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Silica nanoparticles encapsulating near-infrared emissive cyanine dyes

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Abstract

We show that efficient near-infrared (NIR) cyanine fluorophores (1 and 2) can be encapsulated into silica nanoparticles providing a highly versatile and unique platform for *in vivo* diagnostics. Utilizing this platform, multiple fluorophores can be loaded within a single particle allowing the light absorption and emission properties of the nanoparticle to be controlled independent of particle size. Furthermore, such dyed nanoparticles may have extinction coefficients as high as about 100×10^6 L mol^{-1} cm^{-1} in the NIR (on a per mole of particles basis), with quantum yields from about 8–10%. A simple synthetic method for varying particle size and dye-loading level is presented, and a modified Stober synthesis reduces deleterious exposure of the dye to the highly alkaline conditions used. The cyanine dyes are encapsulated in silica in a non-aggregated state and the fluorescence brightness is largely maintained to nominal dye concentrations approaching 50 μM. The ability to control light absorption and emission properties independent of particle size, and convenient access to particle sizes in the range of 20–100 nm (a size regime difficult to access with other nanoparticle approaches such as quantum dots), are important features for anatomical targeting in *in vivo* diagnostics and targeted therapeutic applications.

Keywords: Near-infrared dyes; Nanoparticles; Fluorescent particles; Silica

1. Introduction

Optical methods of imaging and diagnosing human disease have undergone a renaissance in recent years as a result of convergent advances in the understanding of the optical properties of biological tissue, near-infrared laser technology, image reconstruction algorithms and the development of light-emissive "probes" that may potentially be administered *in vivo* [1]. Optical-based methods utilizing the near-infrared spectral region are particularly important because biological tissue is relatively transparent in the 650–900 nm wavelength range [1f], minimizing complications resulting from scattering and absorption and maximizing deep tissue imaging sensitivity. Many types of near-infrared (NIR) luminescent materials are being developed for this field including organic dye fluorophores [2], rare-earth emitters [3], inorganic nanoparticles such as quantum dots [4], gold and silver nanoparticles [5], fluorophore-tagged polymers [6], and fluorescent and bioluminescent proteins [7]. Currently, the organic fluorophore indocyanine green (ICG) is the only NIR fluorophore approved by the FDA for use *in vivo* in humans [1c]. ICG, however, whilst having the required safety-profile and photophysical properties has several inherent limitations, most notably poor photostability [1b], poor hydrolytic stability and a low fluorescence quantum yield, particularly in aqueous media.

Nanoparticle-based approaches to optical probe design present unique opportunities because such particles may potentially carry multiple fluorophores or be composed entirely of a luminescent material, and further may carry molecules rendering them capable of evading the human immune system and even recognizing (targeting) diseased cells. By far the most extensively explored nanoparticle approach has been based upon quantum dots (QDs) composed of II–VI and III–V semiconductors [4,8]. QDs are highly luminescent and relatively photostable and many reports have appeared regarding their application as *in vitro* and *in vivo* diagnostic tools. However,

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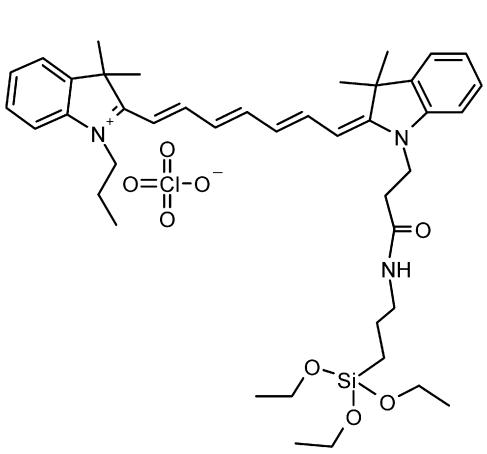
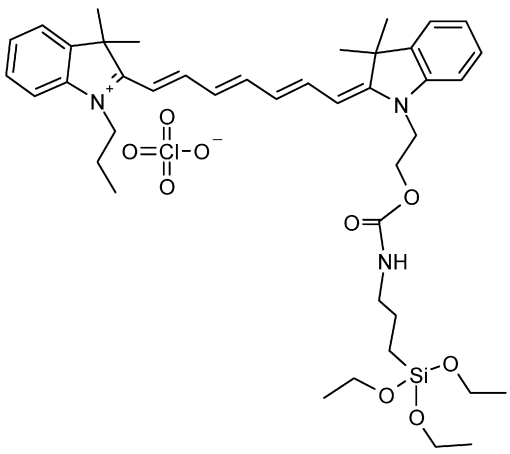


Dye 1



Dye 2

QDs, whilst having many remarkable properties, are arguably not ideally suited for *in vivo* applications. This is true primarily because *in vivo* imaging requires that both the excitation and emissive wavelengths be in the NIR, as visible excitation wavelengths will readily be absorbed by biological tissue. QDs show diminishing molar absorptivity in the NIR [4b,8] as well as diminishing fluorescence quantum yields as the emission is pushed into the NIR [8]. Their absorption and emission properties are also strongly dependent upon particle size and cannot be varied independently [8]. Furthermore, QDs are difficult to surface functionalize [4c], and possible toxicological issues associated with their chemical compositions may prevent their application in *in vivo* diagnostics [4d]. Recent progress toward these limitations was reported by Bawendi et al. [4b] who showed that $InAs_{1-x}P_x$ QDs had improved absorption properties in the NIR, albeit at a lower quantum efficiency (3.5%), and further progress was made by Aharoni et al. [4e] who demonstrated NIR-QDs with quantum efficiencies from 30–70%. Other examples of NIR based nanoparticle approaches include emissive polymerosomes [6a], and cyanine-dye labeled core/shell microgels [6b].

Another approach to building nanoparticle-based luminescent probes involves the encapsulation of luminescent dyes and other materials into silica nanoparticles. Silica represents a convenient platform for probe design as well-controlled, nearly monodisperse and stable nanoparticle suspensions can be readily synthesized via the Stober method [9]. Since the seminal work of van Blaaderen et al. [10], many groups have shown that visible-wavelength fluorophores can be encapsulated in silica and that their quantum yield, photostability and several other properties can be improved [11]. However, to our knowledge NIR fluorophores have not been successfully encapsulated into silica nanoparticles, presumably because they do not survive the very alkaline conditions of the Stober process. Del Monte et al. [12] have reported the encapsulation of NIR fluorophores in dried silica xerogels, without the addition of acid or base catalyst necessary to produce nanoparticle dispersions.

We show the first example of NIR cyanine fluorophores (1 and 2) encapsulated into silica nanoparticles, providing a highly versatile and unique platform for *in vivo* diagnostics. Utilizing this platform, multiple dye molecules can be loaded within a single particle and the absorption and emission properties of the nanoparticles can be controlled independent of particle size. Furthermore, the dyed nanoparticles may have extinction coefficients as high as about 100×10^6 L mol^{-1} cm^{-1} in the NIR (at 750 nm), making them about 100 times more absorptive than QDs (e.g., CdQ (Q = Se, Te) or $InAs_xP_{1-x}$) in this region. Preliminary data show that the quantum yield of the encapsulated cyanine fluorophore in NIR-SiO_2 is around 7 to 14% (solvent dependent).

2. Experimental

2.1. Materials and methods

Tetraethoxyorthosilane (TEOS), 99.999%, was purchased from Aldrich Chemical Co. and used as received. Anhydrous ethanol was dried over molecular sieves before use. The synthesis of the two unsymmetrical dye precursors 1 and 2 will be published elsewhere [13].

2.2. Preparation of NIR-SiO_2

Sample (a): A dye solution was prepared by dissolving 0.020 g of 2 in 500.00 mL of anhydrous ethanol (49.6 μM). To a 500 mL Erlenmeyer flask with stir bar was then added 200.0 mL of the dye solution and the temperature increased to 65 °C using a controlled (±0.2 °C) temperature bath. To the stirred solution was then added 7.62 mL (34.2 mmol) TEOS followed by 12.0 mL distilled water and 6.40 mL of a 28% ammonia solution in water. The contents were stirred at this temperature for 3.0 h and the solution cooled to 15 °C. The contents were then poured into 200.0 mL ethanol. Ethanol and water were removed by rotary evaporation to a volume of about 100 mL, and additional anhydrous ethanol was then added so that the final volume was 250.0 mL.

Samples (b)–(d) were prepared in an identical manner to sample (a) except that a dye solution of 1 was prepared by dissolving 0.160 g of dye in 1.00 L anhydrous ethanol (200.4 μM); aliquots of the dye solution were then diluted with anhydrous ethanol to a total volume of 200.0 mL so that the concentration of 1 was in each case 66.7 μM. The synthetic conditions and