Supporting Information for:

Isozyme Specific Allosteric Regulation of Human Sulfotransferase 1A1

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This file includes: 1. Study of the interaction between EGCG and TAM.
2. Supplementary Figures
3. Supplementary Table

The Interactions of EGCG and 4-Hydroxytamoxifen (TAM). EGCG binds the open and closed forms of SULT1A1 with different affinities (Table 1, Main Text). At saturating PAP and sub-saturating TAM, two major species are present: $E\cdot(PAP)_2$ and $E\cdot(PAP)_2\cdot TAM_x$. Both caps of the $E\cdot(PAP)_2$ complex are open, and they close independently as EGCG binds (see *Results and Discussion*). In contrast, the cap of any subunit to which TAM is bound is "held" in the open position by this large substrate and cannot be closed by EGCG. The affinities of EGCG for the cap-open and -closed forms of SULT1A1 are 17-fold different (Table 1). Consequently, when the enzyme is saturated with PAP and sub-saturated with TAM one expects a bi-phasic EGCG titration, which is what is observed (Fig S5). In the high-affinity phase, EGCG binds to a PAP-occupied subunit, and closes its cap. In the low-affinity phase, EGCG competes with TAM for the cap, and the affinity constant obtained by fitting this region of the titration curve is an apparent constant ($K_{d\ app}$) that is dependent on [TAM]. The algebra that describe this dependence is given by equation 1, which is obtained from equation 2 by simplification after casting each

$$K_{d\ app} = (K_{d1} \cdot K_{d3} + K_{d3} \cdot [TAM]) / ([TAM] + (K_{d1} \cdot K_{d3}/K_{d2})) \qquad (1)$$

$$K_{d\ app} = (E' + E'\cdot TAM) \cdot [EGCG] / (E'\cdot EGCG + E'\cdot TAM\cdot EGCG) \qquad (2)$$

enzyme form in terms of $E'\cdot TAM\cdot EGCG$ (where E' is a nucleotide-bound subunit), a K_d value (according to Fig S6), and [TAM]. Equation 1 predicts that at [TAM] = ∞, $K_{d\ app} = K_{d3}$, which is the K_d for EGCG binding to $E\cdot(PAP)_2\cdot(TAM)_2$. If $K_{d\ app}$ values are obtained at a series of fixed TAM concentrations and the data are plotted in double reciprocal format (i.e., $1/K_{d\ app}$ *vs* 1/[TAM]) a straight line is obtained (Fig S7), and the affinity of EGCG for $E\cdot(PAP)_2\cdot(TAM)_2$ is given by $K_{d\ app}$ at [TAM] = ∞.

Supporting Figure 1

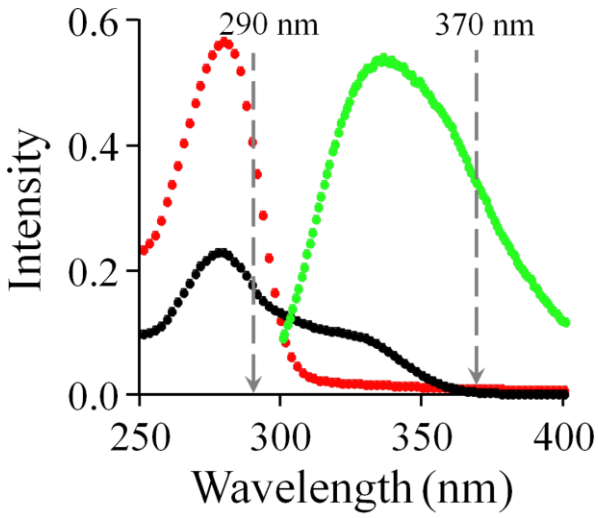

Figure S1. *Absorbance Spectra of EGCG (black), SULT1A1 (red), and Emission Spectrum of SULT1A1 (Green).* To minimize inner filter effects, the excitation and emission wavelengths used for all EGCG titrations were 290 and 370 nM, respectively. Conditions: EGCG (15 µM) or SULT1A1 (10 µM, dimer), $NaPO_4$ (50 mM), pH 7.5, 25 ± 2 °C.

Supporting Figure 2

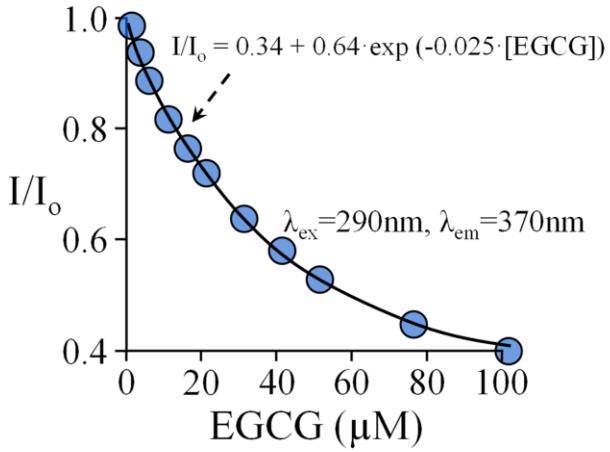

Figure S2. *EGCG Inner Filter Effect Standard Curve.* Construction of the curve is described in *Materials and Methods*. Conditions: SULT1A1 (1.0 μM, dimer), $MgCl_2$ (5.0 mM), $NaPO_4$ (50 mM), pH 7.5, 25 ± 2 °C. I/I_o values were acquired over a series of ATP concentrations (0 – 4.2 mM) whose 290 nm absorbance spanned that of the EGCG concentrations used in titrations in the Main Text. Controls were run to ensure that the ATP did not inhibit the enzyme at the maximum concentrations used. The resulting I/I_o *vs* OD_{ATP} data were converted to I/I_o *vs* [EGCG], as is shown in the figure. The solid line through the data is the behavior predicted by the best-fit to a single-exponential equation.

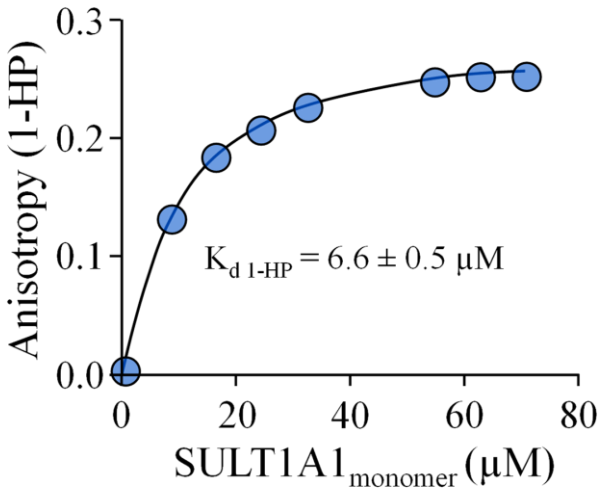

Figure S3. *1-HP Binding to SULT1A1(Anisotropy).* The binding of 1-HP to SULT1A1 was measured *via* changes in its fluorescence anisotropy (λ_{ex} = 385 nm, λ_{em} = 430 nm). Conditions: 1-HP (8.0 µM), $MgCl_2$ (5.0 mM), $NaPO_4$ (50 mM), pH 7.5, 25 ± 2 °C. Each point is the average of two determinations, and the curve through the points is the behavior predicted by a best-fit, single-site binding model.

Supporting Figure 4

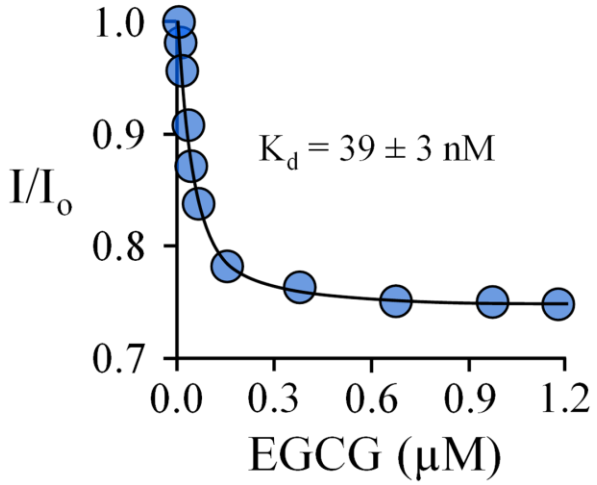

Figure S4. *EGCG binding to SULT1A1·(PAP)$_2$*. Bindings was monitored *via* changes in the intrinsic fluorescence of SULT1A1 (λ_{ex} = 290 nm, λ_{em} = 370 nm). Fluorescence is reported relative to that in the absence of EGCG (i.e., I/I_0). Conditions: SULT1A1 (50 nM, monomer), PAP (0.50 mM, 16 · $K_{d\ PAP\ low\ affinity\ site}$), $MgCl_2$ (5.0 mM), $NaPO_4$ (50 mM), pH 7.5, 25 ± 2 °C. The data were corrected for EGCG inner filter effects (see *Materials and Methods*). Each point represents is average of three determinations. The line through the data is the behavior predicted by a best-fit, single-site binding model.

Supporting Figure 5

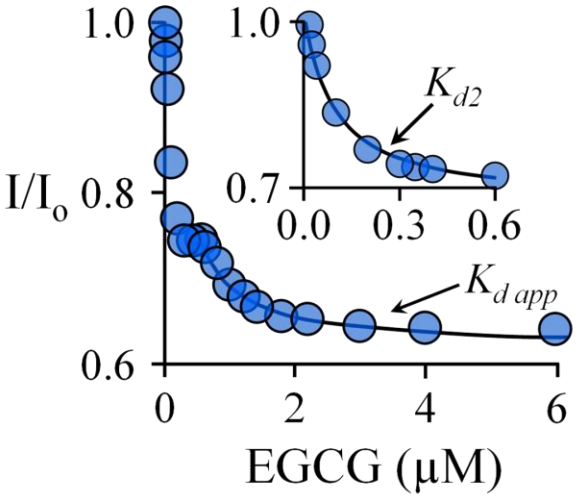

Figure S5. *EGCG Binding to E·(PAP)$_2$·TAM$_x$*. Binding was monitored *via* ligand-induced changes in SULT1A1 intrinsic fluorescence (λ_{ex} = 290 nm, λ_{em} = 370 nm). Fluorescence intensity is reported relative to that in the absence of EGCG (i.e., I/I_0). Conditions: SULT1A1 (0.20 μM, monomer), TAM (2.5 μM, 3.8 · K_d), PAP (0.50 mM), $MgCl_2$ (5.0 mM), $NaPO_4$ (50 mM), pH 7.5, 25 ± 2°C. Each point is the average of two determinations, and the lines through the points are the behaviors predicted by a best-fit, single-site binding model. The insert highlights EGCG binding to the high-affinity subunit, which is not bound to TAM.

Supporting Figure 6

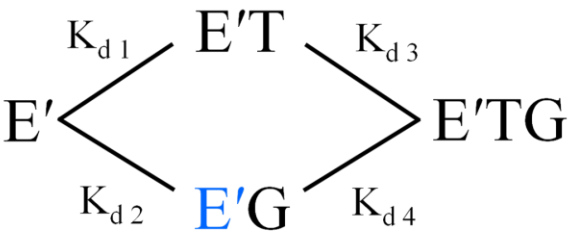

Figure S6. *The Binding of EGCG and TAM at Saturating PAP*. E′ represents a PAP-bound subunit of the SULT1A1 in the E·$(PAP)_2$ complex. T and G represent TAM and EGCG, respectively. A blue E′ indicates that the cap of that subunit is closed, black indicates it is open.

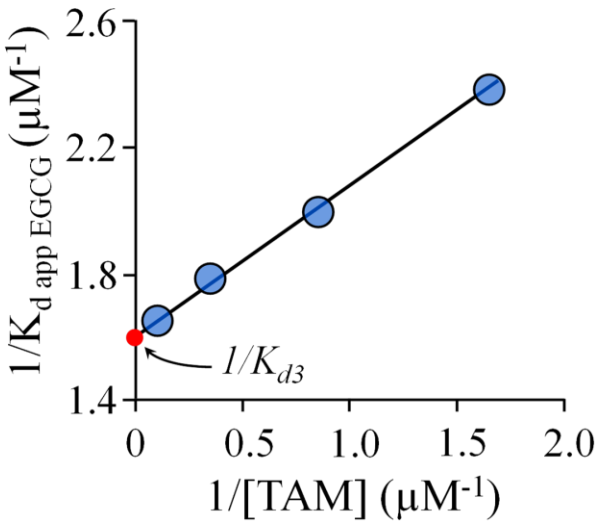

Figure S7. *EGCG Binding to E·(PAP)$_2$·(TAM)$_2$.* The measurements and conditions are identical to those in the Fig S5 Legend except that the TAM concentration is varied.

Table S1. Ligand Binding and Interaction Equilibrium Constants

Reaction #[a]	K_d (μM)[b]	K_{iso}[c]	ΔG (kcal/mole)[b]
1	0.68 (0.05)[e]		-9.8
2	0.35 (0.04)		-10.3
3	0.43 (0.08)[d]		-10.1
4	0.044 (0.004)		-11.7
5	0.038 (0.008)[d]		-11.8
6	0.81 (0.07)		-9.7
7	0.84 (0.09)[d]		-9.7
8	0.021 (0.002)[d]		-12.2
9	0.78 (0.06)		-9.7
10	31 (2)		-7.2
11	31 (2)		-7.2
12	0.66 (0.07)		-9.8
13		17 (3)	-2.0
14	0.66 (0.07)		-9.8
15		17 (3)	-2.0
16	0.68 (0.05)		-9.8
17	0.024 (0.002)		-12.1
18	0.83 (0.14)[d]		-9.7
19	0.66 (0.07)		-9.8
20		17 (3)	-2.0

[a]Numbers correspond to reactions shown in Fig 7 (main text). [b]Values are calculated for reactions written in the upward direction. [c]Cap closure isomerization equilibrium constant. [d]Calculated by *conservation of energy*. [e]Values in parentheses indicate error