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Carbon Nanotubes You Can Live With

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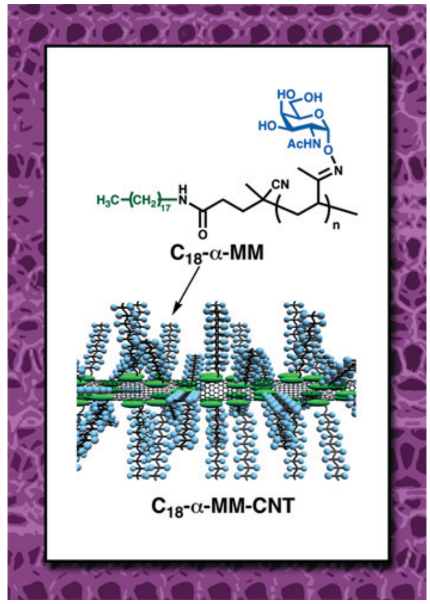
Carbon nanotubes, or CNTs, are hollow wires of pure carbon about 50,000 times narrower than the finest human hair—but stronger than steel. CNTs have enormous potential in a variety of biological applications, including medical diagnostics and treatments. There's a problem, however, and until now it has been what technologists call a "stopper." For reasons not entirely known, CNTs are cytotoxic—contact with them kills cells.

This is one stopper that may have been solved. A team of researchers with Berkeley Lab, the University of California at Berkeley, and the Howard Hughes Medical Institute (HHMI) have developed a means of making CNTs biocompatible. By coating the CNTs with a synthetic polymer that mimics mucin, the substance on cell surfaces that serves as a lubricant, the researchers have been able to safely attach them to biological cells. The polymer coating can also be customized to bind to a specific type of cell.

"CNTs coated with mucin-mimic polymers were not only rendered nontoxic to cells, they were also able to bind to carbohydrate receptors, providing a means for biomimetic interactions with cell surfaces," says chemist Carolyn Bertozzi.

An expert in biomimetics, Bertozzi led the research with physicist Alex Zettl, a leading authority on CNTs. Other members of the team were Xing Chen, a graduate student with both the Bertozzi and Zettl research groups, plus Un Chong Tam, Jennifer Czlapinski, Goo Soo Lee, and David Rabuka.



Carbon nanotubes coated with a polymer, C18-α-MM, are water soluble. The mucin-mimic polymers assemble on the nanotubes via hydrophobic interaction between the polymer's lipid tails and the nanotube surface.

Coating carbon with goo

CNTs are molecule-sized sheets of graphite, rolled up and seamlessly sealed. Their remarkable structural, mechanical, electrical, and thermal properties have made them one of the fundamental building blocks in the burgeoning field of nanotechnology. Because of their scale and broad range of unique properties, biologists covet their use.

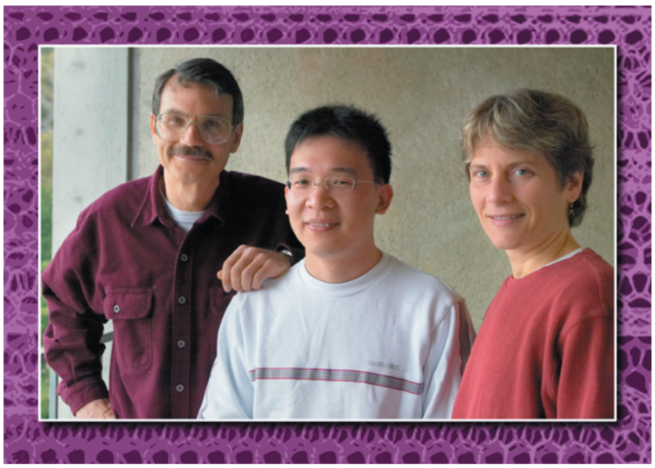
For example, the fluorescent properties of CNTs in near-infrared light have made them outstanding candidates for biological sensing; they could be used to detect proteins or carbohydrates and study physiology at the level of single cells. Carbon nanotubes are also being considered as a means of delivering therapeutic drugs to cancerous tumors or other damaged cells. But the inherent cytotoxicity of CNTs has imposed severe limitations on their use in biological systems.

"We were interested in tailoring the interface between CNTs and cells so as to more accurately reflect physiological interactions at the cell periphery," says Bertozzi.

Earlier research by her and Zettl had shown that CNTs could be coated with synthetic polymers in which sugar molecules called glycans were embedded. These synthetic glycopolymers functioned the same way as natural glycoproteins, key constituents of mucins, which help cells interact with their neighbors.

"Glycans are major determinants of molecular recognition on the cell surface," says Bertozzi. "They participate in diverse processes such as pathogen binding, cell trafficking, endocytosis, and modulation of cell signaling—which means that CNTs functionalized to engage in glycan-receptor interactions would be ideal substrates for more refined cell biology applications."

Bertozzi, Zettl, Chen, and their colleagues customized their mucin-mimicking glycopolymer coatings so that carbon nanotubes would bind only to the surfaces of specific types of cells, via ligand receptors. To do this, they complexed one of their mucin mimics with a sticky cell-surface protein found in snails, called *Helix pomatia* agglutinin, or HPA, which features binding specificity and can crosslink cells and glycoproteins.



Alex Zettl (left), Xing Chen, and Carolyn Bertozzi have developed a technique for safely interfacing carbon nanotubes with biological cells.
(Photo Roy Kaltschmidt)

The researchers then tested their HPA-modified, coated CNTs by incubating them with Chinese hamster ovary (CHO) cells. Fluorescence microscopy revealed that the coated carbon nanotubes modified with HPA interacted with the CHO cells, while CNTs coated with a different glycopolymer—one that lacked the agglutinin modification—did not.

As an alternative pathway, the Berkeley researchers complexed the HPA with CHO cell surfaces rather than with the coated CNTs. Fluorescent labeling revealed that this second approach also resulted in the carbon nanotubes interacting exclusively with the CHO cells. This was the first reported observation of controlled CNT interactions with cells.

"We pursued both methods in parallel because we could perform different control experiments in each pathway that confirmed that the interaction between the nanotubes and cells was mediated by the HPA-carbohydrate interaction," Bertozzi says.

Customizing the interactions

The glycopolymer coating (C18-α-MM, an α-N-acetylgalactosamine sugar with a C18 lipid tail) remained adhered to the carbon nanotubes for several months and kept cells safe from CNT cytotoxicity.

Zettl says, "Choosing different mucins to mimic could produce a wide variety of customized behaviors, such as bonding only to cancer cells or entering specific organelles within the cells. I think this is a huge step forward that will open the door for using nanotubes for biological uses."

Bertozzi adds, "Now that we know our glycopolymers can both solubilize and also biopassivate the CNTs, we are moving to the next step, examining the various ways in which we can employ the coated tubes. One idea that we are contemplating is the use of CNTs as sensors of analytes produced by cells in response to various stimuli."

Bertozzi is director of Berkeley Lab's Molecular Foundry, a faculty scientist with the Lab's Materials Sciences and Physical Biosciences Divisions, the T.Z. and Irmgard Chu Distinguished Professor of Chemistry and a professor of molecular and cell biology at UC Berkeley, and an investigator with the Howard Hughes Medical Institute. Zettl is a senior scientist with Berkeley Lab's Materials Sciences Division, director of the Center of Integrated Nanomechanical Systems at UC Berkeley, and a professor of physics at UC Berkeley.

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