Upconversion nanoparticle-thermometer monitors mitochondrial thermal dynamics *in situ*

Intracellular temperature dynamics is a key parameter reflective of the physiological status of biological cells and organisms. In particular, the mitochondria is an important organelle that regulates critical processes and thermodynamics of the cell, making it essential to have accurate and specific temperature-sensing techniques capable of monitoring mitochondrial thermal dynamics. To this end, Xiangjun Di and her research team from the University of Technology Sydney have developed an upconversion nanoparticle (UCNP)-based thermometer that allows for the *in situ* monitoring of mitochondrial thermal dynamics in living cells. This research was published in a recent issue of *Nano Letters* (https://doi.org/10.1021/acs.nanolett.0c04281).

This novel UCNP-based thermometer exploits the unique optical and thermoresponsive properties of UCNPs for intracellular temperature sensing. UCNPs are a new generation of fluorophores, which are able to convert low-energy photons into shorter wavelength emissions via a nonlinear optical process. This unique capability allows UCNPs to exhibit temperature-dependent fluorescence, following a Boltzmann distribution. In this study, the thermoresponsive properties of UCNPs were validated *in vitro* and were shown to be independent of probe concentration and medium conditions.

To achieve targeting UCNPs to mitochondria so that localized temperature sensing can be achieved, the mitochondria-targeting moiety (3-carboxypropyl)triphenylphosphonium bromide (TPP) was covalently functionalized to the UCNPs, forming UCNPs termed UCNPs@TPP. This allows for targeting as the membrane-potential gradient from the cell plasma to mitochondria allows for the corresponding stepwise accumulation of TPP^+. Using a HeLa cell model, it was demonstrated from microscopy imaging that UCNP@TPPs were co-localized with mitochondria. Mitochondrial isolation also showed that the fluorescence intensity of UCNPs@TPP-treated mitochondria was three times higher than mitochondria treated with control groups of UCNPs, and the relative sensing sensitivity was found to be 3.2% K^{-1}.

More critically, the use of UCNPs@TPP was validated by monitoring the mitochondrial temperature variations of HeLa cells induced by external nutrient conditions and chemical stimulations, including glucose, oleic acid, ionomycin calcium salt and carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP). It was shown that UCNPs@TPP could monitor mitochondrial thermodynamics *in situ*, and interestingly, reflect the different response time and thermal dynamics profiles in response to different stimuli. This highlights the potential use of these novel UCNPs@TPP as a precise quantitative subcellular nanothermometry platform to study key processes involving the mitochondria, such as mitochondrial metabolism activities as well as the interactions between the mitochondria and other organelles in the cell. This work on UCNPs could also be expanded for future developments of various organelle-targeting nanothermometers.

Gang Han of the University of Massachusetts Medical School, whose research interests include the use of UCNPs for biomedical applications, says, "The mitochondrial targeting upconversion nanoparticle as an intracellular thermometer is indeed clever. Such nanoparticles address the daunting photobleaching and photoblinking issues with respect to fluorescent molecules and quantum dot-based nanothermometers." Han was not involved in this study.

Han says that this research work involving UCNPs "paves the way for a new direction for emergence in the nanothermometer field." Furthermore, he says, "I can imagine numerous things that will follow and be inspired from this work. For example, improved cell selectivity and detection sensitivity should be able to be further expected by tailoring nanoparticle compositions and surface modification." Han also anticipates "dynamic tracking of these single upconversion nanothermometers to be achieved soon from the solid ensemble results and the snapshots of the single nanoparticles imaging," as well as "further enhancing endosomal escape [to be] another direction to eventually create the small molecular-like homogeneity."

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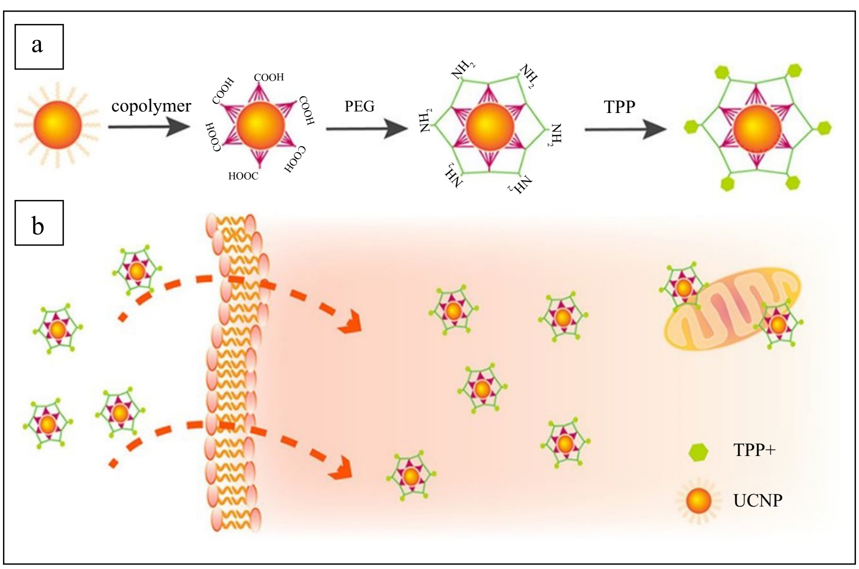

Schematic illustration of design of (a) UCNPs@TPP and (b) intracellular mitochondrial targeting of UCNPs@TPP. UCNPs is upconversion nanoparticles; TPP is (3-carboxypropyl) triphenylphosphonium bromide. Credit: *Nano Letters.*