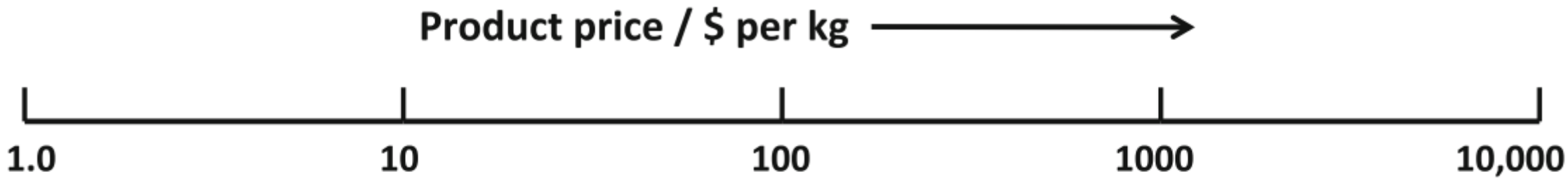
Future of Bioscience and Biotechnology

Topics to be covered today:

- Cell-free biosystems for biomanufacturing
- DNA molecular digital data storage
- Applications of biosensors

Cell-free biosystems for biomanufacturing

- Biomanufacturing : manufacturing the desired products by using living biological organisms (e.g., bacteria, yeasts, plants)
- Potential products that could be produced through biomanufacturing
  - biocommodities ($0.3–several US dollars per kg)
  - specialties and biomaterials (tens of dollars per kg)
  - fine chemicals (hundreds of dollars per kg)
  - pharmaceuticals (thousands of dollars/kg)
  - protein drugs (more than tens of thousands of dollars per kg)



Biocommodities
Hydrogen
Alcohols
Fructose
Synthetic starch as food and feed

Specialties & biomaterials
Xylitol
Synthetic amylose

Fine chemicals
Chiral alcohols
Carbohydrates (natural or non -natural)

Pharmaceuticals
Heparin
Heparan sulfate
Antibodies
Special proteins/peptides

History of Biomanufacturing

- Can be classified into two platforms: living organisms and cell-free biosystems.
- Microorganisms have been utilized for several thousand years to produce a number of products
  - With genetic engineering, protein engineering, systems biology, and synthetic biology living organisms are modified to produce natural products at high yields or produce non-natural products

- In 1897, Eduard Buchner discovered a yeast extract (not living yeast) that can convert glucose to ethanol, leading to his Nobel Prize in Chemistry in 1907.
- Enzyme-based biotransformation became a manufacturing tool approximately 50 years after the discovery of enzymes:
  - Phase 1 (1960s)—one-enzyme biotransformation
    - Production of fructose syrup using glucose isomerase in 1967
  - Phase 2 (1990s)—multienzyme one pot for relatively complicated biotransformation
    - enzymatic hydrolysis of crystalline cellulose requires a synergetic action of endoglucanase, cellobiohydrolase, and beta-glucosidase because a single enzyme cannot hydrolyze cellulose efficiently
  - Phase 3 (2000s)—the utilization of numerous enzymes (i.e., more than three) for implementing very complicated biotransformations.

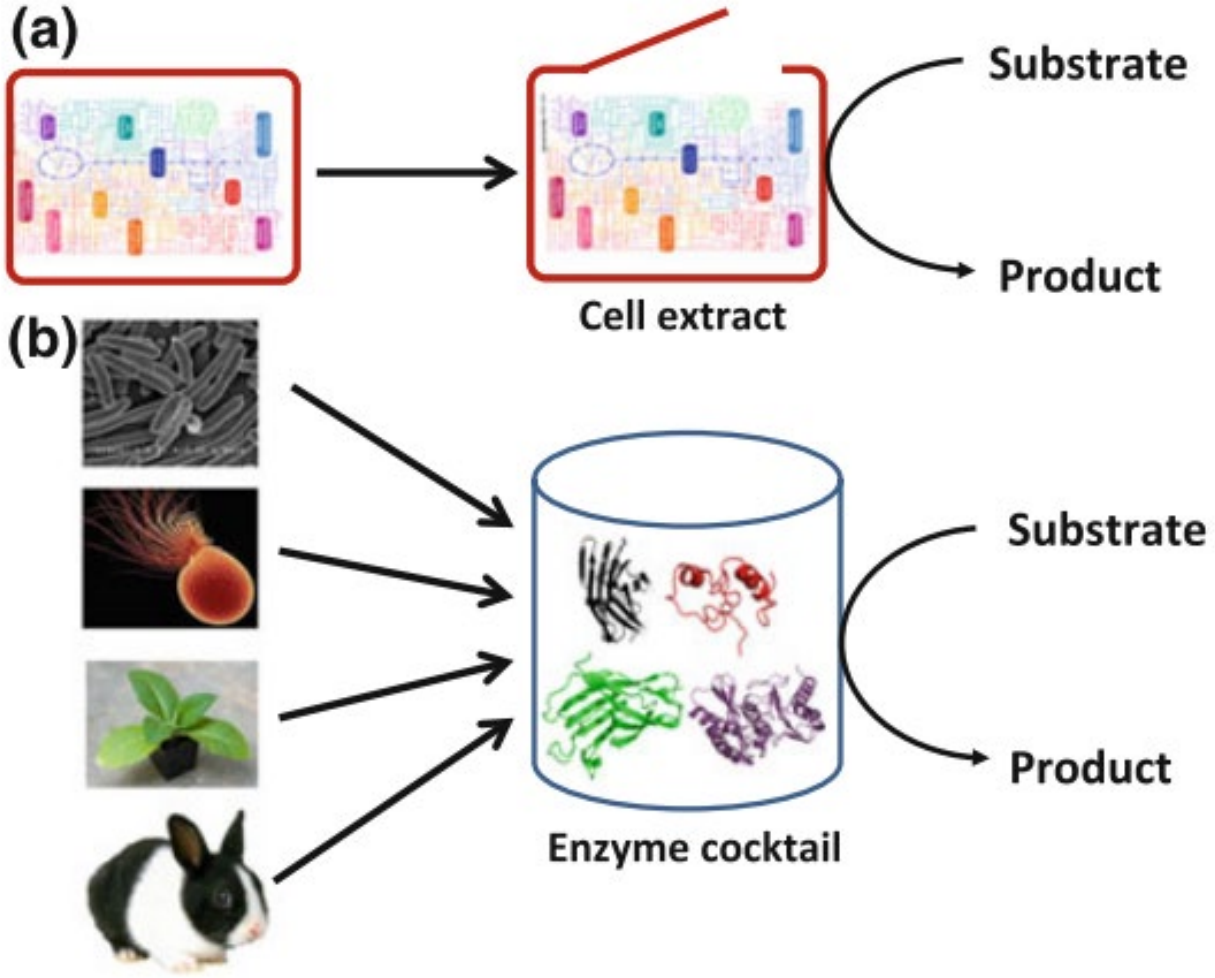
Cell-free biosystems for biomanufacturing

- Cell-free biosystems have many advantages over living-organism biomanufacturing, such as high product yield, fast reaction rate, high product titer, unprecedented level of control and freedom of design, and broad reaction conditions.
- Cell-free biomanufacturing usually gives a yield of the desired product that is often close to a theoretical value
- Higher reaction rates are believed to be possible for cell-free systems than living organisms because
  - (i) neither cell membrane nor wall is present to slow down substrate/product transport,
  - (ii) no energy is needed for transport of substrate/product across the membrane
  - (iii) much higher concentrations of biocatalysts can be present in the reactors and no side reactions "slow" the production of the desired product

Biomanufacturing : organism vs cell-free

Features	Cell-free biosystems	Living organism
Product yield	Theoretic or high	Low or modest
Product titer	High	Low or modest
Reaction rate	Fast	Slow
Process control	Easy	Difficult
Reaction conditions	Broad	Narrow

- Cell-free biosystems may be classified into two distinctive platforms according to their preparation methods:
  i. based on whole cell extract by breaking the cell membrane,
  ii. based on purified enzymes from different sources that are then mixed together



Living organism has complicated feedback control for gene regulation, protein transcription, translation, and metabolite fluxes which inhibit producing high concentration manufacturing

One pot four enzyme example

synthesis of carbohydrates from glycerol and an aldehyde

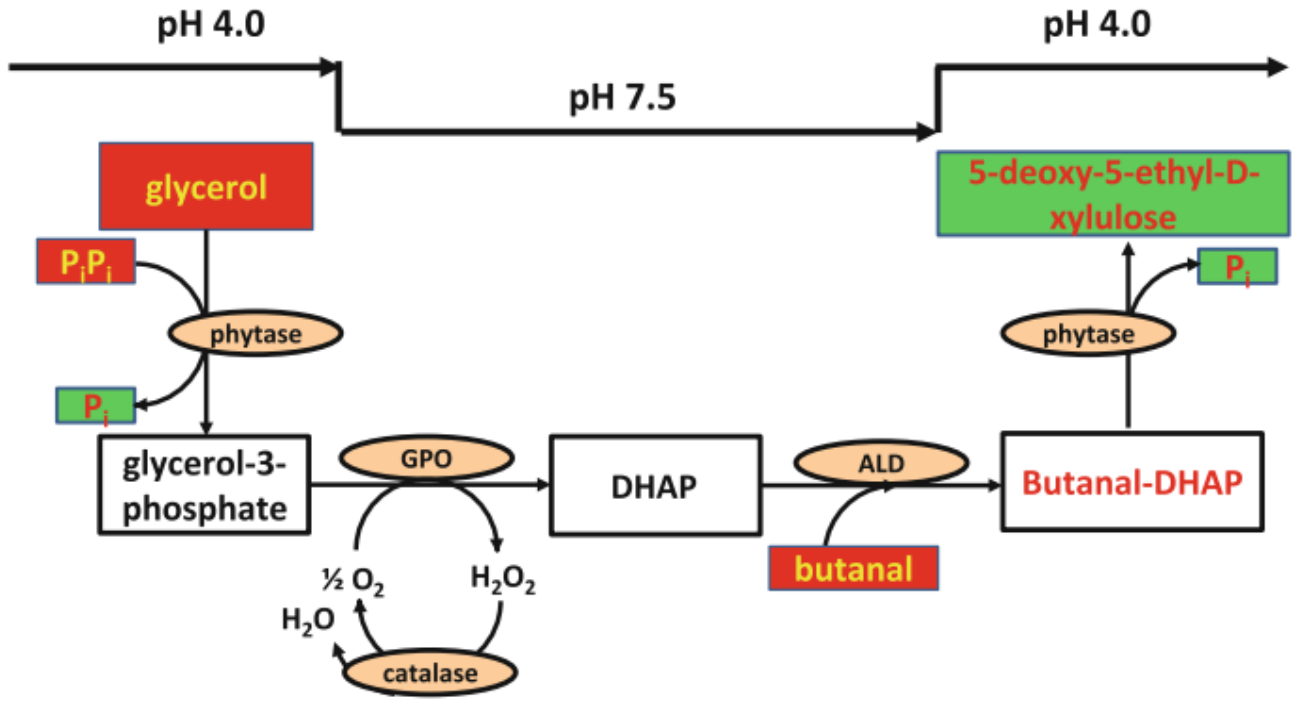
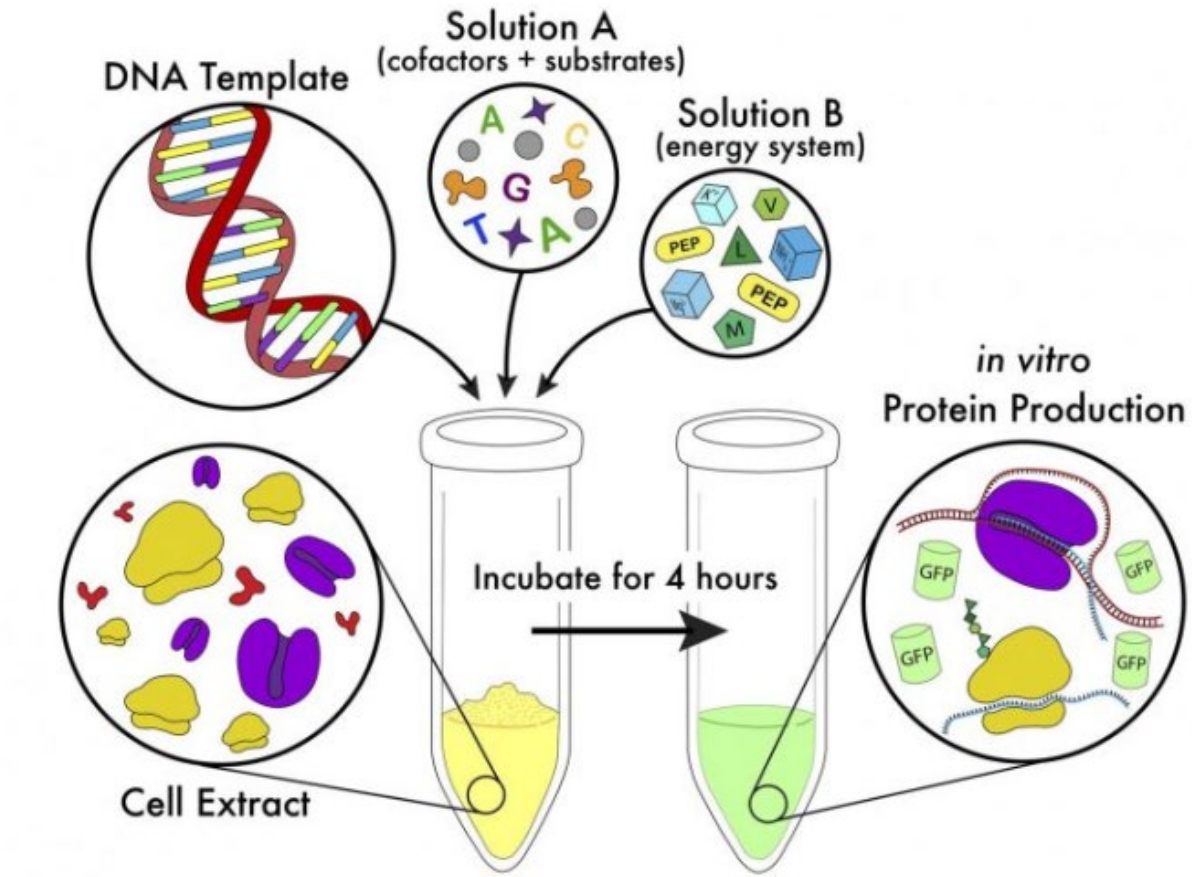


Fig. 6 Schematic representation of enzymatic transformation from glycerol to carbohydrates in one pot with pH shifts. *GPO* glycerol phosphate oxidase; *ALD* fructose-1,6-bisphosphate aldolase. Modified from Ref. [158]

Cell-Free Protein Synthesis (CFPS)



A cartoon schematic of cell-free protein synthesis, a biotechnology that harnesses the cell's genetic code into a test tube. The schematic represents a simplified approach that allows scientists to execute the reactions by mixing a DNA template and two reaction premixes that contain all reagents necessary for protein synthesis to take place. [Nicole E. Gregorio/ Cal Poly San Luis Obispo]

DNA molecular digital data storage

- Digital data production has been growing exponentially , outpacing growth of mainstream storage, including magnetic (for example, tape or hard disk drives), optical (for example, Blu-ray) and solid state (for example, flash).
- Density, durability and energy cost at rest are primary factors for archival storage, which aims to store vast amounts of data for long-term future use.

DNA as storage

- using DNA for data storage offers density of up to 1018 bytes per mm^3 , approximately six orders of magnitude denser than the densest media available today.
- The sheer density also facilitates preservation of the data in molecules for long periods of time at low energy costs.
- A particularly unique advantage is the ease of replication of DNA, for example, using PCR, which offers the ability to copy large amounts of data at very low time and resource cost.
- Data storage can also benefit from fast progress in DNA writing and reading by the biotechnology industry

- DNA is time tested by nature, with DNA sequences having been read from fossils thousands of years old.
- The basic process in DNA data storage involves encoding digital information into DNA sequences (encoding), writing the sequences into actual DNA molecules (synthesis), physically conditioning and organizing them into a library for long-term storage, retrieving and selectively accessing them (random access), reading the molecules (sequencing) and converting them back to digital data (decoding).

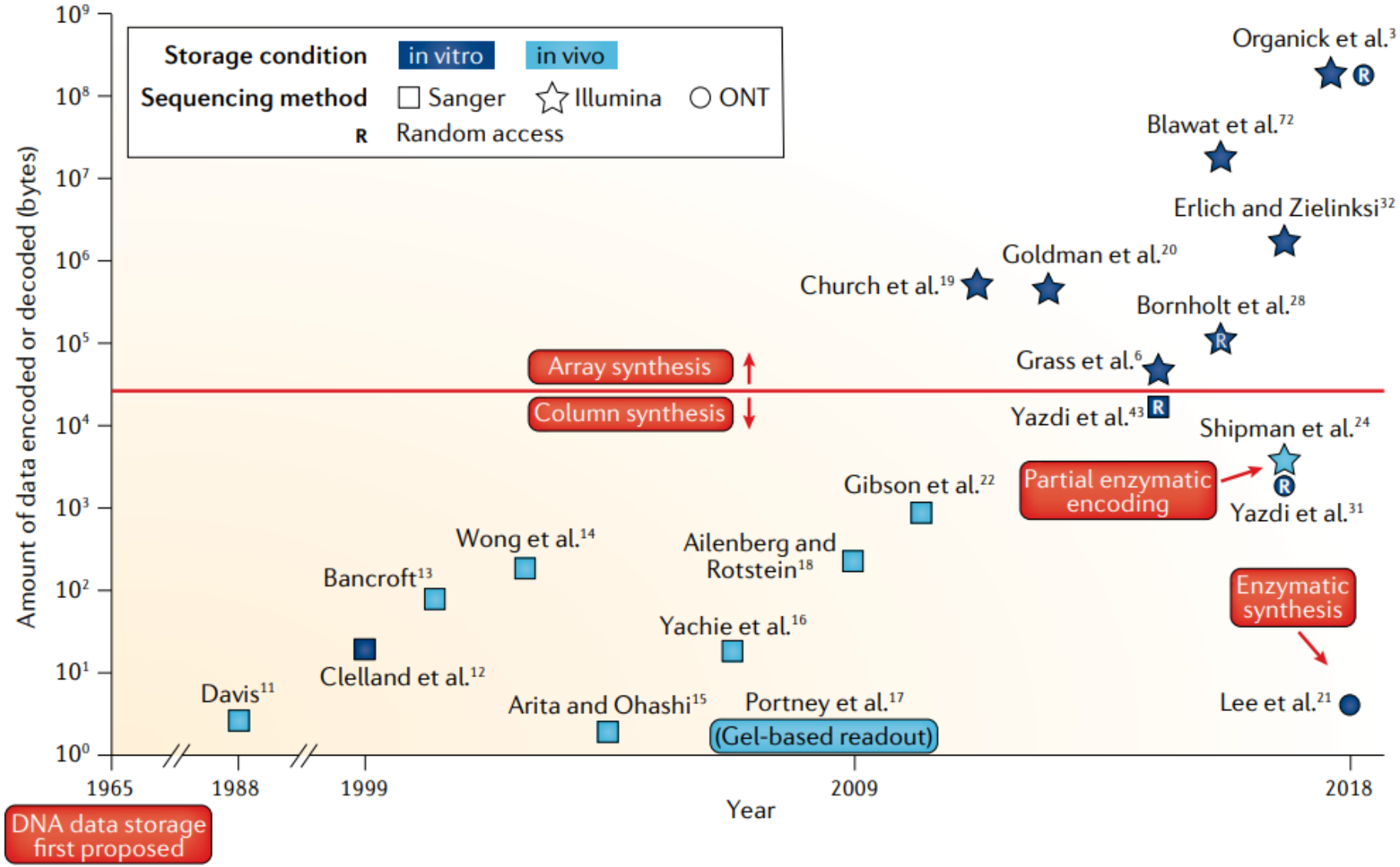
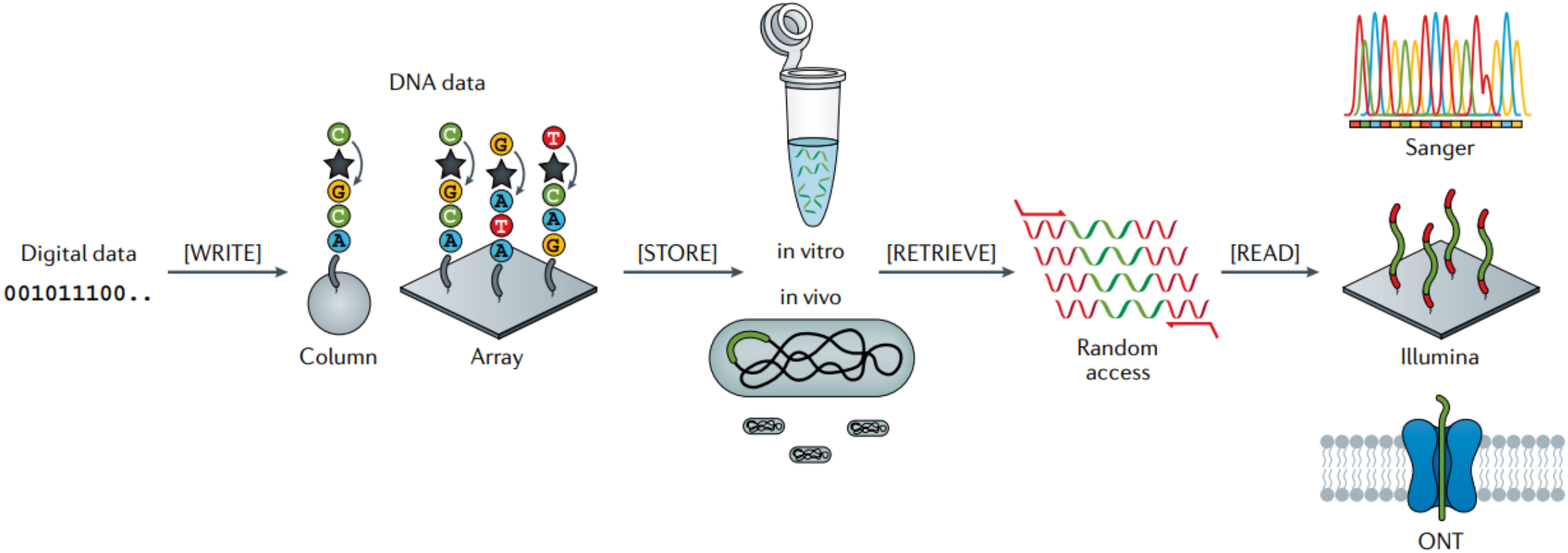


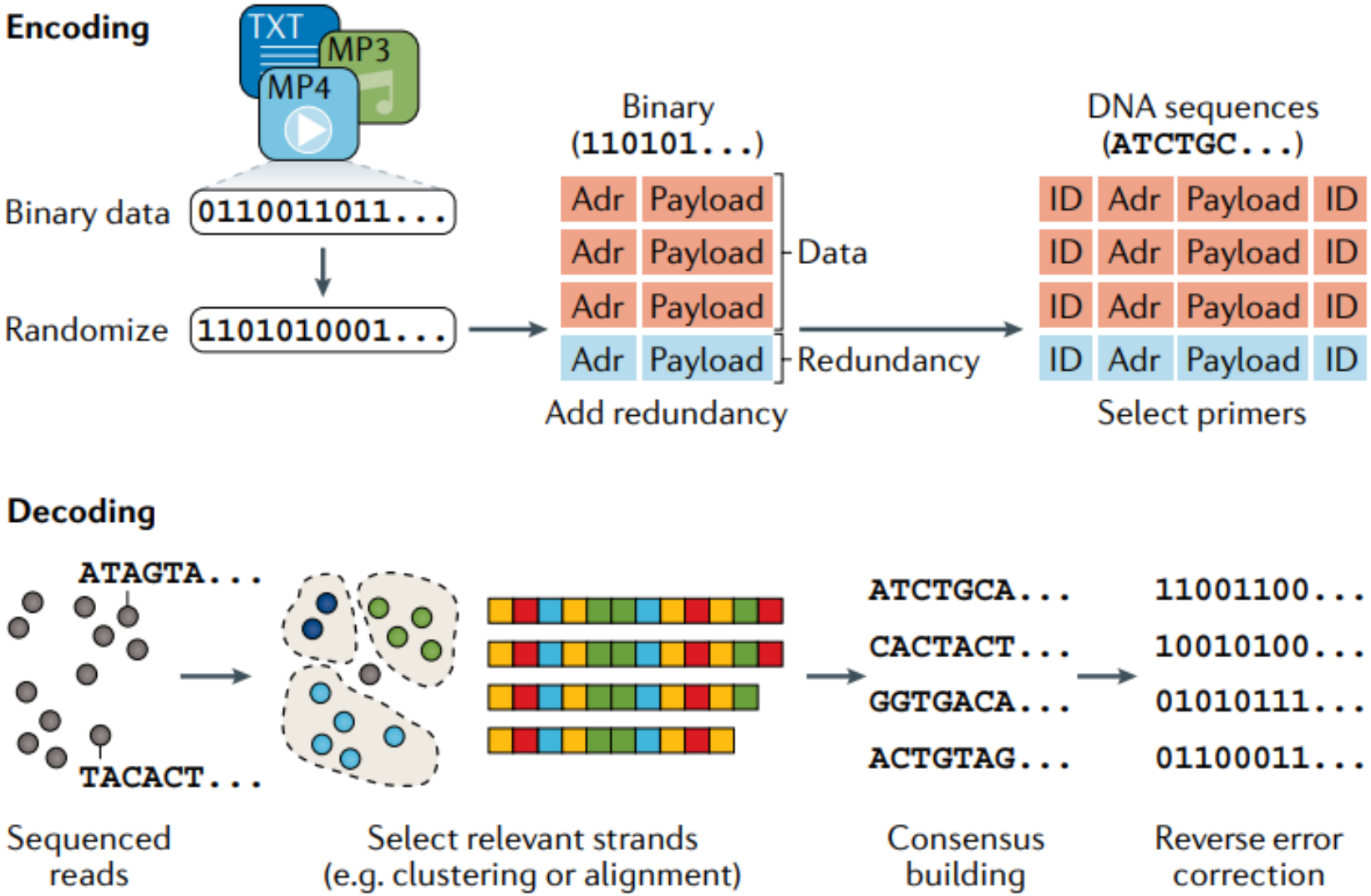
Fig. 1 | **Timeline of major published works on digital data storage with DNA.** The timeline comprises studies that included a wet-laboratory experimental demonstration. Details include how the DNA data were synthesized, stored and read and whether retrieval supported random access. Superscript numbers correspond to citations in the references section. ONT, Oxford Nanopore Technologies.

Basic process in DNA data storage



DNA synthesis and sequencing errors

- DNA synthesis and sequencing are error prone. Several papers on DNA data storage report errors of approximately 1% per base per position.
- Interestingly, modern magnetic media has a raw error rate of approximately 1% as well.
- It would be catastrophic to expose this level of error to end users in storage applications. Therefore, it is paramount to overlay error correcting codes on top of the raw storage media.


Encoding
TXT
MP3
MP4
Binary data
0110011011...
Randomize
1101010001...
Binary
(110101...)
Adr
Payload
Data
Redundancy
Add redundancy
DNA sequences
(ATCTGC...)
ID
Select primers
Decoding
ATAGTA...
TACACT...
Sequenced reads
Select relevant strands (e.g. clustering or alignment)
ATCTGCA...
CACTACT...
GGTGACA...
ACTGTAG...
Consensus building
11001100...
10010100...
01010111...
01100011...
Reverse error correction

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TECH

Scientists Store Data in Synthetic DNA Embedded in a Plastic Bunny

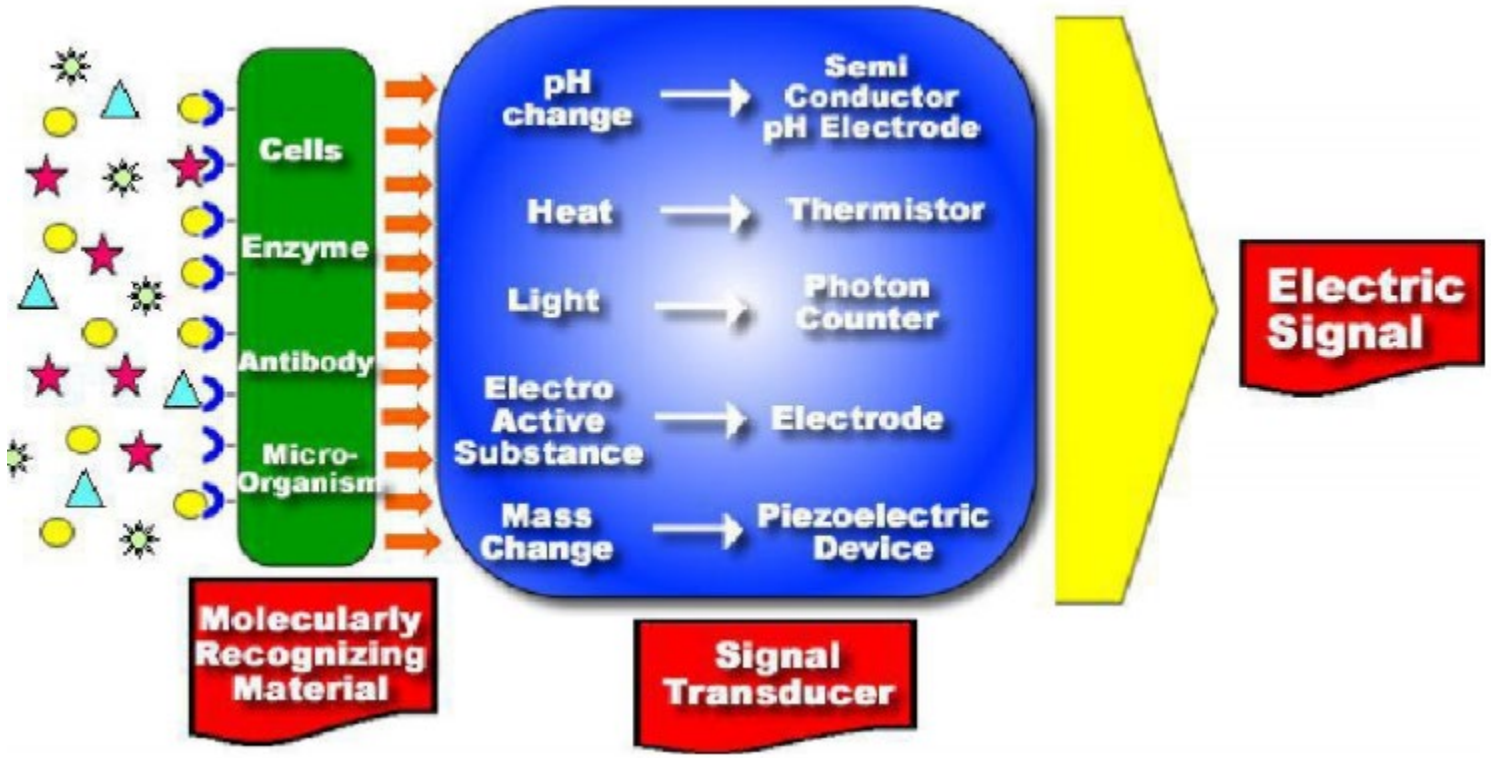
A new method for preserving genetically encoded data into common manufacturing materials is reported

As the data deluge outpaces the capacity to store it on conventional devices, researchers are hoping natural genetic code can be used to contain billions of megabytes of information. Could DNA be the future of digital storage? Photo: New York Genome Center

Applications of Biosensors

- Human beings have at least five sensors i.e., noses, tongues, ears, eyes and skin.
- A sensor is a device that measures a physical quantity and converts it into a signal which can be read by an observer or by an instrument
- Sensors can divide into three types, namely
    a. physical sensors for measuring distance, mass, temperature, pressure, etc. A physical sensor is a device that provides information about a physical property of the system.
    b. chemical sensors which measure chemical substances by chemical or physical responses. It is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal.
    c. biosensors which measure chemical substances by using a biological sensing element.

Principles of biosensors



Biosensors in food industry

- Quality of a food product is evaluated through periodic chemical and microbiological analysis.
- Conventionally use techniques such as, chromatography, spectrophotometry, electrophoresis, titrations and others.
- These methods do not allow an easily continuous monitoring because they are expensive, slow, need well trained operators and in some cases, require steps of extraction or sample pretreatment, elongating the time of analysis.
- There is an increasing demand for instruments suitable for automatic quality control through the process and at the end of the line so that the real time state of the process can be described.

Detection of microbes

- Conventional methods to determine and specify microorganisms are time consuming and laborious, often based on colony counts on solid media
- The confirmation of the identity of the isolated microorganism is achieved by microscope, biochemical and immunological characteristics.
- This leads to total detection times of several days which are the major disadvantage of conventional plating methods.

- Improved analytical methods have been developed which predominantly use the advantages supplied by immunological methods
- Biosensors have become more and more important for the determination of microorganism. Very specific antibodies can be produced against surface antigens of various microorganisms. In this way, an immunosensor can discriminate between different organisms.
- Most applications focus on confirming the absence of pathogenic organism like *Salmonella* species and *Escherichia coli* species.

Quality control of modified atmosphere packages

- Improper package design or temperature abuse during handling may cause fruits and vegetables in modified atmosphere packages to be exposed to low, injurious O_2 levels associated with the production of fermentation volatiles, quality loss and eventually product breakdown.
- The detection of ethanol would provide a sensitive technique for low-O_2 injury identification.
- A commercial ethanol biosensor composed of a chromagen and immobilized enzyme: alcohol oxidase and peroxidase have been tested. Alcohol oxidase catalyses oxidation of ethanol into acetaldehyde and H_2O_2 in the presence of O_2 and peroxidase (an H_2O_2 decomposing enzyme) catalyses oxidation of the chromagen causing a colour change.
- The response of the biosensor was very similar to the one measured by gas chromatography, which is expensive and requires technical expertise.

Fish freshness analysis

- Trimethylamine (TMA) is typical and common fish-odour substance in seafood, and is produced by the decomposition of trimethylamine N-oxide (TMAO) in sea creatures.
- The fact is that fresh marine products contain little TMA.
- Mitsubayashi et al., (2004) constructed aTMA biosensors by immobilizing flavine containing mono oxygenase type 3 (FMO3), as one of drug metabolizing enzymes in human liver, onto a sensitive area of a dissolved oxygen electrode.
- The sensor output induced by FMO3 enzyme reaction was continuously monitored on a computer display and saved on the hard disk for later analysis

Biosensors of dairy products

Table.3 Range of analyte monitored in milk and milk products by biosensors

Analyte	Food matrix	Bio component
Glucose	Milk yoghurt	Glucose oxidase Galactosidase
Fructose	Milk	D-fructose dehydrogenase
Lactose	Milk	Galactosidase, lactozym and *Saccharomyces*
Laculose	Milk	D-fructose dehydrogenase, glucose oxidase
Biotin	Infant formula and milk	Anti- biotin antibody
Folate	Infant formula and milk	Anti folic acid antibody
L- lysine	Milk	Lyase oxidase
Antibiotics	Milk	Antibodies
Pesticides	Milk	Chlinesterase
Cholesterol	Butter, cream	Cholesterol oxidase or Horseradish peroxidase