Ultrafast transient absorption and fluorescence spectroscopy

LAMELIS 2017


School of Chemistry
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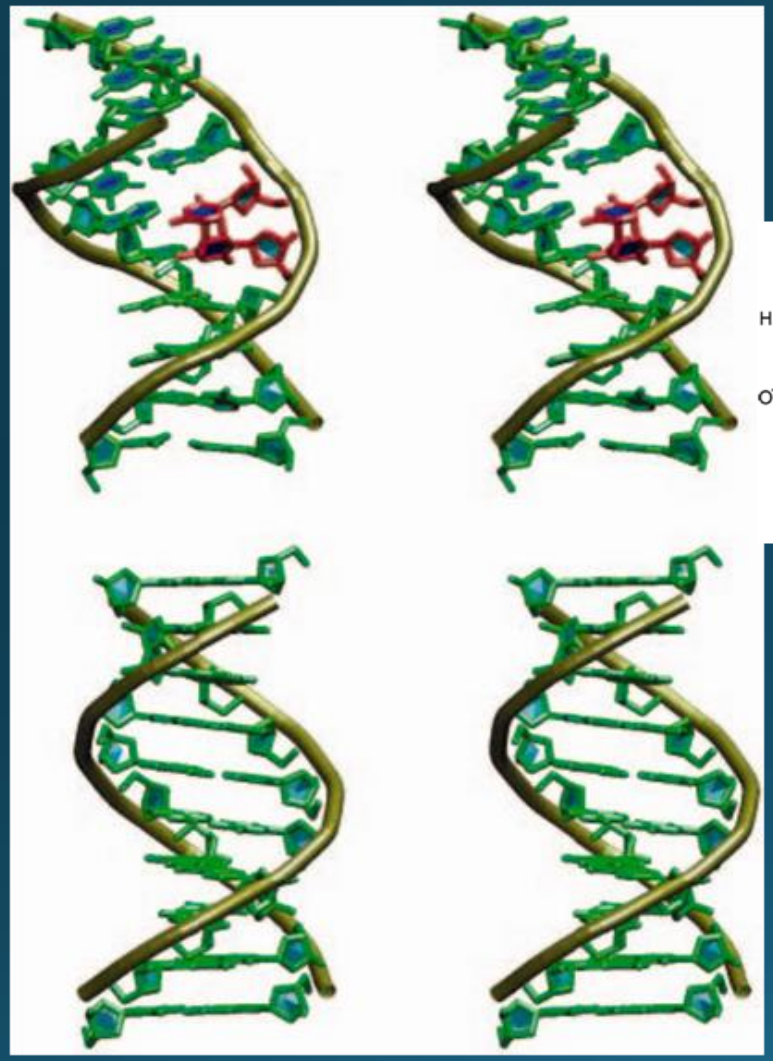
Outline

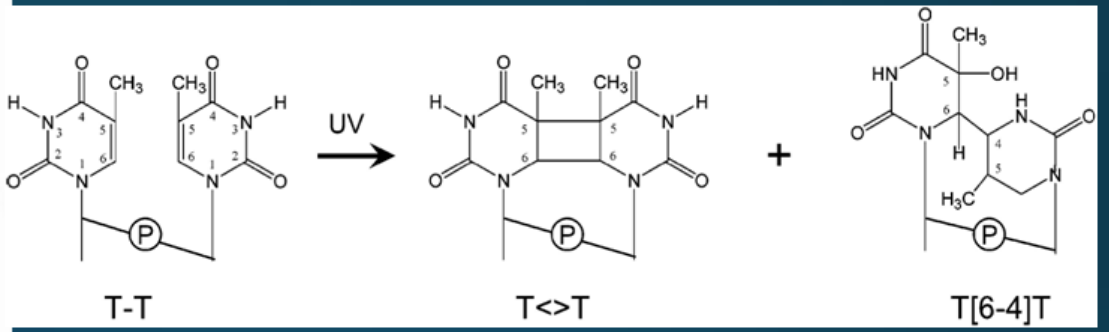
- Why do we need ultrafast laser spectroscopy?
- What do we need for ultrafast laser spectroscopy?
- Visible transient absorption
- Infrared transient absorption
- Fluorescence lifetime measurement using upconversion
- Fluorescence lifetime measurement using Kerr-gate method

Why do we need ultrafast spectroscopy?

- There are many processes which take place on the femtosecond-picosecond timescale
- The primary steps of the photocycle in photolyase and cryptochrome family take place on the fs-ps timescale.
- The primary steps of the photocycle in BLUF domain proteins takes place in tens of picoseconds

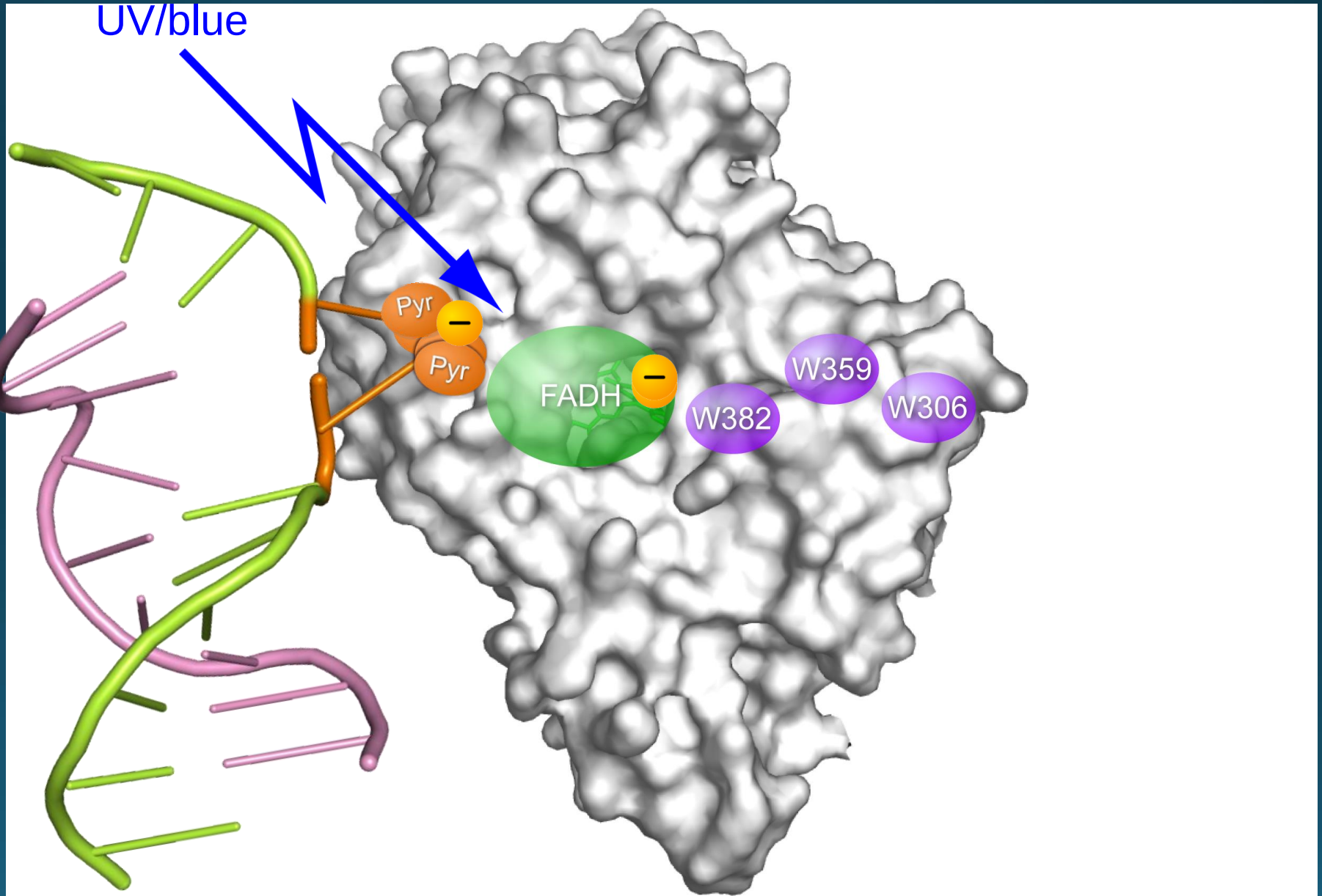
UV induced DNA damage



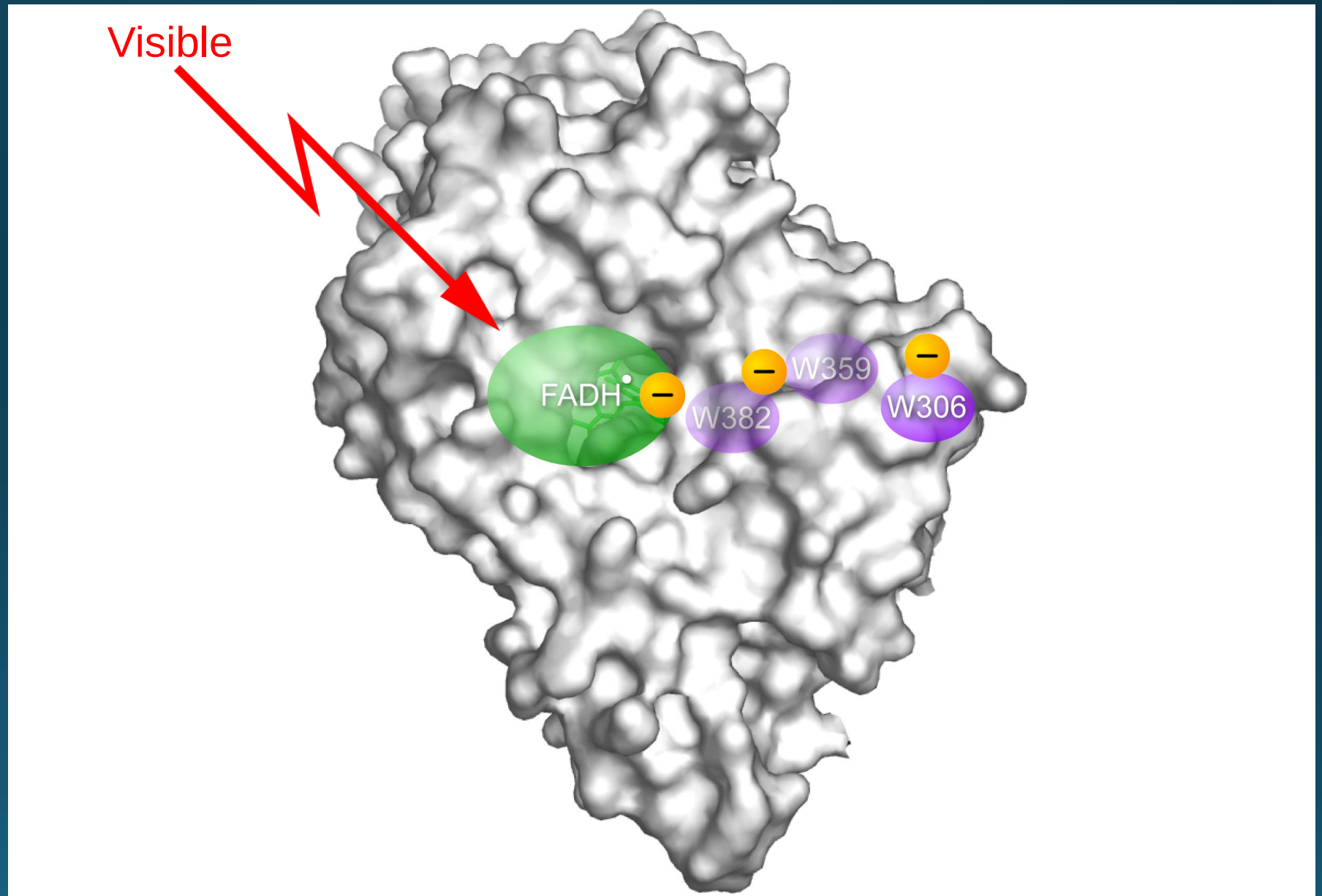


UV light induce two major lesions in DNA: cyclobutane pyrimidine dimers (Pyr<>Pyr) and the pyrimidine-pyrimidone (6-4) photoproduct (Pyr [6-4] Pyr)

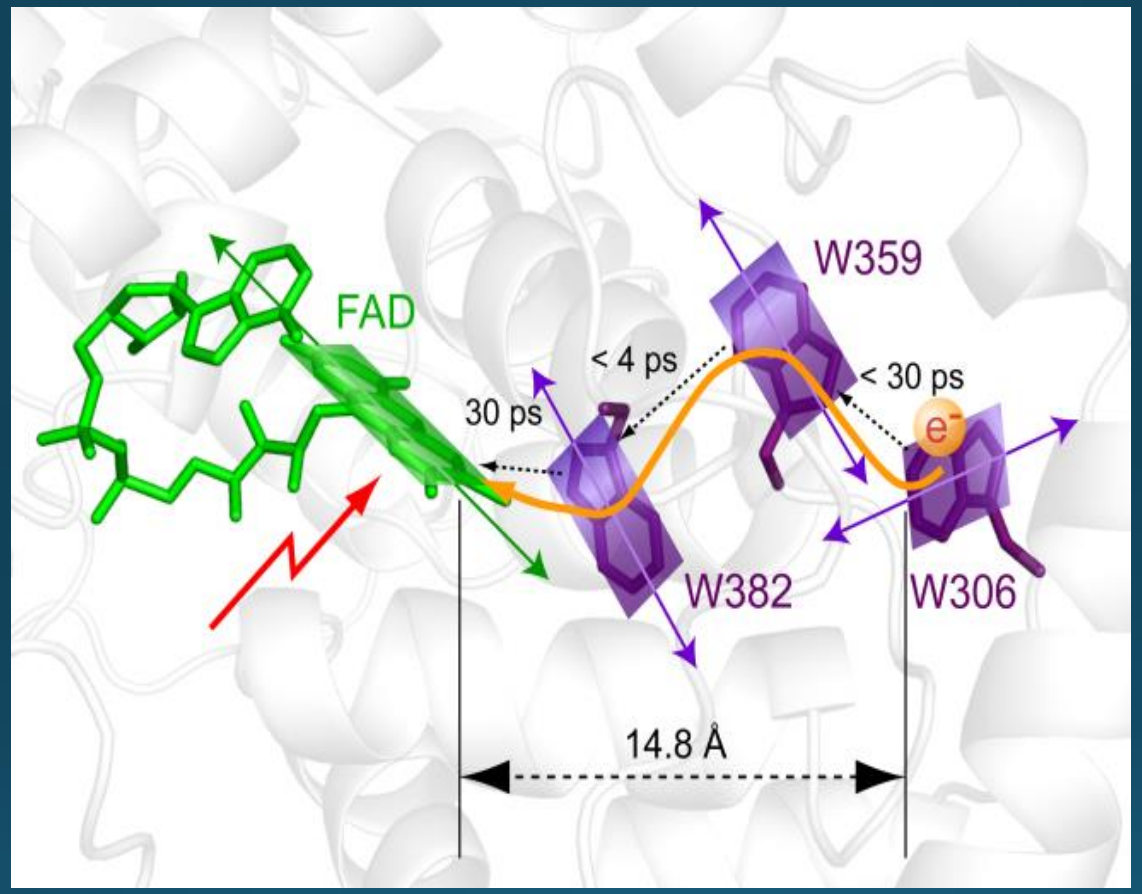
Photorepair in photolyase



Photoactivation in photolyase



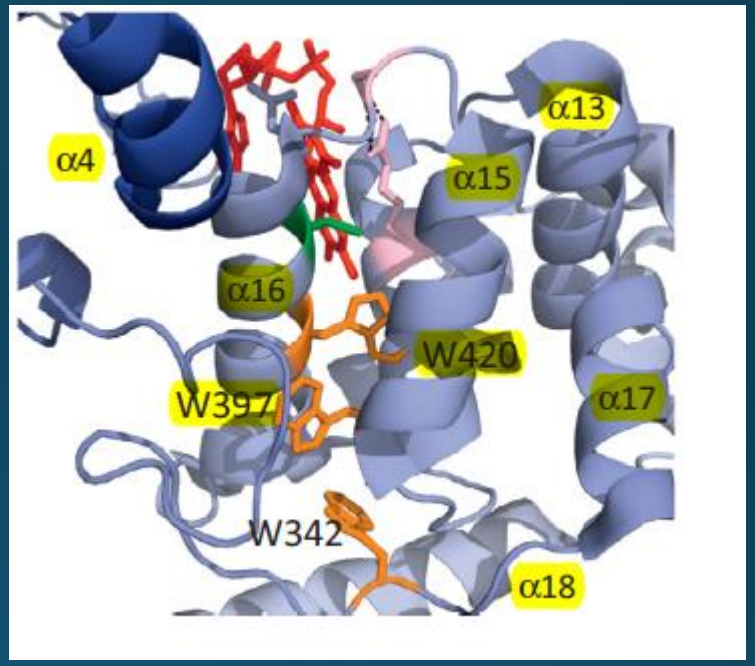
Photoactivation in photolyase



Photoreduction in photolyase (Lukacs et al, *JACS* , 2008)

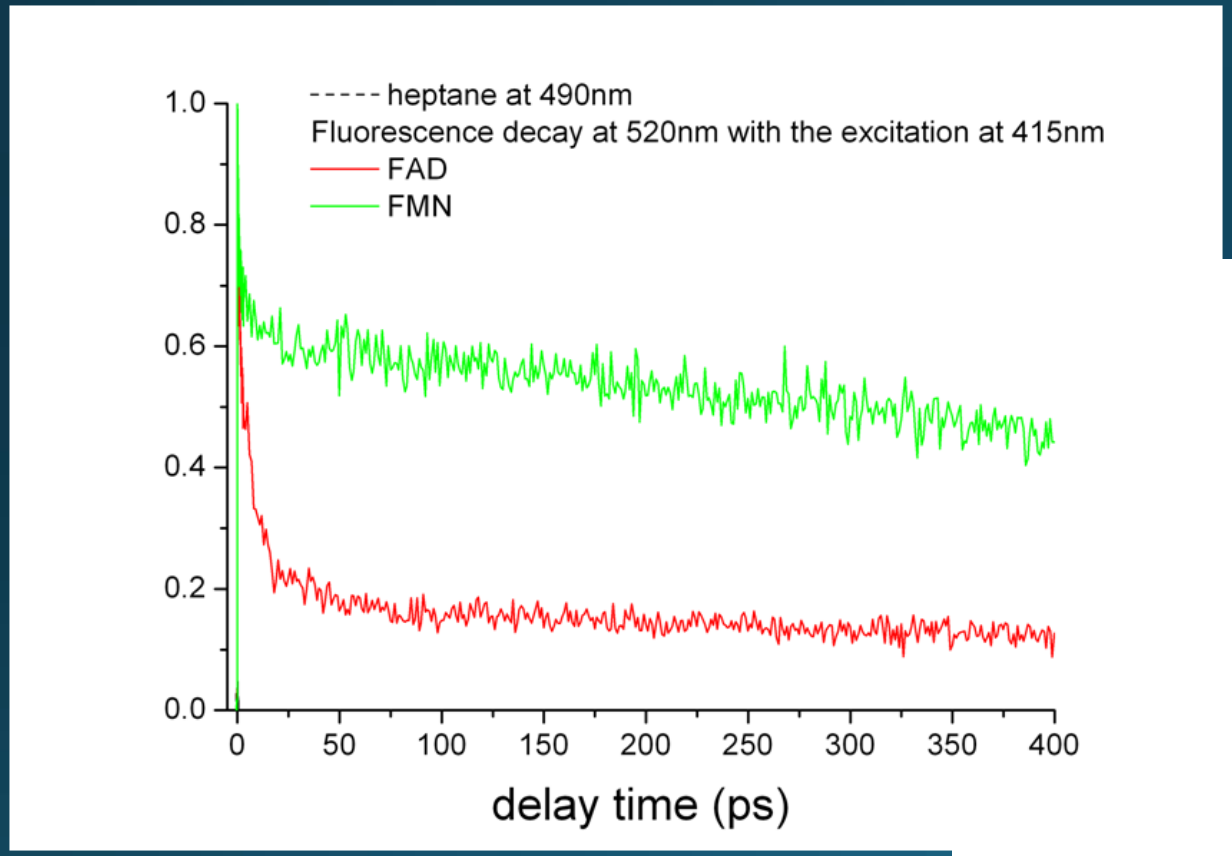
Cryptochromes

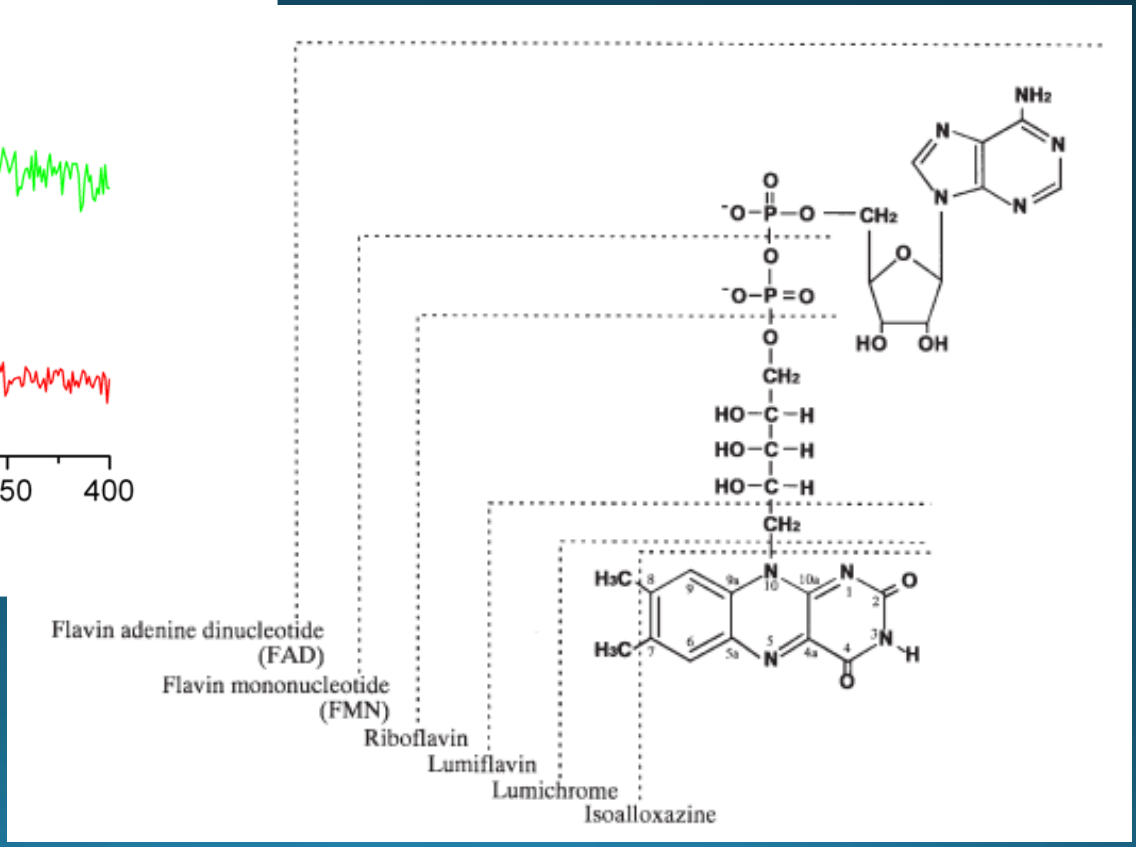
Sylvia borin

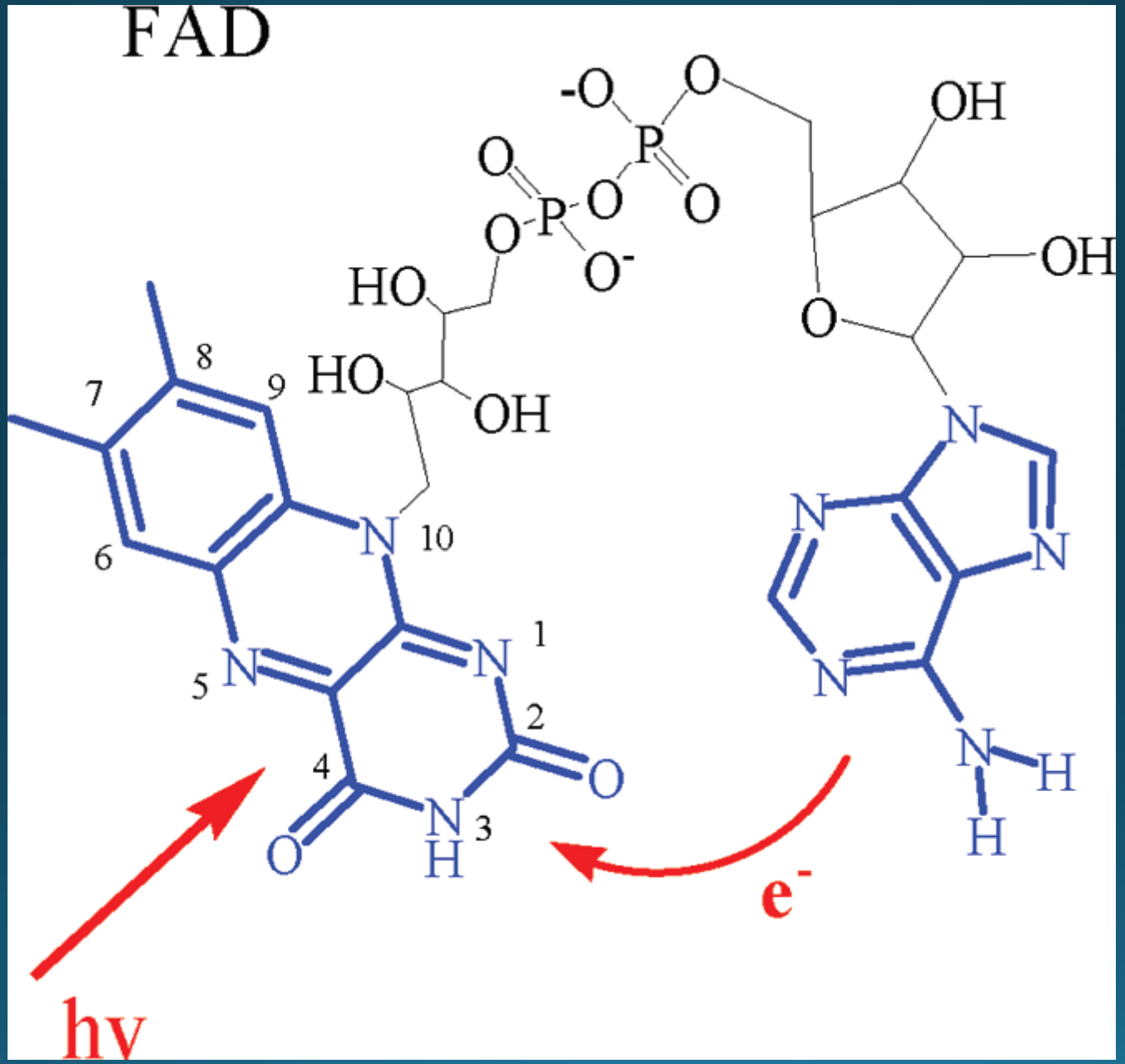


- Photoreception of blue light
- Circadian rythm (insects)
- Sensing the magnetic field

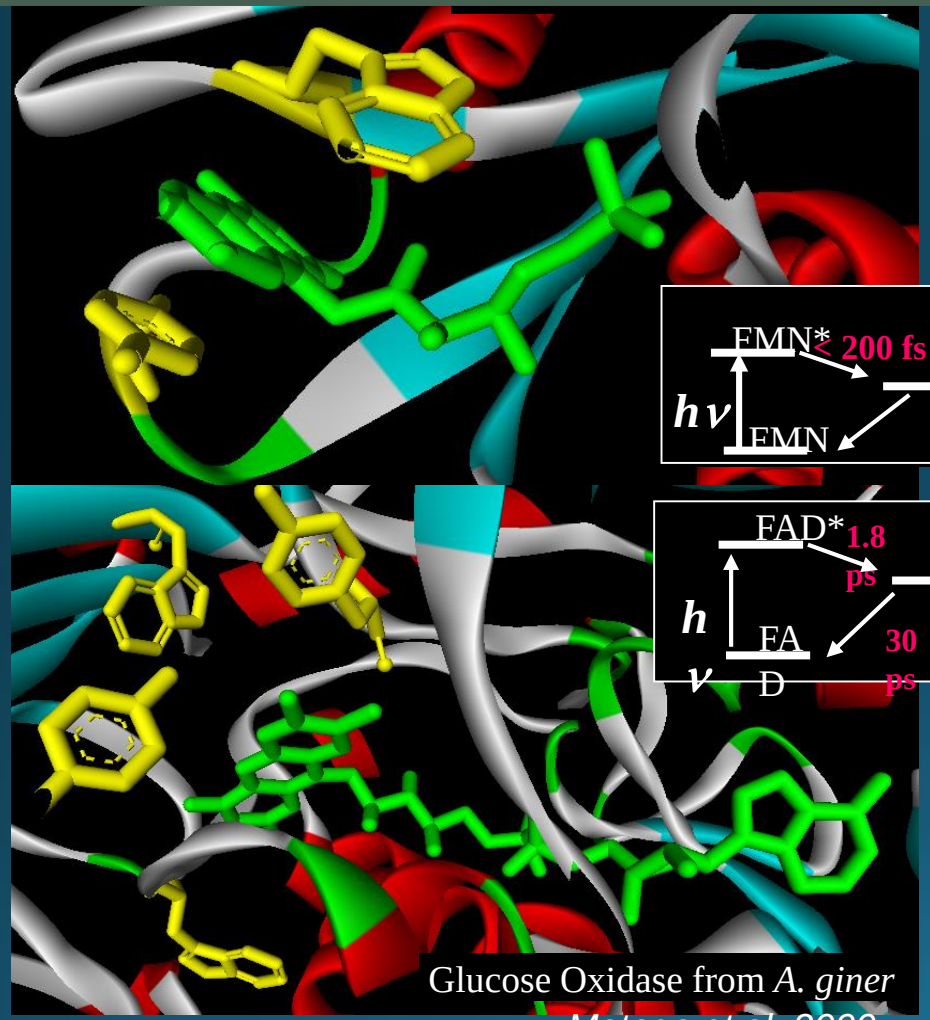
Fluorescence lifetime of FAD and FMN






FAD
hv
e-

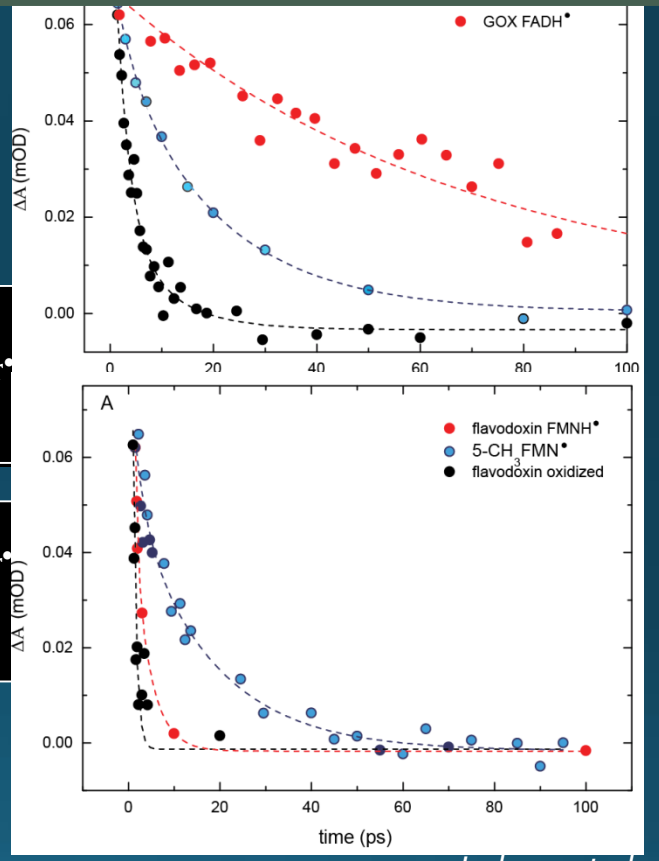
Flavin photochemistry



Glucose Oxidase from *A. giner*

Mataga et al, 2000

Zhong & Zewail, 2001

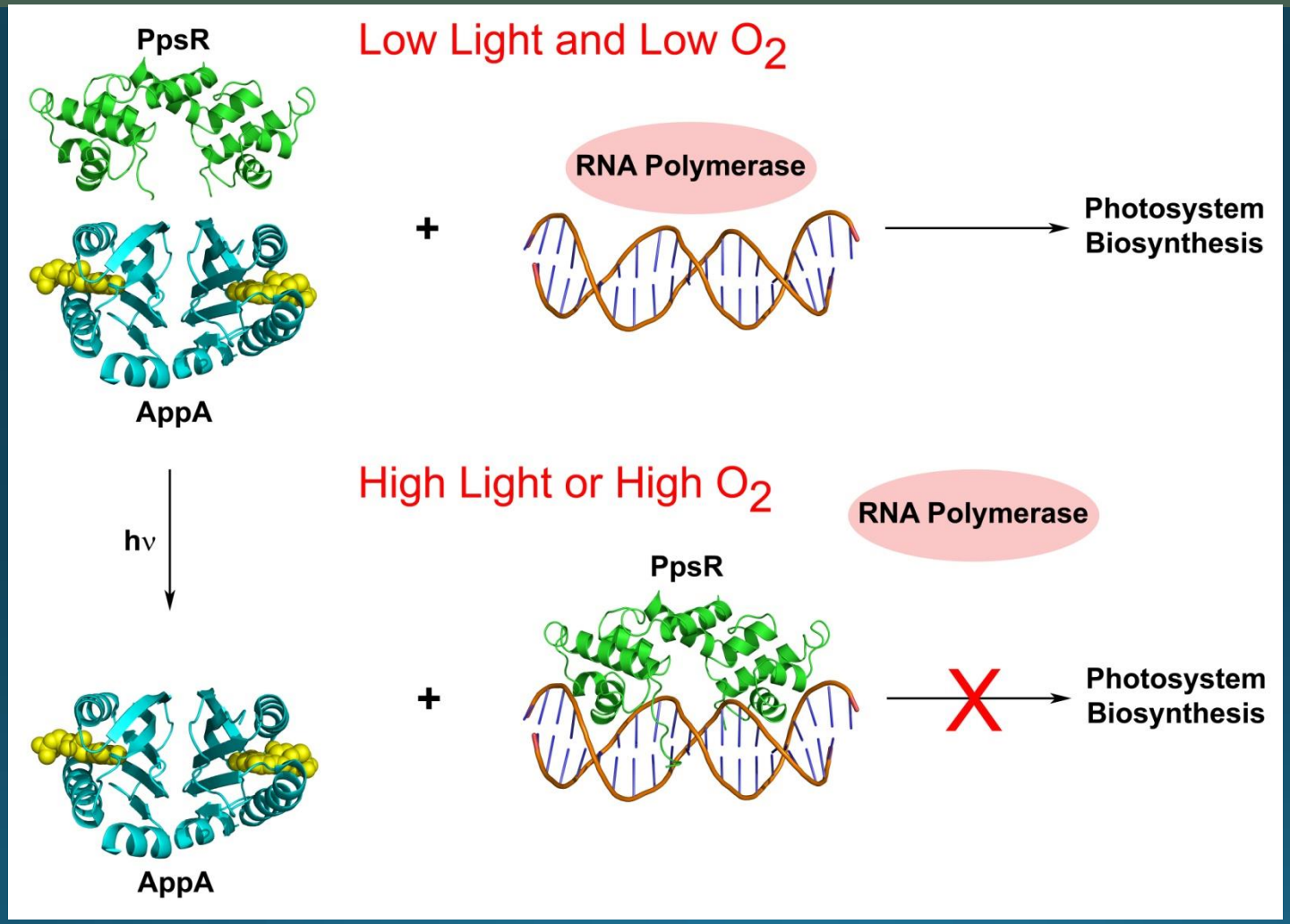


Lukacs et al., 2012

-FAD* lifetime in solution ~4 ns

In proteins: quenching by ET from aromatic residues and subsequent (sub-)picosecond recombination:

Biological Response of the AppA BLUF Domain



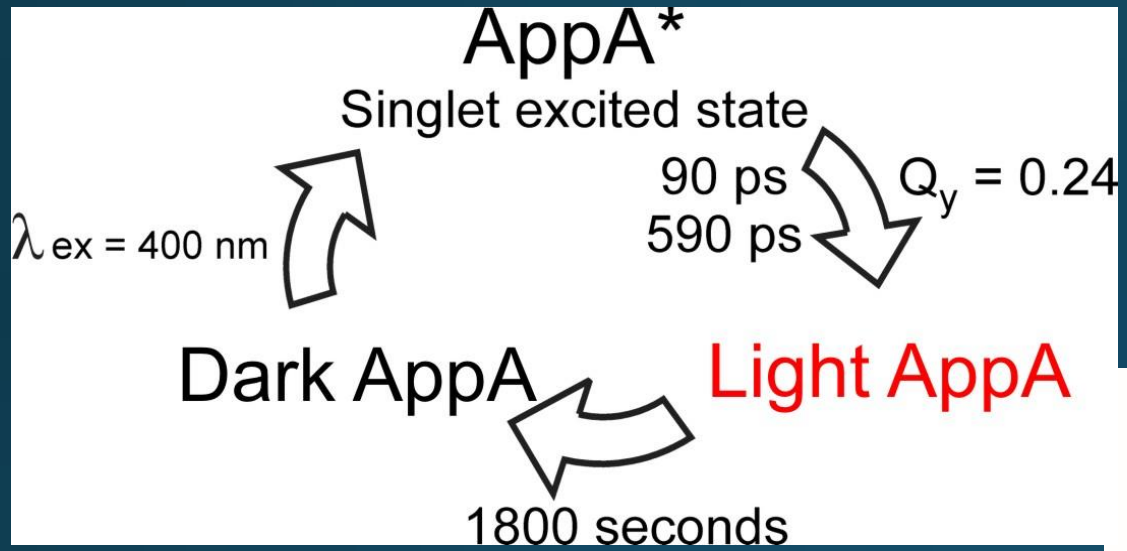
Masuda, S., and Bauer, C. E. Cell, 2002. 110: p. 613-623.

How Does Light Absorption Lead to the Release of PpsR?

AppA is a Multidomain Complex

- N-terminal BLUF domain
- C-terminal oxygen sensing domain that binds PpsR
- AppA acts as a dual sensor : two signals give the same response
  - How are the signals related?
  - How does light absorption by the N-terminal BLUF domain cause a structural change in the C-terminus, leading to the release of PpsR?

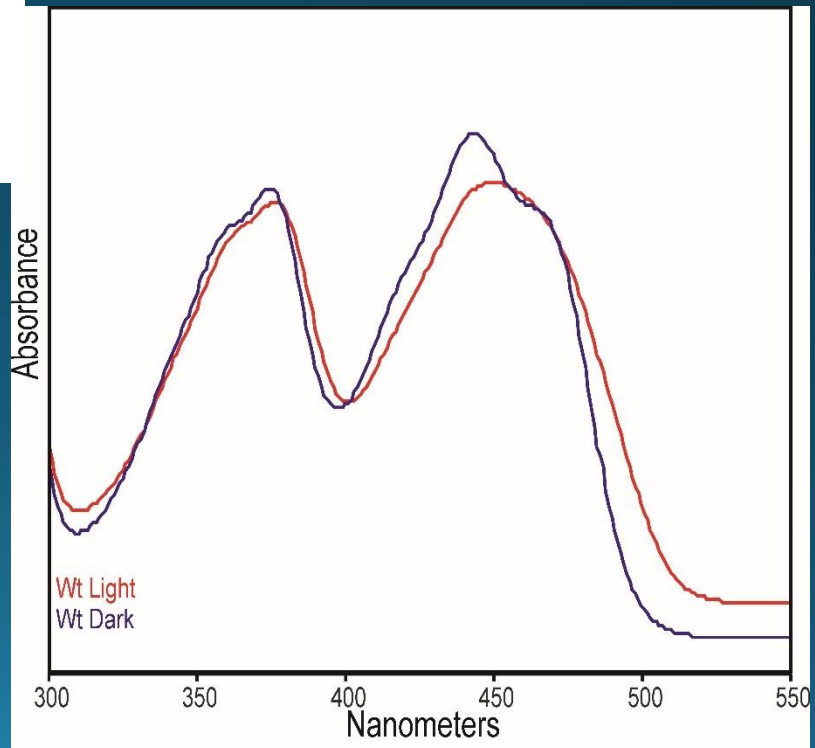
The BLUF Domain of AppA Has Two States: Dark AppA and Light AppA



Formation of the long lived Light state on a picosecond timescale

Gauden M, Y.S., et al. Biochemistry, 2005. 44(10): p. 3653-62.

After excitation with 400 nm light 10 nm red-shifted electronic spectrum

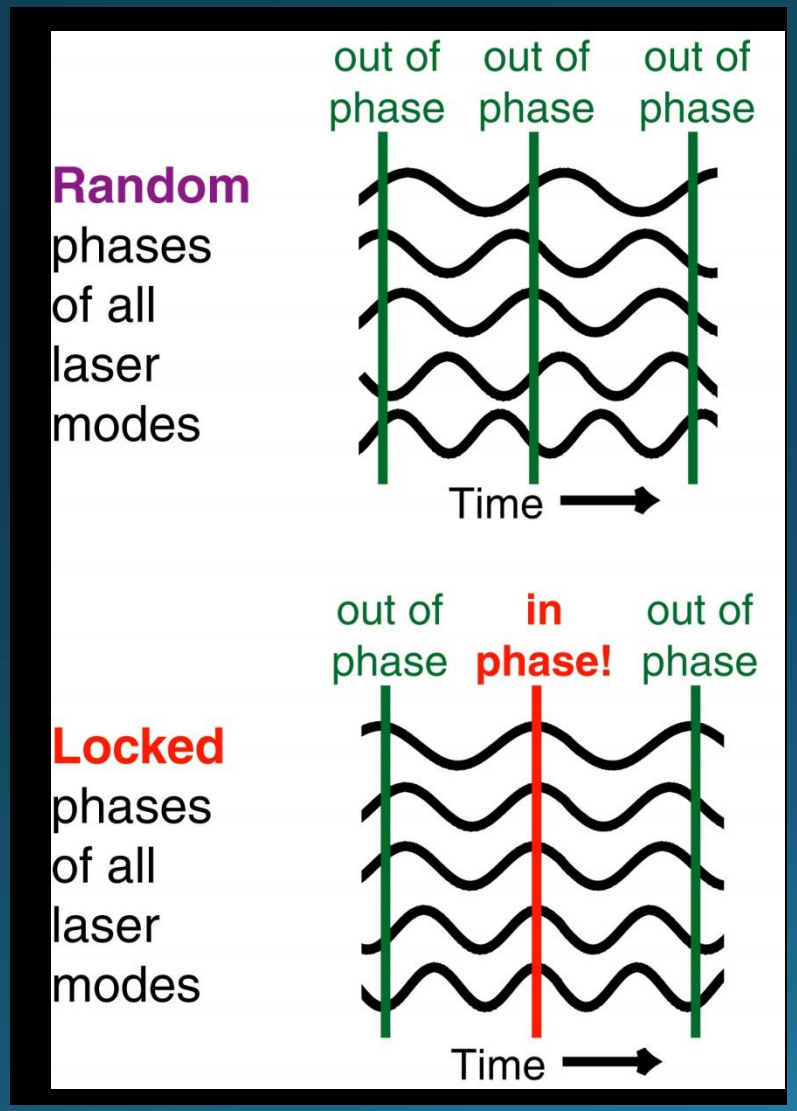


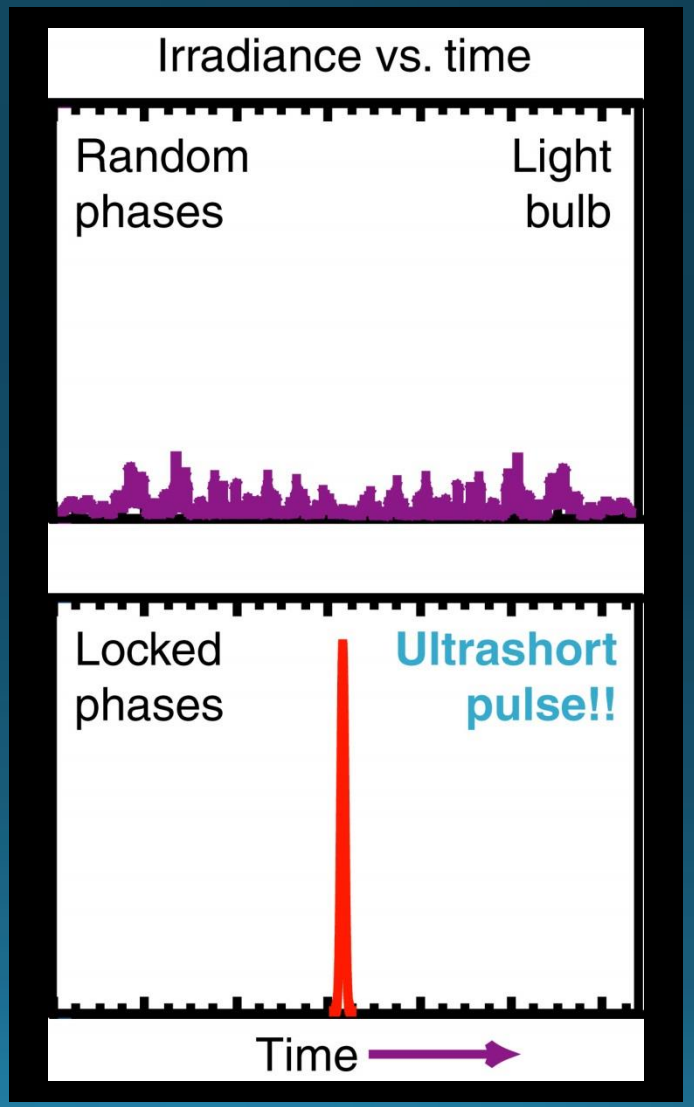
What do we need for ultrafast spectroscopy?

- Ultrafast lasers (oscillator or amplifier)
- Ti:Sa oscillator high repetition rate (100 MHz) and lower energy (1nJ)
- Regenerative amplifier (1-10 kHz) but high (1-5 mJ) energy
- The resolution of the system is limited by the pulse with of the laser
- Typical resolution is between 100-200 fs. There are system (UEA, RIKEN) less than 50 fs

Mode-locking

- Locking the phases of the laser modes yields an ultrashort pulse.



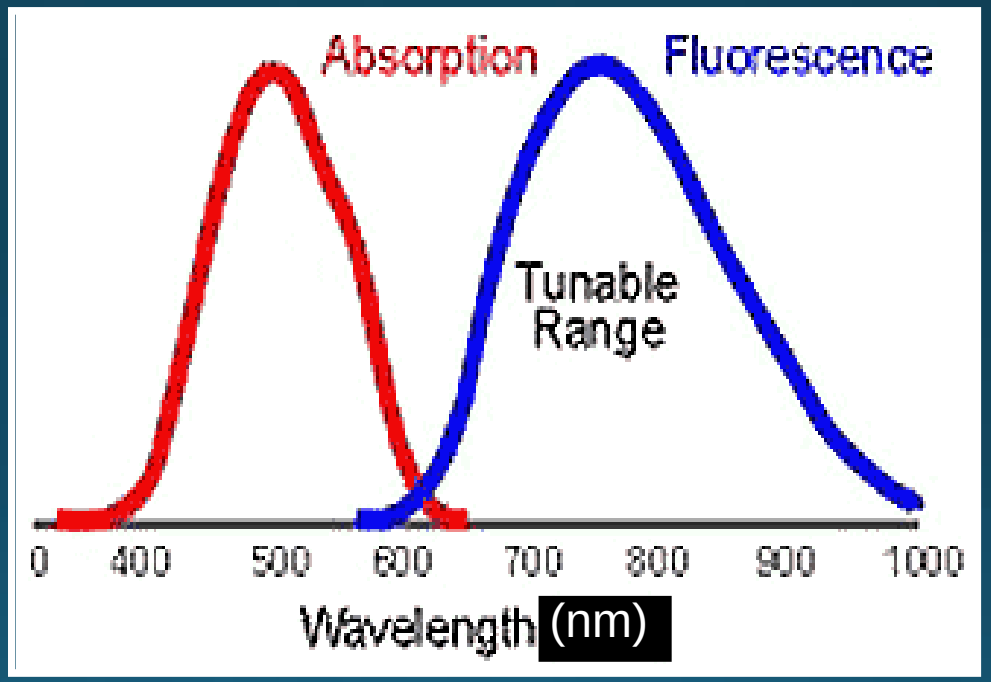


Ti:sapphire laser

It can be pumped with a (continuous) Argon laser (~450-515 nm) or a doubled-Nd laser (~532 nm).

Upper level lifetime:
3.2 μsec

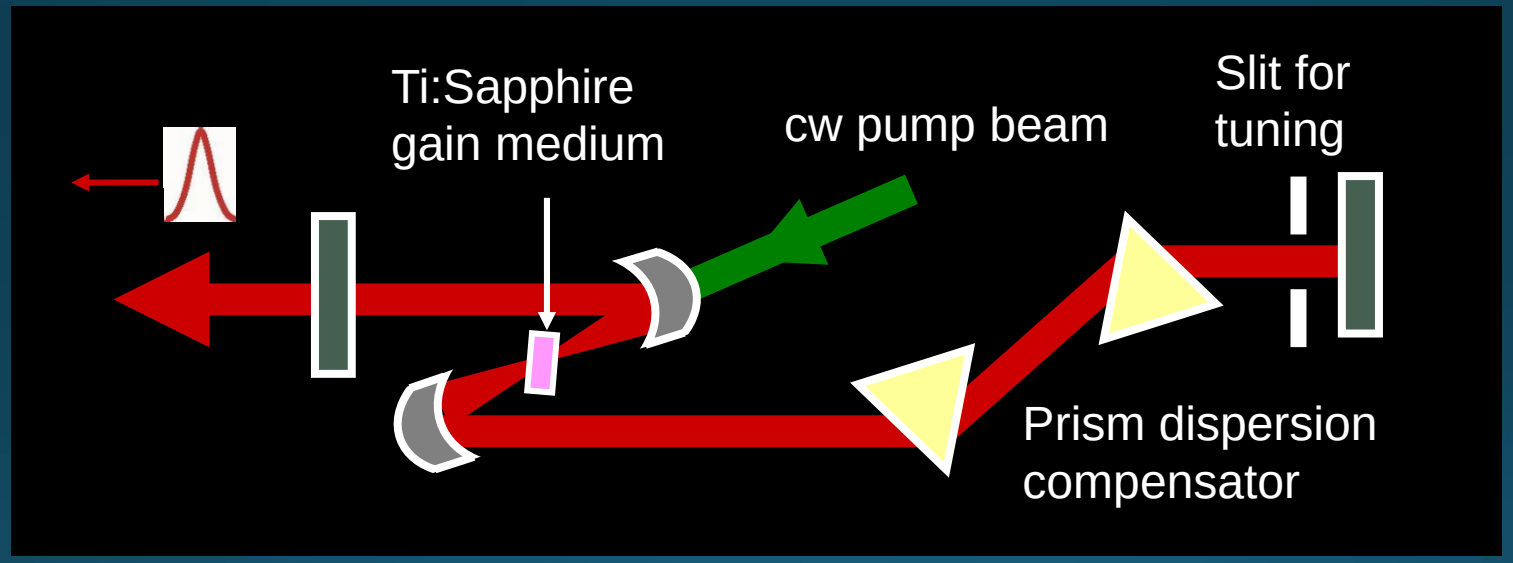
Absorption and emission spectra of Ti:Sapphire



Ti:Sapphire lases from ~700 nm to ~1000 nm.

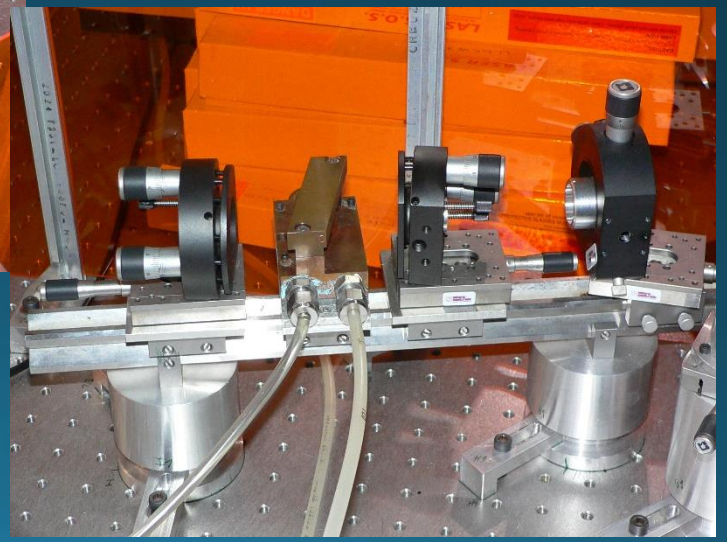
Ti:sapphire laser

Adding two prisms compensates for dispersion in the Ti:Sapphire crystal and mirrors.



This is currently the workhorse laser of the ultrafast optics community.

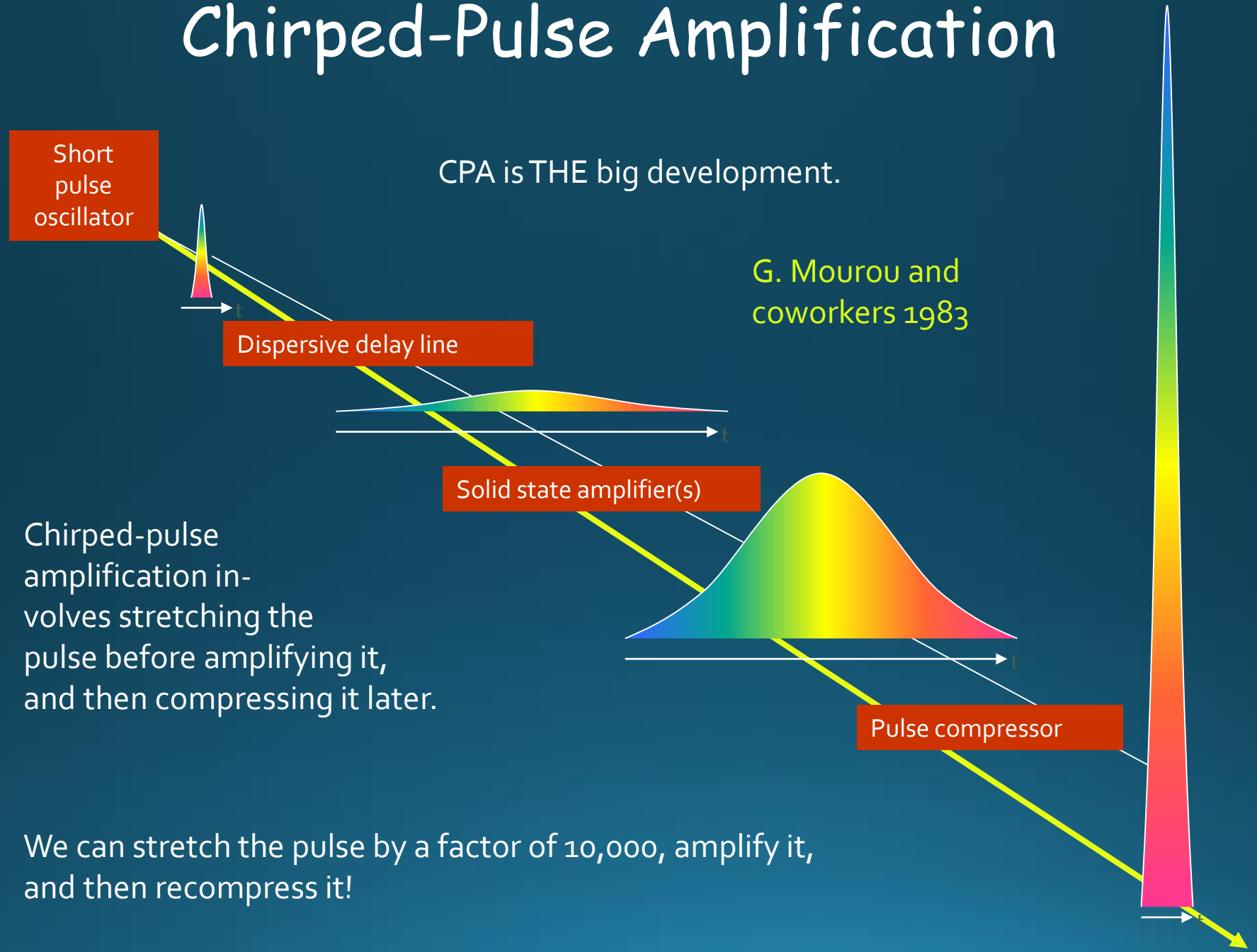
Ti:sapphire laser



Chirped-Pulse Amplification

CPA is THE big development.

G. Mourou and coworkers 1983

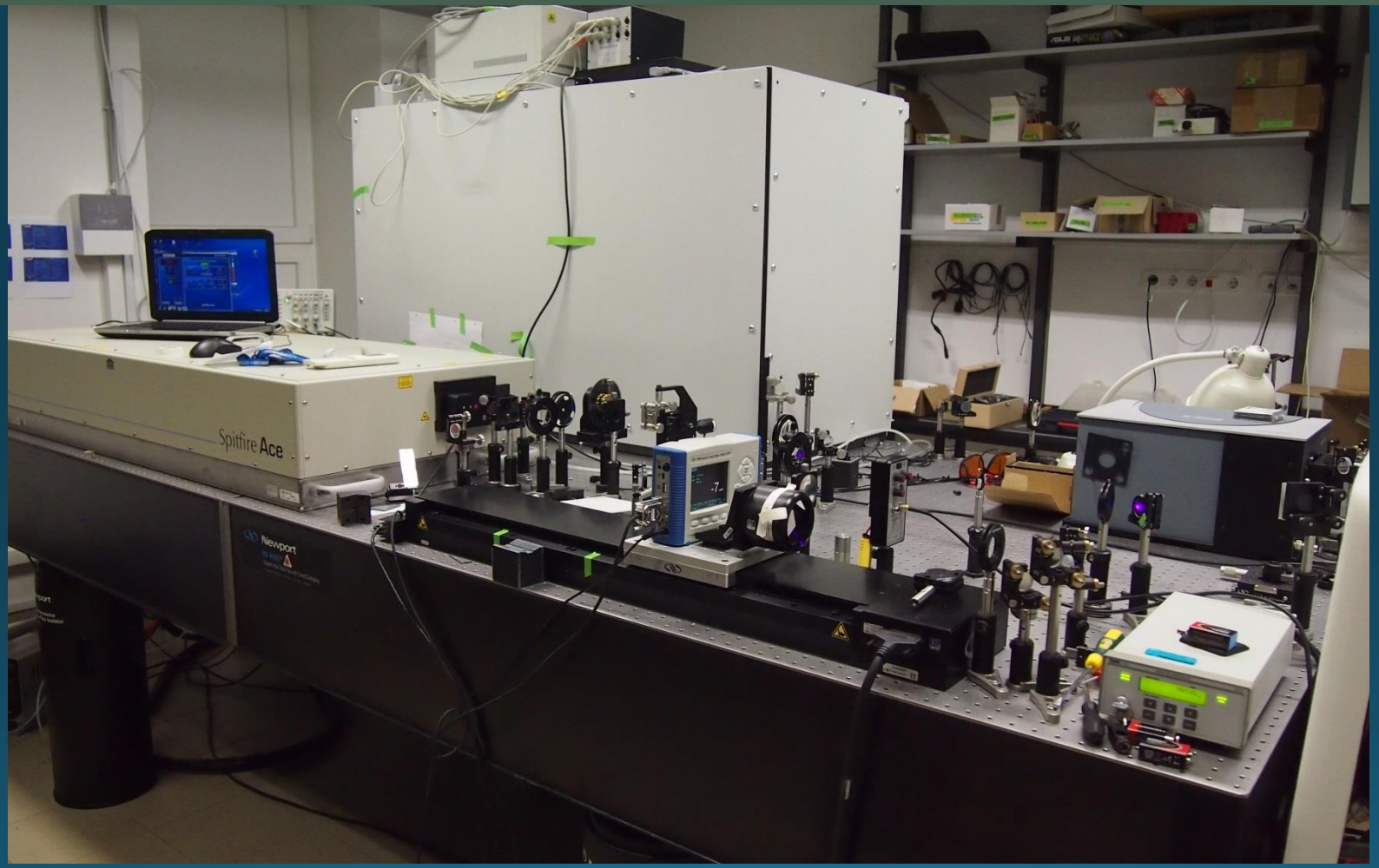


Chirped-pulse amplification involves stretching the pulse before amplifying it, and then compressing it later.

We can stretch the pulse by a factor of 10,000, amplify it, and then recompress it!

What do we need for ultrafast spectroscopy?

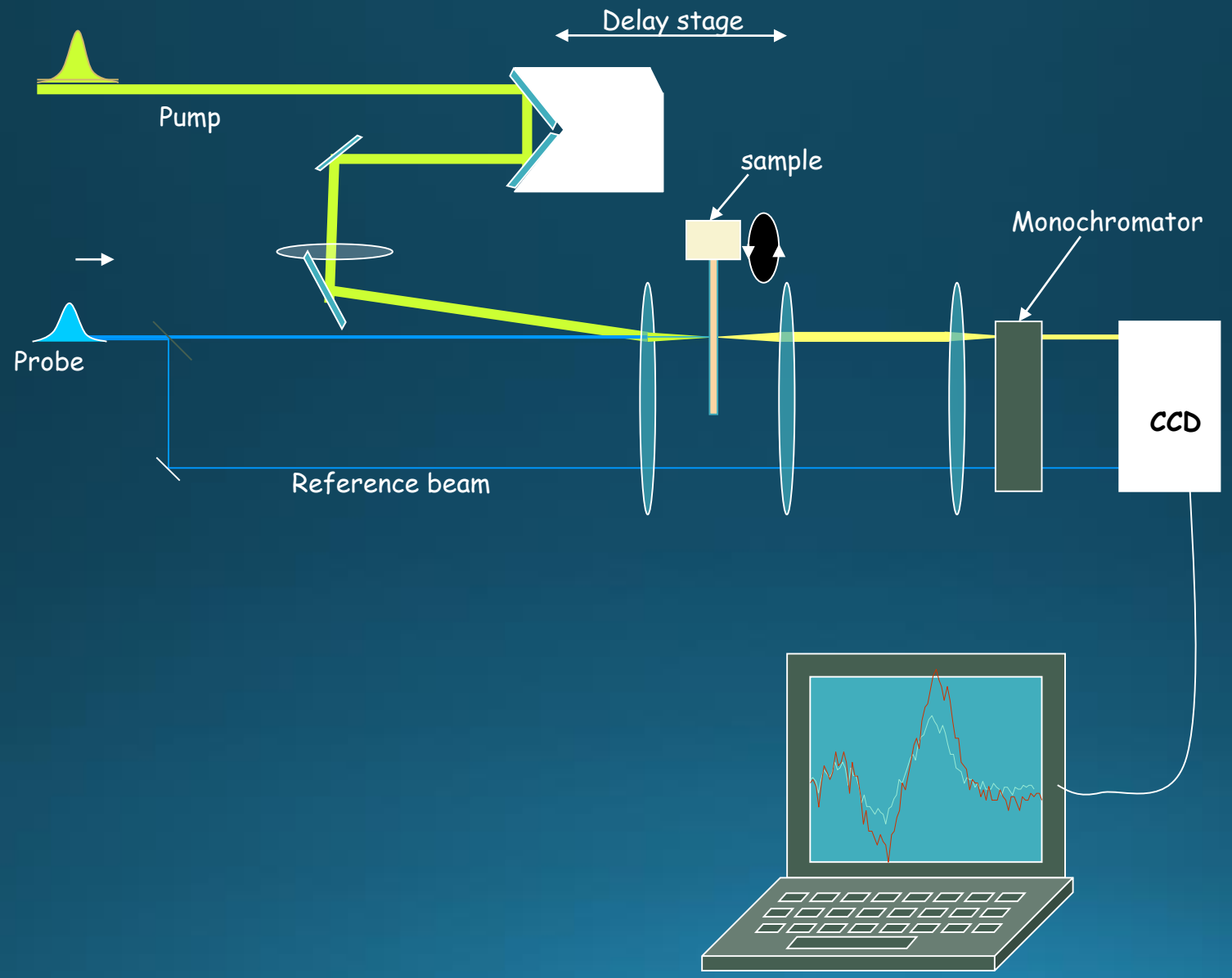
What do we need for ultrafast spectroscopy?



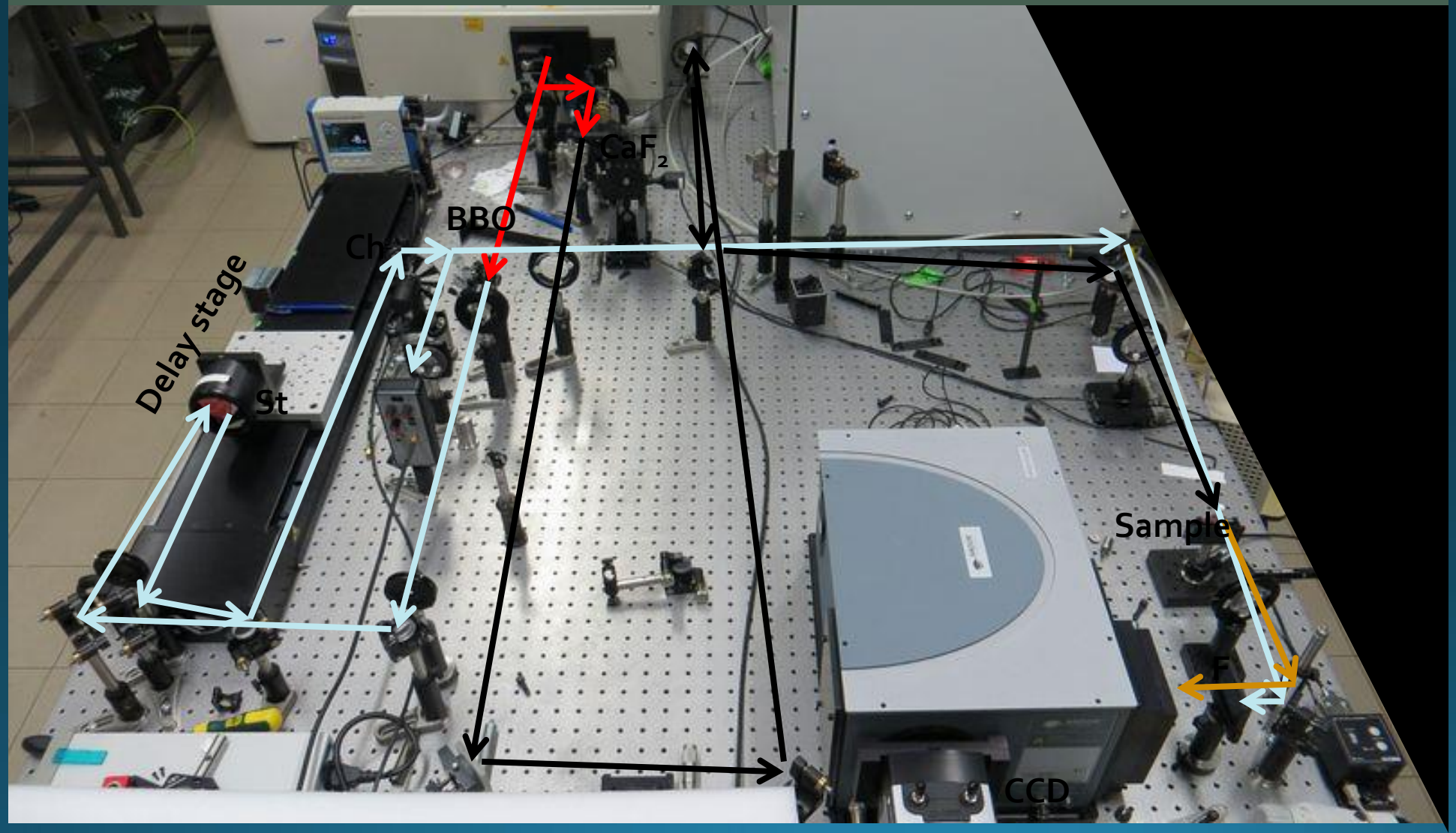
Ultrafast laser spectroscopy

I. Transient absorption spectroscopy

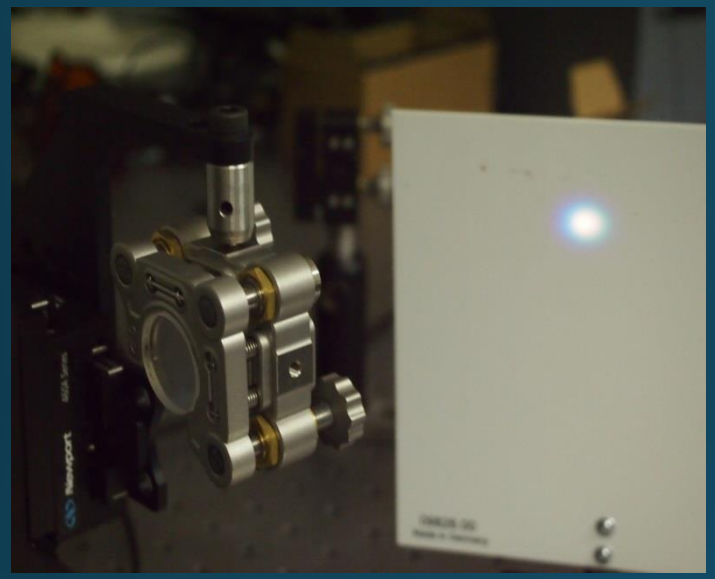
Transient absorption spectroscopy

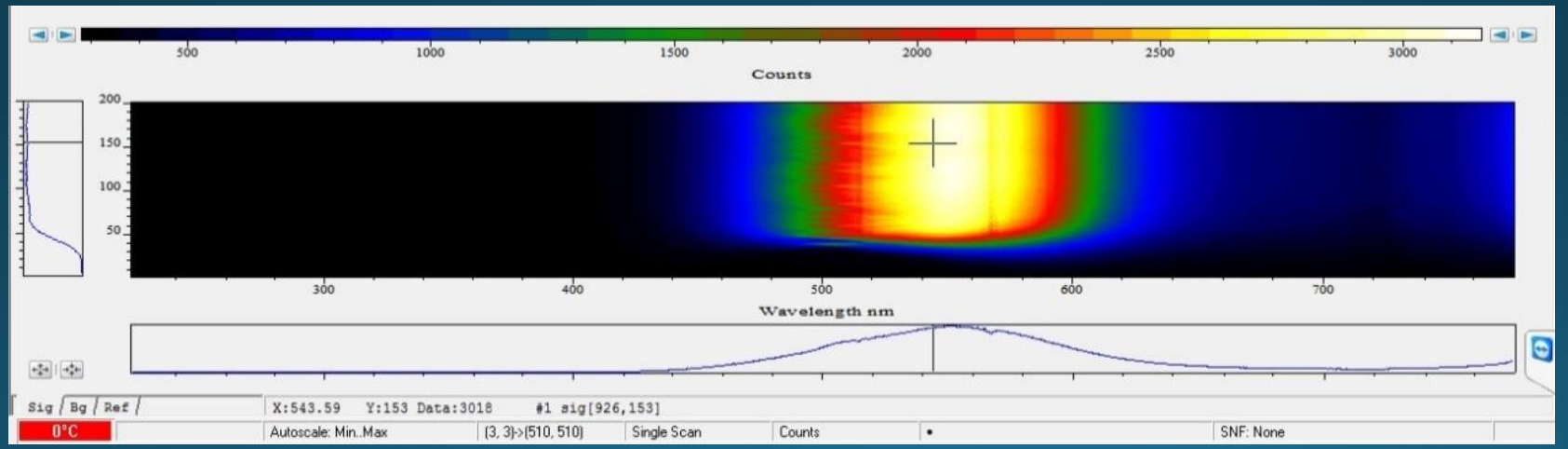


Transient absorption spectroscopy

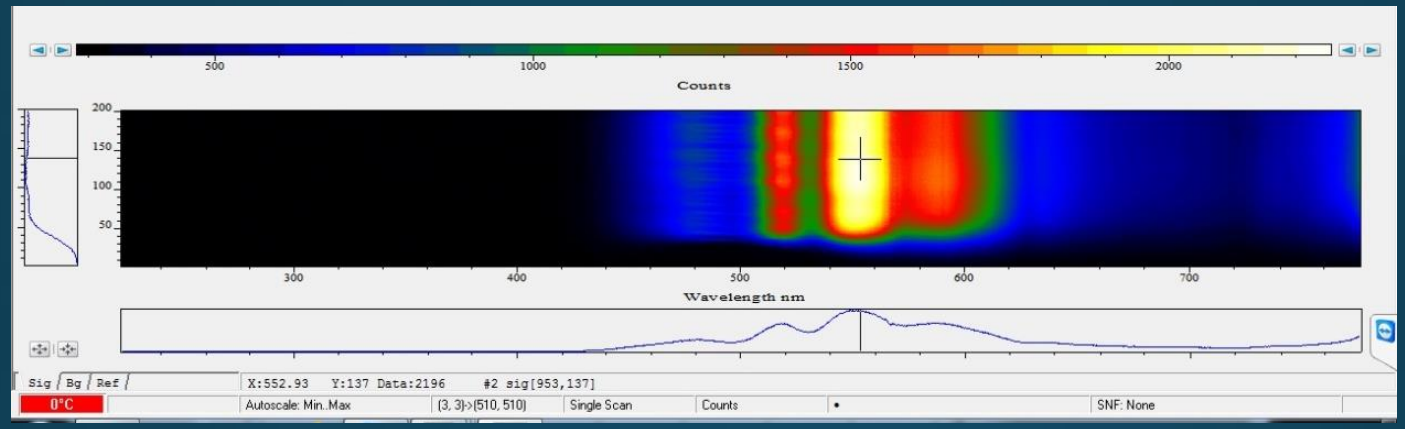


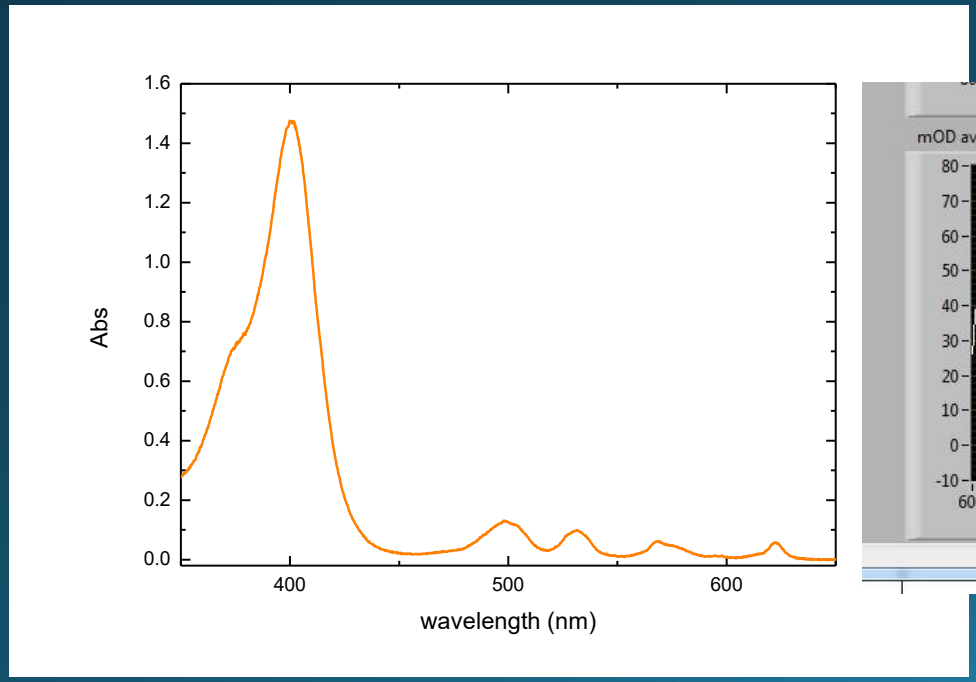
Transient absorption spectroscopy
White light continuum



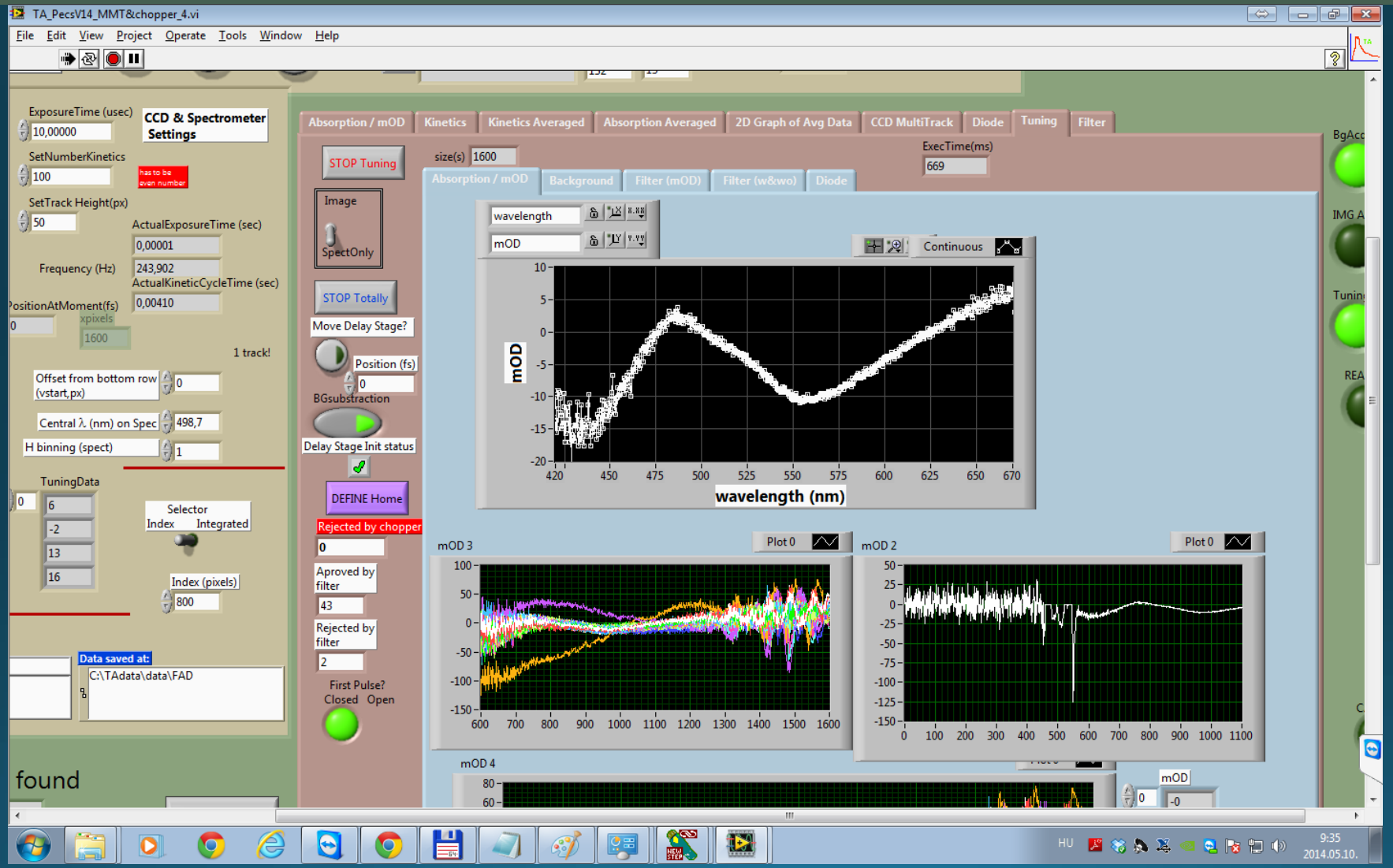


Transient absorption spectroscopy

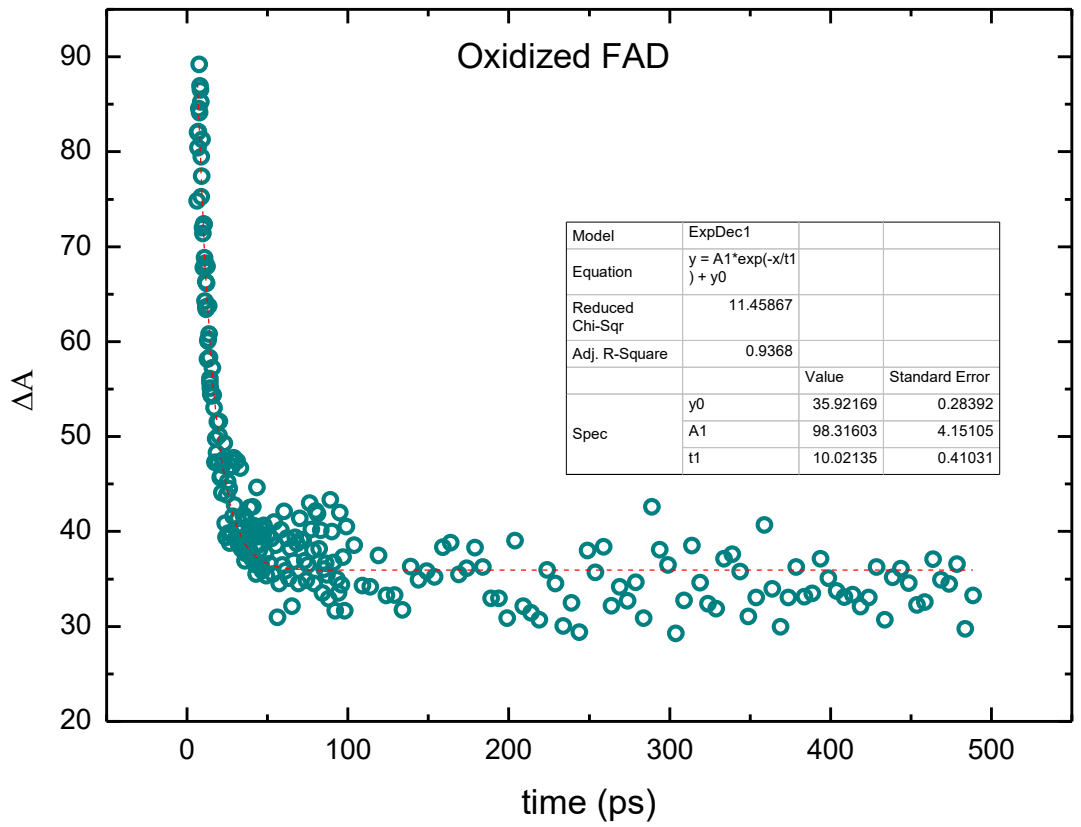




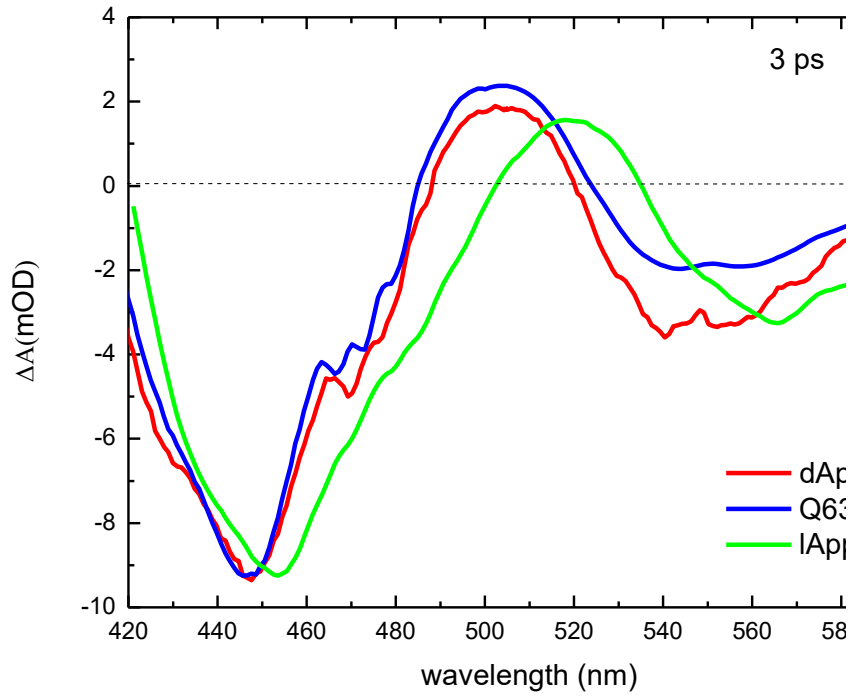
Transient absorption spectroscopy

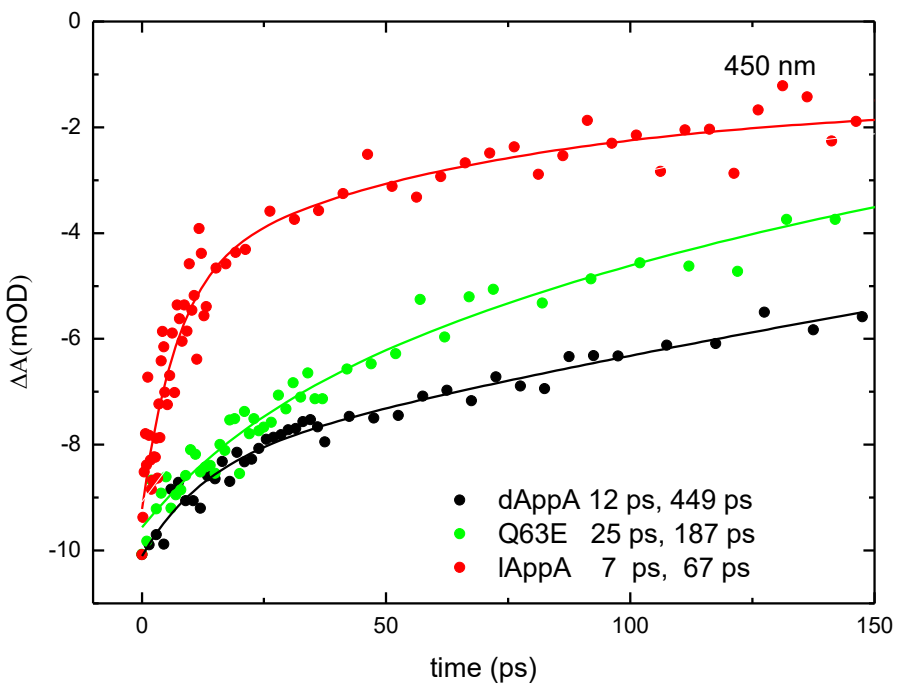


Transient absorption spectroscopy



Transient absorption spectroscopy





Data analysis

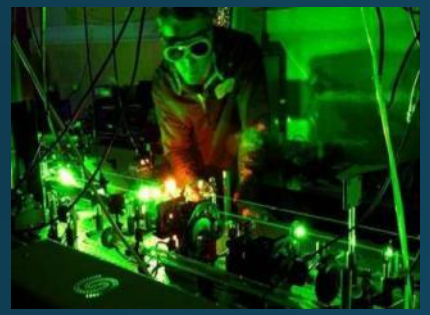
Ultrafast laser spectroscopy

II. Transient infrared absorption spectroscopy

Rutherford Appleton Laboratory

Central Laser Facility

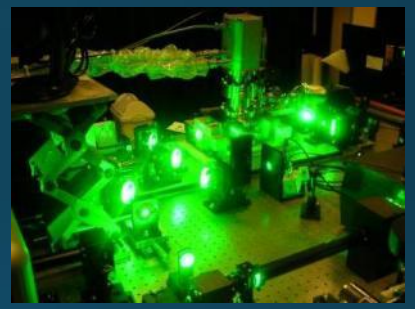
ASTRA



- Ultra-high intensity 2.6 kJ in sub-picosecond pulses, 100 TW → PW
- Fusion and Plasma Research
- Extreme UV generation
- Attosecond pulse generation research

VULCAN

LSF



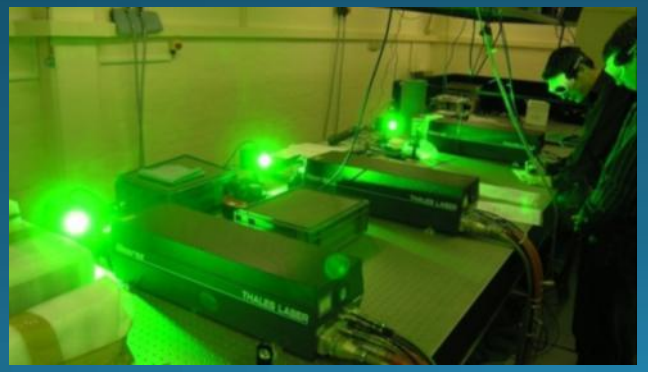
- Smaller scale lasers
- Vibrational spectroscopy
- Imaging; laser tweezers and microscopy

LSF User community

Research Complex

- RCUK Building
- Activity Across "Life and Physical Sciences Interface"
- Short and Long term Research Visitors using facilities across RAL site (Diamond, ISIS CLF)

Research Complex at Harwell

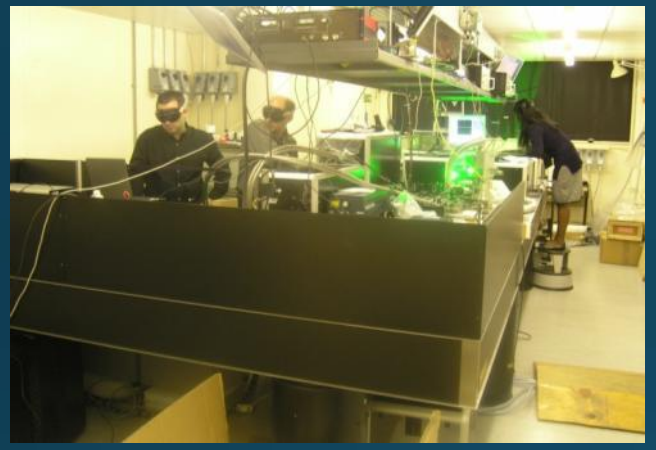


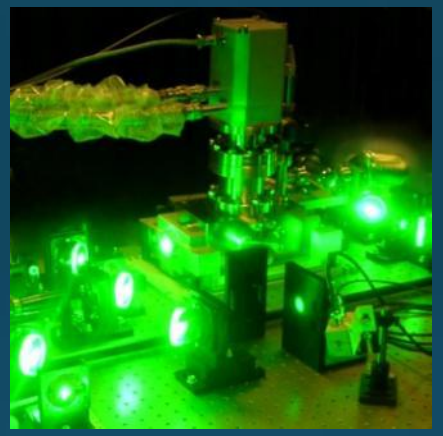
Time-Resolved Spectroscopy

- **Pump – probe** scheme with variable time delays

- Pump pulse drives a chemical reaction or energy transfer
- Probe pulse may observe UV – IR absorption spectrum or Raman spectrum

Dual 10 kHz Ti:S Amp





Touaryx > 65 W

Touaryx > 75 W

Touaryx > 75 W

Oscillator

fs Stretcher with Dazzler control

ps Stretcher with Dazzler control

Regenerative Amplifier

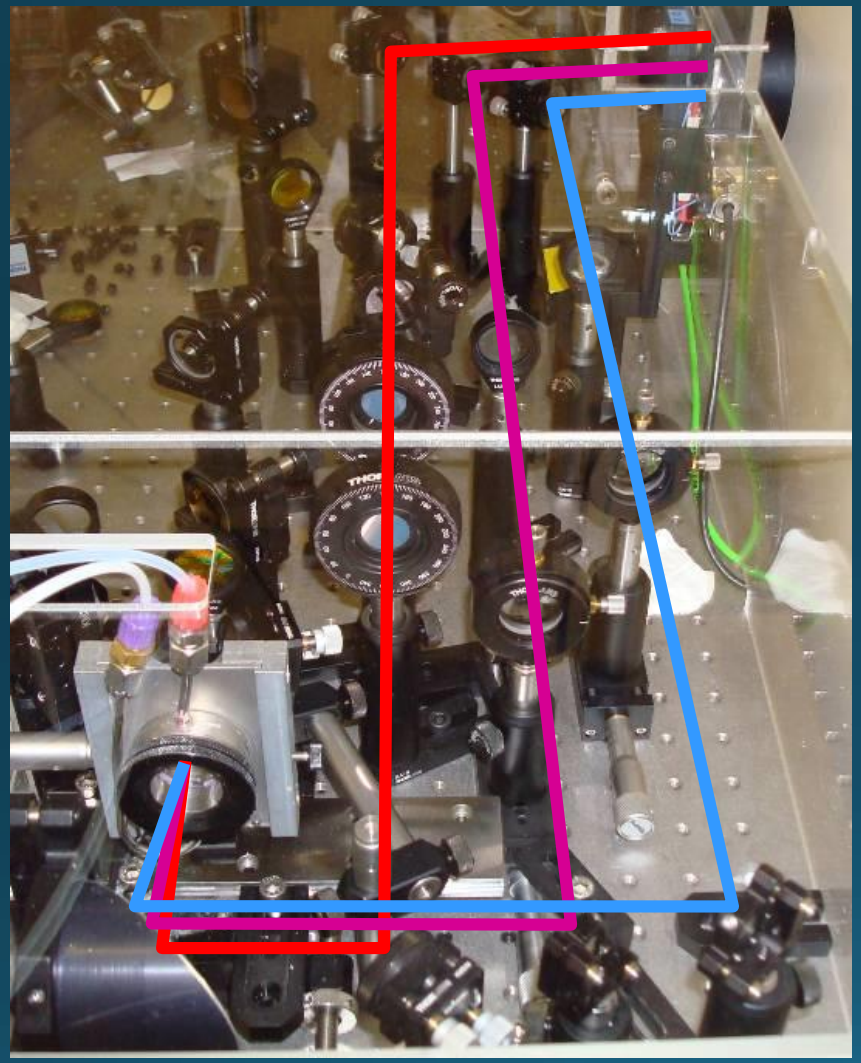
Regenerative Amplifier

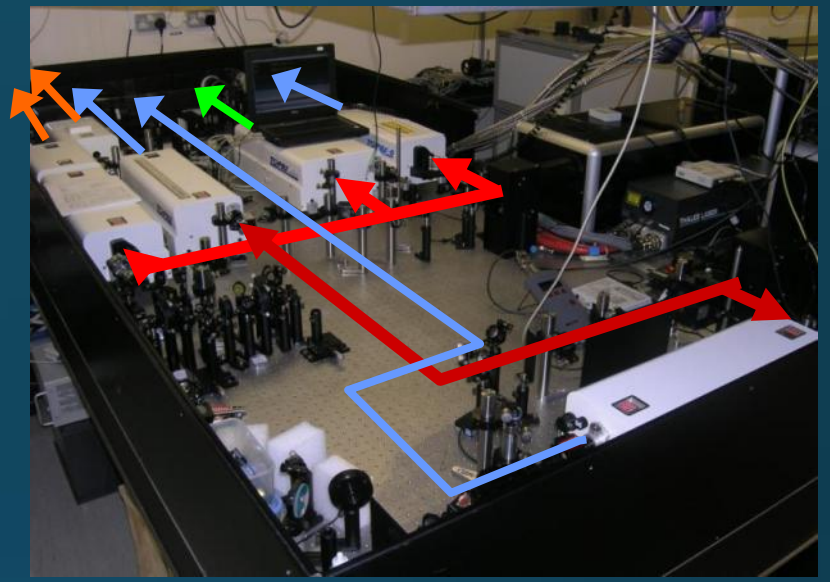
2-pass Amplifier (CRYO)

2-pass Amplifier (CRYO)

Compressor

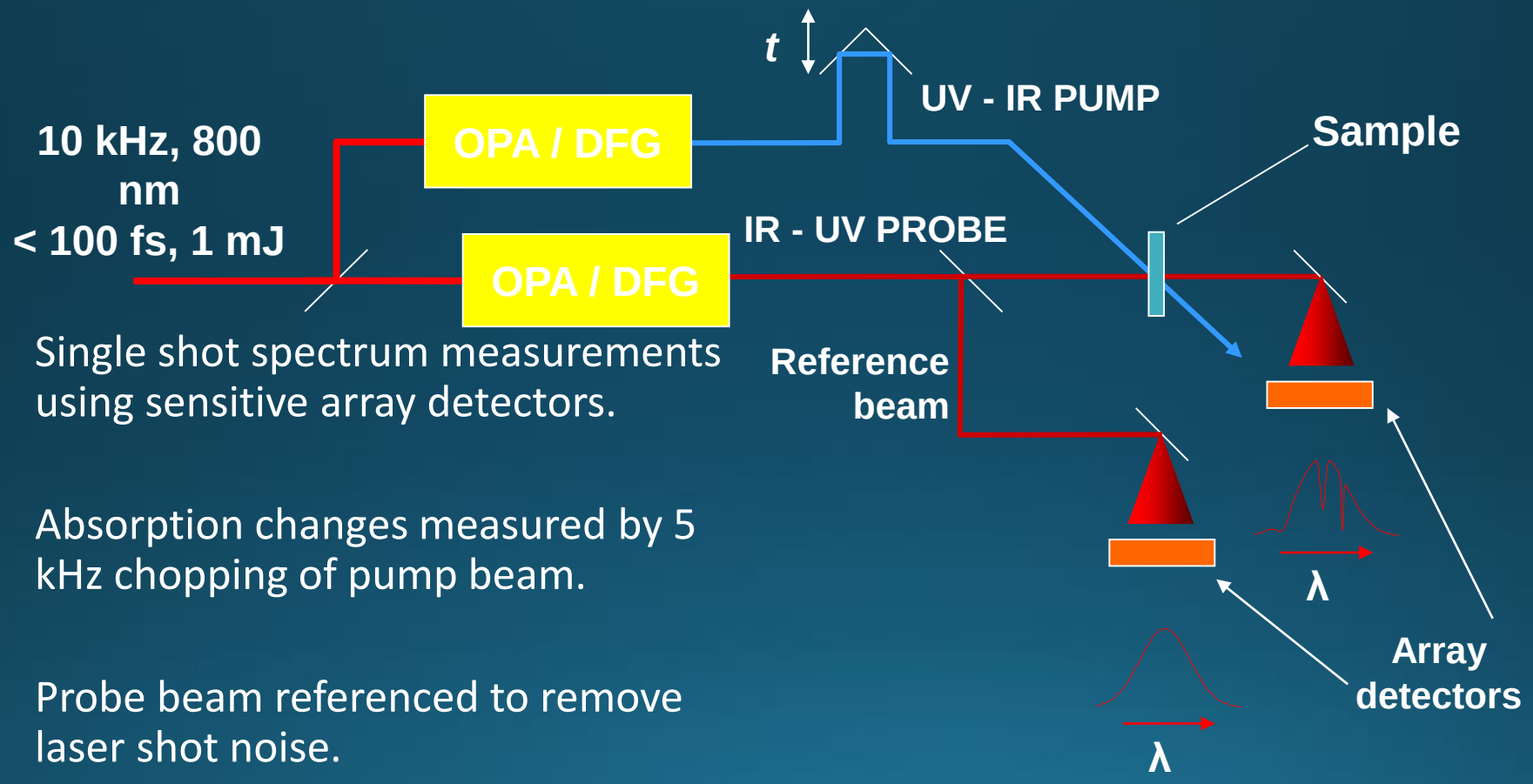
Compressor





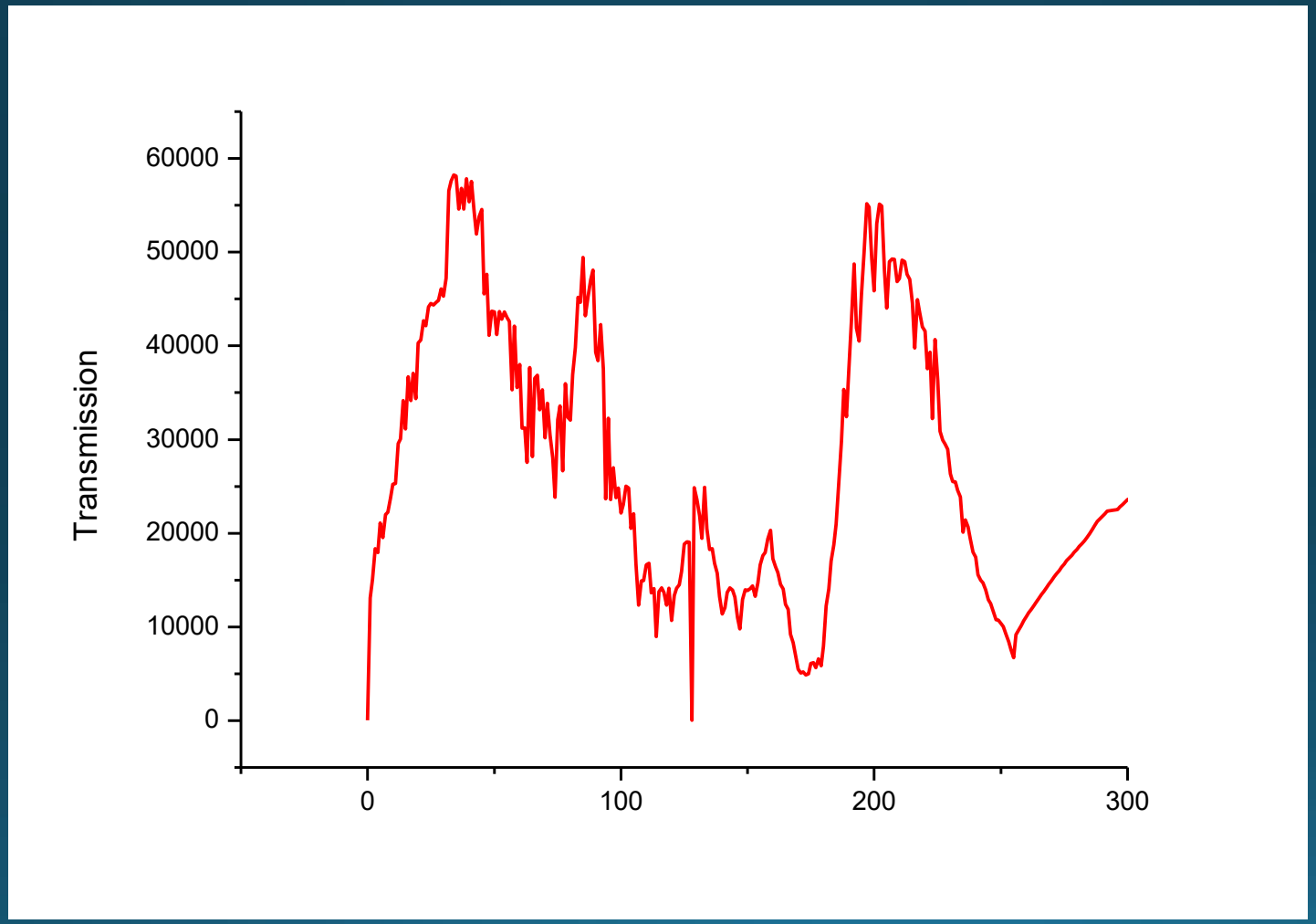
Tunability: 200 – 20000 nm
Pulse durations: 40 fs – 1 nm
Bandwidth: 5 – 600 cm^{-1}

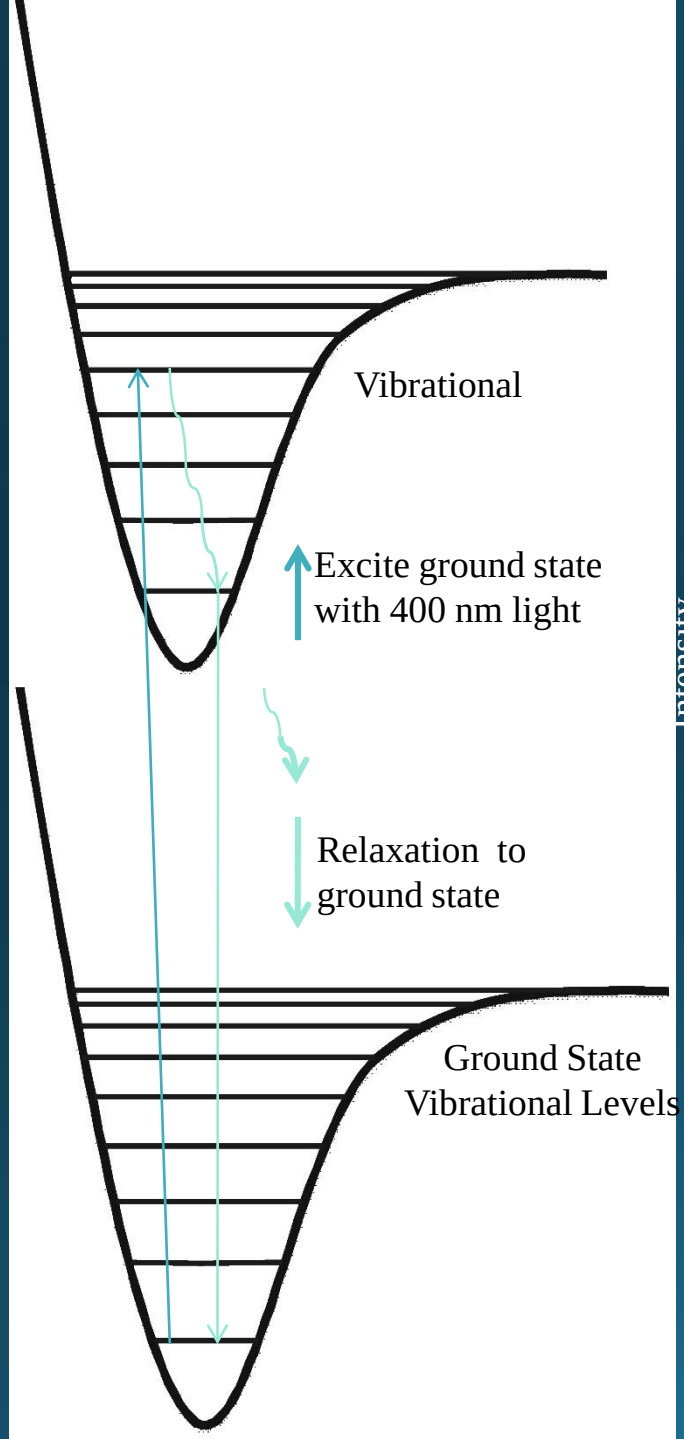
Time-Resolved Absorption Spectroscopy

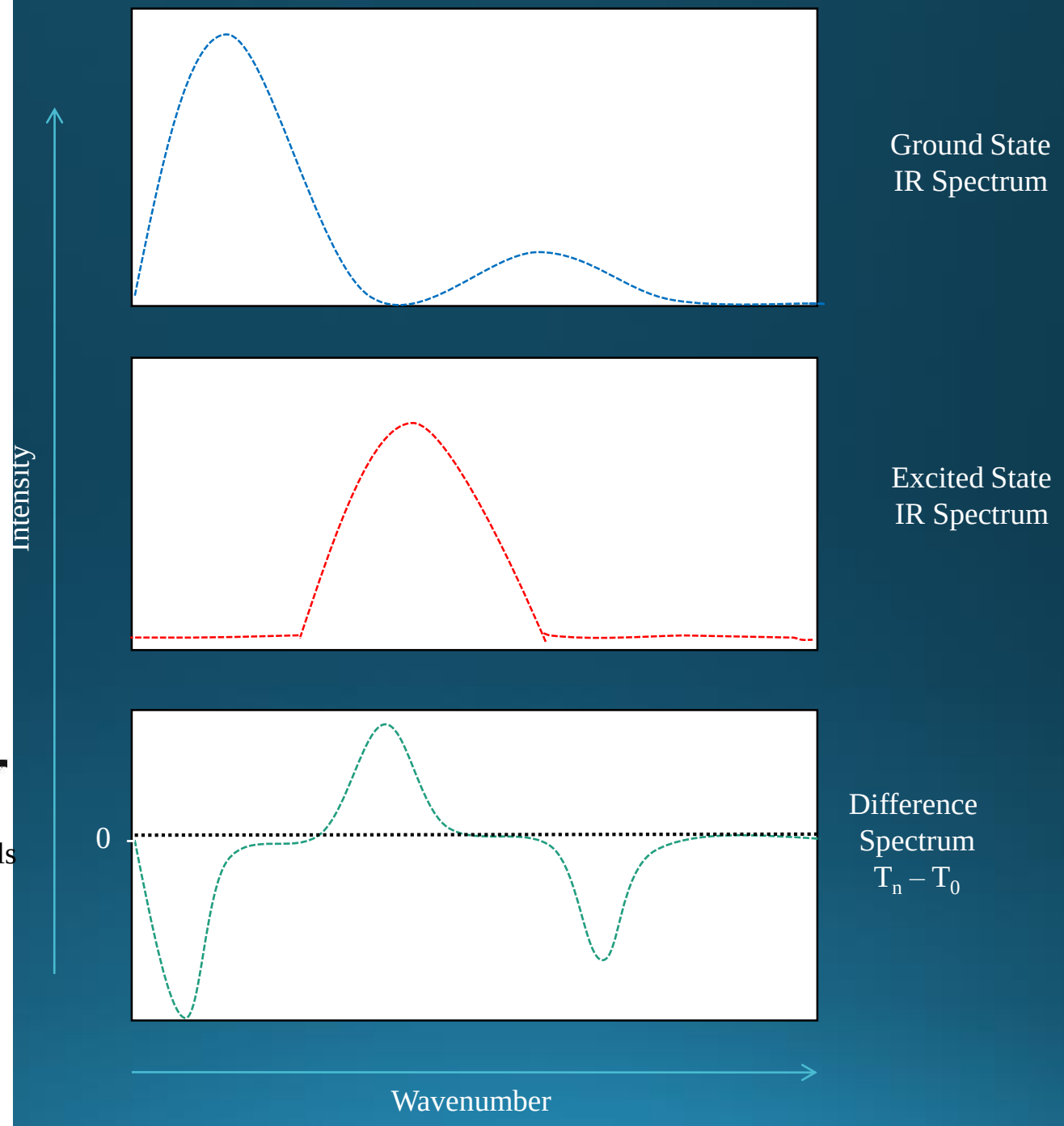


- Single shot spectrum measurements using sensitive array detectors.
- Absorption changes measured by 5 kHz chopping of pump beam.
- Probe beam referenced to remove laser shot noise.

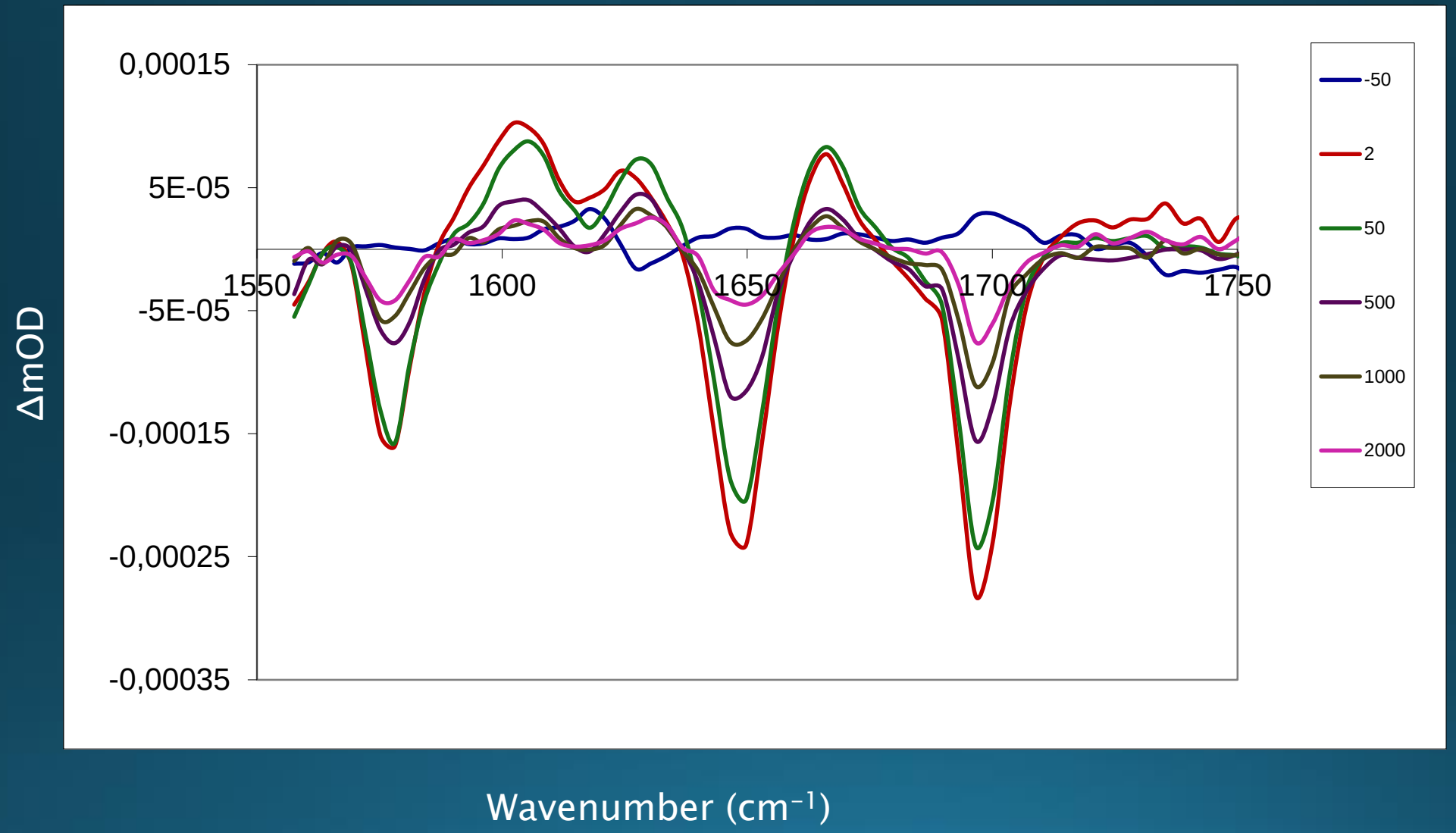
Primary data



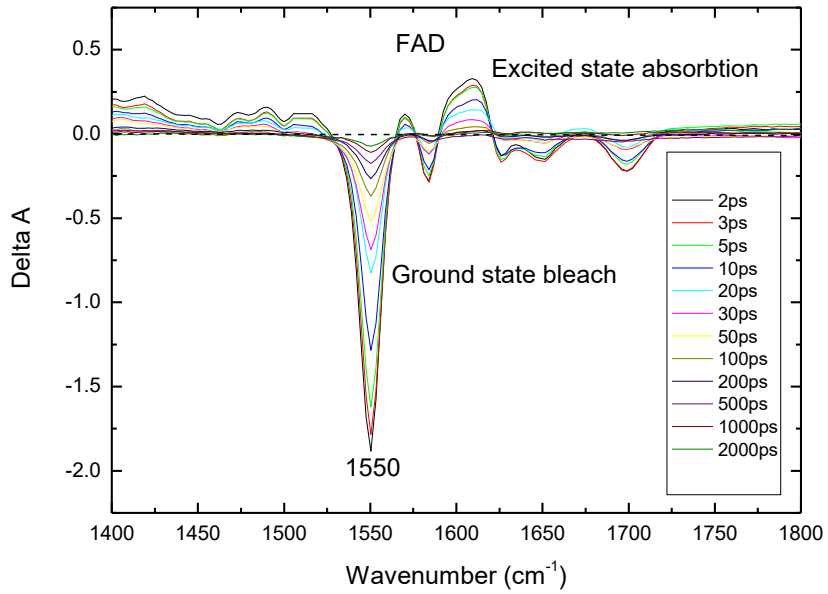

Vibrational
Excite ground state
with 400 nm light
Relaxation to
ground state
Ground State
Vibrational Levels

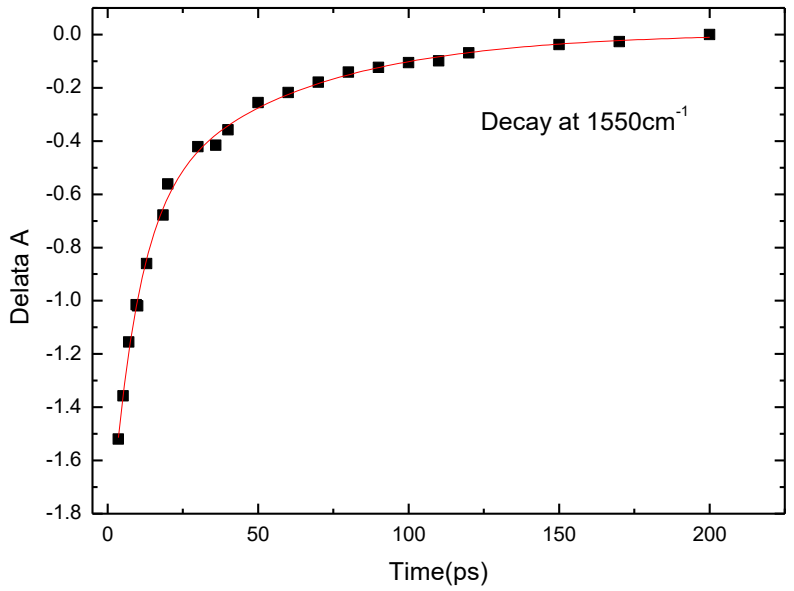

Intensity
Ground State
IR Spectrum
Excited State
IR Spectrum
0
Difference
Spectrum
$T_n - T_0$
Wavenumber

Transient infrared spectra



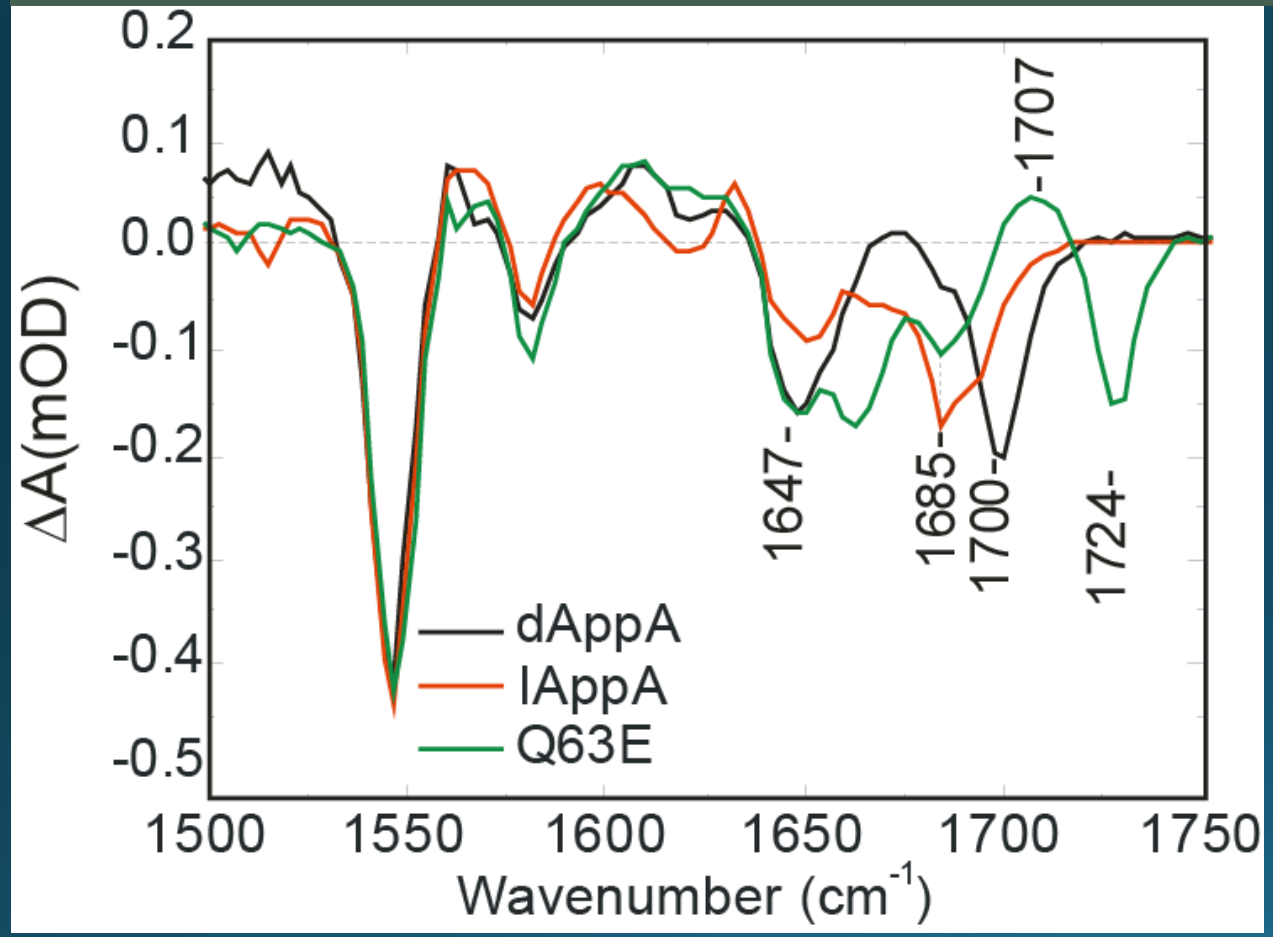
Transient infrared spectra

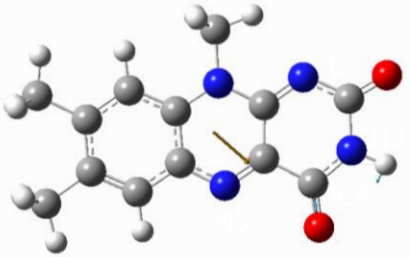




a) TRIR spectra of FAD at various time delays b) Decay curve at 1550 cm^{-1} band

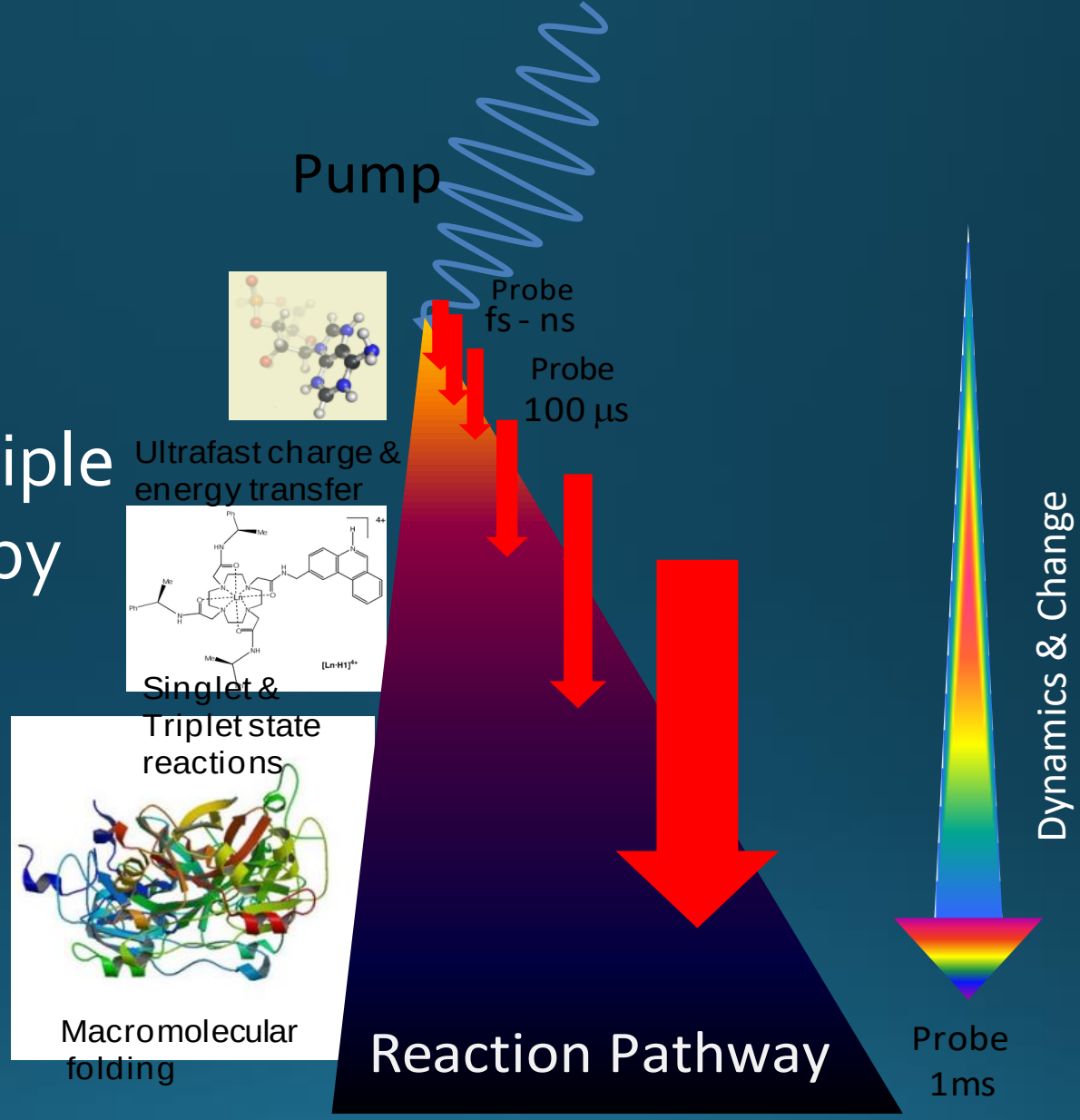
TRIR measurements on AppA



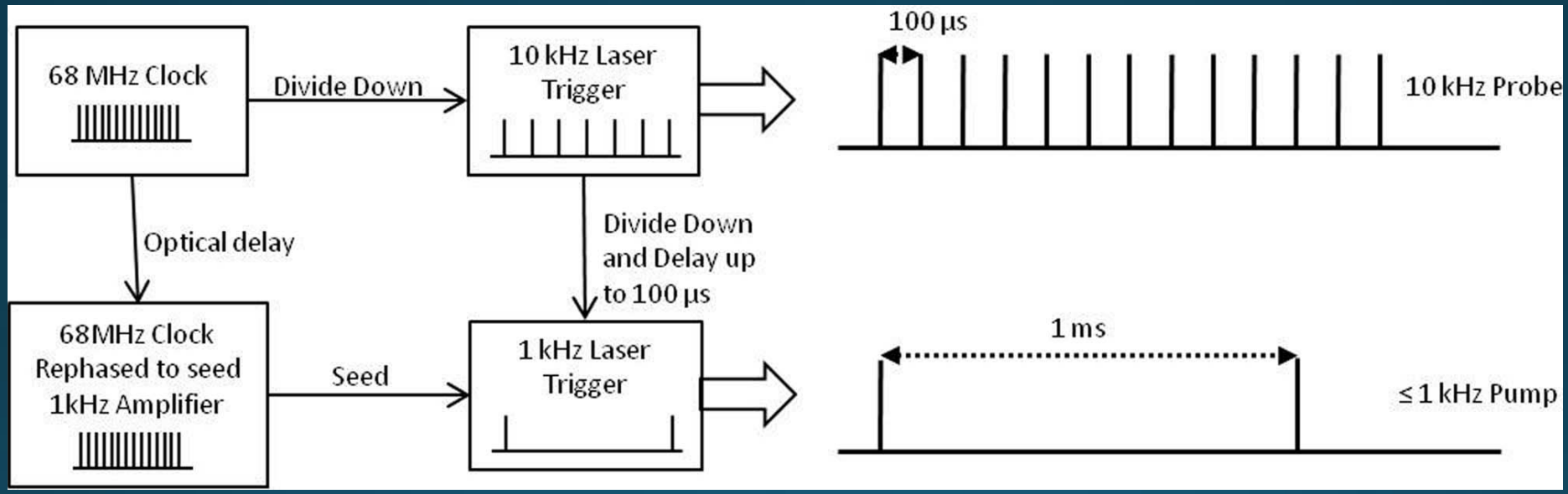


Flavin chromophore

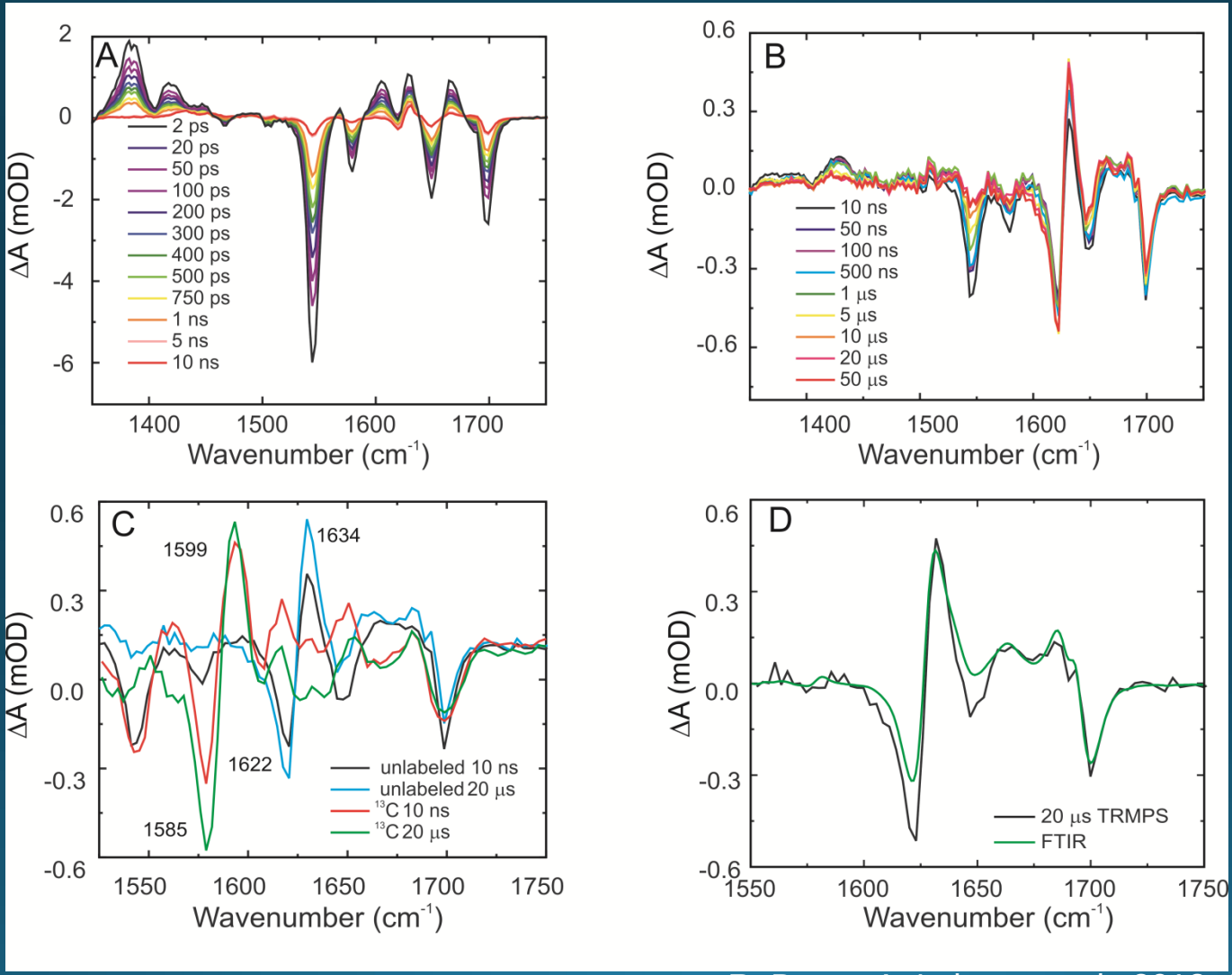
Time Resolved Multiple Probe Spectroscopy



TRMPS concept

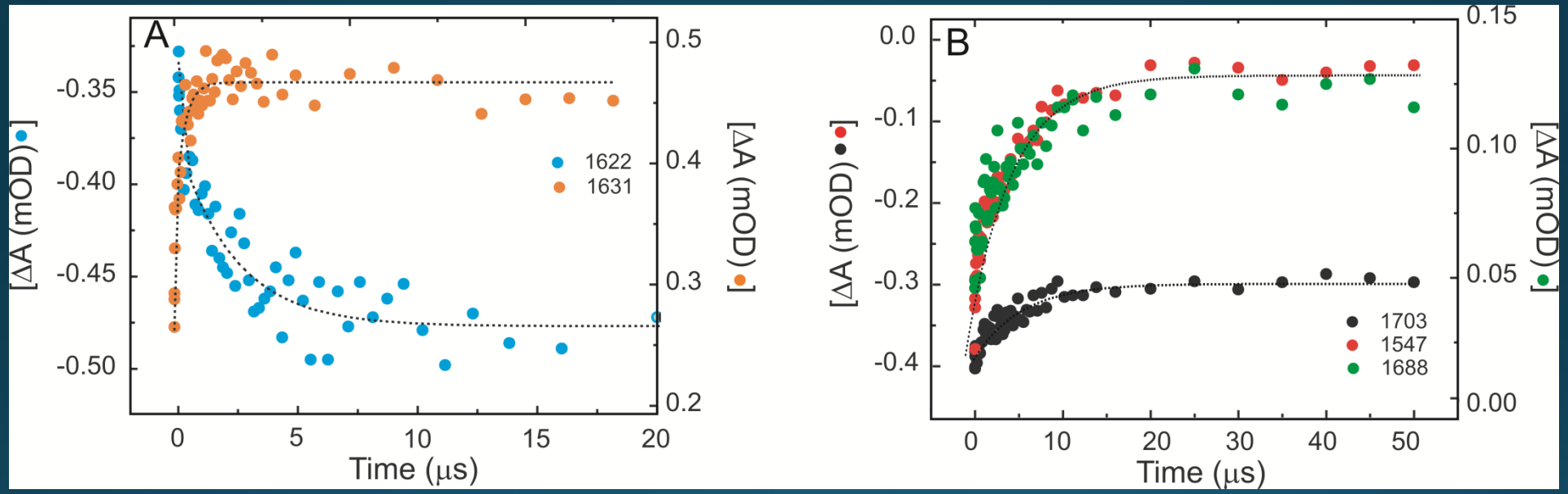


TRMPS on WT AppA



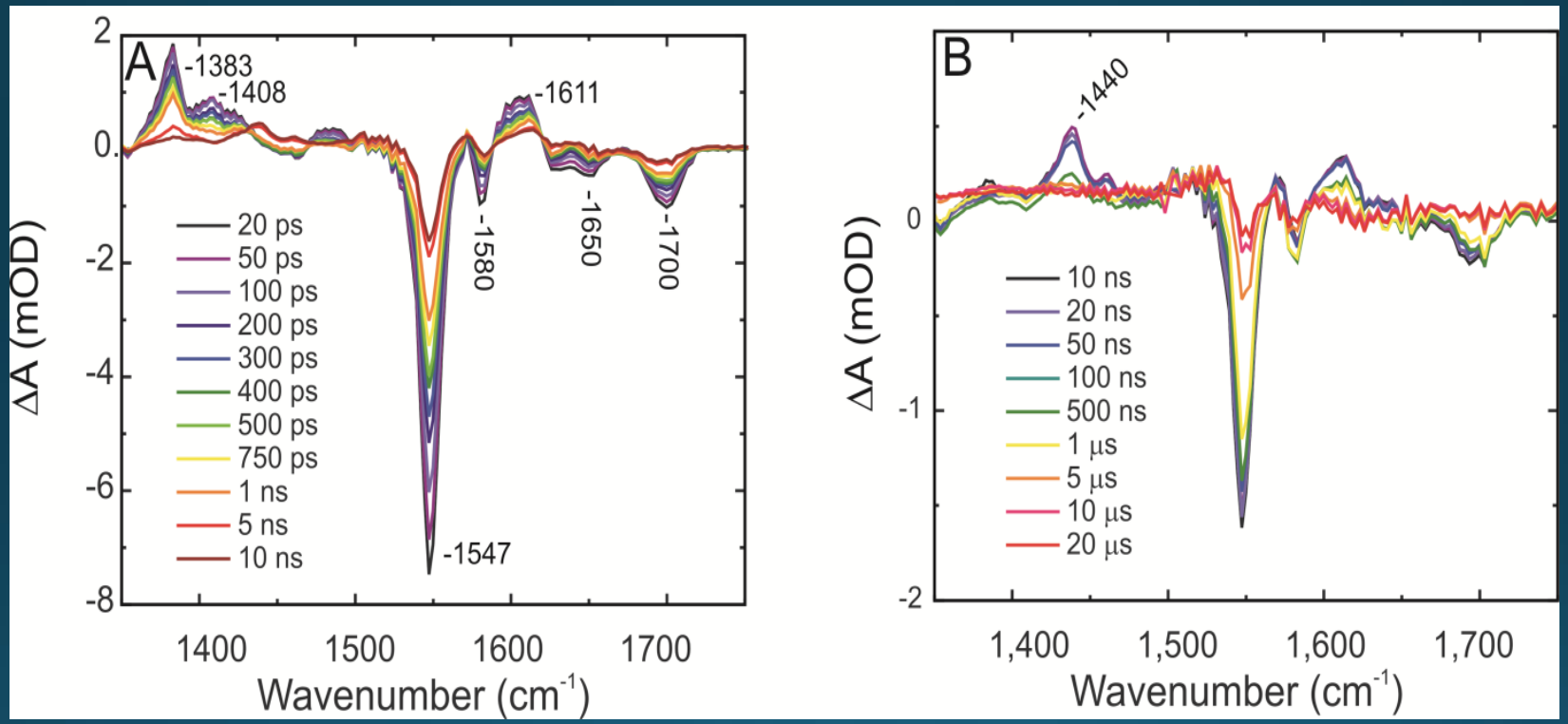
R. Brust , A. Lukacs,et al., 2013, JACS

TRMPS on WT AppA



R. Brust , A. Lukacs,et al., 2013, JACS

TRMPS on FMN



R. Brust , A. Lukacs,et al., 2013, JACS

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Spotlights

pubs.acs.org/JACS

Spotlights on Recent *JACS* Publications

2D π-CONJUGATED POLYMERS: LARGER SIZE, SMALLER BAND GAP

A new computational study brings researchers one step closer to designing tailor-made polymers with desirable electronic properties. The study, performed by Rico Gutzler and Dmitrii Perepichka, sheds light on the relationship between the size of planar two-dimensional π-conjugated polymers and their semiconducting potential (DOI: 10.1021/ja408355p).

Due to a growing interest in 2D materials and their electronic properties, chemists are increasingly exploring new synthetic routes to create semiconducting organic analogues to graphene. To assist in this pursuit, the researchers performed density functional theory calculations to determine the structures and electronic properties of both theoretical and experimentally realized 2D π-conjugated polymers.

The team reports that, compared to their linear, 1D counterparts, 2D polymers that are grown in two directions as planar sheets have smaller band gaps, a quality that is favorable for most electronic device applications. Additionally, the band gap in 2D polymers decreases faster with the number of molecular repeat units in 2D than in 1D polymers. This information may help researchers in the field of band gap engineering design functional organic materials with desirable semiconducting and luminescent properties for applications ranging from optoelectronics to sensing.

Christine Herman, Ph.D.

SHINING A LIGHT ON PROTEIN DYNAMICS

Light can provoke changes in protein structure, which in turn can affect a cell's movement or gene expression. Now a team led by Peter J. Tonge and Stephen R. Meech uses high-speed spectroscopy to reveal how certain proteins change their structure on time scales ranging from 100 fs to 1 ms after the short pulse of light arrives (DOI: 10.1021/ja407265p).

Light is a useful tool for studying protein dynamics because its arrival at the protein is easier to control than other stimuli, such as small molecules. And unlike well-established X-ray studies, infrared observations do not require the proteins to be in crystalline form, so they offer a more natural glimpse of how proteins change their shape in real time.

In this study, the researchers bombard a bacterial protein that naturally responds to light and changing oxygen levels with pulses of blue light. Using a tool called time-resolved multiple probe spectroscopy, the team identifies a hierarchy of activity in the protein's structural changes: residues close to the site of light absorption respond first and then activate more remote parts of the protein. In a mutant version of the protein, the team finds that the response to light is short-circuited. The ability to observe a protein's dynamics across multiple time domains will help test and improve models of protein function and lead to the development of new tools for controlling gene expression with light, the authors say.

Lucas Laursen

NEW CLASS OF HYDROGELS FALLS APART WITH LIGHT

"Smart" biomaterials, which undergo a physical change in response to external stimuli, are of enormous interest to researchers in cellular and biomolecular engineering. A new class of photoresponsive hydrogels, designed by Yan Zhang and co-workers, represents a significant step toward the goal of controlling both the structure and assembly of cellular microenvironments (DOI: 10.1021/ja409000b).

The team synthesizes peptides modified with a small-molecule phototrigger, known as a biaryl-substituted tetrazole. The peptides self-assemble to form a hydrogel that can be used to culture cells in either a 2D or 3D environment. In the presence of UV light, the tetrazole-based phototrigger undergoes an intramolecular ligation that causes the hydrogel to partially disassemble, presumably because the new slightly tilted ring system interrupts the hydrophobic π−π stacking.

To demonstrate the utility of the light-responsive hydrogels in biological applications, the researchers show that they can control the differentiation of a model cell line by using UV light to release a differentiation-inducing protein from within the hydrogel. Efforts to create photopatterned channels that induce different biological behaviors of cultured cells are currently underway, the researchers say.

Christine Herman, Ph.D.

BREAKING DNA, TWICE

A compelling therapeutic strategy for diseases characterized by out-of-control cell growth, like cancer and rheumatoid arthritis, is to kill the cells by inducing DNA damage. Numerous compounds damage DNA by breaking a single strand of the DNA double helix, but molecules that break both strands are more efficient damaging agents. Toward the design of compounds capable of inducing such double strand breaks, Marisa Taverna Porro and Marc Greenberg delineate a chemical pathway that leads to this deadly alteration (DOI: 10.1021/ja409513q).

The authors determine that, in the presence of oxygen, formation of a highly reactive free radical at a particular location within the DNA double-helix structure leads to a double strand break. Specifically, they find that the radical triggers breakage of the first strand, which generates a second radical. This second radical triggers the removal of a hydrogen atom from the double helix, which initiates breakage of the second strand.

These findings provide a starting point for the design of molecules that can produce double strand breaks for potential therapeutic applications. In addition, they may offer insight into the mechanisms by which certain natural products cause double strand breaks in DNA.

Eva J. Gordon, Ph.D.

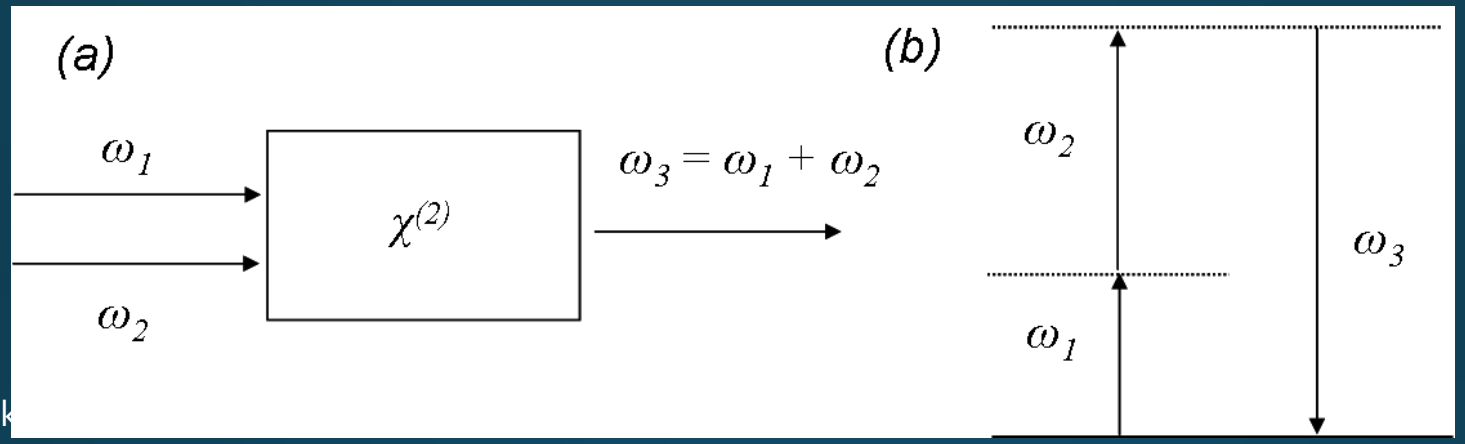
Published: November 13, 2013

dx.doi.org/10.1021/ja41266n | J. Am. Chem. Soc. 2013, 135, 16745−16745

Ultrafast laser spectroscopy

III. Fluorescence upconversion

Frequency mixing



Minak

$\omega_3 = \omega_1 + \omega_2$

$$\vec{k}_3 = \vec{k}_2 + \vec{k}_1$$

$$\Delta k = \vec{k}_3 - \vec{k}_2 - \vec{k}_1$$

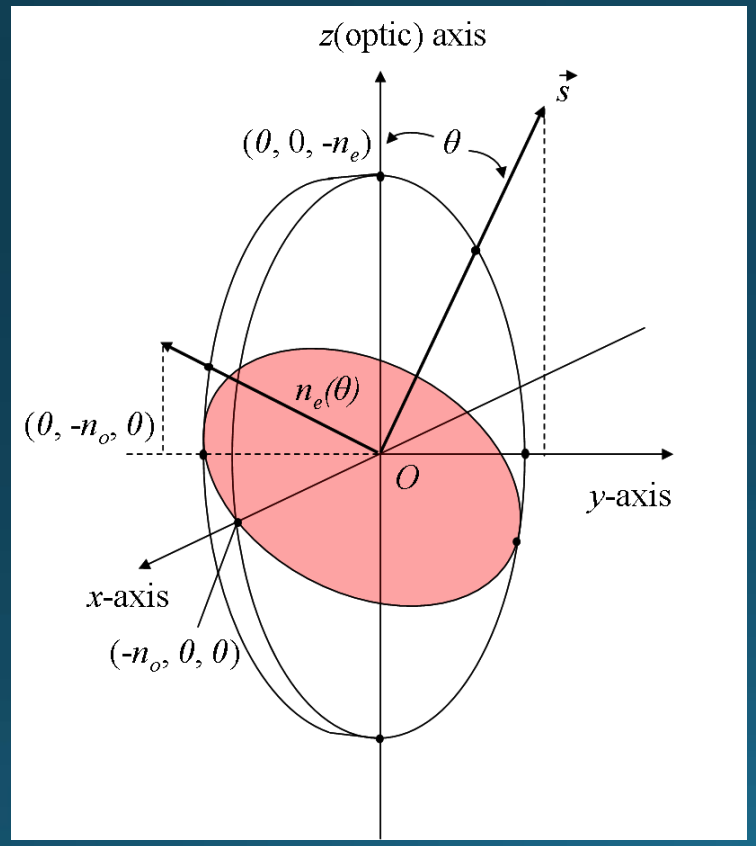
conservation of energy

Conservation of momentum

Phase mismatch

Frequency mixing

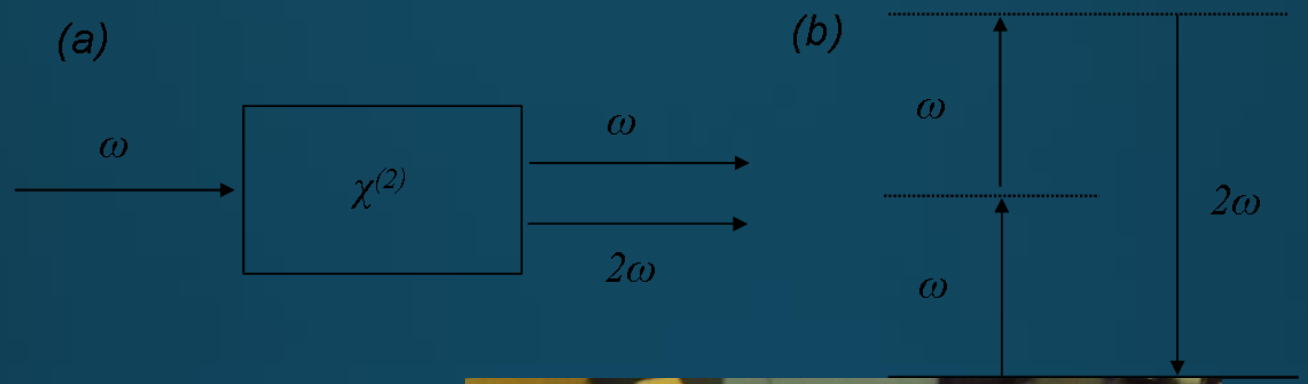
Phase matching in a birefringent crystal (BBO)



$$n_3^e \omega_3 = n_1^o \omega_1 + n_2^o \omega_2$$

$$\sin^2 \theta_m = \frac{(1/n_3^2(\theta_m)) - (1/n_{o3}^2)}{(1/n_{e3}^2) - (1/n_{o3}^2)}$$

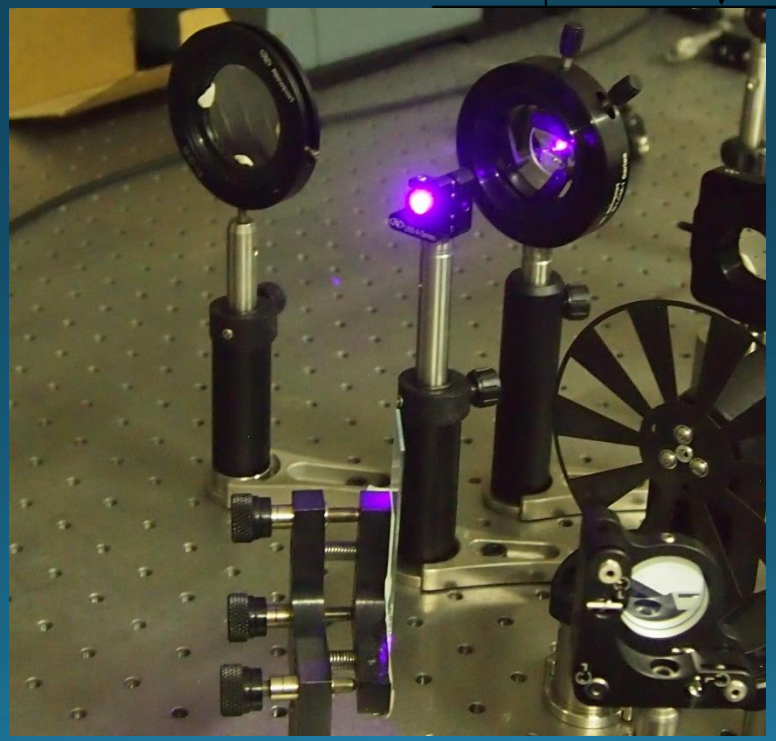
Frequency mixing



Type I. phase matching:

800.0(o)+ 800.0(o)= 400.0(e)

theta = 29.2 deg.



First demonstration of second-harmonic generation

- P.A. Franken, et al, Physical Review Letters 7, p. 118 (1961)

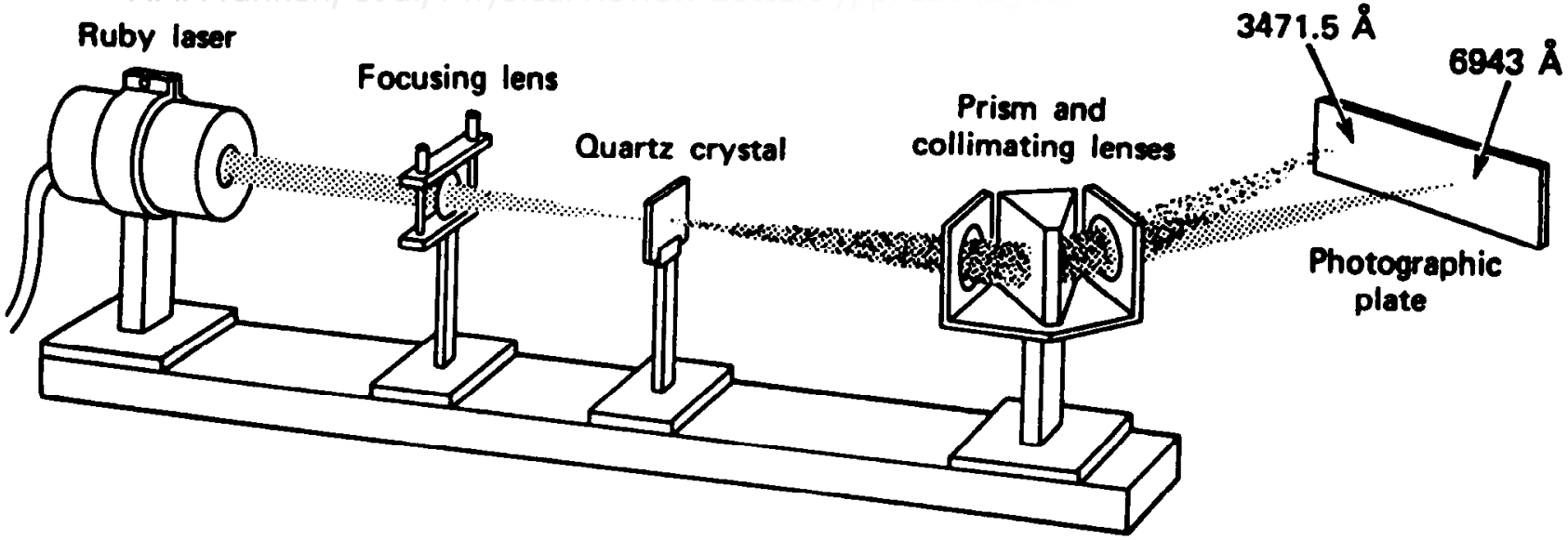
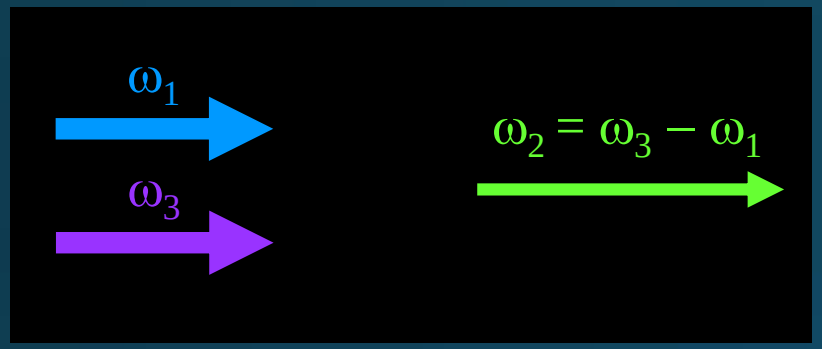


Figure 12.1. Arrangement used in the first experimental demonstration of second-harmonic generation [1]. A ruby-laser beam at $\lambda = 0.694\ \mu$m is focused on a quartz crystal, causing the generation of a (weak) beam at $\frac{1}{2}\lambda = 0.347\ \mu$m. The two beams are then separated by a prism and detected on a photographic plate.

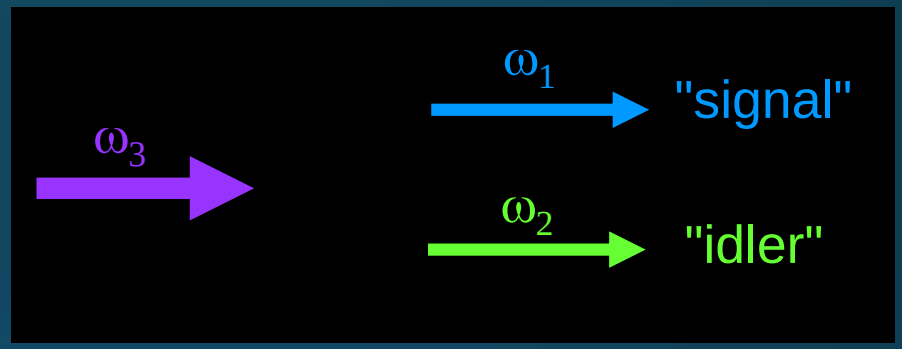
The second-harmonic beam was very weak because the process was not phase-matched.

Difference-Frequency Generation: Optical Parametric Generation, Amplification, Oscillation

Difference-frequency generation takes many useful forms.

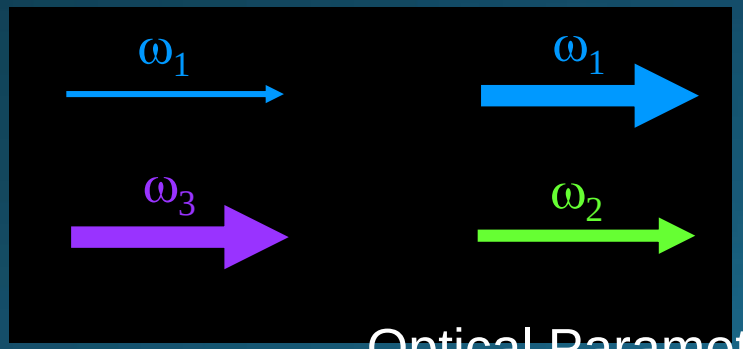


Parametric Down-Conversion
(Difference-frequency generation)

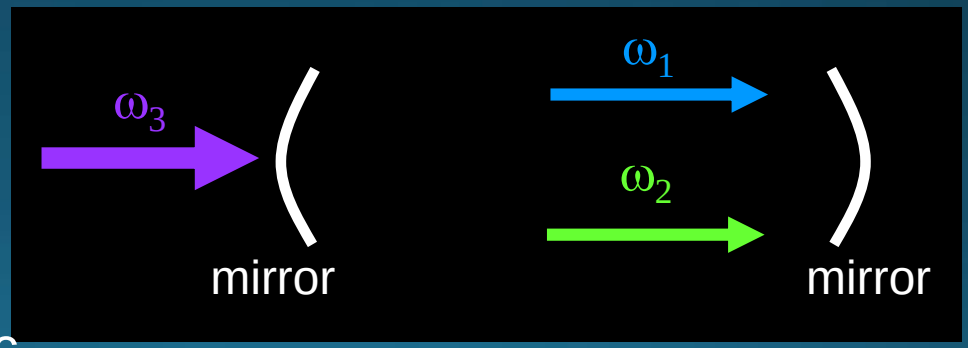


Optical Parametric
Generation (OPG)

By convention:
$\omega_{signal} > \omega_{idler}$



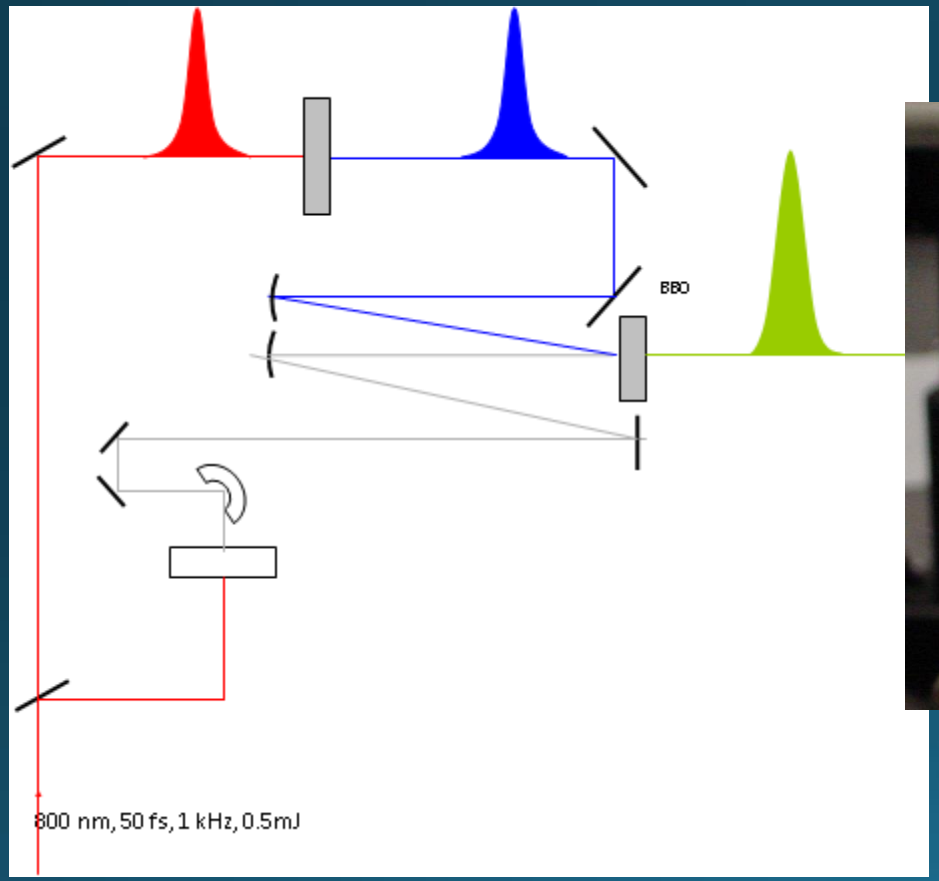
Optical Parametric
Amplification (OPA)

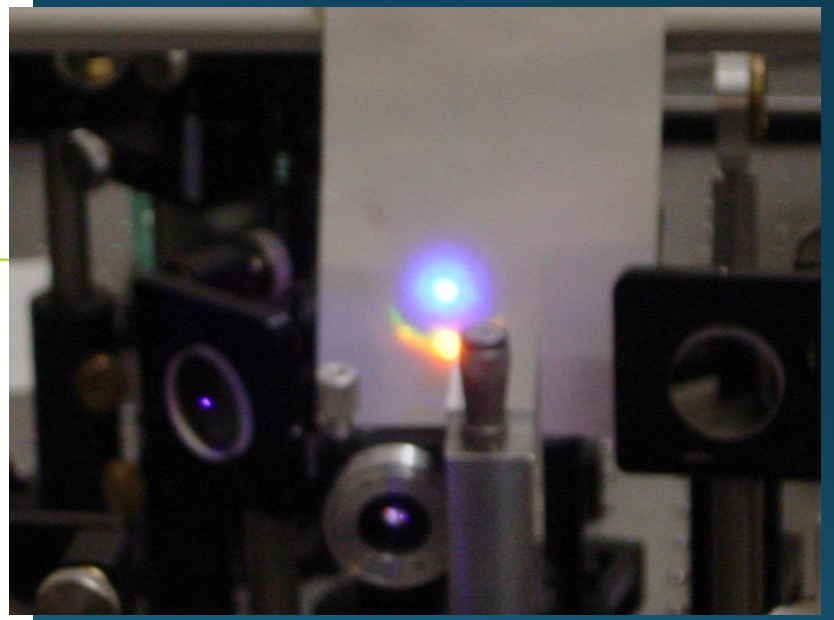


Optical Parametric
Oscillation (OPO)

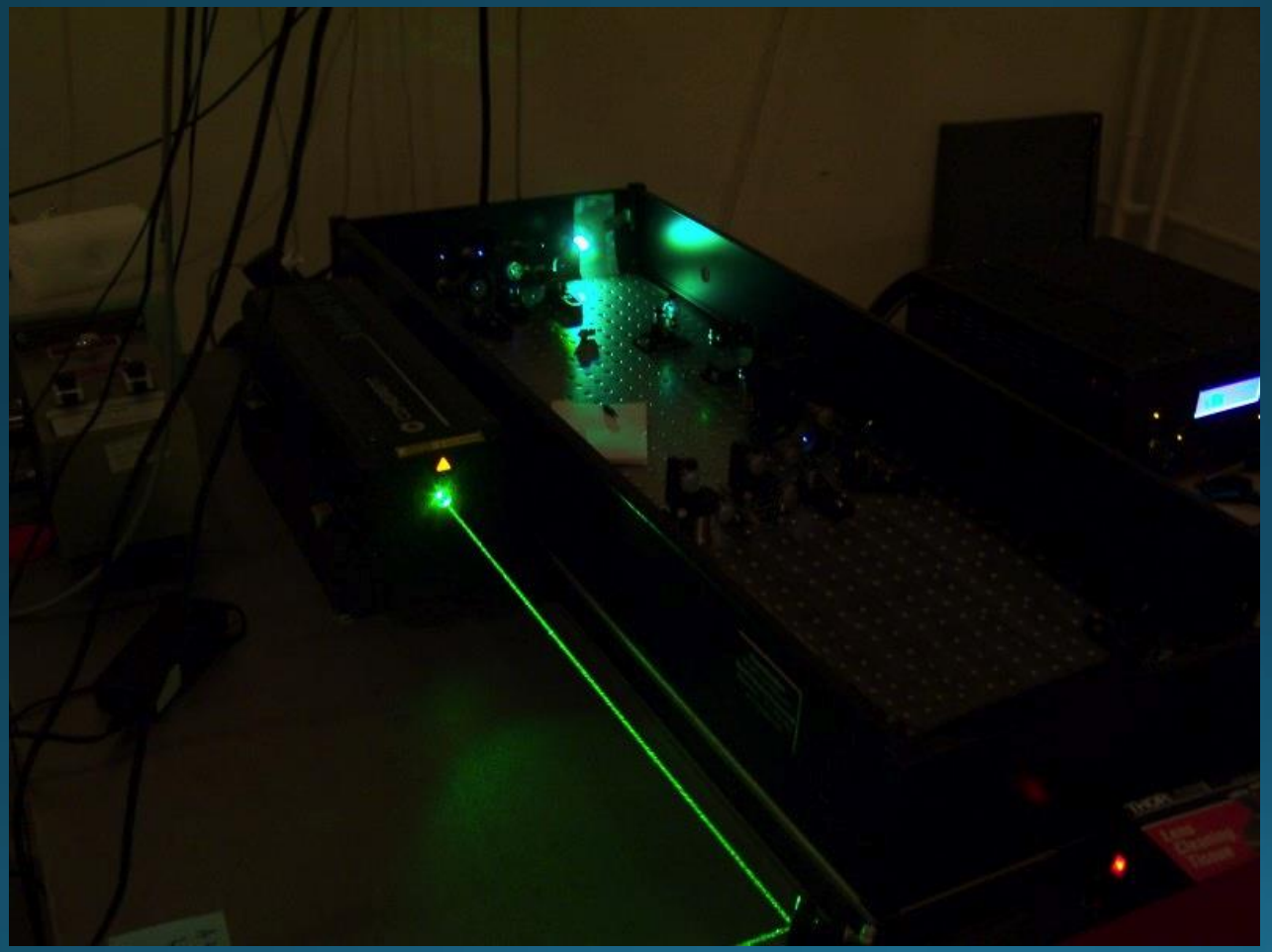
Frequency mixing

Noncollinear optical amplification



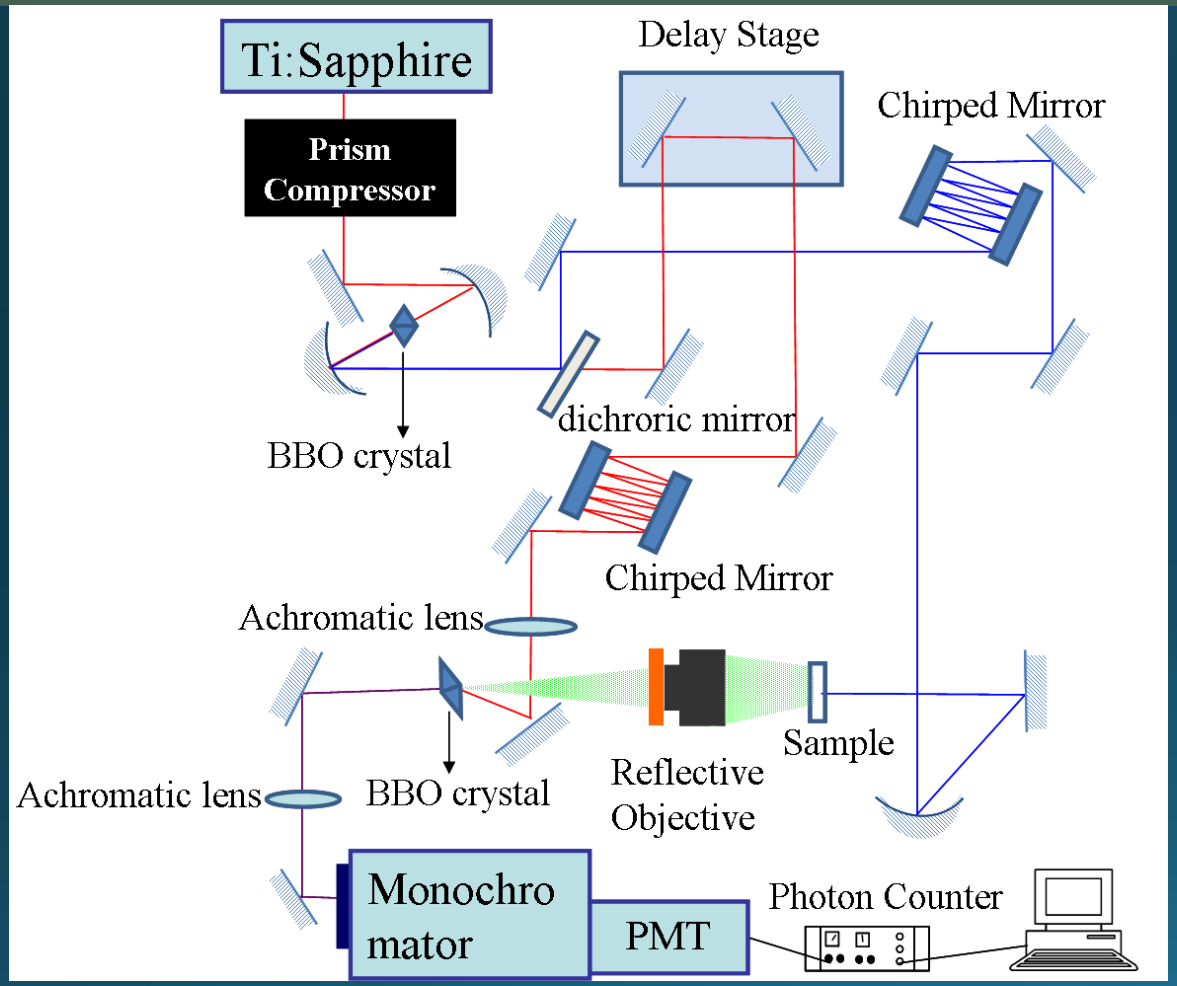


Frequency mixing (OPOs)



Optical parametric oscillators are tunable femtosecond light sources. They are working at the same repetition rate as the oscillator

Fluorescence upconversion

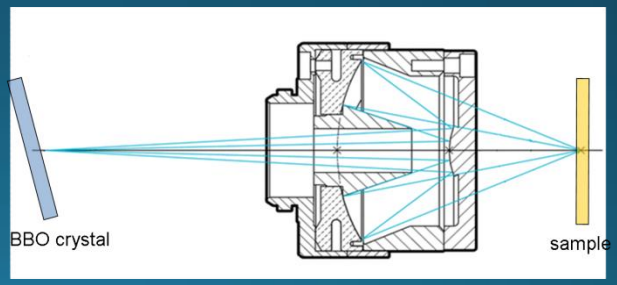


Type I. phase matching:

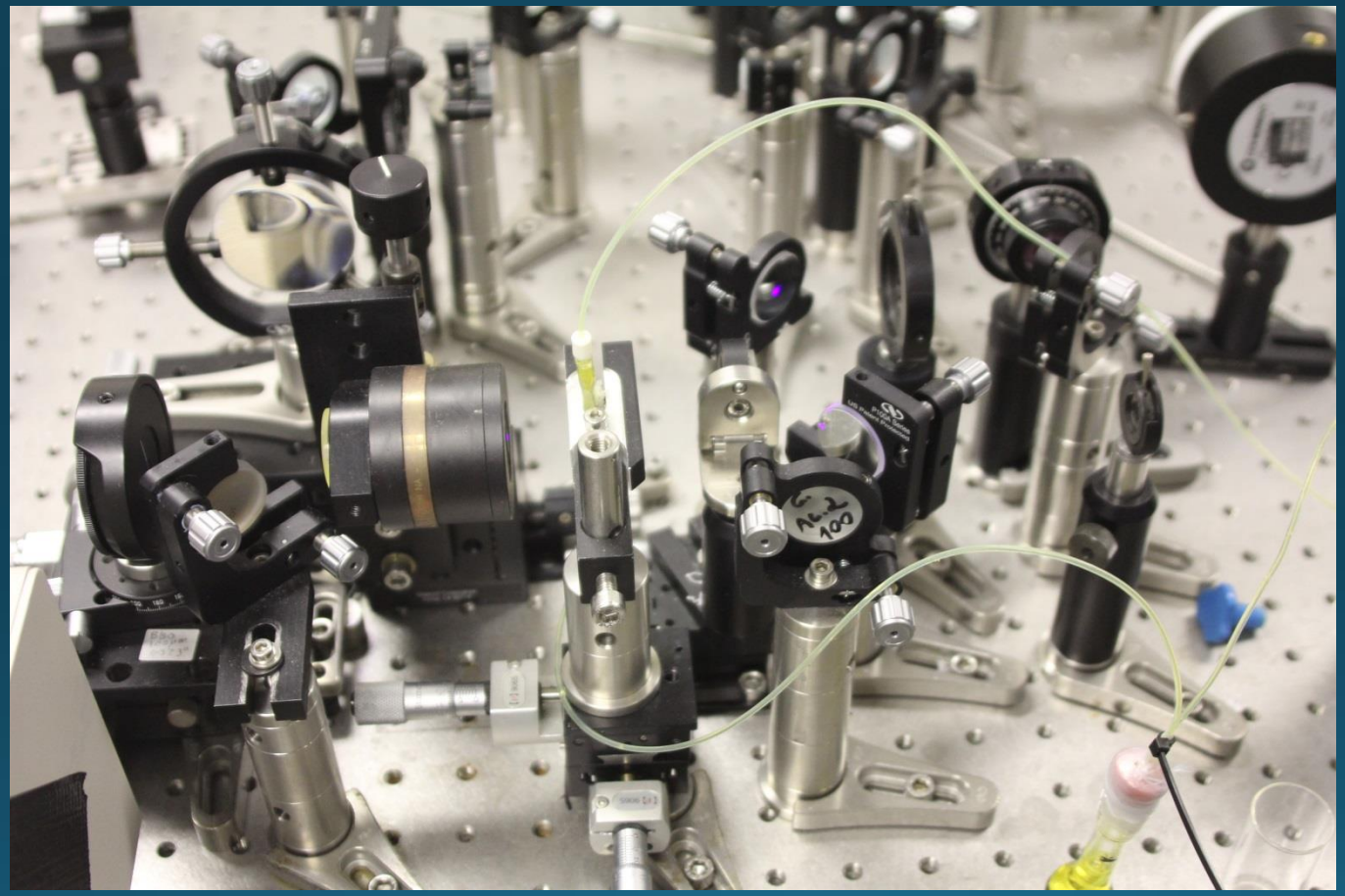
800.0(0)+ 520.0(0)= 315.2 (e)

Theta = 37.2 deg

Cassegrain objective

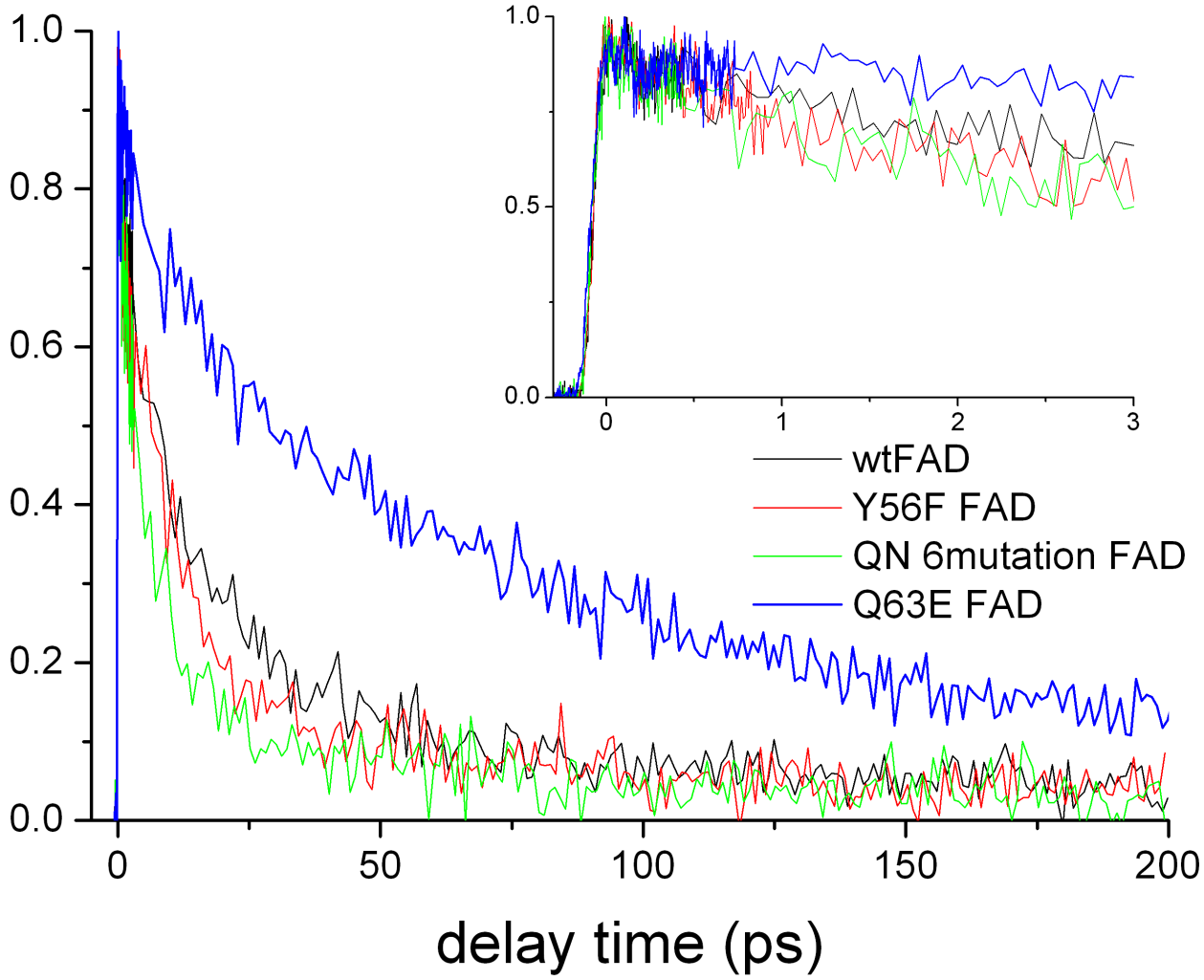


Fluorescence upconversion



Upconversion fluorescence setup, UEA, 2012

Fluorescence lifetime of WT AppA and Y56F, Q63E mutants



Fluorescence lifetime of WT AppA and Y56F, Q63E mutants

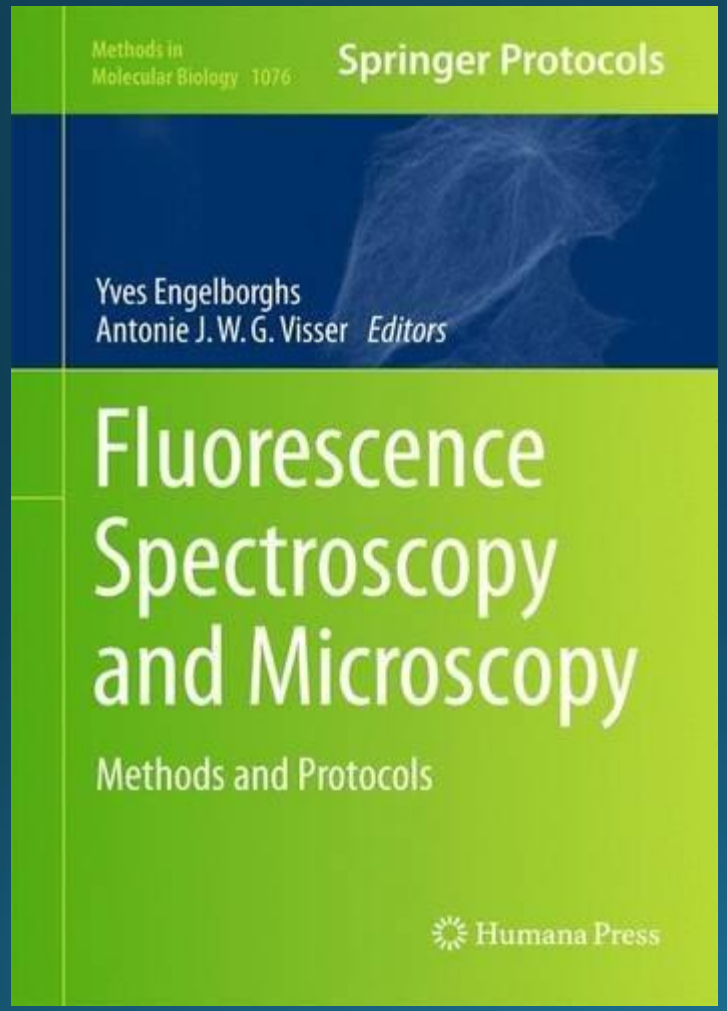
	wtFAD	Y56F FAD	QN 6mutation FAD	Q63E FAD
t_1	1.44	0.39	1.91	5.53
a_1	0.22	0.27	0.29	0.13
t_2	15.17	11.26	8.64	35.52
a_2	0.57	0.65	0.52	0.32
t_3	148.96	156.56	147.66	159.38
a_3	0.15	0.12	0.10	0.44
A_1	0.23	0.26	0.32	0.15
A_2	0.61	0.63	0.57	0.36
A_3	0.16	0.11	0.11	0.49
$<\tau>$ /ps	32.95	24.73	21.82	92.12

	Q63E Rf	wtRf
t_1	1.44	0.70
a_1	0.11	0.32
t_2	26.62	15.71
a_2	0.39	0.54
t_3	153.16	133.02
a_3	0.40	0.07
A_1	0.13	0.34
A_2	0.43	0.58
A_3	0.45	0.08
$<\tau>$ /ps	80.05	19.44

Ultrafast laser spectroscopy

IV. Kerr-gated fluorescence spectroscopy

Kerr-gate fluorescence spectroscopy

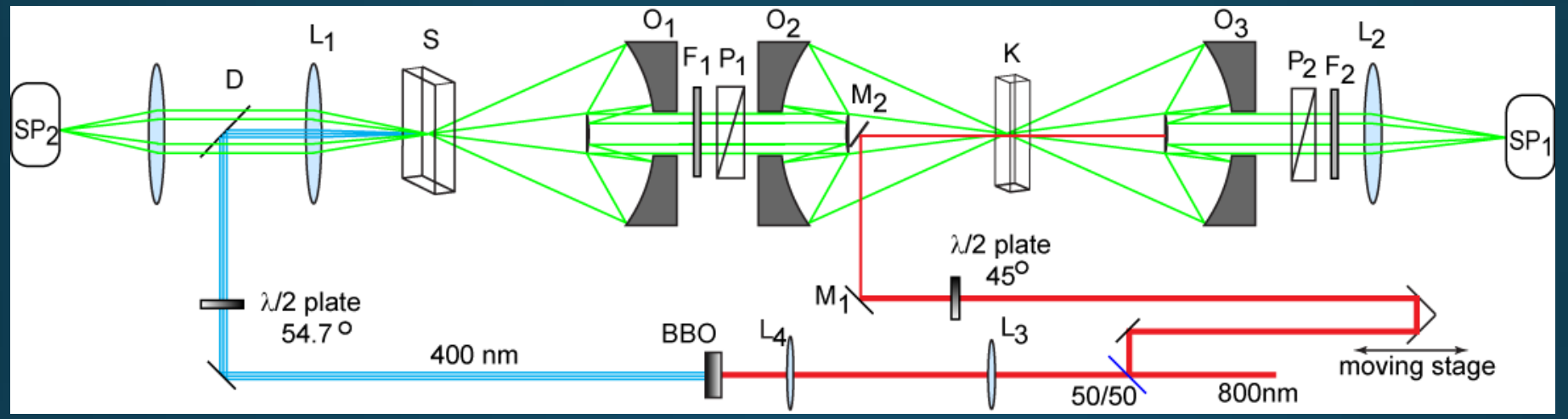


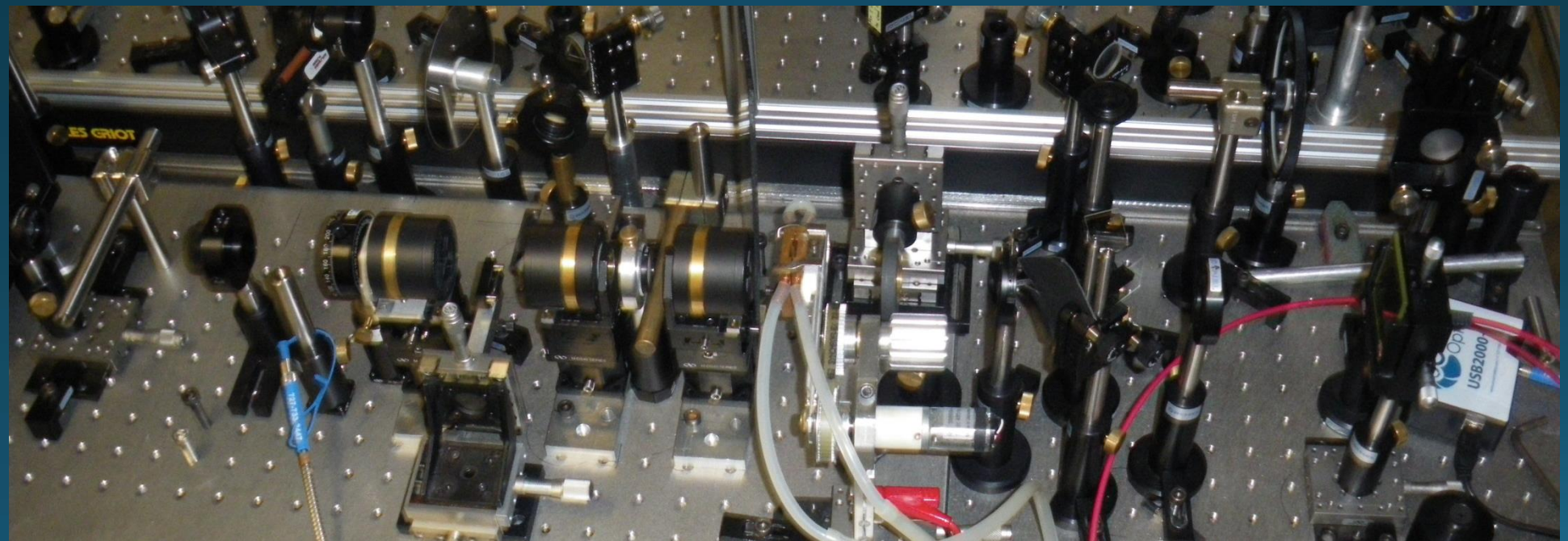
Laptenok SP, Nuernberger P, Lukacs A, Vos MH:
Subpicosecond Kerr-Gate Spectrofluorometry.

in Fluorescence Spectroscopy and Microscopy Methods in Molecular Biology Volume 1076, 2014, pp 321-336 (Springer Protocols)

- Optical Kerr-effect: high laser intensity will change the refractive index of the optical material
- $n=n_0+n_2I$ if the intensity (I) of the laser pulse is high
- The incident laser pulse will induce a change in polarisation

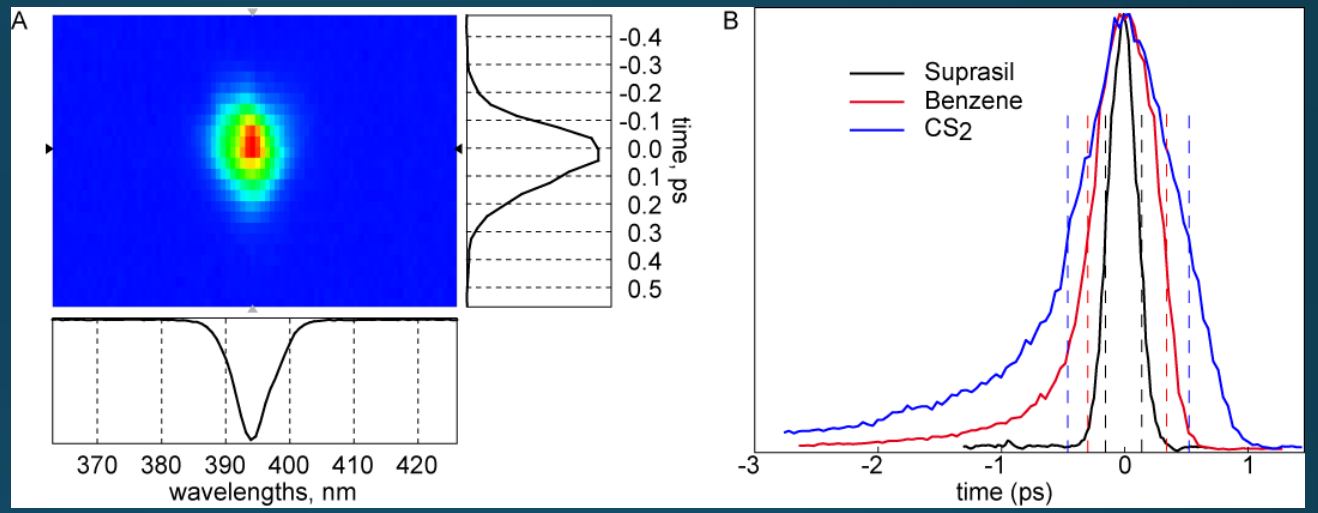
Kerr-gate setup





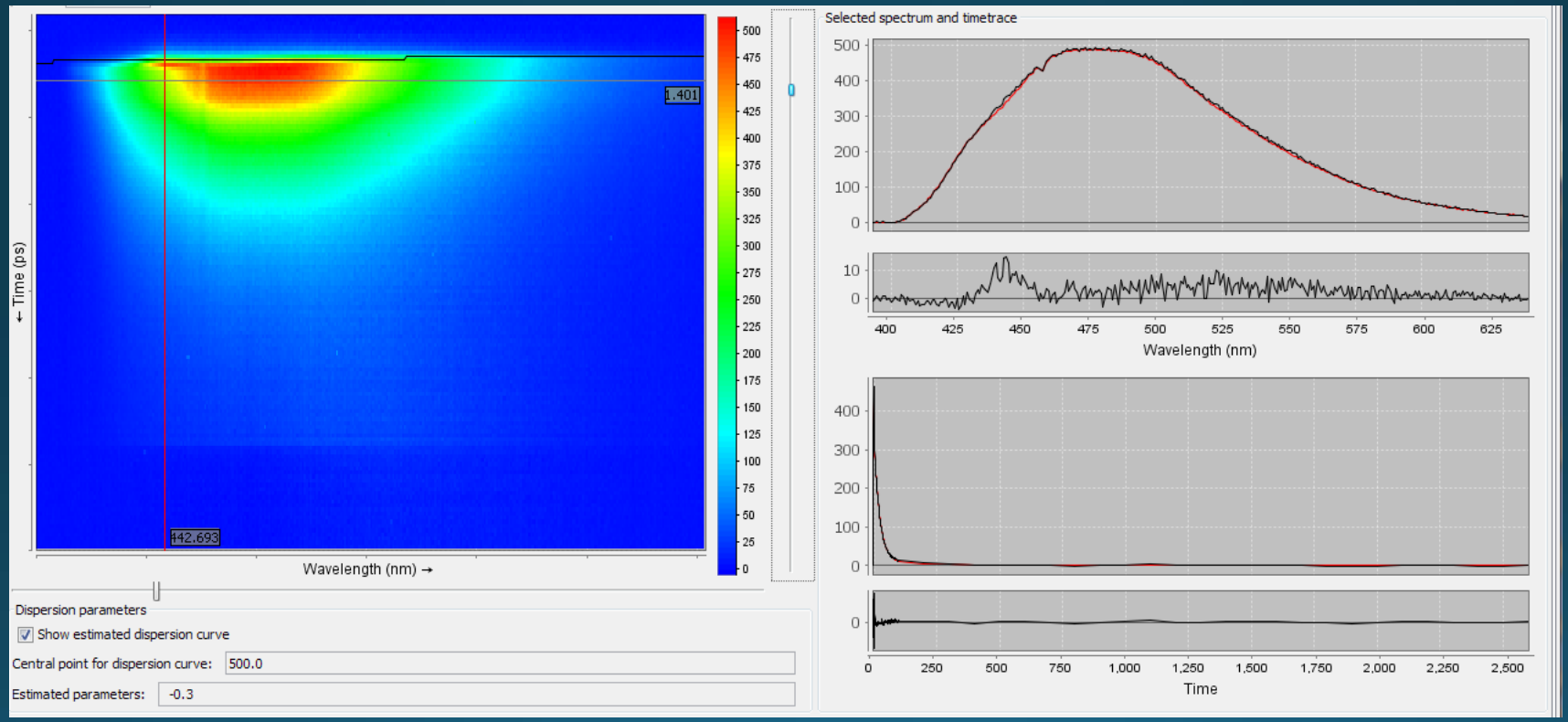
Kerr-gate setup

Pick-up mirror on the Cassegrain objective

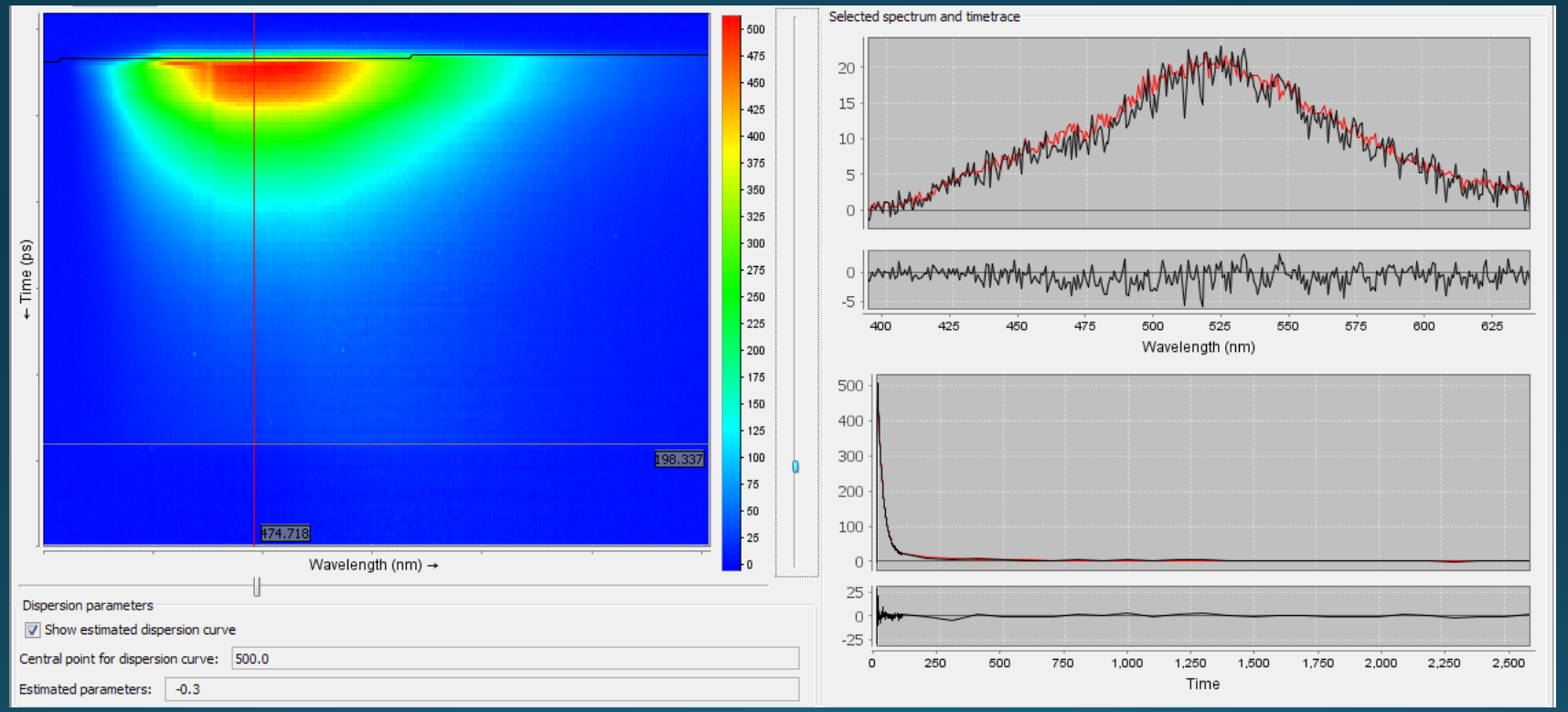


Comparison of Kerr-materials

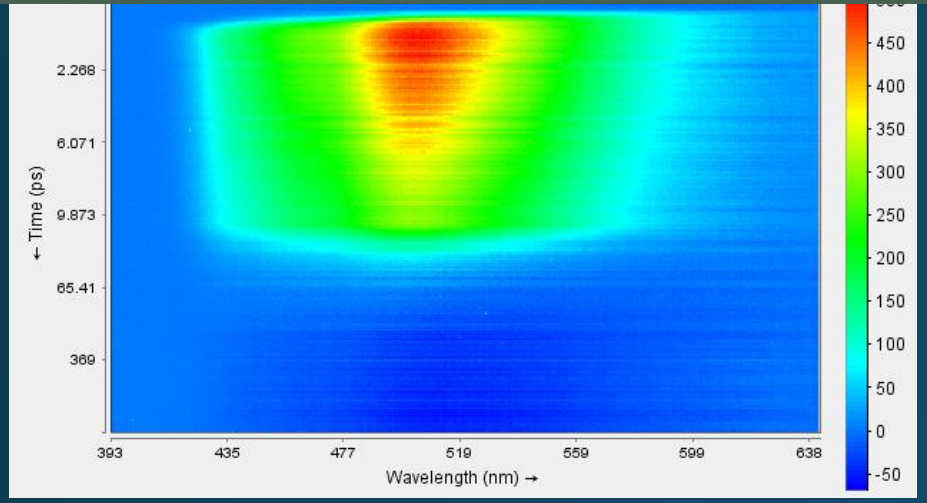
Fluorescence lifetime of MTHF in N378D photolyase

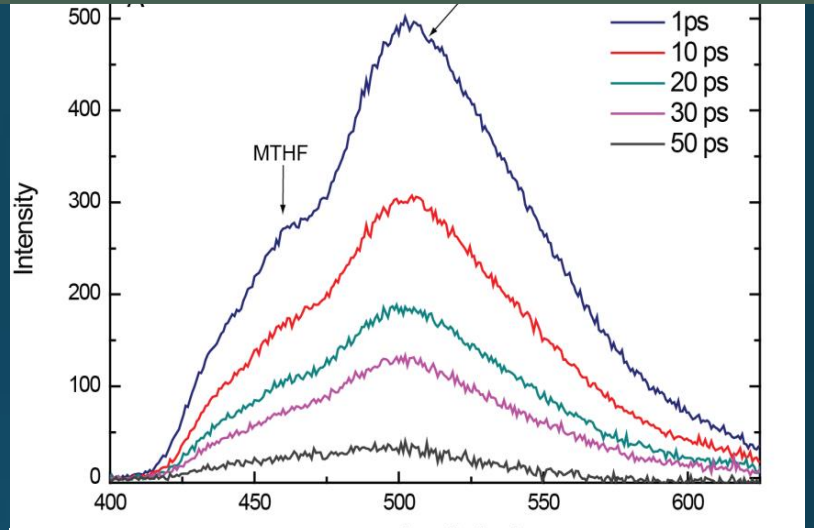


Fluorescence lifetime of MTHF in N378D photolyase



Fluorescence lifetime of MTHF in N378D photolyase





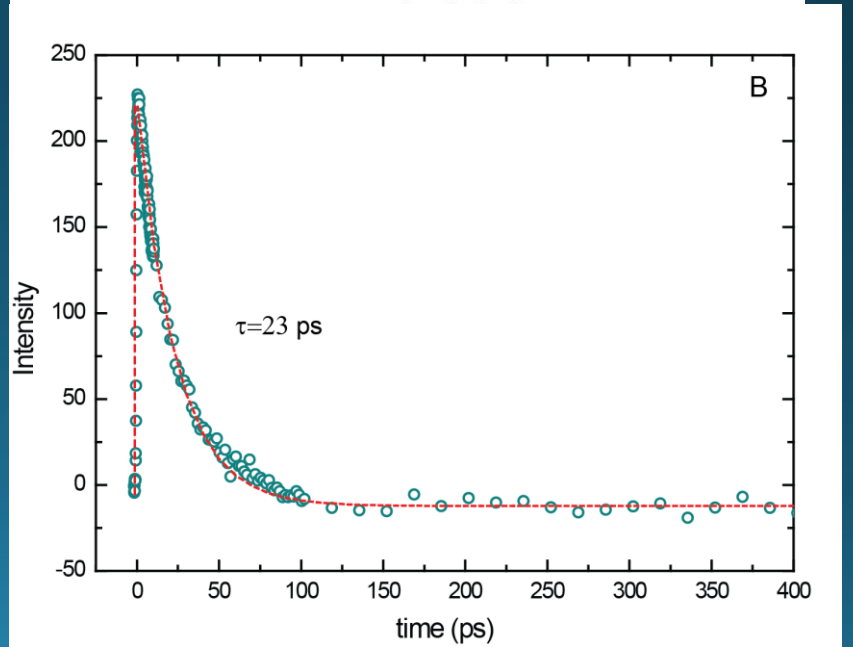
$\tau_{MTHF} = 354$ ps

$\tau_{MTHF\text{-}FAD} = 23$ ps

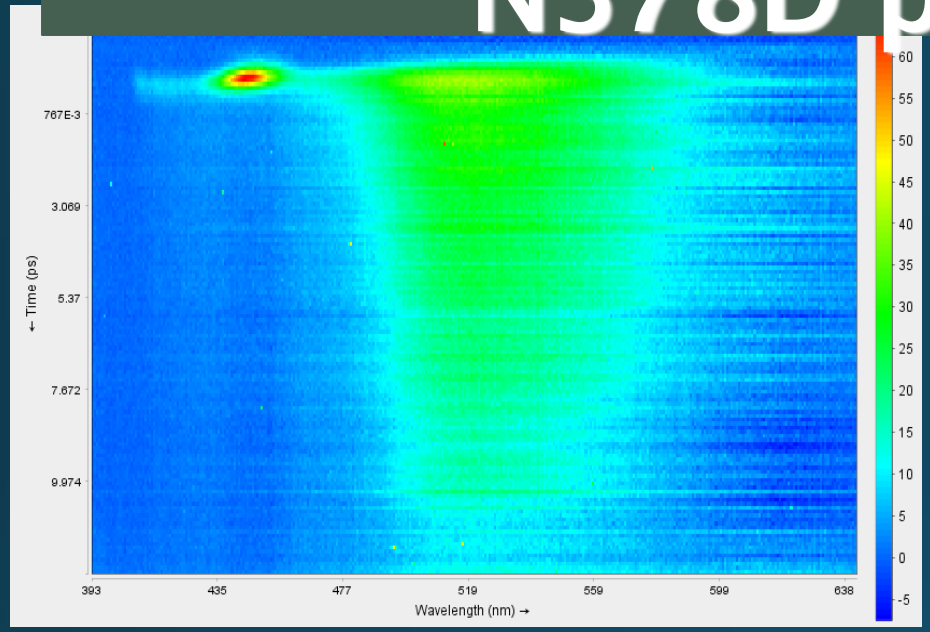
$E = 1 - \frac{\tau_{DA}}{\tau_D} = 0.935$

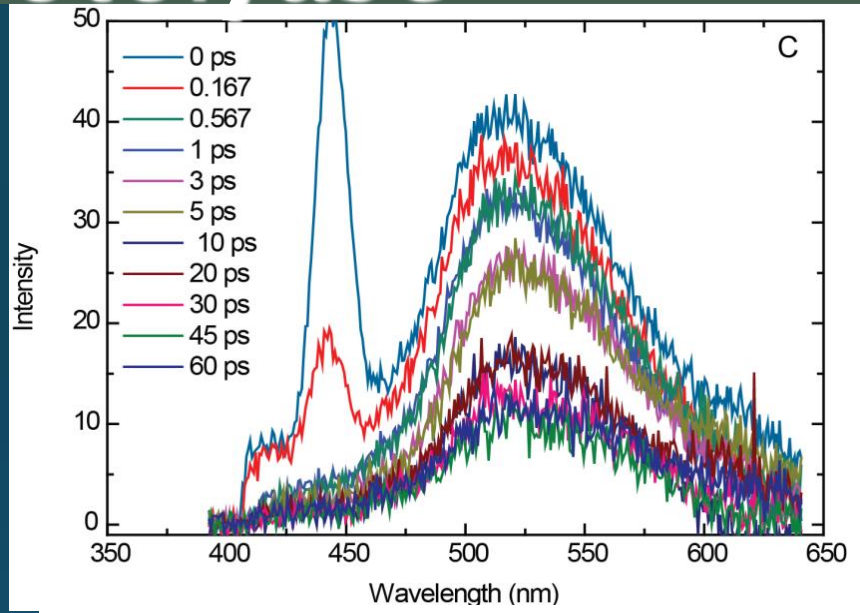
$E = \frac{R_0^6}{R_0^6 + R^6}$

R = 17.53Å



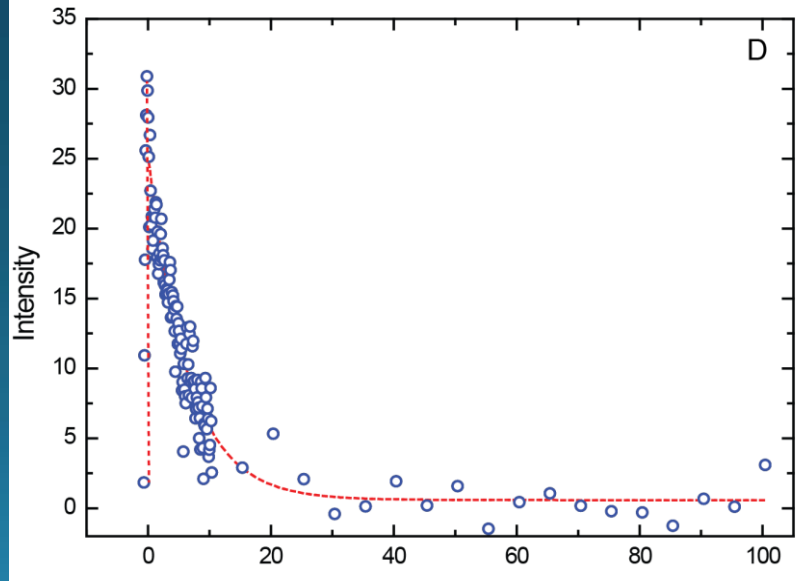
Fluorescence lifetime of FAD in N378D photolyase



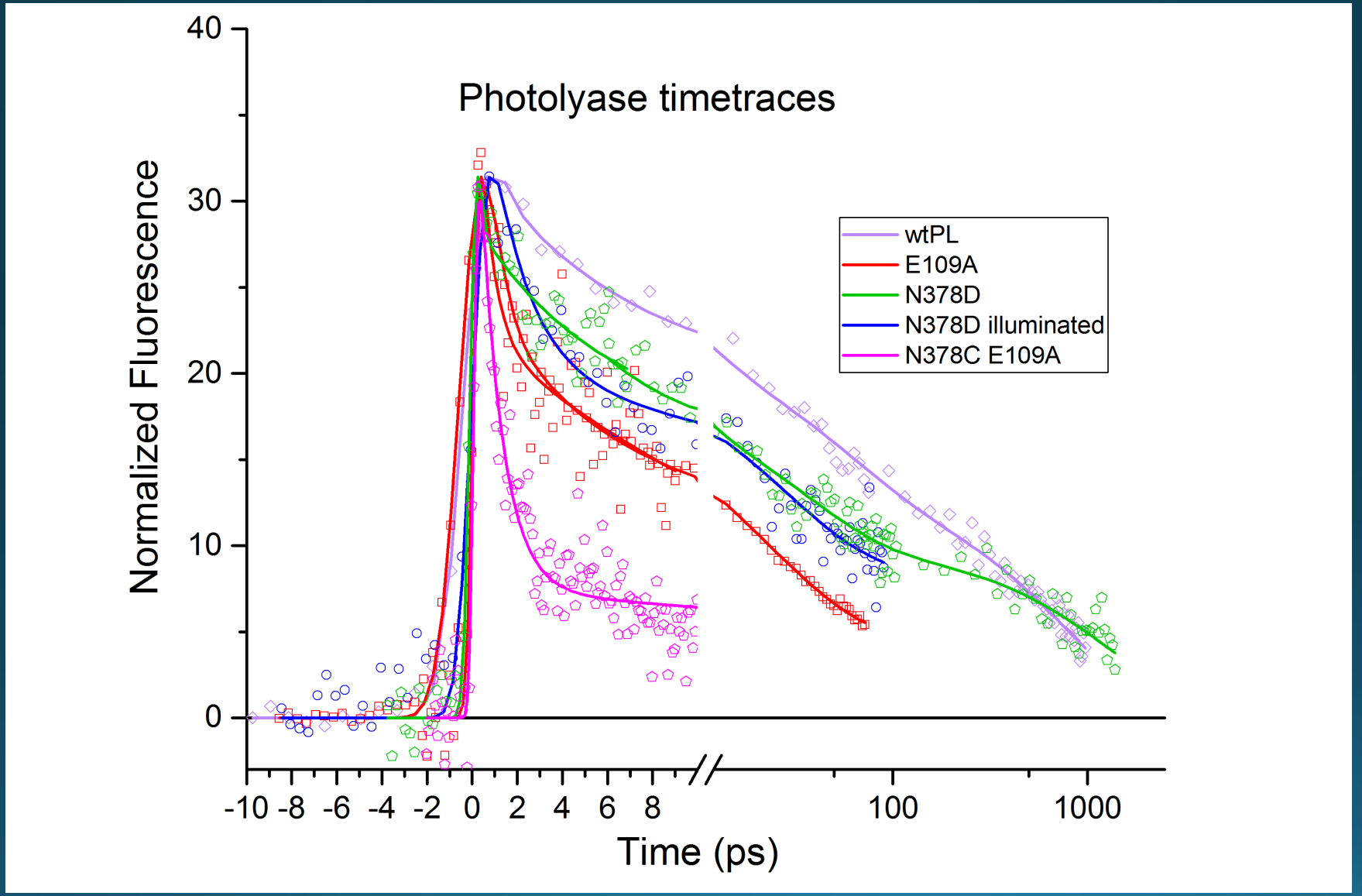


$\tau_{N378D\text{-}FAD} \sim 6$ ps

$\tau_{FAD} \sim 9$ ps



Transient fluorescence of PI mutants



Acknowledgements

University of East Anglia

Prof. Stepen R Meech
Dr. Sergey Laptenok

Stony Brook University

Prof. Peter Tonge
Allison Haigney
Richard Brust
Agnieszka Gil

Ecole Polytechnique

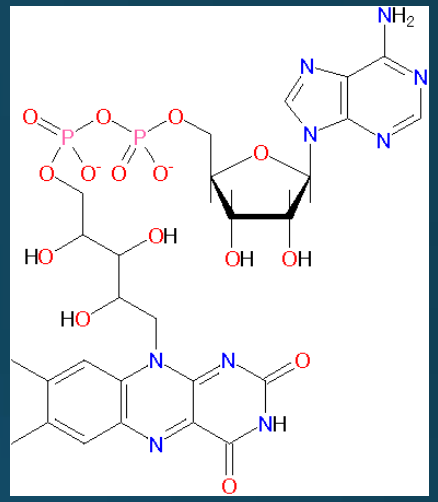
Dr. Marten H. Vos

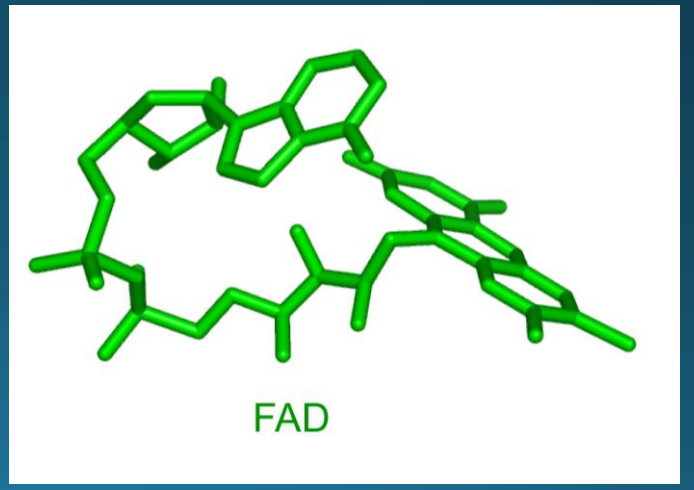
University of Pécs

Prof. Miklós Nyitrai
Dr. László Grama
Dr. József Orbán

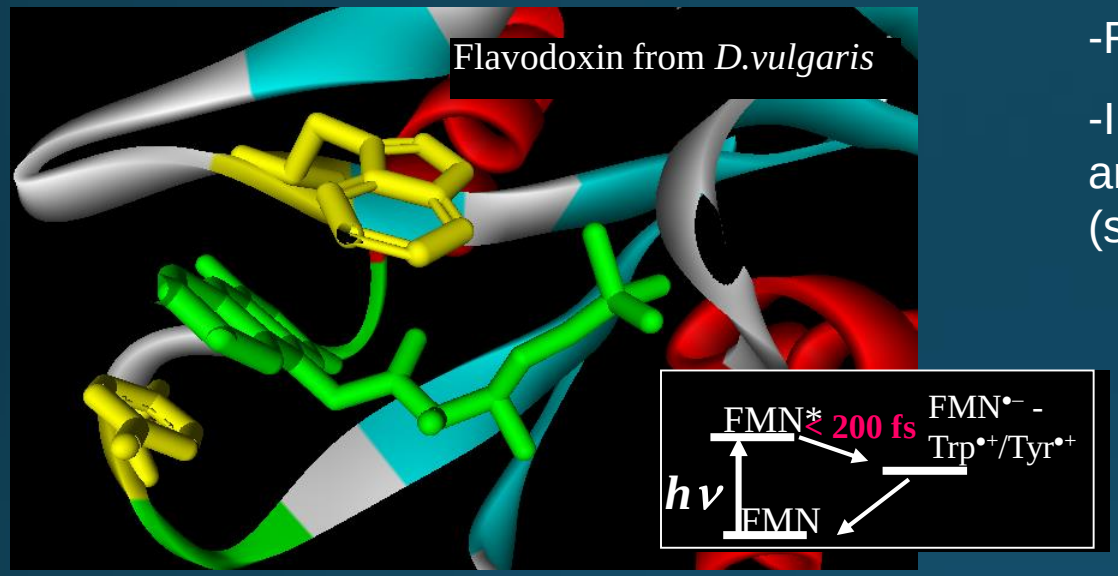
Flavoproteins

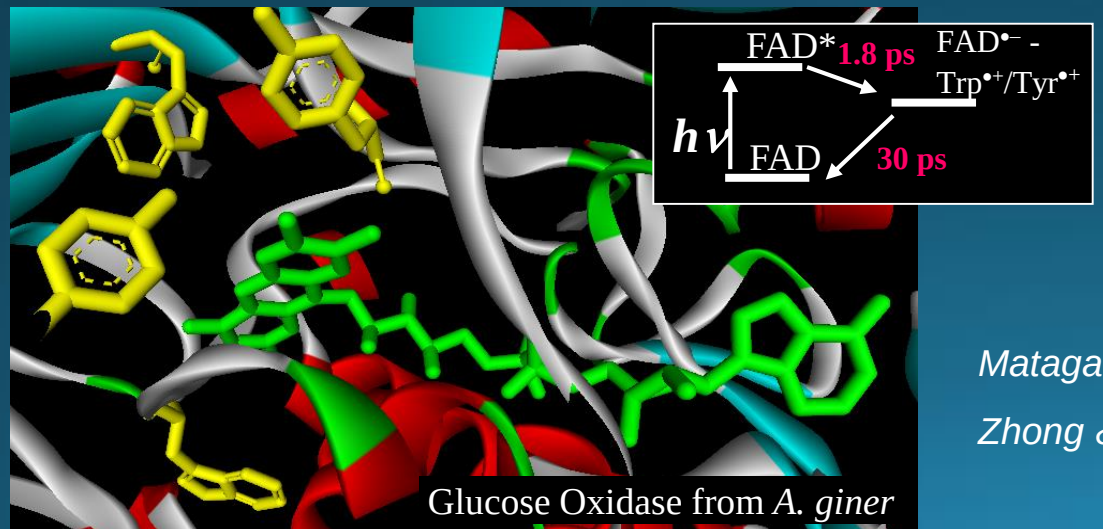
- More than 150 enzymes use flavin (FAD or FMN) as a cofactor
- Essential for many biochemical processes
- Rich redox and proton chemistry
- Most encountered as (light-independent) redox intermediate
- Absorb UV and blue light
- Some flavoproteins are photoactive





Flavin photochemistry



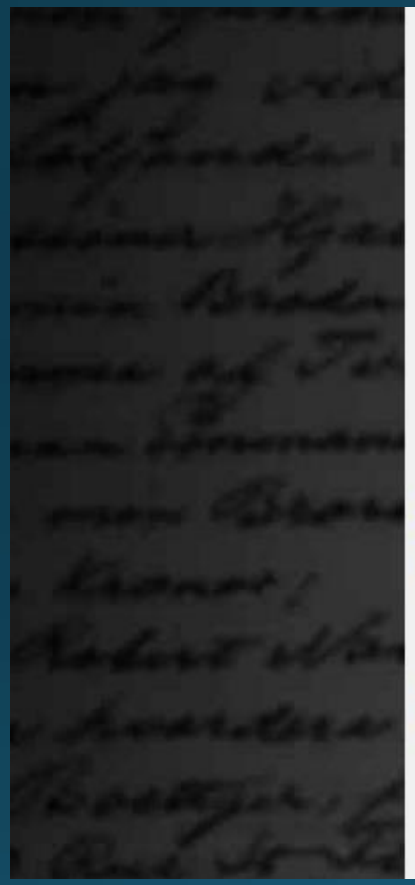


-FAD* lifetime in solution ~4 ns

-In proteins: quenching by ET from aromatic residues and subsequent (sub-)picosecond recombination:

Mataga et al, 2000

Zhong & Zewail, 2001



The Nobel Prize in Chemistry 2015

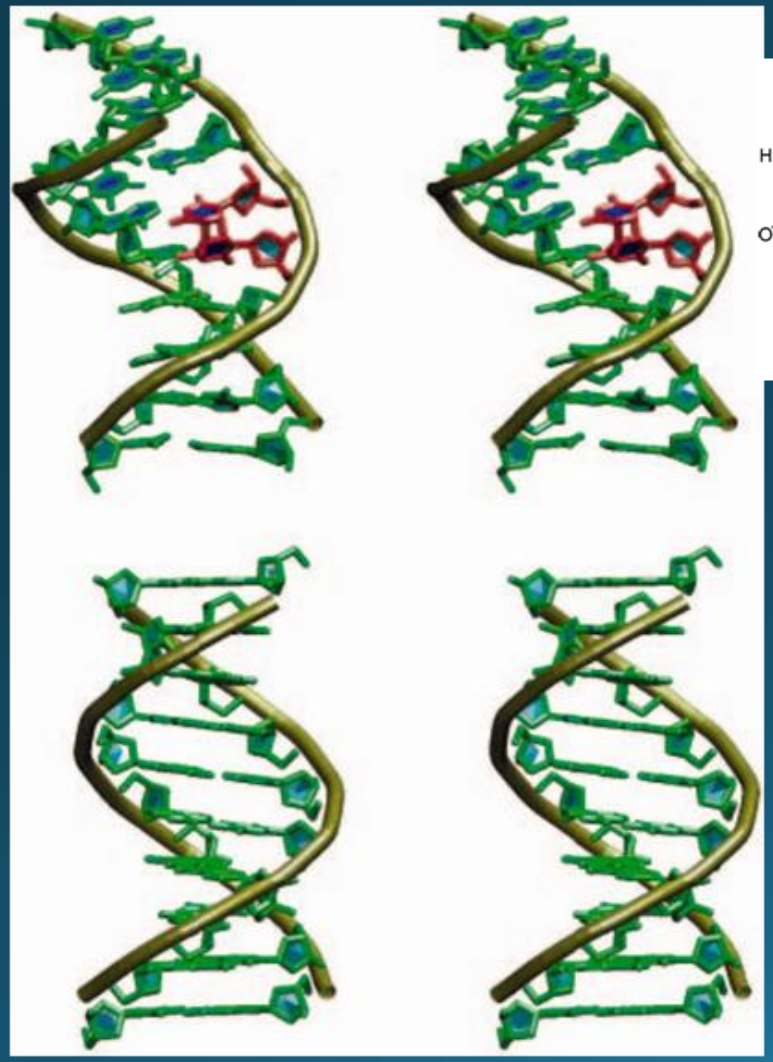
Photo: A. Mahmoud
Tomas Lindahl
Prize share: 1/3

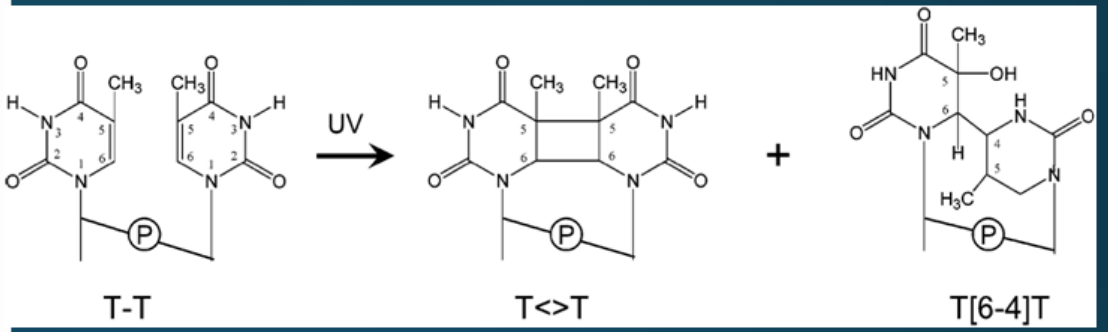
Photo: A. Mahmoud
Paul Modrich
Prize share: 1/3

Photo: A. Mahmoud
Aziz Sancar
Prize share: 1/3

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar *"for mechanistic studies of DNA repair"*.

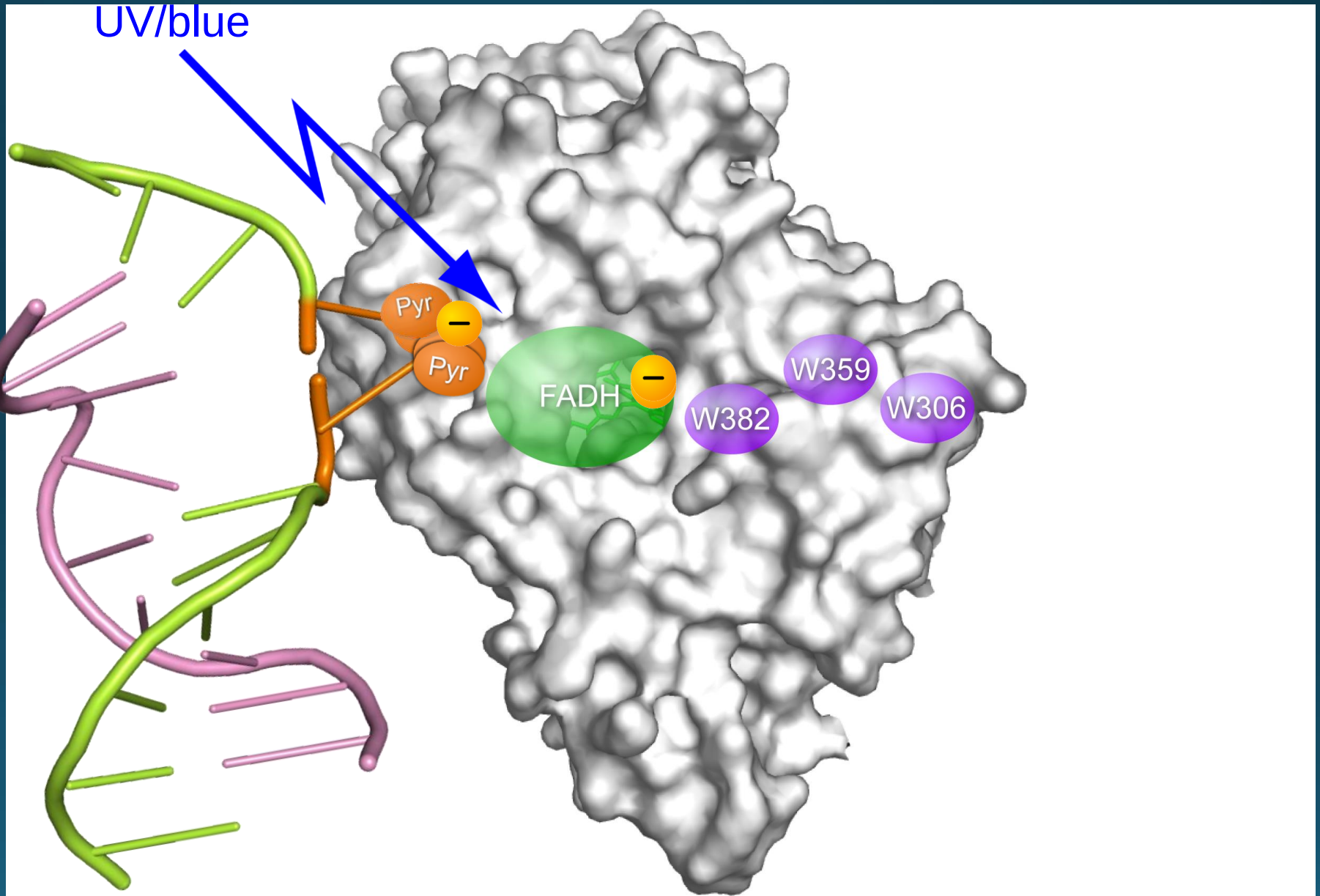
Photoinduced DNA damage



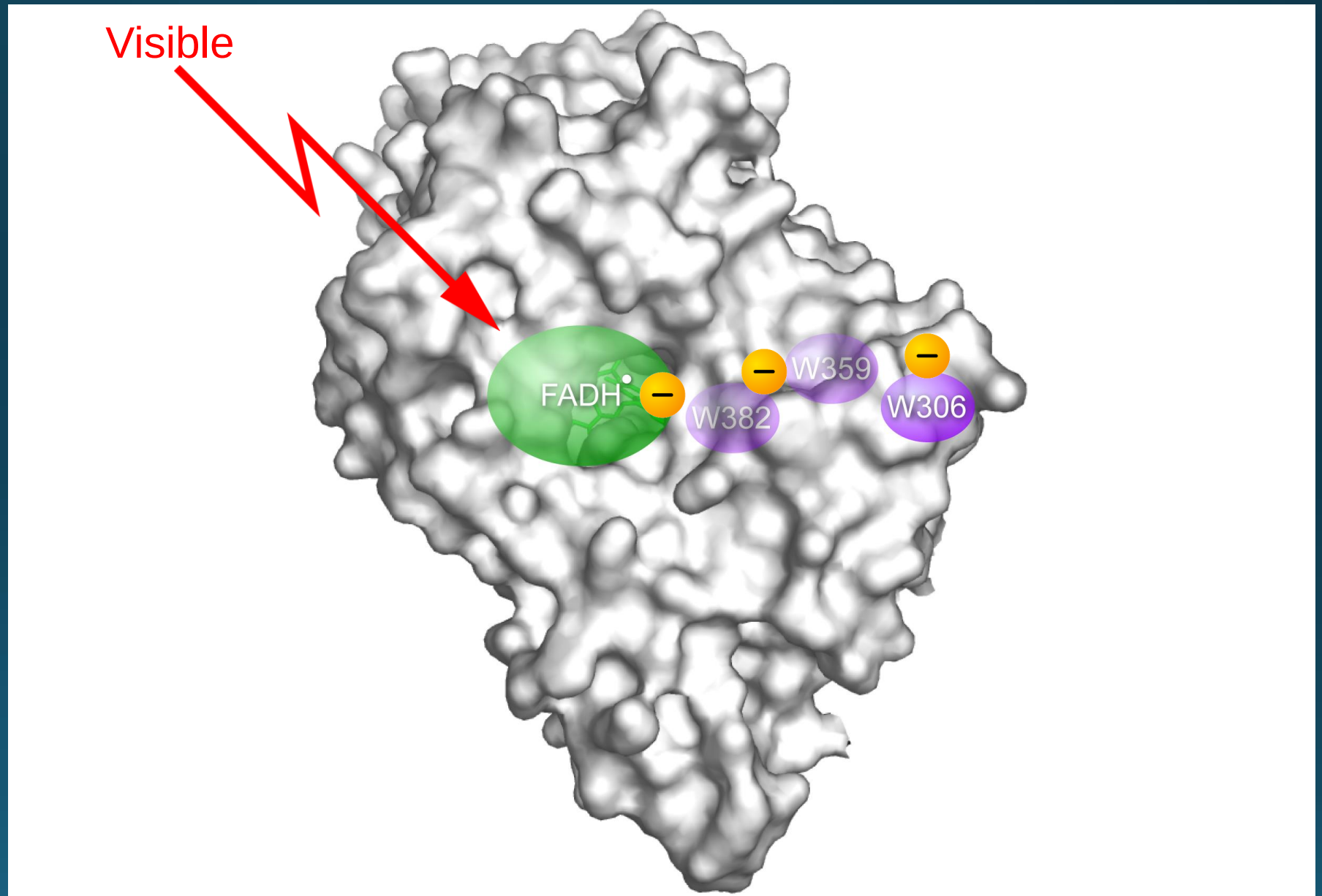


UV light induce two major lesions in DNA: cyclobutane pyrimidine dimers (Pyr<>Pyr) and the pyrimidine-pyrimidone (6-4) photoproduct (Pyr [6-4] Pyr)

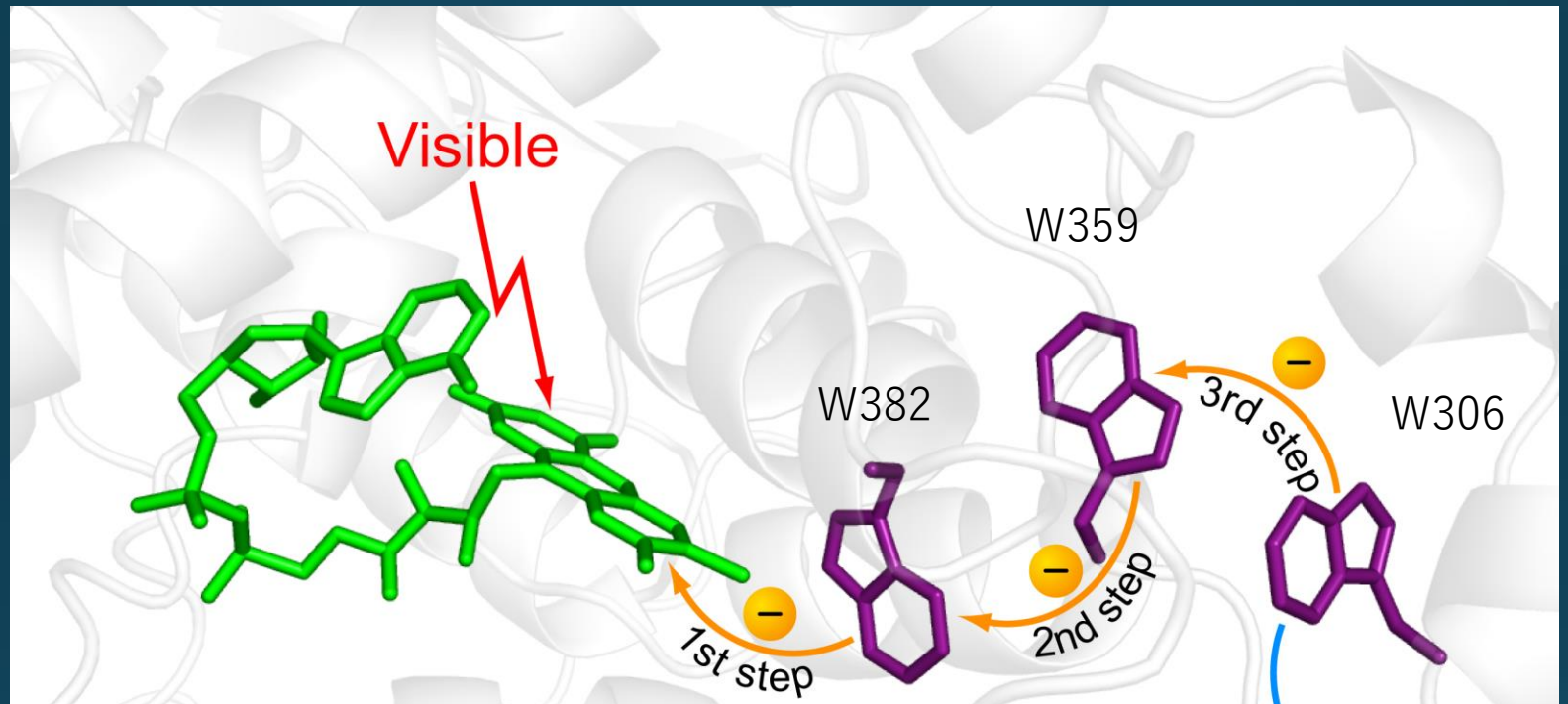
Photorepair



Photoactivation



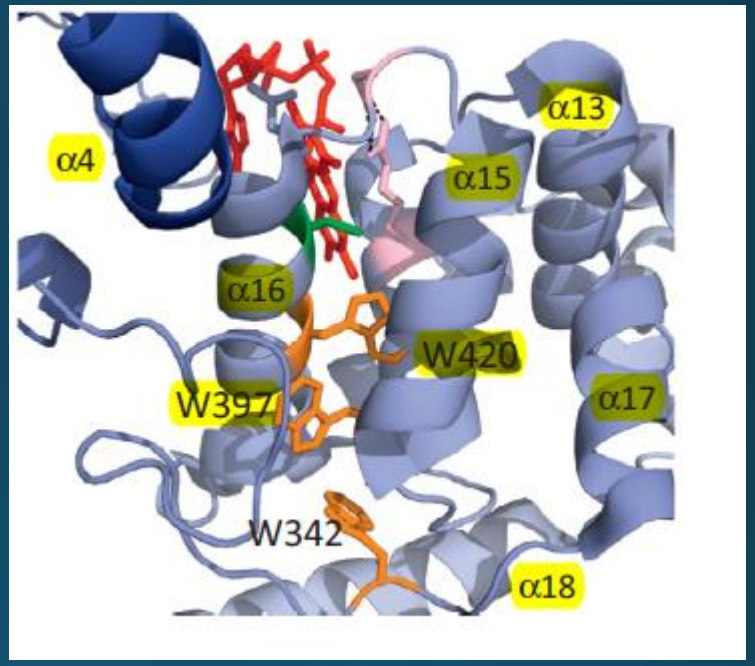
Photoactivation in *E coli* PL



- Excited state of $FADH^{\circ}$ extracts an electron from W382 in ~ 30 ps
- The cation radical $W382^{\circ+}$ is reduced by W359
- $W359^{\circ+}$ is subsequently reduced by the solvent exposed W306 in < 10 ns
- $W306^{\circ+}$ releases a proton into the aqueous phase in ~300 ns

Cryptochromes

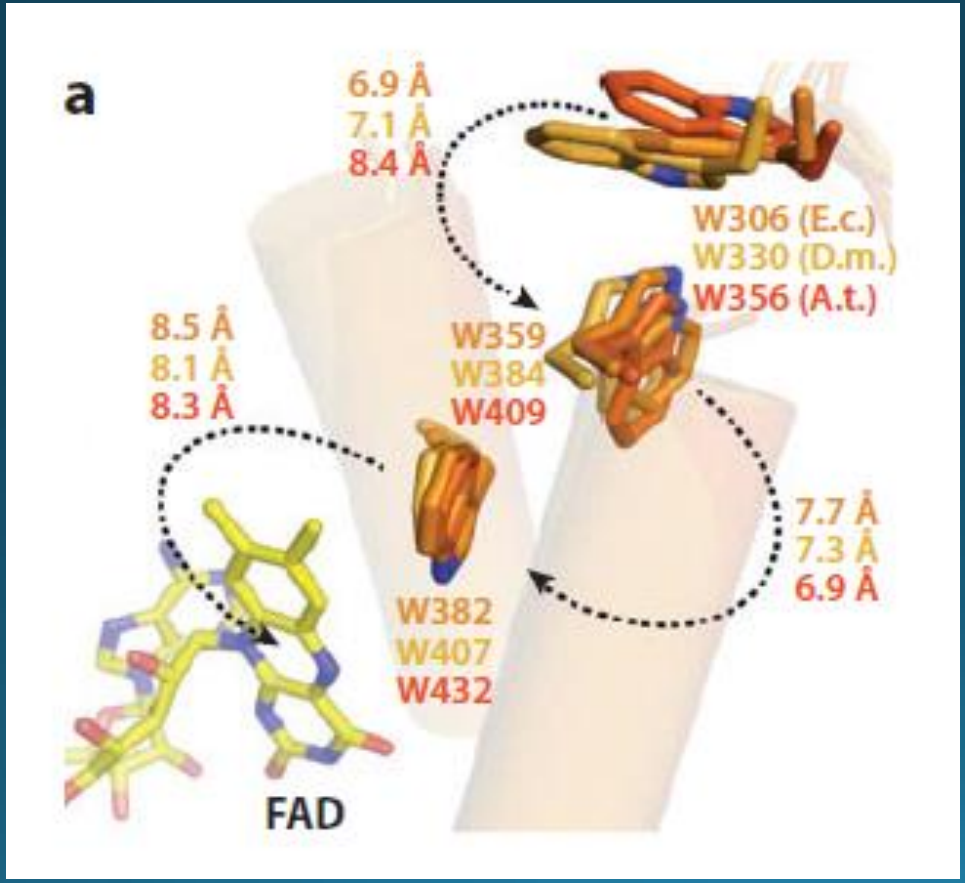
Sylvia borin



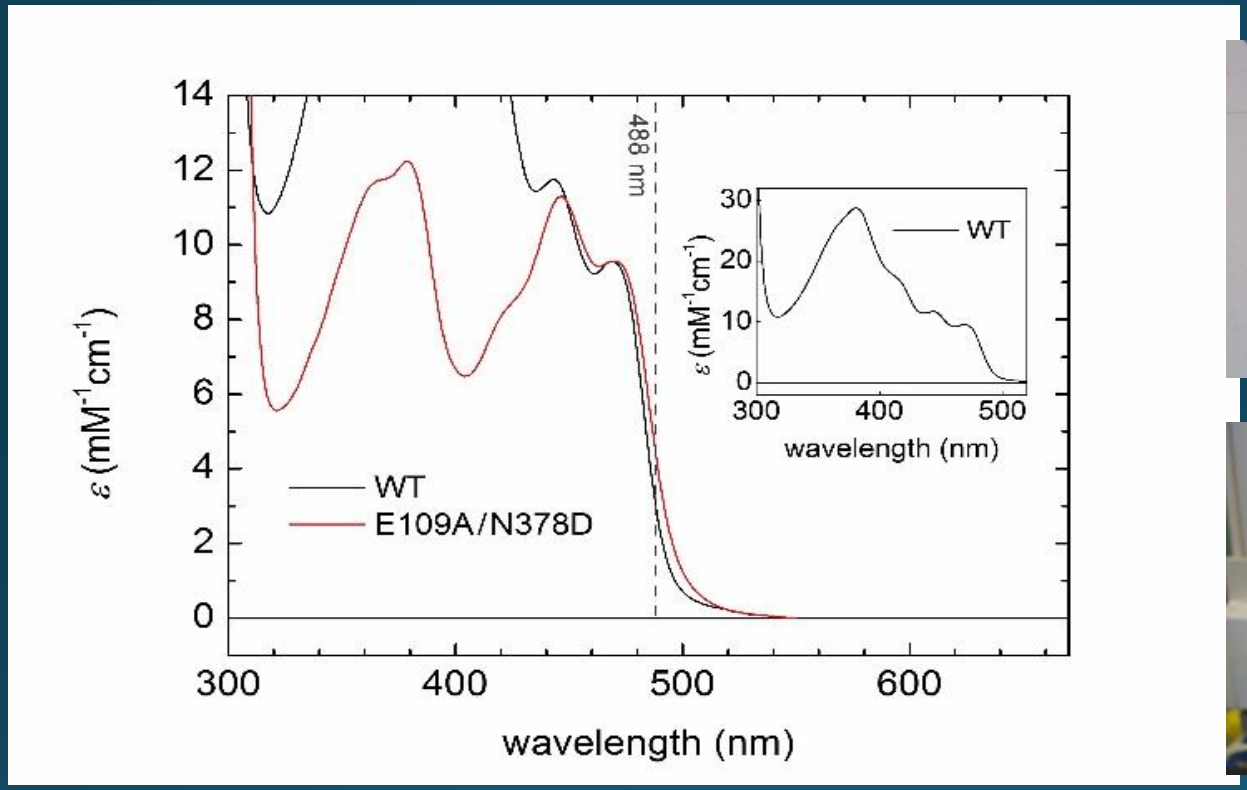
- Photoreception of blue light
- Circadian rythm (insects)
- Sensing the magnetic field

Cryptochrome vs photolyase

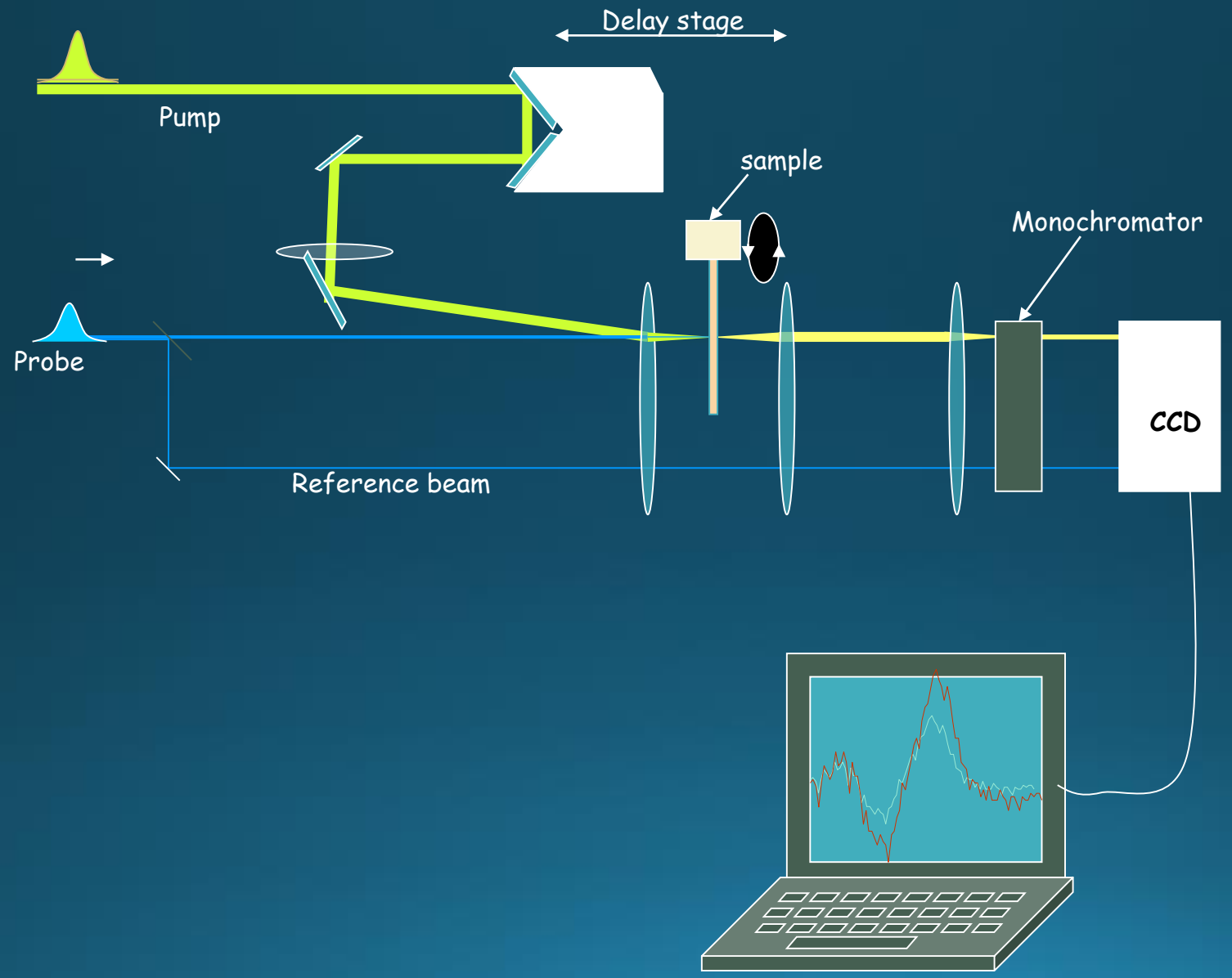
What makes photolyase different from cryptochromes?



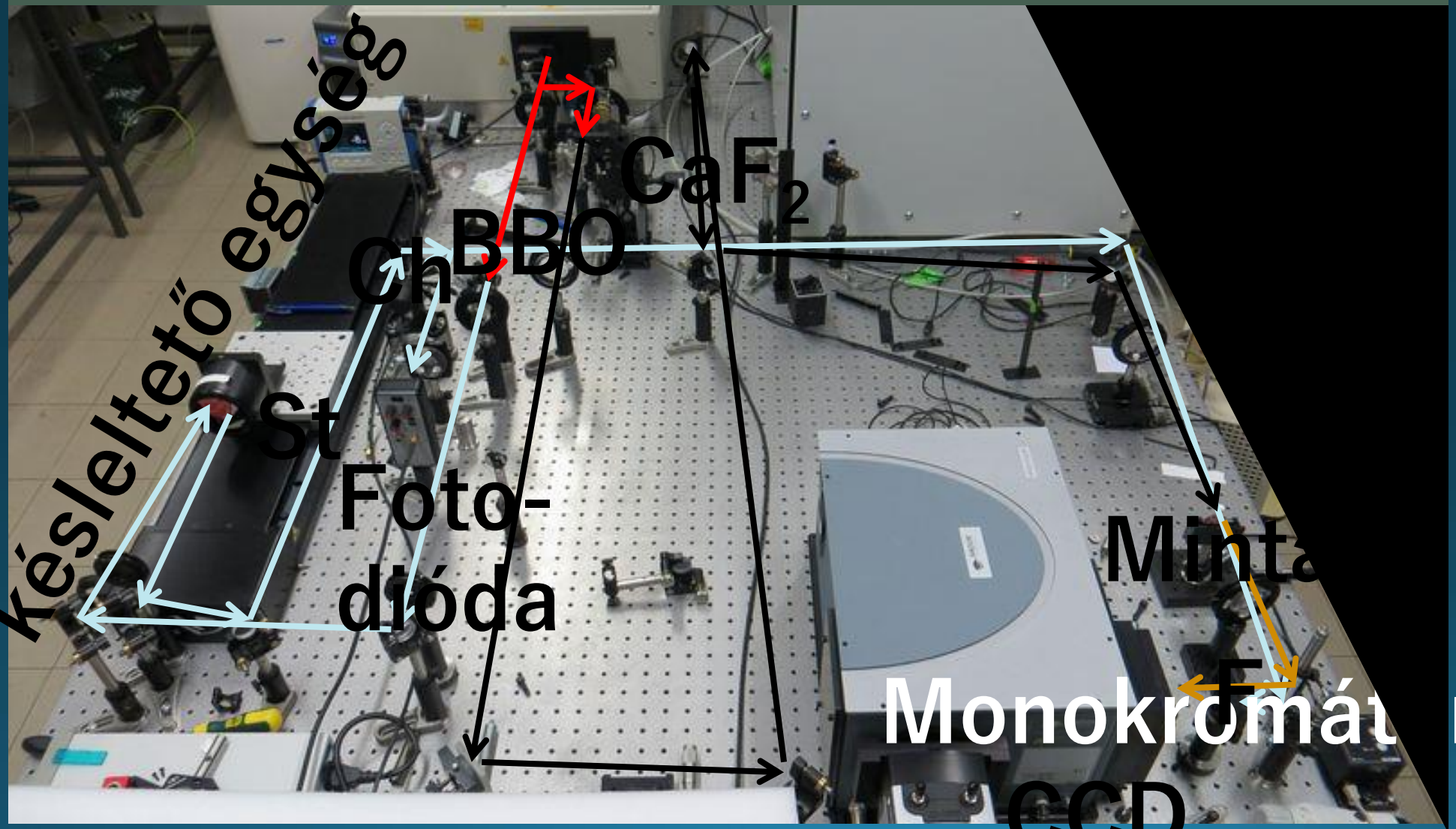
Absorption spectra of WT and N378D mutant



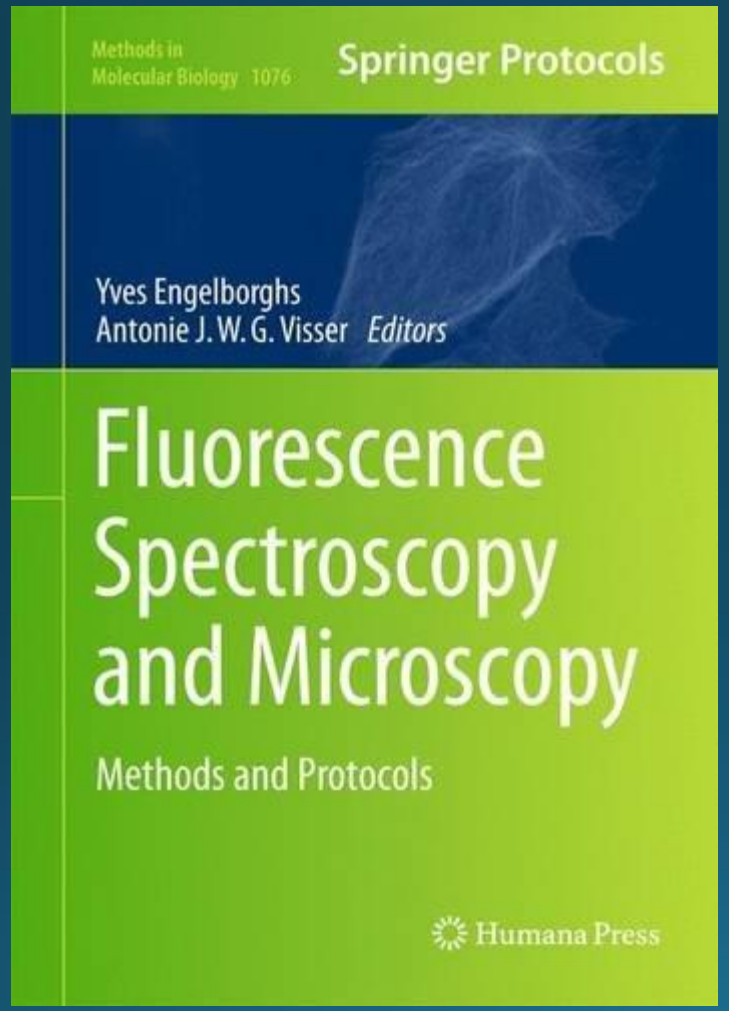
Transient absorption spectroscopy



Transient absorption spectroscopy

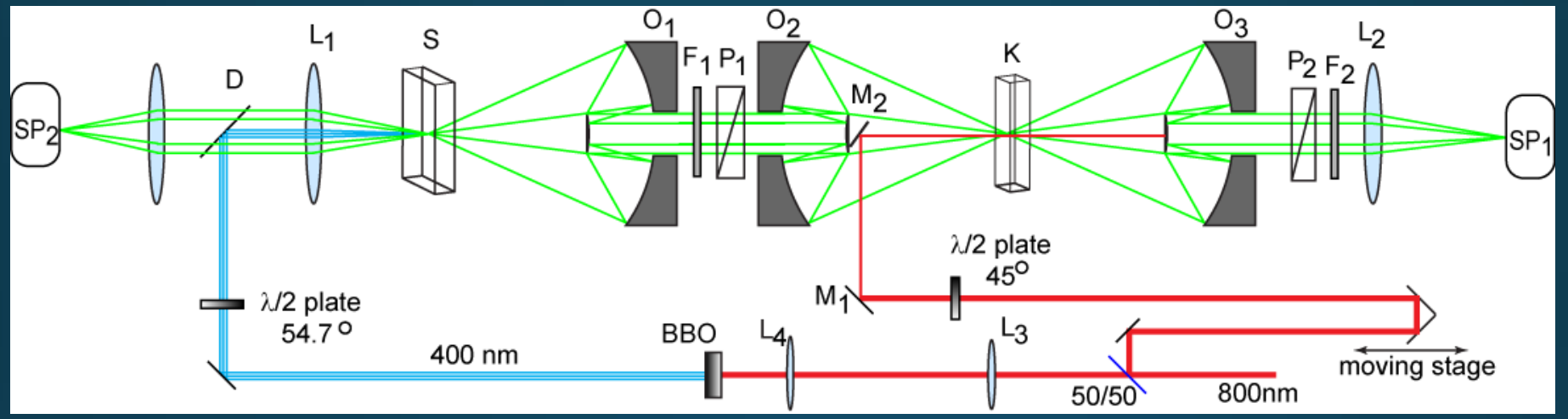


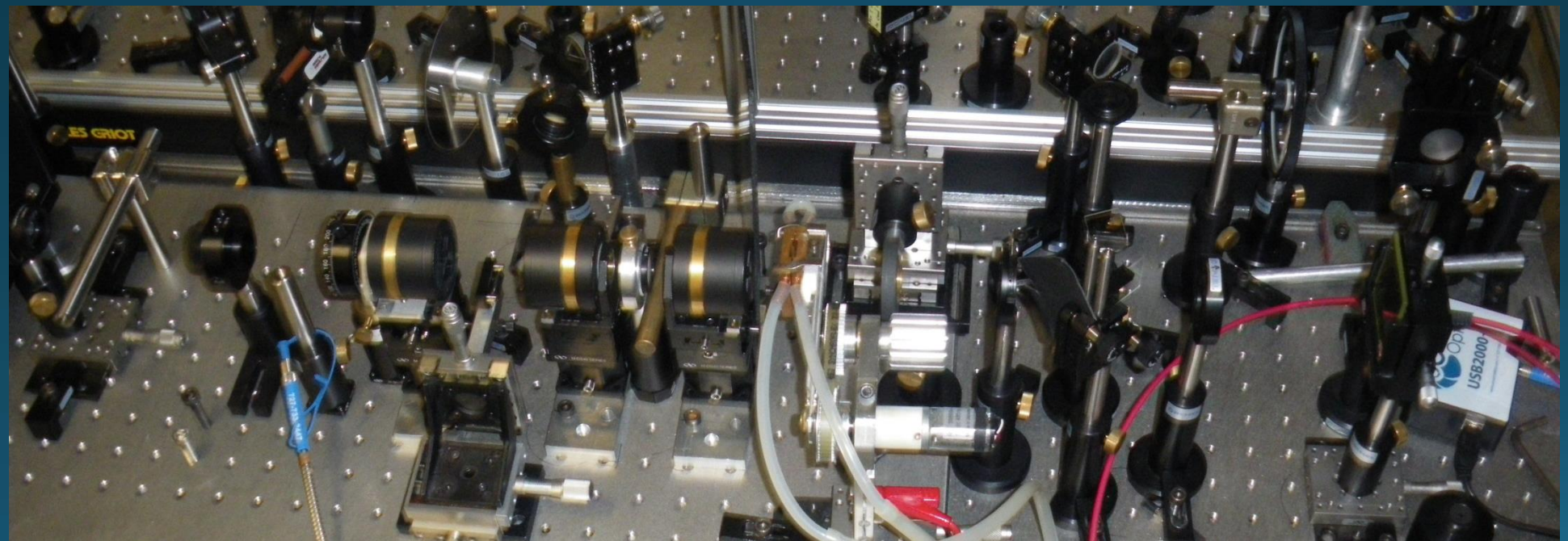
Kerr-gate fluorescence spectroscopy



Laptenok SP, Nuernberger P, Lukacs A, Vos MH: Subpicosecond Kerr-Gate Spectrofluorometry.

Kerr-gate setup

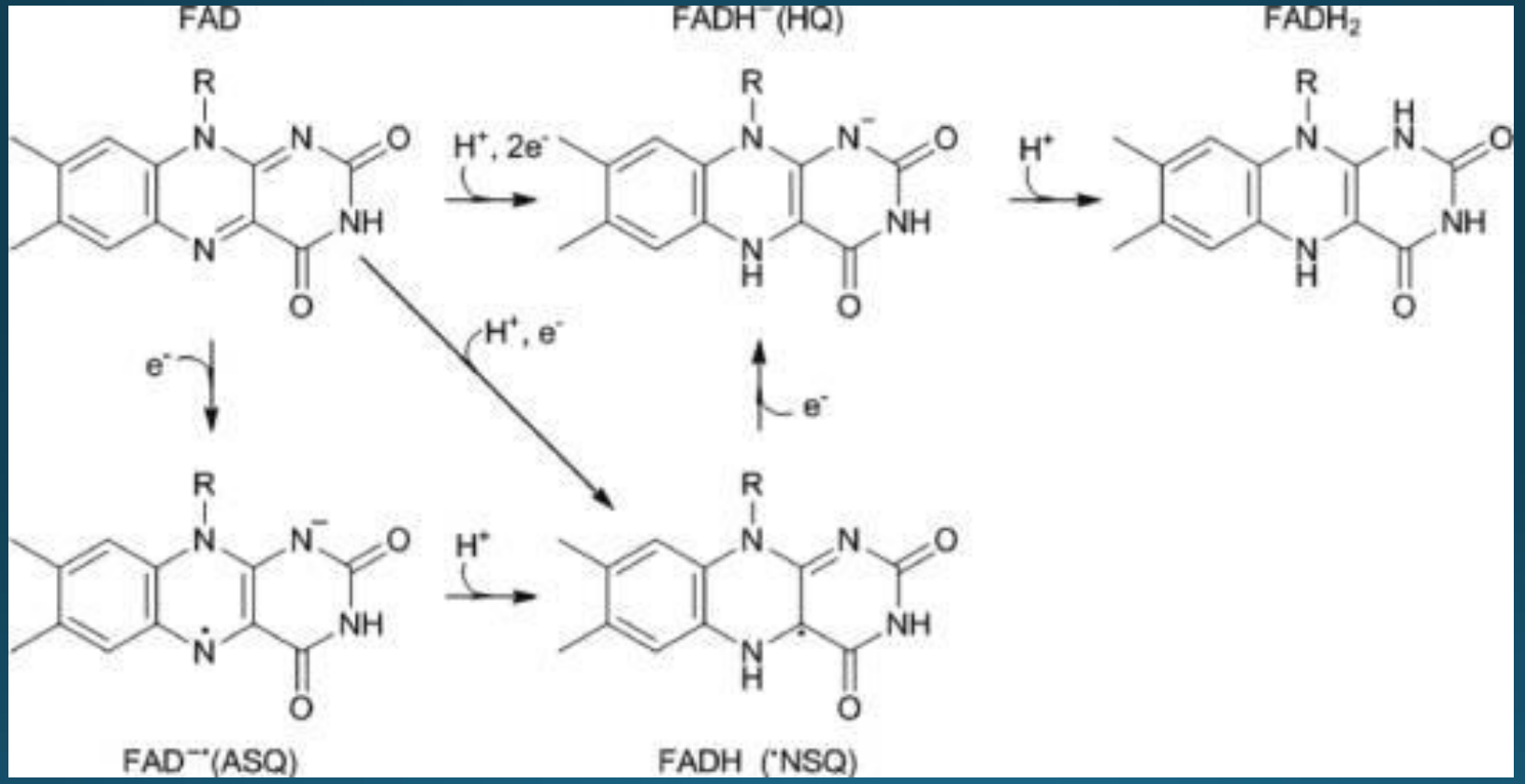




Redox state of FAD in Cry-s

- In plant cryptochromes (Arabidopsis Thaliana) FAD is oxidized after purification. After ligth absorption gets reduced to neutral semiquinone state ($FAHD^{o}$)
- In insects (Drosophila, Monarch butterfly, etc.) FAD is oxidized after purification but can be reduced easily (by light) to the anionic radical state (FAD^{o-})
- In plants there is an aspartic acid facing the N5 of the isoalloxazine ring, in insects this is rather a cysteine residue

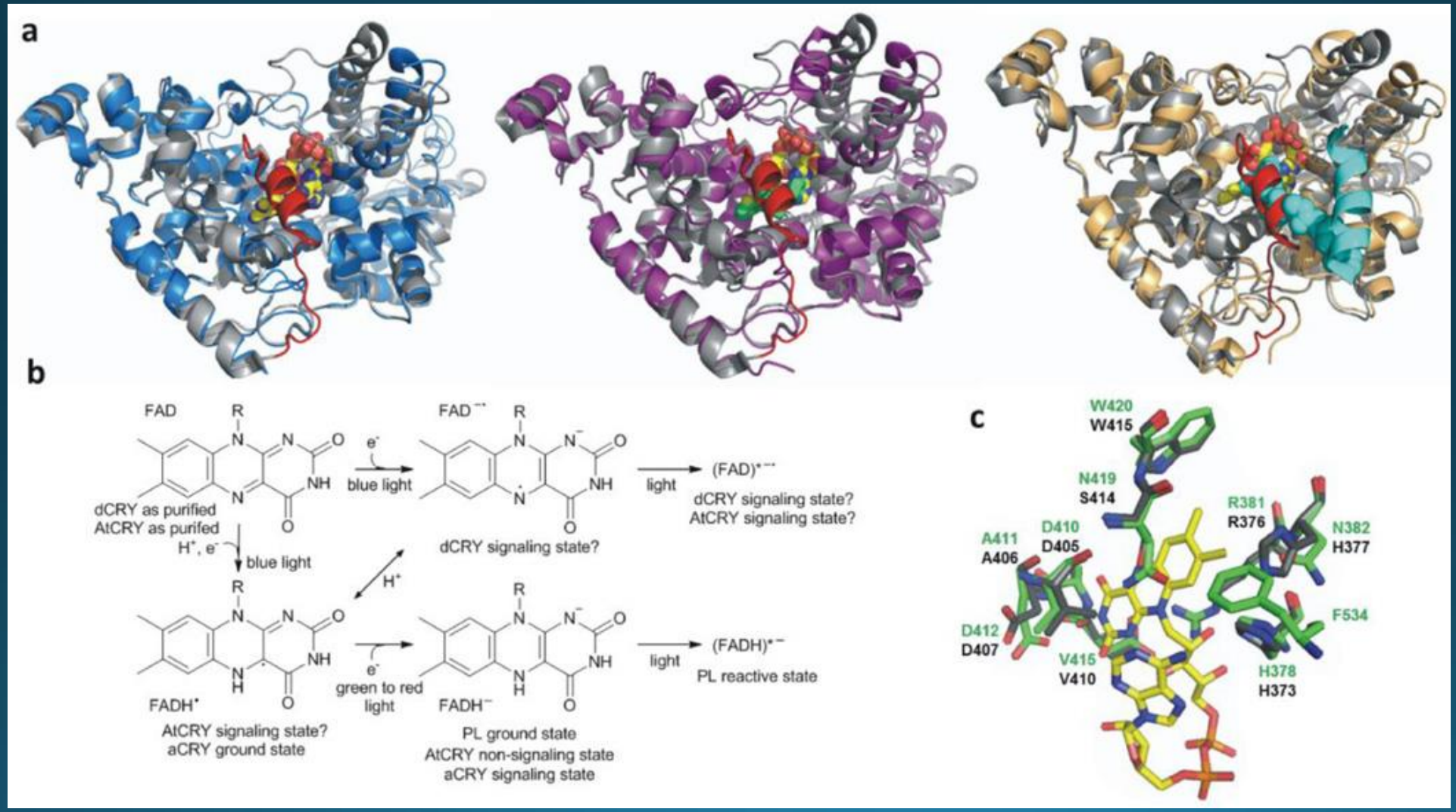
Redox states of flavin



What drives the function ?

- Sancar: photoinduced ET is not needed for the function. Same time if W382 was mutated it abolished the function
- Vaidya et al.: change in the redox state of flavin will alter the conformational change of CTT (this happen also in At Cry)
- The difference in the redox state after light absorption is related to the amino acid facing N5 (Asp, Cys)

What drives the function ?

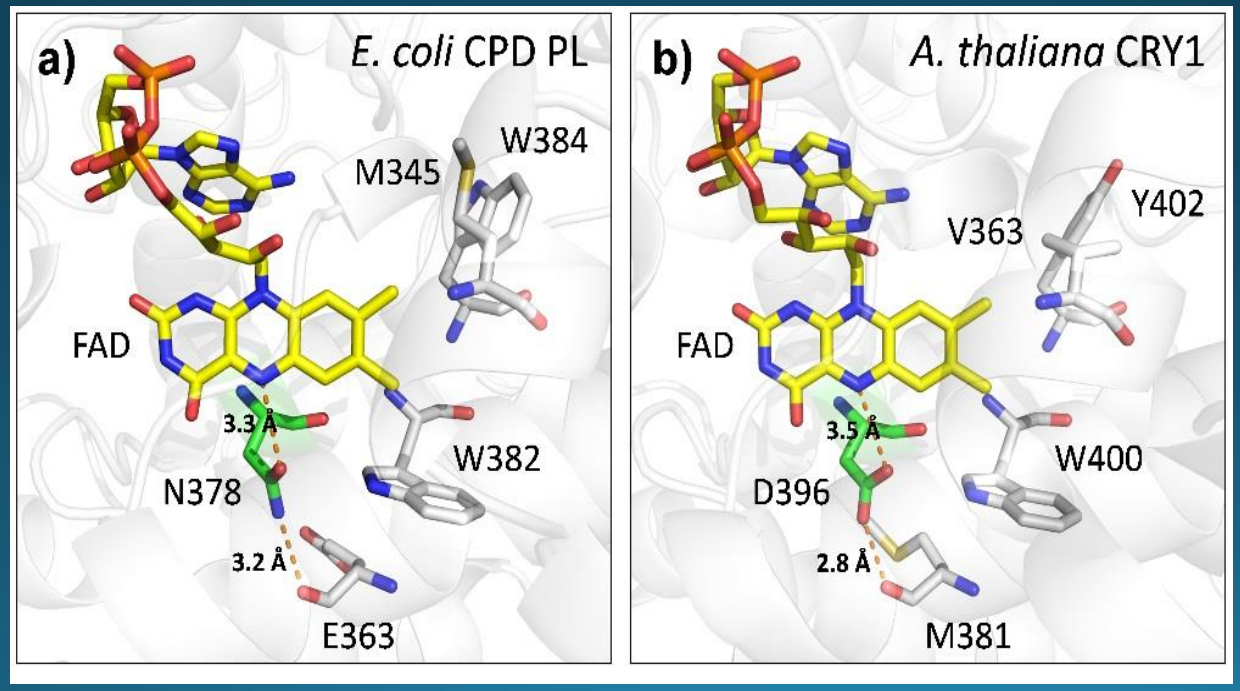


Strategy

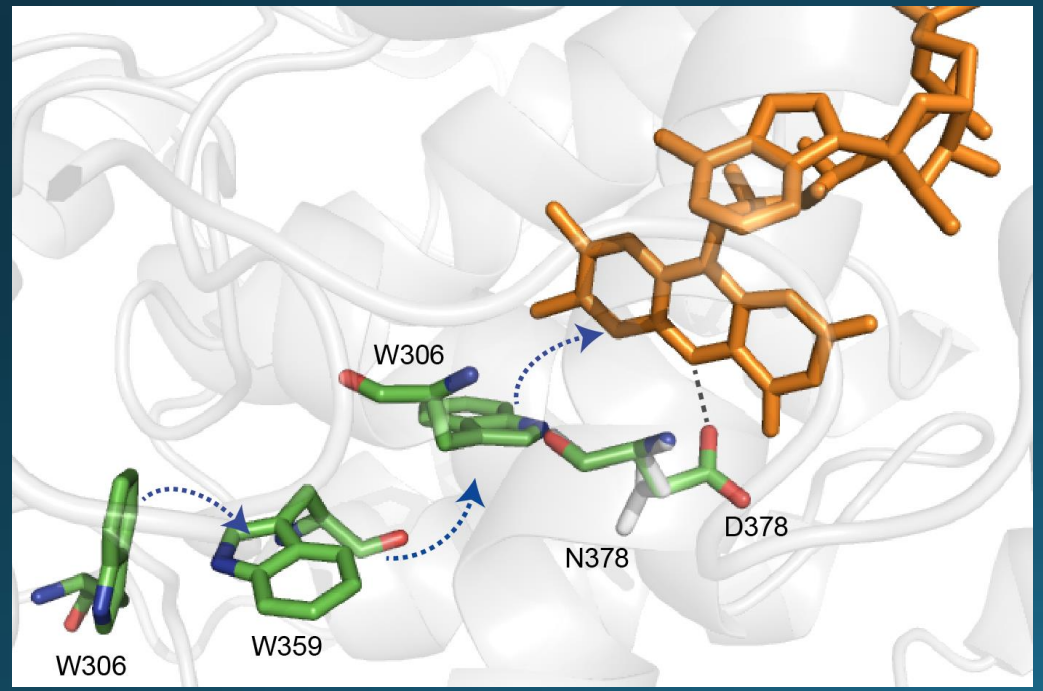
- Check photcycle of At Cry in the case of the short and full length protein
- Test whether mutation of the aspartic acid (D396) will abolish the conformational change in the full length At Cry
- Miscellaneous experiments (Cryptochrome like photolyase mutants)
- Express dCry and check the redox change by ultrafast spectroscopy

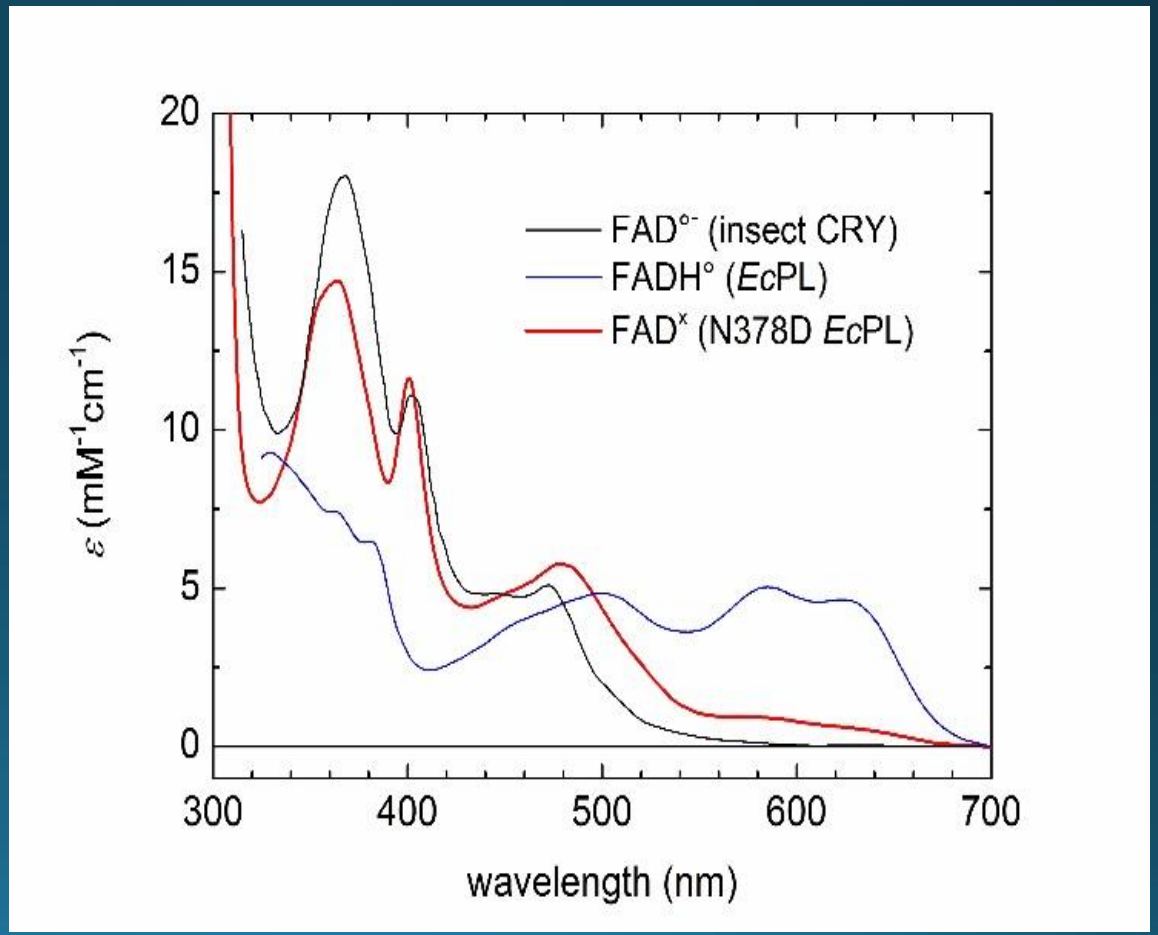
Miscellaneous

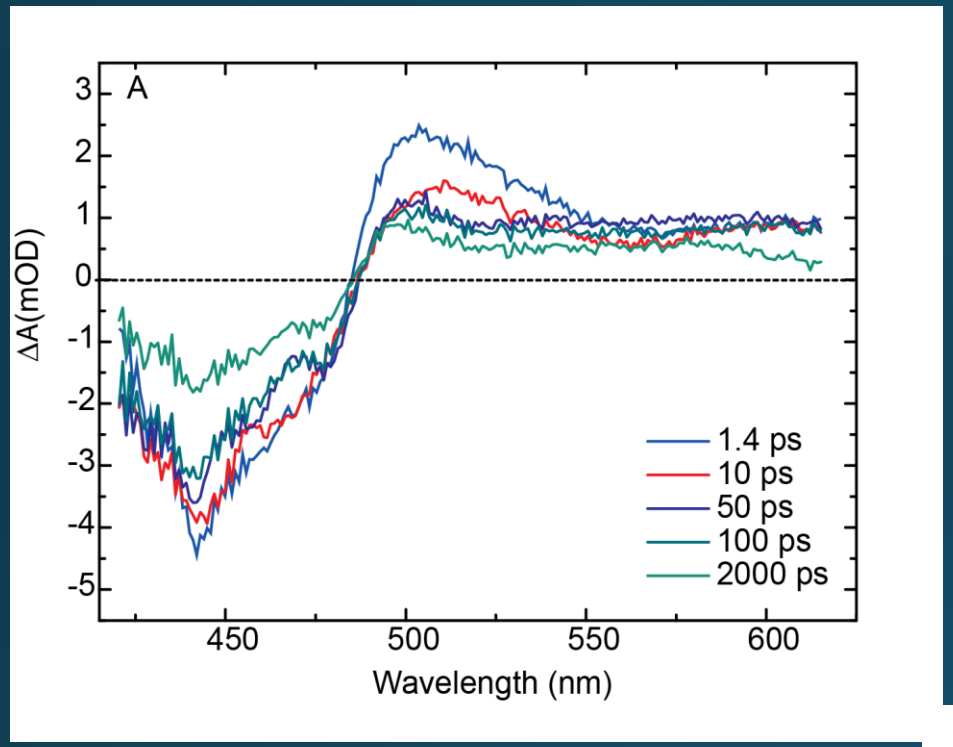
- Amino acid opposite to N5 of FAD is asparagine in photolyase, aspartic acid or cystein in the cases of cryptochromes
- Two mutants (N378D, N378C) were made to mimic the cryptochromes

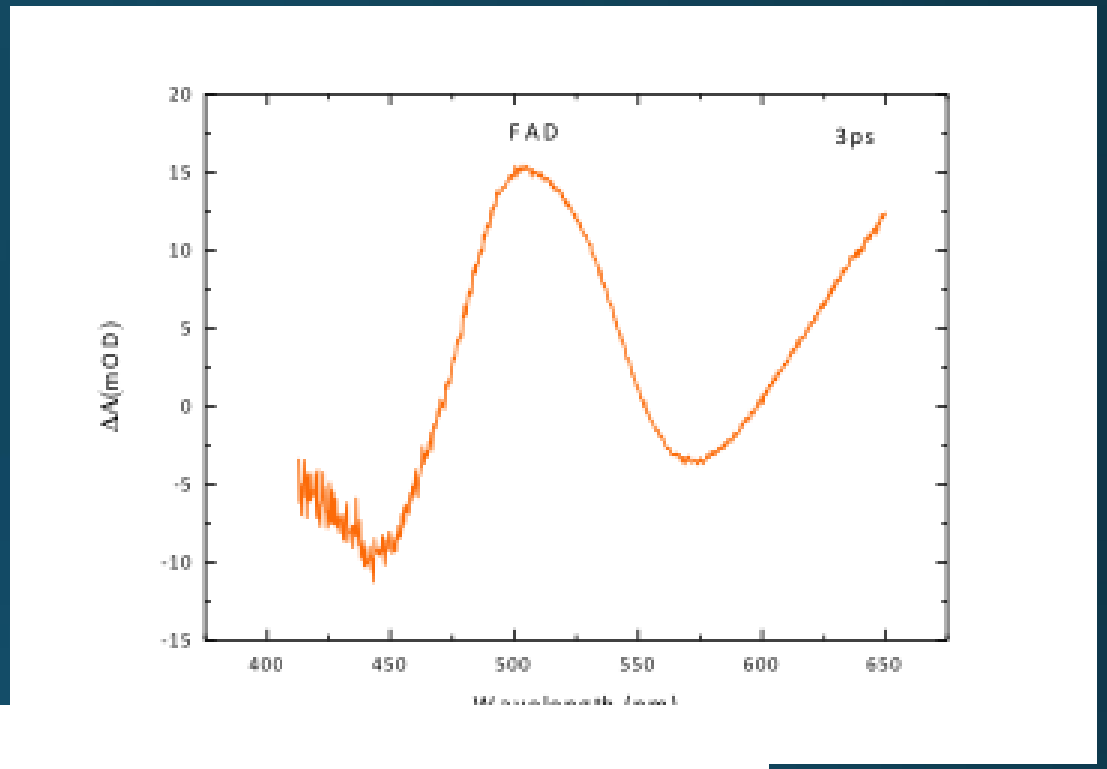


N378D mutant

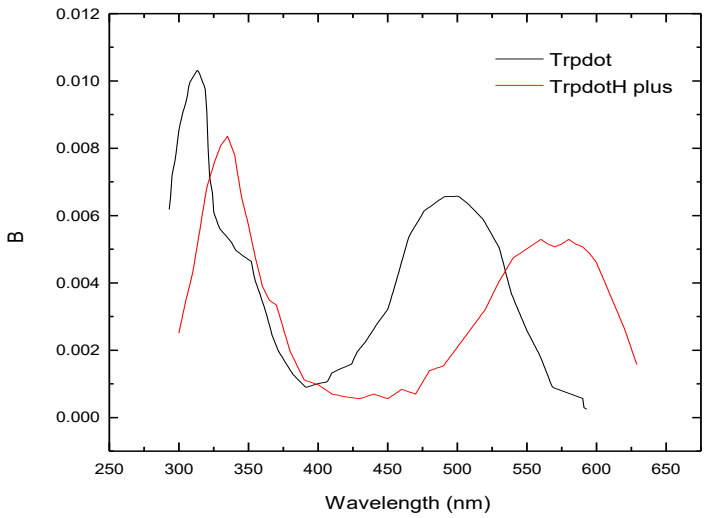


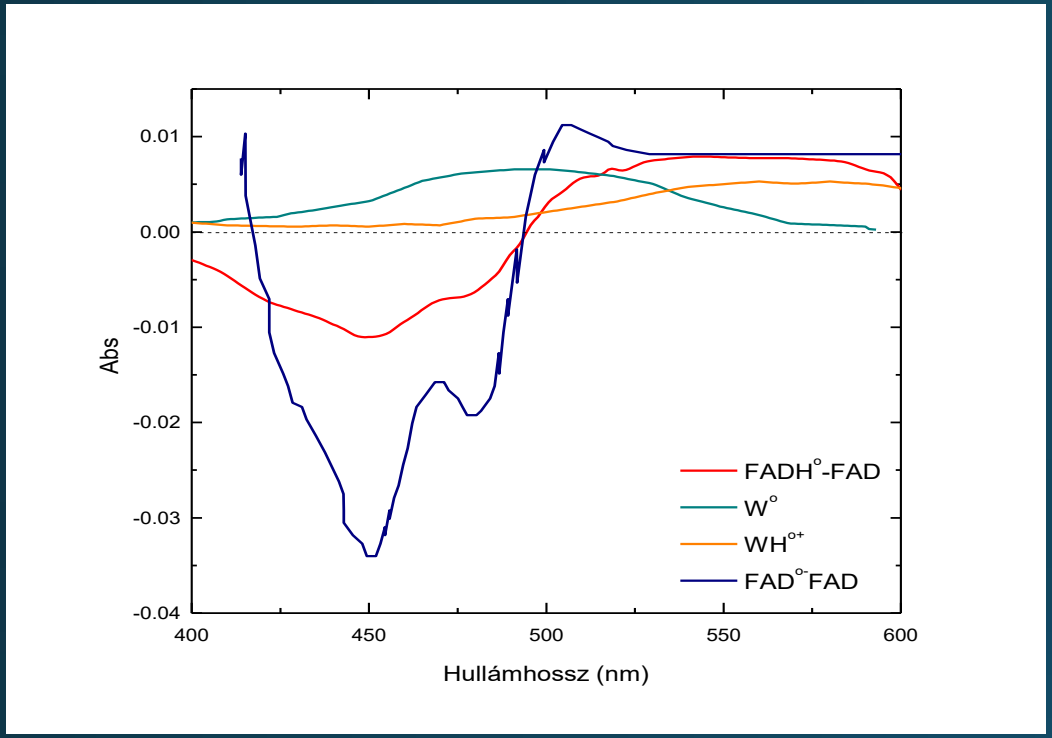


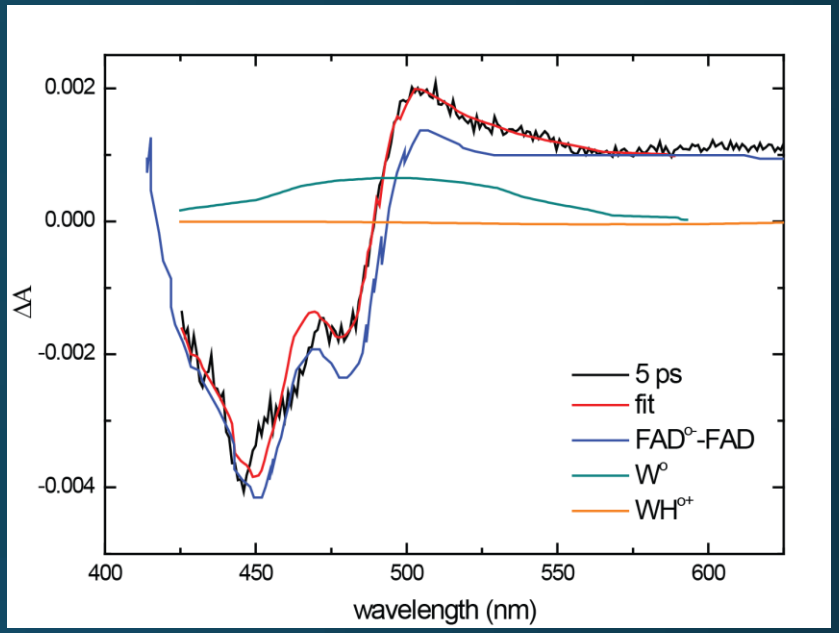




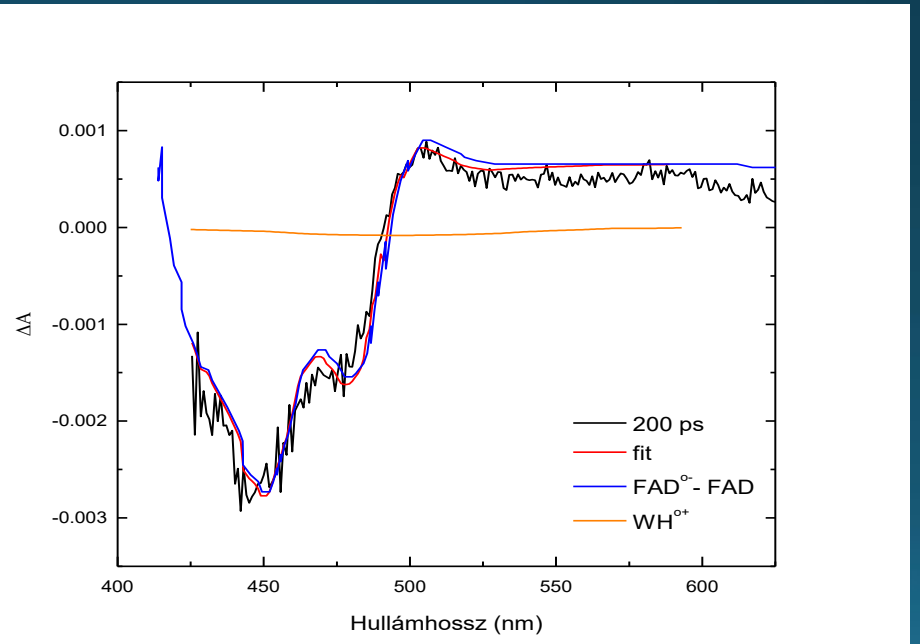
A mutáns tranziens spektruma az oxidált FAD-hez erősen hasonlít, de hiányzik a stimulált emisszió

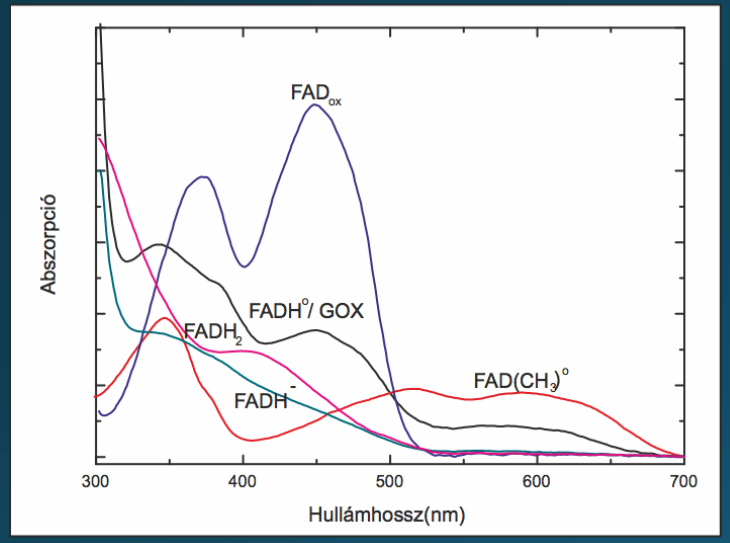


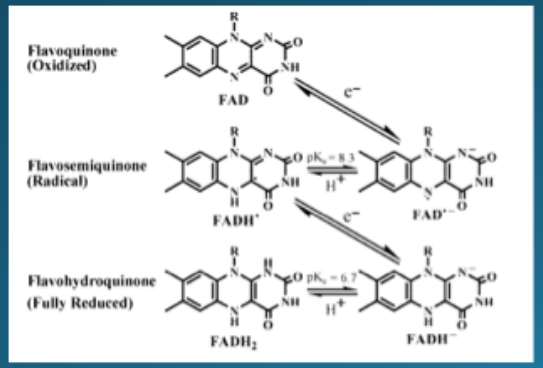




$$FAD^* \longrightarrow FAD^{o-} + TrpH^{o+}$$




FAD$_{ox}$
FADH°/ GOX
FADH$_2$
FADH$^-$
FAD(CH$_3$)°
Abszorpció
300
400
500
600
700
Hullámhossz(nm)


Flavoquinone
(Oxidized)
FAD
Flavosemiquinone
(Radical)
FADH°
FAD°⁻
Flavohydroquinone
(Fully Reduced)
FADH$_2$
FADH$^-$