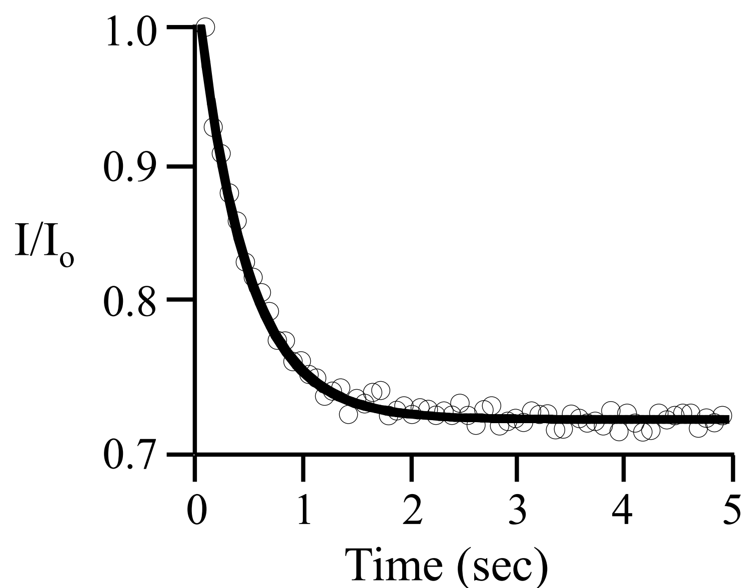

Figure S1. ***Presteady-state binding of PAPS to SULT1A1.*** Reactions were initiated by rapidly mixing 1:1 (v/v) a solution containing SULT1A1 (30 nM, dimer), $MgCl_2$ (5.0 mM) and $NaPO_4$ (25 mM), pH 7.2, with an identical solution that lacked enzyme and contained PAPS (2.0 μM). Binding was monitored *via* nucleotide-induced changed in SULT1A1intrinsic fluorescence (λ_{ex}= 290 nm, $\lambda_{em} \geq 330$ nm). Fluorescence intensity is plotted relative to the protein fluorescence intensity at t = 0 (I/I_o). Solutions were equilibrated at 25 ± 2 ºC prior to mixing. The solid curve represents the best-fit behavior predicted by a single-exponential model.

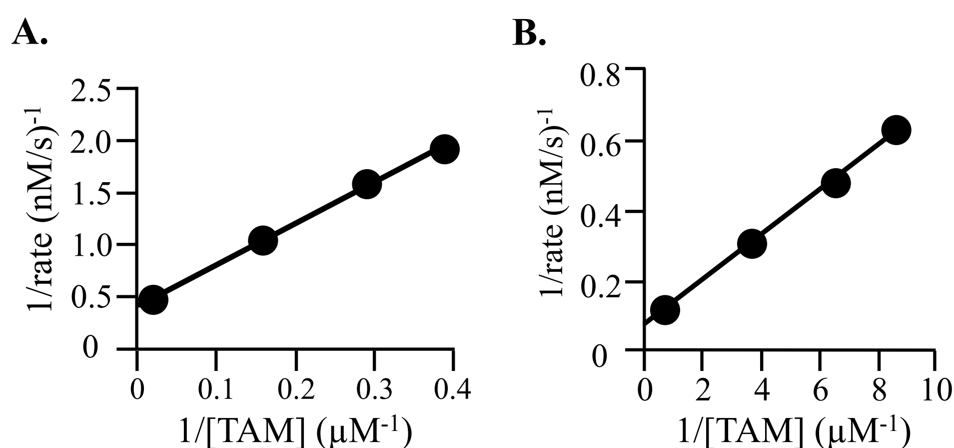

Figure S2.*TAM initial-rate studies.* A.) *The turnover of E•PAPS.* The reaction conditions were SULT1A1 (3.0 nM, dimer), ^{35}S-PAPS (3.5 μM), $MgCl_2$ (5.0 mM), and $NaPO_4$ (50 mM), pH 7.2, T = 25 ± 2 °C. **B.)** *The turnover of E•(PAPS)$_2$.* Conditions were identical to *A* except that [PAPS] = 300 μM. Radiolabelled reactants were separated using reverse phase TLC and quantitated using storm imaging. Each point is the average of three independent determinations. K_m and k_{cat} values are compiled in Table 3. Further details are given in *Materials and Methods*.

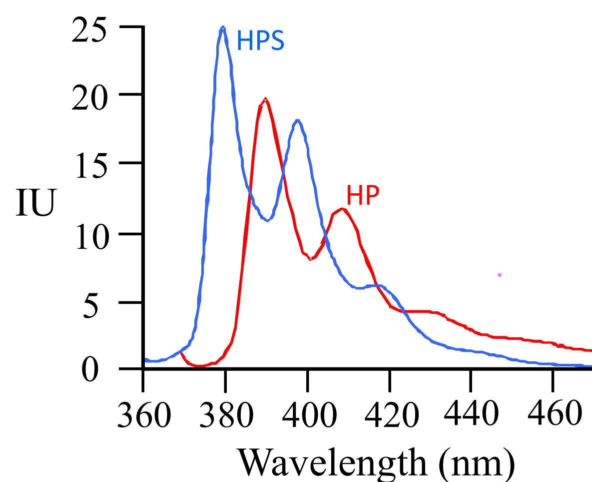

Figure S3. ***Fluorescence spectra of 1-HP & 1-HPS.*** Emission spectra of 1-HP (1-hydroxypyrene) and 1-HPS (1-hydroxypyrene sulfate) at λ_{ex} = 320 nm. Solutions contained 1-HP (1.0 µM) or 1-HPS (1.0 µM), $MgCl_2$ (5.0 mM), $NaPO_4$ (50 mM), pH 7.2, T = 25 ± 1 °C.

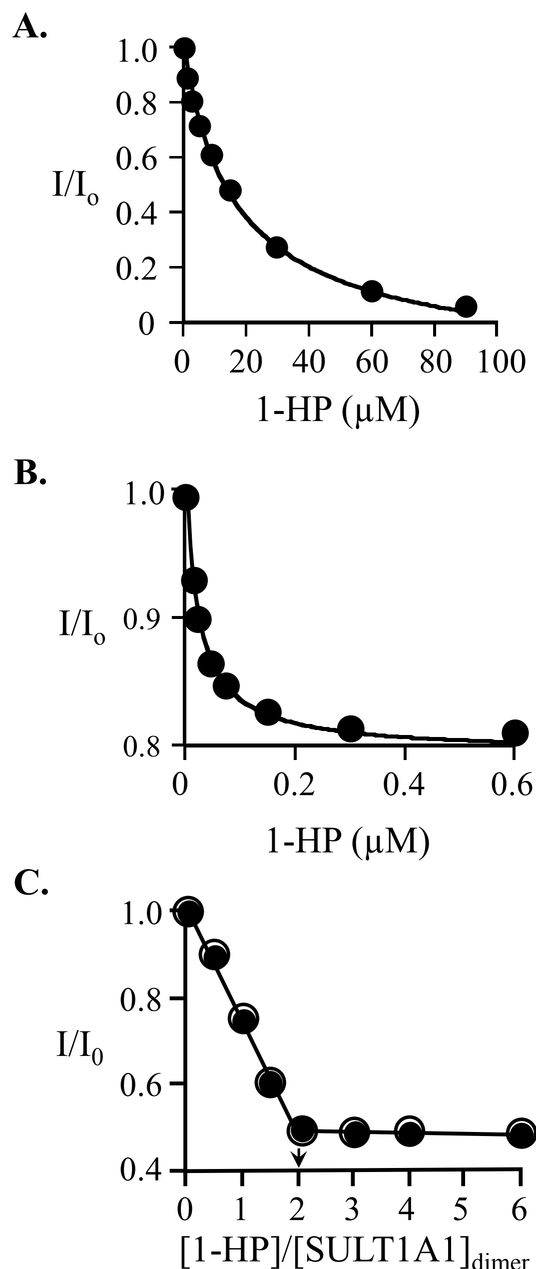

Figure S4. ***1-HP binding to the E and E·(PAP)$_2$ forms of SULT1A1.*** **A.)** *1-HP binding to E.* Ligand binding was monitored *via* changes in SULT1A1 intrinsic fluorescence (λ_{ex} = 280 nm, λ_{em} = 335 nm). The conditions were as follows: SULT1A1 (8.0 μM, dimer), $MgCl_2$ (5.0 mM), $NaPO_4$ (50 mM), pH 7.2, T = 25 ± 2°C. Each point is the average of three independent determinations. The line through the points is the behavior predicted by a best-fit of the data assuming a single binding site *per* subunit. **B.)** *1-HP binding to E·(PAP)$_2$.* The conditions were identical to *A* except SULT1A1 = 50nM (dimer) and [PAP] = 2.0 μM. **C.)** *The stoichiometry of 1-HP binding to E and E·(PAP)$_2$.* Conditions were identical to *A* except that [SULT1A1] = 120 μM (dimer,○), or [SULT1A1] = 1.0 μM (dimer), [PAP] = 1.5 μM (●). 1-HP binds to E and E·(PAP)$_2$ with a stoichiometry of 1.0 *per* subunit.