Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Recent advances in homogenous immunoassays based on resonance energy transfer

Kristiina Takkinen[1] and Aurelija virblienė[2]

Development of homogeneous immunoassays based on Förster resonance energy transfer (FRET), chemiluminescence resonance energy transfer (CRET) or bioluminescence resonance energy transfer (BRET) enables a one-step, rapid and direct detection of analytes as compared to the multistep, time-consuming heterogeneous immunoassays. Antibody fragments such as Fab or $F(ab)_2$ are extensively exploited in both competitive and non-competitive formats to circumvent the size limitations characteristic of full-length antibodies. Semiconductor fluorescent nanocrystals, quantum dots are becoming increasingly popular as energy acceptors due to their beneficial optical properties as compared to organic dyes. These and other technical advances open new ways for using homogenous immunoassays in a variety of bioanalytical applications including detection of protein biomarkers, hormones, drugs of abuse, food and environmental toxins.

Addresses
[1] VTT Technical Research Centre of Finland Ltd, P.O. Box 1000, Espoo FI-02044 VTT, Finland
[2] Institute of Biotechnology, Life Sciences Center, Vilnius University, Sauletekio av. 7, Vilnius LT-10257, Lithuania

Corresponding author: virblienė, Aurelija (aurelija.zvirbliene@bti.vu.lt)

Current Opinion in Biotechnology 2019, **55**:16–22

This review comes from a themed issue on **Analytical biotechnology**

Edited by **Saulius Klimasauskas** and **Linas Mazutis**

https://doi.org/10.1016/j.copbio.2018.07.003

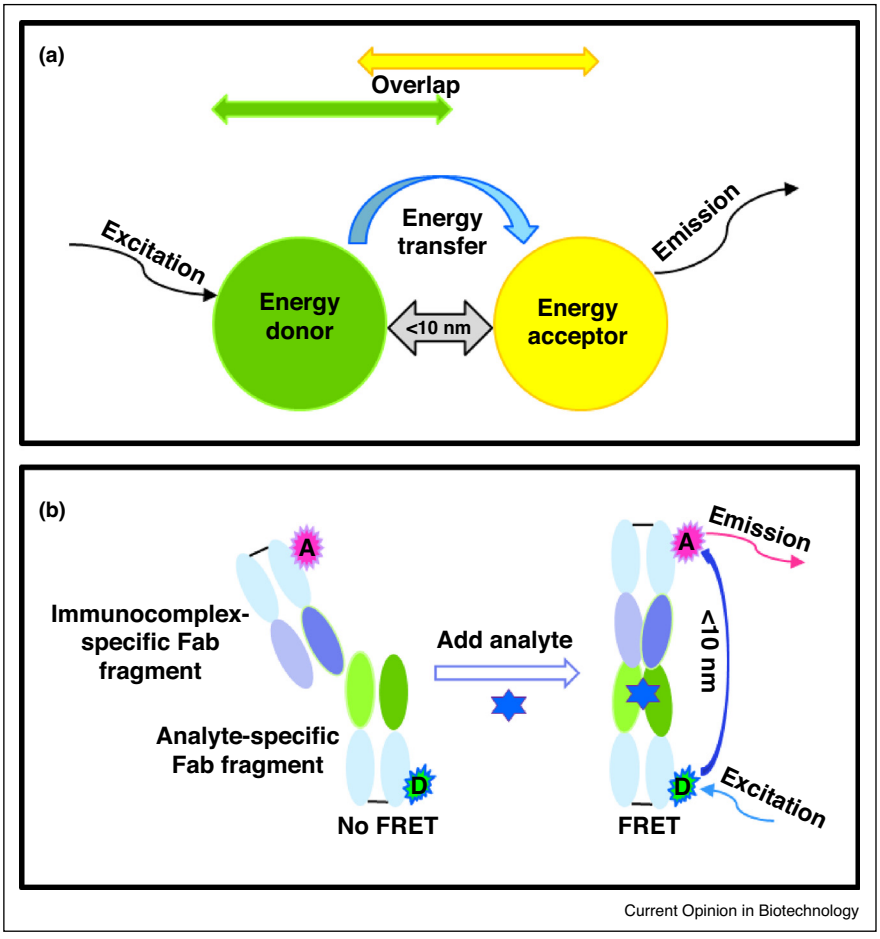
Introduction

Immunoassays are based on antibody–antigen interactions that allow highly specific detection of the analytes. Heterogeneous immunoassays such as ELISA are most widely used in bioanalysis and diagnostics. However, they require multiple incubation and washing steps. In contrast, homogenous immunoassays can be performed directly in solution and require only minimal expertise of the end-user. Recent development of homogenous immunoassays based on resonance energy transfer (RET) enables single-step, rapid and direct detection of the analytes both in a competitive and non-competitive formats. RET involves a non-radiative energy transfer from an energy donor to a fluorescent or nonfluorescent energy acceptor within a suitable distance, usually less than 10 nm. Molecular binding events such as antibody–antigen interactions induce RET signal by changing the distance between the donor and acceptor molecules used as labels. Förster resonance energy transfer (FRET) is based on energy transfer from an excited donor fluorophore to a proximal ground-state acceptor fluorophore [1]. Chemiluminescence RET (CRET) is based on energy transfer from a chemiluminescent donor to a suitable acceptor molecule without an external excitation source [2]. In bioluminescence RET (BRET), sensor proteins are designed such that their interaction induces energy transfer between the light-emitting enzyme and the acceptor fluorophore [3]. Recent advances of the exploitation of FRET, CRET and BRET in homogeneous one-step immunoassays for small analytes and protein biomarkers are reviewed here.

FRET-based immunoassays

FRET based on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule is widely applied in reliable one-step assay formats for protein biomarkers, drugs, environmental and food toxins [1,4,5]. Organic dye molecules are traditional FRET fluorophore pairs with overlapping donor emission and acceptor excitation spectra leading in close proximity (<10 nm) of the labels to observation of the acceptor fluorescence (Figure 1a) [[20•]]. The spectral properties of the FRET donor–acceptor complex enable direct detection of the acceptor emission without a separation step from the unbound partners. The transient background fluorescence of sample molecules present in complex media such as serum or cell lysates can be eliminated using long-lived fluorophore labels and time-resolved FRET. During the past decade, quantum dots (QDs), highly photostable nanocrystals of semiconducting materials with a diameter size of 2–10 nm, have been exploited extensively as labels in FRET [5–8]. Properties of organic dyes and QDs, their application potential and different FRET detectors have been compared in detail in [[20•],7]. Although having beneficial optical properties as compared to organic dyes, QDs pose more challenges in terms of solubility, bioconjugation, particle size and steric properties [9]. Reliable, quick on-site detection of disease biomarkers, pharmaceuticals, drugs of abuse and toxins is a rapidly growing sector addressing the needs of point-of-care, environment and food safety fields. FRET-based one-step immunoassays provide an excellent basis for the development of user-friendly, reliable on-site tests

Figure 1



The principle of FRET (a) and an example of FRET-based homogenous immunocomplex assay for small analytes (b). **(a)** FRET occurs when a donor fluorophore and an acceptor fluorophore are in close proximity to each other (<10 nm) and the emission spectrum of the donor and the excitation spectrum of the acceptor are overlapping; **(b)** schematic presentation of the homogenous one-step FRET immunoassay for a small analyte [10]. The primary, analyte-specific Fab fragment is labelled with a donor fluorophore and the immunocomplex-specific Fab fragment is labelled with an acceptor fluorophore. FRET occurs only when the immunocomplex-specific Fab fragment binds to the primary Fab fragment-analyte complex bringing the donor and acceptor fluorophores in close proximity to each other. In the absence of antibody–antigen interaction, only the emission of the donor fluorophore is observed.

for non-professional end-users. A novel one-step time-resolved FRET-based sandwich immunoassay principle for a sensitive detection of drugs of abuse from saliva samples has been developed based on recombinant antibodies and morphine as the model analyte [10]. A highly specific antibody fragment against the immune complex formed between the recombinant anti-morphine Fab fragment and morphine was selected from a naïve antibody phage display library. In the FRET-based immunoassay, the antibody fragments labelled with organic dyes were incubated with saliva samples spiked with morphine, codeine, or heroin. In this one-step assay, 5 ng/mL of morphine was detected already after 2 min of incubation without any cross-reactivity to structurally homologic opiates, codeine or heroin. The small size of recombinant Fab fragments (~5 nm) enables development of sensitive immunocomplex-based FRET assays for small analytes, such as morphine, buried deeply in the binding pocket leading to a compact packing of the fluorophore-labelled antibody fragments (Figure 1b). Significant benefits of this rapid immunocomplex-based assay for small analytes are the high specificity, sensitivity and increased readout signal with increased analyte concentration. Later this principle has been applied for a sensitive detection of (−)-Δ9-tetrahydrocannabinol (THC) from saliva samples [11], mycotoxin from wheat extracts [12], and in a slightly modified format — for detection of cyanobacterial microcystins and nodularins from water samples [13•,14]. Many recent review articles describe the advantages of combining lanthanide (europium, terbium) complexes as long-decay-time donors with QDs as strong-absorption acceptors [15–17]. The