Description of Additional Supplementary Files

File name- Supplemental Data 1

File description – Numerical data used to generate the plots in Figs. 2–8

File name- Supplemental Data 2

File description – Numerical data used to generate the plots in Supplementary Figs. 2–8.

File name- Supplemental Data 3

File description – Numerical data used to generate the plots in Supplementary Figs. 9–14

File name- Supplemental Movie 1

File description – Unbiased simulation of CYP3A4–DHB binding in trajectory 0-1-3. DHB and the heme group are shown in cyan. The F–F' loop is colored green, with residues R212 and L216 displayed in licorice representation; part of the B–C loop is shown in magenta. From the beginning of the movie to 11 seconds, the DHB molecule diffuses randomly around the protein surface. Between 12 and 28 seconds, DHB becomes stabilized at the entrance of channel 2b. After 29 seconds, the FF' loop shifts upward, accompanied by an upward movement of the R212 side chain, enabling DHB to undergo a conformational change, enter the active site, and become stably bound.

File name- Supplemental Movie 2

File description – Unbiased simulation of CYP3A4–DHB binding in trajectory 0-2-4. From the beginning to approximately 20 seconds, DHB resides at the entrance of channel 2a, while the F–F' loop remains in a downward-facing conformation, with occasional upward fluctuations of the R212 and L216 side chains. At around 23 seconds, the F–F' loop begins shifting upward, although R212 still points downward. Between 31 and 34 seconds, DHB attempts to enter the active site, but its entry is hindered by the downward-facing orientation of R212 and L216. After 37 seconds, the F–F' loop fully transitions to an upward conformation, with both R212 and L216 side chains oriented upward, allowing DHB to enter and become stabilized within the CYP3A4 active site

File name- Supplemental Movie 3

File description – Unbiased simulation of DHB conformational change in CYP3A4 (trajectory 3-5). DHB and the heme group are shown in cyan. The F–F' loop is colored green, with residues R212, F215, and L216 displayed in licorice representation; part of the B–C loop is shown in magenta. At the beginning of the movie, the F–F' loop adopts an upward-facing conformation. Around 8 seconds, the loop partially shifts downward, with the side chains of R212, F215 (most notably), and L216 oriented downward. From 30 seconds to the end of the movie, the psoralen moiety of DHB faces the heme and fluctuates around this conformation, suggesting a dynamic interaction with the active site.

File name- Supplemental Movie 4

File description – Unbiased simulation of DHB conformational change in CYP3A4 (trajectory 3-6). At the beginning of the movie, the F–F' loop adopts an upward-facing conformation. After 12 seconds, the loop partially shifts downward, with the side chain of F215 notably oriented downward. From 32 seconds to the end of the movie, the psoralen moiety of DHB faces the heme and forms a parallel ππ stacking interaction with the heme group.

File name- Supplemental Movie 5

File description - Unbiased simulation of dual-substrate binding in CYP3A4 (trajectory 3-7). The heme group is shown in cyan. The first DHB molecule (DHB1) is displayed in pink, and the second (DHB2) in green. The F–F' loop is colored green, with residues F213, L216, and P218 shown in licorice representation; part of the B–C loop is shown in magenta. At the beginning of the movie, DHB1 is stably bound in the active site. Starting at 6 seconds, DHB2 begins to enter the active site via the pink tunnel and is eventually captured within the active site. From 22 seconds onward, the FF' loop adopts a partially downward-facing conformation with minor fluctuations, and the side chains of F213, L216, and P218 are oriented downward.

File name- Supplemental Movie 6

File description - Unbiased simulation of dual-substrate binding in CYP3A4 (trajectory 4-8). The FF' loop is colored green, with residue L216 shown in licorice representation; part of the B–C loop is colored magenta. At 4 seconds, DHB1 enters the active site, followed by DHB2 entering via the pink tunnel within the next 2 seconds. Afterward, the dual-substrate conformation remains stable through the end of the movie. Similarly, starting at 5 seconds, the F–F' loop begins to move downward, with the side chain of L216 oriented downward; this conformation is maintained for the remainder of the simulation.