‘Omics discussion in *Nature*

http://www.nature.com/news/big-biology-the-omes-puzzle-1.12484?WT.ec_id=NATURE-20130228

SYLICA 2013
Bowater lectures

Synthetic Biology & Nanotechnology: Tomorrow's Molecular Biology?

Bowater Lectures in Brno, Feb. 2013

4 lectures on linked topics will be delivered during the coming week:

- *Contemporary DNA Sequencing Technologies* – 26/2/2013 @ 10:00
- *Using 'Omic Technologies to Investigate Gene Function* – 26/2/2013 @ 14:00
- *Biophysical Methods to Study Molecular Interactions* – 27/2/2013 @ 10:00
- *Synthetic Biology & Nanotechnology: Tomorrow's Molecular Biology?* – 28/2/2013 @ 10:00

Nanotechnology & Synthetic Biology

- Presentation will discuss two overlapping topics:
  - Nanotechnology
  - Synthetic Biology (incorporating metabolic engineering and protein engineering)
- These are emerging disciplines, covering vast areas of science – not just biology!
- Here it is only possible to introduce the topics and provide some brief discussion of specific examples

- *To ensure that we are all clear where the discussion should start, it is useful to include some definitions….*

Nanotechnology

- Nanotechnology….
  - Literally defined as: Technology that is useful on the nanoscale – 1-100 nm (atom scale = 0.1 nm)
  - For biologists, this is more usefully defined as: manipulation of biological molecules/structures to produce useful materials or devices
- Biological molecules used in such technology must be stable for their required use e.g. uses of proteins will provide different opportunities to nucleic acids
- Requires collaboration of molecular biologists with experts in quantum physics, organic chemistry, surface science, computer science….etc.

Synthetic Biology

The combination of engineering with biology to engineer living things to create novel:
Fuels, Medicines , and Materials

The overall aims are to solve the Grand Challenges of the 21st Century

Explanation on Youtube: http://www.youtube.com/watch?v=rD5uNAMbDaQ

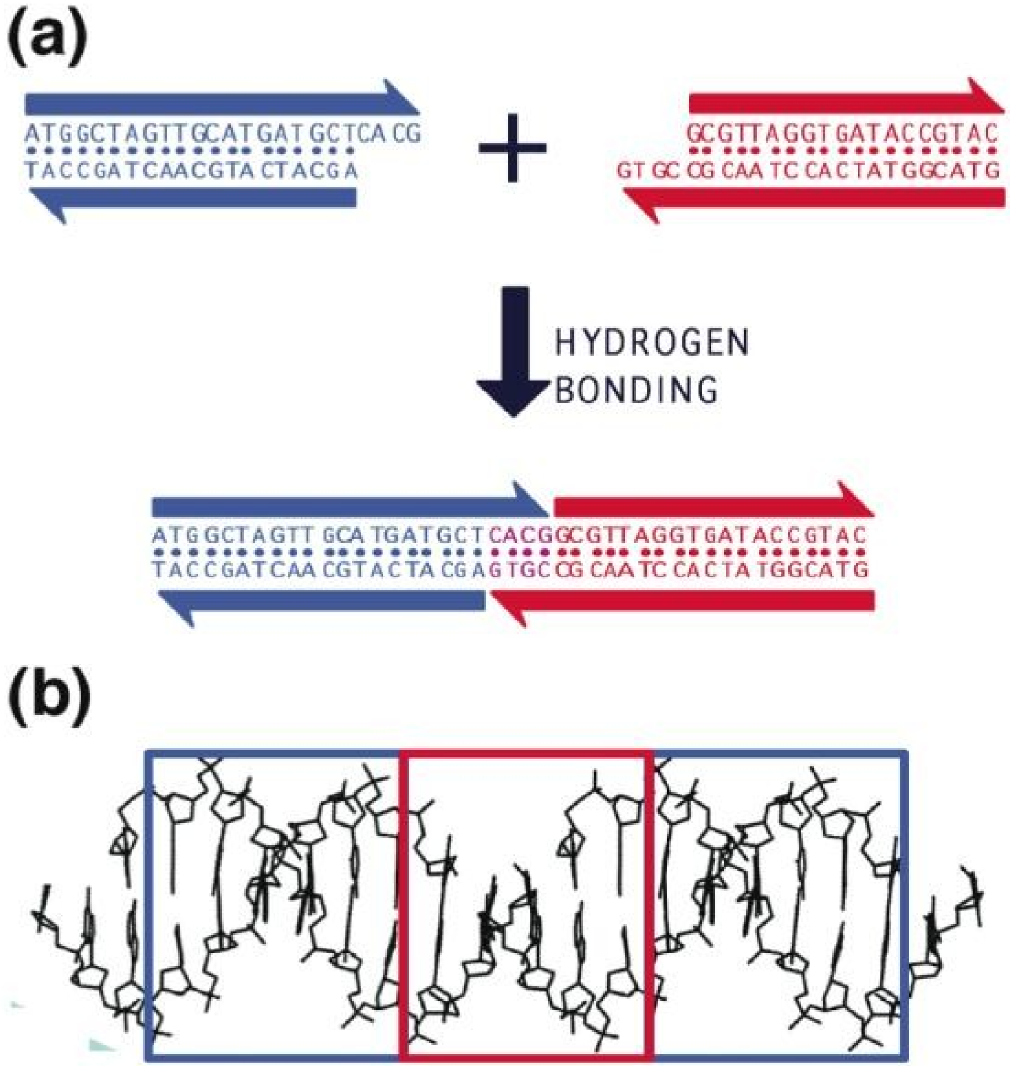
DNA Nanotechnology

- DNA is appropriate for nanotechnological methods for several reasons:
  - It is a (relatively) stable chemical, which exists in different forms (nucleotides, nucleic acids)
  - As a polymer it can form very long molecules
  - It has a well defined, repetitive structure
  - “Rules” for determining the structure are simple and well-understood
  - Within the molecule many atoms are available to form useful interactions/modifications

DNA Origami

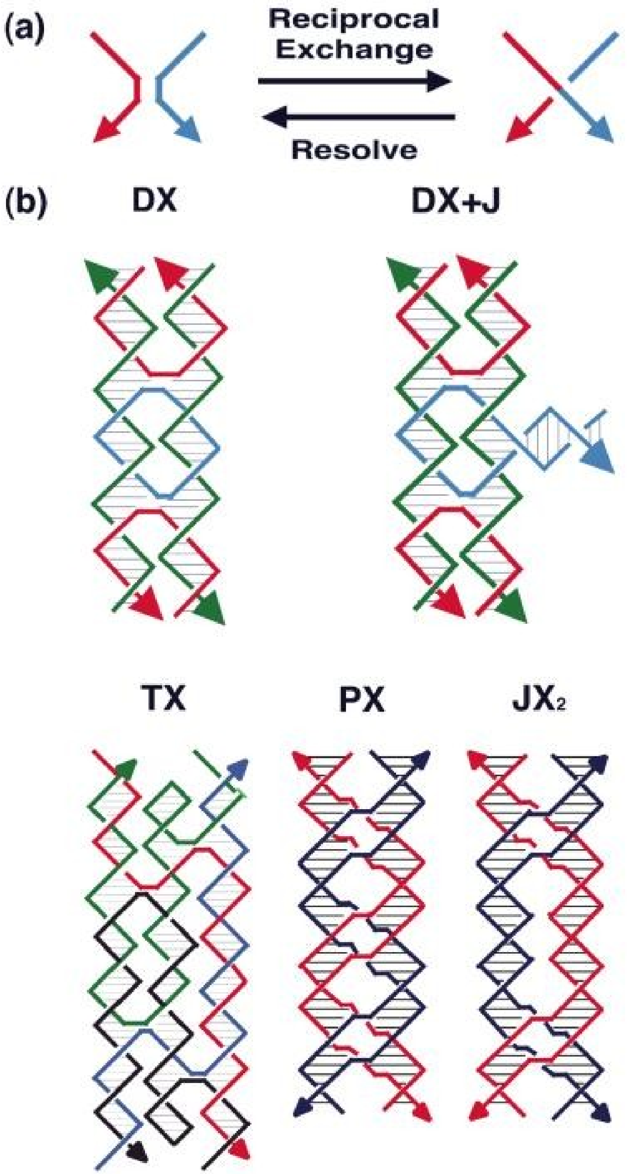
- During the 1980's, studies of DNA highlighted that complex structures could be formed
- Since these structures are stabilised by base pairs in the molecule, it became clear that the complex structures could be created using carefully-designed DNA sequences
- Importantly, the complex structures can be built up from simpler molecules

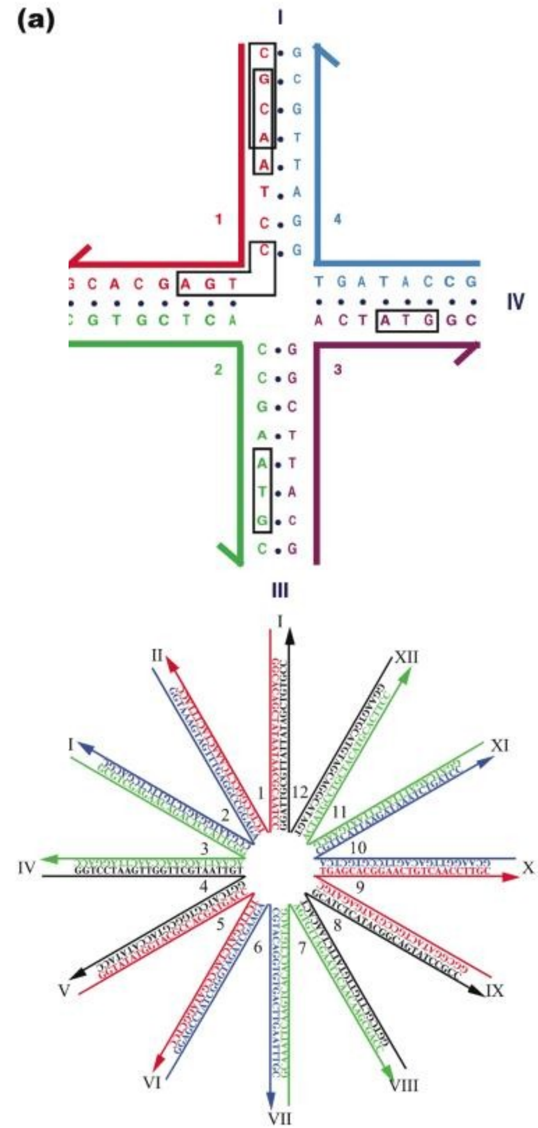
DNA Origami



Seeman, 2010, *Ann. Rev. Biochem.*, **79**, 65-87

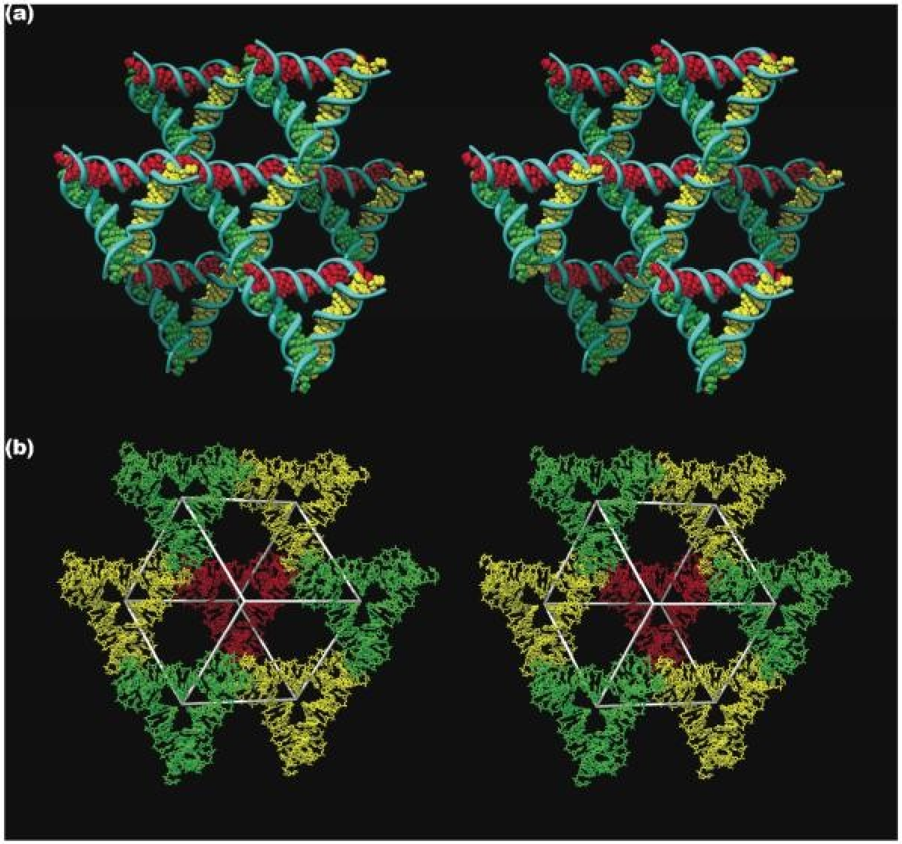
DNA Origami

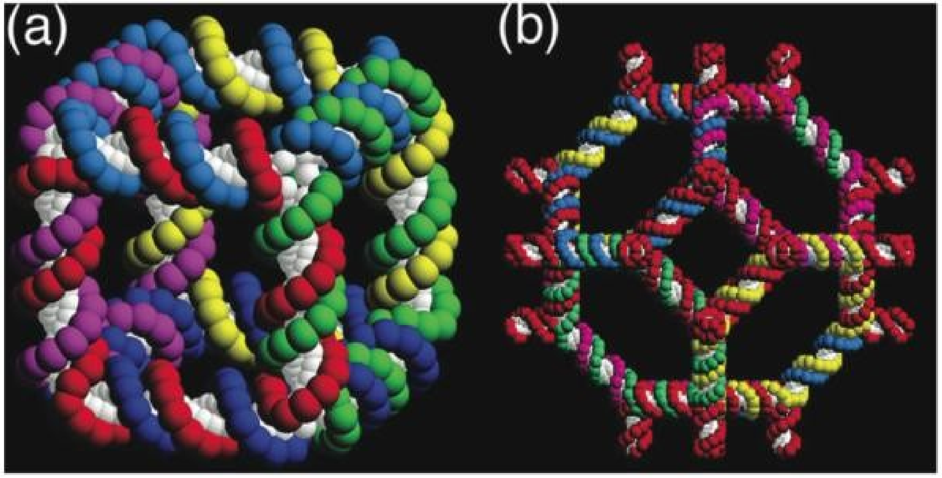




Seeman, 2010, *Ann. Rev. Biochem.*, **79**, 65-87

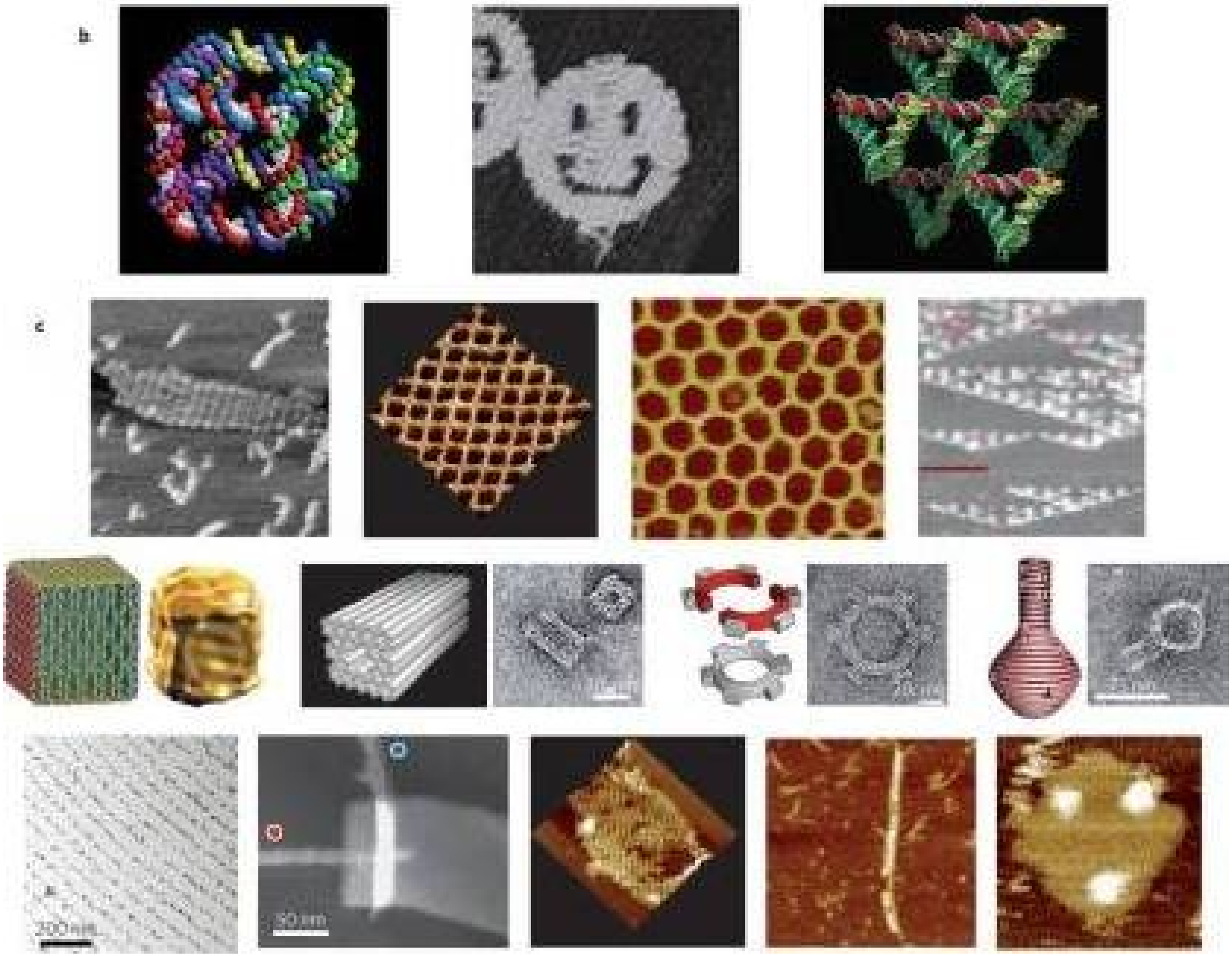
DNA Origami





Seeman, 2010, *Ann. Rev. Biochem.*, **79**, 65-87

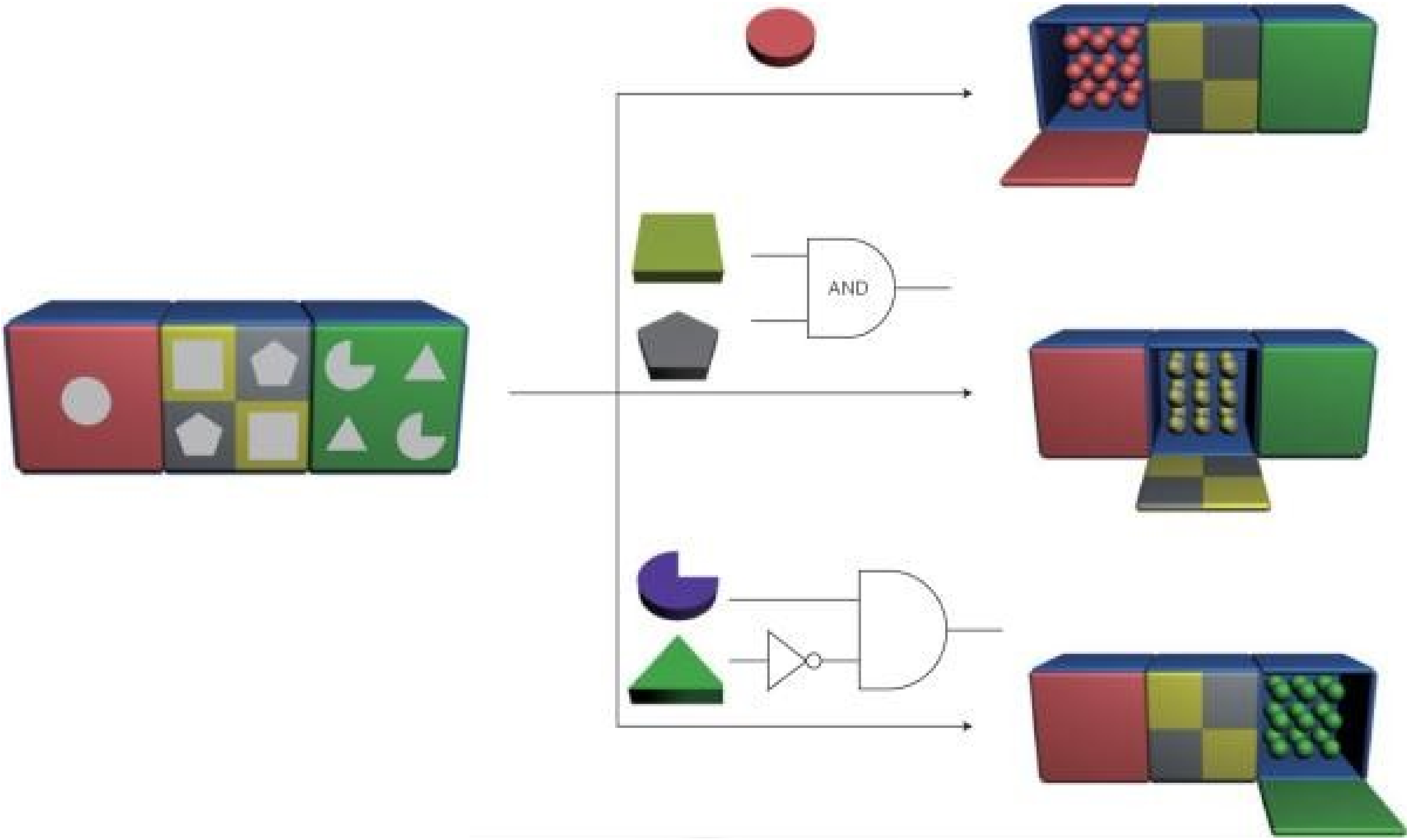
DNA Origami: Examples



Pinheiro *et al.*, 2011, *Nat. Nanotech.*, **6**, 763-772

Applications of DNA Nanotechnology

- Not just for creating beautiful pictures....



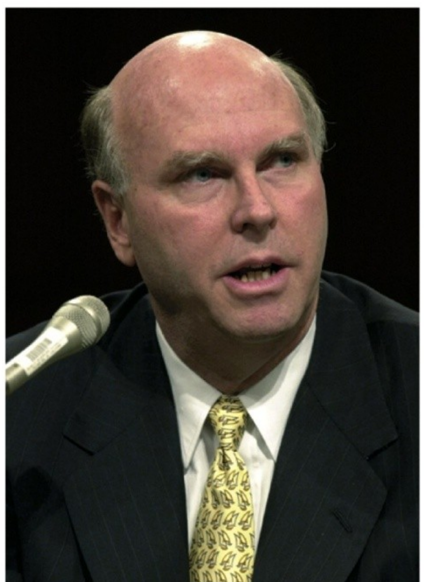
Pinheiro *et al.*, 2011, *Nat. Nanotech.*, **6**, 763-772

Synthetic Biology

General Definition:

A) the design and construction of new biological parts, devices, and systems, and

B) the re-design of existing, natural biological systems for useful purposes.



J. Craig Venter

Unnumbered 9 p322
Lehninger Principles of Biochemistry, Fifth Edition

Synthia: a Synthetic Bacterium

- This paper reported the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome
- The genome was chemically synthesised and transplanted into a *M. capricolum* recipient cell
- The new *M. mycoides* cells are controlled by the synthetic chromosome, which also includes “watermark” sequences, designed gene deletions and polymorphisms, and mutations acquired during the building process
- The new cells have expected phenotypic properties and are capable of continuous self-replication

Gibson *et al.*, 2010, *Science*, **329**, 52-56

Expression Systems

- Mos… …nant prot…
- Nee…
  - E…
  - S…
- Ma… …ins hav…

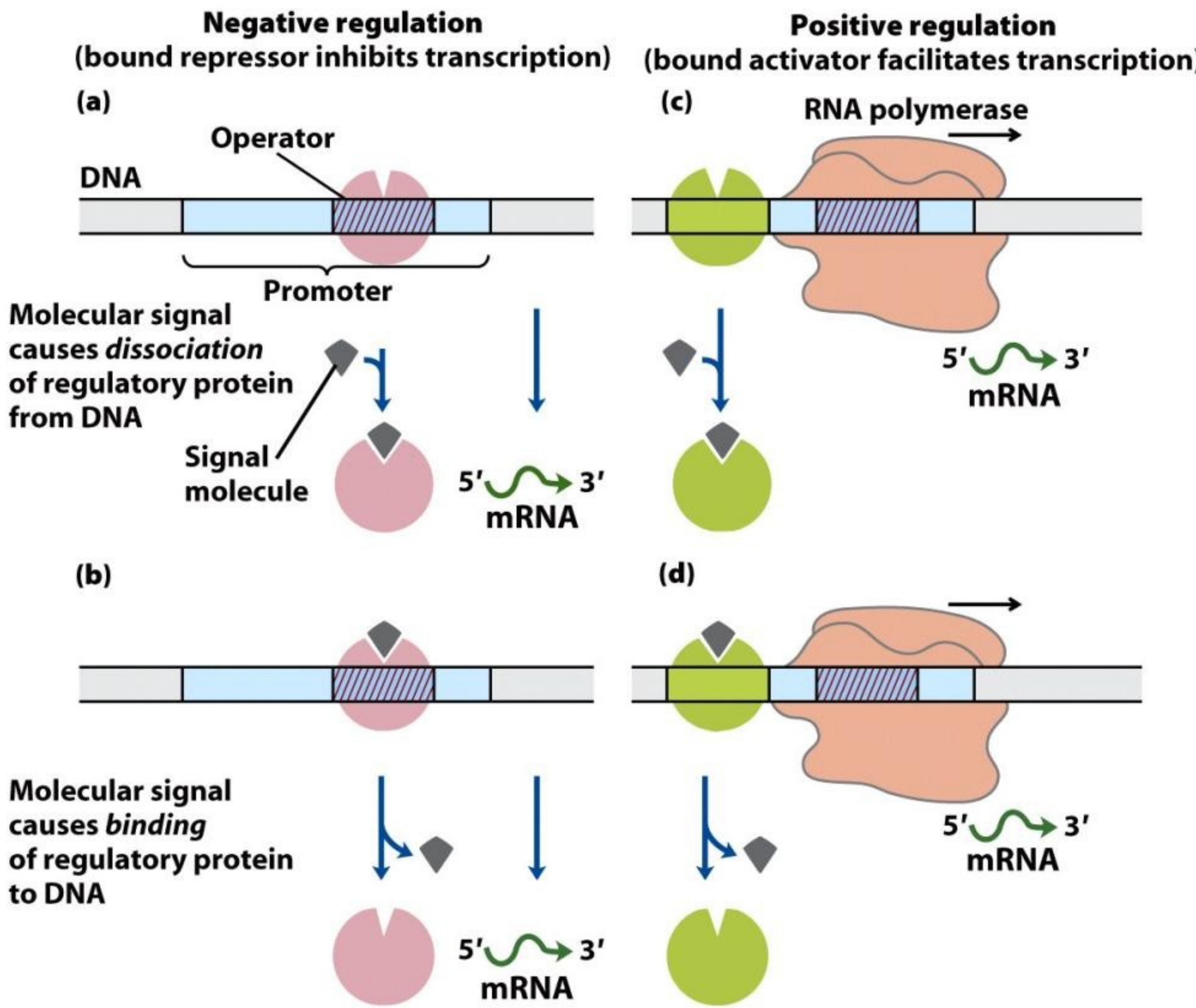
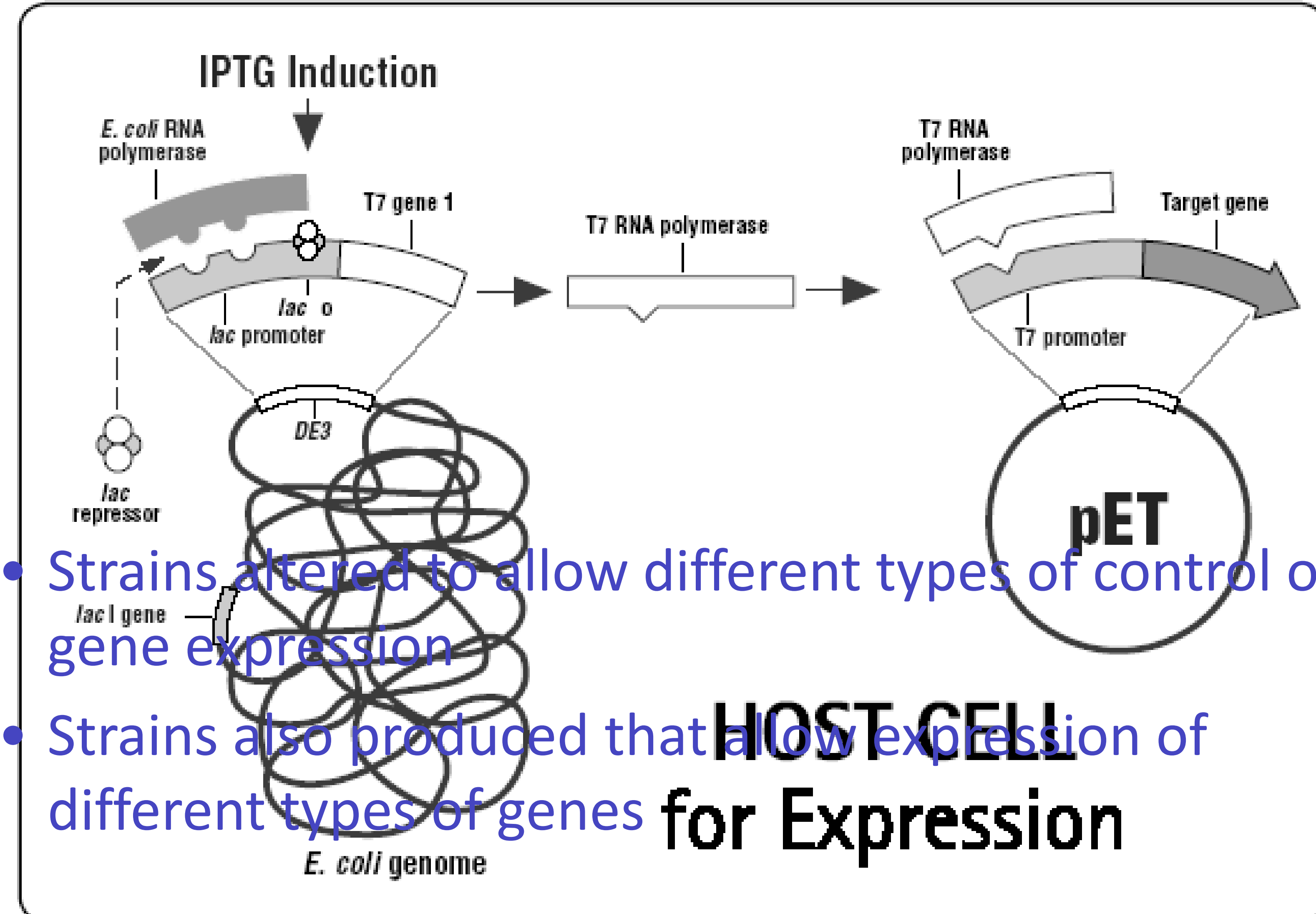


Figure 28-4
Lehninger Principles of Biochemistry, Fifth Edition

Bacterial Expression Strains



- Strains altered to allow different types of control of gene expression
- Strains also produced that allow expression of different types of genes

Novagen pET system manual, 11th ed

Codon Bias

Humans

1st \ 2nd	T	C	A	G
T	TTT 0.43 Phe TTC 0.57 Phe TTA 0.06 Leu TTG 0.12 Leu	TCT 0.18 Ser TCC 0.23 Ser TCA 0.15 Ser TCG 0.06 Ser	TAT 0.42 Tyr TAC 0.58 Tyr TAA 0.22 TERM TAG 0.17 TERM	TGT 0.42 Cys TGC 0.58 Cys TGA 0.61 TERM TGG 1.00 Trp
C	CTT 0.12 Leu CTC 0.20 Leu CTA 0.07 Leu CTG 0.43 Leu	CCT 0.29 Pro CCC 0.33 Pro CCA 0.27 Pro CCG 0.11 Pro	CAT 0.41 His CAC 0.59 His CAA 0.27 Gln CAG 0.73 Gln	CGT 0.09 Arg CGC 0.19 Arg CGA 0.10 Arg CGG 0.19 Arg
A	ATT 0.35 Ile ATC 0.52 Ile ATA 0.14 Ile ATG 1.00 Met	ACT 0.23 Thr ACC 0.38 Thr ACA 0.27 Thr ACG 0.12 Thr	AAT 0.44 Asn AAC 0.56 Asn AAA 0.40 Lys AAG 0.60 Lys	AGT 0.14 Ser AGC 0.25 Ser AGA 0.21 Arg AGG 0.22 Arg
G	GTT 0.17 Val GTC 0.25 Val GTA 0.10 Val GTG 0.48 Val	GCT 0.28 Ala GCC 0.40 Ala GCA 0.22 Ala GCG 0.10 Ala	GAT 0.44 Asp GAC 0.56 Asp GAA 0.41 Glu GAG 0.59 Glu	GGT 0.18 Gly GGC 0.33 Gly GGA 0.26 Gly GGG 0.23 Gly

http://www.kazusa.or.jp/java/codon_table_java/

Other Expression Strains

- *E. coli* expression systems are very powerful but sometimes have problems
- A better approach can be to try to express protein in native cell (or something similar)
  - Different types of bacteria
  - Yeast are widely used e.g. *Pichia pastoris*
  - Insect cells in culture
  - Mammalian cells in culture

Metabolic Engineering

- Metabolic Engineering – or "Biotransformations" – relates to use of biological catalysts to produce specific, desired products
- Usually enzymes, but can be whole organisms
- Industry uses this to produce food, pharmaceuticals, detergents, agricultural chemicals, etc.

Process Development

Basic Biotechnology, 2006, Ratledge & Kristiansen, 3rd edn, Fig. 24.1

Substrates

↓

Screening & selection of biocatalysts

↓

Genetic engineering → Biocatalyst production (fermentation/purification) ← Protein engineering

↓

Medium engineering

↓

Product concentration → Design & scale-up of bio-reactor ← Volumetric productivity

↓

Product isolation & purification

↓

Product ←

- *Quality*
- *Purity*
- *Scale*

Metabolic Engineering - Advantages

- Use of enzymes/organisms has number of possible advantages compared to what can be achieved by chemical industry:
  - Simpler
  - Less raw materials and energy
  - Higher quality products
  - Higher yields
  - Decrease toxic wastes and wastewater
  - Lower costs and environmentally friendly??

Compounds Produced by Commercial-scale Bioprocesses

- Alcohols
- Amino acids
- Antibiotics
- Polymers
  - Starch
  - Polyurethane
- Sweeteners
- Vitamins

Prokaryotes used in Biotransformations

- Wide range of prokaryotes used in biotransformations, including:
  - *Escherichia coli*: Gamma-proteobacteria; widely used in development processes, produce amino acids
  - *Mycobacterium* spp: Actinobacteria; various agricultural and medical compounds
  - *Rhodococcus rhodochrous*: produces acrylamide
  - *Streptomyces coelicolor*: Actinobacteria; antibiotics + wide range of other metabolites

Prokaryotes used in Biotransformations

- A number of diverse bacteria used as models for metabolic engineering
- Microbial genome sequences have revealed many examples of ‘cryptic’ or ‘orphan’ biosynthetic gene clusters
- Have potential to direct the production of novel, structurally complex natural products
- Synthetic biology will provide new mechanisms, roles and specificities for natural product biosynthetic enzymes

Tagged Proteins

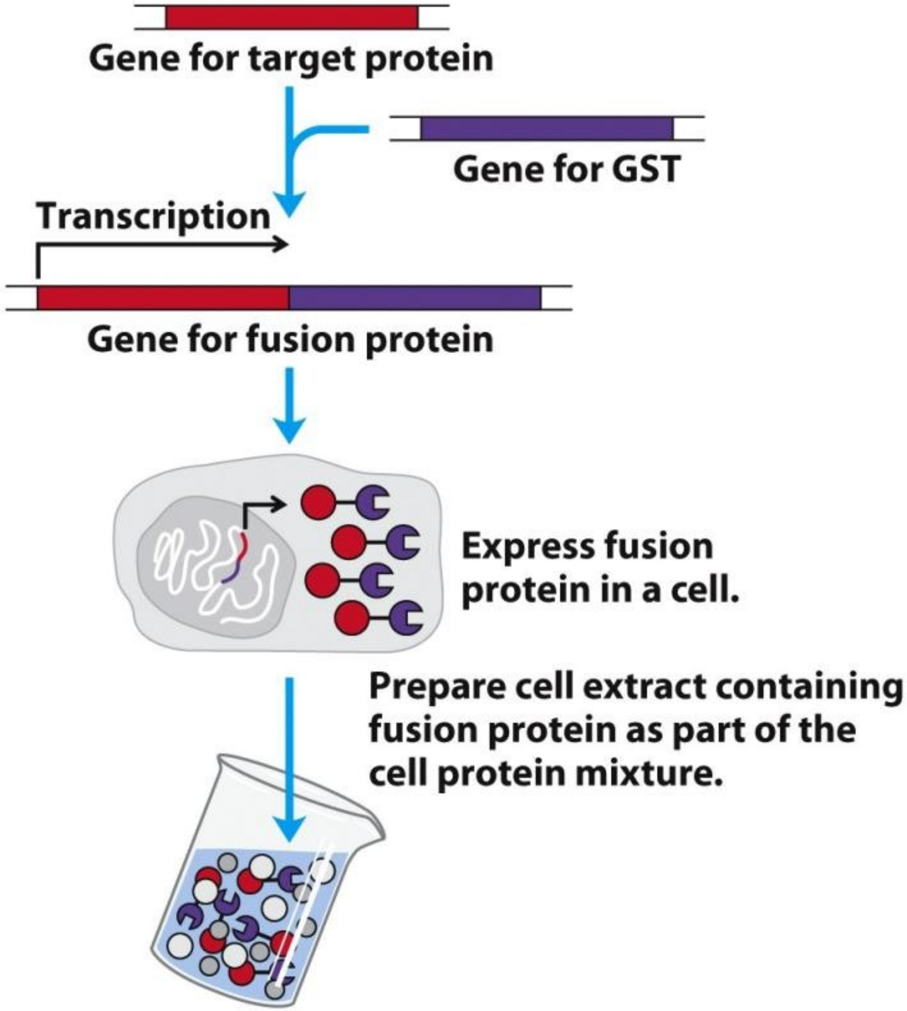


Figure 9-12b part 1
Lehninger Principles of Biochemistry, Fifth Edition

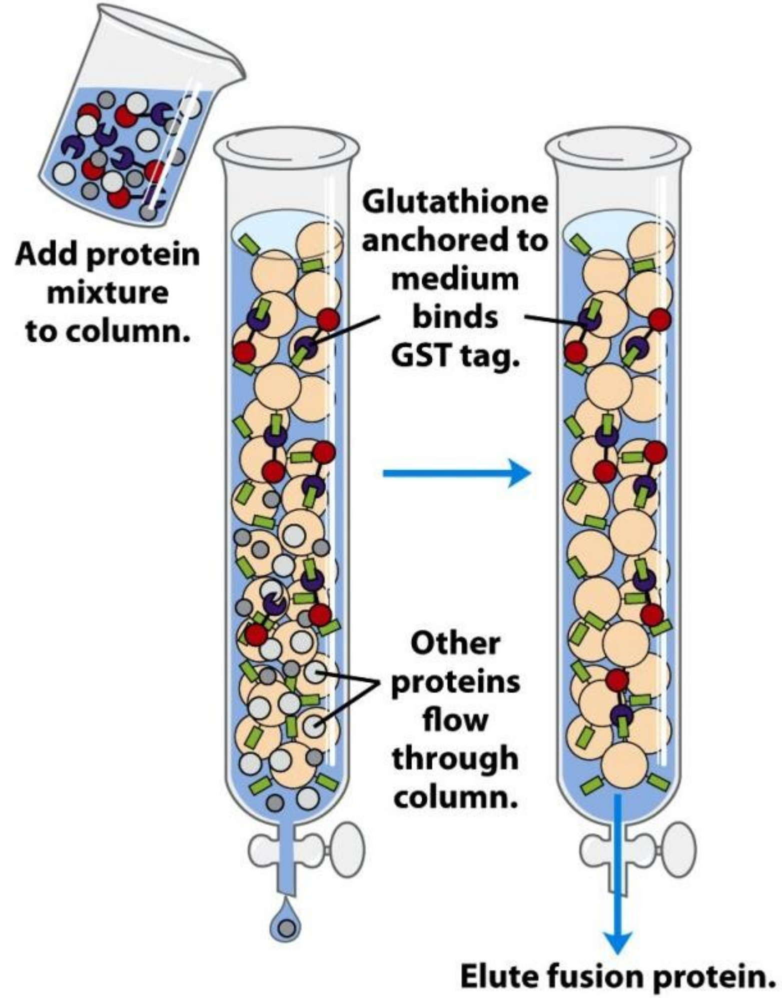


Figure 9-12b part 2
Lehninger Principles of Biochemistry, Fifth Edition

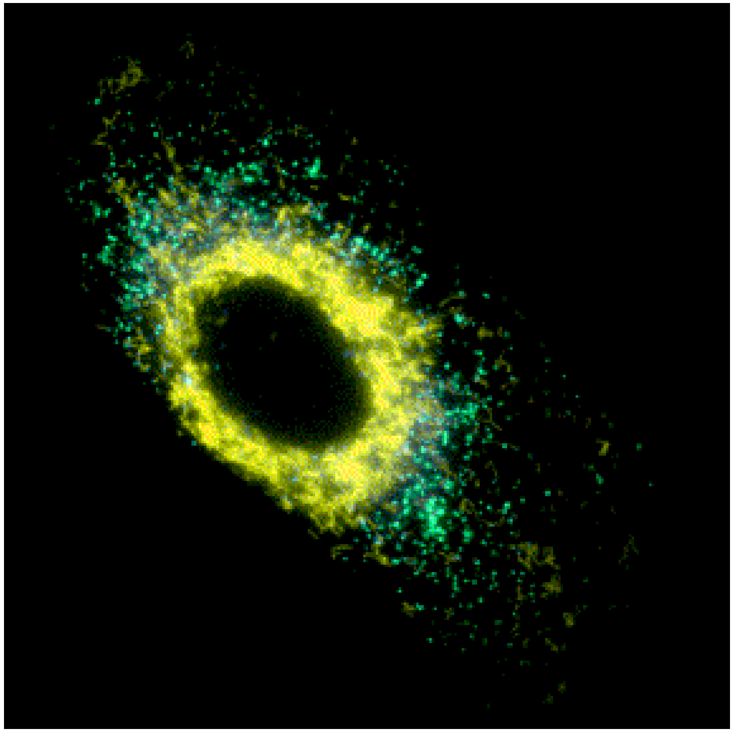
Different Types of Tags

TABLE 9–3 Commonly Used Protein Tags		
Tag protein/peptide	Molecular mass (kDa)	Immobilized ligand
Protein A	59	Fc portion of IgG
$(His)_6$	0.8	Ni^{2+}
Glutathione-*S*-transferase (GST)	26	Glutathione
Maltose-binding protein	41	Maltose
β-Galactosidase	116	*p*-Aminophenyl-*β*-D-thiogalactoside (TPEG)
Chitin-binding domain	5.7	Chitin

Table 9-3
Lehninger Principles of Biochemistry, Fifth Edition

Fluorescently-tagged Proteins

- Combination of molecular and cell biological studies analyse *in vivo* localisation of proteins expressed with a fluorescent "tag"
- Important that "tag" does not interfere with protein activity
- Can examine localisation of proteins containing different fluorophores



Bastiaens & Pepperkok (2000) *TiBS*, **25**, 631-637

GFP–Tagged Protein Localization

(a)

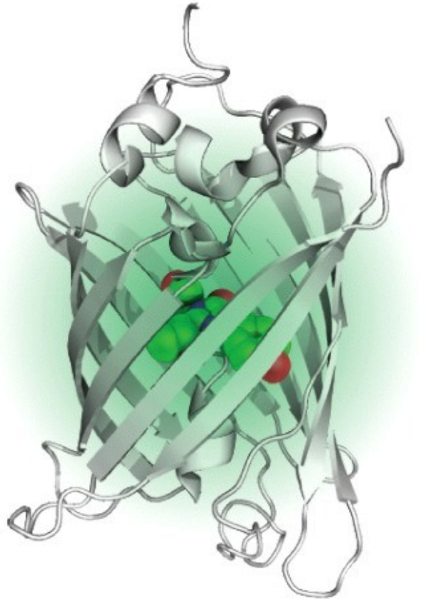


Figure 9-16
Lehninger Principles of Biochemistry, Sixth Edition

Biotechnological Applications

Figure 9-33
Lehninger Principles of Biochemistry, Fifth Edition

Medical Applications

TABLE 9–4	Some Recombinant DNA Products in Medicine
Product category	**Examples/uses**
Anticoagulants	Tissue plasminogen activator (TPA); activates plasmin, an enzyme involved in dissolving clots; effective in treating heart attack patients.
Blood factors	Factor VIII; promotes clotting; it is deficient in hemophiliacs; treatment with factor VIII produced by recombinant DNA technology eliminates infection risks associated with blood transfusions.
Colony-stimulating factors	Immune system growth factors that stimulate leukocyte production; treatment of immune deficiencies and infections.
Erythropoietin	Stimulates erythrocyte production; treatment of anemia in patients with kidney disease.
Growth factors	Stimulate differentiation and growth of various cell types; promote wound healing.
Human growth hormone	Treatment of dwarfism.
Human insulin	Treatment of diabetes.
Interferons	Interfere with viral reproduction; used to treat some cancers.
Interleukins	Activate and stimulate different classes of leukocytes; possible uses in treatment of wounds, HIV infection, cancer, and immune deficiencies.
Monoclonal antibodies	Extraordinary binding specificity is used in: diagnostic tests; targeted transport of drugs, toxins, or radioactive compounds to tumors as a cancer therapy; many other applications.
Superoxide dismutase	Prevents tissue damage from reactive oxygen species when tissues briefly deprived of O_2 during surgery suddenly have blood flow restored.
Vaccines	Proteins derived from viral coats are as effective in "priming" an immune system as is the killed virus more traditionally used for vaccines, and are safer; first developed was the vaccine for hepatitis B.

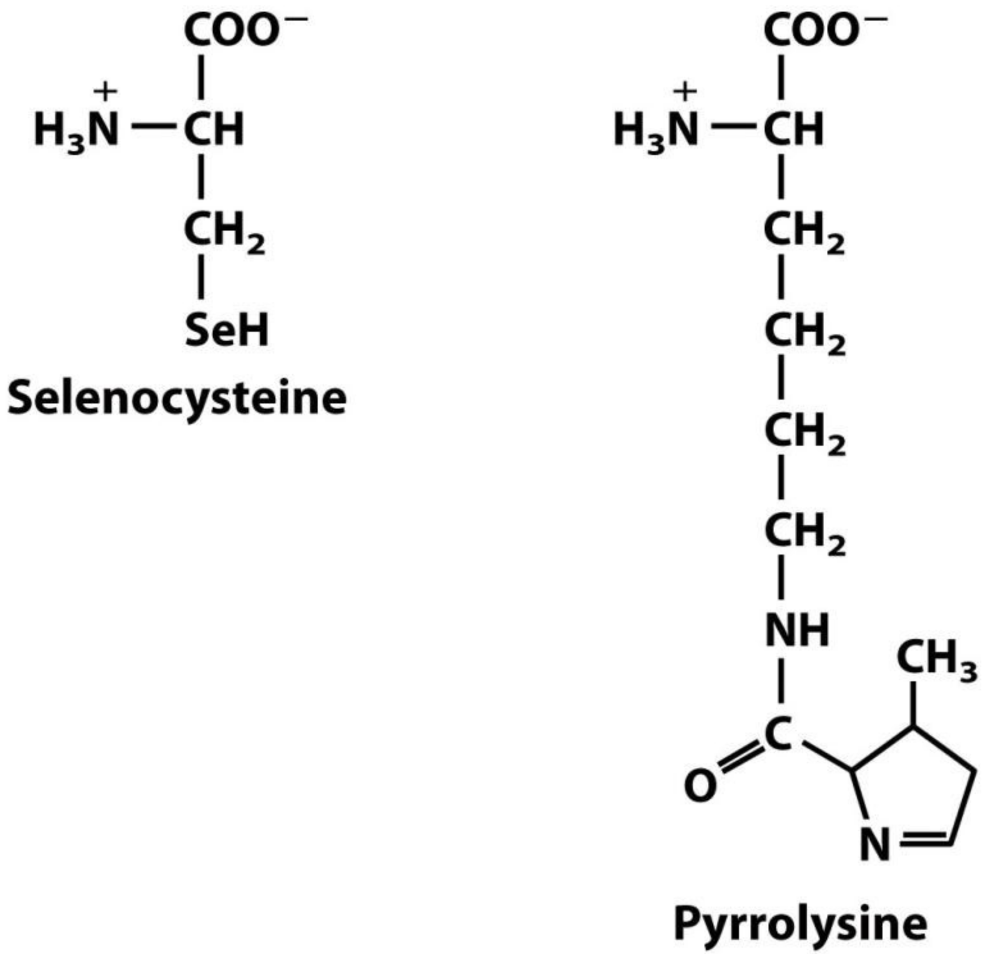
Table 9-4
Lehninger Principles of Biochemistry, Fifth Edition

Protein Engineering

- In all cells proteins have:
  - Enzyme activities
  - Structural roles
- In past 50 years scientists have learned how to prepare large amounts of pure proteins
- Allows detailed *in vitro* studies
- Proteins can also be made to do useful operations both *in vitro* and in cells
- Protein engineering involves processes that modify or improve proteins

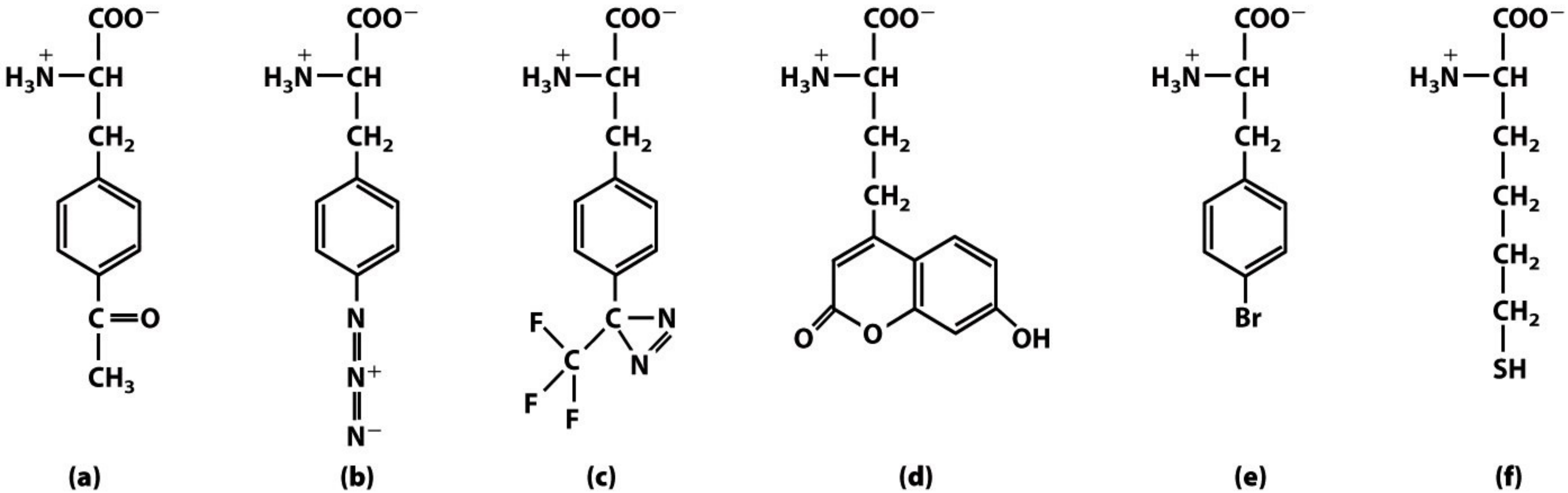
Improving Proteins

- Quite dif … proteins for any p … efficient!
- Can repl … eins
- Recent a … of novel or uncomm
  - Selen … cells (e.g. form … utathione perox
  - Pyrro … oup of Archa



Box 27-3
Lehninger Principles of Biochemistry, Fifth Edition

Uncommon Amino Acids



Box 27-3 figure 2
Lehninger Principles of Biochemistry, Fifth Edition

(a) ketone; (b) azide; (c) photocrosslinker; (d) highly fluorescent; (e) heavy atom for use in crystallography; (f) long-chain cysteine analogue

Tomorrow’s Molecular Biology: Overview

- Biology offers a range of molecules that can be manipulated to produce useful materials or devices
- Synthetic biology incorporates “engineering” approaches to take advantage of and improve biological systems to tackle specific problems
- Protein Engineering manipulates protein production, incorporating modifications to “improve” proteins
- Recombinant proteins can provide much information about protein function both *in vitro* and *in vivo*
- Engineered proteins have huge potentials in biotechnology and medicine
- Important ethical and moral issues to overcome

iGEM: What is it?

A Global Synthetic Biology Competition for Undergraduate Students

What is Involved in iGEM?

- The team (Students + Advisers) develop a Synthetic Biology Project that must be completed during the summer months
- Teams compete to win medals (Gold, Silver or Bronze) and prizes

- Genetic engineering must be perfromed within the project, following quite strict criteria
- Also must involve Human Practices (outreach) and consideration of ethical issues related to the project

In October 2011 the UEA-JIC_iGEM Team attended the iGEM Jamboree in Amsterdam

Find out what we did:

Facebook: www.facebook.com/UEAJIC.IGE

Twitter: www.twitter.com/UEAJIC_IGEM

Wiki: http://2011.igem.org/Team:UEA-JIC_Norwich

NRP-UEA iGEM Team 2012

In 2012 we organised the 2nd iGEM team based on the Norwich Research Park

For info: see http://2012.igem.org/Team:NRP-UEA-Norwich

Our team included 7 undergraduate students from BIO

We were predominantly based at the School of Biological Sciences at UEA