

MICROBIOLOGY

Virus to the rescue

By targeting a noninfectious viral cage to biofilms, researchers developed a system for early detection and eventual destruction of these bacterial communities.

If bacteria had a literary hero, Neil Gaiman would be it. The mantra of the evil little creature in his children's novel *Coraline* exactly captures what microbes in a biofilm would sing: "We are small, but we are many."

The main challenge that biofilms—defined as a microbial community that colonizes a surface—pose for researchers is that they are difficult to target because the bacteria surround themselves with a polymeric substance that prevents access to their surface proteins by anything larger than a few nanometers. This difficulty translates to a clinical problem, in that biofilms are difficult to diagnose at an early stage, and often the only treatment option is surgery.

Mark Young and Trevor Douglas from Montana State University have experience in aiming for other clinically challenging targets—tumors—using viral platforms. Together with Peter Suci, a research fellow

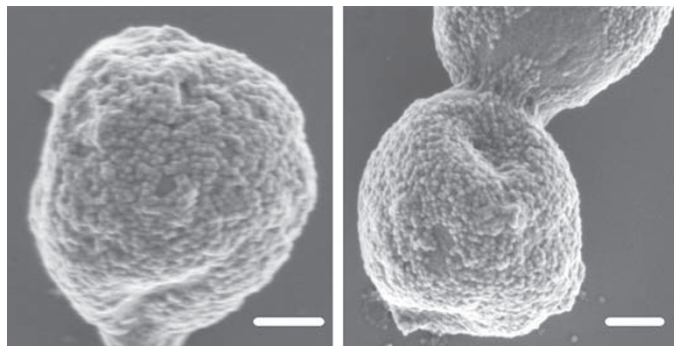


Figure 1 | Virus targets bacteria. *S. aureus* bacteria are covered by a viral cage that specifically targets the surface of the bacteria. Scale bars, 200 nm (left), 100 nm (right). Reprinted with permission from Elsevier.

in their laboratories, they saw parallels in the structure of a tumor and a biofilm, and decided to also try a viral targeting strategy for biofilms. Young describes the basic strategy: "You take a nanoplateform, a virus, put a targeting moiety on the outside and deliver it to a cell."

They used a plant virus that produces a noninfectious symmetrical protein cage

assembled from 180 identical subunits. This gives them exact spatial control over any agent that is linked to these subunits. To target a biofilm made up of *Staphylococcus aureus*, they first bound the protein A on the bacterial cell surface with a biotinylated antibody and then linked a biotinylated viral cage, bearing a magnetic resonance imaging (MRI) contrast agent, to it via a streptavidin molecule.

PROTEIN BIOCHEMISTRY

PINNING DOWN PRIONS

Researchers use peptide arrays to investigate questions about prion recognition, amyloid fibril nucleation and the sequence basis for species barriers.

Ever since the 'mad cow' disease epidemic in Great Britain made big news in 1996 and the 1997 Nobel Prize in Medicine was awarded to Stanley Prusiner for his discovery of prions, the study of these peculiar proteinaceous infectious particles has been a hot area of research.

Peter Tessier, a postdoc in Susan Lindquist's laboratory at the Whitehead Institute was interested in addressing some fundamental questions about the biology of a yeast prion called Sup35. "We were wondering about basic questions like, 'what regions within the protein are involved in nucleation?', 'could we actually see how these proteins interact and could we learn things about how they nucleate into different strains and how species barriers form?'," says Tessier.

Working with prion proteins, however, is not exactly easy. First, as elegantly proposed by Prusiner in his 'prion hypothesis' describing the unique infectious nature of these proteins, prions do not follow the normal rules for biological replication. Second, and more practically speaking, amyloid-forming prions are prone to aggregation during experiments, causing the experimenter much aggravation. "We wanted to do experiments where we looked at the behavior of short peptides in solution, but they often are

simply not soluble, and so there's almost nothing you can do in solution," explains Tessier. To overcome this challenge and create a useful experimental tool, the researchers arrayed synthetic short overlapping peptides from the prion domain of *Saccharomyces cerevisiae* Sup35 on a glass surface.

Upon incubating fluorescently labeled full-length Sup35 with the peptide array, Tessier and Lindquist discovered that only a very small set of peptides interacted with Sup35 and nucleated amyloid fibrils, suggesting that short, specific amino acid sequences are responsible for fibril formation. This finding was somewhat contradictory to the general notion that amyloid formation is governed by low sequence complexity rather than by specific sequence elements.

Of particular interest for the field is to determine how certain prion diseases, such as mad cow, can cross the species barrier (that is, from infected beef to human) while others do not. Tessier and Lindquist were able to reconstitute the species barrier between Sup35 from *S. cerevisiae* and *Candida albicans*, a distantly related yeast, on the peptide array. Upon incubation with full-length Sup35 from *C. albicans*, they observed no association with any of the *S. cerevisiae* peptides and vice versa. This supported their findings that the sequence elements that govern fibril formation are highly specific. They also concluded that the ability of some prions to cross the species barrier could be due to the conservation of specific sequence elements across species.

NEWS IN BRIEF

GENOMICS

Announcing the ENCODE results

The Encyclopedia of DNA Elements (ENCODE) project has been a massive collaborative effort to identify and analyze the functional elements of the human genome. The June issue of *Genome Research* contains 25 research reports describing the validation of results from the pilot ENCODE project, using both experimental and computational methods for characterizing functional elements. A summary of ENCODE's most recent exciting biological findings can be found in *Nature*.

The ENCODE Project Consortium *Nature* **447**, 799–816 (2007).

SPECTROSCOPY

Protein structures from chemical shifts alone

Protein structure determination by NMR spectroscopy typically requires several different kinds of NMR experiments to resolve three dimensional structure. Cavalli *et al.* now show that for 11 diverse small proteins, chemical shift data alone are sufficient for structure determination at 2-Å resolution. Their computational strategy, termed CHESHIRE, is fast and accurate, and may find use in high-throughput structural genomics efforts.

Cavalli, A. *et al. Proc. Natl. Acad. Sci. USA* **104**, 9615–9620 (2007).

RNA INTERFERENCE

Suppressing off-target effects

Along with small interfering RNA, morpholino phosphorodiamidate antisense oligonucleotides (MOs) are effective agents for knocking down gene expression. Off-target effects, however, can be a major problem in the application of both technologies. Robu *et al.* now show that one consequence of MO knockdown is the upregulation of a p53-dependent cell death pathway. They propose the concurrent inhibition of p53 as a viable strategy to minimize off-target effects resulting from MO knockdown.

Robu, M.E. *et al. PLoS Genet.* **3**, 787–801 (2007).

GENOMICS

New classes of regulatory RNAs all over the genome

A study by Kapranov *et al.* shows that almost the entire human genome is transcribed and that most transcripts do not fall within annotated regions. They found distinct categories of non-protein encoding transcripts: short RNAs (<200 nt) and long RNAs (>200 nt). The long RNAs partly act as precursors for the short RNAs, which are distinct from other noncoding RNAs such as microRNAs. The function of these transcripts remains to be determined.

Kapranov, P. *et al. Science* **316**, 1484–1488 (2007).

BIOSENSORS

Endowing yeast with a sense of smell

Radhika *et al.* reconstituted the mammalian olfactory signaling pathway in yeast, coupling olfactory receptor signaling to the expression of GFP as a sensory readout, as a tool to identify odorants targeting orphan olfactory receptors. They identified the specific receptor for an analog of the explosive TNT, demonstrating how engineered yeast could serve as biosensors for warfare agents.

Radhika, V. *et al. Nat. Chem. Biol.* **3**, 325–330 (2007).

They could tightly pack the surface of bacteria with viral cages (Fig. 1) and also achieved deep penetration of the biofilm, demonstrated by MRI. Young says that the plant virus balances the requirements for high cargo capacity to achieve good payload and small size; the viral cage allows multivalent presentation of a binding ligand on a single platform resulting in high affinity and with its 28-nm diameter it is not too large to penetrate the biofilm.

So far Douglas, Young and their teams performed all experiments on a biofilm grown in a test tube, not *in vivo*, but this is clearly the end goal of their research. As Douglas says, “the goal is a multifunctional material, combining both imaging and therapeutic capacity.”

To achieve the latter they are working on delivering an activatable compound that diffuses out of the protein cage and destroys the biofilm. Douglas has no illusions as to the challenges ahead. Since they are working with biomaterials, interactions with the immune system are to be expected, and he cautions: “It is not a ‘plug-and-play’ system at this point; you need to tailor the immune response to the particular pharmacokinetic response that you want.” Young puts their goal in more generic terms: “Our big mantra is control, control in terms of where we put things [on the viral cage], how much we put on, and how and when we release it.”

A virus harnessed to deliver enough imaging agent to visualize even a small biofilm and at the same time deliver a drug to destroy it could usher in the demise of biofilms in the clinic.

Nicole Rusk

RESEARCH PAPERS

Suci, P.A. *et al.* High-density targeting of a viral multifunctional nanoplatform to a pathogenic, biofilm-forming bacterium. *Chem. Biol.* **14**, 387–398 (2007).

To test this hypothesis, the researchers tested an engineered chimeric protein made up of sequences from both *S. cerevisiae* and *C. albicans*. The chimera interacted with Sup35 from both species, and by using the peptide arrays they were able to observe that the interactions were highly sequence-dependent. “In the case of mammalian prions, the sequences of prion proteins [across species] are actually much more closely related than they are between these two yeast species,” remarks Tessier. “Similar mechanisms may be utilized by mammalian prions where a small number of sequence differences in specific regions inhibit or enable proteins to cross species barriers.”

Notably, the researchers observed that at different temperatures, formation of fibrils of one or the other species was favored. According to Tessier, this suggests that “by favoring which regions of the protein are involved in prion nucleation, different amyloid conformations can be formed.”

Tessier and Lindquist foresee the use of peptide arrays to address other questions about prion biology, including screening for compounds that prevent fibril formation. Says Tessier: “If we can understand which [portions of the] sequences are involved in nucleation, then perhaps we can rationally design inhibitors to block this process.”

Allison Doerr

RESEARCH PAPERS

Tessier, P.M. & Lindquist, S. Prion recognition elements govern nucleation, strain specificity and species barriers. *Nature* **447**, 556–561 (2007).