Green Floursecent Protien is a fluorescent protein isolated from *coelenterates*, such as the Pacific jellyfish, *Aequoria victoria,* or from the sea pansy, *Renilla reniformis*.[1] Its role is to transduce the blue chemiluminescence of the protein aequorin into green fluorescent light by the transfer of energy.[2] GFP is an extraordinary protein in many respects: It is fluorescent and its fluorophore is made up of modified amino acid residues. Moreover it is the first known example of a Förster cycle within the core of a protein. Furthermore its crystal structure has recently been solved and the protein turned out to have a new structural motif, called the beta-can.[3] The gene for GFP has been isolated and has become a useful tool for making chimeric proteins of GFP linked to other proteins where it functions as a fluorescent protein tag. GFP tolerates N- and C-terminal fusion to a broad variety of proteins.[2] It has been expressed in bacteria, yeast, slime mold, plants, drosophila, zebrafish, and in mammalian cells. As a noninvasive fluorescent marker in living cells, it allows for a wide range of applications where it may function as a cell lineage tracer, reporter of gene expression, or as a measure of protein-protein interactions.

[1] Chalfie, M.; Tu, Y.; Euskirchen, G.; Ward, W.W.; Prasher, D.C.; Green fluorescent protein as a marker for gene expression. *Science* **2004** 263, 802–805

[2] Morin, X.; Daneman, R.; Zavortink, M; Chia, W; A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in Drosophila. *Proc Natl Acad Sci* **2001** USA 98,15050-15055

[3] Barz, T.; Ackermann, K.; Pyerin, W.; A positive control for the green Fluorescent protein-based one-hybrid system. *Anal Biochem* **2002** 304,117-121

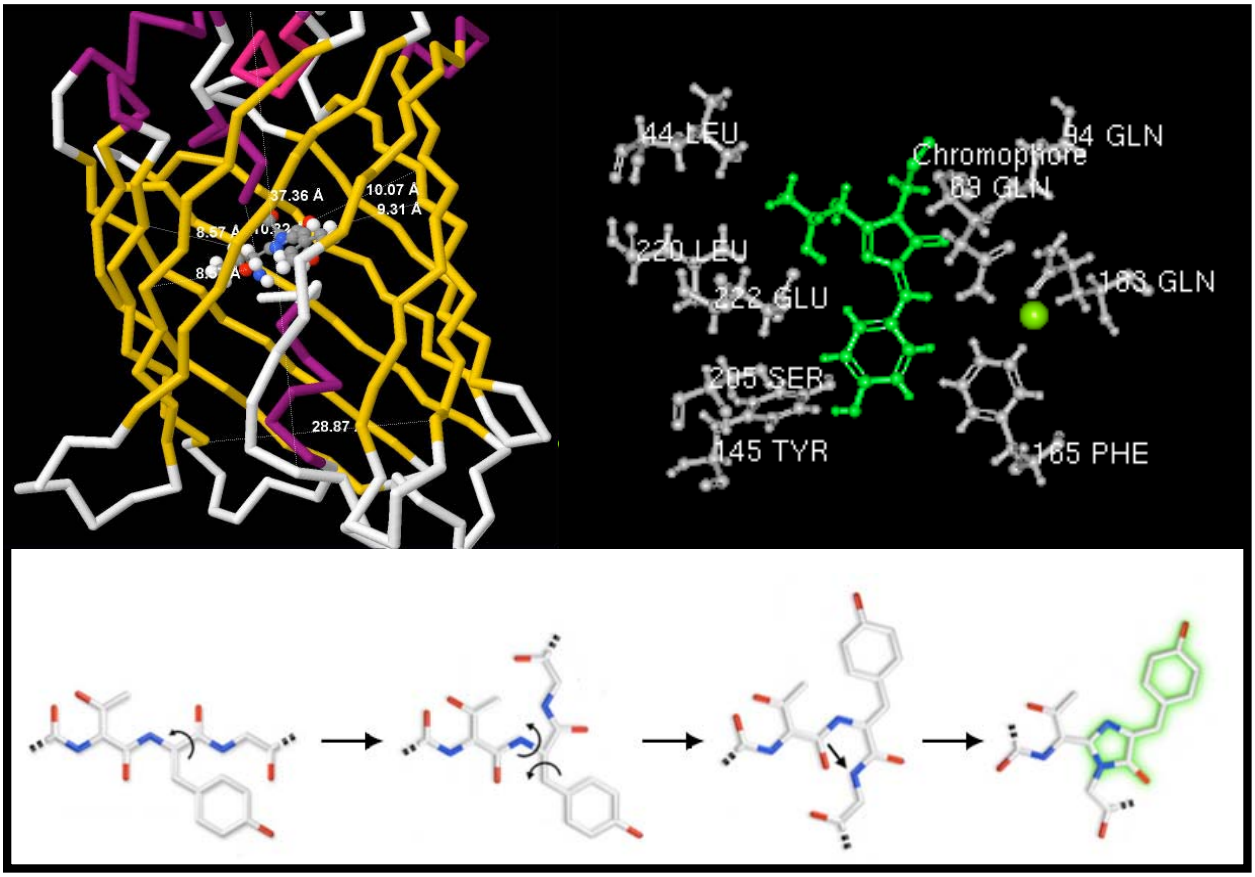

Figure 1. The beta-can which encompasses the fluorophore in about 40 angstroms long with a diameter of about 30 angstroms. Over 20 atoms can be found interacting within 5 angstroms of the fluorophore which is almost 10 angstroms by 8 angstroms. The key angle as indicated by the arrow goes from 108.3˚ to 103.2˚ as the second ring forms.
Source: Protein Workshop. http://www.pdb.org (accessed March 24, 2011); Jmol: an open-source Java viewer for chemical structures in 3D. http://www.jmol.org/ (accessed March 24, 2011).

The GFP's active sight is comprised of the amino acid residues Serine 65, Tyrosine 66, and Glycine 67. Before maturation, it is a simple peptide chain running 40 angrstroms through the center of a beta-can with a diameter of 30 angstroms as seen in Figure 1.[4] Spontaneous post-translation cyclization of the peptide backbone results in a 4-(*p*-hydroxybenzylidene)-imadazolidin-5-one fluorophore within a couple angstroms of the geographic center of the beta-barrel. The fluorophore is held in place by Van der Waal's forces and ionic interactions with the protective beta-can. Fifteen amino residues can be found within 5 angstroms of the fluorophore and keep outside ligands from further modifying the protein.[5]

[4] Yang, F.; Moss, L.; Phillips, G. (1996) The molecular structure of green fluorescent protein. *Nat.Biotechnol*. **1996** 14, 1246-1251.

[5] Wood, T.I.; Barondeau, D.P.; Hitomi, C.; Kassmann, C.J.; Tainer, J.A.; Getzoff, E.D. *Biochemistry* **2005** 44**:** 16211-16220.

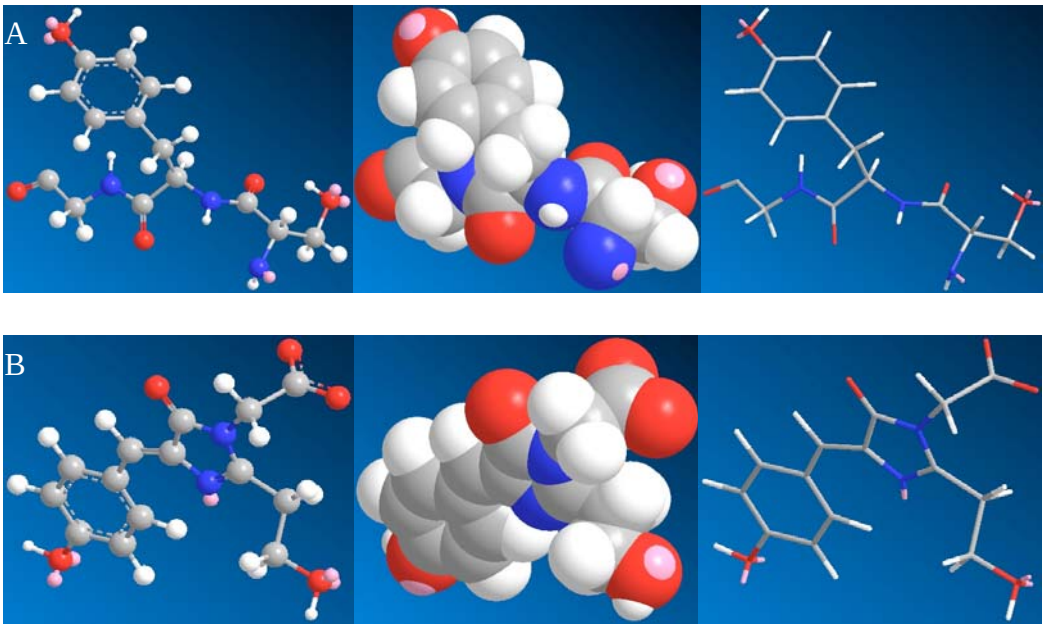

Figure 2. The three slides of A represent the amino residues Ser65, Thr 66, and Gly67 with ball and stick, space fill, and stick models before cyclization and fluorophore formation as seen in B.
Source: ChemBioDraw Ultra 12.0 Suite. CambridgeSoft. (accessed March 21, 2011).

The fluorophore of GFP is generated by a sequential mechanism in an auto-catalytic process.[6] No co-factors or enzymatic components are required. The reaction is initiated by a rapid cyclization between Ser65 and Gly67 to form an imidazolin-5-one intermediate which is followed by a much slower rate-limiting oxygenation of the Tyr66 side chain by O_2 on a timescale of hours (Figure 2).[7] Gly67 is required for formation of the fluorophore, no other amino acid can replace Gly in this role.[7] Molecular oxygen is needed for formation of the double bond between two carbons on the tyrosine to form an extended aromatic system. In contradiction with this requirement, however, oxygen must be excluded from interactions with the fluorophore itself, or else collisional quenching of the fluorescence or damaging photochemistry will occur.[8] The observed low bimolecular quenching rate (0.004 $M^{-1}s^{-1}$) suggests that GFP gives up efficient fluorophore formation for stability and higher quantum yields once fully formed.[9] Steady state and time-resolved fluorescence spectroscopies suggest that proton transfer is involved in

[6] Barondeau, D. P.; Kassmann, C. J.; Tainer, J. A.; Getzoff, E. D. Understanding GFP Chromophore Biosynthesis: Controlling Backbone Cyclization and Modifying Post-translational Chemistry. *Biochemistry***2005** *44* (6), 1960-1970
[7] Hu, Y.; Liang, C.; Tsai, J.; Yap, G.; Chang, Y.; Ong, T.; Zirconium Complexes Supported by Imidazolones: Synthesis, Characterization, and Application of Precatalysts for the Hydroamination of Aminoalkenes. *Organometallics* **2010** *29* (15), 3357-3361
[8] Ma, Y.; Sun, Q.; Zhang, H.; Peng, L.; Yu, J.; Smith, S. The Mechanism of Cyclization in Chromophore Maturation of Green Fluorescent Protein: A Theoretical Study *The Journal of Physical Chemistry***2010** *B 114* (29), 9698-9705
[9] Megley, C.; Dickson, L.; Maddalo, S.; Chandler, G.; Zimmer, M. Photophysics and Dihedral Freedom of the Chromophore in Yellow, Blue, and Green Fluorescent Protein *The Journal of Physical Chemistry* **2009** *B 113* (1), 302-308

interconversions within two ground and two excited states. The extended set of polar interactions around the flourophore can accomodate proton rearrangements. The most likely direct effects is associated with His148 interactions with the hydroxyl of Tyr66, Arg96 interactions with the imidazolidone, and Glu222 interactions with the hydroxyl of Ser65.[10] Mutations at Ser65 and Glu222 results in loss in the 400 nm absorption bands. Thus, the 400 nm band may arise from the abstraction of the Ser65 hydroxyl proton by Glu222.[11]

[10] Pouwels, L.; Zhang, L.; Chan, N.; Dorrestein, P.; Wachter, R. Kinetic Isotope Effect Studies on the de Novo Rate of Chromophore Formation in Fast- and Slow-Maturing GFP Variants *Biochemistry* **2008** *47* (38), 10111-10122

[11] Barondeau, D.; Kassmann, C.; Tainer, J.; Getzoff, E. The case of the missing ring: radical cleavage of a carbon-carbon bond and implications for GFP chromophore biosynthesis. J Am Chem Soc. **2007** Mar 21;129(11):3118-26.