Appl Microbiol Biotechnol (2012) 96:895–902
DOI 10.1007/s00253-012-4449-6

MINI-REVIEW

Fluorescence resonance energy transfer (FRET)-based biosensors: visualizing cellular dynamics and bioenergetics

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Received: 3 August 2012 / Revised: 17 September 2012 / Accepted: 17 September 2012 / Published online: 6 October 2012

Abstract Förster (or fluorescence) resonance energy transfer (FRET) is a process involving the radiation-less transfer of energy from a "donor" fluorophore to an "acceptor" fluorophore. FRET technology enables the quantitative analysis of molecular dynamics in biophysics and in molecular biology, such as the monitoring of protein–protein interactions, protein–DNA interactions, and protein conformational changes. FRET-based biosensors have been utilized to monitor cellular dynamics not only in heterogeneous cellular populations, but also at the single-cell level in real time. Lately, applications of FRET-based biosensors range from basic biological to biomedical disciplines. Despite the diverse applications of FRET, FRET-based sensors still face many challenges. There is an increasing need for higher fluorescence resolution and improved specificity of FRET biosensors. Additionally, as more FRET-based technologies extend to medical diagnostics, the affordability of FRET reagents becomes a significant concern. Here, we will review current advances and limitations of FRET-based biosensor technology and discuss future FRET applications.

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Keywords Förster resonance energy transfer (FRET) · Biosensors · Imaging · Fluorescence · Medical diagnostics

Introduction

Since the emergence of the fluorescence protein in 1992, fluorescence-based biosensors have become critical players in monitoring and identifying cellular molecular dynamics, cellular physiology, and cell–cell interactions, or, more simply, what occurs inside and outside of the cell (Periasamy 2001). Förster or fluorescent resonance energy transfer (FRET) is a process involving the radiation-less transfer of energy from a "donor" fluorophore to an "acceptor" fluorophore. FRET is commonly utilized to demonstrate near-field communication or interaction between two molecules (Day et al. 2001). FRET-based assays are able to transduce a near-field interaction into a far-field signal, a unique optical tool to assess biological phenomena well below the resolution of standard optical microscopy (Roy et al. 2008; Moerner and Fromm 2003). In essence, FRET magnifies microscopic conformational changes by emitting light, which can be captured by a sensor (Ha et al. 1996). Governed by the physics of molecular proximity, FRET can only allow energy transfer to occur when the distance between the donor and the acceptor fluorophores are within 1–10 nanometers (nm) of each other. An excited fluorophore emits an essentially virtual photon, which is then absorbed by a receiving fluorophore. Due to the conservation of energy and momentum, FRET is a mostly radiation-less event where the transfer is largely undetectable (Merchant et al. 2007; Kapanidis and Weiss 2002).

The donor fluorophore and the acceptor fluorophore can be brought together in several ways. For example, one fluorophore can be attached to a substrate while the other could be attached to its binding site, and once bound the

fluorophores would be in close enough proximity to interact via FRET (Joo et al. 2007). Alternatively, two fluorophores can be attached to a single protein and, due to environmental constraints, force the protein to alter its conformation and initiate FRET (Kajihara et al. 2006). Another means through which FRET is stimulated and utilized to monitor cellular dynamics occurs when the FRET fluorophores are simultaneously attached to a molecule at close positions to continually engage in FRET; this interaction will cease when a cellular process causes a cleavage somewhere on the binding site of one of the fluorophores, separating it from the other fluorophores and decreasing the ability of FRET to occur (Kohl et al. 2002).

The ability of FRET to occur is heavily dependent on the FRET efficiency, *E*. *E* is essentially the measurement of every incident of energy transfer during every incident of donor excitation. The efficiency depends on the overlap of the donor and acceptor spectra and the orientation of the dipole moments of the donor and acceptor (Ansbacher et al. 2012). FRET efficiency is also dependent on the distance R_0 between the donor and acceptor. The relationship between FRET efficiency and R_0 is elucidated by the following equation:

$$E = 1 \Big/ \left[1 + (R/R_0)^6\right]$$

where R_0 is the Förster's radius, or the distance between the fluorophores at which E=50 %, and where

$$R_0 = 8.79 \times 10^{-5} \times \left[n^{-4} \times Q \times \kappa 2 \times J(\lambda)\right]$$

R_0 is in Angstrom, n is the refractive index of medium in the range of overlap, Q is the quantum yield of the donor in the absence of acceptor, and $J(\lambda)$ is the spectral overlap. $\kappa 2$ is the orientation factor of the two dipoles. $\kappa 2 = [\cos \theta T - 3 \cos \theta A \cos \theta D]$, where θT is the angle between donor (D) and acceptor (A) moments. The dynamic value of $\kappa 2$ is commonly 2/3 for freely rotating donor and acceptor fluorophores (Jares-Erijman and Jovin 2003; Hoppe et al. 2002).

Usually, the donor and acceptor fluorophores are characteristically different, particularly in emission. In this case, FRET can be detected either by monitoring the fluorescence readout of the acceptor or by acceptor quenching. Both of these cases result in a decrease in the amount of donor fluorescence. FRET donor fluorophores are always fluorescent. Upon excitation, the electrons of the donor fluorophore jump from their ground state to a higher energy level. As they return to the ground state, a particle of light known as a photon is emitted. During FRET, the photon is not emitted from the donor; rather, the energy is transferred from the donor molecule to the acceptor molecule, exciting the acceptor's electrons and causing the acceptor to emit a photon instead (Meyer and Teruel 2003). FRET quenchers have been developed to reduce fluorescence bleed-through between donor and acceptor. Much like traditional FRET-based fluorophores, FRET quenchers act as FRET acceptors; they are capable of similar energy acceptance, but instead of returning to the ground state from the excited state with the emission of light, quenchers return to the ground state via non-radiative decay pathways. Quenchers have a wide absorption spectrum and a high extinction coefficient (Hangauer and Bertozzi 2008; Leriche et al. 2012; Ogawa et al. 2009).

FRET has been a key tool in analyzing biological systems for decades, but it continues to be faced with challenges. In the remainder of this review, we will discuss the current applications of FRET ranging from basic science to biomedicine. We will also address current limitations and possible routes to overcome them.

Design and limitations of FRET probes

Although it is an incredibly useful tool, FRET is not without shortcomings. The first problem that researchers must address is that the donor fluorophore emission range (how much energy is emitted from an excited donor) and the acceptor fluorophore excitation range (how much energy is necessary to excite an acceptor) have to overlap by at least 30 % (Lovell et al. 2009). This means that there are a limited number of florescent tag proteins that can be used in combination with each other. This can hinder the ability to use this technology on certain sensitive cellular mechanisms. Pairs of organic fluorescent tags are often classified as "FRET pairs" (Ai et al. 2008). The combination of cyan fluorescent protein (CFP)- and yellow fluorescent protein (YFP)-labeled fusion proteins has been widely used for FRET measurements in living cells. Other donor and acceptor fluorophore pairs that have been used for FRET include CFP and dsRED, BFP and GFP, GFP (or YFP) and dsRED, Cy3 and Cy5, Alexa488 and Alexa555, Alexa488 and Cy3, and FITC and rhodamine. These pairs are only some of the many probes commonly used in FRET-based assays (Wiedenmann et al. 2009).

There is also a phenomenon called auto-fluorescence that can interfere with the FRET data. When mitochondria and lysosomes are excited by light photons, they release energy in the form of photons, causing auto-fluorescence (Sturmey et al. 2006). This radiation will be measured by sensors in addition to the radiation produced by the cellular mechanisms of interest and can produce significant error when trying to monitor cellular activity. The additional light may cause researchers and clinicians to believe a certain metabolic pathway is changing or not changing when the opposite is actually occurring.

Another disadvantage to the use of fluorophores is that they have a short fluorescence lifetime. This means that once they are excited, they stay excited for only a very short period of time. Because of the brevity of the opportunity to measure fluorophore emissions, auto-fluorescence can take

up a significant percentage of the recorded emissions instead of the emissions produced by the cellular process of interest (Davydov et al. 2008; Fernando et al. 2006). To help alleviate this problem, FRET quenchers were developed to replace certain acceptor fluorophores. These quenchers do not have emission spectra themselves and can transfer the energy they absorb without producing any visual "noise" that can make the data harder to interpret. This also allows for multiple acceptor fluorophores to be used for one assay, greatly increasing efficiency. One further benefit is that these quenchers are more resilient and are therefore easier to synthesize (Linder et al. 2011).

There are many different types of quenchers, just as there are many different types of fluorophores. One of the first reported quenchers was the azobenzene dye, Dabcyl. With a broad absorbance centered around 478 nm, Dabcyl is ideal for quenching dyes by FRET that fluoresce in the blue to green region, such as EDANS or fluorescein (Marras et al. 2002). Recently, the development of quenchers that fall into the red and near-infrared dyes, known as Black Hole Quenchers (BHQs), has further enhanced FRET. BHQ dyes are robust dark quenchers. They exhibit excellent coupling efficiencies, have large extinction coefficients and, like other quenchers, are non-fluorescent. The absorbance capability of BHQ dyes enables BHQs to quench a variety of popular fluorophores, including Cy3 and Cy5 (Johansson 2006).

It is important to note that FRET fluorophores must have proper excitation and emission spectra as well as no effect on the three-dimensional structure of the molecule(s) to which they are attached. For large macromolecules such as large proteins, the aforementioned stipulations are not significant problems, but it is not uncommon for small changes in the conformation of a macromolecule to alter both its structure and behavior (Bujalowski and Jezewska 2012).

Another important concern with regards to the detection of FRET involves analyte concentration (Long et al. 2012). Only molecules that interact with one another will result in FRET. If large amounts of donor and acceptor molecules are present, but do not interact, the amount of FRET taking place would be quite low. In this example, while the donor and the acceptor molecules may be very easy to detect separately, the actual amount of FRET activity may not be sufficient to detect. There are also limitations in the development of more sensitive fluorogenic probes capable of greater solubility, greater absorption coefficients, and greater pairing compatibilities (Kwak et al. 2010). One approach to overcome these current probe limitations is the use of quantum dots, the excitation of which in the ultra violet range does not produce any excitation of its coupled fluorescent protein acceptor (mOrange). Thus quantum dots prolong the functional life and thus the visibility of the fluorescent protein of interest (Liu et al. 2011).

Designing FRET experiments has limitations. The most obvious limitation of current FRET models is the necessity of a close physical proximity between donor and acceptor probes for FRET to even occur. Depending on the assay design, close proximity will either be established or removed during the assay, resulting in a change in signaling that can be measured. Additionally, appropriate donor and acceptor pairs need to have enough spectral overlap for efficient energy transfer to take place or have enough of a difference in spectrums to be visually distinguishable from one another. The choice of filters for fluorescent wavelength selection is also critical, as the excitation filter for the donor has to be able to selectively excite the donor while minimizing excitation of the acceptor. Furthermore, the insertion of biosensors into live cells has had an efficiency rating of around 20–30 % (Komatsua et al. 2011).

FRET-based assays: from the (lab) bench to the (hospital) bedside

The FRET mechanism can be used to monitor cellular activity in a variety of ways. There are many different types of changes occurring in a cell that involve different classes of molecules. FRET donor and acceptor fluorophores have been conjugated to a variety of biomolecules creating functional assays such as protein–protein binding, antigen–antibody binding, ligand–receptor binding, DNA or RNA hybridization and DNA or RNA-protein binding (Okamura and Watanabe 2006; Howell 2006; Li et al. 2011). A recently developed FRET-based biosensor assay is capable of detecting in vivo Bcr-Abl and epidermal growth factor receptor activity and its inhibition by tyrosine kinase inhibitors in real time (Waterhouse et al. 2011). This assay utilizes a specific SH2-phosphotyrosine module separated by a flexible linker, and flanked at each termini by CFP and YFP. When the biosensor is phosphorylated, FRET occurs, and when the biosensor is dephosphorylated, the FRET diminishes. These FRET-biosensor probes can also detect changes in tyrosine kinase activity. A FRET-based assay has also been developed to monitor ligand-dependent interactions of estrogen receptor isoform, ERα and Sp1 in MCF-7 breast cancer cells. Chimeric ERα and Sp1 proteins fused to CFP and YFP have been transfected into MCF-7 cells, and a FRET signal was induced after treatment with 17β-estradiol, 4′-hydroxytamoxifen, or ICI 182,780 (Kim et al. 2005).

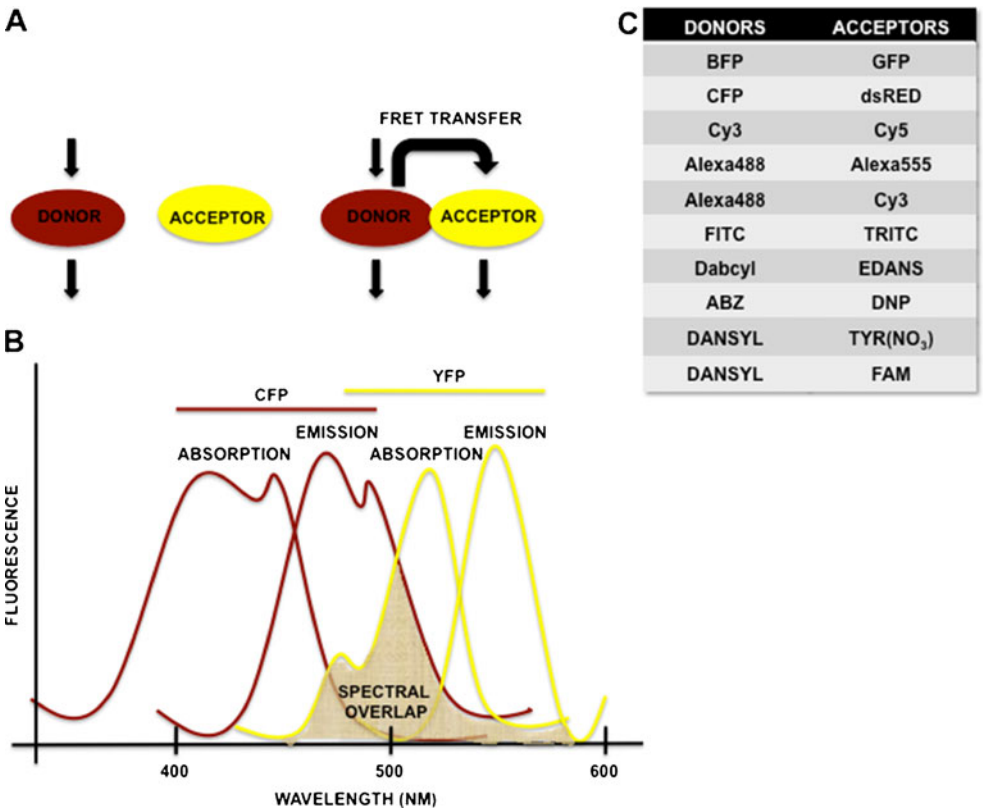
Oligonucleotides (short nucleic acid polymers) with DNA or RNA backbones, labeled with one or several FRET probes, can also form FRET systems. FRET can occur when the oligonucleotide duplex is formed, bringing the donor and acceptor dyes in close proximity. Alternatively, the hairpin configuration of the oligonucleotide labeled dually with both donor and acceptor fluorophores can result in FRET (Tsuji et al. 2001). DNA or RNA-based FRET probes are used in vitro and in vivo in a plethora of applications that monitor various types of DNA and RNA reactions including

PCR, hybridization, ligation, cleavage, recombination, and synthesis (Cardullo et al. 1988; Li et al. 2011; Dietrich et al. 2002). This DNA or RNA FRET-based assay essentially utilizes two oligonucleotide probes hybridized to adjacent DNA or RNA sequences. The probes are end-labeled with FRET probes so that one has a 3′ donor and the other has a 5′ acceptor, or vice versa. Conformation-wise, the probes can be linear, hairpin-shaped, or have more complex configurations. The FRET system is formed after the hybridization of the oligonucleotide probes, which signals the completion of the reaction by quenching the donor and the sensitization of the acceptor fluorescence (Lassaunière et al. 2010). The introduction of FRET-based hybridization probes greatly improves DNA and RNA hybridization biotechnology. FRET-based hybridization assays are speedy and simple assays that enable the signal from the ground state probes to be quenched, permitting observations of hybridization reactions in real time.

FRET-based assays have also been developed to monitor DNA methylation status (Bellau-Pujol et al. 2005; Bailey et al. 2009). A study used genomic DNA from cancer cells and pretreated it with a methylation-sensitive restriction endonuclease, an optically amplifying cationic conjugated polymer, followed by polymerase chain reaction (PCR) amplification, in the presence of fluorescein-labeled dNTP and Taq polymerase. The PCR only occurred for methylated DNA. DNA methylation of the gene sequence of interest is detected as a resulting FRET.

Oligonucleotides with a DNA backbone and one or several chromophore tags have multiple applications as FRET probes. They are especially advantageous for the real-time monitoring of biochemical reactions and in vivo studies (Kehl and Kumar 2009; Hangauer and Bertozzi 2008). FRET has also been utilized to detect DNA hybridization in a microfluidic channel (Crivat et al. 2010; Long et al. 2012). Antibiotics targeting the bacterial rRNA A-site have also been developed by incorporating a new emissive surrogate into the RNA and labeling the aminoglycosides with an appropriate fluorescence acceptor (Means et al. 2005). This allowed for real-time detection of RNA-small molecule binding.

Recently, Marshfield Clinic has developed a FRET-based sensor to detect food-borne illness. This technology utilizes



C

DONORS	ACCEPTORS
BFP	GFP
CFP	dsRED
Cy3	Cy5
Alexa488	Alexa555
Alexa488	Cy3
FITC	TRITC
Dabcyl	EDANS
ABZ	DNP
DANSYL	TYR(NO_3)
DANSYL	FAM

Fig. 1 FRET-based kinetics: **a** FRET occurs between two fluorophores, aptly named the donor and acceptors. When the donor and acceptor are dissociated or far apart, the donor emission is detected upon donor excitation. Alternatively, the two fluorophores interact and the acceptor emission is predominately observed when the donor and acceptor are in proximity (1–10 nm). This acceptor emission is due to intermolecular fluorescence resonance energy transfer (FRET) from the donor to the acceptor. **b** Compatibility of FRET pairs. A compatible acceptor is a molecule whose absorbance spectrum overlaps the emission spectrum of the donor molecule. If the absorbance spectrum of a molecule does not overlap the emission spectrum of the donor, the emitted energy will not be able to excite the potential acceptor. If the absorbance spectrum of the acceptor does overlap the emission spectrum of the donor, energy from the donor will excite the acceptor molecule provided that the two fluorophores are in close proximity. **c** FRET pairs

FRET hybridization probe technology to quickly detect markers for enterohemmorhagic *Escherichia coli* and *Salmonella* (Ellingson et al. 2005). Also, a cell-based high-throughput screening method utilizing FRET kinetics can selectively detect apoptosis and is highly effective in identifying anti-cancer compounds. This FRET-based assay can detect apoptosis, but not necrosis, and is thus more specific than MTT-based cell viability assays (Tian et al. 2007; Zhu et al. 2012) (Fig. 1).

Regulation of cellular bioenergetics has become the subject of increased interest, particularly in cancer biology, biomedical engineering, and medicine. Genetically encoded FRET-glucose nanosensors have been used for imaging glucose flux in single cells. These FRET-based sensors are highly sensitive and thus capable of detecting glucose at a dynamic range of 0.05–11 mM in vivo, permitting analyses in a physiologically relevant range (Deuschle et al. 2006). Single-cell FRET-based biosensors have been developed to monitor cellular energy flux as well (Willemsel et al. 2007).

FRET is very much a quantitative technique that can assist in developing and bringing to fruition previously theoretical models as well. Already FRET has been applied to the observation of transition paths in protein-folding to determine the average transition-path time for a fast- and slow-folding protein from a photon-by-photon analysis of the results of a kinetic model (Wang et al. 2010). In this model, the FRET efficiency trajectory was used to describe the transition path from the unfolded (U) to folded (F) states of a protein exhibiting two-state kinetics and thermodynamics (Wigelsworth et al. 2004).

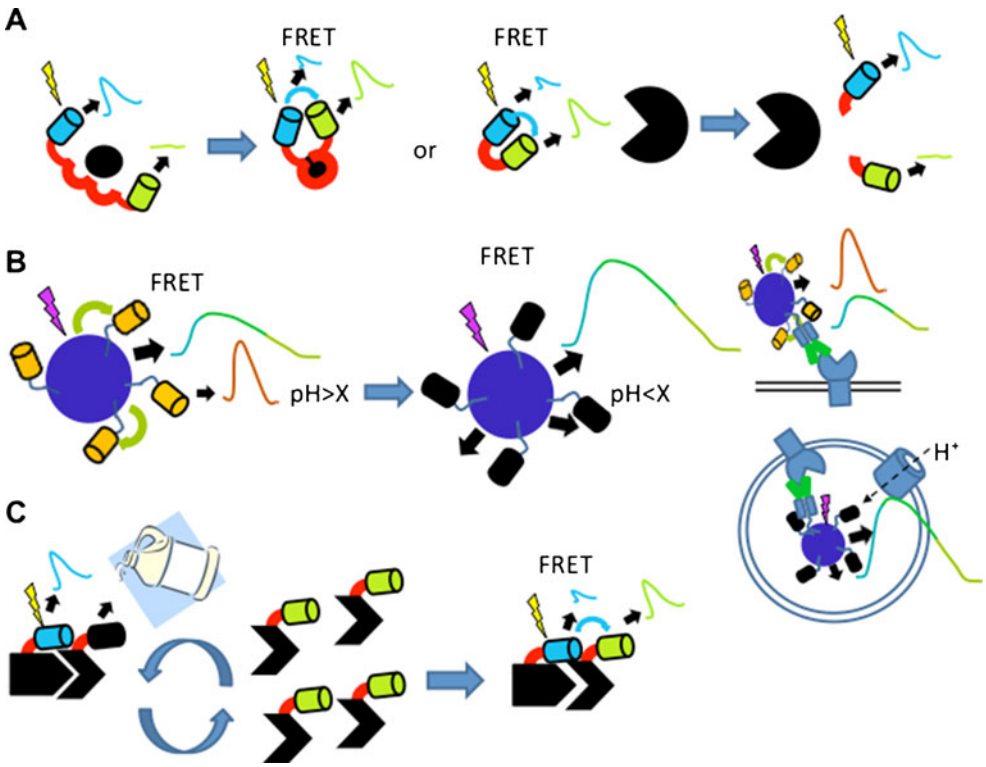


Fig. 2 FRET conceptual schemes: FRET between fluorescent proteins happens if the emission energy of one protein (donor) reasonably matches that of the other protein's excitation energy requirement, and occurs at distances of approximately 10 nm and less. FRET occurs not through emission of photons that excite the acceptor, but energy transfer through electromagnetic dipole–dipole interactions. Thus, the FRET pairs must be in actual physical contact with each other. The different ways of measuring FRET can be quite sophisticated, but conceptually, FRET schemes fall into two basic categories thus far: (1) Those that bring together or separate FRET pairs with various ways of measuring FRET, and (2) those that take advantage of an inherently variable property of a FRET donor or acceptor. Examples from **a** fit the first scheme and examples **b** and **c** fit the second scheme. Genetic chimeras of proteins that collectively bind an ion or substrate and are conformationally transformed upon binding can be linked to donor and acceptor fluorescent proteins. The substrate can bring two FRET pairs together (*left*), or break them apart (*right*). The latter represents a method of choice for characterizing proteolytic enzyme activity. It is accomplished by linking donor and acceptor FPs with an amino acid sequence that is specifically cleaved by a protease of interest. There are now several forms of FPs inherently sensitive to pH. For now, these consist essentially of FPs that are non-fluorescent at a lower pH, and fluoresce at a physiological pH. These can be attached directly to a protein alone or with a FP donor or acceptor. More recently, FPs have been linked to QDs, which improve photo-stability and allow pH measurements ranging from 6 to 8. Additionally QDs can be conjugated to a variety of trafficking and targeting sequences. Exchange among interacting proteins can also be measured by Förster transfer recovery by first exciting the donor and measuring donor emission and FRET, then photobleaching the acceptor, increasing the diminished donor emission by FRET, followed by observing the recovery of FRET

The coupling of more efficient FRET donor and acceptor fluorescent proteins, the optimization of linker-length, the mutation of fluorescent protein amino acid residues that mediate multi-dimerization, and the subsequent coupling to two zinc binding proteins, Atox1 and WD4, that together each contribute two cysteines to form a single tetrahedral zinc binding pocket, now make a sensitive measurement of intracellular zinc concentrations across a wide dynamic range feasible (Lee et al. 2010). Further manipulation of this construct has made it amenable to measuring cadmium as well (Halivni et al. 2012; Gill et al. 2005). One can easily imagine this kind of construct being further coupled to trafficking or subcellular targeting signals to allow discrete characterization of ionic concentrations in specific cellular compartments. A particularly intriguing possibility would be the prospect of measuring zinc-induced FRET in real time with a chimeric construct enabling specific extracellular synaptic targeting in neurons, as zinc concentration affects not only glutamatergic receptors like NMDA receptors, but also synaptic function. Specific glutamatergic agonists, such as α-amino-3-hydroxy5-methyl-4-isoxazolepropionic acid and kainic acid, both increase extracellular zinc concentrations in tissue and these fluctuations affect cellular function. FRET-based assessment and sensitive quantification of protease activity has emerged as probably the most refined means of obtaining real time and single-cell functional measurements, since coupling a donor and acceptor fluorophore between a specific proteolytic consensus sequence for a particular protease provides the opportunity to measure not only the induction or change in protease activation, but also the subcellular loci wherein the protease activation takes place. Such FRET-based constructs have been used to assess Caspase-3 activation (Paulsson et al. 2008), matrix metalloproteinase (MMP) activation (Yang et al. 2007) and *m*-calpain activation (Zadran et al. 2009). Moreover, these can be adapted to high-throughput types of assays for identification of molecules, for instance, that treat cancer (Caspase-3, MMP), or possibly stroke (*m*-calpain) (Fig. 2).

Summary

FRET is an imaging technique widely used to monitor cellular dynamics in living cells in real time. Despite the value of this ability, the accuracy of FRET is still dependent on the FRET efficiency. Despite recent advances in the use of FRET, this technology still requires further investigation and development. The improvement of FRET technology may lead to the development of alternative, non-invasive, and live-cell resonance energy transfer (RET) techniques. Fluorescent RET (FRET) and its variation, bioluminescent RET, can be used to assess the real-time responses without altering cellular protein network, compartmentalization, and spatial arrangement. This allows medical diagnostics to proceed through less invasive procedures, enhancing patient care. Such techniques will undoubtedly be of great importance in the future of fluorescence-based assays for the analysis and real-time monitoring of cellular events in live cells, and thus allow for a more exact and thorough understanding of such cellular events. With the increased understanding that it will produce, the adaptation of FRET will provide a key biological tool in learning critical lessons about cellular processes, disease progression, and drug therapy.

Acknowledgments We are thankful guidance and suggestions provided by Dr. Raphael Levine for this review. We would also like to thank the UCLA Department of Pathology and Laboratory Medicine and the David Geffen School of Medicine for providing Early Career Award and support to SZ. This mini-review is dedicated to the late Professor Paola S. Timiras.

Competing interest Authors declare no competing financial interests.

References

Ai H, Hazelwood K, Davidson M, Campbell R (2008) Fluorescent protein FRET pairs for ratiometric imaging of dual biosensors. Nat Methods 5:401–403. doi:10.1038/nmeth.1207

Ansbacher T, Srivastava HK, Stein T, Baer R, Merkx M, Shurki A (2012) Calculation of transition dipole moment in fluorescent proteins—towards efficient energy transfer. Phys Chem Chem Phys 14(12):4109–4117

Bailey VJ, Easwaran H, Zhang Y, Griffiths E, Belinsky SA, Herman JG, Baylin SB, Carraway HE, Wang TH (2009) MS-qFRET: a quantum dot-based method for analysis of DNA methylation. Genome Res 19(8):1455–1461

Bellau-Pujol S, Vabret A, Legrand L, Dina J, Gouarin S, Petitjean-Lecherbonnier J, Pozzetto B, Ginevra C, Freymuth FJ (2005) Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. Virol Methods 126(1–2):53–63

Bujalowski WM, Jezewska MJ (2012) Using structure–function constraints in FRET studies of large macromolecular complexes. Methods Mol Biol 875:135–164

Cardullo RA, Agrawal S, Flores C, Zamecnik PC, Wolf DE (1988) Detection of nucleic acid hybridization by nonradiative fluorescence resonance energy transfer. Proc Natl Acad Sci U S A 85 (23):8790–8794

Crivat G, Da Silva SM, Reyes DR, Locascio LE, Gaitan M, Rosenzweig N, Rosenzweig Z (2010) Quantum dot FRET-based probes in thin films grown in microfluidic channels. J Am Chem Soc 132 (5):1460–1461

Davydov DR, Davydova NY, Halpert JR (2008) Allosteric transitions in cytochrome P450eryF explored with pressure-perturbation spectroscopy, lifetime FRET, and a novel fluorescent substrate, Fluorol-7GA. Biochemistry 47(43):11348–11359

Day RN, Periasamy A, Schaufele F (2001) Fluorescence resonance energy transfer microscopy of localized protein interactions in the living cell nucleus. Methods 25(1):4–18

Deuschle K, Chaudhuri B, Okumoto S, Lager I, Lalonde S, Frommer WB (2006) Rapid metabolism of glucose detected with FRET glucose nanosensors in epidermal cells and intact roots of

Arabidopsis RNA-silencing mutants[W][OA]. Plant Cell 18 (9):2314–2325. doi:10.1105/tpc.106.044073

Dietrich A, Buschmann V, Müller C, Sauer M (2002) Fluorescence resonance energy transfer (FRET) and competing processes in donor-acceptor substituted DNA strands: a comparative study of ensemble and single-molecule data. J Biotechnol 82(3):211–231

Ellingson JLE, Koziczkowski JJ, Anderson JL, Carlson SA, Sharma VK (2005) Rapid PCR detection of enterohemorrhagic *Escherichia coli* (EHEC) in bovine food products and feces. Mol Cell Probes 213–217

Fernando H, Halpert JR, Davydov DR (2006) Resolution of multiple substrate binding sites in cytochrome P450 3A4: the stoichiometry of the enzyme-substrate complexes probed by FRET and Job's titration. Biochemistry 45:4199–4209

Gill R, Willner I, Shweky I, Banin U (2005) Fluorescence resonance energy transfer in CdSe/ZnS-DNA conjugates: probing hybridization and DNA cleavage. J Phys Chem B 109(49):23715–23719

Ha T, Enderle T, Ogletree DF, Chemla DS, Selvin PR, Weiss S (1996) Probing the interaction between two single molecules: fluorescence resonance energy transfer between a single donor and a single acceptor. Proc Natl Acad Sci U S A 93:6264–6268

Halivni S, Sitt A, Hadar I, Banin U (2012) Effect of nanoparticle dimensionality on fluorescence resonance energy transfer in nanoparticle-dye conjugated systems. ACS Nano 6(3):2758–2765

Hangauer MJ, Bertozzi CR (2008) A FRET-based fluorogenic phosphine for live cell imaging with the Staudinger ligation. Angew Chem Int Ed Engl 47(13):2394–2397. doi:10.1002/anie.200704847

Hoppe A, Christensen K, Swanson JA (2002) Fluorescence resonance energy transfer-based stoichiometry in living cells. Biophys J 83:3652–3664

Howell WM (2006) Detection of DNA hybridization using induced fluorescence resonance energy transfer. Methods Mol Biol 335:33–41

Jares-Erijman EA, Jovin TM (2003) FRET imaging. Nat Biotechnol 21:1387–1395. doi:10.1038/nbt896

Johansson MK (2006) Choosing reporter-quencher pairs for efficient quenching through formation of intramolecular dimers. Methods Mol Biol 335:17–29

Joo C, Ha T, Selvin P (2007) Single-molecule FRET with total internal reflection microscopy. Single Mol Tech Lab Man 3–36

Kajihara D, Abe R, Iijima I, Komiyama C, Sisido M, Hohsaka T (2006) FRET analysis of protein conformational change through position-specific incorporation of fluorescent amino acids. Nat Methods 3(11):923–929

Kapanidis AN, Weiss S (2002) Fluorescent probes and bioconjugation chemistries for single-molecule fluorescence analysis of biomolecules. J Chem Phys 117:10953–10964

Kehl SC, Kumar S (2009) Utilization of nucleic acid amplification assays for the detection of respiratory viruses. Clin Lab Med 29 (4):661–671

Kim K, Barhoumi R, Burghardt R, Safe S (2005) Analysis of estrogen receptor alpha-Sp1 interactions in breast cancer cells by fluorescence resonance energy transfer. Mol Endocrinol 19(4):843–854

Kohl T, Heinze KG, Kuhlemann R, Koltermann A, Schwille P (2002) A protease assay for two-photon crosscorrelation and FRET analysis based solely on fluorescent proteins. Proc Natl Acad Sci U S A 17; 99(19): 12161–12166. doi:10.1073/pnas.192433499

Komatsua N, Aokia K, Yamadac M, Yukinagac H, Fujitac Y, Kamiokac Y, Matsudaa M (2011) Development of an optimized backbone of FRET biosensors for kinases and GTPases. Mol Biol Cell 22(23)

Kwak CK, Kim DM, Lee CS, Lee M, Lee TS (2010) Aldehyde-functionalized, water-soluble poly(*para*-phenylene): synthesis and streptavidin assay using FRET. J Nanosci Nanotechnol 10 (10):6920–6924

Lassaunière R, Kresfelder T, Venter M (2010) A novel multiplex real-time RT-PCR assay with FRET hybridization probes for the detection and quantitation of 13 respiratory viruses. J Virol Methods 165(2):254–260

Lee AJ, Ensign AA, Krauss TD, Bren KL (2010) Zinc porphyrin as a donor for FRET in Zn(II)cytochrome *c*. J Am Chem Soc 132 (6):1752–1753

Leriche G, Budin G, Darwich Z, Weltin D, Mély Y, Klymchenko AS, Wagner A (2012) A FRET-based probe with a chemically deactivatable quencher. Chem Commun 48:3224–3226

Li H, Luo Y, Sun X (2011) Fluorescence resonance energy transfer dye-labeled probe for fluorescence-enhanced DNA detection: an effective strategy to greatly improve discrimination ability toward single-base mismatch. Biosens Bioelectron 27(1):167–171

Linder KE, Metcalfe E, Nanjappan P, Arunachalam T, Ramos K, Skedzielewski TM, Marinelli ER, Tweedle MF, Nunn AD, Swenson RE (2011) Synthesis, in vitro evaluation, and in vivo metabolism of fluor/quencher compounds containing IRDye 800CW and Black Hole Quencher-3 (BHQ-3). Bioconjug Chem 22 (7):1287–1297

Liu H, Liang G, Abdel-Halim ES, Zhu J (2011) A sensitive and selective quantum dots-based FRET biosensor for the detection of cancer marker type IV collagenase. Anal Methods 3:1797–1801

Long F, Gu C, Gu§ AZ, Shi H (2012) Quantum dot/carrier–protein/haptens conjugate as a detection nanobioprobe for FRET-based immunoassay of small analytes with all-fiber microfluidic biosensing platform. Anal Chem 84(8):3646–3653

Lovell JF, Chen J, Jarvi MT, Cao WG, Allen AD, Liu Y, Tidwell TT, Wilson BC, Zheng G (2009) FRET quenching of photosensitizer singlet oxygen generation. J Phys Chem B 113(10):3203–3211

Marras SAE, Kramer FR, Tyagi S (2002) Efficiencies of fluorescence resonance energy transfer and contact-mediated quenching in oligonucleotide probes. Nucleic Acids Res 30(21):e122

Means JA, Hines JV, Merchant KA, Best RB, Louis JM, Gopich IV, Eaton WA (2005) Fluorescence resonance energy transfer studies of aminoglycoside binding to a T box antiterminator RNA. Bioorg Med Chem Lett 15(8):2169–2172

Merchant KA, Best RB, Louis JM, Gopich IV, Eaton WA (2007) Characterizing the unfolded states of proteins using single-molecule FRET spectroscopy and molecular simulations. Proc Natl Acad Sci U S A 104:1528–1533

Meyer T, Teruel MN (2003) Fluorescence imaging of signaling networks. Trends Cell Biol 13:101–106

Moerner WE, Fromm DP (2003) Methods of single-molecule fluorescence spectroscopy and microscopy. Rev Sci Instrum 74:3597–3619

Ogawa M, Kosaka N, Longmire M, Urano Y, Choyke PL, Kobayashi H (2009) Fluorophore-quencher based activatable targeted optical probes for detecting in vivo cancer metastases. Mol Pharm 6 (2):386–395. doi:10.1021/mp800115t

Okamura Y, Watanabe Y (2006) Detecting RNA/DNA hybridization using double-labeled donor probes with enhanced fluorescence resonance energy transfer signals. Methods Mol Biol 335:43–56

Paulsson JF, Schultz SW, Köhler M, Leibiger I, Berggren P, Westermark GT (2008) Real-time monitoring of apoptosis by caspase-3-like protease induced FRET reduction triggered by amyloid aggregation. Exp Diabetes Res Vol 2008. doi:10.1155/2008/865850

Periasamy A (2001) Fluorescence resonance energy transfer microscopy: a mini review. J Biomed Opt 6(3):287–291

Roy R, Hohng S, Ha T (2008) A practical guide to single-molecule FRET. Nat Methods 5:507–516. doi:10.1038/nmeth.1208

Sturmey RG, O'Toole PJ, Leese HJ (2006) Fluorescence resonance energy transfer analysis of mitochondrial:lipid association in the porcine oocyte. Reproduction 132(6):829–837

Tian H, Ip L, Luo H, Chang DC, Lu KQ (2007) A high throughput drug screen based on fluorescence resonance energy transfer (FRET) for anticancer activity of compounds from herbal

medicine. Br J Pharmacol 150(3):321–334. doi:10.1038/sj.bjp.0706988

Tsuji A, Sato Y, Hirano M, Suga T, Koshimoto H, Taguchi T, Ohsuka S (2001) Development of a time-resolved fluorometric method for observing hybridization in living cells using fluorescence resonance energy transfer. Biophys J 81(1):501–515

Wang Z, Elbaz J, Remacle F, Levine RD, Willner I (2010) All-DNA finite-state automata with finite memory. Proc Natl Acad Sci U S A 107(51):21996–22001. doi:10.1073/pnas.1015858107

Waterhouse BR, Gijsen M, Barber PR, Tullis ID, Vojnovic B, Kong A (2011) Assessment of EGFR/HER2 dimerization by FRET-FLIM utilizing Alexa-conjugated secondary antibodies in relation to targeted therapies in cancers. Oncotarget 2(9):728–736

Wiedenmann J, Oswald F, Nienhaus GU (2009) Fluorescent proteins for live cell imaging: opportunities, limitations, and challenges. IUBMB Life 61(11):1029–1042

Wigelsworth DJ, Krantz BA, Christensen KA, Lacy DB, Juris SJ, Collier RJ (2004) Binding stoichiometry and kinetics of the interaction of a human anthrax toxin receptor, CMG2, with protective antigen. J Biol Chem 279:23349–23356

Willemsel M, Janssen E, de Lange F, Wieringa B, Fransen J (2007) ATP and FRET—a cautionary note. Nat Biotechnol 25:170–172. doi:10.1038/nbt0207-170

Yang J, Zhang Z, Lin J, Lu J, Liu BF, Zeng S, Luo Q (2007) Detection of MMP activity in living cells by a genetically encoded surface-displayed FRET sensor. Biochem Biophys Acta 1773(3):400–407

Zadran S, Qin Q, Bi X, Zadran H, Kim Y, Foy MR, Thompson R, Baudry M (2009) 17-Beta-estradiol increases neuronal excitability through MAP kinase-induced calpain activation. Proc Natl Acad Sci U S A 106(51):21936–21941

Zhu X, Fu A, Luo KQ (2012) A high-throughput fluorescence resonance energy transfer (FRET)-based endothelial cell apoptosis assay and its application for screening vascular disrupting agents. Biochem Biophys Res Commun 418(4):641–646