

## UC Davis Partnering opportunities: Arachidonic acid pathway

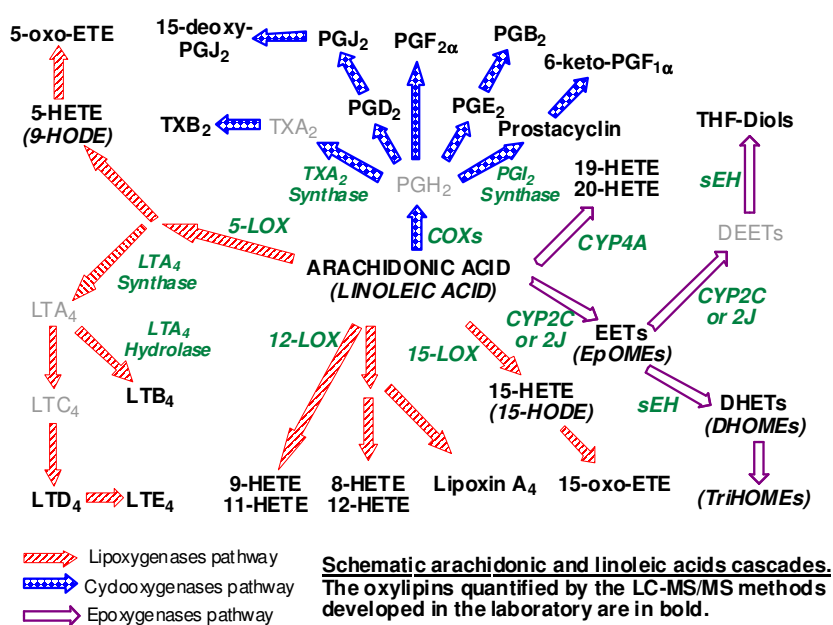
Contact:

<p>Bruce D. Hammock, Professor Department of Entomology University of California, Davis One Shields Avenue Davis, CA 95616-8584 (530)752-8465 bdhammock@ucdavis.edu</p>	<p>Kate Marusina, Ph.D., MBA Manager, Research Facilitation and Industry Alliance, Clinical and Translational Science Center UC Davis School of Medicine TEL: (916)703-9177 CEL: (530)979-1522 EMAIL: kate.marusina@ucdmc.ucdavis.edu</p>
---	---

### Partnering Area 1.

#### Analysis of synergistic effects of epoxide hydrolase inhibitors and FLAP inhibitors.

We are able to monitor eicosanoid profiles in animal models and in clinical samples with and without drug administration. Using our LC-MS technology, Dr. Hammock lab demonstrated that inhibitors of the soluble epoxide hydrolase will dramatically synergize cox inhibitors and other NSAIDs. We also showed that cox 2 inhibitors shifted the prostacyclin/thromboxane ratios leading to reduced platelet stability and accelerated blood clotting while aspirin did the opposite<sup>1</sup>. More recently we have correlated a dramatic increase of a biologically active component of the cascade with Vioxx treatment. Cox inhibitors with similar mode of action to Vioxx could be tested in our in vitro model to identify increased cardiovascular risk with the drug treatment. In a clinical study, a similar analysis would identify a patient population particularly sensitive to the effects of Cox inhibitor treatments.



Subsequently we have found the soluble epoxide hydrolase inhibitors also synergize several other inhibitors of enzymes in the arachidonate cascade such as FLAP. Thus, the oxylipin platform could be successfully utilized to probe the mechanism of action of drugs. This technology is covered in a UC patent (UC Case No. 2005-253).

<sup>1</sup> 581. Schmelzer, K.R., L. Kubala, J.W. Newman, I.-H. Kim, J.P. Eiserich and B.D. Hammock. 2005. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. PNAS. 102:9772-9777.

624. Schmelzer, K.R., B. Inceoglu, L. Kubala, I.-H. Kim, S.L. Jinks, J.P. Eiserich and B.D. Hammock. 2006. Enhancement of antinociception by coadministration of nonsteroidal anti-inflammatory drugs and soluble epoxide hydrolase inhibitors. P. Natl. Acad. Sci. USA. 103:13646-13651.

633. Inceoglu, B., K.R. Schmelzer, C. Morisseau, S.L. Jinks and B.D. Hammock. 2007. Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs). Prostag. Oth. Lipid M. 82:42-49.

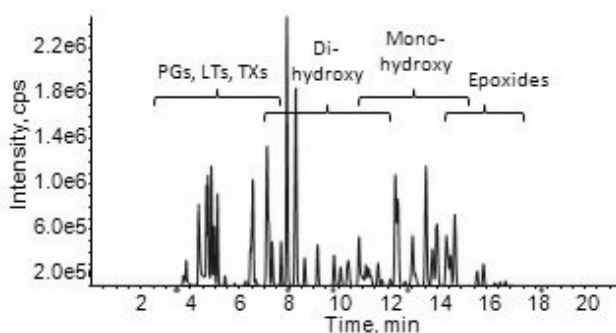
## Oxylipin analysis of fluids and plasma for predictive patient segmentation

We have developed several LC-MS platforms for monitoring biologically active lipids by LC-MS. The major platform provides quantitative data on 80 eicosanoids and related compounds by negative ion LC-MS. The metabolomic approach allows one to look at patterns of bioactive metabolites rather than single analytes and thus test hypotheses of interaction. Some of the major targets for this platform are shown below. We also have other platforms running on positive ion for example leukotrienes, endocannabinoids such as anadamide or 2-AG (the ethanolamide and glycerol derivatives of arachidonic acid), and vitamin D. The later is increasing being suspected of having a major role in inflammation. The vitamin D metabolites include all key intermediates and the difficult to analyze and biologically active dihydro D<sub>2</sub> and D<sub>3</sub>. We will correlate these oxylipin profiles with biological effects resulting from a disease, such as asthma or COPD and the treatments group. The method has also been used to look at the effects of two major components of smog (oxone and nitronaphthalene) in a rodent model<sup>2</sup>. We are expanding our analytical platform to include metabolites of ω3 lipids since they increasingly will be included in human diets as value added products.

Our current method relies on LC-MS/MS using an ABI 4000 linear ion trap. We test up to 77 analytes using 200 μl of plasma or equivalent tissue. We anticipate continual improvements in the method by adding new analytes, reducing the volume of sample needed, reducing the length of the analytical procedure, improving clean up and improving accuracy and precision.

For a limited set of key analytes we develop focused methods to improve sensitivity and to reduce the cost. For example the 200 μl plasma volume needed for a full eicosanoid profile precludes taking multiple samples from rodents. Thus, when we found that the PGE<sub>2</sub> and TXB<sub>2</sub> and 5-HETE levels are good indicators of the LOX5 pathway, we developed a rapid micromethod for these analytes, using the Agilent 'Chip' LC-MS.

Samples are prepared by solid phase extraction using Oasis HLB cartridges followed by reversed phase HPLC analysis utilizing C18 columns. A representative chromatogram of the oxylipin analysis is shown below. The analytes elute according to their polarity with the most polar analytes, prostaglandins and leukotrienes eluting first followed by the hydroxy and epoxy fatty acids (figure below). The separated analytes are then quantified by tandem mass spectrometry in multiple-reaction monitoring mode utilizing negative electrospray ionization for the oxylipin profiling and positive electrospray ionization for the endocannabinoids and related fatty acid amides and esters. Surrogate analytes and internal and external standards will be used to monitor extraction efficiency and ensure accurate quantitation of analytes. When possible we will incorporate heavy atom internal standards. Random selection of 5% of samples for replicate analysis will allow for documentation of method performance in terms of accuracy and precision. In addition, quality control samples are analyzed at a minimum frequency of 10 hours to ensure stability of the analytical calibration throughout a given analysis.



## Partnering Area 2

### Discovery of novel FAAH inhibitors using a novel fluorescent assay.

Most of existing FAAH inhibitors have electrophilic carbonyls that form a covalent complex with the enzyme that mimics substrate binding. It includes carbamates, trifluoromethyl ketones, α-keto

<sup>2</sup> Smith, K.R., K.E. Pinkerton, T. Watanabe, T.L. Pedersen, S.J. Ma and B.D. Hammock. 2005. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. *Proc. Natl. Acad. Sci. USA* 102:2186-2191.

Schmelzer, K.R., A.M. Wheelock, K. Dettmer, D. Morin and B.D. Hammock. 2006. The role of inflammatory mediators in the synergistic toxicity of ozone and 1-nitronaphthalene in rat airways. *Environ. Health Persp.* 114:1354-1360.

heterocycles, sulfonyl fluorides and fluorophosphonates. These mechanistic inhibitors also inhibit other enzymes that are using a reactive serine in their mechanism, such as esterases and serine-proteases. Other developed FAAH inhibitors also lack specificity, for example arachidonoyl diazomethyl ketone, a mixed inhibitor of FAAH, also inhibits 5-lipoxygenase. Furthermore, the most powerful inhibitors of FAAH appear to be irreversible or pseudo-reversible, thus lacking the flexibility granted by a reversible modulation of the endocannabinoid system. While these inhibitors were useful to probe the biological functions of FAAH, their lack of specificity and poor physical properties make them less than therapeutically efficient, underlying the need for novel inhibitor structures. Therefore, the **overall objective of our work is to obtain new FAAH inhibitor structures that are reversible inhibitors with improved specificity, solubility, and metabolic stability that will result in greater *in vivo* potency.**

We recently developed new fluorescent substrates for the FAAH based on the formation of highly fluorescent amino-pyridines following amide hydrolysis by our recombinant human enzyme. Human fatty acid amide hydrolase was cloned and expressed in high yield in the baculovirus expression system. Our HTS assay is two orders of magnitude more sensitive than the coumarin based fluorescent assay used to develop the described FAAH inhibitors. We also used another fluorescent substrate to obtain an end point assay. This assay was used to screen a small defined library (two 96-well plates) of compounds generated in house. In the conditions used, we obtained a signal to background ratio above 8, and a signal to noise ratio above 30. We also observed plate to plate variations smaller than 6%, calculated Z' values above 0.9, and determine an accuracy of above 90%. The early structures of potential inhibitors using this assay are published<sup>3</sup>

In addition, we have cloned and expressed a variety of esterases, lipases and amidases from humans (15 enzymes for the moment), and established high throughput screens for these materials. The FAAH assay is coered by PCT Publication [WO 08/008732](#) published on Jan 17, 2008

#### **Potential areas of collaboration:**

**1. Transferring screening assays for FAAH.** We can provide the substrate and recombinant enzyme for screening FAAH and assistance in establishing the assay. The assay has very high S/B (~150), S/N (~35) and Z' (~0.8) values, indicating that it is very suitable for HTS.

**2. Screening a limited set of compounds for FAAH inhibitors and/or other hydrolase inhibitors.** Moderate throughput screens with two robotic systems could be handled in Dr. Hammock laboratory. We can handle 384 well plates but our chemical libraries and data systems are set up for 96 well format.

**3. Counter-screening against a number of other human esterases, amidases and related enzymes.** We use both classical substrates and esters with novel fluorophores such as the alpha cyano fluorophores<sup>4</sup>. We produce these human and rodent hydrolase enzymes in high yield in the baculovirus system. For the FAAH and 2-AGL enzymes, we can monitor its activity with its natural substrates (arachidonoyl ethanolamide, or 2-glycerol arachidonate) by LC/MS as well as by fluorescent substrates.

**4. Developing SARs in several series of compounds using FAAH and 2-AG lipase as endocannabinoid targets.** Earlier, we have identified a unique inhibitor pharmacophore. Our lab has extensive expertise in organophosphate and carbamate chemistry. We would be happy to work collaboratively to further develop this or other inhibitors, optimizing on the target enzyme and running counter screens.

---

<sup>3</sup> Wheelock CE, Nishi K, Ying A, Jones PD, Colvin ME, Olmstead MM, Hammock BD.

Influence of sulfur oxidation state and steric bulk upon trifluoromethyl ketone (TFK) binding kinetics to carboxylesterases and fatty acid amide hydrolase (FAAH). *Bioorg Med Chem.* 2008 Feb 15;16(4):2114-30. Epub 2007 Nov 26

<sup>4</sup> Huang, H., C.D. Fleming, K. Nishi, M.R. Redinbo and B.D. Hammock. 2005. Stereoselective hydrolysis of pyrethroid-like fluorescent substrates by human and other mammalian liver carboxylesterases. *Chem. Res. Toxicol.* 18:1371-1377.

**5. Monitor endocannabinoid levels *in vivo*.** Similar to partnering goal 2, **we can test investigational compounds that alter** the arachidonate cascade, linoleate cascade or  $\omega$ -3 **cascade** (such as FAAH, 2-AG lipase inhibitors) by administering the compounds *in vivo* and monitoring the eicosanoid profiles in plasma.