ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

# FLUORESCENT PROTEINS - XFPs

Marcel Walser; PhD student






## 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*,

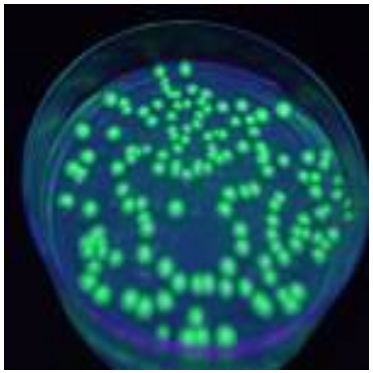
-238 Aminoacids

-26.9 kDa

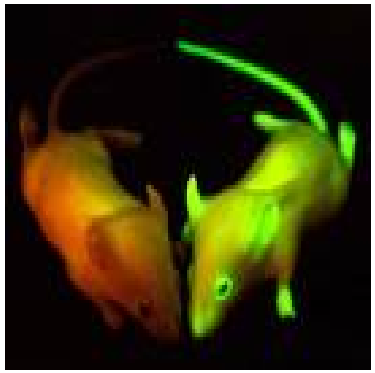
-Fusion-protein Marker since 1994: Doug Prasher et al.

-Unique quarternary structure for fluorescence

## Cell Marker and Fusion Proteins



Fluorescent cells for FACS

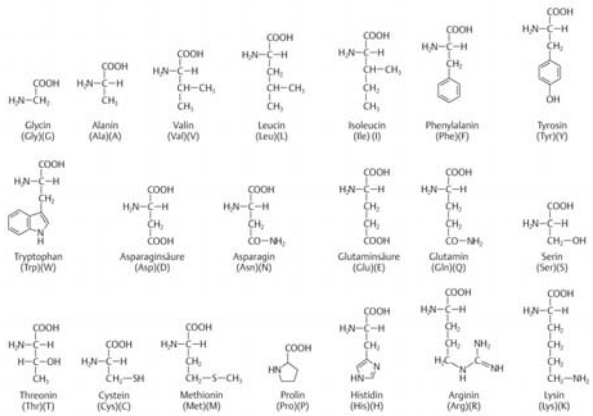


Fusion-proteins



## Short Protein Review

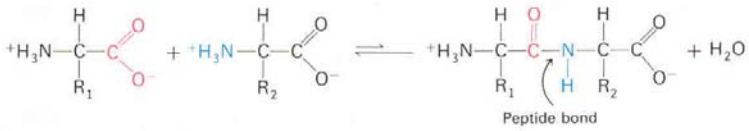
### 20 Amino Acids



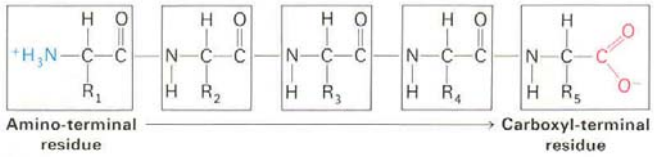



## Primary Structures

Die Carboxy- und die Aminofunktion kondensieren zur Peptidbindung



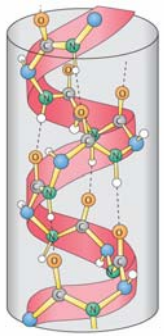

Ein Peptid: eine Aminosäurekette mit “Anfang” und ein “Ende”



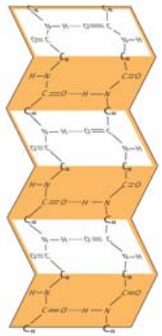

Primärstruktur: Die Aminosäuresequenz



## Secondary Structures



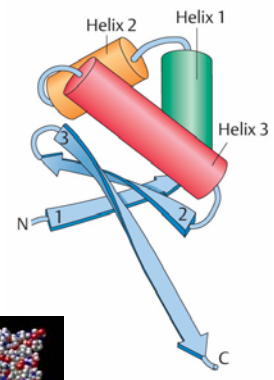
**α-Helix**: Every fourth Aminoacid is connected via H-bonds to form a helical structure.

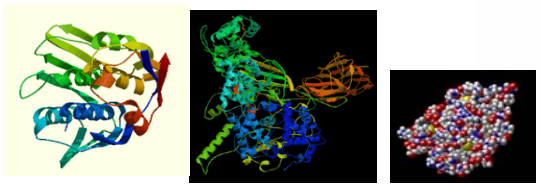


**β-Sheet**: H-Bonds of neighboring Aminoacids build up a sheet like structure.

## Tertiary Structures

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



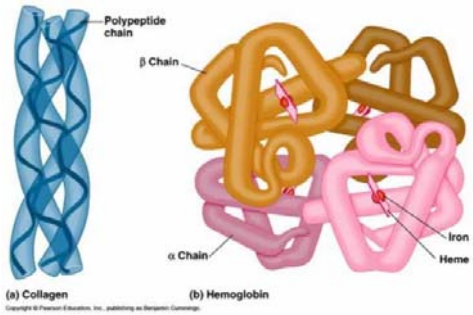



Aus: Internet und *Knippers - Molekulare Genetik*



## Quarternary Structure

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

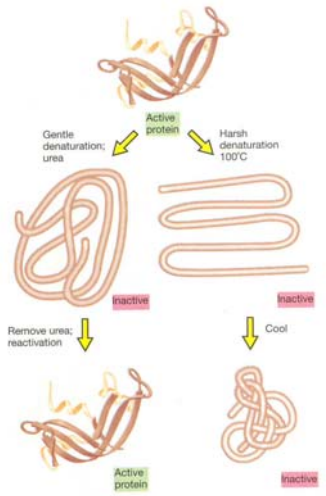





## Denaturation → Renaturation

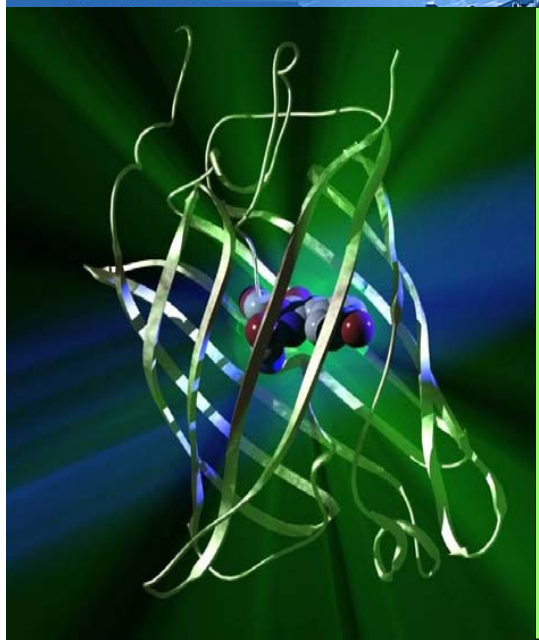
-Denaturation of proteins does not influence primary
-Higher structures are influenced.

→ Plan for folding is in the primary structure!




Example: Ribonuclease

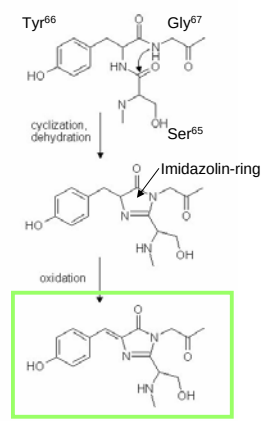




## 3D-Structure of GFP

- "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



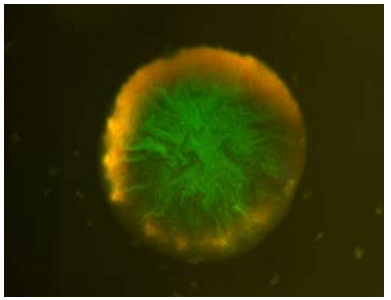

- 3AS: Serine65, Tyrosine66, Glycine67
- 2Step maturation:
  1)Cyclization, dehydration: formation of Imidazolin-ring
  2)Oxidation: Extension of conjugated pi-electron system (reversible with reducing agents)

→ Environment matters:

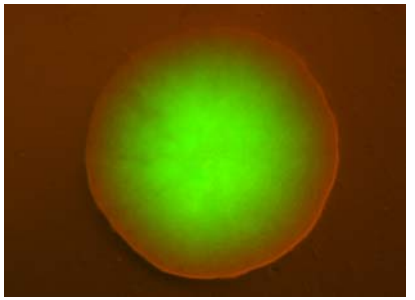
- Oxidizing Environment
- Thermostable but temperature sensitive: Lower temperature →better protein folding.
- Long maturation time (up to 6h)



## 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

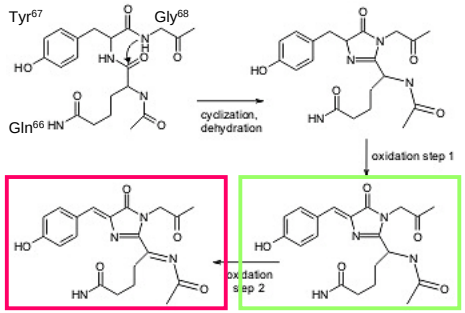
-Human codon optimization for expression in mammalian cell-lines

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

EGFP-Phe64Leu → up to 35 fold

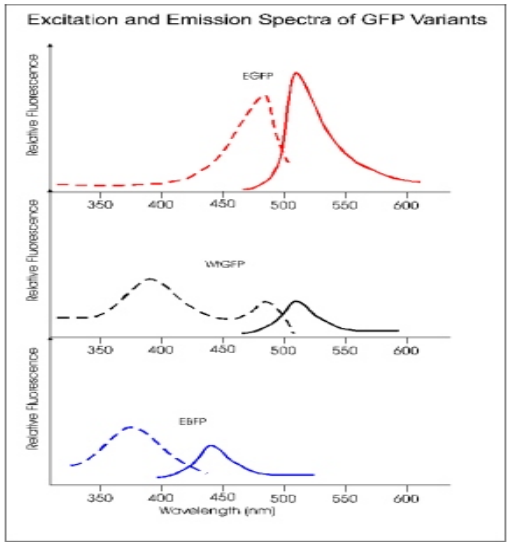


## RFP - dsRED




- Ser66 → Gln66
- 2Step maturation:
  1)Cyclization, dehydration: formation of Imidazolin-ring
  2)First oxidation: Extension of conjugated pi-electron system → GFP
  3)Second oxidation: → RFP shift

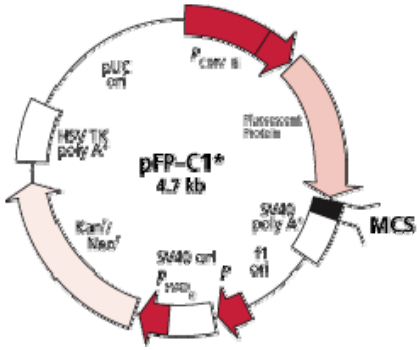
→ Interference between monomers








## XFP – Fusion-Protein-Vectors



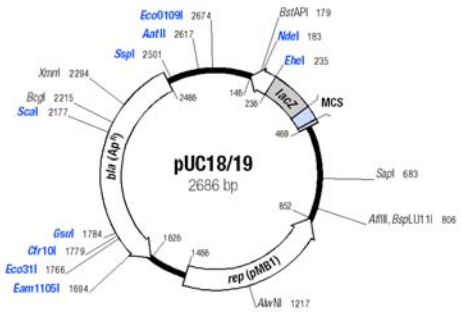

Applications:

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

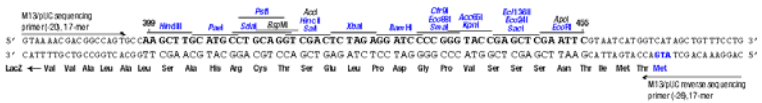




## MCS – Multiple Cloning Site



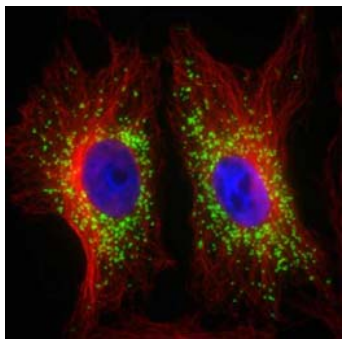

pUC18








## 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

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**Thank you for your attention!**