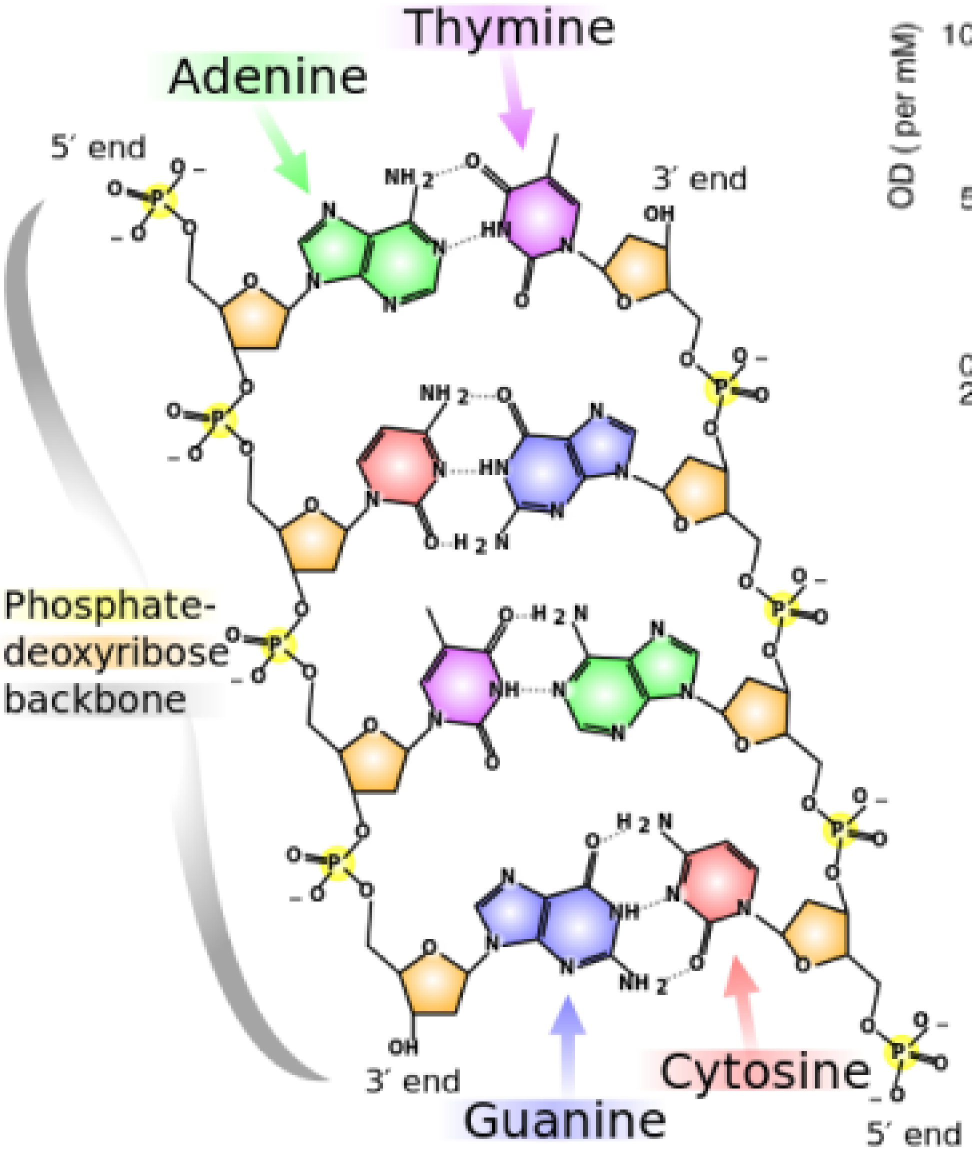
A CIDNP study of the reduction of short-lived thymine radicals by aromatic amino acids

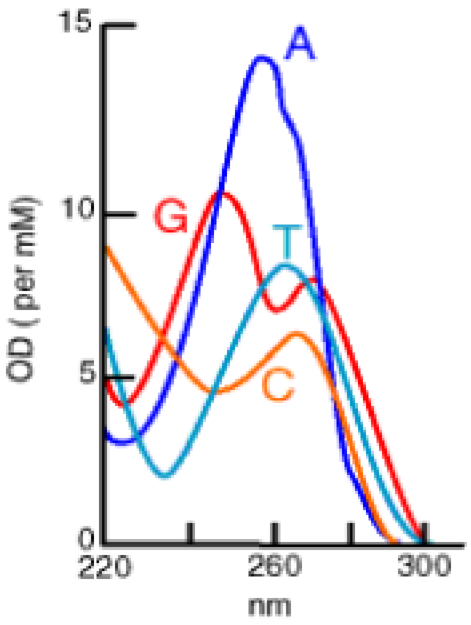
Natalya N. Fishman, Olga B. Morozova, Hans-Martin Vieth,
Alexandra V. Yurkovskaya

Photoinduced damage of DNA

UV light

UVA	400 – 315 nm
UVB	315 – 280 nm
UVC	280 – 100 nm





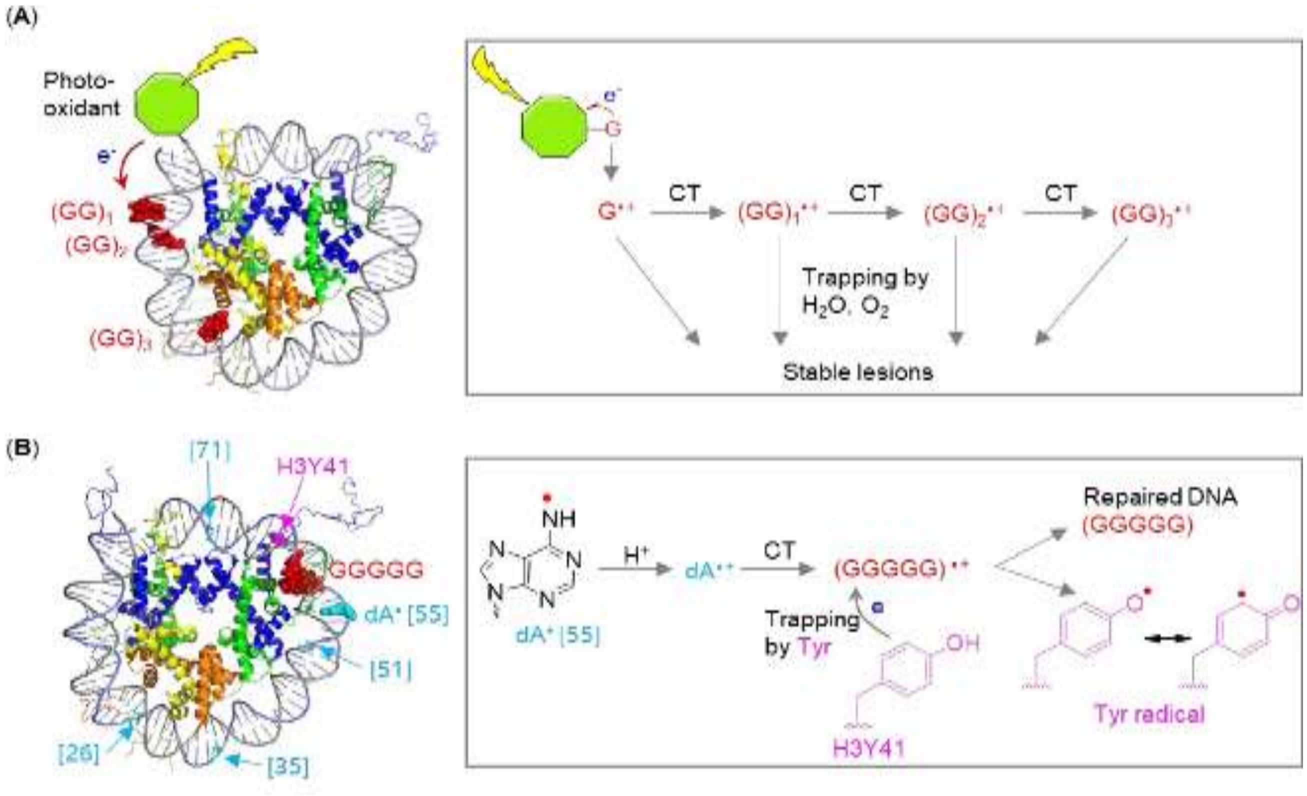
UVA-Induced Damage: photosensitized reactions

Type I photosensitization:
triplet excited photosensitizers – anthraquinone, benzophenone, 1,4-dimethyl-2-naphthoquinone, riboflavin – react with DNA components by one-electron transfer (or hydrogen atom abstraction)

Short-lived radicals of G, A, T, C

“Chemical way” of DNA repair

“Participation of Histones in DNA Damage and Repair within Nucleosome Core Particles: Mechanism and Applications” M. Ren, M. M. Greenberg, C. Zhou. *Acc. Chem. Res.* 2022, 55, 7, 1059–1073

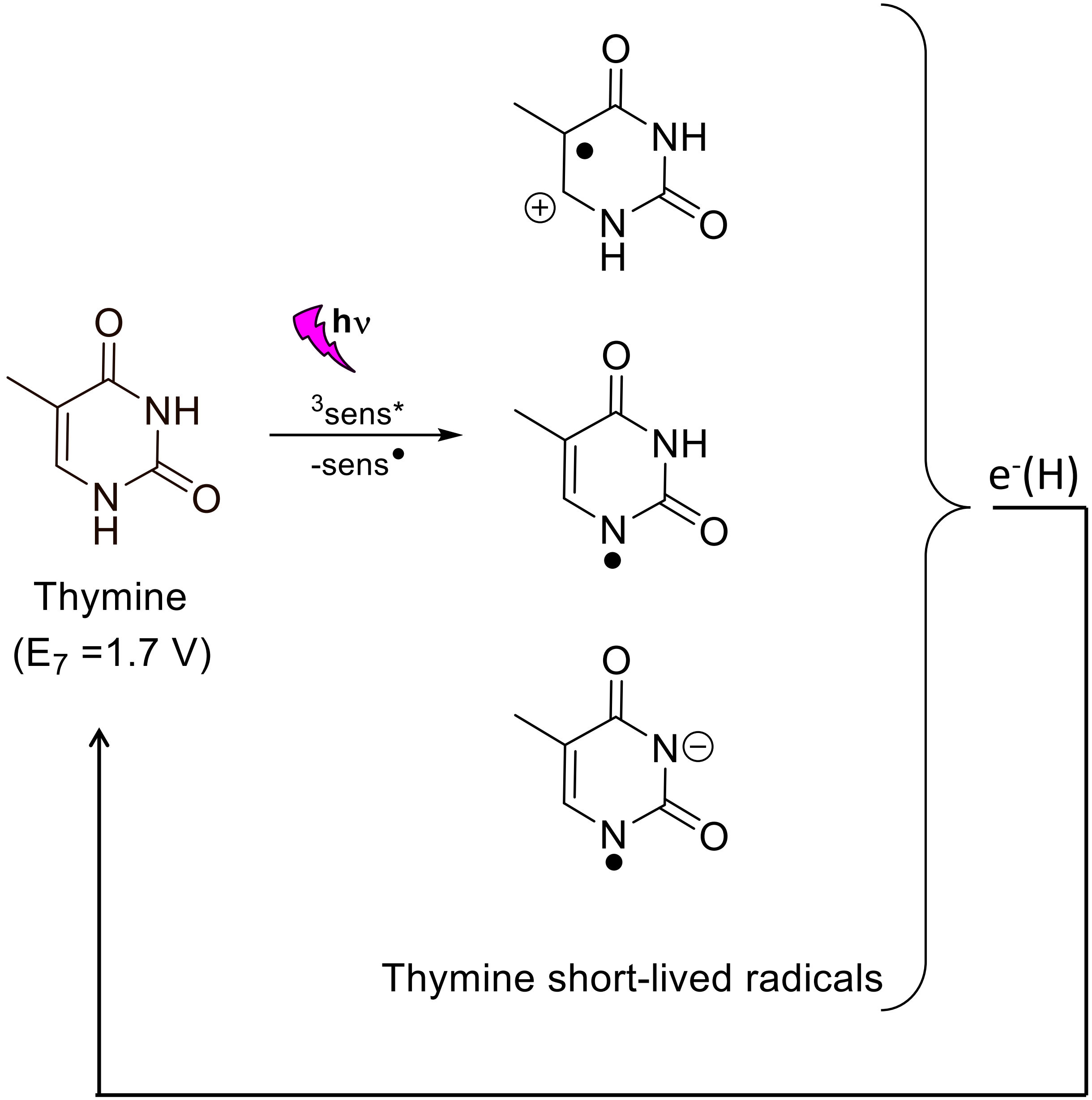


Photooxidant-induced electron transfer in nucleosome.

Histones repair oxidatively damaged DNA via electron transfer

Guanine (E_7 = 1.29 V), adenine, cytosine, thymine (E_7 = 1.7 V)

Reduction of thymine radicals by amino acids



Thymine
(E_7 =1.7 V)

Thymine short-lived radicals

Reductants:

Amino-acids:
Tryptophan (E_7 = 1.0 V)
Tyrosine (E_7 = 0.9 V)

How to detect thymine radicals and to study reduction reaction?

- Time-resolved laser flash photolysis and pulsed radiolysis study

↓

insufficient spectral resolution, do not provide direct information on radicals structure; nucleotide radicals are weak chromophores

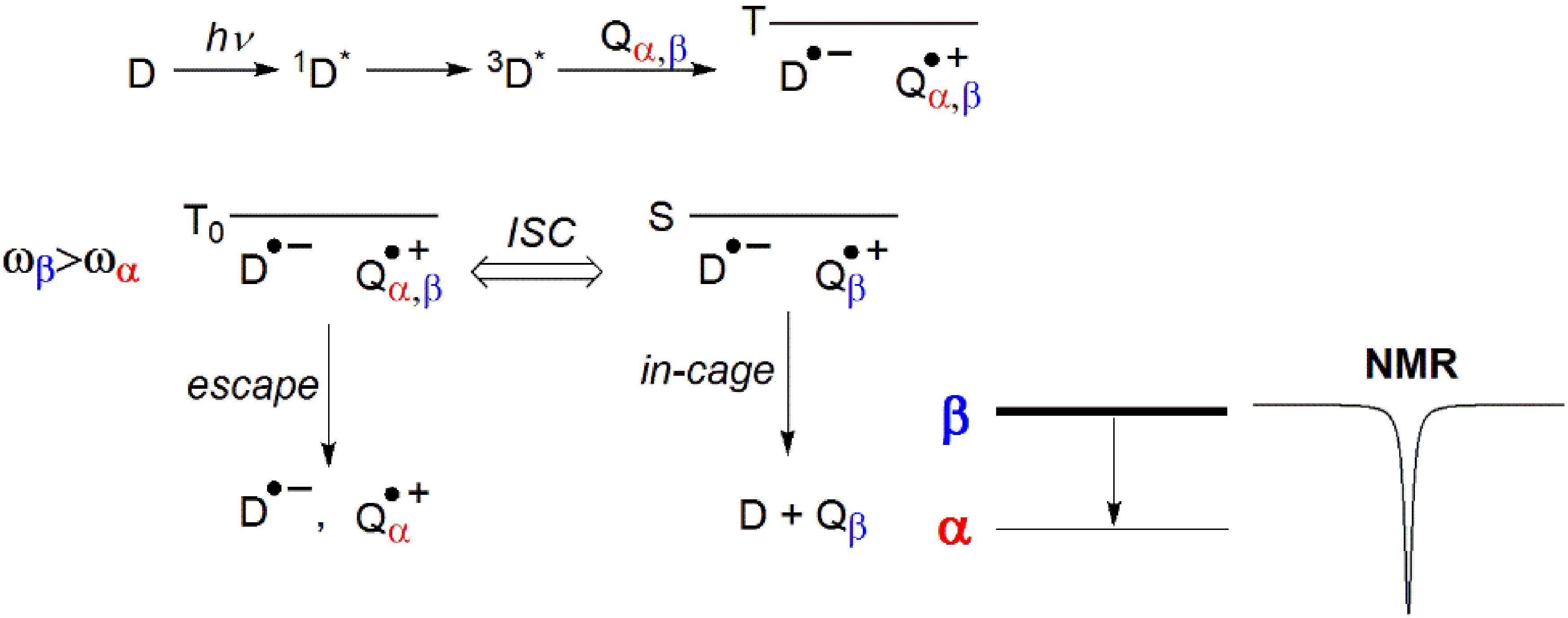
- EPR detection under physiological conditions

↓

problematical because of the very short lifetime of pyrimidine radicals

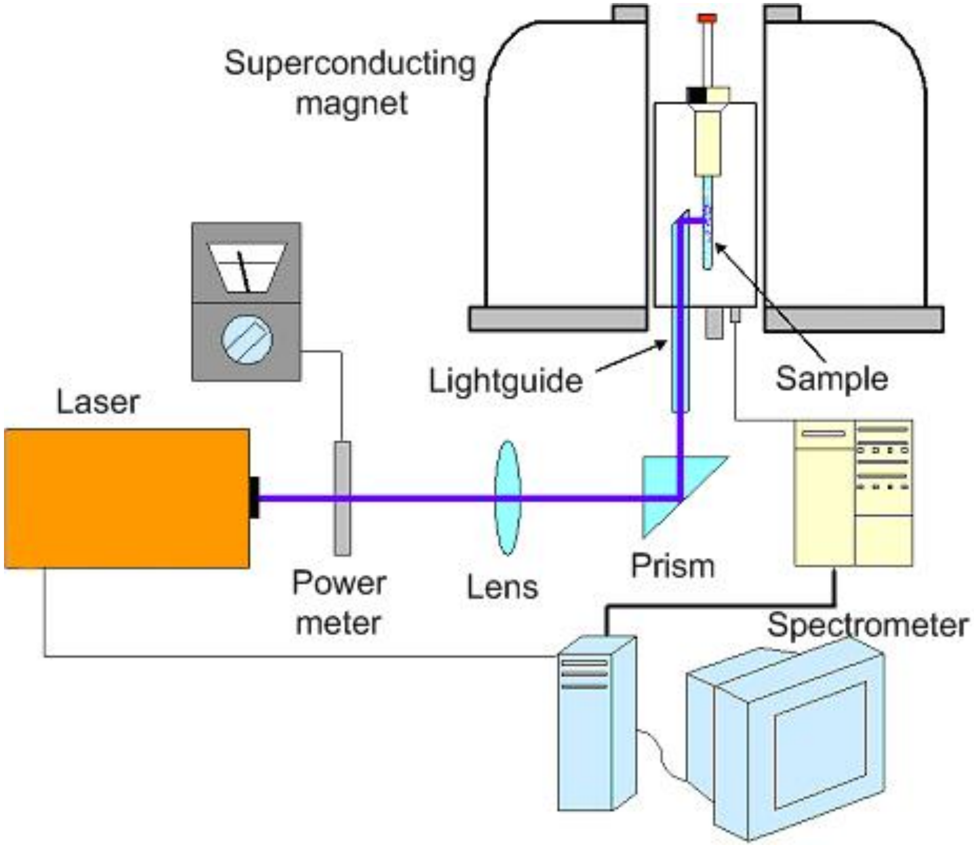
Solution: application of the method of time-resolved Chemically Induced Dynamic Nuclear Polarization allows one to follow the reactions of transient thymine radicals using NMR detection of its product

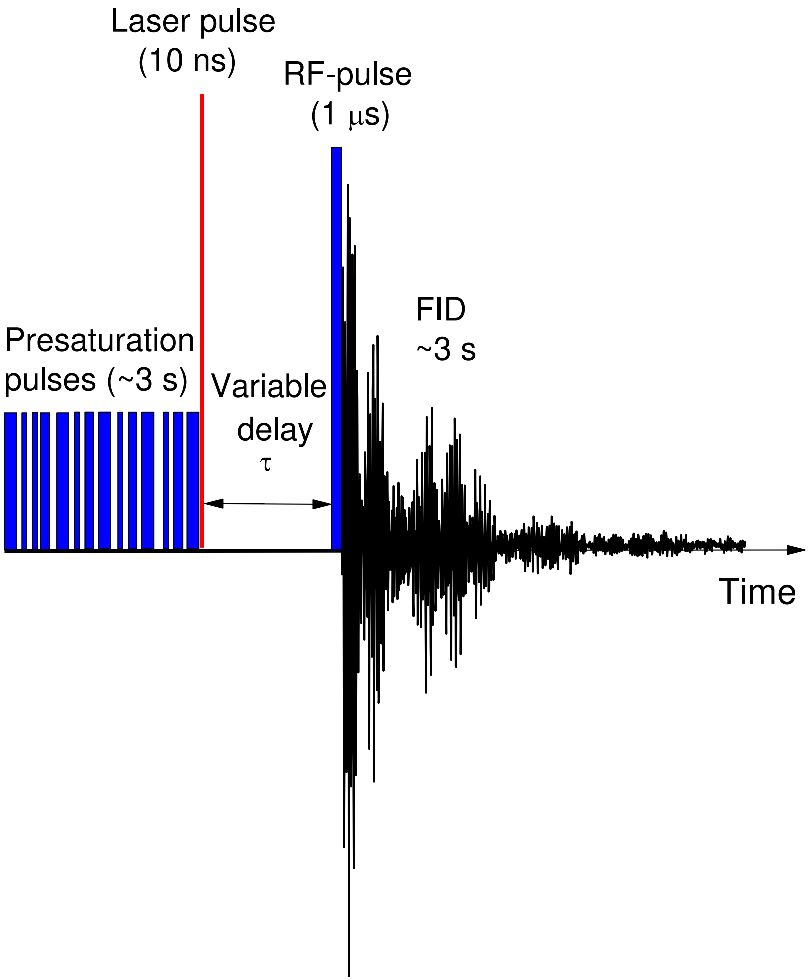
*Spin sorting mechanism of CIDNP in high field**



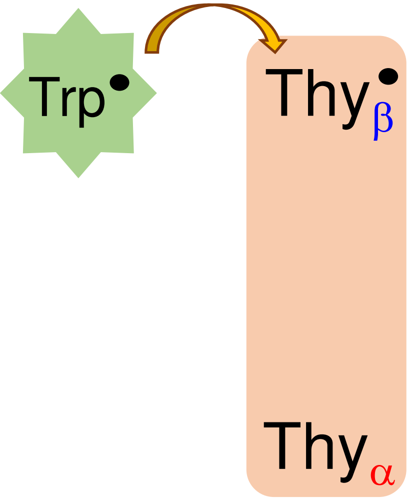
*R. Kaptein, G. Closs, L. J. Oosterhoff, 1969

TR CIDNP experiment

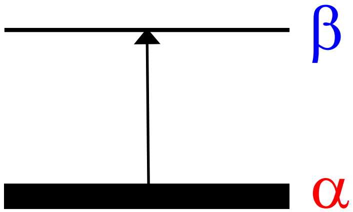


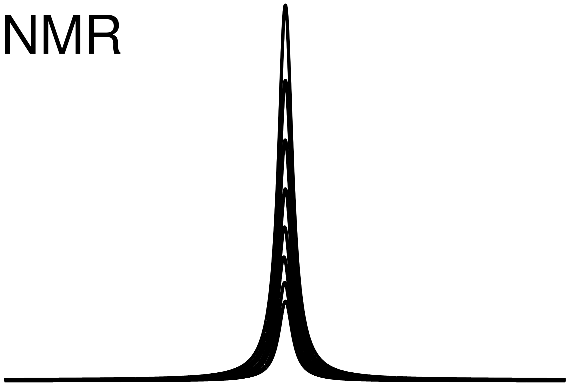


CIDNP in studying radical reduction

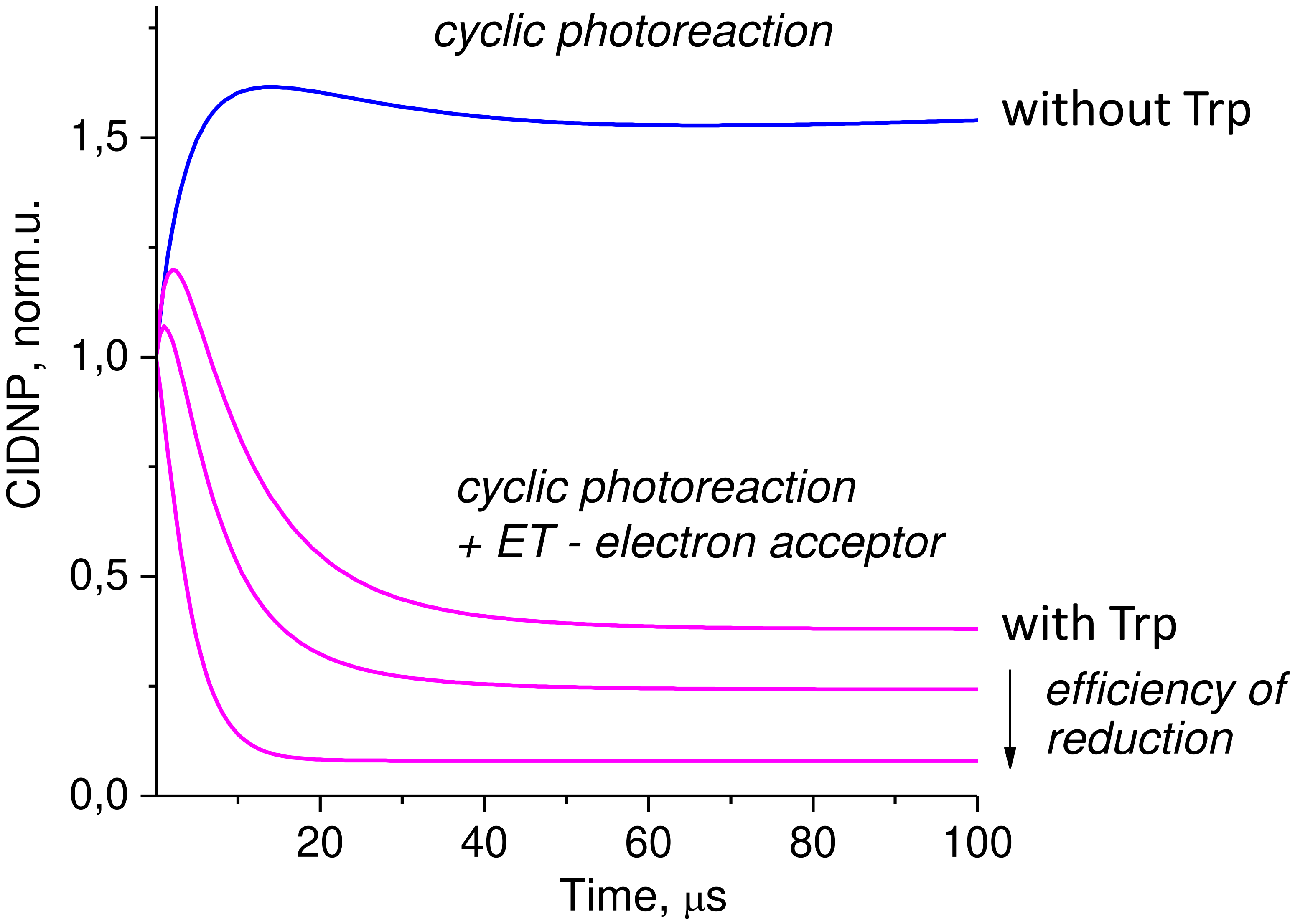


Detected by NMR

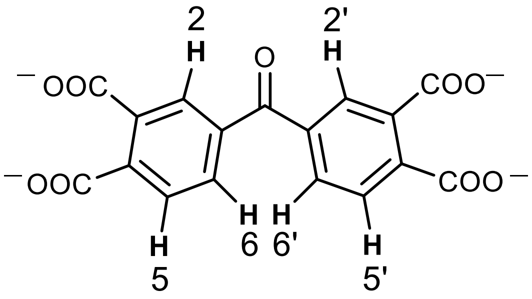




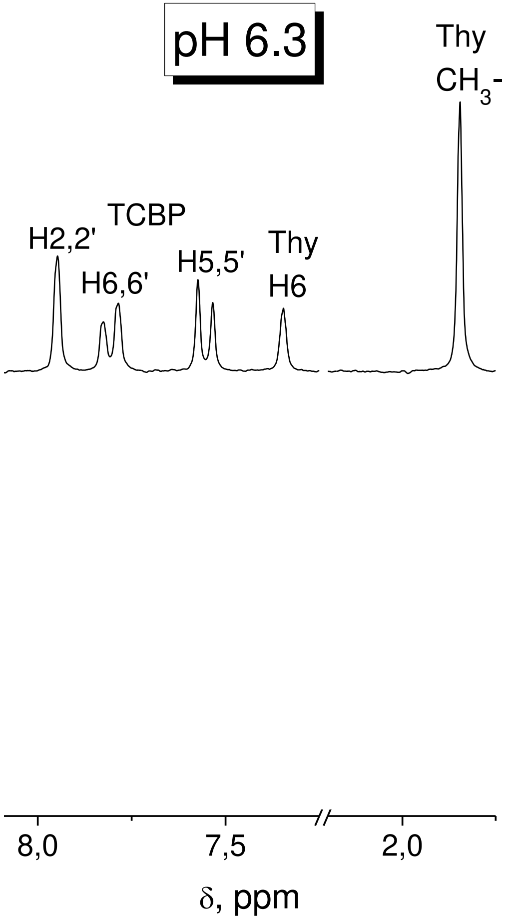
CIDNP kinetics

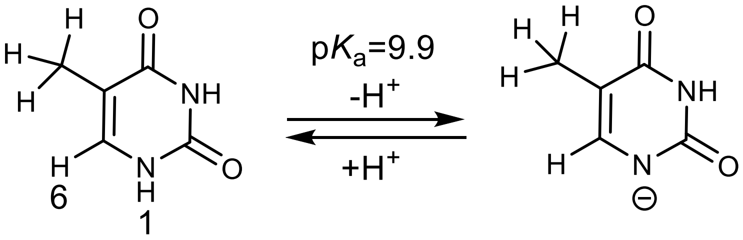


Geminate CIDNP in cyclic photoreaction of TCBP and Thy

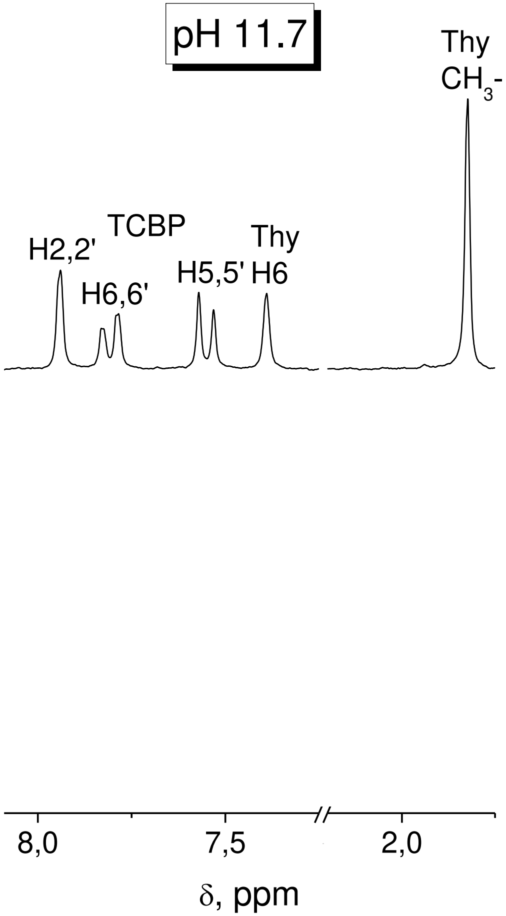


TCBP

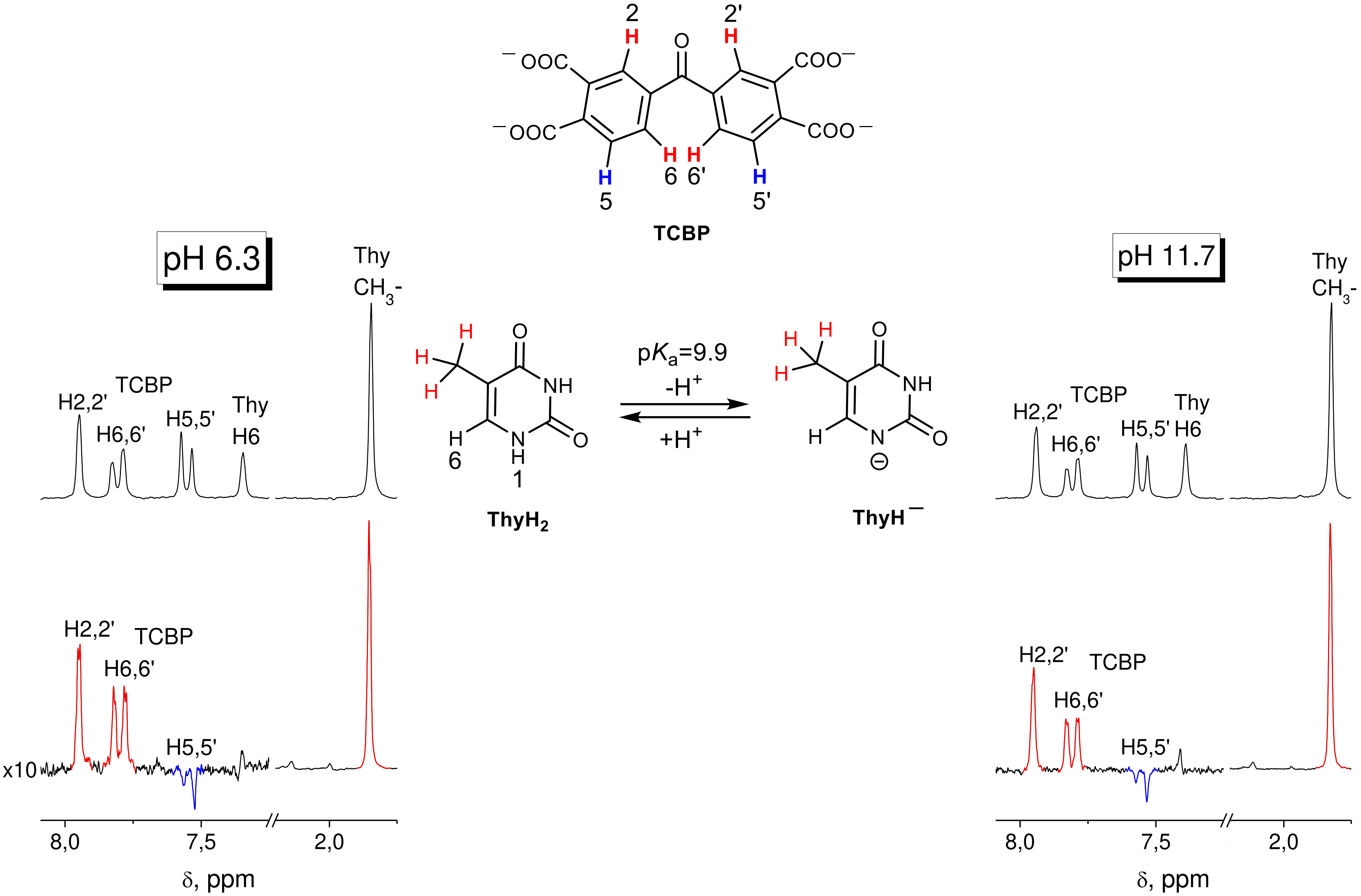




ThyH$_2$ **ThyH$^-$**

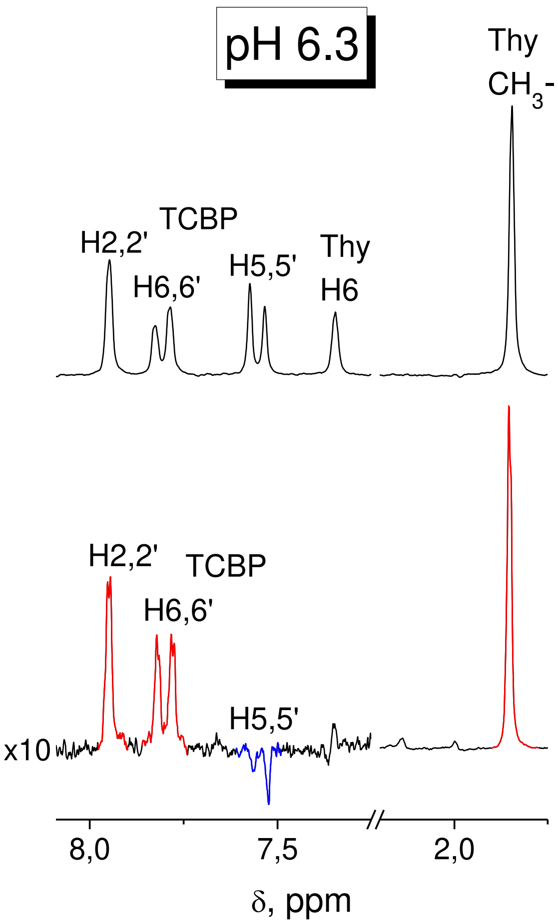


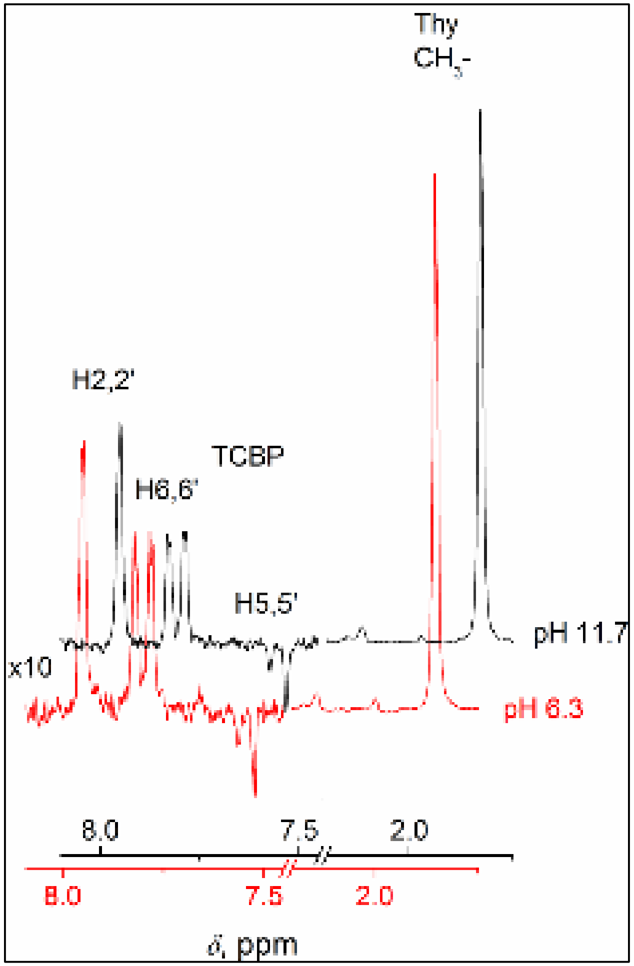
Geminate CIDNP in cyclic photoreaction of TCBP and Thy

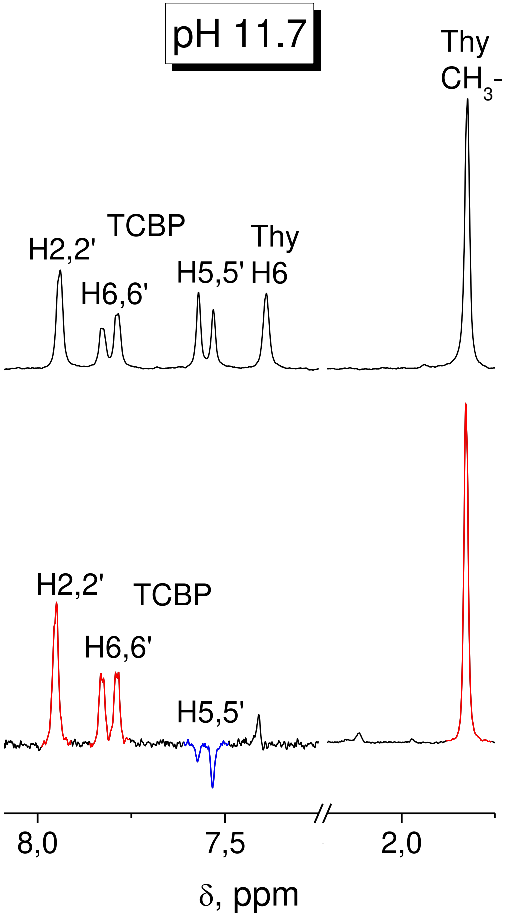


Geminate CIDNP spectrum detected with no delay after the laser pulse

Geminate CIDNP in cyclic photoreaction of TCBP and Thy

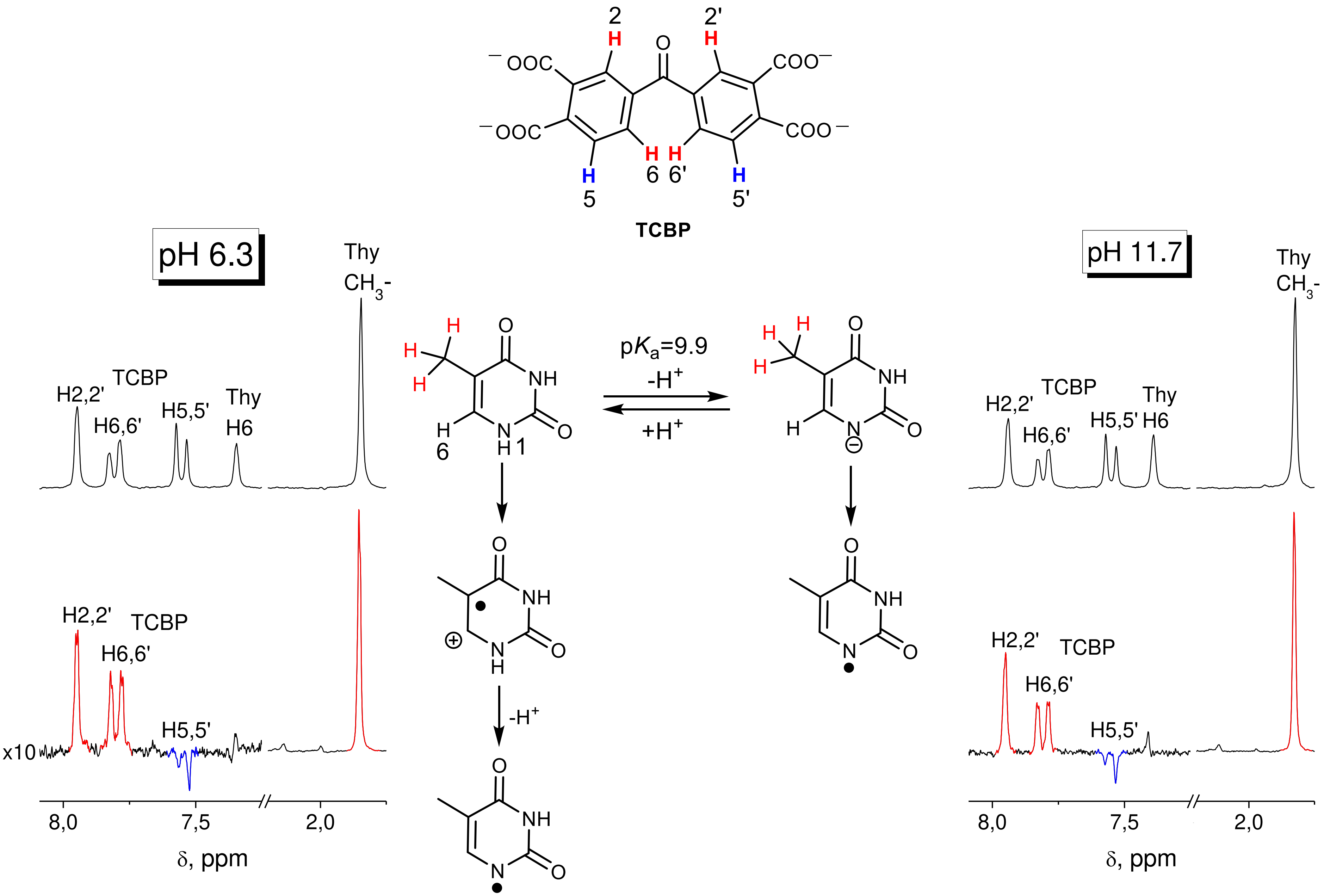






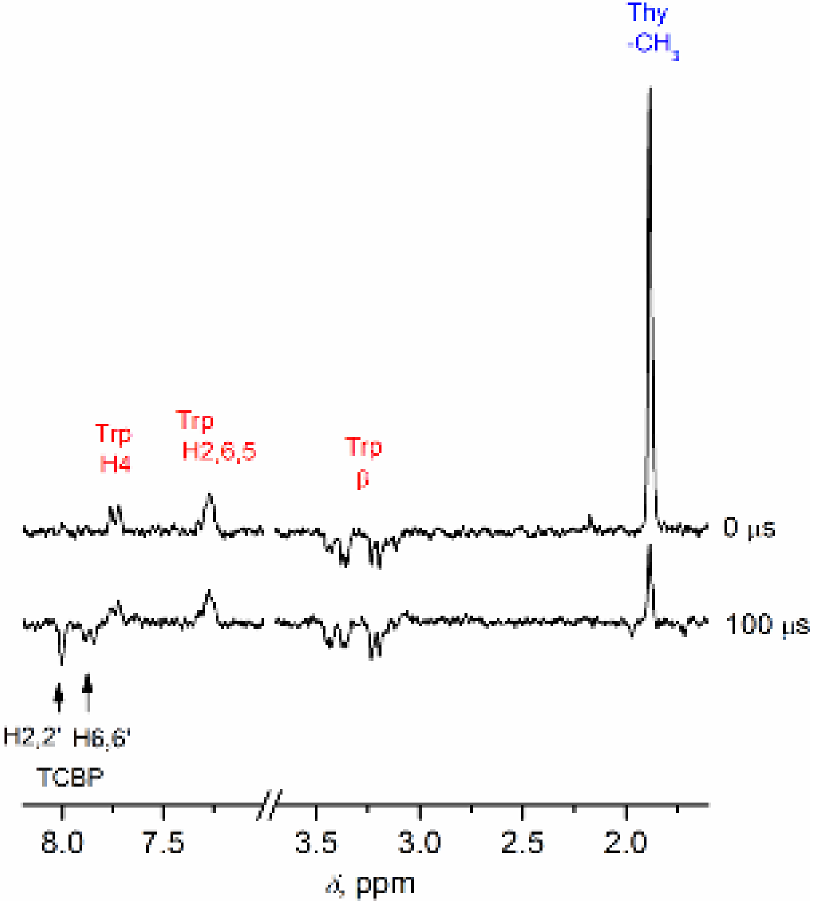
Geminate CIDNP spectrum detected with no delay after the laser pulse τ=0

Geminate CIDNP in cyclic photoreaction of TCBP and Thy

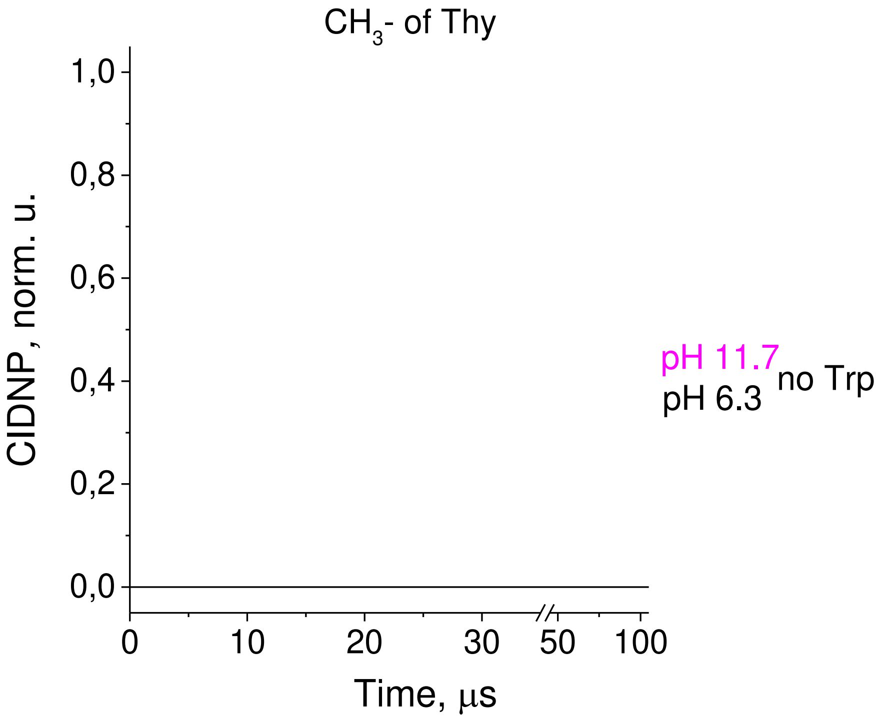


Reduction of Thy radicals by Trp and N-AcTrp

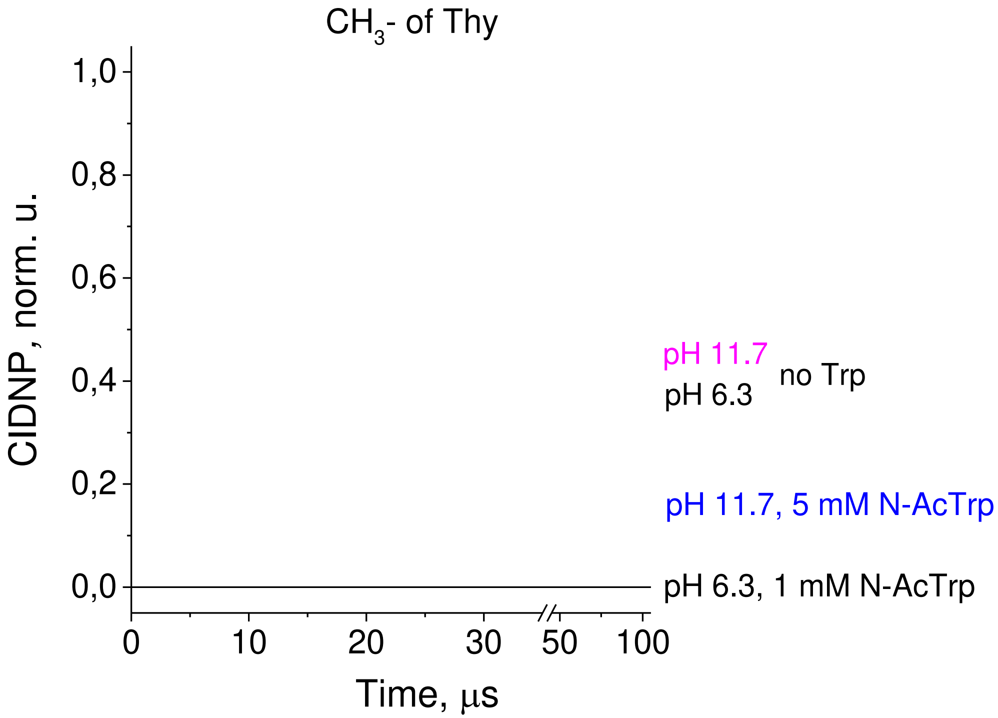
pH 11.7



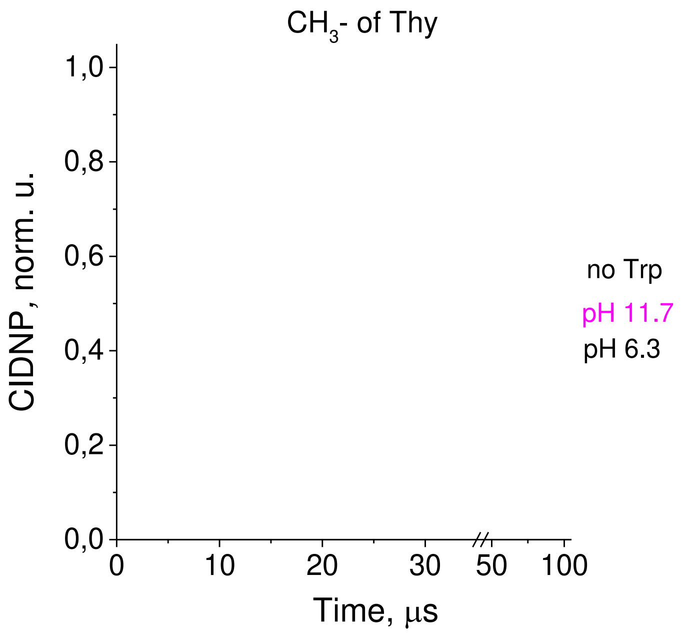
CIDNP kinetics in the photoreaction of TCBP and Thy



Reduction of Thy radicals by N-AcTrp



Reduction of Thy radicals by Trp and N-AcTrp



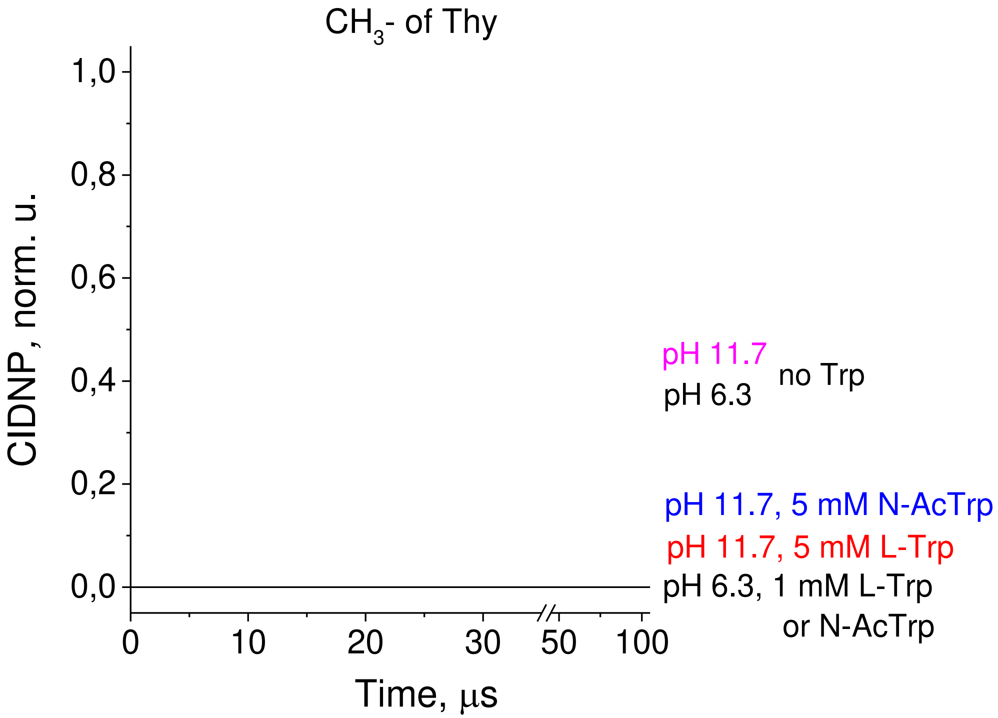
pH 6.3

$$^3\text{TCBP} + \text{ThyH}_2 \xrightarrow{\text{ET}} \text{TCBP}^{\bullet-} + \text{ThyH}_2^{\bullet+} \xrightarrow{\text{PT}} (\text{TCBPH})'^{\bullet} + \text{ThyH}^{\bullet}$$

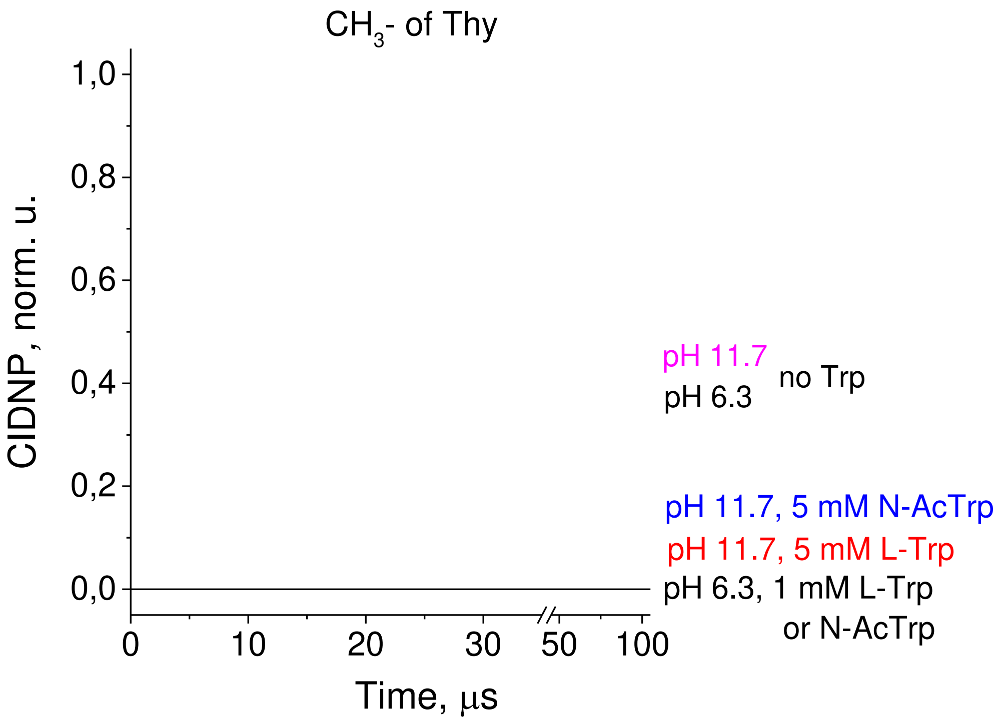
pH 11.7

$$^3\text{TCBP} + \text{ThyH}^- \xrightarrow{\text{ET}} \text{TCBP}^{\bullet-} + \text{ThyH}^{\bullet} \xrightarrow[\text{deprotonation}]{\text{fast}} \text{Thy}^{\bullet-}$$

Reduction of Thy radicals by Trp and N-AcTrp

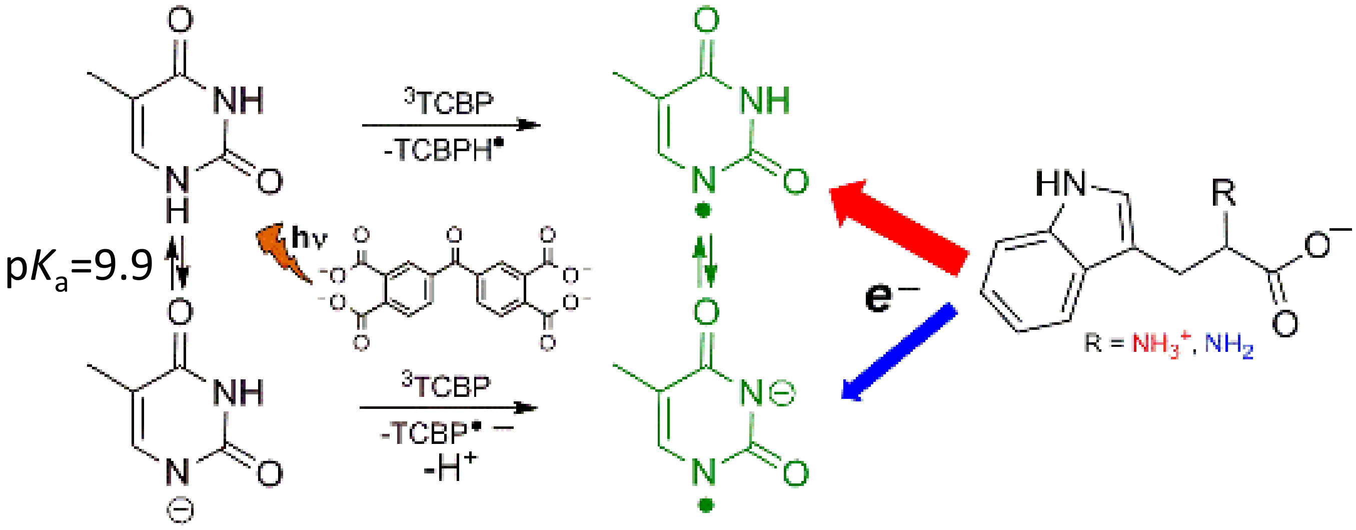


Reduction of Thy radicals by Trp and N-AcTrp



pH	Thy radical	Reducing agent	k_r, $M^{-1}s^{-1}$
6.3	$ThyH^{\bullet}$	$NH_3^{+}TrpH$	$(1.1\pm0.4)\times10^{9}$
6.3	$ThyH^{\bullet}$	N-AcTrpH	$(1.1\pm0.3)\times10^{9}$
11.7	$Thy^{\bullet-}$	NH_2TrpH	$(4.1\pm0.8)\times10^{7}$
11.7	$Thy^{\bullet-}$	N-AcTrpH	$(1.3\pm0.3)\times10^{7}$

Summary



- In neutral aqueous solution (pH 6.3) the neutral thymine radical is formed via PCET to the ^{3}TCBP.
- In strongly basic aqueous solution (pH 11.7) ET leads to the formation of TCBP radical anion and neutral thymine radical, that deprotonate rapidly with the formation of thymine radical anions.
- The reduction of the neutral thymine radical at pH 6.3 and of the thymine radical anion at pH 11.7 by tryptophan and N-acetyl Trp was revealed and the rate constants of these reactions were determined.
- The rate constant of the reduction of the neutral thymine radical is more than an order of magnitude higher than that for the thymine radical anion.

N. Fishman, O. Morozova, H.-M. Vieth, A. Yurkovskaya “Reduction of thymine radicals by Tryptophan: A study of CIDNP kinetics” J. Photochem. Photobiol., A: 426 (2022) 113761

Summary

- Chemically Induced Dynamic Nuclear Polarization (photo-CIDNP) is a hyperpolarization technique that gives information about magnetic interaction in short lived radical intermediates with atomic resolution.

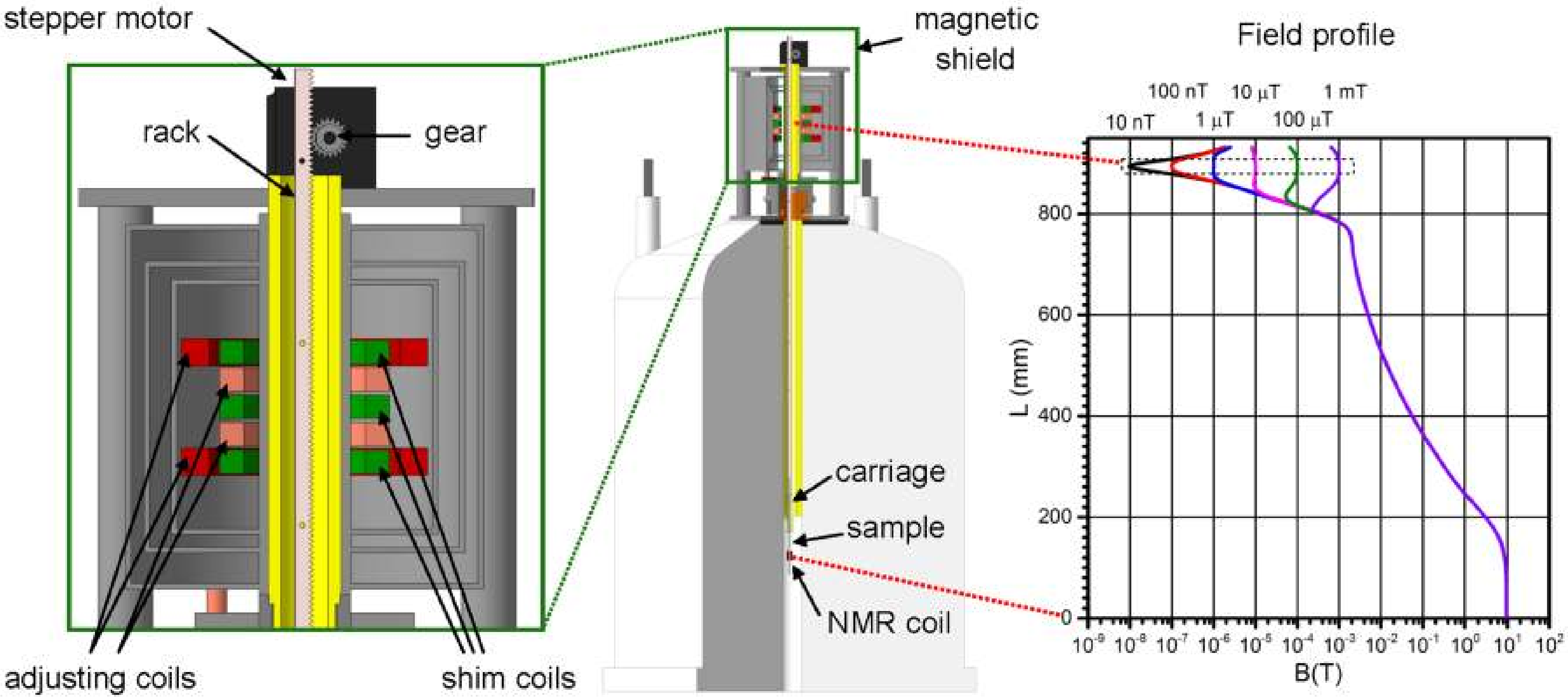
- CIDNP serves as a "fingerprint" of short-lived radicals:

(1) an assignment of the polarization signals in CIDNP spectra of the radical reaction products for the establishing of the structure of the transient radicals;

(2) an analysis of geminate CIDNP patterns in order to define quantitatively (the values and the sign) the HFCCs of the corresponding nuclei in the intermediate radicals;

(3) a kinetic analysis of the time-resolved CIDNP data and revealing of the absence of degenerate electron exchange in order to determine the protonated state of participating thymine radicals.

Field-Cycling NMR Spectrometer
Range 5 nT to 9.4 T



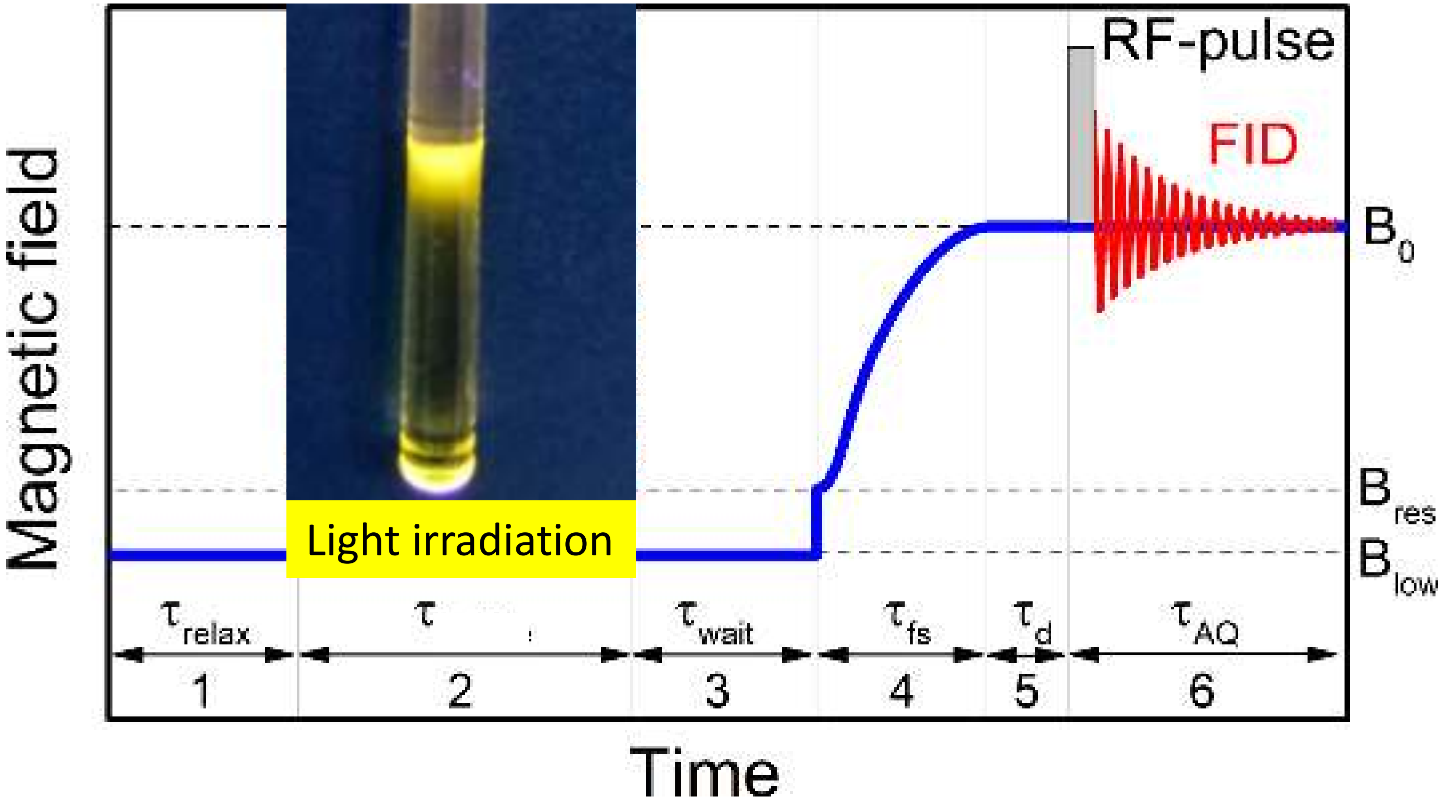
Operation mode: Fast shuttling between observation field and evolution field

1 mT to 9.4 T: position the sample in the fringe field of the NMR magnet;

5 nT to 1 mT: place sample in center of magnetic shield (cylinder made out of µ-metal), where coil system is mounted that serves for setting the field and for shimming

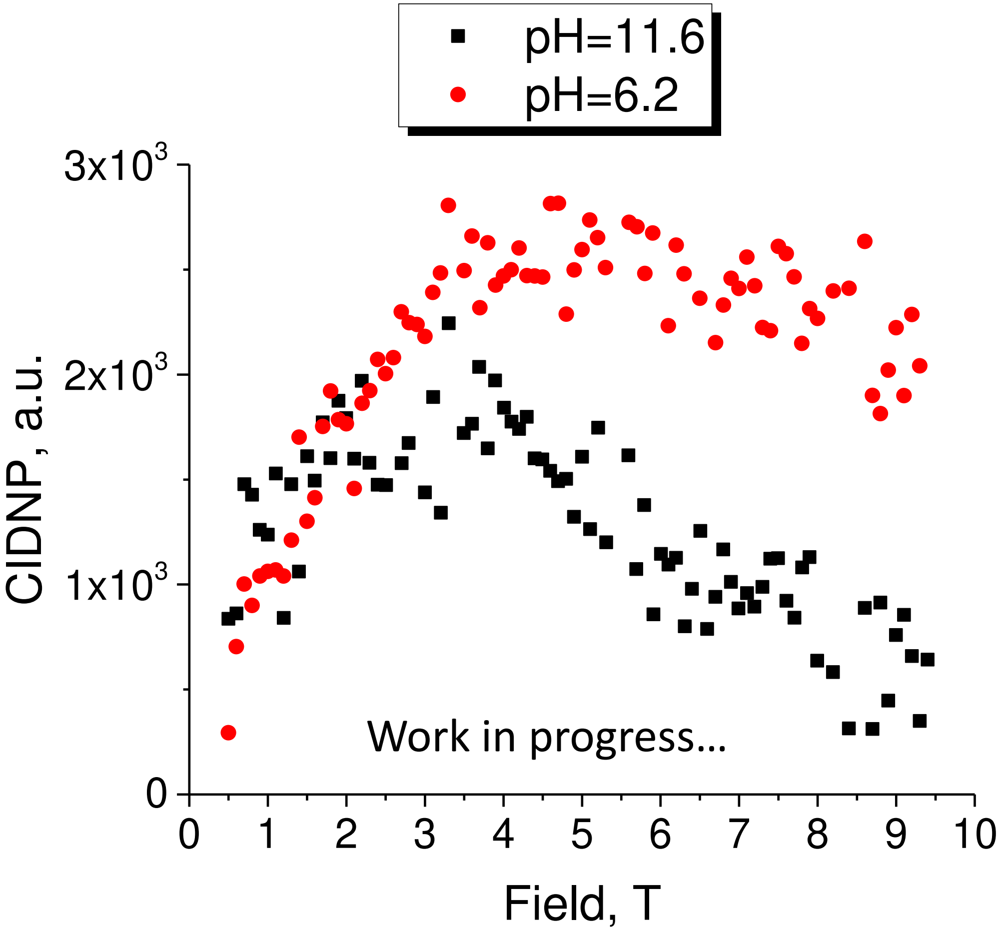
Time resolved PLUS field dependent CIDNP

Experimental protocol for photo-CIDNP magnetic field dependence



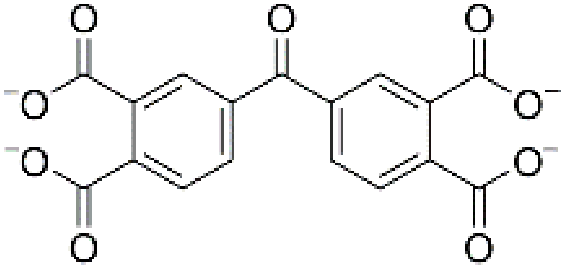
Stages: relaxation at B_{low} (**1**) 10-20s, sample irradiation during τ at B_{low} 1-10s (**2**), evolution at variable field B_{res}, 0-5s , optional (**3**), field switching <0.5 s (**4**), NMR signal detection at 9.4 T after τ_δ =0.1s (**5,6**).

CIDNP field dependence in the photoreaction of TCBP and Thy



CIDNP maximum at high magnetic field B_0 relates to the difference in g-factor $\Delta g = (g_1 - g_2)$

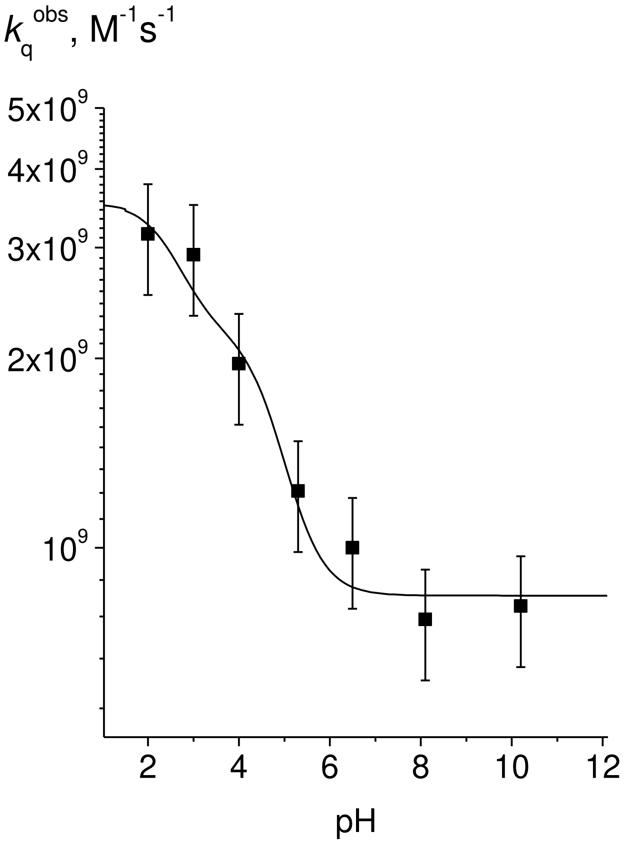
Photoinduced reaction between TCBP and Thy



3,3′,4,4′-tetracarboxy benzophenone (TCBP)

$$\mathrm{TCBP} \xrightarrow{h\nu} {}^{1}\mathrm{TCBP} \longrightarrow {}^{3}\mathrm{TCBP}$$

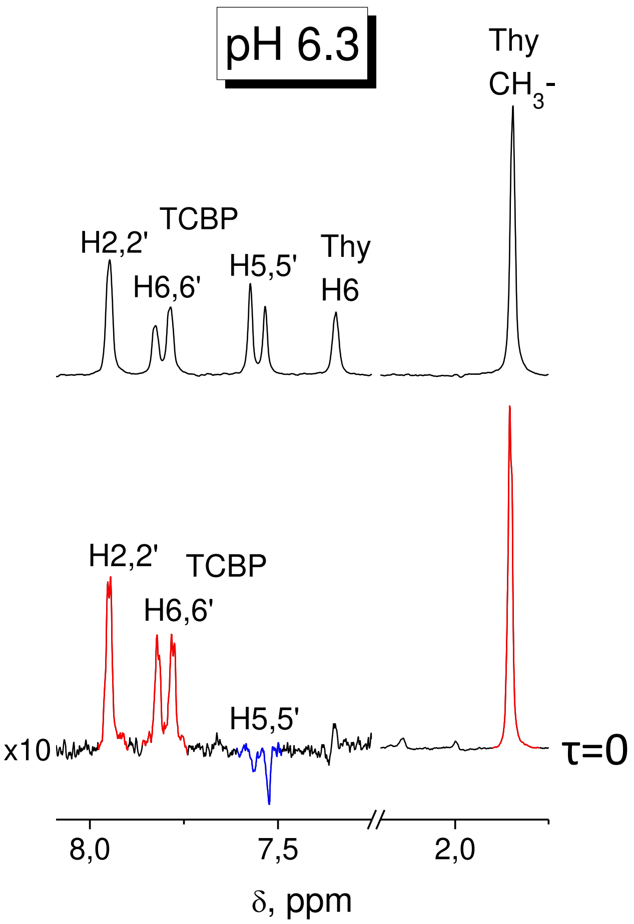
- water-soluble
- high triplet energy
- well studied by LFP, EPR and CIDNP

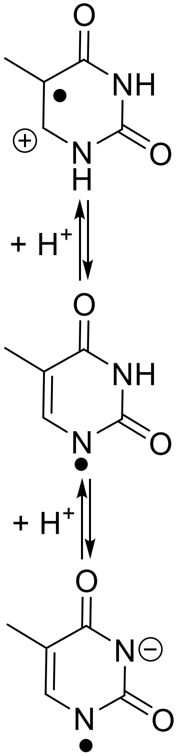


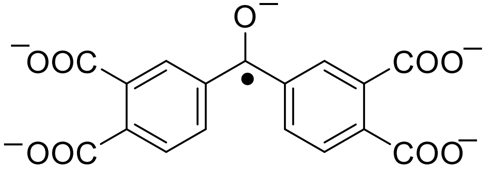
*T.X. Nguyen, D. Kattnig, A. Mansha, G. Grampp, A.V. Yurkovskaya, N. Lukzen, J. Phys. Chem. A 116 (2012) 10668.

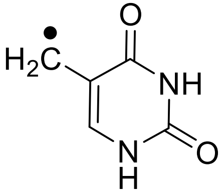
Geminate CIDNP in cyclic photoreaction of TCBP and Thy

Kaptein’s rule: Γ = sgn(Δg) × sgn(A)
for a triplet precursor and for geminate polarization



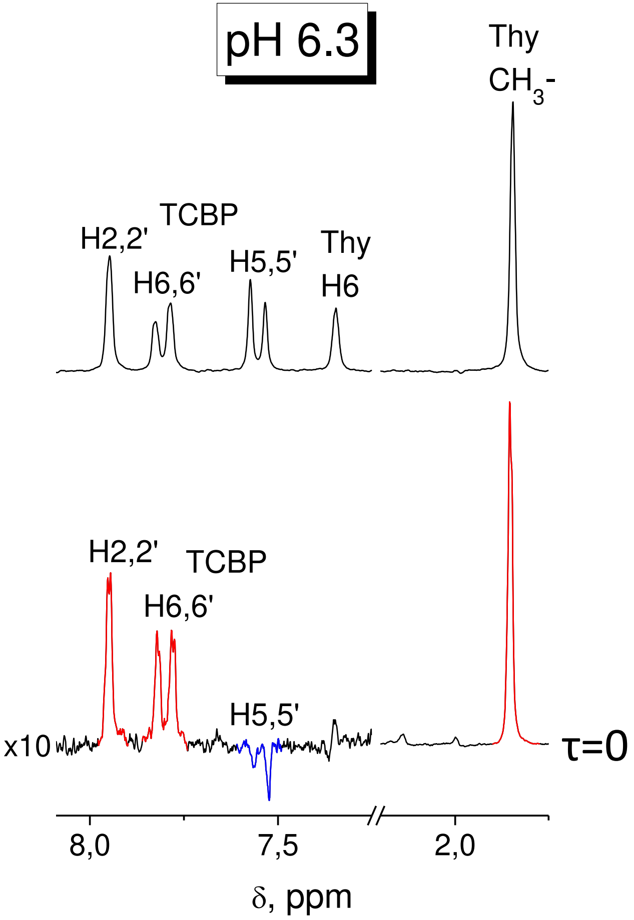


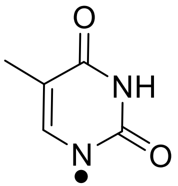




Geminate CIDNP in cyclic photoreaction of TCBP and Thy

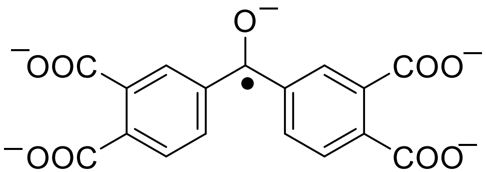
Kaptein's rule: Γ = sgn(Δg) × sgn(A)
for a triplet precursor and for geminate polarization



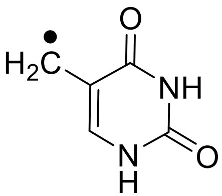


g=2.0046/2.0041
A(CH_3)>0

Γ > 0



g=2.0035
A(H2, H6)<0
A(H5)>0

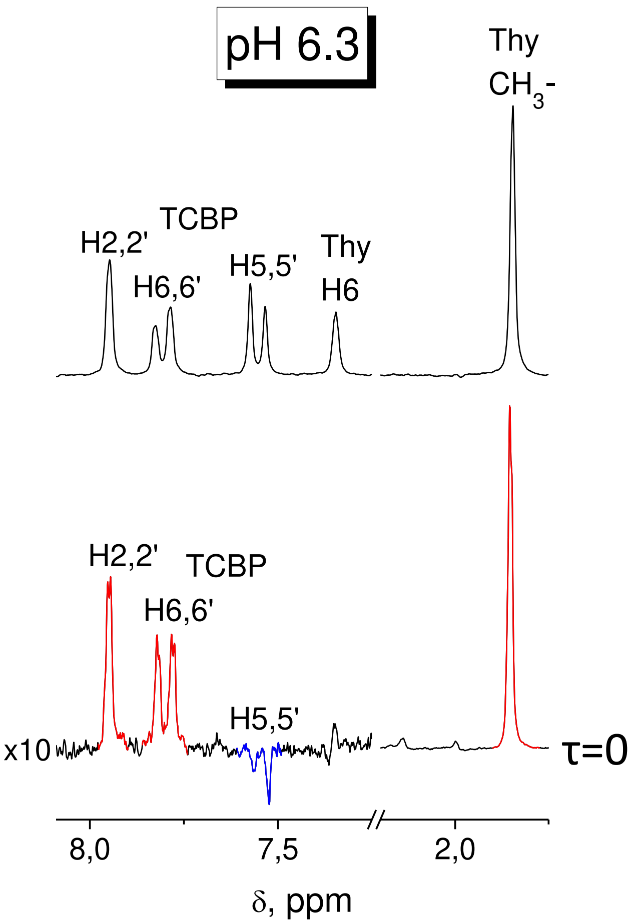


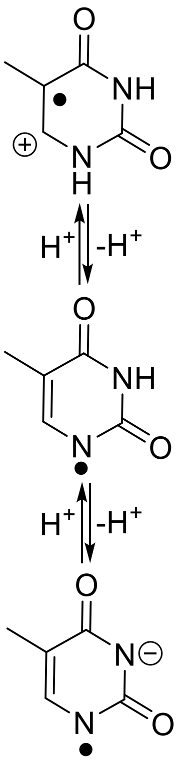
g=2.0023
A(CH_2)>0

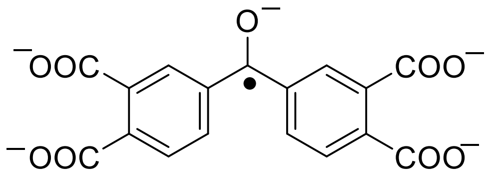
Γ < 0

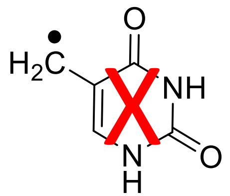
Geminate CIDNP in cyclic photoreaction of TCBP and Thy

Kaptein’s rule: Γ = sgn(Δg) × sgn(A)
for a triplet precursor and for geminate polarization









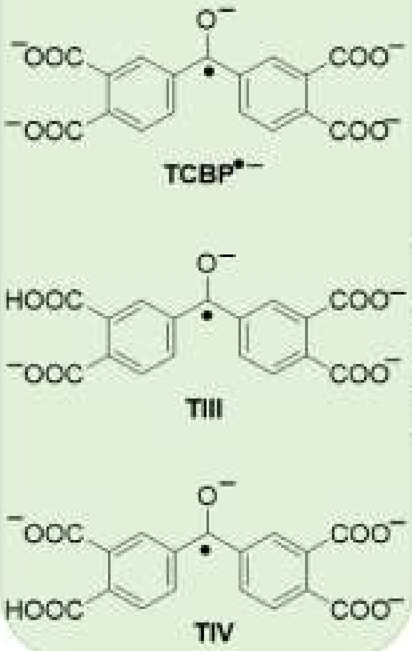
Proportionality between HFCCs and geminate CIDNP

$$\mathrm{CIDNP(Thy)} = CT_{hy} \cdot \mathrm{HFCC\ (Thy)}$$

$$\mathrm{CIDNP(TCBP)} = CT_{CBP} \cdot \mathrm{HFCC\ (TCBP)}$$

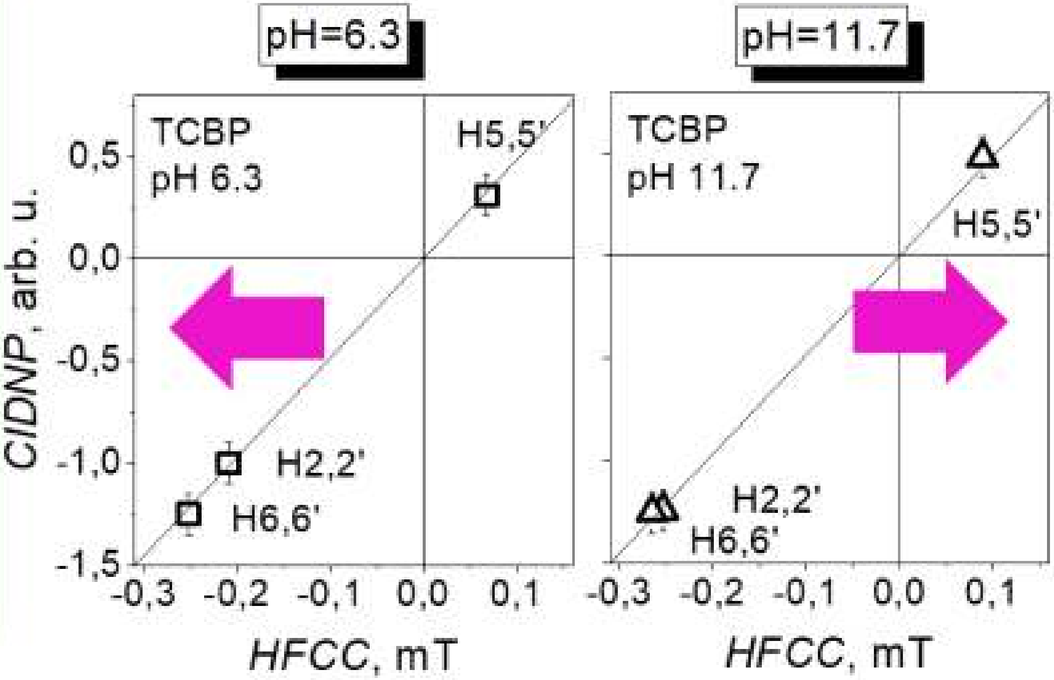
$$C_{Thy} = -C_{TCBP}$$

$R^2 = 0.998$

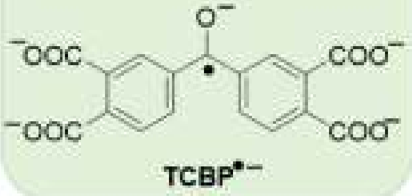


proton-coupled electron transfer

^{3}TCBP+ThyH$_2$



$R^2 = 0.999$



electron transfer

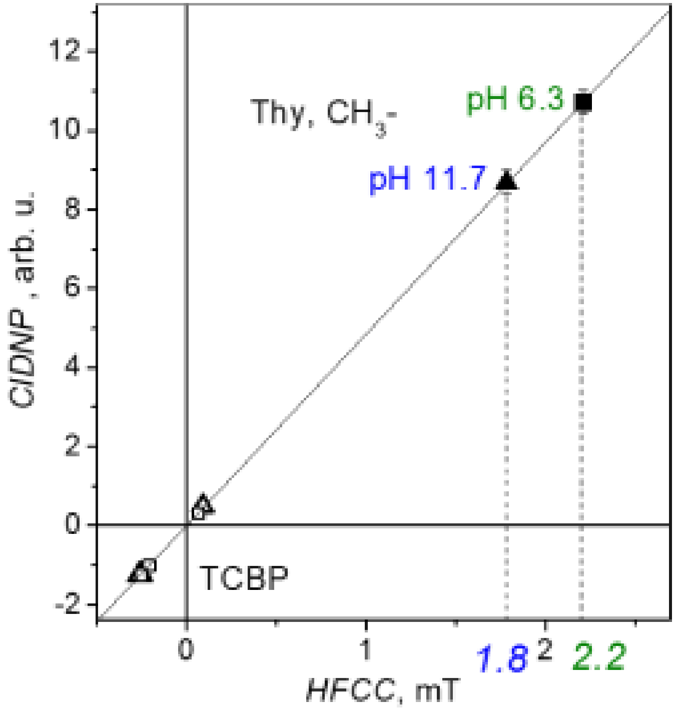
^{3}TCBP+ThyH$^-$

Proportionality between HFCCs and geminate CIDNP

Geminate CIDNP spectra provide HFCCs of Thy radicals: CIDNP(Thy) = CT_{hy} · HFCC (Thy)

CIDNP(TCBP) = CT_{CBP} · HFCC (TCBP)

$C_{Thy} = - C_{TCBP}$



pH 11.7

$$^3\text{TCBP} + \text{ThyH}^- \xrightarrow{\text{ET}} \text{TCBP}^{\bullet-} + \text{ThyH}^\bullet$$

A(CIDNP)=1.8 mT
A(DFT)=1.84 mT

pH 6.3

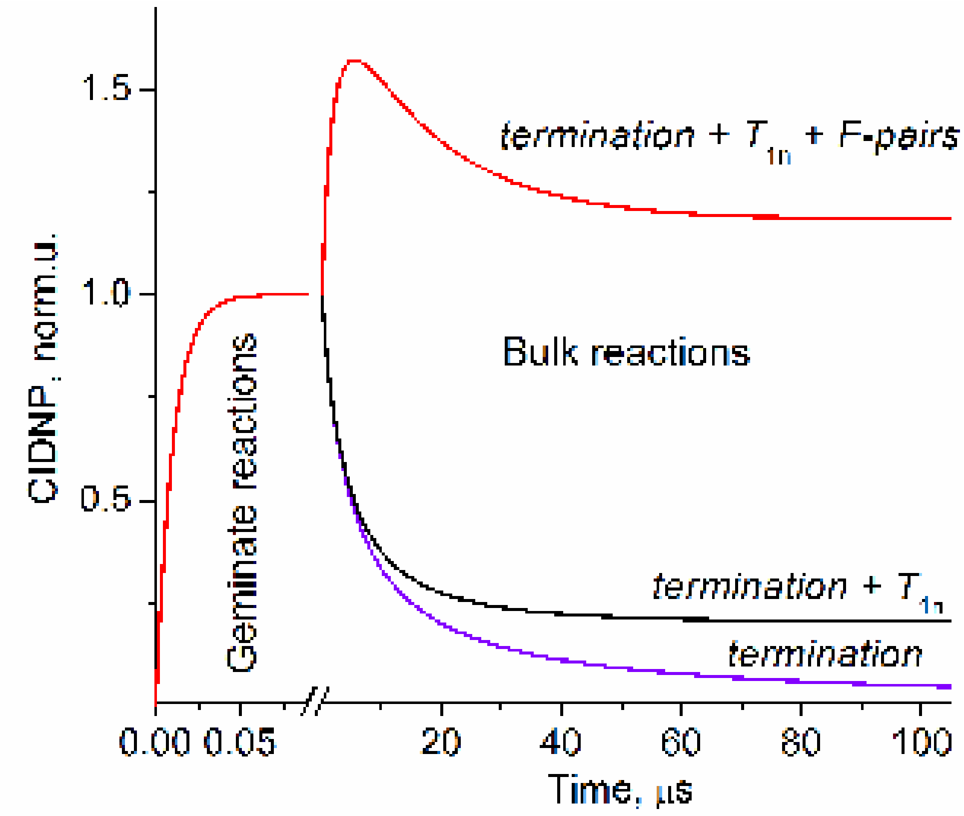
$$^3\text{TCBP} + \text{ThyH}_2 \xrightarrow{\text{ET}} \text{TCBP}^{\bullet-} + \text{ThyH}_2^{\bullet+} \xrightarrow{\text{PT}} (\text{TCBPH})'^\bullet + \text{ThyH}^\bullet$$

A(DFT)=2.37 mT

A(DFT)=1.84 mT

0.68:0.32
A(CIDNP)=2.2 mT

Typical CIDNP time traces



$$\frac{dP_r}{dt} = -k_t R P_r - \beta k_t R^2 - \frac{P}{T_{1n}};$$

$$\frac{dP}{dt} = k_t R P_r + \beta k_t R^2 \,,$$

initial conditions

$$P(t=0) = -P_r(t=0) = P_G$$

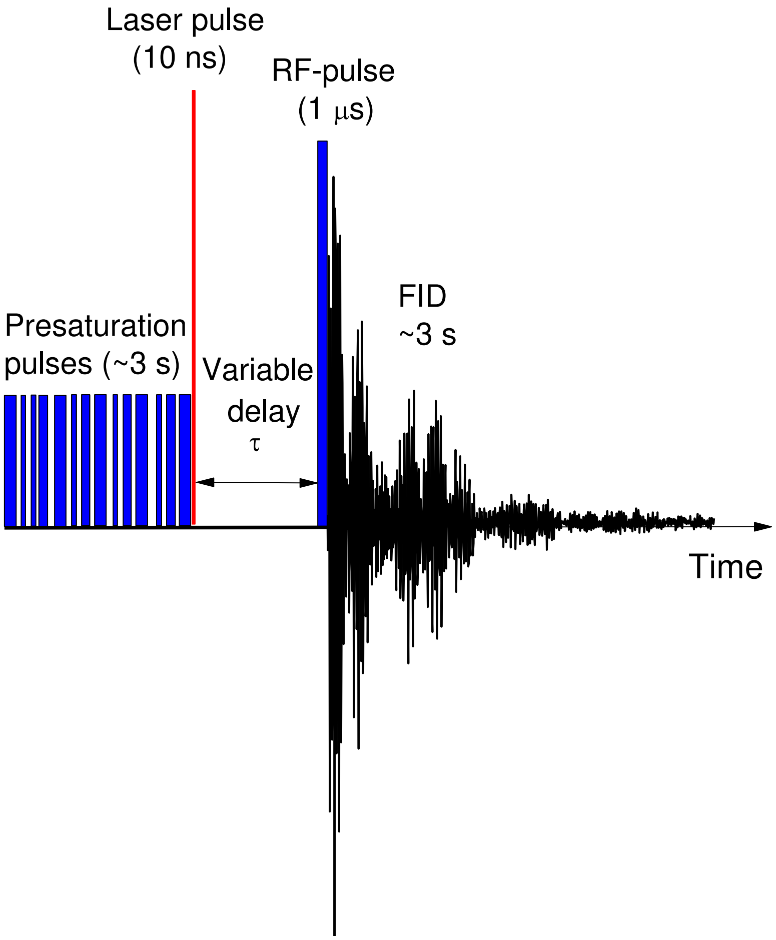
Bimolecular reaction kinetics :

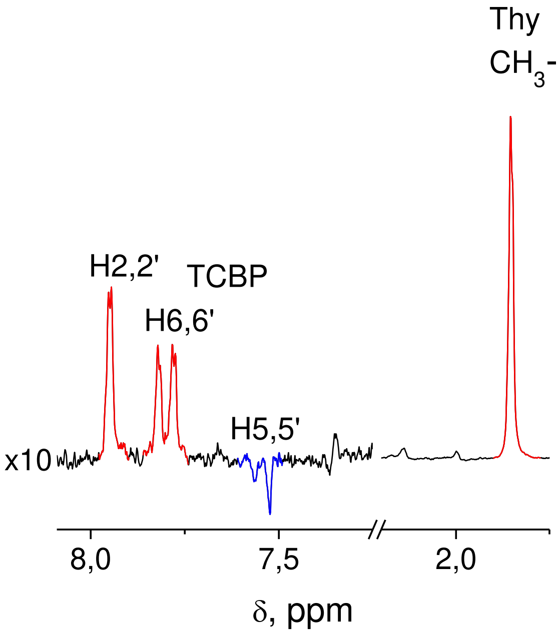
$$R(t) = \frac{R_0}{1+k_t R_0} \quad , \qquad R_0 = R(t=0)$$

$$\gamma = \frac{R_0 \beta}{P_G}$$

$$\gamma = \frac{P_F}{P_G} = \frac{P_{T_0}}{P_{T_0}/3} = 3$$

CIDNP kinetics in the photoreaction of TCBP and Thy





τ=0

τ=1 μs

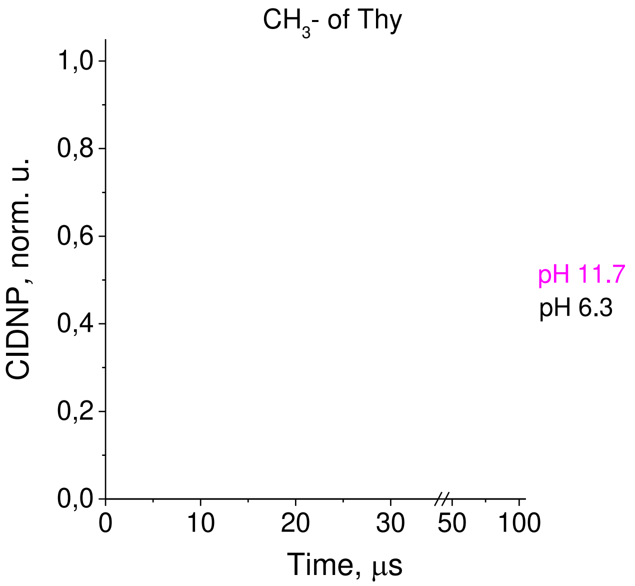
τ=2 μs

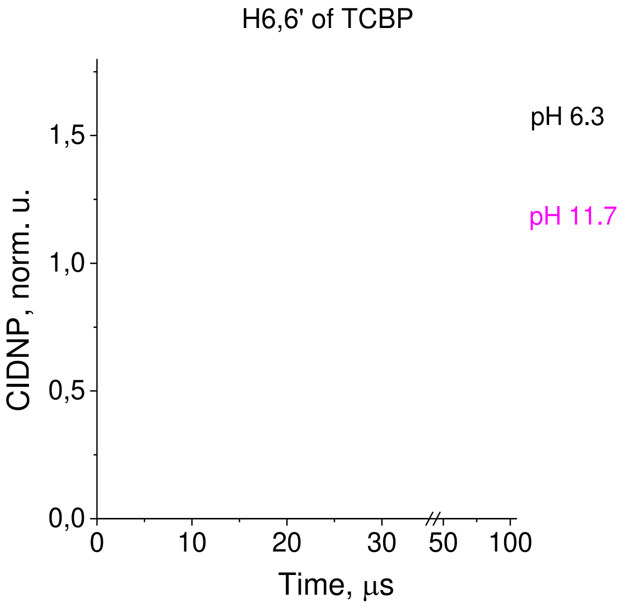
τ=5 μs

…

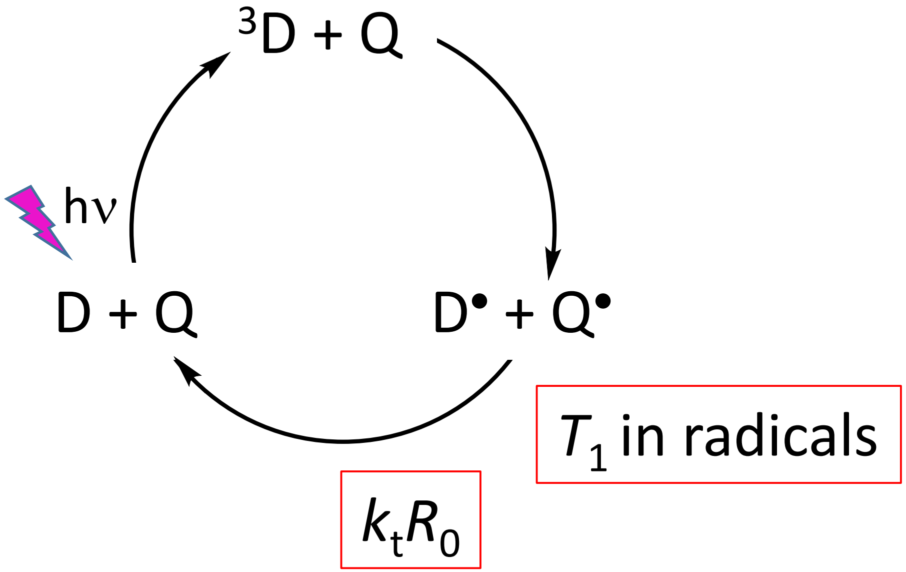
τ=100 μs

CIDNP kinetics in the photoreaction of TCBP and Thy





CIDNP in cyclic photoreactions



^{3}D + Q

hν

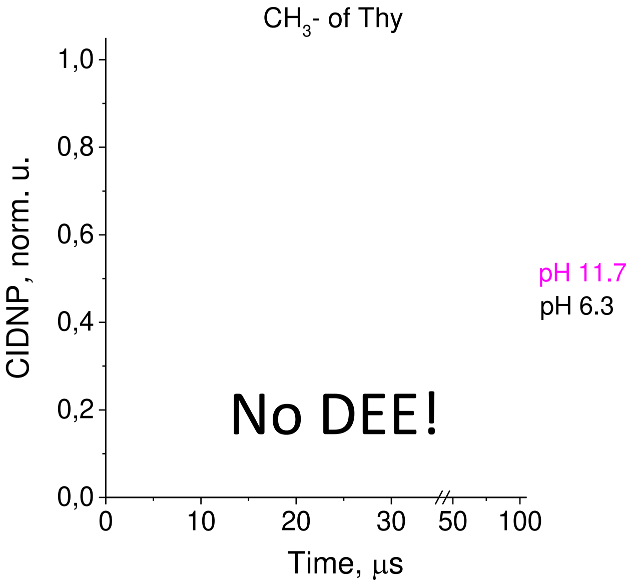
D + Q

D$^\bullet$ + Q$^\bullet$

T_1 in radicals

k_tR_0

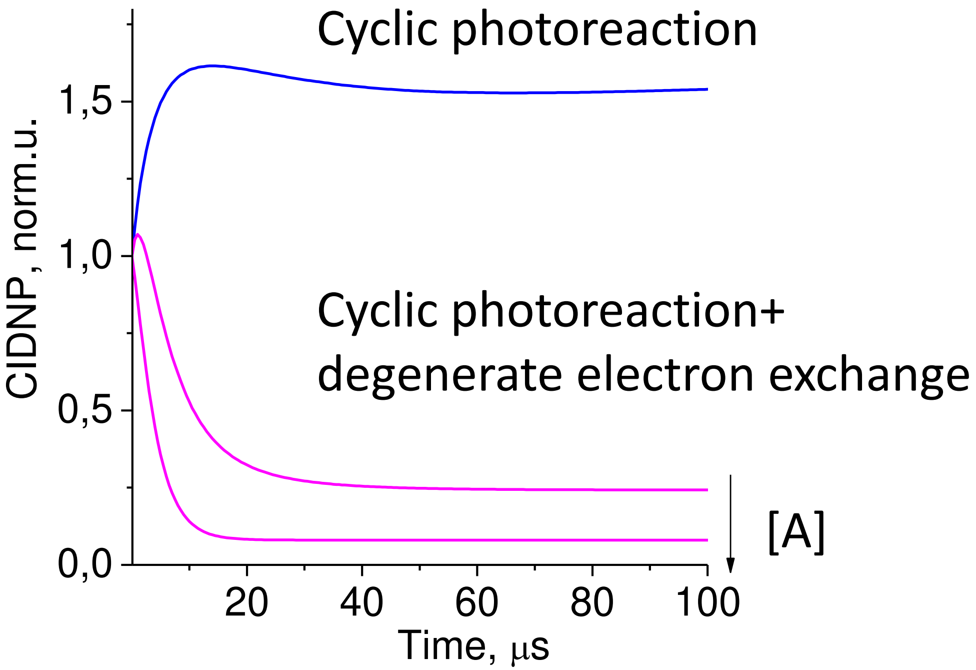
CIDNP kinetics in the photoreaction of TCBP and Thy



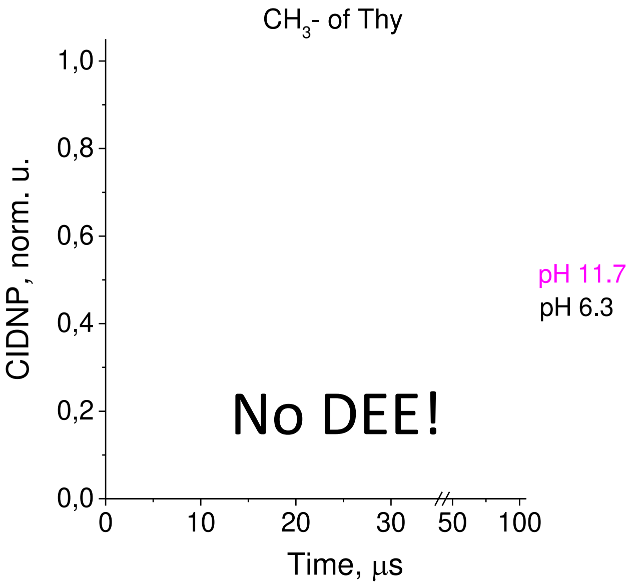
No DEE!

Degenerate electron exchange (DEE):

$$^{P}A^{+\bullet} + A \rightarrow {}^{P}A + A^{+\bullet}$$



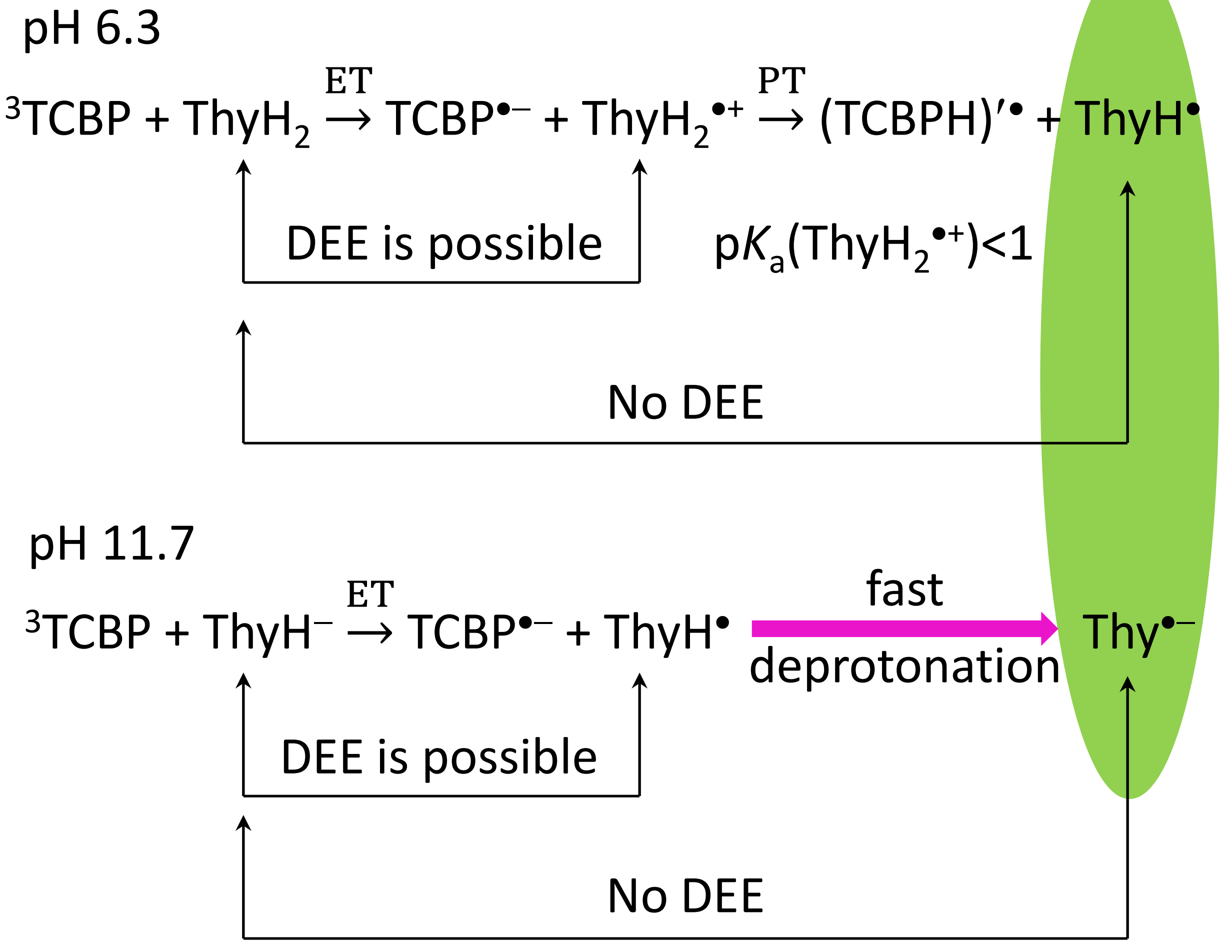
CIDNP kinetics in the photoreaction of TCBP and Thy



No DEE!

No degenerate electron exchange (DEE):

$$^{P}A^{+\bullet} + A \cancel{\rightarrow} {}^{P}A + A^{+\bullet}$$



pH 6.3

$$^{3}TCBP + ThyH_2 \xrightarrow{ET} TCBP^{\bullet-} + ThyH_2^{\bullet+} \xrightarrow{PT} (TCBPH)'^{\bullet} + ThyH^{\bullet}$$

DEE is possible

$pK_a(ThyH_2^{\bullet+})<1$

No DEE

pH 11.7

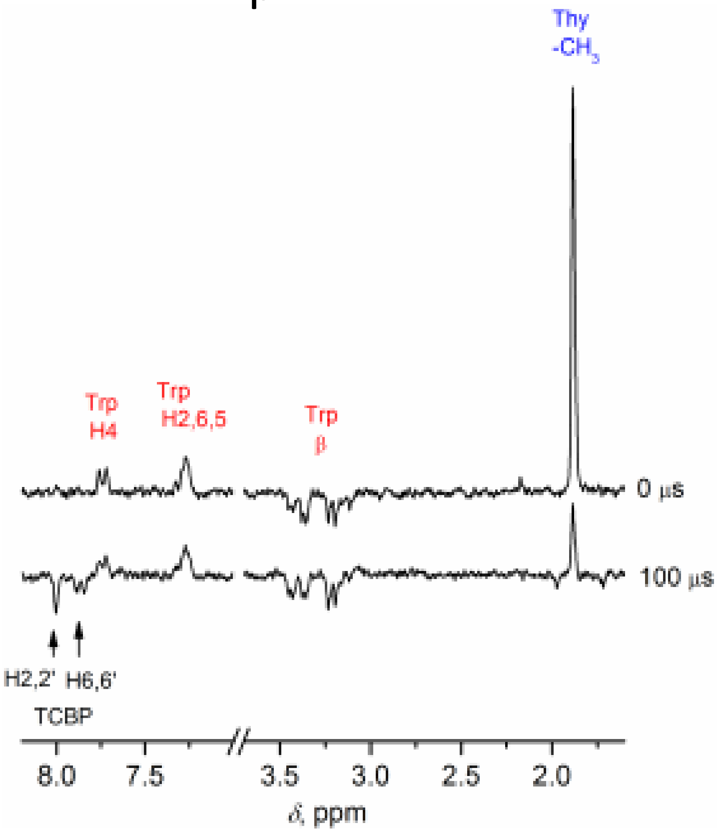
$$^{3}TCBP + ThyH^{-} \xrightarrow{ET} TCBP^{\bullet-} + ThyH^{\bullet} \xrightarrow[\text{deprotonation}]{\text{fast}} Thy^{\bullet-}$$

DEE is possible

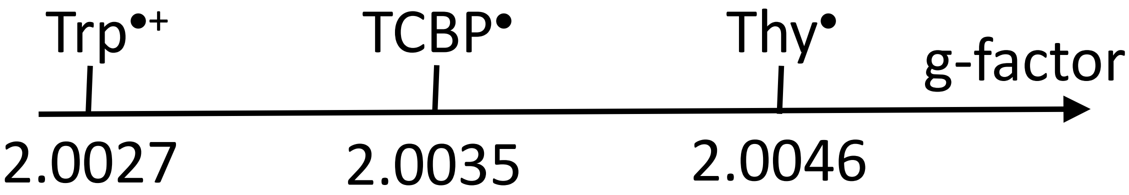
No DEE

Reduction of Thy radicals by Trp and N-AcTrp

pH 11.7



CIDNP sign of TCBP



Reduction of Thy radicals by Trp and N-AcTrp

pH 6.3

$$\mathrm{ThyH^{\bullet} + TrpH \rightarrow ThyH_2 + Trp^{\bullet}}$$

pH 11.7

$$\mathrm{Thy^{\bullet -} + TrpH \rightarrow ThyH^{-} + Trp^{\bullet}}$$