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 aequorea victoria following its photoexcitation with 400 nm light.

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 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*

Refined dynamic model accounting for non-exponential kinetics vibrational energy transfer within the reagent (A*) well and ESPT from A* occur on similar time scales. In addition, torsional motion leads to non-radiative decay via internal conversion.

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

Results & Discussion

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

Vibrational relaxation is slower than proton-transfer.
Consequence of rigid tertiary structure (chromophore seclusion).

Literature