

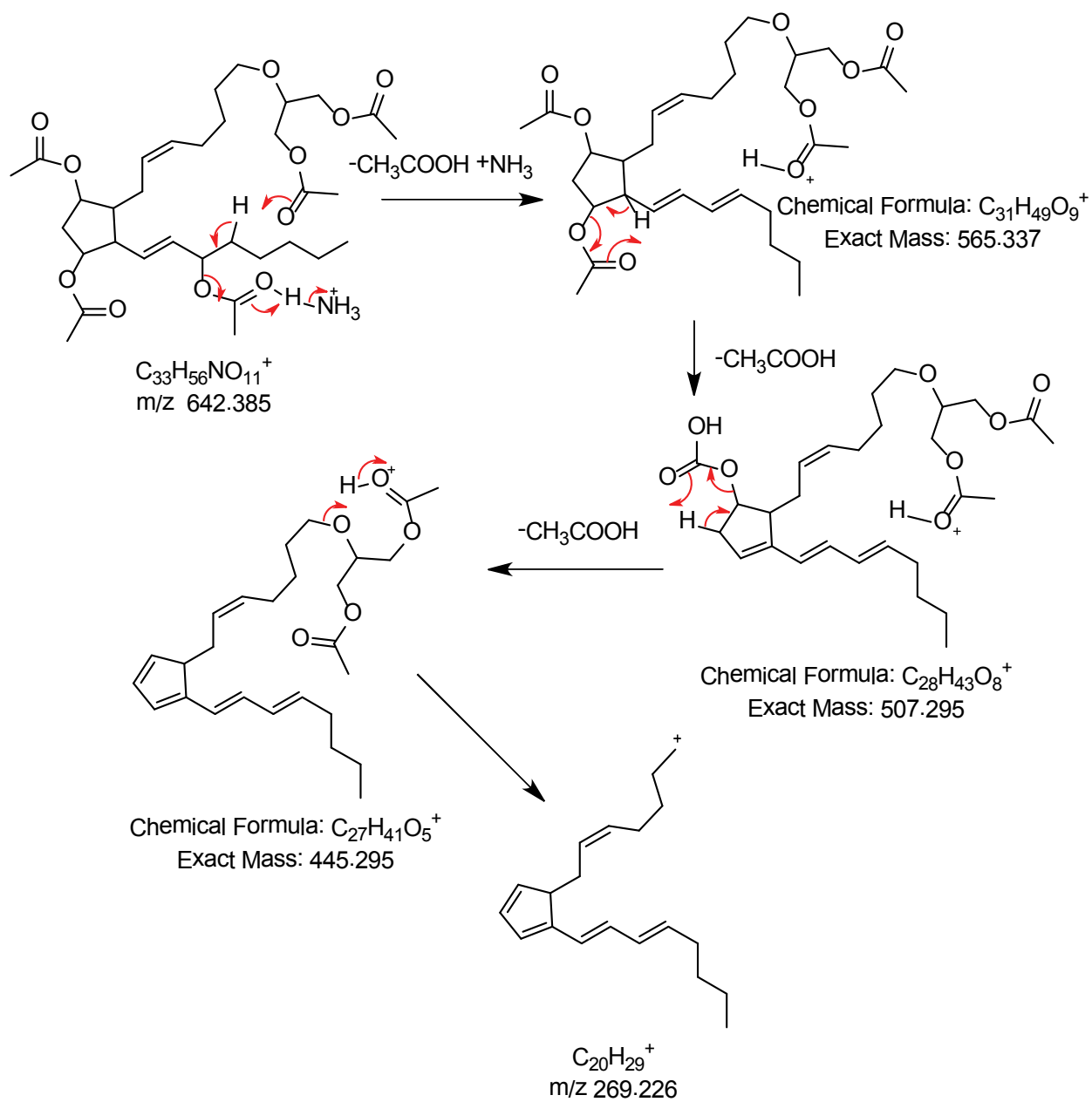
SUPPLEMENTAL INFORMATION:

**Interactions of 2-O-Arachidonylglycerol Ether and Ibuprofen with
The Allosteric and Catalytic Subunits of Human Cyclooxygenase-2**

Liang Dong[#], Hechang Zou[#], Chong Yuan[#], Yu H. Hong[#],
Charis L. Uhlson[&], Robert C. Murphy^{†&} and William L. Smith^{†#}

[#]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109 USA

[&]Department of Pharmacology, University of Colorado-Denver, Aurora, CO 80045



Supplemental Figure S1

Supplemental Fig. S1: Proposed pathway operating to form the major fragment ion from Peak C obtained by MS/MS.

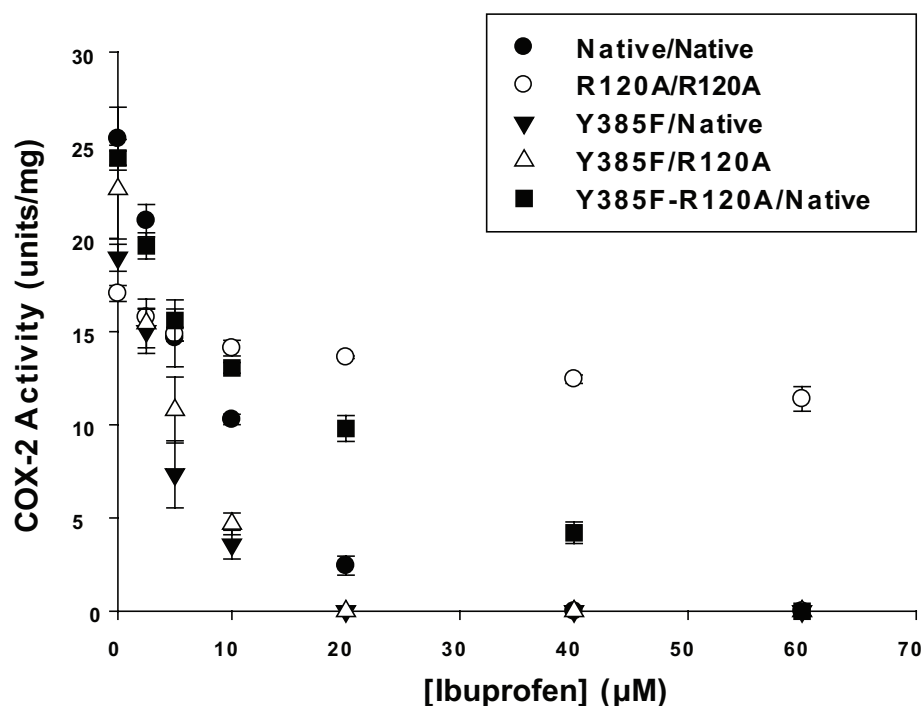


Fig. 2S. Instantaneous inhibition by *S*-ibuprofen of 2-AG ether oxygenation by huPGHS-2 variants having an Arg-120 substitution in E_{allo} or E_{cat} . Assays of O_2 consumption using an O_2 electrode were performed as described in Experimental Procedures on the parent publication using $50 \mu\text{M}$ 2-AG ether as the substrate. huPGHS-2 heterodimer variants were described previously (15). The designations indicate the mutations in the subunits. For example, Y385F R120A/Native indicates a PGHS-2 molecule having one subunit with both Y385F and R120A mutations and the other subunit as having no mutations (i.e. a Native subunit); R120A/R120A indicates a PGHS-2 molecule with mutations in both subunits. Results are shown for a single experiment involving triplicate determinations. Relative COX-2 activities derived from these data in Fig. 2S are compared in Fig. 6 of the parent publication. Specific activities for the huPGHS-2 variants with 2-AG ether ($50 \mu\text{M}$) were as follows: For Native, 25 units/mg; for R120A/R120A, 17 units/mg; for Y385F/Native, 19 units/mg; for Y385F R120A/Native, 24 units/mg and Y385F/R120A, 22 units/mg.