



Fig. 8. Simulations guiding introduction of a new peroxidase activity. A highly-stable peroxidase was engineered for oxidation of a new substrate (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid], ABTS) using PELE simulations for substrate diffusion in wild and mutant enzyme (left) and electron transfer estimation by quantum calculations after proper docking at the active site (right). The substrate binding residues to be introduced were identified by previous substrate diffusion on an active enzyme, and the new activity of the engineered enzyme was experimentally confirmed by PCR mutagenesis, heterologous expression and estimation of kinetic constants. ABTS-heme distances *vs* interaction energies in the model active enzyme (red dots) and the wild and mutated target enzyme (blue dots) during PELE simulations are shown in left. Detail of ribbon-type structure with docked ABTS and heme as CPK-colored sticks (other ABTS molecules during PELE diffusion as blue lines) and solvent access surface in grayish blue are shown in right. Adapted from Acebes et al. (2016).