Drug delivery under investigation

BY **NANCY MILLS**

Drug delivery research at the Australian Synchrotron is a world of membrane mimics, metabolism mapping, anti-microbial weapons and much more.

'No drugs on the market consist of drug alone,' says Ben Boyd from the Monash Institute of Pharmaceutical Sciences. 'Drug delivery is all about what we put in with the drug so that it can go to the right place in the right quantity at the right time to elicit the best therapeutic effect.'

The challenge is to prepare systems with complex functionality, while also ensuring 'that the systems are simple and can be made on a large scale, and that we can readily prove their quality and safety.'

Boyd and colleagues are investigating systems that can be activated to release a drug by an internal mechanism such as enzymatic degradation, or an external stimulus such as light, a magnetic field or heat. Their materials are mostly based on biocompatible lipids that self-assemble in water. The aim is to stimulate – and control – structural changes that lead to drug release, in order to reduce the number of times a drug has to be administered, and to moderate side effects.

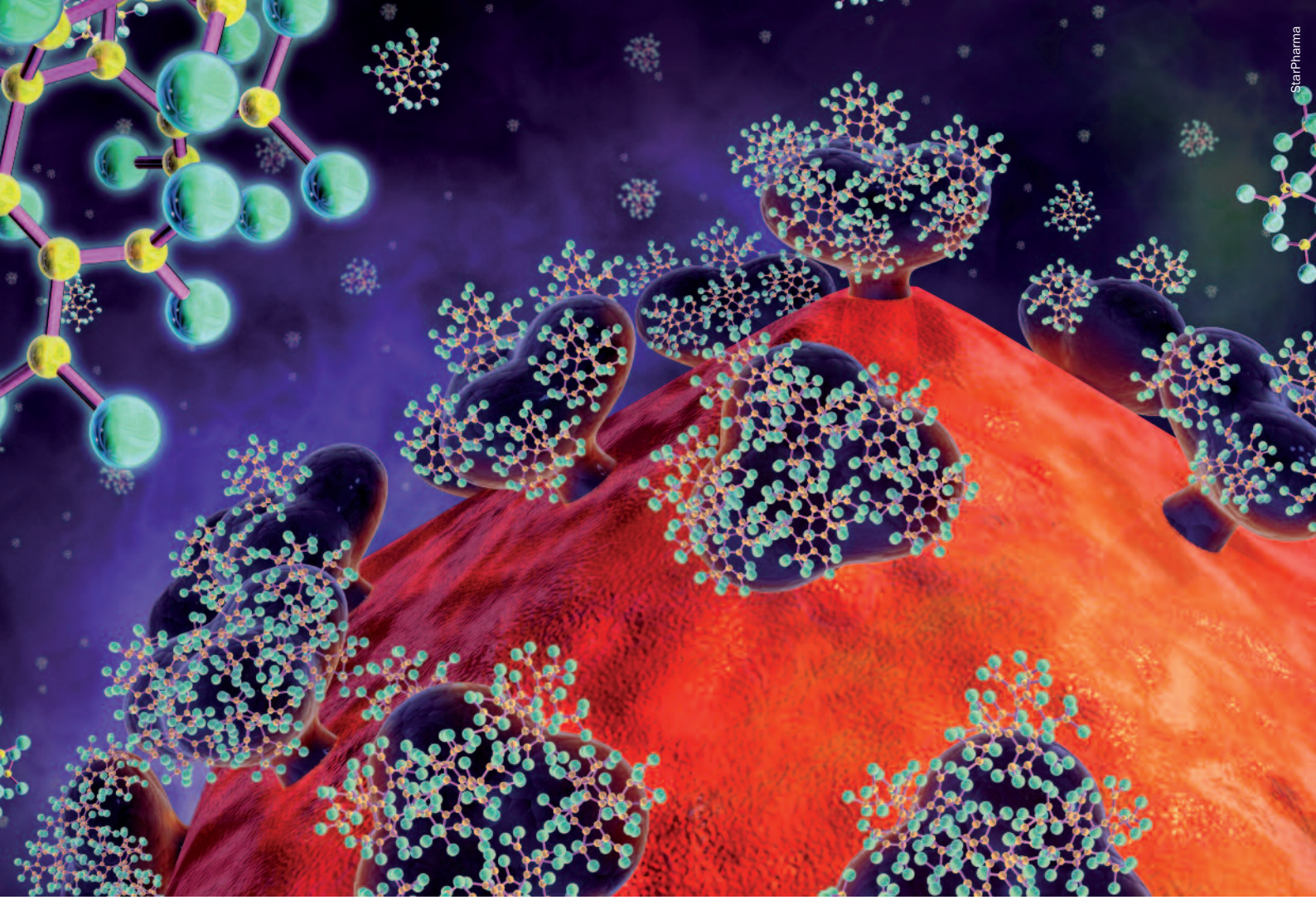
Two medical conditions that stand to benefit from this research are insulin-dependent diabetes, which requires repeated injections daily, and macular degeneration, which requires intravitreal injections, often fortnightly, for life. Being able to repeatedly activate, on demand, the release of a known dose of drug after it has been administered could dramatically cut the number of injections needed.

Boyd's group uses small-angle X-ray scattering (SAXS) to determine the structure of the lipid matrix during activation. The synchrotron is essential because the transitions between structures occur too fast to be measured by lab-based SAXS approaches.

They have shown that they can use light to activate structural changes in lipid systems, with drug release turned on and off by photothermal or photochromic mechanisms. This has important implications for potential treatment of conditions such as macular degeneration, where a laser could stimulate the system and activate drug release.

'Our results look promising, but there's still a lot of work to do,' Boyd says. 'We need to identify more appropriate lipid systems, optimise the design of our systems, and demonstrate how these can be applied to treatment in disease models.'

One way to speed up the process of investigating large numbers of samples and conditions is to use a high-throughput set-up. That's the approach taken by CSIRO's Xavier Mulet, who collaborates with Boyd.



Artist's impression of dendrimer SPL7013 molecules binding to glycoprotein spikes on the outside of HIV viral particles, preventing entry to potential host cells. StarPharma

Membrane power

Mulet and fellow CSIRO researcher Charlotte Conn are using high-throughput SAXS to study advanced, nanostructured, self-assembled materials (lipid cubic phases) for separate drug design and particle-based drug delivery purposes. Developed by CSIRO and Australian Synchrotron beamline scientists, the high-throughput set-up can analyse thousands of samples in a 24-hour shift.

The lipidic materials that Conn and Mulet are studying have an interesting 'bicontinuous' structure that contains both hydrophobic and hydrophilic domains. This makes them ideal for encapsulating many different types of drugs – and for stabilising membrane proteins, which also have hydrophobic and hydrophilic domains.

Mulet is developing a series of complex nanostructured particles, based on naturally occurring lipids, to act as drug delivery vehicles. The particles are created from small amphiphilic molecules that self-assemble when water is added, forming structures with long-range order, such as inverse bicontinuous cubic phases (cubosomes), inverse discontinuous micellar cubic phases (micellosomes) and inverse hexagonal (hexosomes) or lamellar phases (liposomes). While many existing nanomedicine drug delivery systems involve liposomes, cubosomes possess a much higher internal lipid content and interfacial structure, with potential to load increased levels of hydrophobic and amphiphilic drugs.

Mulet is using high-throughput SAXS to investigate how the structure of cubic phase nanoparticles changes when a drug is incorporated. Particle structure is important because it affects how much drug can be loaded, and how quickly or slowly the drugs are released into the body.

More than half of all commercially available drugs target membrane proteins, which are embedded in the lipid bilayer membrane that surrounds living cells. These proteins are important for fundamental cell

… they can use light to activate structural changes in lipid systems, with drug release turned on and off by photothermal or photochromic mechanisms.

processes and in diseases ranging from cardiovascular to neurological complaints such as schizophrenia and Parkinson's disease. However, their structures are not well understood because they are unstable outside the cell membrane environment, and difficult to crystallise. Conn says this lack of structural information is 'reflected in the adverse side effects associated with many commercially available drugs'.

In meso crystallisation utilises bicontinuous lipidic cubic phases to mimic the native cell membrane environment during the crystal growth process. The technique has had some notable successes, particularly for complex human membrane proteins, but has not otherwise lived up to its promise because we do not know a lot about the crystal growth process *in meso*. Conn is addressing this knowledge gap by studying how the structure of the underlying material changes during crystal growth.

Tuning platinum pro-drugs

Platinum antitumour drugs such as cisplatin (*cis*-diamminedichloroplatinum(II) or *cis*-$PtCl_2(NH_3)_2$) are widely used chemotherapy agents effective against testicular cancer and some head, neck and ovarian cancers.

However, Pt^{II} agents are predominantly taken up by cells on the tumour periphery and their effectiveness can be limited by poor drug distribution within tumours, leading to incomplete tumour eradication and drug resistance. In addition, poor selectivity and premature activation can result in severe side effects such as kidney damage, neurotoxicity and hearing loss.

Trevor Hambley and Jenny Zhang from the University of Sydney are developing platinum(IV)-based pro-drugs with 'enormous potential to mitigate the shortcomings of current platinum(II)-based drugs'. Pt^{IV} pro-drugs are less reactive than Pt^{II} complexes and require activation to release the cytotoxic Pt^{II} analogue. A major challenge is to stabilise Pt^{IV}-based antitumour pro-drugs against premature activation.

At the Australian Synchrotron, Hambley and Zhang use X-ray fluorescence microscopy (XFM) for elemental mapping of treated tumour model samples, X-ray absorption fine-edge structure (XANES) mapping to examine platinum speciation in the tumour models, and X-ray absorption spectroscopy to study the metabolism of platinum complexes in whole cells.

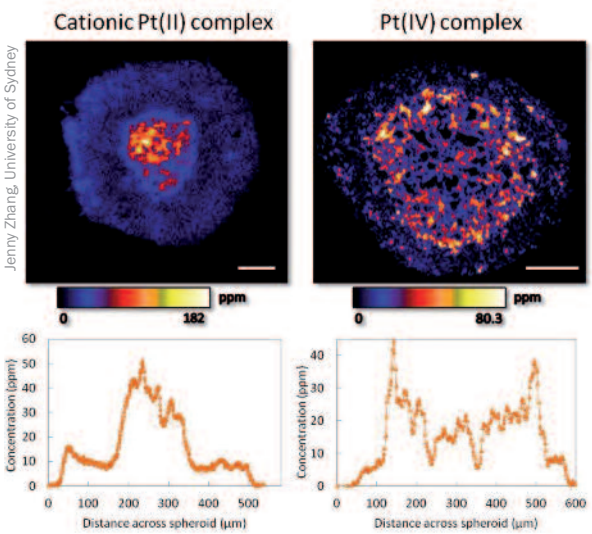
XFM mapping shows how modifying the chemical and physical properties (e.g. charge and reactivity) of the platinum complexes affects platinum distribution in the tumour models, enabling the researchers to 'tune' the tumour penetration abilities of their drug molecules.

XANES has yielded 'invaluable insights' into the metabolism of Pt^{II} and Pt^{IV} complexes in multicellular tumour model systems. It indicates where and when Pt^{IV} pro-drugs are reduced (activated) or further metabolised within the tumour, allowing additional tuning of the complexes to exhibit a more targeted mode of activation.

Overall, synchrotron experiments show that charged platinum complexes can better penetrate into tumour model systems, mainly because their suppressed cellular uptake enables them to bypass cells on the tumour model periphery. The researchers also found that Pt^{IV} complexes can be stabilised by coordination spheres made up of four carboxylate donors and two *cis*-amine donors; these slow the reduction of the complexes until they enter tumour cells.

The next step will involve modifying the Pt^{IV} complexes to target unique characteristics of the tumour micro-environment, such as enzymes that are over-expressed in tumours, and using synchrotron techniques to re-check in-vitro and in-vivo distribution and activation.

Because the Pt^{IV} complexes belong to a proven class of anticancer agent,



XFM mapping is used to show how modifying the chemical and physical properties (e.g. charge and reactivity) of platinum drug complexes affects their ability to penetrate tumour models.

… charged platinum complexes can better penetrate into tumour model systems, mainly because their suppressed cellular uptake enables them to bypass cells on the tumour model periphery.

they could reach the market in as little as 5–10 years. The main issue will be to prove lower toxicity relative to existing Pt^{II} drugs.

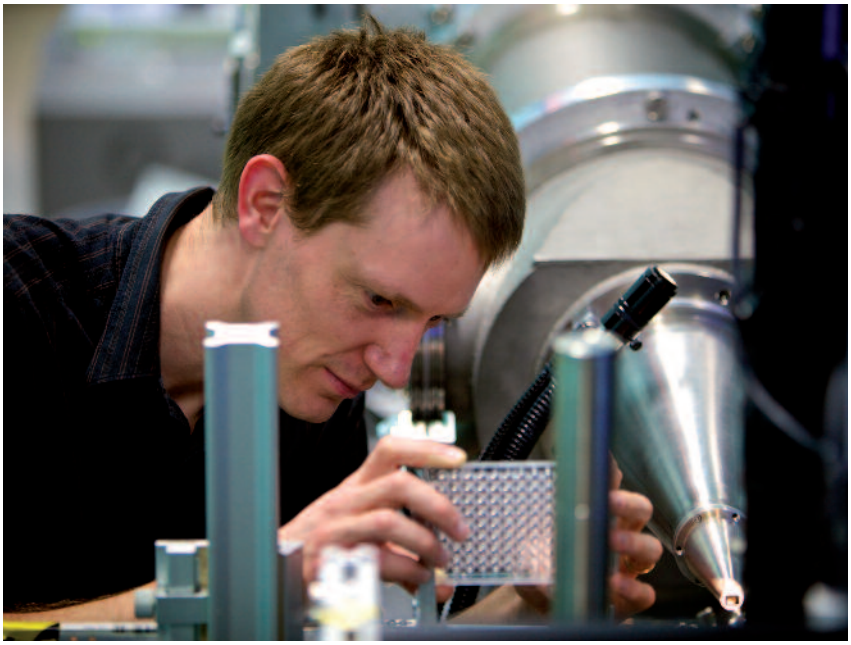
Branching out

Imagine a highly branched molecule that can be designed to interact with target sites on microbial pathogens.

Dendrimers are synthetic molecules about 3–4 nanometres in diameter, comprising a central core and one or more sets of branches, with the outermost branches being capped by biologically active surface groups. A first-generation dendrimer has a single set of three or so branches attached to the core. A second-generation dendrimer has a second set of branches attached to the original branches, and so on up to a tenth-generation dendrimer. Because they can be designed to interact with biological cells and receptors, and to carry other active molecules within or on their branched structure, dendrimers have many potential uses in medicine, as well as numerous industrial applications.

The world's first dendrimer-based drug, developed by Australian-based pharmaceutical company Starpharma with assistance from Gilda Tachedjian of the Burnet Institute, is now in clinical trials. The product is a topically applied microbicide delivered in a mucoadhesive Carbopol® gel formulation that is being developed as a product for women to treat and prevent bacterial vaginosis, and to protect themselves from sexually transmitted HIV-AIDS and genital herpes infections. Significant problems in their own right, bacterial vaginosis and genital herpes are also known to increase the risk of HIV infection.

Interestingly, the active ingredient in this case is the dendrimer itself, a fourth-generation dendrimer known as SPL7013. Burnet postdoc Cath Latham recently used SAXS to investigate how SPL7013 interacts with glycoprotein spikes that are present on the outside of HIV viral particles to understand how this dendrimer can block viral entry into the host cell. Dendrimers are thought to bind to their targets in a multivalent manner that overcomes intrinsically weak monovalent interactions and holds potential for broad-spectrum antiviral activity.



Australian Synchrotron

Stephen Mudie (Australian Synchrotron) checks a high-throughput sample holder on the SAXS beamline, which can analyse thousands of solution phase samples in a 24-hour shift.

According to a 2012 UNAIDS report, 'Together we will end AIDS', around 34 million people were living with HIV in 2011. People aged 15–24 account for 40% of all new adult cases, with up to a sixfold higher prevalence of HIV in young women compared to young men in resource-poor settings.

Sharp as silk

Another intriguing possibility is that fibroin, a protein found in silkworm silk, can be made into 'microneedles' loaded with drug molecules that will diffuse into the skin at a controlled rate. In late 2011, a US research team reported production of a biocompatible, degradable silk microneedle that can incorporate, store and controllably release sensitive drugs – and does not require refrigerated transport or storage. Because microneedles only pierce the outer layer of the skin, they do not cause any pain.

In Australia, CSIRO's Andrew Walker is using SAXS to investigate silk proteins and structures from a surprising range of insect sources that includes honeybees, crickets, lacewings, webspinners, glow-worms, weevils, silverfish, dance-flies and praying mantises. Walker's aim is to identify silk proteins with better potential for artificial production – and new industrial and biomedical applications such as microneedle drug delivery systems – than the long and repetitive silk proteins made by silkworms and spiders.

The big picture

The development of better-targeted, more-effective drug delivery systems that can successfully negotiate the body's digestive system with reduced side effects requires a broad range of expertise, tools and techniques. The Australian Synchrotron provides a variety of techniques – only possible at a synchrotron facility – that complement and extend existing drug delivery research methods.

Nancy Mills is the Australian Synchrotron's science writer.