Observing nano-bio interactions in real time

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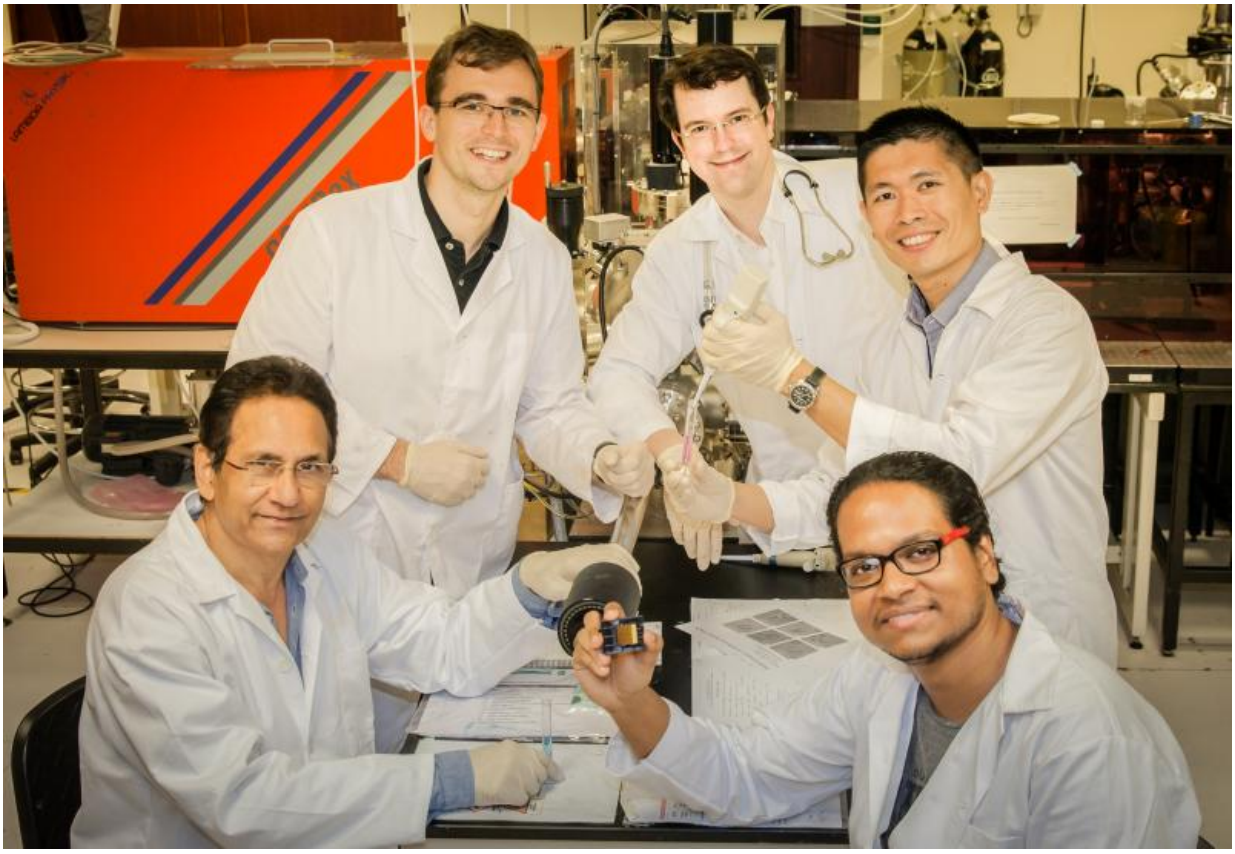

Researchers from the National University of Singapore, comprising (from left to right) Professor T. Venky Venkatesan, Mr Michal Marcin Dykas, Assistant Professor Chester Lee Drum, Assistant Professor James Kah and Mr Abhijeet Patra, have developed a technique to observe, in real time, how individual blood components interact and modify advanced nanoparticle therapeutics. Credit: National University of Singapore

Researchers at the National University of Singapore (NUS) have developed a technique to observe, in real time, how individual blood components interact and modify advanced nanoparticle therapeutics. The method, developed by an interdisciplinary team consisting clinician-scientist Assistant Professor Chester Lee Drum of the Department of Medicine at the NUS Yong Loo Lin School of Medicine, Professor T. Venky Venkatesan, Director of NUS Nanoscience and Nanotechnology Institute, and Assistant Professor James Kah of the Department of Biomedical Engineering at the NUS Faculty of Engineering, helps guide the design of future nanoparticles to interact in concert with human blood components, thus avoiding unwanted side effects.

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Challenges of using nanoparticles in diagnostic and drug delivery systems

With their small size and multiple functionalities, nanoparticles have attracted intense attention as both diagnostic and drug delivery systems. However, within minutes of being delivered into the bloodstream, nanoparticles are covered with a shell of serum proteins, also known as a protein 'corona'.

"The binding of serum proteins can profoundly change the behaviour of nanoparticles, at times leading to rapid clearance by the body and a diminished clinical outcome," said Asst Prof Kah.

Existing methods such as mass spectroscopy and diffusional radius estimation, although useful for studying important nanoparticle parameters, are unable to provide detailed, real-time binding kinetics.

Novel method to understand nano-bio interactions

The NUS team, together with external collaborator Professor Bo Liedberg from the Nanyang Technological University, showed highly reproducible kinetics for the binding between gold nanoparticles and the four most common serum proteins: human serum albumin, fibrinogen, apolipoprotein A-1, and polyclonal IgG.

"What was remarkable about this project was the initiative taken by Abhijeet Patra, my graduate student from NUS Graduate School for Integrative Sciences and Engineering, in conceptualising the problem, and bringing together the various teams in NUS and beyond to make this a successful programme," said Prof Venkatesan. "The key development is the use of a new technique using surface plasmon resonance (SPR) technology to measure the protein corona formed when common proteins in the bloodstream bind to nanoparticles," he added.

The researchers first immobilised the gold nanoparticles to the surface of a SPR sensor chip with a linker molecule. The chip was specially modified with an alginate polymer layer which both provided a negative charge and active sites for ligand immobilisation, and prevented non-specific binding. Using a 6 x 6 microfluidic channel array, they studied up to 36 nanoparticle-protein interactions in a single experiment, running test samples alongside experimental controls.

"Reproducibility and reliability have been a bottleneck in the studies of protein coronas," said Mr Abhijeet Patra. "The quality and reliability of the data depends most importantly upon the design of good control experiments. Our multiplexed SPR setup was therefore key to ensuring the reliability of our data."

Testing different concentrations of each of the four proteins, the team found that apolipoprotein A-1 had the highest binding affinity for the

gold nanoparticle surface, with an association constant almost 100 times that of the lowest affinity protein, polyclonal IgG.

"Our results show that the rate of association, rather than dissociation, is the main determinant of binding with the tested blood components," said Asst Prof Drum.

The multiplex SPR system was also used to study the effect of modification with polyethylene (PEG), a synthetic polymer commonly used in nanoparticle formulations to prevent protein accumulation. The researchers found that shorter PEG chains (2-10 kilodaltons) are about three to four times more effective than longer PEG chains (20-30 kilodaltons) at preventing corona formation.

"The modular nature of our protocol allows us to study any nanoparticle which can be chemically tethered to the sensing surface," explained Asst Prof Drum. "Using our technique, we can quickly evaluate a series of nanoparticle-based drug formulations before conducting in vivo studies, thereby resulting in savings in time and money and a reduction of in vivo testing," he added.

The researchers plan to use the technology to quantitatively study protein corona formation for a variety of nanoparticle formulations, and rationally design nanomedicines for applications in cardiovascular diseases and cancer.

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