Supplemental Information

P450cin Active Site Water: Implications for Substrate Binding and Solvent Accessibility

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Figure S1.S1a-c. Total GC-MS ion counts for selected time points in the extraction of cineole from the NADPH turnover assay for WT, Y81F, and N242A mutants plotted against retention time. The M/Z for fragmentation of cineole, internal standard and hydroxycineole are plotted in the Panel A inset.

Figure S2. The GC-MS peak areas from the total ion counts for cineole (6.30-6.35 minutes retention time) are divided by the peak areas for the internal standard, camphor (8.1-8.2 minute retention time) and labeled “R”. This ratio is plotted against known cineole concentrations to give the standard curve.

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RESI CNL 0.00
GROUP
ATOM  C1  CT1  0.5250  !
ATOM  C6  CT2 -0.1680  !
ATOM  H1  HA   0.0510  !
ATOM  H2  HA   0.0460  !
ATOM  C5  CT2 -0.0780  !
ATOM  H3  HA   0.0220  !
ATOM  H4  HA   0.0500  !
ATOM  C4  CT1 -0.1160  !
ATOM  H5  HA   0.0180  !
ATOM  C8  CT1  0.8400  !
ATOM  C10 CT3 -0.5640  !
ATOM  H6  HA   0.1440  !
ATOM  H7  HA   0.1090  !
ATOM  H8  HA   0.1280  !
ATOM  C9  CT3 -0.5620  !
ATOM  H9  HA   0.1430  !
ATOM  H10 HA   0.1080  !
ATOM  H11 HA   0.1280  !
ATOM  C3  CT2 -0.0790  !
ATOM  H12 HA   0.0220  !
ATOM  H13 HA   0.0500  !
ATOM  C2  CT2 -0.1680  !
ATOM  H14 HA   0.0460  !
ATOM  H15 HA   0.0510  !
ATOM  C7  CT3 -0.5790  !
ATOM  H16 HA   0.1540  !
ATOM  H17 HA   0.1540  !
ATOM  H18 HA   0.1290  !
ATOM  O1  OC3 -0.6030  !
BOND C1 C7  C1 C6  C1 C2  C1 O1
BOND C2 C3  C2 H14  C2 H15
BOND C3 C4  C3 H12  C3 H13
BOND C4 C5  C4 C8  C4 H5
BOND C8 O1  C8 C9  C8 C10
BOND C9 H6  C9 H7  C9 H8
BOND C10 H9  C10 H10  C10 H11
BOND C5 H4  C5 H3  C5 C6
BOND C6 H2  C6 H1
BOND C7 H16  C7 H17  C7 H18
PATCHING FIRS NONE LAST NONE

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```

BONDS
!
!V(bond) = Kb(b - b0)**2
!
!Kb: kcal/mole/A**2
!b0: A
!
!atom type Kb          b0
!
CT1 OC3      360.00      1.415  ! CIN BOND ETHER

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ANGLES
!
!V(angle) = Ktheta(Theta - Theta0)**2
!
!V(Urey-Bradley) = Kub(S - S0)**2
!
!Ktheta: kcal/mole/rad**2
!Theta0: degrees
!Kub: kcal/mole/A**2 (Urey-Bradley)
!S0: A
!
!atom types      Ktheta      Theta0      Kub      S0
!
OC3  CT1  CT3      45.000      109.00
OC3  CT1  CT1      45.000      105.00
OC3  CT1  CT2      45.000      109.00
CT1  OC3  CT1      50.000      111.50
! CNL 0
! CNL 0
! CNL 0
! CNL 0

IMPROPER
!
!V(improper) = Kpsi(psi - psi0)**2
!
!Kpsi: kcal/mole/rad**2
!psi0: degrees
!note that the second column of numbers (0) is ignored
!
!atom types      Kpsi      psi0
!
CC   CT1  CT2  0      55.00      0      0.00 ! SPECIAL IMPROPER TORSION FOR CINEOLE

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Figure S3. CHARMM compatible cineole forcefield.