Research in the Dedon Laboratory: Infection and Inflammation – Biomarkers, Diagnostics, Therapeutics

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Who We Are

The Dedon Laboratory is an interdisciplinary group of biologists, chemists, and engineers from all around the world. We operate full laboratories both at MIT and at the National University of Singapore, though the Singapore-MIT Alliance for Research and Technology. Our MIT lab currently houses three full-time research scientists, six postdoctoral fellows, five graduate students, and two visiting scientists. Our NUS lab adds an additional postdoctoral associate and two graduate students. Our alumni have gone on to have successful careers in both industry and academia, in fields as diverse as public health and analytical chemistry.

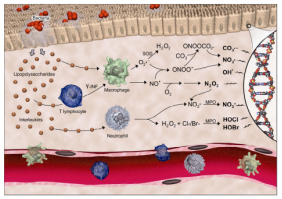
http://dedon.mit.edu/

What We Do

We seek to understand the chemical etiology of human disease, with the long-term goals of developing diagnostic tools and therapies. We apply techniques from organic and analytical chemistry to interrogate biochemical networks and systems in a quantitative manner. Using both data- and hypothesis-driven approaches, we develop ultra-sensitive bioanalytical tools to characterize and quantify normal and damaged biomolecules in cells and tissues. The current research program has a broad theme of nucleic acid chemistry and biology with a focus on microbial pathogens, inflammation and cancer in four major projects, described in greater detail below.

Inflammation and Chemical Immunology

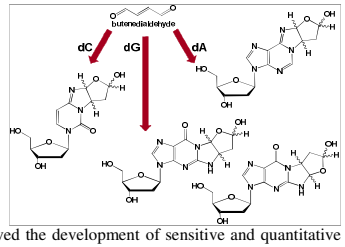
We have a long-standing interest in understanding the link between chronic inflammation and human diseases, such as cancer. Activation of the innate immune system by infection or tissue damage leads to the generation of highly reactive oxygen and nitrogen species, such as nitric oxide (NO), hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), peroxynitrite ($ONOO^-$), and nitrosoperoxycarbonate ($ONOOCO_2^-$). Intended to combat invading microbes, these reactive species also damage virtually all types of biological molecules in surrounding host cells. The hypothesis is that these species cellular damage that eventually leads to causative mutations in cancer.



Current projects embrace three areas. One involves development of damage products as biomarkers, with emphasis on developing ultrasensitive mass spectrometric methods to quantify RNA, DNA, protein and lipid damage. These biomarkers represent surrogates for the short-lived reactive oxygen and nitrogen species, as well as indices of the severity of the inflammatory process. The second research thrust involves metabolomic analyses of serum and urine for biomarkers of inflammation, using an LC-QTOF approach. The third area of research involves defining the chemical mechanisms of damage caused by reactive nitrogen and oxygen species, using a novel NO delivery system and mouse models of inflammation, colitis, gastritis and liver disease.

Fate and Transport of DNA Damage Products

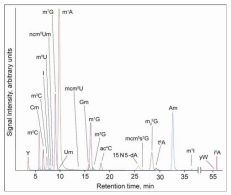
A major hurdle to the development of damaged biomolecules as biomarkers is our lack of understanding of the metabolic fate of the damage products that are formed in tissues. With much of the DNA and RNA damage chemistry now characterized in terms of product structure, we have now turned our attention to the biological fates of these damage products, with the goal of developing biomarkers of mechanism, source and risk. This effort is illustrated in our studies of the spectrum of products arising from oxidation of 2-deoxyribose in DNA, both *in vitro* and *in vivo*. An important facet of this research has involved the development of sensitive and quantitative analytical methods for the various products, including gel-based approaches, GC-MS, and LC-MS.



With the goal of identifying biomarkers of oxidative stress and inflammation, we are shifting our focus from the formation of DNA damage products to their fate in biological systems. For example, the electrophilic species arising from 2-deoxyribose oxidation in DNA are ideal candidates for glutathione conjugation by glutathione transferases, which points to mercapturic acid metabolites as potential biomarkers of oxidatively damaged DNA arising from oxidative stress and inflammation. Given the presence of glutathione transferase activities in mammalian nuclei, such activity may represent a form of DNA protection or repair by reacting with base propenals and other DNA-bound or freely diffusible deoxyribose oxidation products, as well as the more commonly recognized lipid peroxidation targets.

RNA Modifications in Cellular Response Pathways

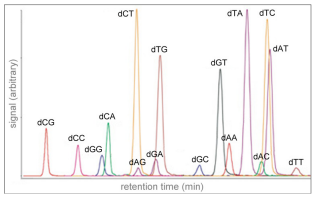
A major new line of research addresses the role of RNA secondary modifications in the cellular responses to toxic exposures. Over 100 different ribonucleoside structures have been identified in both prokaryotes and eukaryotes, most prominently in tRNA and rRNA, with ~20-30 types of modifications present in any one organism. Recent studies suggest an important role for the various nucleoside structures in controlling translation in response to cell stimuli. We have developed a sensitive LC-MS/MS approach to identifying all of the RNA modifications in an organism and quantifying changes in the spectrum of modifications following cellular exposures. Principal component analysis of changes in the modification spectrum following exposure to chemical mediators of information provides a "top down" approach that has led to the identification of RNA modifications that are critical to the cell survival response.



As part of the Infectious Disease group of the Singapore-MIT Alliance for Research and Technology, we have embarked on a study of RNA modifications in tuberculosis and malaria. The goal is to characterize the role of RNA modifications, starting with tRNA, in the response of microbial pathogens to reactive oxygen and nitrogen species generated by phagocytes in the human immune response.

Phosphorothioate Modifications in Bacterial DNA

Phosphorothioate (PT) modification of the DNA backbone involves replacement of a non-bridging phosphate oxygen with a sulfur atom. Following the pioneering work of Eckstein and coworkers, PT modification of DNA has long been known as a synthetic modification that stabilizes oligodeoxynucleotides against nuclease degradation. However, we recently worked with the Deng group at Shanghai Jiao Tong University in our discovery of PT modifications as natural products in DNA from bacteria harboring the five-gene *dnd* cluster, with the PT modification located in G-G and G-A sequence contexts in *Streptomyces lividans* and *E. coli* B7A, respectively. The discovery of sequence-selective PT modification of DNA raises many questions about the function of this enzymatically-mediated, post-synthetic modification of DNA in cell physiology. Is it part of a restriction-modification system? What are the broader sequence and genome contexts for PT? How widespread are the *dnd* genes responsible for the modification?



We have undertaken a series of studies to define the quantity, location and function of PT modifications of DNA in bacterial genomes. Our initial efforts led to the development of an LC-MS/MS method that screen for the presence of PT in genomic DNA, to identify the two-nucleotide sequence context (of 16 possible) and to quantify PT. A second effort has led to the development of an affinity purification technique for the isolation of PT-containing DNA fragments. We exploited the nucleophilicity of PT to biotinylate the modifications as a means to affinity purify PT-containing DNA fragments for subsequent sequence analysis to define larger sequence contexts, genomic location, and bacterial speciation. These techniques have led to identification of a variety of consensus sequences in dozens of bacterial genera.