Postcards From The Edge

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7 January 2009

Combining two genomes in one cell: Stable cloning of the *Synechocystis* PCC6803 genome in the *Bacillus subtilis* 168 genome

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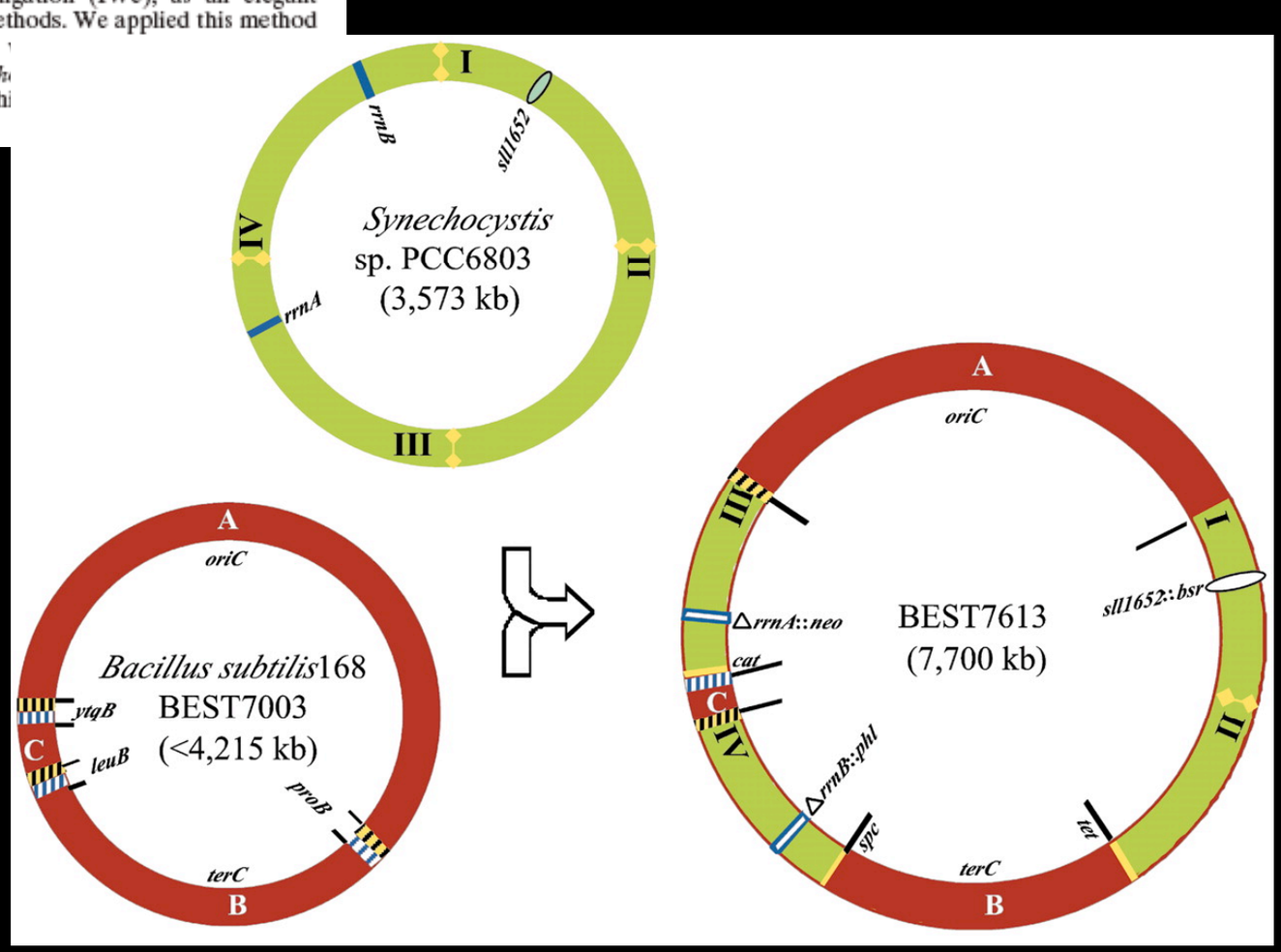
Edited by J. Craig Venter, The J. Craig Venter Institute, Rockville, MD, and approved September 16, 2005 (received for review May 10, 2005)

Cloning the whole 3.5-megabase (Mb) genome of the photosynthetic bacterium *Synechocystis* PCC6803 into the 4.2-Mb genome of the mesophilic bacterium *Bacillus subtilis* 168 resulted in a 7.7-Mb composite genome. We succeeded in such unprecedented large-size cloning by progressively assembling and editing contiguous DNA regions that cover the entire *Synechocystis* genome. The strain containing the two sets of genome grew only in the *B. subtilis* culture medium where all of the cloning procedures were carried out. The high structural stability of the cloned *Synechocystis* genome was closely associated with the symmetry of the bacterial genome structure of the DNA replication origin (*oriC*) and its termination (*terC*) and the exclusivity of *Synechocystis* ribosomal RNA operon genes (*rrnA* and *rrnB*). Given the significant diversity in genome structure observed upon horizontal DNA transfer in nature, our stable laboratory-generated composite genome raised fundamental questions concerning two complete genomes in one cell. Our megasize DNA cloning method, designated megacloning, may be generally applicable to other genomes or genome loci of free-living organisms.

and demonstrated the successful reconstruction of long contiguous DNAs (12–14). Our cloning principle took advantage of features inherent to this bacterium, i.e., the development of natural competence and the subsequent homologous recombination activity in the cytoplasm. Both features are induced because of their association with growth-phase transition (15, 16). The target DNA is guided in the BGM vector by simultaneous homologous recombination at two small flanking DNAs called landing pad sequences (LPS), integrated at the BGM cloning locus before cloning. The two LPS, ordered and oriented correctly, are termed the LPS array (LPA) (13, 14). Sliding the LPA results in elongation of the adjacent target DNA (Fig. 1). We offer such elongation-coupled cloning in the BGM vector, hereafter called inchworm elongation (IWe), as an elegant alternative to current cloning methods. We applied this method in the complete cloning of the
photosynthetic bacterium *Synech*
4.2-Mb genome of the mesophi
(Fig. 2).



Sciencexpress Research Article

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson, Gwynedd A. Benders, Cynthia Andrews-Pfannkoch, Evgeniya A. Denisova, Holly Baden-Tillson, Jayshree Zaveri, Timothy B. Stockwell, Anushka Brownley, David W. Thomas, Mikkel A. Algire, Chuck Merryman, Lei Young, Vladimir N. Noskov, John I. Glass, J. Craig Venter, Clyde A. Hutchison III, Hamilton O. Smith*

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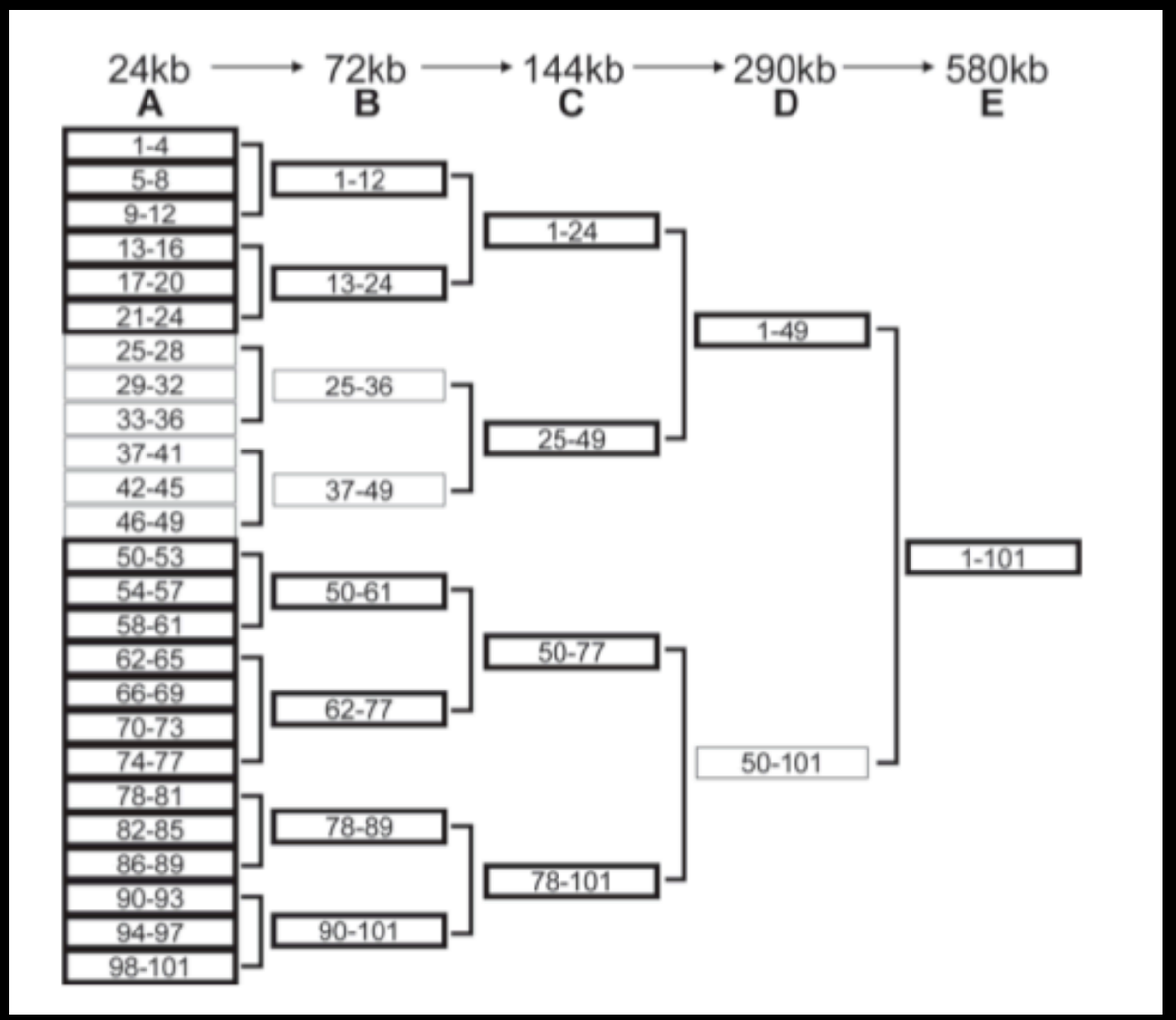
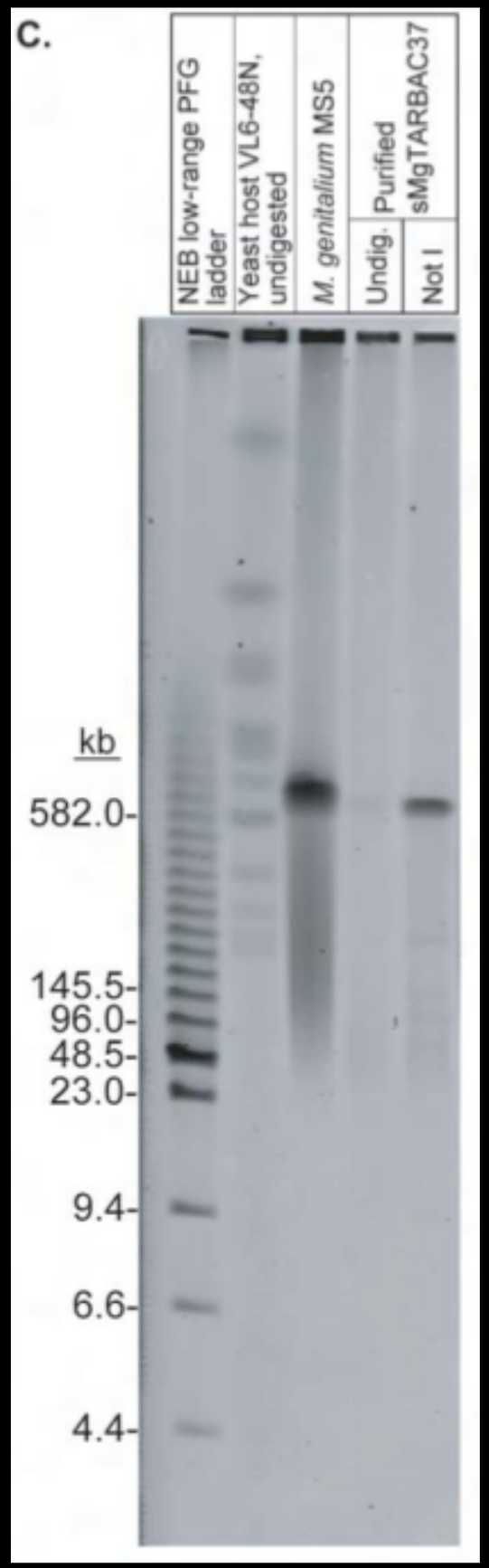
We have synthesized a 582,970 bp *Mycoplasma genitalium* genome. This synthetic genome, named *M. genitalium*

The actual synthesis and assembly of this genome presented a formidable technical challenge. Although





Sciencexpress Research Article

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium*

One-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic *Mycoplasma genitalium* genome

Daniel G. Gibson[a,1], Gwynedd A. Benders[b], Kevin C. Axelrod[a], Jayshree Zaveri[a], Mikkel A. Algire[a], Monzia Moodie[a], Michael G. Montague[a], J. Craig Venter[a], Hamilton O. Smith[b], and Clyde A. Hutchison, III[c]

[a]The J. Craig Venter Institute, Synthetic Biology Group, Rockville, MD 20850 and [b]The J. Craig Venter Institute, Synthetic Biology Group, San Diego, CA 92121

Contributed by Clyde A. Hutchison III, October 30, 2008 (sent for review September 11, 2008)

We previously reported assembly and cloning of the synthetic *Mycoplasma genitalium* JCVI-1.0 genome in the yeast *Saccharomyces cerevisiae* by recombination of six overlapping DNA fragments to produce a 592-kb circle. Here we extend this approach by demonstrating assembly of the synthetic genome from 25 overlapping fragments in a single step. The use of yeast recombination greatly simplifies the assembly of large DNA molecules from both synthetic and natural fragments.

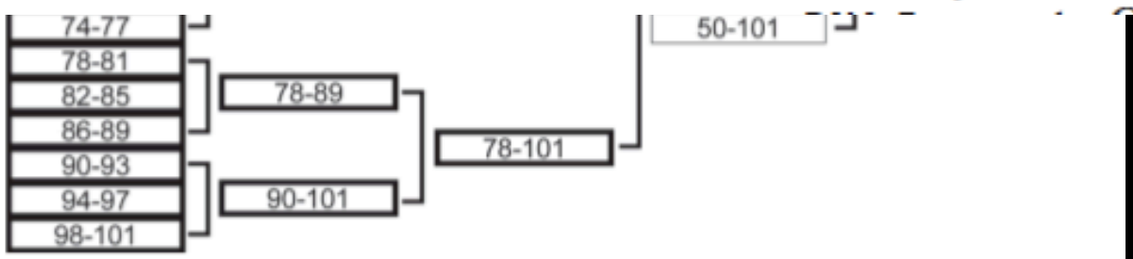
in vivo DNA assembly | genome synthesis | combinatorial assembly | yeast transformation | *Mycoplasma genitalium* | synthetic biology

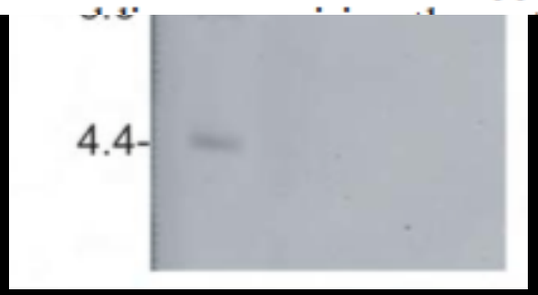
JCVI-1.0 genome. At the time, we wondered whether it would be possible to assemble DNA fragments from earlier stages into a complete genome in a single step in yeast.

The assembly intermediates from our construction of the synthetic JCVI-1.0 genome provided a resource for exploring the limits of yeast uptake and assembly. Here we report the successful assembly of the entire synthetic genome from 25 A-series assemblies in a single step.

Results

Assembly of the *M. genitalium* Genome in Yeast from 25 Overlapping





doi:10.1016/j.jmb.2005.10.076 J. Mol. Biol. (2006) 355, 619–627

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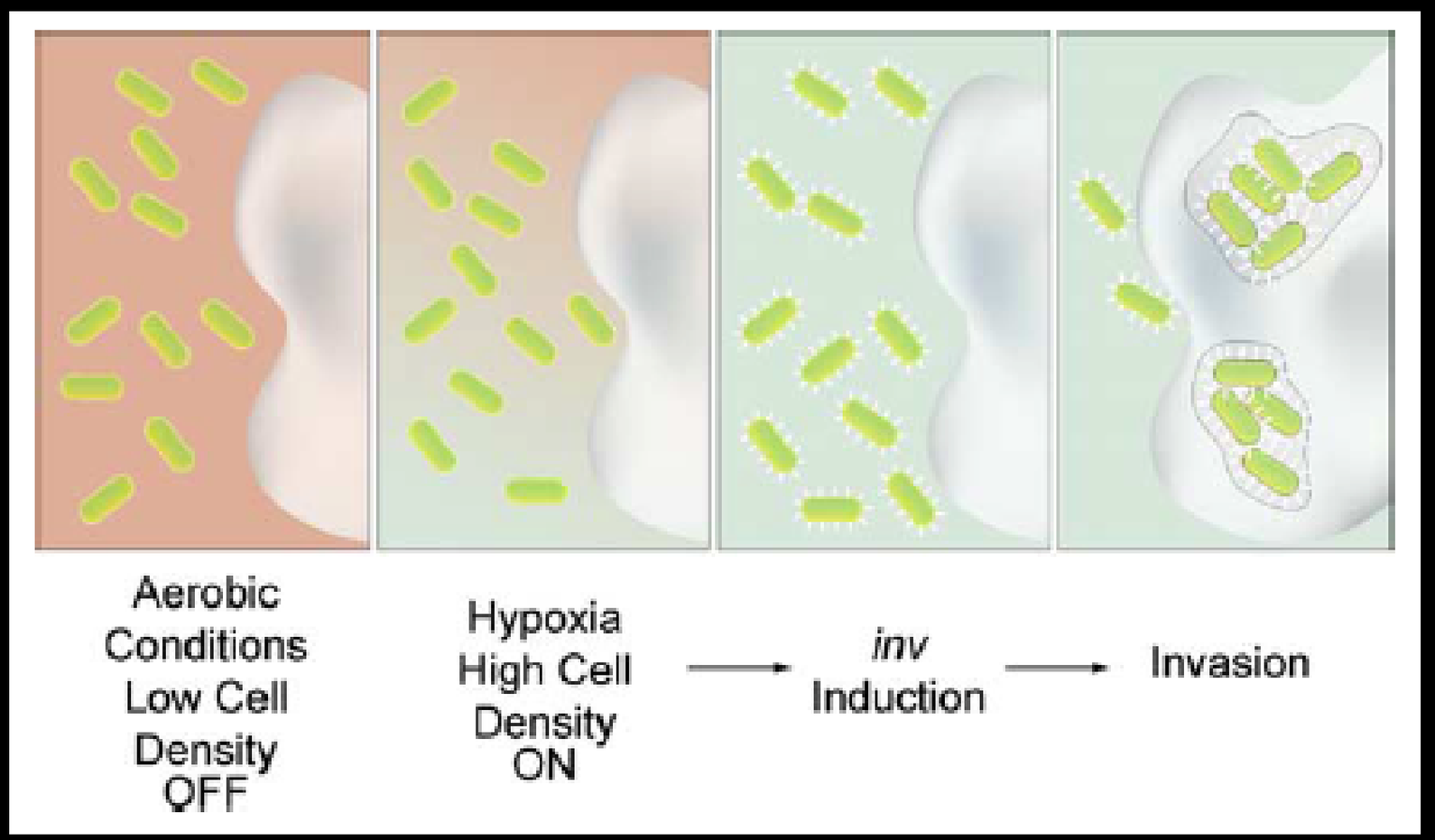
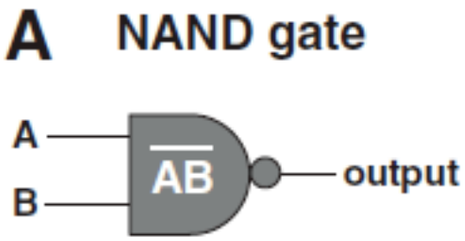
Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria

J. Christopher Anderson[1,3], Elizabeth J. Clarke[3], Adam P. Arkin[1,2*] and Christopher A. Voigt[2,3]

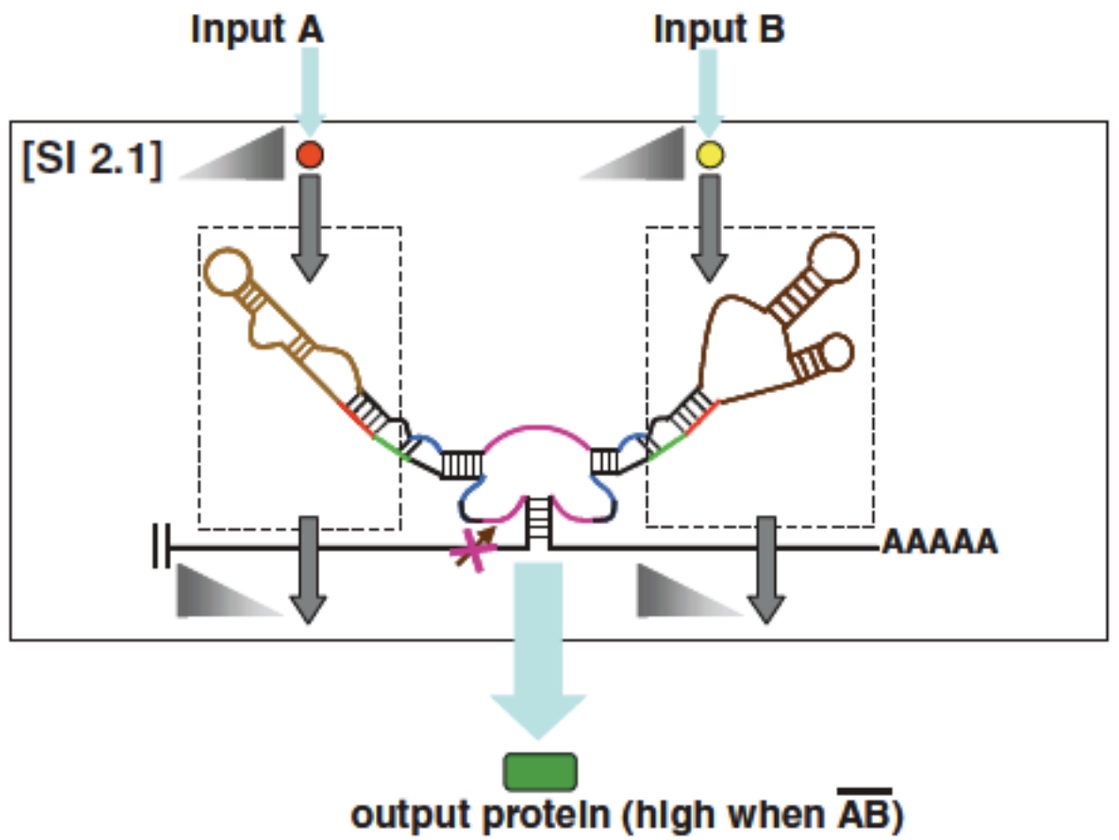


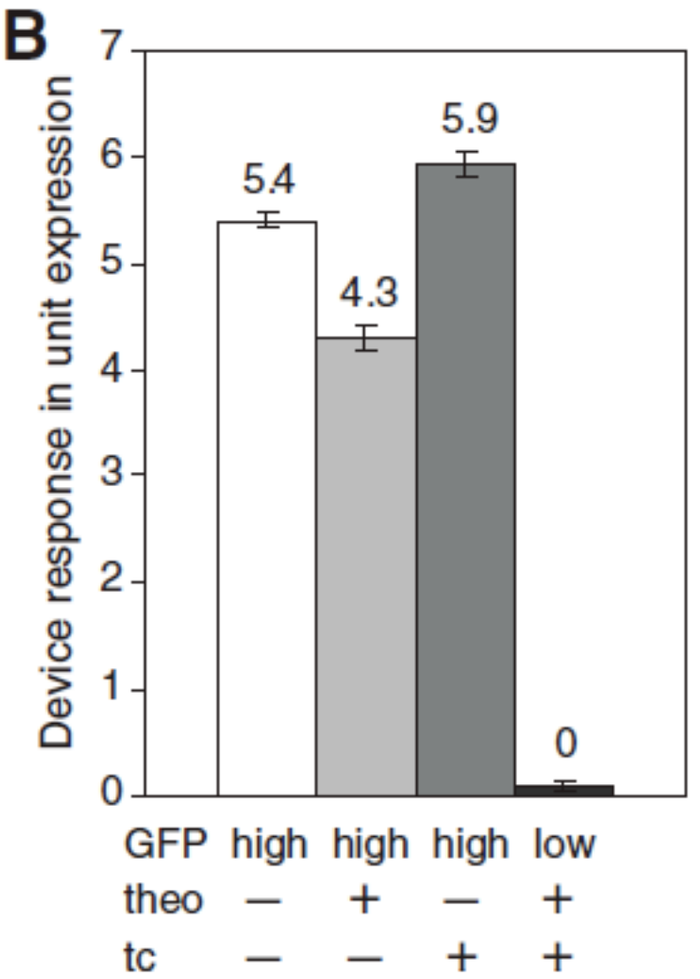
Higher-Order Cellular Information Processing with Synthetic RNA Devices

Maung Nyan Win and Christina D. Smolke*



A theo	B tc	output GFP
0	0	1
0	1	1
1	0	1
1	1	0

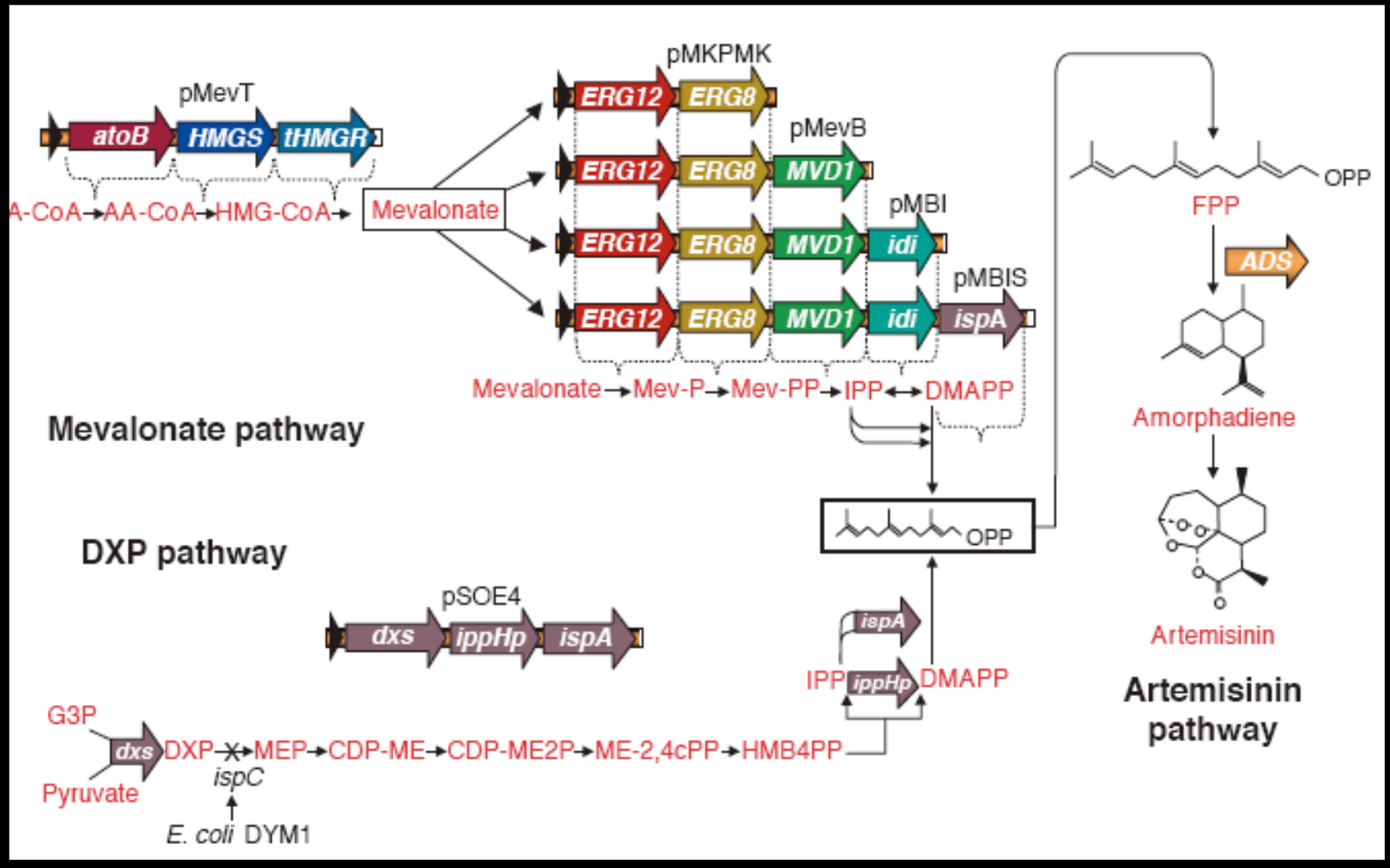




Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids

Vincent JJ Martin[1,2,3], Douglas J Pitera[1,3], Sydnor T Withers[1], Jack D Newman[1] & Jay D Keasling[1]

Isoprenoids are the most numerous and structurally diverse family of natural products. Terpenoids, a class of isoprenoids often isolated from plants, are used as commercial flavor and fragrance compounds and antimalarial or anticancer drugs. Because plant tissue extractions typically yield low terpenoid concentrations, we sought an alternative method to produce high-value terpenoid compounds, such as the antimalarial drug artemisinin, in a microbial host. We engineered the expression of a synthetic amorpha-4,11-diene synthase gene and the mevalonate isoprenoid pathway from *Saccharomyces cerevisiae* in *Escherichia coli*. Concentrations of amorphadiene, the sesquiterpene olefin precursor to artemisinin, reached 24 μg caryophyllene equivalent/ml. Because isopentenyl and dimethylallyl pyrophosphates are the universal precursors to all isoprenoids, the strains developed in this study can serve as platform hosts for the production of any terpenoid compound for which a terpene synthase gene is available.



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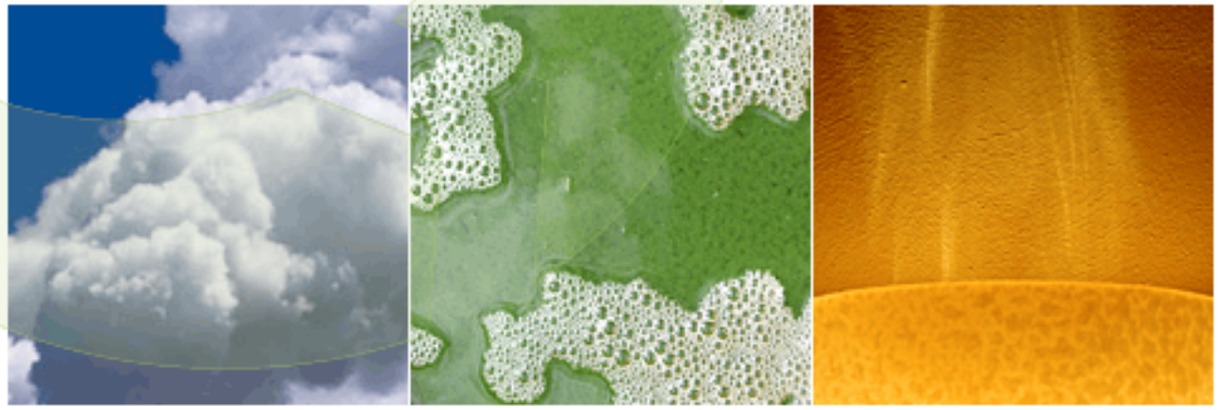

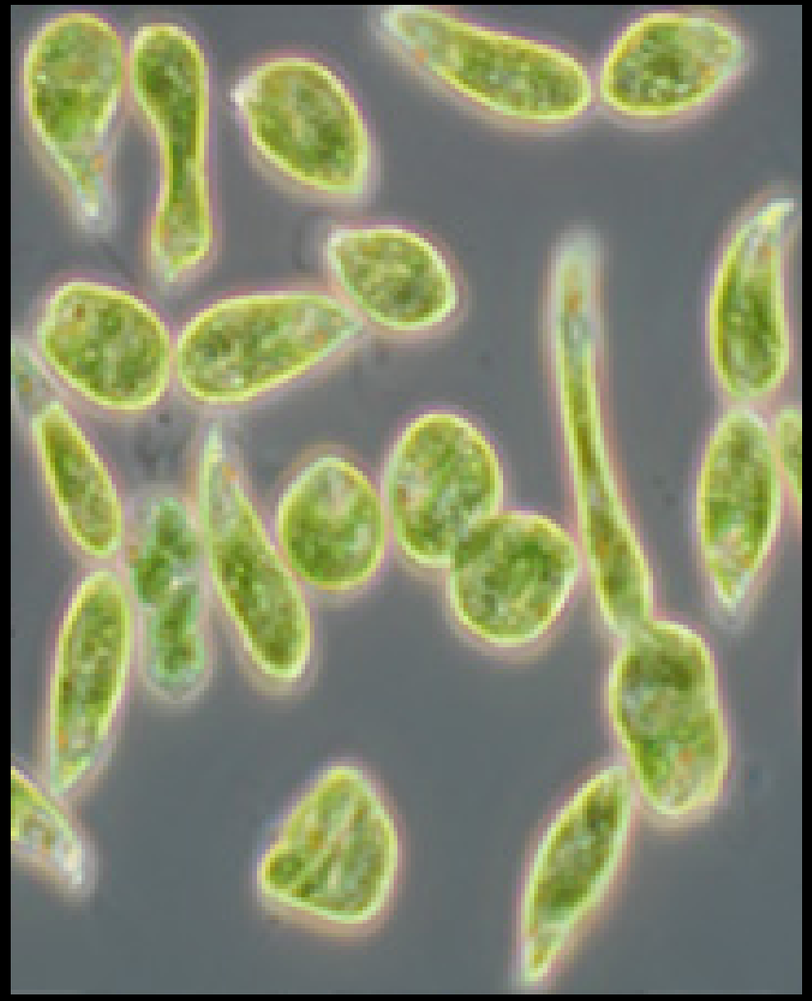

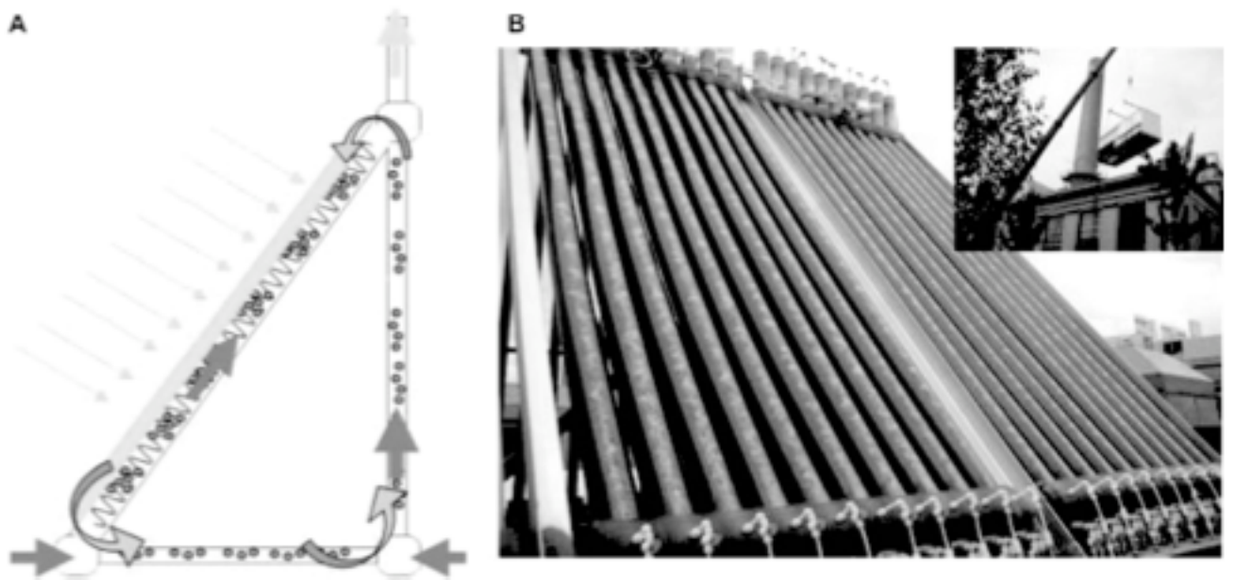

Figure 8. Inclined-tube ALR configuration: (A) schematic presentation of one ALR "triangle". Solid arrows indicate the direction of the gas flow, and open arrows indicate the direction of the liquid flow (B). An array of 30 ALRs, each with a volume of 30 L, with an algal culture grown on a flue gas. Inset: installation of the array of ALRs on the roof of MIT's Cogeneration Power Plant.

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REPORTS

Emergence of Novel Color Vision in Mice Engineered to Express a Human Cone Photopigment

Gerald H. Jacobs,[1*] Gary A. Williams,[1] Hugh Cahill,[2,3,4] Jeremy Nathans[2,3,4,5]

Changes in the genes encoding sensory receptor proteins are an essential step in the evolution of new sensory capacities. In primates, trichromatic color vision evolved after changes in X chromosome–linked photopigment genes. To model this process, we studied knock-in mice that expressed a human long-wavelength-sensitive (L) cone photopigment in the form of an X-linked polymorphism. Behavioral tests demonstrated that heterozygous females, whose retinas contained both native mouse pigments and human L pigment, showed enhanced long-wavelength sensitivity and acquired a new capacity for chromatic discrimination. An inherent plasticity in the mammalian visual system thus permits the emergence of a new dimension of sensory experience based solely on gene-driven changes in receptor organization.

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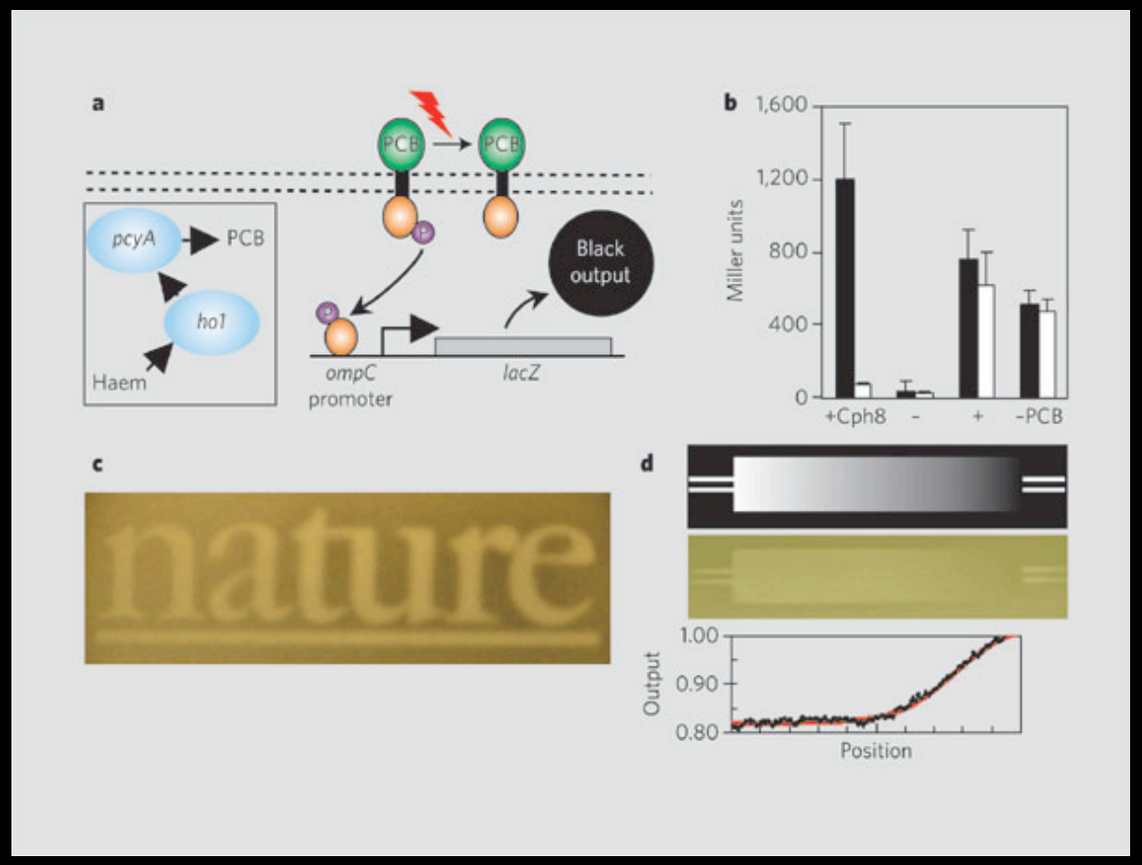
Vol 438 | 24 November 2005 nature

BRIEF COMMUNICATIONS

Engineering *Escherichia coli* to see light

These smart bacteria 'photograph' a light pattern as a high-definition chemical image.

Anselm Levskaya*, Aaron A. Chevalier†, Jeffrey J. Tabor†, Zachary Booth Simpson†, Laura A. Lavery†, Matthew Levy†, Eric A. Davidson†, Alexander Scouras†, Andrew D. Ellington†‡, Edward M. Marcotte†‡, Christopher A. Voigt*§||



A synthetic multicellular system for programmed pattern formation

Subhayu Basu[1], **Yoram Gerchman**[1], **Cynthia H. Collins**[3], **Frances H. Arnold**[3] **& Ron Weiss**[1,2]

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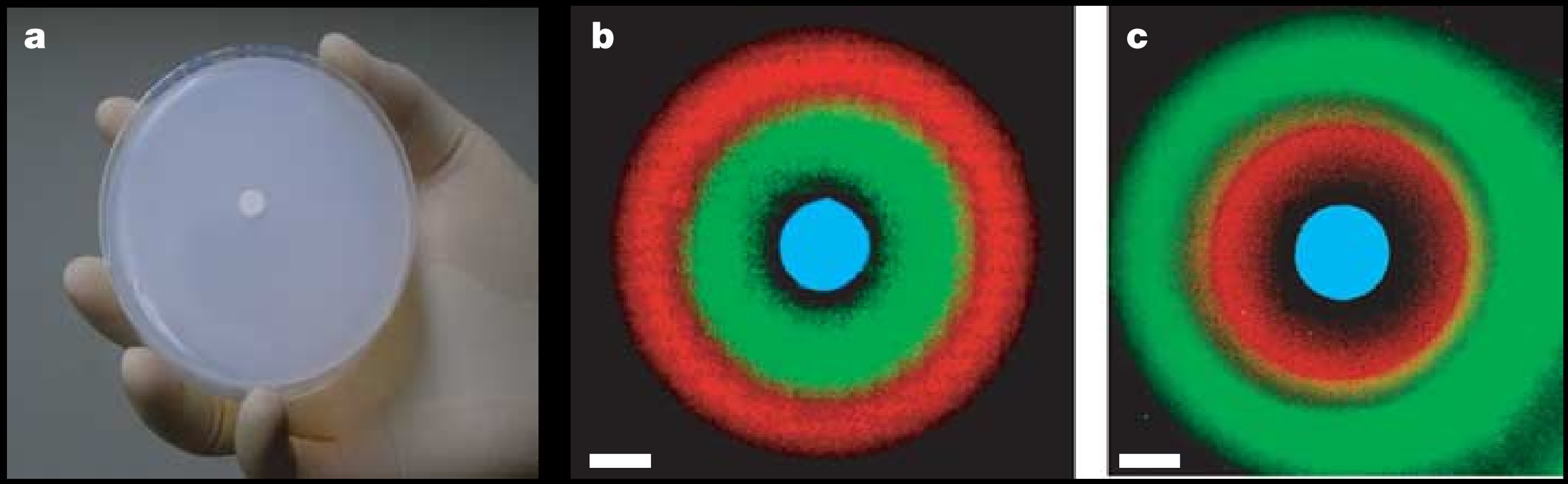
[3]*Division of Chemistry and Chemical Engineering, California Institute of Technology 210-41, Pasadena, California 91125, USA*



Vol 440|16 March 2006|doi:10.1038/nature04586

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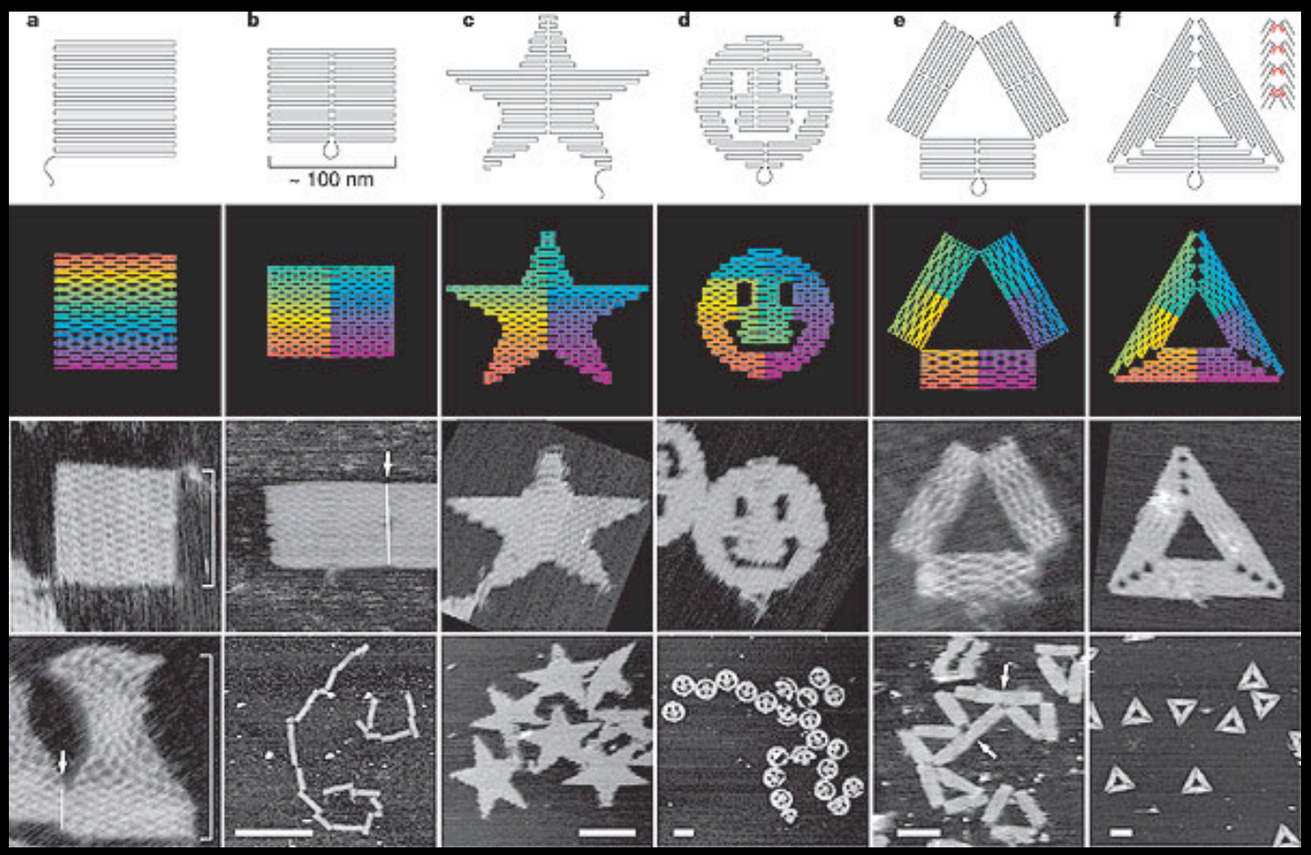
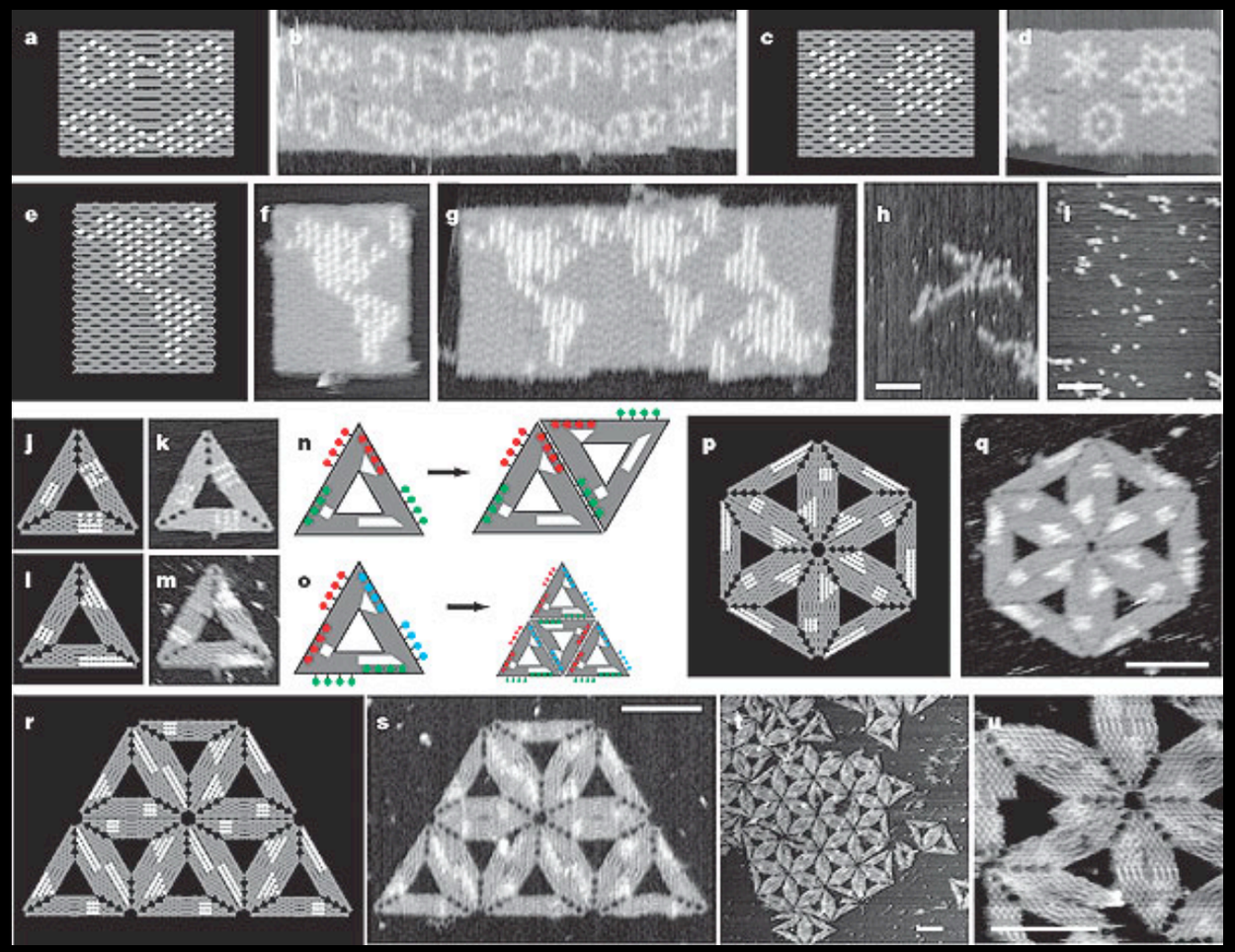
ARTICLES

Folding DNA to create nanoscale shapes and patterns

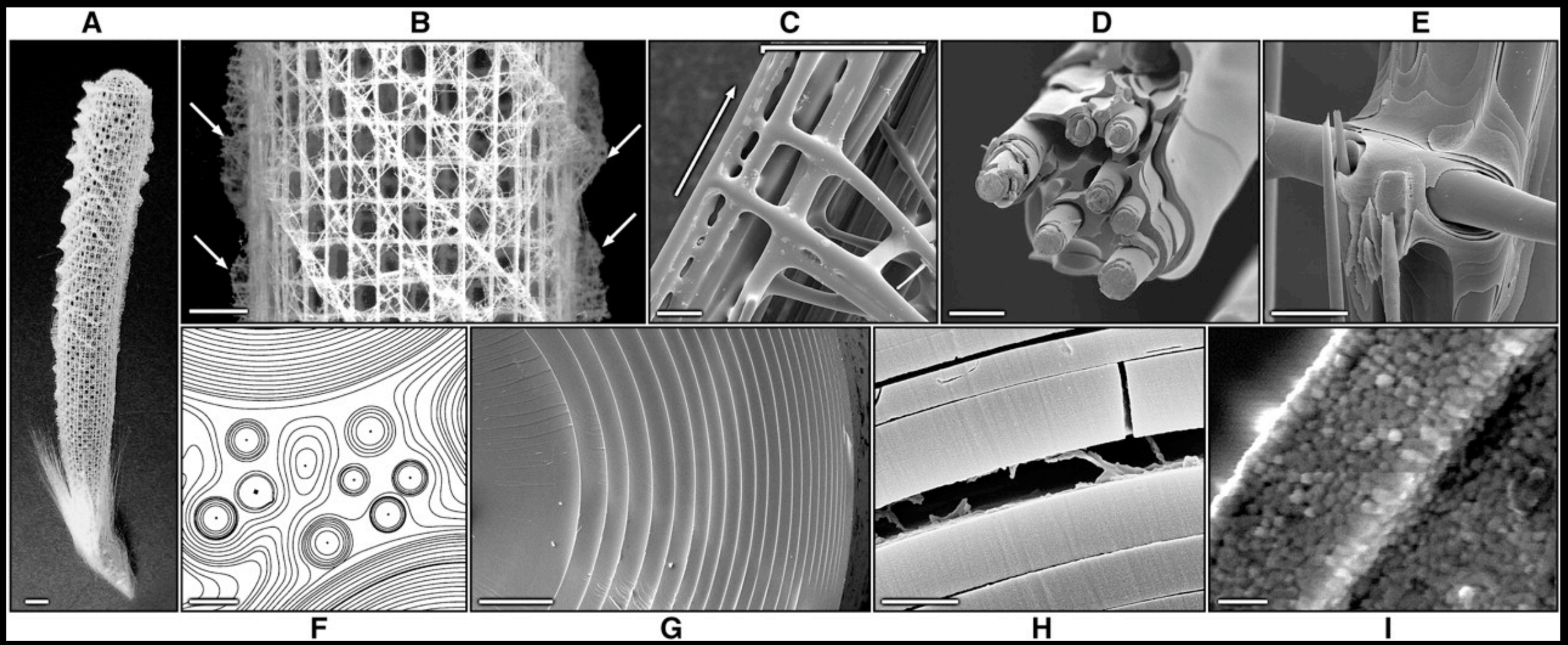
Paul W. K. Rothemund[1]





Skeleton of *Euplectella* sp.: Structural Hierarchy from the Nanoscale to the Macroscale

Joanna Aizenberg,[1*] James C. Weaver,[2] Monica S. Thanawala,[1] Vikram C. Sundar,[1] Daniel E. Morse,[2] Peter Fratzl[3]



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