

1. **Faculty name:** Ewa Skrzypczak-Jankun, Ph. D.
2. **Academic rank:** Associate Professor
3. **Department:** Urology
4. **Name(s), academic rank, and Department of any co-investigators:** Jerzy Jankun, Professor, Urology
5. **Project title:** Testing the role of a naturally occurring single nucleotide polymorphism on activity and function of human 12-lipoxygenase.

6. **Brief summary/description of project:**

Human platelet 12-lipoxygenase (hp-12LOX) plays role in the inflammatory, cardiovascular, cancer, renal and neurodegenerative diseases. It has 663 amino acids, a single non-heme iron cofactor that catalyses oxidation of arachidonic acid to 12S-HpETE. Although hp-12LOX participation in many physiological processes has been confirmed there is no progress in the development of drugs due to the lack of information about enzyme's structure and its relation to the molecular behavior. In vivo this enzyme shows two peaks of activity corresponding to enzyme present in cytosol or when firmly attached to the phospholipid membrane. It has been found that glutathione depletion triggers an increased activity of hp-12LOX, but the relation between those facts could not be explained. We have developed an expression system and the purification protocol to study this recombinant enzyme. We have found that the wild type enzyme (WT) is active as an oligomer, with the smallest aggregate being a dimer. Its aggregation depends on the properties of the environment (reducing or not) and its activity depends on molecular association and pH. Doing selective elimination of cysteines (Cys→Ser mutants) we have proven their role in aggregation. We have also determined that each molecule of hp-12LOX binds 2 molecules of substrate although (based on homology modeling) the active site can accommodate only one. Hence we suspect a presence of an allosteric site but its location has yet to be determined. Utilizing small angle X-ray scattering we have obtained low resolution structure of hp-12LOX dimer in solution suggesting possible modes of association of its monomers. These experimental data together with molecular modeling allow us to pinpoint the domains/subdomains/fragments which might be crucial for the dimer integrity and activity and/or allosteric property. We have localized a fragment that we would like to test by the side directed mutagenesis (deletions or mutations) altering size, shape and charge distribution, thus affecting its hydrophobic/hydrophilic properties and affinity to binding other molecule(s). It has been also confirmed in clinical studies that cancer patients have significantly higher occurrence of single nucleotide polymorphism in that region. The student project will be to create mutant mimicking a natural variant R261Q observed in cancer patients, and test its molecular properties vs. WT. Such project can be accomplished within a duration of the MSSR2008 program and will be a valuable part of our ongoing research on understanding the structure-function relationship of this important enzyme.

7. **Describe students role and responsibilities.**

The student will design experiments and (upon approval) execute them utilizing/learning a common biochemical techniques (expression of recombinant protein, mass spectroscopy, UV-VIS, electrophoresis, chromatography) and learn methods for protein crystallization for X-ray analysis.

8. **Special qualifications required.** A person should be well organized, neat and meticulous in data recording and documentation.