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Review

Luminescent terbium complexes: Superior Förster resonance energy transfer donors for flexible and sensitive multiplexed biosensing

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ABSTRACT

Optical quantification of several biomarkers at very low concentrations and nanometric distances has become an important requirement for many biosensing applications. Förster resonance energy transfer (FRET) and, in particular, luminescent terbium complex (LTC)-based FRET, is a valuable tool for sensitive and versatile multiplexed FRET. Here, we review recent progress in the development of novel LTC-FRET photonic sensors for ultra-sensitive and multiplexed diagnostics of various biomarkers and distances (molecular ruler) in different biological systems. The basic concept of FRET, the exceptional photophysical properties of LTCs, and possibilities and opportunities for multiplexed optical sensing are outlined. Sophisticated FRET systems such as multiplexed LTC-to-dye FRET, LTC-to-quantum dot (QD) FRET, and LTC-to-QD-to-dye FRET relays have been assembled with biological recognition molecules such as antibodies, peptides, and oligonucleotides to permit biosensing applications in the form of homogeneous immunoassays, DNA hybridization and enzyme assays, and molecular logic devices. A perspective on the emerging field of multiplexed LTC-based FRET biosensing is given at the end of this review to highlight the promising future of these nanometric biosensors.

Abbreviations: A, acceptor; AF, Life Technologies AlexaFluor dyes; AFP, alpha-fetoprotein; APC, allophycocyanin; BHQ, Black-Hole quencher; CA15.3, Carbohydrate Antigen 15.3; CARM1, coactivator-associated arginine methyltransferase 1; CEA, carcinoembryonic antigen; Cy, GE Healthcare cyanine dyes; Cyfra21-1, Cytokeratin-19 Fragment 21-1; *D*, donor; DLS, dynamic light scattering; DNA, deoxyribonucleic acid; Dy, dysprosium; EGFR, epidermal growth factor receptor; ELISA, enzyme-linked immunosorbent assay; ER, estrogen receptor; Eu-TBP, europium trisbipyridine cryptate; Eu, europium; $F(ab')_2$, antigen-binding fragment consisting of two F(ab) fragments; F(ab), antigen-binding fragment of an IgG antibody; FP, fluorescent protein; FRET, Förster resonance energy transfer; GFP, green fluorescent protein; HTS, high-throughput screening; IgG, Immunoglobulin G antibody; LOD, limit of detection; LTC, luminescent terbium complex; MLD, molecular logic device; NSE, neuron specific enolase; OG, Life Technologies OregonGreen dye; PEG, polyethylene glycol; PL, photoluminescence; PRB, probe ssDNA; PSA, prostate specific antigen; QD, quantum dot; RNA, ribonucleic acid; SCC, squamous cell carcinoma antigen; Sm, samarium; SNAPFL, semi-naphthalene fluorescein; ssDNA, single-stranded DNA; Tb, terbium; TEM, transmission electron microscopy; TGT, target ssDNA; TR-FIA, time-resolved fluoroimmunoassay; V_HH, single-domain antibodies (or nanobodies); Zn, zinc.

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1. Introduction

Technological advances in the bio- and nanosciences have fostered new methods for detecting a myriad of different chemical and biological processes and interactions at extremely low concentrations and extremely short distances [1–3]. Among these advances, luminescent lanthanide coordination compounds containing the trivalent ions of Eu, Tb, and, less frequently, Sm and Dy, have played an important role in developing very sensitive, time-resolved photoluminescence (PL) biosensing because of their exceptional photophysical properties [4–7]. Well-designed ligand structures (e.g., chelates or cryptates) coordinate a central lanthanide ion and serve as both a light absorbing "antenna" and a "cage" to protect against PL quenching from the outer environment. The antenna structure should be a chromophoric unit with strong absorption and the ability to efficiently transfer the absorbed energy to the central lanthanide ion. The antenna can be incorporated into the cage structure, bind to the cage, or coordinate directly to the lanthanide ion in parallel with the cage. In most cases, the ligand is designed to occupy all of the coordination sites of the lanthanide ion in order to suppress PL quenching from water molecules. However, when a separate antenna unit needs to coordinate to the lanthanide ion, or when coordination of an analyte to the lanthanide ion produces a signal change suitable for sensing, less than full coordination is more appropriate. Once sensitized by the antenna, lanthanide ions provide extremely long excited-state lifetimes (up to milliseconds) and multiple narrow emission bands in the visible region of the spectrum. The long PL decay times allow for efficient suppression of short-lived autofluorescence background from the sample or from other fluorophores by using pulsed excitation and time-gated detection (i.e., measuring the long-lived PL intensity decay in a time-window that opens after the other fluorescent components have already decayed). The emission spectra of lanthanide ions are usually red-shifted by more than a hundred nanometers from the ligand absorption (a large *effective* Stokes shift), which, when combined with time-gating, allows detection of lanthanide emission with almost no background from excitation light. These features enable the very high sensitivity of luminescent lanthanide biosensors.

To exploit luminescent lanthanide complexes for biosensing, Förster resonance energy transfer (FRET) has frequently been utilized and provides two important capabilities. First, when lanthanide complexes are paired as FRET donors with different fluorophores as FRET acceptors, nanometric distance information can be obtained from luminescence measurements [8–13]. Second, FRET biosensors can offer on/off signaling for quantitative analysis of biological processes such as cellular signaling, ligand–receptor binding, protein–protein interactions, DNA/RNA hybridization, and enzymatic reactions [14–30]. Although lanthanide-based FRET biosensors have been reported and applied for decades [5,31–38], and despite the more recent development of lanthanide-based nanoparticles [39], increasing demand for the simultaneous measurement of multiple biological parameters (e.g., concentrations or distances) in a single sample, so-called multiplexing, has stimulated the renewed interest in novel lanthanide-based FRET biosensing approaches. In preference to other lanthanide ions, luminescent Tb complexes (LTCs) have been the major players in this field because of their large PL quantum yields, extremely long PL lifetimes, and multiple spectrally resolved emission bands. Although Eu complexes are also very bright emitters in the visible spectrum, and have arguably been applied for biosensing more often than Tb complexes (because of fewer problems with back-energy transfer from the ion to ligand), the emission bands of Tb are much better separated and therefore superior for spectral multiplexing. In this article, we review recent developments and applications of multiplexed Tb-based FRET biosensors, emphasizing our own research program that focuses on the development of multiplexed molecular recognition and interaction assays. Although there are several very interesting optical imaging applications using LTC-based biosensors and FRET [40–48], this topic has been recently reviewed elsewhere [49] and will not be discussed here. Nevertheless, the recent advances in LTC-based FRET probes and their many advantages for biosensing provide good evidence that multiplexed LTC-based FRET imaging techniques will be available in the near future.

2. LTCs and FRET

Many recent reviews and textbooks cover the general theory and applications of FRET [8,9,11,13–17,19,30,32], including the details for lanthanide complexes in particular [31,32,49]. In order to understand the theoretical background of the multiplexed Tb-based FRET applications presented in this article, we briefly review the most important concepts in this section.

FRET is a non-radiative energy transfer process between two molecules or particles. The energy donor (D) is a luminophore (e.g., organic dye, fluorescent protein (FP), quantum dot (QD), lanthanide complex) in an electronically excited state. FRET can occur if the electronic transitions from this excited state to lower-lying states are in energetic resonance with the electronic transitions, from the ground state to higher-lying states, of a suitable acceptor (A) in close proximity to D (ca. 1–20 nm). This resonance condition requires spectral overlap between D emission and A absorption. A can be a luminophore or a non-luminescent FRET quencher (e.g., Black-Hole quencher or gold nanoparticle). The FRET efficiency (η_{FRET}) is usually determined by the D–A distance, r, and the Förster distance or Förster radius, R_0 (which can be calculated by the D–A orientation, the emission and absorption spectra of D and A, and the luminescence quantum yield of D), or by luminescence intensities (I) or decay times (τ) of D in the absence (subscript D) and in the presence (subscript DA) of A (Eq. (1)).

$$\eta_{FRET} = \frac{1}{1+(r/R_0)^6} = \frac{R_0^6}{R_0^6+r^6} = 1 - \frac{I_{DA}}{I_D} = 1 - \frac{\tau_{DA}}{\tau_D} \quad (1)$$

The Förster distance R_0 (or Förster radius) can be calculated by Eq. (2).

$$R_0 = 0.02108(\kappa^2 \Phi_D n^{-4} J)^{1/6} \quad (2)$$

where κ^2 is the orientation factor between the emission and absorption transition dipole moments of D and A, respectively, Φ_D is the donor PL quantum yield, n is the refractive index of the solvent, and J (in $M^{-1}\,cm^{-1}\,nm^4$) defines the spectral overlap of D emission and A absorption using the intensity normalized emission spectrum of D ($\bar{I}_D(\lambda)$ where $\int \bar{I}_D(\lambda) = 1$) with and the molar absorptivity (or extinction coefficient) spectrum of A ($\varepsilon_A(\lambda)$) as defined by Eq. (3).

$$J = \int \bar{I}_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (3)$$

LTCs such as the macrocyclic ligand-based Lumi4–Tb complex developed in Raymond's group (Fig. 1) [50] are usually applied as FRET donors. There are four major advantages that distinguish LTCs significantly from other FRET donors.

2.1. Unpolarized emission

Tb possesses multiple emission transition dipole moments (unpolarized emission) and therefore a dynamic averaging can be applied for the orientation factor κ^2. Thus, even with a FRET acceptor having a fixed transition dipole moment, κ^2 is limited to values between 1/3 and 4/3. In many biosensing applications, the acceptor (labeled to a biomolecule) has fast isotropic rotation and the