



## Communication

## Long-term super-resolution imaging of mitochondrial dynamics

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## ABSTRACT

Monitoring dynamics of mitochondria has become an essential approach to explore the function of mitochondria in living cells with the emergence of super-resolution fluorescence microscopy. However, long-term super-resolution imaging of mitochondria is still challenging due to the lack of photostable fluorescent probes and stable mitochondria-specific markers which are not affected by the changes of mitochondrial membrane potential. Here, we introduce a method for long-term imaging mitochondrial dynamic through the SNAP-tag fluorogenic probe based on 4-azetidinylnaphthalimide derivatives. Using structured illumination microscopy (SIM), we observed the fusion and fission of mitochondria over a course of 16 min at 109 nm resolution. Furthermore, the interactions as well as fusion between mitochondria and lysosomes were studied during mitophagy at the nanoscale. Convincingly, the combination of SNAP-tag fluorogenic probes and super-resolution fluorescence microscopy will offer a new way to monitor dynamic mitochondria in living cells.

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Mitochondria, which are generally considered as the power house of cell, are crucial and semi-autonomous organelles for energy production, cell respiration, regulation of metabolism and cell signaling [1,2]. Therefore, real-time monitoring mitochondrial states can indicate the tiny changes of physiological process in living cells. Mitochondria can form dynamic network and are endowed with the ability to change their shape (elongation, shortening, branching, buckling, swelling) and location in three dimensions, which make it difficult to real-time monitor mitochondria *in situ* [3–5]. For instance, fusion and fission of mitochondria, closely related to stability of mtDNA and apoptosis, are dynamic events which usually occur in short time [6]. Besides, mitochondria can frequently communicate with other organelles, along with revelation of various physiological processes [7–9].

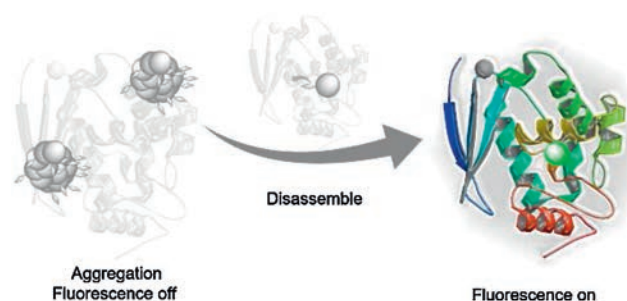
Although electron microscopy (EM), which boasts sub-nanometer resolution, has been widely used to inspect different form of mitochondria, the dynamic visualization of mitochondria in living cells is difficult to be achieved until the appearance of fluorescence microscopy. Conventionally, laser scan confocal microscopy has

become a universal tool to monitor *in situ* events in living cells. However, the far-field optical diffraction limit restricts the spatial resolution beyond 200 nm [10–15]. Recently emerging super-resolution fluorescence microscopes, such as stimulated emission depletion (STED) [16], structured illumination microscopy (SIM) [17,18], and stochastic optical reconstruction microscopy (STORM) [19], can break the conventional resolution limit which allow the visualization of dynamic mitochondria at the nanoscale in cells. Among them, SIM is a widefield microscopy technique with a spatial resolution of up to 100 nm which is realized by illuminating with multiple interfering beams of light. Furthermore, SIM has gradually become one of key technologies in modern cell biology, especially in dynamic changes of mitochondria, for the preferable biocompatibility and no extra demands in experimental procedures. But the ideal long-term dynamic super-resolution imaging of mitochondria requires excellent accuracy and stability of labeling [20], but remains challenging. Nowadays, organic probes with higher brightness and photostability compared with fluorescent proteins are promisingly used to label mitochondria in SIM imaging. Zhang's group used an existing cell-permeable organic fluorescent probes, Atto 647 N dye, to achieve specific labeling of mitochondria in live cells [21]. Through dual-color SIM images of dynamic physical lysosome-mitochondrion interactions, the authors observed consecutive dynamic processes of lysosomal fusion and fission. In addition, Diao and coworkers proposed a strategy to screen and discover drugs by monitoring of

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**Scheme 1.** Schematic illustration of SNAP-tag sensing.

mitochondria and lysosomes interactions at nanoscale level in living cells using Mito-Tracker Green [22]. However, most mitochondria-targeted probes are lipophilic cations with so called covalent linkage sites which are usually considered as mitochondrial membrane potential probes [23–25]. The accuracy and stability of mitochondria labeling mainly depend on the negative mitochondrial membrane potential. As a result, once the mitochondrial membrane potential changed, redundant probes would dissociate to cytoplasm which will greatly increase the background. In another words, existing mitochondrial membrane potential probes can hardly achieve long term and dynamic super-resolution imaging of mitochondria, especially in mitophagy, apoptosis which can induce loss of membrane potential.

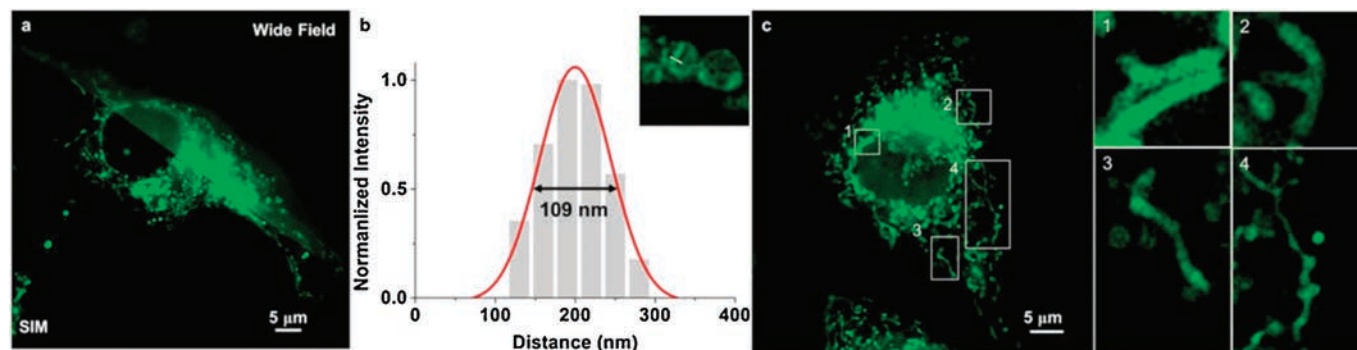
In our previous research, we reported a series of wash-free SNAP-tag fluorogenic probe based on naphthalimide which could specifically label mitochondria through the formation of stable thioether bond between probes and SNAP-Cox8A fusion proteins [26,27]. For this reason, the probes could realize long term and dynamic imaging of mitochondria without diffusion in living cells. Herein, we developed a novel SNAP-tag fluorogenic probe, which could achieve enormous fluorescence enhancement upon labeling SNAP-tag (Scheme 1), based on 4-azetidinylnaphthalimide derivatives. Through the stable fluorescence labeling of mitochondria and high signal-to-noise ratio, dynamic mitochondria were visualized by SIM in living cells without washing. Furthermore, fusion and fission of mitochondria were also monitored at nanoscale level. We also monitored the fusion of mitochondria and lysosomes during mitophagy *via* dual-color SIM imaging with the wash-free SNAP-tag probe. This strategy to visualize dynamic mitochondria at nanoscale level will provide deep understanding of mitochondrial function in physiological process. The experimental procedures can be found in Supporting information.

We first investigated whether the probe could realize wash-free SIM imaging of SNAP-tag fusion proteins in mitochondria.

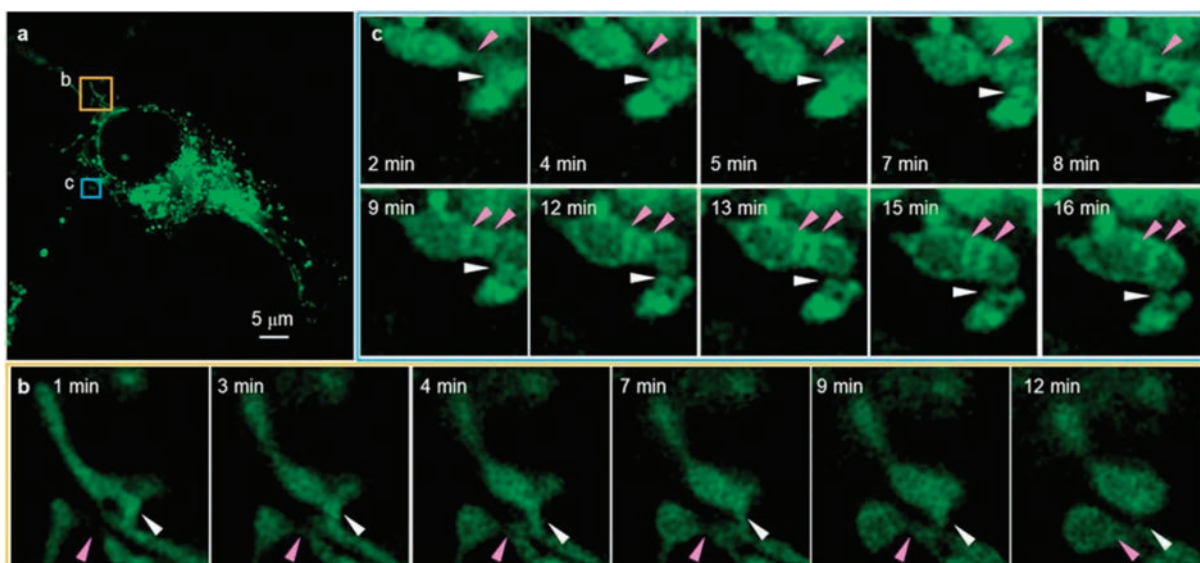
Commercial available plasmids pSNAPf-Cox8A fusing SNAP-tag to cytochrome c oxidase subunit 8 (Cox8A) was transiently transfected to HeLa cells (the cell culture and transfection methods can be found in Supporting information). After incubated with the SNAP-tag probe for 1 h, HeLa cells were directly imaged without washing processes. Strong green fluorescence was observed in different form of mitochondria, while other locations and non-transfected cells showed negligible background under wide-field imaging (Fig. 1a). The SIM images displayed a greatly improved spatial resolution ( $\sim 109$  nm, Fig. 1b) in which mitochondrial cristae were clearly distinguished (Fig. 1c, 1–4). Furthermore, the network of mitochondria, consisted with rounded, clavate and linear mitochondria, was extraordinary coherent. It was revealed that the probe was able to image mitochondria using SIM in living cells without washing.

The excellent performance in SIM imaging inspired us to continuously monitor dynamic changes of mitochondria in living cells. During the time course of over 16 min, the green fluorescence from mitochondria was almost unchanged and the outline of mitochondria were clearly demarcated (Fig. 2). Besides, we observed the shape changes of mitochondria which varied from linear to irregular mitochondria, and to rounded mitochondria finally (Fig. 2b, pink arrow). Moreover, the process of mitochondrial fusion and fission was long term monitored in living cells. As shown in Fig. 2b, as time went on, the site labeled by white arrow changed to slender structure and reached to fracture at the 12th min. So, the single mitochondrion, which exhibited branch at the 1st min, gradually turned into two dumbbell-like mitochondria. Furthermore, the fusion of two isolated mitochondria was viewed in the same cell (Fig. 2c). In the first 5 min, the two mitochondria were found to establish communications through the slender structure labeled by white arrow, which became coarse next. Two obvious mitochondrial cristae were gradually formed along with the mitochondrial fusion, due to the accumulation of matrix compartment during fusion of two mitochondria material of the inner membrane. Through SNAP-tag fluorogenic probe, long-term super-resolution imaging of dynamic mitochondria was realized which might provide functional significance of mitochondria in mtDNA mutations and programmed cell death.

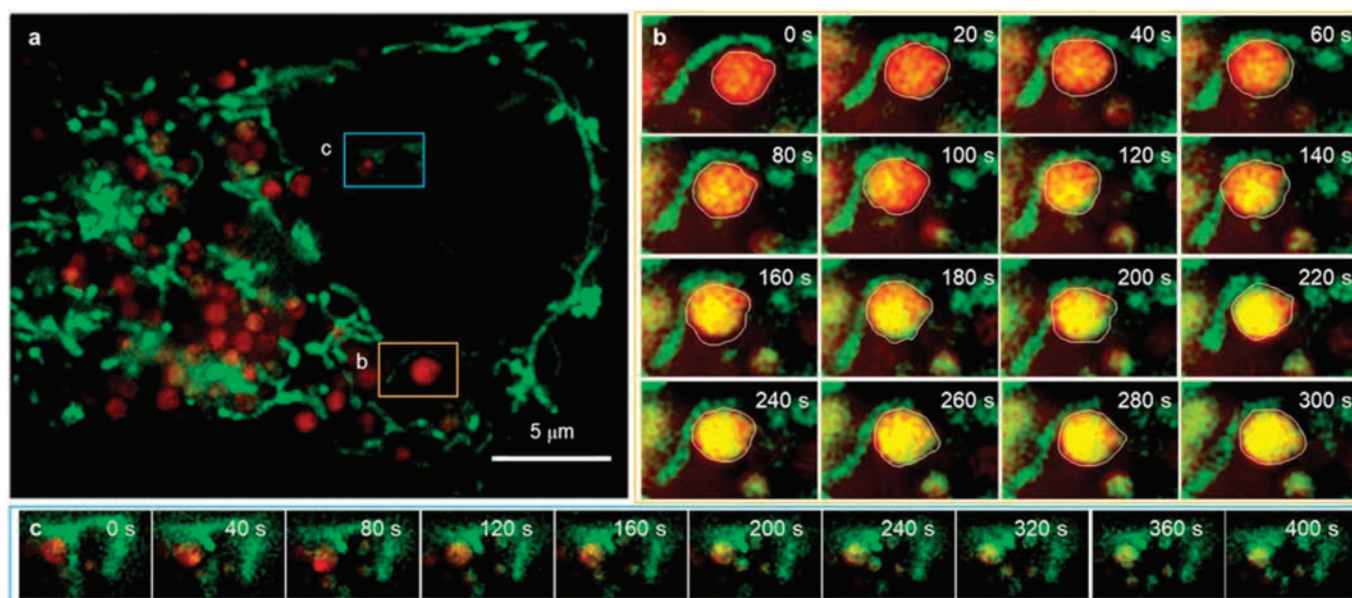
Finally, we intended to monitor the fusion of mitochondria and lysosomes during mitophagy which could maintain homeostasis through selectively degrading damaged mitochondria. Therefore, long-term dual-color SIM imaging of mitochondria and lysosomes in HeLa cells was performed (Fig. 3). In order to facilitate the occurrence of mitophagy, we enhanced the power of laser ( $0.5$  kW/cm<sup>2</sup>) for laser- induce damage was reported as one of effective methods to induce mitophagy. Initially, the lysosome labeled by Lyso-Tracker Red and mitochondria were completely separated (Fig. 3b, 0 s). However,  $\sim 40$  s later, the mitochondria-lysosome



**Fig. 1.** (a) Comparison of SIM image and wild field image of the cell in Fig. 2. (b) Cross-sectional profile of a mitochondrial cristae at 16 min in Fig. 2 at a full-width at the half-maximum (FWHM) of 109 nm. Excitation: 488 nm, collected: 500–545 nm. (c) Wash-free SIM images of living HeLa cells incubated with  $1 \mu\text{mol/L}$  SNAP-tag fluorogenic probe. (1), (2), (3) and (4) Enlargements of the boxed regions in (c), in which the fine structures especially mitochondrial cristae were visible.



**Fig. 2.** Time-lapse SIM images of living HeLa cells stained with SNAP-tag probe. (a) The SIM image of whole living HeLa cells. (b, c) Enlargements of the boxed regions in (a), which revealed different types of dynamic physical process of mitochondria. (b) SIM images of mitochondrial morphologic changes of mitochondria swollen with laser irradiation. (c) SIM images of mitochondria fission (white arrow) and fusion (pink arrow). For the time-lapse images, the time interval between each SIM image was set to 30 s. Excitation: 488 nm, collected: 500–545 nm.



**Fig. 3.** Dual-color SIM images of lysosomes and mitochondria in living HeLa cells. (a) The SIM image of whole live HeLa cells stained with Lyso-Tracker Red (red) and SNAP-tag fluorogenic probe (green). (b) and (c) are representative time-lapse SIM images of different types of dynamic physical interactions between lysosomes and mitochondria in live HeLa cells. For the time-lapse images, the time interval between each SIM image was set to 20 s. Excitation: 488 nm, collected: 500–545 nm (green channel). Excitation: 561 nm, collected: 570–640 nm (red channel).

contact increases, as evidenced by the nearness between mitochondria and lysosomes. Hence, the locally damaged mitochondria could be transported to lysosome which indicated the accomplishment of mitophagy along with the increase of yellow area in circle. In the meanwhile, numbers of mitophagy were captured at nanoscale level in living cells (Fig. 3c).

In conclusion, we introduced a new method for long term monitoring dynamic mitochondria *via* SNAP-tag at nanoscale level. The SNAP-tag fluorogenic probe could realize wash-free SIM imaging of mitochondria in living cells without diffusion due to the formation of stable thioether bond with SNAP-Cox8A fusion

proteins. Furthermore, we recorded consecutive dynamic physical process of mitochondrial fusion and fission with our SNAP-tag probe over a course of 16 min. The formation of mitochondrial cristae, due to the accumulation of matrix compartment, during fusion was also clearly demonstrated. Meanwhile, we successfully applied this method to track the interaction of mitochondria and lysosomes, and captured the fusion between mitochondria and lysosomes during mitophagy in live cells. The method in this paper for nanoscale monitoring dynamic mitochondria in living cells offers a convincing mode to indicate the role of mitochondria in molecular biological mechanisms.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ccl.2020.05.043>.

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