Optimizing Gene Delivery with Quantum Dot-FRET Technology

In this presentation, we will review our studies on using chitosan and polyphosphoramidate (PPA) for oral nonviral gene delivery. In addition we will discuss our recent in vitro and in vivo mechanistic studies on understanding the rate barriers in oral nonviral gene delivery. Particularly we will focus on the steps of DNA nanocomplex (NC) unpacking and DNA degradation. Encapsulation of DNA within nanocomplexes (NC) protects it from enzymatic degradation, but after release, DNA is susceptible to cytosolic nucleases. Previously, we reported that quantum dot-FRET (QD-FRET) is an ultrasensitive method to detect NC dissociation. An integrated approach to study both NC dissociation and DNA degradation may elucidate the contributing roles of these rate-limiting barriers. In this presentation I will discuss the development of two-step QD-FRET using plasmid DNA doubly-labeled with QDs and DNA dyes which are then complexed with a Cy5-labeled cationic polymer. The emission spectra of these NCs showed that the QD donor can drive energy transfer step-wise through the DNA dye (as a relay) to Cy5 on the polymer. NC dissociation is indicated by the elimination of FRET between the DNA dye and Cy5 (FRET-2), while DNA degradation is indicated by the QD and DNA dye (FRET-1). This two-step QD-FRET approach to track DNA release and integrity provides valuable mechanistic and kinetic data that may facilitate gene carrier design.

Wednesday, April 23, 2008
4:00 pm
1000 Micro and Nanotechnology Laboratory

Kam Leong
Department of Biomedical Engineering
Duke University

Kam Leong is a Professor in the Department of Biomedical Engineering, and also is Professor in the Division of Experimental Surgery, School of Medicine, and Director of Bioengineering Initiative, at Duke University. He is Principal Investigator, Duke-NUS Graduate Medical School, Singapore. Prior to joining Duke University, he was a faculty member in the Johns Hopkins University School of Medicine from 1986 to 2005, and directed the Therapeutics and Tissue Engineering Laboratory at the Division of Johns Hopkins in Singapore from 1998-2005. The research focus of his laboratory is on understanding and exploiting the interactions of cells with nanostructures for therapeutic applications. Continuous nanostructures in the form of nanofibers and nanopatterns are applied to scaffolding design to influence cellular response. He has published more than 200 peer-reviewed manuscripts and has more than 30 patents. He is the recipient of the Young Investigator Research Achievement Award of the Controlled Release Society in 1994 and a Fellow of the American Institute for Medical and Biological Engineering. He also serves on the editorial board of the Journal of Controlled Release, Biomaterials, Molecular Therapy, Acta Biomaterialia, Genetic Vaccines and Therapy, International Journal of Nanomedicine, and Nanomedicine.

Co-sponsored by the **NCI-funded Siteman Center for Cancer Nanotechnology Excellence (SCCNE-University of Illinos at Urbana-Champaign: co-location)**