Pharmaceutical Sciences Seminar and GTR seminar

Dr. Jeffrey Keillor

Professor, *Department of Chemistry and Biomolecular Sciences,* University of Ottawa (Canada)

Aug. 2nd , PM 4:00

Bldg. Graduate School of Pharmaceutical Sciences, 2F lecture room

Fluorogenic Protein Labelling

The fluorescent labelling of proteins is a technique widely used to gain insight into their function, localization and trafficking. We have developed a fluorescent labelling method based on the use of small synthetic fluorogenic molecules. These fluorogens contain two maleimide groups that quench the latent fluorescence through an elucidated PeT mechanism, until they both undergo thiol addition reactions. In parallel, we have designed alpha-helical peptide sequences that present two appropriately positioned cysteine residues. Proteins that are genetically encoded to bear one of these *de novo* peptide tags ('dC10a') can then be selectively fluorescently labelled with our synthetic fluorogens.

Recently we have increased the reactivity of the peptide sequence by protein engineering, and tuned the reactivity of the dimaleimide fluorogens through substituent effects. These efforts have allowed us to label specific proteins of interest, in the cytosol and in the nucleus of living cells, without the need for washing before fluorescent imaging. Furthermore, by employing DFT calculations to predict orbital energies, we have designed highly fluorogenic labelling agents whose emission wavelengths are tuned to each of the channels of the fluorescent microscope – blue, green and red.

Kiyotaka HITOMI: hitomi@ps.nagoya-u.ac.jp