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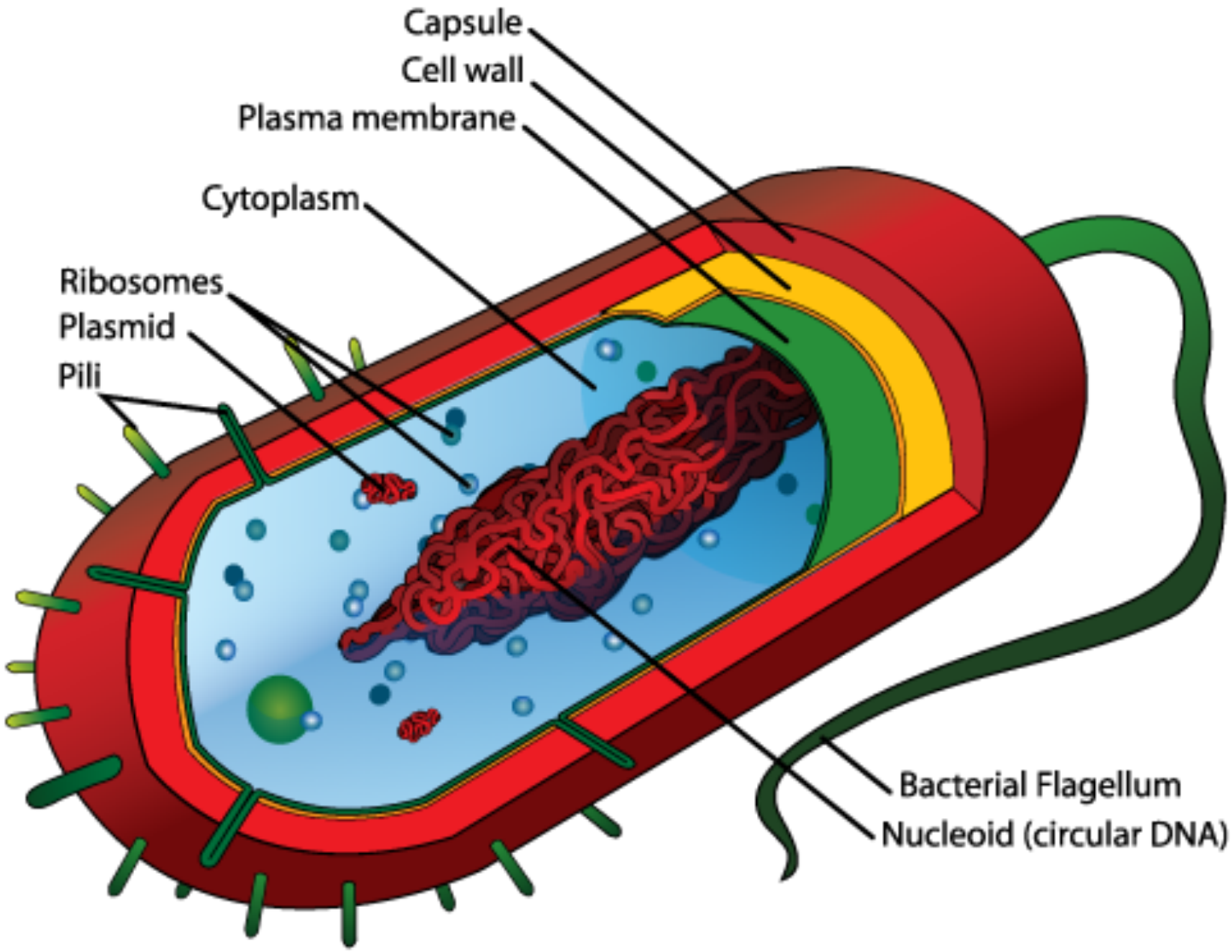
BioHack Academy

A short introduction to

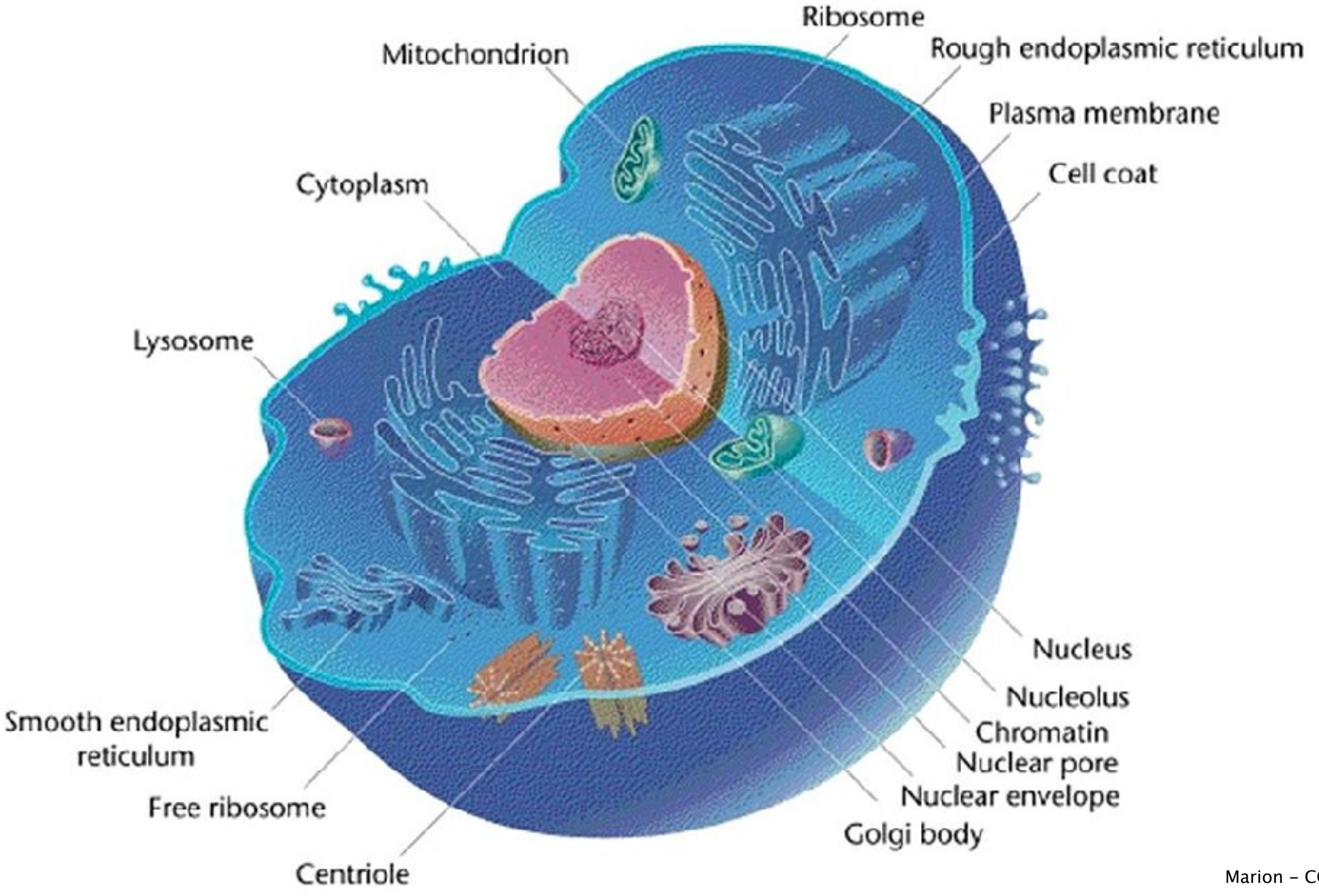
Molecular Biology

Kishony Lab (Harvard Medical School)

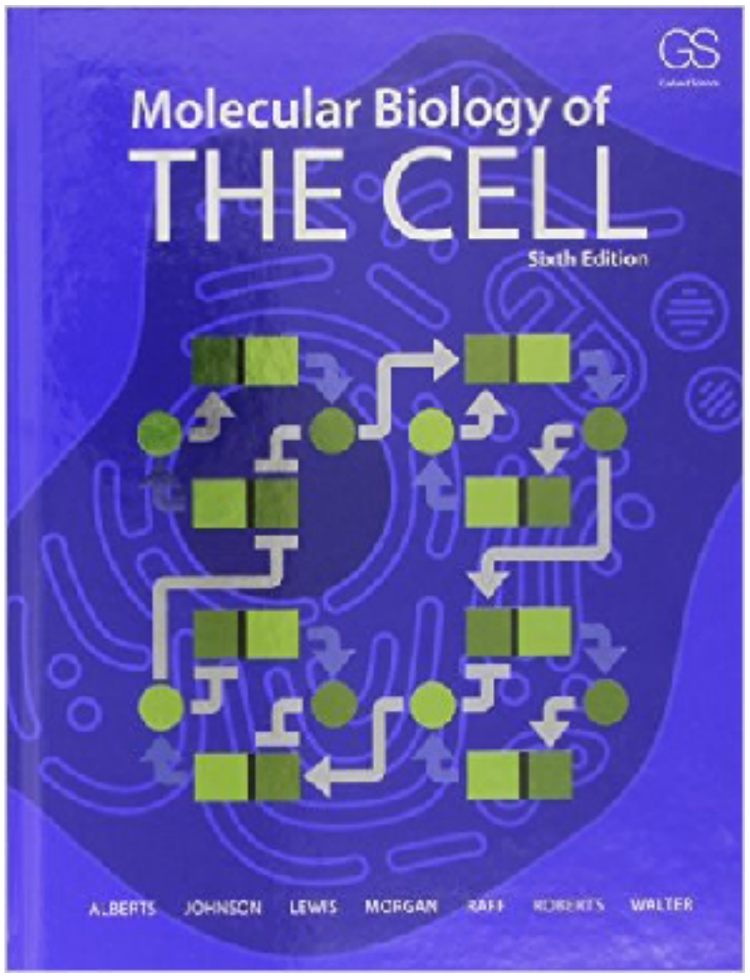
Cells



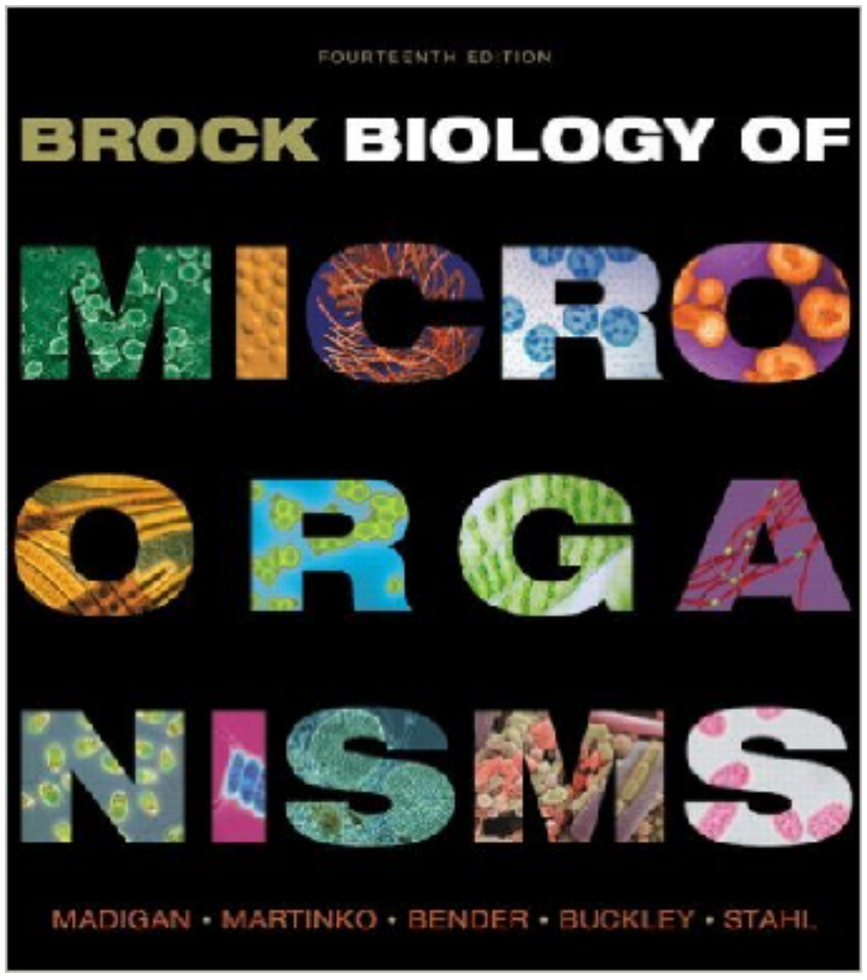
Cells



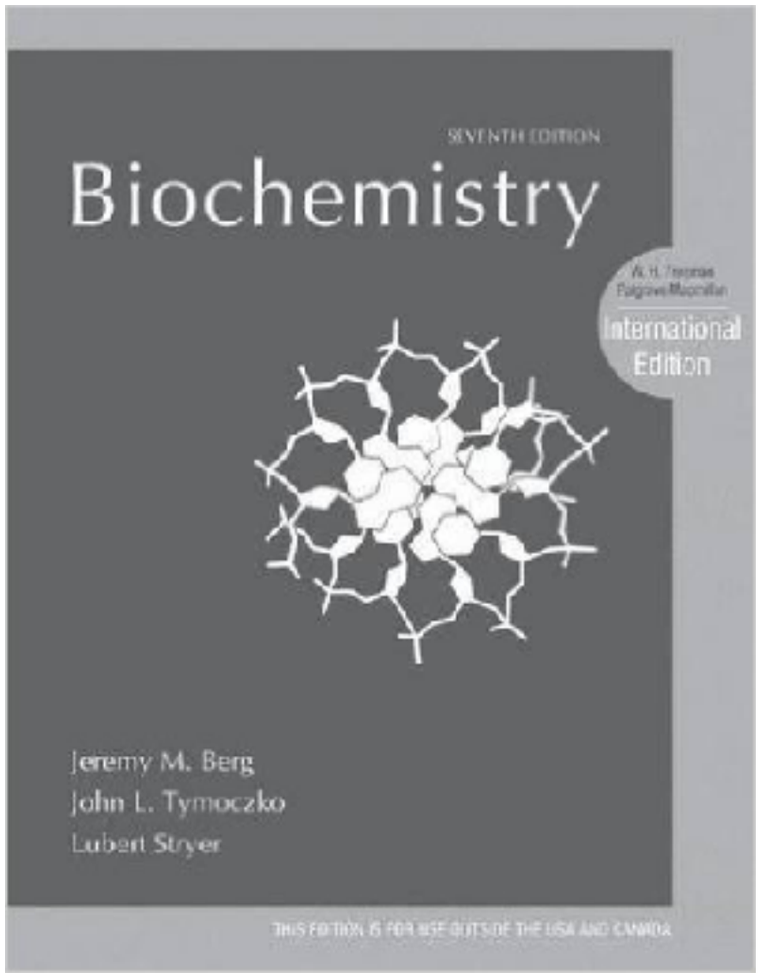
Books



Alberts



Brock



Stryer

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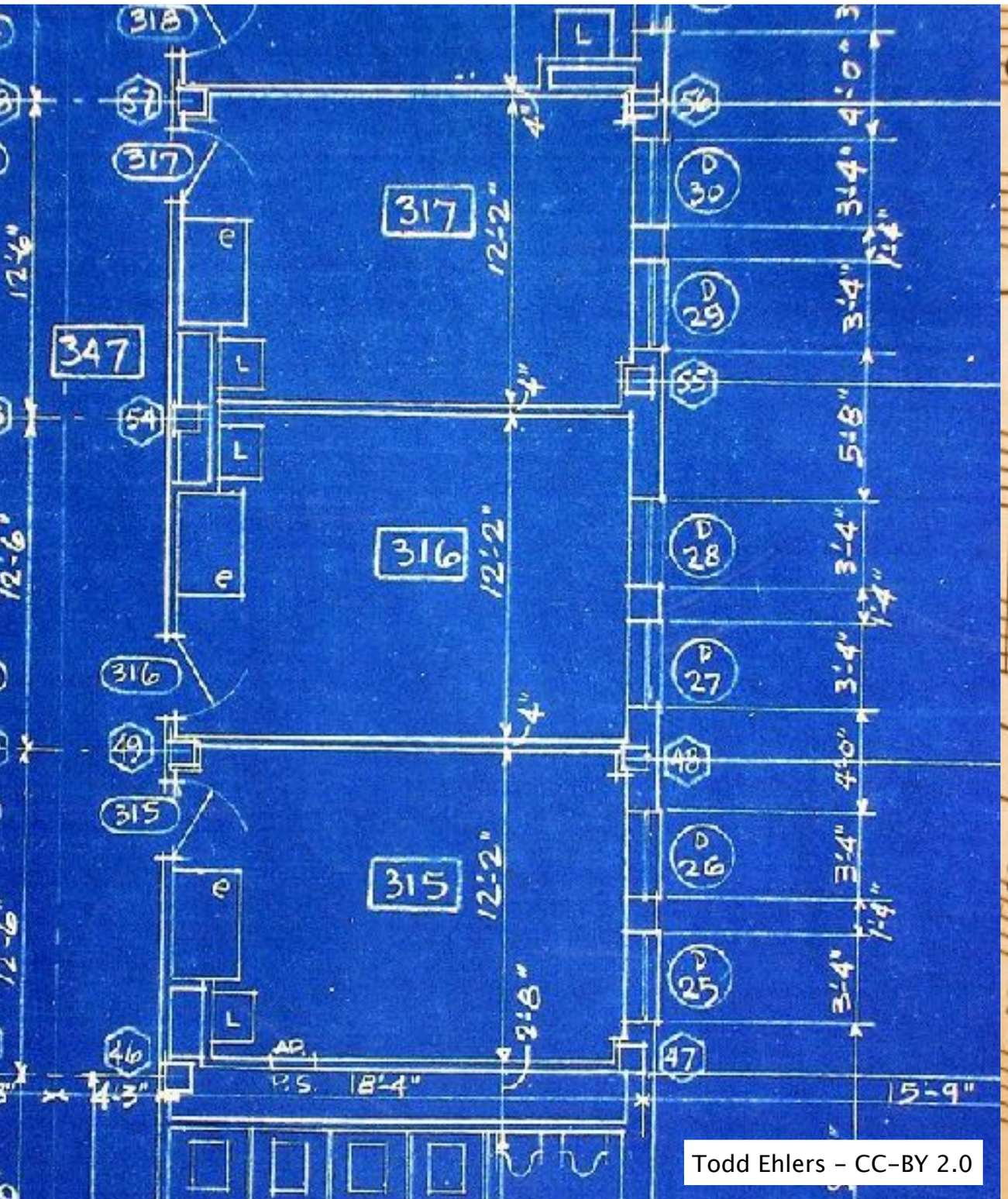
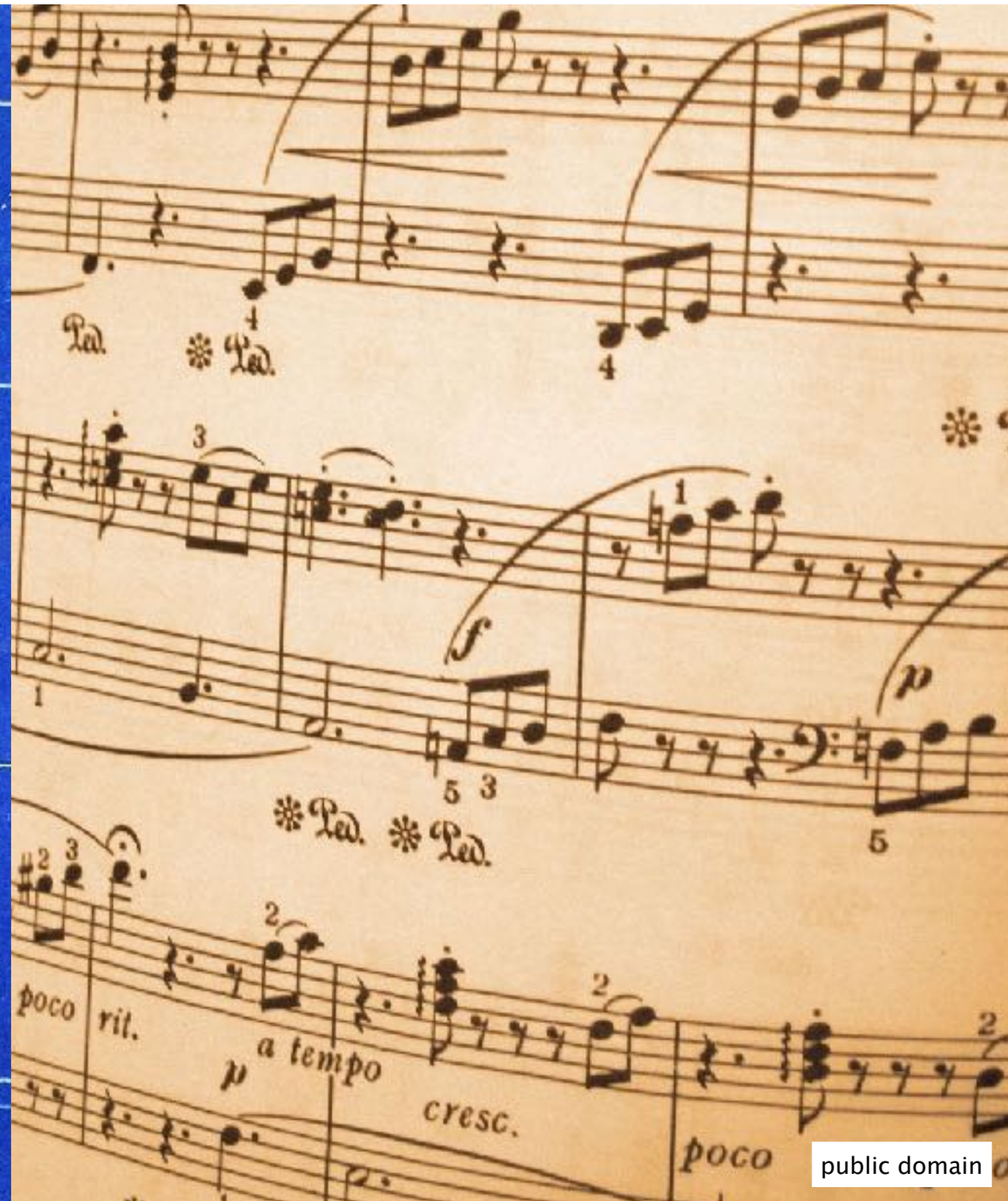
DNA & Chromosomes

Blueprint or music



Todd Ehlers - CC-BY 2.0



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Simon E. Fisher
@ProfSimonFisher

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Your genome is not a blueprint. A thread about misleading metaphors in science communication... 1/11

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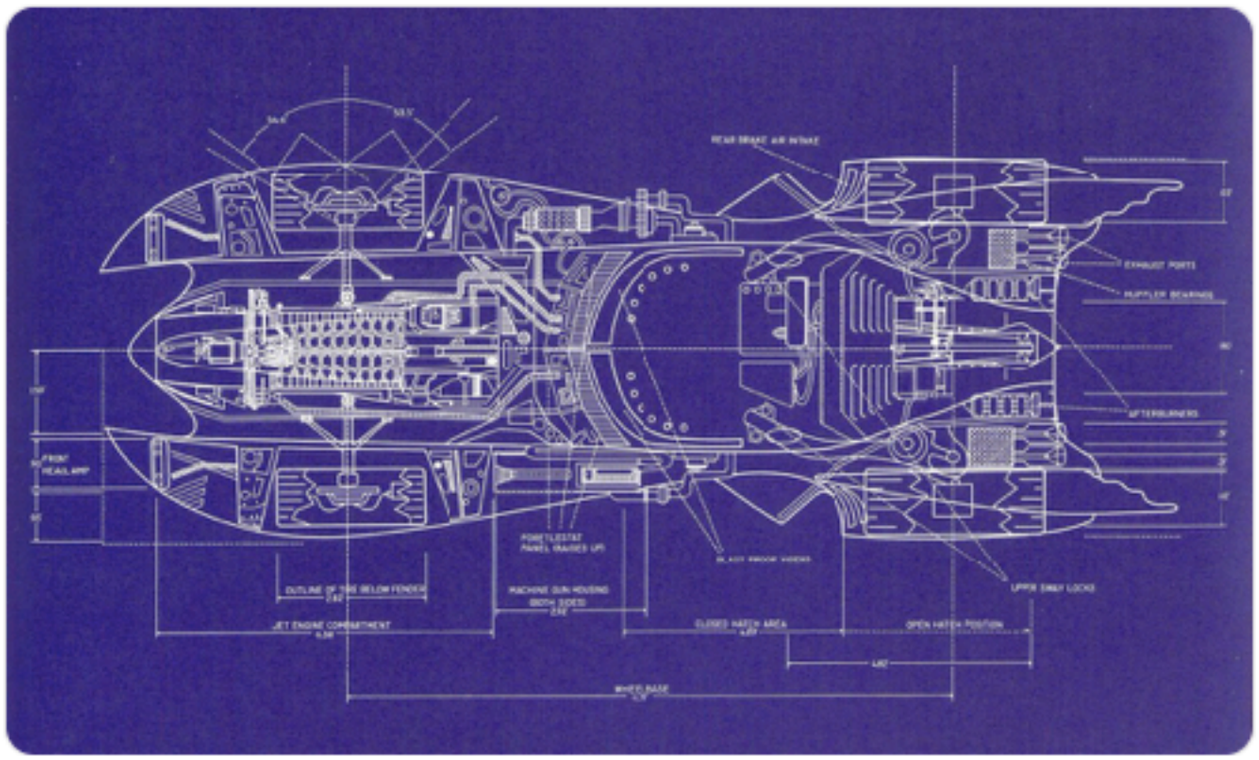
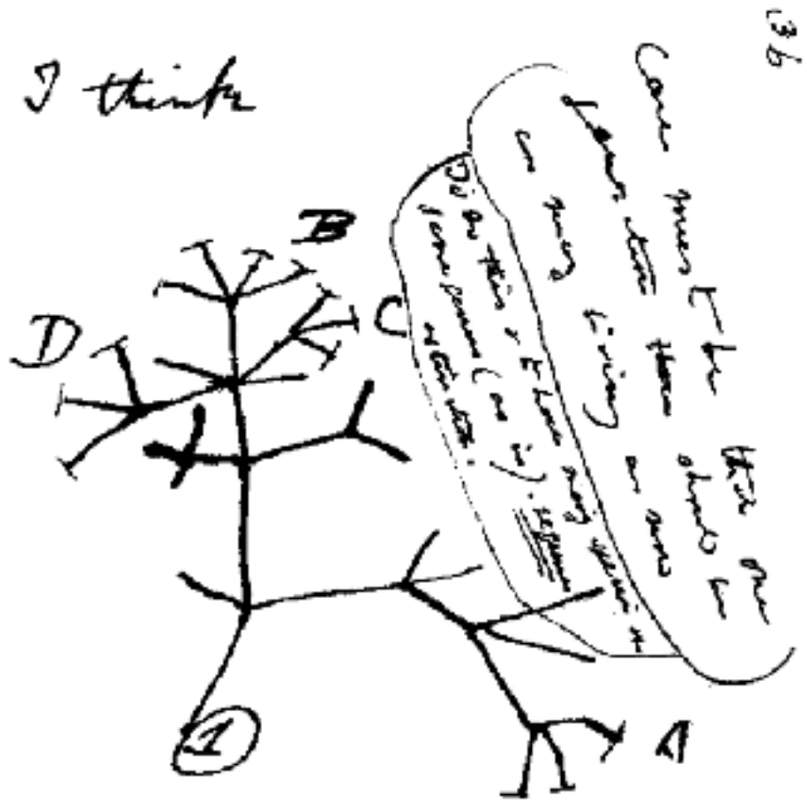
Simon E. Fisher @ProfSimonFisher · 15 Jul 2018

DNA is often referred to as a "blueprint for life". A blueprint is an architect plan, technical drawing or engineering design. Like a blueprint, DNA contains information to guide construction, in this case of a living organism. Beyond that, the analogy rapidly breaks down...2/11



Origin of Species



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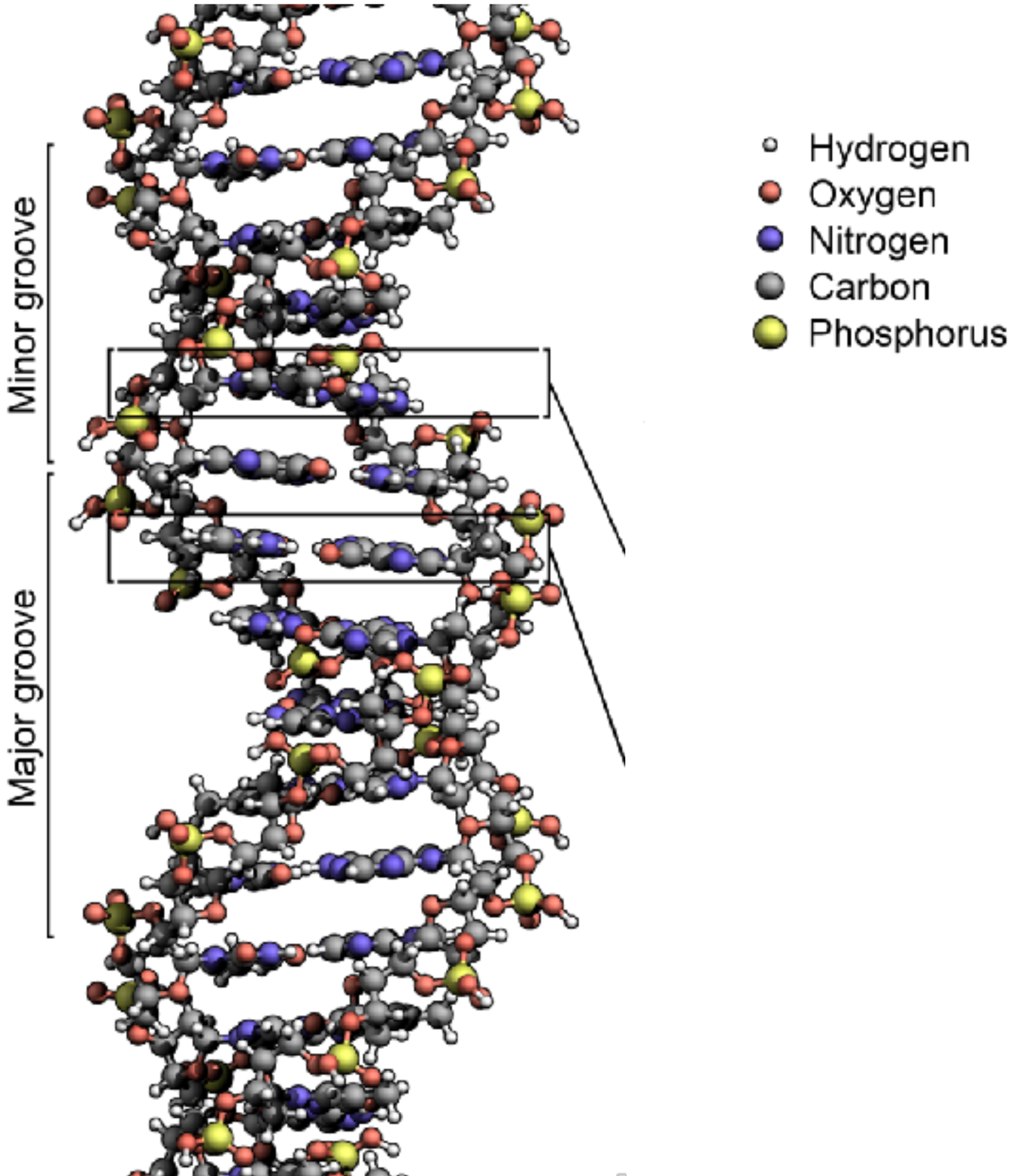
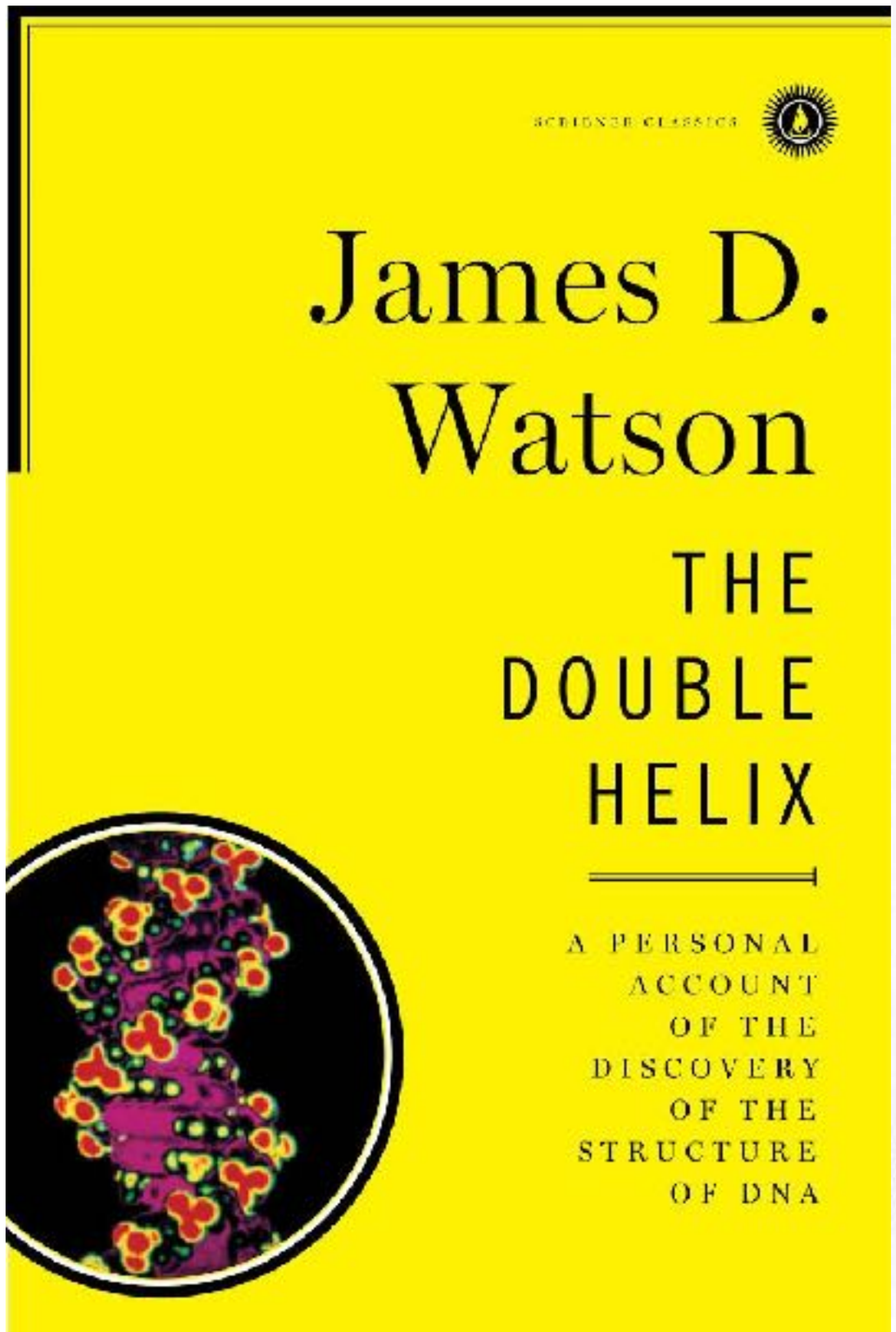
DNA Molecule

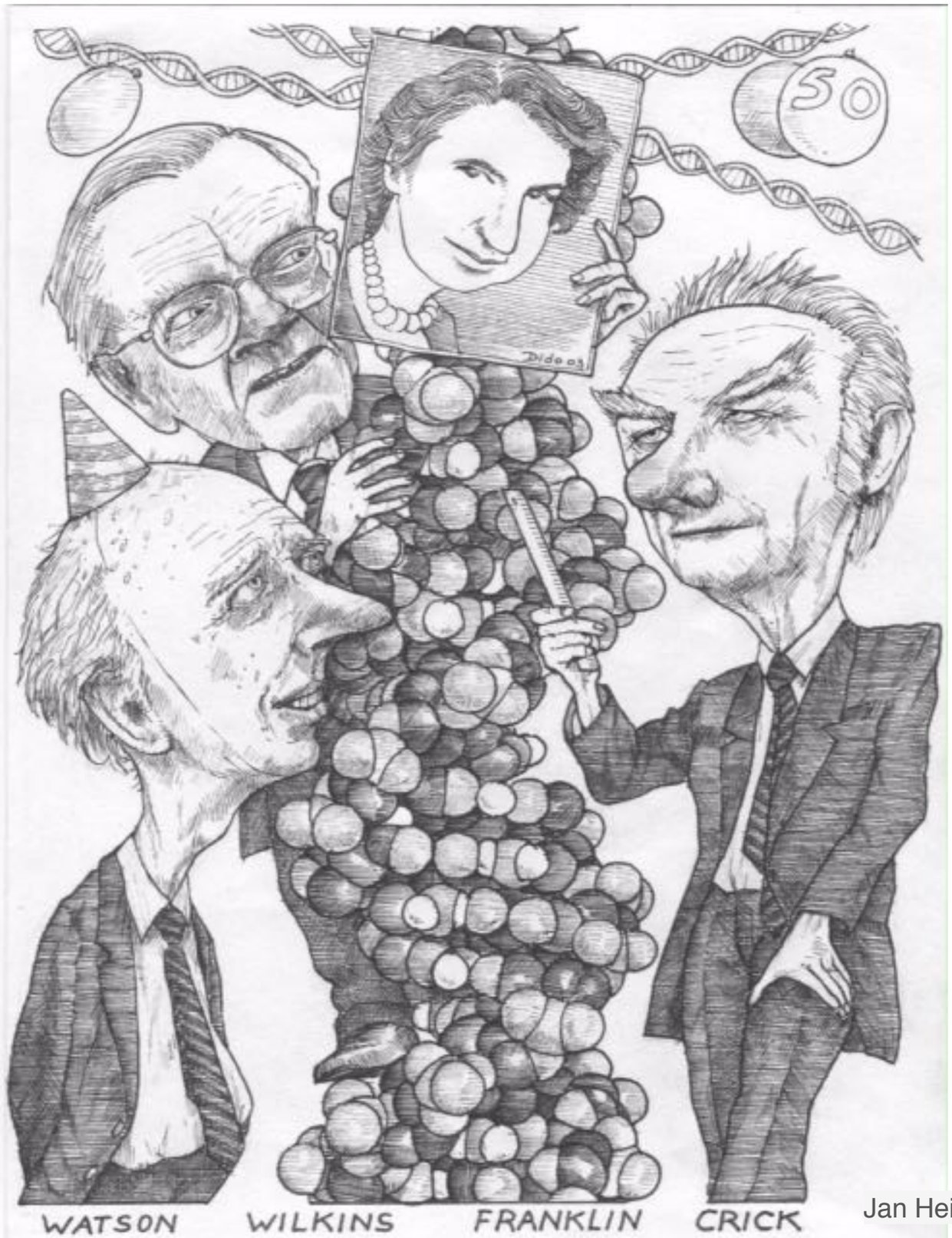


Sequence = Code

AATCGAATTGAGTAATAGGGAACCT

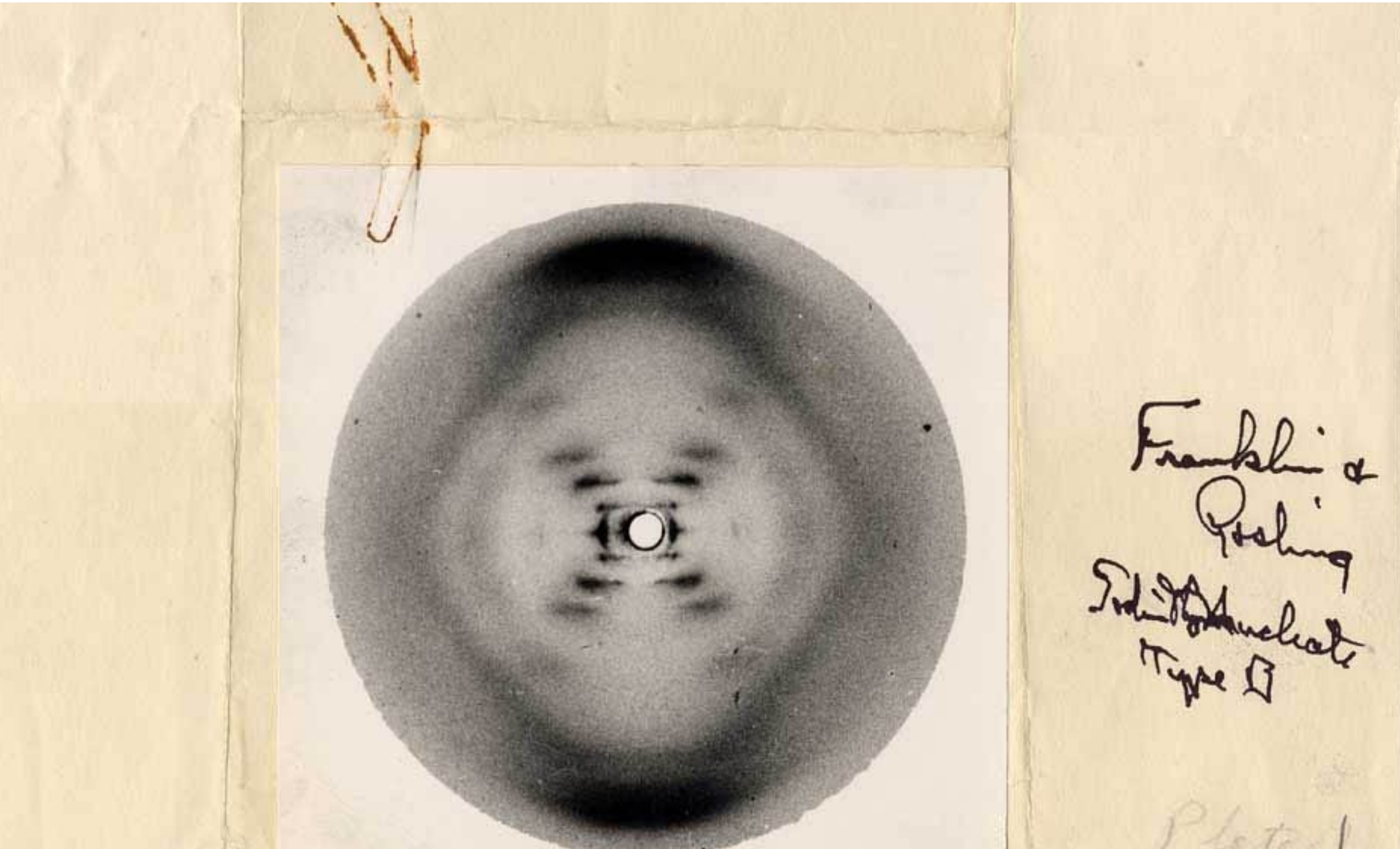
Discovery of the double helix





Jan Hein van Dierendonck

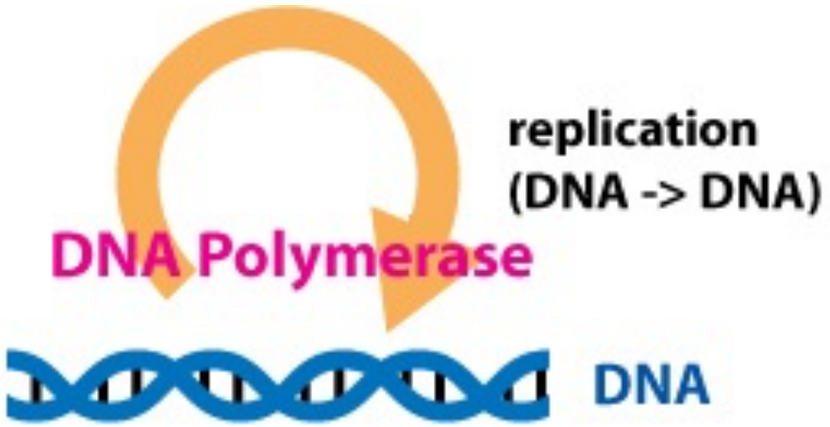
Discovery of the double helix



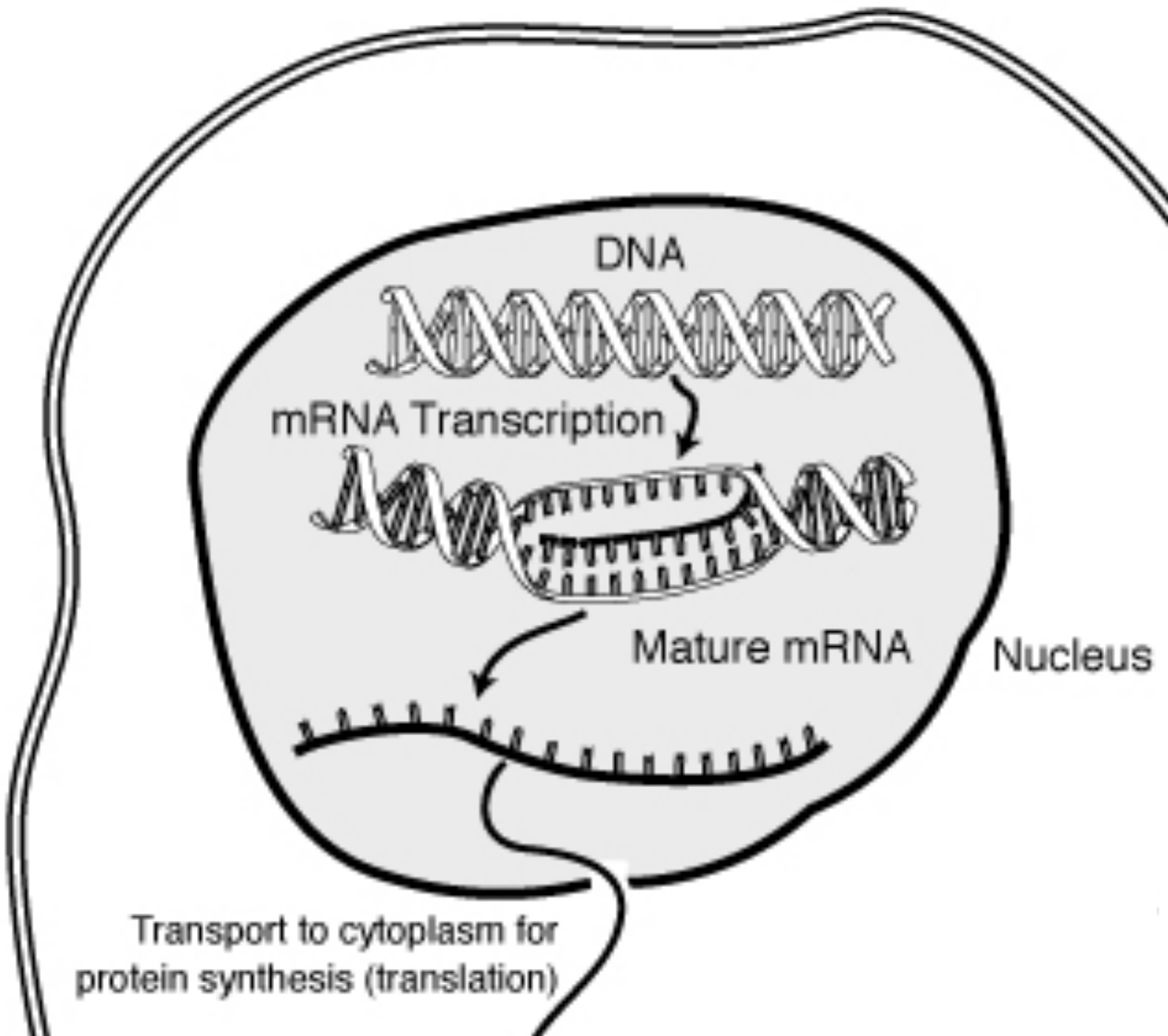

ROSALIND
FRANKLIN
AIRBUS
esa

Discovery of the double helix

“Central Dogma”



“Central Dogma” in the cell



Sequence = Code

DNA TACCGAATTGAGTAATAGGGAACCT

RNA AUGGCUUAACUCAUUAUCCCUUGGA

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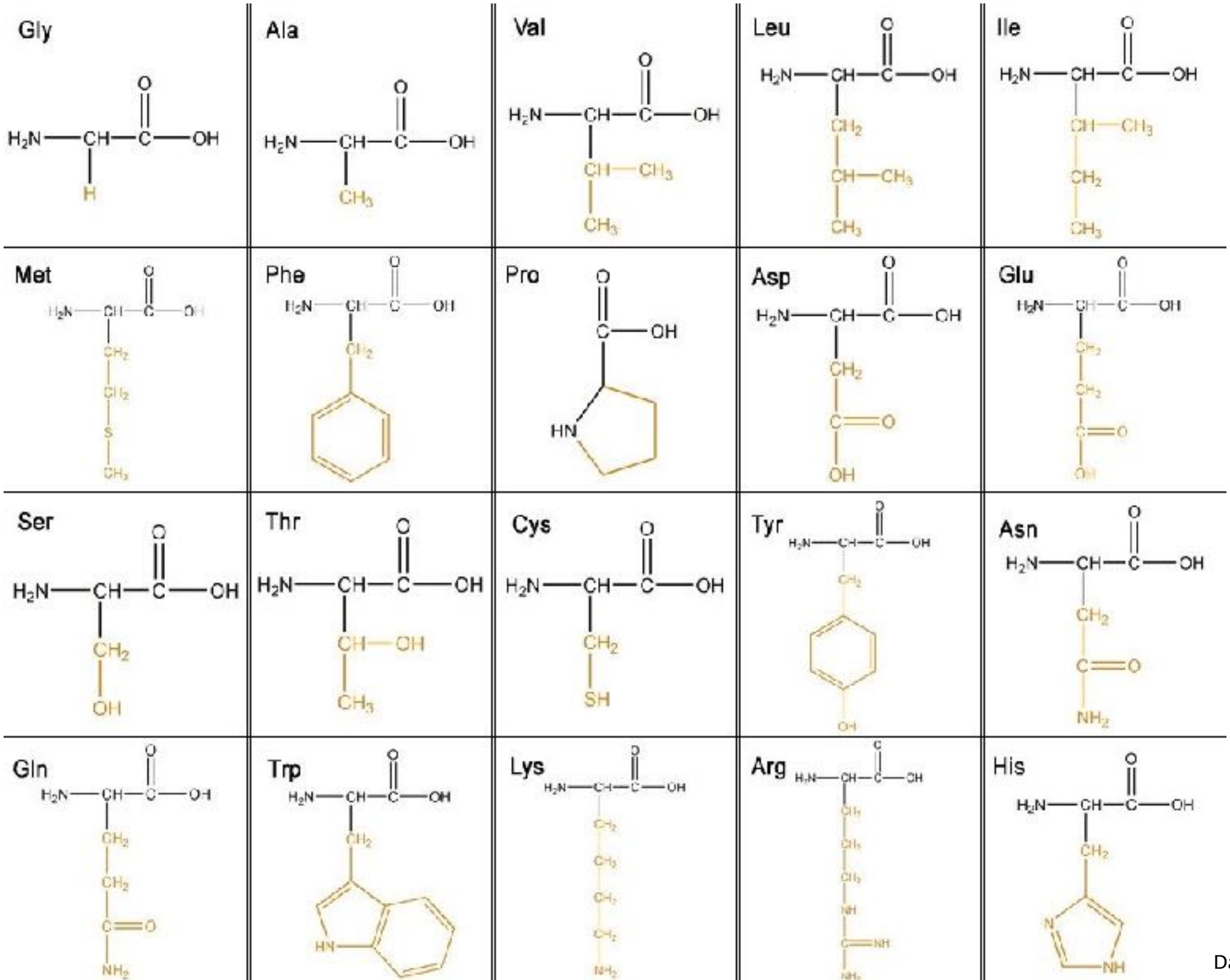
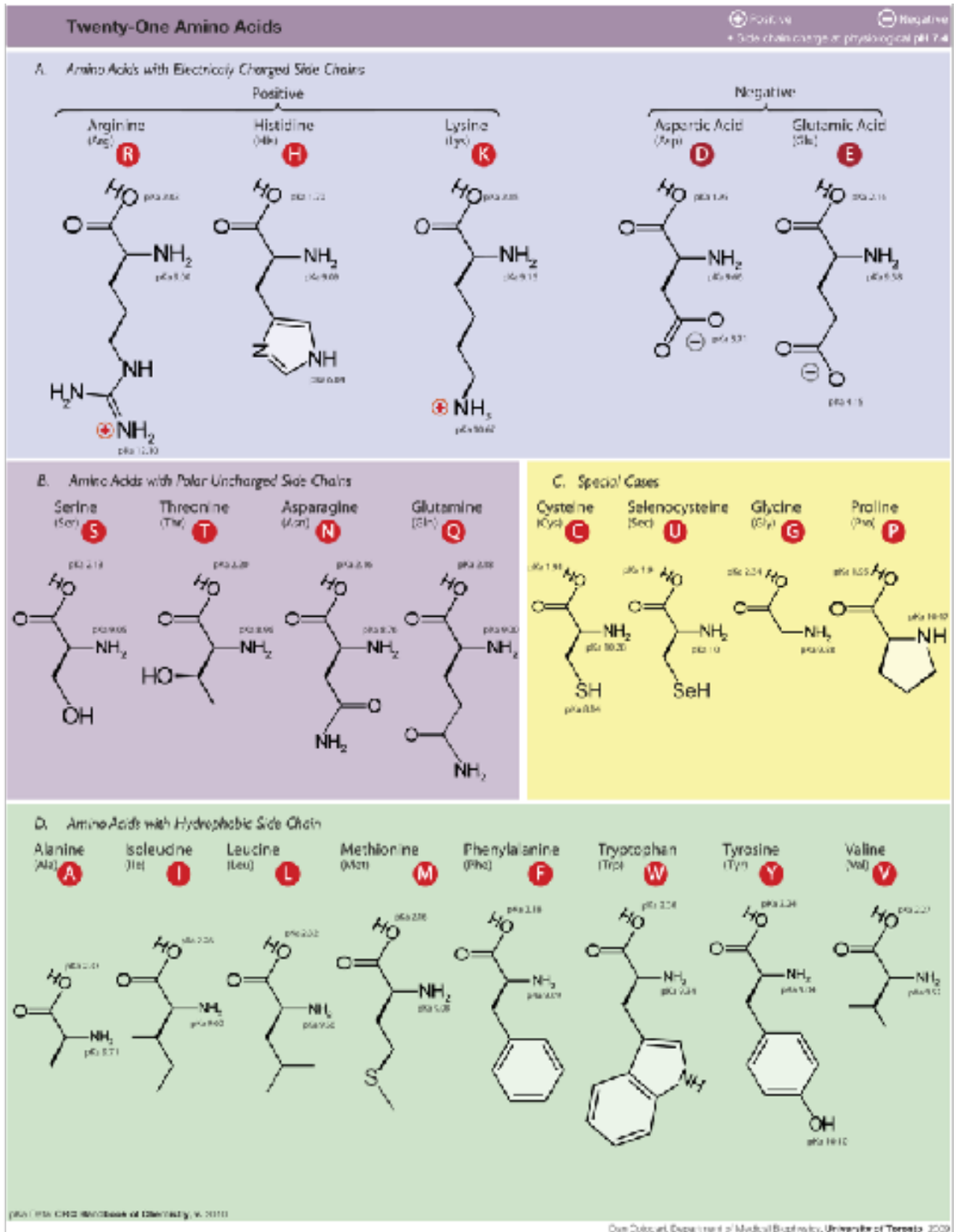
Proteins

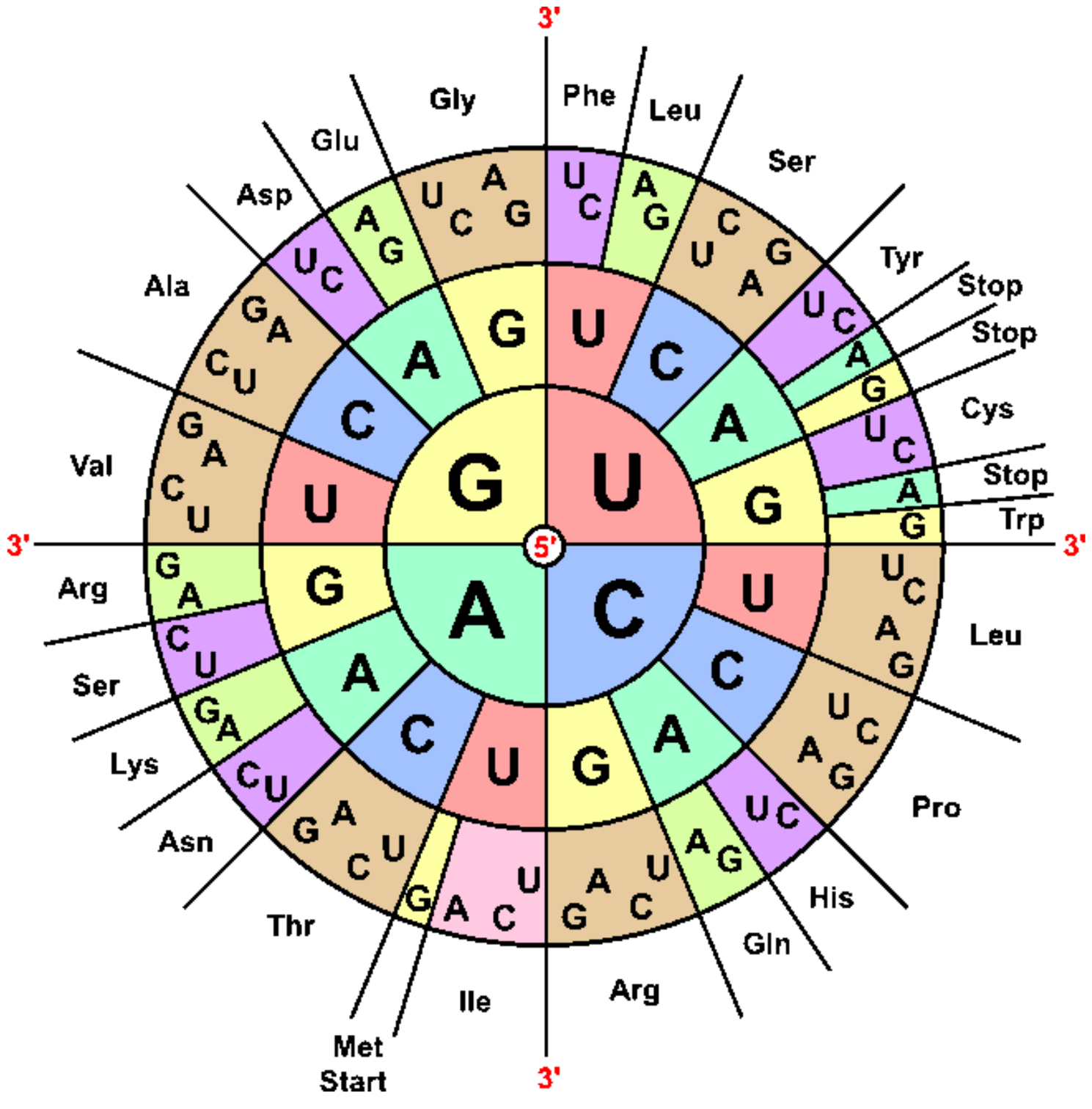
Amino acids, the building blocks



Amino acid groups

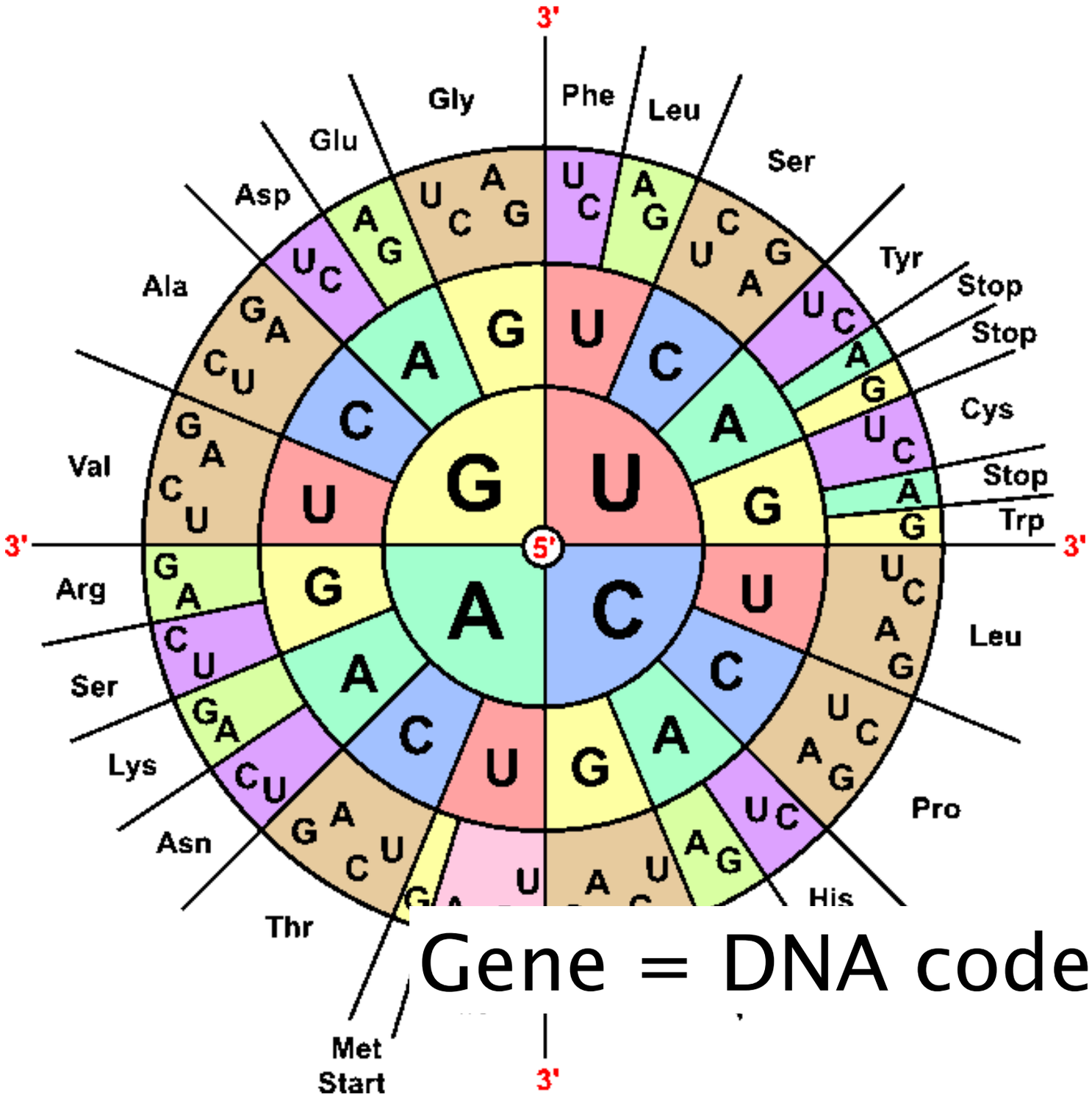


Amino acid rosetta stone




12
3
6
9

Amino acid rosetta stone



DNA TACCGAATT

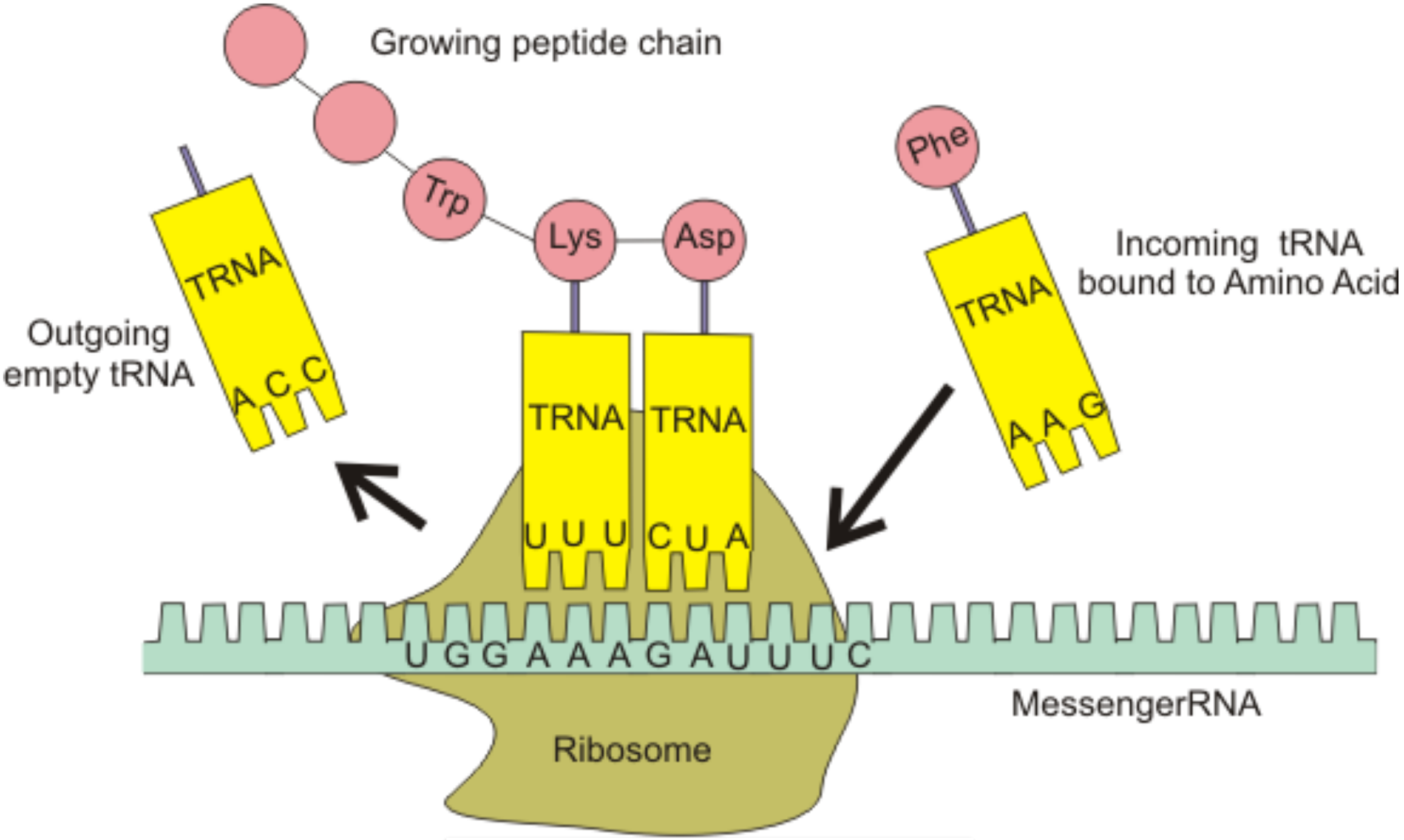
RNA AUGGCUUAA

AUG GCU UAA

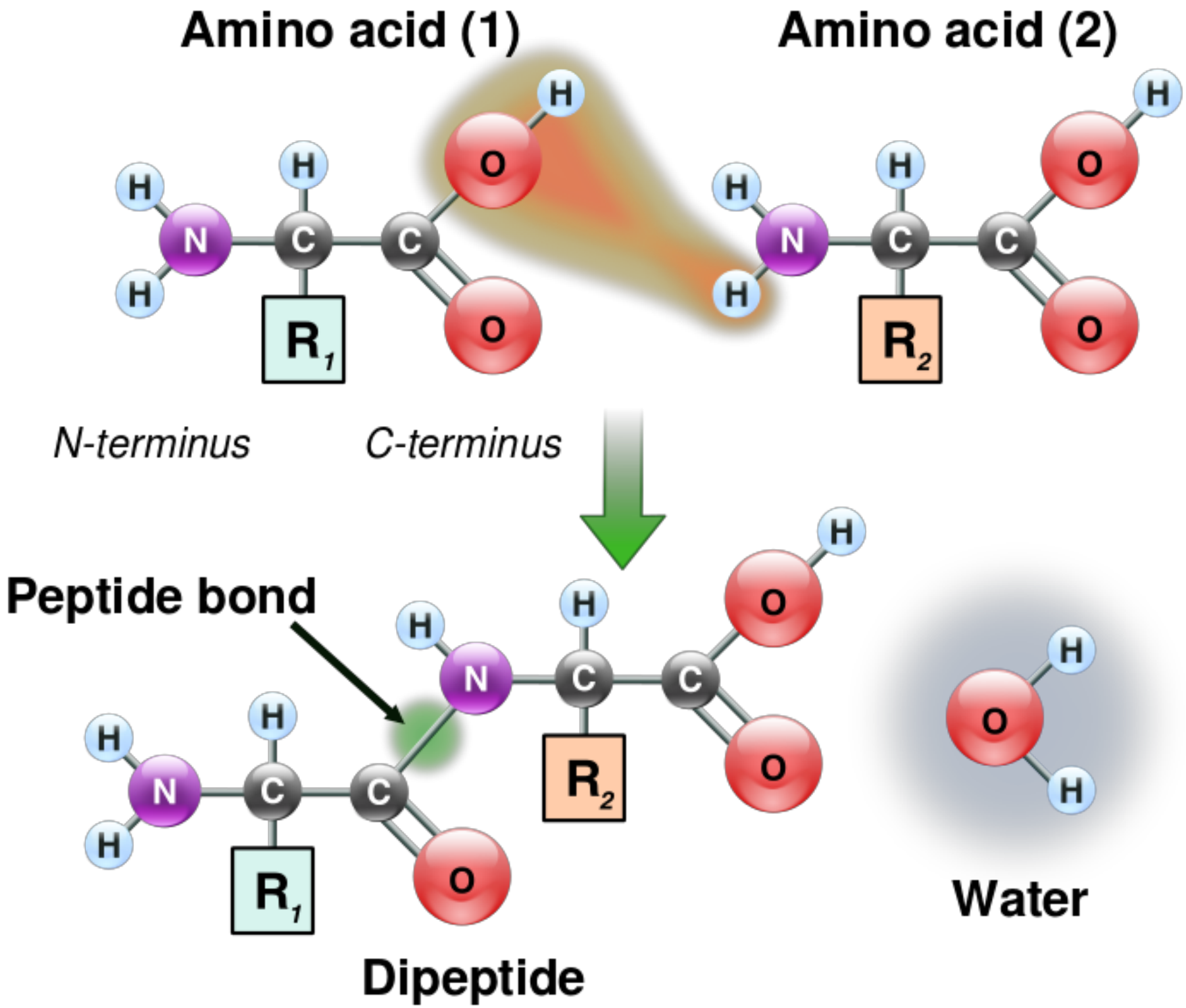
Start Ala Stop

Gene = DNA code from start to stop

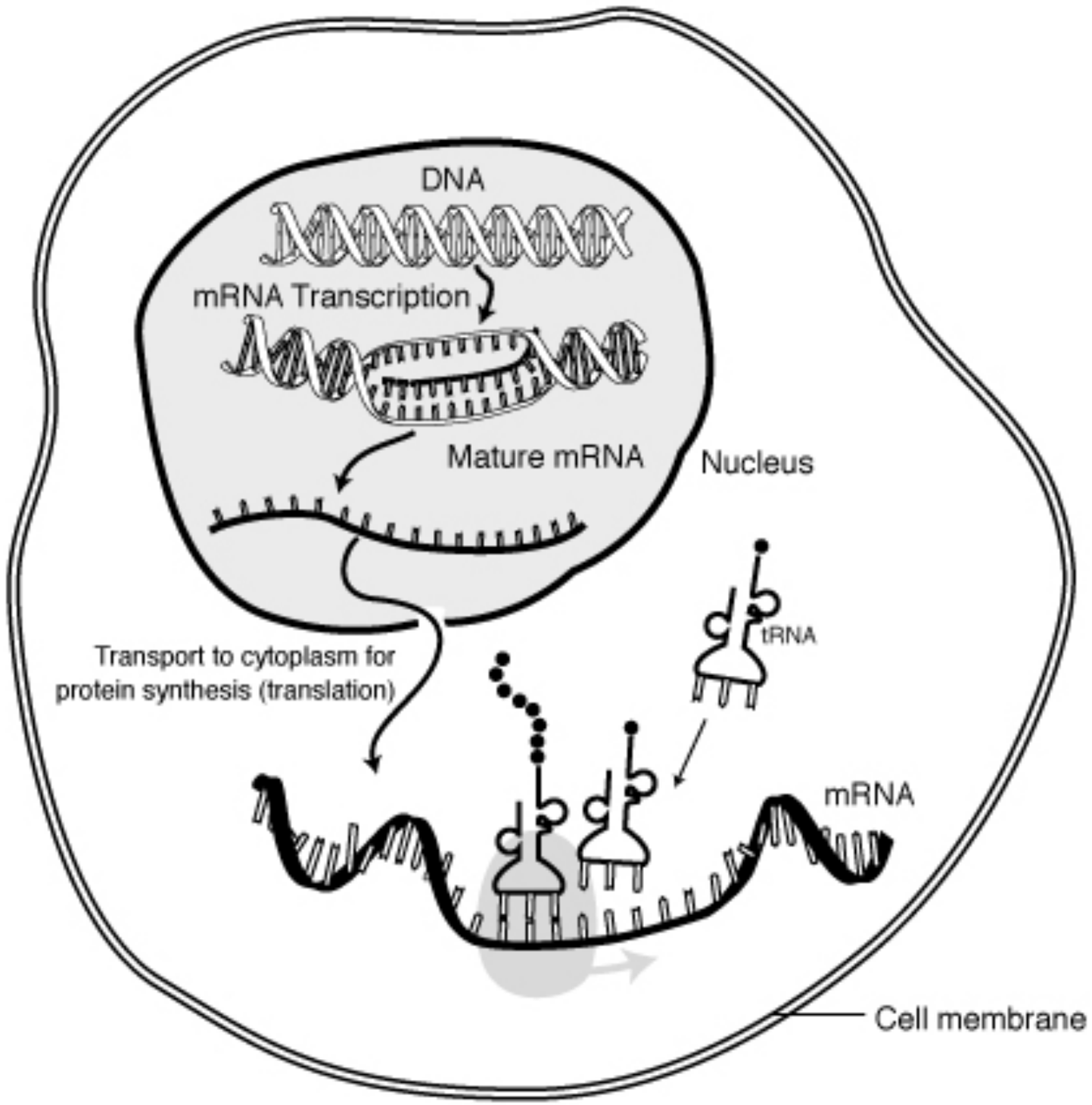
Protein synthesis



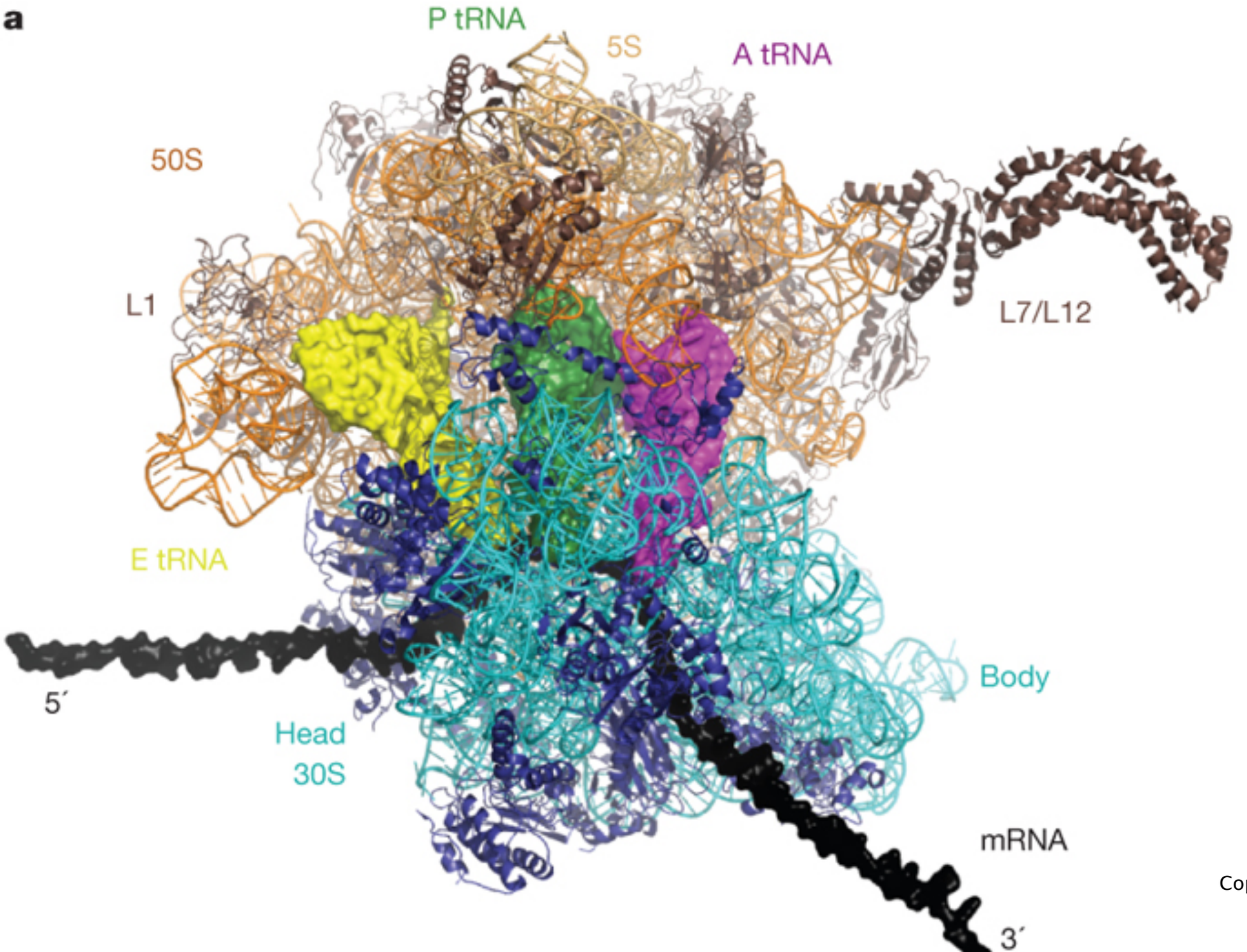
Peptide bond formation



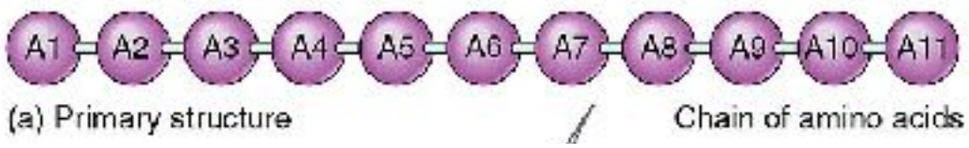
“Central Dogma” in the cell



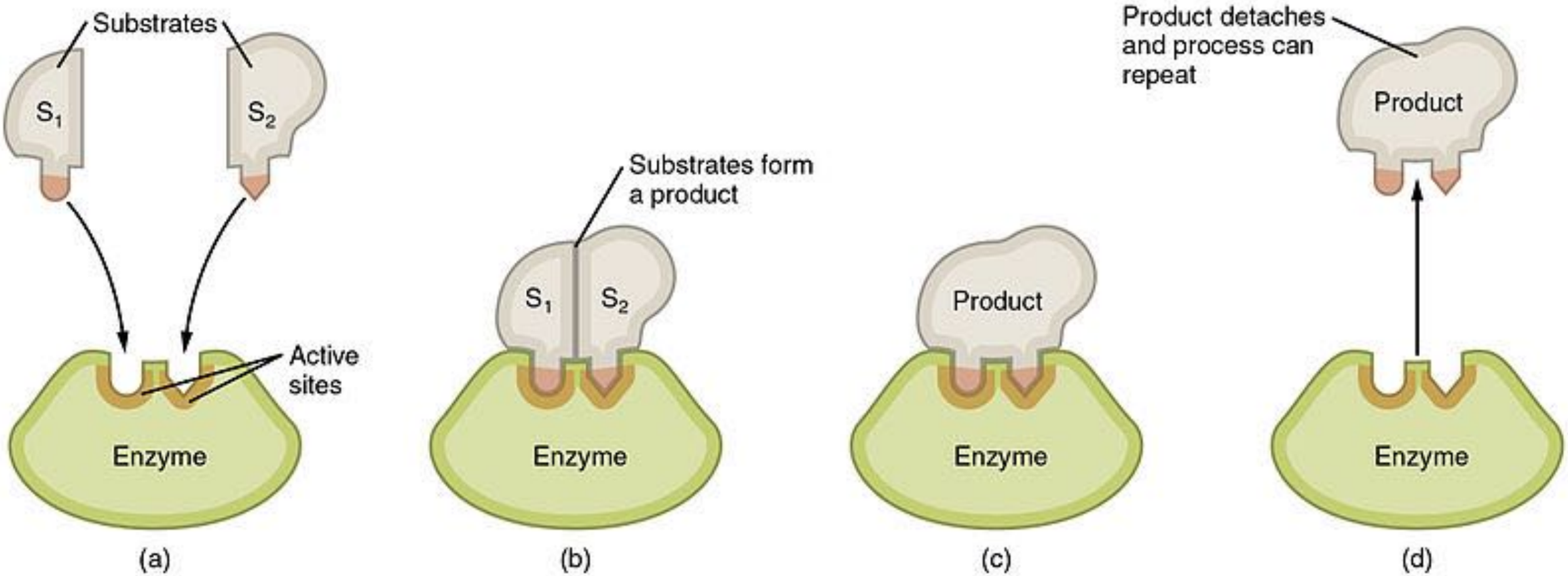
Snapshot of the process in 3D



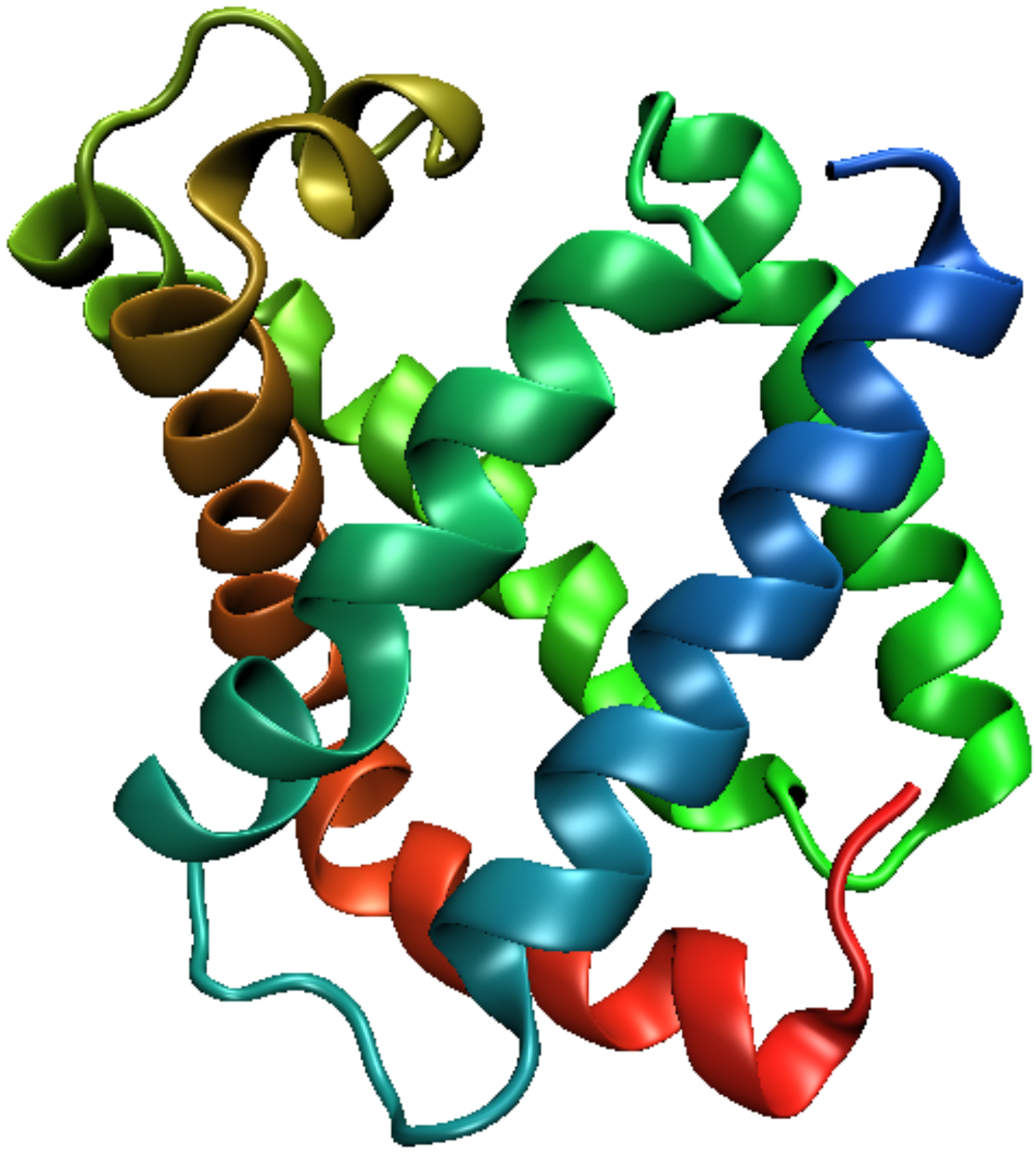
Protein folding



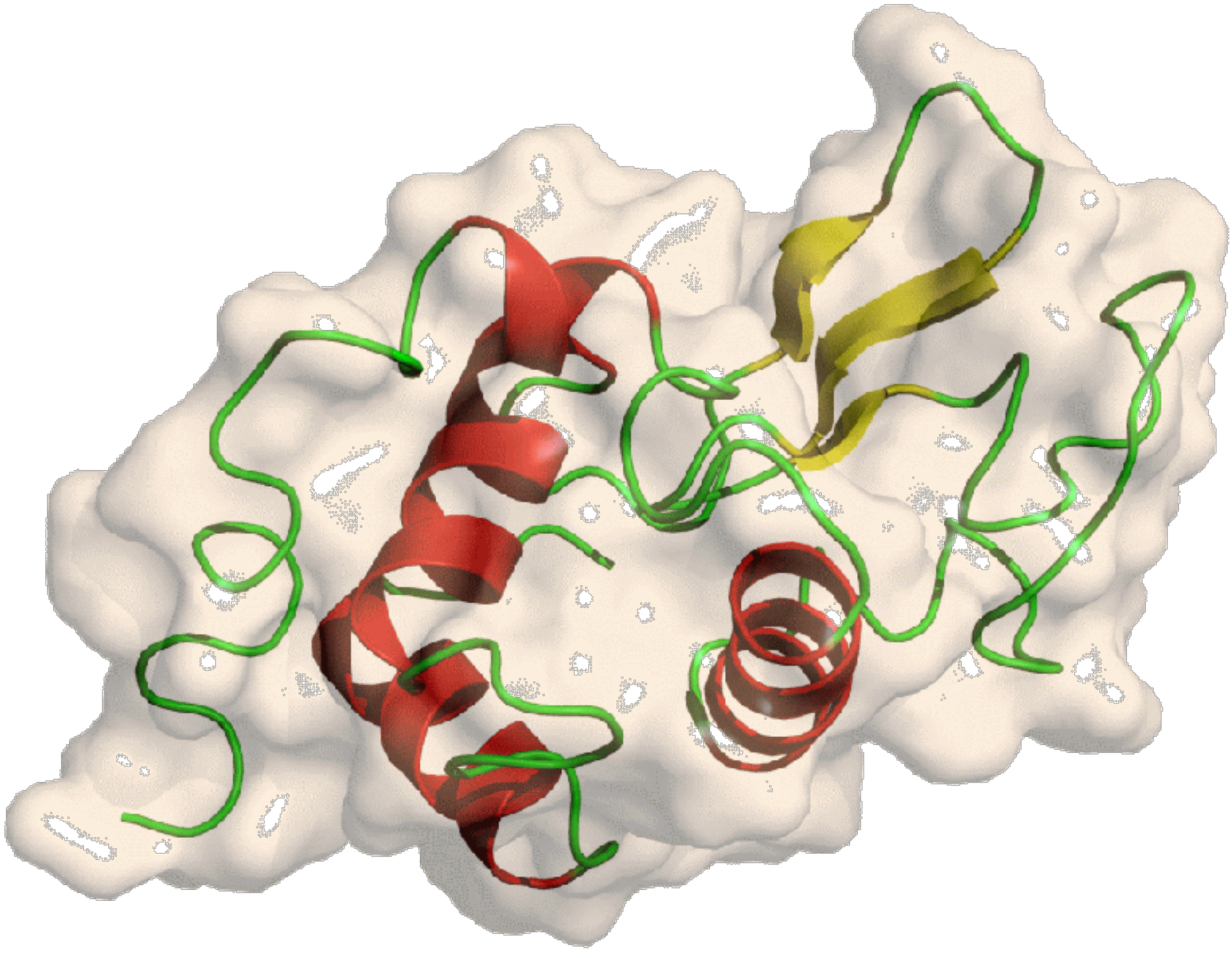
Some proteins are enzymes



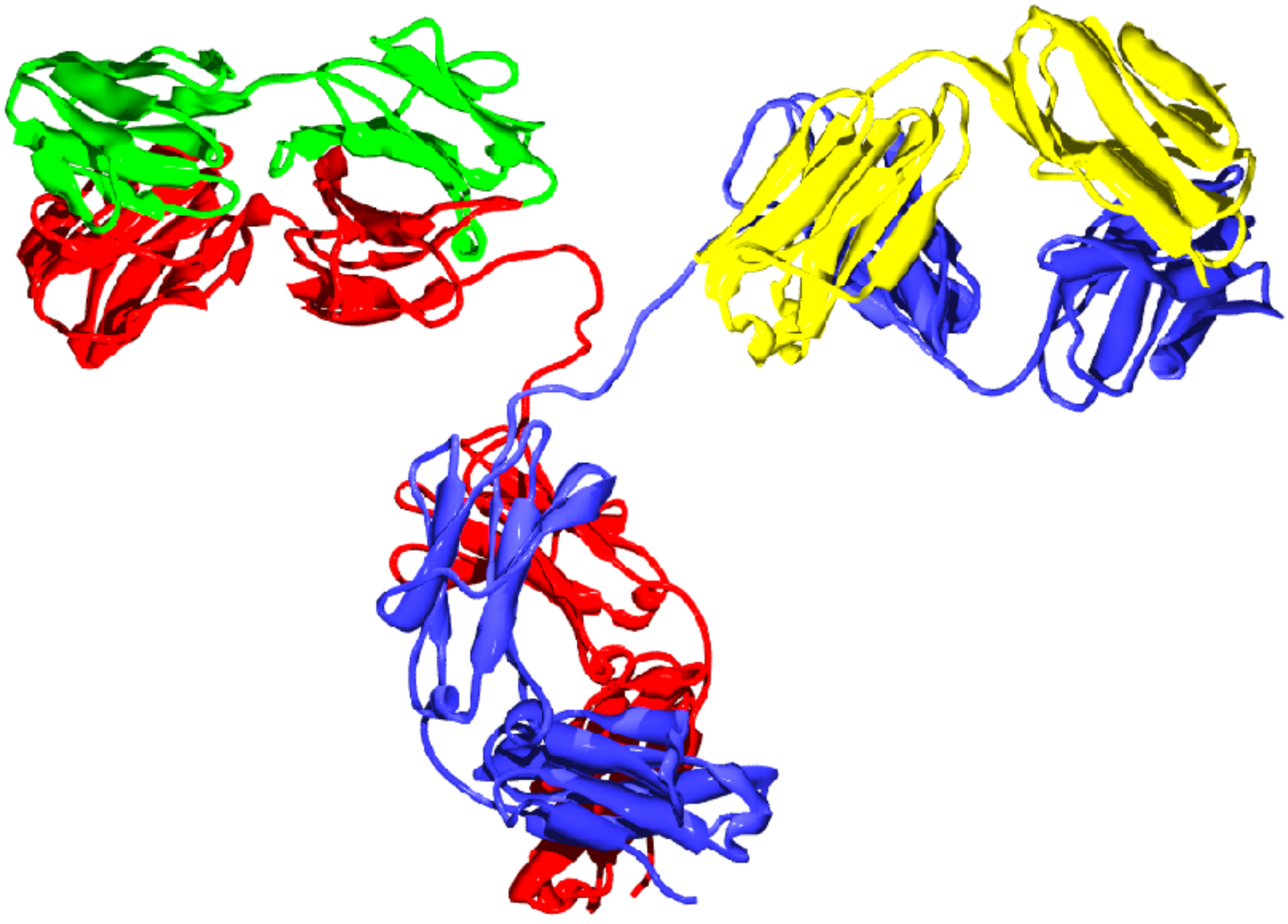
Myoglobin



Lysozyme

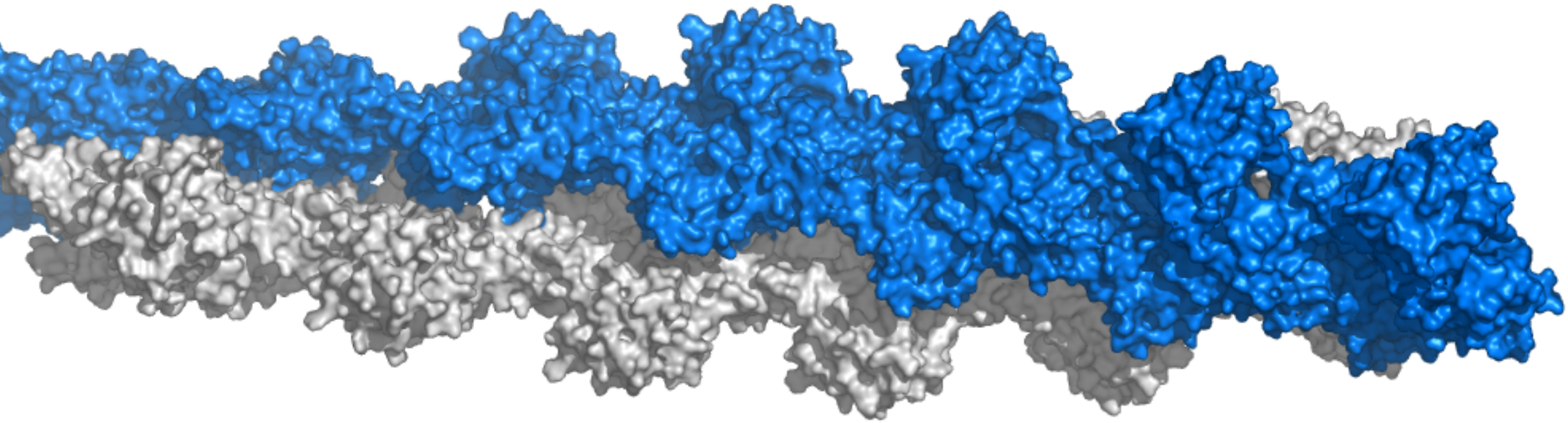


Antibody

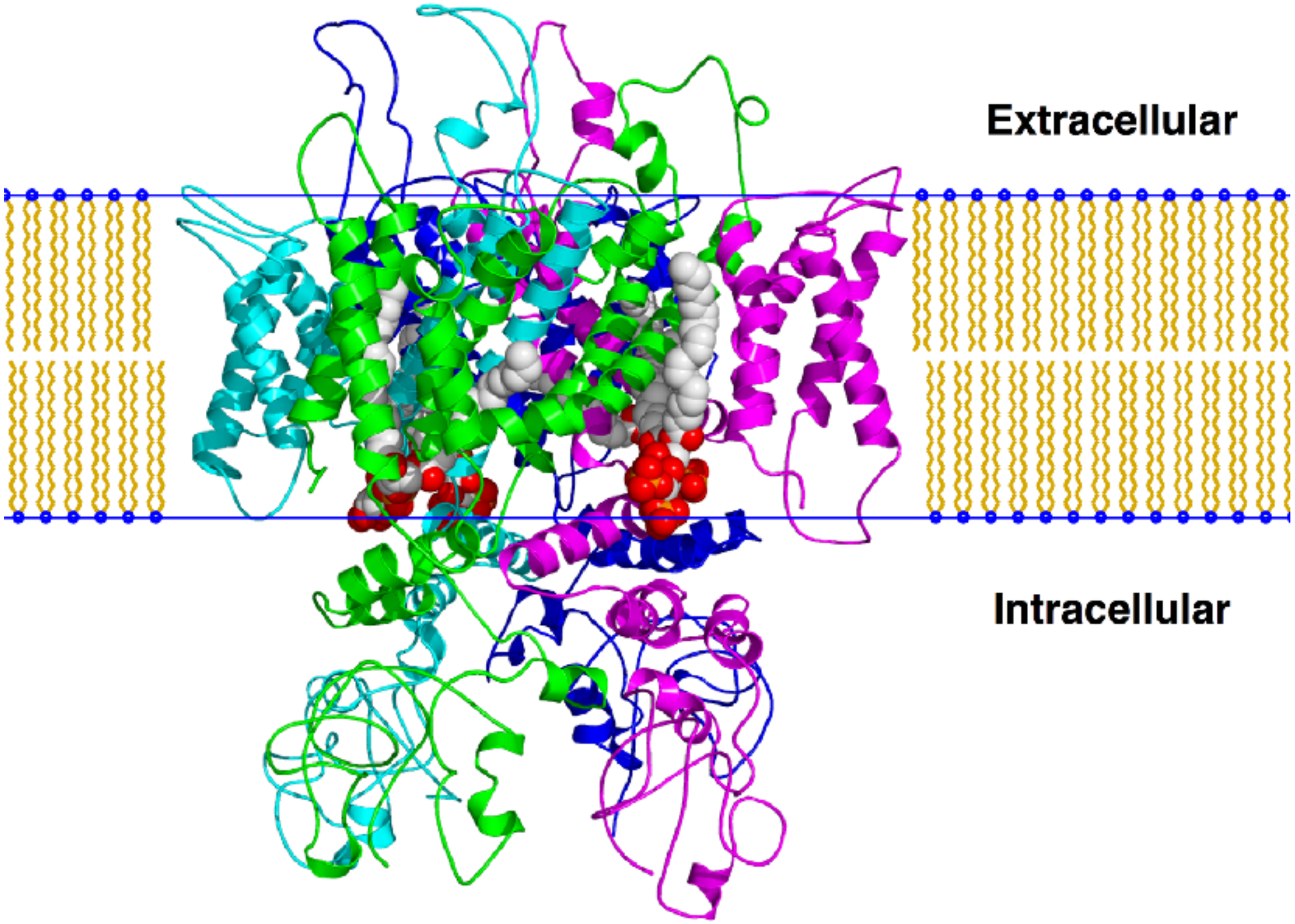


PDB 1IGT, PMID 9048542

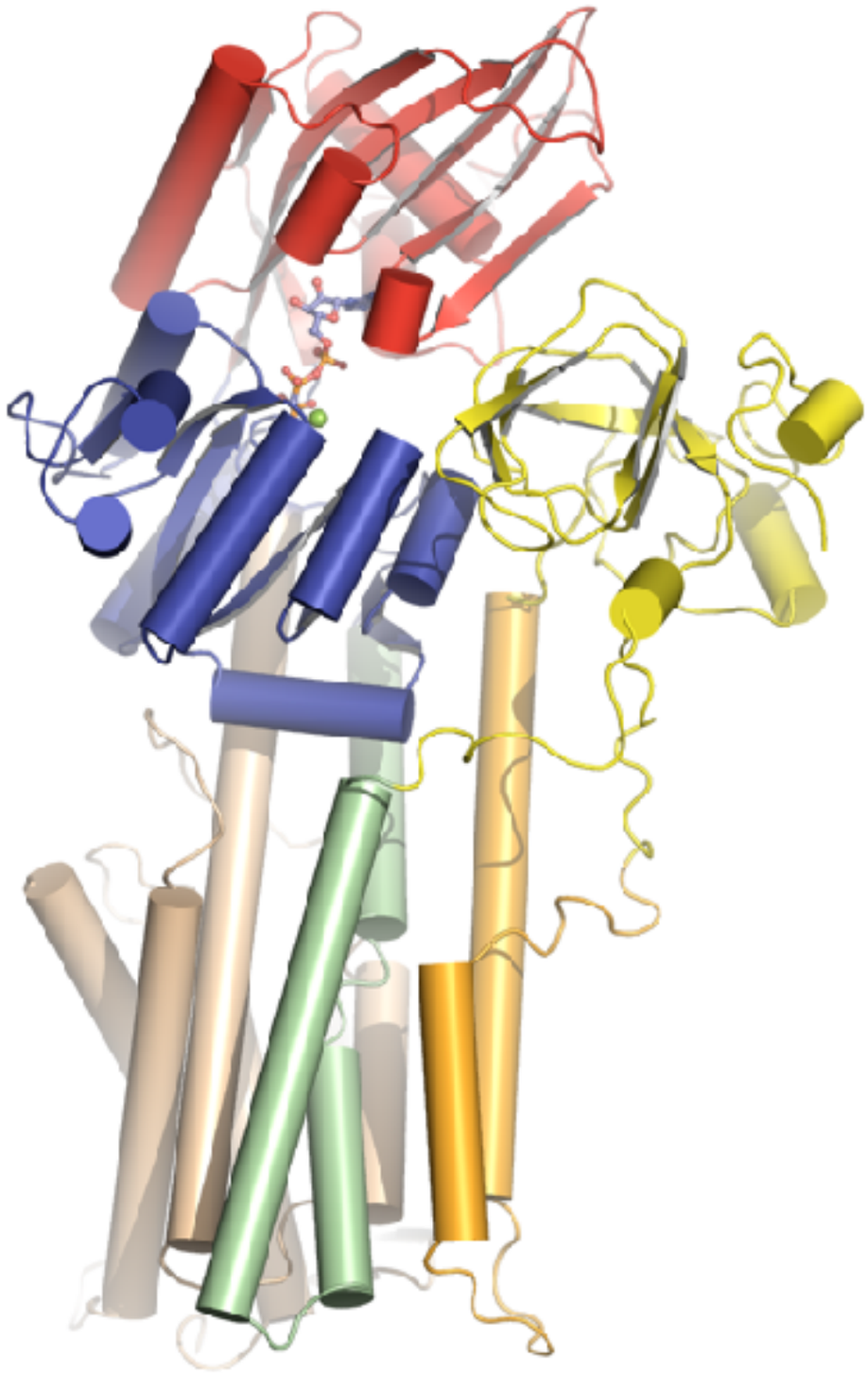
Structural proteins: Actin



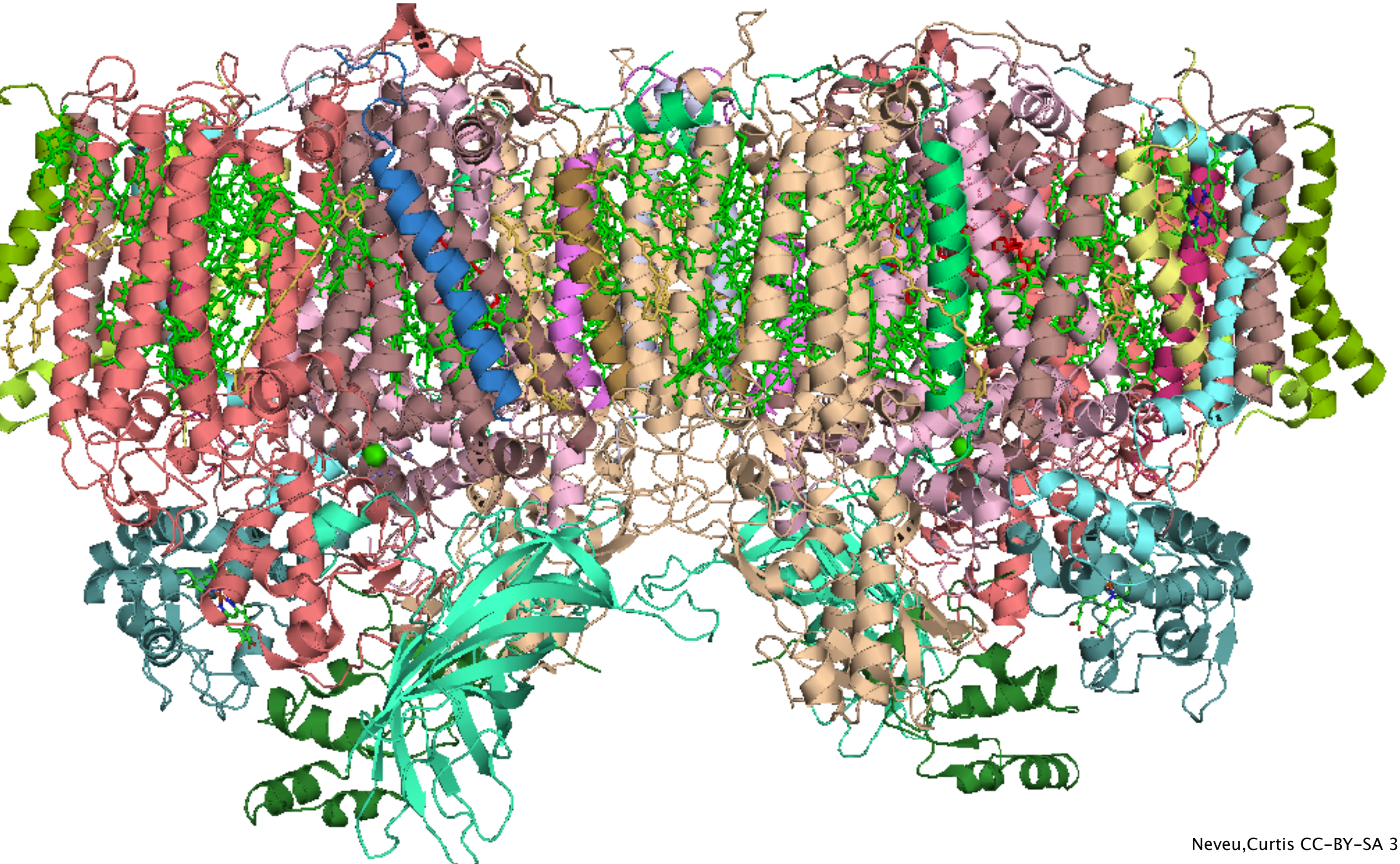
Receptor proteins



ATPase



Photosystem II



Sequence = Code

DNA TACCGAATTGAGTAATAGGGAACCT

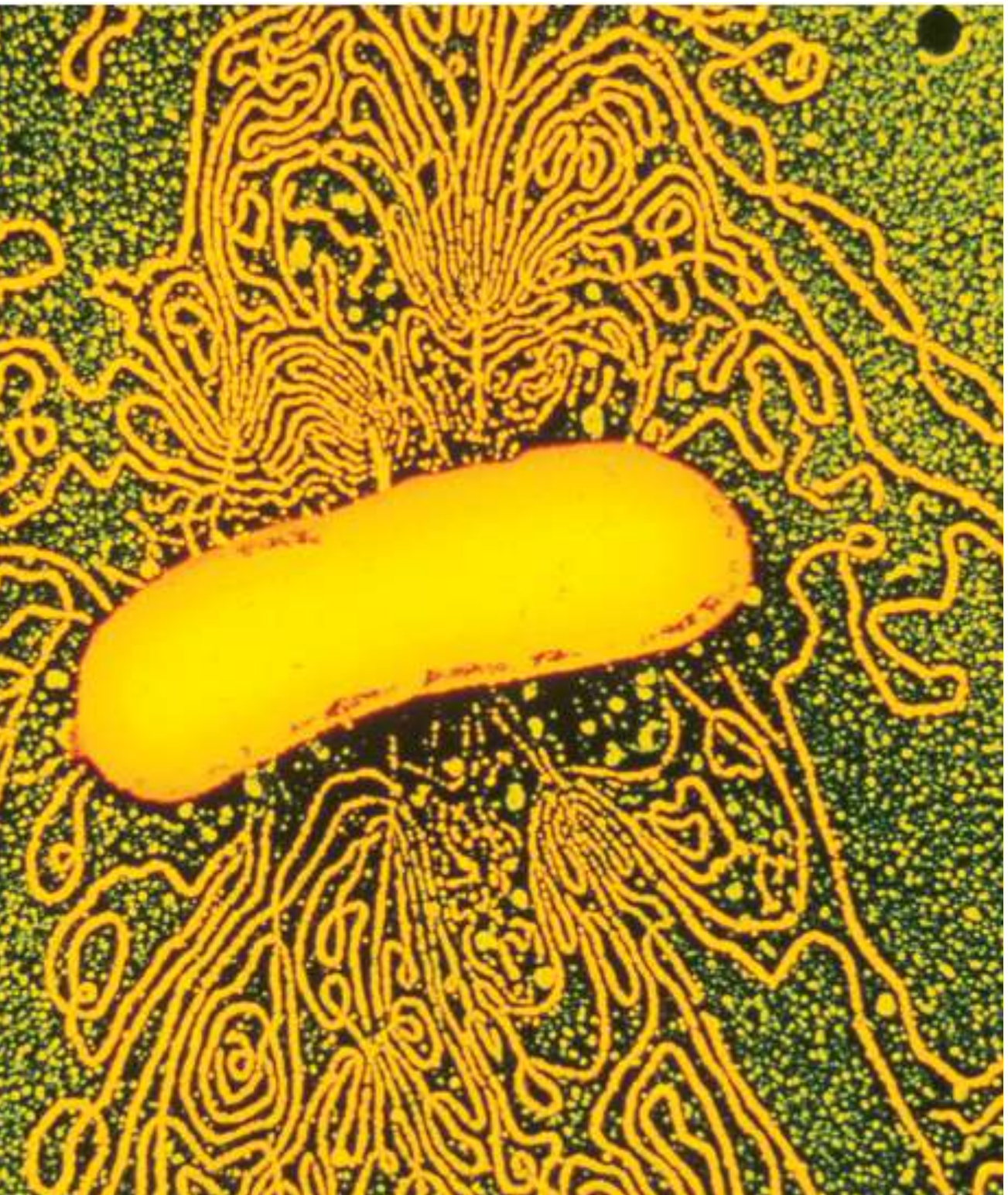
RNA AUGGCUUAACUCAUUAUCCCUUGGA

AA Met Ala Stop

Folded AA = Protein

Shape = Function

5,000 vs 25,000 genes



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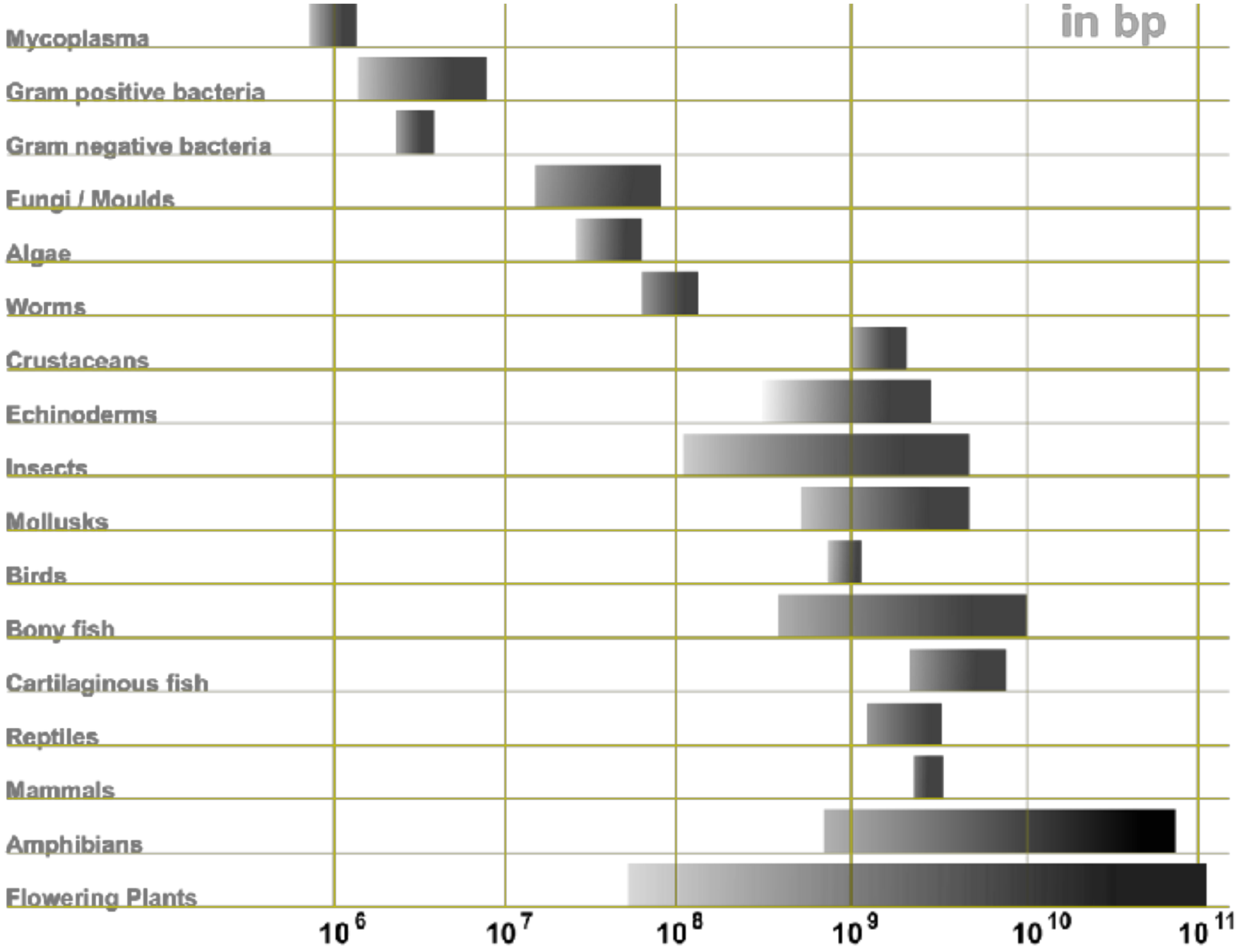
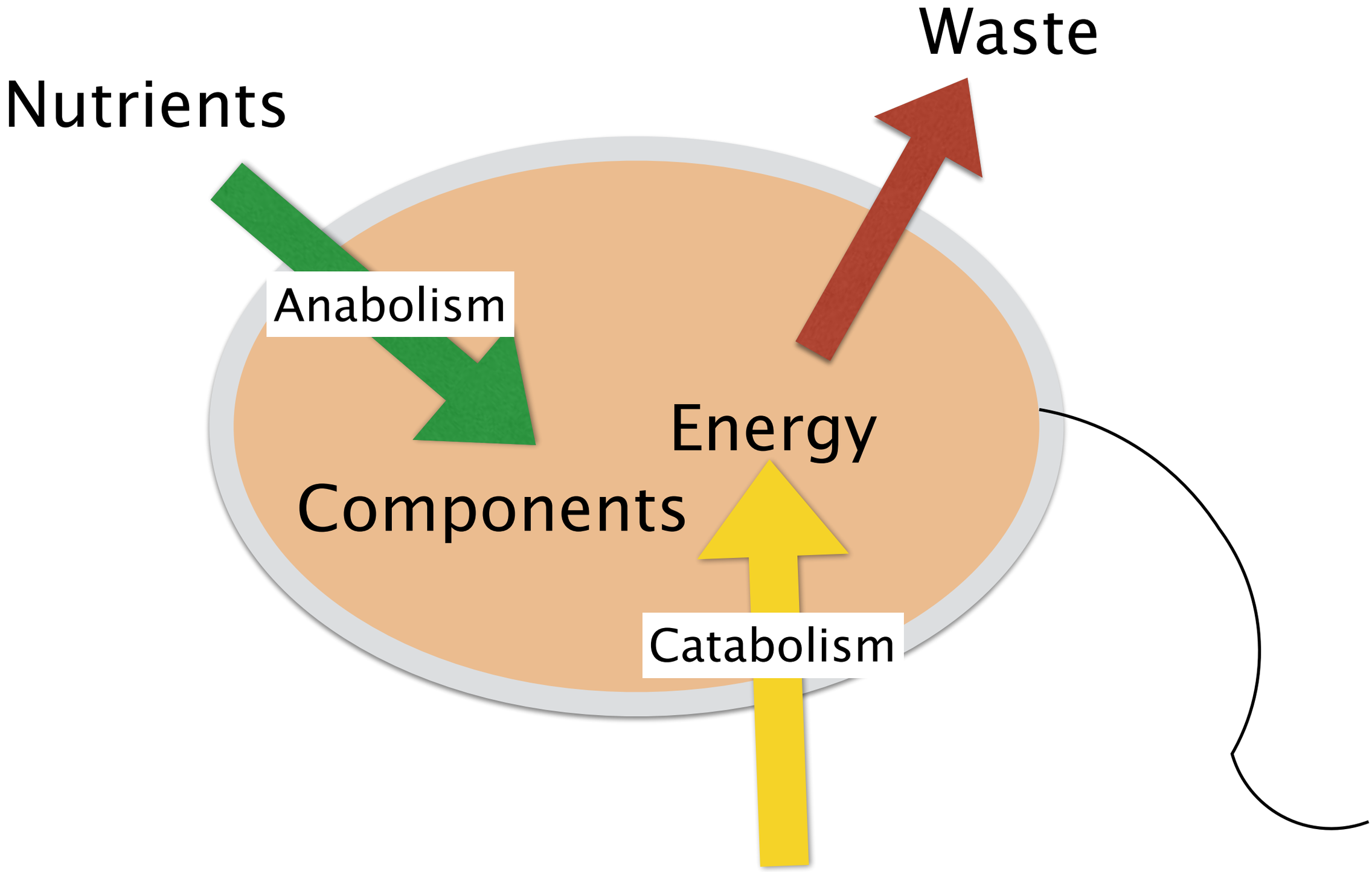
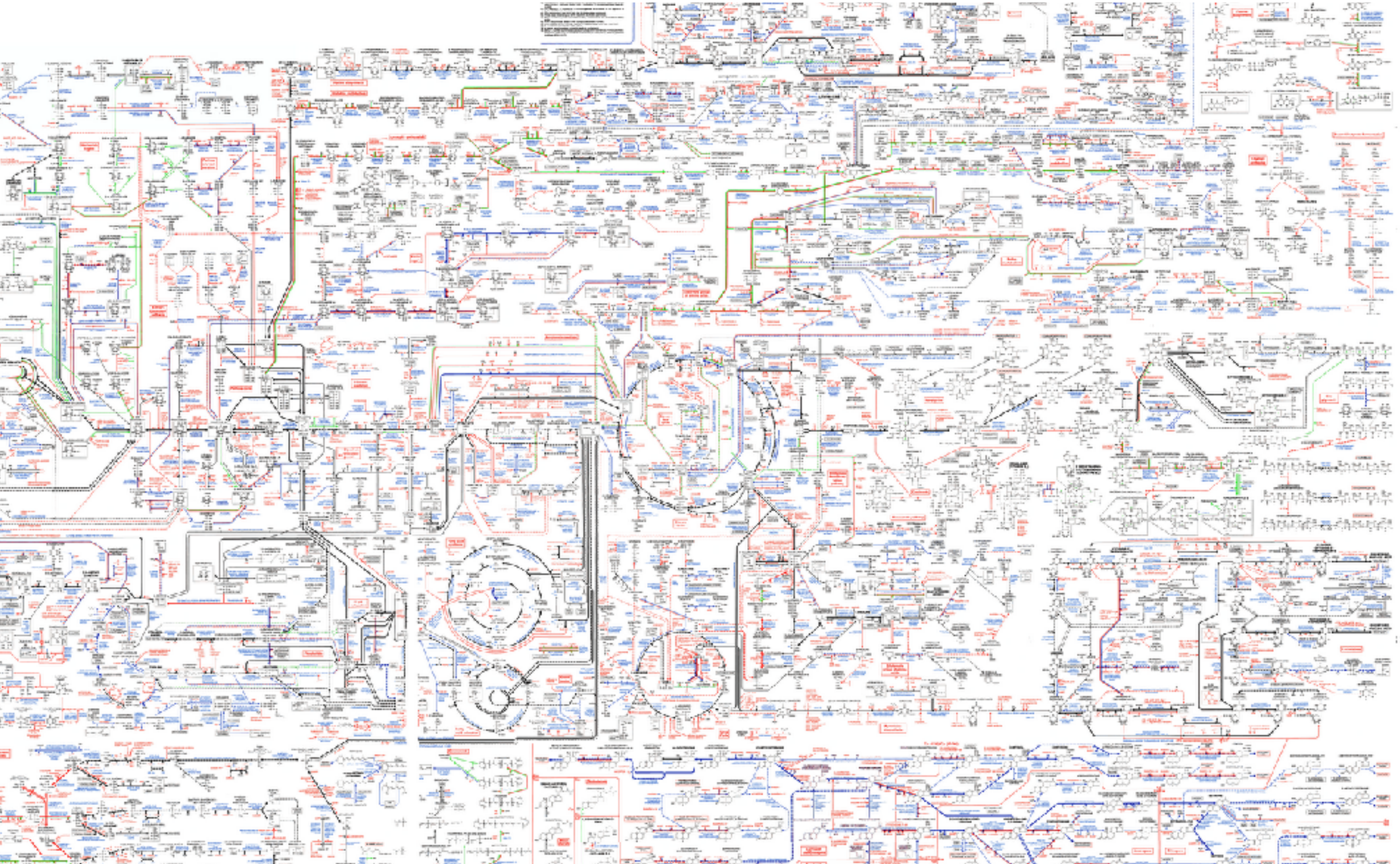
Genome size compared



Black box approach



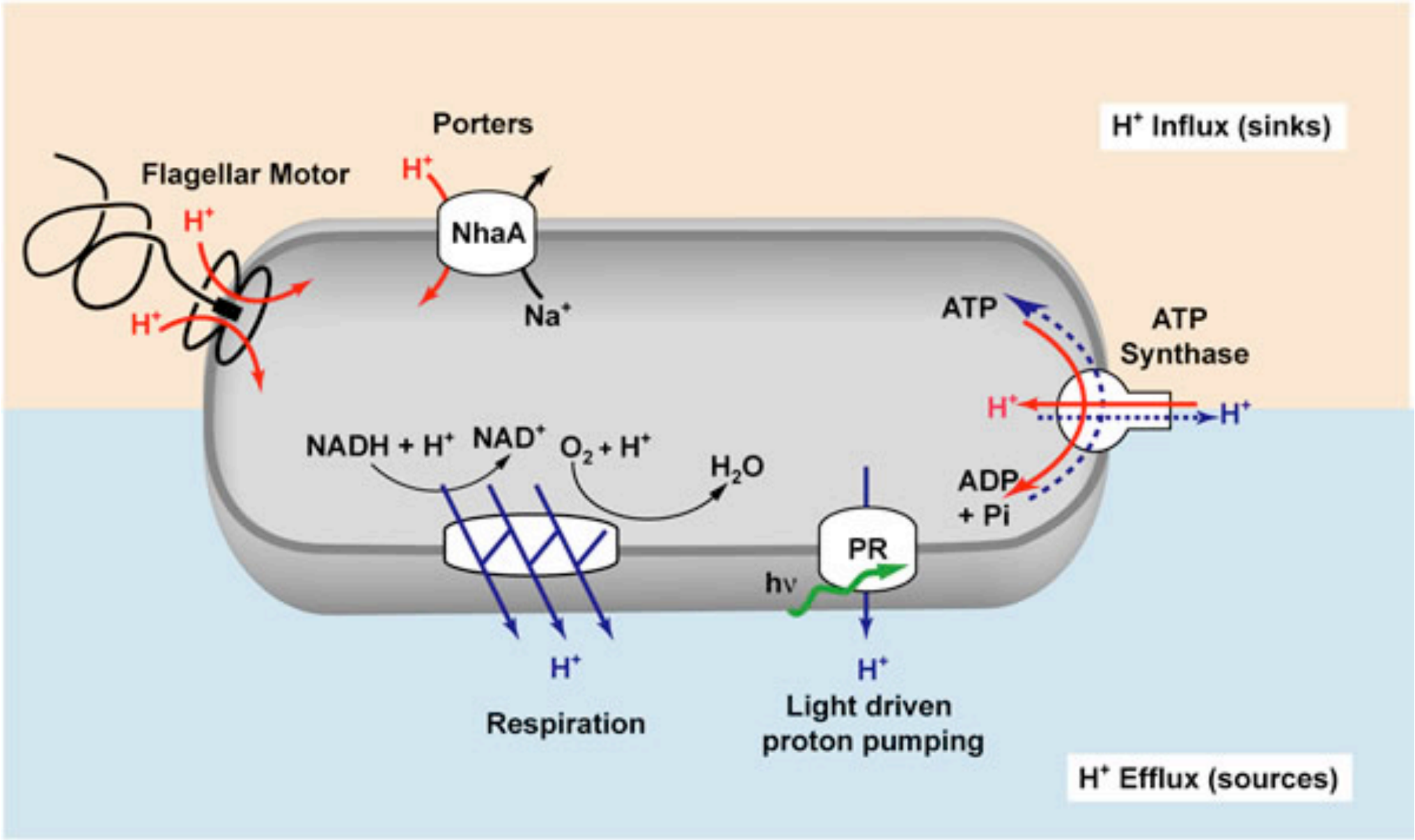
Metabolic Pathways



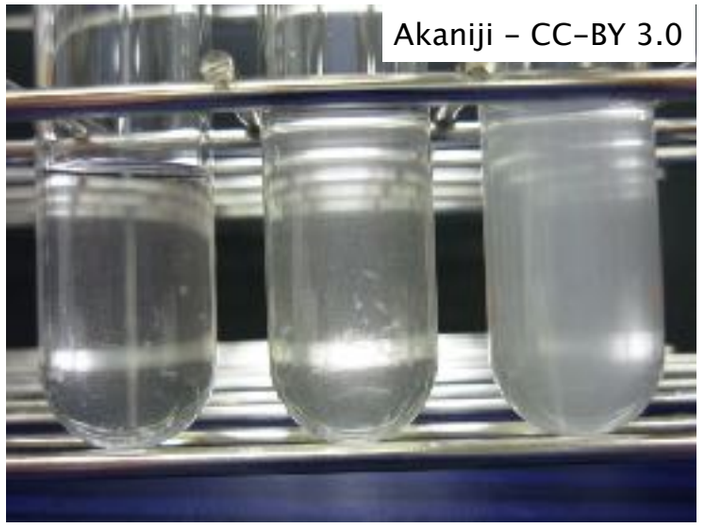
Diversity in Metabolism

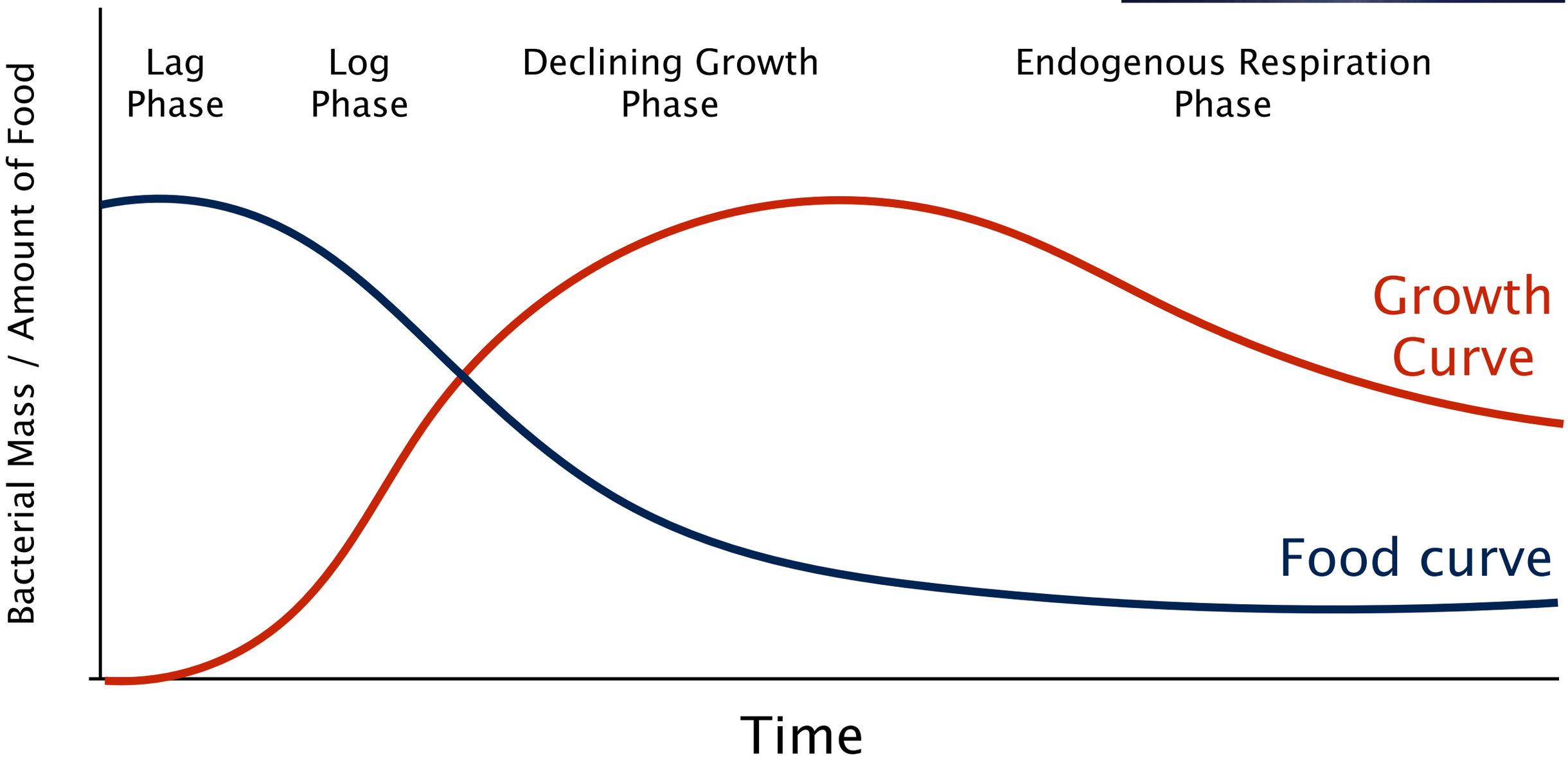
All Organisms

Membranes create potential



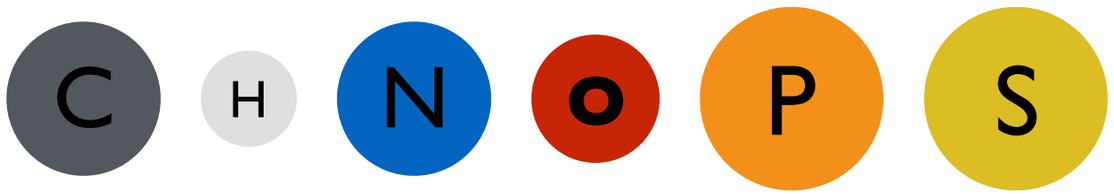
Bacterial growth curve



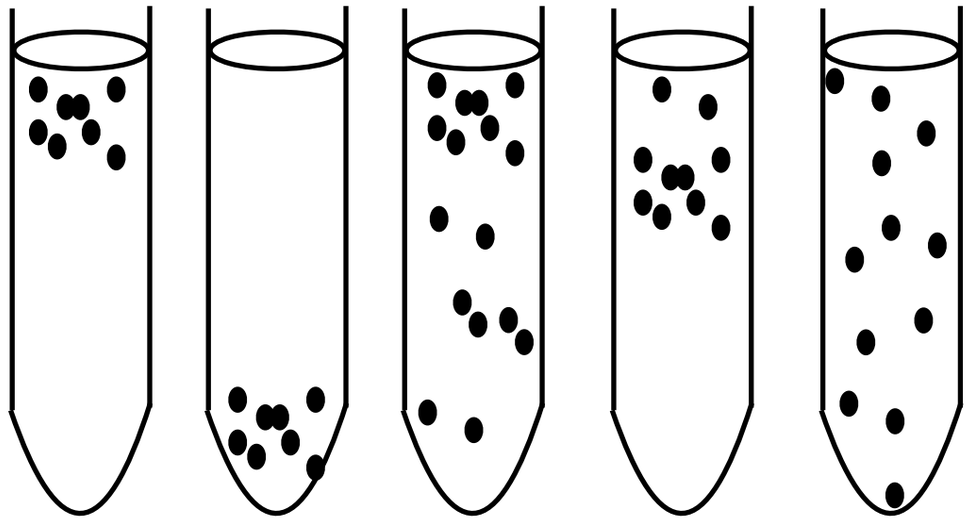


Diversity in growth conditions

Nutrients



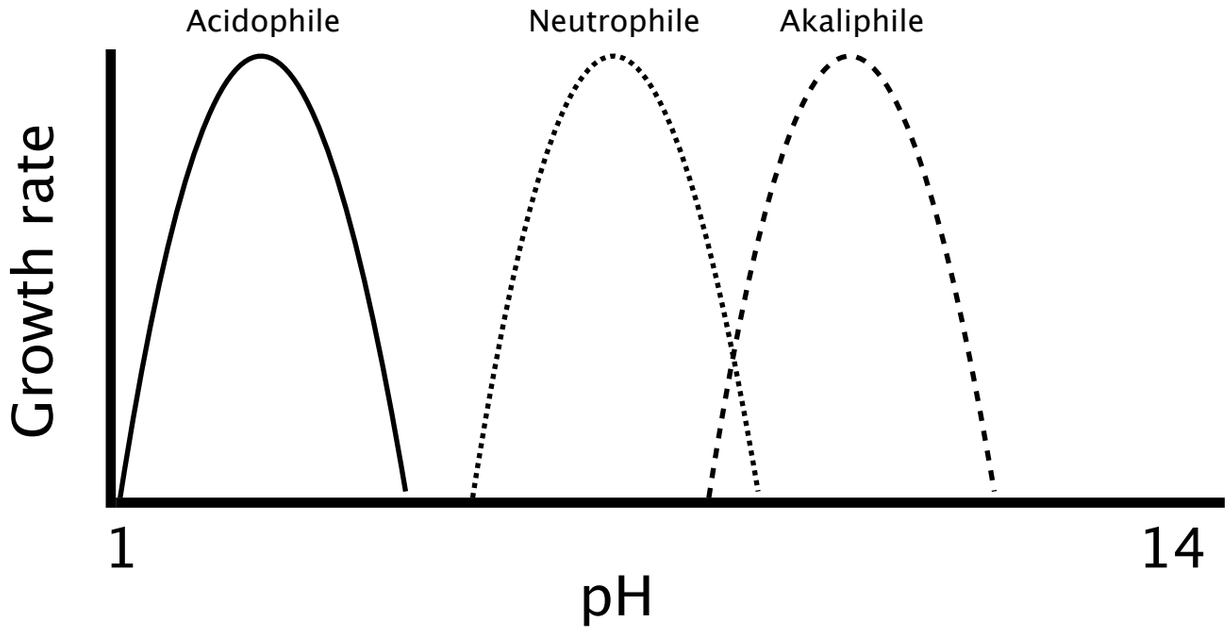
Atmosphere



Temperature

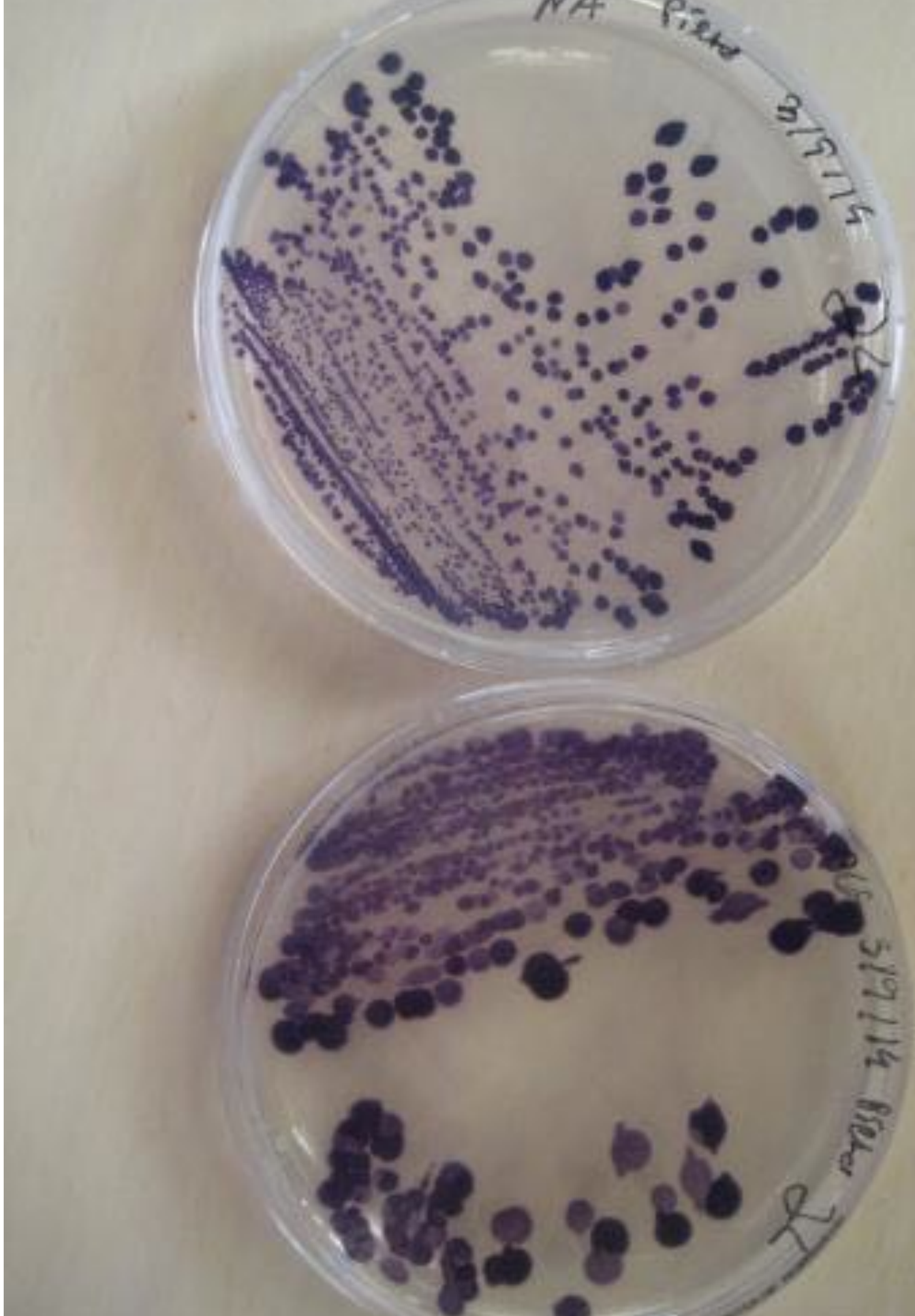
Pixabay - CC0

pH



Non selective

- Plate count agar
- Nutrient agar



Slightly selective

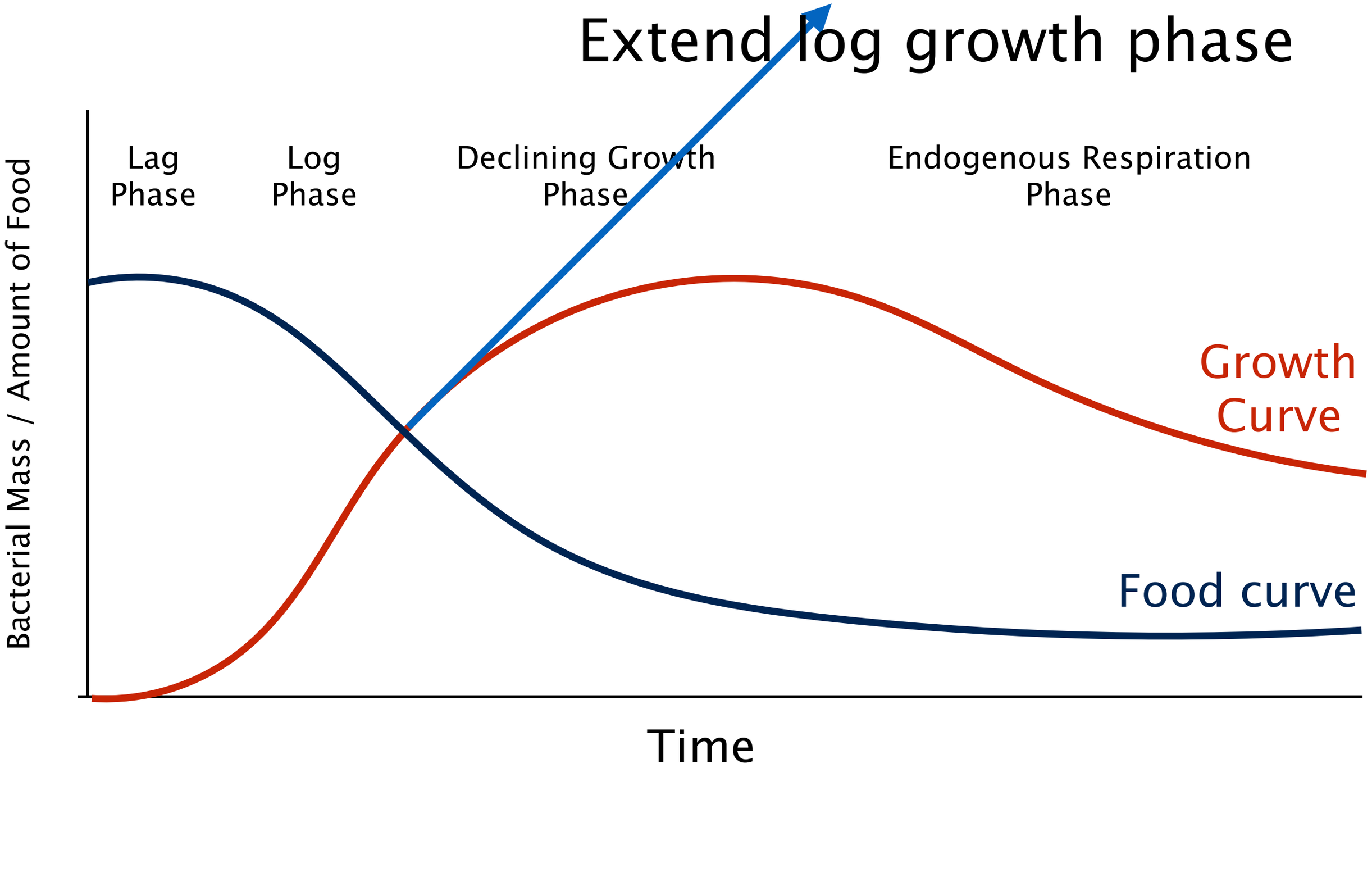
- Malt agar
- MRS agar

- Kombucha medium

Selective

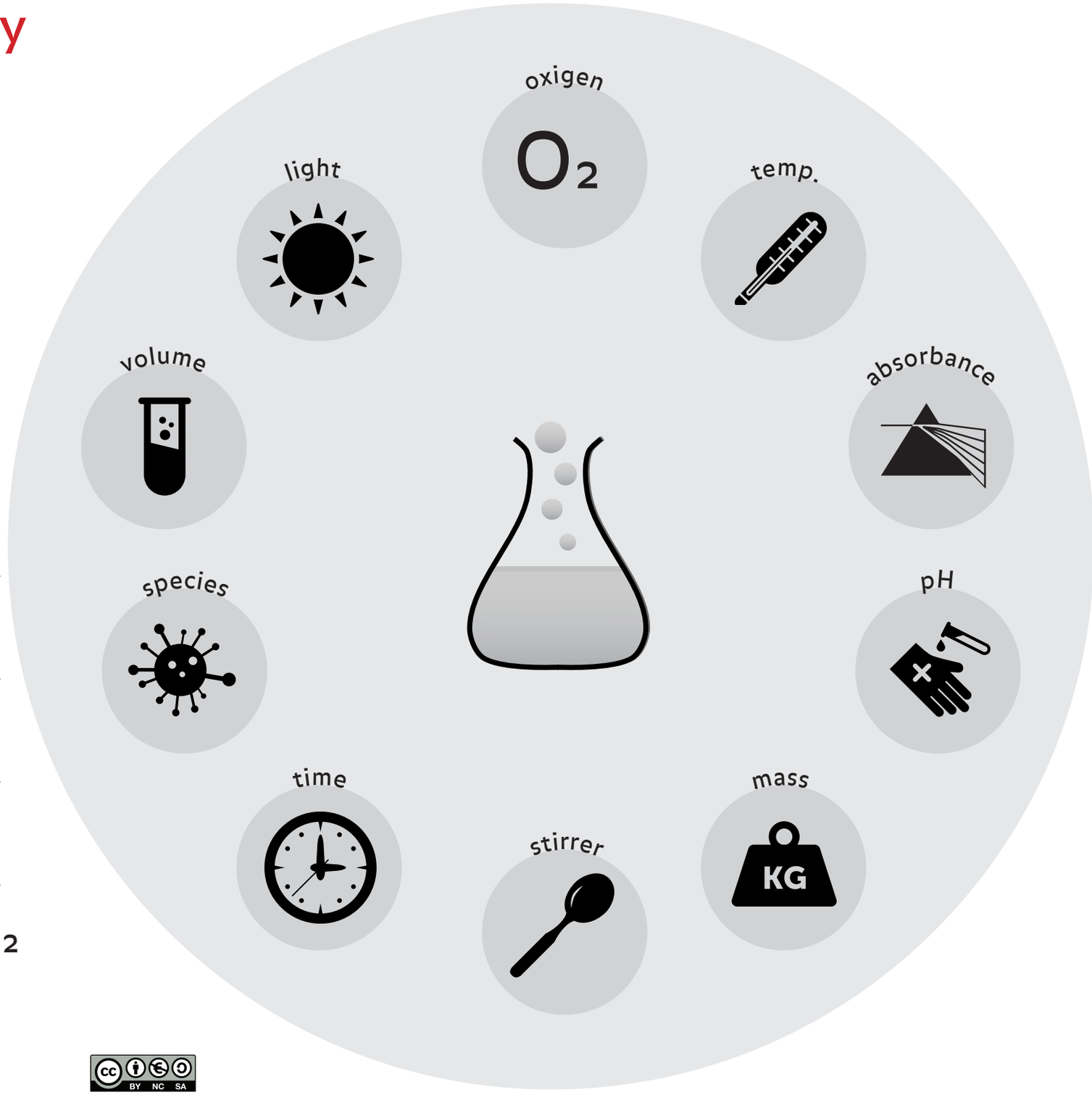
- Spirulina medium

Primary products



BioFactory
canvas

open wetlab
waag society



input

C

N

P

O_2

S

observations

day #	
day #	
day #	
day #	
day #	

material

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Example Production Process Design

Violacein production

Janthinobacterium lividum

My online search for J. lividum

- „Janthinobacterium lividum” +
  - „growth conditions”
  - „violacein pathway”
  - „violacein genes”
  - „patent”
  - „yield”
  - „inhibition”
  - „extraction”

Violacein pricing?

SIGMA-ALDRICH

200,000+ PRODUCTS | 500+ SERVICES | Featured INDUSTRIES | Hello. Sign in. ACCOUNT | 24/7 SUPPORT | 0 items ORDER

Netherlands Home › V9389 - Violacein from *Janthinobacterium lividum*

V9389 SIGMA

Violacein from *Janthinobacterium lividum*

>98% (violacein (minimum 85% violacein) and deoxyviolacein, HPLC)

MSDS | SIMILAR PRODUCTS

CAS Number 543-54-9 | Empirical Formula (Hill Notation) $C_{20}H_{13}N_3O_3$ | Molecular Weight 343.34

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Properties

Related Categories	Apoptosis Inducers, Apoptosis and Cell Cycle, Bioactive Small Molecule Alphabetical Index, Bioactive Small Molecules, Cell Biology, More...
assay	**>98% (violacein (minimum 85% violacein) and deoxyviolacein, HPLC)**
solubility	**H_2O: insoluble**
	acetone: soluble
	ethanol: soluble

Price and Availability

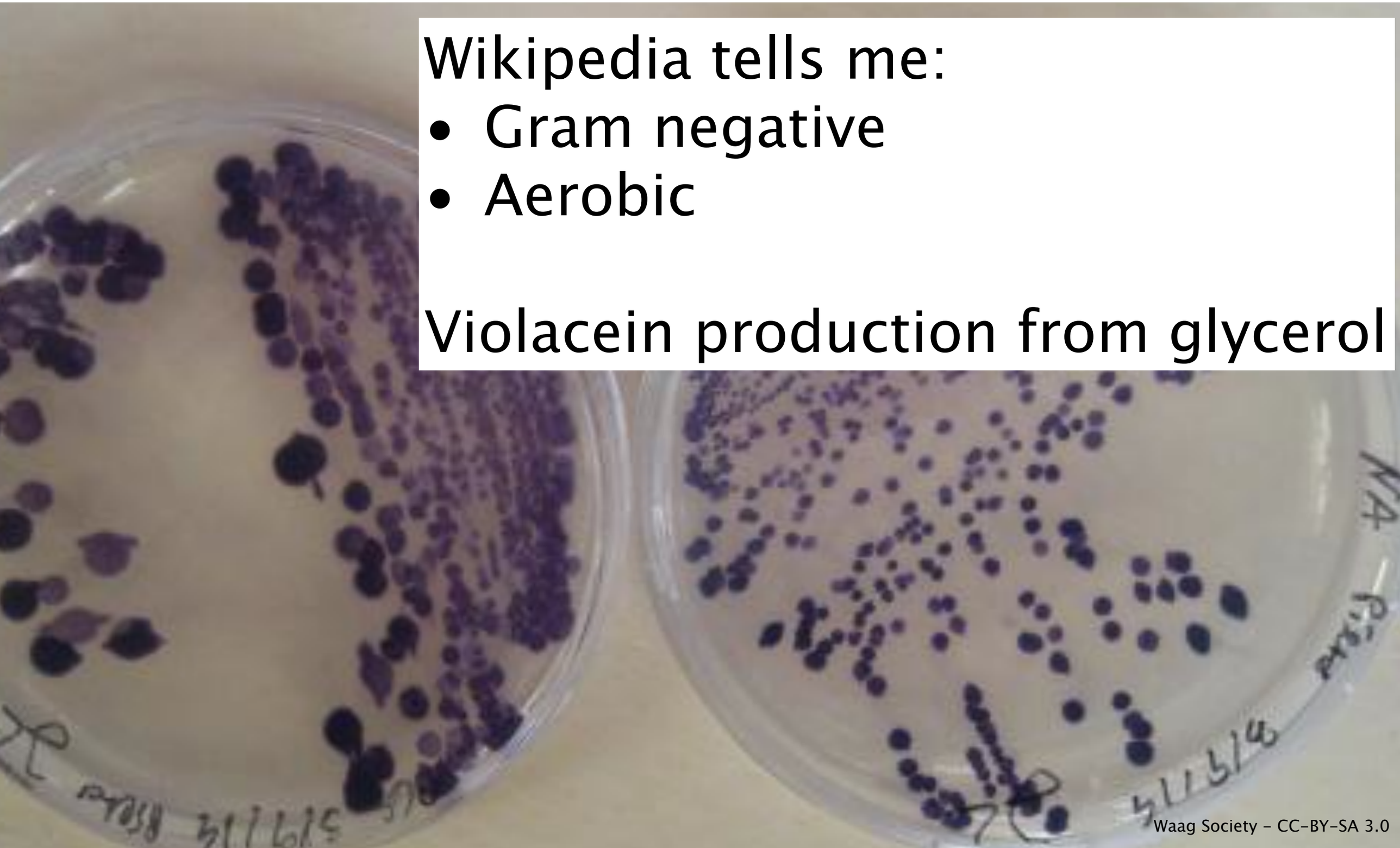
SKU-Pack Size	Availability	Price (EUR)	Quantity
V9389-1MG	1 left in stock. Order soon. - FROM	308.00	0

Bulk orders?

ADD TO CART

Protein-Protein Interaction Webinar Series

Janthinobacterium lividum

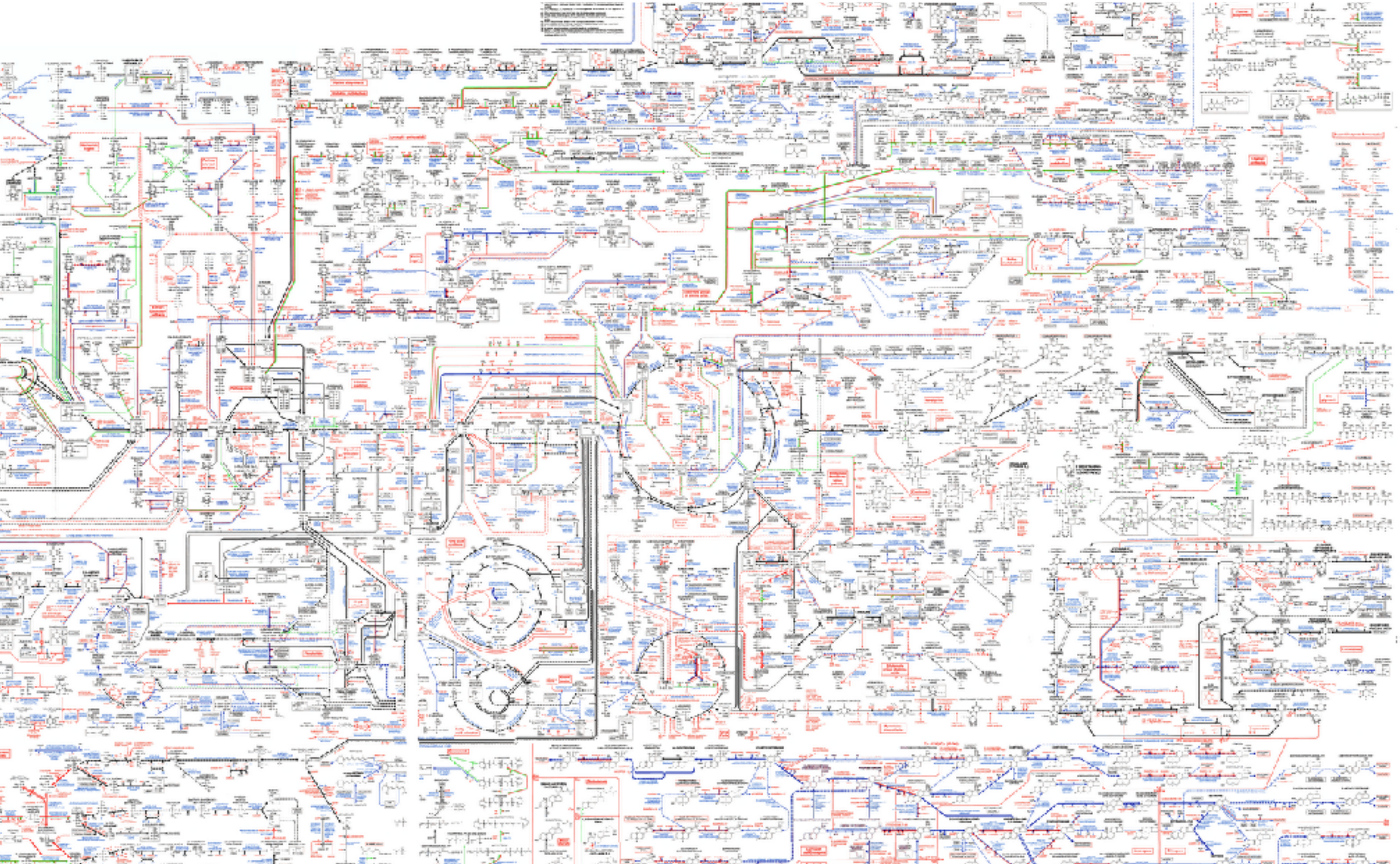


Wikipedia tells me:

- Gram negative
- Aerobic

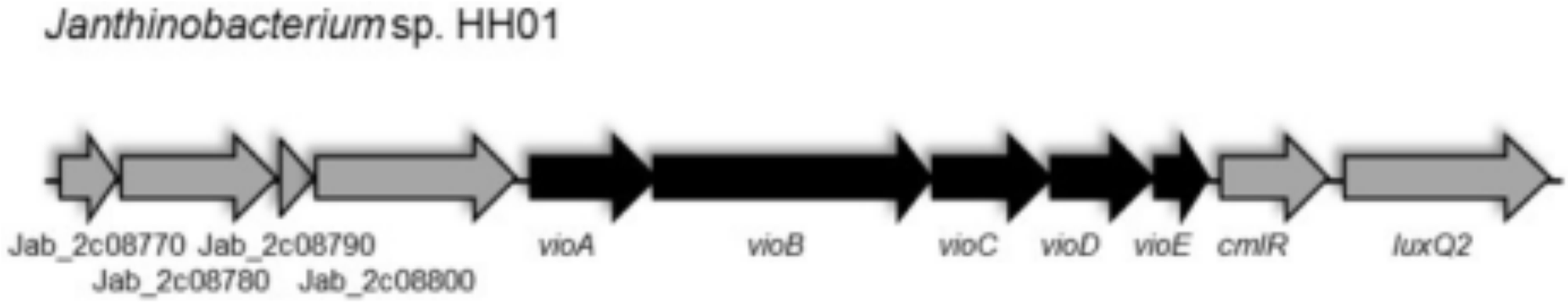
Violacein production from glycerol

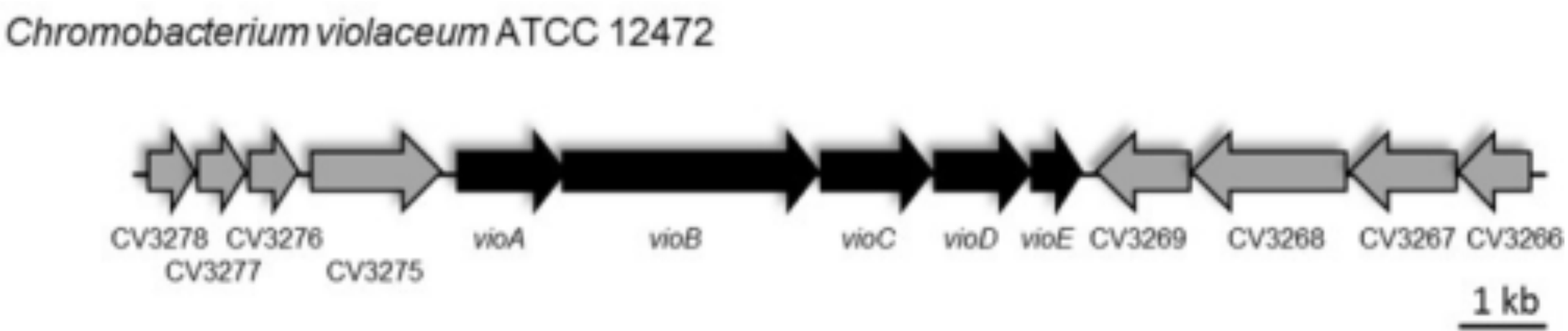
Production pathway?



Violacein genes?

Hornung et al. - The Janthinobacterium sp. HH01 Genome Encodes a Homologue of the V. cholerae CqsA and L. pneumophila LqsA Autoinducer Synthases (2013)





Production pathway?

Tryptophan

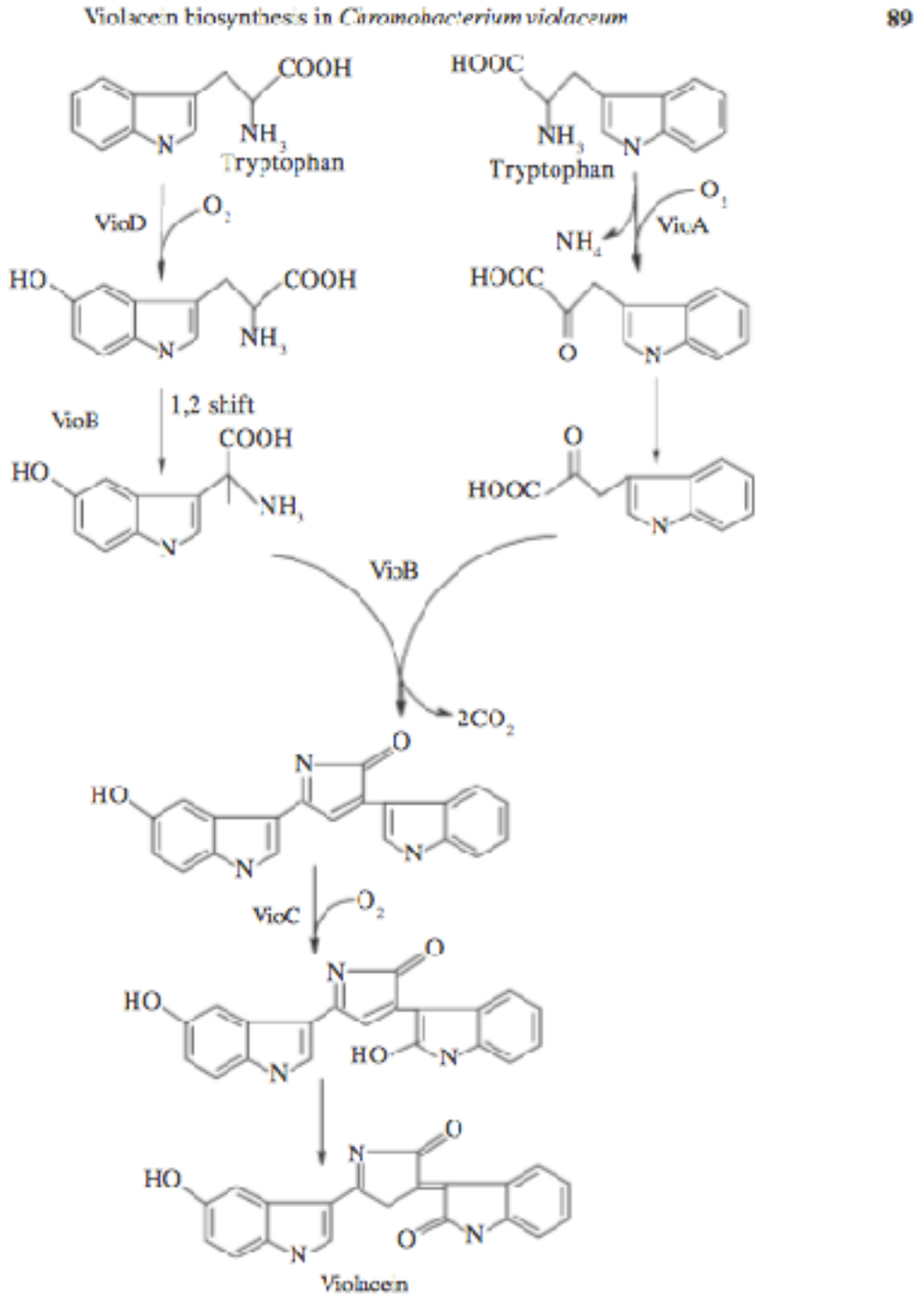


Figure 2. Violacein biosynthesis, as proposed by August et al., 2000. VioA, VioB, VioC, and VioD are the gene products of the biosynthesis operon, encoding nucleotide-dependent monooxygenases and a protein similar to a polyketide synthase (VioB).

Antonio, R.V. and Creczynski-Pasa, T.B. (2004) Genetics analysis of violacein biosynthesis by C. violaceum. Genet Mol Res 3, 85-91.

Other interesting things:

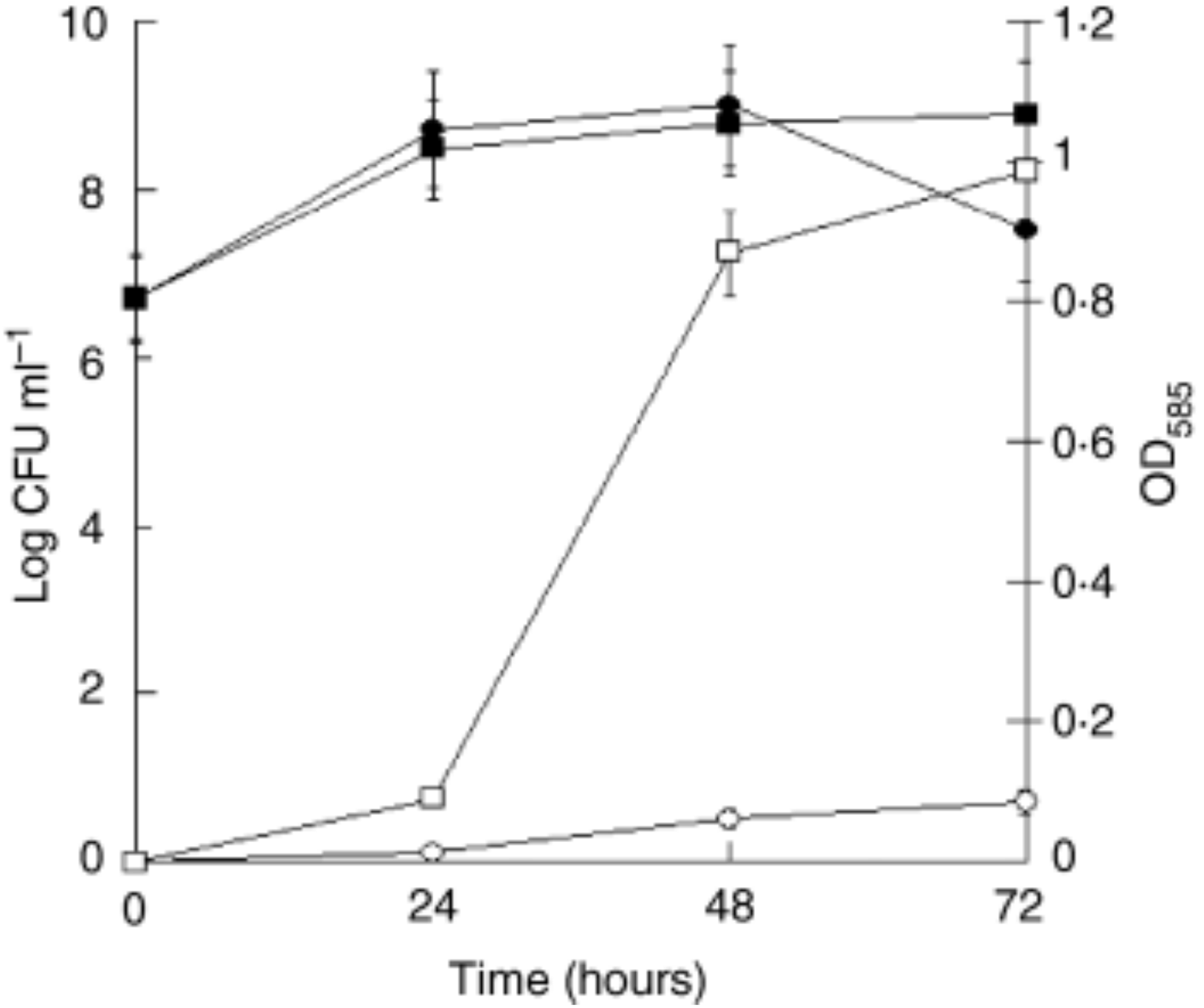
- *J. lividum* produces a metallo-*β*-lactamase conferring resistance to several *β*-lactam antibiotics

Rossolini, G.M., Condemi, M.A., Pantanella, F., Docquier, J.D., Amicosante, G. and Thaller, M.C. (2001) Metallo-β-lactamase producers in environmental microbiota: new molecular class B enzyme in Janthinobacterium lividum. Antimicrob Agents Chemother 45, 837–844.

- Violacein:
  - C_{20}–H_{13}–N_3–O_3
  - molecular weight of 343·33
  - insoluble in water
  - soluble in alcohols as methanol, ethanol and acetone
  - maximal absorption in a solution of methanol is at 585 nm

Blosser, R.S. and Gray, K.M. (2000) Extraction of violacein from Chromobacterium violaceum provides a new quantitative bioassey for N-acyl homoserine lactone autoinducers. J Microbiol Methods 40, 47–55.

Production inhibition



Pantanella, F., Berlutti, F., Passariello, C., Sarli, S., Morea, C. and Schippa, S. (2007), Violacein and biofilm production in *Janthinobacterium lividum*. Journal of Applied Microbiology, 102: 992–999. doi: 10.1111/j.1365-2672.2006.03155.x

Production conditions?

Growing the bacteria in culture took 5 days before the culture would turn purple due to *J. lividum* forming a biofilm in the media. Large culture growth by embedding sterile cotton mats in sterile 2L bottles with nutrient media with the added glycerol and L-tryptophan **(fig. 2)** that showed purple coloring after 48 hour incubation [9]. The mats were extracted after 5 days to harvest the violacein. Yield of violacein from after crude methanol extraction and low was about 10mg.

Figure 2: Violacein optimization. 1% Glycerol and 250μM L-tryptophan were added to the nutrient broth media to enhance pigment development. Cotton mats were used to allow bacteria to become sessile and produce violacein faster than with liquid cultures.

Patent? - USPTO

Process for the production of violacein and its derivative deoxyviolacein containing bioactive pigment from Chromobacterium sp. (MTCC5522)

EXAMPLE 1

PRODUCTION AND EXTRACTION OF THE BIOACTIVE PIGMENT FROM THE CULTURE OF CHROMOBACTERIUM SP. NIIST-CKK-01

A loopful of 24 hrs old pure culture Chromobacterium sp. NIIST-CKK-01 from solid agar medium (LB agar or Nutrient agar) was inoculated with 50 ml of the growth medium (0.5% Yeast extract and 1.5% Peptone) taken in a 250 ml Erlenmeyer flask. Alternatively, 10% (v/v) of 24 hour old pure culture of Chromobacterium sp. NIIST- CKK-01 in LB broth was also used as inoculum. The pH of the medium was 7. The flasks inoculated with Chromobacterium sp. NIIST-CKK-01 were subsequently incubated in a rotary shaker at ambient temperature (30 °C) and 200 rpm for 24 hours. The deep purple purple-blue pigment starts appearing in the medium by about 6 hours of incubation and continued beyond biomass increase (Fig 1).

After 24 hrs of incubation, the bacterial biomass with pigment was centrifuged at 9676.8 x g and 4 °C for 10 minutes. After centrifugation, the clear supernatant was removed. The pellet containing biomass and pigment was mixed thoroughly with 5 ml of extra pure methanol. The mixture was centrifuged again at 9676.8 χ g and 4 °C for 10 minutes to separate the cell pellet from the solvent-pigment mixture. The pigment extraction was repeated twice using fresh solvent as described. All the pigment extracted solvent pooled together and the pigment was concentrated by normal vacuum drying in a desiccator. The quantity of biomass and pigment produced could be accounted by measuring optical density at 600 nm and 575 nm respectively. The yield of pigment by this method was about 1.0 g pigment/g of dry biomass in 24 hrs.

HPLC analysis is carried out for checking the purity of the pigment produced using an ODS column (Lichrospher-100; Merck) with acetonitrile (40%) at lml/min as mobile phase and using UV-VIS detector at 575 nm (Figure 2). UV-VIS absorption spectra indicated maximum absorption at 575 nm, typical of violacein and its derivatives (Figure 3).

EXAMPLE 2

J. Lividum canvas

BioFactory canvas

open wetlab
waag society

Yes

oxigen
O_2

No light

15 temp.

volume
330 ml

absorbance
585

species

pH
?

time
48h

stirrer
No

mass
KG
1 mL?

input

C
N
P
O_2
S

Nutrient Broth
Glycerol
Tryptoph.

observations

day #	
day #	
day #	
day #	

material

Summary

- Life is made out of cells
- Cells are envelopes made out of lipids
- Cells create specialised structures to conduct chemical reactions
  - Structures are made out of standardised blocks
    - DNA out of nucleotides (A, T, C or G)
    - Proteins out of amino acids (20 types)
  - The combination (sequence) of building blocks results in a specific 3D shape
    - Shape = function
    - Shapes interact by docking
- Diversity in metabolism
- Diversity in growth conditions
  - BioFactory canvas: use as a tool
- Example Production Process design