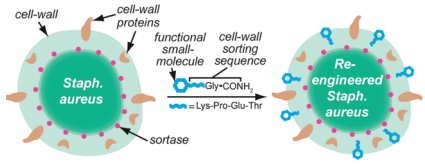
# In this ISSUE

## Re-engineering Gram-Positive Bacterial Cell Walls

Under pressure from the emergence of drug-resistant bacteria, researchers often focus their efforts on the development of novel drugs. However, Nelson *et al.* (DOI: 10.1021/cb100195d) have developed an innovative strategy to combat drug resistance in Gram-positive bacteria. Their method involves "tricking" *Staphylococcus aureus* into incorporating non-natural compounds into its cell wall, thereby making the organism more susceptible to therapeutics. The novel approach presents an opportunity to affect the way bacteria interact with host cells.

The authors employ the endogenous enzyme sortase A (SrtA), which incorporates proteins into the bacterial cell wall. Typically, SrtA recognizes its substrate via a conserved pentapeptide motif, LPETG, proximate to the C-terminus of the secreted protein. Upon substrate recognition, SrtA forms an acyl-enzyme intermediate by cleaving the threonine−glycine bond of the pentapeptide motif. This event is followed by an attack of the peptidoglycan precursor (lipid II) and the formation of an adduct that is eventually incorporated into the bacterial cell wall. Using these properties of SrtA, exogenous small molecules varying in functionality, such as fluorescein, azides, and biotin, were incorporated into the *S. aureus* cell wall. A wide array of experimental techniques such as epifluorescence and electron microscopy, flow cytometry, mass spectrometry, and biochemical cell wall extraction were used to confirm the covalent incorporation of the non-native small molecules into the peptidoglycan layer.

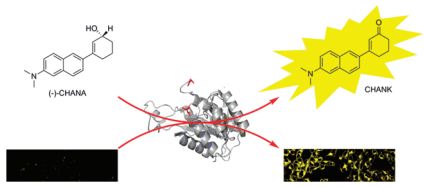
This report presents the first example of cell wall engineering of any pathogenic Gram-positive bacteria not involving the genetic manipulation of the organism. Given the global prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and related nosocomial infections, the importance of new approaches to combat drug-resistant bacteria cannot be overstated. This unique method offers fresh impetus to efforts to develop new antimicrobial tools.




## A Shining New Probe

During the progression of Alzheimer's disease (AD), elevated concentrations of β-amyloid (βA) peptides are found as plaques in the patient's brain. These peptides have been demonstrated to bind and inhibit the mitochondrial alcohol dehyrogenase HSD10, an enzyme implicated in mitochondrial dysfunction linked with AD. However, the association between HSD10 activity and its role in AD in living cells is unclear. In this issue, Muirhead *et al.* (DOI: 10.1021/cb100199m) report the development of a fluorescent probe, (−)-CHANA (cyclohexenyl amino naphthalene alcohol), enabling detection and quantification of HSD10 activity. This single enantiomer probe is a major advancement in the accurate measurement of HSD10 activity in living cells.

*In vivo*, HSD10 binds and oxidizes (−)-CHANA to produce a quantifiable fluorescence that can be studied in real time using fluorescence microscopy. Importantly, the fluorescent signal was found to correlate with the concentration of HSD10. The selectivity of (−)-CHANA for HSD10 was confirmed using a specific inhibitor to the alcohol dehydrogenase, which in turn inhibited the oxidation of the probe *in vivo*. The authors were thus able to measure HSD10 activity in living cells treated with βA. The fluorescence observed in HSD10-expressing cells was significantly lower when βA was present. As the binding site of HSD10 for βA is a therapeutic target for treating AD, (−)-CHANA provides a key tool in analyzing promising pharmaceutical compounds.

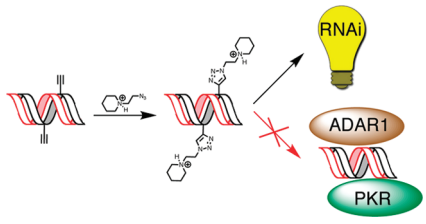



## Making siRNAs Better

Short-interfering RNAs (siRNAs) are a powerful tool in studying gene function and have been implicated as a potential therapeutic via the RNA interference (RNAi) pathway. However, harnessing siRNAs for therapeutic use is impeded by the intrinsic structure of siRNA, which is prone to nuclease degradation and often suppress nonspecific genes. The off-target effects are attributed to the binding of proteins independent of the RNAi pathway. Many of the off-target effects of siRNA can be attributed to the binding of proteins such as RNA-dependent protein kinase (PRK) and adenosine deaminase which acts on RNA 1 (ADAR1)

In this issue, Peacock *et al.* (DOI: 10.1021/cb100245u) show modification of the chemical structure of siRNA significantly reduces nonspecific effects while maintaining native RNAi activity. Site-specific incorporation of $N^2$-propargyl 2-aminopurine and other adenosine analogues into the RNA minor groove partially destabilized the duplex but maintained base pairing. By copper-catalyzed azide−alkyne cycloaddition, the analogues were subsequently converted into bulky adducts. These modified siRNAs were tested for RNAi activity by substituting adenosines in both the guide and passenger strands of siRNA directed to mRNA. The modified duplexes retained full RNAi activity but showed decreased binding to PKR and ADAR1.

By reducing off-target effects, the specificity of siRNA is increased. The chemically modified siRNAs described in this work exhibit an enhanced ability to specifically silence a gene of interest, advancing the development of potential RNAi-derived therapeutics.




Published online December 17, 2010 • 10.1021/cb100366h