**Supporting Information for**

# Structural Analysis of Mammalian Cytochrome P450 2B4 Covalently Bound to the Mechanism-Based Inactivator *tert*-Butylphenylacetylene: Insight into Partial Enzymatic Activity

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**Figure S1**. Confirmation of the chemical modification of 2B4 by tBPA. The molecular mass of 2B4 was determined using ESI-LC/MS after a 20 min of incubation of the reaction mixture in the presence of both tBPA and NADPH. An equal volume of water was substituted for NADPH in the control sample. The tBPA-modified 2B4 showed a mass increase of 172 Da, which is equivalent, within experimental error, to the addition of one tBPA and one oxygen atom (174 Da). Retention times for the unmodified and modified 2B4 were 22.6 and 21.0 min, respectively.

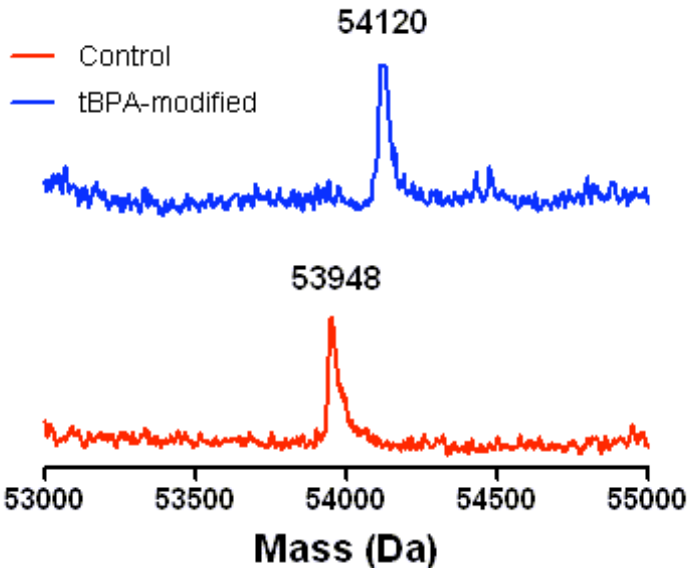

**Figure S2**. Overall structures of tBPA-modified 2B4. A) The closed enzyme form is monomeric and the sequence is colored from the N-terminus (blue) to the C-terminus (red). B) In the open form of tBPA-modified 2B4, the symmetrical dimer is formed predominantly by the insertion of the hydrophobic F' helix of each monomer into the active site of the other, where its closest approach to the heme iron is 9.7 Å. The protein chain on the bottom is colored in rainbow from the N-terminus (blue) to C-terminus (red) and is in the same orientation as the closed monomer in panel A for comparison. The chain on the top is colored gray. For both panels, secondary structural elements are denoted by letters (helices) and numbers (β-sheets) and the heme and tBPA are shown in red and gray sticks, respectively.