Individual variability in drug metabolism by human FMO3

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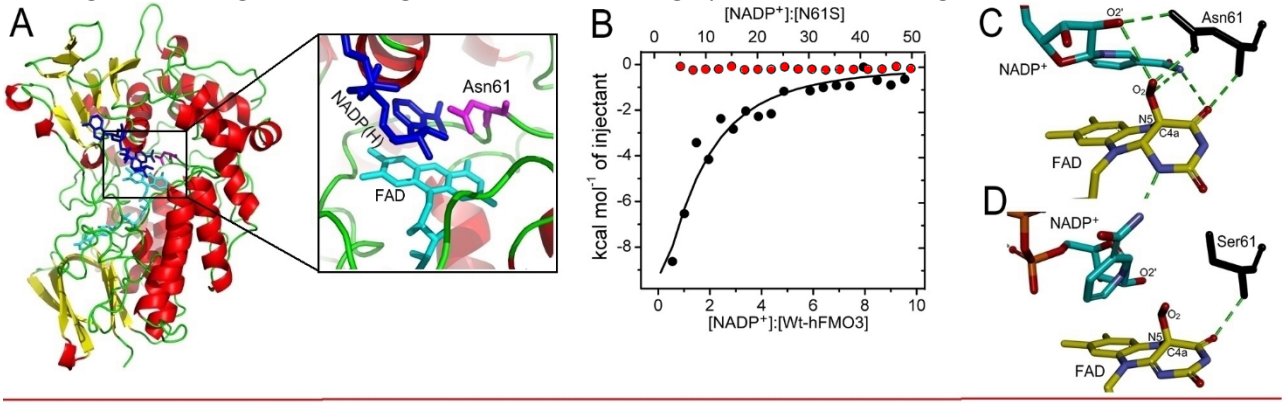
Background: Human flavin-containing monooxygenase isoform 3 (FMO3) is the second most important hepatic monooxygenase, catalyzing the metabolism of drugs, dietary compounds and other xenobiotics. Previous studies have also demonstrated that FMO3 is associated with both fish-odor syndrome (TMAU) and atherosclerosis. In this thesis, FMO3 is investigated both at molecular/functional level as well as possible industrial applications in drug metabolite production.

Objectives: (1) To investigate the molecular basis of the TMAU-causing N61S mutant and other mutations at this site; (2) Construction of an efficient whole-cell biocatalyst for production of human drug metabolites.

Achievements: FMO3 model was constructed by homology modeling [1], showing that Asn61 was located inside the catalytic center (Fig. 1A). ITC data show that N61S mutation results in poor $NADP^+$ binding affinity of FMO3 (Fig. 1B), and disordered binding mode (Fig. 1D), which is unfavorable for the stability of its catalytic intermediate. The unstable intermediate leads to defective TMA *N*-oxygenation and cause TMAU [2, 3]. While for its industrial application, whole-cell catalysis was successfully applied in the synthesis of FMO3-generated high valued drug metabolites with a high yield conversion (Fig. 1E and F) [4].



F

Substrate	N-oxide product (mg/L)	Conversion (%)
TAM	294.5	76.5 ± 4.6
BZD	323.1	99.6± 0.3
Clomiphene	392.4	93.0 ± 0.4
Dasatinib	499.0	99.0 ± 0.7
GSK5182	208.8	49.2 ± 0.7
Tozasertib	201.8	42.0 ± 0.6

Figure 1 (A) FMO3 model constructed by homology modeling. (B) $NADP^+$ binding profiles for FMO3 (black dot) and N61S variant (red dot) were determined by ITC experiments. The H-bond network in the catalytic center of (C) FMO3 is interrupted by (D) N61S mutation. (E) FMO3-based whole cell catalysis of six drugs to synthesize their corresponding *N*-oxide metabolites. (F) Yield conversion of FMO3-based whole cell catalysis.

[1] Gao, C., Catucci, G., Di Nardo, G., Gilardi, G. and Sadeghi, S.J., (2016). *Gene* 593, 91-99.
[2] Gao, C., Catucci, G., Castrignanò, S., Gilardi, G. and Sadeghi, S.J., (2017). *Sci Rep* 7, 14668.
[3] Gao, C., Catucci, G., Gilardi, G. and Sadeghi, S.J., (2018). *Int J Biol Macromol* 118, 460-468.
[4] Gao, C., Catucci, G., Gilardi, G. and Sadeghi, S.J., (2018). *Microb Cell Fact*, Under review.