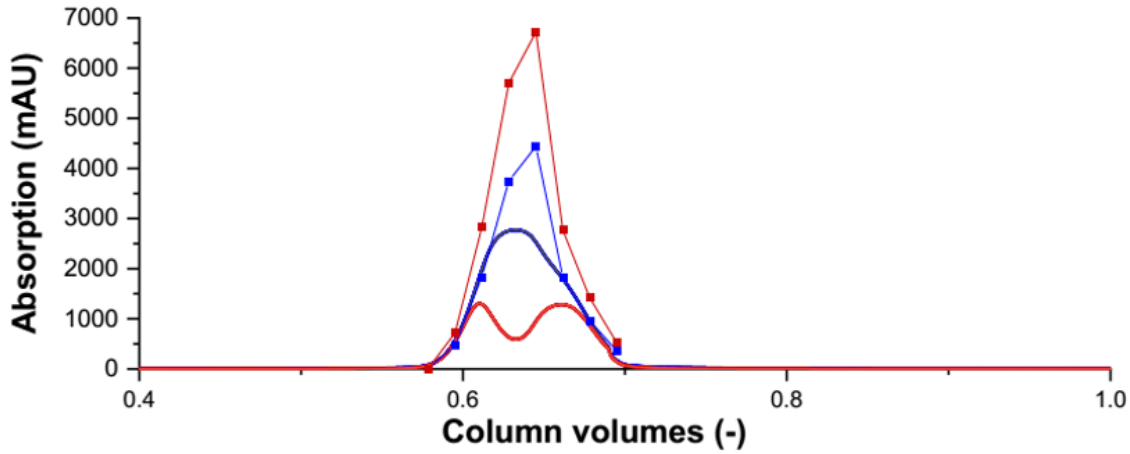
SI Figure S3: Purification of UPOs by hydrophobic interaction chromatography (HIC) using an ammonium sulfate gradient from 1 M to 0 M as indicated by the reduction in conductivity (—). Absorption was determined at 280 nm (—) and heme-specific absorption at 420 nm (—).



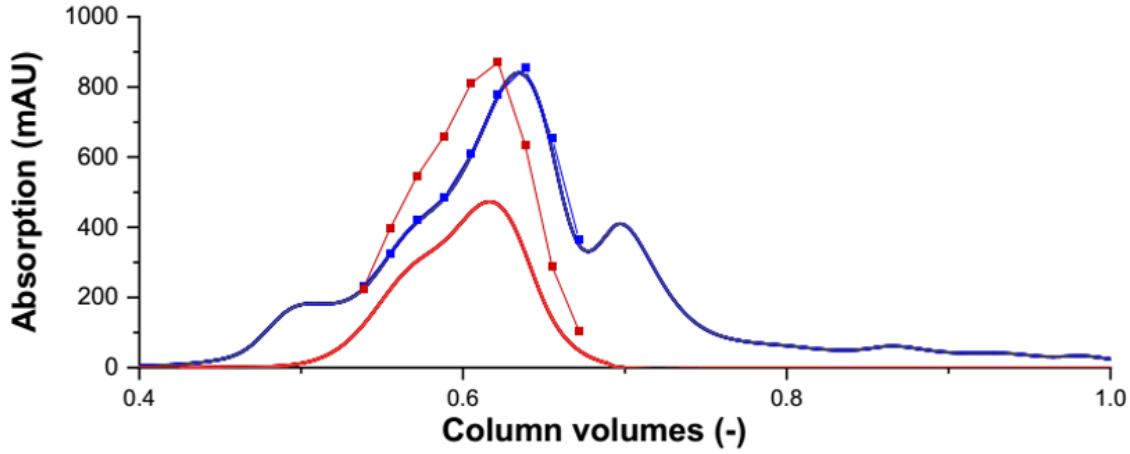
SI Figure S4: SDS-PAGE analysis of pooled fractions after HIC purification. Lanes: **M** PageRuler Prestained (ThermoFisher), 1 rAniUPO, **2** r*Cab*UPO 1 peak 2.4 CV,**3** r*Cab*UPO 1 peak 2.9 CV,**4** r*Cab*UPO 2



(a) r*Cab*UPO1

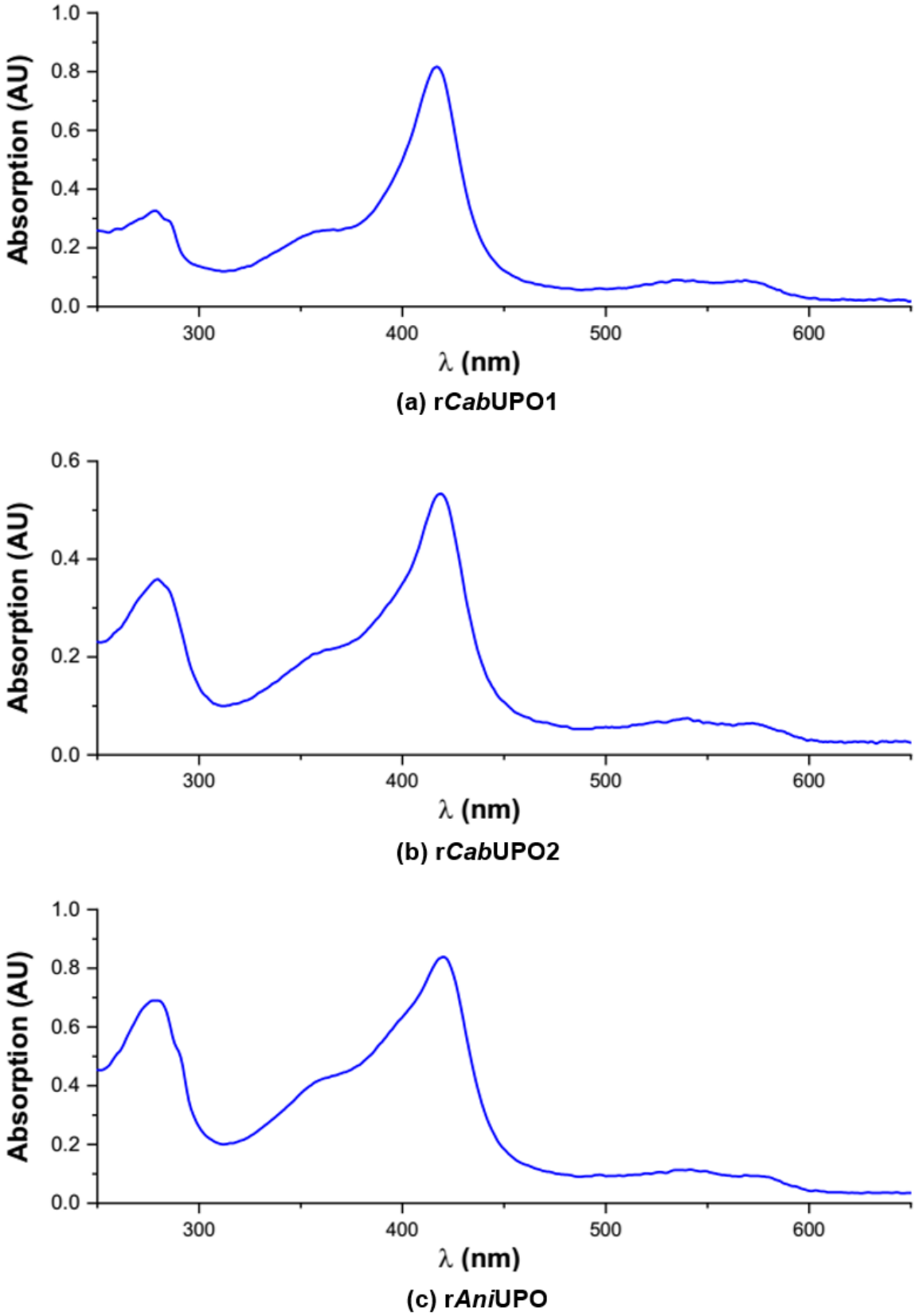


(b) r*Cab*UPO2

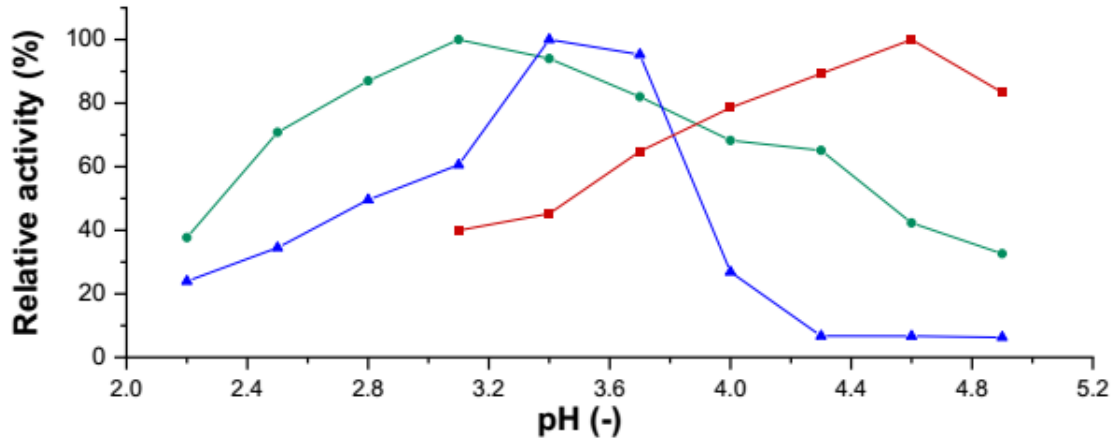


(c) r*Ani*UPO

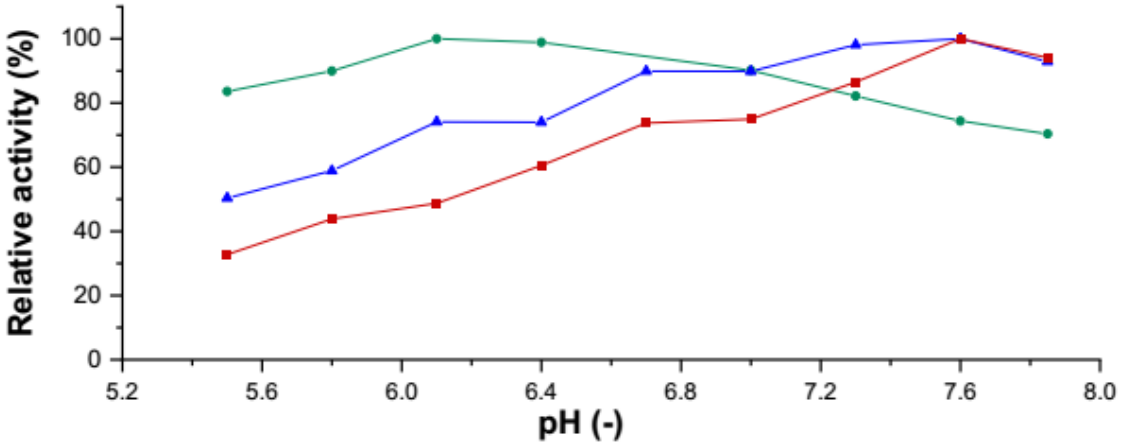
SI Figure S5: Purification of UPOs by size exclusion chromatography. Absorption was determined online (280 nm —, 420 nm —) and offline (280 nm ■, 420 nm ■).



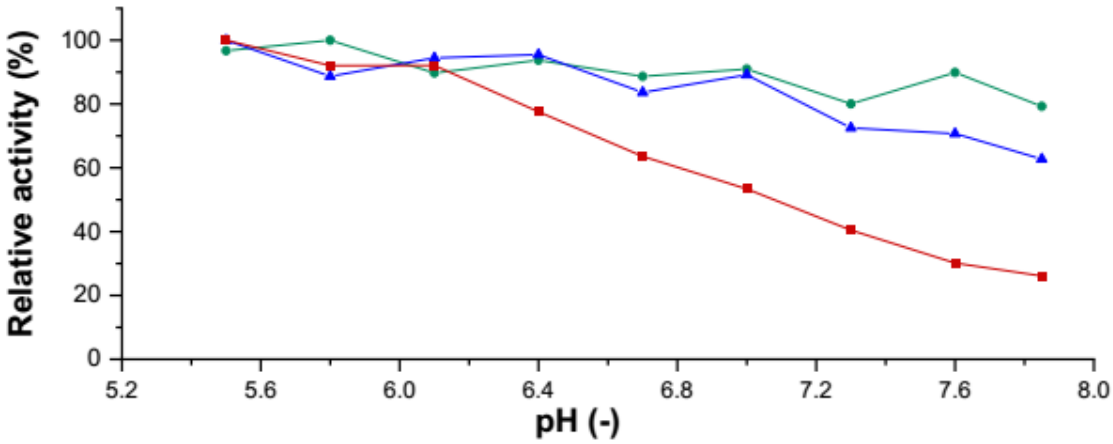
SI Figure S6: Absorption spectra of purified proteins.



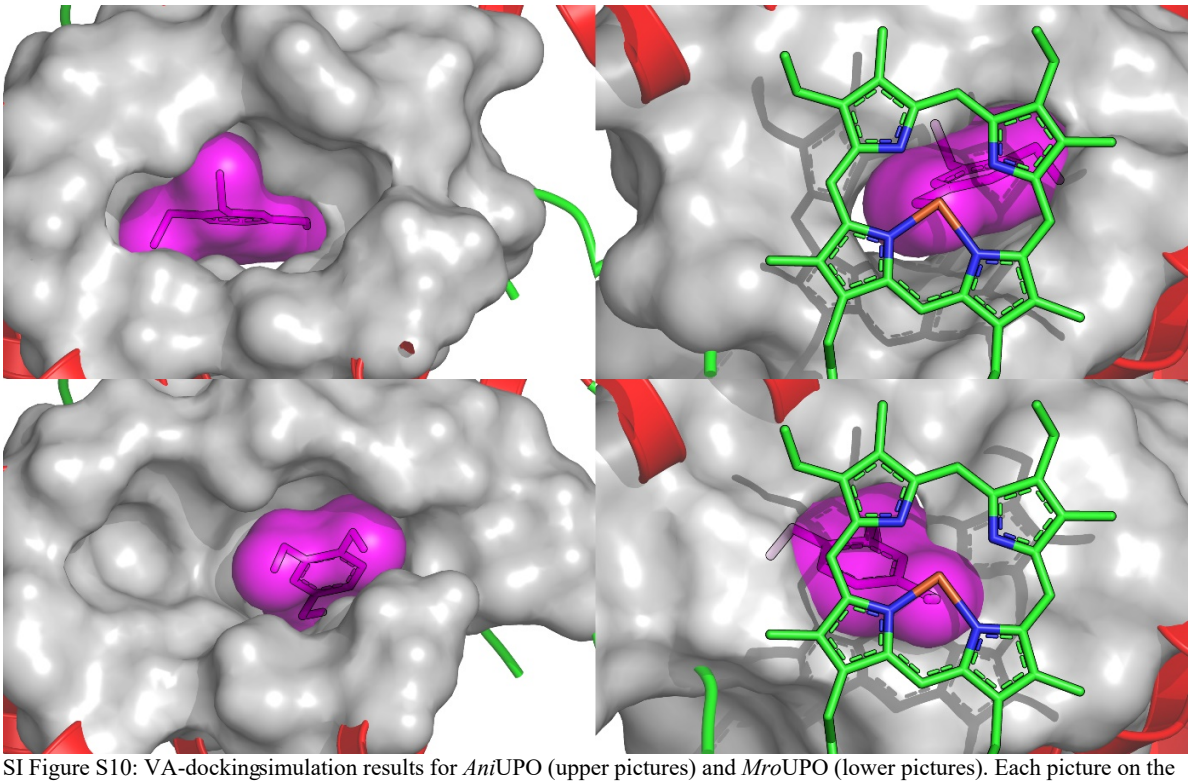
SI Figure S7: Determination of the pH-optimum of r*Cab*UPO 1 (▲), r*Cab*UPO 2 (■) and r*Ani*UPO (●) using ABTS as a substrate. An activity of 100% corresponds to the highest activity measured for each individual enzyme.



SI Figure S8: Determination of the pH-optimum of r*Cab*UPO 1 (▲), r*Cab*UPO 2 (■) and r*Ani*UPO (●) using NBD as a substrate. An activity of 100% corresponds to the highest activity measured for each individual enzyme.



SI Figure S9: Determination of the pH-optimum of r*Cab*UPO 1 (▲), r*Cab*UPO 2 (■) and r*Ani*UPO (●) using veratryl alcohol as a substrate. An activity of 100% corresponds to the highest activity measured for each individual enzyme.



SI Figure S10: VA-dockingsimulation results for *Ani*UPO (upper pictures) and *Mro*UPO (lower pictures). Each picture on the left is a top-down view from the proteins surface into the substrate access channel. Pictures to the right are the respective bottom-up view. VA in magenta as sticks surrounded by its approximated SES. Heme as sticks; carbon = green, nitrogen = blue, iron = brown.

SI Table S1*:* Primers used for the construction of pPpB1-based UPO expression plasmids.

Target	**Primer (5'-33)**	**TM °C**
pPpB1	tcaagaggatgtcagaatgc	
	CGTTTCGaataattagttgttttttgatcttctcaag	60
AniUPO	aagatcaaaaaacaactaattattCGAAACGATGaagaccaccaccc	
	ggcattctgacatcctcttgaTTAgccacgccagga	62
AveUPO	aagatcaaaaaacaactaattattCGAAACGATGaagacccctatcct	
	ggcattctgacatcctcttgaTTAgttatcggaatccttgac	59
CglUPO	aagatcaaaaaacaactaattattCGAAACGATGcgcaccagcctg	
	ggcattctgacatcctcttgaTTAgaagccgaagtggg	62
KdeUPO	aagatcaaaaaacaactaattattCGAAACGATGaagttcgccaccc	
	ggcattctgacatcctcttgaTTAgccgaagatctcgc	62
MfiUPO	aagatcaaaaaacaactaattattCGAAACGATGaagaacctcctctcc	
	ggcattctgacatcctcttgaTTAcacatcagggagctg	61
MgaUPO	aagatcaaaaaacaactaattattCGAAACGATGcagctcaagctga	
	ggcattctgacatcctcttgaTTAatggaagacgatgccg	62
CabC27	aagatcaaaaaacaactaattattCGAAACGATGatcagccagagcttc	
	ggcattctgacatcctcttgaTTAcagctggccgtag	62
CabC32	aagatcaaaaaacaactaattattCGAAACGATGgtgagcaagccc	
	ggcattctgacatcctcttgaTTAcagctggccgtaag	62
ThaUPO1	aagatcaaaaaacaactaattattCGAAACGATGaagtacctgctcagc	
	ggcattctgacatcctcttgaTTAggcgccgatctg	62
ThaUPO2	aagatcaaaaaacaactaattattCGAAACGATGctgctgtccaac	
	ggcattctgacatcctcttgaTTAatcctcggagaacttctg	60

DNA-Sequences of UPO genes

UPO from Aspergillus niger (AniUPO)

ATGaagaccaccaccctcctgtgcctcgccgccgctctcacccaggctaacgccttccctcagcagggcgc
ccctcatcctctcccttggtcccccccggccctaacgatgtgcgcgctccttgccccatgctgaacaccc
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catcatcaacgccaccggcggcgagtccgagttcgaccgtgagctggccaagcgcggcggcgttcatgtgg
gctcctggcgtggcTAA

UPO from Aspergillus versicolor (AveUPO)

ATGaagacccctatcctgtccctggccgtgggcctggccggttgtgctgtcgcttaccctgagtacctcga
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caagtactccaacctgctgcaggaggccgccggcgtcaaggattccgataacTAA

UPO from Chaetomium globosum (CglUPO)

ATGcgcaccagcctgctccccgccctggctgctgtgtcccctgtgctggccggcttcgatacctgggcccc
ccctggtccctacgacgtccgtggtccctgccccatgctgaacaccctcaccaaccatggcttcttccctc
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UPO from Kretzschmaria deusta (KdeUPO)

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UPO from Marasmius fiardii (MfiUPO)

ATGaagaacctcctctccctgatcttcatcgccgtcgccgtcaccgcctccaccgactctcaggtggactg
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AA

UPO from Mycena galopus (MgaUPO)

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UPO 1 from Candolleomyces aberdarensis (CabUPO 1)

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gTAA

UPO 2 from Candolleomyces aberdarensis (CabUPO 2)

ATGgtgagcaagcccttcgccctcctgaccacctacctgctgatcctgggcaccctctccacctccctcgc
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acctctgccgcctgtacgagttcgtggtgggctccgtgcaggagctctaccccaaccctaccggcatcctg
cgccgcaacctgatcaagaacctccattactggtggaccggcgtcaacgtcgccttcggcggctgcgatga
gctcttcccttacggccagctgTAA

UPO 1 from Trichoderma harzianum (ThaUPO 1)

ATGaagtacctgctcagcctggtcgccatcagcaccctgagcaacatcggcgccgcccagctgctggatct
ccctcctctcttcgatccctacgatcctaagttcgacgactggcagcccgccggccctgacgatagccgtg
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cagcgcatccctaccaacctgggcttccgccccaagcccatcgtcctcaacaacgccgtgggcgtcgccac
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A

UPO 2 from Trichoderma harzianum (ThaUPO 2)

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