METHODS IN BRIEF

BIOPHYSICS

Watching proteins in motion

Proteins are not static entities; rather, their structures constantly fluctuate between different conformational states. Detecting and understanding this dynamic motion is crucial to understanding how proteins function. Although nuclear magnetic resonance (NMR) spectroscopy has provided valuable insights into local molecular dynamics, no method so far has been able to detect concerted motion or motion through space. Vögeli *et al.* now show that it is possible to use exact nuclear Overhauser enhancement (NOE) rates to determine ensemble-averaged distance restraints. NOE measurements, which reflect through-space spin-spin coupling between NMR-active nuclei, are commonly applied in NMR-based three-dimensional structure determination of proteins. Vögeli *et al.* now apply their method for calculating exact NOE rates for ensemble-based structure determination to the model protein GB3, obtaining both its structure and a description of its dynamics.

Vögeli, B. *et al. Nat. Struct. Mol. Biol.* **19**, 1053–1057 (2012).

STEM CELLS

Single-cell analysis of reprogramming

Studies of gene expression during cellular reprogramming to pluripotency typically examine populations of cells. But reprogramming is an inefficient and heterogeneous process: only a small fraction of cells are fully reprogrammed to stable induced pluripotent stem cells (iPSCs). Analysis of gene expression in single cells is likely to be required to fully understand the sequence of events during reprogramming. Using their previously established secondary mouse reprogramming system, Buganim *et al.* monitored expression of 48 genes (selected because they are involved in epigenetic regulation, cell cycle control and maintenance of the pluripotent state) in single cells at different stages of the reprogramming process. They identified transcripts that are predictive of the formation of stable iPSCs and thus propose earlier stochastic and later hierarchical phases of the reprogramming process.

Buganim, Y. *et al. Cell* **150**, 1209–1222 (2012).

SYNTHETIC BIOLOGY

Many genes, one starting pistol

Synthetic biology projects often involve wiring a number of genes together, and tight control is needed for the system to function reliably. In theory, the genes can be controlled identically by using the same regulatory element, but differing genomic contexts and interactions between regulatory proteins at promoters, ribosome-binding sites and untranslated regions introduce unpredictable variation. Qi *et al.* get around this by separating genes on the same operon with cleavable sequences. The sequences are derived from the clustered regularly interspaced short palindromic repeat (CRISPR) pathway and only require expression of an inducible endonuclease to eliminate interactions by cutting the expressed operon into discrete transcripts. The researchers show that programmable RNA processing yields predictable and tightly controlled protein expression in complex circuits in bacteria and yeast.

Qi, L. *et al. Nat. Biotechnol.* **30**, 1002–1006 (2012).

SINGLE MOLECULE

Soft bacterial chromosomes

The single, circular chromosome of *Escherichia coli* occupies only a fraction of the space in a bacterial cell. As it segregates during cell division, the chromosome's morphology changes, but to date little is known about the micromechanical properties of the chromosome. Pelletier *et al.* now present a combination of microfluidics, imaging and single-molecule manipulation tools to answer questions such as "How much force is needed to keep the chromosome in its compacted state *in vivo*?" The researchers extracted the chromosome into a microchannel the width of a bacterial cell and visualized the expansion dynamics with mCherry directed against a histone-like associated protein. Using optical-trap micropistons, they then measured the compression force needed to fold the chromosome back to its *in vivo* size. Surprisingly, the force and free energy needed to compact the chromosome is an order of magnitude lower than the turgor pressure inside the cell.

Pelletier, J. *et al. Proc. Natl. Acad. Sci. USA* **109**, E2649–E2656 (2012).