

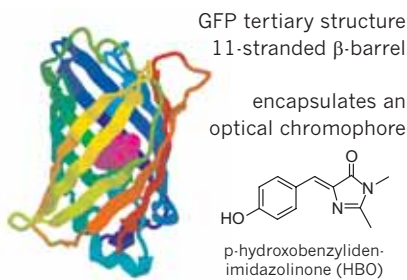
Primary processes in green fluorescent protein (wt) studied by femtosecond UV/VIS pump-probe spectroscopy

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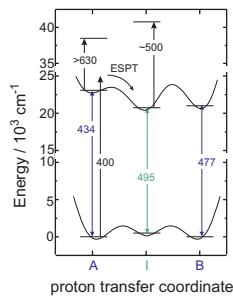
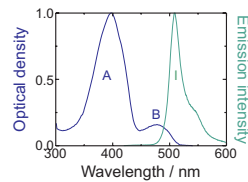
Introduction

The photochemical and photophysical primary events leading to bioluminescence have recently attracted considerable interest. In particular, elucidation of the underlying microscopic molecular mechanisms and their time scales will help establish fundamental principles leading to photon emission from protein environments. Such information can then be used to custom design optical bio-chromophores with optimally adapted spectral properties for a wealth of applications in biomedical imaging. Here, we report fs-time and frequency resolved pump-probe studies as well as femtosecond-to-nanosecond time-resolved emission spectroscopy of the green fluorescent protein (wt-GFP) of *Aequoria victoria* following its photoexcitation with 400 nm light.



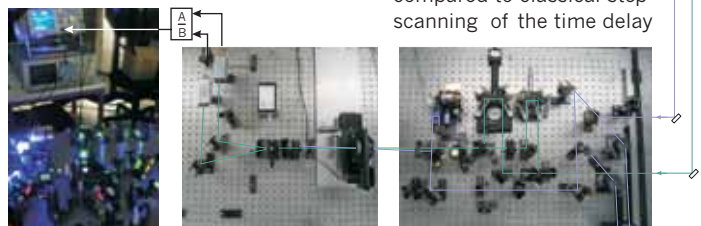
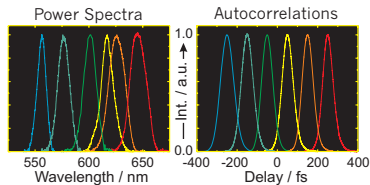
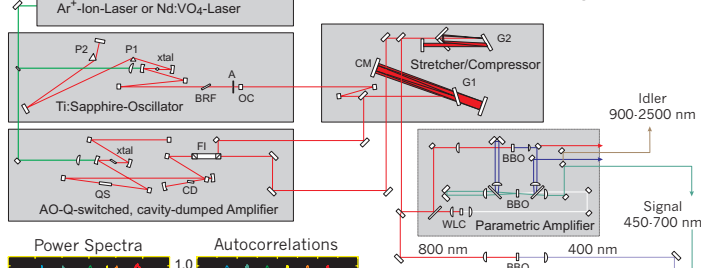
Tertiary structure provides extended H-bond network for the chromophore and isolates it from the aqueous solvent.

Primary processes of GFP can be discussed with extended Förster-cycle for excited state proton transfer (ESPT) incl. protonated (A), deprotonated (B), and intermediate (I) form



Experiment

Laser system: Generation of <70-fs light pulses tunable from the near IR to the near UV. Ti:Sapphire based CPA-laser system incl. a co-linearly phase-matched OPA running @ 250 kHz



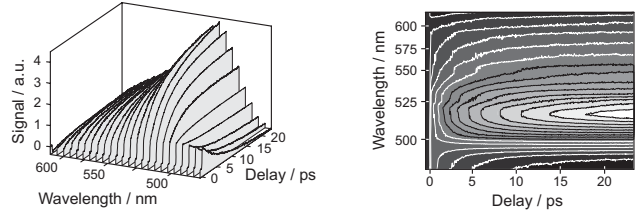
Frequency-resolved pump-probe:
Rapid-scan data acquisition provides superior S/N-ratio compared to classical step-scanning of the time delay

Literature

- [1] K. Winkler, J. Lindner, T.M. Jovin, P. Vöhringer, PCCP 4, 1072 (2002).
[2] K. Winkler, M. Seidel, P. Vöhringer, Ultrafast Phenomena 13, 611 (2003)
[3] K. Winkler, J. Lindner, M. Seidel, P. Vöhringer, Femtochemistry 6, 433 (2004)

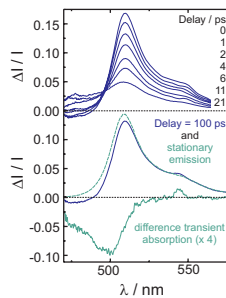
Results & Discussion

Frequency-resolved pump-probe spectroscopy:



- 1.) stimulated emission of the deprotonated form I* grows in on a time scale of ~20 ps.
- 2.) Kinetic are highly multi-exponential
- 3.) finite component of stimulated emission is visible already at the earliest times (~50 fs), "prompt emission"
- 4.) weak transient absorption in the red spectral region decays on a time scale identical to that for build-up of stimulated emission

Femtosecond pump-supercontinuum-probe spectroscopy:



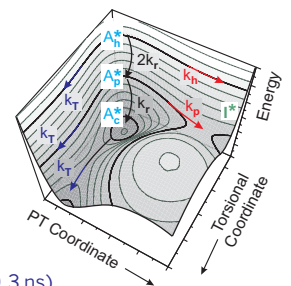
- 1.) prompt emission originates from protonated (A*) and deprotonated (I*) form. Latter due to cross-well excitation!
- 2.) low-frequency edge of transient spectrum is identical to stationary emission of GFP
- 3.) difference between the two must correspond to transient absorption of I*
- 4.) isosbestic point @ 496 nm separates A* from I*

Nonexponential kinetics AND isosbestic point at the same time? Apparent contradiction!

Refined dynamic model accounting for non-exponential kinetics

vibrational energy transfer within the reactant (A*) well and ESPT from A* occur on similar time scales. In addition, torsional motion leads to non-radiative decay via internal conversion.

for incomplete IVR and VET use master equation



$$\frac{d[A(E)]}{dt} = -k_{PT}(E)[A(E)] \quad \text{proton transfer}$$

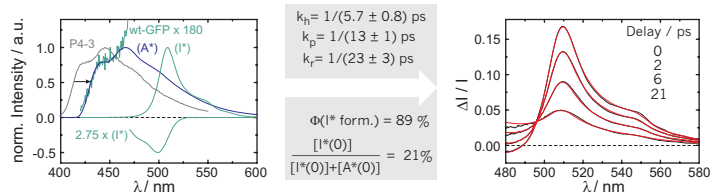
$$-k_T(E)[A(E)] \quad \text{torsion } (k_T = 1/0.3 \text{ ns})$$

$$- \sum k_f(E, E') [A(E)] \quad \text{activation}$$

$$+ \sum k_r(E', E) [A(E')] \quad \text{deactivation}$$

Non-exponentiality built in!
Channel switching built-in!

Analytical solutions are readily available for this simple dynamical model. Three fitting parameters only to fit the entire spectro-temporal response of GFP in the UV/VIS spectral range from femtoseconds to nanoseconds. Required input: Emission spectrum of A* (estimated from mutant P4-3)



- 1.) Vibrational relaxation is slower than proton-transfer.
- 2.) Consequence of rigid tertiary structure (chromophore seclusion).
- 3.) If it were faster, only torsion and no fluorescence!

