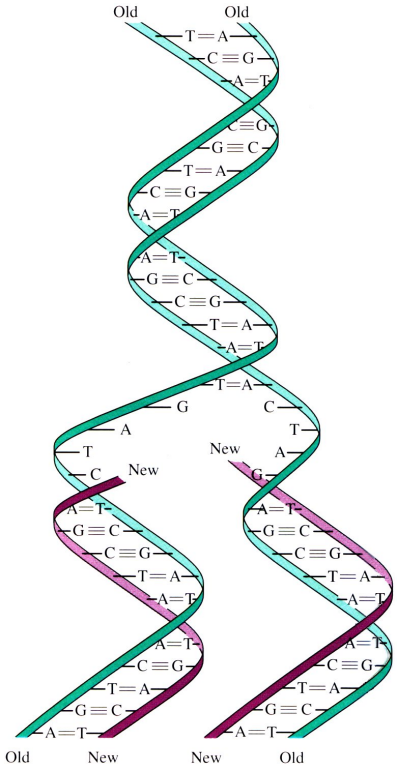
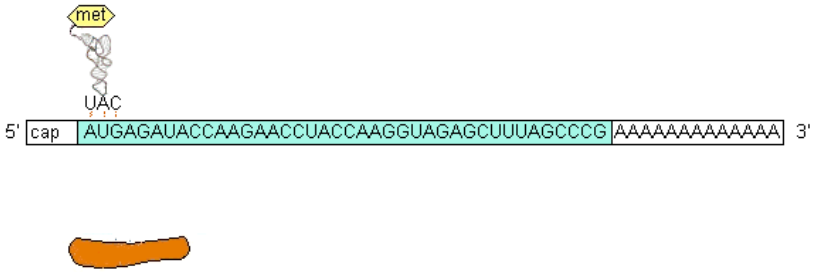
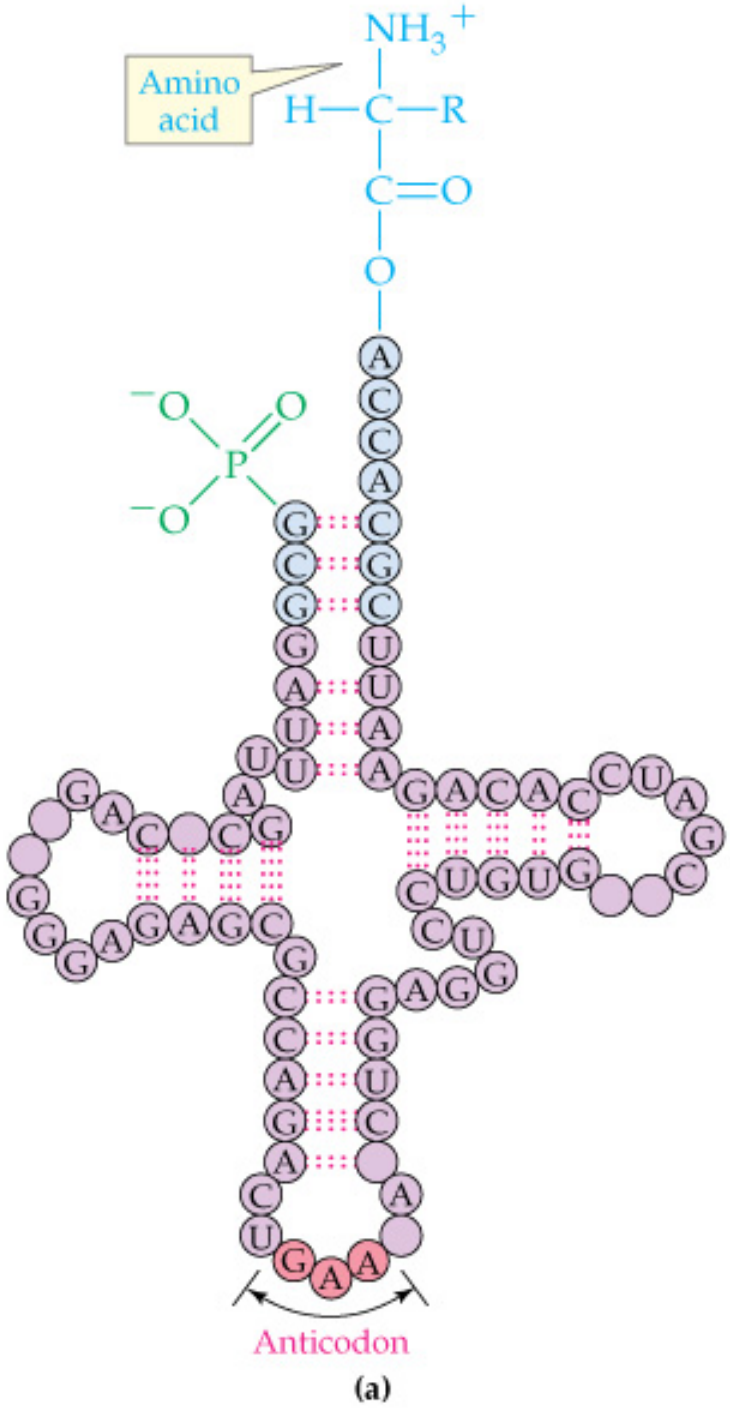
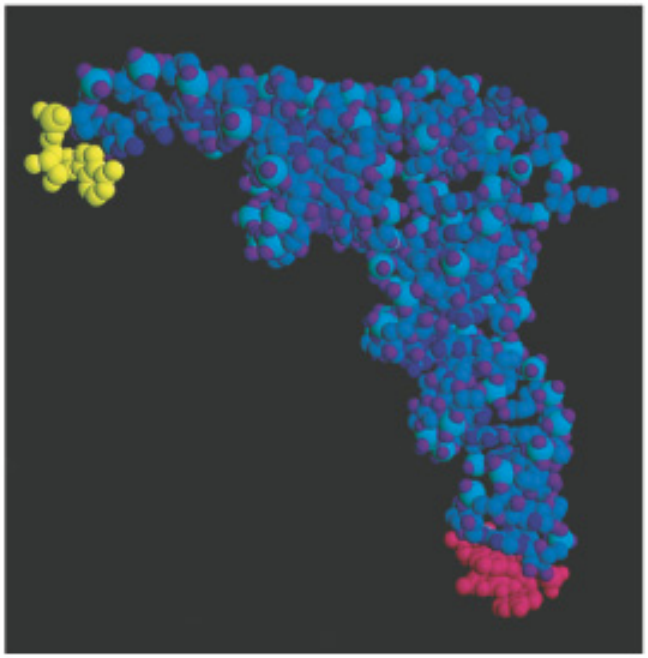
Self-Assembly Process in Nature



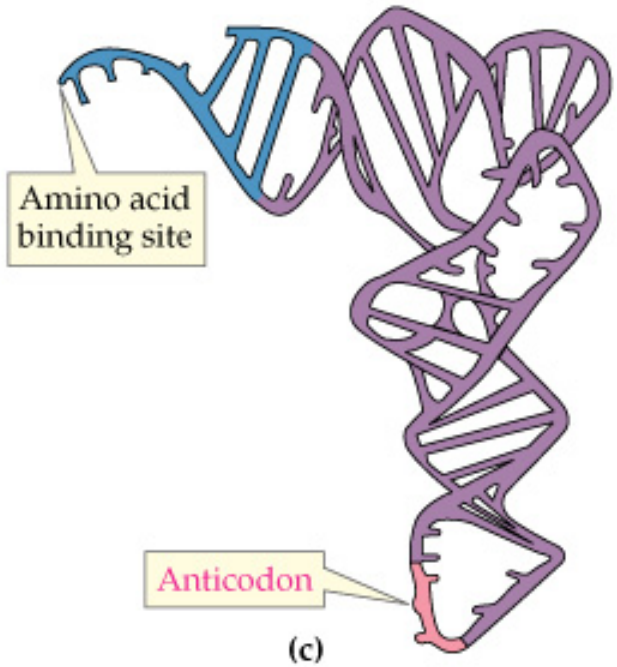



Amino acid
NH_3^+
H—C—R
C=O
O
^-O
O
P
^-O
Anticodon

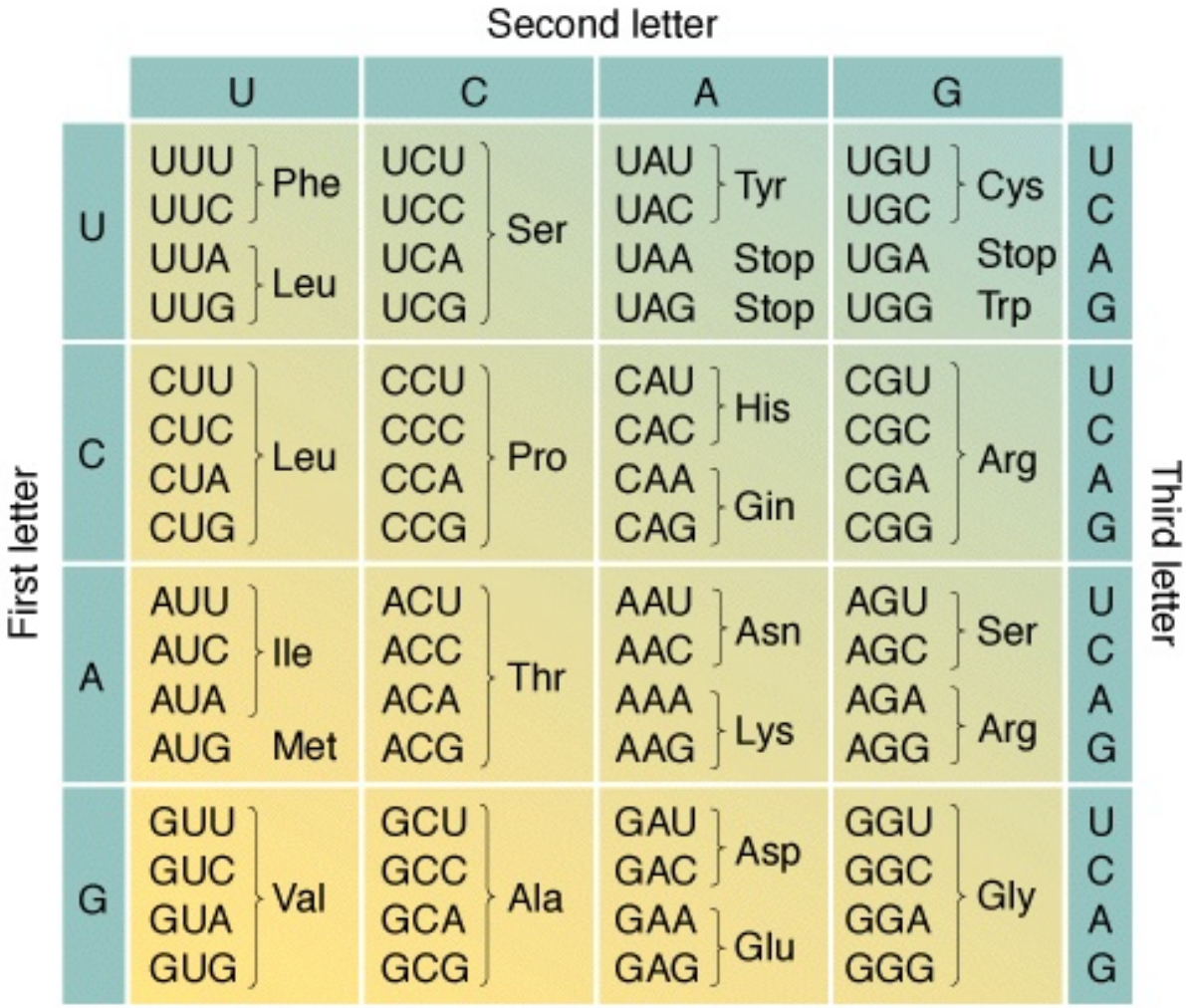
(a)



(b)

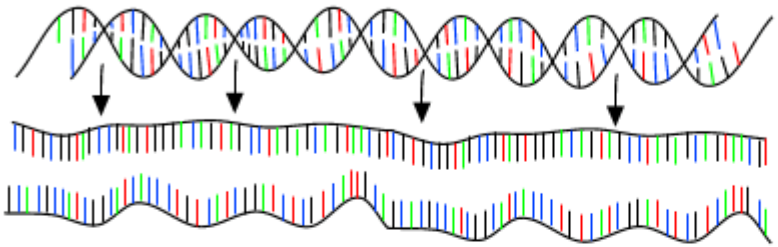

Amino acid binding site
Anticodon

(c)


Second letter
U
C
A
G
First letter
Third letter
U
UUU Phe
UUC
UUA Leu
UUG
UCU Ser
UCC
UCA
UCG
UAU Tyr
UAC
UAA Stop
UAG Stop
UGU Cys
UGC
UGA Stop
UGG Trp
C
CUU Leu
CUC
CUA
CUG
CCU Pro
CCC
CCA
CCG
CAU His
CAC
CAA Gin
CAG
CGU Arg
CGC
CGA
CGG
A
AUU Ile
AUC
AUA
AUG Met
ACU Thr
ACC
ACA
ACG
AAU Asn
AAC
AAA Lys
AAG
AGU Ser
AGC
AGA Arg
AGG
G
GUU Val
GUC
GUA
GUG
GCU Ala
GCC
GCA
GCG
GAU Asp
GAC
GAA Glu
GAG
GGU Gly
GGC
GGA
GGG

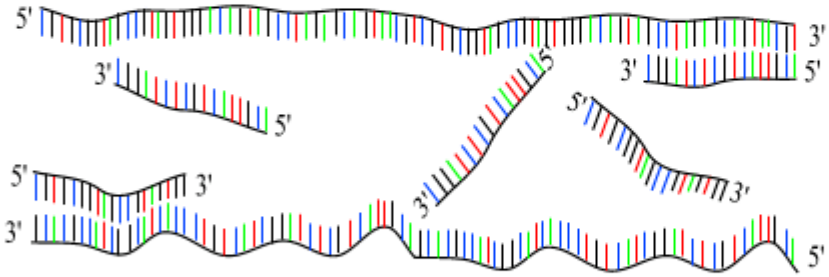
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

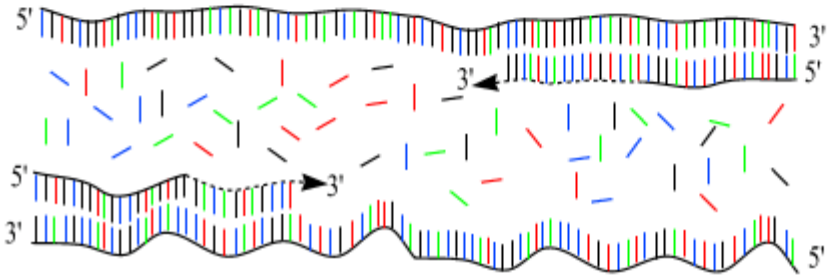
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!

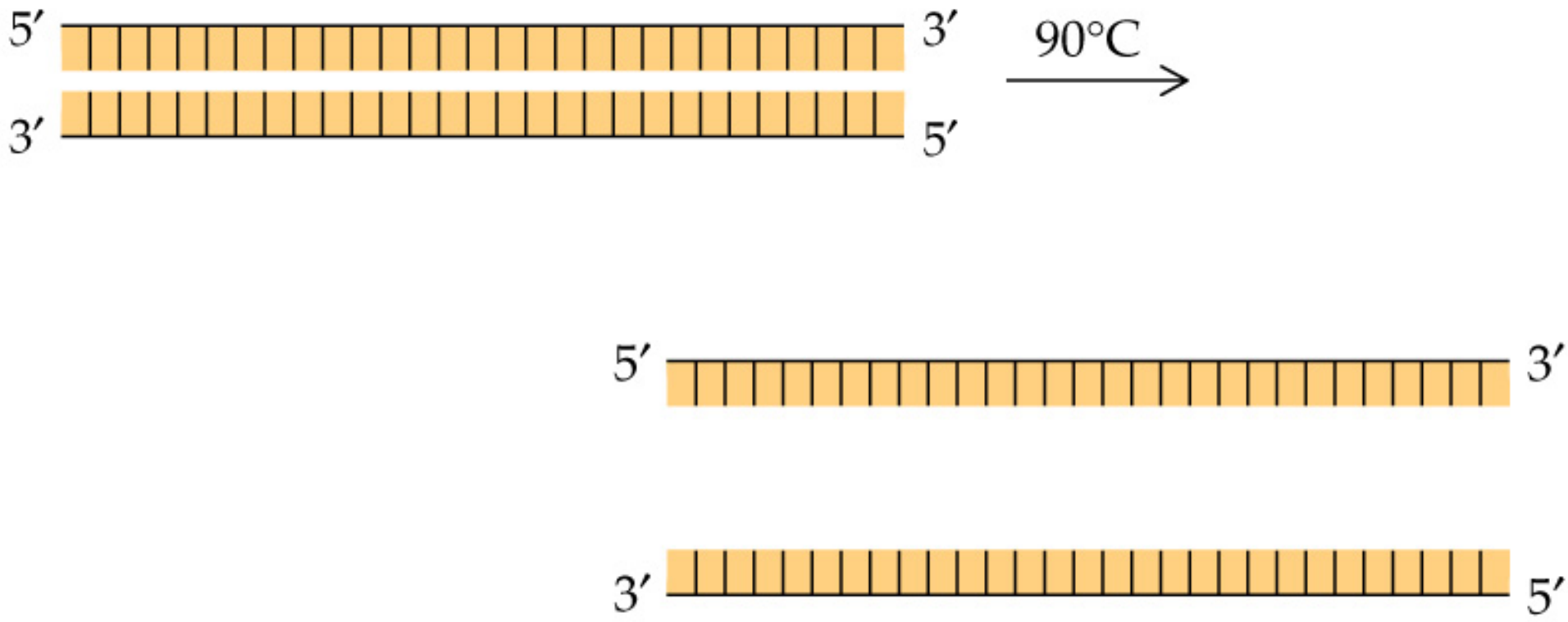


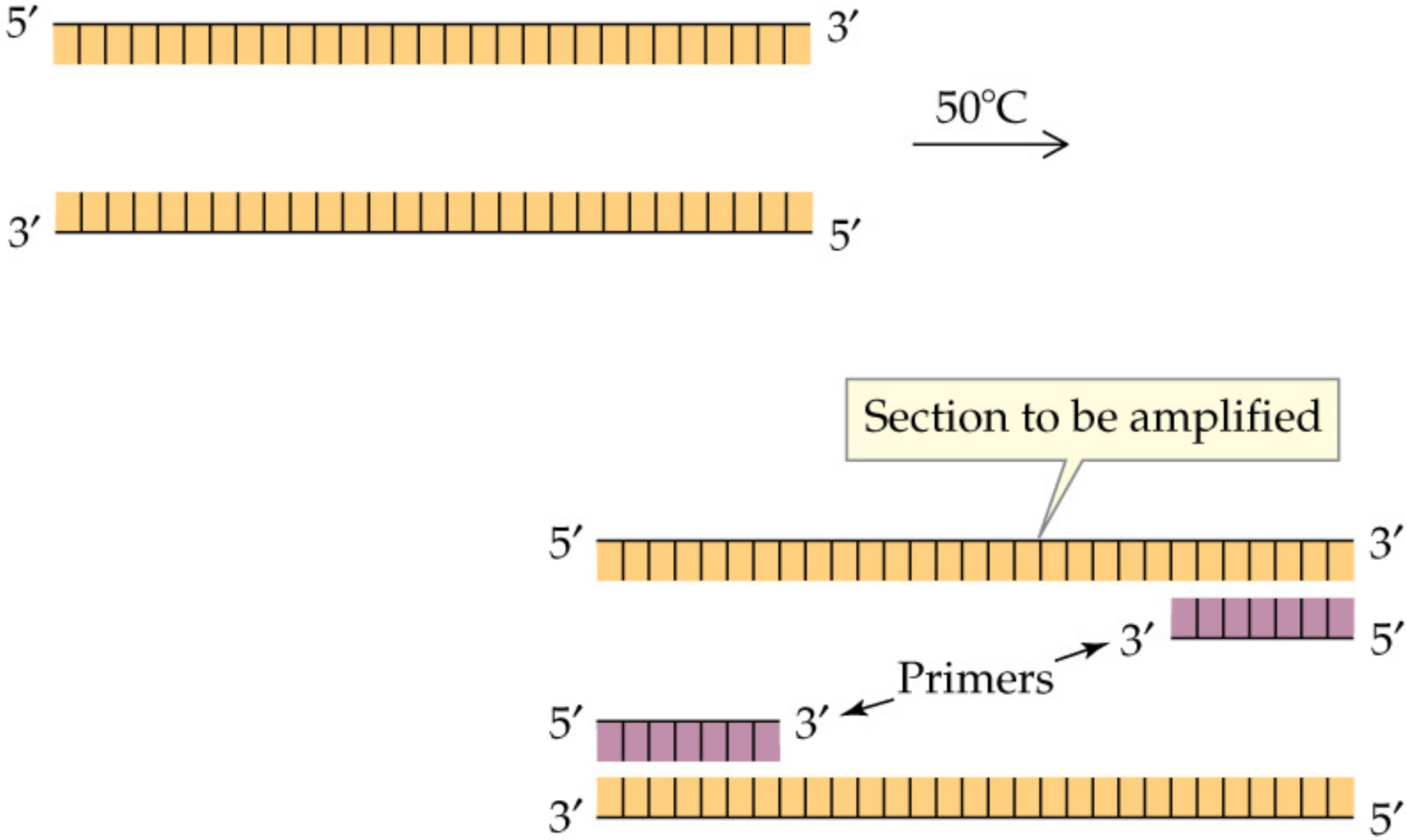
Step 3 : extension

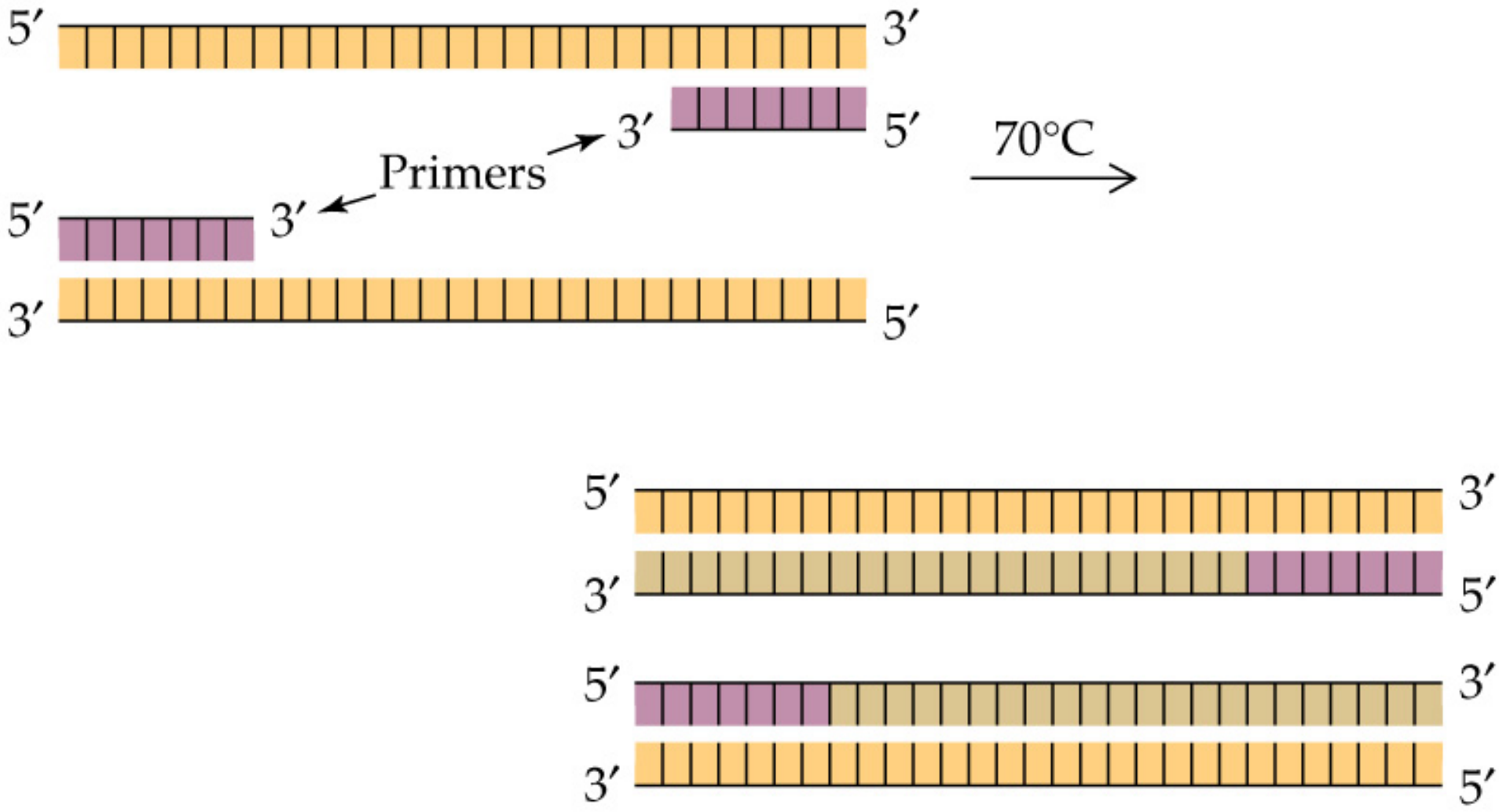
2 minutes 72 °C

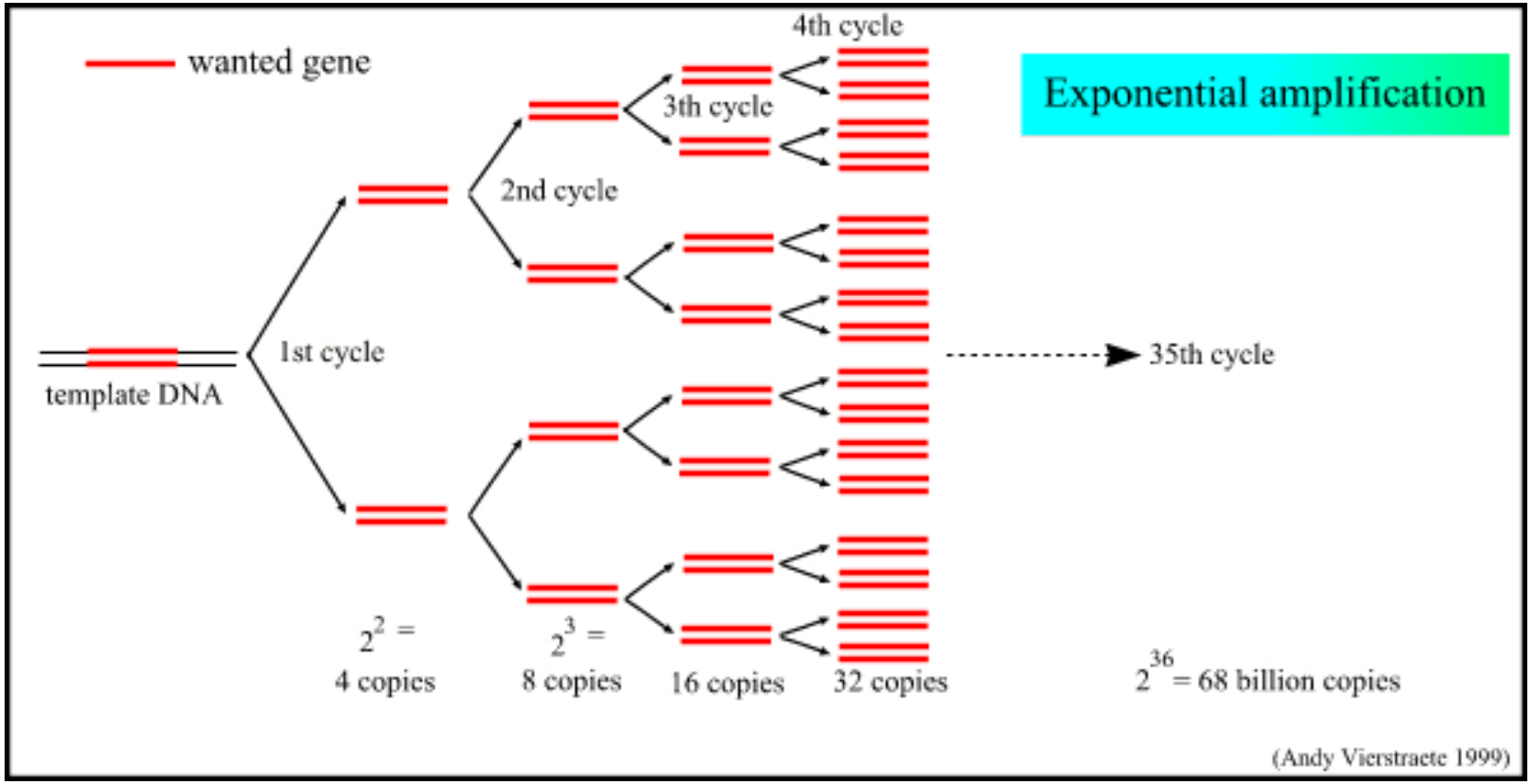
only dNTP's

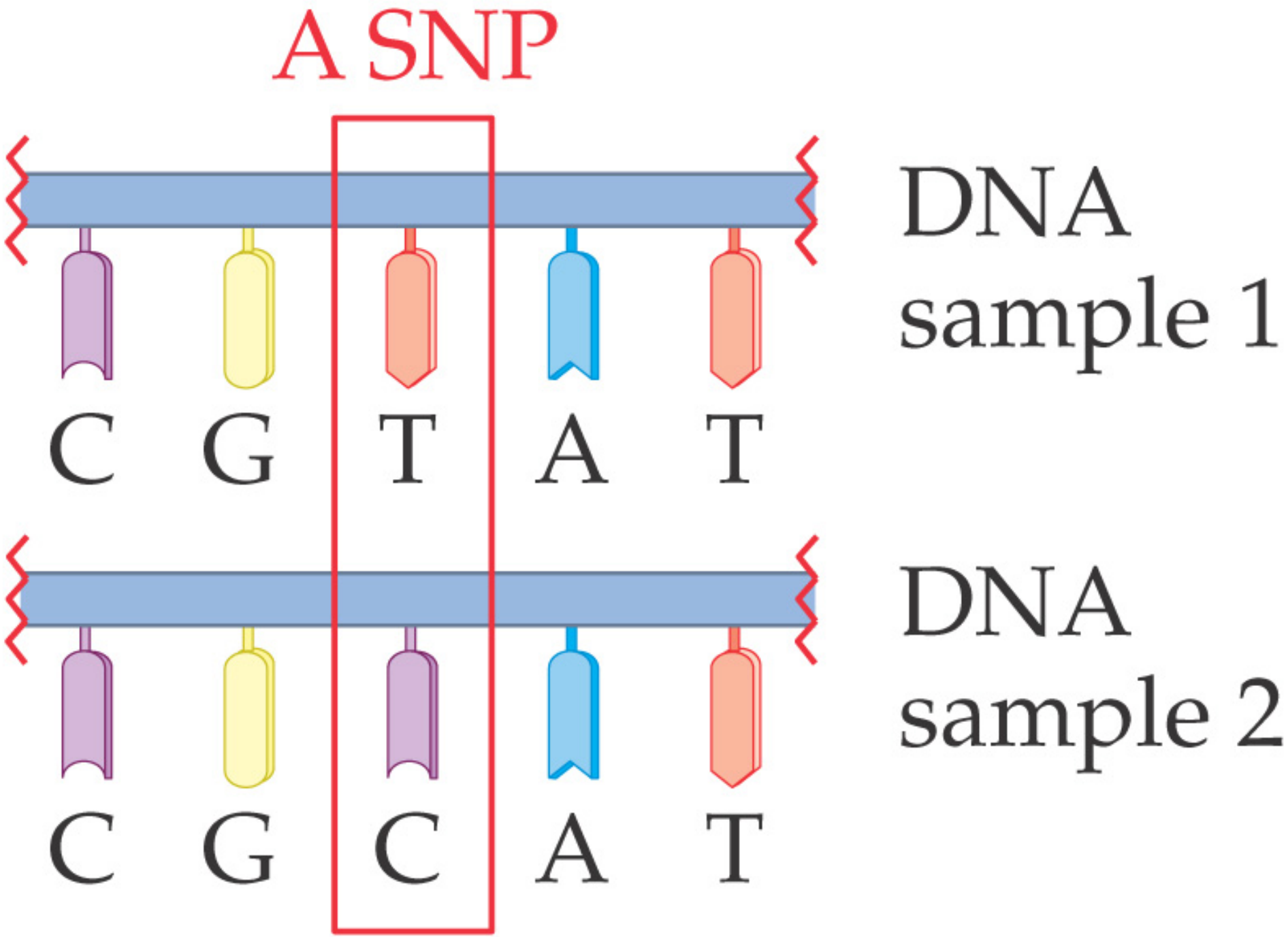
(Andy Vierstraete 1999)

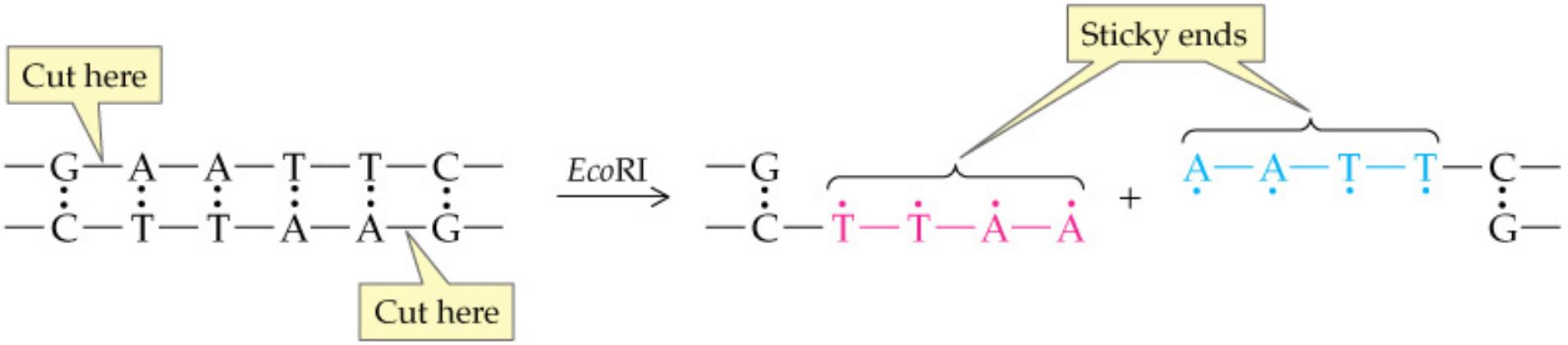

5′
3′
3′
5′
90°C
5′
3′
3′
5′

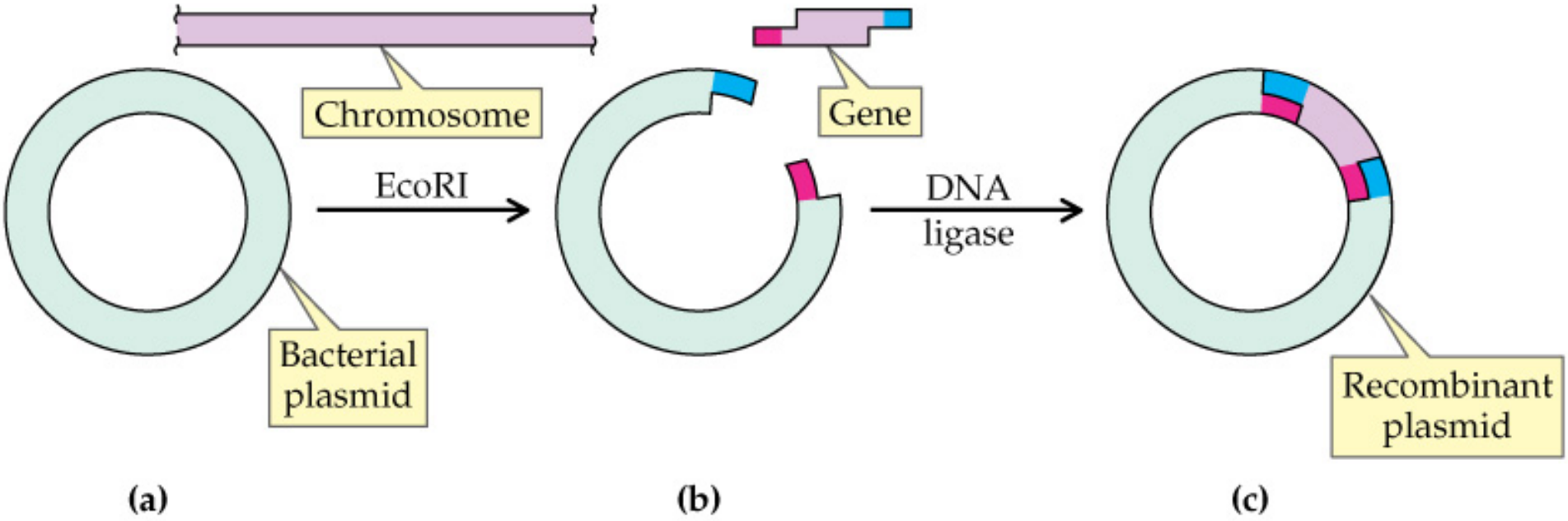

5′
3′
50°C
3′
5′
Section to be amplified
5′
3′
3′
5′
Primers
5′
3′
3′
5′


5′
3′
3′
5′
Primers
70°C
5′
3′
3′
5′
5′
3′
3′
5′
5′
3′
3′
5′


wanted gene
4th cycle
Exponential amplification
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

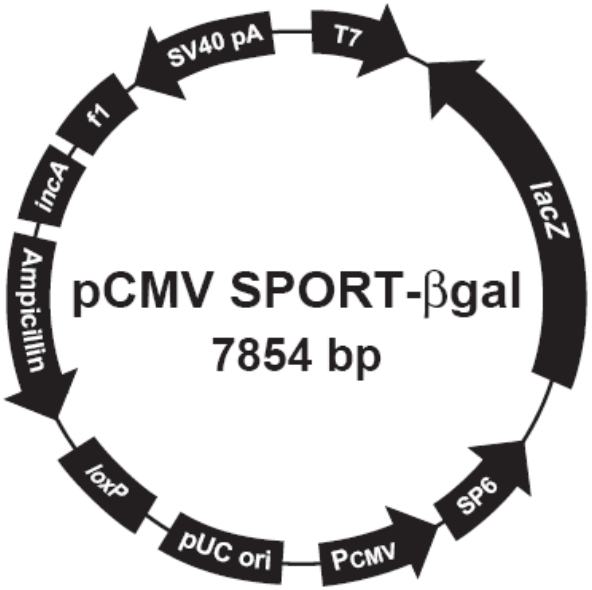

A SNP
C G T A T
C G C A T
DNA sample 1
DNA sample 2

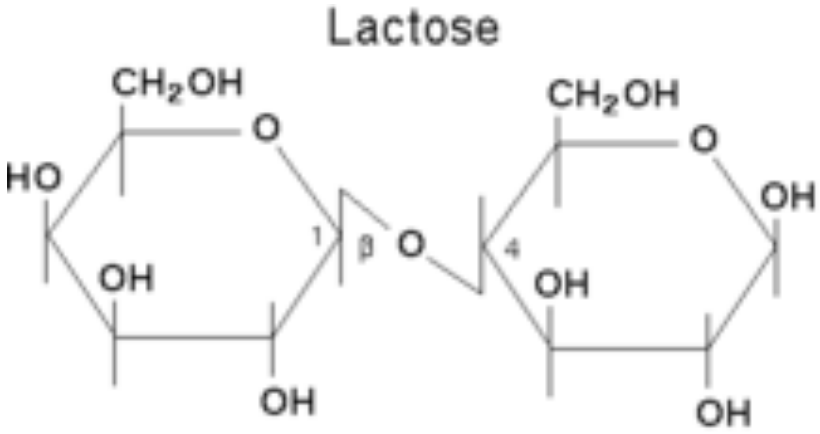

Cut here
—G—A—A—T—T—C—
—C—T—T—A—A—G—
Cut here
*Eco*RI
Sticky ends
—G
—C—T—T—A—A
+
A—A—T—T—C—
G—


Chromosome
Gene
EcoRI
DNA
ligase
Bacterial
plasmid
Recombinant
plasmid
(a)
(b)
(c)

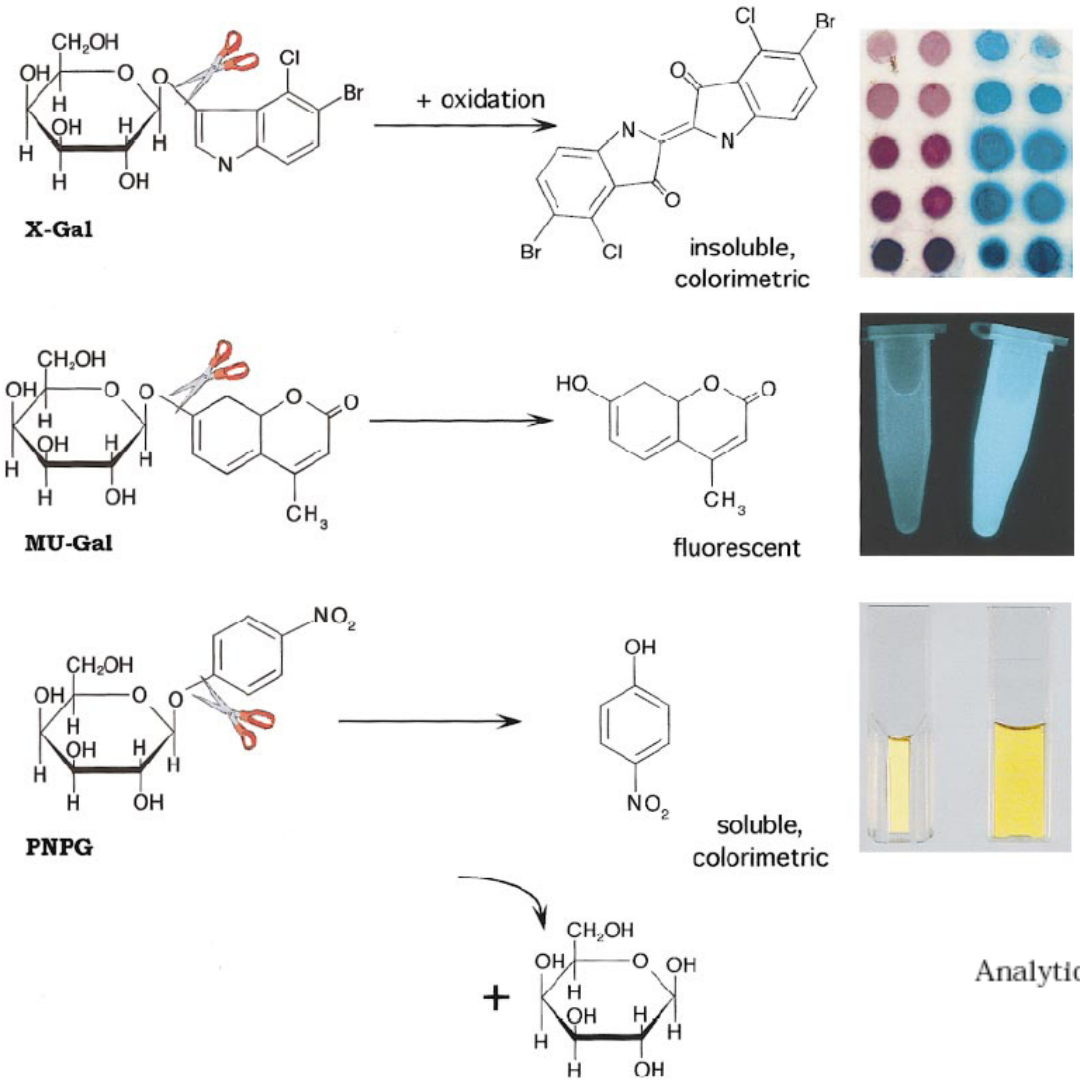
β-Galactosidase

The enzyme that splits lactose into glucose and galactose. Coded by a gene (lacZ) in the lac operon of Escherichia coli.





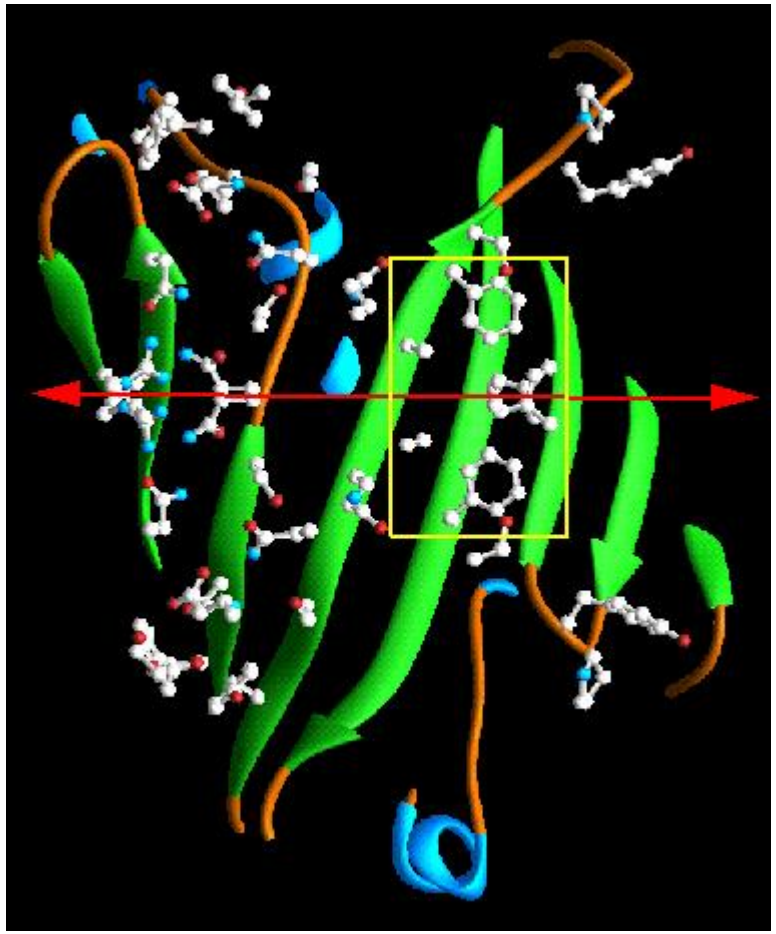
PUC is a family of plasmids that have an ampicillin resistance gene and more importantly a *lacZ* gene. A functional lacZ gene will produce the protein β - galactosidase. Bacterial colonies in which β - galactosidase is produced, will form blue colonies in the presence of the substrate 5 - bromo - 4 - chloro - 3 - indolyl - b - D - galactoside or as it is more commonly referred to, X-gal.



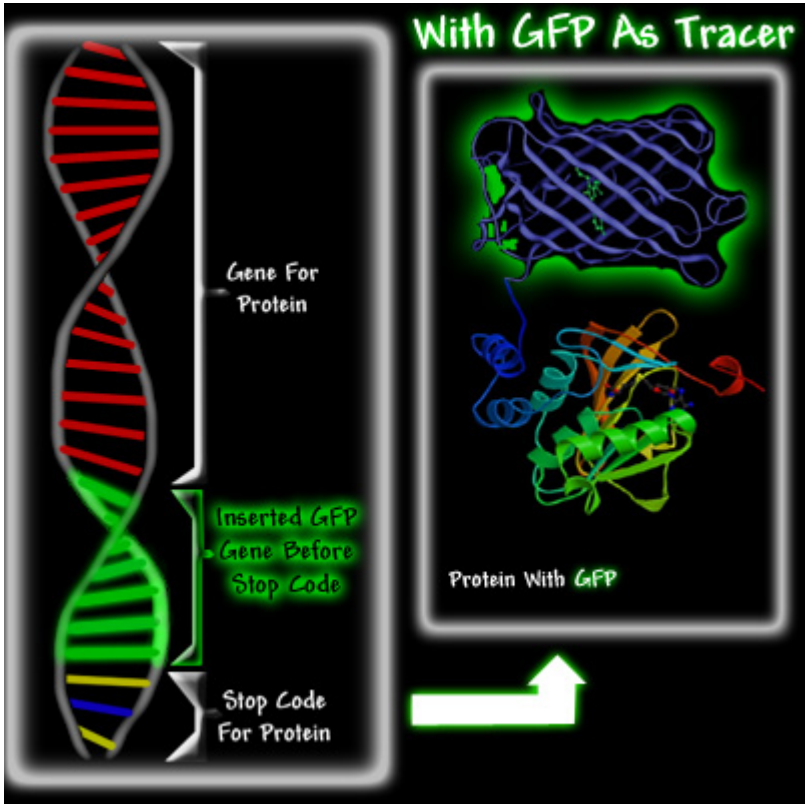
Analytical Biochemistry **285**, 1–15 (2000)

FIG. 1. Enzymatic function of β-galactosidase in cleaving indicator substrates. β-gal cleaves β-D-galactoside containing substrates with a diverse range of aglycone groups, targeting between the glycosyl oxygen and anomeric carbon as indicated (scissors). Substrates shown indicate commonly used indicators for assays on β-gal function on plates (X-Gal) or for liquid assay by measure of fluorescence (MU-Gal or MUG) or color (ONPG). Top left, X-Gal is 5-bromo-4-chloro-3-indolyl-β-D-galactoside, and when cleaved and oxidized produces the insoluble dye 5-bromo-4-chloro-indigo, as described previously (22). Right panel, top, yeast colonies expressing β-gal and exposed to X-Gal (right half) or the closely related compound Magenta-Gal (left half, see Biosynth, Inc., or Diagnostic Chemicals Limited). Middle left, MUG is methylumbelliferyl-β-D-galactoside, and when cleaved by β-gal produces the fluorescent product methylumbelliferone (first described in (102)). Right panel, middle, shows yeast lysates expressing β-gal exposed to MUG, under long-wave UV. Bottom left, PNPG and ONPG are closely related nitrophenol-β-D-galactosides with similar assay properties, e.g., (103), whose cleavage releases the yellow product nitrophenol (right panel, bottom); PNPG is shown.

Green Fluorescent Protein (GFP)

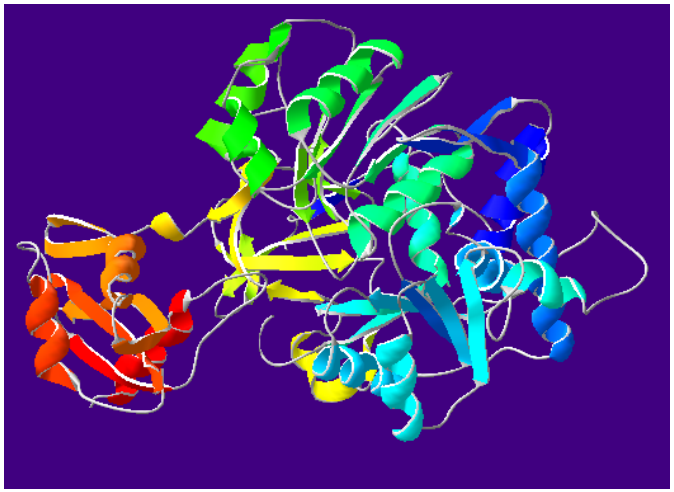


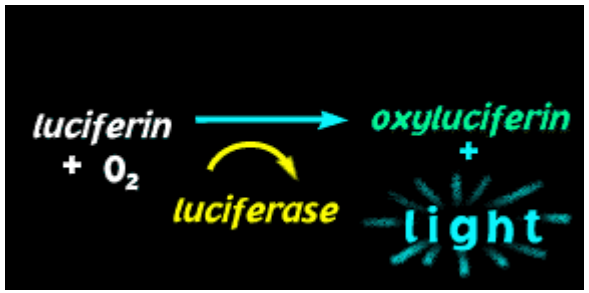
The **green fluorescent protein** (**GFP**) is a protein from the jellyfish *Aequorea victoria* that fluoresces green when exposed to blue light.



GFP Rats

Luciferase



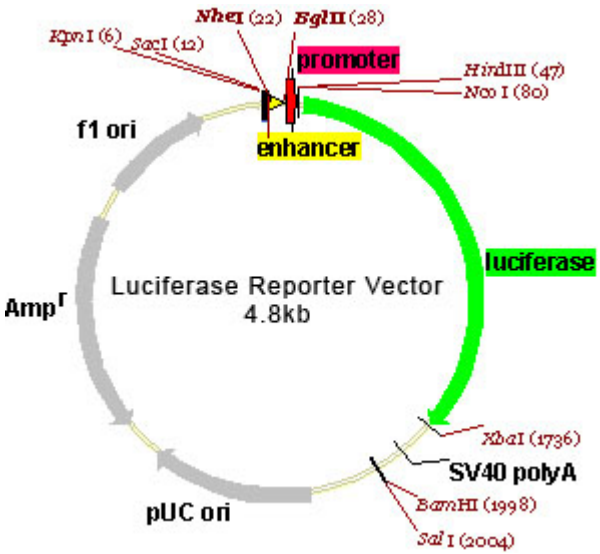


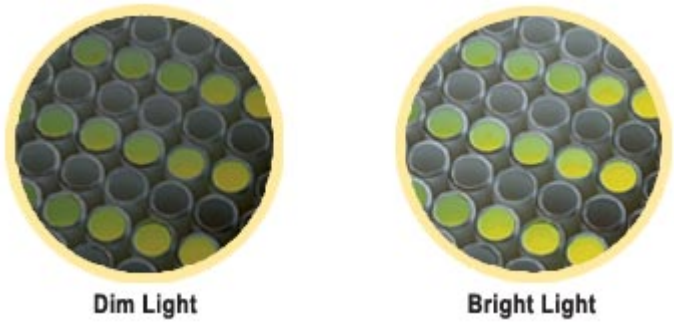
Luciferase is a generic name for enzymes commonly used in nature for bioluminescence. The name itself is derived from *Lucifer*, which means *light-bearer*. The most famous one is firefly luciferase from the firefly *Photinus pyralis*. In luminescent reactions, light is produced by the oxidation of a luciferin (a pigment), sometimes involving Adenosine triphosphate (ATP). The rates of this reaction between luciferin and oxygen are extremely slow until they are catalyzed by luciferase, often mediated by the presence of calcium ions (an analog of muscle contraction). The reaction takes place in two steps:

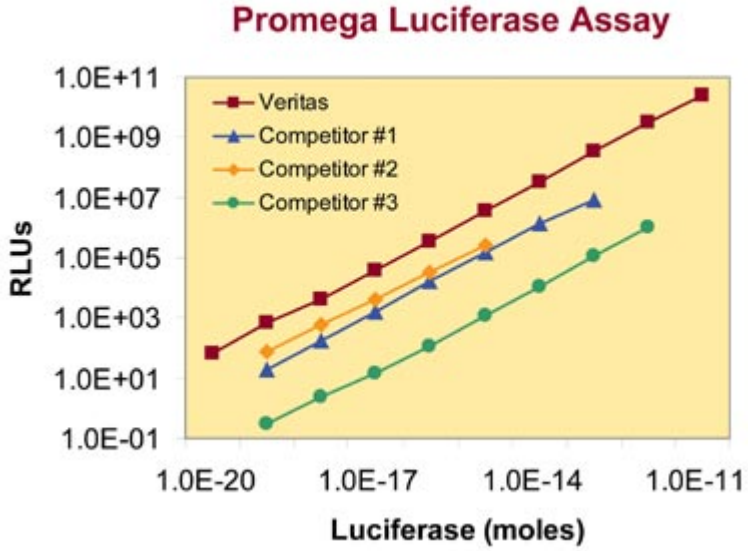
luciferin + ATP → luciferyl adenylate + PPi

luciferyl adenylate + O2 → oxyluciferin + AMP + light

Luciferase


NheI (22)
BglII (28)
KpnI (6)
SacI (12)
promoter
HindIII (47)
NcoI (80)
f1 ori
enhancer
luciferase
Luciferase Reporter Vector
4.8kb
Amp^r
XbaI (1736)
SV40 polyA
pUC ori
BamHI (1998)
SalI (2004)


Dim Light
Bright Light


Promega Luciferase Assay
1.0E+11
1.0E+09
1.0E+07
1.0E+05
1.0E+03
1.0E+01
1.0E-01
RLUs
Veritas
Competitor #1
Competitor #2
Competitor #3
1.0E-20
1.0E-17
1.0E-14
1.0E-11
Luciferase (moles)

DNA Origami

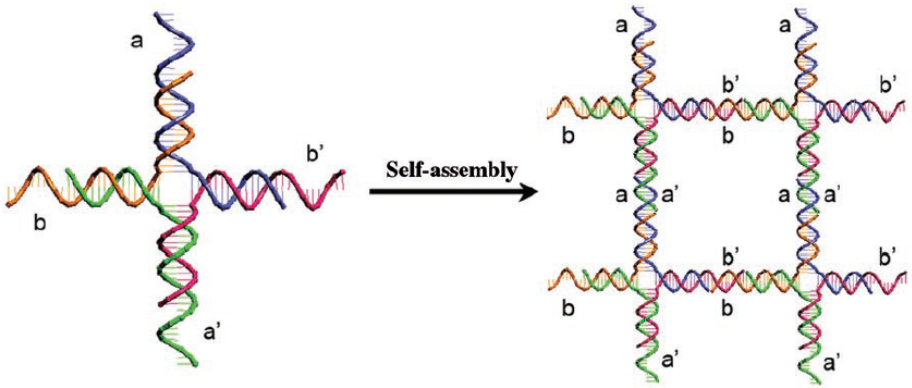
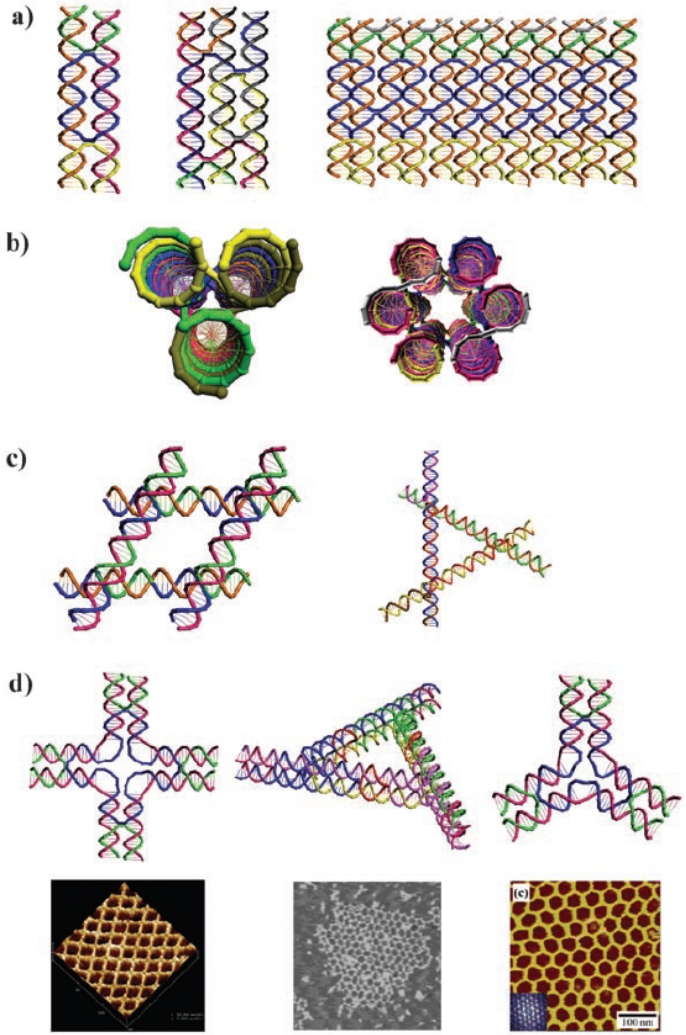


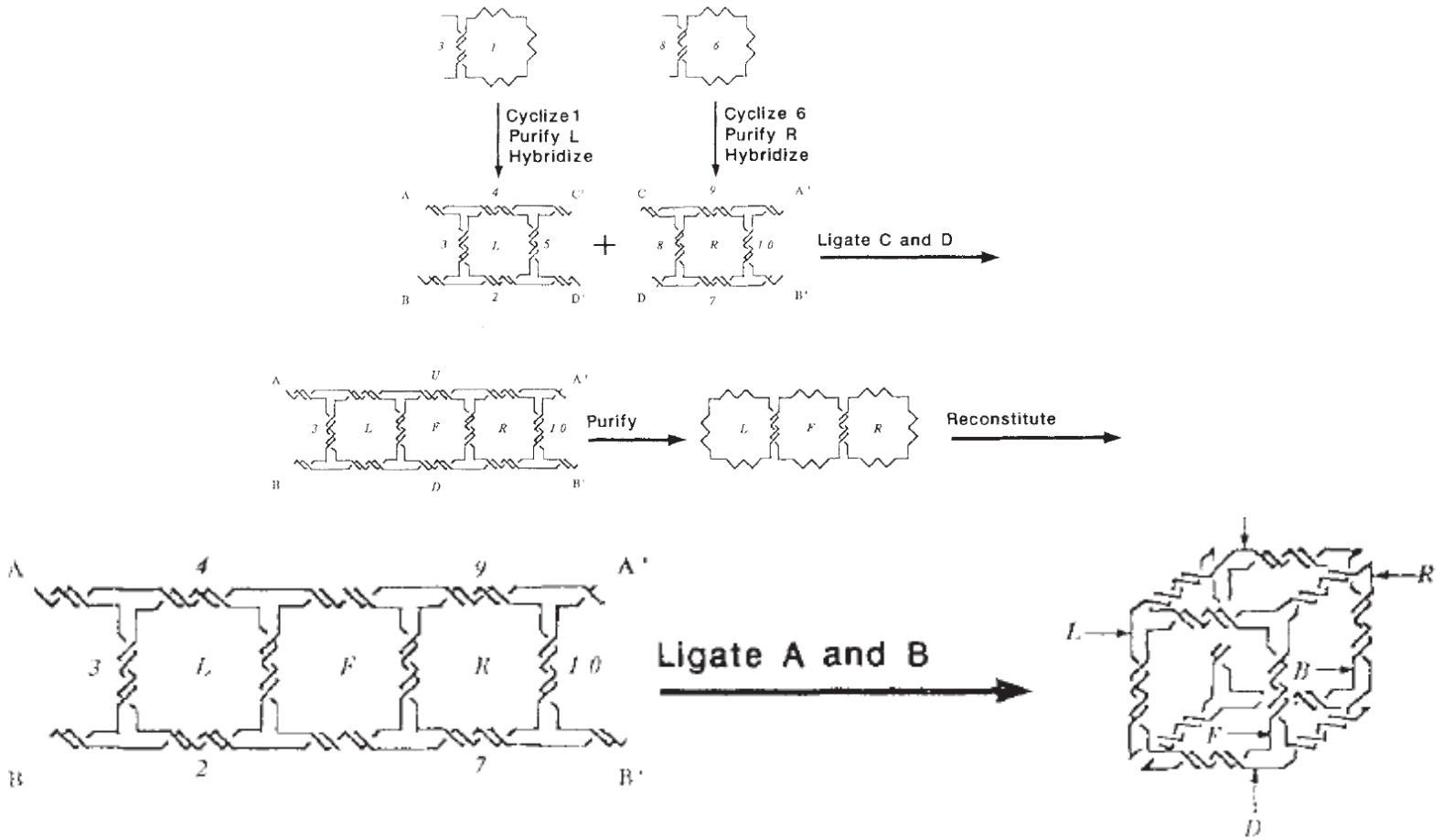
Figure 1. Key concept of DNA tile based self-assembly: combining branched DNA junction with sticky-end associations (e.g. a–a′ and b–b′ pairings) to self-assemble 2D lattices (adapted from ref. [1]). The DNA model was rendered using the Strata program (www.strata.com).



Synthesis from DNA of a molecule with the connectivity of a cube

Junghuei Chen & Nadrian C. Seeman

NATURE · VOL 350 · 18 APRIL 1991



Folding DNA to create nanoscale shapes and patterns

NATURE|Vol 440|16 March 2006

Paul W. K. Rothemund[1]

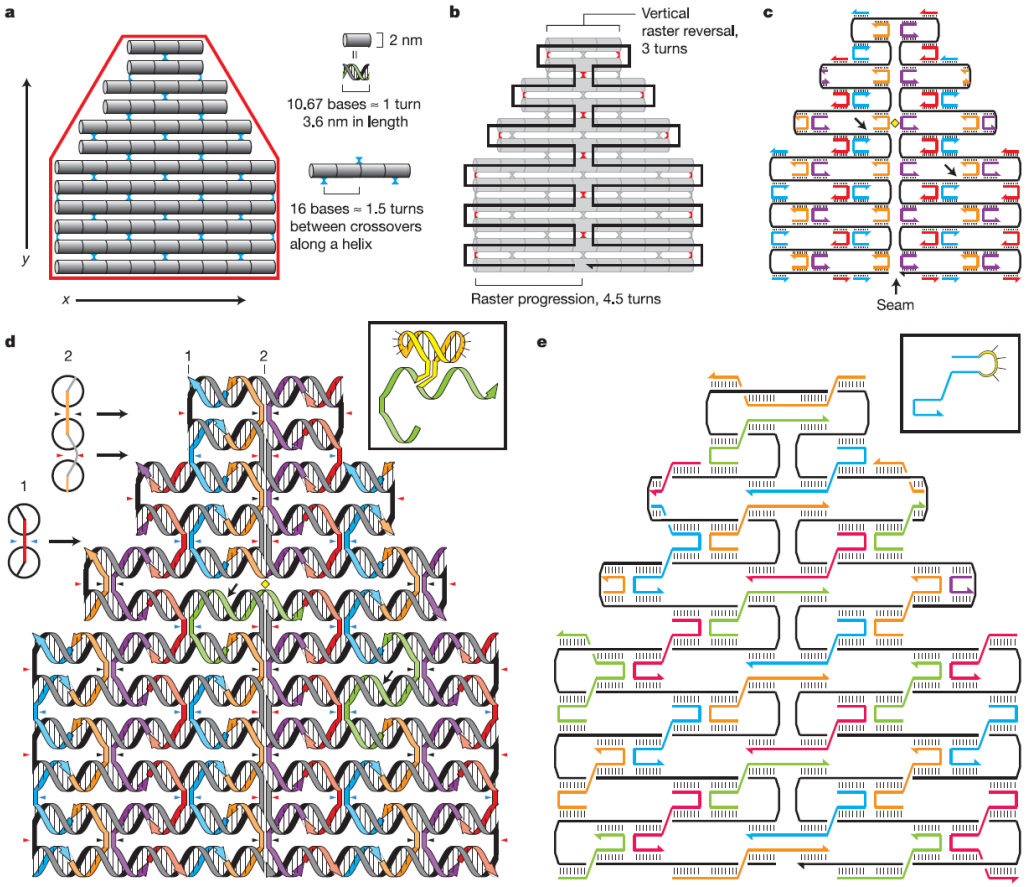
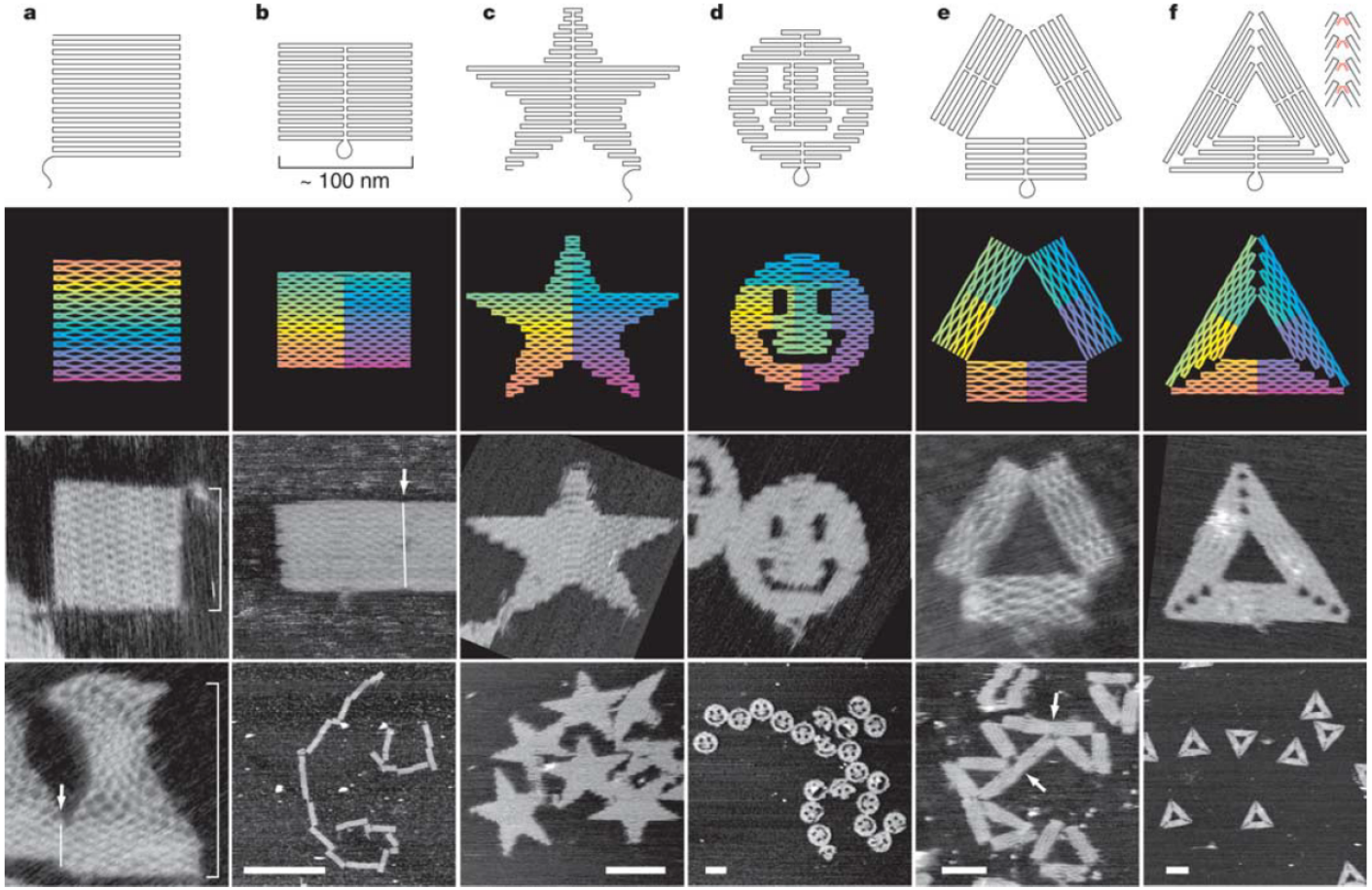
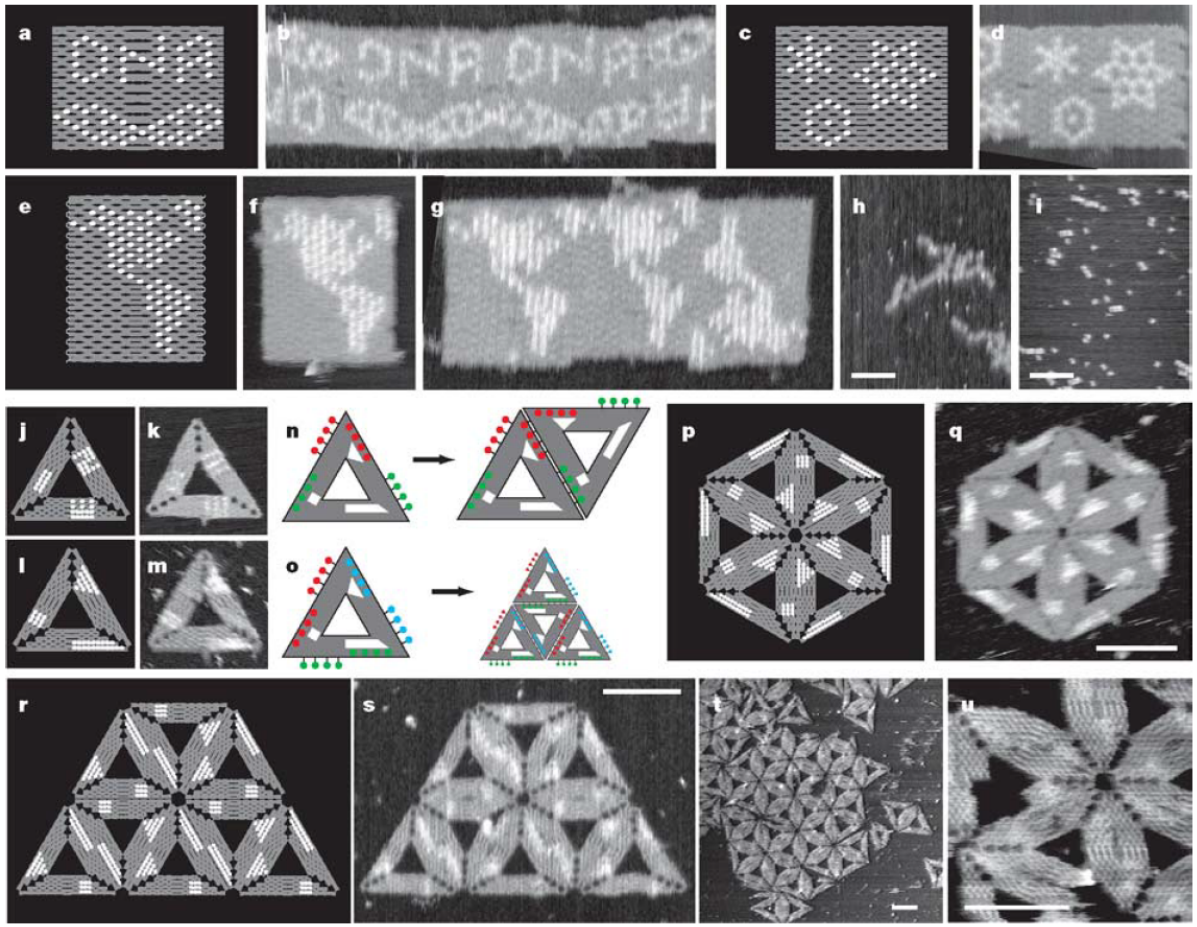


Figure 1 | Design of DNA origami. **a**, A shape (red) approximated by parallel double helices joined by periodic crossovers (blue). **b**, A scaffold (black) runs through every helix and forms more crossovers (red). **c**, As first designed, most staples bind two helices and are 16-mers. **d**, Similar to **c** with strands drawn as helices. Red triangles point to scaffold crossovers, black triangles to periodic crossovers with minor grooves on the top face of the shape, blue triangles to periodic crossovers with minor grooves on bottom. Cross-sections of crossovers (1, 2, viewed from left) indicate backbone positions with coloured lines, and major/minor grooves by large/small angles between them. Arrows in **c** point to nicks sealed to create green strands in **d**. Yellow diamonds in **c** and **d** indicate a position at which staples may be cut and resealed to bridge the seam. **e**, A finished design after merges and rearrangements along the seam. Most staples are 32-mers spanning three helices. Insets show a dumbbell hairpin (**d**) and a 4-T loop (**e**), modifications used in Fig. 3.

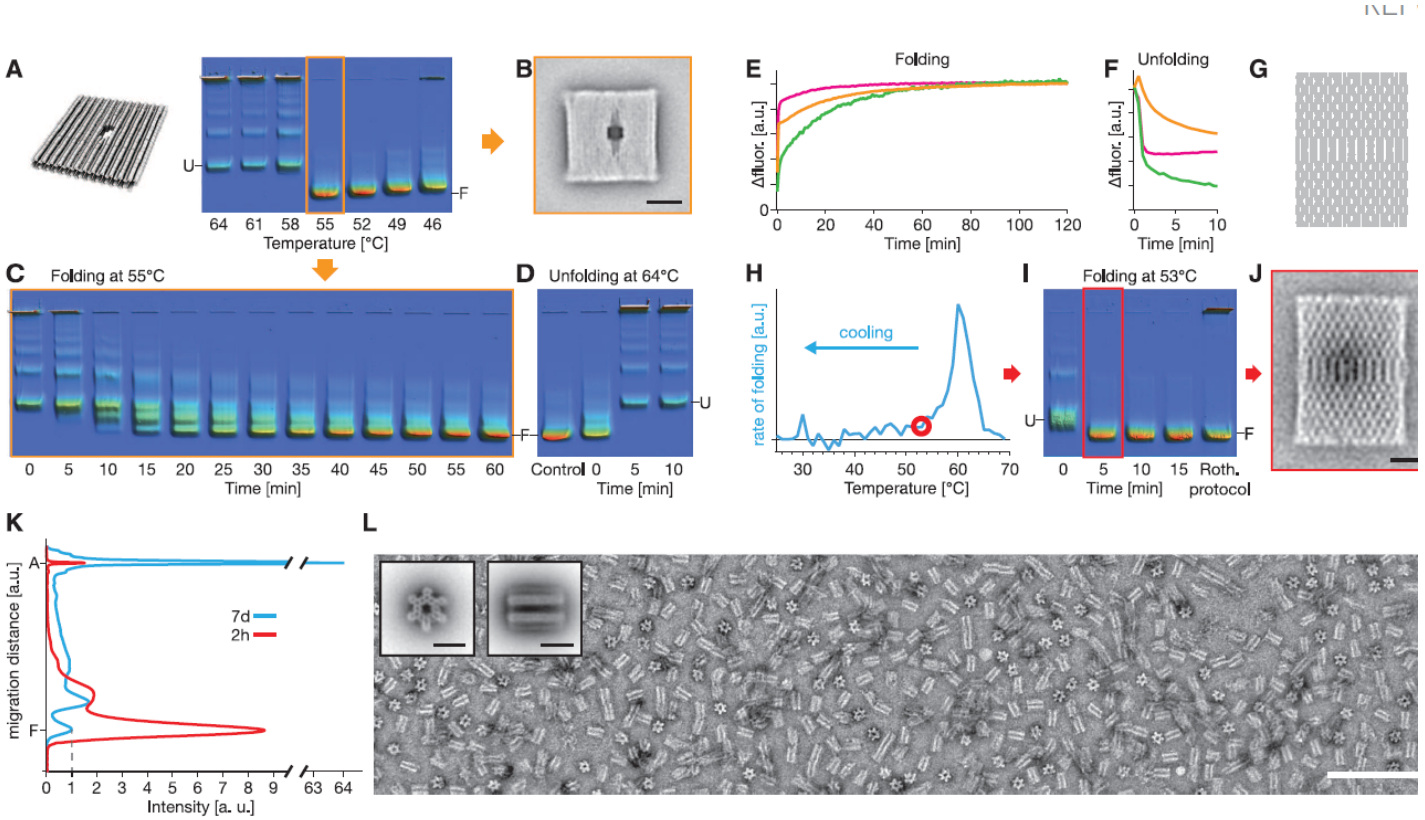

a
b
c
d
e
f
~ 100 nm


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u

Rapid Folding of DNA into Nanoscale Shapes at Constant Temperature

Jean-Philippe J. Sobczak *et al.*

Science **338**, 1458 (2012);
DOI: 10.1126/science.1229919



DNA Origami as a Carrier for Circumvention of Drug Resistance

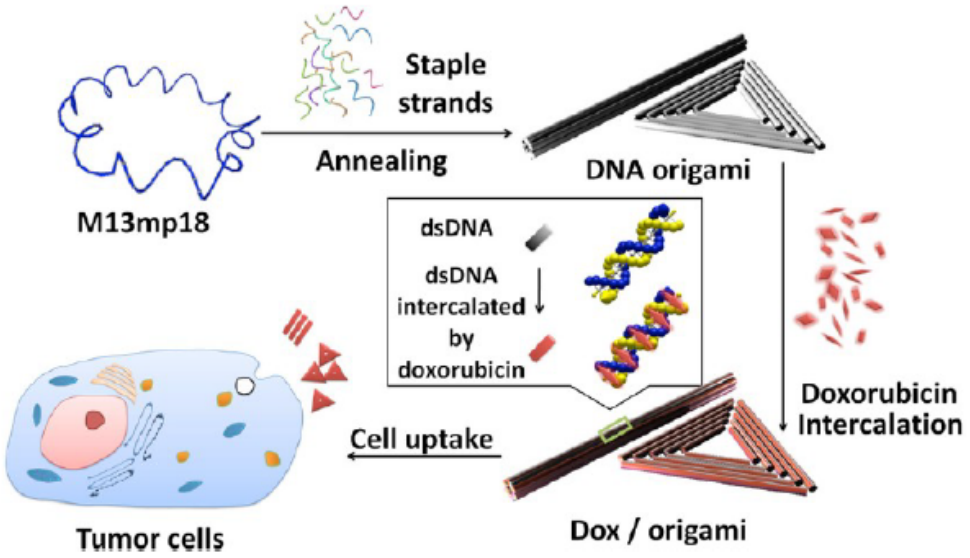
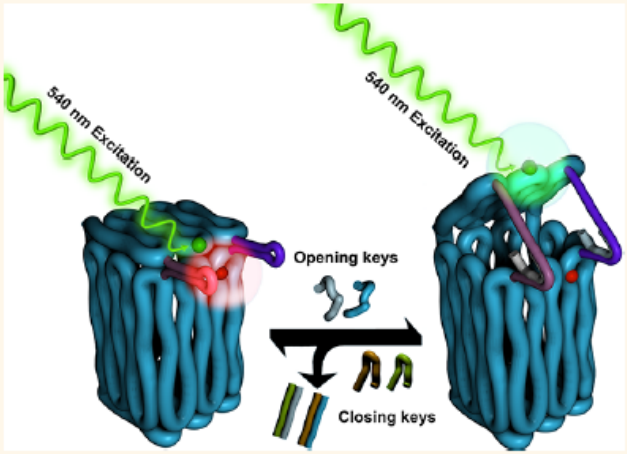


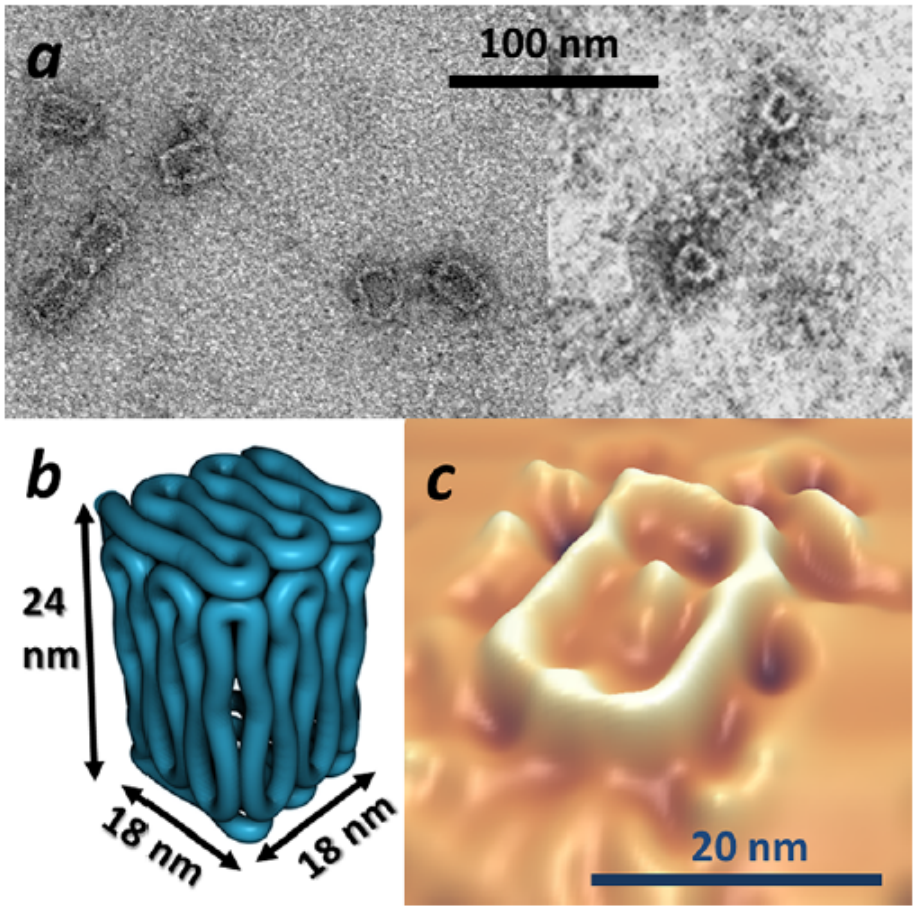
Figure 1. DNA origami and doxorubicin origami delivery system assembly. The long single-strand M13mp18 genomic DNA scaffold strand (blue) is folded into the triangle and tube structures through the hybridization of rationally designed staple strands. Watson–Crick base pairs in the double helices serve as docking sites for doxorubicin intercalation. After incubation with doxorubicin, the drug-loaded DNA nanostructure delivery vessels were administered to MCF 7 cells, and the effects were investigated.

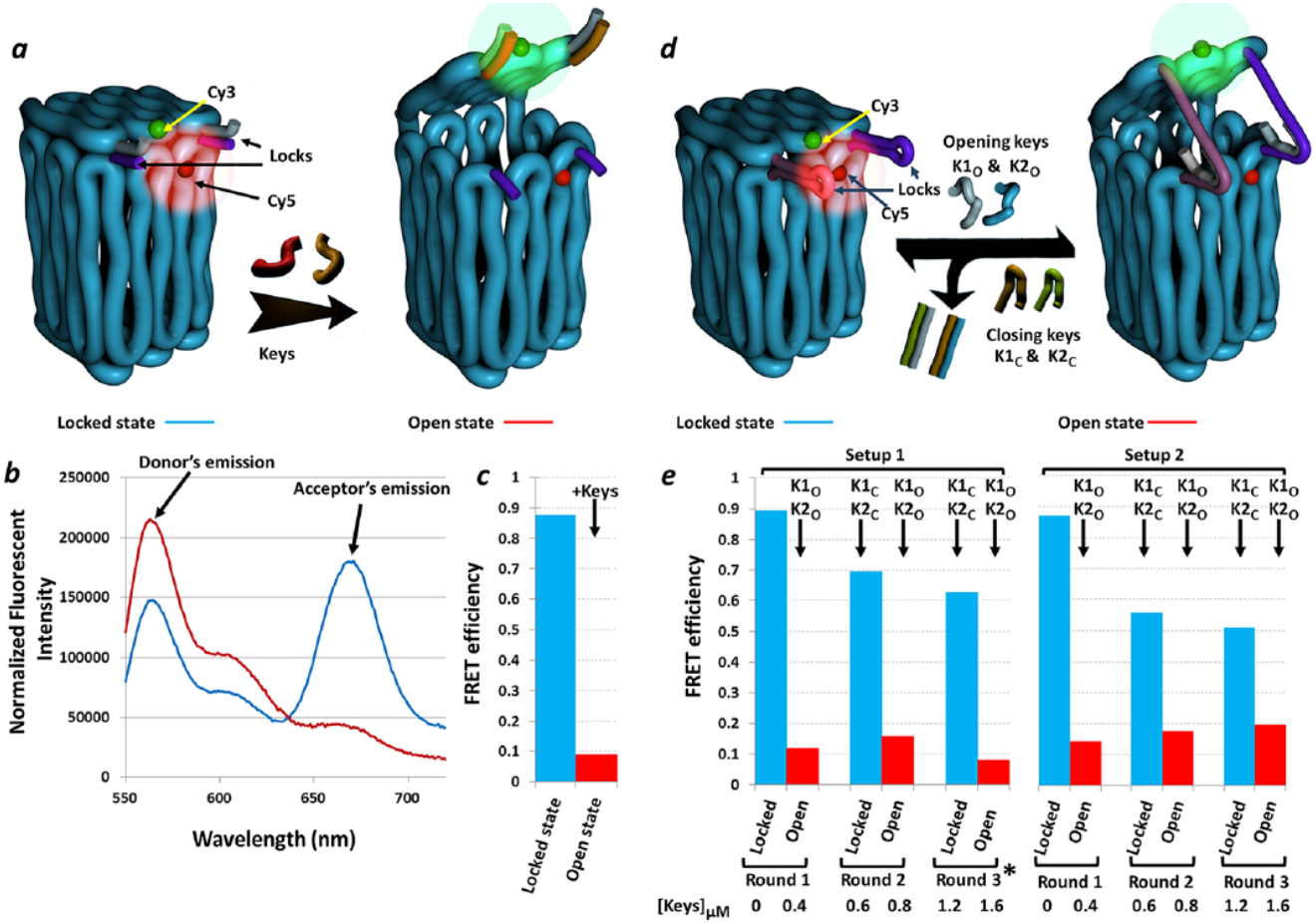
dx.doi.org/10.1021/ja304263n | *J. Am. Chem. Soc.* 2012, 134, 13396–13403

Construction of a 4 Zeptoliters Switchable 3D DNA Box Origami

ABSTRACT The DNA origami technique is a recently developed self-assembly method that allows construction of 3D objects at the nanoscale for various applications. In the current study we report the production of a 18 × 18 × 24 nm^3 hollow DNA box origami structure with a switchable lid. The structure was efficiently produced and characterized by atomic force microscopy, transmission electron microscopy, and Förster resonance energy transfer spectroscopy. The DNA box has a unique reclosing mechanism, which enables it to repeatedly open and close in response to a unique set of DNA keys. This DNA device can potentially be used for a broad range of applications such as controlling the function of single molecules, controlled drug delivery, and molecular computing.



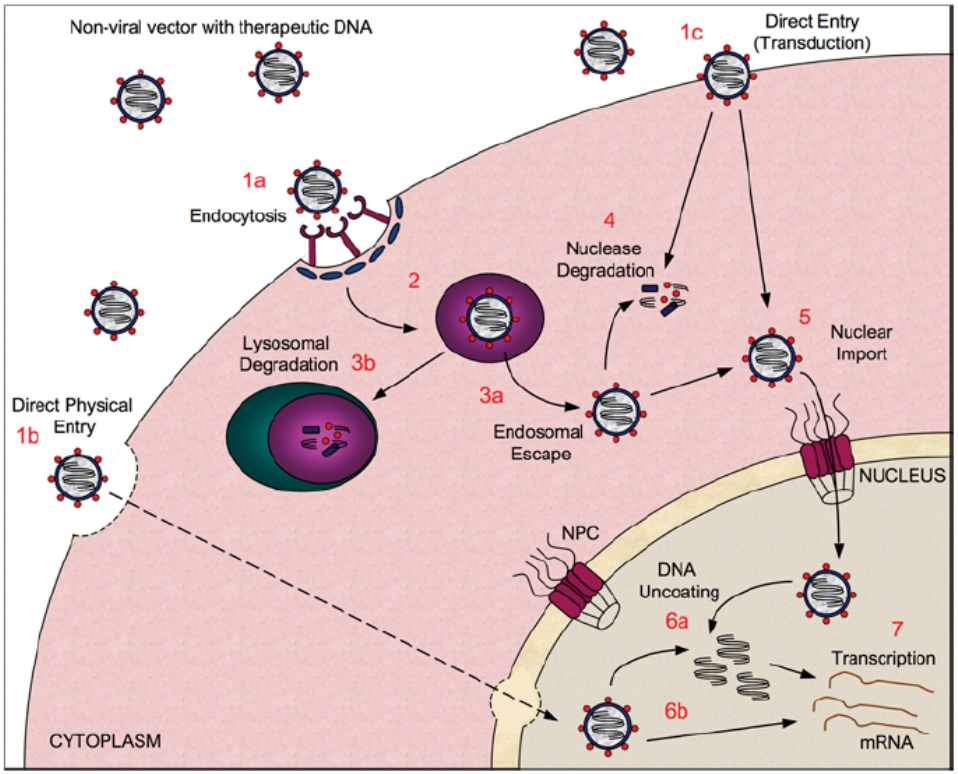

a
100 nm
b
24
nm
18 nm
18 nm
c
20 nm


a
Cy3
Locks
Cy5
Keys
Locked state
Open state
d
Cy3
Locks
Cy5
Opening keys
K1O & K2O
Closing keys
K1C & K2C
Locked state
Open state
b
Donor's emission
Acceptor's emission
Normalized Fluorescent Intensity
250000
200000
150000
100000
50000
0
550
600
650
700
Wavelength (nm)
c
+Keys
FRET efficiency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Locked state
Open state
e
Setup 1
Setup 2
K1O K2O
K1C K2C K1O K2O
FRET efficiency
Locked
Open
Round 1
Round 2
Round 3 *
[Keys]μM
0 0.4 0.6 0.8 1.2 1.6

Gene Delivery

- Transfection- the delivery of foreign molecules such as DNA and RNA into eukaryotic cells
- Naked DNA is not suitable for in-vivo transport of genetic materials-> degradation by serum nucleases
- Ideal gene delivery system
  - Biocompatible
  - Non-immunogenic
  - Stable in blood stream
  - Protect DNA during transport
  - Small enough to extravagate
  - Cell and tissue specific

Barrier to non-viral gene delivery



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NLS-mediated nuclear import

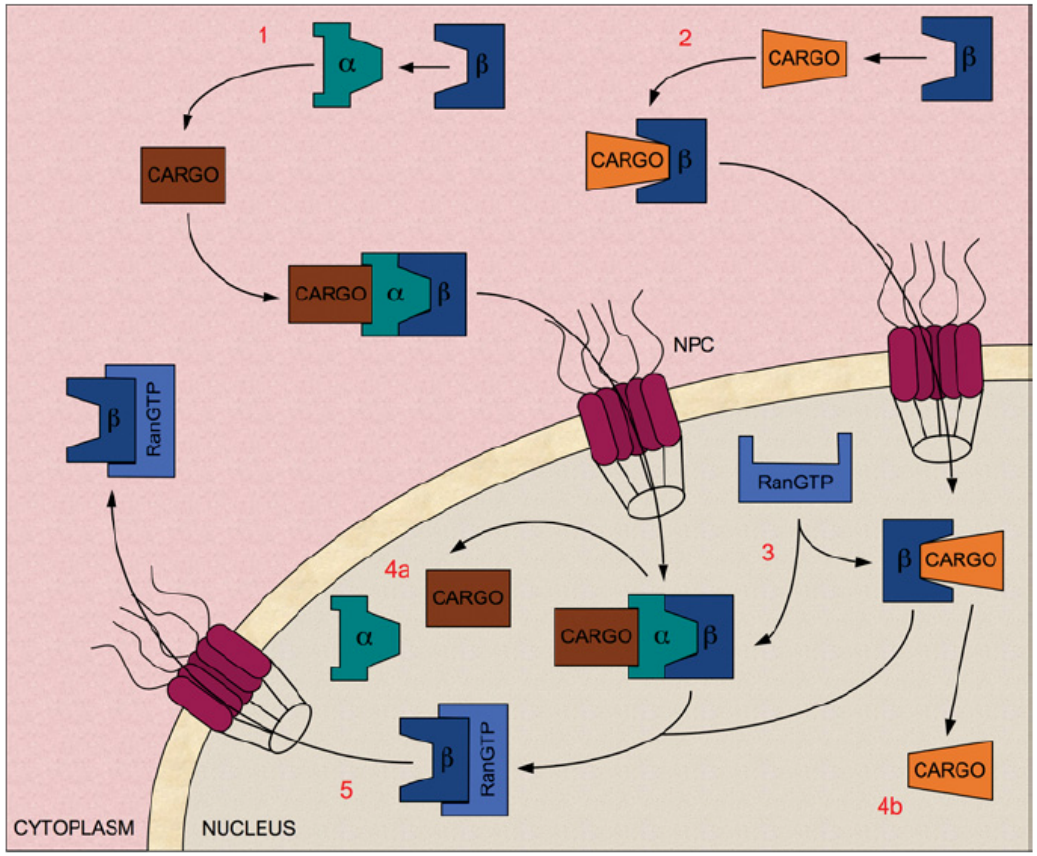


Figure 2 NLS-mediated nuclear import pathways

In classical nuclear import, the NLS found in cargo bound for the nucleus is recognized by the Imp α subunit of the Imp α/β heterodimer (1). However, there are also many examples where Imp β or one of its many homologues can mediate nuclear import or cargo proteins independently of Imp α (2). In both cases, transient interactions between the Imp β and the nucleoporin proteins that line the NE-embedded NPCs mediate translocation into the nucleus. Once inside, RanGTP binds to Imp β (3), releasing Imp α and the cargo into the nucleoplasm (4a and 4b). RanGTP itself is then recycled back to the cytoplasm (5), where it is converted into its RanGDP state (not shown). An animated version of this Figure can be found at http://www.BiochemJ.org/bj/406/0185/bj4060185add.htm

Non-viral Gene Delivery

- Transfection- the delivery of foreign molecules such as DNA and RNA into eukaryotic cells
- Naked DNA is not suitable for in-vivo transport of genetic materials-> degradation by serum nucleases
- Ideal gene delivery system
  - Biocompatible
  - Non-immunogenic
  - Stable in blood stream
  - Protect DNA during transport
  - Small enough to extravagate
  - Cell and tissue specific

CANCER NANOTECHNOLOGY: OPPORTUNITIES AND CHALLENGES

Summary

- Nanotechnology concerns the study of devices that are themselves or have essential components in the 1–1,000 nm dimensional range (that is, from a few atoms to subcellular size).
- Two main subfields of nanotechnology are nanovectors — for the administration of targeted therapeutic and imaging moieties — and the precise patterning of surfaces.
- Nanotechnology is no stranger to oncology: liposomes are early examples of cancer nanotherapeutics, and nanoscale-targeted magnetic resonance imaging contrast agents illustrate the application of nanotechnology to diagnostics.
- Photolithography is a light-directed surface-patterning method, which is the technological foundation of microarrays and the surface-enhanced laser desorption/ionization time-of-flight approach to proteomics. Nanoscale resolution is now possible with photolithography, and will give rise to instruments that can pack a much greater density of information than current biochips.
- The ability of nanotechnology to yield advances in early detection, diagnostics, prognostics and the selection of therapeutic strategies is predicated based on its ability to 'multiplex' — that is, to detect a broad multiplicity of molecular signals and biomarkers in real time. Prime examples of multiplexing detection nanotechnologies are arrays of nanocantilevers, nanowires and nanotubes.
- Multifunctionality is the fundamental advantage of nanovectors for the cancer-specific delivery of therapeutic and imaging agents. Primary functionalities include the avoidance of biobarriers and biomarker-based targeting, and the reporting of therapeutic efficacy.
- Thousands of nanovectors are currently under study. By systematically combining them with preferred therapeutic and biological targeting moieties it might be possible to obtain a very large number of novel, personalized therapeutic agents.
- Novel mathematical models are needed, in order to secure the full import of nanotechnology into oncology.

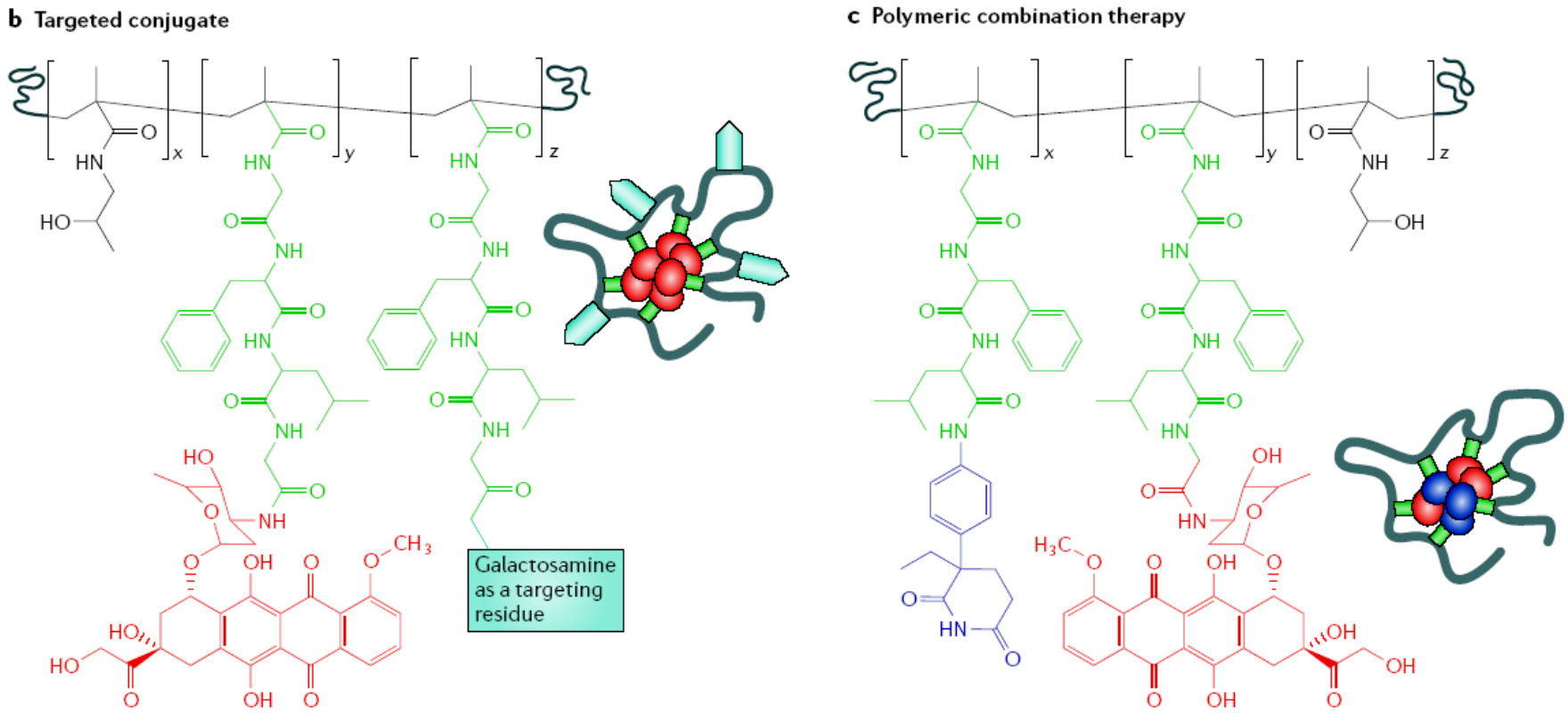


Figure 1 | **Polymer–anticancer drug conjugates.** Each panel shows both the detailed chemical structure and a cartoon of the general structure. The polymer backbone is shown in black, linker region in green, drug in red and additional components (for example, a targeting residue) in blue. **a** | Two examples of more 'simple' polymer–drug conjugates containing doxorubicin (left) and paclitaxel (right) that have progressed to clinical trial. **b** | A multivalent receptor-targeted conjugate containing galactosamine (light blue) to promote liver targeting. **c** | Polymer combination therapy containing the aromatase inhibitor aminogluthethimide (red) and doxorubicin (blue).

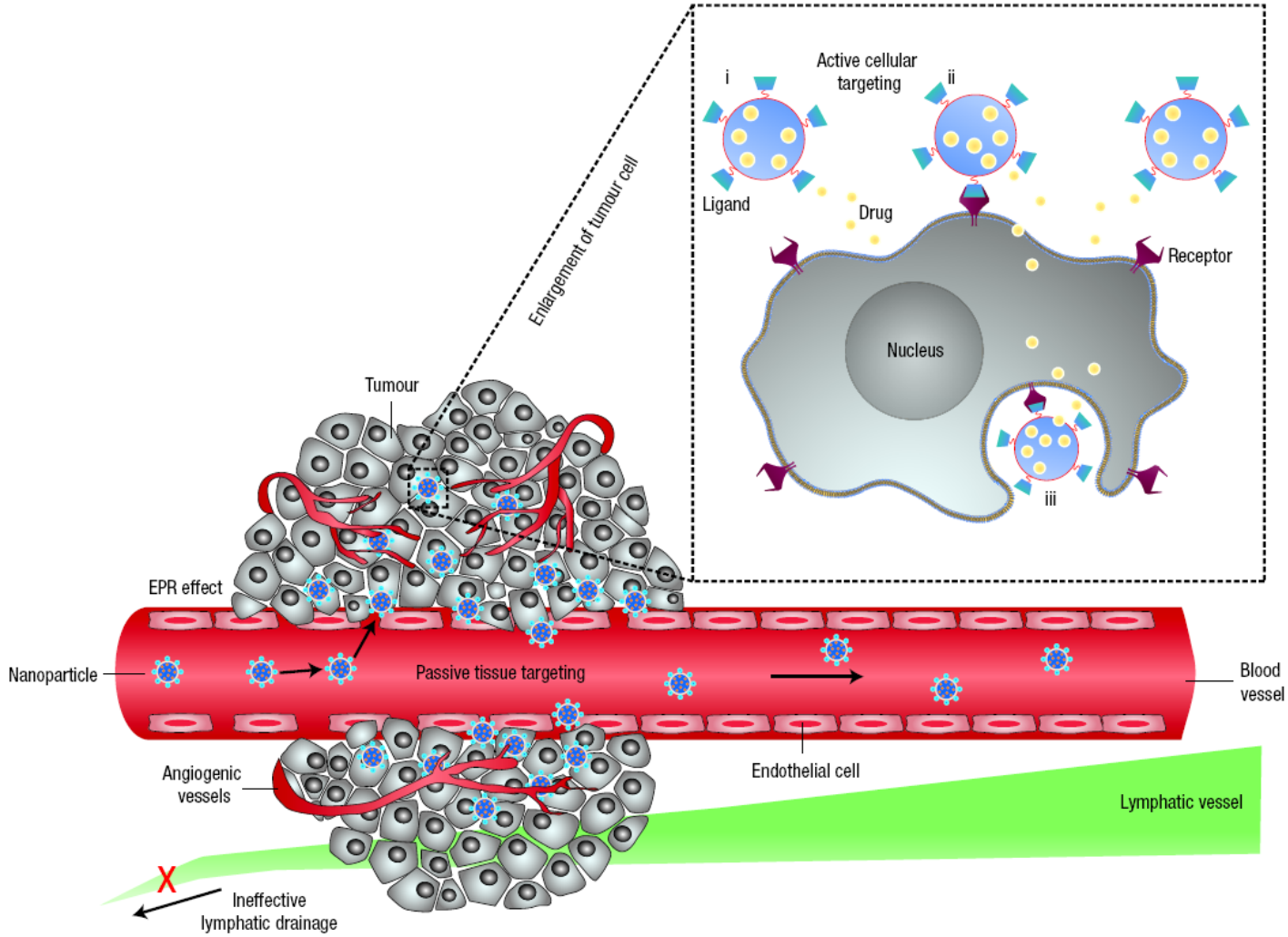
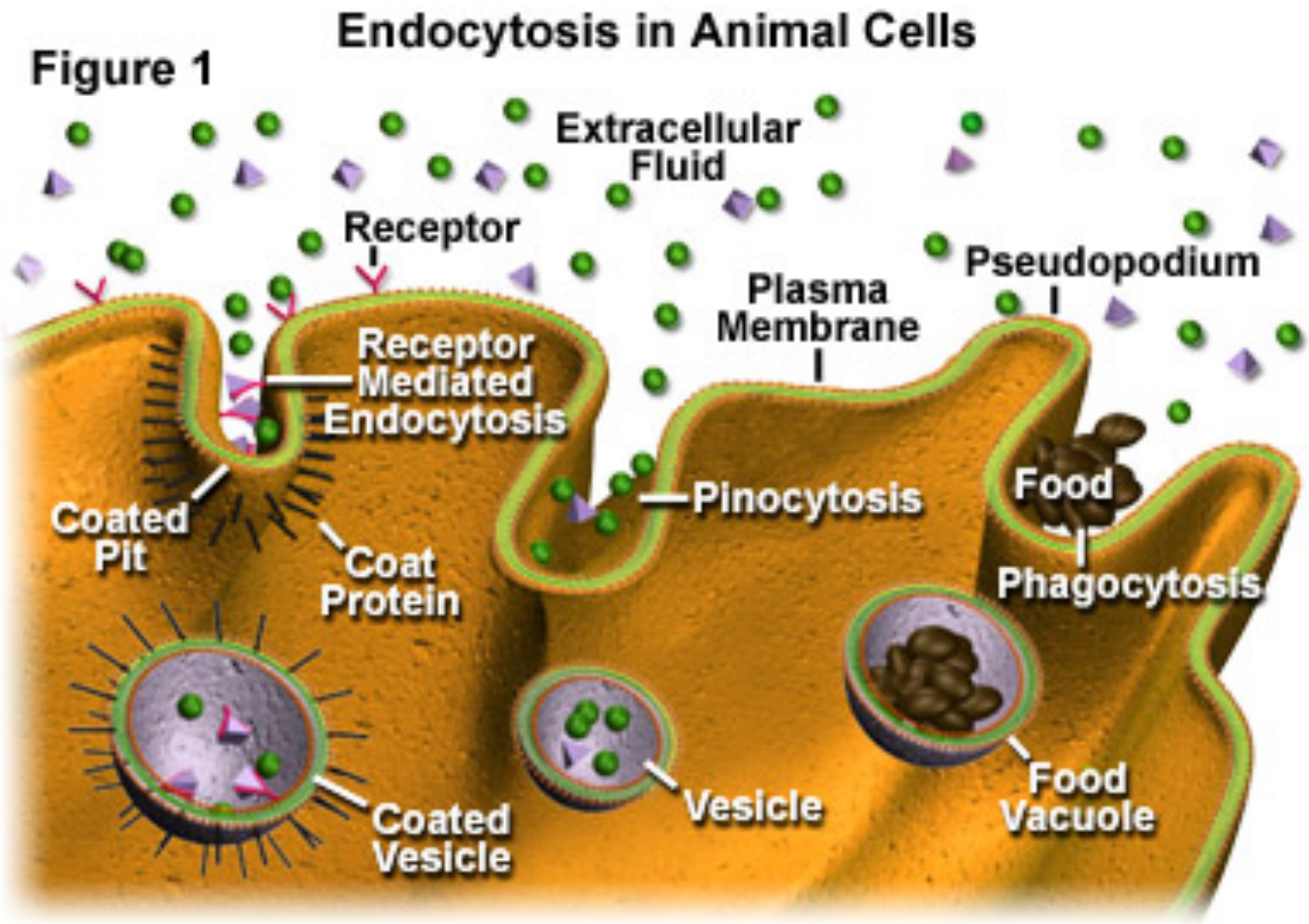


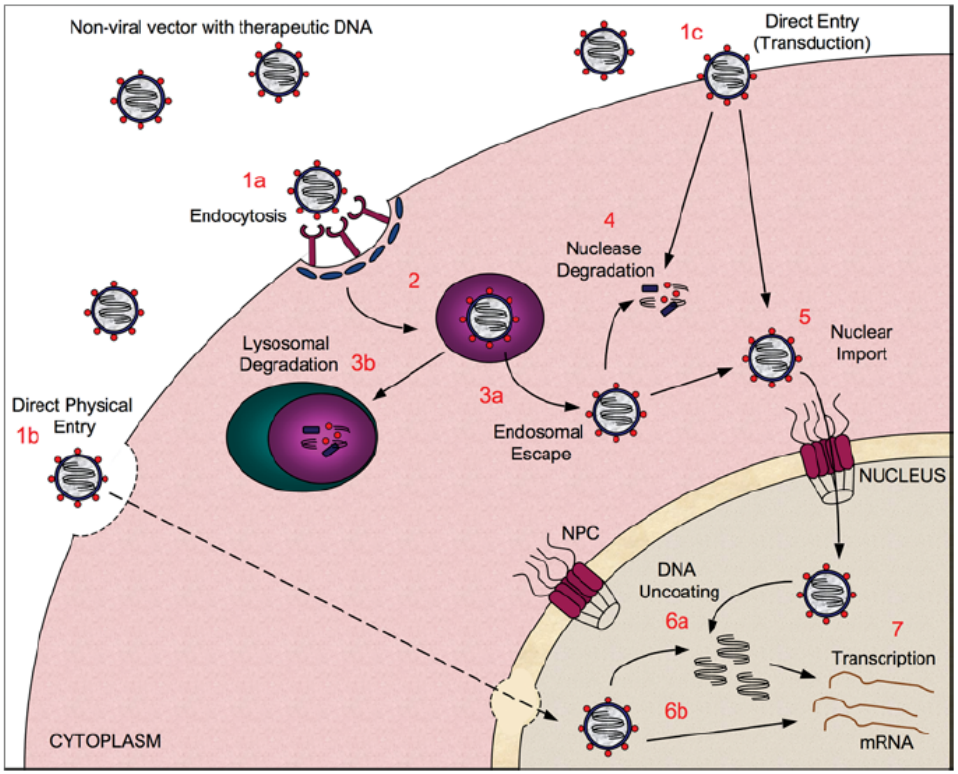
Figure 1 Schematic representation of different mechanisms by which nanocarriers can deliver drugs to tumours. Polymeric nanoparticles are shown as representative nanocarriers (circles). Passive tissue targeting is achieved by extravasation of nanoparticles through increased permeability of the tumour vasculature and ineffective lymphatic drainage (EPR effect). Active cellular targeting (inset) can be achieved by functionalizing the surface of nanoparticles with ligands that promote cell-specific recognition and binding. The nanoparticles can (i) release their contents in close proximity to the target cells; (ii) attach to the membrane of the cell and act as an extracellular sustained-release drug depot; or (iii) internalize into the cell.

Endocytosis



Barrier to non-viral gene delivery

<45 kDa
< 250 bp



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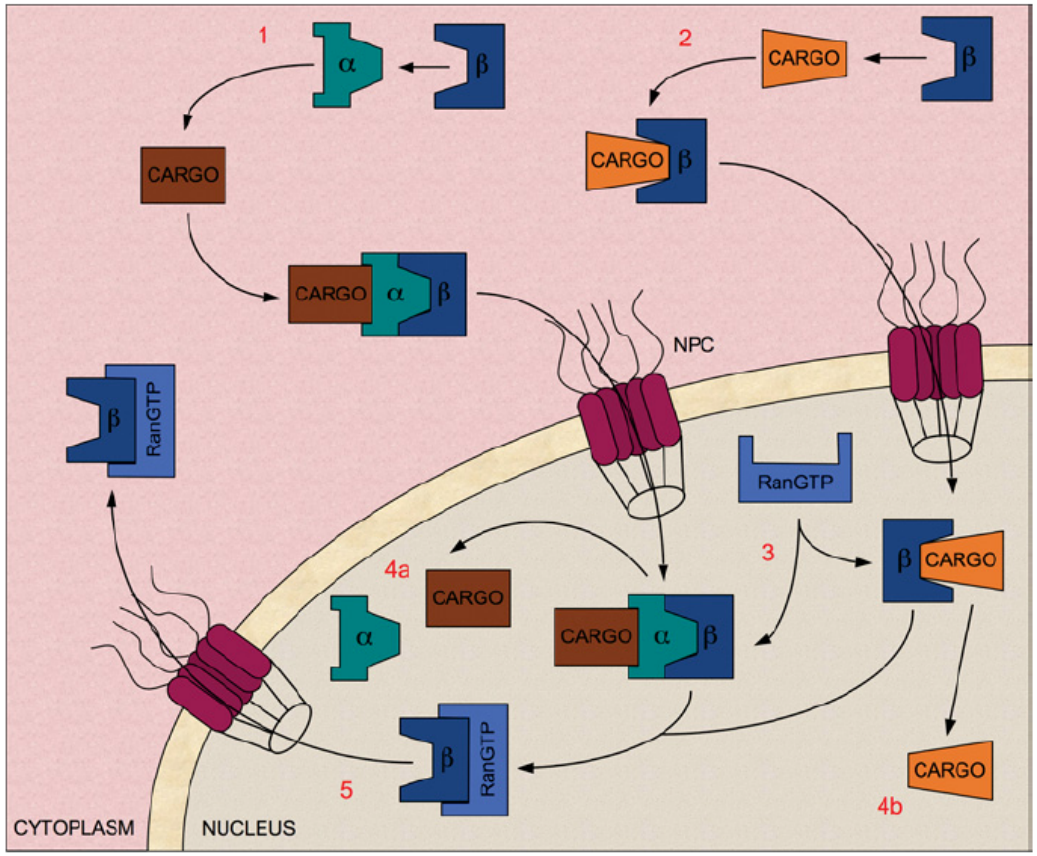

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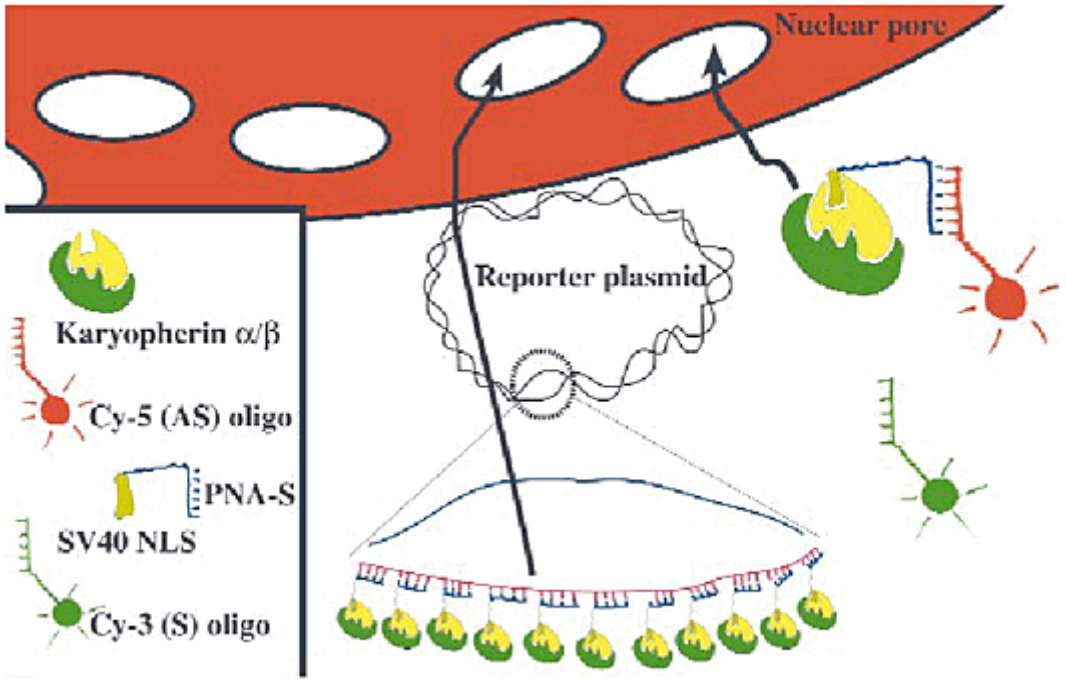
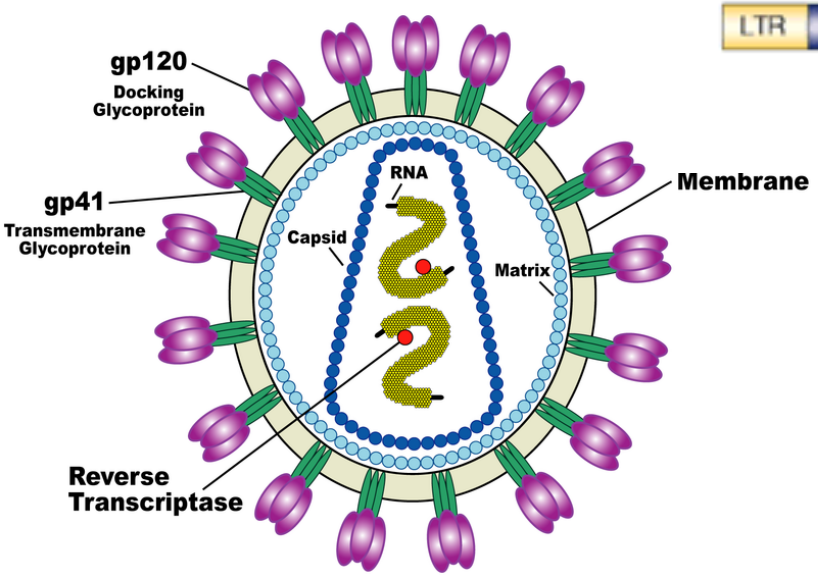
NLS Peptide


Nuclear pore
Reporter plasmid
Karyopherin α/β
Cy-5 (AS) oligo
PNA-S
SV40 NLS
Cy-3 (S) oligo

Tat Protein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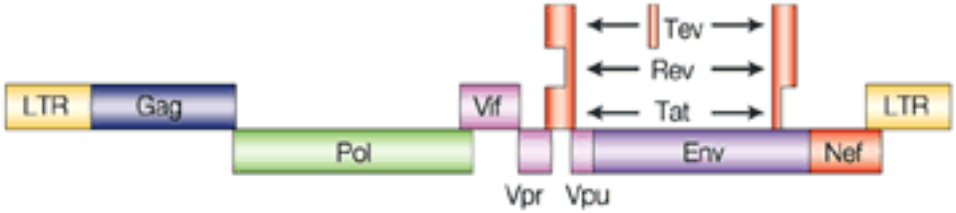
Docking
Glycoprotein
gp41
Transmembrane
Glycoprotein
RNA
Capsid
Matrix
Membrane
Reverse
Transcriptase


LTR
Gag
Pol
Vif
Vpr
Vpu
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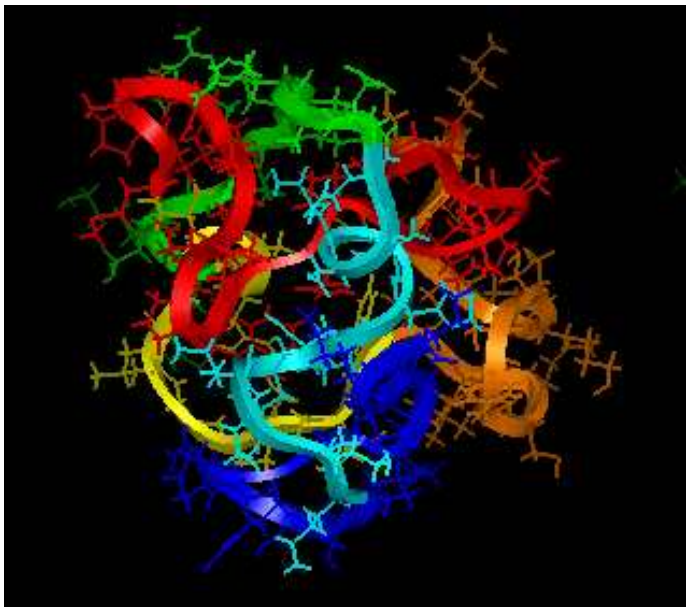
Rev
Tat
Env
Nef
LTR



Nanoparticles

- 1-100 nm
- High surface/volume ratio
- Surface charge and size of nanoparticles
- Advantage
  - Large payload
  - Multiple targeting ligands
  - Multiple drug

Table 1 | **Nanoscaled systems for systemic cancer therapy**

Platform	Latest stage of development	Examples
Liposomes	Approved	DaunoXome, Doxil
Albumin-based particles	Approved	Abraxane
PEGylated proteins	Approved	Oncospar, PEG-Intron, PEGASYS, Neulasta
Biodegradable polymer–drug composites	Clinical trials	Doxorubicin Transdrug
Polymeric micelles	Clinical trials	Genexol-PM*, SP1049C, NK911, NK012, NK105, NC-6004
Polymer–drug conjugate-based particles	Clinical trials	XYOTAX (CT-2103), CT-2106, IT-101, AP5280, AP5346, FCE28068 (PK1), FCE28069 (PK2), PNU166148, PNU166945, MAG-CPT, DE-310, Pegamotecan, NKTR-102, EZN-2208
Dendrimers	Preclinical	Polyamidoamine (PAMAM)
Inorganic or other solid particles	Preclinical (except for gold nanoparticle that is clinical)	Carbon nanotubes, silica particles, gold particles (CYT-6091)

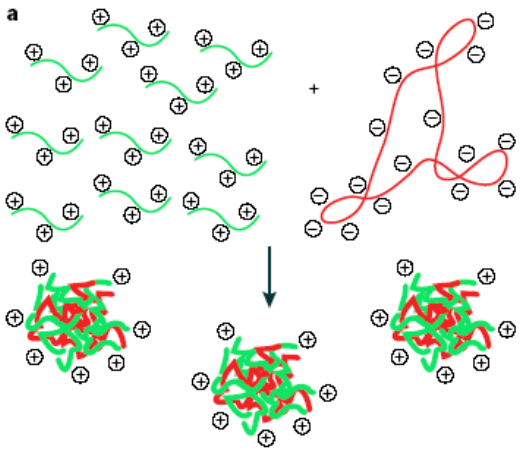


Figure 1 | **Polyplex formation.** Polyplexes are formed by electrostatic interactions between polycations and DNA. **a** | When aqueous solutions of a polycation and DNA are mixed, polyplexes form spontaneously. The interaction is entropically driven. For gene delivery, an excess of polycation is typically used, which generates particles with a positive surface charge. Each particle consists of several plasmid DNA molecules and hundreds of polymer chains and is 100–200 nm in diameter. **b** | Transmission electron micrograph of polyplexes comprising plasmid DNA and a polycation, in this case cyclodextrin-modified, branched polyethylenimine (PEI)[165]. Scale bar = 200 nm.

Box 1 | **Design criteria for non-viral vectors**

- Protection of DNA
- Packaging of large DNA plasmids
- Easy administration
- Serum stability
- Targetability to specific cell types
- Ease of fabrication
- Inexpensive synthesis
- Facile purification
- Robustness/stability
- Internalization
- Endolysosomal escape
- Nuclear transport
- Efficient unpackaging
- Infection of non-dividing cells
- Safety
- Non-toxic
- Non-immunogenic
- Non-pathogenic

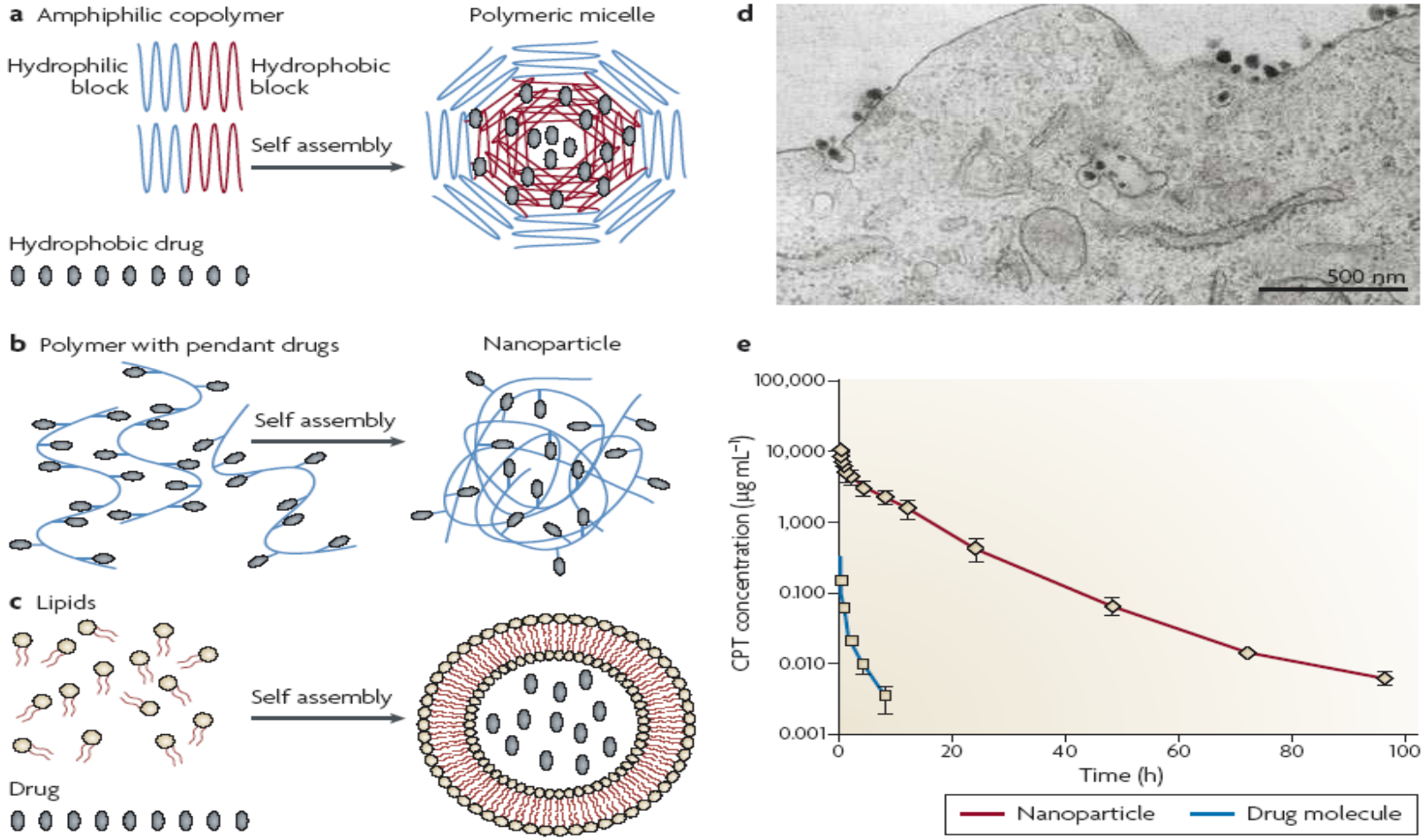


Figure 1 | **Major classes of nanoparticles that are in clinical trials and some of their properties. a** | Nanoparticles formed from therapeutic entities and block copolymers that can form polymeric micelles. **b** | Nanoparticles that form with polymer–drug conjugates. **c** | Nanoparticles formed of liposomes. **d** | Nanoparticles can enter cells via endocytosis. This figure shows a transmission electron micrograph of nanoparticles at the surface of a cancer cell, entering the cell and within endocytic vesicles. **e** | Nanoparticles can have extended pharmacokinetics over the therapeutic entity alone. The data are from a small-molecule drug (CPT) and a nanoparticle containing CPT in rats. Panel (**e**) is adapted with permission from REF. 94 © Springer (2006).

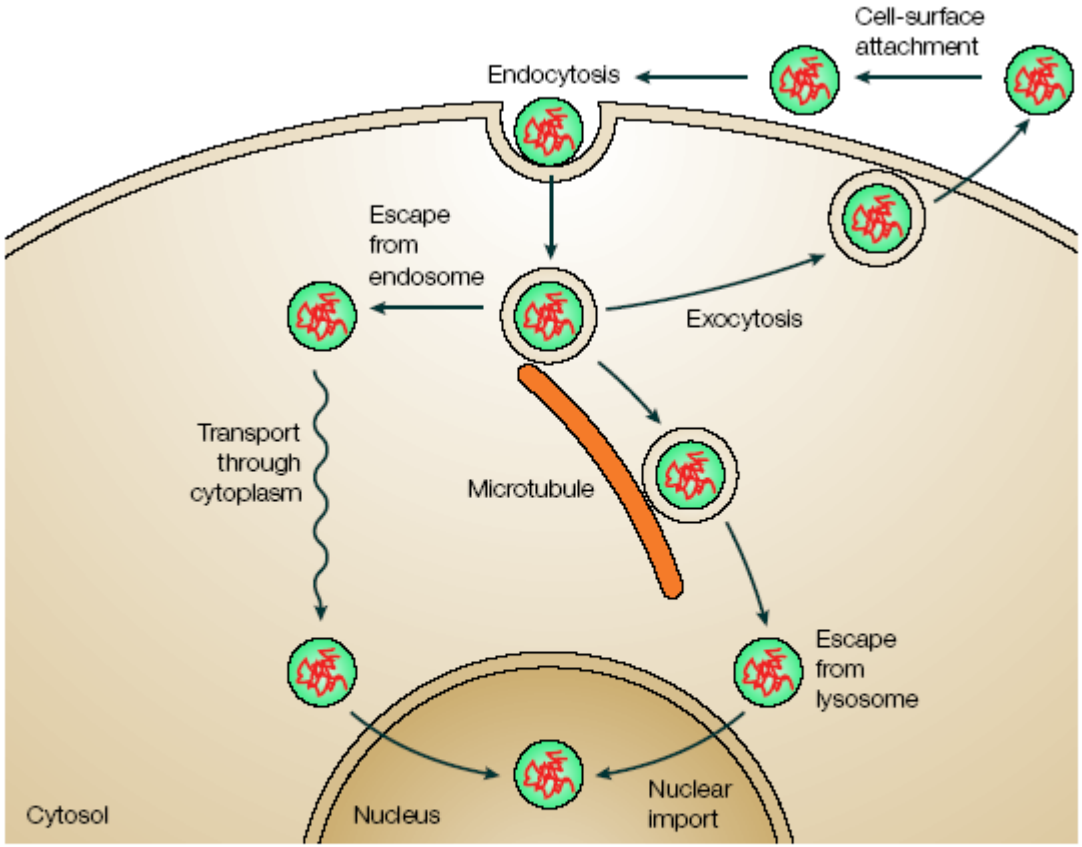


Figure 2 | **Barriers to intracellular trafficking of polyplexes.** Polyplexes must attach to the cell surface, be internalized (by endocytosis), escape from endolysosomes, move through the cytoplasm toward the nucleus and cross the nuclear membrane. Alternative pathways exist for several of these steps. In addition, the polyplexes must unpackage — DNA must be released by the polymer — but where unpackaging occurs is not known.

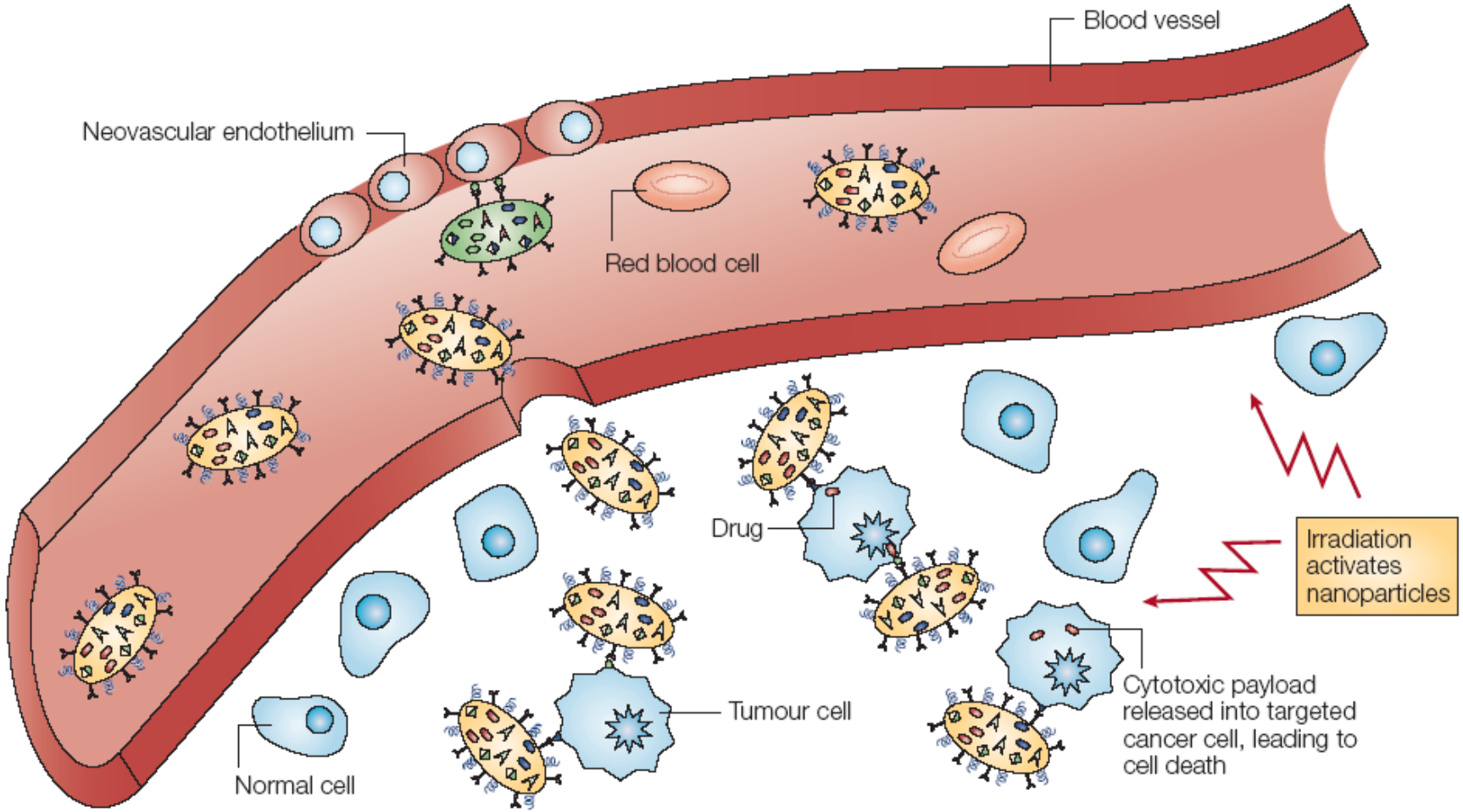


Figure 4 | **Multicomponent targeting strategies.** Nanoparticles extravasate into the tumour stroma through the fenestrations of the angiogenic vasculature, demonstrating targeting by enhanced permeation and retention. The particles carry multiple antibodies, which further target them to epitopes on cancer cells, and direct antitumour action. Nanoparticles are activated and release their cytotoxic action when irradiated by external energy. Not shown: nanoparticles might preferentially adhere to cancer neovasculature and cause it to collapse, providing anti-angiogenic therapy. The red blood cells are not shown to scale; the volume occupied by a red blood cell would suffice to host 1–10 million nanoparticles of 10 nm diameter.

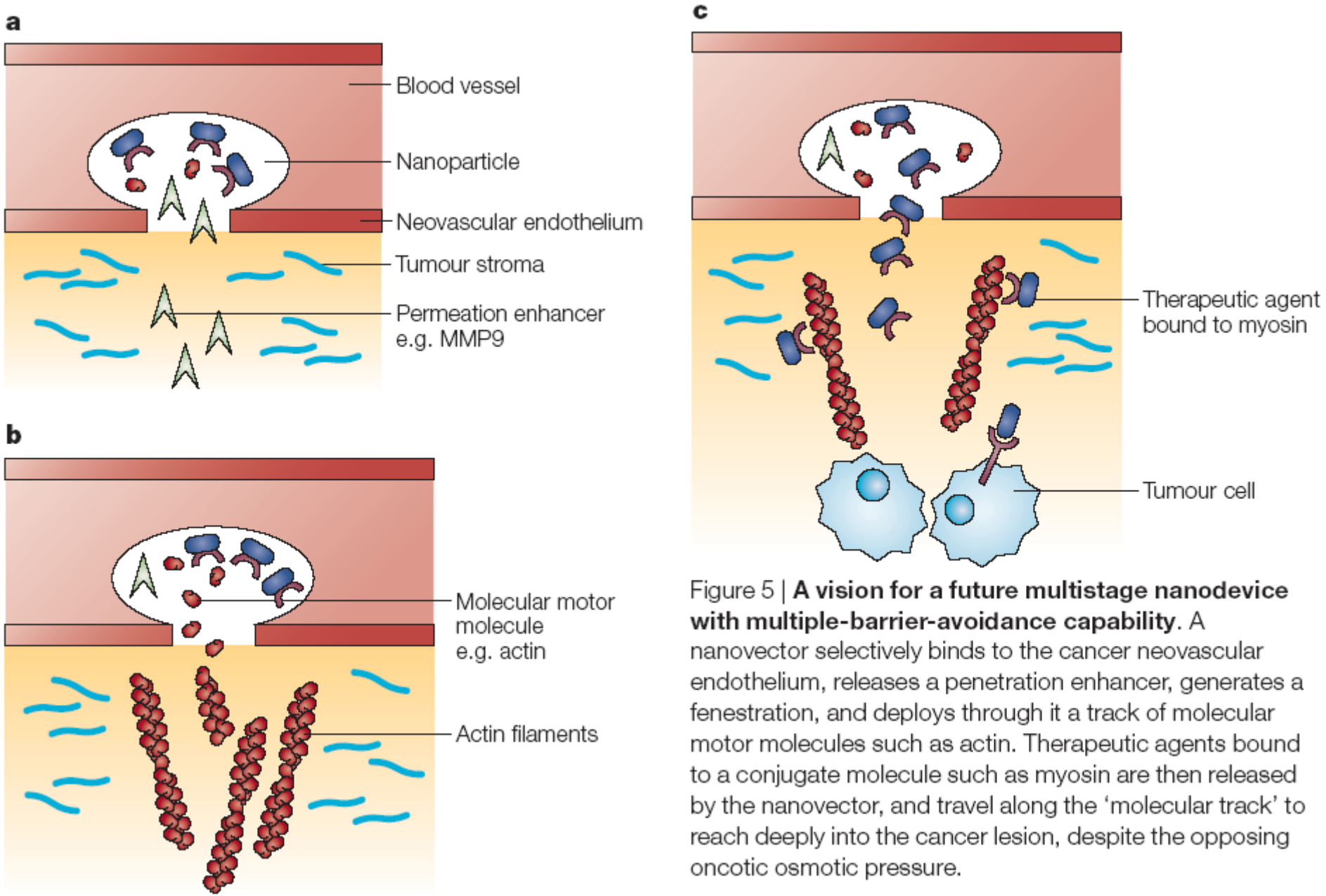


Figure 5 | **A vision for a future multistage nanodevice with multiple-barrier-avoidance capability**. A nanovector selectively binds to the cancer neovascular endothelium, releases a penetration enhancer, generates a fenestration, and deploys through it a track of molecular motor molecules such as actin. Therapeutic agents bound to a conjugate molecule such as myosin are then released by the nanovector, and travel along the 'molecular track' to reach deeply into the cancer lesion, despite the opposing oncotic osmotic pressure.

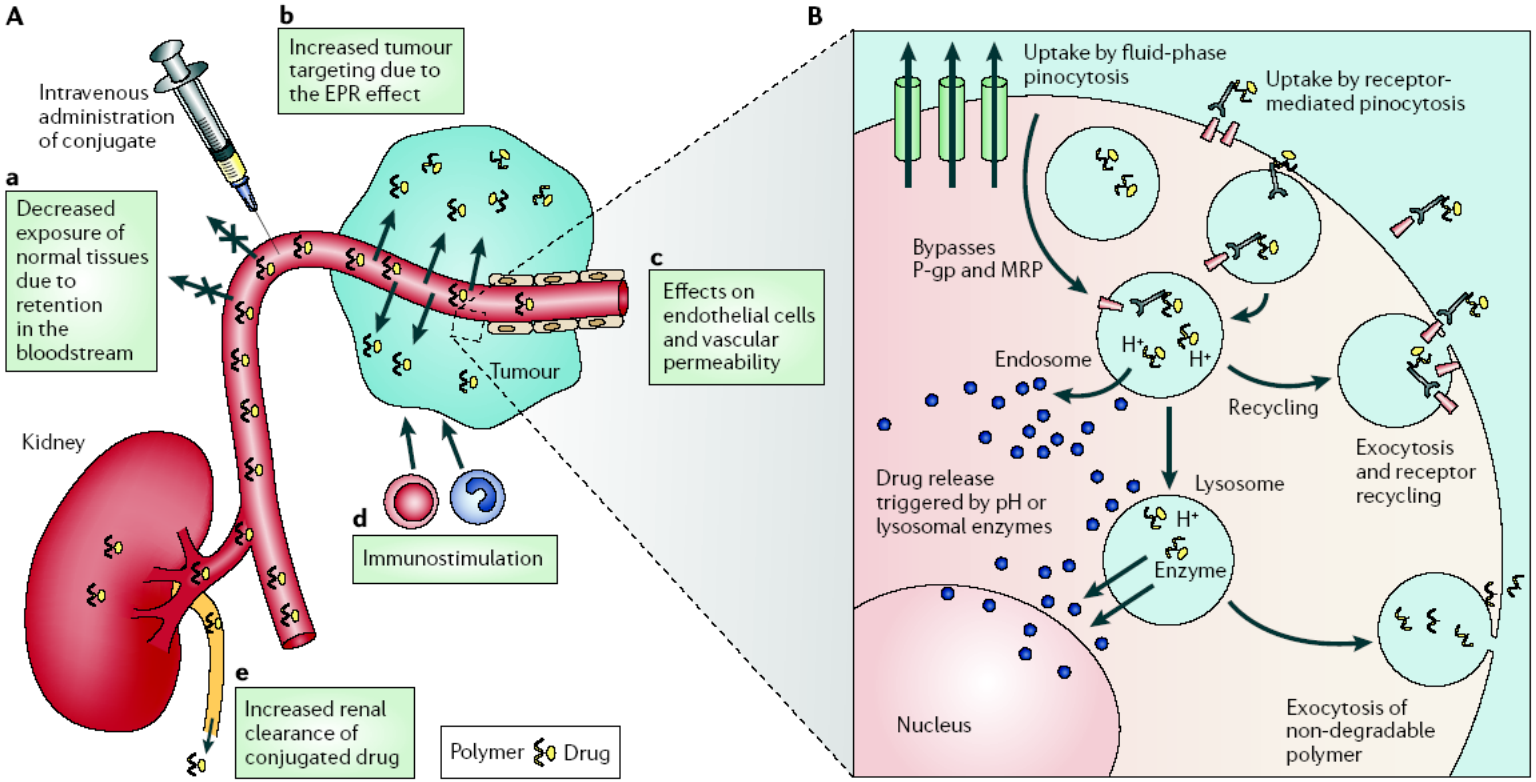
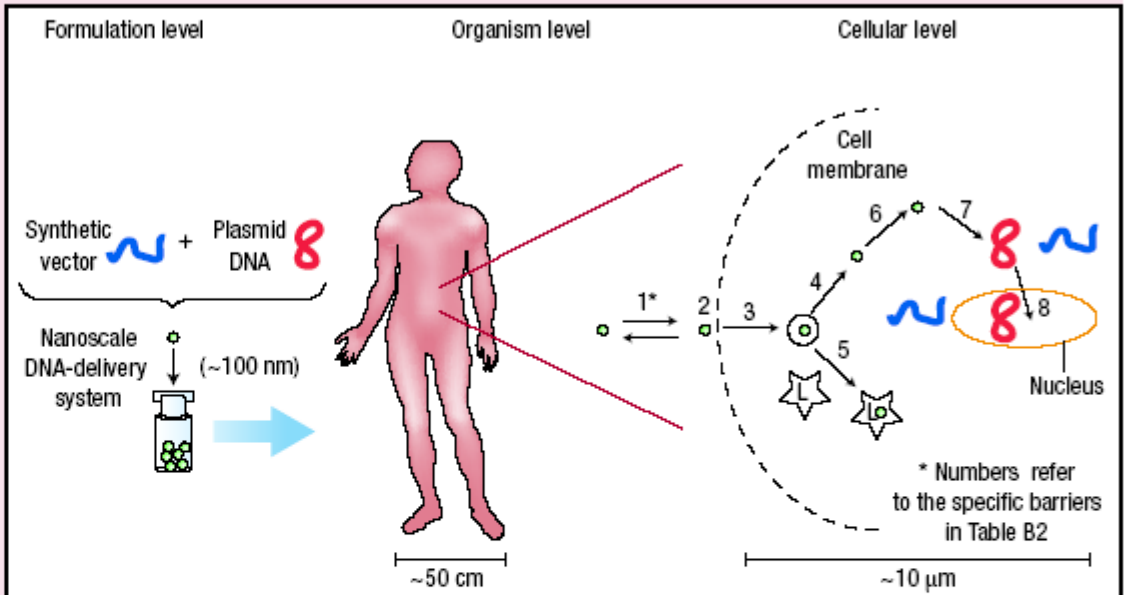


Figure 2 | **Current understanding of the mechanism of action of polymer–drug conjugates. A** | Hydrophilic polymer–drug conjugates administered intravenously can be designed to remain in the circulation — their clearance rate depends on conjugate molecular weight, which governs the rate of renal elimination. **a** | Drug that is covalently bound by a linker that is stable in the circulation is largely prevented fom accessing normal tissues (including sites of potential toxicity), and biodistribution is initially limited to the blood pool. **b** | The blood concentration of drug conjugate drives tumour targeting due to the increased permeability of angiogenic tumour vasculature (compared with normal vessels), providing the opportunity for passive targeting due to the enhanced permeability and retention effect (EPR effect). **c** | Through the incorporation of cell-specific recognition ligands it is possible to bring about the added benefit of receptor-mediated targeting of tumour cells. **d** | It has also been suggested that circulating low levels of conjugate (slow drug release) might additonally lead to immunostimulation. **e** | If the polymer–drug linker is stable in the circulation, for example, *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–Gly-Phe-Leu-Gly–doxorubicin, the relatively high level of renal elimination (whole body $t_{1/2}$ clearance >50% in 24 h) compared with free drug ($t_{1/2}$ clearance ~50% in 4 days) can increase the elimination rate. **B** | On arrival in the tumour interstitium, polymer-conjugated drug is internalized by tumour cells through either fluid-phase pinocytosis (in solution), receptor-mediated pinocytosis following non-specific membrane binding (due to hydrophobic or charge interactions) or ligand–receptor docking. Depending on the linkers used, the drug will usually be released intracellularly on exposure to lysosomal enzymes (for example, Gly-Phe-Leu-Gly and polyglutamic acid (PGA) are cleaved by cathepsin B) or lower pH (for example, a hydrazone linker degrades in endosomes and lysosomes (pH 6.5–<4.0). The active or passive transport of drugs such as doxorubicin and paciltaxel out of these vesicular compartments ensures exposure to their pharmacological targets. Intracellular delivery can bypass mechanisms of resistance associated with membrane efflux pumps such as p-glycoprotein. If >10-fold, EPR-mediated targeting will also enable the circumvention of other mechanisms of drug resistance. Non-biodegradable polymeric platforms must eventually be eliminated from the cell by exocytosis. Rapid exocytic elimination of the conjugated drug before release would be detrimental and prevent access to the therapeutic target. In general, polymeric carriers do not access the cytosol. MRP, multidrug resistance protein.

Barriers to DNA Delivery

BOX 1

A number of challenges and barriers face the successful delivery of therapeutic DNA to target cells in the body. Physicochemical, economic and sterilization challenges complicate formulation; the complex environment of the human body hinders its successful transport to the target cell population; and endocytic pathway barriers hinder its successful transport to the nucleus of the cell (the site of action). Each known and major barrier is listed in Fig. B1, using nanoscale DNA-delivery systems as representative examples. Each barrier exists independent of length scale. L = lysosome. A number of clever systems have been devised to overcome these barriers, the general design criteria of which are given in Tables B1 and B2.



Barriers / challenges / needs

Formulation	Organism	Cellular
Economically viable	Prolonged circulation time	Transport to cell surface
FDA-approvable	Stability in blood circulation	Cellular internalization
Acceptable shelf-life	Access to target tissue/cells	Intracellular transport
	Elimination from body	Enzymatic degradation
	Mininal toxicity	Nuclear entry

Figure B1 Barriers to DNA delivery.

Organism Level			
Barrier/challenge/need	**Rationale**	**Example approaches**	**Materials design criteria**
Prolonged circulation time	Maximize total flux past target cell type	PEG conjugates to minimize interaction with serum proteins	Hydrophilic Uncharged
Stability within blood circulation	Maintenance of designed functionality	Crosslinking to maximize overall stability	Stable crosslinks within bloodstream, but reversible upon entry into target cell
Access to target tissue/cells	Transport from capillary lumen to extracellular space to reach target cell surface	Vaso-active protein conjugates (for example, vascular endothelial growth factor)	Retention of protein activity post conjugation
		Targeting restricted to 'leaky' vessel tissues (for example, tumour, liver, spleen).	Small diameter delivery system (for example, <100 nm)
Elimination from body	Minimal build-up of delivery vector over time	Control over molecular weight	Filterable through kidneys
		Engineered biodegradation sites	Biodegradable
Minimal toxicity and immunogenicity	Safety over treatment duration and beyond that required for FDA-approval	Minimize cation density	Non-cytotoxic
		Avoid protein-based materials/conjugates	Non-immunogenic

Cellular Level

Barrier number (from Fig. B1)	Barrier/challenge/need	Example approaches	Materials design criteria
1, 2 and 3	Transport to cell surface, association with cell membrane, internalization	Receptor/ligand interaction (for example, antibody/polymer conjugates, recombinant protein–polymer fusions, carbohydrate conjugates)	Cell-type specificity, low cross reactivity, if desired
			Promiscuous attachment, high cross reactivity, if desired (for example, positive zeta potential, lipid conjugation)
		Non-specific interaction with cell surface (for example, positive zeta potential, lipid conjugates)	
			Endocytic pathway trigger (for example, clathrin-dependent, clathrin-independent, caveolin-dependent)
4 and 5	Escape endosomal vesicle and avoid transport to lysosome	Buffering capacity between pH ~7.2 and ~5.0	Ability to disrupt endosomal membrane and/or fusion of endosome with lysosome
		Fusogenic peptide conjugate	
6	Transport through cytosol to perinuclear space with minimal degradation	'Higher' molecular weight to maintain complex stability within cytosol	Thermodynamic and kinetic stability of complex within cytosol
			Minimize DNA degradation within cytosol
7	Separation of complex to allow nuclear translocation	Hydrolytically or reductively degradable polymers to reduce molecular weight	'Triggered' degradation of polymer to reduce thermodynamic and kinetic stability of complex. Release of intact DNA at or near nuclear envelope
8	Nuclear entry	Nuclear localization sequence conjugates	Facilitate nuclear uptake of DNA using virus-derived signals
		Mitosis	Facilitate nuclear uptake during mitosis when the nuclear envelope is dissolved.