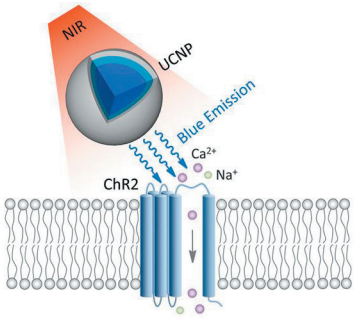
NEUROSCIENCE

# Minimally invasive optogenetics

**Upconversion nanoparticles can serve as intermediaries to illuminate optogenetic tools in the mouse brain.**

Minimally invasive methods to stimulate neurons in the rodent brain are available, but they tend to be temporally imprecise or not cell-type specific. In contrast, optogenetic tools can be targeted to specific cell types and can be precisely controlled in time. However, the technology requires the implantation of devices to illuminate brain areas of interest, which can cause damage.




Upconversion nanoparticles convert NIR light into visible light, which can then activate optogenetics tools such as ChR2. Reproduced with permission from Chen *et al.* (2018).

To overcome these problems, Shuo Chen, a postdoc in the lab of Thomas McHugh from the RIKEN Brain Science Institute in Saitama and the University of Tokyo, initiated a collaboration between the McHugh lab and the lab of Xiaogang Liu from the National University of Singapore and A*STAR in Singapore. The teams explored the feasibility of using upconversion nanoparticles (UCNPs) for optogenetic manipulation of neurons in the mouse brain.

UCNPs can emit in the visible range after stimulation with near-infrared (NIR) light. Their emission wavelength can be tuned to match the action spectra of tools such as channelrhodopsin-2 (ChR2) and archaerhodopsin (Arch). UCNPs have been applied in the context of optogenetics in cell culture, as well as in the roundworm and zebrafish. "Some attempts at in vivo work in rodents were reported," explains McHugh, but they were done in a very invasive way.

The team found that they could inject the UCNPs into the brain area of interest and then illuminate them through the intact skull using 980-nanometer-wavelength NIR light. The

PROTEOMICS

## HEATING UP CELLS TO BRING DOWN COMPLEXES

**A combination of heat denaturation and mass spectrometry is applied to monitor protein-complex dynamics in the cell.**

Biological processes depend on different proteins coming together in complexes that function as molecular machines. Protein complexes are dynamic, associating and dissociating depending on the cellular condition. Understanding how these shifting interactions affect cellular functions is both a major focus of and a major challenge for cell biologists.

In recent work, Pär Nordlund of Karolinska Intitutet in Sweden and Nanyang Technological University in Singapore, along with postdoc Chris Soon Heng Tan and their colleagues, adapted their previously established cellular thermal shift assay (CETSA) method to monitor protein-complex dynamics in the cell. They dubbed this new approach thermal proximity coaggregation, or TPCA.

CETSA was originally designed to detect protein interactions with small molecules, the idea being that proteins are thermally stabilized upon binding. Proteins binding a small molecule of interest exhibit shifted thermal melting curves; such profiles can be analyzed on a proteomic scale through the coupling of heat denaturation with mass spectrometry.

In analyzing CETSA data collected for a different study, Nordlund serendipitously noticed that proteins from some prominent complexes, such as the ribosome, had similar melting profiles. This led his group to develop TPCA, which is based on the hypothesis that proteins that are interacting should have very similar melting curves; that is, they coaggregate upon heat denaturation—the most heat-sensitive member of the complex will drag the other members down with it. TPCA does not provide direct evidence of interacting proteins, but it can be used to predict or confirm interactions.

Nordlund's team validated their hypothesis with the well-studied CDK2–cyclin E1 complex. In isolation, these two proteins had distinct melting curves, but when in complex,

UCNPs could be located as deep as 4.5 millimeters and still generate enough short-wavelength light to activate ChR2 or Arch, and the NIR illumination scheme was designed to keep tissue heating at bay. "We included a large amount of data characterizing this and demonstrating it was safe under the conditions we employed," writes McHugh via email.

The researchers applied their approach first in the ventral tegmental area (VTA), a deeply situated area in the mouse brain. They targeted ChR2 to dopamine neurons in the VTA by using a viral vector in combination with a Cre recombinase line, and they injected blue-emitting UCNPs into this area. Transcranial NIR illumination activated dopamine neurons, as confirmed by higher c-Fos levels and dopamine release. The researchers also targeted ChR2 to parvalbumin-expressing neurons in the medial septum, which is involved in theta rhythmicity, an oscillatory pattern of neural activity in the range of 4–7 Hertz. In this case, the team was able to modulate the frequency of theta oscillations with pulsed NIR illumination. Finally, UCNP-mediated optogenetics can be used to influence the behavior of awake mice, which the authors demonstrated by inducing freezing behavior in response to NIR illumination of ChR2-expressing neurons in the dentate gyrus.

The researchers also showed that UCNPs tuned to emit green light can activate the inhibitory tool Arch. But the strategy is not limited to ChR2 and Arch. "We are working now to expand the use to other opsins," McHugh writes.

Although the technology works well and is promising, it is not without challenges. The illumination with NIR light leads to temperature increases and therefore needs to be tightly controlled. In addition, although NIR light scatters less than visible light, areas beyond about 4 millimeters in depth cannot be reached because of scattering.

Further developments in the UCNP design could improve the efficiency of upconversion. "We will continue our collaboration with the Liu lab to improve the targeting and efficacy of UCNPs and test their performance in vivo," writes McHugh. He would also like to transfer the approach to animals with larger brains, such as rats or nonhuman primates.

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**RESEARCH PAPERS**
Chen, S. *et al.* Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics. *Science* **359**, 679–684 (2018).



they had very similar melting profiles. The researchers then analyzed melting curves for nearly 8,000 proteins in K562 cell lysates. Evaluating their results in light of over 110,000 protein interactions available in public databases, they found that known protein-interaction pairs were statistically more likely to have similar melting curves than random pairs of proteins.

A strong advantage of TPCA over other protein-complex detection methods is that the heating step can be performed not only with lysates but with intact cells. "Proteins are so sensitive," says Nordlund. In lysates, he notes, "it's a big risk that you get artificial interactions; in addition to that you can lose the complexes." Indeed, the researchers found that many protein complexes had different TPCA signatures in intact cells versus in cell lysates, and that those signatures detected in intact cells were statistically more enriched.

The group also applied TPCA to dynamically monitor protein complexes in S-phase synchronized cells compared with those in unsynchronized cells. They identified 18 protein complexes with enhanced TPCA signatures in S-phase, 15 of which were previously implicated in this cell state, thus providing strong support for the method. Finally, they compared TPCA signatures across multiple cell types, and found that although many complexes had similar melting profiles across cell types, a substantial number of other complexes were apparently unique, which suggested differences in their compositions.

Nordlund highlights the information-dense results one can achieve by using TPCA to study protein complexes. "We are looking at the actual biochemistry in the cells," he notes. "This gives you orthogonal information from what we know from transcriptomics and proteomics." In the future, the researchers plan to improve the sensitivity of their proteomics measurements by exploring alternative formats of isobaric-labeling mass spectrometry, and will apply TPCA to study cancer drug resistance.

**Allison Doerr**

**RESEARCH PAPERS**
Tan, C.S.H. *et al.* Thermal proximity coaggregation for system-wide profiling of protein complex dynamics in cells. *Science* **359**, 1170–1177 (2018).