you never quite know whether you can 100% trust it," notes Bax. "You sort of start saying, 'well this is obvious, this is logical, and clearly I shouldn't take this answer because it's ridiculous'. And in the absence of a blind test, you can never be really sure." Therefore they also tested CS-Rosetta on 9 novel protein targets of the NESG, which were in the process of being solved by traditional NOE-based NMR methods. Once the results were in (**Fig. 1**), "this finally made us believers that this was for real because initially I was worried the results were really too good to be true," says Bax.

Bax anticipates that methods like CS-Rosetta and Cheshire will rapidly become accepted in structural genomics because they greatly simplify and shorten the process of structure determination. CS-Rosetta currently is limited, however, to small proteins of about 15 kDa or less. "It appears that the most severe limit is computational; the Rosetta approach really blows up exponentially, and it already takes a humongous amount of computational time," explains Bax. He also notes that as proteins get larger, their folds become more complex. However, Rosetta allows input of any kind of data, so entering additional structural information such as disulfide bond links or just a few long-range NOEs could simplify analyses for larger proteins.

Bax also expects that future developments in NMR spectroscopy will further the application of this technology. He predicts that, "Over the next ten years, we could develop a much more quantitative relationship between chemical shift and structure, at which point one would be able to get atomic-resolution structures better than what we can get from current technology."

Allison Doerr

RESEARCH PAPERS

Cavalli, A. *et al.* Protein structure determination from NMR chemical shifts. *Proc. Natl. Acad. Sci. USA* **104**, 9615–9620 (2007).

Shen, Y. *et al.* Consistent blind protein structure generation from NMR chemical shift data. *Proc. Natl. Acad. Sci. USA* **105**, 4685-4690 (2008).

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simultaneously disrupted at a high frequency." As a result, he estimates that the time required for this approach is less than half of that required for current homologous recombination–based methods, such as those that use adeno-associated virus (AAV)-mediated delivery.

The hardest part of both of these approaches is making the targeting tools, and neither is an off-the-shelf system. "At the moment most labs that find it difficult to make an AAV vector would also find it difficult to make a zinc-finger protein," Gregory notes. "But both are increasingly available through commercial entities and will be available to researchers." Sangamo's zinc-finger proteins and zinc-finger protein nucleases are now being distributed by Sigma-Aldrich.

In the meantime, Sangamo scientists are partnering with Pfizer and others in the industry to use this method to create improved cell lines for manufacturing proteins for human therapeutics. But another direction for this work, according to Gregory, is that it might be a method to create transgenic animals. "Because of the simplicity of the method—the fact that you only have to make a double-stranded break—and the frequency with which you get biallelic targeting, it's an exciting possibility that this will be a method for the creation of transgenic animals."

Irene Kaganman

RESEARCH PAPERS

Santiago, Y. *et al.* Targeted gene knockout in mammalian cells using engineered zinc-finger nucleases. *Proc. Natl. Acad. Sci. USA*, published online 21 March 2008.

NEWS IN BRIEF

IMAGING AND VISUALIZATION

Guidelines for reporting gene expression localization

Deutsch *et al.* present guidelines for authors reporting gene expression localization experiments, the minimum information specification for *in situ* hybridization and immunohistochemistry experiments (MISFISHIE). Six types of information—including experimental design, specimens and treatments, reporters, staining, imaging data, and image characterization—should be provided to allow experiments to be reproduced.

Deutsch, E.W. *et al. Nat. Biotechnol.* **26**, 305–312 (2008).

GENE REGULATION

Transgene combinatorics

For *Caenorhabditis elegans*, researchers primarily rely on endogenous enhancers to regulate transgene expression, but this approach restricts them to the spatiotemporal activity of those enhancers during development. Davis *et al.* created a system to turn on transgene activity when a site-specific recombinase removes an intervening marker gene. Different regulatory regions for the transgene and recombinase create a combinatorial code that can better target transgene expression.

Davis, M.W. *et al. PLoS Genet.* **4**, e1000028 (2008).

PROTEIN BIOCHEMISTRY

Monitoring by SPR transcription

Each step of transcription—from initiation to termination—is dictated by the sequence of the nucleic acids involved and by the proteins comprising the RNA polymerase (RNAP) complex. Greive *et al.* applied surface plasmon resonance (SPR) technology to quantitate these steps in solution and determine their rate constants. Using this method they observed processes including RNAP binding DNA as well as transcript release and RNAP dissociation at terminators.

Greive, S.J. *et al. Proc. Natl. Acad. Sci. USA* **105**, 3315–3320 (2008).

MICROSCOPY

Pushing the resolution of electron cryomicroscopy

Though electron cryomicroscopy does not usually yield structures with resolution as high as crystallography, no crystallization is needed, and structures can be observed under near-native conditions. Jiang *et al.* report a 4.5 Å resolution structure for the infectious epsilon15 virus capsid using single-particle electron cryomicroscopy. They obtained this very high resolution for the 22 MDa structure by collecting 20,000 individual particle images and using distributed computing for image processing.

Jiang, W. *et al. Nature* **451**, 1130–1134 (2008).

MICROBIOLOGY

Complete ORF collection for *Vibrio cholerae*

Cholera, caused by the bacterium *V. cholerae*, is still a major public health issue in many parts of the world where it is easily spread through contaminated water. Rolfs *et al.* report a complete FLEXGene clone set containing full-length open reading frames for a pathogenic *V. cholerae* strain, with each clone verified by sequencing to ensure high quality. The authors hope that this resource will stimulate research on cholera, and perhaps lead to new vaccines and therapeutic strategies.

Rolfs, A. *et al. Proc. Natl. Acad. Sci. USA* **105**, 4364–4369 (2008).