Bakteriorodopsinas: modelinis fotoaktyvus baltymas ir naujausių spektroskopijų “poligonas”

Suvestinė:

- Gana rezultatyvus baltymas:

  – Nature – 23 straipsniai (1990 – 2005)
  – Science – 38 straipsniai (1990 – 2005)
  – PNAS -133 straipsniai (1990 – 2005)

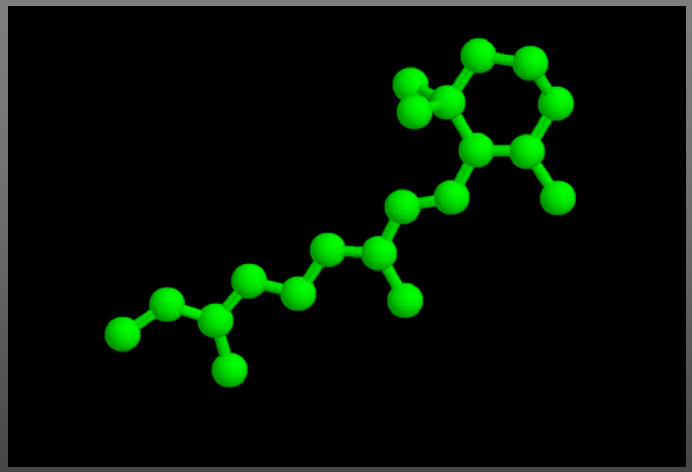
  (Šaltinis: Web of science)

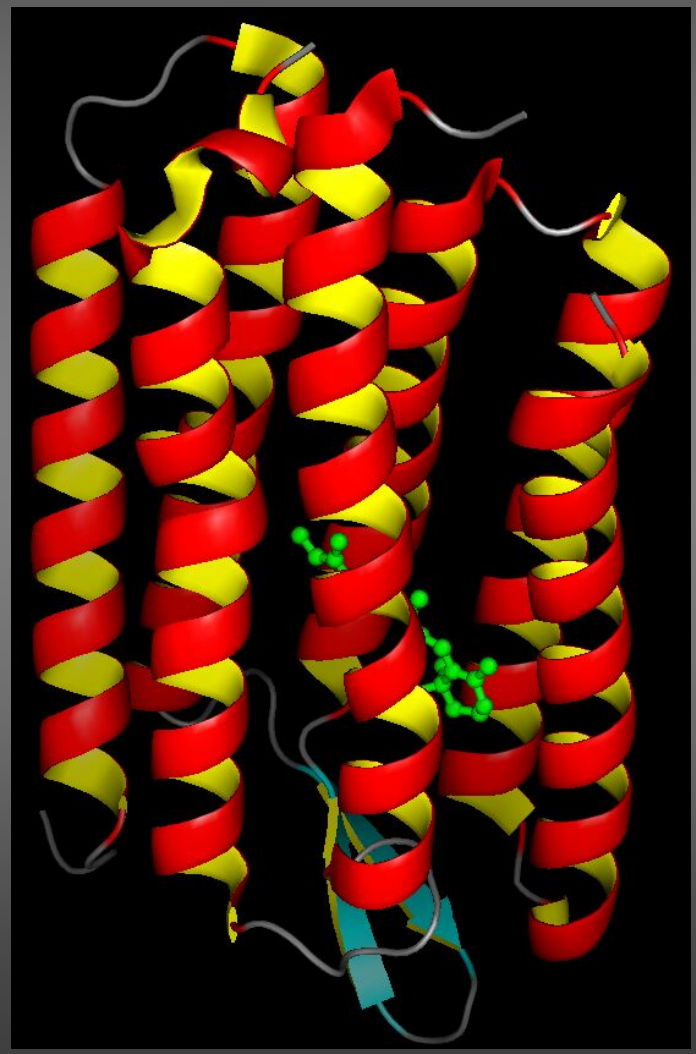
Funkcija:

- Šviesa ‘varomas’ protonų siurblys: pumpuoja protonus per membraną *prieš* elektrinio lauko kryptį.
- Randamas *Halobacterium salinarium* bakterijų purpurinėse membranose, sudaro iki 90% membranos masės

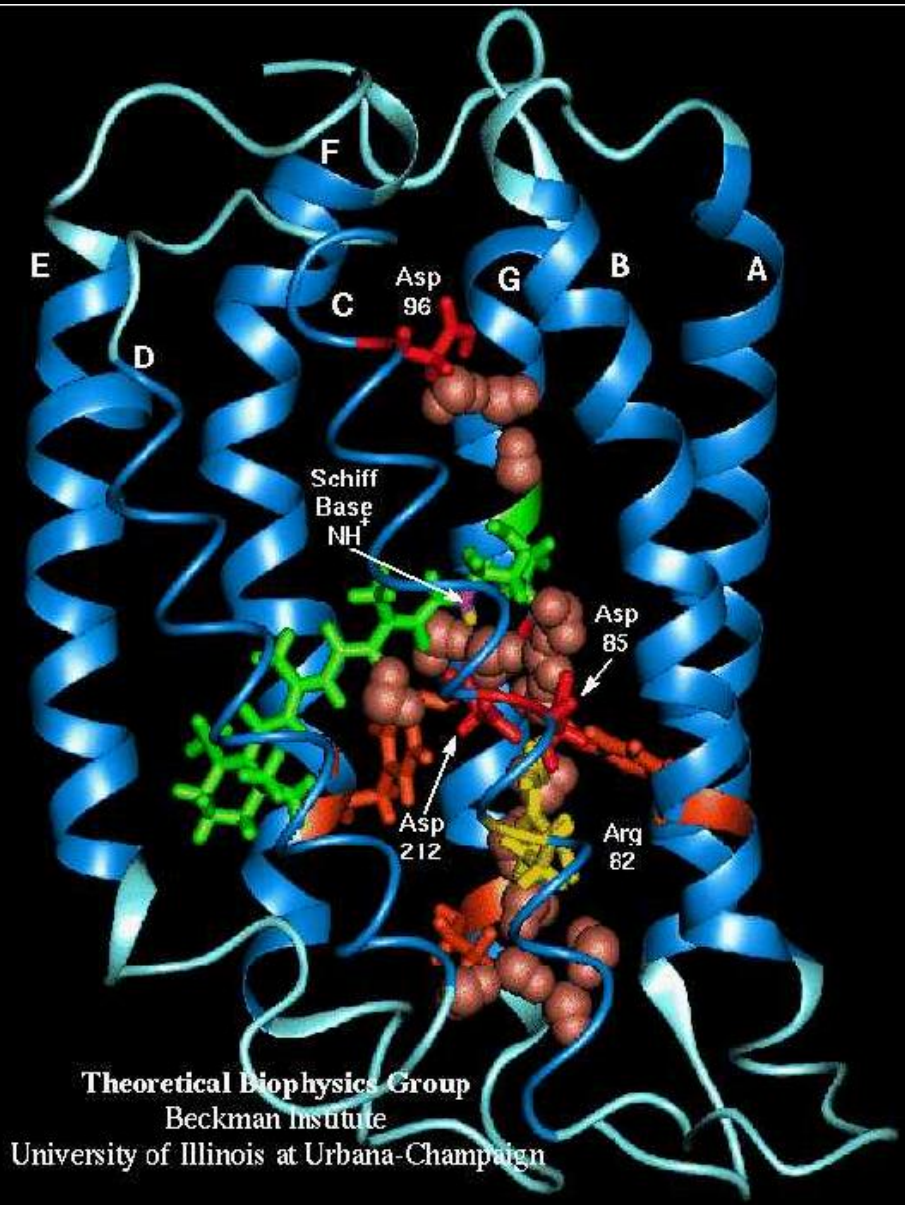
Struktūra: membraninis baltymas

- Membraninis baltymas (tik 30 struktūrų iš viso žinoma), struktūros skyra 1.65 Å.
- 7 alfa-spiralės, kurių viduje pasislėpes chromoforas retinalis.





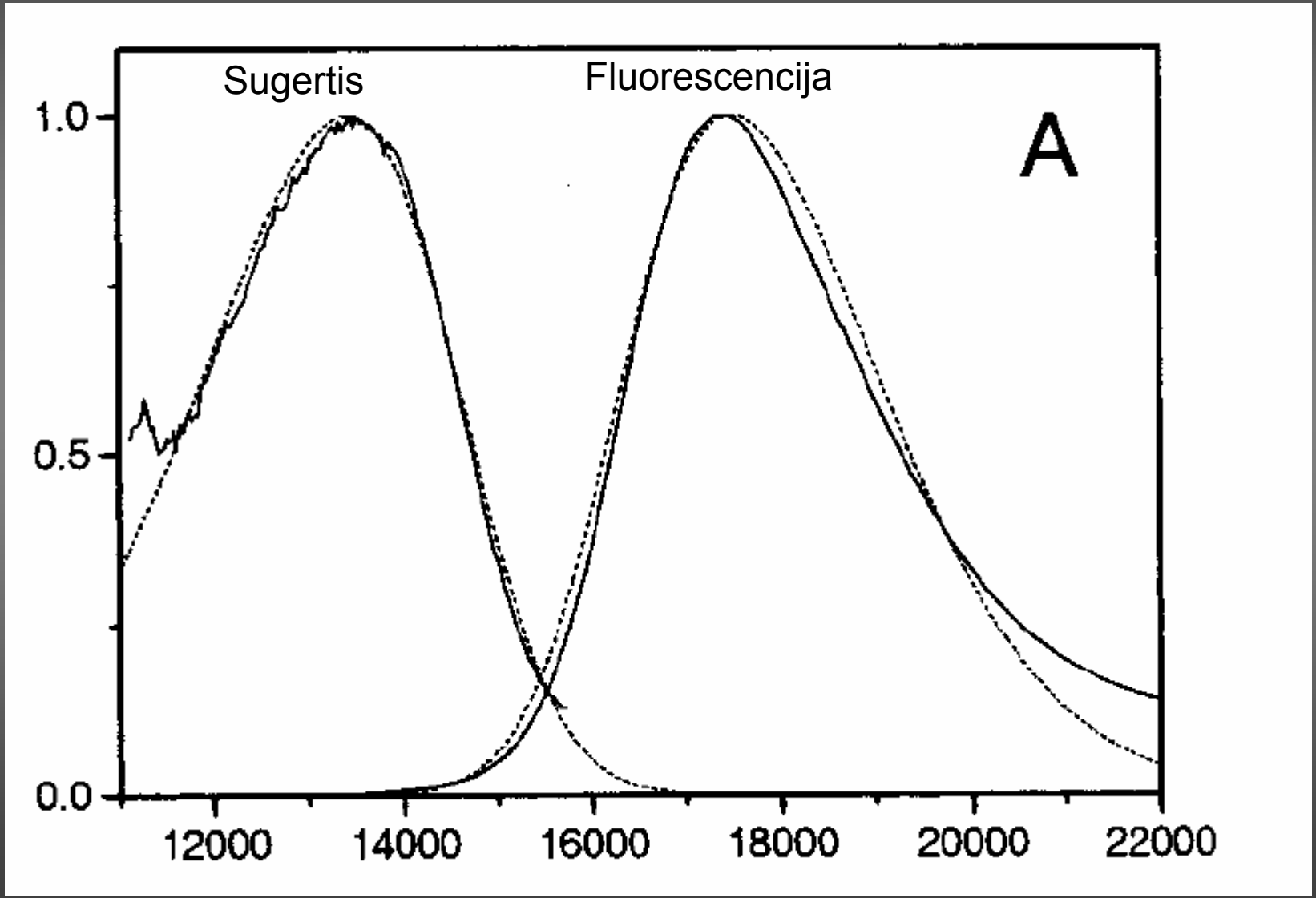
Mechanizmas:



Privalumai:

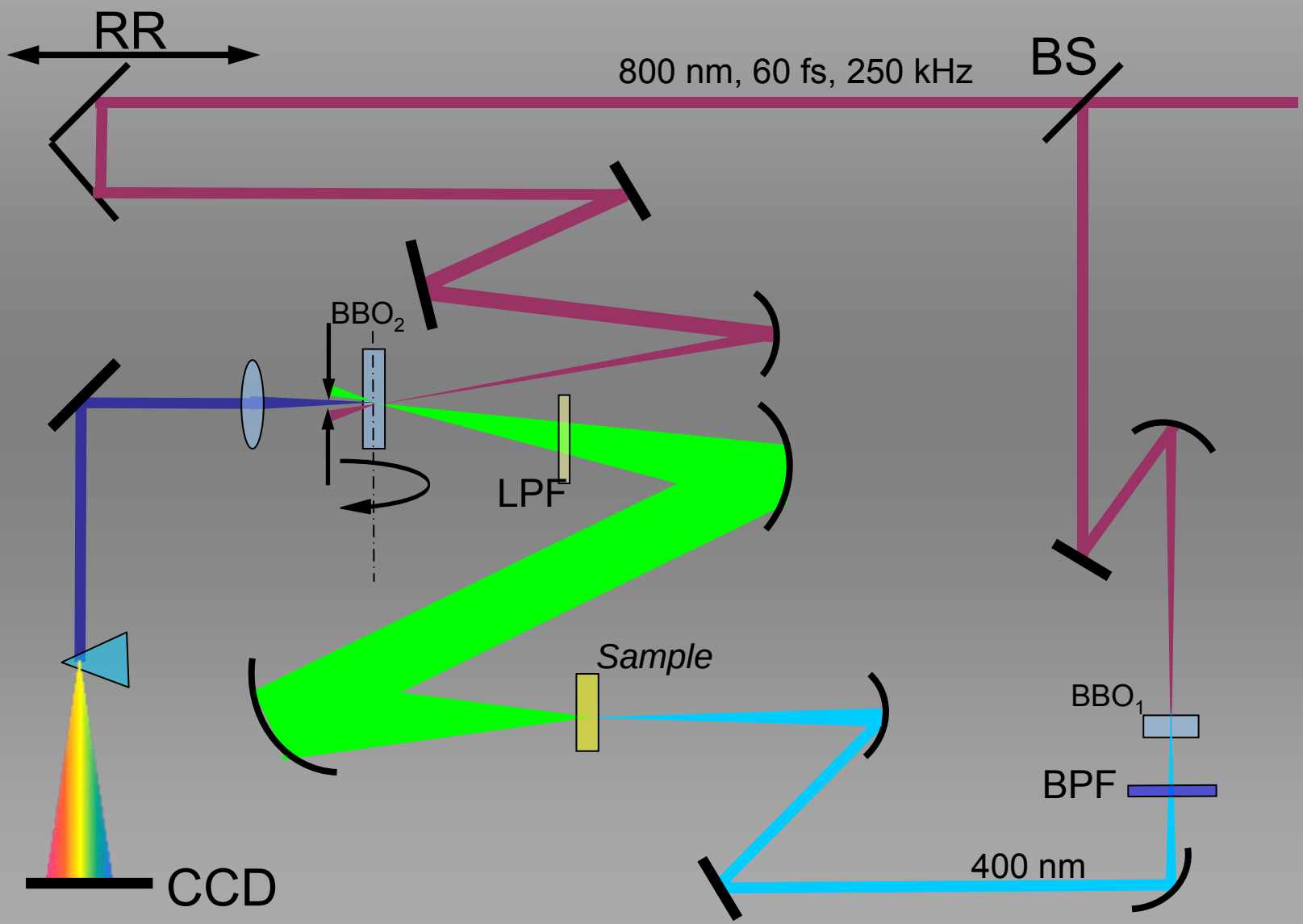
- Stabilus chemiškai ir foto-
- Žinoma struktūra, lengva kristalizuoti
- Bakterijos jo turi daug
- Fotoaktyvus, greitas ir dėl to įdomus.

Sugerties ir fluorescencijos spektrai



Kaip suspėti su tokiais greičiais?

Dispersed Fluorescence Upconversion Setup



Pastebėjimas:

- ***The decays can be well fitted as a sum of three exponential decay components with time constants in the range of 90 fs-240 fs, 0.6 ps-0.9 ps, and 9.0–13.0 ps.***

- **Fluorescencija užgęsta žaibiškai!**

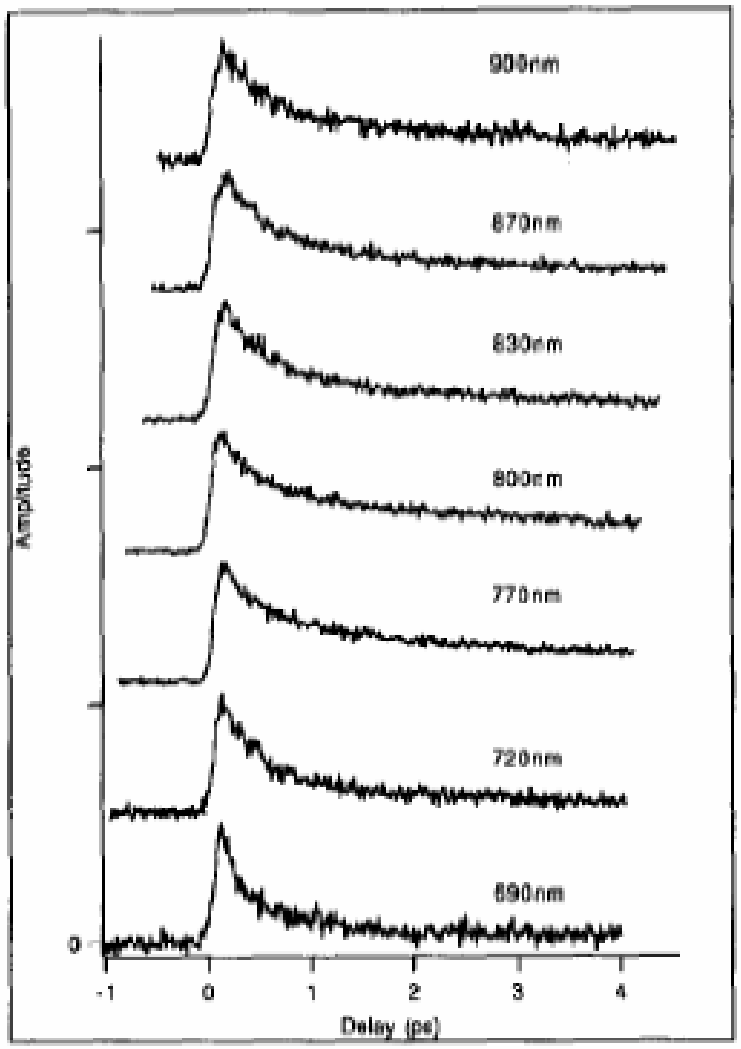
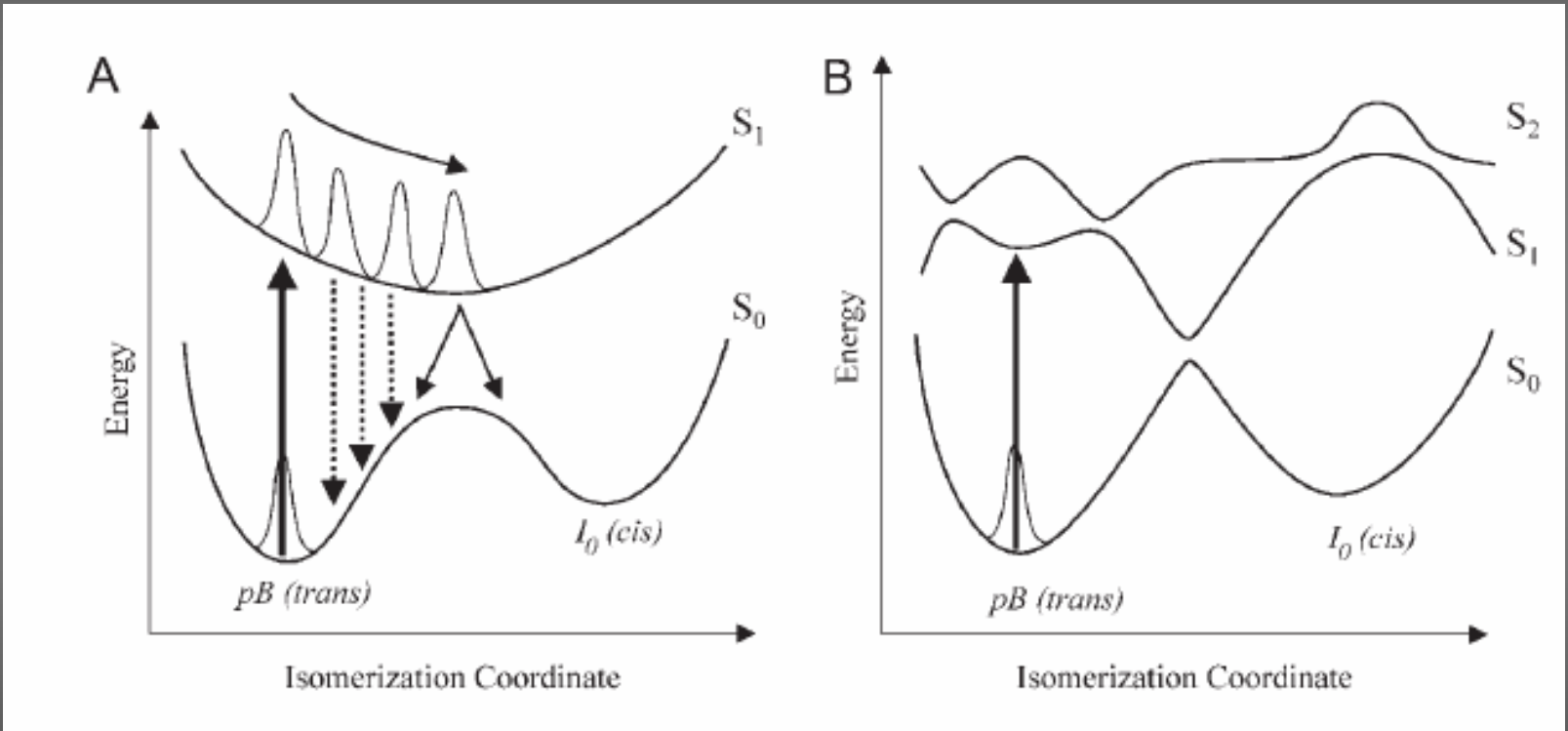


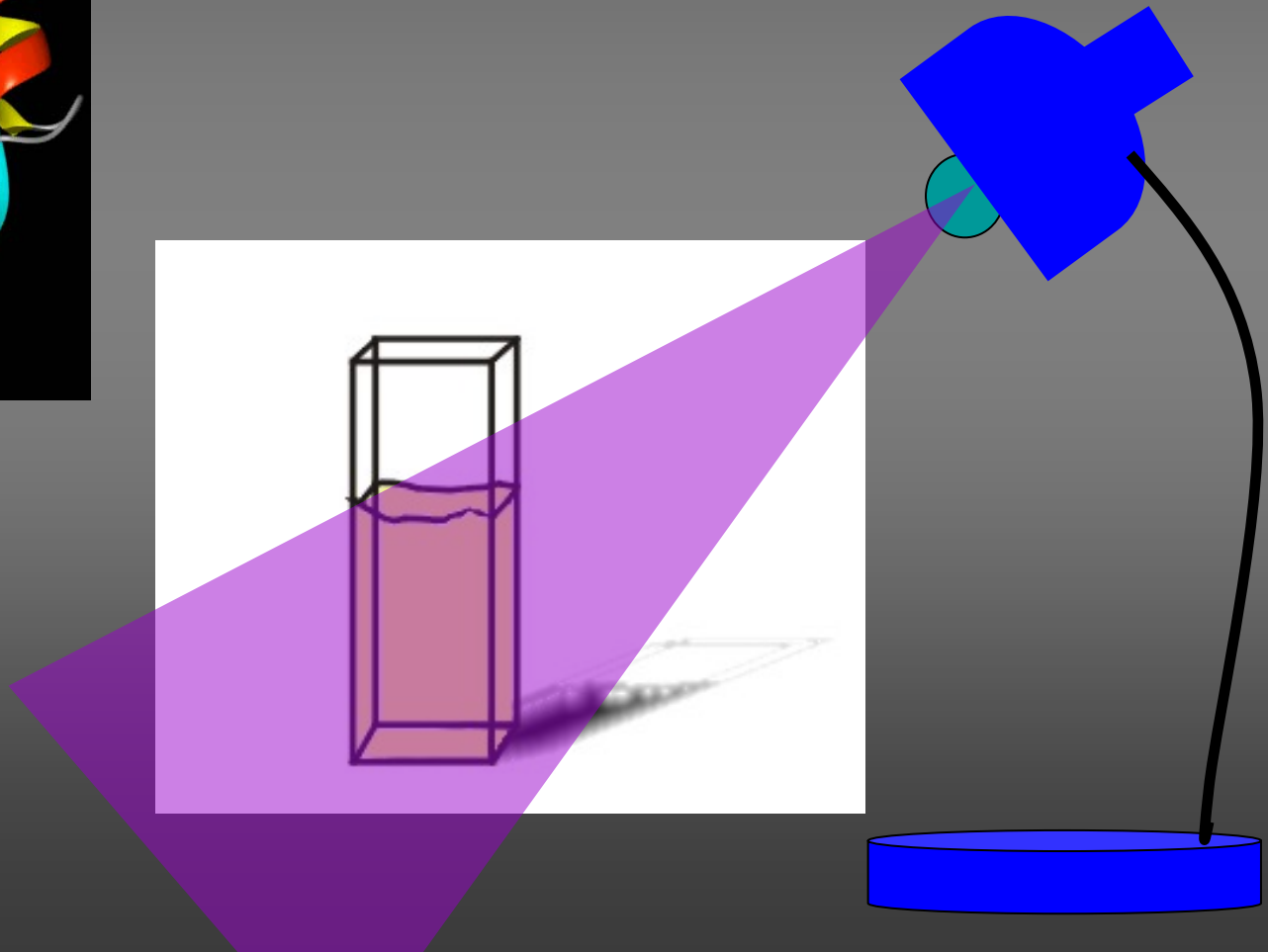
Fig. 3. Fluorescence decays at different detection wavelengths measured over 5 ps scan length.

Du et al. Biophysical chemistry, 1993

Pastebėjimas:



Pašvietus į šviesai jautrius baltymus, jie ima vykdyti savo funkcijas, ir keičiasi jų spalva



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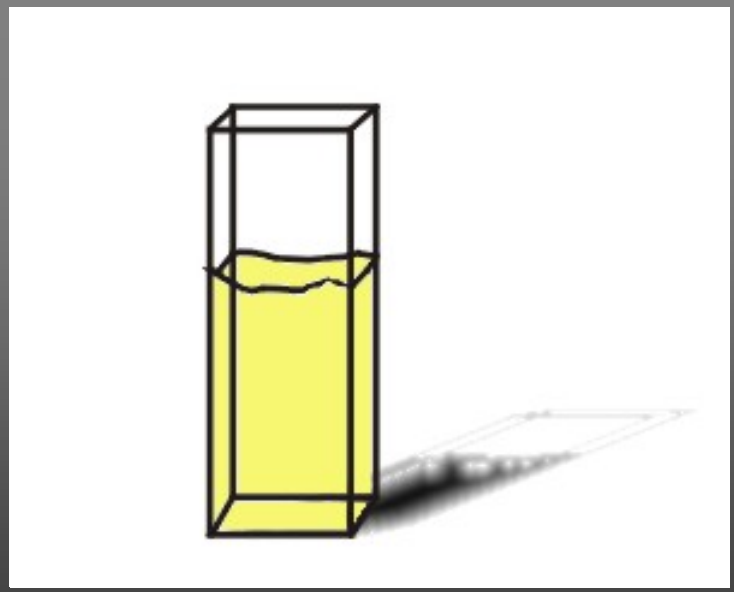
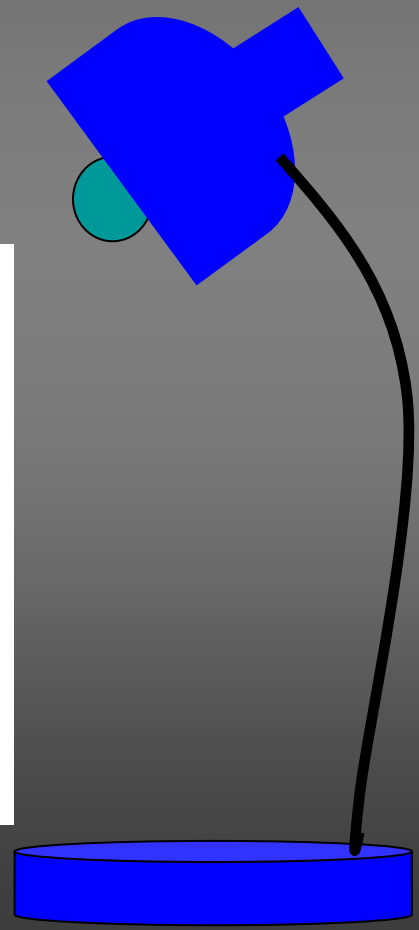


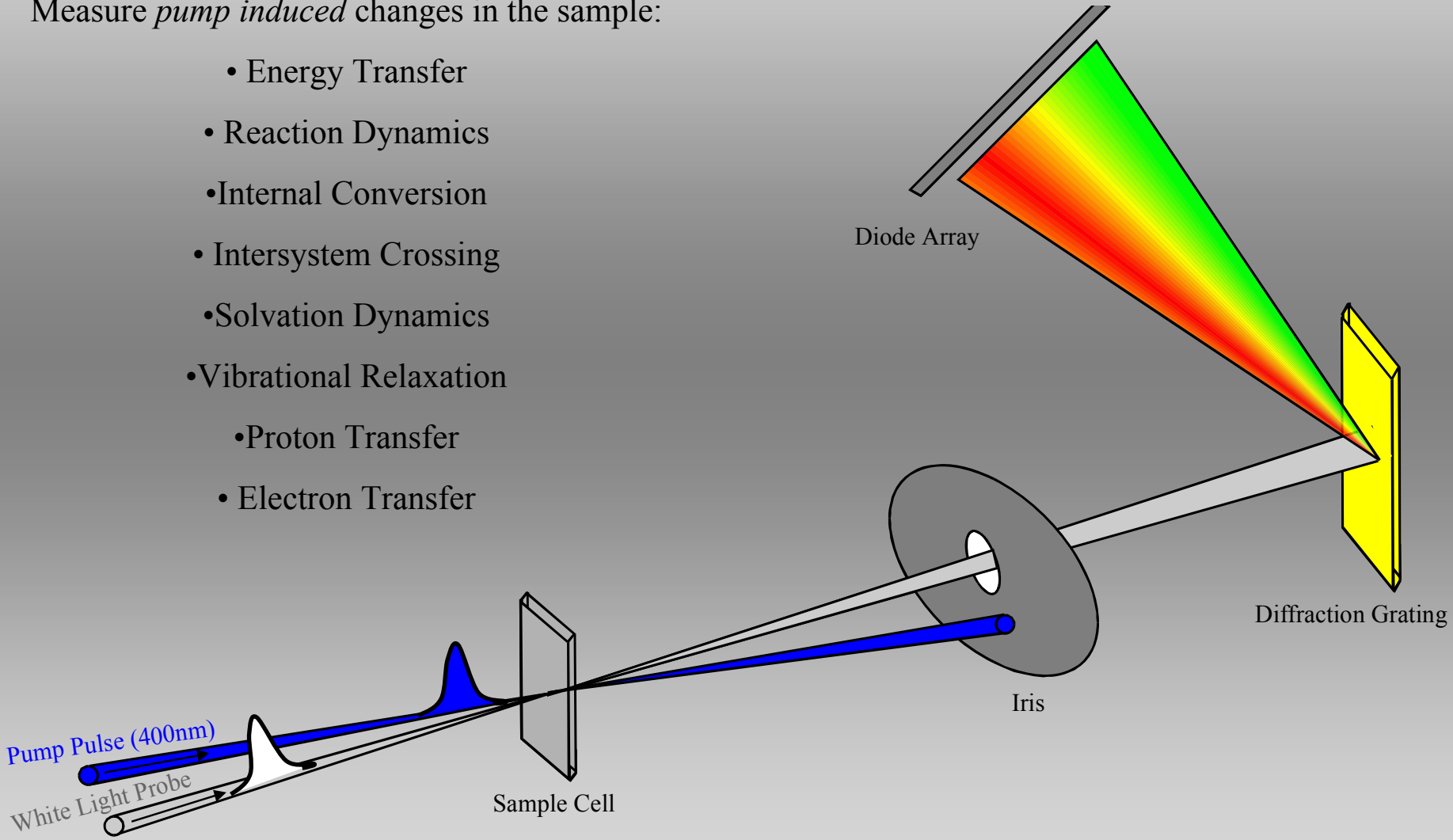


1 s

Dispersed Pump-Probe Experimental Setup

Measure *pump induced* changes in the sample:

- Energy Transfer
- Reaction Dynamics
- Internal Conversion
- Intersystem Crossing
- Solvation Dynamics
- Vibrational Relaxation
- Proton Transfer
- Electron Transfer



Žadinimas – zondavimas

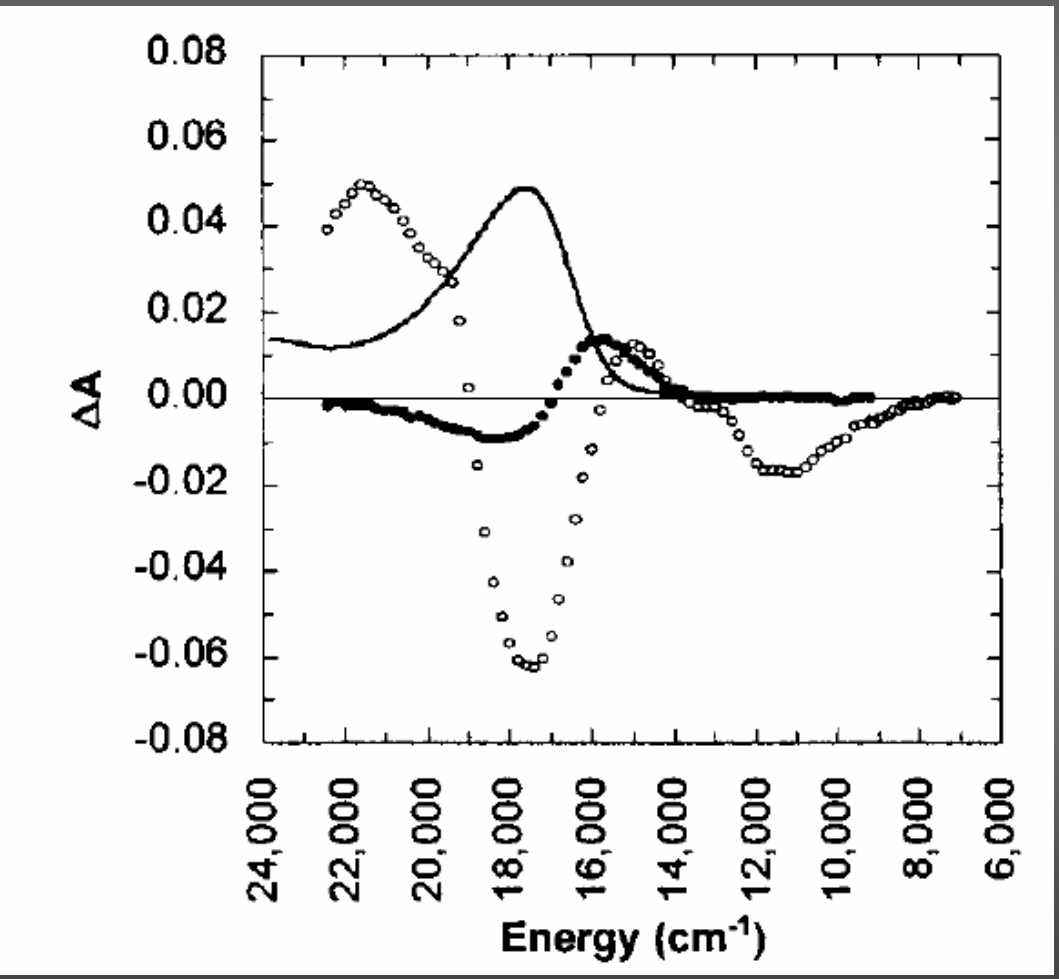


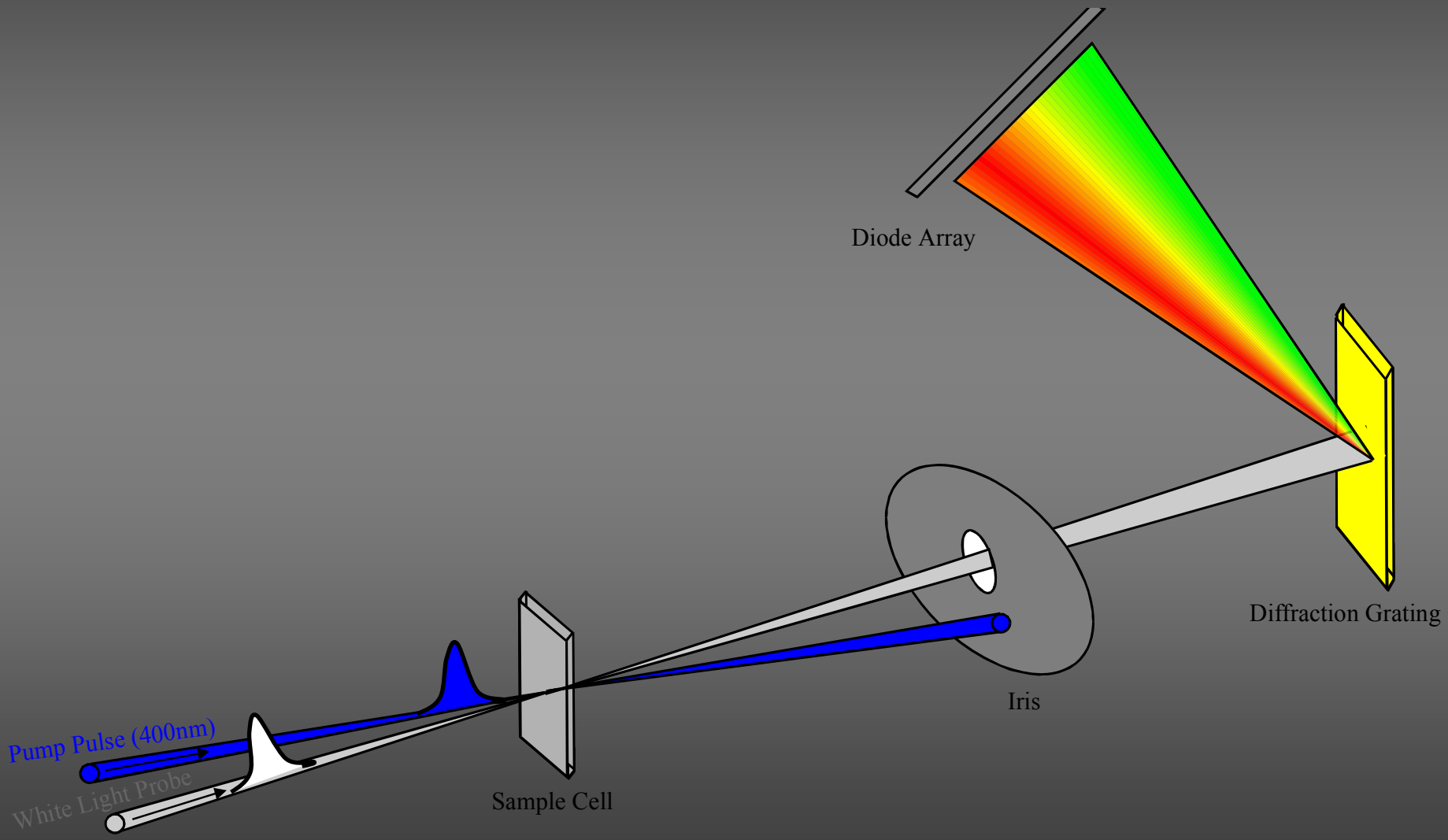
Fig. 3. Time-resolved absorbance spectra of bR recorded (*50*) at 0.316 ps (open circles) and 31.6 ps (filled circles). In the 0.316-ps spectrum, the negative-going feature centered at 17,600 cm^{-1} corresponds to photon-induced depletion of the ground-state absorbance; the negative-going feature peaked near 11,000 cm^{-1} corresponds to stimulated emission. Positive-going features correspond to excited-state or photoproduct absorbances. The 31.6-ps spectrum consists only of depleted ground-state and photoproduct absorbances. For comparison, a scaled equilibrium absorbance spectrum is shown (thick line). [Adapted from figure 2 of (*35*); copyright 1996, U.S. National Academy of Sciences]

Gai et al. Science 1998

Nėra detalios informacijos apie struktūrinius pokyčius ☹

....Sprendimas: go infrared! ☺

Padarius zonduojantį spindulį IR, matomi vibracinio spektro pokyčiai!



Vibraciniai spektrai: izomerizacija per < 1ps!

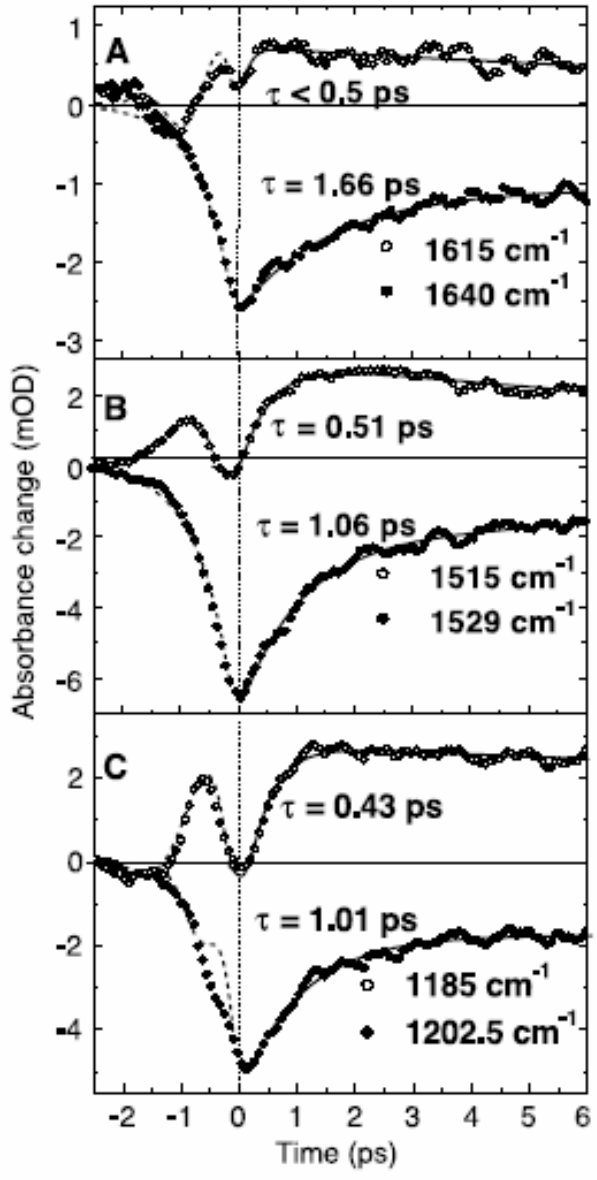
Pastebėta, kad *cis* konformacijos spektras (žinomas iš FTIR matavimų) susiformuoja per <1 ps.

Pirmą kartą patikimai įrodyta, kad fluorescencijos gesimas ir izomerizacija yra susiję!

C—NH

C=C

C—C



Herbst et al. Science, 2002

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Herbst et al. Science, 2002

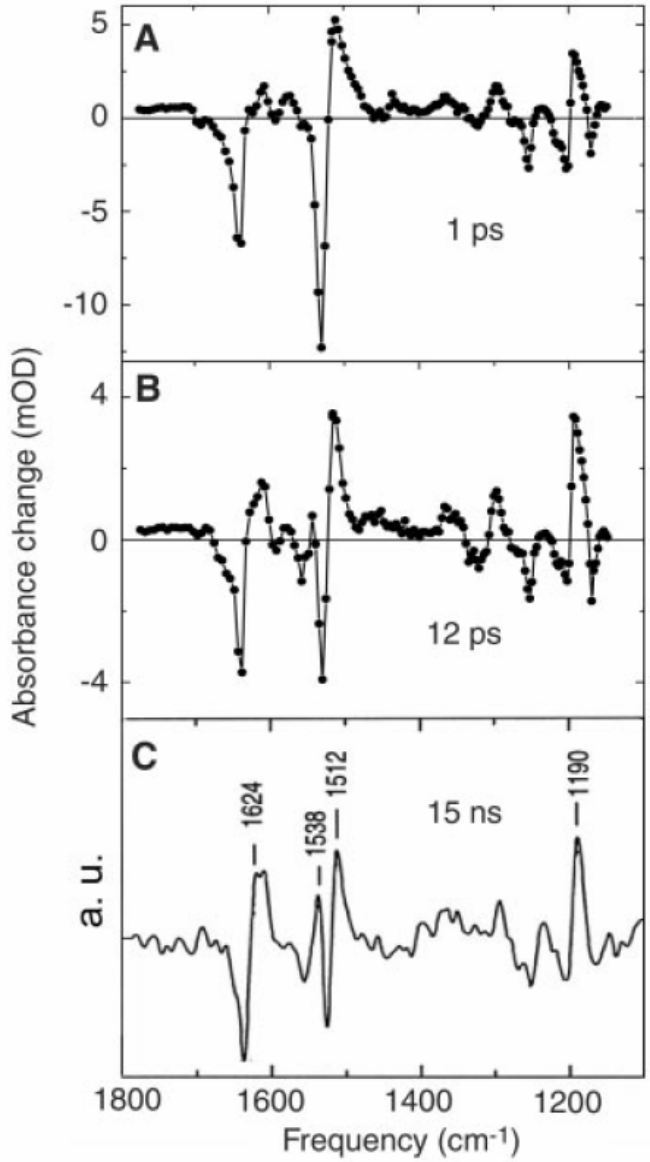


Fig. 2. IR difference spectra of bR with conditions as in Fig. 1 at (**A**) 1 ps and (**B**) 12 ps. (**C**) For comparison, a FTIR bR_{570}-K difference spectrum at 15 ns after photoexcitation at room temperature [from (*15*)].

O ką tuo metu veikia baltymas?...

....Sprendimas: go UV! ☺

O ką tuo metu veikia baltymas?...

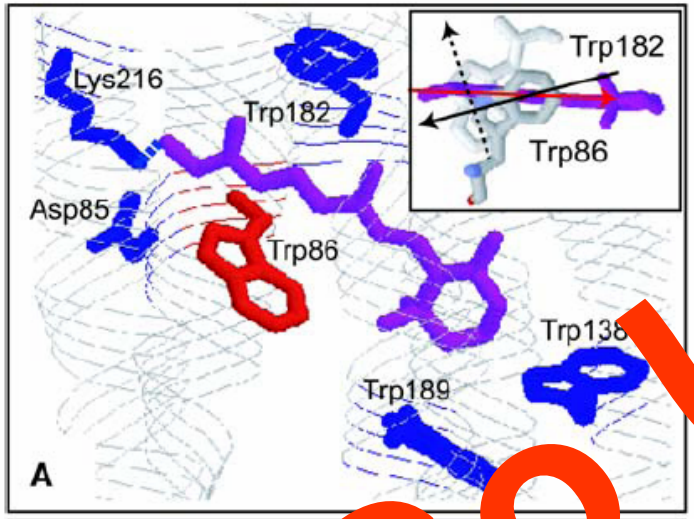
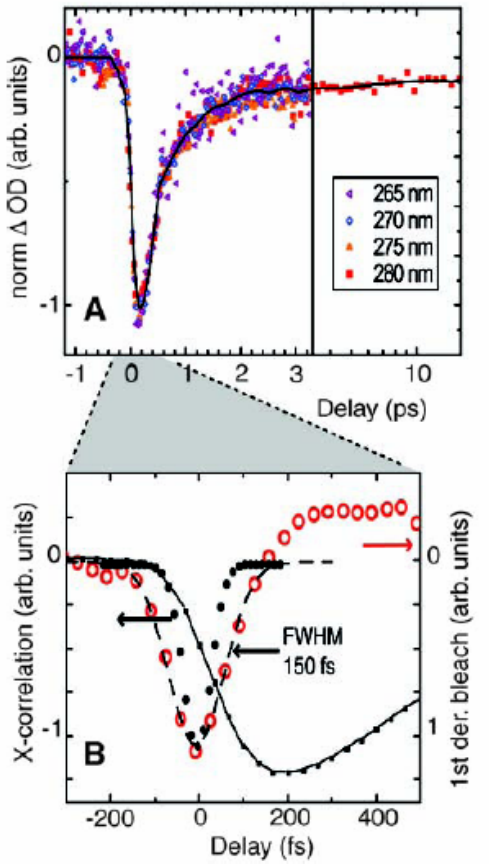


Fig. 1. Structure and optical properties of bR. (**A**) The retinal binding pocket of bR (from PDB entry 1C3W) (*19*). Retinal (purple) is covalently bound to Lys^{216} through a Schiff base linkage. The four nearest Trp residues are shown. Inset: Relative orientations of the difference dipole moments for retinal (red arrow), Trp^{86} (solid black arrow), and Trp^{182} (dashed black arrow). (**B**) Absorption spectrum of bR in the purple membrane. The band with maximum at 568 nm is due to the S_0-S_1 transition of the protonated Schiff base form of retinal, whereas the near-UV band at 280 nm is dominated by the S_0-L_a and S_0-L_b transitions of eight Trps. The vertical and horizontal arrows indicate the excitation wavelength and the range of probe wavelengths, respectively. Inset: Orientations of the transition (solid arrows) and difference (dashed arrows) dipole moments of tryptophan for S_0-L_a (red) and S_0-L_b (blue) (*21*). Arrows point to the positive end of the difference dipole moment.

GO UV!

Schenkl et al. Science 2005

O ką tuo metu veikia baltymas?...



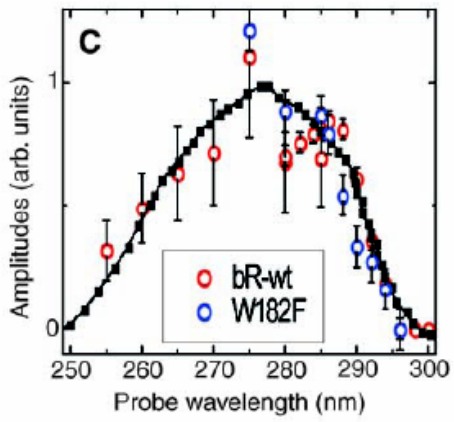


Fig. 2. Ultrafast response of bR in the near-UV. (A) Transient bleach signals at 265 to 280 nm after excitation with 25 fs pulses at 560 nm at 1 kHz repetition rate. UV probe pulses (75 to 85 fs) were obtained by second-harmonic generation of the output of a tunable noncollinear optical parametric amplifier (*23*). The transients are normalized in amplitude, which shows that they are independent of the probe wavelength. Each data point represents the average of 10,000 laser shots, allowing for a differential optical density (ΔOD) sensitivity of 10^{-4}. The time axis of the left panel is linear; that of the right panel is logarithmic. (B) The early time portion of the bleach transient in (A) (black squares) and its temporal derivative (red circles), showing a full width at half maximum (FWHM) of 150 fs. The latter is clearly larger than the experimental time resolution of 85 fs (black dots) determined by the pump-probe cross-correlation (*23*). (C) Maximum amplitude of the bleach transients of wild-type bR and of the W182F mutant as a function of probe wavelength. Experimental data (circles) are compared with the L_a absorption component of Trp in propylene glycol at –50°C (*25*) (black squares). The L_a absorption was shifted by 5 nm to the blue to account for the apolar character of the binding pocket. The data have been normalized to the Trp L_a spectrum at 285 nm.

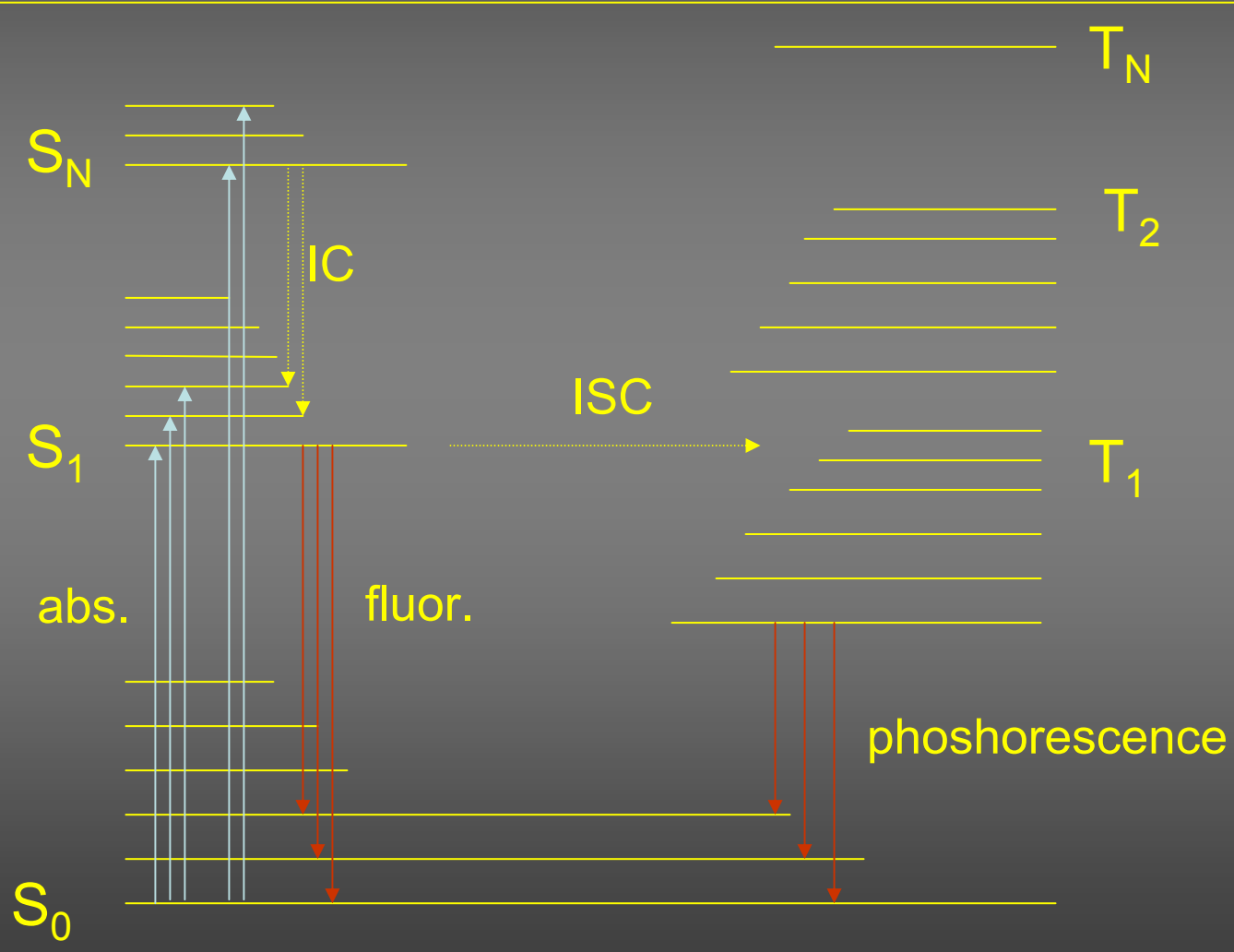
Triptofanas – elektrinio lauko baltyme ‘zondas’. Parodyta, kad vykstant izomerizacijai keičiasi dipolinio momento pasiskirstymas aplink baltymą – gali būti, kad tai yra izomerizacijos varomoji jėga...

Schenkl et al. Science 2005

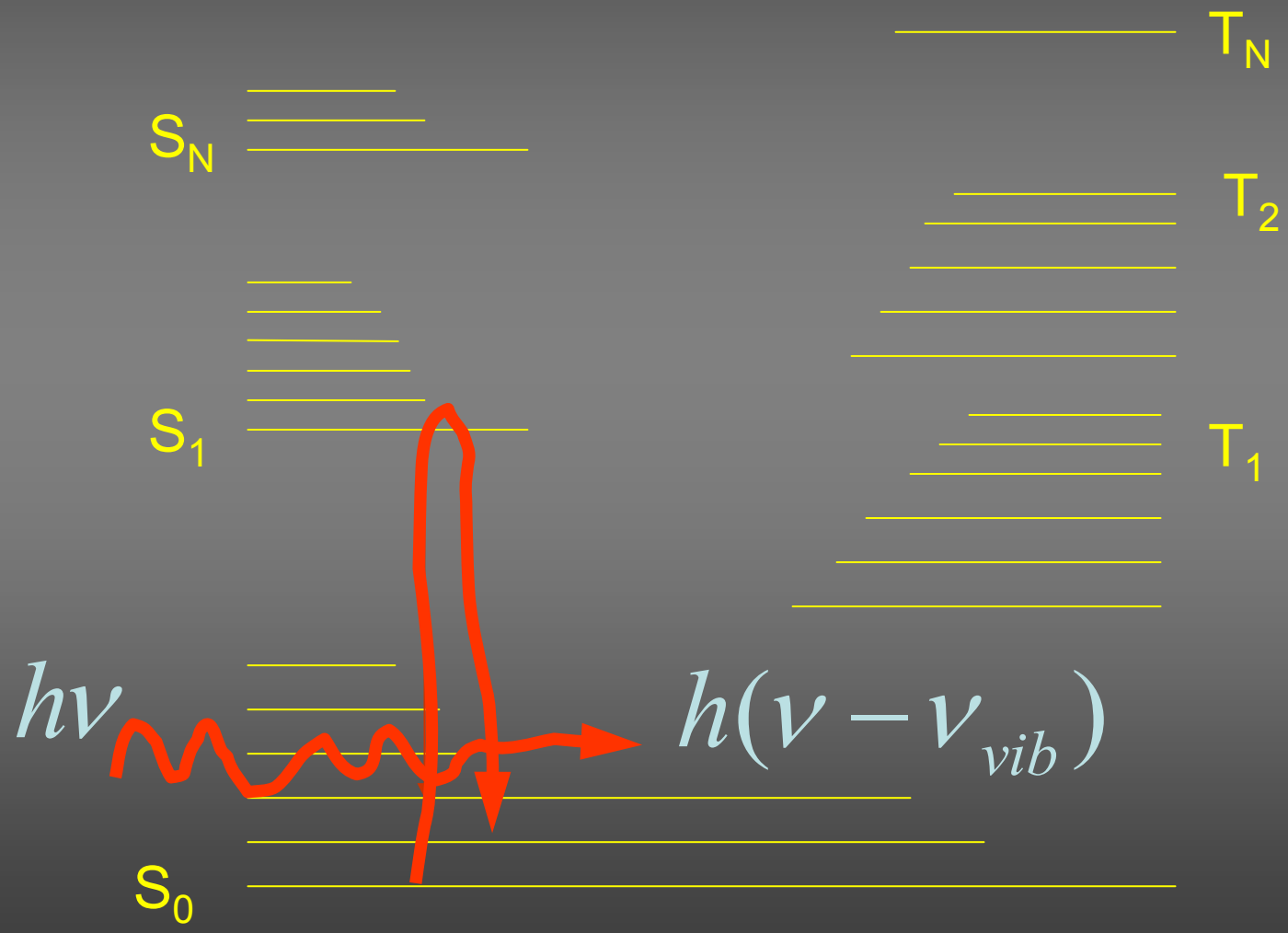
Vibracinė spektroskopija be IR bėdų....

Daugiaimpulsinė spektroskopija!!!!!!!!!

Molekulių energijos lygmenys: Jablonskio diagrama



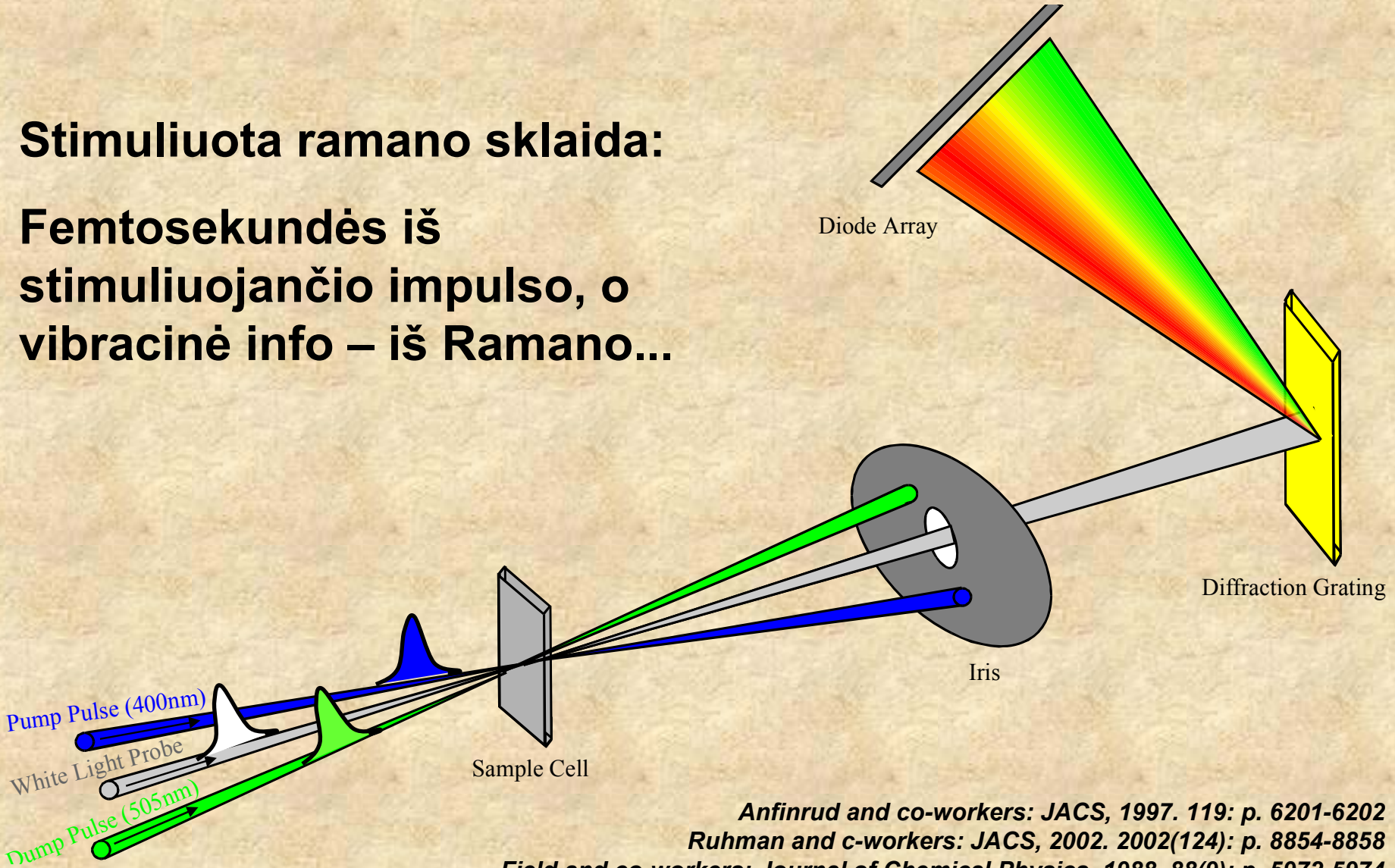
Molekulių energijos lygmenys: Jablonskio diagrama



Femtosekundinė stimuliuota Ramano sklaida:

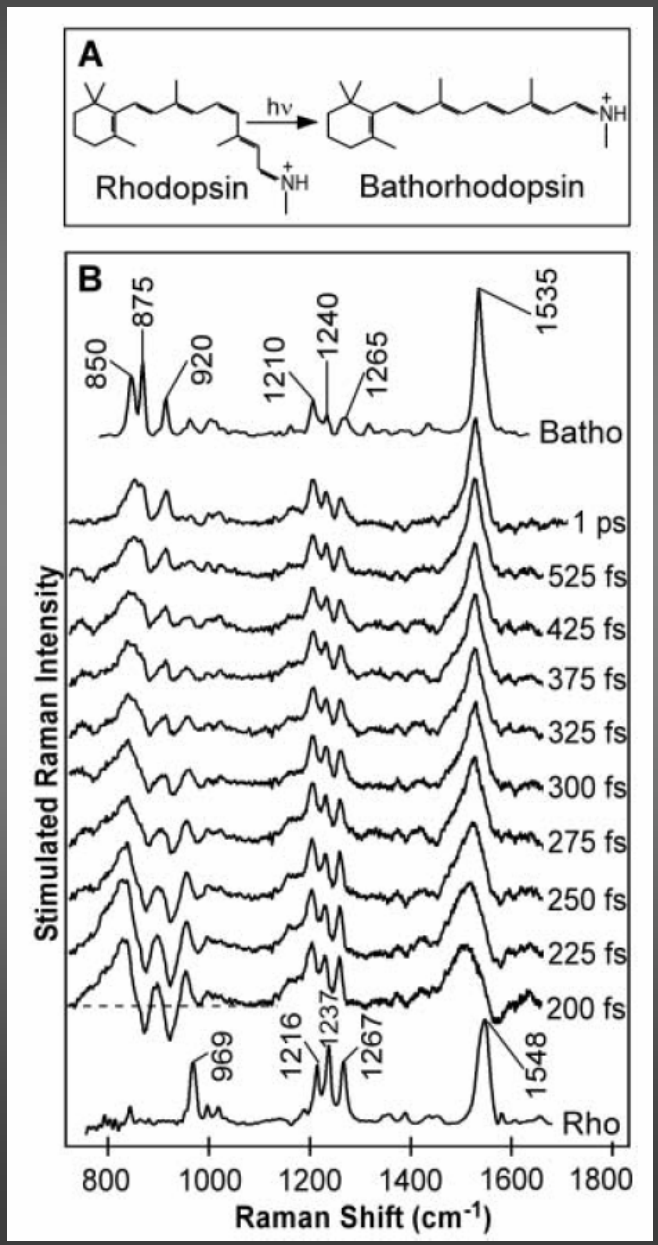
Stimuliuota ramano sklaida:

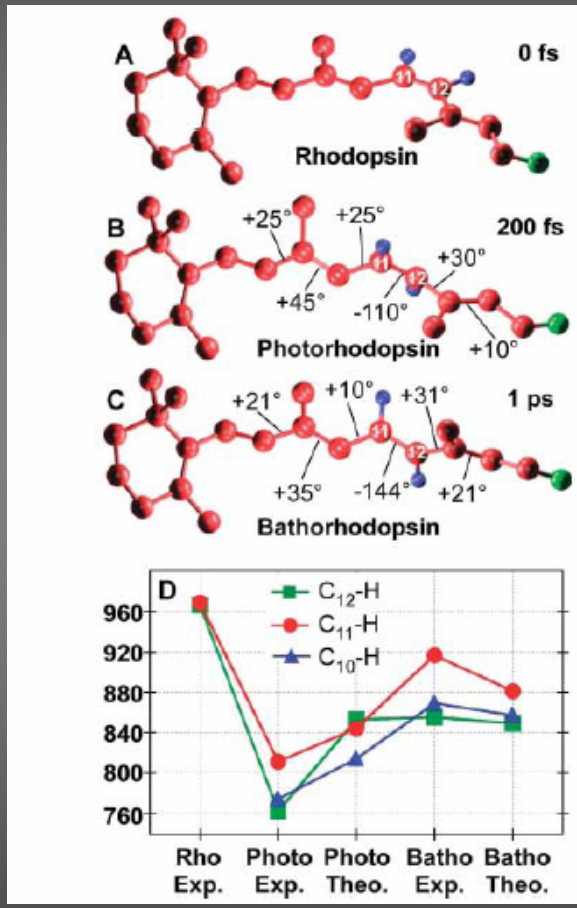
Femtosekundės iš stimuliuojančio impulso, o vibracinė info – iš Ramano...

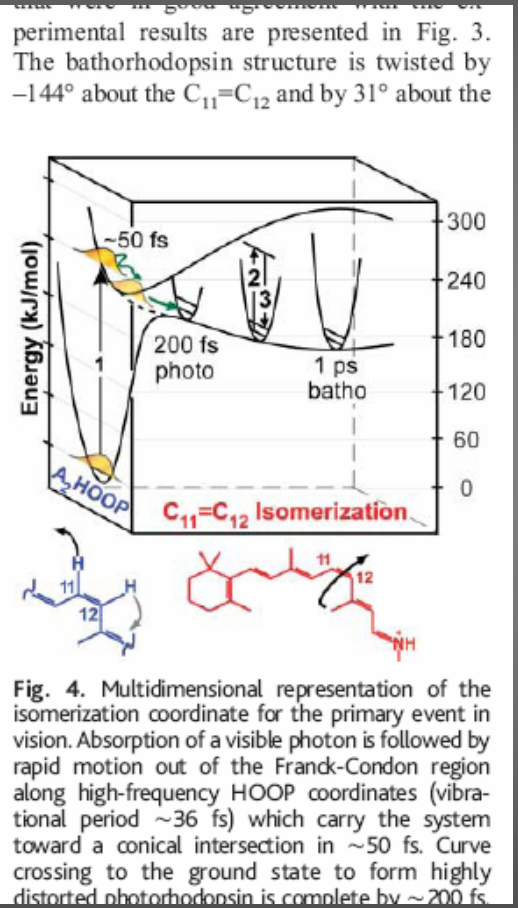


Anfinrud and co-workers: JACS, 1997. 119: p. 6201-6202
Ruhman and c-workers: JACS, 2002. 2002(124): p. 8854-8858
Field and co-workers: Journal of Chemical Physics, 1988. 88(9): p. 5972-5974

Rodopsinas: detalūs izomerizacijos spektrai







Kukura et al. Science 2005

Ir aš ne be nuodėmės...

Biophysical Journal Volume 80 April 2001 1631–1640

Electrical-to-Mechanical Coupling in Purple Membranes: Membrane as Electrostrictive Medium

Povilas Ki[illegible]tis,*† Mikas Vengris,† and L[illegible]onas Valkunas†
*Physics Faculty, Vilnius University, 2054 Vilnius and †Institute of Physics, 2600 Vilnius, Lithuania

ABSTRACT In this paper, we present acousto-electrical measurements performed on dry films of purple membranes (PM) of *Halobacterium salinarium*. The purpose of these measurements is to determine the relation between mechanical and electrical phenomena in bacteriorhodopsin and to define the role of the protein in the proton transfer process. Electrical-to-mechanical coupling in PMs manifests itself as direct and inverse piezoelectric effects. Measurements performed on the samples with different degrees of PM orientation and at various values of the externally applied cross-membrane electric field indicate that piezoelectric phenomena in PMs arise from the electric asymmetry of the membranes, i.e., they originate from electrostriction. Experiments with samples made of oriented PMs allow estimation of the value of the intrinsic cross-membrane electric field, which is $\approx 10^8$ V/m. A hypothetical model of PM is presented where the electrical-to-mechanical coupling is suggested to be the main driving force for the proton translocation against the Coulomb forces acting in the membrane.