

METHODS IN BRIEF

SYNTHETIC BIOLOGY

Tuning expression by numbers

When predicting the regulatory effect of a transcription factor (TF) in bacterial gene expression, many models assume the TF to be in excess of its binding sites on a promoter. But as Brewster *et al.* recently pointed out, this assumption is not always valid, particularly in cases where genes are expressed at high copy numbers or where many genes compete for a TF. The researchers describe a simple regulatory circuit in which a TF represses a reporter to measure the relationship between TF and various reporter copy numbers in the absence or presence of competing TF-binding sites. The resulting model will help fine-tune the output of a genetic circuit without requiring modification of the regulated gene.

Brewster, R.C. *et al. Cell* **156**, 1312–1323 (2014).

MICROSCOPY

Live-cell automated super-resolution microscopy

Following dynamic processes in living cells with photoactivated localization microscopy is extremely challenging. First, there is a limited pool of labeled molecules that is increasingly depleted during each localization step. Second, high irradiation intensities can cause phototoxicity during time-lapse imaging. Holden *et al.* overcome these challenges by performing automated photoactivated localization microscopy on different cells in synchronized populations of dividing bacteria. A μ Manager software module automatically adjusts the intensity of the activation laser to maintain appropriate densities of activated single molecules in an imaging system designed to minimize drift. An external phase-contrast system allows automated bacteria identification and orientation of molecule localizations with the outline of the cell. The methodology permitted nanoscale observation of the role of the protein FtsZ in bacterial cell division and the identification of previously unseen processes.

Holden, S.J. *et al. Proc. Natl. Acad. Sci. USA* **111**, 4566–4571 (2014).

SEQUENCING

5-formylcytosine-seq at single-base resolution

Cytosine methylation is a dynamic form of epigenetic regulation, and demethylation occurs via intermediates such as 5-formylcytosine (5-fC) that may themselves play regulatory roles. The position of methylated cytosines (5-mC) in the genome can be inferred using bisulfite to convert unmodified cytosine to thymine during sequencing (5-fC is also read as thymine in the process). Booth *et al.* have developed reduced bisulfite sequencing (redBS-Seq), which uses sodium borohydride to specifically reduce 5-fC and thereby protect it from bisulfite conversion. redBS-Seq provides single-base resolution and may be more efficient than the related fCABs-Seq (chemically assisted bisulfite sequencing) method, making the genome-wide discovery of this rare mark potentially more practical. Booth *et al.* sequenced 5-fC in addition to 5-mC and another intermediate in mouse embryonic stem cell genomes.

Booth, M.J. *et al. Nat. Chem.* doi:10.1038/nchem.1893 (23 March 2014).

BIONANOTECHNOLOGY

Cell-produced functional biomaterials

Escherichia coli produce an extracellular amyloid material called curli that is involved in biofilm formation. The secreted major curli subunit, CsgA, self-assembles to form fibrils with great mechanical strength. Chen *et al.* harnessed control over this system to produce potentially useful functional materials using synthetic biology techniques. They engineered CsgA to contain a histidine tag and put it under the control of an anhydrotetracycline or acyl-homoserine lactone inducer-responsive riboregulator such that curli fibrils would be produced only in the presence of the cognate inducer. By tuning the delivery of the cognate inducers, they were able to generate curli fibrils that could be labeled with gold nanoparticles or quantum dots via the histidine tag. Such systems could be used to produce 'smart' biomaterials such as an environmentally responsive biofilm-based electrical switch.

Chen, A.Y. *et al. Nat. Mater.* doi:10.1038/nmat3912 (23 March 2014).