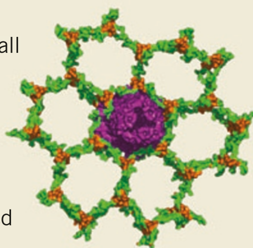
© 2006 Nature Publishing Group http://www.nature.com/naturebiotechnology

## Bacterial cell wall structure



Researchers have solved the long elusive three-dimensional structure of the cell wall of Gram-positive bacteria. Although the cell wall's basic structural unit has long been known to be a peptidoglycan, *N*-acetylglucosamine (NAG)-*N*-acetylmuramic (NAM)-pentapeptide, its three-dimensional structure had remained unsolved due to the inherent complexity of the polymer and the need for pure units for structural studies. Merouch *et al.*, who recently synthesized a NAG-NAM(pentapeptide)-NAG-NAM(pentapeptide) dimer, have now succeeded in determining the structure of this 2-kDa peptidoglycan in solution by NMR spectroscopy. Their analysis reveals an ordered, right-handed, helical saccharide conformation, with a set of repeated glycosidic torsion angles that lead the authors to suggest an oligomer structure with three NAG-NAM repeats per turn. Computer models of the cell wall structure, assuming such threefold symmetry as well as incomplete cross-linking, show a honeycomb pattern with pores ranging in size from 70 to 120 Å. These pores are large enough to accommodate the cell wall's own catalytic machinery as well as channel proteins and other macromolecules (see picture). Furthermore, the orientation of the glycan strand is orthogonal to the membrane and not parallel as had been previously assumed by many, based on the structures of chitin and cellulose, the two other main wall-forming β-1,4-linked glycan biopolymers in nature. The proposed structure sheds new light on our understanding of bacterial cell wall integrity and promises to facilitate research into antibiotics targeting the cell wall. (*Proc. Natl. Acad. Sci. USA* **103**, 4404–4409, 2006) *TM*

## Getting to grips with Fc fragments

The potency of antibody fragments in cancer therapy is dependent on the antibody Fc-region's ability to complex with T lymphocyte Fc γ-receptors (FcγRs), leading to the induction of cytotoxic effector functions. With the aim of optimizing Fc fragment affinity and specificity for FcγRs, Lazar *et al.* combined computational design and high-throughput protein screening to identify amino acids that enhance the *in vitro* affinity of the Fc region for FcγRs more than 100-fold. Incorporation of these amino acid substitutions into alemtuzumab (anti-CD52), trastuzumab (anti-Her2) and rituximab (anti-CD20) enhanced antibody-dependent cell-mediated cytotoxicity. In the case of a trastuzumab Fc variant, a cytotoxic effect was evident at Her2 concentrations well below the normal threshold for a response. What's more, a preclinical trial involving macaques demonstrated that the rituximab Fc variant enhanced levels of B-cell depletion relative to a wild-type control. Incorporation of these variants into antibodies promises to elevate their clinical efficacy in poorly responsive patients and to expand the pool of targets within the reach of therapeutic immunology. (*Proc. Natl. Acad. Sci. USA* **103**, 4005–4010, 2006) *PH*

## Combinatorial drug assessment

With combination therapies increasingly prevalent in anti-cancer and anti-viral regimens, a need exists for rapid means of elucidating drug interactions. By analogy with genetic mutations, two drugs can have an additive effect (no interaction), interact synergistically (larger than additive effect) or interact antagonistically (smaller than additive effect). Kishony and colleagues clustered 21 antibiotics and the anti-cancer agent bleomycin into classes such that any two classes interact either completely synergistically or antagonistically. After determining the nonlethal concentration at which each drug significantly affects growth in *Escherichia coli*, the authors carry out a growth assay to quantify deviation of additivity for every possible drug pair. Subsequently, using an algorithm in which the synergistic or antagonistic interaction between any two classes is measured by a cost term, 19 of the 21 drugs clustered into 7 interacting classes containing at least 2 different drugs, with each class having a distinctive predicted mechanism of action. As one of the drugs tested, bleomycin, failed to fall into an interacting class with at least two drugs, the approach suggests it might enable identification of new drugs with novel mechanisms of action. The same classification concept could potentially be applied to the design of new combination therapies. (*Nat. Genet.* **38**, 489–494, 2006) *JWT*

## NMR takes a new tack

NMR spectroscopy is an increasingly important method for determining the three-dimensional structure of proteins in solution—it is particularly useful in instances where protein crystals are not available or of sufficient quality for X-ray crystallographic study. One drawback of NMR analysis, however, is that the structures of proteins larger than 25 kDa cannot easily be solved because of increased levels of spectral noise arising from the random incorporation of isotopes into proteins. Kainosho *et al.* take a new tack to this problem by introducing stereo-array isotope labeling (SAIL), a modified approach to labeling proteins for NMR analysis. First, the authors synthesize a set of amino acid isotopomers designed to contain $^{13}$C and deuterium ($^{2}$H) at very precise positions so as to minimize the spectral noise due to intramolecular signal coupling. These amino acids are subsequently incorporated into the proteins of interest by a cell-free protein expression system. The authors show that they can successfully solve high-quality solution structures of two proteins, the 17-kDa calmodulin and the 41-kDa maltodextrin-binding protein. The approach promises to facilitate three-dimensional structure determination of larger proteins of relevance to basic science as well as pharmaceutical applications. (*Nature* **440**, 52–57, 2006) *GTO*

## Interfering with flaviviruses

Flaviviruses—such as the Japanese encephalitis virus (JEV) and the West Nile virus (WNV)—cause acute and often fatal infections, with no specific treatments available. Kumar and coworkers have created a series of short interfering RNAs (siRNAs) directed against a flavivirus envelope gene to test their potential for warding off flaviviral infections. siRNAs are known to control viral infections in plants and some preliminary work also suggests an antiviral activity in animal models. The authors transduced neuronal cell cultures with lentivirus expressing short-hairpin RNAs, which are subsequently processed into siRNAs, and achieved suppression of JEV infection. They also show that siRNAs delivered intracranially (via lentiviral vectors or in complex with various lipid formulations) protect mice against infection. By targeting a conserved region of the flavivirus envelope glycoprotein, they were further able to protect mice against both JEV and WNV. More importantly, the siRNA protected mice when given before, during and after (six hours) infection with the virus. These results suggest that, once a suitable delivery method for humans has been identified, siRNAs could have significant therapeutic potential. (*PLOS Medicine [online]* **3**:e96, 2006; doi:10.1371/journal.pmed.0030096) *LD*

*Research Highlights written by Laura DeFrancesco, Peter Hare, Teresa Moogan, Gaspar Taroncher-Oldenburg and Jan-Willem Theunissen.*