

Supplementary Material

Synthesis of Tyrian purple and other indigo-dyes from corresponding indole derivatives by unspecific peroxygenases and their application for *in situ* dyeing

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Table S1. Applied oligomers for *rHin*UPO mutants.

A55L	AACGGATTGAACATCAACAAGACTCTCGCATCCGCGTTGTTGATTTCG
N71Q	CTCATGACATCGCCCAAGCCGCAGGCAACAACCTTCTCCCTCAACG
I154V	TTCATGTCGGAGCTCGGCAACGTCTTCACTTACGGTGAGTCGGTCG
W183I	AACAGGCGCTGGGTCGAGTATATCTTCGAGAACGAGCGCCTCCC
W183V	AACAGGCGCTGGGTCGAGTATGTCTTCGAGAACGAGCGCCTCCC

Figure S1. A – HPLC elution profiles (280 nm) of micro-aerobic reaction solutions (N_2 -flushed) containing 500 μ M indoxyl acetate (**IA**) in 5 % ACN / 50 mM PPB pH 7 (pink line); 20 min after addition of esterase (green line), and after aeration (blue line). Right: photographs of the respective reaction solutions in HPLC-vials. B – HPLC elution profiles of reaction solution A, but in presence of 10% ACN and 500 μ M 2-oxindole (**II**). C – HPLC elution profiles of a reaction solution containing 0.04 U mL⁻¹ *Aae*UPO and 500 μ M indole (**I**) in 5% ACN / 50 mM PPB pH 7 under micro-aerobic conditions (pinkish line) and after addition of 500 μ M H₂O₂ and incubation for 20 min (green line). **III** - indoxyl, **IV** - indigo, **XI**, **XII**, **XV** and **XIV** - unidentified products.

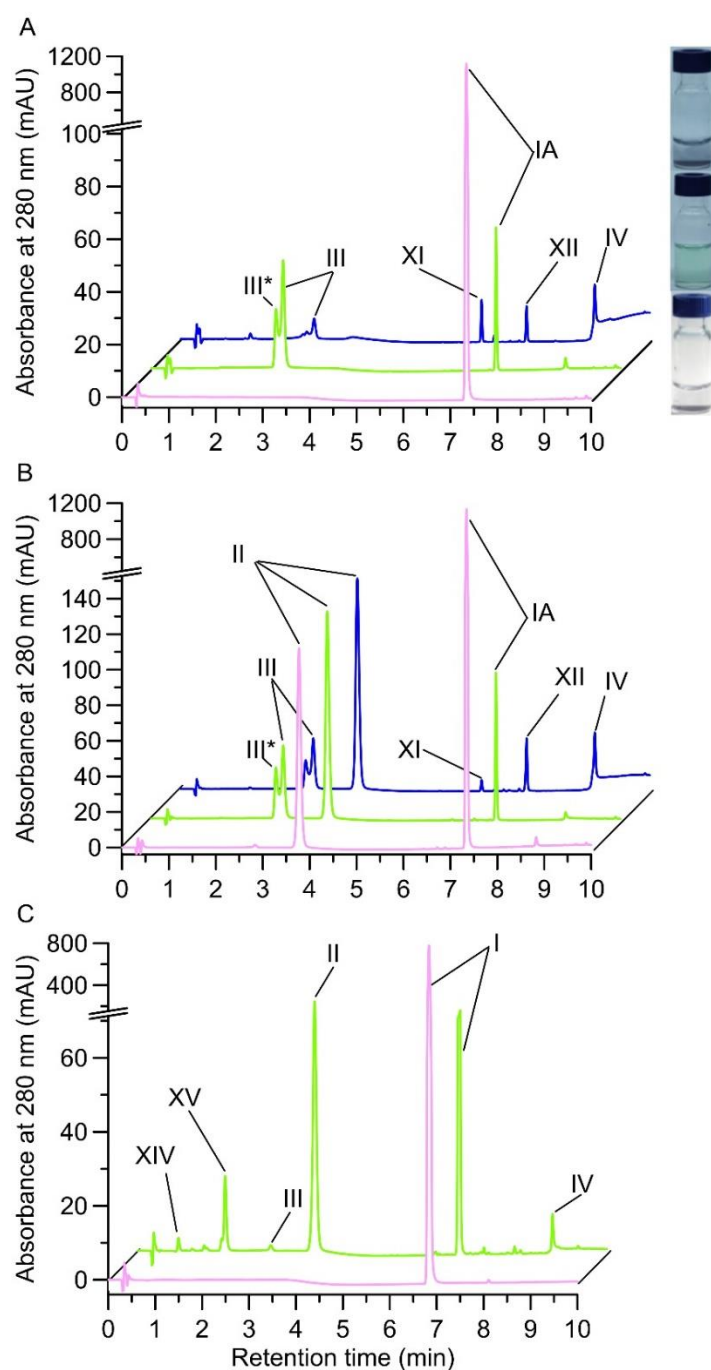
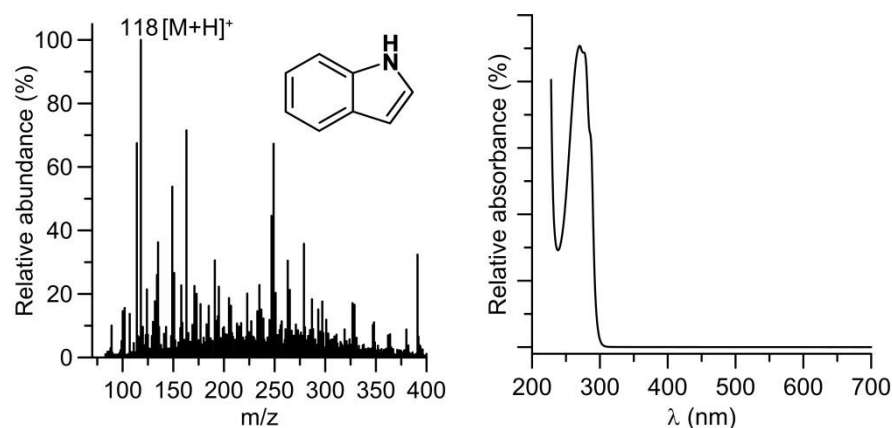
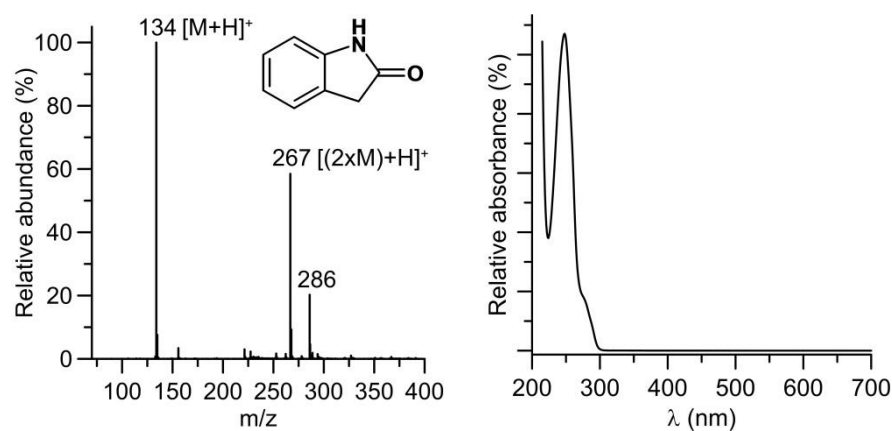


Figure S2. Mass and UV-Vis spectra of an indole standard (I) and several of its oxidation products: 2-oxindole (II), indoxyl (III), putative dimeric indoxyl (III*) and indigo (IV). IA shows the respective spectra of indoxyl acetate (standard), XI-XIII and XV represent unknown byproducts and XIV is a hydroxylated 2-oxindol with unclear position of the hydroxyl group.

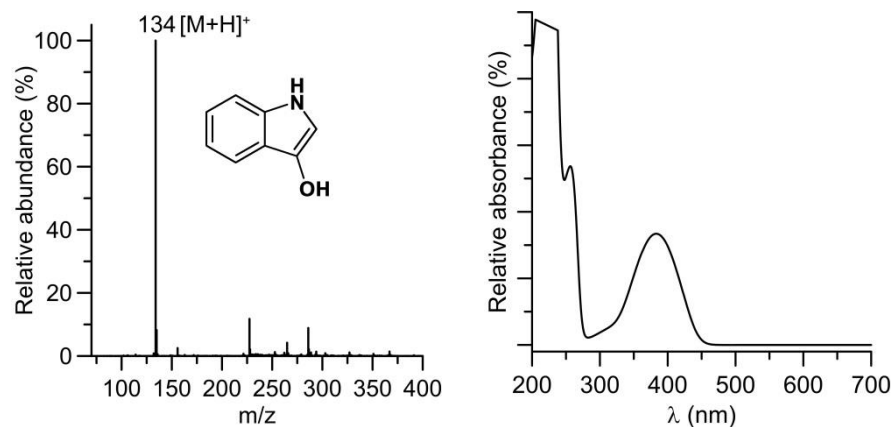
I Indol



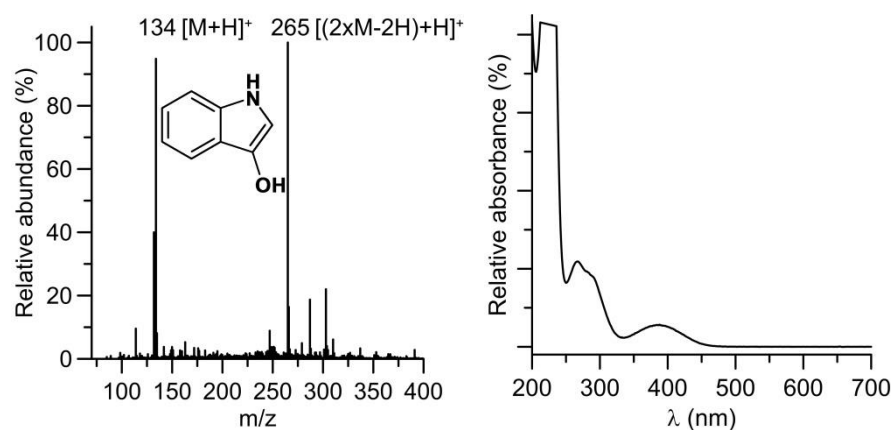
II 2-Oxindol



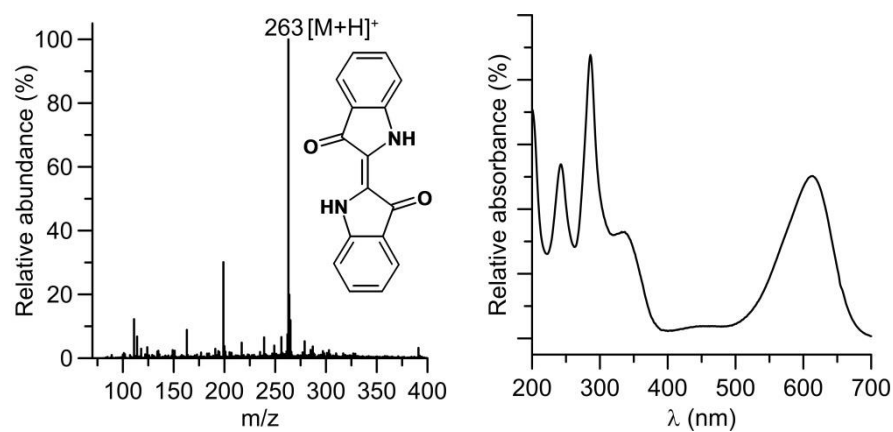
III Indoxyl (2.8 min)



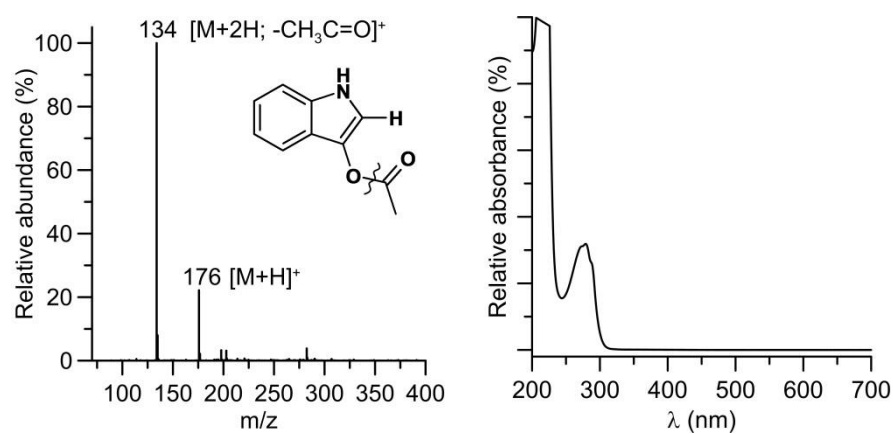
III* dimeric Indoxyl (2.5 min)



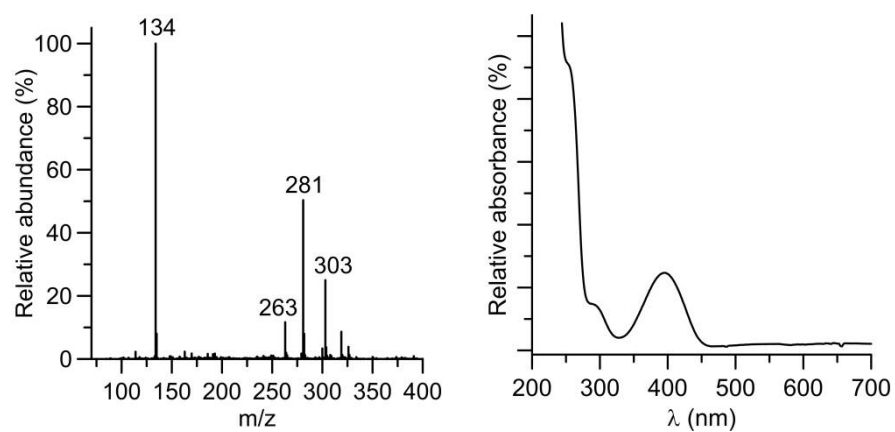
IV Indigo



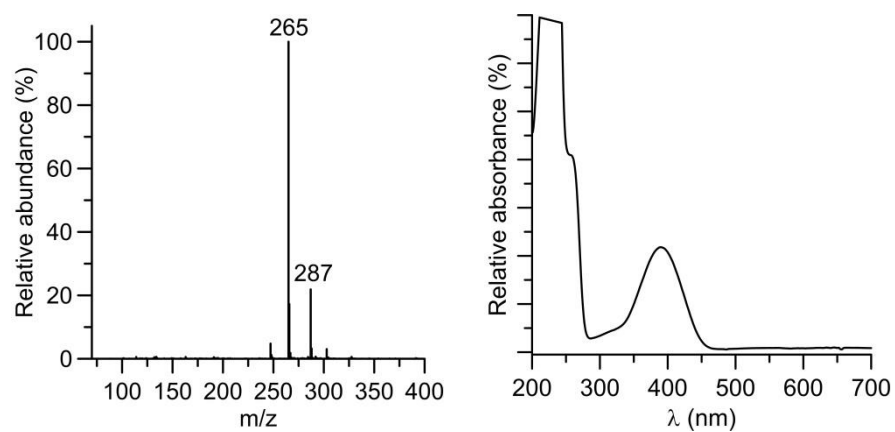
IA Indoxylacetate



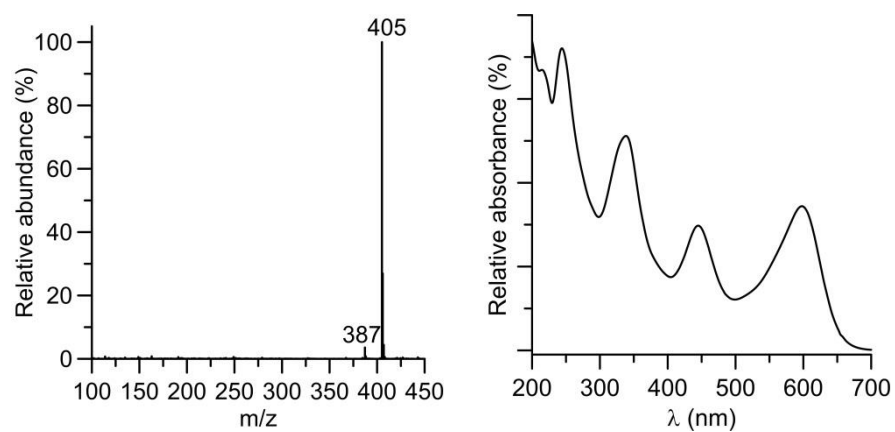
XI product



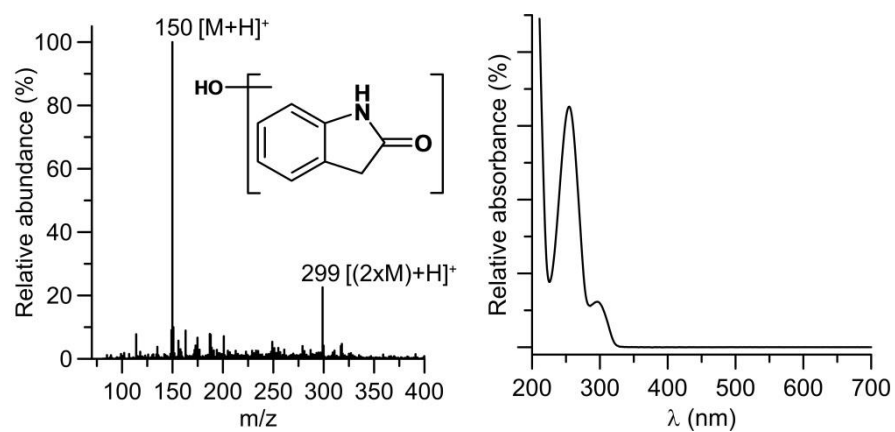
XII product



XIII product (in presence of ascorbic acid)



XIV product (Hydroxy-2-oxindol)



XV product

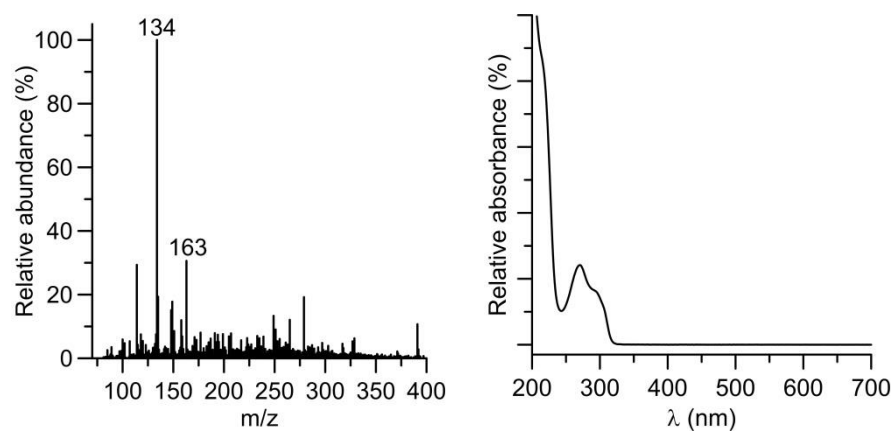


Figure S3. HPLC elution profiles of a reaction solution containing 2 U mL⁻¹ *Aae*UPO and 500 μ M 2-oxindole (**II**) in 5% ACN / 50 mM PPB pH 7 (pink line), after addition of 500 μ M H₂O₂ under micro-aerobic (green line) or aerobic conditions (blue line). Photographs on the right show the respective reaction solutions in HPLC-vials.

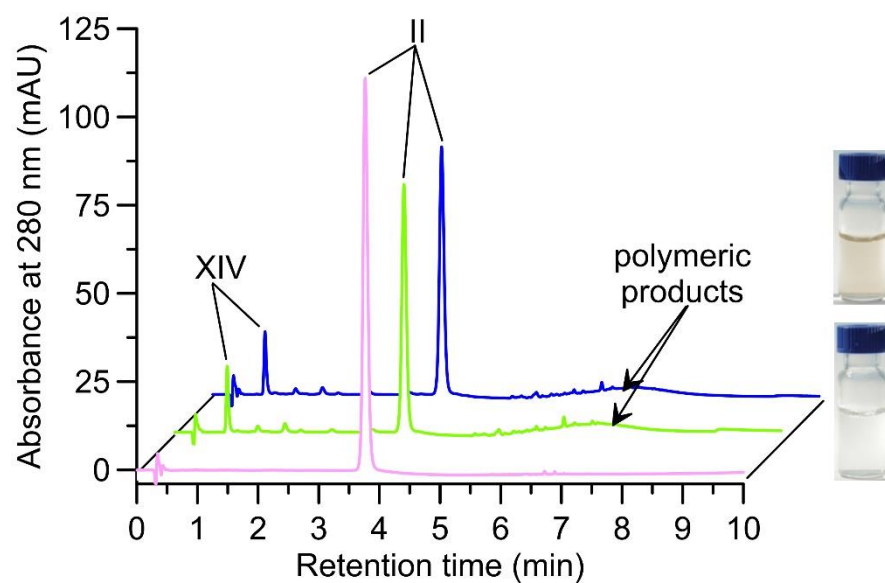


Figure S4. Influence of the pH on the conversion of indole (5.725 mM) by *rHinUPO* (0.01 mg mL⁻¹) in a Britton-Robinson buffered solution. The formation of indigo was calculated using mathematical formula (1). All further details are described in the materials and methods section.

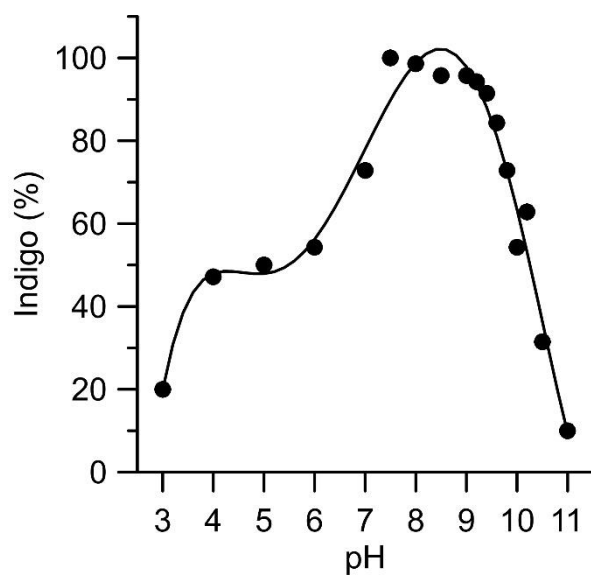


Figure 1 shows a 3x9 grid of microtiter plate wells. The rows are labeled *rHinUPO*, *AaeUPO*, and *rCciUPO*. The columns are grouped by substrate and pH. The first three columns are for 5-Br-indoline at pH 5.0, 7.0, and 9.0. The next three columns are for 5-Br-indole at pH 5.0, 7.0, and 9.0. The color of the wells indicates UPO activity, with blue representing high activity and yellow representing low activity. *rHinUPO* shows high activity (blue) across all conditions. *AaeUPO* and *rCciUPO* show low activity (yellow) at pH 5.0 and 7.0, but high activity (blue) at pH 9.0.

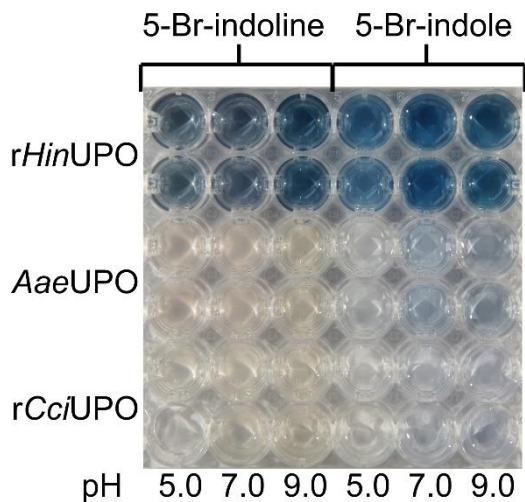


Figure S6. Vis-spectra of dyes produced from 5-bromoindoline (A-C) and 5-bromoindole (D-F) by *Hin*UPO (A;D), *Aae*UPO (B;E) and *Cci*UPO (C;F) (35 mg L⁻¹) at different pH values: 5.0 (red), 7.0 (green) and 9.0 (blue). Reactions were started by addition of H₂O₂ (800 μM in the case of 5-bromoindoline and 400 μM in the case of 5-bromoindole) and proceeded over two hours at 23°C.

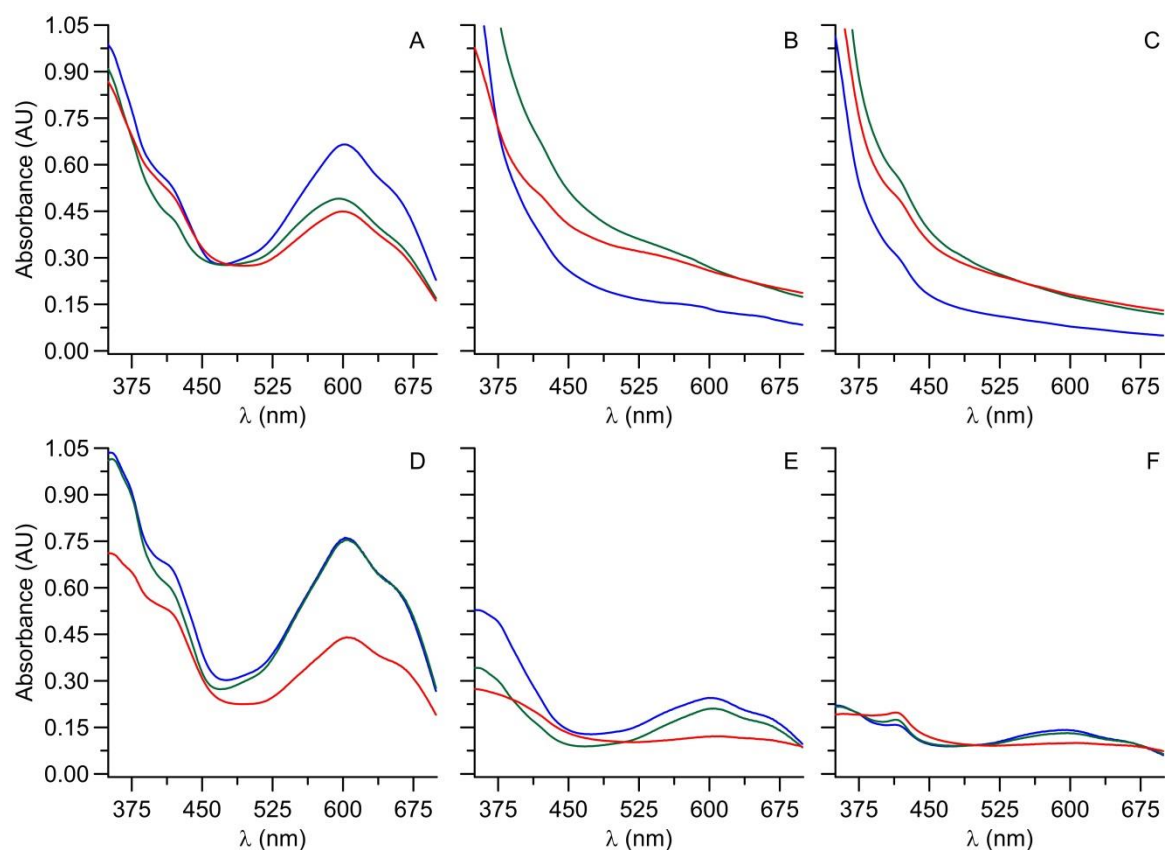


Figure S7. Structure of *Mro*UPO (pdb5FUK) with flanking helices.

