Supplemental materials

Phenylalanine residues in the active site of CYP2E1 participate in determining the binding orientation and metabolism-dependent genotoxicity of aromatic compounds

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**Table S1.** Molecular docking of 1-MP to human CYP2E1 under rigid and flexible settings with regard to the cavity volume, binding energy, and ligand-heme distance

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PDB ID | Resolution | Rigid docking | | |  | PHEs flexible docking | | |
| Volume (Å3) | Binding energy score (kcal/mol) | Cα to Heme (Å) |  | Volume (Å3) | Binding energy score (kcal/mol) | Cα to Heme (Å) |
| 3LC4 | 3.10 | 101.93 | ‒8.24 | 4.4 |  | 101.59 | ‒10.99 | 3.8 |
| 3KOH | 2.90 | 110.40 | ‒9.57 | 3.8 |  | 111.90 | ‒10.71 | 4.0 |
| 3GPH | 2.70 | 94.83 | ‒9.30 | 3.2 |  | 82.77 | ‒10.92 | 4.2 |
| 3E4E | 2.60 | 69.22 | ‒7.56 | Outside\* |  | 78.35 | ‒10.73 | 3.8 |
| 3T3Z | 2.35 | 111.83 | ‒8.82 | 10.4 |  | 95.69 | ‒10.16 | 5.3 |
| 3E6I | 2.20 | 83.38 | ‒7.73 | Outside\* |  | 86.01 | ‒10.89 | 3.0 |
| F298A | - | - | - | - |  | - | ‒10.62 | 4.7 |
| F478A | - | - | - | - |  | - | ‒9.55 | 3.3 |

\* Compound was bound to the enzyme at some position outside of the active site.

**Table S2**. Features of high rank tunnels for the binding of 1-MP to the active center of human CYP2E1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compounds | Tunnels | Occurrence, % | Average bottleneck radius, Å | Maximum bottleneck radius, Å | Average tunnel length, Å |
| 1-MP | 2c | 97.6 | 1.31 ± 0.16 | 1.71 | 21.44 ± 1.73 |
|  | 2b | 74.6 | 1.22 ± 0.16 | 1.72 | 30.12 ± 2.57 |

**Table S3**. The energy decomposition of non-PHE amino acid residues in the active sites of 1-MP-bound wild-type and mutated human CYP2E1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Residues | CYP2E1-WT  kJ/mol | CYP2E1-F298A  kJ/mol | CYP2E1-F478A  kJ/mol | Energy Change |
| ILE115 | −3.18 ± 1.31 | −4.26 ± 0.71 | −5.36 ± 0.89 | ↓ |
| ASN206 | −0.33 ± 0.10 | −6.52 ± 0.98 | −1.44 ± 0.32 | ↓ |
| LEU210 | −0.37 ± 0.16 | 0.16 ± 1.11 | −2.01 ± 0.66 | ↓ |
| ALA299 | −4.16 ± 0.86 | −3.17 ± 1.29 | −2.69 ± 1.27 | ↑ |
| GLU302 | −2.62 ± 1.30 | −3.23 ± 1.21 | −0.94 ± 0.35 | ↑ |
| THR303 | −4.94 ± 1.12 | −0.33 ± 0.09 | −1.74 ± 1.72 | ↑ |
| LEU363 | −2.47 ± 1.04 | −0.95 ± 0.20 | −1.22 ± 0.64 | ↑ |
| VAL364 | −2.85 ± 1.70 | −3.47 ± 0.70 | −3.19 ± 0.91 | ↓ |
| LEU368 | −2.68 ± 0.58 | 1.25 ± 1.08 | −6.20 ± 1.06 | ↓ |

**Table S4.** The molecular descriptors of PCB congeners as candidate factors influencing their binding to human CYP2E1 and orientation

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PCB | MutaG (1-4) | CN | OP | MP | PP | DBES | DtF | L\_HLG | L\_EP | L\_NP | P\_HLG | P\_EP | P\_NP | FODw | FODm | FODp | MW | AHR | Kow | HLF |
| 1 | 0 | 1 | 1 | 0 | 0 | -7.00 | 3.2 | 7.67 | 1.18 | 1.66 | 7.36 | 1.22 | 1.86 | 0.102 | 0.106 | 0.098 | 188.65 | 28.5 | 4.47 | 7.1 |
| 2 | 0 | 1 | 0 | 1 | 0 | -7.23 | 3.9 | 7.17 | 1.32 | 1.86 | 6.92 | 1.37 | 2.00 | 0.146 | 0.163 | 0.102 | 188.65 | 52.3 | 4.64 | 20.2 |
| 3 | 0 | 1 | 0 | 0 | 1 | -5.40 | 10.0 | 7.06 | 1.29 | 1.99 | 7.00 | 1.27 | 2.08 | 0.108 | 0.114 | 0.127 | 188.65 | 38.5 | 4.61 | 35.3 |
| 4 | 1 | 2 | 2 | 0 | 0 | -7.56 | 3.2 | 7.82 | 1.23 | 1.45 | 7.49 | 1.27 | 1.69 | 0.224 | 0.103 | 0.108 | 223.10 | 20.1 | 4.95 | 185.5 |
| 5 | 1 | 2 | 1 | 1 | 0 | -7.71 | 3.9 | 7.74 | 1.31 | 1.49 | 7.28 | 1.32 | 1.77 | 0.165 | 0.144 | 0.120 | 223.10 | 29.3 | 5.02 | 141.4 |
| 6 | 1 | 2 | 1 | 1 | 0 | -7.83 | 3.8 | 7.21 | 1.40 | 1.70 | 7.34 | 1.33 | 1.71 | 0.164 | 0.176 | 0.107 | 223.10 | 30.1 | 5.02 | 185.3 |
| 7 | 0 | 2 | 1 | 0 | 1 | -7.32 | 3.9 | 7.41 | 1.29 | 1.69 | 7.24 | 1.31 | 1.80 | 0.231 | 0.168 | 0.112 | 223.10 | 26.3 | 5.13 | 42.4 |
| 8 | 1 | 2 | 1 | 0 | 1 | -7.19 | 3.9 | 7.42 | 1.28 | 1.71 | 7.20 | 1.31 | 1.84 | 0.149 | 0.212 | 0.098 | 223.10 | 28.4 | 5.09 | 42.8 |
| 9 | 0 | 2 | 1 | 1 | 0 | -7.85 | 3.7 | 7.41 | 1.33 | 1.65 | 7.17 | 1.32 | 1.87 | 0.136 | 0.160 | 0.121 | 223.10 | 30.6 | 5.13 | 239.1 |
| 10 | 1 | 2 | 2 | 0 | 0 | -7.37 | 4.1 | 8.14 | 1.06 | 1.25 | 7.47 | 1.24 | 1.73 | 0.155 | 0.102 | 0.122 | 223.10 | 28.7 | 4.99 | 185.6 |
| 11 | 1 | 2 | 0 | 2 | 0 | -7.49 | 4.6 | 7.03 | 1.47 | 1.76 | 6.99 | 1.45 | 1.83 | 0.164 | 0.172 | 0.104 | 223.10 | 40.5 | 5.28 | 28.5 |
| 12 | 1 | 2 | 0 | 1 | 1 | -7.39 | 4.0 | 6.94 | 1.41 | 1.90 | 6.85 | 1.40 | 2.01 | 0.130 | 0.133 | 0.141 | 223.10 | 31.0 | 5.29 | 48.8 |
| 13 | 0 | 2 | 0 | 1 | 1 | -5.83 | 10.0 | 6.74 | 1.49 | 1.97 | 6.89 | 1.42 | 1.95 | 0.162 | 0.160 | 0.133 | 223.10 | 30.3 | 5.16 | 63.5 |
| 14 | 0 | 2 | 0 | 2 | 0 | -5.97 | 10.0 | 6.78 | 1.61 | 1.84 | 7.02 | 1.46 | 1.80 | 0.126 | 0.137 | 0.127 | 223.10 | 41.3 | 5.41 | 12.9 |
| 15 | 0 | 2 | 0 | 0 | 2 | -7.33 | 8.5 | 6.74 | 1.42 | 2.06 | 6.80 | 1.36 | 2.10 | 0.168 | 0.134 | 0.139 | 223.10 | 20.2 | 5.26 | 12.5 |
| 18 | 1 | 3 | 2 | 1 | 0 | -8.32 | 3.7 | 7.68 | 1.30 | 1.45 | 7.52 | 1.27 | 1.64 | 0.170 | 0.188 | 0.131 | 257.54 | 11.2 | 5.52 | 74.3 |
| 19 | 1 | 3 | 3 | 0 | 0 | -7.65 | 3.0 | 8.04 | 1.18 | 1.29 | 7.48 | 1.18 | 1.81 | 0.118 | 0.130 | 0.114 | 257.54 | 11.2 | 5.48 | 315.2 |
| 20 | 1 | 3 | 1 | 2 | 0 | -8.09 | 4.5 | 7.32 | 1.47 | 1.55 | 7.26 | 1.40 | 1.66 | 0.188 | 0.187 | 0.118 | 257.54 | 11.2 | 5.57 | 122.0 |
| 22 | 1 | 3 | 1 | 1 | 1 | -7.85 | 4.5 | 7.28 | 1.39 | 1.65 | 7.16 | 1.36 | 1.81 | 0.179 | 0.168 | 0.115 | 257.54 | 11.3 | 5.44 | 144.2 |
| 27 | 1 | 3 | 2 | 1 | 0 | -7.87 | 4.5 | 8.03 | 1.17 | 1.28 | 7.41 | 1.31 | 1.68 | 0.179 | 0.175 | 0.114 | 257.54 | 11.2 | 5.64 | 97.7 |
| 28 | 1 | 3 | 1 | 0 | 2 | -7.76 | 5.8 | 7.14 | 1.39 | 1.75 | 7.02 | 1.39 | 1.86 | 0.148 | 0.145 | 0.127 | 257.54 | 11.0 | 5.62 | 75.9 |
| 32 | 1 | 3 | 2 | 0 | 1 | -7.26 | 3.7 | 7.98 | 1.18 | 1.33 | 7.41 | 1.32 | 1.65 | 0.165 | 0.153 | 0.116 | 257.54 | 1.12 | 5.75 | 274.2 |
| 40 | 0 | 4 | 2 | 2 | 0 | -8.56 | 4.5 | 7.91 | 1.34 | 1.20 | 7.45 | 1.29 | 1.66 | 0.159 | 0.155 | 0.133 | 291.98 | 7.68 | 6.04 | 102.6 |
| 46 | 0 | 4 | 3 | 1 | 0 | -8.35 | 4.4 | 7.96 | 1.31 | 1.21 | 7.44 | 1.29 | 1.76 | 0.147 | 0.182 | 0.132 | 291.98 | 7.64 | 6.18 | 153.5 |
| 52 | 1 | 4 | 2 | 2 | 0 | -6.37 | 10.0 | 7.56 | 1.42 | 1.39 | 7.44 | 1.36 | 1.59 | 0.207 | 0.190 | 0.162 | 291.98 | 7.92 | 6.18 | 262.4 |
| 54 | 0 | 4 | 4 | 0 | 0 | -5.60 | 10.0 | 8.06 | 1.24 | 1.22 | 7.54 | 1.20 | 1.67 | 0.171 | 0.148 | 0.134 | 291.98 | 7.44 | 5.94 | 153.3 |
| 56 | 1 | 4 | 1 | 2 | 1 | -8.27 | 4.5 | 7.37 | 1.45 | 1.48 | 7.16 | 1.48 | 1.63 | 0.197 | 0.198 | 0.134 | 291.98 | 8.71 | 6.17 | 295.5 |
| 66 | 1 | 4 | 1 | 1 | 2 | -8.60 | 5.6 | 7.09 | 1.49 | 1.64 | 7.02 | 1.47 | 1.75 | 0.243 | 0.177 | 0.139 | 291.98 | 9.12 | 6.11 | 119.6 |
| 74 | 1 | 4 | 2 | 1 | 1 | -8.11 | 3.8 | 7.71 | 1.36 | 1.37 | 7.51 | 1.32 | 1.66 | 0.169 | 0.201 | 0.141 | 291.98 | 9.13 | 6.66 | 346.9 |
| 77 | 0 | 4 | 0 | 2 | 2 | -8.62 | 8.3 | 6.55 | 1.68 | 1.87 | 6.71 | 1.56 | 1.90 | 0.253 | 0.250 | 0.171 | 291.98 | 10.1 | 6.62 | 22.7 |
| 81 | 0 | 4 | 0 | 2 | 2 | -6.39 | 10.0 | 6.51 | 1.70 | 1.88 | 6.72 | 1.57 | 1.87 | 0.189 | 0.200 | 0.176 | 291.98 | 10.1 | 6.15 | 48.8 |
| 105 | 1 | 5 | 1 | 2 | 2 | -8.50 | 5.3 | 7.08 | 1.57 | 1.55 | 7.02 | 1.54 | 1.66 | 0.283 | 0.245 | 0.160 | 326.42 | 6.49 | 6.79 | 115.7 |
| 118 | 1 | 5 | 1 | 2 | 2 | -8.67 | 5.4 | 7.40 | 1.54 | 1.31 | 6.89 | 1.53 | 1.75 | 0.250 | 0.243 | 0.164 | 326.42 | 6.41 | 7.11 | 154.7 |
| 126 | 0 | 5 | 0 | 3 | 2 | -8.71 | 7.3 | 6.50 | 1.80 | 1.75 | 6.64 | 1.66 | 1.81 | 0.274 | 0.273 | 0.204 | 326.42 | 6.63 | 6.56 | 250.4 |

MutaG, human CYP2E1-activated mutagenicity of a PCB congener (1-4); CN, the number of Cl-substitutions; OP, the number of ortho-Cl-substitutions; MP, the number of meta-Cl-substitutions; PP, the number of para-Cl-substitutions; DBES, binding energy score obtained from molecular docking with PHE478 being set flexible; DtF, the distance from SOM to Fe ion in the heme; L\_HLG,the HOMO-LUMO gap of ligand; L\_EP, the electrophilicity of ligand; L\_NP, the nucleophilicity of ligand; P\_HLG,the HOMO-LUMO gap of ligand-PHE complex; P\_EP, the electrophilicity of ligand-PHE complex; P\_NP, the nucleophilicity of ligand-PHE complex; FODw, the FOD value of ligand bound to the active site of human CYP2E1; FODm, the FOD value of ligand bound to the active site of F478A mutant; FODp, the FOD value of ligand; Mass, the molecular weight; AHR, atmospheric hydroxylation rate (cm3/molecule\*sec); Kow, LogKow: Octanol-Water partition coefficient; HLF, the half-life (by biotransformation and elimination) in fish.



**Figure S1.** Two major tunnels for ligands entering the active site in human CYP2E1

Tunnel 1 indicates the 2b tunnel, which is controlled by PHE478; Tunnel 2 indicates the 2c tunnel, as controlled by PHE298.

References

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