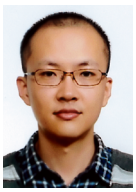
Development of a fast-responding genetically-encoded temperature indicator

Department of Biomolecular Science and Engineering, SANKEN (The Institute of Scientific and Industrial Research)

Specially Appointed Associate Professor Tetsuichi Wazawa Researchmap https://researchmap.jp/wazawa?lang=en

Specially Appointed Researcher Kai Lu Researchmap https://researchmap.jp/ka1lu?lang=en

Professor Takeharu Nagai Researchmap https://researchmap.jp/ng1?lang=en


Abstract

Genetically-encoded temperature indicators (GETIs) are sensor proteins used for measurement and imaging of temperature through the temperature-dependent fluorescence. Here we developed a fast-responding GETI, B-gTEMP, which reports temperature through a ratio between two fluorescence bands for accurate measurement and responds to a temperature change with an unprecedentedly small time constant of <1 ms. We applied B-gTEMP to temperature imaging in mammalian cells and successfully observed intracellular heat diffusion on the millisecond scale. Furthermore, by comparing the imaging data with heat diffusion simulation, we estimated that the heat diffusion coefficient in cells was 1/5 of that of pure water, indicating that intracellular heat diffusion would be considerably slower.

Background & Results

Thermogenesis plays a key role in the homeostasis and regulation of body temperature in animals. In particular, the non-shivering thermogenesis, which occurs in non-muscle tissues, is thought to be closely associated with not only the homeostasis but also inflammation-related fever, cold-adaptation, and obesity. However, the detail of the relationship remains elusive. Fluorescent temperature indicators report temperature through the fluorescence and can be used for temperature imaging on a fluorescence microscope. Among temperature indicators developed from various materials, genetically-encoded temperature indicators (GETIs) are particularly useful for measurements of live biological samples, because GEITs can be introduced into cells for expression as a protein in a minimally invasive manner and can be easily modified on the basis of DNA for versatile applications. However, GETIs developed to date are slow to respond to temperature changes or need time-consuming measurement procedures. Thus, a fast-responding GETI has been required to study transient intracellular heat diffusion.

Here we have developed a GETI, B-gTEMP, from a temperature-responsive red fluorescent protein and a much less temperature-responsive green fluorescent protein. The ratio of red to green fluorescence intensity correlates with temperature, and responds to a temperature change with a time constant of <1 ms. By applying B-gTEMP to temperature imaging of mammalian cells, we successfully observed an intracellular heat diffusion process with sub-millisecond resolution. Furthermore, we compared the imaging data with heat diffusion simulation and estimated that the heat diffusion coefficient in cells was 1/5 of that of pure water. B-gTEMP is expected to contribute to the investigation of non-shivering thermogenesis in relation to the subtle intracellular temperature regulation and diseases and disorders involving intracellular thermogenesis.

Significance of the research and Future perspective

Thermogenesis in homeothermic animals plays an important role in the homeostasis and regulation of body temperature. For the investigation of thermogenesis at the single cell level, it has been necessary to develop a fast-responding GETI capable of observing millisecond intracellular heat diffusion processes occurring in a small volume such as mammalian cells. B-gTEMP should be useful for investigating thermal biology issues such as mechanisms of subtle intracellular temperature regulation and diseases and disorders involving intracellular thermogenesis.

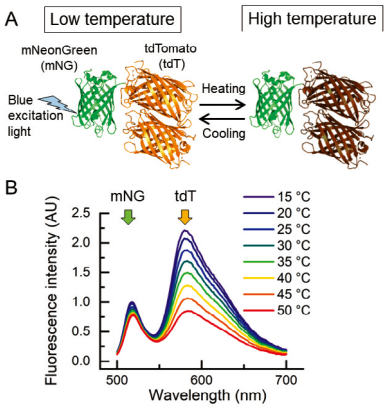


Figure 1. Design of B-gTEMP and its response to temperature. (A) A schematic diagram of the temperature response of B-gTEMP. (B) Temperature-dependent fluorescence emission spectrum of B-gTEMP.

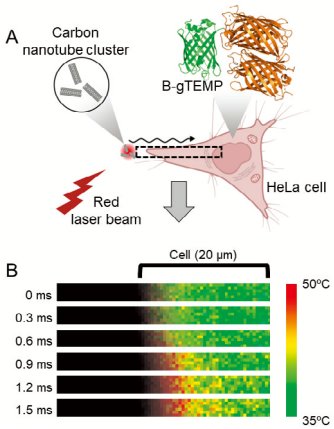


Figure 2. High-speed imaging of heat diffusion in a HeLa cell using B-gTEMP. (A) A schematic diagram of the experimental configuration. A carbon nanotube cluster close to a HeLa cell was irradiated with a red laser beam to generate heat, and the intracellular temperature distribution was imaged through the B-gTEMP fluorescence. (B) Time development of temperature distribution in the HeLa cell after starting to heat the cell.

Patent

Treatise Lu, Kai; Wazawa, Tetsuichi; Nagai, Takeharu et al. Intracellular heat transfer and thermal property revealed by kilohertz temperature imaging with a genetically encoded nanothermometer. Nano Letters, 2022, 22(14), 5698–5707. doi: 10.1021/acs.nanolett.2c00608

URL https://www.sanken.osaka-u.ac.jp/labs/bse/index-E.html

Keyword thermogenesis, heat diffusion, fluorescent protein, fluorescence imaging, protein engineering