

Supplementary figures

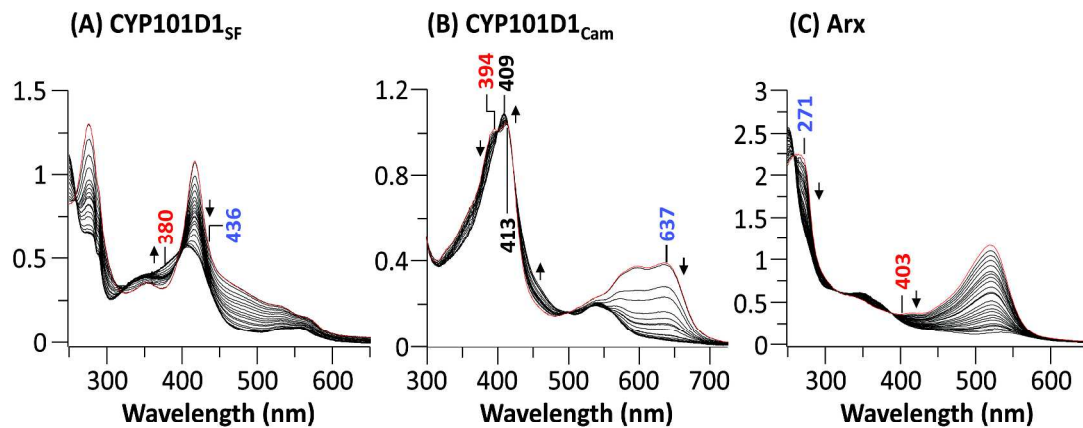


Figure S1. Reductive titration of substrate-free CYP101D1 (A), camphor-bound CYP101D1 (B) and Arx (C) in the presence of neutral red, Nile blue and phenosafranine, respectively. The red traces correspond to the initial spectra prior to the titration. The red and blue numbers represent the wavelengths at which the reduction reactions of the proteins and dyes, respectively, were monitored. The titrations were carried out in a sealed anaerobic cuvette under nitrogen atmosphere. The reaction following the addition of each aliquot of dithionite was allowed to reach equilibrium (~5-6 minutes) before the spectrum was acquired. The samples were prepared in 50 mM Tris (pH 7.4)

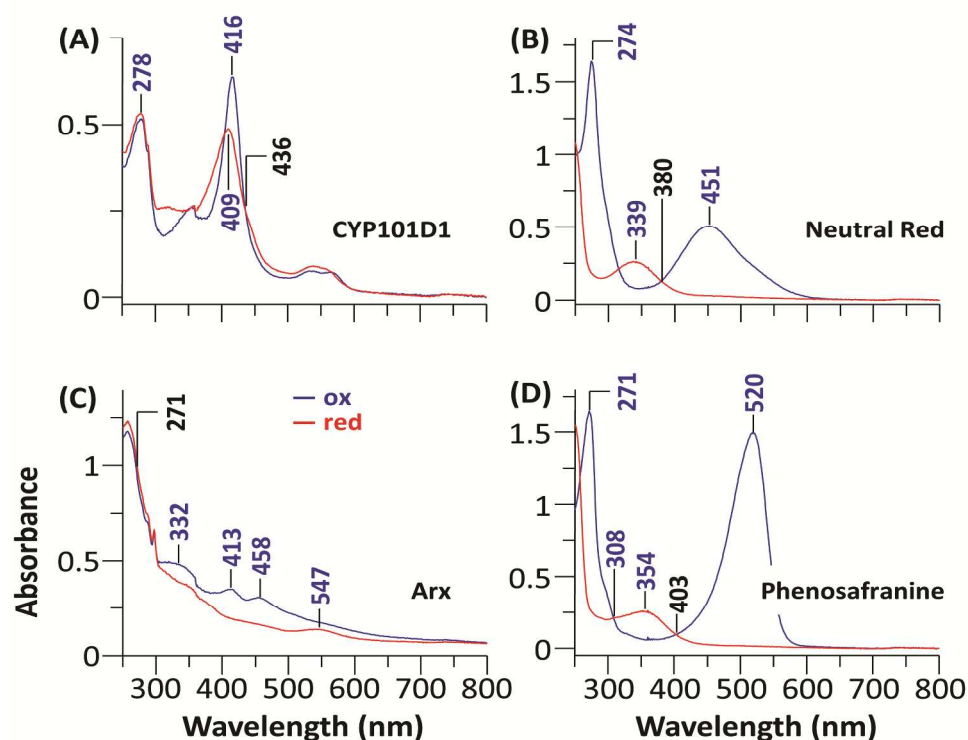


Figure S2. Determination of the isosbestic points of CYP101D1 (A), neutral red (B), Arx (C) and phenosafranine (D) following the reduction with dithionite. The numbers in black represent the isosbestic points, at which the reduction of the enzyme or dye were monitored for the mid-point potential measurements presented in Fig. 3. The reduction reaction was induced by the addition of stoichiometric amounts of sodium dithionite in N_2 purged dye or protein solutions in a sealed anaerobic cuvette. All the samples were prepared in 50 mM Tris (pH 7.4). The determination of the isosbestic points for the camphor-bound CYP101D1 and Nile blue was not required for the mid-point potential measurement due to the absence of spectral overlap.