Introduction

In 1871 fluorescein (1) was firstly synthesized by A. von Baeyer via a Friedel–Crafts acylation of phthalic anhydride (2) and resorcinol (3). Fluorescein is a dark red powder which when dissolved displays excellent fluorescent properties (\(\lambda_{\text{ex}} = 494\) nm and \(\lambda_{\text{em}} = 521\) nm in water) making it widely used in applications ranging from molecular sensing to labeling. It has a high quantum yield, is water soluble, can be excited with the readily available argon ion laser, is prepared in a short synthetic route and is non-toxic [LD\(_{50}\) = 6.7 g/kg (rat)]. A large number of fluorescein derivatives are commercially available while the properties can be modified to tune its fluorescent properties and widen its range of applications.

Abstracts

(A) Metal Sensor:
A number of metals are biologically vital; however, increasing metal concentrations can cause harmful effects. For example copper accumulation has been shown to induce neurodegenerative diseases such as Alzheimer’s. The group of Jung prepared a copper sensor based on fluorescein modified with chelating groups attached onto silica nanoparticles. Addition of copper resulted in chelation quenching of the fluorescence and allowed quantitative copper sensing with a detection limit of 0.5 \(\mu\)M in the presence of other divalent metal ions. The fluorescein-based sensor was applied in measuring Cu\(^{2+}\) concentrations in living cells.

(B) pH Sensor:
The pH is maintained in a narrow window in cells. A variety of applications demand visualization of the pH within cells and require non-invasive sensors. A novel pH sensor was prepared by Unciti-Broceta combining the excellent optical-physical properties of fluorescein with the broad spectral window of anthocyanidins. The sensor detected pH values between 7 and 10, in addition, a diacetylated form was able to cross cell membranes and following intracellular, enzymatic deacetylation allowed determination of the cytosolic pH while acting as a viscosity sensor.
(C) **Reactive Oxygen Species (ROS) Sensor:**
The balanced redox state system is important for the normal physiological function of cells. However, cells are very sensitive to disturbances. The ability to measure the redox state of a cell with a reversible redox sensor was demonstrated by Miller with a fluorescein derivative incorporating a disulfide linkage. The sensor was able to visualize multiple cycles of oxidative stress and reductive repair in cells by fluorescence measurement.

(D) **Small Molecule Sensor:**
Over the years the ability to sense small molecules such as nitric oxide has become more important since they have been identified to be part of cellular signaling pathways and related to diabetes and hypertension. A non-fluorescent ytterbium fluorescein complex was synthesized containing two ortho-amino groups. This complex was able to detect nitric oxide via formation of a fluorescent triazole complex with a detection limit of 90 nM. The incorporation of the ytterbium allowed luminescence detection in the near-infrared region (980 nm) via energy transfer following fluorescein excitation.

(E) **Enzyme Activity:**
The detection of fast and sensitive routes to measure enzyme activity is of importance due to their involvement in various cellular functions. The group of Chujo prepared a phosphate-linked fluorescein polymer for the detection of alkaline phosphatase which has been related to bone and liver diseases. The fluorescein’s fluorescence was caged due to self-quenching of the fluorophore in the polymer structure. Alkaline phosphatase cleaves the phosphoramidite bonds and releases fluorescein whose fluorescence was used to quantify the enzyme activity.

(F) **Protein Labeling:**
Site-selective labeling of proteins remains a challenge due to the large number of functionalities present requiring bioorthogonal methods. Li et al. demonstrated regio- and chemoselective fluorescent labeling of an alkyne-bearing protein with iodinated fluorescein via a copper-free Sonogashira coupling. The bioorthogonality was impressively proven by carrying out the reaction in live bacteria.

(G) **Polymerizable Fluorescein Derivatives:**
The detection of nanoparticles is commonly based on fluorescent labels verifying their location and allowing quantification of cellular loading. In this study, fluorescein was modified with styryl monomers at different attachment points (xanthine and phthalic moiety) and converted into polymer particles. Attachment on the phthalic moiety was advantageous with enhanced fluorescent properties, and demonstrated a five-fold increase in comparison to the commonly employed surface coupled fluorescein approach.

**References**

8. Thielbeer, F.; Chankeshwara, S. V.; Bradley, M. *Biomacromolecules* 2009, 10, 3713.