1,2,3-Triazole-Heme Interactions in Cytochrome P450: Functionally Competent Triazole-Water-Heme Complexes

Supplemental Information

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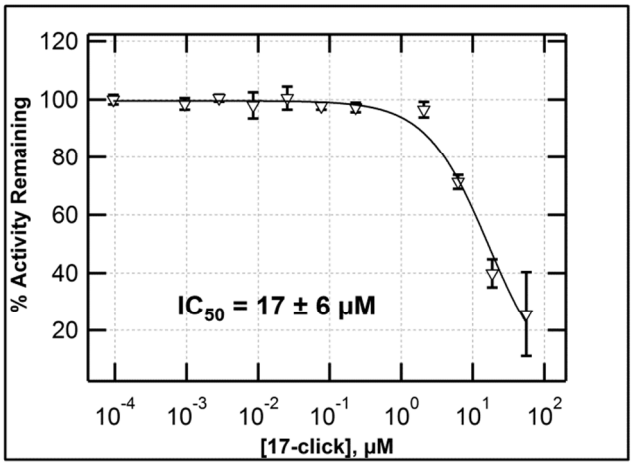
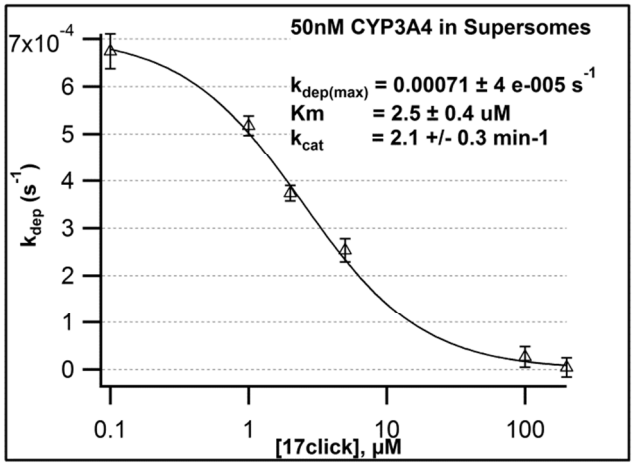
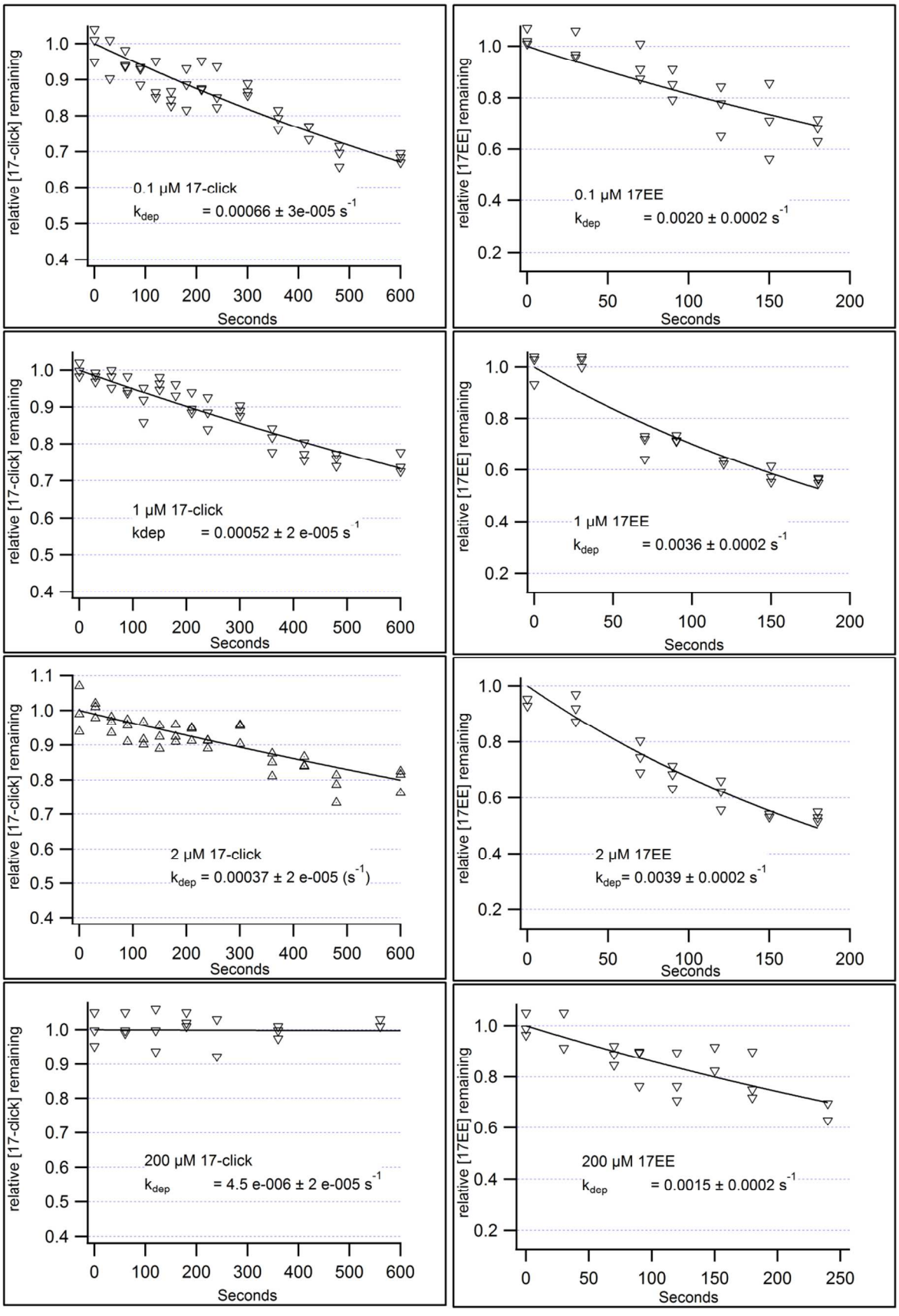
Supplemental Figure S1. IC_{50} measured for 17-click inhibition of CYP3A4-mediated 6β-OH testosterone formation in Supersomes. See Materials and Methods in main text for assay design and conditions.



Supplemental Figure S2. kinetic parameters K_M and V_{Max} for CYP3A4-mediated turnover of 17-click in Supersomes determined by LC-MS/MS assay using the methods of substrate depletion. Assay concentration of CYP3A4 was 50 nM based on 0.5 nmol / 0.5 mL CYP concentration provided by the manufacturer. See Materials and Methods section of the main text for experimental design and conditions.



Supplemental Figure S3. Substrate depletion of 17EE and 17-click in the presence of 50 nM CYP3A4 in Supersomes. Depletion of the 17-click (m/z = 340) and 17EE (dansylated derivative, m/z = 530) were monitored by LC-MS/MS assay as described in the Materials and Methods section of the main text. While accurate determination of kinetic parameters Km and Vmax were only obtained for 17-click (Supplemental Figure S2), comparison of depletion data obtained at low and high concentrations of 17EE suggest metabolism is between 3-10 fold faster for the type I substrate 17EE.


relative [17-click] remaining
Seconds
0.1 μM 17-click
k_{dep} = 0.00066 ± 3e-005 s^{-1}
relative [17EE] remaining
Seconds
0.1 μM 17EE
k_{dep} = 0.0020 ± 0.0002 s^{-1}
relative [17-click] remaining
Seconds
1 μM 17-click
kdep = 0.00052 ± 2 e-005 s^{-1}
relative [17EE] remaining
Seconds
1 μM 17EE
k_{dep} = 0.0036 ± 0.0002 s^{-1}
relative [17-click] remaining
Seconds
2 μM 17-click
k_{dep} = 0.00037 ± 2 e-005 (s^{-1})
relative [17EE] remaining
Seconds
2 μM 17EE
k_{dep}= 0.0039 ± 0.0002 s^{-1}
relative [17-click] remaining
Seconds
200 μM 17-click
k_{dep} = 4.5 e-006 ± 2 e-005 s^{-1}
relative [17EE] remaining
Seconds
200 μM 17EE
k_{dep} = 0.0015 ± 0.0002 s^{-1}

Supplemental Figure S4. qTOF MS/MS analysis of 17click metabolites M3 (Top), M2 (Middle), and M1 (Bottom). Similarity of fragment ion spectra to M4, (predominant fragment ions at m/z = 320, m/z = 292, and m/z = 157) suggests metabolism not occurring on steroid A-ring post 1,2,3-triazole fragment installation (see Results/Discussion).