

CIMIT MEETING SUMMARY 7/19/2005

The meeting was the third in a series of educational forums on molecular imaging. Sanford Simon, PhD, Rockefeller University, spoke on “Dynamics of Proteins at the Cell Surface During Exocytosis and Endocytosis”. The dynamics of molecules at the cell surface were studied to gain insight into the mechanisms of exocytosis and endocytosis. A variety of imaging modalities were used to study single molecules or single exo or endocytic events. Among these was Total Internal Reflectance Fluorescence Microscopy (TIR-FM), which uses the evanescent field that penetrates into a medium adjacent to an optical material, such as a cover slip, in which a wave undergoing total internal reflection propagates, to limit optical excitation of cellular fluorescence to a layer 40-80 nm thick. In this way studies of single events in the cell are possible and lead to different conclusions about the mechanisms than have been drawn from examining macroscopic events in the cell. Observations of both the peak and the integrated fluorescence from a spot can be used to differentiate between secretion of the contents of a vesicle and its lysing, as well to obtain the diffusion coefficients for those contents. Once specific cell surface events have been identified, their mechanisms can be studied further by adding or removing fluorescently-labeled molecules. While molecules such as GFP are frequently used for labeling, they suffer from photobleaching and from overlapping excitation spectra. The use of quantum dots as fluorophores overcomes some of these problems; the questions involved in using the dots as labels for cells (do they alter cell behavior?) are being resolved.

John Frangioni, MD, PhD, BIDMC, spoke on “Inherent Detector Sensitivity and Limitations of Molecular Imaging”. Molecular imaging was considered in light of two clinical problems: cancer detection and stem cell detection and tracking. In the latter case, interest lies in whether stem cells home to the site of injury go after a typical infusion of 10^6 cells. Stem cell tracking may require single cell detection, but currently-available combinations of molecular-imaging contrast agents and detectors are 7 to 9 decades away from such sensitivity. The common element in assessing various imaging modalities lies in the signal to background (S/B) level attainable. Clearance of a contrast agent from normal tissue improves the S/B and depends on the hydrodynamic diameter (HD) of the agent; HDs of < 5 nm, corresponding to < 40 kD MW, facilitate uptake but an $HD < 3$ leads to rapid renal clearance from the body and a decrease of S/B. The calculation of signal from an agent requires consideration of the dilution effects in the form of the number of cells in an imaging voxel. The calculation also requires consideration of the concentration of contrast agent within a cell, together with the signal produced by the agent. Using CT, for example, the signal will depend on the linear absorption coefficient of the agent. MRI using Gd as a contrast agent requires intracellular concentrations of 10-100 μ M. SPECT (Single Photon Emission CT) suffers from low detection sensitivity (0.01 %) linked to the need to use a collimator in front of the detector, while PET's detector sensitivity is degraded by the low absorption coefficient for the 511 keV photons involved. US detection would be optimized by a contrast agent with a 1 micron diameter, larger than optimum for delivery, and additionally US requires direct contact with a transducer. Improvements in contrast agents by multimerization of low affinity ligands was suggested.