Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

FLUORESCENT PROTEINS - XFPs

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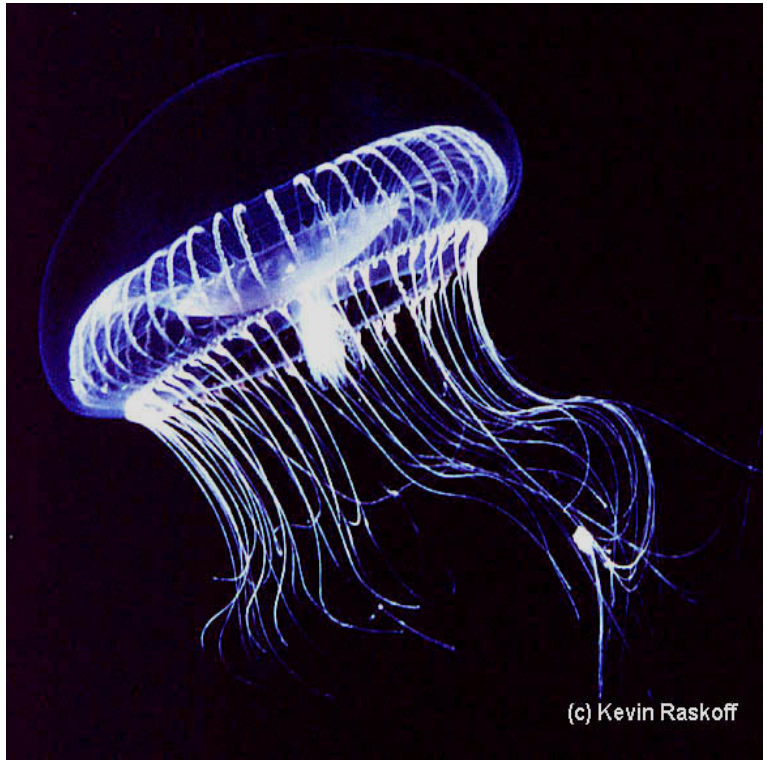
Presentations Content

- Originating organisms
- GFP characteristics
- Applications
- Short protein structure review
- GFP 3-D Structure
- GFP Maturation
- XFP Maturation Problems
- New XFP Generation
- RFP Maturation
- XFP Fusion-Proteins

XFPs originate from reefs

Coral – *Zoanthus*



Jellyfish - *Aequorea*

GFP – Green Fluorescent Protein

-1962: Osamu Shimomura et al.

-*Aequorea victoria*,

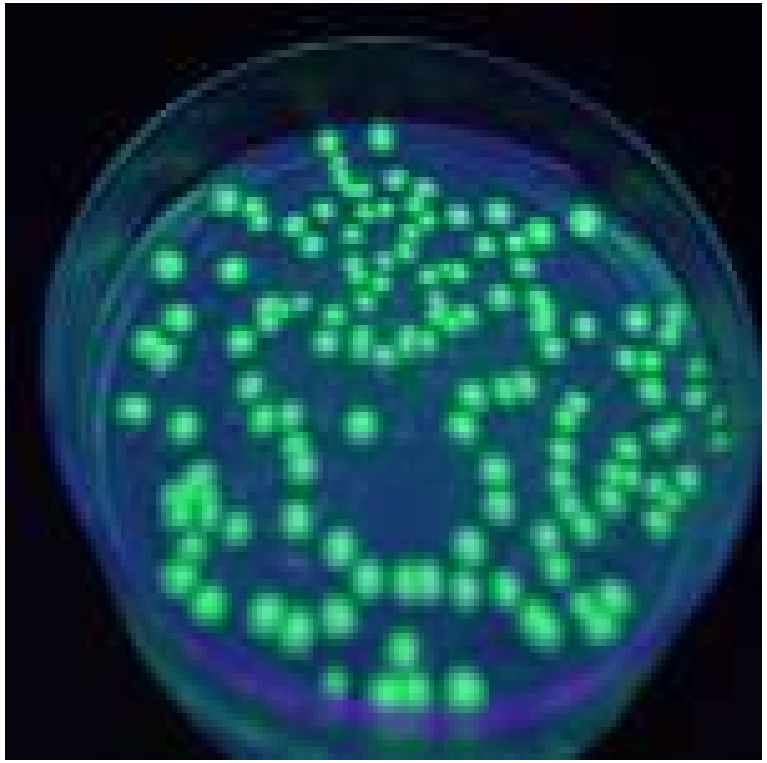
-238 Aminoacids

-26.9 kDa

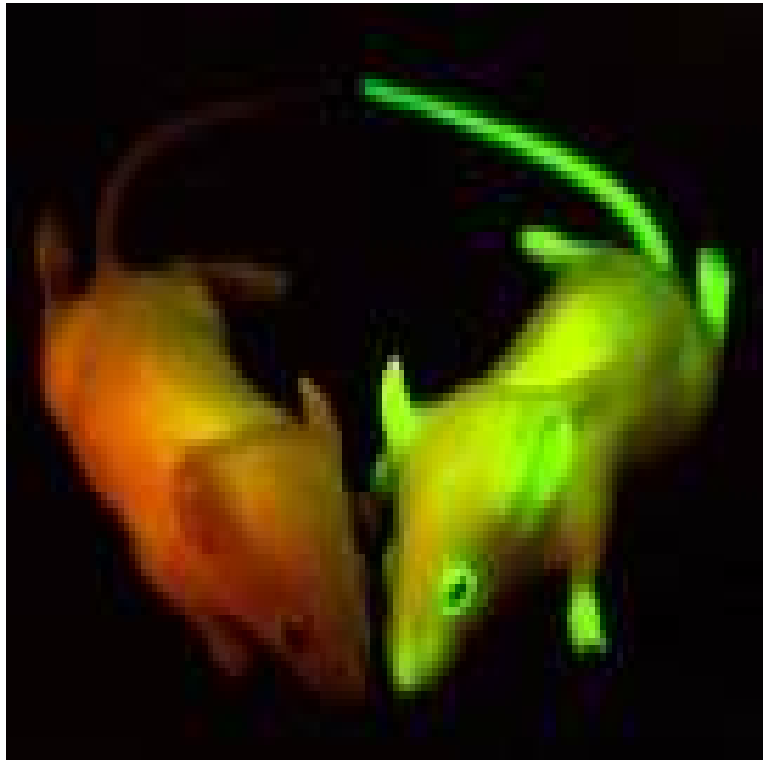
-Fusion-protein Marker since 1994: Doug Prasher et al.

-Unique quarternary structure for fluorescence

Cell Marker and Fusion Proteins



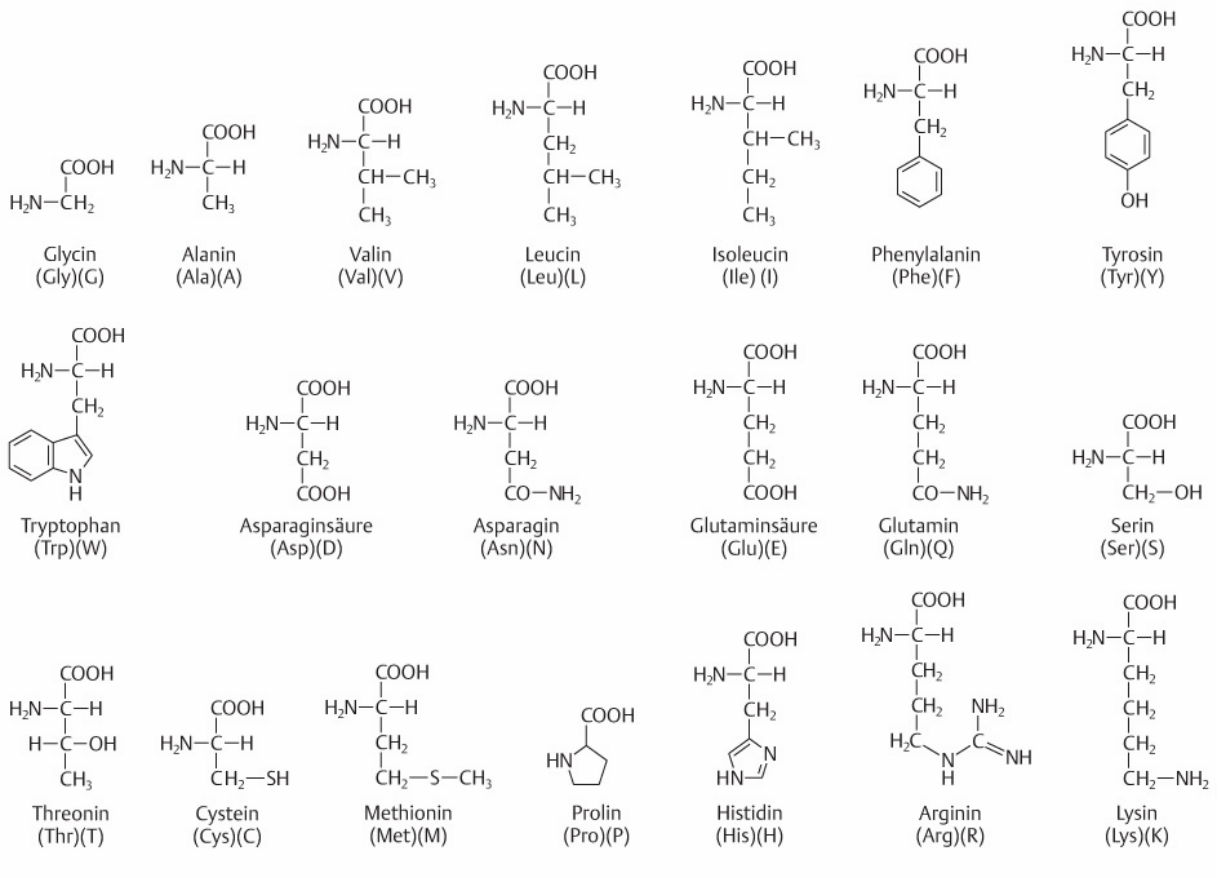
Fluorescent cells for FACS



Fusion-proteins

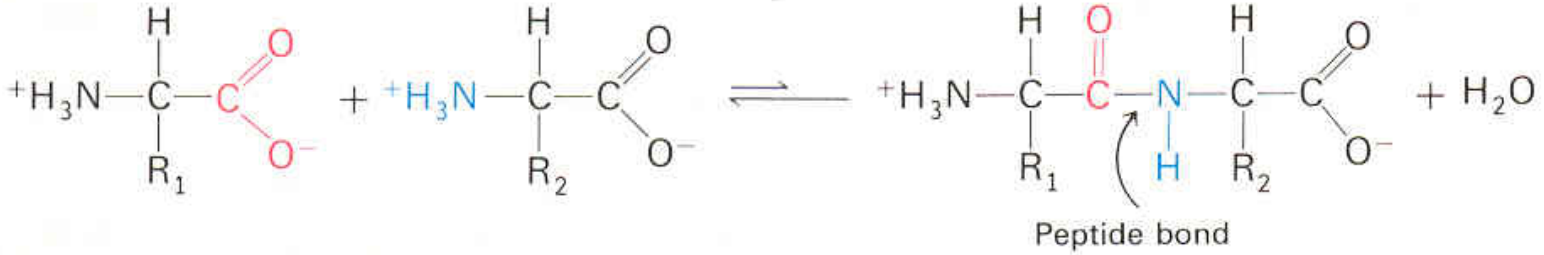
Short Protein Review

20 Amino Acids

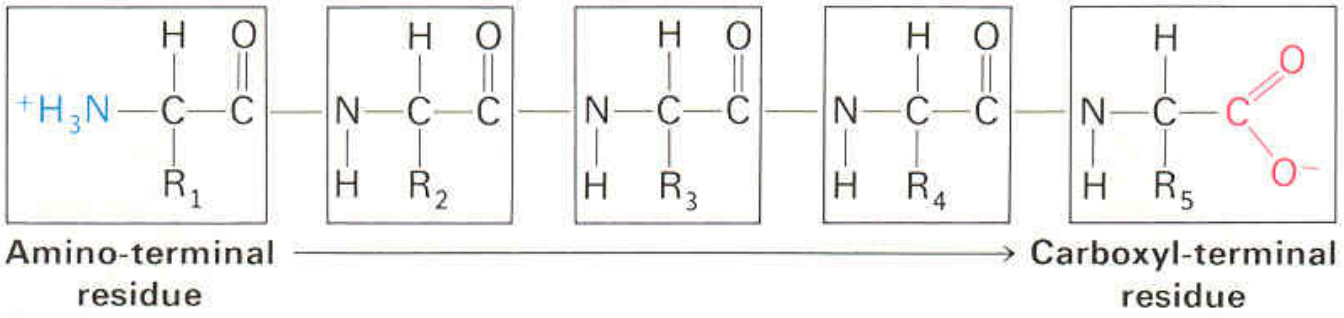


Primary Structures

Die Carboxy- und die Aminofunktion kondensieren zur Peptidbindung

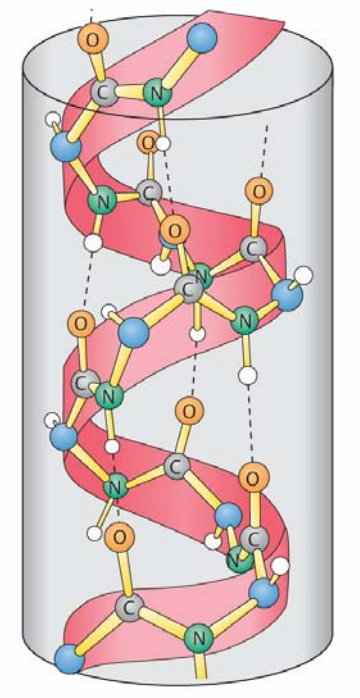


Ein Peptid: eine Aminosäurekette mit “Anfang” und ein “Ende”

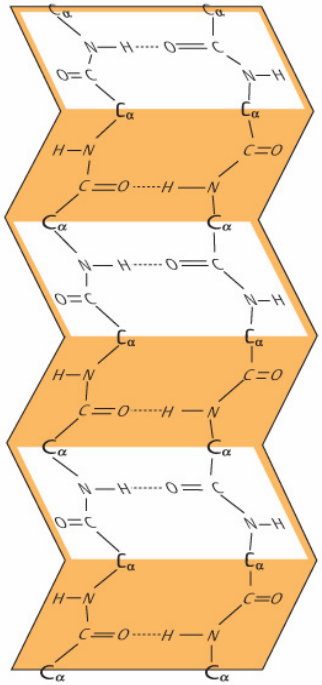


Primärstruktur: Die Aminosäuresequenz

Secondary Structures



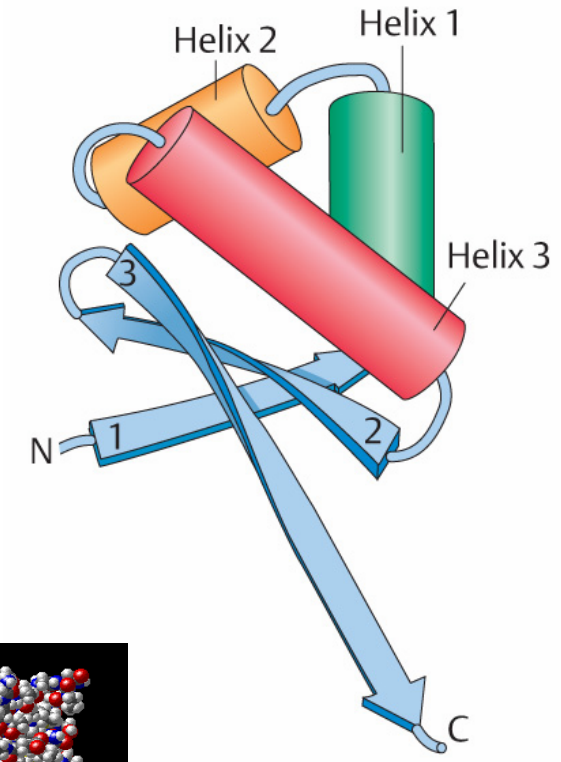
α-Helix:
Every fourth Aminoacid is connected via H-bonds to form a helical structure.

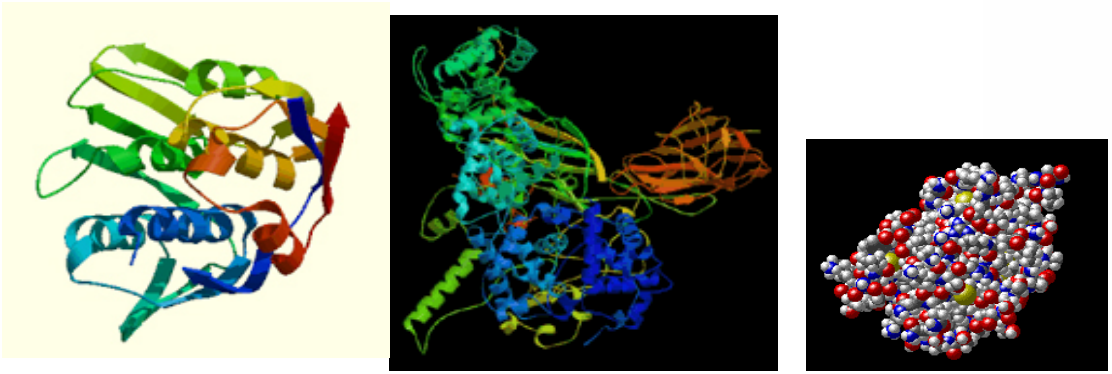


β-Sheet:
H-Bonds of neighboring Aminoacids build up a sheet like structure.

Tertiary Structures

Wechselwirkungen der Polypeptidketten (Ionenbindung, Wasserstoffbrücken, van der Waals-Bindungen, hydrophobe Wechselwirkungen) bewirken eine charakteristische räumliche Anordnung der Polypeptidkette eines Proteins.

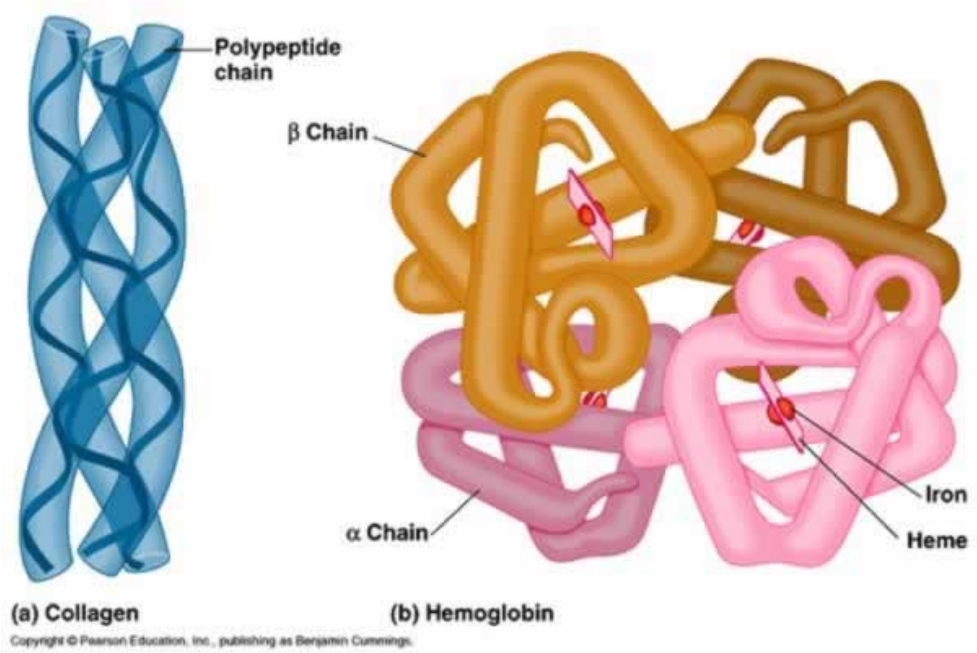




Aus: Internet und *Knippers - Molekulare Genetik*

Quarternary Structure

Struktur, die durch Wechselwirkung und Bindungen mehrerer Polypeptidketten eines Proteinmoleküls zustande kommt.

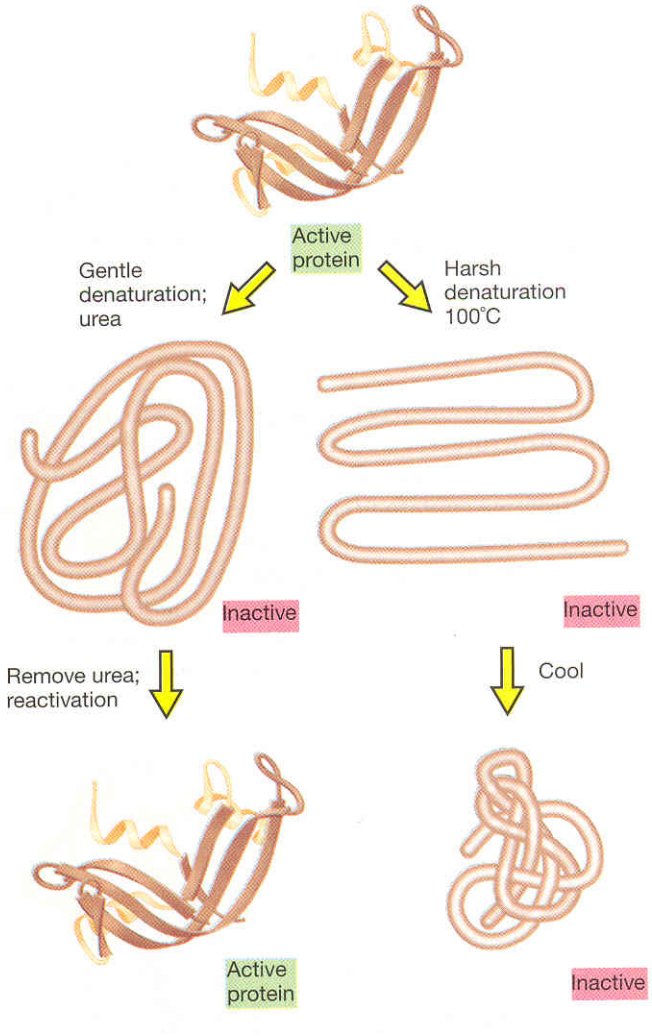


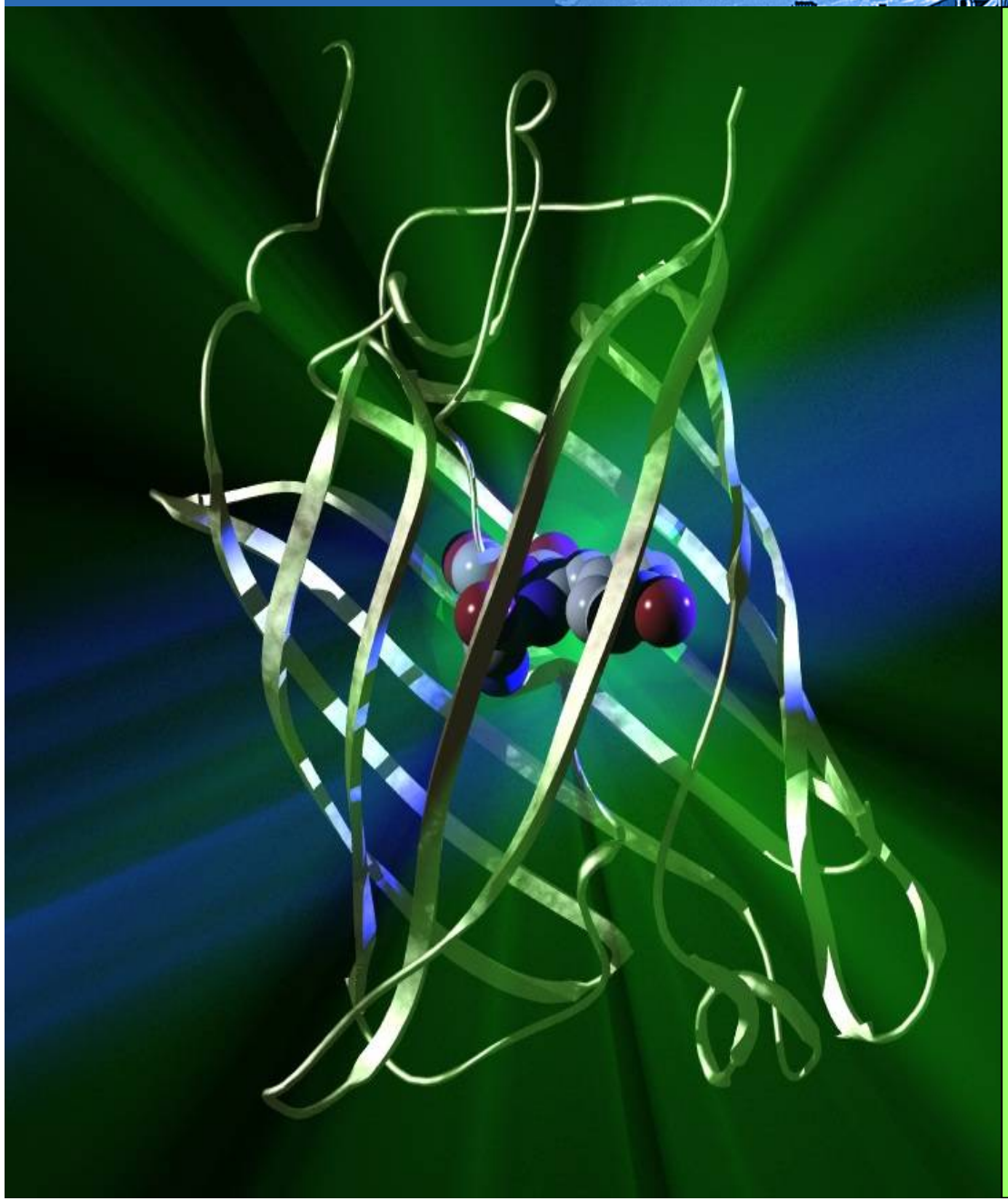
Denaturation → Renaturation

-Denaturation of proteins does not influence primary
-Higher structures are influenced.

→ Plan for folding is in the primary structure!

Example: Ribonuclease

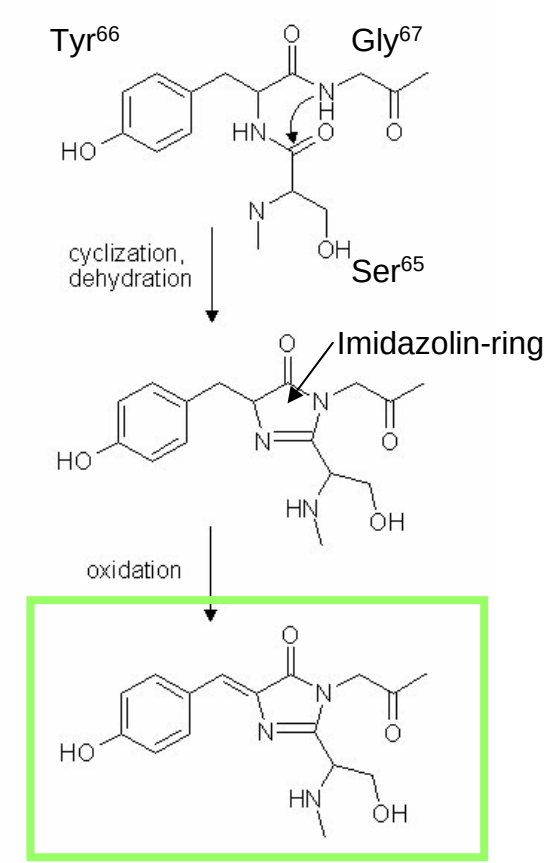




3D-Structure of GFP

- “Paint in a can”
- Each monomer composed of a central helix surrounded by an eleven stranded cylinder of anti-parallel beta-sheets (shields fluorophore from solvent)
- Cylinder has a diameter of about 30A and is about 40A long
- Fluorophore is located on the central helix

The Fluorophore – Protein Maturation



- 3AS: Serine65, Tyrosine66, Glycine67
- 2Step maturation:

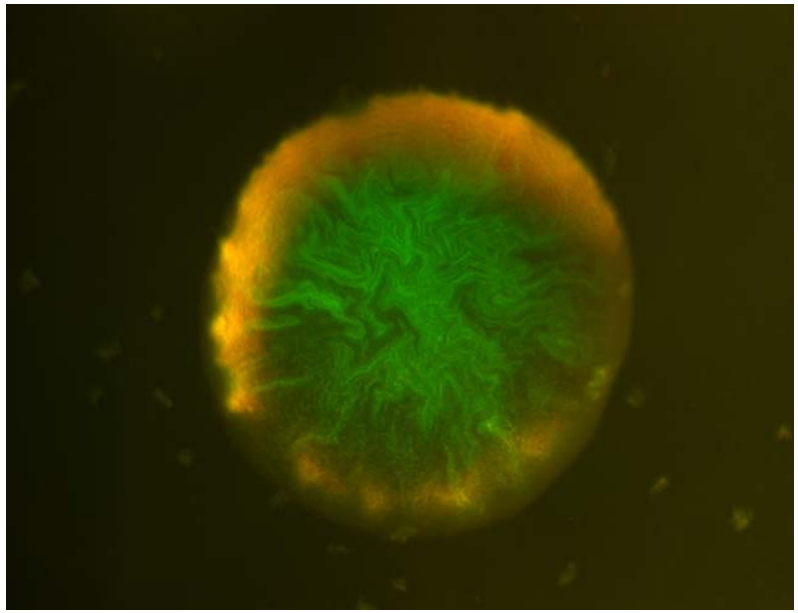
  1)Cyclization, dehydration: formation of Imidazolin-ring

  2)Oxidation: Extension of conjugated pi-electron system (reversible with reducing agents)

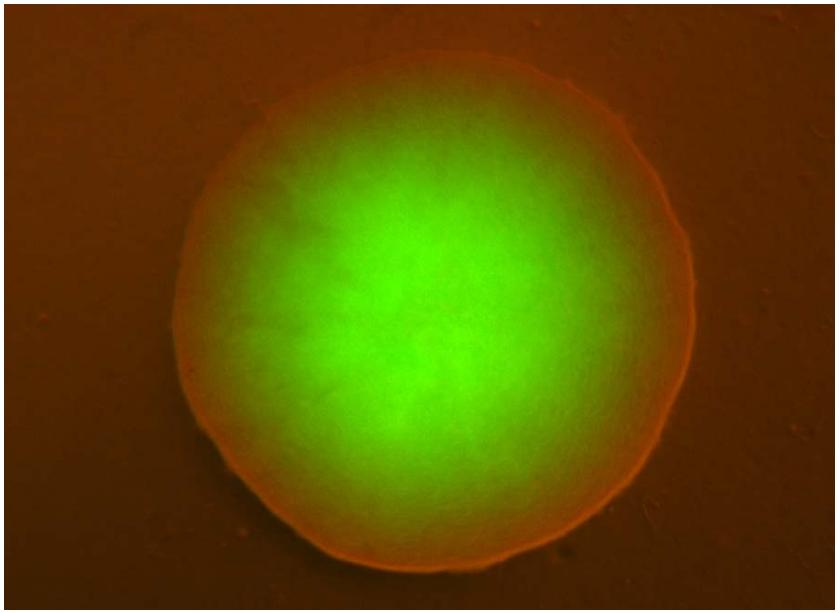
→ Environment matters:

- Oxidizing Environment
- Thermostable but temperature sensitive: Lower temperature →better protein folding.
- Long maturation time (up to 6h)

Temperature Sensitivity of yeGFP: An Example



Bacterial colony grown at 37°C



Bacterial colony grown at 30°C

XFP Mutants

Single AS substitutions make the difference:

-Wavelength shifts for excitation 395nm wtGFP → 488nm EGFP

-Wavelength shifts for emission: RFP, CFP, YFP… → better reporter systems

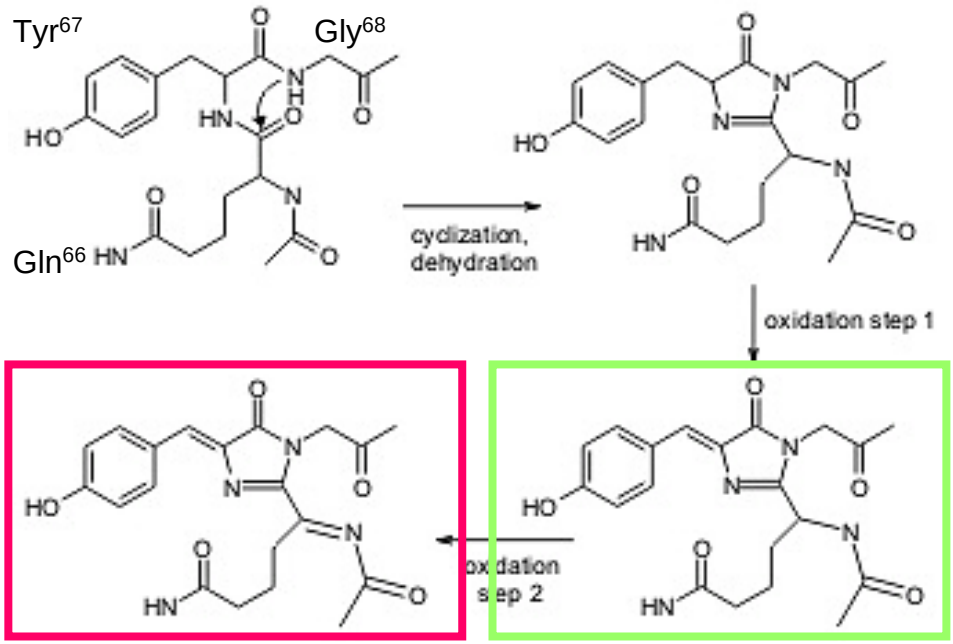
-Enhanced expression in mammalian cells

-Human codon optimization for expression in mammalian cell-lines

-Enhanced fluorescence: GFP-Ser65Thr → 4-6 fold

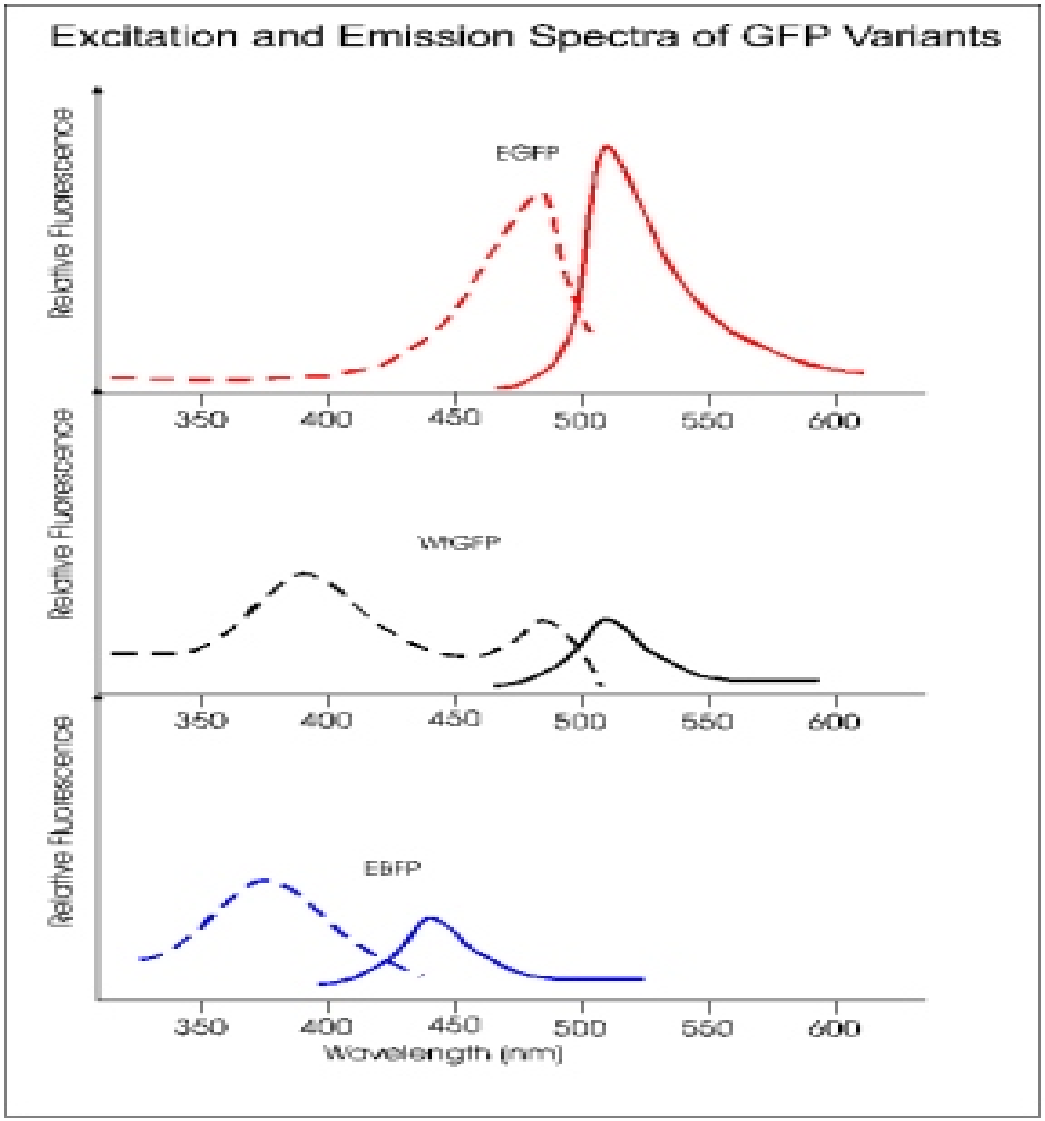
EGFP-Phe64Leu → up to 35 fold

RFP - dsRED

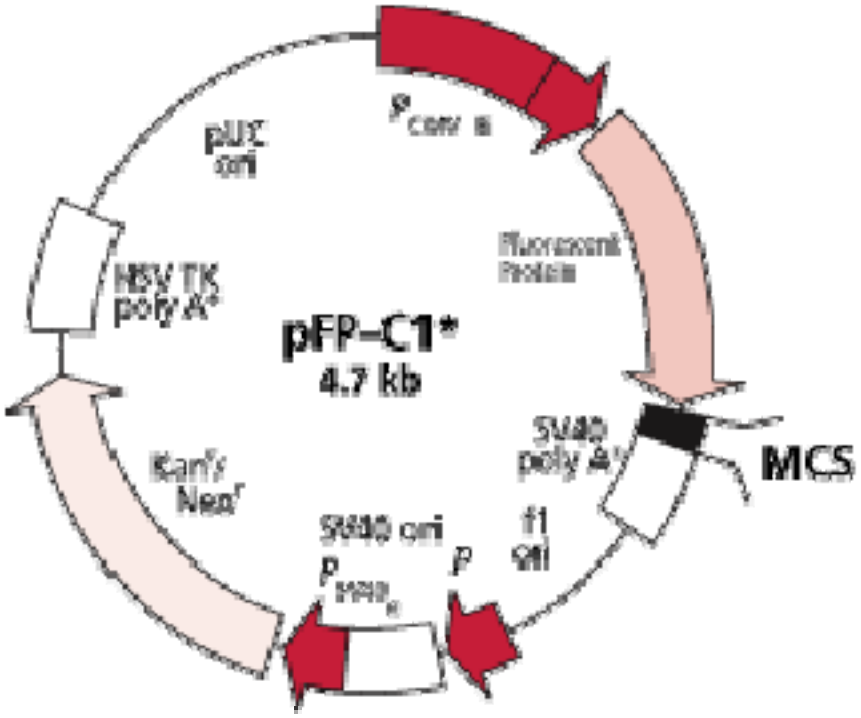


- Ser^{66} → Gln^{66}
- 2Step maturation:
  1)Cyclization, dehydration: formation of Imidazolin-ring
  2)First oxidation: Extension of conjugated pi-electron system → GFP
  3)Second oxidation: → RFP shift

→ Interference between monomers


Excitation and Emission Spectra of GFP Variants
Relative Fluorescence
EGFP
350
400
450
500
550
600
Relative Fluorescence
WtGFP
350
400
450
500
550
600
Relative Fluorescence
EBFP
350
400
450
500
550
600
Wavelength (nm)

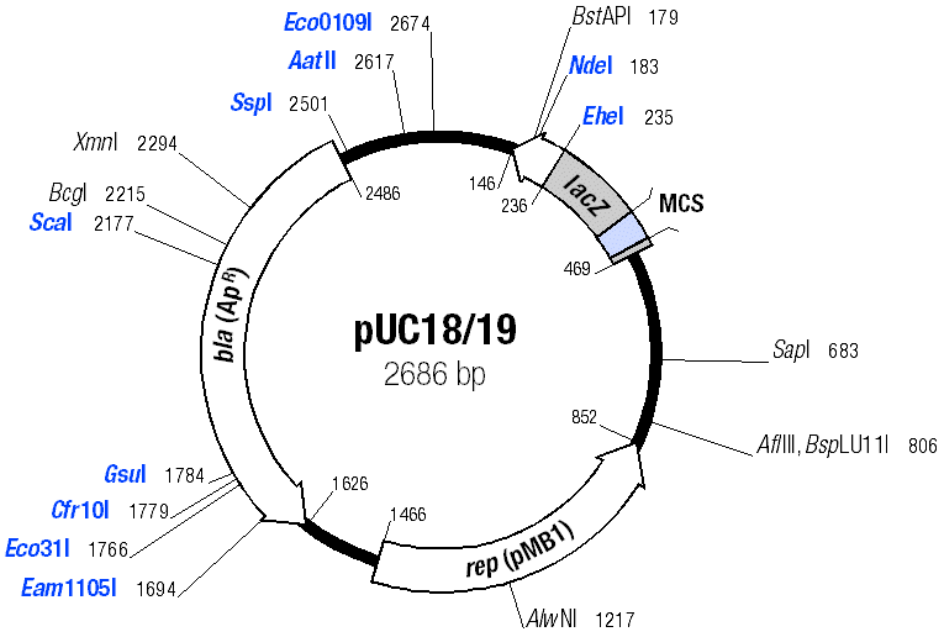
XFP – Fusion-Protein-Vectors



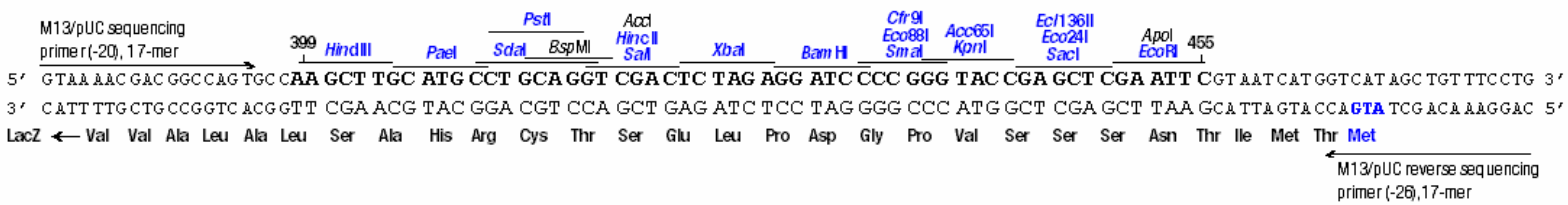
Applications:

- Protein localization
- General reporter for mammalian cells
- Monitoring transfection efficiencies

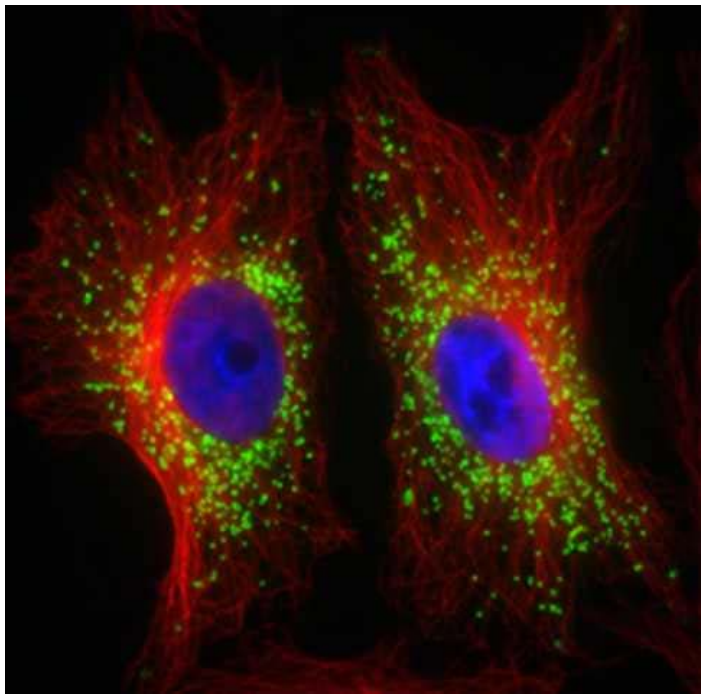
MCS – Multiple Cloning Site



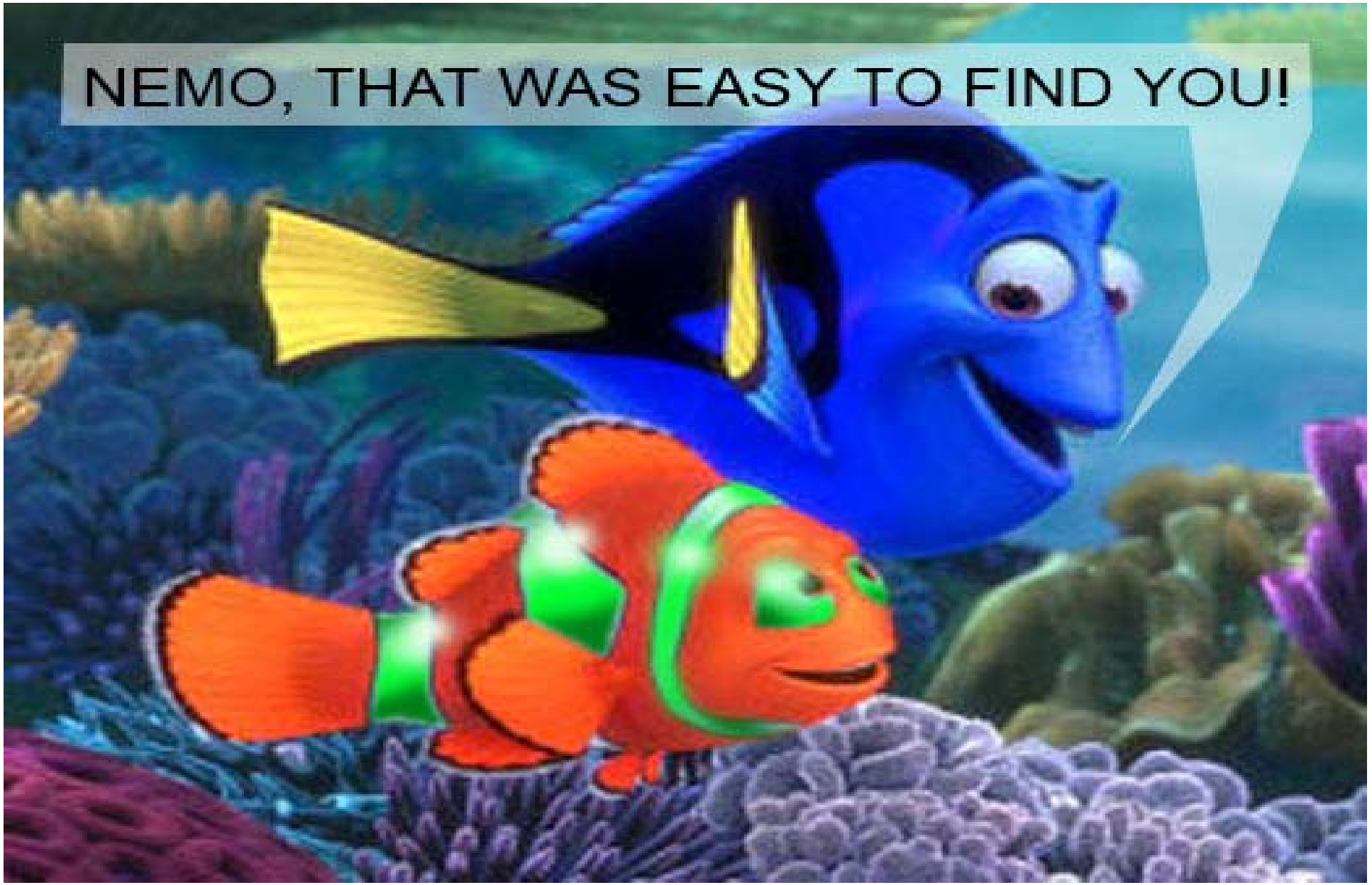
pUC18



Confocal Microscopy



- XFP Tagged Proteins
- Specific antibodies for XFPs
- Excitation filter at 488nm
- Emission filter at various wavelenth
- FRET (fluorescence resonance energy transfer)
- Precise protein localization?
- Organic solvents such as: methanol, ethanol, acetone do not preserve XFPs
- fixing with paraformaldehyde


NEMO, THAT WAS EASY TO FIND YOU!

Thank you for your attention!