Supporting Information

Membrane-embedded substrate recognition by cytochrome P450 3A4

John C Hackett

Department of Physiology and Biophysics and the Massey Cancer Center, School of Medicine, Virginia Commonwealth University, Richmond, Virginia 23298-0035

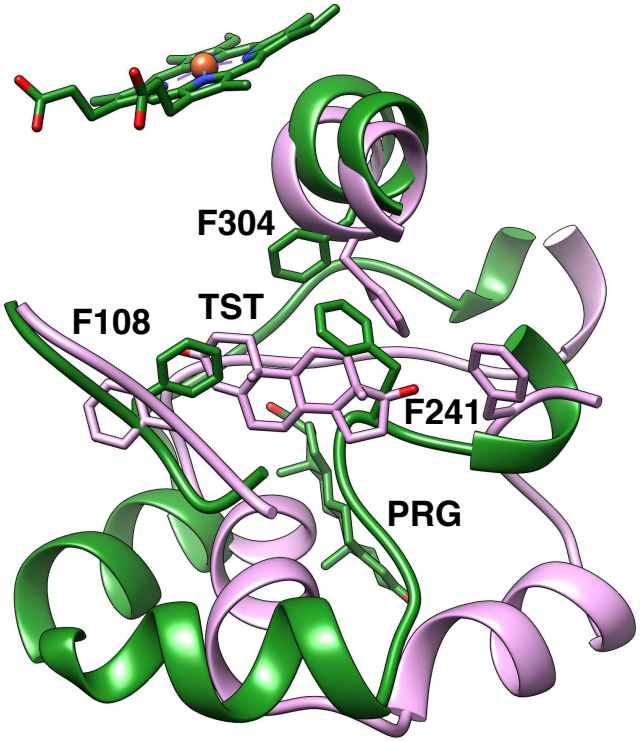

Figure S1. Superimposition of regions of the B-C loop, F'-, G', and I-helices from a representative trajectory snapshot from the intermediate bound state (pink) and the crystal structure of the CYP3A4-PRG complex (PDB ID: 5A1R). (1) The structures were overlaid using UCSF Chimera.(2)

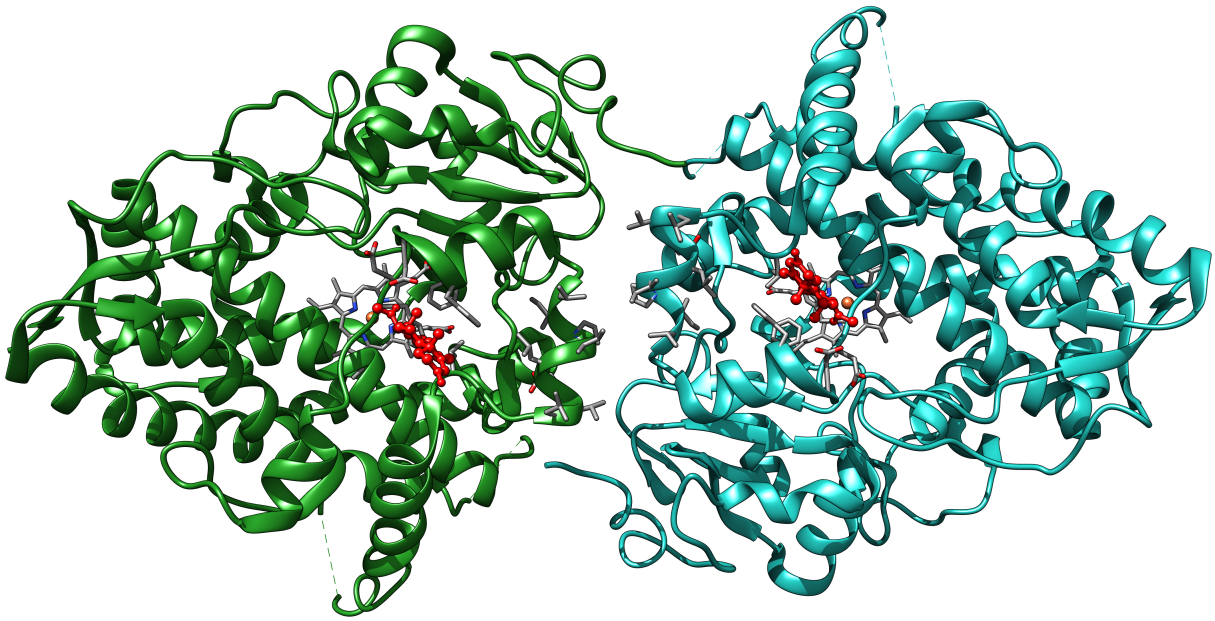

Figure S2. Structure of the CYP3A4-PRG dimer illustrating the interactions between the G'-helices and the position of progesterone (PRG). Coordinates were extracted from PDB ID 5A1R. (1)

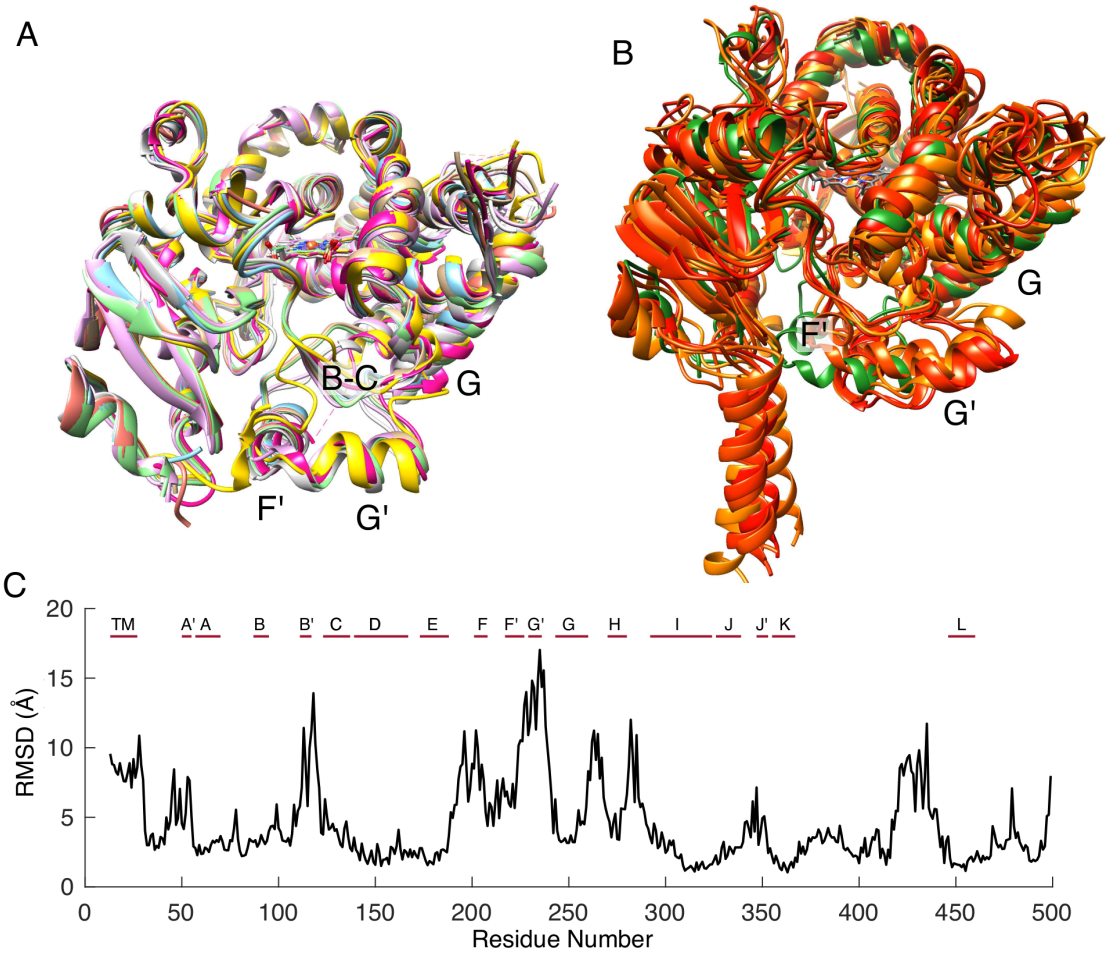

Figure S3. Comparisons between the structural deviations in CYP3A4 crystal structures(1,3-8) and the fluctuations observed in a 1 μs aMD simulation support that the structural changes observed in the simulations are independent of the crystal structure used to build the initial model. The greatest mobility is observed in the F'-G'-G region that simulations predict is critical for extracting TST from the membrane. The extensive conformational changes observed in this region in the simulations far exceed those observed when comparing available crystal structures. (A) Superimposition of CYP3A4 crystal structures with various ligands and their backbone RMSD values relative to the ligand-free 1TQN structure. Metformin (5G5J, light blue, 0.61 Å), Ritonavir (5VC0, pink, 1.55 Å), Bromocriptine (5VCG, green, 0.56 Å), Progesterone (5A1R, orange, 0.64 Å); Ketoconazole (2V0M, silver, 1.69 Å), Erythromycin (2J0D, magenta, 1.21 Å), and Midazolam (5TE8, yellow, 1.88 Å). The RMSDs for the F-F'-G'-G region (residues 200-260) that undergo the most remarkable shifts in the simulations have RMSDs of 0.72 Å (5G5J, metformin) to 3.94 Å (5TE8, midazolam). (B) Representative snapshots from the top 5 most populated clusters determined by the NMRCLUST algorithm(9) are illustrated in shades of red to orange. These are superimposed onto the crystal onto the 1TQN crystal illustrated in green. The RMSDs for the F-F'-G'-G regions range from 6.83 Å to 10.15 Å relatives to the 1TQN structure. All structural comparisons were performed with USCF Chimera.(2)(C) Average RMSD per residue over the 1 μs simulation where TST was observed to bind in the active site.

Supplemental References

1. Sevrioukova, I. F., and Poulos, T. L. (2015) Anion-Dependent Stimulation of CYP3A4 Monooxygenase. *Biochemistry* **54**, 4083-4096
2. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera--a visualization system for exploratory research and analysis. *J .Comput .Chem.* **25**, 1605-1612
3. Yano, J. K., Wester, M. R., Schoch, G. A., Griffin, K. J., Stout, C. D., and Johnson, E. F. (2004) The structure of human microsomal cytochrome P450 3A4 determined by X-ray crystallography to 2.05-A resolution. *J. Biol. Chem.* **279**, 38091-38094
4. Ekroos, M., and Sjogren, T. (2006) Structural basis for ligand promiscuity in cytochrome P450 3A4. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 13682-13687
5. Sevrioukova, I. F., and Poulos, T. L. (2010) Structure and mechanism of the complex between cytochrome P4503A4 and ritonavir. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 18422-18427
6. Sevrioukova, I. F., and Poulos, T. L. (2012) Structural and mechanistic insights into the interaction of cytochrome P4503A4 with bromoergocryptine, a type I ligand. *J. Biol. Chem.* **287**, 3510-3517
7. Guo, Z., Sevrioukova, I. F., Denisov, I. G., Zhang, X., Chiu, T. L., Thomas, D. G., Hanse, E. A., Cuellar, R. A. D., Grinkova, Y. V., Langenfeld, V. W., Swedien, D. S., Stamschror, J. D., Alvarez, J., Luna, F., Galvan, A., Bae, Y. K., Wulfkuhle, J. D., Gallagher, R. I., Petricoin, E. F. R., Norris, B., Flory, C. M., Schumacher, R. J., O'Sullivan, M. G., Cao, Q., Chu, H., Lipscomb, J. D., Atkins, W. M., Gupta, K., Kelekar, A., Blair, I. A., Capdevila, J. H., Falck, J. R., Sligar, S. G., Poulos, T. L., Georg, G. I., Ambrose, E., and Potter, D. A. (2017) Heme Binding Biguanides Target Cytochrome P450-Dependent Cancer Cell Mitochondria. *Cell Chem. Biol.* **24**, 1259-1275 e1256
8. Sevrioukova, I. F., and Poulos, T. L. (2017) Structural basis for regiospecific midazolam oxidation by human cytochrome P450 3A4. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 486-491
9. Kelley, L. A., Gardner, S. P., and Sutcliffe, M. J. (1996) An automated approach for clustering an ensemble of NMR-derived protein structures into conformationally related subfamilies. *Protein Eng.* **9**, 1063-1065