Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Design Strategy for Photoinduced Electron Transfer-Based Small Molecule Fluorescent Probes of Biomacromolecules

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

As cardinal support of innumerable biological processes, biomacromolecules such as proteins, nucleic acids and polysaccharides, are of importance to living systems. The key to understanding biological processes is to realize the role of these biomacromolecules in localization, distribution, conformation and interaction with other molecules. With current development and adaptation of fluorescent technologies in biomedical and pharmaceutical fields, fluorescence imaging (FLI) approach by using small molecule fluorescent probes is becoming an up-to-the-minute method to detect and monitor these imperative biomolecules in life sciences. However, conventional small molecule fluorescent probes may provide undesirable results because of their intrinsic deficiencies such as low signal-to-noise ratio (SNR) and the false-positive error. Recently, small molecule fluorescent probes with a photoinduced electron transfer (PET) "on/off" switch for biomacromolecules are well considered. When recognized by the biomacromolecules, these probes turn on/off the PET switch and change the fluorescence intensity to present a high SNR result. It should be emphasized that these PET-based fluorescent probes could be advantageous for understanding the pathogenesis of various diseases caused by abnormal expression of biomacromolecules. The discussion of this successful strategy involved in this review will be a valuable guide for further development of new PET-based small molecule fluorescent probes for biomacromolecules.

Key words: Fluorescence imaging, photoinduced electron transfer (PET), small molecule fluorescent probes, biomacromolecules.

1. Introduction

Biomacromolecules that include proteins, nucleic acids and polysaccharides often play pivotal roles in living systems, which cause a wide variety of diseases in the case of abnormally expressed. Although the structures of many biomacromolecules are successfully analyzed, it is remaining difficult to understand their complicated positions in biological processes. In view of the vivid visualization of biomolecules, fluorescence imaging (FLI) technology has been a practical and ideal method to detect biological substances.[1] With considerable attempts by scientists, an obvious development of small molecule fluorescent probes for the biosystem in the past few decades.[2, 3] FLI can reveal the localization and distribution of intracellular macromolecules, sometimes at the single-molecule level,[4-6] which allows us to understand the roles of biomacromolecules in living systems.

Fluorescent probes are indispensable tools for bioimaging, and a large number of fluorescent probes have been well designed and synthesized so far. Nevertheless, conventional techniques of imaging biomacromolecules with organic dyes or fluorescent probes regularly consist of painstaking steps. In the concept to design small molecule fluorescent probes for bioimaging, photoinduced electron transfer (PET) effect is one of the most widely used sensing mechanisms.[7-9]

Recently, numerous PET-based fluorescent probes are developed for imaging biomacromolecules. In general, this type of probe is structurally divided into two parts: a fluorophore and a quencher. The fluorescence of probes is suppressed by the quencher at the free conditions and then can be released at the binding conditions by blocking up the PET process when the quencher reacts with the target substrate. By using these PET-based fluorescent probes, the localization, distribution and conformation change of target molecules can be conveniently unveiled. Compared with those regular probes without fluorescence switches, PET-based probes can easily receive a high SNR, which is a tremendously decisive factor to *in vitro* and *in vivo* fluorescence imaging. The present review article summarizes the PET-based fluorescent probes for biomacromolecules and their applications in bioimaging. A brief discussion on fluorescence imaging advantages will be presented herein, as well.

2. Photoinduced electron transfer (PET) Effect

When irradiated by ultraviolet or visible light, certain substances

can emit light with different wavelength and intensity, and if the irradiation is stopped, the emitted light will disappear instantaneously. This emitted light is known as fluorescence. The intensity of fluorescence emission could be weakened by a large number of methods and such a reduction in emission intensity is called fluorescence quenching. As one of the mechanisms that can result in fluorescence quenching, PET effect is a widely used method to diminish fluorescence of fluorescent probes.[7, 8, 10, 11]

A typical PET system consists of an aromatic fluorophore, an aliphatic amine and a short methylene chain as the linker.[12] In other words, a PET-based fluorescent sensor for biomacromolecules often includes three parts: a receptor that serves as an electron donor or a quencher, a fluorophore that acts as the electron acceptor, and a spacer that links the two parts above. In this system, the intramolecular electron transfer from the receptor to the fluorophore will lead to fluorescence quenching. Nevertheless, when the receptor binds upon its targets, this PET process is restricted or totally abolished, and as a result, the sensor retrieves fluorescence [13, 14] (Fig. 1).

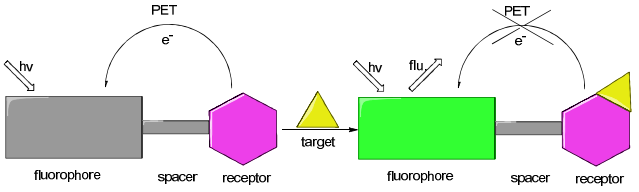

Fig. 1 The mechanism of PET-based fluorescent probes.

The mechanism of PET-based fluorescent probes can be explained by the frontier orbital theory (Fig. 2).[12, 15-18] When the fluorophore is excited by an appropriate light, the electron of the highest occupied molecular orbital (HOMO) is transferred to the lowest unoccupied molecular orbital (LUMO). Because the receptor is adjacent to the fluorophore and HOMO energy level of the receptor is between the LUMO and HOMO levels of the fluorophore, electron transfers from HOMO of receptor to LUMO of fluorophore that prevent the electron in the LUMO of fluorophore from returning to HOMO, and then PET effect occurs to quench the emitted fluorescence. When the receptor binds upon the target, the HOMO energy of the receptor changes to be lower than the HOMO energy level of the fluorophore. Therefore, the PET process is blocked, and the fluorescence is recovered.

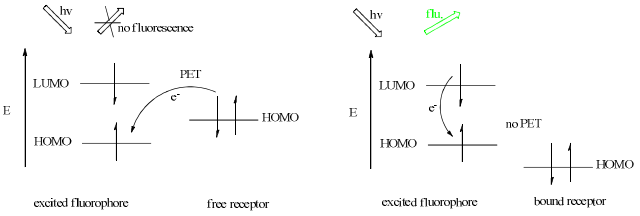

Fig. 2 Frontier orbital theory for PET effect.

3. The advantage of PET-based fluorescence imaging (FLI)

FLI is one of the most convincing techniques for medical imaging. Compared to other imaging methods, such as X-ray radiography, computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US), FLI is more convenient, specific, sensitive, fast-responding and easy-handling. In addition, FLI has been successfully implemented in endoscopy[19] and surgery.[20, 21]

However, the overall FLI has some inadequacies, such as low SNR and the false-positive error. These defects can be overcome by using the "switchable" fluorescent probes, for example, PET-based probes. Switchable fluorescent probes can be turned on only when specifically binding upon the targets of interest, otherwise fluorescence of probes is quenched. Consequently, the imaging result with particularly high target-to-background ratio will be presented.

The PET-based FLI can non-invasively monitor the biomolecules of interest in the living system with high spatial and temporal resolution, opposed to other imaging techniques. Moreover, this PET-based FLI has the high potential on no-wash living cell imaging, thus enabling rapid identification of biomacromolecules in living cells with high SNR.[22] The combination of these advantages would contribute to conveniently revealing the biological roles that the biomolecules of interest perform in the complex organisms. As a result, the PET-based FLI is effective enough to promote the development of life sciences, which will be generally considered in the future of medical imaging.

4. PET-Based small-molecule fluorescent probes

4.1 The fluorophores in PET-based probes

A wide range of small-molecule fluorophores with several core structures, including BODIPY, fluorescein, rhodamine, cyanine, naphthalimide and coumarin (Fig. 3), ranging from near-infrared light to blue light, can be applied to design a PET-based fluorescent probe. These commercially available fluorophores are hydrophilic and contain no net charge, so as to be reasonable candidates for *in vivo* imaging.

A successful PET-based fluorescent probe not only depends on the high sensitivity and selectivity towards the target, but also relies on the appropriate choice of fluorophore. The appropriate fluorophore is bonded to the selected recognition group through chemical reactions to form a PET-based fluorescent probe. For example, Tang and his co-workers designed a PET-based probe for copper (II).[23] N-hydroxyacetamide was chosen as the Cu^{2+} receptor and Tricarbocyanine (Cy) as the NIR fluorophore. This was the first PET-based NIR fluorescent probe for Cu^{2+} and was successfully applied to living cells and tissues to visualize Cu^{2+}.

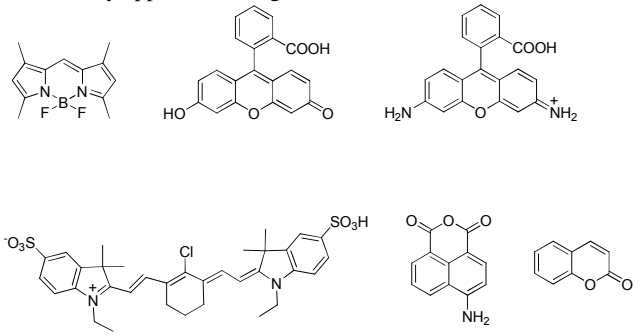

Fig. 3 Small-molecule fluorophores including BODIPY, fluorescein, rhodamine, cyanine (Cy7), naphthalimide and coumarin.

4.2 PET-based Probes for enzymes

PET-based fluorescent probes are particularly profitable for sensitively and selectively monitoring target enzyme activities. In a typical case, a PET-based probe can be recognized by a specific enzyme to cleave the PET quencher, which leads to the disappearance of PET effect. As a result, this system recovers to release the fluorescence. Based on this PET mechanism, so far various fluorescent probes have been well developed for manifesting the functional role of the enzyme in the physiological environment.

4.2.1 Glutathione S-transferase

As a kind of the dimeric enzyme related to detoxification reactions,[24] Glutathione S-transferase (GST) is involved in a large number of biological processes, such as drug metabolism, protecting our body against endogenous reactive oxygen species (ROS) and xenobiotic biotransformation.[25] In other words, GST can catalyze the reaction of reduced glutathione (GSH) with the targets, including both exogenous and endogenous molecules. Recently, Tetsuo Nagano et al. designed and synthesized an "off/on" fluorescent probe for GST based on PET mechanism.[26] On the basis of 3,4-dinitrobenzanilide (NNBA) as a specific substrate for GST, they prepared probes (DNAFs). DNAFs have excellent kinetic parameters on releasing fluorescence catalyzed by GST. However, DNAFs were membrane-impermeable that were inappropriate for living cells. Therefore, they designed and synthesized another fluorogenic substrate, DNAT-Me, which expressed a dramatic fluorescence surge after GST-catalyzed glutathionylation and subsequent denitration (Fig. 4). It was available for imaging GST activities in living cells. (Fig. 5) These results indicated that DNAT-Me was a suitable fluorogenic substrate for fluorescence imaging of intracellular distribution of GST and also for the research on GST activities, including drug resistance in cancer cells and detection of GST-overexpressing preneoplastic foci in diagnosis. This design strategy would serve as a direction for developing probes on the structural basis of enzyme substrates in living cells.

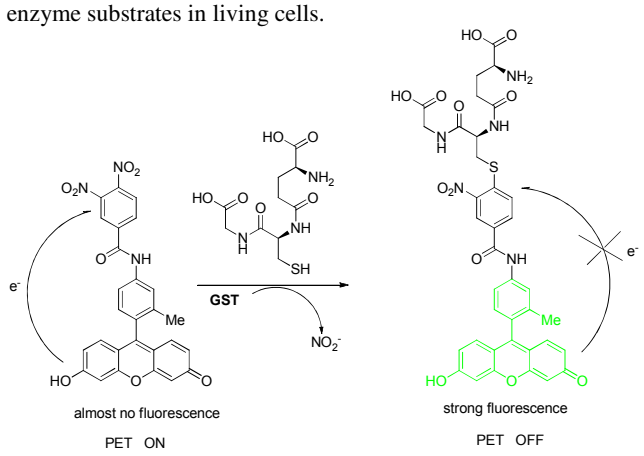

Fig. 4 DNAT-Me catalyzed by GST to release fluorescence.

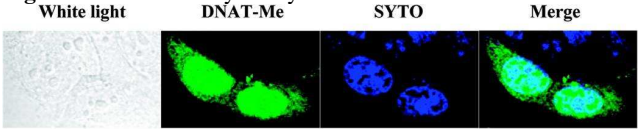

Fig.5 Confocal microscopic imaging of DNAT-Me and nuclear-specific dye SYTO-loaded HuCCT1 cells. The results displayed the imaging efficacy of DNAT-Me to visualize the GST activity in HuCCT1 cells. Reprinted with permission from ref 26. Copyright 2008 American Chemical Society.

4.2.2 Alkaline phosphatase

Alkaline phosphatase (ALP) is a hydrolase with high sensitivity towards numerous types of target molecules by removing the phosphate groups.[27] A conventional utilization of ALP is used to conjugate with a secondary antibody to identify the target protein in Western blot immunoanalysis. The combination of ALP with a fluorescent probe can achieve the discernable signal amplification.[28] However, the application of available fluorescent probes to Western blot analysis is limited in the present time. Recently, Tetsuo Nagano et al. designed and synthesized an "off/on" fluorescent probe for ALP based on PET mechanism that is suitable for Western blot analysis.[29] They obtained almost nonfluorescent TG-Phos by linking the phosphate group to the phenolic group of 2-Me-4-OMe TG, which could be hydrolysed by ALP to release strong fluorescence as well as confirm high affinity for Western blots. (Fig. 6) It should be underlined that this new probe TG-Phos provided suitable sensitivity to ALP for multicolor labeling. In our opinion, this approach may provide a rapid and practical identification of proteins other than ALP on Western blotting analysis.

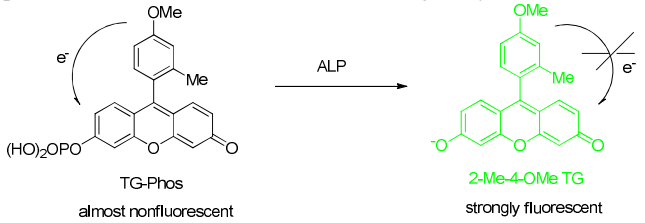

Fig. 6 Reaction of TG-Phos with ALP to produce high fluorescent hydrolyzed products by breaking PET process.

4.2.3 β-Galactosidase

As an exoglycosidase that catalyzes the hydrolysis of β-galactosides to generate monosaccharides, β-Galactosidase (β-gal) is widely recognized as a marker enzyme, especially as a gene expression marker.[30] Though there have been many fluorescent substrates for β-gal,[31] most of them were applied in *in vitro* systems and only a few *in vivo*. Recently, Tetsuo Nagano and Yasuteru Urano developed a unique, sensitive and membrane-permeable PET-based "off/on" fluorescent probe, TG-βGal, for β-gal.[32] TG-βGal has an O-alkylated xanthene as the fluorophore and a 3-methoxytoluene moiety as the PET donor. When hydrolyzed by β-gal, the β-galactoside group was released, and the probe released the fluorescence. (Fig. 7) In view of its reasonable membrane-permeability, this probe enables to image the β-gal activities in living cells in a real-time manner. It needs to be noted that the "off/on" switch of such a probe gives the results with a high SNR, which is crucial to cell imaging.

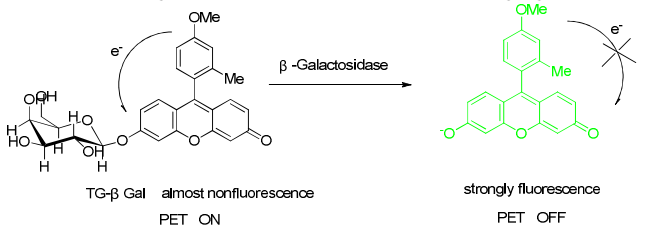

Fig. 7 A highly sensitive fluorescent probe based on PET mechanism for β-Galactosidase.

Depending on these results, such a group kept forward to examine whether the probe, TG-βGal, was applicable for targeted tumor imaging. However, the results were unsatisfactory because the fluorescent hydrolysis product of TG-βGal was hydrophobic, which made the probe be easily washed out from cells to weaken the fluorescence. In other words, the probe TG-βGal was not suitable for targeted tumor imaging. Based on photochemical and photophysical experiments, they modified the previous probe by adding an ester moiety. The new probe, AM-TG-βGal, also based on PET mechanism, manifests a great fluorescence enhancement when hydrolyzed by β-gal and subsequently hydrolyzed the ester moiety by widespread intracellular esterases.[33] (Fig. 8) The final hydrolysis product is hydrophilic and can be well retained in the cells even if being washed, thus the loss of fluorescence is avoided. In tumor imaging experiments, the mouse tumor model was labeled with an avidin-β-galactosidase conjugate and followed incubated by AM-TG-βGal. Afterwards, the intraperitoneal tumors were obviously visualized in fluorescence imaging. The results indicated the bright prospect in the applications of the new probe, such as video-assisted laparoscopic tumor resection. The design strategy provided a unique method to other researchers on tumor imaging by using a two-step procedure: firstly the localization on target cancer cells of target enzyme and subsequently recognition with a highly sensitive fluorescent probe, which results in a high tumor-to-background ratio.

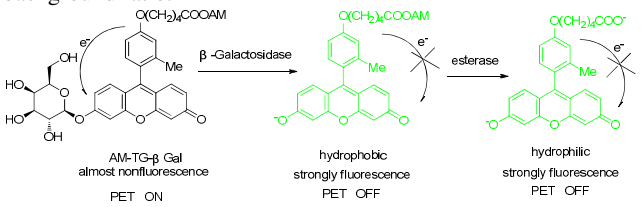

Fig. 8 Reaction of AM-TG-βGal as a β-gal and esterase-sensitive probe based on PET.

4.2.4 Protease

A protease, often related to a number of disease processes, functions as an enzyme to remove a specific peptide from a substrate peptide or protein. Among the proteases, microsomal leucine aminopeptidase (LAP), an exopeptidase to eliminate N-terminal amino acids of unsubstituted oligopeptides, plays essential roles in tumor metastasis.[34] Tetsuo Nagano et al. designed and synthesized some lanthanide-based functional probes for LAP on the basis of "off/on" PET mechanism.[35] Upon enzymatic cleavage of the substrate peptide, a significant difference in luminescence intensity can be observed. (Fig. 9) When the leucine group was removed by LAP, the aniline group acted as a quencher causing the PET process "on" and then no luminescence could be seen. It should be noted that LAP is an enzyme in association with a wide range of clinical diseases, such as liver and pancreas, and therefore, LAP fluorescent probes should own practical values in clinical diagnosis. This report disclosed a novel strategy for developing PET-based probes for all proteases.

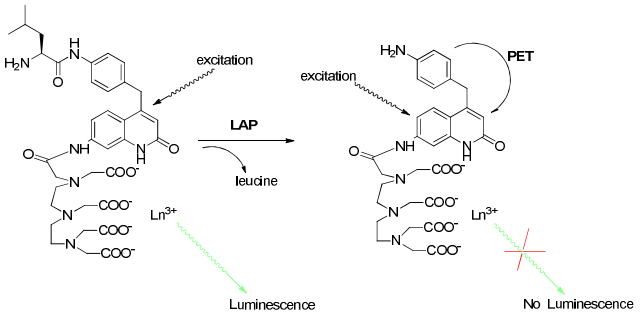

Fig. 9 Reaction scheme of the LAP probe. Ln= Tb, Eu.

4.2.5 Carbonic anhydrase isozymes IX

Carbonic anhydrases (CAs) are ubiquitous metalloenzymes that catalyze rapid physiological reactions including interconversion of carbon dioxide and water to bicarbonate and protons. These enzymes are involved in various pathological and physiological processes, such as lipogenesis, gluconeogenesis and tumorigenicity. As a kind of transmembrane α–CA isoform, human carbonic anhydrase IX (hCA IX) is recognized as a target of cancer diagnosis and treatment.[36] However, hitherto there has no selective fluorescent probe for CA IX. Recently, Qian and his coworkers designed and synthesized a high-sensitive PET-based fluorescent probe with an ideal "off/on" switch for CA IX (Fig. 10), which could release a strong fluorescence when recognized.[37] In this case, saccharin was employed as a recognition motif because of its high specificity to CA IX. When the saccharin group is recognized by the target enzyme, the conformation of this probe will change to overthrow the PET process and, as a result, the quenched fluorescence is released again. It's well known that the hCA IX is highly overexpressed in hypoxia cells, in the meanwhile SiHa cells were singled out for cell imaging on the account of their relatively high expression of endogenous CA IX. Therefore, in cell imaging, two parallel SiHa cells were treated in normoxia and hypoxia condition at 37 °C before incubated with the probe. The imaging results obviously exemplified stronger fluorescence in hypoxia cells than normoxia, in other words, these outputs clarified much higher selectivity towards CA IX than other CA enzymes. In conclusion, the designed probe could be a profitable fluorescent probe to detect CA IX *in vitro* or *in cellulo*. In our opinion, this successful strategy could afford a new avenue to design PET-based probes through changing the conformation of the probe.

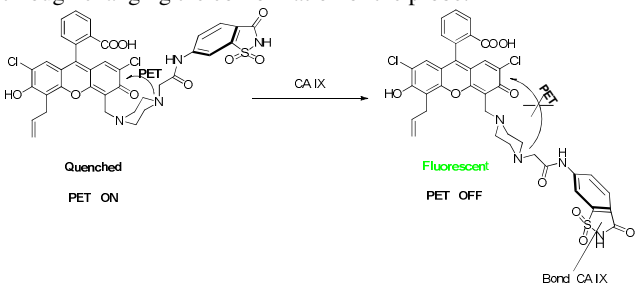

Fig. 10 Strategy of the fluorescent probe for CA IX with space-folded PET mechanism.

4.2.6 Arylamine N-acetyltransferase 2

Arylamine N–acetyltransferases (NATs) are metabolism enzymes

that catalyze an acetylation of aromatic amines or arylhydroxylamines through acetyl-CoA.[38] The NATs often play an imperative role in various significant pharmacological and toxicological reactions, including detoxification of arylamines by N-acetylation and bioactivation reactions of arylamine carcinogens.[39] NAT1 and NAT2 have been reported to be related to several diseases.[40] Therefore, detection of NAT activities is of importance in clinical diagnosis. Because NAT1 is ubiquitously expressed, and NAT2 mainly locates in liver and colorectal tissues, the detection of NAT2 is urgently required for biological study.

Tetsuo Nagano et al. introduced a lanthanide complex "off/on" probe that exposed high specificity to NAT2 over NAT1.[41] They initially synthesized the probe as a product which was obtained from catalyzed by LAP[35] that we discussed above. The aniline group functioned as the quencher of the probe, but the quenched luminescence by PET could be released after N-acetylation by NAT. (Fig. 11) Hereto, a reasonable probe with an "on/off" switch was established for NAT. With the switch, the probe provided high S/N ratios. In the meanwhile, considering that NATs are imperative metabolism enzymes, the probe is expected to be a useful tool for drug discovery or in clinical diagnosis. The product of one enzyme can be a substrate for another enzyme. Therefore, this strategy can be used to develop two-step continuous fluorescent probes for two different enzymes.

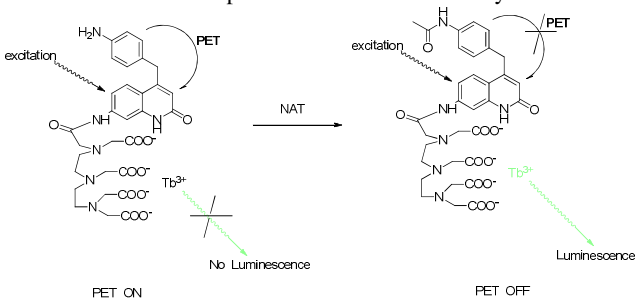

Fig. 11 Schematic of the novel NAT probe based on PET strategy.

Xu and co-workers designed and synthesized a near-infrared "off/on" fluorescent probe for NAT2 with high sensitivity and selectivity.[42] Because of superior tissue penetration and weak auto-fluorescence interference, such a near-infrared probe has important influence in studying biological processes, especially *in vivo*.[23, 43] They employed CY7 as the fluorophore and the arylamines as the quencher, which are linked by a sulphur atom to form the PET system, in which the arylamines functioned as the substrates of NATs. When the probe molecule was catalyzed by NAT to generate an acetylation product, the PET process was blocked and the fluorophore released the fluorescence (Fig. 12). Benefiting from its high sensitivity and selectivity to NAT2, as well as the long emission wavelength, the probe could be applied for real-time fluorescence imaging of NAT2 *in vivo*. The results clearly demonstrated that the fluorescence in the liver is much stronger than in other tissues of mice, which in line with the high expression of NAT2 in liver. To confirm the *in vivo* results, tissue homogenates assays were carried out by incubating the probe with different tissue homogenates. The results also confirmed that the intensity in the liver is the highest in all tissues. Obviously, the near-infrared probe could be a valuable tool for clinical diagnosis and biochemical analysis.

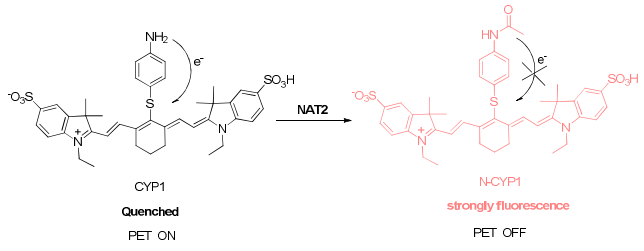

Fig. 12 Schematic representation of PET-based NAT fluorescent probe.

4.2.7 Cyclooxygenase-2 (COX-2)

In the past few years, fluorescence imaging has provided convenience for early diagnosis of cancer because of its high signal-to-background ratio and selectivity. Cyclooxygenase-2 (COX-2) is a biomarker of almost all cancer cell lines, which can be used to distinguish cancer cells from normal ones. Recently, Peng et al. designed and synthesized a novel "off/on" fluorescent probe targeted to COX-2 and localized in the Golgi apparatus of cancer cells.[44] The mechanism of the fluorescent probe depends on PET: when binding to COX-2 in the Golgi apparatus of cancer cells, the probe changes its conformation from folded to unfolded state, which restrains the PET process from the fluorophore ANQ to the recognition group indomethacin (IMC) through release of the fluorescence (Fig. 13).

It needs to be noted that such a PET-quenched fluorescent probe elucidated high selectivity and sensitivity to COX-2 for fluorescence imaging of the Golgi apparatus through normal and two-photon fluorescence microscopy. As a result, the probe could be a practical reagent for early diagnosis of cancers.

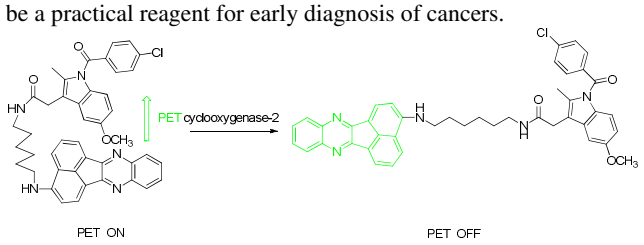

Fig. 13 The strategy of the COX-2 probe.

4.2.8 Human neutrophil elastase

Human neutrophil elastase (HNE) is widely considered to be associated with several human diseases. Recently, Yang and his co-workers designed and synthesized the first non-peptide-based PET "off/on" fluorescent probe which showed highly specific response for HNE.[45] They chose 7-amino-4-trifluoromethyl coumarin (AFC) as the fluorophore and the pentafluoroethylcarbonyl group as the quencher, linked by amide bond. When catalyzed by HNE, the pentafluoroethylcarbonyl group was removed and the fluorescence released.(Figure 14) This small PET-based fluorescent probe can be easily synthesized and requires no particular storage, compared with peptide-based substrates. What's more, it can be utilized to develop an efficient and cost-effective fluorescent assay to determine the enzymatic activity of HNE. It should be emphasized that this assay will promote future high-throughput discovery of HNE inhibitors as well as clinical diagnosis of elastase-related diseases.

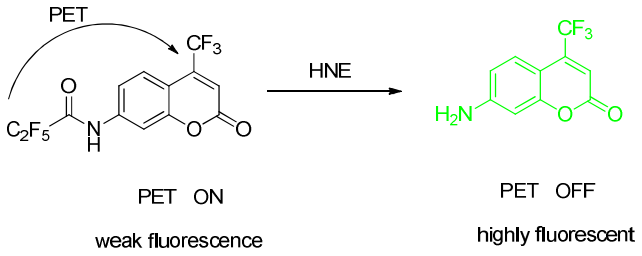

Fig. 14 Mechanism of the fluorescent probe for HNE.

4.2.9 Serum albumin

Tetsuo Nagano and co-workers discovered that the solvent-dependent change of fluorescence on/off switch of BODIPY derivatives is because of the influence of environmental polarity on the PET process. On the basis of the finding, they successfully prepared an environment-sensitive fluorescent probes library of BODIPY derivatives.[46] The probes could be used to assess the polarity of the surface of bovine serum albumin (BSA) in vitro. (Fig. 15) In this case, the fluorescence of the molecule is quenched by the PET process in buffer solutions, but the PET process is less favorable at the surface of BSA because of the hydrophobic environment. Fluorescence switches of these probes are realized based on the polarity of the protein. Hence, the environment-sensitive BODIPY library can be applied to sense the hydrophobicity of the target protein surface, membranes and receptors.

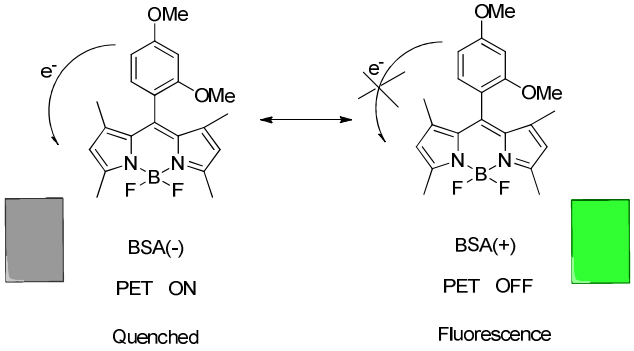

Fig. 15 BSA acts as a switch of fluorescence on/off by blocking PET process.

4.2.10 Human NAD(P)H: quinone oxidoreductase isozyme 1

Human NAD(P)H: quinone oxidoreductase isozyme 1 (NQO1) is intimately involved with cancer and is often present in human tumor cells.[47] What's more, NQO1 is found in the cytosol that catalyzes the reduction of quinones to hydroquinones, thus making this enzyme an ideal target for profluorophores. Recently, Robin L. McCarley and co-workers designed and synthesized an "off/on" fluorescent probe for hNQO1 based on PET mechanism.[48] (Fig. 16) They chose 1,8-naphthalimide as the fluorophore and quinone propionic acid as the quencher. The pharmacophore was attached to the fluorophore via an N-methylethanolamine linker through a carbamate to the amine of the naphthalimide ring. After extensive evaluation, they found that probe Q_3NI fluorescence is controlled by PET quenching. The fluorescence signal of the Q_3NI probe is quenched via oxidative electron transfer (OeT) by the covalently attached quinone propionic acid and once the quinone is reduced to its hydroquinone (HQ_3NI), the fluorescence is quenched by reductive electron transfer (ReT), a common feature with naphthalimide dyes.[49] Subsequently, the fluorescence of NI released after cyclizative cleavage reaction of the hydroquinone via the gem-dialkyl effect. It needs to be noted that the whole process is at a high rate. In total, this profluorogenic probe provides a real-time, highly sensitive and selective human tumor cell analysis and differentiation method. In the meanwhile, the fluorophore dequenching strategy and the entire fast, continuous procedure provide a new direction for us to develop brand new probes.

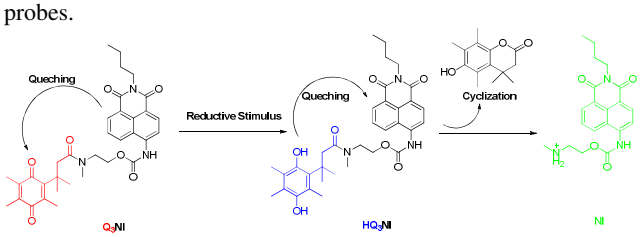

Fig. 16 The utilization of PET quching in the hNQO1 probe Q_3NI

4.3 PET-based Probes for RNA

Fluorescent imaging is considered a useful toolbox for analyzing biomolecules *in vitro* and *in vivo*. Although more and more fluorescent sensors for ions and proteins are being reported,[50, 51] there have been few reasonable fluorescent probes for sequence-specific RNA with high signal to noise ratio and cell-permeability.[52] As a result, there is an urgent demand on developing a desirable fluorescent sensor for sequence-specific RNA that could permeate the cell membrane and restore fluorescence only in the presence of the target RNA. Kazunori Koide and co-workers developed fluorescent probes for sequence-specific RNA on the basis of PET strategy.[15, 53] The probe is composed of the fluorophore (F) and the quencher (Q), and the quencher also plays another indispensable role of the ligand for the target RNA (Fig. 17). When the quencher bounds to the target RNA sequence, the PET process is suppressed, as well as the fluorescence is released.

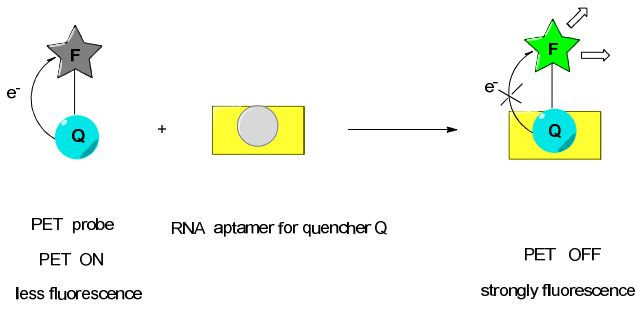

Fig. 17 Restore fluorescence by interaction of quencher with RNA.

Based on this strategy, this group synthesized a series of compounds as fluorescent probes of RNA. They employed 2',7'-dichlorofluorescein (DCF) as the fluorophore, and 1-(4-methoxyphenyl) piperazine (MPP) or its derivatives as the quencher. A NOESY NMR assay indicates that the compounds prefer the closed conformation under the natural condition. In order to find a sequence-specific RNA towards the probes, they immobilized MPP onto agarose resin to isolate aptamers for MPP.

Fluorescence titration was used to examine the ability of the isolated MPP aptamers to enhance fluorescence of the probes. Cyclodextrin assays proved that the aptamer such as cyclodextrin binds to MPP to make the molecule change in conformation and then to suppress the PET process. (Fig. 18) The researchers established a new paradigm for developing fluorescent sensors for specific RNA, which makes it possible to study gene expression at the RNA level using the fluorescent imaging method.

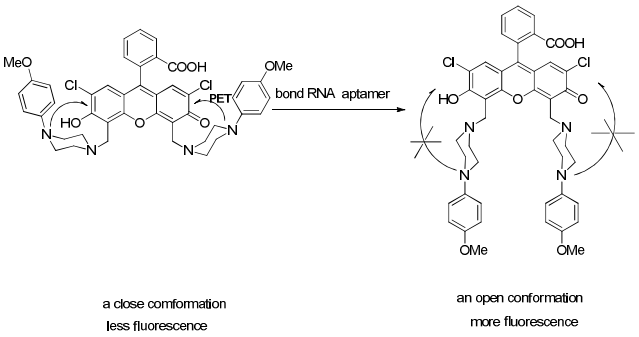

Fig. 18 Fluorescence enhancement by conformation changing upon binding RNA aptamer.

5. Conclusions

Biomacromolecules, including enzymes, proteins and RNAs, are playing significant roles in the complex biological systems. In view that a broad variety of diseases are caused by abnormal expression of biomacromolecules, visualization of their localizations and activities is very important for pathological study and clinical diagnose. Fluorescence imaging is one of the most promising techniques for real-time monitoring of biomolecules in the physiological environment with high temporal and spatial resolution, which helps researchers to recognize the biological roles of biomolecules in the complex systems. And fluorescence imaging is regarded as the future of medical imaging. Furthermore, an increasing number of fluorophores are becoming available with improved properties, such as the superior stability and the reasonable emission wavelength.

Table 1. PET-based fluorescent probes mentioned in this review.

Fluorescent probe	Target	λ_{ex} (nm)	λ_{em} (nm)	Change of quantum yield	Available for *in vitro* test	Available for live cells	Ref.
DNAFs	GST	492	514	34-fold	yes	-	26
DNAT-Me	GST	490	514	23-fold	yes	yes	26
TG-Phos	ALP	492	509	-	yes	-	29
TG-βGal	β-gal	492	509	419-fold	yes	yes	32
AM-TG-βGal	β-gal	453	518	470-fold	yes	tumor imaging	33
LAP probes	LAP	330	547, 614	500-fold	yes	-	35
CA IX probe	CA IX	511	534	-	yes	yes	37
NAT probe	NAT2	330	545	479-fold	yes	-	41
NAT probe	NAT2	790	815	20-fold	yes	fluorescence imaging *in vivo*	42
COX-2 probe	COX-2	457	547	28-fold	yes	yes	44
coumarin probe	HNE	370	495	20-fold	yes	-	45
BODIPY derivatives	BSA	500	515	10 to 100-fold	yes	yes	46
hNQO1 probe	hNQO1	374	490	33-fold	yes	yes	48
RNA probe	RNA	505	525	>7-fold	yes	-	15, 53

As one of the most widely used fluorescence switch strategies, the PET mechanism provided various fluorescent probes for fluorescence imaging. The combination or binding of probes with targets may lower the HOMO energy level of the quencher or increase the distance between the fluorophore and the quencher, so as to result in the PET process "on/off". With this mechanism, we can get superb results with a high signal-to-noise ratio that is very prominent for *in vivo* imaging. So far, several small-molecule fluorescent probes for detecting biomacromolecules have been well developed on the basis of PET mechanism (**Table 1**). It should be emphasized that these PET-based fluorescent probes will be helpful for researchers to determine the quantity of their biomacromolecule targets, understand the pathogenesis of various diseases caused by abnormal expression of biomacromolecules, and effective enough to promote the development of life sciences.

Besides the characteristic advantages, PET probes are rapid, sensitive and selective. In view that the general excitation wavelength of fluorophores is under 600 nm that may lead to tissue autofluorescence, a PET-based near-infrared fluorescent probe is urgently demanded on deep tissue imaging. Due to the PET-based probes are sensitive to pH changes, scientists should take care of the influence of pH during the design process and the entire experimental procedure. As a result, considering that PET-based fluorescent probes for biomacromolecules are fantastic tools to study biomacromolecules activities and to look into relevant diseases, a bright future of these fluorescent probes will be expected in the fields of diagnosis, analysis and medical imaging.

Acknowledgements:

The present work was supported by grants from the National Natural Science Foundation of China (No. 81370085), the Program of New Century Excellent Talents in University (No. NCET-11-0306), the Shandong Natural Science Foundation (No. JQ201019) and the Independent Innovation Foundation of Shandong University, IIFSDU (No. 2010JQ005 and 2012JC002). We also thank Professor A Prasanna de Silva (Queen's University of Belfast) for his helpful comment on the PET mechanism.

Notes and references

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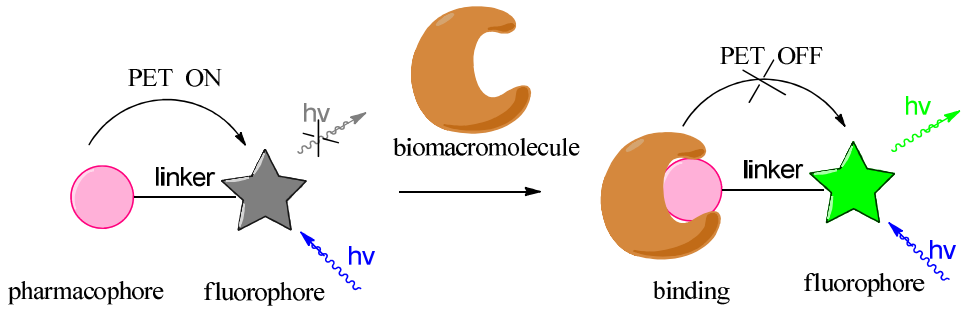
Graphical Abstract



This review article summarizes the advances in fluorescent probes on the basis of photoinduced electron transfer (PET) mechanism towards diverse biomacromolecule targets, as well as their applications in fluorescent imaging.