General Audience Report

Project title
Reactive Metabolites from Drugs: Generation and Impact Prediction by Computational Biophysical Chemistry

HPC System(s) and corresponding centre(s)
JUWELS Booster Module (Ampere A100 GPUs)

Project ID
CYP450

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Of 57 human Cytochrome P450 (CYP) enzymes, twelve metabolize 90% of xenobiotics. Previous work based on crystal structures and computational studies stressed the importance of the F/G-region, consisting of the F-, F'-, G'-, and G-helices, for substrate binding because the region is at the beginning of a tunnel that is considered the substrate entrance channel. Such tunnels can act as filters and have been found to influence both substrate specificity and catalytic mechanism. Enzyme structural dynamics, besides its role in catalysis and allosteric regulation, has also been recognized for other systems as an important mechanism by which promiscuity can be achieved. Surprisingly, no further studies addressed the relation between enzyme dynamics and promiscuity of more than three CYP isoforms comparatively. Furthermore, the approaches did not provide a quantitative relation between substrate scope and structural rigidity of CYP isoforms involved in metabolism.

In this project, Prof. Dr. Holger Gohlke and Daniel Becker show for the largest data set of CYP isoforms investigated in this context that the structural rigidity of the F/G-region is inversely correlated to the enzymes' substrate scope.

The team generated comparative models of the investigated CYP isoforms with TopModel. The globular part and the transmembrane helix were modeled separately, and their positions on or in the membrane were predicted with the CCTop webserver. Afterward, they were docked together using the predicted membrane positions as a spatial restraint. The quality of the models was assessed with TopScoreSingle.

To improve the robustness of the analyses and quantify the statistical uncertainty of the results, the team carried out constraint network analysis (CNA) on ensembles of network topologies generated by molecular dynamics simulations: The generated structural models were embedded by PACKMOL-Memgen into a membrane with a composition similar to the main lipid components of the human endoplasmic reticulum. The GPU particle mesh Ewald implementation of the AMBER18 molecular simulations suite was used to perform the simulations. The large size of the systems, reaching 200,000 atoms, in conjunction with the outstandingly long simulation time of in total more than 1.5 millisecond, required the efficient use of Graphics Processing Units (GPUs) on the JUWELS Booster system.

Exemplarily, constraint dilution trajectories of CYP3A4, CYP3A5, CYP2C8, and CYP1A2 are shown in Figure 1. Differences in the structural stability of the F/G-region were observed that do not only occur between sequentially different CYP isoforms but also for sequentially close ones as exemplarily depicted for CYP3A4 and CYP3A5 (sequence identity (similarity) 84% (90%)). In all CYP isoforms exemplarily shown, the F/G-region is most weakly coupled to the remainder of the globular domain. Between both sequentially different isoforms and sequentially close ones, differences in the structural stability of this region are revealed by rigidity analysis. These differences qualitatively relate to the substrate scope of the isoforms: more promiscuous isoforms such as CYP3A4 show less structurally stable F/G-regions and *vice versa*. To quantify the relation between substrate scope and structural rigidity, the team computed the average chemical potential of rigid contacts in the F/G-region ($\bar{E}_{\mathrm{FG,CNA}}$) as a measure for how well residues in the F/G-region form rigid contacts and correlated it to the promiscuity index I_{cat} introduced by Nath and Atkins. The index is normalized and ranges from 0 (specific) to 1 (promiscuous). A good and significant inverse correlation (R^2 = 0.85 and p < 0.01, Wald test) between the isoform promiscuity based on catalytic efficiency (e) and the structural stability of the F/G-region was obtained for eight CYP isoforms for which e values are available in the literature.

In summary, the team's results signify that characterizing the structural rigidity of the F/G-region can be used to classify CYP isoforms involved in metabolism with respect to their substrate scope.

This model may allow predicting the substrate promiscuity of novel CYP enzymes, e.g., found in meta-genome approaches for potential use in bioorganic chemistry or biotechnology.

Teaser

Prof. Dr. Holger Gohlke and Daniel Becker studied human Cytochrome P450 (CYP) enzymes, focusing on the F/G-region's role in substrate specificity. They found that this region acts as a filter, influencing promiscuity. Enzyme dynamics play a crucial role in achieving promiscuity. The team generated comparative models of CYP isoforms and used constraint network analysis to analyze their stability. They discovered an inverse correlation between the structural rigidity of the F/G-region and substrate scope. These differences were observed not only between distinct CYP isoforms but also between closely related ones. The team quantified this relationship and established a link between structural stability and promiscuity. This knowledge can classify CYP isoforms and predict substrate promiscuity in novel CYP enzymes, with potential applications in bioorganic chemistry and biotechnology, including metagenomics approaches.

In the granting period, the following publication was published:

Becker, D., Bharatam, P.V., Gohlke, H., F/G-region rigidity is inversely correlated to substrate promiscuity of human CYP isoforms involved in metabolism. **J. Chem. Inf. Model. 2021**, 61, 4023–4030.

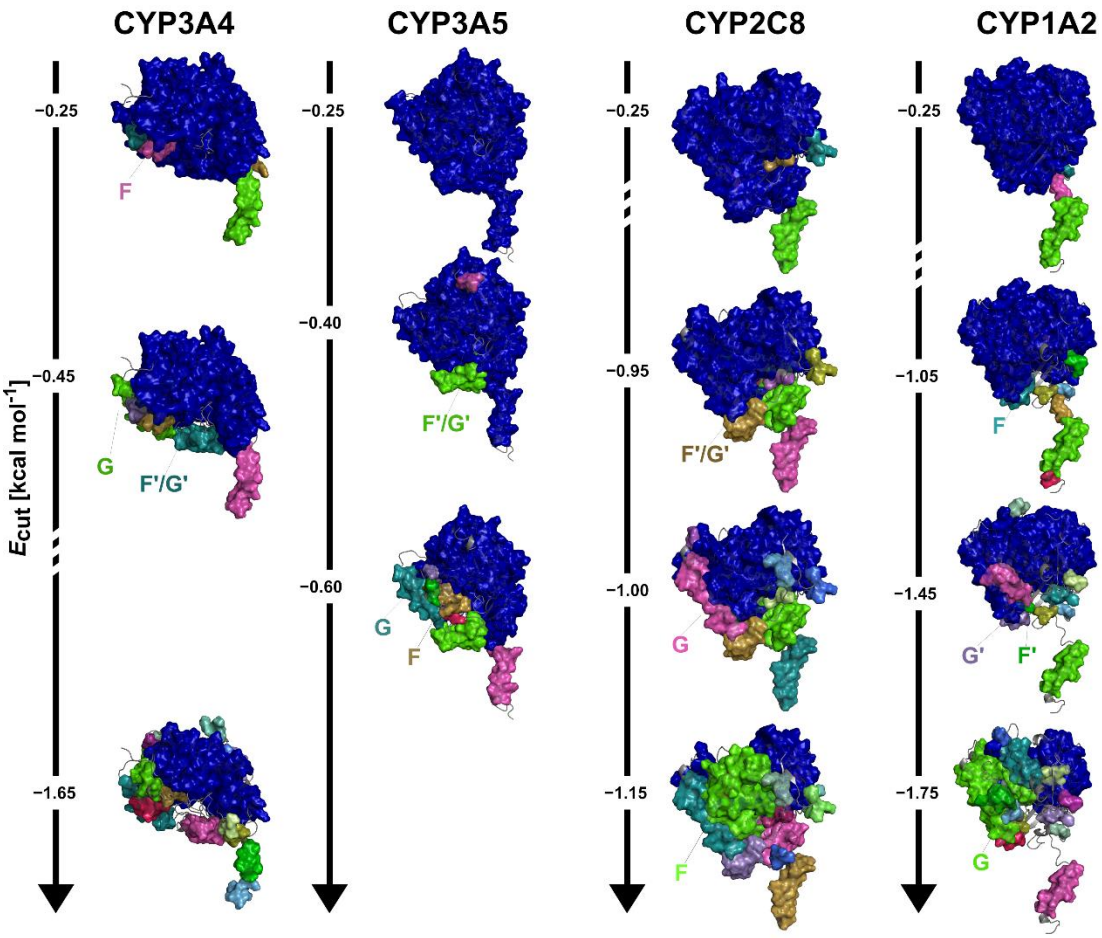

Figure 1: Constraint network analysis of CYP3A4, CYP3A5, CYP2C8, and CYP1A2 and rigid cluster decompositions along constraint dilution trajectories. Rigid clusters of the CYP isoforms at different E_{cut} values are colored blue, green, pink, and cyan in descending order of their size. The examples qualitatively depict that the F/G-region of more promiscuous CYP isoforms, such as CYP3A4 and CYP3A5, are less structurally stable than that of more specific isoforms. Furthermore, differences in structural stability are revealed for sequentially close isoforms. The rigid clusters are calculated based on the neighbor stability map of one exemplary MD trajectory, respectively. Figure taken from ref. **J. Chem. Inf. Model. 2021**, 61, 4023–4030.