Japan Academy Prize to:

Tetsuo NAGANO

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for "Research on General Principles for Modulating the Fluorescence Properties of Bioimaging Probes and Their Applications to Life Science"



Outline of the work:

Bioimaging techniques have become indispensable tools for clarifying the functions of biological systems at the molecular level, because they can provide dynamic information concerning the localization and amount of bioactive molecules of interest *in vitro* and *in vivo*. Dr. Tetsuo Nagano has developed powerful general principles for the rational design of bioimaging fluorescent probes by employing mechanisms such as acceptor-excited photoinduced electron transfer (a-PeT) and donor-excited photoinduced electron transfer (d-PeT) to modulate the fluorescence properties of fluorophores. The a-PeT and d-PeT mechanisms established by Dr. Nagano have been applied for off/on fluorescence switching in the design of a wide range of bioimaging probes based on many fluorophores. First, the mechanisms are introduced below.

1. Mechanisms for modulation of fluorescence properties: a-PeT and d-PeT

Fluorescein is a widely used, highly fluorescent molecule that emits long-wavelength light upon excitation at around 500 nm in aqueous media. Dr. Nagano has developed diaminofluoresceins (DAFs) as novel fluorescein-based nitric oxide (NO) probes. DAFs are weakly fluorescent before reaction with NO, but become highly fluorescent after reaction with NO. The weak fluorescence of DAFs themselves can be explained in terms of the a-PeT mechanism, through which fluorescence is quenched by electron transfer from the benzene moiety of DAFs to the fluorophore.

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, and there appears to be little ground-state interaction between these two parts, 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°. 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 Dr. Nagano's studies 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). If the highest occupied molecular orbital (HOMO) energy level of the benzene moiety is high enough to permit electron transfer to the excited xanthene ring, the Φ_{fl} value is small. In contrast, fluorescein derivatives with high Φ_{fl} values have benzene moieties with low HOMO energy levels, and a-PeT cannot take place.

Dr. Nagano also uncovered the d-PeT mechanism as another principle for modulating the fluorescence properties of the fluorescein molecule, based on electron transfer from the excited fluorophore to the benzene moiety, i.e., in the opposite direction to a-PeT.

By utilizing these mechanisms, Dr. Nagano has developed more than forty bioimaging probes, of which

fourteen are commercially available at present. Fluorescence modulation mechanisms such as a-PeT and d-PeT are essential to enable the rational design of novel fluorescence probes for an enormous range of target molecules.

2. Applications of bioimaging fluorescent probes (DAFs) to living cells

In 1987, it was proposed that endothelium-derived relaxing factor (EDRF) is nitric oxide (NO) or a labile nitroso species. To investigate the biological roles of NO, Dr. Nagano has developed a range of novel fluorescein-based fluorescent NO probes, including diaminofluoresceins (DAFs: DAF-2, DCI-DA Cal, DCI-DA Cal-AM, etc.), based on the a-PeT mechanism. DAFs are converted to a triazole derivative (DAFs-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. Dr. Nagano applied DCI-DA Cal-AM (a DAF derivative) to cultured bovine aortic endothelial cells, and showed that it was possible to visualize the spatiotemporal dynamics of intracellular NO. DCI-DA Cal-AM was introduced as a highly sensitive intracellular bioimaging probe in *Nature Methods*, Research Highlights, in 2009.

3. Applications to screening systems for drug discovery

The fluorescent probes developed by Dr. Nagano are also very effective as screening tools for drug discovery, offering extremely high sensitivity and specificity. For example, autotaxin (ATX) inhibitors have been developed using a sensitive and specific fluorescent probe for ATX, which employed a PeT-based mechanism. ATX is considered to regulate the physiological and pathological roles of lysophosphatidic acid, including angiogenesis, lymphocyte trafficking, tissue fibrosis, and cancer cell invasion and metastasis. Thus, it is a potentially important therapeutic target. The fluorescent probe is suitable for high-throughput screening of ATX inhibitors in large compound libraries and has enabled Dr. Nagano to identify several novel ATX inhibitor scaffolds. These inhibitors are expected to be useful tools to understand the roles of ATX *in vitro* and *in vivo* and could also be candidate anti-ATX therapeutic agents.

Dr. Nagano has published more than 450 papers, which have been cited more than 20,000 times, demonstrating the pivotal role of his work in the field of life science.

He is also the founder of Japanese Society for Chemical Biology and his research on the development of novel fluorescent probes has contributed greatly to the development of chemical biology. In recognition of these achievements, he has been bestowed with numerous awards, including the Medal with Purple Ribbon, Uehara Prize, and Shimadzu Prize.

List of Main Publications

Reviews

- 1. Nagano, T and Yoshimura, T: Bioimaging of nitric oxide. Chem. Rev., 102; 1235-1269, 2002.
- 2. Ueno, T and Nagano, T: Fluorescent probes for sensing and imaging. Nat. Methods, 8; 642-645, 2011.

Original Papers

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- 18. Kawaguchi, M, Okabe, T, Okudaira, S, Nishimasu, H, Ishitani, R, Kojima, H, Nureki, O, Aoki, J, and

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