Review

Development of fluorescent probes for bioimaging applications

By Tetsuo NAGANO[*1,†]

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Abstract: Fluorescent probes, which allow visualization of cations such as Ca^{2+}, Zn^{2+} *etc.*, small biomolecules such as nitric oxide (NO) or enzyme activities in living cells by means of fluorescence microscopy, have become indispensable tools for clarifying functions in biological systems. This review deals with the general principles for the design of bioimaging fluorescent probes by modulating the fluorescence properties of fluorophores, employing mechanisms such as acceptor-excited Photoinduced electron Transfer (a-PeT), donor-excited Photoinduced electron Transfer (d-PeT), and spirocyclization, which have been established by our group. The a-PeT and d-PeT mechanisms are widely applicable for the design of bioimaging probes based on many fluorophores and the spirocyclization process is also expected to be useful as a fluorescence off/on switching mechanism. Fluorescence modulation mechanisms are essential for the rational design of novel fluorescence probes for target molecules. Based on these mechanisms, we have developed more than fifty bioimaging probes, of which fourteen are commercially available. The review also describes some applications of the probes developed by our group to *in vitro* and *in vivo* systems.

Keywords: probe, bioimaging, photoinduced electron transfer, fluorescence, spirocyclization

Introduction

It is generally said that an appeal to the eye is more effective than an appeal to the ear, which may be the basis of the proverb "Seeing is believing". Methods to "see into the body" or "see into cells" are essential for the diagnosis and treatment of disease, as well as for research into the basic processes of life. It is desirable that the methods used should not be invasive, *i.e.*, should not involve cutting into the body or isolating cellular constituents. Therefore, techniques to visualize physiological or pathophysiological changes in the body and cells have become increasingly important in biomedical sciences.

Compared to other technologies such as radioisotope labeling, magnetic resonance imaging (MRI), electron spin resonance (ESR) spectroscopy, and electrochemical detection, fluorescence imaging has many advantages for this purpose, because it enables highly sensitive, less-invasive and safe detection using readily available instruments. Another advantage of fluorescence imaging we should emphasize here is that the fluorescence signal of a molecule can be drastically modulated, so that probes relying on activation, not just accumulation, can be utilized. Today, fluorescent probes based on small organic molecules have become indispensable tools in modern biology because they provide dynamic information concerning the localization and quantity of the molecules of interest, without the need for genetic engineering of the sample. It is also expected that technology using fluorescent probes will play a pivotal role in the field of drug discovery, with applications in both academia and industry. For *in vivo* molecular imaging, fluorescent probes are administered to the subject and emit a signal within the body. It should be clear that, in order to achieve successful imaging, the role of appropriate chemical design for activation of the probes, is extremely important.

At present, activatable probes for cell or *in vivo* imaging, which emit an increased fluorescence signal after reaction with the target biomolecules, are not very common.[1)–3)] Over the past decade, however, we have developed a variety of such probes, based on

[*1] Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan.
[†] Correspondence should be addressed: T. Nagano, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan (e-mail: tlong@mol.f.u-tokyo.ac.jp).

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several design strategies, including the mechanisms listed below.

(1) Photoinduced electron Transfer (PeT) mechanism[2),4)–9)]
   i) acceptor-excited PeT (a-PeT) mechanism[10)]
   ii) donor-excited PeT (d-PeT) mechanism[11)]
(2) Förster Resonance Energy Transfer (FRET) mechanism[12)–14)]
(3) Intramolecular Charge Transfer (ICT) mechanism[15)]
(4) Spirocyclization mechanism[16)]

This review focuses on three mechanisms (a-PeT, d-PeT, and spirocyclization) for control of fluorescence characteristics, which have been established by our group and others, together with some bioimaging applications of probes utilizing these mechanisms.

Principles for modulating the fluorescence properties

Fluorescent probes are excellent sensors for biomolecules, being sensitive, fast-responding, and capable of affording high spatial resolution *via* microscopic imaging. Suitable fluorescent probes are naturally of critical importance for fluorescence imaging, but only a limited range of biomolecules can currently be visualized because of the lack of flexible design strategies. Most fluorescent probes were obtained empirically, not rationally, and novel rational approaches are required for efficient development of practical fluorescent probes. Therefore, our goal is to establish a general strategy to create a wide variety of practical fluorescent probes for certain biomolecules. Rational and practical strategies based on general fluorescence modulation mechanisms would enable us to rapidly develop novel fluorescent probes for target molecules. Here, we present three general principles for modulating the fluorescence properties of fluorophores including fluorescein and rhodamine, with particular emphasis on our own work.

1. Acceptor-excited Photoinduced electron Transfer (a-PeT) mechanism. Fluorescein is a highly fluorescent molecule that emits long-wavelength light upon excitation at around 500 nm in aqueous media. Fluorescein derivatives have been widely used as fluorescent tags for many biological molecules such as protein, DNA, and so on, and fluorescein has been used as a platform for many kinds of fluorescent probes.[17)–20)] We have developed a range of novel fluorescein-based fluorescent probes, including diaminofluoresceins (DAFs).[21)]

DAFs are weakly fluorescent before reaction with nitric oxide (NO), but become highly fluorescent after reaction with NO. I will introduce the a-PeT mechanism using DAF-2 as an example. As shown in Fig. 1, DAF-2 is converted to a triazole compound, DAF-2 T by reaction with NO, and this causes little change of the absorbance maximum, but greatly increases the fluorescence intensity. Notably, the increase of fluorescence intensity is dependent on the concentration of NO.

The reason why DAF-2 is almost non-fluorescent can be explained in terms of the a-PeT mechanism, through which the fluorescence of a fluorophore is quenched by electron transfer from the donor to the acceptor fluorophore.[4),8),10),11)] The fluorescein structure can be divided into two parts, *i.e.*, the benzene moiety as the PeT donor and the xanthene ring as the fluorophore (Fig. 2), because only small alterations in absorbance were observed among fluorescein and its derivatives and the dihedral angle between the benzene moiety and the xanthene ring is almost 90°, which suggests that there is little ground-state interaction between these two parts. In other words, although there is no obvious linker within the fluorescein molecule, it can be understood as a directly linked donor–acceptor system, in which PeT might determine the quantum efficiency of fluorescence (Φ_{fl}). The results of our study using a series of fluorescein derivatives indicated that weakly fluorescent derivatives have benzene moieties that work as electron donors to the excited fluorophore (a-PeT).[22)] If the highest occupied molecular orbital (HOMO) energy level of the benzene moiety is high enough (in other words, if the oxidation potential of the moiety is low enough) for electron transfer to the excited xanthene ring, the Φ_{fl} value is small (Fig. 2, left). In contrast, fluorescein derivatives with high Φ_{fl} values have benzene moieties with low HOMO energy levels, where a-PeT cannot take place (Fig. 2, middle). In the case of DAF-2, the HOMO energy level of benzotriazole, which is the benzene moiety of DAF-2 T, is substantially lower than that of diaminobenzene, the corresponding moiety of DAF-2. Hence, electron transfer from the benzene moiety of DAF-2 T to the fluorophore does not occur on irradiation, which leads DAF-2 T to be highly fluorescent. Thus, the a-PeT process controls the fluorescence properties of fluorescein derivatives, and importantly, these properties can be predicted from the HOMO energy level of the benzene moiety.

Subsequently, to evaluate the relationship between the oxidation potential of the benzene moiety

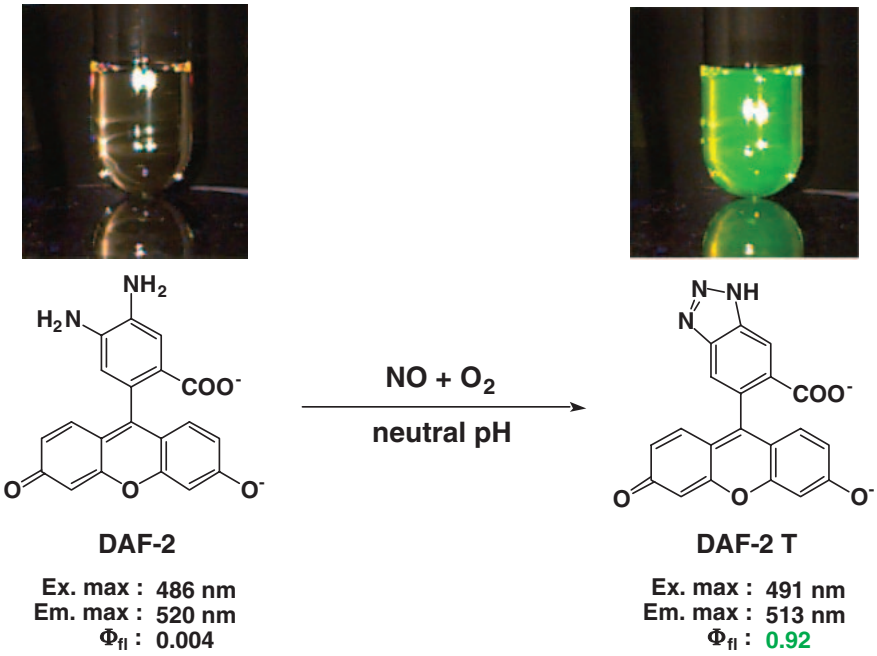

Fig. 1. NO bioimaging probe, DAF-2, and reaction with NO to produce DAF-2 T.

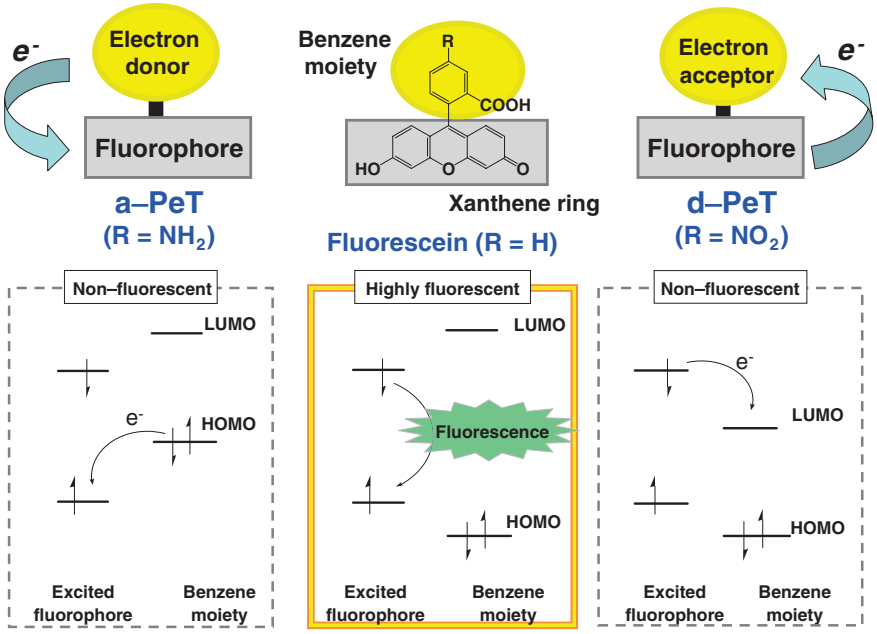

Fig. 2. Schematic illustration of the modulation of fluorescence properties by the acceptor-excited PeT (a-PeT) mechanism (left column) and donor-excited PeT (d-PeT) mechanism (right column). Fluorescein derivatives can be regarded as conjugates of two independent moieties, the benzene moiety and the xanthene ring (fluorophore). Although fluorescein itself (R = H, middle column) is highly fluorescent, some of its derivatives have almost no fluorescence, due to PeT. For example, fluorescence of a derivative with an amino group on the benzene moiety (left column) is quenched by electron transfer *from* the benzene moiety *to* the excited fluorophore (a-PeT). For this to take place, the HOMO (highest occupied molecular orbital) energy level of the benzene moiety must be very high. On the other hand, fluorescence of a derivative with a nitro group on the benzene moiety (right column) is quenched by electron transfer *to* the benzene moiety *from* the excited fluorophore (d-PeT). For this to take place, the LUMO (lowest unoccupied molecular orbital) energy level of the benzene moiety must be very low. See the text for further explanation.

and the Φ_{fl} values of the molecule in detail, we designed and synthesized various derivatives of an analog of fluorescein (TokyoGreen), in which the oxidation potential of the benzene moiety was finely tuned by introducing electron-donating groups into the moiety.[10] The results clearly showed that the fluorescence properties of TokyoGreen derivatives can indeed be finely modulated by varying the oxidation potential of the benzene moiety. We believe that this information provides the basis for a practical strategy for rational design of novel functional fluorescent probes. Importantly, although PeT-based fluorescent probes have been known for a long time,[23),24)] such a detailed study with a visible-wavelength fluorophore has not been performed previously.

2. Donor-excited Photoinduced electron Transfer (d-PeT) mechanism.[11)] The finding that the fluorescence properties of fluorescein derivatives can be modulated by a-PeT from the benzene moiety to the acceptor fluorophore enabled us to design flexibly many kinds of functional fluorescent probes based on the change of the oxidation potential of the benzene moiety upon encountering a target molecule.[10)] This rational strategy made it possible to develop novel fluorescent probes with high efficiency.[25)–28)]

Next, we introduce another principle for controlling the fluorescence properties of the fluorescein molecule, based on electron transfer from the excited fluorophore to the benzene moiety (donor-excited PeT; d-PeT), *i.e.*, in the opposite direction to a-PeT (Fig. 2, right).

We designed and synthesized dicarboxyfluorescein (**1a**) and its trimethyl ester derivative (**1b**), as shown in Fig. 3. Owing to the presence of electron-withdrawing ester groups, the HOMO energy level of the benzene moiety of **1b** should be lower than that of **1a**. According to our previous studies, the oxidation potential, or HOMO energy level, of the benzene moiety is the most important factor determining the Φ_{fl} value of fluorescein. Therefore, we considered that both **1a** and **1b** should be highly fluorescent (Fig. 3). As anticipated, **1a** was highly fluorescent ($\Phi_{fl} = 0.817$), but unexpectedly, the fluorescence of **1b** was significantly quenched ($\Phi_{fl} = 0.001$). From the viewpoint of a-PeT, these results appeared contradictory.

To understand the mechanism underlying the fluorescence quenching further, we focused particularly on the *reduction* potential of the benzene moiety. In consequence, we found that the fluorescence properties of fluorescein derivatives are influenced by not only the oxidation potential of the benzene moiety, but also the reduction potential of the benzene moiety. This is because PeT can take place in the opposite direction to a-PeT, *i.e.* from the excited fluorophore to the lowest unoccupied molecular orbital (LUMO) of an electron acceptor (Fig. 2, right), which we call d-PeT. In this case, if the reduction potential of the benzene moiety is high enough, *i.e.*, if the LUMO energy level is low enough, for electron transfer to occur thermodynamically, the singlet excited state of the fluorophore will be quenched *via* electron transfer and the fluorescence will be diminished. By taking advantage of the intramolecular donor–acceptor system, the fluorescein molecule might become useful as a platform not only for a-PeT probes, but also for d-PeT probes.

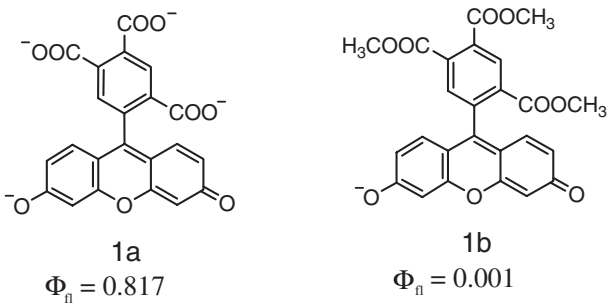

Fig. 3. Structures and fluorescence properties of carboxyfluorescein derivatives (**1a**, **1b**).

Further, to appreciate fully the relationship between the reduction potential of the benzene moiety and the Φ_{fl} value, we designed and synthesized various fluorescein derivatives in which the benzene moieties are replaced with several electron-deficient aromatic rings. Their structures, the absorbance and fluorescence properties, the reduction potentials of their benzene moieties, and the Φ_{fl} value in basic aqueous media were examined.[11)] The absorbance and emission maxima showed no significant change among the derivatives, and thus the ground-state interaction between the benzene moiety and the xanthene moiety was small in each derivative. On the other hand, the Φ_{fl} values varied greatly, depending on the reduction potential of the benzene moiety. In general, the feasibility of electron transfer between an excited-state sensitizer and quencher can be judged from the change in free energy (ΔG_{eT}). The ΔG_{eT} value can be calculated from the Rehm–Weller equation

$$\Delta G_{eT} = E_{ox} - E_{red} - \Delta E_{0,0} - w_p$$

where E_{ox} and E_{red} are the oxidation and reduction potentials of the electron donor (*i.e.* fluorophore) and acceptor (*i.e.* benzene moiety), respectively, $\Delta E_{0,0}$ is the singlet excited energy, and w_p is the work term for the charge separation state.[29] Owing to the similarity of their structures and the alteration in charge which accompanies electron transfer, the synthesized fluorescein derivatives have almost the same values of E_{ox}, $E_{0,0}$, and w_p. Therefore, in this case the E_{red} value plays a primary role in determining the feasibility of electron transfer. Indeed, as described above, the Φ_{fl} values of synthesized fluorescein derivatives were strongly influenced by change of the E_{red} value. Despite the widespread use of a-PeT as a fluorescence-modulating principle of current PeT probes, to our knowledge there are few functional fluorescent probes that utilize d-PeT. If d-PeT-based fluorescence modulation mechanism is applicable as a gerneral design principle as we believe, it would allow the development of novel and useful functional fluorescent probes for biomolecules that could not be visualized with so-far developed a-PeT-based probes.

On the basis of these two design strategies, it is possible to develop novel PeT-based fluorescent probes for a wide range of targets by introducing an appropriate sensor moiety. Now, we can choose either an oxidizable sensor moiety or a reducible sensor moiety, and alteration of the redox potential of the sensor can be used as a fluorescence modulation switch. To summarize, the fluorescence properties of fluorescein derivatives can be controlled by either the a-PeT or d-PeT process, which provides the basis for a practical strategy for rational design of fluorescent probes to detect various biological events. It should also be pointed out that both a-PeT and d-PeT can be used to design probes based on other fluorophores such as BODIPY,[30),31)] cyanine,[32)] and rhodamine.[33)]

3. Spirocyclization mechanism. We previously found that the derivatization of fluorescein does not disrupt the photochemical properties when various functional groups are substituted for the carboxyl group at the 2 position.[10)] We thought that a similar design might be applicable to the tetramethylrhodamine (TMR) fluorophore and have developed new TMR derivatives that show different dependences of their behavior upon the environment. Among them, the fluorescence of hydroxymethyltetramethylrhodamine (HMTMR) showed a very interesting environmental dependence. HMTMR has a large absorbance and fluorescence in protic solvents but little in aprotic or basic solvents. This was found to be because of intramolecular spirocyclization at C9, deconjugating the TMR fluorophore (Fig. 4, upper panel). The spirocyclic structure was confirmed by X-ray crystallography.[16)]

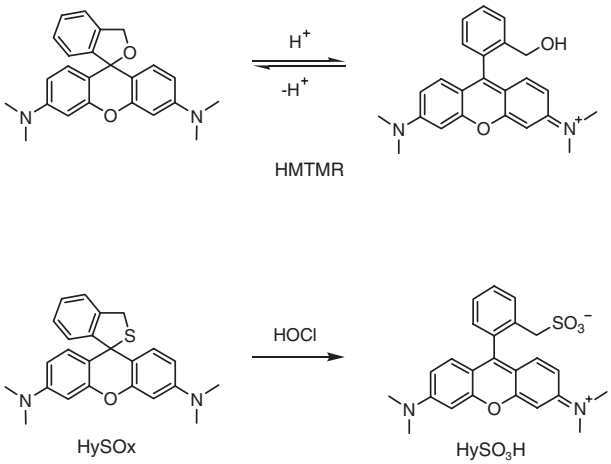

Fig. 4. Structures of HMTMR, HySOx, and $HySO_3H$, and reaction of HySOx with HOCl to produce $HySO_3H$.

Regulation of the spirocyclization of rhodamines provides a new approach to the rational development of novel fluorescent probes, and we successfully developed a new fluorescent probe named HySOx, which can specifically detect hypochlorous acid (HOCl) (Fig. 4, lower panel).[16)] HySOx is almost completely colorless and non-fluorescent in aqueous solutions over a wide range of pH values and in various organic solvents. HySOx favors a spirocyclic form more strongly than HMTMR because HySOx has a thiol group, which is more nucleophilic than the hydroxyl group of HMTMR.

The thiol group of HySOx works not only as a cyclization enhancer, but also as a center of redox reaction. We tested the utility of HySOx as a fluorescence probe for reactive oxygen species (ROS). Various important ROS generated in organisms (*i.e.*, HOCl, hydroxyl radical, peroxynitrite, NO, superoxide, singlet oxygen, and hydrogen peroxide) were added to a solution of HySOx at pH 7.4. The fluorescence intensity increased quickly and quantitatively only upon addition of sodium hypochlorite (NaOCl) to produce $HySO_3H$, and the absorbance also increased. HySOx is able to detect HOCl specifically among various ROS in aqueous solution. Currently, there are other fluorescence probes that can detect HOCl,[34)–36)] but HySOx seems to be the first one that can detect HOCl specifically under biological conditions.

Recently, many fluorescent probes based on rhodamine spirocyclization have been reported,[37)]

Table 1. Bioimaging probes developed by Nagano's group

Nitric Oxide (NO) Probes	DAF-1,[21] DAF-2,[21,38] DAF-2 DA,[21,38] DAF-3,[21] DAF-4,[21,38] DAF-4 M1,[38] DAF-4 M2,[38] DAF-5,[21,38] DAF-5 M1,[38] DAF-5 M2,[38] DAF-6,[21,38] DAF-FM,[38] DAF-FM DA,[38] DAMBO,[39] DAMBO-R,[39] DAMBO-P^H,[39] DAMBO-P^{Me},[39] DAC-P,[32] DAC-S,[32] DCl-DA Cal,[25] DCl-DA Cal-AM[25]
Highly Reactive Oxygen Species (ROS) Probes	HPF,[26] APF,[26] MitoHR,[33] MitoAR,[33] APC,[25] APC-AM[25]
ROS Oxidative Stress Probe	FOSCY-1[40]
Peroxynitrite Probe	NiSPY-1,[30] NiSPY-2,[30] NiSPY-3[30]
Singlet Oxygen Probes	DPAX-1,[41] DPAX-2,[41] DPAX-3,[41] DMAX[22]
Hypochlorous Acid (HOCl) Probe	HySOx[16]
Zinc Probes	ZnAF-1,[42] ZnAF-1F,[43] ZnAF-2,[27,42] ZnAF-2 DA,[27] ZnAF-2F,[43] ZnAF-2M,[27] ZnAF-2MM,[27] ZnAF-3,[27] ZnAF-3 DA,[27] ZnAF-4,[27] ZnAF-5,[27] ZnAR-R1,[44] ZnAF-R2,[44] DIPCY Zinc (NIR probe),[45] ZnIC,[15] Zn probe based on ICT[46]
pH Probes	NIR pH probe,[47] pH probe for cancer imaging[48]
Environment-sensitive Probes	BODIPY-based probe,[31] photosensitizer with off/on switching for singlet oxygen generation[49]
Anion Probe	TC2412-Cd[50]
Esterase Probes	Near-infrared (NIR) esterase probe,[47] Ln-1–12 (Lanthanide complexes probes),[51] ratiometric probe based on BODIPY scaffold[52]
β-Galactosidase Probes	TG-βGal,[28] AM-TG-βGal[28]
Glutathione S-transferase Probes	DNAF-1,[53] DNAF-2,[53] DNAT-Me[53]
Enzyme Activity-based Labeling Probe	CMF β-Gal[54]
Phosphodiesterase Probe	CPF-1,[55] CPF-2,[55] CPF-3,[55] CPF-4[55]
Protein Tyrosine Phosphatase (PTP) Probe	Protein Tyrosine Phosphatase (PTP) Probe[56]
Protein Kinase Probe	Protein Kinase Probe[57]

but the spirocyclization mechanism should be applicable for the development of probes with other fluorophores, too. In contrast to PeT-based probes, spirocyclization-based probes have no absorbance upon irradiation with the excitation light, before reaction with the target molecule. Hence, if designed well, they are expected to provide higher S/N ratios and better photostability than PeT-based probes.

Probes for *in vitro* and *in vivo* imaging developed by our group

Our probes have been designed rationally, based on detailed photochemical investigation of various mechanisms of modulation of fluorescence properties, including a-PeT, d-PeT, FRET, ICT, and spirocyclization. So far, we have developed more than fifty probes for biological applications (Table 1), including those listed below, of which fourteen are now commercially available and are used by cutting-edge biologists all over the world.

Many of the fluorescent probes discovered by our group are useful for cellular and *in vivo* bioimaging. Noninvasive visualization and investigation of interactions among small biomolecules, proteins, DNA, lipids and sugar in living cells are an important goal for biologists, and fluorescent probes are powerful tools for this purpose. In the next section, we will describe bioimaging using DAF and HySOx as examples of biological applications of the probes.

Bioimaging applications of fluorescent probes: DAF and HySOx

1. Bioimaging applications of DAF and DCl-DA Cal. A DAF derivative was applied to NO

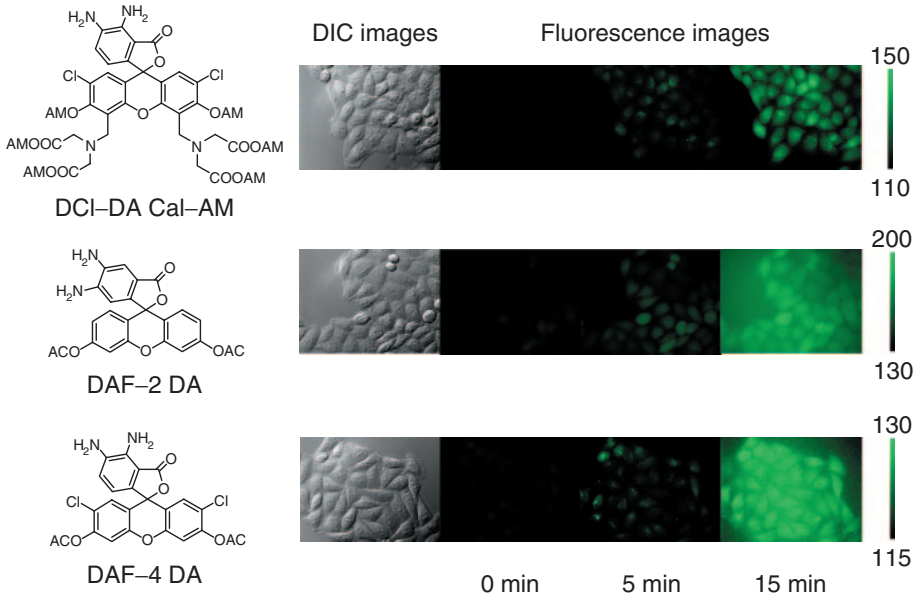

Fig. 5. NO detection by DCl-DA Cal AM, DAF-2 DA and DAF-4 DA in living cells. Fluorescence and differential interference contrast (DIC) images of HeLa cells loaded with 10 µM (0.1% DMSO as a cosolvent) DCl-DA Cal-AM (upper panel), DAF-2 DA (middle panel), or DAF-4 DA (lower panel) for 30 min are shown. The change of fluorescence images after addition of NOC 7 (final 100 µM) was measured at 5 and 15 min.

bioimaging in cultured bovine aortic endothelial cells.[38] After stimulation with bradykinin, the fluorescence intensity in the cells increased and the augmentation of the fluorescence intensity was suppressed by an NOS inhibitor. A DAF derivative was also applied to imaging of NO generated in rat hippocampal slices by exposure to an aglycemic medium.[58] NO production was observed mainly in the CA1 area and was dependent on the concentration of O_2. During exposure to an anoxic–aglycemic medium, NO was hardly produced, while marked elevation of intracellular Ca^{2+} was observed. Production of NO increased sharply as soon as the perfusate was changed to the normal medium. These results suggest that NOS is activated after reperfusion rather than during ischemia.

DAFs are excellent fluorescent probes for NO, but their sensitivity is sometimes insufficient to measure NO in living cells. Hence, we designed and synthesized dichlorodiaminocalcein as a novel fluorescent probe for NO, to confirm that improving the intracellular retention of fluorescent probes generally leads to enhancement of sensitivity.[25] The fluorescence quantum yield of DCl-DA Cal is 0.013 at pH 7.4, indicating that the fluorescence is well quenched *via* the a-PeT mechanism. When DCl-DA Cal reacts with NO in air, the triazole compound, DCl-DA Cal T, is produced and emits strong fluorescence in the same manner as DAF-2. As a membrane-permeable fluorescent probe, we also prepared DCl-DA Cal-AM, in which the phenolic hydroxyl group and carboxyl group are protected as acetoxymethyl (AM) ester (DCl-DA Cal-AM, see Fig. 5 for the structure).

To examine how well DCl-DA Cal is retained in living cells, we compared DCl-DA Cal-AM with our first-generation probes, DAF-2 DA and DAF-4 DA (Fig. 5). The experiment to examine the leakage of these triazole compounds, DCl-DA Cal T, DAF-2 T and DAF-4 T, was done by addition of a NO donor (NOC-7) to living cells. In contrast to rapid leakage of DAF-2 T and DAF-4 T, DCl-DA Cal-T was well retained in the living cells. The results obtained show that DCl-DA Cal and DCl-DA Cal T have excellent intracellular retention. We applied DCl-DA Cal-AM to cultured bovine aortic endothelial cells. The results indicated that visualization of low levels of NO depends critically on preventing leakage of the probe and the fluorescent product from the cells.

In other words, to develop fluorescent probes that offer high sensitivity inside living cells, efficient retention of the probes and products within the cells is required. The iminodiacetic acid group (IAG) of DCl-DA Cal was very effective in improving intracellular retention. It is expected that by introducing IAG into other fluorescein-based probes, intracellular

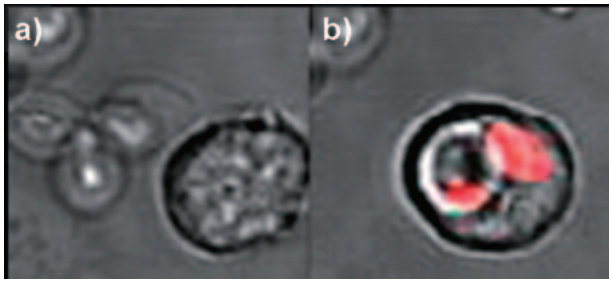

Fig. 6. Confocal microscopic imaging of porcine neutrophil with HySOx (5 µM). (a) Zymosan particles are present near the neutrophil. (b) Phagocytosis is complete.

retention of the probe and the product should be significantly improved, which is advantageous for the visualization of low levels of physiological molecules and for long-term observation in living cells.[59)]

In conclusion, DCl-DA Cal-AM is a useful tool for visualizing the temporal and spatial distribution of intracellular NO.

2. Bioimaging applications of HySOx.[16)] HySOx was applied to visualize phagocytosis by porcine neutrophils, with opsonized zymosan derived from *Saccharomyces cerevisiae* as a target. HySOx detected HOCl generated inside the phagosomes just after the completion of phagocytosis (Fig. 6). In the imaging of phagocytosis, there was little fluorescence increase due to autoxidation under laser excitation before phagocytosis began. Further, there was no fluorescence decrease due to photobleaching after membrane fusion following phagocytosis. In contrast, it is well-known that ROS probes such as dihydrodichlorofluorescein (DCFH) become fluorescent due to autoxidation simply upon exposure to excitation light. Furthermore, photobleaching is a very common problem in fluorescence microscopy. Therefore, HySOx has superior properties as a probe, being tolerant to autoxidation due to its high specificity for HOCl and producing a fluorescent product, $HySO_3H$, which is photostable and shows pH-independent fluorescence owing to the TMR fluorophore.

In conclusion, HySOx is a useful tool for visualizing the temporal and spatial detection of HOCl generated inside phagosomes.

Conclusion

In this review, I have introduced several approaches (a-PeT, d-PeT, and spirocyclization) to the design of bioimaging probes. These strategies should be applicable to develop fluorophore-derived functional probes for a wide range of applications.

PeT is one of the relaxation processes from the excited state to the ground state, and is an important mechanism for fluorescence quenching in photochemistry. It can be applied for design of not only bioimaging fluorescent probes, but also other photofunctional molecules, including photosensitizers with an environment-sensitive off/on switch for singlet oxygen generation,[49)] highly activatable and rapidly releasable caged fluorophores,[60)] and lanthanide complex probes that are applicable for the screening of enzyme inhibitors, as well as for clinical diagnosis.[51)]

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Profile

Dr. Nagano is a full professor at the Department of Chemistry and Biology, Graduate School of Pharmaceutical Sciences, the University of Tokyo, and also Director General of the Chemical Biology Research Initiative for drug discovery at the University of Tokyo. He was elected Dean of the Graduate School of Pharmaceutical Sciences in 2010.

He graduated from the Faculty of Pharmaceutical Sciences, the University of Tokyo, in 1972. After completing his Ph.D. with work on development of bio-active heterocyclic compounds under the supervision of Professor Toshihiko Okamoto at the University of Tokyo, he became an Assistant Professor at the same University. In 1983–1985 he joined Professor Fridovich's group as a research associate in the Department of Biochemistry, Duke University Medical School, and worked on a novel detection method for reactive oxygen species. After his studies at Duke University, he rejoined the University of Tokyo as an Associate Professor and changed his research field, moving from organic chemistry to chemical biology. Recently, his main research interests are focused on the molecular design, synthesis and applications of novel bio-imaging probes for physiologically active species, such as nitric oxide, Zn^{2+}, reactive oxygen, and various enzymes including beta-galactosidase and caspase. He has developed more than 50 fluorescent bioimaging probes by means of rational molecular design methods, employing the mechanisms of photoinduced electron transfer (a-PeT and d-PeT), Förster resonance energy transfer (FRET) and spirocyclization. Fourteen of those probes are now commercially available and widely used by biological researchers throughout the world. He was awarded the Uehara Prize in 2004, Shimadzu Prize in 2005, National Medal with Purple Ribbon in 2006 and the Pharmaceutical Society of Japan Award in 2006. He was also President of the Pharmaceutical Society of Japan in 2008.