

Supplemental materials for

Aldehyde Oxidase Contributes to all-*trans*-Retinoic Acid Biosynthesis in Human Liver

Guo Zhong¹, Chris J. Seaman¹, Erickson M. Paragas², Huaqing Xi¹, Karla-Luise Herpoldt³, Neil King³, Jeffrey P. Jones², Nina Isoherranen^{1,*}

1 Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, WA;

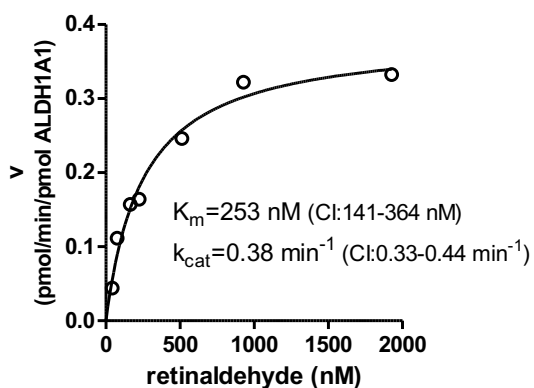
2 Department of Chemistry, Washington State University, Pullman, WA;

3 Department of Biochemistry and Institute for Protein Design, University of Washington, Seattle, WA

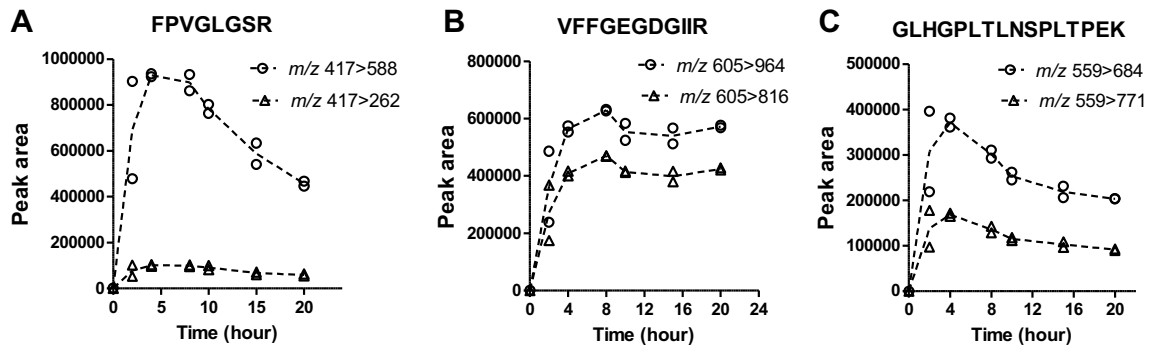
* Corresponding author

Supplemental Table 1: DNA and amino acid sequences for recombinant human CRBP1.

1 (DNA)	ATGCCAGTGGATTTACGGGATACTGGAAAATGTTAGTGAATGAGAATT TTGAGGAGTACCTTCGTGCTTTAGACGTAAATGTTGCGCTTCGTA GAAATCTGTTGAAACCAGACAAAGAAATTGTGCAAGATGGCGACCAC ATGATTATTCGCACCTTGAGTACATTCCGTAATTATATTATGGACTTCA AGTGGGAAAGGAGTTCGAAGAAGACTTGACTGGTATCGACGATCGTAA ATGTATGACAACAGTATCATGGGATGGTGATAAATTGCAATGTGTCCAA AAGGGAGAAAAGGAGGGGCCGCGGGTGGACTCAGTGGATTGAAGGGGA CGAACTTCACTTAGAGATGCGCGTCGAGGGTGTTCGTATGCAAGCAGGTA TTCAAGAAGGTGCAA
2 (amino acid)	MPVDFTGYWKMLVNENFEEYLRALDVNVALRKIANLLKPDKEIVQDGDH MIIRTLSTFRNYIMDFVQVGFKEFEEDLTGIDDRKCMTTVSWDGDKLQCVQKG EKEGRGWTQWIEGDELHLEMVRVEGVVCKQVFKKVQ



Supplemental Figure 1. Representative Michaelis-Menten plot of *atRA* formation by recombinant ALDH1A1. Each symbol is the mean value of duplicate measurement. The assay was performed as described before [14]. CI: 95% confidence interval. Due to adjustments in protein purification protocols, enzyme kinetic parameters of ALDH1A1 with retinaldehyde were remeasured in this study and K_m of retinaldehyde was similar to what has been reported previously (285 nM) while k_{cat} was lower than the reported value (1.1 min^{-1}) [14].



Supplemental Figure 2. Matrix effects on AOX digestion, peptide stability, and detection. Purified AOX of 4 pmol was spiked into mouse liver S9 fractions (20 μ L of 2 mg/mL) and samples were processed and analyzed as described in *Methods and Materials*. The X axis indicates the trypsin digestion time. The time course of peptide formation of three selected AOX peptides was observed in mouse liver S9. VFFGEGDGIIR was selected as the target peptide to quantify AOX due to its good stability in mouse liver S9 during trypsin digestion and its good signal intensity indicated by the peak area.