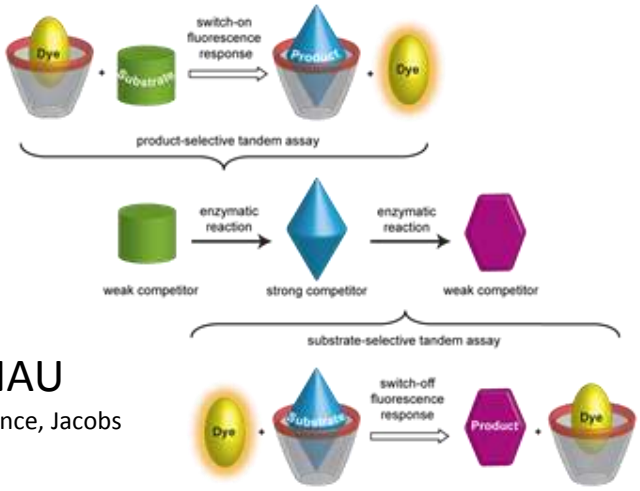
NUS National University of Singapore | Department of Biological Sciences Faculty of Science

BIOLOGY COLLOQUIUM

Friday, 22 November 2013 | 4pm | DBS Conference Room 1

Hosted by A/P K Swaminathan

Supramolecular Tools in Enzyme and Membrane Assay Development



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We have developed a label-free, convenient, and economical fluorescence-based assay method (Supramolecular Tandem Assays). Unpublished is the transfer of the method to monitoring the translocation or transport of analytes through membranes. Published is the method for monitoring a large range of enzymatic reactions.1-3 Our assays rely on the pronounced changes to the photophysical properties of fluorescent dyes upon encapsulation by a water-soluble macrocycle as an artificial receptor, typically a cyclodextrin, calixarene, or cucurbituril, and the indicator displacement principle. Accordingly, an enzymatic reaction can be conducted in the presence of a reporter pair composed of a synthetic macrocyclic host and a fluorescent dye. The macrocycle is selected to have a different affinity for the substrate and product of the enzymatic reaction. As the reaction proceeds, the product either displaces the fluorescent dye from the complex (product-selective assay), or the enzymatic conversion of the more strongly bound substrate allows the uptake of the dye into the complex (substrate-selective assay). Both processes result in a readily detectable fluorescence response, and depending on the photophysical characteristics of the dye, either a fluorescence increase (switch-on response) or a fluorescence decrease (switch-off) is observed (see Scheme). In the case of supramolecular tandem membrane assays, only a high affinity (but no high selectivity) for the analyte is required.

In this presentation we will show our progress made with tandem assays, including the investigation of different enzymes and classes of enzymes, inhibitor and activator screening, domino tandem assays, the implementation of anion- as well as cation-receptor macrocycles, the transfer from low-molecular weight to biopolymeric substrates, and applications to analyte sensing and enantiomeric excess determination. The transfer of the method to monitor the transport and translocation of analytes, for example, protamine as a model of the class of cationic antimicrobial peptides, is also introduced.