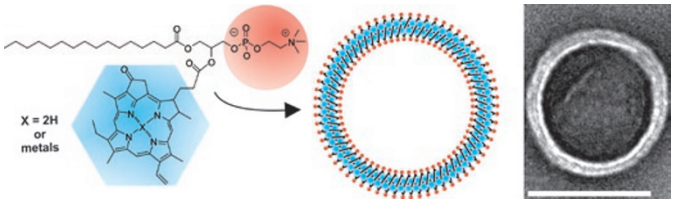
IMAGING

One particle to rule them all?

Nanoparticles made of the natural pigment porphyrin combine desirable properties of both organic and inorganic particles.

The genesis of porphysomes was an accident. Gang Zheng and his colleagues at the University of Toronto wanted to better understand photosensitizing agents and were thus examining insertion of porphyrin, a pigment from chlorophyll, into liposomes.

The problem was that the maximum free porphyrin that could be inserted was only about 15 molar %; beyond that the structures became unstable. But unexpectedly, using porphyrin-phospholipid conjugates, a self-assembly process took over and a stable structure formed. "When my student told me he could load the phospholipid-porphyrin at 50% into the liposome, I said, wait a minute, that's not a liposome any longer," Zheng says. The researchers soon found that they could form stable structures made of even 100% porphyrin-lipid conjugates. "I only believed it when we did the [transmission electron microscopy] and I saw the bilayer," says Zheng. "Then I almost jumped to the roof."



A schematic representation (left) and a transmission electron micrograph (right) of a pyropheophorbide-lipid porphysome. Scale bar, 100 nm. Image adapted from *Nature Materials*.

What they saw in the transmission electron micrograph was a vesicle with two high-density layers, ~5 nanometers (nm) thick in total and separated by a ~2 nm gap. Each layer is thought to correspond to a monolayer of porphyrin-lipid. The vesicles can be made by standard lipid extrusion and form monodisperse 100-nm particles, though they can be made smaller too.

But it is the photonic properties that make porphysomes really interesting, says Zheng. Particles made of pyropheophorbide have two absorption peaks, at 400 nm and at 680 nm, and this can be tuned by using other pigment conjugates or by including metals in the bilayer. Fluorescence is strongly self-quenched, presumably because there are about 80,000 tightly packed porphyrins in each particle. Illumination therefore results in dissipation of the energy as heat, meaning that the particles could be useful reagents for both photothermal therapy and photoacoustic imaging, in which thermal expansion generates a measurable acoustic signal. And finally, unlike inorganic nanoparticles, the porphysomes can be actively loaded with dyes or drugs just like a regular liposome.

This unique combination of properties, says Zheng, means that porphysomes could prove extremely versatile for both research and therapy. "What you have here is quite

GENOMICS

UNDERSTANDING SLEEPING SICKNESS

High-coverage sequencing of RNA interference targets gives insight into parasite phenotypes.

If you live in sub-Saharan Africa, the bite of a fly can have life-threatening consequences. Trypanosomes, transmitted to the blood stream by the bite of the tsetse fly, will proliferate, invade the central nervous system and kill an affected individual if left untreated. For all the hardship it causes, African trypanosomiasis remains an orphan disease with few effective drugs. The only targeted compound, initially developed around 30 years ago for cancer chemotherapy, is eflornithine, which is less toxic but also more expensive than arsenic-based compounds that have a drug-induced fatality rate of 5–10% in recipients.

A better understanding of trypanosome biology and its genetic makeup is key for identifying targets for new compounds. Genome sequencing revealed ~7,500 genes in *Trypanosome brucei*, but the function of 64% of them remains unknown.

In the bloodstream of human hosts, trypanosomes coat themselves in glycoproteins, which are expressed from subtelomeric sequences with rapid switches in expression, thus making the parasites poor targets for antibodies. This antigenic variation first lured David Horn of the London School of Hygiene and Tropical Medicine into the study of trypanosome biology. "But as the genome sequencing projects progressed," he says, "we became very interested in developing technologies to exploit the genome sequences." Starting in 2001 his group began a collaborative project to systematically look at the approximately 200 genes on chromosome 1 using RNA interference (RNAi) screens. This labor-intensive project took 3 years to complete.

It made Horn realize that if they wanted to do a genome-wide screen, a pooled approach would be needed, and the idea of RNAi target sequencing (RIT-seq) was born. His team used an inducible RNAi plasmid library made up of sheared genomic fragments with an average length of 600 base pairs. To ensure tightly inducible, efficient expression, they targeted the RNAi cassettes to a single genomic locus and examined the effects of gene knockdown in four different samples. They isolated genomic DNA from the bloodstream stage of the trypanosome life cycle 3 or 6 days after induction, from the procyclic form at the insect stage and from cells that were induced during the bloodstream stage and allowed to differentiate all the way through to the insect stage. Horn then worked with Matt Berriman's team at The Wellcome Trust Sanger Institute to amplify the RNAi library cassette inserts and to perform high-throughput sequencing. The comparison of aligned sequence reads between the different induced stages and an uninduced control showed them 'cold spots'—genes that were associated with a loss of fitness in various samples.

astonishing: you have a structure self-assembled from one component less than 1,000 daltons in molecular weight; this molecule can be well characterized, and the resulting structure achieves many functions without stacking together different components as with other multi-functional platforms," he says.

Porphysomes could be used to switch from photoacoustic imaging to fluorescence imaging. For instance, when the researchers modified the particles to contain 1% folate and applied them to folate receptor-expressing cells, the fluorescence signal only 'came on' when the particles had been endocytosed and presumably broken apart within the endosome. *In vivo*, upon intradermal injection of porphysomes into rats, the photoacoustic signal could be used to monitor the local lymphatic vasculature and the draining lymph node, and in a tumor model, the particles could be monitored as they accumulated in the tumor and their fluorescence dequenched.

Finally, porphysomes are enzyme-degradable and could therefore turn out to be biodegradable also *in vivo*. "We got the components from nature," says Zheng. "We got the porphyrin from algae that you can actually find in a health-food store." It should be noted that the algal porphyrin is modified in the laboratory, and that long-term toxicity of porphysomes has still to be tested, but rats could tolerate very high intravenous doses over the short term with no apparent ill-effects.

It is an attractive idea that the solution to a knotty problem lies in simplicity. To make multifunctional particles with the capacity for nontoxic drug or contrast-agent delivery, simplicity may indeed be the key.

Natalie de Souza

RESEARCH PAPERS

Lovell, J.F. *et al*. Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic constrast agents. *Nat. Mater.* **10**, 324–332 (2011).

Although Horn acknowledges that the datasets with loss of fitness in all four conditions are more robust, he sees the differentiation-specific genes also as potential drug targets. "A drug could trigger the cells in the patient to differentiate," he says, "and in the process they lose their glycoprotein coat and become subject to complement mediated lysis."

It goes without saying that all these results require experimental follow-up. Horn has released all the screening data on TriTrypDB, a site for kinetoplastid genomics resources. His team is now doing selective drug-resistance screens to get at the pathogen-specific uptake and metabolism of certain compounds.

"We need more resources to move the field forward more rapidly," Horn says. Helpful resources would include a collection of defined RNAi lines to do gain-of-function screens and more precise follow-up.

Horn also points out that the principle behind RIT-seq could be applied to other parasites as well, as long as they have an RNAi machinery.

For the time being, the rich data resource Horn's team created will help with drug target triage and assist in the first steps toward more effective disease treatment. Go to http://www.youtube.com/watch?v=WhdYbrv3YCs to see for yourself how sorely treatment is needed.

Nicole Rusk

RESEARCH PAPERS

Alsford, S. *et al*. High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome. *Genome Res.* advance online publication (1 March 2011).

NEWS IN BRIEF

GENETICS

Temperature-sensitive yeast mutants

Li *et al.* present a collection of temperature-sensitive mutants of almost half of the essential genes in yeast, in a genetic background appropriate for interaction screening using the synthetic genetic array method. Barcoding of the collection permits chemical-genetic suppression screening and combination with fluorescent markers of subcellular structures enables high-content screening of double-mutant phenotypes in single cells.

Li, Z. *et al. Nat. Biotechnol.* **29**, 361–367 (2011).

MASS SPECTROMETRY

HCD without a dedicated collision cell

In proteomics applications, especially for *de novo* sequencing and identifying protein modifications, peptides can be efficiently fragmented by tandem mass spectrometry via high-energy collision-induced dissociation (HCD) involving a specialized collision cell. McAlister *et al.* now show that HCD can be performed in the ion injection pathway of any mass spectrometer with a common atmospheric inlet, without a dedicated collision cell.

McAlister, G.C. *et al. Mol. Cell. Proteomics* advance online publication (10 March 2011).

GENOMICS

GROMIT looks at the regulatory genome

To find regulatory elements in the mouse genome, Ruf *et al.* developed genome regulatory organization mapping with integrated transposons (GROMIT), a strategy that uses the Sleeping Beauty transposon containing a *lacZ* reporter gene driven by a minimal promoter that responds to nearby enhancers. Following the expression patterns of the reporters in single-integrant mouse lines, they found transcriptional activity all over the chromosomes. Currently available lines cover about 10% of the mouse genome.

Ruf, S. *et al. Nat. Genet.* **43**, 379–386 (2011).

CHEMICAL BIOLOGY

Protein control with a photoactivatable split intein

Binschik *et al.* introduce a photoactivatable split-intein system for studying protein function in the living cell. Attaching one half of the split intein to the pathogenic protein staphylocoagulase modulated its binding to prothrombin in human blood plasma. Upon light activation, a bulky caging group is cleaved off the other half of the split intein, facilitating the splicing reaction, and resulting in the cleavage of staphylocoagulase and the restoration of its activity.

Binschik, J. *et al. Angew. Chem. Int. Edn.* **50**, 3249–3252 (2011).

NEUROSCIENCE

Addressing errors in patch clamping

The cell-attached patch clamp configuration is useful for investigating the functional properties of voltage-activated ion channels without substantially disturbing the rest of the cell. Williams and Wozny carefully examine the errors associated with such measurements and introduce simple correction procedures; in particular they found that the amplitude and kinetics of ion-channel activity can be distorted by transmembrane voltage changes associated with current flow.

Williams, S.R. & Wozny, C. *Nat. Commun.* **2**, 242 (2011).