Targeting Fluorescent Sensors in Living Cells, Tissues and Animals

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Fluorescent indicators for monitoring intracellular signal transduction pathways have been designed using Green Fluorescent Protein (GFP) and its color mutants fused to ligand binding sites from proteins. For example, calmodulin (fused to a short substrate-binding peptide) is sandwiched between a cyan and yellow GFP such that, on Ca^{2+} binding, fluorescence resonance energy transfer (FRET) between the GFP's increases, giving a change in fluorescence emission. As these fusion proteins (cameleons) are genetically-encodable, they can be targeted to subcellular locations such as the nucleus, endoplasmic reticulum or synapses to measure localized changes in Ca^{2+} concentrations. Transfection by adenoviral vectors permits transient expression and Ca^{2+} imaging in intact tissues which are difficult with conventional chemical indicators. Transgenic mice and nematodes have been generated permitting non-invasive (one or multi-photon) imaging of living animals.

A further extension of these approaches involves replacement of the GFP fluorophore by a small, membrane-permeable, non-fluorescent dye containing two arsenic atoms (FlAsH). A highly-fluorescent complex is formed upon specific and high-affinity binding to a unique tetracysteine motiv inserted in or appended to the protein of interest. FRET can be demonstrated between FlAsH dyes and GFP tagged with such a tetracysteine-containing peptide in living cells. Chemical modification of the FlAsH ligand should allow a wide range of functionalities such as photosensitizers, dimerizers and MRI-contrast agents to be directed to specific sites in cells.