Introduction

Cyanine dyes are highly conjugated, fluorescent molecules with absorption and emission wavelengths in the near infra-red region (700–900 nm). The simplest synthetic route to heptamethine cyanine dyes was first described by Narayanan and Patonay who heated N-alkylated indolium salts with 2-chloro-1-formyl-3-(hydroxyl methylene) in a Vilsmeier-type reaction.1 These heptamethine cyanine scaffolds can be readily modified through displacement of the labile chloride group by nucleophiles,2,3,4 resulting in fluorescent molecules with varying quantum yields, extinction coefficients, and fluorescence maxima. Conjugation to biomolecules is achieved through chlorine substitution by 3-(4-hydroxyphenyl) propionic acid. The resulting cyanine dye has a carboxylic acid moiety which can be coupled to an amine-containing compound via amide-bond formation. Enhanced aqueous solubility is typically achieved through sulfonation of the indole.5 As biological tissue does not absorb strongly within the near infra-red window, cyanine fluorophores are ideal for in vivo optical imaging application,6,7 while clinically, indocyanine green has been used for over 25 years in fluorescence angiography and ophthalmology (mouse LD50 = 60 mg/kg).8,9

Scheme 1 Synthesis of heptamethine cyanine dyes

Abstract

(A) Necrosis Detection
Necrotic tissue is found in a variety of disease states including cancer and sepsis, where levels of extracellular DNA are increased due to dead or dying cells. Murthy et al. described a hybrid heptamethine (IR-786)–bisbenzimidazole (Hoechst 33258) probe that accumulates in necrotic tissue by binding to extracellular DNA.2 In vivo analysis in mice ischemia–reperfusion models confirmed probe accumulation in necrotic tissue.2

(B) pH Sensor
Nagano and co-workers synthesised a ratiometric, NIR heptamethine pH sensor. By using two excitation wavelengths (670 nm and 750 nm), the relative fluorescence intensities (λem = 780 nm) allowed pH values between 6 and 10 to be readily measured. Incubation of HeLa cells with the sensor resulted in staining of lysosomes and mitochondria with a demonstrable ability to monitor intracellular pH changes.4

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(C) Reactive Oxygen Species Detection

Uncontrolled reactive oxygen species (ROS) are implicated in several inflammatory disease states.\(^{12}\) Nagano and co-workers reported the real-time analysis of ROS by linking two NIR cyanine dyes with different oxidation potentials.\(^{13}\) A turn on fluorescence signal was observed upon oxidation of the more susceptible cyanine dye as this removed the static quenching effect. A strong fluorescence signal was found after incubation with a variety of ROS such as the hydroxyl radical (OH) using Fenton’s reagent and superoxide (O\(_2^-\)) generated from xanthine oxidase.\(^{13}\)

(D) \(\text{H}_2\text{S} \) Molecule Sensor

Hydrogen sulfide is known to be an important gaseous signaling molecule and is key in the regulation of blood pressure.\(^{14}\) Zhang and co-workers developed a real-time NIR sensor for \(\text{H}_2\text{S} \) by incorporating 3-nitrophenol onto the heptamethine dye scaffold which resulted in photo-induced electron transfer (PET)\(^{15}\) and quenching of the cyanine dye fluorescence.\(^13\) This was liberated by nitro group reduction with hydrogen sulfide. Incubation with other reactive sulfide species such as glutathione and cysteine gave a far weaker fluorescence increase.

(E) Silver Sensor

Bioaccumulation of metal ions such as silver can demonstrate adverse biological effects due to binding to functional groups such as thiols.\(^{16}\) Zheng, Jiang and co-workers developed a \(\text{Ag}^+ \) sensor based on a heptamethine cyanine motif that contained an adenine moiety.\(^{17}\) Aggregation\(^{18}\) of the cyanine dye with increasing concentrations of \(\text{Ag}^+ \) ions resulted in a fluorescence shift of 185 nm with a detection limit of 34 nM. High selectivity over other metal ions such as copper and iron was demonstrated.

References