PDF Annex: FIGURES for the Description of the Main S&T Results/Foregrounds

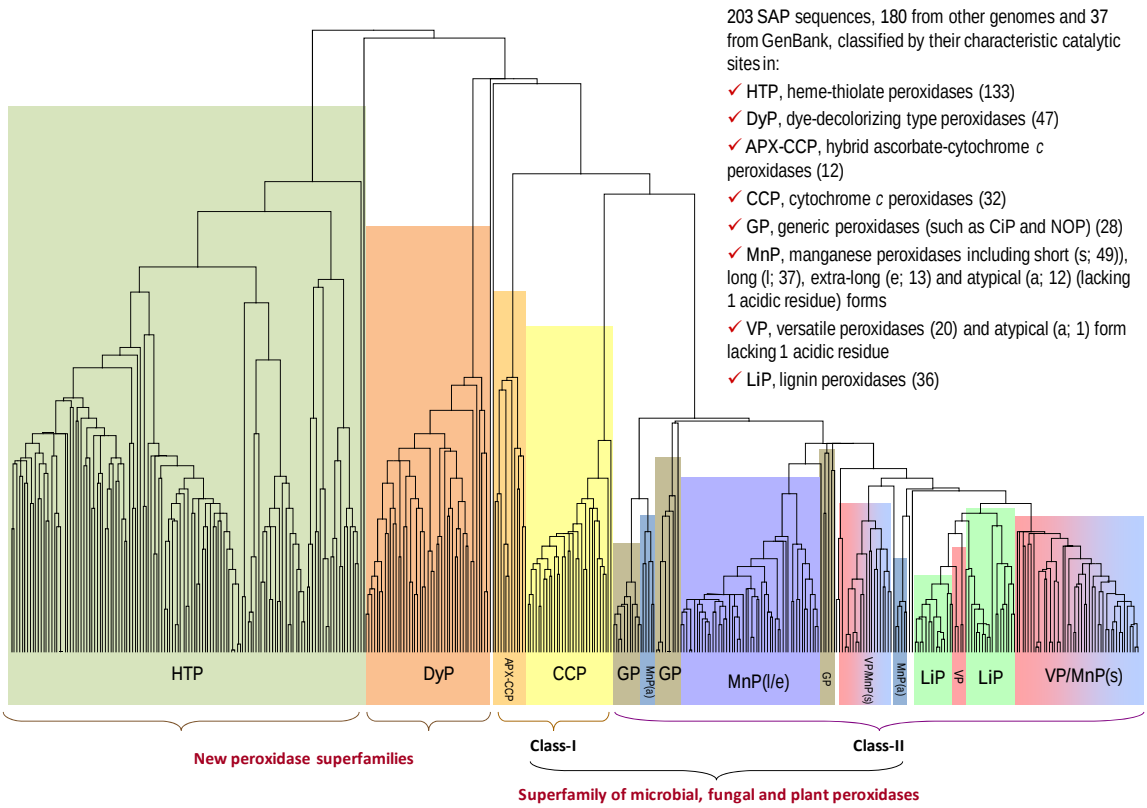

Fig. 1. Evolutionary relationships between 420 basidiomycete peroxidases from SAP (JGI Saprotrophic Agaricomycotina Project) genomes and other sources classified in different superfamilies. Based on Floudas et al. (10).

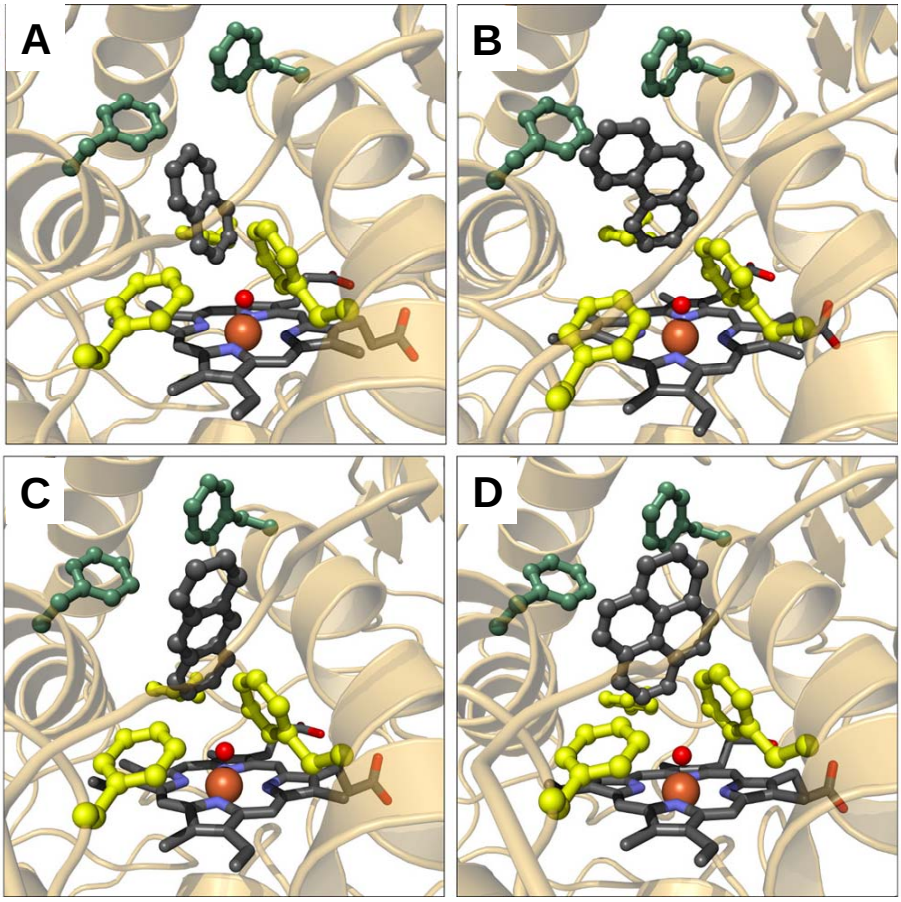

Fig. 2. A-D) Docking of naphthalene, phenanthrene, anthracene and pyrene substrates, respectively, on *A. aegerit*a UPO. Adapted from Piontek et al. (28).

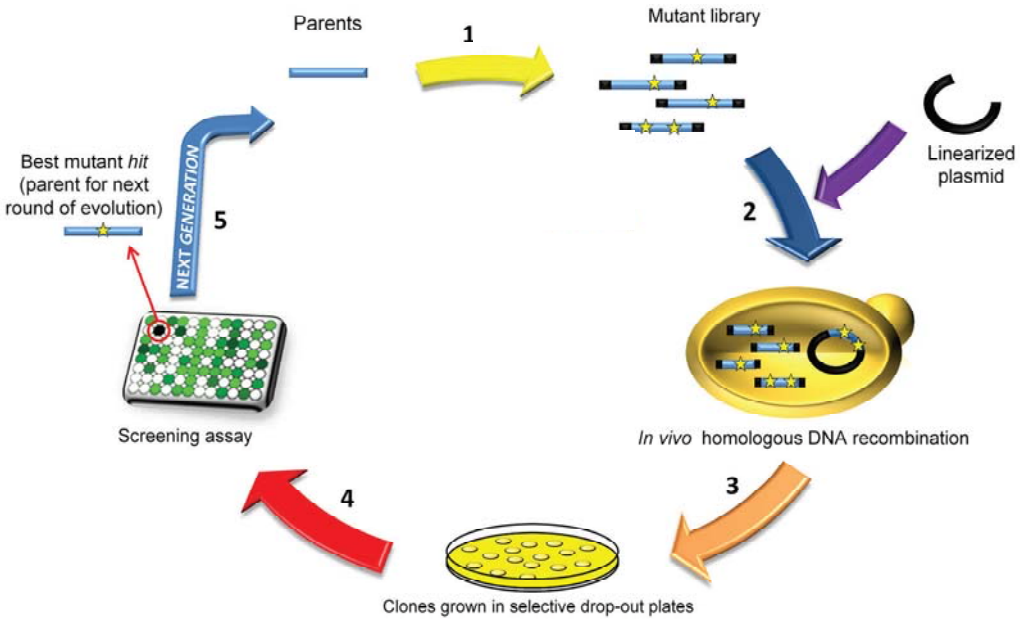

Fig. 3. A typical peroxidase directed evolution experiment using *S. cerevisiae* as a host. The cycle begins with diversity generation by mutagenic PCR (1) (stars indicate single mutations). The mutagenic library is transformed into *S. cerevisiae* (2) and the pool of templates is recombined by "in vivo" DNA shuffling. Each template contains adequate overhangs (in black) that overlap with the linearized plasmid, facilitating "in vivo" cloning to generate the autonomously replicating vector. The clones are grown on selective drop-out plates (3) and transferred to 96-well plates, where the expression is induced. After secretion, the supernatants are subjected to a high throughput assay (4) to select the best enzyme variants. Finally, the best hits are recovered, characterized and their genes subjected to a further generation of directed evolution (5). Adapted from González-Pérez et al. (36).

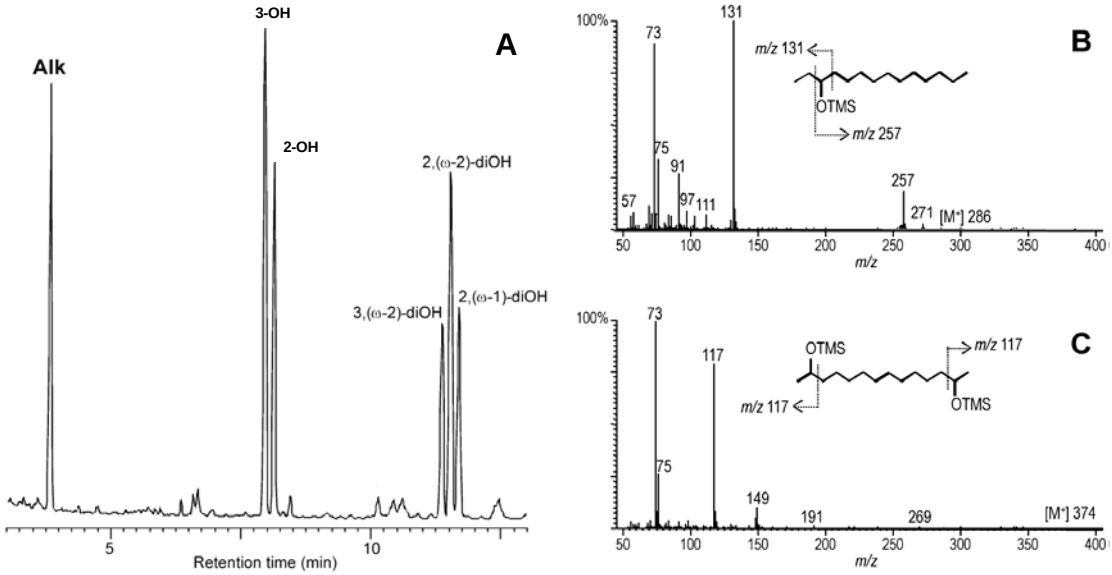

Fig. 4. GC–MS analysis of recombinant *C. cinerea* UPO reaction (in 40% acetone) with tetradecane (Alk). **A)** Chromatogram showing all the possible subterminal monohydroxy (2-OH and 3-OH) and dihydroxy (2[ω-1]-diOH, 2[ω-2]-diOH and 3[ω-2]-diOH) derivatives. **B** and **C)** Example of mass spectra of one monohydroxy (3-OH) and one dihydroxy (2[ω-1]-diOH) derivative, respectively, showing characteristic ions and fragmentation patterns. Adapted from Babot et al. (46).

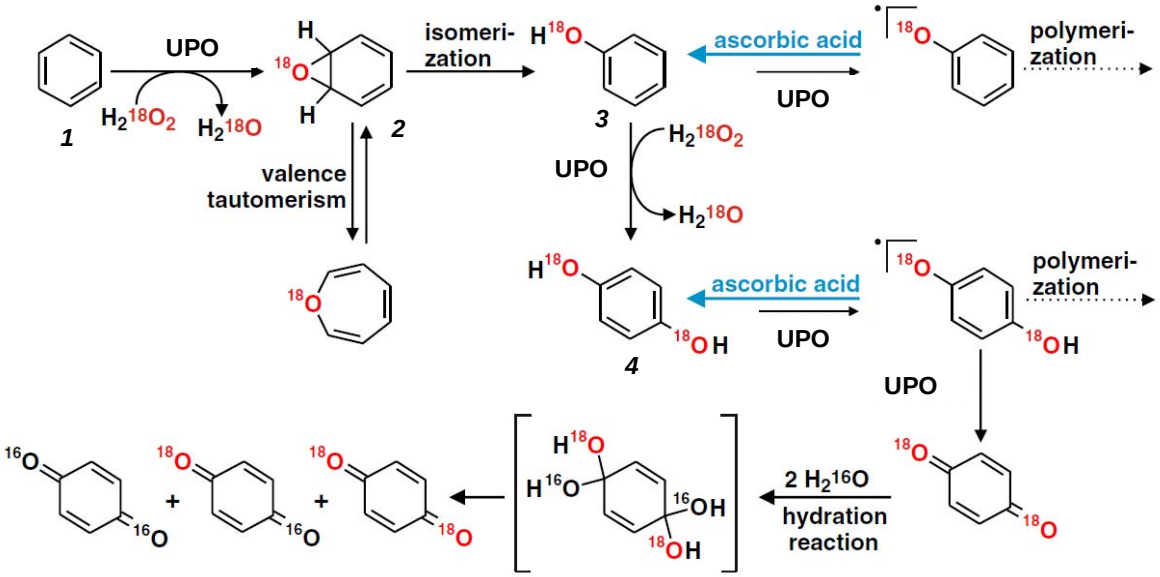

Fig. 5. Scheme for the epoxidation/hydroxylation and further oxidation of benzene *(1)* and its following products phenol *(3)* and *p*-hydroquinone *(4)* by UPO *via* the benzene epoxide intermediate *(2)* (in the same way, catechol may be oxidized to *o*-benzoquinone). Adapted from Karich et al. (57).

Fig. 6. Enzymatic delignification (of whole lignocellulose) and bleaching (of paper pulp) experiments: General view of the multireactor equipment (left) and detail of the pressurized individual reactors outside the equipment (right).