Supporting Information for:

Adrenodoxin allosterically alters human cytochrome P450 11B enzymes to accelerate substrate binding and decelerate release

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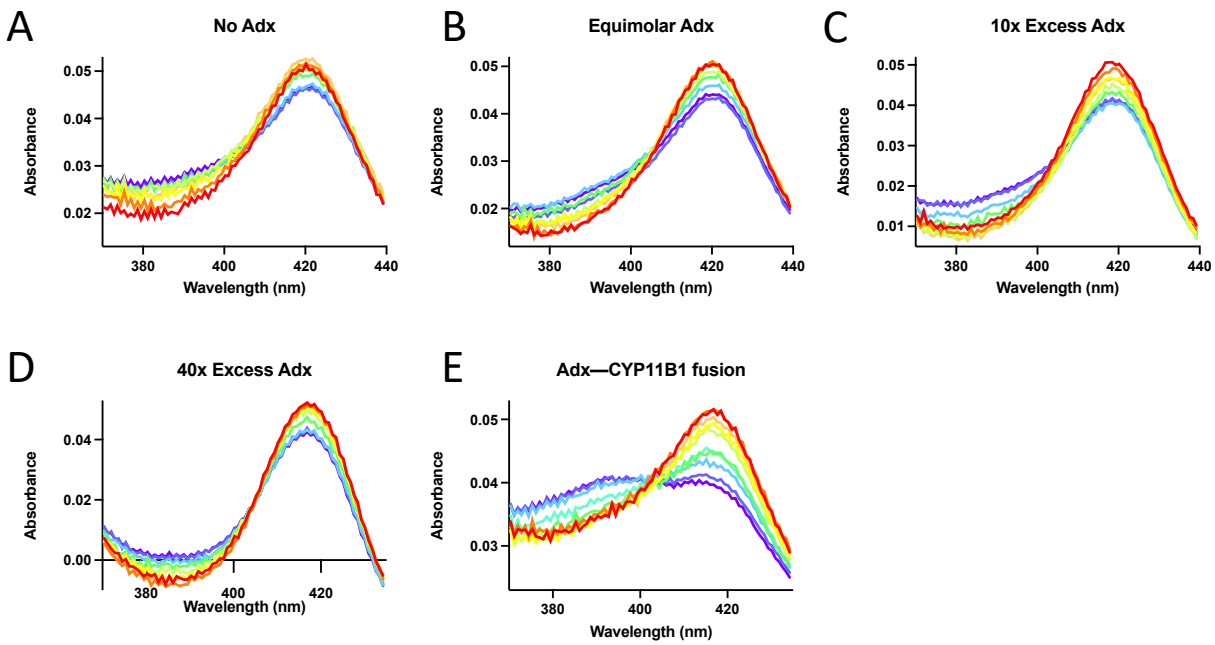
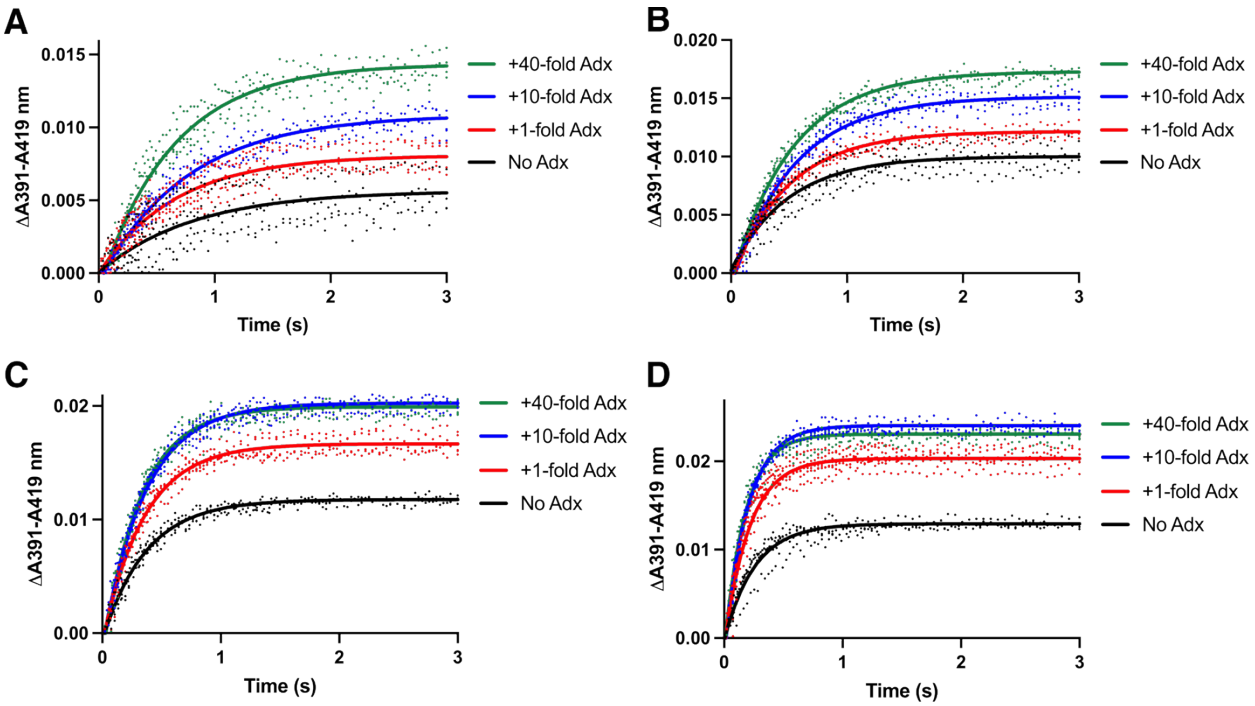
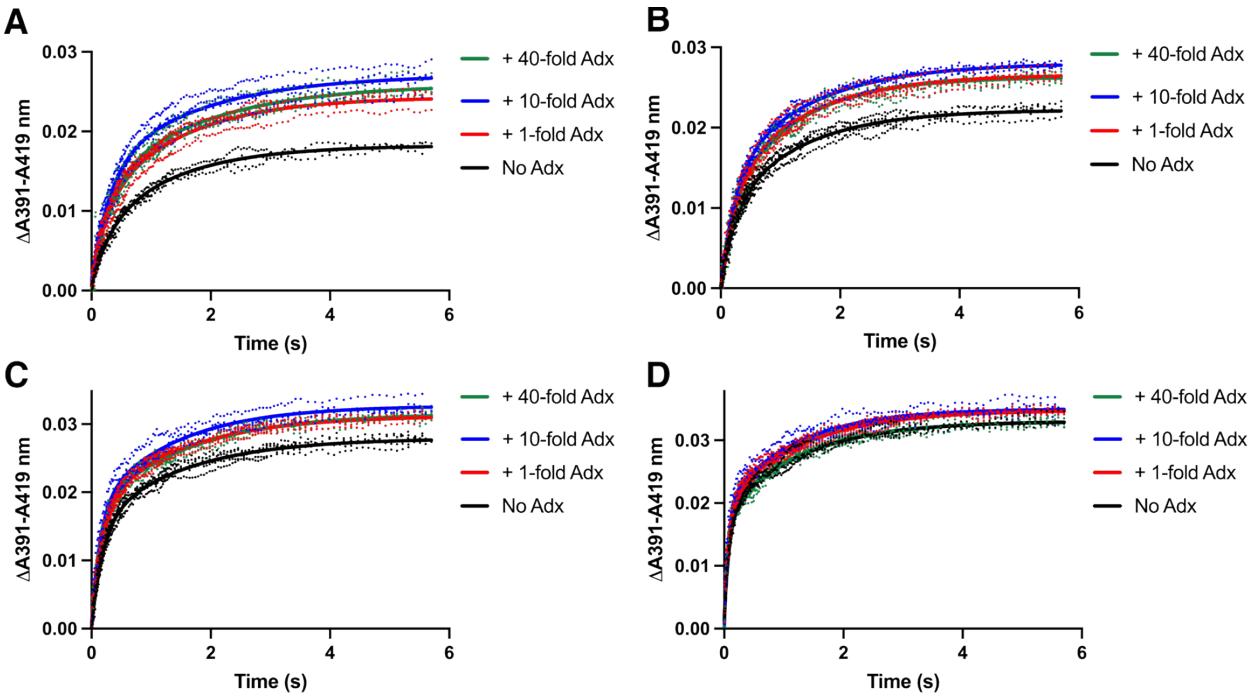


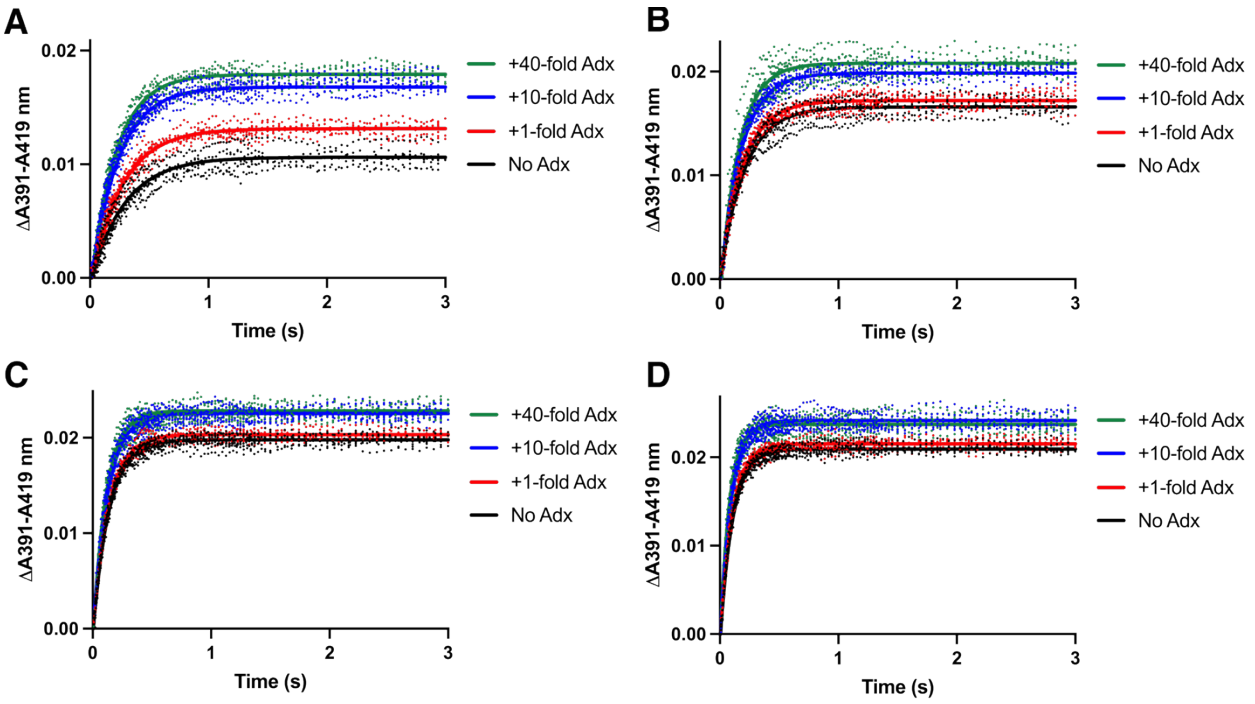
Supporting Figure 1. Spectra during 11-deoxycortisol binding to CYP11B1 in the presence of different adrenodoxin concentrations (A-D) and to the Adx/CYP11B1 fusion (E). All spectra are shown from the earliest time point in red to the latest time point in purple. Individual spectra were normalized by correcting the starting absorbance (700 nm) to zero. To account for variations between experiments and allow better comparison between data, the absorbance at the Soret peak (419 nm) of the first time-point was then corrected to 0.05.



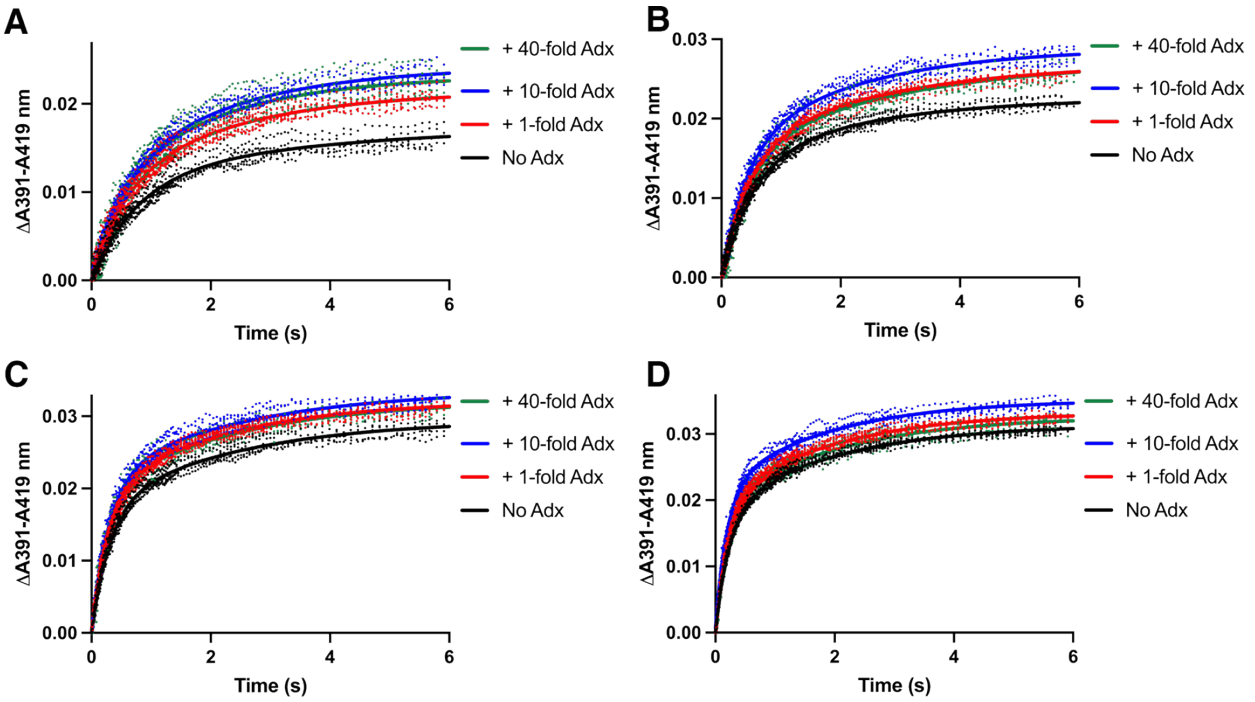
Supporting Figure 2. Series of stopped-flow traces for 11-deoxycortisol binding to CYP11B1 at four different substrate concentrations (A—2.5 μM, B—5 μM, C—10 μM, and D—25 μM) and four different adrenodoxin ratios (0, black; 1x, red; 10x, blue; 40x, green). Some data presented as representative data in Figure 2A and 2B is repeated here to facilitate comparisons within the data sets.



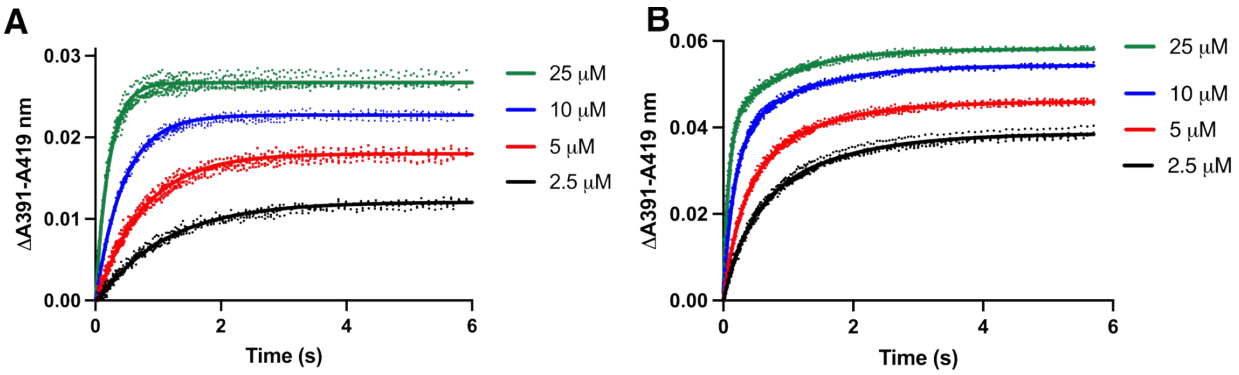
Supporting Figure 3. Series of stopped-flow traces for 11-deoxycorticosterone binding to CYP11B2 at four different substrate concentrations (A—2.5 μM, B—5 μM, C—10 μM, and D—25 μM) and four different adrenodoxin ratios (0, black; 1x, red; 10x, blue; 40x, green). Some data presented as representative data in Figure 3A and 3B is repeated here to facilitate comparisons within the data sets.



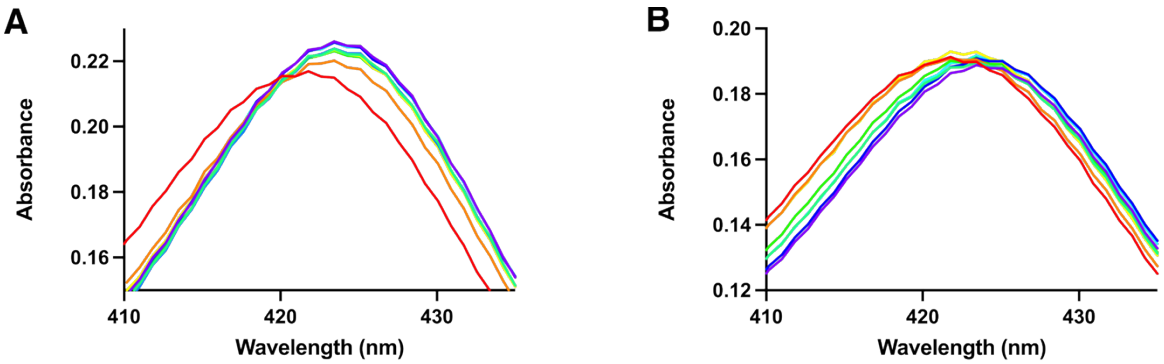
Supporting Figure 4. Series of stopped-flow traces for 11-deoxycorticosterone binding to CYP11B1 at four different substrate concentrations (A—5 μM, B—10 μM, C—17.5 μM, and D—25 μM) and four different adrenodoxin ratios (0, black; 1x, red; 10x, blue; 40x, green). Some data presented as representative data in Figure 4A and 4B is repeated here to facilitate comparisons within the data sets.



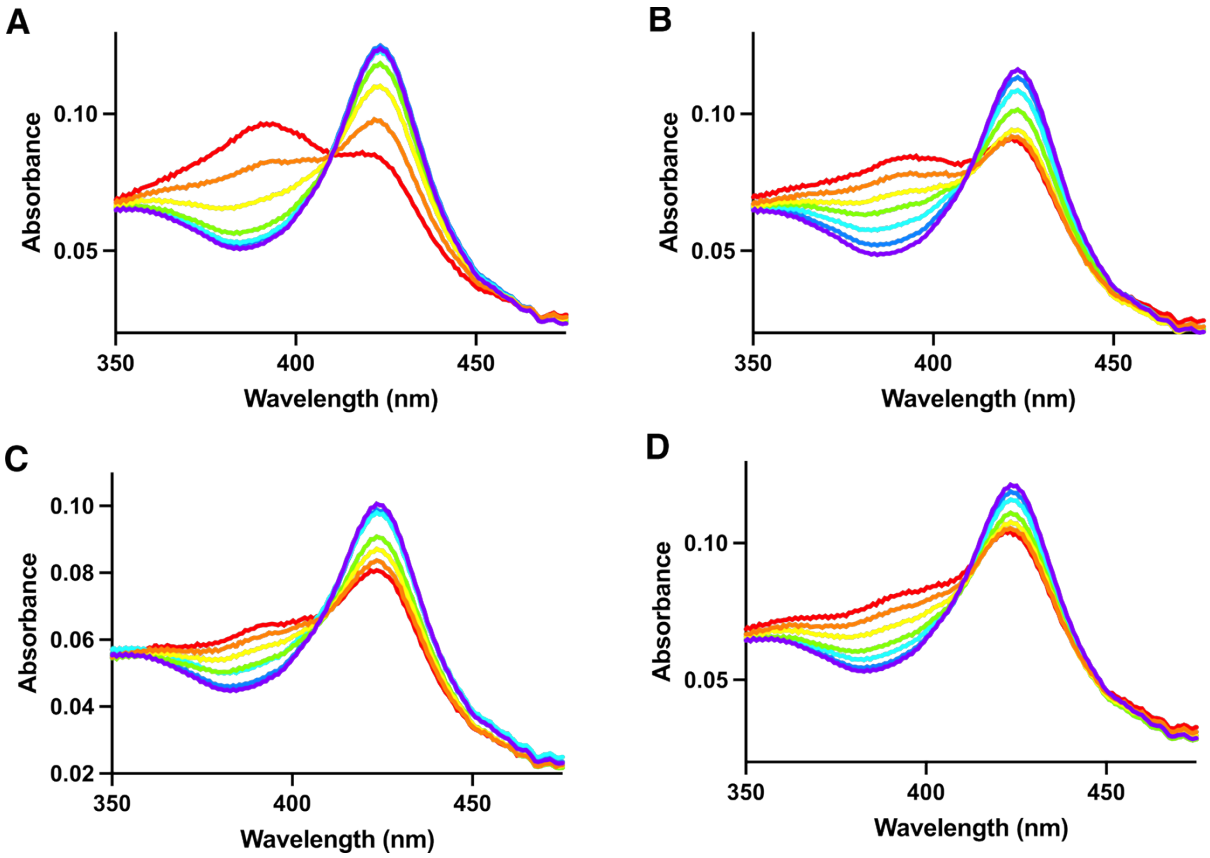
Supporting Figure 5. Series of stopped-flow traces for 11-deoxycortisol binding to CYP11B2 at four different substrate concentrations (A—5 μM, B—10 μM, C—17.5 μM, and D—25 μM) and four different adrenodoxin ratios (0, black; 1x, red; 10x, blue; 40x, green). Some data presented as representative data in Figure 5A and 5B is repeated here to facilitate comparisons within the data sets.



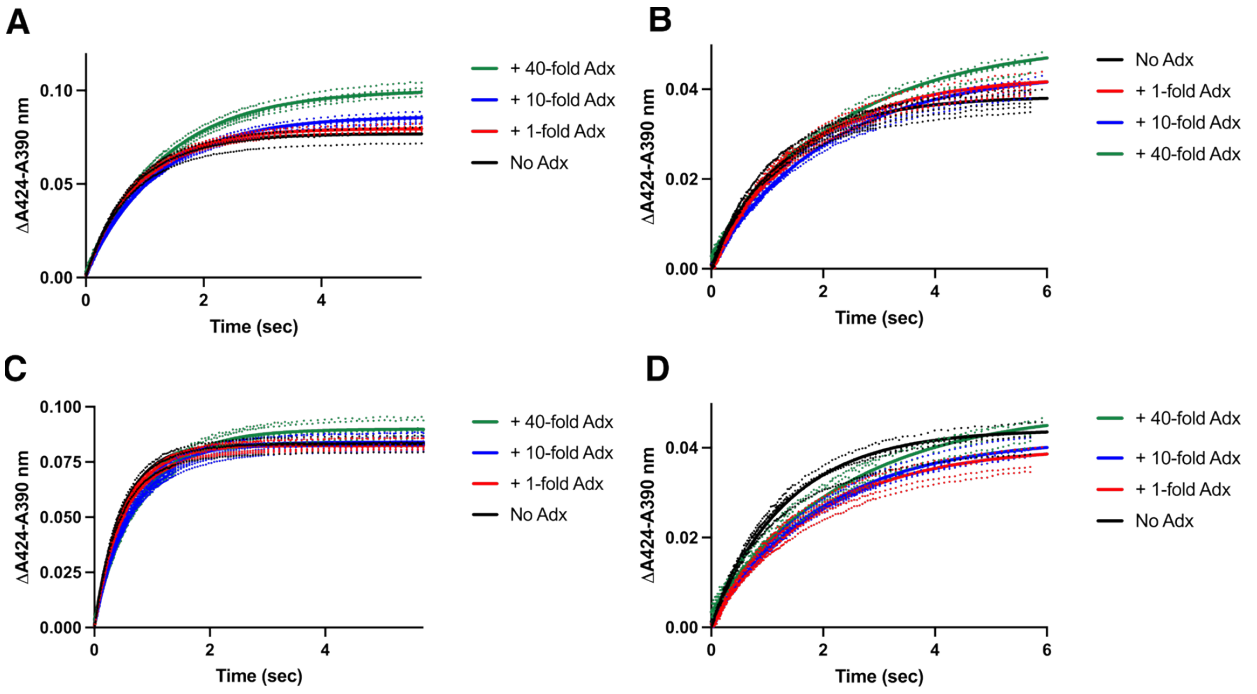
Supporting Figure 6. Series of stopped-flow traces for substrate binding to the adrenodoxin/CYP11B fusion proteins. Adrenodoxin/CYP11B1 fusion protein at four different 11-deoxycortisol concentrations (2.5 μM, black; 5 μM, red; 10 μM, blue; 25μM, green). Adrenodoxin/CYP11B2 fusion protein at four different 11-deoxycorticosterone concentrations (2.5 μM, black; 5 μM, red; 10 μM, blue; 25 μM, green).



Supporting Figure 7. Spectral shifts occurring as unliganded CYP11B1 and CYP11B2 (4 μM) were mixed the type II inhibitor LCI699 (100 μM). CYP11B1 (A) or CYP11B2 (B) mixed demonstrated a shift from 420 nm at the earliest time points (red) to 424 nm at the latest time points (blue, inhibitor bound).



Supporting Figure 8. Spectral shifts observed as CYP11B enzymes (2 µM) saturated with substrate (70 µM) are mixed 1:1 with 100 µM of the type II LCI699 inhibitor. CYP11B1 saturated with 11-deoxycortisol (A) or 11-deoxycorticosterone (B) and CYP11B2 saturated with 11-deoxycortisol (C) or 11-deoxycorticosterone (D) mixed with 100 µM of the type II inhibitor LCI699 all yielding absorbance decreases at 390 nm (substrate bound form) and increases at 424 nm (inhibitor bound form). Spectra are shown from the earliest time point with in red to the latest time point in purple.



Supporting Figure 9. Series of stopped-flow traces for CYP11B1 and CYP11B2 trapping experiments. CYP11B1 or CYP11B2 (2 μM) was saturated with 11-deoxycortisol or 11-deoxycorticosterone (70 μM) and varying amounts of adrenodoxin (0, 1-,10-, or 40-fold excess). The CYP11B/substrate/adrenodoxin was mixed with 100 μM LCI699 via stopped-flow and the change in absorbance from 390 to 424 nm was plotted over time and fit to the one-phase association equation. Traces are shown for (A) CYP11B1 and 11-deoxycortisol, (B) CYP11B2 and 11-deoxycorticosterone, (C) CYP11B1 and 11-deoxycorticosterone, and (D) CYP11B2 and 11-deoxycortisol. Each panel contains four different adrenodoxin concentrations (0, black; 1x, red; 10x, blue; 40x, green). While 15 seconds of total data were collected, only the first 6 seconds is displayed here as representative data.