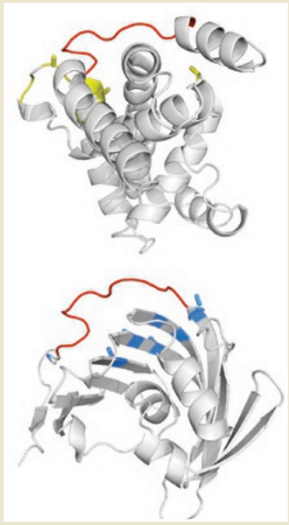
In silico epitope scaffolds



A protein's ability to induce antibodies resides in the structure of individual epitopes, with some more effective at eliciting neutralizing antibodies than others. Ofek *et al.* describe a method for creating and optimizing epitopes on scaffolds, predicted *in silico*, that can be used to generate high-affinity antibodies *in vivo*. Using as a model an epitope from the membrane-proximal external region of HIV-1 gp41 protein, they first searched a protein database for scaffolds that structurally resemble the epitope bound to an antibody (2F5) directed against it, and introduced mutations to improve scaffold-epitope binding. Having designed five scaffolds *in silico*, they analyzed the biophysical interaction of each with 2F5 antibody. They found that they all bound 2F5 with roughly the same affinity as free peptide, but that there were differences among the five scaffolds in how flexible they were, which interestingly translated into differences in their ability to elicit an immune response *in vivo*. This method for recreating and optimizing epitopes may provide a way to generate antibodies to recalcitrant, yet medically important, proteins. In a related study, some of the same researchers achieved similar success with the poorly immunogenic HIV epitope 4E10, another membrane-proximal epitope in gp41. This system may also provide useful insights into the adaptive immune response to particular structures. (*Proc. Natl. Acad. Sci. USA*, published online 27 September 2010; doi:10.1073/pnas.1004728107; *Structure* **18**, 1116–1126, 2010) *LD*

MRI for microfluidics

The potential of microfluidics to increase the throughput and portability of analyses has been limited by the need for a tool that can probe both microscale chemistry and flow dynamics. Conventional magnetic resonance imaging (MRI) cannot sensitively record images of microfluidic flow with the required spatial and temporal resolution. By adapting remotely detected MRI—a variant of conventional MRI in which the signal-encoding phase of MRI is decoupled from the signal detection process—and combining it with JPEG-style compressive sampling, Bajaj *et al.* are able to dramatically increase the speed of acquisition of MRI images. The spatial resolution they achieve is sufficient to capture the results of up to several thousand parallel assays on a microfluidic device. Although the approach is only relevant to nuclear magnetic resonance (NMR)-active analytes or those that can be detected indirectly by contrast agents or other sensors, it may open new opportunities for a range of highly parallel analytical applications involving microfluidic chips. (*Science*, published online 7 October 2010; doi:10.1126/science.1192313) *PH*

Written by Kathy Aschheim, Laura DeFrancesco, Peter Hare & Craig Mak

Identifying transcription factor modulators

How does genetic variation affect gene expression? Approaches to date have used genetic linkage analysis to associate genetic markers with the expression of genes measured across a population of closely related individuals. In a twist on these approaches, Lee and Bussemaker first infer the 'activity' of transcription factors in each individual and then look for markers linked to transcription factor activity. The authors posit that this extra computational step increases the signal-to-noise ratio by combining into a single metric the expression of many genes predicted to be targets of a transcription factor. Transcription factor activity was inferred from gene expression profiles, the promoter sequence of each gene and known DNA-binding specificities of transcription factors. When applied to data from yeast, the approach outperformed previous methods, identifying over six times as many associations between transcription factors and loci that may modulate their activity. Although some of these links are consistent with existing data and could be supported experimentally, much work remains to understand the importance of each association. (*Mol. Syst. Biol.* **6**, 412, 2010) *CM*

Deep sequencing and RNA fitness

The vastness of sequence space—a 20-mer RNA, for example, has ~10^{12} possible sequences—makes it extremely challenging to visualize the relationships between all possible genotypes of a macromolecule and their cognate phenotypes. Pitt and Ferré-D'Amaré demonstrate the potential of next-generation sequencing methods to profile the activities associated with different RNA sequences—so-called fitness landscapes. Working with a 54-nucleotide RNA ligase ribozyme, they use deep sequencing to characterize both the sequences and activities (measured as abundances) of a population of variants of the catalytic RNA both before and after *in vitro* selection. By projecting the empirically determined fitness onto the ribozyme sequence, they are able to identify functionally important residues not previously known to be required for maximal activity. Besides its potential use in such applications as the optimization of aptamer sequences, the same principle could theoretically be used to elucidate the molecular basis of pathogen fitness in patient populations, which might in turn have implications for vaccine and drug development. (*Science* **330**, 376–379, 2010) *PH*

Lentivectors and clinical validation

Gene-therapy vectors based on lentiviruses may be safer than gammaretrovirus vectors, which have caused leukemia in several patients by integrating into the genome near proto-oncogenes. Last year, the first reported clinical trial of a lentiviral vector found no evidence of cancer or clonal cell amplification in two children with adrenoleukodystrophy. A second clinical trial based on a lentiviral vector has now released results on one patient. This study treated beta-thalassemia, a disease of red blood cells, with autologous hematopoietic stem cells genetically modified to express the missing beta-globin chain of hemoglobin. One year after the transplant, the patient's red blood cells had recovered sufficiently that he has not required blood transfusions for 28 months. But the therapy did elicit a clonal amplification: a cell carrying a vector insertion in *HMGA2*, a gene involved in transcriptional activation, expanded to ~50% of transduced cells, although staying below 6% of all blood cells. Why this occurred remains to be determined. Overexpression of full-length and truncated *HMGA2* has been linked to benign and malignant tumors, and, about three years after the transplant, it is unclear whether the clonal expansion seen here is innocuous or "a prelude to multistep leukaemogenesis." (*Nature* **467**, 318–322, 2010) *KA*