APS SCIENCE
Research at the ADVANCED PHOTON SOURCE

Shining Light on a New Gene Therapy

IMPACT: Brilliant x-ray beams from the Advanced Photon Source are playing a crucial role in the development of a novel, light-activated hybrid "nanodevice" that one day may be used to target the defective genes that play a role in cancer, neurological diseases, and other conditions.

A novel, light-activated hybrid "nanodevice" composed of titanium oxide nanocrystals and carefully selected segments of DNA could one day be used to target the defective genes that play a role in cancer, neurological diseases, and other conditions. The work that has opened this possibility was performed by scientists from Argonne National Laboratory and Northwestern University.

Titanium oxide crystals are only a few nanometers across (it takes about 50,000 nanometers to equal the width of a human hair) and can enter a cell and the nucleus carrying one or more segments of DNA (or peptides, or proteins) that can steer the titanium crystals to precise locations on a defective gene. The precise positions of the nanodevices within the cells and nuclei were tracked by using an x-ray fluorescence technique at the X-ray Operation and Research 2-ID-E beamline at Argonne's Advanced Photon Source (APS). In the study, the chosen DNA segments were oligonucleotides that, once in position, could be used to attach themselves to the gene's defective DNA.

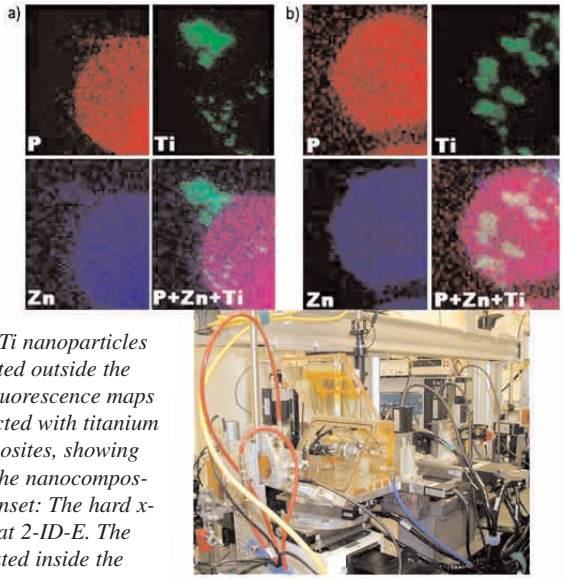
Cells containing the nanodevices were then exposed to ultraviolet light (other kinds of radiation, such as medical x-rays, would also work for clinical applications) that caused the titania crystals to oxidize, which is a chemical reaction that "snips" off the defective DNA to make room for new segments of DNA brought along for repair (delivered by the same nanodevice).

The new technique has several advantages over existing approaches to gene therapy that often use genetically modified viruses and can cause adverse reactions in patients. The hybrid nanodevices don't exist in nature, so they aren't recognized and attacked by the body's immune system. The hybrid nanodevice also offers more focused, site-specific cleaving (or cutting), not just of DNA, but also of RNA – something that existing cleaving methods can't do. This has the advantage of quickly destroying bad genes instead of merely interfering with or slowing their activity.

Titania's chemical oxidation reaction to light and radiation provides a convenient way of triggering the cleaving of the DNA after it has bound to defective genes inside the nucleus.

One obstacle the scientists encountered was the difficulty of following the progress of their nanodevices after the devices had entered the cells. Typically, nanoparticles have very low optical contrast, which prevents the use of conventional imaging methods to observe them. However, use of the extremely bright and focused APS x-ray beams enabled the researchers to track the titania crystals all the way into the cell's nucleus.

The researchers employed an x-ray microscope configured to use "x-ray fluorescence," a technique where primary x-rays induce an atom into an excited state to produce an emission of secondary x-rays. These primary x-rays remove a core electron from the atom; the ensuing "hole" is filled when an electron drops down from a higher energy state. As these higher energy electrons drop down, they emit characteristic fluorescent x-ray photons. In this case, the locations of the nanodevices were mapped by detecting the titanium-specific x-ray fluorescence induced by focusing APS x-ray beams onto a small spot with a known x-ray energy. By employing special optics called zone plates, a spot of 250 nm is achieved for x-rays with a wavelength of 1.3 Å.



*Right: Images from the studies carried out on APS beamline 2-ID-E. **a)** X-ray fluorescence maps of a cell transfected with "free" titanium oxide (Ti) crystals, showing that most of the Ti nanoparticles (green) are located outside the cells. **b)** X-ray fluorescence maps of a cell transfected with titanium oxide nanocomposites, showing introduction of the nanocomposites in the cell. Inset: The hard x-ray microscope at 2-ID-E. The specimen is located inside the orange enclosure.*

This research is in the early stages of development. Yet the fact that these simple titanium oxide hybrid nanoparticles were able to perform complex chemical and biological tasks (including light-induced, site-specific DNA cleaving) is very encouraging. It now appears that these and similar hybrid nanodevices can be produced with unique functions to advance the field of genetic therapy and biotechnology in general.

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See: T. Paunesku, T. Rajh, G. Wiederrecht, J. Maser, S. Vogt, N. Stojicevic, M. Protic, B. Lai, J. Oryhon, M. Thurnauer, and G. Woloschak, Nat. Mat. **2**, 343-346 (01 May 2003).
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