

GFP and it's relatives

Biomol. Structure 2005

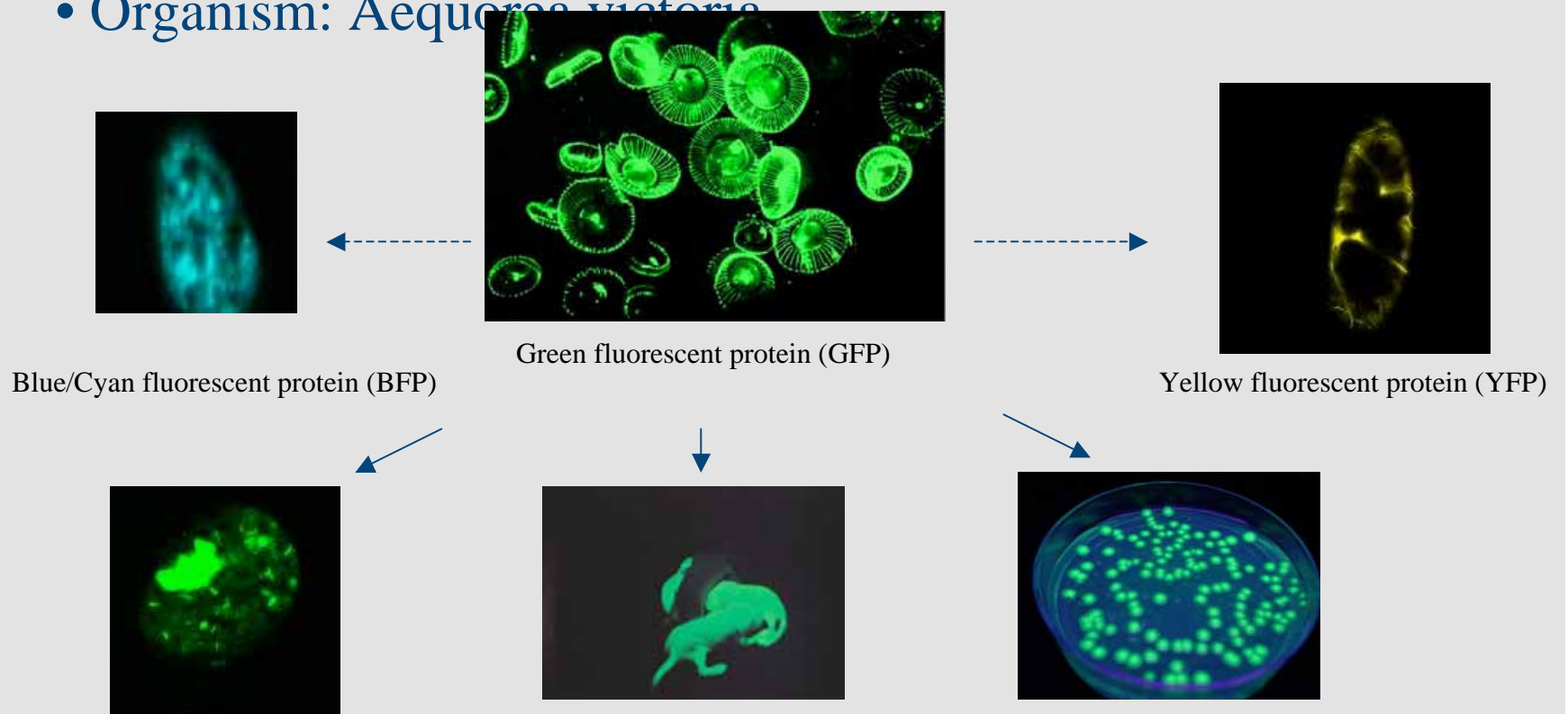
Biomolecular Structure 2005
University of Massachusetts, Amherst

Mathias Stotz

GFP and it's relatives

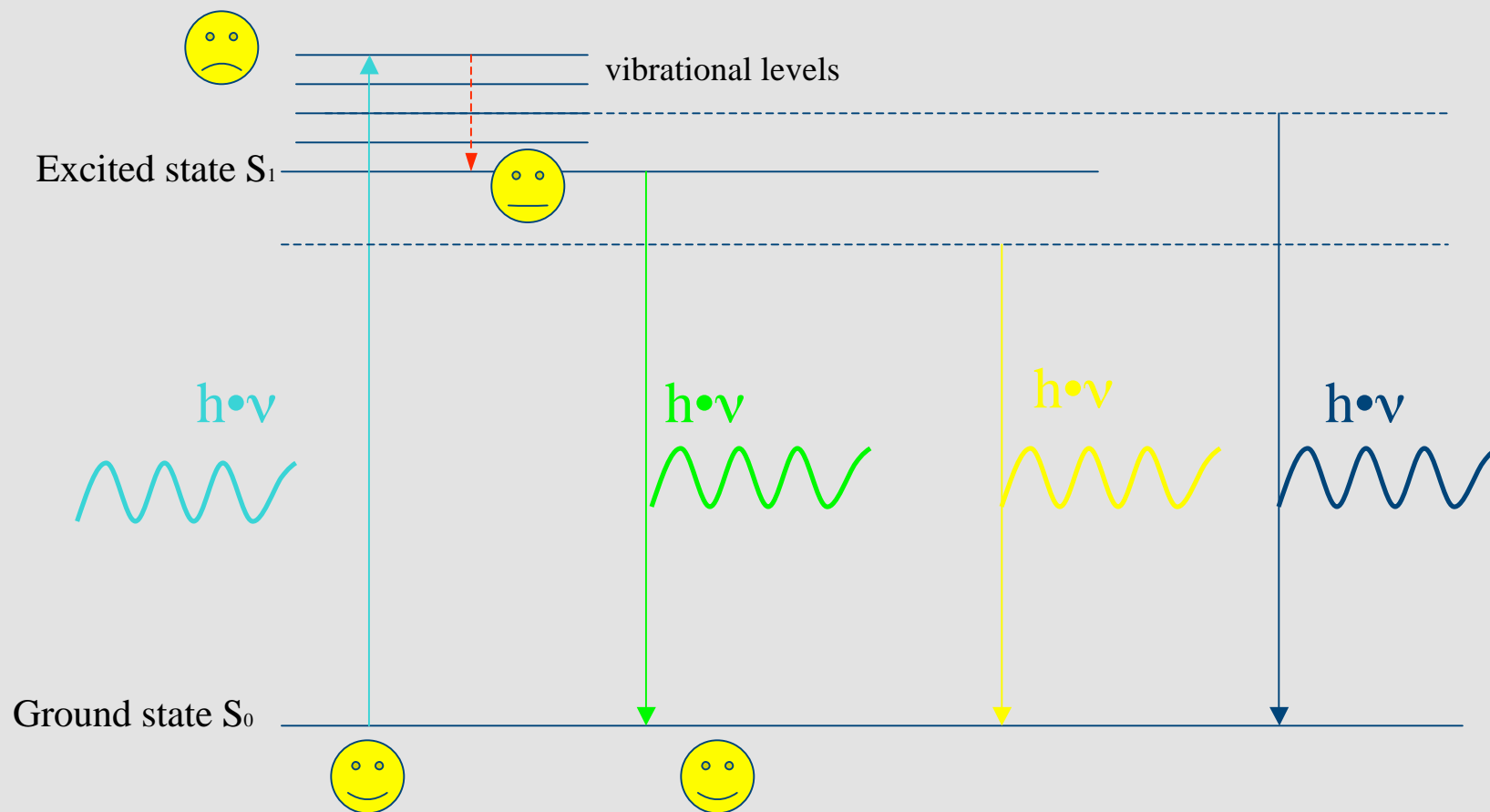
- the basics

- Organism: *Aequorea victoria*



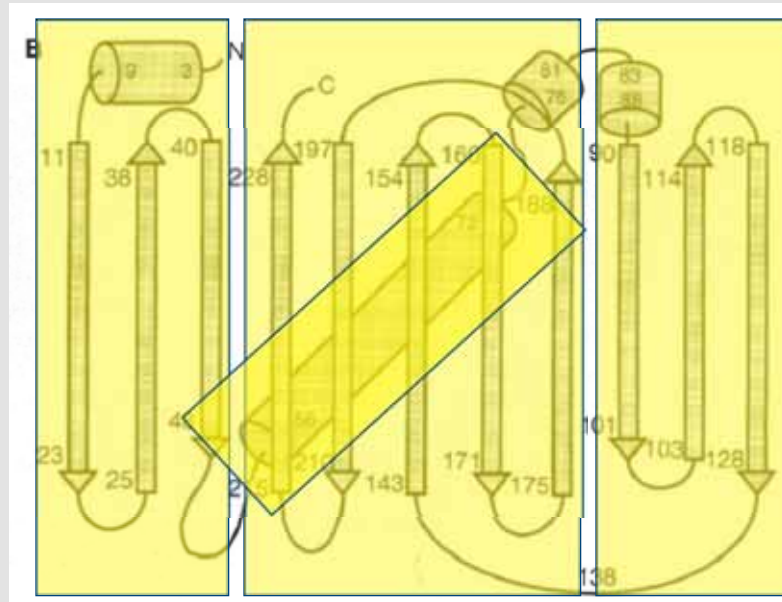
Jablonski diagram for fluorescence

- with a few simplifications



The overall fold of GFP

- schematic drawing of the β -can fold



- 3 antiparallel β -sheets
- the central α -helix
- 3 antiparallel β -sheets
- greek key motif

Picture taken from Science Vol 273, Sep 1996
Ormö, Cubitt, Kallio, Gross, Tsien, Remington
“Crystal structure of the Aequorea Victoria Green Fluorescent Protein“

Chromophore formation in GFP

- cyclization, dehydration, oxidation

Chromophore Formation in GFP

Biochemistry, Vol. 36, No. 22, 1997 6787

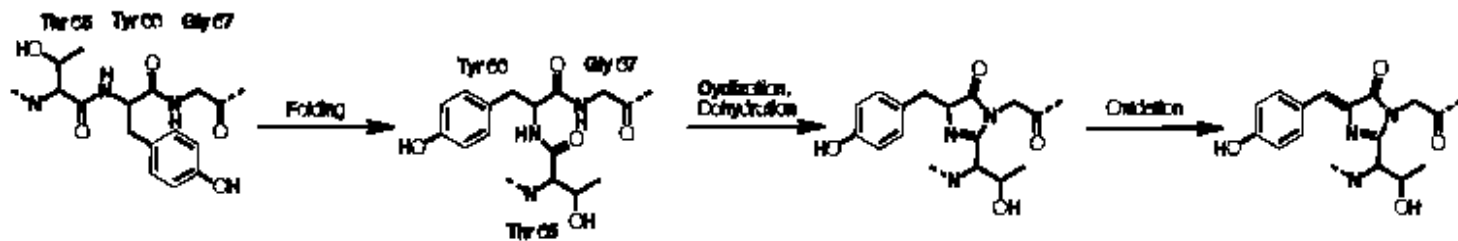


FIGURE 1: Model for chromophore formation in S65T-GFP as proposed by Heim et al. (1994). The chromophore tripeptide is represented in an extended conformation in the denatured state. Folding to the native state promotes chromophore cyclization, possibly by placing the carboxyl group of Thr 65 in close proximity to the amide of Gly 67.

- Involved residues are: Thr 65, Tyr66, Gly67

Conclusions

- the take home messages

- GFP

- β - can fold
- Fluorophore in the central α -helix (T65-Y66-G67)
- Fluorophore shows interactions with Ser 205 and Thr 203

- BFP

- altered fluorophore Y66 \rightarrow H66
- altered interactions with Ser 205 and Thr 203

- YFP

- same fluorophore
- Thr 203 \rightarrow Tyr 203; aromatic stacking

References

- Who reads all that stuff ?

- [1] Rebekka M Wachter, Marc-André Elsliger, Karen Kallio ; George T Hanson and S James Remington; “Structural basis of spectral shifts in the yellow-emission variants of green fluorescent protein” ; Structure 15 October 1998, 6:1267–1277
- [2] Mats Ormo, Andrew B. Cubitt, Karen Kallio, Larry A. Gross, Roger Y. Tsien, S. James Remington; “Crystal Structure of the Aequorea Victoria Green Fluorescent Protein”; Science (273) Sep 1996, 5280, 1392-1395
- [3] Brian G. Reid and Gregory C. Flynn ; “Chromophore Formation in Green Fluorescent Protein” ; Biochemistry 1997, 36, 6786-6791
- [4] Wachter, King, Heim, Kallio, Tsien, Boxer and Remington; “Crystal Structure and Photodynamic behaviour of the blue emission variant Y66H / Y145F of Green Fluorescent protein” ; Biochemistry 1997, 36, 9759-9765
- [5] Crystal structures: 1EMA , 1BFP, 1HUY