Development of a mitochondria-targeting fluorescent probe for visualizing Zn^{2+} flux

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Zinc ion (Zn^{2+}), which is one of the most essential elements in mammalian cells, plays critical roles in enzymatic activities, cellular signaling, and transcription. Dyshomeostasis of Zn^{2+}, particularly within mitochondria, can lead to loss of mitochondrial membrane potential, ATP depletion, increased production of reactive oxygen species, and apoptosis.[1] Therefore, tools for visualizing intracellular Zn^{2+} dynamics are essential to understand the physiological roles of Zn^{2+}.

Previously, we developed a small molecule–protein hybrid probe to quantify labile Zn^{2+} concentration in various organelles by combining **ZnDA-3H** (Figure 1a) with HaloTag technology.[2] In this study, to extend the versatility of our probes, we developed a new fluorescent probe, **ZnDA-3TPP**, which enables the monitoring of Zn^{2+} dynamics in mitochondria without HaloTag. This mitochondria-targeting probe is particularly advantageous for applications in cells that are difficult to transfect, such as neurons, and also allows HaloTag to be used for another purpose in general cell studies. The substitution of the HaloTag ligand with a triphenyl phosphonium (TPP) moiety facilitates the spontaneous probe retention in mitochondria through electrostatic interaction with negatively charged mitochondrial inner membrane. In vitro fluorescence measurement demonstrated that **ZnDA-3TPP** exhibits Zn^{2+}-dependent fluorescence enhancement with a K_d of 0.28 nM, indicating that **ZnDA-3TPP** possesses sufficient binding affinity to visualize labile Zn^{2+} in mitochondria (60 pM^2). Fluorescence imaging of HeLa cells treated with **ZnDA-3TPP** confirmed that the probe localized predominantly to the mitochondria (Figure 1b,c).

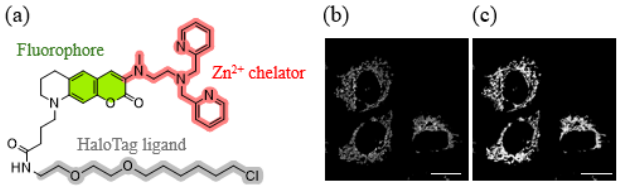

Figure 1. (a) Structure of **ZnDA-3H**. (b,c) Confocal fluorescence microscopic images of **ZnDA-3TPP** (b) and MitoTracker Deep Red FM (c) in HeLa cells. Scale bar: 20 μm.

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