Nanotube Disassembly

Real-time observation with the Revolution DSD

Prof. Dr. Ben L. Feringa and Dr. Wesley R. Browne from the University of Groningen,the Netherlands, are using synthetic chemistry to create new light-responsive nanoscale structures that could one day find use in applications such as smart materials and drug delivery. The photoreactivity of the structures being developed places the Andor Revolution DSD confocal microscope as a key asset in studying the dynamic properties of the materials in real time.

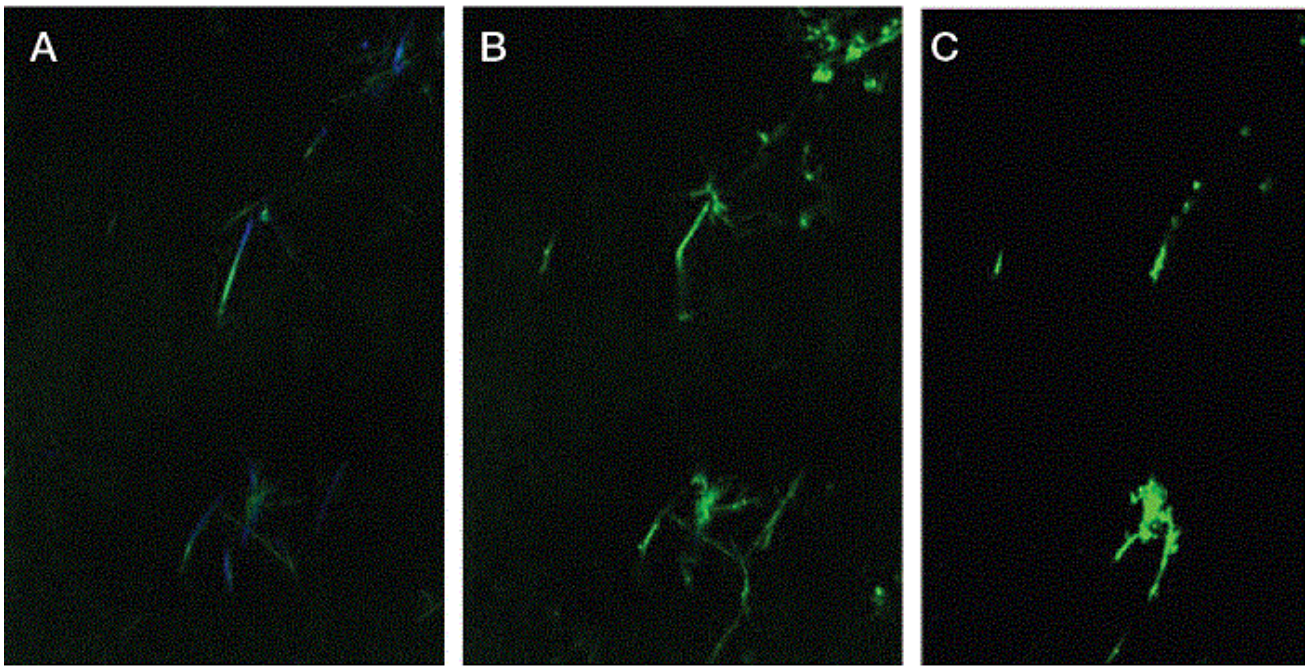

Figure 1. Amphiphilic building blocks. The amphiphile is highly fluorescent in the blue region, excited at 406nm, but at the same time undergoes rapid photochemical conversion at this wavelength to a green fluorescent structure that cannot form the nanotubes. Image A shows mature nanotubes that are already undergoing conversion to green fluorescence owing to the violet excitation of the blue signal. In B, 12 seconds later the majority of tubes have converted to green and by 48 seconds (C) they are disassembling.

Scientists in the field of nanotechnology strive to create smart nano and microscale structures, incorporating switch and motor units that mimic Nature's fascinating mechanical cellular systems. In the research groups of Feringa and Browne, molecular motors and switches are built into new smart materials through self-assembly and give these materials dynamic properties that respond to external stimuli. Recently the Groningen team created micron long nanotubes that are tens of nanometers in diameter using a functional amphiphilic building block. The amphiphile is highly fluorescent in the blue region, but at the same time undergoes rapid photochemical conversion to a green fluorescent structure that cannot form the nanotubes. Hence with light the nanotubes can be forced to disassemble (Figure 1).

Dr. Browne says that their work builds on that of other research groups, in particular that of Takuzo Aida's group at the University of Tokyo, which has developed several beautiful examples of nanotubeforming molecular systems. However, by building a reactive element into their amphiphile, the Groningen team's new nanotube systems exhibit unprecedented multifunctionality. "Not only can they be studied by fluorescence microscopy, but we can use light to control the stability and structure of the tubes, ultimately allowing us to trigger the tubes' disintegration in a controlled manner to form new types of structures," he says.

Following the UV-triggered disassembly of the nanotubes in real time at room temperature was singularly the biggest challenge faced by the team because conventional point-scanning, PMT-based confocal systems could provide images but could not provide the time resolution needed. The rapid switching between excitation/ emission wavelengths allowed for by the DSD together with the speed of image acquisition offered by the Clara CCD camera was critical.

"In this case the 'switching' between the blue tube-forming fluorescence and green tube-disrupting fluorescence is such that we need to be able to rapidly switch between excitation and emission wavelengths to capture the entire image simultaneously," Dr. Browne says. "This is not practical with PMT-based systems that use scanning to produce an image and require much higher total light intensities."

The researchers used the DSD Revolution confocal microscope to monitor a cross-section of a nanotube during irradiation. When exposed to ultraviolet light, they could see the blue fluorescence decreasing and the green fluorescence increasing in intensity. As green fluorescence increased, they observed matching structural changes within the tube until it eventually disassembled. "Furthermore with the DSD we are able to collect a widefield and confocal image with the one system," says Dr. Browne. In widefield mode the DSD can use external excitation sources so it is not restricted to the excitation wavelengths provided for by the interchangeable filter sets.

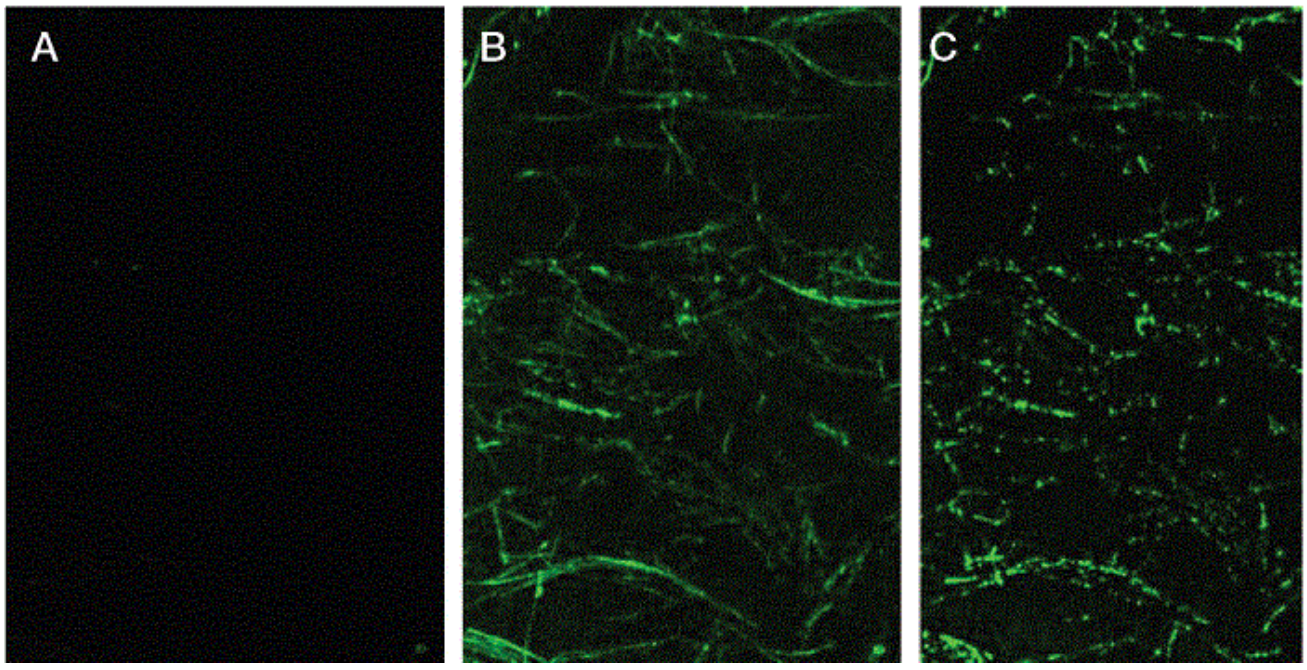

Figure 2. The amphiphile conversion can be regulated with more control using lower intensity and shorter wavelengths. Here the nanotubes are only imaged for green emission (excitation at 494nm) so avoiding excitation at 406nm, whilst shorter wavelength excitation was controlled using an external UV light source. A, shows no evidence of nanotubes undergoing conversion as seen by the lack of green signal. Significant conversion is only observed 41 seconds later in B, with disassembly evident as long as 1 minute and 13 seconds later in C.

The researchers also used the DSD microscope to follow controlled disassembly of the nanotube/vesicle system, which could be accomplished by varying the intensity or wavelength of light (Figure 2.). They found, for example, that irradiation at 365 nm using a UV lamp held above the sample instead of 390 nm light from the DSD's filtered superbright white light source allowed them to slow the disassembly process. "The precise software control of the illumination intensity that is possible with the DSD and the flexibility and speed of switching between excitation wavelengths was central to discovering the functionality of the nanotubes," Dr. Browne says.

For Prof. Feringa's and Dr. Browne's research teams it was important that the DSD could be connected to the side port of any microscope. The researchers use it on a set-up that can also perform widefield imaging and Raman spectroscopy. "The DSD Revolution Confocal system is an ideal workhorse instrument, and a number of projects make use of it," Dr. Browne says. "The key benefit is that at a relatively low cost we have access to a powerful microscopy system that allows optical, widefield, and confocal fluorescence, and together with a Shamrock303 spectrograph and a spectroscopy camera on the second port of the microscope we are able to obtain emission spectra of the fluorophores and carry out Raman microspectroscopy at the flick of a switch on the one sample without any changes in sample position."

In addition, the DSD Revolution confocal microscope uses a bright white light source, which eliminates the expense and safety precautions of working with lasers. "In the future we can easily change the system to a different excitation emission combination - something that would be prohibitively expensive with lasers," Dr. Browne says.