

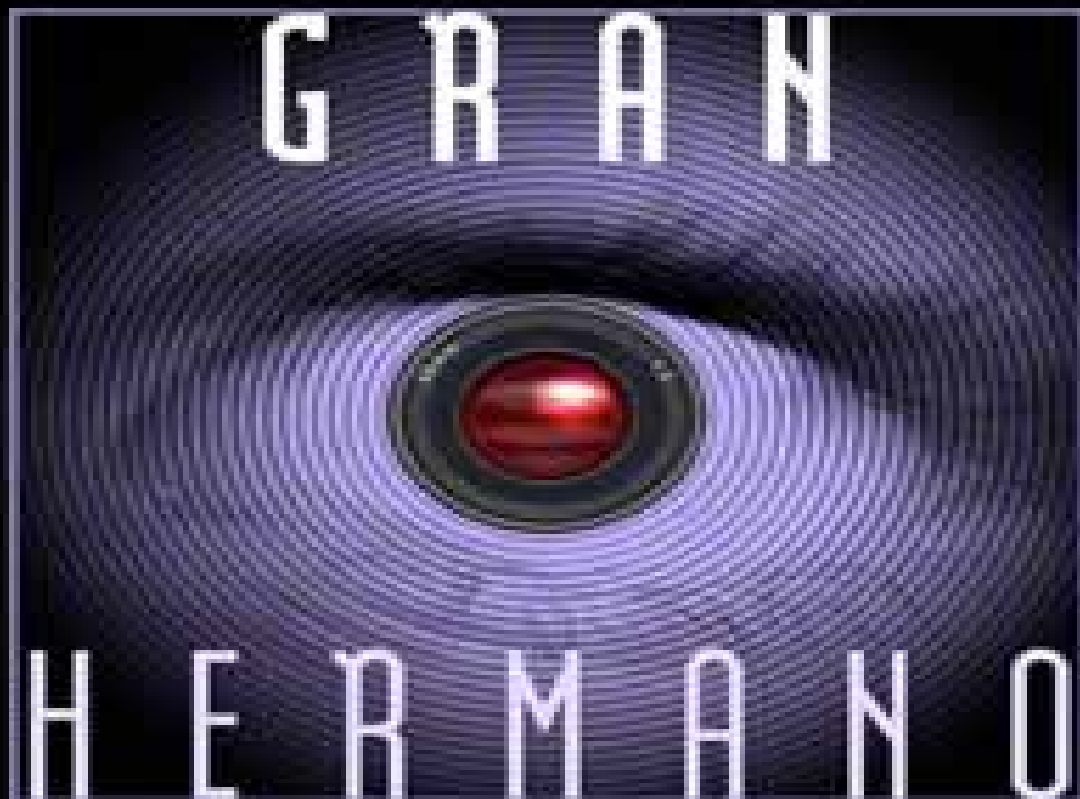
**Intracellular localization and
trafficking of proteins**

or

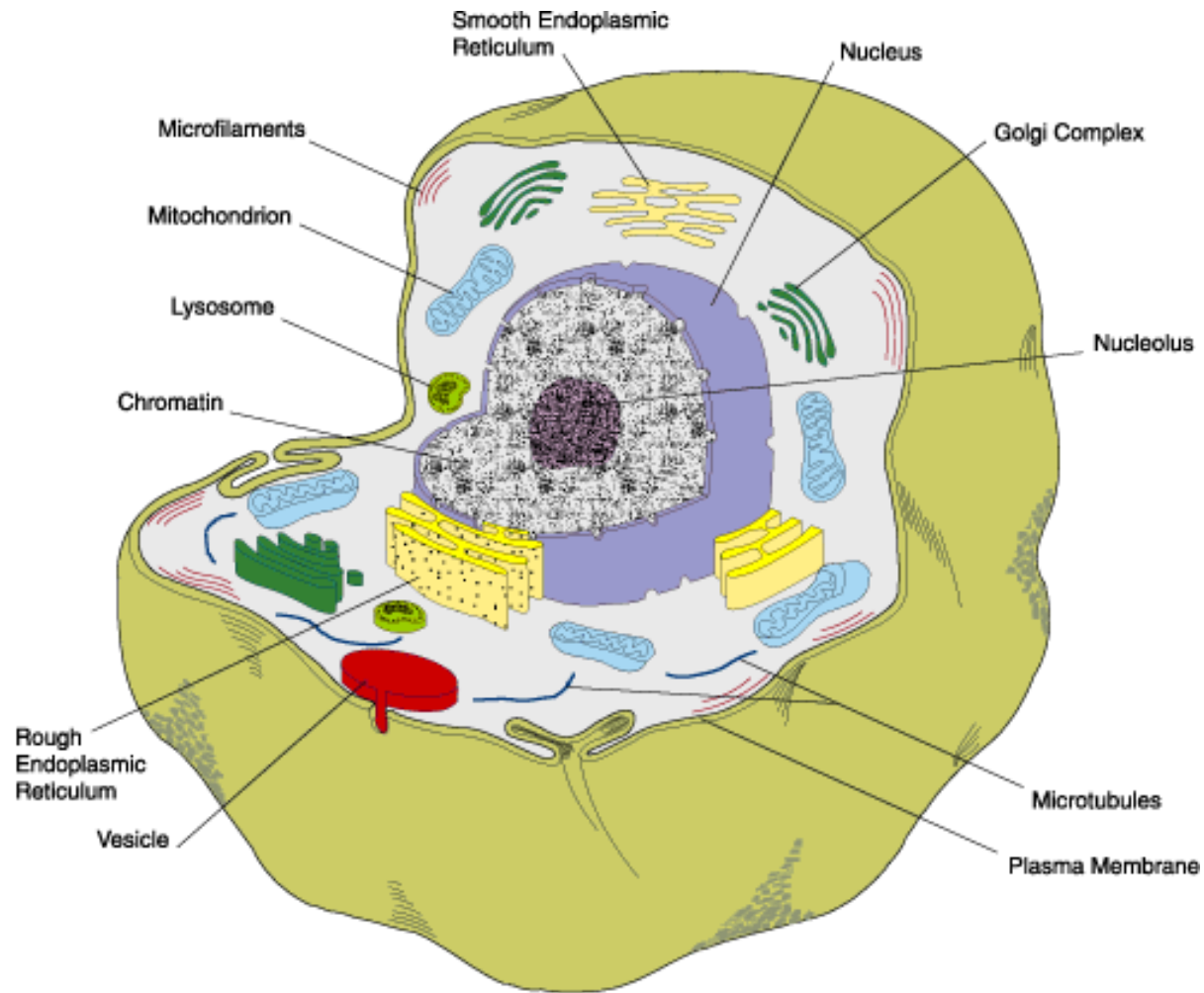
**How (and why) to find a needle in a
haystack**

GRAN

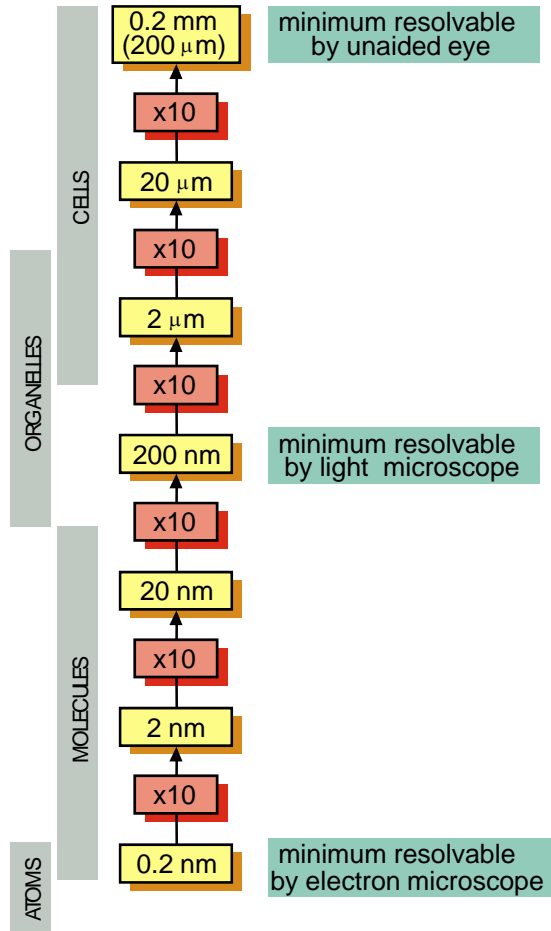
HERMANO



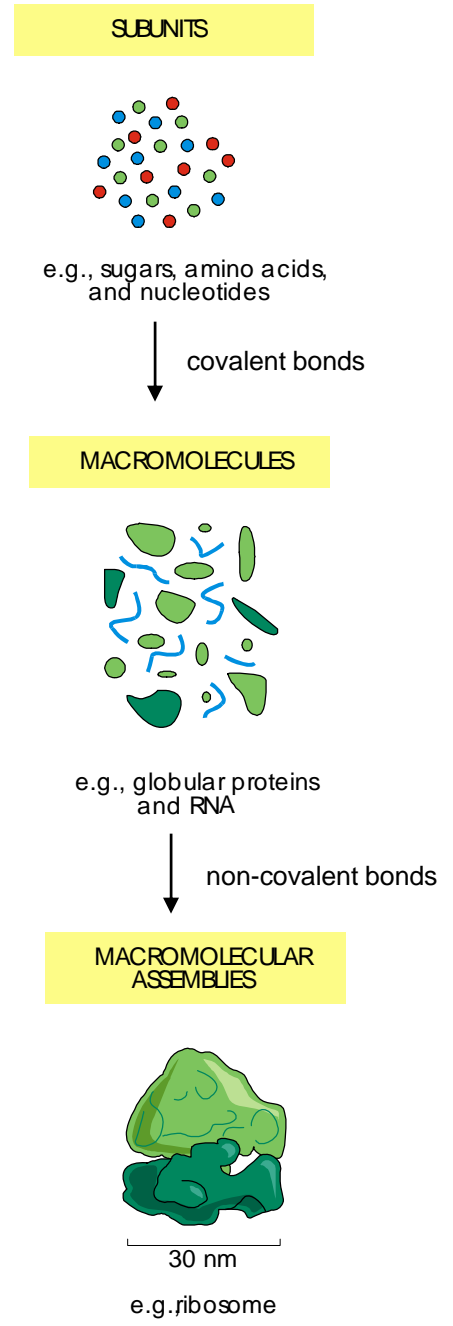
:: The Structure of a Cell



:: Relative sizes

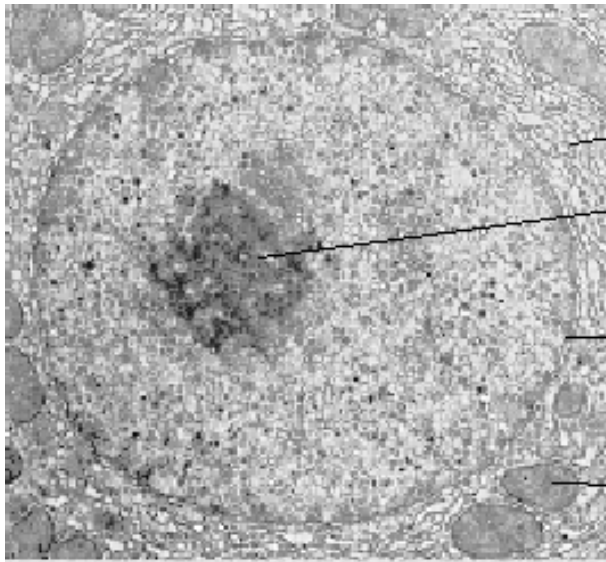


$$\begin{aligned}
 1 \text{ m} &= 10^3 \text{ mm} \\
 &= 10^6 \text{ } \mu\text{m} \\
 &= 10^9 \text{ nm}
 \end{aligned}$$



:: Electronic Microscopy

Nucleus



ER

nucleolus

nuclear membrane

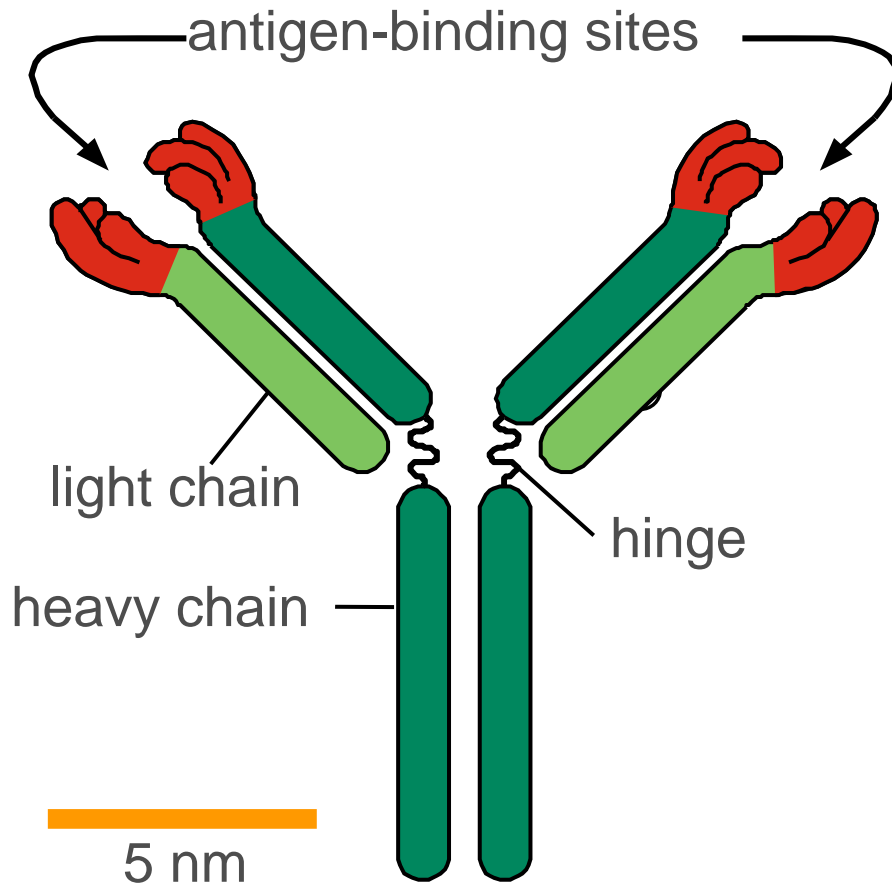
vesicle

Rough endoplasmic reticulum



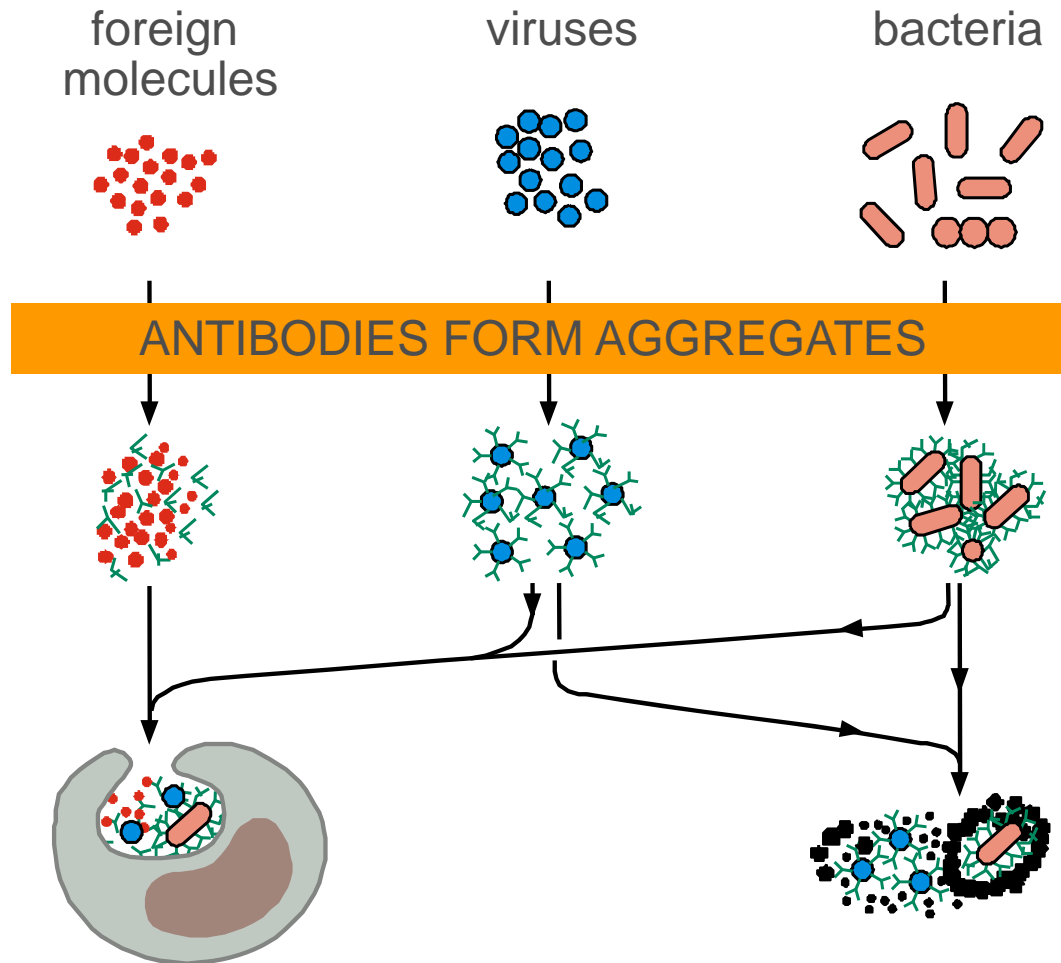
ribosomes

:: The antibody molecule



- Antibodies are proteins that bind very tightly to their targets (antigens).
- They are produced in vertebrates as a defense against infection.
- Each antibody molecule is made of two identical light chains and two identical heavy chains
- The two antigen-binding sites are identical.

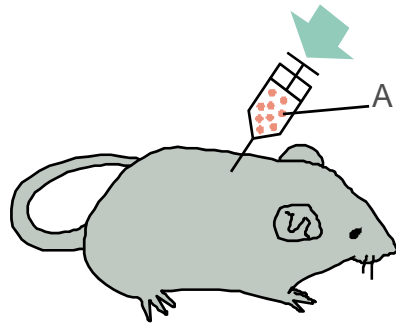
:: Antibodies defend us against infection



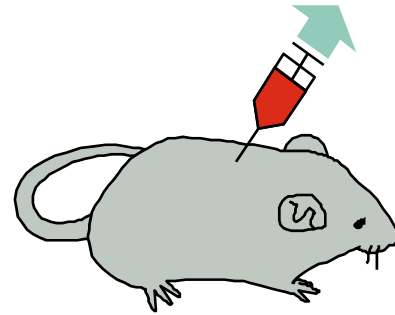
Antibody and antigen aggregates are ingested by phagocytic cells

Special proteins in blood kill antibody-coated bacteria or viruses

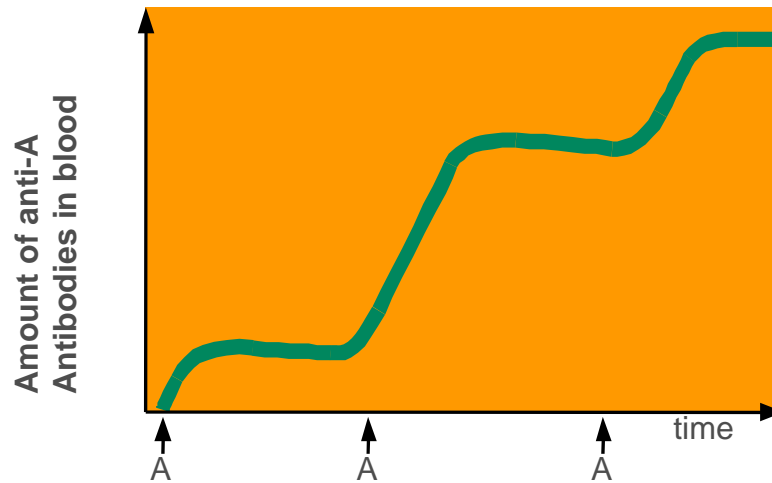
:: Raising antibodies in animals



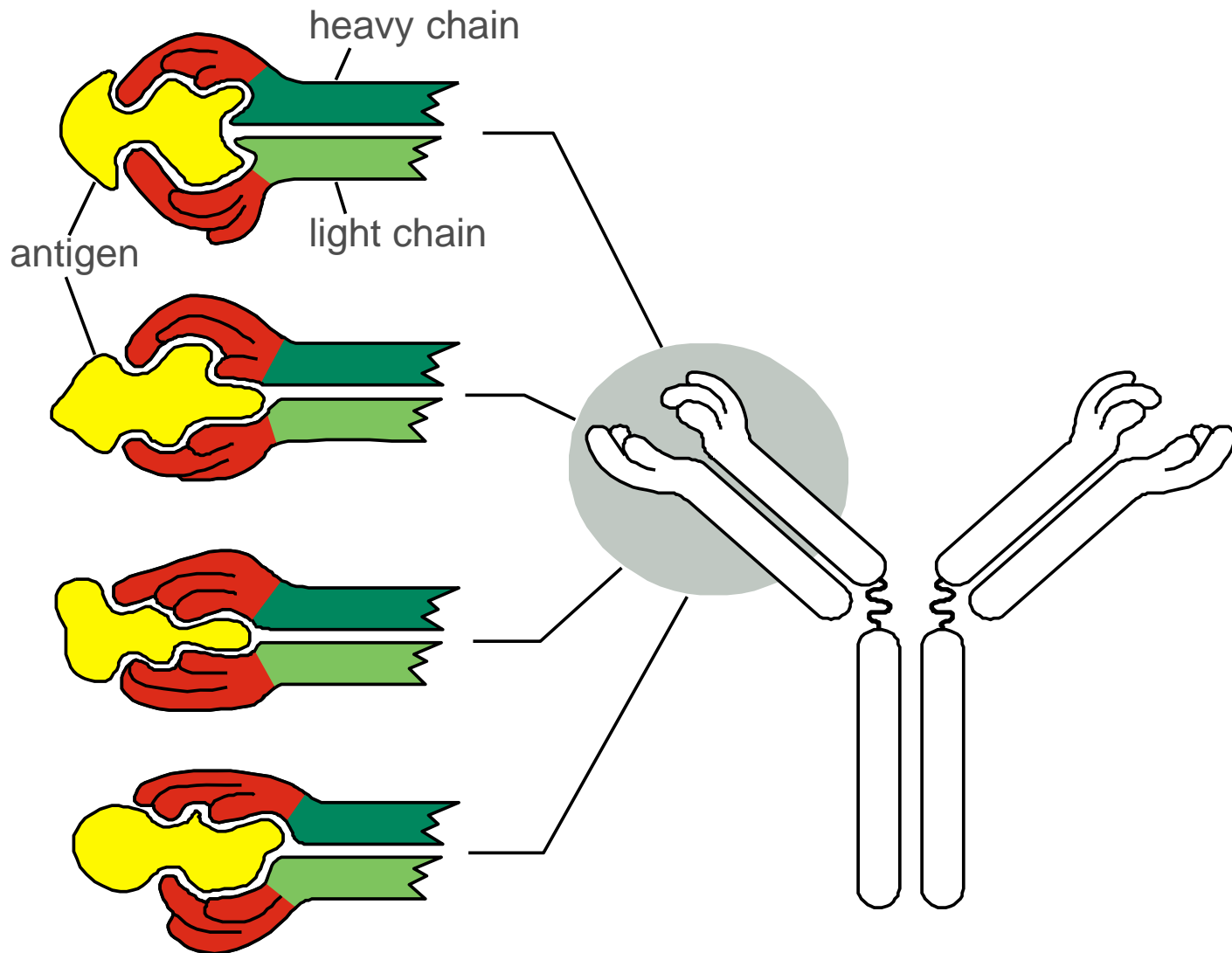
inject antigen A



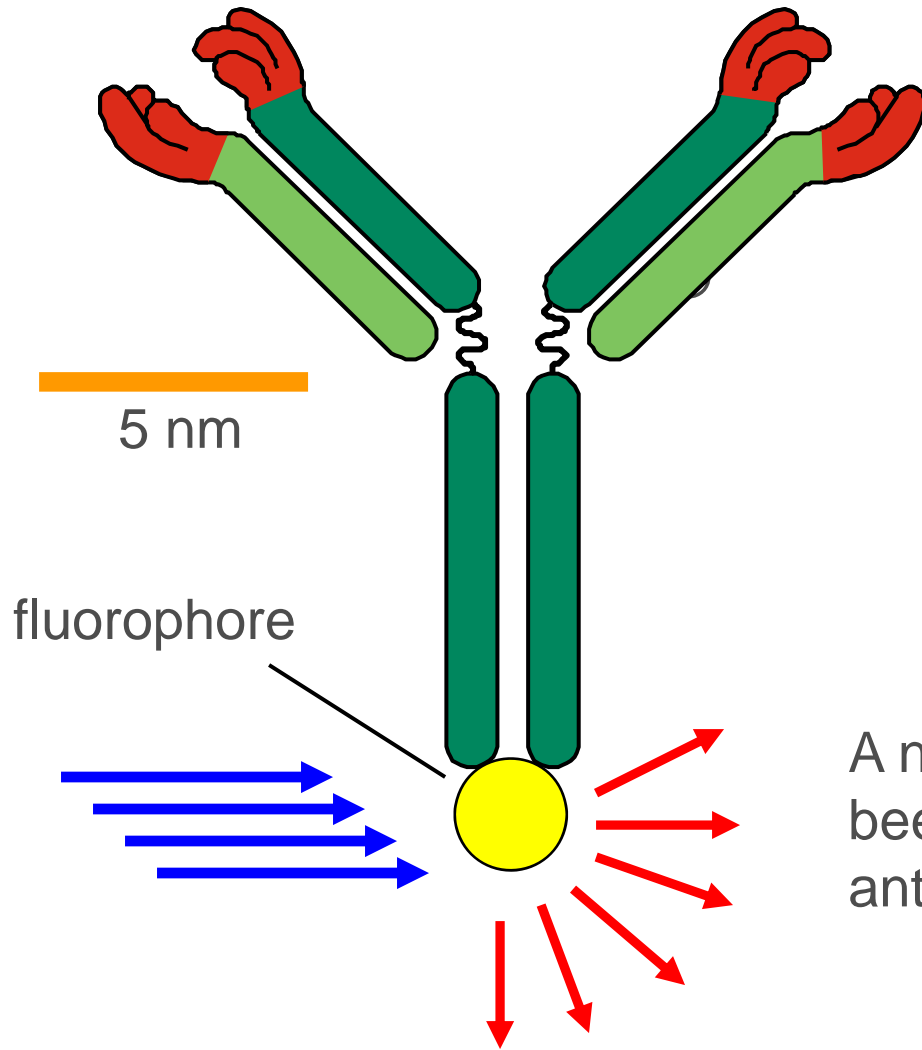
take blood later



:: Antibody specificity



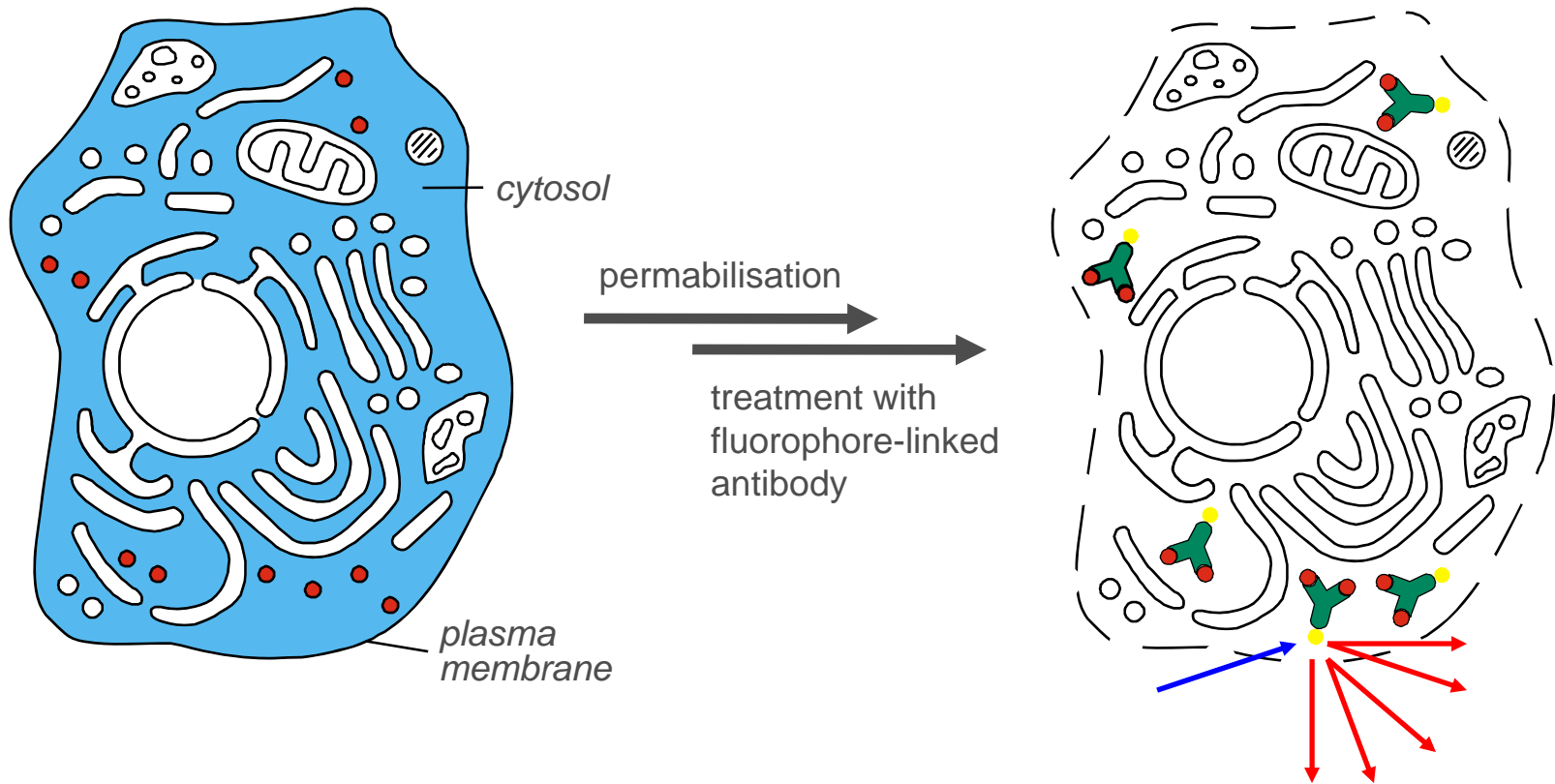
:: Immunofluorescence



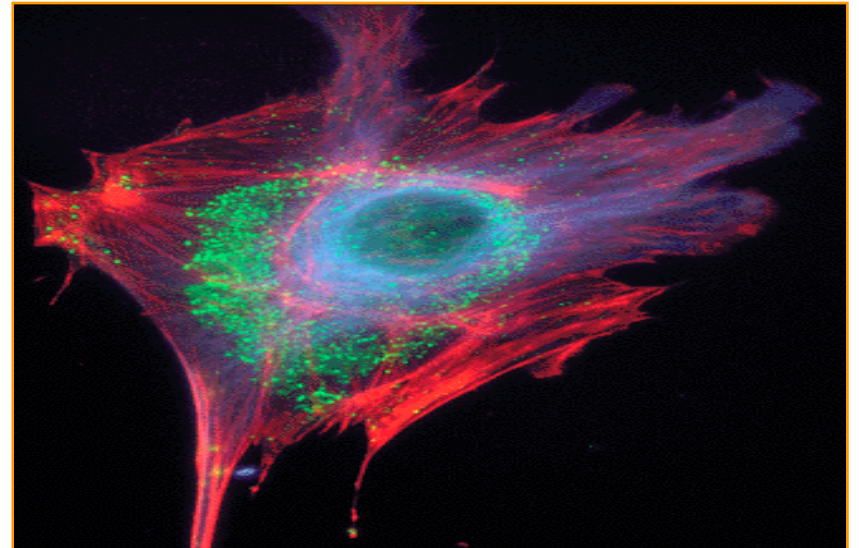
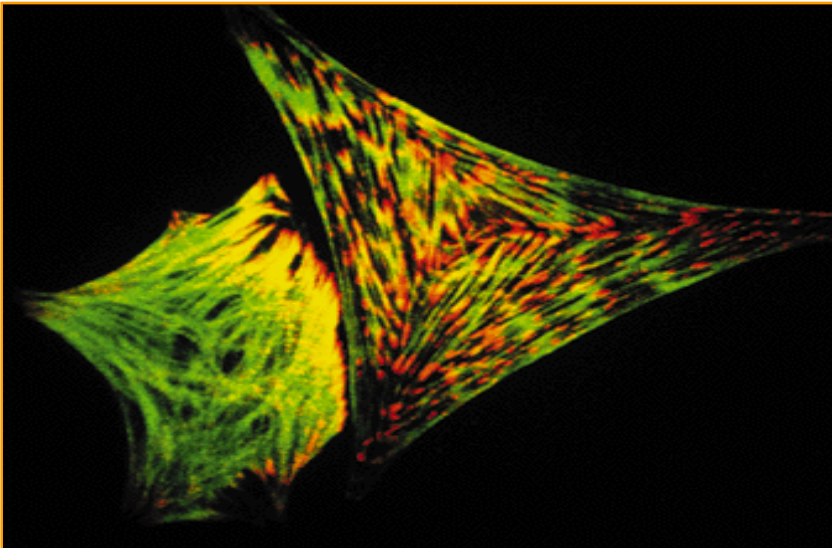
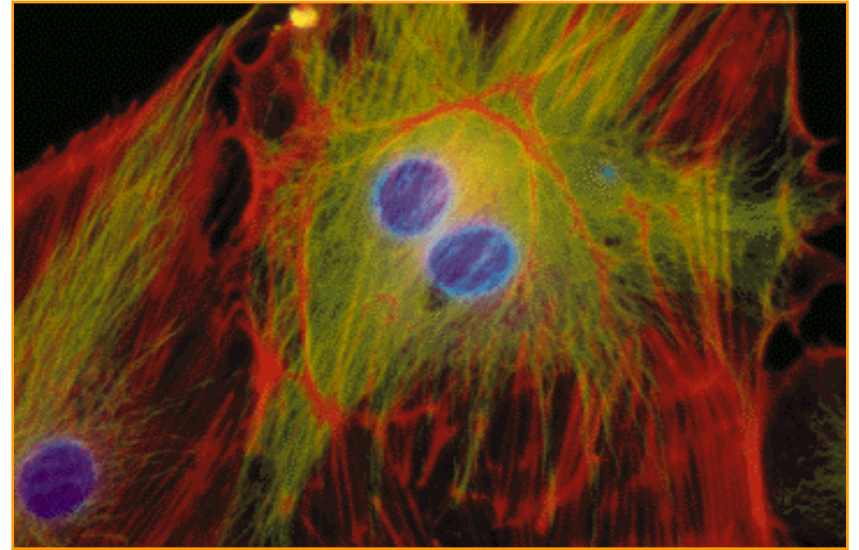
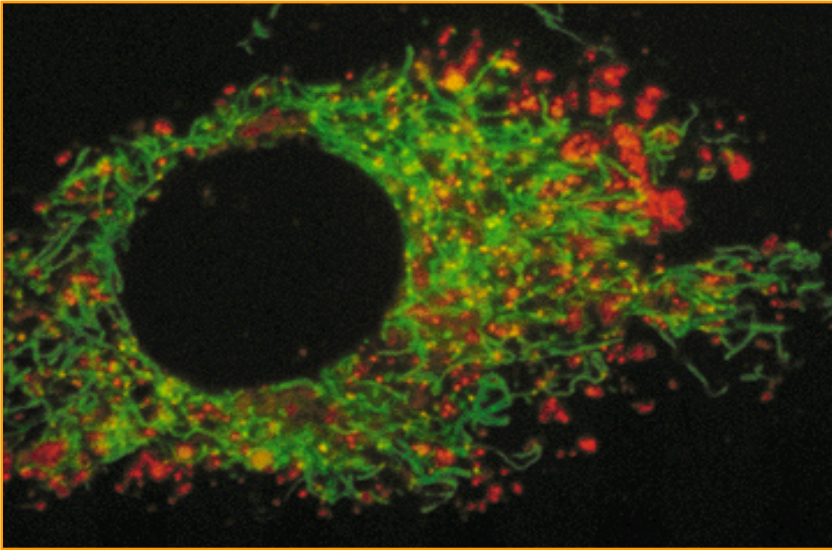
Fluorescence is the property of a molecule illuminated with light of one wavelength (color) that emits light of a longer wavelength (lower energy)

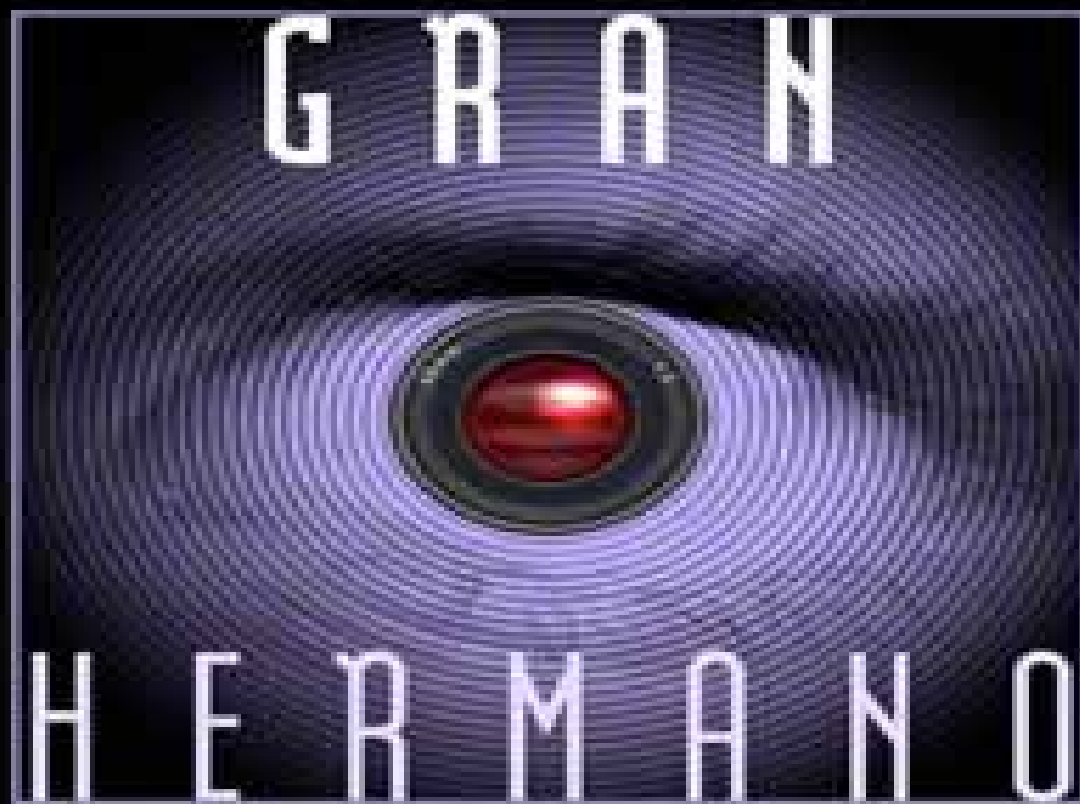
A number of fluorescent probes have been developed that can be linked to antibodies to label specific proteins

:: Immunofluorescence



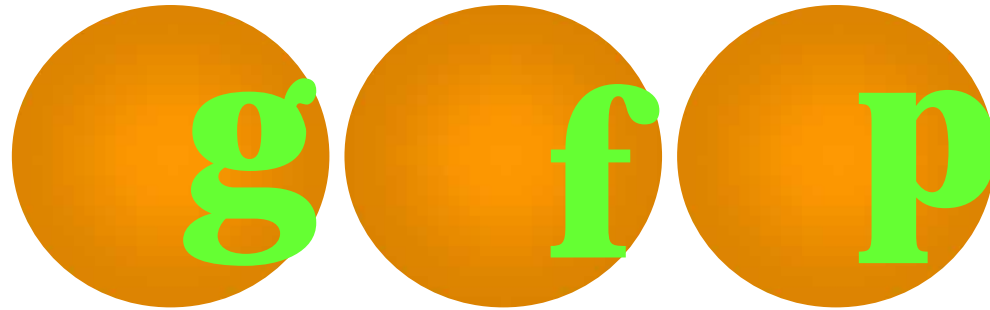
:: Immunofluorescence





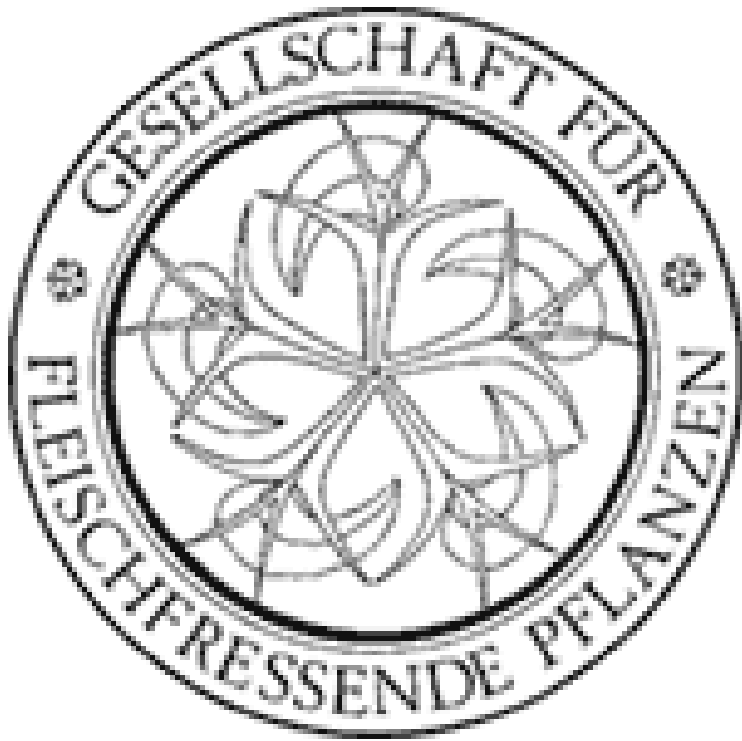
SEGONA PART

Applications of



**in Molecular and
Cell Biology**

g f p



Society for Meat-Eating Plants



g

f

p

Gardening For Profit

BY PETER HENDERSON,
GREAT-GRANDFATHER
OF TRUCK FARMING IN AMERICA



Complete reprint of his 1867 first edition, plus:
* A history of Market Gardening in America
* Special selections from his Garden and Farm Topics
* A Rare Biography

EDITED BY GEORGE DEVAULT

g f p

The screenshot shows the homepage of the Genuine Ford Parts website. At the top, there are navigation links for "Home", "Products", and "Contact Us". The main header reads "Genuine Ford Parts". On the left side, there is a vertical navigation menu with links for "Home", "Accessories", "Parts Center", "Car Care Products", "Shipping Cost", "Policies", "Security", "Privacy", and "Search". The search bar is located at the bottom of this menu. The main content area features a welcome message: "Welcome to Genuine Ford Parts and Accessories!" followed by a paragraph: "We're here to serve you with great customer service, secure ordering, and a large selection of genuine Ford and Motorcraft parts and accessories." Below this is another paragraph: "It's easy to browse through the great selection of genuine Ford parts and accessories. Just click on 'Automotive' or 'Truck/Commercial'." In the center, there is a circular logo with the word "Ford" inside. On the right side, there is a "Featured Product" section titled "Body Color Bed Rails" with a description: "Add a little style to your truck with our Body Color Bed Rails. Available in less than an hour. All hardware and instructions included." Below the text is a photograph of a red pickup truck bed with the rails installed. At the bottom of the page, there is a footer with copyright information: "Copyright © 2002 Genuine Ford Parts. All rights reserved. Visit Ford at www.ford.com." The footer also includes a link to "Ford Motor Company" and a "Home" link.

Home Products Contact Us

Genuine Ford Parts

Welcome to Genuine Ford Parts and Accessories!

We're here to serve you with great customer service, secure ordering, and a large selection of genuine Ford and Motorcraft parts and accessories.

It's easy to browse through the great selection of genuine Ford parts and accessories. Just click on "Automotive" or "Truck/Commercial."

Featured Product:

Body Color Bed Rails

Add a little style to your truck with our Body Color Bed Rails. Available in less than an hour. All hardware and instructions included.

Home | Products | Contact Us

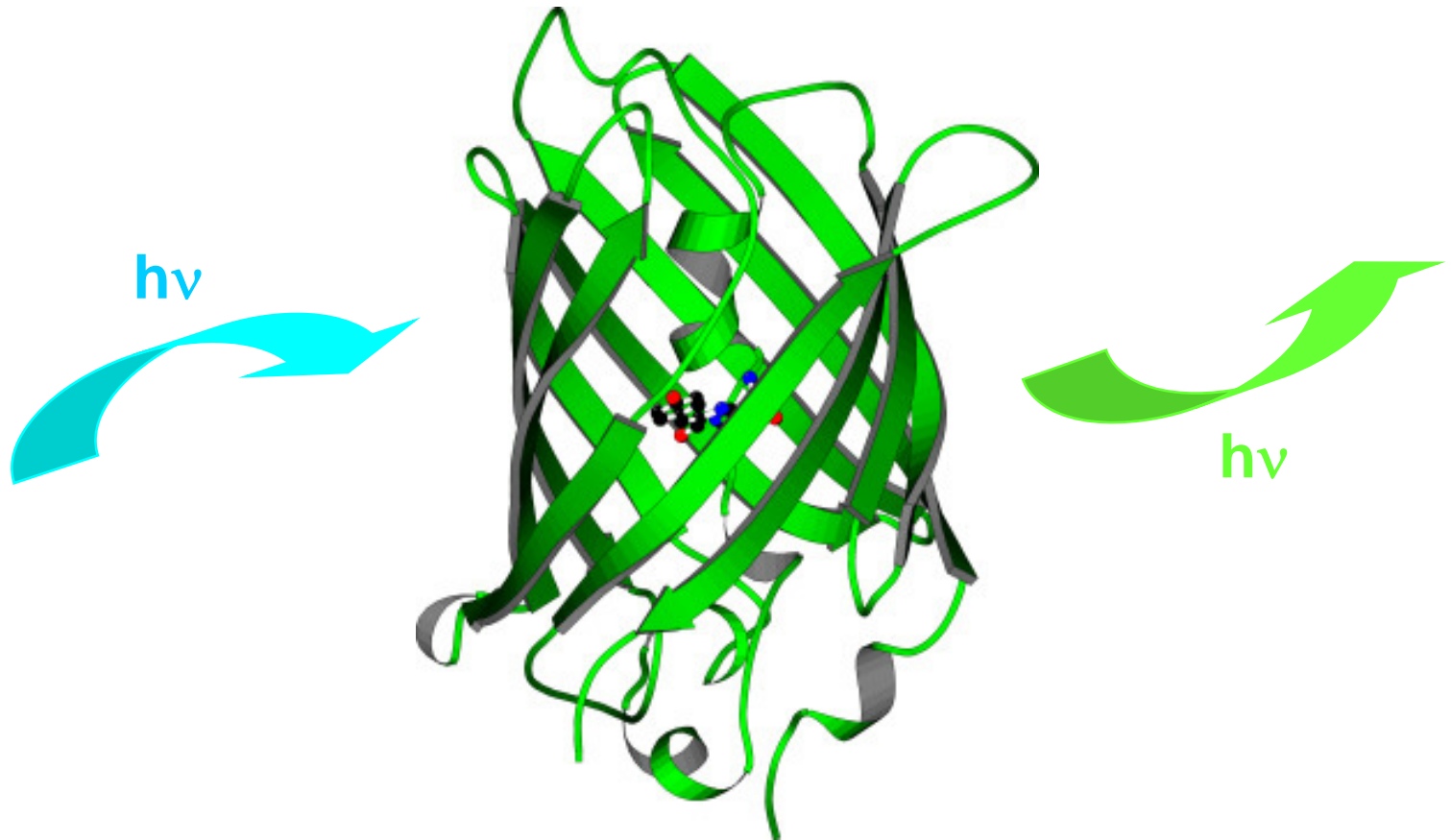
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g f p



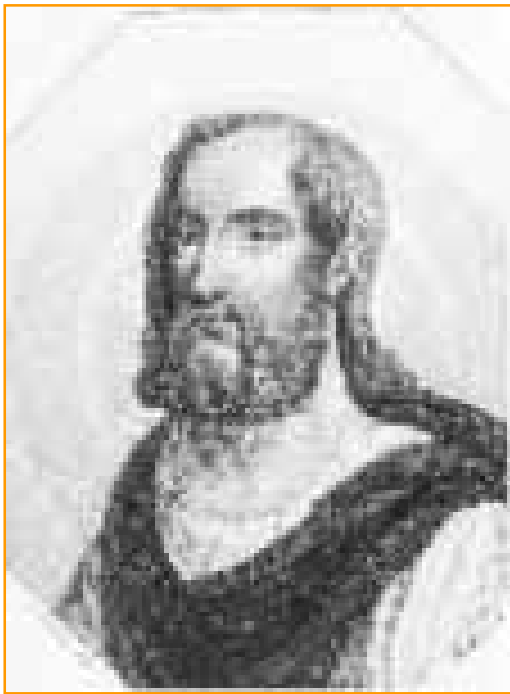
g f p

:: Green Fluorescent Protein



:: Green Fluorescent Protein

Ancient History

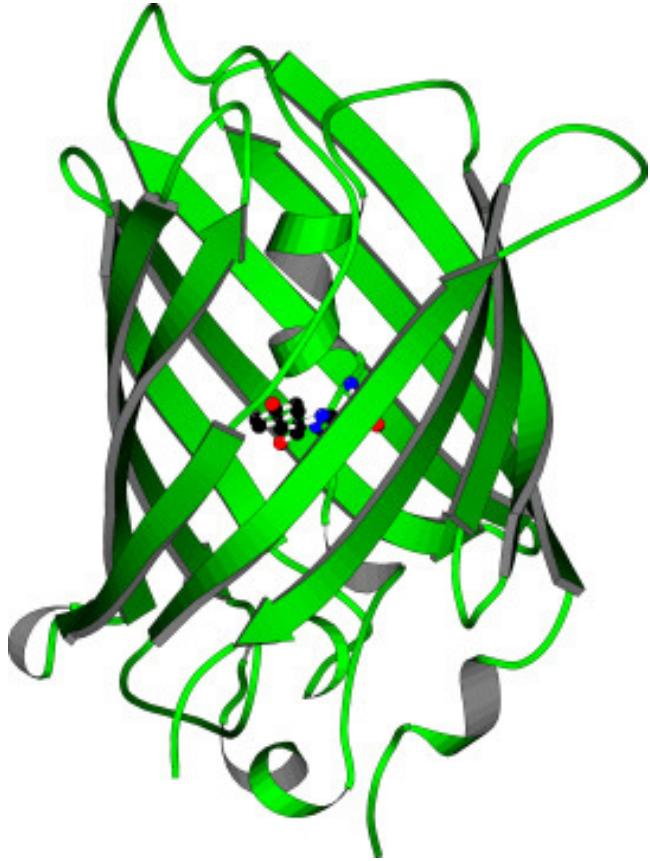


Plinio Secundus Caio
“The Elder” (23/24-79 AC)



:: Green Fluorescent Protein

Time -line



- 1955: Bioluminescence of *Aequorea victoria*
- 1961: Biochemical characterization of GFP
- 1979: Structure of the chromophore
- 1992: GFP cloned (238 aa, 26,9 kDa)
- 1994: Heterologous expression
- 1996: X-ray structure
- 1996-2008: GFP variants
- 2008: Nobel Prize in Chemistry

:: Green Fluorescent Protein

Nobel Prize in Chemistry 2008



Osamu Shimomura

Biochemical characterization of GFP
Structure of the chromophore



Martin Chalfie

Heterologous expression of GFP



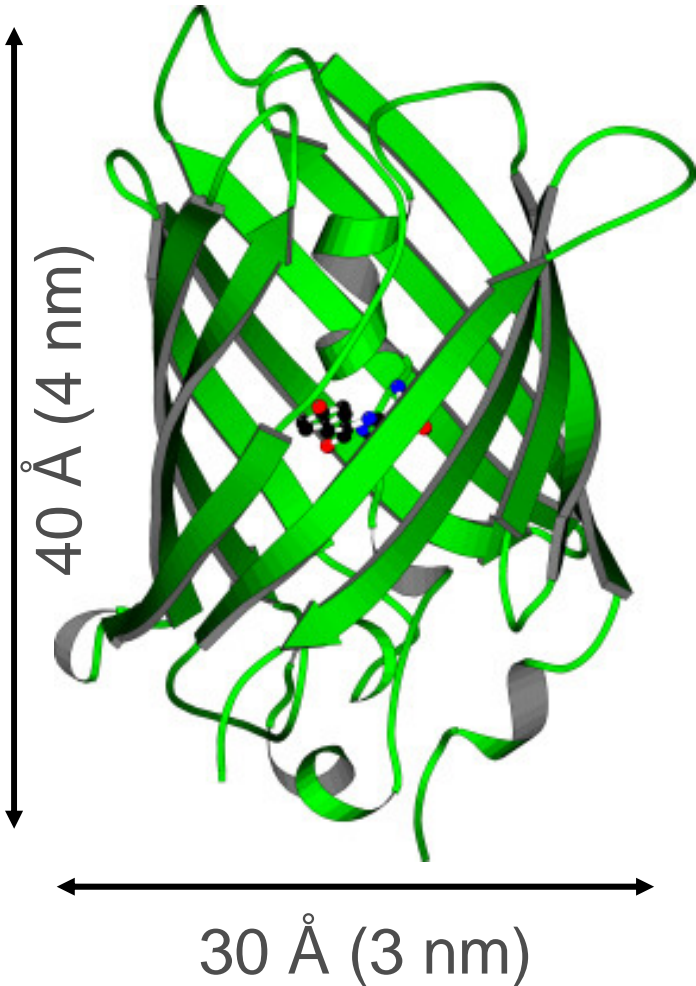
Roger Y Tsien

Everything else



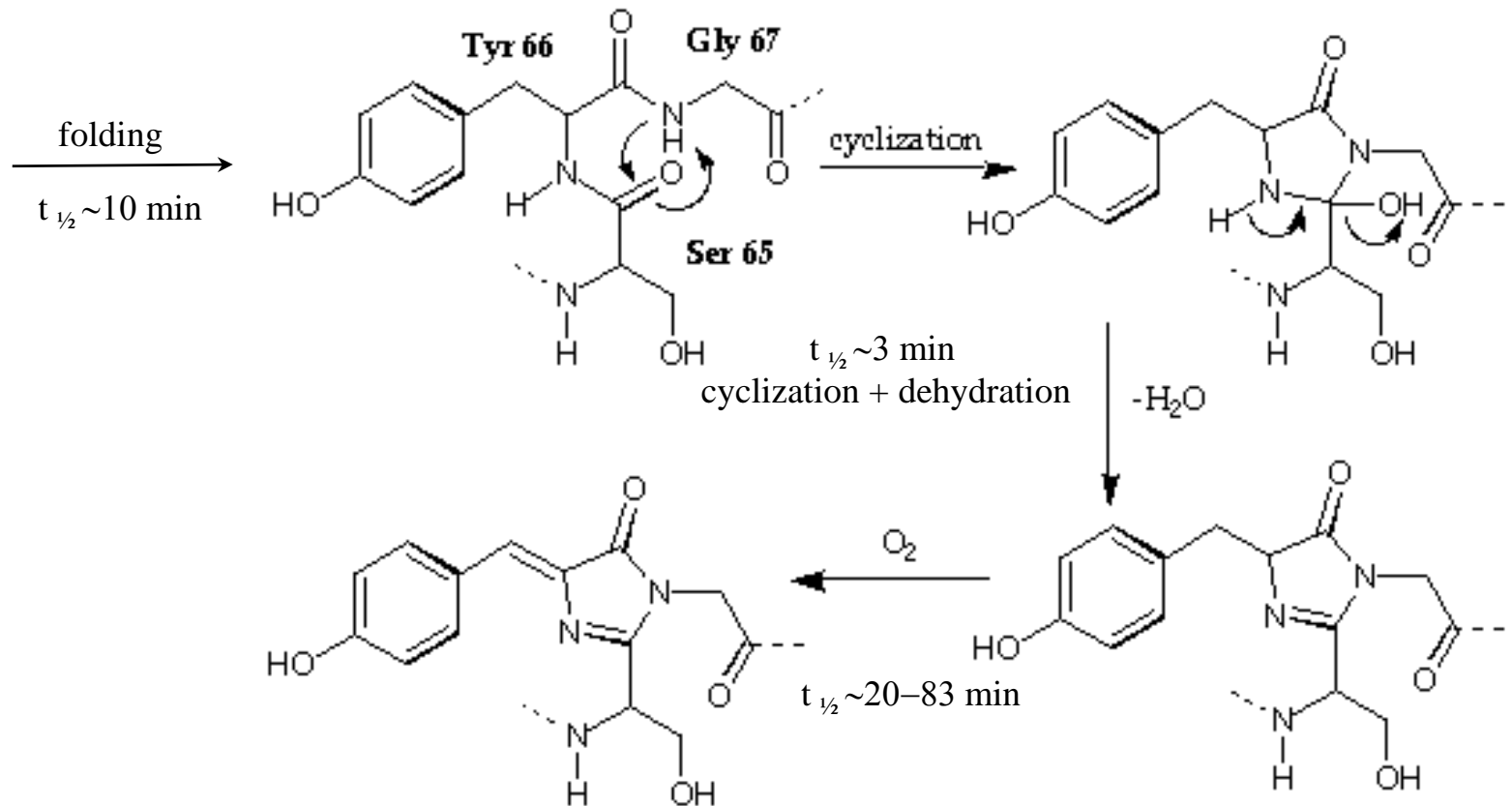
:: Green Fluorescent Protein

Properties



- No need of cofactors for fluorescence
- Highly resistant to denaturation (**Paint** in a can: compact 11 β sheet -can)
- Correct heterologous expression in every cell type
- Genetically engineered variants

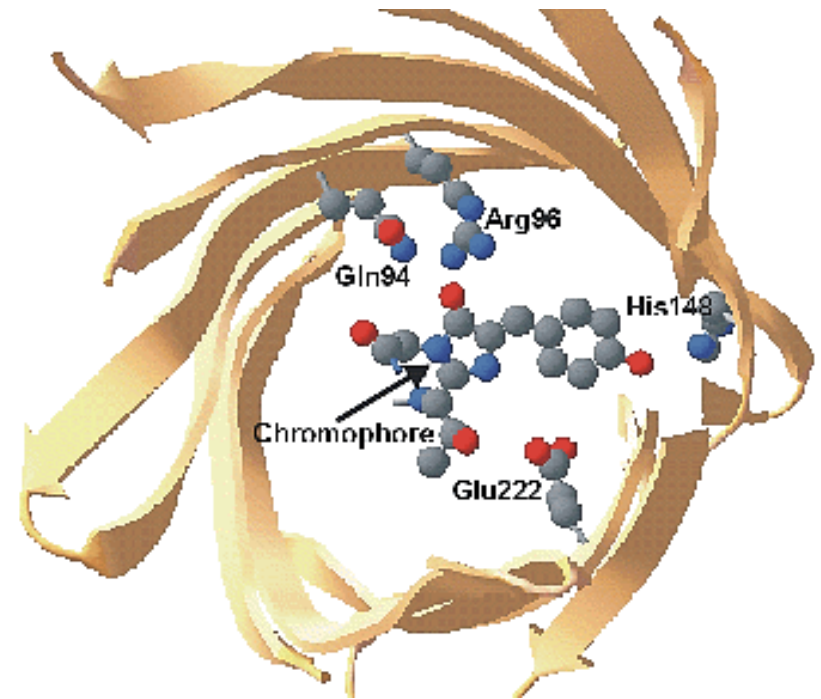
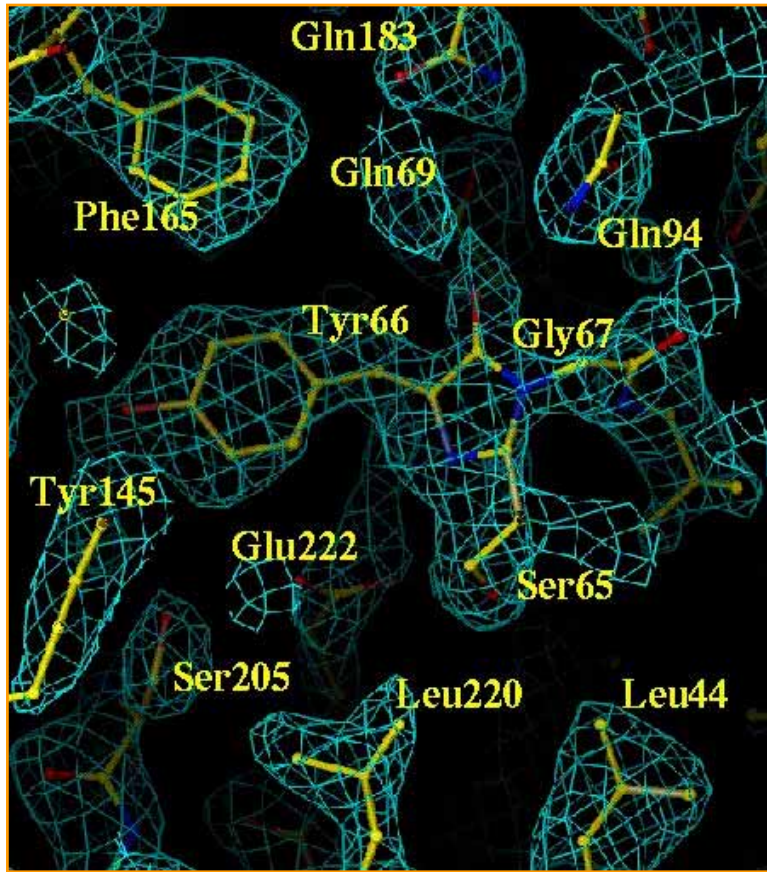
:: Green Fluorescent Protein Fluorophore formation



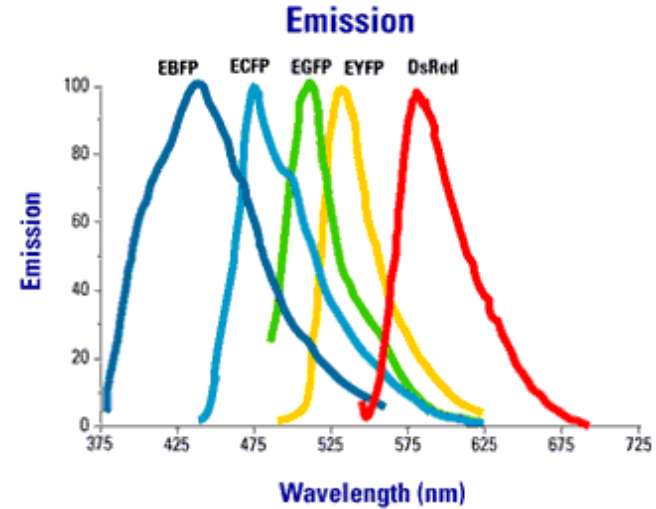
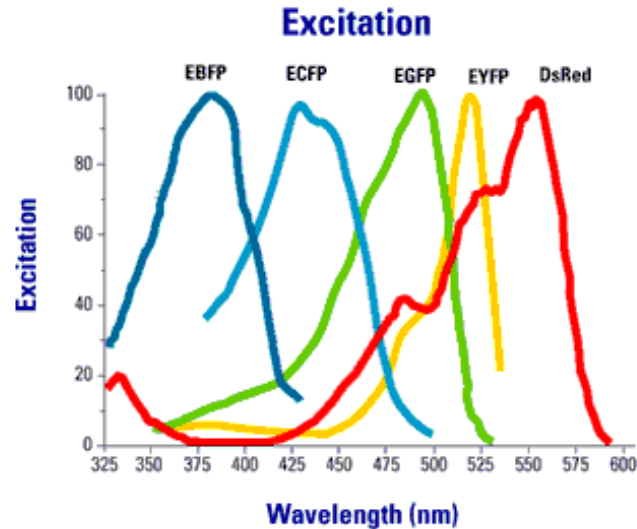
- Autocatalytic cyclization and dehydration
- Requires oxygen in the last, rate-limiting, step

:: Green Fluorescent Protein

Fluorophore properties

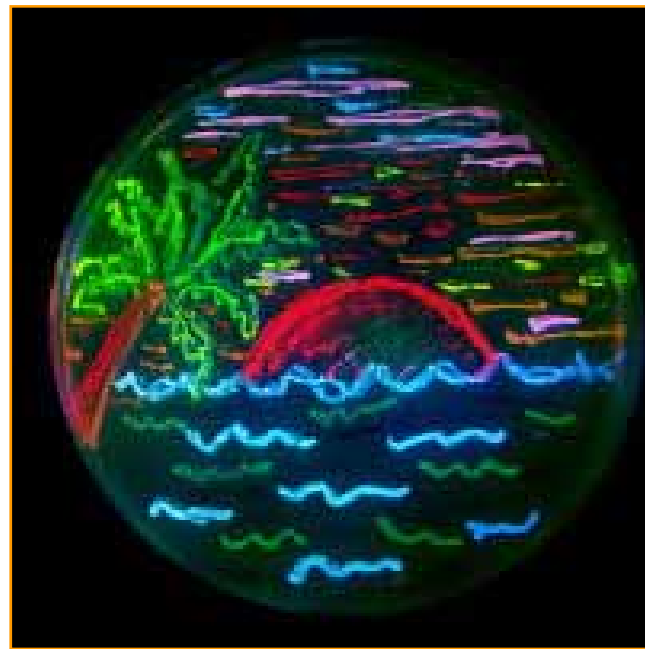
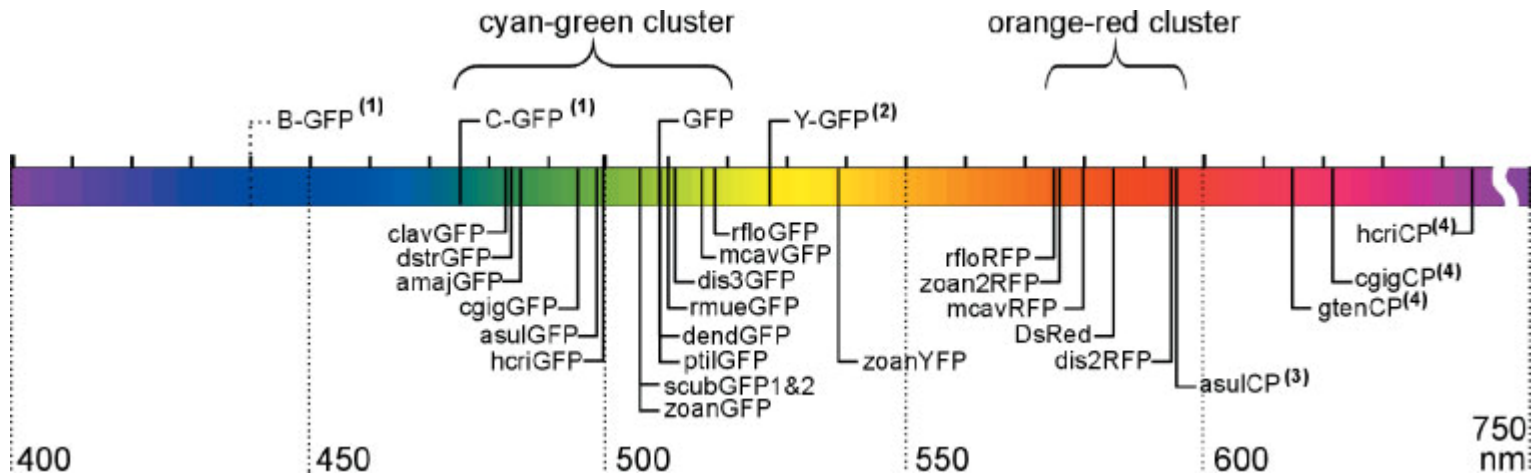


:: GFP Variants



GFP Variant	Fluorescence Intensity	Excitation (nm)	Emission (nm)	Codon Optimization
EYFP	+++	513	527 (Yellow)	Human
EGFP	+++	488	507 (Green)	Human
GFPwt	+	470	509 (Green)	None
ECFP	++	434	477 (Cyan)	Human
GFPuv	++	395	509 (Green)	<i>E. coli</i>
EBFP	+	380	440 (Blue)	Human

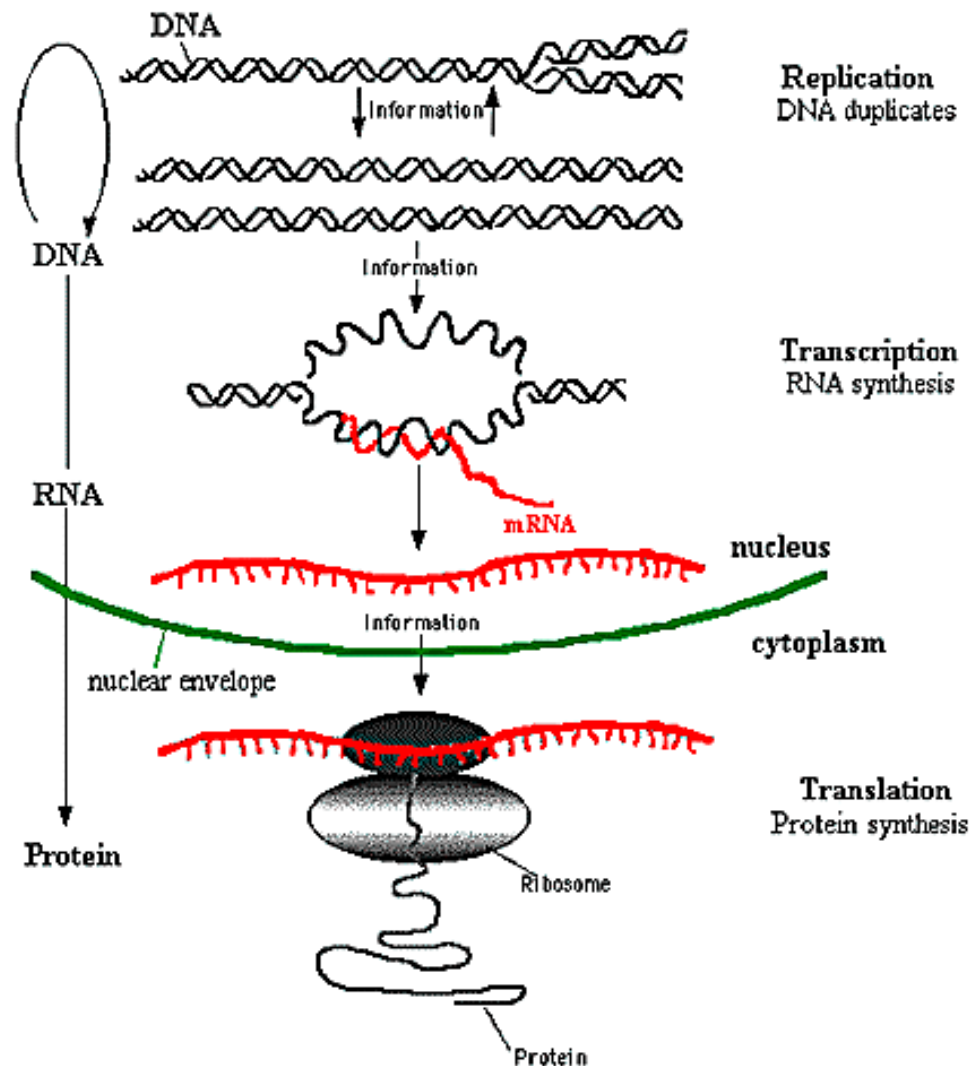
:: GFP Variants



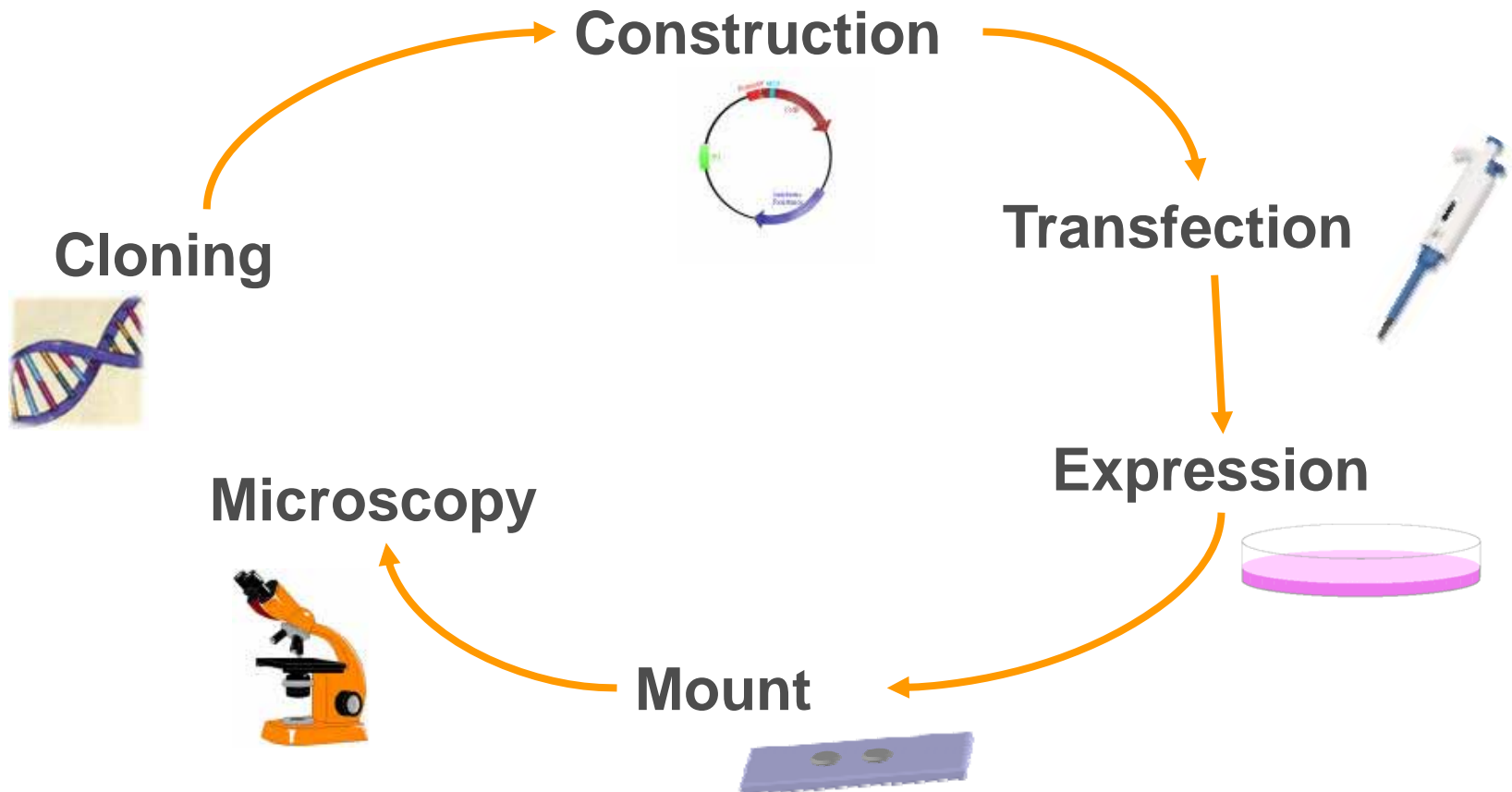
:: GFP applications

- Reporter of gene expression
- Marker of cell lineage
- **Tag for intracellular localization of proteins**
- **Monitor *in vivo* protein-protein interactions (BiFC, FRET)**
- ...

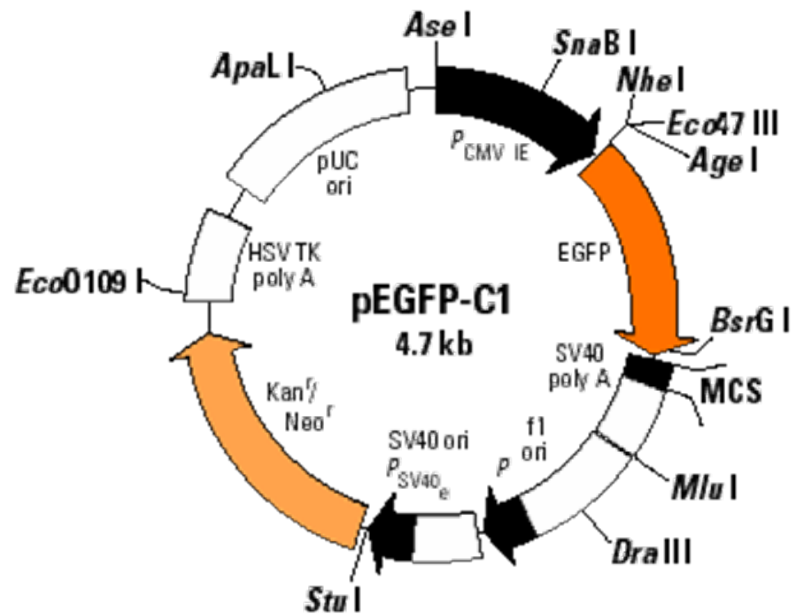
:: The central Dogma of Molecular Biology



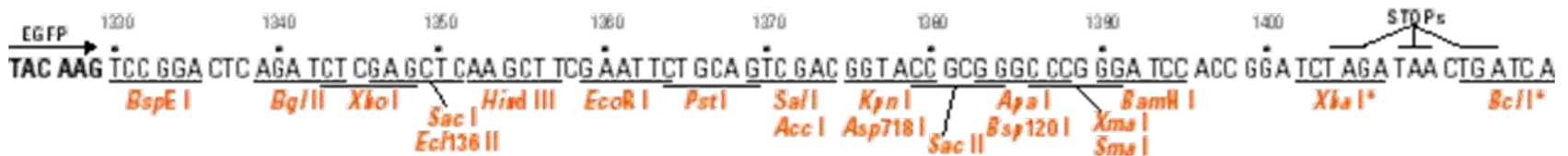
:: GFP protein tagging



:: Construction

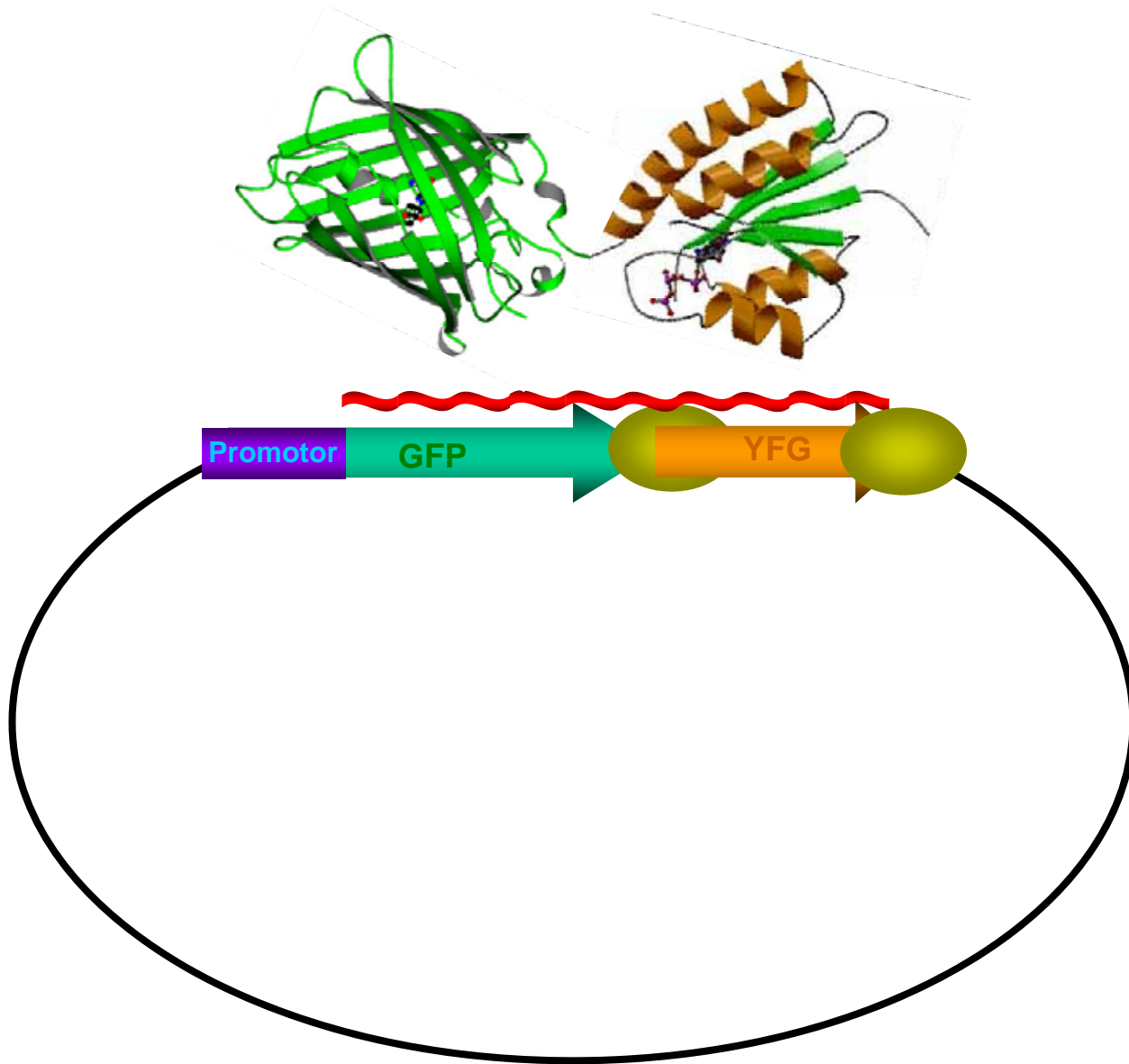


Multicloning Site:

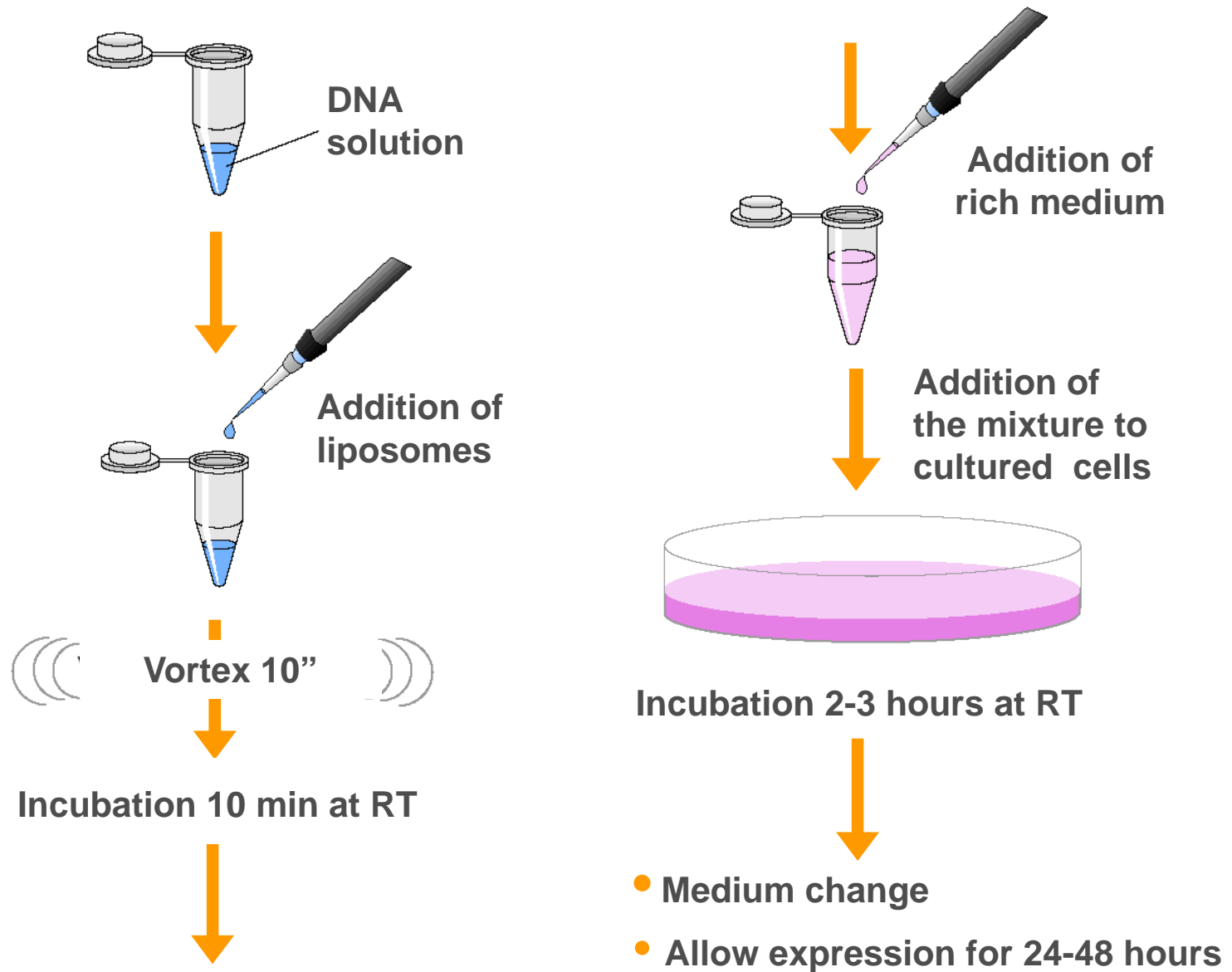


pEGFP-C1, -C2, -C3, -N1, -N2, -N3

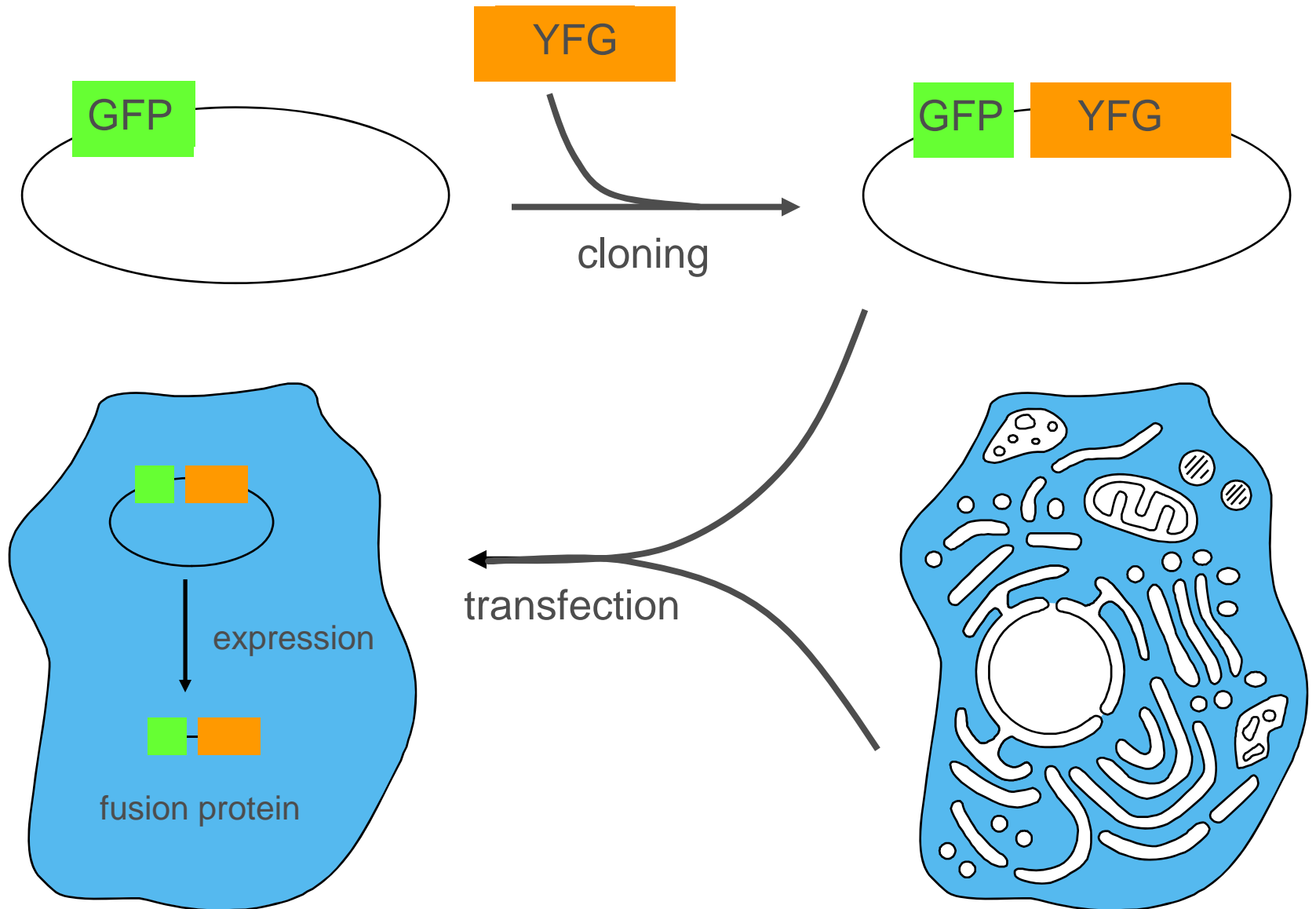
::: Compression



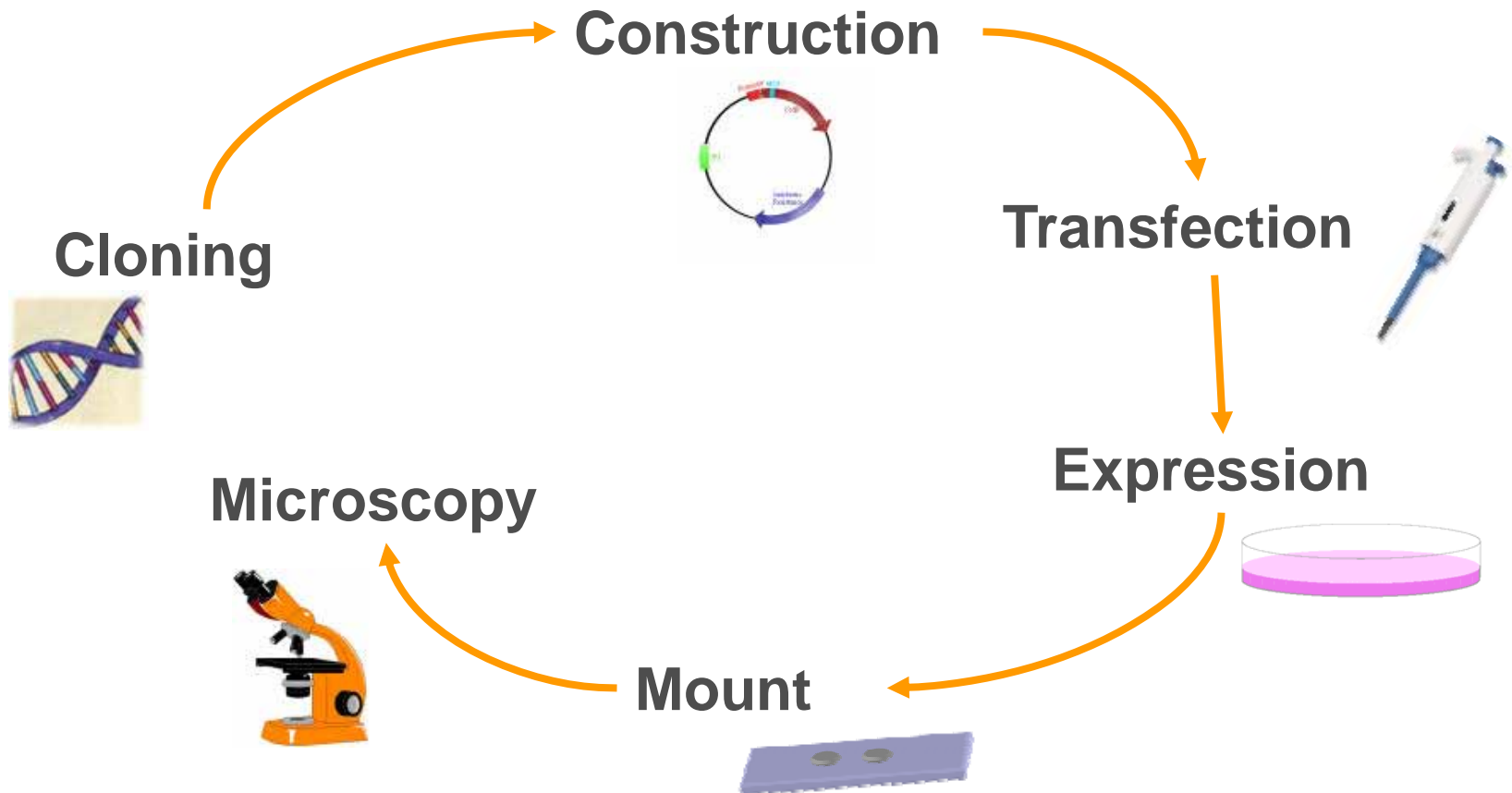
:: Transfection



:: Cloning and Expression



:: GFP tagging in subcellular distribution studies



:: Microscopy

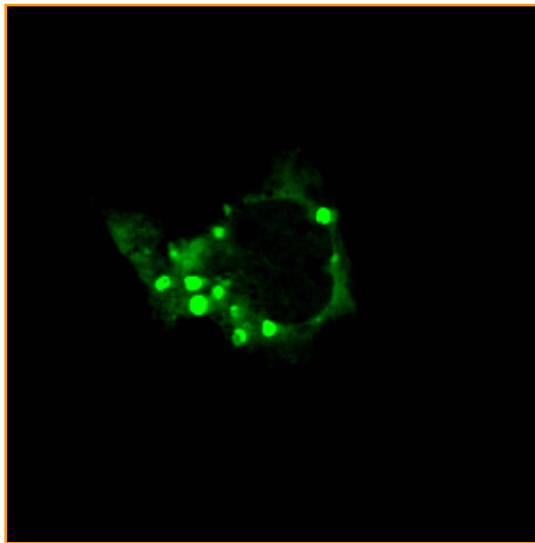
- Fluorescence microscope (**confocal**)

:: Localization of **GFP-MGS** fusion protein

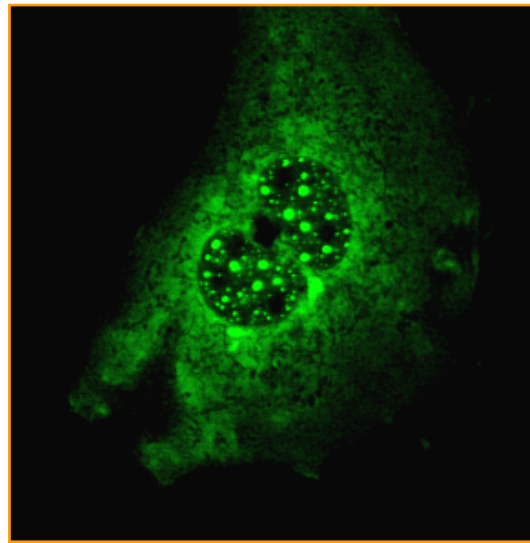
Muscle glycogen synthase fused to GFP (**GFP-MGS**)

Transfected COS-1 cells

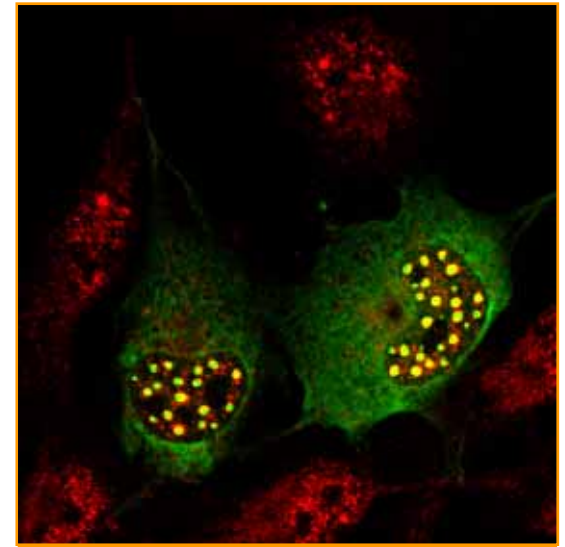
Treated with or without 30 mM **glucose**



+ glucose



- glucose



- glucose
+ p80 immunolabeling

:: GFP Tagging vs. Immunolocalization

- ***In vivo* labeling. Fluorescence develops inside the cell:**
 - No need for post-processing
 - Real-time monitoring
 - Video-microscopy
 - *In vivo* analysis of transgenic organisms
- **Observation of heterologous proteins:**
 - Wild-type and mutant forms
 - Identification of targeting sequences
 - Protein outside its natural environment
- **Caution!!:**
 - Fusion to GFP
 - Overexpression

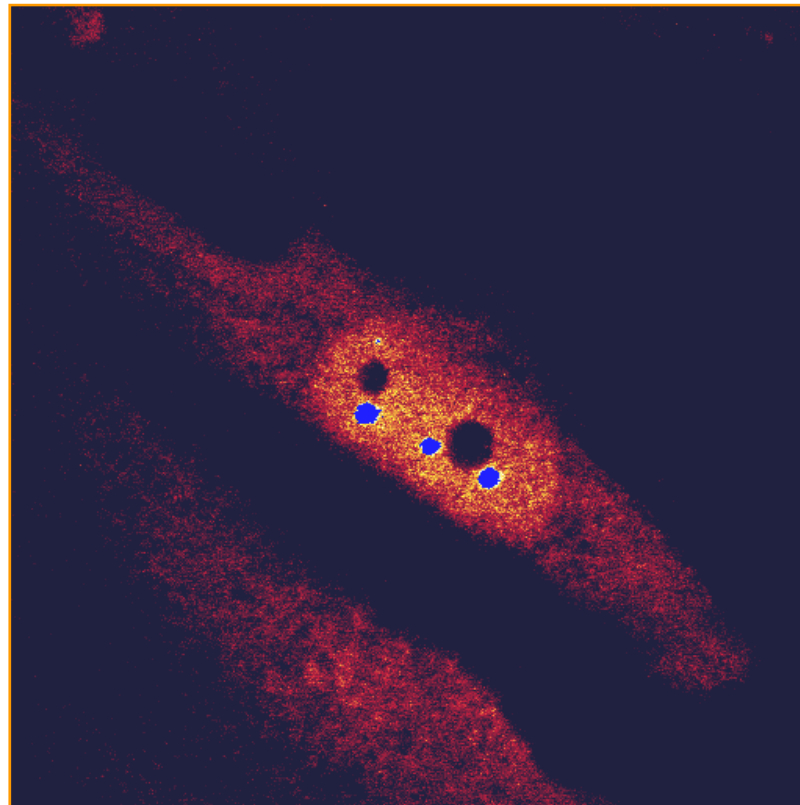


:: Video-Microscopy

Muscle glycogen synthase fused to GFP (**GFP-MGS**)

Transfected C₂C₁₂ cells

Incubated without **glucose** and treated with 30 mM **glucose** at t=0 min



:: Video-Microscopy

- ★ Microtubule dynamics during cell division in the *Drosophila* embryo (GFP-tau fusion protein)
- ★ Cytoskeleton redistribution in chemotaxis of *Dictyostelium discoideum* (GFP-coronin fusion protein)
- ★ Actin dynamics in B-16 melanoma migrating cell (GFP-beta actin fusion protein)

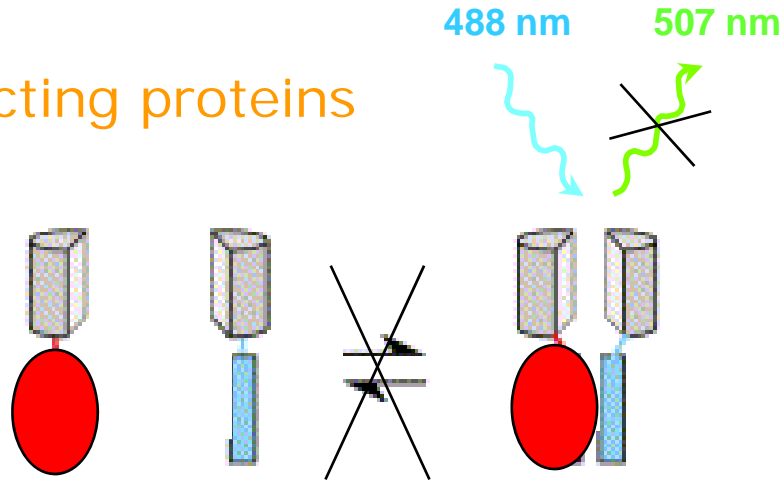
:: *In vivo* Protein-Protein Interactions

- Which ones, where, when?
- Light (fluorescence) microscopy maximal resolution:
200 nm (= 2000 Å)
- Electronic microscopy using metal-labeled antibodies
Resolution: **0.2 nm** (= 2 Å)
- Optical microscopy of **GFP**-derivatives:
 - Bimolecular Fluorescence Complementation
(**BiFC**)
 - Fluorescence Resonance Energy Transfer
(**FRET**)

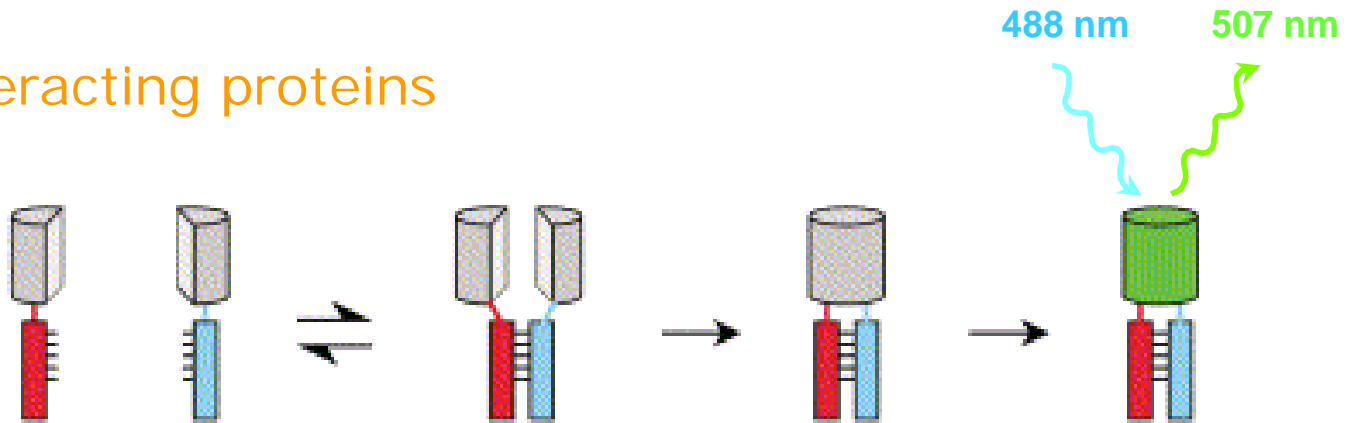
:: Protein-Protein interaction

BiFC Microscopy

a) Non-interacting proteins



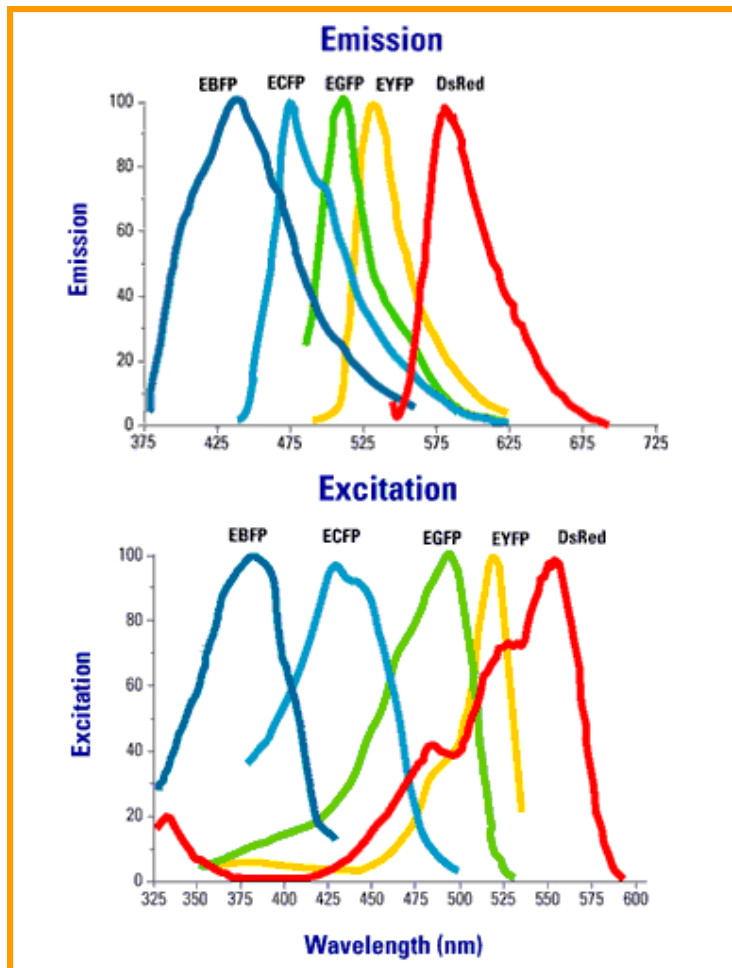
b) Interacting proteins



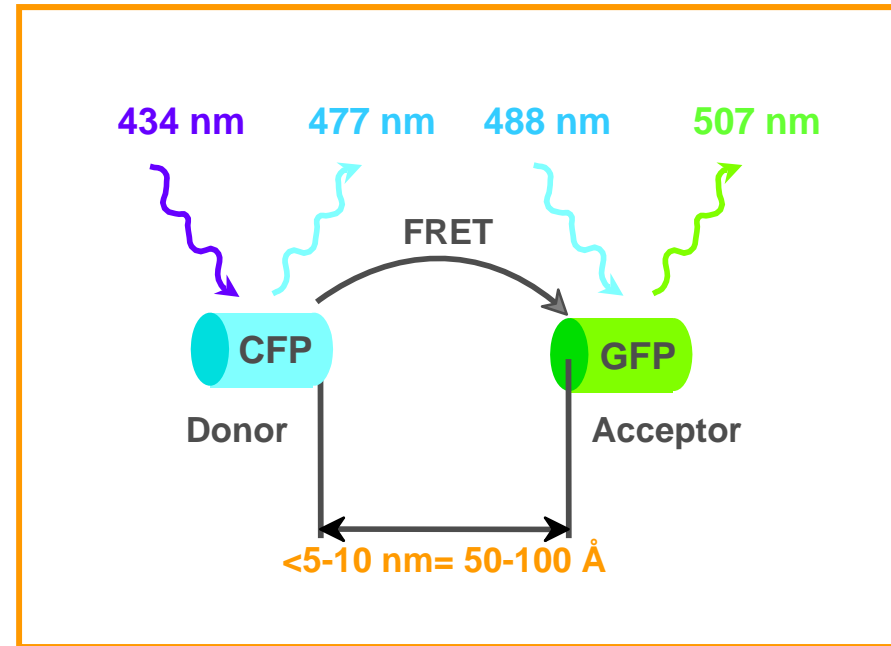
:: Protein-Protein interaction

FRET Microscopy

Spectral overlap



Distance



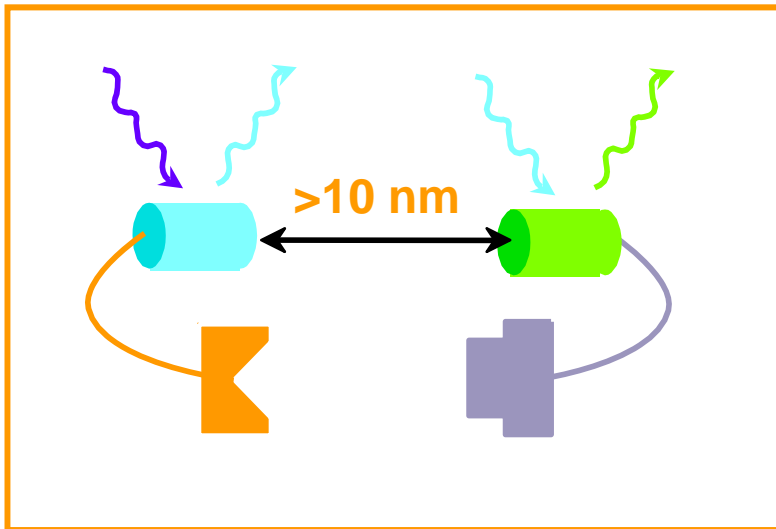
- Conventional microscopy: 200 nm

Orientation

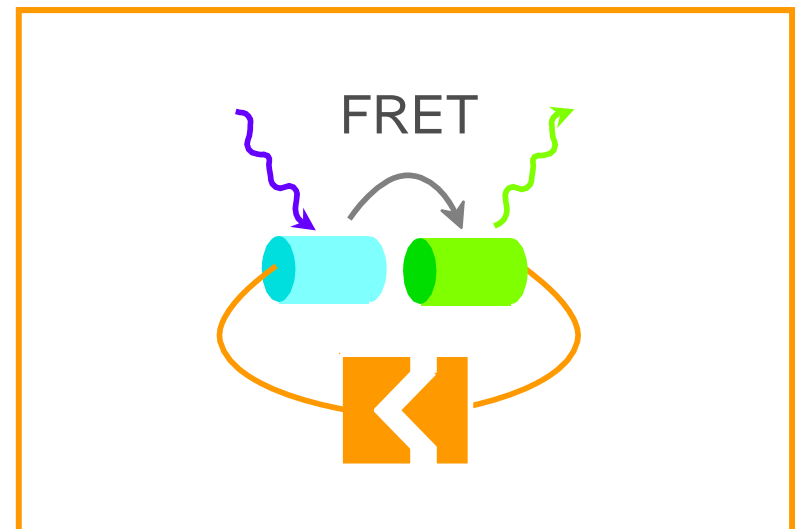
:: Protein-Protein interactions FRET Microscopy

FRET: Fluorescence Resonance Energy Transfer

Non-interacting proteins



Interacting proteins



:: GFP applications

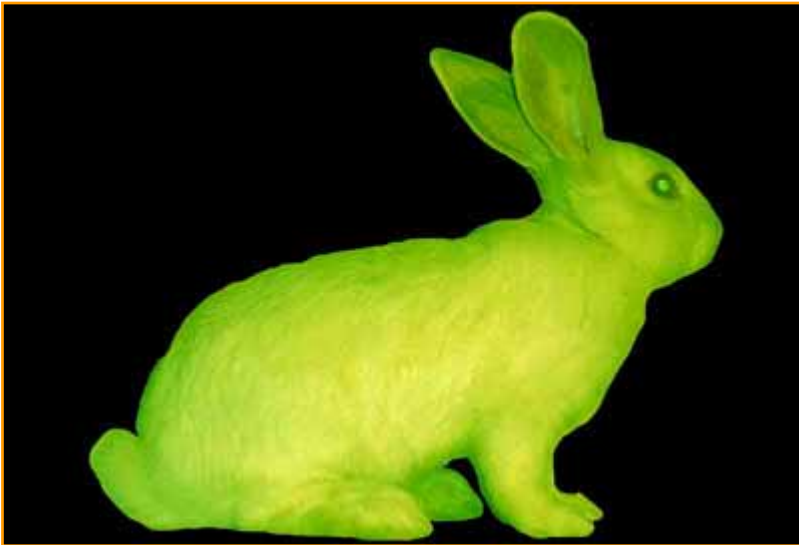
- **Marker of cell lineage:**

- Screenable marker to identify transgenic organisms
- Cell fate during development (tumor genesis, ...)
- Visual selection of cells expressing a given trans-gene



:: Cool applications

Transgenic exotic pets



:: Cool applications

Transgenic art-GFPixel

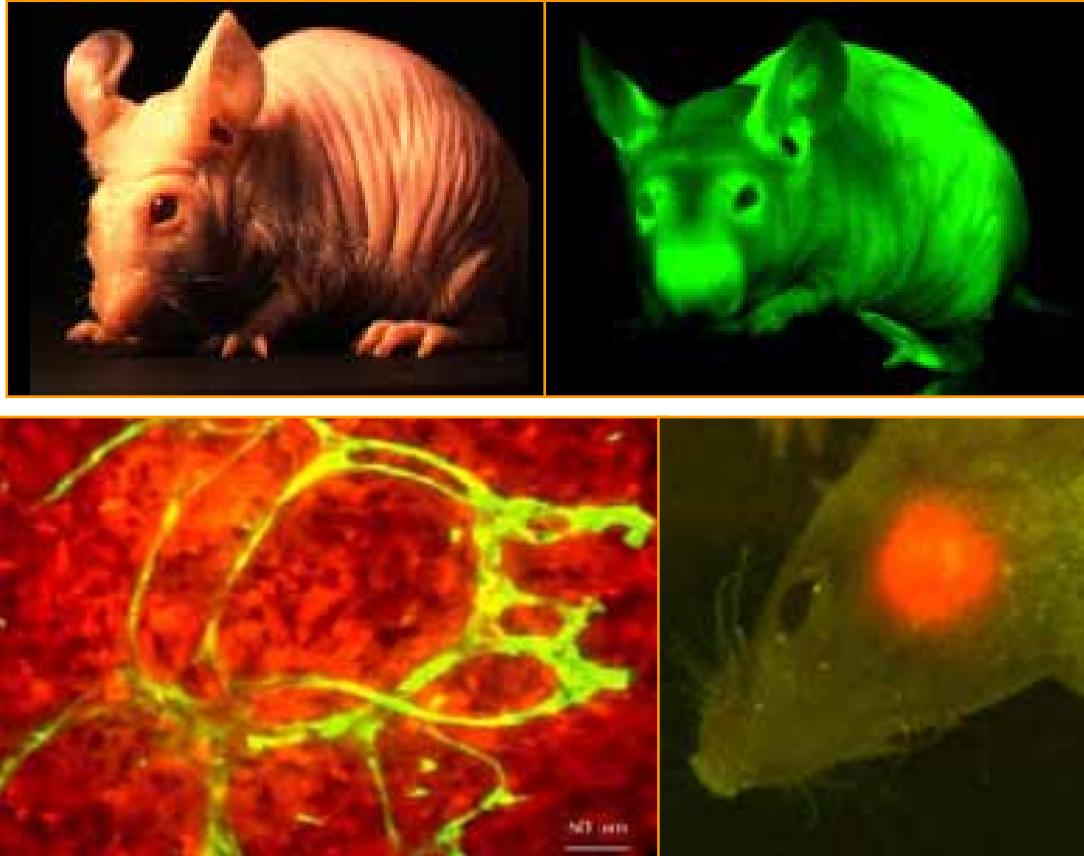


Malaria fighting strategies



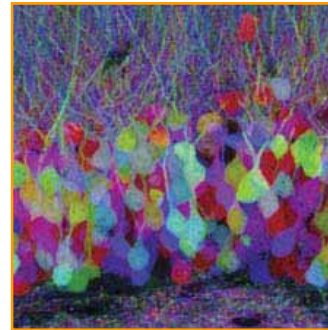
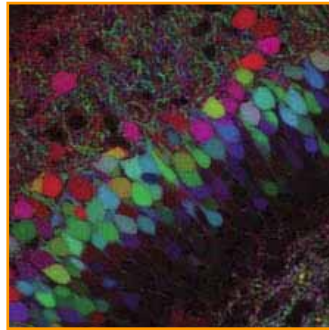
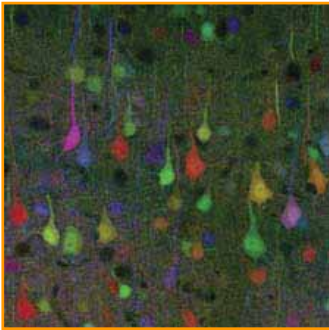
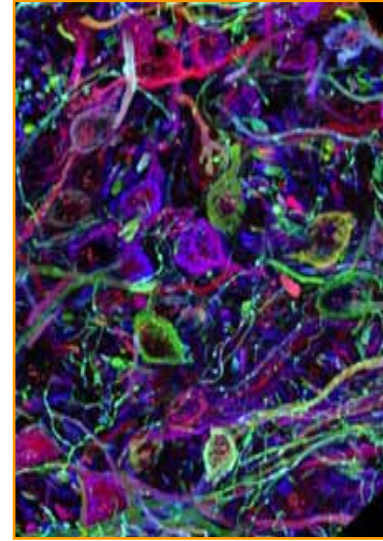
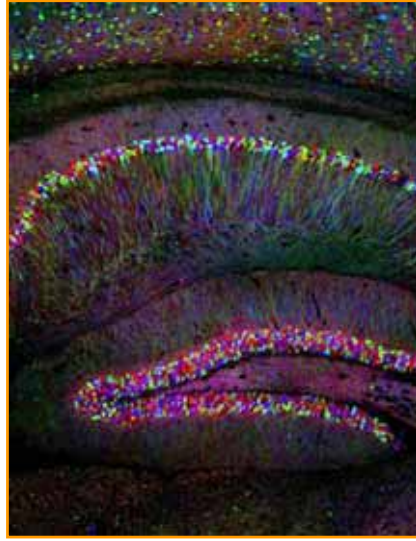
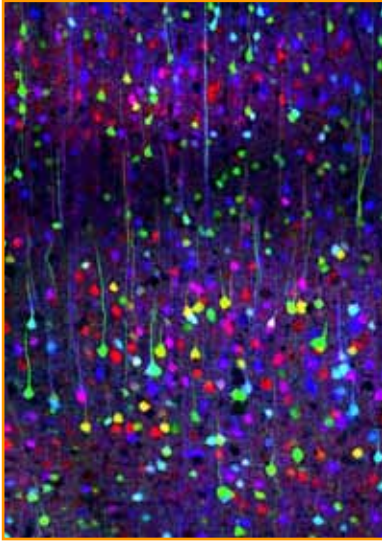
:: Cool applications

Tumor visualization



:: Cool applications

Brainbow



Transgenic mice expressing varying amounts of cyan, green and yellow in the brain neurons

:: GFP and Education

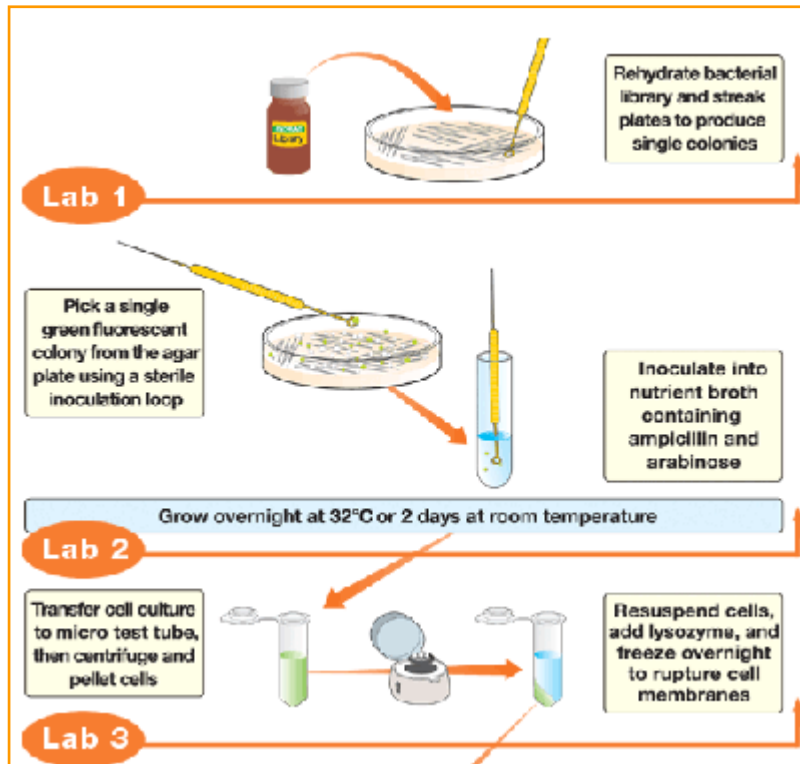


Secrets of the Rainforest Kit

Provides materials for 8 workstations
(2-4 students per workstation)

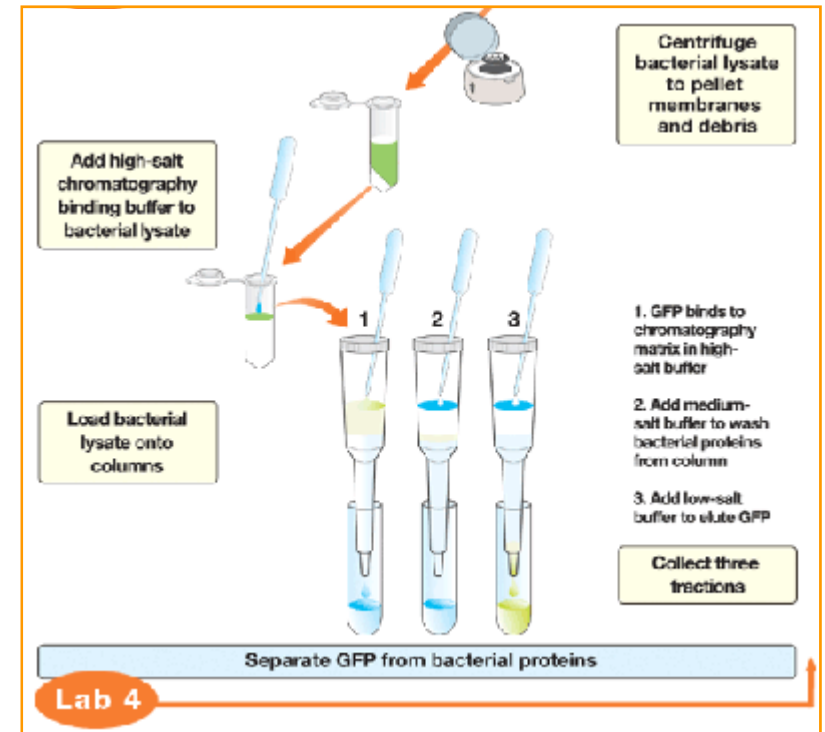
Ref. # 166-0006EDU

:: Secrets of the Rainforest Kit



Required Accessories Not Included in Kit

- UV lamp
- Micro-Centrifuge



Recommended (Optional) Accessories

- Rocking platform
- Microwave oven

Life Science Content Covered by **Secrets of the Rainforest Kit**

Scientific Inquiry

- Use of genetic engineering to produce synthetic proteins
- Cloning genes and screening bacterial libraries
- Interpretation of experimental results

Genetics

- DNA > RNA > protein > trait
- Expression of genes in foreign hosts
- Genetic engineering to create GMOs
- Gene regulation mechanism

Cell and Molecular Biology

- Prokaryotic vs. plant cell structure
- Bacterial growth and cell division
- Plant physiology and medicinal properties

Chemistry of Life

- Chemical and physical properties of proteins
- Protein structure (1°, 2°, 3°, 4°)
- Chromatographic separation of biomolecules

Evolution

- Biodiversity
- Producing medicinal plant proteins in bacteria for human treatment

Environmental and Health Science

- Pharmaceutical drug discovery
- Human physiology and cancer
- GMOs in medicine
- Antibiotic resistance

:: Fluorescent proteins

Further reading and interesting links

- NC Shaner, GH Patterson, MW Davidson. “Advances in fluorescent protein technology” *J. Cell. Sci.* **120** (2007) 4247-60 (Latest review)
- Mark Zimmer. “Glowing Genes: A Revolution In Biotechnology”(2005) Prometheus Books, New York.
- <http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.htm>
- <http://almaz.com/nobel/chemistry/>
- <http://www.microscopyu.com/articles/livecellimaging/fpintro.html>
- <http://www.olympusconfocal.com/java/fpfluorophores/index.html>