ACTIVATABLE SMART PROBES FOR MOLECULAR OPTICAL IMAGING AND THERAPY

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Recent years have seen the design and implementation of many optical activatable smart probes. These probes are activatable because they change their optical properties and are smart because they can identify specific targets. This broad class of detection agents has allowed previously unperformed visualizations, facilitating the study of diverse biomolecules including enzymes, nucleic acids, ions and reactive oxygen species. Designed to be robust in an in vivo environment, these probes have been used in tissue culture cells and in live small animals. An emerging class of smart probes has been designed to harness the potency of singlet oxygen generating photosensitizers. Combining the discrimination of activatable agents with the toxicity of photosensitizers represents a new and powerful approach to disease treatment. This review highlights some applications of activatable smart probes with a focus on developments of the past decade.

Keywords: Optical imaging; photodynamic therapy; activatable imaging probes.

1. Introduction

An activatable optical imaging agent must undergo a change in properties so that different states of the probe can be detected and be spatially resolved. The probes generally produce signals based on fluorescence properties, but some produce phosphorescence or luminescence based signals. Measuring an increase in probe emission intensity is a common measurement parameter, although other useful metrics can include decrease in emission intensity, change in excitation or emission wavelength, change in fluorescence lifetime, and also change in emission or excitation ratio when the optical agent is composed of a Förster resonance energy transfer (FRET) pair. The nature of the changes for activatable smart probes may or may not be reversible.

Activatable imaging agents and targeted imaging agents rely on different mechanisms to identify targets of interest. Activatable agents can be localized nonspecifically, since their detection is based on a change in optical properties brought about by the target. Targeted agents, on the other hand, migrate to the target of interest. An advantage of activatable probes is that probe signal to noise ratio is
not limited by targeting efficiency. Targeted agents often find their target through passive accumulation in damaged vasculature of tumours, or through the use of antibodies. The high affinity of antibodies for their antigens is the basis for the high resolution and contrast of immunofluorescence microscopy. However, for \textit{in vivo} imaging, antibodies are often limited to extracellular protein targets since their large size does not permit entry across the cell membrane. Targeted imaging agents that do not target correctly create misleading and inaccurate results. In theory, both targeted probe and activated probe mechanisms can be combined to create sensors that display an extremely high level of target specificity. For example, certain peptide constructs can be internalized by cells upon cleavage by specific proteases (Jiang \textit{et al.}, 2004). Modification of such a peptide to incorporate a fluorophore and quencher in the correct arrangement would lead to both the internalization (targeting) and dequenching (activation) of the probe upon interaction with the targeted protease.

Generally, activatable smart probes are not well suited for the non-optical imaging modalities of ultrasound, CT, PET and MRI. Since non-optical techniques rely on probe properties such as acoustic density, electron density, positron density, or ability to cause water relaxation, the signal generated by non-optical probes does not readily change intensity on a molecular level. A good introduction to the characteristics of the various imaging modalities with respect to molecular imaging is available elsewhere (Massoud and Gambhir, 2003). There have been some successful efforts to develop activatable MRI contrast agents using molecules that change water relaxation properties upon enzymatic cleavage, but more work is required to expand that field of research (Meade \textit{et al.}, 2003). Thus, to achieve contrast, non-optical modalities usually rely on the targeting rather than the activation of imaging agents.

2. Optical Imaging Methods

Activatable smart probes have greatly benefitted from advances in imaging technology. The resolution of fluorescence microscopy has continued to improve so that it is now possible to optically distinguish individual fluorophores using single molecule imaging (Moerner, 2007). This sensitivity makes it possible to use activatable smart probes to precisely examine the subcellular localization of the activating target. However, there are still challenges in monitoring the precise activation pattern of optical probes since the probes may diffuse quickly throughout the cell. Activatable probes are better suited to monitor changes that occur within whole cells over time. They also hold much potential for \textit{in vivo} imaging, since probes can be activated by groups of similar cells or areas in the body that contain a higher concentration of a target molecule.

A major limitation of \textit{in vivo} optical imaging in tissue is the shallow penetration of light due to absorbance and scattering in body tissue. By using probes that operate in the near infrared (NIR) region of the light spectra, spanning from
600 nm to 1000 nm, water absorbance in the far infrared and light scattering and autofluorescence in the shorter wavelength range are minimized (Richards-Kortum and Sevick-Muraca, 1996). Using an NIR imaging system, the Weissleder group has pioneered in vivo imaging in mice using smart probes that are activated by tumour proteases (Mahmood et al., 1999). Since then, many other targets have been visualized in living organisms using activatable probes. Due to the greater depth of tissue that must be penetrated, optical imaging in humans is still a challenge and may never reach the resolution and depth possible with other imaging modalities. Nevertheless, innovative and refined imaging techniques will improve optical in vivo imaging. One advance in imaging technology is the use of multiphoton excitation. Multiphoton excitation permits light of a longer wavelength to be used to excite fluorophores. The phenomenon is well explained elsewhere (Zipfel et al., 2003). Multiphoton excitation is relevant for in vivo imaging, where longer wavelength light can more effectively penetrate tissues. Fluorescence lifetime imaging microscopy (FLIM) has been developed and offers the advantage over conventional microscopy of being able to perform measurements that are independent of fluorophore concentration. Since FLIM can measure changes in lifetimes brought about by environmental or chemical change, the probes used in certain FLIM experiments can be considered activatable probes. FLIM has been used to measure the change in the endogenous probe NADH in tissue culture (Ramanujan et al., 2005) and in vivo (Skala et al., 2007). New imaging technologies have been combined to create hybrid imaging techniques such as multiphoton FLIM (Peter and Ameer-Bet, 2004).

A promising new approach to fluorescence imaging in vivo has come through the development and commercial availability of fluorescence tomography (Ntziachristos et al., 2002). This technology permits the three dimensional reconstruction of fluorescence distribution. Mice are small enough to be completely imaged using this technique. Fluorescence diffuse optical tomography is a related technique that been used to image breast tumours in humans (Ntziachristos et al., 2000). Furthermore, three dimensional tomography based on fluorescence lifetimes has also been developed (Godavarty et al., 2005). As these new and informative three dimensional imaging techniques are further developed and refined, the usefulness of activatable probes will increase.

3. Mechanism of Activation

There are various routes in which different optical activatable smart probes may be activated. The nature of the activation depends on the characteristics of the optically active component of the probe, as well as the overall probe design. Figure 1 shows some common activation routes that lead to a change in the optical properties of probes. Figure 1A shows the design of a typical probe for sensing protease activity. A fluorophore is linked to a quenching moiety by a peptide linker. The amino acid sequence of the peptide linker is recognized by a specific proteolytic enzyme or enzyme class. Upon linker cleavage by the enzyme, the fluorophore and quencher
Fig. 1. Numerous activation pathways exist for activable smart probes. See text for explanation.

Disassociate, which leads to dequenching and brighter emission. Figure 1B shows the design of a standard beacon for sensing nucleic acids. The linker in this case is a nucleic acid hairpin loop structure. The hairpin portion of the beacon, which consists of about 4 to 6 hybridizing base pairs, physically forces the fluorophore and the quencher together. When a target nucleic acid hybridizes with the loop portion, the hairpin portion of the beacon comes apart and the quencher and fluorophore move apart, resulting in decreased quenching efficiency and greater fluorescence signal.

Figure 1C shows the design of a genetically encoded FRET sensor. This versatile construct consists of two fluorescent proteins, usually CFP and YFP variants,
connected by a linker protein. The genetically encoded linker protein can be a protein that changes conformation upon a substrate binding. Upon conformational change in the linker protein, the positioning of the two FRET proteins changes and results in a change in the FRET efficiency. Figures 1D and 1E represent smaller activatable smart probes that are not composed of amino acid or nucleobase subunits. Figure 1D shows an activatable optical probe that becomes brighter or dimmer upon binding or interacting with a specific substrate. This change can stem from either a substrate enhancement or quenching of the probe emission through contact with the fluorophore. In contrast, Figure 1E shows an activatable optical probe that becomes brighter or dimmer after the removal of part of the fluorophore by the target.

3.1. Enzyme activated optical probes

Because of their abundance, diversity and catalytic activity, enzymes are major targets for activatable smart probes. Proteases, which are involved in many biochemical processes, have been a central focus. One of the first protease activatable imaging agents was developed for the Factor Xa protease by fusing two FRET capable GFP variants linked with a Factor Xa peptide substrate (Mitra et al., 1996). The cleavage of the peptide linker between the two fluorescent proteins causes their dissociation and subsequent loss of FRET. A similar approach of using two fluorescent proteins fused by a specific linker sequence has been applied to detect other proteases including Botulinum toxin (Dong et al., 2004), caspasases (Lin et al., 2006), secretases (Lu et al., 2007), and matrix metalloproteases (Zhang et al., 2008). Some activatable optical probes rely on a conformational change, rather than cleavage, of the linker protein. The conformational change brought about by substrate binding alters the position of the two flanking fluorescent proteins which results in a change in FRET (Figure 1C). By using a protein linker consisting of both a specific phosphorylation substrate and a phosphorylation binding protein, enzymatic substrate phosphorylation and subsequent binding results in a conformational change, which moves the tethered fluorescent proteins closer together and results in higher FRET efficiency (Zhang et al., 2001).

Genetically encoded enzyme sensors have limitations that can be circumvented by using chemically synthesized probes. Genetically encoded sensors display a relatively low change in signal. Typically, the ratio of donor to acceptor emission changes less than two fold, which is a smaller change than most chemically synthesized activatable probes. A second drawback is that genetic probes will not likely be used for human imaging or therapy since this would involve the challenging task of transfecting humans with engineered genes.

A multitude of synthetic activatable enzyme sensing probes have been developed in the past decade. Many have been tested successfully in live mice, showing their potential for eventual use in human diagnostics and therapy. One of the first instances of imaging enzyme activity in vivo used a synthetic activatable optical
probe that detected tumour associated proteases in mice (Weissleder et al., 1999). This study used a polymer based self quenching infrared probe that, upon cleavage by lysine specific proteases, displays a 12 fold increase in emission. As detailed in their review, the Weissleder group has continued to expand this technique, and using similar methodology, has imaged enzyme activity in vivo for proteases associated with a variety of ailments including cardiovascular disease, cancer and HIV (Funovics et al., 2003). Recently, these activatable smart probes have been validated ex-vivo in deceased humans suffering from carotid endarterectomy, where cathepsin protease activity was detected (Jaffer et al., 2007). Protease activity in vivo has also been visualized using peptides linked to a large dendrimer core (McIntyre et al., 2004), as well as smaller non polymeric molecules (Blum et al., 2007). Extracellular proteases are good targets for activatable probes since the beacon does not require cellular uptake and often tumour vasculature permits beacon accumulation and subsequent activation. While most enzyme sensing activatable optical probes have focused on proteases, others have been developed for a range of different enzyme targets. Two probes have been designed for imaging beta-galactosidase activity in vivo (Tung et al., 2004; Wehrman et al., 2006). This enzyme is often used during genetic engineering and such a probe could facilitate identification of successfully modified cells. Another enzyme often used in genetic engineering, beta-lactamase, has also been the target for an infrared activatable probe that has been validated in cells (Xing et al., 2005). By designing an activatable probe with an ester linkage, esterase activity has been imaged in tissue culture (Kim et al., 2007). Lipase is another enzyme that has been examined using activatable optical probes. By using modified fluorescent lipids that are dequenched after lipase cleavage, lipid processing in the digestive system was visualized in live zebra fish (Farber et al., 2001).

3.2. Nucleic acid activated probes

Nucleic acid detection and imaging in living cells using molecular beacons was developed over a decade ago (Tyagi and Kramer, 1996). Molecular beacons have received considerable attention and are reviewed in deeper detail elsewhere (Goel et al., 2004, Tan et al., 2004). A molecular beacon is generally a self folding nucleic acid construct between 20 and 30 bases long, which holds together a fluorophore and a quencher on the extreme ends by 4 to 6 matching bases (Figure 1B). When the central loop region, designed to be complimentary to a target nucleic acid, hybridizes to the target, the beacon unfolds and the separation of the fluorophore and quencher leads to dequenching and stronger fluorescence emission.

Molecular beacons have emerged as a tool to determine the distribution and movement of RNA in living cells. Molecular beacons were used to visualize mRNA movement of developmental mRNAs, highlighting the importance of mRNA distribution in oocytes (Bratu et al., 2003). Molecular beacons have also been used to visualize mRNA transport into the nucleus, (Vargas et al., 2005) and viral mRNA behaviour of the poliovirus (Cui et al., 2005). Since measurement of RNA expression
levels does not require imaging, molecular beacons have not seen an increase in usage comparable to reverse transcriptase Polymerase Chain Reaction (PCR) and genetic microarrays. However, due to their utility at sensing specific nucleic acids, molecular beacons are sometimes used in real time PCR to monitor the amplification of target genes.

Many new reports about molecular beacons describe implementations of new beacon designs. Modified bases, especially 2-O-methyl bases, are used to encourage better hybridization (Molenaar et al., 2002) and to prevent beacon degradation (Bratu et al., 2003). Peptide nucleic acid (PNA) has also successfully been used to construct beacons that can image cellular RNA and offer improved signal to noise and hybridization properties (Xi et al., 2003). Several modifications of overall probe design have also been reported. Dual FRET beacons use two sets of molecular beacons that hybridize to neighbouring RNA sections and generate a FRET based signal (Santangelo et al., 2004). Quenched auto ligating (QUAL) probes function in a similar manner in the sense that two probes hybridize to one contiguous target RNA section. One of the probes then catalyzes the cleavage of the quenching group on the other self quenched probe, resulting in higher emission (Sando et al., 2004).

The use of molecular beacons for in vivo imaging represents an intriguing possibility to image RNA expression in an entire organism, although the challenge of delivery of the beacons must be overcome first.

### 3.3. Ion activated probes

Ion activated probes are by far the most commonly used activatable imaging agents. This is mainly due to the efficiency and importance of calcium imaging. Activatable optical probes have been designed and validated for a wide range of other ions as well, including zinc, iron and hydrogen ions.

The report which describes the creation of the Fluo dyes, which increase fluorescence emission in the presence of calcium, has become the second most highly cited publication in the Journal for Biological Chemistry (Grynkiewicz et al., 1985). Upon binding calcium, these probes display a wavelength shift and an approximate 30 fold increase in emission intensity. These and other classical ion sensing probes, many which have been used for decades, have been thoroughly reviewed elsewhere (Johnson, 1998). Calcium detection has also been examined using a genetically encoded activatable probe. The Cameleon sensor uses a FRET donor CFP linked to a FRET acceptor YFP by calmodulin, a calcium binding protein (Miyawaki et al., 1997). Subsequent work has improved the properties of Cameleon so that it displays a better dynamic range in physiological calcium concentrations (Truong et al., 2001). Upon binding calcium, the sensor undergoes a conformational change and increases the FRET emission ratio approximately 2 fold. Cameleons have been used in transgenic drosophila larvae (Liu et al., 2003) as well as transgenic mice (Hara et al., 2004) to measure neuron activity and calcium distribution. Another genetically encoded ion sensor is the hydrogen ion sensing pHluorin protein (Miesenbock...
et al., 1998). This GFP mutant displays a three fold range in the ratio of two excitation peaks as the pH shifts from 5 to 8. Fluorescence proteins and how they can be used effectively have been the subject of many good reviews (Giepmans et al., 2006, Chudakov et al., 2005, Roessel and Brand, 2001).

Recent research has led to the creation of new small molecule smart probes that can visualize ions. An activatable fluorescent probe, FluoZin3, has been developed for sensing zinc, which is involved in neuron function (Gee et al., 2002). FluoZin3 has a high affinity for zinc and increases emission upon zinc binding 100 fold. This probe is currently restricted to imaging extracellular zinc, and has been used to examine zinc regulation in rat brain slices (Kay, 2003). A chelatable iron specific probe was developed which fuses an iron chelating agent with a rhodamine dye, resulting in the fluorophore quenching upon iron binding (Petrat et al., 2002). A copper specific sensor was developed and validated that displays a 5 fold increase in emission detection when exposed to copper (Yang et al., 2005). Chemists have synthesized probes for a variety of other ions including chloride (Bai et al., 2005) and lead (Kwon et al., 2005), although these probes have not yet been validated in cells.

3.4. Reactive oxygen species (ROS) activated probes

Activatable smart probes that can detect ROS have seen a much new progress. Due to the intrinsic reactivity of ROS, many probes are based on a change in fluorescence properties upon ROS induced chemical modification. The singlet oxygen sensor DanePy is oxidized upon exposure to singlet oxygen and becomes less fluorescent (Hideg et al., 2002). This probe has been used to visualize singlet oxygen production in leaves after stress. Another fluorescent probe, Singlet Oxygen Sensor Green has also been developed and, unlike DanePy, is currently commercially available (Flors et al., 2006). Luminescent probes have also been developed that are suitable for imaging singlet oxygen (Yasui and Sakurai, 2000). Smart probes have been developed for another ROS, hydrogen peroxide, which is involved in cell signalling. The hydrogen peroxide sensing probes show good specificity since they show little response to other ROS. One reported probe is based on linking a fluorophore with a cleavable boron linkage (Miller et al., 2007), while another is based on peroxalate ester luminescent nanoparticles (Lee et al., 2007). Upon interaction with hydrogen peroxide, the linker is cleaved and the probe is unquenched. A genetic technique for an activatable ROS sensor has been developed by fusing a FRET pair of proteins linked by a hydrogen peroxide sensing protein (Belousev et al., 2006). Another important signalling radical, nitric oxide, is the target of an activatable probe capable of a 20 fold increase in emission (Kojima et al., 1998). Oxygen monitoring probes have also been developed and validated recently. A phosphorescence based sensor that is quenched by oxygen has been used to image oxygen in rats (Dunphy et al., 2002). A fluorescence based oxygen sensing smart probe has also been developed to image intracellular oxygen as well (O’Riordan et al., 2007). Both these oxygen sensing probes displayed a 5 fold signal difference upon activation.
3.5. Activatable optical probes for therapy

While activatable smart probes usually incorporate a fluorophore, it is possible to replace the fluorophore with a photosensitizing agent, creating a photodynamic beacon. This approach was first shown using the photosensitizer pyropheophorbide and a peptide linker specific for caspase 3 attached to a carotenoid quencher (Chen et al., 2004). Unlike conventional fluorophores, photosensitizers generate singlet oxygen upon excitation by light. In vivo, singlet oxygen goes on to attack many cellular targets, leading to apoptotic cell death (Oleinick and Evans, 1998). Photosensitizers are the basis of photodynamic therapy (PDT), a form of cancer treatment that has been in use for decades. A photosensitizer behaves similarly to a fluorophore in the sense that it can be excited with visible or NIR light, and its signal can be attenuated by a quencher. Upon activation, photodynamic smart probes therefore become toxic to cells. Photosensitizers not only generate singlet oxygen, but often also generate fluorescence emission. The fluorescence lifetime distribution of photosensitizers has been examined in cells using FLIM (Russell et al., 2007). Furthermore, the fluorescence of photosensitizers can be used to monitor singlet oxygen generation for photodynamic beacons (Stefflova et al., Frontiers in Bioscience, 2007). A PDT based activatable probe has been developed that becomes toxic to tumour cells that express the matrix metalloprotease 7 enzyme (Zheng et al., 2007). This protease is overexpressed in certain tumours. Tumours implanted in the mice showed tumour shrinkage when treated with the probe and light.

Photosensitizing agents have also been used to create molecular beacons that kill cells based on the expression of specific RNA targets using molecular beacon type constructs (Chen et al., 2008, in press). This represents a powerful therapeutic technique that could be widely adaptable to a variety of diseases by simply changing the target mRNA.

Combining activatable optical probes with PDT represents a new and novel therapeutic technique that has the potential to realize previously unachievable layer of specificity to disease treatment. The potential of photodynamic molecular beacons for cancer imaging and therapy has been examined by our group previously (Stefflova et al., Curr Med Chem, 2007).

3.6. Other noteworthy activatable smart probes

While many activatable sensors are specific for detecting enzymes, nucleic acids, ions or ROS, there are probes that can detect many other targets, including peptides, carbohydrates, lipids, and protein behaviour. A probe that can detect amyloid plaques has been developed that displays a 400 fold increase upon binding amyloid. It has been used to image amyloid plaques in mice brains (Nesterov et al., 2005). Carbohydrate sensing using activatable probes has received a great deal attention, although there has not been success yet in creating a suitable chemical in vivo imaging agent (Moschou et al., 2004). Carbohydrate sensing genetically encoded
Table 1. Comparison of selected optical activatable smart probes.

<table>
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<tr>
<th>Activator</th>
<th>Probe name</th>
<th>Type</th>
<th>Activatable Signal</th>
<th>Fold change</th>
<th>Em. λ (nm)</th>
<th>Imaging host</th>
<th>Ref</th>
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<tbody>
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<td>C</td>
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<td>400</td>
<td>610</td>
<td>Transgenic mouse brain slices</td>
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<td>Em. increase</td>
<td>3</td>
<td>660</td>
<td>Mouse</td>
<td>Tung et al., 2004</td>
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<td>A caged luciferin beta-galactoside reporter increase</td>
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<td>Em. increase</td>
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<td>660</td>
<td>C6 cells</td>
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<td>Botulinum toxin</td>
<td>G</td>
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<td>470/530</td>
<td>PC12 cells</td>
<td>Dong et al., 2004</td>
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<td>G</td>
<td>Change in em. ratio</td>
<td>2</td>
<td>480/530</td>
<td>HeLa cells</td>
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<td>475/580</td>
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<td>5</td>
<td>804</td>
<td>Mouse</td>
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<td>Em. increase</td>
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<td>Human (ex-vivo)</td>
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<td>CTAP-1</td>
<td>C</td>
<td>Em. increase</td>
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<td>490</td>
<td>3T3 cells</td>
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Table 1. (Continued)

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<td>5</td>
<td>515/570</td>
<td>Zebrafish</td>
<td>Farber et al., 2001</td>
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<td>Single nucleotide</td>
<td>QUAL probe</td>
<td>C</td>
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<td>7</td>
<td>520/580</td>
<td>E. Coli</td>
<td>Sando et al., 2003</td>
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<td></td>
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<td>Singlet oxygen</td>
<td>DanePy</td>
<td>C</td>
<td>Em. decrease</td>
<td>3</td>
<td>550</td>
<td>Spinach leaves</td>
<td>Hideg et al., 2002</td>
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<td>Tyrosine kinase</td>
<td>Tyrosine kinase</td>
<td>G</td>
<td>Change in em. ratio</td>
<td>1.35</td>
<td>475/530</td>
<td>B82 and MEF cells</td>
<td>Ting et al., 2001</td>
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<td>FRET sensor</td>
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<td>vav oncogene</td>
<td>EDANS –oligo Dabcyl</td>
<td>C</td>
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<td>15</td>
<td>490</td>
<td>TKts13 and K562 cells</td>
<td>Sokol et al., 1998</td>
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<td>Zn</td>
<td>FluoZin-3</td>
<td>C</td>
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<td>100</td>
<td>510</td>
<td>Pancreatic B-cells</td>
<td>Gee et al., 2002</td>
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<tr>
<td>413 nm laser</td>
<td>Photo-activatable GFP</td>
<td>G</td>
<td>Em. increase</td>
<td>100</td>
<td>520</td>
<td>Cos-7 cells</td>
<td>Patterson et al., 2002</td>
</tr>
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<td>activation</td>
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<td>488 nm laser</td>
<td>Dendra</td>
<td>G</td>
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<td>150</td>
<td>575</td>
<td>Hela cells</td>
<td>Gurskaya et al., 2006</td>
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</table>
probes, however, have been successfully developed using a FRET pair of fluorescent proteins and carbohydrate binding linker (Fehr et al., 2002).

Other recent advances in targeted imaging include a photoactivatable GFP mutant that only becomes fluorescent after shorter wavelength laser irradiation (Patterson et al., 2002). By irradiating certain regions of the cell, the movement of tagged proteins from that area can be traced over time. This type of GFP has been used to image drosophila embryo in vivo (Post et al., 2004). Furthermore, different variants have been developed that shift wavelength and are activatable with a longer wavelength irradiation (Gurskaya et al., 2006). Another recent technique in monitoring protein interaction is bifluorescence complementation (Hu et al., 2002). In this technique, two proteins are tagged with partial polypeptides from a fluorescent protein. If the tagged proteins move close together to interact, the nonfluorescent polypeptides fold into the correct structure and fluoresce. This technique overcomes the difficulty of conventional FRET in adjusting for different relative concentrations of acceptor and donor.

3.7. Comparison of different probes

Many diverse smart probes exist that have a wide range of activators and spectral properties. A comparison of some of the probes mentioned in this review is shown in Table 1. The “Type” column refers to whether the probe is genetically encoded (“G”) or chemically synthesized (“C”). It should be noted that in some cases, researchers obtained varying degrees of probe activation in vivo and in vitro, often with the in vitro activation demonstrating greater activation increase. Where possible, the in vivo fold change is reported. In some cases, the fold change and emission properties were estimated from graphs in figures. The original references should be consulted for more accurate information.

4. Conclusion

Numerous optical activatable smart probes have been developed in the past decade that can detect a wide range of targets. Many of these probes are suited for the in vivo imaging of physiological and pathogenic processes in small animals. The usefulness of activatable optical imaging will increase as imaging systems improve resolution and depth capability. Although current smart probes will continue to be used, new generations of activatable optical imaging agents will be developed and will display better contrast and selectivity.

Combining PDT with activatable smart probes is an intriguing therapeutic paradigm. Although this technique is in its infancy, it has already shown to be a successful treatment for xenograft tumours in mice. Many of the synthetic activatable smart probes that have been developed have the potential to be adapted to a photodynamic probe by substituting the fluorophore for a photosensitizing agent. Smart probes that can become activated in certain environments and kill target
cells when treated with light have the potential to treat diseases with unprecedented specificity.

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**References**


