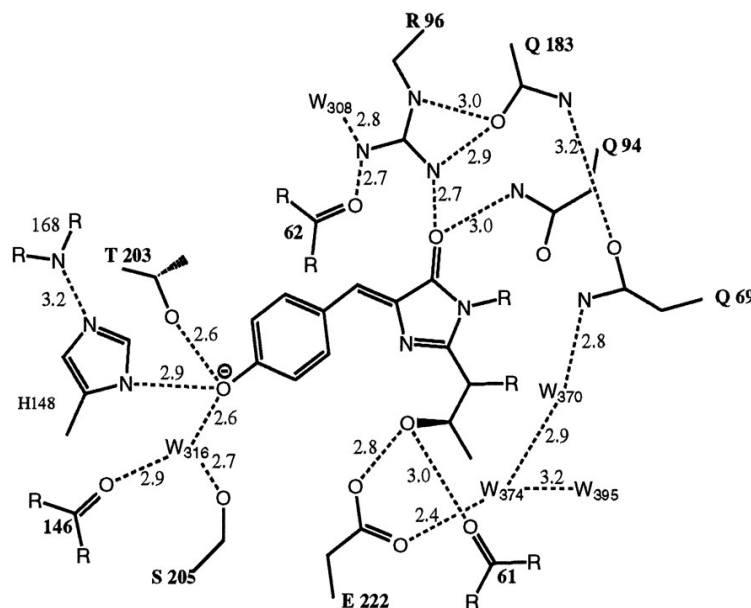


## The Photophysical Properties of Enhanced Green Fluorescent Proteins

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The affect of solvent composition on the fluorescence properties of enhanced green fluorescent protein (EGFP), a mutant (F64L/S65T) of the green fluorescent protein (GFP), was investigated using steady-state and time-resolved fluorescence methods. Specifically, the steady-state fluorescence quantum yields of EGFP were measured as a function of the methanol/buffer composition, ionic strength, and pH. The quantum yields were observed to decrease in a stepwise fashion when the percentage of methanol in the solution was increased. The fluorescence lifetime of EGFP was also measured for different methanol/buffer solutions using time-correlated single photon counting (TCSPC) and was found to remain constant.

These data, together with absorption, emission, and excitation spectra are interpreted in terms of the interactions between the chromophore and the surrounding protein structure and water. It is suggested that slight differences in conformation leads to differences of fluorescence efficiency in the excited state and explains the disparity between the width of the absorption or excitation spectra and the emission spectrum. It can also account for the step-wise change in the quantum yield of EGFP with the increase in methanol composition while the lifetimes remain constant under the same conditions.



**Figure 1**<sup>1</sup> Interactions of EGFP chromophore with surrounding protein structure and solvent waters (denoted by **W**).

<sup>1</sup> Brejc, K., Sixma, T.K., Kitts, P.A., Kain, S.R., Tsien, R.Y., Ormö, M., Remington, S.J. (1997) *Proc. Natl. Acad. Sci. USA* **94** 2306-2311.